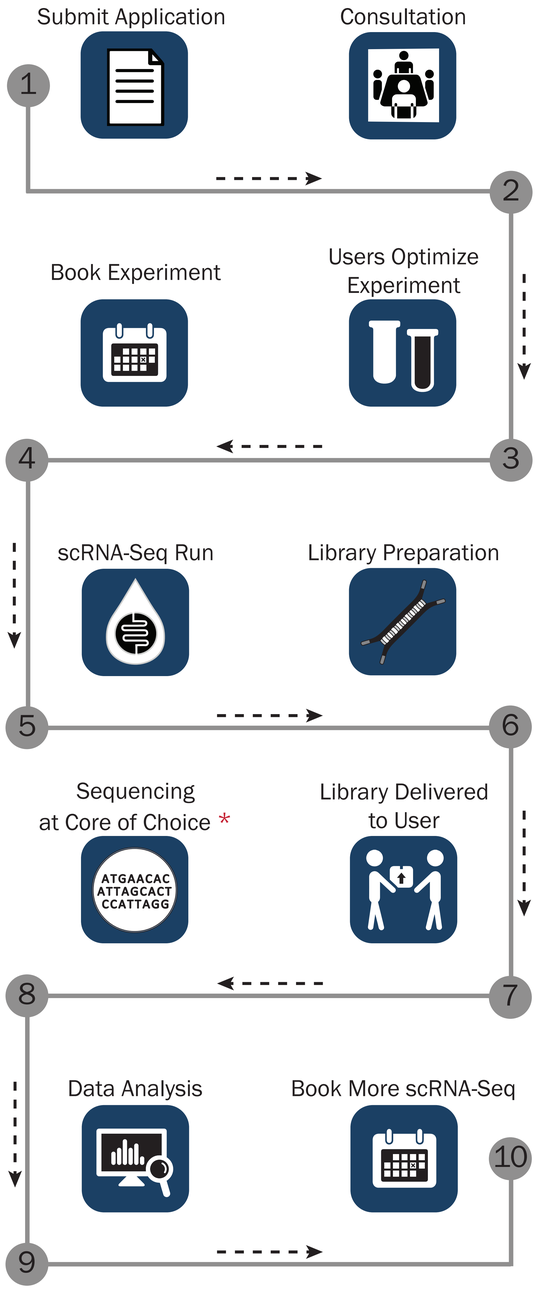
https://projects.iq.harvard.edu/singlecellcore

Single Cell Core @ HARVARD MEDICAL SCHOOL

Mandovi Chatterjee, Alex Ratner, Ian Brenckle, Sarah Boswell

Armenise 561
singlecellcore@listserv.med.harvard.edu

Project Pipeline



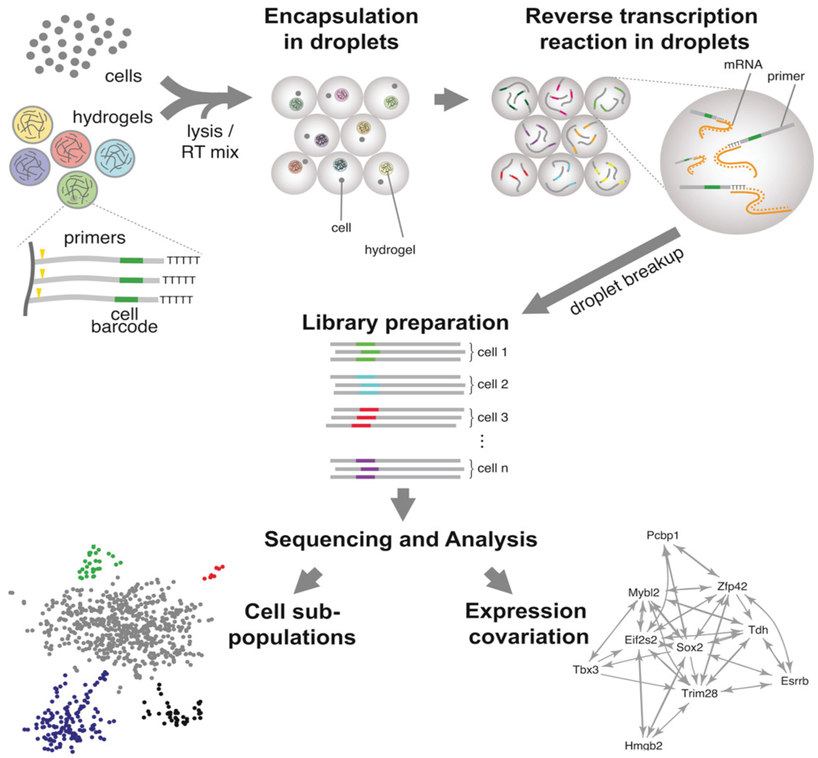
$ Rates

As of July 2018 Users no longer need to bring enzymes for their runs. Enzyme rates have been incorporated into our rates.

Project set up fee: $782

Example: 4 samples in duplicate $3223

See SCC website for full rate schedule
* sequencing costs not covered in SCC fees



InDrops method overview: cells are encapsulated into droplets with lysis buffer, reverse-transcription mix, and hydrogel microspheres carrying barcoded primers. After encapsulation primers are released. cDNA in each droplet is tagged with a barcode during reverse transcription. Droplets are then broken and material from all cells are linearly amplified before sequencing.

How to Succeed with scRNA-Seq?

1) Practice sample dissociation prior to run date.
   a) Gentle cell dissociation: shorter time, lower temperature.
   b) OptiPrep density gradient: cushion cells to avoid pelleting.
   c) Try MACS sorting instead of FACS for increased viability.

2) Ensure cells numbers and viability >90% viable after 1 hour on ice.
   a) Strain cells & confirm cells do not start clumping.
   b) Count cells on hemocytometer. Need 15,000 cells minimum.

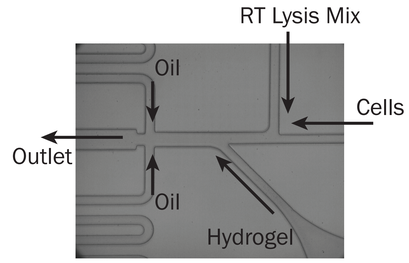
3) Work with SCC to optimize experimental design to address:
   a) Batch effects & number of replicates
   b) Number of single cells to profile

4) Bring cells in buffer WITHOUT calcium, EDTA, or heparin as these will inhibit RT. Magnesium levels should be <3mM.
   a) Suggest PBS with 0.1-1% BSA

Sample Handling

- Cells kept on ice until lysis in droplet
- RT occurs within single cell droplet
- Samples frozen until library preparation

Microfluidic Device



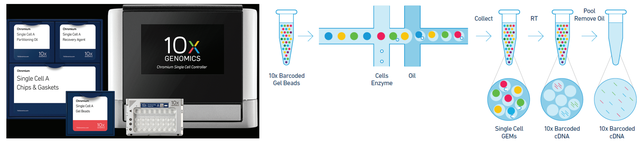
Library Preparation

- Clean up of unused primers from DNA:RNA hybrid
- Second strand synthesis to full length cDNA
- In vitro transcription to amplify product as RNA
- Fragmentation and RT by random hexamers
- PCR adaptor addition and library amplification

Data Analysis

- Experienced informaticists can follow analysis code.
- 10x users can use Cell Ranger Software.
- Suggest work with Chan Bioinformatics Core http://bioinformatics.sph.harvard.edu/

New 10x Chromium Single Cell Controller New



10x Chromium scRNA-Seq is available as a staff assisted or self-service model. Talk to the SCC to learn more!