Regulatory Genomics

Charles Blatti
Research Scientist

Based on the lecture of

Saurabh Sinha
Professor
Biomedical Engineering
Georgia Tech

National Center for Supercomputing Applications
UNIVERSITY OF ILLINOIS URBANA-CHAMPAIGN

The Importance of Gene Regulation

Image Credit: Nick Youngson / Alpha Stock Images

DNA, RNA, Proteins

Gene: a piece of DNA, has the “code” to make a protein

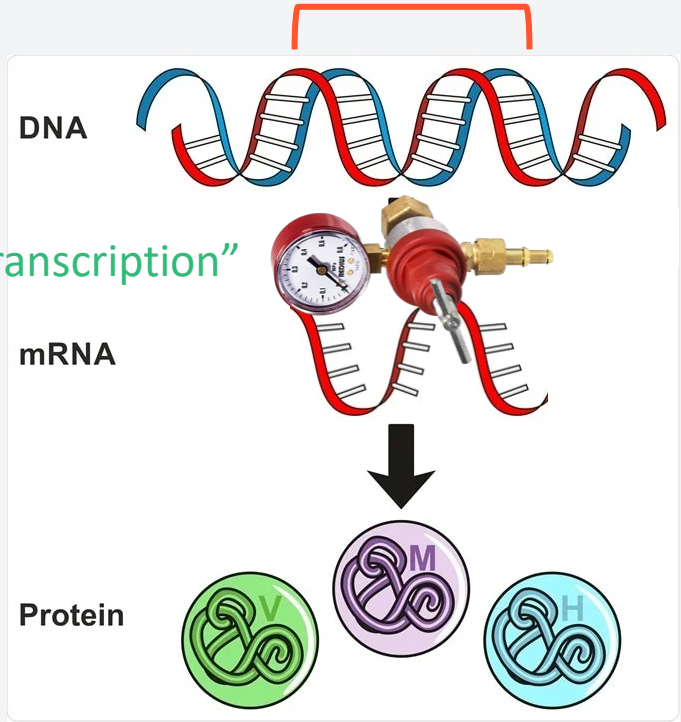


Image Credit: udaix / Shutterstock.com

DNA: a long sequence of nucleotides (a,c,g,t)

GENE EXPRESSION

mRNA: a physical “copy” of gene

CAN BE REGULATED

protein: molecule with important functions in cell

Gene Regulation

- Gene regulation is the process of turning genes on and off.

- Gene regulation ensures that the appropriate genes are expressed in the right cells at the proper times.

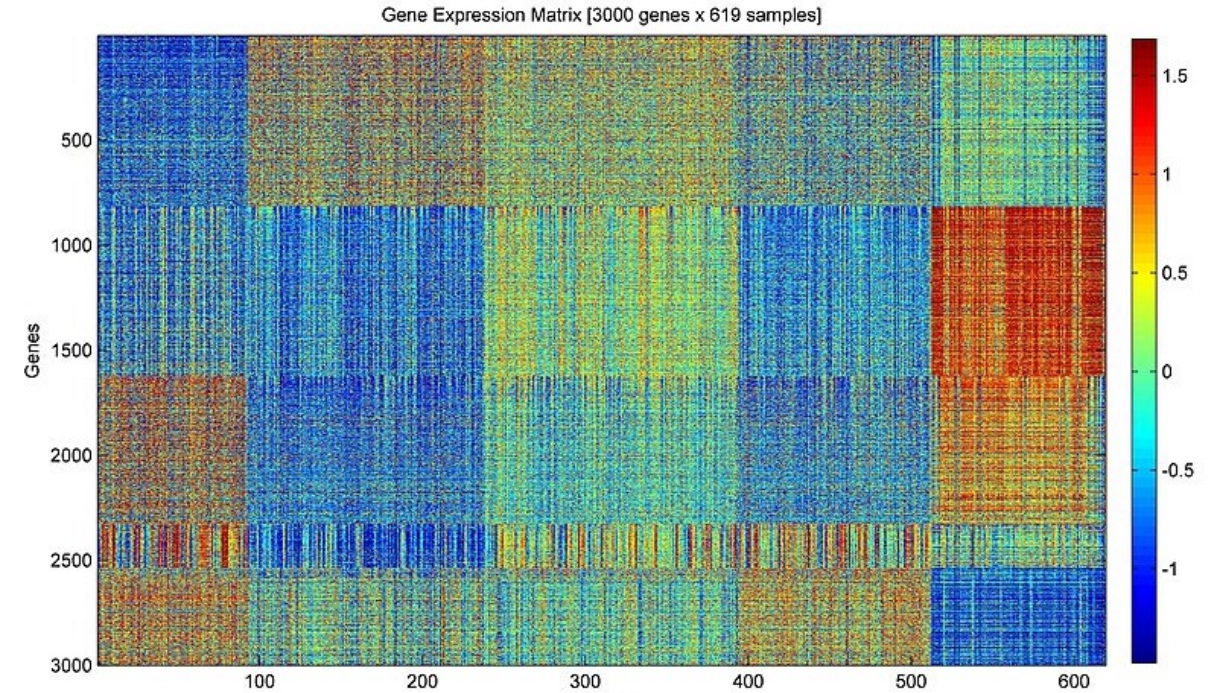
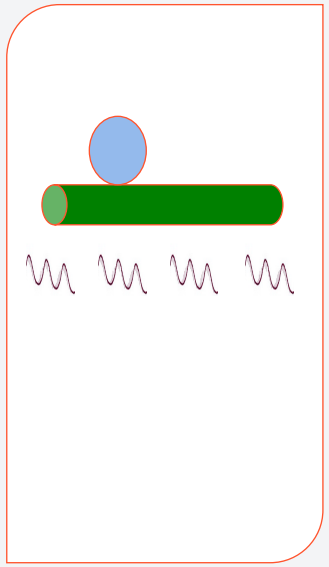
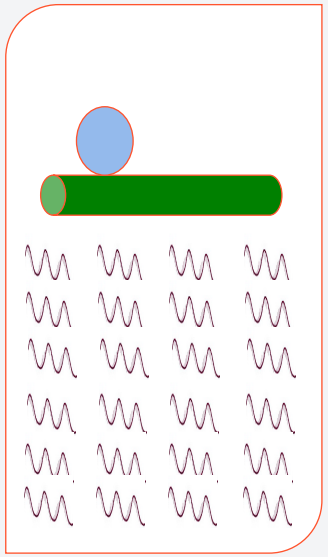


Image Credit: Wikimedia Commons

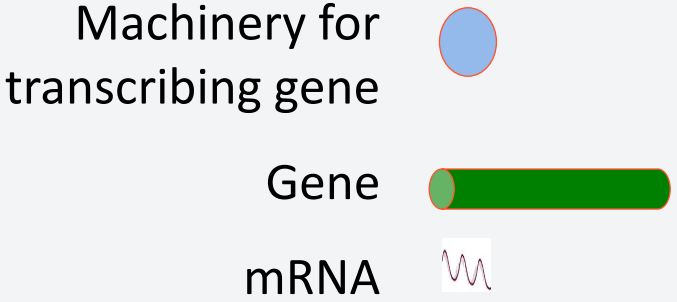
Gene Regulation: fast and slow transcription



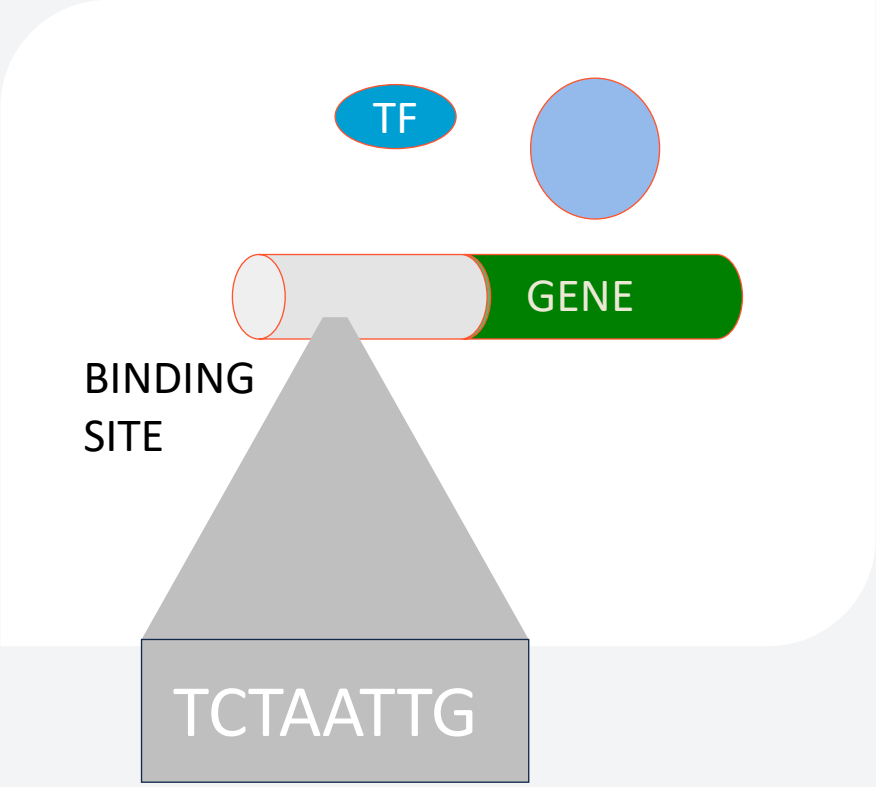
Low gene expression



High gene expression



Regulation by Proteins called Transcription Factors (TFs)

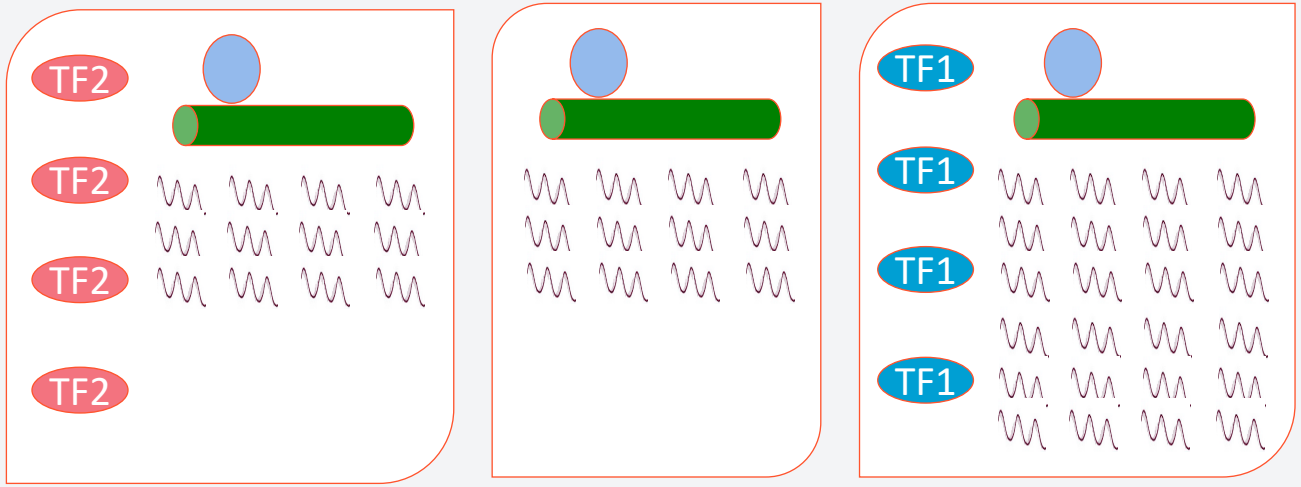


Humans have ~2000 TFs

Different cells may have different TFs

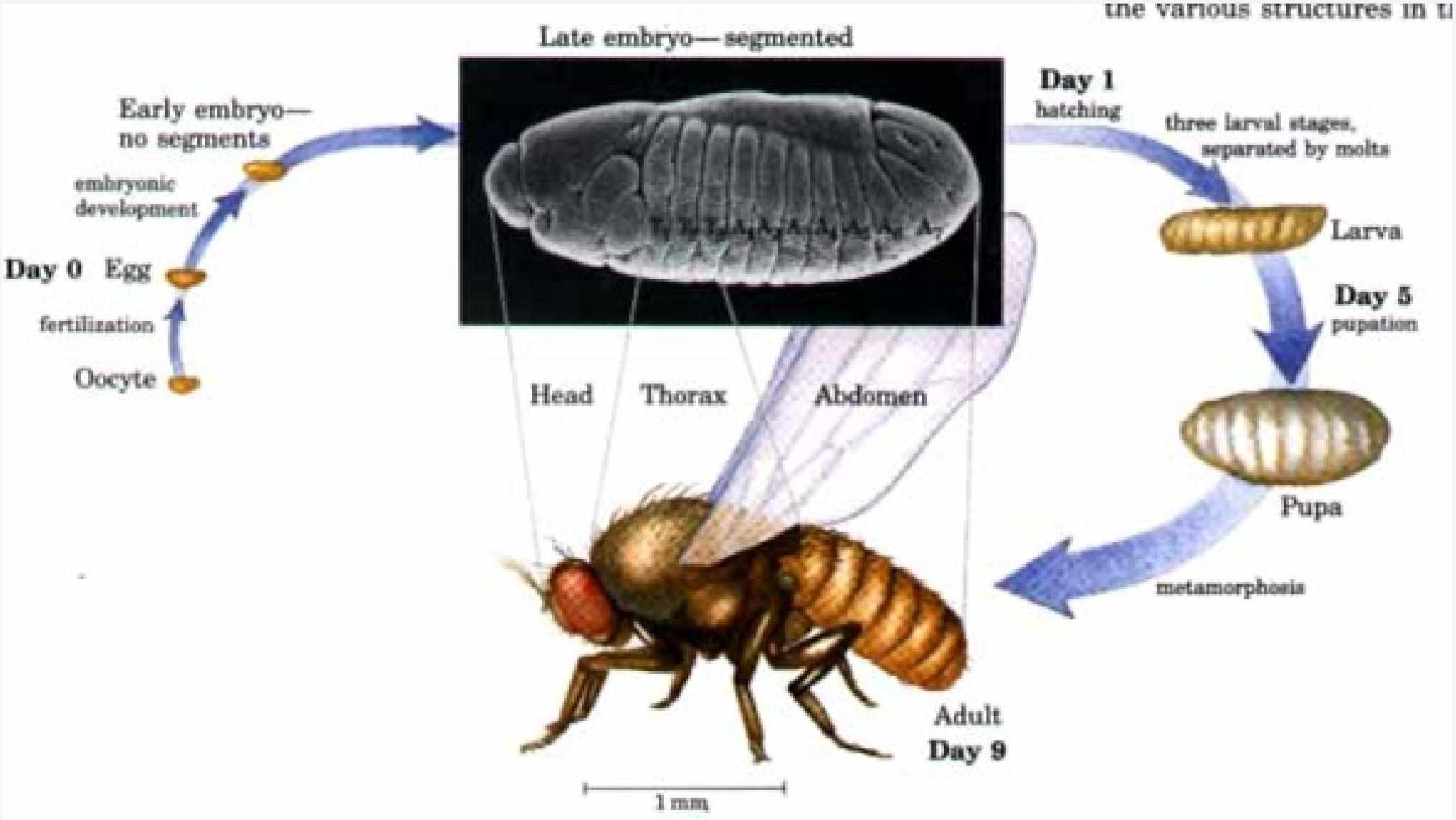
TF2 represses gene.
Low gene expression

TF1 activates gene.
High gene expression



Liver cell Skin cell Heart cell

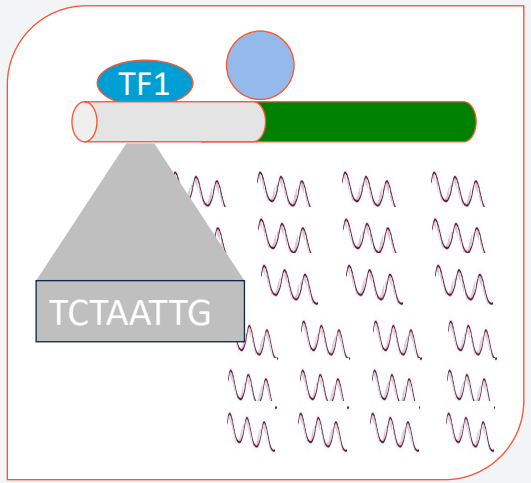
Gene regulation builds bodies



Source: https://thegreatfruitflyescape.weebly.com/fruit-fly-basics.html

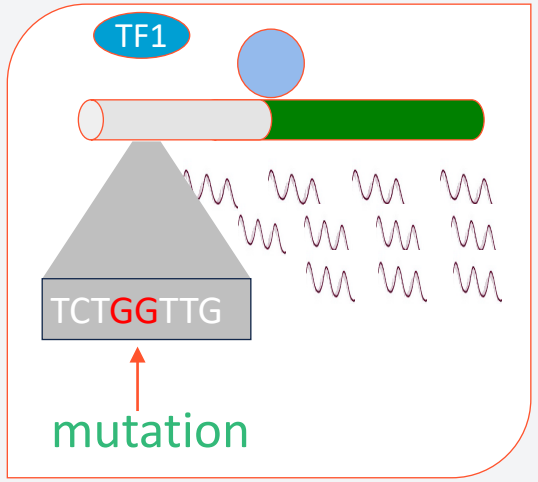
Different cells occasionally have different DNA

TF1 binds DNA and activates gene.
High gene expression



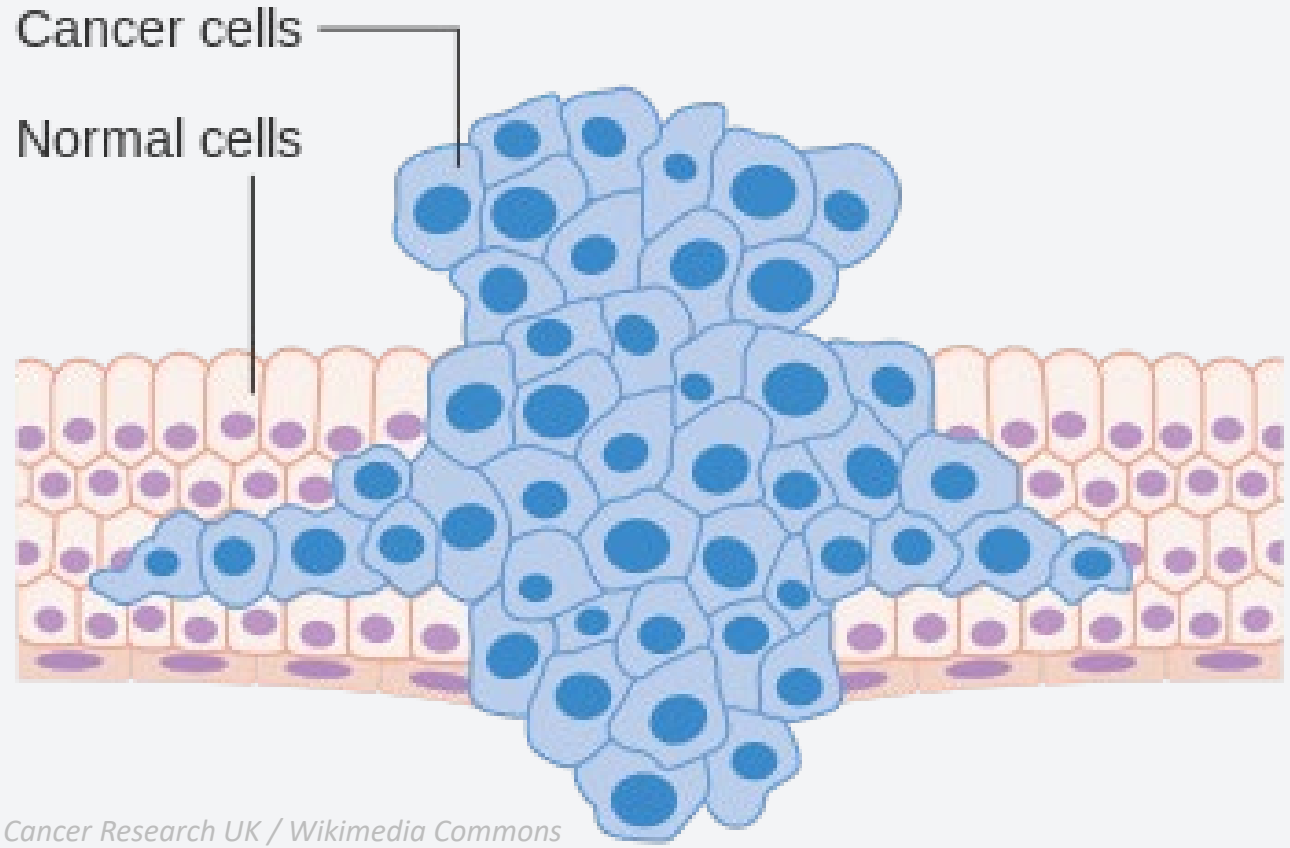
Normal cell

TF1 cannot bind DNA, doesn't activate gene.
Low gene expression



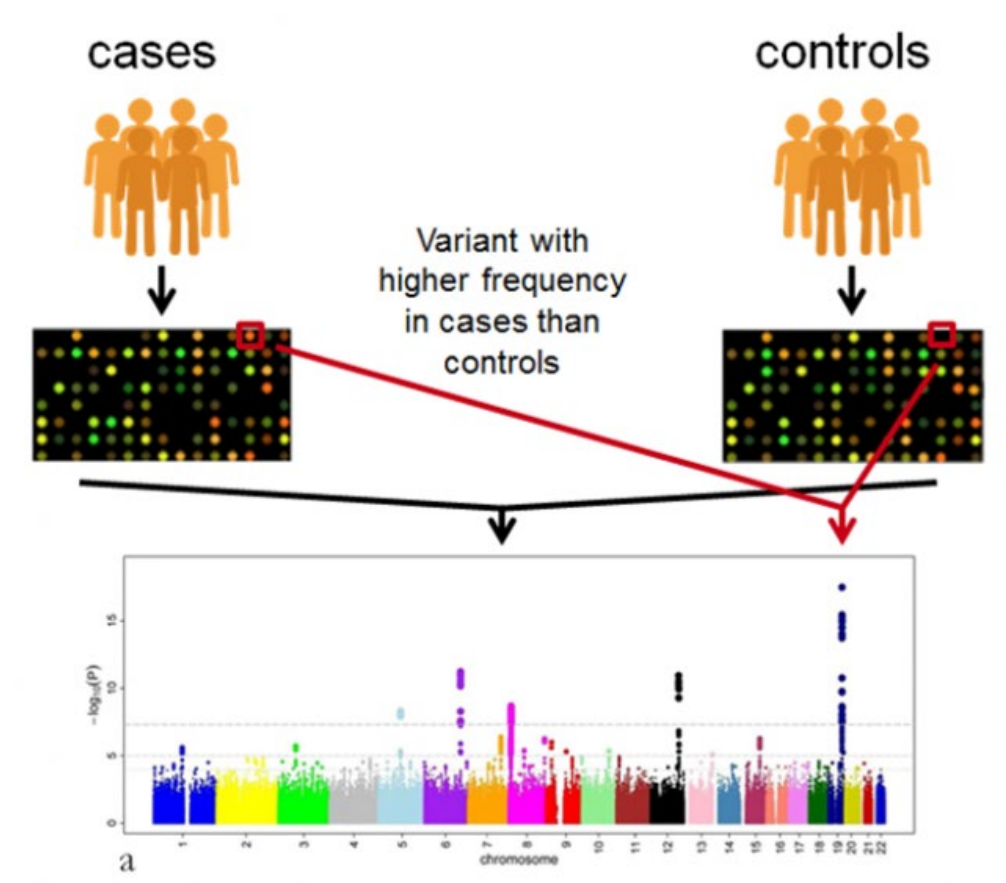
Tumor cell

Gene Regulation is disrupted in cancer



Source: Cancer Research UK / Wikimedia Commons

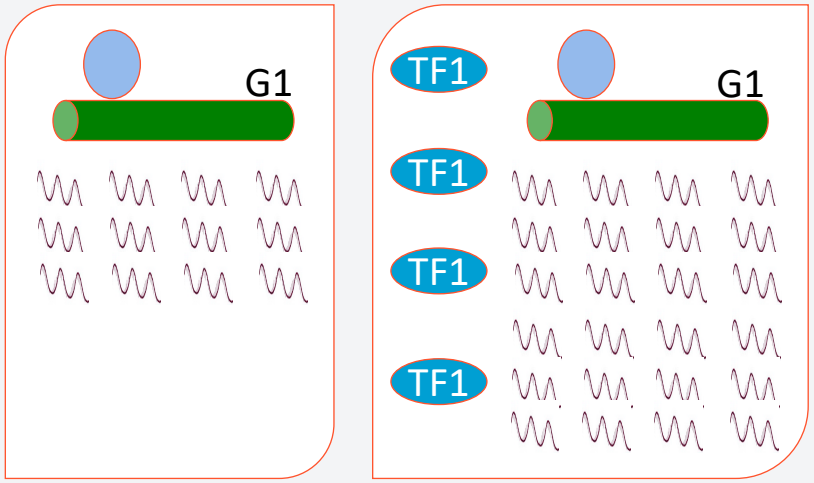
Most disease-related mutations are outside of genes



(impact gene regulation)

Source: https://www.ebi.ac.uk/training/online/courses/gwas-catalogue-exploring-snp-trait-associations/what-is-gwas-catalog/what-are-genome-wide-association-studies-gwas/

Gene Regulatory Networks: TF-gene relationships



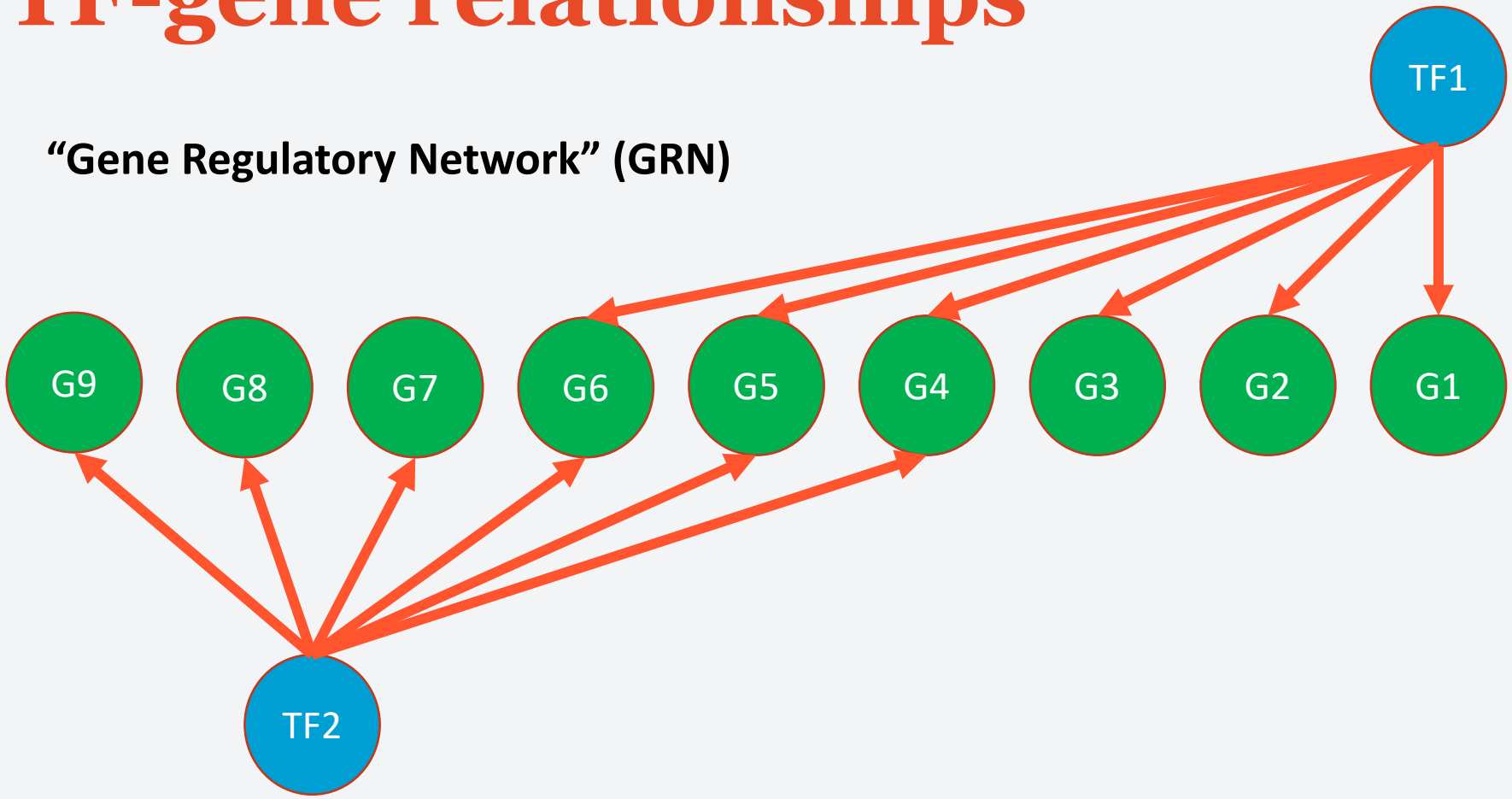
Healthy sample

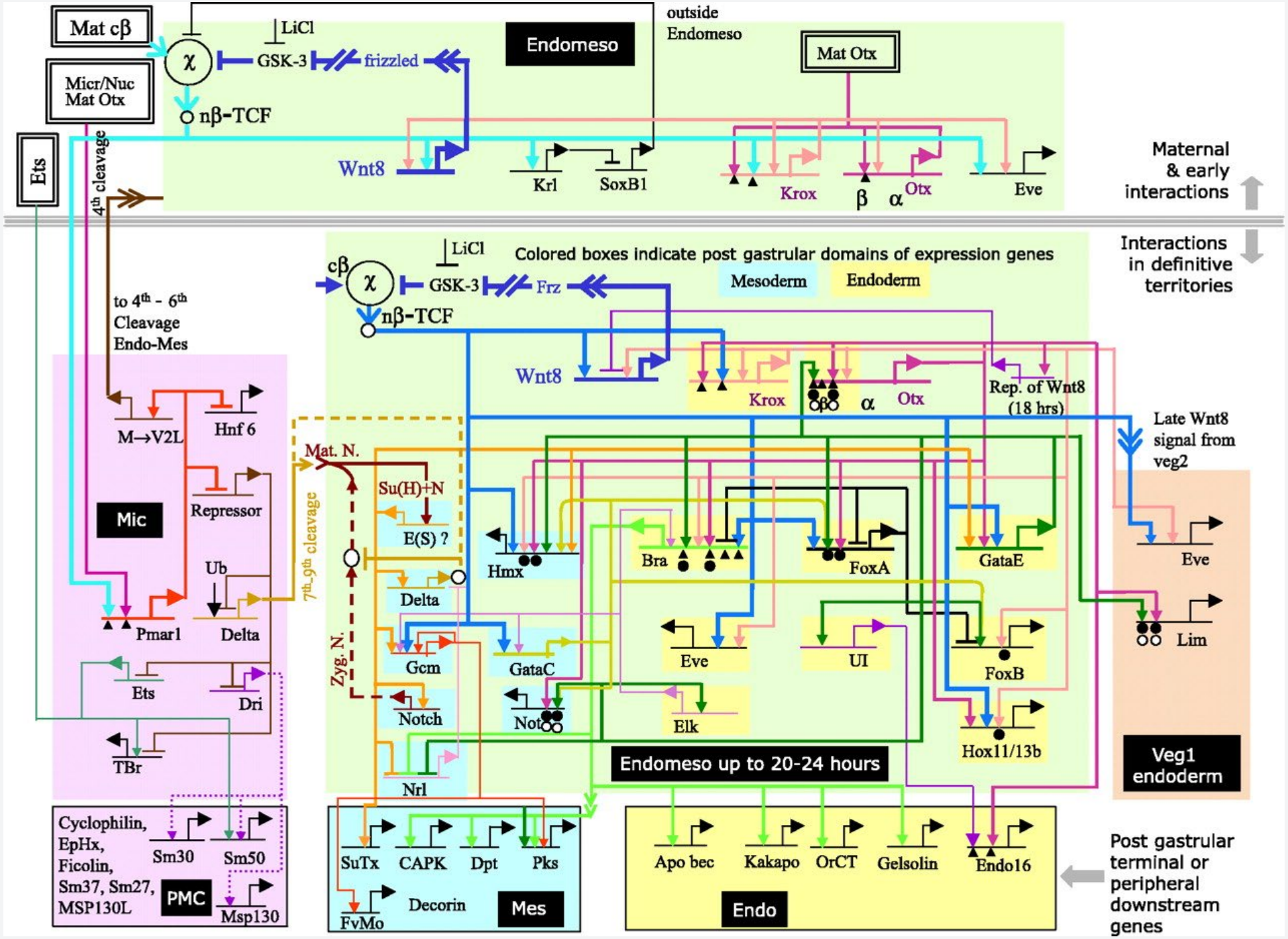
Tumor sample

TF1 activates gene.
High gene expression

Gene Regulatory Networks: TF-gene relationships

"Gene Regulatory Network" (GRN)

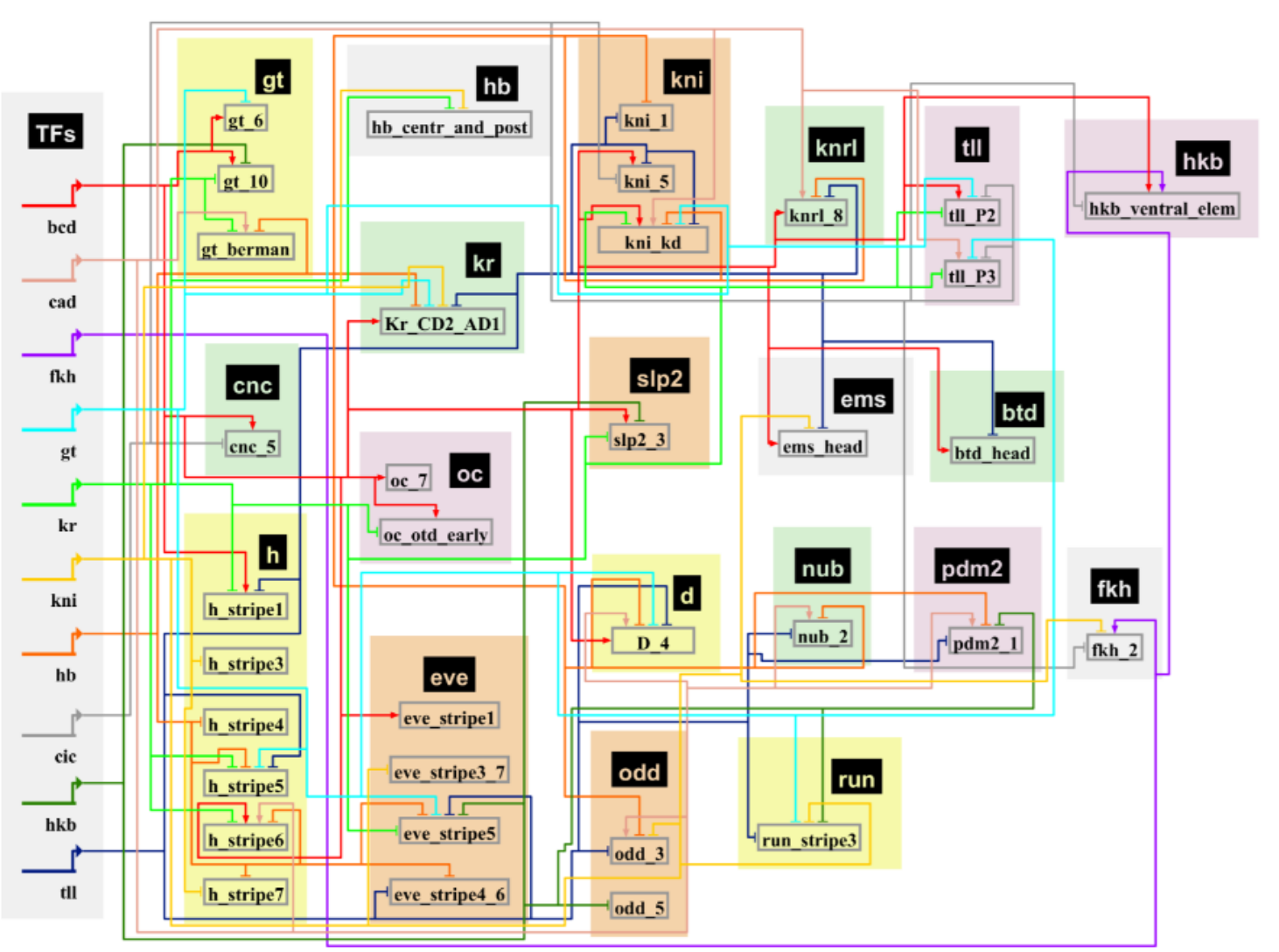




Genetic regulatory network controlling the development of the body plan of the sea urchin embryo.

Davidson et al., Science, 295(5560):1669-1678

GRNs can be reconstructed computationally



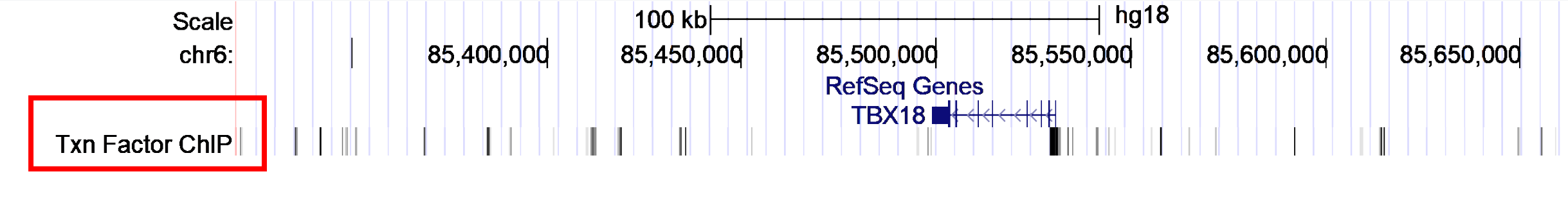
PLOS BIOLOGY

Quantitative Analysis of the *Drosophila* Segmentation Regulatory Network Using Pattern Generating Potentials

Majid Kazemian, Charles Blatti, Adam Richards, Michael McCutchan, Noriko Wakabayashi-Ito, Ann S. Hammonds, Susan E. Celniker, Sudhir Kumar, Scot A. Wolfe, Michael H. Brodsky, Saurabh Sinha

- *Goal: discover the gene regulatory network*
- *Sub-goal: discover the genes regulated by a transcription factor*

Genome-wide assays



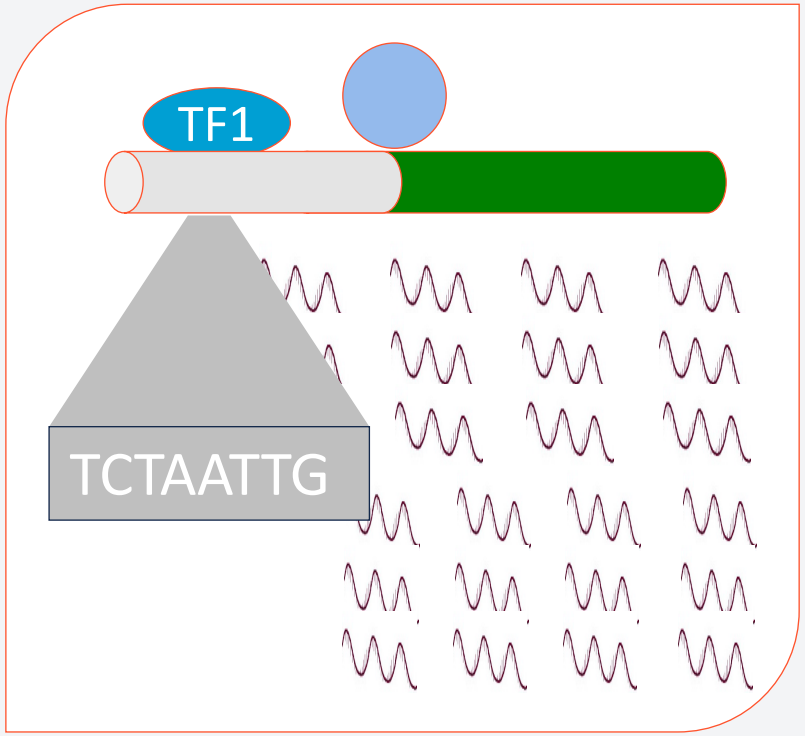
One experiment per cell type AND PER TF

... tells us which TF might regulate a gene of interest

Expensive !

- *Goal: discover the gene regulatory network*
- *Sub-goal: discover the genes regulated by a transcription factor*
- *… by DNA sequence analysis*

The regulatory network is encoded in the DNA



It should be possible to predict where transcription factors bind, by reading the DNA sequence

Motifs and DNA sequence analysis

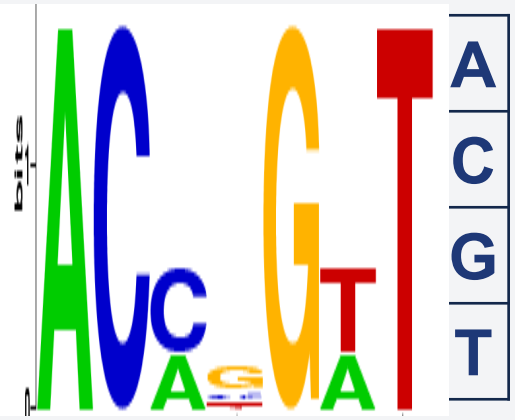
```
GCTCCTCAAG GCTTGTTTACA TAATCACCGT
TCCGCTTCTT GCTTGTTTACA GTTAGGAATG
GTTAGATGTG GCTTGTTTACA TAAGCGATAA
GCTCGCGTCC GCTTGTTTACA CAAACGCCCA
TTTAGTGGCG GCTTGTTTACA GGGTTGCAGC
CATGCCGATC GCTTGTTTACA CACAAGCATG
AGCAGTTTCA GTTTGTTTACA CACCGGAGTC
CACTTTAATG GTTTGTTTACA TCATAAATAA
TGCTGCCTTG GTTTGTTTACA CCAGAATCGA
TTCGCATTTT GCTTGTTTACT TGCGTCAAAC
CGGCAACCAC GCTTGTTTATA ACATACAAAC
CACTCACGCT GTTTGTTTACT TTCGATAAAG
TAGTCACTCT CCTTGTTTACA TTTTGAATGT
TGGAATTTTC CCTTGTTTACA GCTATTATGC
AAGAGTGCCT CCTTGTTTACA CTAACTATTT
CTATTTTTAA GTTTGTTTATA GGAAATAGGT
CTGCACATCT GTTTGTTTATA TTGTAATTGT
CGCCTTCCTT CTTTGTTTACA TTCGTTCTTT
CAACTTCTGC TCTTGTTTACA CTGACGAATG
GATTTGTTCG GGTTGTTTACT GGGATCTCGA
ATCGCTTCTG GCTTGTTTATT TCTGGAGTAG
TCGTCGAGCT GCTTGTTTATT TGCTGCCGTC
CAGCGCCATT GTTTGTTTACG CAACTTTGAT
GTTTTCATGT GCTTGTTTATT TTTTCTGTGG
TCCGTAGCCA GCTTGTTTATT TGTTTGCCTT
GTTGCCGGCG GTTTGTTTACG GACCCGCGGC
TTTTGTCGGT TTTTGTTTACA ATTCGCTTCC
```

Finding TF targets

- Step 1. Determine the binding specificity of a TF
- Step 2. Find motif matches in DNA
- Step 3. Designate nearby genes as TF targets

Step 1. Determine the binding specificity of a TF

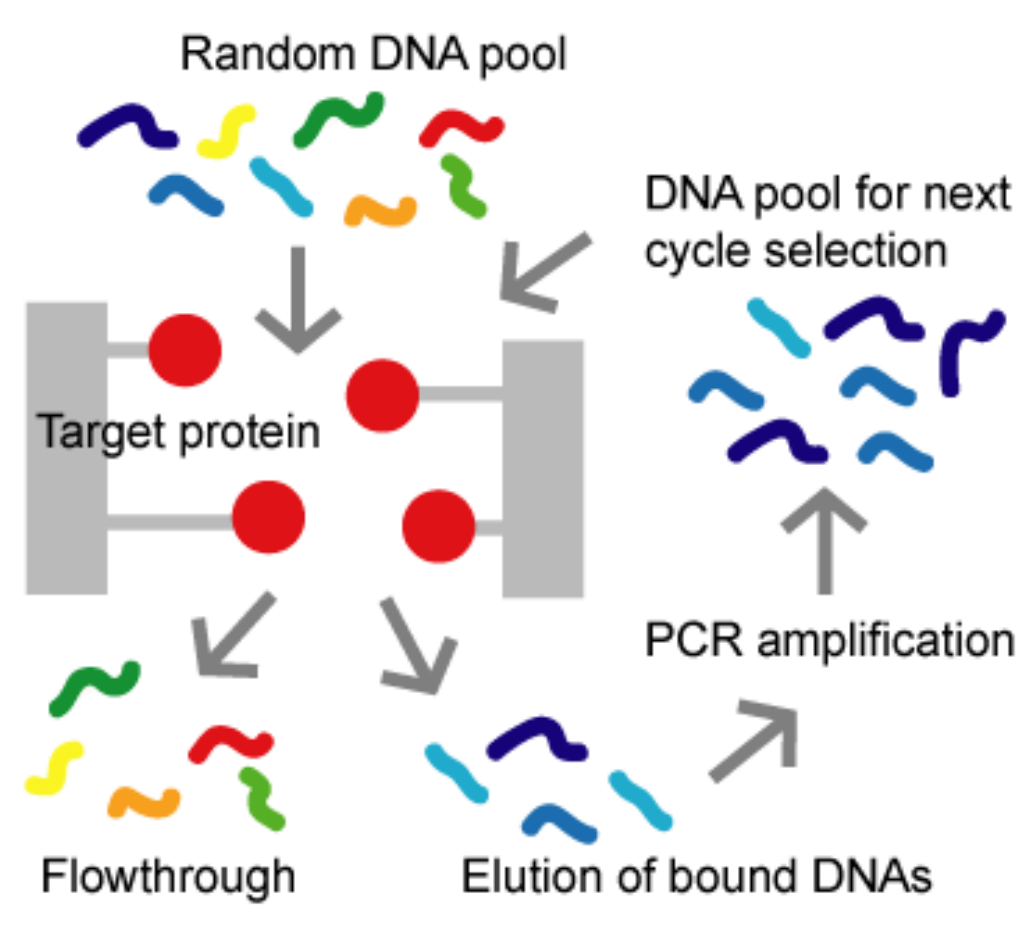
ACCCGTT
ACCGGTT
ACAGGAT
ACCGGTT
ACATGAT



"MOTIF"

How?

- SELEX

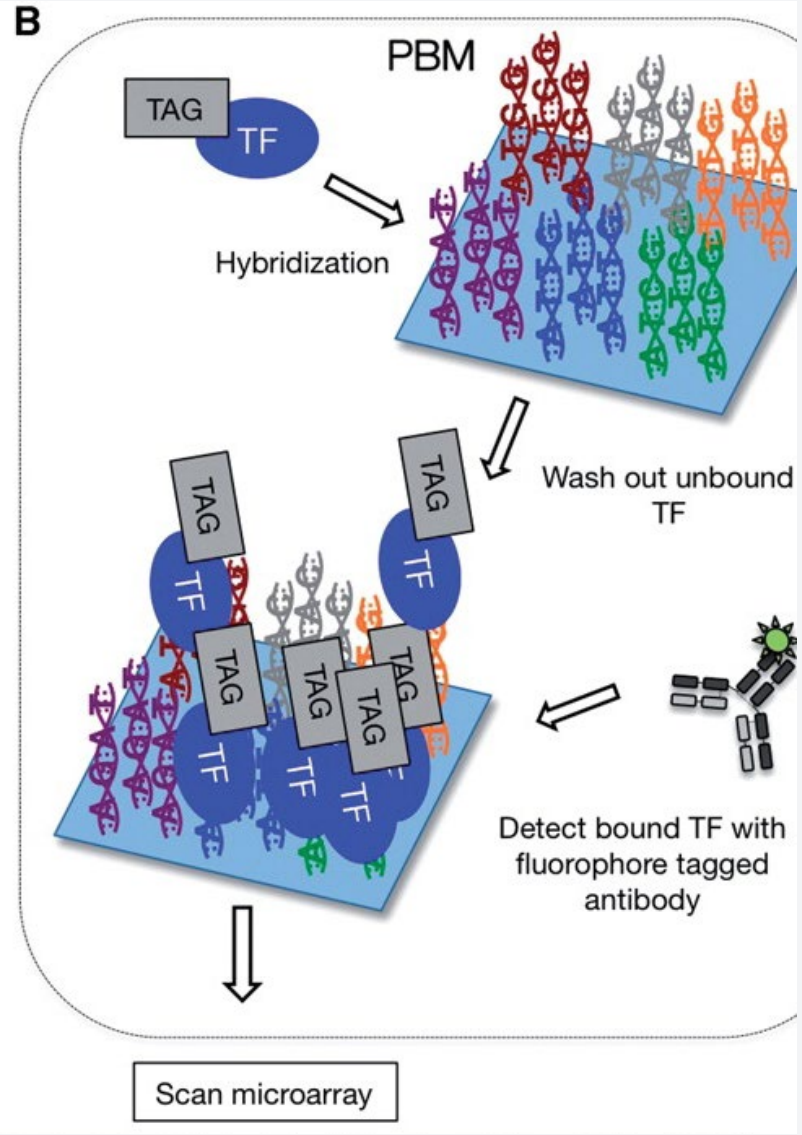


TAACCCGTTC
GTACCGGTTG
ACACAGGATT
AACCGGTTA
GGACATGAT

Source: http://altair.sci.hokudai.ac.jp/g6/Projects/Selex-e.html

How?

- Protein binding microarrays



TAACCCGTTC
GTACCGGTTG
ACACAGGATT
AACCGGTTA
GGACATGAT

Source: https://doi.org/10.1093/bfgp/elq023

Motif Databases

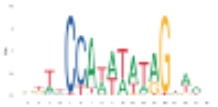
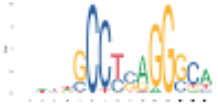
- JASPAR:
- https://jaspar2020.genereg.net/

JASPAR CORE

Total 1964 profiles

Display 10 profiles

Filter:

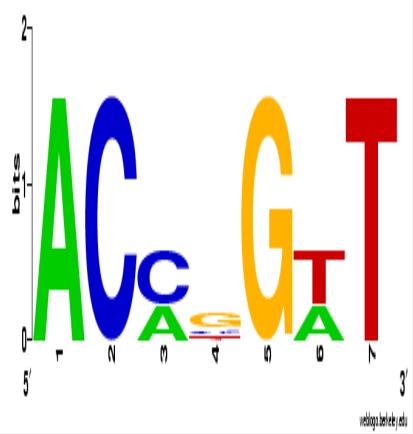
ID	Name	Species	Class	Family	Sequence logo
MA0001.1	AGL3	Arabidopsis thaliana	MADS box factors	MADS	
MA0001.2	AGL3	Arabidopsis thaliana	MADS box factors		
MA0002.1	RUNX1	Homo sapiens	Runt domain factors	Runt-related factors	
MA0002.2	RUNX1	Mus musculus	Runt domain factors	Runt-related factors	
MA0003.1	TFAP2A	Homo sapiens	Basic helix-span-helix factors (bHSH)	AP-2	
MA0003.2	TFAP2A	Homo sapiens	Basic helix-span-helix factors (bHSH)	AP-2	

Motif Databases

- TRANSFAC https://genexplain.com/transfac/
  - Public version and License version
- Cis-BP http://cisbp.ccbr.utoronto.ca/
  - Experimentally determined as well as computationally inferred motifs
- Hocomoco: https://hocomoco11.autosome.org/
  - Human and mouse motifs
- UniProbe: http://thebrain.bwh.harvard.edu/uniprobe/
  - variety of organisms, mostly mouse and human
- Fly Factor Survey: https://pgfe.umassmed.edu/ffs/
  - Drosophila specific

Step 2. Finding motif matches in DNA

- Basic idea:

Motif: 

Match: ACCGGTT
Apprx. Match: ACACGTT

- To score a single site ***s*** for match to a motif ***W***, we use

$$\Pr(s|W)$$

What is Pr (s | W)?

5	0	2	0	0	2	0	**A**
0	5	3	1	0	0	0	**C**
0	0	0	3	5	0	0	**G**
0	0	0	1	0	3	5	**T**

1	0	0.4	0	0	0.4	0	**A**
0	1	0.6	0.2	0	0	0	**C**
0	0	0	0.6	1	0	0	**G**
0	0	0	0.2	0	0.6	1	**T**

Now, say s =ACCGGTT (consensus)

Pr(s|W) = 1 x 1 x 0.6 x 0.6 x 1 x 0.6 x 1 = 0.216.

Then, say s = ACACGTT (two mismatches from consensus)

Pr(s|W) = 1 x 1 x 0.4 x 0.2 x 1 x 0.6 x 1 = 0.048.

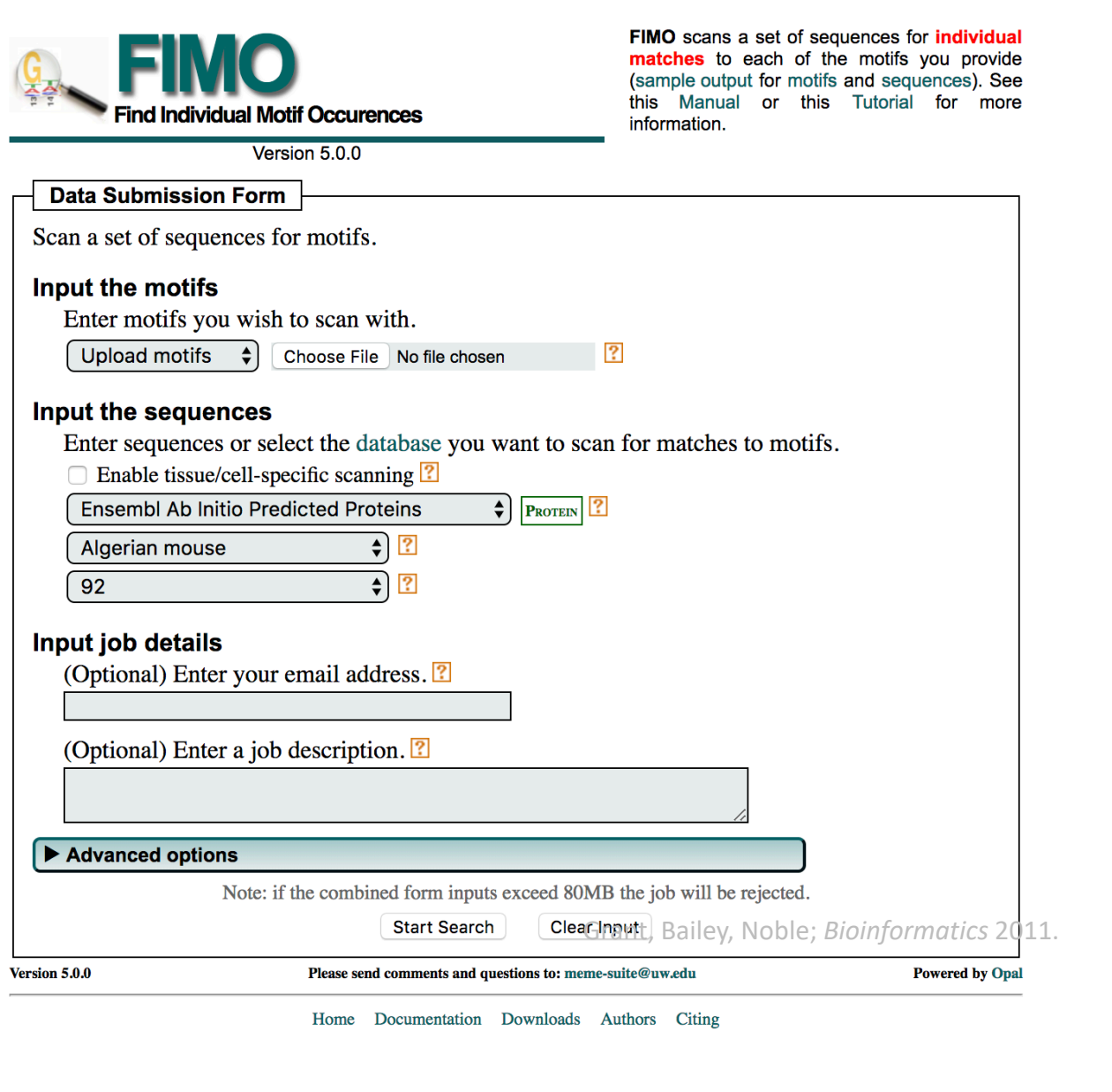
Scoring motif matches with "LLR"

- Pr (s | W) is the key idea.
- However, some statistical massaging is done on this.
- Given a motif W, background nucleotide frequencies W_b and a site s,
- LLR score of *s* =

$$\log \frac{\Pr(s|W)}{\Pr(s|W_b)}$$

- Good scores > 0. Bad scores $\lesssim$ 0.

https://meme-suite.org/meme/tools/fimo



Grant, Bailey, Noble; *Bioinformatics* 2011.

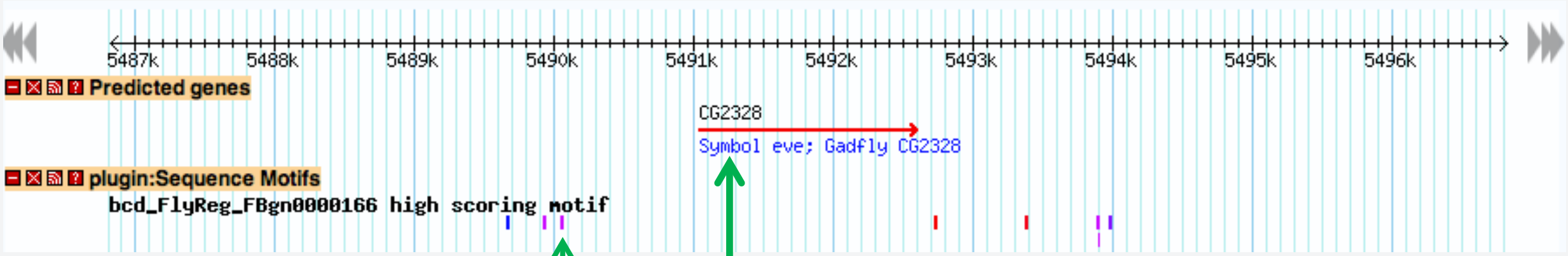
FIMO program

- Takes motif W, background W_b and a sequence *S*.
- Scans every site *s* in *S* and computes its LLR score.
- Uses sound statistics to deduce an appropriate (p-value) threshold on the LLR score. All sites above threshold are predicted as binding sites.

Finding TF targets

- Step 1. Determine the binding specificity of a TF
- Step 2. Find motif matches in DNA
- Step 3. Designate nearby genes as TF targets

Step 3: Designating genes as targets



Predicted binding sites for motif of TF called “bcd”

Designate this gene as a target of the TF

Sub-goal: discover the genes regulated by a transcription factor … by DNA sequence analysis

Computational motif discovery

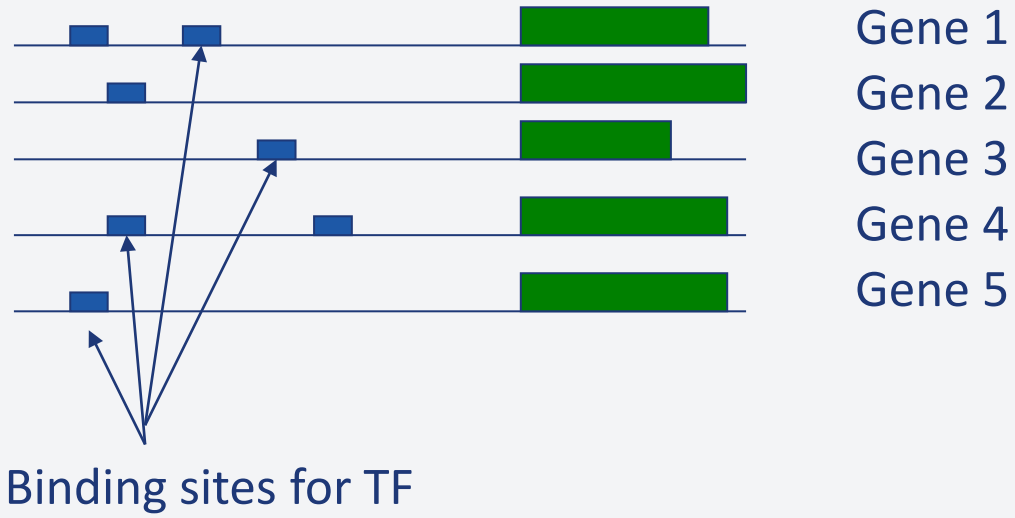
Image Credit: Nick Youngson / Alpha Stock Images

Why?

- We assumed that we have experimental characterization of a transcription factor's binding specificity (motif)
- What if we don't?
- There's a couple of options …

Option 1

- Suppose a TF regulates five different genes
- Each of the five genes should have binding sites for TF in their promoter region

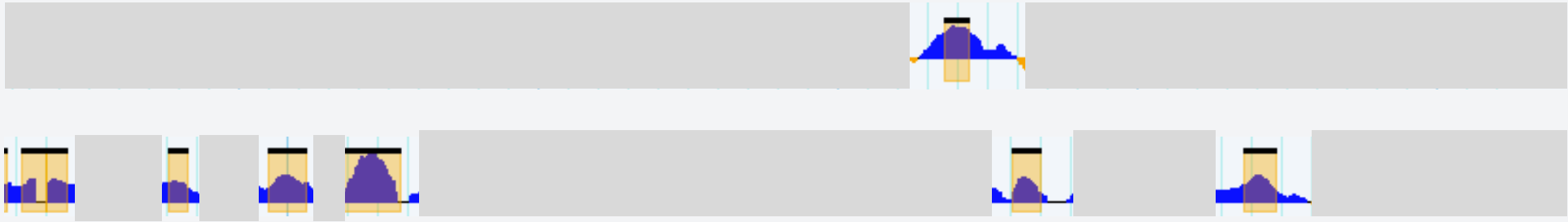


Option 1

- Now suppose we are given the promoter regions of the five genes
  - G1, G2, … G5
- Can we find binding sites of a TF, without knowing them *a priori* ?
- This is the computational ***motif discovery problem***
- Find a motif that represents binding sites of an unknown TF

Option 2

- Suppose we have ChIP-Seq data on binding locations of a transcription factor.



- Collect sequences at the peaks
- Computationally find the motif from these sequences
- This is another version of the motif discovery problem

Motif discovery algorithms

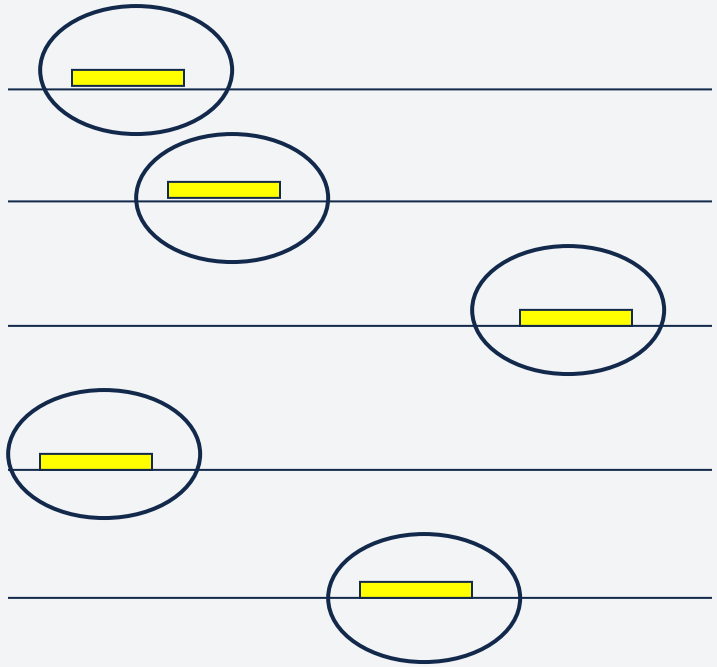
- **Version 1**: Given promoter regions of co-regulated genes, find the motif
- **Version 2**: Given bound sequences (ChIP peaks) of a transcription factor, find the motif
- **Idea**: Find a motif with many (surprisingly many) matches in the given sequences

Motif discovery algorithms

- Gibbs sampling (**MCMC**) : Lawrence et al. 1993
- **MEME** (Expectation-Maximization) : Bailey & Elkan 94. (Very popular, visited in today's lab.)
- **CONSENSUS** (Greedy search) : Stormo lab.
- **Priority** (Gibbs sampling, but allows for additional prior information to be incorporated): Hartemink lab.
- Many many others …

Examining one such algorithm

The “CONSENSUS” algorithm

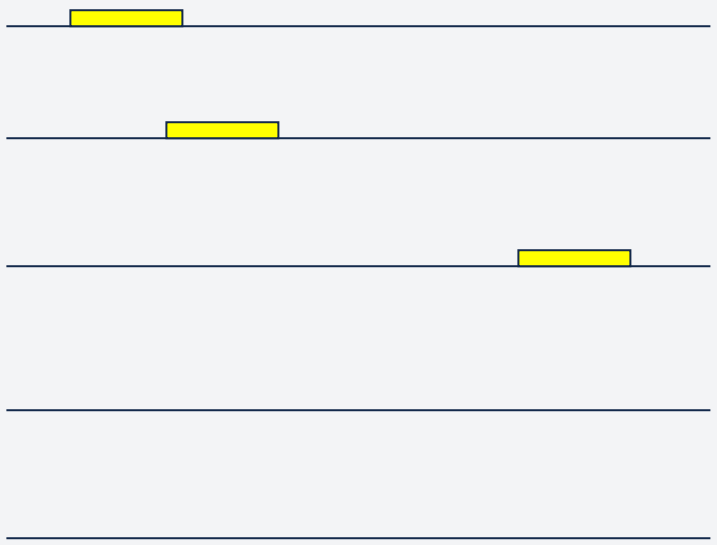


Final goal: Find a set of “substrings” (sites), one in each input sequence

Set of substrings define a motif. Goal: This motif should have high “information content”.

High information content means that sites are identical or similar to each other

The “CONSENSUS” algorithm

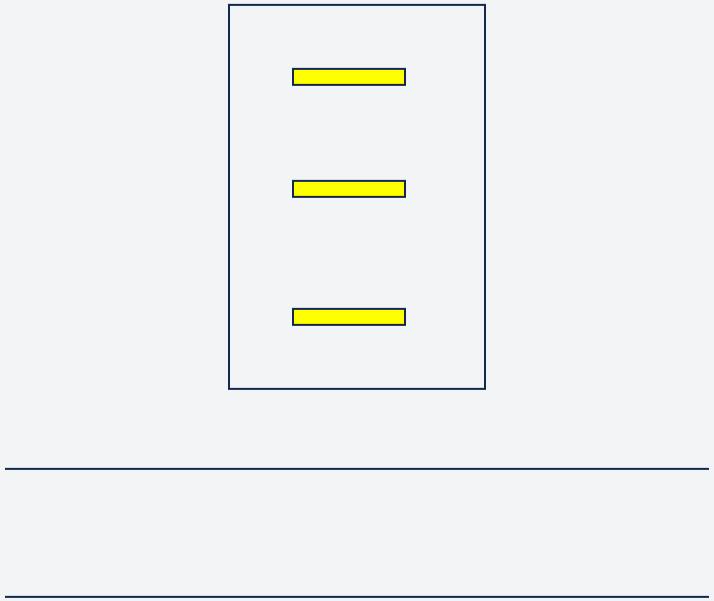


Start with a substring in one input sequence

Build the set of substrings incrementally, adding one substring at a time

The current set of substrings.

The “CONSENSUS” algorithm



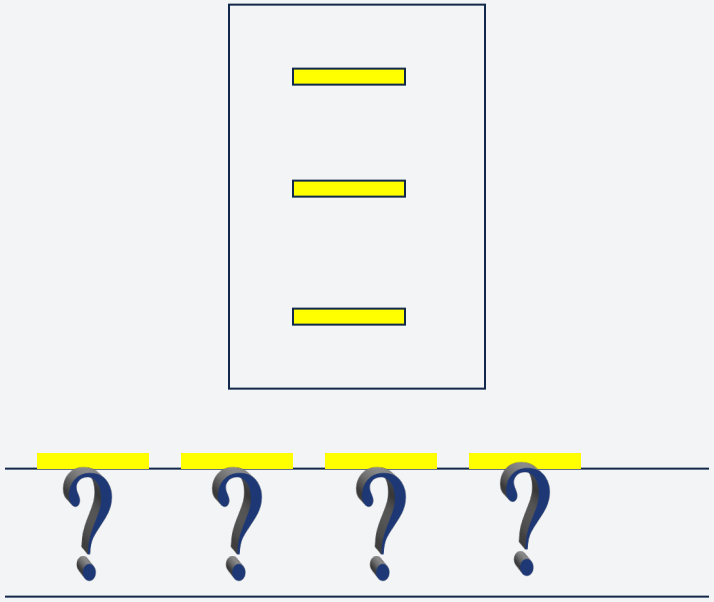
Start with a substring in one input sequence

Build the set of substrings incrementally, adding one substring at a time

The current set of substrings.

The current motif.

The “CONSENSUS” algorithm



Start with a substring in one input sequence

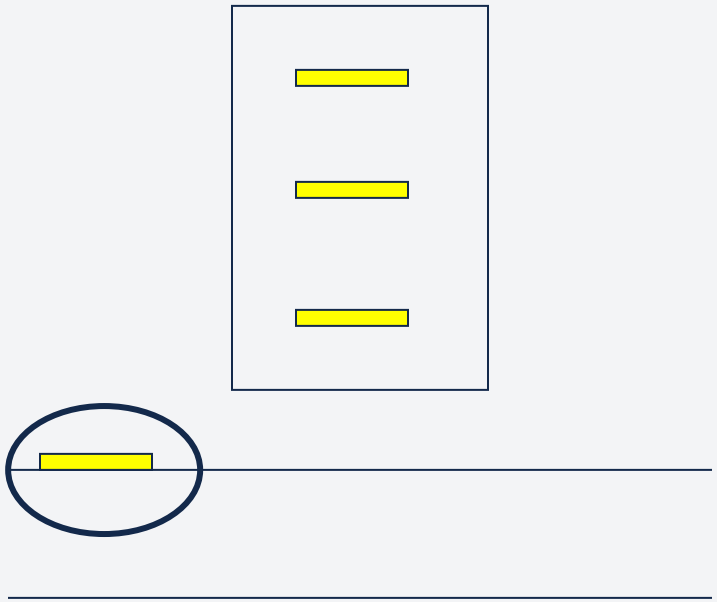
Build the set of substrings incrementally, adding one substring at a time

The current set of substrings.

The current motif.

Consider every substring in the next sequence, try adding it to current motif and scoring resulting motif’s information content

The “CONSENSUS” algorithm



Start with a substring in one input sequence

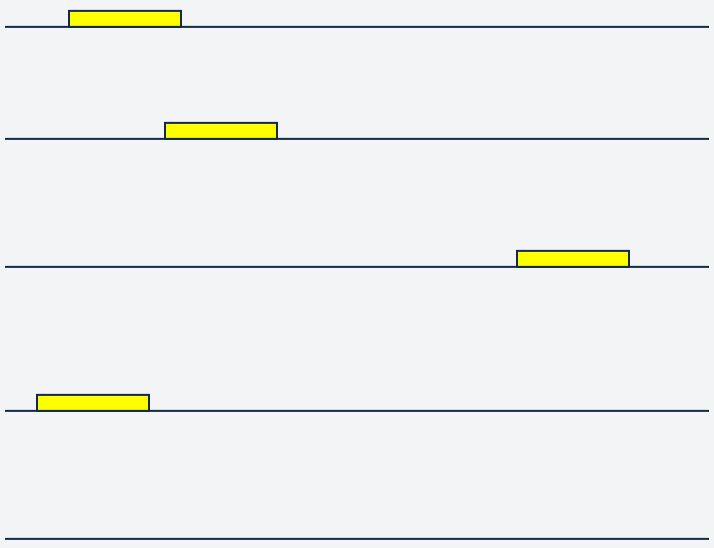
Build the set of substrings incrementally, adding one substring at a time

The current set of substrings.

The current motif.

Pick the best one ….

The “CONSENSUS” algorithm



Start with a substring in one input sequence

Build the set of substrings incrementally, adding one substring at a time

The current set of substrings.

The current motif.

Pick the best one ….

… and repeat

Summary so far

- To find genes regulated by a TF
  - Determine its motif experimentally
  - Scan genome for matches (e.g., with FIMO & the LLR score)

- Motif can also be determined computationally
  - From promoters of co-expressed genes
  - From TF-bound sequences determined by ChIP assays
  - MEME, CONSENSUS, etc.

Further reading

- Introduction to theory of motif discovery
  - Moses & Sinha. Regulatory Motif Analysis. http://www.moseslab.csb.utoronto.ca/Moses_Sinha_Bioinf_Tools_apps_2009.pdf
  - Das & Dai. A survey of DNA motif discovery algorithms. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2099490/pdf/1471-2105-8-S7-S21.pdf

Motif discovery tools

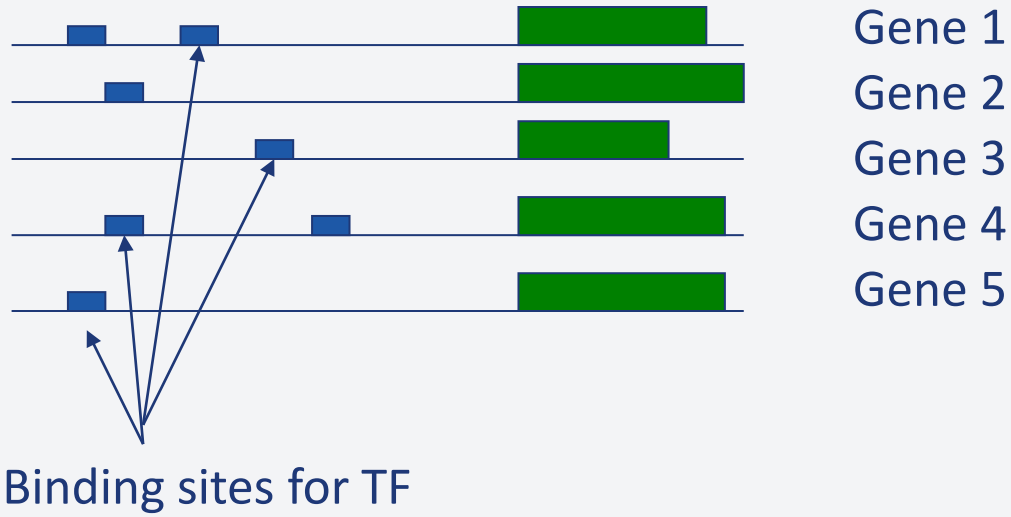
- **MEME**: https://meme-suite.org/
- **RSAT**: http://rsat.sb-roscoff.fr/

Associating sequence analysis and expression data

Image Credit: Nick Youngson / Alpha Stock Images

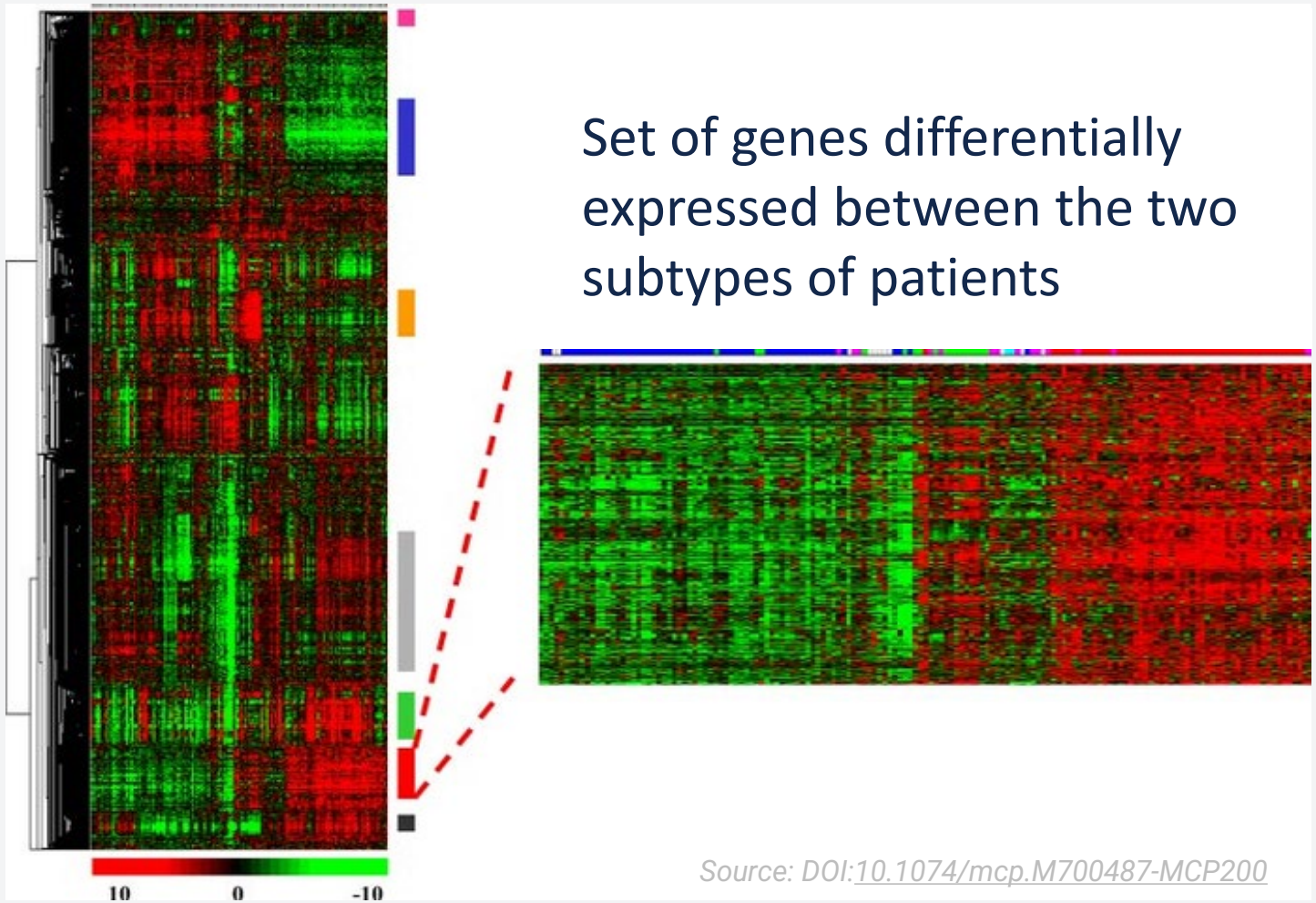
1. Predict regulatory targets of a TF

Motif module: a set of genes predicted to be regulated by a TF (motif)



2. Identify dysregulated genes in phenotype of interest

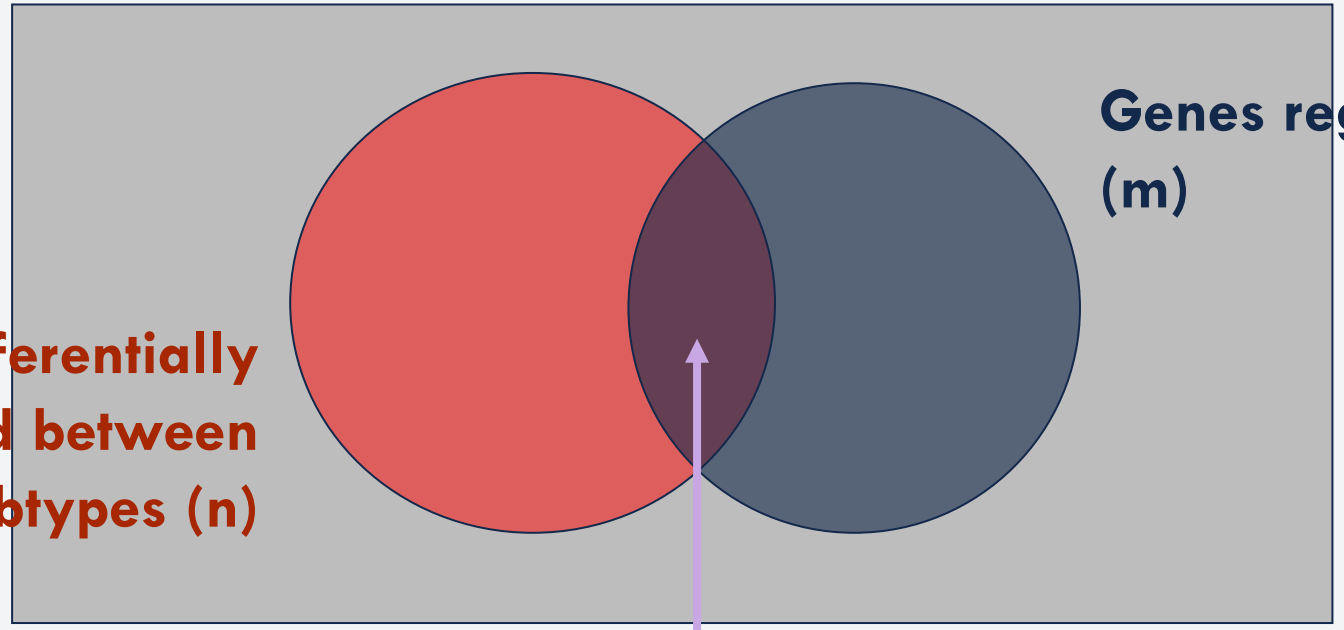
Two “subtypes” of patients



Set of genes differentially expressed between the two subtypes of patients

Source: DOI:10.1074/mcp.M700487-MCP200

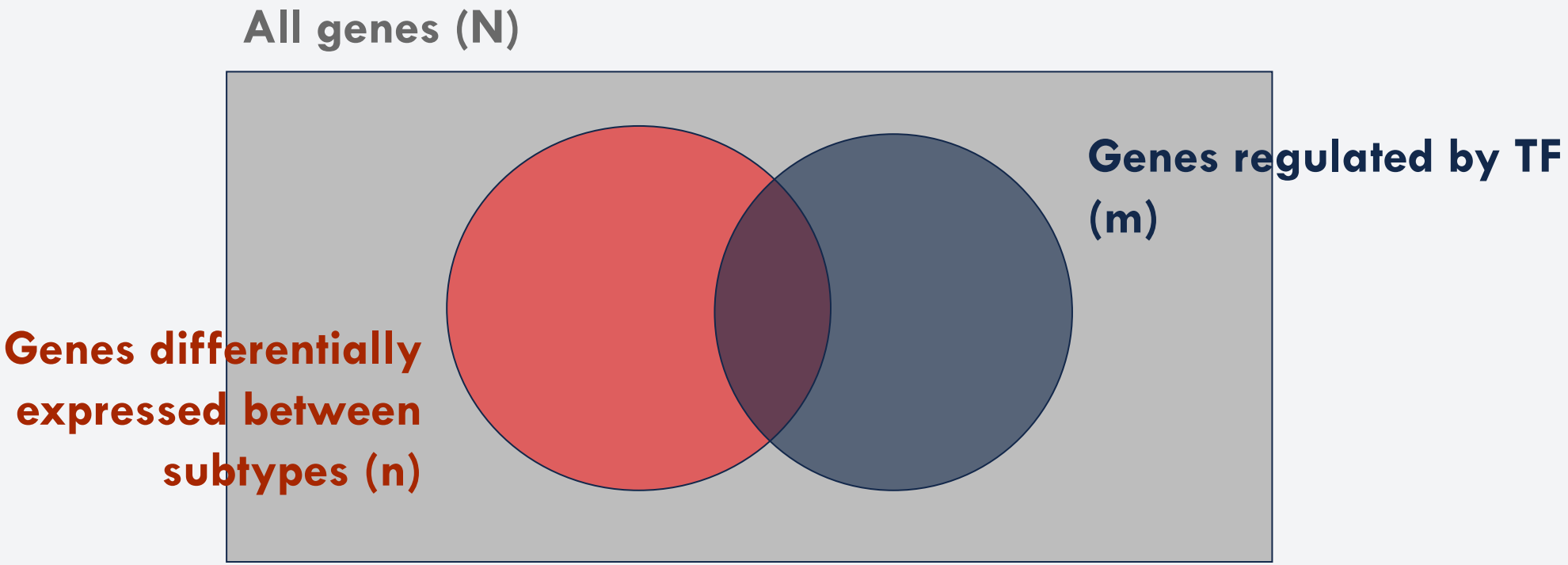
3. Combine motif analysis and gene expression data



Is the intersection (size "k") significantly large, given N, m, n?

Use Hypergeometric test to obtain "p-value"

3. Combine motif analysis and gene expression data



Infer TF may drive subtypes from “association” between motif and condition

Useful tools

- **GREAT**: http://bejerano.stanford.edu/great/public/html/
  - Input a set of genomic segments (e.g., ChIP peaks)
  - Obtain what annotations enriched in nearby genes
  - only for human and mouse

- **DAVID**: https://david.ncifcrf.gov/
  - Input a set of genes
  - Obtain what annotations enriched in those genes
  - Many different species

Quick Break

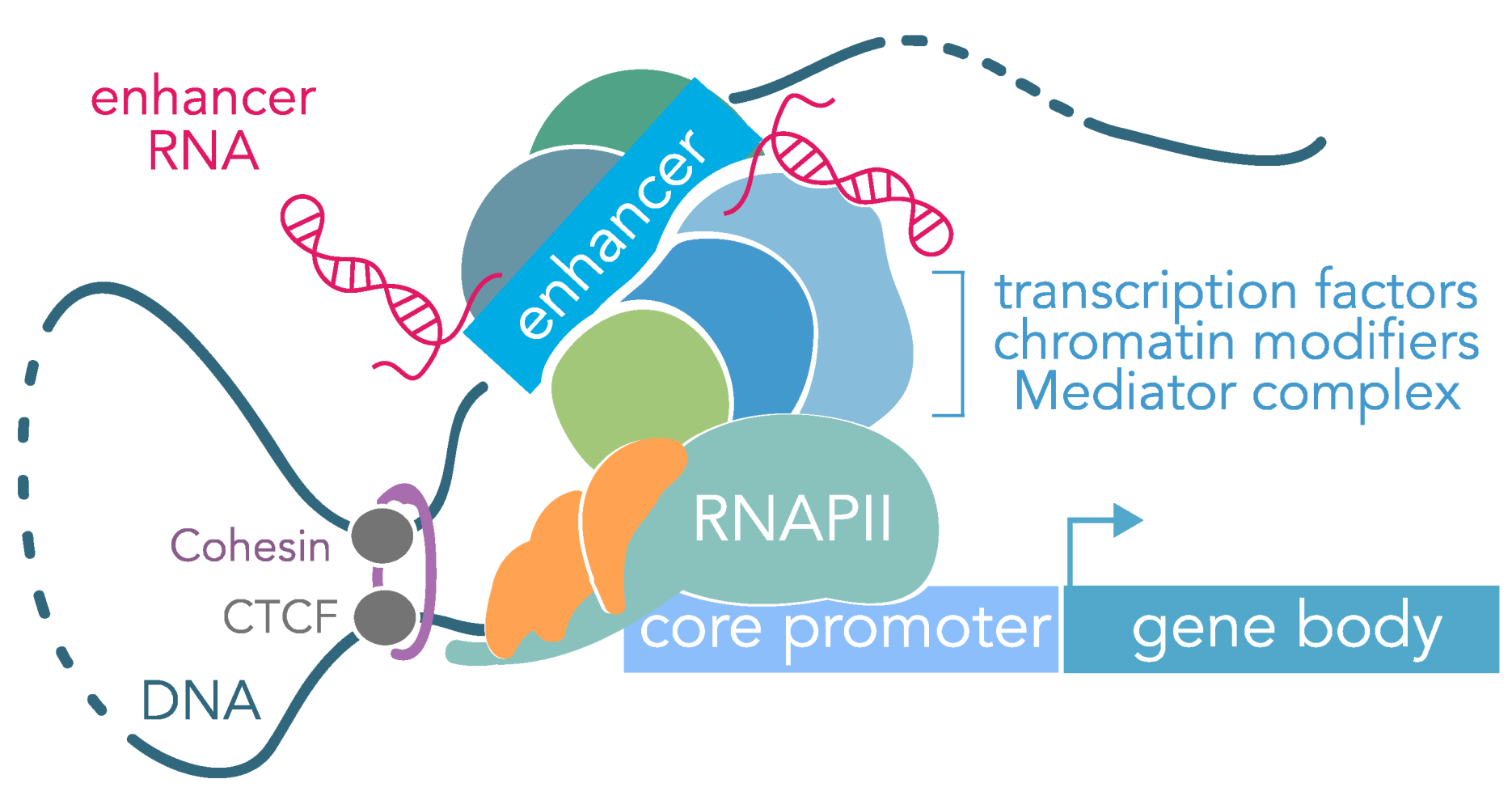
Epigenomics

- Where do TFs bind?
- Which genomic segments actively regulate gene expression?

Outline

- Decorations on the genome
- Experimental assays to profile the decorated genome
- Insights from large scale epigenomics studies

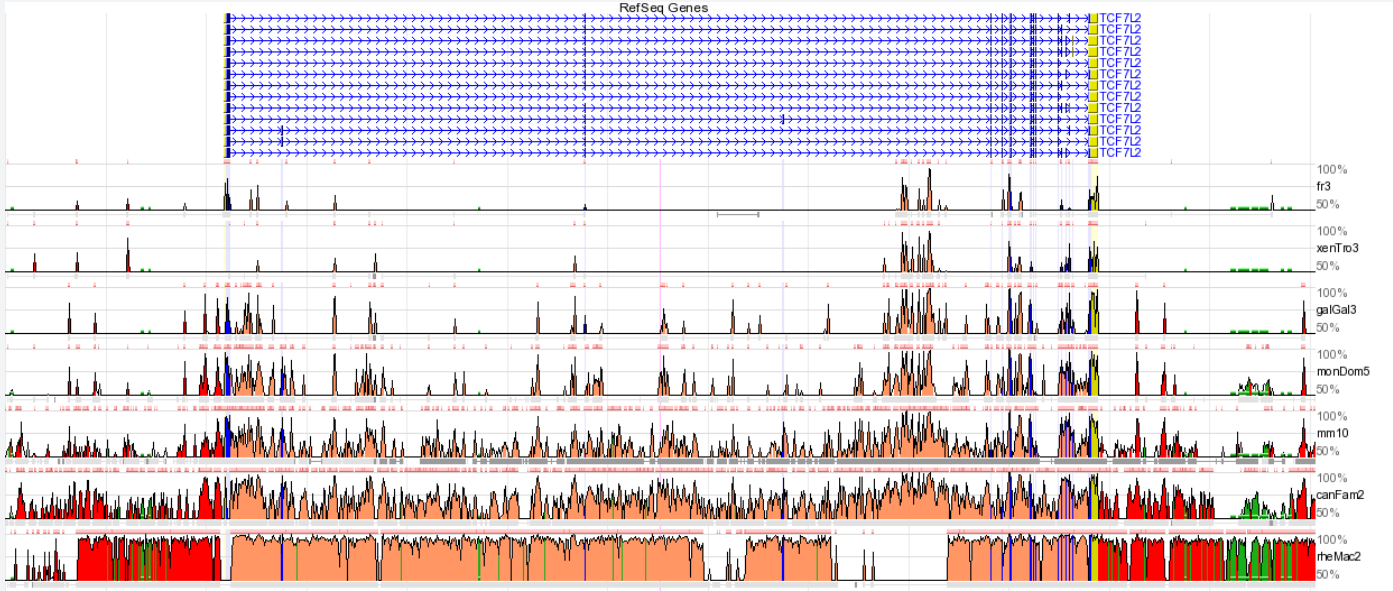
The regulatory genome



Source: *Genomic Enhancers in Brain Health and Disease.*
Carullo & Day. Genes 2019

How to find enhancers?

- Like finding needle in a haystack
- Evolutionary conservation is sometimes used to identify enhancers



- but not all functional elements are conserved at the level that DNA sequence alignments can detect. So how do we find regulatory elements?

- More important question is: which enhancers are *active* in a particular cell type?

Regulatory activity leaves its “mark” on the genome: epigenomics

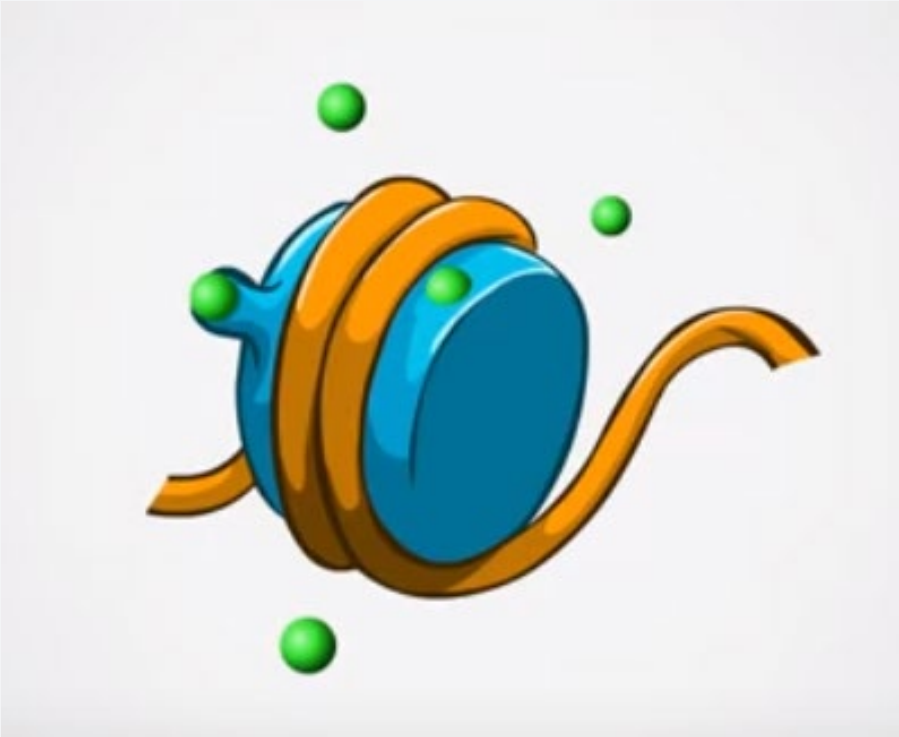
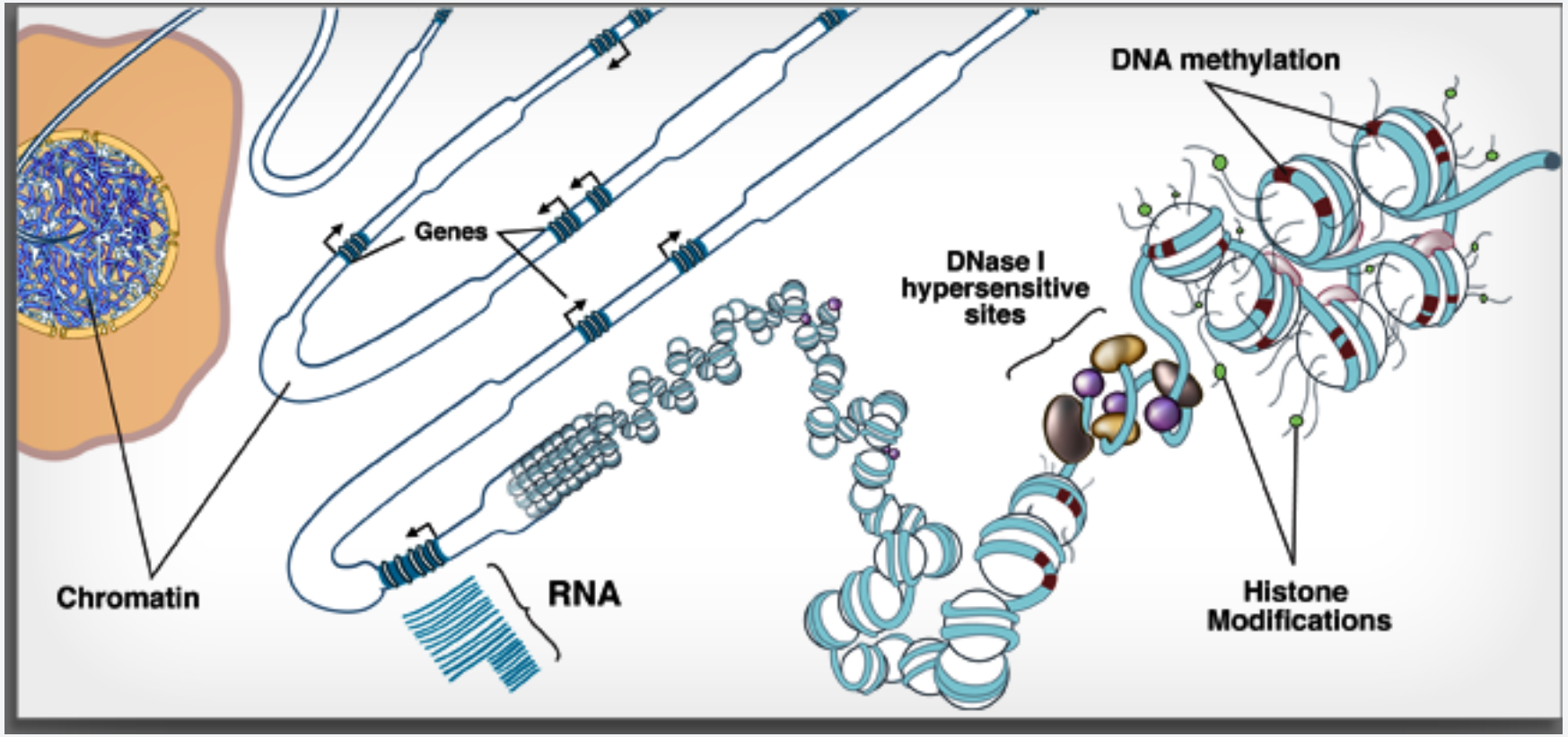


Image Credit: Ahmed.yosri / Wikimedia Commons

Genomes are complex 3D structures

- Comprised of modified and unmodified DNA, RNA and many types of interacting proteins
- Most DNA is wrapped around a "**histone core**". Such wrapped-around DNA is relatively "**inaccessible**" to other molecules such as TFs. But there are "**accessible regions**" as well, can be detected as "**DNase I hypersensitive sites**" (DHS)
- **TFs bind** to their preferred sites (especially in **accessible** regions), or not
- Histone proteins are '**marked**' (like flags), or not
- CpG dinucleotides in DNA are **methylated**, or not



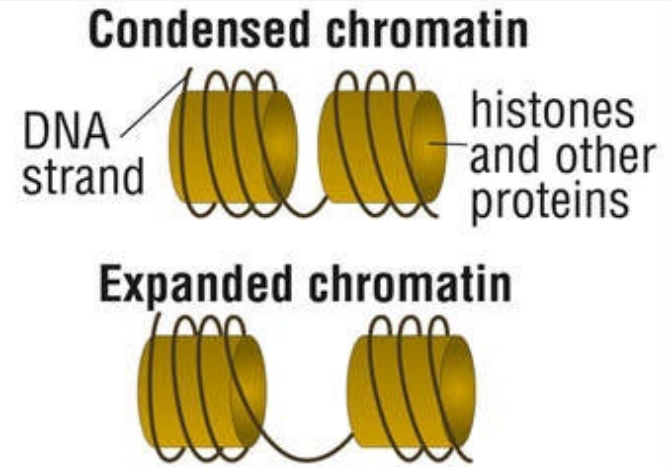
Source: https://www.genome.gov/Pages/Research/ENCODE/Epigenomics_Data_Resource.pdf

Epigenomic clues into regulatory activity

- Look for accessible regions of DNA, that's where active regulatory elements might lie
- Also: specific histone modifications and DNA methylation mark regulatory activity
- If you know a particular TF that is important for regulation, look for its binding sites

Not accessible

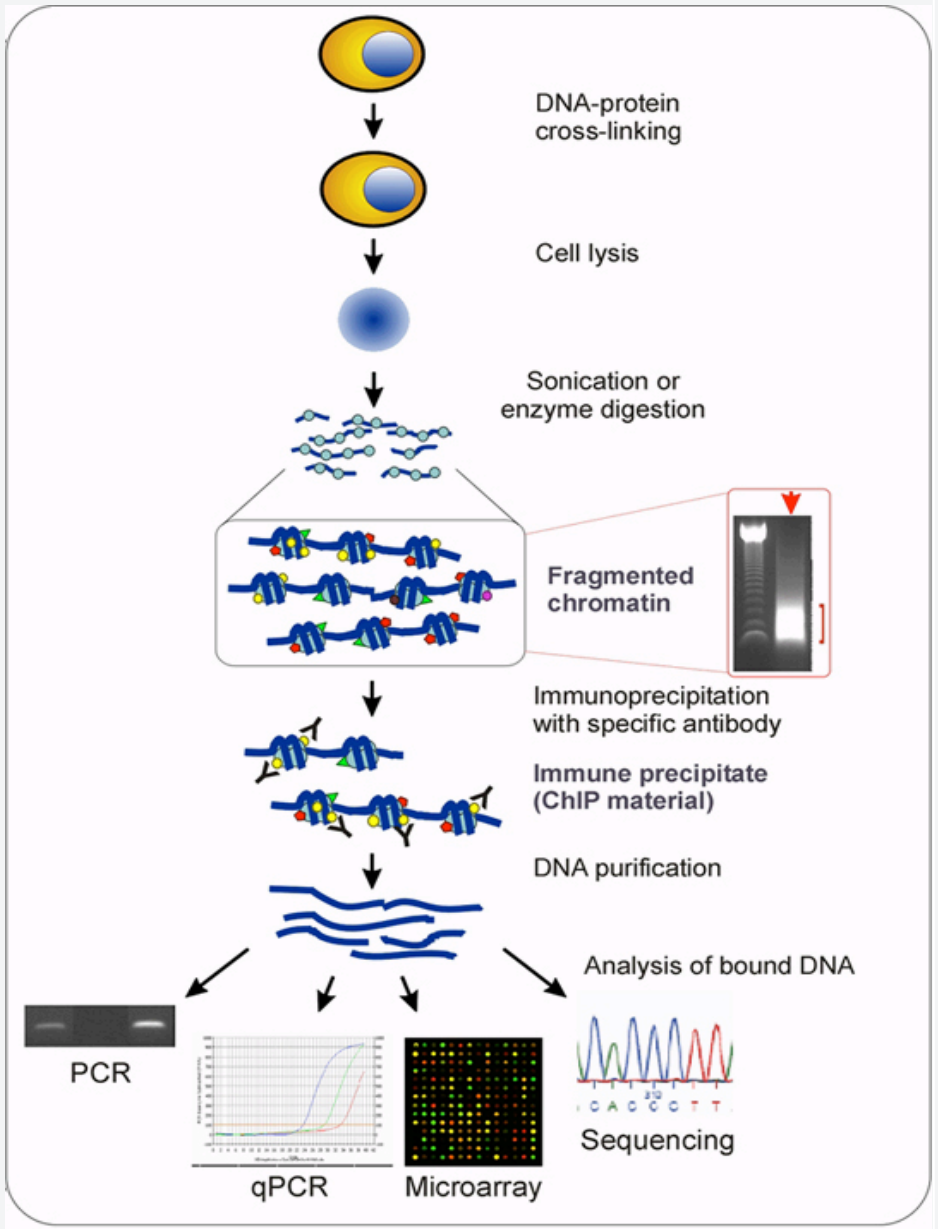
Accessible



Experimental assays

Image Credit: Nick Youngson / Alpha Stock Images

Chromatin Immunoprecipitation (ChIP)

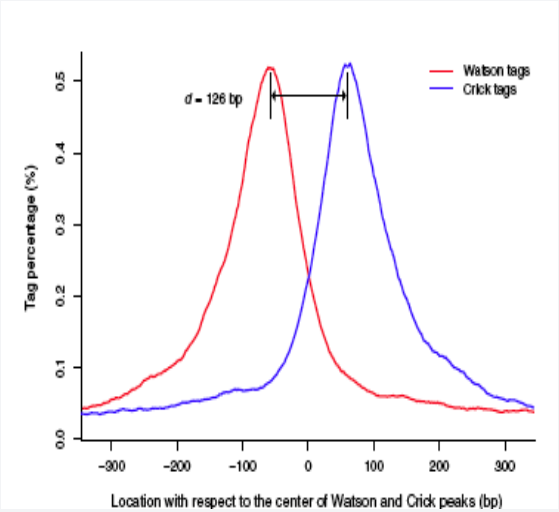


- Antibody to a DNA binding protein is used to "fish out" DNA bound to the protein in a living cell
  - DNA and protein are crosslinked in the cell using formaldehyde
  - Crosslinked chromatin is sheared, usually by sonication, to yield short fragments of DNA+protein complexes
  - Antibody to a TF or other binding protein used to fish out fragments containing that DNA binding protein
  - DNA is then "released" and can be analyzed by sequencing
- Creates a pool of sequences enriched in binding sites for a particular protein
- Requires availability of excellent **antibodies** that can detect the protein *in vivo*

Source: Collas & Dahl. Frontiers in bioscience (2008).

ChIP computational issues

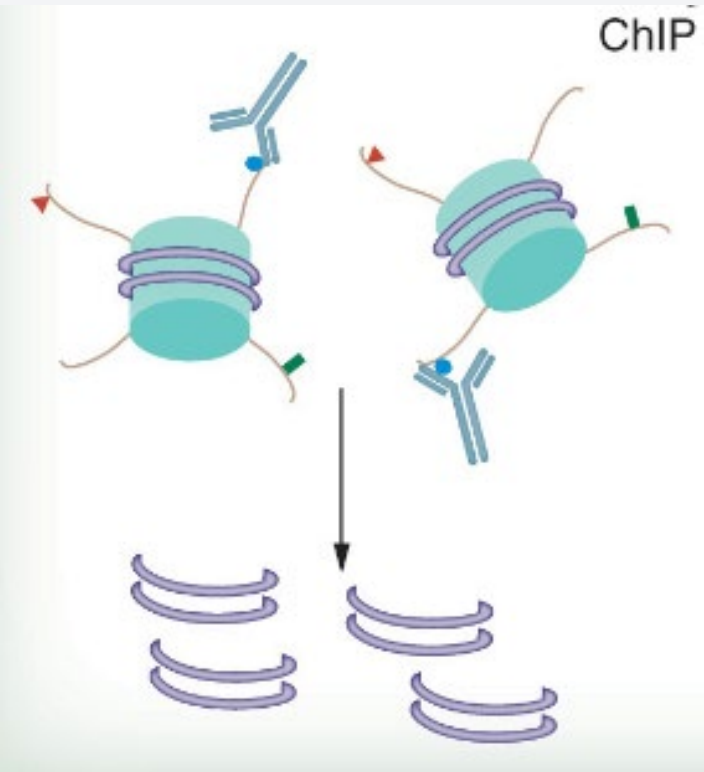
- First step is to map reads: BOWTIE, Novalign, BWA or other
- ChIP-seq reads surround but may not contain the DNA binding site
  - Sequence is generated from the *ends* of *randomly sheared* fragments, which overlap at the protein binding site
- Gives rise to two adjacent sets of read peaks
- Programs like MACS and HOMER automatically subtract your control (genomic input) from sample reads to define a final set of peaks



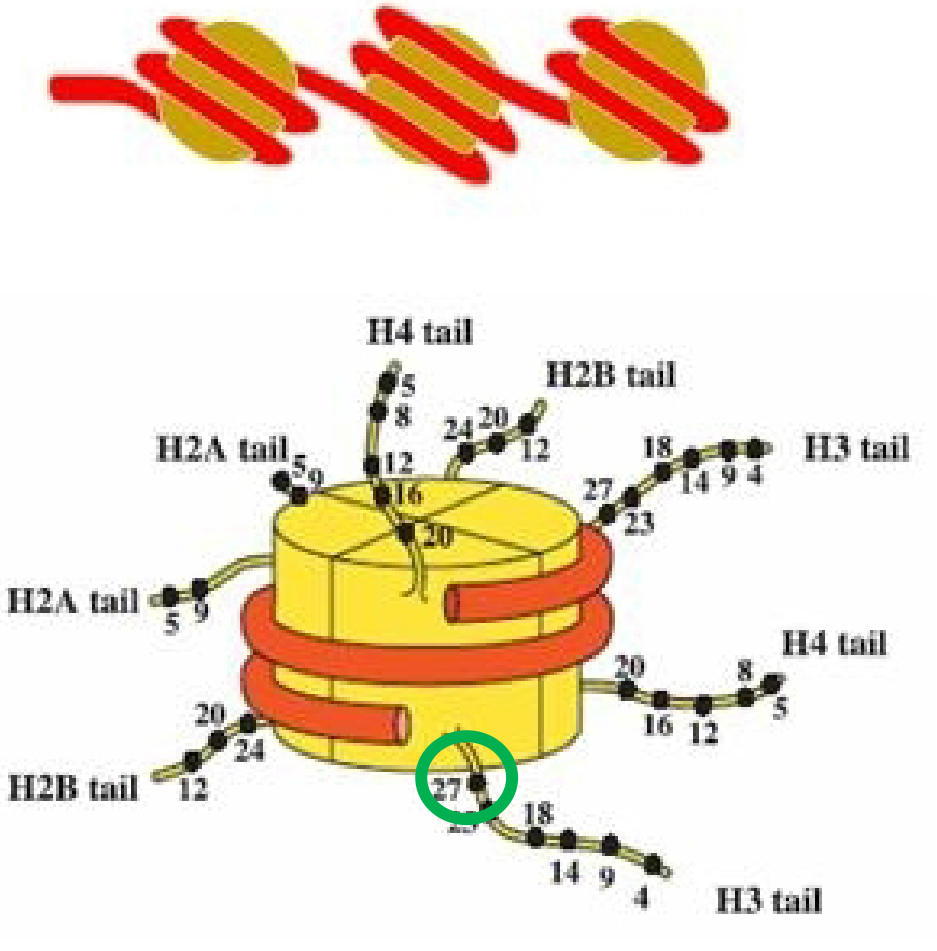
Source: Kungulovski, et al. Epigenomics (2015).

ChIP for histone marks

- ChIP-seq can be used to profile not only TF binding sites but also histone modifications.
- Data/peak characteristics are different depending on what is profiled.
  - TFs are typically sharp peaks; chromatin marks are more diffuse



All histones in the tetramer have "tails" that can be modified in various ways

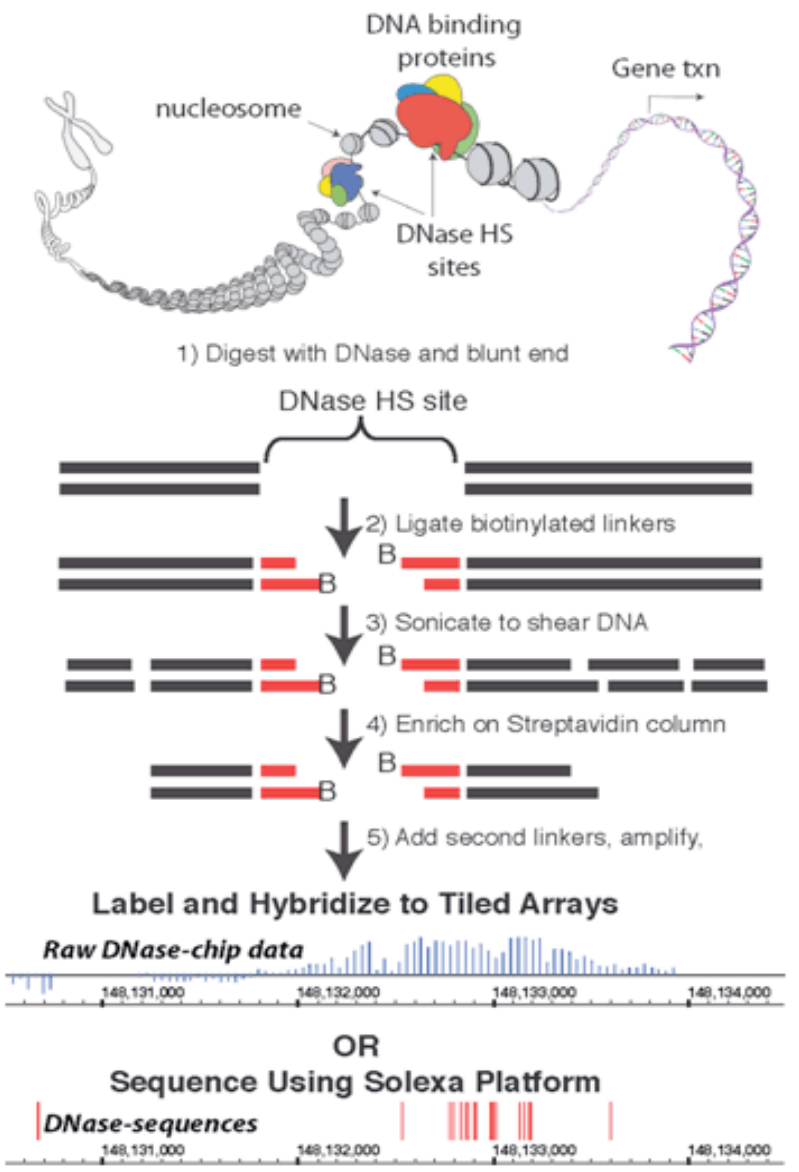


Ac	acetylation
Me	methylation
Ub	ubiquination
SU	sumoylation
P	phosphorylation

Methylation or acetylation of Lysines (K) in histone H3 have an known effect on transcriptional activity

Source: https://www.cs.toronto.edu/~goldenberg/CSC2431/CSC_2431_Machine_Learning_in_Computational_Biology_2015/Schedule_files/Lecture4.pdf

How to find accessible DNA?



Source: [Crawford, et al. Nature methods (2006).]

The first approach:

Crawford et al., Genome Research 16:123, 2006 (Francis Collins' laboratory)

Genome-wide identification of DNase I Hypersensitive sites (DHS)

Later variants also based on DNase I treatment, but different protocol and different philosophy.

ChIP-exo, FAIRE-seq, Mnase-seq, ***ATAC-seq*** etc.

2019 Review covering different methods:

Chromatin accessibility and the regulatory epigenome

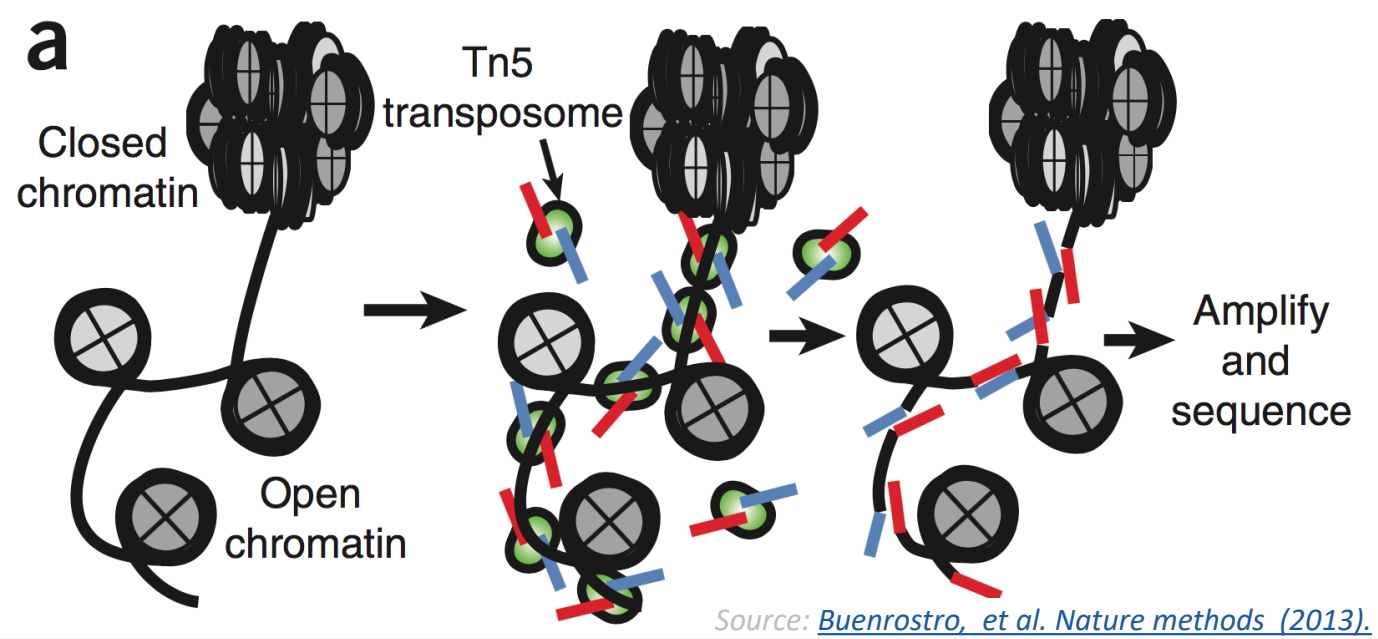
Sandy L. Klemm, Zohar Shipony & William J. Greenleaf

Nature Reviews Genetics **20**, 207–220 (2019) | Cite this article

ATAC-seq: approach to open chromatin

(Assay of Transposase Accessible Chromatin sequencing)

- Uses hyperactive Tn5 transposase to cut and tag accessible DNA
- Transposase "jumps" preferentially (and randomly) into accessible chromatin
- Because of the design the transposase breaks DNA where it jumps in, tagging the site with the primer
- Two insertions close together yield fragments of the size amenable for Illumina sequencing
- PCR amplification between primers is all you need to make a library
- Since it skips library-making steps, it can be done with small amounts of chromatin – e.g. 50K vs 1M cells



Source: Buenrostro, et al. Nature methods (2013).

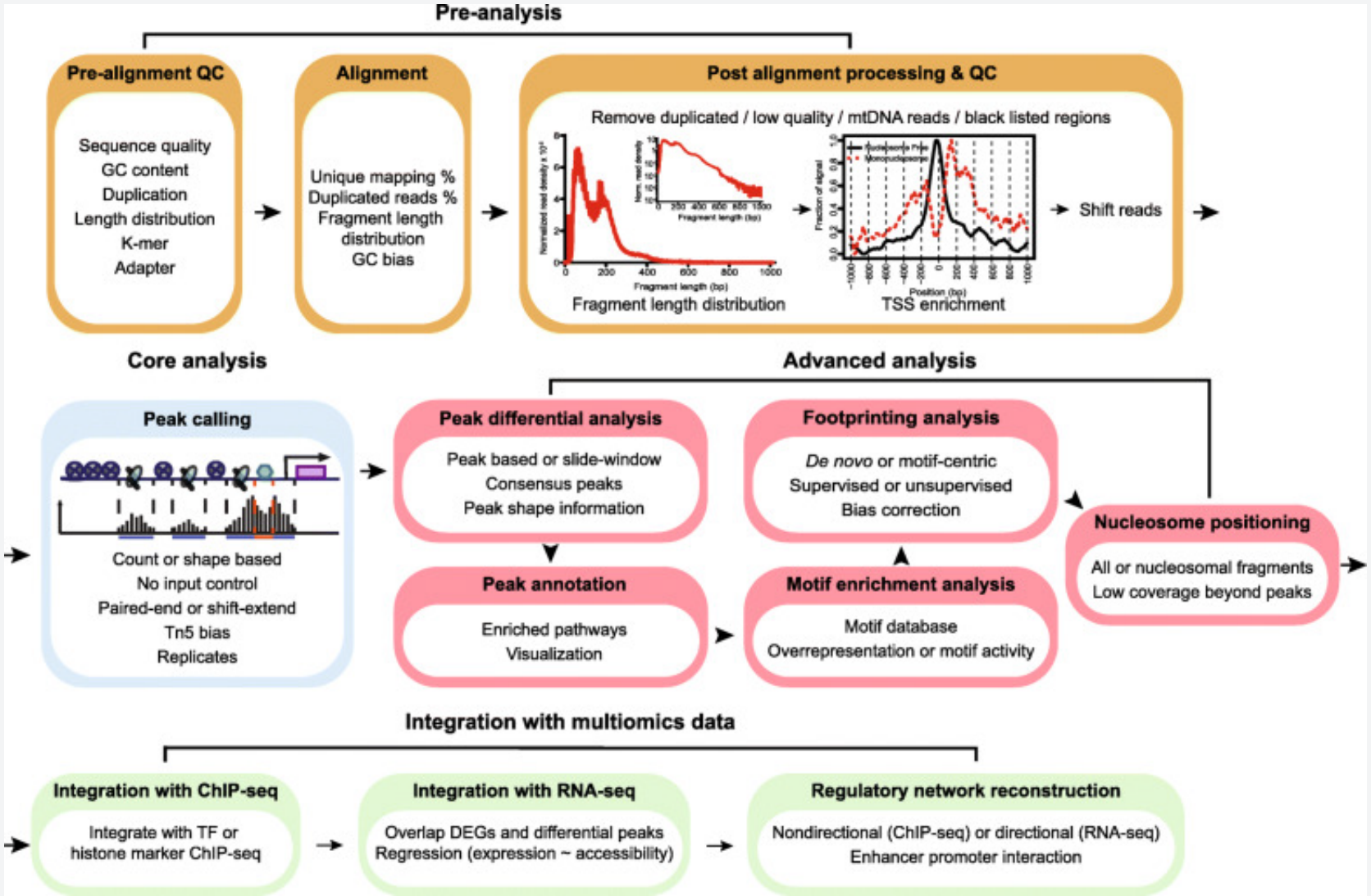
General analysis workflow

- **Upstream Analysis**:
- Alignment, Quality Control, and Peak Calling

- **Downstream Analysis**:
- Mapping peaks to nearby genes (esp. differentially expressed genes)

- Identifying enriched motifs

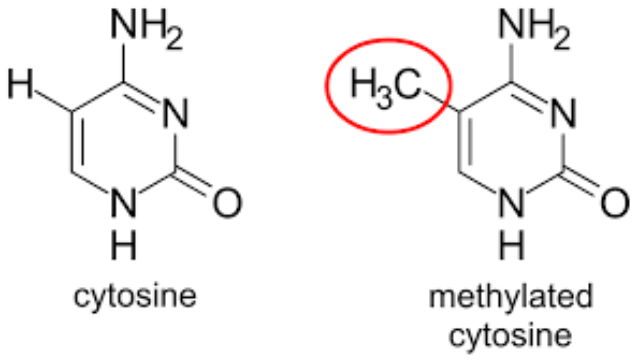
- Overlapping with multi-omics genome features



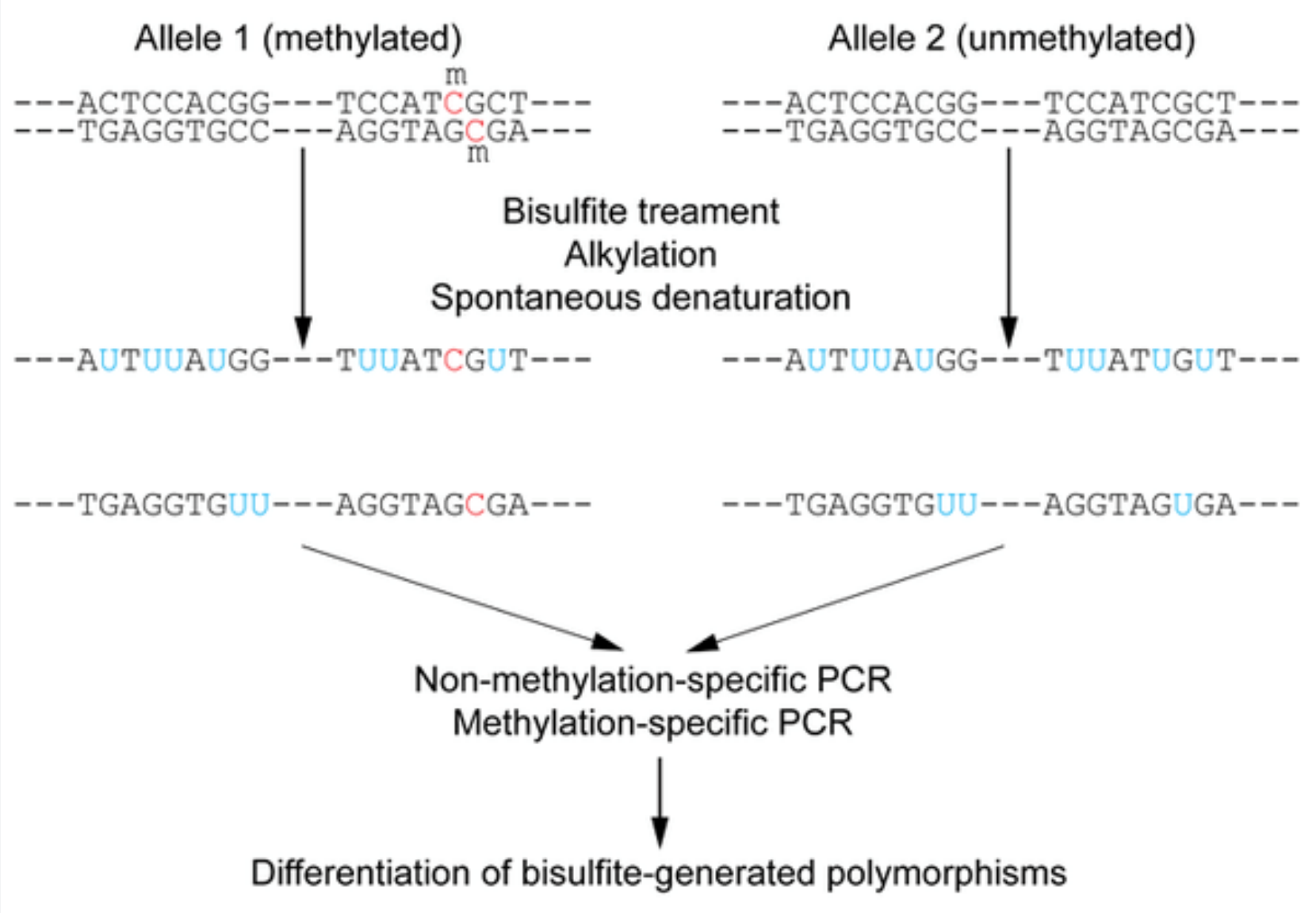
Source: Yan, et al. Genome biology (2020).

DNA Methylation

- Methyl (-CH3) group added to Cytosine ('C')
- CpG (CG dinucleotide) is often methylated
- Methylated CpG may hinder transcription factor binding to DNA at that site
- Methylated CpG may recruit proteins that render local chromatin less accessible
- Roughly speaking, DNA methylation is repressive for gene expression



CpG Methylation profiling



- Bisulfite sequencing

Other methods:

- DNA cleavage by methylation-sensitive restriction enzymes
- Immunoprecipitation with methyl-binding protein

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

Insights from large-scale epigenomics studies

ENCODE

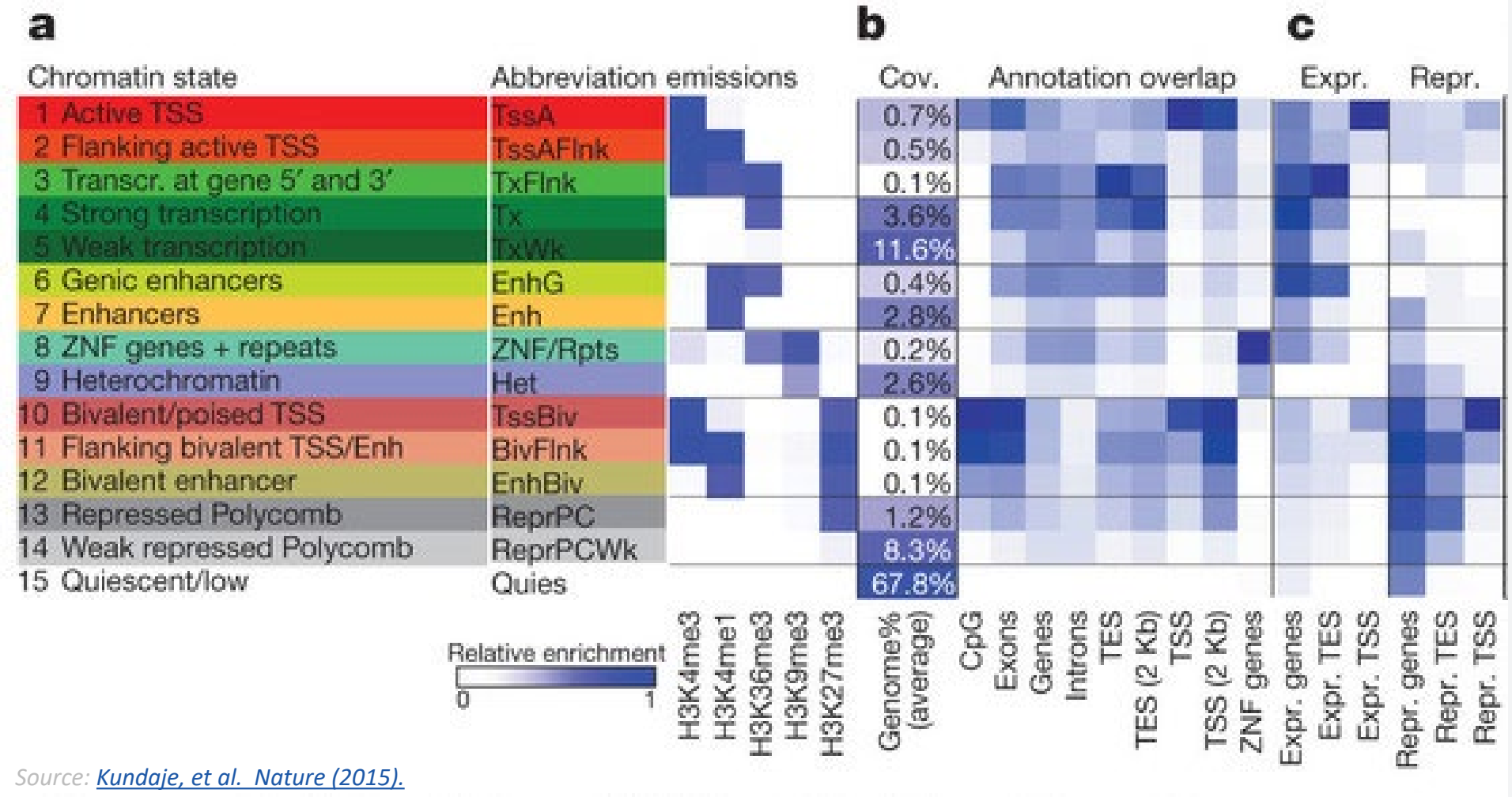
ROADMAP epigenomics PROJECT

Lessons from epigenomics assays

- Massive deep-sequencing of multiple chromatin features in cell lines (ENCODE), primary cell types and tissues (Epigenetics Roadmap)
  - Histone H3 modifications: highlight on H3K4me1, H3K4me3, H3K27Ac, H3K27me3.
  - TFs and other chromatin proteins: e.g. P300 (acetyltransferase)
- H3K4me3 marks are enriched at ***active promoters***
  - H3K4me3 marks are largely the same in all cell lines, with a small fraction of marks being cell-specific
- P300, and H3K4me1 is enriched at *enhancers*
  - Most P300 peaks also contain H3K4me1
  - P300, H3K4me1 marks are highly cell-type specific
  - Most P300 marks are enhancers, but not all enhancers have P300
  - Most enhancers have an H3K4me1 mark, not all H3K4me1 marks are in enhancers
- Other marks: H3K27Ac or H3K27me3
  - Mutually exclusive marks for open (Ac) versus closed (Me3) chromatin regions
  - H3K27Ac may be most general open chromatin mark: promoters and enhancers
  - H3K27Ac often found in combination with H3K4 me1/me3

Application 1: Chromatin "states"

- ChromHMM tool combines information from 38 different histone marks, Pol2 and CTCF profiles to identify different 'states'



Source: Kundaje, et al. Nature (2015).

Application 2: DNA Methylation profiles in cancer and aging

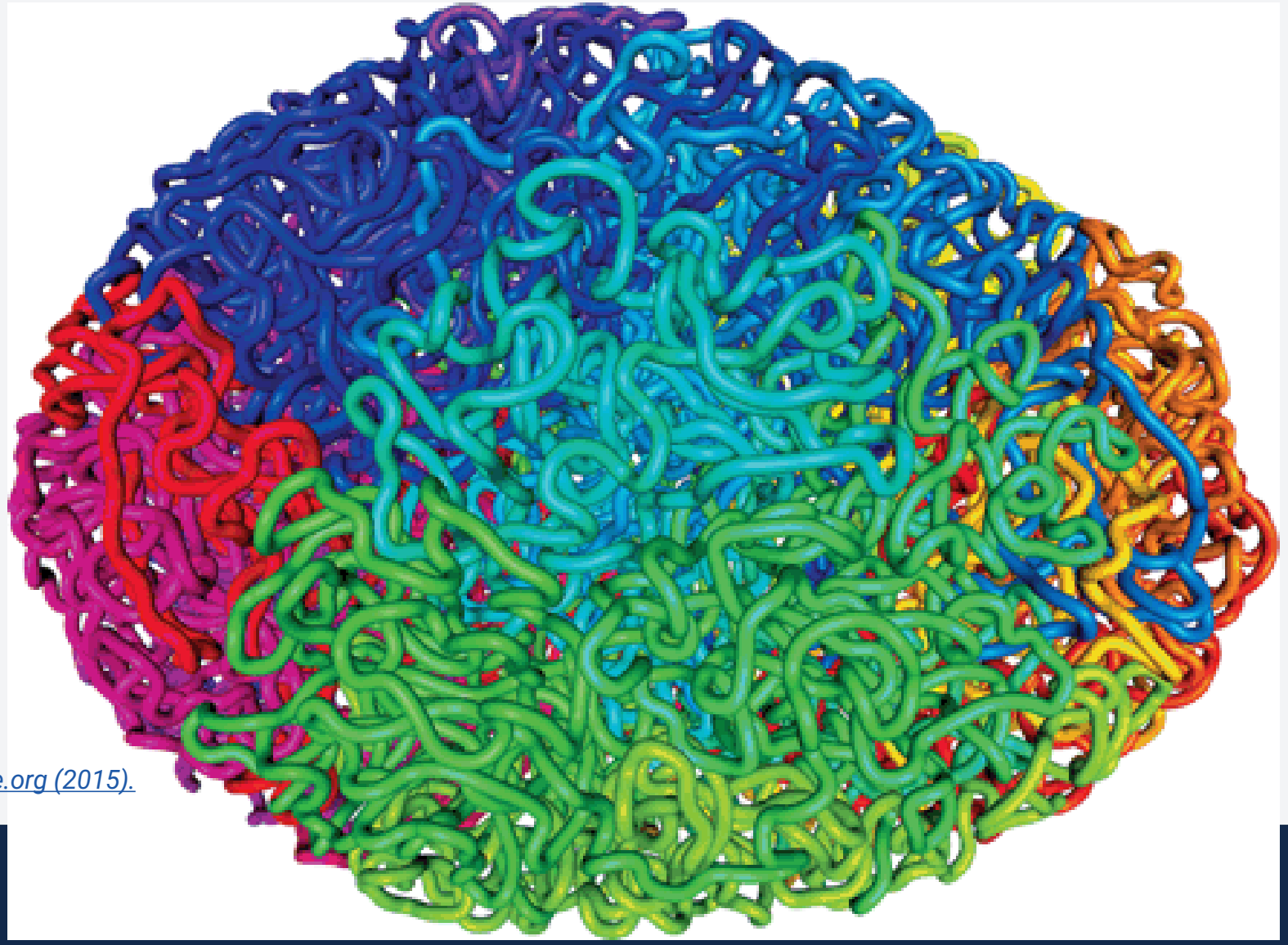
- DNA Methylation levels can be condition-dependent
  - Aberrant methylation patterns in cancer (e.g., hypermethylation of tumor suppressors and hypomethylation of oncogenes)
  - Progressive increase in global methylation levels with age. Also aging-correlated hypomethylation at some genes.

Front Bioinform. 2022; 2: 847629.
Published online 2022 Jun 2. doi: 10.3389/fbinf.2022.847629

DNA Methylation, Aging, and Cancer Risk: A Mini-Review

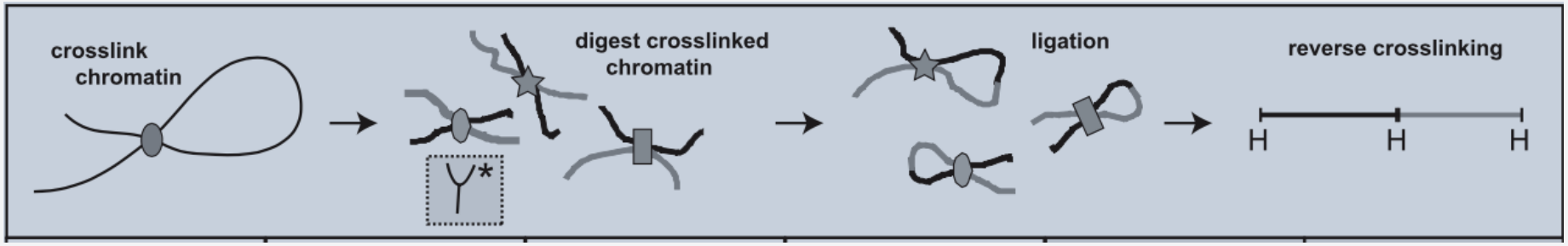
Larry Chen,[1] Patricia A. Ganz,[2,3] and Mary E. Sehl[2,4,*]

3D genome



Source: [Pennisi. Science.org (2015).]

Probing 3-dimensional chromatin structure with conformation capture

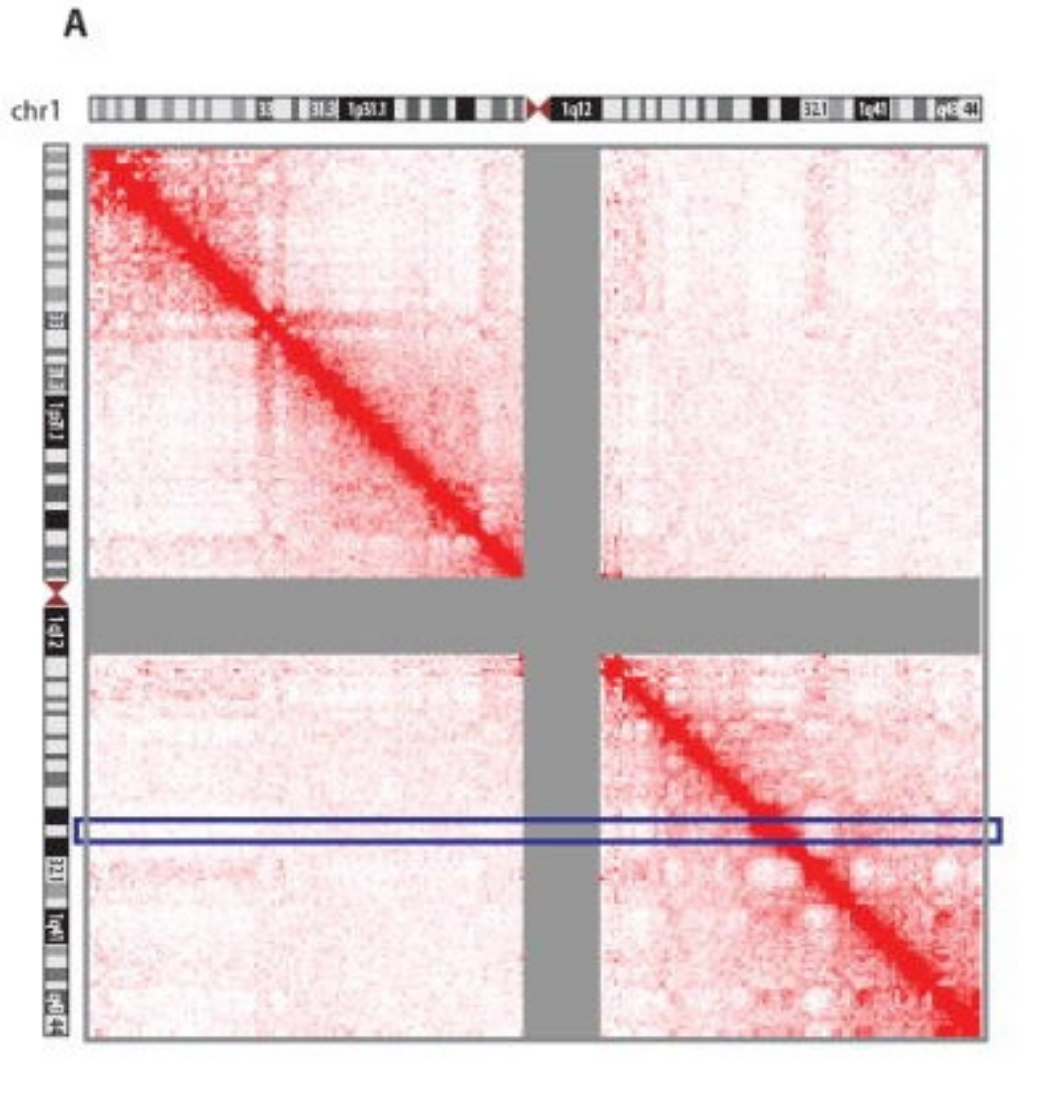


Source: Wit & Laat, 2012

Hi-C “output”

Hi-C: A comprehensive technique to capture the conformation of genomes

Jon-Matthew Belton,[1] Rachel Patton McCord,[1] Johan Gibcus,[1] Natalia Naumova,[1] Ye Zhan,[1] and Job Dekker[1,*]



Heatmap of interactions between all 1 MB bins along chr1 for GM06990 cells.

The intensity of red color corresponds to the number of Hi-C interactions.

Why is 3D information useful?

- The issue is finding out “who is talking to whom?”
  - Enhancers can be shared by multiple genes
  - Alternative promoters for the same gene can have very different regulatory partners
  - Position relative to the TSS is not a reliable indicator in large vertebrate genomes
  - 3D methods are necessary to tie enhancers and promoters (genes) together

Summary (epigenomics)

- Transcription factor binding sites genome-wide
- Histone modification profiles (different marks or combinations of marks can point to different classes of regulatory elements)
- DNA accessibility profiles
- CpG methylation profiles
- Epigenomic profiles are informative about gene expression and regulatory mechanisms

Questions ?

Regulatory Genomics Lab

1. See Pythonic way to process single cell data on sample with both scRNA-seq and scATAC-seq
2. Look at normalization and signatures for scATAC-seq data
3. Identify differentially accessible peak intervals
4. Search for DNA sequence motifs under peaks