BWA-

#Indexing the reference file

bwa index genome.fna

# Alignment using BWA-backtrack [because read length is small (50bp) … backtrack algorithm is preferable when read length is small]

bwa aln genome.fna output-witherror-rate.fastq > aln\_sa.sai

bwa samse genome.fna aln\_sa.sai output-witherror-rate.fastq > aln-se.sam

# Conversion from Sam to BAM

samtools view -bS aln-se.sam > aln-se.bam

# Sort BAM file

samtools sort -O bam -o aln-se.sorted.bam -T temp aln-se.bam

#Index the Sorted BAM file again

samtools index aln-se.sorted.bam

# Get Mapping Statistics

samtools flagstat aln-se.bam

100000 + 0 in total (QC-passed reads + QC-failed reads)

0 + 0 secondary

0 + 0 supplementary

0 + 0 duplicates

100000 + 0 mapped (100.00% : N/A)

0 + 0 paired in sequencing

0 + 0 read1

0 + 0 read2

0 + 0 properly paired (N/A : N/A)

0 + 0 with itself and mate mapped

0 + 0 singletons (N/A : N/A)

0 + 0 with mate mapped to a different chr

0 + 0 with mate mapped to a different chr (mapQ>=5)

# How many reads fall into the target regions ???

bedtools coverage -abam aln-se.sorted.bam -b TargetQC.bed > final\_coverage.bed