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Genome Analysis Facility

Title:	Guidelines Analysis of RNA Quantity and Quality for Next-Generation Sequencing Projects
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Introduction

To ensure successful and rapid processing of total RNA samples for transcriptome or micro-RNA sequencing, the RNA quality has to fulfil a number of requirements to be suitable for use. Properties such as purity, integrity and concentration are of great importance, because they can have a significant impact on the experiment's results.

All the required information for the preparation of the RNA samples is described in this document.

Applications and limitations of the technique

With RNAseq, cDNA is being sequenced and provides information on differential expression of genes, including gene alleles and differently spliced transcripts; non-coding RNAs; post-transcriptional mutations or editing; and gene fusions. These data can be used to answers multiple research questions such as:

- Identification of **genome-wide differential expression of genes** across two or more conditions with statistical significance and/or biological significance (e.g. fold change)
- **Pathway analysis** (a.k.a. gene set analysis) to infer correlation of differential expression evidence in the data with pathway knowledge from established databases
- **Co-expression analysis** and network analysis of RNAseq data are used to investigate potential transcriptional co-regulation and gene interactions
- **Prediction analysis** for clinical practice

The following are points to consider prior to the start of the experiment.

Practical aspects:

- The design of the RNAseq study and the analysis of RNAseq data is rather complex. The data analysis is not supported by the GAF/GCC, thus is the responsibility of the applicant. It is a complicated technology, so ensure sufficient knowledge, computational skills and computer power.
- The GAF offers RNAseq using Illumina TruSeq RNA Sample Preparation Kits, which results in a total RNA prep with fragments of app. 150 bp. The library is sequenced on a single read flowcell with read lengths of 100 bp. Optionally, the library can be run on a paired-end flowcell.
- RNA isolation and quantification: use the same method throughout the whole study to prevent clustering of samples per method.

Limitations of RNAseq:

- Only poly-A tailed RNA transcripts are assessed with standard methods
- Contamination with e.g. abundance of globin mRNAs in whole blood, significantly influence the whole gene expression profile

Points to consider in design of the study and data analyses:

- Resource limitation vs. statistical power: ensure sufficient samples to allow robust results

- The technique is meant for genome-wide expression analysis, not to analyse only a few genes. qPCR is then better suited.
- Replication problems between studies may occur due to diversity of study designs
- Cellular diversity within samples: expression variation is largely within or among cells
- Ensure sufficient sequence depth for analysis of differential expression of low expressed genes and novel splice variants detection, when desired.
- Normalization of the data is necessary
- No consensus on statistical thresholds for differential expression
  - fold-change
  - Bonferroni correction
  - false discovery rate

RNA isolation

For next-generation sequencing experiments, a total of 500 ng RNA is needed.

The quality of RNA isolated by most isolation methods usually meets the criteria for a successful next-generation sequencing experiments. The RNA should be dissolved in Elution buffer or 10 mM Tris-HCL pH 8.0, 1 mM EDTA. We have successfully processed RNA obtained by the Qiagen RNeasy-kit or Trizol reagent.

RNA quantity and quality

The quantity and quality of the isolated DNA should be verified with a spectrophotometer, ideally by the Nanodrop spectrophotometer.

The quantity and quality criteria are:

1. Concentration should be > 10 ng/µl.
2. The OD 260/280 ratio should be above 2.0, the OD 260/230 ratio should be above 2.0
3. For next-generation sequencing experiments, at least 500 ng total RNA is needed (based on nanodrop measurement).
4. The integrity can be measured with the BIO-RAD Experion Bioanalyzer or an Agilent Bio-Analyzer device. The RQI/RIN should be more than 8.0.
5. The quality measurement of RNA samples with the Nanodrop and Experion can be performed by the facility's staff as part of the project. Please contact the Genome Analysis Facility to discuss this if necessary.

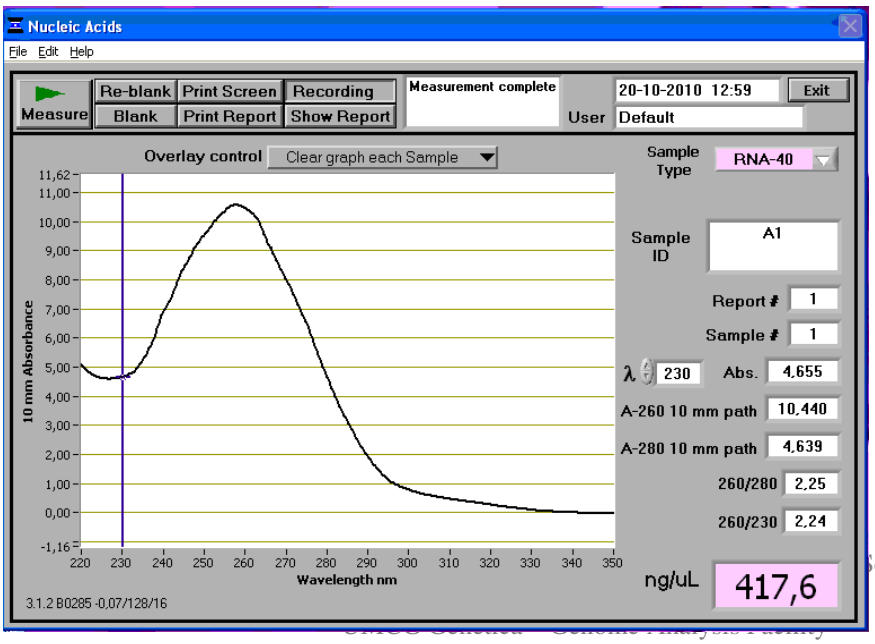

Figure 1. Example of a nanodrop measurement of a good quality RNA-sample.

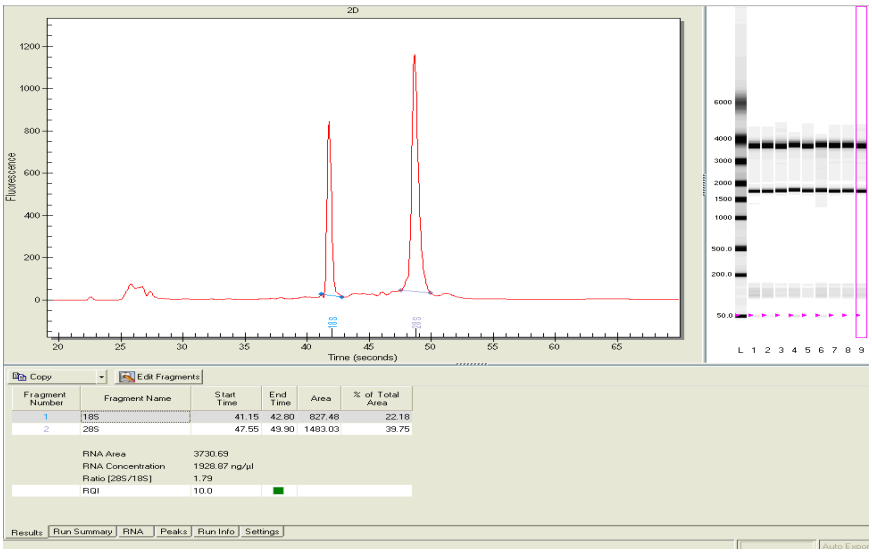

Figure 2.
Example of a BioRad Experion analysis of good quality samples

Preparing your samples and shipping them

1. At least 50 µl of the RNA solution (minimum concentration 10 ng/ul) should be transferred to a screw-capped 1.5 ml vial.
2. The vials should be properly labelled with a unique sample ID and name of the research centre. The facility doesn't accept samples with names of individuals.
3. Deliver the samples on dry ice to the Genome Analysis Facility lab or send your samples by courier to the Genome Analysis Facility laboratory at the Department Genetics, UMCG. The shipment should be placed in boxes containing a sufficient amount of dry ice.
4. To avoid any delay during the weekend, please ship the material at the beginning of the week.

Delivery address: Genome Analysis Facility
Dept. Genetics UMCG
Hanzeplein 1, Ingang 47 Oostersingel
Room E2.030
9713 GZ Groningen
The Netherlands

Phone number: +31 (0)50-3617100
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Before submitting your samples, please contact the Genome Analysis Facility