

## DNA METHYLATION SERVICES

Centre d'expertise et de services Génome Québec

# User Guide: Illumina Infinium HD Technology

Version 7.0

# Table of Contents

# General Guidelines for DNA Sample Preparation

This document describes the procedure to follow when requesting DNA Methylation analysis services using Infinium II Technology from Illumina.

## Preparation of Genomics DNA Samples

Projects can only be performed on genomic DNA samples (gDNA), not on Whole Genome Amplified samples.

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

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.

All samples should have a 100 ng/µL concentration.

A minimum volume of 35 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)

The Illumina protocol allows using samples extracted from paraffin blocks (FFPE). Bisulfite treatment should be done before any other steps to avoid the loss of methylation, which is "erased" after a standard PCR.

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 your 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.

Recommendations in terms of volume and concentration are the following:

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

**The following kits are suggested for FFPE DNA extration :**

- Qiagen QiaAmp DNA FFPE Tissue kit (#56404) combined to the new deparaffinization solution (seems to give better yields than xylen with the same quality) https://www.qiagen.com/us/products/catalog/lab-essentials-and-accessories/deparaffinization-solution/
- Thermo Scientific™GeneJET FFPE DNA : https://www.lifetechnologies.com/order/catalog/product/K0881

## DNA plating

A minimum of 24 samples is required per project.

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)

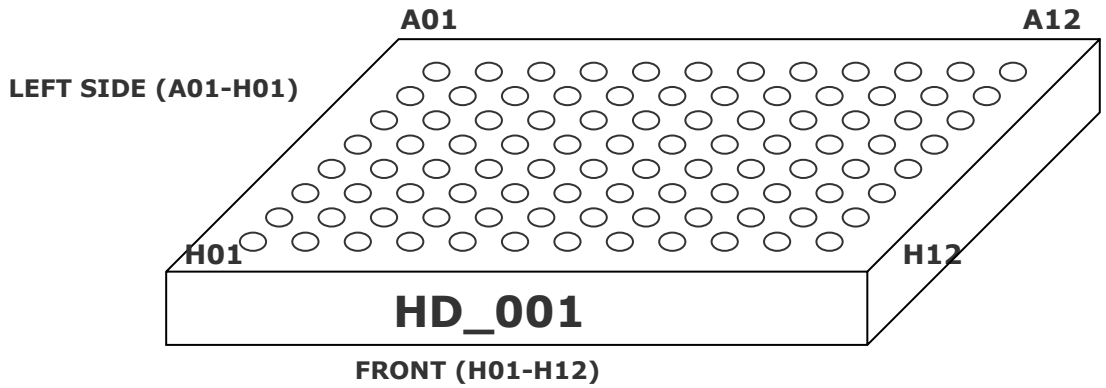
**Note**: Strip-caps must not be used to seal the plates.

Plate samples by filling out rows, not columns (e.g. first 12 samples must be plated from A1 to A12).

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.



IMPORTANT:

Samples must be randomized on 96-well plates to minimize array and position biases.

For projects of 24, 48 or 72 samples please use the examples below:

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A01 | A02 | A03 | A04 | A05 | A06 | A07 | A08 | A09 | A10 | A11 | A12 | | Group A |
| B01 | B02 | B03 | B04 | B05 | B06 | B07 | B08 | B09 | B10 | B11 | B12 | | Group B |
| C01 | C02 | C03 | C04 | C05 | C06 | C07 | C08 | C09 | C10 | C11 | C12 | | |
| D01 | D02 | D03 | D04 | D05 | D06 | D07 | D08 | D09 | D10 | D11 | D12 | | |
| E01 | E02 | E03 | E04 | E05 | E06 | E07 | E08 | E09 | E10 | E11 | E12 | | |
| F01 | F02 | F03 | F04 | F05 | F06 | F07 | F08 | F09 | F10 | F11 | F12 | | |
| G01 | G02 | G03 | G04 | G05 | G06 | G07 | G08 | G09 | G10 | G11 | G12 | | |
| H01 | H02 | H03 | H04 | H05 | H06 | H07 | H08 | H09 | H10 | H11 | H12 | | |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A01 | A02 | A03 | A04 | A05 | A06 | A07 | A08 | A09 | A10 | A11 | A12 | | Group A |
| B01 | B02 | B03 | B04 | B05 | B06 | B07 | B08 | B09 | B10 | B11 | B12 | | Group B |
| C01 | C02 | C03 | C04 | C05 | C06 | C07 | C08 | C09 | C10 | C11 | C12 | | Group C |
| D01 | D02 | D03 | D04 | D05 | D06 | D07 | D08 | D09 | D10 | D11 | D12 | | |
| E01 | E02 | E03 | E04 | E05 | E06 | E07 | E08 | E09 | E10 | E11 | E12 | | |
| F01 | F02 | F03 | F04 | F05 | F06 | F07 | F08 | F09 | F10 | F11 | F12 | | |
| G01 | G02 | G03 | G04 | G05 | G06 | G07 | G08 | G09 | G10 | G11 | G12 | | |
| H01 | H02 | H03 | H04 | H05 | H06 | H07 | H08 | H09 | H10 | H11 | H12 | | |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A01 | A02 | A03 | A04 | A05 | A06 | A07 | A08 | A09 | A10 | A11 | A12 | | Group A |
| B01 | B02 | B03 | B04 | B05 | B06 | B07 | B08 | B09 | B10 | B11 | B12 | | Group B |
| C01 | C02 | C03 | C04 | C05 | C06 | C07 | C08 | C09 | C10 | C11 | C12 | | Group C |
| D01 | D02 | D03 | D04 | D05 | D06 | D07 | D08 | D09 | D10 | D11 | D12 | | Group D |
| E01 | E02 | E03 | E04 | E05 | E06 | E07 | E08 | E09 | E10 | E11 | E12 | | |
| F01 | F02 | F03 | F04 | F05 | F06 | F07 | F08 | F09 | F10 | F11 | F12 | | |
| G01 | G02 | G03 | G04 | G05 | G06 | G07 | G08 | G09 | G10 | G11 | G12 | | |
| H01 | H02 | H03 | H04 | H05 | H06 | H07 | H08 | H09 | H10 | H11 | H12 | | |

For projects of 96 samples and above, please use the examples found below.

Note that wells H12 has to remain empty. Positive controls will be added by the Platform.

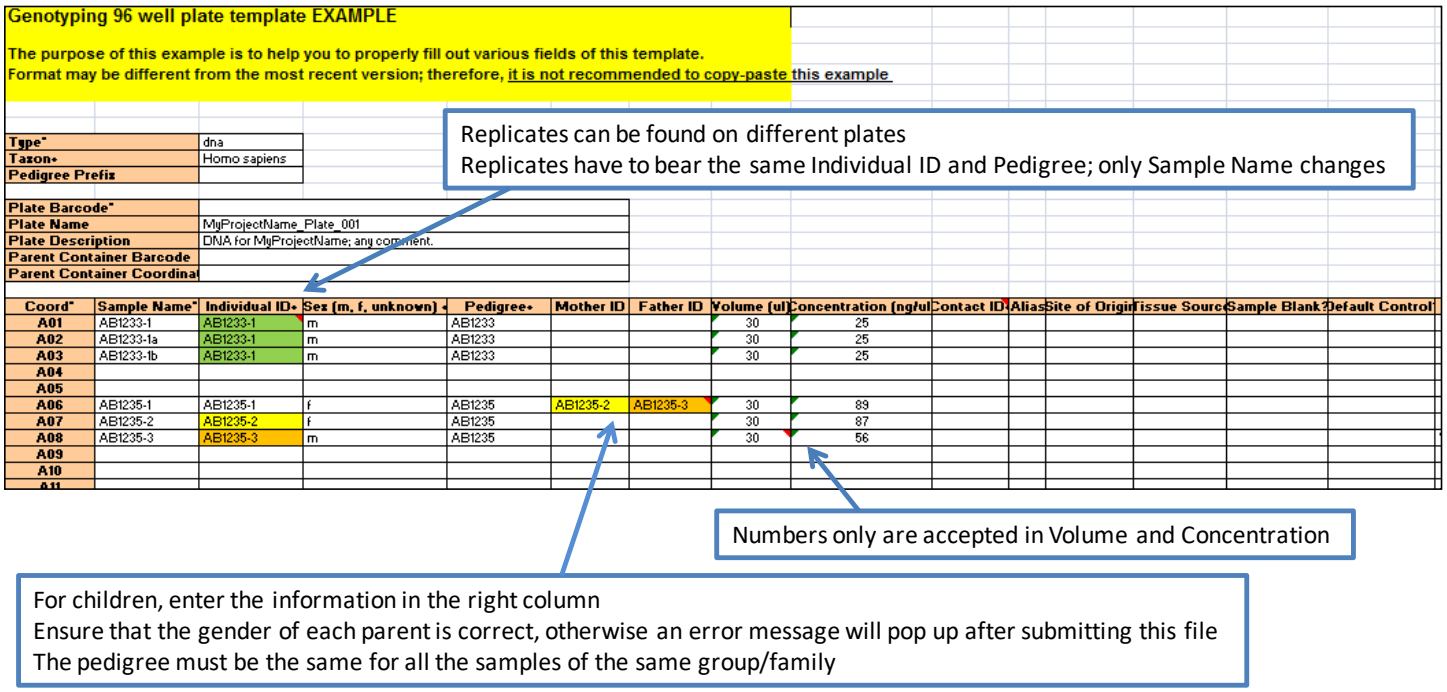
Please e-mail the Client Management Office (infoservices@genomequebec.com) if doubts remain on how plating samples.

## 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).
- The "experimental group" column (not shown here) is mandatory.




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

File format must be .xls and not .xlsx.

# Service Request and Sample Submission

## 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.

## 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* **(Illumina 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 attached customs declaration letter and include with your shipment.

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

## Shipping addresses

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