Systematic analysis of the evolution and conservation of genetic interactions using *C. elegans* as a model system

This dissertation is submitted in accordance with the requirements of the University of Cambridge for the degree of Doctor of Philosophy

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To my parents, with love

Preface

This thesis describes my work undertaken in the laboratory of Andrew G. Fraser at the Wellcome Trust Sanger Institute while member of Clare Hall, University of Cambridge. It is submitted in fulfilment of the requirements for the degree of Doctor of Philosophy. This dissertation is the result of my own work and includes nothing, which is the outcome of work done in collaboration except where specifically indicated in the text. The work described here has not been submitted for any degree, diploma, or other qualification. This thesis does not exceed 300, single-sided pages of double spaced text, not including the bibliography and appendices.

Julia Tischler

Cambridge, September 2007

Abstract

Systematic analyses of loss-of-function phenotypes have been carried out for the majority of genes in *S. cerevisiae, C. elegans,* and *D. melanogaster*. While these studies greatly expand our knowledge of individual gene functions, they do not address redundancy in genetic networks nor do they attempt to identify genetic interactions. Developing tools for the systematic mapping of genetic interactions is thus a crucial step for exploring gene networks.

I established protocols for simultaneously targeting multiple genes by RNA interference (RNAi) in *C. elegans* using bacterial feeding (‘combinatorial RNAi'). This approach allows me to examine interactions between any pair of genes and to detect the great majority of previously known synthetic lethal (SL) and post-embryonic synthetic genetic interactions. I used this technique to provide the first large-scale analysis in any organism of the redundant functions of gene duplicates. Focusing on genes that have been duplicated in the genome of *C. elegans* since divergence from either *S. cerevisiae* or *D. melanogaster,* I identified 16 out of 143 of duplicated gene pairs amenable to analysis by combinatorial RNAi to be at least partially functionally redundant. Intriguingly, the majority of these redundant gene pairs were duplicated before the split of *C. elegans* and *C. briggsae* 80-110 million years ago. My findings support population genetics models, which suggest that redundancy is not just a transient side effect of recent gene duplication but is instead a phenomenon that can be maintained over substantial periods of evolutionary time.

While I have identified functional redundancy between gene duplicates, most redundancy in genetic networks tends to be more complex. The majority of synthetic lethal interactions that were uncovered in *S. cerevisiae* occur between genes unrelated at the sequence level. To date, there is still much debate about how such ‘higher-order’ functional redundancy might arise, whether it is a selectable trait, and whether such redundancy can be conserved throughout evolution. Thus, to shed light on the evolution of genetic interactions, I investigated the conservation of gene networks between *S. cerevisiae* and *C. elegans*. Using an RNAi-based approach, I set out to explore whether

individual synthetic lethal interactions uncovered in *S. cerevisiae* are retained in *C. elegans*. I found synthetic lethal interactions to be poorly conserved between yeast and worm — despite the very high degree of conservation of individual gene functions — demonstrating a substantial evolutionary plasticity of complex gene networks. My results suggest that SL interactions are unlikely to be explained by simple models of genetic redundancy and led me to propose a novel model for the interpretation of SL interactions. In this view ('induced essentiality'), SL interactions represent a special form of conditional essentiality.

Publications

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Tischler, J,, Lehner, B., Chen, N., Fraser, A. G. (2006). Combinatorial RNA interference in *C. elegans* reveals that redundancy between gene duplicates can be maintained for more than 80 million years of evolution. *Genome Biol,* **7**, R69.

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Lehner, B., **Tischler, J.**, Fraser, A. G. (2005). Systems biology: where it's at in 2005. *Genome Biol*,. 6, 338.

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Chapter 1

Introduction

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Introduction

While biological research in the second half of the 20th century embraced 'molecule-centered' approaches to defining the properties of individual components of living systems, this reductionist view has changed with the availability of whole genome sequences for numerous model organisms. By employing integrated computational-experimental approaches, it had become a reachable goal to unravel the molecular functions of each individual gene. These advances led to the appreciation that for a better understanding of living systems, one needs to take a step beyond studying gene functions one at a time. Researchers realized that properties of systems — e.g. organisms, gene networks, protein complexes — are more than merely the sum of their parts. This view led to defining a new area of research, 'Systems Biology' (Ideker *et al.*, 2001; Kitano, 2002a; Kitano, 2002b; Pennisi, 2003). The central aim of Systems Biology is to gain insights into the 'emergent' properties of systems, features that cannot be attributed to any of their individual components. In order to get an understanding of such emergent properties, one needs firstly to identify all of a system's individual components, secondly to unravel the function(s) of each individual component and, finally, to decipher how these individual components interact to result in system-level dynamics (reviewed in Strange, 2006).

1.1. Outline of introduction

In this introduction, I will discuss the major steps that have been taken in the post-genomics era to address the fundamental biological question: 'How does an organism's genotype relate to its phenotype?' Since I used *C. elegans* as model organism for my studies, I will start by introducing 'the worm' and its versatility as model system. I will then describe the various genomics approaches that have been taken to generate genome-scale views of gene function in *C. elegans*, *D. melanogaster*, and *S. cerevisiae*. Moreover, I will discuss the insights gained from large-scale studies of gene functions in worm, fly, and yeast and consequently the hypotheses that have been brought forward. I will conclude by introducing the rationale, aim, and significance of my study.

1.2. *Caenorhabditis elegans* as a model system

The nematode *Caenorhabditis elegans* — also referred to as 'the worm' — has been extensively used as an experimental organism for genetics, development and neurobiology in the 1970s (Brenner, 1974). Numerous unique attributes make *C. elegans* a powerful model system (reviewed in Jorgensen and Mango, 2002; Strange, 2006): Worms have a short life–cycle, with a three-day generation time at room temperature. Animals pass through four larval stages (L1-L4), before they reach adulthood and become fertile. Worms are small in size (~1 mm), produce ~ 300 progeny per animal and can be grown easily and inexpensively in the laboratory on agar plates or in liquid cultures. *C. elegans* feed on bacteria, but can enter a specific developmental programme — called the 'dauer stage' — under limiting food conditions, in which they can survive for months. Furthermore, worms can be kept as frozen stocks.

C. elegans reproduces through self-fertilization in hermaphrodites or by mating with males. Hermaphrodites carry two X chromosomes, whereas males are of X0 karyotype, which arises through occasional meiotic non-disjunction. Self-fertilization allows worms to be propagated clonally and greatly facilitates the isolation of mutants through genetic screens, while males are used for inter-crossing mutant strains.

C. elegans is a highly differentiated animal but of simple anatomy — hermaphrodites comprise of 959 somatic cells only. Nonetheless, worms share many tissues with more complex animals, such as the nervous system, muscles, a reproductive and gastro-intestinal tract and an epidermis. Heroic efforts have been made to trace the fate of every somatic cell in *C. elegans*, starting from the first division (Sulston and Horvitz, 1977; Sulston *et al.*, 1983). This observation culminated in a documentation of the complete cell lineage of the worm, which was found to be relatively invariant. This study was followed by a comprehensive description of the structure of the nervous system, which resulted in a wiring diagram of all 302 *C. elegans* neurons (White *et al.*, 1986).

1.3. Uncovering gene functions in *C. elegans*

1.3.1. Forward genetic screens

Traditionally, ethyl methane sulphonate- (EMS-) or N-ethyl-N-nitrosourea- (ENU-) induced mutagenesis followed by genetic screening has been used to identify genes that function in a biological process or pathway of interest (reviewed in Jorgensen and Mango, 2002). Typically, about one null mutation in any single gene is recovered in ~ 2,000 genomes through such forward genetic approaches. By using mutagenesis screens, Sydney Brenner identified 619 mutants with visible phenotypes (Brenner, 1974). These were instrumental in establishing *C. elegans* as a key model organism, and many similar screens have been performed since then.

1.3.2. Reverse genetic approaches

As an alternative to forward genetic analysis, injection of anti-sense RNAs homologous to any gene of interest has been used as a means to reduce gene expression from endogenous loci to study loss-of-function phenotypes (Fire *et al.*, 1991; Guo and Kemphues, 1995). Surprisingly, Guo and Kemphues found that both anti-sense and sense RNAs were equally efficient for suppressing gene expression (Guo and Kemphues, 1995). This result led Andrew Fire and Craig Mello to their breakthrough. They discovered that introduction of double-stranded RNAs (dsRNAs) into *C. elegans* was substantially more effective in reducing gene expression than introducing single-stranded RNAs (Fire *et al.*, 1998). This potent RNA-mediated interference effect, however, was not observed when dsRNAs homologous to promoters or intronic sequences were used (Fire *et al.*, 1998). These observations, together with the finding that injection of dsRNA resulted in reduced or undetectable levels of corresponding mRNAs, suggested a posttranscriptional gene silencing mechanism. Studies in plant systems further supported this notion (de Carvalho *et al.*, 1992; Jones *et al.*, 2001; Ruiz *et al.*, 1998). Finally, biochemical approaches verified that the observed interference effect resulted from dsRNA-induced degradation of the endogeneous mRNA (Hammond *et al.*, 2000; Kennerdell and Carthew, 1998; Tuschl *et al.*, 1999). This mechanism was termed RNA interference (RNAi).

1.3.2.1. RNA interference phenomena

Strikingly, injection of dsRNA into the germline or extracellular body cavity of *C. elegans* was found to result in an interference effect in a broad region of the animal, demonstrating that dsRNA has the remarkable capacity to cross cell boundaries ('spreading'; Timmons and Fire, 1998). This observation led to the significant discovery that feeding worms on *Escherichia coli* engineered to express dsRNA could also confer specific interference effects; just as worms normally feed on bacteria, dsRNA-expressing bacteria are ingested, dsRNA absorbed through the gut and distributed to somatic tissues and the germ line ('RNAi by feeding'; Timmons and Fire, 1998). Subsequently, it was shown that soaking worms in a solution of dsRNA ('RNAi by soaking') could also induce specific interference with gene expression (Tabara *et al.*, 1998). Finally, it was found that introduction of dsRNA into hermaphrodite worms can also produce a specific and robust interference effect in the progeny (Tabara *et al.*, 1998). Thus, dsRNA-induced gene silencing can be used to study the loss-of-function phenotype of any gene of known sequence. However, RNAi by bacterial feeding or soaking is less effective than direct injection of dsRNA (Tabara *et al.*, 1998; Timmons and Fire, 1998).

1.3.2.2. Mechanism of double-stranded RNA-induced gene silencing

Genetic studies in *C. elegans* and plants together with biochemical approaches using *Drosophila* embryonic extracts or S2 cells have provided fundamental insights into the mechanisms underlying dsRNA-induced gene silencing (reviewed in Hannon, 2002; Joshua-Tor, 2006; Matzke and Birchler, 2005; Zamore and Haley, 2005). In the current model (Figure 1.1.), Dicer, an evolutionarily conserved member of the family of RNase III ribonucleases, recognizes and cleaves dsRNAs into ~ 22-nucleotide fragments, with two-nucleotide 3' overhangs and 5' phosphorylated termini. The protein structure of RNase III enzymes led to the suggestion that the ~22-nucleotide RNAs are generated by association of two Dicer homo-dimers in antiparallel orientation. In this view, one of the two catalytic domains in each homo-dimer is inactive, with the two active catalytic domains being spaced by ~ 22 nucleotides. This configuration results in cleavage of dsRNAs into small interfering RNAs (siRNAs) of ~ 22-nucleotide length. siRNAs are

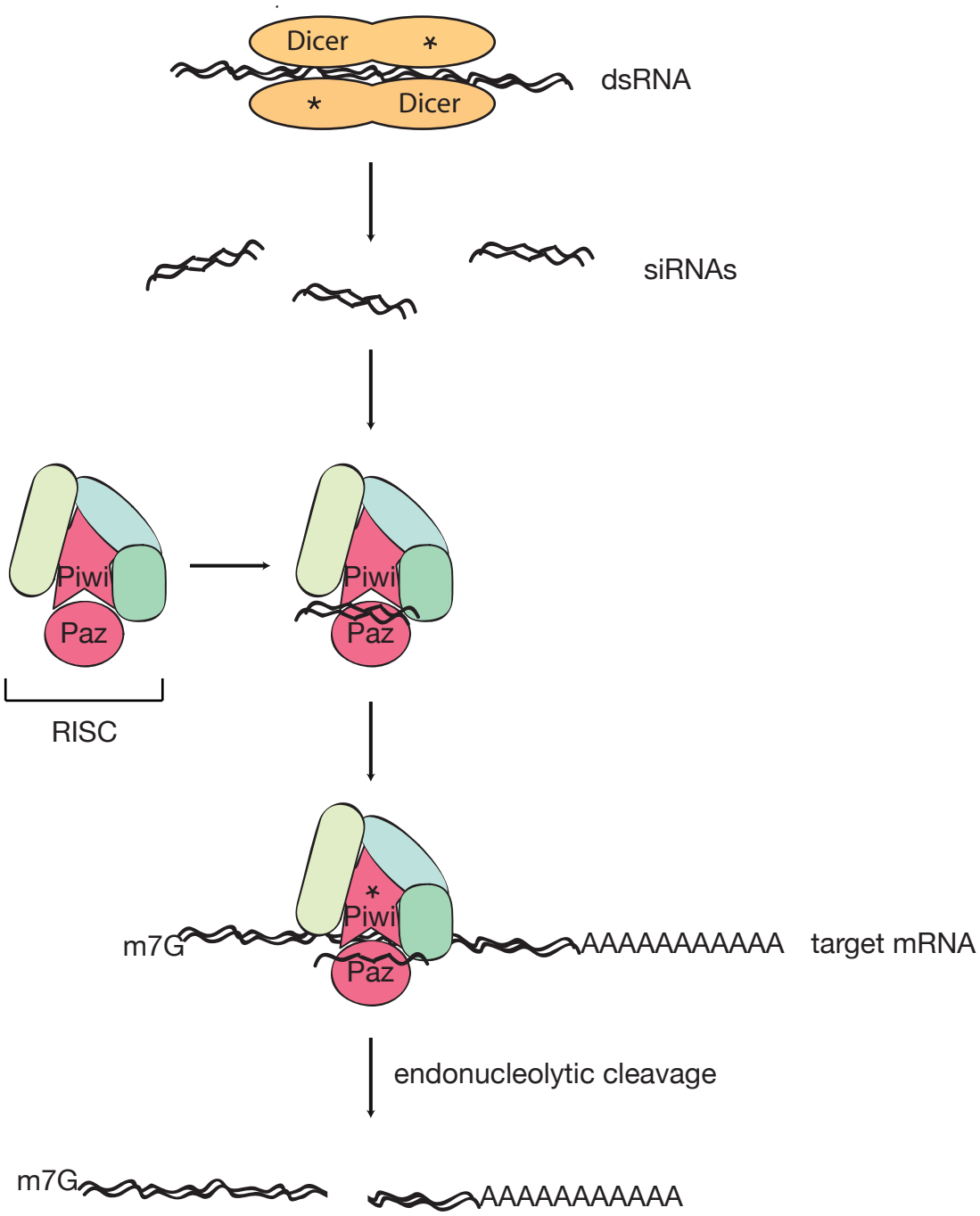

Figure 1.1. Mechanism of RNA interference
Two Dicer homo-dimers associate in anti-parallel orientation to cleave double-stranded RNA (dsRNA). Only one catalytic centre in each Dicer homo-dimer is active (*). Active catalytic domains are spaced by ~ 22 nucleotides (nt), resulting in cleavage products - small interfering RNAs (siRNAs) - of ~ 22 nt length. siRNAs are incorporated into the RNA-induced silencing complex (RISC), a ribonuclease-containing protein complex, which is activated (*) through unwinding of siRNAs. Watson-Crick base-pairing with siRNAs identifies homologous target mRNAs. The Piwi domain of the ribonuclease Slicer (shown in red, comprising of two RNA-binding domains, Piwi and Paz) mediates cleavage of target mRNA.

subsequently incorporated into a ribonuclease-containing protein complex, termed 'RNA-induced silencing complex' (RISC), targeting complementary mRNAs for degradation. Base complementarity with siRNAs identifies target mRNAs, which are subsequently endonucleolytically cleaved by Slicer, the catalytic core of the effector complex RISC. Slicer is a member of the Argonaute protein family, containing two RNA binding domains, the Piwi and PAZ domain. The Piwi domain, which is structurally homologous to ribonuclease H, comprises the endonuclease for cleavage of target mRNAs. *In vitro*, the inactive RISC precursor complex becomes activated upon addition of ATP. Remarkably, a correlation between RISC activation and unwinding of siRNAs was identified.

Intriguingly, in *C. elegans* and plants, RNAi can spread throughout the organism, whereas silencing in *Drosophila* and mammals appears to be cell-autonomous. The requirement of RNA-directed RNA polymerases (RdRPs) for dsRNA-induced gene silencing in *C. elegans* and plants led to the suggestion that siRNAs prime the synthesis for additional dsRNAs by RdRPs, thereby amplifying the silencing signal. While in plants, spreading of dsRNAs can occur by movement through plasmodesmata, in *C. elegans*, a transmembrane protein encoded by *sid-1* is suggested to mediate spreading of the silencing signal. Interestingly, orthologues of SID-1 are also encoded in mammalian genomes, whereas they were not identified in *Drosophila*. However, systemic gene silencing has thus far not been demonstrated in mammals.

1.4. The *C. elegans* genome

Being the first multicellular organism for which a complete genome sequence was available, *C. elegans* set a milestone in genomics in 1998 (The *C. elegans* Sequencing Consortium, 1998). To begin the annotation and analysis of the 97–megabase worm genome, GENEFINDER, an algorithm for the identification of putative coding regions, was used (The *C. elegans* Sequencing Consortium, 1998). By comparing computational gene predictions to experimental genome annotations based on extensive collections of expressed sequence tags (ESTs), over 90% of computationally predicted genes were found to overlap with experimentally verified introns. Additional manual annotation was

used to further refine computational gene structure predictions. With more genomic information becoming available and the development of better genome annotation tools, the analysis and annotation of the *C. elegans* genome is expected to further improve in the future.

The *C. elegans* genome is predicted to encode ~ 19,000 protein-coding genes, with an average density of 1 gene per 5kb, distributed on five autosomes and the X chromosome.

1.5. WormBase

A comprehensive web-accessible database for information on *C. elegans* and related nematodes has been generated for the *C. elegans* research community (www.wormbase.org). This repository is based on the *C. elegans* genome database architecture ('AceDB') that was originally generated for the storage of sequence information (The *C. elegans* Sequencing Consortium, 1998). WormBase is a very navigable database, providing extensive information on the sequence and structure on the genomes of *C. elegans* and its related nematode *C. briggsae*. WormBase also stores information on mutant strains and alleles — these are publicly available from two stock centres, the *C. elegans* Genetics Center, USA (http:// www.cbs.umn.edu/CGC/) and the National Bioresources Project, Japan (http:// shigen.lab.nig.ac.jp/c.elegans/index.jsp). Results from RNAi experiments, gene expression patterns, functional annotations, comparative data, such as orthologues and syntenic regions between species (reviewed in Strange, 2006) are also deposited in WormBase.

1.6. RNA interference in *C. elegans* by bacterial feeding

While the penetrance of the interference effects obtained through delivering dsRNAs by bacterial feeding or soaking are not as strong as those obtained by direct injection of dsRNAs (Tabara *et al.*, 1998; Timmons and Fire, 1998), RNAi by feeding has nonetheless numerous advantages for large-scale applications of RNAi. Firstly, RNAi by feeding is reasonably cost-effective, because it circumvents the need for expensive *in*

vitro synthesis of dsRNA that is necessary when using RNAi by injection or soaking. Secondly, bacterial strains expressing dsRNA can be kept as a reusable resource and thus can be distributed indefinitely. Finally, RNAi by bacterial feeding is less labour-intensive than injection, thereby allowing RNAi experiments to be scaled up to a reasonably high throughput.

This powerful technique led Julie Ahringer's lab to generate a whole-genome RNAi feeding library (also referred to as the 'Ahringer RNAi library'; Fraser *et al.*, 2000; Kamath and Ahringer, 2003; Kamath *et al.*, 2003). In this resource, gene-specific DNA fragments of roughly 1000 – 1500 bp were inserted between inverted repeats of the bacteriophage T7 promoter into a bacterial plasmid vector (L4440; Figure 1.2.). These plasmids were transformed into an RNaseIII-deficient *E. coli* strain (HT115(DE3), also referred to as 'bacterial feeding strain'), which was engineered to express T7 RNA polymerase under an isopropyl-β-D-thiogalactopyranoside- (IPTG-) inducible promoter. Thus, expression of dsRNA could be induced upon addition of IPTG (Timmons *et al.*, 2001; Timmons and Fire, 1998). RNaseIII-deficiency was found to improve the efficacy of RNAi by bacterial feeding, presumably because it results in increased stability of dsRNA that is produced in bacteria (Timmons *et al.*, 2001).

Using this approach, DNA fragments corresponding to ~86% of the predicted *C. elegans* genes were cloned, resulting in a collection of 16,757 dsRNA-expressing bacterial strains (Fraser *et al.*, 2000; Kamath *et al.*, 2003), each targeting one predicted gene. With the generation of this potent resource, RNAi by feeding has become a powerful reverse genetic tool for studying *C. elegans* gene functions on a large scale.

1.6.1. Experimental setup for RNA interference by bacterial feeding

The experimental procedure for RNAi by bacterial feeding, optimized by Kamath and Ahringer (2003), involves three major steps (Figure 1.3.). Firstly, selected bacterial strains of the *C. elegans* whole-genome library are grown to saturation and seeded onto IPTG-containing 12-well assay plates ('plate feeding'). Secondly, late larval-stage worms are placed onto pre-seeded assay plates and are clonally propagated after an appropriate

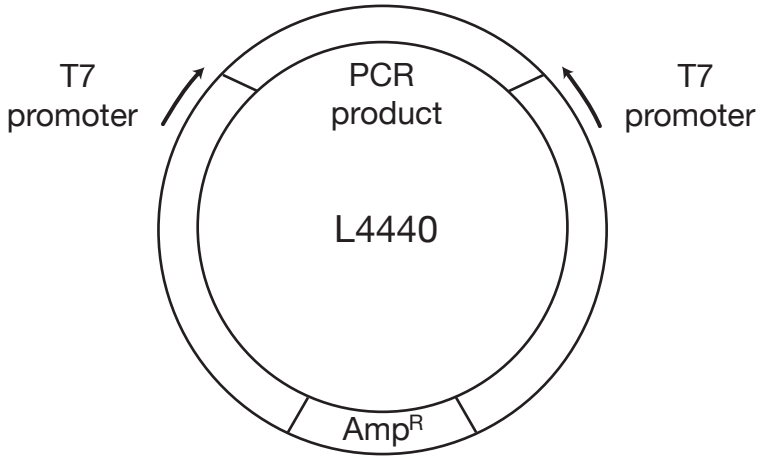

Figure 1.2. L4440 RNA interference feeding vector
A PCR product homologous to a target gene of interest is cloned between inverted T7 promoter sites. Induction of T7 RNA polymerase expression in the bacterial host genome (HT115(DE3)) results in transcription of anti-parallel single-stranded RNAs (ssRNAs). These ssRNAs anneal and form double-stranded RNAs, which trigger RNA interference.

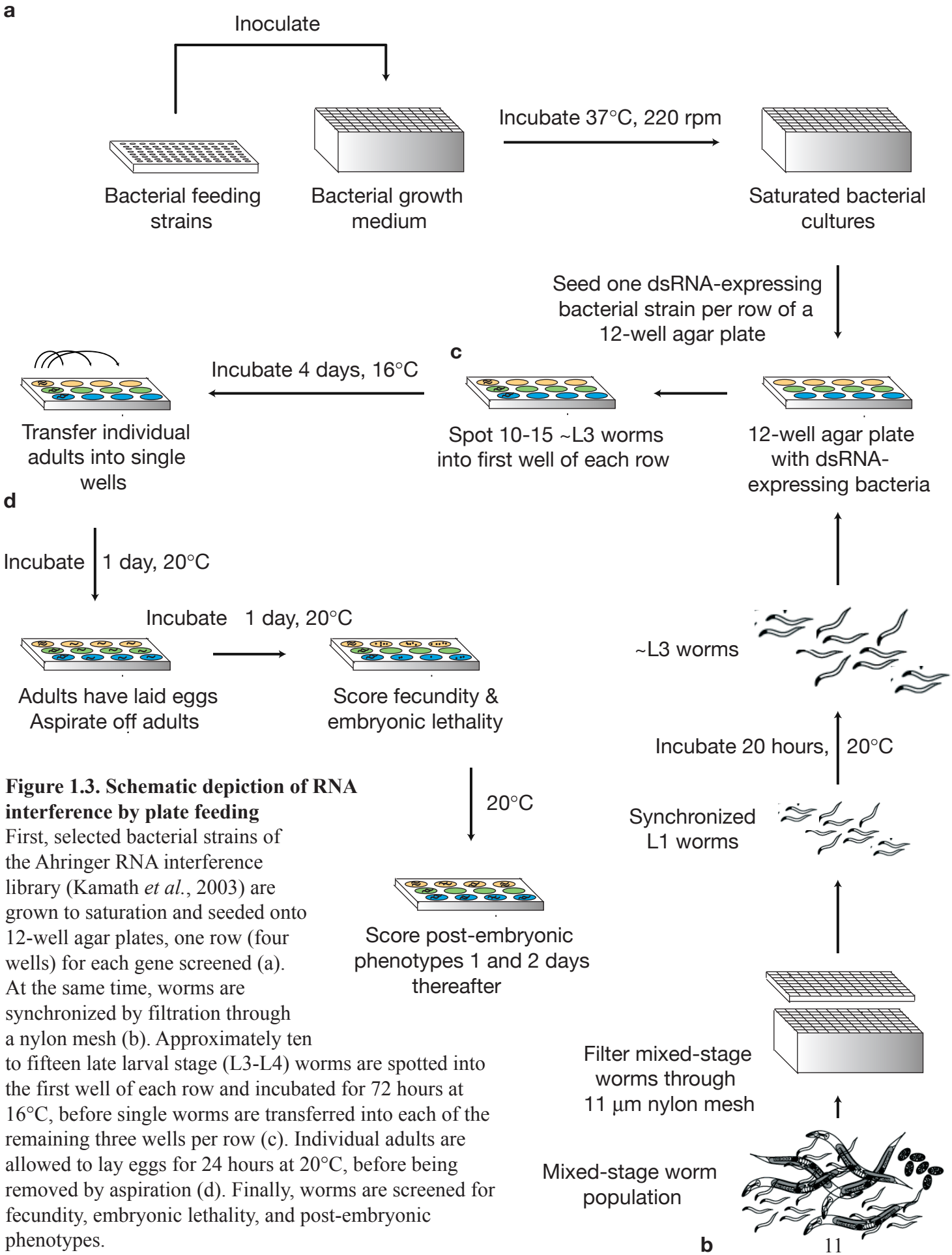

Figure 1.3. Schematic depiction of RNA interference by plate feeding
First, selected bacterial strains of the Ahringer RNA interference library (Kamath *et al.*, 2003) are grown to saturation and seeded onto 12-well agar plates, one row (four wells) for each gene screened (a). At the same time, worms are synchronized by filtration through a nylon mesh (b). Approximately ten to fifteen late larval stage (L3-L4) worms are spotted into the first well of each row and incubated for 72 hours at 16°C, before single worms are transferred into each of the remaining three wells per row (c). Individual adults are allowed to lay eggs for 24 hours at 20°C, before being removed by aspiration (d). Finally, worms are screened for fecundity, embryonic lethality, and post-embryonic phenotypes.

incubation time. Finally, individual adults and their progeny are screened for viability, fecundity, and post-embryonic growth and development. Using this methodology, phenotypes were assigned to 16,757 predicted *C. elegans* genes in wild-type worms (Fraser *et al.*, 2000; Kamath *et al.*, 2003).

1.6.2. Genome-wide RNA interference by bacterial feeding

When using the Ahringer library to target 16,757 predicted *C. elegans* genes in wild-type hermaphrodites, mutant phenotypes were detected for ~10% of targeted genes. While ~7% of genes were found to be essential for viability, knockdown of ~ 3% of genes generated worms with slowed post-embryonic growth or defects in post-embryonic development (Kamath *et al.*, 2003). By assessing the ability to correctly identify the known loss-of-function phenotypes of previously studied loci, the effectiveness of RNAi by bacterial feeding was determined to be, on average, ~64%. The detection rate was higher for loci with non-viable phenotypes (~78%) than for loci with post-embryonic phenotypes (~42%).

Notably, the results obtained by genome-wide RNAi by bacterial feeding correlated well with other previously reported large-scale studies, in which RNAi by injection and soaking, respectively, was used (Gonczy *et al.*, 2000; Maeda *et al.*, 2001). Thus, results obtained by RNAi are highly reproducible regardless of the method used. Most importantly, the false-positive rate of RNAi by bacterial feeding is below 1%, as assessed by targeting 225 genes that were known not to affect viability when deleted. RNAi against one gene only caused a mutant phenotype.

This initial screen was subsequently repeated in a genetic mutant deficient for the putative RNA-directed RNA polymerase RRF-3, which was found to be hypersensitive to RNAi in a forward genetic screen (Simmer *et al.*, 2002). Using the *rrf-3* background, mutant phenotypes could be assigned to an additional 400 genes, thereby increasing the percentage of *C. elegans* genes with a detectable RNAi phenotype to approximately 12% (Simmer *et al.*, 2003).

1.7. Using RNA interference in cell-based approaches for uncovering gene functions in *D. melanogaster* on a large scale

The completion of the *Drosophila* genome sequence (Adams *et al.*, 2000) together with the finding that addition of long dsRNAs to *Drosophila* cells was a potent method to reduce the expression of specific target genes (Clemens *et al.*, 2000; Hammond *et al.*, 2000) led to the assembly of a collection of dsRNAs targeting most of the roughly 13,600 predicted genes in the genome of *D. melanogaster* (Boutros *et al.*, 2004). When using this resource to target over 90% of predicted fly genes in cell-based assays, approximately 3% of genes were found to be indispensable for growth and viability in *D. melanogaster* (Boutros *et al.*, 2004). Recently, further progress has been made to refine the analysis of loss-of-function phenotypes generated by RNAi in cell-based approaches (Bjorklund *et al.*, 2006). Flow cytrometry has been used to study the role of ~70% of *D. melanogaster* genes in cell cycle progression. That way, numerous genes governing cell size, cytokinesis, apoptosis, and cell cycle progression were identified and ordered into known pathways. RNAi reagents have now also become available for genome-scale screens in mammalian cells (reviewed in Moffat and Sabatini, 2006). Thus, cell-based approaches similar to the ones taken for systematically deciphering gene function in *D. melanogaster* will help unravel gene functions on a genome-wide scale in mammals.

1.8. Uncovering gene functions in *S. cerevisiae* on a genome-wide scale

For the systematic unraveling of gene functions in the yeast *S. cerevisiae*, precise start-stop codon deletions were generated for each of the ~ 6,000 predicted genes (Giaever *et al.*, 2002; Winzeler *et al.*, 1999). These were constructed by targeted disruption of each predicted open reading frame (ORF) through homologous recombination (Baudin *et al.*, 1993; Lorenz *et al.*, 1995; Wach *et al.*, 1994). Targeting constructs for each gene were generated through polymerase chain reaction (PCR) amplification of a selectable marker gene, by using primers with a short sequence of homology to the predicted start and stop codons of each gene. In addition, unique 20-bp sequences (‘barcode’ sequences) — referred to as ‘up’ and ‘down’ tags (Hensel *et al.*,

1995; Shoemaker *et al.*, 1996) — flanking the marker gene and universal PCR priming sites just outside the barcodes were included in the targeting cassette. This design allows individual gene deletions to be identified in pools of mutant strains through PCR amplification using primers homologous to the universal sequence and subsequent hybridization of PCR products to a barcode microarray.

Mutant strains are maintained as a collection of heterozygous diploid strains, each of which is carrying a deletion in one copy of a specific gene, whereas the other copy is maintained as wild-type. The accomplishment of precisely deleting every single predicted ORF in the genome of *S. cerevisiae* paved the way for providing a genome-scale view of gene functions in yeast. When analyzing haploid meiotic progeny after induced sporulation, it was found that almost 5,000 of the predicted ~6,000 yeast genes can be eliminated entirely without any apparent deleterious consequences (Giaever *et al.*, 2002).

1.9. Identification of orthologous genes

To be able to transfer functional information between organisms, computational algorithms have been developed that enable the identification of orthologous genes in different species. Traditionally, phylogenetic trees have been constructed for the detection of orthologues (Yuan *et al.*, 1998). However, orthologue assignment by phylogenetic methods is difficult to automate and requires immense computing power. Thus, phylogenetic tree-based approaches are not suitable for assigning orthologues on a genome-wide scale.

The automatic clustering based on two-way best matches provides an alternative method (Chervitz *et al.*, 1998; Mushegian *et al.*, 1998; Rubin *et al.*, 2000; Wheelan *et al.*, 1999). Orthologue assignment, however, can be complicated through the existence of genes in multiple copies (‘co-orthologues’ or ‘paralogues’). While it might be desired to identify paralogues that arose after divergence of one species from another, it had initially not been possible to separate paralogues that predated species split from paralogues that arose after by using conventional clustering approaches based on two-way best pairwise matches.

To overcome this obstacle, an algorithm for the identification of orthologues and paralogues that arose after divergence of one species from another (so-called 'in-paralogues', to distinguish them from 'out-paralogues', paralogues that arose before species split) was devised (Remm *et al.*, 2001). This method (INPARANOID) is based on two-way best pairwise matches for detecting orthologues, with an algorithm added to identify in-paralogues between any two genomes. First, pairwise similarity scores are calculated using BLAST, with the bi-directional best hits being assigned the main orthologue pair. Adjustable cut-off values are applied to separate significant similarity scores from erroneous matches and thus to avoid inclusion of false positives. INPARANOID is based on the assumption that genes within one species that are more similar in sequence to the main orthologue than to any sequence from the other species represent in-paralogues (Figure 1.4.). Consequently, INPARANOID assigns confidence values (on a scale of 0% to 100%) as a measure of sequence similarity of a given in-paralogue to the main orthologue pair (which is assigned 100%).

By comparing orthologue assignments generated by INPARANOID with manual tree-based orthologue detection approaches, INPARANOID was demonstrated to generate data sets with a high degree of confidence.

Thus, while orthologues and in-paralogues have previously been detected through the construction of phylogenetic trees, a rather slow approach that is difficult to automate, alternative methods, based on two-way best pairwise matches, could not separate in-paralogues from out-paralogues. The INPARANOID algorithm, however, provides a powerful method for assigning orthologues and in-paralogues between any two species. Notably, INPARANOID is conservative and rather underpredicts orthologues and paralogues by excluding insignificant hits, though I recognize that this may exclude some true in-paralogues.

1.10. Gene dispensability and its potential underlying causes

The availability of whole genome-sequences for numerous model organisms together with the development of technological platforms has paved the way for the systematic investigation of gene functions on genome-wide scales (Bjorklund *et al.*,

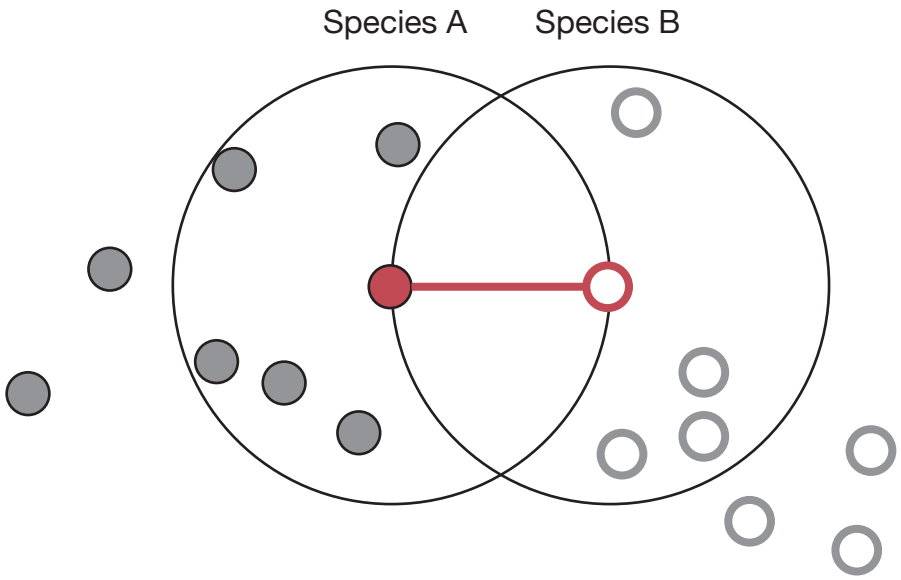

Figure 1.4. Graphical model for orthologue assignment using INPARANOID
Reciprocal best pair-wise matches of genes in two species (here species A and B) are assigned the main orthologue pair (shown as red filled and open circles, respectively). Additional orthologues within each species (co-orthologues) that are more similar in sequence to the main orthologue than to any other gene in the other species are assigned 'in-paralogues' (shown as grey filled and open circles within species A and B, respectively), whereas all other orthologues are assigned 'out-paralogues' (drawn outside the black circles). In-paralogues are considered co-orthologues that arose after divergence of one species from another, whereas out-paralogues represent co-orthologues that predate the species split. Re-drawn based on figure from Remm *et al.*, 2001.

2006; Boutros *et al.*, 2004; Giaever *et al.*, 2002; Kamath *et al.*, 2003). One striking insight gained from these systematic studies was the discovery that the great majority of genes encoded in eukaryotic genomes are dispensable for viability under laboratory conditions. Several theories have been put forward to explain this 'lack-of-phenotype' phenomenon; I will discuss these below.

Although intense theoretical work has been done on this subject, the causes of gene dispensability have remained controversial (reviewed in Wagner, 2005). One hypothesis suggests that apparently dispensable genes might be essential for survival only under specific environmental conditions that have not yet been probed in the laboratory. In this view, gene dispensability might reflect the system's ability to adjust to changing environments ('environmental adaptation'; Papp *et al.*, 2004).

Conversely, another theory postulates functional redundancy as genetic mechanism underlying gene dispensability (Wagner, 2000a; reviewed in Hartman *et al.*, 2001; Wagner, 2005). This view suggests the existence of 'back-up' mechanisms or compensatory pathways, such that loss of one gene has little effect because redundant or alternative pathways can counteract this loss ('mutational robustness').

Both theories suggest that the genetic networks that underlie viability are not constant, but can adjust their mode of operation under different external or internal conditions (reviewed in Kitano, 2004; Wagner, 2005). The two models for gene dispensability, however, might not be mutually exclusive. It has been increasingly noted that mutational robustness might have arisen as a 'by-product' of environmental adaptation (reviewed in Kitano, 2004; Wagner, 2005). I will discuss both theories in more detail below.

1.10.1. Environmental adaptation

While it has long been appreciated that seemingly dispensable genes can have important biological functions under specific environmental conditions, this notion has recently been investigated systematically through the computational modeling of metabolic networks in yeast (Papp *et al.*, 2004). When calculating the metabolic flux

under various nutrient conditions, roughly half of the apparently non-essential genes were predicted to be active under specific growth conditions. Recent experimental support for this computational approach came from large-scale phenotypic analyses, in which yeast deletion mutants were monitored for fitness defects under numerous environmental conditions. These studies led to the suggestion that at least 20% of the approximately 5,000 seemingly dispensable genes in *S. cerevisiae* might have essential functions under specific environmental conditions (Chang *et al.*, 2002; Davis-Kaplan *et al.*, 2004; Dudley *et al.*, 2005; Enyenihi and Saunders, 2003; Kuepfer *et al.*, 2005; Martinez *et al.*, 2004; Smith *et al.*, 2006). Most conditional essential genes were found to be indispensable under a limited number of growth conditions only, further demonstrating their environment-specific essentiality.

It had further been hypothesized that if seemingly dispensable genes were to have essential functions under specific environmental conditions, these genes might not be conserved in species that never encountered these conditions (Papp *et al.*, 2004). In agreement with this theory, enzymes that were predicted to be active under restricted nutrient conditions only by *in silico* modeling of the metabolic flux in yeast were found to have a limited phylogenetic distribution as compared to unconditionally active enzymes (Papp *et al.*, 2004). This computational study was supported by direct experimental evidence from studies on condition-specific genes in *E. coli* (Glasner *et al.*, 2003; discussed in Papp *et al.*, 2004). Furthermore, while the fraction of dispensable genes in yeast is approximated to be above 60%, the proportion of non-essential genes in the parasite *Mycoplasma genitalium* is estimated to be below 25%, suggesting a low number of condition-specific genes, which is consistent with the parasite's narrow host specificity (discussed in Papp *et al.*, 2004).

1.10.2. Functional redundancy and mutational robustness

One obvious source of genetic redundancy is through gene duplication. Duplicated genes that retain at least partially overlapping functions can confer robustness to mutation in the other copy (Force *et al.*, 1999; Lynch and Force, 2000). Gene duplicates can arise through homologous recombination and DNA repair processes, the

action of retrotransposons, or may be relics from whole-genome duplication events. Following a duplication event, both genes are initially performing the same function. However, for proper functions to be maintained, genes need to be under selective pressure. This notion led to the suggestion of various models for the evolutionary fate of duplicated genes.

The classical model for the evolution of gene duplicates predicts that the ancestral gene might be selected to maintain its original function, while its duplicate copy is free to diverge (Ohno, 1970). In this view, the most common fate of the duplicate copy was suggested to be extinction by the accumulation of deleterious mutations ('non-functionalization'; Kimura and King, 1979). Under this model, however, the duplicate copy, while it is not protected against mutations, might acquire new functions through rare beneficial mutations. This scenario has long been considered as the only mechanism for the preservation of gene duplicates and is regarded an important driving force for evolutionary innovation ('neo-functionalization'; Ohno, 1970).

The rationale that the probability for a gene to acquire a degenerative mutation might be higher than the likelihood to acquire a beneficial mutation (Lynch and Walsh, 1998), however, led to the proposal of an alternative model for the evolutionary fate of gene duplicates (Force *et al.*, 1999). This model ('duplication-degeneration-complementation', thereafter also referred to as 'sub-functionalization') suggests degenerative mutations as the central mechanism underlying duplicate gene preservation: Immediately after a duplication event, both copies might experience a period of relaxed selection, in which they might acquire deleterious mutations that impair different ancestral sub-functions. Such partially compromising mutations might result in a duplicate gene pair with complementary functions that can perform the original gene function in combination only. This sub-functionalization model is supported by findings from genome-wide studies, which reveal that, following a duplication event, both copies appear to be under selective pressure, with stronger purifying selection acting on older than on more recent gene duplicates (Kondrashov *et al.*, 2002; reviewed in Wagner, 2002). In addition, available experimental evidence suggests that sub-functionalization through complementary mutations frequently results in the partitioning of tissue-specific expression (Lynch and Force, 2000).

Various theoretical models have been proposed to explain how genes with redundant functions can be evolutionarily stable (Nowak *et al.*, 1997). One model predicts the maintenance of redundancy under the assumption that two genes perform their functions with equal efficiencies and under the same mutation rates. In this model, even if mutation rates differ marginally, thus rendering redundancy evolutionary unstable, it would still require a substantial amount of time until the overlap in function were eliminated, provided mutation rates are low. Conversely, two alternative theories suggest scenarios in which functional redundancy can be maintained indefinitely. While one model predicts the evolutionarily stability of redundancy based on the assumption that two genes perform the same function with different efficacies, with the more efficient gene experiencing higher mutation rates, the other theory relates pleiotropy to redundancy; two genes are maintained by selection because of their individual functions, while they are redundant with respect to another function.

Indirect experimental evidence for the redundant functions of duplicated genes comes from the systematic analysis of loss-of-function phenotypes of single genes: In both yeast and worms, it was found that inactivation of a duplicated gene is less likely to result in a non-viable phenotype than inactivation of a single-copy gene (Conant and Wagner, 2004; Gu *et al.*, 2003; Kamath and Ahringer, 2003). However, there are strong biases in the types of genes that are duplicated in genomes, which complicates the interpretation of these results (Castillo-Davis and Hartl, 2002) and at the time this study began, no attempt had been made to examine the extent of redundancy between duplicated genes *in vivo* directly and systematically.

The first systematic investigation into the mechanistic basis underlying gene dispensability was provided by an *in silico* analysis of the metabolic flux in *S. cerevisiae* (Papp *et al.*, 2004). Notably, while metabolic networks do not contain any redundant biochemical reactions — that is any metabolite is produced by one enzyme only —, approximately half of a system's unique enzymatic reactions can be perturbed without negatively affecting metabolic output (discussed in Wagner, 2005). Computational modeling of yeast metabolic fluxes was used to predict the effects of single-gene deletions under different environmental conditions and the concomitant changes in metabolic flux distribution (Papp *et al.*, 2004). Results from this *in silico* analysis

suggested that compensation for loss of individual gene function by a duplicate copy could account for roughly one quarter of dispensable genes in the yeast metabolic network. In addition, non-essentiality of fewer than 20% of metabolic genes could be explained by a re-routing of the metabolic flux through alternative, unaffected pathways. Remarkably, enzymes with unrelated activities can confer mutational robustness of metabolic networks by co-operation and flux re-organization.

Similar observations were made in developmental biology (reviewed in Wagner, 2005). Regulatory networks, such as the segment polarity network in *D. melanogaster*, do not comprise genes with equivalent functions. These networks, however, can maintain their functions despite perturbations (von Dassow *et al.*, 2000). Thus, genes that do not resemble one another at the sequence level and do not have related molecular roles can nonetheless compensate for loss of one another. This 'higher-order' functional redundancy is often referred to as 'distributed robustness', to distinguish it from genuine functional redundancy of gene duplicates ('redundancy of parts'; reviewed in Wagner, 2005).

1.10.2.1. Systematic experimental approaches for uncovering genetic interactions and functional genetic redundancy

While available evidence supports both conditional essentiality and functional redundancy as potential origins of gene dispensability, direct systematic experimental approaches are needed in order to examine the relative contribution of either source to the high proportion of seemingly non-essential genes. For the scope of this study, I will focus on experimental approaches that have been used to investigate genomes for functional genetic redundancy.

Traditionally, suppressor or enhancement genetics have been used to unravel functional relationships between genes (reviewed in Guarente, 1993; Hartman *et al.*, 2001). Genetic interactions were uncovered by screening with mutant alleles of known genes with defined phenotypes for mutations in other genes that can modulate this phenotype. That way, new genes with functions in the same or related molecular process were uncovered. Suppression, in which mutation in one gene alleviates the effects of

mutation in another gene, can unravel genes with roles in parallel biochemical or genetic pathways, if the second-site mutation increases pathway function ('bypass suppression'). Suppressor screens can also unravel genes functioning in the same pathway and have been instrumental in uncovering regulatory hierarchies. 'Synthetic enhancement', in which mutation in one gene worsens the effects of mutation in another gene, may occur between genes acting in the same biochemical pathways, or in distinct, but functionally redundant pathways. Synthetic lethality represents the most severe form of synthetic enhancement. This phenomenon was first observed by fly geneticists early last century who uncovered mutations in specific pairwise combinations of genes that resulted in lethality, whereas animals carrying mutations in each individual gene were viable (Dobzhansky, 1946; Sturtevant, 1956).

The identification of genetic interactions has provided major insights into key regulatory processes and pathway organization (Avery and Wasserman, 1992; Guarente, 1993; Hartman *et al.*, 2001; Lu and Horvitz, 1998; Thomas, 1993). However, before the availability of complete genome sequences and the feasibility of reverse genetic approaches, genetic interactions have been uncovered on a rather small scale to assist functional biological studies.

Considered a promising approach to uncover functional redundancy on a large scale, high-throughput technological platforms have been developed for the systematic mapping of synthetic lethal (SL) interactions in the yeast *S. cerevisiae*. I will discuss these in more detail below.

1.10.2.1.1. Identifying synthetic lethal interactions in the yeast *S. cerevisiae* on a large scale

Synthetic lethal (SL) interactions represent the most severe form of synthetic enhancement and thus are considerably straightforward to map. Therefore, thus far, most systematic studies focused on synthetic lethality as a framework for identifying non-additive genetic interactions.

Synthetic genetic array (SGA) analysis was the first methodology that had been developed for the genome-scale mapping of synthetic sick or synthetic lethal (SL) interactions in the genome of *S. cerevisiae* (Tong *et al.*, 2001). Using an automated approach, haploid strains carrying defined mutations are intercrossed and, following sporulation, fitness of double mutant meiotic progeny is assessed and compared to fitness of each single mutant. Therefore, a haploid strain of one mating type (α), carrying a mutation in a query gene of interest, is crossed into an array of haploid mutant strains of opposite mating type (a). Mutations in both strains are linked to different antibiotic resistance markers, allowing for selection of double mutant meiotic progeny. Importantly, to prevent mating of meiotic progeny — this would give rise to false-negative results — the query mutant strain is engineered to carry a selectable marker under a mating type a-specific promoter (*MFA1pr-HIS3*), permitting growth of meiotic progeny of one mating type (a) only. This approach has been pioneered by Amy Tong in Charlie Boone's lab and has since then been used extensively to systematically map SL interactions in *S. cerevisiae* (Tong *et al.*, 2004).

SGA analysis has further been extended to screen for genetic interactions between yeast essential genes (Davierwala *et al.*, 2005). Therefore, conditional expression alleles and temperature-sensitive (ts) conditional alleles of essential genes have been created. For the generation of conditional expression alleles, the endogenous promoter is replaced by a tetracycline (tet) – repressible promoter that can be shut off by the addition of doxycycline, a tetracycline analog (Mnaimneh *et al.*, 2004). Ts conditional alleles can be readily created by linking a heat-inducible destabilizing protein ('degron', Arg-DHFR(ts)) to the N-terminus of an essential gene, which results in protein degradation (Dohmen and Varshavsky, 2005). Using these approaches, extensive collections of tet-promoter mutants and degron alleles have been generated (Kanemaki *et al.*, 2003; Mnaimneh *et al.*, 2004). Furthermore, for the systematic construction of hypomorphic alleles, 3' untranslated regions of essential genes are replaced with selectable markers, which results in lower transcript levels through mRNA destabilization ('decreased abundance by mRNA perturbation, DAmP'; Schuldiner *et al.*, 2005). Recently, SGA technology has been coupled with quantitative approaches to allow genetic interactions to be identified more accurately (Schuldiner *et al.*, 2005).

Diploid synthetic lethal analysis by microarray (dSLAM) provides an alternative method to SGA analysis for uncovering SL interactions in the yeast *S. cerevisiae* (Pan *et al.*, 2004). In this approach, a deletion construct for a query gene of interest is transformed into a pool of barcode-tagged heterozygous deletion stains, which are carrying a selectable marker under a mating type-specific promoter (see SGA analysis). Following induced sporulation, DNA from pooled mutants is amplified by polymerase chain reaction (PCR) using a pair of primers binding common sequences outside the barcode tags (as discussed in 'Uncovering gene functions in *S. cerevisiae* on a genome-wide scale'). The abundance of each double mutant in the pool is determined by hybridization of PCR fragments to a barcode miniarray. This approach has been used to uncover SL interactions between genes with functions in the maintenance of DNA integrity (Pan *et al.*, 2006).

While the accuracy of identifying SL interactions by using SGA versus dSLAM approaches has not been assessed systematically, overlaps in data sets are found. Each method, however, also identifies SL interactions not uncovered by one or other approach. Beyond the differences in methodological approach, SGA technology uses haploid deletion strains, whereas dSLAM technology uses the yeast gene deletion collection in heterozygous diploid format. Notably, strains maintained as haploid deletion mutants might be subject to considerable selection pressure, which might lead to the accumulation of compensatory mutations. Results were found to differ when using homozygous haploid and diploid deletion mutants (Pan *et al.*, 2004). In contrast, maintaining mutant strains as heterozygous diploids reduces selection pressures through the existence of a wild-type copy for each deletion allele. Thus — with the exception of ~ 3% haplo-insufficient genes — the great majority of heterozygous yeast deletion mutants show normal growth on rich medium (Deutschbauer *et al.*, 2005).

1.10.2.1.2. Defining non-additive, 'synthetic' genetic interactions by a multiplicative model

For the unambiguous identification of non-additive, 'synthetic' genetic interactions, phenotypes are quantified and subject to statistical analysis. A

'multiplicative model' best describes the contributions of independent genetic loci to a phenotype; that is, the quantitative effects of mutations in individual genes should combine multiplicatively (Phillips *et al.*, 2000; Puniyani *et al.*, 2004). For additive genetic interactions, a double mutant's phenotype is expected to be the product of phenotypes for both individual genes. Divergence of the double mutant phenotype from the expected multiplicative values related to phenotypes for both individual genes is suggestive of a non-additive, synthetic genetic interaction. The multiplicative model also represents an application of Fisher's definition of 'epistacy', in which he describes epistasis as a phenomenon where the double mutant shows an unpredicted phenotype that is deviating from the expected product of phenotypes for both individual genes (Fisher, 1918). Deviations can have either negative or positive values, representing aggravating (fitness of the double mutant is lower than expected, with synthetic lethality as the most dramatic form) and alleviating (fitness of the double mutant is higher than anticipated) synthetic genetic effects. While aggravating interactions often occur between genes acting in distinct, but compensatory pathways ('between-pathway' interactions), alleviating interactions often reflect genes functioning in the same biochemical pathway ('within-pathway' interactions; Segre *et al.*, 2005; St Onge *et al.*, 2007). Notably, databases that store genetic interaction data for model organisms do often not discriminate between additive and non-additive (i.e. aggravating or alleviating) genetic interactions.

1.10.2.1.3. Inferring functional relationships from genetic interaction screens

When systematically mapping genetic interactions in the yeast *S. cerevisiae,* using synthetic genetic array (SGA) or diploid synthetic lethal analysis by microarray (dSLAM) technology, synthetic lethal (SL) interactions were found to be enriched between functionally related genes (Davierwala *et al.*, 2005; Pan *et al.*, 2006; Tong *et al.*, 2001; Tong *et al.*, 2004). Notably, the interaction density of genetic networks was found to exceed that of physical interactions (Tong *et al.*, 2004). Genetic interactions appear to be largely non-overlapping with physical interactions and tend not to occur between components of the same linear biochemical pathway, with the exception of essential

genes. This exception might be explained by the use of hypomorphic alleles when compiling the essential gene network — each mutant allele might partially reduce the flux through a linear pathway, but a combination of both mutant alleles might abolish pathway function (Bader *et al.*, 2003; Kelley and Ideker, 2005; Ye *et al.*, 2005). Most importantly, SL interactions can be used to infer pathway topology. Genes with similar SL interaction partners tend to encode components of the same biochemical pathway (Bader *et al.*, 2003; Kelley and Ideker, 2005; Ye *et al.*, 2005). Thus, genetic interaction and protein interaction networks provide complementary information. Accordingly, by using integrative approaches, such as combining SL data with protein-protein, mRNA co-expression, and phenotype data, the systematic mapping of SL interactions provides a powerful method for inferring functional relationships between genes (Wong *et al.*, 2004).

Together, these findings confirmed the classical interpretation of SL genetic interactions as the results of inactivating two functionally redundant pathways in the cell, either of which is individually dispensable (Figure 1.5.), and led to suggest the existence of abundant 'back-up' pathways conferring robustness to mutation in genetic networks (reviewed in Hartman *et al.*, 2001).

1.10.2.1.4. Features of genetic interaction networks

The systematic mapping of synthetic lethal (SL) interactions in *S. cerevisiae* shed light on the global properties of gene interaction networks. First, genetic interaction networks were found to follow a power-law distribution, with many genes interacting with few others, whereas few genes were found to have numerous interaction partners (Tong *et al.*, 2004). Second, genetic interaction networks appear to have a small-world topology, with short characteristic path length and densely connected local neighborhoods (Tong *et al.*, 2004). The average interaction density of genetic networks was found to be in the range of 1%, with a higher interaction frequency between essential than between non-essential genes (Davierwala *et al.*, 2005; Pan *et al.*, 2006; Tong *et al.*, 2004).

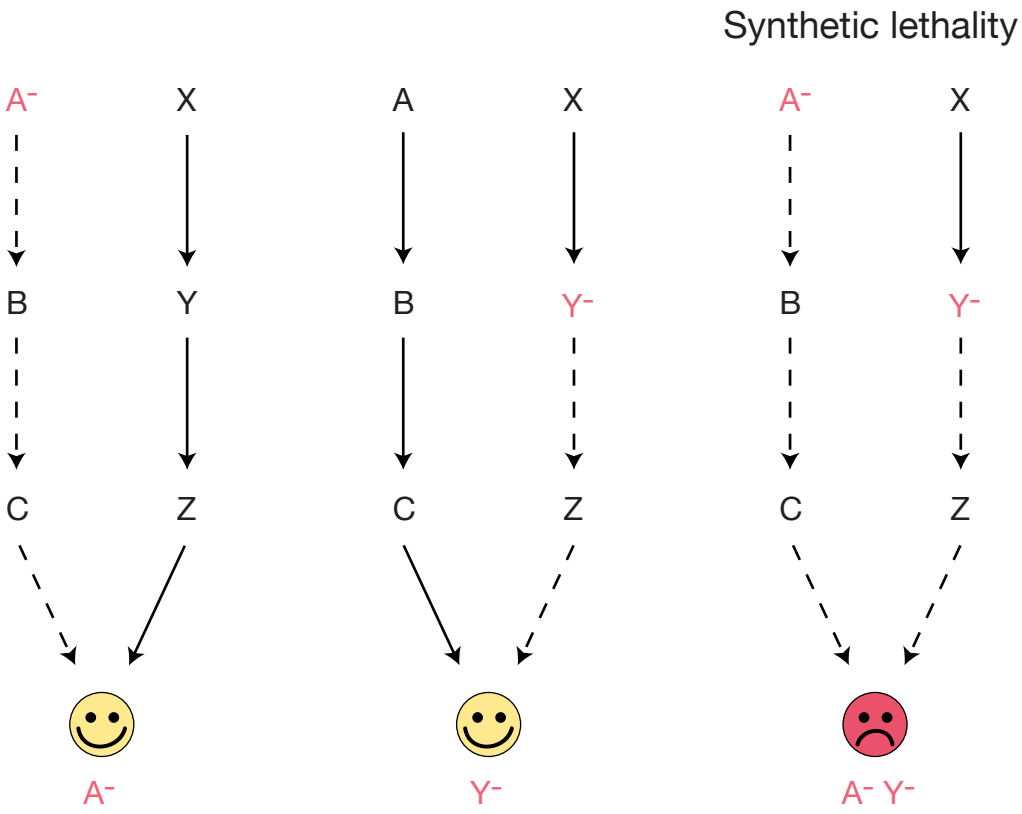

Figure 1.5. Model for the interpretation of synthetic lethal interactions
In the 'classical' model, synthetic lethal interactions are considered the result of inactivating genes with functions in two redundant essential pathways in the cell. In this view, loss of function of one pathway (here A-B-C) can be compensated for by a functionally redundant pathway (here X-Y-Z), and vice versa. Inactivation of both pathways through deleterious mutations (here depicted as A- and Y-, respectively, results in nonviability ('synthetic lethality') of the organism.

Remarkably, although SL interactions were enriched for genes encoding homologous proteins, their overall contribution was negligible (Tong *et al.*, 2004). Notably, however, the proportion of gene duplicates that had been sampled in large-scale SL screens had been fairly low. The majority of genetic interactions were uncovered between pairs of unrelated genes (Tong *et al.*, 2004).

Only recently has the contribution of gene duplicates to the robustness of yeast genetic networks been tested by systematic experimental approaches. Whereas the fraction of duplicate pairs that were found to genetically interact was significantly higher as compared to random pairs in the genome of *S. cerevisiae*, SL interactions were uncovered between ~25% of duplicate gene pairs only (Ihmels *et al.*, 2007). Notably, this fraction relates well to the proportion of duplicate gene pairs in the metabolic yeast network that were predicted to have compensatory capacities by *in silico* flux analysis (Papp *et al.*, 2004).

Taken together, results obtained both from systematic experimental approaches and computational studies led to the suggestion that although gene duplicates can — to some extent — provide robustness to mutation, their overall contribution to gene dispensability is limited. Conversely, most SL interactions take place between genes that do not share sequence similarity.

1.10.2.1.5. Condition-specificity of synthetic lethal interactions

Recently, a computational study has provided insights into the condition-dependence of synthetic genetic interactions. Flux balance analysis of the metabolic networks of *S. cerevisiae* was used to predict the effects of single- and double-gene deletions in 53 different nutritional environments (Harrison *et al.*, 2007). Notably, half of the predicted synthetic lethal (SL) relationships appear to be restricted to one or two nutrient conditions only, as compared to ~14% of SL interactions that appear to take place under all conditions investigated. Conversely, more than half of SL interactions remain undetected if examined in one nutritional environment only. These findings highlight the narrow condition-dependence of many SL interactions. Consequently, numerous SL interactions are likely to be missed by performing SL interactions screens

in one environmental condition only. A fraction of predicted SL interactions was tested experimentally, confirming the feasibility of the *in silico* approach. The study of condition-dependence of SL interactions in the yeast metabolic network was further extended to genetic interactions among non-metabolic genes. SL interaction data from literature were combined with available experimental data on the condition-dependence of single-gene deletion data. Remarkably, more than half of ~2,700 investigated SL interactions were found to take place between gene pairs of which either one or both appeared essential for viability under specific nutrient conditions. Furthermore, when assessing their phylogenetic distribution across species, members of SL pairs were not found to co-occur more frequently than random gene pairs. Thus, the condition-dependent restriction of SL interactions might be — at least for some interactions — explained by the individual essential functions of numerous genes under specific environmental conditions. Together, these findings support the notion that robustness to mutation might have arisen as a by-product during the evolution of adaptive responses to changing environmental conditions (reviewed in Kitano, 2004; Wagner, 2005).

1.10.2.1.6. Synthetic genetic interactions and disease

Enormous advances in genetics have uncovered numerous genes involved in human diseases. While many disorders are caused by mutations in single genes only, increasing numbers of human genetic diseases are identified to result from combinations of mutations in multiple genes (reviewed in Badano and Katsanis, 2002). Thus, many inherited mutations that alone have little effect can combine to result in severe defects. However, predicting and identifying such genetic interactions is a major obstacle in human genetics. Recently, synthetic lethal (SL) interactions have been systematically mapped in the yeast *S. cerevisiae* (Davierwala *et al.*, 2005; Pan *et al.*, 2006; Tong *et al.*, 2004). To date, however, approaches similar to the ones pioneered in yeast are not feasible in mammalian systems. Therefore, it is a major open question in genetics whether individual SL interactions are conserved between species and hence may be directly predicted in humans using interactions identified in simple model organisms. While functional studies in yeast have greatly contributed to our understanding of

individual gene functions in human (reviewed in Dolinski and Botstein, 2007), it remains to be determined whether SL interactions mapped in *S. cerevisiae* can be directly transferred into higher organisms and thus provide us with insights into complex human diseases.

In order to test the power of predicting candidate genetic interactions, comparative experimental approaches are needed. Ultimately, such a comparative study involves a tractable model system that is of higher complexity than yeast and that allows the analysis of systematic genetic perturbations. To date, *C. elegans* is the main animal model in which to carry out systematic functional studies *in vivo* in the context of a developing organism. The ability to inhibit gene function on a genome-wide scale by RNAi by bacterial feeding (Kamath *et al.*, 2003), together with its small size, its simple reproductive cycle and short generation time make the nematode *C. elegans* an ideal animal model system (Brenner, 1974) for the systematic study of synthetic genetic interactions. In particular, the comprehensive mapping of SL interactions in *C. elegans* would allow the comparison of *in vivo* genetic interaction networks between yeast, a single cellular organism and a multicellular animal. Such a study would have major practical implications for the use of yeast SL interaction data to predict SL interactions between genes in complex human diseases. Beyond the direct practical implications, this approach would also increase our understanding of the evolution and conservation of SL interactions.

1.11. Rationale of my study

Studies in *S. cerevisiae*, *C. elegans*, and *D. melanogaster* have shown that inactivation of most genes has little discernable effect on the organisms' fitness under laboratory conditions (Boutros *et al.*, 2004; Giaever *et al.*, 2002; Kamath *et al.*, 2003). These findings have led to various hypotheses explaining the observed high degree of gene dispensability. Whereas many of the apparently non-essential genes might have significant biological functions under specific environmental conditions ('environmental adaptation'; Papp *et al.*, 2004), an alternative theory suggests functional genetic redundancy as the principal source underlying the observed lack of phenotype upon loss

of individual gene function (‘mutational robustness’; Wagner, 2000a). This hypothesis proposes the existence of compensatory pathways as ‘fail-safe’ mechanisms to back-up essential biological pathways. While several lines of evidence support both the ability to adapt to new environmental conditions and the use of genetic compensatory pathways as potential sources underlying the apparent dispensability of a high proportion of genes in eukaryotic genomes, considerably more attention has been paid to the latter mechanism. This is not least because of the likely implications of non-additive, synthetic genetic interactions in complex human diseases (reviewed in Badano and Katsanis, 2002). Recently, enormous efforts have been made to systematically map synthetic lethal (SL) interactions in *S. cerevisiae* (Davierwala *et al.*, 2005; Pan *et al.*, 2006; Tong *et al.*, 2004). These studies appear to have uncovered thousands of gene pairs with redundant functions. However, at the time my study began, the systematic mapping of genetic interactions had not been extended to higher organisms.

The aim of my research was to begin to investigate functional redundancy in the genome of *C. elegans*. Therefore, I wished to systematically map SL interactions in the worm. To do so, it was crucial to establish protocols for simultaneously perturbing the functions of any pairwise combination of genes by using RNAi. First, I sought to investigate whether — and to which extent — *C. elegans* gene duplicates share redundant functions. Second, I wished to study functional redundancy in complex gene networks. In particular, I sought to investigate whether general modes of genetic redundancy are conserved between *S. cerevisiae* and *C. elegans* and whether I can find evidence for parallel pathways and back-up mechanisms in the worm. Therefore, I set out to investigate whether individual SL interactions are conserved between *S. cerevisiae* and *C. elegans*.

Chapter 2

Materials and Methods

2.1. Reagents

2.1.1. *C. elegans*

2.1.1.1. *C. elegans* strains

See Table 2.1. for *C. elegans* strains that were used in this study. The *C. elegans* Genetics Center, USA (http:// www.cbs.umn.edu/CGC/) and the National Bioresources Project, Japan (http:// shigen.lab.nig.ac.jp/c.elegans/index.jsp) provided these strains.

2.1.1.2. Nematode Growth Medium (NGM)

NaCl	3 g
Peptone	2.5 g
Optional[a]: Agar	19 g
dd H_2O	to 1 L

The solution was autoclaved and cooled to 55°C before addition of:

Cholesterol solution (5 mg/ml in ethanol)	1 ml
1M $CaCl_2$	1 ml
1M $MgSO_4$	1 ml
1M KH_2PO_4, pH6.0	25 ml
Fungizone	800 µl

in the order as written, with mixing thoroughly after addition of each component. Solutions were sterile-filtrated through a membrane filter with a pore size of 0.2 µm.

[a] For preparation of agar plates, solution was poured into sterile Petri dishes.

2.1.1.3. M9 Buffer

1M KH_2PO_4	3 g
1M Na_2HPO_4	6 g
1M NaCl	5 g
ddH_2O	to 1 L

1 ml 1M $MgSO_4$ was added after solution had been autoclaved to sterilize.

Gene(allele)	Strain	L1 worms added	Screening temperature
Wild-type	N2	~10	20°C
rrf-3(pk1426)	NL2099	~15	20°C
arx-3	tm1681	~15	20°C
C26E6.3(ok1728)	RB1477	~15	20°C
emb-27(ax81)	DS88	~15	15°C
emb-27(g48)	GG48	~10	15°C
emb-27(ye143)	HY621	~15	15°C
emb-27(g48)	TJ1047	~10-15	15°C
emb-27(g48)	TJ1049	~15	15°C
emb-27(g48)	TJ1061	~15	15°C
mat-1(ax161)	DS77	~15	15°C
mat-1(ax144)	DS80	~15	15°C
mat-1(ye121)	HY604	~15	15°C
cdc-42(ok825)	RB942	~10	20°C
dhc-1(or195)	EU828	~15	15°C
dyn-1(ky51)	CX51	~15	20°C
sel-9(ar22)	GS107	~15	20°C
pes-1(leDf1)	UL768	~10-15	20°C
pfd-4(gk430)	VC1032	~15	20°C
fkh-10(ok733)	RB884	~10	20°C
pqn-19(ok406)	RB674	~15	20°C
C43E11.2	tm1937	~15	20°C
F57C7.2(ok661)	RB836	~15	20°C
pch-2(tm1458)	CA388	~15	20°C
R06F6.2(ok1664)	RB1457	~15	20°C
div-1(or148)	EU548	~10	15°C
div-1(or148)	EU550	~15	15°C
div-1(or345)	EU879	~15	15°C
div-1(or345)	EU880	~15	15°C
xpa-1(ok698)	RB864	~10	20°C
ubc-1(gk14)	VC18	~10	20°C
F58G6.1	tm1060	~10	20°C
him-6(ok412)	VC193	~15	20°C
R07E5.3(ok622)	RB810	~15	20°C
gta-1(ok517)	RB748	~10	20°C
K08E3.5(ok233)	MG278	~10	20°C
C17H12(ok548)	RB769	~10	20°C

Table 2.1. Conditions for high-throughput RNA interference by liquid feeding
For each *C. elegans* strain used in this study, genotype ('Gene(allele)'), the number of L1 worms that were added per well of a 96-well plate, and the incubation temperature for RNA interference screens ('Screening temperature') are shown.

2.1.1.4. Freezing buffer

1M KH_2PO_4	3 g
0.05 M K_2HPO_4	129 ml
0.05 M KH_2PO_4	871 ml
NaCl	5.85 g
Glycerin	30% (v/v)

2.1.1.5. Bleach solution

NaOH	250 µl
Sodium hypochlorite	100 µl
Autoclaved H_2O	to 1000 µl

2.1.2. Bacteria

2.1.2.1. Ahringer RNAi feeding library

Bacterial clones used for RNA interference (RNAi) experiments were selected from the Ahringer RNAi feeding library (Kamath *et al.*, 2003).

2.1.2.2. Luria-Bertani (LB) medium

Bacto-tryptone	10 g
Bacto-yeast extract	5 g
NaCl	10 g
Optional[a]: Bacto-Agar	15 g
ddH_2O	to 1 L

pH was adjusted to 7.2 and solution was autoclaved to sterilize.

[a] For preparation of agar plates, solution was poured into sterile Petri dishes.

2.1.2.3. 2 x Tryptone / yeast extract (TY)

Bacto-tryptone	16 g
Bacto-yeast extract	10 g
NaCl	5 g
dd H_2O	to 1 L

pH was adjusted to 7.2 and solution was autoclaved to sterilize.

2.2. Protocols

2.2.1. Maintenance of *C. elegans* stocks

C. elegans was maintained on NGM agar plates seeded with OP50 *E. coli* according to standard protocols (Brenner, 1974).

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2.2.2. Freezing and recovery of *C. elegans* stocks

For freezing, worms that were approaching starvation — L1 and L2 worms survive freezing best — were washed off plates in M9 buffer, pelleted by centrifugation at 1,000 rpm for 1 minute, and resuspended in an equal volume of M9 and freezing buffer. 1 ml of suspension was aliquoted per 1.8 ml cryovial. Cryovials were placed into freezing boxes filled with isopropanol to allow a gradual 1°C decrease in temperature per minute when placed at -80°C. Cryovials were stored at -80°C.

For thawing, cryovials were placed at room temperature and worms were spotted onto NGM plates seeded with OP50 *E. coli* as soon as all ice had turned to liquid.

2.2.3. High-throughput RNA interference liquid-feeding assay

All RNA interference (RNAi) experiments were performed by using bacterial feeding in liquid cultures in 96-well format.

Preparation of bacteria:

Bacterial glycerol stocks were replica plated onto LB plus 100 µg/ml ampicillin plates using a 96-pin replicating tool and grown overnight at 37°C. The day before starting the screen, bacteria were inoculated in 400 µl 2 x TY containing 100 µg/ml ampicillin in 2-ml 96-well plates and grown overnight to saturation (~15 hours) in a shaking incubator at 220 rpm at 37°C.

Induction of bacteria:

The following morning, expression of double-stranded RNA (dsRNA) was induced by addition of isopropyl-beta-D-thiogalactopyranoside (IPTG) to a final concentration of 4 mM to each well of bacterial cultures and incubated while shaking at 220 rpm for 1 hour at 37°C. Subsequently, bacterial culture medium was replaced by nematode growth medium (NGM). Therefore, bacterial cultures were pelleted by spinning at 3,500 rpm for 5 minutes, the supernatant discarded by quickly inverting the 2-ml 96-well plates, and pelleted bacterial clones were resuspended in 400 µl of NGM plus 100 µg/ml ampicillin and 4 mM IPTG.

Preparation of worms:

96-well RNAi liquid-feeding assays were started with L1 worms. Synchronized populations of L1 worms were obtained by filtration of mixed-stage populations through an 11 µm nylon mesh (MultiScreen^{TM} Nylon Mesh, Millipore) or by bleaching adult worms and allowing their eggs to hatch in M9 buffer.

Filtering: When synchronizing worms by filtration, it is recommended to use worm populations that are approaching starvation, to avoid dilution of dsRNA-expressing bacteria with OP50 *E. coli*. Meshes were sterilized before each filtration with 70% v/v ethanol. Worms were washed off plates in M9 buffer, and transferred into a mesh placed on top of a 2-ml 96-well plate. L1 worms were passed through the mesh by centrifugation at 1,000 rpm for 30 seconds. Synchronized L1s were collected and diluted to a concentration of approximately 10 L1s (for worm strains with a brood size similar to wild-type worms) or 15 L1s (for strains with reduced brood size as compared to wild-type) per 15 µl M9 buffer.

Bleaching: An alternative approach for obtaining synchronized populations of L1 worms was the bleaching of gravid adults and allowing their eggs to hatch overnight in M9 buffer. Therefore, gravid adult worms were washed off plates in M9 buffer and collected by centrifugation at 1,000 rpm for 1 minute. Supernatant was aspirated off, and 1-2 ml bleach solution (depending on the size of the worm pellet) was added. Worms were incubated in bleach solution with occasional vortexing until dissolved and only embryos remained. To remove bleaching solution, eggs were resuspended in 10 ml M9 buffer and subsequently centrifuged at 1,000 rpm for 1 minute. This step was repeated three times. Worms were allowed to hatch overnight in M9 buffer with gentle rocking to allow aeration. The next morning, larvae were pelleted by centrifugation at 1,000 rpm for 1 minute and diluted to a final concentration of 10 to 15 L1s per 15 µl M9 buffer.

RNAi feeding experiments:

Worms in a final concentration of 10 to 15 L1s per 15 µl M9 buffer were pipetted into each well of a 96-well flat-bottom plate from a plastic tray by using a 12-well multi-channel pipette. To avoid settling of worms in the plastic tray, worms were pipetted up and down before aliquoting. Subsequently, 40 µl of resuspended bacterial culture were added to each well. For combinatorial RNAi feeding experiments, resuspended cultures of different bacterial strains were mixed to give a final volume of 40 µl.

Plates were incubated shaking at 150 rpm, 20°C, for 4 days (15°C for 6 days for temperature-sensitive genetic mutants; see Table 2.1. for screening conditions for all strains used in this study). To avoid evaporation of liquid from wells, 96-well plates were stacked in sealable plastic boxes and covered with a wet tissue.

Scoring of phenotypes:

After an appropriate incubation time (4 to 6 days), worms were screened under a dissecting microscope for sterility, embryonic lethality, growth, and developmental defects. Sterility and embryonic lethality were scored semi-quantitatively on a scale from 0 (wild-type) to 3 (100% sterile or embryonic lethal). In cases where sterility or embryonic lethality appeared enhanced after targeting both genes simultaneously by combinatorial RNAi or by RNAi in a genetic mutant as compared to phenotypes of each individual gene, phenotypes were verified by quantification (see below).

2.2.4. Testing post-embryonic additive RNAi phenotypes and known post-embryonic synthetic genetic interactions

To score post-embryonic phenotypes, L1 larvae were collected from the 96-well liquid-feeding assay 4 days after the screen was set up and allowed to develop further on 12-well NGM plates. Therefore, liquid-feeding cultures were filtered through an 11 µm nylon mesh (MultiScreen^{TM} Nylon Mesh, Millipore) by centrifugation and L1 larvae were spotted onto 12-well NGM plates containing 100 µg/ml ampicillin and 1 mM isopropyl-beta-D-thiogalactopyranoside (IPTG), seeded with bacteria expressing a non-targeting dsRNA (Ahringer library clone Y95B8A_84.g). Adult worms were scored after further incubation at 20°C for 72 hours. Since I was assessing second-generation (post-embryonic) phenotypes, I had to exclude genes that resulted in sterility, embryonic lethality, or larval growth arrest after RNAi. Only genes that were (according to the above criteria) amenable to analysis in both wild-type worms and the RNAi-hypersensitive *rrf-3* background could be included in this study.

2.2.5. Investigating dilution effects induced by combinatorial RNAi

To investigate the extent to which combining dsRNA-expressing bacteria leads to a reduced strength of RNAi phenotypes, I evaluated the average failure rate for the successful generation of a phenotypically detectable knockdown for single genes at a given dilution. I therefore added unrelated control dsRNA-expressing bacteria to bacteria expressing dsRNA against genes with previously known non-viable RNAi phenotypes at 2-, 3-, 4-, 5-, and 10-fold dilution. The Ahringer library clone Y95B8A_84.g, expressing dsRNA that does not target an expressed sequence in *C. elegans* and bacteria expressing dsRNA against *lin-31*, respectively, were used as control dsRNA-expressing bacteria.

2.2.6. Estimating the false-negative rate of combinatorial RNAi

Assuming each gene is an independent targeting event in combinatorial RNAi, I estimated the false-negative rate of combinatorial RNAi for identifying multigenic

interactions by calculating the detection rate of n-genic interactions to be x^n, where x is the detection rate of single-gene phenotypes at n-fold dilution.

2.2.7. Identification of *C. elegans* gene duplicates

I used the INPARANOID algorithm (Remm *et al.*, 2001) (version 4.0) to identify genes that have been duplicated in the genome of *C. elegans* since divergence from *S. cerevisiae* and *D. melanogaster*, respectively. I therefore identified single orthologues in *S. cerevisiae* and *D. melanogaster* genomes that correlate to duplicate gene pairs in *C. elegans*.

2.2.8. Identification of *C. elegans* orthologues of *S. cerevisiae* gene pairs with synthetic lethal interactions

The INPARANOID algorithm (Remm *et al.*, 2001) (version 4.0) was used to identify *C. elegans* orthologues of all *S. cerevisiae* gene pairs that were reported to have synthetic lethal or sick (SL) interactions in at least one of three genome-scale screens (Davierwala *et al.*, 2005; Pan *et al.*, 2006; Tong *et al.*, 2004). I only tested for genetic interactions between gene pairs that both had a single orthologue in *C. elegans*.

2.2.9. Identification of synthetic genetic interactions using combinatorial RNAi

For the identification of synthetic genetic interactions between *C. elegans* gene duplicates, I excluded all genes that are targeted by bacterial clones from the *C. elegans* whole-genome RNAi library (Kamath and Ahringer, 2003) with inserts having more than 80% nucleotide identity over 200 bp with multiple predicted genes from the analysis. This is the threshold for cross-reaction used in Kamath *et al.* (2003). Furthermore, genes that resulted in first-generation larval growth arrest after RNAi were not included in any study for synthetic genetic interactions, since this strong phenotype cannot be enhanced any further.

When screening for genetic interactions using combinatorial RNAi, single-gene RNAi phenotypes (as references) were compared with combinatorial RNAi phenotypes side by side. To account for dilution effects arising from combining two dsRNA-expressing bacteria, equal amounts of non-targeting dsRNA-expressing bacteria (Ahringer library clone Y95B8A_84.g) were added to bacteria expressing dsRNA targeting the reference genes. This setup allowed combinatorial RNAi to be performed in triplicates within independent screens, and RNAi against each gene individually in duplicates within independent assays.

Screens for synthetic genetic interactions were performed at least twice independently in duplicate/triplicate within independent assays. Synthetic phenotypes needed to be unambiguous and reproducible in at least two independent RNAi experiments to be scored positive.

Qualitatively observed synthetic lethal phenotypes were further verified by quantification. Therefore, larvae, unhatched eggs, and adults from each RNAi experiment were manually counted. To facilitate counting of worms and dead embryos, respectively, NaN_3 was added to a final concentration of 25mM to each well before worms were spotted onto empty 12-well NGM plates. Quantitative phenotype data were subject to statistical analysis as described under ‘Statistical analysis of quantitative phenotype data under a multiplicative model’.

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2.2.10. Identification of synthetic lethal interactions using RNAi in genetic mutants

When testing for genetic interactions using RNAi to target a single gene in a *C. elegans* strain carrying a homozygous viable loss-of-function allele (see Table 2.1. and Appendix Table 5.2.), RNAi phenotypes seen in the genetic mutants were compared to the RNAi phenotypes of wild-type worms and to the phenotypes of the genetic mutants fed on bacteria expressing a dsRNA that does not target an expressed portion of the *C. elegans* genome (Ahringer library clone Y95B8A_84.g).

Screens for synthetic genetic interactions were performed at least twice independently in duplicate within independent assays. Synthetic phenotypes needed to be

unambiguous and reproducible in at least two independent RNAi experiments to be scored positive.

To exclude false synthetic phenotypic effects caused by non-specific additive effects of genetic mutants and RNAi phenotypes, respectively, when screening for synthetic phenotypes, mutant strains that showed enhanced phenotypes when targeting a second gene by RNAi as compared to the phenotypes observed in wild-type worms were also fed on additional RNAi clones that produce phenotypes similar to the putatively interacting gene in wild-type worms.

2.2.11. Statistical analysis of quantitative phenotype data under a multiplicative model

To be able to unambiguously identify SL interactions, quantitative phenotype data were subject to statistical analysis. Therefore, measurements of brood size and embryonic viability following RNAi were normalized to measurements obtained when worms were fed on bacteria expressing dsRNA against control genes that give no detectable phenotypes (‘wild-type brood size’ and ‘wild-type embryonic survival’). In cases where measurements for brood size and embryonic viability exceeded 100% of wild-type brood and viability, values were set to 100% of wild-type values.

I analysed quantitative phenotype data under a multiplicative model. In this model, the null hypothesis for the contribution of independent genetic loci to a phenotype is that the double mutant RNAi phenotype equals the product of mutant phenotypes associated with each individual gene. Thus, to examine whether the combinatorial RNAi phenotypes were truly synthetic or merely additive, I compared the quantitative phenotypes following combinatorial RNAi with the calculated products of measurements for both individual genes of a pair. I therefore multiplied duplicate brood size and embryonic survival measurements obtained after RNAi against each gene individually (n=2) in two independent experimental setups (n=2) in all possible pairwise combinations. That way I obtained sixteen values (n=4x4) that represented the expected brood size and embryonic survival measurements if genes do not interact. This array of

calculated products was compared to six measurements (n=3x2) obtained after combinatorial RNAi in two independent experiments.

I used a Student's t-Test (two-tailed distribution, two-sample equal variance) to determine whether the observed values differed significantly from the expected values. I considered genes to be synthetic lethal if p-values were below 5.0×10^{-2}.

2.2.12. Evolutionary analysis

The INPARANOID algorithm (version 4.0) was used to identify *C. elegans* orthologues of *C. briggsae* genes (Remm *et al.*, 2001). If both *C. elegans* duplicates had a single identifiable orthologue in *C. briggsae*, this implies that the duplication predates the divergence of *C. elegans* from *C. briggsae*. Protein sequences were aligned using the CLUSTAL W program to determine the percentage of identity between gene duplicates (Thompson *et al.*, 1994). Numbers of synonymous nucleotide substitutions per synonymous site (Ks) and non-synonymous nucleotide substitutions per non-synonymous site (Ka) were estimated using the Maximum Likelihood Method (Goldman and Yang, 1994; Muse and Gaut, 1994).

2.2.13. Comparative analysis of synthetic genetic interactions compiled from literature

Previously known genetic interactions were extracted from BIOGRID (for *S. cerevisiae*; Stark *et al.*, 2006), WormBase (for *C. elegans*; www.wormbase.org), and FlyBase (for *D. melanogaster*; Crosby *et al.*, 2007), respectively, and the INPARANOID algorithm (version 5.1) was used to identify single orthologues between these species.

2.2.14. Imaging

For imaging, worms were pipetted from the 96-well RNAi liquid-feeding assay onto microscope slides. One drop of 25mM NaN_3 was added to worms in liquid before adding a coverslip. Worms with adult lethal phenotypes were very fragile and thus could

not be covered with a coverslip for imaging. Zeiss Stemi SV11 microscope plus Axiovision software version 7.0 were used to capture images.

2.2.15. Amplification of DNA fragments by polymerase chain reaction (PCR)

To confirm the identity of RNAi clones, gene-specific DNA fragments were amplified by PCR and verified by sequencing. For each clone, a separate PCR reaction was performed. Therefore, a small amount of bacterial clones was added to ~ 5 µmol of each primer, 1x Bioline NH_4 reaction buffer, ~37.5 mM $MgCl_2$, 20 mM dNTPs (Boehringer) and 0.2 µl *Taq* DNA polymerase (Bioline) in a 25µl reaction.
PCR Machines were preheated to 94°C for five minutes, followed by 34 cycles of 94°C for 30 seconds, 58°C for 30 seconds, and 72°C for 2 minutes. PCR reactions were stored at -20°C until use. Sequencing of PCR products was performed using standard protocols at The Wellcome Trust Sanger Institute using 5 µmol of sequencing primers for each reaction.

Primers:
L4440 forward primer: 5'—AGCGAGTCAGTGAGCGAGGAAGC—3'
L4440 reverse primer: 5'—GGTTTTCCCAGTCACGACGTTG—3'
Sequencing primer: 5'—TCGAGGTCGACGGTATCG—3'

Chapter 3

Establishing and validating protocols for high-throughput RNA-mediated interference

3.1. Introduction

RNA-mediated interference (RNAi) is a powerful tool for studying the loss-of-function phenotypes of genes. So far, RNAi has been used extensively to generate genome-scale views of gene functions in the nematode *C. elegans* and in fly cells (for examples see Boutros *et al.*, 2004; Bjorklund *et al.*, 2006; Kamath *et al.*, 2003; Kiger *et al.*, 2003; Simmer *et al.*, 2003). In particular in *C. elegans*, the feasibility of generating loss-of-function phenotypes by feeding worms on bacteria expressing double-stranded RNA (dsRNA) against a target gene of interest ('RNAi by bacterial feeding'; Timmons and Fire, 1998) has led to the generation of a whole-genome RNAi feeding library, consisting of 16,757 dsRNA-expressing bacterial clones corresponding to ~86% of predicted *C. elegans* genes (Kamath *et al.*, 2003). This resource allows for the rapid and low-cost analysis of gene functions on a large scale. Therefore, RNAi by bacterial feeding has become the method of choice for performing genome-scale loss-of-function screens in the worm.

While loss-of-function analyses of individual genes give us an unprecedented level of insight into the molecular roles of genes, genome-wide studies revealed that inactivation of most genes in any organism has little discernible effect on fitness under laboratory conditions (Bjorklund *et al.*, 2006; Boutros *et al.*, 2004; Giaever *et al.*, 2002; Kamath *et al.*, 2003). However, inactivating specific rare combinations of such non-essential genes can have profound effects on the organism under exactly the same conditions, the most dramatic being inviability (Dobzhansky, 1946; Sturtevant, 1956; reviewed in Guarente, 1993; Hartman *et al.*, 2001). These combinatorial effects are termed 'synthetic enhancement' or 'synthetic lethal' interactions. Synthetic lethal (SL) genetic interactions are classically interpreted as the result of inactivating two functionally redundant pathways in the cell, each of which is individually dispensable (reviewed in Guarente, 1993; Hartman *et al.*, 2001). Recently, synthetic sick and synthetic lethal (SL) interactions have been mapped systematically in the yeast *S. cerevisiae*. These large-scale studies appear to have uncovered an extensive degree of redundancy in the genome of *S. cerevisiae*. Strikingly, while only ~1,000 of the ~6,000 genes in the yeast genome are essential for viability under standard laboratory conditions and thus show a lethal phenotype when deleted, systematic large-scale studies have

uncovered thousands of SL interactions under identical conditions. These results led to the estimation that, on a genome-wide scale, inactivation of ~ 200,000 pairwise gene combinations might have detrimental effects (reviewed in Boone *et al.*, 2007). These approximations highlight the complexity of biological functions. Thus, while studying the molecular roles of individual genes is a major advance, an understanding of how each phenotype is modulated by the activities of other genes will prove to be just as critical.

Comparable large-scale approaches to identify SL interactions in more complex systems will reveal the extent of redundancy in different genomes, and will also shed light on the evolution and conservation of gene networks (as discussed in the Introduction).

Extrapolating the estimates from large-scale yeast genetic interaction studies to the genome of *C. elegans*, it is evident that high-throughput platforms are needed for the systematic mapping of genetic interactions in the worm. At the time my study began, RNAi by feeding was conventionally performed on 12-well nematode growth medium (NGM) plates (‘plate feeding’; as discussed in the Introduction, see Figure 1.3). However, the throughput of this approach is limited. It thus was critical to establish protocols that allow RNAi experiments to be performed at considerable higher throughput.

In collaboration with Ben Lehner in the lab, I sought to develop a robust high-throughput (HTP) assay for screening RNAi phenotypes in liquid cultures in 96-well format. There are numerous advantages to performing RNAi screens by liquid feeding in 96-well format over conventional plate-feeding protocols. First, all pipetting steps can be done by using multichannel tools, thereby considerably reducing the time it takes to set up screens. Second, when feeding worms in liquid culture, one can increase the amount of food as compared to plate feeding, thereby allowing multiple worms to be screened in each individual RNAi experiment. Thus, in contrast to screening progeny of individual animals — as is done when using conventional plate-feeding methods — RNAi by liquid feeding would allow the screening of populations of worms for loss-of-function phenotypes. First, this would remove the laborious manual step of transferring single worms into individual wells. Most importantly, however, considering the inherent

animal-to-animal variability in RNAi, screening progeny of individual animals can lead to the observation of rather extreme phenotypes and hence to the representation of greatly biased results. In contrast, analysing the loss-of-function phenotypes of pooled adults allows to identifying the mean phenotype. Thus, screening populations of worms would average the animal-to-animal variation of RNAi phenotypes and lead to more standardized results than obtained when using single-animal plate-feeding protocols. Finally, with the smaller size of 96-well in comparison to 12-well plates, incubation space is unlikely to become a limiting factor for performing RNAi screens on a large scale.

Thus, in summary, RNAi by liquid feeding in 96-well format would allow us to study the loss-of-function phenotypes of entire worm populations, thereby avoiding the animal-to-animal variation of RNAi phenotypes. That way, we would obtain an estimate of the mean RNAi phenotype while substantially increasing the throughput.

In this chapter, I will describe the development of an experimental platform for the screening of RNAi phenotypes in liquid culture in 96-well format and demonstrate that this is an efficient and robust method for analyzing loss-of-function phenotypes in *C. elegans*. Moreover, I will discuss how I have adapted these protocols for using RNAi to simultaneously target two genes in the genome of *C. elegans*.

3.2. Establishing a high-throughput RNAi liquid-feeding assay in 96-well format

To set up an HTP platform for RNA interference (RNAi) by liquid feeding, we considered the following criteria. First, we sought to identify an appropriate volume of liquid to ensure adequate aeration of feeding cultures. Second, we needed to identify the number of larval-stage worms that can be accurately pipetted using a multi-channel pipette while being compatible with the volume of bacterial feeding cultures. Taking into account that flat-bottomed 96-well plates can maximally contain 200 µl per individual well, we reasoned that a total volume of 50 µl might be suitable for liquid cultures to be sufficiently oxygenated without requiring excessive shaking. Next, we sought to determine an adequate number of worms to be dispensed into each well. We wanted to start the 96-well liquid-feeding protocol with first larval stage (L1) worms, because it is

straightforward to obtain synchronized cultures of L1 worms by filtering mixed-stage populations of worms through an 11 µm nylon mesh (MultiScreen^{TM} Nylon Mesh, Millipore), or by bleaching adult worms and allowing their eggs to hatch over night in M9 buffer (see Materials and Methods for a detailed description). We found approximately 10 L1 worms (in a volume of 10 µl) to be the minimum number of animals that can be reproducibly distributed. This low quantity also allows L1s to grow to adulthood, lay eggs and these eggs to hatch without food becoming a limiting factor.

3.2.1. Experimental design

For RNAi experiments, selected bacterial strains of the *C. elegans* RNAi feeding library (Kamath *et al.*, 2003) were inoculated in bacterial culture medium containing ampicillin in deep 96-well plates (see Figure 3.1. for an overview of the experimental procedure and Materials and Methods for a more detailed description of the protocol). Bacterial cultures were grown to saturation while shaking at 37°C before inducing dsRNA expression by adding isopropyl-beta-D-thiogalactopyranoside (IPTG) for 1 hour at 37°C. For RNAi by feeding, we exchanged the bacterial culture medium for Nematode Growth Medium (NGM) supplemented with ampicillin and IPTG. Therefore, bacterial cultures were collected by centrifugation, the supernatant discarded, and pelletet bacteria were resuspended in a volume of NGM equal to bacterial culture medium. Approximately 10 L1 worms were distributed into each well of a 96-well flat-bottom plate and 40 µl of the resuspended bacterial feeding cultures were added. For strains with lower brood size as compared to wild-type worms, roughly 15 L1 worms were used. Plates were incubated shaking at 150 rpm, 20°C, for 4 days. This time period allowed L1 worms to grow to adults, lay eggs and for these eggs to hatch and develop into larvae. After 4 days worms had consumed most of their food, which resulted in clearing of the suspension, thereby allowing easy scoring of phenotypes. Worms were manually assessed for viability, fecundity and growth defects using a dissecting microscope (for example phenotypes see Figure 3.2.). Note that at the same time, others have described similar protocols for performing RNAi screens in 96-well format (Nollen *et al.*, 2004; van Haaften *et al.*, 2004).

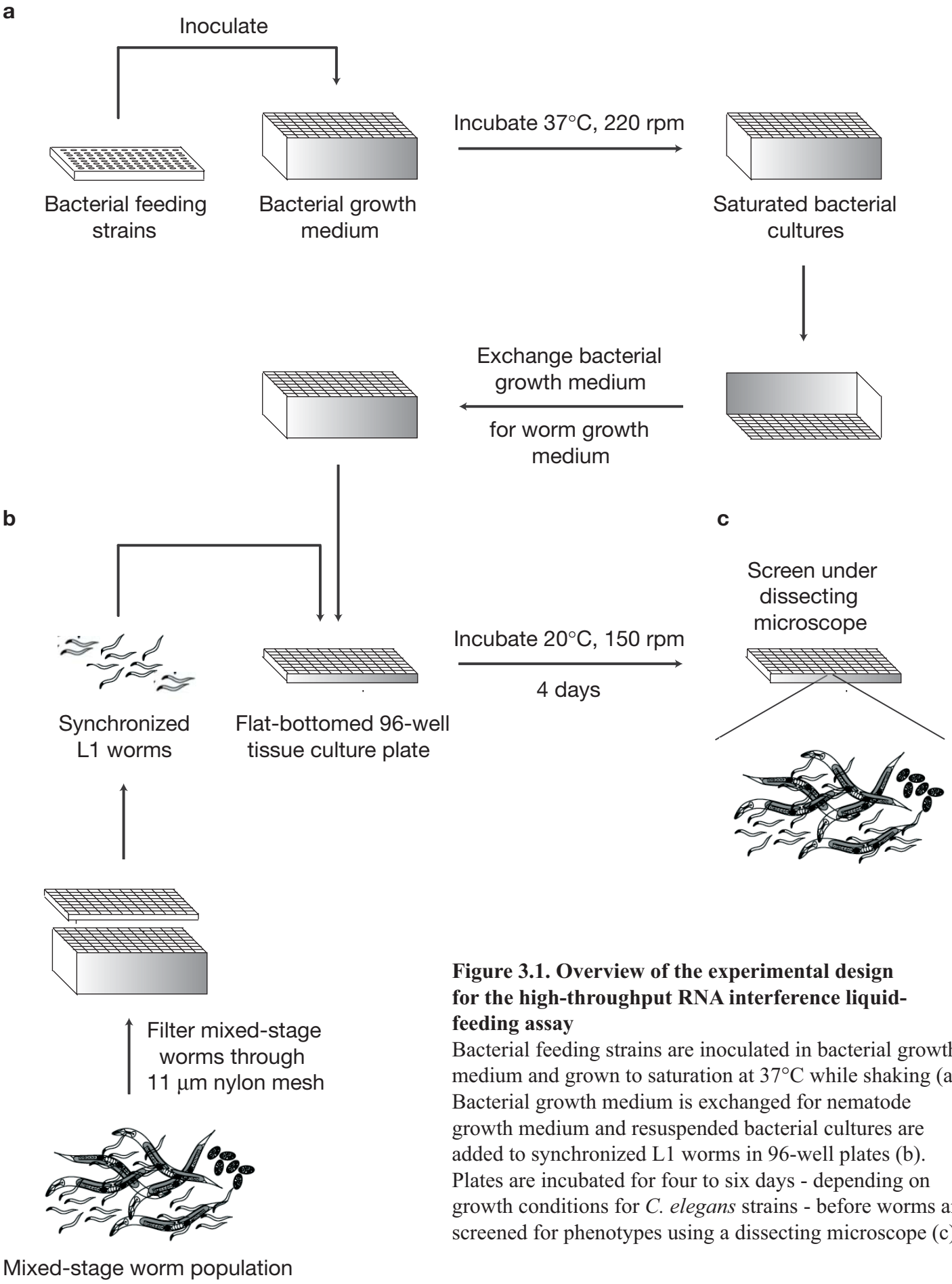

Figure 3.1. Overview of the experimental design for the high-throughput RNA interference liquid-feeding assay

Bacterial feeding strains are inoculated in bacterial growth medium and grown to saturation at 37°C while shaking (a). Bacterial growth medium is exchanged for nematode growth medium and resuspended bacterial cultures are added to synchronized L1 worms in 96-well plates (b). Plates are incubated for four to six days - depending on growth conditions for *C. elegans* strains - before worms are screened for phenotypes using a dissecting microscope (c).

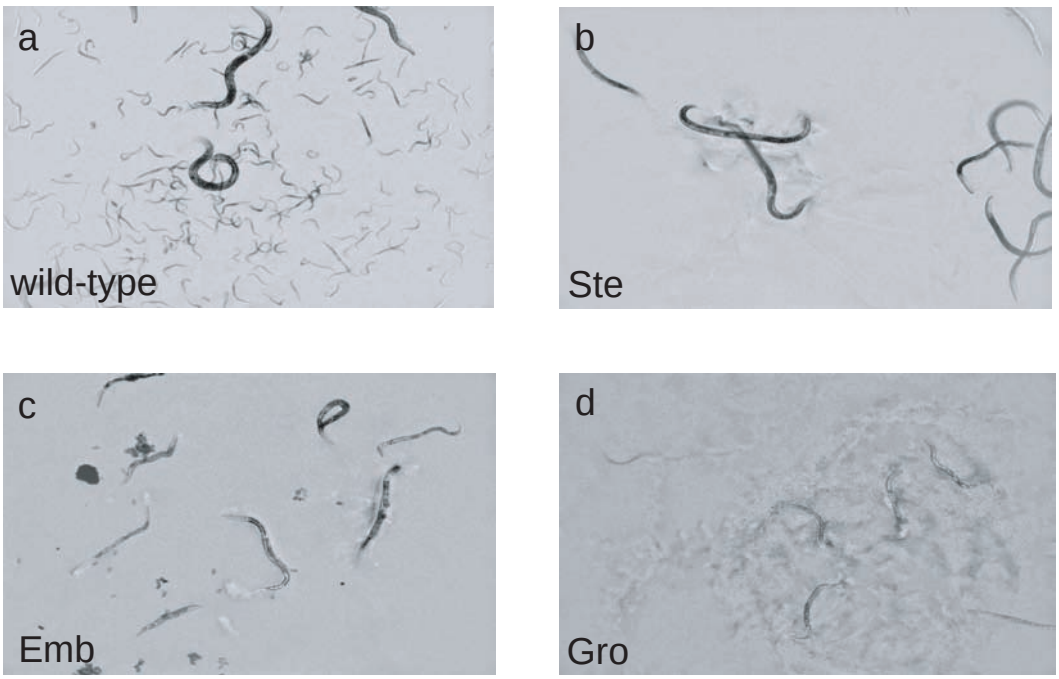

Figure 3.2. Example phenotypes generated by RNA interference by liquid feeding in 96-well format
Representative pictures of wild-type worms (a), sterile (b), embryonic lethal (c, black dots represent dead eggs), and first-generation growth defective (d) worms in 96-well plates are shown.

3.2.2. Determining the sensitivity of RNAi by liquid feeding in 96-well format

To evaluate the sensitivity of RNAi by liquid feeding in 96-well format, I sought to assess the ability of this newly developed assay to recapitulate RNAi phenotypes that have been generated using conventional plate-feeding protocols. Therefore, I selected all 391 genes from *C. elegans* chromosome III that showed an RNAi phenotype in the genome-wide screen performed by Kamath *et al.* (2003) (see Appendix Table 3.1.). I chose to determine the sensitivity of the RNAi liquid-feeding assay in the RNAi-hypersensitive *rrf-3* background, which had previously been found to result in an increased penetrance of RNAi phenotypes as compared to wild-type worms.

First, I tested 282 genes that were reported to give a non-viable (embryonic lethal or sterile) RNAi phenotype and investigated these for non-viable phenotypes in two independent experimental setups. I identified 209 genes (74%) to also result in non-viable RNAi phenotypes in two independent screens by RNAi by liquid feeding. An additional 19 and 10, respectively, genes were found to show non-viable phenotypes in either screen (Table 3.1).

Next, I focused on genes that were known to result in slowed post-embryonic growth. Of 64 genes assayed, 39 genes (61%) gave a detectable RNAi phenotype in two separate screens and a further 9, and 3 genes, respectively, displayed an RNAi phenotype in either screen. The great majority of these genes, however, showed non-viable, rather than slowed growth RNAi phenotypes when screening *rrf-3* worms in my experimental setting (see below for discussion).

Finally, I investigated the detection rate of post-embryonic phenotypes. Of 45 genes with any known post-embryonic RNAi phenotype identified by Kamath *et al.*, 24 genes (53%) showed an RNAi phenotype in both independent screens, and phenotypes were detected for an additional 6, and 4, respectively, genes in either assay. Roughly two thirds of these genes displayed non-viable RNAi phenotypes.

There are two possible explanations for the increased frequency of non-viable RNAi phenotypes that I observed when screening *rrf-3* worms using the 96-well liquid-feeding assay, as compared to the results reported by Kamath and co-workers. First, I used the RNAi-hypersensitive strain *rrf-3*, which is known to result in higher

Kamath *et al.*, 2003 RNAi screen	96 well RNAi liquid feeding assay								
	Detected			Identical			Non-viable		
	Screen 1	Screen 2	Mean	Screen 1	Screen 2	Mean	Screen 1	Screen 2	Mean
Non-viable (n=282)	238 (84%)	232 (82%)	235 (83%)	228 (81%)	219 (78%)	224 (79%)	228 (81%)	219 (78%)	224 (79%)
Growth-defective (n=64)	48 (75%)	42 (66%)	45 (70%)	11 (17%)	8 (13%)	10 (15%)	37 (58%)	33 (52%)	35 (55%)
Post-embryonic (n=45)	30 (67%)	28 (62%)	29 (64%)	4 (9%)	3 (7%)	4 (8%)	20 (44%)	17 (38%)	19 (41%)
Total (n= 391)	316 (81%)	303 (77%)	310 (79%)	243 (62%)	230 (59%)	237 (60%)	285 (73%)	269 (69%)	277 (71%)

Table 3.1. Effectiveness of RNA interference by liquid feeding in 96-well format
RNA interference (RNAi) phenotypes for each gene on *C. elegans* chromosome III that was reported to result in non-viability ('Non-viable'), slowed post-embryonic growth ('Growth-defective') or defects in post-embryonic development ('Post-embryonic') in the genome-wide RNAi screen performed by Kamath *et al*. (2003) were determined when feeding the RNAi-hypersensitive *rrf-3* strain in liquid culture in 96-well format. Worms were assessed for non-viability, slowed post-embryonic growth, and defects in post-embryonic development in two independent experiments ('Screen1', 'Screen 2'). Data shown represent the total number of genes (percentages in brackets) that were detected in each phenotypic category ('Detected'), that had phenotypes identical to the ones reported by Kamath *et al.* ('Identical'), and that resulted in non-viability ('Non-viable') after RNAi, respectively. Mean values for both independent screens are rounded up.

penetrance of RNAi phenotypes as compared to wild-type worms, while Kamath *et al.* have performed their genome-wide screen in wild-type animals. Second, I delivered dsRNA to L1 worms, whereas protocols for RNAi by plate feeding start with L3-stage worms. This early interference of gene expression is likely to lead to more severe developmental defects. When comparing my results to previously reported RNAi phenotypes (www.wormbase.org), I found that the great majority (87%) of genes that resulted in non-viability rather than in slowed post-embryonic growth or other post-embryonic defects — as found by Kamath *et al.* (2003) — were shown to have a non-viable phenotype in other RNAi screens, demonstrating that my results are not false positives.

In summary, RNAi by liquid feeding in 96-well format using the RNAi-hypersensitive *rrf-3* strain allowed, on average, the detection of approximately 80% of genes with a previously identified RNAi phenotype in wild-type worms when using conventional plate-feeding protocols. I was able to re-discover over 80% of previously known non-viable RNAi phenotypes. In addition, approximately 70% of genes conferring growth defects and two thirds of genes resulting in any visible post-embryonic phenotype upon RNAi in wild-type worms were identified by bacterial feeding in liquid culture with roughly 90% reproducibility. Importantly, even when only scoring for non-viable phenotypes using the RNAi-hypersensitive *rrf-3* strain, this HTP RNAi liquid-feeding assay can, on average, capture approximately 60% of any phenotypes that have been detected in wild-type worms when using conventional plate-feeding protocols.

Taken together, these results demonstrate that RNAi by bacterial feeding in liquid culture in 96-well format is a powerful tool for generating loss-of-function phenotypes for *C. elegans* on a large scale. Thus, although RNAi phenotypes for some genes might be missed in this assay, RNAi by liquid feeding in 96-well format makes it feasible to perform roughly two thousand individual RNAi experiments per researcher per day, thereby increasing the throughput as compared to conventional plate-feeding protocols by approximately ten-fold. Most notably, by pooling animals, one can average the animal-to-animal variation of RNAi phenotypes. To obtain similarly standardized results by RNAi by plate feeding, phenotypes for numerous individual adults and their progeny would

need to be assessed. In that respect, the throughput of RNAi by liquid feeding is considerably higher than the estimated 10-fold, but rather ranges around 50-fold.

In summary, I consider the RNAi liquid-feeding assay in 96-well format using the RNAi-hypersensitive *rrf-3* background a HTP screening platform and an efficient and robust method for generating genome-scale views of gene function in *C. elegans*. I will refer to this screening tool as the 'high-throughput (HTP) RNAi liquid-feeding assay' throughout my text.

3.2.3. Targeting multiple genes simultaneously by combinatorial RNAi

While analyzing the loss-of-function phenotypes of individual genes in an essentially wild-type background is a major advance, understanding how each phenotype is modulated by the activities of other genes will give us deeper insights into the complexity of biological functions. If mutations in one gene modulate the mutant phenotype of a second gene, these two genes are said to genetically interact (discussed in Hartman *et al.*, 2001).

To identify genetic interactions and to uncover genetic redundancy systematically in *C. elegans*, it was critical to establish methods for simultaneously perturbing two genes. Therefore, the concurrent targeting of two genes by RNAi would provide a powerful approach for unbiased searches for genetic interactions in the worm. Previously, it had been shown that injection of two dsRNAs targeting two individual loci could effectively reduce the expression of both genes simultaneously (Gotta and Ahringer, 2001; Paradis and Ruvkun, 1998; Pocock *et al.*, 2004). However, because RNAi by injection is both very labour-intensive and costly, its applications are limited. In contrast, targeting two genes simultaneously by RNAi by bacterial feeding would allow the systematic study of bigenic interactions in the genome of *C. elegans*.

Thus, to be able to carry out unbiased screens for genetic interactions in *C. elegans*, I sought to establish conditions for simultaneously targeting two genes using RNAi by bacterial feeding. Therefore, I considered three potential approaches (Figure 3.3.). One possibility would be the generation of a 'two-gene' bacterial feeding library by

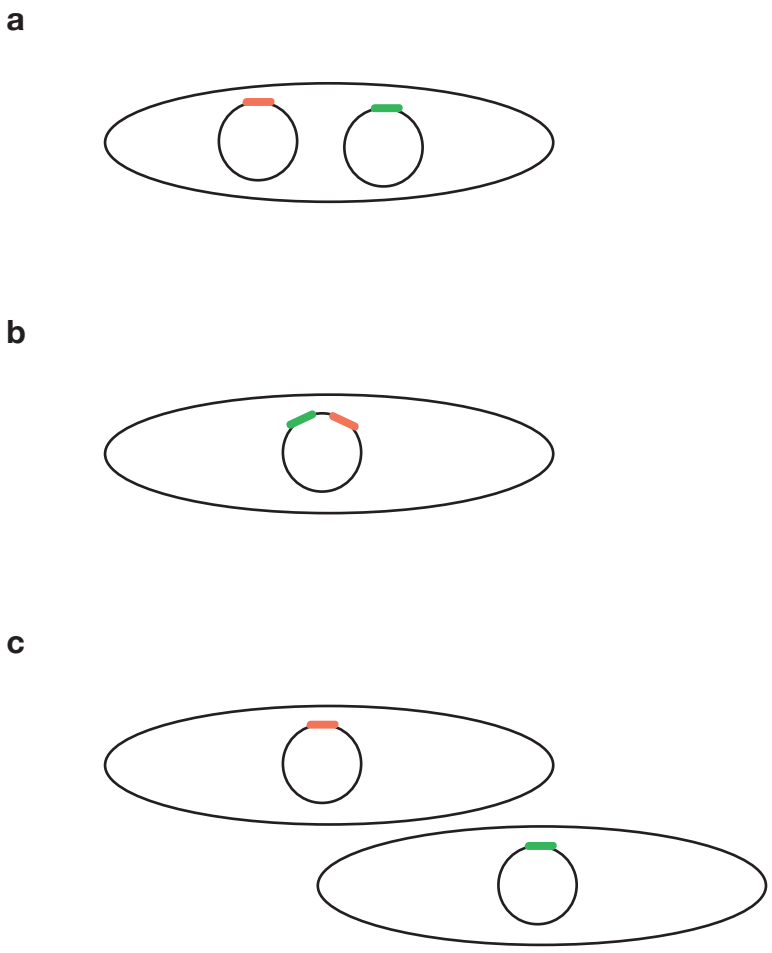

Figure 3.3. Three approaches for using RNA interference by bacterial feeding to simultaneously target two genes
Bacterial strains are transformed with two 'RNA interference (RNAi) feeding vectors' (L4440), each engineered to expressing a dsRNA against a different target gene of interest (a). Bacterial strains are transformed with one RNAi feeding vector, engineered to express two dsRNAs, each targeting a different gene of interest (b). Mixing of two RNAi feeding clones, each expressing a dsRNA targeting a different gene of interest (c). Ellipses, bacterial feeding strains. Circles, RNAi feeding vectors. Red and green rectangles, DNA fragments complementary to the coding regions of two different genes of interest.

co-transforming two ‘RNAi feeding vectors’ (L4440), each harbouring a different selection marker and engineered to express dsRNA against any target gene of interest, into one bacterial feeding strain. Alternatively, one might insert two DNA fragments complementary to the coding regions of any two genes of interest into the same RNAi feeding vector. This approach has previously been reported to result in the concomitant knockdown of two genes (P. Kuwabara, personal communication). However, taking into account that this method would require the laborious cloning of all pairwise combinations of gene-specific DNA fragments, this strategy might not be suitable for an exhaustive screening of bigenic interactions in *C. elegans*. Conversely, the most direct approach would be the feeding of worms on a mixture of two dsRNA-expressing bacterial strains. In principle, this would allow the systematic examination of interactions between any pair of genes on a large scale. Previous studies aimed at the simultaneous targeting of two genes by feeding worms on two different dsRNA-expressing bacteria, however, reported a reduced strength of phenotype produced by either gene (A.G. Fraser and R. Kamath, personal communication). However, these anecdotal negative results have come from a small number of experiments performed on wild-type worms. Given the potential power of this approach for the comprehensive mapping of genetic interactions in the genome of *C. elegans*, I sought to carefully assess the effectiveness of this strategy again, using the RNAi-hypersensitive *rrf-3* background. Therefore, I wished to adapt the high-throughput (HTP) RNAi liquid-feeding assay, which is very efficient and robust for studying the loss-of-function phenotypes of single genes, to targeting two genes simultaneously by mixing two dsRNA-expressing bacterial strains. I will refer to this method as ‘combinatorial RNAi by bacterial feeding’ or simply as ‘combinatorial RNAi’.

3.2.3.1. Testing additive RNAi phenotypes and known synthetic genetic interactions

To investigate whether I could target effectively more than one gene in a single animal by feeding a mixture of two different dsRNA-expressing bacterial strains, I performed three sets of test experiments. In each test set, I sought to determine the effectiveness of combinatorial RNAi both in wild-type worms and in the RNAi-hypersensitive strain *rrf-3*, using the HTP RNAi liquid-feeding assay. First, I sought to

assess whether I could simultaneously target two independent genes, each with a known loss-of-function phenotype, and generate phenotypes for both genes in the same animal — for example, targeting *lin-31* by RNAi generates multivulval worms, targeting *sma-4* results in small worms, and targeting both would be expected to generate small worms with multiple vulvae if combinatorial RNAi was effective. Second, I wished to test whether I can recapitulate genetic interactions between the well-studied 'synthetic multivulval' (synMuv) genes, which have roles in two functionally redundant pathways (see page 61 for a more detailed description). Finally, I sought to investigate whether I can detect a subset of previously described synthetic lethal (SL) interactions.

For combinatorial RNAi feeding experiments, individual clones from the *C. elegans* RNAi feeding library were grown as described above (see Materials and Methods for more detail). After having dispensed first larval stage (L1) worms into each well of a flat-bottomed 96-well plate, equal volumes of two different bacterial feeding cultures were added. To be able to screen for second-generation post-embryonic phenotypes — as I intended to do when screening for additive and synMuv phenotypes — second-generation L1 worms were collected from the 96-well liquid-feeding assay by filtration through an 11 μm nylon mesh (MultiScreen^{TM} Nylon Mesh, Millipore), and allowed to develop further on 12-well Nematode Growth Medium (NGM) plates seeded with bacteria expressing a dsRNA that does not target a transcribed locus of the *C. elegans* genome (Ahringer RNAi feeding library clone Y95B8A_84.g; Figure 3.4.). Growing second-generation L1s to adults on NGM plates by feeding them on non-targeting — as compared to targeting — dsRNA-expressing bacteria ensured that the observed post-embryonic phenotypes were generated by using the HTP RNAi liquid-feeding assay and were not caused by RNAi by plate feeding.

3.2.3.1.1. Generating additive phenotypes by combinatorial RNAi

To examine whether I could generate loss-of-function phenotypes for two genes in the same animal by using combinatorial RNAi, I chose four well-characterized genes with non-overlapping post-embryonic phenotypes (Table 3.2.) to ensure that I could investigate each phenotype independently. Examining all possible pairwise combinations

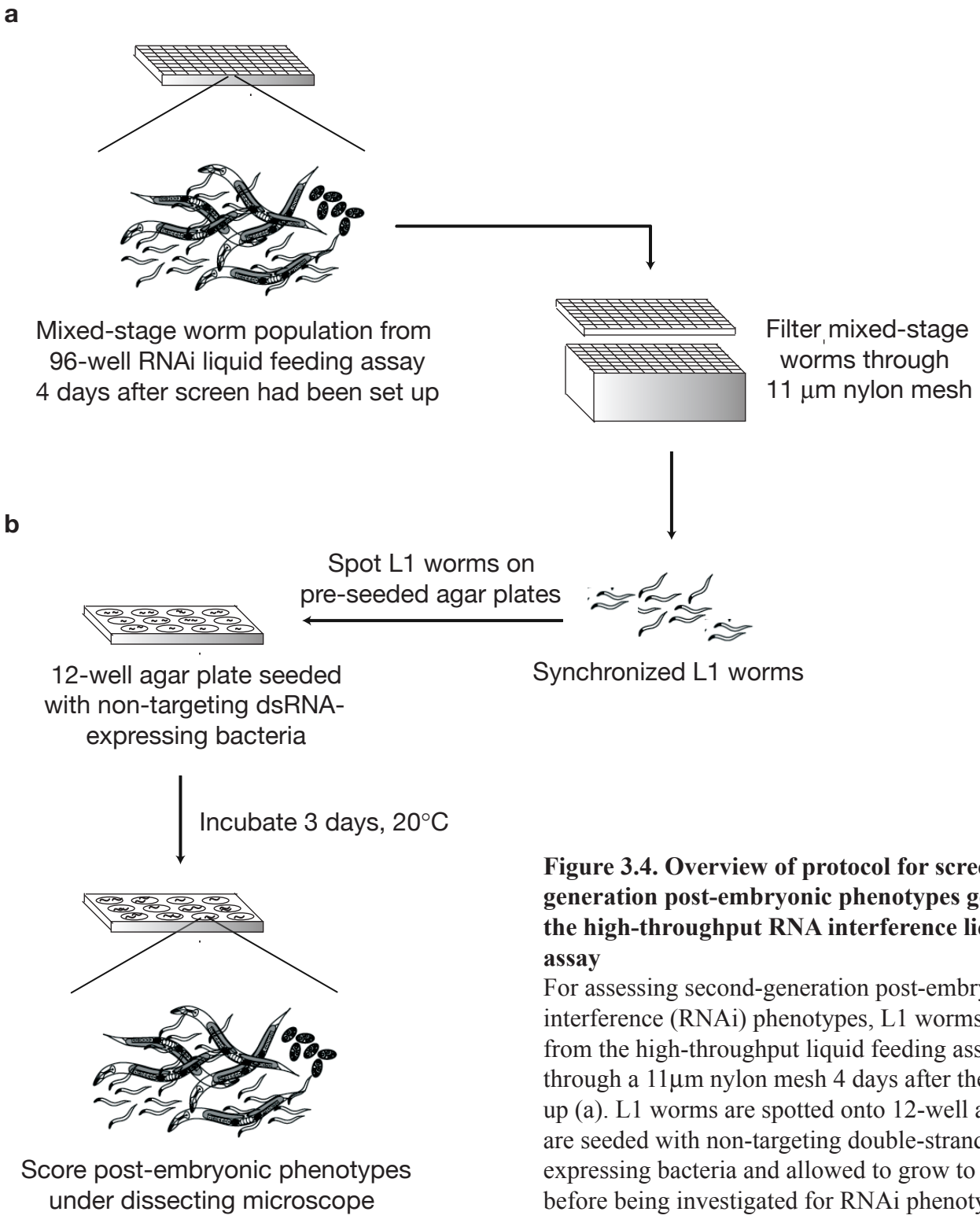

Figure 3.4. Overview of protocol for screening second-generation post-embryonic phenotypes generated in the high-throughput RNA interference liquid-feeding assay

For assessing second-generation post-embryonic RNA interference (RNAi) phenotypes, L1 worms are collected from the high-throughput liquid feeding assay by filtration through a 11µm nylon mesh 4 days after the screen was set up (a). L1 worms are spotted onto 12-well agar plates that are seeded with non-targeting double-stranded RNA-expressing bacteria and allowed to grow to adulthood before being investigated for RNAi phenotypes (b).

Gene1	Gene2	Wild-type		*rrf-3*	
		Pheno Gene1	Pheno Gene2	Pheno Gene1	Pheno Gene2
lin-31	-	5%	-	35%	-
sma-4	-	100%	-	100%	-
unc-22	-	100%	-	100%	-
lon-2	-	100%	-	100%	-
lin-31	*sma-4*	2%	100%	20%	100%
lin-31	*unc-22*	2%	100%	26%	100%
lin-31	*lon-2*	4%	100%	13%	100%
sma-4	*unc-22*	100%	100%	100%	100%
sma-4	*lon-2*	100%	0%	100%	0%
unc-22	*lon-2*	100%	100%	100%	100%

Table 3.2. Combinatorial RNA interference effectively generates additive phenotypes
Wild-type and RNA interference- (RNAi-) hypersensitive *rrf-3* worms, respectively, were fed on selected bacterial strains of the *C. elegans* RNAi feeding library targeting the genes *lin-31*, *sma-4*, *unc-22*, and *lon-2*. Independent RNAi phenotypes ('Pheno Gene1', 'Pheno Gene2') were assessed when each gene was targeted individually and also for all possible pairwise combinations of genes. Percentages represent penetrance of phenotypes.

of my set of test genes and scoring for the known RNAi phenotypes both in wild-type animals and in the RNAi-hypersensitive *rrf-3* background, I could detect five of the five possible additive phenotypes in both wild-type and *rrf-3* worms (Table 3.2.; see Figure 3.5. for an example), demonstrating that it is feasible to target two genes in the same animal by combinatorial RNAi by bacterial feeding. In addition to generating additive phenotypes, I found that the simultaneous targeting of *sma-4* and *lon-2* produced only small worms — the phenotype of *sma-4* alone. Thus, using combinatorial RNAi, it was also possible to recapitulate a previously demonstrated epistatic relationship between SMADs and *lon-2* (Brenner, 1974). Finally, while I could detect additive RNAi phenotypes in wild-type worms, I noted that the penetrance was often higher in the *rrf-3* RNAi-hypersensitive strain, suggesting that this background might be more suitable for combinatorial RNAi. I examine this in more detail below.

3.2.3.1.2. Creating synthetic post-embryonic phenotypes by cmbinatorial RNAi

Next, I investigated whether I could use combinatorial RNAi to recapitulate known genetic interactions that resulted in post-embryonic phenotypes. Therefore, I focused on the well-characterized synthetic multivulval (synMuv) genes (Ferguson and Horvitz, 1989; Poulin *et al.*, 2005). The synMuv genes are organized into two redundant genetic pathways that are required for normal development of the hermaphrodite vulva. Inactivation of either a gene functioning in the synMuv A pathway or a gene functioning in the synMuv B pathway alone does not result in a vulval defect, but inactivation of both a synMuv A and a synMuv B gene in combination leads to the generation of multiple vulvae — the multivulva (Muv) phenotype.

I set out to use combinatorial RNAi to co-target previously identified synMuv A genes (Poulin *et al.*, 2005) with the canonical class B gene *lin-15B*, and synMuv B genes (Poulin *et al.*, 2005) with the canonical synMuv A gene *lin-15A* in both wild-type animals and in the RNAi-hypersensitive strain *rrf-3*, respectively (Table 3.3.). As control, I studied the loss-of-function phenotypes of all synMuv genes individually. In each experiment, I scored progeny for the Muv phenotype; I expected to see this phenotype only if combinatorial RNAi targeted both genes effectively in the same animal. Because I

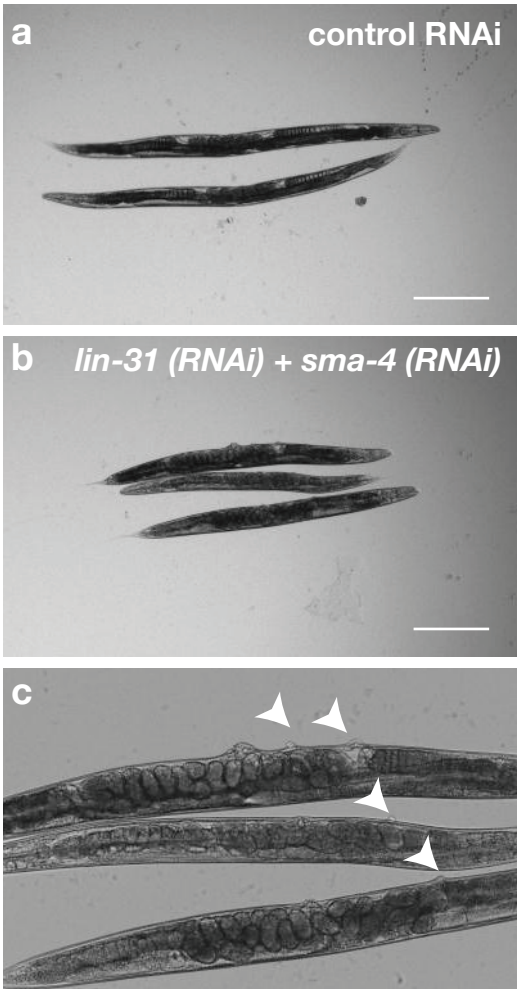

Figure 3.5. Combinatorial RNA interference can target two genes in the same animal
Exposing worms to a mixture of two double-stranded RNA- (dsRNA-) expressing bacterial clones, one targeting *lin-31*, the other one targeting *sma-4*, resulted in small worms with multiple vulvae along their ventral side. Shown are *rrf-3* animals fed on bacteria expressing a non-targeting dsRNA (control, a) and combined bacterial clones expressing dsRNA against *lin-31* and *sma-4* (b and magnified in c). White arrowheads indicate pseudovulvae. Scale bars: 0.1 mm.

lin-15B

Predicted Gene	**Locus**	**synMuv**	**Wild-type**	***rrf-3***
T27C4.4	*egr-1*	A	-	-
ZK678.1	*lin-15A*	A	**Muv**	**Muv**
K12C11.2	*smo-1*	A, B	n.s.	n.s.
W02A11.4	*uba-2*	A, B	**Muv**	**Muv**

lin-15A

Predicted Gene	**Locus**	**synMuv**	**Wild-type**	***rrf-3***
K12C11.2	*smo-1*	A, B	n.s.	n.s.
W02A11.4	*uba-2*	A, B	-	**Muv**
C32F10.2	*lin-35*	B	**Muv**	**Muv**
C47D12.1	*trr-1*	B	n.s.	n.s.
C53A5.3	*hda-1/gon-10*	B	n.s.	n.s.
E01A2.4		B	-	-
F44B9.6	*lin-36*	B	-	**Muv**
JC8.6		B	n.s.	n.s.
K07A1.12	*lin-53/rba-2*	B	n.s.	n.s.
M04B2.1	*mep-1/gei-2*	B	-	**Muv**
R05D3.11	*met-2*	B	-	**Muv**
R06C7.7	*rls-1/lin-61*	B	**Muv**	**Muv**
W01G7.3		B	n.s.	n.s.
W07B3.2	*gei-4*	B	n.s.	n.s.
Y71G12B.9		B	-	**Muv**
Y1O2A5C.18	*efl-1*	B	**Muv**	**Muv**
ZK632.13	*lin-52*	B	**Muv**	**Muv**
ZK637.7	*lin-9*	B	**Muv**	**Muv**
ZK662.4	*lin-15B*	B	**Muv**	**Muv**

Table 3.3. Genetic interactions of synthetic multivulval genes can be recapitulated by combinatorial RNA interference

Previously studied synthetic multivulval (synMuv) genes were targeted by combinatorial RNA interference (RNAi) in wild-type and *rrf-3* worms, respectively. Predicted gene names, their corresponding genetic locus names (if applicable), a definition of the gene as a component of either the synMuv A ('A'), synMuv B ('B'), or both ('A, B') pathways are shown. All synMuv A genes were targeted by RNAi in combination with a double-stranded RNA- (dsRNA-) expressing strain targeting the synMuv B gene *lin-15B*; corresponding experiments were performed with synMuv B genes and a dsRNA-expressing strain targeting *lin-15A*. In both cases, worms were scored for the presence of

was assessing second-generation post-embryonic phenotypes, I had to exclude genes that resulted in sterility, embryonic lethality, or larval growth arrest after RNAi from the screen for synMuv animals (marked as 'n.s.' in Table 3.3.). Of 3 synMuv A genes and 12 synMuv B genes that were amenable to analysis by combinatorial RNAi in both wild-type worms and the RNAi-hypersensitive *rrf-3* background, I observed Muv worms for 13 of 15 test cases in the hypersensitive *rrf-3* background, and for 8 of 15 possible viable combinations in wild-type animals (Table 3.3.).

3.2.3.1.3. Generating known synthetic lethal phenotypes by combinatorial RNAi

As a final test of the efficacy of combinatorial RNAi, I investigated whether I can use combinatorial RNAi to recapitulate a set of known synthetic lethal (SL) interactions compiled from literature (Baugh *et al.*, 2005; Davies *et al.*, 1999; Pocock *et al.*, 2004; Solari *et al.*, 1999; Zhang and Emmons, 2001; Table 3.4.). To do so, I set out to compare the RNAi phenotypes resulting from simultaneously targeting both genes of a SL pair by combinatorial RNAi with the RNAi phenotypes of each gene alone, both in wild-type animals and in the RNAi-hypersensitive strain *rrf-3*, respectively, using the HTP RNAi liquid-feeding assay. To control for mixing two dsRNA-expressing strains when targeting both genes of a pair, I added equal amounts of bacteria expressing a dsRNA that does not target a transcribed portion of the *C. elegans* genome (Ahringer RNAi feeding library clone Y95B8A_84.g) to bacteria expressing dsRNA targeting each gene of a SL pair alone. In order to unambiguously identify SL interactions, I sought to quantify brood sizes per individual adult and embryonic survival rates and assessed these quantitative phenotype data under a multiplicative model (as discussed in the Introduction). Brood size, and embryonic survival rates, respectively, resulting from simultaneously targeting two genes had to be significantly lower than the calculated product of values for both individual genes for a gene pair to be considered SL.

Thus, I first determined brood sizes per individual adult and embryonic survival rates, respectively, following combinatorial RNAi against both genes simultaneously and RNAi against each gene individually in two separate experiments. I manually counted

	Wild-type								
Interaction Gene1 & Gene2	**Gene1**		**Gene2**		**Gene1 & 2**		**SL**	***p*-value**	***p*-value**
	BS	**ES**	**BS**	**ES**	**BS**	**ES**		**BS**	**ES**
mec-8 + sym-1	88	99	82	98	78	92	yes	5.5E-01	1.3E-02
sop-3 + sop-1	91	100	94	99	79	90	yes	2.8E-01	8.5E-04
tbx-8 + tbx-9	83	99	78	97	52	11	yes	7.3E-02	1.4E-24
hlh-1 + unc-120	91	99	76	99	28	91	yes	5.2E-05	1.2E-02
hlh-1 + hnd-1	88	97	75	98	62	81	yes	6.6E-01	5.7E-03
unc-120 + hnd-1	54	100	74	98	36	100	no	6.4E-01	1.9E-01
egl-27 + egr-1	93	99	79	90	90	89	no	6.0E-02	7.4E-01

	rrf-3								
Interaction Gene1 & Gene2	**Gene1**		**Gene2**		**Gene1 & 2**		**SL**	***p*-value**	***p*-value**
	BS	**ES**	**BS**	**ES**	**BS**	**ES**		**BS**	**ES**
mec-8 + sym-1	67	73	61	73	59	16	yes	3.3E-01	3.0E-06
sop-3 + sop-1	82	100	85	96	41	75	yes	3.1E-04	5.7E-06
tbx-8 + tbx-9	96	99	86	92	59	2	yes	8.6E-03	6.3E-27
hlh-1 + unc-120	90	90	31	99	1	64	yes	8.1E-06	2.9E-03
hlh-1 + hnd-1	86	87	82	94	42	24	yes	1.6E-03	8.2E-14
unc-120 + hnd-1	33	100	87	94	7	98	yes	5.7E-04	4.8E-02
egl-27 + egr-1	97	99	83	93	73	62	yes	2.9E-01	5.7E-08

Table 3.4. Combinatorial RNA interference can identify known synthetic lethal interactions

Quantitative analysis of known synthetic lethal interactions ('Interaction Gene1 & Gene2'; see below for references) after combinatorial RNA interference (RNAi) in wild-type and *rrf-3* worms, respectively. Percentages of average wild-type brood size ('BS')

larvae, unhatched eggs and adult worms after combinatorial RNAi (performed in triplicates within independent screens) and RNAi against each gene individually (performed in duplicates within independent assays) in two separate experiments (see Figure 3.6. for an overview of the setup for combinatorial RNAi; see Materials and Methods for a detailed description). I normalized measurements for brood size and embryonic survival to wild-type measurements obtained after feeding worms on non-targeting dsRNA-expressing bacteria (Ahringer feeding library clone Y95B8A_84.g). If brood size or embryonic survival rates after RNAi against individual genes exceeded average wild-type measurements, I set values to 100% of wild-type values.

For statistical analysis, I compared the observed quantitative phenotypes resulting from simultaneously targeting both genes of a synthetic lethal pair with the calculated products of measurements for both genes individually. Therefore, I multiplied duplicate brood size and embryonic survival measurements, respectively, for both individual genes within two independent experiments in all possible pairwise combinations to generate sixteen values; these sixteen calculated products represent the predicted outcome if the double mutant phenotype was merely resulting from a simple additive effect of both single-gene RNAi phenotypes. The sixteen values for the expected quantitative phenotypes under a multiplicative model were compared to six measurements obtained after combinatorial RNAi in two independent experiments, using a Student's t-Test (two-tailed distribution, two-sample equal variance; see Materials and Methods for a detailed description of the statistical analysis). I considered SL interactions to be successfully recapitulated by combinatorial RNAi if p-values were below 5.0×10^{-2}.

Using the above criteria, I was able to detect all seven tested genetic interactions in *rrf-3* animals (Table 3.4., Figure 3.7.). However, in wild-type animals, only five of these interactions could be recapitulated (Table 3.4.). Not only did I fail to detect two out of seven interactions in wild-type worms, the five detected interactions were also weaker than in *rrf-3*, demonstrating that for effective combinatorial RNAi it is often essential to use RNAi-hypersensitive strains.

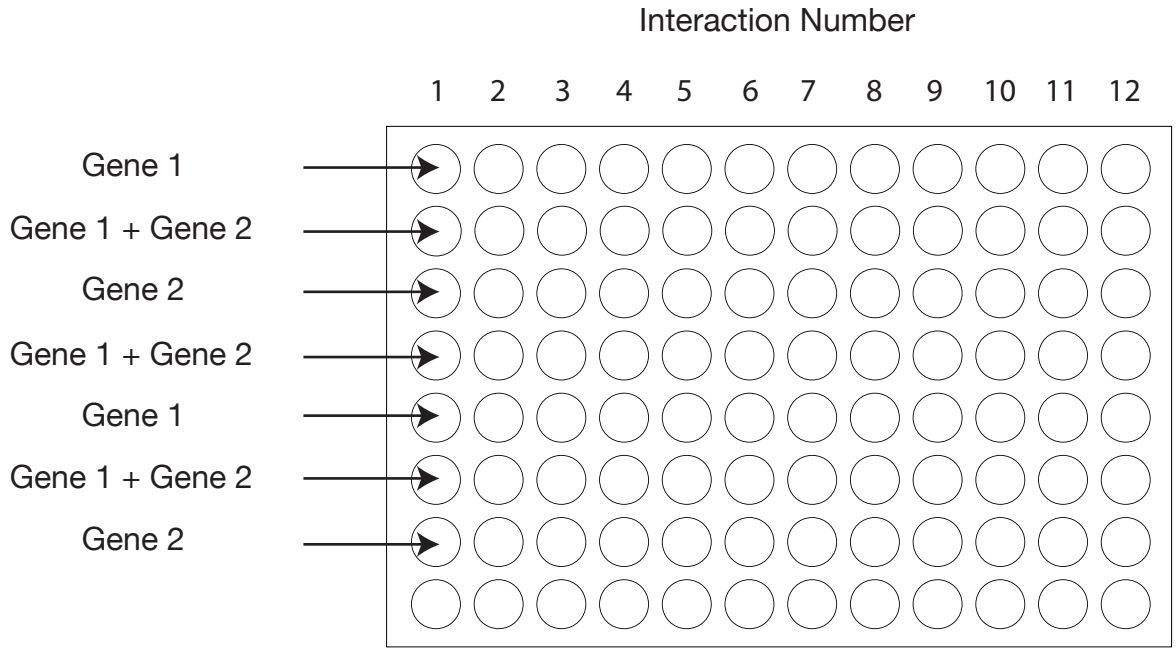

Figure 3.6. Overview of the setup for combinatorial RNA interference screens
When screening for genetic interactions, phenotypes resulting from simultaneously targeting two genes by combinatorial RNA interference (RNAi) were directly compared with the RNAi phenotypes of each gene alone. Genetic interaction screens are therefore set up such that worms were fed with bacteria expressing double-stranded RNA (dsRNA) against one gene ('Gene1'), with equal amounts of bacteria expressing dsRNAs against each of the two genes ('Gene1 + Gene2'), and bacteria expressing dsRNA against the second gene ('Gene2') in alternating rows. Using this setup, combinatorial RNAi was performed in triplicates and RNAi against each gene individually in duplicates within independent screens.

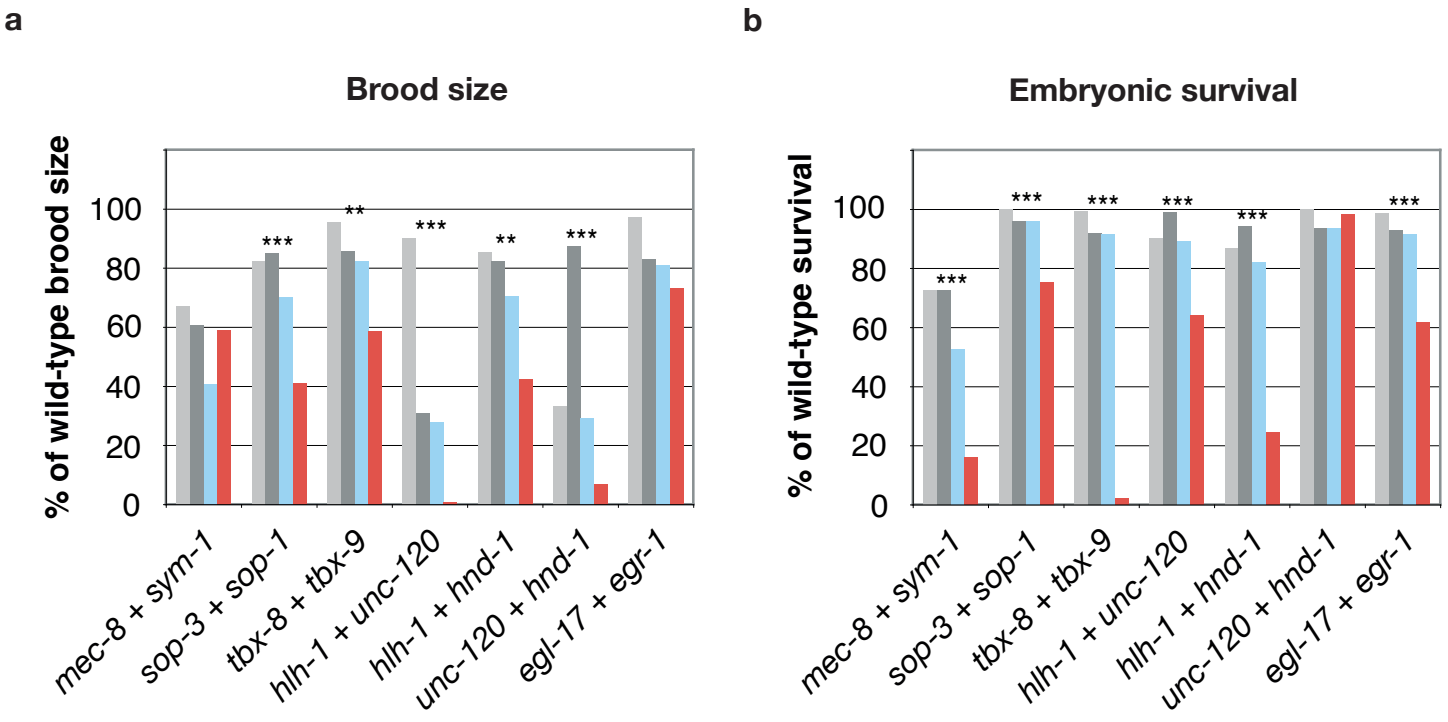

Figure 3.7. Combinatorial RNA interference can recapitulate known synthetic lethal interactions
To test whether combinatorial RNA interference (RNAi) could recapitulate seven synthetic lethal (SL) interactions that were identified from literature (see Table 3.4. for references), brood size (BS, a) and embryonic survival (ES, b) measurements following co-targeting of both genes of a SL pair (red bars) were compared with that following the targeting of each single gene alone (light- and dark-grey bars) and with the calculated product of the single gene brood sizes and embryonic survival measurements, respectively (blue bars). Values plotted represent the percentage of average wild-type brood size and embryonic survival rates, and are the arithmetic mean of two independent experiments performed in the RNAi-hypersensitive strain *rrf-3*. BS and ES measurements, respectively, resulting from combinatorial RNAi against both genes of a pair had to be significantly lower ($P < 5.0E-02$, Student's t-test) than the expected multiplicative values associated with BS and ES rates, respectively, after RNAi against each gene individually for a gene pair to be considered SL. ***, $P < 1.0E-03$; **, $P < 1.0E-02$.

Taken together, these results demonstrate that combinatorial RNAi by feeding using our newly developed HTP liquid-feeding platform is a robust and efficient method to simultaneously perturb the expression of any two genes in the genome of *C. elegans*. I was able to generate additive phenotypes and to detect the great majority of previously described synthetic post-embryonic phenotypes and synthetic lethal interactions. However, for effective combinatorial RNAi, it is often essential to use RNAi-hypersensitive strains. I thus decided to perform all of the following experiments in the RNAi-hypersensitive *rrf-3* background.

3.2.4. Effect of dilution on phenotype strength

When analysing the phenotypes produced through combinatorial RNAi, I and others (Gonczy *et al.*, 2000; Parrish *et al.*, 2000) observed that some of the single-gene phenotypes were qualitatively weaker when two genes were targeted together than when each gene was targeted alone. Since such dilution effects will affect both the false-negative rate in large-scale screens and the possible number of genes that can be co-targeted effectively, I wished to investigate the extent to which combining dsRNA-expressing bacteria leads to a reduced strength of RNAi phenotypes. To do this, I selected 282 genes from chromosome III that were found to have a non-viable (embryonic lethal or sterile) RNAi phenotype (Kamath *et al.*, 2003) (see Appendix Table 3.2.) and examined whether their phenotypes change as the targeting bacteria are diluted with increasing amounts of unrelated dsRNA-expressing bacteria (Figure 3.8.).

Using the HTP liquid-feeding assay, I compared RNAi phenotypes in the RNAi-hypersensitive strain *rrf-3* for each gene alone with RNAi phenotypes generated by diluting individual bacterial feeding cultures two-, three-, four-, five-, and ten-fold, respectively, with bacteria expressing a dsRNA that does not target an expressed portion of the *C. elegans* genome (Ahringer library clone Y95B8A_84.g), and bacteria expressing dsRNA against *lin-31*, respectively. For each gene with a non-viable RNAi phenotype in my experimental setting, I assessed the dilution level that first led to a drop in strength of phenotypes. I observed that the strength of RNAi phenotypes for many genes is indeed reduced when increasing proportions of bacteria expressing unrelated

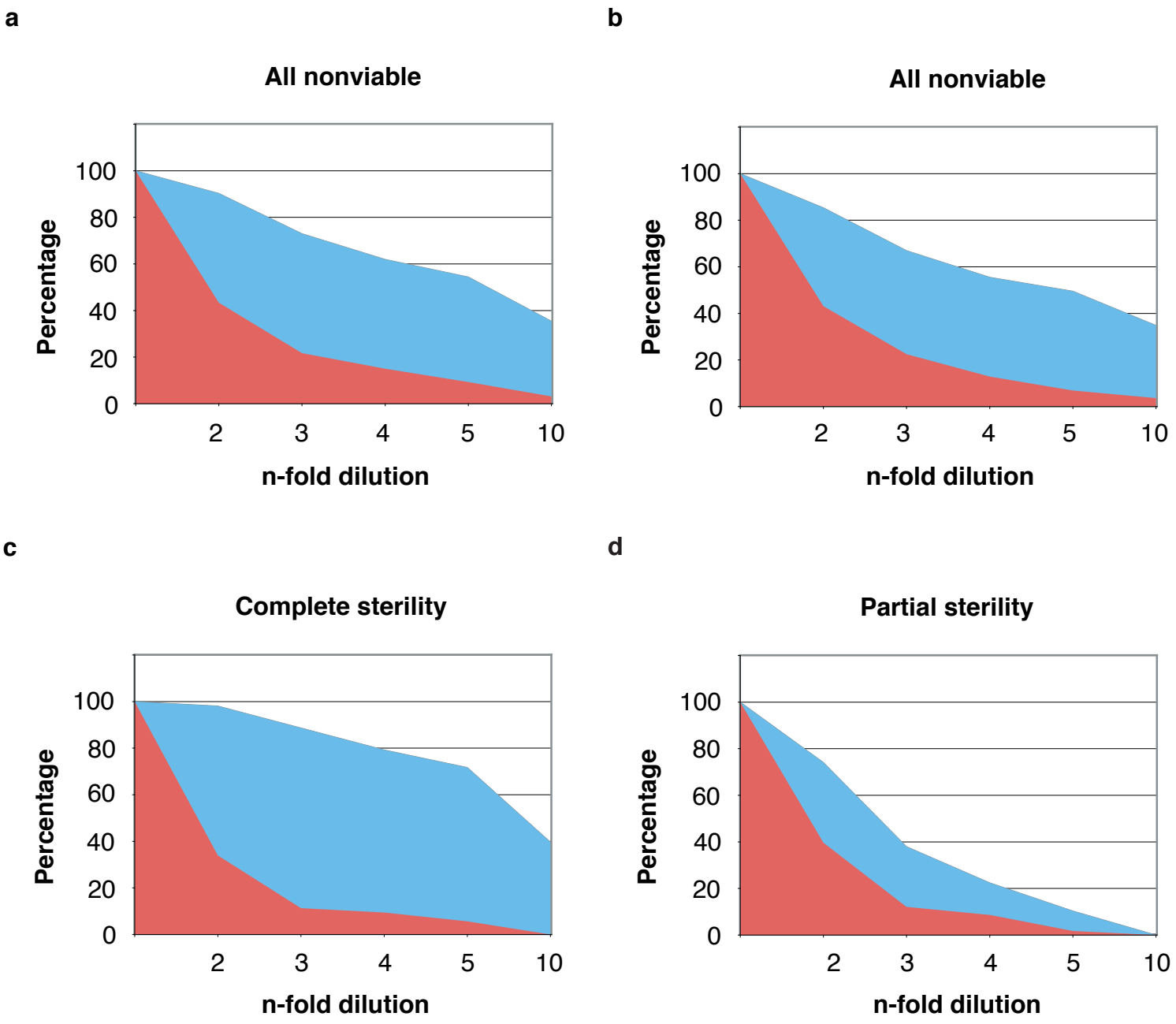

Figure 3.8. Effect of dilution on strength of RNA interference phenotypes
The RNA interference (RNAi) phenotype of each reported non-viable gene on chromosome III (Kamath *et al.*, 2003) was assessed following dilution with increasing amounts of bacteria expressing unrelated double-stranded RNAs (dsRNAs). The percentage of genes with phenotypes that are either identical to that observed when targeted alone (red) or weaker than when targeted alone (blue) is shown for each dilution. This was examined for three phenotypic categories: all non-viable phenotypes (dilution with bacteria expressing a non-targeting dsRNA (Ahringer feeding library clone Y95B8A_84.g; a) and dilution with bacteria expressing dsRNA against *lin-31*; b), complete sterility (no progeny; dilution with non-targeting dsRNA-expressing bacteria; c), and partial sterility (some progeny; dilution with non-targeting dsRNA-expressing bacteria; d). Data shown are representative of two independent experiments performed in the RNAi-hypersensitive *rrf-3* background.

dsRNAs are added. I found essentially identical results when diluting with non-targeting dsRNA-expressing bacteria (Ahringer library clone Y95B8A_84.g; Figure 3.8.a), as when adding increasing amounts of a dsRNA-expressing bacterial strain targeting *lin-31* (Figure 3.8.b), demonstrating that the observed effect is not specific to the diluting dsRNA-expressing strain. I will discuss the observed changes in phenotypic strength in more detail below, focusing on results obtained with increasing dilution of bacteria expressing a non-targeting dsRNA (Ahringer library clone Y95B8A_84.g).

In total, I was able to detect phenotypes for ~90% of genes with non-viable RNAi phenotypes when the targeting strains were diluted with equal amounts of a bacterial strain expressing an unrelated dsRNA. This detection rate dropped further to ~70% at three-fold and to ~60% at four-fold dilution.

I next asked whether the effect of dilution on the observed phenotype is related to phenotypic strength. To this end, I determined the dilution behaviour for genes that have different strengths of brood size defects when targeted alone (Figure 3.8.c and 3.8.d). I found that genes with weak RNAi phenotypes were indeed more likely to appear wild-type following dilution — and thus to be missed in screens — than genes with strong, highly penetrant phenotypes. For example, I could still detect phenotypes for ~80% of genes, that normally have a completely sterile phenotype, at a four-fold dilution (Figure 3.8.c); however, only ~20% of genes conferring partial sterility (i.e. a reduction in brood size) have a detectable phenotype at this dilution (Figure 3.8.d). While this indicates that genes with weaker phenotypes are more likely to appear wild-type when targeted in combination with other genes, I conclude that on average ~90% of genes with a detectable RNAi phenotype still have sufficient knockdown when diluted with equal amounts of a second dsRNA-expressing bacterial strain.

Overall, these experiments allowed me to estimate the false-negative rates induced by dilution effects in combinatorial RNAi (Figure 3.9.; see Materials and Methods for calculation). Assuming each gene behaves independently, I expect that ~80% of bigenic interactions yielding visible RNAi phenotypes will be detectable by combinatorial RNAi using the HTP liquid-feeding assay.

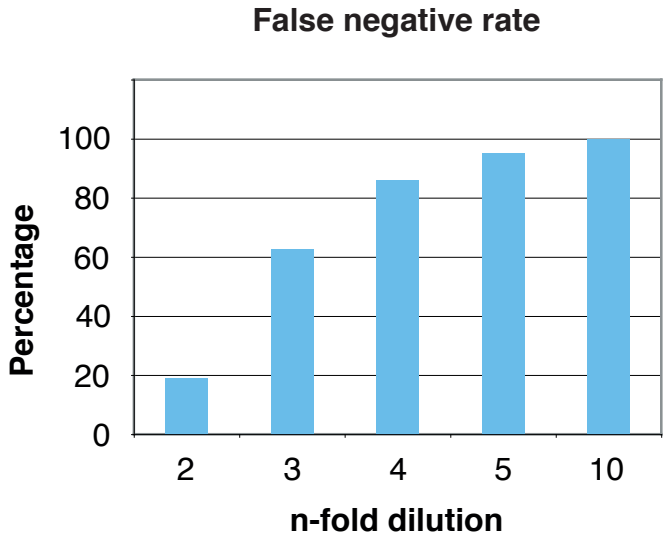

Figure 3.9. False-negative rate of combinatorial RNA interference
The false negative rate (in percent) of combinatorial RNA interference (RNAi) at a given dilution was calculated by assessing the fraction of chromosome III genes with nonviable RNAi phenotypes that resulted in a weaker phenotype when increasing amounts of non-targeting double-stranded RNA- (dsRNA-) expressing bacteria were added to targeting dsRNA-expressing bacteria. Data shown are representative of two independent experiments in the RNAi-hypersensitive strain *rrf-3*.

In summary, I have systematically analysed the effects of diluting bacteria expressing dsRNAs against genes with known non-viable RNAi phenotypes with increasing amounts of unrelated dsRNA-expressing bacteria. I found that combining equal amounts of two dsRNA-expressing bacterial strains frequently results in a reduced strength of phenotypes. The implication of this dilution effect for combinatorial RNAi is that as one increases the number of genes being simultaneously targeted, the efficiency of the knockdown of each individual gene decreases. Thus, one cannot increase the number of genes being co-targeted indefinitely. However, the great majority of genes with sterile or embryonic lethal RNAi phenotype still showed non-viable phenotypes at two-fold dilution. This dilution is critical for the study of bigenic interactions using combinatorial RNAi. Thus, together with the generation of additive phenotypes within the same animal and the detection rate of known synthetic genetic interactions, these findings suggest that combinatorial RNAi using a HTP liquid-feeding assay is a powerful tool for systematically studying the effect of targeting any pairwise combination of genes in the genome of *C. elegans*. I conclude that this approach should allow researchers to explore genetic interactions in the nematode *C. elegans* in a far more systematic manner than has been possible in the past.

3.3. Conclusion

In this chapter, I have shown the development of an experimental platform for using RNAi by bacterial feeding to analyse the loss-of-function phenotypes of single genes on a large scale. By delivering dsRNA-expressing bacteria in liquid cultures in 96-well format, this methodology allows RNAi screens to be performed at considerably higher throughput than has been possible in the past. Notably, this HTP liquid-feeding assay allows the screening of a population of worms in each well, thereby averaging the animal-to-animal variability of RNAi phenotypes that is observed when using single-animal plate-feeding protocols. By using this assay, I was able to identify approximately 80% of previously identified RNAi phenotypes with 90% reproducibility.

I then established protocols to adapt these methods, which are very robust and efficient for analyzing the RNAi phenotypes of single genes, to simultaneously targeting

two genes by mixing two dsRNA-expressing bacterial strains (‘combinatorial RNAi’). Using this methodology, I was able to generate loss-of-function phenotypes for two genes in the same animal and to identify the great majority of a test set of previously known synthetic lethal and synthetic post-embryonic genetic interactions.

When investigating the extent to which combining dsRNA-expressing bacteria can lead to a reduced strength of phenotype, I was able to detect phenotypes for approximately 90% of genes with a non-viable RNAi phenotype at two-fold dilution. This is the critical dilution that is needed for detecting bigenic interactions by using combinatorial RNAi. Assuming that each gene behaves independently in combinatorial RNAi, I expect that approximately 80% of bigenic interactions yielding visible RNAi phenotypes will be detectable by using this approach.

Taken together, I consider combinatorial RNAi by bacterial feeding a powerful tool for examining interactions between any pair of genes in the genome of *C. elegans* on a large scale.

Having established and validated combinatorial RNAi as a robust high-throughput method for simultaneously targeting any pairwise combination of genes by using bacterial feeding in liquid culture, I wished to use this approach to begin to investigate functional redundancy in the genome of *C. elegans*.

Chapter 4

Functional redundancy between *C. elegans* gene duplicates

4.1. Introduction

Having validated combinatorial RNA interference (RNAi) as a robust method to simultaneously perturb the expression of any pairwise combination of genes, I sought to use this approach to uncover functional genetic redundancy in the *C. elegans* genome.

One obvious possible cause of genetic redundancy is through gene duplication (as discussed in the Introduction). Gene duplicates with at least partially overlapping functions can confer robustness to mutation in the other copy (Force *et al.*, 1999; Lynch and Force, 2000). While genome-wide loss-of-function screens provide indirect evidence that gene duplicates may often share redundant functions (Conant and Wagner, 2004; Gu *et al.*, 2003; Kamath *et al.*, 2003), this hypothesis has not been extensively tested with systematic experimental approaches at the time my study began.

I therefore set out to investigate whether *C. elegans* gene duplicates have redundant functions by using combinatorial RNAi. I reasoned that if gene duplicates were genuinely functionally redundant, targeting both genes of a duplicate pair would result in a more severe loss-of-function phenotype than observed when targeting each gene individually. In the most dramatic case, if gene duplicates together confer an essential redundant function, inactivation of both genes of such a pair would be expected to result in synthetic lethality.

4.2. Examining the redundancy of duplicated genes in the genome of *C. elegans*

To investigate the extent of functional redundancy between gene duplicates in the worm, I focused on *C. elegans* gene pairs that correspond to single orthologues in *S. cerevisiae* or *D. melanogaster* genomes. These genes have thus been duplicated in the genome of *C. elegans* since divergence from either species.

Using the INPARANOID algorithm to identify such gene pairs, the *C. elegans* genome was found to comprise a total of 293 gene pairs that have been duplicated since split from yeast or fly (Table 4.1.). To determine whether these gene duplicates share redundant functions, I set out to examine whether targeting both genes of a duplicate pair affected

***C. elegans* gene duplicates**	***S. cerevisiae***	***D. melanogaster***	***S. cerevisiae* & *D. melanogaster***	**Total**
Identified	79	160	54	293
RNAi clones available	53	105	37	195
Amenable to analysis	49	75	29	153

Table 4.1. *C. elegans* duplicate gene pairs that correspond to single orthologues in *S. cerevisiae* and *D. melanogaster* genomes

Gene pairs that have been duplicated in the genome of *C. elegans* since divergence from *S. cerevisiae* and *D. melanogaster*, respectively, were investigated for potential redundant functions. Shown are numbers for *C. elegans* gene duplicates that were identified by using the INPARANOID algorithm (Remm *et al.*, 2001) ('identified'), that could both be targeted by double-strand RNA- (dsRNA-) expressing clones using the Ahringer RNAi feeding library (Kamath *et al.,* 2003; 'RNAi clones available'), and that were amenable to analysis after excluding cross-reacting RNAi clones with inserts having more than 80% nucleotide identity over 200 base pairs with other genes ('amenable to analysis').

viability, fecundity, or growth in a non-additive, synergistic manner compared with the effects of targeting the individual genes.

For 195 out of 293 *C. elegans* gene duplicates that I had identified, RNAi clones were available from the *C. elegans* whole-genome RNAi library to target each gene of a pair. Of these, I excluded all genes that were targeted by bacterial clones with inserts having more than 80% nucleotide identity over 200 bp with the other copy — this is the threshold for cross-reaction used in Kamath *et al.* (2003) — to ensure that I am not targeting both genes of a duplicate pair with one RNAi clone. This left me with 153 duplicate gene pairs amenable to analysis for synthetic phenotypes using combinatorial RNAi (see Appendix Table 4.1.).

For each duplicate gene pair, I compared the RNAi phenotypes for each gene individually with combinatorial RNAi phenotypes side by side, using the HTP liquid-feeding assay, and the RNAi-hypersensitive *rrf-3* strain (as described in Chapter 3 and Materials and Methods; see Figure 3.6). At that stage, 10 duplicate gene pairs had to be excluded from the screen for functional redundancy, because one or other of the individual genes resulted in first-generation larval growth arrest — a phenotype that cannot be enhanced any further — following RNAi.

After two initial rounds of qualitative analysis, all duplicate gene pairs that appeared to show a stronger combinatorial RNAi phenotype as compared to the contributions of each single-gene RNAi phenotype were further verified by quantification (as described in Chapter 3 and Materials and Methods). Quantitative phenotype data were subsequently subject to statistical analysis under a multiplicative model (as described in Chapter 3 and Materials and Methods). Briefly, for each duplicate gene pair, brood size and embryonic survival, respectively, following combinatorial RNAi were compared to the measurements after RNAi against each gene individually, and the expected product associated with single-gene phenotypes using a Student's t-Test (two-tailed distribution, two-sample equal variance). I interpret a synthetic enhancement interaction under a multiplicative model — that is, where the combined phenotype is significantly stronger (as represented by a p-value below 5.0×10^{-2}) than the product of the individual phenotypes — as indicating functional genetic redundancy.

In total, of 143 duplicate gene pairs amenable to analysis by combinatorial RNAi, I identified 16 gene pairs as having synthetic lethal (SL) phenotypes by the criteria discussed above (Table 4.2. and Figure 4.1.). These data thus suggest that these duplicate pairs are — at least in part — functionally redundant. Of these gene pairs, only two have been previously identified as having redundant functions (Koh *et al.*, 2002; Lambie and Kimble, 1991). The pairs of genes that when co-targeted give SL phenotypes encode diverse molecular functions, ranging from structural constituents of the ribosome (e.g. *rpa-2* + C37A2.7, *rpl-25.1 + rpl-25.2*), signaling proteins (e.g. *lin-12 + glp-1*, C13G3.3 + W08G11.4), and transcription factors (e.g. *elt-6 + egl-18*) to polyadenylate-binding proteins (e.g. *pab-1 + pab-2*) (Table 4.3). Thus, the duplicate gene pairs that I have identified to share redundant functions do not appear to be enriched for specific biological function.

4.3. Transferring gene functions between *S. cerevisiae* and *C. elegans*

The duplicated genes that I focused on in the worm corresponded to single genes in either *S. cerevisiae* or *D. melanogaster* genomes. I wished to investigate whether the known function of a single gene in one organism was a good predictor of the synthetic RNAi phenotype identified by co-targeting the corresponding duplicated worm genes with redundant functions. If this were the case, then it is most likely that the redundancy that I observe is due to both duplicates retaining the ancestral molecular function.

As a preliminary to this study I sought to investigate whether the known function of an individual gene in one organism can predict the molecular function of its single orthologue in *C. elegans*. Testing the conservation of individual gene functions between species would allow me to assess the potential of predicting gene functions covered by pairs of redundant genes. I chose to focus on transferring individual gene functions between *S. cerevisiae* and *C. elegans*, because to date, yeast and worm are the main model organisms in which fully systematic functional studies can be performed *in vivo*. Moreover, I will be discussing the conservation of synthetic lethal interactions between yeast and worm in the next chapter.

Interaction Gene1 & Gene2	Gene1		Gene2		Gene1 & 2		p-value	p-value
	BS	ES	BS	ES	BS	ES	BS	ES
pab-1 + *pab-2*	15	100	88	100	0	n.s.	1.9E-04	n.s.
rpl-25.2 + *rpl-25.1*	6	50	17	63	0	n.s.	3.6E-04	n.s.
ptr-2 + *ptr-10*	*	53	*	98	*	n.s.	*	n.s.
unc-78 + *tag-216*	85	96	98	97	0	n.s.	6.4E-15	n.s.
rab-8 + *rab-10*	87	98	70	96	1	n.s.	7.3E-05	n.s
B0495.2 + ZC504.3	84	99	97	99	2	13	6.3E-09	1.4E-17
rpa-2 + C37A2.7	67	74	50	81	1	n.s.	1.9E-07	n.s.
C28H8.4 + *erd-2*	93	95	86	94	10	10	5.6E-08	2.2E-15
lin-12 + *glp-1*	90	95	99	83	16	75	1.2E-13	3.0E-01
C13G3.3 + W08G11.4	73	94	80	97	17	89	1.6E-06	3.5E-01
lin-53 + *rba-1*	74	63	51	5	16	75	1.1E-02	7.3E-17
Y53C12A.4 + R02E12.2	84	81	78	87	32	75	1.3E-03	6.9E-01
F37C12.7 + *acs-17*	95	100	77	98	44	73	9.4E-03	4.2E-06
C05G5.4 + F23H11.3	96	100	94	98	58	72	5.1E-06	1.5E-08
elt-6 + *egl-18*	100	97	86	88	63	73	4.0E-02	6.3E-03
dsh-1 + *dsh-2*	97	98	75	54	58	17	1.6E-02	1.1E-11

Table 4.2. *C. elegans* duplicate gene pairs with at least partially redundant functions
C. elegans duplicate gene pairs ('Interaction Gene1 & Gene2') displaying synthetic phenotypic effects upon combinatorial RNA interference (RNAi) in the RNAi-hypersensitive strain *rrf-3* are listed. Numbers shown are percentages of average wild-type brood size ('BS') and embryonic survival ('ES') rates after RNAi against each gene individually ('Gene1', 'Gene2') as well as after combinatorial RNAi against duplicate gene pairs ('Gene1 & 2'), and are the arithmetic mean of two independent biological repeats. Statistical significance of quantitative phenotype data (BS, ES) was evaluated under a multiplicative model (Phillips *et al.*, 2000; Puniyani *et al.*, 2004); p-values were assigned using a Student's t-test. n.s., given phenotype could not be quantified. * Note

that combinatorial RNAi against the duplicate gene pair *ptr-2 + ptr-10* resulted in an increased number of first generation larval growth arrested worms, rather than in reduced brood size; fraction of population which is wild-type, i.e. that does not arrest at an early larval stage: 70% (*ptr-2*), 100% (*ptr-10*), 0% (*ptr-2 + ptr-10*), *P* = 7.3E-09.

Figure 4.1. Quantitative analysis of synthetic lethal phenotypes following the simultaneous targeting of both genes of a duplicate pair

Phenotypes of duplicate gene pairs that yielded reproducible synthetic effects after combinatorial RNA interference (RNAi) were quantified. For each gene pair, brood size (BS) and embryonic survival (ES) after combinatorial RNAi against both duplicates (red bars), after RNAi against each gene individually (light- and dark-grey bars), and the calculated product of BS and ES measurements, respectively, of both individual genes (blue bars) are shown. Values plotted represent the percentage of average wild-type brood size and embryonic survival rates, respectively, and are the arithmetic mean of two independent RNAi experiments performed in the RNAi-hypersensitive *rrf-3* background. Duplicate gene pairs were considered to be synthetic lethal, if either BS or ES measurements were significantly reduced (P < 5.0E-02; Student's t-test) as compared to the multiplicative values of the single-gene BS and ES measurements, respectively. ***, P < 1.0E-03; **, P < 1.0E-02; *, P < 5.0E-02. Note that combinatorial RNAi against the gene pair *ptr-2 + ptr-10* resulted in a significantly increased number of first-generation larval growth arrested worms (P = 7.3E-09, Student's t-test), rather than a brood size defect, hence these data are not shown.

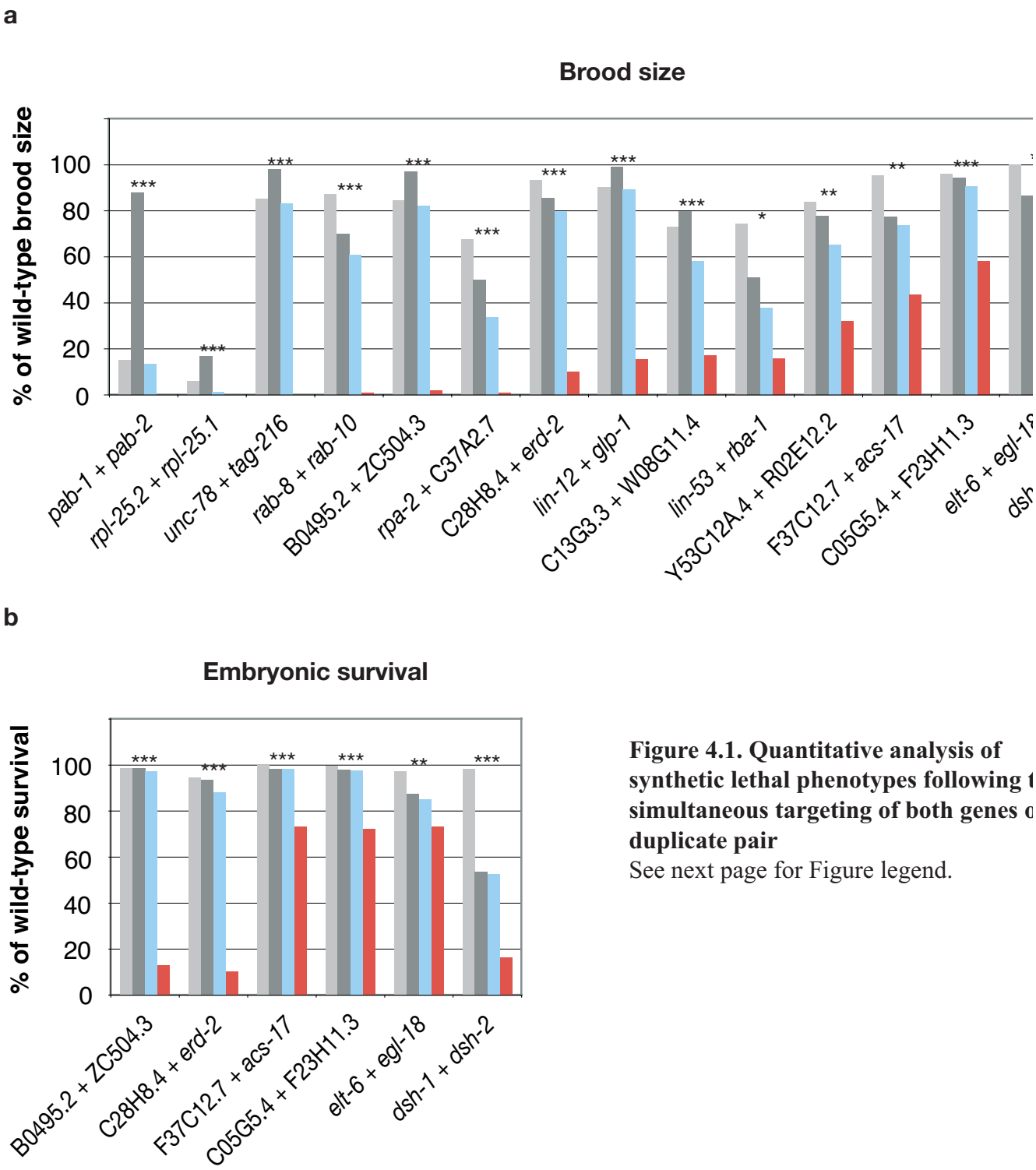

Figure 4.1. Quantitative analysis of synthetic lethal phenotypes following t simultaneous targeting of both genes o duplicate pair

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of the redundant function covered by a pair of duplicated genes in *C. elegans.* Based on the gene deletion phenotypes of the single-copy orthologues in yeast, I split the set of *C. elegans* duplicate gene pairs into those corresponding to essential or non-essential *S. cerevisiae* genes (see Appendix Table 4.2.). I found that five of eighteen worm duplicates (28%), that are orthologous to yeast essential genes, showed synthetic lethal phenotypes by combinatorial RNAi. In contrast, only five of fifty-five *C. elegans* duplicate gene pairs (9%) corresponding to *S. cerevisiae* non-essential genes were found to result in a synthetic viability defect when co-targeted. I thus conclude that duplicated genes in *C. elegans* that are related to an essential gene in yeast are about three times more likely to have an essential redundant function than those related to a non-essential yeast gene. Strikingly, this is the same enrichment for non-viable RNAi phenotypes as for non-duplicated genes: 61% of *C. elegans* single-copy orthologues of *S. cerevisiae* essential genes have non-viable RNAi phenotypes, compared to 20% of orthologues of yeast non-essential genes (Figure 4.2.) Thus, this finding is entirely consistent with a simple model of redundancy, suggesting that the function of a single gene identified in one organism is a good predictor of the redundant function covered by a pair of duplicated genes in a second organism.

4.4. Duplicated genes can maintain redundant functions for more than 80 million years of evolution

Having found that over 10% of genes (16 out of 143) that have been duplicated in the genome of *C. elegans* since the divergence from either *S. cerevisiae* or *D. melanogaster* share at least partially redundant functions, I next sought to address the underlying causes for this redundancy. Therefore, I wished to study the properties of gene duplicates with redundant functions, and whether these differ from duplicated gene pairs that were not identified as having redundant functions. For reasons of compactness, I will refer to these as ‘redundant’ and ‘non-redundant’ duplicate gene pairs, although of course I recognize that failure to detect a phenotype by RNAi does not preclude a genuine function.

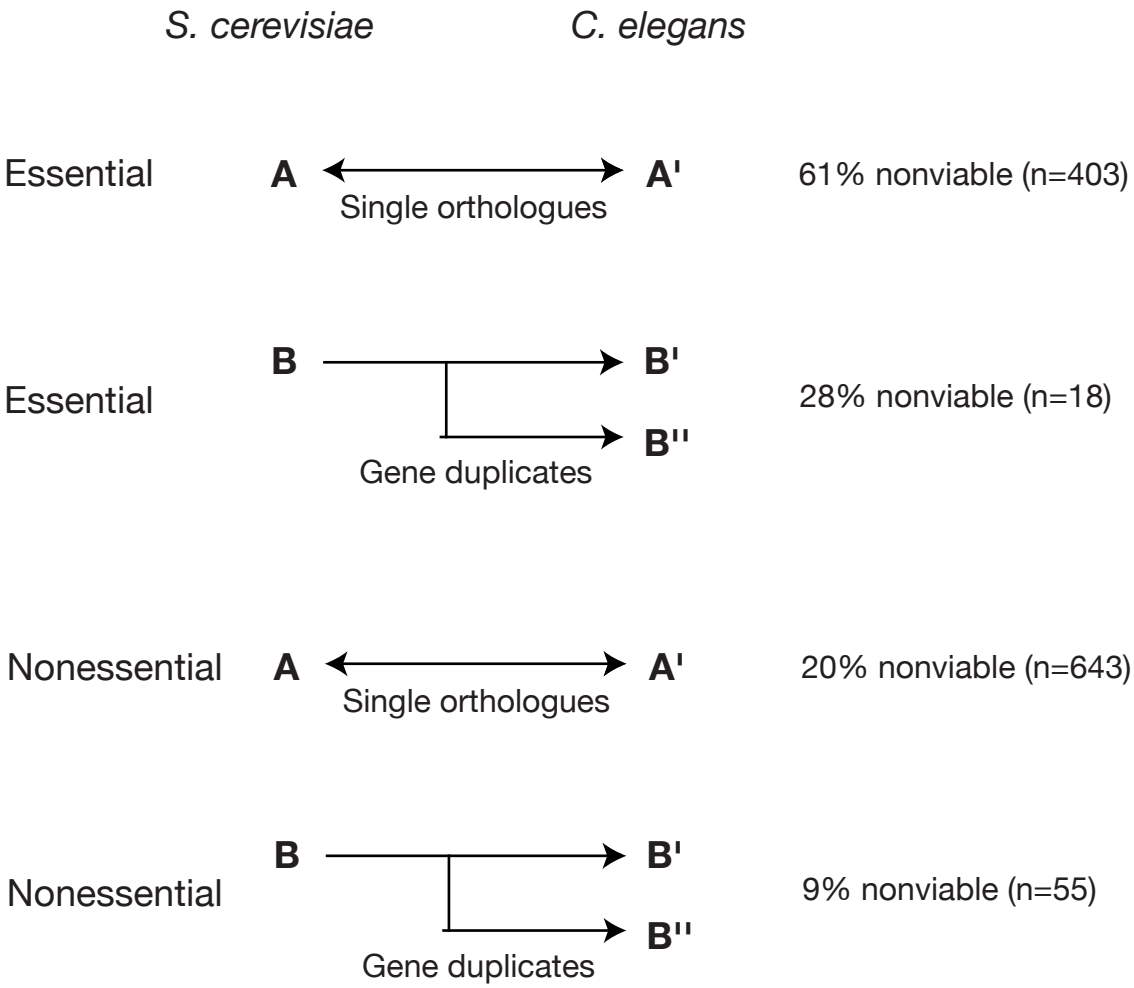

Figure 4.2. Transferring gene functions between *S. cerevisiae* and *C. elegans*
Orthologues of genes with essential functions in *S. cerevisiae* are very likely to have nonviable RNAi phenotypes in *C. elegans* ('Single orthologues'). Likewise, genes that are essential in yeast, but that have duplicated in *C. elegans* are likely to have a nonviable RNAi phenotype in *C. elegans* when both genes are targeted simultaneously by combinatorial RNAi ('Gene duplicates') The numbers indicate the percentage of tested genes or gene pairs with non-viable RNAi phenotypes in two independent experiments performed in the RNAi-hypersensitive strain *rrf-3*.

I considered two simple models that might explain why some duplicated genes appear to have redundant functions (as discussed in the Introduction). First, the redundancy may be a by-product resulting from a recent duplication event and thus represent a transient state; this initial functional overlap might get lost over time by functional divergence (Force *et al.*, 1999; Kimura and King, 1979; Lynch and Force, 2000; Ohno, 1970). In this case, the pairs of genes that I identified as having redundant essential functions would be expected to be more recent duplicates than those for which I found no functional overlap. Alternatively, several groups have established theoretical frameworks suggesting that redundant functions can be maintained by natural selection over substantial periods of evolutionary time (Nowak *et al.*, 1997; Wagner, 2000b). In this case, I would expect no clear difference in age between the sets of redundant and non-redundant duplicate gene pairs. Instead, I anticipated that there would be evidence that the redundant duplicated genes have been maintained relative to their ancestral sequence, thereby retaining their overlapping, redundant functions.

To investigate which of these two models can best explain the redundancy that I observed between some gene duplicates, I first examined whether there is evidence that the redundant gene pairs have duplicated more recently than non-redundant pairs. One would of course anticipate that more recently duplicated genes are more likely to have overlapping functions than more ancient duplicate gene pairs. Intriguingly, when investigating the number of synonymous nucleotide substitutions per synonymous site (Ks) as a measure of the evolutionary age of gene duplicates, I found the average rate of change to be 13.41 for redundant duplicates and 9.48 for non-redundant duplicates, indicating that both redundant and non-redundant duplicate gene pairs are ancient (see Appendix Table 4.3.), and their divergence time can no longer be reliably estimated. Having found no clear evidence that the redundant gene pairs represent more recent gene duplicates than the non-redundant gene pairs, I considered the possibility that the redundancy that I observe might simply be the consequence of a lack of evolutionary time for the duplicates to drift, as very unlikely.

Next, I set out to examine whether the duplicate gene pairs with essential redundant functions also do exist as gene duplicates in the related nematode *C. briggsae*. To do so, the INPARANOID algorithm was used to identify *C. briggsae* orthologues of

C. elegans genes (Remm *et al.*, 2001); I considered the gene duplication to predate the divergence of *C. elegans* from *C. briggsae*, if both *C. elegans* duplicates had a single identifiable orthologue in *C. briggsae*. Remarkably, 14 of the 16 pairs of duplicated genes that I identified as having essential redundant functions in *C. elegans* appear to have also been maintained as gene pairs in the related nematode *C. briggsae*. These findings suggested that these 14 duplicate gene pairs with redundant functions have arisen from a duplication event that predated the split from *C. briggsae*. In contrast, only 100 out of 127 non-redundant duplicate gene pairs also exist as gene pairs in *C. briggsae*. Thus, the frequency of conservation of redundant gene pairs between *C. elegans* and *C. briggsae* is significantly higher than the frequency observed for non-redundant duplicate gene pairs (χ^2 = 8.653, P = 0.0033, 1 degree of freedom; see Appendix Table 4.3.). *C. elegans* and *C. briggsae*, despite being morphologically very similar, last shared a common ancestor 80-110 million years ago (Stein *et al.*, 2003). Taking into account that *C. elegans* and *C. briggsae* only share ~60% of their genes as single orthologues, and a full 10% of genes encoded in either genome has no identifiable match in the other genome (Stein *et al.*, 2003), one would anticipate less than 40% of *C. elegans* duplicate gene pairs to be randomly conserved as pairs in *C. briggsae*. I thus consider the possibility that these 14 duplicate gene pairs with redundant essential functions in *C. elegans* have been retained as duplicate pairs in *C. briggsae* simply as a result of neutral evolution to be very unlikely. Instead, these data suggest that the redundancy between these duplicated genes might have been actively maintained for more than 80 million years of evolution.

Thus, I next sought to investigate whether there is evidence that the overlap in function has been actively retained by natural selection. If there has been selection for the maintenance of redundancy between duplicate gene pairs, then I would expect these duplicates to encode more similar proteins than non-redundant duplicates. To determine the percentage of identity between gene duplicates, protein sequences were aligned using the CLUSTAL W program (Thompson *et al.*, 1994). I found pairs of redundant duplicated genes to be more similar to each other at the amino-acid level ($P = 1.6 \times 10^{-2}$, Wilcoxon rank sum test, Figure 4.3.a) and to also have a greater similarity in alignable protein length ($P = 2.2 \times 10^{-2}$, Figure 4.3.b) than non-redundant duplicates and finally to also show a lower rate of non-synonymous nucleotide substitution per non-synonymous

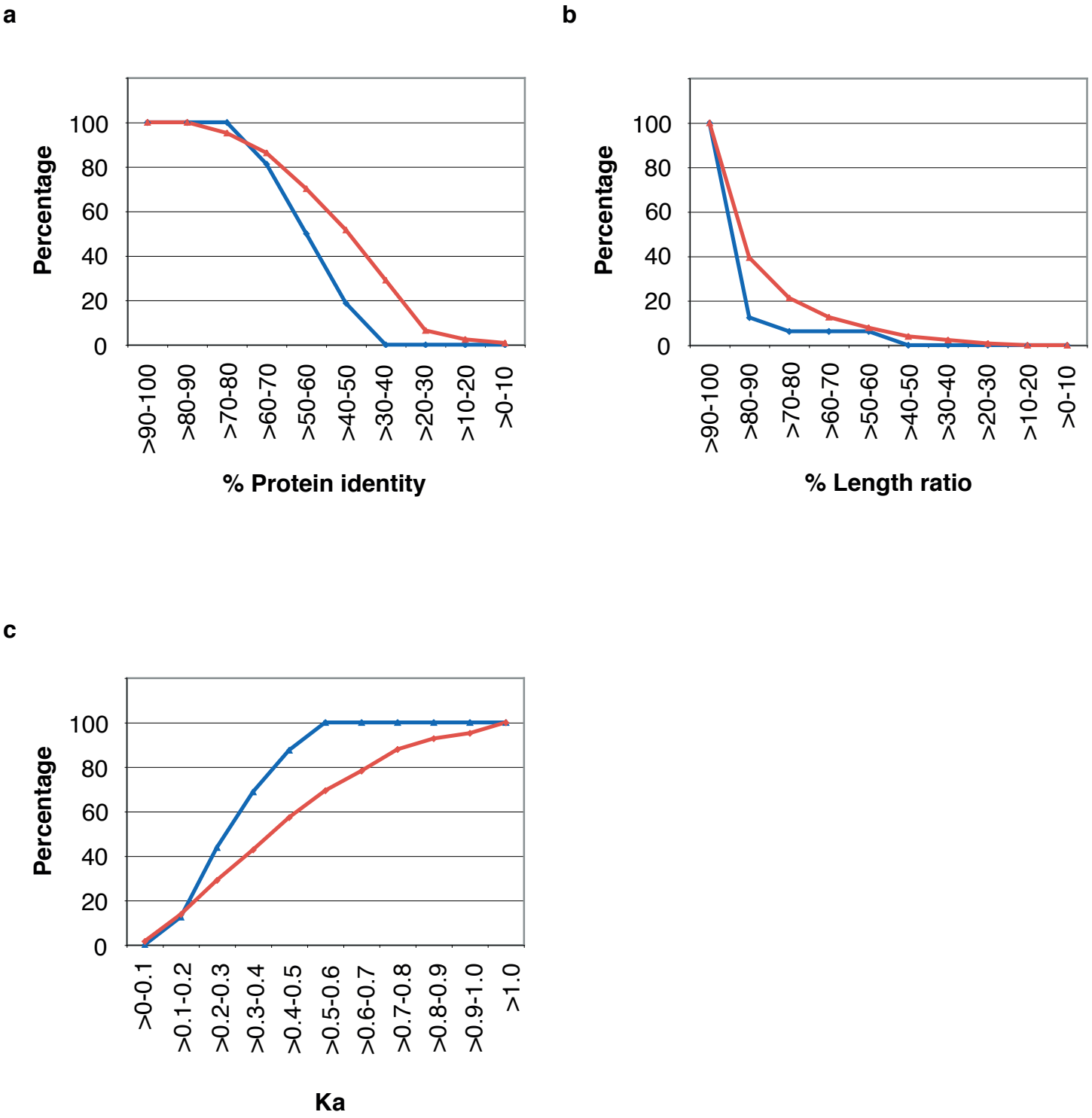

Figure 4.3. Higher sequence similarity between redundant versus non-redundant gene duplicates
Percentage similarity in protein sequence (a) and alignable protein length (b), and rate of non-synonymous nucleotide substitutions per non-synonymous site (Ka; c) are contrasted for gene duplicates with redundant functions (shown in blue) and for duplicate gene pairs that were not identified as having redundant functions (shown in red).

site (mean Ka for redundant duplicates = 0.34; mean Ka for non-redundant duplicates = 0.50; P = 3.8 x 10^{-2}; Figure 4.3.c) than non-redundant duplicates (see Appendix Table 4.3.). While I recognize that the redundant duplicate pairs appear only marginally more similar to each other than non-redundant gene duplicates, I found several independent lines of evidence that together suggest that redundant gene duplicates may have remained a higher degree of similarity as a consequence of stronger purifying selection than duplicate gene pairs that were not identified as having redundant functions.

Several theoretical models have been generated to explain how redundant functions can be maintained by natural selection (as discussed in the Introduction). One theory relates pleiotropy to redundancy. In this model, both copies are only redundant with respect to some sub-functions, while they also perform independent functions and thus are evolutionarily selected (sub-functionalization). While both experimental and theoretical studies support sub-functionalization as a likely evolutionary fate of gene duplicates (Kondrashov *et al.*, 2002; Lynch and Force, 2000) and a means to maintain gene duplicates, one would anticipate that the same mechanisms act on both redundant and non-redundant gene duplicates. Therefore, the sub-functionalization model cannot explain how redundant gene duplicates have maintained a higher degree of sequence similarity as compared to non-redundant gene duplicates. Further two models for the evolutionary stability of genetic redundancy are based on the assumption of very specific mutation rates and efficacies of protein function (Nowak *et al.*, 1997). These theories can — at least mathematically — explain how gene duplicates can maintain sequence similarity and perform the exact same function for very long or even infinite evolutionary timescales. I believe that my findings favour these latter models.

Taken together, I consider it as unlikely that the greater similarity between duplicate gene pairs with redundant functions that I observed is a trivial consequence of their having duplicated more recently. Rather, I suggest that the protein sequences of redundant gene pairs have been maintained relative to each other since duplication as the result of selective pressure to maintain their redundant functions.

4.5. Conclusion

In summary, in this chapter I have described how I have used combinatorial RNAi to systematically investigate whether there is functional redundancy between *C. elegans* gene duplicates. Focusing on genes that have been duplicated in the genome of *C. elegans* since divergence from either *S. cerevisiae* or *D. melanogaster*, I was able to analyse 143 duplicate gene pairs by combinatorial RNAi for their potentially redundant functions. Of these, 16 gene pairs showed unambiguous synthetic RNAi phenotypes, demonstrating that they are at least partially functionally redundant. I found that just as single-copy worm genes are more likely to have a non-viable RNAi phenotype if they are orthologous to an essential gene in *S. cerevisiae*, duplicated worm genes are more likely to have a redundant essential function if they are co-orthologous to an essential yeast gene. It therefore should be possible to predict the redundant functions of many duplicated genes in higher organisms based on the functions of single-copy orthologues in lower organisms.

Most intriguingly, the redundancy that I observed between duplicated genes cannot be explained simply because they are derived from a recent duplication event — 14 of the 16 redundant gene pairs were duplicated before the divergence of *C. elegans* and *C. briggsae* 80-110 million years ago (Stein *et al.*, 2003). The redundancy between these 14 gene pairs has thus been maintained for more than 80 million years of evolution. Therefore, I believe that it is extremely unlikely that the functional overlap between these 14 duplicated genes is present merely due to a lack of evolutionary time since duplication. Not only is the average half-life of a gene duplicate in eukaryotes typically about 4 million years (Lynch and Conery, 2000), but also, over this time period, the *C. elegans* and *C. briggsae* genomes have diverged enormously; they only share ~60% of their genes as single orthologues, and a further 10% of genes are present exclusively in one or other genome (Stein *et al.*, 2003). Rather, my findings are consistent with theoretical models, suggesting that under appropriate — but realistic — conditions it is possible to select, directly or indirectly, for redundancy between duplicates to be maintained (Nowak *et al.*, 1997).

Having provided the first large-scale analysis in any organism of the redundant functions of gene duplicates, I wished to further examine functional redundancy in complex genetic networks.

Chapter 5

Functional redundancy in genetic interaction networks

5.1. Introduction

While I have identified functional redundancy between some duplicate gene pairs, the majority of bigenic interactions that were uncovered when systematically mapping synthetic sick and synthetic lethal (SL) interactions in *S. cerevisiae* do not occur between gene duplicates, but rather between genes unrelated at the sequence level.

To date, however, there is still much debate about how such higher-order functional redundancy might arise, whether it is a selectable trait, and whether such redundancy can be conserved throughout evolution (discussed in Wagner, 2005). Since similar types of non-additive interactions between mutations might underlie multifactorial genetic disease in humans, it is a major open question in genetics whether these individual genetic interactions are conserved between species and thus may be directly predicted in humans using interactions identified in simple model organisms.

I therefore wished to shed light on the evolution of gene networks. To do so, I sought to investigate whether genetic interactions are conserved between the yeast *S. cerevisiae* and the nematode *C. elegans*. Using RNA interference (RNAi) in *C. elegans*, I set out to explore whether individual SL interactions uncovered in yeast are conserved in the worm. Importantly, to date, *C. elegans* is the main animal model in which to carry out systematic functional studies *in vivo* in the context of a developing organism. This study thus allows me to directly compare genetic interaction networks that have been compiled *in vivo* in yeast with *in vivo* genetic interaction networks in the worm.

5.2. Investigating the conservation of synthetic lethal interactions between *S. cerevisiae* and *C. elegans*

I based my study on three *S. cerevisiae* datasets that were compiled using three different technological approaches for the systematic identification of SL interactions: first, a ‘global’ genetic interaction network encompassing ~ 4,000 SL interactions, mapped by using synthetic genetic array (SGA) technology to interrogate synthetic lethality predominantly between deletion alleles of non-essential genes (Tong *et al.*, 2004); second, an essential gene network comprising 567 interactions, including

conditional alleles for almost 300 yeast essential genes, that has been compiled by using SGA analysis (Davierwala *et al.*, 2005); and third, a genome-wide analysis of DNA integrity, providing a network of almost 5,000 SL interactions, which were mapped by employing diploid synthetic lethal analysis by microarray (dSLAM) using the *S. cerevisiae* heterozygous gene deletion collection (Pan *et al.*, 2006). Together, these three screens tested ~850,000 pairwise interactions, covering ~5% of the possible bigenic interaction space, and identified ~9,000 unique interactions including both interactions between null alleles and between hypomorphic mutants. Thus, examining the conservation of these large, diverse and systematically mapped datasets of SL interactions allows me to make firm conclusions about the conservation of genetic interactions between species.

To investigate whether SL interactions are conserved between yeast and worm, I set out to test whether I can detect SL interactions between pairs of *C. elegans* genes that are orthologous to pairs of genes that have been identified as having SL interactions in at least one of these three large-scale screens in *S. cerevisiae*. The INPARANOID algorithm was used to identify *C. elegans* orthologues of *S. cerevisiae* gene pairs (Remm *et al.*, 2001). Considering genetic interactions between yeast gene pairs for which both genes had a single orthologue in *C. elegans* only, 1,148 worm gene pairs were identified.

5.2.1. Using combinatorial RNAi to test whether synthetic lethal interactions are conserved between yeast and worm

Of 1,148 *C. elegans* gene pairs that were orthologous to gene pairs that were reported as having SL interactions in *S. cerevisiae*, 856 gene pairs could be targeted by combinatorial RNAi in the worm using the genome-wide feeding library (Kamath *et al.*, 2003). These gene pairs are listed in Appendix Table 5.1. For each gene pair amenable to analysis by combinatorial RNAi, I compared the phenotype resulting from simultaneously targeting both genes with the phenotypes resulting from targeting each gene individually side by side (as described in Chapter 3 and Materials and Methods; see Figure 3.6). All screens were performed at least twice independently, using the high-

throughput (HTP) RNAi liquid-feeding assay and the RNAi-hypersensitive *rrf-3* background.

First, worms were scored for SL phenotypes in a purely qualitative way. This was done for reasons of throughput. At that stage, thirteen gene pairs had to be excluded from the screen for SL interactions, because RNAi against individual genes resulted in worms that arrested growth at a late larval stage, a phenotype that cannot be enhanced any further. Thus, in total, I was able to screen 843 gene pairs that are orthologous to yeast SL interactions, for synthetic viability defects by using combinatorial RNAi in *C. elegans* (Table 5.1.). These 843 interactions are equivalent to 692 interactions in *S. cerevisiae* between two null alleles, 67 interactions between a hypomorph and a null allele, and 84 interactions between two hypomorphs.

For six out of 843 gene pairs that could be investigated for SL interactions in the worm, phenotypes generated by combinatorial RNAi are qualitatively stronger than the contributions of both individual RNAi phenotypes (Table 5.2.). In four cases, simultaneous targeting of both genes appeared to result either in reduced brood sizes or in reduced embryonic survival rates. RNAi phenotypes for these gene pairs were quantified by manually counting larvae, unhatched eggs, and adults within each experiment and subject to statistical analysis under a multiplicative model, using a Student's t-test (two-tailed distribution, two-sample equal variance; see 'Generating known synthetic lethal phenotypes by combinatorial RNAi' and Materials and Methods for a detailed description) to confirm all four gene pairs as SL interactions (Table 5.3.a and Figure 5.1.a and 5.1.b).

Combinatorial RNAi against two gene pairs (the gene pairs *lis-1* and *cap-1*, and Y6B3A.1 and *tfg-1*) resulted in pronounced synthetic adult lethal phenotypes (Table 5.2., Table 5.3.b and Figure 5.1.c and 5.1.d). Intriguingly, *lis-1* encodes an orthologue of human LIS1, which leads to lissencephaly, a disorder of neural development, when mutated (Online Mendelian Inheritance in Man, OMIM^{TM}; www.ncbi.nlm.nih.gov/omim/). Thus, it would be interesting to investigate whether patients with a severe clinical phenotype also carry a mutant allele of the human orthologue of *C. elegans cap-1*.

Yeast SL dataset	**SL interactions tested in *C. elegans* using combinatorial RNAi**	**SL interactions tested in *C. elegans* using genetic mutants + RNAi**
Tong *et al.*, 2004	**370**[a]	**88**[b]
Null + null	319[a]	71[b]
Hypomorph + null	51	17
Davierwala *et al.*, 2005	**100**	**7**
Null + hypomorph	16	-
Hypomorph + hypomorph	84	7
Pan *et al.*, 2006	**416**[a]	**88**[b]
Null + null	416[a]	88[b]
Total	**843**[a]	**174**[b]

Table 5.1. Overview of synthetic lethal interactions that were tested between *S. cerevisiae* and *C. elegans*
The three yeast data sets my study was based on, the number of interactions between pairs of *C. elegans* genes that are orthologous to pairs of genes identified as having synthetic lethal (SL) interactions in yeast that could be tested by combinatorial RNA interference (RNAi) and by RNAi in a worm strain homozygous for a loss-of-function genetic mutation in a second gene, respectively, and whether these correspond to null or hypomorphic alleles, are shown.
[a] 43 interactions are redundant between the Tong *et al*. and Pan *et al*. datasets.
[b] 9 interactions are redundant between the Tong *et al*. and Pan *et al*. datasets.

Yeast SL dataset	**SL interactions tested in *C. elegans* using combinatorial RNAi**	**Conserved interactions**	**SL phenotype**
Tong *et al.*, 2004	370	*lis-1* + *cap-1* *pfd-6* + C05D11.3	AL BS
Davierwala *et al.*, 2005	100	Y6B3A.1 + *tfg-1*	AL
Pan *et al.*, 2003	416	*rfp-1* + *rack-1* *rfp-1* + *htz1* *rfp-1* + *gfl-1*	BS BS, ES BS, ES
Total	843[a]	6	

Table 5.2. Synthetic lethal interactions are not conserved between *S. cerevisiae* and *C. elegans*

C. elegans gene pairs that were synthetic lethal (SL) in *S. cerevisiae* and that were identified to be SL by combinatorial RNAi are shown ('Conserved interactions'). SL phenotypes are classified as adult lethal ('AL'), reduced brood size ('BS') and reduced embryonic survival ('ES'), respectively.

a)

Interacting gene pairs	Gene1		Gene2		Gene1 & 2		p-value	
	BS	ES	BS	ES	BS	ES	BS	ES
pfd-6 + C05D11.3	49	75	66	72	15	48	6.22E-05	1.04E-01
rfp-1 + *rack-1*	100	90	76	100	28	91	3.81E-19	8.38E-01
rfp-1 + *gfl-1*	96	85	88	98	44	60	1.07E-07	1.51E-05
rfp-1 + *htz-1*	97	94	98	100	60	76	2.30E-10	6.70E-07

b)

Interacting gene pairs	Gene1	Gene2	Gene1 & 2	p-value
	Wt	Wt	Wt	Wt
lis-1 + *cap-1*	100	100	48	6.64E-17
Y6B3A.1 + *tfg-1*	99	95	38	3.93E-18

Table 5.3. Quantitative analysis of synthetic lethal interactions that are conserved between *S. cerevisiae* and *C. elegans*

Synthetic lethal phenotypes in *C. elegans* were verified by quantification and statistical analysis under a multiplicative model (Phillips *et al.*, 2000; Puniyani *et al.*, 2004); percentages of average wild-type brood size ('BS') and embryonic survival ('ES') rate (a), and fractions of animals that appeared wild-type ('Wt'; b) after RNA interference (RNAi) against each gene individually ('Gene1', 'Gene2') and combinatorial RNAi against both genes simultaneously ('Gene1 & 2') are shown. Values presented are the arithmetic mean of two independent experiments performed in the RNAi-hypersensitive *rrf-3* background. A Student's t-test was used to assess the statistical significance of quantitative phenotype data.

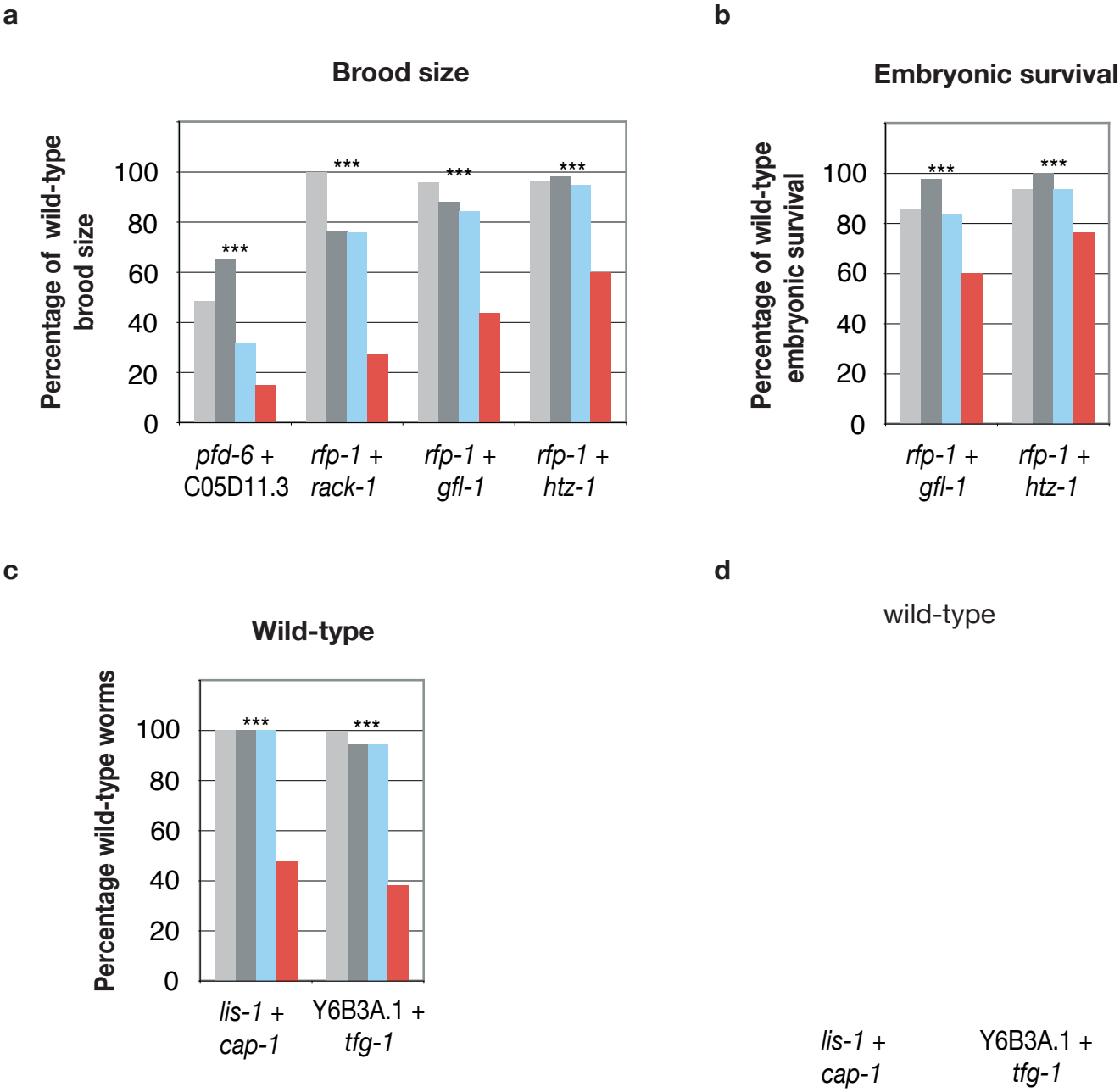

Figure 5.1. Synthetic lethal interactions that are conserved between *S. cerevisiae* and *C. elegans*

For each gene pair that yielded reproducible synthetic effects by combinatorial RNA interference (RNAi), phenotypes were quantified: brood size (a), embryonic survival rates (b), and percentage of wild-type worms (c), resulting from targeting each gene individually (light- and dark-grey bars) were compared with that generated by targeting both genes of a pair simultaneously (red bars) and with the calculated product of the single gene measurements (blue bars). Values plotted in (a) and (b) represent percentages of typical wild-type brood sizes, and embryonic survival rates, respectively. Data shown are the arithmetic mean of two independent experiments. Synthetic lethality was assessed under a multiplicative model (Phillips *et al*., 2000; Puniyani *et al.,* 2004). ***, P < 1.0E-03; Student's t-test. Representative images of synthetic adult lethal phenotypes resulting from combinatorial RNAi against *lis-1* and *cap-1*, and Y6B3A.1 and *tfg-1*, respectively (d). Scale bars: 0.1 mm. All experiments were performed in the RNAi-hypersensitive strain *rrf-3*.

5.2.2. Using RNAi in genetic mutants to test whether synthetic lethal interactions are conserved between yeast and worm

In addition to screening 843 pairwise orthologues of *S. cerevisiae* SL interactions by combinatorial RNAi for synthetic lethality in the worm, I sought to further test all possible gene pairs for synthetic lethality by targeting one gene of a pair by RNAi in a worm strain homozygous for a loss-of-function genetic mutation in the second gene. We have previously used this approach in our laboratory for systematically investigating ~65,000 gene pairs with functions in signal transduction and transcriptional regulation for their ability to genetically interact (Lehner *et al.*, 2006). I was able to analyse 174 gene pairs for synthetic viability defects by using RNAi in 35 *C. elegans* strains carrying defined homozygous genetic mutations; this was the entire set for which a viable mutant strain was publicly available (see Appendix Table 5.2.).

I therefore compared RNAi phenotypes observed in the genetic mutants with the RNAi phenotypes of wild-type worms and with the phenotypes of the genetic mutants fed on bacteria expressing a dsRNA that does not target an expressed portion of the *C. elegans* genome (Ahringer library clone Y95B8A_84.g; see Figure 5.2.) side by side. Worms were screened for SL phenotypes in duplicates in at least two independent experimental setups, using the HTP RNAi liquid-feeding assay.

In few cases, brood size and embryonic survival rates, respectively, after RNAi in a genetic mutant appeared reduced as compared to RNAi against these genes in wild-type worms (Table 5.4.). These homozygous mutant strains, however, had reduced brood sizes and embryonic survival rates, respectively, on their own. I therefore sought to investigate whether the enhanced phenotypes that I observed when targeting a second gene by RNAi in these genetic mutants were the results of true synthetic lethality or rather caused by purely non-specific additive effects of the phenotypes of mutant strains and RNAi phenotypes. To examine this, I fed genetic mutants on additional RNAi clones that produced RNAi phenotypes similar to the phenotype of the putatively interacting gene in wild-type worms. I observed severely enhanced phenotypes after RNAi against all control genes in these four mutant strains carrying defined lesions. I thus considered these putative genetic interactions to be the results of non-specific additive effects between the

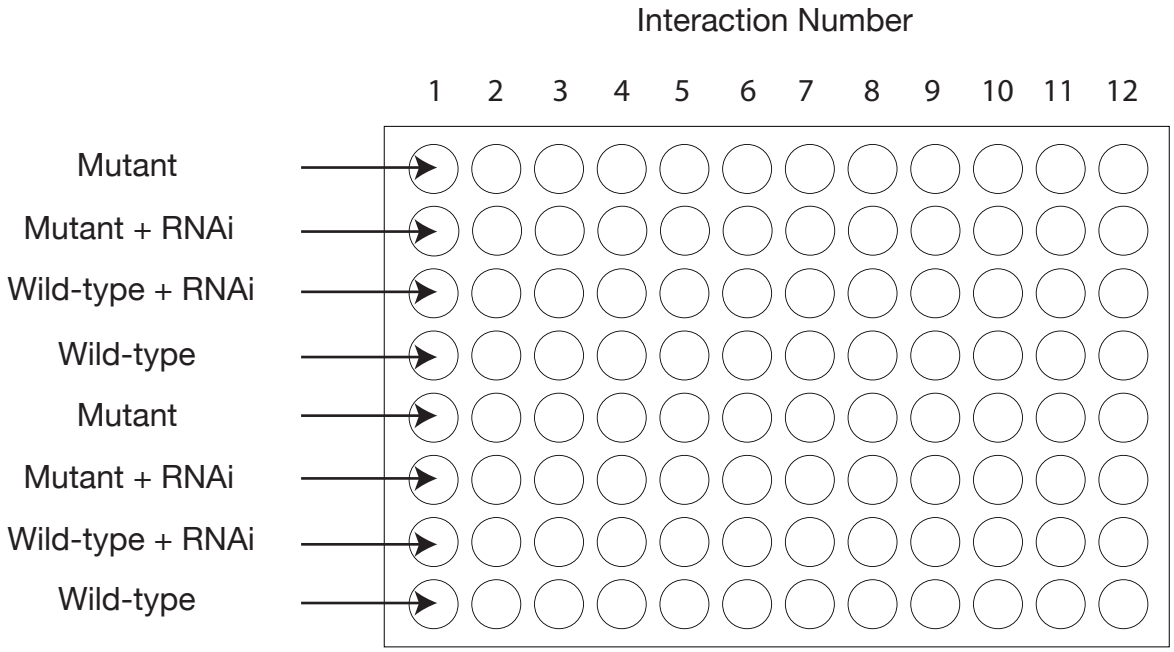

Figure 5.2. Overview of the setup for genetic interaction screens using RNA interference in a genetic mutant
When screening for genetic interactions by targeting one gene by RNA interference (RNAi) in a *C. elegans* strain carrying a homozygous loss-of-function allele of a second gene, RNAi phenotypes of the genetic mutants were compared to the RNAi phenotypes of wild-type worms and to the phenotypes of genetic mutants and wild-type worms, respectively, fed on non-targeting double-stranded RNA-expressing bacteria side by side. By using this setup, genetic interaction screens were performed in duplicates within independent experiments.

C. elegans strain	RNAi clone
TJ1049	C43E11.9
DS77	C39E9.13
CX51	H20J04.d R151.9 T06G6.9 F21C3.5
RB1457	R05D3.4 B0205.3

Table 5.4. *C. elegans* strains with non-specifically enhanced RNA interference phenotypes

C. elegans strains carrying a defined genetic lesion that showed non-specifically enhanced phenotypes when targeted by RNAi clones (represented by Ahringer library RNA interference (RNAi) clone gene pairs names) are shown.

phenotypes of the genetic mutant and the RNAi phenotypes and do not represent informative SL interactions. The logic behind excluding these interactions is analogous to excluding physical interactions between 'sticky' proteins.

5.3. Synthetic lethal interactions are not conserved between *S. cerevisiae* and *C. elegans*

Taken together, I have investigated 843 pairwise orthologues of genes that were identified as having SL interactions in *S. cerevisiae* for synthetic viability defects in *C. elegans* by combinatorial RNAi and a further 174 pairs by single-gene RNAi in worm strains carrying defined homozygous genetic mutations. Strikingly, I only identified 6 gene pairs (0.7%) to show synthetic lethal phenotypes when targeted by combinatorial RNAi in the worm (Table 5.2. and Figure 5.1.). This observed degree of conservation between SL interactions in *S. cerevisiae* and *C. elegans* is not significantly different to the frequency of SL interactions that we have detected in a systematic large-scale study in *C. elegans*: screening for synthetic synthetic lethality between genes with roles in signaling and transcriptional regulation, we found on average 0.6% of tested gene pairs to genetically interact in the worm (Lehner *et al.*, 2006; $\chi^2 = 0.201$, $P = 0.6538$, 1 degree of freedom). These data thus imply that individual SL interactions are not conserved between *S. cerevisiae* and *C. elegans* more than is expected by chance.

I do not see any functional similarities in the small set of genes that interact that distinguish them from the non-conserved interactions (Table 5.5.), nor is there any correlation between which yeast study the SL interaction derived from and whether it is also found in *C. elegans* (Table 5.2.). Therefore, neither interactions between null alleles nor interactions between hypomorphs appear conserved between these two species.

Moreover, the frequency of SL interactions that can be detected in yeast and worm is very similar (Lehner *et al.*, 2006), hence the non-conservation cannot simply be explained by a reduction in the number of SL interactions. Neither can it be explained by increased functional redundancy as a result of gene duplication in the worm, because I only tested for genetic interactions between gene pairs for which both genes had a single orthologue in *C. elegans*.

S. cerevisiae	***C. elegans***	**Gene1**	**Gene2**
PAC1 + CAP1	*lis-1* + *cap-1*	Orthologue of human lissencephaly gene[a] with functions in spindle organization and biogenesis[b]	F-actin capping protein, alpha subunit[c]
SEC7 + LAS17	Y6B3A.1 + *tfg-1*	ADP ribosylation factor (ARF) guanine nucleotide exchange factor[c]	Human TFG related[d], putative apoptotic suppressor in *C. elegans*[e]
YKE2 + PLP1	*pfd-6* + C05D11.3	Prefoldin subunit 6, KE2 family[c]	Essential for proper microtubule organization and function[f]
BRE1 + ASC1	*rfp-1* + *rack-1*	E3 ubiquitin ligase required for H2B ubiquitination[c]	Homolog of mammalian RACK1 (Receptor of Activated C Kinase)[b]
BRE1 + HTZ1	*rfp-1* + *htz-1*	E3 ubiquitin ligase required for H2B ubiquitination[c]	Histone variant H2AZ homolog[d]
BRE1 + YAF9	*rfp-1* + *gfl-1*	E3 ubiquitin ligase required for H2B ubiquitination[c]	Transcription initiation factor IIF, auxiliary subunit[c]

Table 5.5. Molecular functions of *C. elegans* gene pairs with synthetic lethal RNA interference phenotypes

Molecular roles of synthetic lethal gene pairs that are conserved between *S. cerevisiae* and *C. elegans* are shown. *C. elegans* genes ('Gene1', 'Gene2') are represented by their [a] WormBase descriptions (www.wormbase.org); [b] Gene Ontology descriptions (Ashburner *et al.*, 2000); [c] NCBI eukaryotic orthologous groups (Koonin *et al.*, , 2004); [d] WormBase ID (www.wormbase.org); [e] functions as described in Chen *et al.*, 2004; [f] functions as described in Ogawa *et al.*, 2004.

Strikingly, the lack of conservation of non-additive, synthetic genetic interactions between yeast and worms is in stark contrast to the conservation of single gene functions. Using an identical assay, I found 61% of *C. elegans* genes that are orthologous to an essential gene in *S. cerevisiae* to have a non-viable RNAi phenotype (see 'Transferring gene functions between *S. cerevisiae* and *C. elegans*'). Furthermore, 31% of tested protein interactions were found to be conserved between *S. cerevisiae* and *C. elegans* (Matthews *et al.*, 2001). Thus, although the functions of individual genes and the physical interactions between gene products are well conserved between yeast and worms, non-additive, synthetic genetic interactions are not. Consequently, SL interactions identified in *S. cerevisiae* cannot be used to directly predict genetic interactions in the nematode *C. elegans* and thus are very unlikely to be predictive of genetic interactions in humans.

5.4. 'Induced essentiality' as alternative model for the interpretation of synthetic lethal interactions

However, beyond the direct practical implications for using *S. cerevisiae* data to predict SL interactions in humans, I believe these results may also have important implications for the mechanistic interpretation of SL interactions. The classic interpretation of a SL interaction between two genes (gene A and B) is that the genes (or the pathways in which they act) are at least partially functionally redundant (Kelley and Ideker, 2005; reviewed in Hartman *et al.*, 2001). In this model, loss of one gene has little effect since the alternative, redundant pathway can compensate for this loss (see Figure 1.5.). This situation is most apparent for recently duplicated genes that, by their nature, are highly redundant: in some cases the loss of one duplicate has little effect on fitness, but loss of both duplicates together is catastrophic (Ihmels *et al.*, 2007; Tischler *et al.*, 2006). Most SL interactions do not take place between duplicated genes, however, but between genes that do not share sequence similarity (Tong *et al.*, 2004).

Considering the classic model for the interpretation of SL interactions, in which synthetic lethality is considered a consequence of inactivating two functionally redundant genes or pathways, one might expect SL interactions to be conserved if individual gene functions are conserved. My findings, however, do not support this theory, but rather led

me to suggest an alternative model to explain SL interactions. I consider that genetic networks that underlie viability are not constant but flexible to change under different environmental conditions. This flexibility allows biological systems to adopt a range of alternative viable states, each with their set of essential genes. Thus, a gene that is non-essential under normal laboratory growth conditions may be absolutely critical for survival in a different environmental condition. Increasing experimental evidence supports this notion (Chang *et al.*, 2002; Davis-Kaplan *et al.*, 2004; Dudley *et al.*, 2005; Enyenihi and Saunders, 2003; Kuepfer *et al.*, 2005; Martinez *et al.*, 2004; Smith *et al.*, 2006). I propose that loss of gene A results in a rearrangement of the genetic network into an alternative viable state, where gene B is now an essential gene (Figure 5.3.). In this view, gene B is required under a condition caused by the loss of gene A. The functions of gene A and gene B, or the pathways in which they act, are not redundant or related — gene B is simply required under a condition caused by loss of gene A. In this model — which I have termed 'induced essentiality' — I consider SL interactions to represent a special form of conditional lethality, where loss of gene A partly mimics the response of the cell to an environmental condition. In light of this hypothesis, SL interactions are highly unlikely to be conserved — the range of environments that yeast cells need to respond to are very different to those that affect cells in an intact animal. Furthermore, regulatory networks governing cellular responses evolve very rapidly (Maslov *et al.*, 2004; Odom *et al.*, 2007). Thus, while in the classical model, SL interactions are interpreted as the consequence of inactivating functionally redundant genes or pathways, I propose SL interactions to be the consequence of the ability of genetic networks to rearrange into alternative viable states driven by the evolution of adaptive responses to environmental changes.

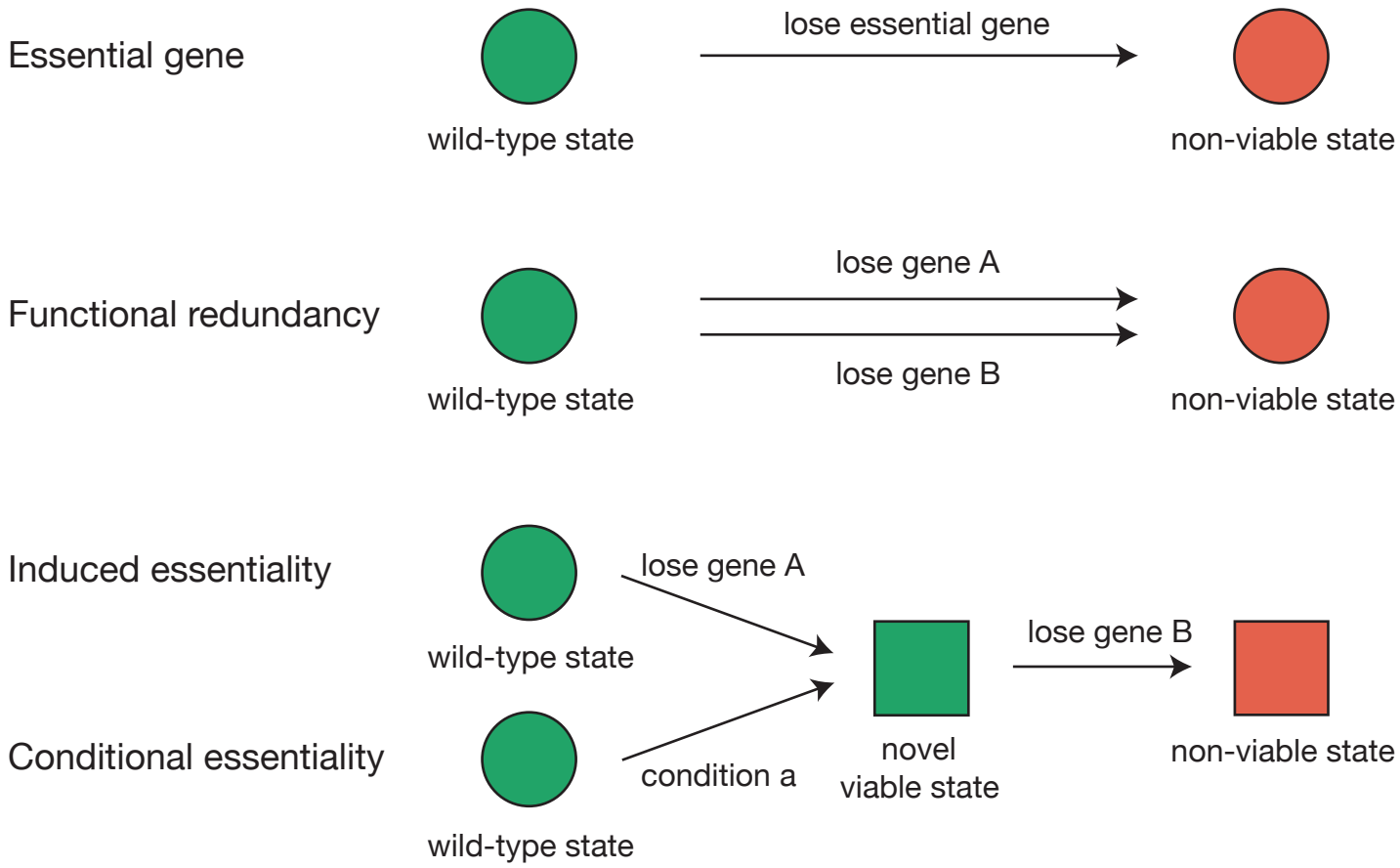

Figure 5.3. Two models for the interpretation of synthetic lethal interactions
In the classical model, SL interactions occur between two redundant genes or pathways ('Functional redundancy'; here gene A and gene B) that can compensate for the loss of one another. In the alternative model ('Induced essentiality'), loss of one gene (gene A) results in a rearrangement of the genetic network into a novel network; this rearrangement may mimic the response to an environmental condition. In this new network, the other gene (gene B) is now an essential gene. The rapid evolution of synthetic lethal interactions compared with individual gene functions favours this second model.

5.5. Investigating higher-order similarities in synthetic lethal interactions between *S. cerevisiae* and *C. elegans*

Having found that SL interactions are not conserved between *S. cerevisiae* and *C. elegans* more than expected by chance despite the very high degree of conservation of individual gene functions, I considered the possibility that there might be higher-order similarities in SL interactions between yeast and animals. I hypothesized that although gene networks might not have been conserved between species at the level of individual SL interactions, they might have been conserved at a higher level, such as at the level of pathways or molecular machines. For example, while in yeast, gene A (e.g. a specific component of the DNA repair pathway) is SL with gene B (e.g. a gene with a role in DNA replication) in worm, another gene (other than the orthologue of yeast gene A) that functions in DNA repair might be SL with another component (other than the orthologue of yeast gene B) that is involved in the process of DNA repair.

In order to test this hypothesis, I set out to screen for genetic interactions in *C. elegans* within the same set of genes that have been screened for SL interactions in *S. cerevisiae* and *C. elegans*. To do so, I selected two query genes and screened for novel genetic interactions between these and all 1,046 single orthologues between *S. cerevisiae* and *C. elegans* that could be targeted by an RNAi clone in the Ahringer library (as discussed in Chapter 4). This approach allowed me to systematically investigate genetic interactions in the worm within the same set of genes that have been screened for SL interactions in yeast. I chose *lis-1*, the *C. elegans* orthologue of *S. cerevisiae PAC1*, which is encoding for a component of the dynein/dynactin pathway and *mdf-2*, orthologous to yeast *MAD2*, a gene encoding for a component of the spindle-assembly checkpoint complex, respectively, as query genes (Table 5.6.). Using our HTP liquid-feeding assay, I compared the phenotypes resulting from simultaneously targeting two genes by combinatorial RNAi with the RNAi phenotypes of both genes individually in the RNAi-hypersensitive strain *rrf-3* in duplicates within two independent experimental setups.

When screening for genetic interactions with *mdf-2* as query, I found combinatorial RNAi against *mdf-2* and *tbg-1*, a gene encoding for gamma-tubulin, to

S. cerevisiae	***C. elegans***	**Gene 1**	**Gene 2**
PAC1 + CAP2	*lis-1* + *cap-2*	Orthologue of human lissencephaly gene[a] with functions in spindle organization and biogenesis[b]	F-actin capping protein, beta subunit[c]
MAD2 + TUB4	*mdf-2* + *tbg-1*	Spindle assembly checkpoint protein[c]	Gamma tubulin[c]

Table 5.6. Molecular functions of novel synthetic lethal interactions between *C. elegans* orthologues of *S. cerevisiae* genes

Molecular roles of novel synthetic lethal gene pairs that were identified in *C. elegans* are shown. *C. elegans* genes ('Gene1', 'Gene2') are represented by their [a] WormBase descriptions (www.wormbase.org); [b] Gene Ontology descriptions (Ashburner *et al*., 2000); [c] NCBI eukaryotic orthologous groups (Koonin *et al*., 2004).

result in reduced brood and embryonic survival rates (Table 5.6., Table 5.7., and Figure 5.4.a and 5.4.b). Using *lis-1* as query gene, I identified both *cap-1* and *cap-2*, encoding for alpha- and beta-subunits, respectively, of the F-actin capping protein hetero-dimer, to result in synthetic adult lethal phenotypes with *lis-1* (Table 5.6., Table 5.7., and Figure 5.4.c and 5.4.d). While both *cap-1* and *cap-2* showed the same combinatorial RNAi phenotype with *lis-1*, combinatorial RNAi against *cap-1* and *cap-2* did not result in an enhanced phenotype as compared to each single-gene RNAi phenotype alone (Figure 5.4.e). This finding supports the notion that SL interactions are mostly uncovered between components of different molecular pathways, rather than between genes functioning within the same pathways (Bader *et al.*, 2003; Kelley and Ideker, 2005; Ye *et al.*, 2005). While I had already verified the SL interaction between *C. elegans lis-1* and *cap-1*, I further confirmed the two novel SL interactions between worm genes *lis-1* and *cap-2*, and *mdf-2* and *tbg-1*, respectively, by quantification and statistical analysis under a multiplicative model (Table 5.7. and Figure 5.4.; as discussed in Chapter 3).

Thus, by systematically screening for SL interactions in *C. elegans* within the same set of genes that have been screened for SL interactions in *S. cerevisiae*, I uncovered two novel genetic interactions and confirmed one previously identified SL interaction in *C. elegans* by using combinatorial RNAi. While the yeast genes *PAC1* and *CAP2*, which are orthologous to the worm genes *lis-1* and *cap-2*, might not have been assayed for synthetic lethality in the large-scale screen by Tong *et al.* (2004), in which the yeast genes *PAC1* and *CAP1* were identified as an SL pair, this novel interaction in *C. elegans* might be explained functionally, considering that *cap-1* and *cap-2* encode for alpha- and beta-subunits, respectively, of a capping protein heterodimer (Table 5.6.).

Intriguingly, when searching BIOGRID, a database for all compiled interaction data for model organisms (Stark *et al.*, 2006), I found *DYN2*, a gene encoding for a microtubule motor protein, to be amongst the reported SL interaction partners of the *S. cerevisiae* gene *CAP2*. Both *PAC1* — the yeast orthologue of *C. elegans lis-1*, which I identified as being SL with both *cap-1* and *cap-2* — and *DYN2* are encoding for components of the dynein pathway. These data support the hypothesis that even though individual SL interactions are not conserved between *S. cerevisiae* and *C. elegans* more

a)

Interacting gene pair	Gene1		Gene2		Gene1 & 2		p-value	
	BS	ES	BS	ES	BS	ES	BS	ES
mdf-2 + tbg-1	77	95	30	38	12	11	2.23E-03	1.82E-03

b)

Interacting gene pair	Gene1	Gene2	Gene1 & 2	p-value
	Wt	Wt	Wt	Wt
lis-1 + cap-2	100	100	25	4.42E-20

Table 5.7. Quantitative analysis of novel synthetic lethal interactions between *C. elegans* orthologues of *S. cerevisiae* genes

Synthetic lethal phenotypes that were identified between *C. elegans* orthologues of *S. cerevisiae* genes were quantified and subject to statistical analysis under a multiplicative model (Phillips *et al.*, 2000; Puniyani *et al.*, 2004). Percentages of average wild-type brood size ('BS'), and embryonic survival ('ES') rates, respectively (a) and fractions of worms with a wild-type ('Wt') phenotype (b) after simultaneously targeting both genes of a pair by combinatorial RNA interference (RNAi) ('Gene1 & 2') and after RNAi against each gene alone ('Gene1', 'Gene2') are shown. Numbers listed are the arithmetic mean of two independent biological repeats performed in the RNAi-hypersensitive strain *rrf-3*. p-values were assigned by comparing measurements obtained after combinatorial RNAi with the calculated product of measurements for both individual genes using a Student's t-test.

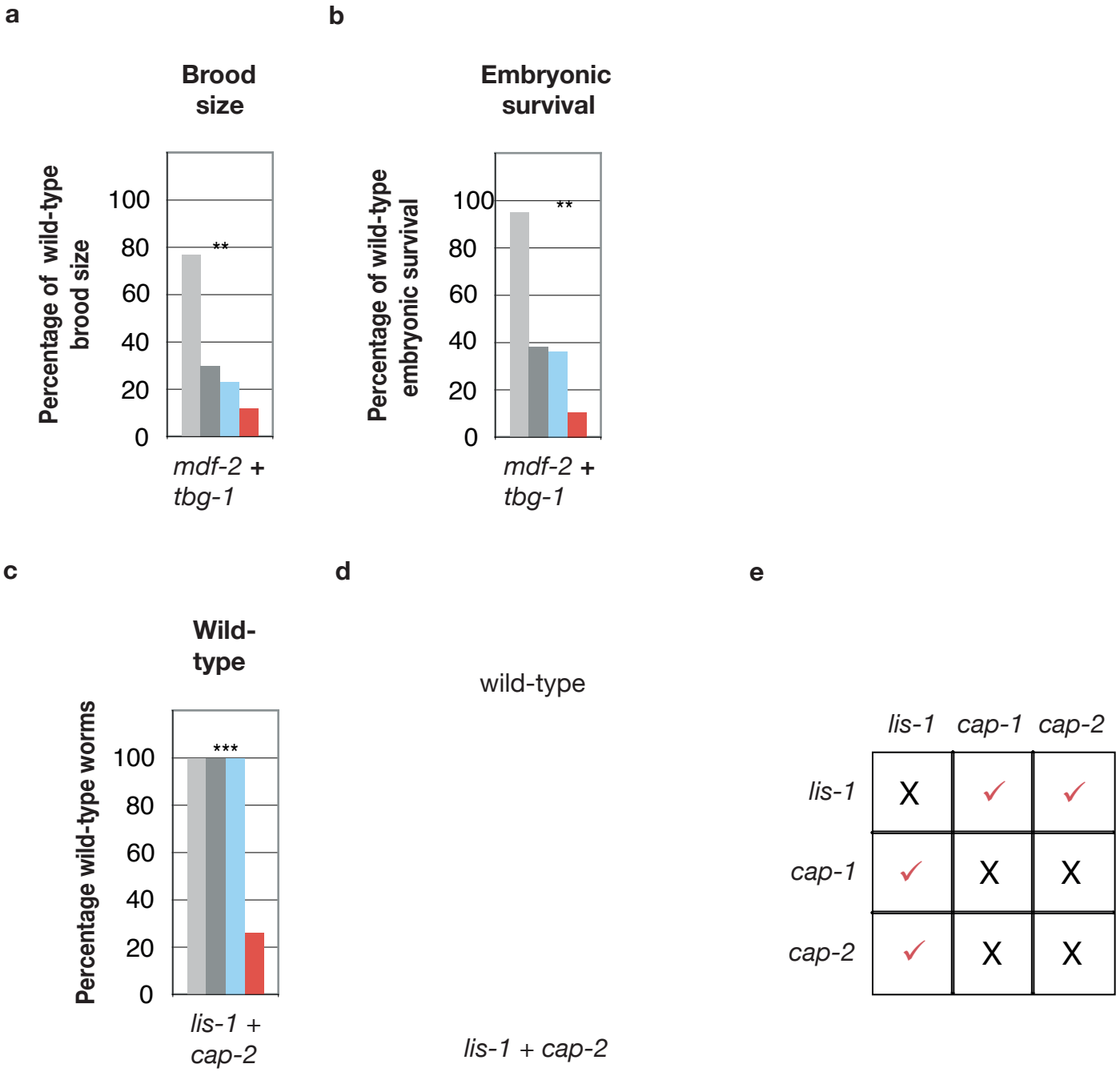

Figure 5.4. Identification of novel synthetic lethal interactions between *C. elegans* orthologues of *S. cerevisiae* genes

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Figure 5.4. Identification of novel synthetic lethal interactions between *C. elegans* orthologues of *S. cerevisiae* genes

Brood size (a) and embryonic survival rates (b) after combinatorial RNA interference (RNAi) against *mdf-2* and *tbg-1* are represented as percentages of typical wild-type measurements. Fraction of wild-type worms after combinatorial RNAi against *lis-1* and *cap-2* are shown (c). Brood size, embryonic survival, and percentages of wild-type worms, respectively, were compared after targeting both genes individually (light- and dark- grey bars), with the values after targeting both genes simultaneously (red bars) and the calculated product of both single-gene measurements (blue bars). Values plotted are the arithmetic mean of two independent experiments. Statistical significance of quantitative phenotype data was assessed under a multiplicative model (Phillips *et al.*, 2000; Puniyani *et al.*, 2004), using the Student's t-test. ***, $P < 1.0E-03$; **, $P < 1.0E-02$. Representation of synthetic adult lethal phenotype generated by the simultaneous targeting of *C. elegans lis-1* and *cap-2* by combinatorial RNAi (d). Scale bars: 0.1 mm. Schematic showing the results of targeting all pairwise combinations of *C. elegans* genes *lis-1*, *cap-1*, and *cap-2* by RNAi (e). ✓ represents a synthetic lethal phenotype. X denotes that combinatorial RNAi phenotypes did not deviate from single-gene RNAi phenotypes. All screens for genetic interactions were performed in the RNAi-hypersensitive *rrf-3* background.

than expected by chance, there might be higher-order similarities in SL interactions between yeast and worm.

Interestingly, a recent study by Cuschieri *et al.* (2006) had uncovered the yeast gene *MAD2* to be synthetic lethal with *TUB4*. These genes are orthologous to *C. elegans mdf-2* and *tbg-1*, respectively, which I had identified as SL gene pair in the worm. Thus, even though on a global scale, SL interactions are not conserved between *S. cerevisiae* and *C. elegans*, isolated individual functional interactions may be conserved throughout evolution.

In summary, while I had found SL interactions to be poorly conserved between *S. cerevisiae* and *C. elegans*, I identified two novel genetic interactions in the worm when screening for SL interactions within *C. elegans* orthologues of *S. cerevisiae* using two genes with roles in mitosis as query genes. One of these, the interaction between *lis-1* and *cap-2* might be similar in function to the genetic interaction between *lis-1* and *cap-1*, which I found to be conserved between yeast and worm. The other one, an interaction between orthologues of the worm genes *mdf-2* and *tbg-1* has lately also been revealed in a small-scale study in the yeast *S. cerevisiae*.

Therefore, extending the search for SL interactions in *C. elegans* between the same set of genes that have been investigated for SL interactions in *S. cerevisiae* to a larger scale might provide further insights into the complexity and global organization of SL interactions in different species.

5.6. Comparison of literature-curated genetic interaction data

Having found that SL interactions between *S. cerevisiae* and *C. elegans* are not conserved more than expected by chance by using a directed systematic approach, I wished to supplement my experimental data with data compiled from literature. I therefore set out to compare all previously known SL data between *S. cerevisiae* and *C. elegans*. To do so, I extracted all 9,175 unique yeast SL interactions from BIOGRID, a database storing genetic interaction data for model organisms (Stark *et al.*, 2006), and all 1,006 known genetic interactions in *C. elegans* from WormBase (www.wormbase.org).

Using the INPARANOID algorithm, I identified single *C. elegans* orthologues for *S. cerevisiae* genes. I found 1,293 pairwise worm orthologues to correlate with yeast SL interactions. None of these gene pairs, however, had been previously shown to genetically interact in *C. elegans*, supporting my experimental data (Table 5.8.).

I further extended this analysis to also include literature data available on genetic interactions in *D. melanogaster* (Crosby *et al.*, 2007). Of 1,575 fly gene pairs that I identified as being orthologous to yeast SL interactions, 3 gene pairs had previously been found to also genetically interact in *D. melanogaster* (see Appendix Table 5.3.).

While I found no overlap between previously known SL interactions in yeast and worm, I next investigated whether I can find an overlap of known genetic interactions between worm and fly. I identified 212 pairwise *D. melanogaster* orthologues corresponding to previously known genetic interactions in *C. elegans*; of these, 23 were reported to also genetically interact in *D. melanogaster* (see Appendix Table 5.3.). However, it has to be noted that the nature of genetic interactions is not specified in the compiled datasets of known genetic interactions both for *C. elegans* and *D. melanogaster*. Hence, these datasets might contain both additive and non-additive genetic interactions. Therefore, the observed higher degree of overlap of genetic interactions between *C. elegans* and *D. melanogaster* than between *S. cerevisiae* and *C. elegans* might — at least in part — be explained by additive genetic interactions.

Moreover, I have to consider likely ascertainment biases in the genes that have been investigated for genetic interactions in either species when comparing literature-curated data that have not been compiled in a comprehensive way. While enormous efforts have been made to map SL interactions on a genome-wide scale in *S. cerevisiae*, SL screens have not yet been extended to genome-scale studies in other model systems. In our laboratory, we have provided the first systematic large-scale analysis of genetic interactions in *C. elegans*. We investigated ~65,000 gene pairs with functions in the signaling and transcriptional networks that regulate development for their ability to genetically interact. Focused small-scale genetic interaction screens have also been performed to gain further insights into DNA repair and posterior patterning in *C. elegans* (Baugh *et al.*, 2005; van Haaften *et al.*, 2004).

S. cerevisiae SL	*C. elegans* orthologous gene pairs	*C. elegans* genetic interactions
9,175	1,293	0

S. cerevisiae SL	*D. melanogaster* orthologous gene pairs	*D. melanogaster* genetic interactions
9,175	1,575	3

C. elegans genetic interactions	*D. melanogaster* orthologous gene pairs	*D. melanogaster* genetic interactions
1,006	212	23

Table 5.8. Comparison of genetic interaction data compiled from literature
Literature-curated synthetic lethal interactions ('SL') from *S. cerevisiae* and previously known genetic interactions between orthologous gene pairs in *C. elegans* and *D. melanogaster* were compared. Numbers for genetic interactions, their respective orthologous gene pairs in another species, and the overlap of genetic interactions are shown.

Thus, while genetic interactions have been mapped on a global scale in yeast, genetic interactions in more complex organisms have — with few exceptions — mostly been compiled on a case-by-case basis. Consequently, even though this comparative study supports my finding that SL interactions between *S. cerevisiae* and *C. elegans* are not conserved more than expected by chance, I cannot make firm conclusions about the considerable degree of conservation of genetic interactions between *C. elegans* and *D. melanogaster*.

5.7. Conclusion

In summary, in this chapter, I have addressed a major open question in genetics: 'Are synthetic lethal interactions evolutionarily conserved?' I set out to investigate whether SL interactions that have recently been mapped on a genome-wide scale in the yeast *S. cerevisiae* are conserved in the nematode *C. elegans*. To do so, I examined whether I can detect SL interactions between pairs of *C. elegans* genes that are orthologous to pairs of genes identified as having SL interactions in at least one of three large-scale screens in *S. cerevisiae*. In total, I investigated 843 pairs of *C. elegans* genes for genetic interactions by using combinatorial RNAi. Of these, I also tested 174 pairs by targeting one gene of a pair by RNAi in a worm strain homozygous for a loss-of-function genetic mutation in the second gene. This was the entire set of yeast SL interactions that could be tested by combinatorial RNAi in *C. elegans* and for which a viable mutant strain was available, respectively. Strikingly, I found that SL interactions are not conserved despite a high degree of conservation of individual gene functions and protein interactions. These results, however, do not rule out the possibility that there might be higher-order similarities in SL interactions between *S. cerevisiae* and *C. elegans*. To test this hypothesis, I extended my search for genetic interactions to all *C. elegans* orthologues of *S. cerevisiae* genes and identified two novel interactions in the worm. I believe that a systematic large-scale analysis of the same set of genes that had been tested for SL interactions in yeast for their ability to genetically interact in the worm will provide deeper insights into the structure and general properties of complex genetic interaction networks.

Together, my findings imply that SL interactions are unlikely to be explained by simple models of redundancy and led me to propose a novel model to explain SL interactions. In this view, synthetic lethality represents a special form of conditional essentiality (‘induced essentiality’). In conclusion, I suggest a substantial evolutionary plasticity in genetic interaction networks.

Chapter 6

Discussion

6.1. Introduction

The availability of whole-genome sequences for numerous model organisms and the development of technological tools for generating loss-of-function phenotypes on a genome-wide scale have given us an unprecedented level of insight into eukaryotic gene function. It was found that inactivation of most genes in *S. cerevisiae*, *C. elegans*, and *D. melanogaster* has little discernable effect on viability under laboratory conditions (Bjorklund *et al.*, 2006; Boutros *et al.*, 2004; Giaever *et al.*, 2002; Kamath *et al.*, 2003). Strikingly, however, inactivating specific rare combinations of such non-essential genes under the exact same conditions can have profound effects on an organism's fitness. Such combinatorial effects are termed 'synthetic enhancement interactions'. Synthetic lethality, where mutation of a gene pair leads to non-viability, while inactivation of each gene individually has no discernible effect, represents the most severe form of synthetic enhancement. Synthetic lethal (SL) genetic interactions are classically interpreted as the result of inactivating two functionally redundant pathways in the cell, either of which is individually dispensable. Recently, enormous progress has been made in the yeast *S. cerevisiae*, where functional genomics tools have been established for the systematic mapping of SL interactions on a genome-wide scale (reviewed in Boone *et al.*, 2007). These studies have identified thousands of genetic interactions in yeast and appear to have uncovered an extensive degree of redundancy. However, similar approaches are not currently feasible in any animal, so alternative strategies are needed.

6.2. Combinatorial RNA interference in *C. elegans*

In the nematode *C. elegans*, RNA-mediated interference (RNAi) by bacterial feeding has emerged as a key technique for the genome-scale analysis of individual gene functions *in vivo* (Timmons and Fire, 1998). So far, however, RNAi has only been used extensively to study the loss-of-function phenotypes of single genes. For the systematic identification of genetic interactions by RNAi, I have established and validated robust methods that allow me to target any pairwise combination of *C. elegans* genes in a high-throughput manner ('combinatorial RNAi'). Using this methodology, I was able to generate loss-of-function phenotypes for two genes in the same animal and to identify the

great majority of previously known SL and synthetic post-embryonic genetic interactions. This approach should therefore allow researchers to explore genetic interactions in the worm in a far more systematic way than has been possible in the past.

6.3. Functional redundancy between *C. elegans* gene duplicates can be maintained for extensive evolutionary timescales

I used combinatorial RNAi to begin to investigate functional redundancy in the genome of *C. elegans*. One obvious cause of functional redundancy is gene duplication; duplicated genes that have retained overlapping functions can compensate for inactivation of one another (Force *et al.*, 1999; Lynch and Force, 2000).

Focusing on *C. elegans* genes that correspond to single orthologues in *S. cerevisiae* or *D. melanogaster* genomes, I have provided the first systematic experimental investigation into the redundancy of duplicated genes in any organism. I have identified 16 out of 143 *C. elegans* duplicate gene pairs to be at least partially functionally redundant. Intriguingly, the majority of these redundant gene pairs also do exist as gene duplicates in the related nematode *C. briggsae*, suggesting that these genes have been duplicated in the genome of *C. elegans* before the split from *C. briggsae* more than 80 million years ago. Thus, my findings strongly support models of gene evolution that suggest that redundancy is not just a transient side effect of recent gene duplication, but is instead a phenomenon that can be maintained over substantial periods of evolutionary time.

6.4. Higher-order redundancy in genetic interaction networks

While I have identified functional redundancy between gene duplicates, most functional redundancy in genetic networks tends to be more complex. The majority of genes that were identified as having SL interactions in genome-scale screens in *S. cerevisiae* do not share sequence similarity, unlike gene duplicates, but rather occur amongst functionally unrelated genes (Tong *et al.*, 2004). This ‘higher-order’ redundancy appears to mechanistically differ from genuine functional redundancy, the redundancy of

gene duplicates. I like to picture this higher-order redundancy as a car. It is possible to tolerate loss of one or other function (i.e. one would be able to prevent an accident, if either the brakes or the steering wheel break), but loss of both functions is catastrophic (i.e. it is probably impossible to direct a car if both brakes and steering wheel are dysfunctional). However, while two functions (i.e. brakes and steering wheel) can somehow compensate for loss of one another, they do not so by simply fulfilling one another's genuine function (i.e. one is not able to steer a car by using the brakes).

6.5. Evolutionary plasticity of genetic interaction networks

In this work, I sought to address a fundamental question in genetics: 'Are SL interactions and thus functional redundant relationships evolutionarily conserved?' I therefore set out to investigate whether SL interactions identified in the yeast *S. cerevisiae* are conserved in the nematode *C. elegans*. I used RNAi to test whether I can detect SL interactions between pairs of *C. elegans* genes that are orthologous to pairs of genes identified as having SL interactions in one of three genome-scale screens in *S. cerevisiae* (Davierwala *et al.*, 2005; Pan *et al.*, 2006; Tong *et al.*, 2004). In total, I screened 843 pairs of *C. elegans* genes for non-additive, synthetic genetic interactions by using combinatorial RNAi. Of these, I also tested 174 pairs by targeting one gene of a pair by RNAi in a worm strain homozygous for a loss-of-function genetic mutation in the second gene; this was the entire set of yeast SL interactions that could be tested by combinatorial RNAi in *C. elegans* and for which a viable mutant strain was available, respectively.

Strikingly, only 6/843 (0.7%) of the tested gene pairs that were SL in *S. cerevisiae* also resulted in a synthetic viability defect in *C. elegans*. This is not significantly different to the frequency of SL interactions that we have detected by systematically investigating ~65,000 *C. elegans* gene pairs with roles in signal transduction and transcription for their ability to genetically interact. Thus, these findings demonstrate that individual SL interactions are not conserved between *S. cerevisiae* and *C. elegans* more than expected by chance.

Moreover, this observed interaction frequency does also not differ from the average interaction density in yeast gene networks. Hence, this non-conservation of genetic interactions between *S. cerevisiae* and *C. elegans* cannot simply be explained by a reduction in the number of SL interactions.

The observed non- conservation of SL interactions between yeast and worms is in marked contrast to the conservation of single gene functions. Using the same experimental platform, I identified 61% of *C. elegans* genes corresponding to an essential gene in yeast to show a non-viable RNAi phenotype, suggesting that these genes also play an essential role in the worm. Moreover, I found 28% of *C. elegans* gene duplicates related to an essential gene in yeast to have a SL RNAi phenotype. Furthermore, 31% of a test set of protein interactions were shown to be conserved between yeast and worm (Matthews *et al.*, 2001). I thus conclude that while the knowledge of an essential gene function in yeast can strongly predict the essential function of an orthologous gene in the worm, and also — albeit to a lesser extent — the essential function covered by a pair of duplicated genes in the genome of *C. elegans*, SL interactions identified in yeast cannot be used to directly predict candidate genetic interactions in the worm.

6.6. Non-conservation of synthetic lethal interactions and its implications for multigenic human disease

Most obviously, if SL interactions are not conserved between yeast and worm, it is highly unlikely that they will be conserved between yeast and human. Thus, while identifying the function of a single gene in yeast is likely to be predictive of the function of its orthologue in humans, one cannot transfer genetic interactions between species so directly. For example, it is highly unlikely that yeast SL data can be used to directly identify genes that when inhibited will selectively kill cancer cells carrying a mutation in a tumour suppressor gene (Kaelin, 2005). Considering that increasing numbers of human diseases are identified as resulting from combinations of mutations in multiple genes that alone have little effect (reviewed in Badano and Katsanis, 2002), alternative integrated approaches will be required to predict modifier genes in complex genetic diseases in humans.

6.7. Synthetic lethal interactions and predictions of gene functions

Finally, I want to emphasize that although I have shown here that SL interactions are not conserved between yeast and a multicellular organism more than expected by chance, genetic interaction screens in *S. cerevisiae* are nonetheless informative for understanding multicellular biology. Clustering yeast genes according to their profiles of genetic interactions is a very powerful method for defining their precise molecular functions (Wong *et al.*, 2004). Thus, despite the lack of direct conservation of SL interactions between yeast and animals, SL screens in yeast are still highly informative for understanding general principles of how genes combine in non-additive modes. (Collins *et al.*, 2007; Pan *et al.*, 2006; Schuldiner *et al.*, 2005; Tong *et al.*, 2004; Wong *et al.*, 2004).

6.8. 'Induced essentiality' model for the interpretation of synthetic lethal interactions

Beyond the direct practical implications for the use of SL interaction data, my findings led me to suggest a novel model for the interpretation of SL interactions. In the classic model, SL interactions between two genes (gene A and B) are considered to be the result of inactivating two functionally redundant genes or pathways in which the genes act (reviewed in Guarente, 1993; Hartman *et al.*, 2001). However, I suggest that there is an alternative explanation for SL interactions, which I have termed 'induced essentiality'. In this view, the phenomenon of synthetic lethality is considered a side-effect of the evolution of adaptive responses to different environmental conditions. In my proposed model, loss of gene A induces the genetic network to rearrange so the organism's viability is maintained. In this novel network, gene B becomes indispensable. Thus, inactivating both gene A and gene B results in synthetic lethality, without gene A and gene B being functionally redundant. I believe that my finding that SL interactions are not conserved favours the 'induced essentiality' model.

6.9. Conclusion

In summary, during my PhD training, I have established robust methods (‘combinatorial RNAi’) to study genetic interactions between any pair of genes in *C. elegans*. I used this technique to provide the first extensive systematic analysis in any organism of the potentially redundant functions of duplicated genes and found that redundancy between some *C. elegans* gene duplicates has been maintained for long evolutionary timescales. When investigating whether SL interactions are conserved between yeast and worms, I found that genetic interaction networks evolve much faster than both the functions of individual genes and protein-protein interaction networks.

I thus consider the current hypothesis that SL interactions represent functional redundant relationships to be very unlikely. Instead, I propose a novel model for the interpretation of SL interactions. In this view, SL interactions are suggested to represent a special form of conditional essentiality (‘induced essentiality’).

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Appendix

Appendix Table 3.1. ***C. elegans*** **chromosome III genes with previously known RNA interference phenotypes**

CE RNAi clone	Pheno Screen 1	Pheno Screen 2	Pheno Kamath *et al.*, 2003
F54C4.1	0	NonV	NonV
F54C4.3	NonV	NonV	Gro
C29F9.1	Gro	0	Gro
C29F9.7	NonV	NonV	NonV
F40G9.1	NonV	NonV	Gro
W10C4.b	NonV	NonV	Gro
W07B3.2	NonV	NonV	NonV
F10C5.1	NonV	NonV	NonV
F10C5.2	NonV	NonV	NonV
F30H5.1	NonV	NonV	NonV
W06E11.1	NonV	NonV	NonV
T17H7.5	NonV	NonV	NonV
B0412.4	NonV	NonV	NonV
T24C4.5	NonV	NonV	NonV
F23H11.5	NonV	NonV	NonV
F58B6.3	NonV	NonV	NonV
Y71D11A.b	NonV	NonV	NonV
F53A3.3	NonV	NonV	NonV
W04B5.4	0	Gro	NonV
H19M22.1	NonV	NonV	NonV
H19M22.2	NonV	NonV	Pep
H19M22.3	NonV	NonV	NonV
H06I04.a	NonV	NonV	NonV
H06I04.f	NonV	NonV	NonV
H06I04.h	NonV	NonV	Gro
H06I04.i	NonV	NonV	Gro
R148.7	0	0	Gro
F59A2.1	NonV	NonV	NonV
C34C12.8	NonV	NonV	NonV
M01F1.3	NonV	NonV	NonV
C54C6.1	NonV	NonV	NonV
C32A3.1	NonV	NonV	NonV
R13G10.1	NonV	NonV	Pep
C36A4.4	0	0	NonV
F13B10.2	NonV	NonV	NonV
ZK1058.2	NonV	NonV	NonV
C36E8.5	NonV	NonV	NonV
T02C12.2	NonV	NonV	Gro
E03A3.3	NonV	NonV	NonV
C03C10.3	NonV	NonV	NonV
C16C10.2	NonV	NonV	Gro
C16C10.6	NonV	NonV	NonV
R74.1	NonV	NonV	NonV
F43C1.2	NonV	NonV	NonV
F43C1.3	NonV	Gro	Gro
F43C1.5	0	Gro	NonV
Y44F5A.1	NonV	NonV	Gro
T08A11.2	NonV	NonV	NonV
R10E4.4	NonV	NonV	NonV
H38K22.2	0	Gro	NonV
B0285.1	NonV	NonV	Gro
B0285.2	NonV	NonV	NonV
R07E5.1	0	Gro	Pep
R07E5.3	NonV	NonV	NonV
R07E5.7	Gro	Gro	Gro
R07E5.10	NonV	NonV	NonV
R07E5.14	NonV	NonV	NonV
F56F3.2	NonV	Gro	NonV
F56F3.5	NonV	NonV	NonV
C07G2.3	NonV	NonV	NonV
M88.2	Gro	0	NonV
M88.6	NonV	NonV	Pep
F35G12.8	NonV	NonV	NonV
F35G12.10	NonV	0	NonV
T04A8.6	NonV	NonV	NonV
T04A8.7	NonV	0	NonV

T04A8.11	NonV	0	NonV
B0393.1	NonV	NonV	NonV
B0393.6	NonV	0	NonV
C38D4.3	NonV	0	NonV
C38D4.6	NonV	NonV	NonV
C35D10.1	0	NonV	NonV
C35D10.5	Gro	0	NonV
C35D10.6	Gro	0	Pep
C35D10.13	0	0	NonV
F26F4.10	NonV	NonV	NonV
F26F4.11	NonV	NonV	NonV
C26E6.4	NonV	NonV	NonV
C26E6.6	NonV	NonV	NonV
C26E6.8	0	0	NonV
C27F2.4	0	0	Gro
C27F2.7	Gro	Gro/Pep	NonV
C27F2.8	NonV	NonV	Gro
R144.2	NonV	NonV	NonV
R144.3	NonV	0	NonV
R144.7	Gro	0	NonV
C45G9.5	NonV	NonV	NonV
F54D8.1	NonV	NonV	Pep
F21H11.4	0	0	NonV
T10F2.1	NonV	NonV	NonV
T10F2.3	Gro	0	Pep
T10F2.4	NonV	NonV	NonV
K10D2.2	Gro	Gro	Gro
K10D2.4	0	Gro	NonV
K10D2.6	NonV	NonV	NonV
C34E10.1	0	0	NonV
C34E10.2	0	0	NonV
C34E10.4	Gro	0	Gro
C34E10.6	NonV	NonV	NonV
ZC395.3	Gro	0	Gro
ZC395.8	0	0	Pep
F48E8.2	0	0	NonV
F48E8.5	0	0	NonV
R02F2.7	NonV	NonV	Gro
F09F7.2	Pep	0	Pep
F09F7.3	NonV	NonV	NonV
F56D2.1	NonV	NonV	NonV
F56D2.6	NonV	NonV	NonV
F54E7.2	NonV	NonV	NonV
F54E7.3	NonV	NonV	NonV
F54E7.4	0	0	NonV
B0336.1	0	0	NonV
B0336.2	NonV	NonV	NonV
B0336.3	Gro	0	Gro
B0336.6	NonV	NonV	NonV
B0336.10	NonV	NonV	NonV
R12B2.1	0	Pep	Pep
R12B2.4	NonV	NonV	NonV
R12B2.5	NonV	NonV	NonV
F01F1.7	NonV	NonV	NonV
F01F1.8	NonV	NonV	NonV
F01F1.12	0	NonV	NonV
C28H8.6	NonV	NonV	NonV
F25B5.4	NonV	NonV	NonV
ZK328.1	0	0	NonV
ZK328.2	NonV	NonV	NonV
ZK328.5	NonV	NonV	NonV
T17E9.1	Gro	Gro	NonV
T17E9.2	NonV	NonV	NonV
Y42G9A.c	Gro	nd	NonV
C23G10.3	NonV	NonV	NonV
C23G10.4	NonV	NonV	NonV
C23G10.8	NonV	NonV	NonV
C23G10.9	NonV	NonV	NonV
T12A2.2	NonV	NonV	NonV

T12A2.7	0	0	NonV
F47D12.4	NonV	NonV	Pep
C56G2.2	0	0	NonV
C56G2.6	NonV	NonV	NonV
C16A3.3	NonV	NonV	Gro
C16A3.4	NonV	NonV	NonV
C16A3.5	NonV	NonV	NonV
C16A3.6	NonV	NonV	NonV
C16A3.9	NonV	NonV	NonV
C05D11.2	0	0	NonV
C05D11.3	0	0	NonV
C05D11.9	0	0	Gro
C05D11.10	0	0	NonV
C05D11.11	0	Gro	NonV
C05D11.12	NonV	NonV	NonV
T26A5.3	NonV	NonV	NonV
T26A5.7	Gro	NonV	Pep
T26A5.9	NonV	NonV	NonV
F23F12.2	0	0	NonV
F23F12.6	NonV	NonV	NonV
F20H11.2	NonV	NonV	Gro
F20H11.3	Gro	0	NonV
F20H11.6	NonV	0	Gro
C13B9.3	NonV	NonV	NonV
F37A4.8	Gro	Gro	NonV
R13F6.1	NonV	NonV	NonV
R13F6.9	Pep	Pep	Pep
R13F6.10	0	0	NonV
K04C2.2	NonV	NonV	Gro
F57B9.2	NonV	NonV	NonV
F57B9.3	NonV	NonV	NonV
F57B9.5	NonV	NonV	NonV
F57B9.6	NonV	NonV	NonV
F57B9.10	NonV	NonV	NonV
F31E3.1	NonV	Gro	Pep
F31E3.3	0	0	NonV
F11H8.4	NonV	NonV	NonV
B0280.9	NonV	NonV	Gro
K04G7.1	NonV	NonV	NonV
K04G7.4	NonV	NonV	NonV
F37C12.1	NonV	NonV	NonV
F37C12.3	Gro	Gro	NonV
F37C12.4	NonV	NonV	NonV
F37C12.9	NonV	NonV	NonV
F37C12.11	NonV	NonV	NonV
F37C12.13	NonV	NonV	NonV
R151.3	NonV	NonV	NonV
R151.9	0	NonV	NonV
T20H4.3	NonV	NonV	NonV
T20H4.5	NonV	NonV	NonV
B0361.5	0	0	Gro
B0361.6	0	0	Gro
B0361.8	NonV	NonV	Gro
B0361.10	NonV	NonV	NonV
F08F8.2	NonV	NonV	NonV
T20B12.1	NonV	NonV	NonV
T20B12.2	NonV	NonV	NonV
T20B12.3	NonV	NonV	Gro
T20B12.7	0	0	NonV
T20B12.8	NonV	NonV	NonV
H14A12.2	0	NonV	NonV
H14A12.6	NonV	NonV	Pep
K07D8.1	NonV	NonV	Pep
C07H6.2	0	0	Gro
C07H6.5	NonV	NonV	NonV
C07H6.7	0	0	Pep
R13A5.5	0	Gro	Pep
R13A5.8	NonV	NonV	NonV
R13A5.12	NonV	NonV	NonV

R13A5.13	NonV	NonV	NonV
ZK783.1	NonV	NonV	Pep
ZK686.1	NonV	NonV	Gro
ZK686.2	NonV	NonV	Gro
ZK686.3	NonV	NonV	NonV
C08C3.4	0	NonV	NonV
C27D11.1	NonV	NonV	NonV
ZK652.1	NonV	NonV	NonV
ZK652.4	NonV	NonV	NonV
C02C2.6	0	0	Pep
C29E4.2	NonV	NonV	NonV
C29E4.8	NonV	NonV	NonV
F54H12.1	NonV	NonV	NonV
F54H12.6	NonV	NonV	NonV
F44B9.7	0	Gro	NonV
K12H4.1	Pep	Pep	Pep
K12H4.3	NonV	NonV	NonV
K12H4.4	NonV	NonV	NonV
K12H4.5	NonV	NonV	NonV
K06H7.1	NonV	NonV	NonV
K06H7.6	NonV	0	NonV
C14B9.4	NonV	NonV	NonV
C14B9.7	NonV	NonV	NonV
D2007.4	0	0	NonV
C30A5.7	0	0	Pep
C02F5.1	NonV	NonV	NonV
C02F5.9	NonV	NonV	NonV
F09G8.3	NonV	NonV	NonV
F10E9.4	NonV	NonV	Gro
F10E9.7	NonV	NonV	NonV
F10E9.8	NonV	NonV	NonV
R05D3.4	NonV	NonV	Gro
R05D3.7	Gro	Gro	Pep
ZK1236.3	NonV	NonV	NonV
ZK1236.5	0	NonV	Gro
C30C11.1	NonV	0	NonV
C30C11.2	NonV	NonV	NonV
C30C11.4	NonV	NonV	NonV
C06E1.10	NonV	NonV	NonV
F22B7.5	NonV	NonV	NonV
B0303.5	NonV	0	Gro
B0303.15	NonV	0	NonV
K02D10.5	NonV	NonV	NonV
F54F2.1	NonV	NonV	Pep
F54F2.8	Gro	Gro	Gro
ZK637.7	0	Gro	Gro
ZK637.8	NonV	NonV	NonV
R08D7.1	NonV	NonV	NonV
R08D7.2	NonV	0	NonV
R08D7.3	NonV	NonV	NonV
R107.6	0	NonV	NonV
R107.8	NonV	NonV	Pep
F02A9.2	NonV	0	Pep
F02A9.4	0	0	NonV
F02A9.6	NonV	NonV	NonV
T23G5.1	NonV	NonV	NonV
K04H4.1	NonV	NonV	NonV
C38C10.4	NonV	NonV	NonV
T26G10.1	NonV	NonV	NonV
F54C8.2	NonV	NonV	NonV
F54C8.3	NonV	0	NonV
F54C8.5	0	Gro	Pep
B0464.1	NonV	NonV	NonV
B0464.7	NonV	NonV	NonV
ZK1098.7	0	NonV	NonV
C48B4.9	NonV	NonV	Gro
F58A4.3	NonV	NonV	NonV
F58A4.4	NonV	NonV	NonV
F58A4.8	NonV	NonV	NonV

F58A4.11	NonV	NonV	Pep
C07A9.2	NonV	NonV	NonV
C07A9.3	NonV	NonV	NonV
T05G5.3	NonV	NonV	NonV
T05G5.6	0	Gro	Gro
T05G5.10	0	NonV	NonV
R10E11.1	NonV	NonV	NonV
R10E11.2	NonV	NonV	NonV
R10E11.8	NonV	NonV	NonV
ZK632.1	NonV	NonV	NonV
ZK632.2	0	0	Pep
K03H1.2	NonV	NonV	NonV
T16G12.5	NonV	NonV	Pep
T16H12.4	NonV	0	NonV
ZK1128.3	NonV	NonV	Gro
ZK1128.5	0	0	Pep
T20G5.1	NonV	NonV	NonV
T20G5.2	NonV	NonV	NonV
T20G5.3	NonV	NonV	NonV
T20G5.6	Pep	0	Pep
R01H10.1	NonV	NonV	NonV
T07C4.1	NonV	0	Gro
T07C4.7	Gro	Gro	NonV
M03C11.7	NonV	NonV	NonV
D2045.1	NonV	0	NonV
D2045.6	NonV	NonV	NonV
F43D9.3	NonV	NonV	NonV
F43D9.5	0	0	Gro
W09D10.1	NonV	Gro	Gro
W09D10.3	0	0	Gro
K01G5.1	NonV	NonV	NonV
K01G5.4	NonV	NonV	NonV
K01G5.7	NonV	NonV	NonV
Y39A1B.3	NonV	Gro/Pep	NonV
K11D9.1	0	0	NonV
K11D9.2	NonV	NonV	NonV
M142.4	Gro	Gro	Pep
M142.6	Gro	Gro	Gro
Y48A6B.3	Gro	0	Gro
Y48A6B.5	0	0	Gro
Y48A6B.11	0	0	NonV
Y48A6C.4	NonV	0	Gro
Y47D3A.aa	Gro	NonV	Gro
Y47D3A.c	NonV	NonV	NonV
Y47D3A.d	NonV	NonV	NonV
Y47D3A.l	0	0	Pep
Y47D3B.7	NonV	NonV	NonV
Y47D3B.10	0	0	Pep
Y66A7A.8	NonV	NonV	NonV
Y41C4A.9	0	0	Pep
Y41C4A.10	0	0	NonV
C24H11.6	0	0	Gro
C24H11.7	NonV	NonV	NonV
C18D11.4	NonV	NonV	Gro
Y56A3A.18	NonV	NonV	Gro
Y56A3A.19	0	0	Gro
Y56A3A.32	0	0	NonV
Y75B8A.2	NonV	NonV	NonV
Y75B8A.7	NonV	NonV	Gro
Y75B8A.25	Gro	Gro	Pep
Y49E10.1	NonV	NonV	NonV
Y49E10.2	0	0	NonV
Y49E10.6	NonV	NonV	NonV
Y49E10.14	NonV	NonV	NonV
Y49E10.15	NonV	NonV	NonV
Y49E10.19	NonV	NonV	NonV
Y49E10.21	NonV	NonV	NonV
Y111B2C.e	NonV	NonV	NonV
Y111B2D.b	NonV	NonV	NonV

Y111B2D.c	NonV	0	Pep
Y111B2D.h	NonV	NonV	NonV
Y37D8A.1	NonV	Pep	Pep
Y37D8A.9	0	0	Pep
Y37D8A.10	NonV	NonV	NonV
Y37D8A.14	NonV	NonV	NonV
Y37D8A.16	NonV	NonV	Gro
Y37D8A.18	NonV	0	NonV
ZK1010.1	NonV	NonV	NonV
ZK1010.3	Gro	Gro	Gro
Y39E4B.1	NonV	NonV	NonV
F56A8.6	NonV	NonV	NonV
Y43F4B.5	NonV	NonV	Gro
Y43F4B.6	NonV	NonV	NonV
F53A2.4	NonV	0	NonV
T03F6.5	NonV	0	NonV
F45G2.5	NonV	NonV	Pep
F45G2.11	NonV	NonV	Pep
Y76A2A.2	NonV	NonV	Pep
T27E9.1	NonV	NonV	NonV
Y76A2B.1	NonV	NonV	NonV
T05D4.4	NonV	NonV	Gro
T25C8.2	NonV	NonV	NonV
T12D8.1	NonV	NonV	NonV
T12D8.6	NonV	NonV	NonV
T12D8.7	0	NonV	NonV
ZK520.1	0	0	Gro
W06F12.1	NonV	NonV	NonV
K08E3.5	NonV	NonV	NonV
K08E3.6	NonV	NonV	NonV
C37G2.7	NonV	NonV	NonV
Y119D3_444.b	NonV	NonV	NonV
Y119D3_446.a	NonV	NonV	NonV
Y119D3_446.c	NonV	NonV	NonV
Y119D3_456.a	NonV	NonV	Gro
Y53G8A_9248.c	NonV	NonV	NonV
Y53G8A_9248.d	0	Gro	NonV
Y53G8B_93.d	NonV	NonV	Gro
Y53G8B_1025.a	NonV	NonV	NonV
Y53G8B_1025.b	NonV	NonV	NonV
Y55B1A_115.c	NonV	0	NonV
Y55B1A_115.e	NonV	NonV	Pep
Y71H2_375.b	NonV	NonV	NonV
Y71H2_378.a	NonV	NonV	NonV
Y71H2_381.a	0	0	Gro
Y71H2_388.c	NonV	NonV	NonV
Y71H2_388.d	NonV	NonV	NonV
Y71H2_388.f	0	0	Pep
Y71H2_389.e	NonV	NonV	NonV

Appendix Table 3.1. *C. elegans* chromosome III genes with previously known RNA interference phenotypes
For each gene on *C. elegans* chromosome III with a previously known RNA interference (RNAi) phenotype (Kamath *et al.*, 2003), the Ahringer library RNAi clone gene pairs name ('CE RNAi clone'), the phenotypes observed in this study in two independent experimental setups ('Pheno Screen 1, 'Pheno Screen 2') by using the high-throughput RNAi liquid feeding assay and the RNAi-hypersensitive strain *rrf-3*), and the phenotype observed in the plate feeding screen performed by Kamath *et al.* (Pheno Kamath *et al.*, 2003), are shown. NonV, non-viable RNAi phenotype (sterility and embryonic lethality, respectively). Gro, slowed post-embryonic growth. Pep, defect in post-embryonic development. 0, no RNAi phenotype observed.

Appendix Table 3.2. ***C. elegans*** **chromosome III genes with non-viable RNA interference phenotypes**

non-targeting (Ahringer library clone Y95B8A_84.g)

CE RNAi clone	Emb	Ste	Lga	Weaker Pheno	No Pheno
F54C4.1	0	0	0	0	0
C29F9.7	0	3	0	3-fold	10-fold
W07B3.2	0	3	0	10-fold	0
F10C5.1	0	2	0	2-fold	4-fold
F10C5.2	0	2	0	4-fold	5-fold
F30H5.1	0	3	0	10-fold	0
W06E11.1	0	2	0	4-fold	10-fold
T17H7.5	0	0	1	2-fold	10-fold
B0412.4	0	0	1	2-fold	0
T24C4.5	0	3	0	2-fold	0
F23H11.5	0	3	0	2-fold	10-fold
F58B6.3	2	2	0	2-fold	0
Y71D11A.b	0	0	1	4-fold	0
F53A3.3	0	0	1	2-fold	0
W04B5.4	0	0	0	0	0
H19M22.1	0	0	1	2-fold	0
H19M22.3	0	3	0	2-fold	0
H06I04.a	0	3	0	5-fold	0
H06I04.f	0	0	1	2-fold	0
F59A2.1	0	3	0	2-fold	10-fold
C34C12.8	0	2	0	3-fold	4-fold
M01F1.3	0	3	0	2-fold	5-fold
C54C6.1	0	0	1	2-fold	0
C32A3.1	2	2	0	2-fold	4-fold
C36A4.4	0	0	0	0	0
F13B10.2	0	0	1	5-fold	0
ZK1058.2	0	0	1	3-fold	0
C36E8.5	0	3	0	3-fold	0
E03A3.3	0	3	0	2-fold	10-fold
C03C10.3	0	3	0	3-fold	0
C16C10.6	3	2	0	2-fold	10-fold
R74.1	0	3	0	2-fold	10-fold
F43C1.2	0	2	0	2-fold	3-fold
F43C1.5	0	0	0	0	0
T08A11.2	0	0	1	2-fold	10-fold
R10E4.4	0	3	0	2-fold	10-fold
H38K22.2	0	0	0	0	0
B0285.2	2	2	0	0	2-fold
R07E5.3	0	3	0	2-fold	10-fold
R07E5.10	0	2	0	2-fold	4-fold
R07E5.14	0	3	0	2-fold	10-fold
F56F3.2	2	2	0	0	2-fold
F56F3.5	0	0	1	10-fold	0
C07G2.3	0	0	1	3-fold	0
M88.2	0	0	0	0	0
F35G12.8	0	3	0	3-fold	0
F35G12.10	0	2	0	0	2-fold
T04A8.6	0	3	0	2-fold	5-fold
T04A8.7	1	1	0	3-fold	5-fold
T04A8.11	0	2	0	0	2-fold
B0393.1	0	0	1	5-fold	0
B0393.6	0	1	0	0	2-fold
C38D4.3	1	2	0	2-fold	3-fold
C38D4.6	3	0	0	2-fold	0
C35D10.1	0	0	0	0	0
C35D10.5	0	0	0	0	0
C35D10.13	0	0	0	0	0
F26F4.10	0	2	0	2-fold	3-fold
F26F4.11	0	2	0	0	3-fold
C26E6.4	0	0	1	2-fold	0
C26E6.6	0	2	0	0	2-fold
C26E6.8	0	0	0	0	0
C27F2.7	0	0	0	0	0
R144.2	0	0	1	2-fold	10-fold

R144.3	0	1	0	0	2-fold
R144.7	0	0	0	0	0
C45G9.5	0	2	0	2-fold	4-fold
F21H11.4	0	0	0	0	0
T10F2.1	0	0	1	2-fold	0
T10F2.4	3	2	0	2-fold	10-fold
K10D2.4	0	0	0	0	0
K10D2.6	2	2	0	2-fold	5-fold
C34E10.1	0	0	0	0	0
C34E10.2	0	0	0	0	0
C34E10.6	0	0	1	4-fold	0
F48E8.2	0	0	0	0	0
F48E8.5	0	0	0	0	0
F09F7.3	0	3	0	2-fold	10-fold
F56D2.1	0	3	0	2-fold	5-fold
F56D2.6	0	3	0	2-fold	3-fold
F54E7.2	0	3	0	2-fold	3-fold
F54E7.3	3	2	0	2-fold	0
F54E7.4	0	0	0	0	0
B0336.1	0	0	0	0	0
B0336.2	0	3	0	2-fold	3-fold
B0336.6	0	2	0	2-fold	4-fold
B0336.10	0	0	1	10-fold	0
R12B2.4	0	2	0	2-fold	3-fold
R12B2.5	0	0	1	2-fold	0
F01F1.7	2	2	0	2-fold	4-fold
F01F1.8	0	0	1	2-fold	10-fold
F01F1.12	0	0	0	0	0
C28H8.6	2	2	0	2-fold	4-fold
F25B5.4	0	0	1	0	0
ZK328.1	0	0	0	0	0
ZK328.2	0	0	1	2-fold	0
ZK328.5	0	0	1	4-fold	0
T17E9.1	0	0	0	0	0
T17E9.2	0	2	0	0	2-fold
Y42G9A.c	0	0	0	0	0
C23G10.3	0	0	1	3-fold	0
C23G10.4	0	0	1	0	0
C23G10.8	0	3	0	10-fold	0
C23G10.9	0	3	0	2-fold	4-fold
T12A2.2	0	0	1	2-fold	10-fold
T12A2.7	0	0	0	0	0
C56G2.2	0	0	0	0	0
C56G2.6	0	0	1	5-fold	0
C16A3.4	0	2	0	0	5-fold
C16A3.5	0	2	0	0	3-fold
C16A3.6	0	3	0	2-fold	4-fold
C16A3.9	0	0	1	2-fold	10-fold
C05D11.2	0	0	0	0	0
C05D11.3	0	0	0	0	0
C05D11.10	0	0	0	0	0
C05D11.11	0	0	0	0	0
C05D11.12	2	0	0	2-fold	3-fold
T26A5.3	0	2	0	0	3-fold
T26A5.9	2	2	0	2-fold	10-fold
F23F12.2	0	0	0	0	0
F23F12.6	0	0	1	10-fold	0
F20H11.3	0	0	0	0	0
C13B9.3	0	0	1	10-fold	0
F37A4.8	0	0	0	0	0
R13F6.1	2	2	0	2-fold	3-fold
R13F6.10	0	0	0	0	0
F57B9.2	0	0	1	4-fold	0
F57B9.3	0	0	1	2-fold	5-fold
F57B9.5	0	3	0	2-fold	4-fold
F57B9.6	0	0	1	3-fold	10-fold
F57B9.10	0	0	1	10-fold	0
F31E3.3	0	0	0	0	0
F11H8.4	0	2	0	0	3-fold

K04G7.1	0	2	0	3-fold	4-fold
K04G7.4	0	2	0	0	10-fold
F37C12.1	2	2	0	2-fold	3-fold
F37C12.3	0	0	0	0	0
F37C12.4	0	3	0	0	2-fold
F37C12.9	0	0	1	5-fold	0
F37C12.11	0	0	1	3-fold	0
F37C12.13	2	2	0	2-fold	4-fold
R151.3	0	0	1	2-fold	0
R151.9	0	0	0	0	0
T20H4.3	0	0	1	4-fold	0
T20H4.5	0	2	0	0	2-fold
B0361.10	0	2	0	2-fold	3-fold
F08F8.2	0	2	0	0	2-fold
T20B12.1	0	2	0	0	5-fold
T20B12.2	0	2	0	0	3-fold
T20B12.7	0	0	0	0	0
T20B12.8	0	2	0	0	5-fold
H14A12.2	0	0	0	0	0
C07H6.5	0	2	0	5-fold	10-fold
R13A5.8	0	0	1	5-fold	0
R13A5.12	0	3	0	3-fold	0
R13A5.13	0	3	0	3-fold	0
ZK686.3	0	2	0	3-fold	4-fold
C08C3.4	0	0	0	0	0
C27D11.1	0	0	1	3-fold	0
ZK652.1	0	0	1	2-fold	10-fold
ZK652.4	0	0	1	3-fold	0
C29E4.2	0	3	0	3-fold	0
C29E4.8	0	2	0	3-fold	10-fold
F54H12.1	0	2	0	2-fold	5-fold
F54H12.6	0	2	0	3-fold	4-fold
F44B9.7	0	0	0	0	0
K12H4.3	0	3	0	2-fold	3-fold
K12H4.4	0	2	0	2-fold	3-fold
K12H4.5	0	2	0	2-fold	3-fold
K06H7.1	0	3	0	2-fold	0
K06H7.6	0	2	0	2-fold	3-fold
C14B9.4	0	3	0	2-fold	0
C14B9.7	0	0	1	5-fold	0
D2007.4	0	0	0	0	0
C02F5.1	0	3	0	4-fold	0
C02F5.9	0	0	1	3-fold	0
F09G8.3	0	2	0	0	3-fold
F10E9.7	0	0	1	2-fold	10-fold
F10E9.8	2	2	0	2-fold	3-fold
ZK1236.3	0	3	0	3-fold	10-fold
C30C11.1	0	2	0	0	2-fold
C30C11.2	0	0	1	3-fold	0
C30C11.4	0	2	0	3-fold	4-fold
C06E1.10	0	2	0	2-fold	5-fold
F22B7.5	0	2	0	0	2-fold
B0303.15	0	2	0	0	2-fold
K02D10.5	0	0	1	4-fold	10-fold
ZK637.8	0	3	0	2-fold	10-fold
R08D7.1	2	2	0	2-fold	4-fold
R08D7.2	0	2	0	0	2-fold
R08D7.3	0	3	0	2-fold	0
R107.6	0	0	0	0	0
F02A9.4	0	0	0	0	0
F02A9.6	2	2	0	2-fold	3-fold
T23G5.1	0	3	0	3-fold	0
K04H4.1	0	2	0	2-fold	3-fold
C38C10.4	0	2	0	2-fold	3-fold
T26G10.1	0	3	0	2-fold	10-fold
F54C8.2	2	2	0	2-fold	3-fold
F54C8.3	0	2	0	0	2-fold
B0464.1	0	0	1	2-fold	0
B0464.7	0	2	0	0	3-fold

ZK1098.7	0	0	0	0	0
F58A4.3	3	2	0	3-fold	10-fold
F58A4.4	0	3	0	3-fold	0
F58A4.8	0	2	0	0	2-fold
C07A9.2	2	2	0	3-fold	5-fold
C07A9.3	2	2	0	0	3-fold
T05G5.3	0	3	0	2-fold	0
T05G5.10	0	0	0	0	0
R10E11.1	0	0	1	10-fold	0
R10E11.2	0	0	1	3-fold	0
R10E11.8	0	0	1	0	0
ZK632.1	0	3	0	3-fold	0
K03H1.2	2	2	0	2-fold	3-fold
T16H12.4	0	1	0	0	2-fold
T20G5.1	0	0	1	0	0
T20G5.2	0	3	0	2-fold	10-fold
T20G5.3	0	0	1	0	0
R01H10.1	1	1	0	0	4-fold
T07C4.7	0	0	0	0	0
M03C11.7	2	2	0	2-fold	3-fold
D2045.1	0	2	0	0	2-fold
D2045.6	1	2	0	0	3-fold
F43D9.3	0	0	1	4-fold	0
K01G5.1	0	3	0	2-fold	3-fold
K01G5.4	0	0	1	4-fold	0
K01G5.7	0	3	0	2-fold	5-fold
Y39A1B.3	1	1	0	2-fold	4-fold
K11D9.1	0	0	0	0	0
K11D9.2	0	0	1	2-fold	0
Y48A6B.11	0	0	0	0	0
Y47D3A.c	0	3	0	5-fold	0
Y47D3A.d	0	3	0	2-fold	10-fold
Y47D3B.7	0	0	1	2-fold	0
Y66A7A.8	2	2	0	2-fold	4-fold
Y41C4A.10	0	0	0	0	0
C24H11.7	0	0	1	2-fold	5-fold
Y56A3A.32	0	0	0	0	0
Y75B8A.2	1	1	0	3-fold	4-fold
Y49E10.1	0	0	1	10-fold	0
Y49E10.2	0	0	0	0	0
Y49E10.6	0	0	1	2-fold	10-fold
Y49E10.14	2	2	0	10-fold	0
Y49E10.15	0	0	1	2-fold	10-fold
Y49E10.19	2	2	0	10-fold	0
Y49E10.21	0	2	0	2-fold	3-fold
Y111B2C.e	0	3	0	2-fold	4-fold
Y111B2D.b	0	0	1	0	0
Y111B2D.h	3	2	0	2-fold	10-fold
Y37D8A.10	0	3	0	2-fold	10-fold
Y37D8A.14	0	3	0	2-fold	4-fold
Y37D8A.18	0	2	0	0	2-fold
ZK1010.1	0	0	1	10-fold	0
Y39E4B.1	1	2	0	0	2-fold
F56A8.6	0	3	0	2-fold	10-fold
Y43F4B.6	1	2	0	2-fold	3-fold
F53A2.4	0	2	0	2-fold	3-fold
T03F6.5	0	1	0	0	2-fold
T27E9.1	0	2	0	0	10-fold
Y76A2B.1	2	2	0	3-fold	10-fold
T25C8.2	0	0	1	0	0
T12D8.1	2	2	0	2-fold	4-fold
T12D8.6	3	2	0	3-fold	0
T12D8.7	0	0	0	0	0
W06F12.1	0	3	0	2-fold	10-fold
K08E3.5	2	2	0	2-fold	4-fold
K08E3.6	0	3	0	3-fold	0
C37G2.7	2	2	0	2-fold	3-fold
Y119D3_444.b	0	2	0	0	3-fold
Y119D3_446.a	0	0	1	2-fold	10-fold

Y119D3_446.c	0	2	0	2-fold	3-fold
Y53G8A_9248.	0	2	0	0	2-fold
c	0	0	0	0	0
Y53G8A_9248.	0	0	1	4-fold	10-fold
d	0	0	1	3-fold	0
Y53G8B_1025.	0	1	0	0	3-fold
a	2	2	0	2-fold	3-fold
Y53G8B_1025.	0	2	0	3-fold	10-fold
b	0	2	0	3-fold	5-fold
Y55B1A_115.c	0	2	0	2-fold	3-fold
Y71H2_375.b	0	0	1	3-fold	0
Y71H2_378.a					
Y71H2_388.c					
Y71H2_388.d					
Y71H2_389.e					

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CE RNAi clone	**Emb**	**Ste**	**Lga**	**Weaker Pheno**	**No Pheno**
F54C4.1	0	2	0	2-fold	2-fold
C29F9.7	3	3	0	3-fold	10-fold
W07B3.2	0	0	1	2-fold	0
F10C5.1	0	2	0	3-fold	3-fold
F10C5.2	0	2	0	2-fold	2-fold
F30H5.1	0	3	0	5-fold	0
W06E11.1	0	2	0	3-fold	3-fold
T17H7.5	nd	nd	nd	nd	nd
B0412.4	0	0	1	2-fold	0
T24C4.5	0	3	0	2-fold	10-fold
F23H11.5	0	3	0	2-fold	4-fold
F58B6.3	2	2	0	2-fold	4-fold
Y71D11A.b	0	0	1	3-fold	0
F53A3.3	0	0	1	3-fold	10-fold
W04B5.4	0	0	0	0	0
H19M22.1	0	0	1	10-fold	10-fold
H19M22.3	0	0	1	2-fold	0
H06I04.a	0	0	1	2-fold	0
H06I04.f	0	0	1	3-fold	0
F59A2.1	0	3	0	2-fold	5-fold
C34C12.8	0	2	0	2-fold	2-fold
M01F1.3	0	2	0	2-fold	3-fold
C54C6.1	0	0	1	2-fold	10-fold
C32A3.1	2	2	0	2-fold	2-fold
C36A4.4	0	0	0	0	0
F13B10.2	0	0	1	0	0
ZK1058.2	0	0	1	2-fold	0
C36E8.5	0	0	1	2-fold	10-fold
E03A3.3	0	0	1	2-fold	3-fold
C03C10.3	0	3	0	2-fold	10-fold
C16C10.6	0	3	0	2-fold	10-fold
R74.1	0	3	0	3-fold	10-fold
F43C1.2	0	2	0	2-fold	2-fold
F43C1.5	0	0	0	0	0
T08A11.2	0	0	1	2-fold	0
R10E4.4	0	3	0	2-fold	5-fold
H38K22.2	0	0	0	0	0
B0285.2	0	2	0	2-fold	2-fold
R07E5.3	0	3	0	2-fold	10-fold
R07E5.10	0	0	0	2-fold	2-fold
R07E5.14	0	3	0	2-fold	5-fold
F56F3.2	0	0	0	0	0
F56F3.5	0	0	1	0	0
C07G2.3	0	0	1	2-fold	10-fold
M88.2	0	0	0	0	0
F35G12.8	0	3	0	4-fold	10-fold
F35G12.10	0	0	0	0	0
T04A8.6	0	3	0	2-fold	3-fold
T04A8.7	0	0	0	0	0
T04A8.11	0	0	0	0	0

B0393.1	0	0	1	3-fold	0
B0393.6	0	0	0	0	0
C38D4.3	0	0	0	0	0
C38D4.6	3	2	0	2-fold	10-fold
C35D10.1	0	2	0	2-fold	4-fold
C35D10.5	0	0	0	0	0
C35D10.13	0	0	0	0	0
F26F4.10	0	2	0	2-fold	3-fold
F26F4.11	0	3	0	2-fold	3-fold
C26E6.4	0	0	1	4-fold	0
C26E6.6	0	2	0	2-fold	2-fold
C26E6.8	0	0	0	0	0
C27F2.7	0	0	0	0	0
R144.2	0	0	1	2-fold	5-fold
R144.3	0	0	0	0	0
R144.7	0	0	0	0	0
C45G9.5	0	2	0	2-fold	4-fold
F21H11.4	0	0	0	0	0
T10F2.1	0	3	0	4-fold	10-fold
T10F2.4	0	3	0	2-fold	10-fold
K10D2.4	0	0	0	0	0
K10D2.6	1	1	0	2-fold	2-fold
C34E10.1	0	0	0	0	0
C34E10.2	0	0	0	0	0
C34E10.6	0	0	1	5-fold	0
F48E8.2	0	0	0	0	0
F48E8.5	0	0	0	0	0
F09F7.3	0	3	0	2-fold	5-fold
F56D2.1	0	0	1	2-fold	5-fold
F56D2.6	0	2	0	3-fold	3-fold
F54E7.2	0	3	0	2-fold	2-fold
F54E7.3	3	2	0	2-fold	10-fold
F54E7.4	0	0	0	0	0
B0336.1	0	0	0	0	0
B0336.2	2	2	0	2-fold	3-fold
B0336.6	1	2	0	2-fold	2-fold
B0336.10	0	0	1	5-fold	0
R12B2.4	0	2	0	2-fold	2-fold
R12B2.5	0	0	1	2-fold	nd
F01F1.7	0	3	0	2-fold	4-fold
F01F1.8	0	0	1	4-fold	0
F01F1.12	2	0	0	2-fold	2-fold
C28H8.6	0	2	0	2-fold	2-fold
F25B5.4	0	0	1	0	0
ZK328.1	0	0	0	0	0
ZK328.2	0	0	1	5-fold	0
ZK328.5	0	0	1	5-fold	0
T17E9.1	0	0	0	0	0
T17E9.2	0	3	0	2-fold	2-fold
Y42G9A.c	nd	nd	nd	nd	nd
C23G10.3	0	0	1	4-fold	0
C23G10.4	0	0	1	0	0
C23G10.8	0	3	0	5-fold	0
C23G10.9	3	2	0	2-fold	4-fold
T12A2.2	0	0	1	2-fold	10-fold
T12A2.7	0	0	0	0	0
C56G2.2	0	0	0	0	0
C56G2.6	0	0	1	5-fold	0
C16A3.4	0	2	0	2-fold	3-fold
C16A3.5	0	2	0	2-fold	3-fold
C16A3.6	0	2	0	3-fold	3-fold
C16A3.9	0	0	1	3-fold	10-fold
C05D11.2	0	0	0	0	0
C05D11.3	0	0	0	0	0
C05D11.10	0	0	0	0	0
C05D11.11	0	0	0	0	0
C05D11.12	1	1	0	2-fold	2-fold
T26A5.3	0	2	0	2-fold	3-fold
T26A5.9	2	2	0	3-fold	4-fold

F23F12.2	0	0	0	0	0
F23F12.6	0	0	1	4-fold	0
F20H11.3	0	0	0	0	0
C13B9.3	0	0	1	3-fold	0
F37A4.8	0	0	0	0	0
R13F6.1	0	2	0	2-fold	5-fold
R13F6.10	0	0	0	0	0
F57B9.2	0	0	1	4-fold	0
F57B9.3	0	0	1	2-fold	4-fold
F57B9.5	0	3	0	2-fold	5-fold
F57B9.6	0	0	1	2-fold	0
F57B9.10	0	0	1	10-fold	0
F31E3.3	0	0	0	0	0
F11H8.4	0	2	0	3-fold	4-fold
K04G7.1	0	2	0	2-fold	4-fold
K04G7.4	0	2	0	4-fold	4-fold
F37C12.1	0	2	0	2-fold	4-fold
F37C12.3	0	0	0	0	0
F37C12.4	0	3	0	2-fold	3-fold
F37C12.9	0	0	1	3-fold	0
F37C12.11	0	0	1	3-fold	0
F37C12.13	0	3	0	2-fold	4-fold
R151.3	0	0	1	4-fold	0
R151.9	0	1	0	2-fold	2-fold
T20H4.3	0	0	1	10-fold	0
T20H4.5	0	2	0	2-fold	3-fold
B0361.10	0	2	0	2-fold	3-fold
F08F8.2	0	1	0	2-fold	2-fold
T20B12.1	0	2	0	3-fold	3-fold
T20B12.2	0	2	0	2-fold	3-fold
T20B12.7	0	0	0	0	0
T20B12.8	0	3	0	2-fold	5-fold
H14A12.2	0	1	0	3-fold	3-fold
C07H6.5	0	2	0	3-fold	4-fold
R13A5.8	0	0	1	5-fold	0
R13A5.12	0	3	0	4-fold	0
R13A5.13	0	3	0	2-fold	10-fold
ZK686.3	0	2	0	3-fold	3-fold
C08C3.4	0	1	0	2-fold	2-fold
C27D11.1	0	0	1	3-fold	0
ZK652.1	0	0	1	2-fold	10-fold
ZK652.4	0	0	1	3-fold	0
C29E4.2	0	3	0	4-fold	0
C29E4.8	0	2	0	2-fold	3-fold
F54H12.1	0	2	0	2-fold	4-fold
F54H12.6	0	3	0	2-fold	3-fold
F44B9.7	0	0	0	0	0
K12H4.3	0	3	0	2-fold	3-fold
K12H4.4	0	2	0	2-fold	3-fold
K12H4.5	0	2	0	2-fold	3-fold
K06H7.1	3	2	0	2-fold	0
K06H7.6	0	0	0	0	0
C14B9.4	0	3	0	3-fold	0
C14B9.7	0	0	1	5-fold	0
D2007.4	0	0	0	0	0
C02F5.1	0	3	0	4-fold	0
C02F5.9	0	0	1	3-fold	0
F09G8.3	0	2	0	3-fold	5-fold
F10E9.7	0	0	1	3-fold	10-fold
F10E9.8	3	2	0	2-fold	3-fold
ZK1236.3	0	3	0	2-fold	10-fold
C30C11.1	0	0	0	0	0
C30C11.2	0	0	1	3-fold	0
C30C11.4	0	2	0	3-fold	3-fold
C06E1.10	0	2	0	2-fold	2-fold
F22B7.5	0	2	0	2-fold	2-fold
B0303.15	0	0	0	0	0
K02D10.5	0	0	1	4-fold	10-fold
ZK637.8	0	2	0	4-fold	10-fold

R08D7.1	2	2	0	2-fold	4-fold
R08D7.2	0	0	0	0	0
R08D7.3	0	3	0	2-fold	10-fold
R107.6	0	2	0	2-fold	3-fold
F02A9.4	0	0	0	0	0
F02A9.6	0	1	0	2-fold	2-fold
T23G5.1	0	3	0	3-fold	0
K04H4.1	2	2	0	2-fold	3-fold
C38C10.4	2	2	0	2-fold	3-fold
T26G10.1	0	2	0	4-fold	10-fold
F54C8.2	2	2	0	2-fold	3-fold
F54C8.3	0	0	0	0	0
B0464.1	0	0	1	2-fold	0
B0464.7	0	2	0	3-fold	4-fold
ZK1098.7	0	2	0	2-fold	2-fold
F58A4.3	3	2	0	3-fold	0
F58A4.4	0	3	0	3-fold	0
F58A4.8	0	2	0	2-fold	3-fold
C07A9.2	2	2	0	4-fold	4-fold
C07A9.3	2	2	0	3-fold	4-fold
T05G5.3	0	3	0	3-fold	0
T05G5.10	0	2	0	2-fold	3-fold
R10E11.1	0	0	1	10-fold	0
R10E11.2	0	0	1	3-fold	0
R10E11.8	0	0	1	0	0
ZK632.1	0	3	0	5-fold	0
K03H1.2	2	2	0	2-fold	2-fold
T16H12.4	0	0	0	0	0
T20G5.1	0	0	1	0	0
T20G5.2	0	2	0	4-fold	5-fold
T20G5.3	0	0	1	0	0
R01H10.1	1	2	0	2-fold	2-fold
T07C4.7	0	0	0	0	0
M03C11.7	2	2	0	2-fold	3-fold
D2045.1	0	0	0	0	0
D2045.6	0	2	0	2-fold	4-fold
F43D9.3	0	0	1	4-fold	0
K01G5.1	2	2	0	2-fold	4-fold
K01G5.4	0	0	1	3-fold	0
K01G5.7	0	0	1	2-fold	4-fold
Y39A1B.3	0	0	0	0	0
K11D9.1	0	0	0	0	0
K11D9.2	0	2	0	10-fold	0
Y48A6B.11	0	0	0	0	0
Y47D3A.c	0	3	0	4-fold	0
Y47D3A.d	0	3	0	2-fold	10-fold
Y47D3B.7	0	0	1	2-fold	0
Y66A7A.8	2	2	0	2-fold	4-fold
Y41C4A.10	0	0	0	0	0
C24H11.7	0	0	1	2-fold	10-fold
Y56A3A.32	0	0	0	0	0
Y75B8A.2	1	1	0	2-fold	0
Y49E10.1	0	0	1	4-fold	0
Y49E10.2	0	0	0	0	0
Y49E10.6	0	0	1	3-fold	10-fold
Y49E10.14	3	2	0	2-fold	0
Y49E10.15	0	0	1	2-fold	0
Y49E10.19	0	3	0	2-fold	0
Y49E10.21	0	2	0	2-fold	2-fold
Y111B2C.e	2	2	0	2-fold	3-fold
Y111B2D.b	0	0	1	10-fold	0
Y111B2D.h	3	2	0	2-fold	0
Y37D8A.10	2	2	0	4-fold	10-fold
Y37D8A.14	0	3	0	2-fold	10-fold
Y37D8A.18	0	0	0	0	0
ZK1010.1	0	0	1	5-fold	0
Y39E4B.1	0	2	0	2-fold	2-fold
F56A8.6	0	2	0	5-fold	5-fold
Y43F4B.6	0	2	0	2-fold	2-fold

F53A2.4	0	0	0	0	0
T03F6.5	0	0	0	0	0
T27E9.1	0	3	0	4-fold	10-fold
Y76A2B.1	0	2	0	3-fold	5-fold
T25C8.2	0	0	1	0	0
T12D8.1	2	2	0	2-fold	3-fold
T12D8.6	3	2	0	2-fold	0
T12D8.7	0	2	0	2-fold	2-fold
W06F12.1	3	2	0	3-fold	0
K08E3.5	2	2	0	2-fold	4-fold
K08E3.6	0	3	0	10-fold	10-fold
C37G2.7	2	2	0	2-fold	2-fold
Y119D3_444.b	0	2	0	2-fold	3-fold
Y119D3_446.a	0	2	0	3-fold	3-fold
Y119D3_446.c	0	2	0	3-fold	3-fold
Y53G8A_9248.c	0	2	0	2-fold	2-fold
Y53G8A_9248.d	0	0	0	0	0
Y53G8B_1025.a	0	0	1	3-fold	0
Y53G8B_1025.b	0	0	1	3-fold	0
Y55B1A_115.c	0	0	0	0	0
Y71H2_375.b	0	2	0	3-fold	3-fold
Y71H2_378.a	0	2	0	3-fold	5-fold
Y71H2_388.c	0	3	0	2-fold	3-fold
Y71H2_388.d	0	2	0	2-fold	2-fold
Y71H2_389.e	0	0	1	5-fold	5-fold

Appendix Table 3.2. *C. elegans* chromosome III genes with non-viable RNA interference phenotypes
For each gene on *C. elegans* chromosome III with a previously reported nonviable RNA interference (RNAi) phenotype (Kamath *et al*., 2003), the Ahringer library RNAi clone gene pairs name ('CE RNAi clone'), its nonviable RNAi phenotype ('Emb', 'Ste', 'Lga') observed in *rrf-3* when using the high-throughput RNAi liquid feeding assay, the n-fold dilution of targeting bacteria with unrelated double-stranded RNA- (dsRNA) expressing bacteria (non-targeting (Ahringer library clone Y95B8A_84.g) and *lin-31*, respectively), which first resulted in a weaker detectable phenotype of nonviable genes ('Weaker phenotype') and the n-fold dilution that resulted in the loss of any detectable phenotype ('No phenotype'). Emb, embryonic lethal. Ste, sterile. Lga, larval growth arrest. Strengths of phenotypes were encoded numerically ranging from 1 (weak), 2 (medium), to 3 (strong). 0, no observed phenotype or no observed dilution effect in a given category, respectively.

Appendix Table 4.1. *C. elegans* duplicate gene pairs corresponding to single orthologues in *S. cerevisiae* and *D. melanogaster* genomes

SC ID/DM ID	SC essentiality	CE RNAi clone	CE Name	Comments
S000000042/CG7758	non-	D1025.2	WBGene00008354	
S000000042/CG7758	essential	F52A8.5	WBGene00009918	
S000000071	non-	F19H6.1	WBGene00008956	
S000000071	essential	Y39G10AR.3	WBGene00021461	
S000000136/CG5183	non-	C28H8.4	WBGene00016195	
S000000136/CG5183	essential	F09B9.3	WBGene00001331	
S000000194	non-	R07B7.5	WBGene00011089	
S000000194	essential	R07B7.4	WBGene00011088	
S000000245	essential	D1009.1	WBGene00017012	
S000000245	essential	F28D1.9	WBGene00009218	
S000000313	non-	T21H3.3	WBGene00000552	Lga
S000000313	essential	C13C12.1	WBGene00000285	
S000000331	non-	F20B6.2	WBGene00006921	Lga
S000000331	essential	Y110A7A.12	WBGene00006921	
S000000339	non-	Y71G12B.27	WBGene00022162	
S000000339	essential	C09G4.3	WBGene00001051	
S000000426	non-	C50H11.1	WBGene00016849	
S000000426	essential	F41C3.3	WBGene00018269	
S000000730	essential	C53B4.6	WBGene00008275	
S000000730	essential	F15B10.1	WBGene00017480	
S000000782	non-	K07A1.12	WBGene00003036	
S000000782	essential	K07A1.11	WBGene00004312	
S000000807	non-	R07E4.4	WBGene00003254	
S000000807	essential	C33H5.14	WBGene00016380	
S000000967/CG5119	essential	Y106G6H.2	WBGene00003902	
S000000967/CG5119	essential	F18H3.3	WBGene00003903	
S000001156	non-	F09E10.8	WBGene00017298	
S000001156	essential	K08E3.3	WBGene00010663	
S000001219	non-	C46H11.2	WBGene00016728	
S000001219	essential	C01H6.4	WBGene00007254	
S000001285	non-	H13N06.5	WBGene00010398	
S000001285	essential	T28F3.3	WBGene00012129	
S000001324/CG9881	non-	M01B12.3	WBGene00000205	
S000001324/CG9881	essential	C46H11.3	WBGene00016729	
S000001327/CG17510	non-	F13B9.8	WBGene00001425	
	essential	F41G3.4	WBGene00001424	
S000001327/CG17510	non-	T25G3.4	WBGene00012031	
	essential	Y50E8A.6	WBGene00013049	
S000001417	non-	K08D10.3	WBGene00004386	
S000001417	essential	K08D10.4	WBGene00004385	
S000001448	non-	K06A9.1	WBGene00019435	
S000001448	essential	H02F09.3	WBGene00019146	
S000001458	essential	C33H5.7	WBGene00016374	
S000001458	essential	C33H5.6	WBGene00016373	
S000001501	non-	C29F7.3	WBGene00007812	
S000001501	essential	F40F8.1	WBGene00009575	
S000001507/CG6092	non-	Y53C12A.4	WBGene00013140	
S000001507/CG6092	essential	R02E12.2	WBGene00019827	
S000001672/CG4083	non-	T09A5.9	WBGene00011637	
S000001672/CG4083	essential	C06A8.6	WBGene00015516	
S000001676	non-	Y51H7C.9	WBGene00021787	
S000001676	essential	K01C8.1	WBGene00010456	
S000001701	non-	F52C12.5	WBGene00001253	
S000001701	essential	F55A8.1	WBGene00001186	
S000001873	non-	B0024.12	WBGene00001646	
S000001873	essential	T23G11.2	WBGene00001647	
S000001877/CG1969	essential	D1037.4	WBGene00004272	
S000001877/CG1969	essential	T23H2.5	WBGene00004273	
S000001889	non-	K08A8.3	WBGene00000591	
S000001889	essential	F10G7.4	WBGene00004737	
S000002161	non-	C25G4.1	WBGene00007729	
S000002161	essential	C49C3.13	WBGene00008203	
S000002195	non-	T06E8.1	WBGene00011543	

S000002195	essential	F59F4.4	WBGene00010339	
S000002210	non-	F57B9.10	WBGene00004462	Lga
S000002210	essential	F59B2.5	WBGene00010309	
S000002255	non-	D2045.6	WBGene00000836	
S000002255	essential	K08E7.7	WBGene00000841	
S000002290/CG1877	non-	B0286.4	WBGene00003825	
S000002290/CG1877	essential	F44A2.1	WBGene00006499	
S000002324	non-	K04D7.4	WBGene00010558	
S000002324	essential	C09D8.1	WBGene00004215	
S000002389	non-	Y22D7AR.6	WBGene00021260	
S000002389	essential	F30A10.3	WBGene00009262	
S000002424	essential	F43H9.2	WBGene00018398	
S000002424	essential	T22G5.5	WBGene00011932	
S000002469	essential	Y57E12AL.1	WBGene00021956	
S000002469	essential	R11H6.2	WBGene00011250	
S000002512/CG4672	essential	F27D9.1	WBGene00006757	
S000002512/CG4672	essential	T07A9.10	WBGene00020298	
S000002571	essential	F40G9.3	WBGene00006715	
S000002571	essential	C06E2.3	WBGene00006716	
S000002584	non-	ZK265.5	WBGene00013957	
S000002584	essential	F36D1.2	WBGene00009462	
S000002727/CG1067	non-	C44C1.3	WBGene00003563	
1	essential	K03E6.3	WBGene00003565	
S000002727/CG1067	non-	F13H8.3	WBGene00017436	
1	essential	Y43F8C.13	WBGene00012834	
S000002781	non-	F21F3.3	WBGene00017673	
S000002781	essential	M01E11.1	WBGene00019710	
S000002808	essential	R151.6	WBGene00020109	
S000002808	essential	F25D7.1	WBGene00009111	
S000002818/CG1126	essential	T01C8.1	WBGene00020142	
8	essential	PAR2.3	WBGene00019801	
S000002818/CG1126	essential	F49E12.9	WBGene00009902	
8	essential	F49E12.10	WBGene00009903	
S000002819	non-	R03A10.4	WBGene00010984	
S000002819	essential	F28H6.3	WBGene00009232	
S000002885	non-	Y110A7A.6	WBGene00022456	
S000002885	essential	K02B2.1	WBGene00019295	
S000003292/CG1998	non-	F35H8.7	WBGene00006938	
S000003292/CG1998	essential	Y53C12A.1	WBGene00006940	
S000003596/CG6950	non-	C15F1.7	WBGene00004930	
S000003596/CG6950	essential	ZK430.3	WBGene00007036	
S000003691/CG3400	essential	R57.1	WBGene00020082	
S000003691/CG3400	essential	C35C5.2	WBGene00007954	
S000003723	essential	C44E4.3	WBGene00016652	
S000003723	essential	C14F11.1	WBGene00015778	
S000003865/CG1179	non-	F59B2.7	WBGene00004269	
3	essential	T25G12.4	WBGene00004270	
S000003865/CG1179	non-	T03F7.7	WBGene00011404	
3	essential	C34C12.6	WBGene00007925	
S000003887	non-	ZK370.4	WBGene00022718	
S000003887	essential	M110.7	WBGene00010915	
S000004017/CG4233	non-	B0280.4	WBGene00003845	
S000004017/CG4233	essential	C34H3.2	WBGene00003846	
S000004252	non-	C04F6.4	WBGene00006810	
S000004252	essential	K08F9.2	WBGene00010685	
S000004372/CG3248	non-	T05H4.13	WBGene00000110	
5	essential	T08B1.3	WBGene00000111	
S000004372/CG3248	essential	F54F7.1	WBGene00006388	
5	essential	Y111B2A.16	WBGene00006389	
S000004524	non-	C42C1.11	WBGene00016589	
S000004524	essential	ZC416.6	WBGene00022610	
S000004640	non-	F17E5.2	WBGene00008924	
S000004640	essential	F55A11.4	WBGene00010077	
S000004698/CG1072	essential	ZK370.3	WBGene00022717	
4	essential	F08A8.6	WBGene00006484	
S000004698/CG1072	non-	C53A5.3	WBGene00001834	
4	essential	R06C1.1	WBGene00001836	
S000004716/CG1114	non-	F52B5.6	WBGene00004439	
0	essential	F55D10.2	WBGene00004438	

S000004716/CG1114	non-	C13G3.3	WBGene00007554	
0	essential	W08G11.4	WBGene00012348	
S000004840/CG2670	non-	C05G5.4	WBGene00007350	
S000004840/CG2670	essential	F23H11.3	WBGene00017759	
S000004990/CG1060	essential	C54G7.2	WBGene00016934	
2	essential	C08F8.4	WBGene00007446	
S000004990/CG1060	non-	F02E8.6	WBGene00003561	
2	essential	F09G8.4	WBGene00003562	
S000005027/CG3210	non-	R12H7.2	WBGene00000217	
3	essential	H22K11.1	WBGene00000216	
S000005027/CG3210	non-	F53C11.7	WBGene00009976	
3	essential	F53C11.8	WBGene00009977	
S000005187/CG1097	non-	K11D2.3	WBGene00006829	
1	essential	F55A12.7	WBGene00000150	Lga
S000005187/CG1097	non-	F55G1.5	WBGene00018901	
1	essential	F20D1.9	WBGene00008979	
S000005274	non-	T14F9.1	WBGene00020507	Lga
S000005274	essential	F52E1.10	WBGene00018698	
S000005487/CG7977	non-	R13A5.10	WBGene00020052	
S000005487/CG7977	essential	Y48A6B.7	WBGene00012968	
S000005540	non-	F26A3.1	WBGene00009140	
S000005540	essential	F11C1.4	WBGene00008693	
S000005668/CG1065	essential	B0513.3	WBGene00004443	
S000005668/CG1065	essential	Y47H9C.14	WBGene00012956	
S000005701/CG1844	non-	T06D8.10	WBGene00011530	
5	essential	C46A5.4	WBGene00016700	
S000005701/CG1844	non-	VF13D12L.3	WBGene00012149	
5	essential	F36A2.3	WBGene00009453	
S000005927	non-	H17B01.1	WBGene00019207	
S000005927	essential	R09B5.11	WBGene00019979	
S000006075	non-	C25B8.3	WBGene00000786	
S000006075	essential	F57F5.1	WBGene00010204	Lga
S000006168	non-	K06B9.5	WBGene00003938	
S000006168	essential	C04G2.7	WBGene00001204	
S000006180	non-	C32E8.8	WBGene00004217	
S000006180	essential	F55F8.1	WBGene00004224	
S000006225	non-	ZK994.3	WBGene00004256	
S000006225	essential	K09C8.5	WBGene00004257	
S000006240	non-	C28C12.7	WBGene00004995	
S000006240	essential	C28C12.5	WBGene00004993	
S000006266	non-	C50B8.2	WBGene00000250	
S000006266	essential	T27F2.3	WBGene00000249	
S000006351/CG9186	non-	T20B3.1	WBGene00011850	
S000006351/CG9186	essential	F41E7.6	WBGene00009622	
S000006437/CG1007	non-	F46E10.1	WBGene00018488	Lga
1	essential	F28F8.2	WBGene00009221	
S000006437/CG1007	non-	B0416.6	WBGene00001638	
1	essential	M01F1.1	WBGene00001639	
CG10211	non-	F26H9.4	WBGene00009176	
CG10211	essential	W06B3.1	WBGene00012295	
CG10512	non-	ZK945.8	WBGene00014170	
CG10512	essential	K10H10.7	WBGene00010763	
CG1086	non-	C47E12.8	WBGene00000964	
CG1086	essential	R06C7.3	WBGene00000963	
CG10992	non-	C44H4.3	WBGene00006366	
CG10992	essential	C44H4.2	WBGene00006370	Lga
CG11049	non-	C03H5.4	WBGene00015406	
CG11049	essential	C07E3.9	WBGene00007419	
CG11212	non-	F27C8.1	WBGene00000002	
CG11212	essential	F52H2.2	WBGene00000004	
CG12002	non-	F16C3.1	WBGene00008885	
CG12002	essential	C24B5.1	WBGene00016044	
CG12070	non-	R06C7.7	WBGene00003041	
CG12070	essential	Y48G1A.6	WBGene00021661	
CG12265	non-	C25H3.3	WBGene00016112	
CG12265	essential	C25H3.14	WBGene00016123	
CG12428	non-	T05A6.1	WBGene00000516	
CG12428	essential	T05A6.2	WBGene00000517	
CG12512	non-	F23H11.1	WBGene00017757	

CG12512	essential	F54B11.6	WBGene00010029	
CG13431	non-	C34F11.9	WBGene00001101	
CG13431	essential	C27A2.6	WBGene00001102	
CG13645	essential	C37H5.3	WBGene00016507	
CG13645	essential	C37H5.2	WBGene00016506	
CG13994	non-	K10B2.5	WBGene00019608	
CG13994	essential	Y43F8C.14	WBGene00012835	
CG1411	non-	C28D4.2	WBGene00000509	
CG1411	essential	C52B9.1	WBGene00000510	
CG14351	non-	ZK688.6	WBGene00022801	
CG14351	essential	ZK112.1	WBGene00003956	
CG14507	non-	C24H12.5	WBGene00016074	
CG14507	essential	C24H12.2	WBGene00016072	
CG1607	non-	H23L24.5	WBGene00004052	
CG1607	essential	F20C5.1	WBGene00004051	
CG16726	non-	F35G2.4	WBGene00004025	
CG16726	essential	Y47D3B.10	WBGene00001077	
CG16975	non-	K06A1.5	WBGene00019427	
CG16975	essential	F02E8.5	WBGene00017178	
CG16986	non-	F45H7.4	WBGene00004183	
CG16986	essential	C06E8.3	WBGene00004182	
CG1772	essential	F57A10.3	WBGene00001813	
CG1772	essential	C30H6.6	WBGene00001811	
CG1815	non-	T07D4.1	WBGene00011578	
CG1815	essential	T23C6.5	WBGene00020727	
CG18361	non-	T05G5.10	WBGene00002064	
CG18361	essential	F54C9.1	WBGene00002065	
CG1882	non-	F46G10.7	WBGene00004801	
CG1882	essential	F46G10.3	WBGene00004802	
CG2092	non-	C50D2.8	WBGene00016811	
CG2092	essential	Y119D3B.12	WBGene00022489	
CG2201	non-	T04G9.4	WBGene00020215	
CG2201	essential	T28H10.1	WBGene00012142	
CG2493	non-	T13G4.3	WBGene00020490	
CG2493	essential	B0416.1	WBGene00015177	
CG2669	non-	C10E2.6	WBGene00015676	
CG2669	essential	T02G5.12	WBGene00020168	
CG2864	non-	F54D8.3	WBGene00000107	
CG2864	essential	K04F1.15	WBGene00000108	
CG31022	non-	C35C5.3	WBGene00007955	
CG31022	essential	F28H7.4	WBGene00009238	
CG31033	non-	C55B7.4	WBGene00016943	
CG31033	essential	K06A5.6	WBGene00019433	
CG3105	non-	R107.8	WBGene00003001	
CG3105	essential	F02A9.6	WBGene00001609	
CG3156	non-	C01H6.9	WBGene00007258	
CG3156	essential	Y18H1A.10	WBGene00021214	
CG31645	non-	Y57A10A.28	WBGene00013268	
CG31645	essential	Y57A10A.10	WBGene00013255	
CG3186	non-	B0495.2	WBGene00015203	
CG3186	essential	ZC504.3	WBGene00013917	
CG3187	non-	D1037.3	WBGene00001501	
CG3187	essential	C54F6.14	WBGene00001500	
CG3198	non-	Y62E10A.1	WBGene00004410	
CG3198	essential	C37A2.7	WBGene00016493	
CG32099	non-	F16B4.8	WBGene00000387	
CG32099	essential	ZK637.11	WBGene00000388	
CG3280	non-	F33D11.11	WBGene00018008	
CG3280	essential	F42G2.5	WBGene00018354	
CG3456	non-	F16H9.1	WBGene00004345	
CG3456	essential	C05B5.7	WBGene00004344	
CG3752	non-	F47G6.4	WBGene00004969	
CG3752	essential	Y66H1A.6	WBGene00002041	
CG3887	non-	F13G3.7	WBGene00008767	
CG3887	essential	Y43C5B.3	WBGene00012786	
CG3902	non-	Y49A3A.1	WBGene00013024	
CG3902	essential	F22E10.5	WBGene00009057	
CG3936	non-	F57C12.5	WBGene00003407	
CG3936	essential	F57C12.4	WBGene00003408	

CG40080	non-essential	T14G11.3	WBGene00020511	
CG40080		W06H3.1	WBGene00012315	
CG4239		C24G6.6	WBGene00016061	
CG4239		F25C8.2	WBGene00000139	
CG4268		F21A10.2	WBGene00008999	
CG4268		F59B10.1	WBGene00004134	Lga
CG4349		C56C10.3	WBGene00016961	Lga
CG4349		C37C3.3	WBGene00016497	Lga
CG4918		F55F3.1	WBGene00010115	
CG4918		Y47D3A.15	WBGene00012928	
CG4965		C09B8.4	WBGene00015623	
CG4965		T10B11.6	WBGene00020402	
CG5014		C55B6.2	WBGene00001025	
CG5014		Y54E10BL.4	WBGene00001046	
CG5036		Y111B2A.19	WBGene00013739	
CG5036		C13C4.5	WBGene00007549	
CG5695		T21D12.9	WBGene00020649	
CG5695		R13.3	WBGene00011258	
CG5805		C44C1.2	WBGene00016642	
CG5805		R09B5.12	WBGene00019980	
CG6016		F37C12.7	WBGene00018152	
CG6016		C46F4.2	WBGene00016716	
CG6214		F53B1.6	WBGene00018738	
CG6214		C03F11.2	WBGene00015388	
CG6455		ZK1098.3	WBGene00014220	
CG6455		ZK1098.8	WBGene00003504	
CG8032		F53C3.12	WBGene00018755	
CG8032		Y46G5A.24	WBGene00012914	
CG3328		F37H8.5	WBGene00009514	
CG3328		K07D4.8	WBGene00004135	
CG8055		Y25C1A.13	WBGene00021296	
CG8055		F58A6.1	WBGene00019022	
CG8057		F42G10.2	WBGene00003368	
CG8057		VZC374L.1	WBGene00012162	
CG8245		C30G12.7	WBGene00004244	
CG8245		W06B11.2	WBGene00004245	
CG8286				
CG8286				
CG8428				
CG8428				
CG8434				
CG8434				
CG8460				
CG8460				
CG8732				
CG8732				
CG9117				
CG9117				
CG9247				
CG9247				
CG9347				
CG9347				
CG9427				
CG9427				
CG9577				
CG9577				
CG9738				
CG9738				
CG9755				
CG9755				

Appendix Table 4.2. ***C. elegans*** **1:1 orthologues of** ***S. cerevisiae*** **genes**

SC Gene	SC essentiality	CE RNAi Clone	CE Name	NonV
2AAA_YEAST	non-	F48E8.5	WBGene00003901	
2ABA_YEAST	essential	F26E4.1	WBGene00006352	x
3HAO_YEAST	non-	K06A4.5	WBGene00010595	
ABC1_YEAST	essential	C35D10.4	WBGene00000767	
ABD1_YEAST	non-	C25A1.3	WBGene00006447	
ABP1_YEAST	essential	K08E3.4	WBGene00010664	
ABPX_YEAST	non-	ZK1058.5	WBGene00014205	
ACBP_YEAST	essential	C44E4.6	WBGene00016655	
ACON_YEAST	essential	F54H12.1	WBGene00000041	x
ADA_YEAST	non-	C06G3.5	WBGene00015551	
ADA1_YEAST	essential	T20B5.1	WBGene00000161	
ADA2_YEAST	non-	F32A5.1	WBGene00017967	
ADB2_YEAST	essential	Y71H2_389.e	WBGene00000160	x
ADB6_YEAST	non-	R11A5.1	WBGene00000163	
ADK_YEAST	essential	R07H5.8	WBGene00011128	
ADPP_YEAST	non-	W02G9.1	WBGene00003579	
ADRO_YEAST	essential	Y62E10A.f	WBGene00013376	
AFG1_YEAST	non-	C30F12.2	WBGene00016261	
AGM1_YEAST	essential	F21D5.1	WBGene00009006	
ALG1_YEAST	non-	T26A5.4	WBGene00020820	
ALG2_YEAST	essential	F09E5.2	WBGene00017282	x
ALG3_YEAST	non-	K09E4.2	WBGene00010720	
ALG6_YEAST	essential	C08B11.8	WBGene00007435	
ALG9_YEAST	non-	C14A4.3	WBGene00007556	
AMDM_YEAST	essential	C34F11.3	WBGene00016415	
AMP2_YEAST	non-	Y116A8A.9	WBGene00003130	
AP10_YEAST	essential	F15H10.3	WBGene00000144	
AP17_YEAST	non-	F02E8.3	WBGene00000157	
AP19_YEAST	essential	F29G9.3	WBGene00000159	
APG6_YEAST	non-	T19E7.3	WBGene00000247	
APG6_YEAST	essential	T19E7.4	WBGene00000247	
APG7_YEAST	essential	M7.5	WBGene00010882	
APG8_YEAST	non-	C32D5.9	WBGene00002980	
APN1_YEAST	essential	T05H10.2	WBGene00000151	
APN2_YEAST	essential	R09B3.1	WBGene00001372	
AR21_YEAST	essential	Y37D8A.1	WBGene00000203	
AR41_YEAST	essential	Y79H2A.6	WBGene00000201	
ARG1_YEAST	non-	D1081.2	WBGene00006844	x
ARGI_YEAST	essential	T21F4.1	WBGene00020658	
ARL1_YEAST	non-	F54C9.10	WBGene00000187	x
ARP2_YEAST	essential	K07C5.1	WBGene00000200	
ARP6_YEAST	non-	C08B11.6	WBGene00007434	
ATC6_YEAST	essential	C10C6.6	WBGene00007514	
ATPB_YEAST	non-	C34E10.6	WBGene00000229	x
ATPD_YEAST	essential	F58F12.1	WBGene00019061	
ATPO_YEAST	non-	F27C1.7	WBGene00017856	x
ATU2_YEAST	essential	Y76A2A.3	WBGene00000834	
ATU2_YEAST	non-	F45G2.11	WBGene00000834	
ATU2_YEAST	essential	Y76A2A.2	WBGene00000834	
ATX1_YEAST	non-	ZK652.2	WBGene00000835	
ATX2_YEAST	essential	T01D3.5	WBGene00011329	
AUT1_YEAST	non-	Y55F3A_746.a	WBGene00021922	
AUT1_YEAST	essential	Y55F3A_746.e	WBGene00021922	
BCS1_YEAST	non-	F54C9.6	WBGene00010042	
BET2_YEAST	essential	B0280.11	WBGene00015099	
BET3_YEAST	non-	ZK1098.5	WBGene00014222	
BET4_YEAST	essential	M57.2	WBGene00019778	
BMS1_YEAST	non-	Y61A9LA_74.b	WBGene00022021	
BMS1_YEAST	essential	Y61A9LA_75.a	WBGene00022021	x
BPH1_YEAST	non-	VT23B5.2	WBGene00012154	
BRX1_YEAST	essential	K12H4.3	WBGene00019678	x
BU31_YEAST	non-	C07A9.2	WBGene00007400	x
BUB2_YEAST	essential	C33F10.2	WBGene00016352	
CAC2_YEAST	non-	Y71G12A_202.	WBGene00022141	

CACM_YEAST	essential	d	WBGene00011122	
CACP_YEAST	non-	R07H5.2	WBGene00007175	
CALX_YEAST	essential	B0395.3	WBGene00000567	
CAP_YEAST	essential	ZK632.6	WBGene00000294	
CAPA_YEAST	non-	F41G4.2	WBGene00000292	x
CAPB_YEAST	essential	D2024.6	WBGene00000293	x
CAT5_YEAST	non-	M106.5	WBGene00000536	
CBF5_YEAST	essential	ZC395.2	WBGene00010478	
CBP3_YEAST	non-	K01G5.5	WBGene00016442	
CC16_YEAST	essential	C35D10.5	WBGene00001281	x
CC27_YEAST	essential	F10B5.6	WBGene00003132	x
CC28_YEAST	non-	Y110A7A.d	WBGene00000405	x
CC42_YEAST	essential	T05G5.3	WBGene00000390	x
CC45_YEAST	non-	R07G3.1	WBGene00009372	
CC45_YEAST	essential	F34D10.2	WBGene00009372	
CC47_YEAST	non-	F34D10.3	WBGene00003159	x
CC54_YEAST	essential	F32D1.10	WBGene00003156	x
CC68_YEAST	non-	Y39G10A_246.	WBGene00018849	
CCHL_YEAST	essential	e	WBGene00011527	
CCL1_YEAST	non-	F55A3.7	WBGene00021714	
CCR4_YEAST	essential	T06D8.6	WBGene00000376	
CDC6_YEAST	non-	Y49F6B.r	WBGene00000382	
CDS1_YEAST	essential	ZC518.3	WBGene00016384	
CEF1_YEAST	non-	C43E11.10	WBGene00008386	x
CEM1_YEAST	essential	C33H5.18	WBGene00008667	
CG48_YEAST	non-	D1081.8	WBGene00015104	x
CH12_YEAST	essential	F10G8.9	WBGene00010676	
CHD1_YEAST	non-	B0280.9	WBGene00010369	
CHL1_YEAST	essential	K08F4.1	WBGene00010839	
CHS5_YEAST	non-	H06O01.2	WBGene00022615	
CLH_YEAST	essential	M03C11.2	WBGene00011867	x
CND1_YEAST	non-	ZC449.5	WBGene00001087	
CNS1_YEAST	essential	T20G5.1	WBGene00015916	
COD1_YEAST	non-	Y39A1B.3	WBGene00021784	x
COD1_YEAST	essential	C17G10.2	WBGene00021784	x
COD1_YEAST	non-	Y51H7C_255.f	WBGene00021784	
COD1_YEAST	essential	Y51H7C_255.g	WBGene00021784	x
COD2_YEAST	essential	Y51H7C_255.b	WBGene00019481	
COPA_YEAST	essential	Y51H7C_255.c	WBGene00022119	x
COPB_YEAST	essential	K07C11.9	WBGene00021292	x
COPD_YEAST	essential	Y71F9A_282.b	WBGene00015734	x
COPG_YEAST	essential	Y25C1A.5	WBGene00011775	x
COPP_YEAST	non-	C13B9.3	WBGene00009542	x
COPZ_YEAST	essential	T14G10.5	WBGene00010333	x
COQ1_YEAST	essential	F38E11.5	WBGene00000761	
COQ1_YEAST	non-	F59E10.3	WBGene00000761	
COQ1_YEAST	essential	C24A11.8	WBGene00000761	
COQ3_YEAST	non-	C24A11.3	WBGene00000763	
COQ4_YEAST	essential	C30H7.1	WBGene00000764	
COQ6_YEAST	non-	Y57G11C.11	WBGene00000766	
CORO_YEAST	essential	T03F1.2	WBGene00000768	
COX6_YEAST	non-	K07B1.2	WBGene00012553	x
COXE_YEAST	essential	R01H10.3	WBGene00018800	
COXG_YEAST	non-	Y37D8A.14	WBGene00022170	x
COXG_YEAST	essential	F54D8.2	WBGene00022170	
COXW_YEAST	non-	Y71H2_388.c	WBGene00011526	
COXX_YEAST	essential	Y71H2_388.f	WBGene00012895	
COXZ_YEAST	non-	T06D8.5	WBGene00010437	
CRC1_YEAST	essential	Y46G5.a	WBGene00000996	
CRD1_YEAST	non-	JC8.5	WBGene00017763	
CSE1_YEAST	essential	F49E8.5	WBGene00002079	x
CSE1_YEAST	non-	F23H11.9	WBGene00002079	x
CTK1_YEAST	essential	Y48G1A_54.b	WBGene00007135	x
CTK1_YEAST	non-	Y48G1A_54.c	WBGene00007135	x
CW41_YEAST	essential	B0285.1	WBGene00008775	
CW43_YEAST	essential	B0285.2	WBGene00011413	
CY1_YEAST	non-	F13H10.4	WBGene00000869	
CYAA_YEAST	essential	T04A8.12	WBGene00009647	
CYB2_YEAST	essential	C54G4.8	WBGene00018286	

DAN4_YEAST	essential	F43C1.1	WBGene00021761	
DAP1_YEAST	essential	F41E6.5	WBGene00006890	
DBP5_YEAST	essential	Y51B11A.a	WBGene00011580	
DBP9_YEAST	essential	K07E3.8	WBGene00016073	x
DBR1_YEAST	essential	T07D4.4	WBGene00000937	
DCAM_YEAST	essential	C24H12.4	WBGene00004875	
DCTD_YEAST	essential	C55B7.8	WBGene00014034	
DF10_YEAST	essential	F47G4.7	WBGene00007102	
DHH1_YEAST	non-	ZK643.2	WBGene00000479	x
DHR1_YEAST	essential	B0024.13	WBGene00015525	x
DHSB_YEAST	essential	C07H6.5	WBGene00006433	x
DIE2_YEAST	non-	C06E1.10	WBGene00011987	
DIM1_YEAST	essential	F42A8.2	WBGene00008455	x
DIP2_YEAST	essential	T24D1.4	WBGene00017435	
DLDH_YEAST	essential	E02H1.1	WBGene00010794	x
DNA2_YEAST	essential	F13H8.2	WBGene00001016	
DNLI_YEAST	non-	LLC1.3	WBGene00002985	x
DNM1_YEAST	essential	F43G6.1	WBGene00001093	
DNPE_YEAST	non-	C29A12.3	WBGene00017163	
DO34_YEAST	essential	T12E12.4	WBGene00011280	
DOA1_YEAST	non-	F01F1.9	WBGene00007333	
DOR1_YEAST	essential	R74.6	WBGene00011736	
DPB2_YEAST	non-	C05C10.6	WBGene00017237	
DPB3_YEAST	essential	T12D8.9	WBGene00013150	
DPD2_YEAST	non-	F08B4.5	WBGene00008722	x
DPD3_YEAST	essential	Y53F4B.d	WBGene00011016	
DPH2_YEAST	non-	F12F6.7	WBGene00007488	
DPH5_YEAST	essential	R04F11.3	WBGene00007194	
DPO2_YEAST	non-	C09G5.3	WBGene00001002	x
DPOA_YEAST	essential	B0491.7	WBGene00012936	x
DPOA_YEAST	essential	R01H10.1	WBGene00012936	x
DPOD_YEAST	essential	Y47D3A.c	WBGene00008645	x
DPOE_YEAST	essential	Y47D3A.d	WBGene00009368	x
DPOG_YEAST	essential	F10C2.4	WBGene00013258	
DPP3_YEAST	essential	F33H2.5	WBGene00008532	
DR48_YEAST	essential	Y57A10A.m	WBGene00004118	
DRS1_YEAST	non-	F02E9.8	WBGene00022148	
DRS1_YEAST	essential	F10F2.9	WBGene00022148	
DSK2_YEAST	essential	Y71G12A_201.	WBGene00008852	
DTD_YEAST	essential	a	WBGene00004151	
DUR3_YEAST	essential	Y71G12A_203.	WBGene00000501	
DYHC_YEAST	essential	c	WBGene00000962	x
DYL1_YEAST	essential	F15C11.2	WBGene00001005	x
DYR_YEAST	essential	T16G1.10	WBGene00007974	
E2BA_YEAST	non-	C48D1.3	WBGene00014221	
E2BD_YEAST	essential	T21E12.4	WBGene00008670	x
E2BE_YEAST	non-	T26A5.9	WBGene00008428	x
EF2_YEAST	essential	C36B1.7	WBGene00001167	x
EFG1_YEAST	non-	ZK1098.4	WBGene00009246	x
EFTU_YEAST	essential	F11A3.2	WBGene00007000	x
EGD2_YEAST	non-	D2085.3	WBGene00022042	x
EGD2_YEAST	essential	F25H5.4	WBGene00022042	x
EM24_YEAST	non-	F29C12.4	WBGene00004766	
END1_YEAST	essential	Y71H2_378.a	WBGene00011067	
ENP1_YEAST	non-	Y65B4B_10.b	WBGene00000276	x
ER19_YEAST	essential	Y65B4B_10.d	WBGene00012984	
ERF1_YEAST	non-	W02D7.7	WBGene00020269	x
ERS1_YEAST	essential	R06F6.2	WBGene00008052	
ERV1_YEAST	non-	F57B9.5	WBGene00018955	
ESA1_YEAST	essential	Y48B6A.13a	WBGene00007029	x
ETFA_YEAST	non-	T05H4.6	WBGene00009187	x
ETFB_YEAST	essential	C41C4.7	WBGene00017734	
ETFD_YEAST	non-	F56C11.3	WBGene00002855	x
F16P_YEAST	essential	VC5.4	WBGene00001404	
FAB1_YEAST	non-	F27D4.1	WBGene00004089	
FAB1_YEAST	essential	F23C8.5	WBGene00004089	
FAD1_YEAST	non-	C05D11.12	WBGene00011271	
FBRL_YEAST	essential	K07A3.b	WBGene00001423	x
FKB2_YEAST	non-	C05E7.5	WBGene00001426	

FKH2_YEAST	essential	VF11C1L.1	WBGene00003976	
FLX1_YEAST	non-	R53.1	WBGene00010459	
FMS1_YEAST	essential	T01C3.7	WBGene00000137	
FMS1_YEAST	non-	F36H1.1	WBGene00000137	
FOLE_YEAST	essential	T28H11.4	WBGene00017777	
FOX2_YEAST	non-	K01C8.7	WBGene00000991	
FRDA_YEAST	essential	R13G10.3	WBGene00001486	
FTHC_YEAST	essential	R13G10.2	WBGene00013708	
FU26_YEAST	essential	F25B5.6	WBGene00009499	
FUMH_YEAST	non-	M03A8.1	WBGene00001503	x
G6PD_YEAST	essential	F59G1.7	WBGene00007108	
G6PI_YEAST	non-	Y106G6E.4	WBGene00013597	
GAA1_YEAST	essential	F36H2.2	WBGene00018006	
GALX_YEAST	non-	H14A12.2	WBGene00008132	
GALY_YEAST	essential	B0035.5	WBGene00004106	
GALY_YEAST	non-	Y87G2A.q	WBGene00004106	
GAR1_YEAST	essential	F33D11.9	WBGene00022046	
GATA_YEAST	non-	C47B2.6	WBGene00001794	
GATH_YEAST	essential	C24A8.2	WBGene00021508	
GBLP_YEAST	essential	C24A8.3	WBGene00010556	x
GC14_YEAST	non-	Y66H1A.4	WBGene00012192	
GC20_YEAST	essential	K04D7.3	WBGene00018339	
GCH1_YEAST	non-	Y41D4A_3192.	WBGene00000298	
GCN2_YEAST	essential	a	WBGene00013591	
GCN5_YEAST	non-	K04D7.1	WBGene00021636	
GCR3_YEAST	essential	W02A11.1	WBGene00018156	x
GCS1_YEAST	essential	F42A10.1	WBGene00010500	
GCSP_YEAST	essential	F32G8.6	WBGene00020022	
GCST_YEAST	non-	Y81G3A.3	WBGene00017765	
GDA1_YEAST	essential	Y47G6A_241.b	WBGene00010697	
GDIR_YEAST	non-	F37E3.1	WBGene00004356	
GEF1_YEAST	essential	K02B12.7	WBGene00000532	
GIR2_YEAST	non-	R12C12.1	WBGene00012037	
GLGB_YEAST	essential	F25B4.1	WBGene00011409	
GLN3_YEAST	non-	K08H10.4	WBGene00001249	x
GLO3_YEAST	essential	F46H6.1	WBGene00017217	
GLPK_YEAST	non-	C07H4.2	WBGene00020007	
GLT1_YEAST	essential	T26E3.4	WBGene00012326	x
GLT1_YEAST	essential	T04A8.7	WBGene00012326	
GLY1_YEAST	non-	W09C2.1	WBGene00011291	
GLYC_YEAST	essential	F07F6.4	WBGene00003214	x
GP12_YEAST	non-	R11F4.1	WBGene00013131	
GPI1_YEAST	essential	W07E11.3	WBGene00008504	
GPI3_YEAST	essential	W07E11.1	WBGene00008431	
GPI8_YEAST	essential	R102.4	WBGene00011482	
GRPE_YEAST	non-	C05D11.11	WBGene00007927	
GSH1_YEAST	essential	Y52B11C.1	WBGene00001527	
GSHB_YEAST	essential	F01G4.5	WBGene00010941	
GSHR_YEAST	essential	D2085.6	WBGene00008117	
GTR1_YEAST	non-	T05E11.6	WBGene00006414	
GTR2_YEAST	essential	C34C12.8	WBGene00012497	
GUAA_YEAST	non-	F37B12.2	WBGene00010912	
GUAD_YEAST	essential	M176.2	WBGene00000775	
GUF1_YEAST	non-	C46F11.2	WBGene00022862	
GYP1_YEAST	essential	T24F1.1	WBGene00009322	
GYP2_YEAST	non-	Y24F12A.a	WBGene00012868	
GYP2_YEAST	essential	M106.4	WBGene00012868	
H2AV_YEAST	non-	F38E11.3	WBGene00019947	
HAP2_YEAST	essential	ZK1236.1	WBGene00013178	
HAS1_YEAST	essential	F32B6.8	WBGene00015232	x
HAT1_YEAST	non-	Y38H8A.1	WBGene00010841	
HCM1_YEAST	essential	Y45F10A.6	WBGene00001442	
HDA1_YEAST	essential	R08C7.3	WBGene00018319	
HMCS_YEAST	non-	Y53H1A.d	WBGene00017769	x
HMT1_YEAST	essential	B0511.6	WBGene00013766	
HNT1_YEAST	non-	M03C11.4	WBGene00009002	
HR25_YEAST	essential	C25A1.2	WBGene00002202	
HS49_YEAST	non-	F41H10.6	WBGene00004723	x
HST2_YEAST	essential	F25B4.6	WBGene00004800	

HUL5_YEAST	essential	Y113G7B.17	WBGene00003898	
IDH2_YEAST	essential	F21C3.3	WBGene00009664	x
IF1A_YEAST	essential	C03C10.1	WBGene00019162	x
IF2B_YEAST	essential	C08B11.5	WBGene00010560	x
IF2G_YEAST	essential	R11A8.4	WBGene00021466	x
IF2G_YEAST	non-	Y39A1C.2	WBGene00021466	x
IF2M_YEAST	essential	F43G9.1	WBGene00009771	
IF32_YEAST	non-	H06H21.3	WBGene00001232	x
IF34_YEAST	essential	K04G2.1	WBGene00001230	x
IF39_YEAST	non-	Y39G10A_246.	WBGene00001225	x
IF3A_YEAST	essential	h	WBGene00001209	x
IF3X_YEAST	essential	Y39G10A_246.	WBGene00000550	
IF3Y_YEAST	essential	c	WBGene00014120	
IF5_YEAST	non-	F46B6.6	WBGene00016496	
IF6_YEAST	essential	Y39G10A_237.	WBGene00001234	x
IKI3_YEAST	non-	b	WBGene00022463	
IM13_YEAST	essential	F22B5.2	WBGene00006574	
IM17_YEAST	non-	Y54E2A.11	WBGene00017119	x
IM44_YEAST	essential	C27D11.1	WBGene00020383	x
IMA1_YEAST	non-	F55H2.6	WBGene00002074	x
IMB2_YEAST	essential	ZK858.7	WBGene00002076	x
IMB3_YEAST	non-	C37C3.2	WBGene00002077	x
IMB3_YEAST	essential	C47B2.5	WBGene00002077	x
IME2_YEAST	essential	Y110A7A.e	WBGene00010860	
IMP3_YEAST	non-	DY3.1	WBGene00016740	x
IMP4_YEAST	essential	E04A4.5	WBGene00014083	x
INO1_YEAST	essential	T09B4.9	WBGene00012148	
IPYR_YEAST	essential	F32E10.4	WBGene00008149	x
IRE1_YEAST	non-	R06A4.4	WBGene00002147	
ISC1_YEAST	essential	C53D5.a	WBGene00012105	
ISY1_YEAST	non-	C53D5.i	WBGene00009966	x
KAD1_YEAST	essential	M04C9.5	WBGene00016205	x
KAPR_YEAST	non-	C48B6.2	WBGene00002190	
KAR3_YEAST	essential	ZK795.3	WBGene00002216	
KC2C_YEAST	non-	VF13D12L.1	WBGene00002196	
KEM1_YEAST	essential	C47E12.4	WBGene00012730	
KEM1_YEAST	non-	C41C4.4	WBGene00012730	
KGUA_YEAST	essential	T27F6.6	WBGene00020190	
KIME_YEAST	non-	F53B7.3	WBGene00021534	
KIP1_YEAST	essential	C29E4.8	WBGene00000257	
KYNU_YEAST	non-	R07E4.6	WBGene00015802	
LA17_YEAST	essential	T09A5.2	WBGene00006565	x
LAH1_YEAST	essential	T01G9.6	WBGene00016653	x
LCB1_YEAST	essential	Y39G8C.1	WBGene00016020	x
LCP5_YEAST	essential	Y39G8C.b	WBGene00003059	x
LEO1_YEAST	non-	T03F1.8	WBGene00007110	
LIP5_YEAST	essential	Y42G9A.c	WBGene00010809	x
LONM_YEAST	essential	F23B12.8	WBGene00016391	
LOS1_YEAST	essential	C15H9.7	WBGene00002080	
LSM1_YEAST	non-	Y63D3A.5	WBGene00003076	
LSM2_YEAST	essential	C44E4.4	WBGene00001808	
LSM3_YEAST	non-	C23H3.4	WBGene00003077	
LSM4_YEAST	essential	C48E7.3	WBGene00003078	
LSM5_YEAST	non-	B0035.11	WBGene00003079	
LSM6_YEAST	essential	M01F1.3	WBGene00003080	
LSM7_YEAST	non-	C34B2.6	WBGene00003081	
LYS9_YEAST	essential	C49H3.10	WBGene00019819	
MAD2_YEAST	non-	F40F8.9	WBGene00003161	
MAF1_YEAST	essential	T10G3.6	WBGene00016622	
MAK3_YEAST	non-	Y62E10A.l	WBGene00015074	
MAK5_YEAST	essential	F32A5.7	WBGene00018890	x
MAOX_YEAST	essential	F28F8.3	WBGene00012983	
MCM2_YEAST	essential	Y71G12A_187.	WBGene00003154	x
MCM5_YEAST	non-	b	WBGene00003157	x
MCX1_YEAST	essential	ZK593.7	WBGene00008412	
MDHM_YEAST	non-	R02D3.1	WBGene00003162	x
MDN1_YEAST	essential	Y69A2A_2326.	WBGene00018898	x
MDN1_YEAST	non-	a	WBGene00018898	x
MED7_YEAST	essential	C43H8.2	WBGene00002324	

MEU1_YEAST	non-	B0238.10	WBGene00015064	
MK16_YEAST	essential	F55F8.2	WBGene00015811	x
MK21_YEAST	non-	Y48B6A.12	WBGene00009084	x
MLH1_YEAST	essential	Y17G7B.5	WBGene00003373	
MMS2_YEAST	non-	R10E4.4	WBGene00006730	
MOD5_YEAST	essential	D2030.2	WBGene00001740	
MOT1_YEAST	non-	F20H11.3	WBGene00000274	
MP10_YEAST	essential	F55F10.1	WBGene00013544	x
MPG1_YEAST	non-	F55F10.2	WBGene00016583	x
MPPB_YEAST	essential	Y54E5B.3	WBGene00013880	
MR11_YEAST	non-	B0228.7	WBGene00003405	
MSH2_YEAST	essential	C16A3.6	WBGene00003418	
MSH4_YEAST	non-	F23B12.7	WBGene00001872	
MSH4_YEAST	essential	T28A8.7	WBGene00001872	
MSH5_YEAST	non-	F39B2.2	WBGene00003421	
MSH5_YEAST	essential	ZC395.6	WBGene00003421	
MSH6_YEAST	non-	F15D4.1	WBGene00003422	
MSN4_YEAST	essential	Y75B8A.7	WBGene00018990	
MSP1_YEAST	non-	C42C1.5	WBGene00010557	
MSRA_YEAST	essential	ZC410.2	WBGene00018393	
MSS1_YEAST	essential	ZC302.1	WBGene00009557	
MSS4_YEAST	non-	H26D21.2	WBGene00004087	x
MTHS_YEAST	essential	ZK1127.11	WBGene00015512	
MTO1_YEAST	non-	ZK1127.1	WBGene00009944	
MTR4_YEAST	essential	F09E8.3	WBGene00012342	x
MTRA_YEAST	non-	F09E8.4	WBGene00006647	x
N145_YEAST	essential	Y47G6A_242.c	WBGene00003796	x
NAB4_YEAST	essential	F56F11.3	WBGene00003423	
NADE_YEAST	non-	K04D7.2	WBGene00007698	
NAH2_YEAST	essential	F43E2.5	WBGene00003733	
NAP1_YEAST	non-	F39B2.7	WBGene00017075	
NAT1_YEAST	essential	F55A12.3	WBGene00021754	
NAT1_YEAST	non-	C06A8.1	WBGene00021754	
NB35_YEAST	essential	F52H3.2	WBGene00008664	
NCL1_YEAST	essential	W08D2.7	WBGene00021686	
NCPR_YEAST	non-	F53G2.6	WBGene00019632	x
NDK_YEAST	essential	ZK328.5	WBGene00009119	
NEP1_YEAST	essential	R10E9.1	WBGene00012652	
NFU1_YEAST	non-	C24F3.4	WBGene00003064	
NHP2_YEAST	essential	F57C7.2	WBGene00012964	x
NHPX_YEAST	non-	D2096.8	WBGene00010896	x
NIP7_YEAST	essential	Y50D7_162.b	WBGene00016607	x
NMD3_YEAST	non-	Y50D7_164.a	WBGene00012030	x
NMT_YEAST	essential	F10G8.6	WBGene00020549	x
NOG1_YEAST	non-	Y48G8A_2614.	WBGene00020297	x
NOP2_YEAST	essential	a	WBGene00021073	x
NOP4_YEAST	non-	K10D2.6	WBGene00011043	x
NOP5_YEAST	essential	F25H2.5	WBGene00020915	
NOT1_YEAST	non-	Y39A1A.14	WBGene00003824	
NPR2_YEAST	essential	R10H10.1	WBGene00018635	
NRD1_YEAST	non-	Y48A6B.3	WBGene00017004	
NRK1_YEAST	essential	M28.5	WBGene00001526	
NTF2_YEAST	non-	C43E11.9	WBGene00004305	x
NU49_YEAST	essential	T25G3.3	WBGene00003790	x
O14467	non-	T17E9.2	WBGene00003148	
OAT_YEAST	essential	T07A9.9	WBGene00015814	
ODO1_YEAST	non-	W07E6.1	WBGene00020679	x
ODO2_YEAST	essential	R05H10.2	WBGene00020950	x
ODP2_YEAST	non-	W01B11.3	WBGene00009082	x
ODPA_YEAST	essential	F57B9.2	WBGene00011510	x
ODPB_YEAST	non-	F49E8.1	WBGene00015413	
OM20_YEAST	essential	D1007.7	WBGene00009092	
OM40_YEAST	non-	T19A5.2	WBGene00007686	x
ORC2_YEAST	essential	R05D11.3	WBGene00003882	
ORN_YEAST	non-	Y54E5A.4	WBGene00007429	
OSTA_YEAST	essential	H21P03.1	WBGene00020683	
OSTB_YEAST	non-	C16A3.10	WBGene00011638	x
OSTE_YEAST	essential	T22B11.5	WBGene00000896	x
OSTG_YEAST	non-	W02F12.5	WBGene00022793	x

OXA1_YEAST	essential	F23B12.5	WBGene00007215	x
PAC1_YEAST	non-	T05H10.6	WBGene00003047	
PAC2_YEAST	essential	C04C3.3	WBGene00019503	
PAF1_YEAST	non-	F23H12.2	WBGene00008338	x
PAN3_YEAST	essential	C18E9.6	WBGene00014015	
PBP2_YEAST	essential	F59E10.1	WBGene00017816	
PBS2_YEAST	non-	C08B6.8	WBGene00003186	x
PBS2_YEAST	essential	T22D1.4	WBGene00003186	x
PCH2_YEAST	essential	T09A5.11	WBGene00008641	
PDAT_YEAST	essential	F57B10.10	WBGene00010872	
PDS5_YEAST	essential	ZK686.3	WBGene00001352	
PDX3_YEAST	non-	C01A2.3	WBGene00018996	
PE11_YEAST	essential	T03F6.5	WBGene00014234	
PEP3_YEAST	non-	K07H8.1	WBGene00021058	
PESC_YEAST	essential	C55A6.9	WBGene00003063	x
PESC_YEAST	non-	ZK632.7	WBGene00003063	x
PET8_YEAST	essential	F26B1.2	WBGene00008364	
PEX1_YEAST	non-	Y54E10B_152.	WBGene00004191	
PEX1_YEAST	essential	e	WBGene00004191	
PEX5_YEAST	non-	Y54E10B_152.	WBGene00004194	x
PEX6_YEAST	essential	b	WBGene00004195	
PEXC_YEAST	non-	F10B5.5	WBGene00004197	
PEXD_YEAST	essential	M05B5.4	WBGene00004198	
PFD2_YEAST	non-	H38K22.1	WBGene00019220	
PFD3_YEAST	essential	F57B9.1	WBGene00006889	x
PFD4_YEAST	non-	ZK1128.8	WBGene00007107	
PFD5_YEAST	essential	W06B4.3	WBGene00020112	
PFD6_YEAST	non-	R13A5.12	WBGene00009004	x
PGK_YEAST	essential	R13A5.13	WBGene00020185	
PHSG_YEAST	non-	D1046.3	WBGene00020696	
PHSG_YEAST	essential	C11H1.4	WBGene00020696	
PHSG_YEAST	non-	C11H1.6	WBGene00020696	
PHSG_YEAST	essential	C34C6.6	WBGene00020696	
PHSG_YEAST	non-	F39G3.7	WBGene00020696	
PHSG_YEAST	essential	F08B12.2	WBGene00020696	
PHSG_YEAST	non-	F32A5.6	WBGene00020696	
PIF1_YEAST	essential	H20J04.d	WBGene00004028	
PIF1_YEAST	essential	T06G6.9	WBGene00004028	
PIF1_YEAST	non-	B0035.4	WBGene00004028	
PIK1_YEAST	essential	R151.9	WBGene00018076	
PIS_YEAST	non-	F21C3.5	WBGene00012897	
PLC1_YEAST	essential	T03F1.3	WBGene00004038	x
PMM_YEAST	non-	F40C5.b	WBGene00009925	x
PNPH_YEAST	essential	F40C5.c	WBGene00019298	
POP1_YEAST	essential	F40C5.d	WBGene00015486	
POP2_YEAST	non-	F40C5.a	WBGene00000369	
POP4_YEAST	essential	F40C5.e	WBGene00007603	
PPE1_YEAST	non-	F40C5.h	WBGene00007188	x
PPT1_YEAST	essential	T22F3.3	WBGene00012665	
PR16_YEAST	non-	Y18H1A_67.d	WBGene00003389	x
PR17_YEAST	essential	Y18H1A_67.f	WBGene00018625	x
PR21_YEAST	essential	Y18H1A_67.c	WBGene00004188	x
PR22_YEAST	non-	F35H12.4	WBGene00003393	x
PR28_YEAST	essential	Y46G5.e	WBGene00017162	x
PR31_YEAST	non-	T01E8.3	WBGene00022458	x
PR39_YEAST	essential	F52B11.2	WBGene00017768	
PR40_YEAST	non-	K02D7.1	WBGene00014218	
PR40_YEAST	essential	C05D11.9	WBGene00014218	
PR43_YEAST	essential	Y56A3A.20	WBGene00018967	x
PR46_YEAST	essential	C15C6.4	WBGene00006481	x
PRI2_YEAST	essential	B0464.7	WBGene00004181	x
PROA_YEAST	essential	Y39B6B.ff	WBGene00011938	
PROC_YEAST	non-	K03H1.2	WBGene00010924	
PROF_YEAST	essential	F49D11.1	WBGene00003991	
PRP3_YEAST	essential	W07E6.4	WBGene00010844	x
PRP4_YEAST	essential	EEED8.5	WBGene00007972	x
PRP9_YEAST	essential	F01F1.7	WBGene00011758	x
PRS6_YEAST	essential	Y110A7A.m	WBGene00004503	x
PRS7_YEAST	non-	F25B4.5	WBGene00004501	x

PRSA_YEAST	essential	ZK1098.7	WBGene00004505	x
PSA1_YEAST	essential	ZK1098.1	WBGene00003927	x
PSA2_YEAST	essential	F56D2.6	WBGene00003923	
PSA5_YEAST	essential	D1054.15	WBGene00003926	x
PSA7_YEAST	non-	W02D9.1	WBGene00003925	x
PSB1_YEAST	essential	T22H6.2	WBGene00003952	x
PSB2_YEAST	non-	M153.1	WBGene00003950	x
PSB3_YEAST	essential	K03E6.6	WBGene00003949	x
PSB4_YEAST	essential	M03C11.7	WBGene00003953	x
PSB5_YEAST	essential	C36B1.5	WBGene00003951	x
PSB7_YEAST	essential	T13H5.4	WBGene00003948	x
PSD1_YEAST	non-	F23F12.6	WBGene00015159	
PSD9_YEAST	essential	C52E4.4	WBGene00016623	
PSDA_YEAST	essential	F56H1.4	WBGene00018239	
PSDA_YEAST	essential	CD4.6	WBGene00018239	x
PSF2_YEAST	non-	D1054.2	WBGene00009287	x
PSP2_YEAST	essential	F25H2.9	WBGene00020726	
PT56_YEAST	essential	C36B1.4	WBGene00012864	
PT91_YEAST	essential	C02F5.3	WBGene00012483	
PUF3_YEAST	non-	T20F5.2	WBGene00004245	
PUR1_YEAST	essential	Y38A8.2	WBGene00011407	
PUR2_YEAST	essential	F39H11.5	WBGene00018174	
PUR4_YEAST	non-	K05C4.1	WBGene00008654	
PUR8_YEAST	essential	C47B2.4	WBGene00011064	x
PURA_YEAST	non-	B0361.5	WBGene00016509	
PUS3_YEAST	essential	C44B7.1	WBGene00006473	
PUT2_YEAST	non-	F40G9.1	WBGene00000112	
PUT2_YEAST	essential	W10C4.b	WBGene00000112	
PWP2_YEAST	non-	F31C3.5	WBGene00018891	x
PYR1_YEAST	essential	T23C6.2	WBGene00004259	x
PYRD_YEAST	non-	Y45F3A.9	WBGene00020932	
PYRF_YEAST	essential	Y18D10A.16	WBGene00011559	
Q02804	non-	W06B11.2	WBGene00021841	
Q02875	essential	T04A8.5	WBGene00016002	
Q02889	non-	F38B6.4	WBGene00008860	
Q02890	essential	F10F2.2	WBGene00010160	
Q02908	non-	R06C7.5	WBGene00014123	
Q03195	essential	C37H5.6	WBGene00012714	x
Q03201	non-	E02H1.3	WBGene00012556	
Q03390	essential	Y57G7A.10	WBGene00018290	
Q03761	essential	F56D12.1	WBGene00006396	
Q03778	essential	F55F8.3	WBGene00011224	
Q03786	non-	D2085.1	WBGene00017818	
Q03920	essential	W02D3.2	WBGene00016341	
Q03940	non-	T07C4.1	WBGene00007784	x
Q04048	essential	Y54E10B_159.	WBGene00016837	x
Q04049	non-	i	WBGene00018721	
Q04081	essential	C18H9.3	WBGene00007137	
Q04149	non-	F15D4.3	WBGene00016602	
Q04311	essential	Y53H1C.a	WBGene00019457	
Q04396	essential	ZK863.3	WBGene00020895	
Q04430	essential	Y39E4B.1	WBGene00004068	
Q04600	non-	Y37D8A.18	WBGene00016113	
Q05498	essential	F41E6.9	WBGene00009266	x
Q05521	non-	Y56A3A.4	WBGene00018756	
Q05583	essential	R10H10.6	WBGene00012479	
Q05946	non-	F26D11.1	WBGene00013143	x
Q06102	essential	C33C12.9	WBGene00008689	x
Q06106	non-	C27H6.2	WBGene00004315	x
Q06132	essential	C50F2.3	WBGene00013129	
Q06143	non-	F53A3.2	WBGene00019656	
Q06152	essential	B0285.4	WBGene00011451	
Q06338	essential	C43E11.2	WBGene00022851	x
Q06344	essential	K06H7.3	WBGene00010231	x
Q06385	essential	T28D9.3	WBGene00021644	x
Q06505	essential	C10G11.5	WBGene00008729	
Q06510	non-	C25H3.4	WBGene00006491	
Q06632	essential	F30A10.9	WBGene00022301	x
Q06632	non-	F53C3.13	WBGene00022301	x

Q06672	essential	Y18D10A.9	WBGene00013109	x
Q06685	non-	Y53C12B.1	WBGene00018508	
Q06696	essential	F11A10.3	WBGene00008919	
Q07381	non-	T23F6.4	WBGene00006497	x
Q07457	essential	Y52B11A.10	WBGene00007008	
Q07508	non-	K11G12.5	WBGene00015207	
Q07830	essential	T04H1.5	WBGene00016159	
Q07896	non-	ZK1127.4	WBGene00016508	x
Q07914	essential	F58B3.4	WBGene00001039	
Q08004	essential	Y47G6A_247.a	WBGene00013236	
Q08023	non-	F13B12.1	WBGene00010768	
Q08444	essential	ZK809.2	WBGene00021843	
Q08444	essential	Y76B12C_65.a	WBGene00021843	
Q08685	essential	Y76B12C_66.c	WBGene00010304	
Q08723	non-	Y51H4A.m	WBGene00004464	x
Q08726	essential	F46F11.1	WBGene00015029	
Q08920	non-	F17C11.8	WBGene00009141	x
Q08951	essential	F10G7.1	WBGene00000162	
Q08952	essential	R05D3.4	WBGene00018700	
Q08963	essential	B0495.8	WBGene00019223	
Q08971	non-	C27A12.9	WBGene00013200	
Q12000	essential	C37H5.5	WBGene00009189	
Q12004	non-	T19B4.4	WBGene00014230	
Q12028	essential	Y56A3A.18	WBGene00000158	x
Q12029	essential	K11D2.1	WBGene00005150	
Q12035	essential	Y54E10B_159.	WBGene00021715	
Q12049	non-	e	WBGene00017158	
Q12052	essential	Y54E10B_159.	WBGene00011631	x
Q12059	non-	g	WBGene00006735	
Q12090	essential	F59A2.4	WBGene00004095	
Q12102	non-	R12E2.3	WBGene00017313	x
Q12118	essential	B0207.6	WBGene00019893	
Q12142	essential	F26A3.2	WBGene00020706	
Q12186	essential	W09G10.4	WBGene00013808	x
Q12199	essential	F52E1.13	WBGene00022803	
Q12275	essential	H20J04.c	WBGene00017882	x
Q12280	non-	Y54E5A.5	WBGene00003980	
Q12309	essential	F27D4.4	WBGene00019762	
Q12311	non-	ZK1128.4	WBGene00003034	
Q12354	essential	Y105E8C.n	WBGene00010564	
Q12368	non-	C41C4.2	WBGene00022794	
Q12383	essential	Y49F6B.q	WBGene00013026	
Q12395	non-	F01F1.1	WBGene00010428	x
Q12400	essential	T08G11.4	WBGene00009131	
Q12449	non-	C26E6.8	WBGene00007235	
Q12453	essential	F52C9.4	WBGene00012337	
Q12463	non-	F09G2.4	WBGene00022107	
Q12463	essential	R05F9.10	WBGene00022107	
Q12464	non-	T22H9.2	WBGene00020687	x
Q12468	essential	Y116A8C.32	WBGene00000817	
Q12481	non-	C02C2.6	WBGene00020705	
Q12483	essential	F28B3.1	WBGene00016167	
Q12500	non-	F09C3.1	WBGene00020822	
Q12511	essential	M03F8.3	WBGene00022832	
Q874G8	non-	F42A9.2	WBGene00013025	x
Q92317	essential	K04G2.5	WBGene00001092	
Q99190	non-	ZK686.4	WBGene00000198	
Q99207	essential	Y49A3A.3	WBGene00021660	x
Q99216	essential	H38K22.2	WBGene00013144	
Q99247	non-	F25H8.1	WBGene00009441	
Q99344	essential	C01G10.8	WBGene00004341	
QOR_YEAST	non-	W07G4.3	WBGene00009554	
QRI7_YEAST	essential	Y71F9A_270.a	WBGene00007237	
R10A_YEAST	essential	Y71F9A_270.b	WBGene00004412	x
R161_YEAST	non-	T22D1.10	WBGene00010272	
R167_YEAST	essential	B0547.1	WBGene00020209	
R167_YEAST	essential	T22H9.1	WBGene00020209	
RA14_YEAST	essential	C27F2.5	WBGene00006963	
RA23_YEAST	essential	T26A5.6	WBGene00013924	

RA27_YEAST	non-	ZK973.a	WBGene00000794	x
RA50_YEAST	essential	Y49A3A.2	WBGene00004296	
RA51_YEAST	non-	F53A2.5	WBGene00004297	
RA54_YEAST	essential	C15F1.c	WBGene00004298	
RAD1_YEAST	non-	Y48G1A_54.d	WBGene00008140	
RAD2_YEAST	essential	Y53C12B.2	WBGene00019004	
RAD5_YEAST	non-	F35G12.4	WBGene00010061	
RAM1_YEAST	essential	F11H8.1	WBGene00009083	
RAM2_YEAST	essential	F39B2.3	WBGene00019823	
RBSK_YEAST	non-	C01G10.10	WBGene00008548	
RBX1_YEAST	essential	Y71F9A_294.c	WBGene00004320	x
RCC1_YEAST	non-	F58G6.1	WBGene00004304	x
RCL1_YEAST	essential	T04C9.1	WBGene00022852	x
RER1_YEAST	non-	ZK328.3	WBGene00009783	
REV1_YEAST	essential	K07G5.2	WBGene00014066	x
RF1M_YEAST	essential	ZK20.3	WBGene00020993	
RFA1_YEAST	non-	Y47G6A_247.i	WBGene00017546	x
RFC1_YEAST	essential	T04H1.4	WBGene00004337	
RFC2_YEAST	essential	Y43C5A.6	WBGene00004340	
RFC4_YEAST	non-	W06D4.6	WBGene00004338	
RFC5_YEAST	essential	C47D12.8	WBGene00004339	
RGD1_YEAST	essential	F57B10.6	WBGene00001559	
RGD1_YEAST	essential	F54E12.2	WBGene00001559	
RIM2_YEAST	essential	F23B12.6	WBGene00011662	
RIO1_YEAST	essential	R02D3.5	WBGene00019698	
RIR2_YEAST	essential	F07A11.5	WBGene00004392	x
RL10_YEAST	essential	ZK287.5	WBGene00004421	x
RL12_YEAST	essential	C26D10.1	WBGene00004424	x
RL18_YEAST	essential	ZK1127.5	WBGene00004430	x
RL19_YEAST	non-	F46C5.8	WBGene00004431	x
RL2_YEAST	essential	ZK675.2	WBGene00004413	x
RL20_YEAST	essential	W03F8.3	WBGene00004432	x
RL20_YEAST	essential	F18A1.5	WBGene00004432	x
RL23_YEAST	essential	C54G10.2	WBGene00004435	x
RL3_YEAST	essential	F31E3.3	WBGene00004414	x
RL30_YEAST	non-	F58F6.4	WBGene00004444	x
RL35_YEAST	essential	C39E9.13	WBGene00004449	x
RL38_YEAST	non-	F45H7.2	WBGene00004452	x
RL39_YEAST	essential	F45H7.3	WBGene00004453	
RL43_YEAST	non-	T09F3.2	WBGene00004456	
RL5_YEAST	essential	M01B12.5	WBGene00004416	x
RLA0_YEAST	non-	C03C10.3	WBGene00004408	x
RLR1_YEAST	essential	F10B5.1	WBGene00015813	
RM09_YEAST	non-	JC8.3	WBGene00016142	x
RM19_YEAST	essential	Y45F10D.12	WBGene00015133	
RN15_YEAST	non-	C09D4.5	WBGene00000774	x
RNH1_YEAST	essential	B0250.1	WBGene00019088	
ROK1_YEAST	non-	E04A4.8	WBGene00011032	
ROX1_YEAST	essential	Y17G9B.e	WBGene00001560	
ROX1_YEAST	non-	B0336.10	WBGene00001560	
RPA2_YEAST	essential	F13B10.2	WBGene00008781	x
RPA9_YEAST	essential	Y106G6H.3	WBGene00007616	x
RPB1_YEAST	essential	ZK652.4	WBGene00000123	
RPB2_YEAST	non-	C06B8.8	WBGene00016140	x
RPB3_YEAST	essential	C26F1.9	WBGene00007971	x
RPB6_YEAST	essential	Y48B6A.2	WBGene00007355	
RPB7_YEAST	essential	F54C9.5	WBGene00021845	x
RPB8_YEAST	essential	F25H2.10	WBGene00017830	x
RPBY_YEAST	non-	C16A3.8	WBGene00012187	x
RPC1_YEAST	essential	C26E6.6	WBGene00004411	x
RPC2_YEAST	non-	B0303.15	WBGene00017300	x
RPC5_YEAST	essential	F56A8.6	WBGene00019275	x
RPC6_YEAST	non-	F59A6.6	WBGene00021112	
RPC9_YEAST	essential	R05D11.4	WBGene00010230	
RPCY_YEAST	non-	T22H6.6	WBGene00014111	x
RPCZ_YEAST	essential	C52G5.1	WBGene00022309	
RPF1_YEAST	non-	F14B4.3	WBGene00009711	x
RPIA_YEAST	essential	C15H11.8	WBGene00015101	
RPN1_YEAST	non-	F36A4.7	WBGene00004458	x

RPN2_YEAST	essential	C26E6.4	WBGene00004459	x
RPN3_YEAST	non-	C36B1.3	WBGene00004460	x
RPN5_YEAST	essential	C06A1.5	WBGene00004461	
RPN7_YEAST	non-	Y54E10B_159.	WBGene00004463	
RPN9_YEAST	essential	c	WBGene00004465	
RPNA_YEAST	non-	F26F4.11	WBGene00004466	
RPNC_YEAST	essential	W01G7.3	WBGene00004468	
RR40_YEAST	non-	C42D4.8	WBGene00010325	x
RR41_YEAST	essential	F09F7.3	WBGene00007201	x
RR44_YEAST	non-	H43I07.2	WBGene00001001	x
RR45_YEAST	essential	W09C3.4	WBGene00018154	x
RRN3_YEAST	essential	F58A4.9	WBGene00007980	
RRP1_YEAST	non-	ZK856.10	WBGene00008151	x
RRP3_YEAST	essential	Y77E11A_3443	WBGene00012059	x
RRP5_YEAST	non-	.q	WBGene00015808	
RRP6_YEAST	essential	F44G4.1	WBGene00000796	x
RRS1_YEAST	non-	B0280.3	WBGene00007617	x
RS11_YEAST	essential	T22D1.9	WBGene00004480	x
RS13_YEAST	essential	C23G10.4	WBGene00004482	
RS15_YEAST	essential	C30C11.2	WBGene00004484	x
RS16_YEAST	non-	F10G7.8	WBGene00004485	x
RS18_YEAST	essential	F49C12.8	WBGene00004487	x
RS2_YEAST	non-	T06D8.8	WBGene00004471	x
RS20_YEAST	essential	B0205.3	WBGene00004489	x
RS21_YEAST	non-	ZK20.5	WBGene00004490	x
RS22_YEAST	essential	F59C6.4	WBGene00004491	x
RS23_YEAST	non-	B0564.1	WBGene00004492	x
RS24_YEAST	essential	C04G2.6	WBGene00004493	x
RS25_YEAST	non-	F37C12.13	WBGene00004494	
RS28_YEAST	essential	C36E8.1	WBGene00004497	
RS3_YEAST	non-	C47E12.7	WBGene00004472	x
RS30_YEAST	essential	T26G10.1	WBGene00004499	
RS37_YEAST	non-	C16A3.3	WBGene00006725	x
RS37_YEAST	essential	C14A4.4	WBGene00006725	x
RS37_YEAST	non-	C15H11.9	WBGene00006725	x
RS37_YEAST	essential	F40F11.1	WBGene00006725	x
RS5_YEAST	non-	C16A3.9	WBGene00004474	x
RS8_YEAST	essential	F36A2.6	WBGene00004477	x
RS8_YEAST	non-	T01C3.6	WBGene00004477	x
RSD1_YEAST	essential	Y57G11C.16	WBGene00009264	x
RSMB_YEAST	non-	C49H3.11	WBGene00004915	x
RSP5_YEAST	essential	Y105E8C.e	WBGene00007009	
RSP5_YEAST	non-	F37C12.11	WBGene00007009	
RSP5_YEAST	essential	F53A3.3	WBGene00007009	x
RT04_YEAST	non-	F28D1.7	WBGene00020718	
RTF1_YEAST	essential	T07A9.11	WBGene00009103	x
RTS2_YEAST	non-	K02B2.5	WBGene00013128	x
RUXE_YEAST	essential	Y41D4A_3613.	WBGene00004919	x
RUXF_YEAST	non-	a	WBGene00004918	x
RUXG_YEAST	essential	C23G10.3	WBGene00004920	x
S160_YEAST	non-	C26F1.4	WBGene00007463	
S3B1_YEAST	essential	H06I04.a	WBGene00011605	x
SAHH_YEAST	non-	H06I04.f	WBGene00019322	x
SAR1_YEAST	essential	Y53G8B_1025.	WBGene00022678	x
SC10_YEAST	non-	a	WBGene00016376	
SC13_YEAST	essential	Y53G8B_1025.	WBGene00003806	x
SC15_YEAST	non-	b	WBGene00016188	
SC17_YEAST	essential	T05E11.1	WBGene00017016	
SC18_YEAST	non-	F42C5.8	WBGene00003818	x
SC18_YEAST	essential	F42C5.1	WBGene00003818	x
SC22_YEAST	non-	F30A10.6	WBGene00018853	
SC23_YEAST	essential	W08E3.1	WBGene00004754	x
SC62_YEAST	non-	Y65B4B_10.a	WBGene00007683	
SC65_YEAST	essential	Y65B4B_10.e	WBGene00018159	
SCD6_YEAST	non-	Y65B4B_11.a	WBGene00012484	
SCS7_YEAST	essential	T23B12.3	WBGene00007707	x
SEC6_YEAST	essential	F25B3.6	WBGene00017284	x
SEC7_YEAST	essential	Y52B11A.9	WBGene00012386	x
SEC7_YEAST	non-	Y49E10.15	WBGene00012386	x

SED5_YEAST	essential	ZK652.1	WBGene00006373	x
SEH1_YEAST	essential	Y71F9B_286.b	WBGene00003804	
SERB_YEAST	non-	C08H9.2	WBGene00013379	
SET2_YEAST	essential	T08A11.2	WBGene00021515	
SET2_YEAST	essential	K02F2.2	WBGene00021515	
SFT2_YEAST	non-	ZK180.4	WBGene00007690	
SFT2_YEAST	essential	C33H5.9	WBGene00007690	x
SGPL_YEAST	essential	Y77E11A_3670	WBGene00004981	
SGS1_YEAST	non-	.c	WBGene00001865	
SGT1_YEAST	essential	C28G1.3	WBGene00008371	
SIK1_YEAST	non-	D1014.3	WBGene00010627	x
SIN3_YEAST	essential	ZK1014.1	WBGene00004117	
SIS1_YEAST	essential	H15N14.1	WBGene00001031	
SKI2_YEAST	non-	F55A4.1	WBGene00008502	
SLU7_YEAST	essential	Y113G7A.3	WBGene00010629	x
SLY1_YEAST	essential	C18E9.2	WBGene00009654	x
SMC1_YEAST	non-	F37F2.2	WBGene00001860	x
SMC2_YEAST	essential	Y18D10A.17	WBGene00003367	
SMC2_YEAST	essential	C25A1.5	WBGene00003367	x
SMC3_YEAST	essential	F09E5.6	WBGene00004873	
SMC4_YEAST	essential	Y87G2A.y	WBGene00004874	x
SMD1_YEAST	essential	Y6B3A.1	WBGene00004916	x
SMD2_YEAST	essential	F55A11.2	WBGene00004917	x
SMD3_YEAST	essential	Y43F4B.4	WBGene00004914	x
SMM1_YEAST	essential	Y62E10A.m	WBGene00013201	
SMP2_YEAST	essential	Y41D4A_2615.	WBGene00010425	
SMT3_YEAST	non-	a	WBGene00004888	x
SNF4_YEAST	essential	Y41D4A_3457.	WBGene00013732	
SNF5_YEAST	essential	b	WBGene00011111	x
SNX3_YEAST	essential	C18E9.10	WBGene00006503	
SNX4_YEAST	essential	C18E9.4	WBGene00003086	
SNX4_YEAST	essential	Y66H1B.4	WBGene00003086	
SOF1_YEAST	essential	T04A11.6	WBGene00022742	x
SOK1_YEAST	essential	D1054.3	WBGene00010875	
SP11_YEAST	essential	K07C5.4	WBGene00004985	
SP14_YEAST	non-	F02E9.4	WBGene00004040	
SP14_YEAST	essential	F54D5.8	WBGene00004040	
SP14_YEAST	essential	F01G4.3	WBGene00004040	
SPB1_YEAST	essential	K07C5.6	WBGene00019168	x
SPC3_YEAST	essential	F43D9.3	WBGene00019679	x
SPEE_YEAST	essential	F28B3.7	WBGene00012909	
SPT4_YEAST	essential	M106.1	WBGene00005014	
SPT5_YEAST	essential	R06F6.10	WBGene00005015	x
SR40_YEAST	essential	Y47D3A.aa	WBGene00000931	
SR54_YEAST	essential	F35G12.8	WBGene00009012	
SR68_YEAST	essential	T28D9.10	WBGene00010097	x
SRPR_YEAST	essential	C52E4.3	WBGene00009521	x
SSL1_YEAST	non-	Y116A8C.42	WBGene00011814	x
ST20_YEAST	essential	Y54E5A.6	WBGene00003911	
ST22_YEAST	non-	H37A05.1	WBGene00015658	
ST24_YEAST	essential	K12C11.2	WBGene00001405	
STI1_YEAST	non-	Y111B2C.h	WBGene00019983	
STT3_YEAST	essential	R07E5.3	WBGene00020437	x
SUB2_YEAST	non-	W06D4.5	WBGene00001840	x
SUCB_YEAST	essential	Y37A1B.2a	WBGene00009812	
SUG2_YEAST	essential	Y37A1B.3	WBGene00004504	x
SULX_YEAST	non-	ZK430.7	WBGene00017464	
SUV3_YEAST	essential	M05D6.2	WBGene00007444	
SYAC_YEAST	non-	T05E11.4	WBGene00000197	x
SYC_YEAST	essential	C04G6.5	WBGene00000800	x
SYDC_YEAST	non-	C04G6.2	WBGene00001094	x
SYDM_YEAST	essential	C04G6.3	WBGene00001095	
SYEM_YEAST	non-	H06I04.h	WBGene00001338	
SYFA_YEAST	essential	K12H4.4	WBGene00001497	x
SYFB_YEAST	non-	Y46G5.w	WBGene00001498	x
SYH_YEAST	essential	F54C4.2	WBGene00002001	x
SYIC_YEAST	non-	K08E4.1	WBGene00002152	x
SYIM_YEAST	essential	C25A1.10	WBGene00002153	x
SYKC_YEAST	non-	F21D5.7	WBGene00002238	x

SYLC_YEAST	essential	F55C5.8	WBGene00003073	x
SYMC_YEAST	essential	F38A1.8	WBGene00003415	x
SYNC_YEAST	non-	T16H12.4	WBGene00003815	x
SYPC_YEAST	essential	C09B8.7	WBGene00004190	
SYQ_YEAST	non-	C09G12.9	WBGene00001336	x
SYSC_YEAST	essential	C04F12.10	WBGene00005663	x
SYTC_YEAST	non-	R09E12.3	WBGene00006617	x
SYV_YEAST	essential	T12A2.2	WBGene00006936	x
SYWC_YEAST	non-	C26D10.2	WBGene00006945	x
SYWC_YEAST	essential	F47B10.1	WBGene00006945	
SYWM_YEAST	essential	F23F1.8	WBGene00006946	
SYYM_YEAST	non-	F14D12.5	WBGene00006968	x
T145_YEAST	essential	C08F8.2	WBGene00006382	x
T145_YEAST	non-	F28H1.3	WBGene00006382	x
T145_YEAST	essential	Y23H5A.7	WBGene00006382	x
T145_YEAST	non-	B0464.1	WBGene00006382	
T2D4_YEAST	essential	F10C2.6	WBGene00006386	x
T2D5_YEAST	non-	T07A9.2	WBGene00006387	
T2EA_YEAST	essential	T08B2.9	WBGene00013998	x
T2FA_YEAST	non-	F22B5.9	WBGene00015296	
T2FB_YEAST	essential	T11G6.1	WBGene00012694	
TAD2_YEAST	non-	R11A8.6	WBGene00010436	
TBG_YEAST	essential	C25A1.7	WBGene00006540	x
TBP_YEAST	non-	T02G5.9	WBGene00006542	x
TBP7_YEAST	essential	R74.1	WBGene00008682	
TCPA_YEAST	non-	F58B3.5	WBGene00000377	x
TCPB_YEAST	essential	F22D6.3	WBGene00011889	x
TCPD_YEAST	essential	T27F6.5	WBGene00000379	x
TCPE_YEAST	essential	Y41E3.4	WBGene00000380	x
TCPG_YEAST	non-	C47E12.1	WBGene00018782	x
TCPH_YEAST	essential	C47D12.6	WBGene00020391	x
TCPQ_YEAST	essential	Y87G2A.l	WBGene00021934	x
TCPQ_YEAST	essential	Y80D3A.a	WBGene00021934	x
TCPZ_YEAST	non-	Y80D3A.b	WBGene00000381	x
TCTP_YEAST	essential	C34E10.4	WBGene00009122	x
TEL1_YEAST	non-	K08F11.4	WBGene00000227	
TF3B_YEAST	essential	W04A8.7	WBGene00000271	x
TFC5_YEAST	essential	Y71A12B.a	WBGene00015091	x
TFS2_YEAST	essential	Y71A12B.b	WBGene00012000	
TLG2_YEAST	non-	Y71A12B.c	WBGene00022534	
TOP1_YEAST	essential	F30F8.8	WBGene00006595	
TPIS_YEAST	non-	W09B6.2	WBGene00006601	
TR20_YEAST	essential	ZK550.4	WBGene00021046	
TRM8_YEAST	non-	C01F1.1	WBGene00012205	
TRMU_YEAST	essential	Y39B6A.f	WBGene00007114	
TWF1_YEAST	non-	JC8.4	WBGene00018187	
TXTP_YEAST	essential	F58A4.8	WBGene00010780	
TYDP_YEAST	non-	T20B12.2	WBGene00018678	
TYSY_YEAST	essential	F11A10.1	WBGene00022455	
UBA1_YEAST	essential	T05C12.7	WBGene00006699	
UBA2_YEAST	non-	T21B10.7	WBGene00006700	
UBC2_YEAST	essential	K01C8.10	WBGene00006701	
UBC3_YEAST	essential	C07G2.3	WBGene00006702	
UBC9_YEAST	non-	F54A3_31.e	WBGene00006706	
UBPE_YEAST	essential	T10B5.5	WBGene00020839	
UBR1_YEAST	essential	Y55F3A_750.c	WBGene00016326	
UCR7_YEAST	essential	Y55F3A_750.d	WBGene00020181	
UDPG_YEAST	essential	F01F1.8	WBGene00010665	x
UFD1_YEAST	essential	F25H2.11	WBGene00006733	x
UFD2_YEAST	essential	Y48G1C_55.a	WBGene00006734	
UGA2_YEAST	non-	F45E12.2	WBGene00000113	
ULP1_YEAST	essential	B0261.1	WBGene00006736	
UME3_YEAST	non-	T24H10.1	WBGene00000506	
UME5_YEAST	essential	ZC155.7	WBGene00000409	
UNG_YEAST	essential	M01E5.5	WBGene00013241	
UT11_YEAST	essential	Y17G7B.7	WBGene00007623	x
UTR4_YEAST	non-	W05H7.3	WBGene00010286	
VATC_YEAST	essential	W02B12.10	WBGene00006920	
VATD_YEAST	essential	B0035.16	WBGene00010130	

VATE_YEAST	non-	F38E9.5	WBGene00006917	x
VATF_YEAST	essential	K11H3.3	WBGene00006918	x
VATG_YEAST	essential	F52C12.1	WBGene00006919	x
VATO_YEAST	essential	Y110A7A.q	WBGene00011347	x
VP13_YEAST	non-	C47E12.5	WBGene00011629	
VP15_YEAST	essential	W02A11.4	WBGene00014151	
VP15_YEAST	non-	C35B1.1	WBGene00014151	
VP16_YEAST	essential	Y71G12A_187.	WBGene00006516	
VP26_YEAST	non-	a	WBGene00006931	
VP27_YEAST	essential	F29B9.6	WBGene00004101	x
VP28_YEAST	essential	T27A3.2	WBGene00013598	x
VP34_YEAST	non-	C32E8.11	WBGene00006932	
VP35_YEAST	essential	T02H6.11	WBGene00006933	
VP45_YEAST	non-	K08E3.5	WBGene00016643	
VP45_YEAST	essential	F19B6.2	WBGene00016643	
VPS4_YEAST	essential	T05H10.5	WBGene00021334	x
VPS5_YEAST	essential	F45H10.1	WBGene00004927	
VPS9_YEAST	essential	T10F2.3	WBGene00012644	
WEB1_YEAST	non-	H14E04.5	WBGene00011338	
XPO1_YEAST	essential	F39H11.3	WBGene00002078	x
Y08L_YEAST	non-	Y56A3A.29	WBGene00011303	
YAB9_YEAST	essential	C16C10.2	WBGene00010845	
YAD2_YEAST	essential	F58H1.3	WBGene00004806	x
YAD6_YEAST	essential	Y38F2A_5743.	WBGene00012126	
YAD6_YEAST	essential	f	WBGene00012126	
YAE2_YEAST	essential	F55H2.2	WBGene00010725	
YAE6_YEAST	essential	C17H12.14	WBGene00013671	
YAH3_YEAST	non-	ZK970.4	WBGene00017683	
YAK1_YEAST	essential	F46F11.5	WBGene00003149	
YB78_YEAST	non-	T01H3.1	WBGene00018909	
YB85_YEAST	essential	T08G11.1	WBGene00020207	
YBA4_YEAST	non-	ZK930.1	WBGene00017558	
YBD6_YEAST	essential	ZK930.7	WBGene00017286	
YBF5_YEAST	non-	C05D11.2	WBGene00000795	
YBF7_YEAST	essential	T20D3.7	WBGene00016062	
YBN5_YEAST	non-	C07G1.5	WBGene00012344	
YBS0_YEAST	essential	Y87G2A.s	WBGene00019725	
YBV8_YEAST	non-	B0025.1	WBGene00004110	
YBY3_YEAST	essential	F59G1.3	WBGene00016408	
YCE5_YEAST	essential	C44C1.1	WBGene00019209	
YCF9_YEAST	non-	C44C1.4	WBGene00015461	x
YCT4_YEAST	essential	Y34D9A_152.a	WBGene00019806	
YCT7_YEAST	non-	C05D9.1	WBGene00016166	
YCU1_YEAST	essential	Y39A1A.5	WBGene00009376	
YCU9_YEAST	essential	T01G1.3	WBGene00013122	
YCW2_YEAST	non-	ZK742.1	WBGene00021074	x
YD13_YEAST	essential	R166.3	WBGene00011275	x
YD23_YEAST	non-	M03C11.8	WBGene00014017	
YD61_YEAST	essential	T27F2.1	WBGene00020296	
YD66_YEAST	non-	Y47D3A.gg	WBGene00016400	
YD83_YEAST	essential	T28D6.6	WBGene00015482	
YDAK_YEAST	non-	K09E9.2	WBGene00017301	
YDB6_YEAST	essential	Y105E8C.j	WBGene00008458	
YEC0_YEAST	essential	F21H12.1	WBGene00007143	
YEJ4_YEAST	non-	T04C10.1	WBGene00016353	
YEJ6_YEAST	essential	F56A3.2	WBGene00003821	x
YEM6_YEAST	non-	T04B8.5	WBGene00012097	
YEM6_YEAST	essential	F18C5.3	WBGene00012097	
YEO1_YEAST	essential	F09E5.8	WBGene00020821	
YEQ8_YEAST	non-	CD4.2	WBGene00020088	
YER2_YEAST	essential	C24G6.8	WBGene00009211	x
YET7_YEAST	non-	W08E3.3	WBGene00003803	x
YEV6_YEAST	essential	M02B7.4	WBGene00012351	
YEX0_YEAST	non-	C37A2.2	WBGene00009172	
YEY6_YEAST	essential	C34E10.5	WBGene00007787	
YEZ3_YEAST	essential	H17B01.4	WBGene00017724	
YFD0_YEAST	non-	C05C8.2	WBGene00011767	
YFH5_YEAST	essential	R01B10.4	WBGene00017280	x
YFH6_YEAST	non-	C27F2.4	WBGene00019673	

YFI8_YEAST	essential	F34D10.6	WBGene00010418	
YG12_YEAST	essential	Y52B11A.2	WBGene00013676	x
YG1D_YEAST	non-	W07E6.2	WBGene00021757	
YG1D_YEAST	essential	R53.6	WBGene00021757	
YG1W_YEAST	non-	ZK632.10	WBGene00022126	
YG1W_YEAST	essential	T07A9.8	WBGene00022126	
YG22_YEAST	non-	C34D4.4	WBGene00008480	
YG25_YEAST	essential	C05D11.3	WBGene00019770	
YG2M_YEAST	non-	F09F7.4	WBGene00017534	
YG2M_YEAST	essential	E02H1.6	WBGene00017534	
YG2O_YEAST	non-	B0334.3	WBGene00016588	
YG3J_YEAST	essential	C33F10.3	WBGene00017989	x
YG3Y_YEAST	non-	K01C8.9	WBGene00015346	x
YG4W_YEAST	essential	Y76A2A.1	WBGene00004015	
YG58_YEAST	non-	T27E9.7	WBGene00012676	x
YG5F_YEAST	essential	T26A5.5	WBGene00007622	
YG5O_YEAST	essential	R119.2	WBGene00013191	
YG5O_YEAST	non-	F28D1.1	WBGene00013191	
YG5U_YEAST	essential	F10G8.3	WBGene00002957	x
YG5Y_YEAST	essential	W09C5.1	WBGene00004788	x
YGA4_YEAST	non-	F26F2.7	WBGene00021467	
YGB8_YEAST	essential	K08H10.8	WBGene00001033	
YGE7_YEAST	non-	F22F7.7	WBGene00011193	
YGG8_YEAST	essential	T14D7.1	WBGene00012361	
YGJ9_YEAST	non-	F09D1.1	WBGene00016907	x
YGL1_YEAST	essential	K12C11.1	WBGene00011538	x
YGO2_YEAST	non-	H27A22.1	WBGene00020868	x
YGR1_YEAST	essential	Y105E8C.d	WBGene00021294	
YGS4_YEAST	essential	Y50D7_165.b	WBGene00011142	x
YGW1_YEAST	essential	Y50D7_165.d	WBGene00017928	
YH04_YEAST	essential	Y71F9B_275.b	WBGene00008148	
YHA2_YEAST	essential	Y71F9B_297.d	WBGene00004109	
YHB3_YEAST	non-	E04D5.1	WBGene00009007	
YHG4_YEAST	essential	M04F3.4	WBGene00018474	
YHH1_YEAST	non-	F17A9.2	WBGene00005662	
YHI0_YEAST	essential	F17A9.3	WBGene00004189	x
YHN0_YEAST	non-	C42C1.10	WBGene00008263	
YHN6_YEAST	essential	F32E10.1	WBGene00012362	
YHP9_YEAST	non-	C02F5.2	WBGene00007028	x
YHR1_YEAST	essential	T24H7.1	WBGene00018357	
YHW8_YEAST	non-	Y39B6B.a	WBGene00010805	
YHY6_YEAST	essential	C16C10.1	WBGene00007510	
YHY8_YEAST	non-	Y54E2A.6	WBGene00008918	
YIE2_YEAST	essential	Y54E2A.7	WBGene00022719	
YIE4_YEAST	non-	F33A8.1	WBGene00000565	
YIG4_YEAST	essential	C54H2.5	WBGene00017919	
YII3_YEAST	non-	Y39G10A_246.	WBGene00022171	
YIJ1_YEAST	essential	b	WBGene00012756	
YIJ7_YEAST	non-	K08D10.2	WBGene00012366	
YIK3_YEAST	essential	R10D12.12	WBGene00007576	
YIK4_YEAST	non-	W09D10.3	WBGene00021655	
YIL0_YEAST	essential	C53H9.2	WBGene00010450	
YIL3_YEAST	non-	T06E6.1	WBGene00006923	x
YIS5_YEAST	essential	T27F7.3	WBGene00016245	
YJ14_YEAST	non-	Y25C1A.7a	WBGene00016244	
YJ40_YEAST	essential	R08D7.1	WBGene00007555	
YJ42_YEAST	non-	F29C4.6	WBGene00001661	x
YJ54_YEAST	essential	C47E12.3	WBGene00016171	
YJ72_YEAST	non-	C34G6.7	WBGene00012193	
YJ76_YEAST	essential	F21D5.2	WBGene00003571	
YJ80_YEAST	non-	F45E12.1	WBGene00003478	
YJ89_YEAST	essential	W03B1.4	WBGene00004319	
YJ95_YEAST	non-	T20H4.3	WBGene00004682	
YJ99_YEAST	essential	C53A5.2	WBGene00011148	
YJE6_YEAST	non-	W09D10.4	WBGene00001590	
YJG2_YEAST	essential	C47D12.1	WBGene00009204	
YJG8_YEAST	non-	F42G8.6	WBGene00013018	
YJJ1_YEAST	essential	M01E5.2	WBGene00022447	
YJJ1_YEAST	essential	C10C5.6	WBGene00022447	

YJJ1_YEAST	non-	F17C11.7	WBGene00022447	
YJJ7_YEAST	essential	ZK370.5	WBGene00020517	
YJK0_YEAST	essential	Y17G7B.15	WBGene00008346	
YJK9_YEAST	essential	F29B9.1	WBGene00022739	x
YJK9_YEAST	essential	Y71H2_388.b	WBGene00022739	x
YJU1_YEAST	non-	Y41C4A.9	WBGene00012203	
YJX8_YEAST	essential	W09G3.2	WBGene00016500	
YJY3_YEAST	non-	C14B1.5	WBGene00007189	
YJZ4_YEAST	essential	Y48A5A.1	WBGene00013870	
YK10_YEAST	non-	K01A11.2	WBGene00011555	
YK18_YEAST	essential	F08B1.1	WBGene00022166	
YK31_YEAST	essential	C30B5.4	WBGene00009574	
YK50_YEAST	essential	C30B5.2	WBGene00019255	
YK59_YEAST	essential	C14A4.1	WBGene00001983	
YKA2_YEAST	essential	C34E10.2	WBGene00012903	
YKA9_YEAST	essential	C27F2.7	WBGene00017347	
YKE1_YEAST	non-	W02A11.2	WBGene00020866	
YKE1_YEAST	essential	C07A9.4	WBGene00020866	
YKF4_YEAST	non-	F53A2.8	WBGene00001081	
YKF9_YEAST	essential	ZK593.4	WBGene00009477	x
YKI2_YEAST	non-	C34E11.1	WBGene00022765	x
YKJ5_YEAST	essential	R08D7.4	WBGene00018149	
YKL7_YEAST	essential	C45G3.3	WBGene00022599	
YKP1_YEAST	essential	F28C6.4	WBGene00011298	
YKQ0_YEAST	essential	Y48G10B.b	WBGene00013219	
YKQ5_YEAST	non-	Y110A2A_54.a	WBGene00021840	
YKQ5_YEAST	essential	Y110A2A_1898	WBGene00021840	
YKT6_YEAST	non-	.d	WBGene00015164	
YKV5_YEAST	essential	Y110A2A_1898	WBGene00021429	
YL01_YEAST	non-	.e	WBGene00021377	
YL05_YEAST	essential	T15B7.2	WBGene00016674	
YL09_YEAST	non-	C56A3.8	WBGene00012887	x
YL16_YEAST	essential	ZK430.1	WBGene00016323	x
YL22_YEAST	non-	T12C9.2	WBGene00021063	
YL27_YEAST	essential	W02B12.8	WBGene00012666	x
YL34_YEAST	non-	C37C3.8	WBGene00003119	
YL51_YEAST	essential	B0491.1	WBGene00003585	
YL53_YEAST	non-	ZC373.5	WBGene00008410	
YL86_YEAST	essential	T07A5.2	WBGene00010565	
YM62_YEAST	essential	Y71H2_385.b	WBGene00016311	
YM8L_YEAST	essential	F40E10.6	WBGene00012803	
YM8T_YEAST	non-	K02B2.2	WBGene00018270	x
YMB4_YEAST	essential	E04A4.4	WBGene00016448	
YMD6_YEAST	non-	Y46G5.m	WBGene00020994	
YME1_YEAST	essential	F10E7.5	WBGene00010842	
YME9_YEAST	non-	T27F7.1	WBGene00019323	x
YMI0_YEAST	essential	F59A6.7	WBGene00009452	
YMJ3_YEAST	non-	F47A4.2	WBGene00017855	
YMJ3_YEAST	essential	F36F2.3	WBGene00017855	x
YMJ6_YEAST	essential	ZK546.14	WBGene00010889	
YMN1_YEAST	essential	F37C12.14	WBGene00010911	
YMO2_YEAST	non-	ZC395.10	WBGene00007630	
YMO9_YEAST	essential	R107.2	WBGene00009636	
YMT1_YEAST	non-	Y54G11A.11	WBGene00003176	
YMT8_YEAST	essential	M01B12.2	WBGene00017738	
YMT9_YEAST	non-	Y54E10B_159.	WBGene00012978	x
YMW7_YEAST	essential	a	WBGene00022027	
YMW7_YEAST	essential	B0361.10	WBGene00022027	x
YN03_YEAST	non-	Y38F2A_6126.	WBGene00010178	
YN15_YEAST	essential	b	WBGene00009920	
YN26_YEAST	essential	Y37E11B.5	WBGene00022025	
YN26_YEAST	non-	C45G9.2	WBGene00022025	
YN26_YEAST	essential	Y45F10D.8	WBGene00022025	
YN28_YEAST	non-	C32E8.5	WBGene00016139	
YN48_YEAST	essential	W06E11.4	WBGene00010044	x
YN53_YEAST	essential	Y39B6B.ee	WBGene00020600	x
YN65_YEAST	non-	Y48C3A.i	WBGene00007912	
YN8K_YEAST	essential	Y87G2A.b	WBGene00011391	
YN8Q_YEAST	essential	D2023.6	WBGene00007312	

YN8U_YEAST	essential	K04G2.6	WBGene00003596	x
YN8V_YEAST	essential	C32D5.3	WBGene00019005	
YNA6_YEAST	essential	Y43F4B.5	WBGene00015697	
YNC2_YEAST	essential	F41C3.4	WBGene00013151	
YNC2_YEAST	essential	C35D10.12	WBGene00013151	
YNC3_YEAST	essential	W03F8.4	WBGene00015812	
YNC6_YEAST	essential	M03C11.5	WBGene00001662	
YNC7_YEAST	essential	K02F2.3	WBGene00007772	
YNK7_YEAST	essential	F36A2.2	WBGene00001585	
YNL0_YEAST	essential	C24A11.9	WBGene00011408	x
YNM9_YEAST	essential	F27C1.6	WBGene00020842	
YNN2_YEAST	essential	M18.3	WBGene00018866	x
YNQ8_YEAST	essential	M106.3	WBGene00022798	
YNR5_YEAST	essential	C16C10.11	WBGene00007111	x
YNU1_YEAST	non-	F42F12.4	WBGene00018285	x
YNU1_YEAST	essential	F37C12.12	WBGene00018285	
YNU1_YEAST	essential	F23C8.9	WBGene00018285	
YNW7_YEAST	essential	Y48B6A.1	WBGene00001035	
YNZ3_YEAST	essential	Y65B4A_182.b	WBGene00009341	
YNZ5_YEAST	essential	Y65B4A_182.c	WBGene00019537	
YNZ5_YEAST	essential	F57A8.2	WBGene00019537	
YO06_YEAST	non-	F52B5.1	WBGene00018679	
YO26_YEAST	essential	Y65B4A_174.a	WBGene00007413	x
YO7T_YEAST	non-	Y65B4A_174.b	WBGene00007101	
YOJ8_YEAST	essential	Y65B4A_179.b	WBGene00015481	
YOZ1_YEAST	essential	C26E6.3	WBGene00008316	
YP18_YEAST	essential	F54C9.9	WBGene00007236	
YP46_YEAST	essential	T20B12.1	WBGene00012692	x
YP59_YEAST	non-	C34B7.2	WBGene00013029	
YP67_YEAST	essential	T03D8.2	WBGene00013218	
YPT1_YEAST	essential	C04H5.1	WBGene00004266	x
YPT7_YEAST	essential	T19A6.2	WBGene00004271	x
YRB1_YEAST	essential	F57B10.8	WBGene00003795	x
YTM1_YEAST	essential	C10H11.8	WBGene00018893	x
ZPR1_YEAST	essential	Y53F4B.e	WBGene00020999	
ZUO1_YEAST	non-	Y53F4B.f	WBGene00001029	
	essential	C16A3.7		
	essential	C34E10.1		
	non-	C27C12.2		
	essential	M04B2.3		
	essential	T04A8.6		
	essential	T27A3.6		
	non-	F55A12.8		
	essential	ZK688.3		
	non-	B0035.12		
	essential	F41E6.3		
	essential	F41E6.4		
	essential	F41E6.10		
	non-	T03F6.2		
	essential	F32H2.4		
	non-	K08D12.h		
	essential	K08D12.c		
	non-	F52C12.2		
	essential	C07E3.2		
	non-	B0024.11		
	essential	C05D11.1		
	non-	C54G4.6		
	essential	C01G10.9		
	non-	Y39B6B.o		
	essential	Y49E10.2		
	essential	Y54G11A.9		
	non-	C39F7.4		
	essential	W03C9.3		
	non-	F59A2.1		
	essential	F55F8.5		
	non-	W03F9.1		
	essential	F38A5.13		
	non-			
	essential			

	non- essential essential non- essential non- essential non- essential non- essential essential essential essential non- essential non- essential essential essential essential essential non- essential essential essential essential essential essential essential essential essential essential non- essential essential essential non- essential non- essential non- essential essential essential essential essential non- essential non- essential non- essential non- essential non- essential non- essential non- essential non- essential non- essential essential essential non- essential essential non-			

Appendix Table 4.3. Features of *C. elegans* duplicate gene pairs

CE Name	CB Name	Prot_id	Length	Ka	Ks
WBGene00008354	WBGene00029517	69.86	148	0.233	13.691
WBGene00009918	WBGene00032946	69.86	146	3	9
WBGene00008956	WBGene00023610	32.2	302	0.233	13.691
WBGene00021461	WBGene00026730	32.2	998	3	9
WBGene00016195	WBGene00030711	68.08	213	0.760	3.4968
*	WBGene00036885	68.08	213	7	3.4968
WBGene00001331	WBGene00041735	41.69	461	0.760	14.567
*	WBGene00041735	41.69	464	7	1
WBGene00011089	WBGene00023673	50.93	655	0.259	14.567
WBGene00011088	WBGene00026207	50.93	650	2	1
WBGene00017012	WBGene00033086	54.44	118	0.259	7.3706
WBGene00009218	WBGene00035798	54.44	94	2	7.3706
WBGene00022162	WBGene00035798	69.7	517	0.560	18.767
WBGene00001051	WBGene00039574	69.7	505	1	4
WBGene00016849	WBGene00040435	40.51	318	0.560	18.767
WBGene00018269	WBGene00027856	40.51	324	1	4
WBGene00008275	WBGene00026512	54.79	417	0.440	18.928
WBGene00017480	WBGene00026513	54.79	412	3	2
WBGene00003036	WBGene00035179	45.45	552	0.440	18.928
*	WBGene00037242	45.45	485	3	2
WBGene00004312	WBGene00025293	66.3	646	0.443	2.8373
*	WBGene00029486	66.3	692	9	2.8373
WBGene00003254	WBGene00036366	31.66	592	0.443	5.0746
WBGene00016380	WBGene00037717	31.66	608	9	5.0746
WBGene00003902	WBGene00032079	52.23	408	0.189	20.823
*	WBGene00040600	52.23	423	6	9
WBGene00003903	WBGene00029678	n.d.	462	0.189	20.823
*	WBGene00023775	n.d.	393	6	9
WBGene00017298	WBGene00026926	50	152	0.629	16.399
WBGene00010663	WBGene00032071	50	146	1	3
WBGene00016728	WBGene00041223	29.71	138	0.629	16.399
WBGene00007254	WBGene00032315	29.71	143	1	3
WBGene00010398	WBGene00032933	46.35	722	0.367	11.586
WBGene00012129	WBGene00027197	46.35	673	2	3
WBGene00000205	WBGene00034641	52.45	217	0.367	11.586
WBGene00016729	WBGene00034640	52.45	206	2	3
WBGene00001425	WBGene00025033	34.72	1032	0.514	15.025
WBGene00001424	WBGene00025033	34.72	1275	6	6
WBGene00012031	WBGene00037234	37.96	326	0.514	15.025
WBGene00013049	WBGene00037235	37.96	248	6	6
WBGene00004386	WBGene00027801	81.15	191	0.268	18.806
WBGene00004385	WBGene00025918	81.15	191	1	7
WBGene00019435	WBGene00024343	71.01	338	0.268	18.806
WBGene00019146	WBGene00034868	71.01	634	1	7
WBGene00016374	WBGene00026186	43.53	326	0.888	n.d.
WBGene00016373	WBGene00033815	43.53	349	6	n.d.
WBGene00007812	WBGene00029321	52.51	448	0.888	2.7613
WBGene00009575	WBGene00024026	52.51	499	6	2.7613
WBGene00013140	WBGene00034303	46.25	367	0.368	10.565
*	WBGene00034302	46.25	376	3	4
WBGene00019827	WBGene00031144	51.88	165	0.368	10.565
*	WBGene00038538	51.88	347	3	4
WBGene00011637	WBGene00033040	65.5	211	n.d.	16.900
WBGene00015516	WBGene00033549	65.5	201	n.d.	5
WBGene00021787	WBGene00036571	30.82	652	0.384	16.900
WBGene00010456	WBGene00027003	30.82	645	5	5
WBGene00001253	WBGene00026215	30.54	708	0.384	17.638
*	WBGene00023788	30.54	481	5	9
WBGene00001186	WBGene00027436	32.82	282	0.869	17.638
*	WBGene00036088	32.82	262	3	9
WBGene00001646	WBGene00037818	50.21	780	0.869	9.1
WBGene00001647	WBGene00037818	50.21	729	3	9.1
WBGene00004272	WBGene00026485	19.18	444	0.457	18.25
*	WBGene00038506	19.18	497	4	18.25
WBGene00004273	WBGene00026268	24.89	1156	0.457	2.9571

*	WBGene00025942	24.89	2180	4	2.9571
WBGene00000591	WBGene00032812	34.16	323	0.364	16.810
WBGene00004737	WBGene00042593	34.16	332	0.364	3
WBGene00007729	WBGene00032511	48.05	558	1.158	16.810
WBGene00008203	WBGene00032560	48.05	512	6	3
WBGene00011543	WBGene00038350	67.19	459	1.158	13.150
WBGene00010339	WBGene00027221	67.19	442	6	3
WBGene00000836	WBGene00036613	38.66	591	0.633	13.150
WBGene00000841	WBGene00034298	38.66	562	4	3
WBGene00003825	WBGene00035871	53.27	199	0.633	11.256
WBGene00006499	WBGene00035728	53.27	260	4	2
WBGene00010558	WBGene00042950	70.11	383	0.113	11.256
WBGene00004215	WBGene00042950	70.11	359	2	2
WBGene00021260	WBGene00029945	64.02	191	0.113	6.895
WBGene00009262	WBGene00029932	64.02	199	2	6.8952
WBGene00018398	WBGene00025632	38.81	374	0.196	9.2763
WBGene00011932	WBGene00041901	38.81	338	1	9.2763
WBGene00021956	WBGene00041901	71.33	295	0.196	19.587
WBGene00011250	WBGene00035270	71.33	293	1	19.587
WBGene00006757	WBGene00023959	n.d.	227	0.625	8.633
WBGene00020298	WBGene00025286	n.d.	245	8	8.633
WBGene00006715	WBGene00036253	51.18	624	0.625	10.831
WBGene00006716	WBGene00029106	51.18	606	8	9
WBGene00013957	WBGene00024008	59.11	269	0.429	10.831
WBGene00009462	WBGene00024007	59.11	286	1	9
WBGene00003563	WBGene00029668	62.74	441	0.429	9.4114
WBGene00003565	WBGene00029668	62.74	437	1	9.4114
WBGene00017436	WBGene00032099	63.82	450	0.531	6.3726
WBGene00012834	WBGene00031037	63.82	457	9	6.3726
WBGene00017673	WBGene00031037	35.42	468	0.531	3.4483
WBGene00019710	WBGene00024344	35.42	677	9	3.4483
WBGene00020109	WBGene00032360	68.54	180	0.387	13.468
WBGene00009111	WBGene00032360	68.54	178	2	1
WBGene00020142	WBGene00030030	38.15	770	0.387	13.468
WBGene00019801	WBGene00036974	38.15	745	2	1
WBGene00009902	WBGene00040842	66.18	419	0.268	10.173
WBGene00009903	WBGene00027571	66.18	414	8	10.173
WBGene00010984	WBGene00029059	78.92	205	0.268	16.577
WBGene00009232	WBGene00036043	78.92	205	8	9
WBGene00022456	WBGene00041573	20.39	368	0.868	16.577
WBGene00019295	WBGene00031308	20.39	392	5	9
WBGene00006938	WBGene00029118	46.64	1342	0.868	11.296
WBGene00006940	WBGene00024017	46.64	880	5	5
WBGene00004930	WBGene00036484	43.72	242	1.036	11.296
WBGene00007036	WBGene00035233	43.72	254	6	5
WBGene00020082	WBGene00034778	69.12	611	1.036	14.588
WBGene00007954	WBGene00041133	69.12	600	6	8
WBGene00016652	WBGene00038231	76.73	493	0.747	14.588
WBGene00015778	WBGene00038231	76.73	437	2	8
WBGene00004269	WBGene00035774	43.75	254	0.747	14.889
WBGene00004270	WBGene00026403	43.75	236	2	3
WBGene00011404	WBGene00028571	48.76	609	0.408	14.889
WBGene00007925	WBGene00030119	48.76	625	2	3
WBGene00022718	WBGene00023609	67.74	531	0.408	2.9108
WBGene00010915	WBGene00041654	67.74	588	2	2.9108
WBGene00003845	WBGene00035644	59.56	927	1.247	7.1603
WBGene00003846	WBGene00035644	59.56	362	2	7.1603
WBGene00006810	WBGene00027230	60.35	461	1.247	5.8279
*	WBGene00038066	60.35	465	2	5.8279
WBGene00010685	WBGene00026823	70.55	146	0.981	1.2764
*	WBGene00034993	70.55	147	5	1.2764
WBGene00000110	WBGene00041499	54.84	557	0.981	n.d.
WBGene00000111	WBGene00041499	54.84	542	5	n.d.
WBGene00006388	WBGene00029579	76.18	322	0.771	10.044
WBGene00006389	WBGene00035499	76.18	321	8	6
WBGene00016589	WBGene00035192	42.74	480	0.771	10.044
WBGene00022610	WBGene00023764	42.74	540	8	6
WBGene00008924	WBGene00034955	30.9	1383	0.497	2.5368
WBGene00010077	WBGene00036534	30.9	1274	7	2.5368

WBGene00022717	WBGene00027753	47.31	444	0.497	16.716
WBGene00006484	WBGene00032145	47.31	398	7	8
WBGene00001834	WBGene00032590	49.08	478	0.23	16.716
WBGene00001836	WBGene00032589	49.08	388	0.23	8
WBGene00004439	WBGene00028243	64.08	309	0.581	16.227
*	WBGene00029624	64.08	300	7	4
WBGene00004438	WBGene00030797	66.01	153	0.581	16.227
*	WBGene00037681	66.01	168	7	4
WBGene00007554	WBGene00040807	42.77	306	0.378	15.751
*	WBGene00036716	42.77	166	2	7
WBGene00012348	WBGene00040965	72.58	62	0.378	15.751
*	WBGene00040965	72.58	87	2	7
WBGene00007350	WBGene00025898	45.16	1490	0.191	1.1986
*	WBGene00037226	45.16	1432	3	1.1986
WBGene00017759	WBGene00026061	54.89	400	0.191	19.587
*	WBGene00033328	54.89	372	3	3
WBGene00016934	WBGene00029242	50.2	492	0.249	19.587
WBGene00007446	WBGene00028696	50.2	516	8	3
WBGene00003561	WBGene00034255	87.54	351	0.249	16.249
WBGene00003562	WBGene00034255	87.54	289	8	9
WBGene00000217	WBGene00033020	51.95	933	0.709	16.249
WBGene00000216	WBGene00035294	51.95	900	2	9
WBGene00009976	WBGene00024751	52.88	997	0.709	16.486
WBGene00009977	WBGene00037136	52.88	1328	2	7
WBGene00018901	WBGene00037909	26.79	238	0.184	16.486
WBGene00008979	WBGene00037906	26.79	402	7	7
WBGene00020052	WBGene00032644	33.77	308	0.184	11.431
WBGene00012968	WBGene00041676	33.77	155	7	8
WBGene00009140	WBGene00042135	34.77	608	n.d.	11.431
WBGene00008693	WBGene00036912	34.77	557	n.d.	8
WBGene00004443	WBGene00036196	57.97	449	0.431	18.643
WBGene00012956	WBGene00031306	57.97	437	3	4
WBGene00011530	WBGene00038684	45.41	223	0.431	18.643
WBGene00016700	WBGene00036217	45.41	220	3	4
WBGene00012149	WBGene00034158	65.14	109	0.304	3.2719
WBGene00009453	WBGene00034158	65.14	132	9	3.2719
WBGene00019207	WBGene00026309	72.16	520	0.304	12.577
WBGene00019979	WBGene00040591	72.16	489	9	1
WBGene00003938	WBGene00040591	37.41	160	0.263	12.577
WBGene00001204	WBGene00025733	37.41	153	6	1
WBGene00004217	WBGene00039142	69.25	493	0.263	2.376
*	WBGene00025215	69.25	493	6	2.376
WBGene00004224	WBGene00033192	32.04	399	0.264	5.098
*	WBGene00038480	32.04	427	3	5.098
WBGene00004256	WBGene00040585	40.69	491	0.264	9.3943
WBGene00004257	WBGene00040585	40.69	544	3	9.3943
WBGene00004995	WBGene00025527	82.52	148	0.646	18.369
WBGene00004993	WBGene00025527	82.52	143	4	1
WBGene00000250	WBGene00033806	30.39	184	0.646	18.369
WBGene00000249	WBGene00033805	30.39	258	4	1
WBGene00011850	WBGene00035494	n.d.	214	0.208	1.1239
WBGene00009622	WBGene00035494	n.d.	183	7	1.1239
WBGene00001638	WBGene00025421	44.07	539	0.208	10.058
WBGene00001639	WBGene00025391	44.07	654	7	8
WBGene00009176	WBGene00030548	77.18	444	0.654	10.058
WBGene00012295	WBGene00030548	77.18	355	0.654	8
WBGene00014170	WBGene00025657	53.24	1009	0.238	11.509
WBGene00010763	WBGene00025657	53.24	709	3	1
WBGene00000964	WBGene00035361	43.06	474	0.238	11.509
WBGene00000963	WBGene00034909	43.06	429	3	1
WBGene00015406	WBGene00033234	40.08	507	0.134	18.331
WBGene00007419	WBGene00023546	40.08	565	0.134	18.331
WBGene00000002	WBGene00029229	85.21	610	1.078	10.737
WBGene00000004	WBGene00029229	85.21	142	2	8
WBGene00008885	WBGene00036378	54.04	485	1.078	10.737
WBGene00016044	WBGene00036378	54.04	781	2	8
WBGene00003041	WBGene00028556	56.45	539	0.465	9.4187
WBGene00021661	WBGene00033991	56.45	559	7	9.4187
WBGene00016112	WBGene00025675	44.03	464	0.465	10.314

WBGene00016123	WBGene00034951	44.03	578	7	8
WBGene00000516	WBGene00031372	35.24	441	0.587	10.314
WBGene00000517	WBGene00036415	35.24	566	2	8
WBGene00017757	WBGene00042202	52.01	733	0.587	1.9277
WBGene00010029	WBGene00023795	52.01	586	2	1.9277
WBGene00001101	WBGene00026107	34.83	393	0.218	19.193
*	WBGene00036051	34.83	362	1	8
WBGene00001102	WBGene00031496	83.85	195	0.218	19.193
*	WBGene00023941	83.85	161	1	8
WBGene00016507	WBGene00029720	75.26	287	0.149	12.798
WBGene00016506	WBGene00029720	75.26	287	3	12.798
WBGene00019608	WBGene00029166	34.86	319	0.149	11.463
WBGene00012835	WBGene00035475	34.86	302	3	5
WBGene00000509	WBGene00029977	34.39	297	0.601	11.463
WBGene00000510	WBGene00041856	34.39	299	9	5
WBGene00022801	WBGene00026875	41.05	1285	0.601	17.454
WBGene00003956	WBGene00036193	41.05	1203	9	3
WBGene00016074	WBGene00036248	69.93	594	0.470	17.454
WBGene00016072	WBGene00036248	69.93	556	8	3
WBGene00004052	WBGene00041442	82.16	510	0.470	0.6483
WBGene00004051	WBGene00041442	82.16	537	8	0.6483
WBGene00004025	WBGene00036973	49.54	255	0.221	6.59
WBGene00001077	WBGene00036973	49.54	216	9	6.59
WBGene00019427	WBGene00033562	68.26	427	0.221	14.074
WBGene00017178	WBGene00033551	68.26	419	9	5
WBGene00004183	WBGene00029035	47.88	1429	0.278	14.074
WBGene00004182	WBGene00029022	47.88	1295	6	5
WBGene00001813	WBGene00025031	69.21	920	0.278	9.7792
WBGene00001811	WBGene00025031	69.21	440	6	9.7792
WBGene00011578	WBGene00039831	55.04	313	0.311	0.3888
WBGene00020727	WBGene00039835	55.04	258	8	0.3888
WBGene00002064	WBGene00033834	53.23	719	0.311	14.752
WBGene00002065	WBGene00033834	53.23	763	8	8
WBGene00004801	WBGene00033041	78.82	170	0.243	14.752
WBGene00004802	WBGene00024156	78.82	170	5	8
WBGene00016811	WBGene00024970	65.09	110	0.243	8.1971
WBGene00022489	WBGene00033667	65.09	107	5	8.1971
WBGene00020215	WBGene00033667	36.42	480	0.372	2.3893
WBGene00012142	WBGene00030028	36.42	316	5	2.3893
WBGene00020490	WBGene00026765	47.74	245	0.372	5.6259
WBGene00015177	WBGene00025735	47.74	374	5	5.6259
WBGene00015676	WBGene00023576	65.27	169	0.181	12.892
WBGene00020168	WBGene00031423	65.27	201	6	8
WBGene00000107	WBGene00040884	64.01	1219	0.181	12.892
WBGene00000108	WBGene00034297	64.01	1219	6	8
WBGene00007955	WBGene00040564	43.4	373	0.569	10.778
WBGene00009238	WBGene00037222	43.4	341	9	4
WBGene00016943	WBGene00027270	59.45	465	0.569	10.778
WBGene00019433	WBGene00023571	59.45	406	9	4
WBGene00003001	WBGene00030001	73.85	1528	0.773	4.2335
*	WBGene00030002	73.85	1525	0.773	4.2335
WBGene00001609	WBGene00025199	33.23	679	0.517	5.8458
*	WBGene00042487	33.23	654	8	5.8458
WBGene00007258	WBGene00030975	28.87	527	0.517	12.699
WBGene00021214	WBGene00027980	28.87	483	8	9
WBGene00013268	WBGene00029442	58.36	269	0.457	12.699
WBGene00013255	WBGene00034008	58.36	274	2	9
WBGene00015203	WBGene00033580	41.18	289	0.457	10.454
*	WBGene00026690	41.18	325	2	3
WBGene00013917	WBGene00032209	53.07	491	0.262	10.454
*	WBGene00026912	53.07	494	4	3
WBGene00001501	WBGene00026406	39.68	518	0.262	11.391
WBGene00001500	WBGene00041750	39.68	531	4	8
WBGene00004410	WBGene00041068	15.08	881	0.277	11.391
*	WBGene00037195	15.08	525	9	8
WBGene00016493	WBGene00029946	33.06	371	0.277	13.631
*	WBGene00028823	33.06	388	9	4
WBGene00000387	WBGene00030164	52.59	731	0.537	13.631
WBGene00000388	WBGene00035178	52.59	718	2	4

WBGene00018008	WBGene00034705	9.39	421	0.537	8.9133
WBGene00018354	WBGene00035101	9.39	362	2	8.9133
WBGene00004345	WBGene00031541	33.68	784	0.253	1.0433
WBGene00004344	WBGene00031539	33.68	910	7	1.0433
WBGene00004969	WBGene00037863	71.46	530	0.253	9.4085
WBGene00002041	WBGene00037848	71.46	529	7	9.4085
WBGene00008767	WBGene00025907	32.13	277	0.482	n.d.
WBGene00012786	WBGene00042179	32.13	319	3	n.d.
WBGene00013024	WBGene00038818	45.49	297	0.482	11.261
WBGene00009057	WBGene00025405	45.49	278	3	3
WBGene00003407	WBGene00025104	63.51	363	0.413	11.261
WBGene00003408	WBGene00025104	63.51	359	2	3
WBGene00020511	WBGene00033942	41.28	535	0.413	1.4609
WBGene00012315	WBGene00035164	41.28	703	2	1.4609
WBGene00016061				0.438	6.7028
WBGene00000139				1	6.7028
WBGene00010115				0.438	9.8317
WBGene00012928				1	9.8317
WBGene00015623				0.075	4.2682
WBGene00020402				7	4.2682
WBGene00001025				0.075	0.1931
WBGene00001046				7	0.1931
WBGene00013739				0.402	2.9597
WBGene00007549				9	2.9597
WBGene00020649				0.402	19.841
WBGene00011258				9	5
WBGene00016642				0.390	19.841
WBGene00019980				2	5
WBGene00018152				0.390	9.8457
*				2	9.8457
WBGene00016716				0.905	12.016
*				1	1
WBGene00018738				0.905	12.016
WBGene00015388				1	1
WBGene00014220				0.769	8.8637
WBGene00003504				4	8.8637
WBGene00018755				0.769	9.7753
WBGene00012914				4	9.7753
WBGene00009514				0.734	8.6519
WBGene00004135				7	8.6519
WBGene00021296				0.734	0.9746
WBGene00019022				7	0.9746
WBGene00003368				0.312	4.577
WBGene00012162				2	4.577
WBGene00004244				0.312	15.803
WBGene00004245				2	8
				0.477	15.803
				7	8
				0.477	8.9123
				7	8.9123
				0.311	1.4083
				4	1.4083
				0.311	3.7686
				4	3.7686
				0.181	4.1253
				1	4.1253
				0.181	13.371
				1	9
				0.688	13.371
				9	9
				0.688	17.120
				9	8
				0.232	17.120
				0.232	8
				0.789	0.831
				9	0.831
				0.789	1.6525
				9	1.6525
				0.591	10.796

Appendix Table 5.1. ***S. cerevisiae*** **synthetic lethal interactions and their pairwise** ***C. elegans*** **orthologues**

Gene 1			Gene 2			Refs
SC Name	CE Name	CE RNAi clone	SC Name	CE Name	CE RNAi clone	
ALG6	WBGene00007435	C08B11.8	OST3	WBGene00022793	ZK686.3	Tong
ALG8	WBGene00007464	C08H9.3	OST3	WBGene00022793	ZK686.3	Tong
ALG8	WBGene00007464	C08H9.3	PER1	WBGene00019806	R01B10.4	Tong
ARC40	WBGene00000201	Y79H2A.6	ARC18	WBGene00000203	Y37D8A.1	Tong
ARC40	WBGene00000201	Y79H2A.6	CHS5	WBGene00022615	ZC449.5	Tong
ARC40	WBGene00000201	Y79H2A.6	GIM3	WBGene00007107	B0035.4	Tong
ARC40	WBGene00000201	Y79H2A.6	GIM4	WBGene00019220	H20J04.d	Tong
ARC40	WBGene00000201	Y79H2A.6	GLO3	WBGene00017217	F07F6.4	Tong
ARC40	WBGene00000201	Y79H2A.6	SEC22	WBGene00018853	F55A4.1	Tong
ARC40	WBGene00000201	Y79H2A.6	SPF1	WBGene00007514	C10C6.6	Tong
ARC40	WBGene00000201	Y79H2A.6	STE24	WBGene00001405	C04F12.10	Tong
ARC40	WBGene00000201	Y79H2A.6	VRP1	WBGene00020094	R144.4 & R144.8	Tong
ARC40	WBGene00000201	Y79H2A.6	YKE2	WBGene00009004	F21C3.5	Tong
ARL1	WBGene00000187	F54C9.10	COG6	WBGene00019481	K07C11.9	Tong
ARL1	WBGene00000187	F54C9.10	COG8	WBGene00011736	T12D8.9	Tong
ARL1	WBGene00000187	F54C9.10	GLO3	WBGene00017217	F07F6.4	Tong
ARL1	WBGene00000187	F54C9.10	PER1	WBGene00019806	R01B10.4	Tong
ARL1	WBGene00000187	F54C9.10	TLG2	WBGene00022534	ZC155.7	Tong
ARL1	WBGene00000187	F54C9.10	VPS29	WBGene00014234	ZK1128.8	Tong
ARL1	WBGene00000187	F54C9.10	VPS35	WBGene00006933	F59G1.3	Tong
ARL1	WBGene00000187	F54C9.10	VPS5	WBGene00004927	C05D9.1	Tong
ARL1	WBGene00000187	F54C9.10	YPT6	WBGene00009880	F49C12.11	Tong
ARL3	WBGene00021841	Y54E10B_159.i	COG6	WBGene00019481	K07C11.9	Tong
ARL3	WBGene00021841	Y54E10B_159.i	COG8	WBGene00011736	T12D8.9	Tong
ARL3	WBGene00021841	Y54E10B_159.i	GDA1	WBGene00010697	K08H10.4	Tong
ARL3	WBGene00021841	Y54E10B_159.i	GLO3	WBGene00017217	F07F6.4	Tong
ARL3	WBGene00021841	Y54E10B_159.i	GYP1	WBGene00009322	F32B6.8	Tong
ARL3	WBGene00021841	Y54E10B_159.i	OST2	WBGene00000896	F57B10.10	Davierwala
ARL3	WBGene00021841	Y54E10B_159.i	TLG2	WBGene00022534	ZC155.7	Tong
ARL3	WBGene00021841	Y54E10B_159.i	TRS20	WBGene00021046	W05H7.3	Davierwala
ARL3	WBGene00021841	Y54E10B_159.i	VPS5	WBGene00004927	C05D9.1	Tong
ARL3	WBGene00021841	Y54E10B_159.i	YPT6	WBGene00009880	F49C12.11	Tong
ARP2	WBGene00000200	K07C5.1	ABD1	WBGene00006447	C25A1.3	Davierwala
ARP2	WBGene00000200	K07C5.1	CHS5	WBGene00022615	ZC449.5	Tong
ARP2	WBGene00000200	K07C5.1	CWC22	WBGene00002957	F33A8.1	Davierwala
ARP2	WBGene00000200	K07C5.1	GIM3	WBGene00007107	B0035.4	Tong
ARP2	WBGene00000200	K07C5.1	GIM4	WBGene00019220	H20J04.d	Tong
ARP2	WBGene00000200	K07C5.1	HRT1	WBGene00004320	ZK287.5	Davierwala
ARP2	WBGene00000200	K07C5.1	LAS17	WBGene00006565	Y63D3A.5	Davierwala
ARP2	WBGene00000200	K07C5.1	MAK5	WBGene00018890	F55F8.2	Davierwala
ARP2	WBGene00000200	K07C5.1	NOP14	WBGene00021660	Y48G1A_54.d	Davierwala
ARP2	WBGene00000200	K07C5.1	PAC10	WBGene00006889	T06G6.9	Tong
ARP2	WBGene00000200	K07C5.1	PFY1	WBGene00003991	K03E6.6	Davierwala

ARP2	WBGene00000200	K07C5.1	PRP9	WBGene00011758	T13H5.4	Davierwala
ARP2	WBGene00000200	K07C5.1	RGD1	WBGene00001559	F45H7.2 & F45H7.3	Tong
ARP2	WBGene00000200	K07C5.1	RVS161	WBGene00010272	F58G6.1	Tong
ARP2	WBGene00000200	K07C5.1	RVS167	WBGene00020209	T04C9.1 & ZK328.3	Tong
ARP2	WBGene00000200	K07C5.1	SPF1	WBGene00007514	C10C6.6	Tong
ARP2	WBGene00000200	K07C5.1	STE24	WBGene00001405	C04F12.10	Tong
ARP2	WBGene00000200	K07C5.1	VRP1	WBGene00020094	R144.4 & R144.8	Tong
ARP2	WBGene00000200	K07C5.1	YKE2	WBGene00009004	F21C3.5	Tong
ARP6	WBGene00007434	C08B11.6	BRE1	WBGene00007008	R05D3.4	Tong, Pan
ARP6	WBGene00007434	C08B11.6	BUD13	WBGene00011142	R08D7.1	Tong
ARP6	WBGene00007434	C08B11.6	COG6	WBGene00019481	K07C11.9	Tong
ARP6	WBGene00007434	C08B11.6	DEG1	WBGene00006473	E02H1.3	Tong
ARP6	WBGene00007434	C08B11.6	GIM3	WBGene00007107	B0035.4	Tong
ARP6	WBGene00007434	C08B11.6	GIM4	WBGene00019220	H20J04.d	Tong
ARP6	WBGene00007434	C08B11.6	GIM5	WBGene00020112	R151.9	Tong
ARP6	WBGene00007434	C08B11.6	GLO3	WBGene00017217	F07F6.4	Tong
ARP6	WBGene00007434	C08B11.6	HCM1	WBGene00001442	C25A1.2	Tong
ARP6	WBGene00007434	C08B11.6	LEO1	WBGene00007110	B0035.11	Tong
ARP6	WBGene00007434	C08B11.6	PAC10	WBGene00006889	T06G6.9	Tong
ARP6	WBGene00007434	C08B11.6	PAC2	WBGene00019503	K07H8.1	Tong
ARP6	WBGene00007434	C08B11.6	RTF1	WBGene00009103	F25B3.6	Tong
ARP6	WBGene00007434	C08B11.6	SEC22	WBGene00018853	F55A4.1	Tong
ARP6	WBGene00007434	C08B11.6	YDL033C	WBGene00007114	B0035.16	Tong
ARP6	WBGene00007434	C08B11.6	YPT6	WBGene00009880	F49C12.11	Tong
BRE1	WBGene00007008	R05D3.4	ARC18	WBGene00000203	Y37D8A.1	Pan
BRE1	WBGene00007008	R05D3.4	ASC1	WBGene00010556	K04D7.1	Pan
BRE1	WBGene00007008	R05D3.4	CCR4	WBGene00000376	ZC518.3	Pan
BRE1	WBGene00007008	R05D3.4	COG6	WBGene00019481	K07C11.9	Pan
BRE1	WBGene00007008	R05D3.4	COG8	WBGene00011736	T12D8.9	Pan
BRE1	WBGene00007008	R05D3.4	CTK1	WBGene00007135	B0285.1 & B0285.2	Pan
BRE1	WBGene00007008	R05D3.4	HTZ1	WBGene00019947	R08C7.3	Pan
BRE1	WBGene00007008	R05D3.4	LEA1	WBGene00019223	H20J04.c	Pan
BRE1	WBGene00007008	R05D3.4	LSM1	WBGene00003076	F40F8.9	Pan
BRE1	WBGene00007008	R05D3.4	MRE11	WBGene00003405	ZC302.1	Pan
BRE1	WBGene00007008	R05D3.4	NAT1	WBGene00021754	Y50D7_162.b & Y50D7_164.a	Pan
BRE1	WBGene00007008	R05D3.4	PEP3	WBGene00021058	W06B4.3	Pan
BRE1	WBGene00007008	R05D3.4	PEP5	WBGene00011067	R06F6.2	Pan
BRE1	WBGene00007008	R05D3.4	POL32	WBGene00011016	R04F11.3	Pan
BRE1	WBGene00007008	R05D3.4	POP2	WBGene00000369	Y56A3A.20	Pan
BRE1	WBGene00007008	R05D3.4	RAD27	WBGene00000794	Y47G6A_247.i	Pan
BRE1	WBGene00007008	R05D3.4	RAD51	WBGene00004297	Y43C5A.6	Pan
BRE1	WBGene00007008	R05D3.4	RAD54	WBGene00004298	W06D4.6	Pan
BRE1	WBGene00007008	R05D3.4	RPN10	WBGene00004466	B0205.3	Pan
BRE1	WBGene00007008	R05D3.4	SAC1	WBGene00009264	F30A10.6	Pan
BRE1	WBGene00007008	R05D3.4	SCS7	WBGene00007707	C25A1.5	Pan
BRE1	WBGene00007008	R05D3.4	SIN3	WBGene00004117	F02E9.4	Pan
BRE1	WBGene00007008	R05D3.4	SMP2	WBGene00010425	H37A05.1	Pan
BRE1	WBGene00007008	R05D3.4	SNF5	WBGene00011111	R07E5.3	Pan

BRE1	WBGene00007008	R05D3.4	TUF1	WBGene00007000	Y71H2_378.a	Pan
BRE1	WBGene00007008	R05D3.4	VIP1	WBGene00018508	F46F11.1	Pan
BRE1	WBGene00007008	R05D3.4	VMA7	WBGene00006918*	ZK970.4	Pan
BRE1	WBGene00007008	R05D3.4	VPS15	WBGene00014151	ZK930.1 & ZK930.7	Pan
BRE1	WBGene00007008	R05D3.4	VPS20	WBGene00022027	Y65B4A_182.b & Y65B4A_182.c	Pan
BRE1	WBGene00007008	R05D3.4	VPS34	WBGene00006932	B0025.1	Pan
BRE1	WBGene00007008	R05D3.4	VPS36	WBGene00008919	F17C11.8	Pan
BRE1	WBGene00007008	R05D3.4	VPS45	WBGene00016643	C44C1.1 & C44C1.4	Pan
BRE1	WBGene00007008	R05D3.4	VPS9	WBGene00012644	Y39A1A.5	Pan
BRE1	WBGene00007008	R05D3.4	VRP1	WBGene00020094	R144.4 & R144.8	Pan
BRE1	WBGene00007008	R05D3.4	YAF9	WBGene00001585	M04B2.3	Pan
BRE1	WBGene00007008	R05D3.4	YJL046W	WBGene00001590	C45G3.3	Pan
CAC2	WBGene00022141	Y71G12A_202.d	CTF18	WBGene00010676	K08F4.1	Pan
CAC2	WBGene00022141	Y71G12A_202.d	ISC1	WBGene00012105	T27F6.6	Pan
CAC2	WBGene00022141	Y71G12A_202.d	MRE11	WBGene00003405	ZC302.1	Pan
CAC2	WBGene00022141	Y71G12A_202.d	POL32	WBGene00011016	R04F11.3	Pan
CAC2	WBGene00022141	Y71G12A_202.d	RAD50	WBGene00004296	T04H1.4	Pan
CCR4	WBGene00000376	ZC518.3	ARP6	WBGene00007434	C08B11.6	Pan
CCR4	WBGene00000376	ZC518.3	ATP1	WBGene00010419*	H28O16.1	Pan
CCR4	WBGene00000376	ZC518.3	ATP5	WBGene00017856*	F27C1.7	Pan
CCR4	WBGene00000376	ZC518.3	CTF18	WBGene00010676	K08F4.1	Pan
CCR4	WBGene00000376	ZC518.3	CTK1	WBGene00007135	B0285.1 & B0285.2	Pan
CCR4	WBGene00000376	ZC518.3	DID4	WBGene00012903	Y46G5.m	Pan
CCR4	WBGene00000376	ZC518.3	GCS1	WBGene00010500	K02B12.7	Pan
CCR4	WBGene00000376	ZC518.3	KAR3	WBGene00002216	T09A5.2	Pan
CCR4	WBGene00000376	ZC518.3	LSM1	WBGene00003076	F40F8.9	Pan
CCR4	WBGene00000376	ZC518.3	LSM6	WBGene00003080	Y71G12A_187.b	Pan
CCR4	WBGene00000376	ZC518.3	LSM7	WBGene00003081	ZK593.7	Pan
CCR4	WBGene00000376	ZC518.3	MRE11	WBGene00003405	ZC302.1	Pan
CCR4	WBGene00000376	ZC518.3	MSY1	WBGene00006968	K08F11.4	Pan
CCR4	WBGene00000376	ZC518.3	MUS81	WBGene00016602	C43E11.2	Pan
CCR4	WBGene00000376	ZC518.3	NAT1	WBGene00021754	Y50D7_162.b & Y50D7_164.a	Pan
CCR4	WBGene00000376	ZC518.3	NHX1	WBGene00003733	F57C7.2	Pan
CCR4	WBGene00000376	ZC518.3	PIF1	WBGene00004028	Y18H1A_67.c & Y18H1A_67.d & Y18H1A_67.f	Pan
CCR4	WBGene00000376	ZC518.3	PIM1	WBGene00016391	C34B2.6	Pan
CCR4	WBGene00000376	ZC518.3	RAD27	WBGene00000794	Y47G6A_247.i	Pan
CCR4	WBGene00000376	ZC518.3	RAD50	WBGene00004296	T04H1.4	Pan
CCR4	WBGene00000376	ZC518.3	RAD51	WBGene00004297	Y43C5A.6	Pan
CCR4	WBGene00000376	ZC518.3	RAD54	WBGene00004298	W06D4.6	Pan
CCR4	WBGene00000376	ZC518.3	RAD6	WBGene00006701	C35B1.1	Pan
CCR4	WBGene00000376	ZC518.3	RVS161	WBGene00010272	F58G6.1	Pan
CCR4	WBGene00000376	ZC518.3	RVS167	WBGene00020209	T04C9.1 & ZK328.3	Pan
CCR4	WBGene00000376	ZC518.3	SAC1	WBGene00009264	F30A10.6	Pan
CCR4	WBGene00000376	ZC518.3	SGS1	WBGene00001865	T04A11.6	Pan
CCR4	WBGene00000376	ZC518.3	SIN3	WBGene00004117	F02E9.4	Pan
CCR4	WBGene00000376	ZC518.3	SNF8	WBGene00016167	C27F2.5	Pan
CCR4	WBGene00000376	ZC518.3	STP22	WBGene00015658	C09G12.9	Pan
CCR4	WBGene00000376	ZC518.3	TFP1	WBGene00013025*	Y49A3A.2	Pan

CCR4	WBGene00000376	ZC518.3	VAC14	WBGene00010565	K04G2.6	Pan
CCR4	WBGene00000376	ZC518.3	VPS20	WBGene00022027	Y65B4A_182.b & Y65B4A_182.c	Pan
CCR4	WBGene00000376	ZC518.3	VPS24	WBGene00020866	F59A6.7 & T27F7.1	Pan
CCR4	WBGene00000376	ZC518.3	VPS30	WBGene00000247	T19E7.3 & T19E7.4	Pan
CCR4	WBGene00000376	ZC518.3	VPS36	WBGene00008919	F17C11.8	Pan
CCR4	WBGene00000376	ZC518.3	VPS4	WBGene00021334	Y34D9A_152.a	Pan
CCR4	WBGene00000376	ZC518.3	VPS45	WBGene00016643	C44C1.1 & C44C1.4	Pan
CCR4	WBGene00000376	ZC518.3	YAF9	WBGene00001585	M04B2.3	Pan
CDC2	WBGene00008645	F10C2.4	CSM3	WBGene00017738	F23C8.9	Tong
CDC2	WBGene00008645	F10C2.4	POL32	WBGene00011016	R04F11.3	Tong
CDC2	WBGene00008645	F10C2.4	TRM10	WBGene00009131	F25H8.1	Tong
CDC40	WBGene00018625	F49D11.1	GPI10	WBGene00020868	T27F7.3	Davierwala
CDC40	WBGene00018625	F49D11.1	GWT1	WBGene00022447	Y110A2A_1898.d & Y110A2A_1898.e &	Davierwala
CDC40	WBGene00018625	F49D11.1	PRP16	WBGene00003389	Y110A2A_54.a	Davierwala
CDC40	WBGene00018625	F49D11.1	PSF2	WBGene00009287	K03H1.2	Davierwala
CDC40	WBGene00018625	F49D11.1	RTS2	WBGene00013128	F31C3.5	Davierwala
CDC40	WBGene00018625	F49D11.1	YGL047W	WBGene00011193	Y52B11A.9	Davierwala
CDC42	WBGene00000390	R07G3.1	CAP1	WBGene00000292	R10D12.12	Tong
CDC42	WBGene00000390	R07G3.1	CAP2	WBGene00000293	D2024.6	Tong
CDC42	WBGene00000390	R07G3.1	ELP3	WBGene00014123	M106.5	Tong
CDC42	WBGene00000390	R07G3.1	FAD1	WBGene00011271	ZK863.3	Davierwala
CDC42	WBGene00000390	R07G3.1	HRT1	WBGene00004320	R53.1	Davierwala
CDC42	WBGene00000390	R07G3.1	RPC40	WBGene00019275	ZK287.5	Davierwala
CDC42	WBGene00000390	R07G3.1	UBA4	WBGene00018357	H43I07.2	Tong
CDC42	WBGene00000390	R07G3.1	YGL211W	WBGene00017928	F42G8.6	Tong
CDC45	WBGene00009372	F34D10.2 & F34D10.3	BUB2	WBGene00016352	F29C4.6	Tong
CDC45	WBGene00009372	F34D10.2 & F34D10.3	CSM3	WBGene00017738	C33F10.2	Tong
CDC45	WBGene00009372	F34D10.2 & F34D10.3	CTF18	WBGene00010676	F23C8.9	Tong
CDC45	WBGene00009372	F34D10.2 & F34D10.3	DPB3	WBGene00013150	K08F4.1	Tong
CDC45	WBGene00009372	F34D10.2 & F34D10.3	FAB1	WBGene00004089	Y53F4B.d	Tong
CDC45	WBGene00009372	F34D10.2 & F34D10.3	GIM4	WBGene00019220	C05E7.5 & VF11C1L.1	Tong
CDC45	WBGene00009372	F34D10.2 & F34D10.3	GIM5	WBGene00020112	H20J04.d	Tong
CDC45	WBGene00009372	F34D10.2 & F34D10.3	HAT1	WBGene00010841	R151.9	Tong
CDC45	WBGene00009372	F34D10.2 & F34D10.3	HTZ1	WBGene00019947	M03C11.4	Tong
CDC45	WBGene00009372	F34D10.2 & F34D10.3	KAR3	WBGene00002216	R08C7.3	Tong
CDC45	WBGene00009372	F34D10.2 & F34D10.3	KEM1	WBGene00012730	T09A5.2	Tong
CDC45	WBGene00009372	F34D10.2 & F34D10.3	LEO1	WBGene00007110	Y39G8CY39G8C.1 & Y39G8C.b.b	Tong
CDC45	WBGene00009372	F34D10.2 & F34D10.3	LSM1	WBGene00003076	B0035.11	Tong
CDC45	WBGene00009372	F34D10.2 & F34D10.3	MAD2	WBGene00003161	F40F8.9	Tong
CDC45	WBGene00009372	F34D10.2 & F34D10.3	NPR2	WBGene00018635	Y69A2A_2326.a	Tong
CDC45	WBGene00009372	F34D10.2 & F34D10.3	POL32	WBGene00011016	F49E8.1	Tong
CDC45	WBGene00009372	F34D10.2 & F34D10.3	RAD54	WBGene00004298	R04F11.3	Tong
CDC45	WBGene00009372	F34D10.2 & F34D10.3	RTF1	WBGene00009103	W06D4.6	Tong
CDC45	WBGene00009372	F34D10.2 & F34D10.3	SCS7	WBGene00007707	F25B3.6	Tong
CDC45	WBGene00009372	F34D10.2 & F34D10.3	SEC22	WBGene00018853	C25A1.5	Tong
CDC45	WBGene00009372	F34D10.2 & F34D10.3	TOP1	WBGene00006595	F55A4.1	Tong
CDC45	WBGene00009372	F34D10.2 & F34D10.3	YJR070C	WBGene00007555	M01E5.5	Tong
CHL1	WBGene00010839	M03C11.2	GIM3	WBGene00007107	C14A4.1	Tong

CHL1	WBGene00010839	M03C11.2	GIM5	WBGene00020112	B0035.4	Tong
CHL1	WBGene00010839	M03C11.2	MAD2	WBGene00003161	R151.9	Tong
CHL1	WBGene00010839	M03C11.2	RAD27	WBGene00000794	Y69A2A_2326.a	Tong, Pan
CHL1	WBGene00010839	M03C11.2	YKE2	WBGene00009004	Y47G6A_247.i	Tong
CHS5	WBGene00022615	ZC449.5	ARC18	WBGene00000203	F21C3.5	Tong
CHS5	WBGene00022615	ZC449.5	ASC1	WBGene00010556	Y37D8A.1	Tong
CHS5	WBGene00022615	ZC449.5	BRE1	WBGene00007008	K04D7.1	Tong
CHS5	WBGene00022615	ZC449.5	COX11	WBGene00010437	R05D3.4	Tong
CHS5	WBGene00022615	ZC449.5	EMP24	WBGene00004766	JC8.5	Tong
CHS5	WBGene00022615	ZC449.5	FAB1	WBGene00004089	W02D7.7	Tong
CHS5	WBGene00022615	ZC449.5	HSE1	WBGene00004109	C05E7.5 & VF11C1L.1	Tong
CHS5	WBGene00022615	ZC449.5	LAT1	WBGene00009082	C34G6.7	Tong
CHS5	WBGene00022615	ZC449.5	LEA1	WBGene00019223	F23B12.5	Tong
CHS5	WBGene00022615	ZC449.5	LSM6	WBGene00003080	H20J04.c	Tong
CHS5	WBGene00022615	ZC449.5	MRE11	WBGene00003405	Y71G12A_187.b	Tong
CHS5	WBGene00022615	ZC449.5	PET8	WBGene00008364	ZC302.1	Tong
CHS5	WBGene00022615	ZC449.5	PRE9	WBGene00003924	D1046.3	Tong
CHS5	WBGene00022615	ZC449.5	RVS161	WBGene00010272	Y110A7A.f & Y110A7A.j	Tong
CHS5	WBGene00022615	ZC449.5	SEC22	WBGene00018853	F58G6.1	Tong
CHS5	WBGene00022615	ZC449.5	VPS24	WBGene00020866	F55A4.1	Tong
CHS5	WBGene00022615	ZC449.5	YDL033C	WBGene00007114	F59A6.7 & T27F7.1	Tong
CNE1	WBGene00000567	ZK632.6	PEX1	WBGene00004191	B0035.16	Tong
CNE1	WBGene00000567	ZK632.6	YNR036C	WBGene00011391	C11H1.4 & C11H1.6	Tong
CSM3	WBGene00017738	F23C8.9	BUB2	WBGene00016352	T03D8.2	Tong
CSM3	WBGene00017738	F23C8.9	CSN12	WBGene00016171	C33F10.2	Tong
CSM3	WBGene00017738	F23C8.9	DOC1	WBGene00000144	C27F2.7	Pan
CSM3	WBGene00017738	F23C8.9	GIM3	WBGene00007107	F15H10.3	Tong
CSM3	WBGene00017738	F23C8.9	GIM4	WBGene00019220	B0035.4	Tong
CSM3	WBGene00017738	F23C8.9	GIM5	WBGene00020112	H20J04.d	Tong, Pan
CSM3	WBGene00017738	F23C8.9	GUF1	WBGene00022862	R151.9	Pan
CSM3	WBGene00017738	F23C8.9	ISC1	WBGene00012105	ZK1236.1	Tong
CSM3	WBGene00017738	F23C8.9	LSM7	WBGene00003081	T27F6.6	Pan
CSM3	WBGene00017738	F23C8.9	MAK3	WBGene00015074	ZK593.7	Pan
CSM3	WBGene00017738	F23C8.9	MRE11	WBGene00003405	B0238.10	Pan
CSM3	WBGene00017738	F23C8.9	NAT1	WBGene00021754	ZC302.1	Pan
CSM3	WBGene00017738	F23C8.9	NPR2	WBGene00018635	Y50D7_162.b & Y50D7_164.a	Pan
CSM3	WBGene00017738	F23C8.9	PAC10	WBGene00006889	F49E8.1	Tong
CSM3	WBGene00017738	F23C8.9	POL32	WBGene00011016	T06G6.9	Tong, Pan
CSM3	WBGene00017738	F23C8.9	POP1	WBGene00015486	R04F11.3	Davierwala
CSM3	WBGene00017738	F23C8.9	RAD5	WBGene00010061	C05D11.9	Tong, Pan
CSM3	WBGene00017738	F23C8.9	RAD50	WBGene00004296	F54E12.2	Pan
CSM3	WBGene00017738	F23C8.9	RAD51	WBGene00004297	T04H1.4	Pan
CSM3	WBGene00017738	F23C8.9	RAD54	WBGene00004298	Y43C5A.6	Pan
CSM3	WBGene00017738	F23C8.9	RAD6	WBGene00006701	W06D4.6	Pan
CSM3	WBGene00017738	F23C8.9	RPN10	WBGene00004466	C35B1.1	Pan
CSM3	WBGene00017738	F23C8.9	VIP1	WBGene00018508	B0205.3	Pan
CSM3	WBGene00017738	F23C8.9	YKE2	WBGene00009004	F46F11.1	Tong
CTF18	WBGene00010676	K08F4.1	ARP6	WBGene00007434	F21C3.5	Tong

CTF18	WBGene00010676	K08F4.1	ASC1	WBGene00010556	C08B11.6	Pan
CTF18	WBGene00010676	K08F4.1	BUB2	WBGene00016352	K04D7.1	Pan
CTF18	WBGene00010676	K08F4.1	CHD1	WBGene00010369	C33F10.2	Pan
CTF18	WBGene00010676	K08F4.1	CHL1	WBGene00010839	H06O01.2	Tong, Pan
CTF18	WBGene00010676	K08F4.1	CSM3	WBGene00017738	M03C11.2	Tong, Pan
CTF18	WBGene00010676	K08F4.1	CTK1	WBGene00007135	F23C8.9	Pan
CTF18	WBGene00010676	K08F4.1	DPB3	WBGene00013150	B0285.1 & B0285.2	Pan
CTF18	WBGene00010676	K08F4.1	GIM3	WBGene00007107	Y53F4B.d	Tong, Pan
CTF18	WBGene00010676	K08F4.1	GIM4	WBGene00019220	B0035.4	Tong, Pan
CTF18	WBGene00010676	K08F4.1	GIM5	WBGene00020112	H20J04.d	Tong, Pan
CTF18	WBGene00010676	K08F4.1	HTZ1	WBGene00019947	R151.9	Tong, Pan
CTF18	WBGene00010676	K08F4.1	ISC1	WBGene00012105	R08C7.3	Pan
CTF18	WBGene00010676	K08F4.1	KAR3	WBGene00002216	T27F6.6	Pan
CTF18	WBGene00010676	K08F4.1	LSM1	WBGene00003076	T09A5.2	Pan
CTF18	WBGene00010676	K08F4.1	LSM7	WBGene00003081	F40F8.9	Pan
CTF18	WBGene00010676	K08F4.1	MAD2	WBGene00003161	ZK593.7	Tong, Pan
CTF18	WBGene00010676	K08F4.1	MRE11	WBGene00003405	Y69A2A_2326.a	Tong, Pan
CTF18	WBGene00010676	K08F4.1	NAT1	WBGene00021754	ZC302.1	Pan
CTF18	WBGene00010676	K08F4.1	PAC10	WBGene00006889	Y50D7_162.b & Y50D7_164.a	Tong, Pan
CTF18	WBGene00010676	K08F4.1	PEP5	WBGene00011067	T06G6.9	Pan
CTF18	WBGene00010676	K08F4.1	POL32	WBGene00011016	R06F6.2	Tong, Pan
CTF18	WBGene00010676	K08F4.1	POP2	WBGene00000369	R04F11.3	Pan
CTF18	WBGene00010676	K08F4.1	RAD23	WBGene00013924	Y56A3A.20	Tong
CTF18	WBGene00010676	K08F4.1	RAD5	WBGene00010061	ZK20.3	Tong, Pan
CTF18	WBGene00010676	K08F4.1	RAD50	WBGene00004296	F54E12.2	Tong, Pan
CTF18	WBGene00010676	K08F4.1	RAD51	WBGene00004297	T04H1.4	Tong, Pan
CTF18	WBGene00010676	K08F4.1	RAD54	WBGene00004298	Y43C5A.6	Tong, Pan
CTF18	WBGene00010676	K08F4.1	RAD6	WBGene00006701	W06D4.6	Pan
CTF18	WBGene00010676	K08F4.1	RPN10	WBGene00004466	C35B1.1	Pan
CTF18	WBGene00010676	K08F4.1	SNF8	WBGene00016167	B0205.3	Pan
CTF18	WBGene00010676	K08F4.1	STP22	WBGene00015658	C27F2.5	Pan
CTF18	WBGene00010676	K08F4.1	UBP14	WBGene00020839	C09G12.9	Pan
CTF18	WBGene00010676	K08F4.1	VIP1	WBGene00018508	T27A3.2	Pan
CTF18	WBGene00010676	K08F4.1	VPS16	WBGene00006516	F46F11.1	Pan
CTF18	WBGene00010676	K08F4.1	VPS20	WBGene00022027	C05D11.2	Pan
CTF18	WBGene00010676	K08F4.1	VPS25	WBGene00012193	Y65B4A_182.b & Y65B4A_182.c	Pan
CTF18	WBGene00010676	K08F4.1	VPS34	WBGene00006932	W02A11.2	Pan
CTF18	WBGene00010676	K08F4.1	VPS36	WBGene00008919	B0025.1	Pan
CTF18	WBGene00010676	K08F4.1	VPS45	WBGene00016643	F17C11.8	Pan
CTF18	WBGene00010676	K08F4.1	YAF9	WBGene00001585	C44C1.1 & C44C1.4	Pan
CWH41	WBGene00008775	F13H10.4	EMP24	WBGene00004766	M04B2.3	Tong
CWH41	WBGene00008775	F13H10.4	LEA1	WBGene00019223	W02D7.7	Tong
CWH41	WBGene00008775	F13H10.4	PER1	WBGene00019806	H20J04.c	Tong
CWH41	WBGene00008775	F13H10.4	SNF4	WBGene00013732	R01B10.4	Tong
CWH41	WBGene00008775	F13H10.4	YBR235W	WBGene00020207	Y111B2C.h	Tong
DIE2	WBGene00011987	T24D1.4	OST3	WBGene00022793	T04B8.5	Tong
DPB3	WBGene00013150	Y53F4B.d	HTZ1	WBGene00019947	ZK686.3	Pan
DYN1	WBGene00000962	T21E12.4	GIM3	WBGene00007107	R08C7.3	Tong

DYN1	WBGene00000962	T21E12.4	GIM4	WBGene00019220	B0035.4	Tong
DYN1	WBGene00000962	T21E12.4	GIM5	WBGene00020112	H20J04.d	Tong
DYN1	WBGene00000962	T21E12.4	HCM1	WBGene00001442	R151.9	Tong
DYN1	WBGene00000962	T21E12.4	KAR3	WBGene00002216	C25A1.2	Tong
DYN1	WBGene00000962	T21E12.4	PAC10	WBGene00006889	T09A5.2	Tong
DYN1	WBGene00000962	T21E12.4	YKE2	WBGene00009004	T06G6.9	Tong
DYN2	WBGene00001130	C02C6.1	GIM3	WBGene00007107	F21C3.5	Tong
DYN2	WBGene00001130	C02C6.1	GIM4	WBGene00019220	B0035.4	Tong
DYN2	WBGene00001130	C02C6.1	GIM5	WBGene00020112	H20J04.d	Tong
DYN2	WBGene00001130	C02C6.1	PAC10	WBGene00006889	R151.9	Tong
DYN2	WBGene00001130	C02C6.1	YKE2	WBGene00009004	T06G6.9	Tong
GIM3	WBGene00007107	B0035.4	BUB2	WBGene00016352	F21C3.5	Tong
GIM3	WBGene00007107	B0035.4	FAB1	WBGene00004089	C33F10.2	Tong
GIM3	WBGene00007107	B0035.4	HTZ1	WBGene00019947	C05E7.5 & VF11C1L.1	Tong
GIM3	WBGene00007107	B0035.4	NCL1	WBGene00021686	R08C7.3	Tong
GIM3	WBGene00007107	B0035.4	PAN3	WBGene00014015	Y48G8A_2614.a	Tong
GIM3	WBGene00007107	B0035.4	PER1	WBGene00019806	ZK632.7	Tong
GIM3	WBGene00007107	B0035.4	PLP1	WBGene00015482	R01B10.4	Tong
GIM3	WBGene00007107	B0035.4	STE24	WBGene00001405	C05D11.3	Tong
GIM3	WBGene00007107	B0035.4	UBA4	WBGene00018357	C04F12.10	Tong
GIM3	WBGene00007107	B0035.4	VAC14	WBGene00010565	F42G8.6	Tong
GIM3	WBGene00007107	B0035.4	VPS29	WBGene00014234	K04G2.6	Tong
GIM3	WBGene00007107	B0035.4	VRP1	WBGene00020094	ZK1128.8	Tong
GIM3	WBGene00007107	B0035.4	YBR108W	WBGene00004110	R144.4 & R144.8	Tong
GIM3	WBGene00007107	B0035.4	YGR054W	WBGene00008480	C37A2.2	Tong
GIM3	WBGene00007107	B0035.4	YTA7	WBGene00008682	E04D5.1	Tong
GIM4	WBGene00019220	H20J04.d	BUB2	WBGene00016352	F11A10.1	Tong
GIM4	WBGene00019220	H20J04.d	CAP2	WBGene00000293	C33F10.2	Tong
GIM4	WBGene00019220	H20J04.d	CHL1	WBGene00010839	M106.5	Tong
GIM4	WBGene00019220	H20J04.d	DNM1	WBGene00001093	M03C11.2	Tong
GIM4	WBGene00019220	H20J04.d	FAB1	WBGene00004089	T12E12.4	Tong
GIM4	WBGene00019220	H20J04.d	HSE1	WBGene00004109	C05E7.5 & VF11C1L.1	Tong
GIM4	WBGene00019220	H20J04.d	HTZ1	WBGene00019947	C34G6.7	Tong
GIM4	WBGene00019220	H20J04.d	ISC1	WBGene00012105	R08C7.3	Tong
GIM4	WBGene00019220	H20J04.d	MMS2	WBGene00006730	T27F6.6	Tong
GIM4	WBGene00019220	H20J04.d	NCL1	WBGene00021686	F39B2.2	Tong
GIM4	WBGene00019220	H20J04.d	PAC2	WBGene00019503	Y48G8A_2614.a	Tong
GIM4	WBGene00019220	H20J04.d	PAN3	WBGene00014015	K07H8.1	Tong
GIM4	WBGene00019220	H20J04.d	PER1	WBGene00019806	ZK632.7	Tong
GIM4	WBGene00019220	H20J04.d	STE24	WBGene00001405	R01B10.4	Tong
GIM4	WBGene00019220	H20J04.d	UBA4	WBGene00018357	C04F12.10	Tong
GIM4	WBGene00019220	H20J04.d	VAC14	WBGene00010565	F42G8.6	Tong
GIM4	WBGene00019220	H20J04.d	VPS29	WBGene00014234	K04G2.6	Tong
GIM4	WBGene00019220	H20J04.d	VRP1	WBGene00020094	ZK1128.8	Tong
GIM4	WBGene00019220	H20J04.d	YJR129C	WBGene00011148	R144.4 & R144.8	Tong
GIM4	WBGene00019220	H20J04.d	YTA7	WBGene00008682	R08D7.4	Tong
GIM5	WBGene00020112	R151.9	BUB2	WBGene00016352	F11A10.1	Tong
GIM5	WBGene00020112	R151.9	CAF40	WBGene00016139	C33F10.2	Tong

GIM5	WBGene00020112	R151.9	CAP2	WBGene00000293	C26E6.3	Tong
GIM5	WBGene00020112	R151.9	DNM1	WBGene00001093	M106.5	Tong
GIM5	WBGene00020112	R151.9	FAB1	WBGene00004089	T12E12.4	Tong
GIM5	WBGene00020112	R151.9	HTZ1	WBGene00019947	C05E7.5 & VF11C1L.1	Tong
GIM5	WBGene00020112	R151.9	KEM1	WBGene00012730	R08C7.3	Tong
GIM5	WBGene00020112	R151.9	MAK3	WBGene00015074	Y39G8C.Y39G8C.1 & Y39G8C.bb	Tong
GIM5	WBGene00020112	R151.9	MMS2	WBGene00006730	B0238.10	Tong
GIM5	WBGene00020112	R151.9	NCL1	WBGene00021686	F39B2.2	Tong
GIM5	WBGene00020112	R151.9	PAN3	WBGene00014015	Y48G8A_2614.a	Tong
GIM5	WBGene00020112	R151.9	PLP1	WBGene00015482	ZK632.7	Tong
GIM5	WBGene00020112	R151.9	SPF1	WBGene00007514	C05D11.3	Tong
GIM5	WBGene00020112	R151.9	STE24	WBGene00001405	C10C6.6	Tong
GIM5	WBGene00020112	R151.9	UBA4	WBGene00018357	C04F12.10	Tong
GIM5	WBGene00020112	R151.9	UGA1	WBGene00001794	F42G8.6	Tong
GIM5	WBGene00020112	R151.9	VAC14	WBGene00010565	K04D7.3	Tong
GIM5	WBGene00020112	R151.9	VPS29	WBGene00014234	K04G2.6	Tong
GIM5	WBGene00020112	R151.9	VPS35	WBGene00006933	ZK1128.8	Tong
GIM5	WBGene00020112	R151.9	VRP1	WBGene00020094	F59G1.3	Tong
GIM5	WBGene00020112	R151.9	YBR108W	WBGene00004110	R144.4 & R144.8	Tong
GIM5	WBGene00020112	R151.9	YGR054W	WBGene00008480	C37A2.2	Tong
GIM5	WBGene00020112	R151.9	YTA7	WBGene00008682	E04D5.1	Tong
GYP1	WBGene00009322	F32B6.8	ARL1	WBGene00000187	F11A10.1	Tong
GYP1	WBGene00009322	F32B6.8	COG6	WBGene00019481	F54C9.10	Tong
GYP1	WBGene00009322	F32B6.8	COG8	WBGene00011736	K07C11.9	Tong
GYP1	WBGene00009322	F32B6.8	SCS7	WBGene00007707	T12D8.9	Tong
GYP1	WBGene00009322	F32B6.8	SEC22	WBGene00018853	C25A1.5	Tong
GYP1	WBGene00009322	F32B6.8	YPT6	WBGene00009880	F55A4.1	Tong
KAR3	WBGene00002216	T09A5.2	ARP6	WBGene00007434	F49C12.11	Tong
KAR3	WBGene00002216	T09A5.2	BUB2	WBGene00016352	C08B11.6	Tong
KAR3	WBGene00002216	T09A5.2	CHL1	WBGene00010839	C33F10.2	Tong
KAR3	WBGene00002216	T09A5.2	CSM3	WBGene00017738	M03C11.2	Tong, Pan
KAR3	WBGene00002216	T09A5.2	GIM3	WBGene00007107	F23C8.9	Tong
KAR3	WBGene00002216	T09A5.2	GIM4	WBGene00019220	B0035.4	Tong
KAR3	WBGene00002216	T09A5.2	GIM5	WBGene00020112	H20J04.d	Tong
KAR3	WBGene00002216	T09A5.2	KEM1	WBGene00012730	R151.9	Tong
KAR3	WBGene00002216	T09A5.2	MAD2	WBGene00003161	Y39G8C.1 & Y39G8C.b	Tong
KAR3	WBGene00002216	T09A5.2	PAC10	WBGene00006889	Y69A2A_2326.a	Tong
KAR3	WBGene00002216	T09A5.2	PRE9	WBGene00003924	T06G6.9	Tong
KAR3	WBGene00002216	T09A5.2	RPN10	WBGene00004466	Y110A7A.f & Y110A7A.j	Tong, Pan
KAR3	WBGene00002216	T09A5.2	RRD2	WBGene00022185	B0205.3	Tong
KAR3	WBGene00002216	T09A5.2	YKE2	WBGene00009004	Y71H2_388.b & Y71H2_388.c & Y71H2_388.f	Tong
LAS21	WBGene00009204	F28C6.4	LEA1	WBGene00019223	F21C3.5	Tong
LAS21	WBGene00009204	F28C6.4	YPR045C	WBGene00017158	H20J04.c	Tong
LIA1	WBGene00007555	C14A4.1	AAH1	WBGene00015551	F01F1.1	Tong
LIA1	WBGene00007555	C14A4.1	RTF1	WBGene00009103	C06G3.5	Tong
MAD2	WBGene00003161	Y69A2A_2326.a	GIM3	WBGene00007107	F25B3.6	Tong
MAD2	WBGene00003161	Y69A2A_2326.a	GIM4	WBGene00019220	B0035.4	Tong
MAD2	WBGene00003161	Y69A2A_2326.a	GIM5	WBGene00020112	H20J04.d	Tong

MAD2	WBGene00003161	Y69A2A_2326.a	HCM1	WBGene00001442	R151.9	Tong
MAD2	WBGene00003161	Y69A2A_2326.a	HTZ1	WBGene00019947	C25A1.2	Tong
MAD2	WBGene00003161	Y69A2A_2326.a	PAC10	WBGene00006889	R08C7.3	Tong
MAD2	WBGene00003161	Y69A2A_2326.a	YKE2	WBGene00009004	T06G6.9	Tong
MRE11	WBGene00003405	ZC302.1	CTK1	WBGene00007135	F21C3.5	Pan
MRE11	WBGene00003405	ZC302.1	KEM1	WBGene00012730	B0285.1 & B0285.2	Pan
MRE11	WBGene00003405	ZC302.1	LSM1	WBGene00003076	Y39G8C.1 & Y39G8C.b	Pan
MRE11	WBGene00003405	ZC302.1	LSM6	WBGene00003080	F40F8.9	Pan
MRE11	WBGene00003405	ZC302.1	LSM7	WBGene00003081	Y71G12A_187.b	Pan
MRE11	WBGene00003405	ZC302.1	MUS81	WBGene00016602	ZK593.7	Pan
MRE11	WBGene00003405	ZC302.1	NAT1	WBGene00021754	C43E11.2	Pan
MRE11	WBGene00003405	ZC302.1	POL32	WBGene00011016	Y50D7_162.b & Y50D7_164.a	Pan
MRE11	WBGene00003405	ZC302.1	POP2	WBGene00000369	R04F11.3	Pan
MRE11	WBGene00003405	ZC302.1	RAD5	WBGene00010061	Y56A3A.20	Pan
MRE11	WBGene00003405	ZC302.1	RAD6	WBGene00006701	F54E12.2	Pan
MRE11	WBGene00003405	ZC302.1	RPN10	WBGene00004466	C35B1.1	Pan
MRE11	WBGene00003405	ZC302.1	SGS1	WBGene00001865	B0205.3	Pan
MRE11	WBGene00003405	ZC302.1	STP22	WBGene00015658	T04A11.6	Pan
MRE11	WBGene00003405	ZC302.1	VPS15	WBGene00014151	C09G12.9	Pan
MRE11	WBGene00003405	ZC302.1	VPS34	WBGene00006932	ZK930.1 & ZK930.7	Pan
MRE11	WBGene00003405	ZC302.1	YDR140W	WBGene00016341	B0025.1	Pan
MRE11	WBGene00003405	ZC302.1	YER087W	WBGene00004190	C33C12.9	Pan
MUS81	WBGene00016602	C43E11.2	POL32	WBGene00011016	T27F6.5	Pan
MUS81	WBGene00016602	C43E11.2	POP2	WBGene00000369	R04F11.3	Pan
MUS81	WBGene00016602	C43E11.2	RAD54	WBGene00004298	Y56A3A.20	Pan
MUS81	WBGene00016602	C43E11.2	RAD6	WBGene00006701	W06D4.6	Pan
NAT1	WBGene00021754	Y50D7_162.b & Y50D7_164.a	ARC18	WBGene00000203	C35B1.1	Pan
NAT1	WBGene00021754	Y50D7_162.b & Y50D7_164.a	ARP6	WBGene00007434	Y37D8A.1	Pan
NAT1	WBGene00021754	Y50D7_162.b & Y50D7_164.a	BUD13	WBGene00011142	C08B11.6	Pan
NAT1	WBGene00021754	Y50D7_162.b & Y50D7_164.a	CBC2	WBGene00009141	R08D7.1	Pan
NAT1	WBGene00021754	Y50D7_162.b & Y50D7_164.a	COX6	WBGene00012553	F26A3.2	Pan
NAT1	WBGene00021754	Y50D7_162.b & Y50D7_164.a	CRD1	WBGene00017763	Y37D8A.14	Pan
NAT1	WBGene00021754	Y50D7_162.b & Y50D7_164.a	DOA1	WBGene00007333	F23H11.9	Pan
NAT1	WBGene00021754	Y50D7_162.b & Y50D7_164.a	DPB3	WBGene00013150	C05C10.6	Pan
NAT1	WBGene00021754	Y50D7_162.b & Y50D7_164.a	FAB1	WBGene00004089	Y53F4B.d	Pan
NAT1	WBGene00021754	Y50D7_162.b & Y50D7_164.a	GIM3	WBGene00007107	C05E7.5 & VF11C1L.1	Pan
NAT1	WBGene00021754	Y50D7_162.b & Y50D7_164.a	GLO3	WBGene00017217	B0035.4	Pan
NAT1	WBGene00021754	Y50D7_162.b & Y50D7_164.a	HTZ1	WBGene00019947	F07F6.4	Pan
NAT1	WBGene00021754	Y50D7_162.b & Y50D7_164.a	IFM1	WBGene00009771	R08C7.3	Pan
NAT1	WBGene00021754	Y50D7_162.b & Y50D7_164.a	IST3	WBGene00016245	F46B6.6	Pan
NAT1	WBGene00021754	Y50D7_162.b & Y50D7_164.a	LEA1	WBGene00019223	C30B5.4	Pan
NAT1	WBGene00021754	Y50D7_162.b & Y50D7_164.a	LSM1	WBGene00003076	H20J04.c	Pan
NAT1	WBGene00021754	Y50D7_162.b & Y50D7_164.a	LSM6	WBGene00003080	F40F8.9	Pan
NAT1	WBGene00021754	Y50D7_162.b & Y50D7_164.a	LSM7	WBGene00003081	Y71G12A_187.b	Pan
NAT1	WBGene00021754	Y50D7_162.b & Y50D7_164.a	MIP1	WBGene00013258	ZK593.7	Pan
NAT1	WBGene00021754	Y50D7_162.b & Y50D7_164.a	MRF1	WBGene00020993	Y57A10A.m	Pan
NAT1	WBGene00021754	Y50D7_162.b & Y50D7_164.a	PAC10	WBGene00006889	W03F8.3	Pan
NAT1	WBGene00021754	Y50D7_162.b & Y50D7_164.a	PEP3	WBGene00021058	T06G6.9	Pan

NAT1	WBGene00021754	Y50D7_162.b & Y50D7_164.a	PEP5	WBGene00011067	W06B4.3	Pan
NAT1	WBGene00021754	Y50D7_162.b & Y50D7_164.a	PEX1	WBGene00004191	R06F6.2	Pan
NAT1	WBGene00021754	Y50D7_162.b & Y50D7_164.a	PEX12	WBGene00004197	C11H1.4 & C11H1.6	Pan
NAT1	WBGene00021754	Y50D7_162.b & Y50D7_164.a	PEX13	WBGene00004198	F08B12.2	Pan
NAT1	WBGene00021754	Y50D7_162.b & Y50D7_164.a	PEX5	WBGene00004194	F32A5.6	Pan
NAT1	WBGene00021754	Y50D7_162.b & Y50D7_164.a	PEX6	WBGene00004195	C34C6.6	Pan
NAT1	WBGene00021754	Y50D7_162.b & Y50D7_164.a	PHB2	WBGene00004015	F39G3.7	Pan
NAT1	WBGene00021754	Y50D7_162.b & Y50D7_164.a	PIM1	WBGene00016391	T24H7.1	Pan
NAT1	WBGene00021754	Y50D7_162.b & Y50D7_164.a	POL32	WBGene00011016	C34B2.6	Pan
NAT1	WBGene00021754	Y50D7_162.b & Y50D7_164.a	POP2	WBGene00000369	R04F11.3	Pan
NAT1	WBGene00021754	Y50D7_162.b & Y50D7_164.a	PPA1	WBGene00011347*	Y56A3A.20	Pan
NAT1	WBGene00021754	Y50D7_162.b & Y50D7_164.a	RAD27	WBGene00000794	T01H3.1	Pan
NAT1	WBGene00021754	Y50D7_162.b & Y50D7_164.a	RAD5	WBGene00010061	Y47G6A_247.i	Pan
NAT1	WBGene00021754	Y50D7_162.b & Y50D7_164.a	RAD50	WBGene00004296	F54E12.2	Pan
NAT1	WBGene00021754	Y50D7_162.b & Y50D7_164.a	RAD51	WBGene00004297	T04H1.4	Pan
NAT1	WBGene00021754	Y50D7_162.b & Y50D7_164.a	RAD54	WBGene00004298	Y43C5A.6	Pan
NAT1	WBGene00021754	Y50D7_162.b & Y50D7_164.a	RVS161	WBGene00010272	W06D4.6	Pan
NAT1	WBGene00021754	Y50D7_162.b & Y50D7_164.a	RVS167	WBGene00020209	F58G6.1	Pan
NAT1	WBGene00021754	Y50D7_162.b & Y50D7_164.a	SAC1	WBGene00009264	T04C9.1 & ZK328.3	Pan
NAT1	WBGene00021754	Y50D7_162.b & Y50D7_164.a	SCS7	WBGene00007707	F30A10.6	Pan
NAT1	WBGene00021754	Y50D7_162.b & Y50D7_164.a	SER2	WBGene00013379	C25A1.5	Pan
NAT1	WBGene00021754	Y50D7_162.b & Y50D7_164.a	SET2	WBGene00021515	Y62E10A.m	Pan
NAT1	WBGene00021754	Y50D7_162.b & Y50D7_164.a	SIN3	WBGene00004117	Y41D4A_2615.a & Y41D4A_3457.b	Pan
NAT1	WBGene00021754	Y50D7_162.b & Y50D7_164.a	SMP2	WBGene00010425	F02E9.4	Pan
NAT1	WBGene00021754	Y50D7_162.b & Y50D7_164.a	SNF8	WBGene00016167	H37A05.1	Pan
NAT1	WBGene00021754	Y50D7_162.b & Y50D7_164.a	STP22	WBGene00015658	C27F2.5	Pan
NAT1	WBGene00021754	Y50D7_162.b & Y50D7_164.a	SWD1	WBGene00017683	C09G12.9	Pan
NAT1	WBGene00021754	Y50D7_162.b & Y50D7_164.a	VMA4	WBGene00006917*	F21H12.1	Pan
NAT1	WBGene00021754	Y50D7_162.b & Y50D7_164.a	VMA8	WBGene00010130	C17H12.14	Pan
NAT1	WBGene00021754	Y50D7_162.b & Y50D7_164.a	VPS20	WBGene00022027	F55H2.2	Pan
NAT1	WBGene00021754	Y50D7_162.b & Y50D7_164.a	VPS25	WBGene00012193	Y65B4A_182.b & Y65B4A_182.c	Pan
NAT1	WBGene00021754	Y50D7_162.b & Y50D7_164.a	VPS27	WBGene00004101	W02A11.2	Pan
NAT1	WBGene00021754	Y50D7_162.b & Y50D7_164.a	VPS36	WBGene00008919	C07G1.5	Pan
NAT1	WBGene00021754	Y50D7_162.b & Y50D7_164.a	VPS45	WBGene00016643	F17C11.8	Pan
NAT1	WBGene00021754	Y50D7_162.b & Y50D7_164.a	VPS9	WBGene00012644	C44C1.1 & C44C1.4	Pan
NAT1	WBGene00021754	Y50D7_162.b & Y50D7_164.a	VRP1	WBGene00020094	Y39A1A.5	Pan
NAT1	WBGene00021754	Y50D7_162.b & Y50D7_164.a	YAF9	WBGene00001585	R144.4 & R144.8	Pan
NAT1	WBGene00021754	Y50D7_162.b & Y50D7_164.a	YER087W	WBGene00004190	M04B2.3	Pan
NAT1	WBGene00021754	Y50D7_162.b & Y50D7_164.a	YGR046W	WBGene00022126	T27F6.5	Pan
NAT1	WBGene00021754	Y50D7_162.b & Y50D7_164.a	YKE2	WBGene00009004	Y71F9B_275.b & Y71F9B_297.d	Pan
NAT1	WBGene00021754	Y50D7_162.b & Y50D7_164.a	YMR293C	WBGene00021508	F21C3.5	Pan
NIP7	WBGene00016607	C43E11.9	ABD1	WBGene00006447	Y41D4A_3192.a	Davierwala
NIP7	WBGene00016607	C43E11.9	BET3	WBGene00014222	C25A1.3	Davierwala
NIP7	WBGene00016607	C43E11.9	CDC16	WBGene00001281	ZK1098.5	Davierwala
NIP7	WBGene00016607	C43E11.9	CFT2	WBGene00017313	F10B5.6	Davierwala
NIP7	WBGene00016607	C43E11.9	CWC22	WBGene00002957	F09G2.4	Davierwala
NIP7	WBGene00016607	C43E11.9	MCD4	WBGene00021840	F33A8.1	Davierwala
NIP7	WBGene00016607	C43E11.9	MVD1	WBGene00012984	M01B12.2 & Y54E10B_159.a	Davierwala

NIP7	WBGene00016607	C43E11.9	PDS5	WBGene00001352	Y48B6A.13a	Davierwala
NIP7	WBGene00016607	C43E11.9	POL12	WBGene00001002	H38K22.1	Davierwala
NIP7	WBGene00016607	C43E11.9	PRO3	WBGene00010924	R01H10.1	Davierwala
NIP7	WBGene00016607	C43E11.9	PRP16	WBGene00003389	M153.1	Davierwala
NIP7	WBGene00016607	C43E11.9	RIM2	WBGene00011662	K03H1.2	Davierwala
NIP7	WBGene00016607	C43E11.9	RIX7	WBGene00003119	T09F3.2	Davierwala
NIP7	WBGene00016607	C43E11.9	RNA15	WBGene00000774	Y48C3A.i	Davierwala
NOP1	WBGene00001423	T01C3.7	BET3	WBGene00014222	F56A8.6	Davierwala
NOP1	WBGene00001423	T01C3.7	CWC22	WBGene00002957	ZK1098.5	Davierwala
NOP1	WBGene00001423	T01C3.7	LCP5	WBGene00003059	F33A8.1	Davierwala
NOP1	WBGene00001423	T01C3.7	MAS1	WBGene00013880	C48E7.3	Davierwala
NOP1	WBGene00001423	T01C3.7	MSS4	WBGene00004087	ZC410.2	Davierwala
NOP1	WBGene00001423	T01C3.7	PRP16	WBGene00003389	F55A12.3	Davierwala
NOP1	WBGene00001423	T01C3.7	PRP28	WBGene00017162	K03H1.2	Davierwala
NOP1	WBGene00001423	T01C3.7	RKI1	WBGene00015101	F01F1.7	Davierwala
NOP1	WBGene00001423	T01C3.7	SEC17	WBGene00017016	B0280.3	Davierwala
NOP1	WBGene00001423	T01C3.7	TAF1	WBGene00006382	D1014.3	Davierwala
NOP1	WBGene00001423	T01C3.7	UGP1	WBGene00010665	W04A8.7 & Y71A12B.a & Y71A12B.b &	Davierwala
NOP1	WBGene00001423	T01C3.7	UTP11	WBGene00007623	Y71A12B.c	Davierwala
NOP1	WBGene00001423	T01C3.7	YNR054C	WBGene00019005	K08E3.5	Davierwala
NOP1	WBGene00001423	T01C3.7	YOR287C	WBGene00020705	C16C10.2	Davierwala
PAC1	WBGene00003047	T03F6.5	CAP1	WBGene00000292	F57B10.8	Tong
PAC1	WBGene00003047	T03F6.5	FAB1	WBGene00004089	T22H9.1	Tong
PAC1	WBGene00003047	T03F6.5	GIM3	WBGene00007107	D2024.6	Tong
PAC1	WBGene00003047	T03F6.5	GIM4	WBGene00019220	C05E7.5 & VF11C1L.1	Tong
PAC1	WBGene00003047	T03F6.5	GIM5	WBGene00020112	B0035.4	Tong
PAC1	WBGene00003047	T03F6.5	KAR3	WBGene00002216	H20J04.d	Tong
PAC1	WBGene00003047	T03F6.5	PAC10	WBGene00006889	R151.9	Tong
PAC1	WBGene00003047	T03F6.5	YHR168W	WBGene00010805	T09A5.2	Tong
PAC1	WBGene00003047	T03F6.5	YKE2	WBGene00009004	T06G6.9	Tong
PAC10	WBGene00006889	T06G6.9	AFG1	WBGene00016261	M01E5.2	Tong
PAC10	WBGene00006889	T06G6.9	BUB2	WBGene00016352	F21C3.5	Tong
PAC10	WBGene00006889	T06G6.9	CAF40	WBGene00016139	C30F12.2	Tong
PAC10	WBGene00006889	T06G6.9	CAP2	WBGene00000293	C33F10.2	Tong
PAC10	WBGene00006889	T06G6.9	CHL1	WBGene00010839	C26E6.3	Tong
PAC10	WBGene00006889	T06G6.9	DNM1	WBGene00001093	M106.5	Tong
PAC10	WBGene00006889	T06G6.9	FAB1	WBGene00004089	M03C11.2	Tong
PAC10	WBGene00006889	T06G6.9	HSE1	WBGene00004109	T12E12.4	Tong
PAC10	WBGene00006889	T06G6.9	HTZ1	WBGene00019947	C05E7.5 & VF11C1L.1	Tong
PAC10	WBGene00006889	T06G6.9	KEM1	WBGene00012730	C34G6.7	Tong
PAC10	WBGene00006889	T06G6.9	MAK3	WBGene00015074	R08C7.3	Tong
PAC10	WBGene00006889	T06G6.9	MMS2	WBGene00006730	Y39G8C.Y39G8C.1 & Y39G8C.bb	Tong
PAC10	WBGene00006889	T06G6.9	NCL1	WBGene00021686	B0238.10	Tong
PAC10	WBGene00006889	T06G6.9	PER1	WBGene00019806	F39B2.2	Tong
PAC10	WBGene00006889	T06G6.9	PLP1	WBGene00015482	Y48G8A_2614.a	Tong
PAC10	WBGene00006889	T06G6.9	SPF1	WBGene00007514	R01B10.4	Tong
PAC10	WBGene00006889	T06G6.9	STE24	WBGene00001405	C05D11.3	Tong
PAC10	WBGene00006889	T06G6.9	UBA4	WBGene00018357	C10C6.6	Tong

PAC10	WBGene00006889	T06G6.9	UTR4	WBGene00010286	C04F12.10	Tong
PAC10	WBGene00006889	T06G6.9	VPS29	WBGene00014234	F42G8.6	Tong
PAC10	WBGene00006889	T06G6.9	VRP1	WBGene00020094	F58H1.3	Tong
PAC10	WBGene00006889	T06G6.9	YBR108W	WBGene00004110	ZK1128.8	Tong
PAC10	WBGene00006889	T06G6.9	YGR054W	WBGene00008480	R144.4 & R144.8	Tong
PAC10	WBGene00006889	T06G6.9	YJR129C	WBGene00011148	C37A2.2	Tong
PAC2	WBGene00019503	K07H8.1	CTF18	WBGene00010676	E04D5.1	Tong
PAC2	WBGene00019503	K07H8.1	GIM3	WBGene00007107	R08D7.4	Tong
PAC2	WBGene00019503	K07H8.1	GIM5	WBGene00020112	K08F4.1	Tong
PAC2	WBGene00019503	K07H8.1	MAD2	WBGene00003161	B0035.4	Tong
PAC2	WBGene00019503	K07H8.1	PAC10	WBGene00006889	R151.9	Tong
PAC2	WBGene00019503	K07H8.1	YKE2	WBGene00009004	Y69A2A_2326.a	Tong
POL32	WBGene00011016	R04F11.3	ARC18	WBGene00000203	T06G6.9	Tong
POL32	WBGene00011016	R04F11.3	ARP6	WBGene00007434	F21C3.5	Pan
POL32	WBGene00011016	R04F11.3	CKB2	WBGene00002196	Y37D8A.1	Pan
POL32	WBGene00011016	R04F11.3	FKH2	WBGene00003976	C08B11.6	Pan
POL32	WBGene00011016	R04F11.3	HTZ1	WBGene00019947	T01G9.6	Pan
POL32	WBGene00011016	R04F11.3	ISC1	WBGene00012105	T28H11.4	Pan
POL32	WBGene00011016	R04F11.3	LSM1	WBGene00003076	R08C7.3	Pan
POL32	WBGene00011016	R04F11.3	MSH2	WBGene00003418	T27F6.6	Pan
POL32	WBGene00011016	R04F11.3	PEP3	WBGene00021058	F40F8.9	Pan
POL32	WBGene00011016	R04F11.3	RAD27	WBGene00000794	H26D21.2	Tong, Pan
POL32	WBGene00011016	R04F11.3	RAD5	WBGene00010061	W06B4.3	Pan
POL32	WBGene00011016	R04F11.3	RAD50	WBGene00004296	Y47G6A_247.i	Tong, Pan
POL32	WBGene00011016	R04F11.3	RAD51	WBGene00004297	F54E12.2	Tong, Pan
POL32	WBGene00011016	R04F11.3	RAD54	WBGene00004298	T04H1.4	Tong, Pan
POL32	WBGene00011016	R04F11.3	RAD6	WBGene00006701	Y43C5A.6	Pan
POL32	WBGene00011016	R04F11.3	RPN10	WBGene00004466	W06D4.6	Pan
POL32	WBGene00011016	R04F11.3	RVS161	WBGene00010272	C35B1.1	Pan
POL32	WBGene00011016	R04F11.3	RVS167	WBGene00020209	B0205.3	Pan
POL32	WBGene00011016	R04F11.3	STP22	WBGene00015658	F58G6.1	Pan
POL32	WBGene00011016	R04F11.3	VIP1	WBGene00018508	T04C9.1 & ZK328.3	Pan
POL32	WBGene00011016	R04F11.3	VPS34	WBGene00006932	C09G12.9	Pan
POL32	WBGene00011016	R04F11.3	YAF9	WBGene00001585	F46F11.1	Pan
POP2	WBGene00000369	Y56A3A.20	ARP6	WBGene00007434	B0025.1	Pan
POP2	WBGene00000369	Y56A3A.20	ATP1	WBGene00010419*	M04B2.3	Pan
POP2	WBGene00000369	Y56A3A.20	ATP5	WBGene00017856*	C08B11.6	Pan
POP2	WBGene00000369	Y56A3A.20	CTK1	WBGene00007135	H28O16.1	Pan
POP2	WBGene00000369	Y56A3A.20	DID4	WBGene00012903	F27C1.7	Pan
POP2	WBGene00000369	Y56A3A.20	GCS1	WBGene00010500	B0285.1 & B0285.2	Pan
POP2	WBGene00000369	Y56A3A.20	KAR3	WBGene00002216	Y46G5.m	Pan
POP2	WBGene00000369	Y56A3A.20	LSM1	WBGene00003076	K02B12.7	Pan
POP2	WBGene00000369	Y56A3A.20	LSM6	WBGene00003080	T09A5.2	Pan
POP2	WBGene00000369	Y56A3A.20	LSM7	WBGene00003081	F40F8.9	Pan
POP2	WBGene00000369	Y56A3A.20	MSY1	WBGene00006968	Y71G12A_187.b	Pan
POP2	WBGene00000369	Y56A3A.20	NHX1	WBGene00003733	ZK593.7	Pan
POP2	WBGene00000369	Y56A3A.20	PIF1	WBGene00004028	K08F11.4	Pan
POP2	WBGene00000369	Y56A3A.20	PIM1	WBGene00016391	F57C7.2	Pan

POP2	WBGene00000369	Y56A3A.20	RAD50	WBGene00004296	Y18H1A_67.c & Y18H1A_67.d & Y18H1A_67.f	Pan
POP2	WBGene00000369	Y56A3A.20	RAD51	WBGene00004297	C34B2.6	Pan
POP2	WBGene00000369	Y56A3A.20	RAD54	WBGene00004298	T04H1.4	Pan
POP2	WBGene00000369	Y56A3A.20	RAD6	WBGene00006701	Y43C5A.6	Pan
POP2	WBGene00000369	Y56A3A.20	RVS161	WBGene00010272	W06D4.6	Pan
POP2	WBGene00000369	Y56A3A.20	RVS167	WBGene00020209	C35B1.1	Pan
POP2	WBGene00000369	Y56A3A.20	SAC1	WBGene00009264	F58G6.1	Pan
POP2	WBGene00000369	Y56A3A.20	SGS1	WBGene00001865	T04C9.1 & ZK328.3	Pan
POP2	WBGene00000369	Y56A3A.20	SIN3	WBGene00004117	F30A10.6	Pan
POP2	WBGene00000369	Y56A3A.20	SNF5	WBGene00011111	T04A11.6	Pan
POP2	WBGene00000369	Y56A3A.20	SNF8	WBGene00016167	F02E9.4	Pan
POP2	WBGene00000369	Y56A3A.20	STP22	WBGene00015658	R07E5.3	Pan
POP2	WBGene00000369	Y56A3A.20	TFP1	WBGene00013025*	C27F2.5	Pan
POP2	WBGene00000369	Y56A3A.20	VAC14	WBGene00010565	C09G12.9	Pan
POP2	WBGene00000369	Y56A3A.20	VPS20	WBGene00022027	Y49A3A.2	Pan
POP2	WBGene00000369	Y56A3A.20	VPS24	WBGene00020866	K04G2.6	Pan
POP2	WBGene00000369	Y56A3A.20	VPS30	WBGene00000247	Y65B4A_182.b & Y65B4A_182.c	Pan
POP2	WBGene00000369	Y56A3A.20	VPS36	WBGene00008919	F59A6.7 & T27F7.1	Pan
POP2	WBGene00000369	Y56A3A.20	VPS4	WBGene00021334	T19E7.3 & T19E7.4	Pan
POP2	WBGene00000369	Y56A3A.20	VPS45	WBGene00016643	F17C11.8	Pan
POP2	WBGene00000369	Y56A3A.20	YAF9	WBGene00001585	Y34D9A_152.a	Pan
PRT1	WBGene00001225	Y54E2A.11	PRO3	WBGene00010924	C44C1.1 & C44C1.4	Davierwala
RAD27	WBGene00000794	Y47G6A_247.i	ARP6	WBGene00007434	M04B2.3	Pan
RAD27	WBGene00000794	Y47G6A_247.i	CAC2	WBGene00022141	M153.1	Tong, Pan
RAD27	WBGene00000794	Y47G6A_247.i	CKB2	WBGene00002196	C08B11.6	Pan
RAD27	WBGene00000794	Y47G6A_247.i	CSM3	WBGene00017738	Y71G12A_202.d	Tong, Pan
RAD27	WBGene00000794	Y47G6A_247.i	CTF18	WBGene00010676	T01G9.6	Tong, Pan
RAD27	WBGene00000794	Y47G6A_247.i	CTK1	WBGene00007135	F23C8.9	Pan
RAD27	WBGene00000794	Y47G6A_247.i	DOA1	WBGene00007333	K08F4.1	Pan
RAD27	WBGene00000794	Y47G6A_247.i	DOC1	WBGene00000144	B0285.1 & B0285.2	Tong
RAD27	WBGene00000794	Y47G6A_247.i	GUF1	WBGene00022862	C05C10.6	Pan
RAD27	WBGene00000794	Y47G6A_247.i	HTZ1	WBGene00019947	F15H10.3	Pan
RAD27	WBGene00000794	Y47G6A_247.i	ISC1	WBGene00012105	ZK1236.1	Pan
RAD27	WBGene00000794	Y47G6A_247.i	LSM1	WBGene00003076	R08C7.3	Pan
RAD27	WBGene00000794	Y47G6A_247.i	LSM6	WBGene00003080	T27F6.6	Pan
RAD27	WBGene00000794	Y47G6A_247.i	LSM7	WBGene00003081	F40F8.9	Pan
RAD27	WBGene00000794	Y47G6A_247.i	MRE11	WBGene00003405	Y71G12A_187.b	Tong, Pan
RAD27	WBGene00000794	Y47G6A_247.i	MUS81	WBGene00016602	ZK593.7	Tong, Pan
RAD27	WBGene00000794	Y47G6A_247.i	NHX1	WBGene00003733	ZC302.1	Pan
RAD27	WBGene00000794	Y47G6A_247.i	PEP3	WBGene00021058	C43E11.2	Pan
RAD27	WBGene00000794	Y47G6A_247.i	PEP5	WBGene00011067	F57C7.2	Pan
RAD27	WBGene00000794	Y47G6A_247.i	PIF1	WBGene00004028	W06B4.3	Pan
RAD27	WBGene00000794	Y47G6A_247.i	POP2	WBGene00000369	R06F6.2	Pan
RAD27	WBGene00000794	Y47G6A_247.i	RAD23	WBGene00013924	Y18H1A_67.c & Y18H1A_67.d & Y18H1A_67.f	Pan
RAD27	WBGene00000794	Y47G6A_247.i	RAD5	WBGene00010061	Y56A3A.20	Pan
RAD27	WBGene00000794	Y47G6A_247.i	RAD50	WBGene00004296	ZK20.3	Tong, Pan
RAD27	WBGene00000794	Y47G6A_247.i	RAD51	WBGene00004297	F54E12.2	Tong, Pan
RAD27	WBGene00000794	Y47G6A_247.i	RAD54	WBGene00004298	T04H1.4	Tong, Pan

RAD27	WBGene00000794	Y47G6A_247.i	RAD6	WBGene00006701	Y43C5A.6	Pan
RAD27	WBGene00000794	Y47G6A_247.i	RPN10	WBGene00004466	W06D4.6	Pan
RAD27	WBGene00000794	Y47G6A_247.i	RTF1	WBGene00009103	C35B1.1	Pan
RAD27	WBGene00000794	Y47G6A_247.i	RVS167	WBGene00020209	B0205.3	Pan
RAD27	WBGene00000794	Y47G6A_247.i	SAC1	WBGene00009264	F25B3.6	Pan
RAD27	WBGene00000794	Y47G6A_247.i	SNF8	WBGene00016167	T04C9.1 & ZK328.3	Pan
RAD27	WBGene00000794	Y47G6A_247.i	SSN8	WBGene00000506	F30A10.6	Pan
RAD27	WBGene00000794	Y47G6A_247.i	STP22	WBGene00015658	C27F2.5	Pan
RAD27	WBGene00000794	Y47G6A_247.i	UBP14	WBGene00020839	H14E04.5	Pan
RAD27	WBGene00000794	Y47G6A_247.i	VPS34	WBGene00006932	C09G12.9	Pan
RAD27	WBGene00000794	Y47G6A_247.i	VPS5	WBGene00004927	T27A3.2	Pan
RAD27	WBGene00000794	Y47G6A_247.i	YAF9	WBGene00001585	B0025.1	Pan
RAD27	WBGene00000794	Y47G6A_247.i	YTA7	WBGene00008682	C05D9.1	Pan
RAD5	WBGene00010061	F54E12.2	BRE1	WBGene00007008	M04B2.3	Pan
RAD5	WBGene00010061	F54E12.2	MUS81	WBGene00016602	F11A10.1	Pan
RAD5	WBGene00010061	F54E12.2	RAD51	WBGene00004297	R05D3.4	Pan
RAD5	WBGene00010061	F54E12.2	RAD54	WBGene00004298	C43E11.2	Pan
RAD5	WBGene00010061	F54E12.2	SGS1	WBGene00001865	Y43C5A.6	Pan
RAD50	WBGene00004296	T04H1.4	BRE1	WBGene00007008	W06D4.6	Tong, Pan
RAD50	WBGene00004296	T04H1.4	CDC45	WBGene00009372	T04A11.6	Davierwala
RAD50	WBGene00004296	T04H1.4	CDC9	WBGene00002985	R05D3.4	Davierwala
RAD50	WBGene00004296	T04H1.4	CTK1	WBGene00007135	F34D10.2 & F34D10.3	Pan
RAD50	WBGene00004296	T04H1.4	KEM1	WBGene00012730	C29A12.3	Pan
RAD50	WBGene00004296	T04H1.4	LSM1	WBGene00003076	B0285.1 & B0285.2	Pan
RAD50	WBGene00004296	T04H1.4	LSM6	WBGene00003080	Y39G8C.1 & Y39G8C.b	Pan
RAD50	WBGene00004296	T04H1.4	LSM7	WBGene00003081	F40F8.9	Pan
RAD50	WBGene00004296	T04H1.4	MUS81	WBGene00016602	Y71G12A_187.b	Pan
RAD50	WBGene00004296	T04H1.4	NPR2	WBGene00018635	ZK593.7	Tong
RAD50	WBGene00004296	T04H1.4	RAD5	WBGene00010061	C43E11.2	Tong, Pan
RAD50	WBGene00004296	T04H1.4	RAD6	WBGene00006701	F49E8.1	Pan
RAD50	WBGene00004296	T04H1.4	REV1	WBGene00014066	F54E12.2	Pan
RAD50	WBGene00004296	T04H1.4	RFC2	WBGene00004340	C35B1.1	Davierwala
RAD50	WBGene00004296	T04H1.4	RPN10	WBGene00004466	ZK675.2	Pan
RAD50	WBGene00004296	T04H1.4	STP22	WBGene00015658	F31E3.3	Pan
RAD50	WBGene00004296	T04H1.4	SWD1	WBGene00017683	B0205.3	Tong
RAD50	WBGene00004296	T04H1.4	VPS15	WBGene00014151	C09G12.9	Pan
RAD50	WBGene00004296	T04H1.4	VPS34	WBGene00006932	F21H12.1	Pan
RAD50	WBGene00004296	T04H1.4	YDR140W	WBGene00016341	ZK930.1 & ZK930.7	Pan
RAD50	WBGene00004296	T04H1.4	YER087W	WBGene00004190	B0025.1	Pan
RAD51	WBGene00004297	Y43C5A.6	CTK1	WBGene00007135	C33C12.9	Pan
RAD51	WBGene00004297	Y43C5A.6	DIA4	WBGene00005662	T27F6.5	Pan
RAD51	WBGene00004297	Y43C5A.6	LSM1	WBGene00003076	B0285.1 & B0285.2	Pan
RAD51	WBGene00004297	Y43C5A.6	LSM6	WBGene00003080	W03B1.4	Pan
RAD51	WBGene00004297	Y43C5A.6	LSM7	WBGene00003081	F40F8.9	Pan
RAD51	WBGene00004297	Y43C5A.6	RAD6	WBGene00006701	Y71G12A_187.b	Pan
RAD51	WBGene00004297	Y43C5A.6	REV1	WBGene00014066	ZK593.7	Pan
RAD51	WBGene00004297	Y43C5A.6	RPN10	WBGene00004466	C35B1.1	Pan
RAD51	WBGene00004297	Y43C5A.6	SGS1	WBGene00001865	ZK675.2	Pan

RAD51	WBGene00004297	Y43C5A.6	STP22	WBGene00015658	B0205.3	Pan
RAD51	WBGene00004297	Y43C5A.6	VPS15	WBGene00014151	T04A11.6	Pan
RAD51	WBGene00004297	Y43C5A.6	VPS34	WBGene00006932	C09G12.9	Pan
RAD51	WBGene00004297	Y43C5A.6	YER087W	WBGene00004190	ZK930.1 & ZK930.7	Pan
RAD54	WBGene00004298	W06D4.6	CTK1	WBGene00007135	B0025.1	Pan
RAD54	WBGene00004298	W06D4.6	KEM1	WBGene00012730	T27F6.5	Pan
RAD54	WBGene00004298	W06D4.6	LSM1	WBGene00003076	B0285.1 & B0285.2	Pan
RAD54	WBGene00004298	W06D4.6	LSM6	WBGene00003080	Y39G8C.1 & Y39G8C.b	Pan
RAD54	WBGene00004298	W06D4.6	LSM7	WBGene00003081	F40F8.9	Pan
RAD54	WBGene00004298	W06D4.6	RAD6	WBGene00006701	Y71G12A_187.b	Pan
RAD54	WBGene00004298	W06D4.6	RPN10	WBGene00004466	ZK593.7	Pan
RAD54	WBGene00004298	W06D4.6	SGS1	WBGene00001865	C35B1.1	Pan
RAD54	WBGene00004298	W06D4.6	STP22	WBGene00015658	B0205.3	Pan
RAD54	WBGene00004298	W06D4.6	VPS34	WBGene00006932	T04A11.6	Pan
RAD54	WBGene00004298	W06D4.6	YDR140W	WBGene00016341	C09G12.9	Pan
RAD54	WBGene00004298	W06D4.6	YER087W	WBGene00004190	B0025.1	Pan
RAD6	WBGene00006701	C35B1.1	ARC18	WBGene00000203	C33C12.9	Pan
RAD6	WBGene00006701	C35B1.1	ARP6	WBGene00007434	T27F6.5	Pan
RAD6	WBGene00006701	C35B1.1	BUB2	WBGene00016352	Y37D8A.1	Pan
RAD6	WBGene00006701	C35B1.1	CEM1	WBGene00008667	C08B11.6	Pan
RAD6	WBGene00006701	C35B1.1	COQ6	WBGene00000766	C33F10.2	Pan
RAD6	WBGene00006701	C35B1.1	COX6	WBGene00012553	F10G8.9	Pan
RAD6	WBGene00006701	C35B1.1	CTK1	WBGene00007135	K07B1.2	Pan
RAD6	WBGene00006701	C35B1.1	DIA4	WBGene00005662	Y37D8A.14	Pan
RAD6	WBGene00006701	C35B1.1	FAB1	WBGene00004089	B0285.1 & B0285.2	Pan
RAD6	WBGene00006701	C35B1.1	FKH2	WBGene00003976	W03B1.4	Pan
RAD6	WBGene00006701	C35B1.1	GUA1	WBGene00010912	C05E7.5 & VF11C1L.1	Pan
RAD6	WBGene00006701	C35B1.1	HTZ1	WBGene00019947	T28H11.4	Pan
RAD6	WBGene00006701	C35B1.1	ISC1	WBGene00012105	M106.4	Pan
RAD6	WBGene00006701	C35B1.1	KAR3	WBGene00002216	R08C7.3	Pan
RAD6	WBGene00006701	C35B1.1	KEM1	WBGene00012730	T27F6.6	Pan
RAD6	WBGene00006701	C35B1.1	MRPL9	WBGene00016142	T09A5.2	Pan
RAD6	WBGene00006701	C35B1.1	PEP3	WBGene00021058	Y39G8C.1 & Y39G8C.b	Pan
RAD6	WBGene00006701	C35B1.1	PPA1	WBGene00011347*	C26E6.6	Pan
RAD6	WBGene00006701	C35B1.1	RAD14	WBGene00006963	W06B4.3	Pan
RAD6	WBGene00006701	C35B1.1	RAD23	WBGene00013924	T01H3.1	Pan
RAD6	WBGene00006701	C35B1.1	RVS161	WBGene00010272	K07G5.2	Pan
RAD6	WBGene00006701	C35B1.1	RVS167	WBGene00020209	ZK20.3	Pan
RAD6	WBGene00006701	C35B1.1	SAC1	WBGene00009264	F58G6.1	Pan
RAD6	WBGene00006701	C35B1.1	SIN3	WBGene00004117	T04C9.1 & ZK328.3	Pan
RAD6	WBGene00006701	C35B1.1	SMP2	WBGene00010425	F30A10.6	Pan
RAD6	WBGene00006701	C35B1.1	SPF1	WBGene00007514	F02E9.4	Pan
RAD6	WBGene00006701	C35B1.1	UBP14	WBGene00020839	H37A05.1	Pan
RAD6	WBGene00006701	C35B1.1	VMA5	WBGene00006920*	C10C6.6	Pan
RAD6	WBGene00006701	C35B1.1	VMA7	WBGene00006918*	T27A3.2	Pan
RAD6	WBGene00006701	C35B1.1	VMA8	WBGene00010130	Y38F2A_5743.f	Pan
RAD6	WBGene00006701	C35B1.1	VPS16	WBGene00006516	ZK970.4	Pan
RAD6	WBGene00006701	C35B1.1	VPS25	WBGene00012193	F55H2.2	Pan

RAD6	WBGene00006701	C35B1.1	VPS36	WBGene00008919	C05D11.2	Pan
RAD6	WBGene00006701	C35B1.1	VRP1	WBGene00020094	W02A11.2	Pan
RAD6	WBGene00006701	C35B1.1	YAF9	WBGene00001585	F17C11.8	Pan
RAD6	WBGene00006701	C35B1.1	YER087W	WBGene00004190	R144.4 & R144.8	Pan
RAD6	WBGene00006701	C35B1.1	YGR257C	WBGene00007622	M04B2.3	Pan
RAD6	WBGene00006701	C35B1.1	ZUO1	WBGene00001029	T27F6.5	Pan
RFC5	WBGene00004339	C39E9.13	ALG2	WBGene00017282	C16C10.1	Davierwala
RFC5	WBGene00004339	C39E9.13	CDC27	WBGene00003132	F38A5.13	Davierwala
RFC5	WBGene00004339	C39E9.13	CDC45	WBGene00009372	F09E5.2	Davierwala
RFC5	WBGene00004339	C39E9.13	CDC9	WBGene00002985	Y110A7A.d	Davierwala
RFC5	WBGene00004339	C39E9.13	DPS1	WBGene00001094*	F34D10.2 & F34D10.3	Davierwala
RFC5	WBGene00004339	C39E9.13	MOT1	WBGene00000274	C29A12.3	Davierwala
RFC5	WBGene00004339	C39E9.13	NOP14	WBGene00021660	B0464.1	Davierwala
RFC5	WBGene00004339	C39E9.13	POL1	WBGene00012936	F15D4.1	Davierwala
RFC5	WBGene00004339	C39E9.13	RFC2	WBGene00004340	Y48G1A_54.d	Davierwala
RPN10	WBGene00004466	B0205.3	CBC2	WBGene00009141	Y47D3A.c & Y47D3A.d	Pan
RPN10	WBGene00004466	B0205.3	NHX1	WBGene00003733	F31E3.3	Pan
RPN10	WBGene00004466	B0205.3	PEP3	WBGene00021058	F26A3.2	Pan
RPN10	WBGene00004466	B0205.3	PEP5	WBGene00011067	F57C7.2	Pan
RPN10	WBGene00004466	B0205.3	RAD23	WBGene00013924	W06B4.3	Pan
RPN10	WBGene00004466	B0205.3	RAD6	WBGene00006701	R06F6.2	Pan
RPN10	WBGene00004466	B0205.3	RVS167	WBGene00020209	ZK20.3	Pan
RPN10	WBGene00004466	B0205.3	SIN3	WBGene00004117	C35B1.1	Pan
RPN10	WBGene00004466	B0205.3	SNF8	WBGene00016167	T04C9.1 & ZK328.3	Pan
RPN10	WBGene00004466	B0205.3	STP22	WBGene00015658	F02E9.4	Pan
RPN10	WBGene00004466	B0205.3	SWD1	WBGene00017683	C27F2.5	Pan
RPN10	WBGene00004466	B0205.3	VPS20	WBGene00022027	C09G12.9	Pan
RPN10	WBGene00004466	B0205.3	VPS25	WBGene00012193	F21H12.1	Pan
RPN10	WBGene00004466	B0205.3	VPS36	WBGene00008919	Y65B4A_182.b & Y65B4A_182.c	Pan
RPN10	WBGene00004466	B0205.3	VPS45	WBGene00016643	W02A11.2	Pan
RPN10	WBGene00004466	B0205.3	YDR117C	WBGene00016113	F17C11.8	Pan
RVS161	WBGene00010272	F58G6.1	CAP1	WBGene00000292	C44C1.1 & C44C1.4	Tong
RVS161	WBGene00010272	F58G6.1	CAP2	WBGene00000293	C25H3.4	Tong
RVS161	WBGene00010272	F58G6.1	DOA1	WBGene00007333	D2024.6	Tong
RVS161	WBGene00010272	F58G6.1	GIM3	WBGene00007107	M106.5	Tong
RVS161	WBGene00010272	F58G6.1	GIM4	WBGene00019220	C05C10.6	Tong
RVS161	WBGene00010272	F58G6.1	GIM5	WBGene00020112	B0035.4	Tong
RVS161	WBGene00010272	F58G6.1	PAC10	WBGene00006889	H20J04.d	Tong
RVS161	WBGene00010272	F58G6.1	SEC22	WBGene00018853	R151.9	Tong
RVS161	WBGene00010272	F58G6.1	SIN3	WBGene00004117	T06G6.9	Tong
RVS161	WBGene00010272	F58G6.1	SPF1	WBGene00007514	F55A4.1	Tong
RVS161	WBGene00010272	F58G6.1	YKE2	WBGene00009004	F02E9.4	Tong
RVS167	WBGene00020209	T04C9.1 & ZK328.3	CAP1	WBGene00000292	C10C6.6	Tong
RVS167	WBGene00020209	T04C9.1 & ZK328.3	CAP2	WBGene00000293	F21C3.5	Tong
RVS167	WBGene00020209	T04C9.1 & ZK328.3	CHS5	WBGene00022615	D2024.6	Tong
RVS167	WBGene00020209	T04C9.1 & ZK328.3	DOA1	WBGene00007333	M106.5	Tong
RVS167	WBGene00020209	T04C9.1 & ZK328.3	GIM3	WBGene00007107	ZC449.5	Tong
RVS167	WBGene00020209	T04C9.1 & ZK328.3	GIM4	WBGene00019220	C05C10.6	Tong

RVS167	WBGene00020209	T04C9.1 & ZK328.3	GIM5	WBGene00020112	B0035.4	Tong
RVS167	WBGene00020209	T04C9.1 & ZK328.3	PAC10	WBGene00006889	H20J04.d	Tong
RVS167	WBGene00020209	T04C9.1 & ZK328.3	SEC22	WBGene00018853	R151.9	Tong
RVS167	WBGene00020209	T04C9.1 & ZK328.3	SIN3	WBGene00004117	T06G6.9	Tong
RVS167	WBGene00020209	T04C9.1 & ZK328.3	SPF1	WBGene00007514	F55A4.1	Tong
RVS167	WBGene00020209	T04C9.1 & ZK328.3	YKE2	WBGene00009004	F02E9.4	Tong
SEC15	WBGene00016188	C28G1.3	ALG14	WBGene00019725	C10C6.6	Davierwala
SEC15	WBGene00016188	C28G1.3	BET3	WBGene00014222	F21C3.5	Davierwala
SEC15	WBGene00016188	C28G1.3	CDS1	WBGene00016384	M02B7.4	Davierwala
SEC15	WBGene00016188	C28G1.3	ECM16	WBGene00015525	ZK1098.5	Davierwala
SEC15	WBGene00016188	C28G1.3	HRT1	WBGene00004320	C33H5.18	Davierwala
SEC15	WBGene00016188	C28G1.3	MPE1	WBGene00009477	C06E1.10	Davierwala
SEC15	WBGene00016188	C28G1.3	MRT4	WBGene00017347	ZK287.5	Davierwala
SEC15	WBGene00016188	C28G1.3	NOC2	WBGene00007413	F36F2.3	Davierwala
SEC15	WBGene00016188	C28G1.3	NOP15	WBGene00011408	F10E7.5	Davierwala
SEC15	WBGene00016188	C28G1.3	PDS5	WBGene00001352	C07E3.2	Davierwala
SEC15	WBGene00016188	C28G1.3	PFY1	WBGene00003991	T04A8.6	Davierwala
SEC15	WBGene00016188	C28G1.3	PRP16	WBGene00003389	H38K22.1	Davierwala
SEC15	WBGene00016188	C28G1.3	RPC11	WBGene00022309	K03E6.6	Davierwala
SEC15	WBGene00016188	C28G1.3	RPC40	WBGene00019275	K03H1.2	Davierwala
SEC15	WBGene00016188	C28G1.3	RPF1	WBGene00009711	Y77E11A_3443.q	Davierwala
SEC15	WBGene00016188	C28G1.3	YGL047W	WBGene00011193	H43I07.2	Davierwala
SEC15	WBGene00016188	C28G1.3	YNL313C	WBGene00020600	F44G4.1	Davierwala
SEC15	WBGene00016188	C28G1.3	ZPR1	WBGene00020999	R10D12.12	Davierwala
SEC18	WBGene00003818	H15N14.1 & ZK1014.1	HRT1	WBGene00004320	T20B12.1	Davierwala
SEC18	WBGene00003818	H15N14.1 & ZK1014.1	MRT4	WBGene00017347	W03F9.1	Davierwala
SEC18	WBGene00003818	H15N14.1 & ZK1014.1	PFY1	WBGene00003991	ZK287.5	Davierwala
SEC18	WBGene00003818	H15N14.1 & ZK1014.1	RPC40	WBGene00019275	F10E7.5	Davierwala
SEC18	WBGene00003818	H15N14.1 & ZK1014.1	RPF1	WBGene00009711	K03E6.6	Davierwala
SEC18	WBGene00003818	H15N14.1 & ZK1014.1	TFG2	WBGene00012694	H43I07.2	Davierwala
SEC7	WBGene00012386	Y6B3A.1 & Y87G2A.y	CDC45	WBGene00009372	F44G4.1	Davierwala
SEC7	WBGene00012386	Y6B3A.1 & Y87G2A.y	DRS1	WBGene00022148	Y39B6A.f	Davierwala
SEC7	WBGene00012386	Y6B3A.1 & Y87G2A.y	LAS17	WBGene00006565	F34D10.2 & F34D10.3	Davierwala
SEC7	WBGene00012386	Y6B3A.1 & Y87G2A.y	NOC2	WBGene00007413	Y71G12A_201.a & Y71G12A_203.c	Davierwala
SEC7	WBGene00012386	Y6B3A.1 & Y87G2A.y	NOG2	WBGene00003596	Y63D3A.5	Davierwala
SEC7	WBGene00012386	Y6B3A.1 & Y87G2A.y	NOP15	WBGene00011408	C07E3.2	Davierwala
SEC7	WBGene00012386	Y6B3A.1 & Y87G2A.y	NUG1	WBGene00003821	T19A6.2	Davierwala
SEC7	WBGene00012386	Y6B3A.1 & Y87G2A.y	PFY1	WBGene00003991	T04A8.6	Davierwala
SEC7	WBGene00012386	Y6B3A.1 & Y87G2A.y	RFT1	WBGene00022677	K01C8.9	Davierwala
SEC7	WBGene00012386	Y6B3A.1 & Y87G2A.y	RPF1	WBGene00009711	K03E6.6	Davierwala
SEC7	WBGene00012386	Y6B3A.1 & Y87G2A.y	THS1	WBGene00006617	ZK180.3	Davierwala
SEC7	WBGene00012386	Y6B3A.1 & Y87G2A.y	TRM5	WBGene00008263	F44G4.1	Davierwala
SET2	WBGene00021515	Y41D4A_2615.a &	ARP6	WBGene00007434	C47D12.6	Tong
SET2	WBGene00021515	Y41D4A_3457.b	BET3	WBGene00014222	C53A5.2	Davierwala
SET2	WBGene00021515	Y41D4A_2615.a &	BRE1	WBGene00007008	C08B11.6	Tong
SET2	WBGene00021515	Y41D4A_3457.b	GIM3	WBGene00007107	ZK1098.5	Tong
SET2	WBGene00021515	Y41D4A_2615.a &	GIM5	WBGene00020112	R05D3.4	Tong
SET2	WBGene00021515	Y41D4A_3457.b	HTZ1	WBGene00019947	B0035.4	Tong

SET2	WBGene00021515	Y41D4A_2615.a &	LEO1	WBGene00007110	R151.9	Tong
SET2	WBGene00021515	Y41D4A_3457.b	RTF1	WBGene00009103	R08C7.3	Tong
SET2	WBGene00021515	Y41D4A_2615.a &	SEC22	WBGene00018853	B0035.11	Tong
SET2	WBGene00021515	Y41D4A_3457.b	SED5	WBGene00006373	F25B3.6	Davierwala
SET2	WBGene00021515	Y41D4A_2615.a &	SLU7	WBGene00010629	F55A4.1	Davierwala
SET2	WBGene00021515	Y41D4A_3457.b	TFG1	WBGene00015296	F55A11.2	Davierwala
SET2	WBGene00021515	Y41D4A_2615.a &	VAC14	WBGene00010565	K07C5.6	Tong
SET2	WBGene00021515	Y41D4A_3457.b	YDL033C	WBGene00007114	C01F1.1	Tong
SGS1	WBGene00001865	Y41D4A_2615.a &	CSM3	WBGene00017738	K04G2.6	Tong, Pan
SGS1	WBGene00001865	Y41D4A_3457.b	CTF18	WBGene00010676	B0035.16	Tong, Pan
SGS1	WBGene00001865	Y41D4A_2615.a &	LSM1	WBGene00003076	F23C8.9	Pan
SGS1	WBGene00001865	Y41D4A_3457.b	MUS81	WBGene00016602	K08F4.1	Tong, Pan
SGS1	WBGene00001865	Y41D4A_2615.a &	POL32	WBGene00011016	F40F8.9	Tong, Pan
SGS1	WBGene00001865	Y41D4A_3457.b	RAD27	WBGene00000794	C43E11.2	Tong, Pan
SGS1	WBGene00001865	Y41D4A_2615.a &	RAD50	WBGene00004296	R04F11.3	Tong, Pan
SGS1	WBGene00001865	Y41D4A_3457.b	SLX1	WBGene00018909	Y47G6A_247.i	Tong, Pan
SGS1	WBGene00001865	Y41D4A_2615.a &	TOP1	WBGene00006595	T04H1.4	Tong
TOP1	WBGene00006595	Y41D4A_3457.b	MRE11	WBGene00003405	F56A3.2	Tong
TOP1	WBGene00006595	Y41D4A_2615.a &	RAD50	WBGene00004296	M01E5.5	Tong
YKE2	WBGene00009004	Y41D4A_3457.b	ARP6	WBGene00007434	ZC302.1	Tong
YKE2	WBGene00009004	Y41D4A_2615.a &	BUB2	WBGene00016352	T04H1.4	Tong
YKE2	WBGene00009004	Y41D4A_3457.b	CAF40	WBGene00016139	C08B11.6	Tong
YKE2	WBGene00009004	T04A11.6	CAP2	WBGene00000293	C33F10.2	Tong
YKE2	WBGene00009004	T04A11.6	CBP3	WBGene00016442	C26E6.3	Tong
YKE2	WBGene00009004	T04A11.6	CTF18	WBGene00010676	M106.5	Tong, Pan
YKE2	WBGene00009004	T04A11.6	FAB1	WBGene00004089	C35D10.5	Tong
YKE2	WBGene00009004	T04A11.6	HAP2	WBGene00013178	K08F4.1	Tong
YKE2	WBGene00009004	T04A11.6	HTZ1	WBGene00019947	C05E7.5 & VF11C1L.1	Tong
YKE2	WBGene00009004	T04A11.6	ISC1	WBGene00012105	Y53H1A.d	Tong
YKE2	WBGene00009004	T04A11.6	KEM1	WBGene00012730	R08C7.3	Tong
YKE2	WBGene00009004	T04A11.6	MMS2	WBGene00006730	T27F6.6	Tong
YKE2	WBGene00009004	M01E5.5	NCL1	WBGene00021686	Y39G8C.1 & Y39G8C.b	Tong
YKE2	WBGene00009004	M01E5.5	PAN3	WBGene00014015	F39B2.2	Tong
YKE2	WBGene00009004	F21C3.5	PCH2	WBGene00008641	Y48G8A_2614.a	Tong
YKE2	WBGene00009004	F21C3.5	PET8	WBGene00008364	ZK632.7	Tong
YKE2	WBGene00009004	F21C3.5	PLP1	WBGene00015482	F10B5.5	Tong
YKE2	WBGene00009004	F21C3.5	SPF1	WBGene00007514	D1046.3	Tong
YKE2	WBGene00009004	F21C3.5	STE24	WBGene00001405	C05D11.3	Tong
YKE2	WBGene00009004	F21C3.5	UBA4	WBGene00018357	C10C6.6	Tong
YKE2	WBGene00009004	F21C3.5	UGA1	WBGene00001794	C04F12.10	Tong
YKE2	WBGene00009004	F21C3.5	VAC14	WBGene00010565	F42G8.6	Tong
YKE2	WBGene00009004	F21C3.5	VPS29	WBGene00014234	K04D7.3	Tong
YKE2	WBGene00009004	F21C3.5	VRP1	WBGene00020094	K04G2.6	Tong
YKE2	WBGene00009004	F21C3.5	YDL033C	WBGene00007114	ZK1128.8	Tong
YKE2	WBGene00009004	F21C3.5	YTA7	WBGene00008682	R144.4 & R144.8	Tong
		F21C3.5			B0035.16	
		F21C3.5			F11A10.1	
		F21C3.5				

		F21C3.5 F21C3.5 F21C3.5 F21C3.5 F21C3.5 F21C3.5 F21C3.5 F21C3.5 F21C3.5 F21C3.5 F21C3.5				

Appendix Table 5.1. *S. cerevisiae* synthetic lethal interactions and their pairwise *C. elegans* orthologues
For each *S. cerevisiae* synthetic lethal gene pair ('Gene1', 'Gene 2') with both single orthologues in *C. elegans*, *S. cerevisiae* standard names ('SC Name'), their orthologous *C. elegans* WormBase gene names ('CE Name') & Ahringer library RNA interference (RNAi) clone gene pairs names ('CE RNAi clone') together with references for yeast synthetic lethal screens are shown. * designates genes that resulted in first-generation larval growth arrest after RNAi & were excluded from the study for genetic interactions. Tong, Davierwala, and Pan denote yeast datasets from Tong *et al.*, 2003, Davierwala *et al.*, 2005, and Pan *et al.*, 2006, respectively.

Appendix Table 5.2. ***C. elegans*** **orthologues of** ***S. cerevisiae*** **synthetic lethal interactions with homozygous viable loss-of-function alleles**

Gene 1 (homozygous viable loss-of-function allele)				Gene 2 (RNAi)			References
SC Name	CE Name	CE RNAi clone	CE Strain	SC Name	CE Name	CE RNAi clone	
ARC40	WBGene00000201	Y79H2A.6	tm1681	ARC18	WBGene00000203	Y37D8A.1	Tong
ARC40	WBGene00000201	Y79H2A.6	tm1681	CHS5	WBGene00022615	ZC449.5	Tong
ARC40	WBGene00000201	Y79H2A.6	tm1681	GIM3	WBGene00007107	B0035.4	Tong
ARC40	WBGene00000201	Y79H2A.6	tm1681	GIM4	WBGene00019220	H20J04.d	Tong
ARC40	WBGene00000201	Y79H2A.6	tm1681	GLO3	WBGene00017217	F07F6.4	Tong
ARC40	WBGene00000201	Y79H2A.6	tm1681	SEC22	WBGene00018853	F55A4.1	Tong
ARC40	WBGene00000201	Y79H2A.6	tm1681	SPF1	WBGene00007514	C10C6.6	Tong
ARC40	WBGene00000201	Y79H2A.6	tm1681	STE24	WBGene00001405	C04F12.10	Tong
ARC40	WBGene00000201	Y79H2A.6	tm1681	VRP1	WBGene00020094	R144.4 & R144.8	Tong
ARC40	WBGene00000201	Y79H2A.6	tm1681	YKE2	WBGene00009004	F21C3.5	Tong
CAF40	WBGene00016139	C26E6.3	RB1477	GIM5	WBGene00020112	R151.9	Tong
CAF40	WBGene00016139	C26E6.3	RB1477	PAC10	WBGene00006889	T06G6.9	Tong
CAF40	WBGene00016139	C26E6.3	RB1477	YKE2	WBGene00009004	F21C3.5	Tong
CDC16	WBGene00001281	F10B5.6	DS88	NIP7	WBGene00016607	C43E11.9	Davierwal
CDC16	WBGene00001281	F10B5.6	GG48	NIP7	WBGene00016607	C43E11.9	a
CDC16	WBGene00001281	F10B5.6	HY621	NIP7	WBGene00016607	C43E11.9	Davierwal
CDC16	WBGene00001281	F10B5.6	TJ1047	NIP7	WBGene00016607	C43E11.9	a
CDC16	WBGene00001281	F10B5.6	TJ1049	NIP7	WBGene00016607	C43E11.9	Davierwal
CDC16	WBGene00001281	F10B5.6	TJ1061	NIP7	WBGene00016607	C43E11.9	a
CDC27	WBGene00003132	Y110A7A.d	DS77	RFC5	WBGene00004339	C39E9.13	Davierwal
CDC27	WBGene00003132	Y110A7A.d	DS80	RFC5	WBGene00004339	C39E9.13	a
CDC27	WBGene00003132	Y110A7A.d	HY604	RFC5	WBGene00004339	C39E9.13	Davierwal
CDC42	WBGene00000390	R07G3.1	RB942	CAP1	WBGene00000292	D2024.6	a
CDC42	WBGene00000390	R07G3.1	RB942	CAP2	WBGene00000293	M106.5	Davierwal
CDC42	WBGene00000390	R07G3.1	RB942	ELP3	WBGene00014123	ZK863.3	a
CDC42	WBGene00000390	R07G3.1	RB942	FAD1	WBGene00011271	R53.1	Davierwal
CDC42	WBGene00000390	R07G3.1	RB942	HRT1	WBGene00004320	ZK287.5	a
CDC42	WBGene00000390	R07G3.1	RB942	RPC40	WBGene00019275	H43I07.2	Davierwal
CDC42	WBGene00000390	R07G3.1	RB942	UBA4	WBGene00018357	F42G8.6	a
CDC42	WBGene00000390	R07G3.1	RB942	YGL211W	WBGene00017928	F29C4.6	Davierwal
DYN1	WBGene00000962	T21E12.4	EU828	GIM3	WBGene00007107	B0035.4	a
DYN1	WBGene00000962	T21E12.4	EU828	GIM4	WBGene00019220	H20J04.d	Tong
DYN1	WBGene00000962	T21E12.4	EU828	GIM5	WBGene00020112	R151.9	Tong
DYN1	WBGene00000962	T21E12.4	EU828	HCM1	WBGene00001442	C25A1.2	Tong
DYN1	WBGene00000962	T21E12.4	EU828	KAR3	WBGene00002216	T09A5.2	Davierwal
DYN1	WBGene00000962	T21E12.4	EU828	PAC10	WBGene00006889	T06G6.9	a
DYN1	WBGene00000962	T21E12.4	EU828	YKE2	WBGene00009004	F21C3.5	Davierwal
DYN2	WBGene00001130	C02C6.1	CX51	GIM3	WBGene00007107	B0035.4	a
DYN2	WBGene00001130	C02C6.1	CX51	GIM4	WBGene00019220	H20J04.d	Davierwal
DYN2	WBGene00001130	C02C6.1	CX51	GIM5	WBGene00020112	R151.9	a
DYN2	WBGene00001130	C02C6.1	CX51	PAC10	WBGene00006889	T06G6.9	Tong

DYN2	WBGene00001130	C02C6.1	CX51	YKE2	WBGene00009004	F21C3.5	Tong
EMP24	WBGene00004766	W02D7.7	GS107	CHS5	WBGene00022615	ZC449.5	Tong
EMP24	WBGene00004766	W02D7.7	GS107	CWH41	WBGene00008775	F13H10.4	Tong
FKH2	WBGene00003976	T28H11.4	UL768	POL32	WBGene00011016	R04F11.3	Tong
FKH2	WBGene00003976	T28H11.4	UL768	RAD6	WBGene00006701	C35B1.1	Tong
GIM3	WBGene00007107	B0035.4	VC1032	ARC40	WBGene00000201	Y79H2A.6	Tong
GIM3	WBGene00007107	B0035.4	VC1032	ARP2	WBGene00000200	K07C5.1	Tong
GIM3	WBGene00007107	B0035.4	VC1032	ARP6	WBGene00007434	C08B11.6	Tong
GIM3	WBGene00007107	B0035.4	VC1032	BUB2	WBGene00016352	C33F10.2	Tong
GIM3	WBGene00007107	B0035.4	VC1032	CHL1	WBGene00010839	M03C11.2	Tong
GIM3	WBGene00007107	B0035.4	VC1032	CSM3	WBGene00017738	F23C8.9	Tong
GIM3	WBGene00007107	B0035.4	VC1032	CTF18	WBGene00010676	K08F4.1	Tong
GIM3	WBGene00007107	B0035.4	VC1032	DYN1	WBGene00000962	T21E12.4	Tong
GIM3	WBGene00007107	B0035.4	VC1032	DYN2	WBGene00001130	C02C6.1	Tong
GIM3	WBGene00007107	B0035.4	VC1032	FAB1	WBGene00004089	C05E7.5 & VF11C1L.1	Tong
GIM3	WBGene00007107	B0035.4	VC1032	HTZ1	WBGene00019947	R08C7.3	Pan
GIM3	WBGene00007107	B0035.4	VC1032	KAR3	WBGene00002216	T09A5.2	Pan
GIM3	WBGene00007107	B0035.4	VC1032	MAD2	WBGene00003161	Y69A2A_2326.a	Tong
GIM3	WBGene00007107	B0035.4	VC1032	NAT1	WBGene00021754	Y50D7_162.b & Y50D7_164.a	Tong
GIM3	WBGene00007107	B0035.4	VC1032	NCL1	WBGene00021686	Y48G8A_2614.a	Tong
GIM3	WBGene00007107	B0035.4	VC1032	PAC1	WBGene00003047	T03F6.5	Tong
GIM3	WBGene00007107	B0035.4	VC1032	PAC2	WBGene00019503	K07H8.1	Tong
GIM3	WBGene00007107	B0035.4	VC1032	PAN3	WBGene00014015	ZK632.7	Tong
GIM3	WBGene00007107	B0035.4	VC1032	PER1	WBGene00019806	R01B10.4	Tong, Pan
GIM3	WBGene00007107	B0035.4	VC1032	PLP1	WBGene00015482	C05D11.3	Tong
GIM3	WBGene00007107	B0035.4	VC1032	RVS161	WBGene00010272	F58G6.1	Tong
GIM3	WBGene00007107	B0035.4	VC1032	RVS167	WBGene00020209	T04C9.1 & ZK328.3	Tong
GIM3	WBGene00007107	B0035.4	VC1032	SET2	WBGene00021515	Y41D4A_2615.a &	Tong
GIM3	WBGene00007107	B0035.4	VC1032	STE24	WBGene00001405	Y41D4A_3457.b	Tong
GIM3	WBGene00007107	B0035.4	VC1032	UBA4	WBGene00018357	C04F12.10	Tong
GIM3	WBGene00007107	B0035.4	VC1032	VAC14	WBGene00010565	F42G8.6	Pan
GIM3	WBGene00007107	B0035.4	VC1032	VPS29	WBGene00014234	K04G2.6	Tong
GIM3	WBGene00007107	B0035.4	VC1032	VRP1	WBGene00020094	ZK1128.8	Tong
GIM3	WBGene00007107	B0035.4	VC1032	YBR108W	WBGene00004110	R144.4 & R144.8	Tong
GIM3	WBGene00007107	B0035.4	VC1032	YGR054W	WBGene00008480	C37A2.2	Tong
GIM3	WBGene00007107	B0035.4	VC1032	YTA7	WBGene00008682	E04D5.1	Tong
HCM1	WBGene00001442	C25A1.2	RB884	ARP6	WBGene00007434	F11A10.1	Tong
HCM1	WBGene00001442	C25A1.2	RB884	DYN1	WBGene00000962	C08B11.6	Tong
HCM1	WBGene00001442	C25A1.2	RB884	MAD2	WBGene00003161	T21E12.4	Tong
HSE1	WBGene00004109	C34G6.7	RB674	CHS5	WBGene00022615	Y69A2A_2326.a	Tong
HSE1	WBGene00004109	C34G6.7	RB674	GIM4	WBGene00019220	ZC449.5	Tong
HSE1	WBGene00004109	C34G6.7	RB674	PAC10	WBGene00006889	H20J04.d	Tong
MUS81	WBGene00016602	C43E11.2	tm1937	CCR4	WBGene00000376	T06G6.9	Tong
MUS81	WBGene00016602	C43E11.2	tm1937	MRE11	WBGene00003405	ZC518.3	Tong
MUS81	WBGene00016602	C43E11.2	tm1937	POL32	WBGene00011016	ZC302.1	Tong
MUS81	WBGene00016602	C43E11.2	tm1937	POP2	WBGene00000369	R04F11.3	Tong
MUS81	WBGene00016602	C43E11.2	tm1937	RAD27	WBGene00000794	Y56A3A.20	Tong
MUS81	WBGene00016602	C43E11.2	tm1937	RAD5	WBGene00010061	Y47G6A_247.i	Tong

MUS81	WBGene00016602	C43E11.2	tm1937	RAD50	WBGene00004296	F54E12.2	Tong
MUS81	WBGene00016602	C43E11.2	tm1937	RAD54	WBGene00004298	T04H1.4	Tong
MUS81	WBGene00016602	C43E11.2	tm1937	RAD6	WBGene00006701	W06D4.6	Tong
MUS81	WBGene00016602	C43E11.2	tm1937	SGS1	WBGene00001865	C35B1.1	Tong
NHX1	WBGene00003733	F57C7.2	RB836	CCR4	WBGene00000376	T04A11.6	Tong
NHX1	WBGene00003733	F57C7.2	RB836	POP2	WBGene00000369	ZC518.3	Tong
NHX1	WBGene00003733	F57C7.2	RB836	RAD27	WBGene00000794	Y56A3A.20	Pan
NHX1	WBGene00003733	F57C7.2	RB836	RPN10	WBGene00004466	Y47G6A_247.i	Pan
PCH2	WBGene00008641	F10B5.5	CA388	YKE2	WBGene00009004	B0205.3	Pan
PEP5	WBGene00011067	R06F6.2	RB1457	BRE1	WBGene00007008	F21C3.5	Pan
PEP5	WBGene00011067	R06F6.2	RB1457	CTF18	WBGene00010676	R05D3.4	Tong, Pan
PEP5	WBGene00011067	R06F6.2	RB1457	NAT1	WBGene00021754	K08F4.1	Pan
PEP5	WBGene00011067	R06F6.2	RB1457	RAD27	WBGene00000794	Y50D7_162.b & Y50D7_164.a	Pan
PEP5	WBGene00011067	R06F6.2	RB1457	RPN10	WBGene00004466	Y47G6A_247.i	Pan
POL12	WBGene00001002	R01H10.1	EU548	NIP7	WBGene00016607	B0205.3	Pan
POL12	WBGene00001002	R01H10.1	EU550	NIP7	WBGene00016607	C43E11.9	Tong, Pan
POL12	WBGene00001002	R01H10.1	EU879	NIP7	WBGene00016607	C43E11.9	Pan
POL12	WBGene00001002	R01H10.1	EU880	NIP7	WBGene00016607	C43E11.9	Pan
RAD14	WBGene00006963	K07G5.2	RB864	RAD6	WBGene00006701	C43E11.9	Pan
RAD6	WBGene00006701	C35B1.1	VC18	ARC18	WBGene00000203	C35B1.1	Pan
RAD6	WBGene00006701	C35B1.1	VC18	ARP6	WBGene00007434	Y37D8A.1	Tong
RAD6	WBGene00006701	C35B1.1	VC18	BUB2	WBGene00016352	C08B11.6	Pan
RAD6	WBGene00006701	C35B1.1	VC18	CCR4	WBGene00000376	C33F10.2	Pan
RAD6	WBGene00006701	C35B1.1	VC18	CEM1	WBGene00008667	ZC518.3	Pan
RAD6	WBGene00006701	C35B1.1	VC18	COQ6	WBGene00000766	F10G8.9	Pan
RAD6	WBGene00006701	C35B1.1	VC18	COX6	WBGene00012553	K07B1.2	Pan
RAD6	WBGene00006701	C35B1.1	VC18	CSM3	WBGene00017738	Y37D8A.14	Davierwal
RAD6	WBGene00006701	C35B1.1	VC18	CTF18	WBGene00010676	F23C8.9	a
RAD6	WBGene00006701	C35B1.1	VC18	CTK1	WBGene00007135	K08F4.1	Davierwal
RAD6	WBGene00006701	C35B1.1	VC18	DIA4	WBGene00005662	B0285.1 & B0285.2	a
RAD6	WBGene00006701	C35B1.1	VC18	FAB1	WBGene00004089	W03B1.4	Davierwal
RAD6	WBGene00006701	C35B1.1	VC18	FKH2	WBGene00003976	C05E7.5 & VF11C1L.1	a
RAD6	WBGene00006701	C35B1.1	VC18	GUA1	WBGene00010912	T28H11.4	Davierwal
RAD6	WBGene00006701	C35B1.1	VC18	HTZ1	WBGene00019947	M106.4	a
RAD6	WBGene00006701	C35B1.1	VC18	ISC1	WBGene00012105	R08C7.3	Pan
RAD6	WBGene00006701	C35B1.1	VC18	KAR3	WBGene00002216	T27F6.6	Pan
RAD6	WBGene00006701	C35B1.1	VC18	KEM1	WBGene00012730	T09A5.2	Pan
RAD6	WBGene00006701	C35B1.1	VC18	MRE11	WBGene00003405	Y39G8C.1 & Y39G8C.b	Pan
RAD6	WBGene00006701	C35B1.1	VC18	MRPL9	WBGene00016142	ZC302.1	Pan
RAD6	WBGene00006701	C35B1.1	VC18	MUS81	WBGene00016602	C26E6.6	Pan
RAD6	WBGene00006701	C35B1.1	VC18	PEP3	WBGene00021058	C43E11.2	Pan
RAD6	WBGene00006701	C35B1.1	VC18	POL32	WBGene00011016	W06B4.3	Pan
RAD6	WBGene00006701	C35B1.1	VC18	POP2	WBGene00000369	R04F11.3	Pan
RAD6	WBGene00006701	C35B1.1	VC18	PPA1	WBGene00011347	Y56A3A.20	Pan
RAD6	WBGene00006701	C35B1.1	VC18	RAD14	*	T01H3.1	Pan
RAD6	WBGene00006701	C35B1.1	VC18	RAD23	WBGene00006963	K07G5.2	Pan
RAD6	WBGene00006701	C35B1.1	VC18	RAD27	WBGene00013924	ZK20.3	Pan
RAD6	WBGene00006701	C35B1.1	VC18	RAD50	WBGene00000794	Y47G6A_247.i	Pan

RAD6	WBGene00006701	C35B1.1	VC18	RAD51	WBGene00004296	T04H1.4	Pan
RAD6	WBGene00006701	C35B1.1	VC18	RAD54	WBGene00004297	Y43C5A.6	Pan
RAD6	WBGene00006701	C35B1.1	VC18	RPN10	WBGene00004298	W06D4.6	Pan
RAD6	WBGene00006701	C35B1.1	VC18	RVS161	WBGene00004466	B0205.3	Pan
RAD6	WBGene00006701	C35B1.1	VC18	RVS167	WBGene00010272	F58G6.1	Pan
RAD6	WBGene00006701	C35B1.1	VC18	SAC1	WBGene00020209	T04C9.1 & ZK328.3	Pan
RAD6	WBGene00006701	C35B1.1	VC18	SIN3	WBGene00009264	F30A10.6	Pan
RAD6	WBGene00006701	C35B1.1	VC18	SMP2	WBGene00004117	F02E9.4	Pan
RAD6	WBGene00006701	C35B1.1	VC18	SPF1	WBGene00010425	H37A05.1	Pan
RAD6	WBGene00006701	C35B1.1	VC18	UBP14	WBGene00007514	C10C6.6	Pan
RAD6	WBGene00006701	C35B1.1	VC18	VMA5	WBGene00020839	T27A3.2	Pan
RAD6	WBGene00006701	C35B1.1	VC18	VMA7	WBGene00006920	Y38F2A_5743.f	Pan
RAD6	WBGene00006701	C35B1.1	VC18	VMA8	*	ZK970.4	Pan
RAD6	WBGene00006701	C35B1.1	VC18	VPS16	WBGene00006918	F55H2.2	Pan
RAD6	WBGene00006701	C35B1.1	VC18	VPS25	*	C05D11.2	Pan
RAD6	WBGene00006701	C35B1.1	VC18	VPS36	WBGene00010130	W02A11.2	Pan
RAD6	WBGene00006701	C35B1.1	VC18	VRP1	WBGene00006516	F17C11.8	Pan
RAD6	WBGene00006701	C35B1.1	VC18	YAF9	WBGene00012193	R144.4 & R144.8	Pan
RAD6	WBGene00006701	C35B1.1	VC18	YER087W	WBGene00008919	M04B2.3	Pan
RAD6	WBGene00006701	C35B1.1	VC18	YGR257C	WBGene00020094	T27F6.5	Pan
RAD6	WBGene00006701	C35B1.1	VC18	ZUO1	WBGene00001585	C16C10.1	Pan
RVS161	WBGene00010272	F58G6.1	tm1060	ARP2	WBGene00004190	F38A5.13	Pan
RVS161	WBGene00010272	F58G6.1	tm1060	CAP1	WBGene00007622	K07C5.1	Pan
RVS161	WBGene00010272	F58G6.1	tm1060	CAP2	WBGene00001029	D2024.6	Pan
RVS161	WBGene00010272	F58G6.1	tm1060	CCR4	WBGene00000200	M106.5	Pan
RVS161	WBGene00010272	F58G6.1	tm1060	CHS5	WBGene00000292	ZC518.3	Pan
RVS161	WBGene00010272	F58G6.1	tm1060	DOA1	WBGene00000293	ZC449.5	Pan
RVS161	WBGene00010272	F58G6.1	tm1060	GIM3	WBGene00000376	C05C10.6	Pan
RVS161	WBGene00010272	F58G6.1	tm1060	GIM4	WBGene00022615	B0035.4	Pan
RVS161	WBGene00010272	F58G6.1	tm1060	GIM5	WBGene00007333	H20J04.d	Pan
RVS161	WBGene00010272	F58G6.1	tm1060	NAT1	WBGene00007107	R151.9	Pan
RVS161	WBGene00010272	F58G6.1	tm1060	PAC10	WBGene00019220	Y50D7_162.b & Y50D7_164.a	Pan
RVS161	WBGene00010272	F58G6.1	tm1060	POL32	WBGene00020112	T06G6.9	Pan
RVS161	WBGene00010272	F58G6.1	tm1060	POP2	WBGene00021754	R04F11.3	Pan
RVS161	WBGene00010272	F58G6.1	tm1060	RAD6	WBGene00006889	Y56A3A.20	Pan
RVS161	WBGene00010272	F58G6.1	tm1060	SEC22	WBGene00011016	C35B1.1	Pan
RVS161	WBGene00010272	F58G6.1	tm1060	SIN3	WBGene00000369	F55A4.1	Pan
RVS161	WBGene00010272	F58G6.1	tm1060	SPF1	WBGene00006701	F02E9.4	Tong
RVS161	WBGene00010272	F58G6.1	tm1060	YKE2	WBGene00018853	C10C6.6	Tong
SGS1	WBGene00001865	T04A11.6	VC193	CCR4	WBGene00004117	F21C3.5	Tong
SGS1	WBGene00001865	T04A11.6	VC193	CSM3	WBGene00007514	ZC518.3	Pan
SGS1	WBGene00001865	T04A11.6	VC193	CTF18	WBGene00009004	F23C8.9	Tong
SGS1	WBGene00001865	T04A11.6	VC193	LSM1	WBGene00000376	K08F4.1	Tong
SGS1	WBGene00001865	T04A11.6	VC193	MRE11	WBGene00017738	F40F8.9	Tong
SGS1	WBGene00001865	T04A11.6	VC193	MUS81	WBGene00010676	ZC302.1	Tong
SGS1	WBGene00001865	T04A11.6	VC193	POL32	WBGene00003076	C43E11.2	Tong
SGS1	WBGene00001865	T04A11.6	VC193	POP2	WBGene00003405	R04F11.3	Pan
SGS1	WBGene00001865	T04A11.6	VC193	RAD27	WBGene00016602	Y56A3A.20	Tong

SGS1	WBGene00001865	T04A11.6	VC193	RAD5	WBGene00011016	Y47G6A_247.i	Pan
SGS1	WBGene00001865	T04A11.6	VC193	RAD50	WBGene00000369	F54E12.2	Pan
SGS1	WBGene00001865	T04A11.6	VC193	RAD51	WBGene00000794	T04H1.4	Pan
SGS1	WBGene00001865	T04A11.6	VC193	RAD54	WBGene00010061	Y43C5A.6	Tong
SGS1	WBGene00001865	T04A11.6	VC193	SLX1	WBGene00004296	W06D4.6	Tong
SGS1	WBGene00001865	T04A11.6	VC193	TOP1	WBGene00004297	F56A3.2	Tong
SNF5	WBGene00011111	R07E5.3	RB810	BRE1	WBGene00004298	M01E5.5	Tong
SNF5	WBGene00011111	R07E5.3	RB810	POP2	WBGene00018909	R05D3.4	Pan
UGA1	WBGene00001794	K04D7.3	RB748	GIM5	WBGene00006595	Y56A3A.20	Tong, Pan
UGA1	WBGene00001794	K04D7.3	RB748	YKE2	WBGene00007008	R151.9	Tong, Pan
UGP1	WBGene00010665	K08E3.5	MG278	NOP1	WBGene00000369	F21C3.5	Pan
VMA4	WBGene00006917	C17H12.14	RB769	NAT1	WBGene00020112	T01C3.7	Pan
					WBGene00009004	Y50D7_162.b & Y50D7_164.a	Tong, Pan
					WBGene00001423		Tong, Pan
					WBGene00021754		Pan
							Tong, Pan
							Pan
							Tong, Pan
							Pan
							Pan
							Tong, Pan
							Tong
							Pan
							Pan
							Tong
							Tong
							Davierwala
							Pan

Appendix Table 5.2. *C. elegans* orthologues of *S. cerevisiae* synthetic lethal interactions with homozygous viable loss-of-function alleles
C. elegans orthologues of *S. cerevisiae* synthetic lethal interactions that were used for the study of synthetic lethal interactions by RNAi in a mutant carrying a defined genetic lesion ('CE_Strain') are shown. For each gene pair ('Gene 1', 'Gene 2'), *S. cerevisiae* standard names ('SC Name'), *C. elegans* WormBase names ('CE Name') & Ahringer library RNA interference (RNAi) clone gene pairs names ('CE RNAi clone') together with references for yeast synthetic lethal data are listed. * designates genes that resulted in first-generation larval growth arrest after RNAi & were not be included in the screen for synthetic lethal interactions. Tong, Davierwala, and Pan denote yeast datasets from Tong *et al.*, 2003, Davierwala *et al.*, 2005, and Pan *et al.*, 2006, respectively.

Appendix Table 5.3. Overlap of literature-curated genetic interactions between *S. cerevisiae*, *D. melanogaster*, and *C. elegans*

Overlap *S. cerevisiae* and *D. melanogaster*

SC Name Gene 1	SC Name Gene 2	DM Name Gene 1	DM_Name Gene 2
S000002575	S000000364	FBgn0011573	FBgn0004106
S000006323	S000002314	FBgn0000405	FBgn0015625
S000000364	S000006323	FBgn0004106	FBgn0000405

Overlap *C. elegans* and *D. melanogaster*

CE Name Gene 1	CE Name Gene 2	DM Name Gene 1	DM Name Gene 2
WBGene00000090	WBGene00003965	FBgn0015279	FBgn0020386
WBGene00004297	WBGene00004985	FBgn0003479	FBgn0002716
WBGene00002335	WBGene00004214	FBgn0003205	FBgn0000382
WBGene00000453	WBGene00006870	FBgn0025360	FBgn0005558
WBGene00002299	WBGene00002335	FBgn0003731	FBgn0003205
WBGene00001163	WBGene00006868	FBgn0040324	FBgn0025936
WBGene00000554	WBGene00006527	FBgn0015614	FBgn0011826
WBGene00002299	WBGene00004947	FBgn0003731	FBgn0001965
WBGene00002299	WBGene00003043	FBgn0003731	FBgn0036844
WBGene00002694	WBGene00003196	FBgn0026181	FBgn0005536
WBGene00001648	WBGene00001678	FBgn0001122	FBgn0001104
WBGene00000478	WBGene00000857	FBgn0016797	FBgn0004009
WBGene00006957	WBGene00006958	FBgn0024273	FBgn0041781
WBGene00003024	WBGene00006796	FBgn0003339	FBgn0001235
WBGene00000443	WBGene00006796	FBgn0000611	FBgn0001235
WBGene00000406	WBGene00000870	FBgn0016131	FBgn0010315
WBGene00000870	WBGene00001061	FBgn0010315	FBgn0011763
WBGene00006745	WBGene00006746	FBgn0034013	FBgn0015774
WBGene00002694	WBGene00003372	FBgn0026181	FBgn0003514
WBGene00000870	WBGene00000871	FBgn0010315	FBgn0010382
WBGene00002335	WBGene00002363	FBgn0003205	FBgn0004177
WBGene00003196	WBGene00003776	FBgn0005536	FBgn0005634
WBGene00000962	WBGene00003047	FBgn0010349	FBgn0015754

Appendix Table 5.3. Overlap of literature-curated genetic interactions between *S. cerevisiae*, *D. melanogaster*, and *C. elegans*
Pair-wise ('Gene 1, Gene 2') orthologues of *S. cerevisiae*, *D. melanogaster*, and *C. elegans* genes with reported genetic interactions are shown. *S. cerevisiae*, *D. melanogaster*, and *C. elegans* genes are listed as *Saccharomyces* Genome Database identities ('SC Name'), FlyBase gene names ('DM Name'), and WormBase gene names ('CE Name'), respectively.