Supplementary File 1. Bioinformatics sequence alignment R code.

library(data.table)

library(magrittr)

library(dplyr)

library(plyr)

#5'-TGASTCA-3' (JUN)

NFkB.seq = "TGA[CG]TCA"

out.path = "data/JUN\_AT30\_CG20"

tfbs.path = "http://jaspar.genereg.net/download/bed\_files/MA0488.1.bed"

length.NFkB.seq = 13

#0. Build gene ref

if (file.exists("data/Gene\_Location.RData")) {load("data/Gene\_Location.RData")} else {

 library(BSgenome.Hsapiens.UCSC.hg19)

 library(TxDb.Hsapiens.UCSC.hg19.knownGene)

 HGNC.URL = "http://www.genenames.org/cgi-bin/download?col=gd\_hgnc\_id&col=gd\_app\_sym&col=gd\_app\_name&col=gd\_prev\_sym&col=gd\_aliases&col=gd\_pub\_eg\_id&status=Approved&status\_opt=2&where=&order\_by=gd\_app\_sym\_sort&format=text&limit=&hgnc\_dbtag=on&submit=submit"

 HGNC.ref = fread(HGNC.URL, data.table = FALSE)

 HGNC.ref = HGNC.ref[,c(6, 2, 3)]

 txdb <- TxDb.Hsapiens.UCSC.hg19.knownGene

 gn <- sort(genes(txdb))

 GN = data.table(gene.num = gn@ranges@NAMES,

 chr = as.data.frame(gn)[,1],

 start = gn@ranges@start,

 end = gn@ranges@start + gn@ranges@width - 1,

 width = gn@ranges@width)

 GN = GN %>% filter(as.character(as.data.frame(gn)[,1]) %in% paste0("chr", c(1:22, "X", "Y"))) %>% setDT()

 GN = GN %>% mutate(chr = mapvalues(chr, paste0("chr", c(1:22, "X", "Y")), 1:24)) %>% setDF()

 GN[,1] = as.integer(GN[,1])

 GN[,2] = as.integer(GN[,2])

 GN[,4] = as.integer(GN[,4])

 GN = merge(GN, HGNC.ref, by.x = "gene.num", by.y = "Entrez Gene ID", all.x = TRUE)

 GN = GN[order(GN[,3]),]

 GN = GN[order(GN[,2]),]

 colnames(GN)[6:7] = c("Short.name", "Full.name")

 URL = "https://www.ncbi.nlm.nih.gov/gene/"

 pb = txtProgressBar(max = nrow(GN), style = 3)

 for (i in 1:nrow(GN)) {

 if (is.na(GN[i,6])) {

 sep.URL = paste0(URL, GN[i,1])

 txt = scan(sep.URL, what="character", encoding="UTF-8", quiet = TRUE)

 txt = paste(txt, sep="", collapse=" ")

 pos.1 = regexpr('Gene symbol </dt> <dd class=\"noline\">', txt)[1] + 37

 subtxt.1 = substr(txt, pos.1, pos.1 + 200)

 pos.2 = regexpr('</dd>', subtxt.1)[1] - 1

 GN[i,6] = substr(subtxt.1, 1, pos.2)

 pos.3 = regexpr('Gene description </dt> <dd>', txt)[1] + 27

 subtxt.2 = substr(txt, pos.3, pos.3 + 200)

 pos.4 = regexpr('</dd>', subtxt.2)[1] - 1

 GN[i,7] = substr(subtxt.2, 1, pos.4)

 }

 setTxtProgressBar(pb, i)

 }

 close(pb)

 pb = txtProgressBar(max = nrow(GN), style = 3)

 for (i in 1:nrow(GN)) {

 if (GN[i,6] == "") {

 sep.URL = paste0(URL, GN[i,1])

 txt = scan(sep.URL, what="character", encoding="UTF-8", quiet = TRUE)

 txt = paste(txt, sep="", collapse=" ")

 pos.1 = regexpr('Official Symbol</dt> <dd class=\"noline\">', txt)[1] + 40

 subtxt.1 = substr(txt, pos.1, pos.1 + 200)

 pos.2 = regexpr('<span', subtxt.1)[1] - 1

 GN[i,6] = substr(subtxt.1, 1, pos.2)

 pos.3 = regexpr('Official Full Name</dt> <dd>', txt)[1] + 28

 subtxt.2 = substr(txt, pos.3, pos.3 + 200)

 pos.4 = regexpr('<span', subtxt.2)[1] - 1

 GN[i,7] = substr(subtxt.2, 1, pos.4)

 }

 setTxtProgressBar(pb, i)

 }

 close(pb)

 save(GN, file = "data/Gene\_Location.RData")

 write.csv(GN, "data/Gene\_Location.csv", quote = FALSE, na = "", row.names = FALSE)

}

#0. Build tfbs ref

if (file.exists(paste0(out.path, "/tfbs.RData"))) {load(paste0(out.path, "/tfbs.RData"))} else {

 tfbs = fread(tfbs.path)

 colnames(tfbs) = c("Chrom", "start", "end", "name", "V5", "strand")

 tfbs = tfbs %>% mutate(Chrom = as.integer(factor(Chrom, levels = paste0("chr", c(1:22, "X", "Y"))))) %>% setDF()

 tfbs$gene = NA

 tfbs$gene.num = NA

 tfbs$gene.start = NA

 tfbs$gene.end = NA

 tfbs$coding = NA

 for (k in 1:nrow(tfbs)) {

 subGN = GN[GN[,2] == tfbs[k,1],]

 pos1 = which(diff((tfbs[k,2] - subGN[,3]) < 0) == 1)[1] + 1

 pos2 = which(diff((tfbs[k,2] - subGN[,4]) < 0) == 1)[1] + 1

 if (!is.na(pos2)) {

 tfbs[k,11] = (subGN[pos2,3] < tfbs[k,2] & subGN[pos2,4] > tfbs[k,3])

 if (tfbs[k,11]) {

 POS = pos2

 } else {

 POS = pos1

 }

 tfbs[k,7] = subGN[POS,6]

 tfbs[k,8] = subGN[POS,1]

 tfbs[k,9] = subGN[POS,3]

 tfbs[k,10] = subGN[POS,4]

 } else {

 tfbs[k,11] = FALSE

 }

 }

 tfbs$POS = tfbs[,2] - tfbs[,9]

 tfbs = tfbs[,-c(4:5)]

 save(tfbs, file = paste0(out.path, "/tfbs.RData"))

}

###################################################################

NGS.list = list()

for (j in 24:1) {

 t1 = Sys.time()

 #1. Read data

 load(paste0("data/TWBNGS-Seq\_chr", j, ".RData"))

 #2. Data processing

 FINAL.DATA = FINAL.DATA %>% mutate(Seq.1 = gsub("[", "", Seq.1, fixed = TRUE),

 Seq.2 = gsub("[", "", Seq.2, fixed = TRUE),

 Seq.3 = gsub("[", "", Seq.3, fixed = TRUE),

 Seq.4 = gsub("[", "", Seq.4, fixed = TRUE)) %>% setDT()

 FINAL.DATA = FINAL.DATA %>% mutate(Seq.1 = gsub("]", "", Seq.1, fixed = TRUE),

 Seq.2 = gsub("]", "", Seq.2, fixed = TRUE),

 Seq.3 = gsub("]", "", Seq.3, fixed = TRUE),

 Seq.4 = gsub("]", "", Seq.4, fixed = TRUE)) %>% setDT()

 FINAL.DATA = FINAL.DATA %>% mutate(Seq.1 = gsub("-", "", Seq.1, fixed = TRUE),

 Seq.2 = gsub("-", "", Seq.2, fixed = TRUE),

 Seq.3 = gsub("-", "", Seq.3, fixed = TRUE),

 Seq.4 = gsub("-", "", Seq.4, fixed = TRUE)) %>% setDT()

 FINAL.DATA = FINAL.DATA %>% mutate(Seq.1 = gsub("<(A|T|C|G)\*>", "", Seq.1),

 Seq.2 = gsub("<(A|T|C|G)\*>", "", Seq.2),

 Seq.3 = gsub("<(A|T|C|G)\*>", "", Seq.3),

 Seq.4 = gsub("<(A|T|C|G)\*>", "", Seq.4)) %>% setDT()

 FINAL.DATA.SNP = FINAL.DATA %>% filter(VarType == "SNP") %>% setDT()

 FINAL.DATA.INS = FINAL.DATA %>% filter(VarType == "INS") %>% setDT()

 FINAL.DATA.DEL = FINAL.DATA %>% filter(VarType == "DEL") %>% setDT()

 FINAL.DATA.DEL = FINAL.DATA.DEL %>% mutate(NCHAR.1 = nchar(Seq.1)-1,

 NCHAR.2 = nchar(Seq.2)-1,

 NCHAR.3 = nchar(Seq.3)-1,

 NCHAR.4 = nchar(Seq.4)-1) %>% setDT()

 FINAL.DATA.DEL = FINAL.DATA.DEL %>% mutate(Seq.1 = substr(Seq.1, 1, NCHAR.1),

 Seq.2 = substr(Seq.2, 1, NCHAR.2),

 Seq.3 = substr(Seq.3, 1, NCHAR.3),

 Seq.4 = substr(Seq.4, 1, NCHAR.4)) %>% setDT()

 FINAL.DATA.DEL = FINAL.DATA.DEL[, 1:18, with = FALSE]

 NGS.DATA = bind\_rows(FINAL.DATA.SNP, FINAL.DATA.INS, FINAL.DATA.DEL)

 #2. Matching

 NGS.DATA.Comparison = NGS.DATA %>% mutate(Seq1.bind = grepl(NFkB.seq, Seq.1),

 Seq2.bind = grepl(NFkB.seq, Seq.2),

 Seq3.bind = grepl(NFkB.seq, Seq.3),

 Seq4.bind = grepl(NFkB.seq, Seq.4)) %>% setDT()

 NGS.DATA.Comparison = NGS.DATA.Comparison %>% mutate(L1 = nchar(Seq.1), L2 = nchar(Seq.2), L3 = nchar(Seq.3), L4 = nchar(Seq.4)) %>% setDT()

 NGS.DATA.Comparison = NGS.DATA.Comparison %>% mutate(Included.bind.500 = (Seq1.bind == "TRUE"|Seq2.bind == "TRUE"|Seq3.bind == "TRUE"|Seq4.bind == "TRUE")) %>% setDT()

 NGS.DATA.Comparison = NGS.DATA.Comparison %>% mutate(Seq.1 = paste0(substr(Seq.1, 500 - length.NFkB.seq + 2, 500), substr(Seq.1, 501, L1 - 500), substr(Seq.1, L1 - 499, L1 - (500 - length.NFkB.seq + 1))),

 Seq.2 = paste0(substr(Seq.2, 500 - length.NFkB.seq + 2, 500), substr(Seq.2, 501, L2 - 500), substr(Seq.2, L2 - 499, L2 - (500 - length.NFkB.seq + 1))),

 Seq.3 = paste0(substr(Seq.3, 500 - length.NFkB.seq + 2, 500), substr(Seq.3, 501, L3 - 500), substr(Seq.3, L3 - 499, L3 - (500 - length.NFkB.seq + 1))),

 Seq.4 = paste0(substr(Seq.4, 500 - length.NFkB.seq + 2, 500), substr(Seq.4, 501, L4 - 500), substr(Seq.4, L4 - 499, L4 - (500 - length.NFkB.seq + 1)))) %>% setDT()

 NGS.DATA.Comparison = NGS.DATA.Comparison %>% mutate(Seq1.bind = grepl(NFkB.seq, Seq.1),

 Seq2.bind = grepl(NFkB.seq, Seq.2),

 Seq3.bind = grepl(NFkB.seq, Seq.3),

 Seq4.bind = grepl(NFkB.seq, Seq.4)) %>% setDT()

 NGS.DATA.Comparison = NGS.DATA.Comparison %>% mutate(L1 = nchar(Seq.1), L2 = nchar(Seq.2), L3 = nchar(Seq.3), L4 = nchar(Seq.4)) %>% setDT()

 NGS.DATA.Comparison = NGS.DATA.Comparison %>% mutate(diff.bind = ((n.Ale == 2 & !(Seq1.bind == Seq2.bind))|(n.Ale == 3 & !(Seq1.bind == Seq2.bind & Seq2.bind == Seq3.bind))|(n.Ale == 4 & !(Seq1.bind == Seq2.bind & Seq2.bind == Seq3.bind & Seq3.bind == Seq4.bind)))) %>% setDT()

 NGS.DATA.Comparison = NGS.DATA.Comparison %>% mutate(Seq.1 = paste0(substr(Seq.1, 1, length.NFkB.seq - 1), "[", substr(Seq.1, length.NFkB.seq, L1 - length.NFkB.seq + 1), "]", substr(Seq.1, L1 - length.NFkB.seq + 2, L1)),

 Seq.2 = paste0(substr(Seq.2, 1, length.NFkB.seq - 1), "[", substr(Seq.2, length.NFkB.seq, L2 - length.NFkB.seq + 1), "]", substr(Seq.2, L2 - length.NFkB.seq + 2, L2)),

 Seq.3 = paste0(substr(Seq.3, 1, length.NFkB.seq - 1), "[", substr(Seq.3, length.NFkB.seq, L3 - length.NFkB.seq + 1), "]", substr(Seq.3, L3 - length.NFkB.seq + 2, L3)),

 Seq.4 = paste0(substr(Seq.4, 1, length.NFkB.seq - 1), "[", substr(Seq.4, length.NFkB.seq, L4 - length.NFkB.seq + 1), "]", substr(Seq.4, L4 - length.NFkB.seq + 2, L4))) %>% setDT()

 NGS.DATA.Comparison = NGS.DATA.Comparison %>% mutate(Seq.1 = mapvalues(Seq.1, "[]", ""),

 Seq.2 = mapvalues(Seq.2, "[]", ""),

 Seq.3 = mapvalues(Seq.3, "[]", ""),

 Seq.4 = mapvalues(Seq.4, "[]", "")) %>% setDT()

 NGS.DATA.Comparison = NGS.DATA.Comparison %>% mutate(Seq1.bind = as.character(Seq1.bind),

 Seq2.bind = as.character(Seq2.bind),

 Seq3.bind = as.character(Seq3.bind),

 Seq4.bind = as.character(Seq4.bind)) %>% setDT()

 NGS.DATA.Comparison.2 = NGS.DATA.Comparison %>% filter(n.Ale == 2) %>% setDT()

 NGS.DATA.Comparison.3 = NGS.DATA.Comparison %>% filter(n.Ale == 3) %>% setDT()

 NGS.DATA.Comparison.4 = NGS.DATA.Comparison %>% filter(n.Ale == 4) %>% setDT()

 NGS.DATA.Comparison.2 = NGS.DATA.Comparison.2 %>% mutate(MAF = as.numeric(Freq.1)\*(Seq1.bind=="TRUE") +

 as.numeric(Freq.2)\*(Seq2.bind=="TRUE")) %>% setDT()

 NGS.DATA.Comparison.3 = NGS.DATA.Comparison.3 %>% mutate(MAF = as.numeric(Freq.1)\*(Seq1.bind=="TRUE") +

 as.numeric(Freq.2)\*(Seq2.bind=="TRUE") +

 as.numeric(Freq.3)\*(Seq3.bind=="TRUE")) %>% setDT()

 NGS.DATA.Comparison.4 = NGS.DATA.Comparison.4 %>% mutate(MAF = as.numeric(Freq.1)\*(Seq1.bind=="TRUE") +

 as.numeric(Freq.2)\*(Seq2.bind=="TRUE") +

 as.numeric(Freq.3)\*(Seq3.bind=="TRUE") +

 as.numeric(Freq.4)\*(Seq4.bind=="TRUE")) %>% setDT()

 NGS.DATA.Comparison.2 = NGS.DATA.Comparison.2 %>% mutate(Seq3.bind = "", Seq4.bind = "") %>% setDT()

 NGS.DATA.Comparison.3 = NGS.DATA.Comparison.3 %>% mutate(Seq4.bind = "") %>% setDT()

 NGS.Comparison = bind\_rows(NGS.DATA.Comparison.2, NGS.DATA.Comparison.3, NGS.DATA.Comparison.4) %>% setDT()

 NGS.Comparison = NGS.Comparison %>% dplyr::select(-L1) %>% dplyr::select(-L2) %>% dplyr::select(-L3) %>% dplyr::select(-L4) %>% setDT()

 #NGS.Comparison = NGS.Comparison %>% mutate(MAF = 0.5 - abs(0.5 - MAF)) %>% setDT()

 #3. Annotation

 subGN = GN[GN[,2] == j,]

 Coding.regien = list()

 Promoter.regien = list()

 for (i in 1:nrow(subGN)) {

 Coding.regien[[i]] = subGN[i,3]:subGN[i,4]

 Promoter.regien[[i]] = (subGN[i,3] - 1000):(subGN[i,3] - 1)

 }

 Coding.regien = unlist(Coding.regien)

 Promoter.regien = unlist(Promoter.regien)

 subtfbs = tfbs[tfbs[,1] == j,]

 if (nrow(subtfbs)==0) {

 Big.regien = NULL

 Cis.regien = NULL

 Trans.regien =NULL

 } else {

 Big.regien = list()

 Cis.regien = list()

 Trans.regien = list()

 for (i in 1:nrow(subtfbs)) {

 Big.regien[[i]] = (subtfbs[i,2]-100):(subtfbs[i,3]+100)

 if (!is.na(subtfbs[i,4])) {

 if (subtfbs[i,4] == "+") {

 Cis.regien[[i]] = subtfbs[i,2]:subtfbs[i,3]

 } else {

 Trans.regien[[i]] = subtfbs[i,2]:subtfbs[i,3]

 }

 }

 }

 Big.regien = unlist(Big.regien)

 Cis.regien = unlist(Cis.regien)

 Trans.regien = unlist(Trans.regien)

 }

 NGS = NGS.Comparison %>% mutate(Coding = Position %in% Coding.regien \* 2 + Position %in% Promoter.regien) %>% setDT()

 NGS = NGS %>% mutate(Chip = Position %in% Cis.regien \* 3 + Position %in% Trans.regien \* 2 + Position %in% Big.regien) %>% setDT()

 save(NGS, file = paste0(out.path, "/chr", j, ".RData"))

 NGS.list[[j]] = NGS

 t2 = Sys.time()

 cat(paste0("The ",j," Chrom was end, we cost ",formatC(as.numeric(t2-t1, units = "secs"), 2, format = "f")," secs in this loop\n"))

 NGS.list[[j]] = NGS

}

NGS.Final = bind\_rows(NGS.list)

save(NGS.Final, file = paste0(out.path, "/NGS\_all.RData"))

rm(list = ls()[which(!ls()%in%"NGS.Final")])

###################################################################

#source("https://bioconductor.org/biocLite.R")

#biocLite("BSgenome.Hsapiens.UCSC.hg19")

library(data.table)

library(magrittr)

library(dplyr)

library(plyr)

out.path = "data/MYOG\_sarcopenia"

load(paste0(out.path, "/NGS\_all.RData"))

#0. Build gene ref

if (file.exists("data/Gene\_Location.RData")) {load("data/Gene\_Location.RData")} else {

 library(BSgenome.Hsapiens.UCSC.hg19)

 library(TxDb.Hsapiens.UCSC.hg19.knownGene)

 HGNC.URL = "http://www.genenames.org/cgi-bin/download?col=gd\_hgnc\_id&col=gd\_app\_sym&col=gd\_app\_name&col=gd\_prev\_sym&col=gd\_aliases&col=gd\_pub\_eg\_id&status=Approved&status\_opt=2&where=&order\_by=gd\_app\_sym\_sort&format=text&limit=&hgnc\_dbtag=on&submit=submit"

 HGNC.ref = fread(HGNC.URL, data.table = FALSE)

 HGNC.ref = HGNC.ref[,c(6, 2, 3)]

 txdb <- TxDb.Hsapiens.UCSC.hg19.knownGene

 gn <- sort(genes(txdb))

 GN = data.table(gene.num = gn@ranges@NAMES,

 chr = as.data.frame(gn)[,1],

 start = gn@ranges@start,

 end = gn@ranges@start + gn@ranges@width - 1,

 width = gn@ranges@width)

 GN = GN %>% filter(as.character(as.data.frame(gn)[,1]) %in% paste0("chr", c(1:22, "X", "Y"))) %>% setDT()

 GN = GN %>% mutate(chr = mapvalues(chr, paste0("chr", c(1:22, "X", "Y")), 1:24)) %>% setDF()

 GN[,1] = as.integer(GN[,1])

 GN[,2] = as.integer(GN[,2])

 GN[,4] = as.integer(GN[,4])

 GN = merge(GN, HGNC.ref, by.x = "gene.num", by.y = "Entrez Gene ID", all.x = TRUE)

 GN = GN[order(GN[,3]),]

 GN = GN[order(GN[,2]),]

 colnames(GN)[6:7] = c("Short.name", "Full.name")

 URL = "https://www.ncbi.nlm.nih.gov/gene/"

 pb = txtProgressBar(max = nrow(GN), style = 3)

 for (i in 1:nrow(GN)) {

 if (is.na(GN[i,6])) {

 sep.URL = paste0(URL, GN[i,1])

 txt = scan(sep.URL, what="character", encoding="UTF-8", quiet = TRUE)

 txt = paste(txt, sep="", collapse=" ")

 pos.1 = regexpr('Gene symbol </dt> <dd class=\"noline\">', txt)[1] + 37

 subtxt.1 = substr(txt, pos.1, pos.1 + 200)

 pos.2 = regexpr('</dd>', subtxt.1)[1] - 1

 GN[i,6] = substr(subtxt.1, 1, pos.2)

 pos.3 = regexpr('Gene description </dt> <dd>', txt)[1] + 27

 subtxt.2 = substr(txt, pos.3, pos.3 + 200)

 pos.4 = regexpr('</dd>', subtxt.2)[1] - 1

 GN[i,7] = substr(subtxt.2, 1, pos.4)

 }

 setTxtProgressBar(pb, i)

 }

 close(pb)

 pb = txtProgressBar(max = nrow(GN), style = 3)

 for (i in 1:nrow(GN)) {

 if (GN[i,6] == "") {

 sep.URL = paste0(URL, GN[i,1])

 txt = scan(sep.URL, what="character", encoding="UTF-8", quiet = TRUE)

 txt = paste(txt, sep="", collapse=" ")

 pos.1 = regexpr('Official Symbol</dt> <dd class=\"noline\">', txt)[1] + 40

 subtxt.1 = substr(txt, pos.1, pos.1 + 200)

 pos.2 = regexpr('<span', subtxt.1)[1] - 1

 GN[i,6] = substr(subtxt.1, 1, pos.2)

 pos.3 = regexpr('Official Full Name</dt> <dd>', txt)[1] + 28

 subtxt.2 = substr(txt, pos.3, pos.3 + 200)

 pos.4 = regexpr('<span', subtxt.2)[1] - 1

 GN[i,7] = substr(subtxt.2, 1, pos.4)

 }

 setTxtProgressBar(pb, i)

 }

 close(pb)

 save(GN, file = "data/Gene\_Location.RData")

 write.csv(GN, "data/Gene\_Location.csv", quote = FALSE, na = "", row.names = FALSE)

}

#1. combine gene information

#load(paste0(out.path, "/NGS\_all.RData"))

NGS.500 = NGS.Final %>% filter(Included.bind.500 == TRUE & VarType == "SNP" & call > 0.9) %>% setDT()

NGS.diff = NGS.500 %>% filter(diff.bind == TRUE) %>% setDT()

NGS.5 = NGS.diff %>% filter(MAF <= 0.95 & MAF >= 0.05) %>% setDT()

NGS.Chip = NGS.5 %>% filter(Chip > 1) %>% setDT()

NGS.Chip$gene = NA

NGS.Chip$gene.num = NA

NGS.Chip$gene.start = NA

NGS.Chip$gene.end = NA

NGS.Chip$coding.reg = NA

for (k in 1:nrow(NGS.Chip)) {

 subGN = GN[GN[,2] == NGS.Chip[k,1],]

 pos1 = which(diff((NGS.Chip[k,2] - subGN[,3]) < 0) == 1)[1] + 1

 pos2 = which(diff((NGS.Chip[k,2] - subGN[,4]) < 0) == 1)[1] + 1

 if (!is.na(pos2)) {

 NGS.Chip[k,32] = (subGN[pos2,3] < NGS.Chip[k,2] & subGN[pos2,4] > NGS.Chip[k,2])

 if (NGS.Chip[k,32]) {

 POS = pos2

 } else {

 POS = pos1

 }

 NGS.Chip[k,28] = subGN[POS,6]

 NGS.Chip[k,29] = subGN[POS,1]

 NGS.Chip[k,30] = subGN[POS,3]

 NGS.Chip[k,31] = subGN[POS,4]

 } else {

 NGS.Chip[k,32] = FALSE

 }

}

NGS.Chip$POS = NGS.Chip$Position - NGS.Chip$gene.start