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## This code was used to generate the m6Aclusters. ##

library(TCGAbiolinks)

require(dplyr)

require(tidyr)

library(mclust)

#Download of RNA-Seq TCGA-KIRC data

expquery <- GDCquery(project = "TCGA-KIRC",

                     data.category = "Transcriptome Profiling",

                     data.type = "Gene Expression Quantification",

                     workflow.type = "HTSeq - Counts"

)

GDCdownload(expquery)

expquery2 <- GDCprepare(expquery)

expdat <- TCGAanalyze_Preprocessing(expquery2)

#Convert read count to TPM

eff_length <- read.csv("eff_length.csv", row.names = 1, header = T)

eff_length$ensembl_id <- rownames(eff_length)

rownames(eff_length) <- do.call(rbind, strsplit(eff_length$ensembl_id, '\\.')[,1])

expdat <- expdat[rownames(expdat) %in% rownames(eff_length),]

eff_length <- eff_length[match(rownames(expdat), rownames(eff_length)), ]
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k <- expdat / eff_length$eff_length

expdat_tpm <- t( t(k) / colSums(k) ) * 1e6

#Convert Ensembl gene IDs to gene symbols

#The file "gencode.v22.annotation.gtf" can be download from
https://www.gencodegenes.org/human/release_22.html

expdat_tpm <- as.data.frame(expdat_tpm)

expdat_tpm <- cbind(gene_id=rownames(expdat_tpm),expdat_tpm)

expdat_tpm$gene_id <- as.character(expdat_tpm$gene_id)

gtf1 <- rtracklayer::import('gencode.v22.annotation.gtf')

gtf_df <- as.data.frame(gtf1)

exprSet <- gtf_df %>%

  dplyr::filter(type=="gene") %>%

  dplyr::select(c(gene_name,gene_id)) %>%

  dplyr::inner_join(expdat_tpm,by ="gene_id") %>%

  tidyr::unite(gene_id,gene_name,gene_id,sep = "|")

rownames(exprSet) <- exprSet[,1]

exprSet <- exprSet[,-1]

#Extraction of TPM expression level of m6A genes

m6A_gene <-

c("YTHDC1|ENSG00000083896","IGF2BP1|ENSG00000159217","IGF2BP2|ENSG0000
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0073792","IGF2BP3|ENSG00000136231","YTHDF1|ENSG00000149658",  
  
"YTHDF3|ENSG00000185728","YTHDC2|ENSG00000047188","HNRNPA2B1|ENSG000  
00122566","YTHDF2|ENSG00000198492","HNRNPC|ENSG00000092199",  
  
"RBMX|ENSG00000147274","METTL3|ENSG00000165819","METTL14|ENSG00000145  
388","WTAP|ENSG00000146457","KIAA1429|ENSG00000164944",  
  
"RBM15|ENSG00000162775","RBM15B|ENSG00000259956","ZC3H13|ENSG00000123  
200","FTO|ENSG00000140718","ALKBH5|ENSG00000091542",  
  
"CBLL1|ENSG00000105879","FMR1|ENSG00000102081","LRPPRC|ENSG0000013809  
5","ELAVL1|ENSG00000066044")  
m6A_expr<-t(exprSet[m6A_gene,])  
  
#Extraction of the cases containing immune subtypes in TCGA-KIRC dataset  
  
#Reference: Thorsson V, Gibbs DL, Brown SD, et al. The Immune Landscape of Cancer.  
Immunity. 2018;48(4): 812-830 e814.  
  
immunesubtype <- read.csv("immunesubtype.csv",row.names = 1, header = T)  
  
share<-intersect(rownames(immunesubtype),rownames(m6A_expr))  
  
m6A_expr<-m6A_expr[share,]
```

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#log2 transform
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rt<-log2(m6A_expr+1)
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#mclust
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m_clust <- Mclust(as.matrix(rt))
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```
m6Acluster<-m_clust$classification
```