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UDA-Multiome-protocol

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Human Cell Atlas Method …

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We use this protocol and it's working

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Abstract

Droplet microfluidics-based single-cell combinatorial indexing sequencing represents an attractive way to balance cost, scalability, robustness, and accessibility. However, current methods need a tailored protocol for specific modality respectively, which may limit their potential for automation. We introduce UDA-seq, universal droplet microfluidics-based combinatorial indexing for massive-scale single-cell multimodal sequencing. We demonstrate that when necessary, UDA-seq enables effectively generating more than 100,000 single-cell data in a single-channel experiment of droplet microfluidics. Meanwhile, UDA-seq provides a universal workflow for accomplishing several multimodal tasks, including single-cell co-assay of RNA and VDJ, RNA and ATAC, and RNA and CRISPR guide RNA.

Transposition

1 Prepare Transposition Mix

1.1 Prepare Transposition Mix (7ul ATAC Buffer B (2000193); 3ul ATAC Buffer B (2000193) each sample) on ice. Pipette mix 10x and centrifuge briefly.

1.2 Add 10 µl Transposition Mix to a tube of a PCR 8-tube strip for each sample.

1.3 Refer to Nuclei Concentration Guidelines to calculate the volume of Nuclei Stock and Diluted Nuclei Buffer for a total volume of 5 µl.

1.4 Add the calculated volume of Diluted Nuclei Buffer to the Transposition Mix. Pipette mix. Centrifuge briefly.

2 Isothermal Incubation

2.1 Incubate in a thermal cycler using the follows: 30˚C for 30min, 37˚C for 30min, 4˚C hold.

2.2 immediately proceed to the next step。

GEM Generation & Barcoding

3 Prepare Master Mix & Load Chromium Next GEM Chip J

3.1 A certain number of nuclei were added to Master Mix (49.5ul Barcoding Reagent Mix (2000267); 1.9ul Reducing Agent B (2000087); 1.1ul Template Switch Oligo (3000228) and 7.5ul Barcoding Enzyme Mix (2000266/
2000273) each sample) .

3.2 The microfluidic Chromium Next GEM Chip J(2000264) was loaded with 70 µl of cells or nuclei in thermoligation mix (inlet 1), 50 µl of Single Cell Multiome Gel Beads (inlet 2, 10x Genomics catalog no. 2000261) and 45 µl of Partitioning Oil (inlet 3, 10x Genomics catalog no. 2000190) and run on the Chromium system.

3.3 The PCR mix was incubate in a thermomixer to perform enrichment PCR as follows: 37˚C for 45min, 25˚C for 30min, 4˚C hold.

4 Quenching Reaction

4.1 Add 5 µl Quenching Agent to each sample to stop the reaction.

4.2 Slowly pipette mix 10x (pipette set to 90 µl). The solution will be viscous.
Ensure that no liquid remains along the tube sidewalls and pipette tips. If necessary, aspirate the entire volume and dispense back slowly into the tube.

GEM clean-up

5 Cleanup – Dynabeads

5.1 Add 125 µl Recovery Agent to each sample at room temperature.
DO NOT pipette mix or vortex the biphasic mixture. Gently invert tube 10x to mix. Centrifuge briefly.

5.2 Slowly remove and discard 125 µl Recovery Agent/Partitioning Oil (pink) from the bottom of the tube.
DO NOT aspirate any aqueous sample.

5.3 Add 160ul 1x Nuclei buffer to the remaining aqueous phase (to total 230ul), mix well then dispense the liquid evenly into 96-well plate, each well add 2ml.
<u>After brief centrifugation, the products can be stored at −80 °C for at least 2 weeks.</u>

5.4 Add 4ul EB and 1ul of Proteinase K (Tiangen #W9527).
<u>Do not touch the liquid.</u>

5.5 Vortex for 15 sec to mix thoroughly then brief centrifugation, repeat 3X.

5.6 Incubate 55˚C for 5 min.

5.7 Pure the samples with 14ul Dynabeads Cleanup Mix(1365ul Cleanup Buffer(2000088); 97.5ul Dynabeads MyOne SILANE(2000048) and 37.5ul Reducing Agent B(2000087)), vortex for 15 sec to mix thoroughly. Incubate 10 min at room temperature.

5.8 Prepare Elution Solution I(1960ul Buffer EB; 20ul 10% Tween 20 and 20ul Reducing Agent B(2000087)). Vortex and centrifuge briefly.

5.9 At the end of 10 min incubation, brief centrifugation, and then place on a 96-well plate magnet until the solution clears.

5.10 Remove the supernatant.

5.11 Add 150 µl 80% ethanol to the pellet while on the magnet. Wait 30 sec.
Remove the ethanol, repeat 2X

5.12 Centrifuge briefly. Place on the magnet. Remove remaining ethanol.

5.13 Remove from the magnet. Immediately add 14.5 µl Elution Solution I.

5.14 Incubate 1 min at room temperature.

5.15 Place on the magnet until the solution clears.

5.16 Transfer 14 µl sample to a **new 96-well plate**.

6 Cleanup – SPRIselect

6.1 Vortex the SPRIselect reagent until fully resuspended. Add 25.2 µl SPRIselect (1.8x) reagent to each sample. Vortex for 15 sec to mix thoroughly.

6.2 Incubate 5 min at room temperature.

6.3 Centrifuge briefly. Place on the magnet until the solution clears.

6.4 Remove the supernatant.

6.5 Add 150 µl 80% ethanol to the pellet while on the magnet. Wait 30 sec.
Remove the ethanol, repeat 2X

6.6 Centrifuge briefly. Place on the magnet.

6.7 Remove any remaining ethanol.
Residual ethanol can inhibit Pre-Amplification PCR and impact assay performance.

6.8 Remove the tube strip from the magnet. Immediately add 14.5 µl Buffer EB.

6.9 Incubate 2 min at room temperature.

6.10 Centrifuge briefly. Place on the magnet until the solution clears.

6.11 Transfer 14 µl sample to a new tube strip.

Pre-Amplification PCR

7 Index Amplification

7.1 Prepare and add 26ul linear amplification mix(NEBNext UltraII Q5 Master Mix(NEB# M0544S); 0.5uM Truseq-i5 index primer; 0.5uM Partial TSO/IS; 0.5uM P5 primer; 0.5uM Nextare-i7 index primer). Pipette 10X to mix thoroughly. Centrifuge briefly.

7.2 PCR mix was incubate in a thermomixer to perform enrichment PCR as follows: 72˚C for 5min , 98˚C for 3min , and then 7 cycles of [98˚C for 20s, 63 °C for 30s, 72˚C for 1min]; 72˚C for 1min.

7.3 Amplification: half of the PCR product were purified with 1.8x XP beads and elute in N*80 µl buffer EB.

8 **FREE primer removal**

8.1 Prepare and add ***primer removal*** mix(10ul 10x NEBBuffer r3.1; 5ul Thermolabile Exonuclease I(M0568S) and 5ul Exonuclease I (M0293L)) to each tube strip.

8.2 Pipette 10X to mix thoroughly. Centrifuge briefly.

8.3 PCR mix was incubate in a thermomixer to perform enrichment PCR as follows: 37˚C for 30min , 80˚C for 15min.

8.4 PCR product were purified with 1.6x XP beads and elute in 160 µl buffer EB.

ATAC-seq amplification

9 ***ATAC-seq amplification***

9.1 Prepare and add 60ul ATAC-seq amplification PCR Mix(2X KAPA HiFi HotStart Ready Mix; 0.5uM Partial P5; 0.5uM P7 primer) in 40ul Pre-Amplification product.

10 Pipette 10X to mix thoroughly. Centrifuge briefly.

11 Incubate in a thermal cycler with the following protocol. 98˚C for 45s, and then 7-9 cycles of [98˚C for 20s, 67 °C for 30s, 72˚C for 20s]; 72˚C for 1 min.

12 cDNA PCR product were purified with 0.5x-1.4x XP beads and elute in 25.5 µl H2O.

RNA Library Construction

13 **cDNA-biotin amplification**

13.1 Prepare and add **cDNA-biotin** amplification PCR Mix(2X KAPA HiFi HotStart Ready Mix; 0.5uM Partial P5 and 0.5uM Bio-Tso/ISPCR oligo) in 40ul Pre-Amplification product.

13.2 Incubate in a thermal cycler with the following protocol. 98˚C for 3min, and then 5-6 cycles of [98˚C for 15s, 63 °C for 20s, 72˚C for 1min]; 72˚C for 1 min.

14 **Pre-Amplification Cleanup**

14.1 **prepare Dynabead 2× 'Binding and Wash' buffer(10mM** Tris–HCL (pH 7.5); 1mM EDTA and 2M NaCl**)**.
This buffer can be stored at 4 °C for up to 1 month.

14.2 Prepare **Dynabead 1× 'Binding and Wash' buffer** by diluting the 2× solution in a 1:1 ratio with nuclease-free water.

14.3 Use 20µl Dynabeads MyOne Streptavidin C1 (Invitrogen) beads for each sample.

14.4 Wash the beads once with 100µl 2X B&W buffer, re-suspend beads with 100µl 2X B&W buffer.

14.5 Add 100µl re-suspended beads to 100µl PCR product, incubate on rotator at room temperature for 45min~1h. Place the tube on a magnet for 3 min or until the solution clears. Remove the supernatant.

14.6 Add 100µl 1x B&W buffer to wash beads, then add 100µl elution buffer to wash beads again.

15 ***RNA-seq amplification***

15.1 Re-suspend beads with 100ul RNA-seq amplification PCR Mix (2X KAPA HiFi HotStart Ready Mix; 0.5uM Partial P5 and 0.5uM Tso/ISPCR oligo in nuclease-free water)

15.2 Incubate in a thermal cycler with the following protocol. 98˚C for 3min, and then 4 cycles of [98˚C for 15s, 63 °C for 20s, 72˚C for 1min]; 72˚C for 1 min.

15.3 cDNA PCR product were purified with 0.6x XP beads and elute in 40.5µl H2O.

16 ***Gene Expression (GEX) Library Construction***

16.1 50 ng mass of cDNA products (35µl) were mixed with 15 µl of i7-only TN5 Tagmentation Mix(10ul 5x Reaction Buffer (vazyme L buffer) and 5ul Self-i7 TN5).

16.2 Pipette mix 15x (pipette set to 30 µl) on ice. Centrifuge briefly.

16.3 Incubate in a thermal cycler with the following protocol. 55 ˚C for 15min.

16.4 PCR product were purified with 0.8x XP beads and elute in 40.5 µl EB.

17 ***GEX Sample Index PCR***

17.1 Prepare and add 60ul Sample Index PCR Mix(NEBNext µltra II Q5 Master Mix(NEB# M0544S); 0.5uM Partial P5 and 0.5uM Nextera P7-index).

17.2 Pipette 10X to mix thoroughly. Centrifuge briefly.

17.3 Incubate in a thermal cycler with the following protocol. 72 ˚C for 5 min, 98 ˚C for 45 s, 8-9 cycles of [98 ˚C for 20 s, 60 ˚C for 30 s, 72 ˚C for 1min], 72 ˚C for 5 min in thermocycler.

17.4 PCR product were selected size with 0.6-0.75x XP beads and elute in 25.5 µl EB.