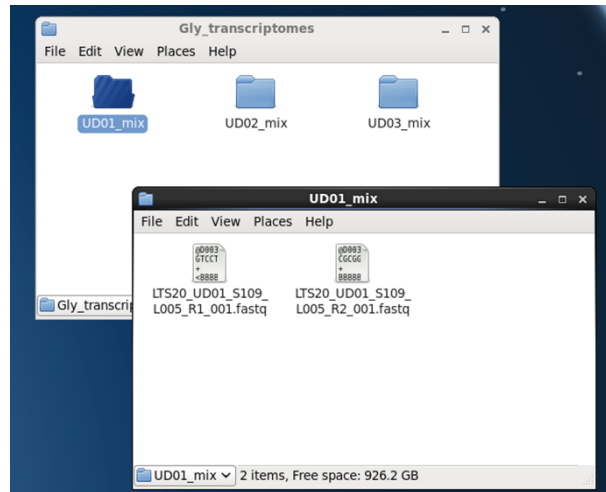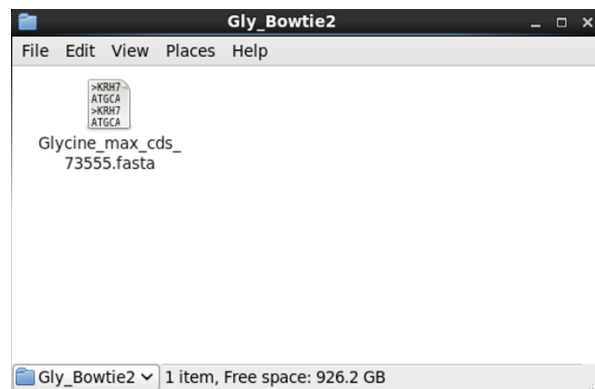# SOP of Running Bowtie2BuildAndCal.R

I. Preparing transcriptome data and cds.fasta for building Bowtie2 index.

    1. Saving all transcriptomes in a folder. For example: here is the folder "Gly_transcriptomes".
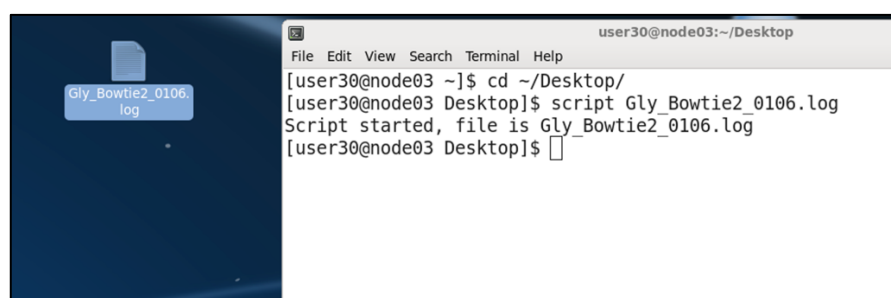

    2. Create a file with cds.fasta (without descriptions) for building Bowtie2 index. For example, here is "Gly_Bowtie2".

II. Recording print out information when running Bowtie2

    A. Create a log file.

        ■ Code: script [file_name].log

III. Running Bowtie2BuildAndCal.R

    A.   Go to the file which is going to build Bowtie2 index.

        ■ Code: cd [path of filename]

    B.   See the usage and argument of Bowtie2BuildAndCal.R

        ■ Code: Rscript [path of Bowtie2BuidAndCal.R] -h

```
[user30@node03 Desktop]$ Rscript Bowtie2BuildAndCal.R -h
Usage: Bowtie2BuildAndCal.R [-[-input|i] [<character>]] [-[-output|o] [<character>]] [-[-help|h]]
    -i|--input     Folder name of all transcriptomes(default_path:~/Desktop).
    -o|--output    Create folder for saving bowtie2 results(default_path:~/Desktop).
    -h|--help      Help:explanation of arguments.
```

    C.   Start running Bowtie2BuildAndCal.R

        ■ Code: Rscript [path of Bowtie2BuildAndCal.R] -i [folder name of transcriptome data] -o [folder name of Bowtie2 result]

```
[user30@node03 Gly_Bowtie2]$ Rscript ~/Desktop/Bowtie2BuildAndCal.R -i Gly_sample -o Gly_Bowtie2Result_0106
[1] "Gly_sample"
[1] "Gly_Bowtie2Result_0106"
[1] "Glycine_max_cds_73555.fasta"
Settings:
  Output files: "Bowtie2_index.*.bt2"
```

IV. Finishing and checking Bowtie2 record

    1.   Finishing record

        ■ Code: exit

    2.   Checking Bowtie2 record, including Bowtie2 build index and total alignment rate of each sample.

        ■ Code: more -n/UD01 Bowtie2_Result/Gly_Bowtie2_record.log

◆ **Code explanation:**

1. Showing the folder name of transcriptome data and the folder name which is going to saving Bowtie2 result(A).
2. Create the folder which named "Gly_Bowtie2Result_0106" on the Desktop for saving Bowtie2 result(B).
3. Showing the CDS fasta file name it catches (C) and starting to build index for calculation (D).
4. Grab all transcriptome samples' names (E).
5. In the for loop, for each sample: first, grab R1.fastaq and R2.fastaq files and save them as R1 and R2 (F); second, show the id of each samples (G); third, run Bowtie2 by put in variables including index name, cds, id, R1, R2 (H); fourth, create a file named with its id and move the Bowtie2 result to this file (I&J) .

```
library(getopt)
args=matrix(
 c( "input", "i", 2, "character", "Folder name of all transcriptomes.",
    "output", "o", 2, "character", "Create folder for saving bowtie2 results.",
    "help", "h", 0, "logical", "Help:explanation of arguments."),
    byrow=TRUE,ncol=5 )
#print(args)
opt=getopt(args)

if(!is.null(opt$help) || is.null(opt$input) || is.null(opt$output)){
  cat(paste(getopt(args,usage=T), "\n"))
  quit()}
Samples=opt$input
Bowtie2_Result=opt$output
```
A
```
print(Samples)
print(Bowtie2_Result)
```
```
#create a file to save Bowtie2 result
```
B
```
system(paste("mkdir ~/Desktop/", Bowtie2_Result,sep=""),wait=T)
```
```
##build_index
cds=Sys.glob("*")
```
C
```
print(cds)
index_name="Bowtie2_index"
```
D
```
system(paste("bowtie2-build",cds,index_name,sep=" "),wait=T)
#print(paste("bowtie2-build",cds,index_name,sep=" "))
```
```
##grab_sample_file_name
```
E
```
samples=as.data.frame(Sys.glob(paste("~/Desktop/",Samples,"/*",sep="")))
colnames(samples)=c("name")
```
```
#Bowtie2_calculation
for (i in samples$name){
```
F
```
  R1=Sys.glob(paste(i,"/*R1*",sep = ""))
  R2=Sys.glob(paste(i,"/*R2*",sep = ""))
  id=basename(i)
```
G
```
  print(id)
```
H
```
  system(paste("bowtie2 -x ",index_name," -p 16"," -1 ",R1 ," -2 ",R2," | express ",cds," > express_",id,".log", sep = ""),wait = T)
```
I & J
```
  system(paste("mkdir ~/Desktop/", Bowtie2_Result,"/", id,sep = ""),wait = T)
  system(paste("mv express ", id , ".log results.xprs params.xprs", " ~/Desktop/", Bowtie2_Result,"/",id,sep = ""),wait = T)
}
```

```
[user30@node03 Gly_Bowtie2]$ Rscript ~/Desktop/Bowtie2BuildAndCal.R -i Gly_sample -o Gly_Bowtie2Result_0106
```
A
```
[1] "Gly_sample"
[1] "Gly_Bowtie2Result_0106"
```
C
```
[1] "Glycine_max_cds_73555.fasta"
Settings:
  Output files: "Bowtie2_index.*.bt2"
```