

New Service - mRNA SMART-Seq2

The Product

- Modified SMART-Seq2 cDNA and Illumina Nextera XT library construction
- Protocol based on *Preparation of Single-Cell RNA-Seq Libraries for Next Generation Sequencing* - **Trombetta et al** 2017
- NextSeq500 sequencing

Preferred Input

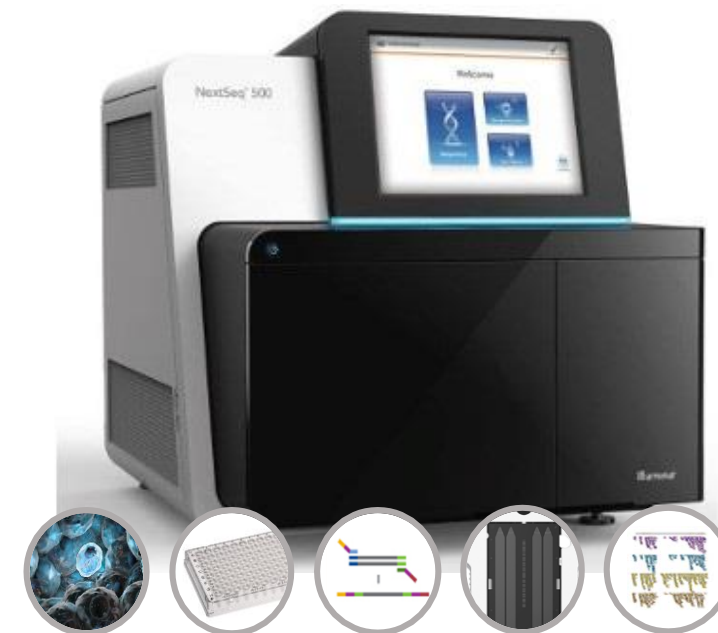
- Sorted lysed cells in 96-well Eppendorf twin-tec barcoded plates
- 5ul Buffer TCL + 1% beta-mercaptoethanol (BME) in each well
- Stored at -80 °C
- 384 sample batching [4 plates] preferred

Coverage Options

- Low Coverage (single cell): 1 Million reads/sample
- High Coverage (populations): 4 Million reads/sample

Output/Deliverable

- Demultiplex fastq files



Single-cell Sorting & Shipping Instructions

Recommended Materials

- Eppendorf twin.tec® PCR 96-well plate LoBind, FULL skirted, (Eppendorf, cat. no. 0030129512)
 - Microseal® 'F' Foil (Bio-Rad Laboratories, Inc., cat. no. MSF-1001)
 - 2-mercaptoethanol, ≥ 99.0% (Sigma-Aldrich, cat. no. M3148-25ML)
 - Buffer TCL (Qiagen, cat. no. 1031576)
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Preparation of single-cell lysates

1. Prepare a solution of 1% 2-mercaptoethanol (by volume) in TCL buffer and distribute 5 µL of this solution into each well of a skirted-side 96-well PCR plate. Cover this plate with Microseal F, [Spin down the plate](#) and keep at room temperature until ready for single-cell isolation.
2. Prepare a cell suspension in complete media and use a FACS machine to sort a single cell into each well of the above mentioned 96-well plate containing Buffer TCL. Once sorting is completed, seal plate with Microseal F and centrifuge (800g, 1 min). Immediately freeze plate on dry ice and keep at -80°C until ready for lysate cleanup.

Note: To improve yield, adding a cell death marker is recommended so that only live cells are sorted into the plate. (e.g., the membrane impermeant DNA stain EthD-1, Life Technologies; 7AAD (7-Aminoactinomycin D, Thermo Fisher Scientific, cat A1310) at 1:500 dilution.

3. Please leave one well per plate blank which the platform will be used for a positive control (recommend H12-please list in the SK manifest).
 4. Place the SK-id on top of the plate seal and the 'Cellular Transcriptome' label on the side of the plate. It is also recommended that the SK-id be labeled by pen somewhere on the plate as well.
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1. **Make sure the plates are sealed well!** If the plates are going to be shipped on dry ice, make sure they are packaged securely in plastic bags. We recommend saving the bags that the plates originally came in, putting the same number of plates back into the bags. We have seen seals come off in shipping when sent any other way.
2. **Do not wrap them in Parafilm, tape, or foil.** These substances do not withstand the dry ice shipping very well when used with plates.

SHIPPING & SPREADSHEET INSTRUCTIONS

The PCR plates for your physical specimens should now be labeled with a Sample Kit ID (SK-XXXX) and a Cellular Transcriptome label. These are accompanied by an excel spreadsheet reflecting the layout of your PCR plate. Once you label your plate, you must fill out the sample data in the corresponding spreadsheets. It is important that this information is filled out correctly so that your data is correct in our databases. Please do not delete or modify any existing header or tab names in the spreadsheets.

When the spreadsheets are completed, please e-mail to your Broad Project Manager. Once the plate has been labeled and the spreadsheets have been properly filled out, please either drop the kits off in person (directions to our location on other attachment) or ship the kit overnight on dry ice to the following address:

Broad Institute
Attn: Genomics Platform – Samples Lab
320 Charles St – Lab 181
Cambridge, MA 02141
Phone: (617) 714-8952

TAB 1: SAMPLE INFO: *REQUIRED FIELDS*

Column A [Well Position]: Columns A is **pre-filled** containing the well position of the PCR plate.

Column B [Plate Barcode]: List the SK-ID associated. This is the plate barcode.

Column C [Alias Collaborator Participant ID]: Collaborator (i.e. your) participant ID. This ID *must be the same for all samples that come from the same patient*. Please use only Alpha-Numeric ID's. DO NOT use only numeric ID's

Column D [Alias Collaborator Sample ID]: Collaborator (i.e. your) Sample ID. This ID should be unique for each sample. Please use Alpha-Numeric ID's. DO NOT use only numeric ID's. If the sample is another aliquot of a sample already existing in the Genomics Platform Samples repository, please add the suffix “_2, _3, etc. to your Collaborator Sample ID's (depending on which aliquot is being submitted.)

Example: You have two samples that come from one participant/patient/or root sample: AB003_1 and AB003_2. The Collaborator Participant ID (Column C) would be the same, AB003, for both of these samples. The Collaborator Sample ID's (Column D) would be AB003-1 and AB003-2 respectively.

Column E [Types of Cell]: List the type of cell for each well.

Column F [Cells per Well]: List the number of cells per well.

Column G [Volume]: Enter the volume in ‘uL’ per well [note: this must be the same for all wells]

Column H [Species]: List the species the cell was derived from.

Column I [Collected after 01/25/2015]: Enter ‘yes’, ‘no’, or unknown as to whether the samples were collected after 1/25/2015.

Column J [Control]: List “positive control” or “negative control” in the same row as the corresponding well position **[Column A]**.