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Tagmentation and library generation for human placental bulk ATACseq

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This protocol describes the generation and amplification/indexing of tagmented libraries from freshly isolated nuclei for bulk ATACseq. It is adapted from Buenrostro et al., 2015, PMID: 25559105.

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Equipment:

Eppendorf ThermoMixer
Thermal cycler that will accommodate 96-well plate
Pipettes (including multichannel)
Magnetic stand
Qubit fluorometer
Agilent bioanalyzer

Supplies/reagents:

AMPure XP beads (Beckman Coulter, cat # A63881)
200 proof ethanol
Nuclease-free water
Tagment DNA TDE1 Enzyme (Illumina, cat # 20034197)
MinElute PCR Purification Kit (Qiagen, cat # 28004)
Sodium Acetate Solution, 3M, pH 5.2 (Fisher, cat # FERR1181)
IDT for Illumina Nextera DNA UD Index set (Illumina)
NEBNext High-Fidelity 2X PCR Master Mix (VWR, cat # 102500-096)
LoBind Eppendorf tubes (Eppendorf, cat # 022431021)
Qubit dsDNA HS Assay kit (Fisher, cat # Q32854)
Agilent High Sensitivity DNA Kit (Agilent, cat # 5067-4626)

Preparation

1 Pre-heat Eppendorf ThermoMixer to **37 °C** .

2 Bring AMPure XP beads to room temperature.

3 Prepare 70% ethanol using 200 proof ethanol and nuclease-free water.

Tagmentation and purification

4 Add **0.5 µL Tagment DNA TDE1 Enzyme** to 50,000 freshly prepared nuclei (5,000 nuclei/ul in tagmentation buffer), and mix by pipetting gently.

5 Incubate in Eppendorf ThermoMixer **500 rpm, 37°C, 00:30:00** .

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6 After completion, add **100 µL PB buffer (Qiagen)** and **5 µL 3M Na-acetate, pH 5.2** . Purify using MinElute PCR Purification Kit and elute in **10 µL Buffer EB (Qiagen)** .

This is a safe stopping point. Store tagmented DNA at **-20 °C** until all samples are ready for library construction.

Amplification and indexing 7m 10s

7 Thaw index adapter plate at room temperature, and spin briefly before use. Assemble PCR reactions as follows:

10 µL tagmented DNA

25 µL NEBNext High-Fidelity 2X PCR Master Mix

10 µL IDT for Illumina Nextera DNA UDIs

5 µL nuclease-free water

Each well of the Illumina Nextera DNA UDI 96-well plate is single use and contains a little over 10 ul pre-paired i5/i7 index adapters. The Nextera adapters add 140 bp to the libraries.

8 Perform a limited-cycle amplification of the tagmented DNA: 7m 10s

Step 1: **72 °C** **00:05:00**

Step 2: **98 °C** **00:00:30**

Step 3: **98 °C** **00:00:10**

Step 4: **63 °C** **00:00:30**

Step 5: **72 °C** **00:01:00**

[Repeat steps 3-5 for a total of 8 cycles]

Step 6: **12 °C hold**

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9 After completion, add **250 µL PB buffer** and **10 µL 3M Na-acetate, pH 5.2** . Purify using MinElute PCR Purification Kit and elute in **20 µL Buffer EB** .

Size selection 10m

10 Add **180 µL Buffer EB** and **110 µL AMPure XP beads** (0.55x sample volume) and mix by pipetting. Incubate **Room temperature** **00:05:00** . 5m

11 Separate on magnetic stand, and transfer **300 µL** supernatant to a new tube.

12 Add **250 µL AMPure XP beads** (1.8x sample volume) and mix by pipetting. Incubate **Room temperature** **00:05:00** . 5m

13 Separate on magnetic stand and wash beads twice with **200 µL 70% ethanol** .

14 Resuspend beads in **20 µL Buffer EB** by pipetting.

15 Separate on magnetic stand and transfer **17 µL** of size-selected library to a new tube.

Quality control

16 Quantitate libraries using the Qubit DNA High Sensitivity Assay, and check library distribution by running the DNA High Sensitivity Assay on an Agilent Bioanalyzer. A typical trace is shown below.

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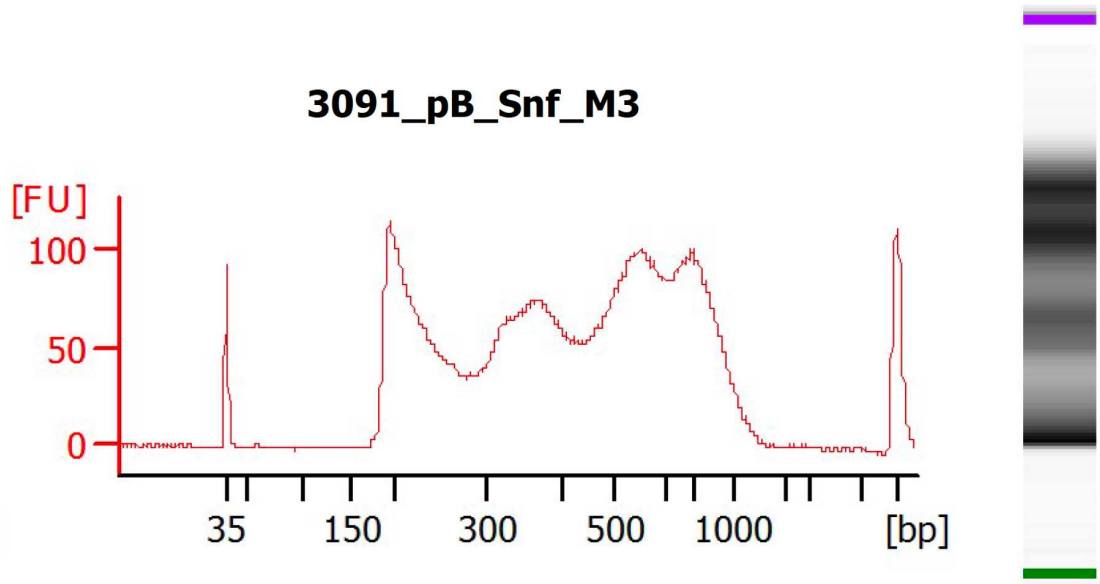

Note the nucleosomal laddering pattern, which corresponds to nucleosome-free, mononucleosomal, dinucleosomal, and trinucleosomal fragments enriched at ~200, 350, 550, 750bp, respectively.

Pay attention to the presence of any primer dimers, which will appear at ~50-80 bp. These can occupy space on the flow cell and reduce the number of useful reads generated from a sequencing run. If primer dimers are present (even at low levels), perform another 1.8x cleanup using AMPure XP beads.

17 If multiplexing samples, first perform a balancing run to ensure equal representation of all samples in the pool. For the balancing run, prepare an "equal volume" pool by combining **2 µL** each library together. Run the pool on a MiSeq instrument using a MiSeq Reagent Kit Nano. Based on the proportion of reads assigned to each index during the Nano run, prepare a balanced pool that will yield an equal read depth for all samples in the pool.

18 Prior to submitting for sequencing, quantitate the pool using the Qubit High Sensitivity DNA assay. Determine the average fragment size for each library from the Bioanalyzer traces.

Tip: set a region from 200bp to 1000bp in the region table tab (fragments over ~1kb don't cluster efficiently on the flow cell) and the Agilent software will calculate the average fragment size.

Determine the average fragment size in the balanced pool, and use the following formula to determine the nM concentration:

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$$\frac{(\text{concentration in ng}/\mu\text{l})}{(660\ \text{g/mol} \times \text{average library size in bp})} \times 10^6 = \text{concentration in nM}$$

Submit the pool to your sequencing facility, noting the nM concentration.

For HuBMAP bulk ATAC-seq samples, the multiplexed pool was sequenced on a NovaSeq 6000 S4 lane using a 100bp paired-end run configuration.

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