

**aNon-target analysis in R (R138)**  
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**20151209**

```
```{r}
#Gene list has been generated

setwd("~/TheShell/SeqResults/R138_Jin_RiPr")
library(data.table)
library(ggplot2)
cov <- fread("coverage_matrix_small")
setkey(cov, symbol) #setkey based on gene symbol

# Import genelists, from separate csv files
genelist <-
read.table("06_visualization/Coverage_Nontarget_forloop_20151209/genelist_nontarget_noago.csv",
header=T, sep=",")

nontarget_down_in_tg <- as.vector(genelist$nontarget_down_in_tg)
nontarget_down_in_tg <- nontarget_down_in_tg[nontarget_down_in_tg != ""]
noago_down_in_tg <- as.vector(genelist$noago_down_in_tg)
noago_down_in_tg <- noago_down_in_tg[noago_down_in_tg != ""]
nontarget_up_in_tko <- as.vector(genelist$nontarget_up_in_tko)
nontarget_up_in_tko <- nontarget_up_in_tko[nontarget_up_in_tko != ""]
noago_up_in_tko <- as.vector(genelist$noago_up_in_tko)
noago_up_in_tko <- noago_up_in_tko[noago_up_in_tko != ""]

...

#WT-TG comparison first
```{r}

#WT --> nontarget_down_in_tg
wt.start <- matrix(0, nrow=150, ncol=0)
for(i in nontarget_down_in_tg) {
  g <- i
  cg <- cov[g]
  strand <- cg$strand[1]
  if(is.na(strand) == TRUE) {
    next
  }
  if(strand == "+") {
    cg <- cg[order(cg$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 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 (increase speed)
for(i in nontarget_down_in_tg) {
    g <- i
    cg <- cov[g]
    strand <- cg$strnd[1]
    if(is.na(strand) == TRUE) { # this is for avoid non-gene name matched ones
        next
    }
    if(strand == "+") {
        cg <- cg[order(cg$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))

```

```

#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$strnd[1]
  if(is.na(strand) ==TRUE) {
    next
  }
  if(strand == "+") {
    cg <- cg[order(cg$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 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$cdsutr ==1), n=50)
  cdsregion <- c(beforestart, afterstart)
  tg1r <- (cov[c(g), sum(tg1)])
  tg2r <- (cov[c(g), sum(tg2)])
  tg3r <- (cov[c(g), sum(tg3)])
  tg.nrm.strt <-cg$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))

#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 --> nontarget\_up\_in\_tko

wt.start <- matrix(0, nrow=150, ncol=0)

for(i in nontarget\_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 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 (increase speed)

for(i in nontarget\_up\_in\_tko) {

  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)
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] == 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
  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$cdsutr ==1), n=50)
  cdsregion <- c(beforestart, afterstart)
  tko1r <- (cov[c(g), sum(tko1)])
  tko2r <- (cov[c(g), sum(tko2)])
  tko3r <- (cov[c(g), sum(tko3)])
  tko.nrm.strt <- cg$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] == 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

```

...