

Forward genetics approach reveals host-genotype dependent importance of accessory chromosomes in the fungal wheat pathogen *Zymoseptoria tritici*

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Supplementary text

Reference mapping of Illumina reads

Paired-end reads of 150 bp were mapped directly to the genome of the reference isolate IPO323 [1]. Processing of the reads were carried out using the below listed pipeline:

- 1) Quality filtering using Trimmomatic V0.30 [2]

```
java -jar /trimmomatic-0.30.jar PE -phred33 R1.fastq R2.fastq R1_paired.fastq  
R1_unpaired.fastq R2_paired.fastq R2_unpaired.fastq HEADCROP:2 CROP:149  
LEADING:3 TRAILING:3 SLIDINGWINDOW:4:15 MINLEN:50
```

<http://www.usadellab.org/cms/?page=trimmomatic>

- 2) Mapping to IPO323 reference genome using Bowtie 2 version 2.1.0 [3]

```
bowtie2 -p 6 -q -x IPO323_reference -1 R1_paired.fastq -2 R2_paired.fastq -S R.sam
```

<http://bowtie-bio.sourceforge.net/bowtie2/index.shtml>

- 3) Converting to BAM and sorting using Picard 1.141

```
java -jar /picard.jar SortSam INPUT=R.sam OUTPUT=R.bam  
SORT_ORDER=coordinate
```

<http://broadinstitute.github.io/picard>

4) Removing duplicates using Picard 1.141

```
java -XX: +UseParallelGC -XX:ParallelGCThreads=6 -jar /picard.jar MarkDuplicates  
INPUT=      R.bam      OUTPUT=R_dedup.bam      METRICS_FILE=metrics.txt  
REMOVE_DUPLICATES=true ASSUME_SORTED=true
```

5) Index Bamfile using samtools 0.1.19 [4]

```
samtools index R_dedup.bam
```

<http://github.com/samtools/samtools>

6) SNP calling using samtools 0.1.19 [4]

```
samtools mpileup -E -C50 -Q20 -q20 -uf IPO323.fa R_dedup.bam | ./bcftools call --  
ploidy-file ploidyfile -vc -O u -o R_dedup.bcf
```

7) BCF-VCF conversion using bcftools 0.1.19 [4]

```
bcftools view R_dedup.bcf > R_dedup.vcf
```

<http://github.com/samtools/bcftools>

8) Filtering using bcftools 0.1.19 [4]

```
bcftools filter -o R_filtered.vcf -s LOWQUAL -e'QUAL<20 | DP<10 | AF1<0.8'  
R_dedup.vcf
```

References

1. Goodwin SB, Ben M'Barek S, Dhillon B, Wittenberg, Alexander H. J., Crane CF, Hane JK, et al. Finished Genome of the Fungal Wheat Pathogen *Mycosphaerella graminicola* Reveals Dispensome Structure, Chromosome Plasticity, and Stealth Pathogenesis. *PLoS Genet.* 2011; 7: e1002070. doi: 10.1371/journal.pgen.1002070.
2. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics.* 2014; 30: 2114–2120. doi: 10.1093/bioinformatics/btu170.
3. Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. *Nat Methods.* 2012; 9: 357–359. doi: 10.1038/nmeth.1923.
4. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, et al. The Sequence Alignment/Map format and SAMtools. *Bioinformatics.* 2009; 25: 2078–2079. doi: 10.1093/bioinformatics/btp352.