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# CS681: Advanced Topics in Computational Biology

Week 9 Lecture 1

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Can Alkan

EA224

[calkan@cs.bilkent.edu.tr](mailto:calkan@cs.bilkent.edu.tr)

<http://www.cs.bilkent.edu.tr/~calkan/teaching/cs681/>

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# EPIGENETICS

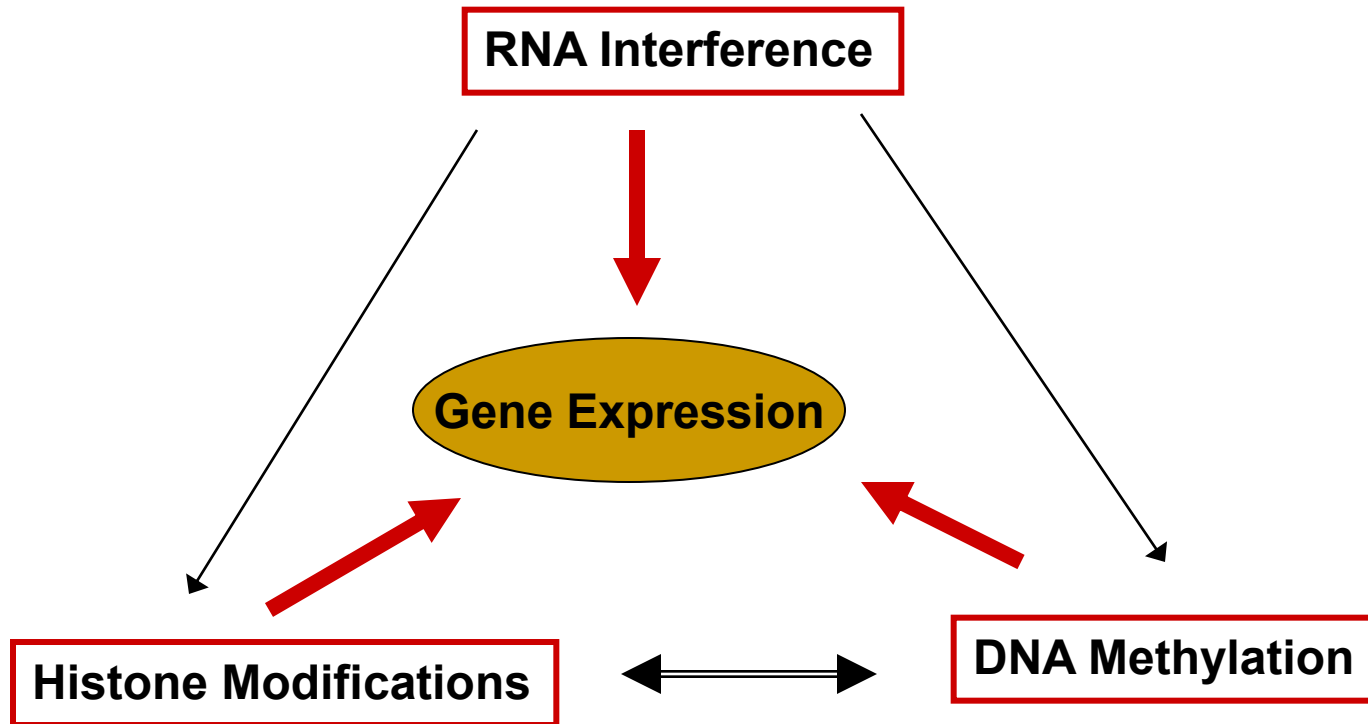
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# Definition

- Epigenetics: study of all meiotically and mitotically heritable changes in gene expression that are not coded in the DNA sequence itself
  - DNA methylation
  - RNA associated silencing
  - Histone modification

# Epigenetics Mechanisms



# Importance

- Development of multicellular organism
- Environment-organism interaction

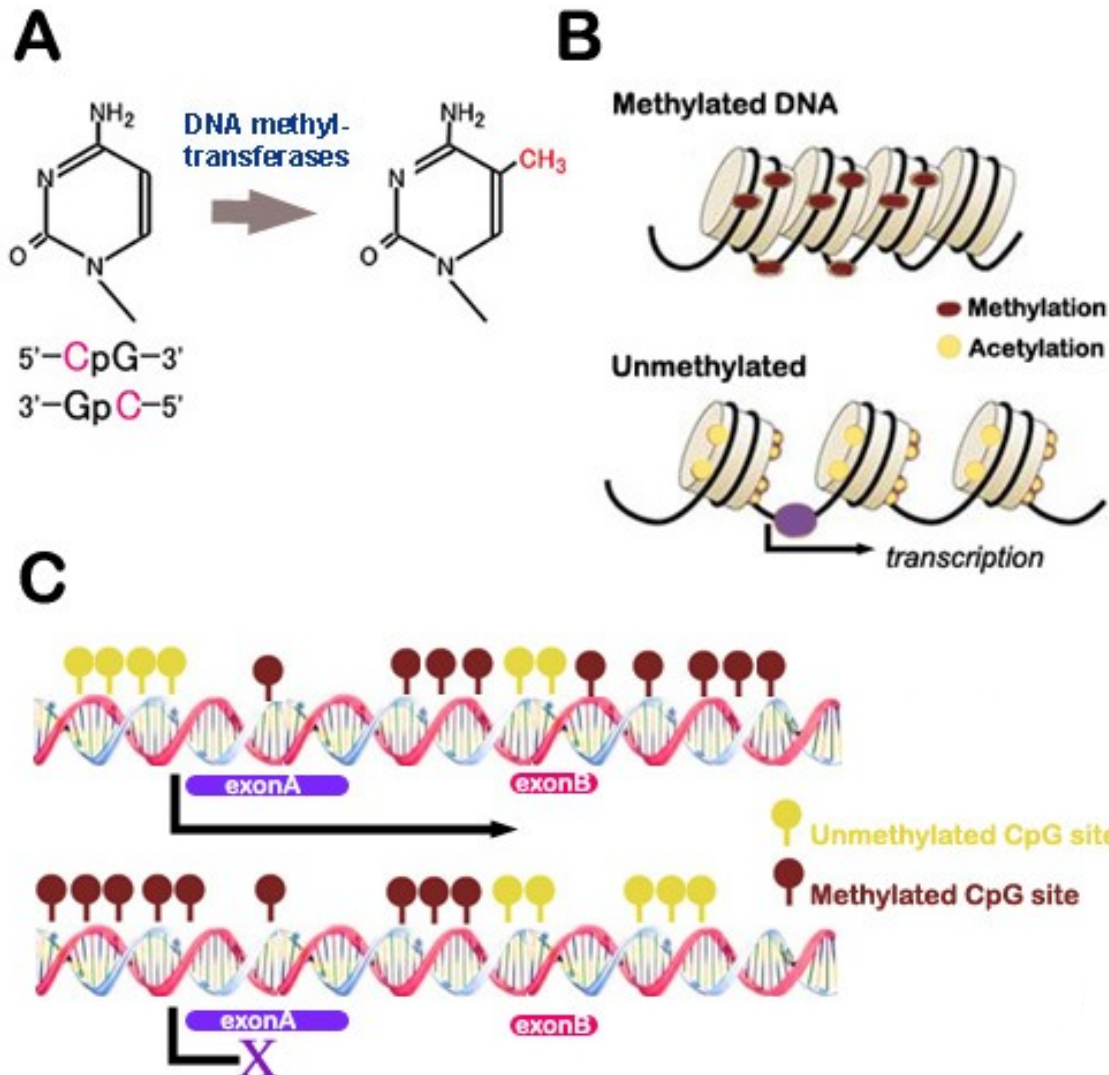
For example: Nutrition supplements and environmental toxins



Image: Randy Jirtle

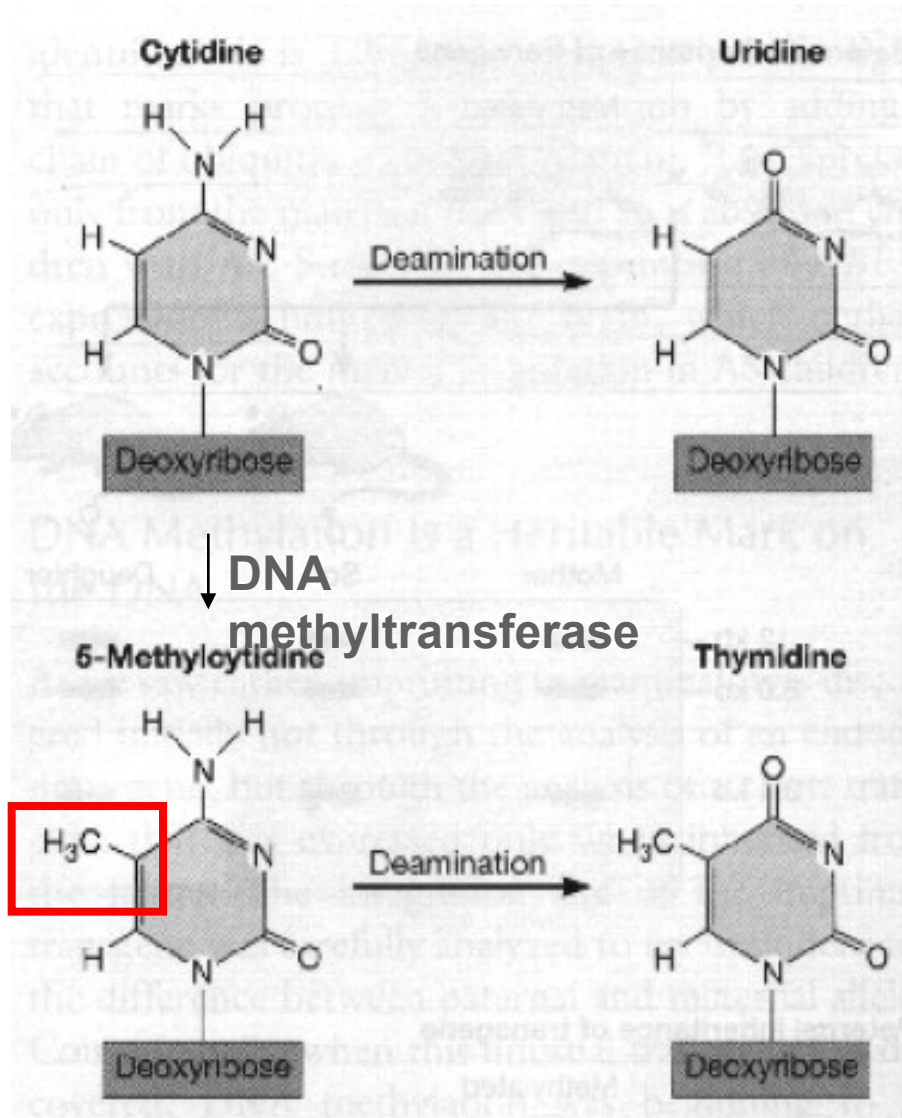
- Pathogenesis of diseases

# DNA Methylation



Hypomethylation  
Hypermethylation

# DNA Methylation



- At promoter, DNA methylation suppresses transcription
- With deamination, DNA methylation induces C → T mutation

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# DNA Methylation

- DNA methylation is the addition of methyl group to cytosine generally in CpG dinucleotides
  - 26.7 million CpG sites in the autosomal genome
  - CpG rich regions known CpG Islands (CGIs) are generally located near to the start of genes and associate with promoters
  - Previously thought to be the key site of epigenetic regulation of gene expression, and have been the main focus of epigenetic research
  - Recently (2009) methylation changes in regions outside CGIs known as CGI shores have been shown to be more significantly associated with gene regulation
  - Shores are defined as the regions 2kb up and downstream of the CGI
  - Only 7% of CpGs reside within CGIs, the majority of CpG sites reside within repeat regions, many of which are un-assayable by microarray technologies
  - New technologies such as next generation sequencing combined with techniques such as MeDIP will allow for the 1<sup>st</sup> time to assay changes in whole genome methylation (methylome)
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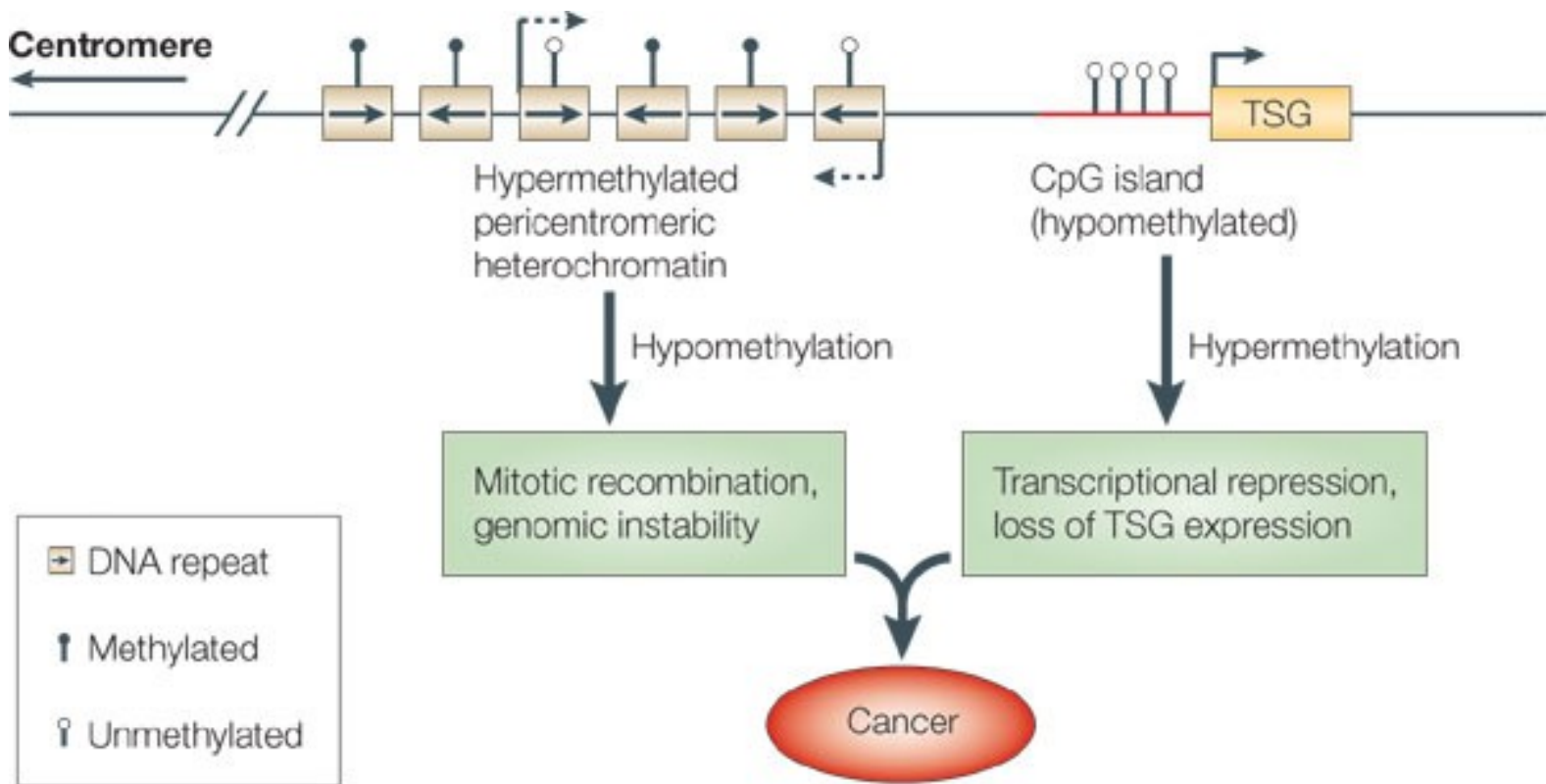


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# Roles of DNA Methylation in Mammals

- Imprinting
    - Expression of genes in a parent-of-origin manner
  - X chromosome inactivation
    - In females, one of the X chromosomes is inactive
  - Heterochromatin maintenance
  - Developmental controls
  - Tissue specific expression controls
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# DNA Methylation and Cancer



Nature Reviews | **Genetics**

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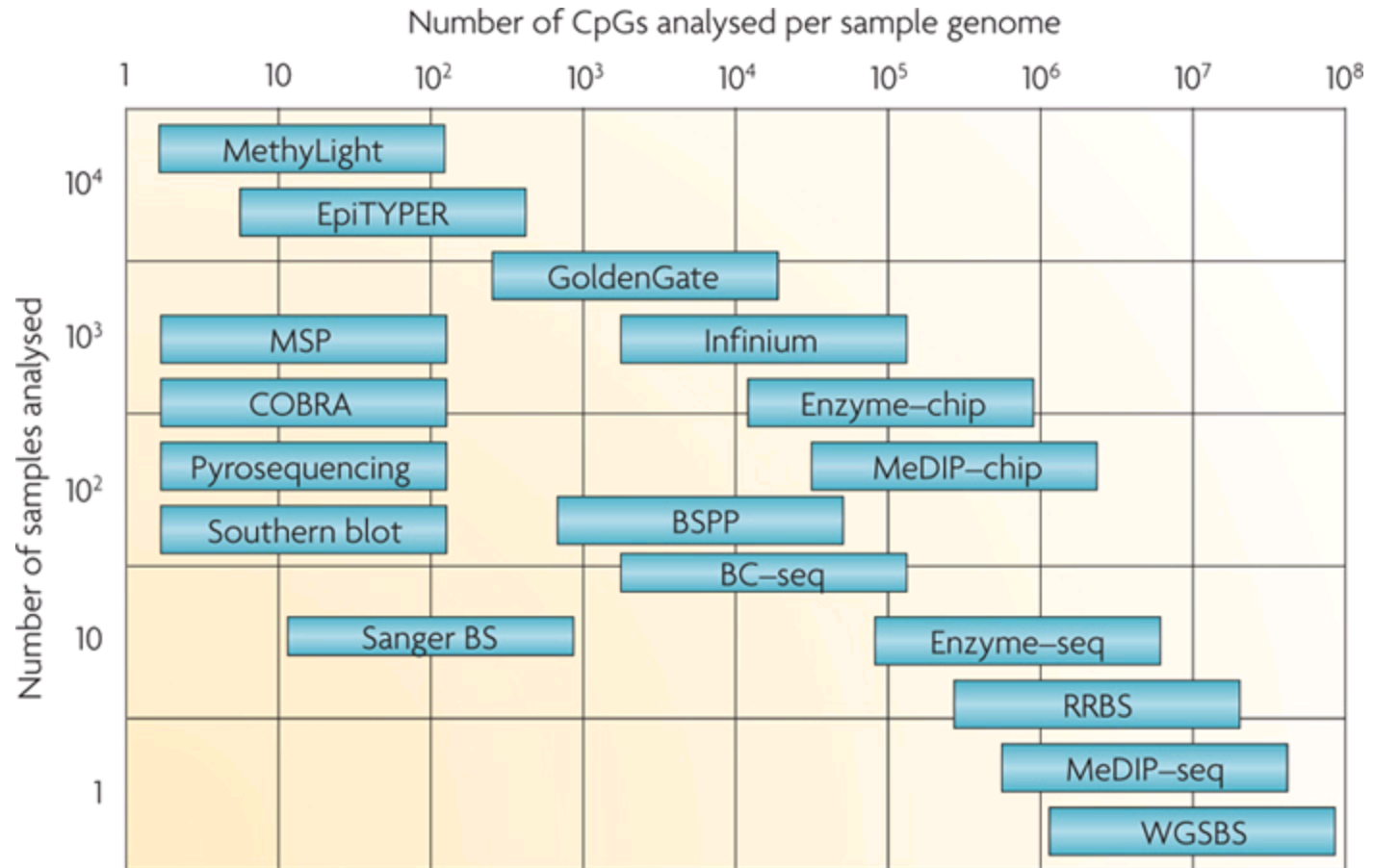
# Other Human Diseases

- Imprinting Disorders:
    - Beckwith-Wiedemann syndrome (BWS)
    - Prader-Willi syndrome (PWS)
    - Transient neonatal diabetes mellitus (TNDM)
  - Repeat-instability diseases
    - Fragile X syndrome (FRAXA)
    - Facioscapulohumeral muscular dystrophy
  - Defects of the methylation machinery
    - Systemic lupus erythematosus (SLE)
    - Immunodeficiency, centromeric instability and facial anomalies (ICF) syndrome
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# Technologies for Studying Methylation

- PCR-based locus specific analysis
  - Fragmentation of DNA with methylation sensitive enzymes
  - Follow with sequencing: Methyl-seq
- Immunoprecipitation
  - MeDIP: Methylated DNA immunoprecipitation
    - Enrich methylated regions using specific antibodies
    - Array: MeDIP-chip; sequencing: MeDIP-Seq
- Bisulfite treatment
  - BS-seq

# Technologies for Studying Methylation



Nature Reviews | **Genetics**

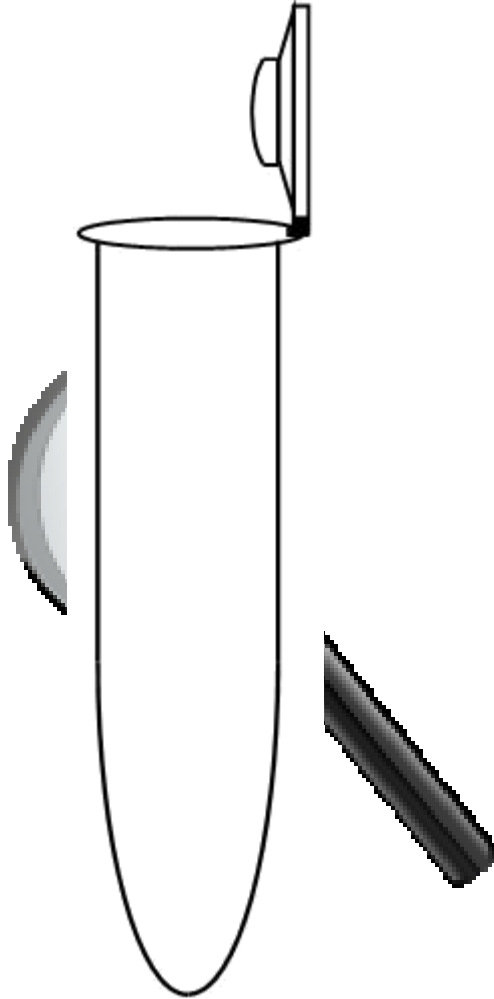
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Animation by Gareth Wilson

**MEDIP**

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## The *MeDIP* reaction begins...

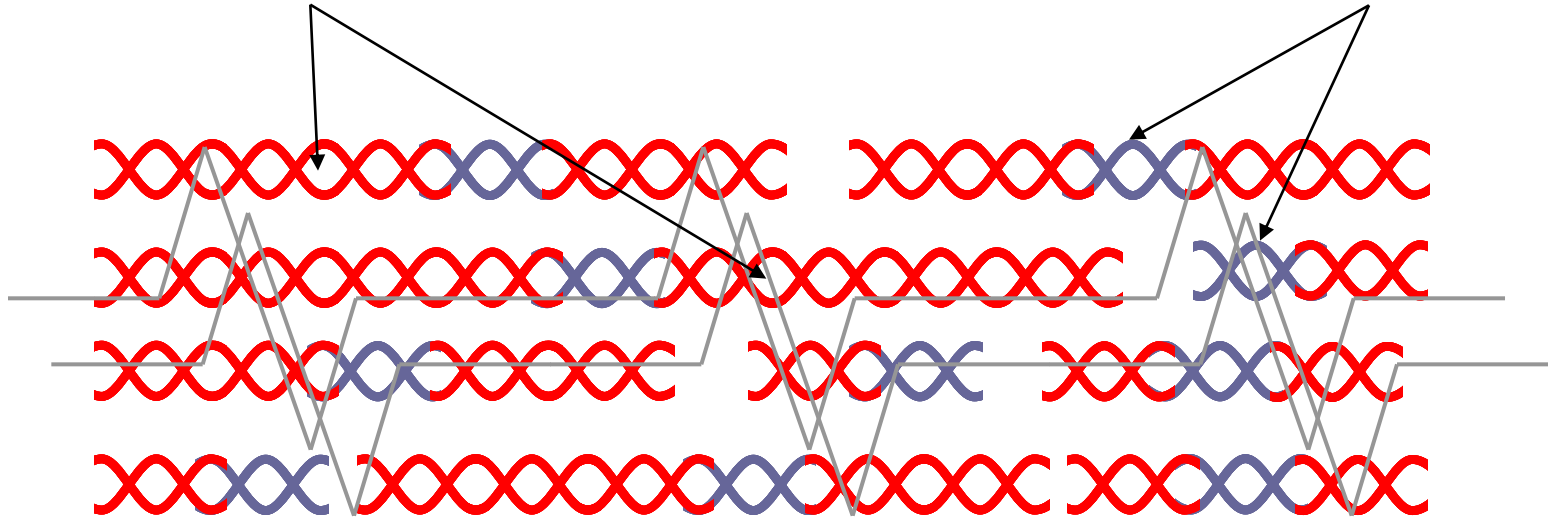


... with high molecular-weight, double-stranded DNA.

This contains methylated and unmethylated cytosines residues.

Unmethylated DNA

Methylated DNA

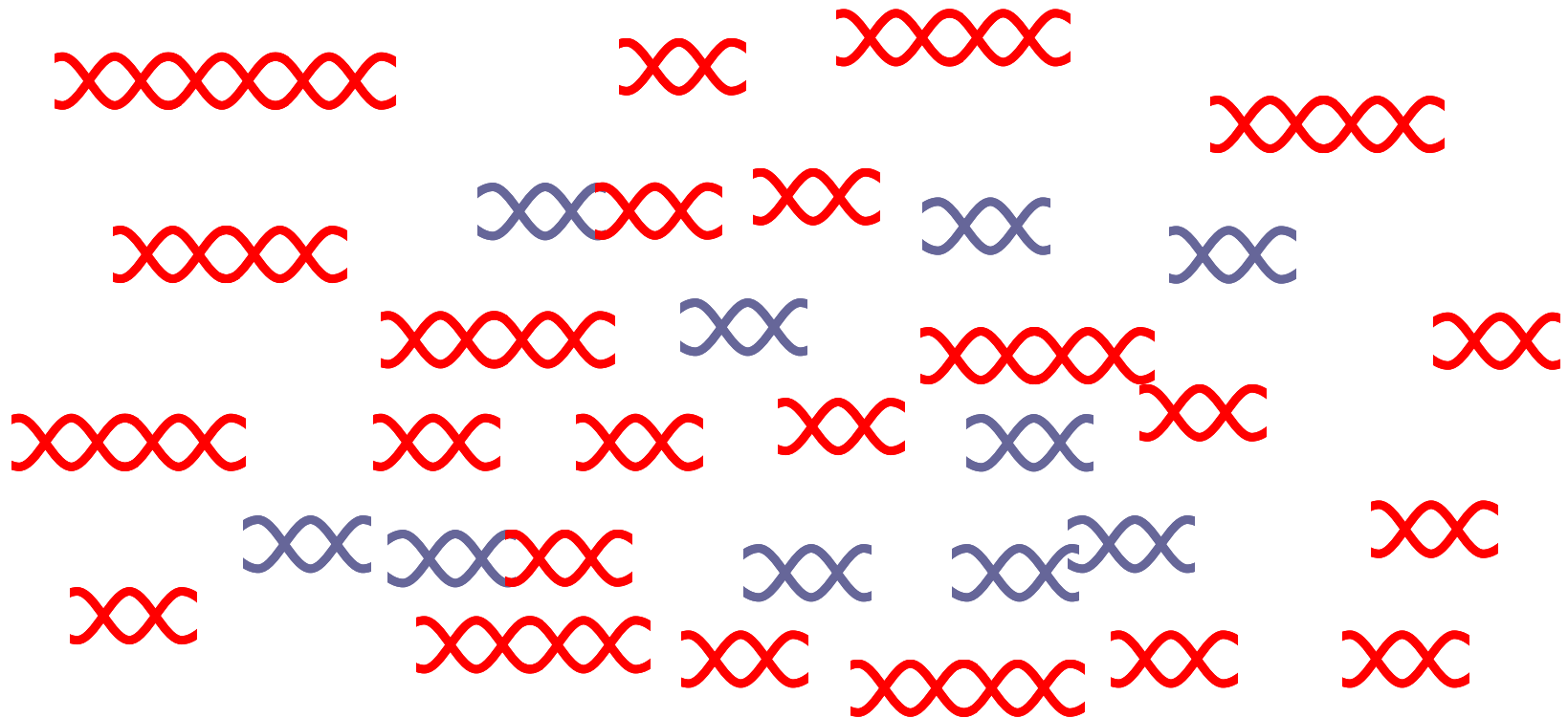


For *MeDIP*, the DNA is first sonicated...



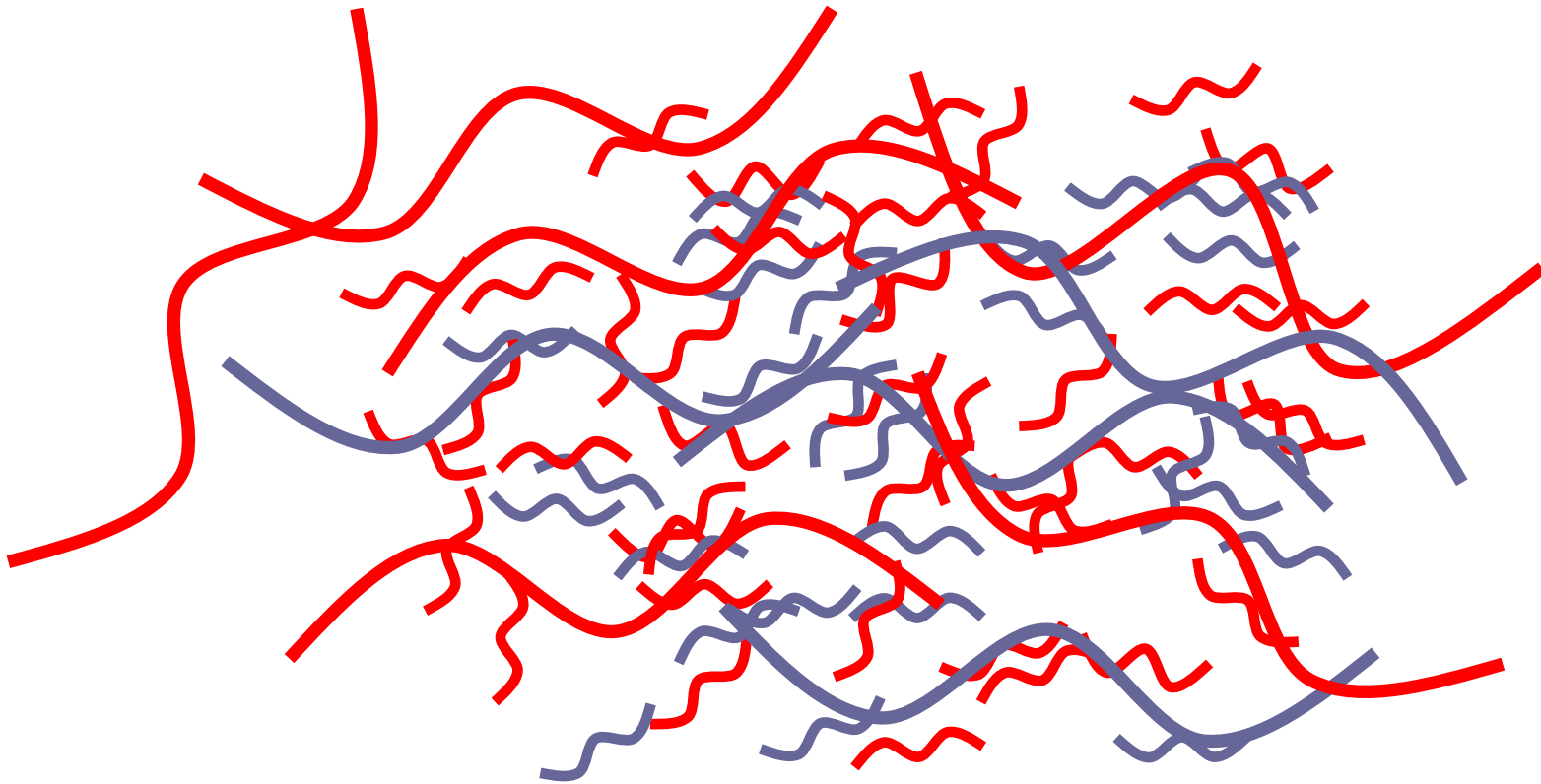
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... to create short fragments...



...then denatured to create single-stranded DNA fragments.

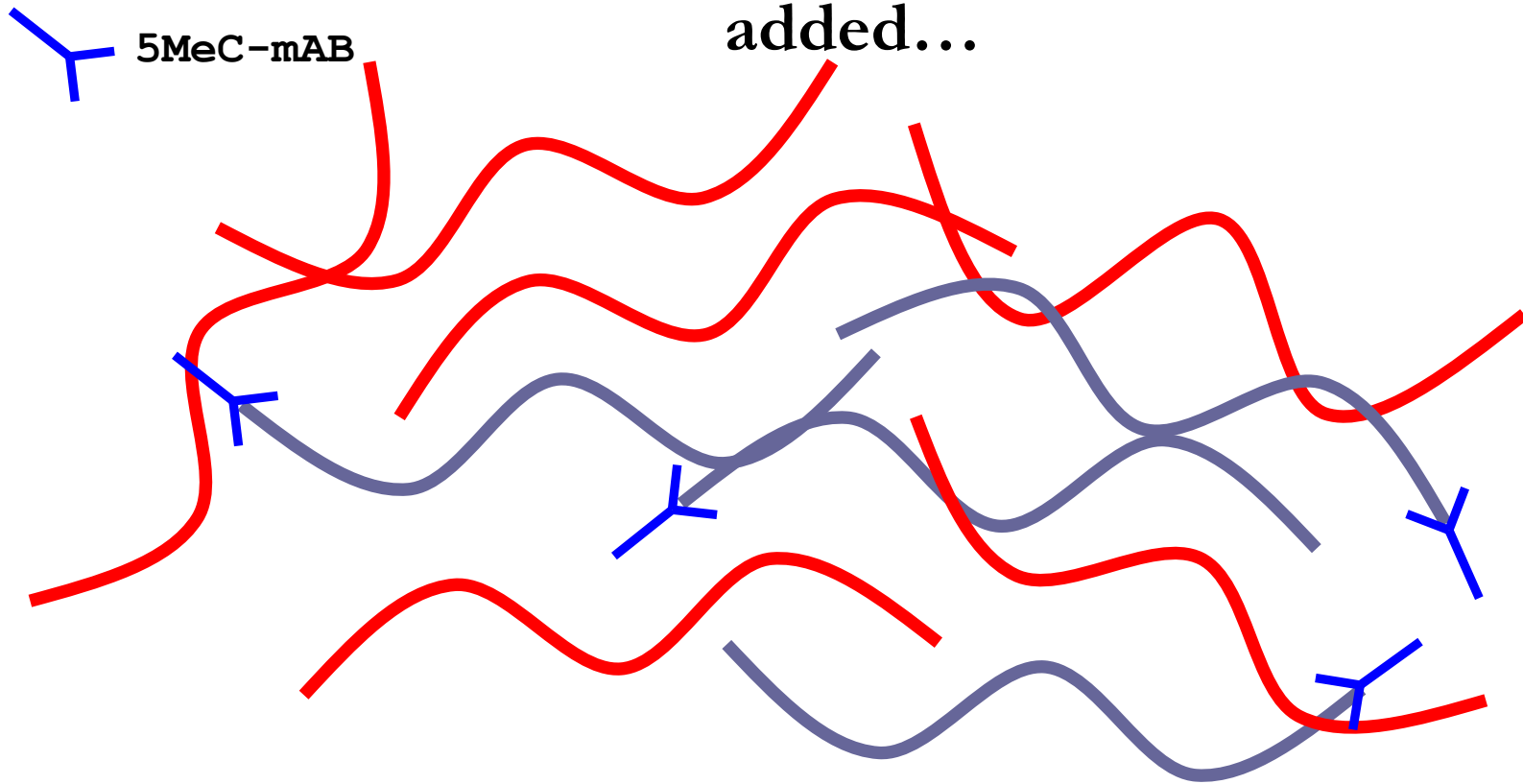
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**...then denatured to create single-stranded DNA fragments.**

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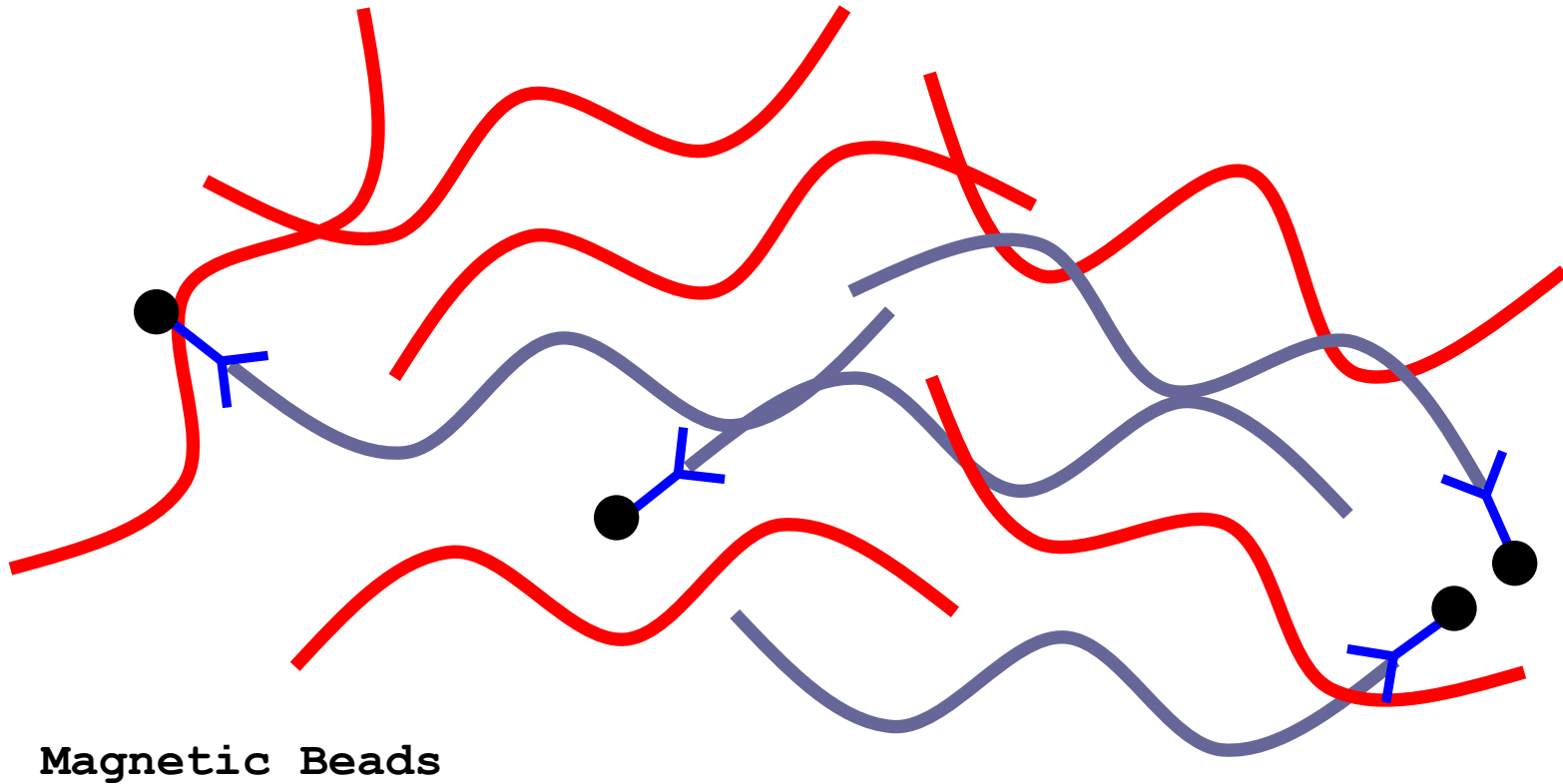
Antibody directed against 5-methylcytidine is



... that binds to the *methylated fraction* of the  
genome

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Magnetic beads are added...

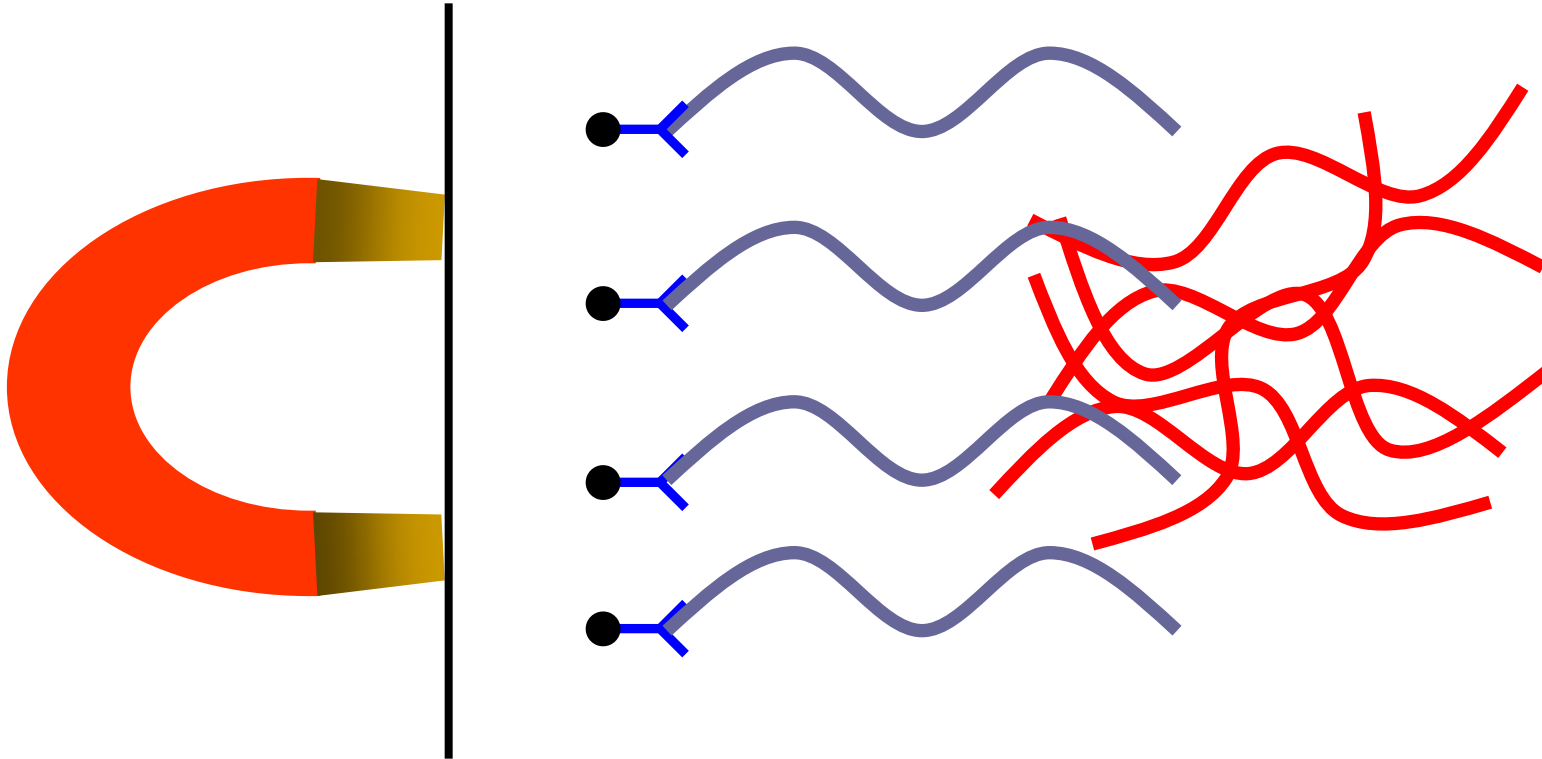


... that bind to the *antibody*...

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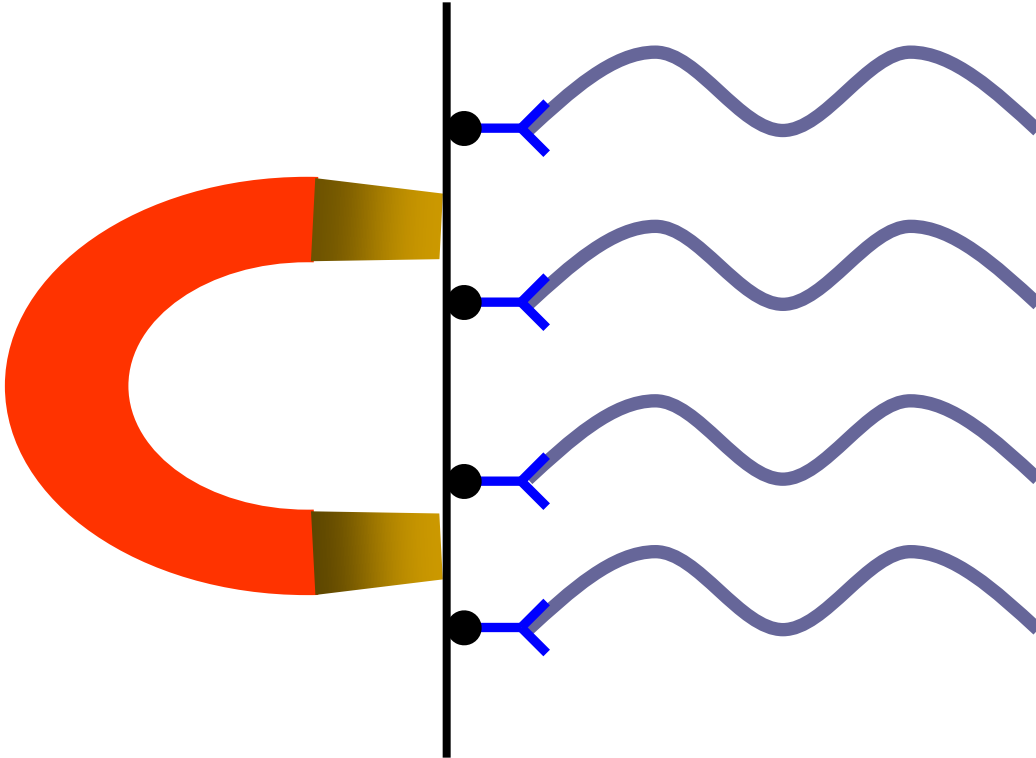
... allowing the *methylated fraction* to be captured with magnets.

... allowing the *methylated fraction* to be captured with magnets.

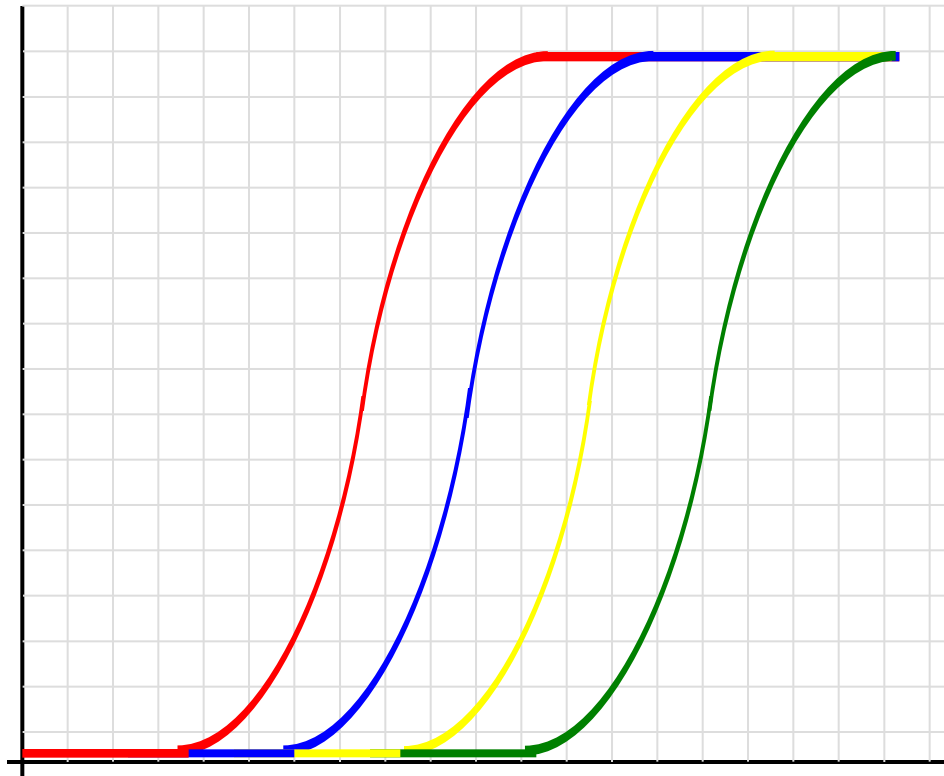


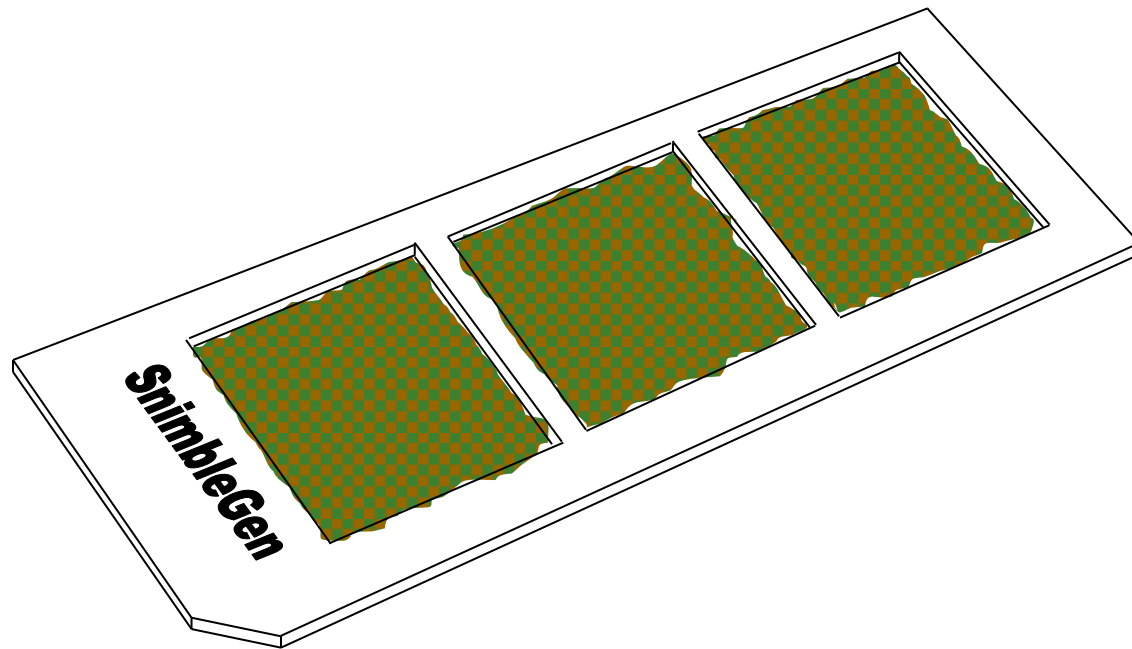
The methylated fraction can then be isolated using...

... proteinase K...



Once isolated, methylated DNA can be...  
... analyzed at candidate loci using qPCR...



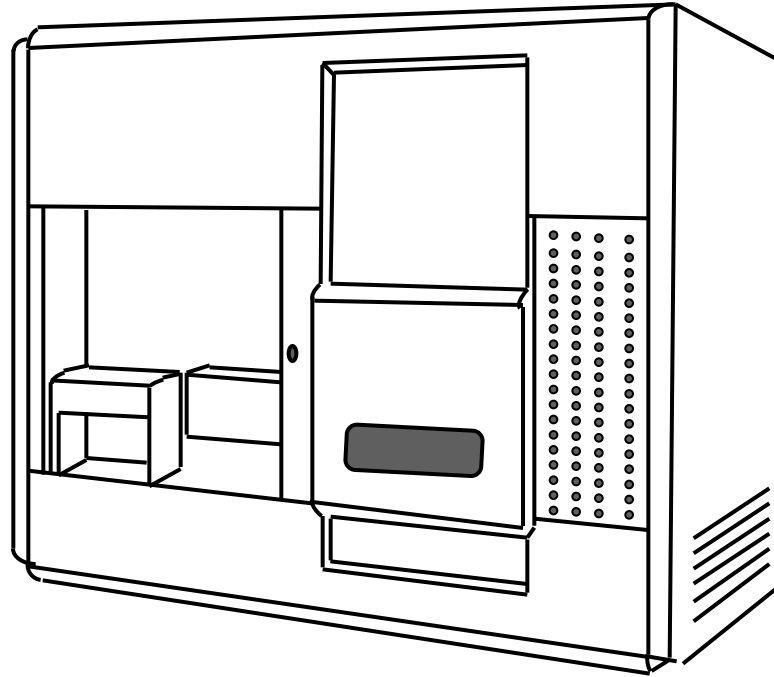


... hybridised to microarrays for genome-wide testing...

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... or high-throughput sequenced for whole-genome analysis.



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# Tools for MeDIP-seq analysis

## ■ BATMAN

- Bayesian Tool for Methylation Analysis
  - See Down *et al.* Nat Biotechnol. 2008 Jul;26(7):779-85 for a description of the algorithm.
  - Used in the analysis of the first mammalian methylome.
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# BATMAN

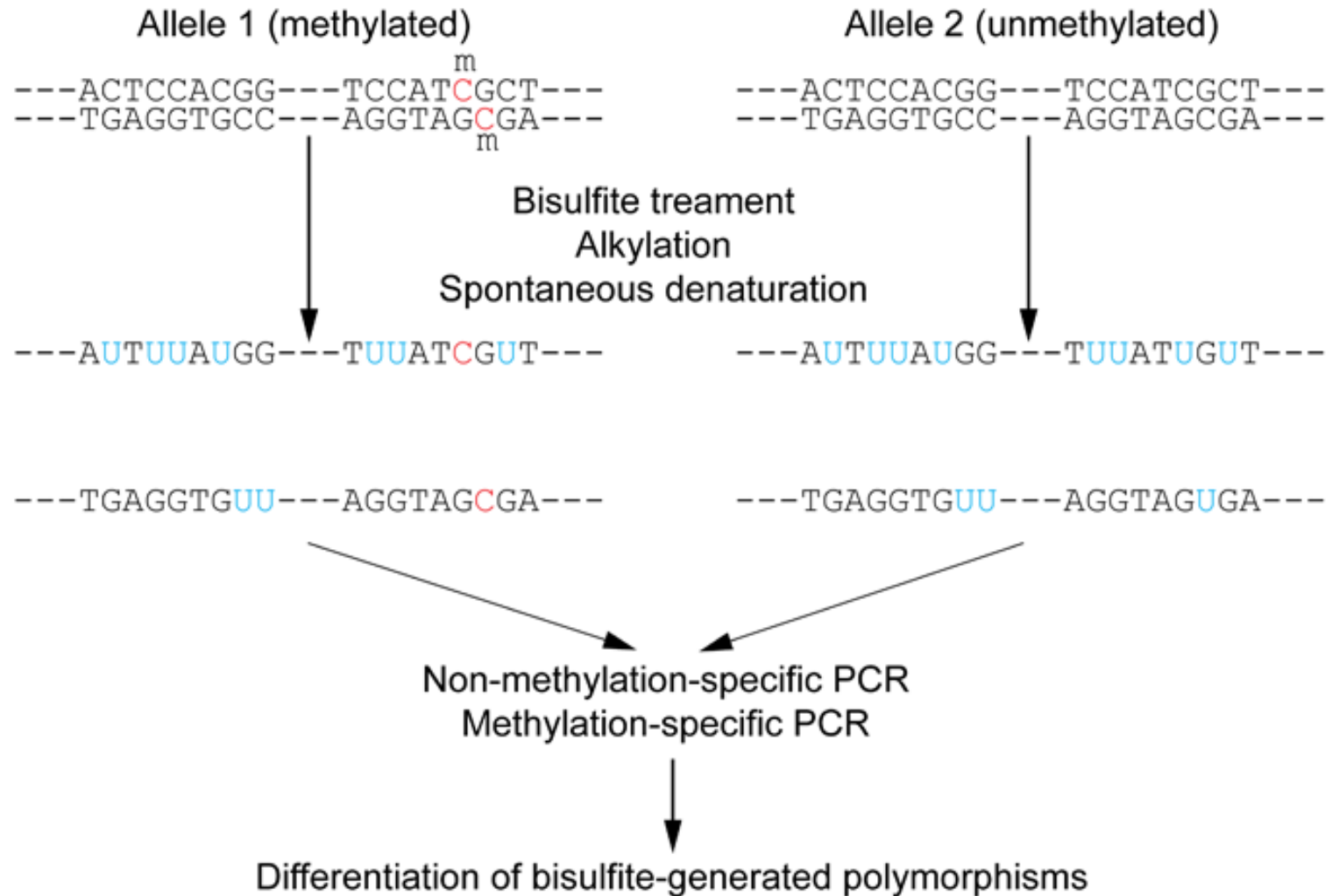
- Bayesian Tool for Methylation Analysis
  - The algorithm is used to provide a quantitative estimation of absolute methylation values from Methylated DNA Immunoprecipitation (MeDIP) profiles.
  - Takes into account the 'neighbourhood' in which each CpG is found when determining absolute methylation levels.
  - Output provides methylation score (0-1)
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# BISULFITE SEQUENCING

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# Bisulfite Sequencing



# Bisulfite mappers

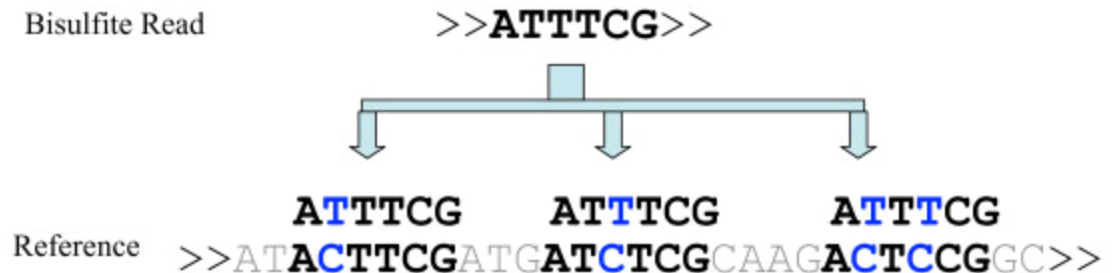
- After bisulfite treatment, unmethylated Cytosines turn into Uracils
  - PCR amplification: U->T
  - Sequenced as Thymines
- Mappers should be able to allow C->T mismatches
  - C-C matches give methylated sites
- Mappers
  - BSMAP, BSseeker, BRAT, Bismark, GSNAP, CokusAlignment, PASH, RMAP, SOCS, etc.

# Bisulfite mappers

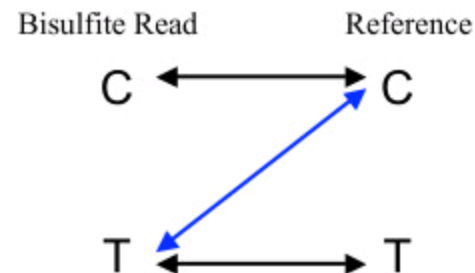
## ■ Challenges

- Multiple mapping
- Mapping asymmetry

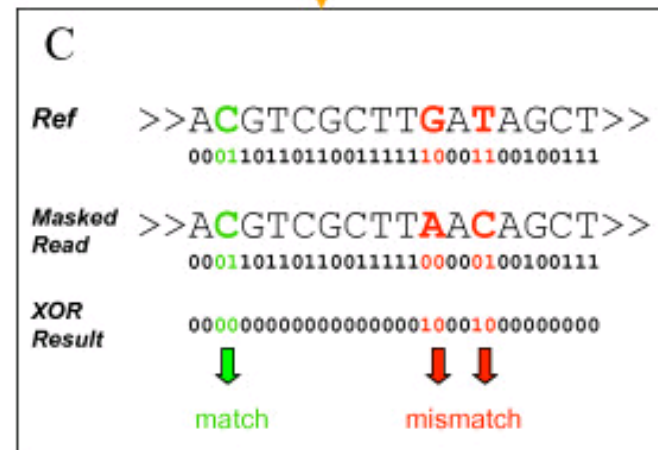
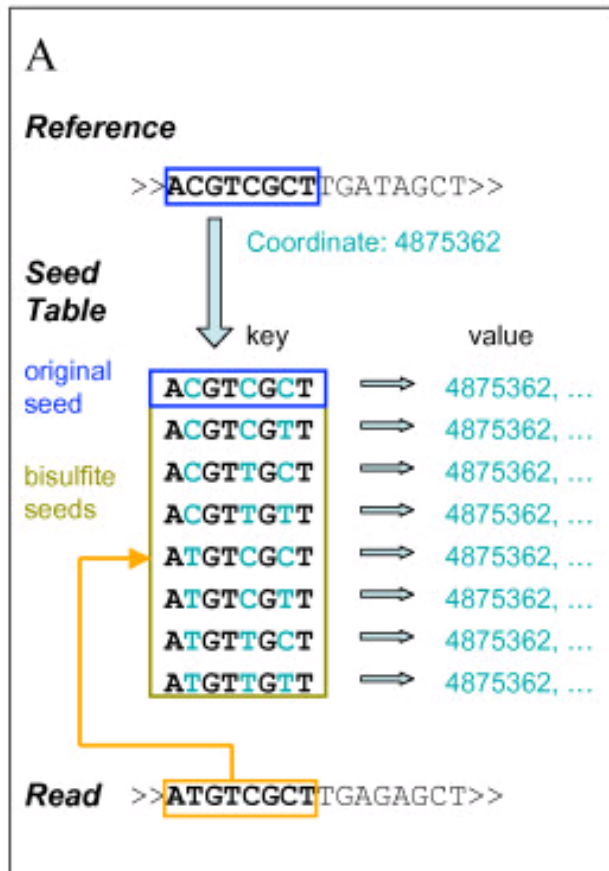
### 1) Multiple Mapping



### 2) Mapping Asymmetry

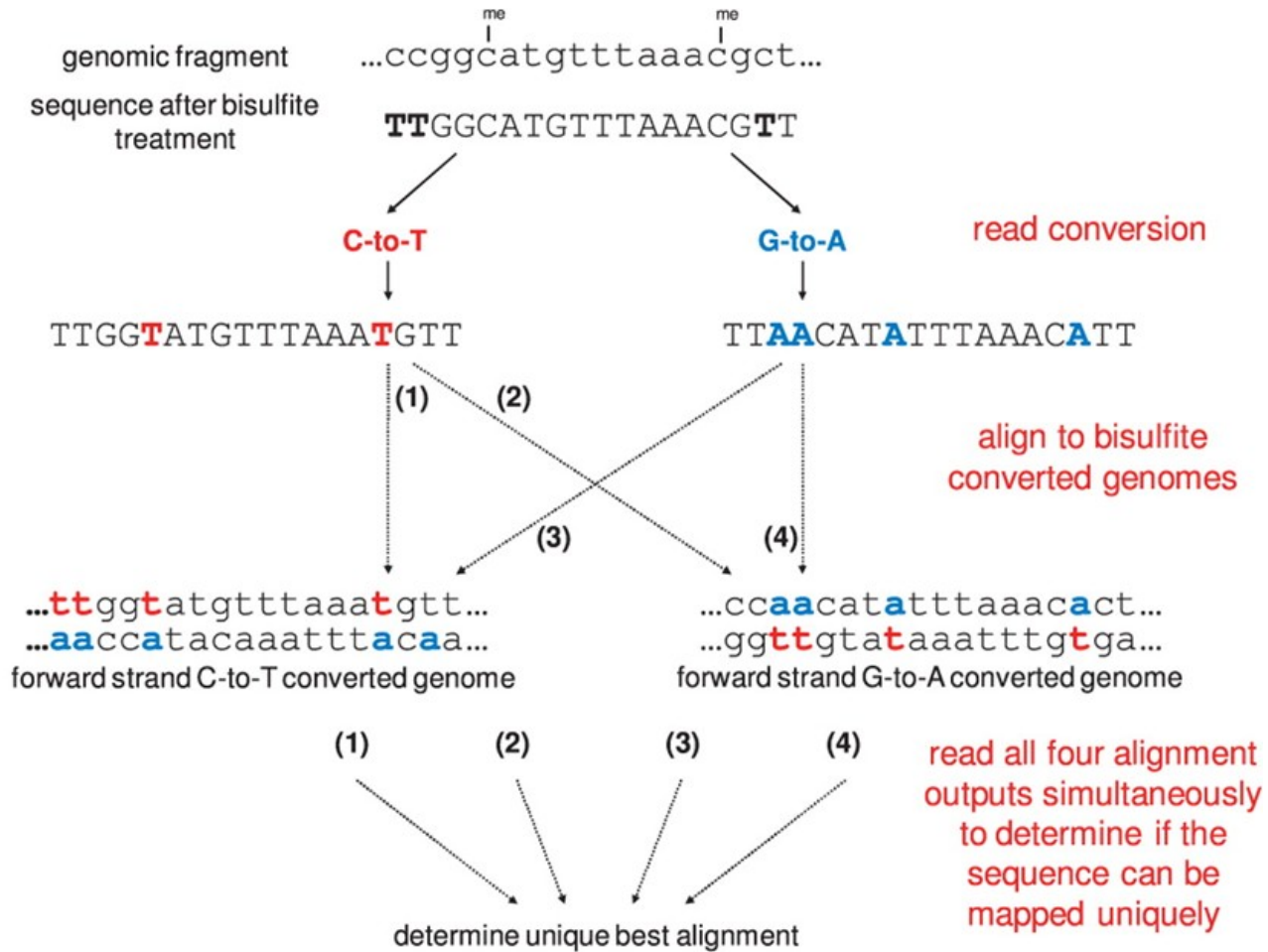


# BSMAP





# Bismark



# Bismark

BS-read corresponds to converted original top strand

