

```
#make a directory to hold the data
mkdir data
```

```
#make a directory to hold the analysis
mkdir rnaseq
```

```
#go into the data directory and copy all of the data into it
cd data
cp /srv/data/day3_shared/* .
```

```
#uncompress the fastq files for the first sample
gunzip HUES64.mRNA*
```

```
#explore the fastq files
head HUES64.mRNA.chr20.R1.fastq
head HUES64.mRNA.chr20.R2.fastq
```

```
cd ..
```

```
#go into the analysis directory
cd rnaseq
```

```
#create the hisat index for chromosome 20, name it chr20
hisat2-build ../data/hs_ref_GRCh38_chr20.fa chr20
```

```
#run hisat on the HUES64 data
hisat2 -x chr20 --dta-cufflinks -1 ../data/HUES64.mRNA.chr20.R1.fastq -2 ../
data/HUES64.mRNA.chr20.R2.fastq -S HUES64.sam
```

```
#make a bam file from the sam file
samtools view -b HUES64.sam -o HUES64.bam
```

```
#sort the bam file by chromosome name and position
samtools sort HUES64.bam -o HUES64_sorted.bam
```

```
#index the sorted bam file for viewing
```

```
samtools index HUES64_sorted.bam
```

```
#repeat the alignment and samtools processing with the ectoderm data
```

```
gunzip ../data/ectoderm.mRNA*
```

```
hisat2 -x chr20 --dta-cufflinks -1 ../data/ectoderm.mRNA.chr20.R1.fastq -2 ../  
data/ectoderm.mRNA.chr20.R2.fastq -S ectoderm.sam
```

```
samtools view -b ectoderm.sam -o ectoderm.bam
```

```
samtools sort ectoderm.bam -o ectoderm_sorted.bam
```

```
samtools index ectoderm_sorted.bam
```

```
#calculate differential expression between the HUES64 and ectoderm alignments
```

```
mkdir HUES64_ectoderm
```

```
cuffdiff -o HUES64_ectoderm ../data/chr20.gff3 HUES64_sorted.bam  
ectoderm_sorted.bam
```

```
#look at the results
```

```
head HUES64_ectoderm/gene_exp.diff
```

```
#extract any lines that have significant differential expression
```

```
grep yes HUES64_ectoderm/gene_exp.diff
```

```
#download the _sorted.bam and _sorted.bam.bai files. Load them into IGV on  
#your computer, making sure the genome is hg38.
```

```
#Navigate to a gene that has differential expression between the two  
conditions.
```

```
#your exercise now is to repeat this for the mesoderm and endoderm samples,  
#finding the differential expression between them
```