hw1

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# NGS data simulation using wgsim

#### Type in the following command:

wgsim lambda.fa -e 0 -d 500 -N 5000 -1 100 -2 100 -r 0.01 -R 0 -X 0 -S 1234567 -h l1.read1.fq l1.read2.fq

# Download Reference from EnsemblPlants

Maize [reference genome](https://plants.ensembl.org/Zea_mays/Info/Index)

#### Change to largedata\lab4 folder:

cd largedata  
mkdir Thetas\_Fst  
cd Thetas\_Fst

#### Then use wget to download the reference genome:

wget ftp://ftp.ensemblgenomes.org/pub/plants/release-46/fasta/zea\_mays/dna/Zea\_mays.B73\_RefGen\_v4.dna.chromosome.Mt.fa.gz  
  
### then unzip it  
gunzip Zea\_mays.B73\_RefGen\_v4.dna.chromosome.Mt.fa.gz

# NGS data simulation using wgsim

#### Type in the following command:

wgsim Zea\_mays.B73\_RefGen\_v4.dna.chromosome.Mt.fa \  
-e 0 -d 500 -N 5000 -1 100 -2 100 -r 0.01 \  
-R 0 -X 0 -S 1234567 l1.read1.fq l1.read2.fq

* Reference (about 500k)
  + Zea\_mays.B73\_RefGen\_v4.dna.chromosome.Mt.fa
* 20x coverage
  + N 5000
* PE 100bp
  + -1 100 -2 100
* Only SNP no Indel
  + -R 0 -X 0
* Mutation rate is low
  + -r 0.01

# NGS data simulation using wgsim

## simulate 10 individals population 1

for i in {1..10}; do wgsim Zea\_mays.B73\_RefGen\_v4.dna.chromosome.Mt.fa -e 0 -d 500 -N 50000 -1 100 -2 100 -r 0.1 -R 0 -X 0 p1l$i.read1.fq l$i.read2.fq; done  
mv l1.read1.fq p1l1.read1.fq #(Change name pop 1. all 10 idividuals)

## simulate 10 individals population 2

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for i in {1..10}; do wgsim Zea\_mays.B73\_RefGen\_v4.dna.chromosome.Mt.fa -e 0 -d 500 -N 50000 -1 100 -2 100 -r 0.1 -R 0 -X 0 l$i.read1.fq l$i.read2.fq; done  
mv l1.read1.fq p2l1.read1.fq #(Change name pop 2. all 10 idividuals)

|  |
| --- |
| #### check how many reads |
| bash wc -l p1l1.read1.fq wc -l p2l1.read1.fq # suppose to be 200,000 lines = 50,000 reads |

# A procedure to calculate and values

### 1. Align the NGS reads to the reference genome

* [bwa](https://github.com/lh3/bwa)
* [samtools](https://github.com/samtools/samtools)

### 2. Calculate SFS

* [ANGSD](http://www.popgen.dk/angsd/index.php/ANGSD)

### 3. Calculate the thetas and Fst for each site

* ANGSD

# Submit a Slurm job (I couldn’t do it)

* We wrap our jobs in little batch scripts, which is nice because these also help make steps reproducible.
* To keep your directory organized, I usually keep a scripts directory (i.e., slurm-script/ ) and log dir (i.e., slurm-log ) for Slurm’s logs.
  + Tip: use these logs, as these are very helpful in debugging. I separate them from my project because they fill up directories rather quickly.
* D sets your project directory.
* o sets where standard output (of your batch script) goes.
* e sets where standard error (of your batch script) goes.
* J sets the job name.
* t sets the time limit for the job, 24:00:00 indicates 24 hours.
* --mail: will email you if the job is “END” or “FAIL”

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vi my\_theta.sh  
  
 #!/bin/bash -l  
 #SBATCH -D /home/agro932/sybarreral/Agro932hw  
 #SBATCH -o /home/agro932/sybarreral/Agro932hw/slurm-log/stdout-%j.txt  
 #SBATCH -e /home/agro932/sybarreral/Agro932hw/slurm-log/stderr-%j.txt  
 #SBATCH -J theta  
 #SBATCH -t 1:00:00  
 #SBATCH --mail-user=sybarreral@huskers.unl.edu  
 #SBATCH --mail-type=END #email if ends  
 #SBATCH --mail-type=FAIL #email if fails  
 set -e  
 set -u  
  
# insert your script here  
module load bwa samtools  
# alignment pop.1  
cd largedata/Thetas\_Fst/bam\_files  
for i in {1..10}; do bwa mem Zea\_mays.B73\_RefGen\_v4.dna.chromosome.Mt.fa p1l$i.read1.fq p1l$i.read2.fq | samtools view -bSh - > p1l$i.bam; done  
# alignment pop.2  
for i in {1..10}; do bwa mem Zea\_mays.B73\_RefGen\_v4.dna.chromosome.Mt.fa p2l$i.read1.fq p2l$i.read2.fq | samtools view -bSh - > p2l$i.bam; done  
# sort  
for i in \*.bam; do samtools sort $i -o sorted\_$i; done  
# index them  
for i in sorted\*.bam; do samtools index $i; done

# A procedure to calculate values

### 2. Calculate SFS using ANGSD

#### Install ANGSD first

cd ~/bin/ # if you don't have one, do `mkdir bin`  
git clone https://github.com/samtools/htslib.git  
git clone https://github.com/ANGSD/angsd.git   
cd htslib; make;   
cd ../angsd;  
make HTSSRC=../htslib

#### run angsd

#write the bam files to a txt file  
mkdir bam\_files  
mv sorted\*.bam bam\_files  
cd bam\_files/  
ls sorted\_p1\*.bam > bam.txt  
#see file 1  
cat bam\_p1.txt   
ls sorted\_p2\*.bam > bam.txt  
#see file 2  
cat bam\_p2.txt

# A procedure to calculate values

#calculate Sfs   
../../../../angsd/angsd -bam bam.txt -doSaf 1 -anc ../Zea\_mays.B73\_RefGen\_v4.dna.chromosome.Mt.fa -GL 1 -out out  
# use realSFS to calculate sfs  
../../../../angsd/misc/realSFS out.saf.idx > out.sfs

#### Copy the result to cache/ folder

## cp sfs to the cache/ folder  
cp out.sfs ../../../cache/

### 3. Calculate the thetas for each site

The output from the above command are two files out.thetas.gz and out.thetas.idx. A formal description of these files can be found in the doc/formats.pdf in the angsd package. It is possible to extract the logscale persite thetas using the ./thetaStat print program.

../../../../angsd/angsd -bam bam.txt -out out -doThetas 1 -doSaf 1 -pest out.sfs -anc ../Zea\_mays.B73\_RefGen\_v4.dna.chromosome.Mt.fa -GL 1  
../../../../angsd/misc/thetaStat print out.thetas.idx > theta.txt  
## cp theta to the cache/ folder  
cp theta.txt ../../../cache/

# A procedure to calculate

#### Create two list bam files

cp bam\_p1.txt pop1.txt  
cp bam\_p2.txt pop2.txt

#### Two population Fst

# first calculate per pop saf for each populatoin  
../../../../angsd/angsd -b pop1.txt -anc ../Zea\_mays.B73\_RefGen\_v4.dna.chromosome.Mt.fa -out pop1 -dosaf 1 -gl 1  
../../../../angsd/angsd -b pop2.txt -anc ../Zea\_mays.B73\_RefGen\_v4.dna.chromosome.Mt.fa -out pop2 -dosaf 1 -gl 1  
# calculate the 2dsfs prior  
../../../../angsd/misc/realSFS pop1.saf.idx pop2.saf.idx > pop1.pop2.ml  
# prepare the fst for easy window analysis etc  
../../../../angsd/misc/realSFS fst index pop1.saf.idx pop2.saf.idx -sfs pop1.pop2.ml -fstout out  
  
# get the global estimate  
../../../../angsd/misc/realSFS fst stats out.fst.idx  
# below is not tested that much, but seems to work  
../../../../angsd/misc/realSFS fst stats2 out.fst.idx -win 500 -step 100 > fst\_win.txt

#### Copy the result to cache/ folder

## cp sfs to the cache/ folder  
cp fst\_win.txt ../../../cache/

# Visualize the results

In local computer, using R:

#### Barplot for SFS

#Barplot for SFS  
sfs <- scan('cache/out.sfs')  
sfs <- sfs[-c(1,length(sfs))]  
sfs <- sfs/sum(sfs)  
  
barplot(sfs,names=1:length(sfs), main='SFS')  
barplot(sfs, col="#cdc0b0", xlab="No. of segregating sites",   
 ylab="Minor allele frequency",   
 names=1:length(sfs))

#### Histgram distribution of the theta values

library("data.table")  
#Histgram distribution of the theta values  
#theta <- read.table("cache/theta.txt", header=TRUE)  
theta <- fread("cache/theta.txt", data.table =FALSE)  
hist(theta$Pairwise)   
hist(theta$Pairwise, col="green", xlab="Theta pairwise values (log10)")

#### Scatter plot of the Fst values

#Scatter plot of the Fst values  
fst <- read.table("cache/fst\_win.txt", skip=1, header=FALSE)  
names(fst)[c(3,5)] <- c("midp", "fst")  
plot(fst$midp, fst$fst, xlab="Physical position", ylab="Fst", col="#5f9ea0", pch=16)

# General feature format (GFF) from EnsemblPlants

Maize [reference genome](https://plants.ensembl.org/Zea_mays/Info/Index)

change to largedata\lab4 folder:

wget ftp://ftp.ensemblgenomes.org/pub/plants/release-46/fasta/zea\_mays/dna/Zea\_mays.B73\_RefGen\_v4.dna.chromosome.Mt.fa.gz  
  
### then unzip it  
gunzip Zea\_mays.B73\_RefGen\_v4.dna.chromosome.Mt.fa.gz

Similarly, we will download and unzip the [Mt GFF3](ftp://ftp.ensemblgenomes.org/pub/plants/release-46/gff3/zea_mays/Zea_mays.B73_RefGen_v4.46.chromosome.Mt.gff3.gz) file

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#### Use R to process the GFF3 file

# install.package("data.table")  
library("data.table")  
## simply read in wouldn't work  
gff <- fread("largedata/Zea\_mays.B73\_RefGen\_v4.46.chromosome.Mt.gff3", skip="#", header=FALSE, data.table=FALSE)  
## grep -v means select lines that not matching any of the specified patterns  
gff <- fread(cmd='grep -v "#" largedata/Zea\_mays.B73\_RefGen\_v4.46.chromosome.Mt.gff3', header=FALSE, data.table=FALSE)

# General feature format (GFF) version 3

V1 V2 V3 V4 V5 V6 V7 V8  
1 Mt Gramene chromosome 1 569630 . . .  
2 Mt ensembl gene 6391 6738 . + .  
3 Mt NCBI mRNA 6391 6738 . + .  
4 Mt NCBI exon 6391 6738 . + .  
5 Mt NCBI CDS 6391 6738 . + 0  
6 Mt ensembl gene 6951 8285 . + .  
 V9  
1 ID=chromosome:Mt;Alias=AY506529.1,NC\_007982.1;Is\_circular=true  
2 ID=gene:ZeamMp002;biotype=protein\_coding;description=orf115-a1;  
3 ID=transcript:ZeamMp002;Parent=gene:ZeamMp002;  
4 Parent=transcript:ZeamMp002;Name=ZeamMp002.exon1;constitutive=1;ensembl\_end\_phase=0;  
5 ID=CDS:ZeamMp002;Parent=transcript:ZeamMp002;  
6 ID=gene:ZeamMp003;biotype=protein\_coding;description=orf444

# Work with GFF

names(gff) <- c("seq", "source", "feature", "start", "end", "score", "strand", "phase", "att")  
table(gff$feature)

### Get genes and upstream and downstream 5kb regions

g <- subset(gff, feature %in% "gene")  
g$geneid <- gsub(".\*gene:|;biotype.\*", "", g$att)  
  
### + strand  
gp <- subset(g, strand %in% "+")   
# nrow(gp) 75  
  
### get the 5k upstream of the + strand gene model  
gp\_up <- gp  
gp\_up$end <- gp\_up$start - 1  
gp\_up$start <- gp\_up$end - 5000   
  
### get the 5k downstream of the + strand gene model  
gp\_down <- gp  
gp\_down$start <- gp\_down$end + 1  
gp\_down$end <- gp\_down$start + 5000

### Get genes and upstream and downstream 5kb regions

### - strand  
gm <- subset(g, strand %in% "-")   
dim(gm) # 82  
  
fwrite(g, "cache/mt\_gene.txt", sep="\t", row.names = FALSE, quote=FALSE)

## Intepret the theta results

library("data.table")  
library("GenomicRanges")  
library("plyr")  
  
  
theta <- fread("cache/theta.txt", data.table=FALSE)  
names(theta)[1] <- "seq"  
  
up5k <- read.table("cache/mt\_gene\_up5k.txt", header=TRUE)  
  
### define the subject file for theta values  
grc <- with(theta, GRanges(seqnames=seq, IRanges(start=Pos, end=Pos)))  
  
### define the query file for genomic feature  
grf <- with(up5k, GRanges(seqnames=seq, IRanges(start=start, end=end), geneid=geneid))  
   
### find overlaps between the two  
tb <- findOverlaps(query=grf, subject=grc)  
tb <- as.matrix(tb)  
   
out1 <- as.data.frame(grf[tb[,1]])  
out2 <- as.data.frame(grc[tb[,2]])  
### for each genomic feature, find the sites with non-missing data  
out <- cbind(out1, out2[, "start"])   
names(out)[ncol(out)] <- "pos"

## Intepret the theta results

#define unique identifier and merge with the thetas  
out$uid <- paste(out$seqnames, out$pos, sep="\_")  
theta$uid <- paste(theta$seq, theta$Pos, sep="\_")  
  
df <- merge(out, theta[, c(-1, -2)], by="uid")  
# for each upstream 5k region, how many theta values  
  
mx <- ddply(df, .(geneid), summarise,  
 Pairwise = mean(Pairwise, na.rm=TRUE),  
 thetaH = mean(thetaH, na.rm=TRUE),  
 nsites = length(uid))

## Intepret the theta results

Copy and paste everything above, and pack it into an R function:

get\_mean\_theta <- function(gf\_file="cache/mt\_gene\_up5k.txt"){  
 # gf\_file: gene feature file [chr, ="cache/mt\_gene\_up5k.txt"]  
   
 theta <- fread("cache/theta.txt", data.table=FALSE)  
 names(theta)[1] <- "seq"  
  
 up5k <- read.table(gf\_file, header=TRUE)  
  
 ### define the subject file for theta values  
 grc <- with(theta, GRanges(seqnames=seq, IRanges(start=Pos, end=Pos)))  
  
 ### define the query file for genomic feature  
 grf <- with(up5k, GRanges(seqnames=seq, IRanges(start=start, end=end), geneid=geneid))  
   
 ### find overlaps between the two  
 tb <- findOverlaps(query=grf, subject=grc)  
 tb <- as.matrix(tb)  
   
 out1 <- as.data.frame(grf[tb[,1]])  
 out2 <- as.data.frame(grc[tb[,2]])  
 ### for each genomic feature, find the sites with non-missing data  
 out <- cbind(out1, out2[, "start"])   
 names(out)[ncol(out)] <- "pos"  
  
 #define unique identifier and merge with the thetas  
 out$uid <- paste(out$seqnames, out$pos, sep="\_")  
 theta$uid <- paste(theta$seq, theta$Pos, sep="\_")  
  
 df <- merge(out, theta[, c(-1, -2)], by="uid")  
 # for each upstream 5k region, how many theta values  
  
 mx <- ddply(df, .(geneid), summarise,  
 Pairwise = mean(Pairwise, na.rm=TRUE),  
 thetaH = mean(thetaH, na.rm=TRUE),  
 nsites = length(uid))  
 return(mx)  
}

## Plot the results

Run the customized R function

### apply the function  
up5k <- get\_mean\_theta(gf\_file="cache/mt\_gene\_up5k.txt")  
down5k <- get\_mean\_theta(gf\_file="cache/mt\_gene\_down5k.txt")  
gene <- get\_mean\_theta(gf\_file="cache/mt\_gene.txt")

And then plot the results:

library("ggplot2")  
  
up5k$feature <- "up 5k"  
down5k$feature <- "down 5k"  
gene$feature <- "genic"  
res <- rbind(up5k, down5k)  
res\_nongenic <- res  
res\_nongenic$feature <- "intergenic"  
res\_t <- rbind(res, gene, res\_nongenic)  
ggplot(res\_t, aes(x=feature, y=Pairwise, fill=feature)) +   
 geom\_violin(trim=FALSE)+  
 labs(title="Theta value", x="", y = "Log10 (theta)")+  
 geom\_boxplot(width=0.1, fill="white")+  
 scale\_fill\_brewer(palette="Blues") +   
 theme\_classic()

## Intepret the Fst results

get\_mean\_Fst <- function(gf\_file="cache/mt\_gene\_up5k.txt"){  
 # gf\_file: gene feature file [chr, ="cache/mt\_gene\_up5k.txt"]  
   
 fst <- fread("cache/fst\_win.txt", data.table=FALSE)  
 names(fst)[2] <- "seq"  
 up5k <- read.table(gf\_file, header=TRUE)  
 ### define the subject file for Fst values  
 grc\_f <- with(fst, GRanges(seqnames=seq, IRanges(start=chr, end=chr)))  
 ### define the query file for genomic feature  
 grf <- with(up5k, GRanges(seqnames=seq, IRanges(start=start, end=end), geneid=geneid))  
   
 ### find overlaps between the two  
 tb\_f <- findOverlaps(query=grf, subject=grc\_f)  
 tb\_f <- as.matrix(tb\_f)  
   
 out1\_f <- as.data.frame(grf[tb\_f[,1]])  
 out2\_f <- as.data.frame(grc[tb\_f[,2]])  
 ### for each genomic feature, find the sites with non-missing data  
 out\_f <- cbind(out1\_f, out2\_f[, "start"])   
 names(out\_f)[ncol(out\_f)] <- "pos"  
 #define unique identifier and merge with the Fst  
 out\_f$uid <- paste(out\_f$seqnames, out\_f$pos, sep="\_")  
 fst$uid <- paste(fst$seq, fst$chr, sep="\_")  
 df\_f <- merge(out\_f, fst[, c(-1, -2, -3, -4)], by="uid")  
 # for each upstream 5k region, how many Fst values  
 names(df\_f)[ncol(df\_f)] <- "fst"  
   
 mx\_f <- ddply(df\_f, .(geneid), summarise,  
 Fst = mean(fst, na.rm=TRUE),  
 nsites = length(uid))  
 return(mx\_f)  
}

## Plot the results

Run the customized R function

### apply the function  
up5k\_f <- get\_mean\_Fst(gf\_file="cache/mt\_gene\_up5k.txt")  
down5k\_f <- get\_mean\_Fst(gf\_file="cache/mt\_gene\_down5k.txt")  
gene\_f <- get\_mean\_Fst(gf\_file="cache/mt\_gene.txt")

And then plot the results:

library("ggplot2")  
up5k\_f$feature <- "up 5k"  
down5k\_f$feature <- "down 5k"  
gene\_f$feature <- "genic"  
res\_f <- rbind(up5k\_f, down5k\_f)  
res\_nongenic\_f <- res\_f  
res\_nongenic\_f$feature <- "intergenic"  
res\_t\_f <- rbind(res\_f, gene\_f, res\_nongenic\_f)  
ggplot(res\_t\_f, aes(x=feature, y=Fst, fill=feature)) +   
 geom\_violin(trim=FALSE)+  
 labs(title="Fst distribution", x="", y = "Fst")+  
 geom\_boxplot(width=0.1, fill="white")+  
 scale\_fill\_brewer(palette="Reds") +   
 theme\_classic()