

Guidelines for Sequencing Single Cell 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.
- Do NOT mix these custom primers into the standard Illumina primer mix. This will cause sequencing failure for InDrop libraries. V2 custom primers must be kept separate.

Sequencing

Our suggested method for sequencing is the Illumina NextSeq. Users typically pool 10,000 to 30,000 cells on one NextSeq run. The amount of reads per cell is typically between 50k-100k, this will vary based on cell type and depth required. For loading concentrations please refer to Illumina's guidelines.

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:
 - 37 cycles on read 1
 - 50 cycles on read 2

- 6 cycles on Index read
- Read 1 can be longer if desired as this end reads into the gene

For HiSeq 2500 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

Sequencer	Multiplexing?	Kit	Read1	Index read	Read2
MiSeq	Yes	MiSeq Reagent Kit v3 (150 cycles)	89 cycles	6 cycles	55 cycles
NextSeq	Yes	NextSeq 500/550 High-Output v2 Kit (75 cycles) ^a	37 cycles	6 cycles	51 cycles
NextSeq	No	NextSeq 500/550 High-Output v2 Kit (75 cycles) ^a	43 cycles	0 cycles	51 cycles
HiSeq 2500	Yes	HiSeq SBS Kit v4 (2 × 50 cycles)	61 cycles	6 cycles	51 cycles

^a92-94 cycles can actually be performed using the '75 cycle' Kit.

Table 1. Sequencing parameters for NGS platforms

Frequently Asked Questions

Q: Is the library dual or single index?

A: Single index

Q: What Illumina adapters are used in the 1CellBio protocol?

A: TruSeq Single Indices. There is a document of all the indices on our website called "Primer Sequences for inDrop". You can also refer to the Illumina support document on Page 20.

https://support.illumina.com/content/dam/illumina-support/documents/documentation/chemistry_documentation/experiment-design/illumina-adapter-sequences-100000002694-09.pdf

Q: What percent PhiX spike in should I use?

A: Percent can vary, 1%-3% is typical

Q: What is the concentration of the 1CellBio Custom Sequencing Primers?

A: 100um

Resources

Loading Custom Sequencing Primers

MiSeq

https://support.illumina.com/content/dam/illumina-support/documents/documentation/system_documentation/miseq/miseq-system-custom-primers-guide-15041638-01.pdf

NextSeq

https://support.illumina.com/content/dam/illumina-support/documents/documentation/system_documentation/nextseq/nextseq-custom-primers-guide-15057456-01.pdf

HiSeq

https://support.illumina.com/content/dam/illumina-support/documents/documentation/system_documentation/hiseqkits/hiseq-custom-primers-guide-15061846-03.pdf