

Regulatory Genomics II: Introduction to Epigenomics

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Introduction to Epigenomics

- Epigenetics and Epigenomic
- Chromosomal Territories
- Chromatin organization
 - Open Chromatin and Transcription
- Histone modifications
 - Impact on gene regulation
- Epigenomic codes
- 3D Architecture of chromatin
 - Long distance chromatin interactions with Hi-C
 - A-B compartments
 - TADs
- Open Chromatin with ATAC-seq
- Cellular Regulomes

Overview

- Understand computational biology methods, tools and resources that explore DNA in the context of chromatin
- Detecting open and closed chromatin
- Functional transcriptional regions
- Long Distance interactions
- 3D structure of chromatin
- Cellular Regulomes

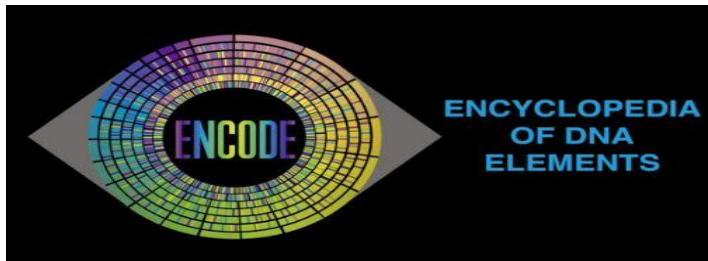
Epigenetics and Epigenomics

- **Epigenetics** encompasses processes that lead to heritable change in gene expression without changes to the DNA itself.
- DNA is packaged into chromatin. This nucleoprotein structure is highly dynamic and plays a role in gene regulation. Chromatin states can vary between conditions, cells and tissue types and even within a single chromosome. The **Epigenome** refers to these chromatin states at a whole genome level. A multicellular organism has a single genome but many epigenomes.
- Paradox: Although overall rates of cardiovascular disease increase with rising national prosperity, the least prosperous residents of a wealthy nation suffer the highest rates.

Developmental origin of health and disease

- The dutch famine (“Hongerwinter”) 1944-45 in German occupied Netherlands towards the end of the WWII affected 4.5 million people and led to ~22000 deaths.
- “People ate grass and tulip bulbs, and burned every scrap of furniture they could get their hands on, in a desperate effort to stay alive.”
- The Dutch Hunger Winter study, from which results were first published in 1976, provides an almost perfectly designed, although tragic, human experiment in the effects of intrauterine deprivation on subsequent adult health.
- Critical windows during development where epigenetic modification will affect adult health.
- Those exposed during early gestation experienced elevated rates of obesity, altered lipid profiles, and cardiovascular disease. In contrast, markers of reduced renal function were specific to those exposed in mid-pregnancy. Those who were exposed to the famine only during late gestation were born small and continued to be small throughout their lives, with lower rates of obesity as adults than in those born before and after the famine.

Large-scale epigenomic studies



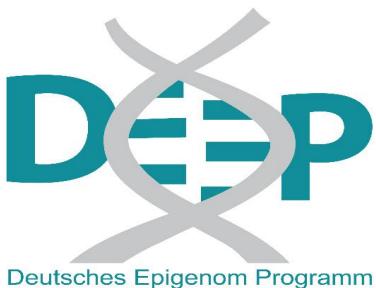
Histone and TF ChIP-seq,
Transcriptomics, Hi-C



Epigenomes of 100 blood cell
types



Stem cells, fetal tissues, adult
tissues



Various human, mouse tissues



Methylomes

Chromosome territories

- “**Chromosomal Territory**” model - *Theodor Boveri* in 1885 and Carl Rabl in 1909 (Cremer T, Cremer M. 2010. Cold Spring Harb. Perspect Biol 2:1–22)
- These early observations were superseded when electron microscopy showed evidence of chromosome intermingling during interphase.
- “**Spaghetti**” model of the interphase nucleus — chromatin fibers from different chromosomes are interwoven.



- More recently methods such as
 1. Fluorescent in Situ hybridization (FISH)
 2. Chromosomal Conformation Capture (3C)demonstrated genome compartmentalization of chromosomes.

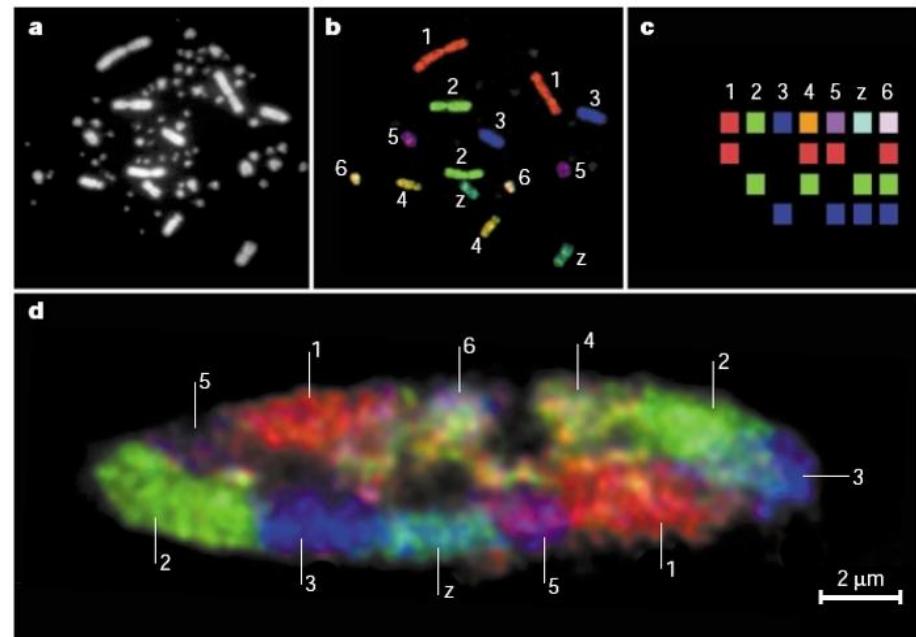
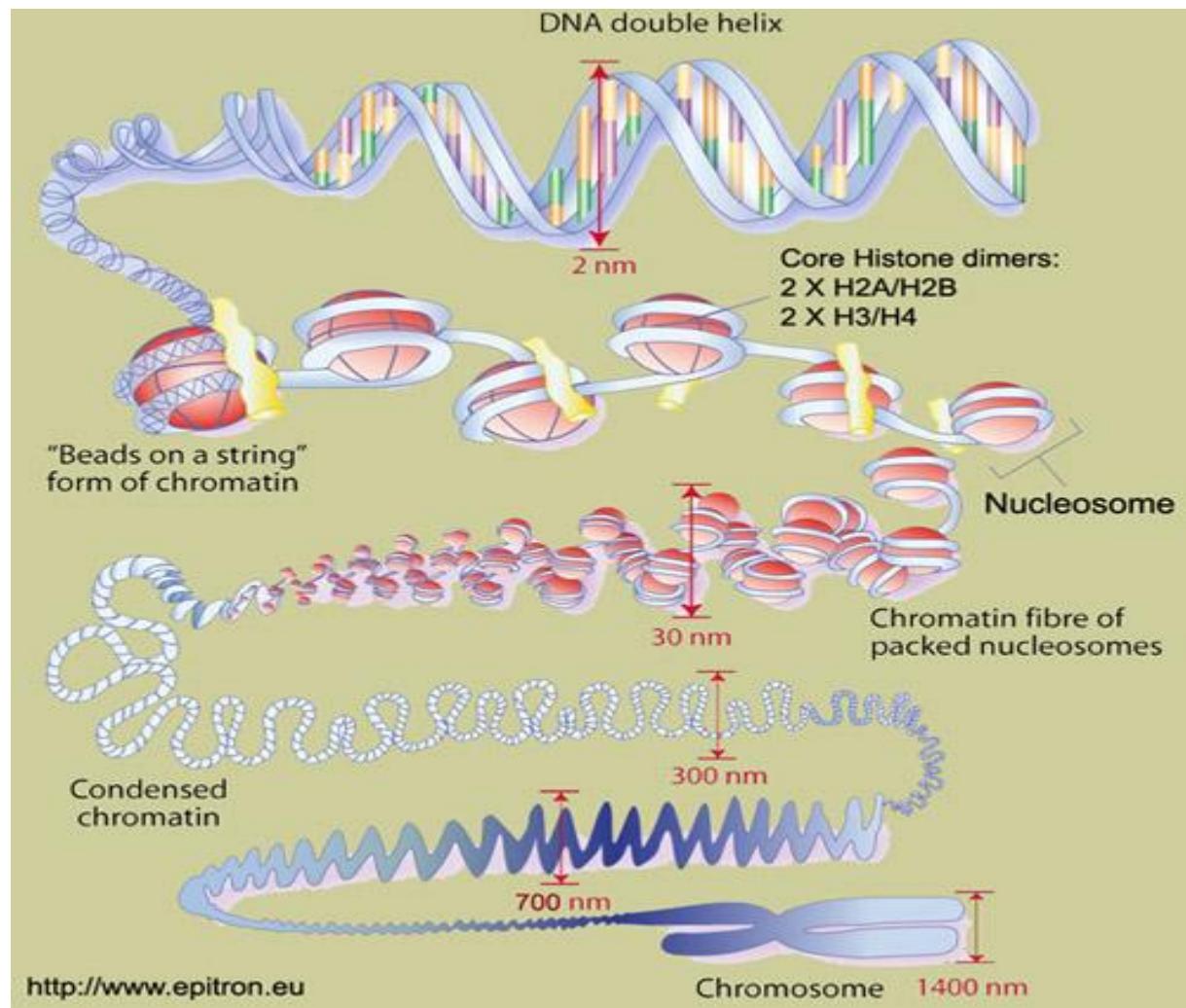
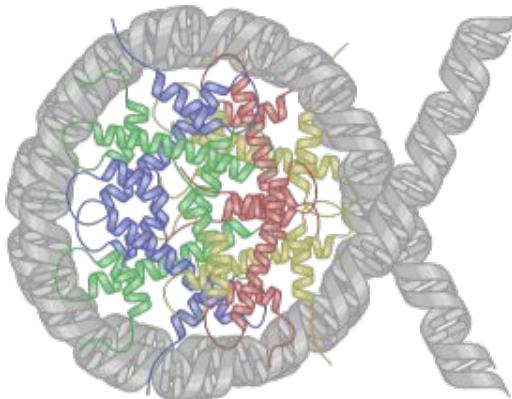


Fig: Multicolour FISH labelled chicken nucleus. (Misteli, T. 2008 Nature Education 1(1):167)

Chromatin Organization

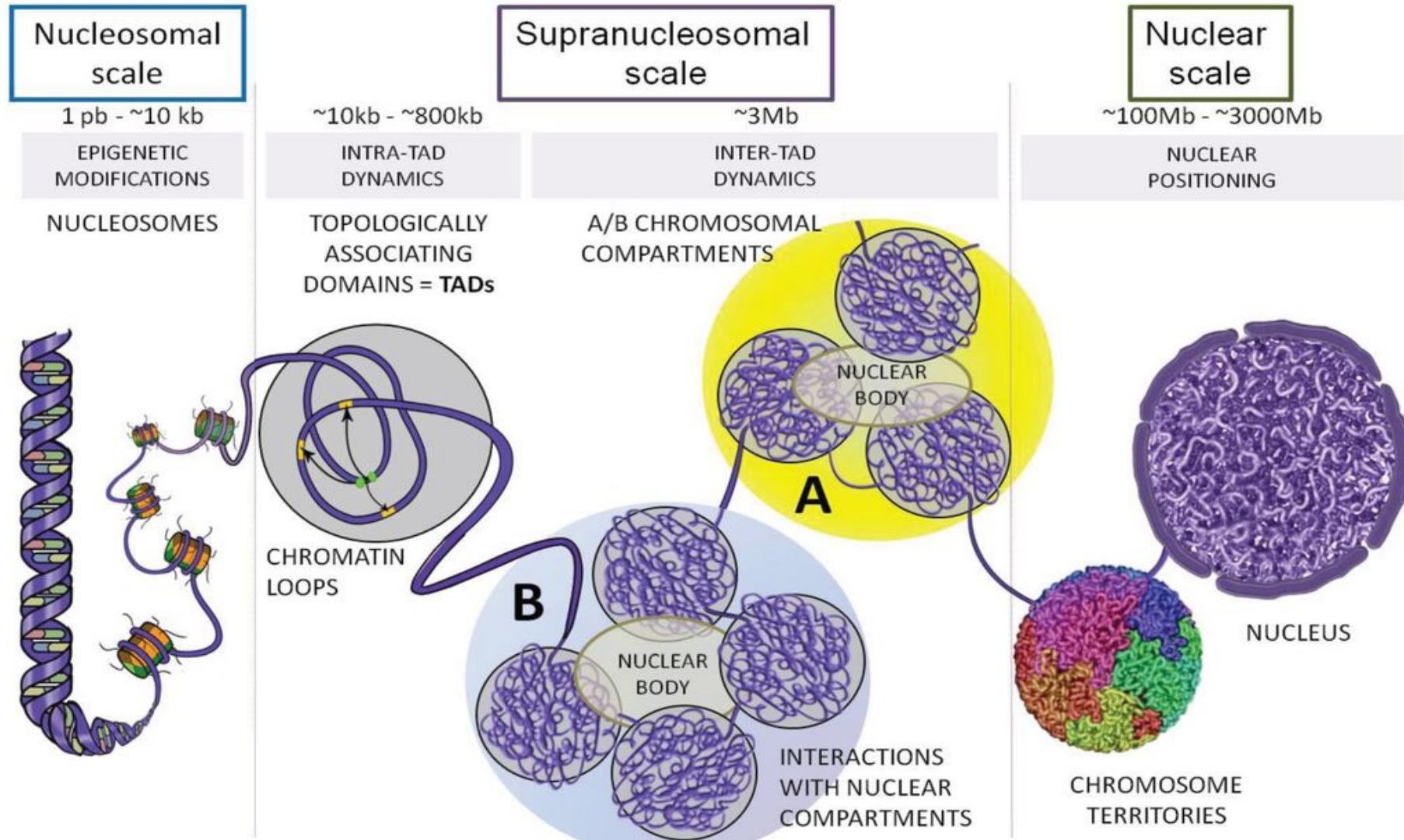
- Histone octamer core wrapped around by 147 bp of DNA and separated by linker DNA.

Complete Histone With DNA



The DNA double helix wrapped around four histone proteins, in a structure called a nucleosome. By Richard Wheeler (Zephyris)
[CC-BY-SA-3.0]]/Wikimedia Commons

Current model of chromatin organization

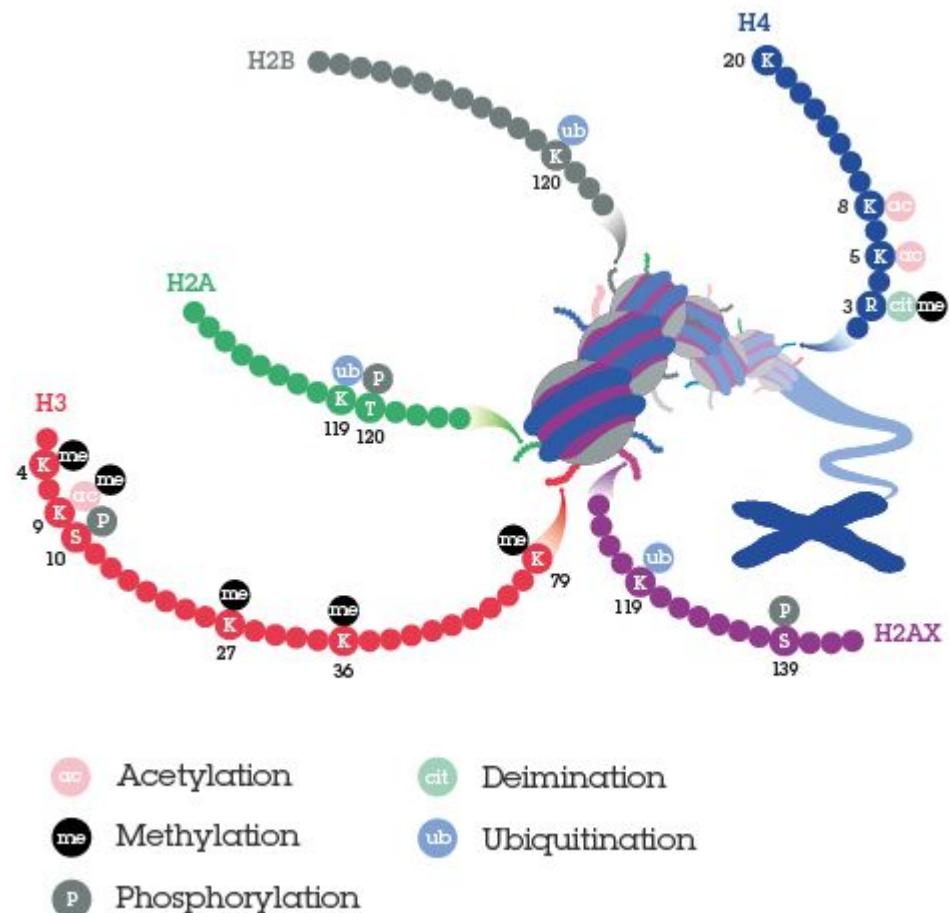


Histone Modifications

- Nucleosomes consist of 2x H2A/H2B and 2x H3/H4 histones.
- 80 known covalent modifications

H3K4me3 →

H3	Histone 3
K	Residue is lysine, K
4	4 th residue.
me3	Trimethylation



The most common histone modifications

Histone Modifications

Some examples:

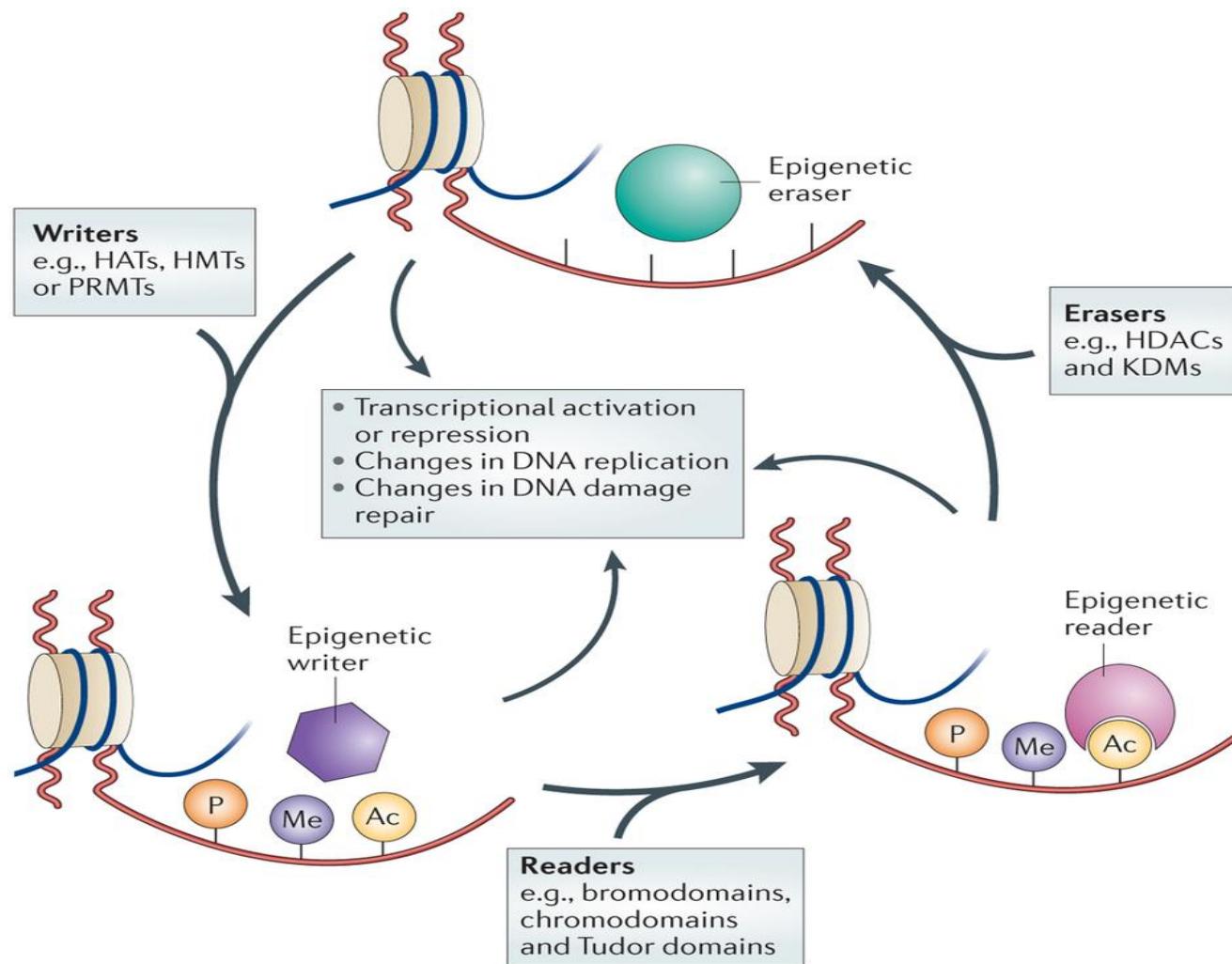
- H3K4me3 - active promoters
- High H3K4me1 and H3k27Ac, low H3K4me3 - active enhancers
- H3K27me3 -repression at promoters
- H3K9me3 - Heterochromatin (inactive, condensed chromatin)

More information at:

<http://epigenie.com/key-epigenetic-players/histone-proteins-and-modifications>

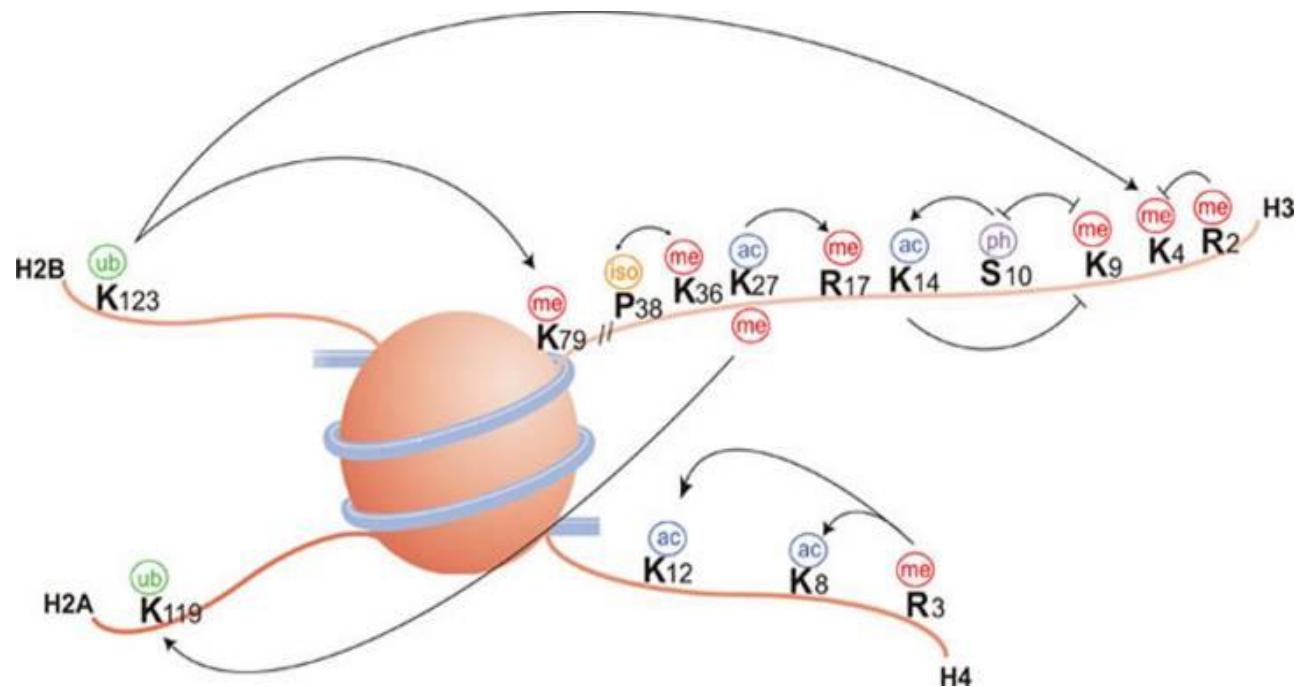
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Epigenetic Readers, Writers and Erasers



Nature Reviews | Drug Discovery

Combinations of marks can have different effects



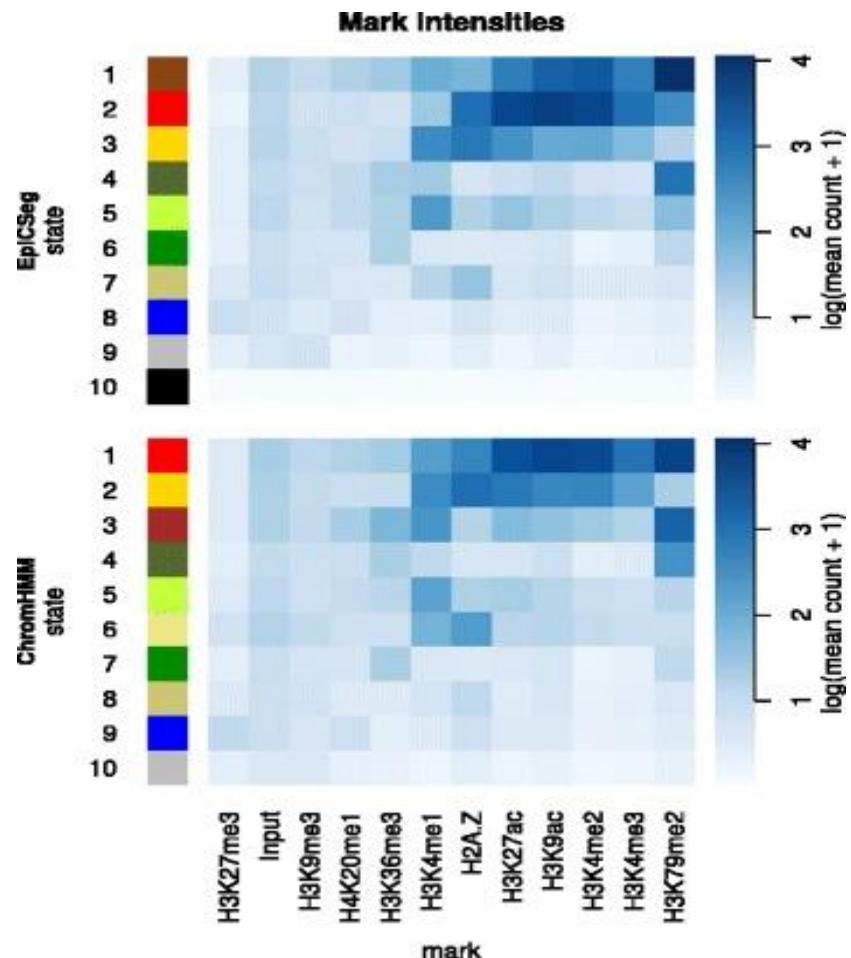
Bannister and Kouzarides (Cell Res. 2011)

To understand the entire code need to ChIP-seq each mark. This information has to be integrated and simplified.

Simplifying histone marks

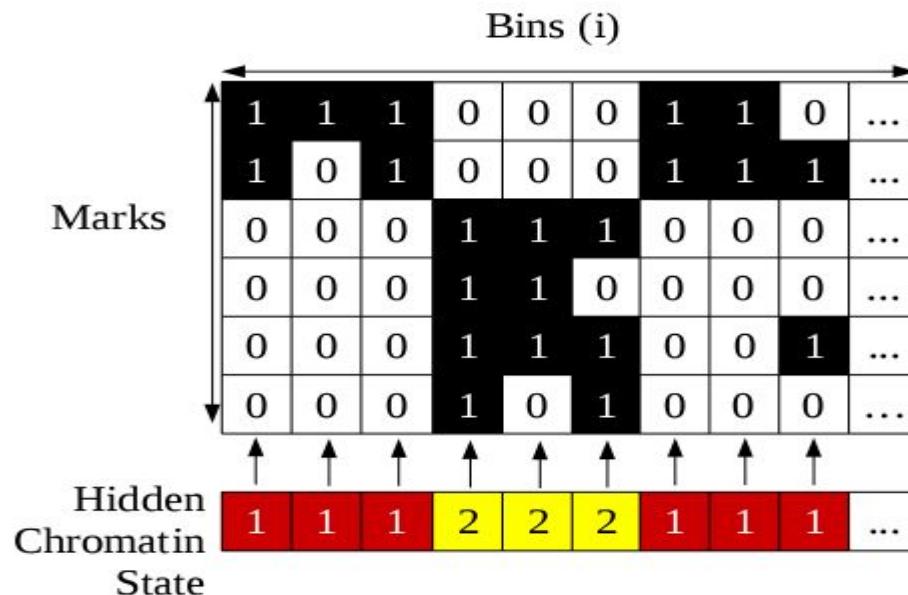
Unsupervised learning methods for *segmentation*;

- ChromHMM (Ernst et al., 2011)
- Segway (Hoffman et al., 2012)
- EpiCSeg (Mammana and Chung, 2015)
- GenoSTAN (Zacher et al., 2016)



Chromatin Segmentation Algorithms

- Genome divided into 200bp bins
- Adjust read position (shift 5' of each read 5'->3' by 0.5 the fragment length)
- Count reads in each bin for each mark and generate count matrix
- HMM with specified states is used to model the count matrices and derive segmentation



Chromatin Segmentation

Advantages:

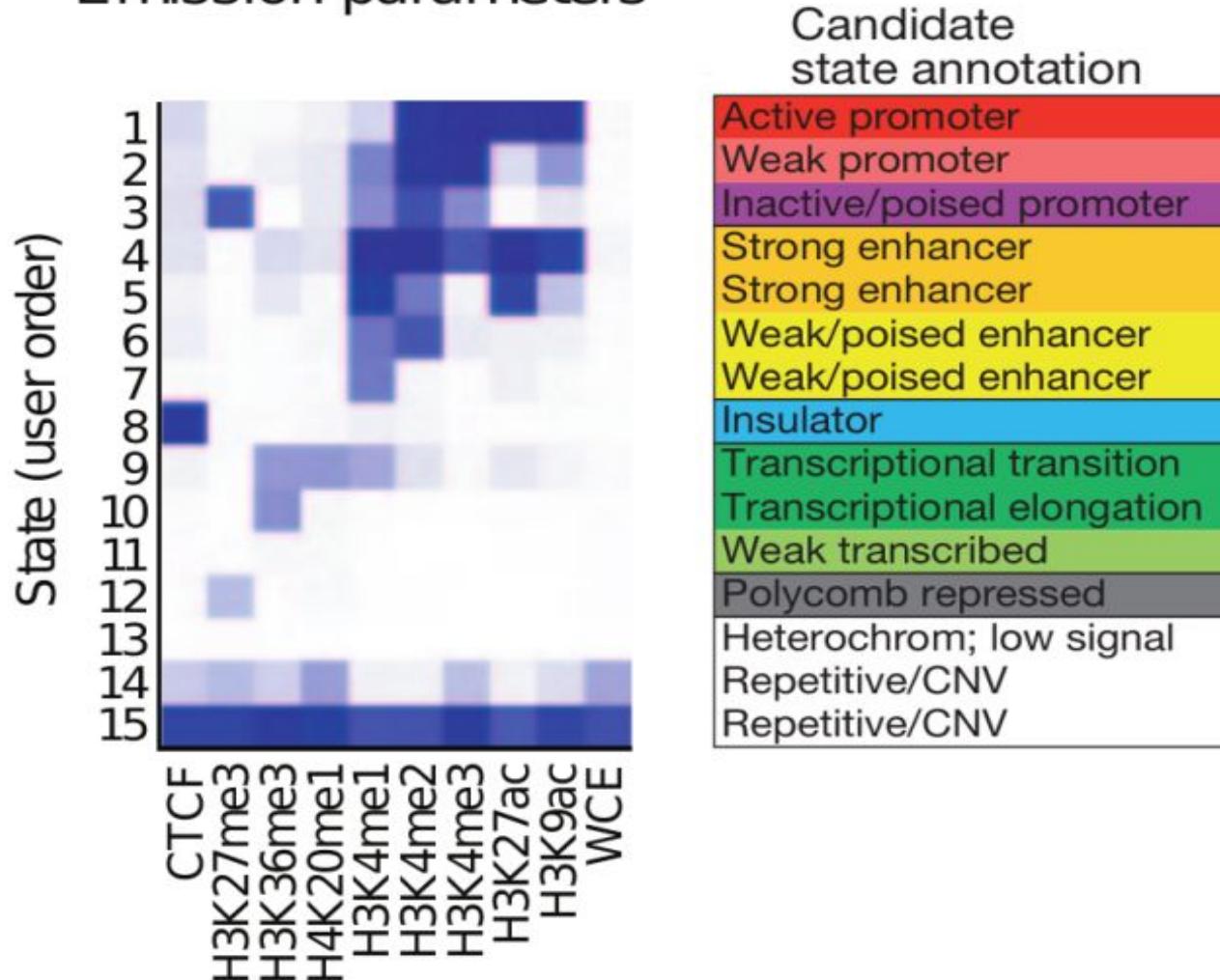
- Derived states, not vectors of chromatin marks -easier to determine genome wide properties.
- Can train on one set and apply to another.

Disadvantages:

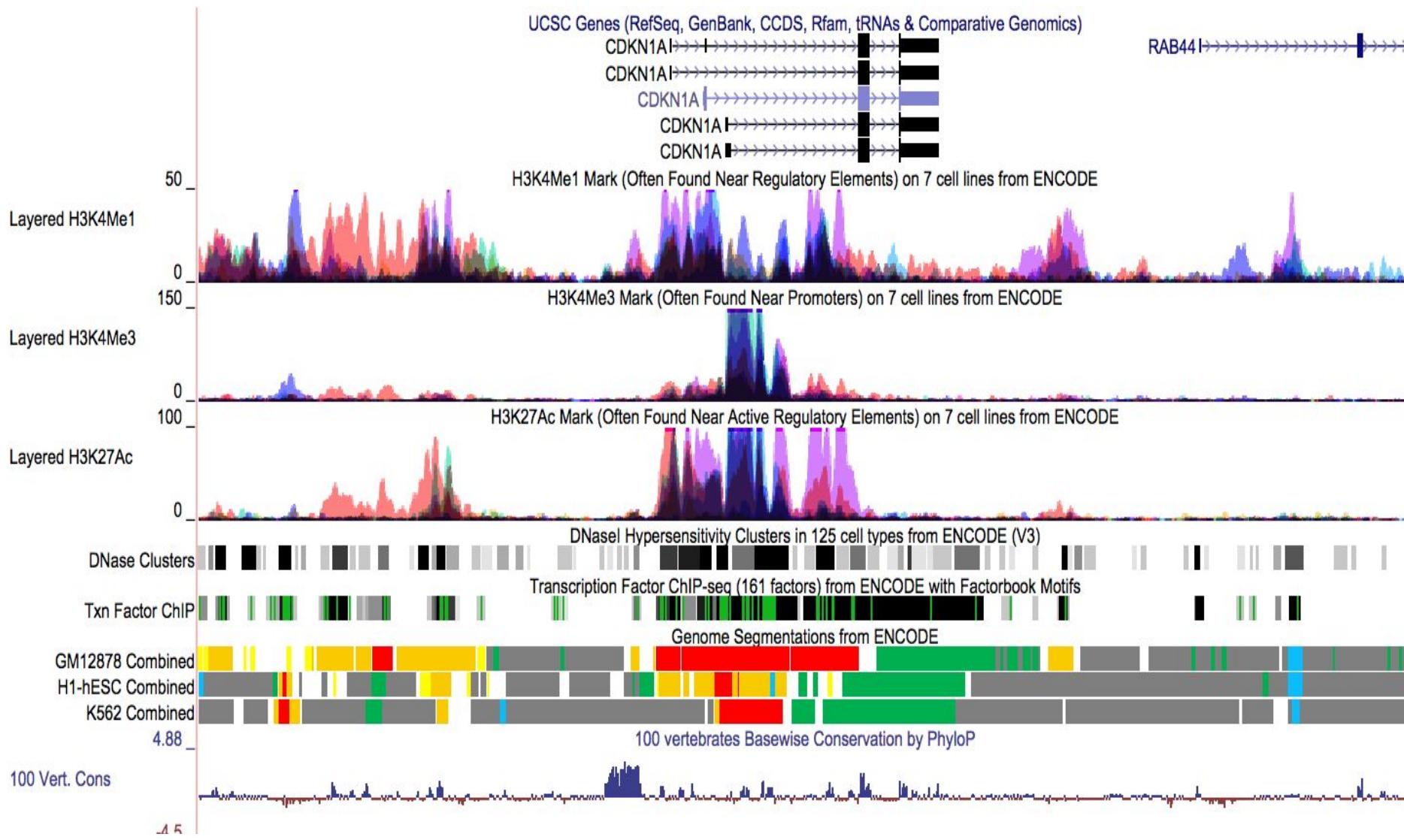
- How many states?
- Histone states binary -lose information (except in EpiCSeq)
- Causality unknown

Chromatin Colours

Emission parameters



Visualizing Chromatin Marks



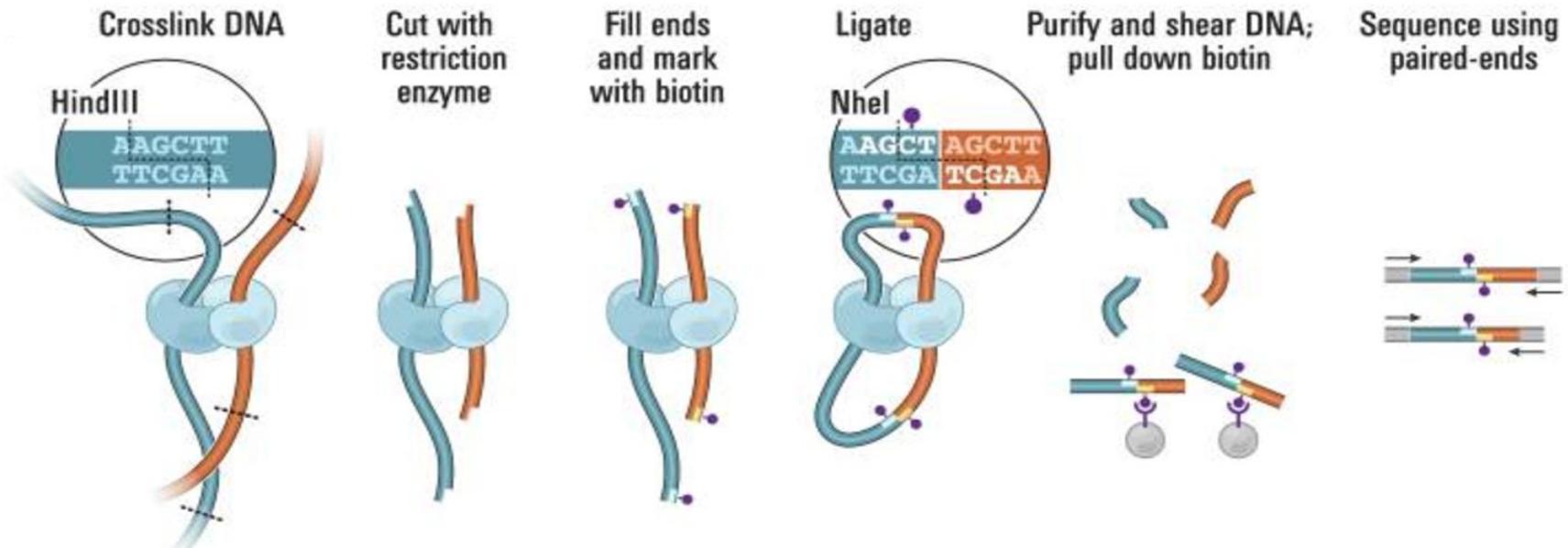
Chromosomal Conformation Capture

- 3C methods identify all possible chromatin interactions between two distinct genomic regions such as promoter-enhancer interactions.

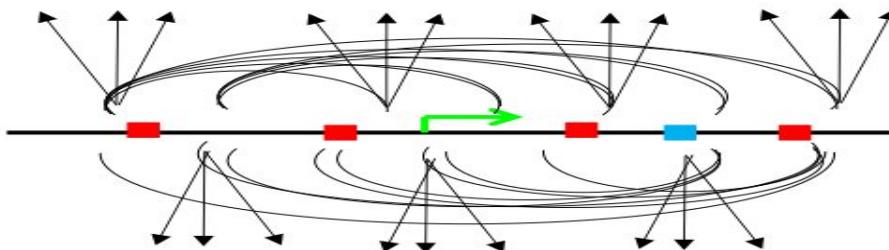
Table 1. Advantages and limits of 3C-derived methods.

Method	Genomic Scale Investigated	Advantages	Limits
3C-qPCR	~250 kilobases	Very high dynamic range (highly quantitative), easy data analysis	Very low throughput: limited to few viewpoints in a selected region
4C	Complete genome	Good sensitivity at large separation distances	Genome-wide contact map limited to a unique viewpoint (few viewpoints if multiplex sequencing is used)
5C	Few megabases	Good dynamic range, complete contact map (all possible viewpoints) of a specific locus	The contact map obtained is limited to a selected region
Hi-C	Complete genome	Very high throughput (complete contact map)	Poor dynamic range, complex data processing

Hi-C: long distance interactions

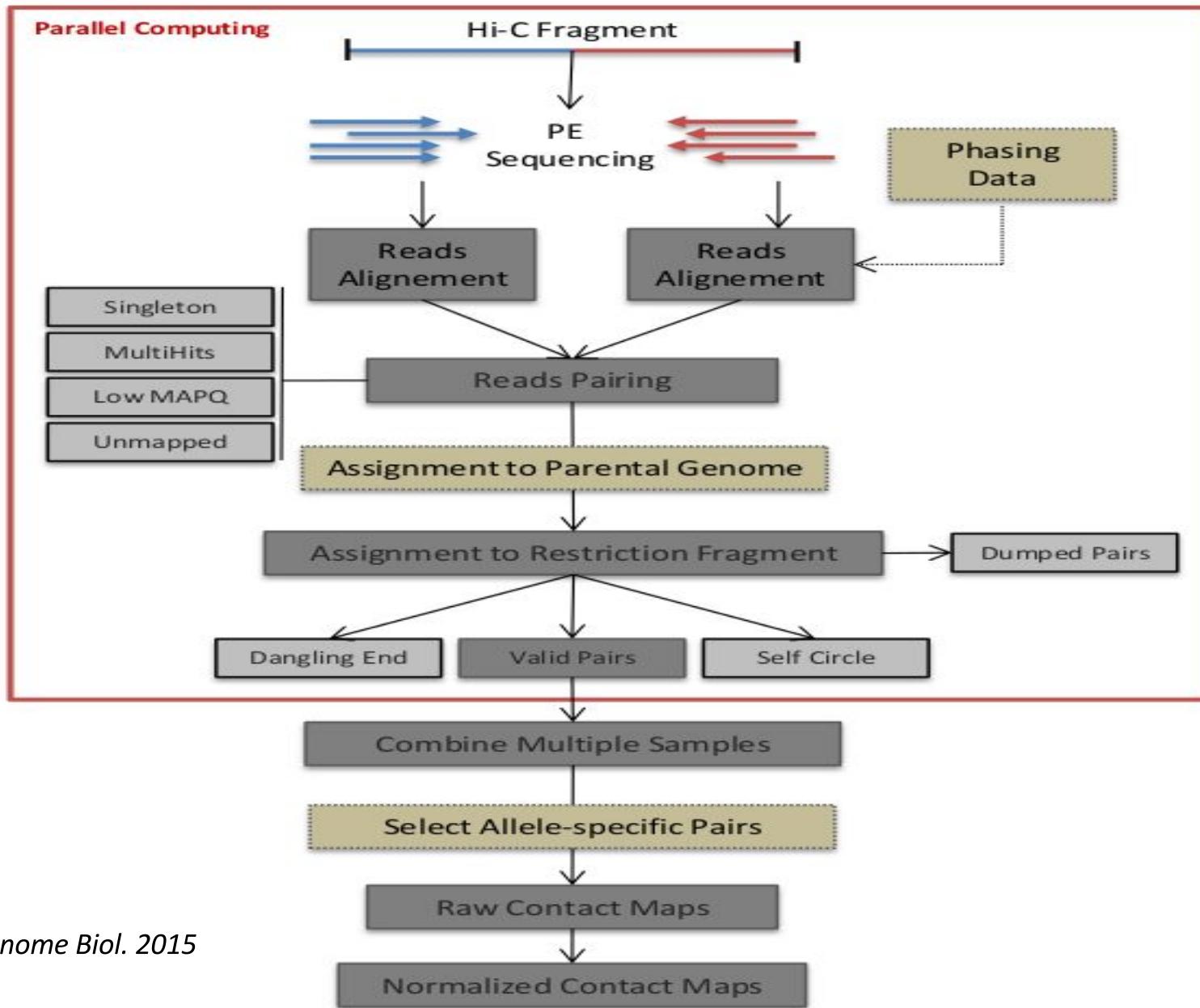


Lieberman-Aiden, Science 2009



Principle	All against all
Coverage	Genome-wide *
Detection	Paired end HT-sequencing
Resolution	Low *
Limitations	
Examples	All intra- and inter- chromosomal associations

HiC: Computational workflow



Hi-C: technical and biological biases

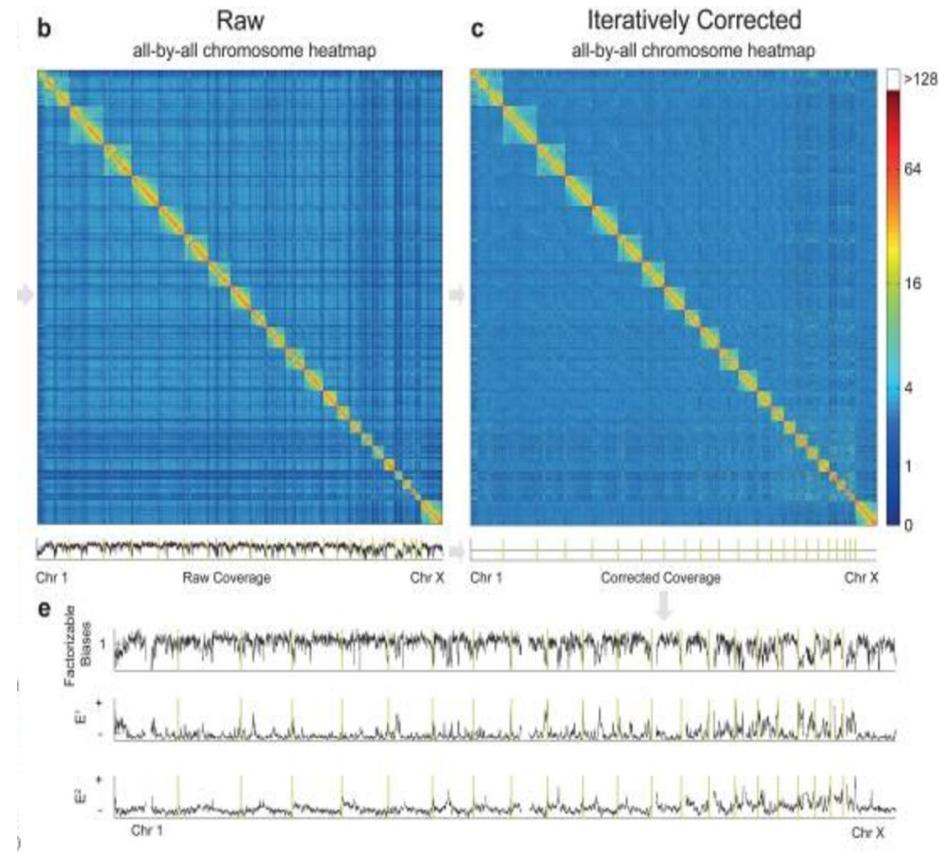
- Hi-C experiments are designed to measure the contact probability between different chromosomal loci on a genome-wide scale.
- This is done by cleaving fixed chromosomes into restriction fragments using six-cutter restriction enzymes and ligating fragment ends to form ligation junctions connecting two loci that are nearby in three-dimensional space.

Biases:

- Hi-C sequence pairs that represent ligation products between nonspecific cleavage sites rather than restriction fragment ends.
- The length of restriction fragments (in other words, the distance between adjacent cutter sites).
- GC bias.
- Mappability (genomic Uniqueness)
- Sequence depth & Coverage.

Normalization and bias correction

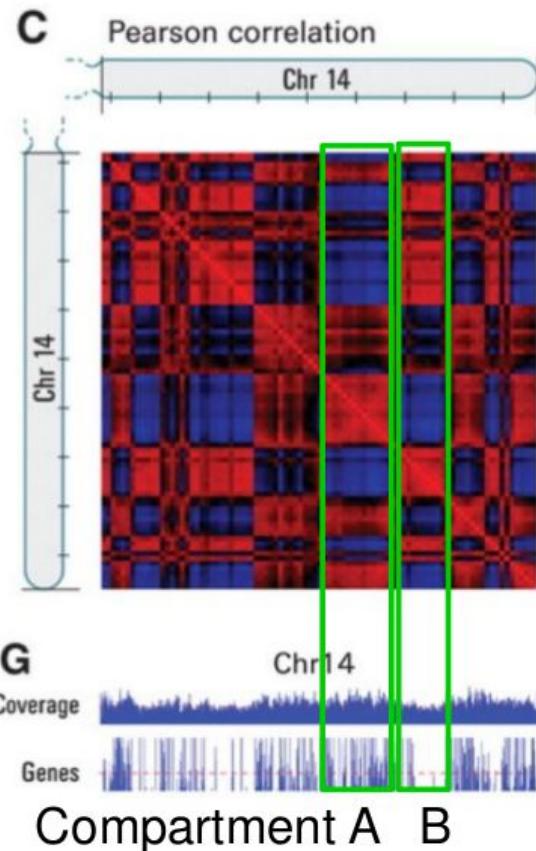
- **Iterative Correction and Eigenvector decomposition (ICE)**
- A method of iterative correction, which eliminates biases and is based on the assumption that all loci should have equal visibility.
- Iterative correction leverages the unique pairwise and genome-wide structure of Hi-C data to decompose contact matrices into a set of biases and a map of relative contact probabilities between any two genomic loci.
- The obtained corrected interaction maps can then be further decomposed into a set of genome-wide tracks (eigenvectors) describing several levels of higher-order chromatin organization



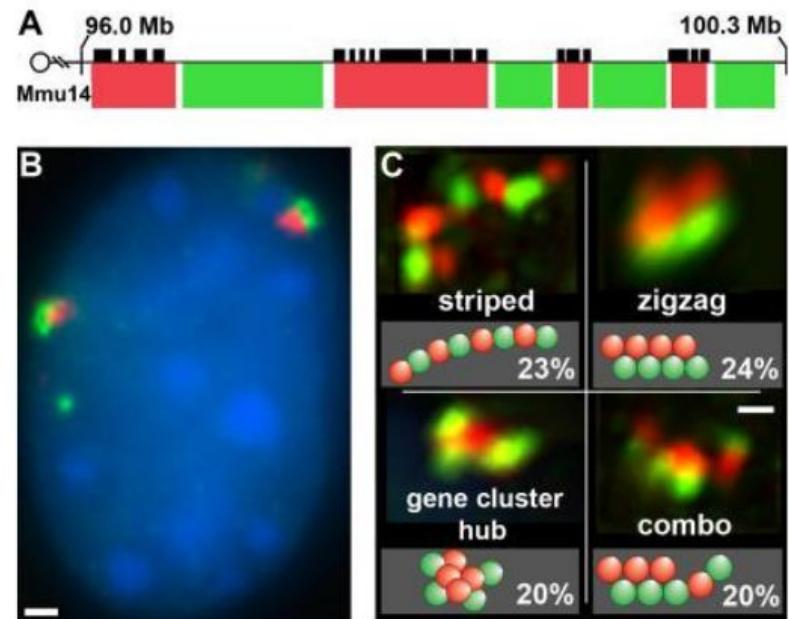
Chromatin: A-B compartments

- The genome (at the Mb scale) can be divided into cell type or condition specific A/B compartments that are associated with open and closed chromatin.
- The A compartment is associated with gene rich, transcriptionally active, open chromatin state regions.
- Interactions are more likely between A-A or B-B regions and not A-B regions.
- A and B regions can change into the other.

A-B compartments



Compartment A is gene rich



Compartment A

Genes Spearman's $\rho = 0.431$

Expression Spearman's $\rho = 0.476$

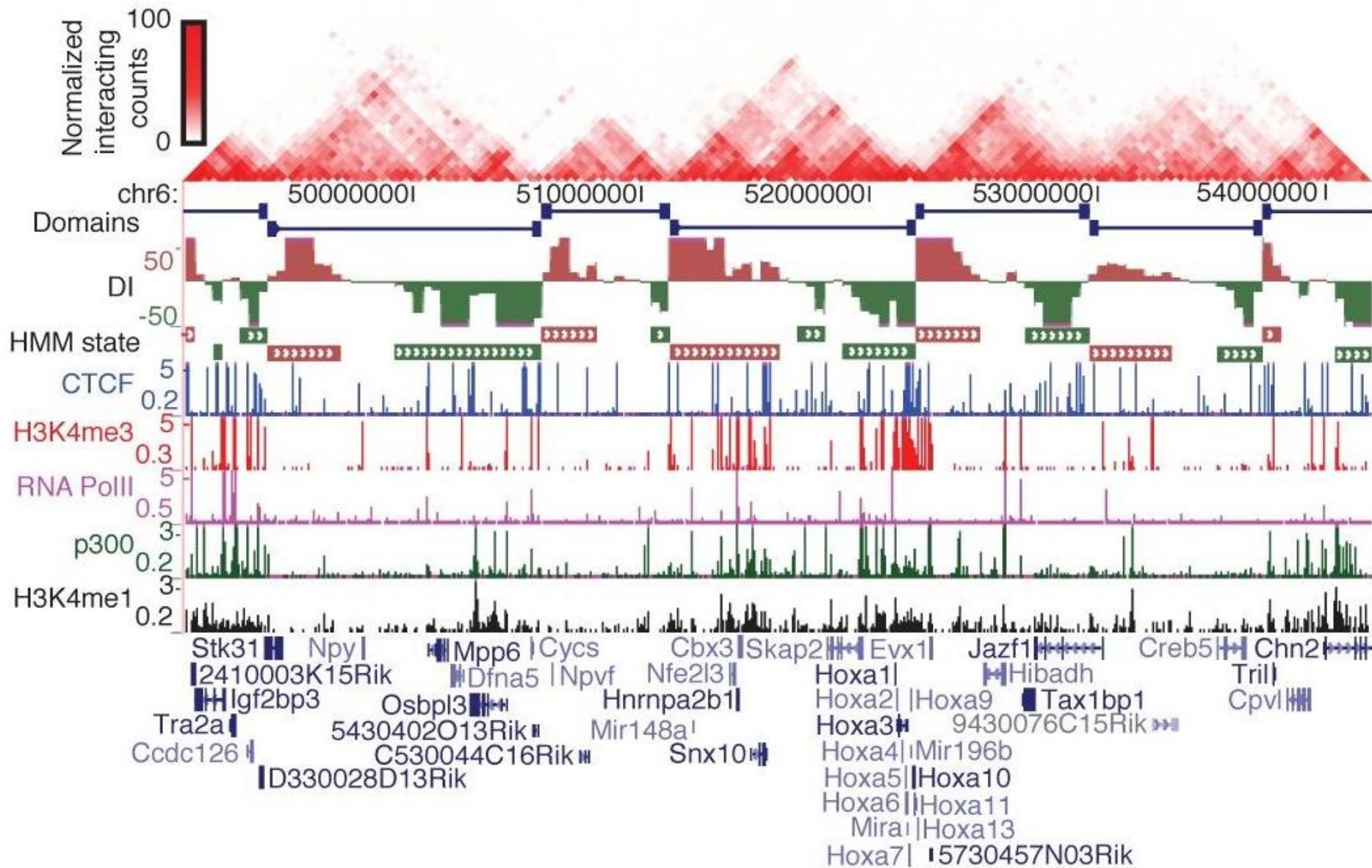
Accessible chromatin, Spearman's $\rho = 0.651$

H3K36 trimethylation, Spearman's $\rho = 0.601$ (active)

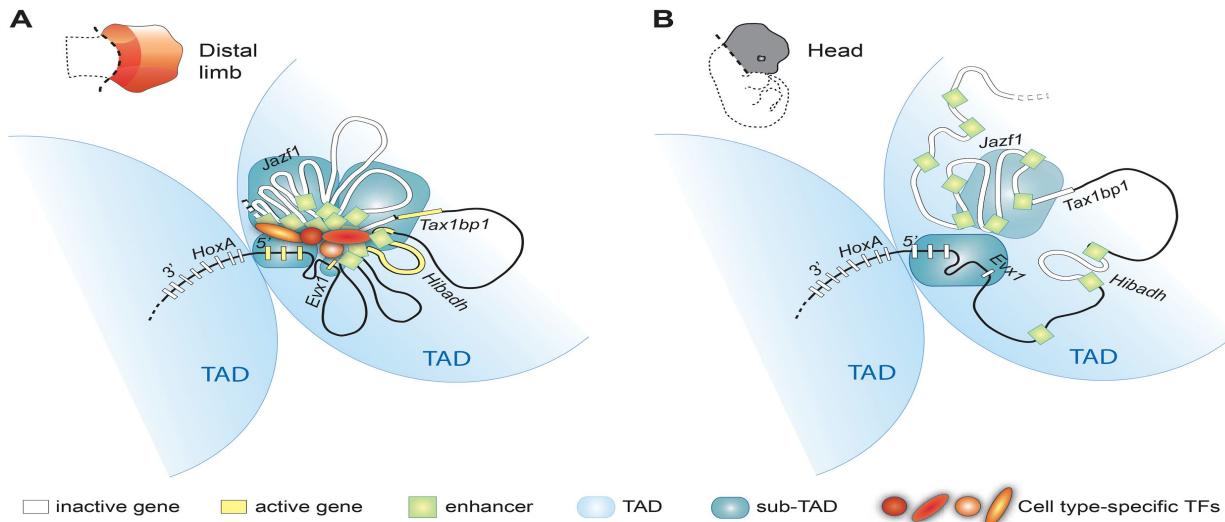
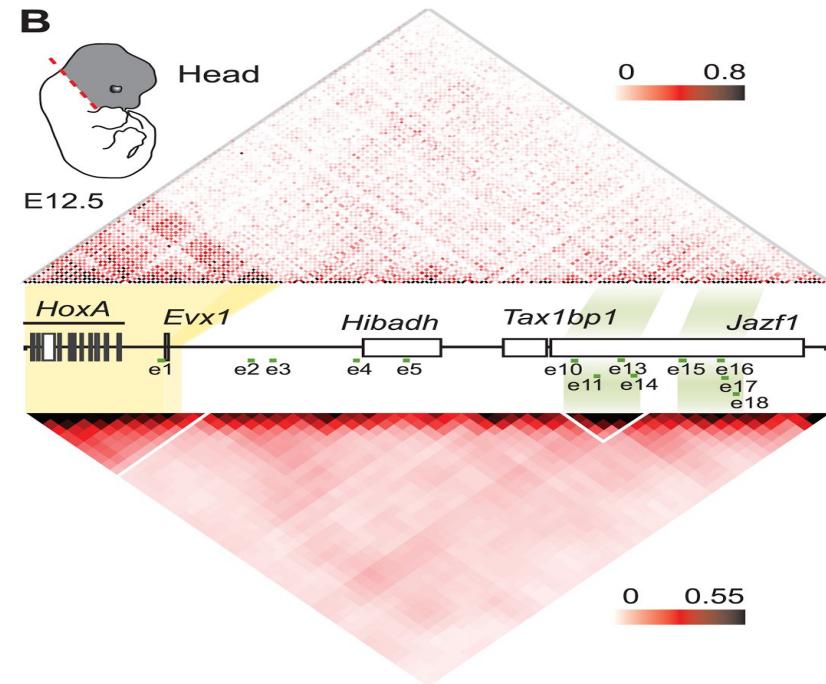
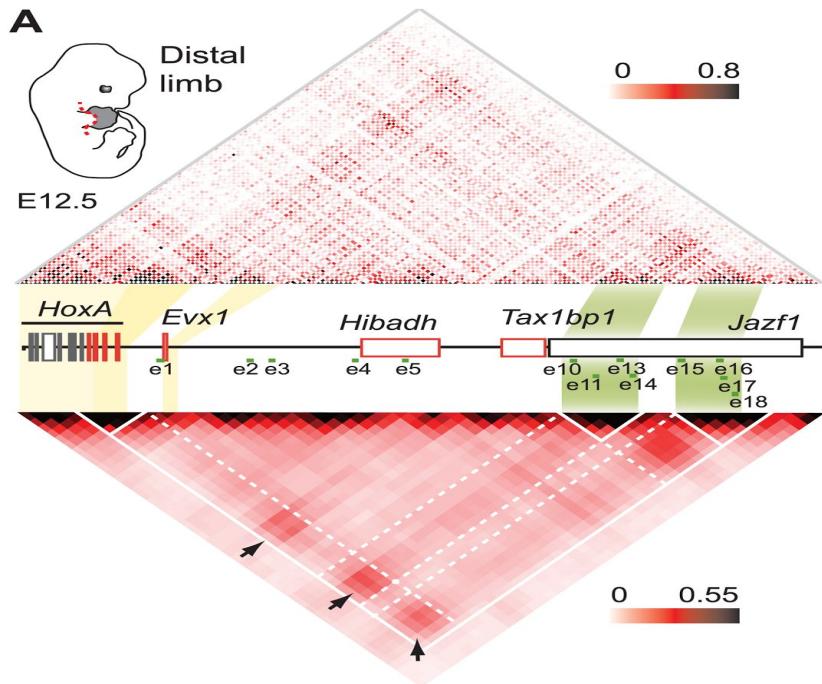
H3K27 trimethylation, Spearman's $\rho = 0.282$ (repressive)

A is more closely associated with open, accessible, actively transcribed chromatin.

Topologically Associated Domains

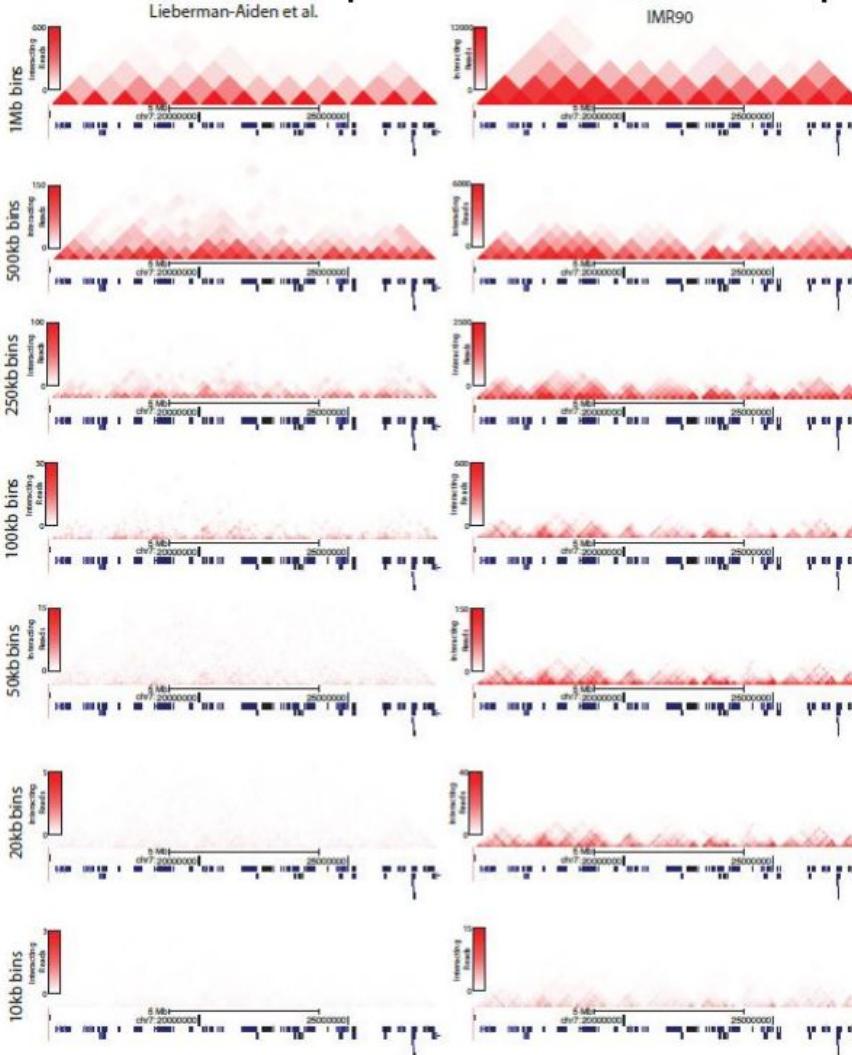


Long distance interactions

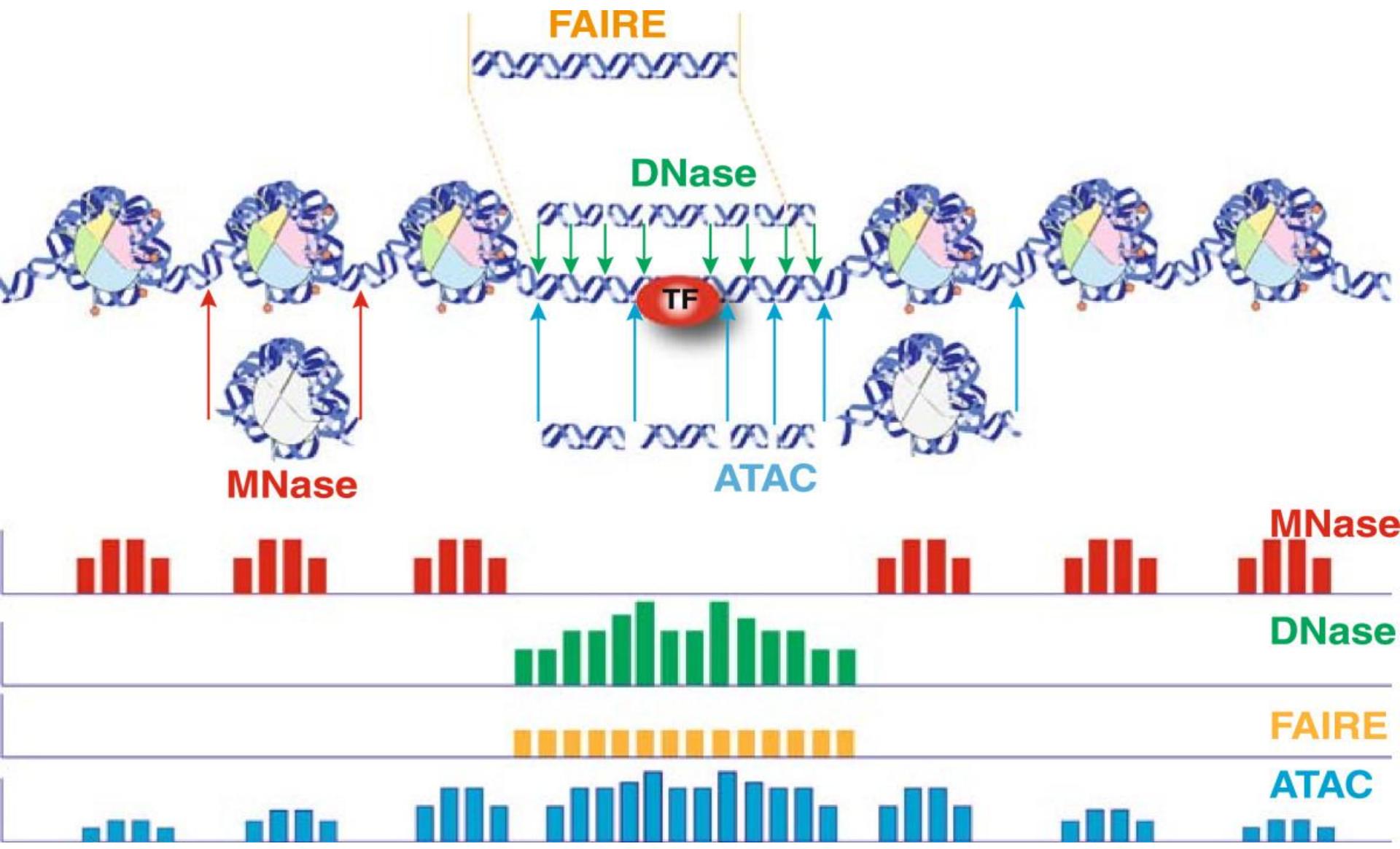


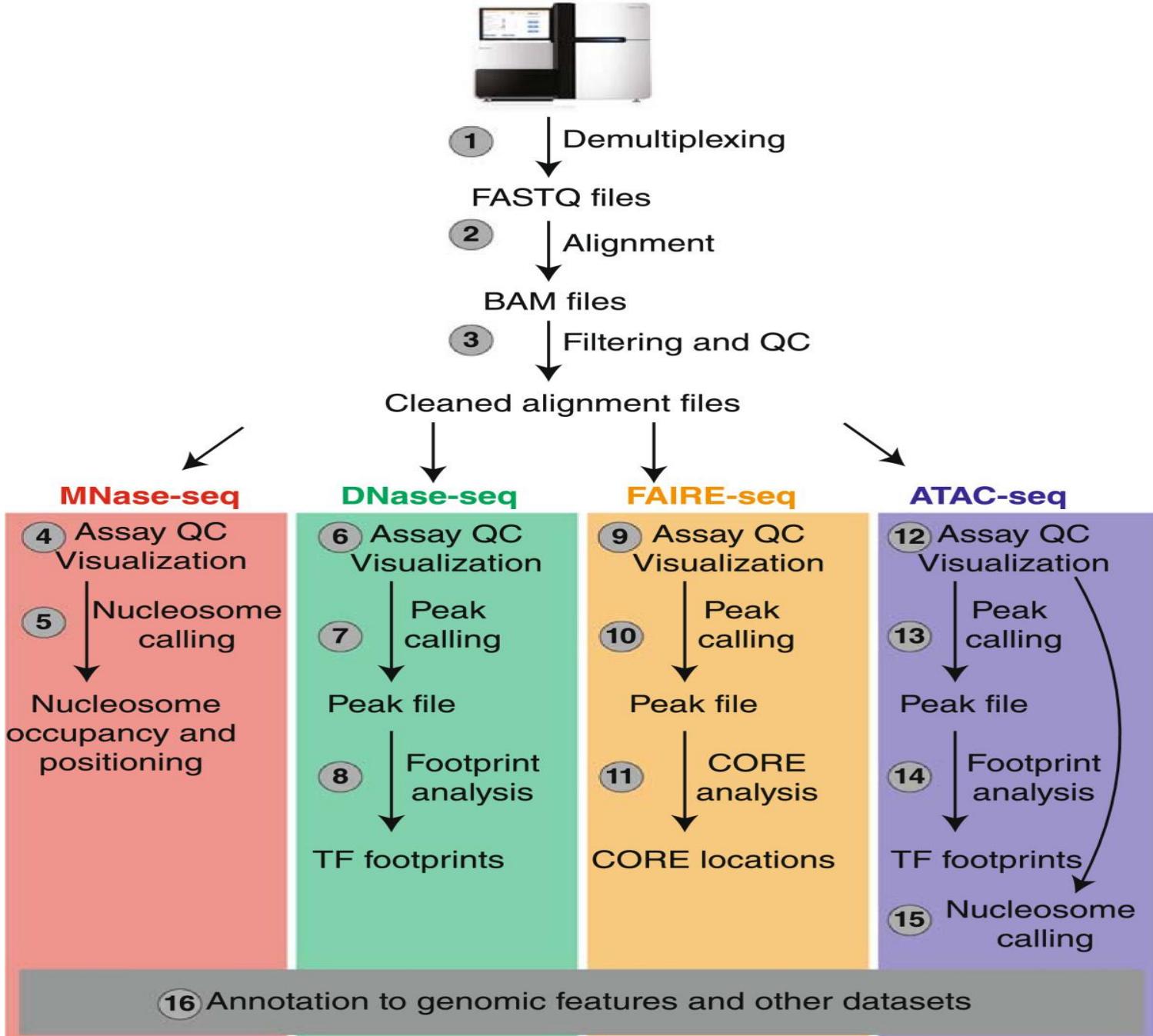
Read depth and bin size

~ 20 million read pairs ~ 1 billion read pairs

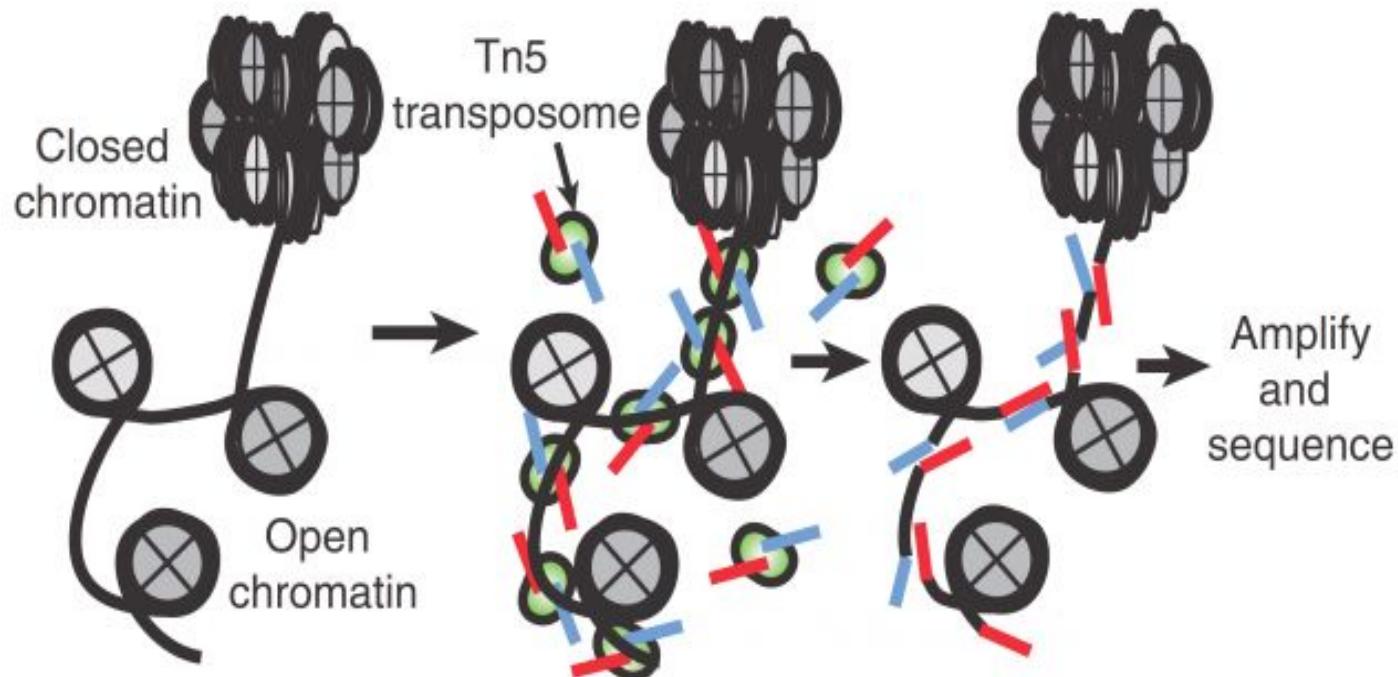


Detecting Chromatin Accessibility





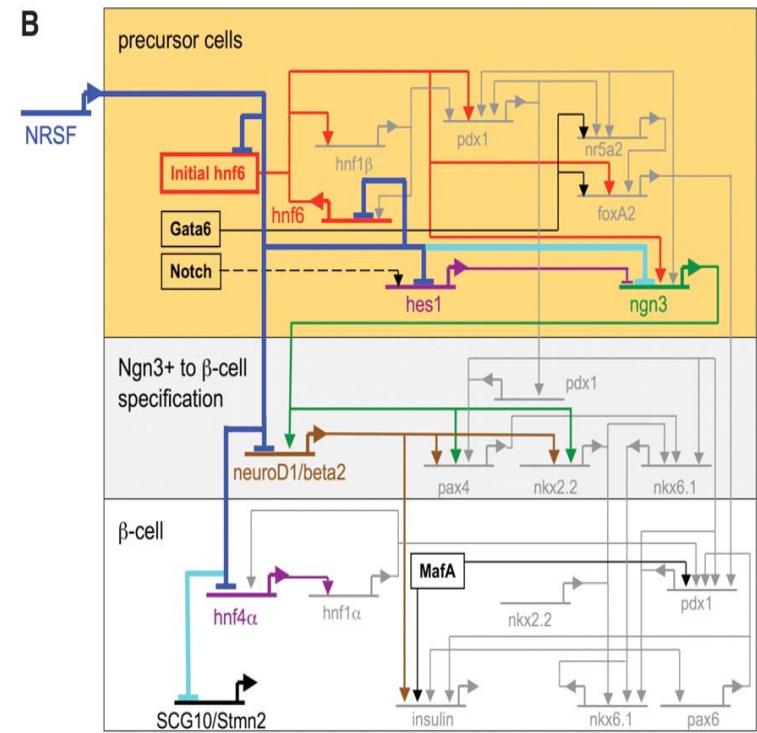
ATAC-seq



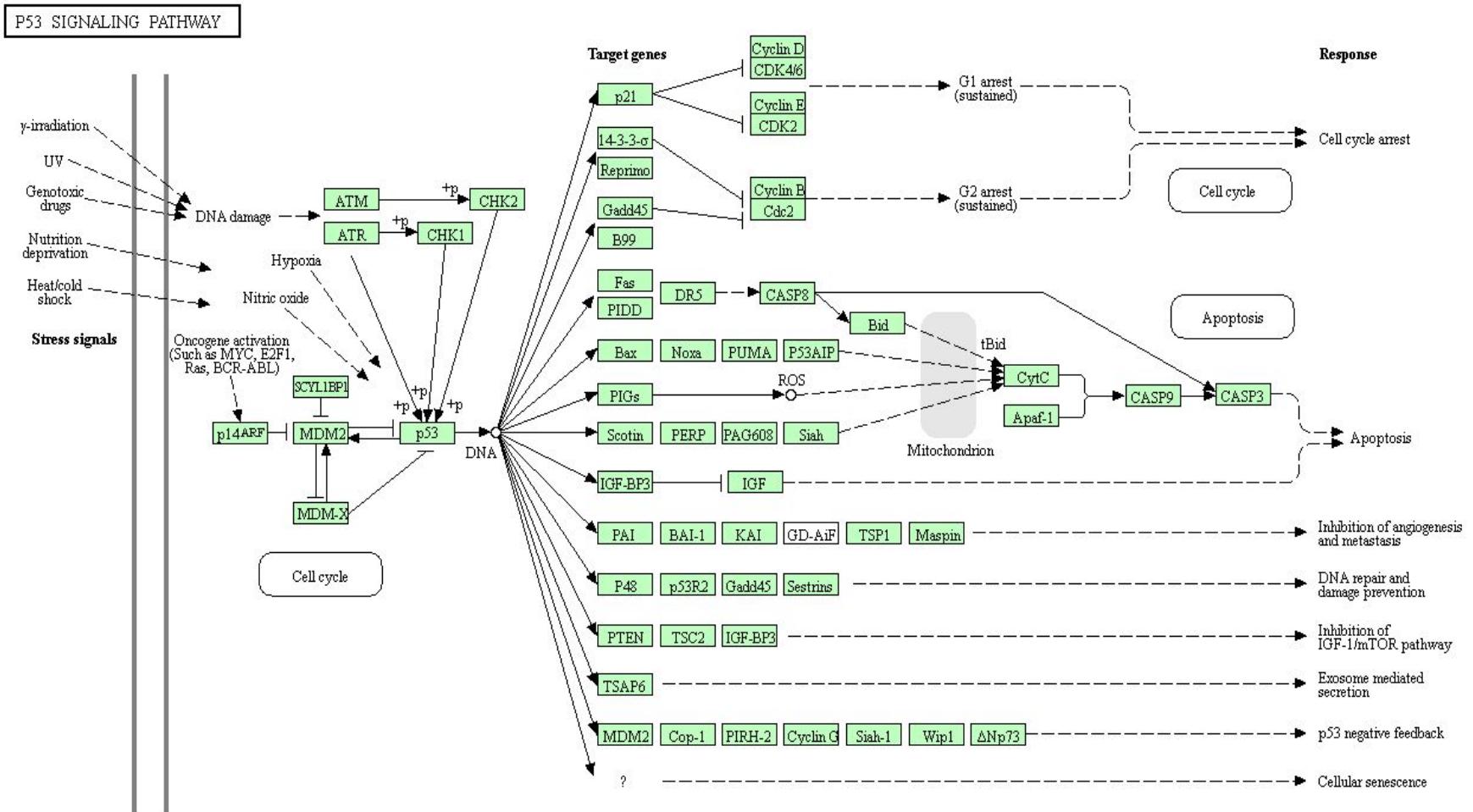
- Directly measures the effect of chromatin structure modifications (open chromatin) on gene transcription.
- Does not require antibodies or tags that can introduce potential bias.
- Hyperactive Tn5 transposase is used to fragment DNA and integrate into active regulatory regions.
- During ATAC-seq, 500–50,000 unfixed nuclei are tagged *in vitro* with sequencing adapters by purified Tn5 transposase.
- Can also detect nucleosome packing, positioning and TF footprints.

Regulomes: from active regulatory elements to networks

- Not all TF binding sites are transcriptionally active. The collection of transcriptionally active targets of a TF is it's **regulome**.
- Regulomes can be used to “explain” the phenotype under consideration and understand aspects of biological systems.
- Regulomes in combination with pathway and network modelling approaches can then be used decipher the networks underlying phenotypes.
- These networks provide information on connectivity, information flow, and regulatory, signaling and other interactions between cellular components.

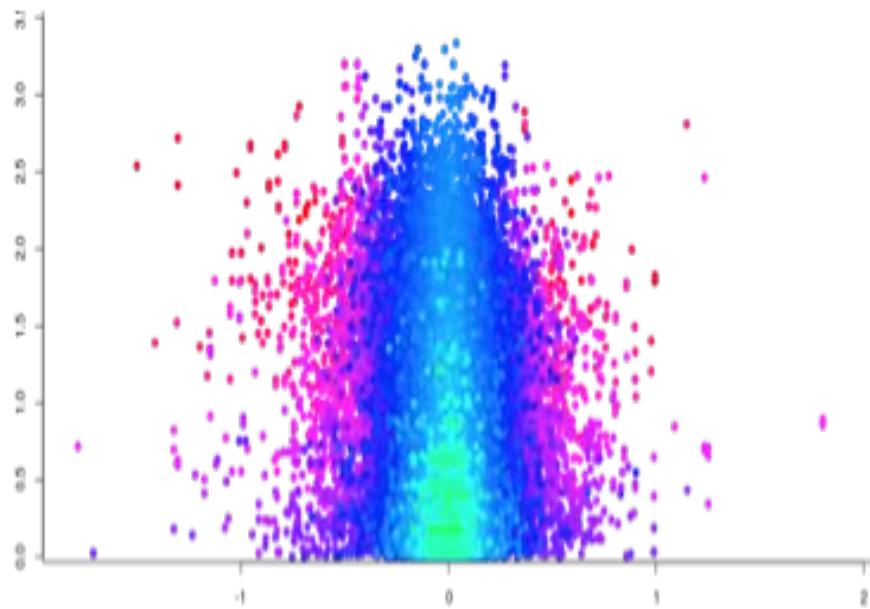
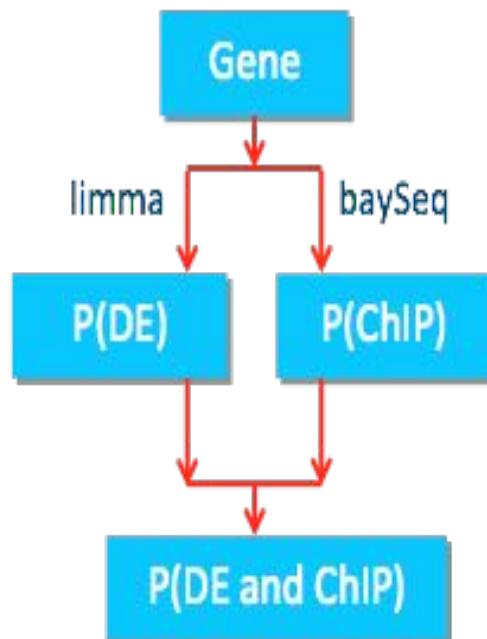


KEGG: p53 signaling pathway

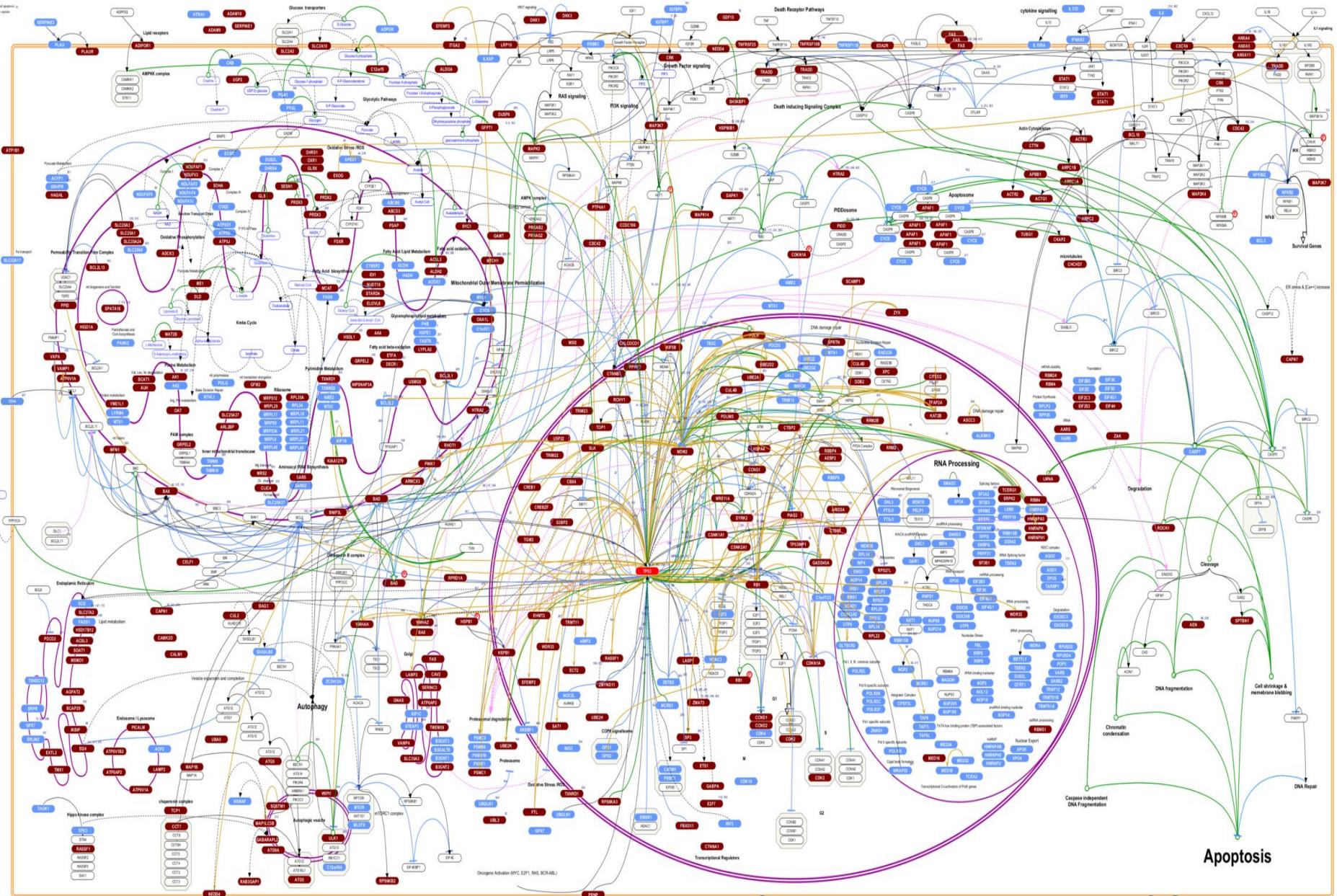


Rcade

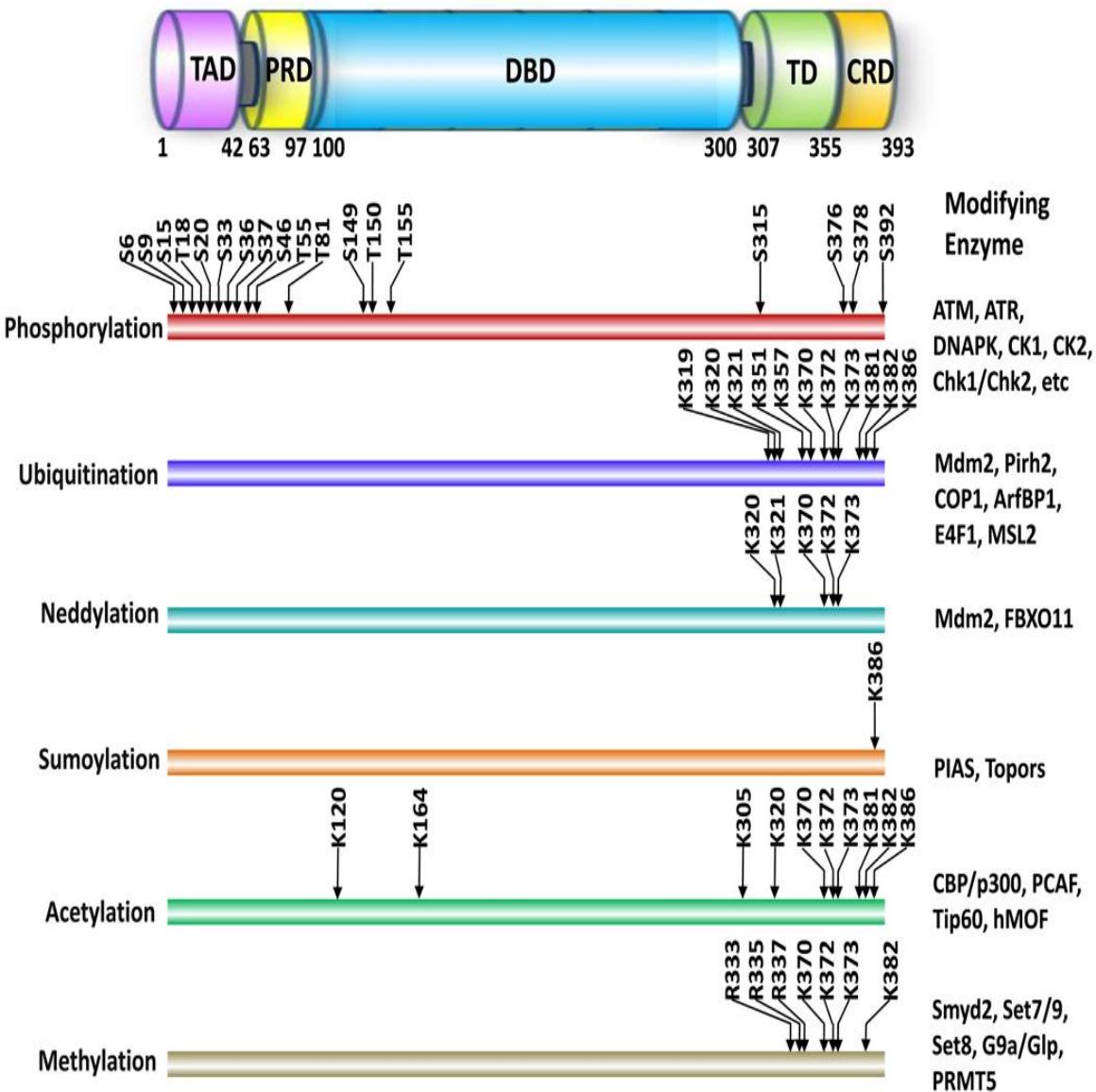
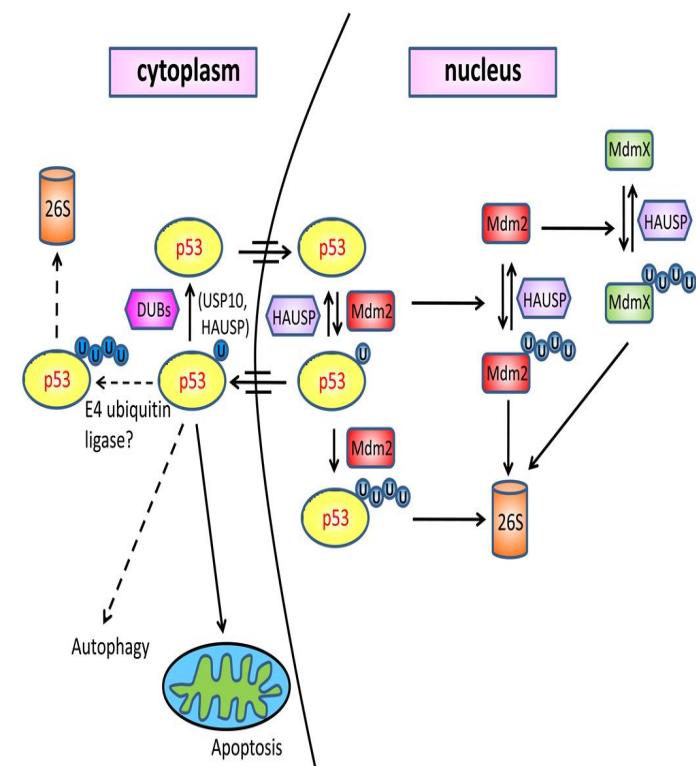
- **Rcade: R based analysis of ChIPseq And Differential Expression**
- Bayesian approach used to integrate ChIP-seq with differential expression to identify direct transcriptional targets of transcription factors.



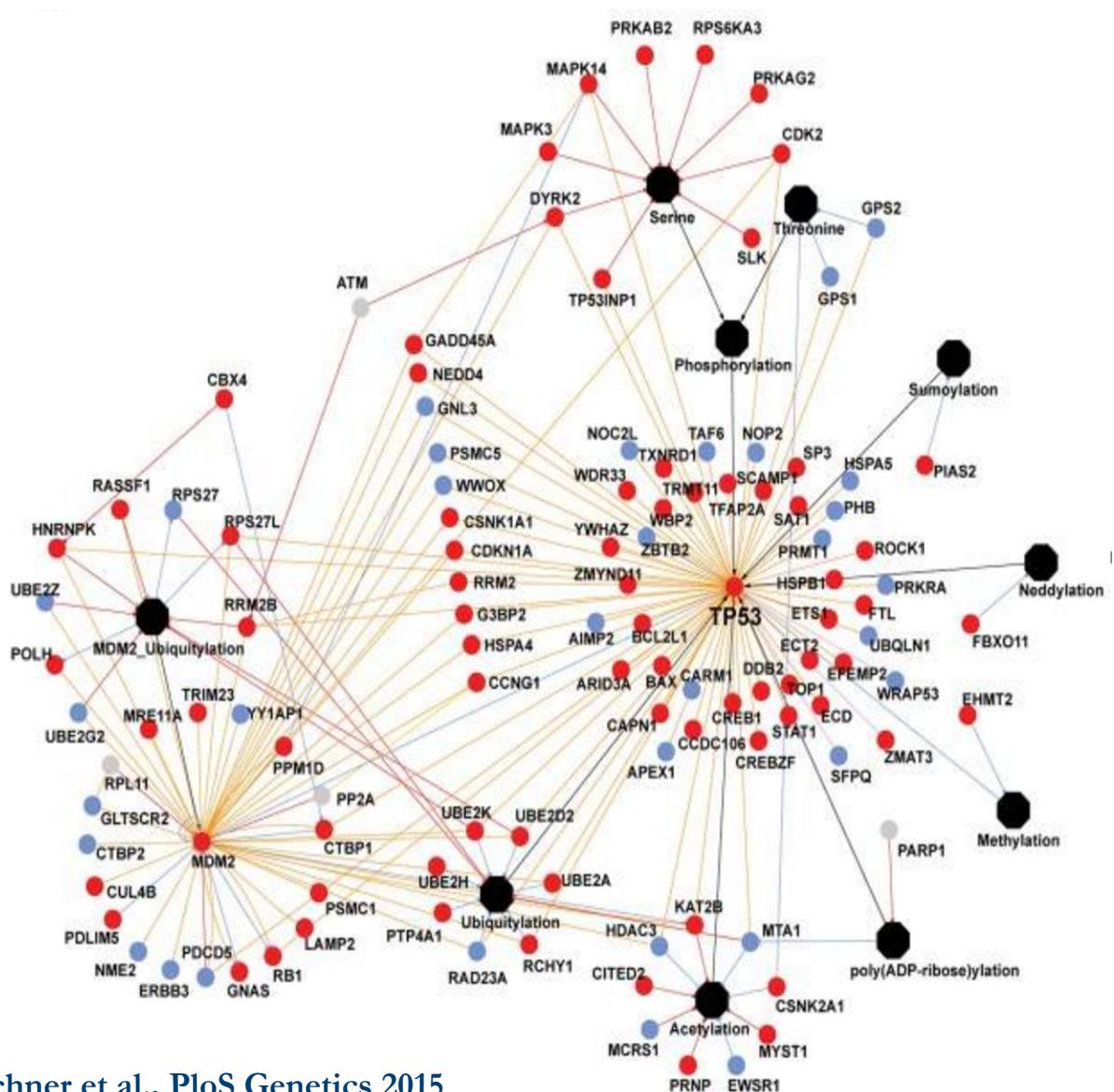
The TP53 Regulome



Fine tuning regulation: post-translational modifications



The Self-Regulatory TP53 Network



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Jonathan Cairns
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