Supplemental Code. Custom script for Oxford Nanopore sequencing data analysis

```{r setup, include=FALSE}
knitr::opts_chunk$set(echo = TRUE)
knitr::opts_knit$set(root.dir = "/mnt/raid5/Personal/minion/cooperation/YaoLab/")

library(Rsamtools)
library(GenomicRanges)
library(GenomicAlignments)
library(pheatmap)
library(dplyr)
library(parallel)
setwd("/mnt/raid5/Personal/minion/cooperation/YaoLab/")
```

```{linux}
## ONT seq
rawPath="/var/lib/minknow/data/YaoshaohuaLab_Jiao/TD_19sam/20221213_2206_MN29097_F
AS82028_7e8ad3e7/fastq_pass"
fastq="/mnt/raid5/Personal/minion/cooperation/YaoLab/02fastq"

#01 cat fastq
cd $fastq
zcat $rawPath/barcode01/* > F1.fq
zcat $rawPath/barcode02/* > F2.fq
zcat $rawPath/barcode03/* > F3.fq
zcat $rawPath/barcode04/* > F_Ctr.fq
zcat $rawPath/barcode05/* > E1.fq
zcat $rawPath/barcode06/* > E2.fq
zcat $rawPath/barcode07/* > E3.fq
zcat $rawPath/barcode08/* > E_Ctr.fq
zcat $rawPath/barcode09/* > A1.fq
zcat $rawPath/barcode10/* > A2.fq
zcat $rawPath/barcode11/* > A3.fq
zcat $rawPath/barcode12/* > A_Ctr.fq
zcat $rawPath/barcode13/* > H1.fq
zcat $rawPath/barcode14/* > H2.fq
zcat $rawPath/barcode15/* > H3.fq
zcat $rawPath/barcode16/* > H4.fq
zcat $rawPath/barcode17/* > H_Ctr.fq
zcat $rawPath/barcode18/* > E2_2.fq
zcat $rawPath/barcode19/* > E2_2_Ctr.fq
```

```{linux}
#02 minimap2:alignment
ref="/mnt/raid64/ref_genomes/HomSap/release101/"
fasta="/mnt/raid64/ref_genomes/HomSap/release101/Homo_sapiens.GRCh38.dna.primary_assem
bly.fa"
gtf="/mnt/raid64/ref_genomes/HomSap/release101/Homo_sapiens.GRCh38.101.sorted.gtf"

cd $fastq
for i in $(ls | awk -F '.' '{print $1}');
do
  minimap2 -t 30 --secondary=no -ax map-ont
/mnt/raid5/Personal/minion/cooperation/YaoLab/01Reference/split/${i}-TD_split.fa \
  ./${i}.fq | samtools sort -@ 30 | samtools view -hb      >    ../bam/${i}_spl.bam
  samtools view -hb -q 1 -F 2048    ../bam/${i}_spl.bam >    ../bam/${i}_splf.bam
  samtools index ../bam/${i}_splf.bam
done
```

#03 pheatmap: visualization
```{r fig.width=4,fig.height=4}
files <- list.files("/mnt/raid5/Personal/minion/cooperation/YaoLab/07new_sample/bam/", "f.bam$",
full.names = TRUE)

Mat0 <- parallel::mclapply(files, function(x) {
   map0 <- readGAlignments(x,use.names = T)
},mc.cores = 10)
names(Mat0) <- gsub("_splf.bam","",basename(files))

seq_l <- lapply(Mat0, function(x) {
   table(seqnames(x))
})
seq_m <- seq_l[names(seq_l)[grep("Ctr",names(seq_l),invert = T)]] %>% do.call(rbind,.)
colnames(seq_m) <- paste0(2:10,"X.fa")

pheatmap(t(seq_m),scale="column",
             cluster_cols = F,
               cluster_rows = F)
```

#04 statistics
```{r fig.width=4,fig.height=4}
library(ShortRead)
fastq <-  list.files("/mnt/raid5/Personal/minion/cooperation/YaoLab/07new_sample/fastq/",  ".fq$",
full.names = TRUE)
```

```
Fastq0 <- parallel::mclapply(fastq, function(x){
   readFastq(x)
},mc.cores = 10)
names(Fastq0) <- gsub(".fq","",basename(fastq))
fq_l <- lapply(Fastq0, length)
fq_l <- t(data.frame(fq_l))

seq_m1 <- data.frame(RawReads=fq_l[grep("Ctr",rownames(fq_l),invert = T),],seq_m)
seq_m1$Map_all <- rowSums(seq_m1[,-1])
tab2 <- apply(seq_m1[,-1], 2,function(x){
   round(x/seq_m1$RawReads,3)
})
tab3 <- apply(seq_m1[,-1], 2,function(x){
   round(x/seq_m1$Map_all,3)
})

write.csv(seq_m1,"/mnt/raid5/Personal/minion/cooperation/YaoLab//00analysis/new_sample_map
_reads_num.csv",row.names = T)
write.csv(tab2,"/mnt/raid5/Personal/minion/cooperation/YaoLab//00analysis/new_sample_map_R
awreads.csv",row.names = T)
write.csv(tab3,"/mnt/raid5/Personal/minion/cooperation/YaoLab//00analysis/new_sample_map_M
apsum.csv",row.names = T)

```
```
```