Next-Generation Sequencing (NGS) Wet-Lab Workflow

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Applications of Genomics and Bioinformatics to Infectious Diseases
GABRIEL Network
Agenda

• Lab Workflow
  o Material extraction
  o Amplification
  o Barcoding/multiplexing
  o Library preparation
Material Extraction
Amplification
Barcoding/Multiplexing
Library Preparation
NGS Library Prep

1. DNA fragments
2. Blunting by Fill-in and exonuclease
3. Phosphorylation
4. Addition of A-overhang
5. Ligation to adapters
Illumina Workflow

- Library Prep
  - Shear DNA
  - Ligate Adapters
- Cluster generation
  - Bridge PCR
  - Clonal amplification
- Sequencing
Cluster Generation

- Library hybridizes to the oligos attached to the FC surface
- Bridge amplification
- Strands are linearized & seq primer is hybridized
- Clusters contain ~1000 molecules
Illumina Sequencing By Synthesis

Clusters on Flow Cell Surface

Anneal Read 1 primer

Single Molecule View

3' 5'
Sequencing - Imaging
Primary Analysis

- Matrix Calculation – what base is being imaged?
  - Determined during cycles 1-12 of both reads
  - Cross talk
  - Corrected intensities

- Phasing Correction – how accurate is the chemistry?
  - Determined during cycles 1-12 of both reads
  - Phasing
  - Prephasing

- Quality Filtering – is this a high quality cluster?
  - Determined at cycle 25
  - Phred Quality Scores – is the sequence high quality? (Cycle 25 onward)
Real-Time Run Monitoring
ION Torrent PGM

- **Library Construction**: shear DNA and ligate barcoded adapters
  - 1/2 day
- **qPCR**
  - 2 hours
  - template quantitation
- **Emulsion PCR on the One Touch 2**
  - 5 hours
  - adapter attached to ion sphere particles (ISP)
- **Enrich for template-positive beads on the One Touch ES**
  - 1 hour
  - Magnetic beads
- **Initialize PGM & sequence**
  - 4-6 hours

![Preparing an Ion amplicon library by the ligation method](image)

*Figure 2A Ion amplicon library design by the ligation method*
ION Torrent Sequencing

- 314 chip: 2h 20m
  - 1.2 million wells
  - ~80 Mbp (covers 10 flu viruses at 500x)
- 316 chip: 3h 05m – 6.3 million wells
  - ~500Mbp
- 318 chip: 4h 30m
  - 12 million wells
  - ~800Mbp (covers 96 flu viruses at 600x)
ION Torrent Results

Fast Direct Detection

DNA $\rightarrow$ Ions $\rightarrow$ Sequence
- Nucleotides flow sequentially over Ion semiconductor chip
- One sensor per well per sequencing reaction
- Direct detection of natural DNA extension
- Millions of sequencing reactions per chip
- Fast cycle time, real time detection
## Sample Prep Comparison

<table>
<thead>
<tr>
<th>Prep Name</th>
<th>Advantages</th>
<th>Disadvantages</th>
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<tbody>
<tr>
<td>SISPA</td>
<td>inexpensive, can start from RNA or DNA, 288 barcodes currently available, can be used for viral discovery, compatible with any sequencing technology</td>
<td>Barcode can bias amplification, potential creation of chimeras</td>
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<tr>
<td>Nextera</td>
<td>low input (1ng, XT kit), very robust and reproducible, 192 dual index barcodes currently available</td>
<td>You must start from dsDNA, expensive, only compatible with Illumina, ends will always be missing</td>
</tr>
<tr>
<td>Ion Torrent IonXpress Plus Fragment Library Kit</td>
<td>very robust and reproducible, 384 barcodes currently available</td>
<td>You must start from dsDNA, expensive, only compatible with Ion Torrent sequencing</td>
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Questions?

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