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UDA-5'RNA-protocol

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Yun Li¹

¹Beijing Institute of Genomics



Yun Li

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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.

GEM Generation & Barcoding.

- 1 Prepare Master Mix & Load Chromium Next GEM Chip K
 - 1.1 A certain number of cells or nuclei were added to Master Mix (18.8ul RT Reagent B (2000165); 7.3ul 1.1ul Template Switch Oligo (3000228); 1.9ul Reducing Agent B (2000087) and 8.3ul RT Enzyme C(2000085/2000102) each sample).
 - 1.2 The microfluidic Chromium Next GEM Chip K (2000182) was loaded with 70 µl of cells or nuclei in thermoligation mix (inlet 1), 50 µl of Single Cell VDJ 5' Gel Bead (inlet 2, 10x Genomics catalog no. 1000264/1000267) and 45 µl of Partitioning Oil (inlet 3, 10x Genomics catalog no. 2000190) and run on the Chromium system.
 - 1.3 The PCR mix was incubate in a thermomixer to perform enrichment PCR as follows: 53°C for 45min, 4°C hold.

GEM clean-up

- 2 Cleanup – Dynabeads
 - 2.1 Add 125 µl Recovery Agent to each sample (post GEM-RT incubation) at room temperature. DO NOT pipette mix or vortex the biphasic mixture. Wait 2 min.
 - 2.2 Slowly remove and discard 125 µl Recovery Agent/Partitioning Oil (pink) from the bottom of the tube. DO NOT aspirate any aqueous sample.
 - 2.3 Add 150ul PBS to the remaining aqueous phase, mix well then dispense the liquid evenly into 96-well plate, each well add 2ul.
After brief centrifugation, the products can be stored at -80 °C for at least 2 weeks.
 - 2.4 Incubate 85°C for 5min.
 - 2.5 Add 6ul EB.
 - 2.6 Pure the samples with 16ul Dynabeads Cleanup Mix(40ul Nuclease-free Water; 1465ul Cleanup Buffer(2000088); 64ul Dynabeads MyOne SILANE(2000048) and 40ul Reducing Agent B(2000087), vortex for 15 sec to mix thoroughly.



- 2.7 Incubate 10 min at room temperature.
- 2.8 Prepare Elution Solution I(1960ul Buffer EB; 20ul 10% Tween 20 and 20ul Reducing Agent B(2000087)). Vortex and centrifuge briefly.
- 2.9 At the end of 10 min incubation, place on a on a 96-well plate magnet until the solution clears.
- 2.10 Remove the supernatant.
- 2.11 Add 300 μ l 80% ethanol to the pellet while on the magnet. Wait 30 sec.
- 2.12 Remove the ethanol.
- 2.13 Add 200 μ l 80% ethanol to pellet. Wait 30 sec.
- 2.14 Remove the ethanol.
- 2.15 Centrifuge briefly. Remove remaining ethanol. Air dry for 2 min.
- 2.16 Remove from the magnet. Immediately add 17.5 μ l Elution Solution I.
- 2.17 Vortex for 15 sec to mix thoroughly.
If beads still appear clumpy, continue pipette mixing until fully resuspended.
- 2.18 Incubate 1 min at room temperature.
- 2.19 Place on the magnet•Low until the solution clears.
- 2.20 Transfer 17 μ l sample to a Pre-Amplification PCR.



Pre-Amplification PCR

- 3 cDNA index amplification
 - 3.1 Prepare linear amplification mix(2X KAPA HiFi HotStart Ready Mix; 0.5uM Truseq-i5 index primer; 0.5uM Partial TSO/IS and 0.5uM P5 primer).
 - 3.2 Pipette 10X to mix thoroughly. Centrifuge briefly.
 - 3.3 PCR mix was incubate in a thermomixer to perform enrichment PCR as follows: 98°C for 45s , and then 14 cycles of [98°C for 20s, 63 °C for 30s, 72°C for 1min]; 72°C for 1min.
 - 3.4 cDNA PCR product were purified with 0.6x XP beads and elute in 300 ul EB.
 - 3.5 Purified with 0.7x XP beads and elute in 55 ul EB.

5' Gene Expression (GEX) Library Construction

- 4 Fragmentation
 - 4.1 50 ng mass of cDNA products (35ul) were mixed with 15 µl of i7-only TN5 Tagmentation Mix(10ul 5x Reaction Buffer (vazyme L buffer) and 5ul Self-i7 TN5).
 - 4.2 Pipette mix 15x (pipette set to 30 µl) on ice. Centrifuge briefly.
 - 4.3 Incubate in a thermal cycler with the following protocol. 55 °C for 10min.
 - 4.4 PCR product were purified with 0.8x XP beads and elute in 40.5 ul EB.
- 5 GEX Sample Index PCR



- 5.1 Prepare and add Sample Index PCR Mix(NEBNex High-Fidelity 2X PCR Master Mix (NEB#M0541S); 0.5uM Partial P5 and 0.5uM Nextera P7-index).
- 5.2 Pipette mix and centrifuge briefly.
- 5.3 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, storage at 4 °C.
- 5.4 PCR product were selected size with 0.6-0.8x XP beads and elute in 25.5 ul EB.

VDJ Capture

- 6 VDJ capture 1
- 6.1 Place a tube strip on ice and transfer 5 µl cDNA product.
- 6.2 Prepare V(D)J Amplification 1 Reaction Mix(Amp Mix (2000047/2000103); 0.5uM Partial P5 and 0.5uM T/B VDJ outer primer in nuclease-free water) on ice. Vortex and centrifuge briefly.
- 6.3 Add 75 µl V(D)J Amplification 1 Reaction Mix to each tube containing 5 µl sample.
- 6.4 Pipette mix 5x (pipette set to 90 µl). Centrifuge briefly.
- 6.5 Incubate in a thermal cycler with the following protocol. 98 °C for 45 s, 10 cycles for T cells /8 cycles for B cells of [98 °C for 20 s, 62 °C for 30 s, 72 °C for 1min], 72 °C for 1 min in thermocycler, storage at 4 °C.
- 6.6 Store at 4°C for up to 72 h or proceed to the next step.
- 6.7 PCR product were selected size with 0.5X-0.8x XP beads and elute in 30.5 ul EB.



7 VDJ capture 2

- 7.1 Prepare and add 50ul V(D)J Amplification 2 Reaction Mix(Amp Mix (2000047/2000103); 0.5uM Partial P5 and 0.5uM T/B VDJ inner primer) .
- 7.2 Pipette mix 5x (pipette set to 90 µl). Centrifuge briefly.
- 7.3 Incubate in a thermal cycler with the following protocol. 98 °C for 45 s, 8 cycles for T cells /8 cycles for B cells of [98 °C for 20 s, 62 °C for 30 s, 72 °C for 1min], 72 °C for 1 min in thermocycler, storage at 4 °C.
- 7.4 Store at 4°C for up to 72 h or proceed to the next step.
- 7.5 PCR product were selected size with 0.5X-0.8x XP beads and elute in 30.5 ul EB.

VDJ (GEX) LibraryConstruction

8 Fragmentation

- 8.1 50 ng mass of VDJ capture products (35ul) were mixed with 15 µl of i7-only TN5 Tagmentation Mix(10ul 5x Reaction Buffer (vazyme L buffer) and 5ul Self-i7 TN5).
- 8.2 Pipette mix 15x (pipette set to 30 µl) on ice. Centrifuge briefly.
- 8.3 Incubate in a thermal cycler with the following protocol. 55 °C for 5min.
- 8.4 PCR product were purified with 0.8x XP beads and elute in 40.5 ul EB.

9 GEX Sample Index PCR



- 9.1 Prepare and add Sample Index PCR Mix(NEBNex High-Fidelity 2X PCR Master Mix (NEB#M0541S); 0.5uM Partial P5 and 0.5uM Nextera P7-index).
- 9.2 Pipette mix and centrifuge briefly.
- 9.3 Incubate in a thermal cycler with the following protocol. 72 °C for 5 min, 98 °C for 45 s, 8 cycles of [98 °C for 20 s, 60 °C for 30 s, 72 °C for 1min], 72 °C for 5 min in thermocycler, storage at 4 °C.
- 9.4 PCR product were selected size with 0.8x XP beads and elute in 25.5 ul EB.