ATAC-seq: from experimental design to computational analysis

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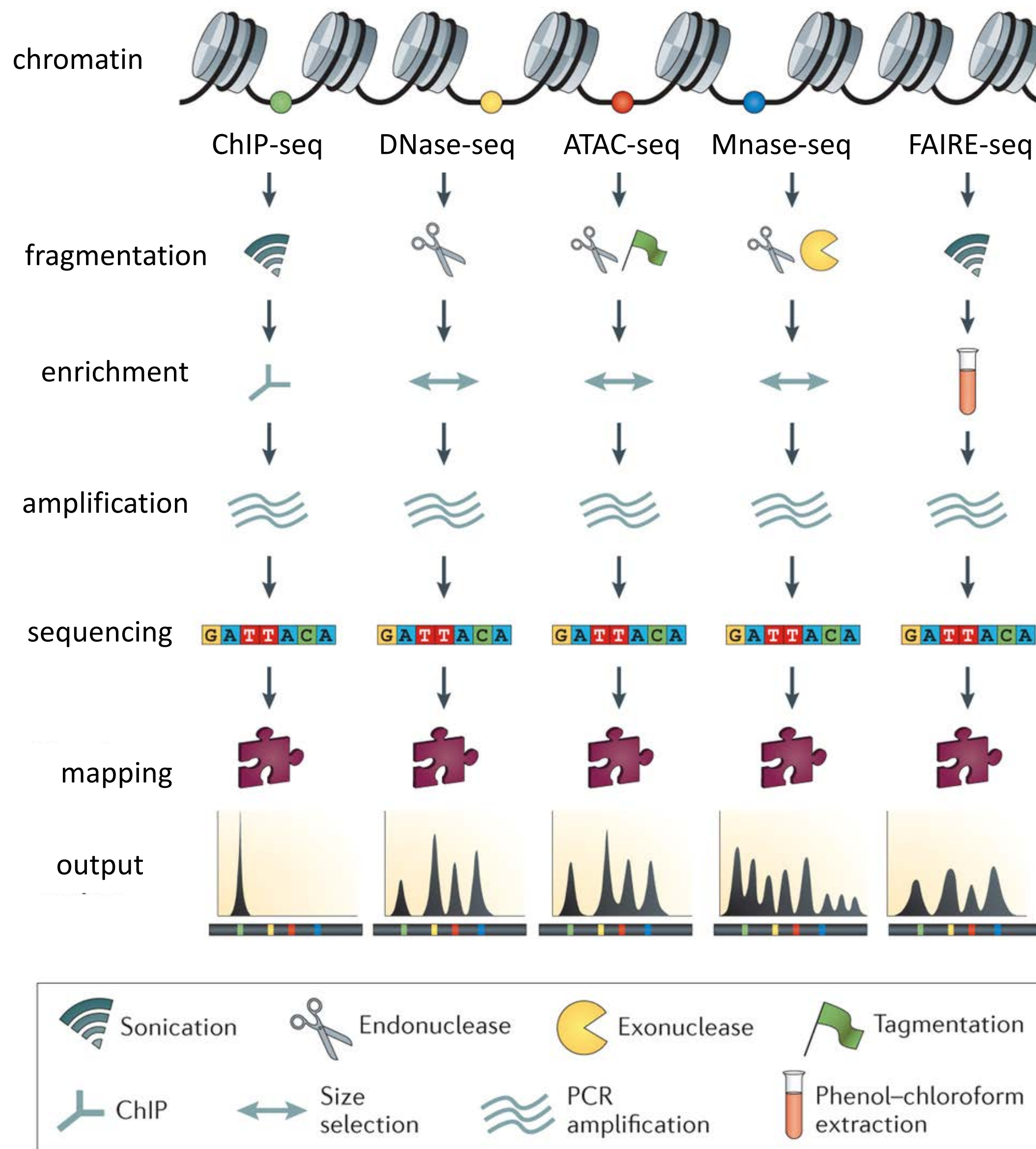
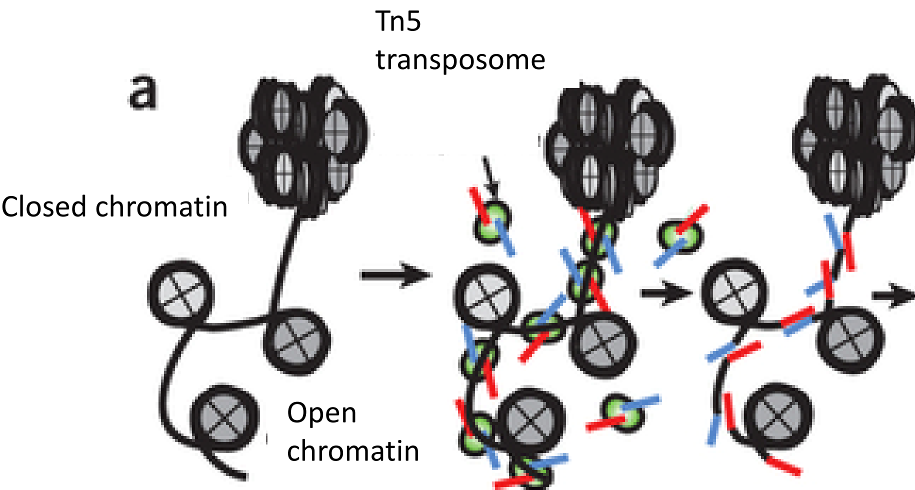
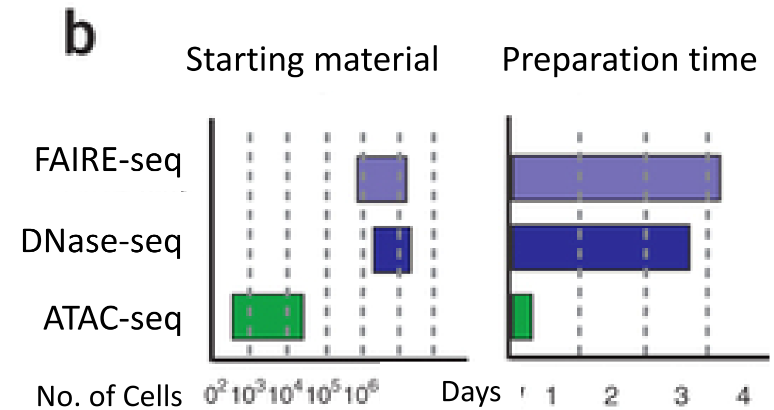


Meyer and Liu, NRG, 2014

Why use Assay for Transposase-Accessible Chromatin (ATAC)-seq





Buenrostro et al., Nature Methods, 2013

ATAC-seq protocol

- Isolation of intact nuclei (Day 1)
- Fragment and tag chromatin (Day 1)
- Amplify library (Day 2)

Isolation of intact nuclei

- Use any method that generates intact nuclei
- Method must be gentle
- Plants:
  - We froze the leaves and gently ground the tissue with mortal and pestle.
  - Remove chloroplasts by repeat washing.
  - Method from Jiang Lab at U. Wisconsin (Hexylene glycol)
- We started with a large amount of tissue (100µl) and performed serial dilutions of nuclei

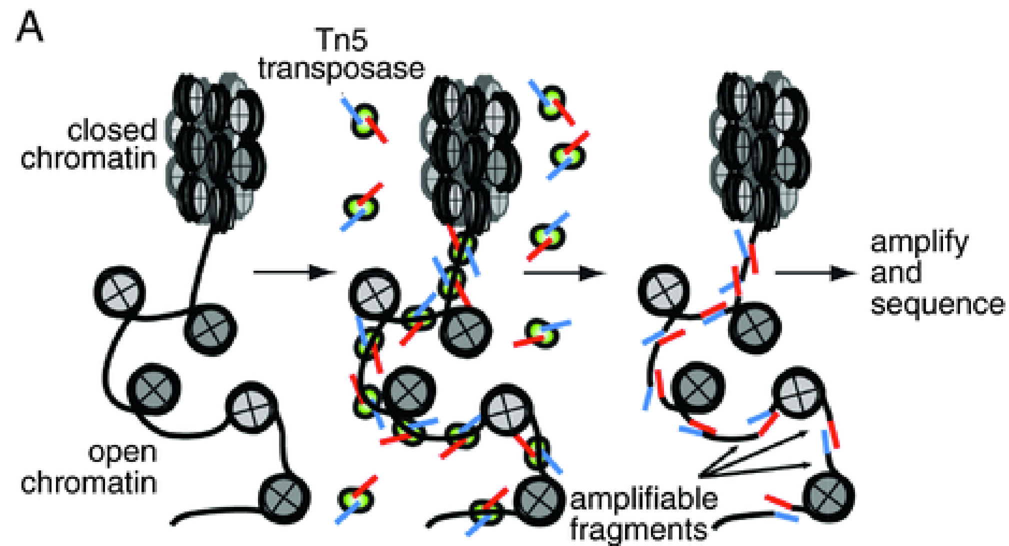
Tagmentation Reaction

- Uses the Nextera DNA Sample Preparation Kit (FC-121-1030)
  - 2X TD Buffer
  - Tn5 Transposase
- The 24 reaction kit can be used to generate around 70 ATAC-seq libraries.
- Or you can make your own: Picelli *et al.* Genome Research, 2014

Tagmentation Reaction

- Starting material 500 – 50,000 nuclei
  - Single cell ATAC-seq method published in July 2015 (Buenrostro *et al.*, Nature, 2015)
- Incubate at 37°C for 30 minutes:
  - Nuclei
  - 25µl 2X TD Buffer
  - 2.5µl Tn5 Transposase
  - 22.5µl water
- Clean with MinElute column
- Can stop here (freeze at -20°C)

Schematic of Tagmentation



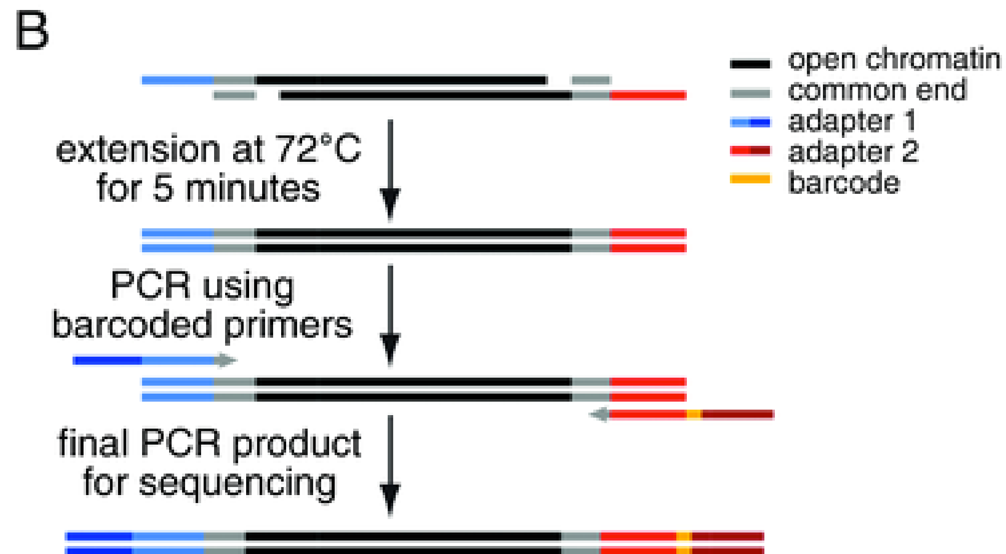
Buenrostro et al., Curr. Prot. Mol. Biol., 2015

PCR amplification of the ATAC-seq library

- Done in two stages:
  1. Preliminary amplification
     1. 5 minute incubation at 72°C to allow extension of both strands.
     2. 5 cycles of amplification

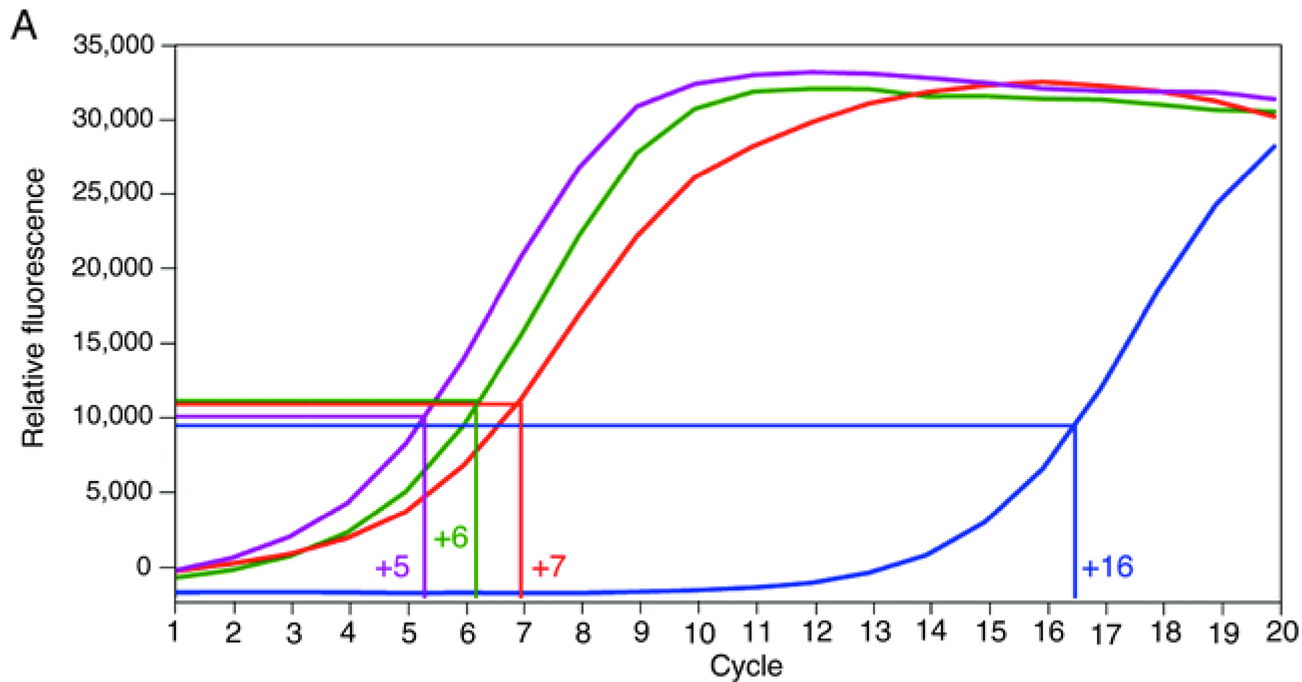
     PAUSE – amplify a subset using qPCR to determine optimal cycle number
  2. Final amplification
     1. Experimentally determined number of additional cycles

PCR Amplify Library



Buenrostro et al., Curr. Prot. Mol. Biol., 2015

qPCR quantification of library



- Identify cycle at which the relative fluorescence is 1/3 maximum fluorescence.
- Add this number of PCR cycles to your original samples

Buenrostro et al., Curr. Prot. Mol. Biol., 2015

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Quantify libraries by KAPA kit

- This is performed exactly as it is for RNA-seq libraries.
- VERY important to balance the samples that you pool in a multiplex.
  - This must take into account the average fragment length for your library.

Our experimental design

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Tadukan

Treatment	0.25h	1h	2h
Control	X	X	X
Heat shock	X	X	
Water deficit		X	X

Sequencing

- 10 libraries per lane (probably too many)
- RapidRun v2 2X 50bp

Where things can go wrong

1. Intact nuclei
2. Ratio of Tn5 enzyme to nuclei
3. Over amplification of the library
4. Unequal pooling of libraries for sequencing
5. (Chloroplasts)