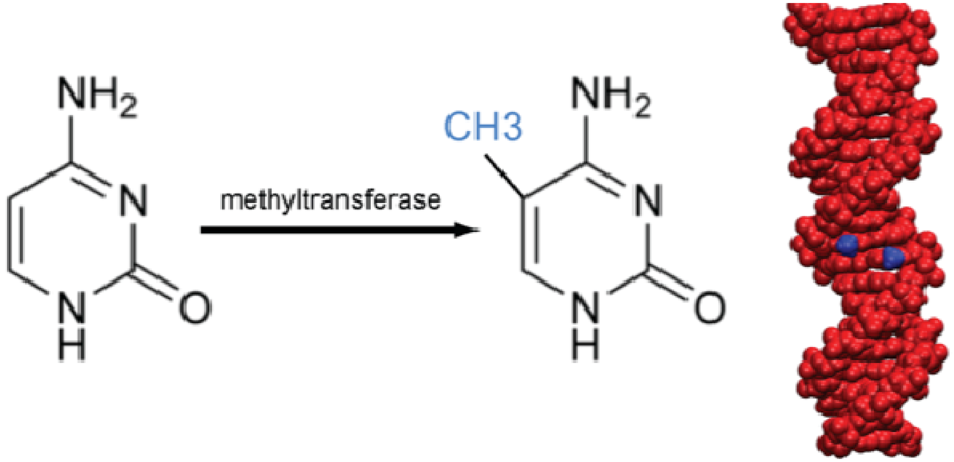
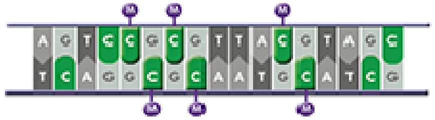
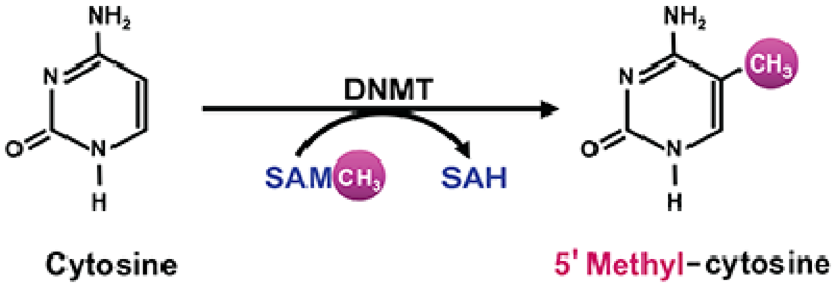
Presente in Mammiferi e altri vertebrati (prevalentemente CpG) e
Piante CpG, CpHpG e siti CpHpH (H = A, C or T)
limitata in alcuni insetti e assente nel lievito S.cerevisiae



DNA Methylation

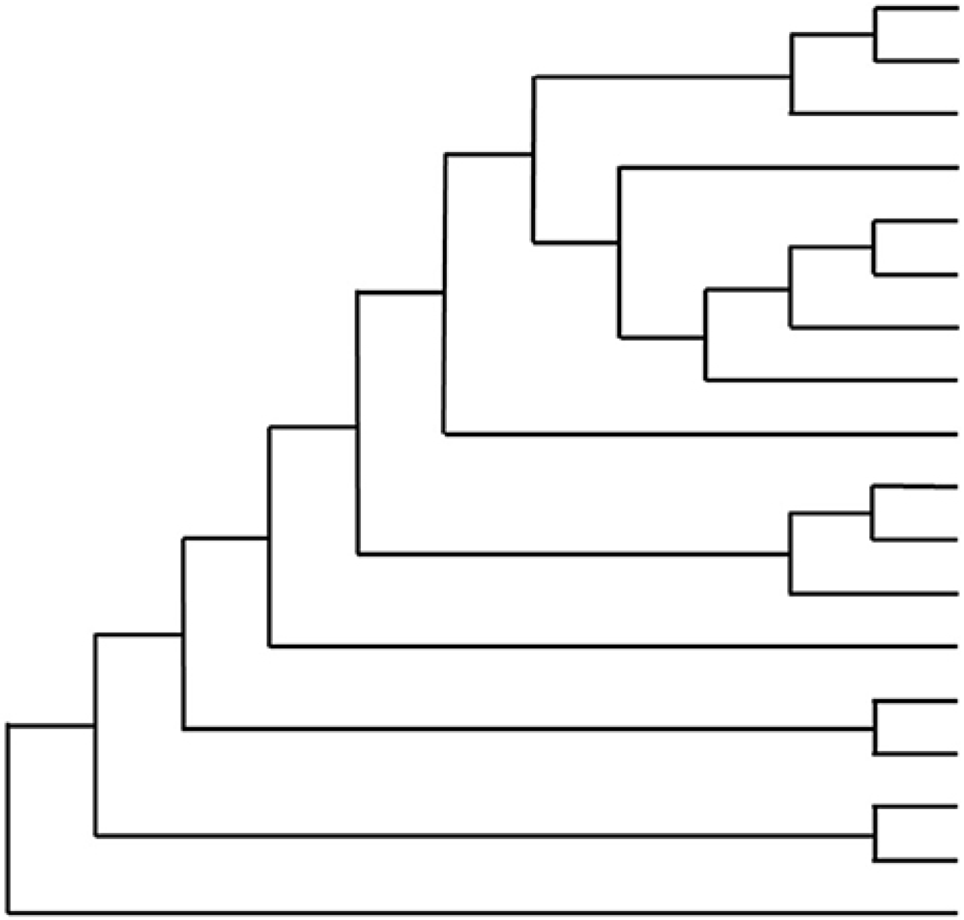
Methylating the cytosine of a CpG motif silences genes



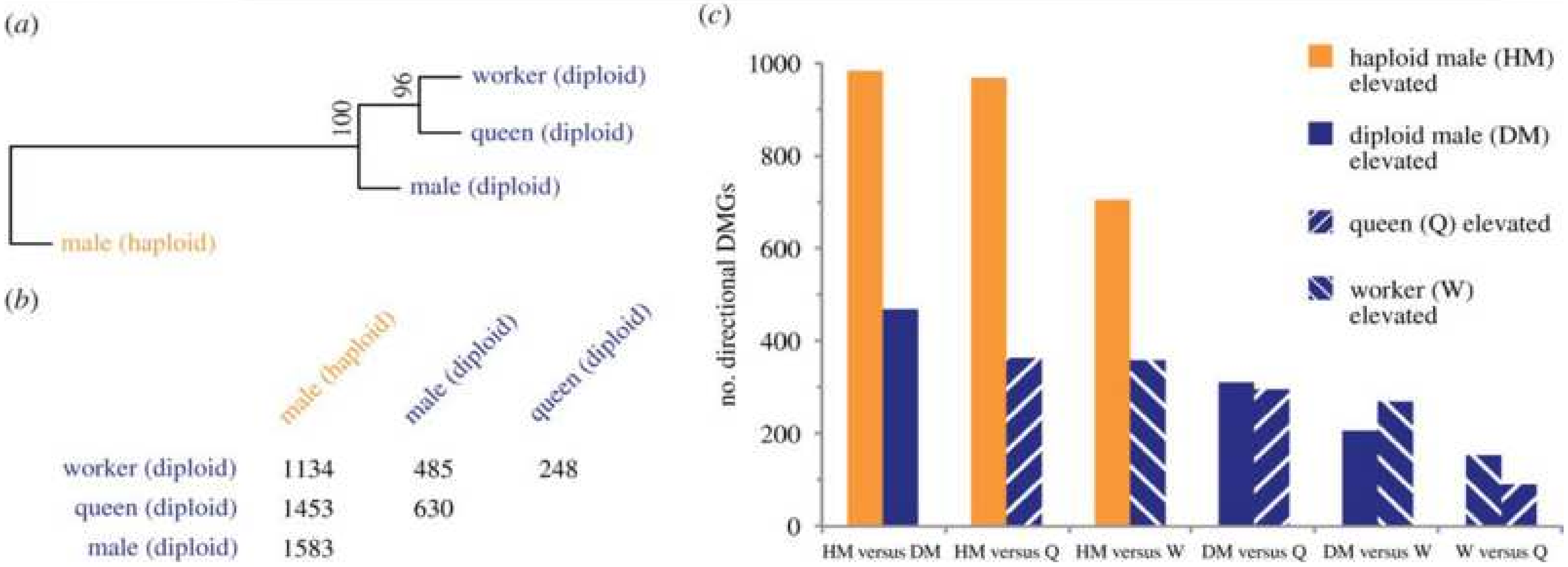


Metilazione delle citosine

Mus musculus 7,5%
Arabidopsis 14% (non solo CpG ma anche CpH e CpHpH)
Fungi 0,1-0,5%
(assente in Saccharomyces e Scizosaccharomyces ma 5% in Neurospora)
Drosophila 0,04%
Presente nelle api nelle sequenze codificanti (effetto sullo slicing)

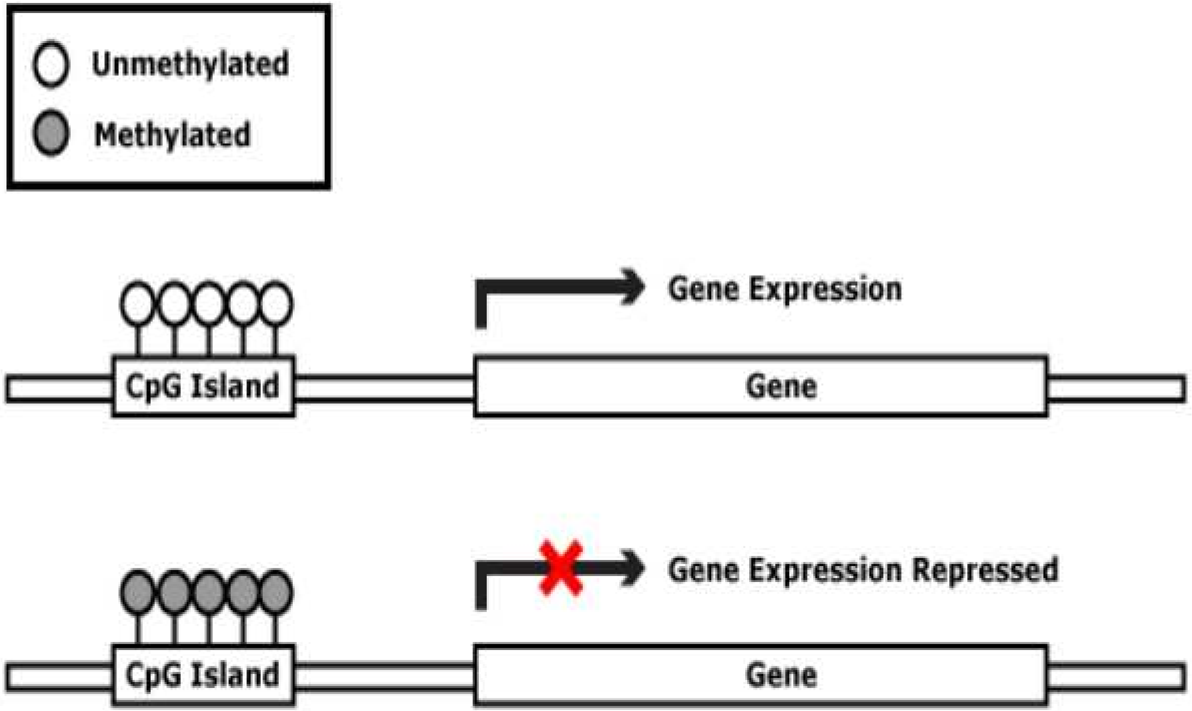


	DNMT1	DNMT2	DNMT3	TET
Apis mellifera	☆ ☆	☆	☆	☆
Bombus terrestris	☆	—	☆	☆
Lasioglossum albipes	☆ ☆	☆	☆	☆
Polistes canadensis	☆	☆	—	☆
Solenopsis invicta	☆	☆	☆	☆
Pogonomyrmex barbatus	☆	☆	☆	☆
Camponotus floridanus	☆	☆	☆	☆
Harpegnathos saltator	☆	☆	☆	☆
Nasonia vitripennis	☆☆☆	☆	☆	☆
Aedes aegypti	—	—	—	—
Drosophila melanogaster	—	☆	—	☆
Bombyx mori	☆	☆	—	☆
Tribolium castaneum	—	☆	—	☆
Acyrthosiphon pisum	☆ ☆	☆	☆	☆
Myzus persicae	☆	—	☆	—
Zootermopsis nevadensis	☆	☆	☆	☆
Locusta migratoria	☆	☆	☆	☆
Homo sapiens	☆	☆	☆☆☆	☆☆☆



	male (haploid)	male (diploid)	queen (diploid)
worker (diploid)	1134	485	248
queen (diploid)	1453	630	
male (diploid)	1583		

DNA Cytosine Methylation

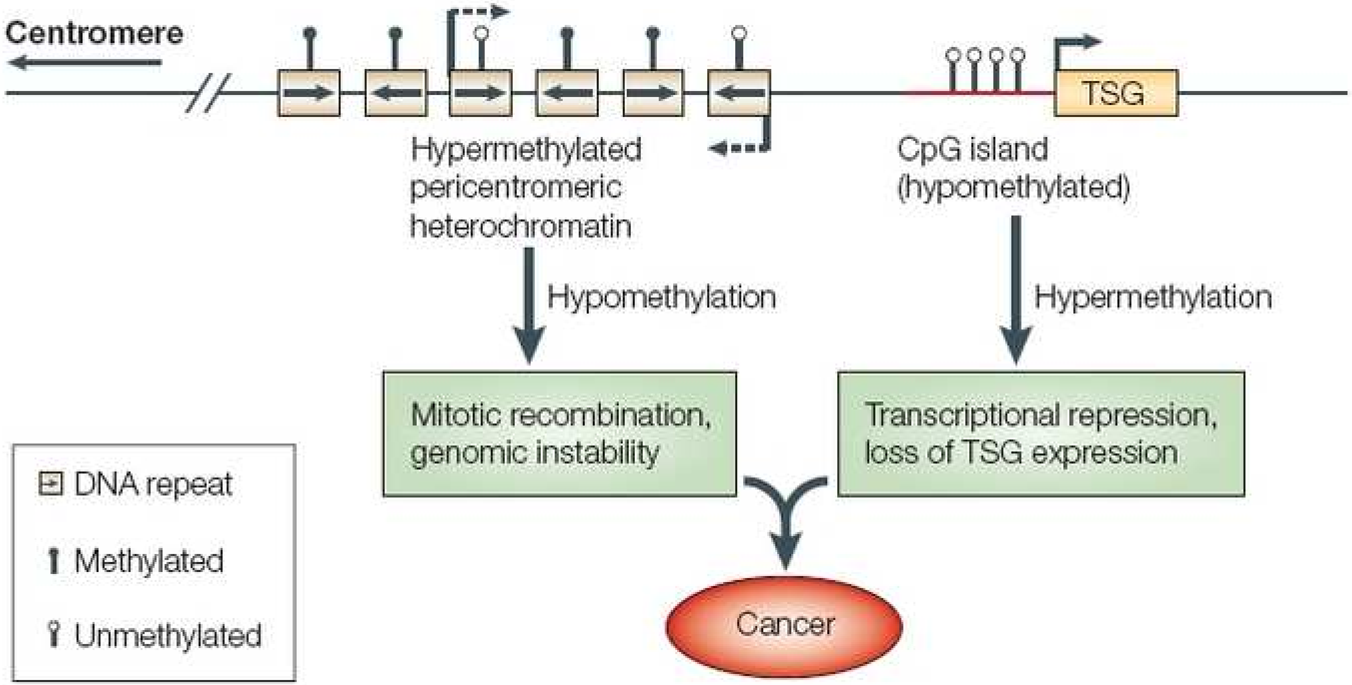


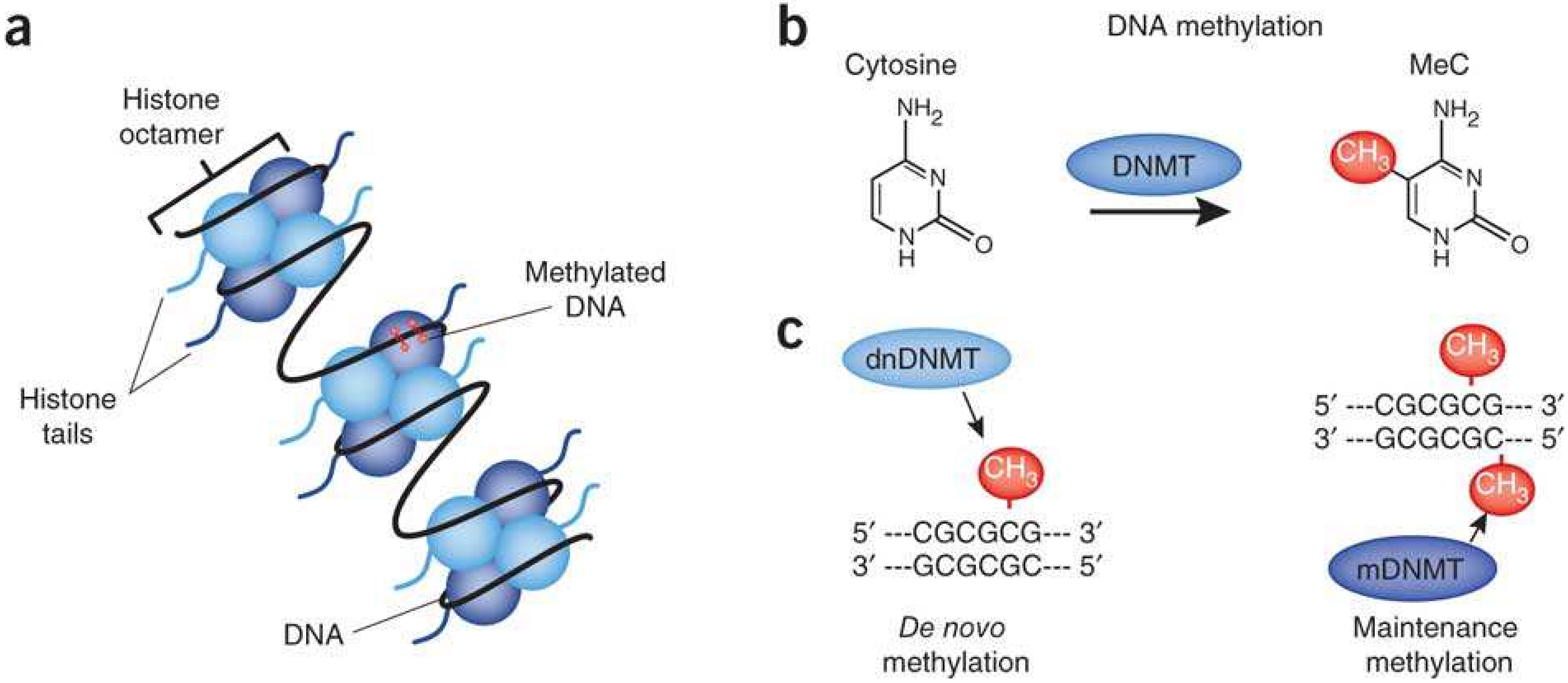
. Metivier, R. *et al.* Cyclical DNA methylation of a transcriptionally active promoter. *Nature* **452**, 45–50 (2008).

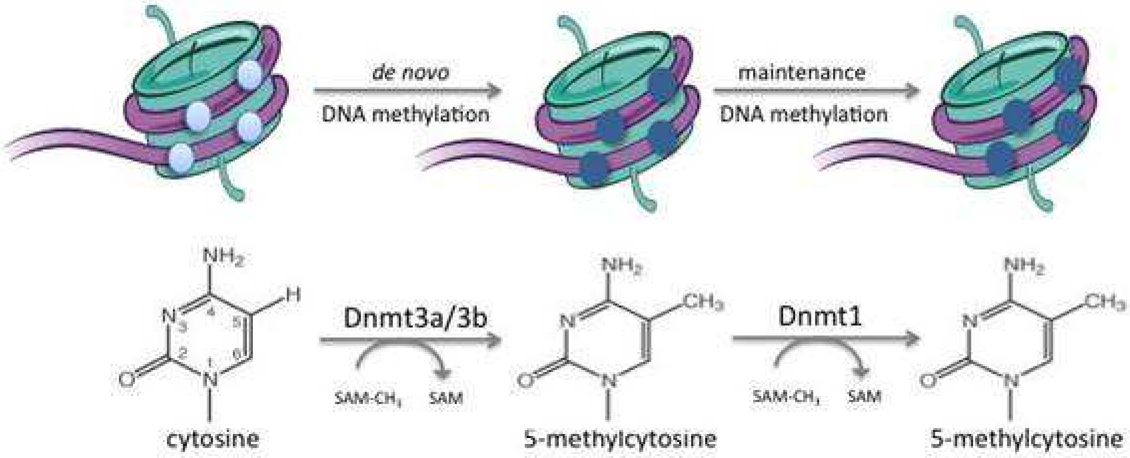
Le principali funzioni della metilazione sono collegate alla repressione della trascrizione:

- **Difesa contro i trasposoni**: la metilazione e' fondamentale per mantenere silenti i genomi dei trasposoni e dei retrotrasposoni

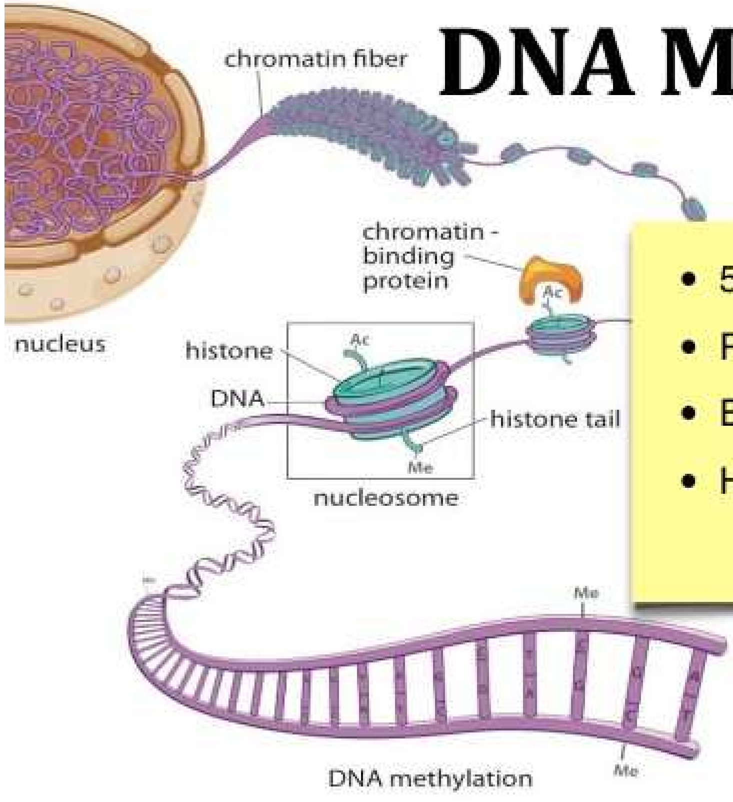
- **Regolazione genica:** la metilazione contribuisce a stabilire e mantenere uno stato trascrizionalmente inattivo (eterocromatina)


Centromere
TSG
Hypermethylated pericentromeric heterochromatin
CpG island (hypomethylated)
Hypomethylation
Hypermethylation
Mitotic recombination, genomic instability
Transcriptional repression, loss of TSG expression
Cancer
DNA repeat
Methylated
Unmethylated

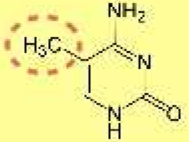

a
Histone octamer
Methylated DNA
Histone tails
DNA
b
DNA methylation
Cytosine
NH2
N
N
H
O
DNMT
MeC
CH3
NH2
N
N
H
O
c
dnDNMT
CH3
5′ ---CGCGCG--- 3′
3′ ---GCGCGC--- 5′
De novo methylation
CH3
5′ ---CGCGCG--- 3′
3′ ---GCGCGC--- 5′
CH3
mDNMT
Maintenance methylation

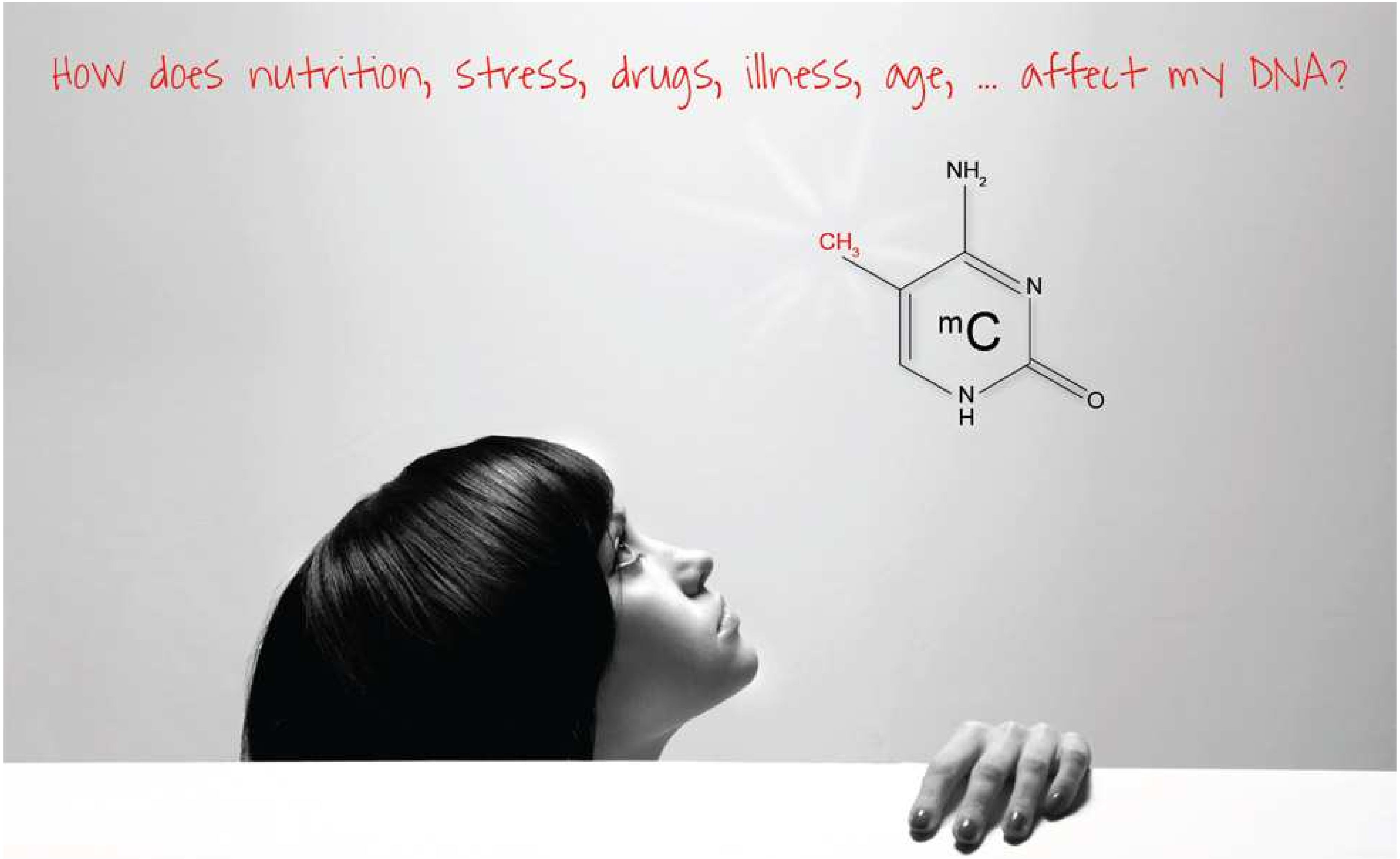

de novo
DNA methylation
maintenance
DNA methylation
NH2
H
N
3
4
5
2
1
6
O
N
cytosine
Dnmt3a/3b
SAM-CH3
SAM
NH2
CH3
N
O
N
5-methylcytosine
Dnmt1
SAM-CH3
SAM
NH2
CH3
N
O
N
5-methylcytosine

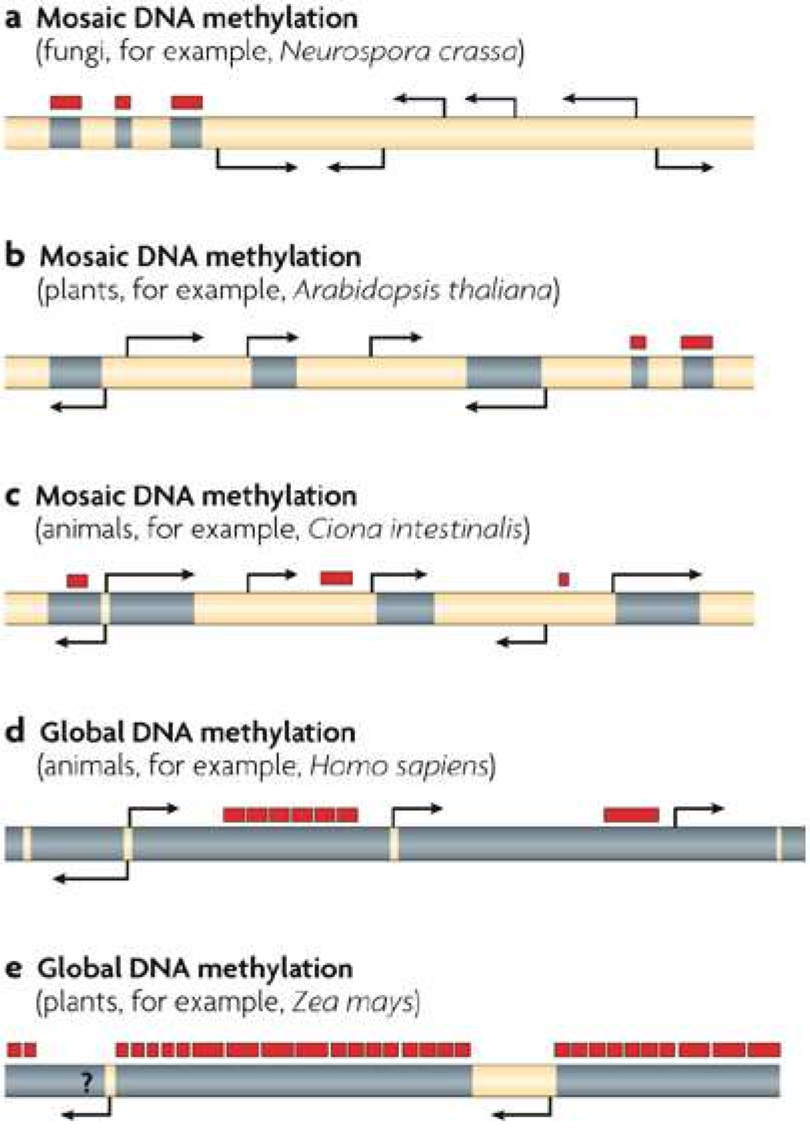
DNA Methylation



- 5 position of cytosine
- Regulation of gene expression
- Environment effect
- Heritable mark




How does nutrition, stress, drugs, illness, age, ... affect my DNA?
NH_2
CH_3
N
mC
N
H
O



a Mosaic DNA methylation
(fungi, for example, *Neurospora crassa*)

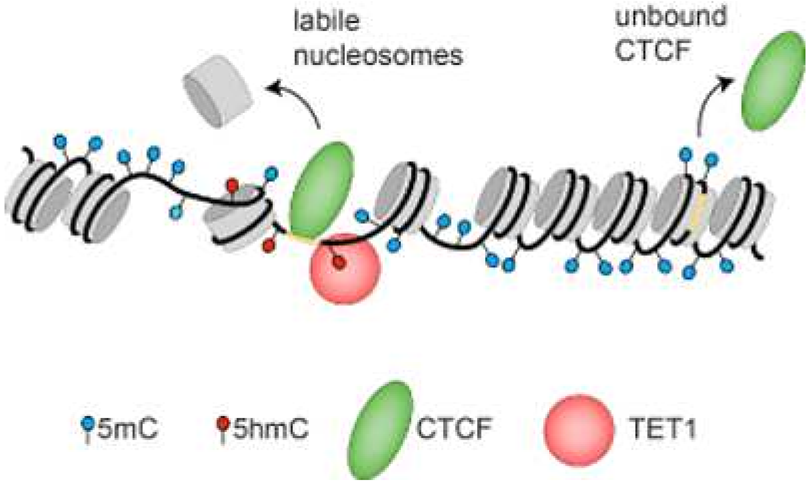
b Mosaic DNA methylation
(plants, for example, *Arabidopsis thaliana*)

c Mosaic DNA methylation
(animals, for example, *Ciona intestinalis*)

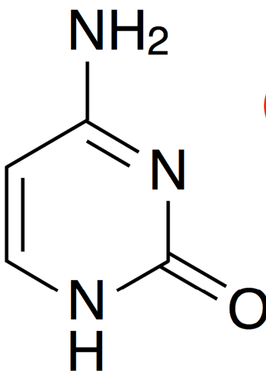
d Global DNA methylation
(animals, for example, *Homo sapiens*)

e Global DNA methylation
(plants, for example, *Zea mays*)

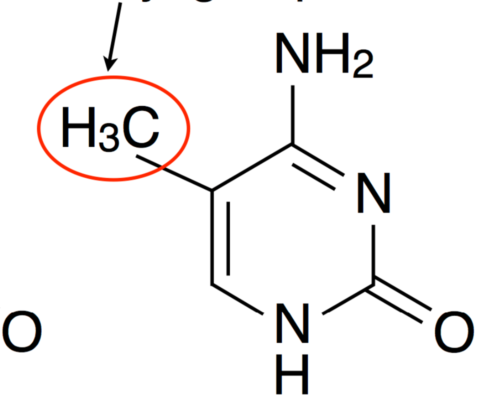
Nature Reviews | Genetics



Methyl group

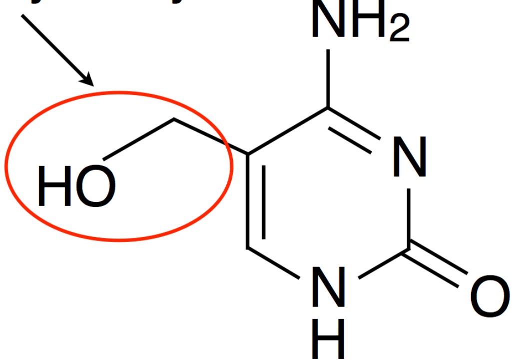


Cytosine

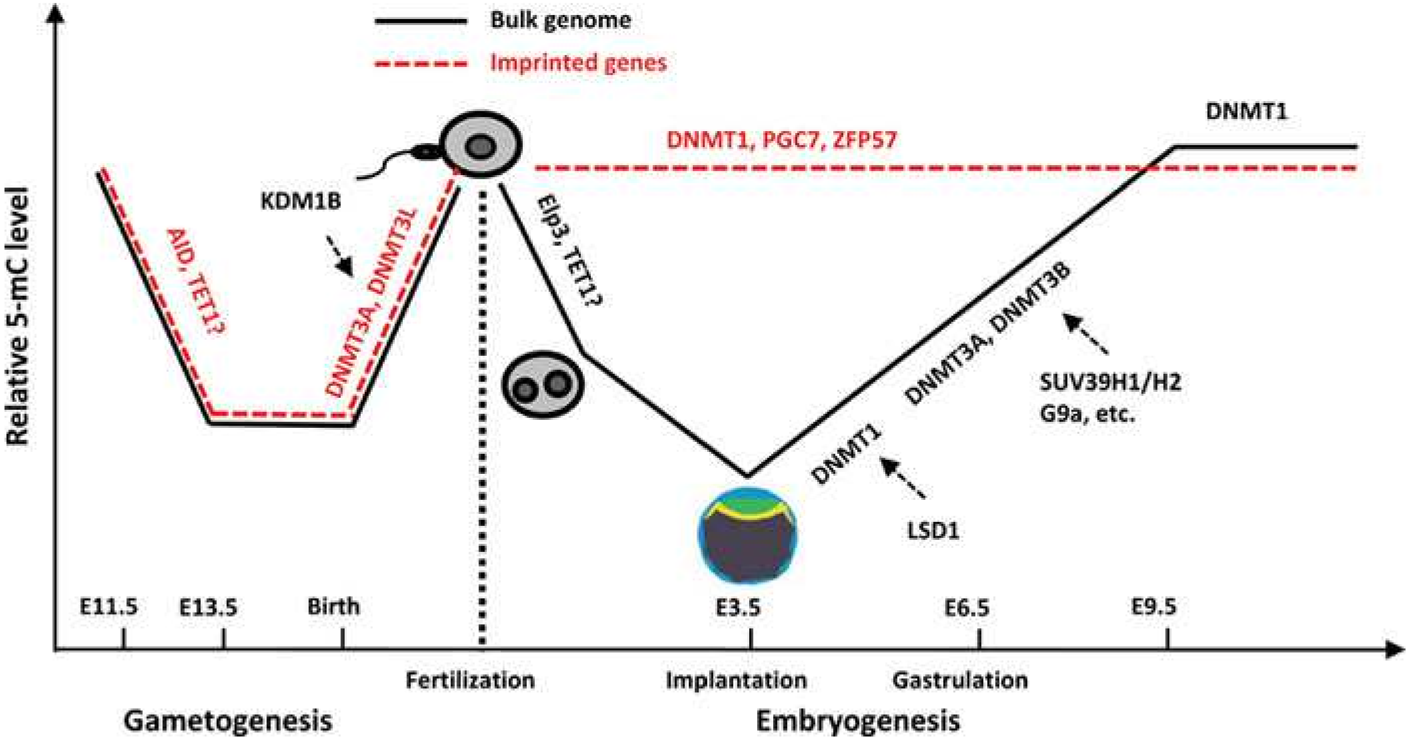


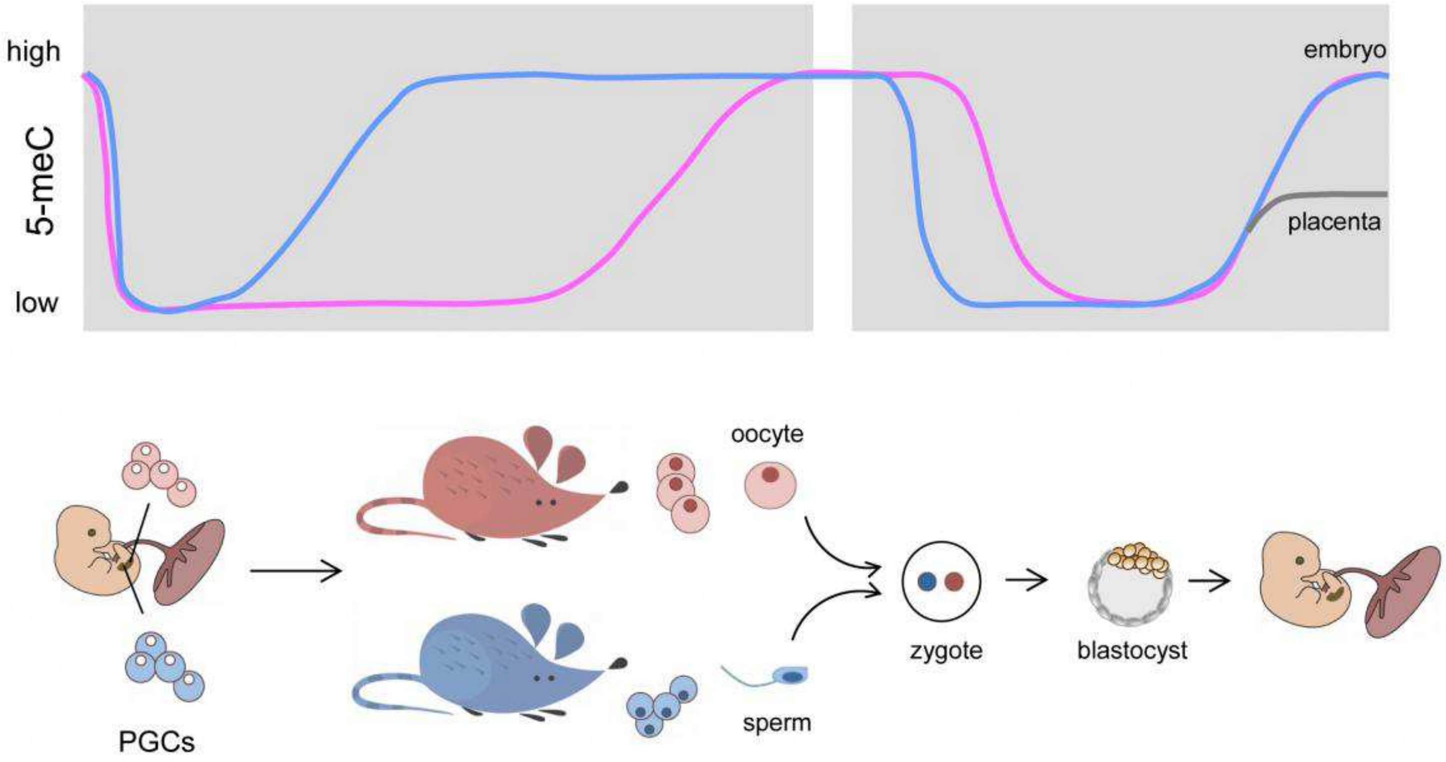
Methylcytosine

Hydroxymethyl group



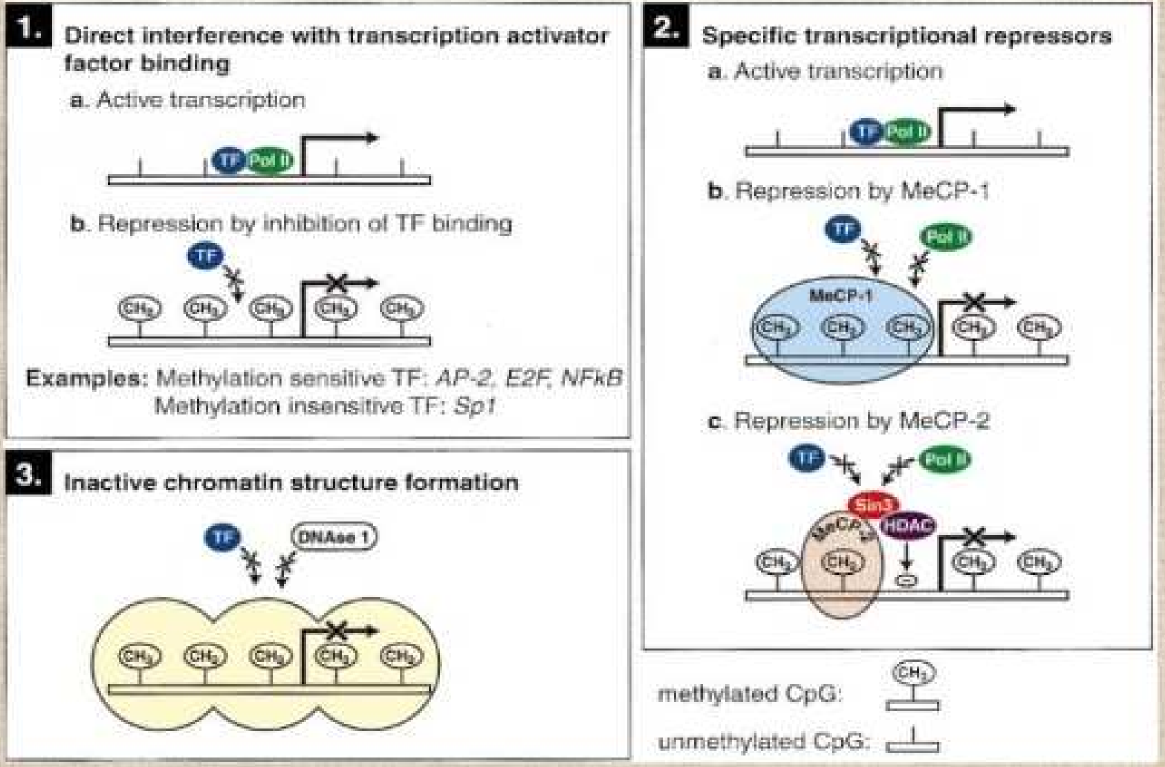
Hydroxymethylcytosine


Bulk genome
Imprinted genes
Relative 5-mC level
AID, TET1?
KDM1B
DNMT3A, DNMT3L
DNMT1, PGC7, ZFP57
Elp3, TET1?
DNMT1
DNMT3A, DNMT3B
SUV39H1/H2
G9a, etc.
DNMT1
LSD1
E11.5
E13.5
Birth
E3.5
E6.5
E9.5
Fertilization
Implantation
Gastrulation
Gametogenesis
Embryogenesis

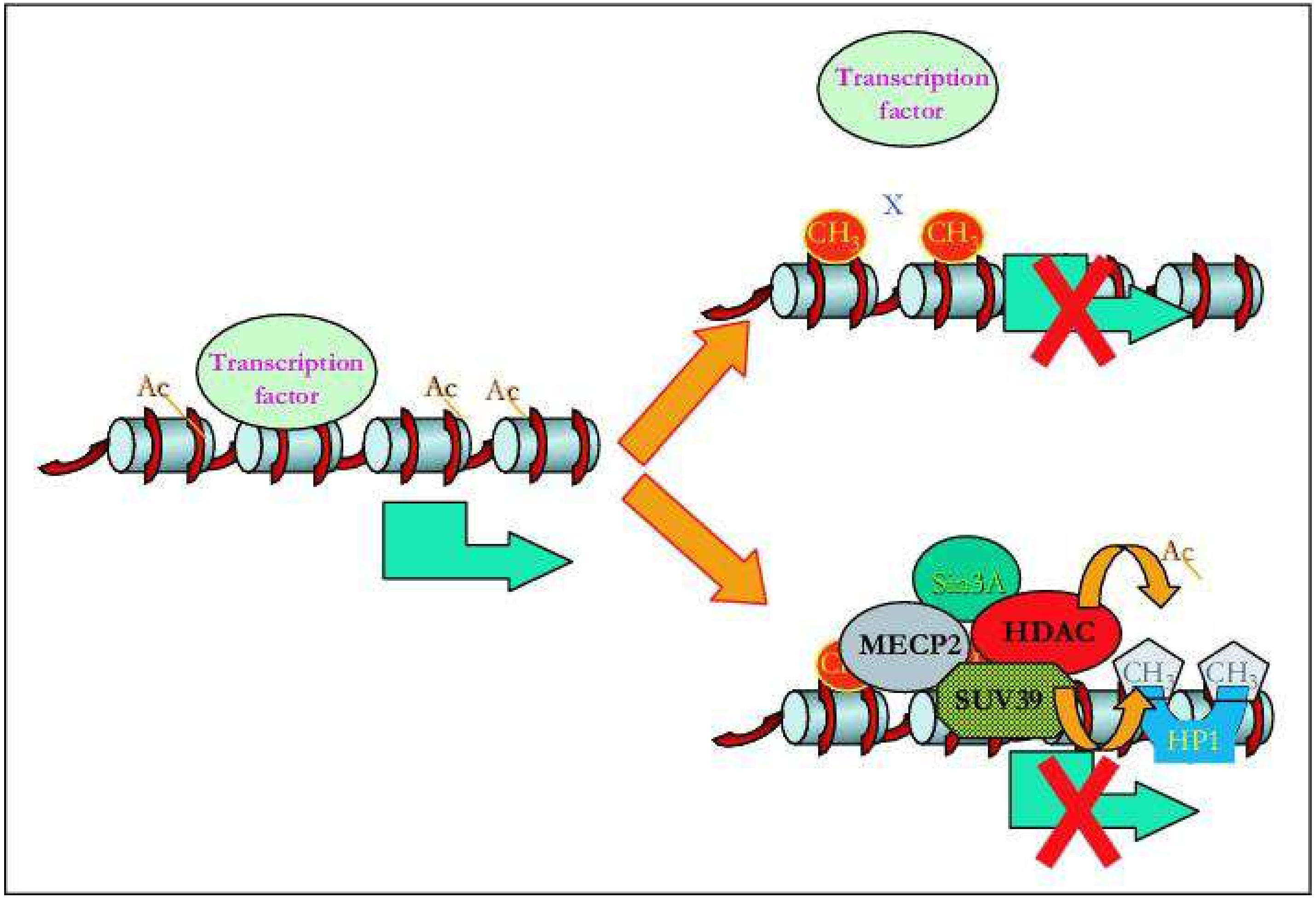


Dynamics of DNA methylation in germ cells and early mouse development

DNA methylation and transcriptional repression

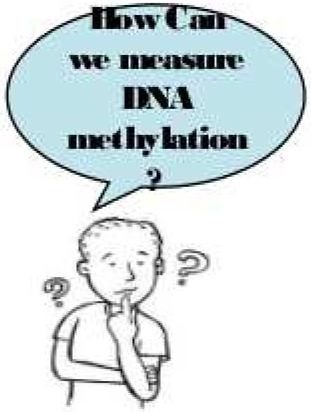


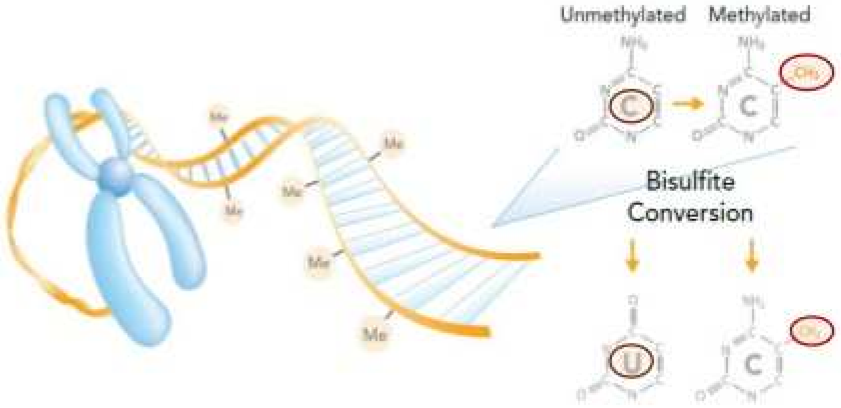
Singal and Ginder, 1999

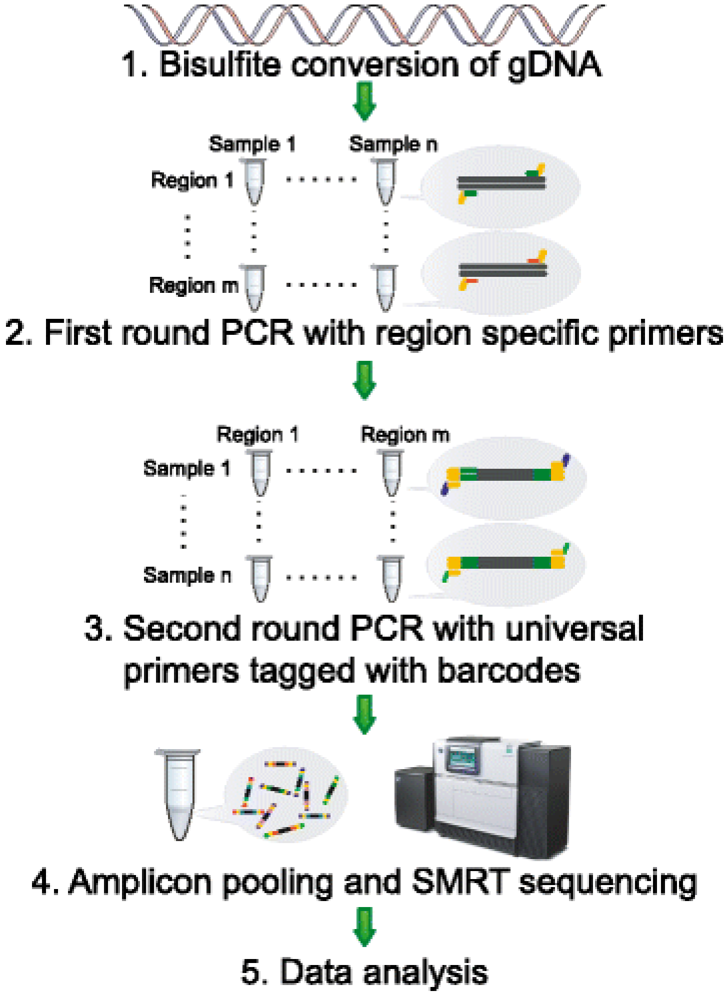

Transcription factor
X
CH_3
CH_3
Ac
Transcription factor
Ac
Ac
Ac
Sin3A
MECP2
HDAC
CH_3
CH_3
SUV39
HP1

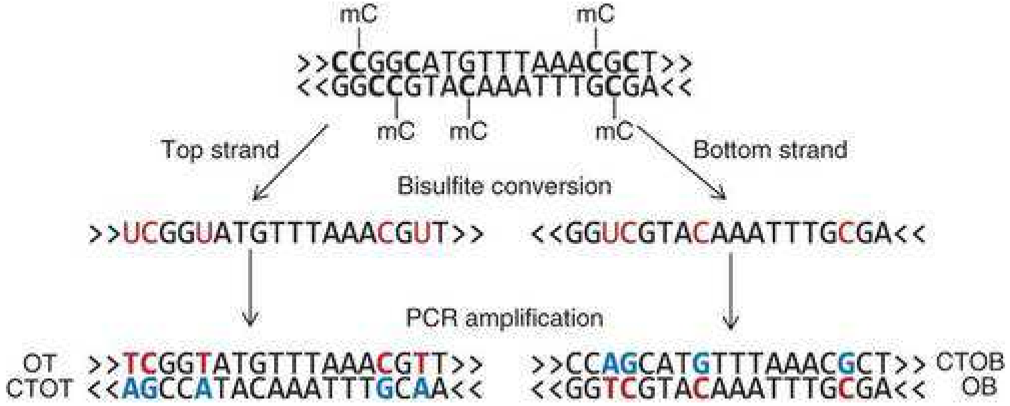
Introduction

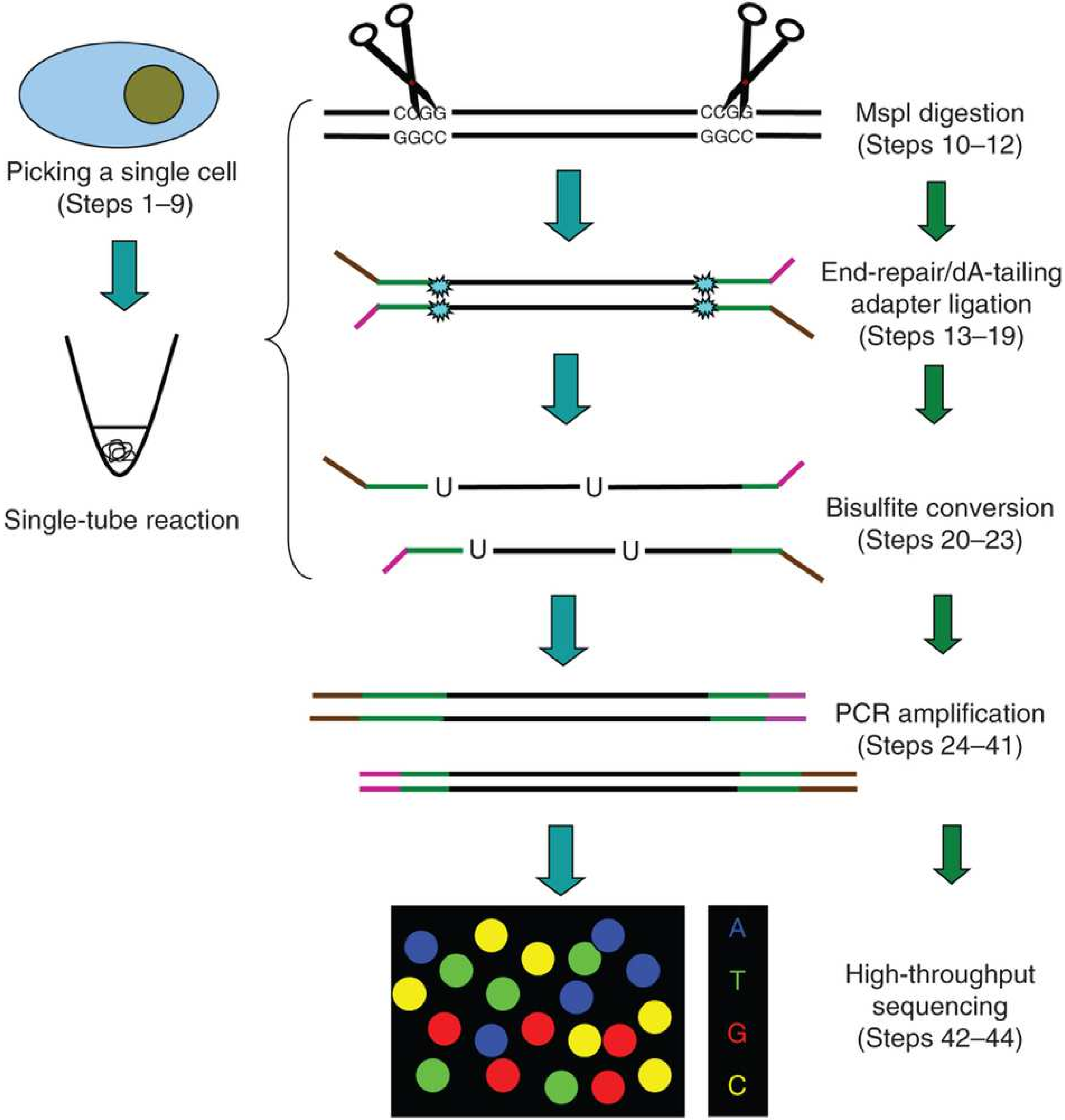
- Measuring DNA Methylation



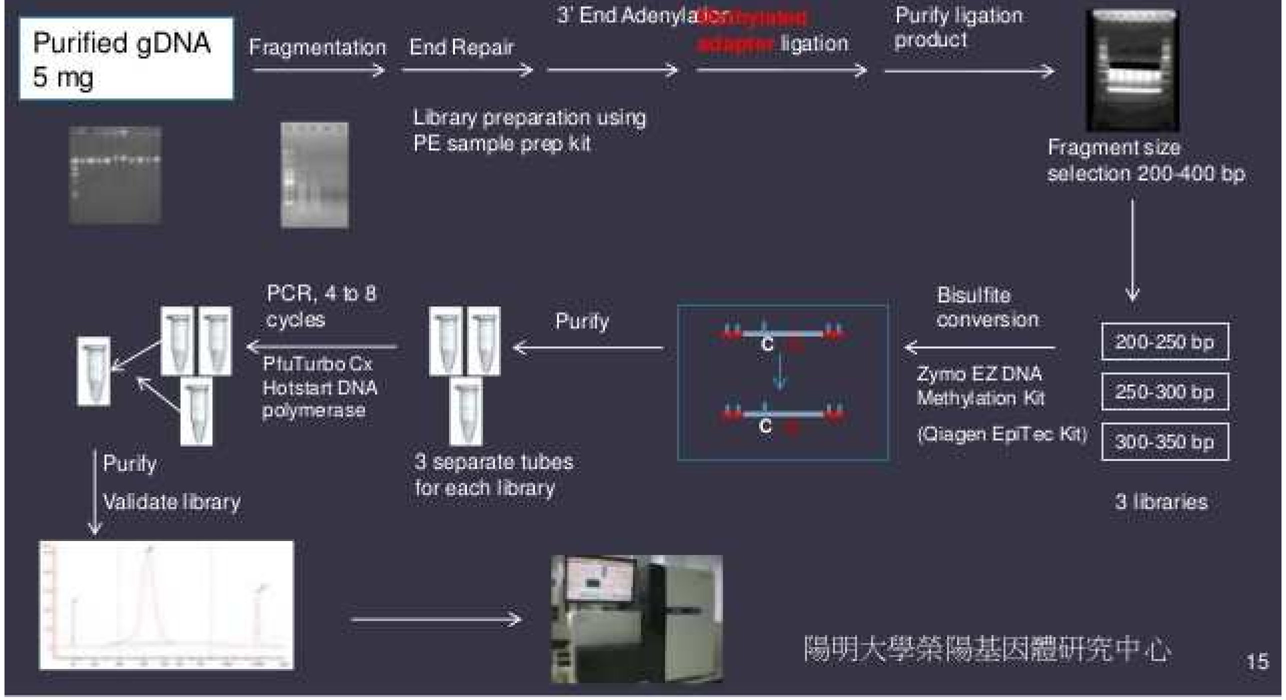



1. Bisulfite conversion of gDNA
Sample 1
Sample n
Region 1
Region m
2. First round PCR with region specific primers
Region 1
Region m
Sample 1
Sample n
3. Second round PCR with universal primers tagged with barcodes
4. Amplicon pooling and SMRT sequencing
5. Data analysis


mC
mC
>>CCGGCATGTTTAAACGCT>>
<<GGCCGTACAAATTTGCGA<<
mC
mC
mC
Top strand
Bottom strand
Bisulfite conversion
>>UCGGUATGTTTAAACGUT>>
<<GGUCGTACAAATTTGCGA<<
PCR amplification
OT
CTOT
>>TCGGTATGTTTAAACGTT>>
<<AGCCATACAAATTTGCAA<<
>>CCAGCATGTTTAAACGCT>>
<<GGTCGTACAAATTTGCGA<<
CTOB
OB


Picking a single cell
(Steps 1–9)
Single-tube reaction
CCGG
GGCC
CCGG
GGCC
MspI digestion
(Steps 10–12)
End-repair/dA-tailing
adapter ligation
(Steps 13–19)
U
U
U
U
Bisulfite conversion
(Steps 20–23)
PCR amplification
(Steps 24–41)
A
T
G
C
High-throughput
sequencing
(Steps 42–44)

Whole Genome Bisulfite Sequencing Library Construction



Purified gDNA
5 mg

Fragmentation

End Repair

3' End Adenylation

Methylated adapter ligation

Purify ligation product

Library preparation using PE sample prep kit

Fragment size selection 200-400 bp

200-250 bp

250-300 bp

300-350 bp

3 libraries

Bisulfite conversion

Zymo EZ DNA Methylation Kit

(Qiagen EpiTec Kit)

Purify

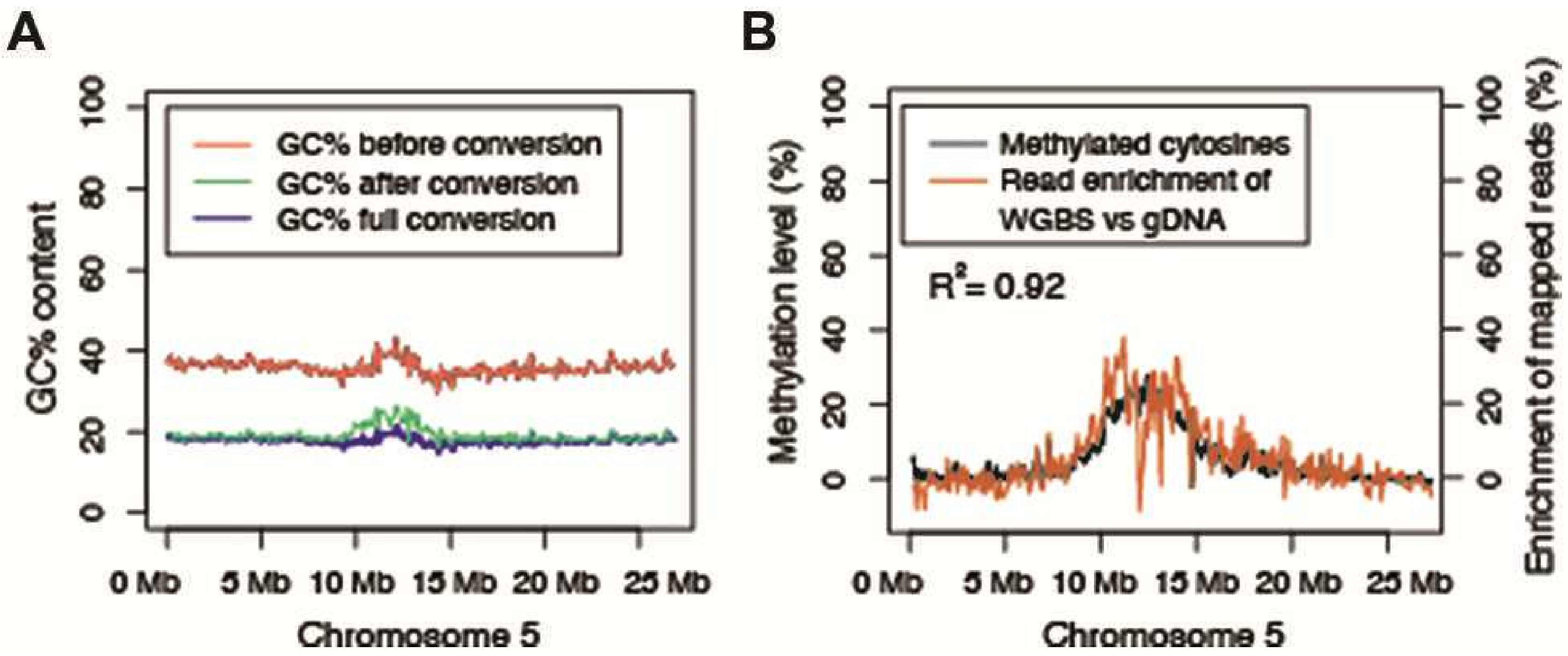
3 separate tubes for each library

PCR, 4 to 8 cycles

PfuTurbo Cx Hotstart DNA polymerase

Purify

Validate library


A
GC% content
GC% before conversion
GC% after conversion
GC% full conversion
0
20
40
60
80
100
0 Mb
5 Mb
10 Mb
15 Mb
20 Mb
25 Mb
Chromosome 5
B
Methylation level (%)
Enrichment of mapped reads (%)
Methylated cytosines
Read enrichment of WGBS vs gDNA
R^2= 0.92
0
20
40
60
80
100
0 Mb
5 Mb
10 Mb
15 Mb
20 Mb
25 Mb
Chromosome 5