Epigenomics: Bisulfite sequencing

John Garza
Washington University in St. Louis

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

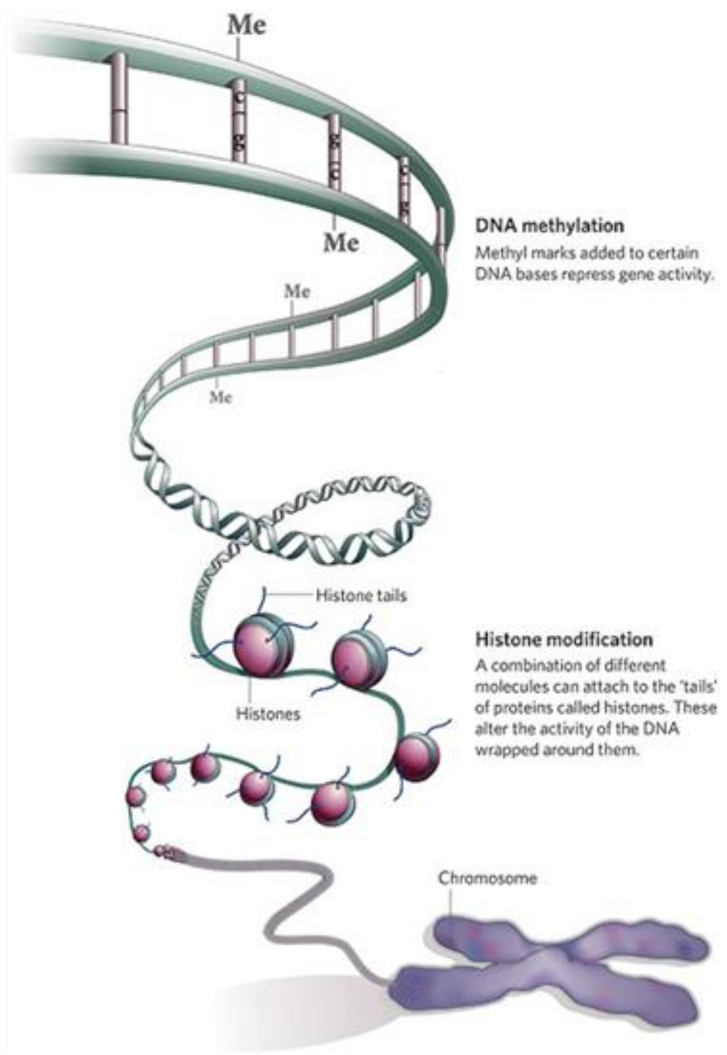
Epigenomics

- Alterations of DNA state or accessibility

- Wrapped around histones

- Bound by transcription factors

- etc

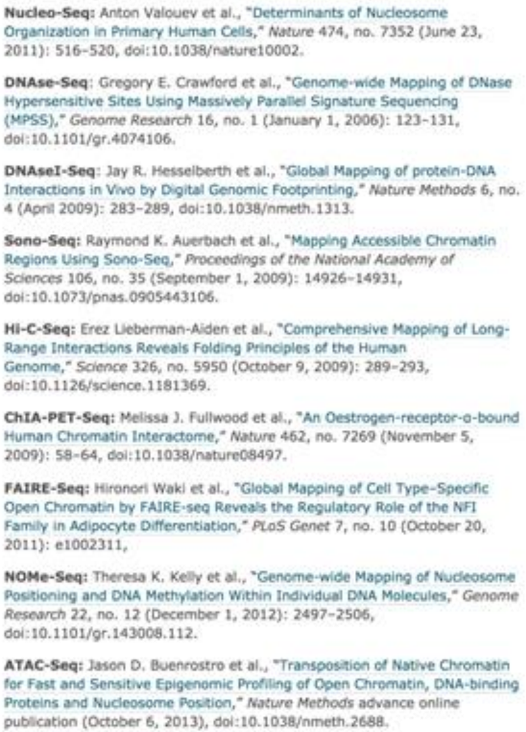


DNA methylation
Methyl marks added to certain DNA bases repress gene activity.

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.

105+ *-seq assays

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.

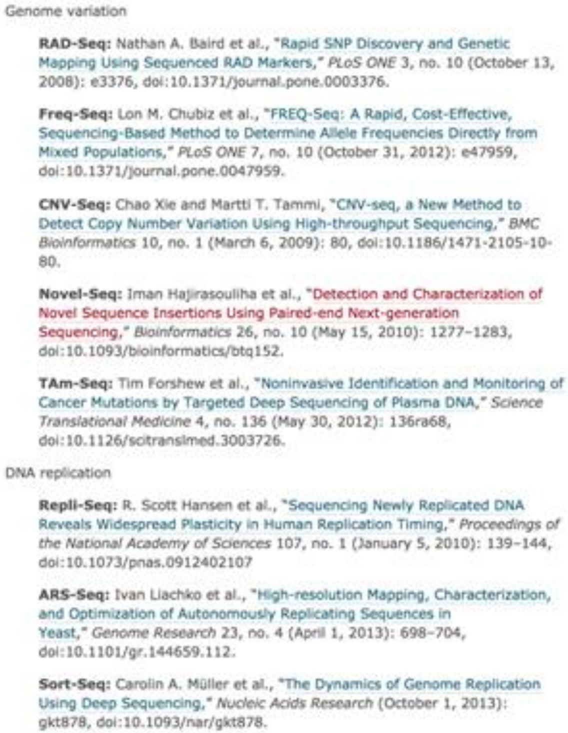
Hi-C-Seq: Erez Lieberman-Aiden et al., "Comprehensive Mapping of Long-Range Interactions Reveals Folding Principles of the Human Genome," *Science* 326, no. 5950 (October 9, 2009): 289–293, doi:10.1126/science.1181369.

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.

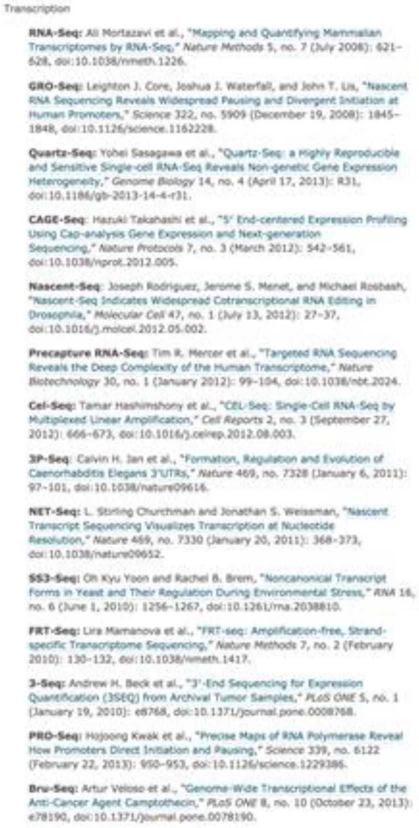
TAm-Seq: Tim Forshew et al., "Noninvasive Identification and Monitoring of Cancer Mutations by Targeted Deep Sequencing of Plasma DNA," *Science Translational Medicine* 4, no. 136 (May 30, 2012): 136ra68, doi:10.1126/scitranslmed.3003726.

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.

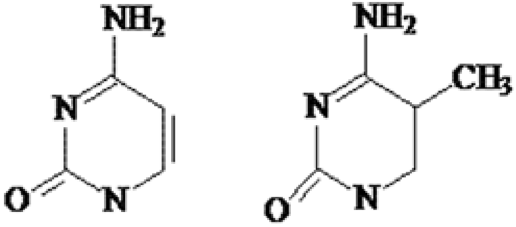
FRT-Seq: Lira Mamanova et al., "FRT-seq: Amplification-free, Strand-specific Transcriptome Sequencing," *Nature Methods* 7, no. 2 (February 2010): 130–132, doi:10.1038/nmeth.1417.

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.

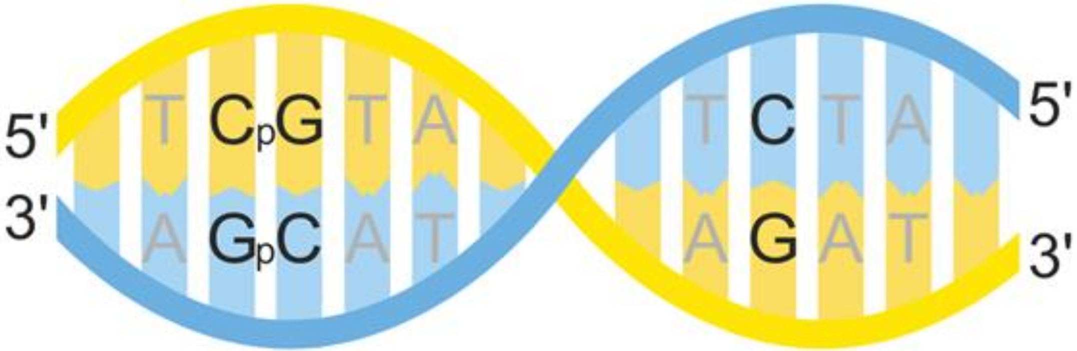
Bru-Seq: Artur Veloso et al., "Genome-Wide Transcriptional Effects of the Anti-Cancer Agent Camptothecin," *PLoS ONE* 8, no. 10 (October 23, 2013): e78190, doi:10.1371/journal.pone.0078190.

DNA Methylation



- Mostly happens at CpGs

- About 25 million CpGs in human genome



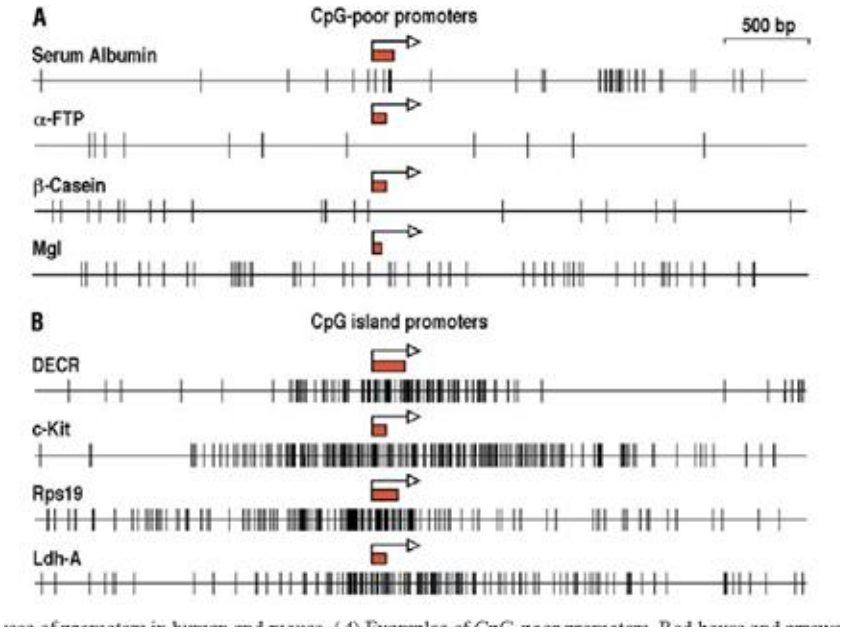
- https://en.wikipedia.org/wiki/CpG_site#/media/File:CpG_vs_C-G_bp.svg

DNA Methylation

- CpG Islands

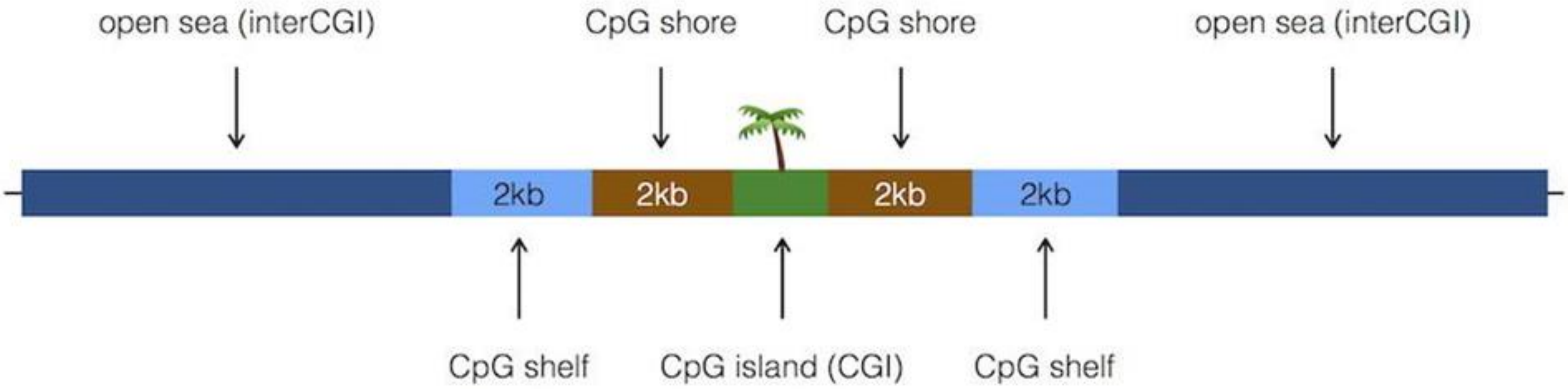
- Length >= 200 bp
  GC% > 50%
  o/e CpG ratio > 60%

- Selective pressure/
  Evolutionary constraint



- DOI:10.1007/s00018-003-3088-6

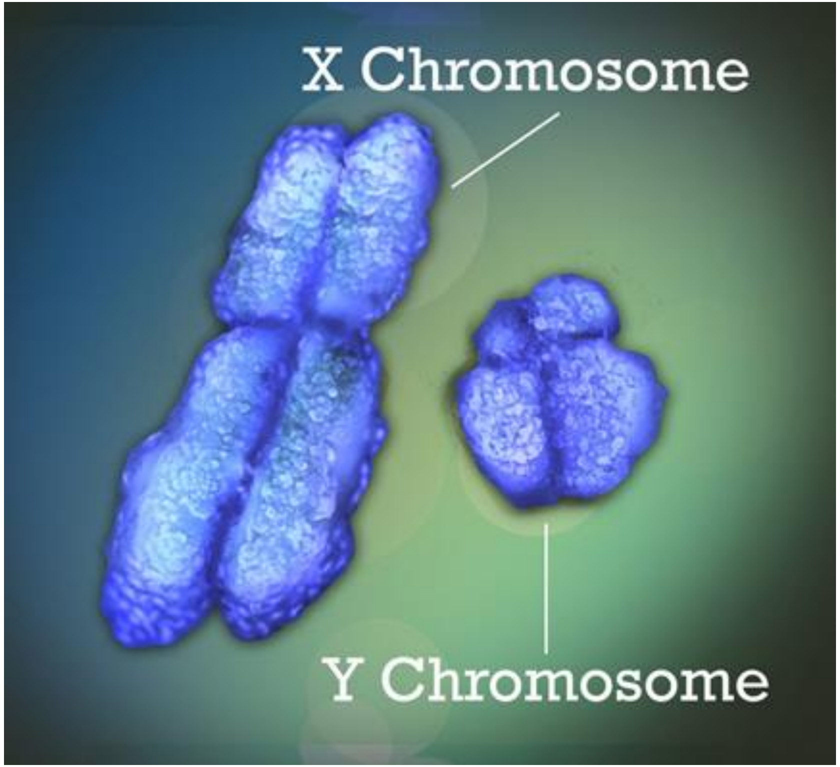
Islands, shores, and shelves



https://bioconductor.org/packages/release/bioc/vignettes/annotatr/inst/doc/annotatr-vignette.html

What does DNA methylation do?

- The short answer: It depends!
- X-chromosome inactivation
- Silencing of transposable elements
- Cellular differentiation
- Cancer - hypo/hypermethylation

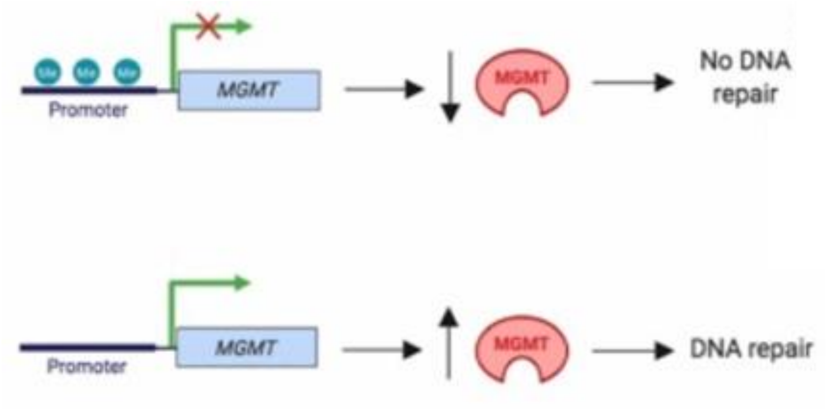


MGMT and Temozolomide

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

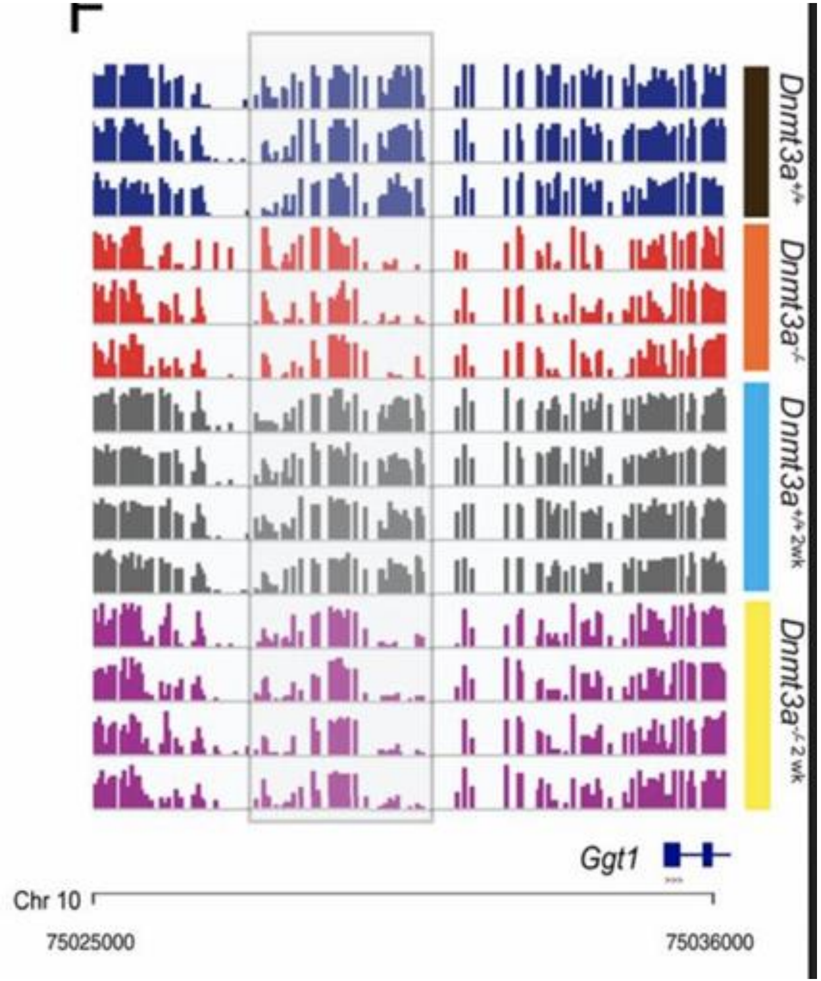
- MGMT “cleans up” the damage

- Methylation of the MGMT promoter is linked to better outcomes!



Methylation Patterns

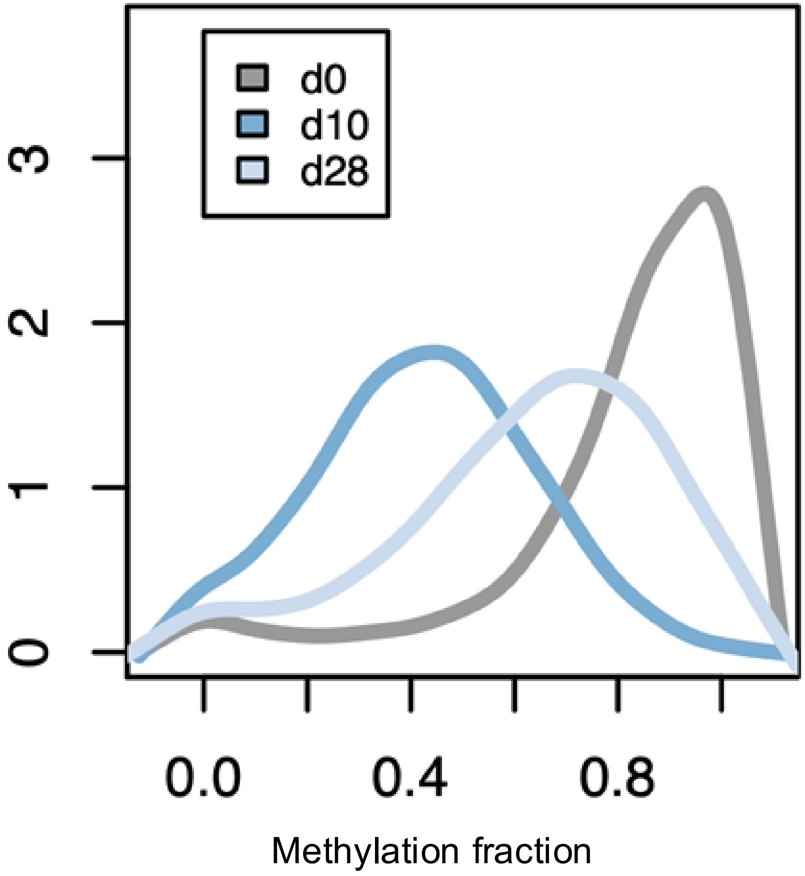
- Methyltransferases that act locally



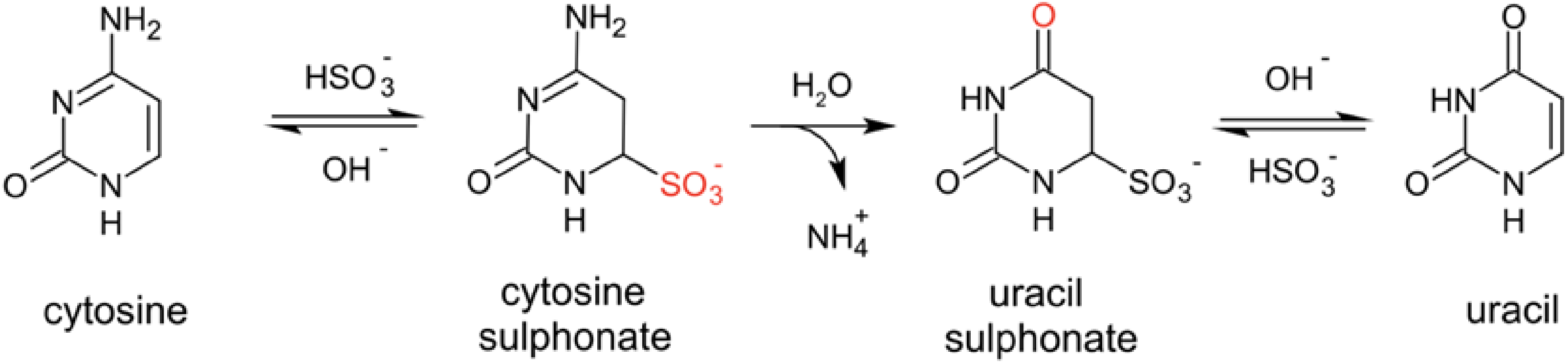
Methylation Patterns

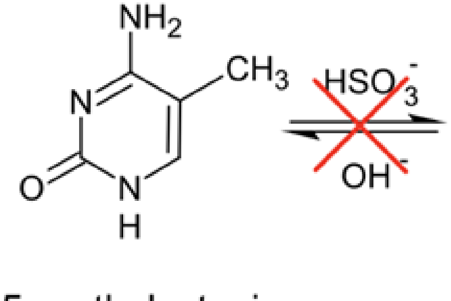
- Methyltransferases that act locally

- Other alterations (or treatments) that act globally

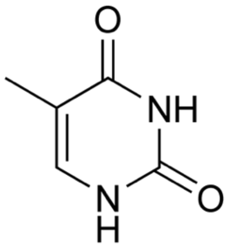


Bisulfite sequencing





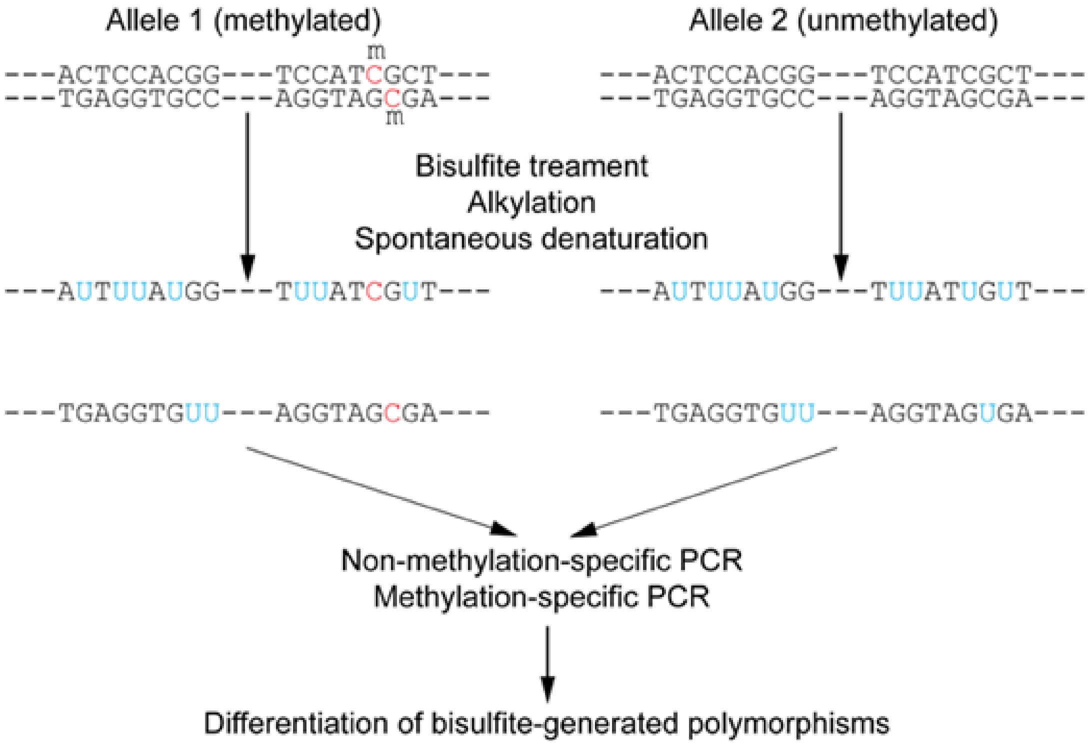
5-methylcytosine



thymine

https://en.wikipedia.org/wiki/File:Bisulfite_conversion.svg

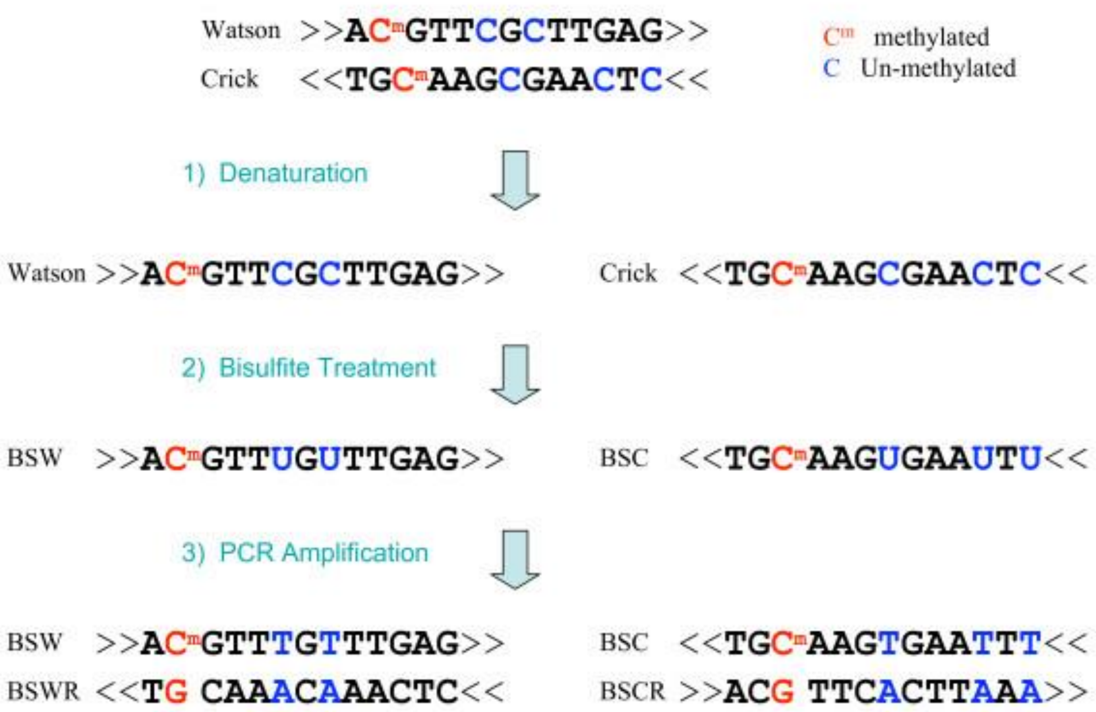
Bisulfite sequencing



Allele 1 (methylated)
Allele 2 (unmethylated)
m
---ACTCCACGG---TCCATCGCT---
---TGAGGTGCC---AGGTAGCGA---
m
---ACTCCACGG---TCCATCGCT---
---TGAGGTGCC---AGGTAGCGA---
Bisulfite treament
Alkylation
Spontaneous denaturation
---AUTUUAUGG---TUUATCGUT---
---AUTUUAUGG---TUUATUGUT---
---TGAGGTGUU---AGGTAGCGA---
---TGAGGTGUU---AGGTAGUGA---
Non-methylation-specific PCR
Methylation-specific PCR
Differentiation of bisulfite-generated polymorphisms

https://en.wikipedia.org/wiki/File:Wiki_Bisulfite_sequencing_Figure_1_small.png

Bisulfite sequencing



Watson >>AC^{m}GTTCGCTTGAG>>

Crick <<TGC^{m}AAGCGAACTC<<

C^{m} methylated

C Un-methylated

1) Denaturation

Watson >>AC^{m}GTTCGCTTGAG>>   Crick <<TGC^{m}AAGCGAACTC<<

2) Bisulfite Treatment

BSW >>AC^{m}GTTUGUTTGAG>>   BSC <<TGC^{m}AAGUGAAUTU<<

3) PCR Amplification

BSW >>AC^{m}GTTTGTTTGAG>>   BSC <<TGC^{m}AAGTGAATTT<<

BSWR <<TG CAAACAAACTC<<   BSCR >>ACG TTCACTTAAA>>

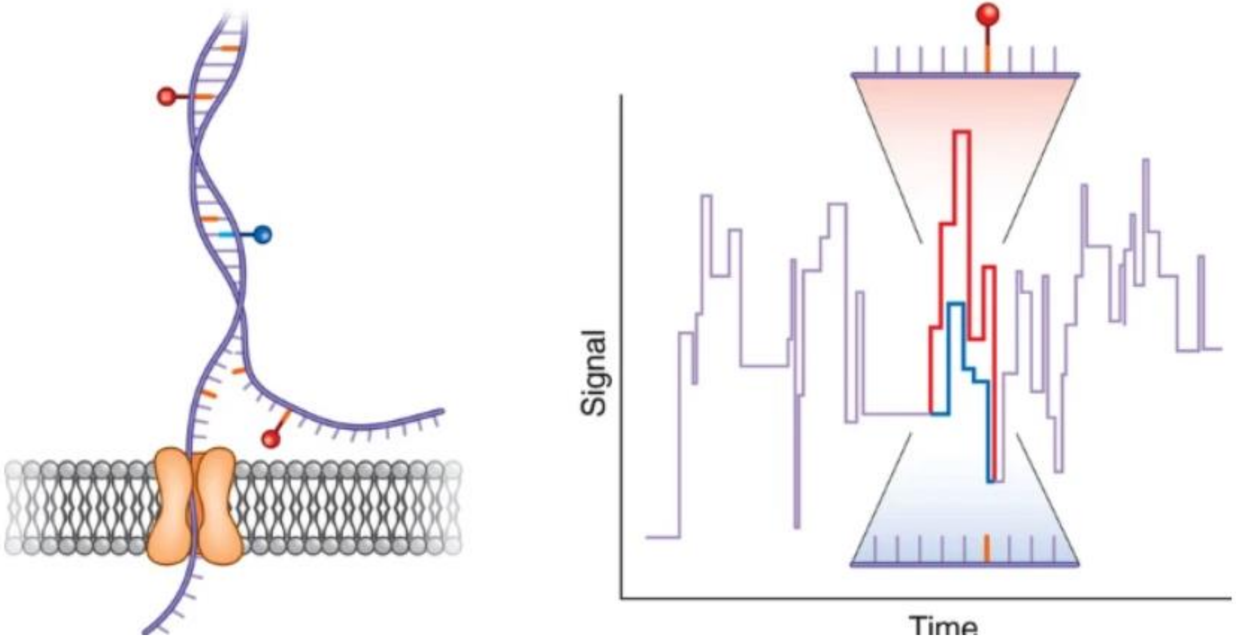
BSMAP: whole genome bisulfite sequence MAPping program. BMC Bioinformatics 10:232

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

-

Methylation calling

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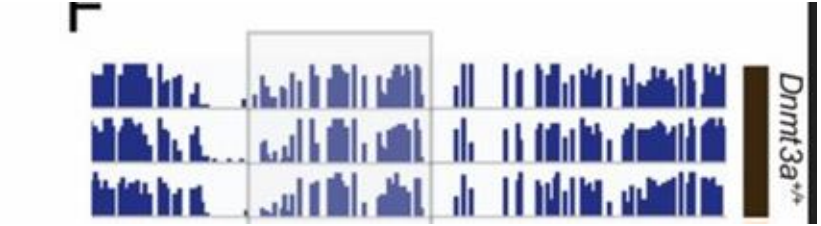
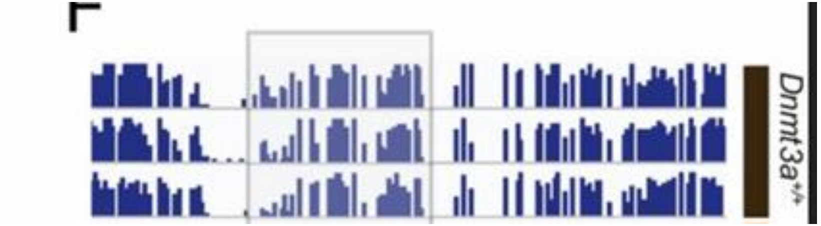
- Why isn't every position 0%, 50% or 100%?



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!



Workflow/File formats

- Aligning: FASTQ > BAM/CRAM

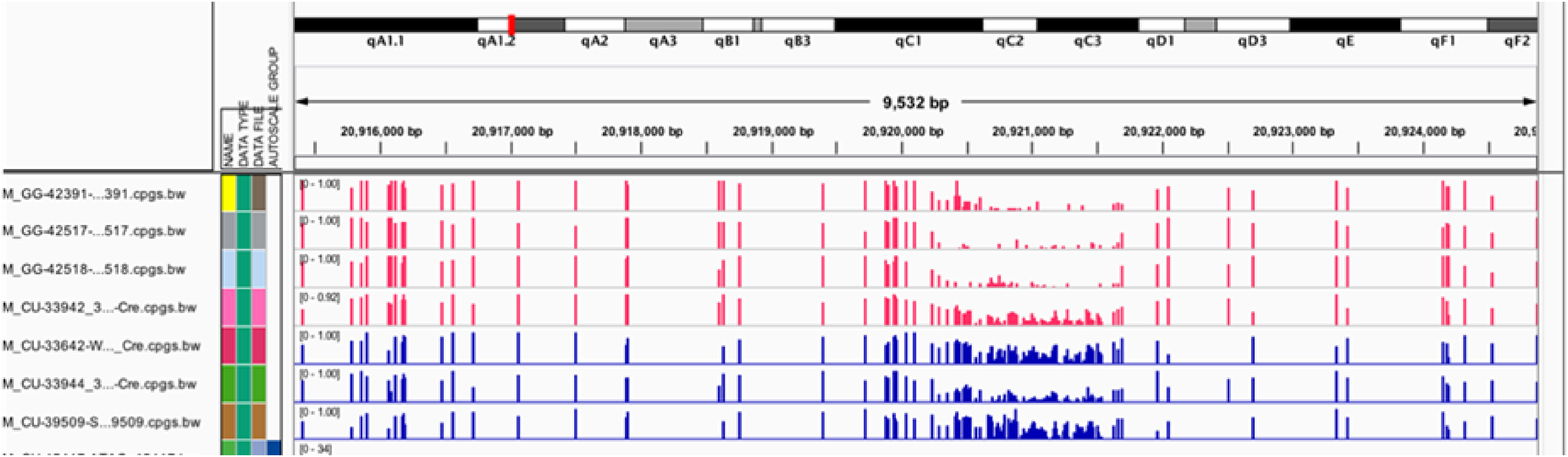
- Pileup: BAM/CRAM > VCF
    - (entries for every site, allele frequencies)

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

- bedgraph > bigwig (for visualization in IGV)

- There are workflows for this!

IGV visualization

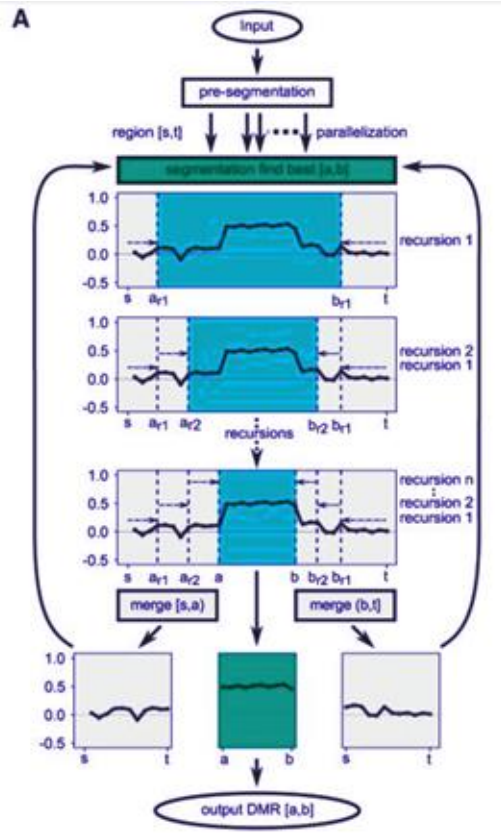


Differentially methylated regions

- Comparing two groups to find changes

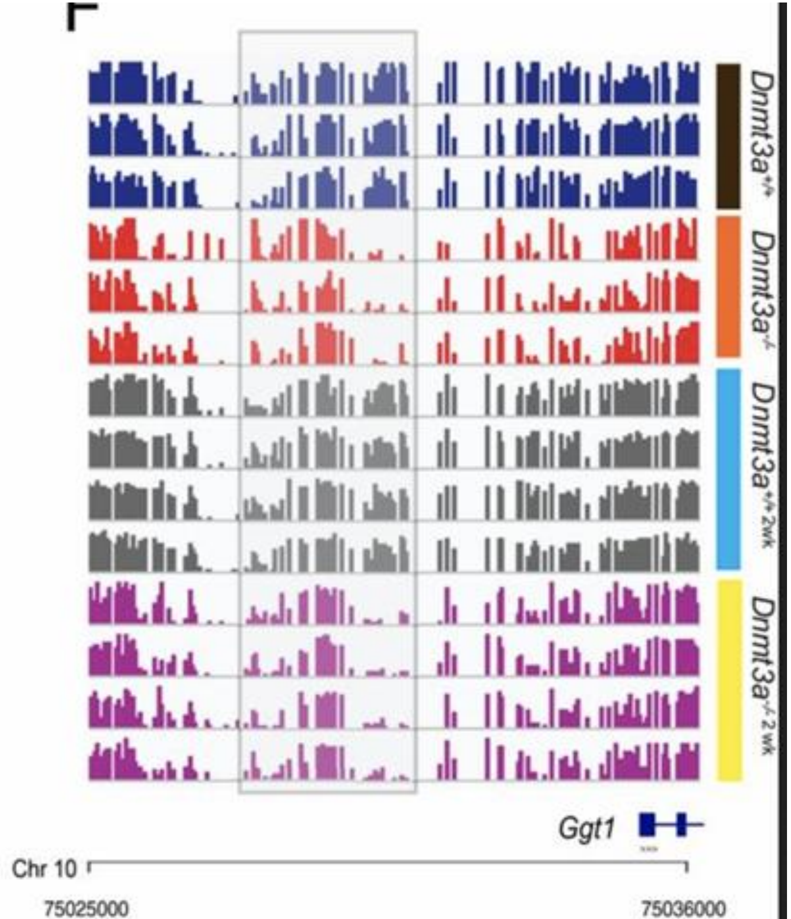
- Finding DMRs is a segmentation problem

- We use a tool called metilene

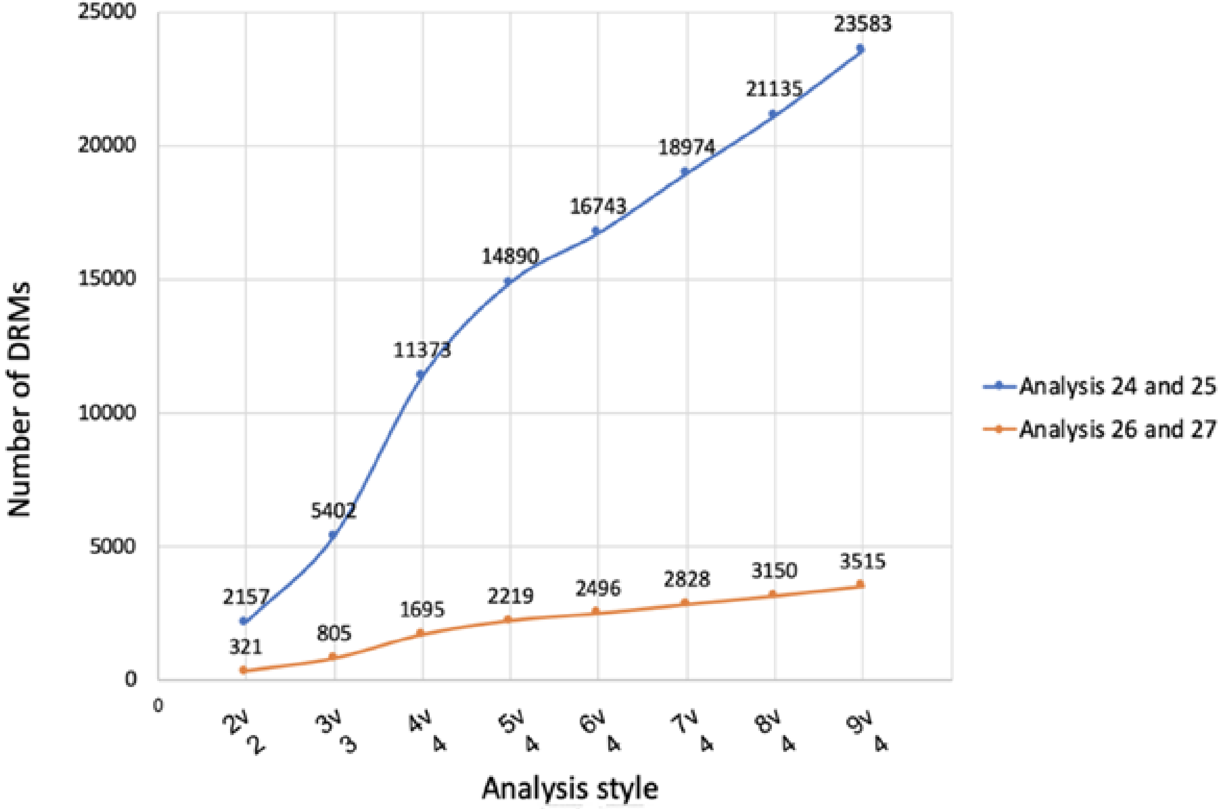


https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4728377/

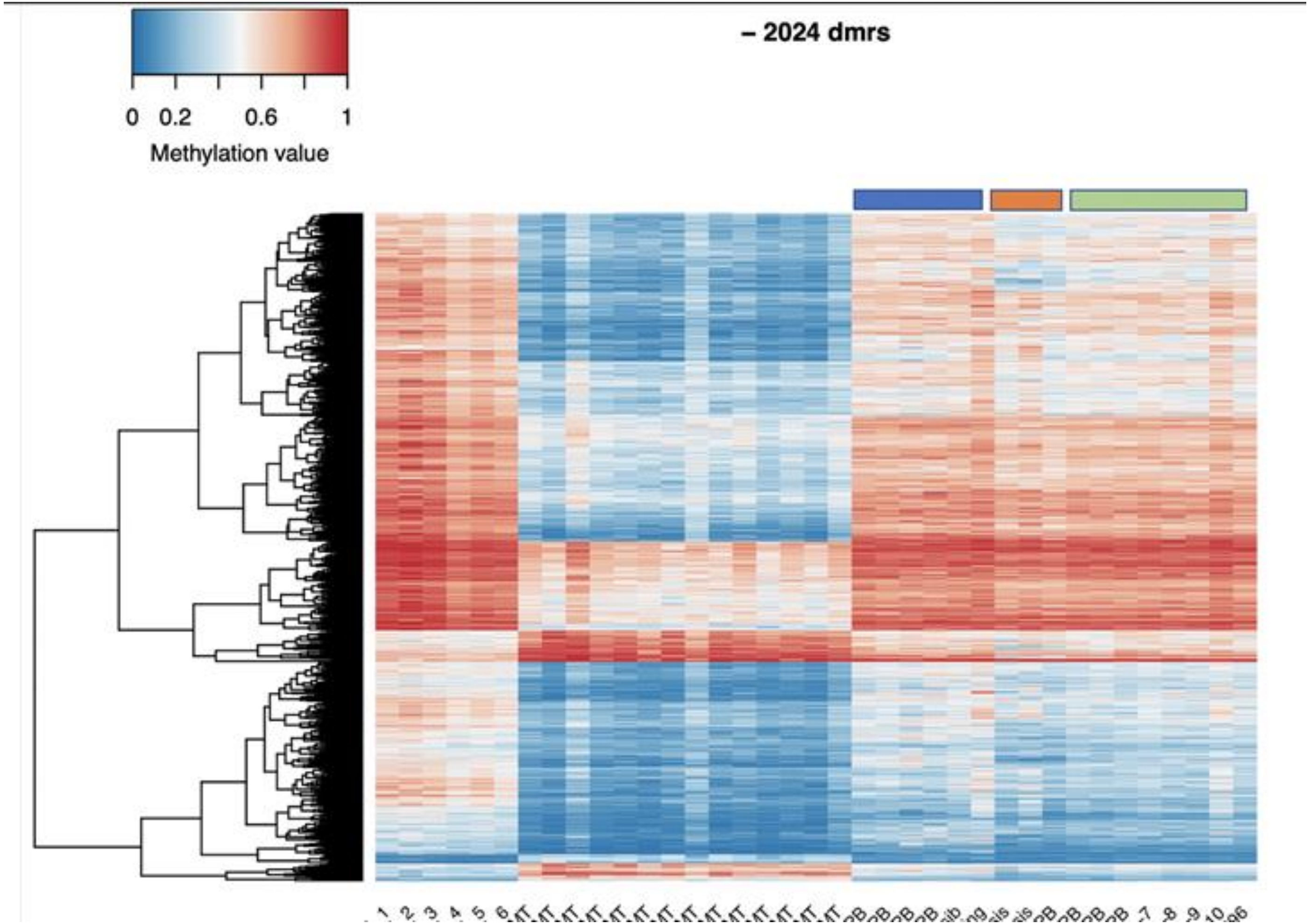
Differentially methylated regions



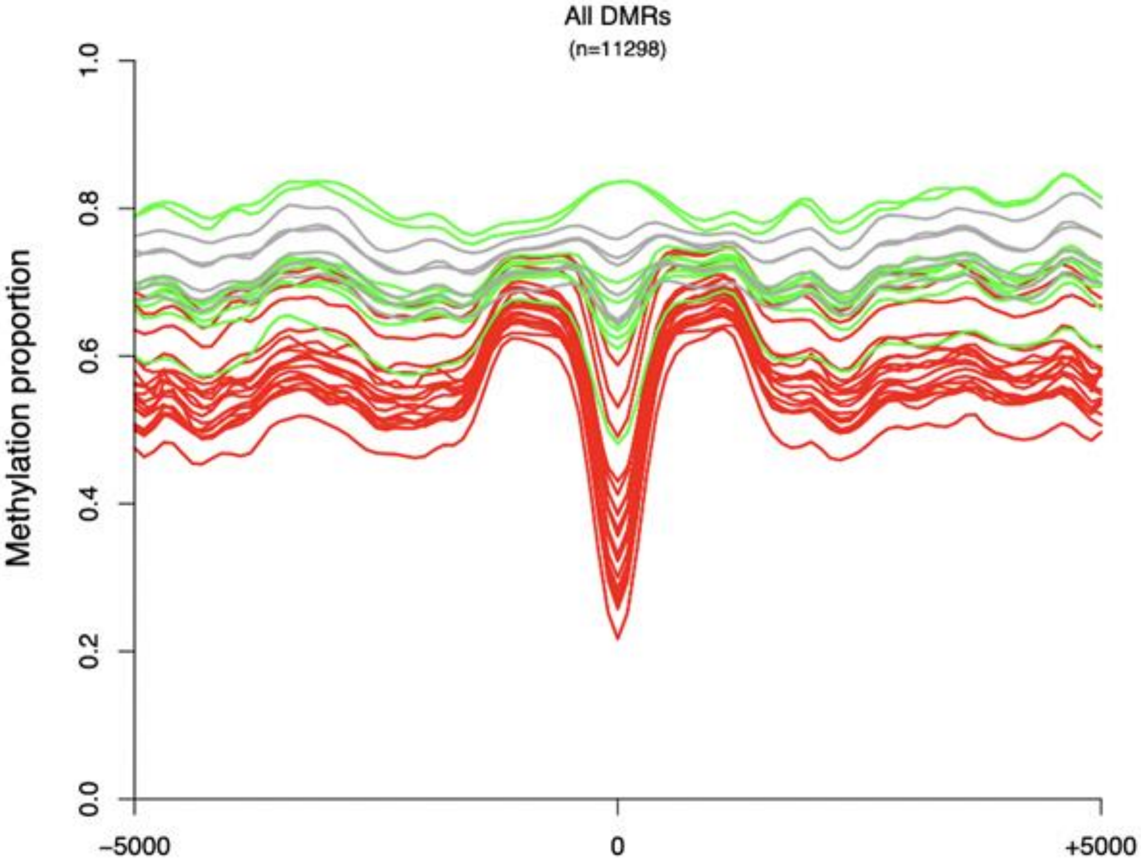
Number of samples matters!



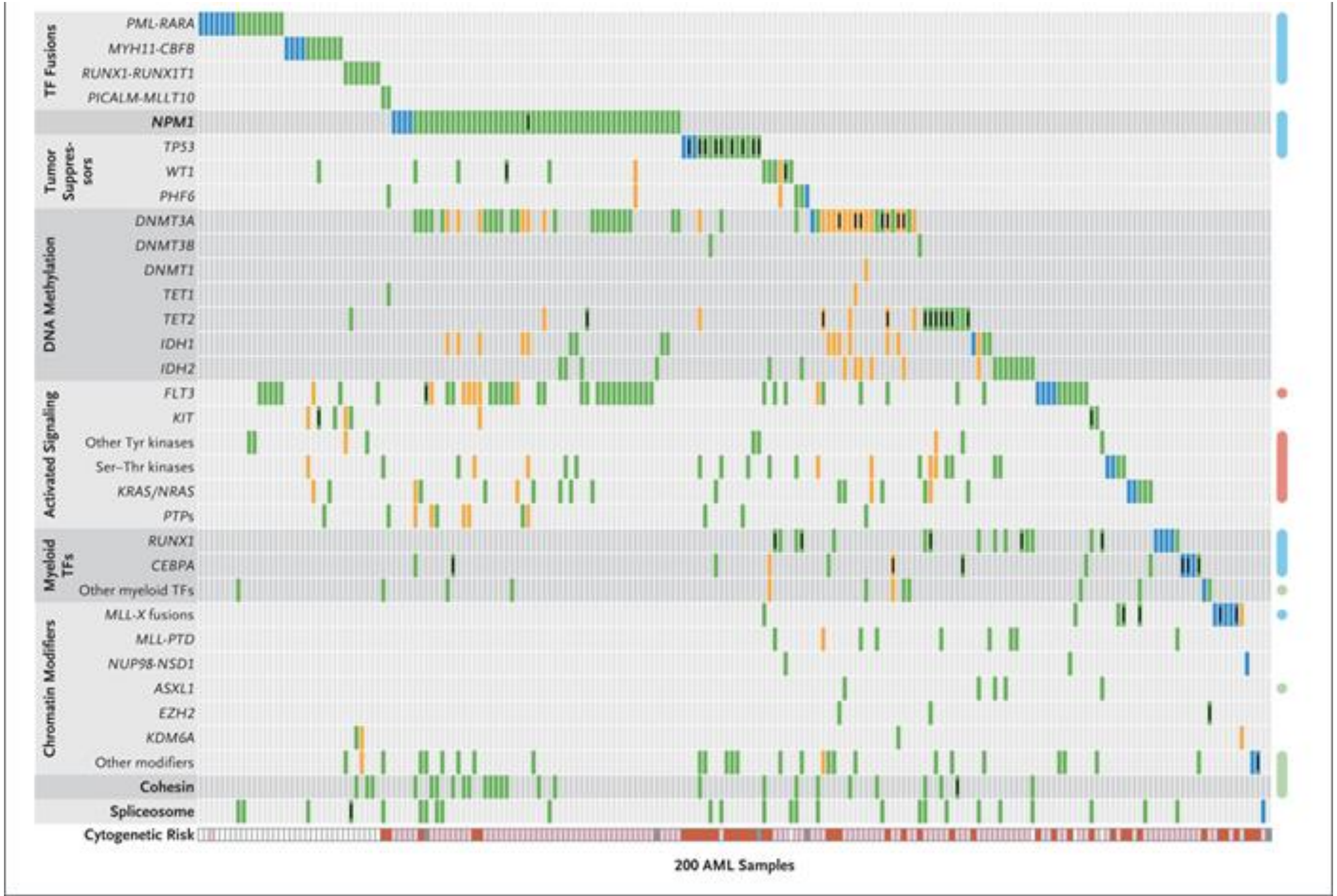
Heatmaps



Canyon Plots



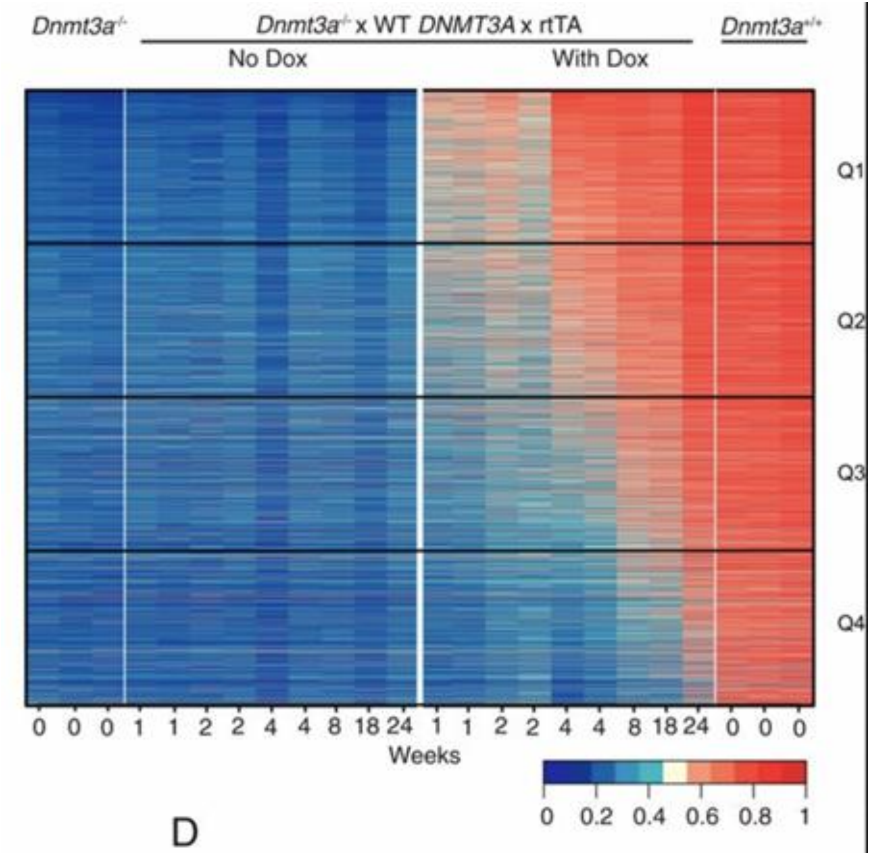
DNMT3A deficiency



DNMT3A deficiency

- Mouse models (and human data)

- Looking at context, effects, and reversibility



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

DNMT3A deficiency

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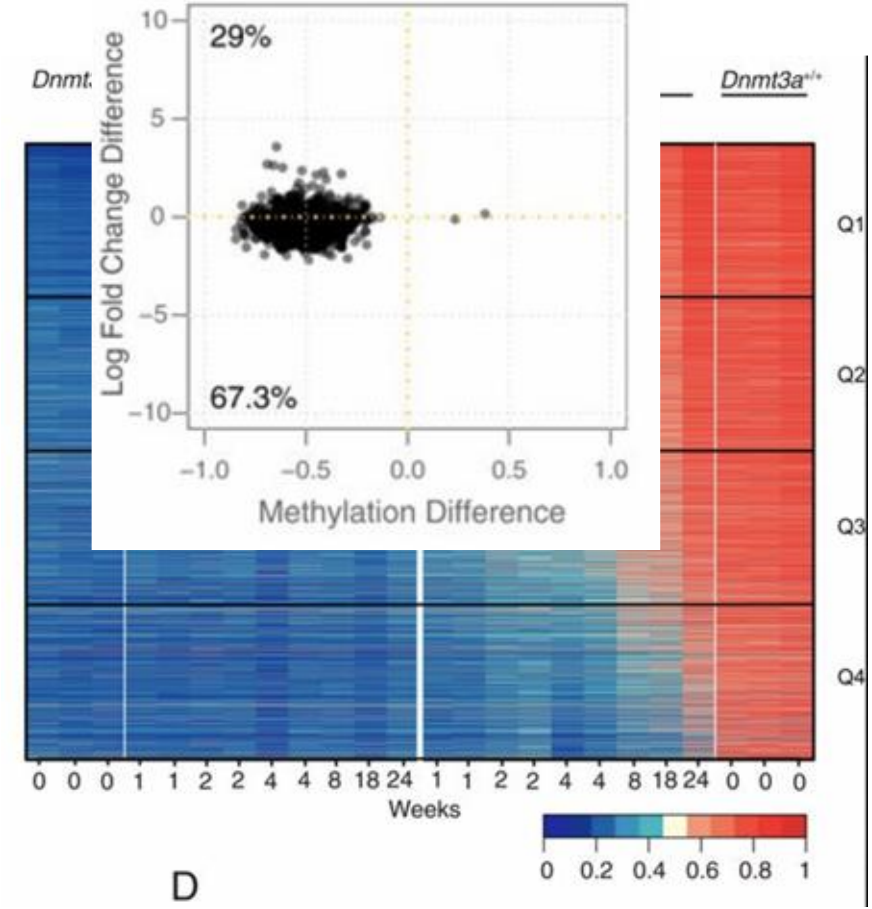


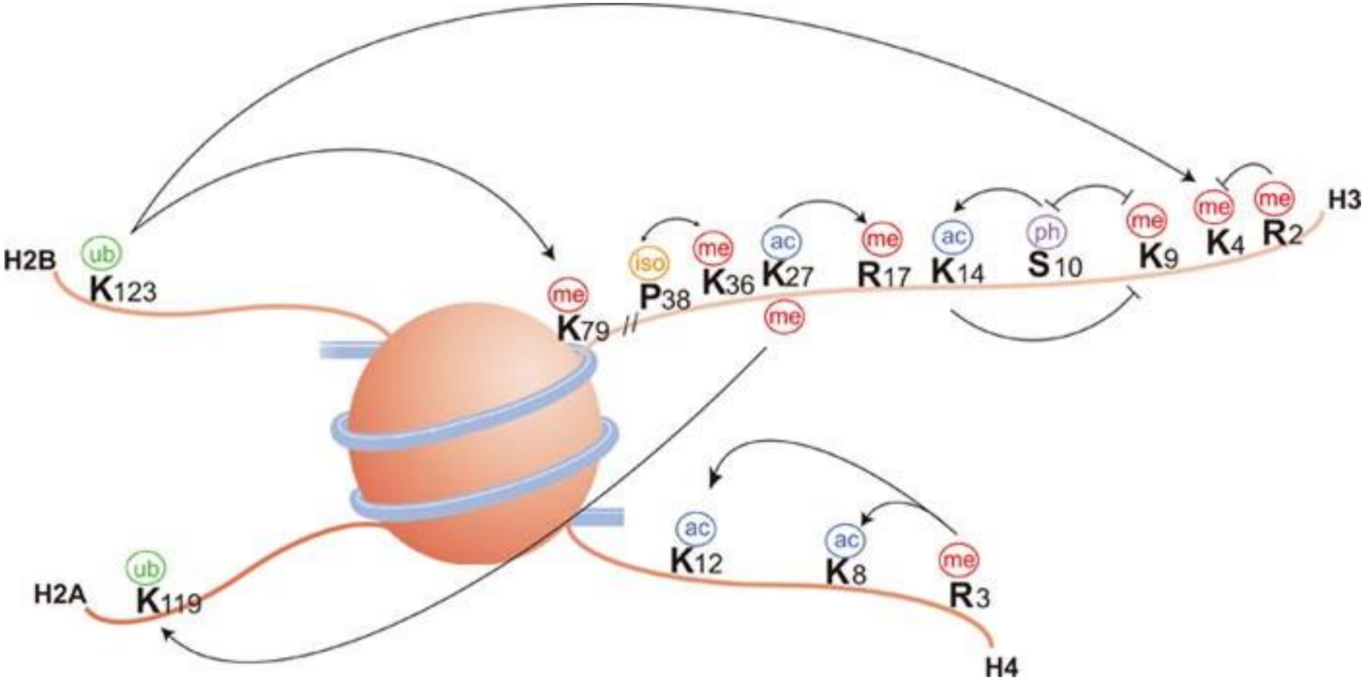
https://www.pnas.org/content/117/6/3123.full

Epigenomics: ChIP-seq and ATAC-seq

John Garza
Washington University in St. Louis

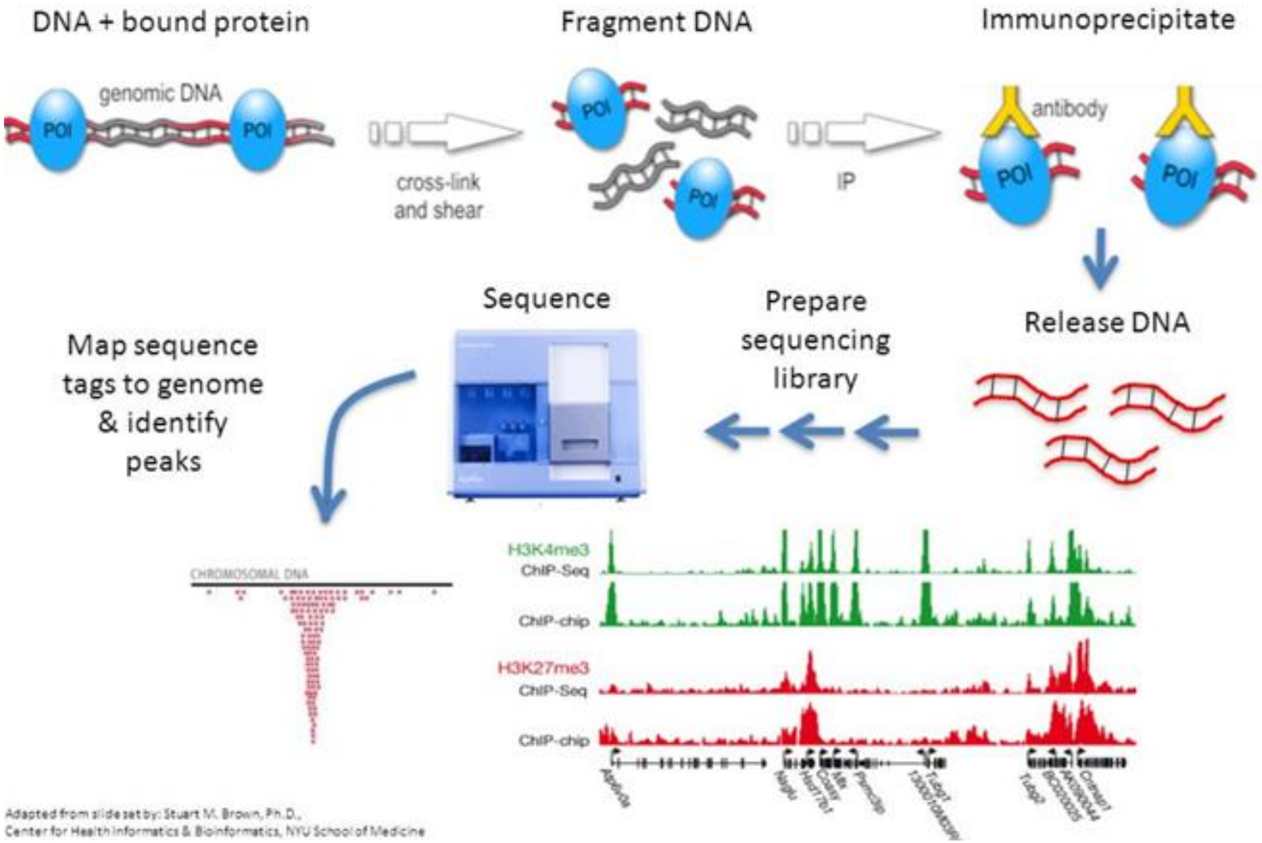
Some slides adapted from:
https://github.com/genome/bfx-workshop
https://github.com/quinlan-lab/applied-computational-genomics

Histone Modifications



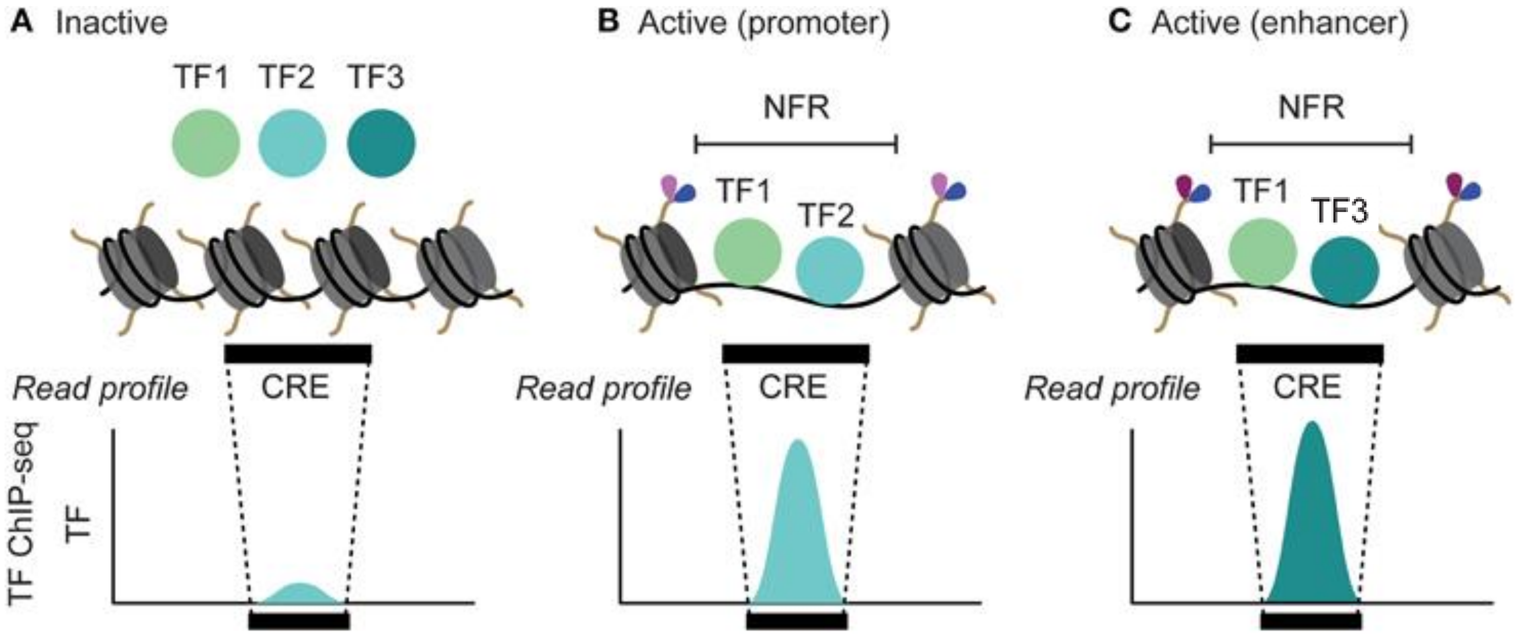
https://www.nature.com/articles/cr201122

ChIP-seq



Adapted from slide set by: Stuart M. Brown, Ph.D.,
Center for Health Informatics & Bioinformatics, NYU School of Medicine

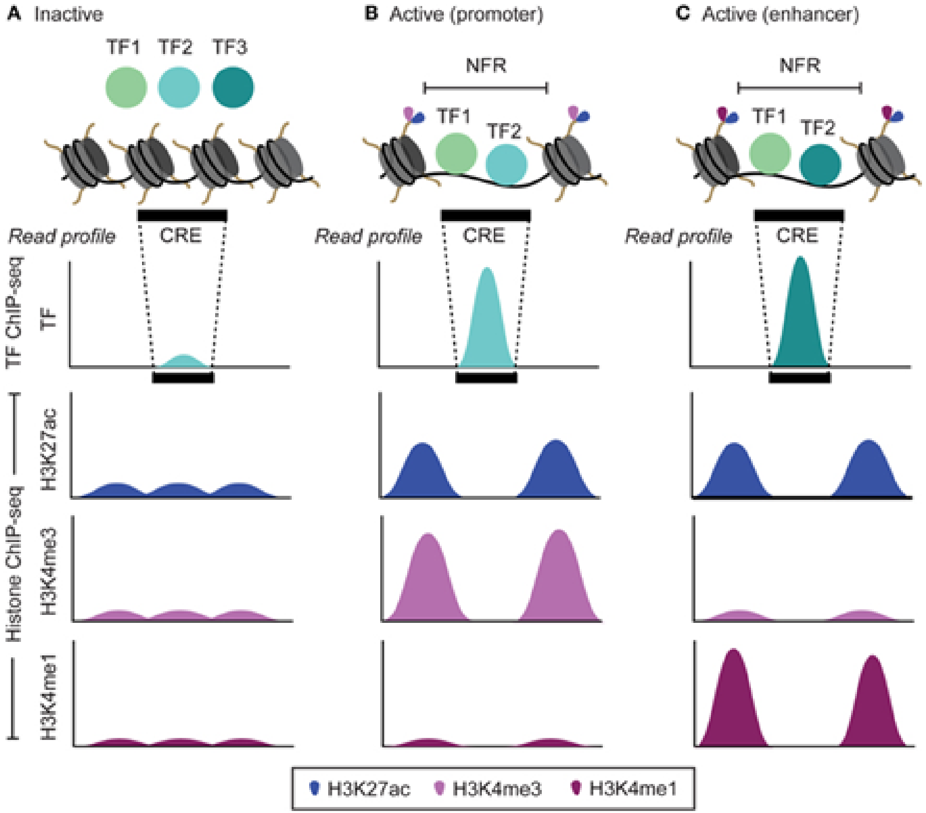
Mapping transcription-factor binding locations



NFR = nucleosome free region
CRE = Cis regulatory element

http://journal.frontiersin.org/article/10.3389/fgene.2015.00188/full

Mapping histone modifications



H3K4me3:

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

NFR = nucleosome free region
CRE = Cis regulatory element

http://journal.frontiersin.org/article/10.3389/fgene.2015.00188/full

CUT&RUN

Potentially less off-target/background signal

Very similar, from a data perspective

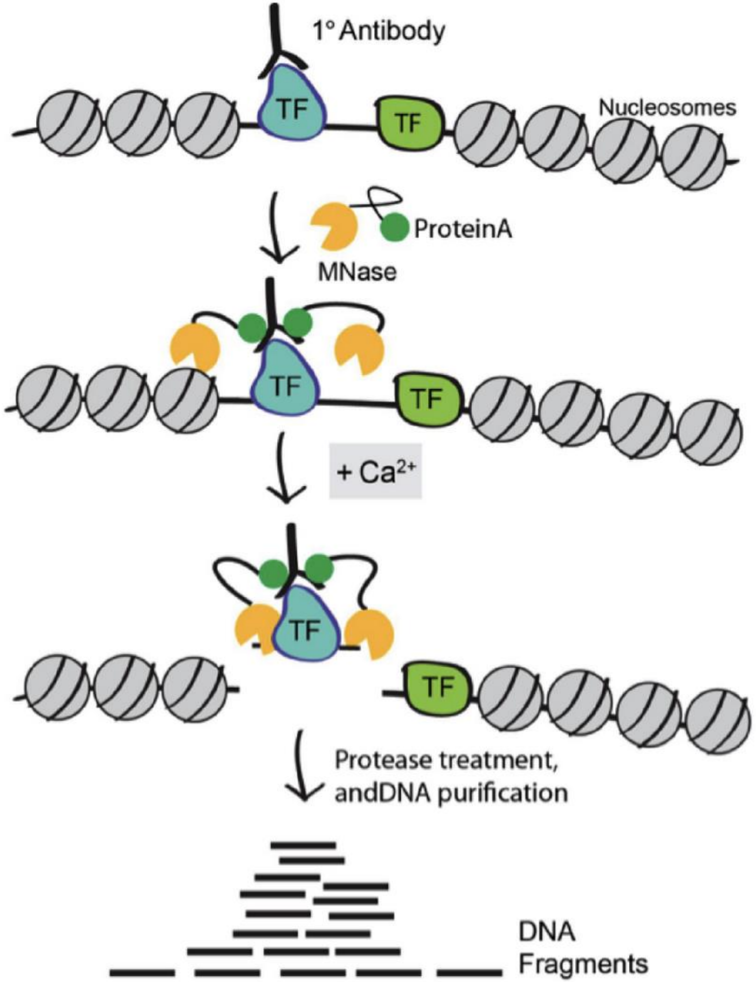
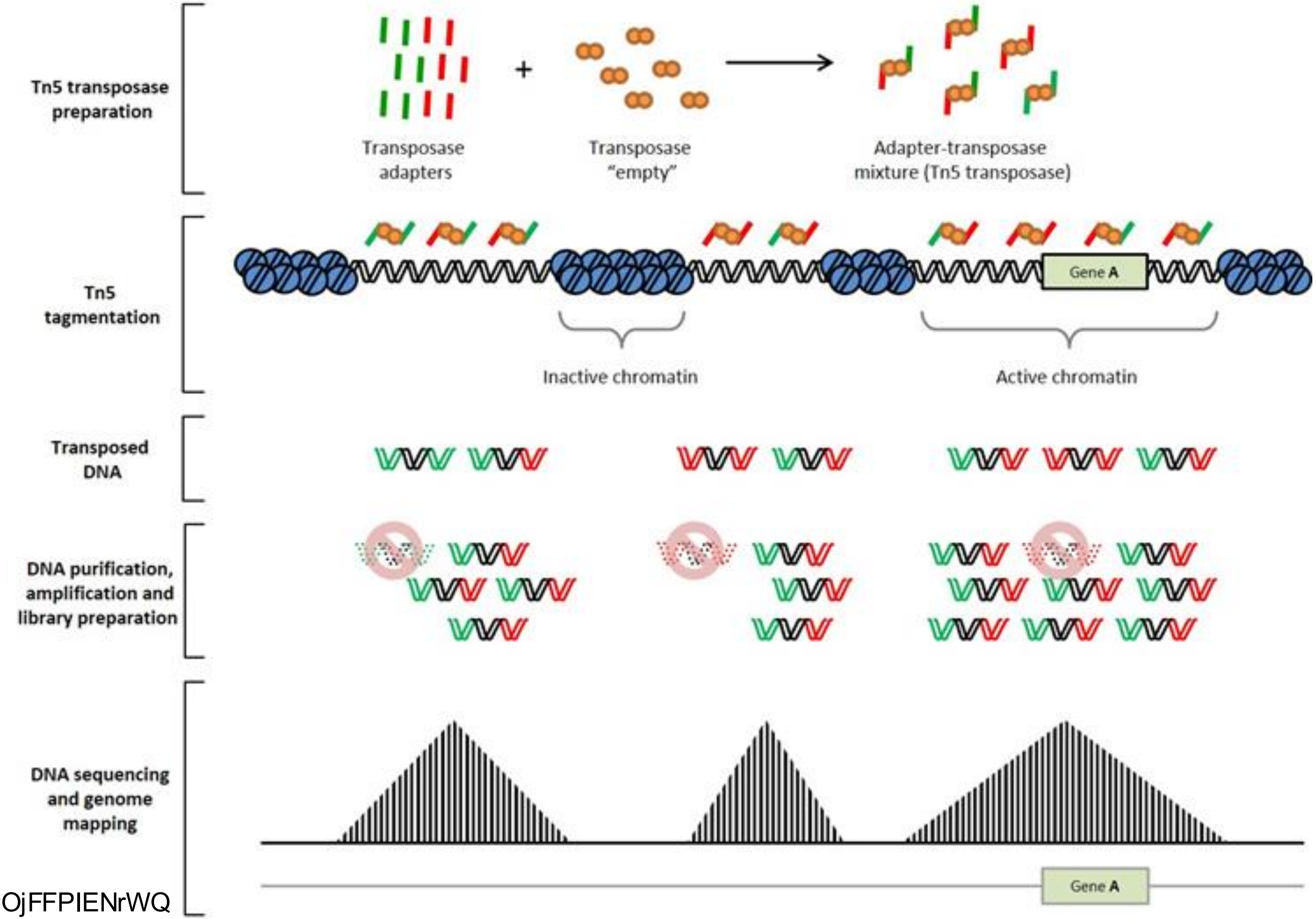


Figure 1. CUT&RUN schematic (see text for details).

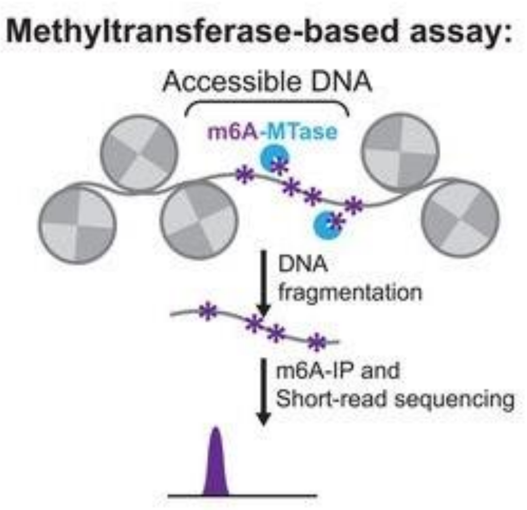
ATAC-seq

DNA accessibility

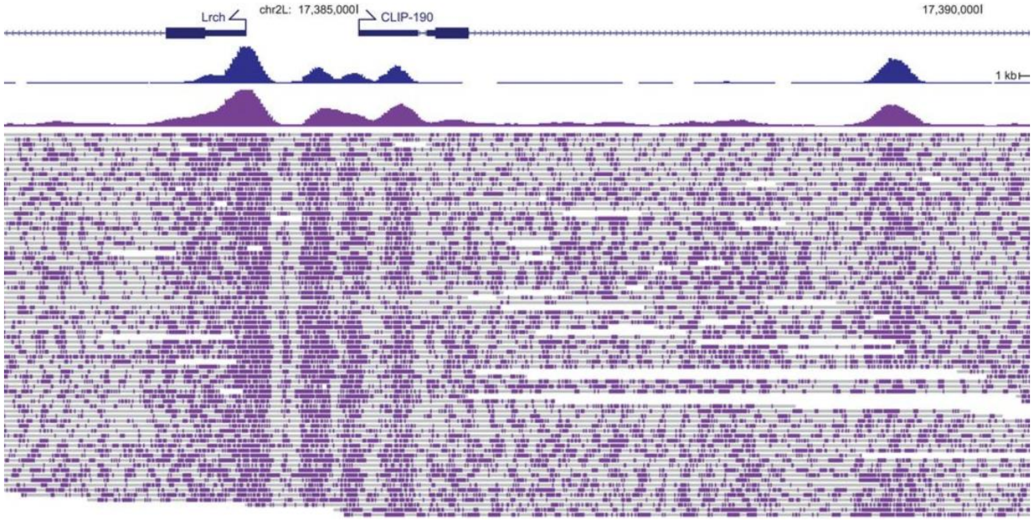

Tn5 transposase preparation
Transposase adapters
+
Transposase "empty"
Adapter-transposase mixture (Tn5 transposase)
Tn5 tagmentation
Gene A
Inactive chromatin
Active chromatin
Transposed DNA
DNA purification, amplification and library preparation
DNA sequencing and genome mapping
Gene A

https://www.youtube.com/watch?v=OjFFPIENrWQ

Fiber-seq

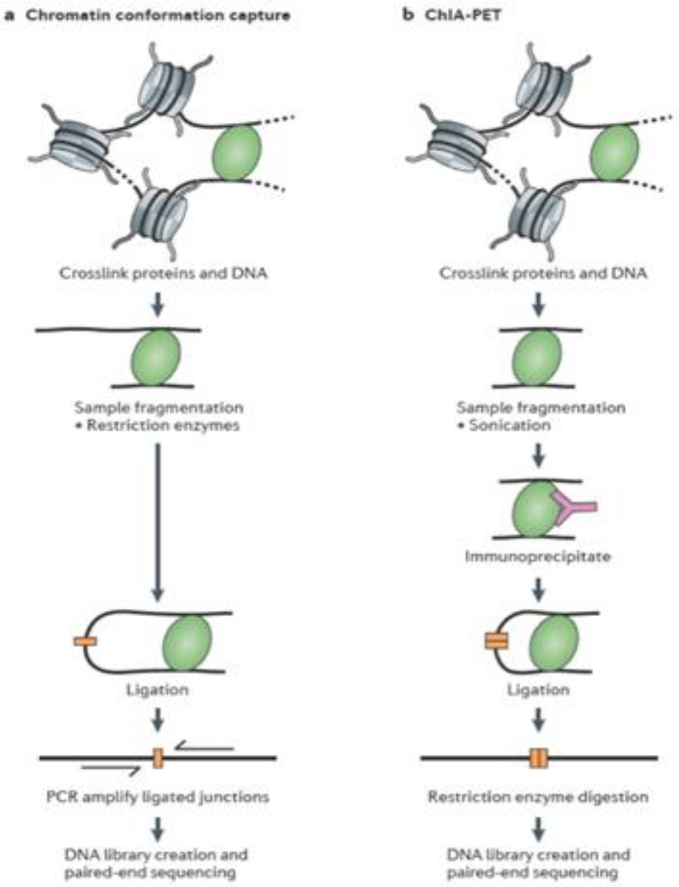

Methyltransferase-based assay:
Accessible DNA
m6A-MTase
DNA fragmentation
m6A-IP and Short-read sequencing

Long-read sequencing (PacBio/ONT)



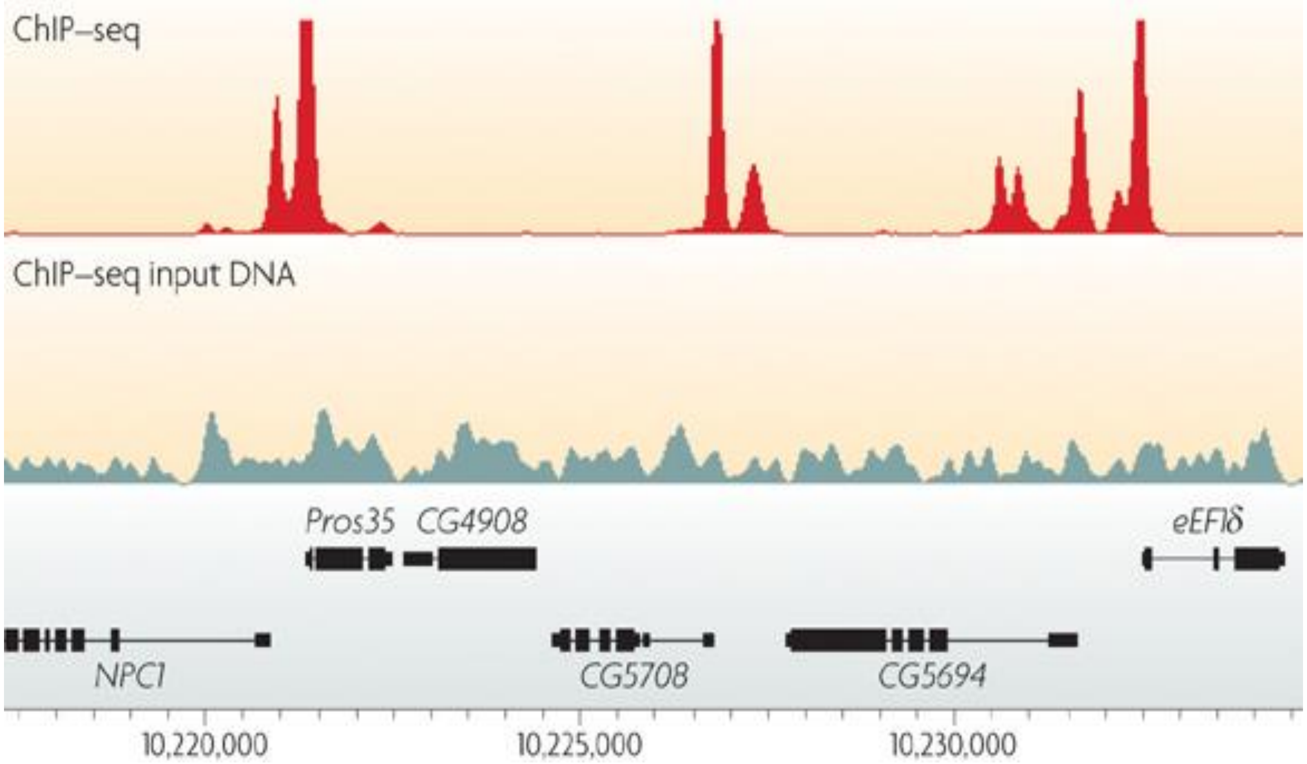
https://www.science.org/doi/10.1126/science.aaz1646

Examining 3-D DNA interactions in the nucleus


a Chromatin conformation capture
b ChIA-PET
Crosslink proteins and DNA
Crosslink proteins and DNA
Sample fragmentation
• Restriction enzymes
Sample fragmentation
• Sonication
Immunoprecipitate
Ligation
Ligation
PCR amplify ligated junctions
Restriction enzyme digestion
DNA library creation and paired-end sequencing
DNA library creation and paired-end sequencing

Nature Reviews Genetics 2012 13:840

Peak-calling

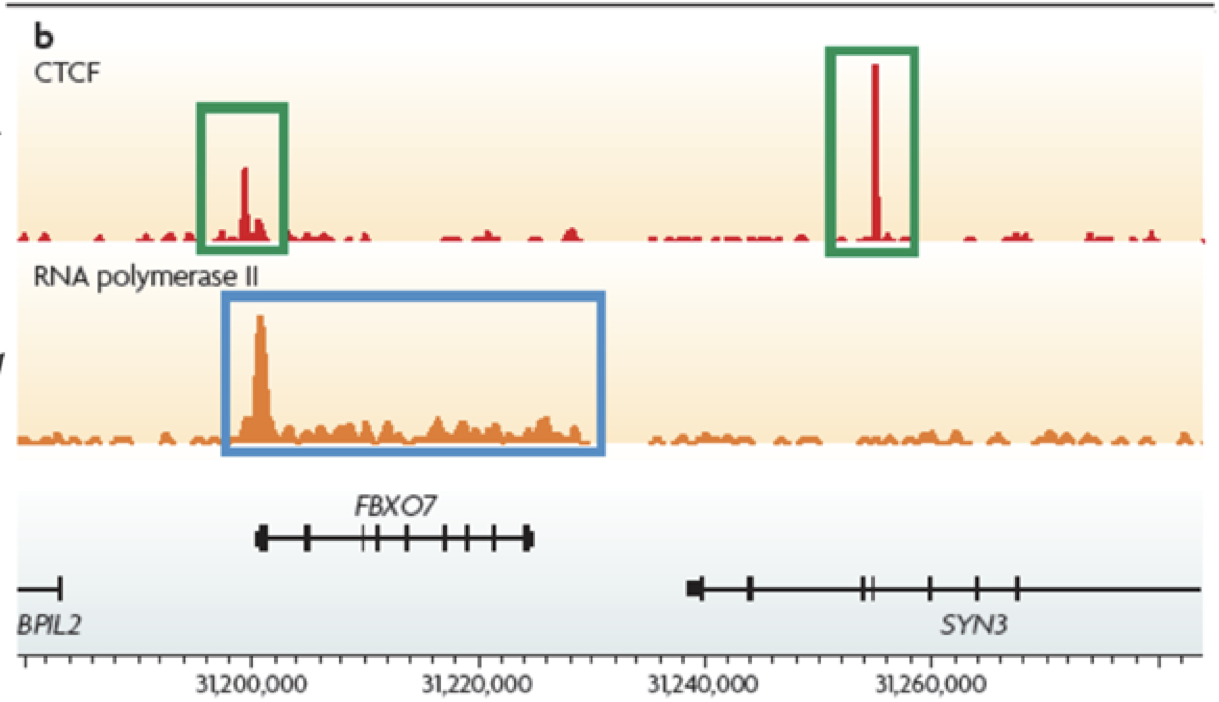


Fundamentally a
signal vs noise problem

Nature Reviews Genetics 10, 669-680 (October 2009)

Proteins bind in different ways

Transcription factor – tight, highly-peaked binding region

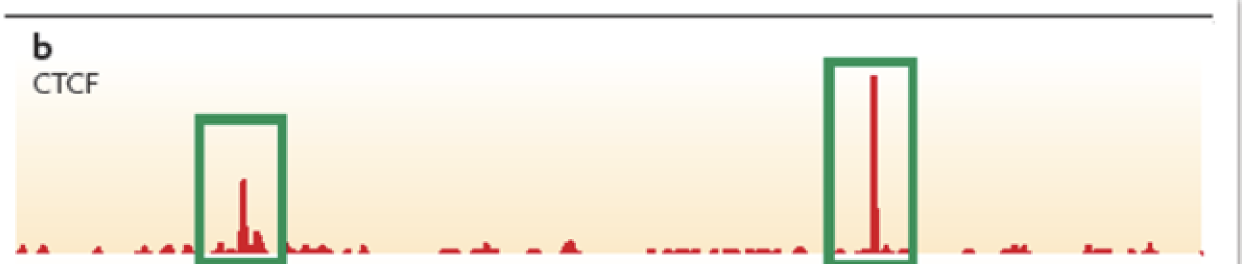


RNA PolII – enriched at TSS but bound throughout gene body

https://www.ebi.ac.uk/sites/ebi.ac.uk/files/content.ebi.ac.uk/materials/2013/131021_HTS/introduction_to_chip_seq_data_and_analysis-b.gerle_.pdf

How much sequence coverage do we need?

Transcription factor – tight, highly-peaked binding region



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).

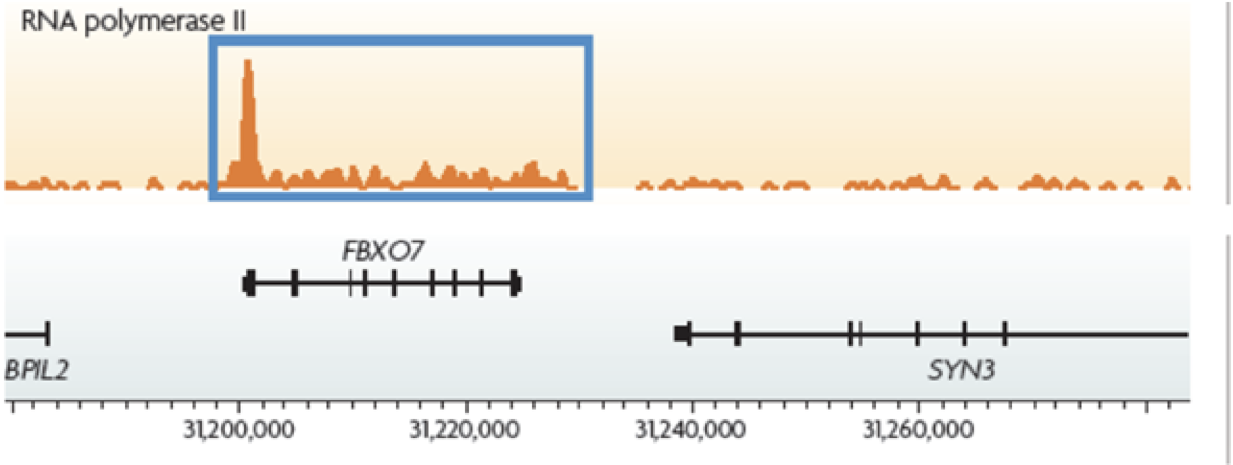
https://www.ebi.ac.uk/sites/ebi.ac.uk/files/content.ebi.ac.uk/materials/2013/131021_HTS/introduction_to_chip_seq_data_and_analysis-b.gerle_.pdf

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.

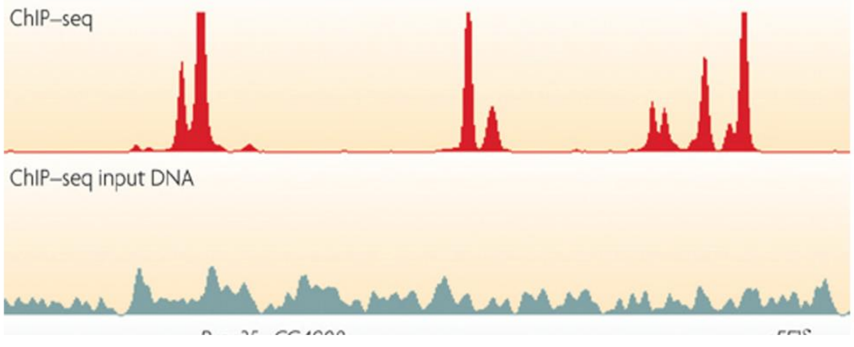
RNA PolII – enriched at TSS but bound throughout gene body



https://www.ebi.ac.uk/sites/ebi.ac.uk/files/content.ebi.ac.uk/materials/2013/131021_HTS/introduction_to_chip_seq_data_and_analysis-b.gerle_.pdf

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 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".

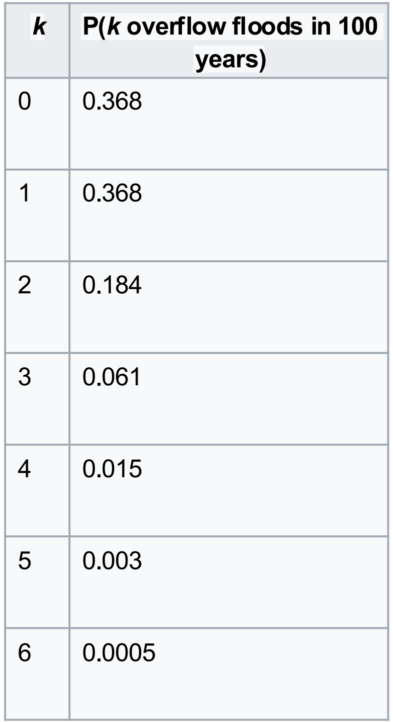
Thus ƛ=1 (1 catastrophic flood every 100 years

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

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

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

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



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

https://en.wikipedia.org/wiki/Poisson_distribution

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

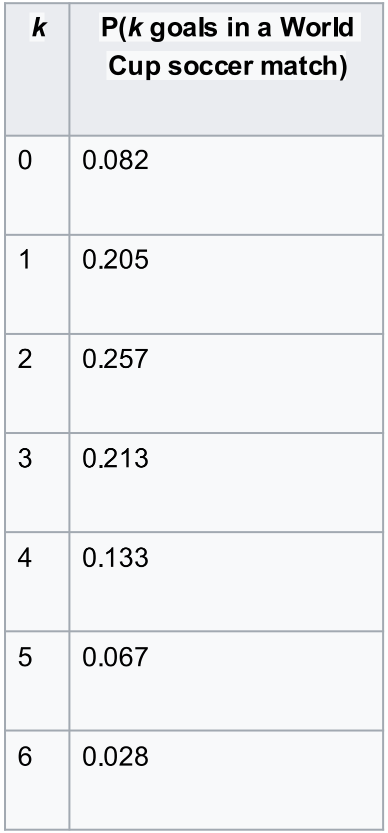
Average number of goals is 2.5

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

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

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

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

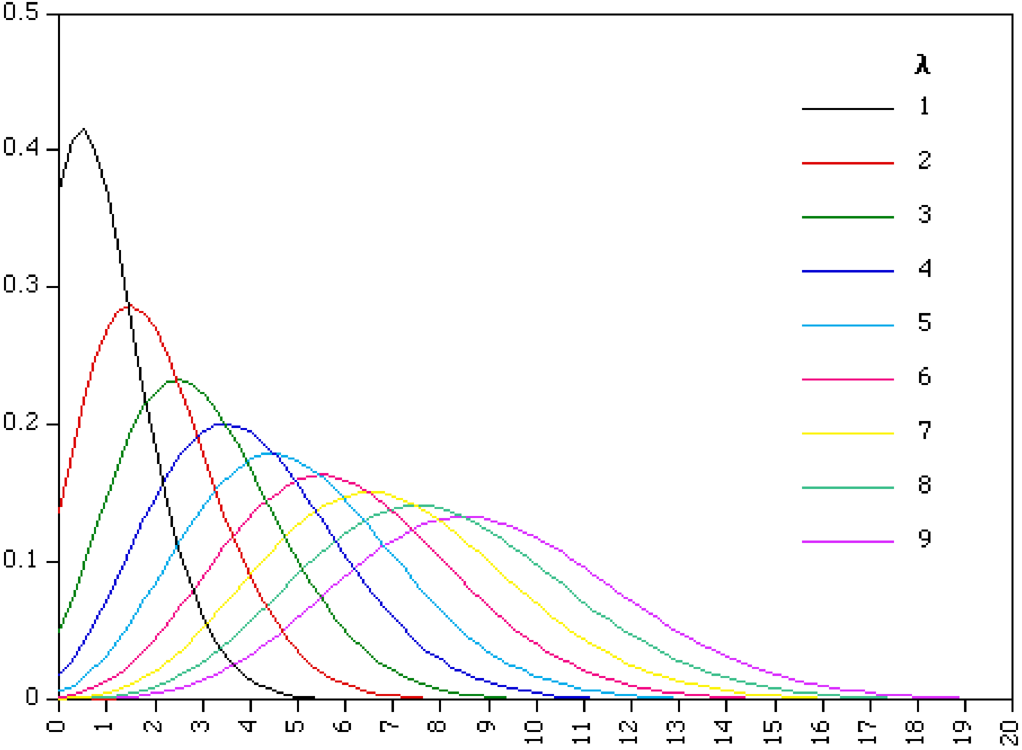


k	**P(*k* goals in a World Cup soccer match)**
0	0.082
1	0.205
2	0.257
3	0.213
4	0.133
5	0.067
6	0.028

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

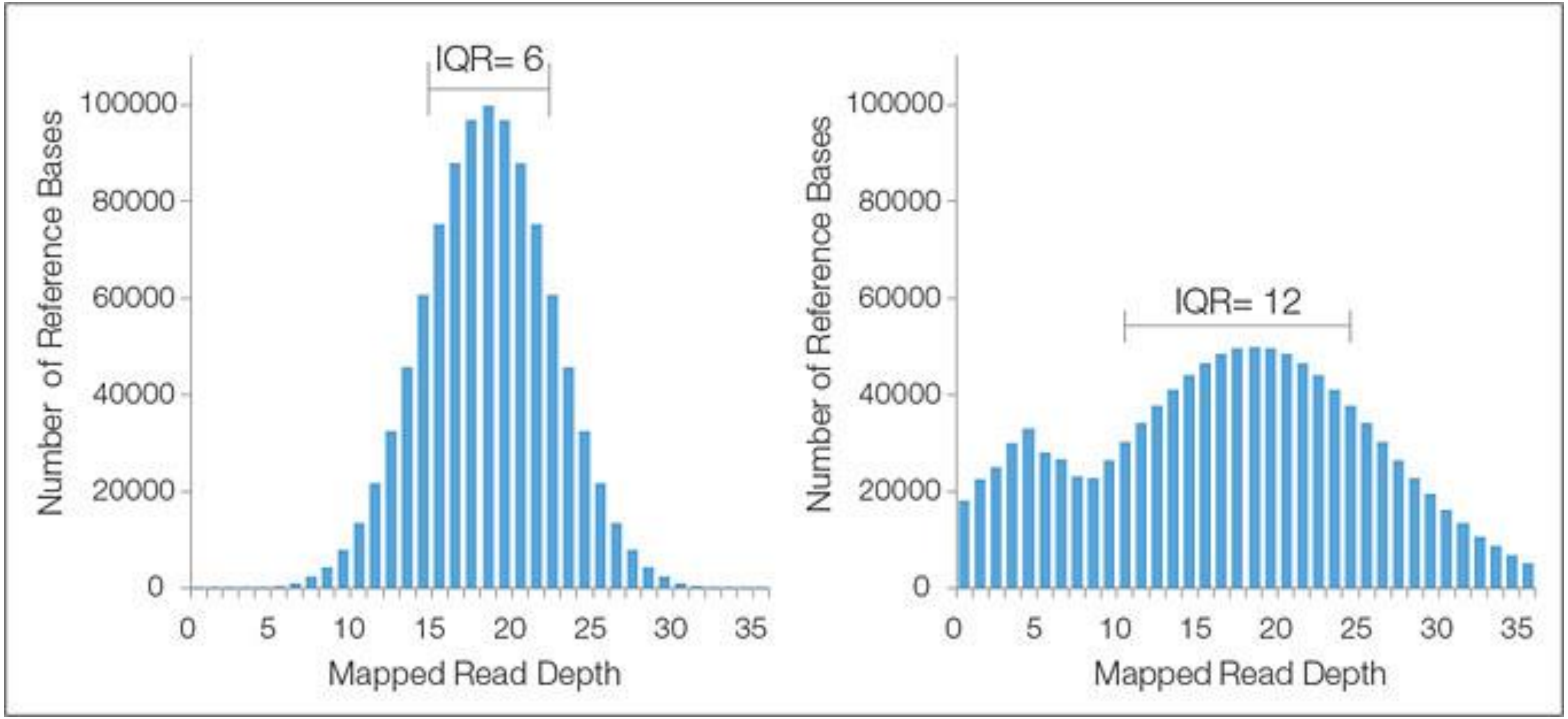
https://en.wikipedia.org/wiki/Poisson_distribution

Poisson distribution with different values of ƛ



https://www.umass.edu/wsp/resources/poisson/

Ideally, sequencing coverage will follow a Poisson distribution. But...

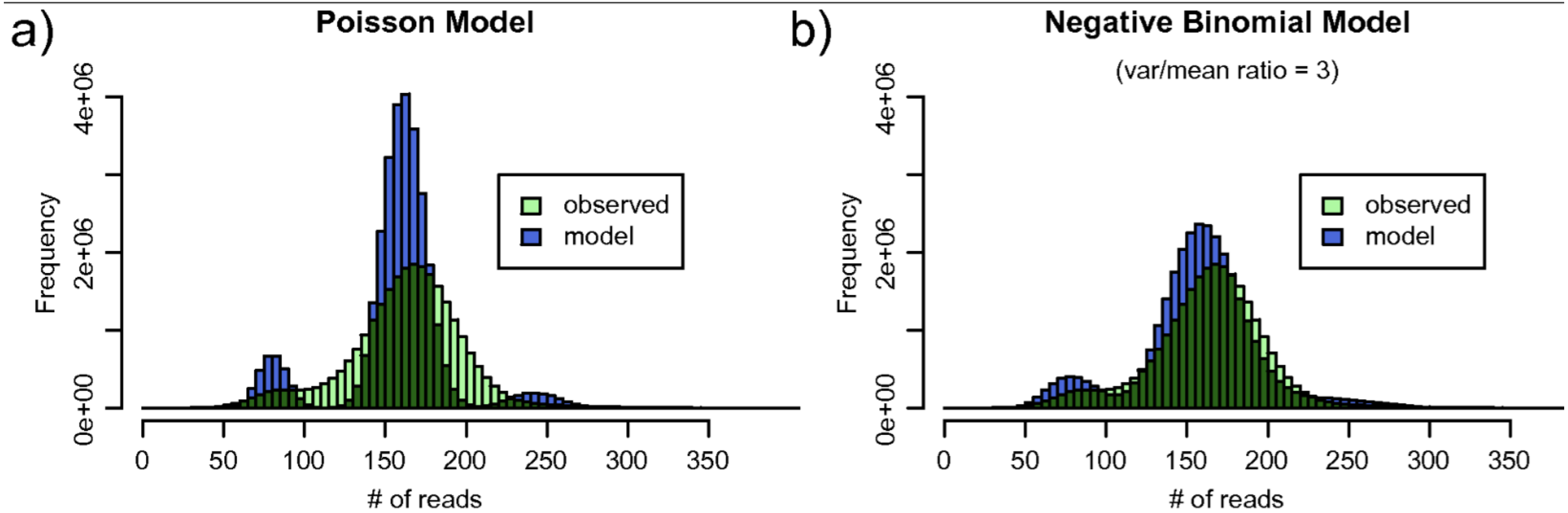


Poisson

Not Poisson.
Overly "dispersed"

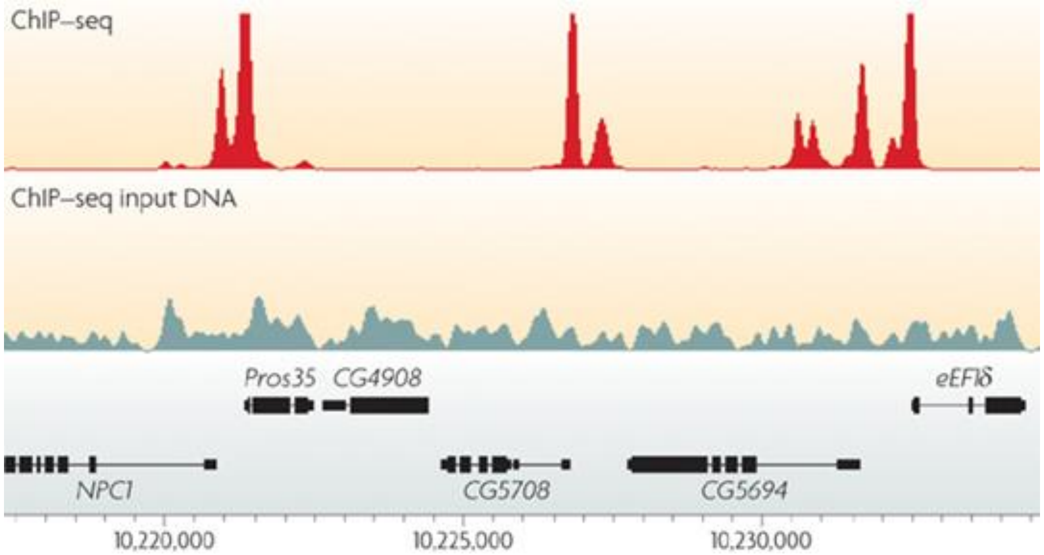
https://www.umass.edu/wsp/resources/poisson/

Negative binomial fits sequencing coverage data much better



https://www.umass.edu/wsp/resources/poisson/

Comparative ChIP-seq: scaling and normalizing



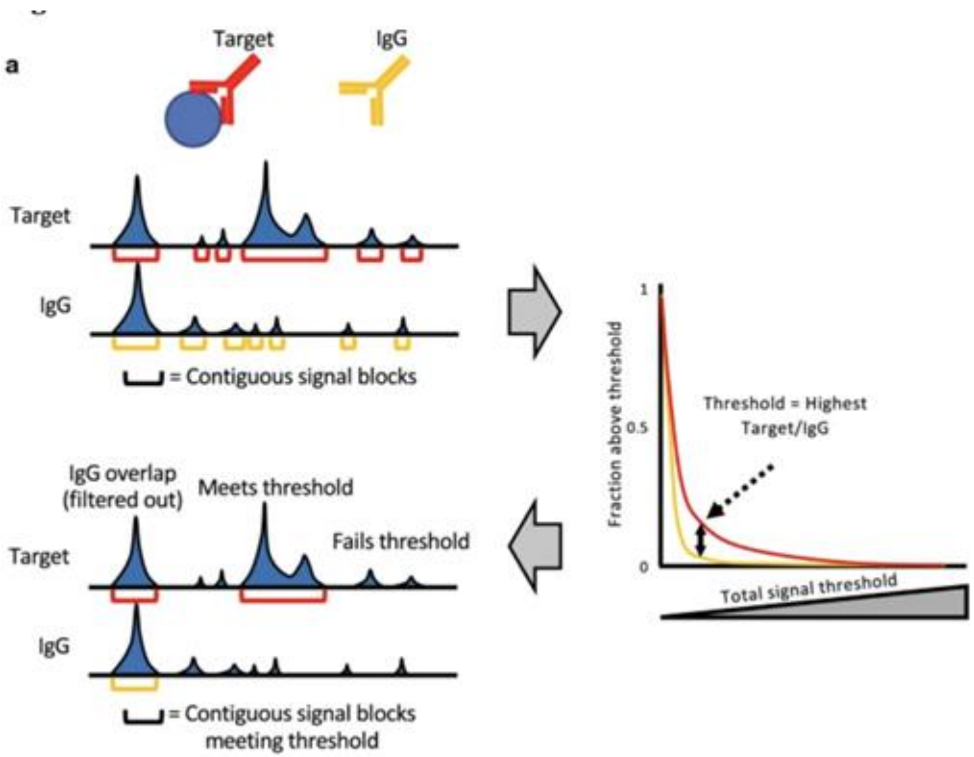
Sequencing depths from TF ChIP should be compared to control (input DNA) to get a sense of the noise.

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.**

Nature Reviews Genetics 10, 669-680 (October 2009)

Peak-calling

MACS2, HOMER, SEACR, etc


a
Target
IgG
Target
IgG
= Contiguous signal blocks
1
0.5
0
Fraction above threshold
Threshold = Highest Target/IgG
Total signal threshold
IgG overlap (filtered out)
Meets threshold
Fails threshold
Target
IgG
= Contiguous signal blocks meeting threshold

ChIP-seq peak callers

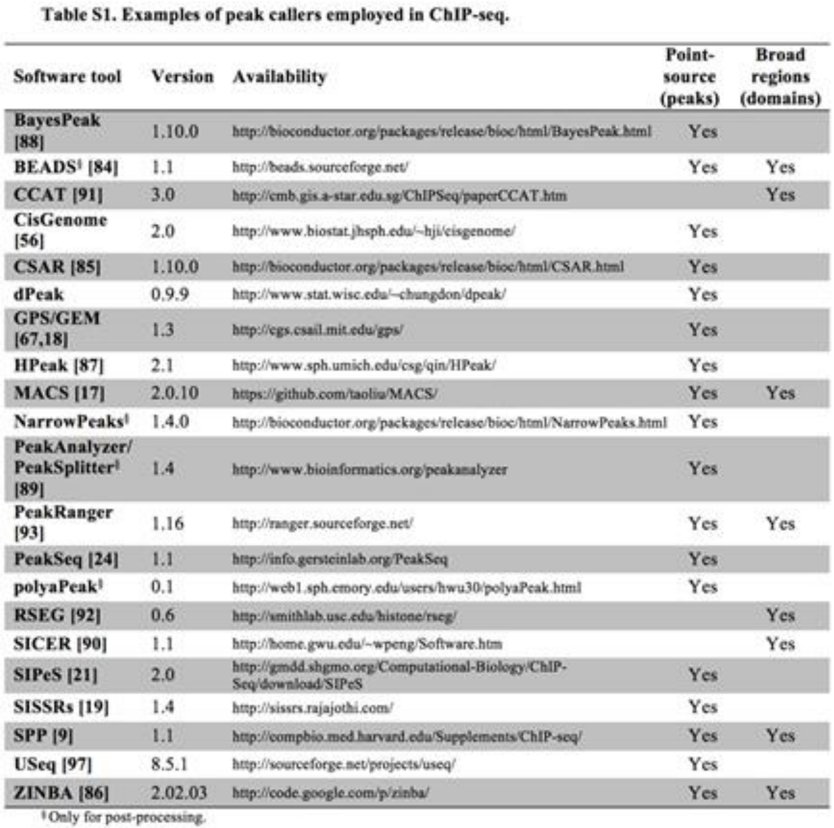


Table S1. Examples of peak callers employed in ChIP-seq.

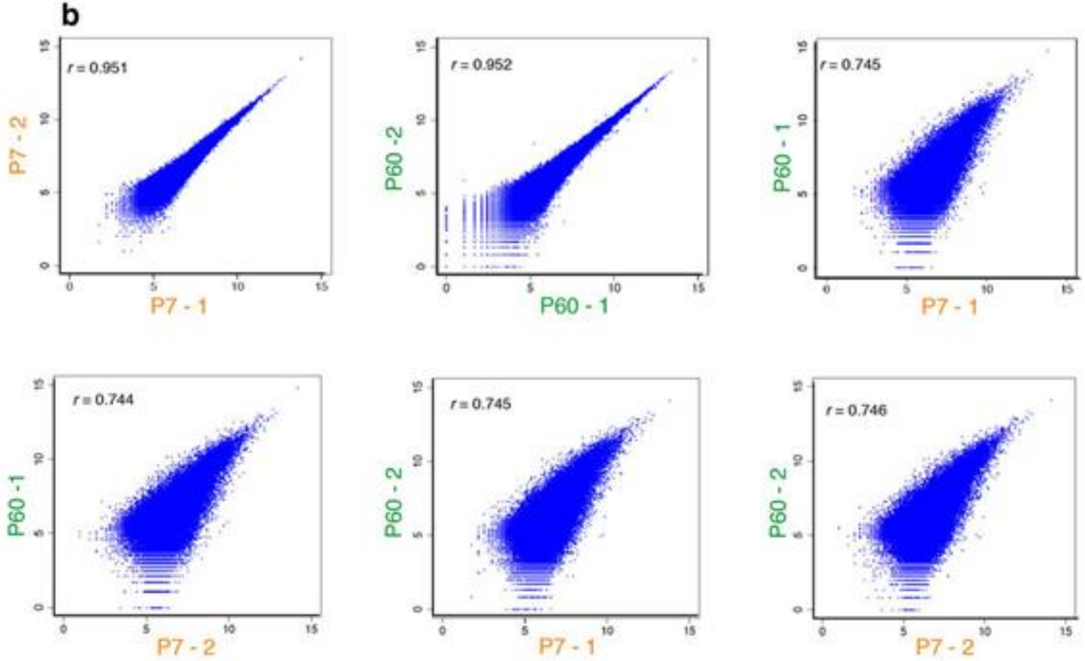
Software tool	Version	Availability	Point-source (peaks)	Broad regions (domains)
BayesPeak [88]	1.10.0	http://bioconductor.org/packages/release/bioc/html/BayesPeak.html	Yes	
BEADS‡ [84]	1.1	http://beads.sourceforge.net/	Yes	Yes
CCAT [91]	3.0	http://cmb.gis.a-star.edu.sg/ChIPSeq/paperCCAT.htm		Yes
CisGenome [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

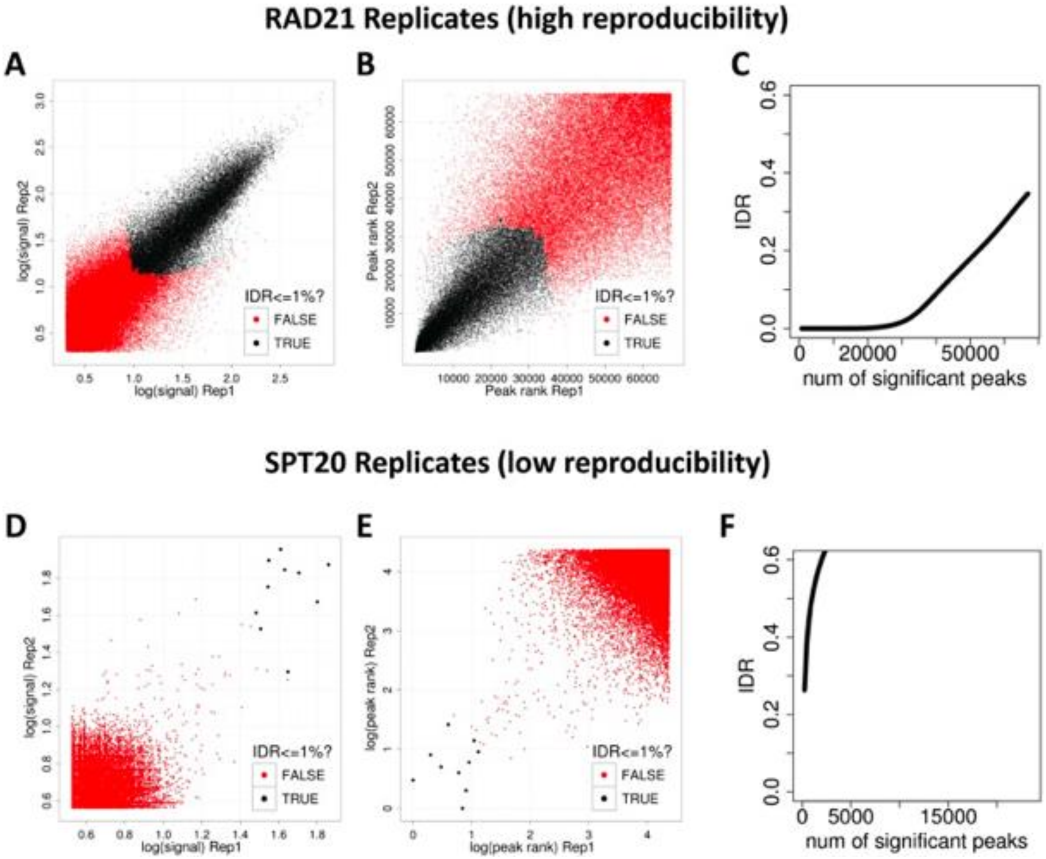


(**b**) Scatterplots of pairwise Zic ChIP-seq replicates with Pearson correlation (*r*) displayed. **Note the correlations are much higher between biological replicates of the same developmental stage than between P7 and P60 cerebellum**.

Pearson Correlation is one metric indicative of overall reproducibility

http://www.nature.com/neuro/journal/v18/n5/full/nn.3995.html

Irreproducibility Discovery Rate (IDR)

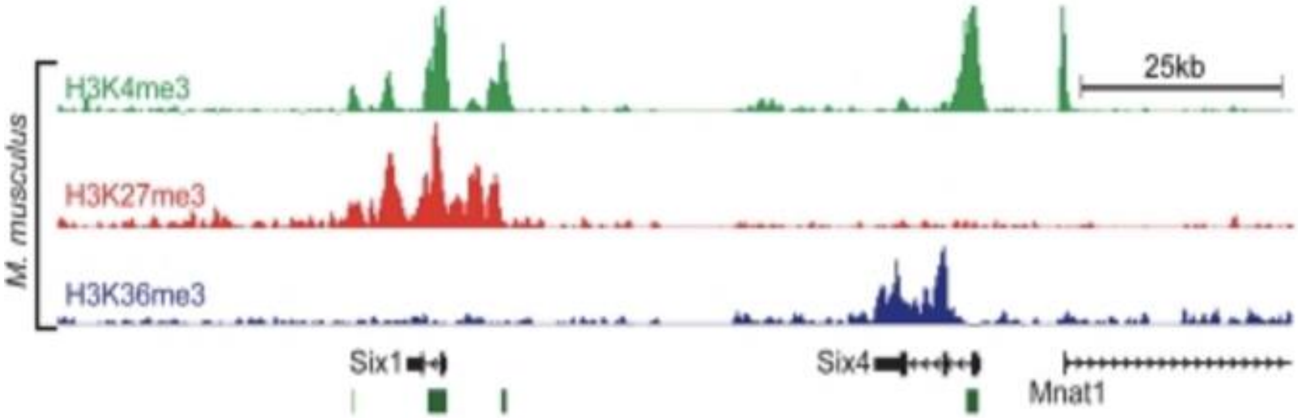


Peaks that show up consistently among replicates are more likely to be real!

Fantastic resource for learning ChIP-seq analysis

https://github.com/hbctraining/Intro-to-ChIPseq

Interpretation

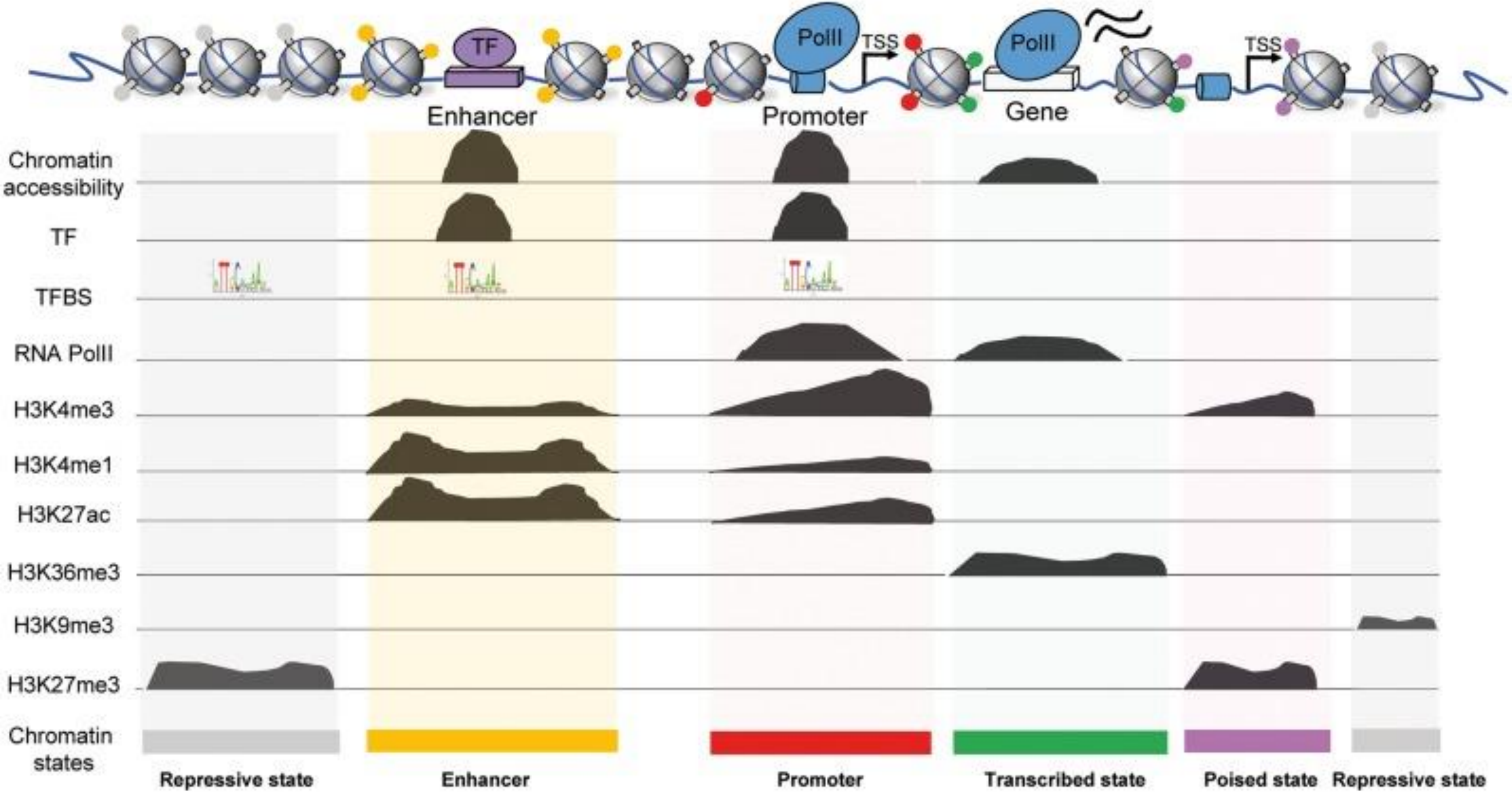


Activating mark (near TSS)

Peaks within body of inactive genes

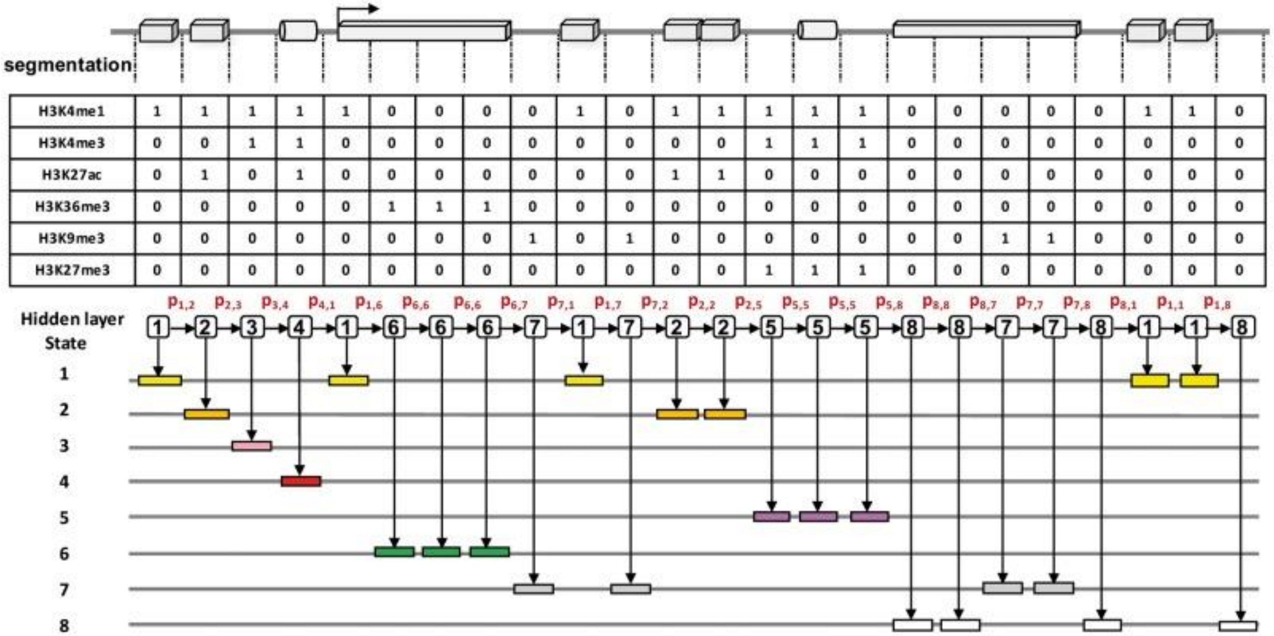
Peaks within body of active genes

https://www.ebi.ac.uk/sites/ebi.ac.uk/files/content.ebi.ac.uk/materials/2013/131021_HTS/introduction_to_chip_seq_data_and_analysis-b.gerle_.pdf



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5888983/

Genome Segmentation with ChromHMM


segmentation
Hidden layer State
p1,2 p2,3 p3,4 p4,1 p1,6 p6,6 p6,6 p6,7 p7,1 p1,7 p7,2 p2,2 p2,5 p5,5 p5,5 p5,8 p8,8 p8,7 p7,7 p7,8 p8,1 p1,1 p1,8
1 2 3 4 1 6 6 6 7 1 7 2 2 5 5 5 8 8 7 7 8 1 1 8
1
2
3
4
5
6
7
8

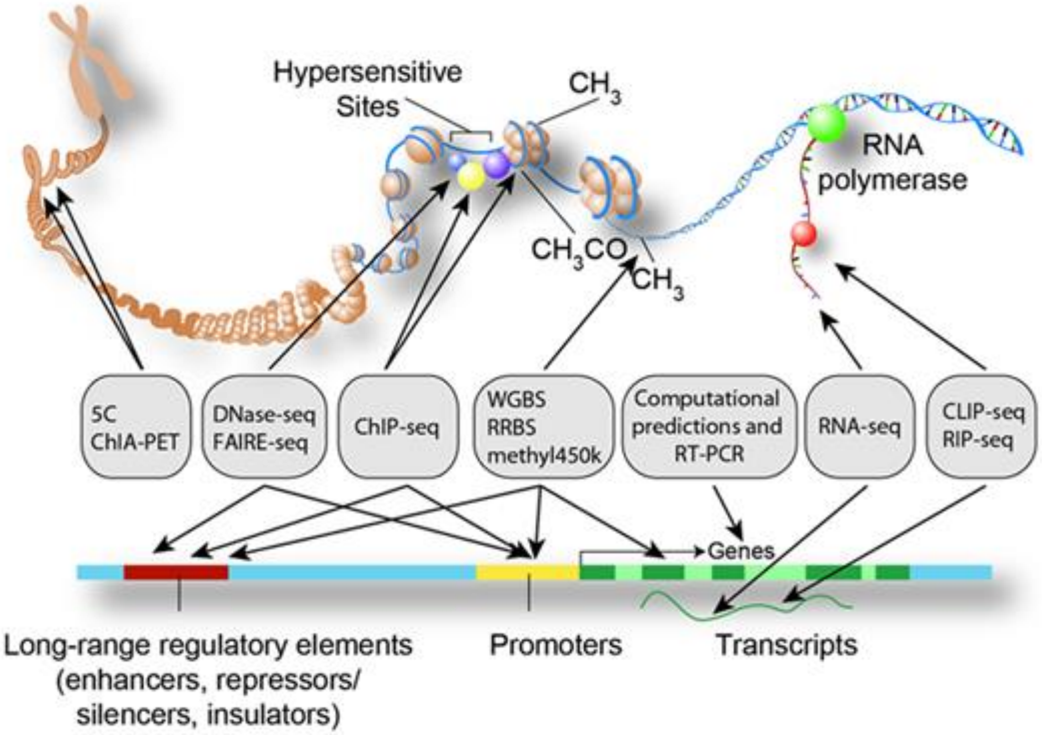
H3K4me1	1	1	1	1	1	0	0	0	0	1	0	1	1	1	1	1	0	0	0	0	0	1	1	0
H3K4me3	0	0	1	1	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0
H3K27ac	0	1	0	1	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0
H3K36me3	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
H3K9me3	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	1	1	0	0	0	0
H3K27me3	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0

Chromatin state	State annotation
1	Poised enhancer
2	Active enhancer
3	Weak promoter
4	Active promoter
5	Poised promoter
6	Transcriptional elongation
7	Repressive state
8	Low signal state

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

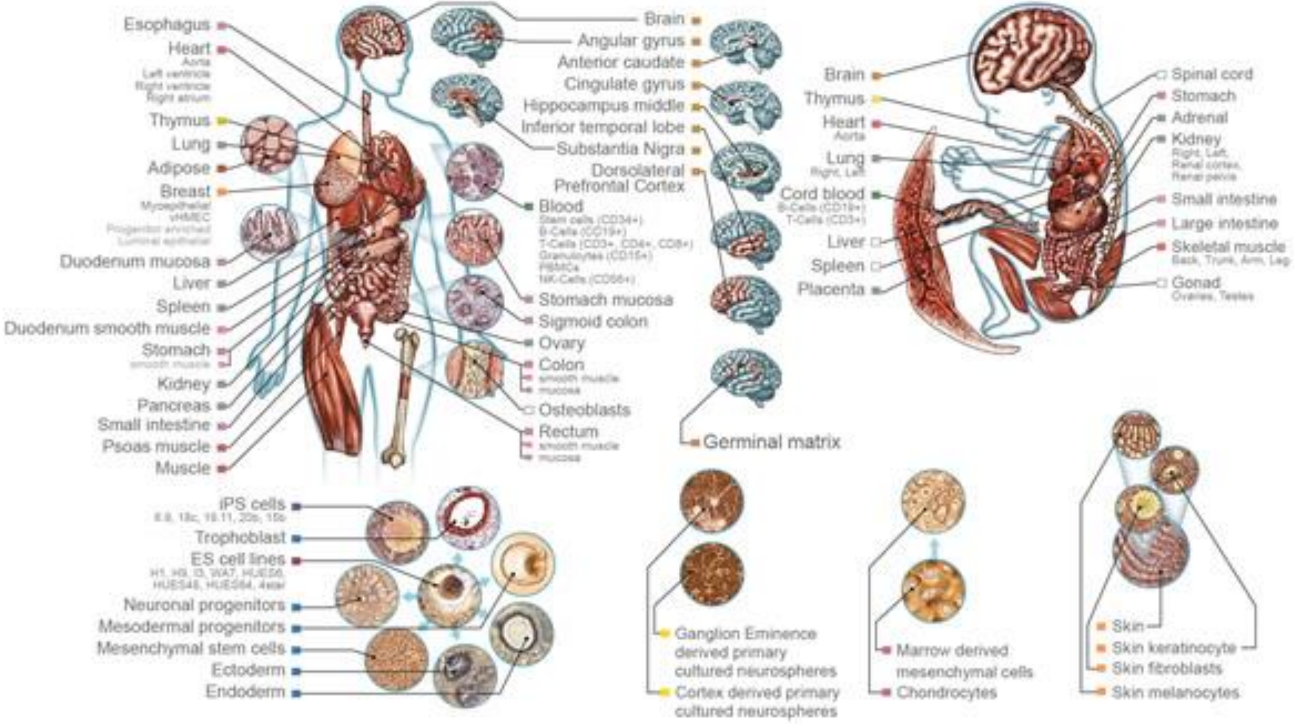
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5888983/

The Encyclopedia of DNA Elements Project



https://www.encodeproject.org/

Roadmap Epigenomics Project



http://commonfund.nih.gov/epigenomics/index