Jones Lab Illumina Microarray Q&A

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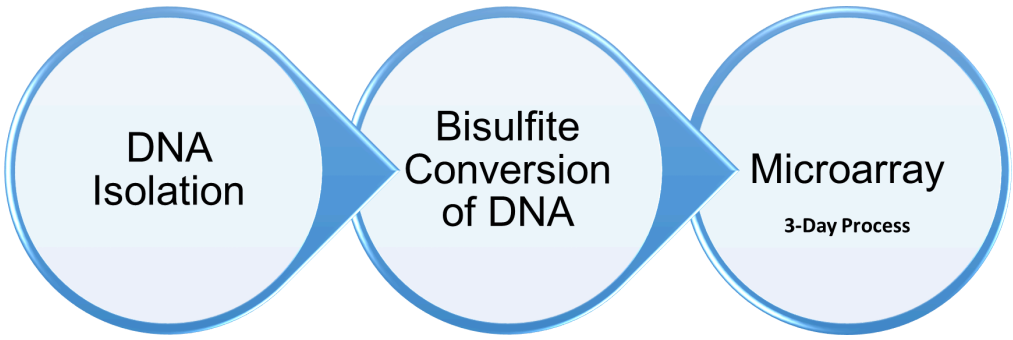
Overview of Microarray Workflow



Note: time taken to process samples prior to the microarray differs based on on number of samples submitted for microarray and at what stage samples are submitted (ie. Has DNA been extracted yet?)

Sample Instructions

Number of Samples Required

It is important to determine the number of samples that you would like to send in for the microarray. In one week, we are able to do a maximum of two runs but typically perform only one run per week.

- For **human** samples, we require a **minimum** of 32 samples and **maximum** of 64 samples for 1 array run, maximum of 64 samples processed per week of the arrays.
- For **mice and other mammal** samples, we require a **minimum** of 48 samples and **maximum** of 96 for 1 run of the array.

Starting Material

If giving tissues as starting material

For blood samples, please send a minimum of 2 mL volume. For tissue samples, please send at least 25 mg of sample, preferably 50mg if possible in case of sample loss.

- Please ensure that tissues are packaged in appropriate freezer containers (please transport in a freezing vial that can withstand -80℃)
- When sending multiple samples, ensure that the positions of the samples are being noted in the sample sheet to make it easier to locate the samples
- Send us a sample sheet containing a list of sample names, tissue type, approximate sample volume being shipped, position of samples in the shipping container (box layout), and either sample variables to use for randomisation, or a randomization layout (see below).

If giving DNA as starting material

For higher reproducibility, we require a minimum of 750 ng of DNA per 20µL elution buffer or water. We recommend sending **3000 ng of DNA in 80uL** buffer or water if possible to account for cases where DNA has a lower quality since we may have to repeat the bisulfite conversion protocol for selected samples.

- Please quantify your DNA (and volumes) being shipped and check the sample qualities
- When sending multiple samples, ensure that the positions of the samples are being noted in the sample sheet to make it easier to locate the samples
- Send us a sample sheet containing a list of sample names, DNA quantity, 260/280, 260/230, sample volume being shipped, position of samples, and either sample variables to use for randomisation, or a randomization layout (see below).

If giving bisulfite converted DNA as starting material *NOT recommended

This step assumes that bisulfite conversion has been done. We do not recommend shipping bcDNA, as it is less stable than genomic DNA, but there are times when it may be required. If

using the 96-well kit (see below for recommended bisulfite conversion kits), **please elute in 12 µL elution buffer.**

We require at least **12 µL** of bisulfite converted DNA. No specific concentration is required but we recommend at minimum 10ng/uL up to a maximum of 40ng/uL if possible.

- Please quantify your bisulfite converted DNA (and volumes) being shipped and check the sample qualities
- When sending multiple samples, ensure that the positions of the samples are being noted in the sample sheet to make it easier to locate the samples
- Send us a sample sheet containing a list of sample names, bisulfite converted DNA quantity, 260/280, 260/230, sample volume being shipped, position of samples, and either sample variables to use for randomisation, or a randomization layout (see below).

Randomisation layout of samples on the chips

Prior to running the microarrays for your samples, we randomise the samples for each run. If you are doing plate randomisation, please send us a plate layout for your samples to be loaded onto the chips.

The plate randomisation layout consists of all your samples in each microarray run. This includes the chip that samples would be placed on, location that the samples would be on the chip and the column on the plate where the samples would be on. Mouse and HorvathMammal array chips have 12 sample slots on each chip whereas the EPIC human array chips have 8 sample slots on each chip.

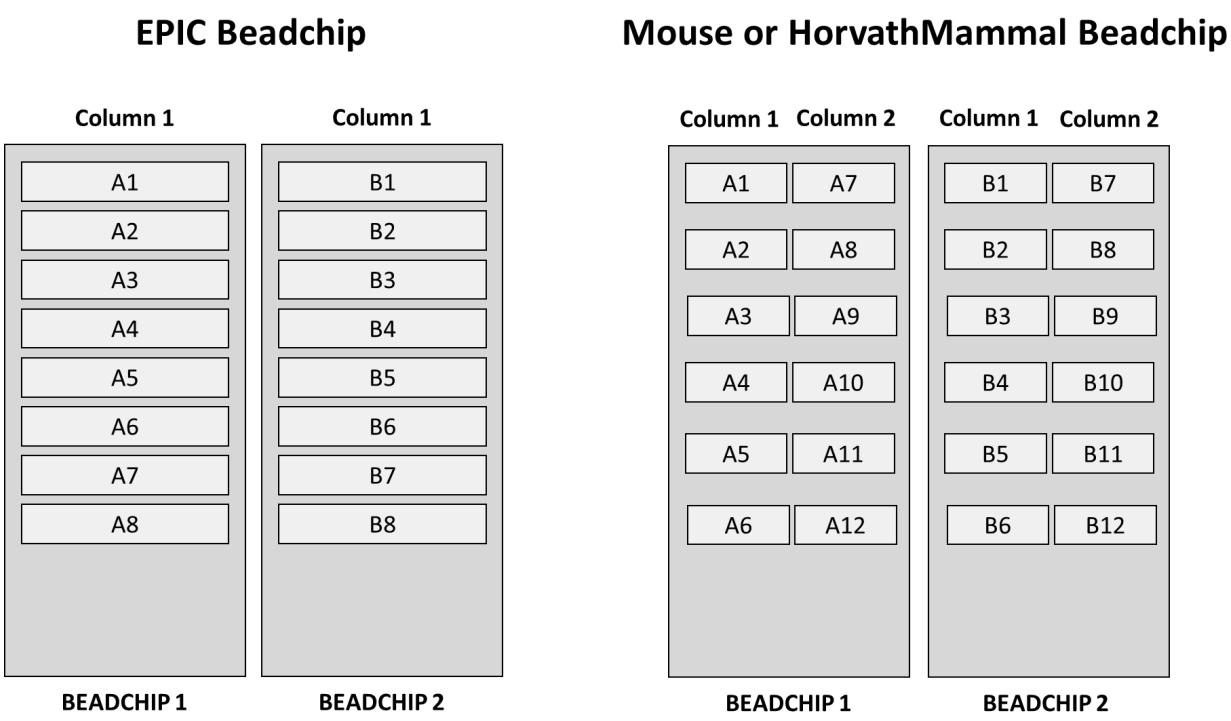


Figure1. Layout of the types of chips available for randomisation of samples. The letters represent the rows of the plate (and also tell us which samples are on the same chip) and the numbers refer to the position of the sample on each chip.

Considering Groups When Randomizing Samples on the Chips

We usually consider any innate groups that the samples have prior to randomisation. This is done to minimise any introduced variability for samples from the same group by trying to place these samples on the same chip(s). Innate groups can include but are not limited to the following:

1. Biological or technical replicates (samples from the same treatment group, cell type, etc)
2. Sex of sample
3. Samples from the same organism that has been collected over time (longitudinal studies)
4. Study type - if you are submitting a collection of samples from various different studies, we try to keep samples for the same study on the same chip(s) as much as possible

If unsure of groupings for your samples, please discuss with us and we can advise further.

Ordering Instructions

DNA Isolation Kits

To order DNA isolation kits

We recommend using either one of the following kits

Kit	Canadian Distributor	List Price[1]
Invitrogen PureLink™ Genomic DNA Mini Kit (Cat# K182002)	Life Technologies Inc.	K182002 - CAD$1016
DNeasy Blood & Tissue Kit (50 or 250) 50 samples - Cat# 69504 250 samples - Cat# 69506	Qiagen	69504 - USD$215 69506 - USD$876

[1] Refers to the last checked (18 Sep 2023) list price on Cedarlane's punch out catalogue on EPIC. May differ depending on date last checked and if there are any company/institutional discounts/offers.

Bisulfite Conversion Kits

To order bisulfite conversion kits

We recommend either one of the following kits:
Red refers to the human arrays (EPIC)
Blue refers to the mouse and mammal arrays (Horvath)

Number of Samples	Kit	Canadian Distributor	List Price[1]
minimum 32 or **48** ≥ **N** ≱ **96**	EZ DNA Methylation-Gold Kit (Cat# D5005 or D5006)	Cedarlane Corporation	D5005 - CAD$304 D5006 - CAD$982
minimum 96 (or any denominations of 96)	EZ-96 DNA Methylation-Gold Kit (Cat# D5007)	Cedarlane Corporation	D5007 - CAD$776

[1] Refers to the last checked (18 Sep 2023) list price on Cedarlane's punch out catalogue on EPIC. May differ depending on date last checked and if there are any company/institutional discounts/offers.

Illumina Microarray Kits

We get discounts on human and mouse arrays so recommend that we order them for you and then either invoice you for them (if external to UM) or charge to your FOP (if internal to UM). For mammal arrays you can order yourselves if preferred, and ship to:

Meaghan J. Jones
University of Manitoba
744 Bannatyne Ave
RM 336 Basic Medical Sciences Bldg
Winnipeg, MB R3E 0W2
Canada

Shipping Instructions

1. Send us a sample sheet containing a list of sample names, DNA quantity, 260/280, 260/230, sample volume being shipped, position of samples, groupings of samples to consider for randomisation on the microarray chips
2. Randomisation layout (if any done) - this can be included in the sample sheet as well but please set column name as **“Plate Randomisation”**.
3. If possible, send us the tracking number for your samples after shipping

If using 1.5mL tubes for samples, please arrange samples in a cardboard freezer box (if you have randomised the samples, please place them in the order of randomisation). If shipping samples in a PCR plate (bisulfite converted DNA), place plate(s) in a ziplock bag. Samples should be shipped on dry ice to minimise sample degradation during shipping (especially important for bisulfite converted DNA samples).

Shipping Address:
Meaghan J. Jones
University of Manitoba
744 Bannatyne Ave
RM 336 Basic Medical Sciences Bldg
Winnipeg, MB R3E 0W2
Canada

Scheduling Microarray Runs

Microarray runs **will only be scheduled** once we have received the appropriate materials (ie. microarray kits, bisulfite conversion kits etc.) and the full set of samples that you would like to run.

We require at least **one month's notice** that you are planning to ship samples and/or microarrays.

Downloading Data

Once the array run for the week has been completed, we will send the intensity data (IDAT) files together with the randomised sample sheet to you. Please take note of your own research ethics requirements when considering data transfer methods. There are a few options for data sharing:

- If you have a secure data space we can access, we can upload your data to it.
- We can upload the data onto a OneDrive folder which we will send you a unique link to download the files. Data in the OneDrive folder would be stored for two weeks after the run has been completed, after which we will remove the data from the drive
- If you send us a hard drive or appropriately sized flash drive we can mail your data to you.

- For internal UM collaborators you can come visit us with a hard drive or flash drive to collect your data.