**NGSC Quick Start**

### Sequencers

<table>
<thead>
<tr>
<th>Model</th>
<th>Reads or read-pairs / lane</th>
</tr>
</thead>
<tbody>
<tr>
<td>new hiSeq 2500</td>
<td>$250 \times 10^6$</td>
</tr>
<tr>
<td>NextSeq 500</td>
<td>$130, 400 \times 10^6$</td>
</tr>
<tr>
<td>miSeq</td>
<td>$1, 4, 20 \times 10^6$</td>
</tr>
<tr>
<td>old hiSeq 2500</td>
<td>$150 \times 10^6$</td>
</tr>
<tr>
<td>hiSeq 2000</td>
<td>$200 \times 10^6$</td>
</tr>
</tbody>
</table>

### Other Equipment

- Covaris LE220 sonicator
- Agilent Bravo liquid handler
- Fluidigm C1 Single Cell RNA-Seq or Whole-Genome Seq

### Bringing In Samples

1. Make sure you have a confirmed investigation set up first.
2. Make sure your samples meet our concentration and volume requirements.
3. Check calendar to see when we can accept samples.
4. Bring in all of the sample you have. We will return what we do not use.
5. Do not predilute.
6. Read further in this document for more info.

### Scheduling Sequencing

The miSeq and NextSeq 500 serve one sample or pool at a time. We run them as often as we can.

A hiSeq2500 has two independent lanes, so requires one or two samples to run. They run often as possible.

The hiSeq2000 sequencers sequence eight lanes and we put together sets of eight lanes to fill the sequencers.

You do not have to have eight lanes worth to sequence with us.

### For Locals

- We are located on the 12th floor of the Smilow Center (see below).
- See the map above.
- Check the Lab Calendar at the website for samples drop off times

### For Distant Collaborators

- Ship **overnight** in **plenty of dry ice**.
- Do **not** ship on Friday!
- The shipping address is:
  
  Room 12-160  
  Smilow Center for Translational Research  
  3400 Civic Center Blvd Bldg 421  
  Philadelphia, PA 19104-5156
When Will My Sample Be Sequenced?

Check the sample queue to see how your samples fit into all the samples we have.

Check the Runs Calendar to see if we have scheduled it for sequencing and/or when the first open slot is.

Sample Volume and Concentration

**RNA-Seq**  As follows:

<table>
<thead>
<tr>
<th>Library Prep</th>
<th>RNA Conc</th>
<th>Volume</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Illumina TruSeq Total stranded</td>
<td>50-100ng/µl</td>
<td>10 µl</td>
<td></td>
</tr>
<tr>
<td>Illumina TruSeq mRNA stranded</td>
<td>50-100ng/µl</td>
<td>10 µl</td>
<td></td>
</tr>
</tbody>
</table>

**RNA to cDNA Amplification**

| NuGEN Ovation RNA-Seq System V2       | 0.1-1ng/µl | 10 µl  |       |
| Clontech SMARTer Ultra Low Input RNA Kit V3 | 20pg-1ng/µl | 10 µl  |       |

Notes:

- We will use RNA for QC, library prep, and return unused portion.
- If you cannot meet above requirements, please contact NGSC to discuss different options.
- It is very important to use high-quality RNA as the starting material.
- **If your concentrations are not in the appropriate ranges we will have to charge extra for dilutions.**
  - Illumina recommends that you check total RNA integrity following isolation using an Agilent Technologies 2100 Bioanalyzer with an **RNA Integrity Number (RIN) value greater than or equal to 8**.
  - RNA that has DNA contamination will result in an under-estimation of the amount of RNA used and reads from genomic DNA.
  - Illumina recommends including a DNase step with the RNA isolation method.
  - Use of degraded RNA can result in low yield, over-representation of the 3’ ends of the RNA molecules, or failure of the protocol.

**RNA-Extraction Kits**

The following kits from **QIAGEN** worked well have worked well for ‘normal’ amounts of cells.

- miRNeasy Mini Kit (217004) For purification of microRNA and total RNA from tissues and cells
- RNaseasy Plus Mini Kit (74134) For purification of up to 100 µg total RNA from cells/tissues using gDNA Eliminator columns

See this or a tool for the appropriate kit selection


and here for a list of products:


These kits from **Applied Biosystem** have worked well for very small cell counts.

- PicoPure RNA Isolation Kit (KIT0204)
  - PicoPure RNA Isolation Kit is designed to recover high-quality total RNA consistently from fewer than ten cells, even from a single cell.

See here for a list of products:

**Accepting New Samples**

**Before You Bring Samples**

1. Check Progress // My Investigations to make sure we have a condition and assay for each of your samples.
2. If not, either begin a New Experiment or Request a New Condition or Assay
3. Check the guidelines to make sure you have enough material to bring us.
4. Plan your barcodes so that they are compatible.
5. Think about using source IDs to link samples together for automatic analysis.
6. We always do QC and confer with you before sequencing.
7. Generally, do not dilute samples before bringing them in.
Check List For Loading Samples

1. What QC will the NGSC perform?
   NGSC to skip these QC steps. qubit bioAnalyzer Kapa

2. Does a sample require custom sequencing primers?
   A custom primer is anything other than the standard truSeq primer. custom primers may be used for custom library types or for skipping over fixed sequence in the insert. some custom primers are older, non-standard Solexa primers, e.g., SSP1. in many cases, clients need to provide a vial of the primers if we do not have some.
   Samples need custom primers? yes no
   Core has possession of the right primers? yes no
   Client will give the core the right primers? yes no

3. Are the barcodes in normal sense or reverse complement?
   Many protocols inadvertently specify the barcodes in reverse complement by giving the oligos that need to be synthesized. The data we actually sequence is the reverse complement of the synthesized oligos. Give the barcode number as well as sequence.
   Barcodes are correct? yes no

4. Are you using source IDs?
   If we are only generating FASTQ files, you do not need to use source IDs. source IDs connect samples back to biological and/or technical replicates so that we can perform automatic data analysis. For example, if you ChIP for H3K4me3 and provide an input sample from the same mouse, then use the same source ID for both libraries. The same logic applies across assays, for example when combining RNA-Seq and ChIP-Seq or the mRNA and miRNA components of an Ago HITS-CLIP experiment.
   Client is using source IDs? yes no

5. How to pool samples?
   Put text like POOL A in the comments for all samples that will go in a pool together. continue with POOL B, etc. for other pools. The pool name will be the first word after pool. if you need to pool across investigations, then put an exclamation mark at the end of the pool name, e.g., POOL MyBigPool!
   The NGSC/FGC will do pooling? yes no
   Pools are indicated? yes no

6. Are the barcodes compatible for the samples in a pool?
   For best barcode decoding, follow Illumina’s rules to make sure you have either a A or C and a G or T in each position. Also it is good to make sure that barcodes differ by at least two positions to ensure optimal read counts.
   Barcodes are compatible? yes no

7. Where are the barcodes located in the library?
   If the barcodes are located in the adapter(s) then the samples can be demultiplexed in the usual manner. If the barcodes are located at the 5 or 3 end of the insert, then demultiplexing requires a special process.
   Barcode location? adapter(s) insert

8. Are the pools premixed?
   Premixed pools (pools that the core did not make) should be marked as have it as an individual. The virtual samples in premixed pools should be marked as have it in a pool.
   Premixed pools annotated correctly? yes no

9. What sequencing dimensions?
   This should have been specified in the experiment description (and placed in the comment field) but it is good to confirm. dimensions may vary by assay which is not captured by the automatic system.
   Sequencing dimensions confirmed? yes no

10. Double Check Everything!!!!

   We did! Investigator NGSC Staff Date