Epigenomics: ChIP-seq and ATAC-seq

Chris Miller, Ph.D.
Washington University in St. Louis

Some slides adapted from:
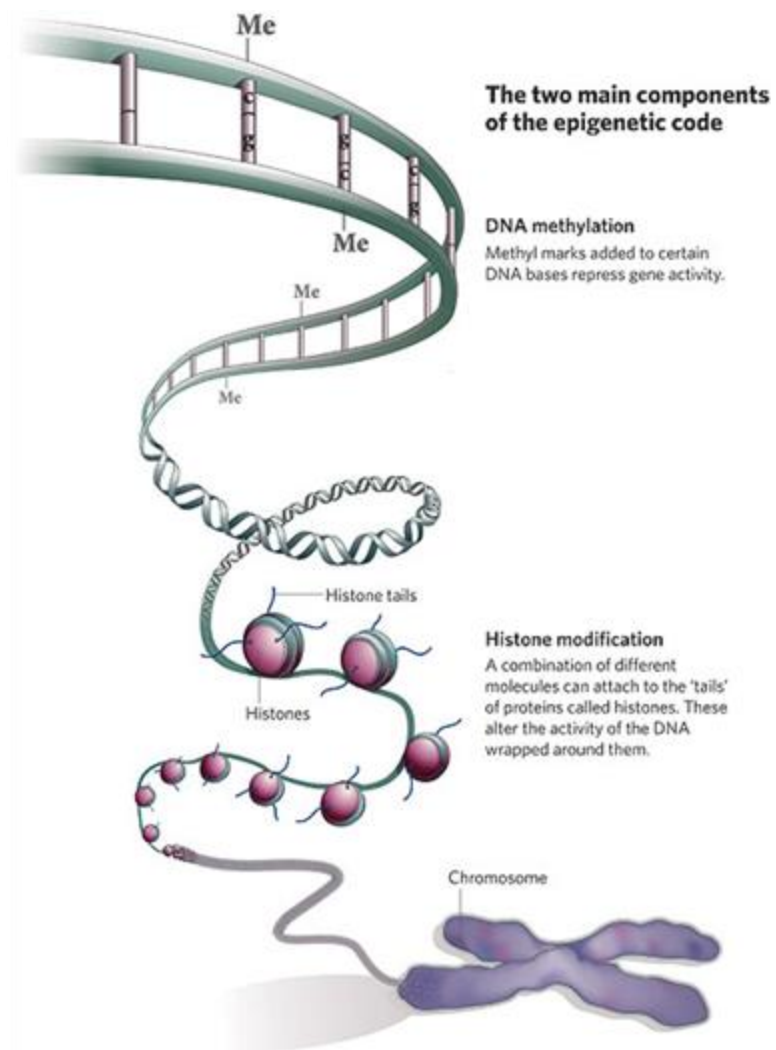https://github.com/genome/bfx-workshop
https://github.com/quinlan-lab/applied-computational-genomics

Epigenomics

- Alterations of DNA state or accessibility

- Wrapped around histones

- Bound by transcription factors

- etc

The two main components of the epigenetic code

DNA methylation
Methyl marks added to certain DNA bases repress gene activity.

Histone modification
A combination of different molecules can attach to the 'tails' of proteins called histones. These alter the activity of the DNA wrapped around them.

Me

Me

Me

Me

Histone tails

Histones

Chromosome

105+ *-seq assays

(as of several years ago)

from Lior Pachter's blog

Nucleo-Seq: Anton Valouev et al., "Determinants of Nucleosome Organization in Primary Human Cells," *Nature* 474, no. 7352 (June 23, 2011): 516–520, doi:10.1038/nature10002.

DNAse-Seq: Gregory E. Crawford et al., "Genome-wide Mapping of DNase Hypersensitive Sites Using Massively Parallel Signature Sequencing (MPSS)," *Genome Research* 16, no. 1 (January 1, 2006): 123–131, doi:10.1101/gr.4074106.

DNAseI-Seq: Jay R. Hesselberth et al., "Global Mapping of protein-DNA Interactions in Vivo by Digital Genomic Footprinting," *Nature Methods* 6, no. 4 (April 2009): 283–289, doi:10.1038/nmeth.1313.

Sono-Seq: Raymond K. Auerbach et al., "Mapping Accessible Chromatin Regions Using Sono-Seq," *Proceedings of the National Academy of Sciences* 106, no. 35 (September 1, 2009): 14926–14931, doi:10.1073/pnas.0905443106.

Hi-C-Seq: Erez Lieberman-Aiden et al., "Comprehensive Mapping of Long-Range Interactions Reveals Folding Principles of the Human Genome," *Science* 326, no. 5950 (October 9, 2009): 289–293, doi:10.1126/science.1181369.

ChIA-PET-Seq: Melissa J. Fullwood et al., "An Oestrogen-receptor-α-bound Human Chromatin Interactome," *Nature* 462, no. 7269 (November 5, 2009): 58–64, doi:10.1038/nature08497.

FAIRE-Seq: Hironori Waki et al., "Global Mapping of Cell Type-Specific Open Chromatin by FAIRE-seq Reveals the Regulatory Role of the NFI Family in Adipocyte Differentiation," *PLoS Genet* 7, no. 10 (October 20, 2011): e1002311,

NOMe-Seq: Theresa K. Kelly et al., "Genome-wide Mapping of Nucleosome Positioning and DNA Methylation Within Individual DNA Molecules," *Genome Research* 22, no. 12 (December 1, 2012): 2497–2506, doi:10.1101/gr.143008.112.

ATAC-Seq: Jason D. Buenrostro et al., "Transposition of Native Chromatin for Fast and Sensitive Epigenomic Profiling of Open Chromatin, DNA-binding Proteins and Nucleosome Position," *Nature Methods* advance online publication (October 6, 2013), doi:10.1038/nmeth.2688.

Genome variation

RAD-Seq: Nathan A. Baird et al., "Rapid SNP Discovery and Genetic Mapping Using Sequenced RAD Markers," *PLoS ONE* 3, no. 10 (October 13, 2008): e3376, doi:10.1371/journal.pone.0003376.

Freq-Seq: Lon M. Chubiz et al., "FREQ-Seq: A Rapid, Cost-Effective, Sequencing-Based Method to Determine Allele Frequencies Directly from Mixed Populations," *PLoS ONE* 7, no. 10 (October 31, 2012): e47959, doi:10.1371/journal.pone.0047959.

CNV-Seq: Chao Xie and Martti T. Tammi, "CNV-seq, a New Method to Detect Copy Number Variation Using High-throughput Sequencing," *BMC Bioinformatics* 10, no. 1 (March 6, 2009): 80, doi:10.1186/1471-2105-10-80.

Novel-Seq: Iman Hajirasouliha et al., "Detection and Characterization of Novel Sequence Insertions Using Paired-end Next-generation Sequencing," *Bioinformatics* 26, no. 10 (May 15, 2010): 1277–1283, doi:10.1093/bioinformatics/btq152.

TAm-Seq: Tim Forshew et al., "Noninvasive Identification and Monitoring of Cancer Mutations by Targeted Deep Sequencing of Plasma DNA," *Science Translational Medicine* 4, no. 136 (May 30, 2012): 136ra68, doi:10.1126/scitranslmed.3003726.

DNA replication

Repli-Seq: R. Scott Hansen et al., "Sequencing Newly Replicated DNA Reveals Widespread Plasticity in Human Replication Timing," *Proceedings of the National Academy of Sciences* 107, no. 1 (January 5, 2010): 139–144, doi:10.1073/pnas.0912402107

ARS-Seq: Ivan Liachko et al., "High-resolution Mapping, Characterization, and Optimization of Autonomously Replicating Sequences in Yeast," *Genome Research* 23, no. 4 (April 1, 2013): 698–704, doi:10.1101/gr.144659.112.

Sort-Seq: Carolin A. Müller et al., "The Dynamics of Genome Replication Using Deep Sequencing," *Nucleic Acids Research* (October 1, 2013): gkt878, doi:10.1093/nar/gkt878.

Transcription

RNA-Seq: Ali Mortazavi et al., "Mapping and Quantifying Mammalian Transcriptomes by RNA-Seq," *Nature Methods* 5, no. 7 (July 2008): 621–628, doi:10.1038/nmeth.1226.

GRO-Seq: Leighton J. Core, Joshua J. Waterfall, and John T. Lis, "Nascent RNA Sequencing Reveals Widespread Pausing and Divergent Initiation at Human Promoters," *Science* 322, no. 5909 (December 19, 2008): 1845–1848, doi:10.1126/science.1162228.

Quartz-Seq: Yohei Sasagawa et al., "Quartz-Seq: a Highly Reproducible and Sensitive Single-cell RNA-Seq Reveals Non-genetic Gene Expression Heterogeneity," *Genome Biology* 14, no. 4 (April 17, 2013): R31, doi:10.1186/gb-2013-14-4-r31.

CAGE-Seq: Hazuki Takahashi et al., "5' End-centered Expression Profiling Using Cap-analysis Gene Expression and Next-generation Sequencing," *Nature Protocols* 7, no. 3 (March 2012): 542–561, doi:10.1038/nprot.2012.005.

Nascent-Seq: Joseph Rodriguez, Jerome S. Menet, and Michael Rosbash, "Nascent-Seq Indicates Widespread Cotranscriptional RNA Editing in Drosophila," *Molecular Cell* 47, no. 1 (July 13, 2012): 27–37, doi:10.1016/j.molcel.2012.05.002.

Precapture RNA-Seq: Tim R. Mercer et al., "Targeted RNA Sequencing Reveals the Deep Complexity of the Human Transcriptome," *Nature Biotechnology* 30, no. 1 (January 2012): 99–104, doi:10.1038/nbt.2024.

Cel-Seq: Tamar Hashimshony et al., "CEL-Seq: Single-Cell RNA-Seq by Multiplexed Linear Amplification," *Cell Reports* 2, no. 3 (September 27, 2012): 666–673, doi:10.1016/j.celrep.2012.08.003.

3P-Seq: Calvin H. Jan et al., "Formation, Regulation and Evolution of Caenorhabditis Elegans 3'UTRs," *Nature* 469, no. 7328 (January 6, 2011): 97–101, doi:10.1038/nature09616.

NET-Seq: L. Stirling Churchman and Jonathan S. Weissman, "Nascent Transcript Sequencing Visualizes Transcription at Nucleotide Resolution," *Nature* 469, no. 7330 (January 20, 2011): 368–373, doi:10.1038/nature09652.

SS3-Seq: Oh Kyu Yoon and Rachel B. Brem, "Noncanonical Transcript Forms in Yeast and Their Regulation During Environmental Stress," *RNA* 16, no. 6 (June 1, 2010): 1256–1267, doi:10.1261/rna.2038810.
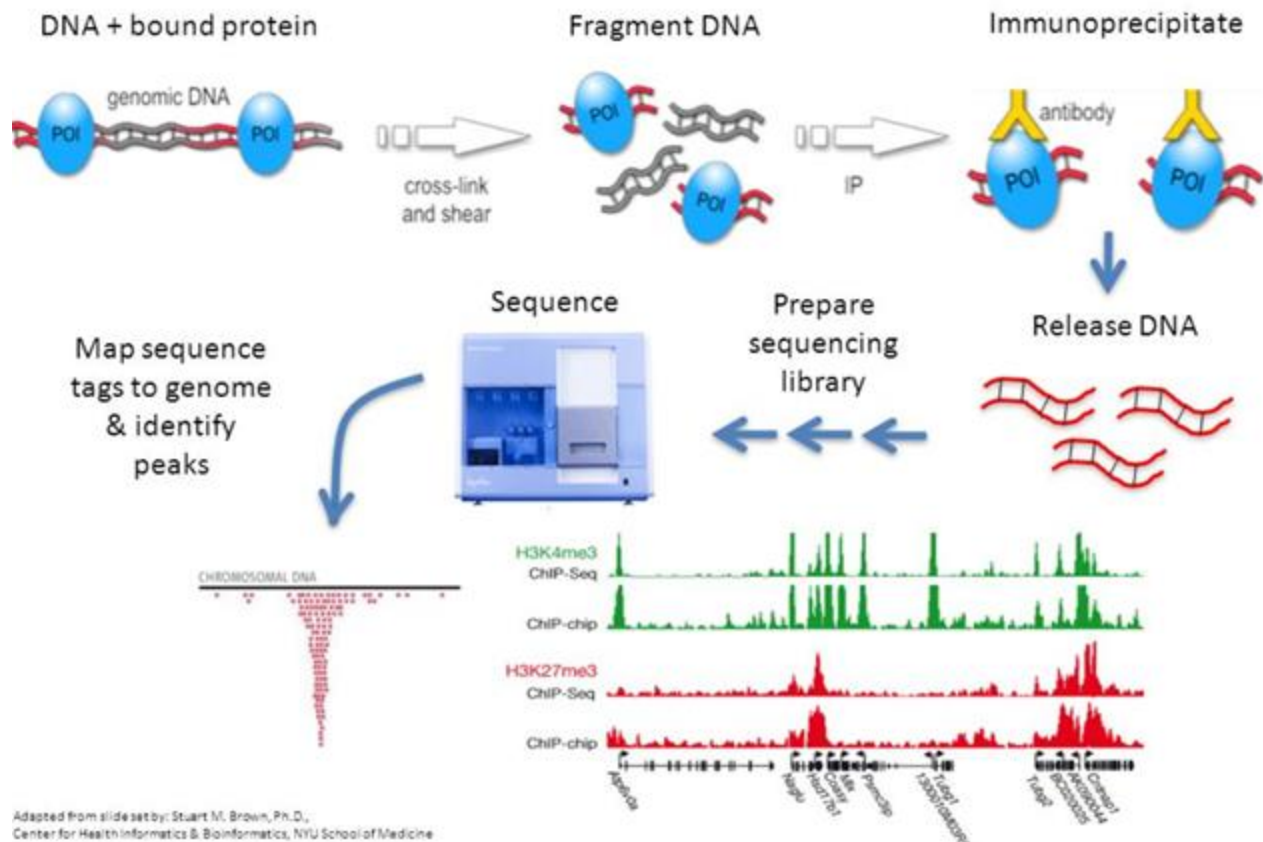
FRT-Seq: Lira Mamanova et al., "FRT-seq: Amplification-free, Strand-specific Transcriptome Sequencing," *Nature Methods* 7, no. 2 (February 2010): 130–132, doi:10.1038/nmeth.1417.

3-Seq: Andrew H. Beck et al., "3'-End Sequencing for Expression Quantification (3SEQ) from Archival Tumor Samples," *PLoS ONE* 5, no. 1 (January 19, 2010): e8768, doi:10.1371/journal.pone.0008768.
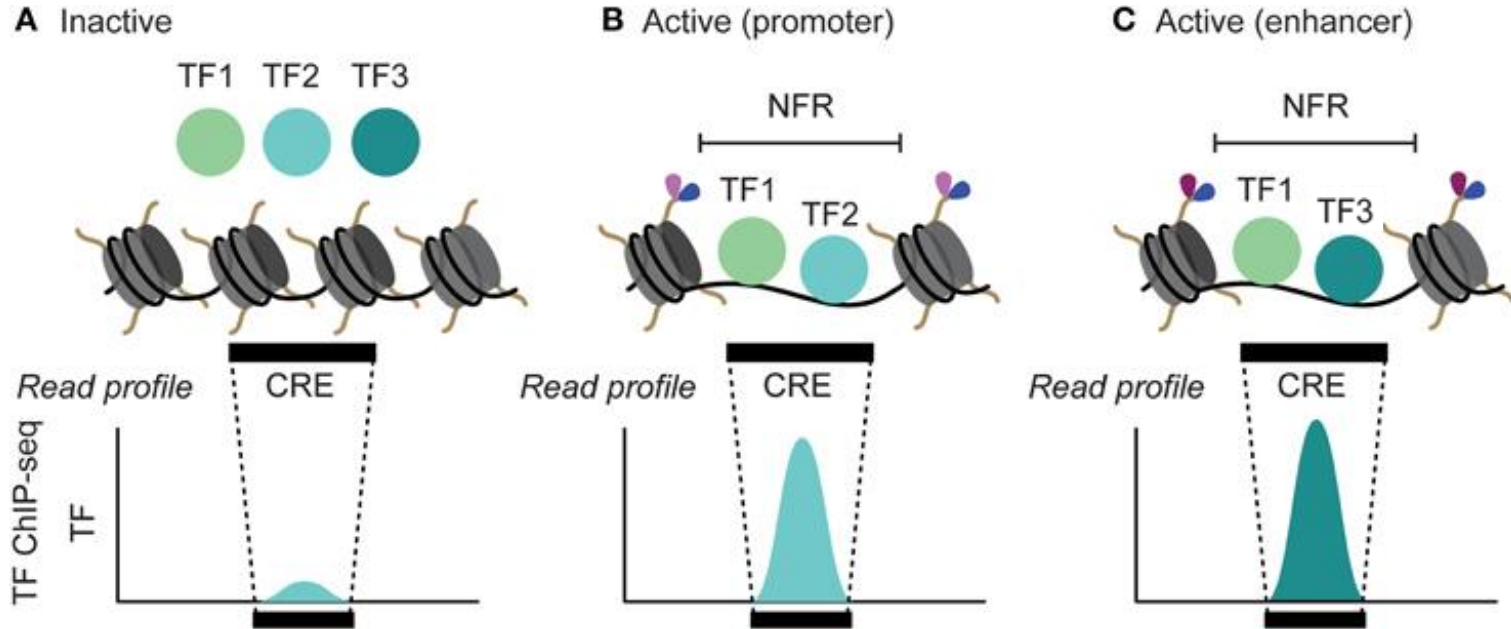
PRO-Seq: Hojoong Kwak et al., "Precise Maps of RNA Polymerase Reveal How Promoters Direct Initiation and Pausing," *Science* 339, no. 6122 (February 22, 2013): 950–953, doi:10.1126/science.1229386.

Bru-Seq: Artur Veloso et al., "Genome-Wide Transcriptional Effects of the Anti-Cancer Agent Camptothecin," *PLoS ONE* 8, no. 10 (October 23, 2013): e78190, doi:10.1371/journal.pone.0078190.

ChIP-seq

Adapted from slide set by: Stuart M. Brown, Ph.D.,
Center for Health Informatics & Bioinformatics, NYU School of Medicine
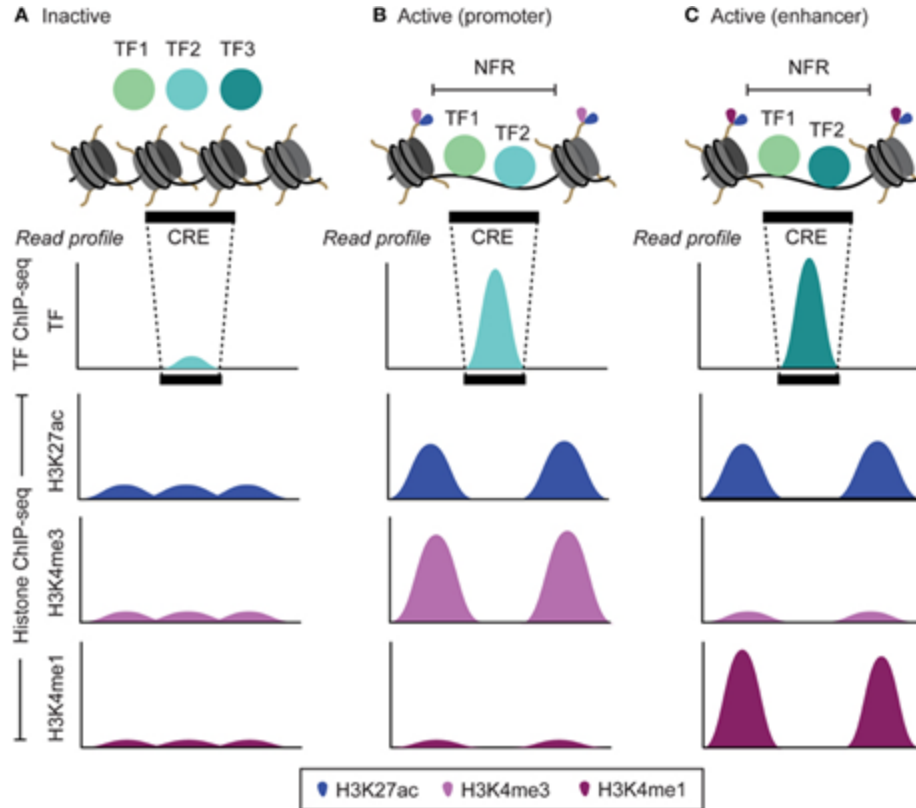
Mapping transcription-factor binding locations

NFR = nucleosome free region
CRE = Cis regulatory element

Mapping histone modifications

H3K4me3:

H3 = name of histone
K4 = 4th lysine residue
me3 = tri-methylation

NFR = nucleosome free region
CRE = Cis regulatory element

CUT&RUN

Potentially less off-target/background signal

Very similar, from a data perspective
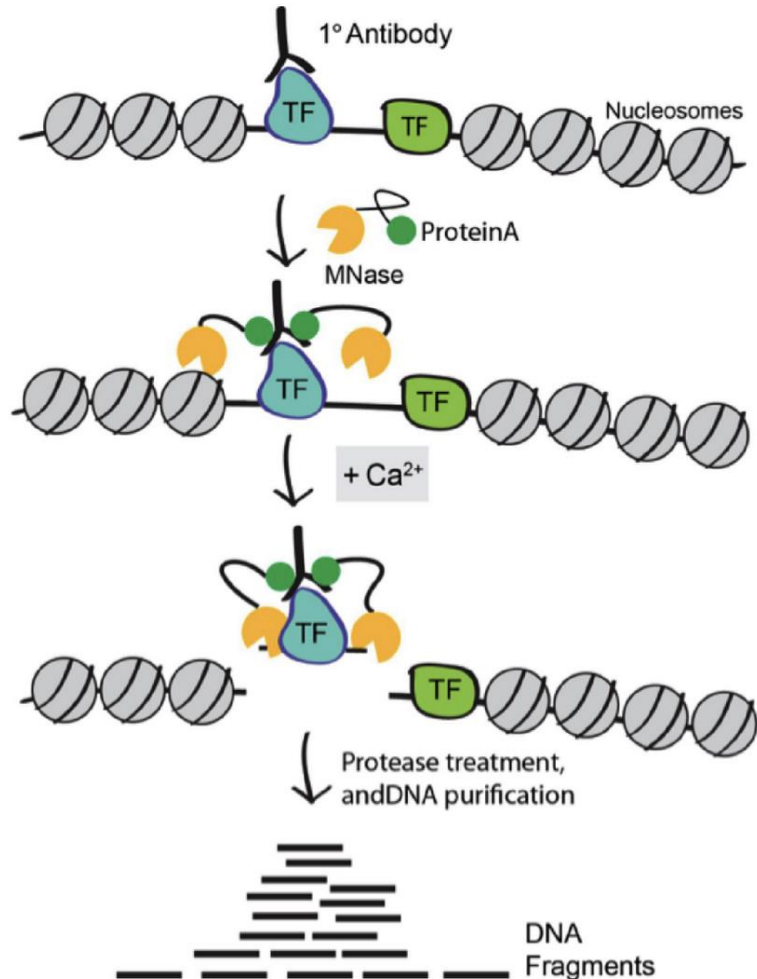
Figure 1. CUT&RUN schematic (see text for details).

ATAC-seq

DNA accessibility
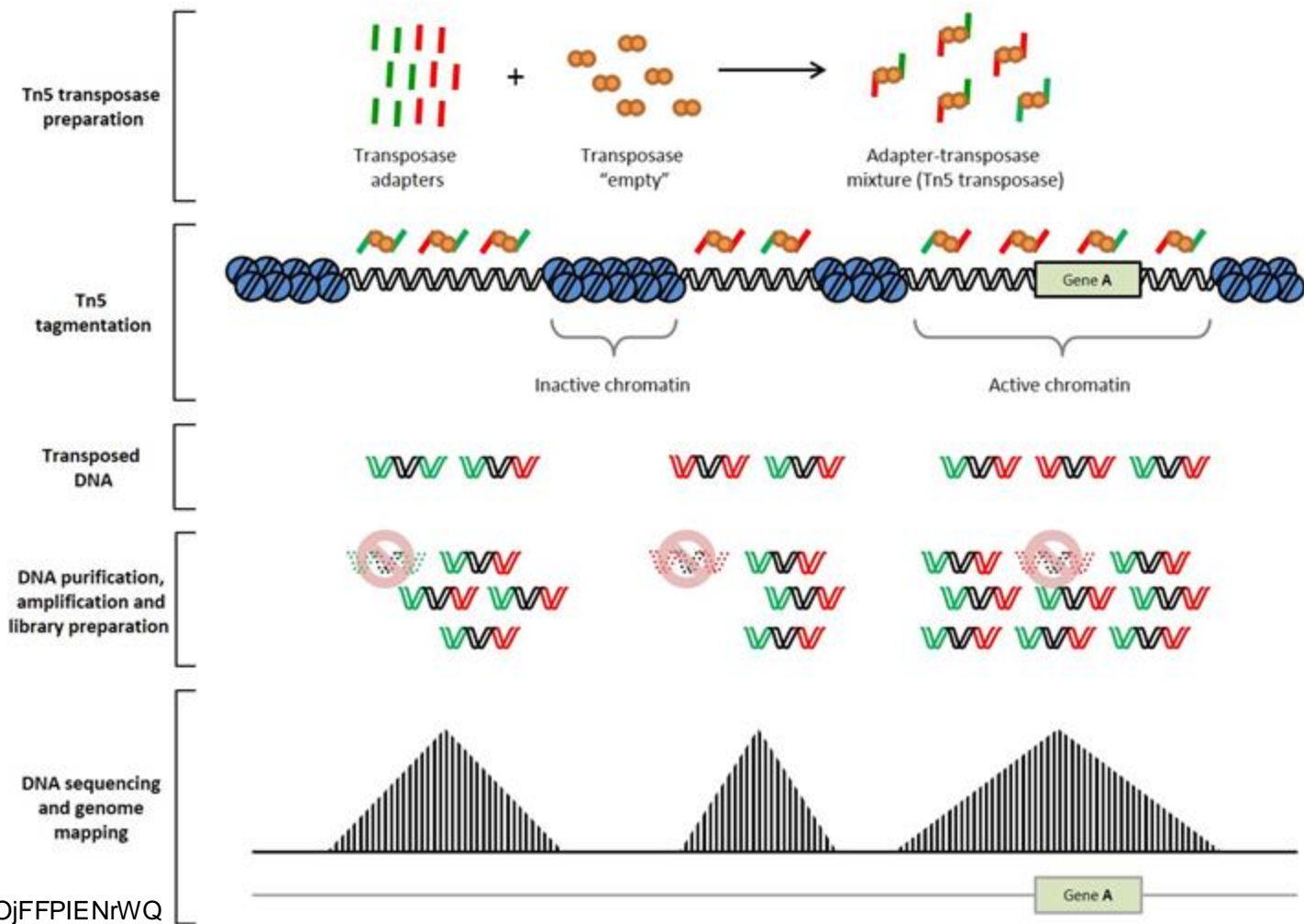
Examining 3-D DNA interactions in the nucleus

a Chromatin conformation capture

Crosslink proteins and DNA
↓
Sample fragmentation
• Restriction enzymes
↓
Ligation
↓
PCR amplify ligated junctions
↓
DNA library creation and paired-end sequencing

b ChIA-PET

Crosslink proteins and DNA
↓
Sample fragmentation
• Sonication
↓
Immunoprecipitate
↓
Ligation
↓
Restriction enzyme digestion
↓
DNA library creation and paired-end sequencing

Fiber-seq

Methyltransferase-based assay:
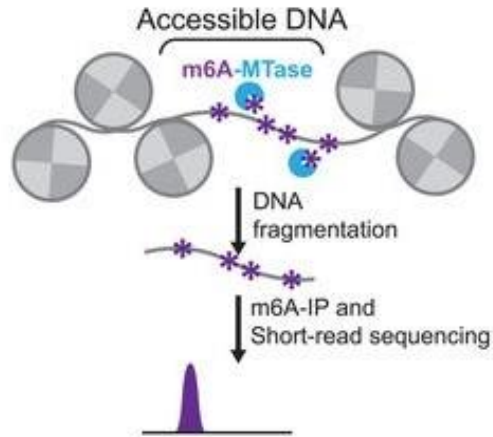
Long-read sequencing (PacBio/ONT)
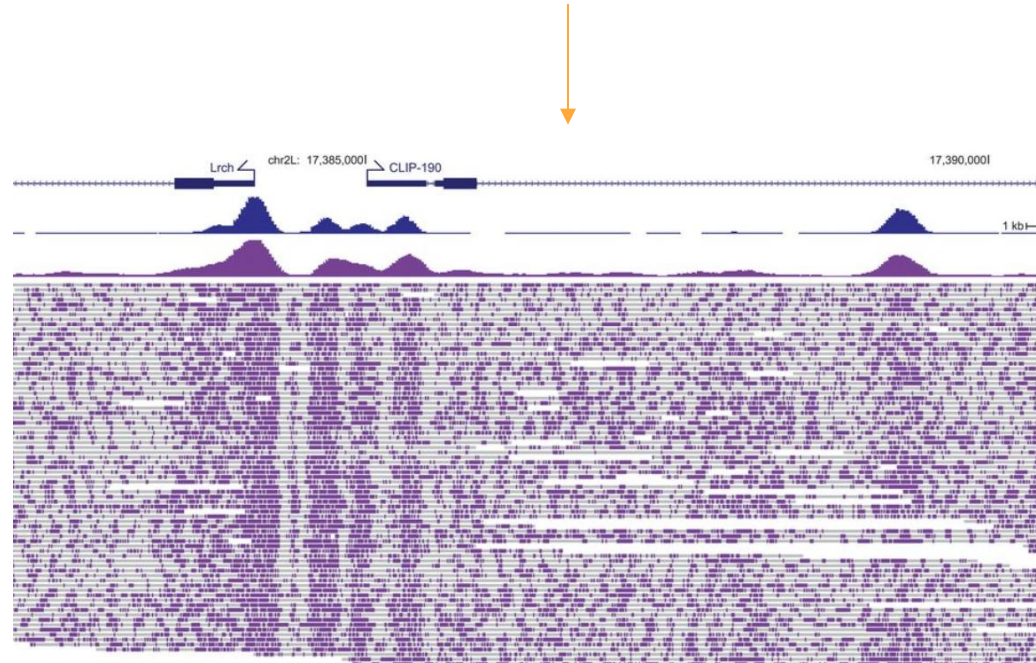
Peak-calling
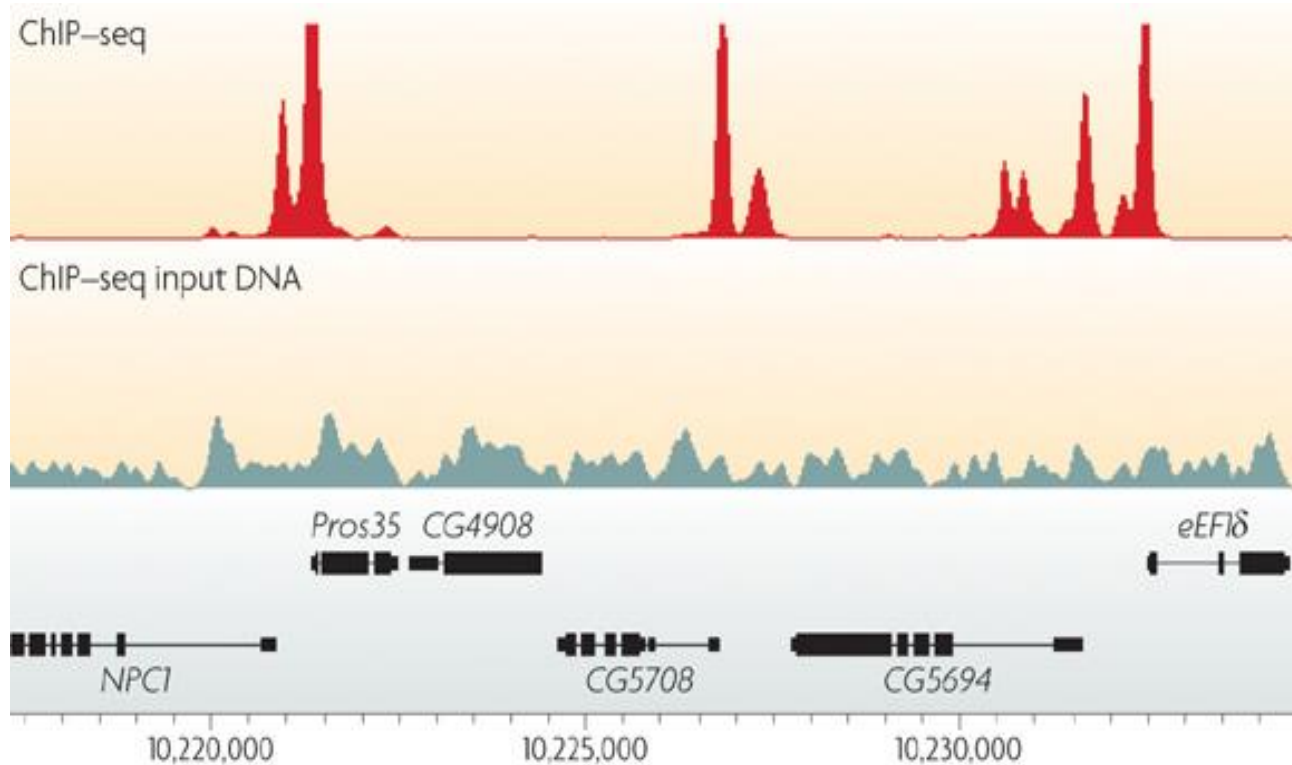
Fundamentally a signal vs noise problem

Proteins bind in different ways

Transcription factor – tight, highly-peaked binding region
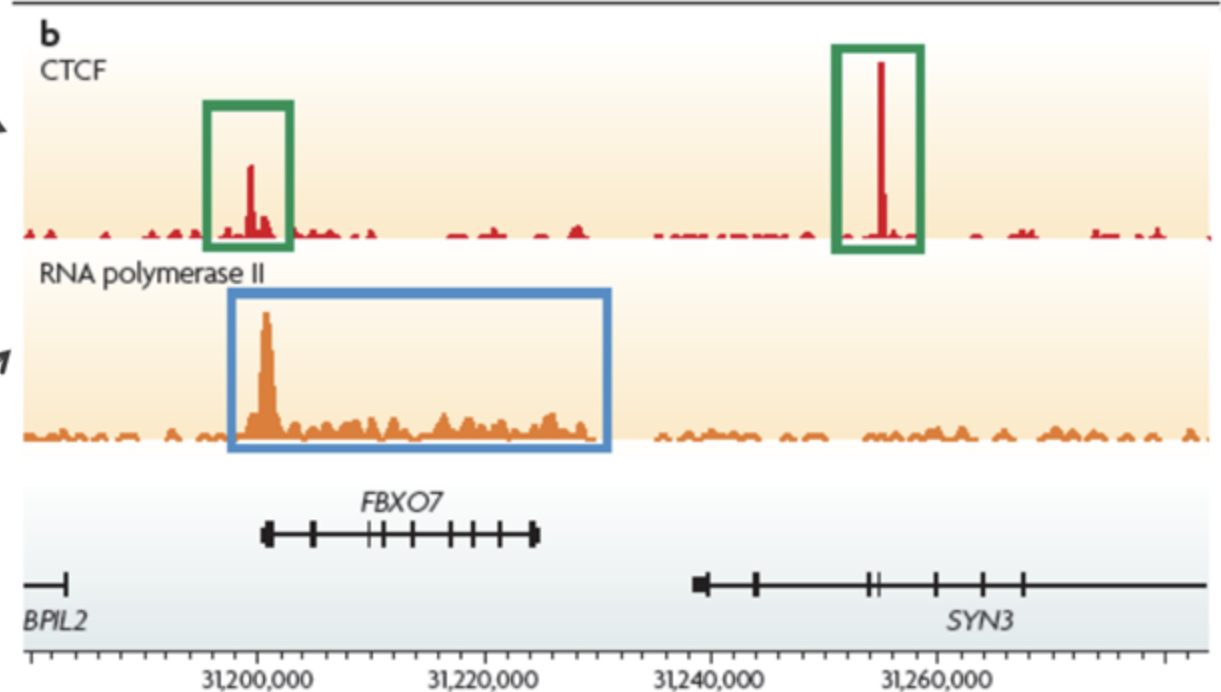
RNA PolII – enriched at TSS but bound throughout gene body

How much sequence coverage do we need?

Transcription factor – tight, highly-peaked binding region

b
CTCF

For mammalian TFs, other chromatin mods like enhancer-associated histone marks:

- have on the order of thousands of binding sites,
- 20 million reads may be adequate
- (4 million reads for worm and fly TFs).

How much sequence coverage do we need?

More binding sites (e.g., RNA Pol II) or broader factors, including most histone marks,

- require more reads, up to 60 million for mammalian ChIP-seq.

RNA PolII – enriched at TSS but bound throughout gene body
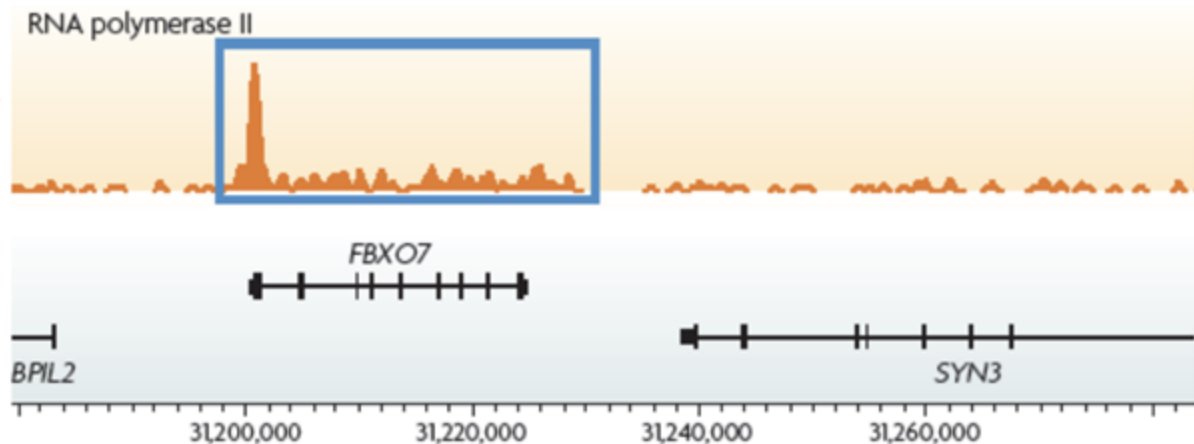
How much sequence coverage do we need?

- In TF experiments, Control samples should be sequenced **more deeply** than the ChIP-seq samples

  - True for assays involving diffused broad-domain chromatin data.

- This is to ensure sufficient coverage of a substantial portion of the genome and non-repetitive autosomal DNA regions.
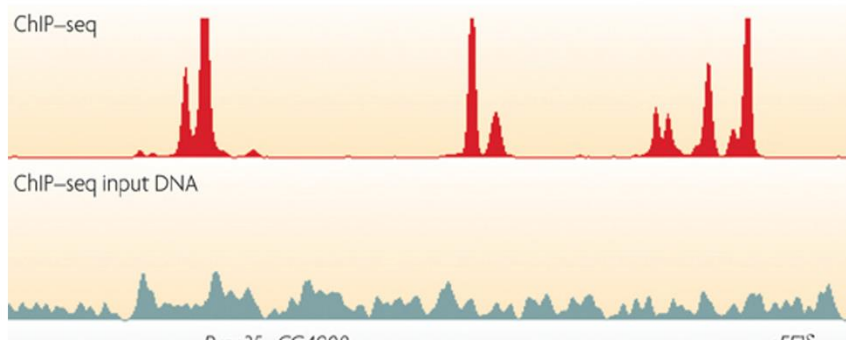
ChIP-seq statistics

Continuous variables:
- Your exact height
- Your dog's exact weight
- The winning time in a race
- Exact distance between stars
- Your exact age
- Time it takes a computer to complete a task.

Discrete variables:
- The number of lightbulbs that burn out in a warehouse in a given week.
- The number of heads when flipping a coin 50 times.
- The number of students in a class
- The number of times you forget the attachment to an email on Fridays.
- The number of green M&Ms in a bag
- **The number of times a given base is sequenced.**

The Poisson distribution:
discrete distribution to model coverage

$$P(k \text{ discrete events}) = \lambda^k e^k / k!$$

Where e is Euler's constant (2.718), and ƛ is the average number of occurrences of an event

Example: the "hundred year flood". Thus ƛ=1 (1 catastrophic flood every 100 years

Example: the "hundred year flood".
Thus ƛ=1 (1 catastrophic flood every 100 years

$$P(k \text{ overflow floods in 100 years}) = \frac{\lambda^k e^{-\lambda}}{k!} = \frac{1^k e^{-1}}{k!}$$

$$P(k = 0 \text{ overflow floods in 100 years}) = \frac{1^0 e^{-1}}{0!} = \frac{e^{-1}}{1} = 0.368$$

$$P(k = 1 \text{ overflow flood in 100 years}) = \frac{1^1 e^{-1}}{1!} = \frac{e^{-1}}{1} = 0.368$$

$$P(k = 2 \text{ overflow floods in 100 years}) = \frac{1^2 e^{-1}}{2!} = \frac{e^{-1}}{2} = 0.184$$

k	P(k overflow floods in 100 years)
0	0.368
1	0.368
2	0.184
3	0.061
4	0.015
5	0.003
6	0.0005

Example: expected number of goals in a World Cup game.

Average number of goals is 2.5

$$P(k \text{ goals in a match}) = \frac{2.5^k e^{-2.5}}{k!}$$

$$P(k = 0 \text{ goals in a match}) = \frac{2.5^0 e^{-2.5}}{0!} = \frac{e^{-2.5}}{1} = 0.082$$

$$P(k = 1 \text{ goal in a match}) = \frac{2.5^1 e^{-2.5}}{1!} = \frac{2.5 e^{-2.5}}{1} = 0.205$$

$$P(k = 2 \text{ goals in a match}) = \frac{2.5^2 e^{-2.5}}{2!} = \frac{6.25 e^{-2.5}}{2} = 0.257$$

Note that λ does not have to be a countable integer.

k	P(k goals in a World Cup soccer match)
0	0.082
1	0.205
2	0.257
3	0.213
4	0.133
5	0.067
6	0.028

Poisson distribution with different values of λ
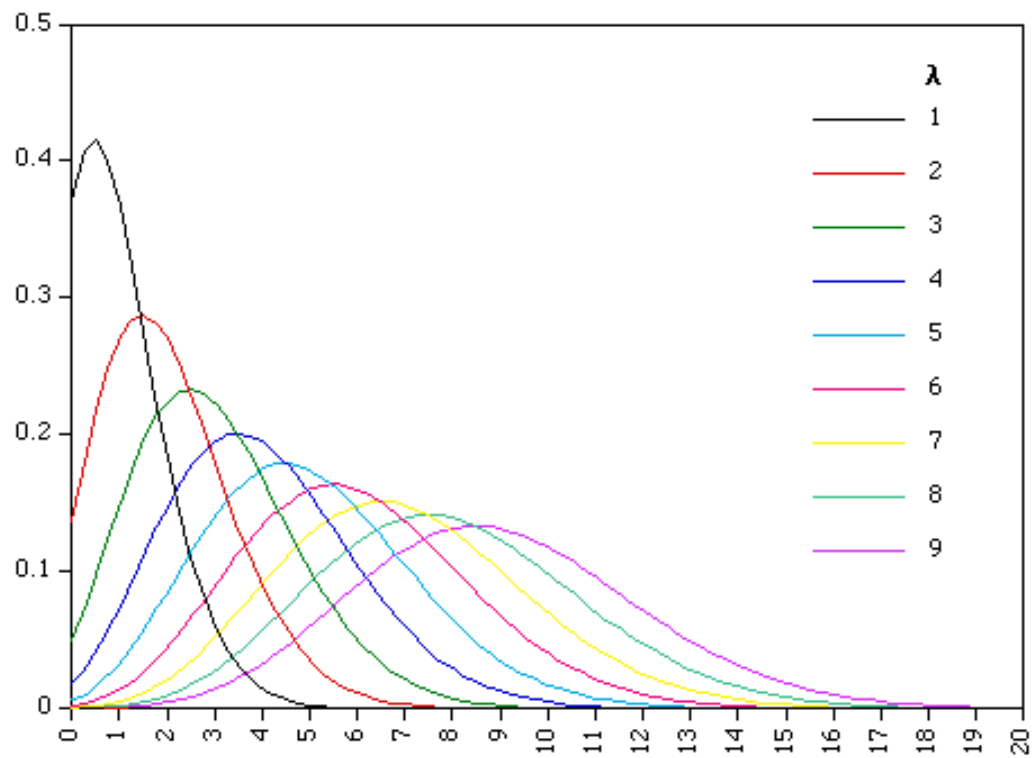
Ideally, sequencing coverage will follow a Poisson distribution. But...
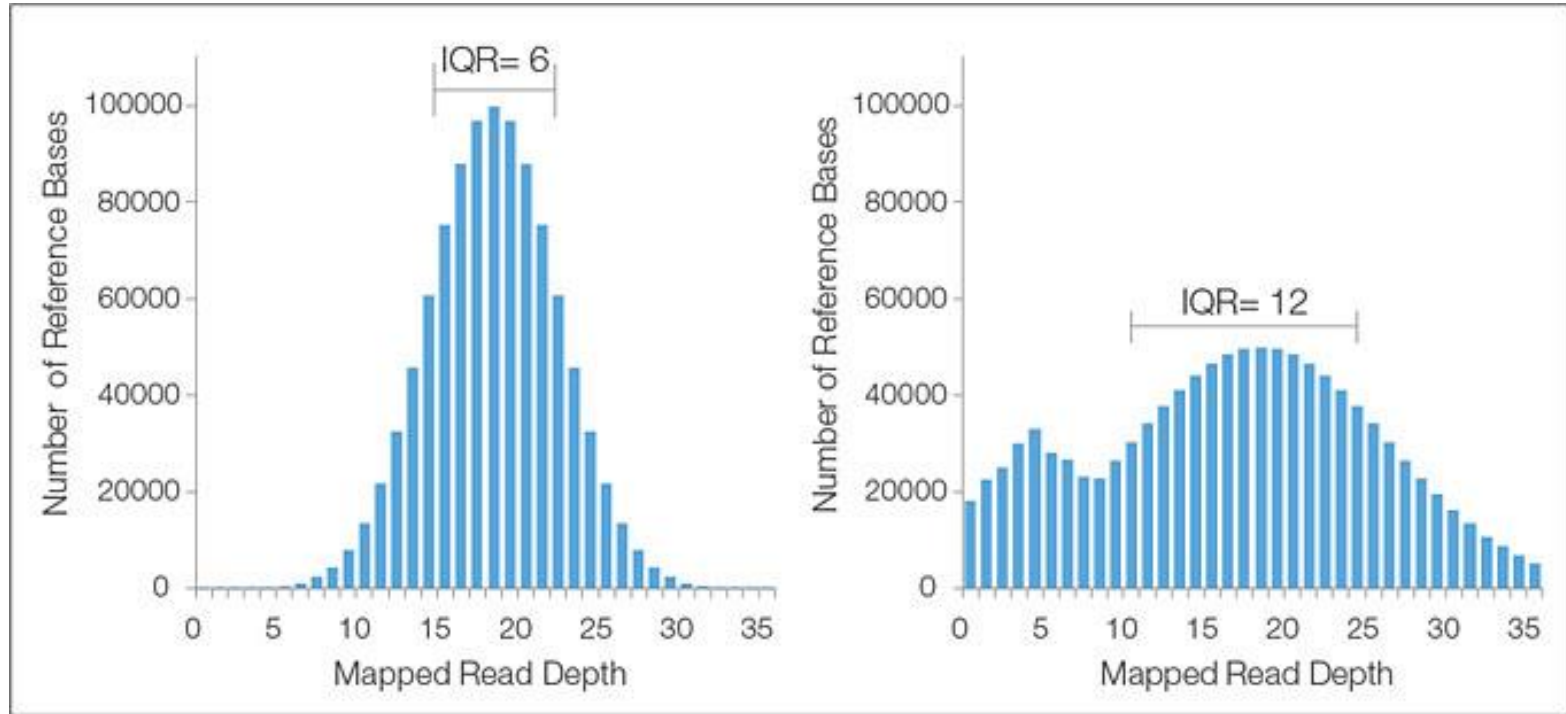
Poisson

Not Poisson.
Overly "dispersed"

Negative binomial fits sequencing coverage data much better

Miller, et al. ReadDepth
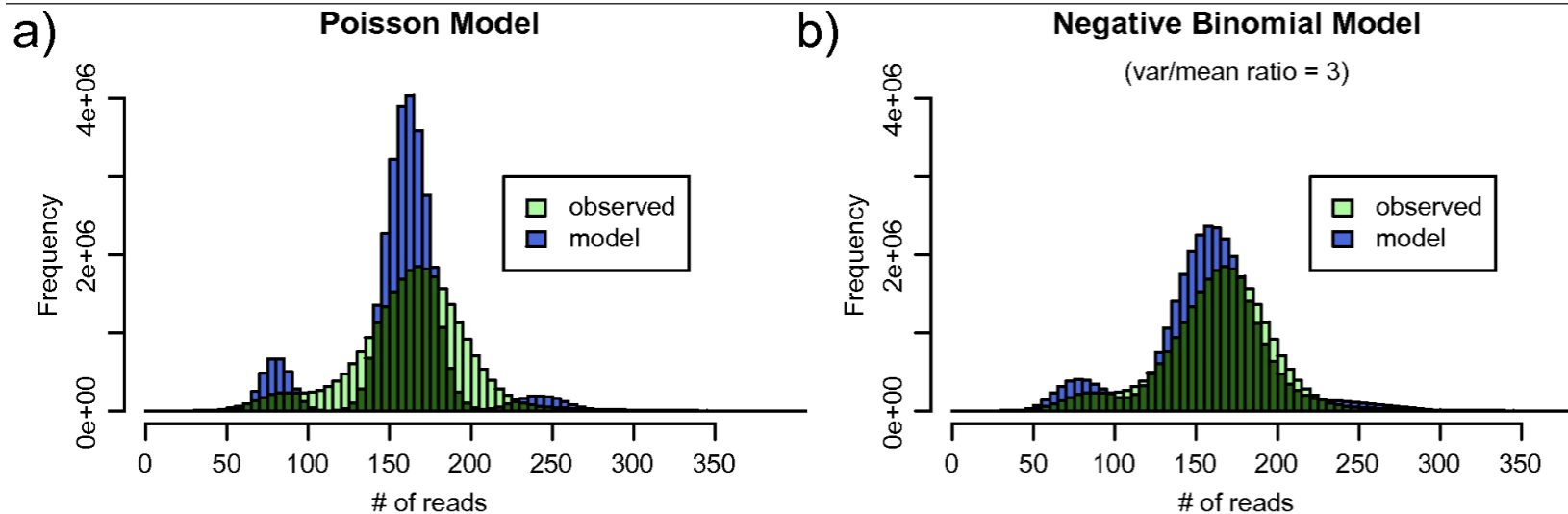
Comparative ChIP-seq: scaling and normalizing
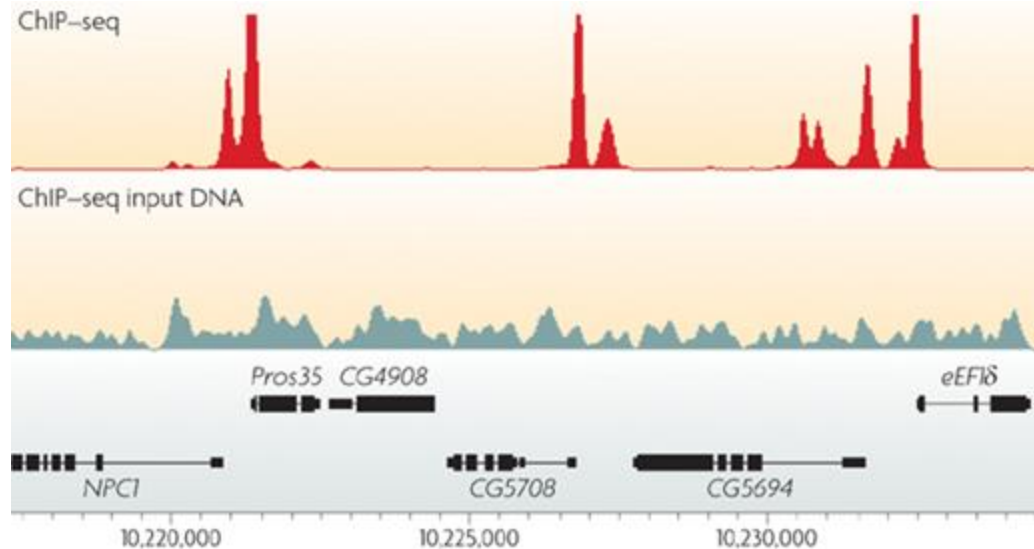
Sequencing depths from TF ChIP should be compared to control (input DNA) to get a sense of the noise.

When comparing two ChIP-seq experiments, you need to normalize the counts / peaks before doing so. **E.g., comparing experiments where one had 10 million reads and the other had 100 million reads.**

Peak-calling

MACS2, HOMER, SEACR, etc
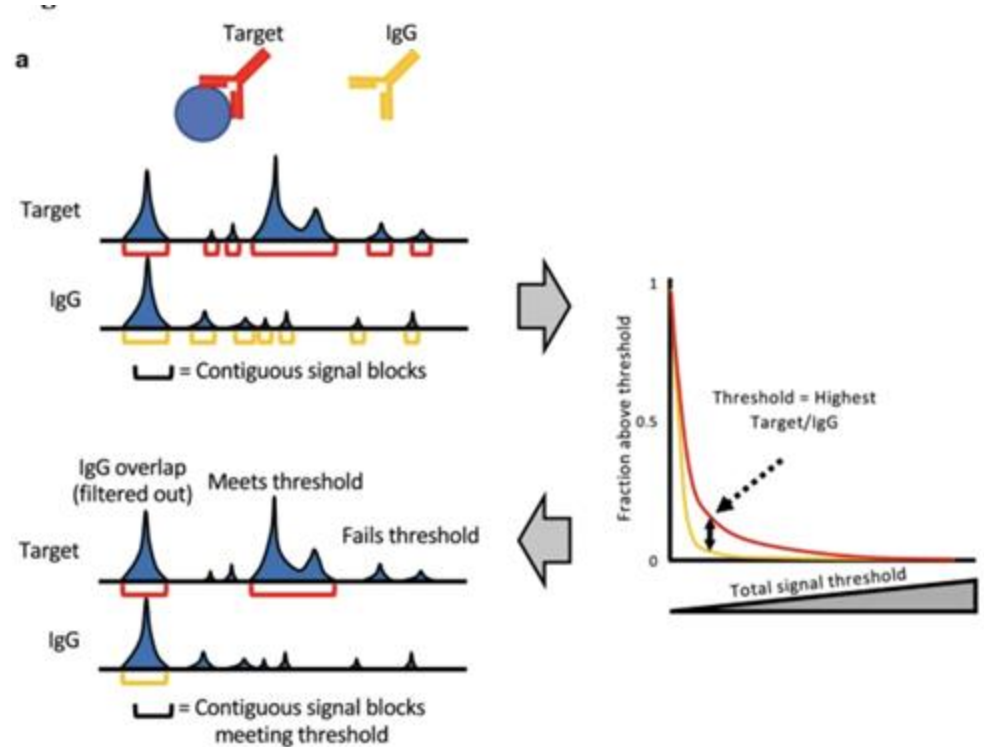
ChIP-seq peak callers

Table S1. Examples of peak callers employed in ChIP-seq.

Software tool	Version	Availability	Point-source (peaks)	Broad regions (domains)
BayesPeak [88]	1.10.0	http://bioconductor.org/packages/release/bioc/html/BayesPeak.html	Yes	
BEADS[‡] [84]	1.1	http://beads.sourceforge.net/	Yes	Yes
CCAT [91]	3.0	http://cmb.gis.a-star.edu.sg/ChIPSeq/paperCCAT.htm		Yes
CisGenome [56]	2.0	http://www.biostat.jhsph.edu/~hji/cisgenome/	Yes	
CSAR [85]	1.10.0	http://bioconductor.org/packages/release/bioc/html/CSAR.html	Yes	
dPeak	0.9.9	http://www.stat.wisc.edu/~chungdon/dpeak/	Yes	
GPS/GEM [67,18]	1.3	http://cgs.csail.mit.edu/gps/	Yes	
HPeak [87]	2.1	http://www.sph.umich.edu/csg/qin/HPeak/	Yes	
MACS [17]	2.0.10	https://github.com/taoliu/MACS/	Yes	Yes
NarrowPeaks[‡]	1.4.0	http://bioconductor.org/packages/release/bioc/html/NarrowPeaks.html	Yes	
PeakAnalyzer/ PeakSplitter[‡] [89]	1.4	http://www.bioinformatics.org/peakanalyzer	Yes	
PeakRanger [93]	1.16	http://ranger.sourceforge.net/	Yes	Yes
PeakSeq [24]	1.1	http://info.gersteinlab.org/PeakSeq	Yes	
polyaPeak[‡]	0.1	http://web1.sph.emory.edu/users/hwu30/polyaPeak.html	Yes	
RSEG [92]	0.6	http://smithlab.usc.edu/histone/rseg/		Yes
SICER [90]	1.1	http://home.gwu.edu/~wpeng/Software.htm		Yes
SIPeS [21]	2.0	http://gmdd.shgmo.org/Computational-Biology/ChIP-Seq/download/SIPeS	Yes	
SISSRs [19]	1.4	http://sissrs.rajajothi.com/	Yes	
SPP [9]	1.1	http://compbio.med.harvard.edu/Supplements/ChIP-seq/	Yes	Yes
USeq [97]	8.5.1	http://sourceforge.net/projects/useq/	Yes	
ZINBA [86]	2.02.03	http://code.google.com/p/zinba/	Yes	Yes

[‡] Only for post-processing.

MACS is probably the most widely used

http://journals.plos.org/ploscompbiol/article?id=10.1371/journal.pcbi.1003326

Replicates, replicates, replicates
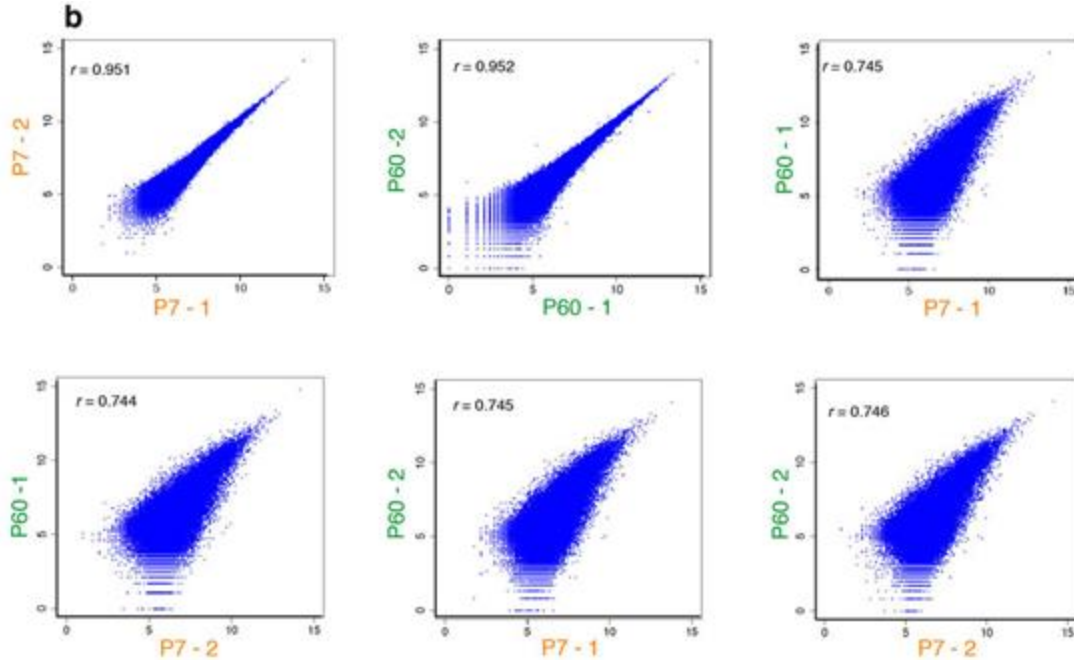
Pearson Correlation is one metric indicative of overall reproducibility

(**b**) Scatterplots of pairwise Zic ChIP-seq replicates with Pearson correlation (*r*) displayed. **Note the correlations are much higher between biological replicates of the same developmental stage than between P7 and P60 cerebellum**.
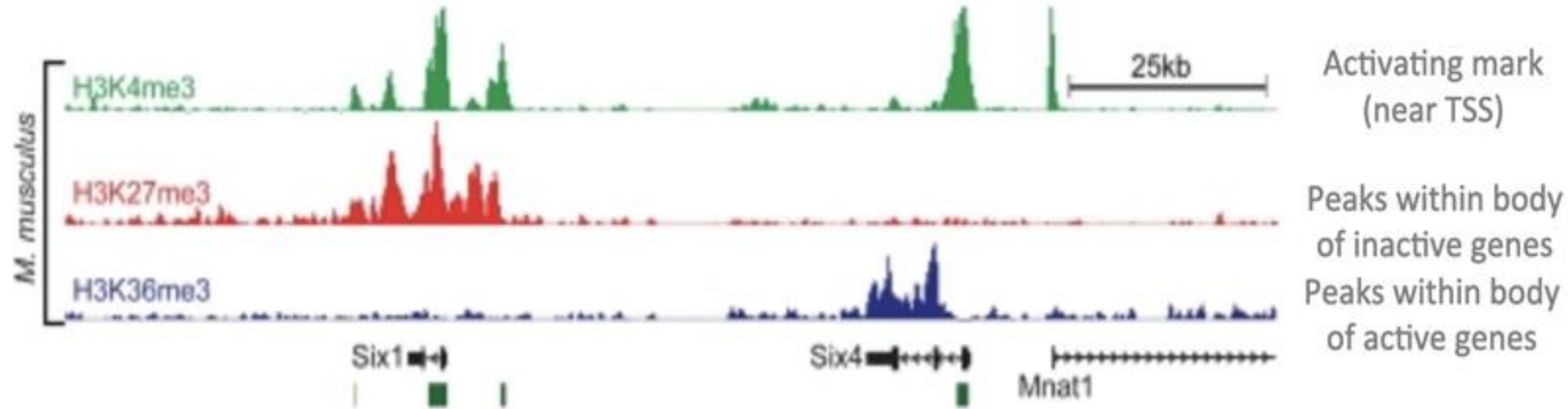
Irreproducibility Discovery Rate (IDR)
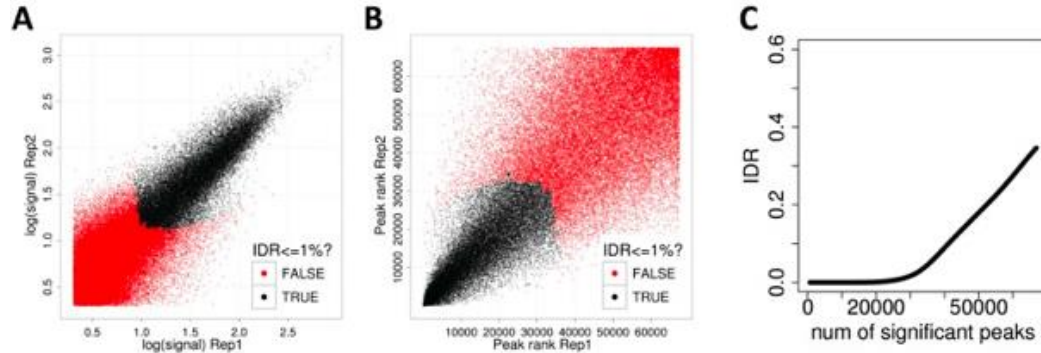
Peaks that show up
consistently among replicates
are more likely to be real!
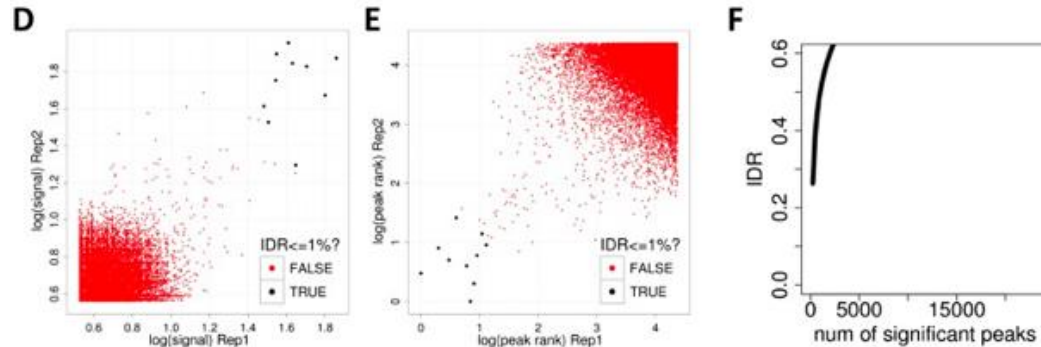
Fantastic resource for
learning ChIP-seq analysis
https://github.com/hbctraining/Intro-to-ChIPseq

Interpretation

Genome Segmentation with ChromHMM

Be aware that segmentation may be tissue or cell-type specific!

The Encyclopedia of DNA Elements Project
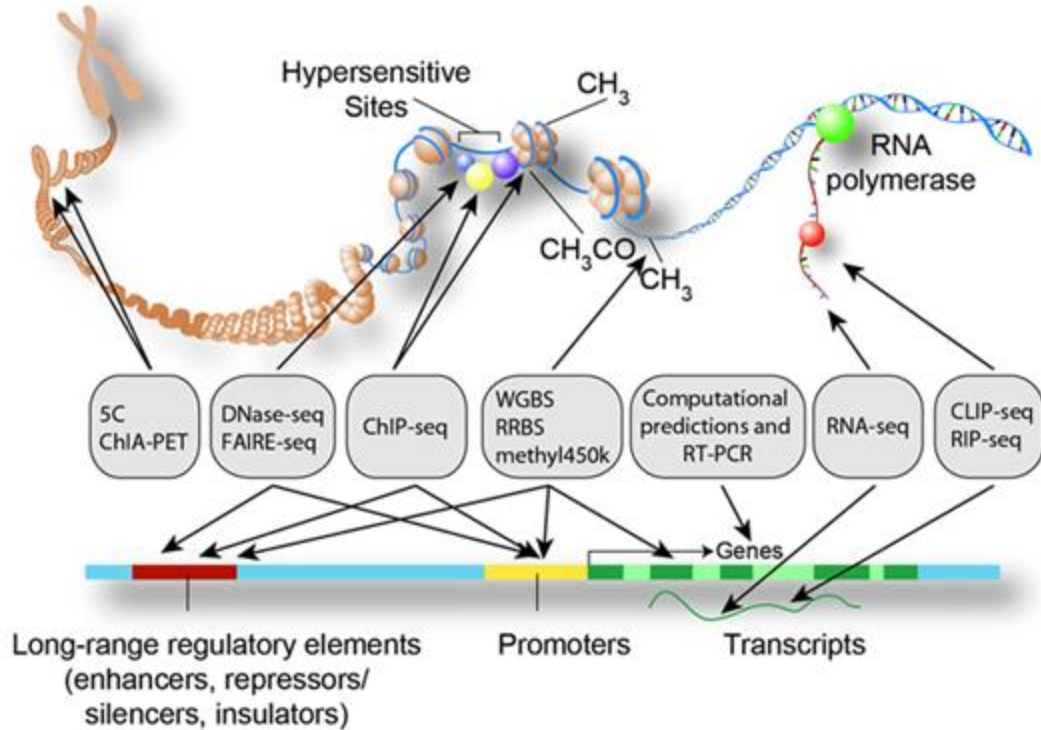
Roadmap Epigenomics Project
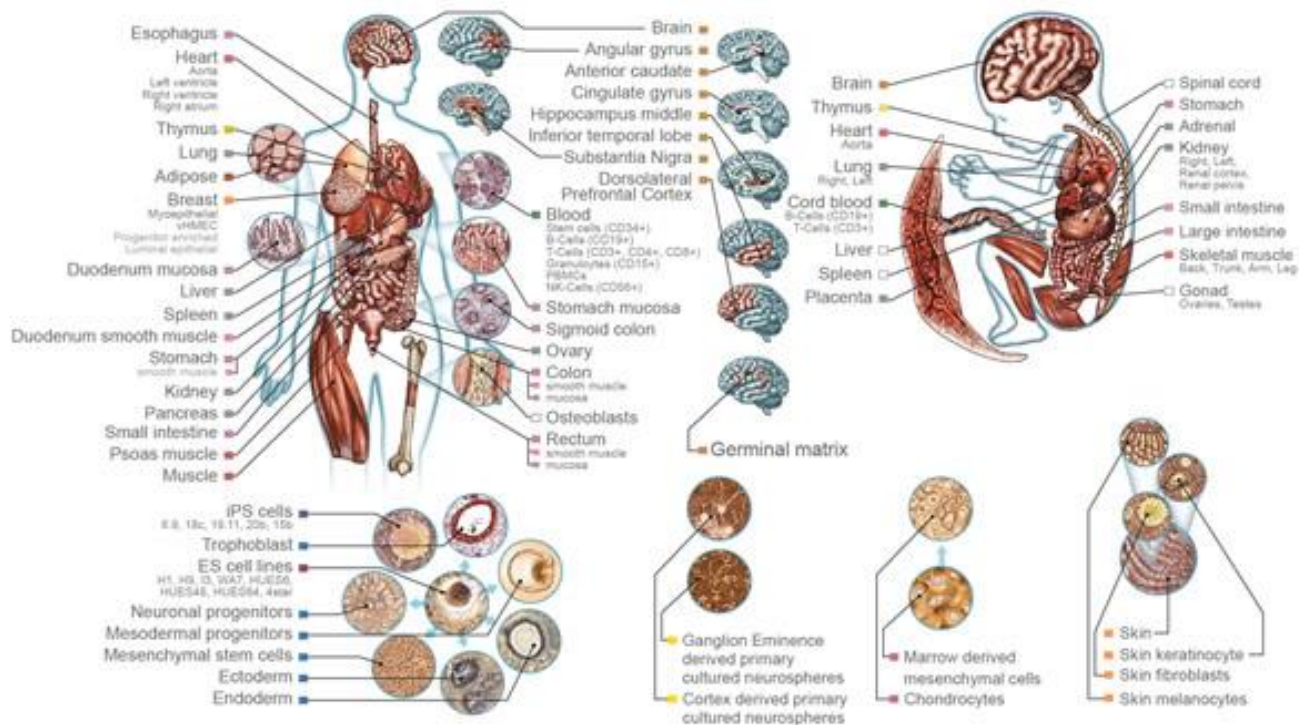
Epigenomics: Bisulfite sequencing

Chris Miller, Ph.D.
Washington University in St. Louis

Some slides adapted from:
https://github.com/genome/bfx-workshop
https://github.com/quinlan-lab/applied-computational-genomics

Epigenetics

The two main components of the epigenetic code

DNA methylation
Methyl marks added to certain DNA bases repress gene activity.

Histone modification
A combination of different molecules can attach to the 'tails' of proteins called histones. These alter the activity of the DNA wrapped around them.
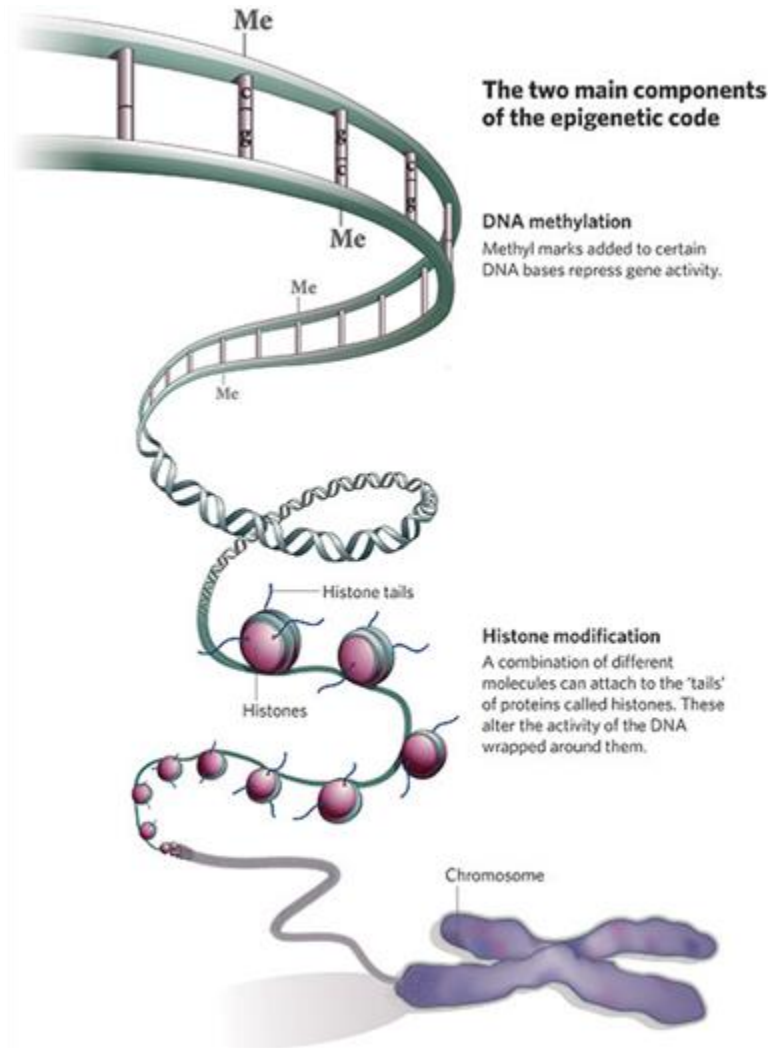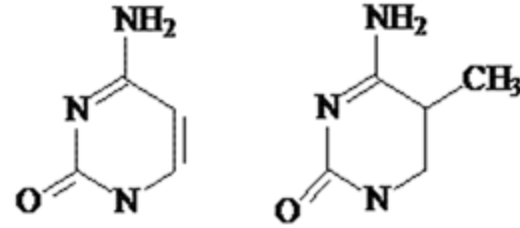
DNA Methylation

- Mostly happens at CpGs

- About 25 million CpGs in
  human genome

DNA Methylation

- CpG Islands

- Length >= 200 bp
  GC% > 50%
  o/e CpG ratio > 60%

- Selective pressure/
  Evolutionary constraint
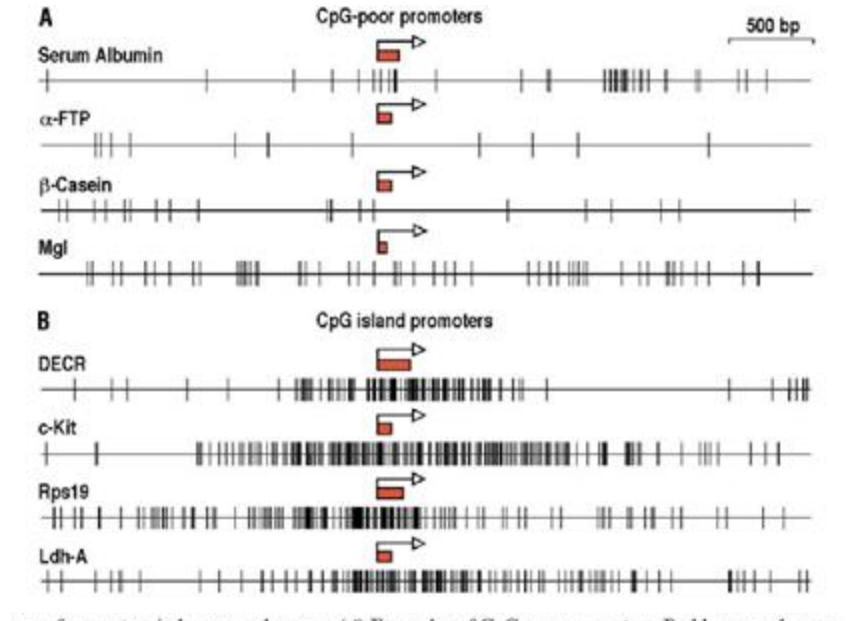
Islands, shores, and shelves
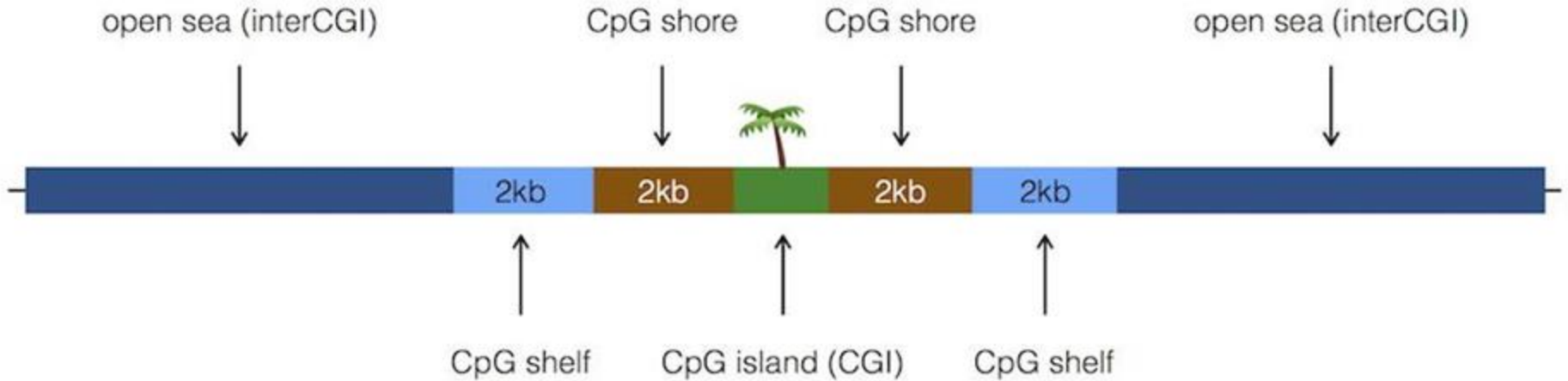
What does DNA methylation do?

- The short answer: It depends!

- X-chromosome inactivation

- Silencing of transposable elements

- Cellular differentiation

- Cancer - hypo/hypermethylation
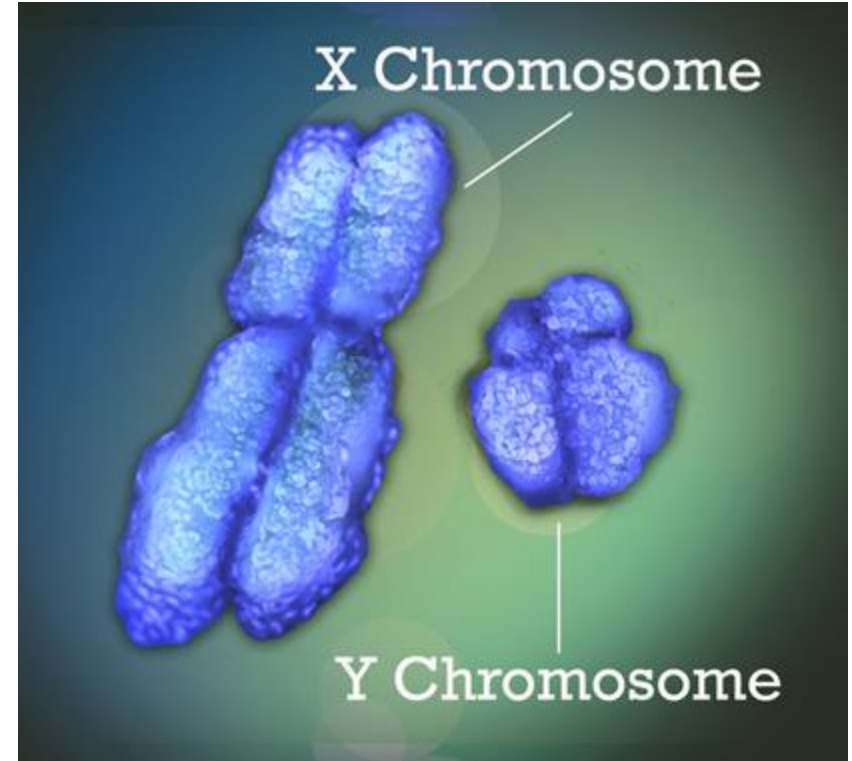
MGMT and Temozolomide

- TMZ is an alkylating agent -
  damages DNA, causes cell death

- MGMT "cleans up" the damage

- Methylation of the MGMT

  promoter is linked to better

  outcomes!
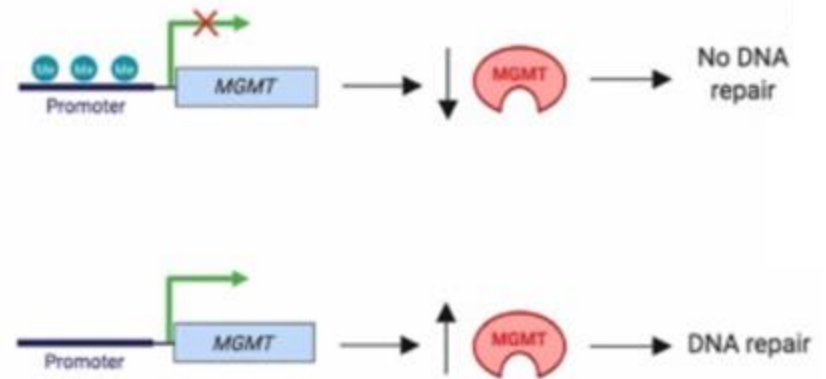
Methylation Patterns

- Methyltransferases that act locally
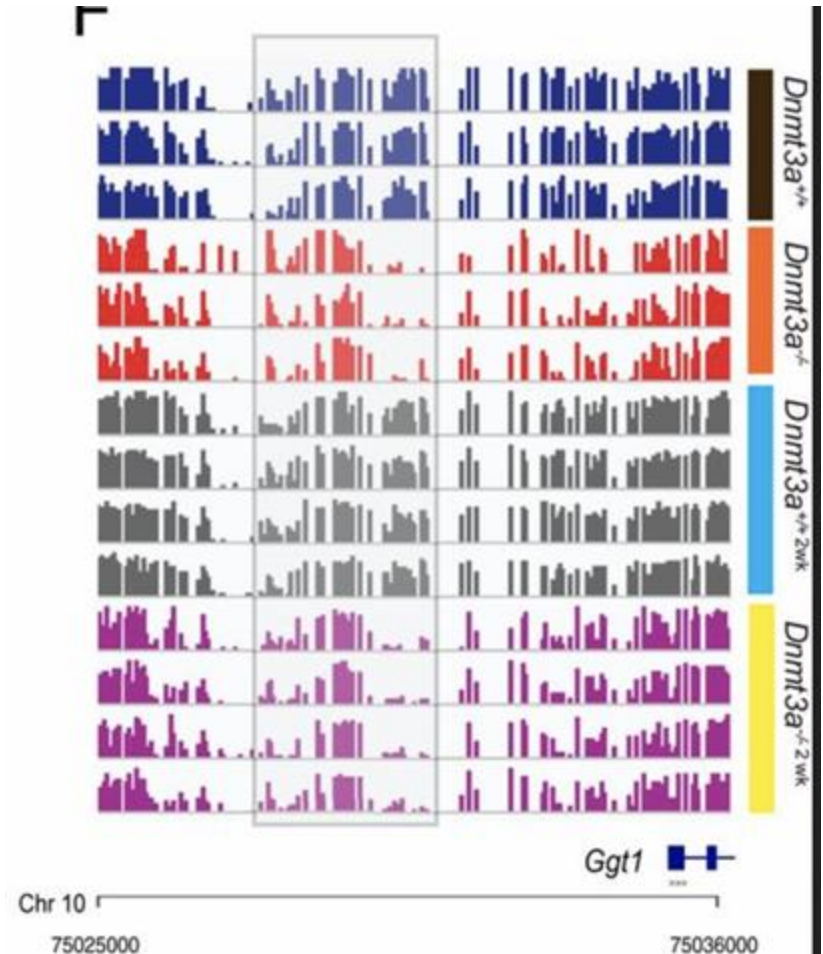
Methylation Patterns

- Methyltransferases that act locally

- Other alterations (or treatments) that act globally
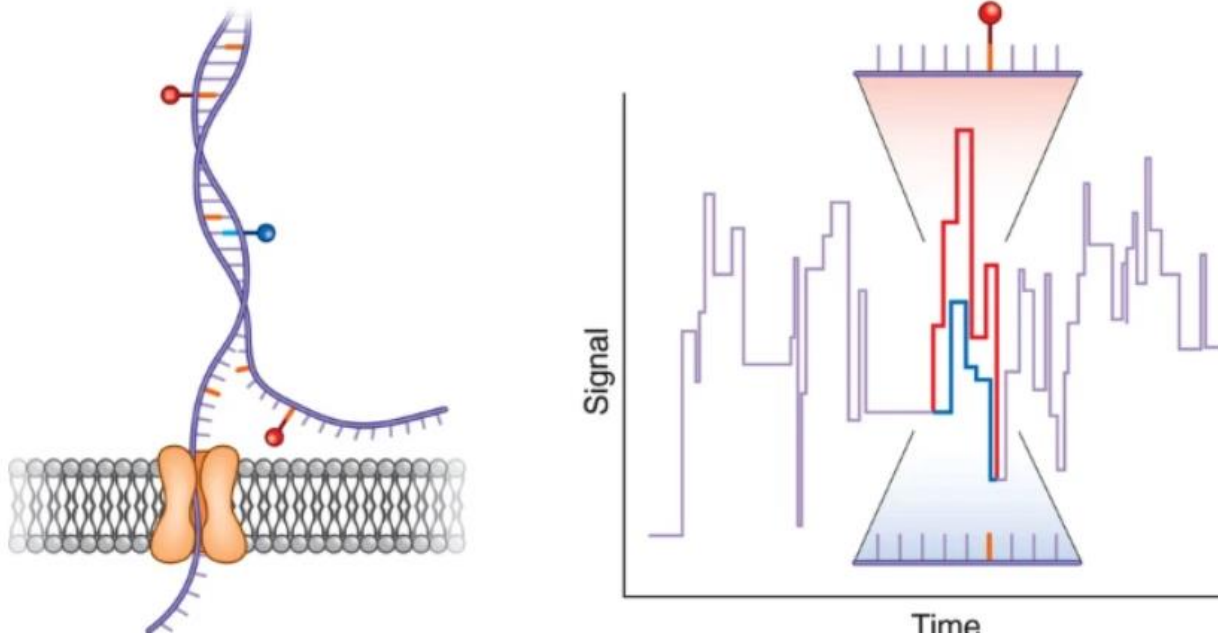

Methylation fraction

Bisulfite sequencing

cytosine → cytosine sulphonate → uracil sulphonate → uracil
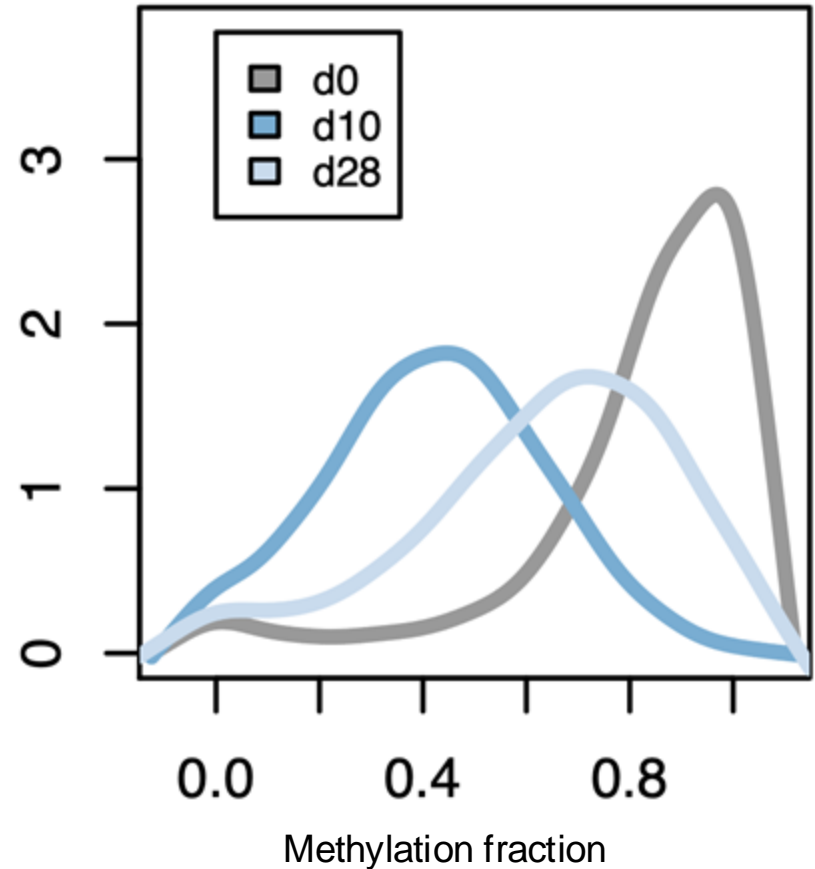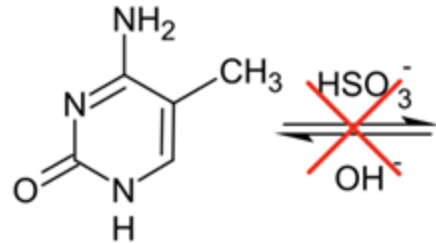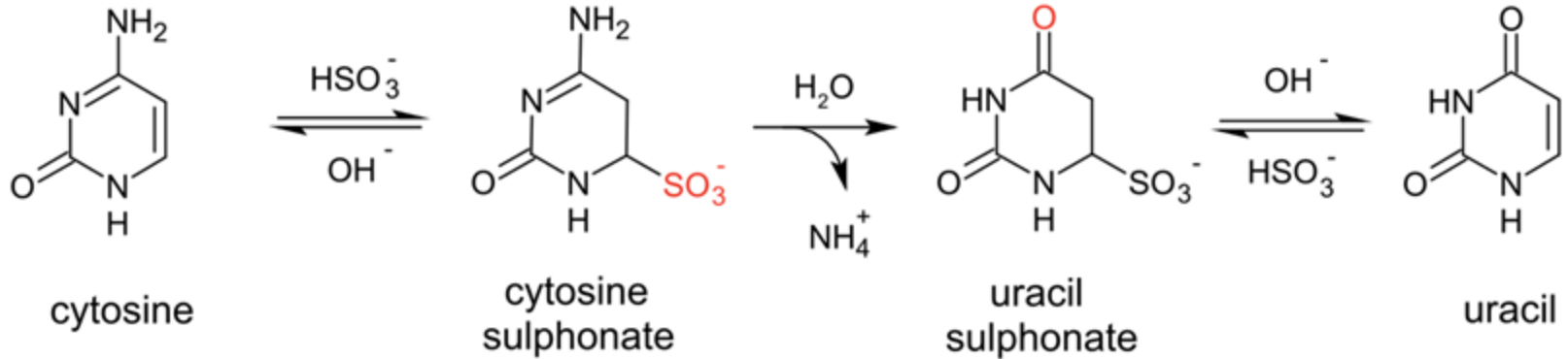
5-methylcytosine

Bisulfite sequencing

Bisulfite sequencing

BSMAP: whole genome bisulfite sequence MAPping program. BMC Bioinformatics 10:232
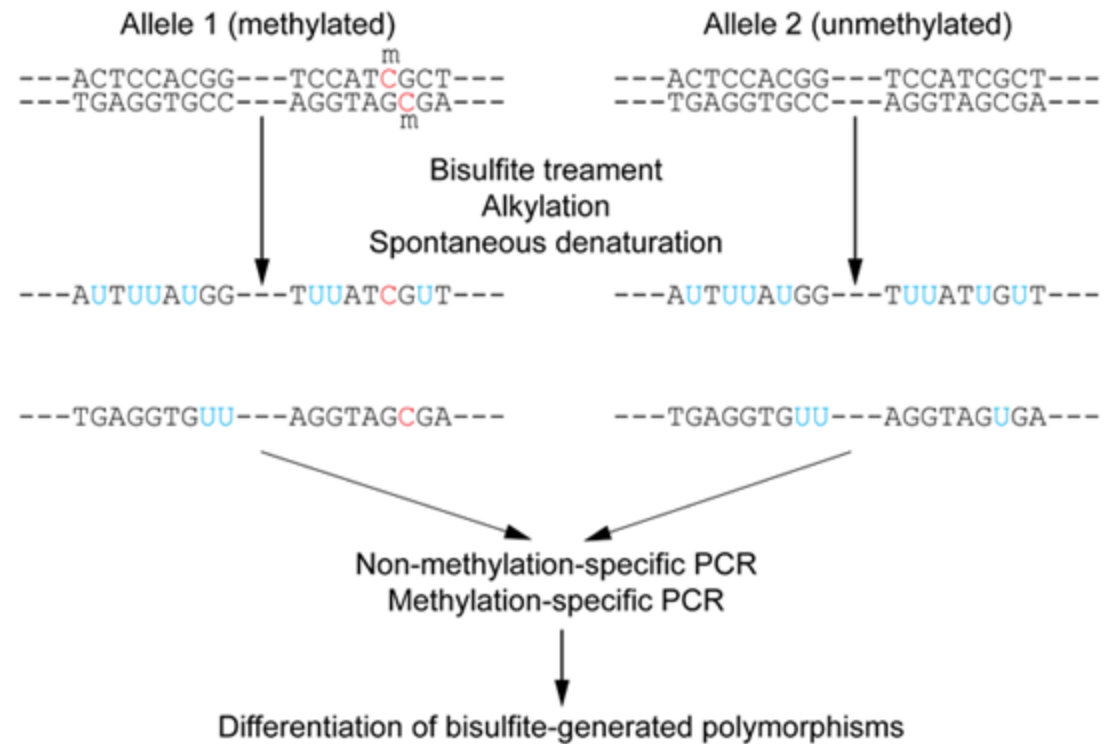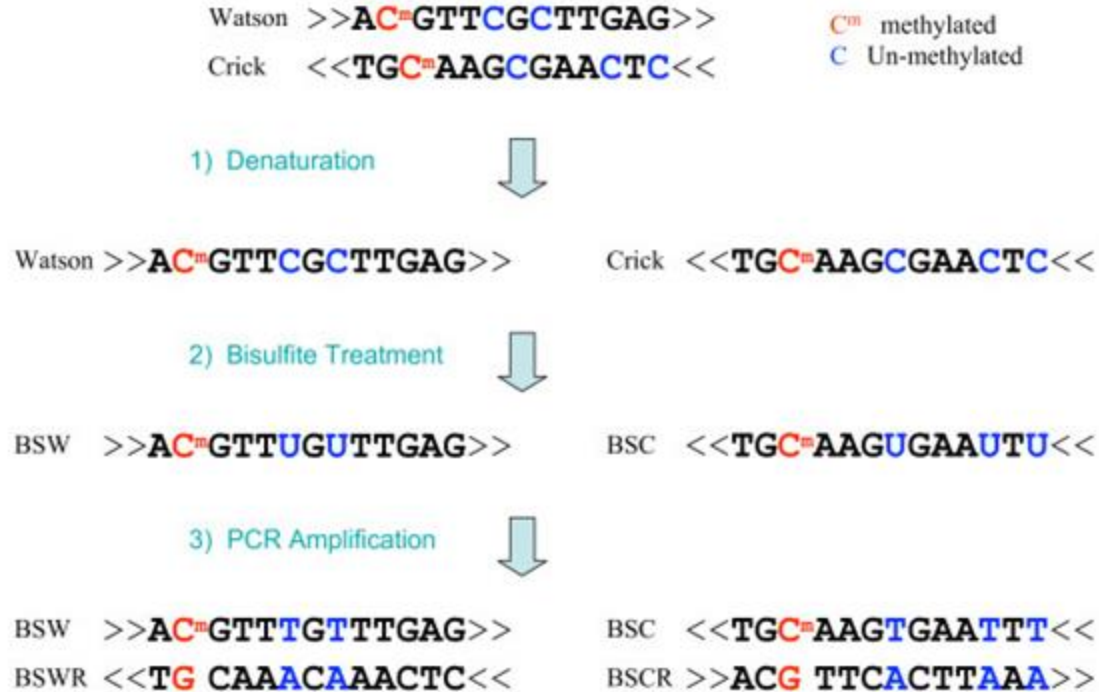
Whole-genome Bisulfite Sequencing (WGBS)

- Need a special aligner - has to expect many C > T mismatches!

- BSMAP
- bismark
- BWA-meth
- biscuit

Direct detection with long read sequencing

Can be used for 5mC as well as m6A in direct RNAseq

Methylation calling

- Determine methylation fraction at each site in the genome

  - Count the Cs and Ts, taking strandedness into account

  - Some tools account for SNPs while doing this

-

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Dnmt3a⁺/⁺

Methylation calling

- Determine methylation fraction at each site in the genome

    - Count the Cs and Ts, taking strandedness into account

    - Some tools account for SNPs while doing this

- Why isn't every position 0%, 50% or 100%?
    - we're sequencing a population of cells!

Dnmt3a⁺/⁺

Workflow/File formats

- Aligning: FASTQ > BAM/CRAM

- Pileup: BAM/CRAM > VCF
  - (entries for every site, allele frequencies)

- VCF > bedgraph
  - chr, start, stop, beta_value (methylation fraction)

- bedgraph > bigwig (for visualization in IGV)

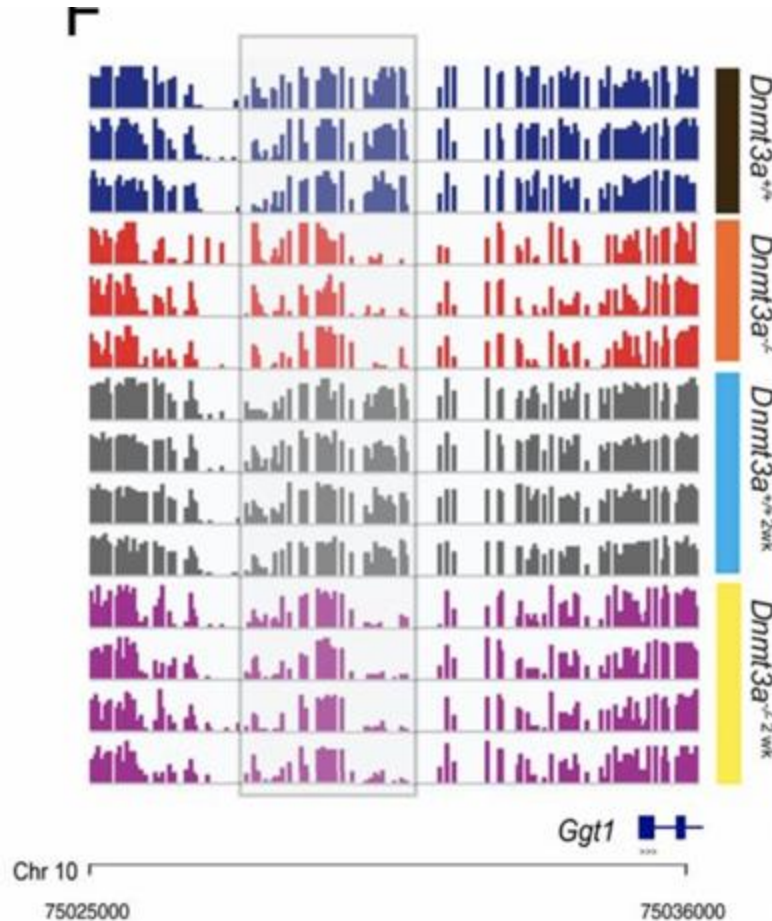- There are workflows for this!

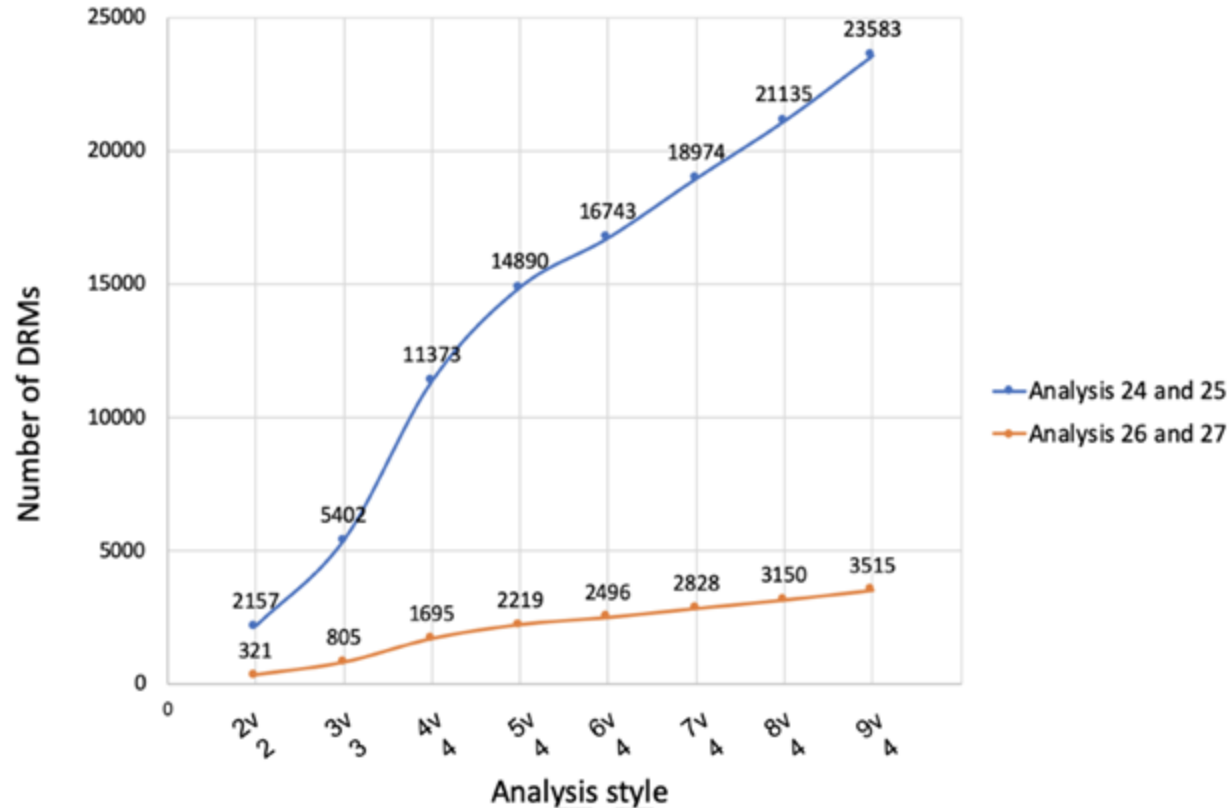# IGV visualization
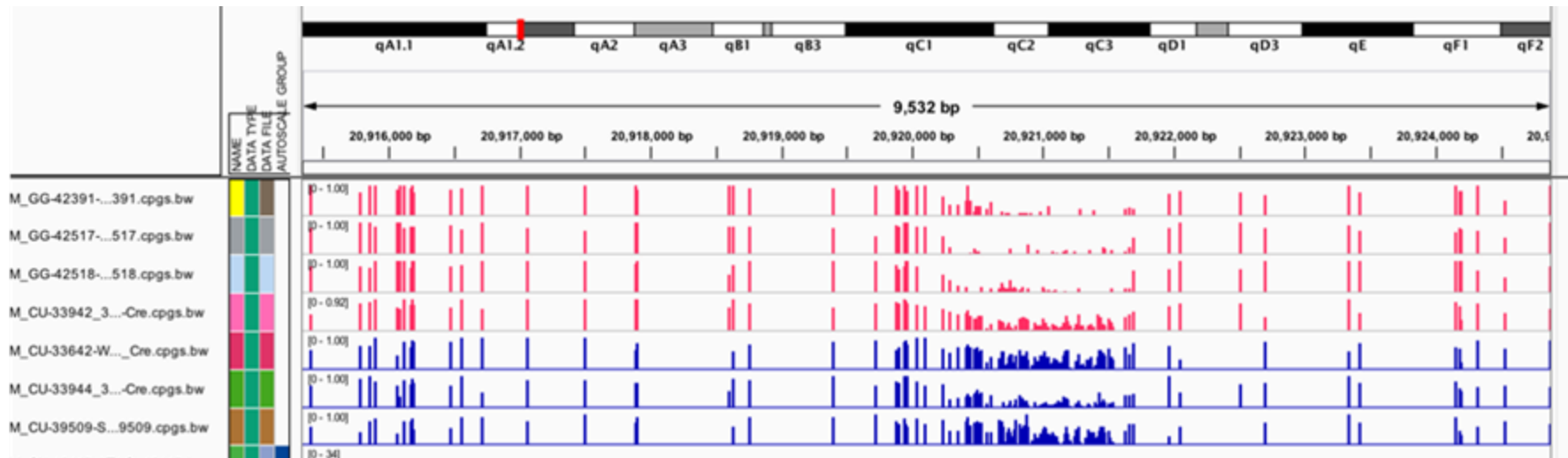
Differentially methylated regions

- Comparing two groups to find changes

- Finding DMRs is a segmentation problem
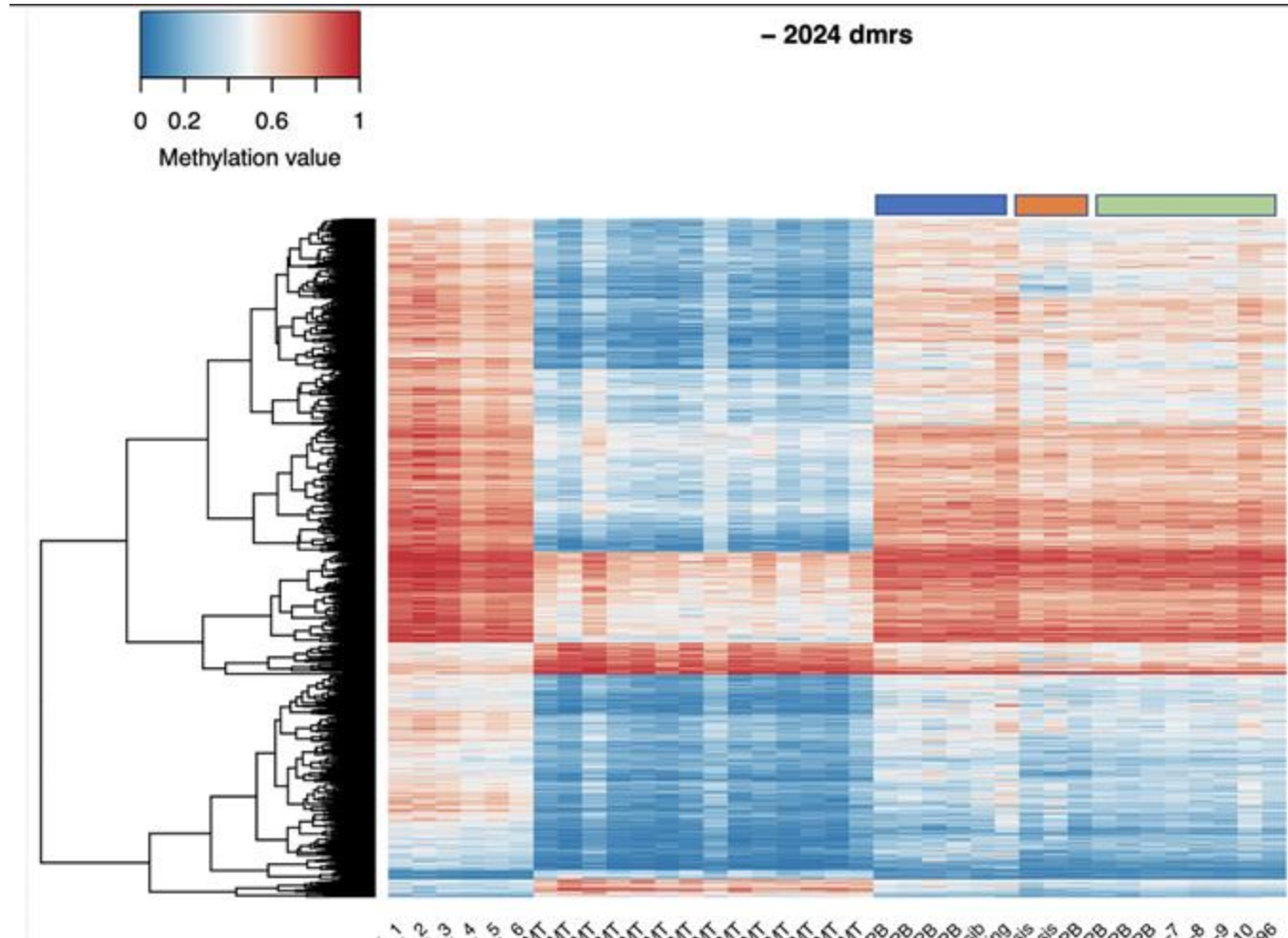
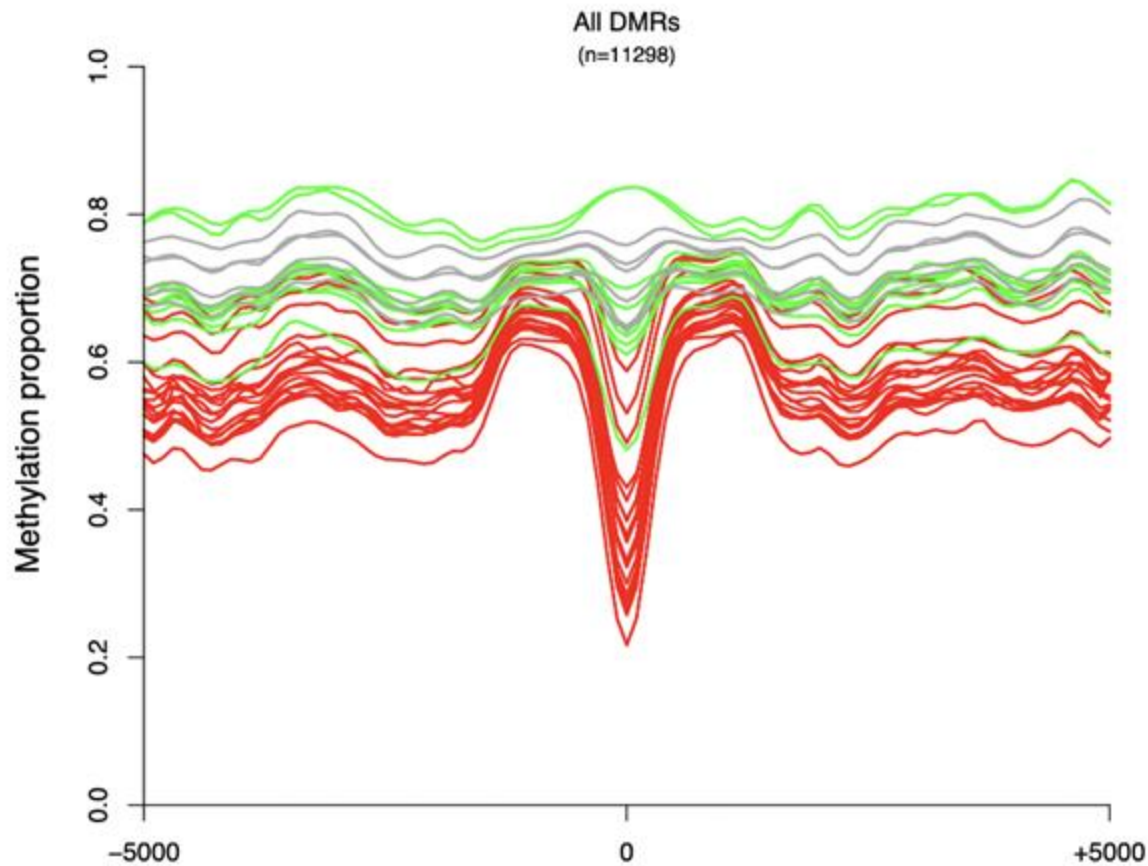- We use a tool called metilene
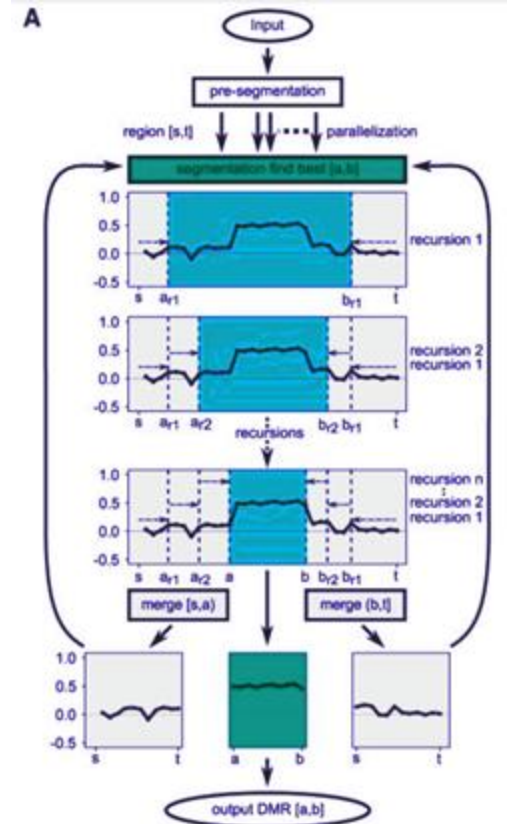
Differentially methylated regions
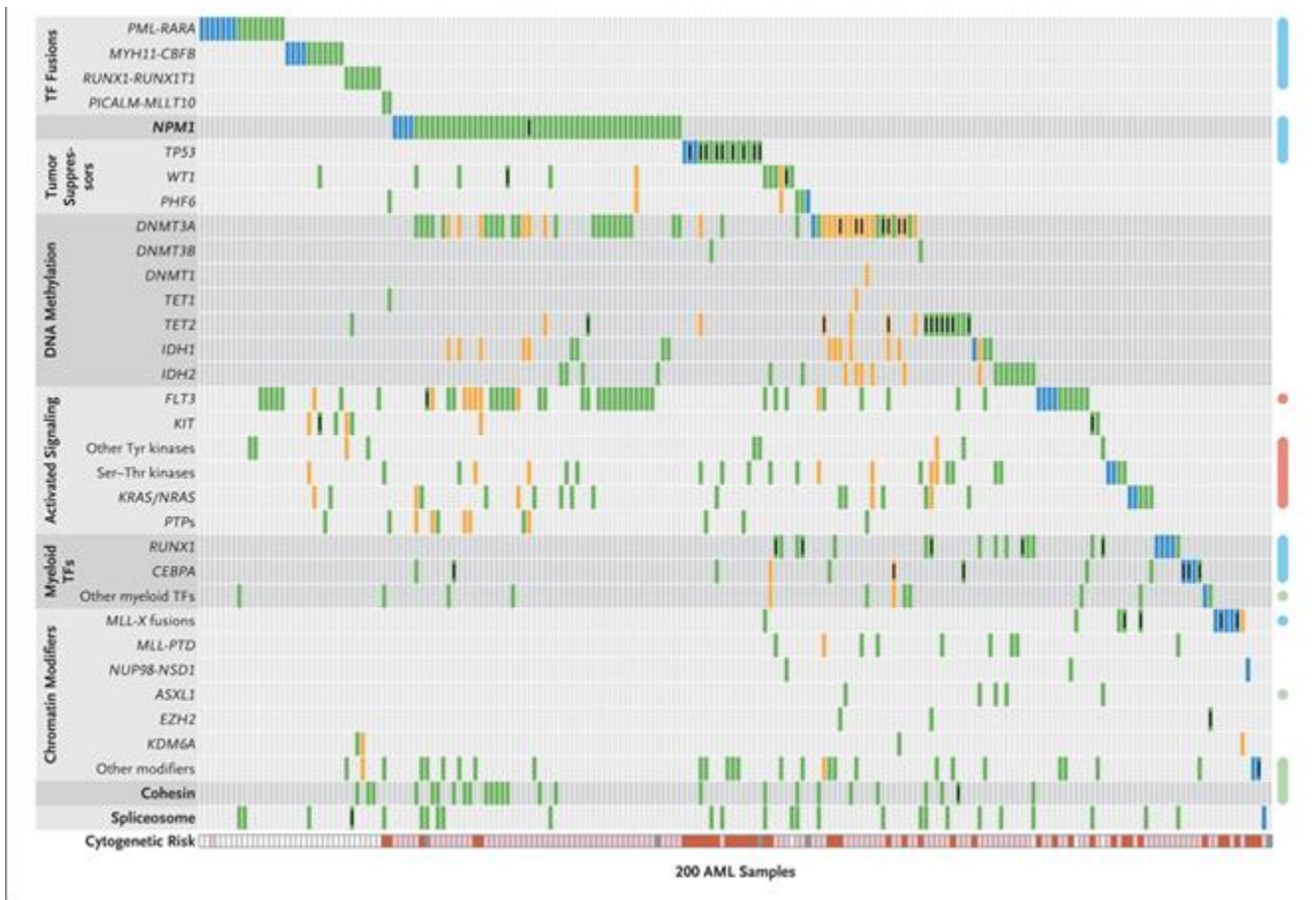
Number of samples matters!

Heatmaps

Canyon Plots

All DMRs
(n=11298)

DNMT3A deficiency

200 AML Samples
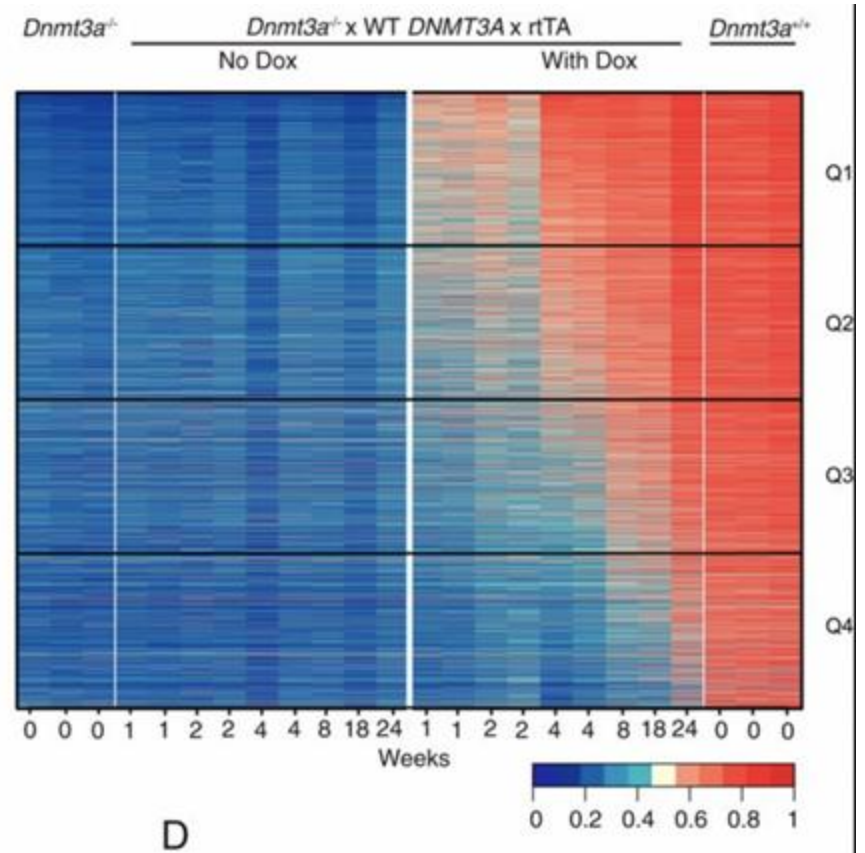
DNMT3A deficiency

- Mouse models (and human data)

- Looking at context, effects, and reversibility

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https://www.pnas.org/content/117/6/3123.full