```
#make a directory to hold the analysis
mkdir chipseq
cd chipseq

#build the indexes needed by the bowtie2 aligner
bowtie2-build ../data/hs_ref_GRCh38_chr20.fa chr20

#use bowtie2 to align the single reads to the chr20 index, for H3K27 and input
bowtie2 -x chr20 -U ../data/HUES64_rep1.H3K27me3.chr20.fastq.gz -S
HUES64_rep1.H3K27.sam
bowtie2 -x chr20 -U ../data/HUES64_rep1.input.chr20.fastq.gz -S HUES64_rep1.input.sam

#use samtools to create a sorted, indexed bam file for each output file
samtools view -b HUES64_rep1.H3K27.sam -o HUES64_rep1.H3K27.bam
samtools sort HUES64_rep1.H3K27.bam -o HUES64_rep1.H3K27_sorted.bam
samtools index HUES64_rep1.H3K27_sorted.bam
samtools view -b HUES64_rep1.input.sam -o HUES64_rep1.input.bam
samtools sort HUES64_rep1.input.bam -o HUES64_rep1.input_sorted.bam
samtools index HUES64_rep1.input_sorted.bam

#repeat the process for the second replicates for each sample
bowtie2 -x chr20 -U ../data/HUES64_rep2.H3K27me3.chr20.fastq.gz -S
HUES64_rep2.H3K27.sam
bowtie2 -x chr20 -U ../data/HUES64_rep2.input.chr20.fastq.gz -S HUES64_rep2.input.sam
samtools view -b HUES64_rep2.H3K27.sam -o HUES64_rep2.H3K27.bam
samtools sort HUES64_rep2.H3K27.bam -o HUES64_rep2.H3K27_sorted.bam
samtools index HUES64_rep2.H3K27_sorted.bam
samtools view -b HUES64_rep2.input.sam -o HUES64_rep2.input.bam
samtools sort HUES64_rep2.input.bam -o HUES64_rep2.input_sorted.bam
samtools index HUES64_rep2.input_sorted.bam

#use MACS2 callpeak to call peaks in the aligned reads. First look at the help
#so that you can see what options exist
macs2 callpeak -h

#now call peaks, using the inputs as controls and the H3K27 as treatments
macs2 callpeak -t HUES64_rep1.H3K27_sorted.bam HUES64_rep2.H3K27_sorted.bam -c
HUES64_rep1.input_sorted.bam HUES64_rep2.input_sorted.bam -n HUES64_H3K27
```

```
#download all of the sorted bam and bai files, as well as HUES64_H3K27_summits.bed,
#and look at chromsome 20 with all of this data in IGV.
#navigate to a MACS2 peak and take a screenshot. You should see a pileup of reads
#in the enriched conditions relative to the controls
```