DNA Requirements For Sequencing

Each of our sequencing platforms and their sequencing library construction protocols has differing requirements in terms of the quality and quantity of DNA/RNA that each uses. Please consult, and where possible, adhere to the guidelines provided below. Both quality and quantity of starting material can have a critical affect on the success of a sequencing project. Where possible DNA should be analysed by gel electrophoresis and quantified relative to markers of known concentration.

Quantitation and quality assessment of DNA and RNA

DNA Spectrophotometric methods can be rendered inaccurate by small nucleic acids and contaminating chemicals and their use is therefore discouraged. However spectrophotometric analysis of DNA should show a peak at 260nm with 260:280 ratio of 1.8. Quantitation based on nanodrop readings can not be trusted. It is therefore recommended that the Invitrogen Qubit device is used for Quantitation (Qubit fluorimeter, Invitrogen order code Q32857) along with the QUANT-IT dsDNA HS assay (Invitrogen order code Q32854). Alternatively a pico green assay could be used. In addition it is advised that 1ul of each DNA sample be run on an agarose gel alongside a quantitative DNA marker (e.g. using 50ng NEB lambda ladder (order code N3012S)), to confirm concentration and assess integrity.

RNA: RNA should be supplied pure and free of DNA (DNAse treated) and chemical contaminants.
RNA should have a Bioanalyser RIN value of 7 or higher, and spectrophotometric analysis should show a peak at 260nm with: 260:280 ratio of >1.8 and 260:230 ratio close to 2.0.
Where Trizol and other similar organic methods are used for preparation of RNA the RNA should be purified on a Qiagen RNeasy column afterwards to remove all traces of organic solvents.

For queries relating to quality and quantity of DNA for sequencing contact mq1@sanger.ac.uk .

DNA Requirements for Illumina Sequencing

Paired-end sequencing
Ideally 2-5µg of double stranded DNA in TE buffer at a concentration greater than 20 ng/µl, in a volume of 20-100µl, and in fragments >500bp, is required. (Libraries can be made with as little as 50ng, if material is limiting).
Minimum amount of input DNA for guaranteed results is 1µg.

NoPCR libraries
6µg of double stranded DNA in TE buffer at a concentration greater than 20 ng/µl, in a volume of 20-100µl, and in fragments >500bp, is required.
Minimum amount of input DNA for guaranteed results is 5µg for inserts 300bp and below, and 10µg for inserts >300bp.

Paired-end double size selected libraries
For paired end libraries with inserts of 400bp, or greater, double size selection should be requested to avoid significant levels of smaller inserts. 5µg of double stranded DNA in TE buffer at a concentration greater than 20 ng/µl, in a volume of 20-100µl, and in fragments >500bp, is required.
Minimum amount of input DNA for guaranteed results is 5µg.

High-complexity libraries

10µg of double stranded DNA in TE buffer at a concentration greater than 20 ng/µl, in a volume of 20-100µl, and in fragments >500bp, is required.
Minimum amount of input DNA for guaranteed results is 10µg.

Large-insert paired end protocol

10µg of double stranded DNA in TE buffer at a concentration greater than 200 ng/µl, in a volume of 20-100µl, and in fragments >10kb, is required.
Minimum amount of input DNA for guaranteed results is 10µg.

CHiP-Seq libraries

Ideally 200ng-1ug of double stranded DNA in TE buffer at a concentration greater than 20 ng/ul, in a volume of 20-100ul, and in fragments >500bp, is required. (Libraries can be made with as little as 50ng, if material is limiting).
Minimum amount of input DNA for guaranteed result is 200ng.

Indexed libraries with PCR

2ug of double stranded DNA in TE buffer at a concentration of 20 ng/ul and in a volume of 100ul, and in fragments >10kb, is required. Samples must be properly quantified and accompanied by a gel photograph showing 1ul of each sample run alongside a quantitative marker.
Minimum amount of input DNA for guaranteed result is 1ug.

Indexed libraries (noPCR)

2ug of double stranded DNA in TE buffer at a concentration of 20 ng/ul and in a volume of 100ul, and in fragments >10kb, is required. Samples must be properly quantified and accompanied by a gel photograph showing 1ul of each sample run alongside a quantitative marker.
Minimum amount of input DNA for guaranteed result is 2ug.

RNAseq and miRNA libraries

Preferably 10ug (and a minimum of 2ug) of total RNA (DNA free) in a volume of 50ul is required.

Minimum amount of input RNA (with RIN > 7.0) for guaranteed result is 5ug.

Note: The stated quantities are for a single attempt. To minimise delays should the library fail and a repeat library needs to be attempted, it is recommended that twice the quantities above be supplied, if material is available.

DNA Requirements for 454 Sequencing

Shotgun Library

>500ng of double stranded DNA in TE buffer at a concentration greater than 50 ng/ul, and in fragments >1.5 kb (or 70-500bp for low mw procedure), is required.

Standard protocol

Ideally >2.5ug of double stranded DNA in TE buffer at a concentration greater than 300 ng/ul, and in fragments >1.5 kb (or 70-500bp for low mw procedure), is required. Libraries can be made with as little as 1.3ug.

8kb Large-insert paired end libraries

15ug of double stranded DNA in TE buffer at a concentration greater than 300 ng/ul, and in fragments >24kb, is required.

20kb Large-insert paired end libraries

30ug of double stranded DNA in TE buffer at a concentration greater than 300 ng/ul, and in fragments >60kb, is required.

For queries relating to quality and quantity of DNA for sequencing by 454 contact Richard Rance; rr1@sanger.ac.uk.

DNA Requirements for Capillary Sequencing

Quantity

Ideally we will require:
- approx. 1mg of genomic DNA for sequencing and finishing Eukaryotic genomes
- 0.5mg for sequencing and finishing Prokaryote genomes
- Between 1 and 5ug DNA when only shotgun sequencing is to be carried out

Note: If DNA is in limiting supply we can proceed with less but success cannot be guaranteed.

Quality

Wherever possible DNA should be prepared from a single individual or clonal population.
DNA should be as high a quality as possible and preferably >50kb average. Methods that have worked well include traditional organic extraction with DNA spooling, Qiagen genomic DNA columns and for gram positive bacteria the Flowgen Gram +VE DNA isolation kit.