**# Filtering variants:**

bcftools mpileup -Ou -f 0140J.fasta $sorted\_bam\_files | bcftools call -O b -cv --threads 24 --ploidy 1 -V indels | bcftools filter --threads 24 -i 'MIN(INFO/DP)>=10 & %QUAL>=30||MQ>30'> Suberis.vcf

**# Mask the bases that failed filtering parameter with “N”**

bgzip -c Suberis.vcf > Suberis.vcf.gz

bcftools query -f'%CHROM\t%POS0\t%END\n' Suberis.vcf.gz > variants.bed

bedtools genomecov -bga -ibam $sorted\_bam\_files | awk '$4 < 10' > low\_coverage\_sites.bed

bedtools subtract -a low\_coverage\_sites.bed -b variants.bed > mask.bed

bcftools consensus -f 0140J.fasta -m mask.bed -i 'FORMAT/VAF > 0.90 & INFO/MQ >= 20 & FORMAT/DP >= 10' Suberis.vcf.gz > bcftools.consensus.fa

**Note**: I need to replace all the bases that failed the filtering metrics of DP>=10, QUAL>=30 and MQ>30, with the character “N”.