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Genetica
Genome Analysis Facility

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

To ensure a successful and rapid processing of the DNA samples, the DNA quality needs to meet various criteria. There are also special demands with respect to the format of the sample delivery.

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

Applications and limitations of the technique

Next generation sequencing of DNA can be applied for many purposes, but only whole genome resequencing or targeted resequencing (e.g. exome sequencing) are fully supported in the GAF.
There are no limitations for whole genome resequencing, but the following are points to consider:

- ensure sufficient sequence depth
- it is possible to apply paired-end sequencing with different insert-sizes, which may aid in genome-assembly
- for small genomes, you can use our commercial software package CLCBio for genome assembly and data analysis. *De novo* genome assembly of larger genomes (>1000Mb) requires much computational skills and power. Ensure this in your project team.
- the GAF is not responsible for long term storage of research samples. After finishing the experiment it is possible to receive te residue of the research samples. 6 Months after finishing the experiments, the samples will be destroyed.

For exome-sequencing:

- the capturing-kit the GAF fully supports from sample preparation to generation of SNP reports is the Agilent SureSelect Human All Exon V4 and is designed to capture ~50Mb of exonic sequence. If you are interested in a particular genomic region, ensure this region is covered in the kit. Otherwise it is possible to design a custom-kit. Contact the GAF for further advise about this.
- the exome-capturing kit does not give equal sequence depth for all targets. Our quality criterium is a 20x sequence depth for 80% of the targets in the kit. It is however possible that you will miss variants in the remaining 20%.

DNA isolation

For next-generation sequencing experiments we need 3 µg high molecular weight DNA, or 400 ng fragmented DNA.

The quality of DNA isolated by most isolation methods usually meets the criteria for successful next-generation sequencing experiments. Because of the high yield of pure DNA obtained, a manual salt/chloroform method is recommended

The DNA should be dissolved in Elution buffer or 10 mM Tris-HCL pH 8.0, 1 mM EDTA.

DNA quantity and quality

The quantity and quality of the isolated DNA should be verified with a spectrophotometer, ideally by the Nanodrop spectrophotometer. The criteria for non-fragmented DNA samples are:

1. Concentration > 25 ng/µl
2. The OD 260/280 ratio should be between 1.8 and 2.0
3. The OD 260/230 ratio should be above 1.5
4. The DNA should be in high molecular fragments of > 5kb. This can by checked using agarose gel electrophoresis on the samples.

Alternatively, fragmented DNA can be submitted for next-generation sequencing. Please contact the Genome Analysis Facility to discuss this.

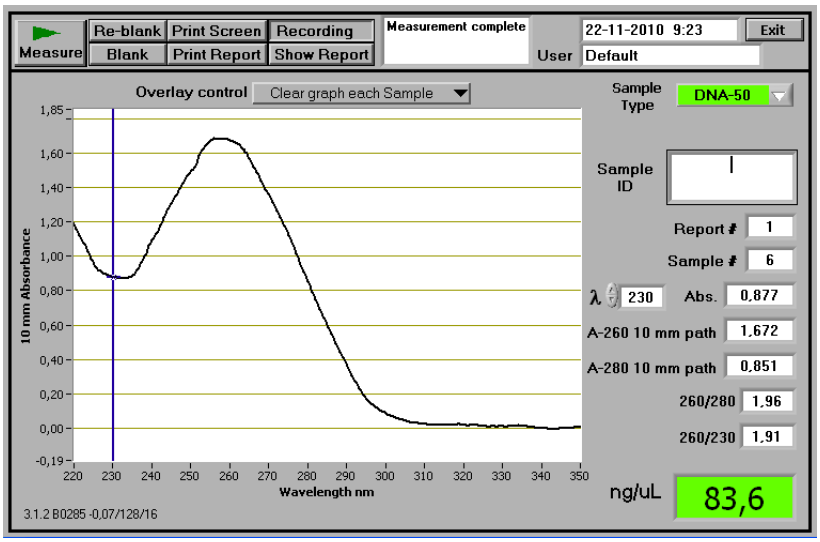

Figure 1.
Example of a nanodrop measurement of a good quality DNA sample.

Preparing your samples and shipping them

1. At least 3 µg high molecular DNA in a maximum volume of 130 µl of the DNA solution 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 or send your samples by courier to the Genome Analysis Facility laboratory at the Department of 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.

Before submitting your samples, please contact the Genome Analysis Facility

Delivery Address: Genome Analysis Facility
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