Low input RNA-seq from FACS sorted cells

Flow sort cells

Sort into tubes with cell buffer

cDNA generation

Library Generation

Directly processes into libraries

Sequence

Health Sciences Sequencing Core

http://nextgen.pitt.edu/

William MacDonald – Assistant Director

w.a.macdonald@pitt.edu
Overview of Smart-Seq protocol
Overview of Smart-Seq protocol

- Direct cell lysis (no RNA extraction)
- Oligo(dT) primer (no gDNA contamination)
- Smart-Seq template switching oligo (TSO)
- cDNA amplification using universal primer
Overview of Smart-Seq protocol

**Smart-Seq cDNA**
- Fragment cDNA and add sequencing adapters
- Paired-end sequencing recommended (unstranded library)
- ~30-40 million reads per sample recommended
Why select this protocol?

- Low input: 1-1000 cells (500-1000 cells recommended)

- Targeted bulk RNA-seq if you are interested in transcriptional profiles of specific cell types
  - follow-up sequencing after 10X single-cell for greater sequencing depth on cell types of interest
Sample considerations

- Need FACS surface markers available for cells of interest
  - cells cannot be fixed

- High cell viability required, cell damage or degradation during the sort can lead to sample failure
  - some cell types are more sensitive
  - double sorts can also be harsh on cells

- High quality low input extracted RNA also possible (10 pg - 1 ng)
Flow sort 1-1000 cells

Cell Lysis & cDNA generation

Library Generation (Nextera XT)

Sequence

1) Sorting cells directly into wells is critical, lysis buffer contains RNase inhibitors
2) cDNA generation and amplification happened on the same day as the sort (safe pause point)
3) QC of cDNA and Nextera XT library generation on successful samples
4) Sequence libraries
Examples of Smart-Seq generated cDNA

Full length cDNA

Fragment size (bp)

Poor quality cDNA

Fragment size (bp)
Examples of Smart-Seq generated cDNA

Full length cDNA

<table>
<thead>
<tr>
<th>Fragment size (bp)</th>
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<tbody>
<tr>
<td>2500</td>
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<td>500</td>
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Poor quality cDNA

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<tr>
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</tbody>
</table>

Intact RNA

- AAAAAAAAAAAAA
  - Oligo-dT

Degraded RNA

- AAAAAAAAAAAAA
  - Oligo-dT
Flow sort 1-1000 cells

Cell Lysis & cDNA generation

Library Generation (Nextera XT)

Sequence

FLOW core dependent

$105

$105

$325

- 2x75
- 40M

*2 - 4 weeks turnaround time
Contact Information

Health Sciences Sequencing Core

http://nextgen.pitt.edu/
*New* Submission Forms and Current Prices are on our website!

Amanda Poholek - Director
poholeka@pitt.edu

William MacDonald – Assistant Director
w.a.macdonald@pitt.edu

Rania Elbakri – Research Technician
Rania.Elbakri@chp.edu

Genomics Research Core

https://www.genetics.pitt.edu/

Janette Lamb – Director
jal18@pitt.edu

Debby Hollingshead – Assistant Director
hollings@pitt.edu