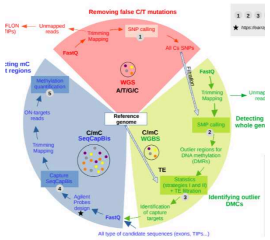
Jun 20, 2024

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



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

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Manuscript citation:
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

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Keywords: DNA Methylation, Epigenetics, Epigenomics, Methylome, Natural population, Oak, Poplar, Transposon Insertion Polymorphism, SeqCapBis, WGS, WGBS, quantifying epigenetic diversity, epigenetic diversity in natural population, bioinformatics manual for population epigenomic, population epigenomic, variations of dna methylation, variable dna methylation, dna methylation, genotypes representative of the biological diversity, sequencing capture bisulphite, genome sequencing, whole genome, genotypes representative, genome, bioinformatics manual, biological diversity, sequencing, combining whole genome, bioinformatics, natural population, population by wgb, targeted region, capture bisulphite

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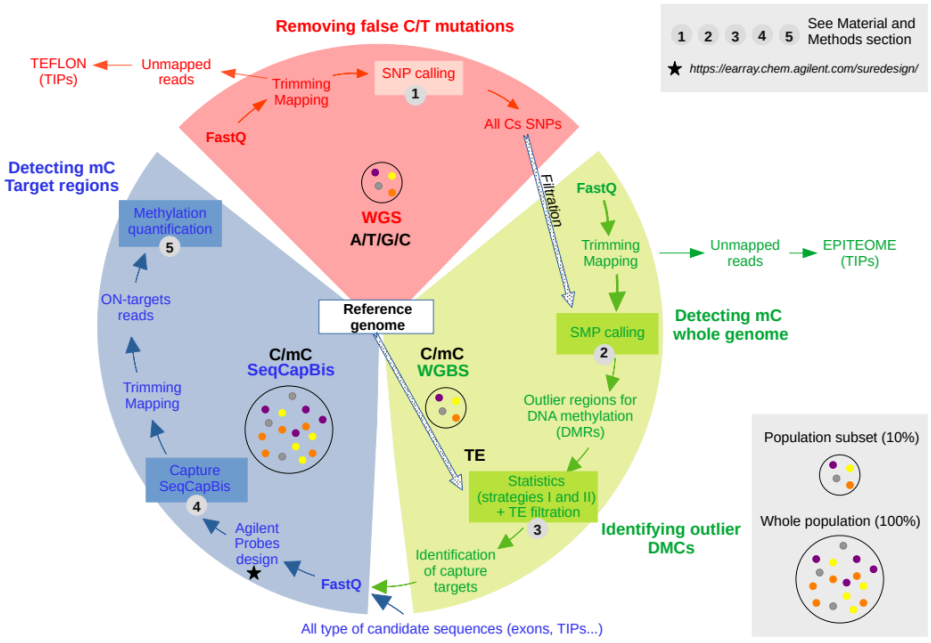
Abstract

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).

Troubleshooting

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..

https://doi.org/10.1093/bioinformatics/btu170 LINK

Command

new command name

```
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 (Invalid date)
. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM.
arXiv:1303.3997 [q-bio.GN].

https://doi.org/10.48550/arXiv.1303.3997 LINK

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)..

https://doi.org/ LINK

Command

new command name

```
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..

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

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..

https://doi.org/10.1093/gigascience/giab008

LINK

Command

new command name

```
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..

https://doi.org/10.12688/f1000research.6924.1 LINK

Step1: Interleave reads
Parameters: Python-3.6.3

Command

new command name

```
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

new command name

```
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

new command name

```
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

new command name

```
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..

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

Command

new command name

```
## 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

new command name

```
#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..

https://doi.org/10.1093/gigascience/giab008 LINK

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..

https://doi.org/10.1093/bioinformatics/btr509 LINK

Command

new command name

```
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 (Invalid date)
. Haplotype-based variant detection from short-read sequencing.
arXiv preprint arXiv:1207.3907 [q-bio.GN] 2012.

https://doi.org/10.48550/arXiv.1207.3907 LINK

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

Command

new command name

```
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..

https://doi.org/10.1093/bioinformatics/btr330 LINK

Command

new command name

```
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..

https://doi.org/10.1093/gigascience/giab008 LINK

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

Command

new command name

```
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 (2021)
. From Methylome to Integrative Analysis of Tissue Specificity..

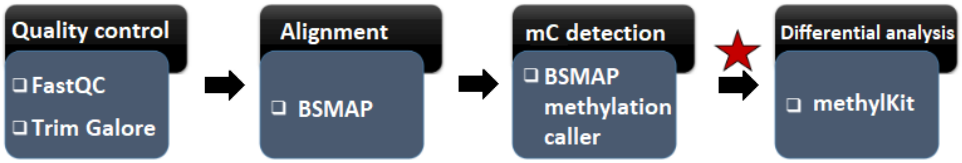
https://doi.org/10.1007/978-1-0716-2349-7_16 LINK

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
(Invalid date)
. Epigenetic Variation in Tree Evolution: a case study in black poplar (Populus nigra).
bioRxiv 2023.07.16.549253.

https://doi.org/10.1101/2023.07.16.549253 LINK

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 (Invalid date). FelixKrueger/TrimGalore: v0.6.10. Zenodo.

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

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..

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

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..

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

Poplar genome: *Populus trichocarpa* v3.1

Command

new command name

```
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..

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

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

Command

new command name

```
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

new command name

```
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

new command name

```
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..

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

Input files: pre-filtered SMPs in each context.

Command

new command name

```
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

new command name

```
#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

new command name

```
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

new command name

```
#$Id$

###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

new command name

```
#$Id$

###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..

https://doi.org/10.1093/bioinformatics/btq033 LINK

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
(Invalid date). FelixKrueger/TrimGalore: v0.6.10. Zenodo.

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

Command

new command name

```
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

new command name

```
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..

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

- 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

new command name

```
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..

https://doi.org/10.1093/gigascience/giab008 LINK

Command

new command name

```
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..

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

- 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

new command name

```
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..

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

Command

new command name

```
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

new command name

```
#!/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..

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

Functions: getMethylationStats(), getCoverageStats()

Command

new command name

```
# 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
(Invalid date). FelixKrueger/TrimGalore: v0.6.10. Zenodo.

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

Command

new command name

```
#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

NAME	OS	DEVELOPER
BWA	Linux	Heng Li

Citation

Heng Li; Richard Durbin (Invalid date)
. Fast and accurate short read alignment with Burrows-Wheeler transform.
bioinformatics.

https://doi.org/10.1093/bioinformatics/btp324 LINK

Command

new command name

```
#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 (Invalid date). Twelve years of SAMtools and BCFtools. GigaScience, Volume 10.

https://doi.org/10.1093/gigascience/giab008

LINK

Command

new command name

```
#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 (Invalid date). nf-core/bamtofastq: 2.1.0. Zenodo.

https://doi.org/10.5281/zenodo.4710628 LINK

Command

new command name

```
#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 (Invalid date) . Genome-wide estimates of transposable element insertion and deletion rates in *Drosophila melanogaster*. Genome Biology and Evolution.

https://doi.org/10.1093/gbe/evx050 LINK

Software

RepeatMasker	NAME
Linux	OS
Robert Hubley	DEVELOPER

Command

new command name

```
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 (Invalid date)
. Bismark: a flexible aligner and methylation caller for Bisulfite-Seq applications.
Bioinformatics.

https://doi.org/10.1093/bioinformatics/btr167 LINK

Command

new command name

```
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 (Invalid date)
. EpiTEome: Simultaneous detection of transposable element insertion sites and their DNA methylation levels.
Genome Biology.

https://doi.org/10.1186/s13059-017-1232-0 LINK

Command

new command name

```
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

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https://doi.org/10.1093/gigascience/giab008

Step 13

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Step 14

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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
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