BLOOD- ATAC-SOP-Vinesh Vinayachandran SB-LAB JHU

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Prior to Transposition: Make sure your cells are viable!

We recommend viability above 90% and preferably around 95%. If you are using cells directly from culture without fluorescence activated cell sorting enrichment for viable cells, you should try to clean up dead cells by one or more of the
following:

1.For samples with 5-15% dead cells, treat cells in culture medium with **DNase (Worthington cat# LS002007) at a final concentration of 200 U/ml.** Resuspend DNase in Hanks Balanced Salt Solution. DNase needs divalent cations so treat cells in culture media that **lacks EDTA**. Treat for **30 minutes at 37°C.** Wash thoroughly with PBS to remove DNase prior to proceeding to ATAC-seq transposition reaction.

2. For samples with more than 15-20% dead cells, separate viable cells over ficoll (GE cat# 17-1440-02). Make sure Ficoll and centrifuge are at room temp and that the brake has been switched to off. Exact conditions are dependent on cell type and cell number. A standard spin is for 25 minutes at 400 RCF with no brake. Prior to Ficoll, it may help to treat cells with DNase as above.

3. If viability is still a problem, either sort or use a magnetic bead depletion based on Annexin V (Miltenyi cat# 130-090-201).

Buffers and Reagents:
ATAC-RSB

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. Avoid more than 5 freeze thaw cycles. Can be kept at -20°C for up to 6 months. (I keep one use aliquots)

Tween-20 – (Sigma/Roche cat# 11332465001) Tween-20 is supplied at 10%. Use at this concentration (100x stock). Store at 4°C.

NP40 – (Sigma/Roche cat# 11332473001) NP40 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

This protocol has been optimized for blood cells. We note that digitonin is a gentle detergent and this protocol may not be ideal for cell lines and other cell types that are more resistant to lysis. 50,000 cells were pelleted by centrifugation at 500 RCF for 5 minutes at 4C in a pre-cooled fixed-angle centrifuge. All supernatant was removed using two pipetting steps being careful to not disturb the not visible cell pellet.

Wash out with 500 ul PBS if DNase is used and with1 ml of cold ATAC-RSB (to remove DMSO and a buffer exchange for transposition. I saw it better than RPMI media while comparing fragment size distribution (an indicator of efficient transposition), but I never had a direct or deep comparison other than this) containing 0.1% Tween-20 and invert tube 3 times to mix

Pellet nuclei at 500 RCF for 10 min at 4°C in a fixed angle centrifuge.
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).
Resuspend cell pellet in 50 ul of transposition

50 ul transposase mixture 25 ul of 2x TD buffer,

2.5 ul of TDEnzyme-1,

0.5 ul of 1% digitonin,

22 ul of nuclease-free water (I use PBS 16.5ul +5.5 ul HOH instead which can reduce background)

was added to the cells and the pellet was disrupted by pipetting.

Transposition reactions were incubated at 37°C for 30 minutes in an Eppendorf Thermomixer with agitation at 300 RPM I do at 1000 @ 300 RPM with 50ul reaction vol in 1.5 ml tube I don't think it's enough mixing).

Transposed DNA was purified 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) and purified DNA was eluted in 21 ul elution buffer (10 mM Tris-HCl, pH 8) (I do it with warm elution buffer 65^0C). I do check the concentration of elute in Nano-drop (not needed)

Transposed fragments were amplified

Transposed fragments were amplified for 5 cycles, then a 5ul aliquot was used to determine the number of additional cycles to add by qPCR, according to Buenostro et al ATAC-Seq protocol. (but generally, 6-8 cycles are the rule and result, if PCR more face the ire of duplication rate. I do the same for WGBS (100ng DNA)

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°

and purified as described previously

I use SPRI select or Ampure XP for purification (I generally pool 4 samples based on nano-drop or qubit readings. And per sequencing 8 samples (i.e. 4 samples/pool * 2)

Final libraries are again checked for size and quantity using Bioanalyser High sensitivity chip. (this is little tricky you might have to play around with the dilutions of library say ½,1/5, 1/10 etc as high salt may kill you run.

All Fast-ATAC libraries were sequenced using paired-end, dual-index sequencing on a NextSeq.