ATAC-Seq protocol for fresh tissue

Wold Lab, Caltech

This protocol follows very closely the protocol from Buenrostro et al. (2015) but has some modifications.


Fresh tissue preparation:

Excise small piece (~20 uL) volume of fresh tissue.

Add 100 uLs ice cold cell lysis buffer:

Cell lysis buffer (10 mLs)
9.67 mLs UltraPure H2O (Life Technologies catalog 10977-015)
100 uLs Tris-HCl pH 7.5 (US Biochemical catalog 22639)
100 uLs 1M NaCl (make from stock and filter sterilize with 0.2 um filter)
30 uLs 1M MgCl2 (US Biochemical catalog 78641)
100 uLs 10% IGEPAL-360 (Sigma catalog I3021) in UltraPure H2O

Attempt to triturate the tissue in lysis buffer with a P200 pipet tip (5-10 strokes).

If this fails, let the tissue sit in the lysis buffer for 5 minutes on ice, then use a 21g needle on a 1 mL syringe and shear the tissue through the needle 5 strokes.

Spin 10 minutes, 2500 RPM on microfuge at 4C.

Decant lysis buffer and immediately add tagmentation reaction.

Tagmentation reaction:

22.5 uLs UP H2O
25 uLs Nextera TD buffer
2.5 uLs Nextera transposome mixture

Incubate for 30 minutes at 37C

Cleanup with Qiagen MinElute Reaction cleanup kit (Catalog# 28204), following the manufacturer’s protocol. At the final step, elute in 21.5 elution buffer (EB), and collect only 20 uLs.
Amplification reaction:
5 uLs N500 primer from Nextera tagmentation kit
5 uLs N700 primer from Nextera tagmentation kit
5 uLs PCR primer cocktail from Nextera tagmentation kit
15 uLs PCR master mix from Nextera tagmentation kit
20 uLs tagedmented DNA eluted from minelute column

Amplify:
Step 1: 5 min 72°C
Step 2: 30 sec 98°C
Step 3: 10 sec 98°C
Step 4: 30 sec 63°C
Step 5: 1 min 72°C.
Return to step 3, 11 more times
Step 6: 10°C indefinitely

SPRI beads cleanup:
Add 60 uLs (1.2X) SPRI beads (AMPURE XP beads)
Cleanup according to manufacturer's protocol
Elute in 31.5 uLs Qiagen Elution Buffer (EB)
Quantify on Qubit
Run 3 ngs on Agilent BioAnalyzer HS DNA chip