Guidelines for sequencing SCC libraries

Libraries are prepared with custom primers that include a 6-base long library index. **Read 1 adapter is on the 5'/cDNA end and reads the gene, and Read 2 adapter is on the 3' barcoded end and reads the BC/UMI**.

General requirements for all Illumina sequencers:

- Give your core facility the average library size (which will be given to you with your final libraries) and make sure the core facility performs library quantification qPCR before run. This will result in optimal cluster generation.
- *Provide the sequencing core with custom primers (for Read 1, Index Read, and Read 2), or check to see if they already have them in stock. Check with the facility to see how much they need.*

Sequencing

Our suggested method for sequencing is the Illumina NextSeq. Users typically pool 10,000 to 30,000 cells on one NextSeq run.

For NextSeq run:

- Use high-yield 75 cycle kit (which comes with 92 cycles)
- 36 cycles on read 1
- 6 cycles on index read
- Remaining 50 cycles on read 2

For diagnostic MiSeq run:

- Use v3 150 cycle kit (all kits comes with +15 cycles for index reads)
- Up to 103 cycles on read 1
- 6 cycles on index read
- 55 cycles on read 2

For HiSeq 2000 run:

- Samples must be multiplexed for this machine, so you are restricted to standard read lengths (be sure to include an index read)
- Use v3 reagents (v4 reagents may be incompatible with our library prep)
- Minimum required reads are Index, 37 cycles R1 and 50 cycles R2. Read 1 can be longer if desired as this end reads into the gene

For HiSeq 2500 run:

- Same as MiSeq