Isolation of homozygous mutant mouse embryonic stem cells by selection for copy number increase

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Abstract and declaration

Forward genetic screens are a powerful method to determine which genes are responsible for a particular phenotype in many model organisms. However, a simple method to conduct genetic screens in a mammalian system has been difficult to develop, due to the problem of making random homozygous mutations in the diploid mammalian genome. Mouse embryonic stem (ES) cells provide a convenient model for mammalian cell biology. Previous studies showed that heterozygous mutants in ES cells with mutations in the *Blm* gene segregate homozygous mutants at a low rate, due to an increase in mitotic crossovers.

Using the piggyBac DNA transposon (PB) for initial single copy heterozygous mutagenesis, I describe a method to isolate the rare homozygous cells based on selection for transposon copy number, which increases to two in homozygotes. I successfully isolated homozygous mutants using this system, but my experiments revealed aneuploidy as an alternative copy number gain pathway in ES cells. By extensive engineering of the ES cell line and PB transposase, I developed a method to allow many different homozygous mutants to be generated in a pooled format. This minimises the problem of background from aneuploidy and allows isolation of clonally pure mutants suitable for genetic screens.

I also investigated the properties of the PB transposon. By sequencing and mapping thousands of insertion sites I have investigated the insertion site preferences of PB. This method can also be used to fully define coverage of mutant libraries. I showed that precise excision of PB from the genome depends on the nonhomologous end joining pathway, and present data indicating that transposition can occur throughout the cell cycle.

The methods and tools presented will be useful for study of gene function in mammalian cells, and are also applicable for the study of DNA double strand break repair and copy number stability.

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.

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