

Sample preparation and submission Guideline for Single cell sequencing experiments

Sample Requirement:

1. Cells must be in a single cell suspension and free of debris and cell aggregates.
2. Cell viability should be >70%, lower viability may produce compromised results
3. Cell stock should be between 500-1200 cells/ul in 1.5ml Eppendorf tubes, volume 30-50ul
4. Cells should be re-suspended in PBS+0.04% BSA or complete media,
5. Sample should be delivered on ice with completed sample submission form by 2pm.
6. Please provide information about desired target cell recovery (100-10000 cells/sample) and assay (3'GEX, 5'GEX, ATACseq, TCR/BCR)

How many cells to submit?

10x platform has a capture rate of ~60% so for example if desired target cell recovery is 10,000 cells, then 16,000 cells are loaded into the system. The chart provides information about number of cells required for different target cell recoveries. We also need 10ul cell suspension for QC.

A practical example:

Cell concentration: 600 cells/ul
Target cell recovery: 5,000 cells

In this case we will need 10ul of cell suspension for counting and QC (6,000 cells) and 13.8 ul of cell suspension will be loaded into the Chromium system (8,280 cells). We will need minimum 23.8ul cell suspension (14,280 cells). We request at least double the amount of minimum required to make sure we have enough cell to re-load if there was an issue with chromium run. For example, in this case we will request 50ul cell suspension or 30,000 cells.

Step 1 GEM Generation & Barcoding

Cell Suspension Volume Calculator Table
(for step 1.2 of Chromium Next GEM Single Cell 3' v3.1 protocol)

Volume of Cell Suspension Stock per reaction (µl) | Volume of Nuclease-free Water per reaction (µl)

Cell Stock Concentration (Cells/µl)	Targeted Cell Recovery										
	500	1000	2000	3000	4000	5000	6000	7000	8000	9000	10000
100	8.3 35.0	16.5 26.7	33.0 10.2	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
200	4.1 39.1	8.3 35.0	16.5 26.7	24.8 18.5	33.0 10.2	41.3 2.0	n/a	n/a	n/a	n/a	n/a
300	2.8 40.5	5.5 37.7	11.0 32.2	16.5 26.7	22.0 21.2	27.5 15.7	33.0 10.2	38.5 4.7	n/a	n/a	n/a
400	2.1 41.1	4.1 39.1	8.3 35.0	12.4 30.8	16.5 26.7	20.6 22.6	24.8 18.5	28.9 14.3	33.0 10.2	37.1 6.1	41.3 2.0
500	1.7 41.6	3.3 39.9	6.6 36.6	9.9 33.3	13.2 30.0	16.5 26.7	19.8 23.4	23.1 20.1	26.4 16.8	29.7 13.5	33.0 10.2
600	1.4 41.8	2.8 40.5	5.5 37.7	8.3 35.0	11.0 32.2	13.8 29.5	16.5 26.7	19.3 24.0	22.0 21.2	24.8 18.5	27.5 15.7
700	1.2 42.0	2.4 40.8	4.7 38.5	7.1 36.1	9.4 33.8	11.8 31.4	14.1 29.1	16.5 26.7	18.9 24.3	21.2 22.0	23.6 19.6
800	1.0 42.2	2.1 41.1	4.1 39.1	6.2 37.0	8.3 35.0	10.3 32.9	12.4 30.8	14.4 28.8	16.5 26.7	18.6 24.6	20.6 22.6
900	0.9 42.3	1.8 41.4	3.7 39.5	5.5 37.7	7.3 35.9	9.2 34.0	11.0 32.2	12.8 30.4	14.7 28.5	16.5 26.7	18.3 24.9
1000	0.8 42.4	1.7 41.4	3.3 39.9	5.0 38.3	6.6 36.6	8.3 35.0	9.9 33.3	11.4 31.7	13.2 30.0	14.9 28.4	16.5 26.7
1100	0.8 42.5	1.5 41.7	3.0 40.2	4.5 38.7	6.0 37.2	7.5 35.7	9.0 34.2	10.5 32.7	12.0 31.2	13.5 29.7	15.0 28.2
1200	0.7 42.5	1.4 41.8	2.8 40.5	4.1 39.1	5.5 37.7	6.9 36.3	8.3 35.0	9.6 33.4	11.0 32.2	12.4 30.8	13.8 29.5
1300	0.6 42.6	1.3 41.9	2.5 40.7	3.8 39.4	5.1 38.1	6.3 36.9	7.6 35.6	8.9 34.3	10.2 33.0	11.4 31.8	12.7 30.5
1400	0.6 42.6	1.2 42.0	2.4 40.8	3.5 39.7	4.7 38.5	5.9 37.3	7.1 36.1	8.3 35.0	9.4 33.8	10.6 32.6	11.8 29.5
1500	0.6 42.7	1.1 42.1	2.2 41.0	3.3 39.9	4.4 38.8	5.5 37.7	6.6 36.6	7.7 35.5	8.8 34.4	9.9 33.3	11.0 32.2