

## 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.**

The barcode read must have at least 56 reads to sequence the full barcode.

We do not recommend using a MiSeq as it does not produce enough sequencing depth to generate useful information from single cell libraries.

### General requirements for all Illumina sequencers:

- Before sequencing, all quality control checks should be carried out by sequencing operator.
- Using the Kapa Library Quantification qPCR kit is strongly recommended to ensure proper cluster generation.
- Provide the sequencing core with custom primers (for Read 1, Index Read, and Read 2).
- When multiplexing libraries, provide your sequencing operator the sequences for the inDrop indices used and follow Illumina multiplexing guidelines.
- Illumina recommends using the NextSeq500/550 for single cell RNA sequencing applications but other instruments, such as the NovaSeq6000, can be used for higher throughput experiments.

### Custom Sequencing Primers

1CellBio's V2 Barcode Hydrogel Microspheres (BHM)s require custom sequencing primers; Custom Read 1, Custom Index Read, and Custom Read 2 (provided by 1CellBio)

If spiking-in PhiX to your run, the custom Read 1 and Read 2 primers can be mixed directly into the respective Illumina primer ports to a final concentration equivalent to the Illumina sequencing primers. The custom index primer can be added to Position 9 on the reagent cartridge.

Alternatively, if PhiX is not being spiked-in, Illumina's guidelines can be followed for using custom primers. Note: PhiX cannot be sequenced when following Illumina's guidelines for custom primer usage.

Our suggested method for sequencing is the Illumina NextSeq. Users typically pool 10,000 to 30,000 cells on one NextSeq run. We recommend paired end sequencing using the NextSeq 500/550 High Output Kit 150 cycles (2x75) for best results. 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.**

Read Format (NextSeq500/550 and NovaSeq 6000):

Read 1: 76b  
Index 1: 6b  
Index 2: 0  
Read 2: 76b

## Frequently Asked Questions

**Q:** Is the library dual or single index?

**A:** Single index, on the i7 end of the library

**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](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, 5-10% is typical

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

**A:** 100um

## Resources

### Loading Custom Sequencing Primers

NextSeq

[https://support.illumina.com/content/dam/illumina-support/documents/documentation/system\\_documentation/nextseq/nextseq-custom-primers-guide-15057456-01.pdf](https://support.illumina.com/content/dam/illumina-support/documents/documentation/system_documentation/nextseq/nextseq-custom-primers-guide-15057456-01.pdf)

Library Quantification qPCR kit

<https://rochesequencingstore.com/catalog/kapa-library-quantification-kit/>

# V2 Library Structure

