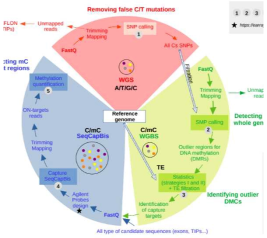
Jun 20, 2024

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



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DOI: dx.doi.org/10.17504/protocols.io.8epv5xw4ng1b/v1

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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We use this protocol and it's working

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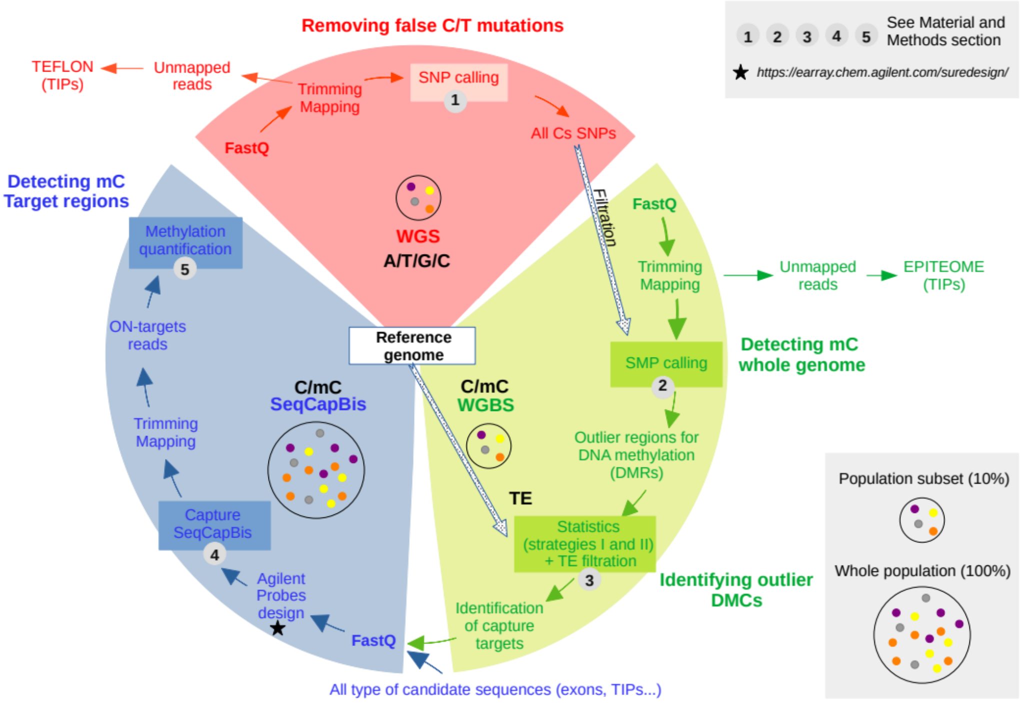
Abstract

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.


Removing false C/T mutations
1 2 3 4 5 See Material and Methods section
★ https://earray.chem.agilent.com/suredesign/
TEFLON (TIPs)
Unmapped reads
Trimming Mapping
SNP calling
1
FastQ
All Cs SNPs
WGS
A/T/G/C
Detecting mC Target regions
Methylation quantification
5
ON-targets reads
Trimming Mapping
Capture SeqCapBis
4
Agilent Probes design
★
FastQ
C/mC
SeqCapBis
Reference genome
C/mC
WGBS
TE
Filtration
FastQ
Trimming Mapping
Unmapped reads
EPITEOME (TIPs)
Detecting mC whole genome
SMP calling
2
Outlier regions for DNA methylation (DMRs)
Statistics (strategies I and II) + TE filtration
3
Identifying outlier DMCs
Identification of capture targets
All type of candidate sequences (exons, TIPs...)
Population subset (10%)
Whole population (100%)

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

GATK NAME

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

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

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

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

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

bcftools NAME

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

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

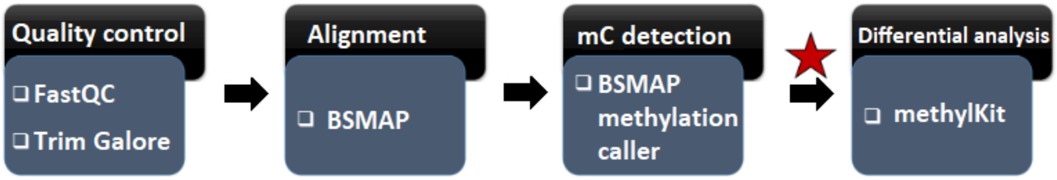
CITATION

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

TrimGalore	NAME
Linux	OS

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

samtools	NAME
Linux	OS

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	
Bismark	NAME
Felix Krueger	DEVELOPER

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

epiTEome	NAME
Josquin Daron	DEVELOPER

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.
https://doi.org/10.1186/1471-2105-10-232

Step 12.1

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

Step 13

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

Step 14

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

Step 16

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

Step 17

Felix Krueger; Frankie James; Phil Ewels; Ebrahim Afyounian; Michael Weinstein; Benjamin Schuster-Boeckler;Gert Hulselmans; sclamons. FelixKrueger/TrimGalore: v0.6.10
https://doi.org/10.5281/zenodo.5127898

Step 18.1

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

Step 2

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

Step 3

Heng Li. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM
https://doi.org/10.48550/arXiv.1303.3997

Step 3

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. The genome of black cottonwood, Populus trichocarpa (Torr. & Gray).
https://doi.org/

Step 3.1

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. Oak genome reveals facets of long lifespan.
https://doi.org/10.1038/s41477-018-0172-3

Step 3.2

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

Step 4.1

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. The khmer software package: enabling efficient nucleotide sequence analysis.
https://doi.org/10.12688/f1000research.6924.1

Step 4.3

McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, Garimella K, Altshuler D, Gabriel S, Daly M, DePristo MA. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data.
https://doi.org/10.1101/gr.107524.110

Step 4.5

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

Step 4.5

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

Step 4.7

Erik Garrison and Gabor Marth. Haplotype-based variant detection from short-read sequencing
https://doi.org/10.48550/arXiv.1207.3907

Step 4.8

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. The variant call format and VCFtools.
https://doi.org/10.1093/bioinformatics/btr330

Step 4.9

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

Step 6

Dugé de Bernonville T, Daviaud C, Chaparro C, Tost J, Maury S. From Methylome to Integrative Analysis of Tissue Specificity.
https://doi.org/10.1007/978-1-0716-2349-7_16

Step 6

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. Epigenetic Variation in Tree Evolution: a case study in black poplar (Populus nigra)
https://doi.org/10.1101/2023.07.16.549253

Step 6.2

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

Step 6.3

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

Step 7

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

Step 8.1

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

Step 8.1

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

Step 8.5

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