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Centre d'expertise et de services Génome Québec

SEPTEMBER 8, 2020

GENOTYPING SERVICES

Centre d'expertise et de services Génome Québec

User Guide: ThermoFisher Scientific and Illumina Genotyping Technologies

Version 8.0

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General Guidelines for Sample Preparation

Detailed instructions for the submission of samples and information on available technologies and products are also provided.

Projects can be performed on various types of Microarrays: commercial, semi-custom or custom.

For projects with customized content, a list of markers has to be provided prior to DNA samples. A User Guide for the submission of markers list is available.

For ThermoFisher Scientific or Illumina technologies, it is recommended to submit genomics DNA (gDNA) for genotyping. However, in certain cases, Whole Genome Amplification (WGA) must be performed prior to genotyping. It is recommended to consult genotyping platform personnel to verify if WGA is required.

Preparation of Genomics DNA Samples

It is recommended to measure DNA content using PicoGreen DNA Measurement.

All samples should have a concentration of 100 ng/µL.

It is recommended to dilute DNA in either 1X TE buffer (10 mM Tris-HCl pH 8.0 / 1 mM EDTA) or in nuclease-free water.

A minimum volume of 20 µL must be sent for each sample.

If the same samples are destined to be analyzed on several BeadChips, a minimum of 50 µL has to be provided.

Preparation of WGA DNA Samples

WGA methods recommended for amplifying genomics DNA are REPLI-g (Qiagen) or OmniPlex (Rubicon Genomics).

At least 50 ng of intact DNA or at least 100-200 ng of DNA suspected or known to have undergone some degradation should be used as starting materials for the amplification.

Once WGA has been performed, it is recommended to quantify DNA using PicoGreen DNA measurements.

It is recommended to test WGA samples prior to genotyping using techniques such as PCR or Taqman.

All samples should have a concentration of 100 ng/µL.

It is recommended to dilute DNA in either 1X TE buffer (10 mM Tris-HCl pH 8.0 / 1 mM EDTA) or in nuclease-free water.

A minimum volume of 20 µL must be sent for each sample.

If the same samples are destined to be analyzed on several BeadChips, a minimum of 50 µL has to be provided.

Preparation of samples extracted from paraffin (FFPE) (Illumina only)

The Illumina protocol allows using samples extracted from paraffin blocks (FFPE).

For this a quality control step (real-time PCR) is done to validate the sample DNA integrity. Then a « restore step » is performed before the usual Illumina protocol.

If you are shipping FFPE and non-FFPE samples, they should be split in two distinct plates with a specific randomization for each plate. FFPE samples should be properly identified in the file TEMPLATE (ex.: FFPE_samples1 etc.).

It is recommended to measure DNA concentration using PicoGreen DNA Measurement.

A minimum volume of 20 µL is needed with a concentration of 60 ng/µL (PicoGreen).

Preparation of DNA plates

DNA Plating

A minimum of samples is required for projects with semi-custom or custom Microarrays.

- 1152 for Illumina technology
- 1920 for ThermoFisher Scientific technology (if ≤50K SNPs)
- 480 for ThermoFisher Scientifictechnology (if ≥50K SNPs)

All DNA samples must be sent in 96-well plates in the appropriate plate type. Plates must be clear or transparent.

Recommended 96-well plates are:

- Full-skirt PCR plates (e.g. Microseal PCR plates; Bio-Rad, cat# MSP9601)
- Half-skirt PCR plates
- Deep-well plates (e.g. ABgene, cat#AB-0859)

After the plating has been completed, 96-well plates must be sealed properly. Recommended seals are:

- MicroAmp Clear Adhesive Films (Applied Biosystems, cat# 4306311)
- MicroSeal 'F' Foil (Bio-Rad, cat# MSF-1001)

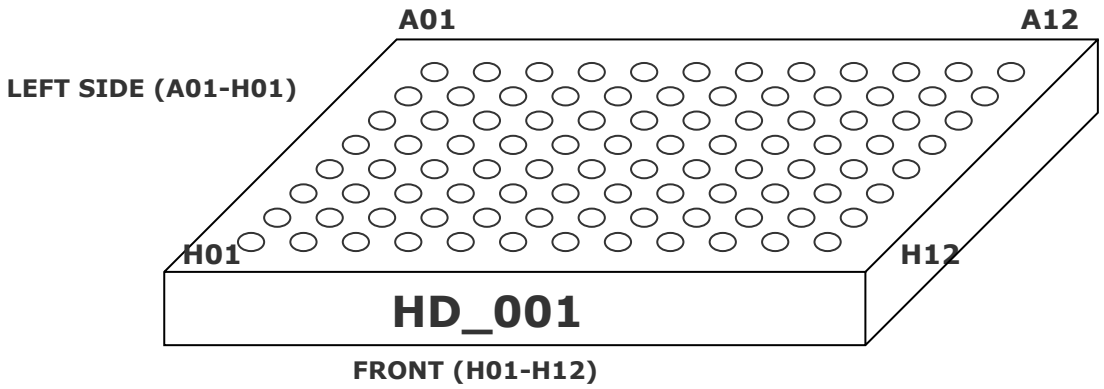
Note: Do not use Strip-caps or aluminium seal to seal the plates.

Samples with common characteristics such as ethnicity, DNA sources or extraction methods, must be grouped together in successive rows or plates.

DNA plates must be clearly labeled on the left side (A01-H01) and on the front (H01-H12) of the plate.

The name of the plate has to be written with a marker pen even when stickers or barcodes are used.

The suggested naming convention is to incorporate the project name. For instance, if the samples are related to heart disease, one way to name the plates would be HD_001 (Heart Disease 1) up to HD_(n), according to the total number of plates for this project.



One well per 96-well plate (H12 positions) must be left empty for positive control.

Consequently, a quote for the genotyping of 96 samples emitted by the Client Management Office means that 94 project samples and two positive controls will be genotyped.

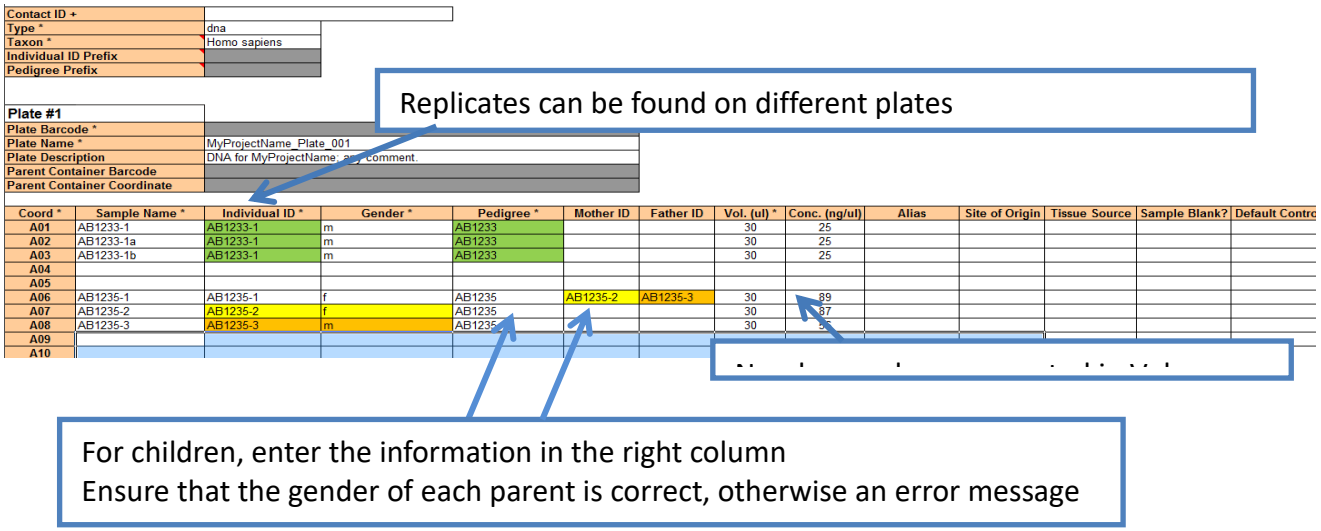
Users will add their own positive controls in the above mentioned wells when projects are done on species other than mouse and human.

Filling out the Plating Layout template

Each file can contain relevant information of samples plated onto up to 12 plates.

The template must be filled using the guidelines described below:

- Mandatory fields are indicated with an asterisk (*) and a plus sign (+).
- Each individual must have a unique *Individual_ID.*
- The individual IDs of the parents must be indicated: *Mother_ID* (*Mother Individual ID*) and *Father_ID* (*Father Individual ID*) (columns F and G) (if applicable).
- It must be specified if the sample is a control by writing "*YES*" under "*Default Control?" (*column N, if applicable).



Each file must be named using this convention: *Project_Name-Plate_layout-00X* (example: HD_001)

File format must be .xls and not .xlsx.

Submission of a List of Markers

Creating the List of Markers

Only bi-allelic markers such as SNPs (Single Nucleotide Polymorphisms) and indels (insertion/deletion polymorphisms) with a single localization may be genotyped.

By contrast, MNPs (Multiple Nucleotide Polymorphisms), SSRs (Simple Sequence Repeats) or SNPs with ambiguous or multiple localizations cannot be genotyped.

For Illumina custom BeadChips the number of loci can either be:

- iSelect-24**:** from 3,072 to 90,000 markers can be chosen for the panel composition.
- iSelect-12: from 90,001 to 250,000 markers can be chosen for the panel composition.
- iSelect-4: from 2,510,001 to 1,000,000 markers can be chosen for the panel composition.

For semi-custom BeadChips, the number of custom loci can either be:

BeadChip	Nb markers per sample	Nb custom markers that can be added to an existing array
GSA (Global Screening Array)	~ 640,000	50,000
GSA-MD (Global Screening Array + Multi Disease)	~ 670,000	20,000
GDA (Global Diversity Array)	~1,800,000	N/A
OncoArray	~ 499,000	120,000
CanineHD	~ 172,000	N/A
BovineHD	~ 777,000	N/A
BovineSNP50	~ 53,000	600,000
BovineLD	~ 8,000	80,000
PorcineSNP60	~ 64,000	25,000
MaizeSNP50	~ 56,000	N/A

For ThermoFisher custom array the number of loci can either be:

- 384 arrays plate: from 1,500 to 50K markers can be chosen for the panel composition.
- 1x96 arrays plate: from 50K to 675K markers can be chosen for the panel composition.
- 2x48 arrays plate: from 675K to 1.3M markers can be chosen for the panel composition.
- 3x32 arrays plate: from 1.3M to 2M markers can be chosen for the panel composition.
- 4x24 arrays plate: from 2M to 2.6M markers can be chosen for the panel composition.

Creating a list of markers

A UserGuide for creating a list of markers is available.

Service Request Form and Sample Submission (new Request)

The new Request Form functionalities are used for new projects only.

Login to your Nanuq account here.

In the Request Section, click Add new Request and follow the instructions.

Do not use the Back button of your Browser to go back to previous pages. Use the menu on the left-hand side of the screen.

The Request Form is completed when the "Submit" button is used, otherwise, its status will remain as Draft.

Incomplete new Requests will become accessible in future sessions using Request List .

The work in the laboratory will only start when all required documentation is provided and approved.

Service Request Form and Sample Submission (Request)

Login to your Nanuq account here.

In the Request Section, click Request List.

This is used when 1) new samples or replacement samples are submitted as part of an existing project or 2) to continue entering out new information in a Draft Request.

To submit new samples, go to the Sample Submission Section. From there, you can:

- Download empty templates for submitting new samples.
- Review previous sample submissions.

Do not add new samples or replacement samples to an existing template file.

Do not use the Back button of your Browser to go back to previous pages. Use the menu on the left-hand side of the screen.

Shipment of Samples

An indication on the outside of the box should mention the *Project Name* **(Project Name),** the *Recipient's Name* **(Daniel Vincent)** and the *Platform Name* **(High Throughput Genotyping).**

DNA plates should be properly sealed and placed in re-sealable plastic bags (Ziploc type). Place the racks in a freezer so that DNA is frozen prior to packaging for shipment.

Fill all empty space in the Styrofoam shipping container with dry ice pellets to ensure that the DNA remains frozen and the racks remain secure during shipment.

The sender must pay for the shipping. Do not ship on a day that would result in the package arriving in Montreal on a weekend.

A paper copy of the electronic Final Sample Manifest should be included with each shipment.

Please fill out the customs declaration letter (provided by the broker) and include with your shipment.

The Tracking number of the shipment should be emailed to **dvincent@genomequebec.com**

It is strongly recommended to e-mail authorized personnel prior to bringing samples at the Centre d'expertise et de services Génome Québec.

If you send us samples, please provide the waybill with your shipment.

To print the waybill:

- Go to the Samples submission tab in the Request form
- Select the appropriate samples submission form by clicking in the box
- Click on "Print Waybill"

Shipping addresses

Please refer to the waybill for instructions on how to ship your samples.

For More Information

Client Management Office

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