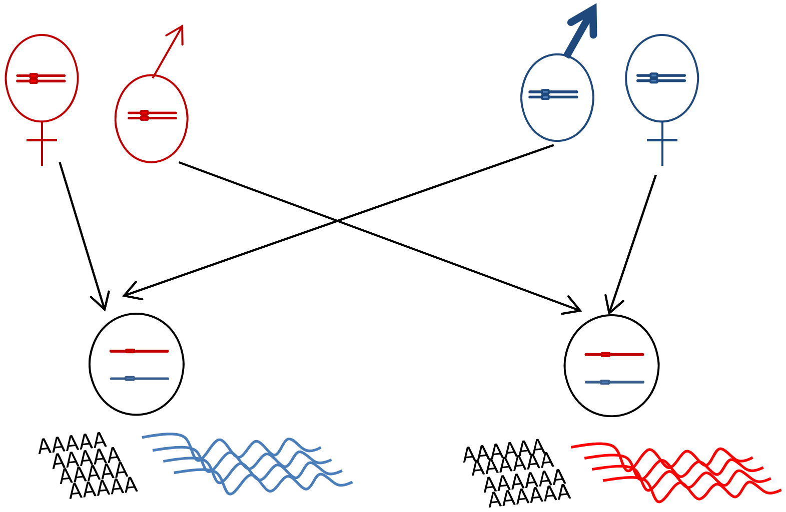
Analyse de méthylation de l'ADN dans l'embryon de poulet par séquençage bisulfite

Frédérique Pitel – Céline Noirot

Experimental design enabling to detect parent-of-origin dependent expression

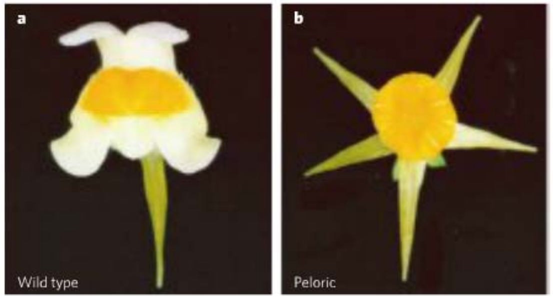
- Parental SNPs to deduce origin of the allele

- Reciprocal cross
  - 12 embryos (E 4.5) from each cross (6 males and 6 females)
  - RNASeq



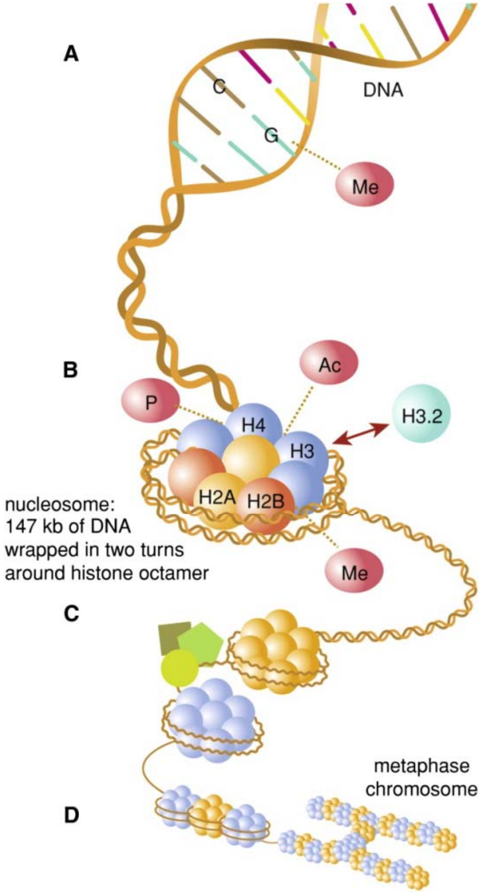
- Search for regions with transcripts presenting a bias in expression
  - Imprinting ?
  - Allele Specific Expression

Methylation analyses



Cubas et al, 1999 (Bird 2007)

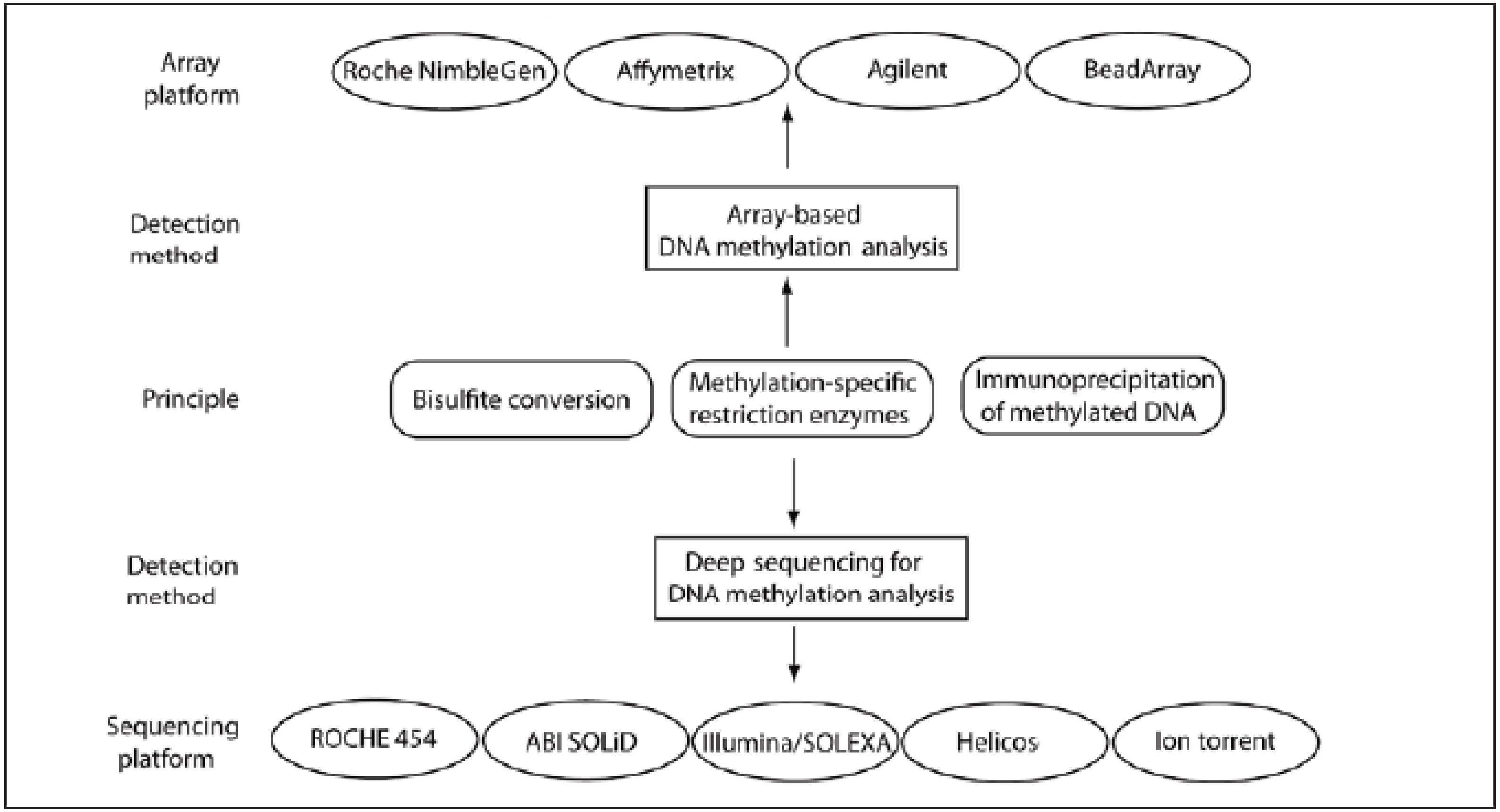
Morgan et al, 1999



Denk & McMahon 2012

- Genome-wide investigation of DNA methylation at single-base resolution in the chicken embryo
    - Methylome description
    - Comparison expression / methylation level

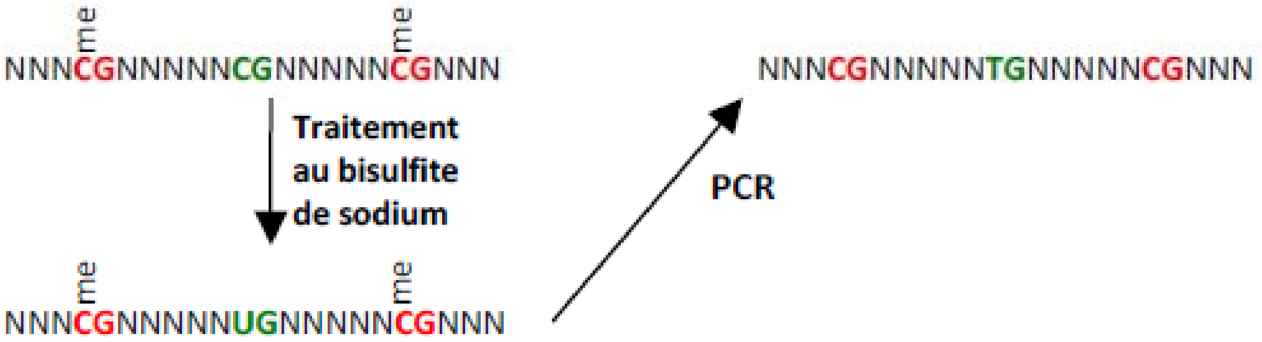
Technologies for methylation studies



Bisulfite Sequencing (BS-seq)

- Two embryos as a test (RNA + DNA Seq)
  - NextFlex bisulfite sequencing kit (BIOO Scientific Corp)
  - Control DNA: pUC19 DNA (Zymo Research), methylated or not

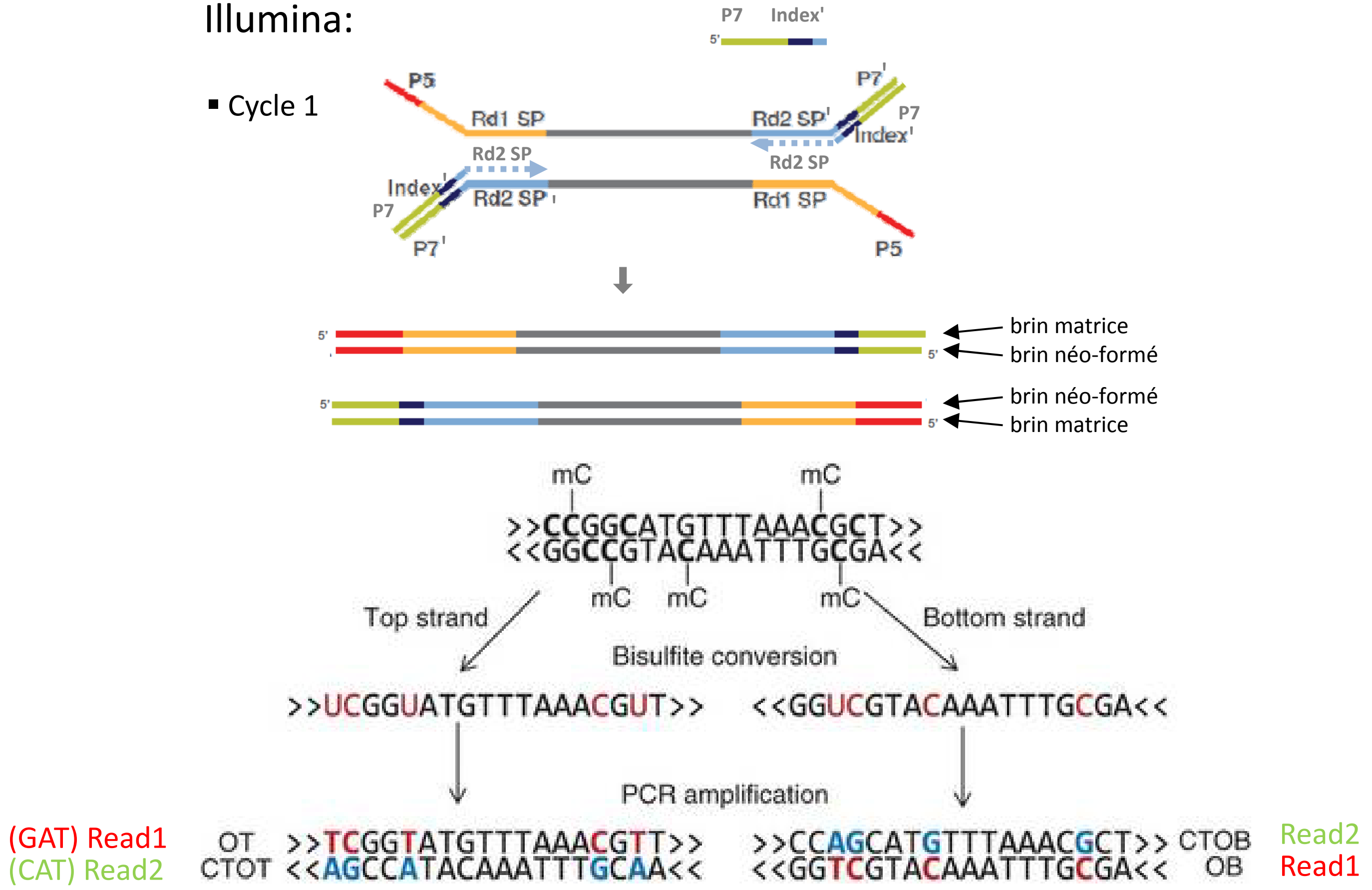
- Methylated C not converted by bisulfite



Methylation analysis (adapters ligation – conversion – PCR)

Illumina:

- Cycle 1



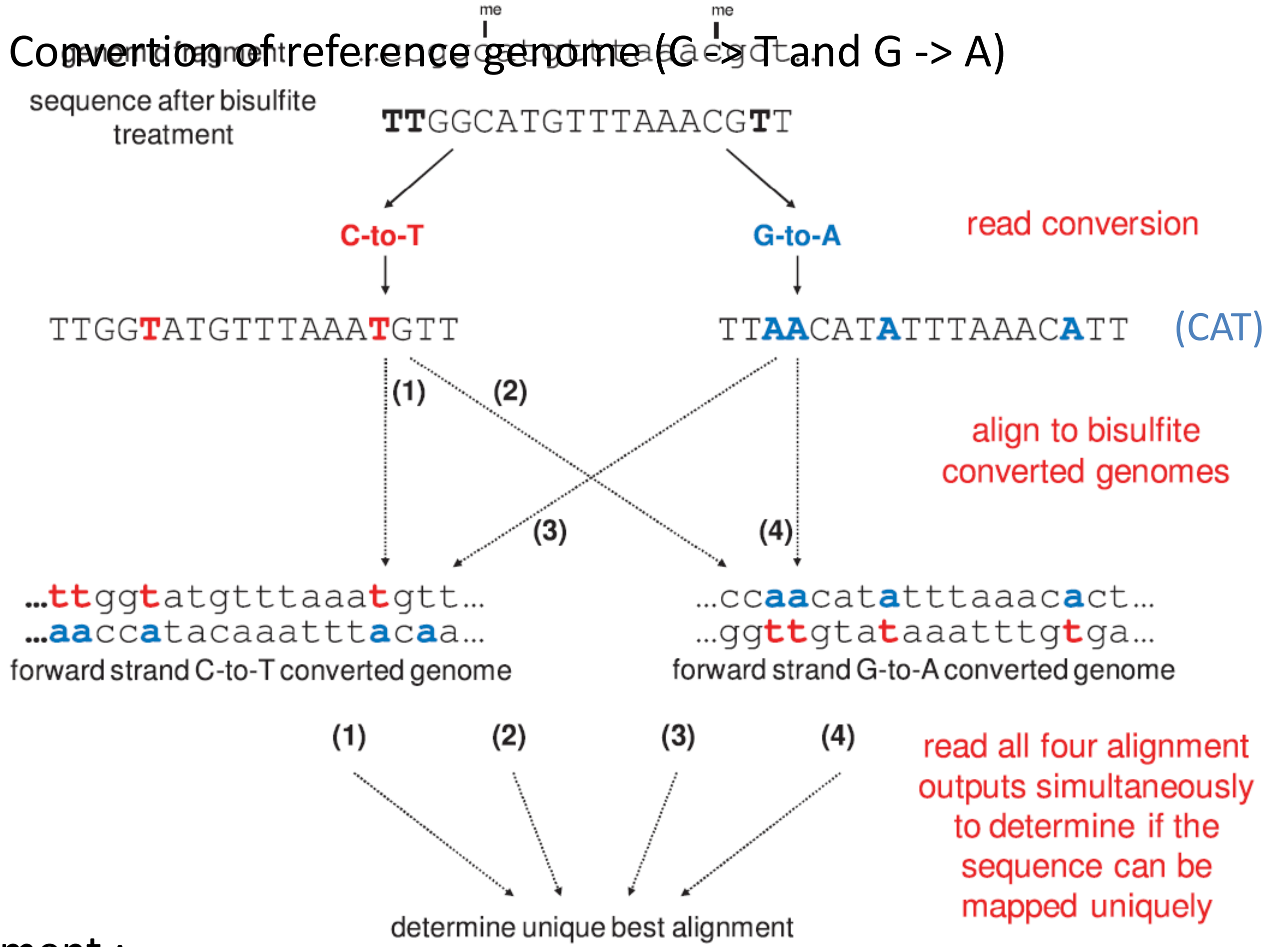
Krueger et al 2012

OT : Original Top
OB : Original Bottom

CTOT : Complementary to Original Top
CTOB : Complementary to Original Bottom

Bismark

Convertion of reference genome (C -> T and G -> A)



(GAT)

(CAT)

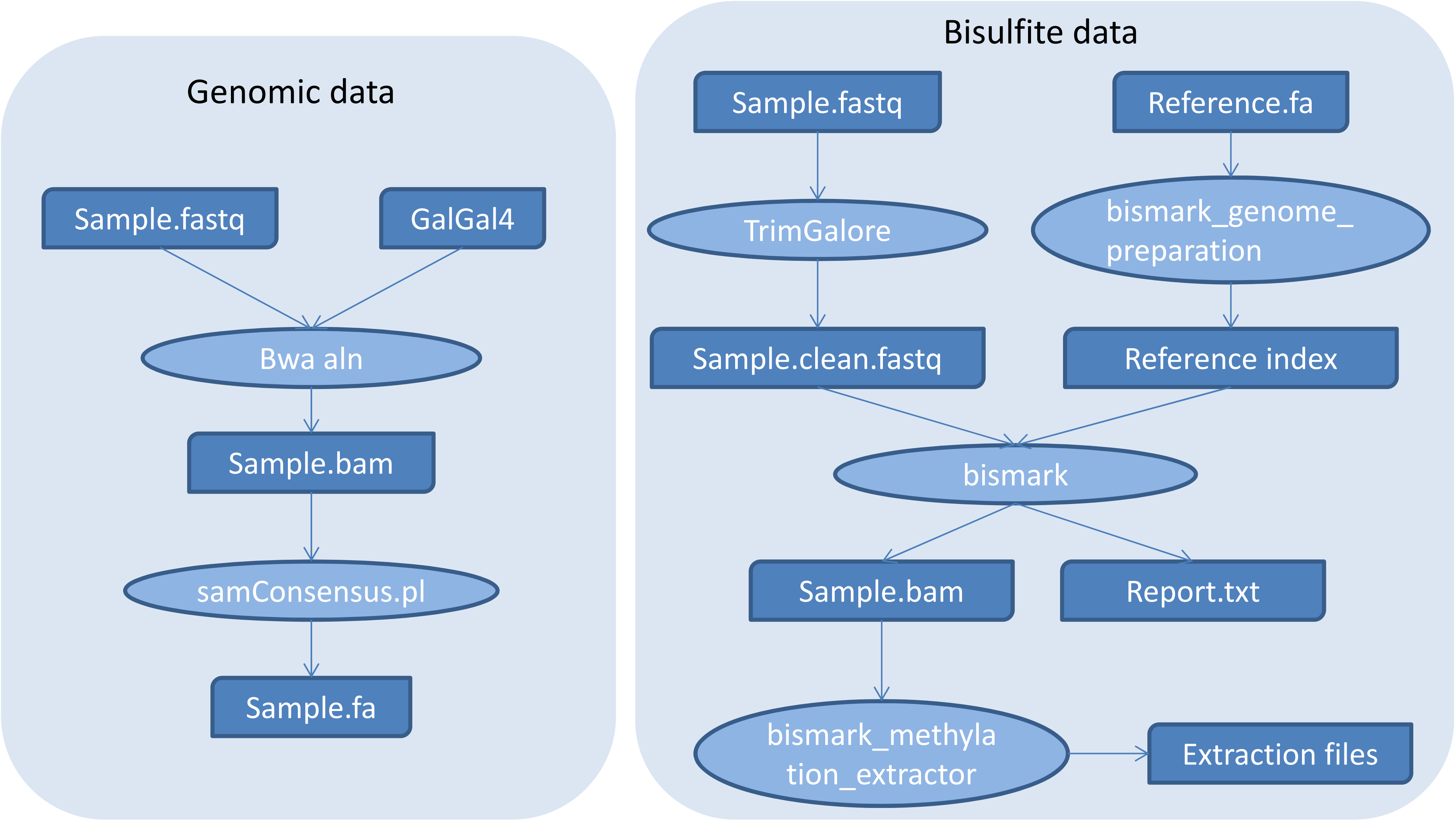
Directionnal paired alignment :

Read1 (OB or OT)

Read2 (CTOB or CTOT)

Krueger et al 2012

Bioinformatics traitments


Genomic data

Sample.fastq

GalGal4

Bwa aln

Sample.bam

samConsensus.pl

Sample.fa

Bisulfite data

Sample.fastq

Reference.fa

TrimGalore

bismark_genome_preparation

Sample.clean.fastq

Reference index

bismark

Sample.bam

Report.txt

bismark_methylation_extractor

Extraction files

Bioinformatics traitments (DNA)

- Reference genome : ICGSC Gallus_gallus-4.0/galGal4

- Genomic sample for both embryos
  - Emb1 : 180M reads paired (93.4% mapped)
  - Emb2 : 209M reads paired (93.1% mapped)

- Generation of new reference sample specific
  - > Reduce putative biais due to SNPs

Bioinformatics traitments (BS-seq)

- Cleanning : TrimGalore
  - Based on Cutadapt
  - Discard reads which length < 20bp

- BS-seq Data (nb reads paired)

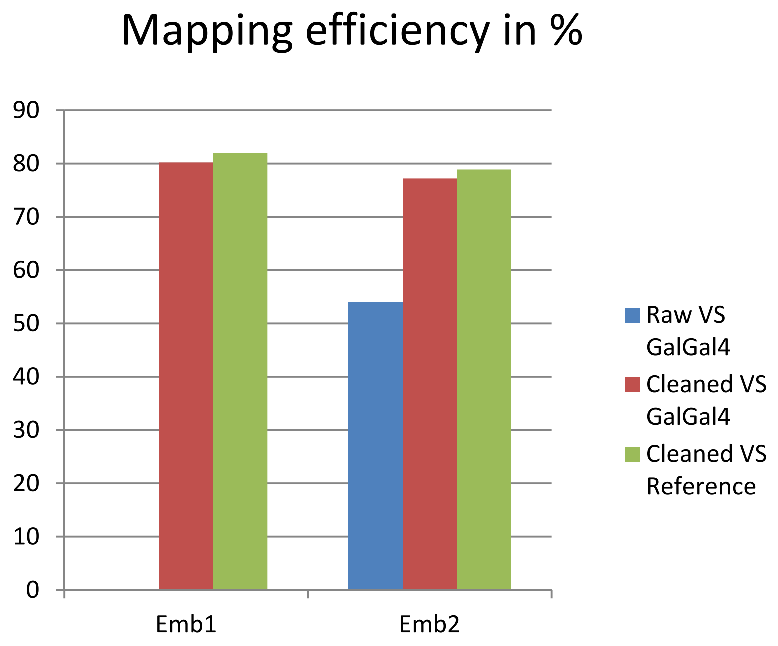
	Raw	Trimmed	Removed
Emb 1	157,230,624	68,430,229	1,099,914
Emb 2	164,728,172	70,697,424	918,796

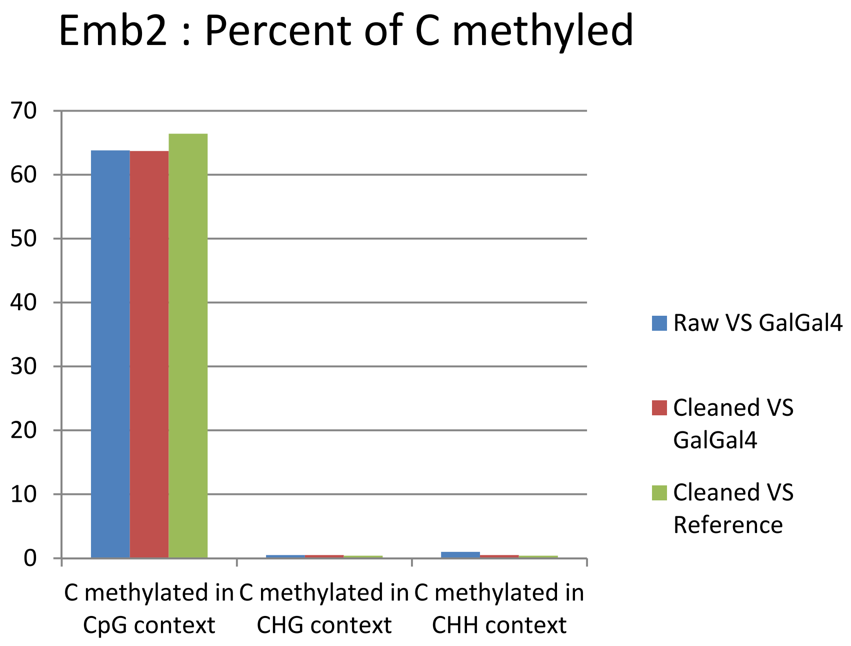
Bioinformatics traitments (BS-seq)

- Tests realized :
  - Alignment of raw data on GalGal4 (Emb2)
  - Alignment of cleaned data on GalGal 4 (Emb 1 & 2)
  - Alignment of cleaned data on sample reference (Emb 1 & 2)

Alignment results

- Outputs : Bam + Report.txt
- Depth = 14.3 x





Methylation calling results

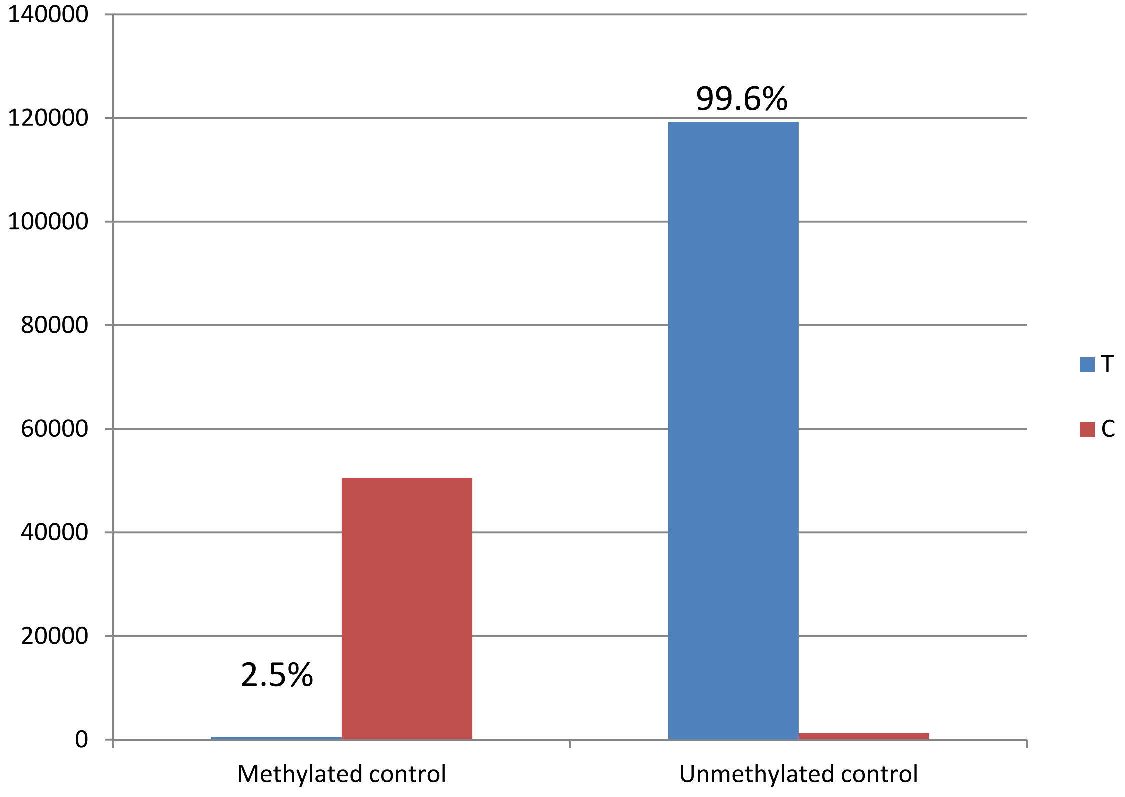
- Extraction of methylation with bismark_methylation_extractor
- Options :

  --report --bedGraph --counts

  --cytosine_report --no_overlap --CX_context
- Outputs:
  - report.txt
  - BEDgaph
  - CHG_OB.txt, CHG_OT.txt, CHH_OB.txt, CHH_OT.txt, CpG_OB.txt, CpG_OT.txt
  - CX_report.txt

Controls

pUC19 DNA (Zymo Research), methylated or not



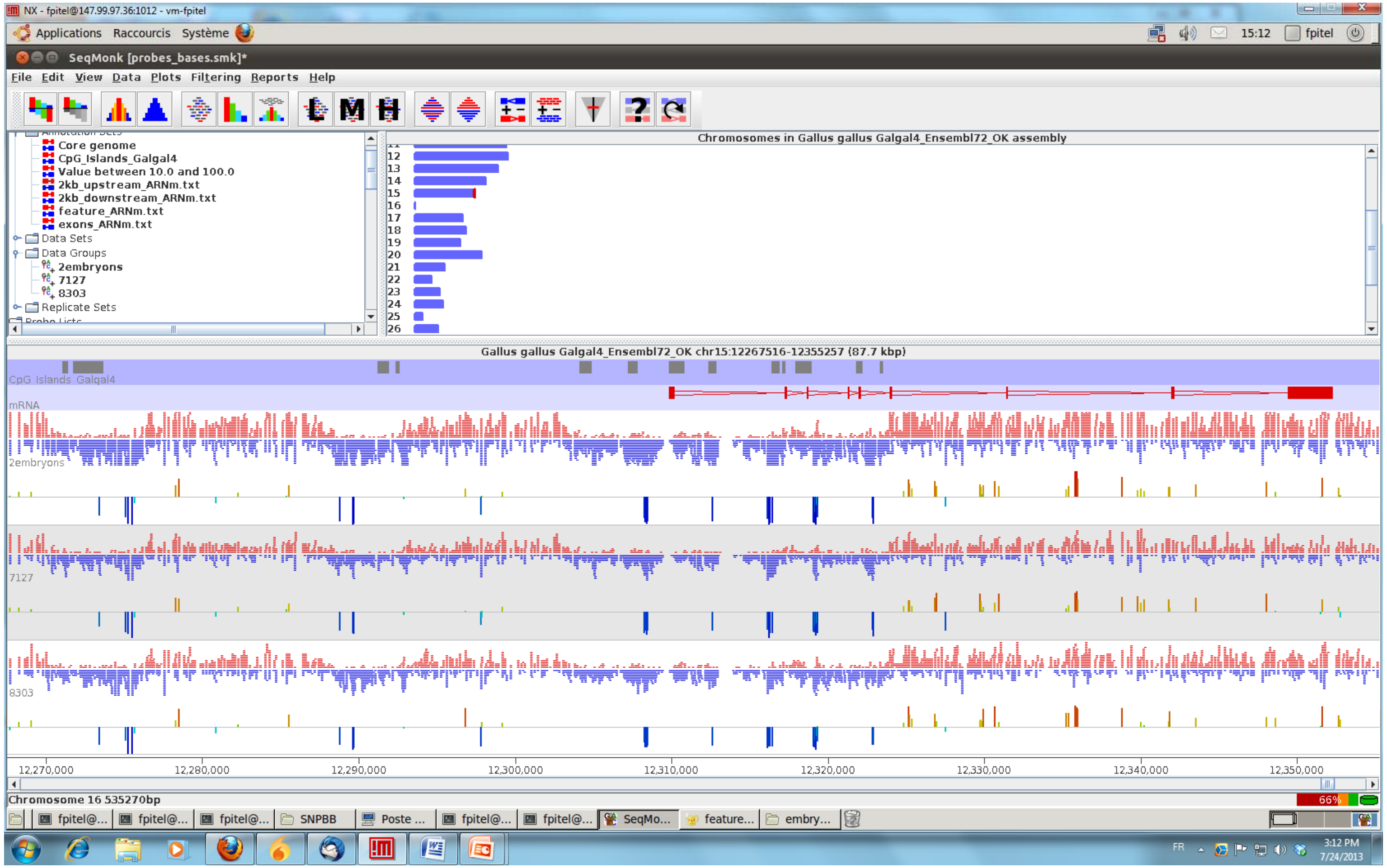
CpG

Overconversion

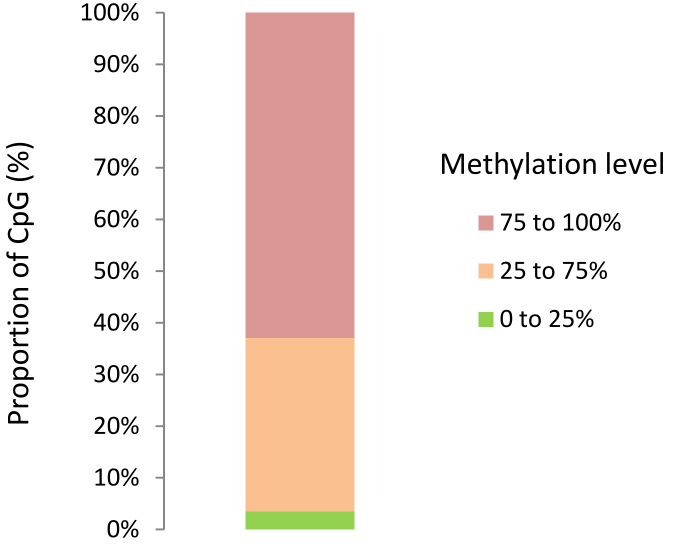
Conversion

Non CpG		
Non converted C	*2593*	*0.40228432*
Total	*644569*	

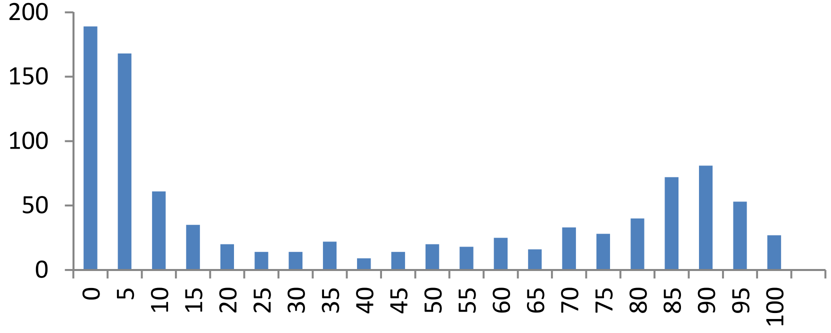
SeqMonk visualization



Methylation level per genomic feature

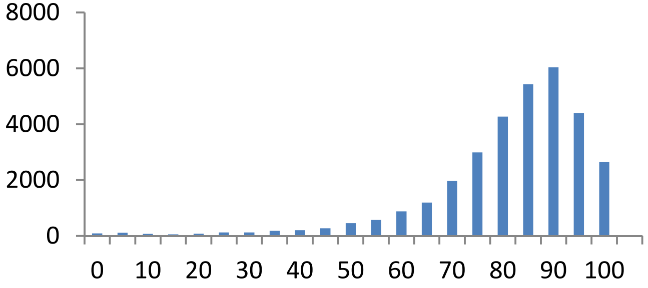


CpG islands

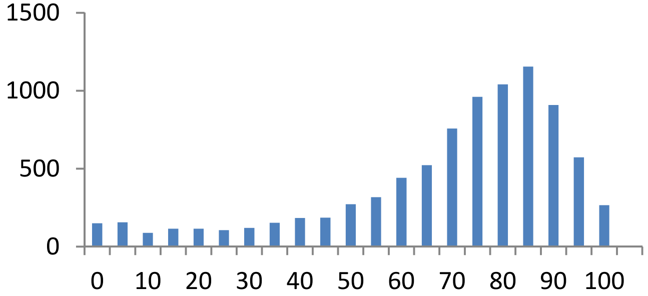


- Similar to other vertebrates

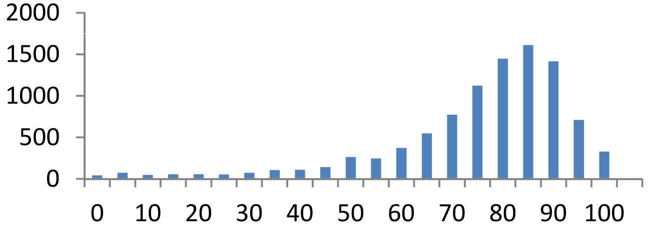
Exons



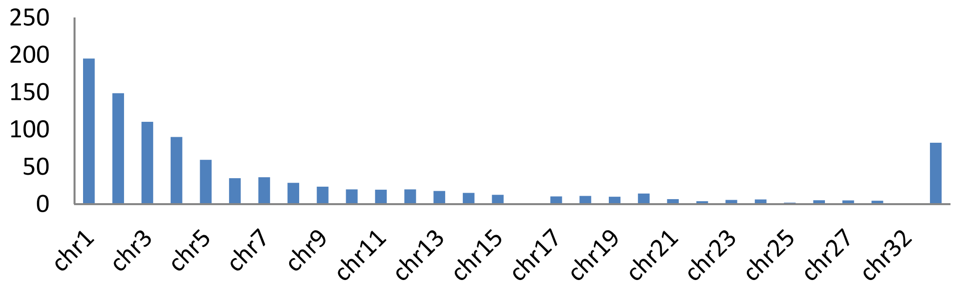
Upstream

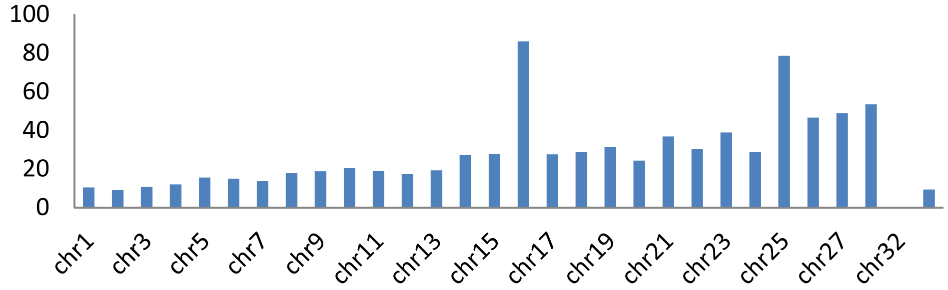


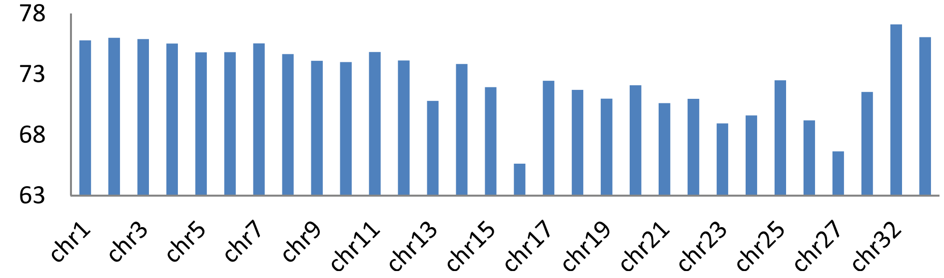
Downstream



Methylation level per chromosome


Chromosome length (Mb)


Genes / Mb


Methylation level

- Expected distribution along the genome

Conclusion

- Method OK
    - Many thanks to Genotoul (PlaGe & Bioinfo)
    - High quality DNA
    - RRBS for sufficient depth

- Pipeline (NG6) in progress

- Confirmation methods available at GeT-PlaGe (Pyromark,…)

- Other embryos to be sequenced

- EpiTherm program underway

Acknowledgments

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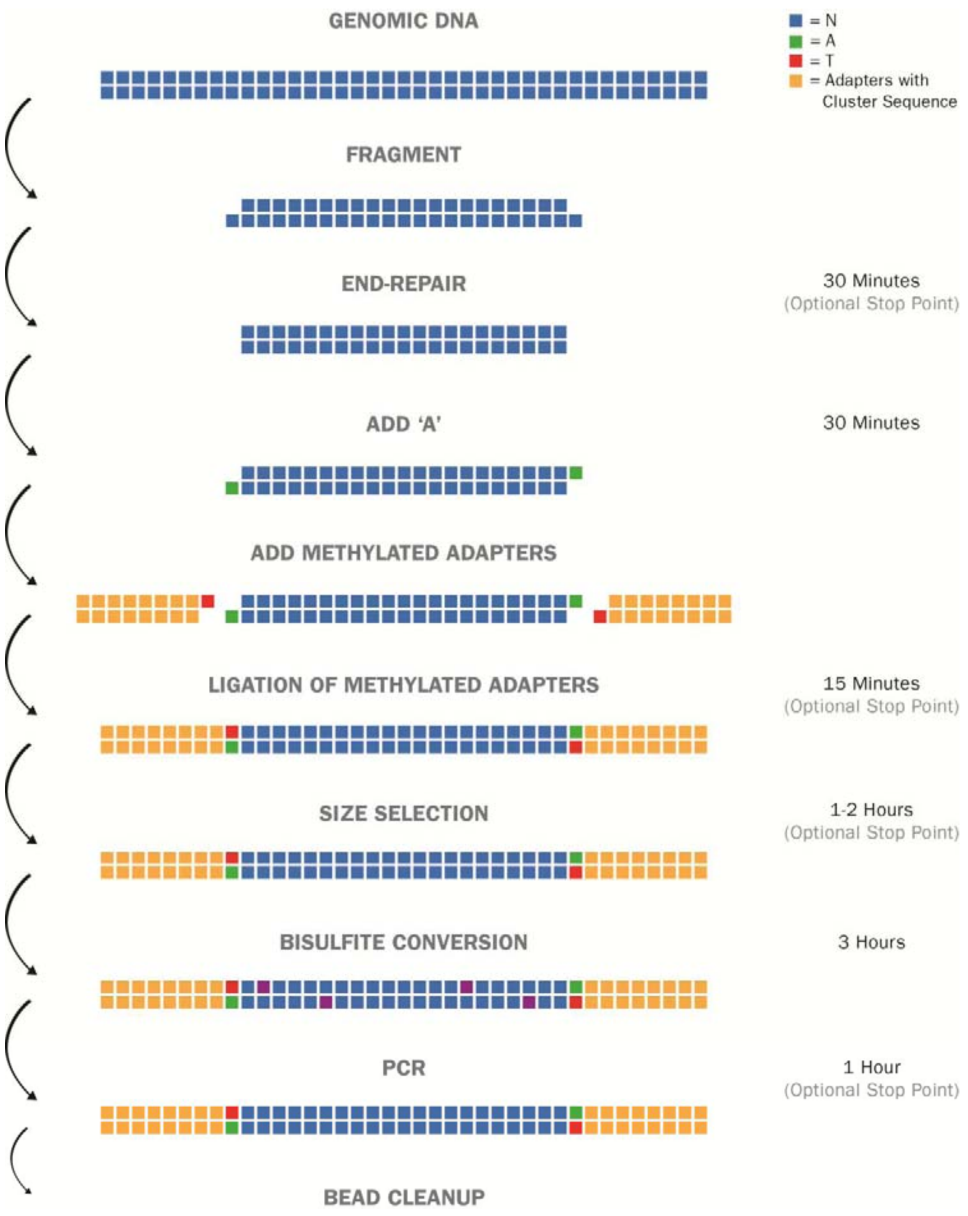


🟦 = N
🟩 = A
🟥 = T
🟧 = Adapters with Cluster Sequence

GENOMIC DNA

FRAGMENT

END-REPAIR
30 Minutes
(Optional Stop Point)

ADD 'A'
30 Minutes

ADD METHYLATED ADAPTERS

LIGATION OF METHYLATED ADAPTERS
15 Minutes
(Optional Stop Point)

SIZE SELECTION
1-2 Hours
(Optional Stop Point)

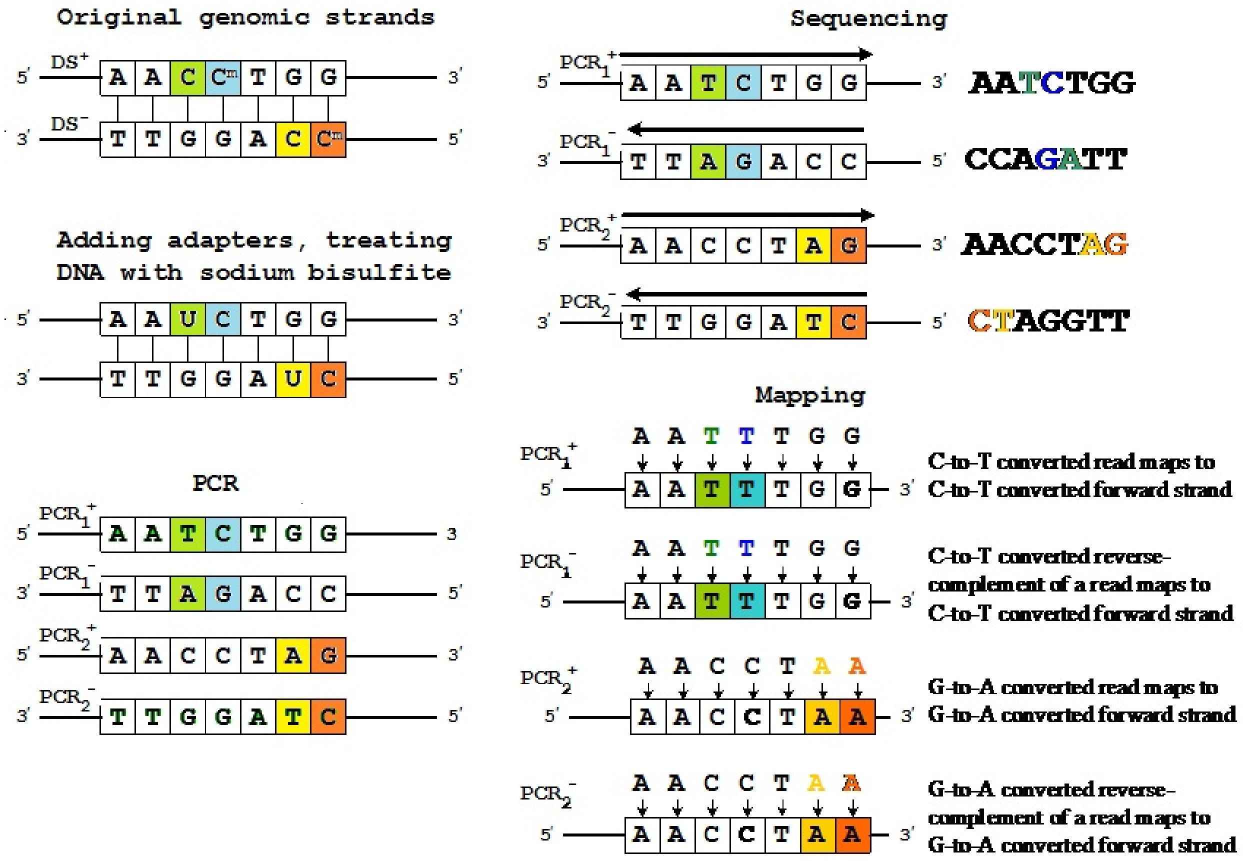
BISULFITE CONVERSION
3 Hours

PCR
1 Hour
(Optional Stop Point)

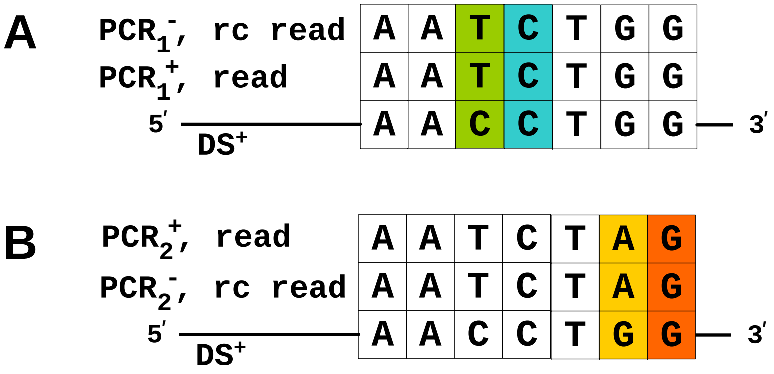
BEAD CLEANUP

BIOO Scientific Corp

BS-seq


Original genomic strands
DS+ 5' A A C Cm T G G 3'
DS- 3' T T G G A C Cm 5'
Adding adapters, treating DNA with sodium bisulfite
5' A A U C T G G 3'
3' T T G G A U C 5'
PCR
PCR1+ 5' A A T C T G G 3
PCR1- 3' T T A G A C C 5'
PCR2+ 5' A A C C T A G 3'
PCR2- 3' T T G G A T C 5'
Sequencing
PCR1+ 5' A A T C T G G 3' AATCTGG
PCR1- 3' T T A G A C C 5' CCAGATT
PCR2+ 5' A A C C T A G 3' AACCTAG
PCR2- 3' T T G G A T C 5' CTAGGTT
Mapping
PCR1+ A A T T T G G 5' A A T T T G G 3'
C-to-T converted read maps to C-to-T converted forward strand
PCR1- A A T T T G G 5' A A T T T G G 3'
C-to-T converted reverse-complement of a read maps to C-to-T converted forward strand
PCR2+ A A C C T A A 5' A A C C T A A 3'
G-to-A converted read maps to G-to-A converted forward strand
PCR2- A A C C T A A 5' A A C C T A A 3'
G-to-A converted reverse-complement of a read maps to G-to-A converted forward strand

BS-seq



A

PCR$_1^-$, rc read	A	A	T	C	T	G	G
PCR$_1^+$, read	A	A	T	C	T	G	G
5′ DS$^+$	A	A	C	C	T	G	G	3′

B

PCR$_2^+$, read	A	A	T	C	T	A	G
PCR$_2^-$, rc read	A	A	T	C	T	A	G
5′ DS$^+$	A	A	C	C	T	G	G	3′