**Experiment outline:**

For general info on the Illumina platform, check out the short informational videos on their website, <http://support.illumina.com/training/online-courses/sequencing.html>.

There are 3 videos worth watching (even if you don’t plan to use TruSeq kits):

Sequencing:Illumina Technology, NextSeq500 Overview, TruSeq:RNA Sample Prep

For an RNAseq experiment, the ultimate goal is to prepare a library of cDNA fragments inserted between adapters of known sequence that facilitate PCR amplification and sequencing on the Illumina FlowCell. Total RNA is prepared from cells or tissues, then a commercially available kit to prepare RNA-Seq libraries is employed. DNA from prepared libraries is carefully sized via agarose gel and quantified via fluorometer in order to calculate molarity. The final concentration of sample library submitted to Brian Golitz should be 15nM.

Multiple samples can be combined per run on the NextSeq (up to 96). To do this, each sample is barcoded with a 6-mer or 8-mer DNA index contained in the adapter DNA. These adapters come standard with most Illumina kits, or can be easily purchased through any standard oligo provider, like IDT or Operon.

**Sample preparation:**

- Prep RNA with Trizol or equivalent method.

- Run 250ng on 2% agarose gel to verify integrity

- 1 to 4ug of RNA per replicate is sufficient for any RNAseq expt.

- once RNA has been obtained, decide on appropriate indices/sample pools and make cDNA library using kit

**Standard RNAseq kits:**

Kapa KK8420 Stranded RNAseq kit with dT beads (need to purchase adapter oligos separately). 24 Samples.

Illumina RS-122-2101 TruSeq Stranded mRNA LT 48 samples, no need to purchase adapter oligos, but need other incidentals, like Superscript II reverse transctiptase.

**Magnetic stand:**

Invitrogen CS15000 MagnaRack™ Magnetic Separation Rack or equivalent (Promega or Biorad, etc. any rack compatable with Dynabeads will work).

**DNA purification beads:**

Gold standard:

Beckman Coulter A63880 Agencourt AMPure XP

Cheaper alternative (no data yet with this product):

VWR M1386-00 MAG BIND RXNPURE PLUS 5ML

**NextSeq 500 reagents:**

|  |  |  |  |
| --- | --- | --- | --- |
| To sequence: | NextSeq Kit name: | Max read output: | Cat #: |
| single-end 150 OR paired-end 75 | NextSeq 500 Mid Output Kit (150 cycles) | 1.30E+08 | FC-404-1001 |
| single-end 150 OR paired-end 75 | NextSeq 500 High Output Kit (150 cycles) | 4.00E+08 | FC-404-1002 |
| paired-end 150 | NextSeq 500 Mid Output Kit (300 cycles) | 1.30E+08 | FC-404-1003 |
| paired-end 150 | NextSeqTM 500 High Output Kit (300 cycles) | 4.00E+08 | FC-404-1004 |
| single-end 75 | NextSeqTM 500 High Output Kit (75 cycles) | 4.00E+08 | FC-404-1005 |

**Other protocols, adapter sequences, information on our lab webpage (including quotes for ordering RNA-Seq kits and NextSeq500 reagents):**

https://www.med.unc.edu/pharm/calabreselab/protocols