Practical Course: Natural Selection [Answers]

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Directory

```
mkdir EMBO_course_2025
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Goal

> Our goal is to explore approaches and methods, which seek to identify regions of the genome with signatures of natural selection. We will use real genomic data and two classes of tests: one based on population differentiation and another based on extended haplotype homozygosity.

Dataset

> Whole genome sequencing data by NGS (WG-NGS) from the 1000 Genomes Project phase III can be accessed through the link: ftp://ftp.1000genomes.ebi.ac.uk/vol1/ftp/release/20130502/

> **The files to download are at:** https://github.com/HunemeierLab/EMBO_Practical_Course_2024

Data pre-processing

> To optimize our time, we will analyze a pre-processed dataset for chromosome 2 corresponding to individuals sampled from the African (504 individuals), European (503 individuals), and East Asian (504 individuals) populations of the 1000 Genomes).

For now, repeating these filters is unnecessary, but here are the commands used:

```
input=/Users/patriciopezo/Desktop/EMBO_course_2025/input
out=/Users/patriciopezo/Desktop/EMBO_course_2025/data_process

#I. Removing INDELS and Singletons
time vcftools --gzvcf $input/ALL.chr2.phase3_shapeit2_mvncall_integrated_v5b.20130502.genotypes.vcf.gz --remove-ind
els --min-alleles 2 --max-alleles 2 --maf 0.001 --max-maf 0.999 --recode --out $out/SNPs_Chr2_filter

#II. Selecting samples of individuals from the AFR, EAS and EUR populations (~ 30min) and filter to maf 0.05
vcftools --vcf SNPs_Chr2_filter.recode.vcf --keep pop_AFR_EAS_EUR_1000g.txt --min-alleles 2 --max-alleles 2 --maf 0.05
--max-maf 0.95 --recode --out SNPs_Chr2_AFR_EUR_EAS_maf

#III. Select individual samples for each population (for pairwise Fst comparison)
vcftools --vcf SNPs_Chr2_AFR_EUR_EAS_maf.recode.vcf --keep pop_AFR_1000g.txt --recode --out SNPs_Chr2_AFR_maf
&

vcftools --vcf SNPs_Chr2_AFR_EUR_EAS_maf.recode.vcf --keep pop_EAS_1000g.txt --recode --out SNPs_Chr2_EAS_maf
&

vcftools --vcf SNPs_Chr2_AFR_EUR_EAS_maf.recode.vcf --keep pop_EUR_1000g.txt --recode --outSNPs_Chr2_EUR_maf &

#IV. Estimating the Fst index between pairs of populations (~20 min each)
/vcftools --vcf ./dados/SNPs_Chr2_AFR_EUR_EAS_maf.recode.vcf --out AFR_EAS_maf --chr 2 --weir-fst-pop ./dados/pop
_AFR_1000g.txt --weir-fst-pop ./dados/pop_EAS_1000g.txt &

vcftools --vcf ./dados/SNPs_Chr2_AFR_EUR_EAS_maf.recode.vcf --out AFR_EUR_maf --chr 2 --weir-fst-pop ./dados/pop_
AFR_1000g.txt --weir-fst-pop ./dados/pop_EUR_1000g.txt &

vcftools --vcf ./dados/SNPs_Chr2_AFR_EUR_EAS_maf.recode.vcf --out EAS_EUR_maf --chr 2 --weir-fst-pop ./dados/pop_
EAS_1000g.txt --weir-fst-pop ./dados/pop_EUR_1000g.txt &
```

Part I | Genetic Differentiation as Evidence of Selection (FST-based Methods)

Through the exercises, discuss and answer the following questions:

1. The estimate of Fst by the Weir and Cockerham metric can sometimes generate negative values and “NA.” What does that mean? How can this interfere with the results?
2. The Fst values observed between pairs of populations for the SNP rs3827760 (position 109513601) fall within which distribution quantiles of Fst values for the studied chromosome? Can they be considered outliers?
3. From the observed Fst values between population pairs and the significance estimates, what can we say about the rs3827760 SNP differentiation between populations?
4. Discuss how these results justify performing another type of analysis based on PBS (population branch statistics).

What does the PBS analysis reveal? What is the difference between PBS and FST analysis?

Practice: Fst

```
input="/Users/patriciopezo/Desktop/EMBO_course_2025/input/"
out="/Users/patriciopezo/Desktop/EMBO_course_2025/data_process/"

#I. Read the files with the Fst estimates (AFR_EUR.weir.fst, AFR_EAS.weir.fst and EAS_EUR.weir.fst)
names_header ← c("CHROM","POS","WEIR_AND_COCKERHAM_FST","NUM","DEN")
```

```
FST_AFR_EAS ← read.table(paste0(input,"AFR_EAS.weir.fst"), header=F, skip=1, col.names=names_header, fill = TRUE) #5
82,963 SNPs
FST_AFR_EUR ← read.table(paste0(input,"AFR_EUR.weir.fst"), header=F, skip=1, col.names=names_header, fill = TRUE) #5
82,964 SNPs
FST_EAS_EUR ← read.table(paste0(input,"EAS_EUR.weir.fst"), header=F, skip=1, col.names=names_header, fill = TRUE) #5
82,964 SNPs




#II. Remove duplicated positions
FST_AFR_EAS_filter ← FST_AFR_EAS[!duplicated(FST_AFR_EAS$POS),] #582,963 SNPs
FST_AFR_EUR_filter ← FST_AFR_EUR[!duplicated(FST_AFR_EUR$POS),] #582,964 SNPs
FST_EAS_EUR_filter ← FST_EAS_EUR[!duplicated(FST_EAS_EUR$POS),] #582,964 SNPs




#III. Take a look at the weir.fst file

head(FST_AFR_EAS_filter)
head(FST_AFR_EUR_filter)
head(FST_EAS_EUR_filter)



#IV. Exclude NAs position in Fst estimations
FST_AfrEas_data ← FST_AFR_EAS_filter[-which(is.na(FST_AFR_EAS_filter[,3])),] #582,694 SNPs
FST_AfrEur_data ← FST_AFR_EUR_filter[-which(is.na(FST_AFR_EUR_filter[,3])),] #582,363 SNPs
FST_EasEur_data ← FST_EAS_EUR_filter[-which(is.na(FST_EAS_EUR_filter[,3])),] #566,650 SNPs



#V. Overlaping SNPs
#565,779 SNPs

overlap_AfrEas_AfrEur ← FST_AfrEas_data[FST_AfrEas_data$POS %in% FST_AfrEur_data$POS,]
overlap_AfrEasEur_EasEur ← overlap_AfrEas_AfrEur[overlap_AfrEas_AfrEur$POS %in% FST_EasEur_data$POS,]

FST_AfrEas_data_clean ← FST_AfrEas_data[FST_AfrEas_data$POS %in% overlap_AfrEasEur_EasEur$POS,]
FST_AfrEur_data_clean ← FST_AfrEur_data[FST_AfrEur_data$POS %in% overlap_AfrEasEur_EasEur$POS,]
FST_EasEur_data_clean ← FST_EasEur_data[FST_EasEur_data$POS %in% overlap_AfrEasEur_EasEur$POS,]



#VI. Convert negative values to zero
FST_AfrEas_data_clean[which(FST_AfrEas_data_clean[,3]<0),3] ← 0
FST_AfrEur_data_clean[which(FST_AfrEur_data_clean[,3]<0),3] ← 0
FST_EasEur_data_clean[which(FST_EasEur_data_clean[,3]<0),3] ← 0



#VII. Check if the SNP rs3827760 (pos 109513601) is a candidate for natural selection
#1. Check if the SNP rs3827760, located at position 109513601, is an outlier in the FST distribution for any of the populatio
n pairs
```

```
POS ← 109513601

FST_AfrEas_data_clean[FST_AfrEas_data_clean$POS==POS,]
##       CHROM      POS WEIR_AND_COCKERHAM_FST      NUM      DEN
## 266093     2 109513601              0.872881 0.762039 0.873016

FST_AfrEur_data_clean[FST_AfrEur_data_clean$POS==POS,]
##       CHROM      POS WEIR_AND_COCKERHAM_FST        NUM      DEN
## 266093     2 109513601            0.00997189 0.000108929 0.0109237

FST_EasEur_data_clean[FST_EasEur_data_clean$POS==POS,]
##       CHROM      POS WEIR_AND_COCKERHAM_FST      NUM      DEN
## 266093     2 109513601              0.859066 0.743056 0.864958
```

#2. Check which quartile percentile the rs3827760 distribution fall in each analyzed population pair?

```
FST_AfrEas_distr ← sort(FST_AfrEas_data_clean[,3])
FST_AfrEur_distr ← sort(FST_AfrEur_data_clean[,3])
FST_EasEur_distr ← sort(FST_EasEur_data_clean[,3])

FST_AfrEas_distrQT ← quantile(FST_AfrEas_distr, c(0.01, 0.05, 0.1, .25, .50,  .75, .90, 0.95, .99))

FST_AfrEas_distrQT
##          1%          5%         10%         25%         50%         75%
## 0.0000000000 0.0000079206 0.0031241100 0.0262112250 0.1056270000 0.2222135000
##         90%         95%         99%
## 0.3675160000 0.4685402500 0.6521344200



FST_AfrEur_distrQT ← quantile(FST_AfrEur_distr, c(0.01, 0.05, 0.1, .25, .50,  .75, .90, 0.95, .99))
FST_AfrEur_distrQT
##          1%          5%         10%         25%         50%         75%
## 0.000000000 0.000000000 0.002228373 0.018979525 0.081086300 0.186617500
##         90%         95%         99%
## 0.308151200 0.395242000 0.551998730



FST_EasEur_distrQT ← quantile(FST_EasEur_distr, c(0.01, 0.05, 0.1, .25, .50,  .75, .90, 0.95, .99))
FST_EasEur_distrQT
##          1%          5%         10%         25%         50%         75%
## 0.0000000000 0.0000000000 0.0004382527 0.0092101950 0.0476573500 0.1294852500
##         90%         95%         99%
## 0.2363264000 0.3175715000 0.4790830000
```

#3. Ploting FST values in a 10,000 base pair region adjacent to the SNP at position 109513601. Highlight the SNPs that are outliers in the 95th percentile in each population pair.

```
#Delimit the region of interest to 10000bp adjacent
SNPfrom_BP ← POS - 10000
SNPto_BP ← POS + 10000


#How many SNPs are in this bounded region?

SNPfrom_id_AfrEas ← max(which(FST_AfrEas_data_clean[,2]<=SNPfrom_BP))
SNPto_id_Afr_Eas ← min(which(FST_AfrEas_data_clean[,2]>=SNPto_BP))
length(FST_AfrEas_data_clean[SNPfrom_BP:SNPto_BP, 2])
## [1] 20001


SNPfrom_id_AfrEur ← max(which(FST_AfrEur_data_clean[,2]<=SNPfrom_BP))
SNPto_id_Afr_Eur ← min(which(FST_AfrEur_data_clean[,2]>=SNPto_BP))
length(FST_AfrEur_data_clean[SNPfrom_BP:SNPto_BP, 2])
## [1] 20001


SNPfrom_id_EasEur ← max(which(FST_EasEur_data_clean[,2]<=SNPfrom_BP))
SNPto_id_EasEur ← min(which(FST_EasEur_data_clean[,2]>=SNPto_BP))
length(FST_EasEur_data_clean[SNPfrom_BP:SNPto_BP, 2])
## [1] 20001


#Select from the FST_AfrEur_data the region of interest
FSTdata_SNP_AfrEas ← FST_AfrEas_data_clean[SNPfrom_id_AfrEas:SNPto_id_Afr_Eas, ]
head(FSTdata_SNP_AfrEas)
##        CHROM       POS WEIR_AND_COCKERHAM_FST        NUM       DEN
## 266063     2 109503245             0.0826870 0.00859760 0.1039780
## 266064     2 109503631             0.0747407 0.00718525 0.0961357
## 266066     2 109503778             0.0668349 0.00590110 0.0882937
## 266067     2 109503862             0.1239490 0.02692580 0.2172320
## 266068     2 109504022             0.0448158 0.00616223 0.1375010
## 266069     2 109504287             0.2989630 0.12097500 0.4046480



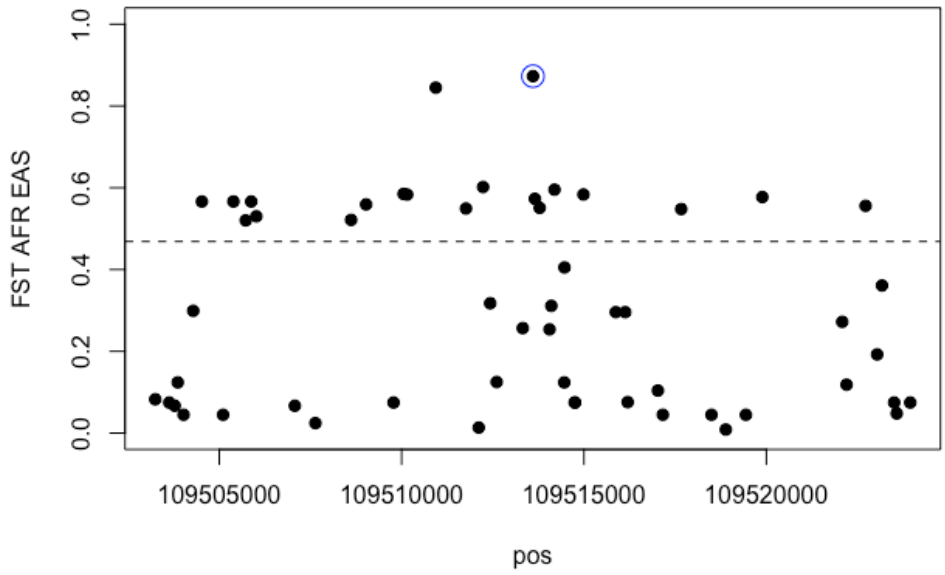
FSTdata_SNP_AfrEur ← FST_AfrEur_data_clean[SNPfrom_id_AfrEur:SNPto_id_Afr_Eur, ]
FSTdata_SNP_EasEur ← FST_EasEur_data_clean[SNPfrom_id_EasEur:SNPto_id_EasEur, ]



#4. PLOT
#a. AfrEas
plot(ylim=c(0,1), x=FSTdata_SNP_AfrEas[,2], y=FSTdata_SNP_AfrEas[,3], xlab='pos', ylab='FST AFR EAS', pch=20, cex=1.
5)

points(x=FSTdata_SNP_AfrEas[which(FSTdata_SNP_AfrEas[,2]==POS),2],  y=FSTdata_SNP_AfrEas[which(FSTdata_SNP_Af
rEas[,2]==POS),3], col='blue', cex=2)

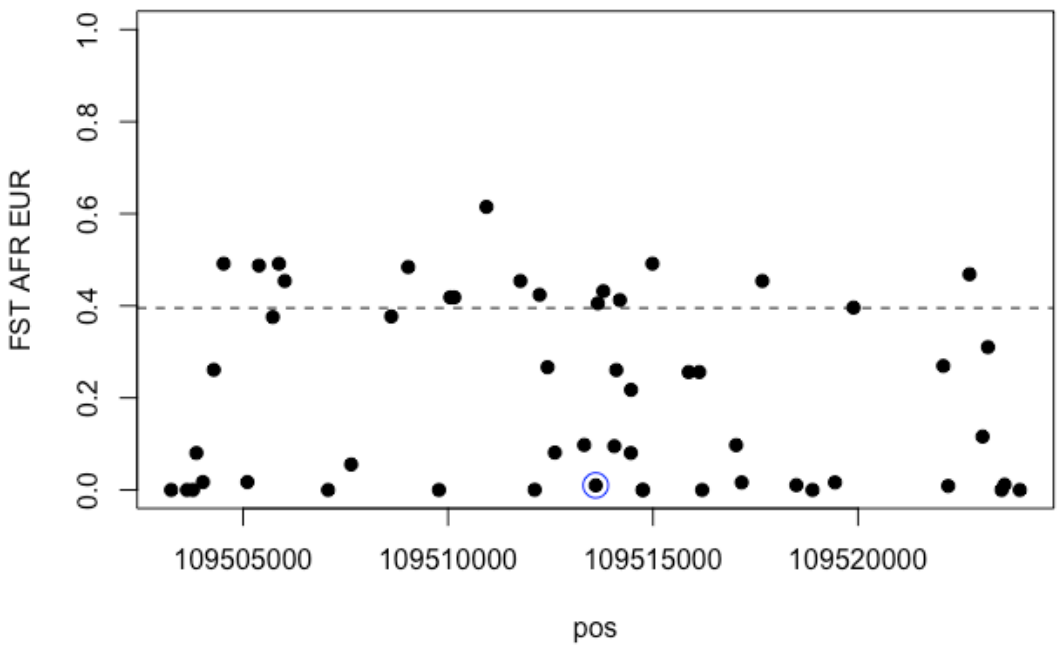
abline(h=FST_AfrEas_distrQT[[8]], lty=2)
```




```
#b.  AfrEur
plot(ylim=c(0,1), x=FSTdata_SNP_AfrEur[,2], y=FSTdata_SNP_AfrEur[,3], xlab='pos', ylab='FST AFR EUR', pch=20, cex=1.5)

points(x=FSTdata_SNP_AfrEur[which(FSTdata_SNP_AfrEur[,2]==POS),2],  y=FSTdata_SNP_AfrEur[which(FSTdata_SNP_AfrEur[,2]==POS),3], col='blue', cex=2)

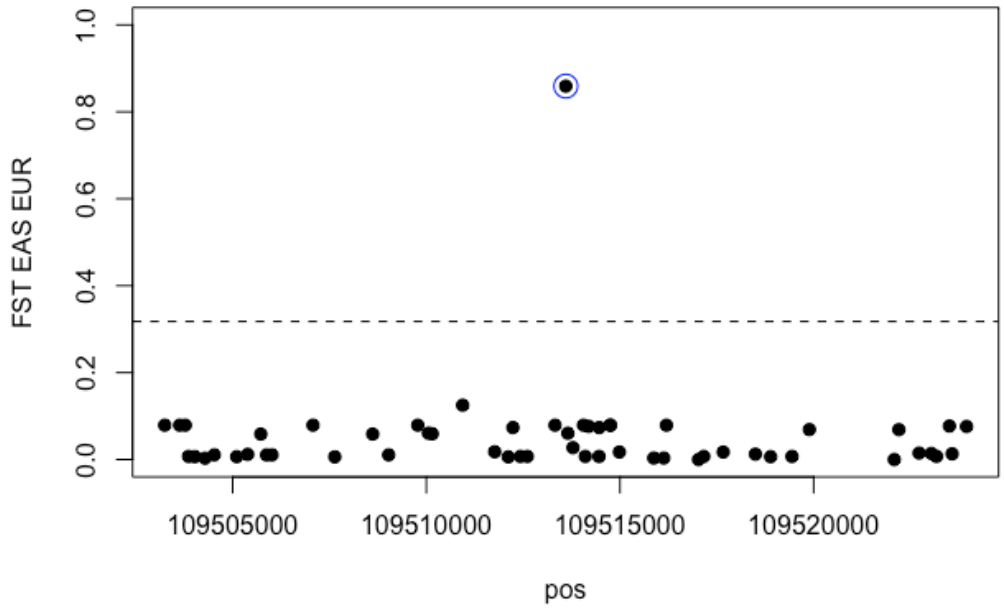
abline(h=FST_AfrEur_distrQT[[8]], lty=2)
```




```
#EasEur
plot(ylim=c(0,1), x=FSTdata_SNP_EasEur[,2], y=FSTdata_SNP_EasEur[,3], xlab='pos', ylab='FST EAS EUR', pch=20, cex=1.
5)

points(x=FSTdata_SNP_EasEur[which(FSTdata_SNP_EasEur[,2]==POS),2],  y=FSTdata_SNP_EasEur[which(FSTdata_SNP_E
asEur[,2]==POS),3], col='blue', cex=2)

abline(h=FST_EasEur_distrQT[[8]], lty=2)
```




```
#VIII. Can the candidate SNP be considered an outlier in all populations? What is the interpretation of this result?
#1. Estimate the p-value for the candidate SNP from the distribution of FST values for each population pair
#  AfrEas
p_value_out_FST_AfrEas ← sum(FST_AfrEas_data_clean$WEIR_AND_COCKERHAM_FST>=FST_AfrEas_data_clean[FST_AfrEas_data_clean$POS==109513601,3])/nrow(FST_AfrEas_data_clean)
p_value_out_FST_AfrEas #0.0004772181


#AfrEur
p_value_out_FST_AfrEur ← sum(FST_AfrEur_data_clean$WEIR_AND_COCKERHAM_FST>=FST_AfrEur_data_clean[FST_AfrEur_data_clean$POS==109513601,3])/nrow(FST_AfrEur_data_clean)
p_value_out_FST_AfrEur #0.8143781


#EurEas
p_value_out_FST_EasEur ← sum(FST_EasEur_data_clean$WEIR_AND_COCKERHAM_FST>=FST_EasEur_data_clean[FST_EasEur_data_clean$POS==109513601,3])/nrow(FST_EasEur_data_clean)
p_value_out_FST_EasEur #5.302424e-06
```

Practice: Population Branch Statistic (PBS)

$$PBS = \frac{((-log(1 - FST\ AB)\ + (-log(1 - FST\ AC))\ -\ (-log(1 - FST\ BC))}{2}$$

Selection signals using the PBS

```
#Path
input="/Users/patriciopezo/Desktop/EMBO_course_2025/input/"
out="/Users/patriciopezo/Desktop/EMBO_course_2025/data_process/"


#1. Perform PBS test, using EAS as candidate population for selection
#Build the PBS Topology and why is it important to measure the distance
PBS_EAS ← ((-log(1-FST_AfrEas_data_clean$WEIR_AND_COCKERHAM_FST))+(-log(1-FST_EasEur_data_clean$WEIR_AND_
COCKERHAM_FST))-(-log(1-FST_AfrEur_data_clean$WEIR_AND_COCKERHAM_FST)))/2


#2. Convert negative PBS values to O
PBS_EAS[which(PBS_EAS<0)] ← 0



#3. Add to the data.table with FST values, a new column with PBS values
fst_pbs←as.data.frame(cbind(FST_EasEur_data_clean$POS, FST_AfrEas_data_clean$WEIR_AND_COCKERHAM_FST, FST_
AfrEur_data_clean$WEIR_AND_COCKERHAM_FST, FST_EasEur_data_clean$WEIR_AND_COCKERHAM_FST, PBS_EAS), stri
ngsAsFactors=FALSE)

head(fst_pbs)
##      V1      V2      V3        V4      PBS_EAS
## 1 10554 0.318827 0.334988 0.00000e+00 0.0000000000
## 2 10560 0.317773 0.333938 0.00000e+00 0.0000000000
## 3 10566 0.315969 0.333938 2.24832e-06 0.0000000000
## 4 10574 0.116785 0.132890 4.42702e-04 0.0000000000
## 5 10587 0.368198 0.355929 0.00000e+00 0.0096164573
## 6 10595 0.205058 0.204892 0.00000e+00 0.0001043992



#4. Check the PBS value for the candidate SNP.
pbs_EDAR←fst_pbs[fst_pbs$V1==POS,]

pbs_EDAR
##            V1      V2        V3      V4  PBS_EAS
## 259224 109513601 0.872881 0.00997189 0.859066 2.006037




#5.   In which quartile of the distribution does the PBS value for the SNP rs3827760 fall?
PBS_distrQT ← quantile(PBS_EAS, c(0.01, 0.05, 0.1, .25, .50,  .75, .90, 0.95, .99))
PBS_distrQT # estimar os quantils
##        1%        5%       10%       25%       50%       75%       90%
## 0.00000000 0.00000000 0.00000000 0.00000000 0.02506831 0.10666149 0.23044290
##       95%       99%
## 0.33611959 0.60783061



#6.   Plot the PBS values in a 10,000 base pair region adjacent to the SNP at position 109513601.
#Highlight the SNPs that are outliers in the 95th percentile.
```

```
#Select 10000bp adjacent to candidate SNP
SNP_FROM ← POS - 10000
SNP_TO ← POS + 10000

SNPfrom_PBS ← max(which(fst_pbs[,1]<=SNP_FROM))
SNPto_PBS ← min(which(fst_pbs[,1]>=SNP_TO))
length(fst_pbs[SNPfrom_PBS:SNPto_PBS, 1])
## [1] 55



#7. Subset the candidate SNP region
subset_fst_PBS ← fst_pbs[SNPfrom_PBS:SNPto_PBS, ]
head(subset_fst_PBS)
##             V1       V2       V3        V4    PBS_EAS
## 259198 109503245 0.0826870 0.0000000 0.07902310 0.08431343
## 259199 109503631 0.0747407 0.0000000 0.07902310 0.08000079
## 259200 109503778 0.0668349 0.0000000 0.07902310 0.07574673
## 259201 109503862 0.1239490 0.0803297 0.00715918 0.02788793
## 259202 109504022 0.0448158 0.0171244 0.00664388 0.01762220
## 259203 109504287 0.2989630 0.2609700 0.00305409 0.02791831



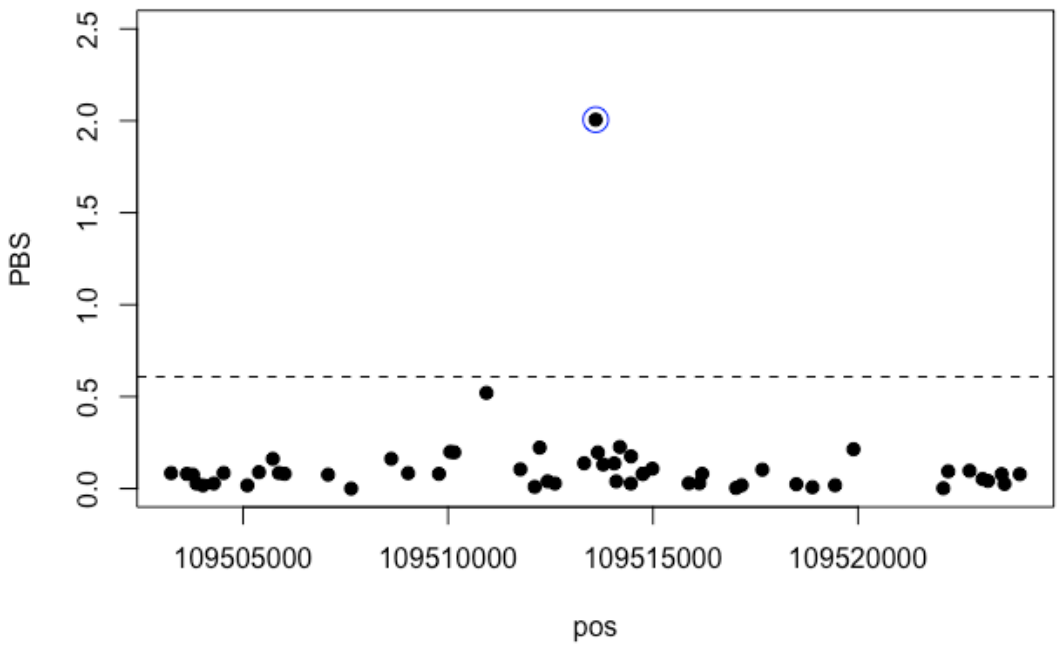
#8. subset_fst_PBS[subset_fst_PBS$V1==109513601,]
##             V1       V2        V3       V4  PBS_EAS
## 259224 109513601 0.872881 0.00997189 0.859066 2.006037



#9. Plot PBS values
plot(ylim=c(0,2.5), x=subset_fst_PBS[,1], y=subset_fst_PBS[,5], xlab='pos', ylab='PBS', pch=20, cex=1.5)

points(x=subset_fst_PBS[which(subset_fst_PBS$V1==POS),1],  y=subset_fst_PBS[which(subset_fst_PBS$V1==POS),5], col
='blue', cex=2)

abline(h=PBS_distrQT[[9]], lty=2)
```



Part II: Extended Haplotype Homozigosity (EHH)

Different approaches are able to detect genomic signatures of selection at different timescales. More recent selection signals can be detected from the extended haplotype homozygosity approach.

Practice: EHH

```
#Path
input="/Users/patriciopezo/Desktop/EMBO_course_2025/input/"
out="/Users/patriciopezo/Desktop/EMBO_course_2025/data_process/"

#I. Install the rehh R package
install.packages("rehh")

#II. Load rehh R package
library("rehh")



#III. Use the following files
#Chr2_EDAR_LWK_500K.recode.vcf #(African population)
#Chr2_EDAR_CHS_500K.recode.vcf # (East Asian population)
```

```
# IV. What is the profile of ancestral and derived haplotypes of the rs3827760 SNP in AFR and EAS?

#1.   Convert the data to haplohh format
data1←data2haplohh(hap_file = paste0(input,"Chr2_EDAR_LWK_500K.recode.vcf"), polarize_vcf = F, vcf_reader = "data.ta
ble")
## * Reading input file(s) *
## Using package 'data.table' to read vcf.
## Extracting map information.
## Extracting haplotypes.
## Number of individuals which are
## Haploid Diploid Triploid, ... :
## 1 2
## 0 99
## * Filtering data *
## Discard markers genotyped on less than 100 % of haplotypes.
## No marker discarded.
## Data consists of 198 haplotypes and 29016 markers.
## Number of mono-, bi-, multi-allelic markers:
## 1 2
## 21289 7727

data2←data2haplohh(hap_file = paste0(input, "Chr2_EDAR_CHS_500K.recode.vcf"), polarize_vcf = F, vcf_reader = "data.ta
ble")
## * Reading input file(s) *
## Using package 'data.table' to read vcf.
## Extracting map information.
## Extracting haplotypes.
## Number of individuals which are
## Haploid Diploid Triploid, ... :
## 1 2
## 0 105
## * Filtering data *
## Discard markers genotyped on less than 100 % of haplotypes.
## No marker discarded.
## Data consists of 210 haplotypes and 29016 markers.
## Number of mono-, bi-, multi-allelic markers:
## 1 2
## 24709 4307


#2.   Calculate the EHH for the candidate SNP (rs3827760) in AFR
ehh_calc_AFR←calc_ehh(data1,mrk = "rs3827760")
ehh_calc_AFR
## An object of class "ehh"
## [[1]]
## [1] "rs3827760"
##
## [[2]]
## FREQ_A FREQ_D
##      1      0
##
## [[3]]
##              POSITION      EHH_A EHH_D
## rs552689611 109506332 0.05081270     0
## rs188449710 109506337 0.05081270     0
```

```
## rs191146014 109506387 0.05081270     0
## rs562458938 109506482 0.05081270     0
## rs182888230 109506509 0.05081270     0
## rs187445332 109506534 0.05081270     0
## rs113027039 109506633 0.05081270     0
## rs569087080 109506722 0.05081270     0
## rs561388021 109506760 0.05081270     0
## rs557899408 109506776 0.05081270     0

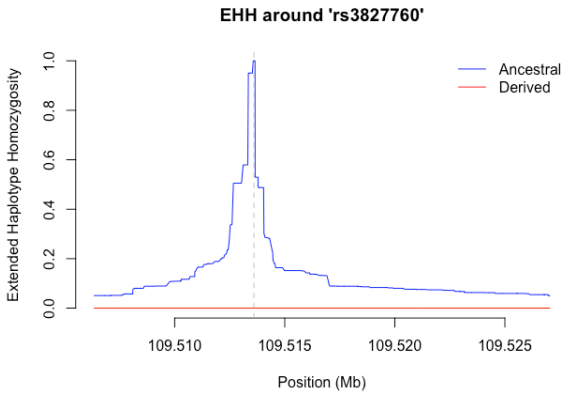
## rs535613872 109527004 0.05004358     0
## rs138769166 109527018 0.05004358     0
## rs201588688 109527031 0.05004358     0
## rs142670672 109527045 0.05004358     0
## rs535840595 109527050 0.05004358     0
##
## [[4]]
##    IHH_A    IHH_D
## 1767.692    0.000

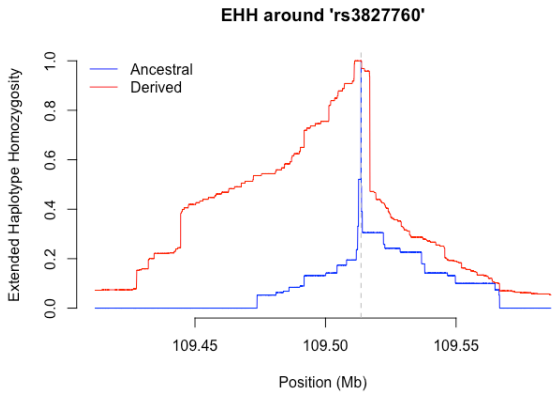

#3.  Calculate the EHH for the candidate SNP (rs3827760) in EAS
ehh_calc_EAS←calc_ehh(data2,mrk = "rs3827760")
ehh_calc_EAS
## An object of class "ehh"
## [[1]]
## [1] "rs3827760"
##
## [[2]]
##    FREQ_A    FREQ_D
## 0.0952381 0.9047619
##
## [[3]]
##             POSITION      EHH_A      EHH_D
## rs144341651 109411690 0.00000000 0.07234754
## rs546981503 109411706 0.00000000 0.07234754
## rs568352591 109411793 0.00000000 0.07234754
## rs529232887 109411800 0.00000000 0.07234754
## rs550870176 109412087 0.00000000 0.07234754
## rs569104695 109412193 0.00000000 0.07234754
## rs113683672 109412248 0.00000000 0.07234754
## rs79168135  109412332 0.00000000 0.07234754
## rs75277911  109412390 0.00000000 0.07234754
## rs147448762 109412402 0.00000000 0.07234754

## rs573491349 109586154 0.00000000 0.05519354
## rs534276564 109586174 0.00000000 0.05329992
## rs554546487 109586260 0.00000000 0.05329992
## rs574390523 109586275 0.00000000 0.05329992
## rs260694    109586313 0.00000000 0.05329992
## rs563073581 109586323 0.00000000 0.05329992
## rs534812588 109586359 0.00000000 0.05329992
##
## [[4]]
##     IHH_A     IHH_D
##  9979.001 55231.782
```

#4. Plot EHH around “rs3827760” in AFR
plot(ehh_calc_AFR)

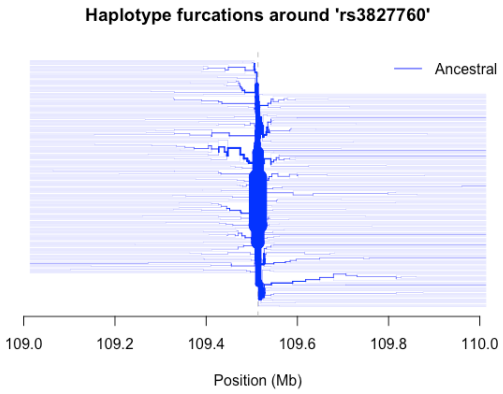
#5. Plot EHH around “rs3827760” in EAS
plot(ehh_calc_EAS)

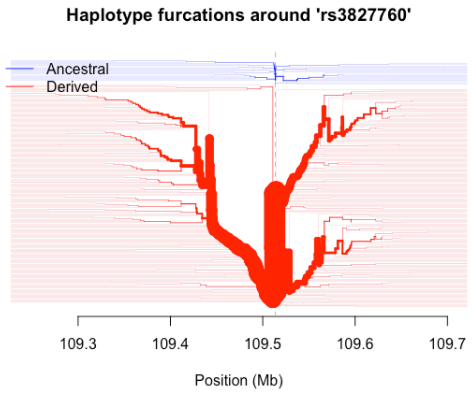




#6. Calculate furcation trees around a candidate SNP in AFR
furcation←calc_furcation(data1, mrk="rs3827760")
plot(furcation)

#7. Calculate furcation trees around a candidate SNP in AFR
furcation←calc_furcation(data2, mrk="rs3827760")
plot(furcation)





Practice 2: integrated Haplotype score (iHS)

iHS is a measure of the amount of extended haplotype homozygosity at a given SNP along the ancestral allele relative to the derived allele. This measure is typically standardized empirically to the distribution of observed iHS scores over a range of SNPs with similar derived allele frequencies.

```
#1.  Calculate the iHS for all SNPs in the file (~5min) for AFR
AFR←scan_hh(data1)

#2.  Calculate the iHS for all SNPs in the file (~5min) for AFR
EAS←scan_hh(data2)

#3.  Check eHH statistics for candidate SNP for AFR
AFR[AFR$POSITION==109513601,]
##          CHR  POSITION FREQ_A FREQ_D NHAPLO_A NHAPLO_D    IHH_A IHH_D      IES
## rs3827760   2 109513601      1      0      198        0 1767.692     0 1767.692
##              INES
## rs3827760 1767.692

#4.  Check eHH statistics for candidate SNP for AFR
EAS[EAS$POSITION==109513601,]
##          CHR  POSITION    FREQ_A    FREQ_D NHAPLO_A NHAPLO_D    IHH_A    IHH_D
## rs3827760   2 109513601 0.0952381 0.9047619       20      190 9979.001 55231.78
##              IES    INES
## rs3827760 43767.3 54737.7

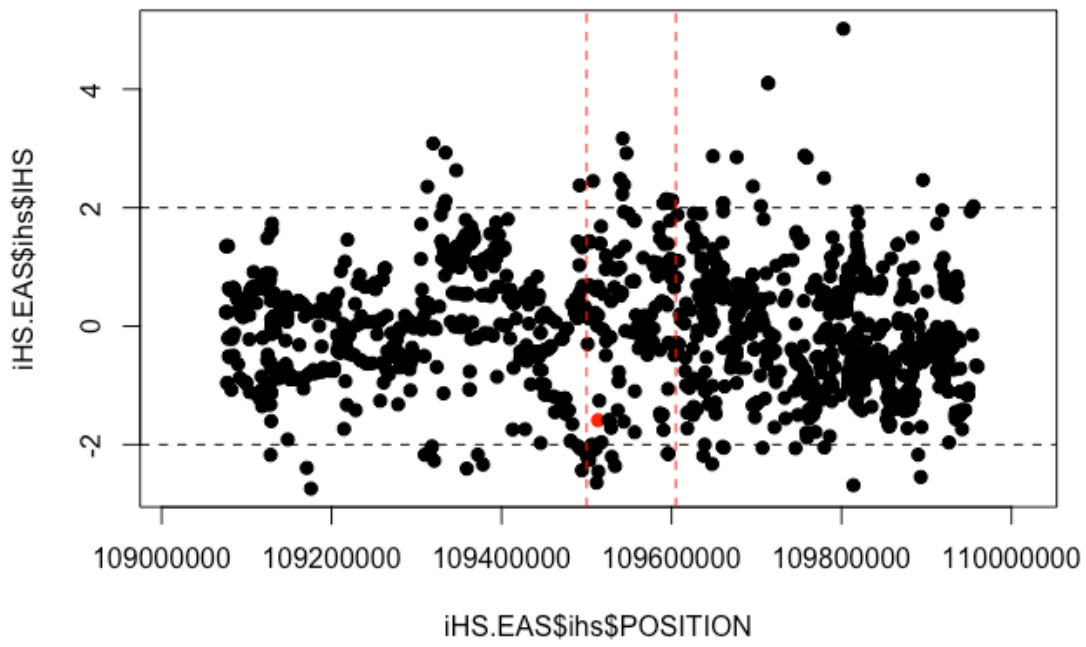
#5.  Estimate the iHS in AFR (use min_maf = 0.02, freqbin = 0.01)
iHS.AFR←ihh2ihs(AFR, min_maf = 0.02, freqbin = 0.01)

#6.  Estimate the iHS in EAS (use min_maf = 0.02, freqbin = 0.01)
iHS.EAS←ihh2ihs(EAS, min_maf = 0.02, freqbin = 0.01)

#7.  Check the iHS score for the candidate SNP in AFR
iHS.AFR$ihs[iHS.AFR$ihs$POSITION==109513601,]
## [1] CHR       POSITION  IHS       LOGPVALUE
## <0 rows> (or 0-length row.names)

#8.  Check the iHS score for the candidate SNP in EAS
iHS.EAS$ihs[iHS.EAS$ihs$POSITION==109513601,]
##          CHR  POSITION       IHS LOGPVALUE
## rs3827760   2 109513601 -1.588263 0.9499032

#9.  Plot the iHS score in EAS
plot(iHS.EAS$ihs$POSITION, iHS.EAS$ihs$IHS, col=ifelse(iHS.EAS$ihs$POSITION==109513601, "red", "black"), pch=19)
abline(h=c(2,-2), lty=2 )
abline(v=c(109500000,109605000), col=c("red", "red"), lty=c(2,2), lwd=c(1, 1))
```



Make windows of SNPs

```
#1.   Create a function to estimate the mean in sliding windows.
slideFunct ← function(data, window, step){
 total ← length(data)
 spots ← seq(from = 1, to = (total - window + 1), by = step)
 result ← vector(length = length(spots))
 for(i in 1:length(spots)){
  result[i] ← mean(abs(data[spots[i]:(spots[i] + window - 1)]),na.rm=TRUE)
 }
 return(result)
}

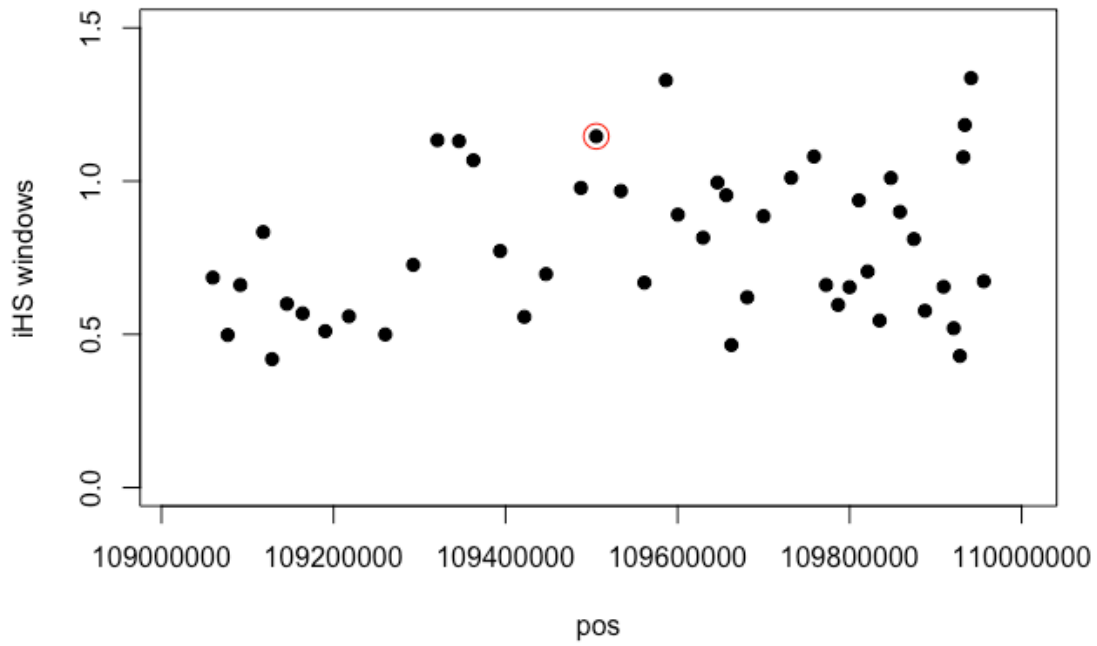
#2.   Estimate the mean over a window of 50 SNPs with steps of 40 SNPs in EAS.
mean_iHS ← slideFunct(iHS.EAS$ihs$IHS, 50,40)

#3.   Identify the starting position of each window
slidePos ← function(data, window, step){
 total ← length(data)
 spots ← seq(from = 1, to = (total - window + 1), by = step)
 result ← vector(length = length(spots))
 for(i in 1:length(spots)){
  result[i] ← data[spots[i]]
 }
 return(result)
}
pos_wind_Eas ← slidePos(iHS.EAS$ihs$POSITION, 50,40)
```

```
#4.  Put the position information and average iHS in a table
wind_iHS ← as.data.frame(cbind(pos_wind_Eas, mean_iHS), stringsAsFactors=FALSE)

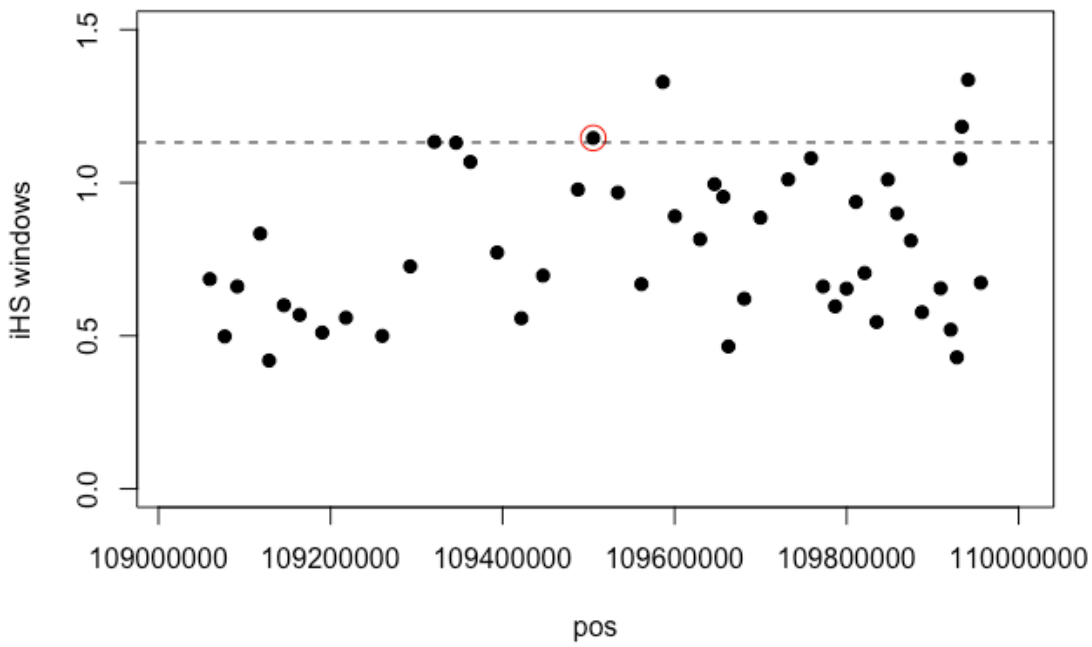
#5.  Identify the window which contains the candidate SNP
Row_WIND_iHS ← wind_iHS[wind_iHS$pos_wind_Eas<=109513601,]
POS_WIND_iHS←max(wind_iHS[nrow(Row_WIND_iHS),])
wind_iHS[wind_iHS$pos_wind_Eas==POS_WIND_iHS,]
##    pos_wind_Eas mean_iHS
## 21    109505388 1.146079

#6.  Plot the mean iHS per window
plot(ylim=c(0,1.5), x=wind_iHS[,1], y=wind_iHS[,2], xlab='pos', ylab='iHS windows', pch=20, cex=1.5)
points(x=wind_iHS[which(wind_iHS[,1]==POS_WIND_iHS),1],  y=wind_iHS[which(wind_iHS[,1]==POS_WIND_iHS),2], col='red',
```




```
#6.  Check the distribution of iHS window in quantiles and check if the candidate SNP is an outlier.
windiHS_distrQT ← quantile(wind_iHS$mean_iHS, c(0.01, 0.05, 0.1, .25, .50,  .75, .90, 0.95, .99), na.rm=T)
windiHS_distrQT
##       1%       5%      10%      25%      50%      75%      90%      95%
## 0.4240205 0.4766102 0.5071474 0.5913277 0.7159912 0.9820098 1.1314710 1.1701018
##      99%
## 1.3328222

#7.  Add the cut line for the quartile to the graph
plot(ylim=c(0,1.5), x=wind_iHS[,1], y=wind_iHS[,2], xlab='pos', ylab='iHS windows', pch=20, cex=1.5)
points(x=wind_iHS[which(wind_iHS[,1]==POS_WIND_iHS),1],  y=wind_iHS[which(wind_iHS[,1]==POS_WIND_iHS),2], col='red',
abline(h=windiHS_distrQT[[7]], lty=2)
```



Practice 3: XP-EHH analysis in the candidate SNP?

Cross-population extended haplotype homozygosity (xp-EHH) method was developed to detect selective sweeps in which the selected allele has approached or achieved fixation in one population but remains polymorphic in the other.
Our candidate SNP is not polymorphic in Africans, but for the purposes of the exercise, let's perform windowed xp-EHH analysis on SNPs adjacent to rs3827760.

```
#1.  Calculate the xp-EHH between EAS e AFR
xpEHH.EAS.AFR←ies2xpehh(EAS,AFR)

## Scan of pop1 contains 29016 markers.
## Scan of pop2 contains 29016 markers.
## Merged data contains 29016 markers.

#2.  Calculate the average xp-EHH per 50 SNP window with 40 SNP steps
mean_xpEHH ← slideFunct(xpEHH.EAS.AFR$XPEHH, 50,40)

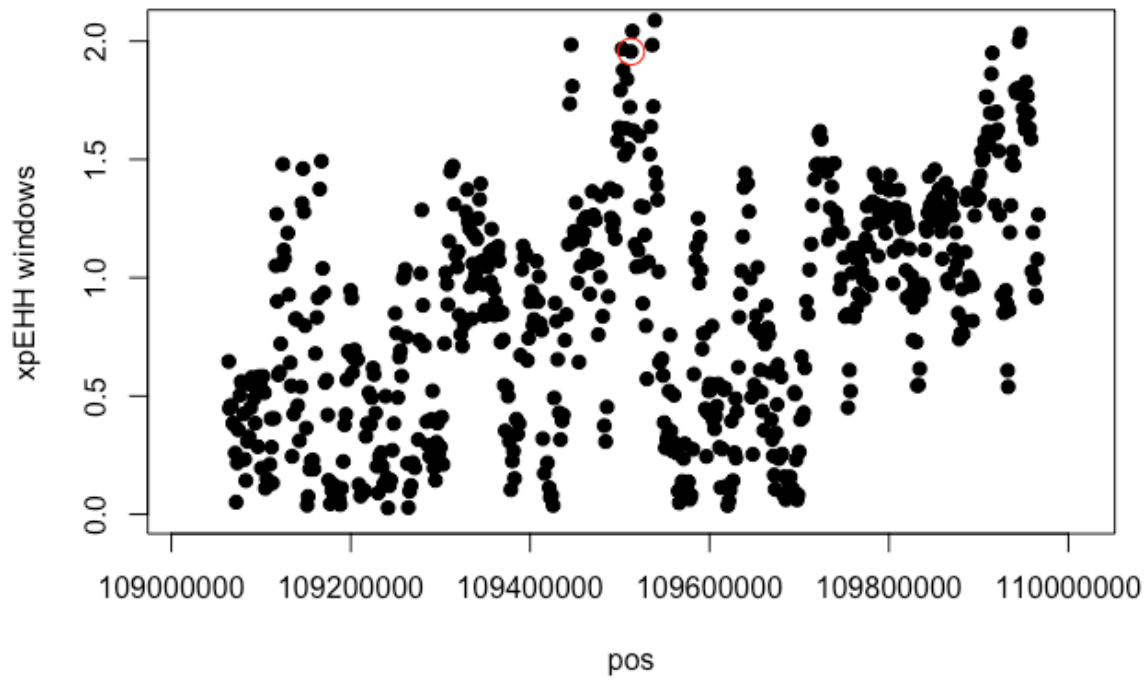
#3.  Identify the starting position of each window
pos_wind_Eas ← slidePos(xpEHH.EAS.AFR$POSITION, 50,40)

#4.  Put the position information and average xpEHH in a table
wind_xpEHH ← as.data.frame(cbind(pos_wind_Eas, mean_xpEHH), stringsAsFactors=FALSE)

#5.  Identify the window which contains the candidate SNP
Row_WIND_xpEHH ← wind_xpEHH[wind_xpEHH$pos_wind_Eas<=109513601,]
POS_WIND_xpEHH←max(wind_xpEHH[nrow(Row_WIND_xpEHH),])
```

```
wind_xpEHH[wind_xpEHH$pos_wind_Eas==POS_WIND_xpEHH,]
##     pos_wind_Eas mean_xpEHH
## 344    109512468   1.956037

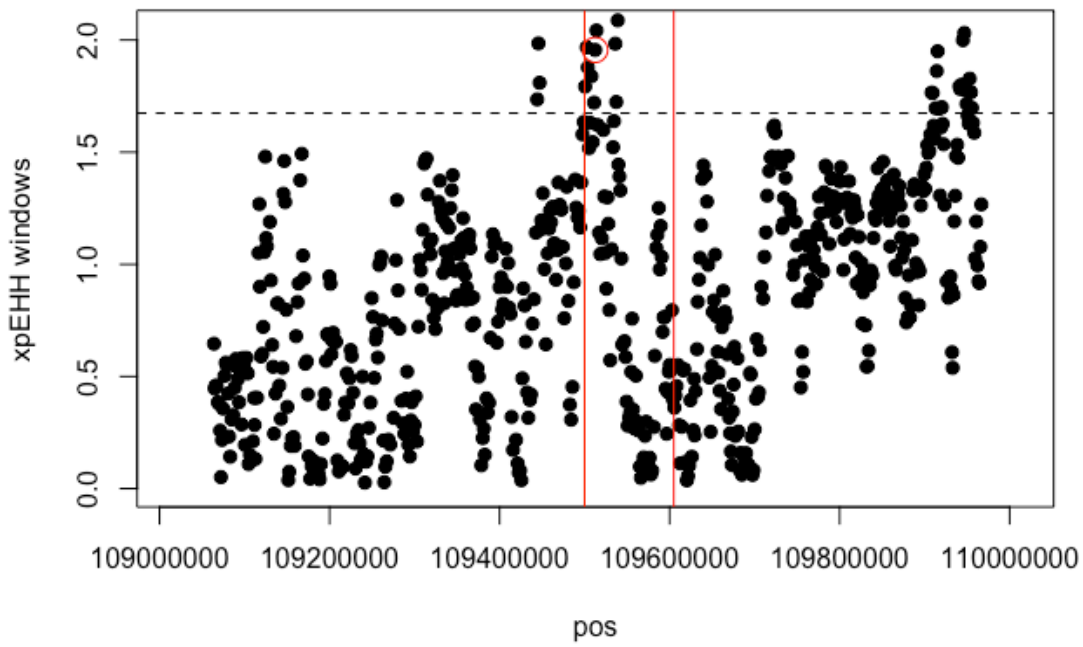
#6.   Plot the mean xpEHH per window
plot(ylim=c(0,2.05), x=wind_xpEHH[,1], y=wind_xpEHH[,2], xlab='pos', ylab='xpEHH windows', pch=20, cex=1.5)
points(x=wind_xpEHH[which(wind_xpEHH[,1]==POS_WIND_xpEHH),1],  y=wind_xpEHH[which(wind_xpEHH[,1]==POS_WIND_x
```




```
#7.   Check the distribution of xpEHH window in quantiles and check if the candidate SNP is an outlier.
windxpEHH_distrQT ← quantile(wind_xpEHH$mean_xpEHH, c(0.01, 0.05, 0.1, .25, .50,  .75, .90, 0.95, .99), na.rm=T)
windxpEHH_distrQT
##        1%        5%       10%       25%       50%       75%       90%
## 0.04627655 0.10333280 0.16789186 0.42277950 0.88493217 1.23910534 1.47871451
##       95%       99%
## 1.67418581 1.99188441

#8.   Add the cut line for the quartile to the graph and outline the candidate gene region
plot(ylim=c(0,2.05), x=wind_xpEHH[,1], y=wind_xpEHH[,2], xlab='pos', ylab='xpEHH windows', pch=20, cex=1.5)
points(x=wind_xpEHH[which(wind_xpEHH[,1]==POS_WIND_xpEHH),1],  y=wind_xpEHH[which(wind_xpEHH[,1]==POS_WIND_x

abline(h= windxpEHH_distrQT [[8]], lty=2)
abline(v=c(109500000,109605000), col="red")
```


xpEHH windows
0.0
0.5
1.0
1.5
2.0
109000000
109200000
109400000
109600000
109800000
110000000
pos