Nuclei preparation from frozen tissue for ATAC-Seq
(no detergent version)
Crawford lab 9/27/2016

1. Grind small amount (50-100mg) of frozen tissue to fine powder using Cellcrusher (cellcrusher.com) and liquid nitrogen to keep everything frozen. Grinding smaller amounts of tissue is possible, but due to static, smaller amounts of tissue is difficult to remove from the pulverizer. Place tissue sample into the pulverizer, give 5 strokes with hammer and visually check your sample. Continue hammering until the tissue powder looks evenly fine and no more chunks are visible. This will depend on tissue type as some tissues are harder than others.
2. Transfer about 20mg of frozen tissue powder into a chilled 1.5ml tube that is on dry ice. Quickly check weight on precision scale, adjust amount of tissue powder if necessary. Make sure to transfer frozen tissue powder with cooled/frozen spatula that pre-cooled in dry ice. If working with multiple samples keep all tubes on dry ice until you are ready to go to step 3.
3. Add 1mL of ice cold NIB (Nuclei isolation Buffer) buffer with or w/o detergent, suspend by inverting tube 5 times (make sure all tissue flecks are separated) and place on wet ice. Note: removing detergent reduces amount of mitochondrial contamination by 50% (e.g., brain, liver, kidney, lung). However, some tissues or cell types (e.g., fat and some cell types) may require the addition of 1% IGEPAL detergent in NIB buffer in order to achieve optimal signal/noise.
4. Position tube horizontally on wet ice and agitate for 5 min (place ice bucket on orbital shaker at 120 rpm).
5. Filter the solution through Miracloth (Calbiochem) into another 1.5ml tube that is in wet ice. Squeeze fabric to get most of the liquid. Larger debris will remain in the Miracloth

6. Centrifuge at 1,100 x g for 10 min at 4˚C.
7. Remove the supernatant, and keep tube on wet ice until next step.
8. Suspend pellet with 50ul RSB buffer, pipet up and down 5 times using wide bore tip (this is reduce shearing of nuclei; cut off end of p200 pipet tip with scissors or razor blade). Pellet is small might not be visible. Centrifuge at 500x g for 5 min at 4˚C.
9. After removing all the supernatant, place tube back on wet ice. The nuclei pellet is ready for transposase reaction (see below).

NIB***: 20mM Tris-HCl, 50mM EDTA, 5mM Spermidine, 0.15mM Spermine, 0.1% mercaptoethanol, 40% Glycerol, pH 7.5.
RSB: 10mM Tris-HCl, 10mM NaCl, 3mM MgCl2, pH 7.4
*****For liver tissue add 1mM EGTA and 60mM KCl to NIB. We have noticed that not adding EGTA and KCl to NIB during liver preps resulted in poor ATAC-seq results.**

Directions below are from Buenrostro et al., Nature Methods 2013 paper describing ATAC-seq protocol. The only difference is purification using 1X Ampure beads instead of gel purification.

Transposase reaction

Immediately following the nuclei prep, suspend the pellet in the transposase reaction mix (25ul 2X TD Buffer, 2.5ul transposase, 22.5ul nuclease-free water), pipet up and down 5 times using wide bore pipet tip. Incubate 30 minutes at 37C. Directly following transposition, purify sample using a Qiagen MinElute kit.

PCR

PCR conditions: 72C for 5min; 98C for 30s and thermocycling at 98C for 10s, 63C for 30s and 72C for 1min.

PCR cocktail: 1.25ul primer 1 (50uM), 1.25ul primer 2 (50uM), 10ul eluted DNA, 25ul NEB Next PCR mix, 12.5ul H2O

Amplify the library for 5 cycles, take a 5ul aliquot of the PCR reaction (keep the rest at 4C) and add 15ul of the PCR cocktail plus SYBR green at a final concentration of 0.6X. Run this reaction for 20 cycles to determine the additional number of cycles needed for the remaining 45ul reaction.

Purification

Purify library using 1X AMPure beads:

Add 45ul of beads to the PCR product. Mix by pipetting 10 times.

Incubate 10 minutes at Room Temp.

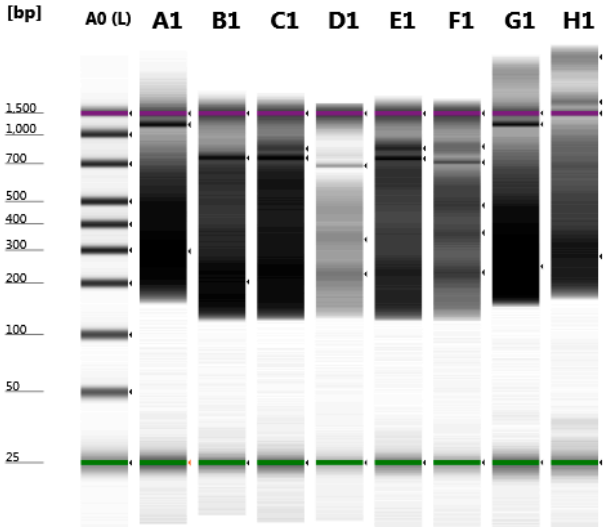
Separate on magnet for 2 minutes. Discard supernatant.

Leaving tubes on magnet wash with 200ul fresh 70% EtOH twice

Elute with 20ul 10mM Tris

Run on tape station or bioanalyzer to determine fragment sizes. Much of the fragment sizes are around 100-500bp in size, but there is usually a substantial amount of DNA fragment sizes that are larger. Note: for tissues, we do not often see typical nucleosome pattern of fragment sizes. This does not necessarily mean that the sample failed.

Example of Tapestation

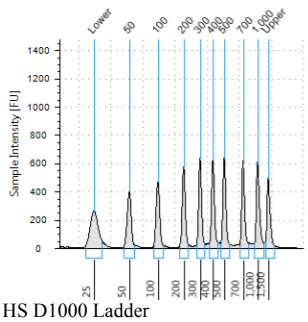


HS D1000
Contrast: 0.50

Sample Info

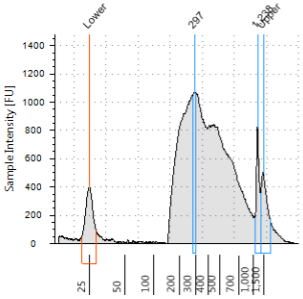
Well	Conc. [pg/µl]	Sample Description	Alert	Observations
A0	2350	HS D1000 Ladder	PCR cycles	Ladder
A1	425	Liver#4	12	ATAC-BD1
B1	733	Liver#42	12	ATAC-BD2
C1	364	Kidney#124	10	ATAC-BD3
D1	206	Lung#6	10	ATAC-BD4
E1	363	Kidney#25	9	ATAC-BD5
F1	773	Lung#46	12	ATAC-BD6
G1	716	Liver#24	11	ATAC-BD7
H1	2200	Kidney#112	10	ATAC-BD8

A0

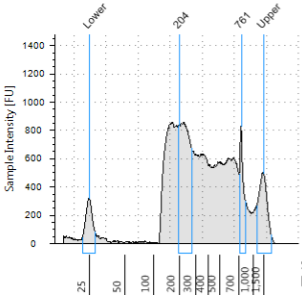


HS D1000 Ladder

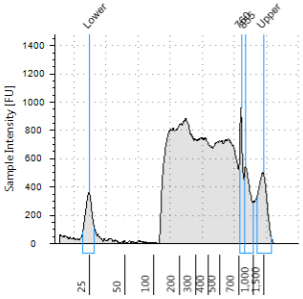
A1



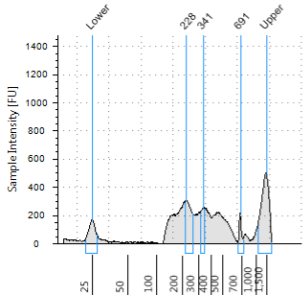
B1



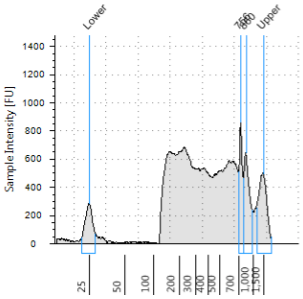
C1



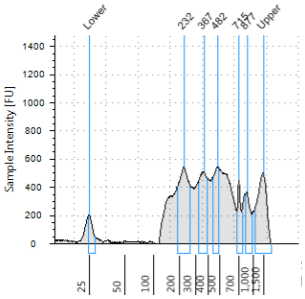
D1



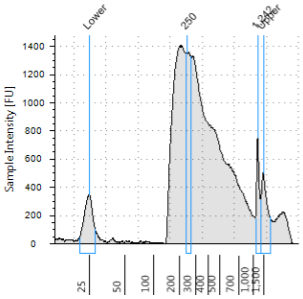
E1



F1



G1



H1