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Tackling data analysis challenges in genome informatics using HPC

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Physiological Genomics & Medicine Group
Clinical Genome Informatics Facility
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MRC Clinical Sciences Centre

THE MAGDI YACOUB INSTITUTE

British Heart Foundation

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Overview

- Genome sequencing: *why & how?*
- Informatics challenges in genome data analysis
- From genome to epigenome:

  Genetic control of inter-individual variation in DNA methylation

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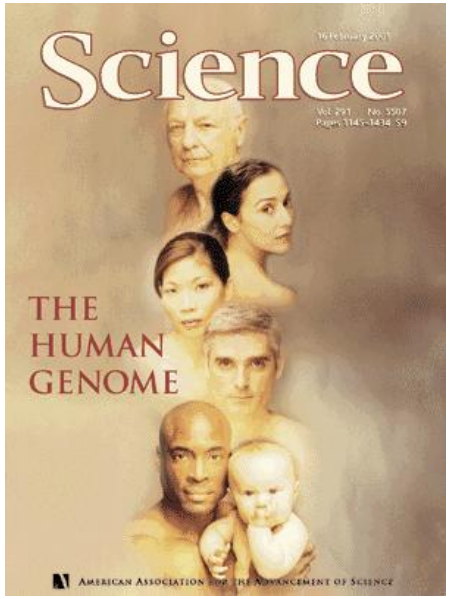
The Human Genome

The Human Genome Project
1990 - 2003

determine the sequence of chemical bases (nucleotides) which make up DNA



articles

Finishing the euchromatic sequence of the human genome

International Human Genome Sequencing Consortium*

* *A list of authors and their affiliations appears in the* Supplementary Information

2001

2003

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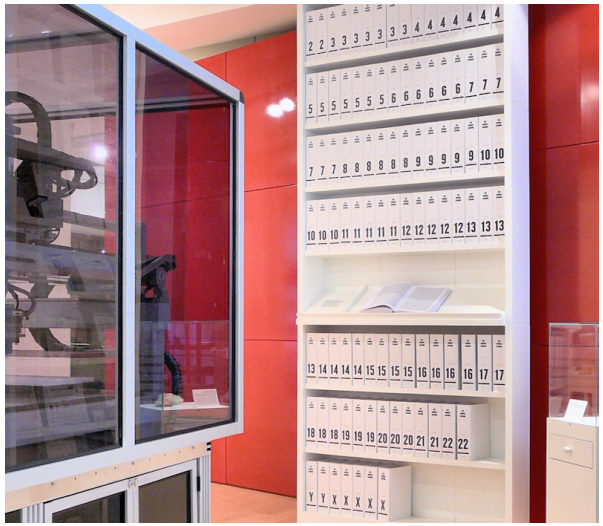
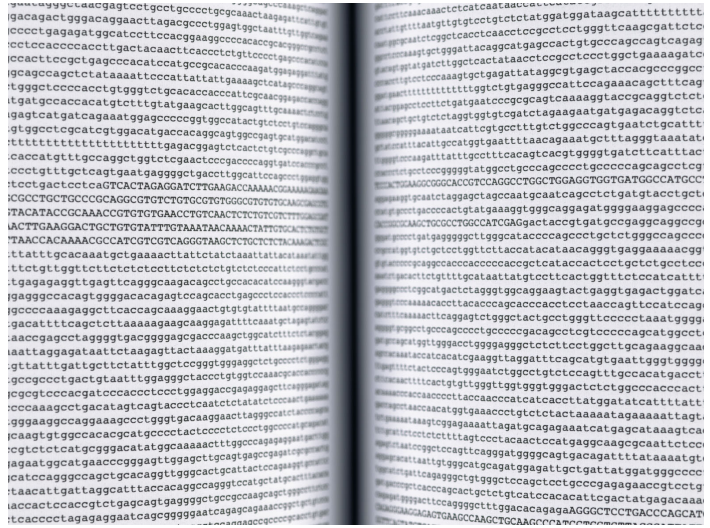
The Human Genome

3.1 billion nucleotides (A,C,T,G)





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The Human Genome

Functional annotation of the genome sequence

The Human Genome Project
identify and map genes

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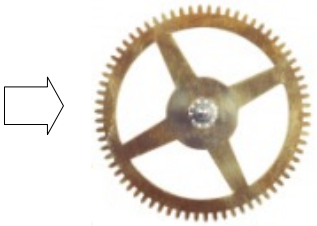

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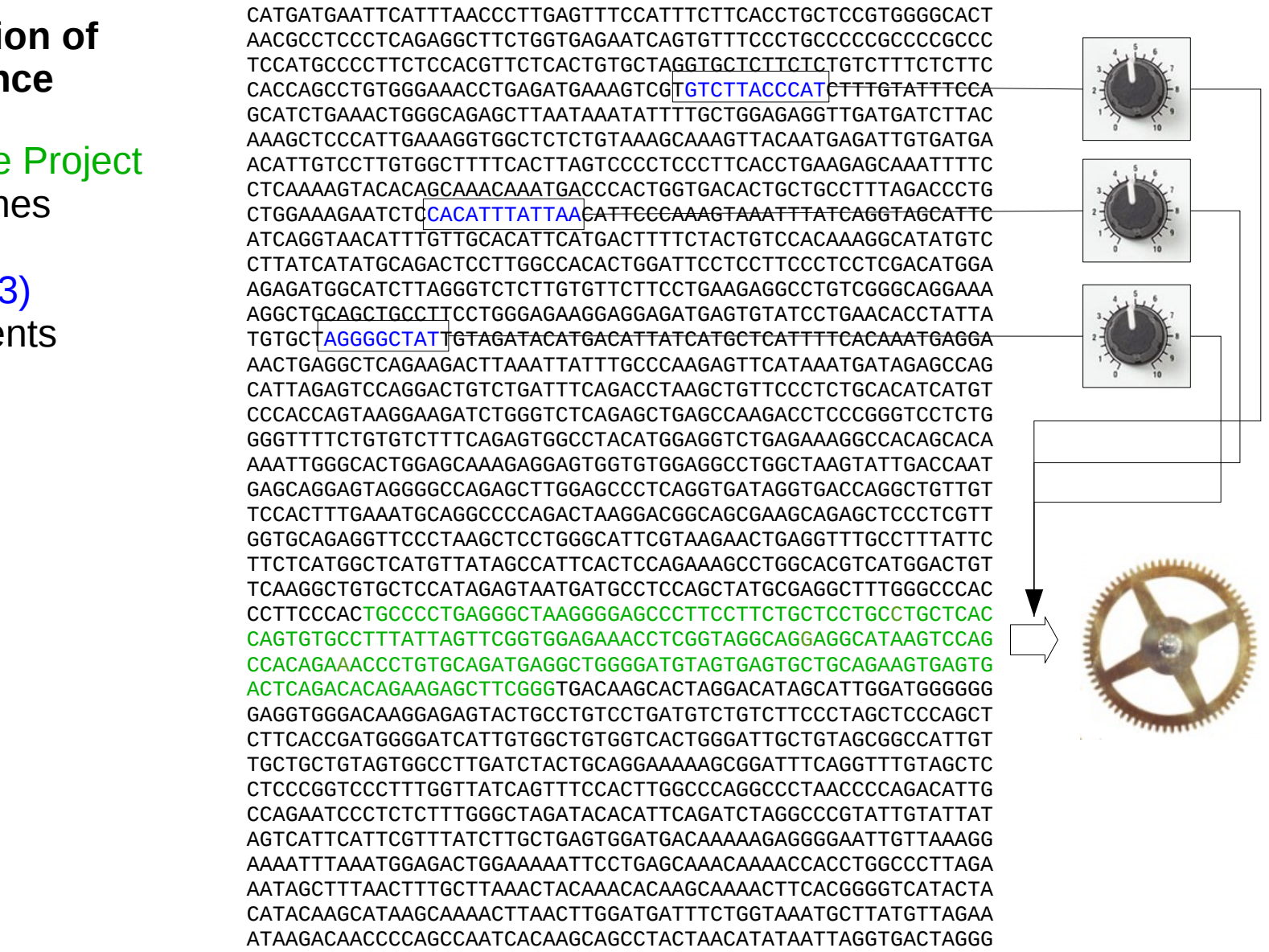
The Human Genome

Functional annotation of the genome sequence

The Human Genome Project
identify and map genes

Encode project (2003)
find functional elements



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The Human Genome

Functional annotation of the genome sequence

The Human Genome Project
identify and map genes

Encode project (2003)
find functional elements

1000 genomes project (2008)
identify inter-individual sequence variation

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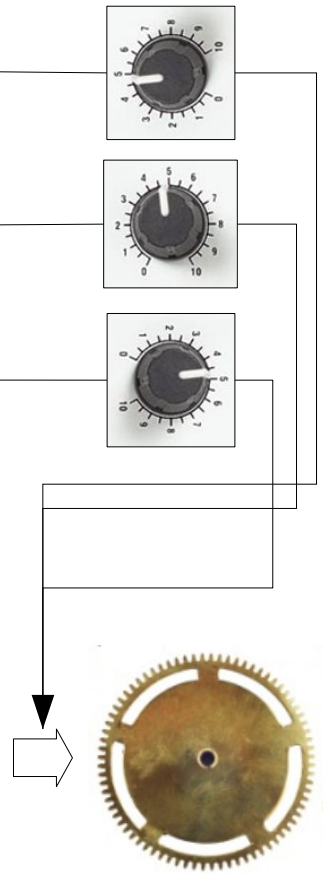

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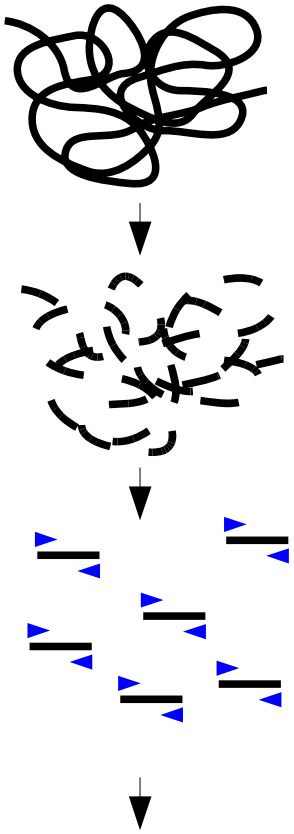
Variant detection by next-generation sequencing

genomic DNA

fragmented DNA

paired-end sequencing

read mapping



Illumina HiSeq 2000

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genomic DNA

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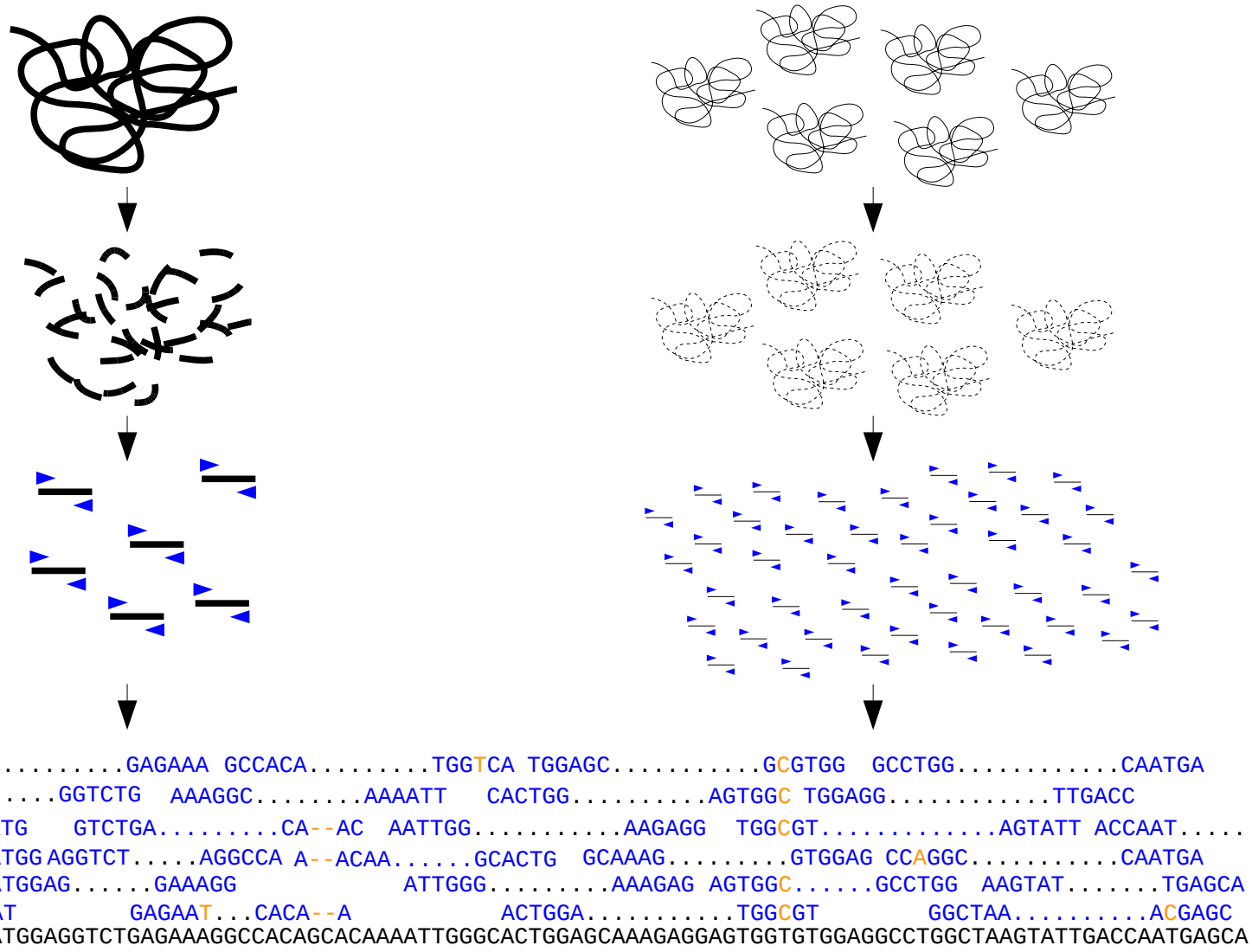

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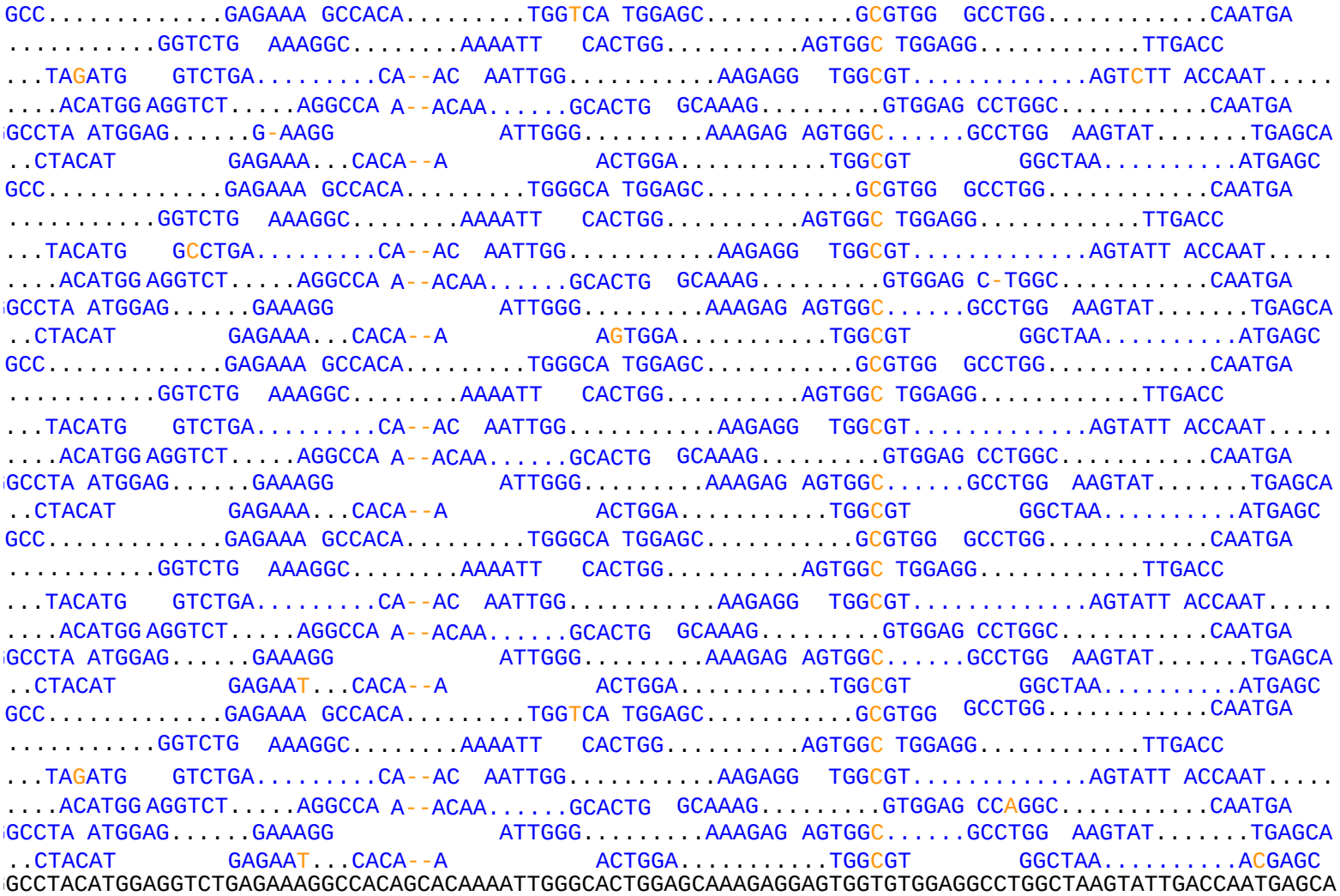
Variant detection by next-generation sequencing

read mapping



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Next-Generation Sequencing (NGS)

Throughput

Illumina HiSeq 2000 run

- 10 days runtime
- ~6 billion 100bp paired-end reads
- ~30x coverage of 6 human genome
- ~220Gb of (compressed) raw data

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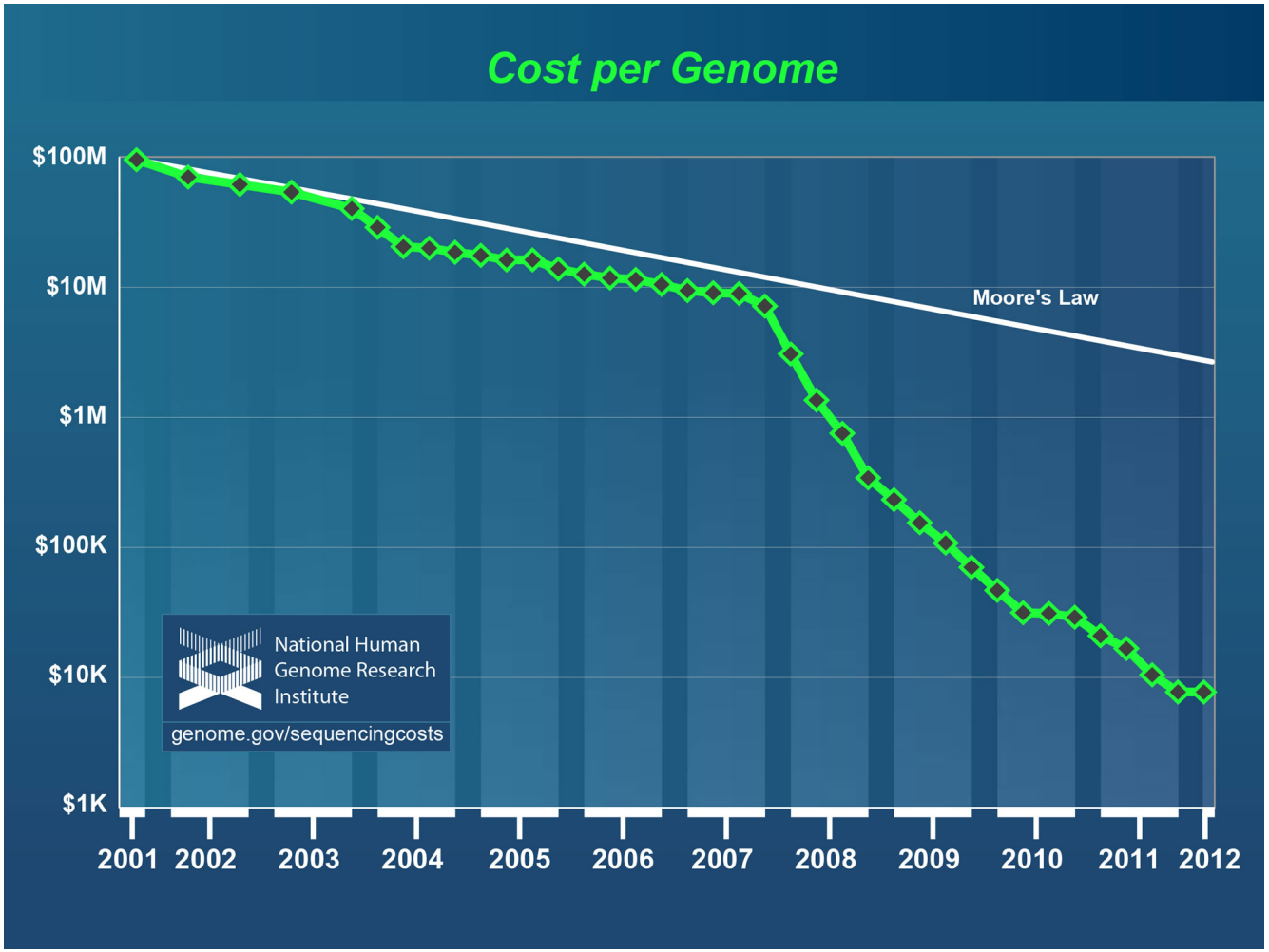
Next-Generation Sequencing (NGS)

Reduction in DNA sequencing costs



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Next-Generation Sequencing (NGS)

Informatics Challenges

- **data storage & I/O**
  - ~7TB raw data per sequencer per year
- **mapping algorithm efficiency**
  - 1st generation: hash-based
  - 2nd generation: Burrows-Wheeler transform
    - 10 times faster at similar sensitivity
- **CPU time**
  - 720 CPU hours (30 days) for ~1 billion reads (30x coverage of the human genome)

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Next-Generation Sequencing (NGS)

NGS data analysis using Imperial College HPC service

- dedicated access to 112 CPU cores on PC cluster
- access to SGI Altix UV system with 256 CPU cores and up to 3TB of shared memory
- 60TB storage

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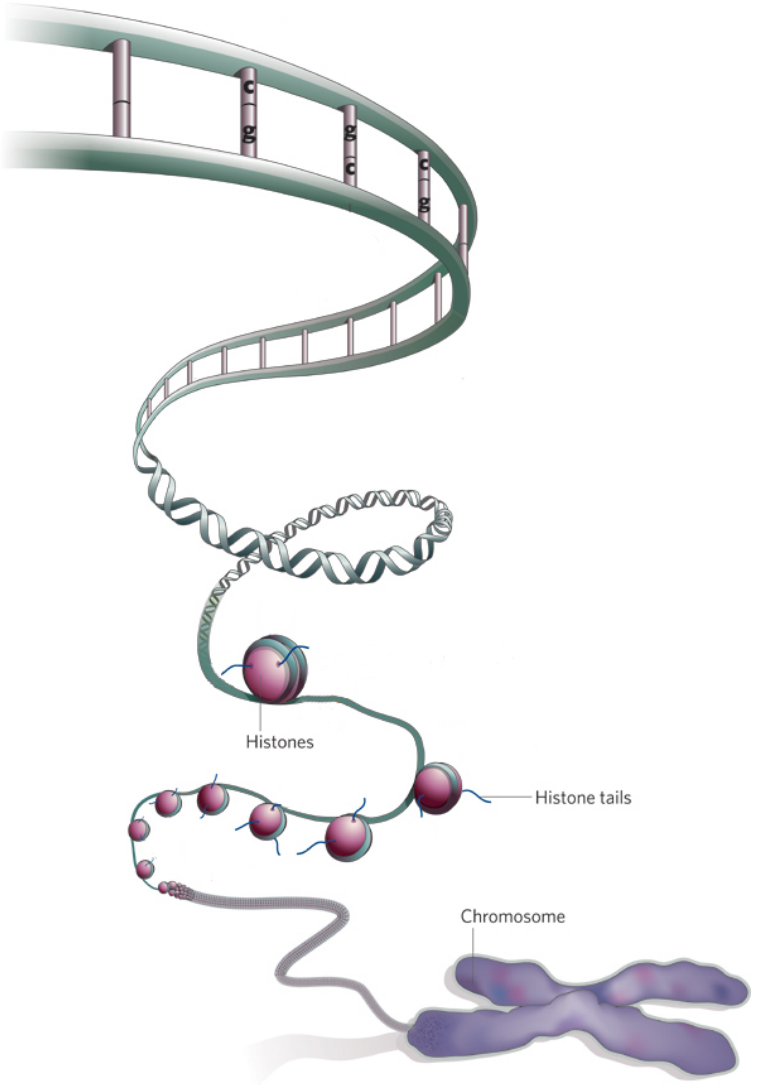
From genome to epigenome



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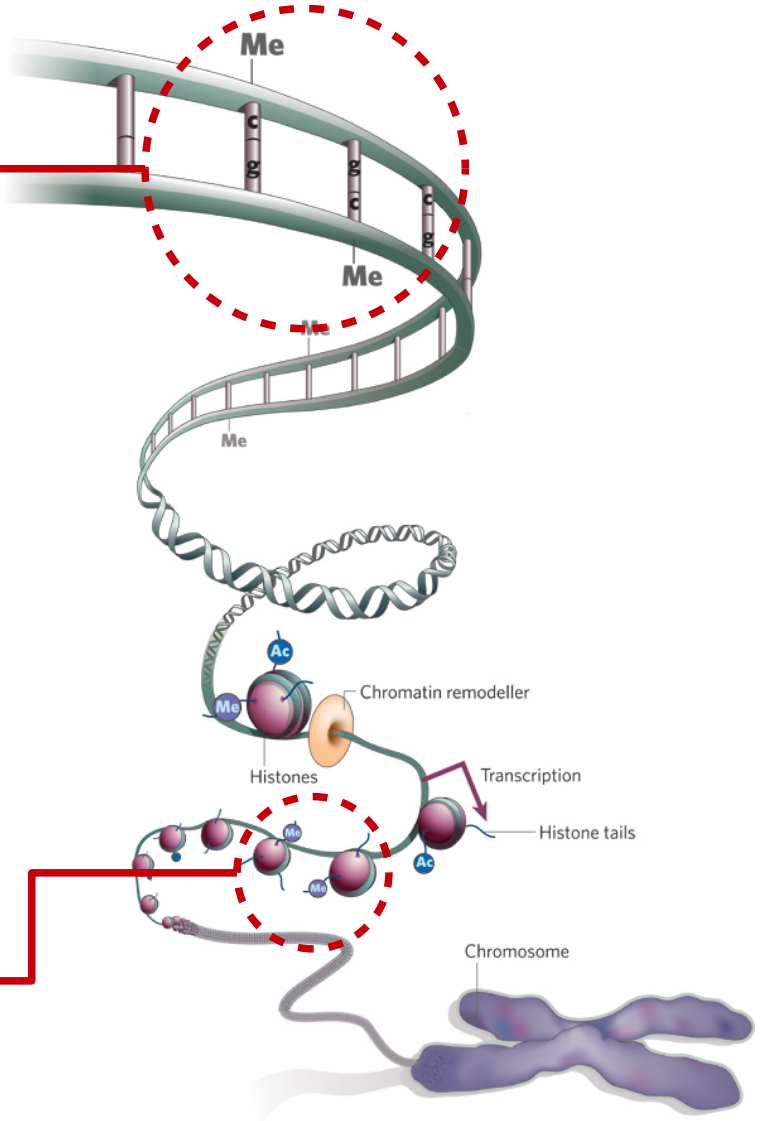
From genome to epigenome

DNA methylation



histone modification

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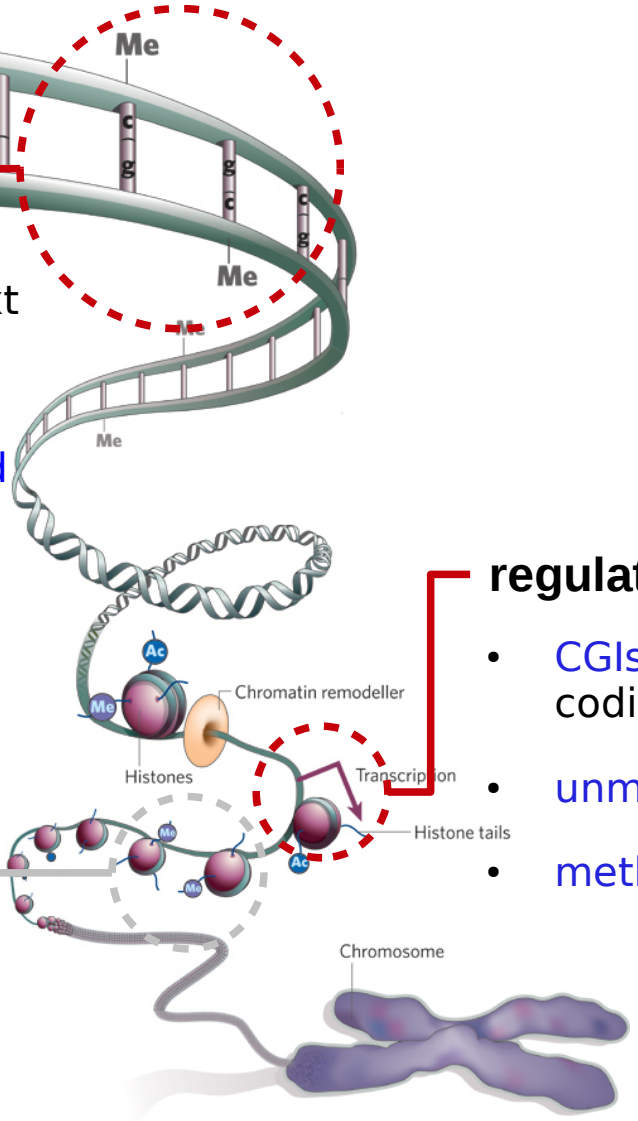
DNA methylation & gene expression

DNA methylation

- occurs in CpG dinucleotides context
- CpGs cluster in CpG Islands (CGI)
- genome globally highly methylated
- CGIs mostly unmethylated



regulation of transcription

- CGIs found in 50% of protein coding gene promoters
- unmethylated promoters active
- methylated promoters repressed

histone modification

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Genetic control of inter-individual variation in DNA methylation

Aims

- measure DNA methylation levels at single nucleotide resolution in two rat inbred strains (Brown Norway & Spontaneously Hypertensive Rat)
- identify inter-strain differences in DNA methylation
- determine inheritance and genetic regulators of DNA methylation

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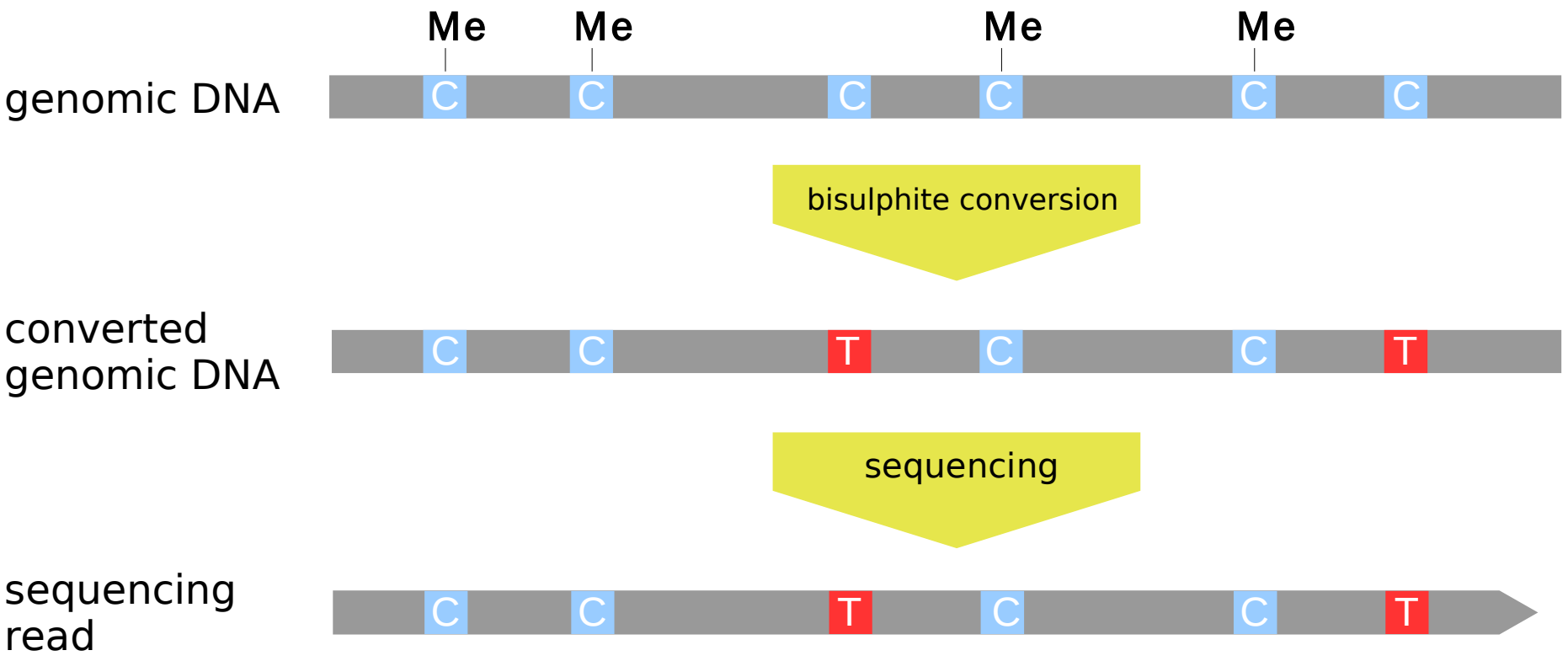
Measuring DNA methylation by bisulphite sequencing



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Measuring DNA methylation by bisulphite sequencing

bisulphite sequencing reads

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Measuring DNA methylation by bisulphite sequencing

bisulphite sequencing reads

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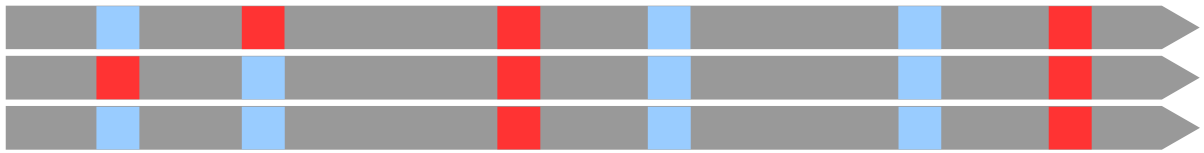
Measuring DNA methylation by bisulphite sequencing

bisulphite sequencing reads



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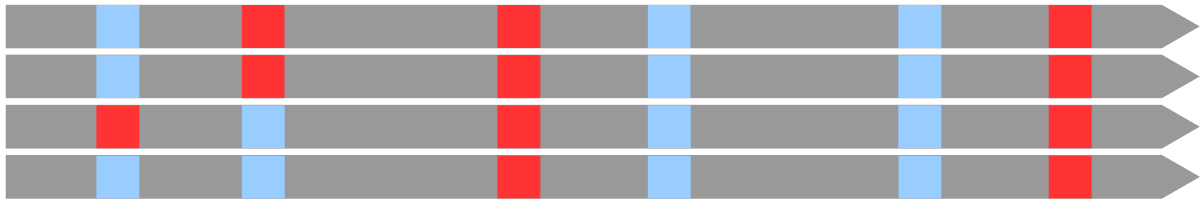
Measuring DNA methylation by bisulphite sequencing

genomic DNA

bisulphite sequencing reads



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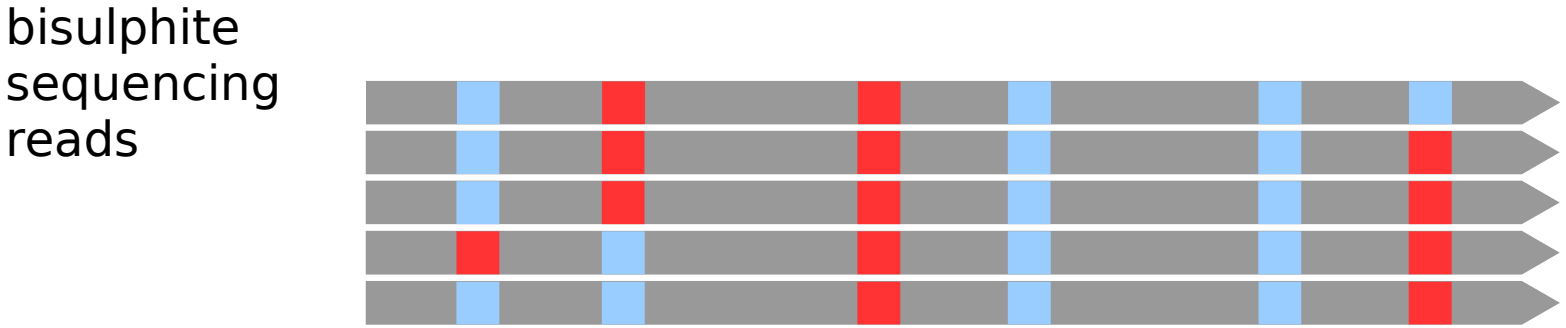
Measuring DNA methylation by bisulphite sequencing



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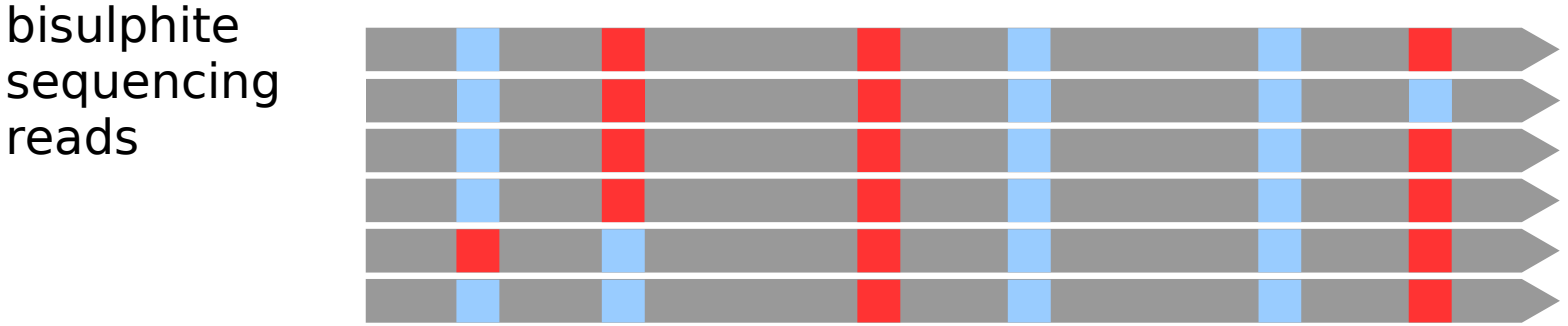
Measuring DNA methylation by bisulphite sequencing



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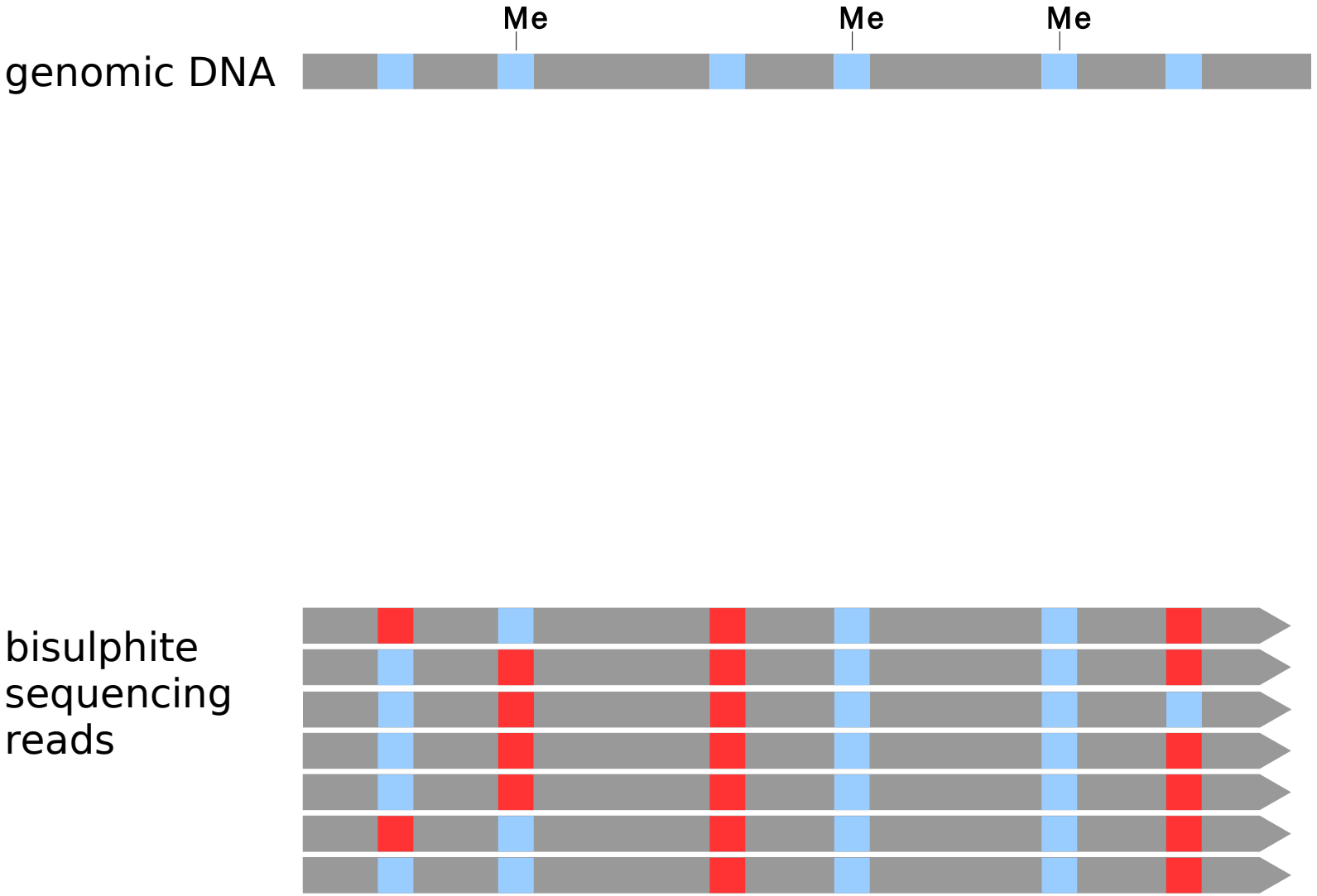
Measuring DNA methylation by bisulphite sequencing



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Measuring DNA methylation by bisulphite sequencing

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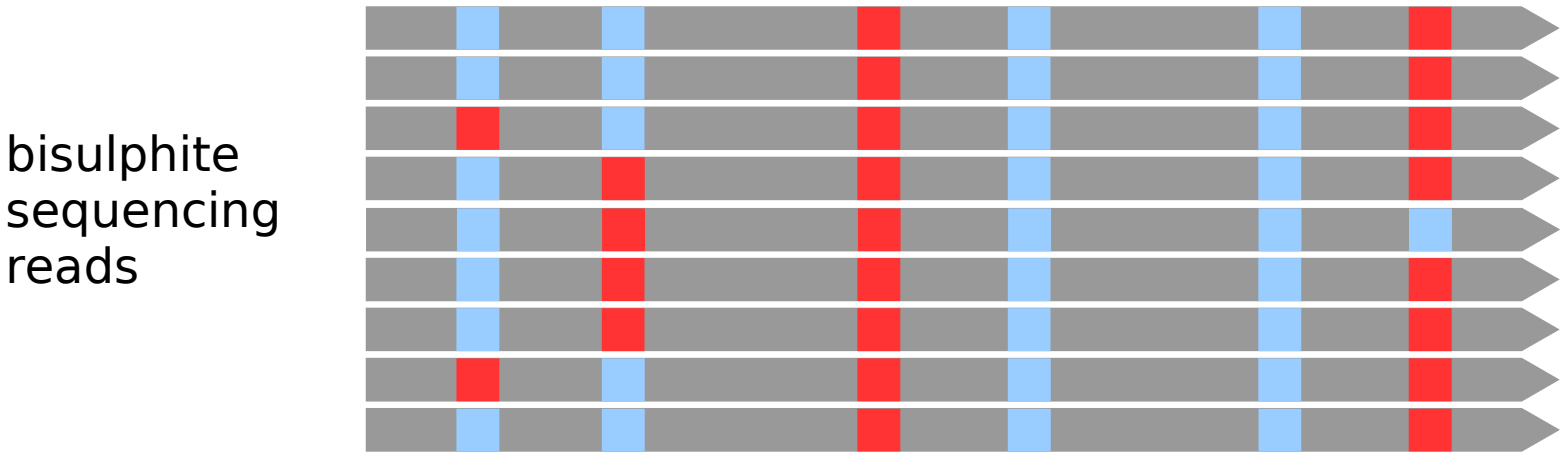

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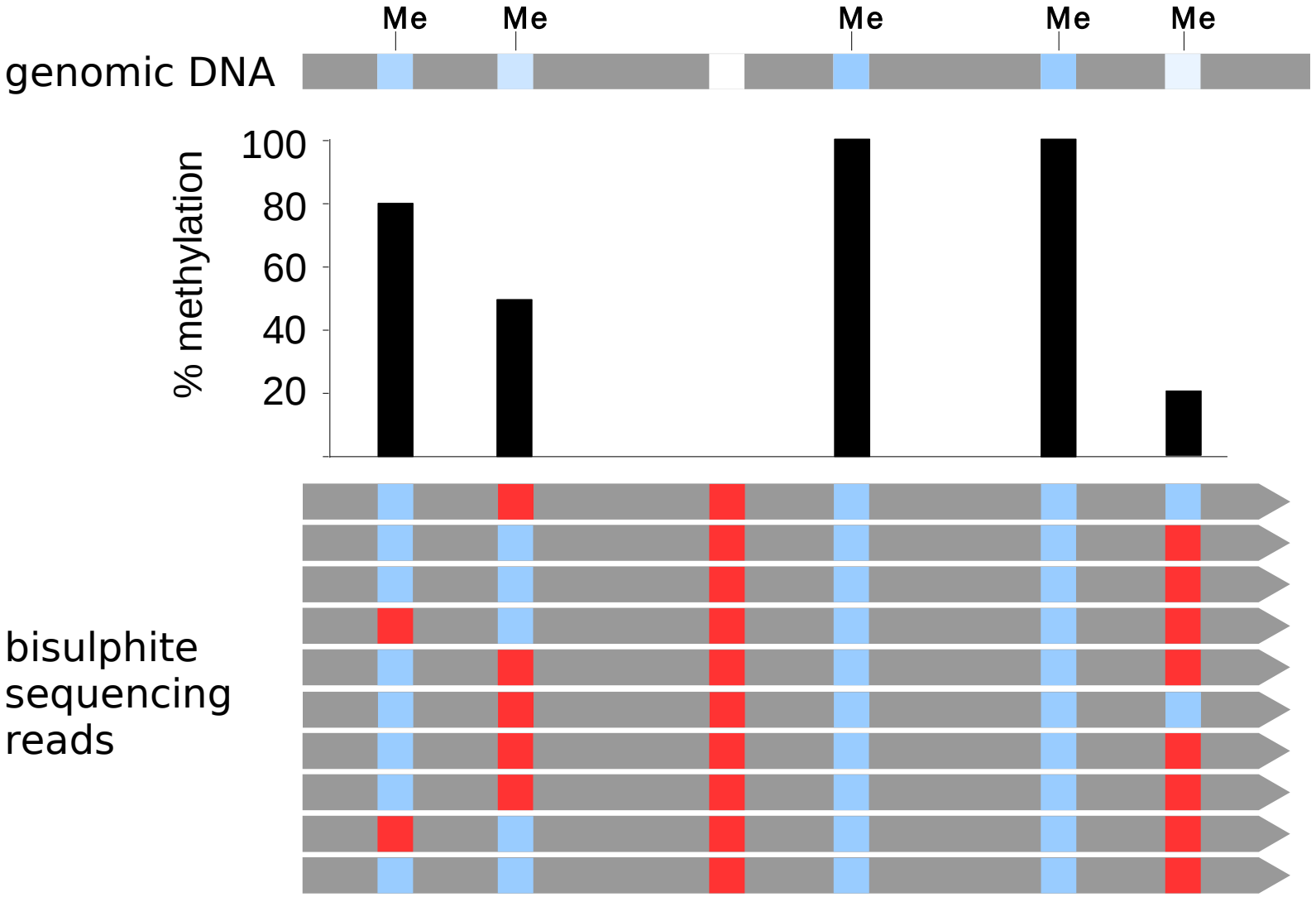
Measuring DNA methylation by bisulphite sequencing



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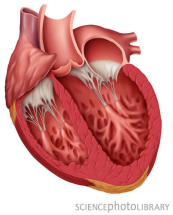
Sequencing the SHR & BN methylomes

- Illumina paired-end sequencing
- 100 bp read length
- 4 lanes per sample



- left ventricle of the heart

- SHR/Ola, BN-Lx, reciprocal F1 crosses
- 4 biological replicates
- six weeks old

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Sequencing the rat methylome

sample	reads	mapped reads		depth of coverage	depth of coverage after filtering*
	[million]	[million]	[%]	[x-fold]	[x-fold]
BN2	258	230	89	7	4
BN4	413	361	87	13	7
BN5	454	404	89	11	8
BN7	370	340	92	12	9
BN total	**1,495**	**1,336**	**89**	**42**	**29**
SHR6	497	448	91	15	10
SHR7	452	398	89	12	8
SHR8	421	369	90	11	8
SHR9	391	356	91	12	8
SHR total	**1,762**	**1,571**	**89**	**50**	**34**

* filtering by read clonality, uniqueness, mapping quality and strand specificity

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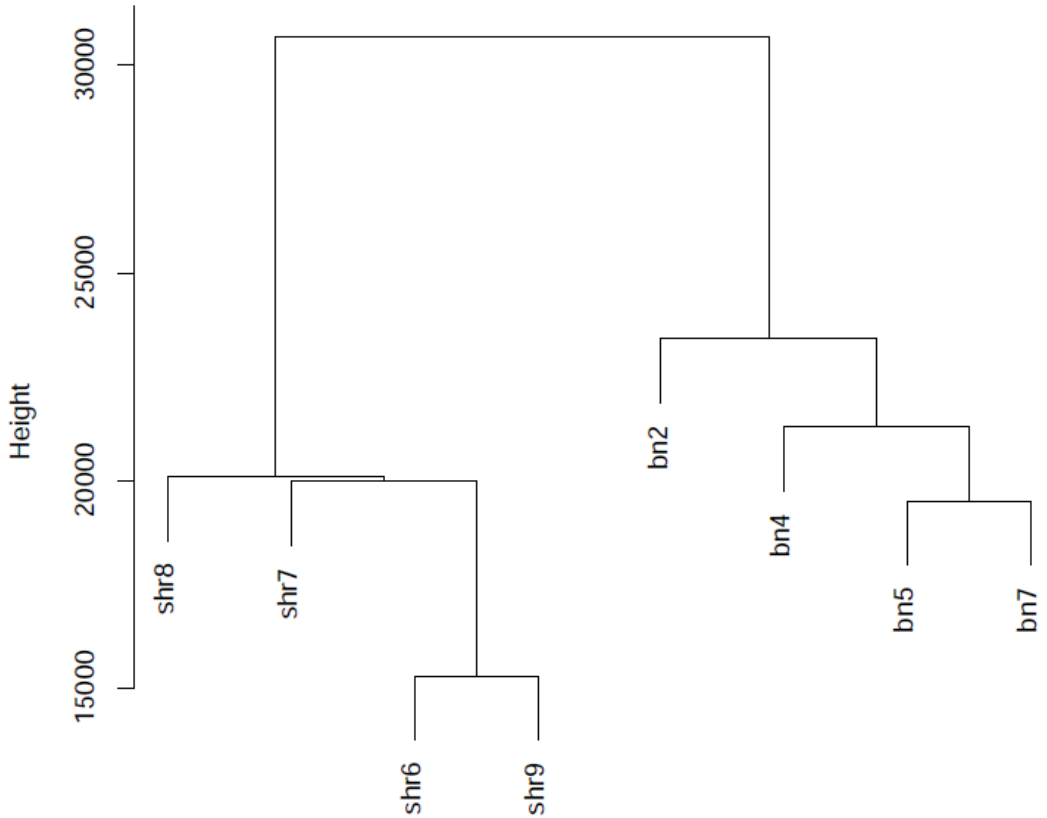
Inter- vs intra-strain differences in CpG methylation

Variability in CpG methylation greater between than within inbred strains



Distance measure: Euclidean
Clustering method: Ward

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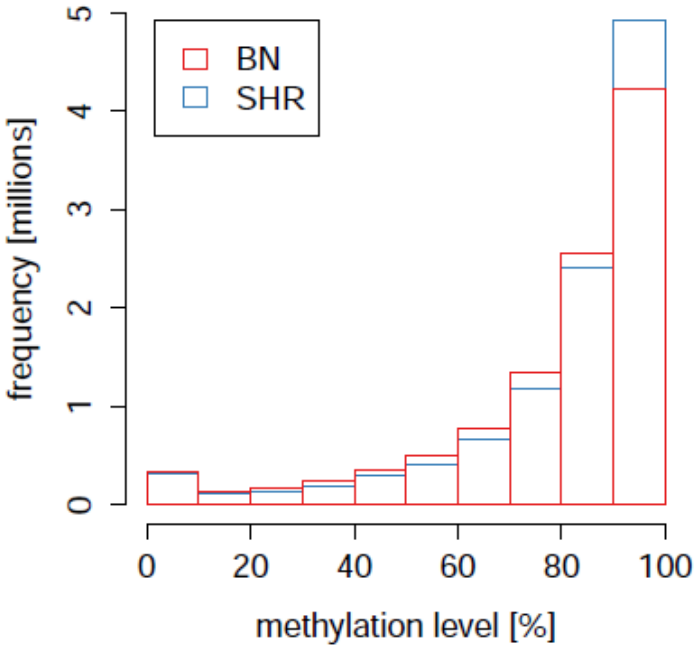
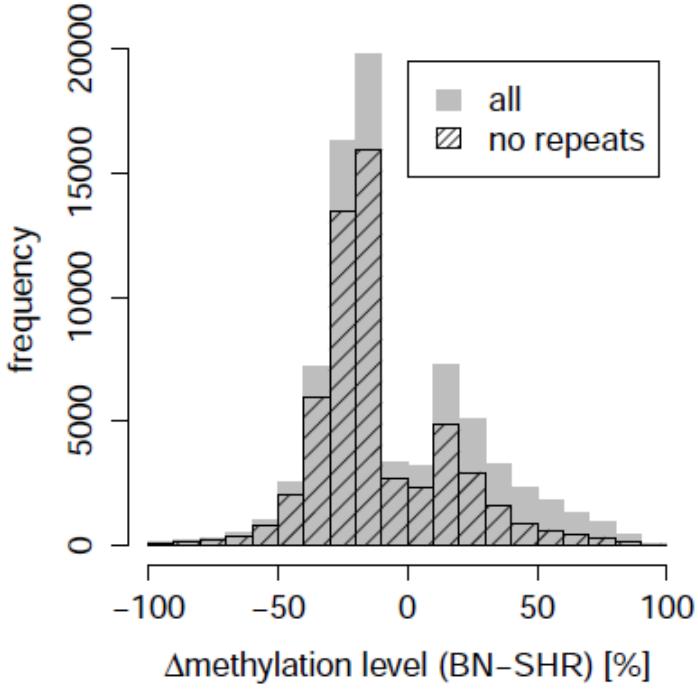
Distribution of methylation levels and inter-strain differences in methylation

analysis of 10.6 million CpG dinucleotides identified
77,088 differentially methylated CpGs (<1%)

The rat genome is globally highly methylated



The majority of methylation differences are small



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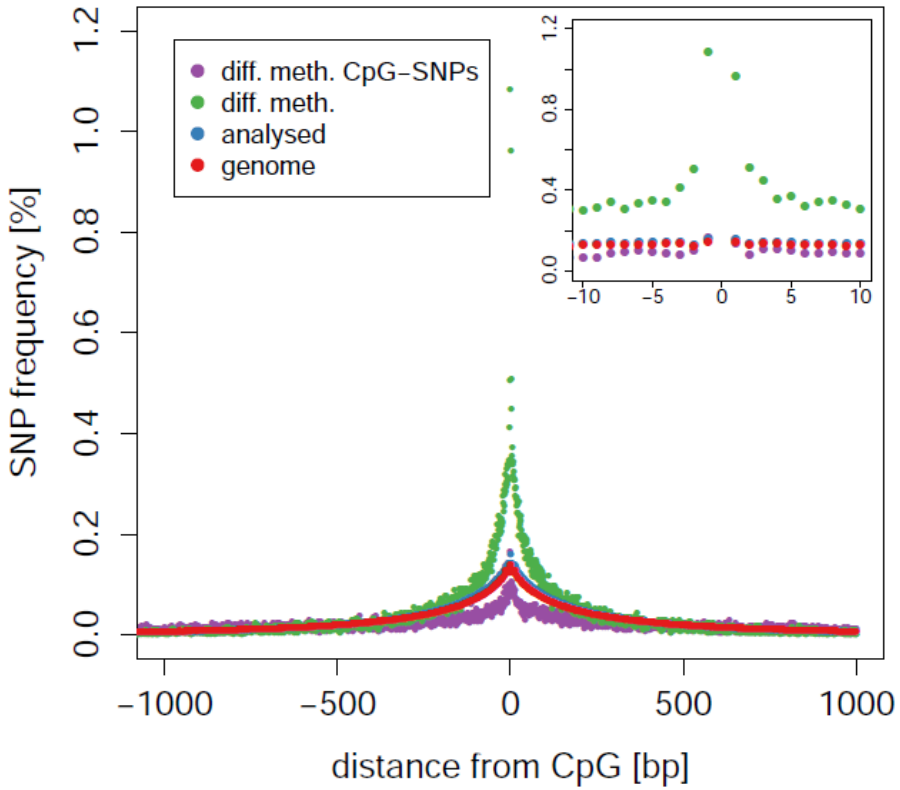
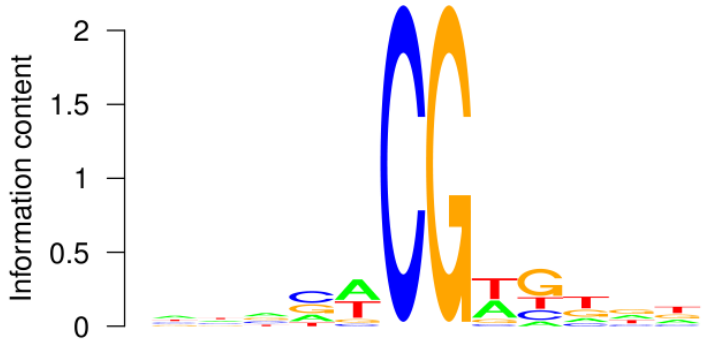
Sequence variation at differentially methylated CpGs

Sequence variation is higher in the proximity of differentially methylated CpGs

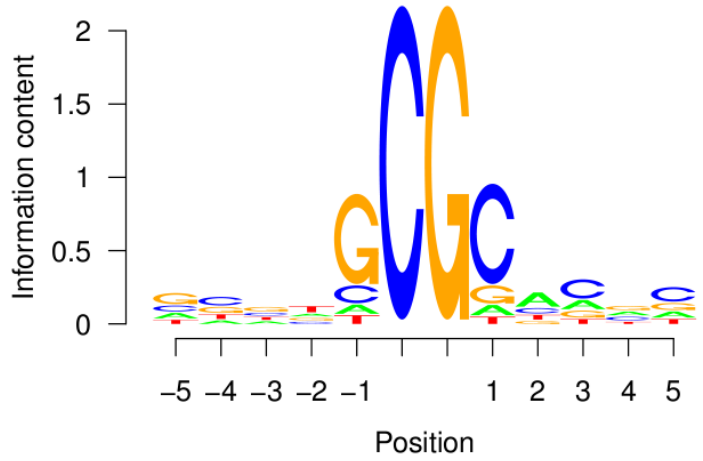


Increased and decreased methylation is associated with specific sequence motifs

increased methylation



decreased methylation



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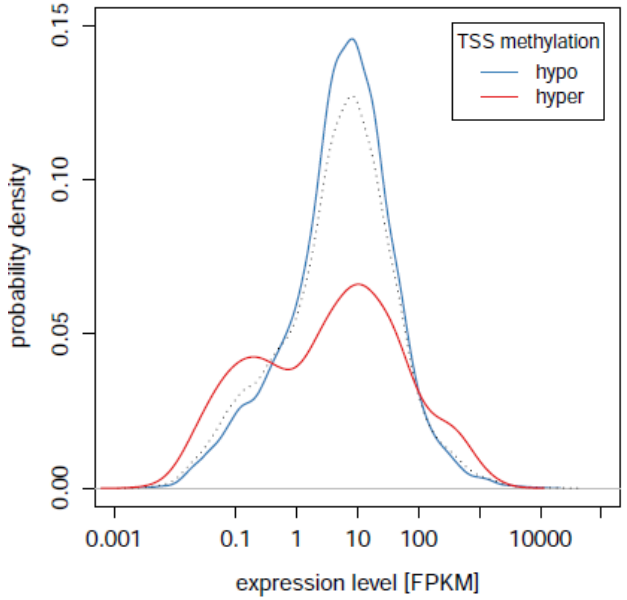
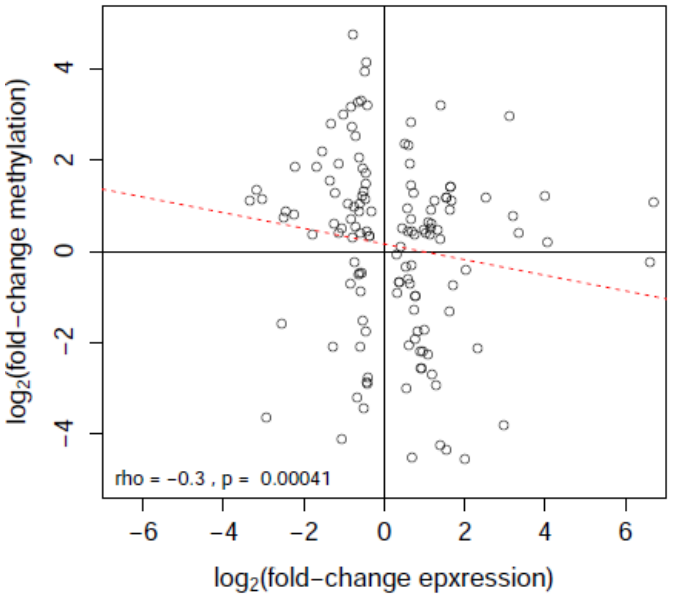
Methylation and gene expression

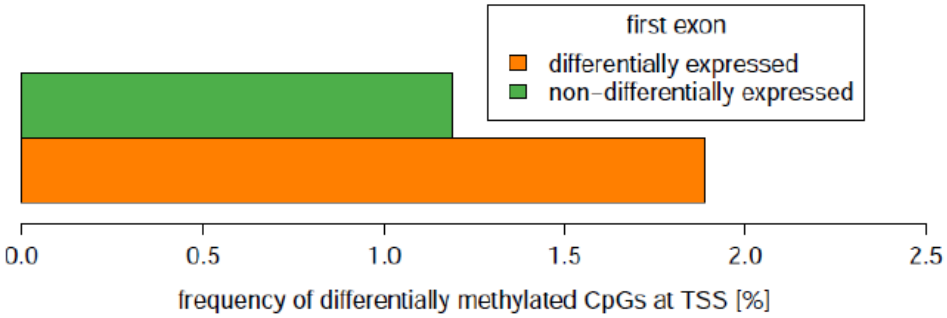
Genes with highly and lowly methylated promoters have different distribution of expression levels



Methylation and gene expression differences are negatively correlated



Promoters of differentially expressed genes are enriched in differentially methylated CpGs



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Genetic control of inter-individual variation in DNA methylation

Summary

- generated whole-genome methylation profiles at single nucleotide resolution in two rat strains (SHR & BN)
- quantified methylation differences between SHR & BN
  - inter-strain methylation differences are greater than intra-strain differences
  - majority of methylation differences are small
  - SNP frequency in proximity of differential methylation CpGs is increase
  - increased/decreased methylation associated with specific sequence motifs
- integrated methylation and gene expression profiles
  - clear but complex association of differential methylation and differential expression

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