4-23 Recitation

Prof. Gifford L18

Analysis of Chromatin Structure

Announcements

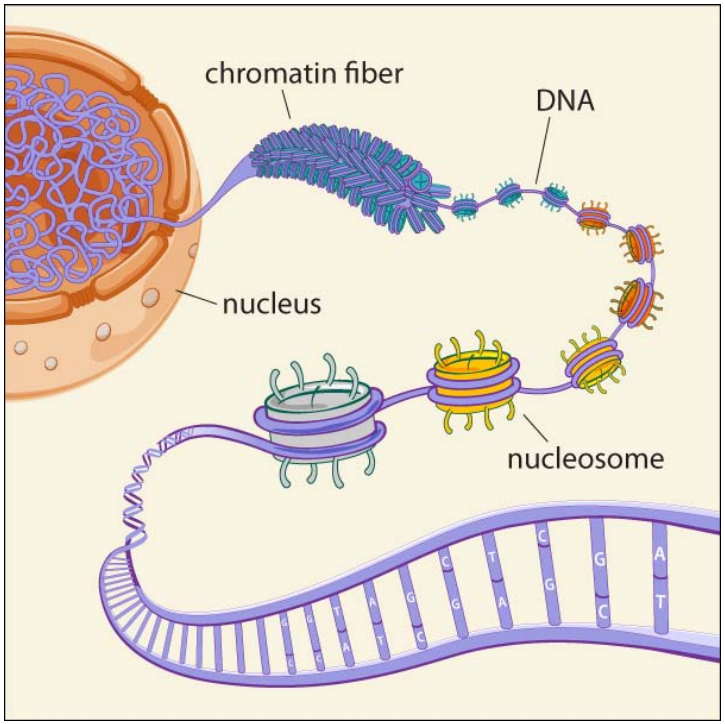
- Problem Set 5 due next Thursday (May 1st)
- 2 more lectures from Prof. Gifford (including today), then 2 guest lectures - Ron Weiss from MIT (Synthetic Bio), George Church from Harvard (Genome Engineering & Systems Biology)
- 2nd exam – Tuesday, May 6th

Outline

- Chromatin Structure
- Dynamic Bayesian Networks / Segway
- DNAse-seq & Protein Interaction Quantitation (PIQ)
- ChIA-PET reveals 3D interactions in the genome

Introduction to Chromatin

- DNA in one cell is 3 meters long, yet fits into a tiny nucleus
- To facilitate the packaging, DNA is wrapped around nucleosomes, and this fiber is wrapped into higher order structures up to the level of a chromosome
  - "chromatin" refers to the structure of DNA + nucleosomes
  - Each nucleosome is an octamer composed of 4 pairs of different histone proteins: H2A, H2B, H3, and H4



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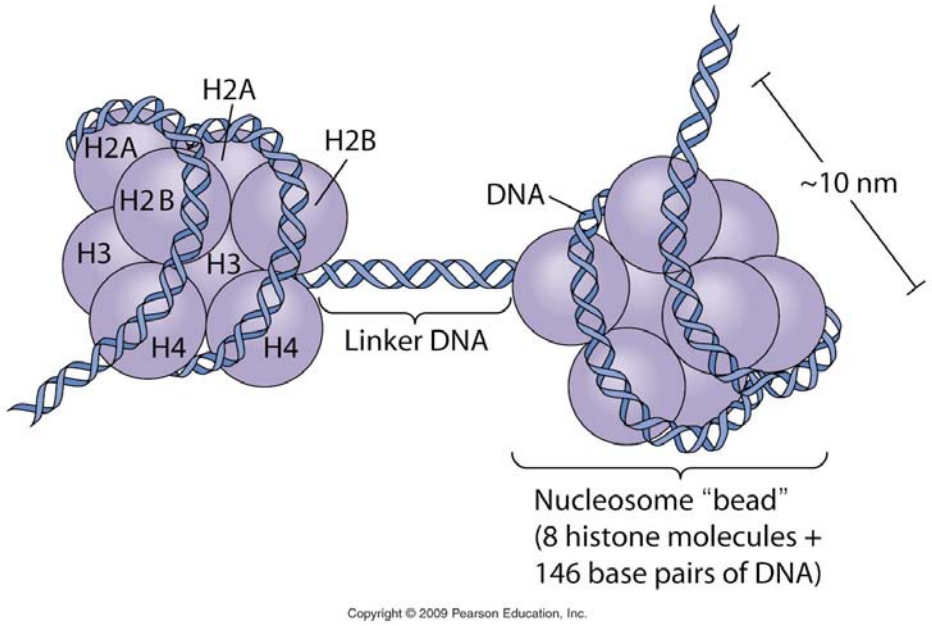
https://www.broadinstitute.org/files/news/images/2010/chromatin_states_2a.png



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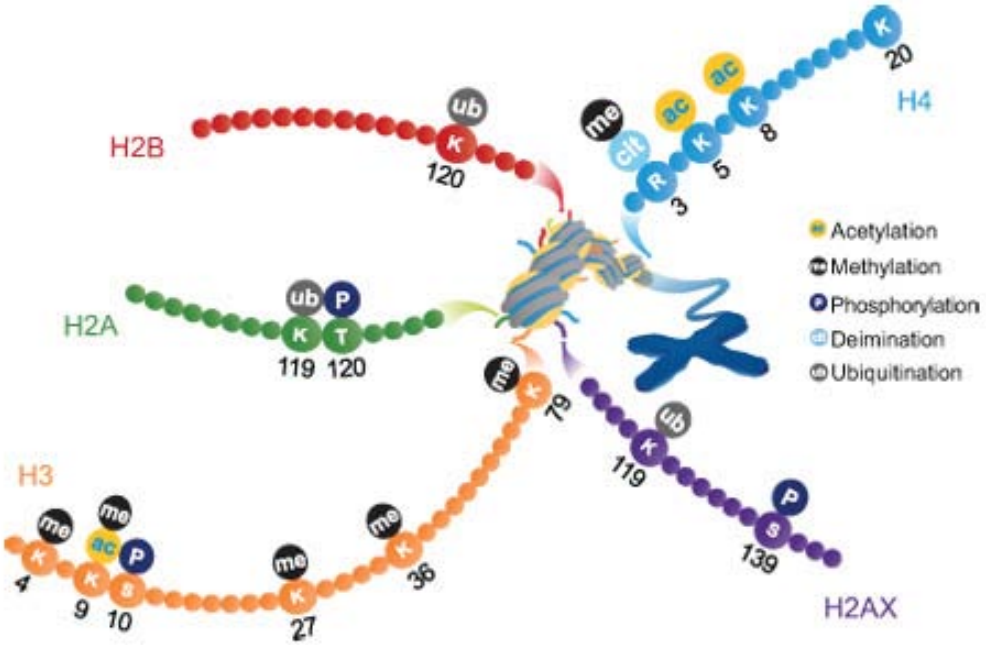
http://www.mun.ca/biology/desmid/brian/BIOL2060/BIOL2060-18/1820.jpg

Histone modifications

– Particular residues on the tails of these histones commonly undergo post-translational chemical modifications



http://a.static-abcam.com/CmsMedia/Media/common-histone-modification-1.jpg

– Some of these modifications are associated with functions – different combinations of marks and their meaning compose the "histone code"

Most common in papers →

Histone modification or variant	Signal characteristics	Putative functions
H2A.Z	Peak	Histone protein variant (H2A.Z) associated with regulatory elements with dynamic chromatin
H3K4me1	Peak/region	Mark of regulatory elements associated with enhancers and other distal elements, but also enriched downstream of transcription starts
H3K4me2	Peak	Mark of regulatory elements associated with promoters and enhancers
H3K4me3	Peak	Mark of regulatory elements primarily associated with promoters/transcription starts
H3K9ac	Peak	Mark of active regulatory elements with preference for promoters
H3K9me1	Region	Preference for the 5′ end of genes
H3K9me3	Peak/region	Repressive mark associated with constitutive heterochromatin and repetitive elements
H3K27ac	Peak	Mark of active regulatory elements; may distinguish active enhancers and promoters from their inactive counterparts
H3K27me3	Region	Repressive mark established by polycomb complex activity associated with repressive domains and silent developmental genes
H3K36me3	Region	Elongation mark associated with transcribed portions of genes, with preference for 3′ regions after intron 1
H3K79me2	Region	Transcription-associated mark, with preference for 5′ end of genes
H4K20me1	Region	Preference for 5′ end of genes

ENCODE Consortium *Nature* 2012

Source: ENCODE Project Consortium. "An Integrated Encyclopedia of DNA Elements in the Human Genome." *Nature* 489, no. 7414 (2012): 57-74.

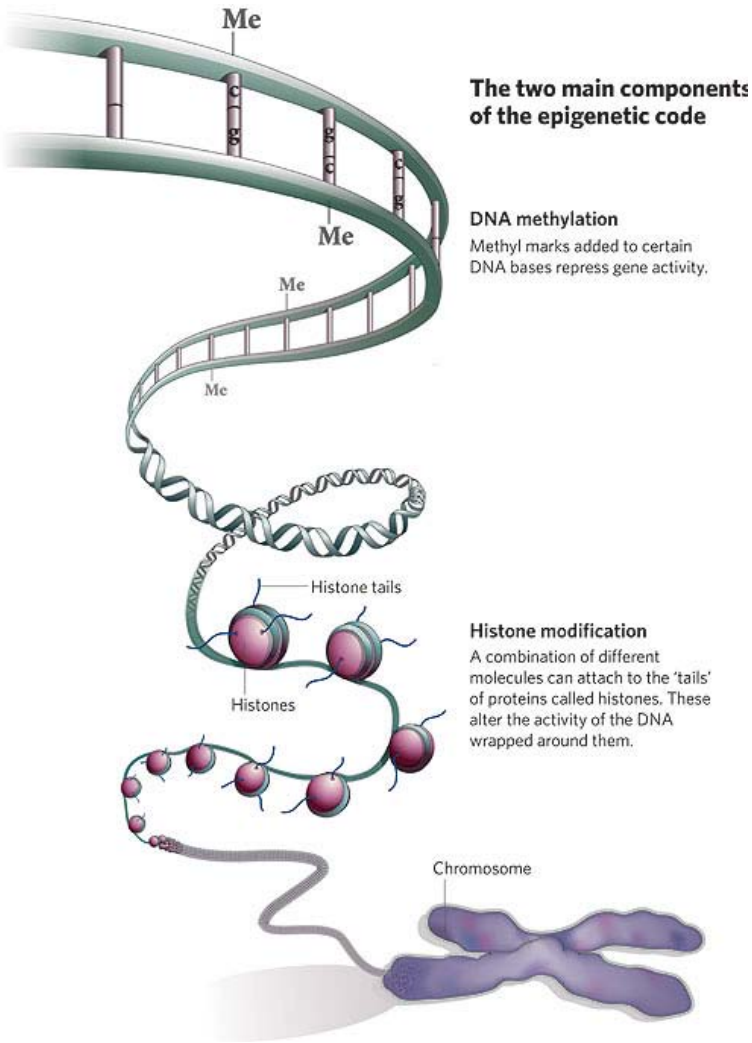
Histone code & DNA methylation regulate gene expression

- In addition to histone modifications, gene expression can be affected by DNA methylation: the 5 carbon of cytosines in DNA can be methylated.
  - In metazoans, only C's before G's can be methylated (the C's of CpG). Hundred or thousands of base-long stretches rich in methylated cytosines form "CpG islands" at some promoters to repress gene expression

- "Epigenetic" changes are changes to DNA not at the level of primary sequence which are reversible & heritable

- Epigenetic marks are often cell-type and/or disease state-specific
  - For example, the pluripotency gene *Nanog* is demethylated during reprogramming of differentiated cells into iPSCs

- Enzymes actively regulate the epigenetic marks
  - Chromatin modifiers and nucleosome remodelers are enzymes that actively regulate chromatin marks, nucleosome positioning & turnover
  - DNA methyltransferases to methylate DNA



Profiling histone modifications

- ChIP-seq w/ antibodies specific to a particular type of modified nucleosome can map histone modifications genome-wide

- We'd like to come up with a way to combine these combinations of histone marks throughout the genome into functional annotations
  - Based on an observed pattern of marks at a locus, we'd like to label it w/ 'enhancer', 'promoter', 'inactive' or 'active gene body region', etc.

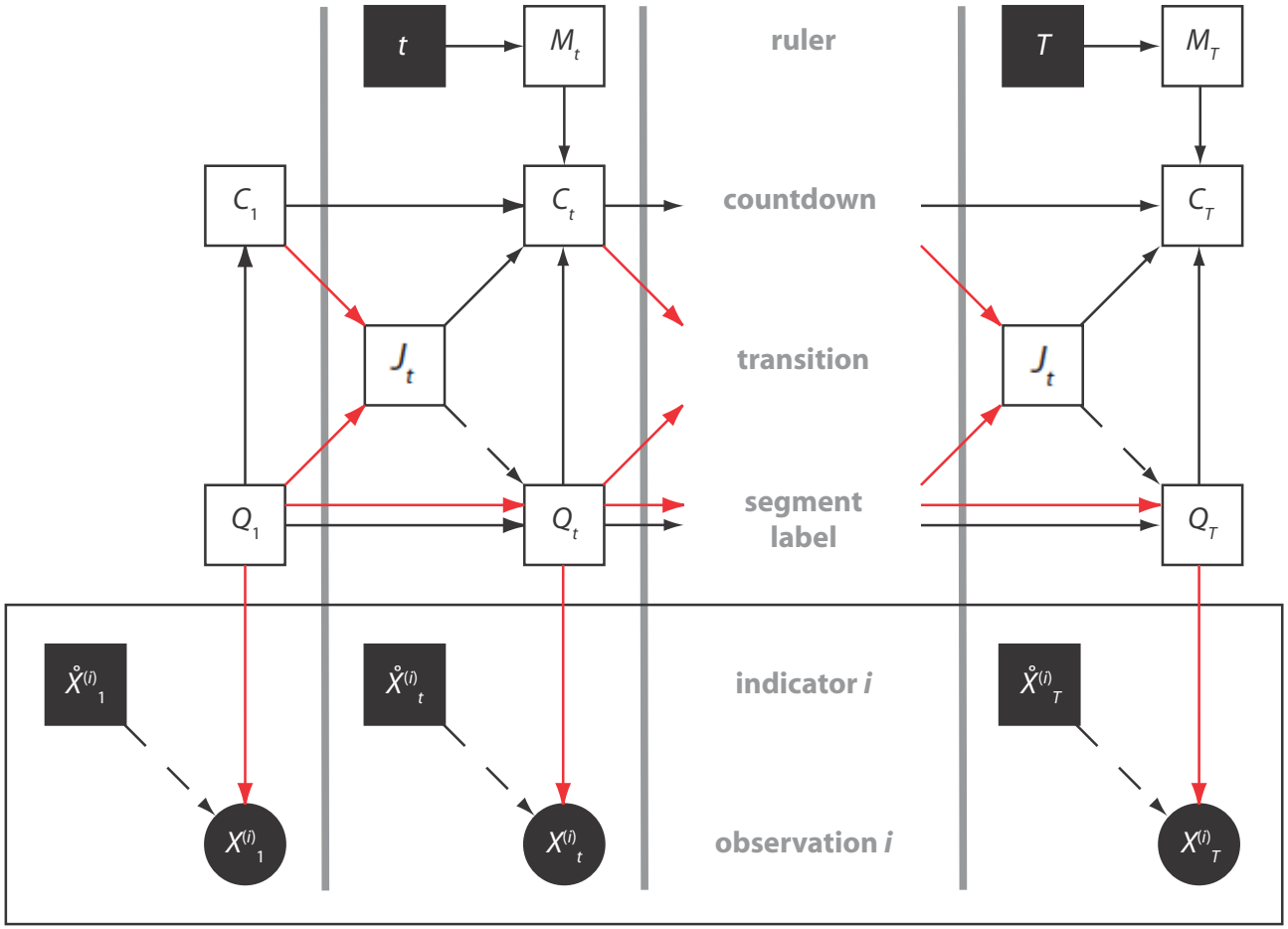
- 2 approaches to functional annotation of the genome:
  - Hidden Markov Model (ChromHMM)
  - Dynamic Bayesian Network (Segway)

Dynamic Bayesian Networks

- A Bayesian network (directed graphical model where arcs/ edges represent conditional dependencies) that models a dynamic process (sequential data, either temporal or spatial – e.g. along the genome)

- Similar to Hidden Markov Models, but include additional random variables that allow tuning (e.g., hard limits on segment lengths)

Segway: Dynamic Bayesian Network



$i \in [1, n]$

Black arcs (edges) = deterministic conditional dependence, red = stochastic conditional dependence

Variable's parents are indicated by its direct predecessor in the directed graph

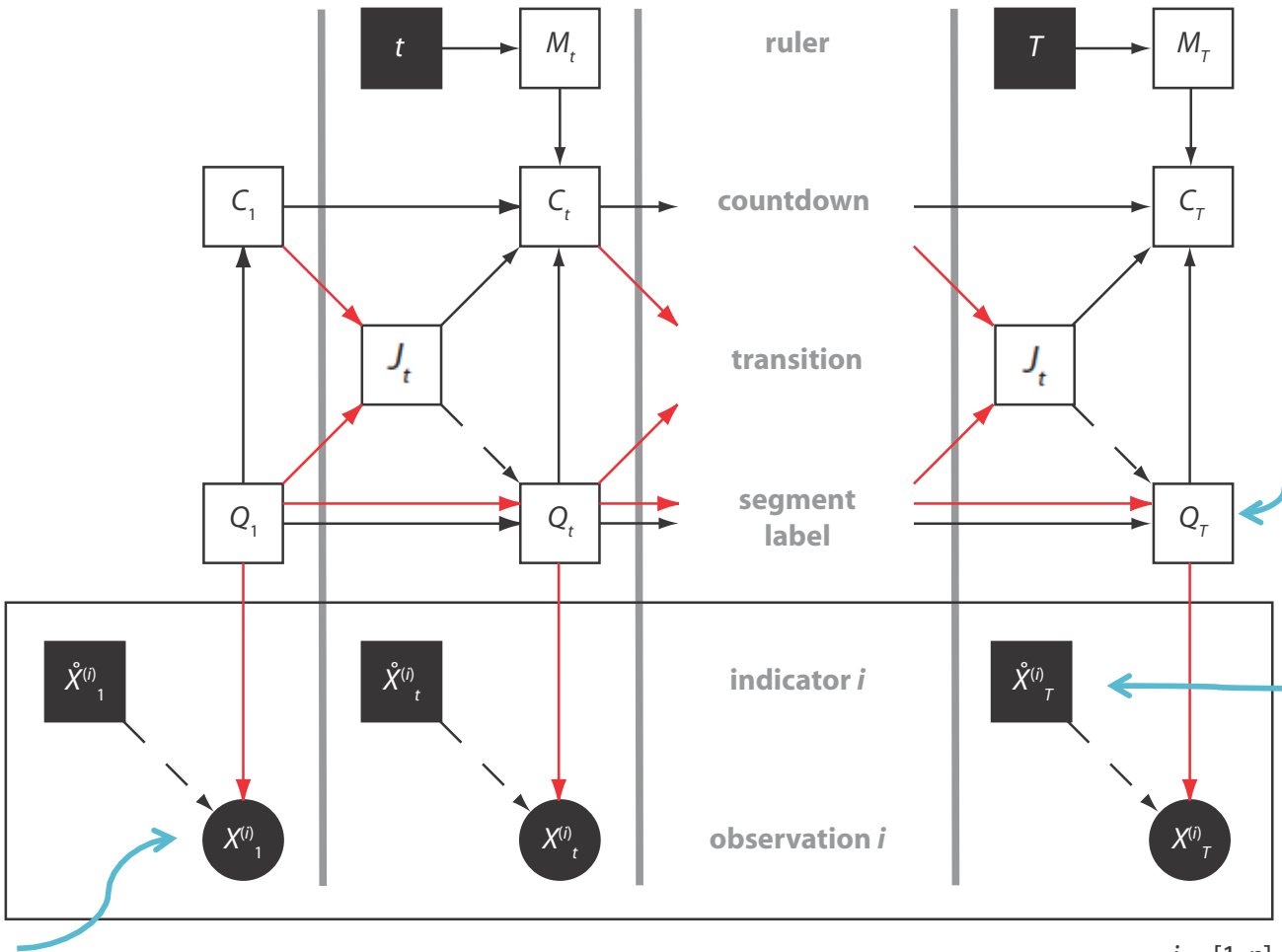
Every variable is conditionally independent of all variables in the model given its parents

n observation tracks
T: sequence length

Square: discrete random variable
Circle: continuous random variable

White: hidden variable
Black: observed variable

Segway: Dynamic Bayesian Network

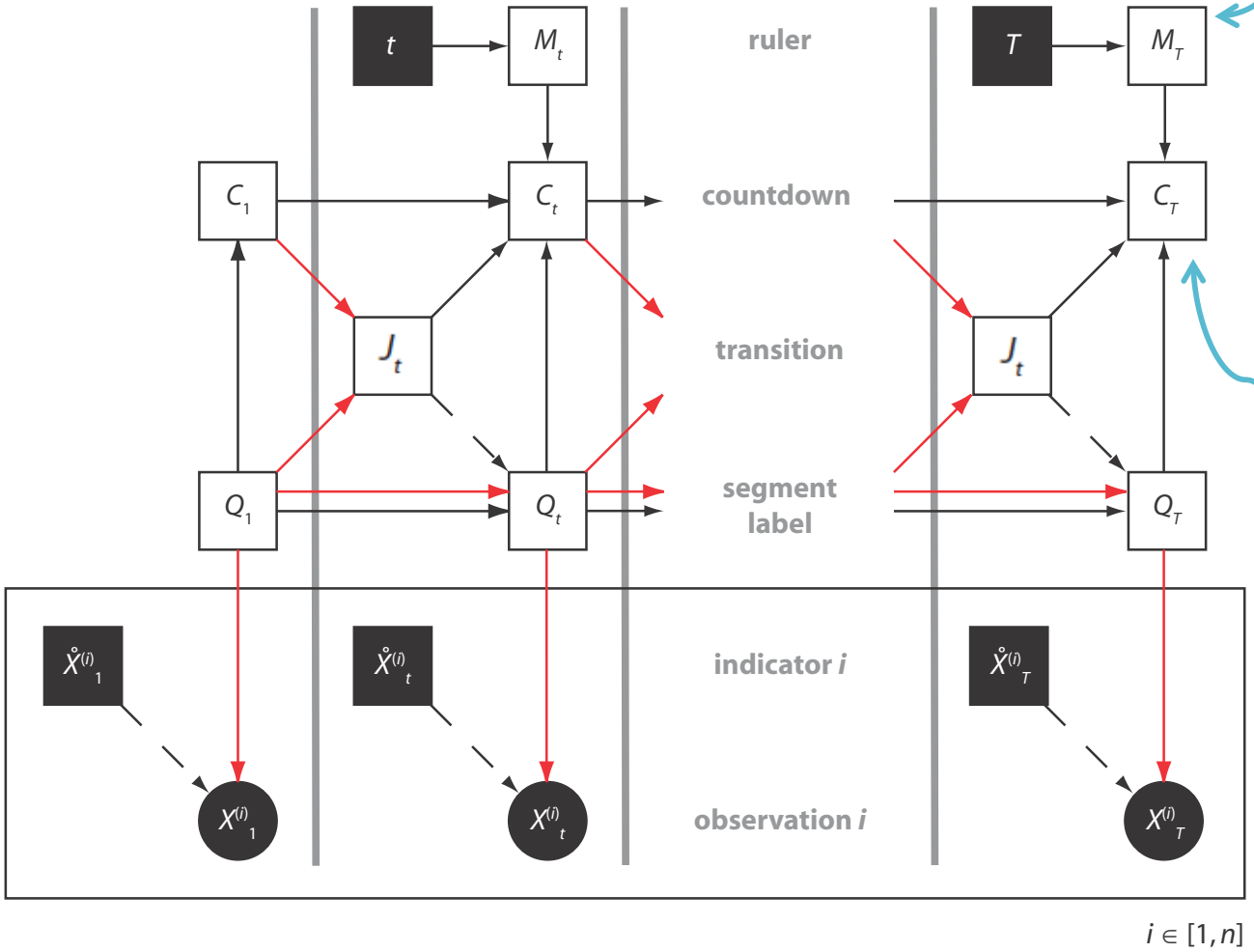


Segment label: The hidden annotation you're trying to infer (e.g. promoter).

Indicator: 1/0 whether or not the assay produced any results for that region (=0 if assay can't map reads to that region. In this case, the edge from Q_t to $X_t^{(i)}$ is edited out)

Observation track: assay output (e.g., density of H3K4me3 ChIP-seq reads – one track for each of *n* experiments) $i \in [1, n]$

Segway: Dynamic Bayesian Network

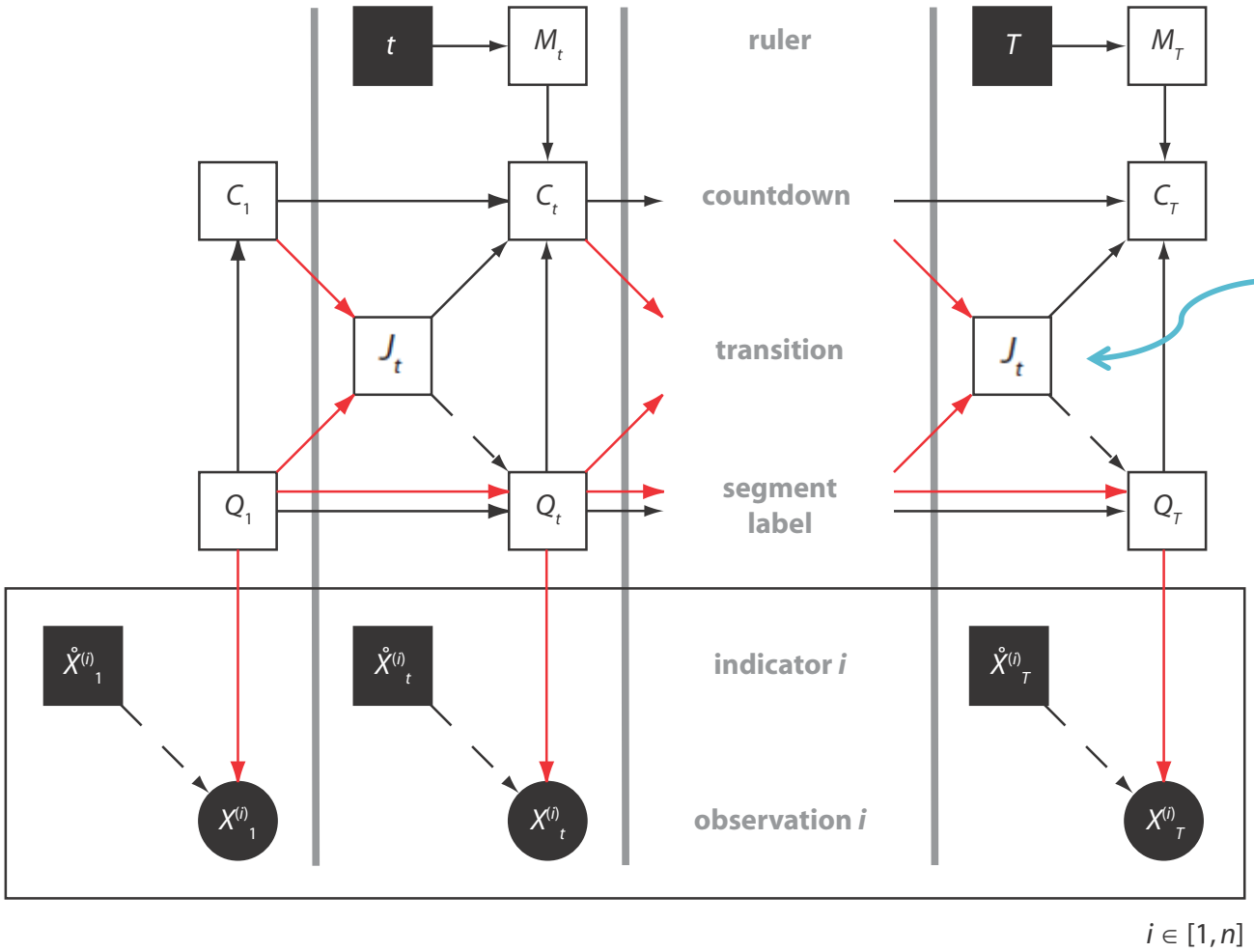


Ruler marker:

=1 every 10th position, 0 otherwise (every 10th position, we update the countdown variable as to how long we've been in that label)

Countdown: Discrete variable that allows the specification of minimum or maximum segment length. Starts at initial value dependent on Q_T (might want TSS to be short but intergenic region to be long) and decreases where ruler marker M_T=1.

Segway: Dynamic Bayesian Network



Transition: binary segment transition label that either forces the segment label to change at the current position (J_t=1) or prevent it from changing (J_t=0).

Segway generates a conditional probability table $P(J_t=1 \mid Q_{t-1}, C_{t-1})$ that maps each (Q_{t-1}, C_{t-1}) to one of three rules that determine the value of J_t:

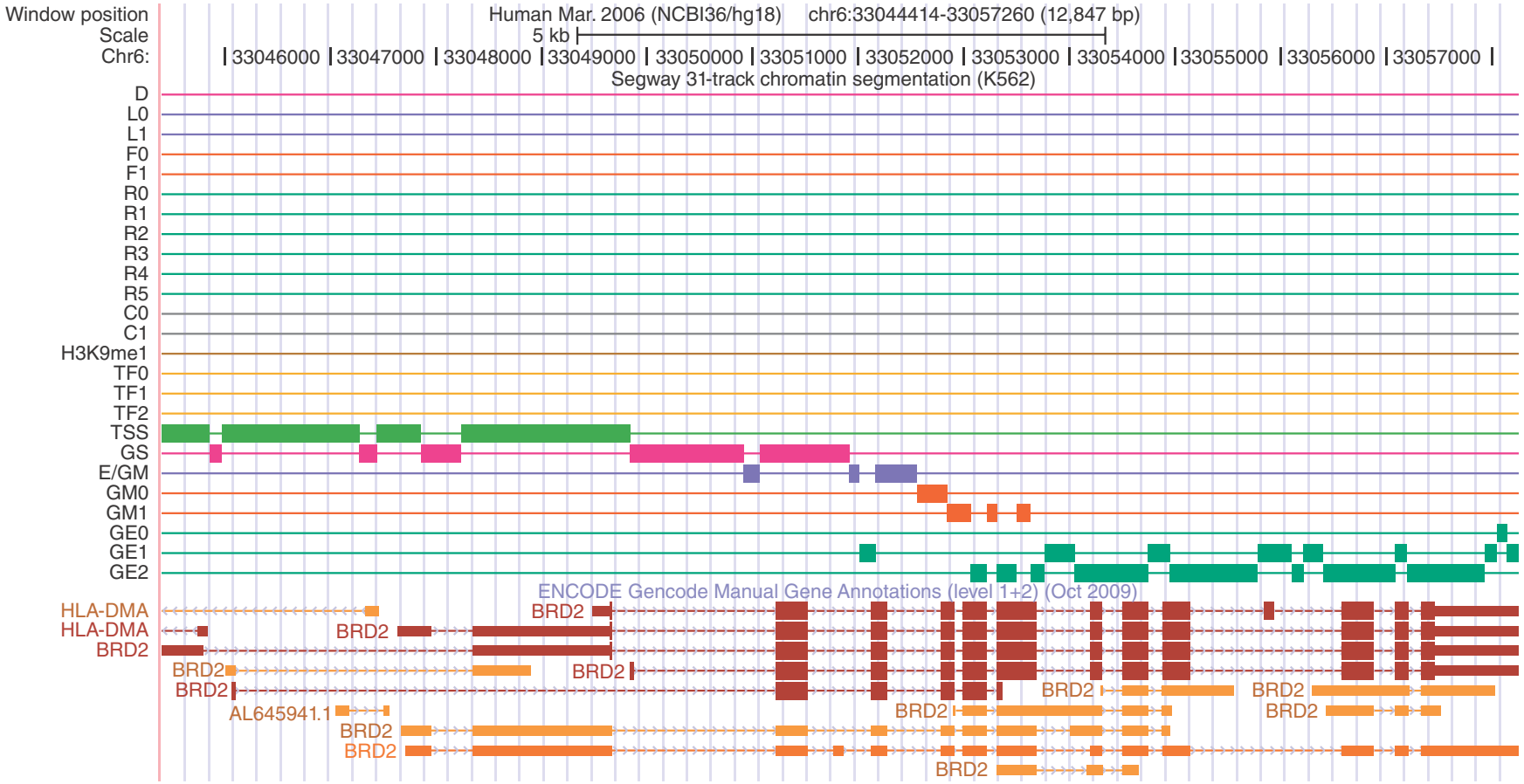
1. Force: $P(J_t=1) = 1$
2. Prevent: $P(J_t=1) = 0$
3. Allow: $P(J_t=1)=1/(1+L)$

"Allow" rule models geometric distribution w/ expected length L

Segway: Dynamic Bayesian Network

- Train on 1% of the Genome
  - Assign equal probability (=1/n) of each label to the starting position, then use Expectation-Maximization (EM) algorithm to learn model parameters (contributions of each track (experimental assay) to each label)
  - Starting from different initial conditions (i.e., contributions of each track to a particular label) gave similar results
- Then use these parameters to segment the rest of the genome using Viterbi decoding (similar to what we discussed for HMMs)

Example of Segway's segmentation for a gene



-Arbitrarily chose there to be 25 labels (so that they would remain interpretable by biologists)

The authors gave names to the resulting 25 labels:

D: "dead" – no activity

GS: gene start

GM: gene middle

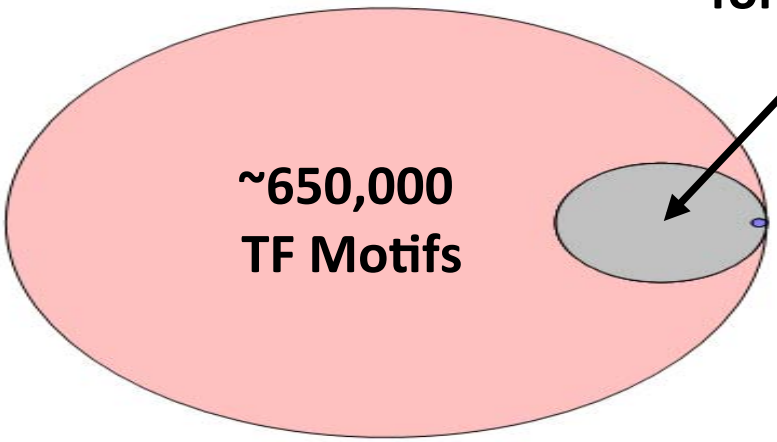
GE: gene end

E: enhancer

Transcription Factor Binding

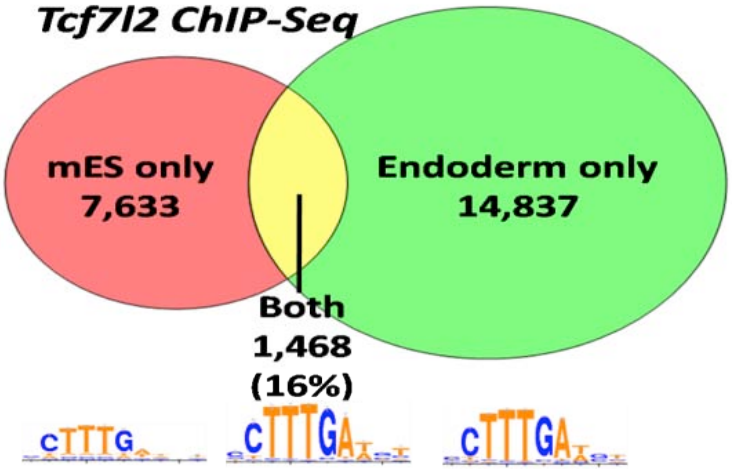
- Many more possible binding sites in genome than are actually occupied
- Binding sites are different in cell types and across time

Motifs are insufficient to predict binding



~50,000 binding sites for a typical TF

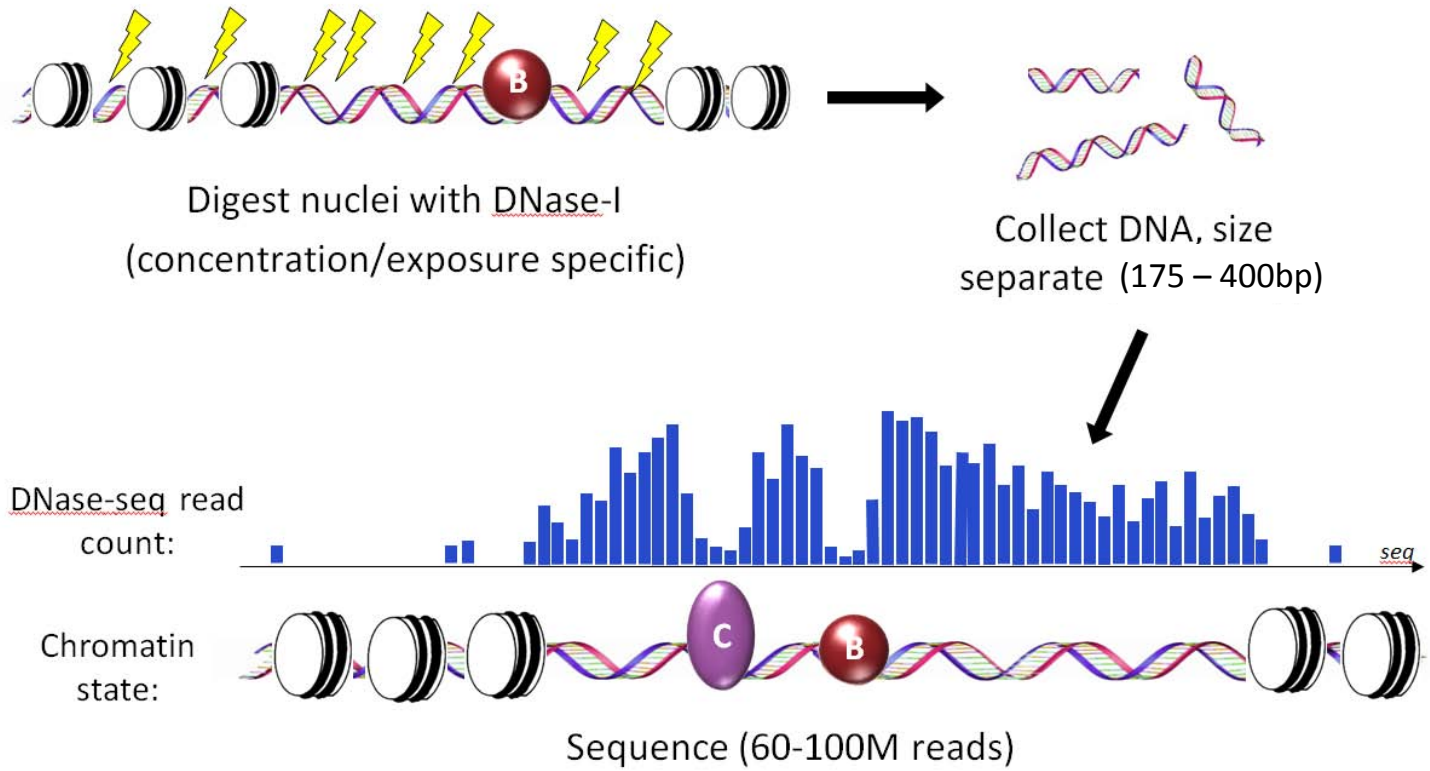
Binding sites change across time



- One key determinant of whether or not a TF binds is the local chromatin landscape: is the DNA accessible?

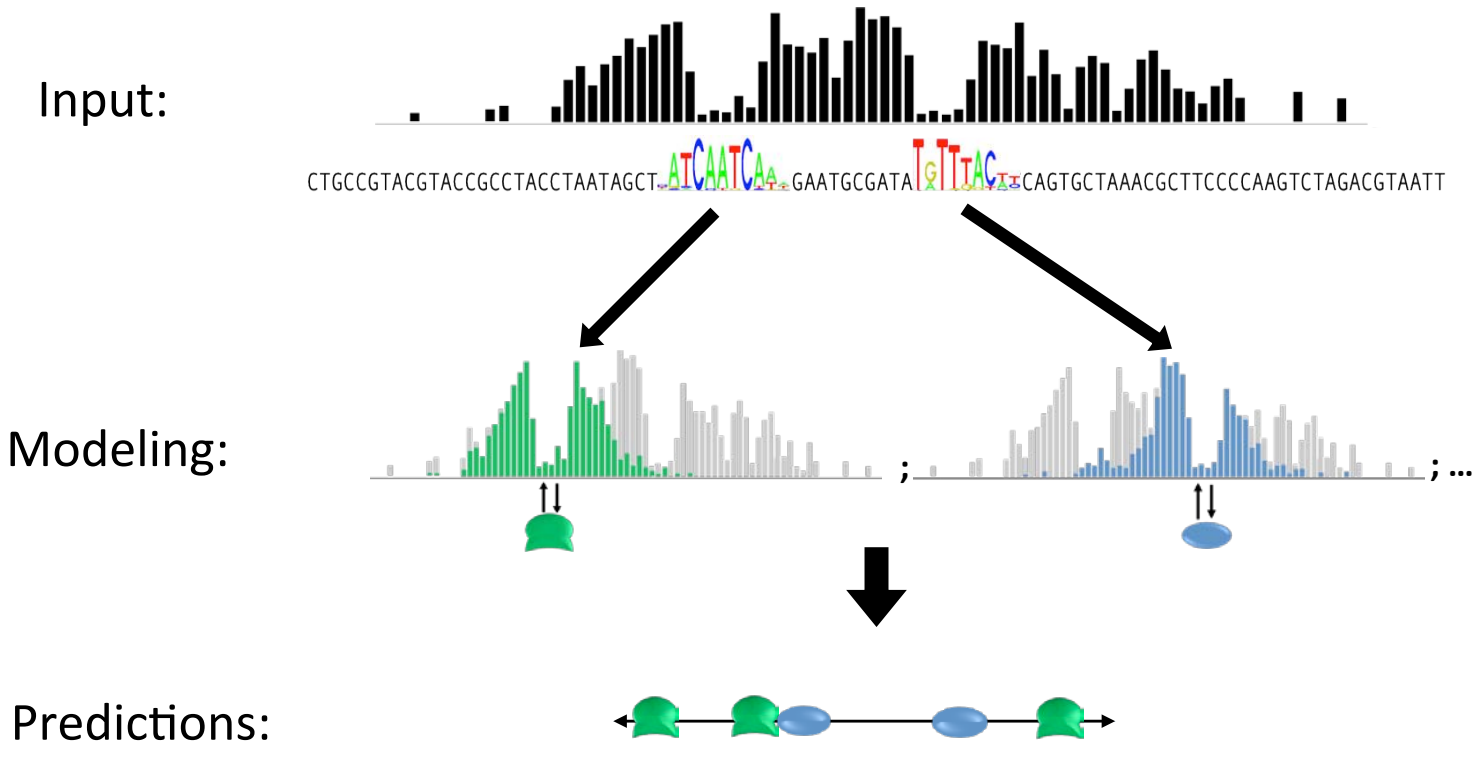
Dnase-seq reveals protected regions of the genome

- DNase-I cleaves at unprotected regions
  - Regions of open chromatin
  - Not wrapped around nucleosomes or bound strong by TFs



Protein Interaction Quantitation (PIQ)

- Predicts TF binding from DNase-seq + sequence motif preferences of TFs



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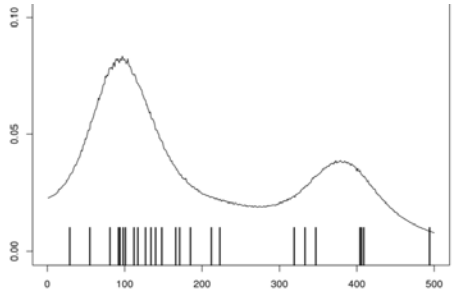
Source: Sherwood, Richard I., Tatsunori Hashimoto, et al. "Discovery of Directional and Nondirectional Pioneer Transcription Factors by Modeling DNase Profile Magnitude and Shape." *Nature Biotechnology* 32, no. 2 (2014): 171-8.

- Can get predictions for hundreds of TFs (need a motif for that TF) – no need for antibodies specific to proteins

3 Steps of PIQ algorithm

- 1. Identification of candidate sites using TF motifs from TF databases

- 2. Smoothing of raw reads from each DNase-seq experiment. DNase-seq reads are modeled as arising from a Gaussian process to remove noise by adaptively smoothing the reads from neighboring bases

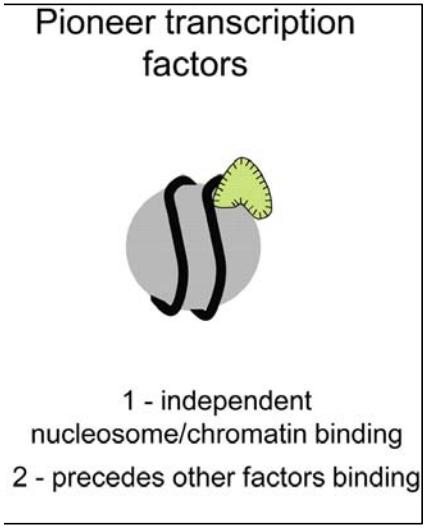


- 3. Identify binding sites of TF by iteratively combining direct evidence of binding (DNase-seq) with computer-generated model of DNaseI hypersensitivity that includes that event (uses TF-signature profile shapes and magnitudes for each TF to build a model of the expected DNaseI hypersensitivity)

- Use log-likelihood ratio to test each region for TF binding, calling those above 1% of null distribution as binary “bound” regions

Pioneer Transcription Factors

- Region of "closed" chromatin that's inaccessible to most TFs can be opened by pioneer TF binding

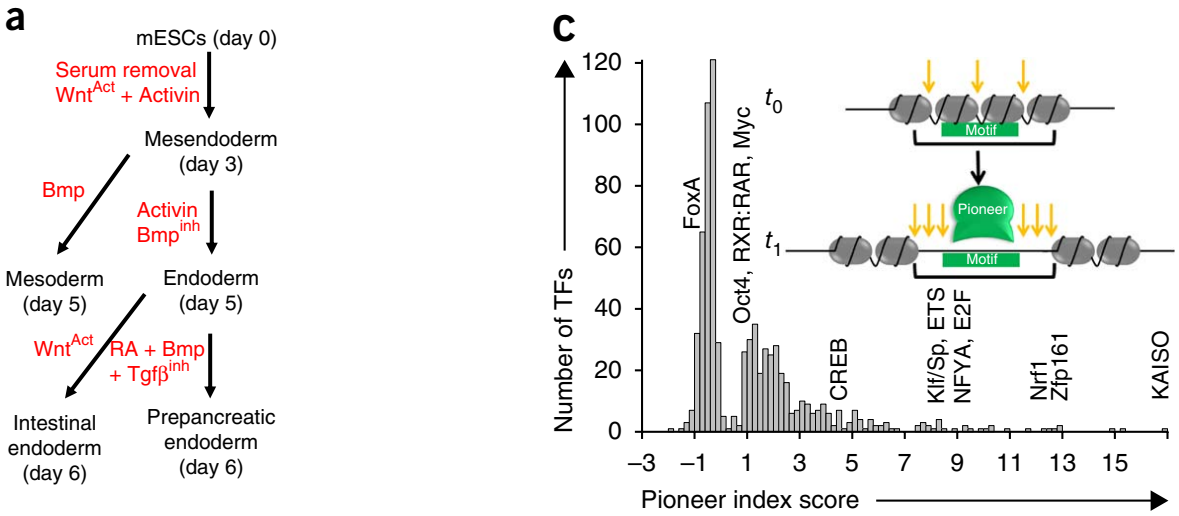


Zaret 2011

- Then once chromatin is opened, other "settler" TFs can bind

Identification of Pioneer TFs

- Apply PIQ to a developmental lineage model that involves stepwise differentiation of mouse stem cells
  - Collect DNase-seq data at six-cell states at different timepoints
  - "Pioneer index" measures motif-specific expected increase in DNaseI accessibility at sites whose binding changes at successive timepoints
  - Most motifs showed little pioneer activity, while a small number of motifs (TFs) open chromatin substantially upon binding

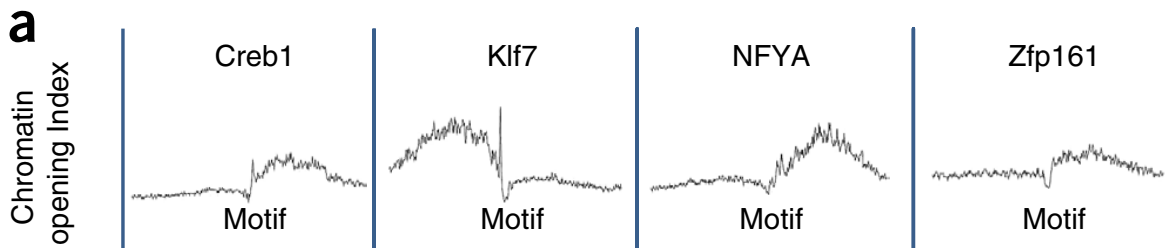


  - Settler TFs can bind once pioneers have opened the chromatin; loss of pioneer binding causes chromatin to return to a closed state

Asymmetrical chromatin opening by directional pioneers

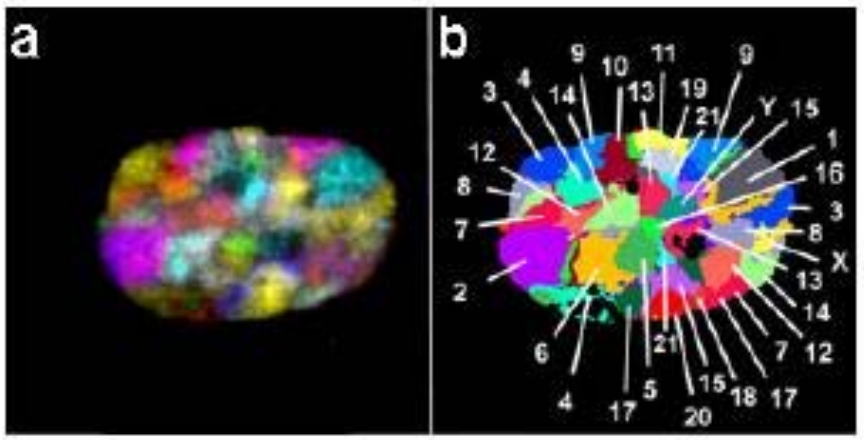
- For non-palindromic motifs (e.g. AATTCG), we know which strand (+/-) the motif is on and therefore in which direction the TF is binding

- Some pioneer TFs tend to open chromatin more strongly in one direction – could inform mechanisms of pathways how TFs deposit histone marks

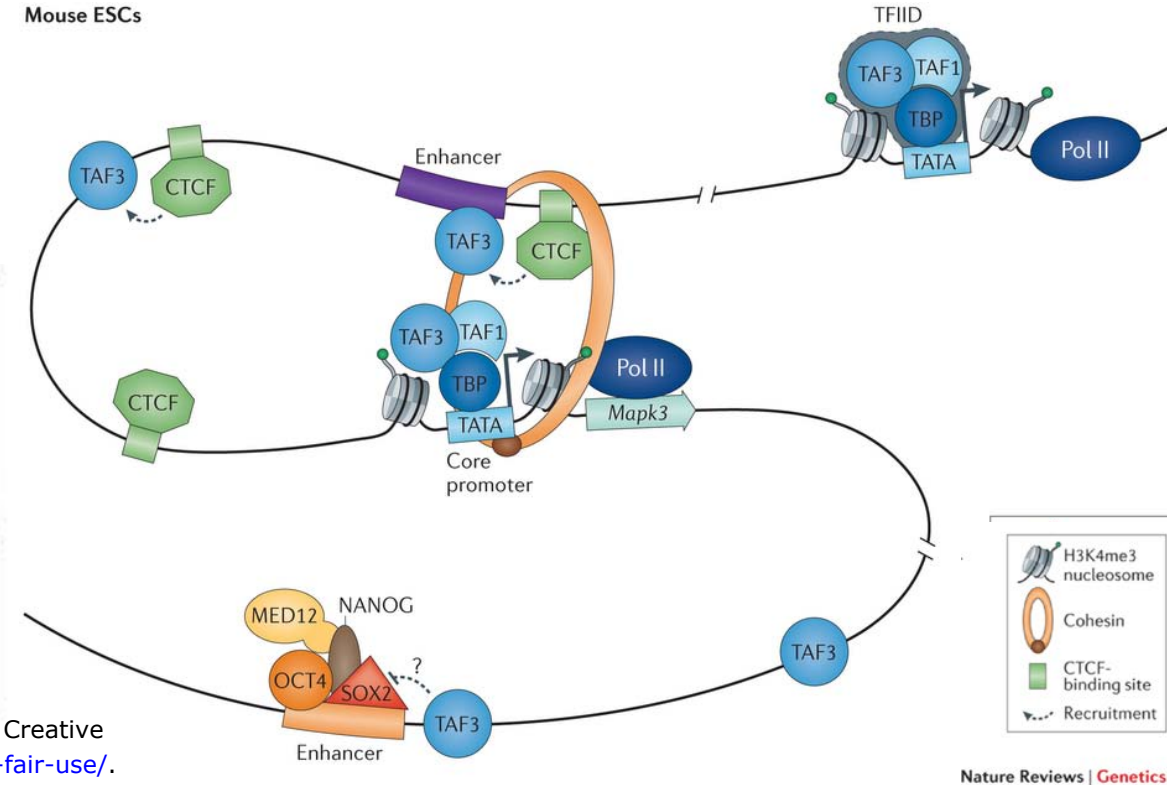


3D structure of the genome & enhancer looping

- DNA is packaged tightly in 3D space in the nucleus
  - This structure dictates which elements far apart on the genome (Mb away) can physically interact due to close proximity in 3D space
- Important for formation of promoter-enhancer interactions
  - Enhancers: distal regulatory elements that, when bound by specific TFs, enhance the expression of an associated gene



http://www.science.ngfn.de/images/S31T04_fig1.JPG



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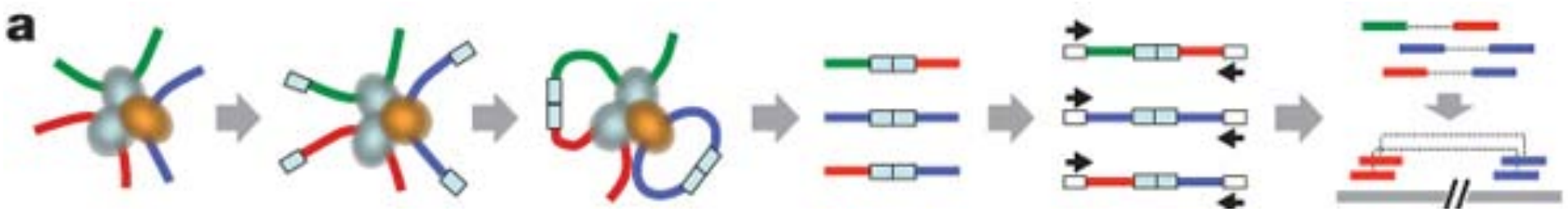
Source: Ong, Chin-Tong, and Victor G. Corces. "CTCF: An Architectural Protein Bridging Genome Topology and Function." *Nature Reviews Genetics* (2014).

http://www.nature.com/nrg/journal/v15/n4/images/nrg3663-f4.jpg

ChIA-PET reveals 3D interactions of the genome

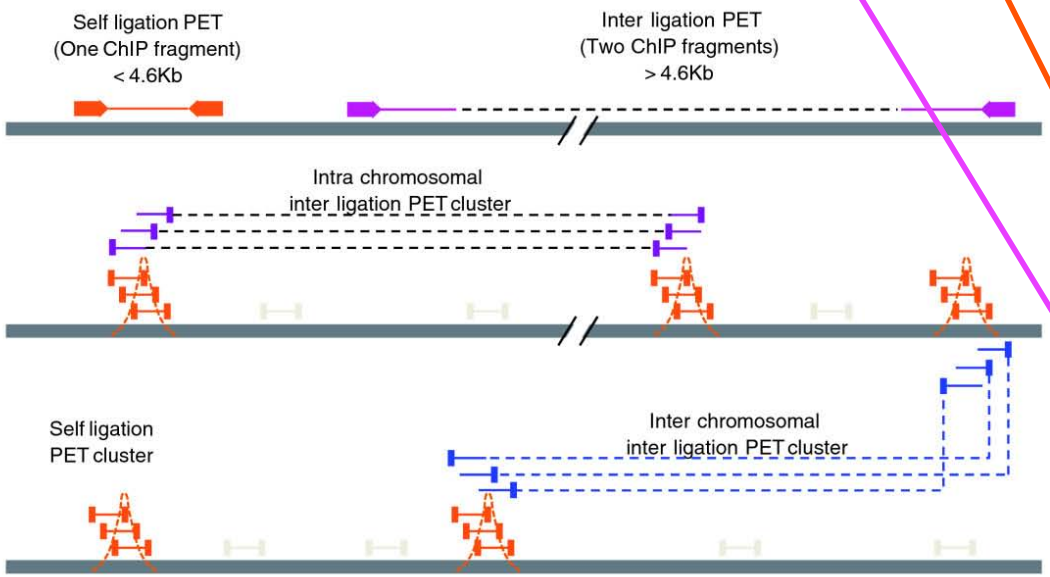
Chromatin Interaction Analysis by Paired-End Tagging



-Crosslink DNA & proteins, ChIP on protein of interest (e.g. RNA Pol II), and shear DNA

-Attach linkers w/ restriction enzyme sites & perform ligation in dilute conditions to favor ligation within each complex

- Perform restriction digest & PCR amplify fragments, then sequence



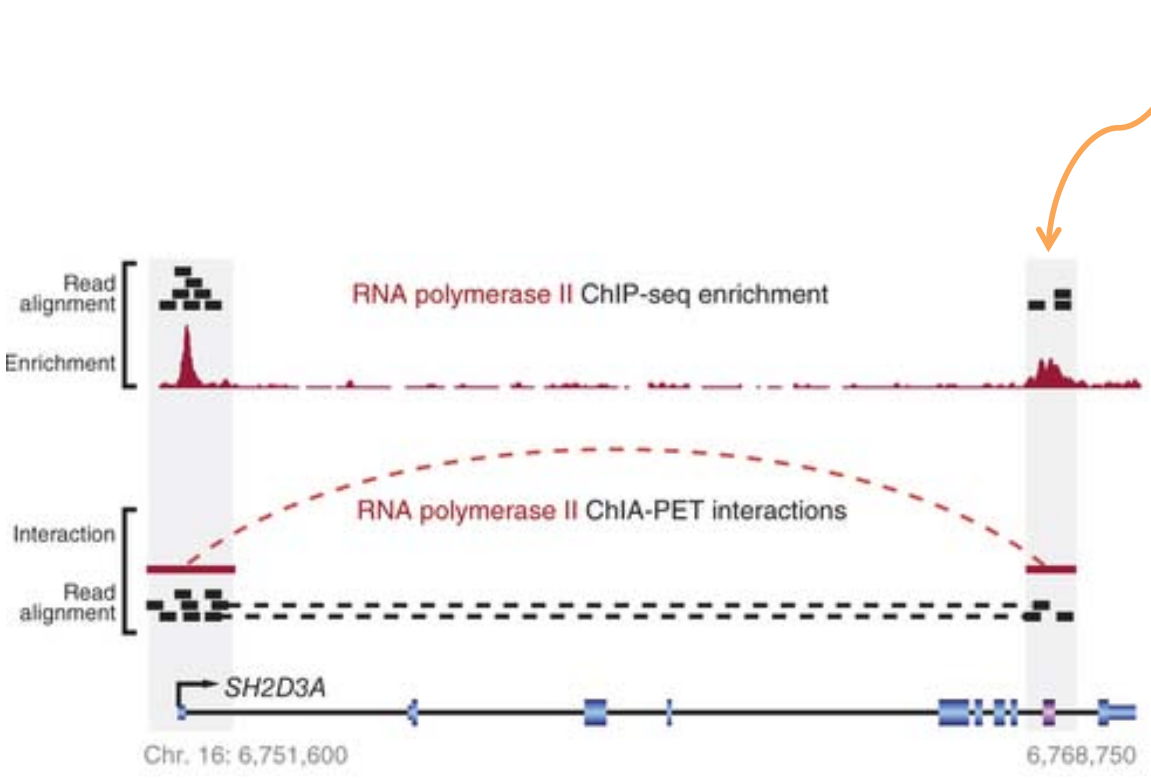
- Two types of ligation events:
  1. Self-ligation (e.g., both tags are near the promoter) – these map near each other on the genome & we throw these out
  2. Inter-ligation (e.g., 1 tag from promoter & 1 from enhancer) reveal interactions

 http://genomebiology.com/content/figures/gb-2010-11-2-r22-1-l.jpg

http://www.nature.com/nature/journal/v462/n7269/images/nature08497-f1.2.jpg

ChIA-PET reveals 3D interactions of the genome

-ChIA-PET sequence tags that pair with tags from known promoter regions reveal RNA PolII ChIP peaks that are at enhancer regions



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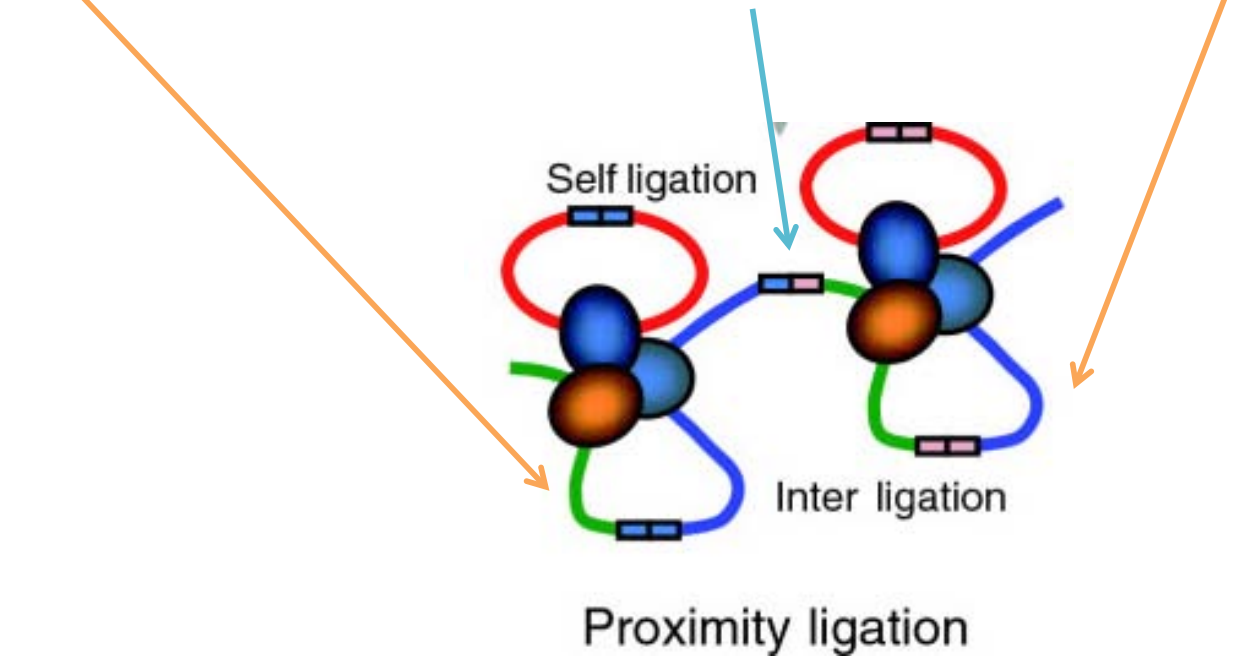
Source: Mercer, Tim R., Stacey L. Edwards, et al. "DNase I-hypersensitive Exons Colocalize with Promoters and Distal Regulatory Elements." *Nature Genetics* (2013).

http://www.nature.com/ng/journal/v45/n8/images/ng.2677-F2.jpg

Assessing significance of ChIA-PET interactions

- Inter-ligation events (e.g. between a putative enhancer and promoter) could arise from two sources
  - 1. Within the same cluster – these are true 3D interactions
  - 2. From ligation between 2 different clusters – these are false positives



- We need to assess if the inter-ligation events are significantly enriched for having occurred from within the same cluster (true interaction events)

Assessing significance of ChIA-PET interactions

- Hypergeometric test for significance
  - $I_{A,B}$: # of inter-ligation events between loci A and B (paired-tags mapping to A & B)
  - c_A , c_B: total number of ligation events associated with A , B (single tags mapping to A or B)
  - N: total number of ligation events (total single tags)

Probability of observing exactly your observed number of inter-ligation events under null hypothesis that each sticky end has an equal probability of ligating to any other end:

$$P(I_{A,B}|N, c_A, c_B) = \frac{\binom{c_A}{I_{A,B}}\binom{N-c_A}{c_B-I_{A,B}}}{\binom{N}{c_B}}$$

P-value is probability of your observation plus anything more extreme:

$$p = \sum_{i=I_{A,B}}^{\min\{c_A,c_B\}} P(i|N, c_A, c_B)$$

ChIA-PET has a high false negative rate

- Due to heterogeneous starting population of cells (transient promoter-enhancer interactions), complex protocol & stringent P-values to cut down on false-positives, ChIA-PET has a high false negative rate

- So, given an a number of promoter-enhancer interactions observed in ChIA-PET experiment 1 & in ChIA-PET experiment 2 with each capturing only a subset of the total events, we'd like to estimate the true number of interactions occurring in the cell

Estimating the total number of events from overlap

- Again, we can use the hypergeometric model
  - Given two observed sample sizes *m* & *n* along with their overlap *k*, we'd like to estimate the total number events *N*

$$\hat{N} = \underset{N}{\operatorname{argmax}} \left[P(X = k; N, m, n) \right]$$

  - The maximum likelihood estimate of *N* is approximately:

$$\hat{N}(m, n, k) = \frac{mn}{k}$$

  - Example: Experiment 1: 100 events

    Experiment 2: 200 events

    - overlap is only 20. It seems like we must be sampling only a small fraction of the total events each time!

    - Indeed, maximum likelihood estimate is 1000 total events that each sample came from

Estimating the total number of events from overlap

- The previous model assumes all events were true positives, while in reality some are false positives.
  - We overestimate the total event count since the observed *m* and *n* are larger than they truly are without the false positives
  - Assume the overlapping events are true positives and the non-overlapping events have false positive rate *f* (so 1-*f* of the events are true positives). Then we can update estimates of *m* and *n*:

$$m' = (1 - f)(m - k) + k$$
$$n' = (1 - f)(n - k) + k$$

  - With the previous example (100 and 200 events w/ 20 overlapping), let's say there is a false positive rate of 5%. Then:
    - *m*' = (0.95)(80) + 20 = 96
    - *n*' = (0.95)(180) + 20 = 191
    - The modified estimate of the total # of events if therefore:
      (96)(191)/20 ≈ 869 (vs. 1000 without considering false-positives)

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Spring 2014