EASIH Sequencing Services Information Sheet

- Sample shipping

Samples must be labelled with the EASIH barcodes

Send samples for Sequencing to:
EASIH Sequencing Laboratory,
Level 4, Laboratory Block
Addenbrooke's Hospital, Hills Road
Cambridge, CB2 0QQ, UK

- Quantitation and quality assessment of DNA, RNA and Library

Genomic DNA

Genomic DNA should be supplied RNA-free. RNAse treatment must be performed either during or after the DNA extraction procedure. Customer must supply a gel picture of the genomic DNA showing no DNA degradation and an effective removal of RNA and any other contaminants. RNase treatment and DNA extraction procedures must be specified.

Sample should be supplied in lo-bind tube in water or low TE in a final volume not higher than 100µl.

EASIH laboratory requires

- 4 µg of genomic DNA for Exome Enrichment sample preparation
- 2 µg of genomic DNA for DNA sample preparation

See below for shipping conditions.

RNA

RNA should be supplied pure and free of DNA and chemical contaminants.
RNA should have a Bioanalyser RIN (RNA Integrity Number) value of 8 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.

Sample should be supplied in lo-bind tube in water in a final volume not higher than 100µl.

EASIH laboratory requires

- 1 µg of total RNA for RNA protocols

See below for shipping conditions.

DNA/RNA Requirements for Illumina GAIIx Sequencing

Illumina Library

20µl of 2nM library in EB buffer should be supplied in addition to library size and concentration information. Gel picture must be provided.

Paired-end Sequencing

2 µg of double stranded DNA in water or TE buffer at a concentration greater than 20ng/µl, in a volume of 20-100µl and in fragments >300bp and < 700bp, are required.

High-complexity libraries
ex. Leishmania (high GC content), Plasmodium falciparum (high AT content)

5 µg of double stranded DNA in TE buffer at a concentration greater than 20ng/µl, in a volume of 20-100µl, and in fragments >300bp and < 700bp, are required.

Total RNA

0.1- 4 µg of high quality total RNA (DNA free) are required.

mRNA libraries

100-400 ng of mRNA are required.

small RNA

1 µg of high quality total RNA (DNA free) is required.

ChIP samples

>10ng of target DNA in a volume of 10-80 µl are required.

DNA/RNA requirements for ROCHE Junior Sequencing

Library

20 µl of 1x10^9molecules/µl of library in TE should be supplied.

cDNA Rapid Protocol

>200 ng of total high quality RNA (DNA free) in a volume ≤ 19 µl are required. OD260/280 of approximately 1.8 is required. If the RNA of interest is mRNA, ribosomal RNA must be removed.

This protocol is not designed for preparing small RNA molecules as snoRNA, microRNA, tRNA etc.

Rapid Protocol

500 ng of double stranded DNA in TE buffer at a concentration greater than 5 ng/µl in fragment > 1.5 Kb are required.

Large-insert Paired End protocol (3Kb, 8 Kb and 20Kb Span)

5 µg, 15 µg or 30 µg of double stranded DNA in TE are required for the 3Kb, 8Kb or 20 Kb Span, respectively.

Amplicon Protocol

This procedure requires 5-20 ng of genomic DNA or 1-2 ng of plasmid DNA or similar of starting DNA material in 5 µl of molecular biology grade water.

DNA/RNA requirements for Ion Torrent Sequencing

DNA

6µg of DNA in TE buffer at a greater concentration that 60ng/µl are required.

RNA

100-500 ng of poly(A) RNA or 200-500 ng of rRNA-depleted total RNA in <20µl are required .

Amplicons

DNA/RNA requirements for SOLiD4 Sequencing

Library

6 µl of 10nM library in E1 elution buffer (E1, SOLiD purification kit)

Fragment library

5µg of DNA in TE buffer at a greater concentration that 100ng/µl are required.

Mate paired library

Mate paired library with insert size of 600bp-2kb can be generated from 5µg-20µg of DNA supplied in 100µl TE buffer.
Mate paired library with insert of size of 3-6kb or >6Kb can be generated from 40µg-60µg or 100µg of DNA, respectively.

ChIP samples

10-20ng of target DNA in 50µl elution buffer (from MAGnify™ kit) recommended by SOLiD are required

Total RNA

200-500ng ribosomal depleted RNA or 100-500ng poly(A) enriched RNA in <20µl TE buffer is required.

Small RNA

10-200ng 15-40bp size selected RNA in 100µl TE buffer is required.

Shipping Conditions

Material	**Shipping condition**
Genomic DNA	Normal ice or ice pack
RNA	Dry ice
Amplicons	Normal ice or dry ice
Library	Dry ice

Note: The stated quantities are for a single attempt. To minimize delays it is recommended that twice the quantities above is supplied, if material is available.

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

The details in this sheet are only a guide. Further details on how to prepare your sample will be included in Section 2 of the Service Description within your quotation.

To obtain a quotation please contact us via the enquiry form at **www.easih.ac.uk**