HDBR
HUMAN DEVELOPMENTAL BIOLOGY RESOURCE

# Extraction of DNA and RNA from tissue and generating cDNA

Tissue and stage requests can be discussed with registered HDBR users and specific extractions will be undertaken where possible.

At time of dissection, samples are taken that are stage and organ or tissue specific and frozen at -80$^{o}$C.

10 - 30mg tissue aliquots are then defrosted and homogenised using Precelly's bead-beating technology.

If a tissue has been divided into many aliquots, the lysate is re-pooled to be representative of the original whole organ or tissue of interest. An aliquot of this lysate is then extracted on a QIAcube which is a robotic workstation for automated purification of genomic DNA, total RNA including microRNAs, or proteins using QIAGEN spin-column kits.

Unless protein is specifically requested, DNA and RNA will be extracted only and then analysed on a ThermoScientific Nanodrop

*Normal QA Measurements*
DNA – A260/A280 = 1.7 – 1.9
RNA – A260/A280 = 1.9 – 2.1

DNA is eluted in RNAse/DNAse free water and stored at -20$^{o}$C and aliquots given per request.

Aliquots are shipped on cold packs and should be frozen upon delivery.

Applications
Sequencing
SNP analysis
Epigenetics

HDBR contact details
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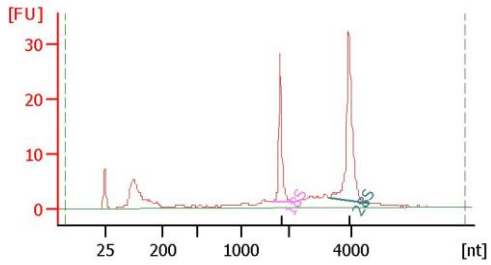
## RNA

The quality of eluted RNA is further analysed on an Agilent Bioanalyser and results are given to the user as a PDF file (example shown):




Overall Results for the sample:

RNA Area: 157.5
RNA Concentration: 133 ng/µl
rRNA Ratio [28s / 18s]: 1.9
**RNA Integrity Number (RIN): 9.3** (B.02.02)

All RNA we provide has a RIN value of at least 7.0. However, RIN numbers are typically over 8.0 for the majority of tissues.

RNA is eluted in The RNA Storage solution (Ambion Life Technologies cat. AM7000 – 1mM Sodium citrate pH 6.4) which gives greater stability. Samples are stored at -80C and aliquots given per request. The amount of RNA that can be supplied to a user will vary depending on the tissue and stage of the starting material.

RNA aliquots are shipped on dry ice and should be frozen at -80$^{o}$C upon delivery.

Applications

RNAseq
Generation of cDNA
Microarray studies
qPCR

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## cDNA

Once RNA is extracted, cDNA is generated using a Roche or Promega first strand cDNA synthesis kit using a mixture of anchored oligo(dT)18 primers AND random hexamer primers.

cDNA is stored in the RT kit buffer at -20°C and aliquots (up to 5ug) given per request. Samples are shipped on cold packs and should be stored at -20°C upon delivery.

- Test primers are used to check the quality of cDNA and gDNA using PCR with reverse transcribed cDNA template

Primers- ACSL4 QC2

PCR conditions 53.5°C 30 cycles

| 100bp ladder | gDNA | cDNA | water control |
|---|---|---|---|

## Applications

Following synthesis of cDNA, gene specific primers can be used to determine the level of gene expression. This is particularly useful for looking at expression levels at different stage embryos/ fetuses where little is known about the gene and can aid the design of future experiments.

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