

SPEAQeasy Differential Expression Analysis

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1 Analysis

The following analysis explores a `RangedSummarizedExperiment` object from the SPEAQeasy pipeline. Note that we will use a modified version of the object, which resolved sample identity issues which were present in the raw output from SPEAQeasy. This object also includes phenotype data added after resolving identity issues. Though SPEAQeasy produces objects for several feature types (genes, exons, exon-exon junctions), we will demonstrate an example analysis for just genes. We will perform differential expression across some typical variables of interest (e.g. sex, age, race) and show how to perform principal component analysis (PCA) and visualize findings with plots.

1.1 Load required libraries

```
library("SummarizedExperiment")
library("recount")
library("edgeR")
library("limma")
library("jaffelab") # GitHub: LieberInstitute/jaffelab
library("RColorBrewer")
library("clusterProfiler")
library("org.Hs.eg.db")
library("pheatmap")
library("here")
```

1.2 Load data and prepare directories to place outputs in

For those who ran SPEAQeasy from the example FASTQ data set, the `RangedSummarizedExperiment` will have a different path, as specified with the `--output` flag. Set the working directory according to where the `SPEAQeasy-example` repository is cloned.

```
# Load the RSE gene object
load(here("rse_speaqeasy.RData"), verbose = TRUE)
```

```
## Loading objects:
##   rse_gene
```

```
# Create directories to organize outputs from this analysis
dir.create(here("DE_analysis", "pdfs"), showWarnings = FALSE)
dir.create(here("DE_analysis", "tables"), showWarnings = FALSE)
dir.create(here("DE_analysis", "rdas"), showWarnings = FALSE)
```

1.3 cell PCs

```
col_names = c('trimmed', 'numReads', 'numMapped', 'numUnmapped', 'overallMapRate', 'concordMapRate', 'totalMapped', 'mitoMapped', 'mitoRate', 'totalAssignedGene')
cellPca = prcomp(as.data.frame(colData(rse_gene)))
rse_genePC = cellPca[,1]
getPcaVars(cellPca)[1] # 87.3
round(cellPca$rot[,1],3) # fetal quiescent and adult neuron increase
```

1.4 filter for expressed

```
rse_gene = rse_gene[rowMeans(getRPKM(rse_gene,"Length")) > 0.2,]
```

1.5 metrics

1.6 check if ratios of cell changed by batch

```
pdf(file = here("DE_analysis", "pdfs", "Region_Race_cellcheck.pdf"))
boxplot(rse_gene$RNA_rate ~ rse_gene$BrainRegion, xlab="")
boxplot(rse_gene$mitoRate ~ rse_gene$BrainRegion, xlab="")
boxplot(rse_gene$gene_assigned ~ rse_gene$BrainRegion, xlab="")
boxplot(rse_gene$mitoRate ~ rse_gene$Race, las=3, xlab="")
boxplot(rse_gene$gene_assigned ~ rse_gene$Race, las=3, xlab="")
dev.off()
```

```

1.6.0.1 explore human geneExprs = log2(getRPKM(rse_gene,"Length")+1) pca = prcomp(t(geneExprs))
pca_vars = getPcaVars(pca) pca_vars_lab = paste0("PC", seq(along=pca_vars), ":", pca_vars, "% Var Expl")

pdf(here("DE_analysis", "pdfs", "PCA_plotsExprs.pdf"), w=9) par(mar=c(8,6,2,2),cex.axis=1.8,cex.lab=1.8)
palette(brewer.pal(4,"Dark2"))

```

1.7 pc1 vs pc2

```

plot(pca$x, pch = 21, bg = factor(rse_gene$PrimaryDx), cex=1.2, xlab = pca_vars_lab[1], ylab =
pca_vars_lab[2]) legend("bottomleft", levels(rse_gene$PrimaryDx), col=1:2, pch=15, cex=2)

```

1.8 by line

```

for(i in 1:10) { boxplot(pca$x[,i] rse_gene$Sex, ylab=pca_vars_lab[i], las = 3, xlab="Sex", outline=FALSE)
points( pca$x[,i] jitter(as.numeric(factor(rse_gene$Sex))), pch = 21, bg = rse_gene$PrimaryDx, cex=1.2) }

```

1.9 by experiment

```

for(i in 1:10) { boxplot(pca$x[,i] rse_gene$Race, ylab=pca_vars_lab[i], las = 3, xlab="Race", outline=FALSE)
points( pca$x[,i] jitter(as.numeric(factor(rse_gene$Race))), pch = 21, bg = rse_gene$PrimaryDx, cex=1.2)
} dev.off()

```

1.10 modeling

```

dge = DGEList(counts = assays(rse_gene)$counts, genes = rowData(rse_gene)) dge = calcNormFactors(dge)

```

1.11 mean-variance

```

mod = model.matrix(~PrimaryDx + PC + BrainRegion, data=colData(rse_gene)) pdf(file = "DE_analysis/pdfs/vGene.pdf")
vGene = voom(dge, mod, plot=TRUE) dev.off()

gene_dupCorr = duplicateCorrelation(vGene, mod, block = colData(rse_gene)$SAMPLE_ID) save(gene_dupCorr,
file = "DE_analysis/rdas/gene_dupCorr_neurons.rda")

fitGeneDupl = lmFit(vGene, correlation=gene_dupCorr$consensus.correlation, block = colData(rse_gene)$SAMPLEID)
ebGeneDupl = eBayes(fitGeneDupl) outGeneDupl = topTable(ebGeneDupl, coef=2, p.value = 1, number=nrow(rse_gene), sort="none")

pdf(file = here("DE_analysis", "pdfs", "hist_pval.pdf")) hist(outGeneDupl$P.Value) dev.off() table(outGeneDupl$adj.P.Val < 0.05)
table(outGeneDupl$adj.P.Val < 0.1)

sigGeneDupl = topTable(ebGeneDupl, coef=2, p.value = 0.1, number=nrow(rse_gene))

sigGeneDupl[,c("Symbol", "logFC", "P.Value", "AveExpr")] sigGeneDupl[sigGeneDupl$logFC > 0, c("Symbol", "logFC", "P.Value")]
sigGeneDupl[sigGeneDupl$logFC < 0, c("Symbol", "logFC", "P.Value")]

write.csv(outGeneDupl, file = here("DE_analysis", "tables", "de_stats_allExprs.csv")) write.csv(sigGeneDupl,
file = here("DE_analysis", "tables", "de_stats_fdr10_sorted.csv"))

```

1.12 check plots

```
exprs = vGene$E[rownames(sigGeneDupl),] #exprsClean = cleaningY(exprs, mod, 2)
```

1.12.1 make boxplots

2 cleanGeneExprs = cleaningY(geneExprs, mod[,!is.na(eBGene\$p.value[1,])] P=3)

```
pdf(here("DE_analysis", "pdfs", "DE_boxplots_byDiagnosis.pdf", w=10)) par(mar=c(8,6,4,2),cex.axis=1.8,cex.lab=1.8,  
cex.main=1.8) palette(brewer.pal(4,"Dark2")) for(i in 1:nrow(sigGeneDupl)) { yy = exprs[i,] boxplot(yy ~  
rse_genePrimaryDx, outline = FALSE, ylim = range(yy), ylab = "Normalizedlog2Exprs", xlab =  
"", main = paste(sigGeneDuplSymbol[i], "-", sigGeneDuplencodeID[i])) points(yy jitter(as.numeric(rse_genePrimaryDx)),  
pch = 21, bg = rse_genePrimaryDx, cex = 1.3) ll = ifelse(sigGeneDupllogFC[i] > 0, "topleft", "topright")  
legend(ll, paste0("p=", signif(sigGeneDupl$P.Value[i],3)), cex=1.3) } dev.off()
```

2.0.0.1 RPKM e = geneExprs[rownames(sigGeneDupl),]

```
pdf(here("DE_analysis", "pdfs", "DE_boxplots_byGenome_log2RPKM.pdf", w=10)) par(mar=c(8,6,4,2),cex.axis=1.8,cex.lab=1.8,  
cex.main=1.8) palette(brewer.pal(4,"Dark2")) for(i in 1:nrow(sigGeneDupl)) { yy = e[i,] boxplot(yy ~  
rse_genePrimaryDx, las = 3, outline = FALSE, ylim = range(yy), ylab = "log2(RPKM + 1)", xlab =  
"", main = paste(sigGeneDuplSymbol[i], "-", sigGeneDuplencodeID[i])) points(yy jitter(as.numeric(rse_genePrimaryDx)),  
pch = 21, bg = rse_genePrimaryDx, cex = 1.3) ll = ifelse(sigGeneDupllogFC[i] > 0, "topleft", "topright")  
legend(ll, paste0("p=", signif(sigGeneDupl$P.Value[i],3)), cex=1.3) } dev.off()
```

no rat astrocyte differences

2.0.1 gene ontology

2.1 get significant genes by sign

```
sigGene = outGeneDupl[outGeneDupl$P.Value < 0.005,] sigGeneList = split(as.character(sigGeneEntrezID), sign(sigGeneList))  
sigGeneList = lapply(sigGeneList, function(x) x[!is.na(x)]) geneUniverse = as.character(outGeneDupl$EntrezID)  
geneUniverse = geneUniverse[!is.na(geneUniverse)]
```

2.2 do GO and KEGG

```
goBP_Adj <- compareCluster(sigGeneList, fun = "enrichGO", universe = geneUniverse, OrgDb =  
org.Hs.eg.db, ont = "BP", pAdjustMethod = "BH", pvalueCutoff = 1, qvalueCutoff = 1, readable = TRUE)
```

```
goMF_Adj <- compareCluster(sigGeneList, fun = "enrichGO", universe = geneUniverse, OrgDb =  
org.Hs.eg.db, ont = "MF", pAdjustMethod = "BH", pvalueCutoff = 1, qvalueCutoff = 1, readable = TRUE)
```

```
goCC_Adj <- compareCluster(sigGeneList, fun = "enrichGO", universe = geneUniverse, OrgDb =  
org.Hs.eg.db, ont = "CC", pAdjustMethod = "BH", pvalueCutoff = 1, qvalueCutoff = 1, readable = TRUE)
```

```
kegg_Adj <- compareCluster(sigGeneList, fun = "enrichKEGG", universe = geneUniverse, pAdjustMethod =  
"BH", pvalueCutoff = 1, qvalueCutoff = 1)
```

```
save(goBP_Adj, goCC_Adj, goMF_Adj, kegg_Adj, file = here("DE_analysis", "rdas", "gene_set_objects_p005.rda"))
```

```
goList = list(BP = goBP_Adj, MF = goMF_Adj, CC = goCC_Adj, KEGG = kegg_Adj) goDf =  
dplyr::bind_rows(lapply(goList, as.data.frame), .id = "Ontology") goDf = goDf[order(goDf$pvalue),]
```

```

write.csv(goDf, file = here("DE_analysis", "tables", "geneSet_output.csv"), row.names=FALSE)
options(width=130) goDf[goDf$p.adjust < 0.05, c(1:5,7)]
#make heatmap of differentially expressed genes# #####
exprs_heatmap = vGene$E[rownames(sigGene),]
df <- as.data.frame(colData(rse_gene)[c("PrimaryDx")]) rownames(df) <- colnames(exprs_heatmap)
colnames(df)<-"diagnosis"
pdf(file=here("DE_analysis", "pdfs", "de_heatmap.pdf")) pheatmap(exprs_heatmap, cluster_rows=TRUE,
show_rownames=FALSE, cluster_cols=TRUE, annotation_col=df) dev.off()

```