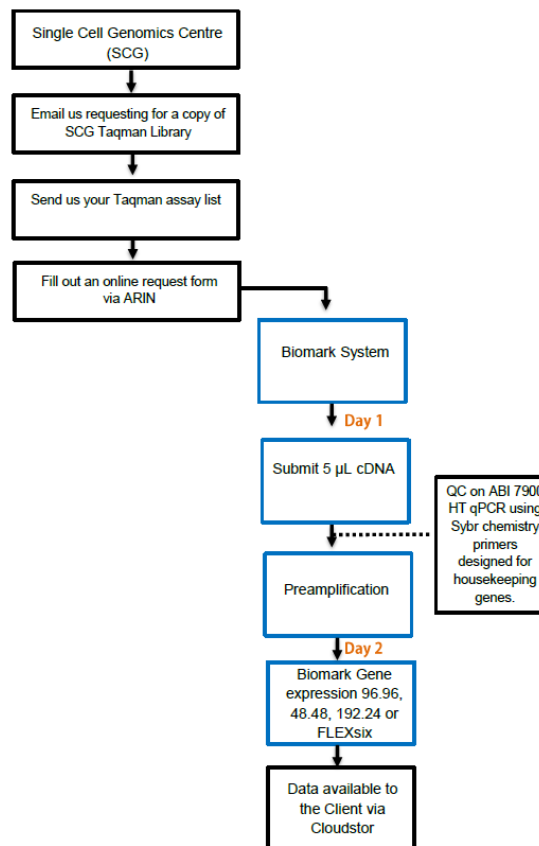


MHTP - Single Cell Genomics Centre

BioMark HD system for Gene Expression - Sample Submission Guide

The Single Cell Genomics Centre hosts the [Fluidigm BioMark HD system](#) that enables automated high throughput qPCR of over 9,000 reactions in a single run. Together, the C1 and BioMark systems enable high-throughput single-cell mRNA sequencing, targeted gene expression, mRNA sequencing, miRNA expression profiling, digital PCR, Copy number studies/genotyping, targeted DNA sequencing, whole exome sequencing and whole genome sequencing. Kindly note that [Fluidigm C1 Single Cell Auto Prep](#) and [Fluidigm BioMark HD system](#) are two different service platforms that can also be used together. The Sample submission guide is designed to assist researchers in sample submission for [Fluidigm BioMark HD system](#).

The flowchart below summarises the procedure followed from sample submission to processing on the [Fluidigm BioMark HD system](#).



Fluidigm BioMark HD system

- Fill in the project summary form to help us understand your project better, offer valuable suggestions and also try delivering good service and data.
- The Single cell genomics Taqman library consists of a comprehensive collection of more than 2000 taqman assays from different species (Human, Mouse, Rat etc) for a wide spectrum of research like the Stem Cell Biology, Developmental biology, Immunology, Cancer genetics, Cardiovascular Endocrinology, Reproduction and Development etc. Clients providing their own assays, aliquot 5 µL of Taqman or Deltagene or other assays in a 96 well PCR plate labelled "ASSAYS_Name of the client_Date of submission" and sealed using an appropriate storage film to prevent evaporation. Please note that the facility takes no responsibility for loss of poorly sealed assays. Kindly refer to Taqman assay instruction guide for further information
- Taqman gene expression assays are provided as 20X forward and reverse primer and probe mixes with each primer at a concentration of 18µM. Kindly note that Deltagene assays are provided as forward and reverse primer mixes with each primer at a concentration of 100 µM. For primers from another source, combine forward and reverse primer for each assay so that the concentration of each primer is 100 µM.
- Please find below information on Gene expression Dynamic array IFCs available in different sizes to accommodate variable number of samples and assays.

Dynamic IFC	Samples	Assays	Data Points
96.96	96	96	9216
48.48	48	48	2304
192.24	192	24	4608
FLEXsix	6*12	6*12	144

- Kindly note that a No Template Control (NTC) is included in the dynamic IFCs on the [Fluidigm BioMark HD system](#). Hence this will leave us with 95, 47, 191 and 71 samples.
- Fill in the request form for [Fluidigm BioMark HD system](#) service and select the respective application (Gene Expression or Digital PCR or Genotyping etc).
- After RNA preparation, store them in smaller aliquots to minimize any freeze-thaw cycles. For RNA samples from sensitive tissues or after long storage (more than 6 months), it is suggested to assess the integrity of the RNA before reverse transcription. Agilent 2100 Bioanalyser in the facility is very sensitive and requires only small amounts (detection down to 200 pg/ µL of total RNA) of material and provides a numerical quality value for RNA. Please note that cDNA samples from RNA samples with RIN value more than 7.0 is ideal for the Gene expression studies.
- Uniform concentration of RNA is taken for cDNA preparation and the RNA should be of the highest quality and measured to have a 260:280 ratio between 1.5 and 1.8. Also for the reverse transcription 2.5pg to 250ng of RNA is suggested- 100ng is a good starting point.
- It is important to have a minimum of 3-4 biological replicates for the qPCR experiment. This can assist in analysis of the qPCR data with higher statistical power.
- 6 µL of cDNA sample suspended in nuclease free and PCR inhibitor free water or TE buffer is a prerequisite for sample submission. The samples are aliquotted by rows and not columns in a 96 well PCR plate labelled "SAMPLES_Name of the client_Date of submission" and sealed using an appropriate storage film to prevent evaporation. Please note that the facility takes no responsibility for loss of poorly sealed samples.

- In addition, minus-reverse transcriptase (“-RT”) control samples are submitted to determine genomic DNA contamination in the cDNA samples. For instance if 48 RNA samples were extracted over 3 or 4 batches, one RT negative for each batch of extraction is required to evaluate the gDNA contamination. Typically, the “-RT” control is a mock reverse transcription containing all the RT-PCR reagents, except the reverse transcriptase. The presence of an amplification product in the “-RT” control is indicative of contaminating DNA in the sample. This control is very important when using taqman assays designed to a single exon (assay IDs with suffix “-g” or “-s”) as they will detect genomic DNA. Kindly note that –RT samples are for quality check only and these samples will be processed on the [Fluidigm BioMark HD system](#) only upon client request.
- Perform a DNase treatment (mandatory) on RNA preparations to degrade any trace amounts of genomic DNA that would otherwise result in false positive signals in subsequent RT-PCR.
- Upon sample arrival, all the cDNA and –RT samples will be quality checked by qPCR on ABI 7900HT using predesigned primers in combination with Sybr chemistry (Quality Check 1). Kindly note that we use one primer set predesigned for an endogenous housekeeping gene suitable for your project design. The facility will not be process the cDNA samples further on the [Fluidigm BioMark HD system](#) if a –RT control sample has a C_T value difference of less than 10 cycles with the cDNA samples, indicating genomic DNA contamination.
- After the Fluidigm BioMark HD Gene expression experiment, the data and the Fluidigm qPCR software for analysis is transferred via cloudstor and the preamplified cDNA in 96 well PCR plate (Client) is handed over to the client.
- The quality report detailing methodologies used for the Fluidigm BioMark HD Gene expression project is emailed in 5 days to the client.