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Genetica
Genome Analysis Facility

Title:	Guidelines Analysis of DNA Quantity and Quality for Infinium and GoldenGate projects
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Author(s):	P. van der Vlies, C.C. van Diemen

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Introduction

To ensure successful and rapid processing of the DNA samples the DNA quality needs to meet a few criteria. There are also special demands about the format of the sample delivery. Al the required information for the preparation of the DNA samples is described in this document.

DNA isolation

To isolate genomic DNA out of multiple blood samples the *Qiagen QiaAmp DNA 96 blood kit* is recommended.
Alternatively many other DNA isolation methods can be used. The quality of DNA isolated by most isolation methods usually meets the criteria for a successful Infinium or GoldenGate assay. The Infinium or GoldenGate assay does not work properly with Amplified DNA.
The DNA should be dissolved in Elution buffer or 10 mM Tris-HCL pH 8.0, 1 mM EDTA

Applications and limitations of the techniques

GoldenGate Custom Genotyping interrogate 48, 96, 192, or from 384 to 3,072 SNP loci simultaneously with the proven GoldenGate Genotyping Assay. Custom Panels can be designed for many species with user-defined content.
Goldengate assays are your choice when you aim to genotype only a limited number of SNPs in a high-throughput fashion.

Illumina Infinium genotyping platforms are used for genome-wide SNP genotyping. Genome-wide association studies are the most common application for genome-wide SNP genotyping, but other applications exist as well. In addition to SNP information, CNV analyses can be performed, which can be useful for e.g. tumor profiling. Infinium arrays are also used for genotype concordance checks for next generation sequencing data.
There are multiple options for density of the genotyping, depending on the application and the budget. The current platforms can be found on the Illumina website:
http://www.illumina.com/applications/detail/snp_genotyping_and_cnv_analysis/whole_genome_genotyping_and_copy_number_variation_analysis.ilmn

Some important pitfalls of genome-wide association studies are:

- Data analysis requires thorough quality control and is more complicated than custom low-scale SNP typing. Please ensure to arrange proper knowledge and guidance with these analyses.
- The SNPs on the platform are marker SNPs: causal variation still unknown in most cases.
- Region of association can be large due to linkage disequilibrium, containing many candidate genes.
- For genome-wide association studies statistical power is crucial in success of the study. Ensure the power is sufficient at the start of the study (for example: with 1000

cases, 2000 controls 65% power to detect association of SNP with MAF 0.30 and OR 1.3 (Genetic power calculator: http://pngu.mgh.harvard.edu/~purcell/gpc/))

- Availability of a cohort to replicate associations.

DNA quantity and quality

The quantity and quality of the isolated DNA should be determined with a spectrophotometer, ideally by the Nanodrop spectrophotometer.
The quality criteria are:

1. Concentration should be between 50 ng/µl and 100 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 high molecular fragments of > 5kb. (This can by checked using agarose gel electrophoresis of a subset of the samples)

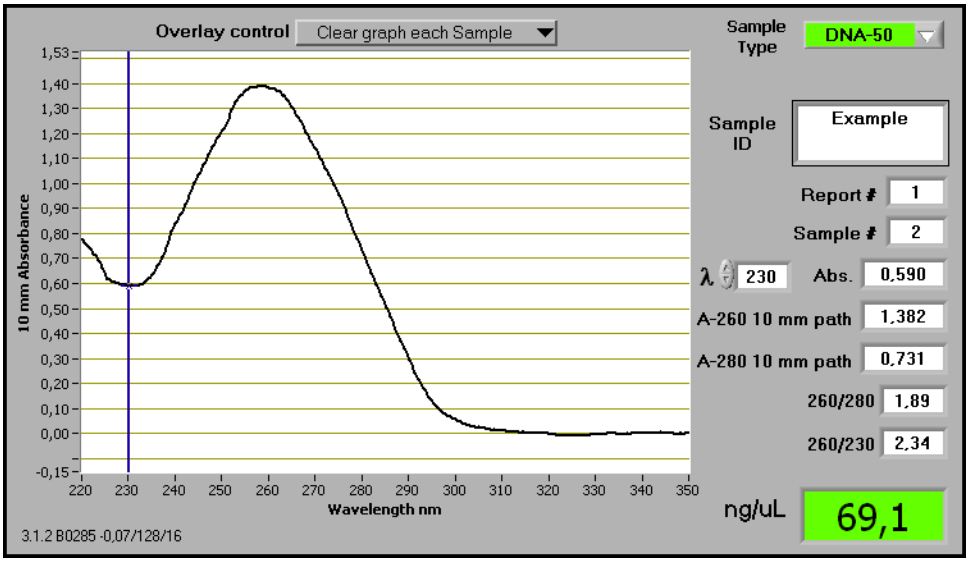


Figure 1: Example of a nanodrop measurement of a good quality DNA-sample.

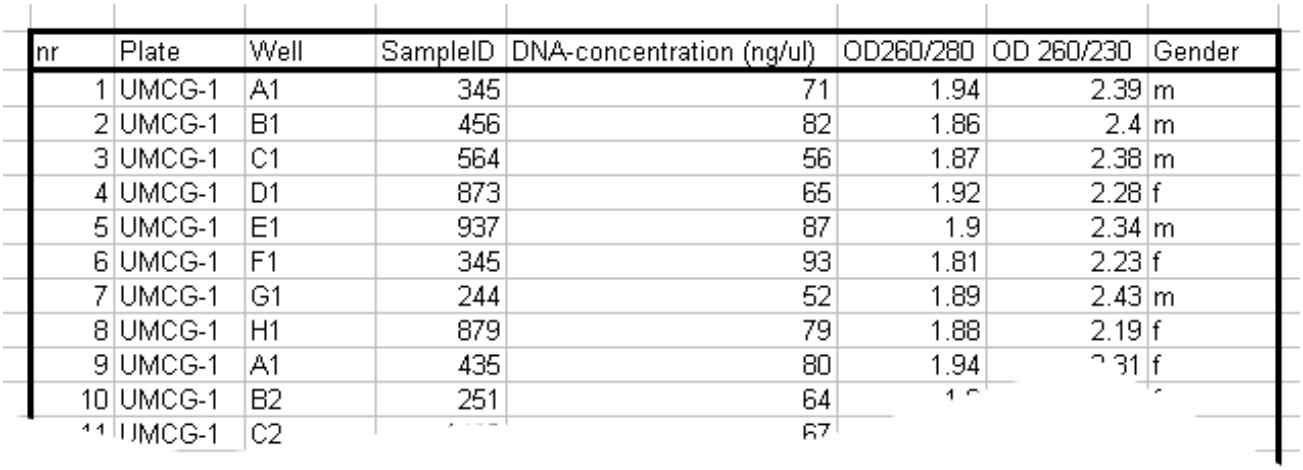
Sample preparation

1. At least 10 µl of the DNA-solution (50 ng/µl-100 ng/µl) should be transferred to 96-well plates (Greiner Bio-One, PCR-plate full skirted, order nr. 652270).
2. Add samples in the well-order A1, B1, C1, D1 etc, don't leave any well blank between samples. Administrate the sample IDs in a special XLS-file according to the guidelines below. A unique plate name should be given to each 96-well plate, using a permanent marker to annotate each plate on two sides.
3. The 96-well plates should be firmly closed preferentially with a Thermo-seal (*Thermo scientific order nr. AB-0559*) using a Heat plate sealer. Alternatively, if a heat plate sealer is not available, adhesive PCR Foil seals (*Thermo scientific order nr. AB-0626*) can be used.
4. After the DNA-solution is transferred to a 96-well plate and the plate is sealed properly, the plates should be stored and transported at -20°C.
5. The quality measurement of DNA-samples with the Nanodrop spectrophotometer and the transfer to 96-well plates can be performed by employees of the facility as part of the project. Please contact the Genome Analysis Facility to discuss this if necessary/requested.

Documentation of the sample and plate layout

All sample-IDs and corresponding wells in the 96-well plate should be administrated in a XSL-file (Excel) (figure 2). In this XSL-file all required information is summarized:

- Number
- Plate name
- Well with corresponded a unique sample ID. The facility doesn't accept samples with names of individuals.
- Information of the DNA quality; DNA concentration, OD 260/280 ratio, OD 260/230 ratio.
- For verification reasons the gender should also be administrated.



nr	Plate	Well	SampleID	DNA-concentration (ng/ul)	OD260/280	OD 260/230	Gender
1	UMCG-1	A1	345	71	1.94	2.39	m
2	UMCG-1	B1	456	82	1.86	2.4	m
3	UMCG-1	C1	564	56	1.87	2.38	m
4	UMCG-1	D1	873	65	1.92	2.28	f
5	UMCG-1	E1	937	87	1.9	2.34	m
6	UMCG-1	F1	345	93	1.81	2.23	f
7	UMCG-1	G1	244	52	1.89	2.43	m
8	UMCG-1	H1	879	79	1.88	2.19	f
9	UMCG-1	A1	435	80	1.94	2.31	f
10	UMCG-1	B2	251	64			
11	UMCG-1	C2		67			

Figure 2: Example of the attached XSL-file.

Please email the XLS-file with the sample-information to: p.van.der.vlies@umcg.nl

Sample shipment

Before submitting your samples, please contact the Genome Analysis Facility.

Please deliver the samples on dry ice on the lab of the Genome Analysis Facility or send your samples by a courier-company to the laboratory of the Genome Analysis Facility of the department Genetics of the UMCG. The shipment should be carried out in boxes containing a sufficient amount of dry-ice.

To avoid any delay during the weekend, please ship the material in the beginning of the week.

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

Phone number: 050-3617100