Protocol updated from phone conversation with Robert Hamanaka and Angelo Meliton by Claudia Lalancette

BLOOD- ATAC-SEQ from Cryopreserved blood cells

December 2018

Notes:

1. This protocol is based on the Fast ATAC-Seq publication, which was optimized for blood cells. However the original protocol uses flow sorted cells. The modifications below are for processing samples from cryopreserved blood cells frozen in freezing media containing DMSO.
2. This protocol uses digitonin, which is a gentle detergent. The authors note that their protocol may not be ideal for cell lines and other cell types that are more resistant to lysis.
3. Protocol modified according to MWG discussion to incorporate processing of cells for ChIP-Seq along preparation of Fast ATAC libraries.

Buffers and Reagents:

Ice cold HBSS (Hanks Balanced Salt Solution) or PBS

ATAC-RSB (ice cold)

Reagent	Final Concentration	Volume for 50 ml
1M Tris-HCl pH 7.4	10 mM	500 ul
5M NaCl	10 mM	100 ul
1M MgCl2	3 mM	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% (100x) stock solution on the day of the experiment. Avoid more than 5 freeze thaw cycles of your DMSO stocks. Can be kept at -20°C for up to 6 months. (I keep single use aliquots)
Tween-20 – (Sigma/Roche cat# 11332465001) Tween-20 is supplied at 10%. Use at this concentration (100x stock). Store at 4°C.

2x TD Buffer

Reagent	Final Concentration	Volume for 100 ml
1M Tris-HCl pH 7.6	20 mM	2 ml
1M MgCl2	10 mM	1 ml
Dimethyl Formamide	20%	20 ml
Sterile water	NA	Bring up to 100 ml

*Before the addition of DMF, adjust pH to 7.6 with 100% acetic acid

Procedure:

1. Take cryovial out; slowly add 1 volume of HBSS to the frozen cells, and gently pipette with a P1000 until the sample thawed. (Angelo does this on ice)
2. Count cells and take aliquot for FastATAC protocol (Robert/Angelo and I used 60K cells). Process the remains of the cells for ChIP-Seq according to Tiffany's protocol.
3. Bring the volume of the aliquot for FastATAC (from step2) to 1mL with ATAC-RSB containing 0.1% Tween-20.
4. Pellet nuclei at 500 RCF for 10 min at 4°C in a fixed angle centrifuge.
5. 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).
6. Resuspend cell pellet in 50 ul of transposition buffer, pipette 6 times:
   25 ul of 2x TD buffer,
   2.5 ul of TDE1,
   0.5 ul of 1% digitonin,
   16.5 ul of PBS
   5.5 ul H2O
7. Incubate the reactions were incubated at 37°C for 30 minutes in an Eppendorf Thermomixer with agitation at 1000 RPM
8. Purify the DNA using a QIAgen MinElute Reaction Cleanup kit (Cat# 28204) or Zymo kit (Zymo kit is cheap and I like the pointed elution tubes as the wetting surface is less elution volumes can be manipulated without compromising yield). Elute the purified DNA 21 ul elution buffer (10 mM Tris-HCl, pH 8).
9. PCR amplification of the transposed fragments:

   PCR Mix
   2.5 ul 25uM Primer Ad1
   2.5 ul 25 uM Primer Ad2
   2x NEBNext Master Mix 25 ul
   Transposed Sample 20 ul

   Cycling Conditions
   72°C ---5 min
   98°C --- 30 sec
   Then 8 cycles of: 98°C ---10 sec
   63°C--- 30 sec
   72°C--- 1 min
   Hold at 4°

10. Purify the libraries using the double-sided AMPure XP protocol used for ATAC-Seq on 5mo liver samples:
    a. Add 150 µl EB to the PCR reaction, then add 110 µl beads (0.55 x sample volume) and mix 10 times by pipetting.
    b. Incubate 5 min at room temperature. Separate on magnetic stand.

Commented [c1]: I use PBS; cells are usually frozen in 1ml of cryomedia, so I add 1ml of PBS

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c. Transfer supernatant (310 µl) to a new tube.
d. Add 190 µl beads (1.5 x sample volume).
e. Incubate 5 min at room temperature. Separate on magnetic stand.
f. Wash beads 2 times with 200 µl ethanol (80 %).
g. After the second wash, remove ethanol completely and dry beads on a 37°C heat block for ~1min, then resuspend beads in 10 µl EB by pipetting 10 times.
h. Separate on magnetic stand and transfer the final library to a new tube.

11. Quantify final libraries using Qubit HS dsDNA kit, and TapeStation HS D1000 (or Bioanalyzer High Sensitivity chip).
12. Sequence as paired-end libraries. (We pool 4 libraries per lane of a HiSeq 4000)

Commented [CL2]: I always do this step before I do the TapeStation, so that I can dilute the libraries if necessary