

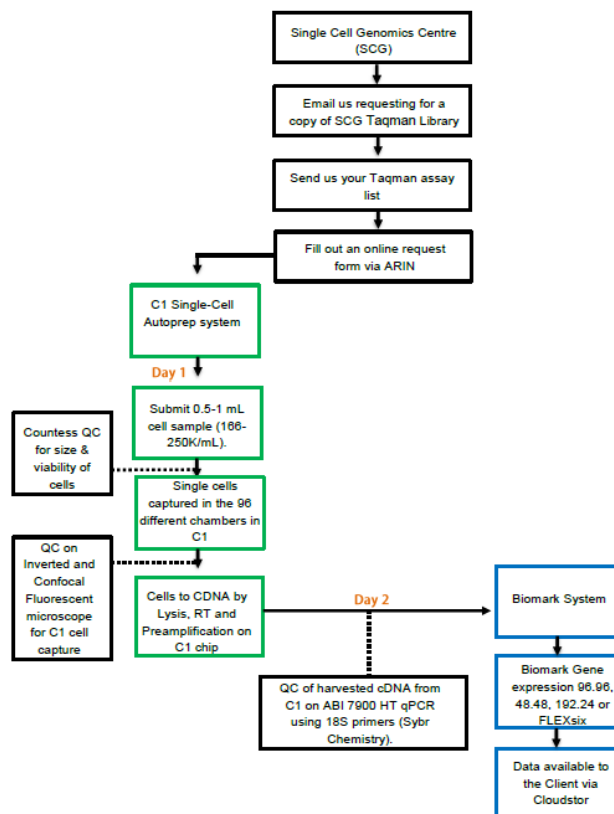
MHTP - Single Cell Genomics Centre

Fluidigm Single Cell Auto Prep - Sample Submission Guide

The Single Cell Genomics Centre hosts the [Fluidigm C1 Single Cell Auto Prep](#) that is based on Fluidigm's innovative microfluidic technology enabling researchers to rapidly and reliably isolate, process, and profile individual cells for genome and transcriptome analysis. The Centre also hosts a [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 C1 Single Cell Auto Prep](#) and [Fluidigm BioMark HD system](#).

The flowchart below summarises the procedure followed from sample submission to processing on the [Fluidigm C1 Single Cell Auto Prep](#) and [Fluidigm BioMark HD system](#).



Fluidigm C1 Single Cell Auto Prep

- Fill in the project summary form to help us understand your project better, offer valuable suggestions and also try delivering good service and data.
- Cells must meet Fluidigm's specification for a successful single cell capture. A copy of Fluidigm's single cell preparation guide can be downloaded from the Medical Genomics Facility home page.
- The Single cell genomics Taqman library consists of a comprehensive collection of more than 1000 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.
- Fill in the request form [Fluidigm C1 Single Cell Auto Prep service](#) (for cDNA samples from cells only) or for both the [Fluidigm C1 Single Cell Auto Prep](#) and [Fluidigm BioMark HD system](#) service (for processing cell samples to cDNA and continue qPCR Gene expression studies) via ARIN.
- 0.5-1ml of flow sorted cell sample suspended in native medium (166-350K/mL) filled in a sterile eppendorf tube is a prerequisite for sample submission. Please provide an aliquot (2mL) of the native medium.
- Kindly provide sample details such as cell size distribution (average cell diameter of cell population and variability within the population), Cell viability and Cell count (cell concentration per ml) in the service request form.
- Upon sample arrival, the cell samples are immediately quality checked on a countess using trypan blue stain to confirm cell size, concentration and viability. Cell samples with low viability (below 75%) will not be processed further on the Fluidigm C1 Single Cell Auto prep as we require approximately 500-1000 viable cells to ensure high capture of cells on the C1 IFC. Occasionally upon client's request, as few as 200 cells total, from 66K/mL may be loaded in the IFC; however fewer cells loaded may yield fewer captured cells.
- Based on the Countess data (Quality check 1), the most appropriate C1 IFC is selected. Please find below information on C1 IFC available in different size and compatible for different cell types.

Cell Type	Size of C1 IFC (microns)
Stem cells, White Blood Cells	5-10
Progenitor cells, neurons, iPS	10-17
Fibroblasts, Keratinocytes, Cardiomyocytes and smooth muscle cells	17-25

- The Cell capture on C1 IFC is determined by image analysis using inverted microscope and then by confocal microscopy (Quality Check 2). Immediately, a summary of the rate of live single cell capture is reported to the client. Kindly note that for cells with no fluorescent markers, optionally live/dead@ Viability/Cytotoxicity Kit *for mammalian cells* (Molecular probes, MP03224) can be used.

- Following C1 automation (lysis, reverse transcription and preamplification) on the captured live single cells, C1 harvest amplicons (cDNAs) will be harvested from the C1 IFC into a 96 well PCR plate (Client) and diluted 1:5 by adding 25 µL C1 DNA dilution reagent to 3-5 µL of each harvest amplicon volume for a total volume of 28 µL.
- Kindly note that 6 µL out of the 28 µL is aliquoted into another 96 well microtitre plate (Core facility) as a working stock to avoid multiple freeze thaws, which is later used for qPCR using Sybr 18S primers (Quality Check 3) and Biomark Gene expression project.
- 25 C1 harvest amplicons (cDNAs) is randomly picked for qPCR using Sybr 18S primers (Quality Check 3) to confirm that the C1 Single cell autoprep process has worked before we process the cDNA samples on the Biomark for Gene expression study.
- Exceptionally for only Fluidigm C1 Single Cell Auto Prep service, 4 µL out of the 28 µL is aliquoted into another 96 well microtitre plate (Core facility) as a working stock to avoid multiple freeze thaws, which is later used for qPCR using Sybr 18S primers (Quality Check 3) and the preamplified cDNA in 96 well PCR plate (Client) is handed over to the client.
- However if the client requests to proceed with [Fluidigm BioMark HD](#) Gene expression experiment, the cDNA samples are run on the biomark. 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

- Data and the Fluidigm qPCR software for analysis is transferred via cloudstor and the preamplified cDNA in 96 well microtitre plate (Client) is handed over to the client.
- The quality report detailing methodologies used for the Fluidigm C1 Single Cell Auto Prep and Fluidigm BioMark HD Gene expression project is emailed in 3 days to the client.