Arming Pulmonary Macrophages for Pathogen Defense Program Project

SOP for cell preparation for ATAC-seq
Version 1.0

I. Objective of the SOP

The objective of this document is to provide detailed guidelines for preparation of cells for ATAC-seq, beginning with lysis of sorted cells.

II. Version History

v1.0 Prepared by Jason Weinstein *et al*. This version is our current stable as of December 2019.

Prior to Transposition:

Make sure your cells are viable! We recommend viability above 90% and preferably around 95%. If sorting use a viability dye as dead cells will add significant background when analyzing final data. Sort cells into tubers containing RPMI with 10% FBS

Buffers and Reagents:

ATAC-RSB

Reagent	Final Concentration	Volume for 50 ml
1M Tris-HCl pH 7.4	10mM	500 ul
5MNaCl	10mM	100 ul
1M $MgCl_2$	3mM	150 ul
Sterile water	NA	49.25 ml

Detergents: All detergents are resuspended as 100x stock solutions

Digitonin - (Promega cat# G9441) Digitonin is supplied at 2% in DMSO. Dilute 1:1 with water to make a 1%(l00x) stock solution. Avoid more than 5 freeze thaw cycles. Can be kept at -20°C for up to 6 months.

Tween- - (Sigma/Roche cat# 11332465001) Tween-20 is supplied at 10%. Use at this concentration (l00x stock). Store at 4°C.

NP40- Sigma/Roche (cat# 11332473001) NP40 is supplied at 10%. Use at this concentration (l00x stock). Store at 4°C.

Omni-ATAC: Optimized Transposition reaction cont.

1. Pellet 50,000 viable cells at 500 RCF at 4°C for 5 min in a fixed angle centrifuge **or** 5000 RPM if in 1.5mL tube.
2. Aspirate all supernatant, carefully avoiding visible cell pellet, using two pipetting steps (aspirate down to 100 ul with a p1000 pipette and remove final 100 ul with a p200 pipette).
3. Add 50 ul cold ATAC-Resuspension Buffer (RSB) containing 0.1% NP40, 0.1% Tween- 20, and 0.01% Digitonin and pipette up and down 3 times.
4. Incubate on ice for 3 minutes.
5. Wash out lysis with 1 ml of cold ATAC-RSB containing 0.1% Tween-20 but ***NO*** NP40 or digitonin and invert tube 3 times to mix and spin down.
6. Pellet nuclei at 500 RCF for 10 min at 4°C in a fixed angle centrifuge **or** 5000 RPM if in 1.5mL tube.
7. Aspirate all supernatant, carefully avoiding visible cell pellet, using two pipetting steps (aspirate down to 100 ul with a p1000 pipette and remove final 100 ul with a p200 pipette).
8. Resuspend cell pellet in 50 ul of transposition mixture by gently pipetting up and down 6 times. Transposition mix =(25 ul 2x TD buffer, 2.5 ul transposase (lOOnM final), 16.5 ul PBS, 0.5 ul 1% digitonin, 0.5 ul 10% Tween-20, 5 ul H2O). (cat# 20034197 Illumina -small kit does 50 rxn).
9. Incubate reaction at 37°C for 30 minutes in a thermomixer with 1000 RPM mixing **or** if using regular thermal cycler gently pipette up and down ~3 times after 15 min.

   Clean with Qiagen MinElute column and elute in 24uL.

Assemble PCR reaction:

25 uL KAPA HiFi 2x mix (KAPA HiFi HotStart ReadyMix PCR Kit; cat #KK2601/KK2602)

24 uL of Tagmented DNA from step 10

1 uL primer mix (12.5 uM each of AD1 and AD2 from indices listed at the end of the protocol)

PCR for 5 cycles: 98^0C/45s + 5 x (98^0C/15s + 63^0C/30s + 72^0C/30s) + 72^0C/1min

1. Clean with Qiagen MinElute column and elute in 50 uL
2. Thermo magJetNGS cleanup following manufacture protocol (cat #K2821).

Q-PCR library amplification test

1. Number of amplification cycles (**Y**) should be determined and PCR library amplification as follows:

   PCR Mix: 5 uL size-selected DNA + 1 uL SYBR + 1 uL G3306/3307 primers + 3 uL H_2O + 10 uL 2x KAPA HiFi mix

   PCR for 20 cycles: 95^0C/45s + 20 x (95^0C/15s + 63^0C/30s + 72^0C/30s)

Determine # of cycles (Y) to reach center of slope (prior to plateau)

Primers: G3306 5'-AATGATACGGCGACCACCGA-3' and G3307 5'-CAAGCAGAAGACGGCATACGA-3'

2. PCR library amplification as follows:

PCR Mix: 5 uL size-selected DNA + 1 uL G3308/3309 primers + 19 uL H_2O + 25 uL 2x KAPA HiFi mix

PCR for Y cycles (determined in step 13): 98^0C/45s + **Y** x (98^0C/15s + 63^0C/30s + 72^0C/30s) + 72^0C/1min

Primers: G3308 5'- AATGATACGGCGACCACCGAGATCTACA*C-3' and G3309 5'-CAAGCAGAAGACGGCATACGAGA*T-3' with *phosphorothioate bond

3. Clean with MinElute columns and elute with 12 uL **(OK) >>>>>>>**

Better >>>> Clean with 0.9V AMPure Beads

- Add 0.9 vol of Agencourt AMPure XP magnetic beads (Beckman Coulter A63880) to PCR rxns
- Mix and incubate @ RT for 15 min
- Put into magnetic stand, remove and discard supernatant
- Keeping tubes in magnetic stand, wash beads 2x with 80% ethanol
- Remove beads from magnetic stand and dry beads completely (~ 15 min)
- Add 15 uL elution buffer (TE or EB from Qiagen kits) and pipette up-and-down 20-30 times
- Put into magnetic stand
- Carefully collect the supernatant without disturbing the beads

4. QC sample –Bioanalyzer
5. Send for sequencing (150bp pair end)

Ad1_noMX:
Ad2.1_TAAGGCGA
Ad2.2_CGTACTAG
Ad2.3_AGGCAGAA
Ad2.4_TCCTGAGC
Ad2.5_GGACTCCT
Ad2.6_TAGGCATG
Ad2.7_CTCTCTAC
Ad2.8_CAGAGAGG
Ad2.9_GCTACGCT
Ad2.10_CGAGGCTG
Ad2.11_AAGAGGCA
Ad2.12_GTAGAGGA
Ad2.13_GTCGTGAT
Ad2.14_ACCACTGT
Ad2.15_TGGATCTG
Ad2.16_CCGTTTGT
Ad2.17_TGCTGGGT
Ad2.18_GAGGGGTT
Ad2.19_AGGTTGGG
Ad2.20_GTGTGGTG
Ad2.21_TGGGTTTC
Ad2.22_TGGTCACA
Ad2.23_TTGACCCT
Ad2.24_CCACTCCT