## Non-target analysis in R (R138) Hyun Yong Jin (hyjin@scripps.edu) 20151209

```
```{r}
#Gene list has been generated
setwd("~/TheShell/SeqResults/R138_Jin_RiPr")
library(data.table)
library(ggplot2)
cov <- fread("coverage_matrix_small")</pre>
setkey(cov, symbol) #setkey based on gene symbol
# Import genelist, from separate csv files
genelist <-
read.table("06_visualization/Coverage_Nontarget_forloop_20151209/geneli
st_nontarget_noago.csv", header=T, sep=",")
nontarget_down_in_tg <- as.vector(genelist$nontarget_down_in_tg)</pre>
nontarget_down_in_tg
<-nontarget down in tg[nontarget down in tg!=""]
noago_down_in_tg <- as.vector(genelist$noago_down_in_tg)
noago_down_in_tg <-noago_down_in_tg[noago_down_in_tg !=""]
nontarget_up_in_tko <- as.vector(genelist$nontarget_up_in_tko)</pre>
nontarget_up_in_tko <-nontarget_up_in_tko[nontarget_up_in_tko !="" ]</pre>
noago_up_in_tko <- as.vector(genelist$noago_up_in_tko)</pre>
noago_up_in_tko <-noago_up_in_tko[noago_up_in_tko!=""]
#WT-TG comparison first
```{r}
#WT -->notarget_down_in_tg
wt.start <-matrix(0, nrow=150, ncol=0)
```

```
for(i in nontarget_down_in_tg) {
    g <- i
    cg <- cov[g]
    strand <- cq$strd[1]
    if(is.na(strand) ==TRUE) {
      next
    if(strand == "+") {
        cg <- cg[order(cg$cdsutr, cg$exonid, cg$nt),]
     } else {
        cg <- cg[order(cg$cdsutr, -cg$exonid, -cg$nt),]
    beforestart <- tail(which(cg$cdsutr ==0), n=100)
    afterstart <-head(which(cg$cdsutr ==1), n=50)
    cdsregion <- c(beforestart, afterstart)
    wt1r <- (cov[c(g), sum(wt1)])
    wt2r <- (cov[c(g), sum(wt2)])
    wt3r < -(cov[c(g), sum(wt3)])
    wt.nrm.strt <-cq$wt1[cdsregion[1]:tail(cdsregion,
n=1)]/(wt1r[wt1r[,symbol] == g, V1]+1) +cg$wt2[cdsregion[1]:tail(cdsregion,
n=1)]/(wt2r[wt2r[,symbol] == q, V1]+1) +cq$wt3[cdsregion[1]:tail(cdsregion,
n=1]/(wt3r[wt3r[,symbol] == g, V1]+1)
    if(length(cdsregion) == 150) 
    } else {
    cdsregion \leftarrow append(rep(c(0), each=150-length(cdsregion)),
cdsregion)
    wt.nrm.strt <- append(rep(c(0), each=150-length(wt.nrm.strt)),
wt.nrm.strt)
  wt.start <- cbind(wt.start, wt.nrm.strt)
dim(wt.start) # supposed to 1044 but matched ones are 976
wt.start.trim<- apply(wt.start, 1, mean, trim=0.15)
#TG -->notarget_down_in_tg
tg.start <-matrix(0, nrow=150, ncol=0) #empty numeric vector (inclease
speed)
```

```
for(i in nontarget_down_in_tg) {
    g <- i
    cg <- cov[g]
    strand <- cg$strd[1]
    if(is.na(strand) ==TRUE) { # this is for avoid non-gene name
matched ones
       next
    if(strand == "+") {
        cg <- cg[order(cg$cdsutr, cg$exonid, cg$nt),]
     } else {
        cg <- cg[order(cg$cdsutr, -cg$exonid, -cg$nt),]
    beforestart <- tail(which(cg$cdsutr ==0), n=100)
    afterstart <-head(which(cg$cdsutr ==1), n=50)
    cdsregion <- c(beforestart, afterstart)
    tg1r <- (cov[c(g), sum(tg1)])
    tg2r <- (cov[c(g), sum(tg2)])
    tg3r <- (cov[c(g), sum(tg3)])
    tg.nrm.strt <-cg$tg1[cdsregion[1]:tail(cdsregion,
n=1)/(tg1r[tg1r[,symbol] == g, V1]+1) +cg$tg2[cdsregion[1]:tail(cdsregion,
n=1)]/(tg2r[tg2r[,symbol] == g, V1]+1) +cg$tg3[cdsregion[1]:tail(cdsregion,
n=1)/(tg3r[tg3r[,symbol] == g, V1]+1)
    if(length(cdsregion) == 150) {
    } else {
    cdsregion <- append(rep(c(0), each=150-length(cdsregion)),
cdsregion)
    tg.nrm.strt <- append(rep(c(0), each=150-length(tg.nrm.strt)),
tg.nrm.strt)
  tg.start <- cbind(tg.start, tg.nrm.strt)
dim(tg.start)
tg.start.trim<- apply(tg.start, 1, mean, trim=0.15)
#generate matrix for graph
plot <- as.data.frame(cbind(wt.start.trim, tg.start.trim))</pre>
```

```
#Plotting: WT vs TG --> tg_res_targets
ggplot(plot, aes(x=as.numeric(row.names(plot))))+
  geom_line(aes(y=wt.start.trim), colour="black", size=2)+
  geom_line(aes(y=tg.start.trim), colour="green", size=2)+
  geom_vline(xintercept=100, linetype="dashed", size=2, color="gray")+
  ggtitle("Nontargets_down_in_tg")+
  labs(x="Start Codon", y="Relative Ribosome Occupancy")+
  geom_segment(mapping=aes(x=100, xend=150, y=-0.0002,
yend=-0.0002), size=12, color="dark grey") +
  geom segment(mapping=aes(x=0, xend=150, y=-0.0002, yend=-0.0002),
size=4, color="dark grey")+
  theme (panel.background = element_rect(fill='white'), axis.text.x=
element_text(color="black"), axis.text.y = element_blank(), plot.title =
element_text(face="italic", size=14), axis.title = element_text(size=15)) +
  scale_y_continuous(limit=c(-0.0003, 0.0045))
Save as 6x8
Save as 4x6
#WT -->noago down in tg
wt.start <-matrix(0, nrow=150, ncol=0)
for(i in noago_down_in_tg) {
    g <- i
    cg <- cov[g]
    strand <- cg$strd[1]
    if(is.na(strand) ==TRUE) {
      next
    if(strand == "+") {
       cg <- cg[order(cg$cdsutr, cg$exonid, cg$nt),]
     } else {
       cg <- cg[order(cg$cdsutr, -cg$exonid, -cg$nt),]
    beforestart <- tail(which(cg$cdsutr ==0), n=100)
    afterstart <-head(which(cg$cdsutr ==1), n=50)
    cdsregion <- c(beforestart, afterstart)
    wt1r < -(cov[c(g), sum(wt1)])
```

```
wt2r <- (cov[c(g), sum(wt2)])
    wt3r <- (cov[c(g), sum(wt3)])
    wt.nrm.strt <-cg$wt1[cdsregion[1]:tail(cdsregion,
n=1)]/(wt1r[wt1r[,symbol] == g, V1]+1) +cg$wt2[cdsregion[1]:tail(cdsregion,
n=1)]/(wt2r[wt2r[,symbol] == g, V1]+1) +cg$wt3[cdsregion[1]:tail(cdsregion,
n=1)]/(wt3r[wt3r[,symbol] == g, V1]+1)
    if(length(cdsregion) == 150) {
    } else {
    cdsregion <- append(rep(c(0), each=150-length(cdsregion)),
cdsregion)
    wt.nrm.strt \leftarrow append(rep(c(0), each=150-length(wt.nrm.strt)),
wt.nrm.strt)
  wt.start <- cbind(wt.start, wt.nrm.strt)
dim(wt.start) # supposed to 755 but matched ones are 687
wt.start.trim<- apply(wt.start, 1, mean, trim=0.15)
#TG -->nogo_down_in_tg
tg.start <-matrix(0, nrow=150, ncol=0) #empty numeric vector (inclease
speed)
for(i in noago_down_in_tg) {
    g <- i
    cg <- cov[g]
    strand <- cg$strd[1]
    if(is.na(strand) ==TRUE) { # this is for avoid non-gene name
matched ones
      next
    if(strand == "+") {
        cg <- cg[order(cg$cdsutr, cg$exonid, cg$nt),]
     } else {
        cg <- cg[order(cg$cdsutr, -cg$exonid, -cg$nt),]
    beforestart <- tail(which(cg$cdsutr ==0), n=100)
    afterstart <-head(which(cg$cdsutr ==1), n=50)
    cdsregion <- c(beforestart, afterstart)
```

```
tg1r <- (cov[c(g), sum(tg1)])
    tg2r \leftarrow (cov[c(g), sum(tg2)])
    tg3r <- (cov[c(g), sum(tg3)])
    tg.nrm.strt <-cg$tg1[cdsregion[1]:tail(cdsregion,
n=1)]/(tg1r[tg1r[,symbol] == g, V1]+1) +cg$tg2[cdsregion[1]:tail(cdsregion,
n=1)]/(tg2r[tg2r[,symbol] == g, V1]+1) +cg$tg3[cdsregion[1]:tail(cdsregion,
n=1)/(tg3r[tg3r[,symbol] == g, V1]+1)
    if(length(cdsregion) == 150) {
    } else {
    cdsregion \leftarrow append(rep(c(0), each=150-length(cdsregion)),
cdsregion)
    tg.nrm.strt <- append(rep(c(0), each=150-length(tg.nrm.strt)),
tg.nrm.strt)
  tg.start <- cbind(tg.start, tg.nrm.strt)
dim(tg.start)
tg.start.trim<- apply(tg.start, 1, mean, trim=0.15)
#generate matrix for graph
plot <- as.data.frame(cbind(wt.start.trim, tg.start.trim))
#Plotting: WT vs TG
ggplot(plot, aes(x=as.numeric(row.names(plot))))+
  geom_line(aes(y=wt.start.trim), colour="black", size=2)+
  geom_line(aes(y=tg.start.trim), colour="green", size=2)+
  geom_vline(xintercept=100, linetype="dashed", size=2, color="gray")+
  ggtitle("No_ago_down_in_tg")+
  labs(x="Start Codon", y="Relative Ribosome Occupancy")+
  geom_segment(mapping=aes(x=100, xend=150, y=-0.0002,
yend=-0.0002), size=12, color="dark grey") +
  geom segment(mapping=aes(x=0, xend=150, y=-0.0002, yend=-0.0002),
size=4, color="dark grey")+
  theme (panel.background = element rect(fill='white'), axis.text.x=
element_text(color="black"), axis.text.y = element_blank(), plot.title =
element_text(face="italic", size=14), axis.title = element_text(size=15)) +
  scale_y_continuous(limit=c(-0.0003, 0.0048))
```

```
Save as 6x8
Save as 4x6
```

. . .

```
#WT-TKO comparison next
```{r}
#WT -->notarget_up_in_tko
wt.start <-matrix(0, nrow=150, ncol=0)
for(i in nontarget_up_in_tko) {
    q <- i
    cg <- cov[g]
    strand <- cq$strd[1]
    if(is.na(strand) ==TRUE) {
      next
    if(strand == "+") {
        cg <- cg[order(cg$cdsutr, cg$exonid, cg$nt),]
     } else {
        cg <- cg[order(cg$cdsutr, -cg$exonid, -cg$nt),]
    beforestart <- tail(which(cg$cdsutr ==0), n=100)
    afterstart <-head(which(cg$cdsutr ==1), n=50)
    cdsregion <- c(beforestart, afterstart)
    wt1r <- (cov[c(g), sum(wt1)])
    wt2r <- (cov[c(g), sum(wt2)])
    wt3r < -(cov[c(g), sum(wt3)])
    wt.nrm.strt <-cg$wt1[cdsregion[1]:tail(cdsregion,
n=1)]/(wt1r[wt1r[,symbol] == g, V1]+1) +cg$wt2[cdsregion[1]:tail(cdsregion,
n=1)]/(wt2r[wt2r[,symbol] == g, V1]+1) +cg$wt3[cdsregion[1]:tail(cdsregion,
```

```
n=1]/(wt3r[wt3r[,symbol] == g, V1]+1)
    if(length(cdsregion) == 150) {
    } else {
    cdsregion <- append(rep(c(0), each=150-length(cdsregion)),
cdsregion)
    wt.nrm.strt <- append(rep(c(0), each=150-length(wt.nrm.strt)),
wt.nrm.strt)
  wt.start <- cbind(wt.start, wt.nrm.strt)
dim(wt.start) # supposed to 781 but matched ones are 730
wt.start.trim<- apply(wt.start, 1, mean, trim=0.15)
#TKO -->nontarget_up_in_tko
tko.start <-matrix(0, nrow=150, ncol=0) #empty numeric vector (inclease
speed)
for(i in nontarget_up_in_tko) {
    q < -i
    cg <- cov[g]
    strand <- cq$strd[1]
    if(is.na(strand) ==TRUE) { # this is for avoid non-gene name
matched ones
      next
    if(strand == "+") {
        cg <- cg[order(cg$cdsutr, cg$exonid, cg$nt),]
     } else {
        cg <- cg[order(cg$cdsutr, -cg$exonid, -cg$nt),]
    beforestart <- tail(which(cg$cdsutr ==0), n=100)
    afterstart <-head(which(cg$cdsutr ==1), n=50)
    cdsregion <- c(beforestart, afterstart)
    tko1r <- (cov[c(g), sum(tko1)])
    tko2r <- (cov[c(g), sum(tko2)])
    tko3r <- (cov[c(g), sum(tko3)])
    tko.nrm.strt <-cg$tko1[cdsregion[1]:tail(cdsregion,
n=1)/(tko1r[tko1r[,symbol] == g, V1]+1)
```

```
+cg$tko2[cdsregion[1]:tail(cdsregion, n=1)]/(tko2r[tko2r[,symbol] == g,
V1]+1) + cg$tko3[cdsregion[1]:tail(cdsregion, n=1)]/(tko3r[tko3r[, symbol] == 1])
g, V1]+1)
    if(length(cdsregion) == 150) {
    } else {
    cdsregion <- append(rep(c(0), each=150-length(cdsregion)),
cdsregion)
    tko.nrm.strt <- append(rep(c(0), each=150-length(tko.nrm.strt)),
tko.nrm.strt)
  tko.start <- cbind(tko.start, tko.nrm.strt)
dim(tko.start)
tko.start.trim<- apply(tko.start, 1, mean, trim=0.15)
#generate matrix for graph
plot <- as.data.frame(cbind(wt.start.trim, tko.start.trim))
#Plotting: WT vs TKO
ggplot(plot, aes(x=as.numeric(row.names(plot))))+
  geom line(aes(y=wt.start.trim), colour="black", size=2)+
  geom line(aes(y=tko.start.trim), colour="red", size=2)+
  geom_vline(xintercept=100, linetype="dashed", size=2, color="gray")+
  ggtitle("Nontargets_up_in_ko")+
  labs(x="Start Codon", y="Relative Ribosome Occupancy")+
  geom_segment(mapping=aes(x=100, xend=150, y=-0.0002,
yend=-0.0002), size=12, color="dark grey") +
  geom_segment(mapping=aes(x=0, xend=150, y=-0.0002, yend=-0.0002),
size=4, color="dark grey")+
  theme (panel.background = element rect(fill='white'), axis.text.x=
element_text(color="black"), axis.text.y = element_blank(), plot.title =
element_text(face="italic", size=14), axis.title = element_text(size=15)) +
  scale_y_continuous(limit=c(-0.0003, 0.0045))
Save as 6x8
Save as 4x6
```

```
#WT -->noago_up_in_tko
wt.start <-matrix(0, nrow=150, ncol=0)
for(i in noago_up_in_tko) {
    g <- i
    cg <- cov[g]
    strand <- cg$strd[1]
    if(is.na(strand) ==TRUE) {
       next
    if(strand == "+") {
        cg <- cg[order(cg$cdsutr, cg$exonid, cg$nt),]
     } else {
        cg <- cg[order(cg$cdsutr, -cg$exonid, -cg$nt),]
    beforestart <- tail(which(cg$cdsutr ==0), n=100)
    afterstart <-head(which(cg$cdsutr ==1), n=50)
    cdsregion <- c(beforestart, afterstart)
    wt1r <- (cov[c(g), sum(wt1)])
    wt2r <- (cov[c(g), sum(wt2)])
    wt3r <- (cov[c(g), sum(wt3)])
    wt.nrm.strt <-cg$wt1[cdsregion[1]:tail(cdsregion,
n=1)]/(wt1r[wt1r[,symbol] == g, V1]+1) +cg$wt2[cdsregion[1]:tail(cdsregion,
n=1)]/(wt2r[wt2r[,symbol] == g, V1]+1) +cg$wt3[cdsregion[1]:tail(cdsregion,
n=1)/(wt3r[wt3r[,symbol] == g, V1]+1)
    if(length(cdsregion) == 150) {
    } else {
    cdsregion <- append(rep(c(0), each=150-length(cdsregion)),
cdsregion)
    wt.nrm.strt <- append(rep(c(0), each=150-length(wt.nrm.strt)),
wt.nrm.strt)
  wt.start <- cbind(wt.start, wt.nrm.strt)
}
dim(wt.start) # supposed to 627 matched 578
wt.start.trim<- apply(wt.start, 1, mean, trim=0.15)
```

```
#TKO -->noago_up_in_tko
tko.start <-matrix(0, nrow=150, ncol=0) #empty numeric vector (inclease
speed)
for(i in noago_up_in_tko) {
    g <- i
    cq <- cov[q]
    strand <- cg$strd[1]
    if(is.na(strand) ==TRUE) { # this is for avoid non-gene name
matched ones
      next
    if(strand == "+") {
        cg <- cg[order(cg$cdsutr, cg$exonid, cg$nt),]
     } else {
        cg <- cg[order(cg$cdsutr, -cg$exonid, -cg$nt),]
    beforestart <- tail(which(cg$cdsutr ==0), n=100)
    afterstart <-head(which(cg$cdsutr ==1), n=50)
    cdsregion <- c(beforestart, afterstart)
    tko1r <- (cov[c(g), sum(tko1)])
    tko2r <- (cov[c(g), sum(tko2)])
    tko3r <- (cov[c(g), sum(tko3)])
    tko.nrm.strt <-cq$tko1[cdsregion[1]:tail(cdsregion,
n=1]/(tko1r[tko1r[,symbol] == g, V1]+1)
+cg$tko2[cdsregion[1]:tail(cdsregion, n=1)]/(tko2r[tko2r[,symbol] == g,
V1]+1) + cg$tko3[cdsregion[1]:tail(cdsregion, n=1)]/(tko3r[tko3r[, symbol] == 
g, V1]+1)
    if(length(cdsregion) == 150) {
    } else {
    cdsregion <- append(rep(c(0), each=150-length(cdsregion)),
cdsregion)
    tko.nrm.strt <- append(rep(c(0), each=150-length(tko.nrm.strt)),
tko.nrm.strt)
  tko.start <- cbind(tko.start, tko.nrm.strt)
dim(tko.start)
tko.start.trim<- apply(tko.start, 1, mean, trim=0.15)
```

```
#generate matrix for graph
plot <- as.data.frame(cbind(wt.start.trim, tko.start.trim))
#Plotting: WT vs TKO
ggplot(plot, aes(x=as.numeric(row.names(plot))))+
  geom_line(aes(y=wt.start.trim), colour="black", size=2)+
  geom line(aes(y=tko.start.trim), colour="red", size=2)+
  geom_vline(xintercept=100, linetype="dashed", size=2, color="gray")+
  ggtitle("Noago up in ko")+
  labs(x="Start Codon", y="Relative Ribosome Occupancy")+
  geom_segment(mapping=aes(x=100, xend=150, y=-0.0002,
yend=-0.0002), size=12, color="dark grey") +
  geom_segment(mapping=aes(x=0, xend=150, y=-0.0002, yend=-0.0002),
size=4, color="dark grey")+
  theme (panel.background = element_rect(fill='white'), axis.text.x=
element_text(color="black"), axis.text.y = element_blank(), plot.title =
element_text(face="italic", size=14), axis.title = element_text(size=15)) +
  scale y continuous(limit=c(-0.0003, 0.0045))
Save as 6x8
Save as 4x6
```

...