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aa-Onepot-seq

Dongju Shin[1], JungWon Choi[1], Ji Hyun Lee[2,3], Duhee Bang[1]

[1]Department of Chemistry, Yonsei University, Seoul, Korea;
[2]Department of Clinical Pharmacology and Therapeutics, College of Medicine, Kyung Hee University, Seoul, Korea;
[3]Department of Biomedical Science and Technology, Kyung Hee University, Seoul, Korea

aa-Onepot-seq follows protocol below:
1. Cells and beads preparation and incubation
2. Transfer and Scatter Cell-bead complexes
3. Cell lysis and beads isolation
4. cDNA synthesis
5. cDNA library amplification
6. NGS library preparation

Dongju Shin, JungWon Choi, Ji Hyun Lee, Duhee Bang 2022. aa-Onepot-seq. **protocols.io** https://protocols.io/view/aa-onepot-seq-b7mtrk6n

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This protocol is modified based on Onepot-seq protocol.
https://www.protocols.io/view/onepot-seq-b5u3q6yn

Onepot-seq protocol follows steps below:
1. Cells and beads preparation
2. Scatteration of beads and cells in well
3. Cell lysis and beads isolation
4. cDNA synthesis
5. cDNA library amplification
6. NGS library preparation

aa-Onepot-Seq protocol requires additional steps in step1 and 2 from Onepot-Seq protocol
The subsequent process is same as Onepot-seq protocol.

aa-Onepot-seq follows protocol below:
1. **Cells and beads preparation and incubation**
2. **Transfer and Scatter Cell-bead complexes**
3. Cell lysis and beads isolation
4. cDNA synthesis
5. cDNA library amplification
6. NGS library preparation

Therefore, this protocol describes only the parts added to step 1 and 2.

- Poly T beads (Chemgene, MACOSKO-2011-10(V+))

- PBSB : PBS supplemented with 0.1% BSA

- incubation buffer : 6% Ficoll PM-400, 20mM EDTA, 0.2M Tris pH 7.5

A	B
1ml of Incubation buffer composition	
H20	460 µl
20% Ficoll PM-400 (GE healthcare)	300 µl
500mM EDTA (Life Technologies)	40 µl
1M Tris pH 7.5 (Sigma)	200 µl
Total	1000 µl

- Lysis buffer : 1:1 mixture of incubation buffer and 20% Sarkosyl

- 6X SSC

- TE-SDS : TE buffer + 0.5% SDS

- TE-TW : TE buffer + 0.01% Tween-20

- 10mM Tris pH8.0

- DNase free Water (DW)

- Reverse Transcription mix (RT mix)

A	B
RT mix composition	
H20	80 µl
Maxima 5X RT buffer	40 µl
20% Ficoll PM-400	40 µl
10mM dNTPs	20 µl
100uM TSO	5 µl
RNase inhibitor	5 µl
Maxima H- RTase	10 µl
Total	200 µl

- Exonuclease I mix (Exo I mix)

A	B
Exonuclease mix composition	
10X ExoIbuffer	20 µl
H20	170 µl
ExoI	10 µl
Total	200 µl

- AMPure XP beads

- pluriSelect strainer (20µm)

- 10ml syringe

Cells and beads preparation and incubation

1 Prepare 1X10^6 cells to be stained

2 Add **100 µL** of staining buffer to cell pellet and incubate for **00:05:00** at **4 °C** 5m

30m

3 Add **2 µL** ADT Antibody and incubate for **00:30:00** at **4 °C**

4 Centrifuge and discard staining buffer
(Determine the centrifugation rcf depending on the cell line)

5 Wash twice with **1 mL** PBS

6 Cell count using hemocytometer

7 Dilute cells with PBSB (1,000 cells/µl is recommended)
(PBSB : PBS supplemented with 0.1% BSA)

8 Wash Poly T beads 3 times with **1 mL** of PBSB

9 Suspend beads in PBSB (20,000 beads in **100 µL** PBSB)

10 Add 20,000 beads to 96 well plate

11 Add 10,000 cells to beads and incubate for 1h at 4C
※ In PBMC experiment, considering the ratio of each cell types of PBMC
(Since antibody efficiencies vary by cell type, the number of cells should be adjusted for each experiment)

Transfer and Scatter Cell-bead complexes

12 Transfer **100 µL** of solutions (containing bead-cell complexes) to 20um pluriSelect 20µm strainer
(Check the picture below for strainer and sytinge setting)

13 Wash 3 times with **2 mL** PBSB using syringe
(Pulling piston with low pressure to prevent bead-cell complex dissociation)

14 Invert the strainer and transfer bead-cell complexes to 12 well plate with adding of **1 mL** incubation buffer
(Incubation buffer : 6% Ficoll PM-400, 20mM EDTA, 0.2M Tris pH 7.5)

15 Gently pipette **1 mL** of incubation buffer to spread bead-cell complexes evenly.

16 Wait **00:05:00** to sink the bead-cell complexes down to the bottom 5m

Subsequent process

17 The subsequent process is same as Onepot-seq protocol.