twoBitToFa reference.2bit reference.fa

bwa index reference.fa

bwa mem -R '@RG\tID:A01\_31\tSM:A01\_31' reference.fa A01\_31.fastq > A01\_31.sam

samtools sort A01\_31.sam > A01\_31.bam

freebayes -f reference.fa \*.bam --genotype-qualities | vcfallelicprimitives -kg > calls.vcf

vcffilter calls.vcf -f “QUAL > 20” > calls\_sorted.vcf

snpEff -v reference.fa calls\_sorted.vcf > ann\_calls.vcf