There are two states of chromatin

Buenrostro (2013)
ATAC-seq probes the conformation of chromatin

Buenrostro (2013)
Open conformations create peaks

Fragmentation  Size selection  PCR  Sequencing  Mapping  Output

Meyer (2014)
Chromatin states vary between cell types

Thurman (2012)
The resolution of ATAC-seq is 500 cells

Buenrostro (2015)
One method of scATAC-seq uses combinatorial indexing

Cusanovich (2015)
A second method of scATAC-seq uses microfluidics

Buenrostro (2015)
The microfluidic device uses values to compartmentalize

Prakadan (2017)
Aggregate single-cell accessibility profiles closely recapitulate profiles of DNase-seq and ATAC-seq

Buenrostro (2015)
Differences in cellular states leads to differential chromatin accessibility

Buenrostro (2015)
Analysis infrastructure to calculate TF deviations and variability from scATAC-seq data

Buenrostro (2015)
Observed cell-to-cell variability within sets of genomic features

Discovery of set of trans-factors associated with high variability.

Buenrostro (2015)
High-variance trans-factors are variable independent of the cell cycle.

Buenrostro (2015)
Calculated changes in associated variability of factors when present together versus independently.

Single cell accessibility profiles nominate distinct *trans*-factors that, in combination, induce or suppress cell-to-cell regulatory variation.

Buenrostro (2015)
Chemical perturbations affect variability on a per-cell basis

Buenrostro (2015)
Mixtures of cells can be deconvolved by trans-factor variability

Buenrostro (2015)
Cell lines have corresponding highly variable *trans*-factors
Covariability across *cis*-elements, sliding windows per-cell

Buenrostro (2015)
scATAC-seq used to assess chromosome conformation

b

Chromosome conformation capture
<ref. 27>

scATAC-seq
cis-correlation

Buenrostro (2015)
In summary...

• Single cell data recapitulates bulk
• Variability in accessibility is associated with
  • Trans-factors: traced by cell type
  • Cis-elements: traced by chromosome conformation
Single cell ATAC-seq by cellular indexing

Multiplex single-cell profiling of chromatin accessibility by combinatorial cellular indexing

Darren A. Cusanovich, Riza Daza, Andrew Adey, Hannah A. Pliner, Lena Christiansen, Kevin L. Gunderson, Frank J. Steemers, Cole Trapnell, Jay Shendure

Technical advances have enabled the collection of genome and transcriptome data sets with single-cell resolution. However, single-cell characterization of the epigenome has remained challenging. Furthermore, because cells must be physically separated before biochemical processing, conventional single-cell preparatory methods scale linearly. We applied combinatorial cellular indexing to measure chromatin accessibility in thousands of single cells per assay, circumventing the need for compartmentalization of individual cells. We report chromatin accessibility profiles from more than 15,000 single cells and use these data to cluster cells on the basis of chromatin accessibility landscapes. We identify modules of coordinately regulated chromatin accessibility at the level of single cells both between and within cell types, with a scalable method that may accelerate progress toward a human cell atlas.
Cellular Indexing vs. Microfluidics

Key steps in ATAC-seq:
- Cell lysis
- Transposition
- Amplification

scATAC-seq based on cellular indexing (Gusanovich et al.):
- Total of 96 pools
- Index 1: combine pools
- FACS sorting total of 96 pools with 15–25 cells
- Index 2: amplify pools and combine for sequencing

scATAC-seq based on microfluidics (Buenrostro et al.):
- Capture cell and assess viability (96 chambers in parallel)
- Lysis and transposition
- Fragment release; initial amplification
- Elution and final amplification

Pott (2015)
Significance of Single Cell ATAC-sequencing

Buenrostro (2015)
Single-cell epigenomic variability reveals functional cancer heterogeneity

Litzenburger (2017)
Questions?


