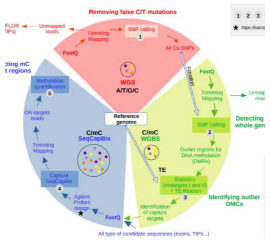
Jun 20, 2024

Bioinformatics manual for population epigenomics combining whole-genome and target genome sequencing



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OPEN ACCESS

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External link: https://epitree-project.hub.inrae.fr/

Protocol Citation: Odile Rogier, Isabelle Lesur Kupin, Mamadou Dia Sow, Christophe Boury, Alexandre Duplan, Abel Garnier, Abdeljalil Senhaji rachik, Peter Civan, Josquin Daron, Alain Delaunay, Ludovic Duvaux, Vanina Benoit, Erwan Guichoux, Gregoire Le Provost, Edmond Sanou, Christophe Ambroise, Christophe Plomion, Jérôme Salse, Vincent Segura, Jorg Tost, Stéphane Maury 2024. Bioinformatics manual for population epigenomics combining whole-genome and target genome sequencing. **protocols.io** **https://dx.doi.org/10.17504/protocols.io.8epv5xw4ng1b/v1**

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A Strategy for Studying Epigenetic Diversity in Natural Populations: Proof of Concept in Poplar and Oak
Lesur I., Rogier O., Sow M-D., Boury C., Duplan A., Garnier A., Senhaji-Rachik A., Civan P., Daron J., Delaunay A., Duvaux L., Benoit V., Guichoux E., Le Provost G., Sanou E., Ambroise C., Plomion C., Salse J., Segura V., Tost J., Maury S. 2024. J. Exp. Bot.

Protocol status: Working
We use this protocol and it's working

Created: March 14, 2024

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Protocol Integer ID: 96705

Keywords: DNA Methylation, Epigenetics, Epigenomics, Methylome, Natural population, Oak, Poplar, Transposon Insertion Polymorphism, SeqCapBis, WGS, WGBS

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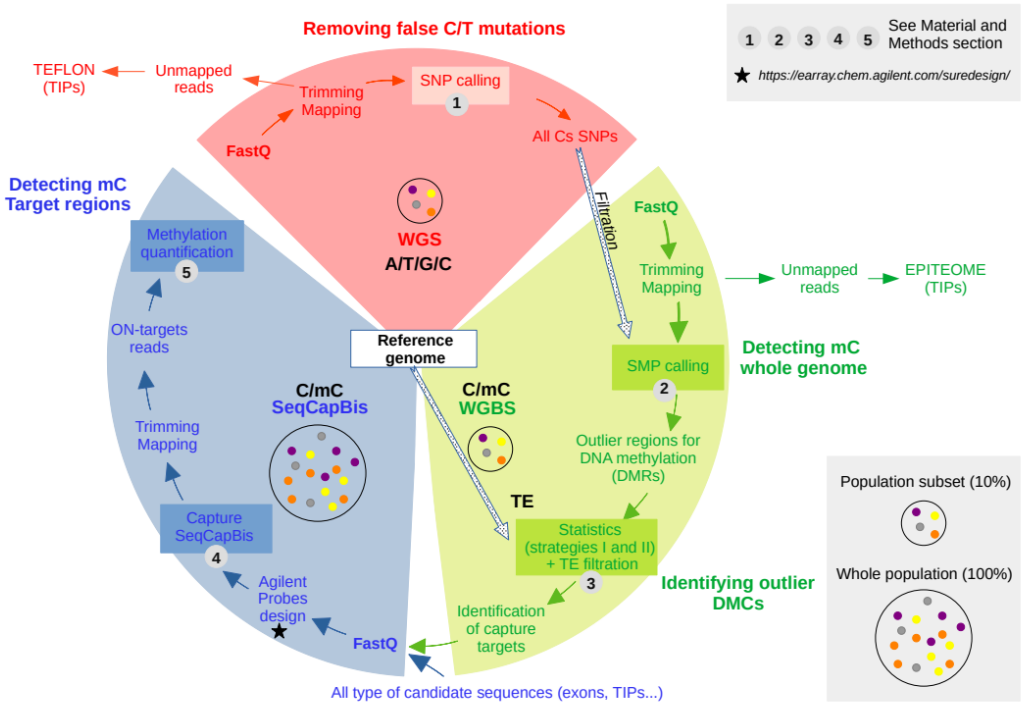
Abstract

We developed a strategy and a workflow for quantifying epigenetic diversity in natural populations combining whole genome and targeted capture sequencing for DNA methylation.
We first identified regions of highly variable DNA methylation in a representative subset of genotypes representative of the biological diversity in the population by WGBS. We then analysed the variations of DNA methylation in these targeted regions at the population level by Sequencing Capture Bisulphite (SeqCapBis).

Whole Genome Sequencing - Removing false C/T mutations

1 A preliminary Whole Genome Sequencing (WGS) step was considered for filtering purposes, to prevent C/T Single Nucleotide Polymorphisms (SNP) being interpreted as bisulfite conversions of unmethylated sites (i.e. false-positive calls). However, this C/T SNPs identification step is not required to study epigenetics levels along genomes.



Strategy for population epigenomics combining whole-genome and target genome sequencing.

2 **Trimming**

Software

Trimmomatic	NAME
https://doi.org/10.1093/bioinformatics/btu170	DEVELOPER
http://www.usadellab.org/cms/?page=trimmomatic	SOURCE LINK

Publication: Bolger et al., 2014

Version: 0.38
Github: https://github.com/usadellab/Trimmomatic

CITATION

Bolger AM, Lohse M, Usadel B (2014). Trimmomatic: a flexible trimmer for Illumina sequence data..
LINK
https://doi.org/10.1093/bioinformatics/btu170

Command

```
java -Xmx4G -jar trimmomatic.jar PE -threads 12 file_R1.fastq.gz
file_R2.fastq.gz
file_trimmed_1.fastq.gz  file_unpaired_1.fastq.gz
file_trimmed_2.fastq.gz
file_unpaired_2.fastq.gz ILLUMINACLIP:TruSeq3-PE.fa:2:30:10 LEADING:3
TRAILING:3 SLIDINGWINDOW:4:15 MINLEN:35
```

3 Mapping

Software

BWA	NAME
Unix	OS
Li, H., Durbin, R.	DEVELOPER
http://bio-bwa.sourceforge.net/	SOURCE LINK

Publication: Li H, 2013
Version: 0.7.17

CITATION

Heng Li (2013). Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. arXiv:1303.3997 [q-bio.GN].
LINK
https://doi.org/10.48550/arXiv.1303.3997

Poplar genome: *Populus trichocarpa* v3.1
Publication: Tuskan GA et al., 2006.

CITATION

Tuskan GA, Difazio S, Jansson S, Bohlmann J, Grigoriev I, Hellsten U, Putnam N, Ralph S, Rombauts S, Salamov A, Schein J, Sterck L, Aerts A, Bhalerao RR, Bhalerao RP, Blaudez D, Boerjan W, Brun A, Brunner A, Busov V, Campbell M, Carlson J, Chalot M, Chapman J, Chen GL, Cooper D, Coutinho PM, Couturier J, Covert S, Cronk Q, Cunningham R, Davis J, Degroeve S, Déjardin A, Depamphilis C, Detter J, Dirks B, Dubchak I, Duplessis S, Ehlting J, Ellis B, Gendler K, Goodstein D, Gribskov M, Grimwood J, Groover A, Gunter L, Hamberger B, Heinze B, Helariutta Y, Henrissat B, Holligan D, Holt R, Huang W, Islam-Faridi N, Jones S, Jones-Rhoades M, Jorgensen R, Joshi C, Kangasjärvi J, Karlsson J, Kelleher C, Kirkpatrick R, Kirst M, Kohler A, Kalluri U, Larimer F, Leebens-Mack J, Leplé JC, Locascio P, Lou Y, Lucas S, Martin F, Montanini B, Napoli C, Nelson DR, Nelson C, Nieminen K, Nilsson O, Pereda V, Peter G, Philippe R, Pilate G, Poliakov A, Razumovskaya J, Richardson P, Rinaldi C, Ritland K, Rouzé P, Ryaboy D, Schmutz J, Schrader J, Segerman B, Shin H, Siddiqui A, Sterky F, Terry A, Tsai CJ, Uberbacher E, Unneberg P, Vahala J, Wall K, Wessler S, Yang G, Yin T, Douglas C, Marra M, Sandberg G, Van de Peer Y, Rokhsar D (2006). The genome of black cottonwood, Populus trichocarpa (Torr. & Gray)..
LINK
https://doi.org/

Command

```
bwa mem genome.fa file_trimmed_1.fastq.gz file_trimmed_2.fastq.gz -t
12 -M > file.sam
```

3.1 Mapping adjustments for *Q. petraea*

Oak genome: *Quercus robur* Haplome V2.3
Publication: Plomion C et al., 2018

CITATION

Plomion C, Aury JM, Amselem J, Leroy T, Murat F, Duplessis S, Faye S, Francillonne N, Labadie K, Le Provost G, Lesur I, Bartholomé J, Faivre-Rampant P, Kohler A, Leplé JC, Chantret N, Chen J, Diévart A, Alaeitabar T, Barbe V, Belser C, Bergès H, Bodénès C, Bogeat-Triboulot MB, Bouffaud ML, Brachi B, Chancerel E, Cohen D, Couloux A, Da Silva C, Dossat C, Ehrenmann F, Gaspin C, Grima-Pettenati J, Guichoux E, Hecker A, Herrmann S, Hugueney P, Hummel I, Klopp C, Lalanne C, Lascoux M, Lasserre E, Lemainque A, Desprez-Loustau ML, Luyten I, Madoui MA, Mangenot S, Marchal C, Maumus F, Mercier J, Michotey C, Panaud O, Picault N, Rouhier N, Rué O, Rustenholz C, Salin F, Soler M, Tarkka M, Velt A, Zanne AE, Martin F, Wincker P, Quesneville H, Kremer A, Salse J (2018). Oak genome reveals facets of long lifespan..

LINK
https://doi.org/10.1038/s41477-018-0172-3

3.2 Mapping conversion, sorting & statistics

Software

SAMtools	NAME
Li et al.	DEVELOPER
https://github.com/samtools/	SOURCE LINK

Publication: Danecek et al., 2021
Version: 1.8
Github: https://github.com/samtools/samtools

CITATION

Danecek P, Bonfield JK, Liddle J, Marshall J, Ohan V, Pollard MO, Whitwham A, Keane T, McCarthy SA, Davies RM, Li H (2021). Twelve years of SAMtools and BCFtools..
LINK
https://doi.org/10.1093/gigascience/giab008

Command

```
samtools view -Sb file_trimmed.sam > file_trimmed.bam
samtools sort file_trimmed.bam  -o file_trimmed_sorted.bam
samtools flagstat file_trimmed_sorted.bam > file_flagstats.txt
samtools stats file_trimmed_sorted.bam > file_stats.txt
```

4 Variant calling

4.1 Adjustment for *Q. petraea* : Digital normalization

Computational limitations associated with GATK and FreeBayes due to the very deep sequencing in oak (100X on average) necessitated a reduction of the complexity of each dataset. To reduce redundancy within the WGS dataset, we randomly downsampled sequencing reads over genome regions that are over-covered.

Software

KHMER	NAME
Linux	OS
Titus Brown	DEVELOPER
https://khmer.readthedocs.io/en/latest/	SOURCE LINK

Publication: Crusoe et al., 2015

Version: 2.1.2
Github: https://github.com/dib-lab/khmer

CITATION

Crusoe MR, Alameldin HF, Awad S, Boucher E, Caldwell A, Cartwright R, Charbonneau A, Constantinides B, Edvenson G, Fay S, Fenton J, Fenzl T, Fish J, Garcia-Gutierrez L, Garland P, Gluck J, González I, Guermond S, Guo J, Gupta A, Herr JR, Howe A, Hyer A, Härpfer A, Irber L, Kidd R, Lin D, Lippi J, Mansour T, McA'Nulty P, McDonald E, Mizzi J, Murray KD, Nahum JR, Nanlohy K, Nederbragt AJ, Ortiz-Zuazaga H, Ory J, Pell J, Pepe-Ranney C, Russ ZN, Schwarz E, Scott C, Seaman J, Sievert S, Simpson J, Skennerton CT, Spencer J, Srinivasan R, Standage D, Stapleton JA, Steinman SR, Stein J, Taylor B, Trimble W, Wiencko HL, Wright M, Wyss B, Zhang Q, Zyme E, Brown CT (2015). The khmer software package: enabling efficient nucleotide sequence analysis..
LINK
https://doi.org/10.12688/f1000research.6924.1

Step1: Interleave reads
Parameters: Python-3.6.3

Command

```
interleave-reads.py file_R1.fastq file_R2.fastq -o
file_interleave_R1_R2.fastq
```

Step2: Digital normalization
Parameters: Python-3.6.3; -k 20 --> kmer size = 20bp; -C 30 --> maximal coverage; -N 4 -x 4e9 --> 16Gb

Command

```
normalize-by-median.py -k 20 -C 30 -N 4 -x 4e9
file_interleave_R1_R2.fastq  -o file_normalize_by_median_R1_R2.fastq
```

Step3: Paired reads extraction
Parameters: Python-3.6.3

Command

```
extract-paired-reads.py file_normalize_by_median_R1_R2.fastq -f --
output-paired file_diginorm_paired --output-single
file_diginorm_single
```

4.2 **Duplicates removing**

Software

picardtools NAME

Publication: “Picard Toolkit.” 2019. Broad Institute, GitHub Repository.
https://broadinstitute.github.io/picard/; Broad Institute
Version: 2.18.2
Github: https://github.com/broadinstitute/picard

Command

```
java -Xmx16g -jar picard.jar MarkDuplicates I=file_trimmed_sorted.bam
O=file_trimmed_sorted_rmdup.bam CREATE_INDEX=true
REMOVE_DUPLICATES=true M=file_output.metrics
```

4.3 **Variant Caller 1: GATK** (Genome Analysis ToolKit)

Software

NAME

GATK

Publication: McKenna et al., 2010
Version: 4.0.11.1
Github: https://github.com/broadinstitute/gatk
Poplar genome: *Populus trichocarpa* v3.1

CITATION

McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, Garimella K, Altshuler D, Gabriel S, Daly M, DePristo MA (2010). The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data..

LINK

https://doi.org/10.1101/gr.107524.110

Command

```
## HaplotypeCaller
gatk --java-options "-Xmx16G" HaplotypeCaller -R genome.fa -I
file_trimmed_sorted_rmdup.bam -ERC GVCF -O
file_trimmed_sorted_rmdup.g.vcf
## GenomicsDBImport
gatk --java-options "-Xmx96G -Xms96G" GenomicsDBImport -V
file1_trimmed_sorted_rmdup.g.vcf -V file2_trimmed_sorted_rmdup.g.vcf -
-genomicsdb-workspace-path my_database -L list_Chr+scaff.list --batch-
size 50 -ip 500
## GenotypeGVCFs
gatk GenotypeGVCFs -R genome.fa -V gendb://my_database  -new-qual
true -O all_trimmed_sorted_rmdup_gVCF_GATK.snps.indels.vcf
```

4.4 **GATK adjustments for *Q. petraea***

Version: GATK 3.8

Download: https://console.cloud.google.com/storage/browser/_details/gatk-software/package-archive/gatk/GenomeAnalysisTK-3.8-0-ge9d806836.tar.bz2;tab=live_object
Oak reference genome: *Quercus robur* Haplome V2.3
Parameters: java 1.8.0_72 ; HaplotypeCaller; GenotypeGVCFs

Command

```
#HaplotypeCaller
GATK -R haplome_v2.3.fa -T HaplotypeCaller -nct 20 -I
sample1_trimmed_vs_haploV23.bam -I sample2_trimmed_vs_haploV23.bam -I
sample3_trimmed_vs_haploV23.bam -I sample4_trimmed_vs_haploV23.bam -I
 sample5_trimmed_vs_haploV23.bam -I sample6_trimmed_vs_haploV23.bam -
I sample7_trimmed_vs_haploV23.bam -I sample8_trimmed_vs_haploV23.bam -
I sample9_trimmed_vs_haploV23.bam -I sample9_trimmed_vs_haploV23.bam
--emitRefConfidence GVCF -o gatk_nct20_slurm_1node-c20_snps.vcf

#GenotypeGVCFs
GATK -T GenotypeGVCFs -R haplome_v2.3.fa --variant sample1.vcf --
variant sample2.vcf --variant sample3.vcf --variant sample4.
vcf --variant sample5.vcf --variant sample6.vcf --variant sample7.vcf
--variant sample8.vcf --variant sample9.vcf --variant sample10.vcf -o
gatk_all10samples_SNPs.vcf
```

4.5 Variant Caller 2: samtools / bcftools

Software

SAMtools	NAME
Linux	OS
Wellcome Trust Sanger Institute	DEVELOPER
https://github.com/samtools/samtools	SOURCE LINK

Publication: Danecek et al., 2021
Version: 1.8
Github: https://github.com/samtools/samtools

Poplar genome: *Populus trichocarpa* v3.1

CITATION

Danecek P, Bonfield JK, Liddle J, Marshall J, Ohan V, Pollard MO, Whitwham A, Keane T, McCarthy SA, Davies RM, Li H (2021). Twelve years of SAMtools and BCFtools..
LINK
https://doi.org/10.1093/gigascience/giab008

Software

bcftools NAME

https://github.com/samtools/bcftools SOURCE LINK

Publication: Li H, 2011
Version: 1.8
Github: https://github.com/samtools/bcftools

CITATION

Li H (2011). A statistical framework for SNP calling, mutation discovery, association mapping and population genetical parameter estimation from sequencing data..
LINK
https://doi.org/10.1093/bioinformatics/btr509

Command

```
samtools mpileup -uf genome.fa
mapping_file_sort_without_duplicate.bam | bcftools call -mv -Oz >
file_bcftools_noduplicate.vcf.gz
```

4.6 **bcftools adjustments for *Q. petraea***

Oak genome*: Q. robur* haplome V2.3
bcftools version: 1.6
Download: **https://sourceforge.net/projects/samtools/files/samtools/1.6/**

4.7 **Variant Caller 3: FreeBayes**

Software

NAME	DEVELOPER	SOURCE LINK
freebayes	Garrison and Marth	https://github.com/freebayes/freebayes

Publication: Garrison and Marth, 2012
Version: 1.2.0-2
Github: https://github.com/freebayes/freebayes

CITATION

Erik Garrison and Gabor Marth (2012). Haplotype-based variant detection from short-read sequencing. arXiv preprint arXiv:1207.3907 [q-bio.GN] 2012.
LINK
https://doi.org/10.48550/arXiv.1207.3907

Poplar genome: *Populus trichocarpa* v3.1
Oak genome*: Q. robur* haplome V2.3

Command

```
freebayes -f genome.fa all_samples.bam > freebayes_all_samples.vcf
```

4.8 **SNP filtering**

For poplar, we considered only biallelic intra-nigra SNPs with quality threshold ≥ 30.

Software

NAME	DEVELOPER	SOURCE LINK
VCFtools	Adam Auton, Petr Danecek, Anthony Marcketta	https://vcftools.github.io/man_latest.html

Publication: Danecek et al., 2011
Version: 0.1.15
Github: https://vcftools.github.io/man_latest.html

CITATION

Danecek P, Auton A, Abecasis G, Albers CA, Banks E, DePristo MA, Handsaker RE, Lunter G, Marth GT, Sherry ST, McVean G, Durbin R, 1000 Genomes Project Analysis Group (2011). The variant call format and VCFtools..
LINK
https://doi.org/10.1093/bioinformatics/btr330

Command

```
vcftools --vcf all_tool.snps.indels.vcf --out all_filtered_tool.vcf --
remove-indels --max-alleles 2 --min-alleles 2 --minQ 30--recode --
recode-INFO-all
```

For oak, we considered bi-allelic SNPs, depth >= 20, maf >= 30% and <= 70%

4.9 **SNP identification**

Only SNPs identified by at least 2 callers were selected to obtain the final set of SNPs.

Software

NAME	SOURCE LINK
bcftools	https://github.com/samtools/bcftools

Publication: Danecek P, et al. 2021
Version: 1.8
Github: https://github.com/samtools/bcftools

CITATION

Danecek P, Bonfield JK, Liddle J, Marshall J, Ohan V, Pollard MO, Whitwham A, Keane T, McCarthy SA, Davies RM, Li H (2021). Twelve years of SAMtools and BCFtools..
LINK
https://doi.org/10.1093/gigascience/giab008

Parameters: tabix-0.2.5, samtools-1.8, bcftools-1.8

Command

```
bcftools index sample1_diginorm_gatk3.8_depth20_maf30.vcf.gz
bcftools index sample1_diginorm_FreeBayes_depth20_maf30.vcf.gz
bcftools index sample1_samtools_depth20_maf30.vcf.gz

bcftools isec -n +3 sample1_diginorm_gatk3.8_depth20_maf30.vcf.gz
sample1_diginorm_FreeBayes_depth20_maf30.vcf.gz
sample1_samtools_depth20_maf30.vcf.gz -O v -o
common_SNPs_sample1_GATK_FreeBayes_samtools_depth20_maf30_bcftools.txt
```

5 **Selection of C/T SNP**
SMPs colocalizing with a C/T SNP (see the WGS and SNP detection section of the manuscript) will be removed at step #7 "SMPs filtering".

Whole Genome Bisulfite Sequencing - Detecting mC whole genome and Identifying outlier DMCs

6 Galaxy pipeline

SMPs were identified with the GALAXY (The Galaxy Community, 2022) pipeline (Dugé de Bernonville et al., 2022; Sow et al., 2023).

CITATION

Dugé de Bernonville T, Daviaud C, Chaparro C, Tost J, Maury S (2022). From Methylome to Integrative Analysis of Tissue Specificity..

LINK

https://doi.org/10.1007/978-1-0716-2349-7_16

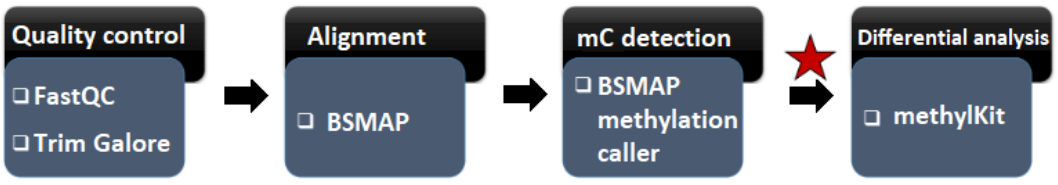
CITATION

Sow MD, Rogier O, Lesur I, Daviaud C, Mardoc E, Sanou E, Duvaux L, Civan P, Delaunay A, Lesage-Descauses MC, Benoit V, Le-Jan I, Buret C, Besse C, Durufle H, Fichot R, Le-Provost G, Guichoux E, Boury C, Garnier A, Senhaji-Rachik A, Jorge V, Ambroise C, Tost J, Plomion C, Segura V, Maury S, Salse J (2023). Epigenetic Variation in Tree Evolution: a case study in black poplar (Populus nigra). bioRxiv 2023.07.16.549253.

LINK

https://doi.org/10.1101/2023.07.16.549253

Following Sow et al., 2023:



mC detection using the Galaxy pipeline

6.1 Trimming

Software

TrimGalore

NAME

Felix Krueger

DEVELOPER

https://github.com/FelixKrueger/TrimGalore

SOURCE LINK

Publication: Krueger F et al., 2023. FelixKrueger/TrimGalore: v0.4.3.1
Version: v0.4.3.1
Github: https://github.com/FelixKrueger/TrimGalore
Parameters: --paired read1.fastq read2.fastq --clip_R1 10 --clip_R2 30

CITATION

Felix Krueger; Frankie James; Phil Ewels; Ebrahim Afyounian; Michael Weinstein; Benjamin Schuster-Boeckler;Gert Hulselmans; sclamons (2023). FelixKrueger/TrimGalore: v0.6.10. Zenodo.

LINK

https://doi.org/10.5281/zenodo.5127898

6.2 Mapping

Software

BSMAP

NAME

https://github.com/genome-vendor/bsmap/

SOURCE LINK

Publication: Xi Y and Li W, 2009
Version: v1.0.0
Github: **https://github.com/genome-vendor/bsmap/**
Parameters: default options

CITATION

Xi Y, Li W (2009). BSMAP: whole genome bisulfite sequence MAPping program..
LINK
https://doi.org/10.1186/1471-2105-10-232

Poplar genome: *Populus trichocarpa* v3.1
Mapping adjustments for *Q. petraea*
Oak genome: *Quercus robur* Haplome V2.3

6.3 **Methylation calling (SMP)**

Software

BSMAP methylation caller NAME

Greg Zynda DEVELOPER

Publication: Xi Y and Li W, 2009
Version: v1.0.0
Github: **https://github.com/genome-vendor/bsmap/**

CITATION

Xi Y, Li W (2009). BSMAP: whole genome bisulfite sequence MAPping program..
LINK
https://doi.org/10.1186/1471-2105-10-232

Poplar genome: *Populus trichocarpa* v3.1

Command

```
methratio.py   --ref ref_genome.fa --zero-meth TRUE --trim-fillin 2 --
combine-CpG --min-depth 8 --context all  bsmap_sample*.sam
```

Mapping adjustments for *Q. petraea*
Oak genome: *Quercus robur* Haplome V2.3

7 **SMP filtering**
Each methylation context (CpG, CHG, CHH) was considered separately.

Software

methylKit	NAME
Alexander Blume	DEVELOPER
https://github.com/al2na/methylKit/releases	SOURCE LINK

Publication: Akalin et al., 2012
Version: Methylkit R package v0.99.2
Github: https://github.com/al2na/methylKit/releases
Site: https://bioconductor.org/packages/release/bioc/html/methylKit.html
Parameters: R (v3.5.1), library(methylKit)

CITATION

Akalin A, Kormaksson M, Li S, Garrett-Bakelman FE, Figueroa ME, Melnick A, Mason CE (2012). methylKit: a comprehensive R package for the analysis of genome-wide DNA methylation profiles..
LINK
https://doi.org/10.1186/gb-2012-13-10-r87

Step1: Forward and reverse strands were merged for the CG context only and 30% missing data were tolerated for each context.

Command

```
meth.CpG <- unite(CpG, destrand = TRUE, min.per.group = 7L)
meth.CHG <- unite(CHG, destrand = FALSE, min.per.group = 7L)
meth.CHH <- unite(CHH, destrand = FALSE, min.per.group = 7L)
```

Step2: Positions corresponding to C/T SNPs were removed.

Command

```
SNPdat <- read.delim("SNP_file.txt", header = F)

#with SNP_file.txt:
#    ScaffoldID     position     allele1     allele2

SNPdat$Scaff_Pos <- paste(SNPdat$Scaff, SNPdat$Pos, sep="_")
SNPdat$SNP <- paste(SNPdat$Ref, SNPdat$Alt, sep ="/")
MethPos2 <- paste(meth.CpG2$chr, meth.CpG2$start, sep = "_")
MethPosMatchSNP2 <-which(MethPos2 %in% SNPdat$Scaff_Pos)
SNPMeth2 <- subset(SNPdat, Scaff_Pos %in% MethPos2[MethPosMatchSNP2])
SNPMethOk <- subset(SNPMeth2, SNP == "C/T")
CpG.posOK2 <- select(meth.CpG2, which (!MethPos2 %in%
SNPMethOk$Scaff_Pos))
```

Step3: A minimum coverage of 7X per sample was considered.

Command

```
for (i in 1:19) {
cov <- getData(meth.CHG.filtind.filtSNP.filtCov)
[,colnames(meth.CHG.filtind.filtSNP.filtCov) == paste0("coverage", i)]
cov_filt <- sort(c(which(cov < 7), which(is.na(cov))))
meth.CHG.filtind.filtSNP.filtCov[cov_filt,
colnames(meth.CHG.filtind.filtSNP.filtCov) == paste0("numCs", i)] <-
NA meth.CHG.filtind.filtSNP.filtCov[cov_filt,
colnames(meth.CHG.filtind.filtSNP.filtCov) == paste0("numTs", i)] <-
NA
     rm(cov, cov_filt)
}
```

8 Identification of target regions for the SeqCapBis design

We first grouped SMPs into 1kb sliding windows of 250bp for each methylation context. Following the calculation of the methylation levels in each window, the outlier DMRs were identified using two strategies (see 8.2 and 8.3) with homemade scripts (given as examples). Finally, target sequences correspond to outlier DMRs identified by the two strategies.

8.1 Grouping SMPs in windows and DMRs identification

Software

methylKit	NAME
Alexander Blume	DEVELOPER
https://github.com/al2na/methylKit/releases	SOURCE LINK

Publication: Akalin et al., 2012
Version: 1.18.0
Github: https://github.com/al2na/methylKit/releases
Site: https://bioconductor.org/packages/release/bioc/html/methylKit.html
Parameters: MethylKit package

CITATION

Akalin A, Kormaksson M, Li S, Garrett-Bakelman FE, Figueroa ME, Melnick A, Mason CE (2012). methylKit: a comprehensive R package for the analysis of genome-wide DNA methylation profiles..

LINK

https://doi.org/10.1186/gb-2012-13-10-r87

Input files: pre-filtered SMPs in each context.

Command

```
meth.CpG.window <-
tileMethylCounts(meth.CpG.filtind.filtSNP.filtTE.filtCov.filtNA,win.si
ze = 1000, step.size = 250)
meth.CHG.window <-
tileMethylCounts(meth.CHG.filtind.filtSNP.filtTE.filtCov.filtNA,win.si
ze = 1000, step.size = 250)
meth.CHH.window <-
tileMethylCounts(meth.CHH.filtind.filtSNP.filtTE.filtCov.filtNA,win.si
ze = 1000, step.size = 250)
```

8.2 **Strategy I: STANDARD DEVIATION OF THE MEANS**

Calculate average C-methylation by averaging the methylation level across all (pre-filtered) cytosines in each window for each individual. Then calculate standard deviation of this average across individuals.

Command

```
#Identification of windows to remove
percmeth.CpG.window.sd <- rowSds(percmeth.CpG.window, na.rm = TRUE)
sum(percmeth.CpG.window.sd == 0)

# Removal of windows showing the less variable levels of methylation
percmeth.CpG.window <-
percmeth.CpG.window[which(percmeth.CpG.window.sd != 0), ]
dim(percmeth.CpG.window)

#Identification of the windows associated with the most variable
methylation levels
percmeth.CpG.window.sd <- rowSds(percmeth.CpG.window, na.rm = TRUE)
layout(matrix(c(rep(1, 2), 2), nrow = 1))
hist(percmeth.CpG.window.sd, col = "grey", main = "")
bp <- boxplot(percmeth.CpG.window.sd, col = "grey")
length(bp$out)
bp$stats
```

8.3 **Strategy II: MEAN OF THE STANDARD DEVIATIONS**

For each (pre-filtered) cytosine, calculate the standard deviation of methylation across individuals. Then calculate the mean standard deviation from all cytosines in a window.

Command

```
dag_window_size=1000
dag_step=250

load("meth.CHG.filtind.filtSNP.filtTE.filtCov.filtNA.Rdata")
y<-x[,c("chr","start","end","strand")]

for (i in 1:length(colnames(x)[colnames(x) %like% "coverage"])){  #
To recover the C/coverage values
  j=5+3*(i-1)
  print(paste0(j," ",j+1))
  y[,paste0("in",i)]<-x[,j+1]/x[,j]
}
yy<-x[,c("chr","start","end","strand")]
rm(x)

z<-rowSds(as.matrix(y[,5:ncol(y)]),na.rm=TRUE) # Calculate row
standard deviations
yy$STDEV<-z
rm(z)
y<-yy
rm(yy)


# Do last adaptations and launch
dag_window=dag_window_size/dag_step
colnames(y)<-c("CHR","START","END","STRAND","STDEV")
y$MEAN<-(y$START+y$END)/2
y$CHR<-gsub("Chr0","Chr",y$CHR,perl=TRUE)
y$WINDOW<-floor(y$MEAN/dag_step)+1


stdev_counts = data.table(
   CHR = character(),
   WIN = numeric(),
   POS = numeric(),
   STDEV = numeric()
)

count=0
for (i in unique(y[y$CHR %like% "Chr" | y$CHR %like%
"scaffold",]$CHR)){
  window_size=dag_window_size
```

```
  step=dag_step
  #i<-paste0("Chr",i)
  z<-y[y$CHR==i,]
  min=0
  max=max(z$WINDOW)
  #print(paste(i,min,max,min(z$MEAN),max(z$MEAN)))
  count=count+1

print(paste(i,min,max,min(z$MEAN),max(z$MEAN),count,length(unique(y[y$
CHR %like% "Chr" | y$CHR %like% "scaffold",]$CHR))))
  zz<-data.frame(matrix(ncol=2,nrow=max*step))
  colnames(zz)<-c("MEAN","STDEV")
  zz$MEAN<-rownames(zz)

  zz[zz$MEAN %in% z$MEAN,]$STDEV<-z[z$MEAN %in% zz$MEAN,]$STDEV

 # Sliding window
  total <- nrow(zz)
  if (max(z$MEAN)<window_size){ # Adapted to avoid problems with
scaffolds smaller than window_size
    spots <- 1
  }
  else {
    spots <- seq(from=1, to=(total-window_size), by=step)
  }

  if (spots[length(spots)]<=total-window_size){spots<-c(spots,
(spots[length(spots)]+step))} # Adapted to recover the last bits
inside smaller window
  result <- vector(length = length(spots))
  for(j in 1:length(spots)){
    if (j%%50000==0){print(paste(j,length(spots)))}
    if ((spots[j]+window_size)>=total){window_size=(total-spots[j])}
# Adapted to recover the last bits inside last smaller window
    result[j] <- mean(zz[spots[j]:(spots[j]+window_size-
1),"STDEV"],na.rm=TRUE)
  }

  stdev_counts<-
rbind(stdev_counts,data.frame(CHR=i,WIN=1:length(spots),POS=spots,STDE
V=result))
}

x<-stdev_counts
write.table(x,file=paste0(save_file_name))
```

8.4 **Outlier threshold**

The threshold for DMRs is defined as (Q3+1.5*(Q3-Q1)) where Q1 and Q3 are the first and third quartiles (i.e. the threshold is not defined by a percentile, but instead depends on the length of the boxplot box)

*** Strategy I**

Parameters: Python 3.7

Command

```
#$Id$

###run with python get_threshold_over_all_windows_calc1.py
OUTPUT_FILE_from_calc1_get_mean_and_stdv_for_each_window.py >
threshold_calc1.txt


import os
import re
import string
import sys
import glob
import numpy

file1 = sys.argv[1]
file1_stream = open(file1)
list_of_means = []

for line1 in file1_stream.readlines():
        if (line1.count('start') == 0):
                line1 = line1.replace('\n','')
                splitted_line1 = line1.split('\t')
                scaffold = splitted_line1[0]
                start = splitted_line1[1]
                end = splitted_line1[2]

                mean = splitted_line1[13]
                mean = float(mean)
                list_of_means.append(mean)

list_of_means.sort()
nbre_de_means = len(list_of_means)
##XXX corresponds to the first half of the dataset
##YYY corresponds to the second half of the dataset
Q1 = numpy.median(list_of_means[:XXX])
Q3 = numpy.median(list_of_means[YYY:])

##for CHH context, hreshold = (Q3 + 3*(Q3- Q1))
threshold = (Q3 + 1.5*(Q3- Q1))
threshold = round(threshold,5)

print 'threshold = ',threshold
```

*** Strategy II**

Parameters: Python 3.7

Command

```
#$Id$

###run with python get_threshold_stdv_over_all_windows_calc2.py
OUTPUT_FILE_from_get_stdv_between_individuals_for_each_window_calc2.py
 > threshold_calc2.txt


import os
import re
import string
import sys
import glob
import numpy

file1 = sys.argv[1]
file1_stream = open(file1)
list_of_stdv = []

for line1 in file1_stream.readlines():
        if (line1.count('start') == 0):
                line1 = line1.replace('\n','')
                splitted_line1 = line1.split('\t')
                scaffold = splitted_line1[0]
                start = splitted_line1[1]
                end = splitted_line1[2]

                stdv = splitted_line1[4]
                stdv = float(stdv)
                list_of_stdv.append(stdv)

list_of_stdv.sort()
nbre_de_stdv = len(list_of_stdv)
##XXX corresponds to the first half of the dataset
##YYY corresponds to the second half of the dataset
Q1 = numpy.median(list_of_stdv[:XXX])
Q3 = numpy.median(list_of_stdv[YYY:])

##for CHH context, hreshold = (Q3 + 3*(Q3- Q1))
threshold = (Q3 + 1.5*(Q3- Q1))
threshold = round(threshold,5)

print 'threshold = ',threshold
```

8.5 **Identification of capture targets**

Target sequences correspond to outlier DMRs identified by the two strategies. This is a two-steps strategy where the 3 contexts are first merged and, then, sequence redundancy between the three methylation contexts is removed.

Software

bedtools	NAME
Linux	OS

Publication: Quinlan AR and Hall IM, 2010
Version: 2.27.1
Github: https://github.com/arq5x/bedtools2
Parameters: intersect, merge

CITATION

Quinlan AR, Hall IM (2010). BEDTools: a flexible suite of utilities for comparing genomic features..
LINK
https://doi.org/10.1093/bioinformatics/btq033

SeqCapBis - Detecting mC Target regions

9 **Agilent Probes design and sequencing**

A set of 120 bp probes was selected to capture 18 Mb of each genome (Agilent, https://earray.chem.agilent.com/suredesign/). The targeted regions corresponded to the regions identified as differentially methylated between populations. Custom targeted genome bisulfite sequencing was performed with SureSelect XT Methyl-Seq Target Enrichment (Agilent, Santa Clara, CA, USA) according to the manufacturer's recommendations.

For poplar, in total, 17.84 Mb of sequence corresponding to the 25,434 DMRs was covered by 339,658 probes. Regarding oak, a set of 140,249 probes (120 bp) was designed by

Agilent to cover 16.15 Mb DMRs.

10 **Trimming**

Software

NAME	OS
TrimGalore	Linux

Publication: Krueger F et al., 2023. FelixKrueger/TrimGalore: v0.6.5
Version: 0.6.5
Github: https://github.com/FelixKrueger/TrimGalore

CITATION

Felix Krueger; Frankie James; Phil Ewels; Ebrahim Afyounian; Michael Weinstein; Benjamin Schuster-Boeckler;Gert Hulselmans; sclamons (2023). FelixKrueger/TrimGalore: v0.6.10. Zenodo.

LINK
https://doi.org/10.5281/zenodo.5127898

Command

```
trim_galore input_R1.fastq.gz input_R2.fastq.gz --paired ADAPTER1 -a2
ADAPTER2 -o output_directory --gzip -j {threads}
```

11 **Quality control**

Software

FastQC	NAME
Linux	OS
Simon Andrews	DEVELOPER

Publication: Andrews, S. (2010). FastQC: A Quality Control Tool for High Throughput Sequence Data [Online]. Available online at:
http://www.bioinformatics.babraham.ac.uk/projects/fastqc/
Version: 0.11.9
Github: https://github.com/s-andrews/FastQC

Command

```
fastqc trimmed_reads.fq.gz -o fastQC_output_directory -t {threads}
```

12 **Mapping**

Software

BsmapZ	NAME
Linux	OS

Publications:
- Xi Y, Li W, 2009

CITATION

Xi Y, Li W (2009). BSMAP: whole genome bisulfite sequence MAPping program..
LINK
https://doi.org/10.1186/1471-2105-10-232

- Zynda G. 2018. BSMAPz. https://github.com/zyndagj/BSMAPz

Version: 1.1.3
Github: https://github.com/zyndagj/BSMAPz
Poplar genome: *Populus trichocarpa* v4.1

Command

```
bsmapz -a fileR1.fq.gz -b fileR2.fq.gz -o {output.out} -d
mapped_file.bam -d ref_genome.fa -p threads
```

Mapping adjustments for *Q. petraea*
Oak genome: *Quercus robur* Haplome V2.3

12.1 **Duplicate Removing**

Software

NAME	OS
samtools	Linux

Publication: Danecek et al., 2021
Version: 1.11
Github: https://github.com/samtools/samtools
Parameters: stat, fixmate, sort, markdup
Poplar genome: *Populus trichocarpa* v4.1

CITATION

Danecek P, Bonfield JK, Liddle J, Marshall J, Ohan V, Pollard MO, Whitwham A, Keane T, McCarthy SA, Davies RM, Li H (2021). Twelve years of SAMtools and BCFtools..
LINK
https://doi.org/10.1093/gigascience/giab008

Command

```
samtools stats sample_bsmapz_sorted.bam -r ref_genome.fa -@ {threads}
> sample.statics
samtools fixmate -@ {threads} -O BAM -m  sample_bsmapz_sorted.bam
sample_fixmate.bam
samtools sort -@ {threads} -O BAM sample_fixmate.bam  -o
sample_fixmate_sort.bam
samtools markdup -r  ref_genome.fa -@ {threads} -s -f sample.statics
sample_fixmate_sort.bam sample_fixmate_sort_temp.bam
```

Mapping adjustments for *Q. petraea*
Oak genome: *Quercus robur* Haplome V2.3

13 **Detection of methylated cytosines (mC)**

Software

BsmapZ	NAME
Linux	OS

Publications:
- Xi Y and Li W, 2009.

CITATION

Xi Y, Li W (2009). BSMAP: whole genome bisulfite sequence MAPping program..
LINK
https://doi.org/10.1186/1471-2105-10-232

- Zynda G. 2018. BSMAPz. https://github.com/zyndagj/BSMAPz

Version: 1.1.3
Github: https://github.com/zyndagj/BSMAPz
Poplar genome: *Populus trichocarpa* v4.1
Parameters: methratio.py, python 2.7, samtools 1.11, pysam 0.16.0.1

Command

```
python methratio.py sample.dedup.bam -o meth_sample.txt -d
ref_genome.fa -N {threads} -I
```

Mapping adjustments for *Q. petraea*
Oak genome: *Quercus robur* Haplome V2.3

14 **10X sequencing filtering**

Software

methylKit	NAME
Alexander Blume	DEVELOPER
https://github.com/al2na/methylKit/releases	SOURCE LINK

Publication: Akalin A et al, 2012.
Version: 1.18.0
Parameters: MethylKit package
Github: https://github.com/al2na/methylKit/releases
Site: https://bioconductor.org/packages/release/bioc/html/methylKit.html

CITATION

Akalin A, Kormaksson M, Li S, Garrett-Bakelman FE, Figueroa ME, Melnick A, Mason CE (2012). methylKit: a comprehensive R package for the analysis of genome-wide DNA methylation profiles..

LINK

https://doi.org/10.1186/gb-2012-13-10-r87

Command

```
SeqCapBis_CHG = methRead(location = path_to_the_files, sample.id =
sample.ids, assembly = "quercus", mincov = 10, context = "CHG",
treatment = rep(0,10))
```

15 **Splitting context**

We set up a homemade bash script (splitting.sh) to obtain methylation files for each sample in the three contexts (CG, CHG and CHH).

Command

```
#!/bin/bash
# Splitting context:

usage()
{
cat << EOF
usage: $0 <options>
splitting context.
OPTION:
  -h      show this Help message.
  -o      Output.
  -i      Input.
EOF
}

# Get options
while getopts "ho:i:" OPTION
do
  case $OPTION in
    h)  usage; exit 1;;
    o)  output=$OPTARG;;
    i)  input=$OPTARG;;
    ?)  usage; exit;;
  esac
done

# Check that all options were passed
if [[ -z $output ]] || [[ -z $input ]]
then
  printf "\n=========================\n ERROR: missing
options\n=========================\n\n"
  usage
  exit 1
fi

#in_file = snakemake.input["isoforms"]
#out_file = snakemake.output["plot"]

# Fail on the first error
set -e

######################
```

```
file=$(echo $output|rev|cut -d "/" -f 1 |rev)
path=$(echo $output|rev|cut -d "/" -f 2- |rev)

for context in "CHH" "CG" "CHG"; do

   awk "NR<=1 || \$4~/$context/" $input  > $path/$context-$file ;
done
```

16 **Methylation quantification**

Software

methylKit	NAME
Alexander Blume	DEVELOPER
https://github.com/al2na/methylKit/releases	SOURCE LINK

Publication: Akalin A et al, 2012.
Version: 1.18.0
Parameters: MethylKit package
Github: https://github.com/al2na/methylKit/releases
Site: https://bioconductor.org/packages/release/bioc/html/methylKit.html

CITATION

Akalin A, Kormaksson M, Li S, Garrett-Bakelman FE, Figueroa ME, Melnick A, Mason CE (2012). methylKit: a comprehensive R package for the analysis of genome-wide DNA methylation profiles..
LINK
https://doi.org/10.1186/gb-2012-13-10-r87

Functions: getMethylationStats(), getCoverageStats()

Command

```
# Read methylation using methylkit function methRead
myobj <- methRead(location = files, sample.id = sample_id, assembly =
"populus tricharpa v3.1", mincov = 1, context = context,treatment =
rep(0, length(files)), pipeline = list(fraction=TRUE, chr.col=1,
start.col=2, end.col=2, coverage.col=6, strand.col=3, freqC.col=5 ))

# Concatenate all samples tables into one unique table
finalFrame <- mergeMethylkitOutput(myobj)

#Write the final table as a csv2 file
write.csv2(finalFrame,file = table,)

# head(myobj)

# plots for statistcs and coverage simple :
pdf(file = XXX)
getMethylationStats(myobj[[1]],plot=TRUE,both.strands=FALSE)
getCoverageStats(myobj[[1]],plot=TRUE,both.strands=FALSE)
dev.off()
```

Transposon insertion polymorphisms (TIPs)

17 **Trimming**

Eliminate unwanted or irrelevant parts of the read. Data trimming may include removing low quality bases or adapters used during sequencing.

Software

TrimGalore	NAME
Linux	OS
Felix Krueger	DEVELOPER

CITATION

Felix Krueger; Frankie James; Phil Ewels; Ebrahim Afyounian; Michael Weinstein; Benjamin Schuster-Boeckler;Gert Hulselmans; sclamons (2023). FelixKrueger/TrimGalore: v0.6.10. Zenodo.

LINK

https://doi.org/10.5281/zenodo.5127898

Command

```
#Trim the paired sequences
trim_galore -q 30 --paired -o paired_1.fastq  paired_2.fastq
```

18 Detection of TIPs on whole genome sequencing (WGS) data with TEFLoN

18.1 Mapping

Alignment of DNA sequences to a reference genome.

Software

BWA	NAME
Linux	OS
Heng Li	DEVELOPER

CITATION

Heng Li; Richard Durbin (2009). Fast and accurate short read alignment with Burrows-Wheeler transform. bioinformatics.
LINK
https://doi.org/10.1093/bioinformatics/btp324

Command

```
#Index Genome
bwa index genome_ref.fa

#Align
bwa mem -Y genome_ref.fa  paired_trimmed_1.fastq
paired_trimmed_2.fastq > whole.sam
```

18.2 **Extracting unmapped reads**

Search for TIPs from reads not aligning with the reference genome. It is interesting to choose non-mapped sequences, because we hypothesize that the insertion of a transposable element is one of the reasons which prevented the alignment of certain reads to their reference genome.

Software

samtools NAME

https://github.com/samtools/samtools SOURCE LINK

CITATION

Petr Danecek, James K Bonfield, Jennifer Liddle, John Marshall, Valeriu Ohan, Martin O Pollard, Andrew Whitwham, Thomas Keane, Shane A McCarthy, Robert M Davies, Heng Li (2021). Twelve years of SAMtools and BCFtools. GigaScience, Volume 10.
LINK
https://doi.org/10.1093/gigascience/giab008

Command

```
#From SAM2BAM
samtools view -S -b whole.sam -o whole.bam

#Extract Unmapped reads

#An unmapped read whose mate is mapped.
samtools view -u  -f 4 -F264 whole.bam  > tmps1.bam

#Both reads of the pair are unmapped
samtools view -u -f 12 -F 256 whole.bam > tmps2.bam

#merge
samtools merge unmapped.bam tmps1.bam tmps2.bam
```

Software

BamToFastq	NAME
Linux	OS
Maxime U Garcia	DEVELOPER

CITATION

Friederike Hanssen, SusiJo, Gisela Gabernet, Maxime U Garcia, Matilda Åslin, nf-core bot (2023). nf-core/bamtofastq: 2.1.0. Zenodo.
LINK
https://doi.org/10.5281/zenodo.4710628

Command

```
#Extract the reads in FASTQ format (paired)
bamToFastq -bam unmapped.bam -fq1 unmapped_reads1.fastq -fq2
unmapped_reads2.fastq
```

18.3 **TIPs detection**

Search for TIPs from reads not aligning with the reference genome. It is interesting to choose non-mapped sequences, because we hypothesize that the insertion of a transposable element is one of the reasons which prevented the alignment of certain reads to their reference genome.

Software

TEFLoN	NAME
Linux	OS
Jeffrey Adrion	DEVELOPER

CITATION

Adrion, J.R., M.J. Song, D.R. Schrider, M.W. Hahn, and S. Schaack (2017). Genome-wide estimates of transposable element insertion and deletion rates in *Drosophila melanogaster*. Genome Biology and Evolution.
LINK
https://doi.org/10.1093/gbe/evx050

Software

RepeatMasker	NAME
Linux	OS
Robert Hubley	DEVELOPER

Command

```
WD="path/to/working/_directory"
PREFIX="prefix_you_want"

##For each samples
python teflon_prep_custom.py -wd ${WD}reference -g genome_ref -l
path/to/TE_LIBRARY -p ${PREFIX}

bwa index ${WD}reference/${PREFIX}.prep_MP/${PREFIX}.mappingRef.fa

bwa mem -Y ${WD}reference/${PREFIX}.prep_MP/${PREFIX}.mappingRef.fa
${READS1} ${READS2} > ${WD}reference/${PREFIX}.sam

samtools view -Sb ${WD}reference/${PREFIX}.sam | samtools sort -o
${WD}reference/${PREFIX}.sorted.bam

samtools index ${WD}reference/${PREFIX}.sorted.bam

#Run Teflon
#For each samples
python teflon.v0.4.py -wd ${WD} -d ${WD}reference/${PREFIX}.prep_TF/ -
s path/to/samples -i unique_ID -l1 family -l2 class

#Teflon collapse
##Only once
python teflon_collapse.py -wd ${WD} -d
${WD}reference/${PREFIX}.prep_TF/ -s path/to/samples -n1
minimum_reads_to_support_TE_in_one_sample -n2
minimum_reads_to_support_TE_in_all_samples

#Teflon Count
#For each samples
python teflon_count.py -wd ${WD} -d ${WD}reference/${PREFIX}.prep_TF/
-s path/to/samples -i unique_ID

#Teflon genotype
##Only once
python teflon_genotype.py -wd ${WD} -d
${WD}reference/${PREFIX}.prep_TF/ -s path/to/samples -dt pooled
```

19 Detection of TIPs on whole genome bisulfite sequencing (WGBS) data with epiTEome

19.1 Mapping and extracting unmapped reads

Alignment of DNA sequences to a reference genome. Search for TIPs from reads not aligning with the reference genome. We choose non-mapped sequences, because we hypothesize that the insertion of a transposable element is one of the reasons which prevented the alignment of certain reads to their reference genome.

Software

NAME	DEVELOPER
Bismark	Felix Krueger

CITATION

Felix Krueger, Simon R Andrews (2011). Bismark: a flexible aligner and methylation caller for Bisulfite-Seq applications. Bioinformatics.
LINK
https://doi.org/10.1093/bioinformatics/btr167

Command

```
bismark_genome_preparation --verbose genome_ref.fa

bismark --genome genome_ref.fa paired_trimmed_1.fastq
paired_trimmed_2.fastq  --un
```

19.2 TIPs detection

Search for TIPs from reads not aligning with the reference genome. It is interesting to choose non-mapped sequences, because we hypothesize that the insertion of a

transposable element is one of the reasons which prevented the alignment of certain reads to their reference genome.

Software

NAME	DEVELOPER
epiTEome	Josquin Daron

CITATION

Josquin Daron & R. Keith Slotkin (2017). EpiTEome: Simultaneous detection of transposable element insertion sites and their DNA methylation levels. Genome Biology.
LINK
https://doi.org/10.1186/s13059-017-1232-0

Command

```
idxEpiTEome.pl -l 100 -gff genome_ref.gff -t /path/to/TE_LIBRARY -
fasta genome_ref.fa

epiTEome.pl -gff genome_ref.gff -ref genome_ref.epiTEome.masked.fasta
-un unmapped_reads.fastq -t /path/to/TE_LIBRARY
```

Citations

Step 12

Xi Y, Li W. BSMAP: whole genome bisulfite sequence MAPping program.
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Step 12.1

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Step 13

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Step 14

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