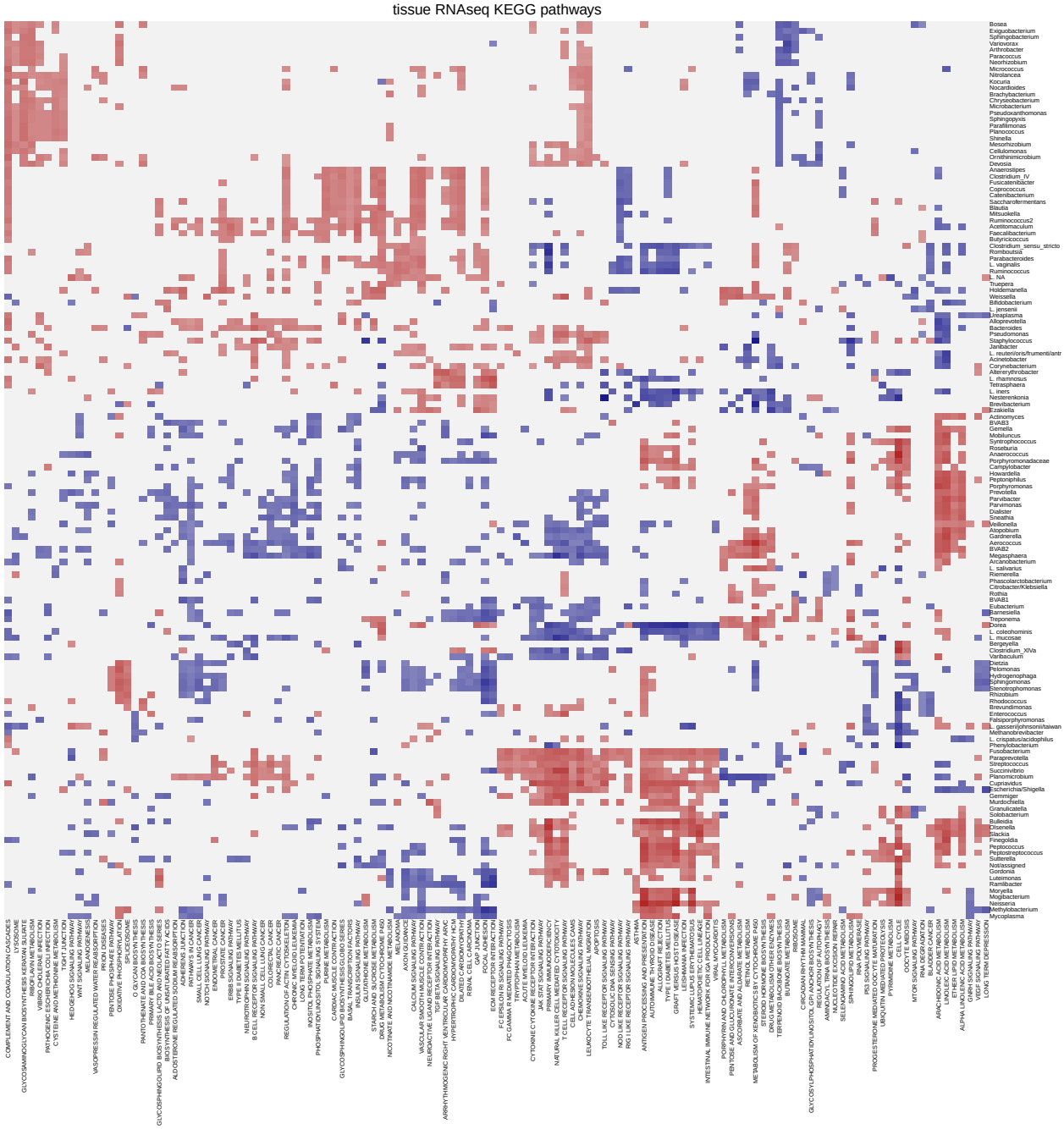
Suppl. Figure 3. & 4. Mirobiome-KEGG correlations.

```
knitr::opts_chunk$set(
  fig.path="./Figures/",
  fig.process = function(filename){
    new_filename <- stringr::str_remove(string = filename,
                                        pattern = "-1")
    fs::file_move(path = filename, new_path = new_filename)
    ifelse(fs::file_exists(new_filename), new_filename, filename)
})
# setwd("/Users/vilkal/work/Brolidens_work/Projects/Gabriella_repo/reports/rmarkdown/manuscript")
```

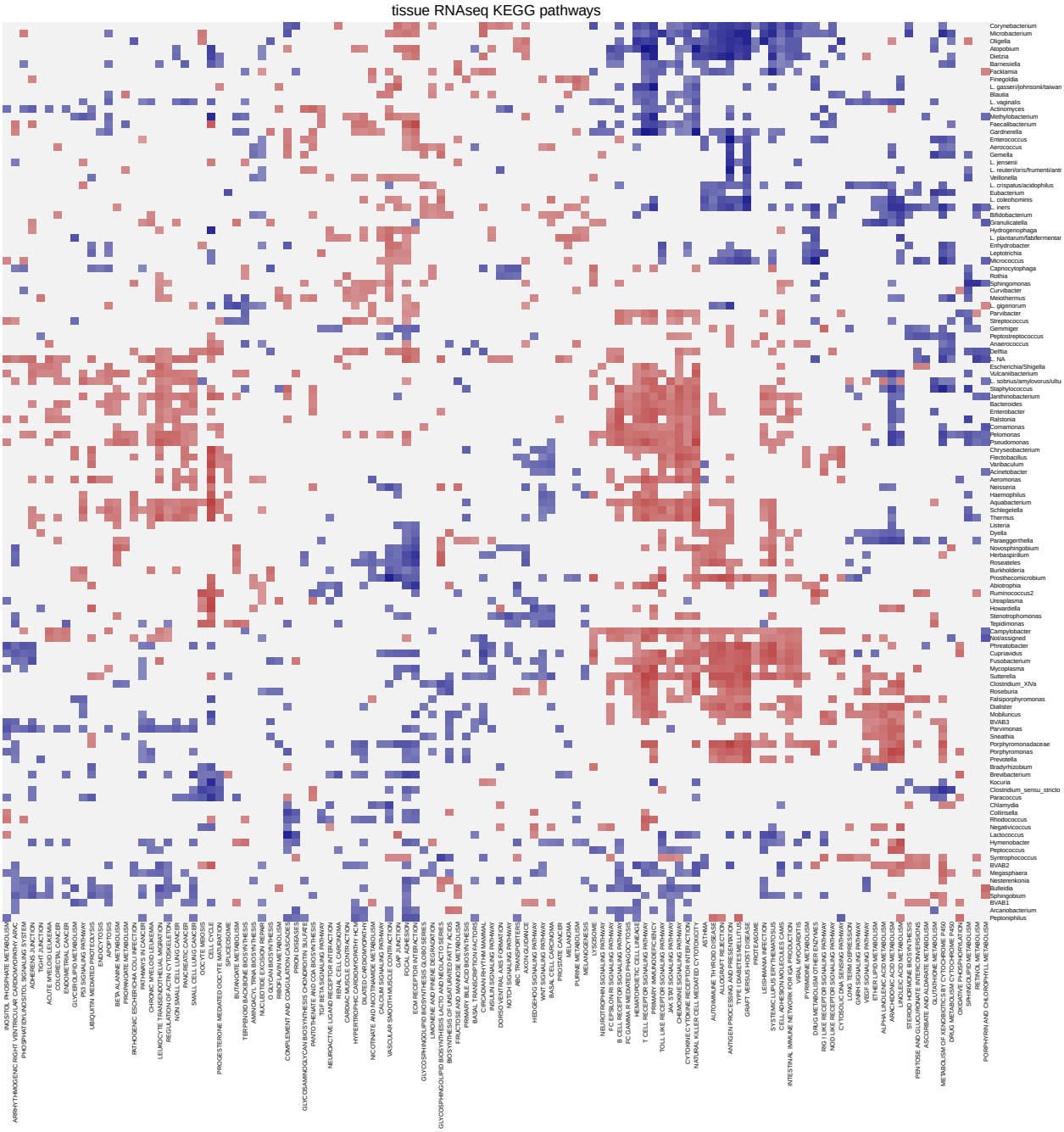
```
myletter <- letters
#reprod. <- FALSE
reprod. <- TRUE

# ASV <- list(c("Luminal","ASV_CVL_V3_normalized_batch_corrected"),
#             c("Tissue","ASV_tissue_V3_normalized_batch_corrected")
#             )
ASV <- list(c("Luminal","ASV_Luminal_normalized"),
            c("Tissue","ASV_Tissue_normalized")
            )
# i <- ASV[[1]]
for(i in ASV){
  cat(paste0("Correlation between RNAseq and ",i[1], " 16S","\n"))

  # because hclust is non reproducible
  if(reprod. == TRUE){
    NESes <- read.csv2(paste0(reprod_files,i[1],"_TRX_Bact_norm_enrichment_scores",".csv"), row.names =
  }else{

    TRX <- datasets_all_samples[["Tissue_RNAseq_V3_normalized"]]
    bac_dataset <- datasets_all_samples[[i[2]]]
    samples_TRX_and_CVL3 <- colnames(counts) [ colSums(counts[c("Tissue_RNAseq_V3_normalized",i[2]),] >

    #filter TRX dataset
    TRX <- TRX [ , samples_TRX_and_CVL3 ]
    TRX <- TRX [ rowSums(TRX>0)>= 2 , ]
    # only top 5000 most variabe genes are included in the correlation
    top_vars_TRX <- names(sort(apply(TRX,1,var),decreasing = T)[1:5000] )
    TRX <- TRX[ top_vars_TRX , ]
    dim(TRX)

    #filter bac_dataset dataset
    bac_dataset <- bac_dataset [ , samples_TRX_and_CVL3 ]
    # keeping taxa that is present in at least two samples :
    bac_dataset <- bac_dataset [ rowSums(bac_dataset>0)>= 2 , ]
    top_vars_bacs <- names(sort(apply(bac_dataset,1,var),decreasing = T)[1:100] )
    dim(bac_dataset)

    cors <- cor( t(rbind(TRX) ) ,  t(rbind(bac_dataset) ) )
```

```
    bacteria_use <- colnames(cors)
    gmt <- gmtPathways(paste0(PATH, "/supplementary_files/c2.cp.kegg.v6.2.symbols.gmt.txt"))

    enrichments <- lapply(bacteria_use,gmt=gmt,cors=cors,function(x,gmt,cors){
      res <- fgsea(pathways = gmt,stats = sort(cors[,x],decreasing = T),nper=10000)
      return(res)
    })
    names(enrichments) <- bacteria_use

    pvalues <- lapply(enrichments,function(x) setNames(x$pval,x$pathway) )
    pvalues <- t(as.data.frame(pvalues))
    pvalues <- -log10( pvalues )
    top_pathways <- names(sort(apply(pvalues,1,median),T))[1:50]

    NESes <- lapply(enrichments,function(x) setNames(x$NES,x$pathway) )
    NESes <- t(as.data.frame(NESes))
    NESes[is.na(NESes)] <- 0
    # replace all NESes values with 0 if they don't have a significant p-value < 0.05:
    NESes[ pvalues < -log10(0.05) ] <- 0
    # filter out bacterium and pathways with <10 significant NES scores
    NESes <- NESes[rowSums(NESes!=0) >= 10 , colSums(NESes!=0) >= 10]

    set.seed(1)
    o_kegg <- hclust( as.dist( (1-cor(NESes))/2 ),"ward.D2")$order
    o_bacs <- hclust( as.dist( (1-cor(t(NESes) ))/2 ),"ward.D2")$order
    NESes <- NESes[o_bacs, o_kegg]

    bact <- gsub("\\.{1}", "/", rownames(NESes))
    bact <- gsub("\\/{2}", ". ", bact)
    rownames(NESes) <- bact

    #write.csv2(NESes, paste0("../../../results/",i[1],"_TRX_Bact_norm_enrichment_scores",".csv"))

  }

  terms <- gsub("_", " ", colnames(NESes))
  terms <- gsub("(GO |KEGG )", "", terms, perl = TRUE)
  colnames(NESes) <- terms

  ### Suppl. Figure 3-4
  ########################################
  # FUNCTIONAL ASSOCIATION BACT AND TRX #
  ########################################
  par(mfrow=c(1,1),mar=c(12,0,2,4)) #b,l,t,r
  image( t(NESes[nrow(NESes):1,]),col=colorRampPalette(c("navy","grey95","firebrick"))(91),breaks=seq(-3
         axes=F,border=NA,main="tissue RNAseq KEGG pathways",xlab="",ylab=i,line=.4,cex.main=1,font.mai
  text(  par("usr")[c(4)] , seq(1,0,length.out = nrow(NESes)),
        labels = rownames(NESes), srt = 0, adj = c(0,.5), xpd = TRUE, cex=.4)
  text( seq(0,1,length.out = ncol(NESes)) , par("usr")[c(1)],
        labels = colnames(NESes), srt = 90, adj = c(1,.5), xpd = TRUE, cex=.4)

}
```

Correlation between RNAseq and Luminal 16S



Correlation between RNAseq and Tissue 16S



Suppl. Figure 3-4. Heatmaps showing the functional association of microbiome datasets with the expression profiles in the RNAseq dataset. Briefly, bacterial abundances from each dataset were correlated with the gene expression of the top 5000 highly variable genes from the RNAseq dataset, generating a correlation matrix between bacteria and genes. Then for each bacteria, we rank genes based on their correlation to that bacteria and perform gene set enrichment anlaysis (GSEA) using the KEGG gene annotation database. This, in turn, will result in a matrix associating every bacteria with every KEGG process in the tissue. The heatmap shows the normalized enrichment score (NES). Only enrichments with pvalue below 0.05 are shown.Only bacterium and pathways with at least 10 significant NES scores were included in the heatmap.