Workflow-BS: an integrative workflow for RRBS and WGBS data

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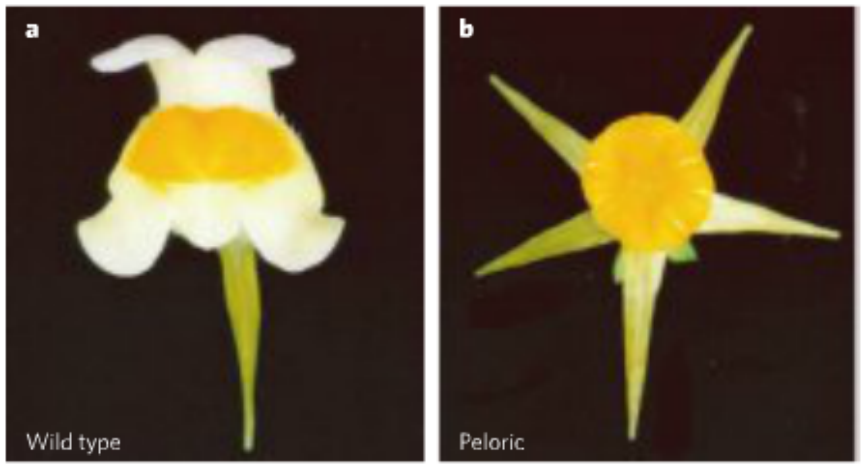
Summary

Summary

Epigenetics

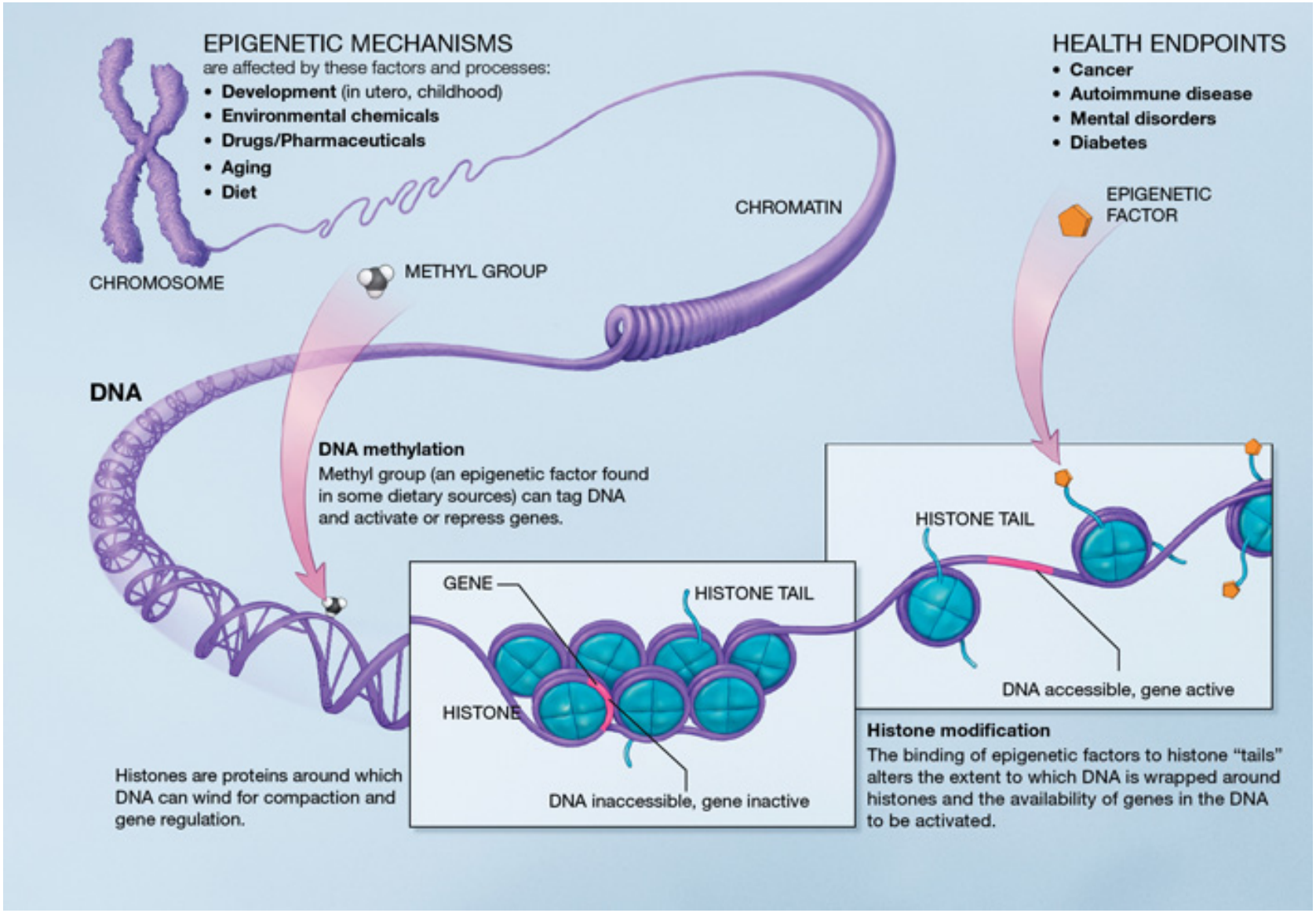
The term epigenetics refers to heritable changes in gene expression (active versus inactive genes) that does not involve changes to the underlying DNA sequence; a change in phenotype without a change in genotype

Morgan et al, 1999

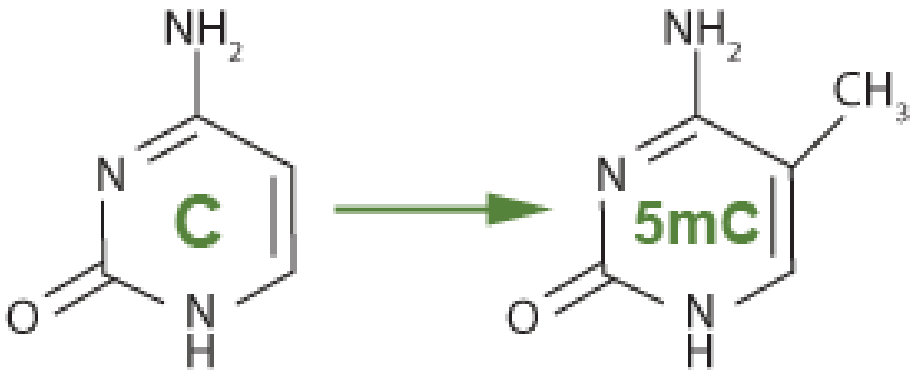


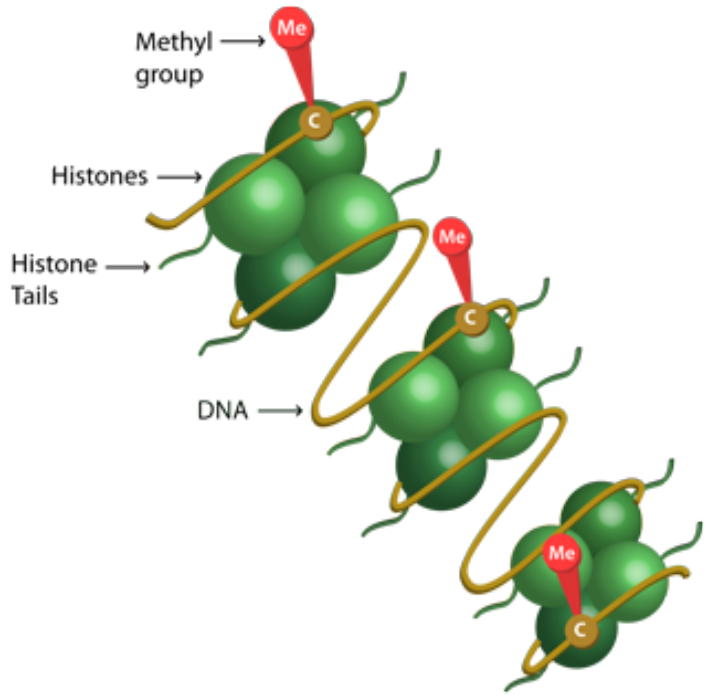
Cubas et al, 1999

Epigenetics



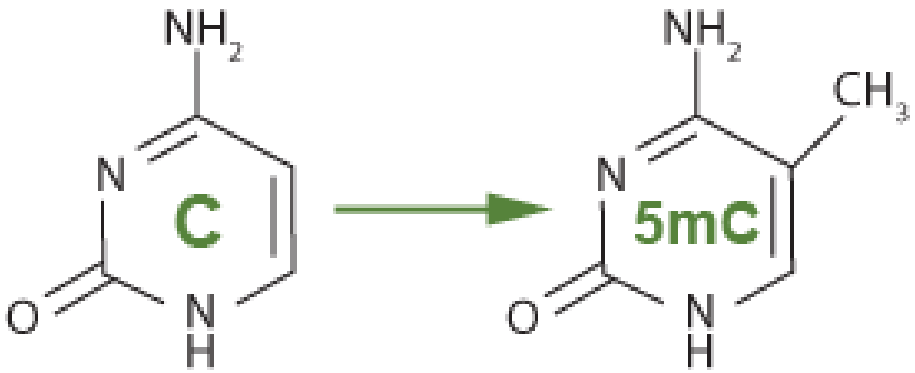
DNA methylation

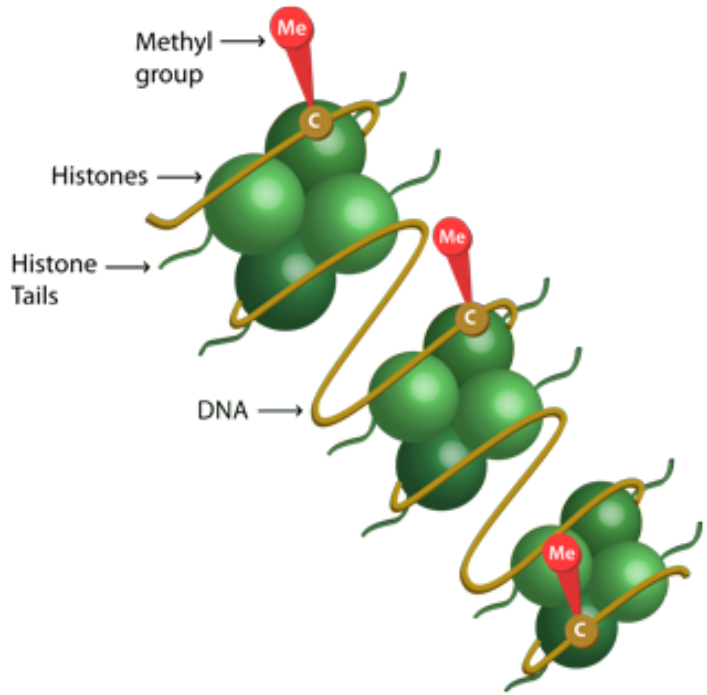




1. The predominant epigenetic modification of DNA in mammalian genomes is methylation of cytosine nucleotides (5-MeC).

DNA methylation





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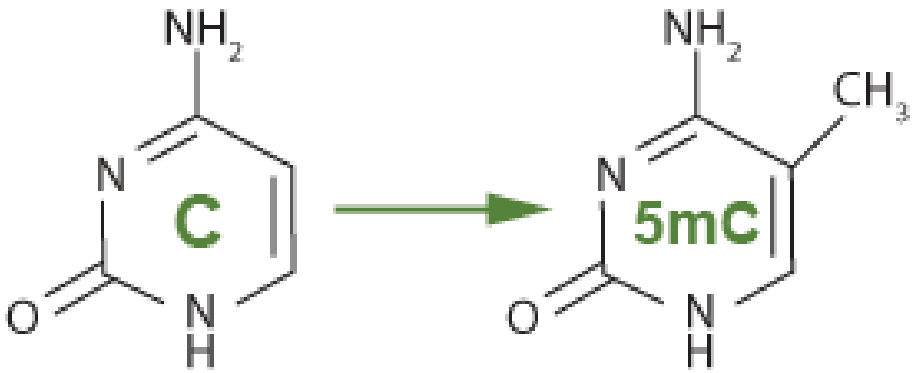
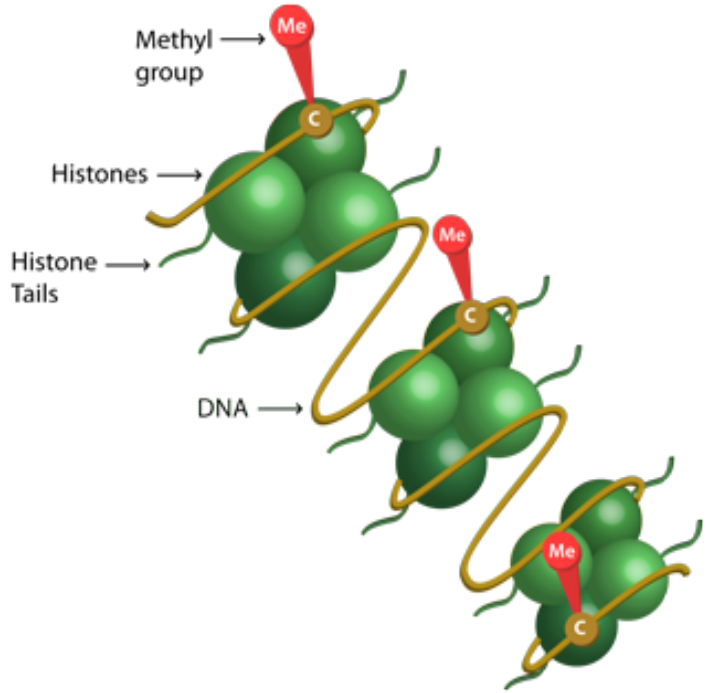
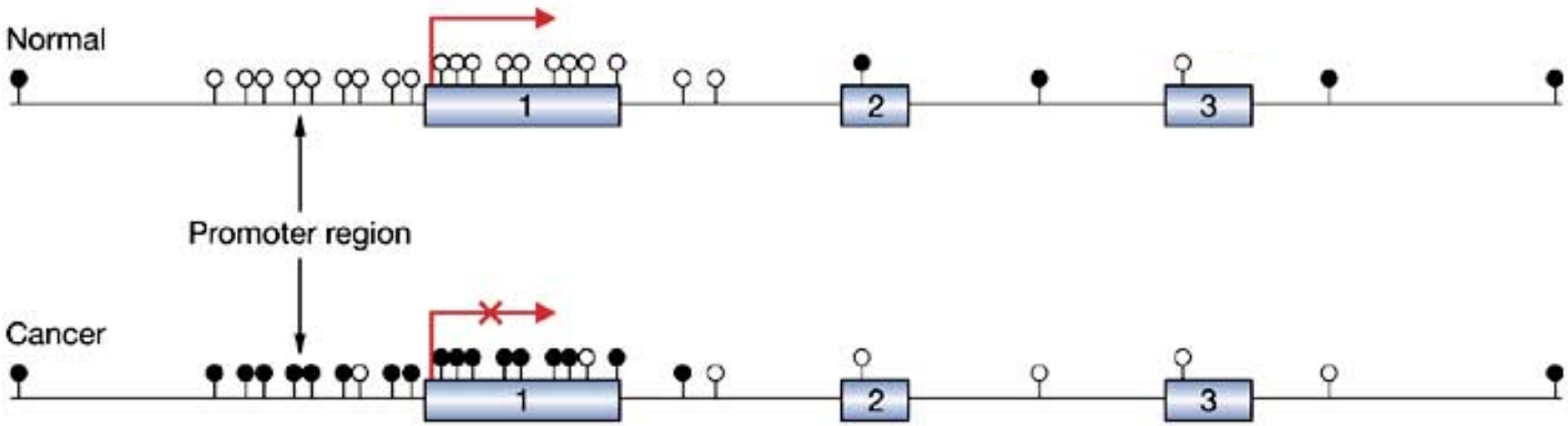




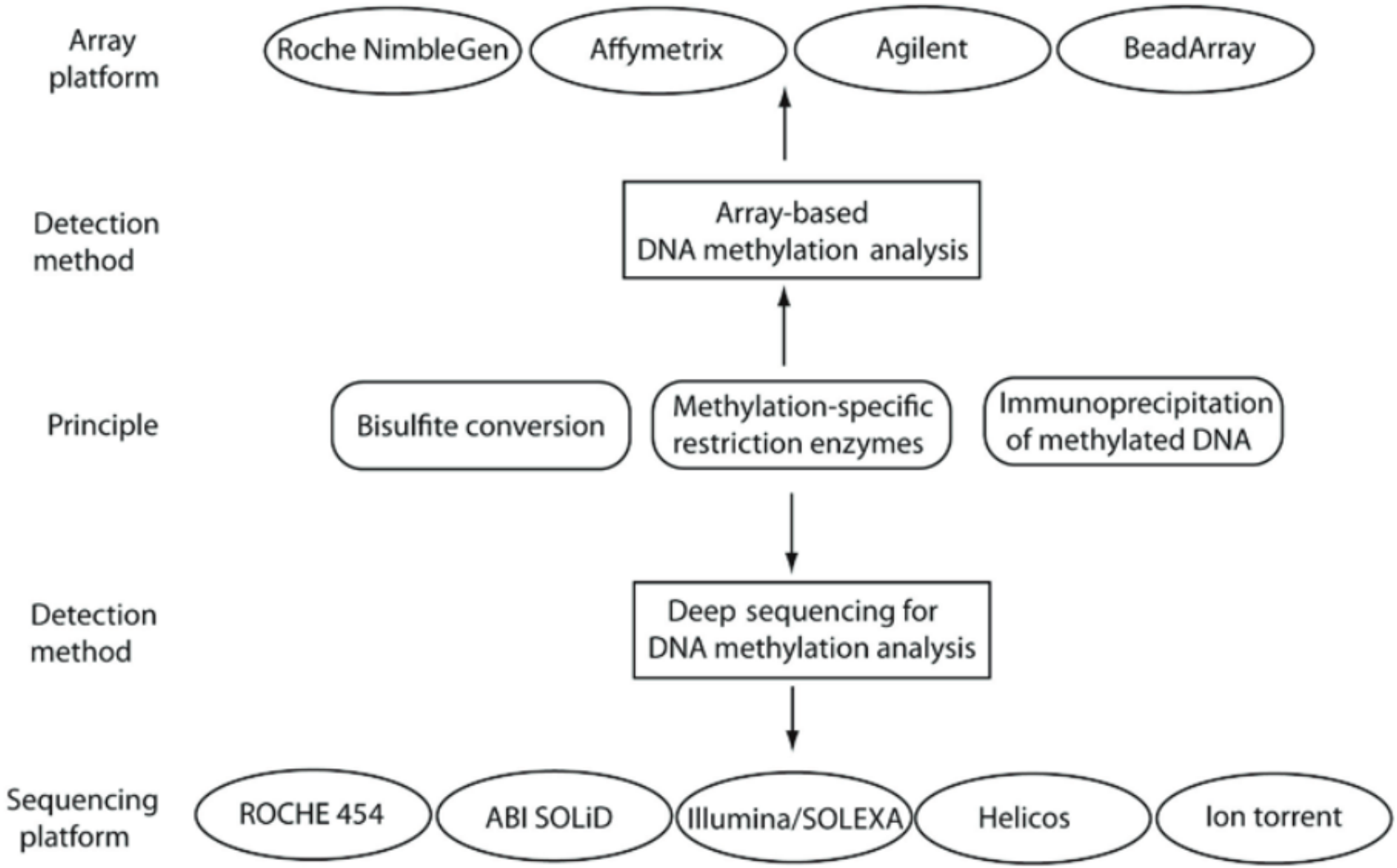
1. The predominant epigenetic modification of DNA in mammalian genomes is methylation of cytosine nucleotides (5-MeC).
2. Mammalian main modification is on 5'-CpG-3' dinucleotides
3. In plant also occurs on CHG and CHH context (where H can be A,C or T)

https://www.epigentek.com/catalog/dna-methylation-c-75_21.html

DNA methylation

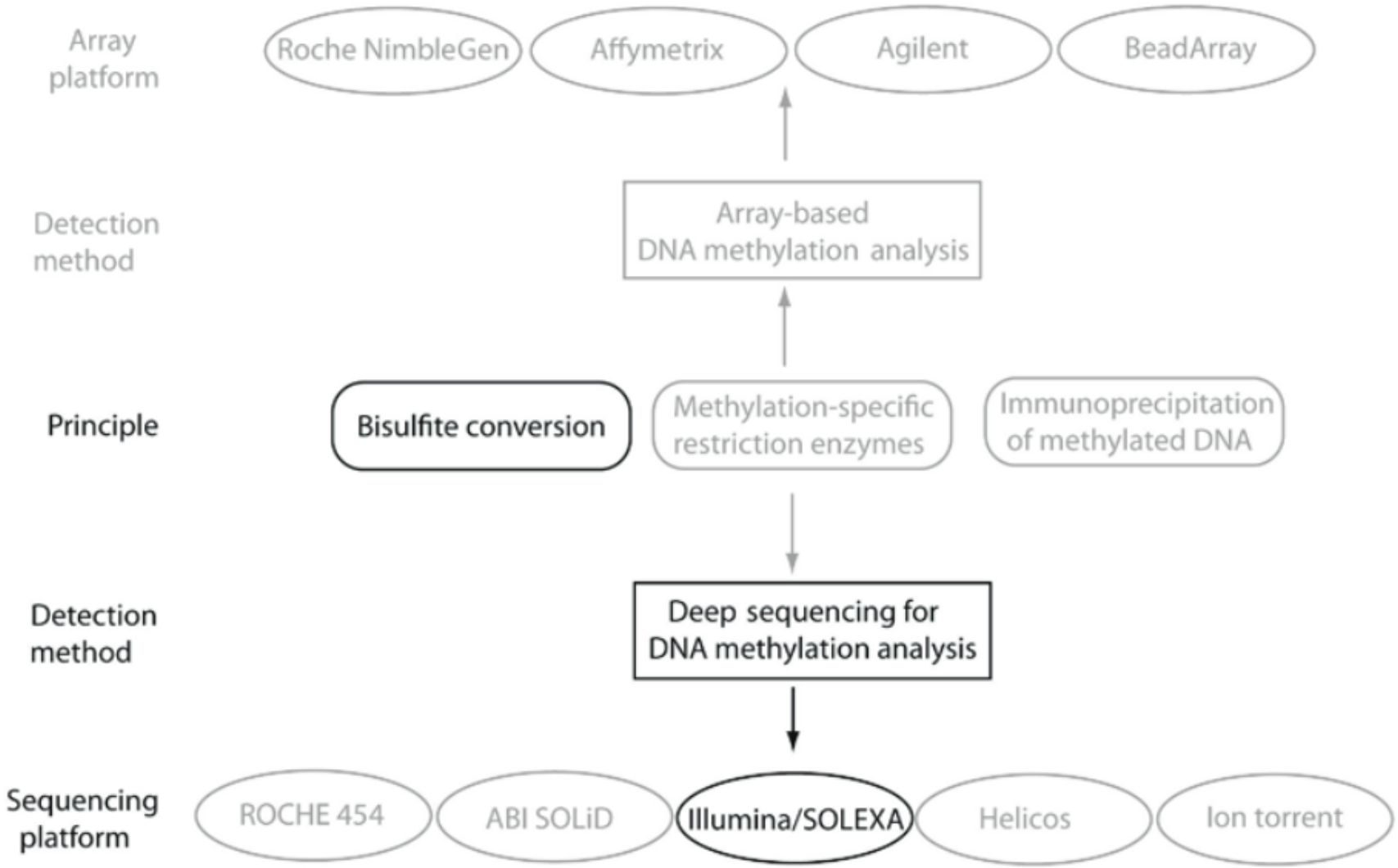


DNA methylation analysis methods



Gupta et al, Review BioTecnique, 2010

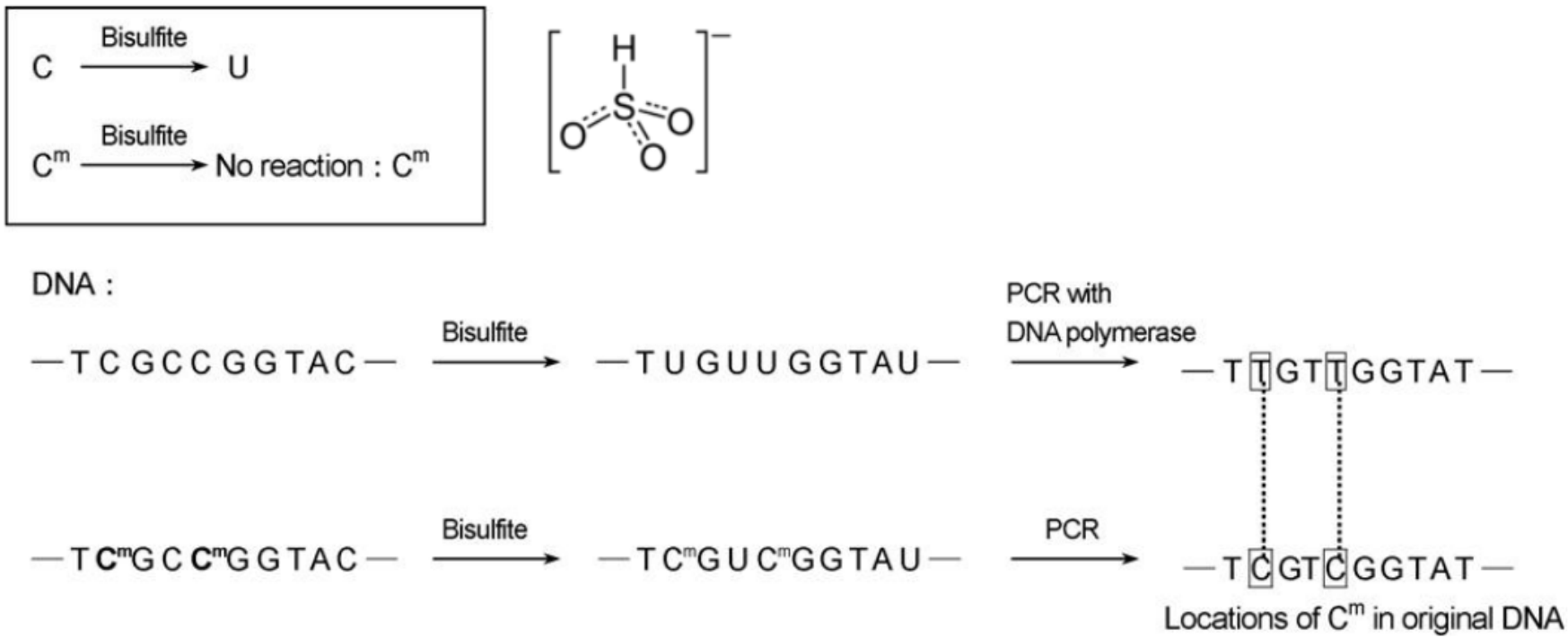
DNA methylation analysis methods



Gupta et al, Review BioTecnique, 2010

Bisulfite treatment

What is bisulfite treatement?

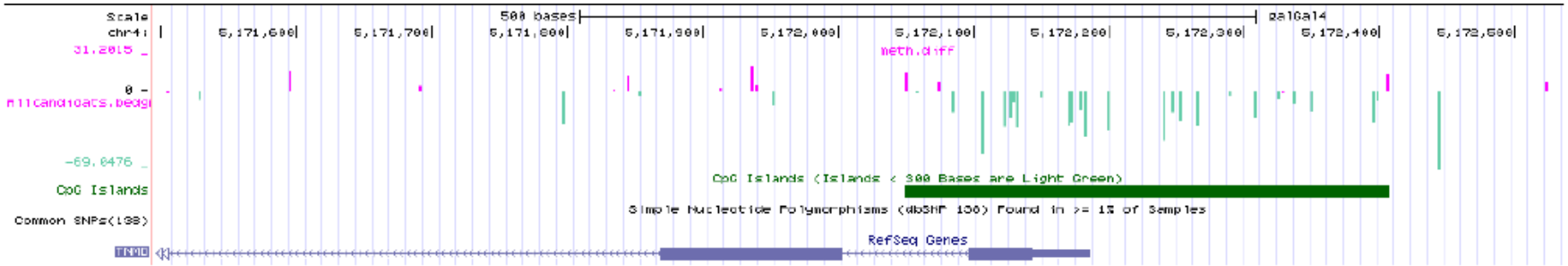


https://www.promega.com/~/media/files/promega%20worldwide/north%20america/promega%20us/webinars%20and%20events/epigeneticwebinarsept2012.pdf

Summary

Purpose

Identification of differential methylated regions.



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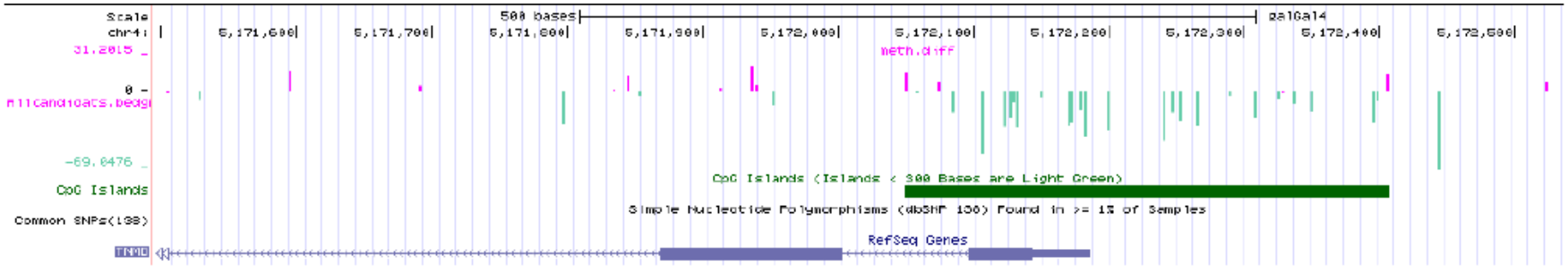
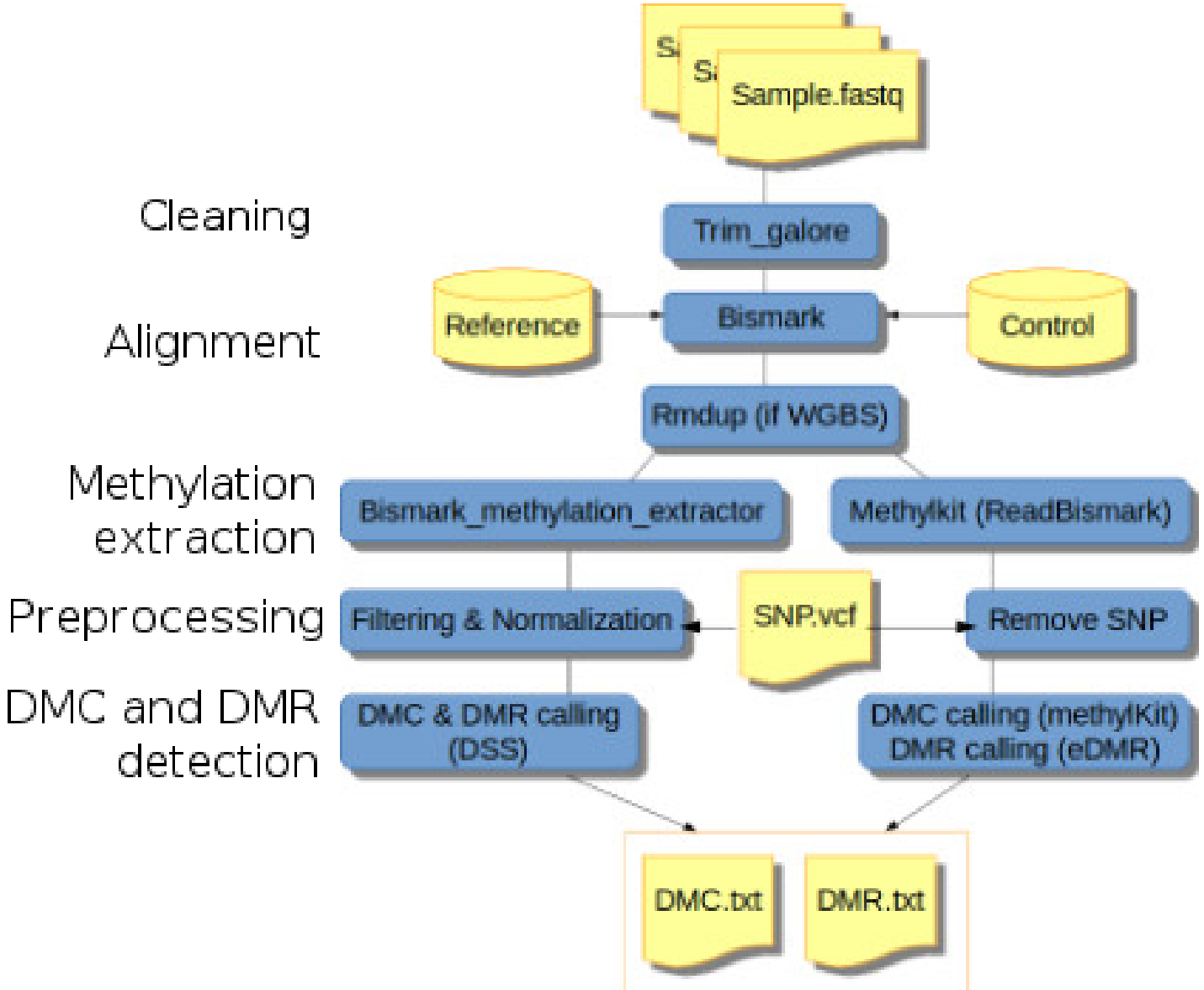


- Projects: Epitherm/Epibird (GenPhySE), Epinod (LIPM), FrAG-ENCODE (SelGen), Mitomics (submitted)
- Kind of data: WGBS, sequence capture, soon RRBS
- Community: France Genomic Methylation WG, FAANG
- Needs: bioinformatics and biostatistics tools

Pipeline overview



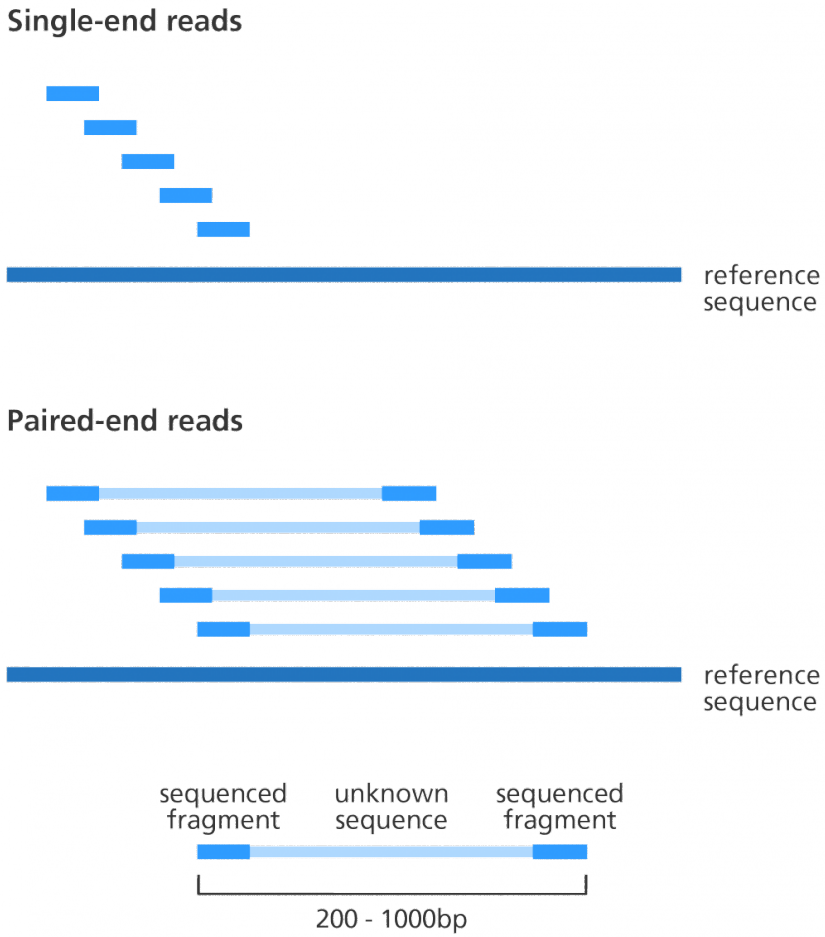
Supported data

- Fastq files from illumina sequencing
  - Bisulfite treated
  - For example a Hiseq 2500 run :
    - ★ up to 2 billion single read or 4 billion paired-end reads
    - ★ read length: from 36bp up to 150bp

```
@HWI-ST314:257:D1WGCACXX:2:1101:1130:51866_1:N:0:TCGAAG
TATGAAAATAAGGTGGATAAGTGTTTTTTGATGTTTATTAGTGATATGTTATTTGAGTTTTGAGTATTTGTATTAGAAAGTTAGATTGATTATTTTAGTT
+
B@@DDFFFHGHHFGHIEHIJJHIIHIJJJGBHIIIJFIJFDFHGIGIGIJJJJJFIJFGIIIGHFHGGIGIJIIGCHHHHCED?@DFCEEEECEEEECCC
@HWI-ST314:257:D1WGCACXX:2:1101:1137:35617_1:N:0:TCGAAG
GGATTTATAGAATTATTTGTTTTCGAAATGCGGGAGGGTATGTTTTTGTTTAATATAGTGAGATAAATAGTAAGTTTGTTGTTTTTTAGGGAGTATATTT
+
BBBDFFFFHHHGHJIJJJHHIJJJEHJJJDHHIGFHIIFCEGHIIJJFHIIIIJHHHEEEEBEDDDEDECEEEEF>CAACACDDDDDD9:?CB?ACCDEE
@HWI-ST314:257:D1WGCACXX:2:1101:1151:27741_1:N:0:TCGAAG
AGGTATAGTGTTTGTTTTGTGTTTTTTTTTTAGTATTGGTTTAGTTGTGTTTTGTGGTTGTATATGTTTTATAGAATATTTTTTTTGTGGTTATGATGGT
+
BBBDDEFFHHHHHGHHIJHGGIIJJJJJJJJJDB=@CGAFHGGECHHAHAHEHFB?C?CC?>CDDD:CCEC>@C>ACCCCFFEDDDD&8<0?B@C>3>@>:
@HWI-ST314:257:D1WGCACXX:2:1101:1160:46348_1:N:0:TCGAAG
AGAAAAAGGTATGAGAGAAAGAATAGGGAAGGAATTATTATTTTAGGTGTTTGGAAATAGATAAGATTAGATTAGTTTTTTAGTGAAGATGATTTTTTTT
+
CCCFFFFFHFFHHGHGIJJJJJJJJJJJJJFCDGHIJJJJIJJJJIGHFHIHHFGIIGIJJJIJJHIHIJIJJHHHHEHHFDDACCDDDDDDDEEEDDDDD
@HWI-ST314:257:D1WGCACXX:2:1101:1186:63430_1:N:0:TCGAAG
ATTTTTTTTTTATTTGTTTGTTTTTTTTTTTTGTGTTAAATTAGGAGGAGAAGGGAAGTTTGAATGAAGTAGTTTATTT
+
CC@FFFFFHHH?FHJ4?FH@GHHIJJJJHFDD',,5?ADA;((,((30((22<A8<88>>@:3:@>C:+:4(4>A4:@C
@HWI-ST314:257:D1WGCACXX:2:1101:1224:90588 1:N:0:TCGAAG
```

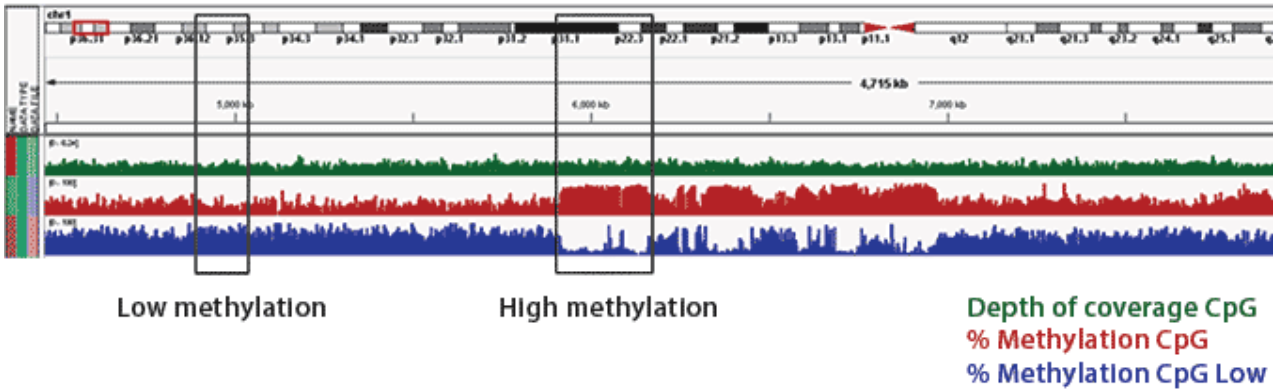
Supported data

- Fastq files from illumina sequencing
- Single or paired reads

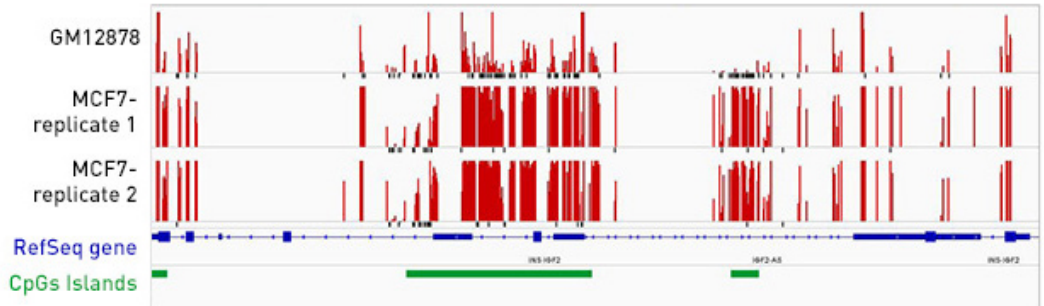


Supported data

- Fastq files from illumina sequencing
- Single or paired reads
- Protocol
  - WGBS: Whole Genome Bisulfite sequencing



  - RRBS: Reduced Representation Bisulfite sequencing

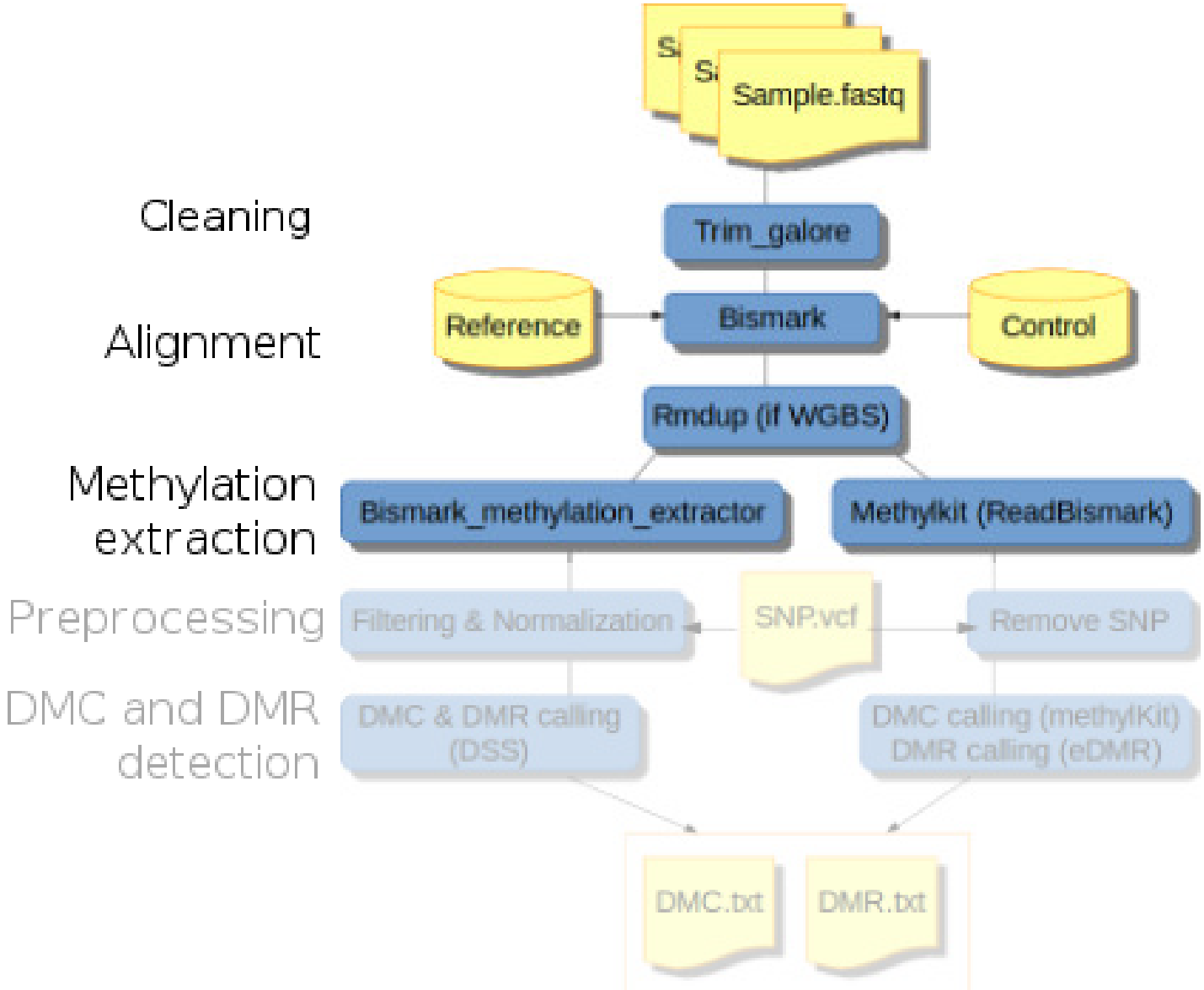


Data from epibird project

- 4 male vs. 4 female chicken embryos
- Sequenced by HiSeq3000
- Whole Genome Bisulfite sequencing

Summary

Bioinformatics steps



Quality control and cleaning

- Trim adapters
- Trim bad quality

Software: Trim_galore

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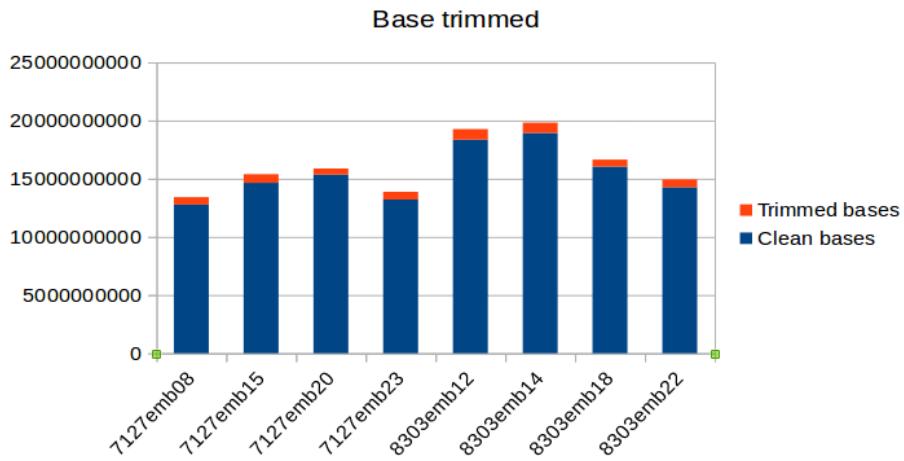


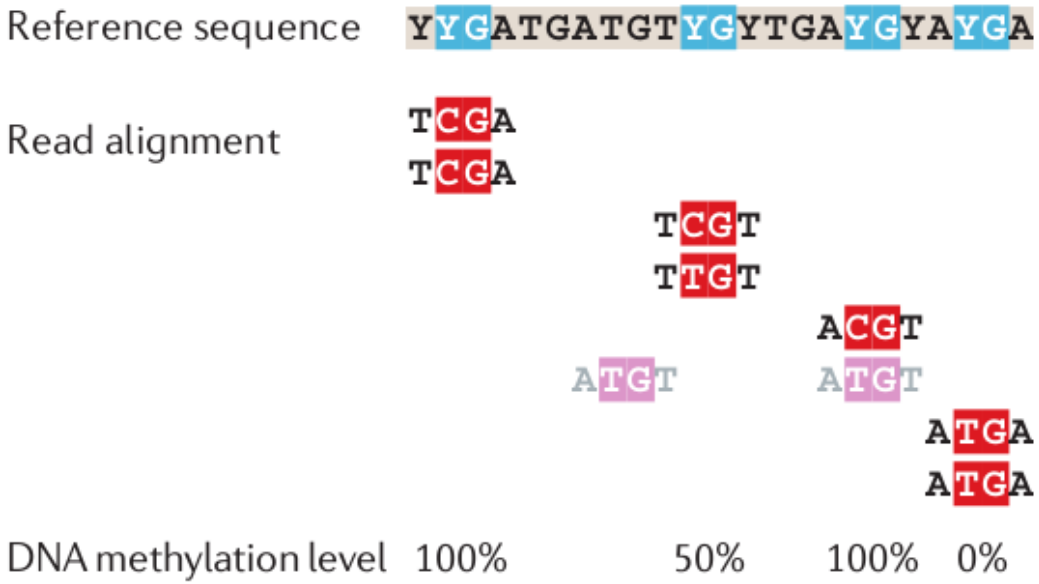
- Reads: about 40% of reads are trimmed
- Bases: about 4% of total bases

Alignment


Genomic DNA sequence
CCGATGATGTCGCTGACGCACGA
DNA methylation level
100%
50%
50%
0%

Alignment

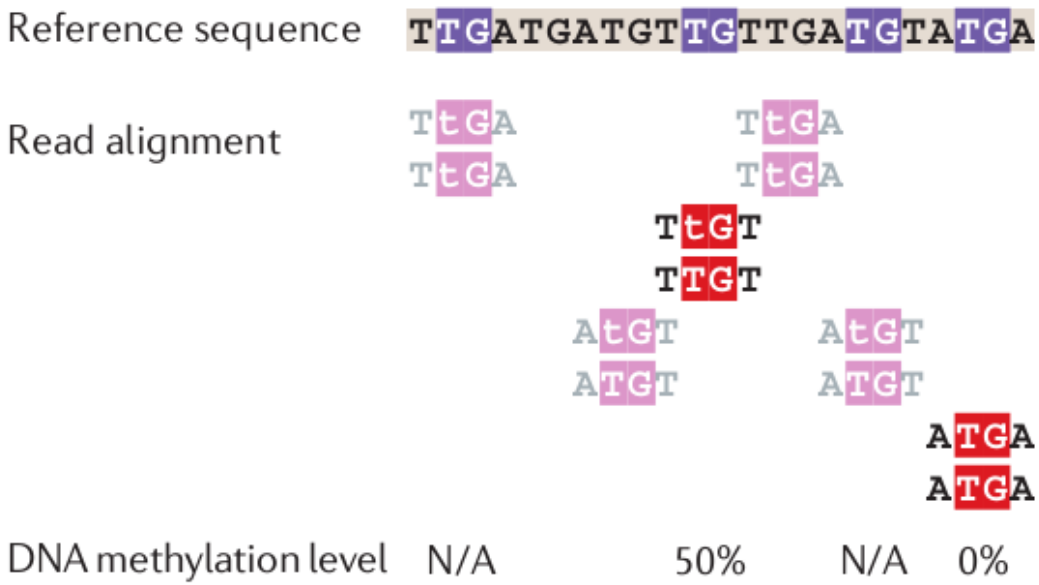
- wild-card alignment



Existing software: BSMAP, GSNAP, Last, Pash, RMAP, RRBSMAP, segemehl...

Alignment

- wild-card alignment
- 3-base encoding



Existing software: **Bismark**, BRAT, BS-Seeker, MethylCoder...

Alignment

Mapping efficiency (percent of mapped reads) depends

- read length
- single or paired end library
- genome composition and variability

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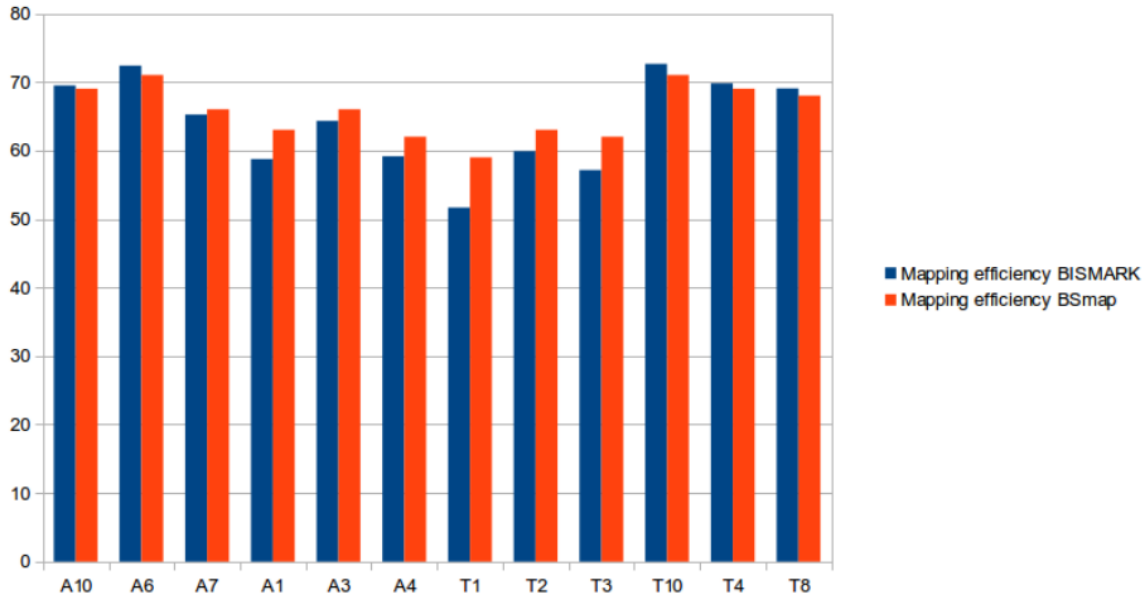
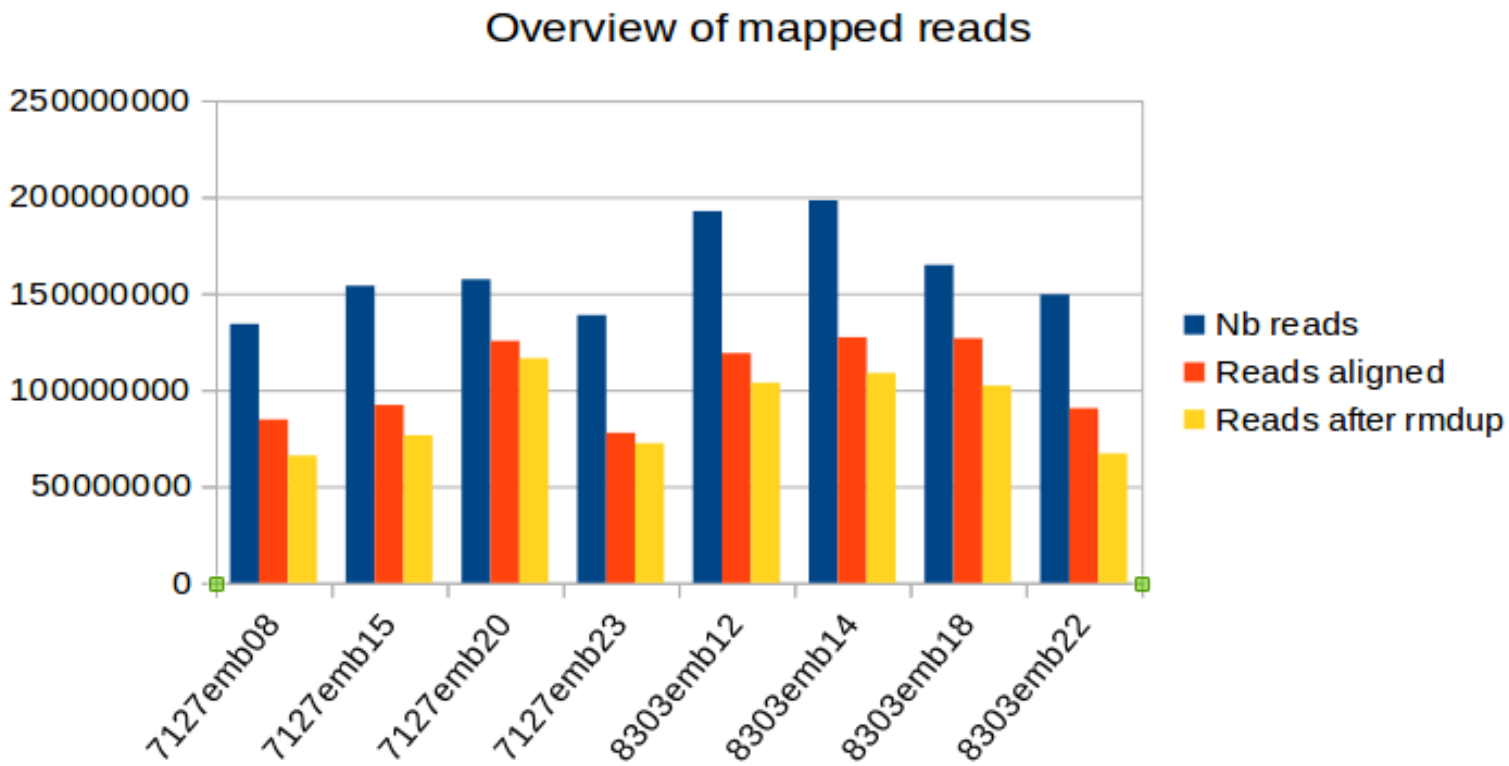
Comparison of 2 strategies :



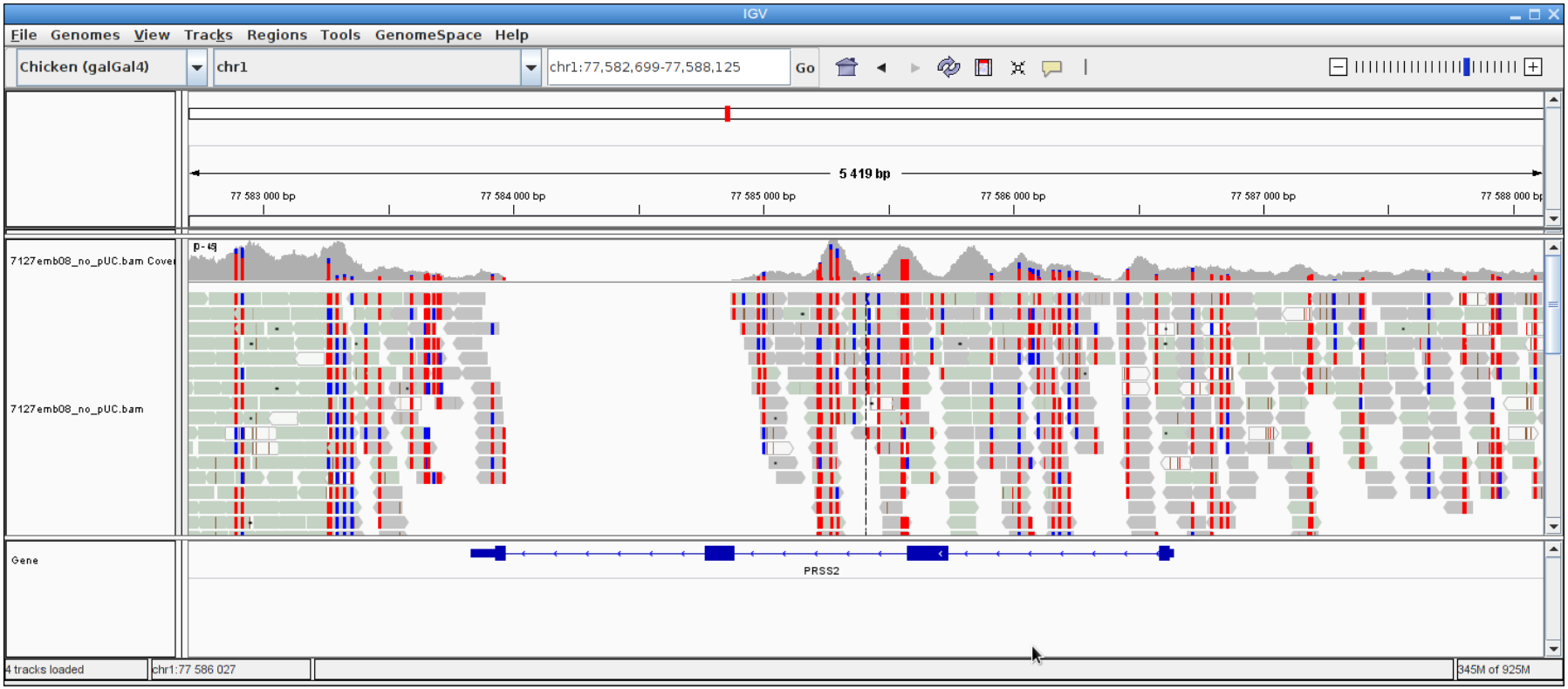
M2 Bioinfo 2014 - Julien Plennecassagne - Projet Epiterm

Alignment: Epibird results



- Bismark: 61 to 81% of mapping efficiency
- Rmdup: 73 to 93% of reads kept after rmdup

Per base methylation extraction



Per base methylation extraction

chrBase	chr	base	strand	coverage	freqC	freqT
chr1.913	chr1	913	R	1	100.00	0.00
chr1.417	chr1	417	R	3	100.00	0.00
chr1.258	chr1	258	F	1	100.00	0.00
chr1.699	chr1	699	F	3	100.00	0.00
chr1.589	chr1	589	R	6	83.33	16.67
chr1.718	chr1	718	R	6	0.00	100.00
chr1.573	chr1	573	F	8	87.50	12.50
chr1.832	chr1	832	R	3	100.00	0.00
chr1.755	chr1	755	R	7	85.71	14.29
chr1.233	chr1	233	F	1	100.00	0.00
chr1.403	chr1	403	R	3	100.00	0.00
chr1.608	chr1	608	F	5	40.00	60.00
chr1.684	chr1	684	R	4	100.00	0.00
chr1.700	chr1	700	R	3	100.00	0.00
chr1.831	chr1	831	F	5	100.00	0.00
chr1.931	chr1	931	F	1	100.00	0.00
chr1.739	chr1	739	F	6	83.33	16.67
chr1.252	chr1	252	F	1	100.00	0.00
chr1.633	chr1	633	R	3	33.33	66.67
chr1.717	chr1	717	F	4	0.00	100.00

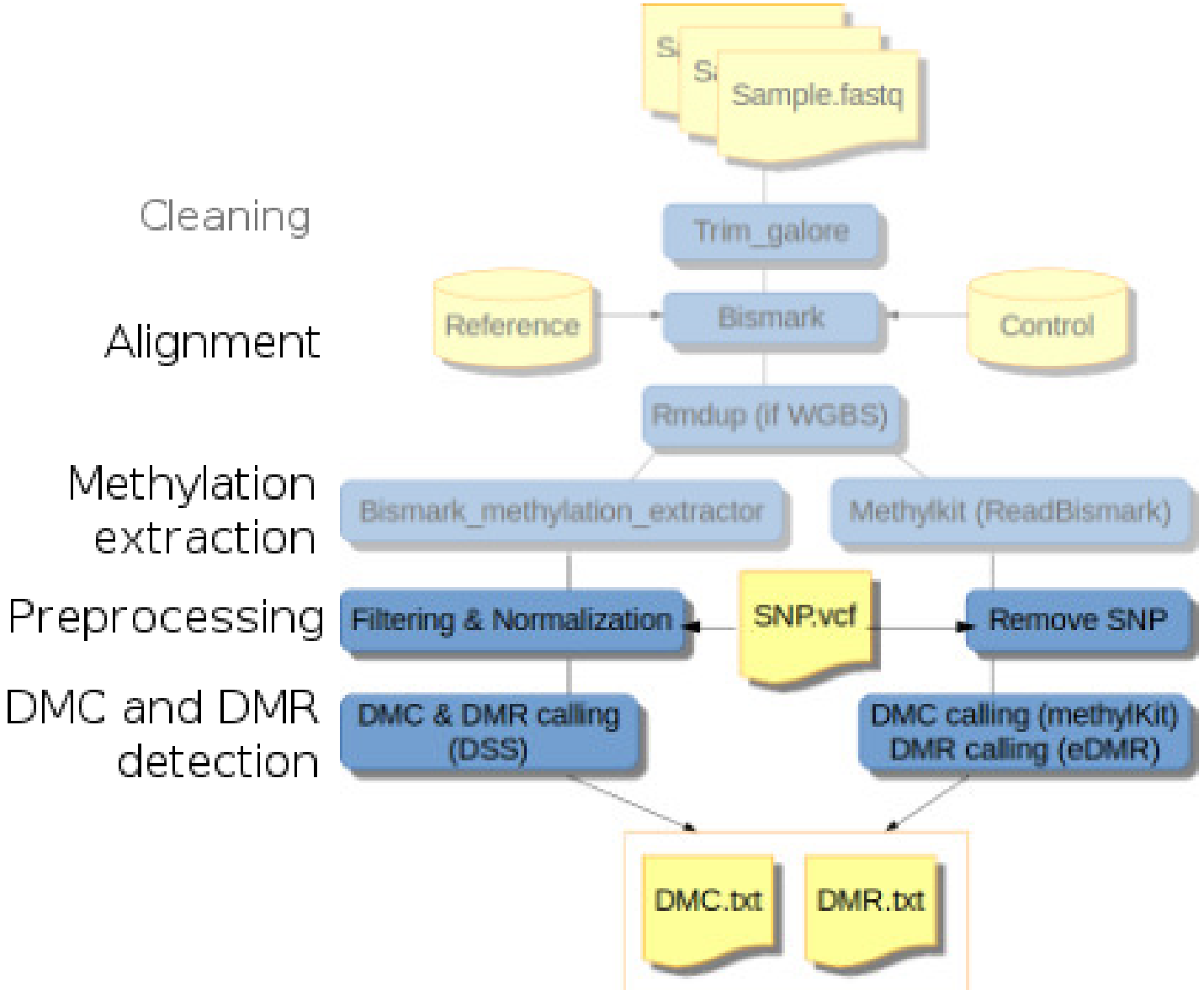
Per sample extract :

1. C in specific context (CpG, CHG, CHH)
2. choose coverage threshold

Existing software: **methylKit**, bismark_methylation_extraction ...

Summary

Biostatistics steps



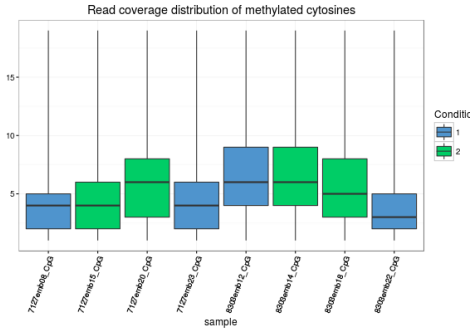
Steps

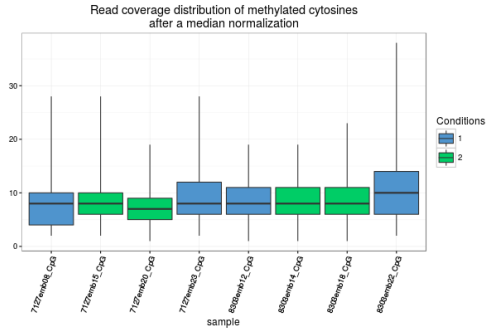
1. Normalization and filter on coverage
2. Identification of differentially methylated cytosines (DMCs)
3. Identification of differentially methylated regions (DMRs)

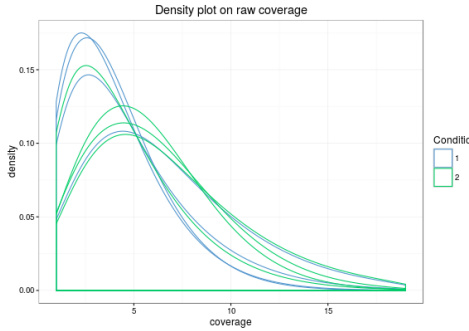
Step 1: Preprocessing on methylation data

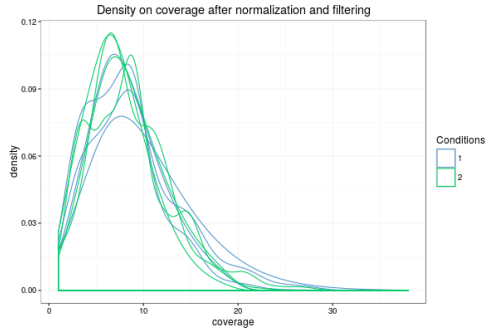
1. Remove known SNPs
2. Remove bases with a very high read coverage
3. Normalize read coverage of each cytosine (5 methods: libsize, median, upper-quartile, RLE and LR)
4. Remove bases with a very low read coverage (a minimum coverage of 5x is recommended)

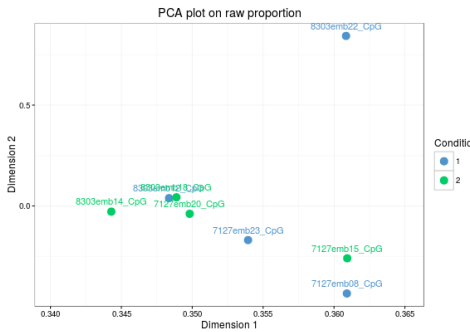
Normalization diagnostics plots

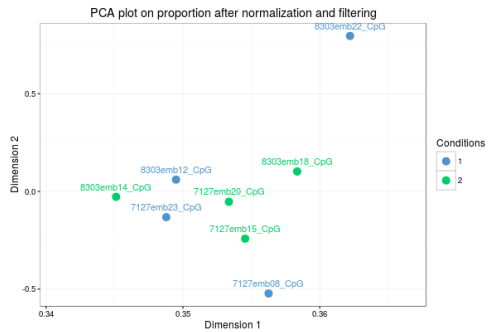


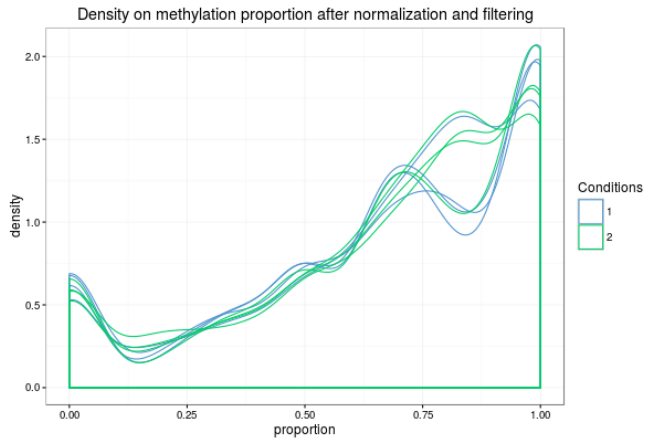












Step 2: identification of DMCs

Used methods

☹ Fisher exact test: all replicates are pooled (methylKit without replicates)

😐 Logistic regression: the hypothesis is that all data is from the same distribution (methylKit)

☺ Beta-binomial model: take into account of the biological variability between samples (DSS)
Case without replicats: nearby cytosines can be used to estimate variability (DSS)

☺ *Hidden Markov model: take into account of the spatial correlation between nearby cytosines*

Beta-binomial model (from DSS package)

Model

$$X_{ijk}|p_{ijk}, N_{ijk} \sim \text{Binomial}(N_{ijk}, p_{ijk})$$

with

$$p_{ijk} \sim \text{Beta}(\mu_{ij}, \phi_{ij})$$

and

$$\phi_{ij} \sim Log - Normal(m_{0j}, r_{0j}^2)$$

Estimation Empirical Bayes approach

Test Wald test on mean methylation levels μ_{ij}

Comparison of normalization methods

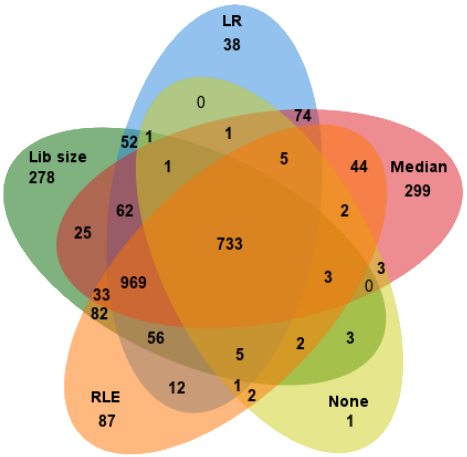


Figure : DMCs identified with DSS depending on the normalization method

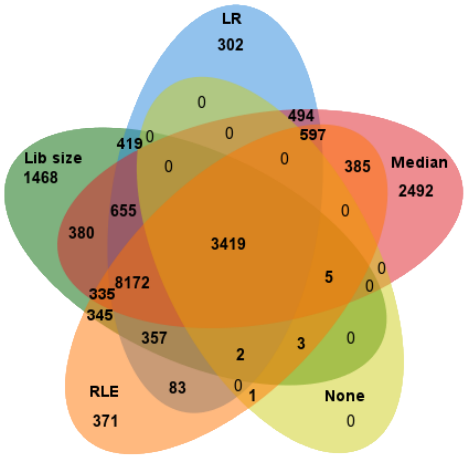


Figure : DMCs identified with methylKit depending on the normalization method

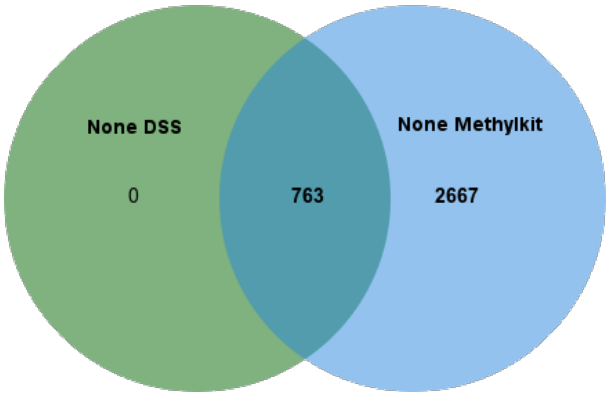


Figure : DMCs identified with methylKit and DSS without normalization

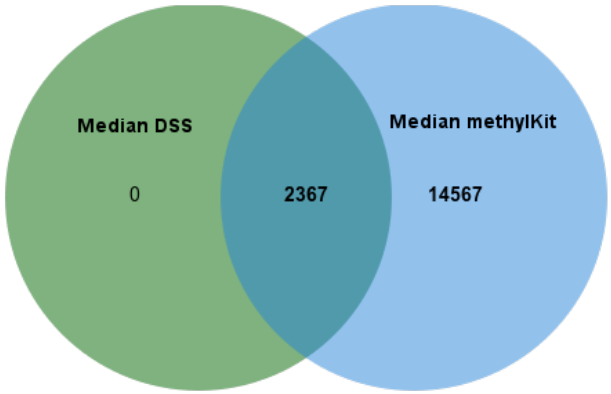


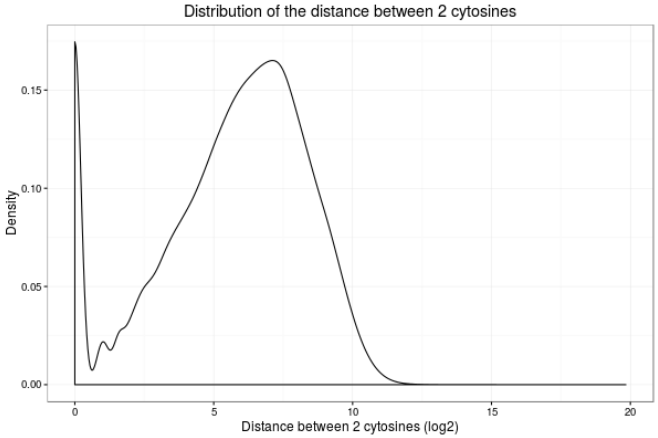
Figure : DMCs identified with methylKit and DSS with median normalization

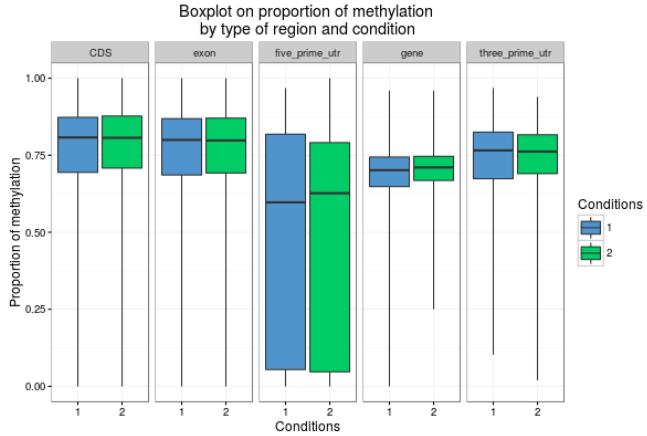
Step 3: identification of DMRs

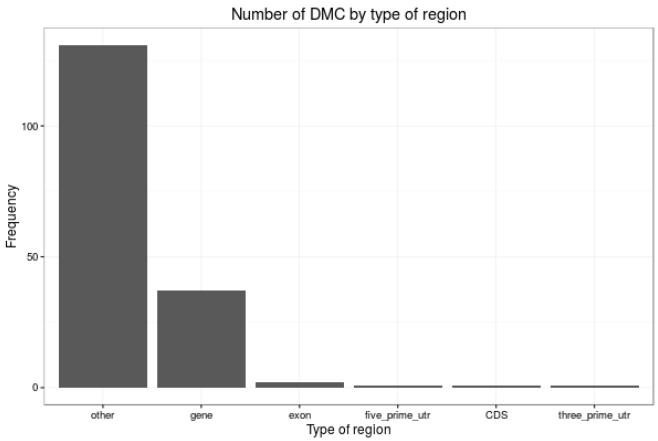
Used methods

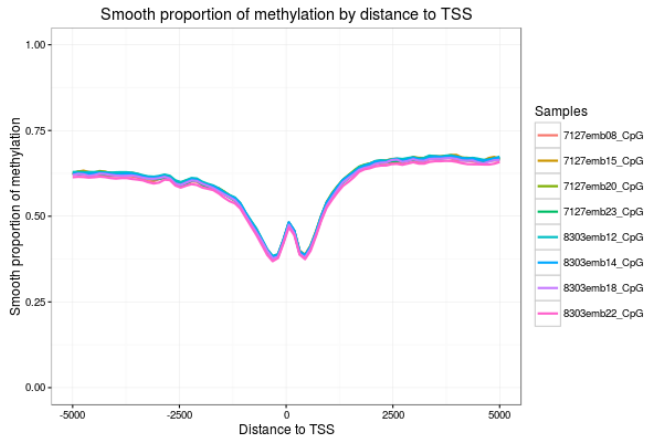
- Sliding windows or predefined regions (MethylKit, DMRcaller...)
- From results on DMCs (DSS, eDMR...)
- With a hidden Markov model (Bisulfighter)

Differential analysis results and other plots









Summary

Pipeline conclusion

- based on Jlow: error recovery, use most HPC (SGE, Condor, ...), extensible
- configuration with one config file
- include all steps (bioinfo and biostats) within a single command line
- re-runable after main step : alignment and methylation extraction

Available :

- github FAANG consortium
- mulcyber: `https://mulcyber.toulouse.inra.fr/plugins/mediawiki/wiki/jflow-toolshed/index.php/Accueil`

Coming soon ...

- Normalization and DSS
- New aligners
- A web server

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- Normalization and DSS
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Next step :

- Link methylation regions with transcriptomics data ...

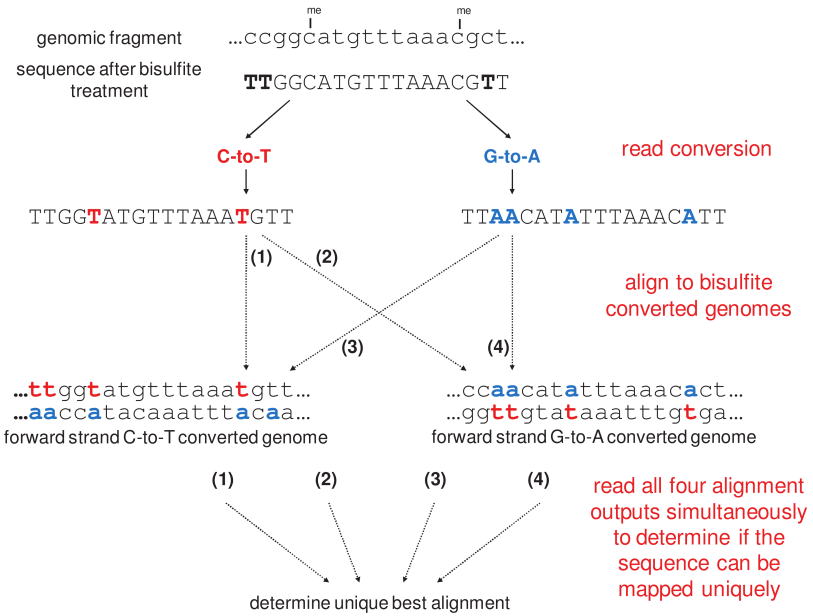
Acknowledgement

- Nathalie Villa-Vialaneix (MIAT)
- Frédérique Pitel, Gérald Salin, Sylvain Foissac, Marjorie Merch, Julien Plennecassagne, Diane Esquerre (GenPhySE)
- Erika Sallet, Pascal Gamas (LIPM)
- Monique Falières (Genotoul)

Thank you for your attention

Questions ?

Bismark


me
me
genomic fragment
...ccggcatgtttaaacgct...
sequence after bisulfite treatment
TTGGCATGTTTAAACGTT
C-to-T
G-to-A
read conversion
TTGGTATGTTTAAATGTT
TTAACATATTTAAACATT
(1)
(2)
(3)
(4)
align to bisulfite converted genomes
...ttggtatgtttaaatgtt...
...aaccatacaaatttacaa...
forward strand C-to-T converted genome
...ccaacatatttaaacact...
...ggttgtataaatttgtga...
forward strand G-to-A converted genome
(1)
(2)
(3)
(4)
read all four alignment outputs simultaneously to determine if the sequence can be mapped uniquely
determine unique best alignment

Config file

```
########################################################################
#Input files

--reference-genome
workflows/methylseq/data/Gallus_gallus.Galgal4.dna.chromosome.7.fa

#--control-genome
#/path/to/control.fa

--snp-reference
workflows/methylseq/data/snp.vcf

########################################################################
#Sample description

--sample
sample-name=sample1
read1=workflows/methylseq/data/sample1.R1.fastq.gz
read2=workflows/methylseq/data/sample1.R2.fastq.gz
#bam=/nosave/cnoirot/work/methylseq/wf000083/Bismark_paired/7127emb20.bam
#methylkit=

--sample
sample-name=sample2
read1=workflows/methylseq/data/sample2.R1.fastq.gz
read2=workflows/methylseq/data/sample2.R2.fastq.gz

########################################################################
#Protocol parameters :
#IF BAM PROVIDED : Set true if you provide alignment of single-end library
#--is-single
#
#Type of methylation context to extract and analyze :
#--rrbs
#
#To set if the libraries are non directional (Default : False)
#--non-directional
#
```

Config file

```
########################################################################
#Bismark alignment parameters
#Sets the number of mismatches to allowed in a seed alignment during multi-seed alignment
#--alignment-mismatch
#10
#
#The maximum insert size for valid paired-end alignments.
#--max-insert-size
#800
#
#Use bowtie1 instead of bowtie2 (longer, better for reads < 50bp) - default False
#--bowtie1
#
#Force to not perform rmdup :
#--no-rmdup
#
########################################################################
# Comment following lines if you do not want to perform statistical analysis
#Methylation extraction parameters (methylkit)
#For extraction of C define min coverage
--coverage
5

#Type of methylation context to extract and analyze [CpG|CHG|CHH]
#specifie several times if you want to analyze several contect
--context
CpG
#--context
#CHH

########################################################################
#DMC parameters will be perform on each context (you can specify several --test)
--test
test-name=sex
pool1=sample1,sample4,sample5,sample8
pool2=sample2,sample3,sample6,sample7
#perform methylKit logical normalization 0/1
normalization=1
#filter position with coverage less than 5 and with coverage above 99% quantile.
filter=1
#method to adjust p-values for multiple testing [BH|bonferroni]
correct=BH
#significance level of the tests (i.e. acceptable rate of false-positive in the list of DMC)
alpha=0.05
```