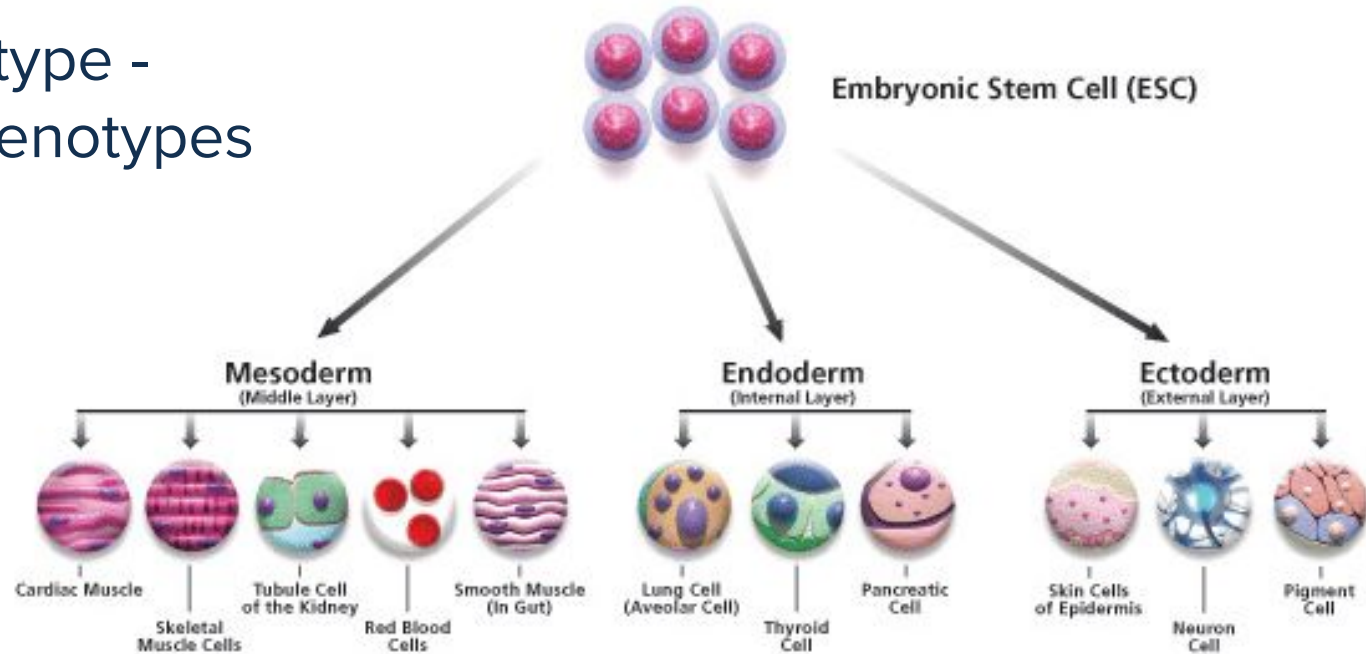# SevenBridges

Epigenetic control

May 2021

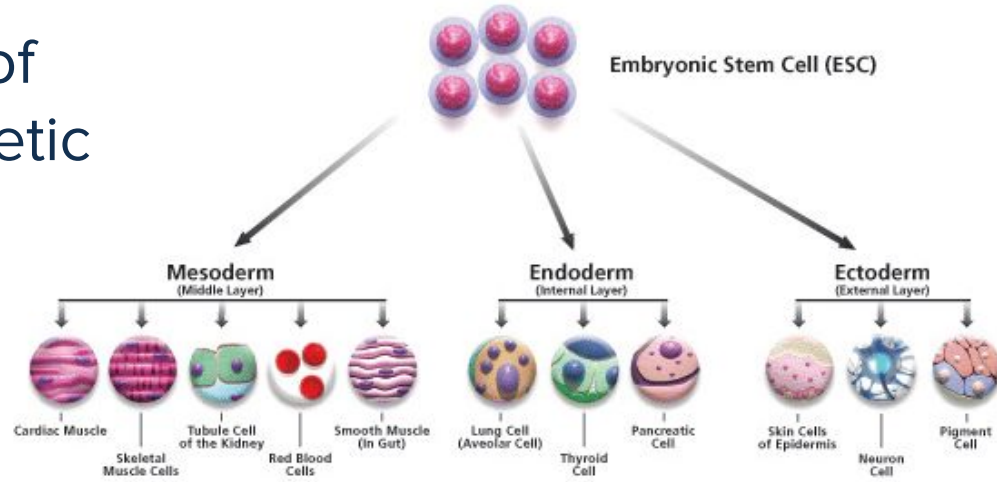Boris Majić, presenter

boris.majic@sbgenomics.com

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

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)
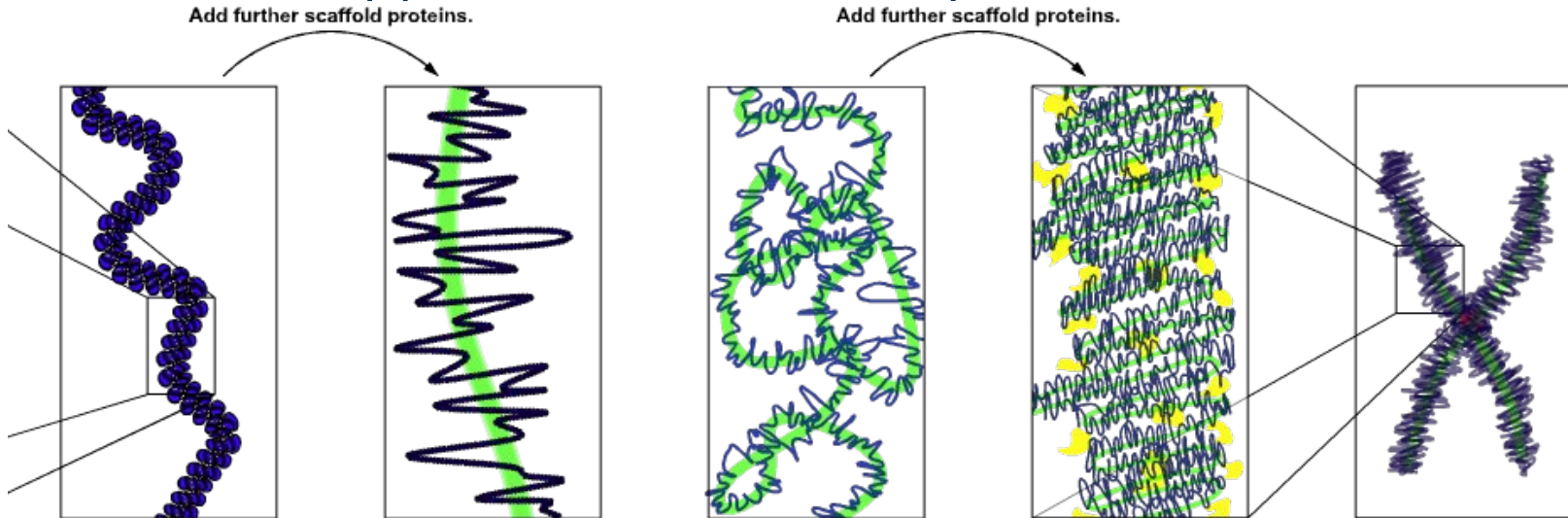 - genes being not expressed (silent)

DNA structure refresher

- DNA is compacted into chromatin

- DNA is wrapped around histone proteins

Add core histones.   Add histone H1.   Add further scat

DNA structure refresher

- DNA is compacted into chromatin

- DNA is wrapped around histone proteins

Add further scaffold proteins.
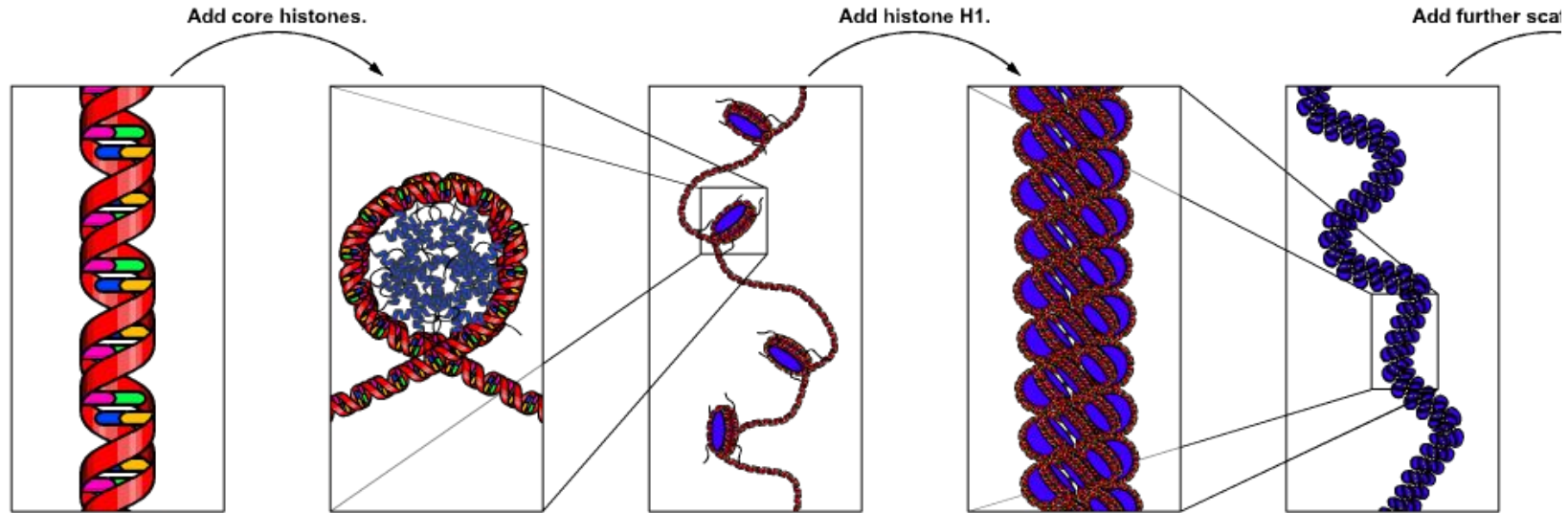
Add further scaffold proteins.

Nucleosome

- 146bp of DNA wrapped around a histone octamer

- Positively charged histones bind to negatively charged DNA

octamer of core histones:
H2A, H2B, H3, H4 (each one ×2)

core DNA

histone H1

linker DNA
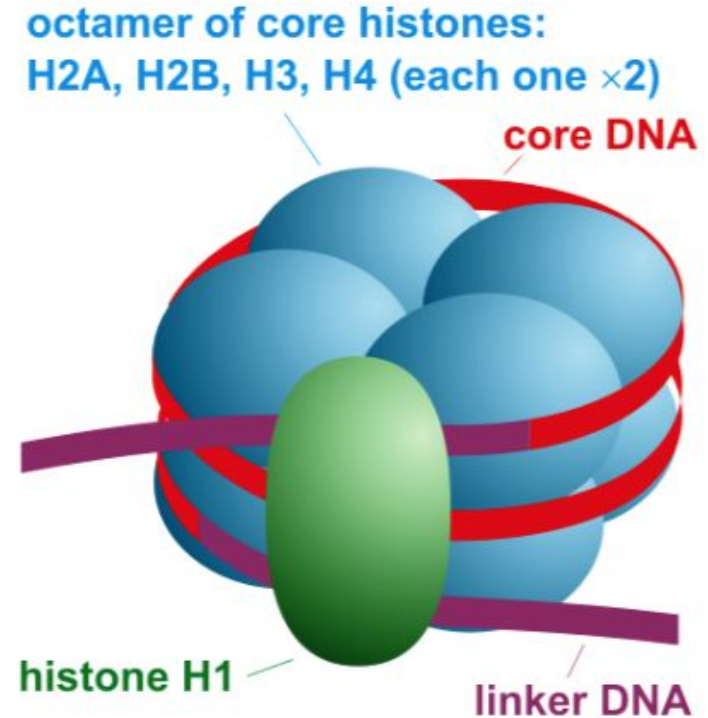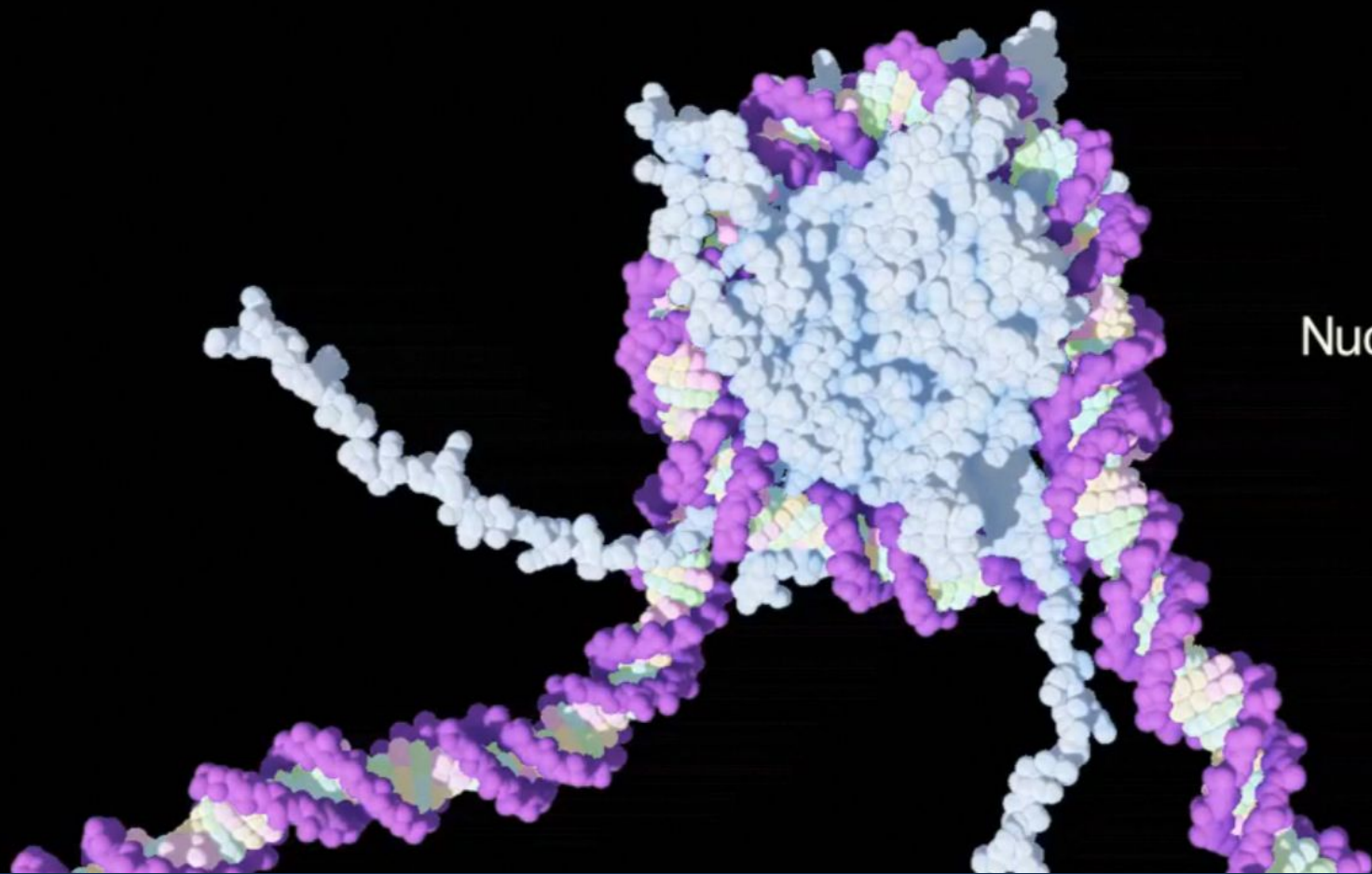
Nucleosome

Heterochromatin versus euchromatin

- Heterochromatin - closed 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 - open chromatin

  - DNA accessible for transcription

Specific epigenetic modifications

- **DNA methylation**

- **Histone modifications**

- Chromatin remodelling
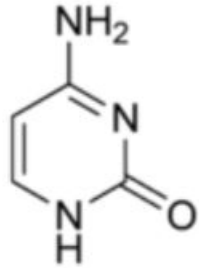
- Histone variation

- Noncoding RNAs

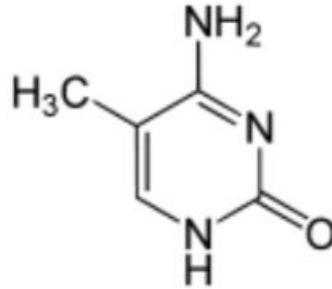# DNA methylation

Changes to DNA amino acids

DNA methylation

- Methylation of cytosine nucleotide

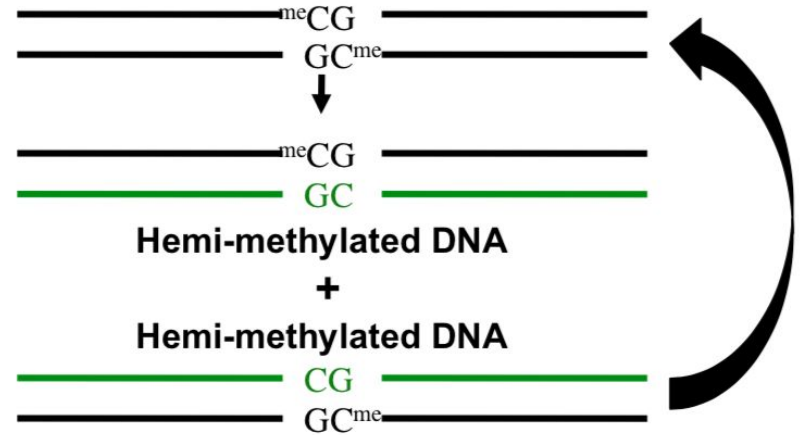- Almost exclusively on CpG dinucleotides

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
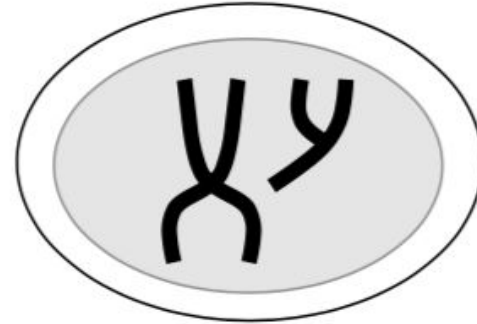   - Methylated ➡ gene expression silenced

X inactivation

- Epigenetic dosage compensation mechanism in mammals -males and females have the same dose of genes on the X chromosome

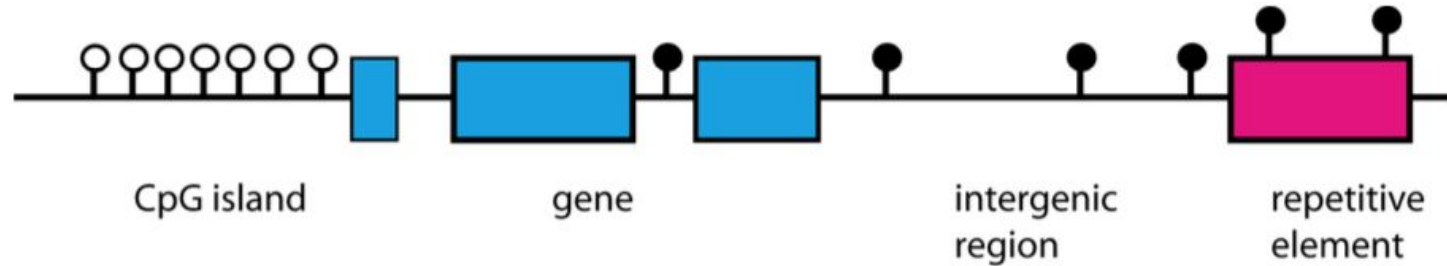- Inactive X chromosome shows DNA methylation of CpG islands
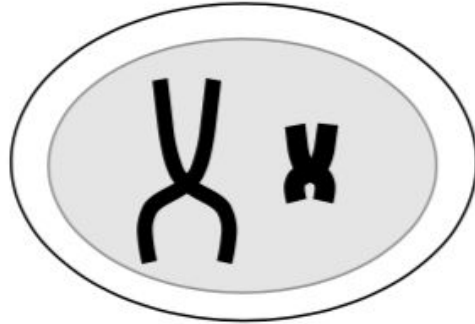
Female XX

Male XY

X inactivation

DNA methylation - where does it occur?

- CpG islands – usually unmethylated

- Intergenic regions – usually methylated

- Repetitive elements – usually methylated

CpG island   gene   intergenic region   repetitive element
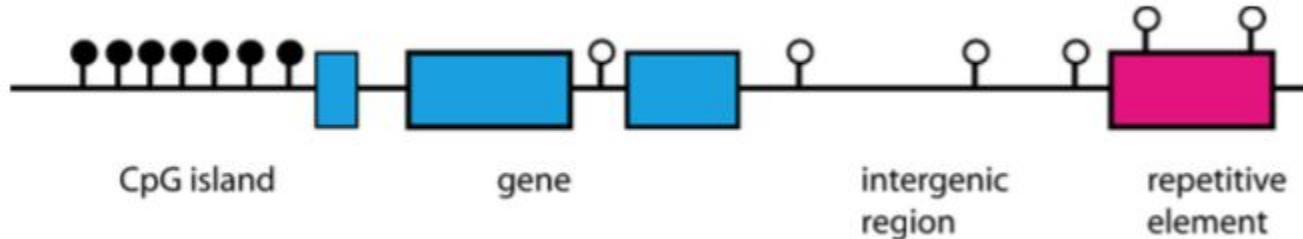
DNA methylation - function

- Intergenic regions - Maintain genomic stability

- Repetitive regions

  - Silence repeats to prevent transposition

  - Mutate transposable elements
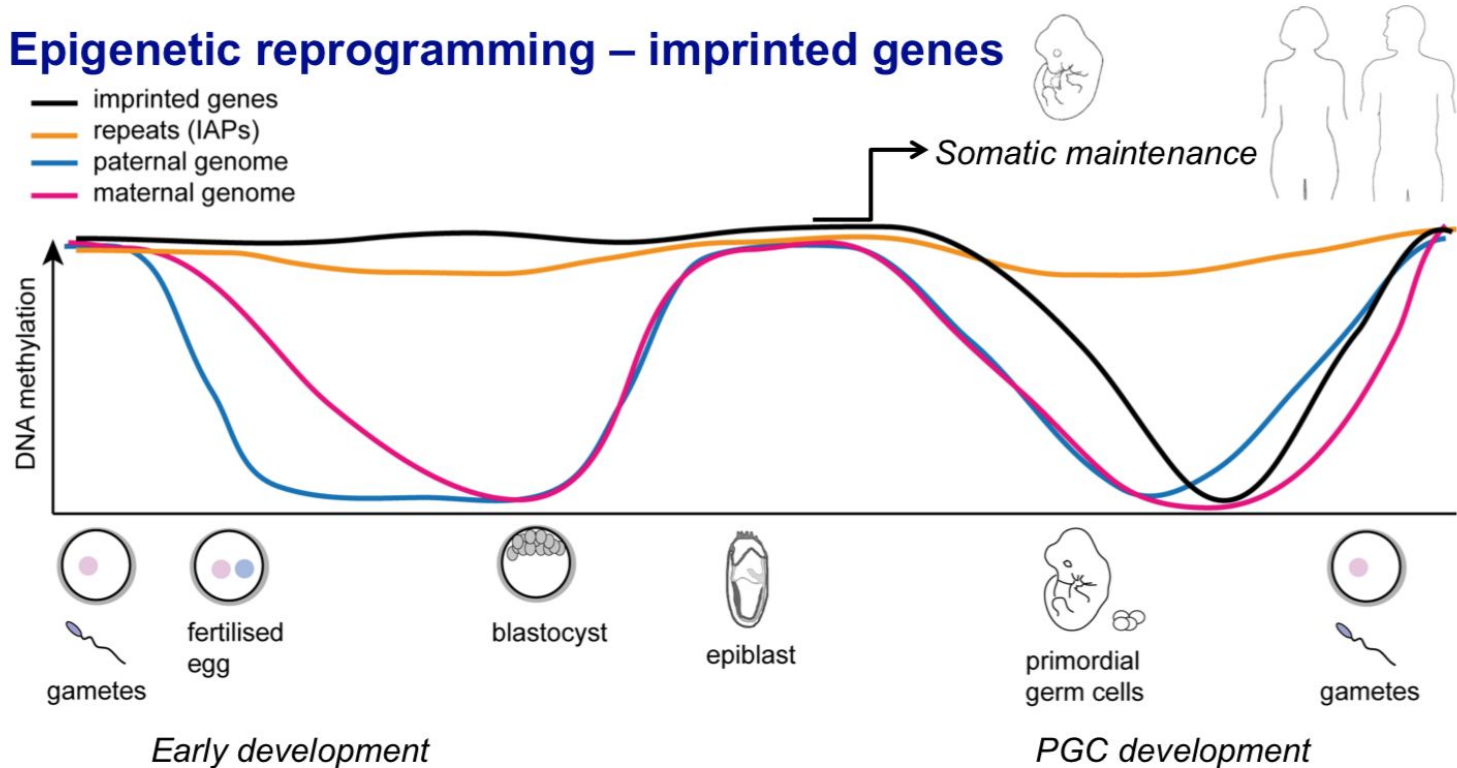
- Oncogenes

  - Silence expression

DNA methylation in cancer

- **Hypermethylation** of CpG islands
  - Disables tumor suppressors

- **Hypomethylation** of genome
  - Leads to genomic instability

CpG island   gene   intergenic region   repetitive element
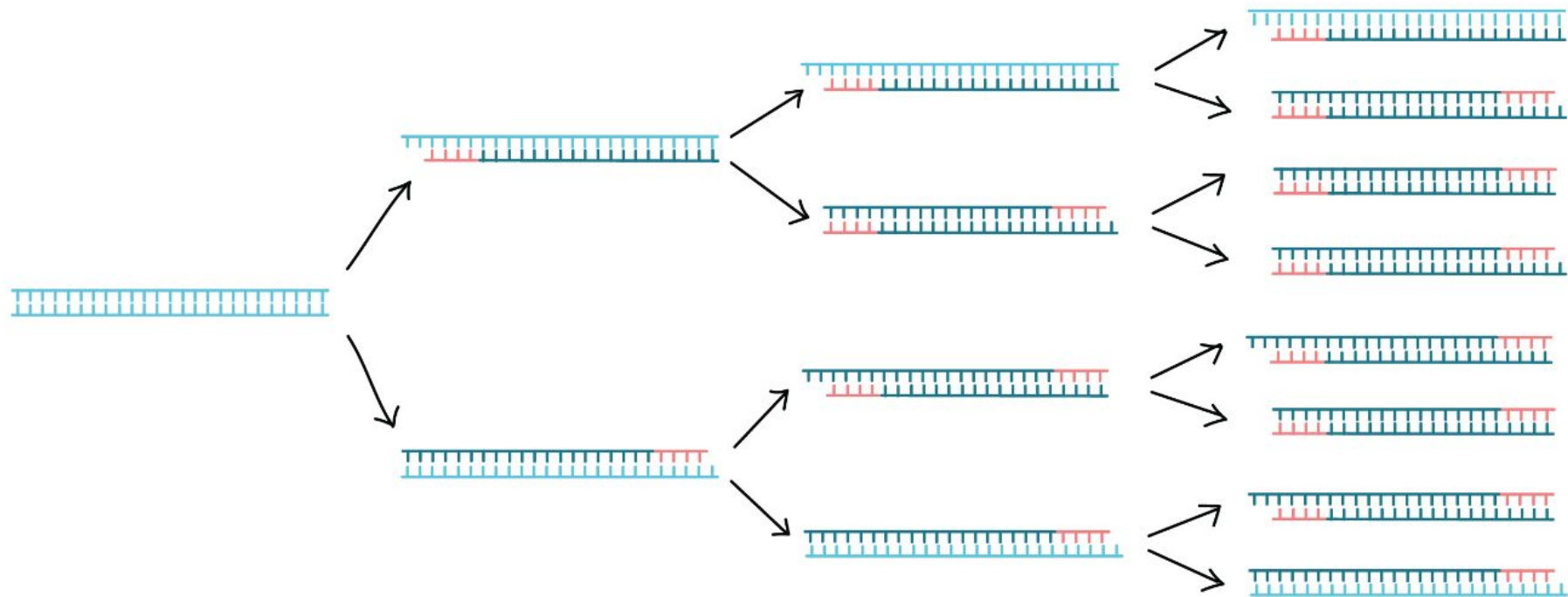
DNA methylation through aging

Bisulfite sequencing

- Bisulfite modification converts non-methylated cytosines to uracils

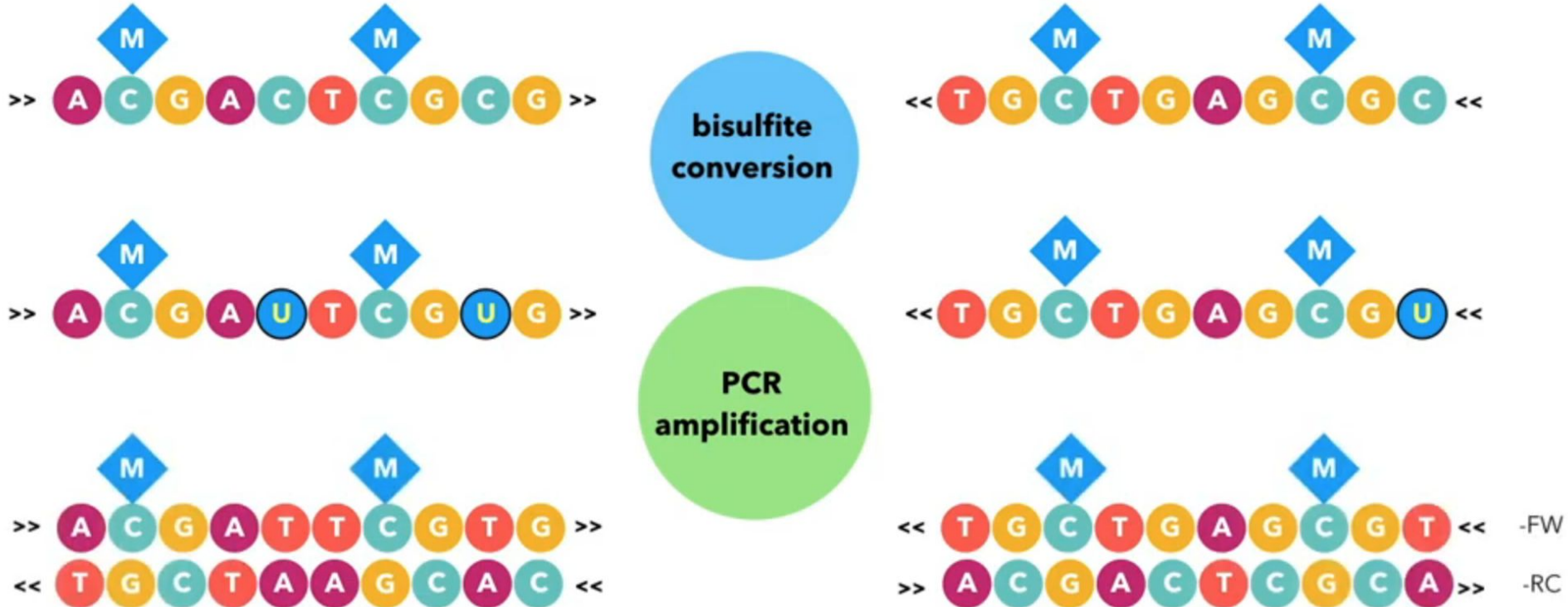- PCR amplification results in replacement of uracils with thymines

PCR

Recap

Cycle: 1   2   3

Bisulfite sequencing

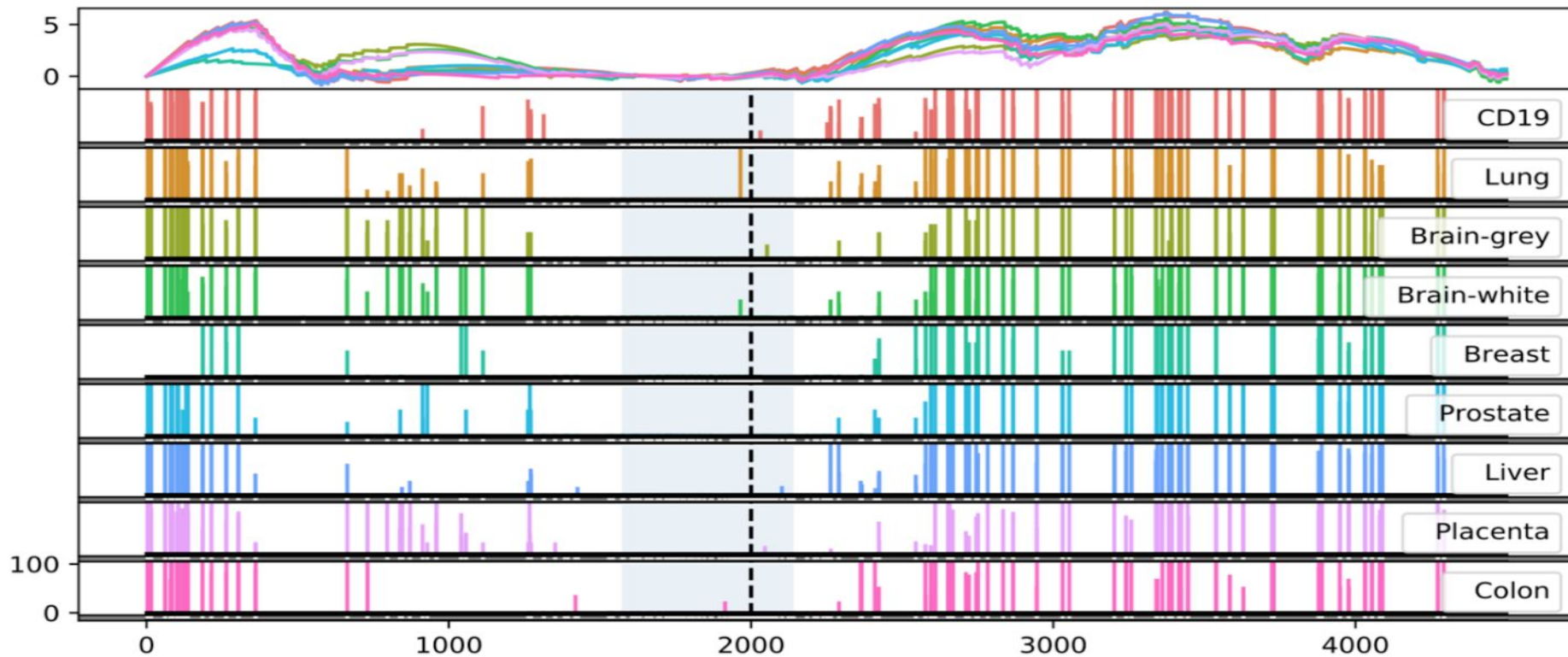# Bisulfite sequencing - alignment

- 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
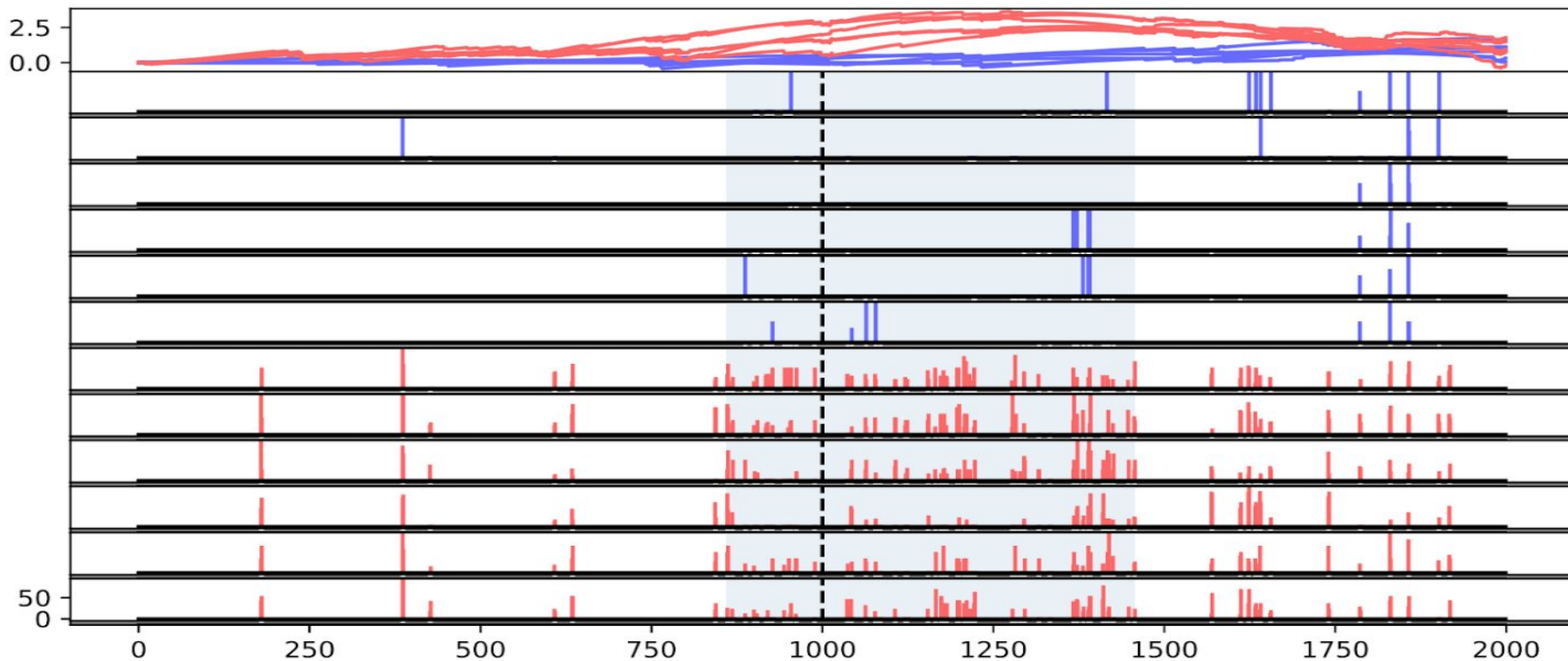
Differential methylation - between cell types

GUSB

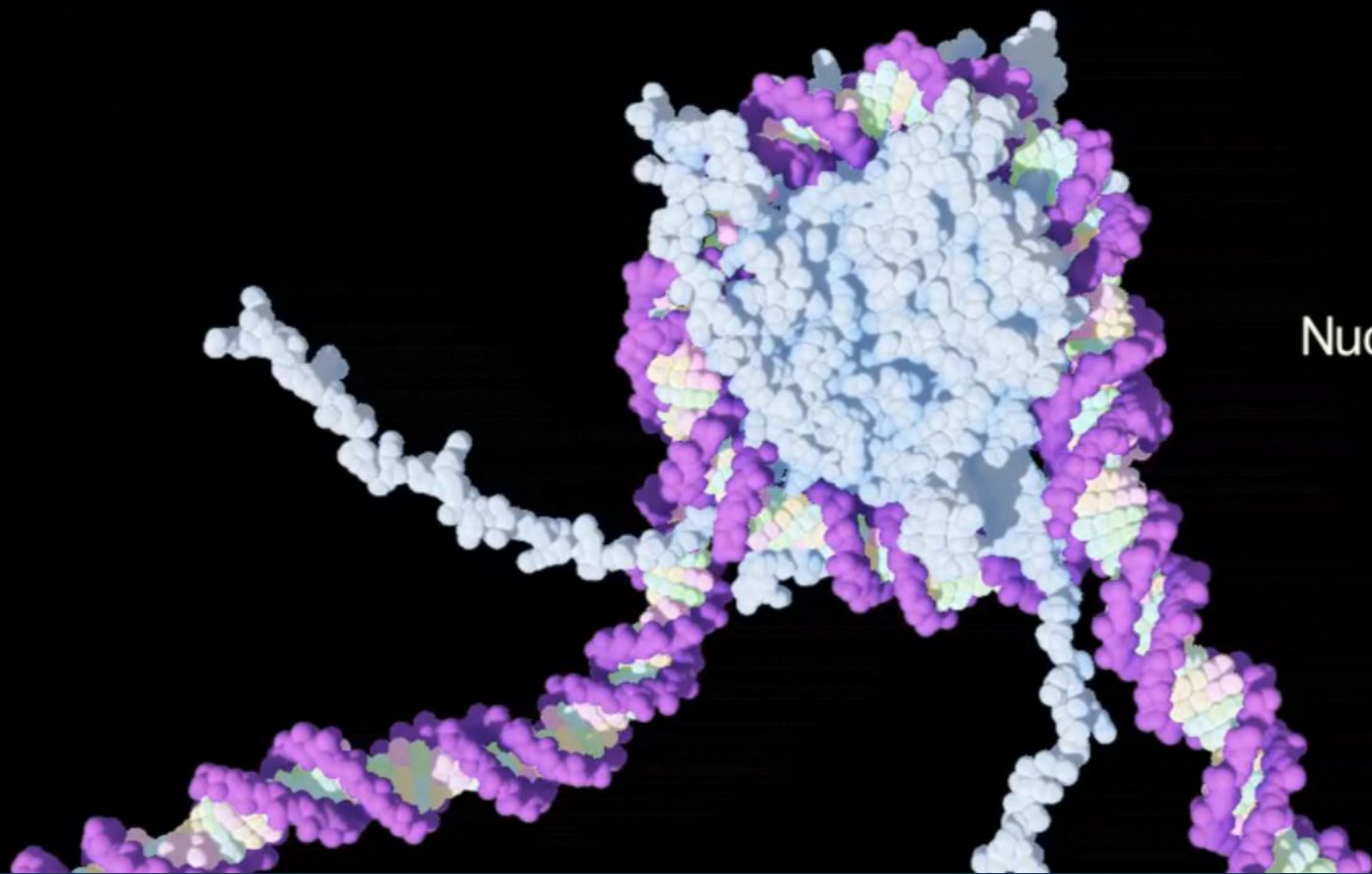# Differential methylation - in tumor

PCDHGB6_1

Histone modification

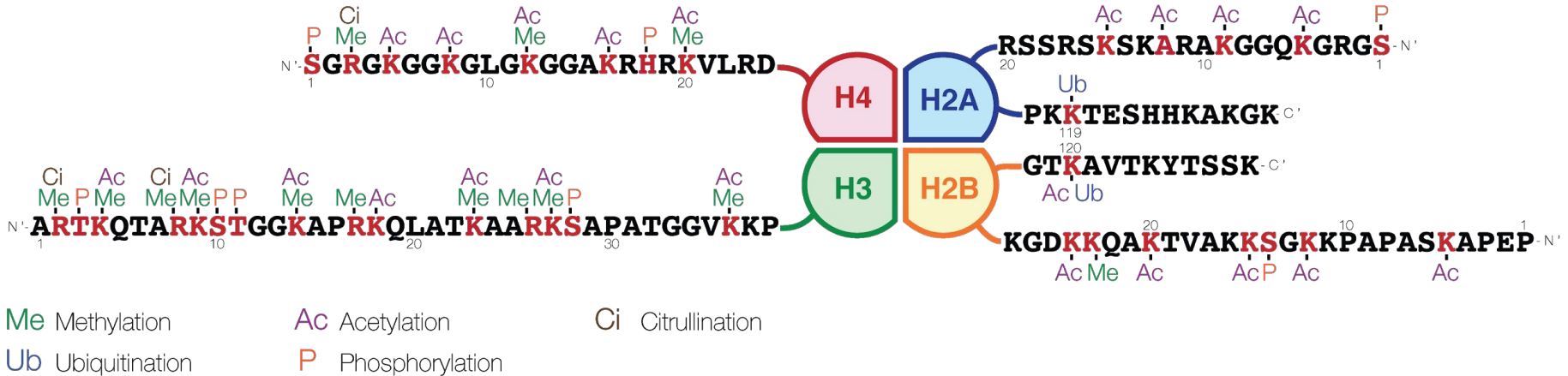Modifications to structure holding DNA

Nucleosome

Histone modifications

- Chemical modifications of histone tails

- More than 50 different modifications

Me Methylation Ac Acetylation Ci Citrullination
Ub Ubiquitination P Phosphorylation

ChIP-seq

- Chromatin ImmunoPrecipitation

Cells are fixed with formaldehyde to cross-link histone and non-histone proteins to DNA.

Chromatin is digested with Micrococcal Nuclease into 150–900 bp DNA/protein fragments.

Antibodies specific to histone of non-histone proteins are added and the complex co-precipitates and is captured by Protein G Agarose or Protein G magnetic beads.

Cross-links are reversed, and DNA is purified and ready for analysis.

ChIP-seq

sevenbridges.com

Other epigenetic modifications

- Chromatin remodeling - dependent on ATP

- Histone variants

- Noncoding RNAs
  - piRNAs
  - siRNAs
  - lncRNAs

- RNA modifications

Literature

- [Epigenetics review](#)

- [Bisulfite sequencing](#)

- [Bismark - bisulfite-seq aligner](#)

- [Differential methylation](#)

- [Chip-seq](#)

Differential methylation exercise

```python
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)
```