Technical Bulletin

MULTI-seq Lipid Modified Oligos

For Single Cell Analysis

LMO001

Storage Temperature: -20 °C

Product Description

MULTI-seq, multiplexing using lipid-tagged indices, is a novel tool that facilitates multiplex single-cell and single-nucleus RNA sequencing[1]. This technology provides sample barcoding that is compatible with any cell type or nucleus containing an accessible plasma membrane and allows for streamlined, pooled single-cell sample processing. The indices provide data quality advantages over non-indexed analysis methods in the form of doublet identification and retention of data from cells with low RNA content.

MULTI-seq increases the efficiency and decreases the reagent cost of droplet-based single-cell RNA sequencing. Compatible with any droplet generation platform, the indexing system enables users to run and analyze up to 96 barcoded samples simultaneously in an 8-lane droplet platform.

The MULTI-seq Lipid Modified Oligos kit is comprised of a lignoceric amide-modified anchor DNA oligo solution and a palmitic amide-modified co-anchor DNA oligo solution. Together, these lipid-modified oligos embed into cell or nuclei membranes and provide a landing pad for DNA barcodes with a complementary 5′ sequence. The MULTI-seq Lipid Modified Oligos kit is intended for upstream sample preparation only, before pooling and single cell analysis.

NOTE: Unique barcode oligos are not included and must be purchased separately.

Barcode 3′ oligo design with poly-A tail for mRNA enrichment[1]:
5′-CCTTGGCACCCGAGAATTCCA-8-**base index**-A30-3′

Barcode oligo design for 5′ cDNA library synthesis:
5′-CCTTGGCACCCGAGAATTCCA-8-**base index**-CCCATATAAGAAA-3′

Custom barcode oligos may be ordered from SigmaAldrich.com/nextgenoligos. For minimal cross-contamination of barcode primers, order Next-Gen Sequencing Oligos with NGSO-Silver or NGSO-Gold quality.

Reagents Provided

- **LMO001A** Lignoceric Anchor with DNA Oligo
- **LMO001B** Palmitic Co-anchor with DNA Oligo

Each lipid-modified oligo is supplied at a concentration of 50 µM in water.

Materials and reagents required but not provided

- Trypsin (product number T2601, T2605, or T2610)
- PBS (-), calcium and magnesium free PBS (product number D8537)
- BSA (product number A4737, A3983, or similar)
- Barcodes, 50 µM (custom ordered, NGSO-Silver or NGSO-Gold quality)
- Microcentrifuge and PCR tubes

Precautions and Disclaimer

This product is for R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Store at -20 °C. At the supplied concentration, MULTI-seq Lipid Modified Oligos are stable for 1 year. Repeated freeze-thaws of MULTI-seq Lipid Modified Oligos are not recommended and may adversely affect functionality. Aliquot and freeze smaller volumes if multiple freeze-thaws are required.

Procedure for Using MULTI-seq Lipid Modified Oligos to Barcode Samples[1]

Preparation of Sample Tissue

1. Culture cells or obtain tissue (~500k or fewer cells per labeling condition).
2. Wash cells with PBS (-) twice.
3. Dissociate or lift cells to obtain single cell suspension (will vary depending on sample type). **Note:** Do not over-trypsinize cells for adherent cells. Over-trypsinized cells may break down during droplet generation after LMO labeling.
4. If cells are adherent, rinse and aspirate on plate.
5. If cells are already in suspension, centrifuge and rinse in tubes.
6. Ensure that cells are resuspended in buffer at single cell suspension prior to addition of barcodes. LMOs partition quickly into cells. Cells must be in suspension prior to labeling or heterogeneous labeling can occur.

Preparation of MULTIseq Lipid Modified Oligos

7. Create a unique Anchor: Barcode solution for each sample by combining 1 µL of the anchor oligo and 1 µL individual barcode (at a concentration of **50 µM**) with 23 µL of PBS, trypsin or desired medium (without FBS or BSA). Place on ice.
8. Create a Co-Anchor solution in the same fashion by combining 1 µL Co-Anchor with 24 µL of PBS, trypsin or desired medium (without FBS or BSA). Place on ice.
9. Prepare 1% (w/v) BSA in PBS (-) and place on ice. User will need 3 mL of 1% BSA per sample.

Barcoding

10. Suspend cells in 180 µL of PBS (or desired buffer without BSA or serum).
11. Note: Avoid FBS or BSA because they sequester LMOs and prevent effective cell labeling.
12. Add 20 µL Anchor: Barcode solution (premixed) and pipette gently to mix.
13. Incubate on ice for 5 minutes.
14. Add 20 µL Co-Anchor solution and pipette gently to mix. Incubate another 5 minutes on ice.
15. Add 1 mL of ice-cold 1% BSA in PBS (-) to the mixture.
16. Transfer each cell sample to its own well of a 96-well round bottom plate or microcentrifuge tube. Place cells on ice immediately after labeling and keep samples on ice for the remainder of the barcoding procedure. Labeling can be done on ice or up to 37 °C, but barcodes are lost more rapidly if cells are maintained at higher temperatures post-labeling.
17. Centrifuge cells at 4 °C. Wash at least 2 times with ice cold 1% BSA in PBS.
18. Combine all samples and filter through cell strainer. Count cells and continue with scRNA-seq procedure according to manufacturer instructions for endogenous transcripts.

References

1. McGinnis, C. S. *et. al*. (2019). Multi-seq: Sample multiplexing for single-cell RNA sequencing using lipid-tagged indices. *Nature Methods*, *16*(7), 619–626.

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