

SevenBridges

Epigenetic control

May 2024

Darko Cucin

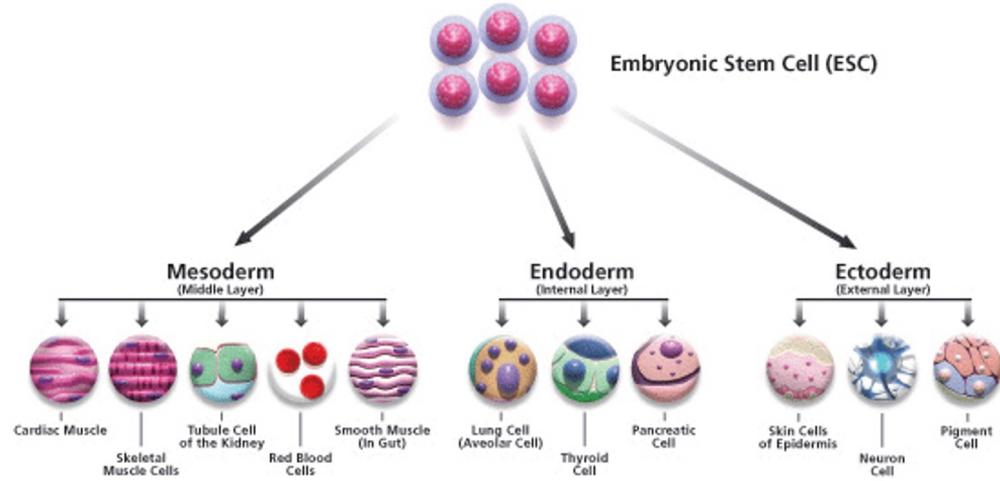
Genomic variation

Recap



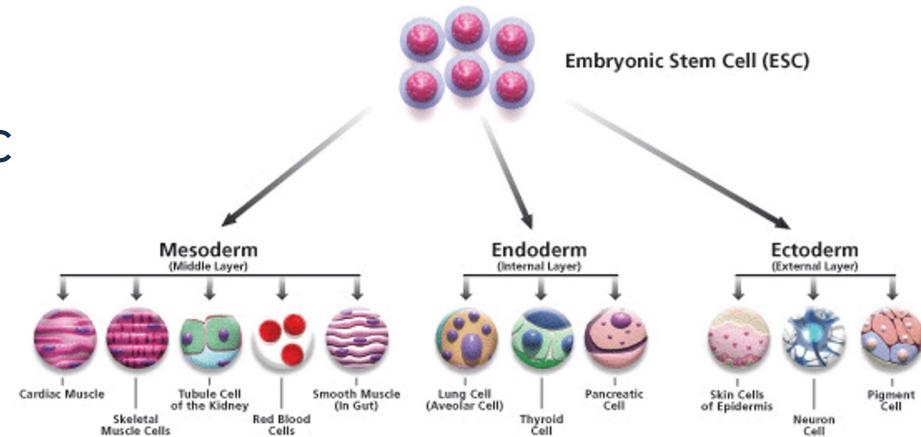
Epigenetic control

- Same genotype - different phenotypes
- How?



Epigenetic control

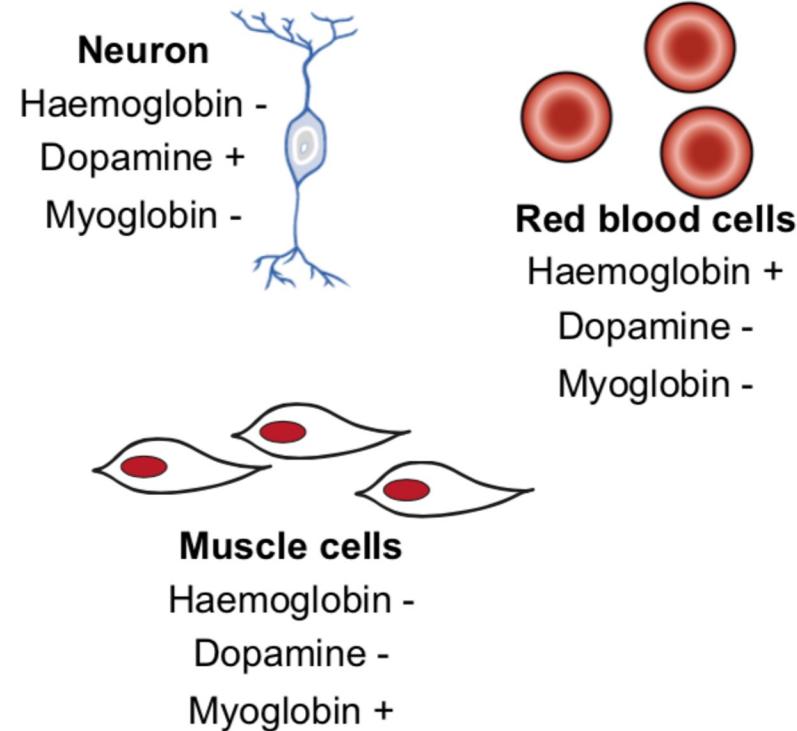
- **Epigenetics** - extra layer of information on top of genetic information
- Each cell type is defined by genes that are **expressed** in the cell



Gene expression

DNA → RNA → protein

Single cell fertilised egg
1 cell type



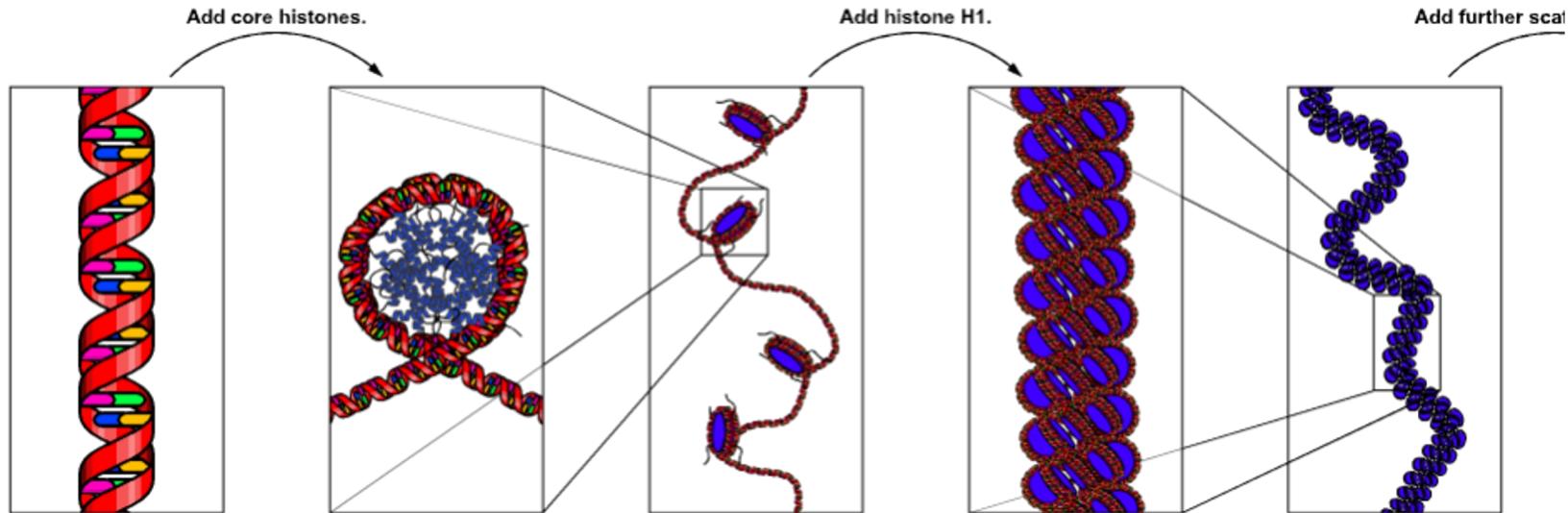
Each cell type only expresses a restricted subset of genes.

Epigenetic modifications

- Demarcate the start and end of genes
- Provide structure to the chromosome
- Alter how we read each and every gene
 - genes being expressed (active)
 - genes being not expressed (silent)

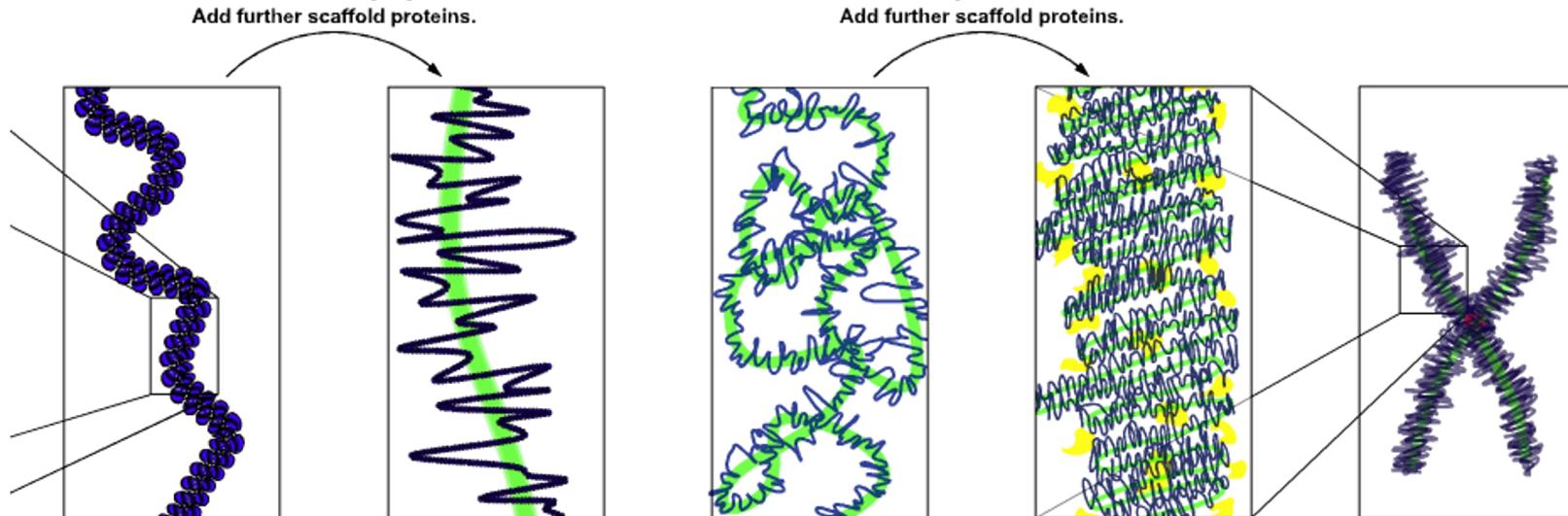
DNA structure refresher

- DNA is compacted into chromatin
- DNA is wrapped around histone proteins



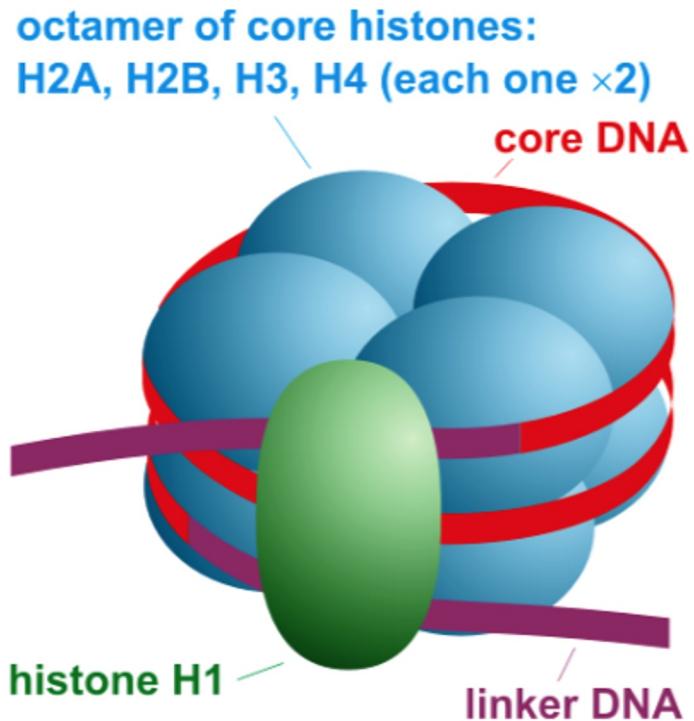
DNA structure refresher

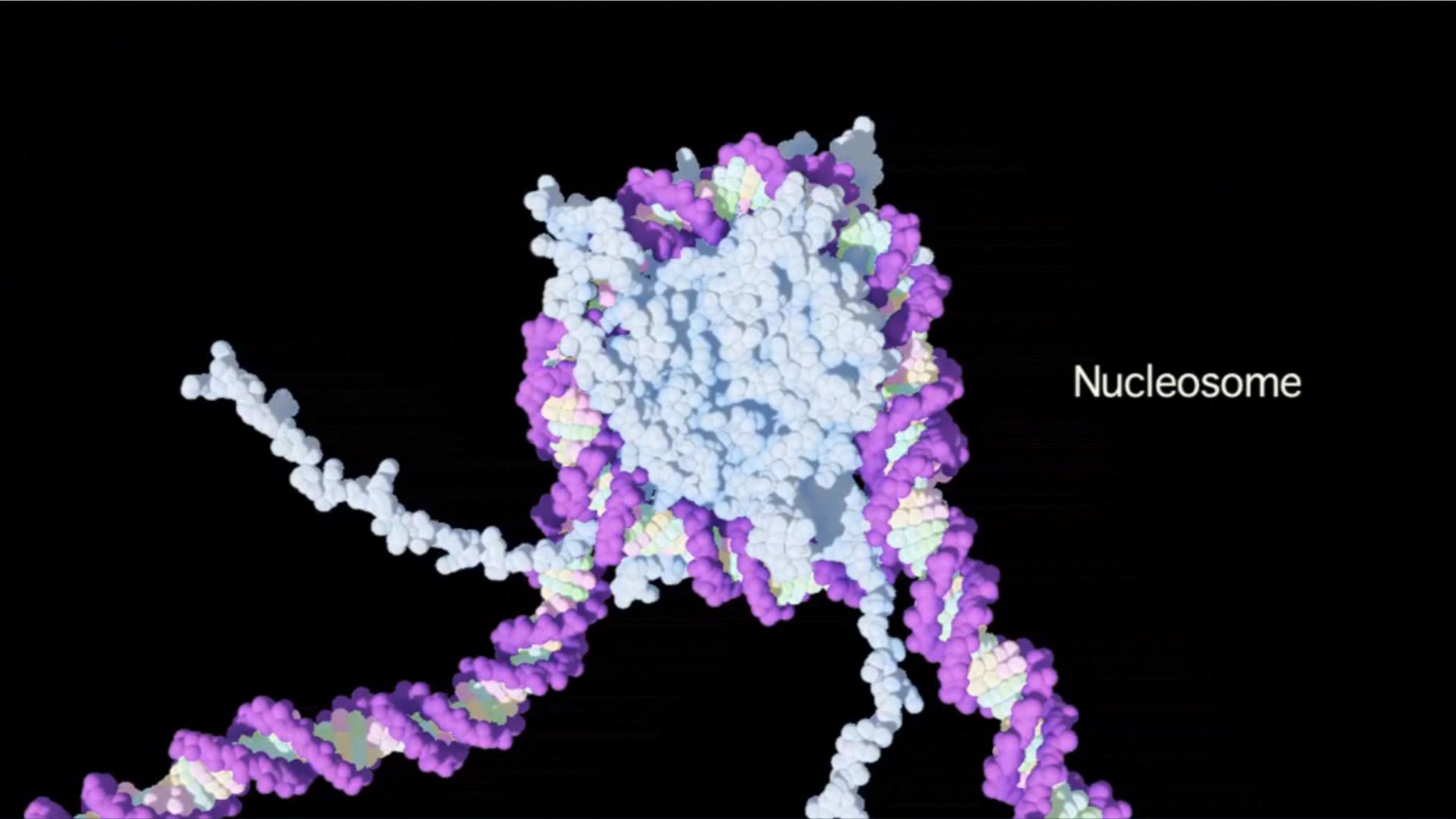
- DNA is compacted into chromatin
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Nucleosome

- 146bp of DNA wrapped around a histone octamer
- Positively charged histones bind to negatively charged DNA



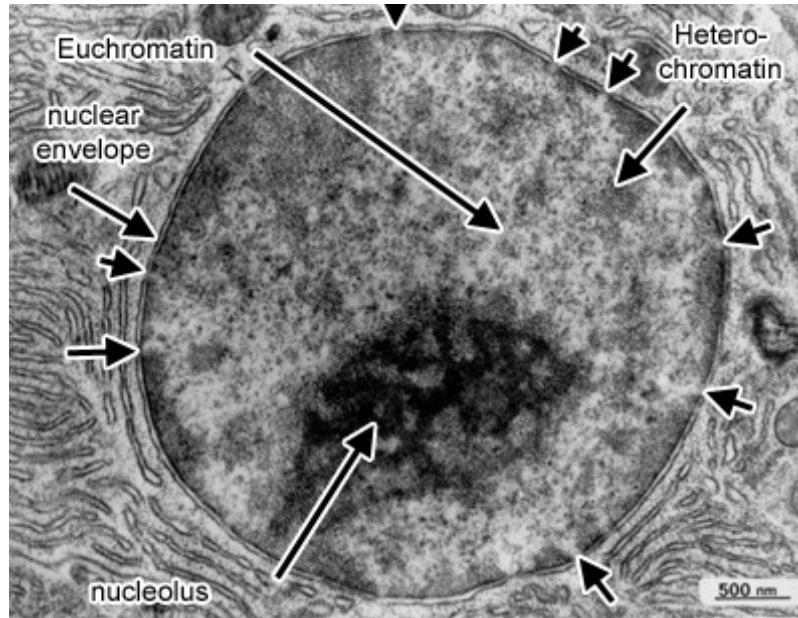
A 3D molecular model of a nucleosome, a complex of DNA and proteins. The nucleosome core is represented by a dense cluster of light blue and purple spheres, forming a roughly spherical shape. A single DNA molecule is wrapped twice around this core. The DNA is shown as a series of connected spheres, primarily colored purple and light blue, with some yellow and green spheres interspersed along its length. The model is set against a solid black background.

Nucleosome

Heterochromatin versus euchromatin

- Heterochromatin - inactive chromatin
 - **Facultative** - can differ by cell type or time
 - tissue specific genes
 - parts of X chromosome
 - **Constitutive** - same in all cell types - structural role
 - Centromeres
 - Telomeres
 - Parts of sex chromosomes
- Euchromatin - active chromatin
 - DNA accessible for transcription

Heterochromatin vs. Euchromatin



Source: [The histology guide: University of Leeds](#)

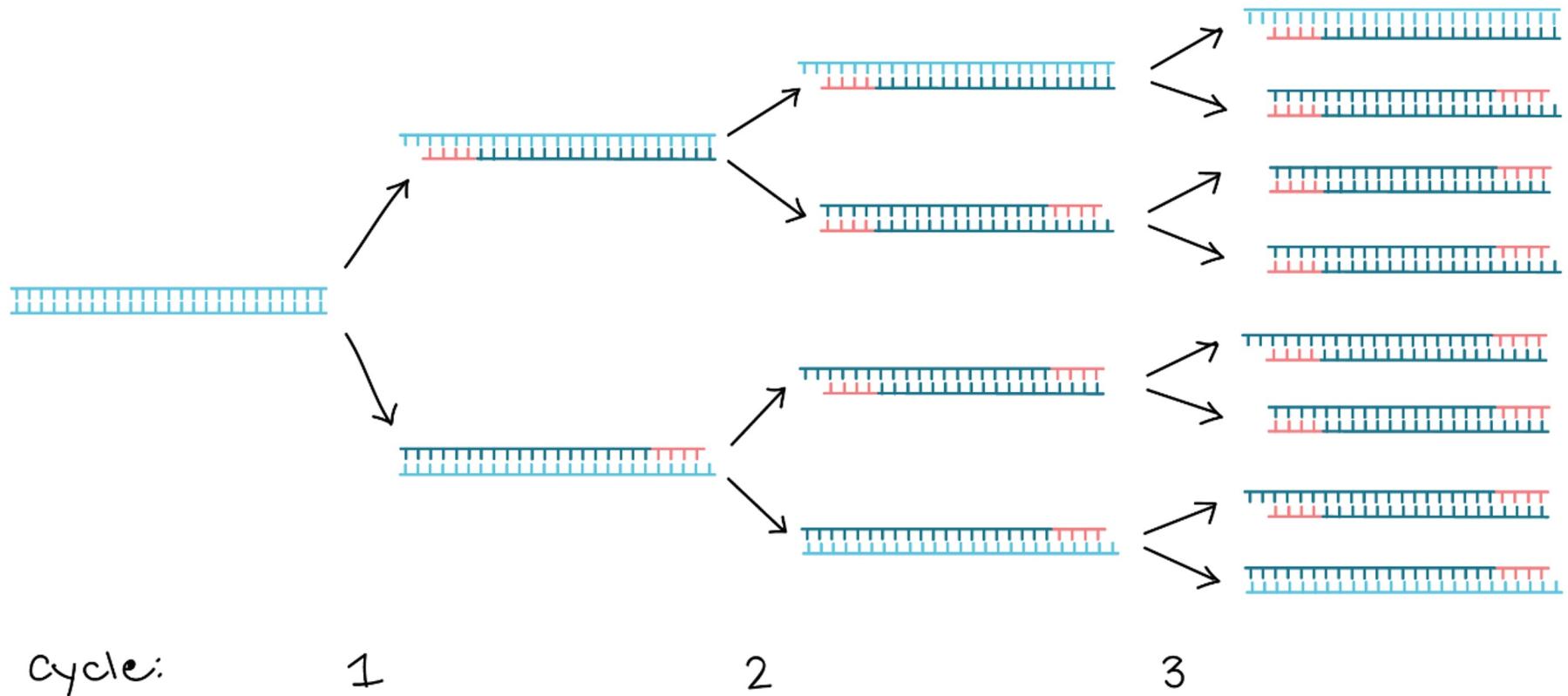
Specific epigenetic modifications

- **DNA methylation**
- **Histone modifications**
- Chromatin remodelling
- Histone variation
- Noncoding RNAs

PCR

Recap





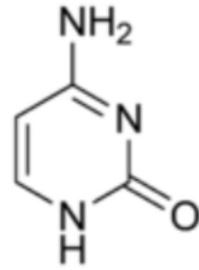
DNA methylation

Changes to DNA amino acids

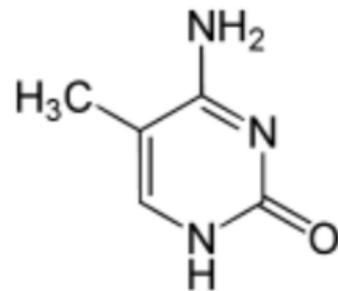


DNA methylation

- Methylation of cytosine nucleotide
- Almost exclusively on CpG dinucleotides



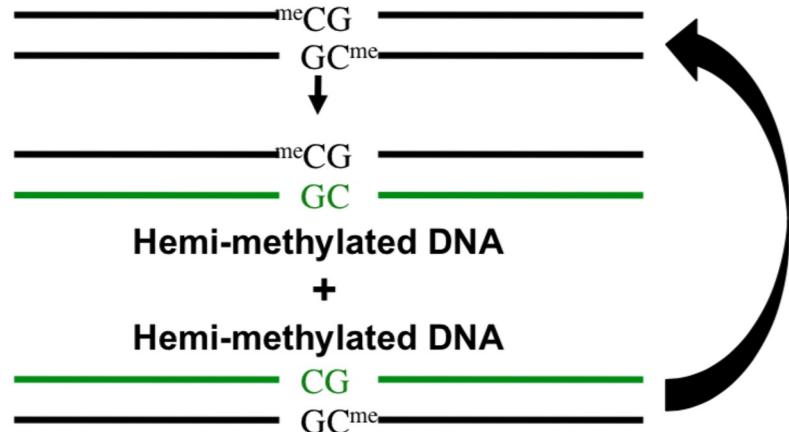
Cytosine



5- methyl cytosine

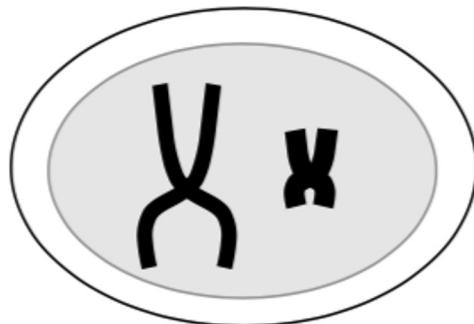
CpG site

- CpG - just a G following a C in sequence
- **CpG islands**
 - Why are they rare?
- CpG islands at gene promoters
 - Methylated → gene expression silenced

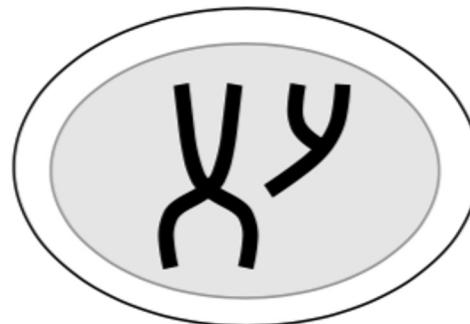


X inactivation

- Epigenetic dosage compensation mechanism in mammals -males and females have the same dose of genes on the X chromosome
- Inactive X chromosome shows DNA methylation of CpG islands



Female XX



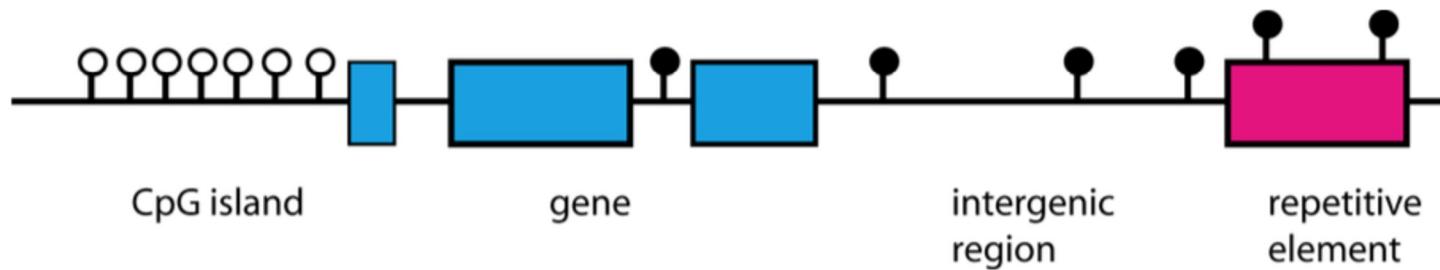
Male XY

X inactivation



DNA methylation - where does it occur?

- CpG islands – usually unmethylated
- Intergenic regions – usually methylated
- Repetitive elements – usually methylated

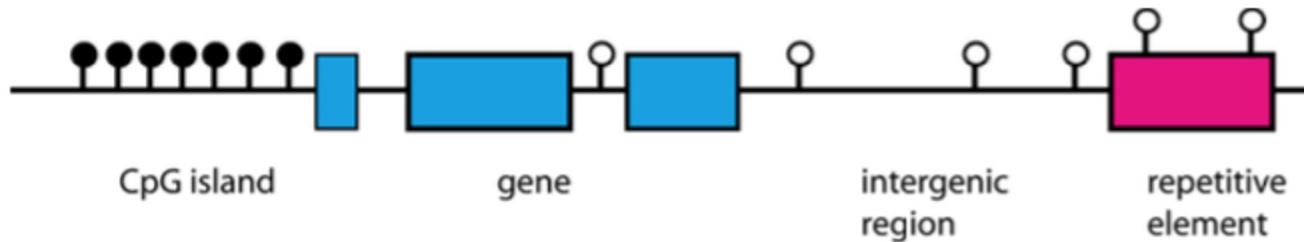


DNA methylation - function

- Intergenic regions - Maintain genomic stability
- Repetitive regions
 - Silence repeats to prevent transposition
 - Mutate transposable elements
- Oncogenes
 - Silence expression

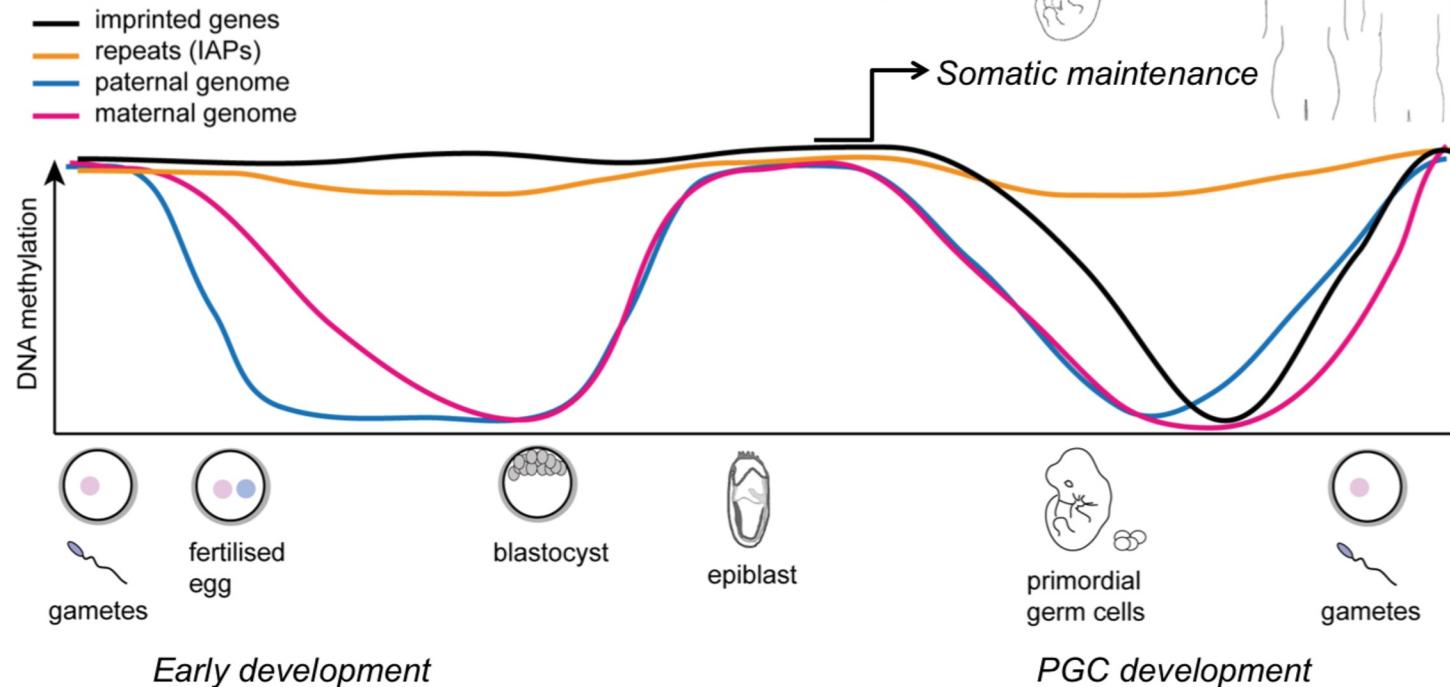
DNA methylation in cancer

- **Hypermethylation** of CpG islands
 - Disables tumor suppressors
- **Hypomethylation** of genome
 - Leads to genomic instability



DNA methylation pattern during development

Epigenetic reprogramming – imprinted genes



Bisulfite sequencing

- Bisulfite modification converts non-methylated cytosines to uracils
- PCR amplification results in replacement of uracils with thymines

Bisulfite sequencing



Bisulfite sequencing - alignment

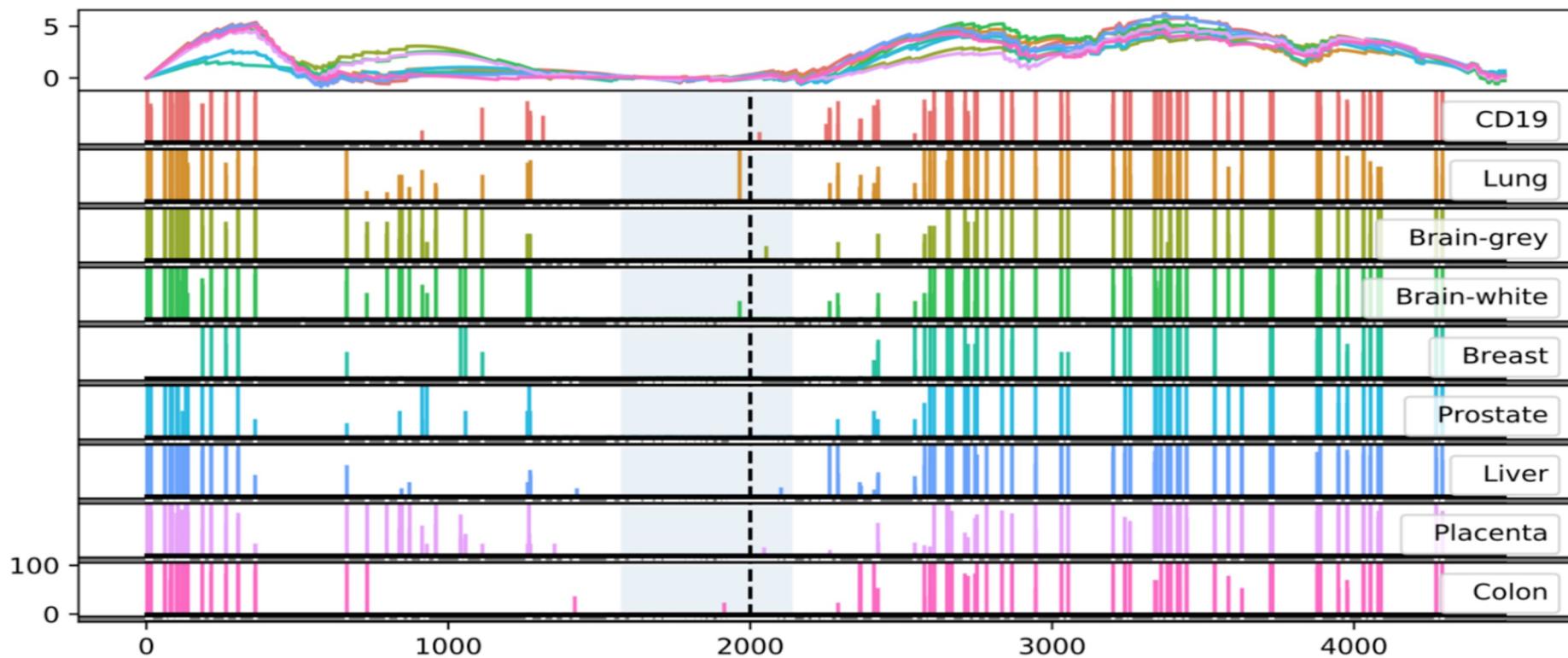
- Similar to regular alignment
- Differences?

Bisulfite sequencing - alignment

- Dependant on library preparation protocol
 - Case when single strand is used
 - Case when both strands are used

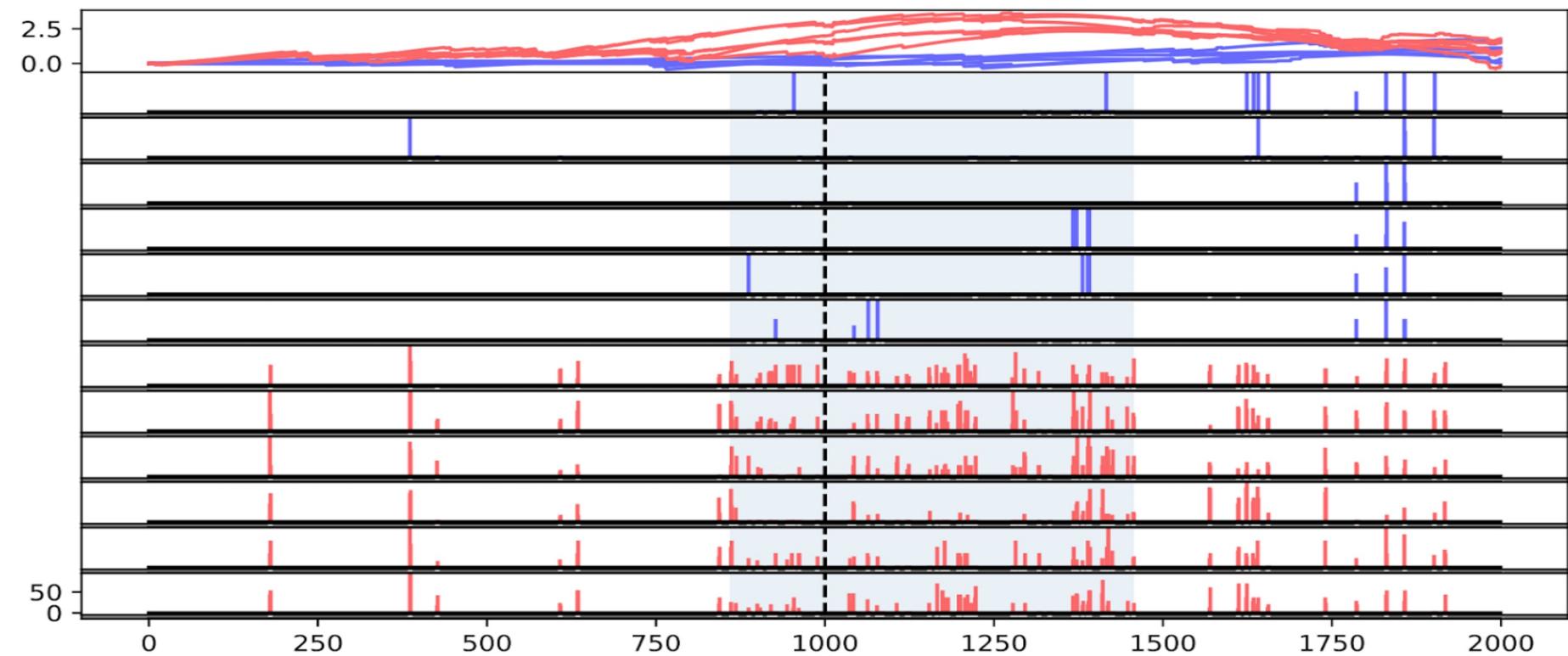
Differential methylation - between cell types

GUSB



Differential methylation - in tumor

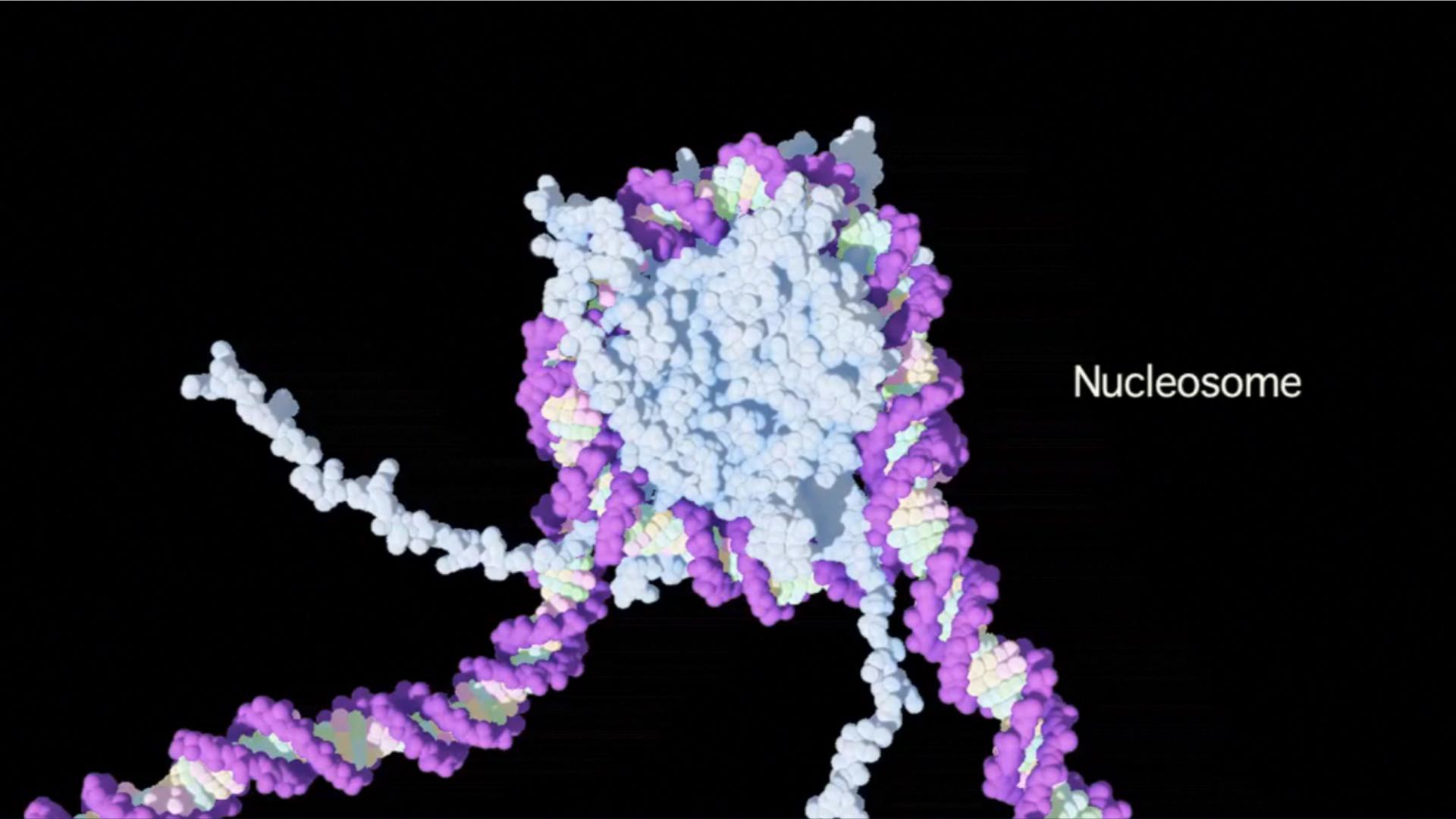
PCDHGB6_1



Histone modification

Modifications to structure holding DNA

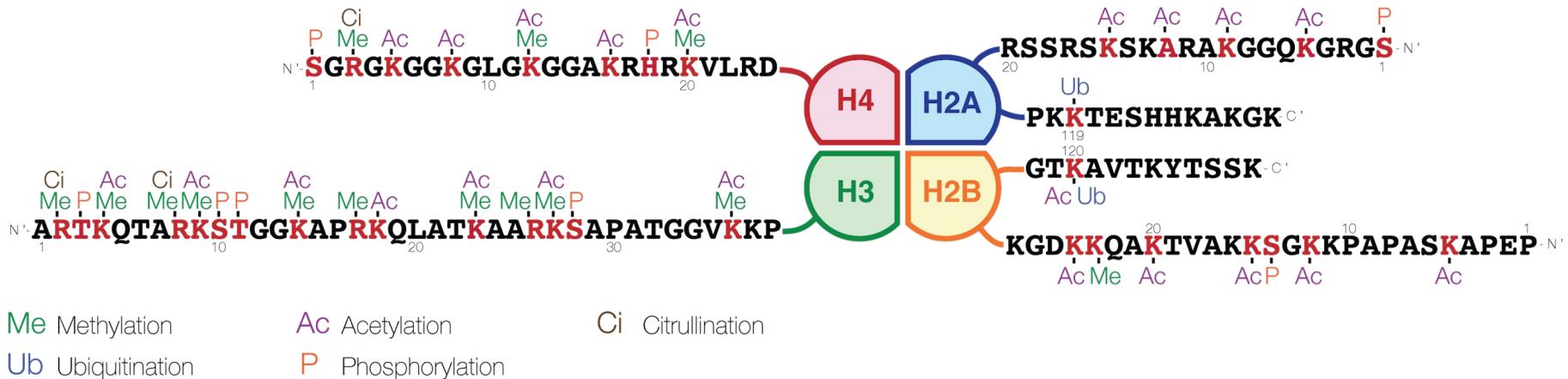


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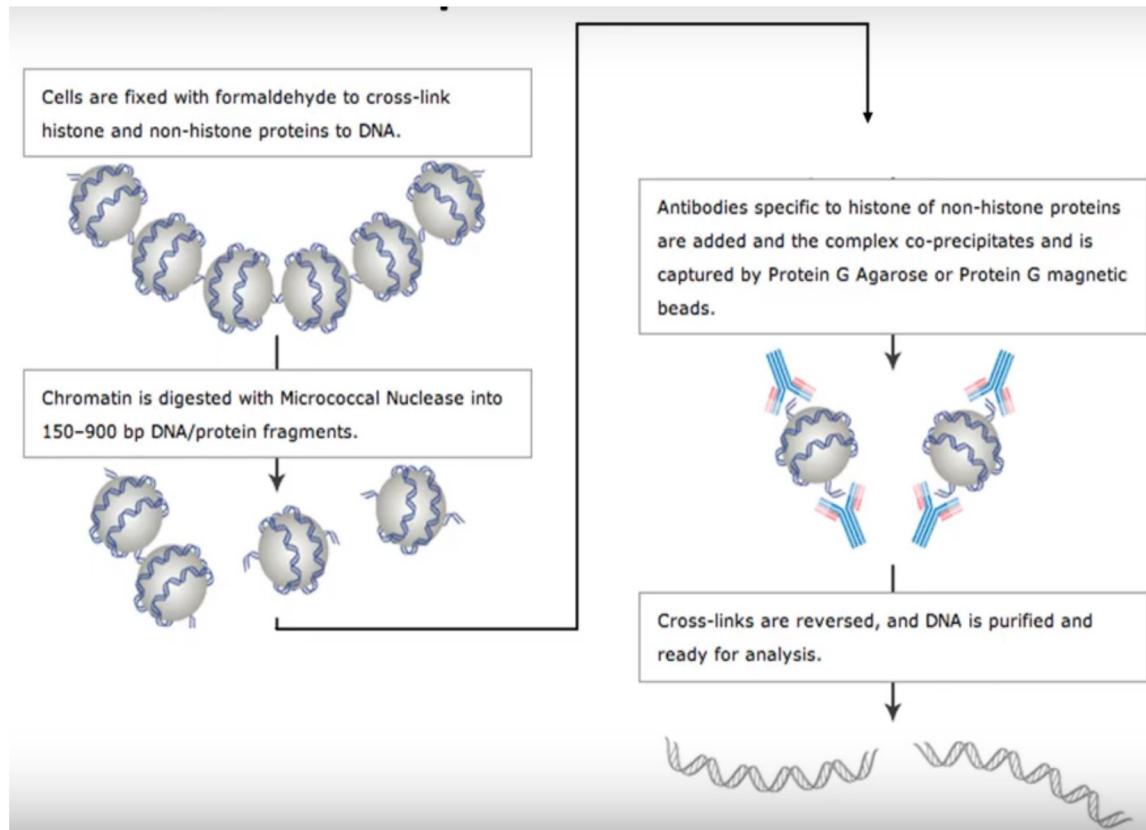
Nucleosome

Histone modifications

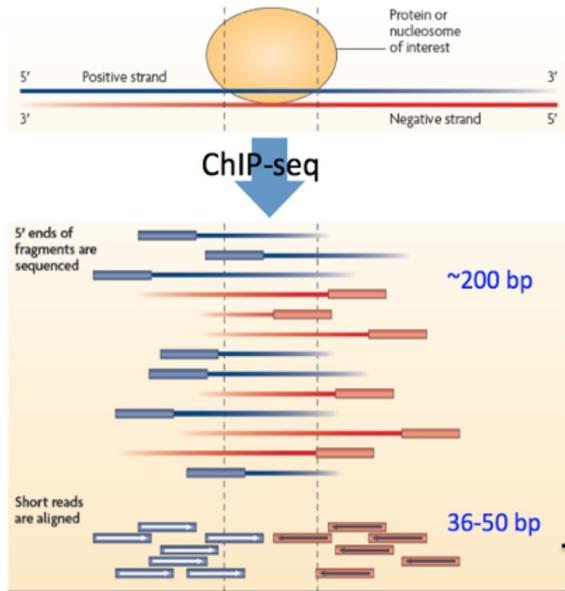
- Chemical modifications of histone tails
- More than 50 different modifications



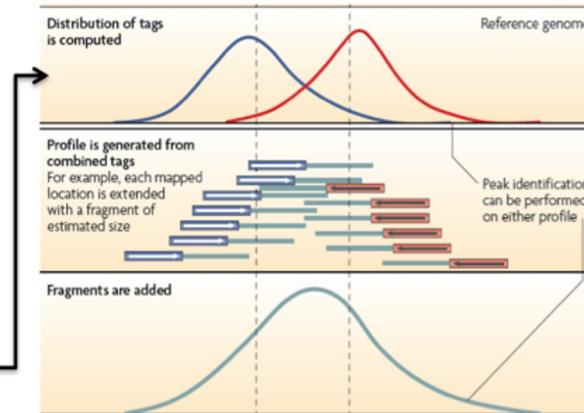
- Chromatin ImmunoPrecipitation



ChIP-seq



- Alignment
- Filtering and QC metrics
- Peak Calling



Other epigenetic modifications

- Chromatin remodeling - dependent on ATP
- Histone variants
- Noncoding RNAs
 - piRNAs
 - siRNAs
 - lncRNAs

Literature

- [Epigenetics review](#)
- [Bisulfite sequencing](#)
- [Bismark - bisulfite-seq aligner](#)
- [Differential methylation](#)
- [Chip-seq](#)

Differential methylation exercise

```
import re
from Bio import SeqIO

reference = SeqIO.parse(open("/sbgenomics/project-files/example_human_reference.fasta"),'fasta')
fasta_1 = SeqIO.parse(open("/sbgenomics/project-files/example_1.fasta"),'fasta')
fasta_2 = SeqIO.parse(open("/sbgenomics/project-files/example_2"),'fasta')
ref_chromosome = list(reference)[0]
f1_chromosome = list(fasta_1)[0]
f2_chromosome = list(fasta_2)[0]

ref_sequence = str(ref_chromosome.seq)
f1_sequence = str(f1_chromosome.seq)
f2_sequence = str(f2_chromosome.seq)
```

SevenBridges

THANK YOU!

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