Providing large scale single-cell RNA-seq in the Genomics Platform at the Broad Institute

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Overview
scRNA-Seq
Single-cell RNA sequencing is a powerful technique to study gene expression, cellular heterogeneity, and delineation of cell states.

Full Scale Automation
Broad Genomics has expanded our portfolio to include single-cell services that utilize automated workflows and integrated sample tracking to support 10X Genomics Chromium Single-Cell 3’ single-cell and a modified SMART-Seq2 mRNA library construction.

SMART-Seq2 is a 96-well protocol where a single cell or a population of cells are sorted into each well of a plate containing lysis buffer by the researcher. The output is a full-length whole transcriptome library.

10X Single Cell is a droplet based protocol that can partition thousands of cells in about 8 minutes providing a 3’ whole transcriptome library. The cells are dissociated immediately before handing off to GP for chip loading.

Data Analysis
Data delivery and analysis from single-cell SMART-Seq2 sequencing is made available through our cloud based platform, Firecloud.

Each cell receives a best practices QC and analysis workflow, mirroring the methods being made publicly available via the Human Cell Atlas consortium, consisting of alignment with HISAT2, sequencing quality assessment with Picard tools, and determination of expression with RSEM. This data is then aggregated and run through a second workflow to visualize the results at the plate level allowing for troubleshooting of lab processes and identification of systematic biases.

SMART-Seq2
Workflow
- Researchers sort cells into 96-well plate containing lysis buffer and BME
- The following occur at the Genomics Platform
  - RNA cleanup
  - Reverse Transcription
  - PCR
- Capacity: 16 plates/week

Challenges
- 4ul elution after RNA cleanup in 96-well plate
- 3D Printed skirt to convert 384-well peg magnet to 96-well low elution magnet
- Limited space on Bravo during cleanups
- Created protocols with ability to stack inactive plates to allow low elution magnet
- Aspirations as low as 0.625ul
- Custom liquid classes are used to slow down aspirations and dispenses, including a post aspiration delay

10X Genomics 3’ Single-Cell RNA
Workflow
- Researchers dissociate cells immediately before handoff to Genomics Platform
- The following occur at the Genomics Platform
  - Chip Loading and Unloading
  - Reverse Transcription
  - Sample Cleanup
  - Bead Breaking
- Capacity: 40 channels/week

Challenges
- Master mix and Emulsions require steps to be done on chill blocks
- Agilent Bravos have been lifted and outfitted with chill blocks
- 10X chip requires custom labware to be placed on the Bravo deck
- Limited space on Bravo during cleanups
- Created protocols with ability to stack inactive plates to allow double-sided SPR
- Custom liquid classes to handle Gel Beads, GEMS, and Silane bead elution.

Evaluating Library Quality
Number of genes detected and percent mitochondrial genes from (A) HEK293T and (B) T-cells

Graphs from the best practices QC and analysis workflow (A-C)
(A) mRNA Region percentage helps monitor 3’ bias and successful polyA selection
(B) Mapping percentage is used to ensure correct alignment and quality
(C) Reads vs genes detected monitors sequencing depth compared to gene count
(D) Electropherograms monitor successful cDNA generation

All 10X and SMART-Seq2 sequencing runs are monitored for number of reads, Q30 scores, and orphan rates among other metrics prior to pipeline analysis to ensure sequencing quality. 10X libraries need to be run through a BCL to FastQ converter before analysis in Cell Ranger. The FastQ can be also run through the Human Cell Atlas’ Optimus Prime Pipeline.

Acknowledgments
Data used in this poster was generated at the Broad Institute, for more information please visit: http://genomics.broadinstitute.org/