High Throughput Sample Preparation for Single Cell RNA Sequencing

Overview

Chromium Single Cell Gene Expression solutions enable the generation of dual index libraries to study gene expression profiles, cell surface protein expression, and/or CRISPR screening in million-cell experiments. Single cell samples may be multiplexed with the 10x Genomics 3' CellPlex Kit, which provides a species agnostic sample multiplexing solution through the use of a set of 12 Feature Barcode oligonucleotides each conjugated to a lipid.

This protocol provides guidance for preparing samples for Cell Multiplexing Oligo labeling in a high-throughput, plate based format. High throughput formats enable sample labeling at scales more appropriate for applications such as drug and CRISPR screening. Prior experience working with 96-well plates is highly encouraged for this protocol. Pilot experiments are advised to ensure minimal cell loss at each wash step.

Additional Guidance

Consult Demonstrated Protocol Cell Preparation Guide (Document CG000053) for Tips & Best Practices on handling cells and Technical Note Guidelines on Accurate Target Cell Counts (Document CG000091) for determining accurate cell counts.

Cells carry potentially hazardous pathogens. Follow material supplier recommendations and local laboratory procedures and regulations for the safe handling, storage and disposal of biological materials.

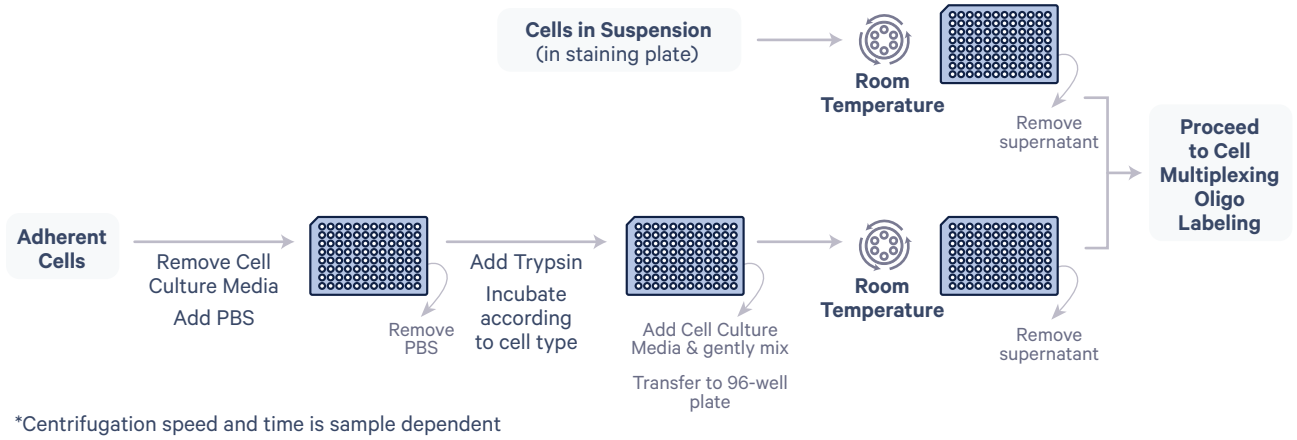
Preparation – Buffer

- Chilled (4°C): PBS + 1% BSA

Specific Reagents and Consumables

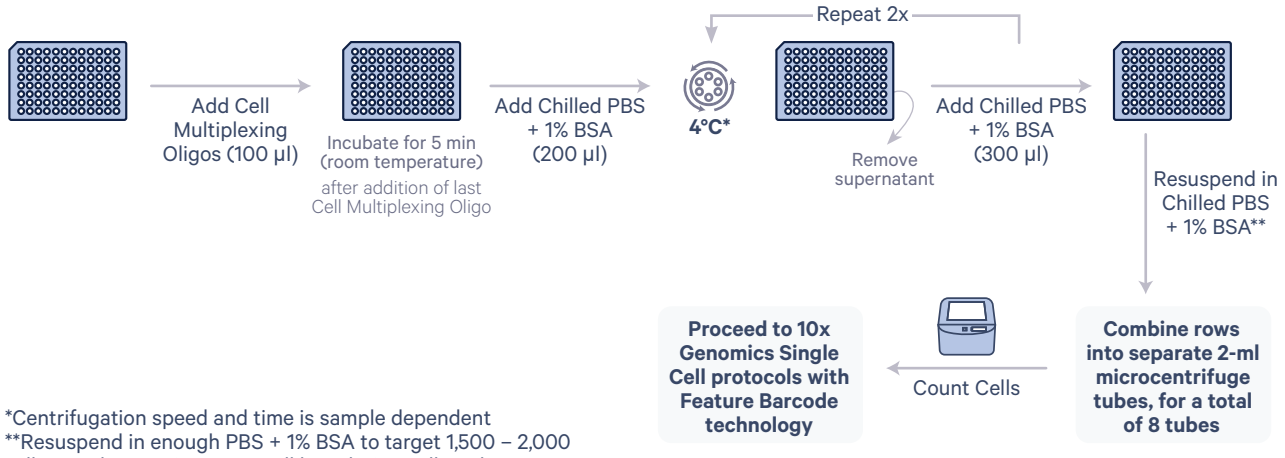
Vendor	Item	Part Number
10x Genomics	3' CellPlex Kit Set A	1000261
Greiner Bio-One	Microplate, 96 well, pp, v-bottom, (chimney well), natural	651201
Thermo Fisher Scientific	UltraPure Bovine Serum Albumin (BSA, 50 mg/ml)	AM2616
	Trypan Blue Stain (0.4%)	T10282
	Fetal Bovine Serum, qualified, heat inactivated	16140071
	Foxx Life Sciences Vactrap™ Vacuum Trap System	601351712
	Trypsin-EDTA	25200056
Corning	Phosphate-Buffered Saline, 1X without Calcium and Magensium	21-040-CV
	Costar Assay Plate, 96 Well, Clear Round Bottom, With Lid, Non-treated Polystyrene	3788
Millipore Sigma	Chemical Duty Pump, 115 V/60 Hz	WP6111560
	Bovine Serum Albumin	A1595
SP Bel-Art	Flowmi 40 micron cell strainers for 1000 microliter pipette tips	13680-0040

Cell Preparation



*Centrifugation speed and time is sample dependent

Cell Multiplexing Oligo Labeling



*Centrifugation speed and time is sample dependent
**Resuspend in enough PBS + 1% BSA to target 1,500 – 2,000 cells per µl, assuming ~20% cell loss during cell washes.

Cell Preparation & Sourcing

All cells were acquired from AllCells, ATCC, DLS, C&M Lab Pro, and iQ Biosciences. 3' CellPlex Kit Set A (PN-1000261) was used for cell multiplexing. Cell Multiplexing Oligos are supplied at **-20°C**.

High Throughput Cell Multiplexing Oligo Labeling Protocol

Prepare Cell Multiplexing Oligo Plate:
Before use, thaw Cell Multiplexing Oligo at **room temperature**. Vortex **5 sec** at maximum speed and centrifuge briefly for **5 sec**. The 3' CellPlex Kit contains 12 Cell Multiplexing Oligos, one for each column of a 96-well plate. Two 3' CellPlex Kits are required for an entire 96-well plate. If a full plate is not ncessary, distribute Cell Multiplexing Oligos such that each sample in a given row has a different Cell Multiplexing Oligo.

Dispense **105 µl** of Cell Multiplexing Oligo into its appropriate column according to the image below.

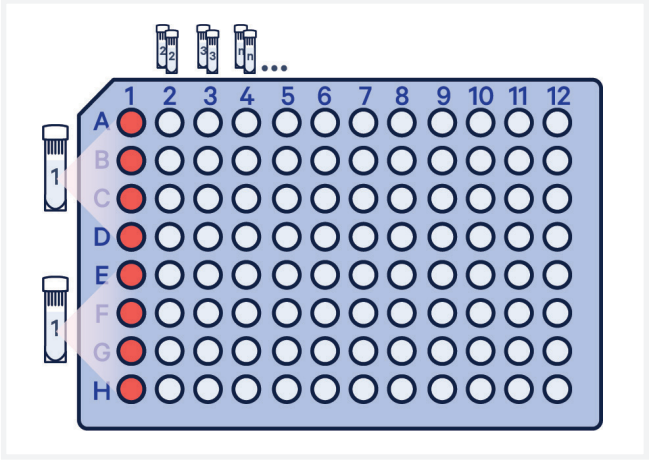


Figure 1. Cell Multiplexing Oligos are distributed in columns (two tubes per column) in the Cell Multiplexing Oligo plate.

Seal plate with a plate sealer and leave at **room temperature** until use.

Plate should be prepared shortly before use.

TIPS When using the vacuum pump, place the 96-well plate at a 45° angle and touch the side walls with the pipette tip DO NOT touch the pellet. Remove all supernatant.

Cells:
This protocol was demonstrated using 0.1 – 2 x 10^5 cells per well. Use 0.1 – 2 x 10^5 cells per well for cells in suspension, or 0.1 – 1 x 10^5 cells per well for adherent cells.

a. Transfer cells to a 96-well plate, with the same number of cells in each well. If transferring adherent cells, wash cells 1x with PBS, trypsinize, add cell culture media, and pipette mix 7-10x. Transfer cell suspension into a v or round-bottom staining plate.

b. Centrifuge cells at **room temperature.** Use of a swinging-bucket rotor is recommended for higher cell recovery. Centrifugation speed and time depends upon the sample type.

c. Remove the supernatant using a multichannel vacuum pump without disturbing the pellet.

d. Using a multichannel pipette, add **100 µl** Cell Multiplexing Oligo (**room temperature**) from the Cell Multiplexing Oligo plate to the samples. Gently pipette mix 10 – 15x to resuspend.

e. After resuspending the last samples with Cell Multiplexing Oligos, incubate for **5 min** at **room temperature**.

f. Wash by adding **200 µl** PBS + 1% BSA to the samples. Gently pipette mix and briefly centrifuge at **4°C**.

g. Remove supernatant with a multichannel vacuum pump.

h. Add **300 µl** PBS + 1% BSA to the samples. Gently pipette mix and briefly centrifuge.

i. Remove supernatant with a multichannel vacuum pump.

j. **Repeat h-i** for a total of two washes.

k. Resuspend in enough PBS + 1% BSA to target 1,500 – 2,000 cells per µl, assuming ~20% cell loss during cell washes. To avoid an over-diluted sample, start with a lower volume of PBS + 1% BSA. The final volume can be adjusted after sample pooling.

l. Combine* each row into a separate 2-ml microcentrifuge tube. Filter pooled sample with a 30-40 µm filter to avoid chip clogs.

*Pool samples based on starting cell counts. If cell counts between samples are the same, pool equal volumes.

m. Determine cell concentration and viability of the pooled sample using a Countess II Automated Cell counter or hemocytometer. Adjust volume if necessary.

n. Proceed immediately to relevant Chromium Single Cell RNA Sequencing protocols with Feature Barcode technology for Cell Multiplexing (see References).

Appendix

Sample Quality Improvements for Pooled Samples

High quality samples should maximize cell viability and minimize debris and cell aggregates. If necessary, clean up the sample by:

- Flow sorting for viable cells
- Using a dead cell removal kit
- Centrifuge at low speed to remove debris
- Sample filtration

References

The Cell Multiplexing Oligo Labeling protocol outlines labeling cells/nuclei with Cell Multiplexing Oligo for use with:

1. Chromium Next GEM Single Cell 3' Reagent Kits v3.1 (Dual Index) with Feature Barcode technology for Cell Multiplexing User Guide (CG000388)
2. Chromium Next GEM Single Cell 3' Reagent Kits v3.1 (Dual Index) with Feature Barcode technology for CRISPR Screening and Cell Multiplexing User Guide (CG000389)
3. Chromium Next GEM Single Cell 3' HT Reagent Kits v3.1 User Guide with Feature Barcode technology for Cell Multiplexing (CG000419)
4. Chromium Next GEM Single Cell 3' HT Reagent Kits v3.1 User Guide with Feature Barcode technology for Cell Surface Protein and Cell Multiplexing (CG000420)

Document Revision Summary

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