

```

# !/bin/bash

### This is installing the NCBI's SRA toolkit, which will help us download the
data
# wget
https://ftp-trace.ncbi.nlm.nih.gov/sra/sdk/3.1.0/sratoolkit.3.1.0-ubuntu64.tar.
gz .
# tar -vzxf sratoolkit.3.1.0-ubuntu64.tar.gz
# chmod +777 sratoolkit.3.1.0-ubuntu64/bin/fastq-dump

###Step Cinco: install hisat2 and subread
# conda install bioconda::hisat2
# conda install bioconda::subread

### Downloading the data from Project 2; subsampling 5M Reads #can line up all
files if you like
# sratoolkit.3.1.0-ubuntu64/bin/fastq-dump -X 5000000 SRR24206824
# sratoolkit.3.1.0-ubuntu64/bin/fastq-dump -X 5000000 SRR24206825
# sratoolkit.3.1.0-ubuntu64/bin/fastq-dump -X 5000000 SRR24206826
# sratoolkit.3.1.0-ubuntu64/bin/fastq-dump -X 5000000 SRR24206827

###Rename Files
# mv SRR24206824.fastq SC2YoungSRR24.fastq
# mv SRR24206825.fastq SC2MidAgeSRR25.fastq
# mv SRR24206826.fastq PBSYoungSRR26.fastq
# mv SRR24206827.fastq PBSMidAgeSRR27.fastq

###Step Uno: FastQC in order to quality control the data
# fastqc *.fastq

###Step Dos: Housekeeping, new directory for output of FastQC
# mkdir FQC_Results
# mv *.html FQC_Results
# mv *.zip FQC_Results

#Step Tres: Trim Using cutadapt, determined 15 from QC results, 28 wiggle room
for ideal 30
cutadapt --cut 200 -q 28 -o trimmed_SC2YoungSRR24.fastq SC2YoungSRR24.fastq
cutadapt --cut 200 -q 28 -o trimmed_SC2MidAgeSRR25.fastq SC2MidAgeSRR25.fastq
cutadapt --cut 200 -q 28 -o trimmed_PBSYoungSRR26.fastq PBSYoungSRR26.fastq
cutadapt --cut 200 -q 28 -o trimmed_PBSMidAgeSRR27.fastq PBSMidAgeSRR27.fastq

#Step Cuatro: Transfer trimmed files into its own folder
mkdir FQC_trimmed
mv trimmed*.fastq FQC_trimmed

#Step Seis: Alignment using hisat2, decompress the .gz to .tar
wget ftp://ftp.ccb.jhu.edu/pub/infphilo/hisat2/data/mm10.tar.gz
tar -xzf mm10.tar.gz

```

#Step Siete: use hisat2 using the mouse indexed reference genome, and trimmed files

```
hisat2 -x mm10/genome -U FQC_trimmed/trimmed_SC2YoungSRR24.fastq -S  
SC2YoungSRR24.sam
```

```
hisat2 -x mm10/genome -U FQC_trimmed/trimmed_SC2MidAgeSRR25.fastq -S  
SC2MidAgeSRR25.sam
```

```
hisat2 -x mm10/genome -U FQC_trimmed/trimmed_PBSYoungSRR26.fastq -S  
PBSYoungSRR26.sam
```

```
hisat2 -x mm10/genome -U FQC_trimmed/trimmed_PBSMidAgeSRR27.fastq -S  
PBSMidAgeSRR27.sam
```

#Step Ocho: create counts table of expression profiles ig, output txt file
wget

```
https://ftp.ensembl.org/pub/release-102/gtf/mus\_musculus/Mus\_musculus.GRCm38.102.gtf.gz
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```
gunzip Mus_musculus.GRCm38.102.gtf.gz
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featureCounts -a Mus_musculus.GRCm38.102.gtf -o counts.txt *.sam
```