SPEAQeasy Differential Expression Analysis

July 10, 2020

Contents

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")
library("devtools")
library("BiocStyle")
```

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.

```
# 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 statistics PCs

Here we are using principal component analysis to control for the listed variables impact on expression. This will be later added into our linear model.

```
col_names = c('trimmed', 'numReads', 'numMapped', 'numUnmapped',
              'overallMapRate', 'concordMapRate', 'totalMapped', 'mitoMapped',
              'mitoRate', 'totalAssignedGene')
statsPca = prcomp(as.data.frame(colData(rse_gene)[,col_names]))
rse_gene$PC = statsPca$x[,1]
getPcaVars(statsPca)[1]
```

```
## [1] 87.3
```

1.4 Stats vs. race and brain region

```
# Filter for expressed
rse_gene = rse_gene[rowMeans(getRPKM(rse_gene,"Length")) > 0.2,]

# Check if certain statistics changed by race or region

# Display box plots here
boxplot(rse_gene$rRNA_rate ~ rse_gene$BrainRegion,xlab="")
```
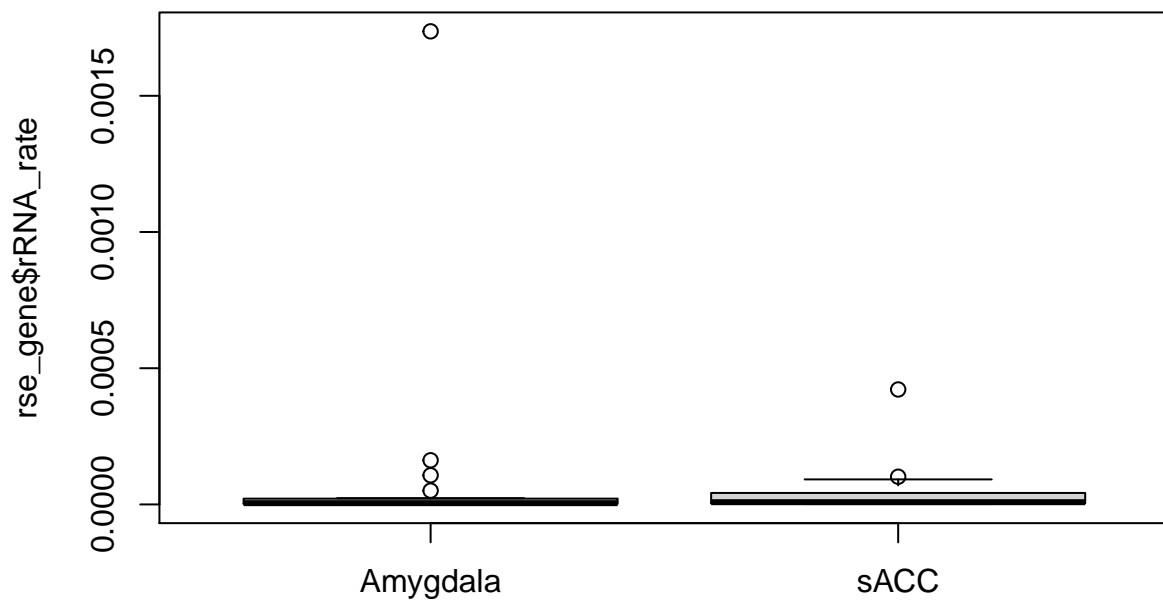
```r
boxplot(rse_gene$mitoRate ~ rse_gene$BrainRegion,xlab="")
```

```
boxplot(rse_gene$gene_Assigned ~ rse_gene$BrainRegion,xlab="")
```

```r
boxplot(rse_gene$mitoRate ~ rse_gene$Race,las=3,xlab="")
```

```r
boxplot(rse_gene$gene_Assigned ~ rse_gene$Race,las=3,xlab="")
```
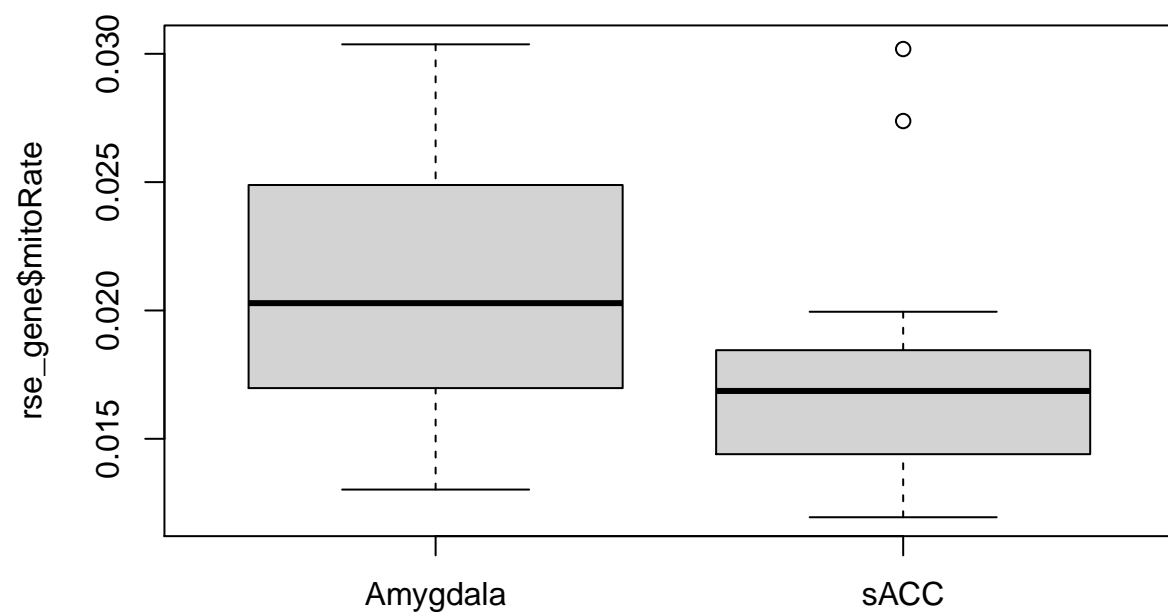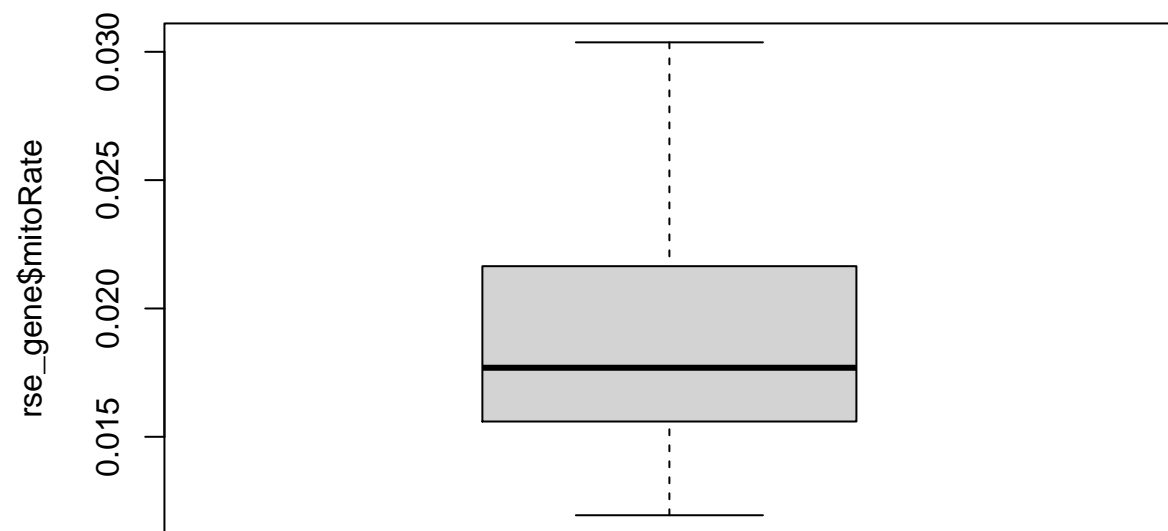
```r
# Save box plots to PDF
pdf(file = here("DE_analysis", "pdfs", "Region_Race_cellcheck.pdf"))
boxplot(rse_gene$rRNA_rate ~ rse_gene$BrainRegion,xlab="")
boxplot(rse_gene$mitoRate ~ rse_gene$BrainRegion,xlab="")
boxplot(rse_gene$gene_Assigned ~ rse_gene$BrainRegion,xlab="")
boxplot(rse_gene$mitoRate ~ rse_gene$Race,las=3,xlab="")
boxplot(rse_gene$gene_Assigned ~ rse_gene$Race,las=3,xlab="")
dev.off()
```
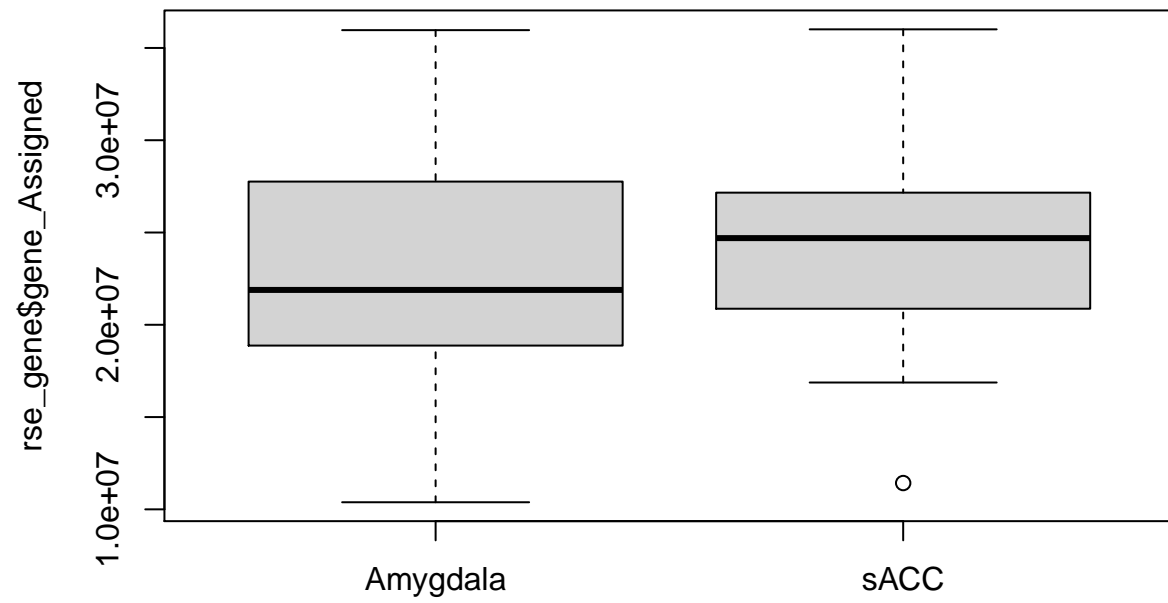
```
## pdf
##   2
```

1.5 Explore and visualize gene expression

```r
# Explore gene expression
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")

# Group together code for generating plots of interest
generate_plots = function() {
```

```r
    par(mar=c(8,6,2,2),cex.axis=1.8,cex.lab=1.8)
    palette(brewer.pal(4,"Dark2"))

    # 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)


    # 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)
    }

    # 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)
    }
}

# Display plots
generate_plots()
```

Race
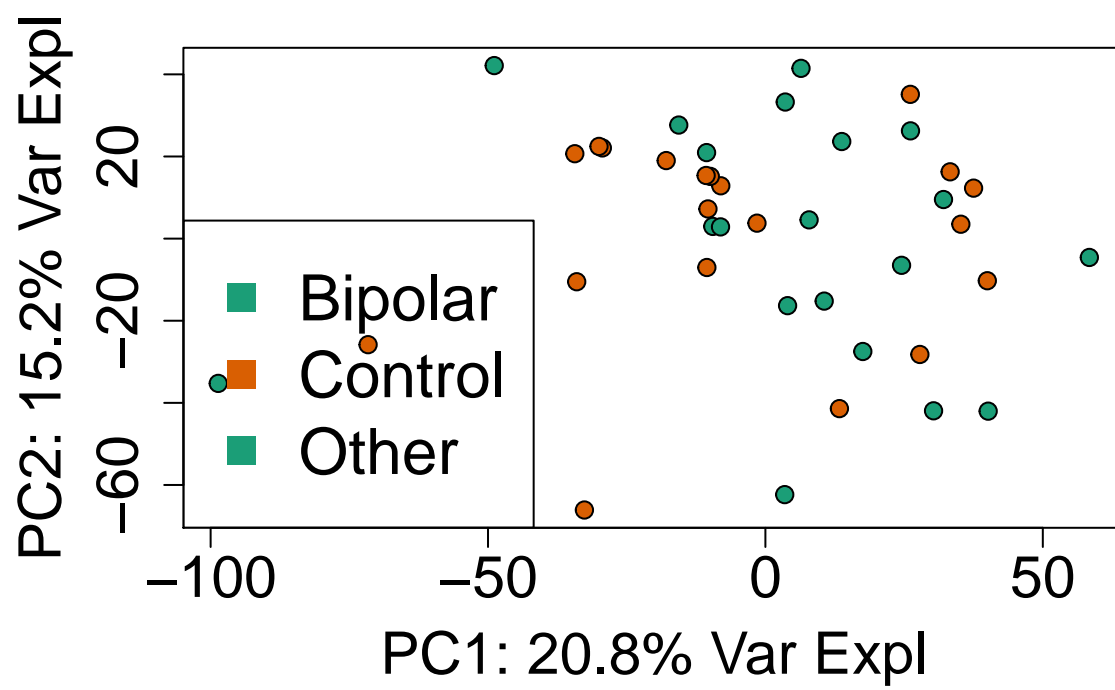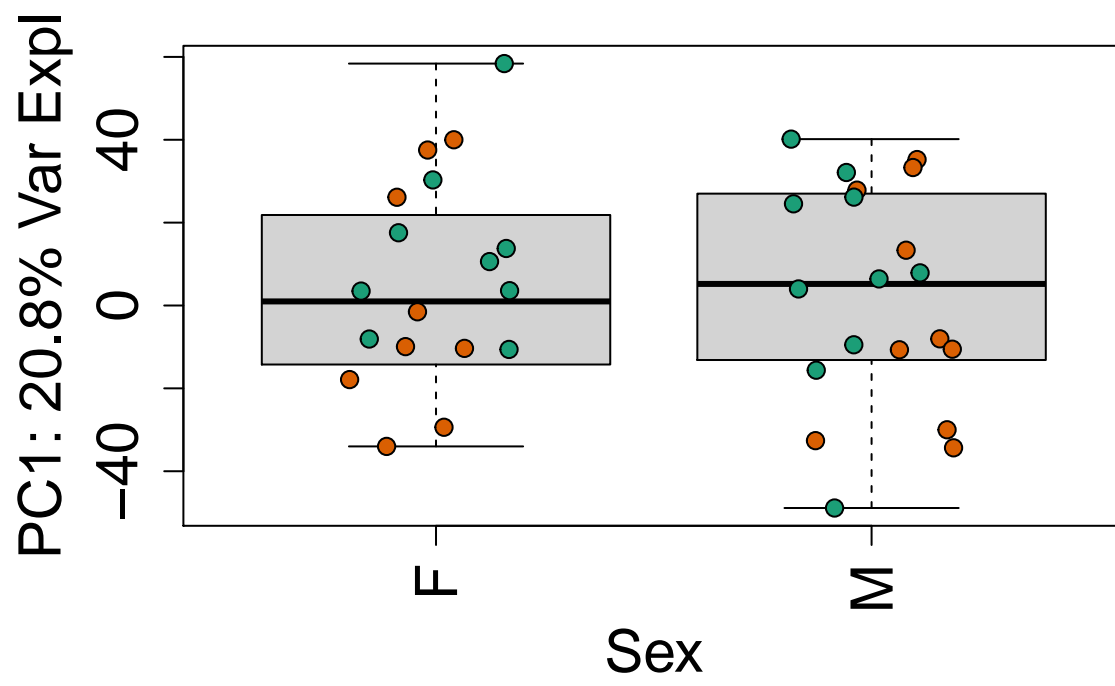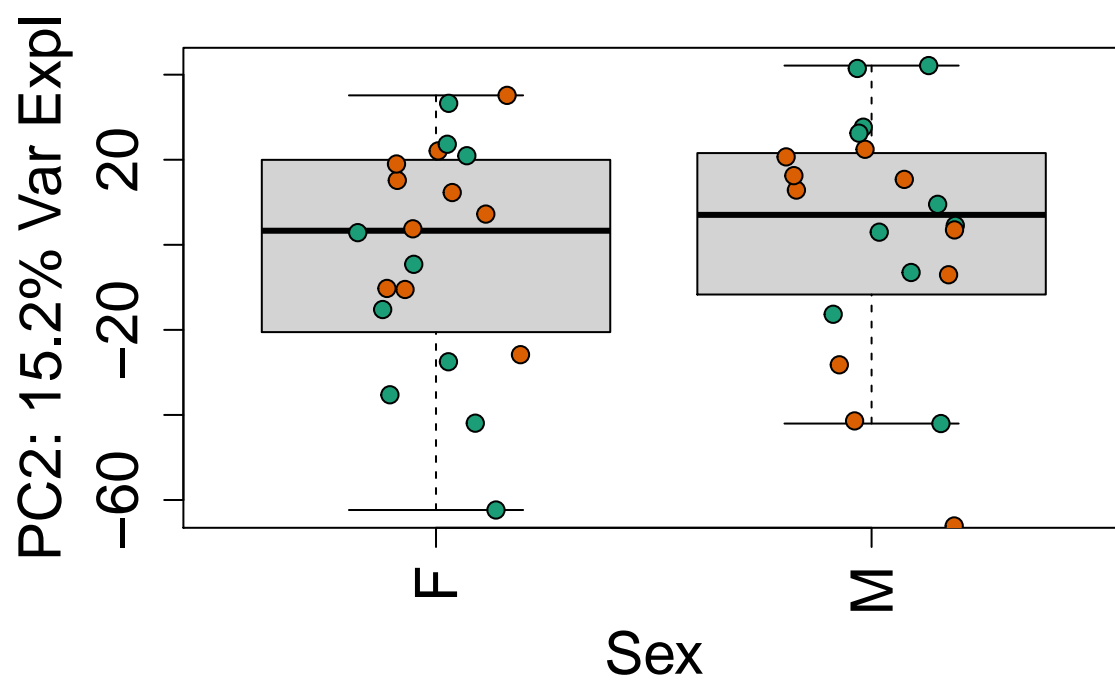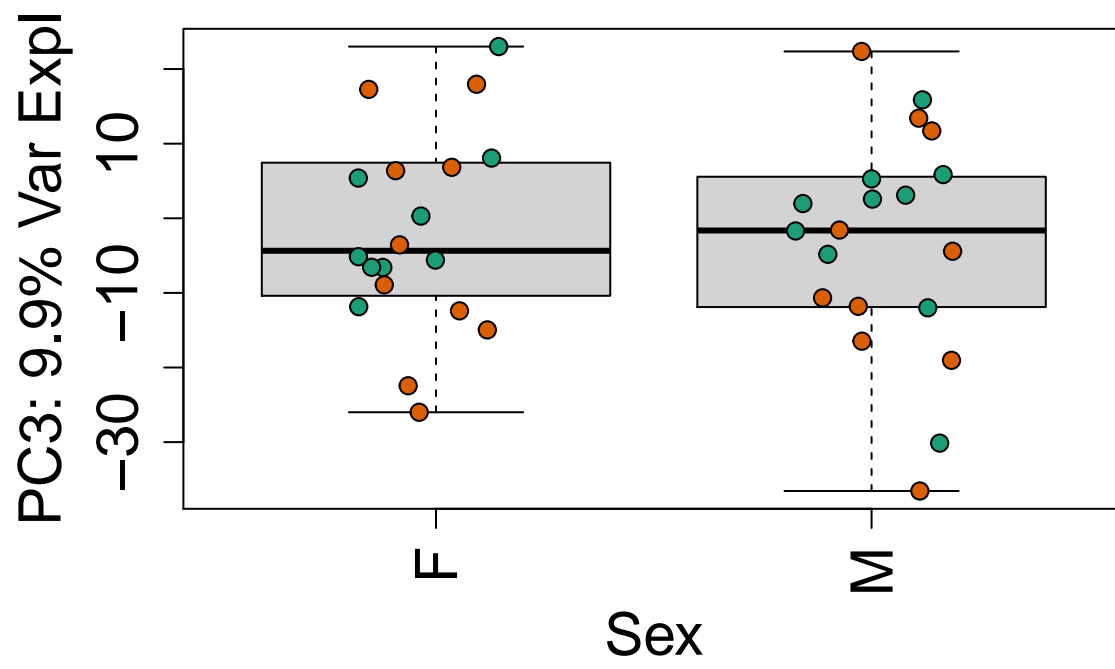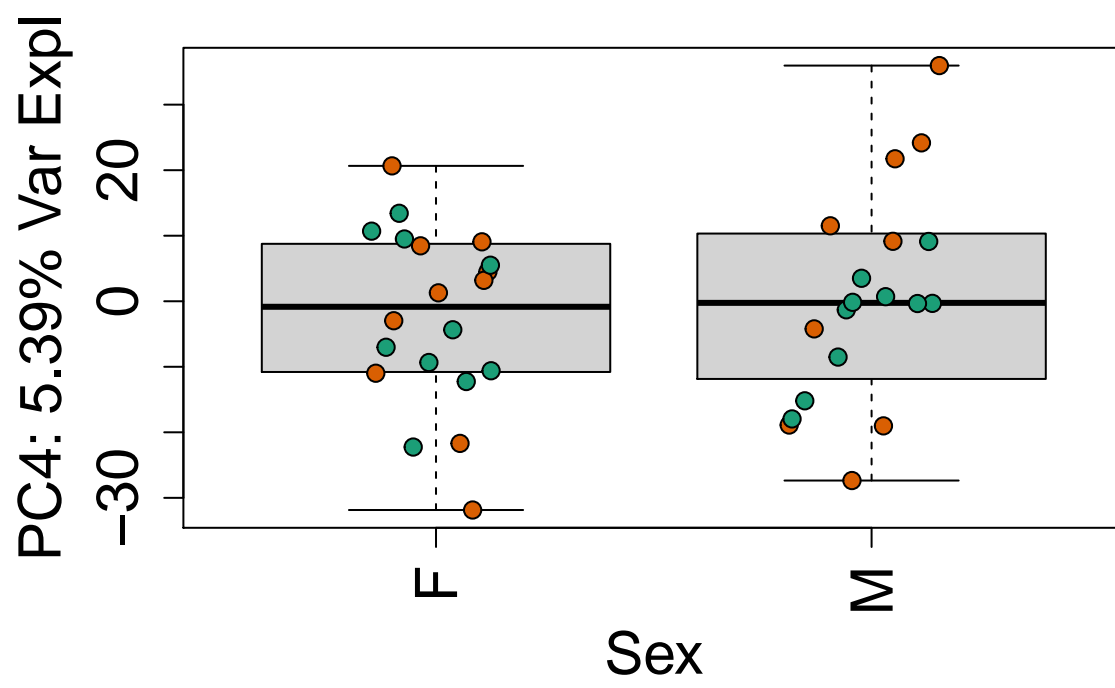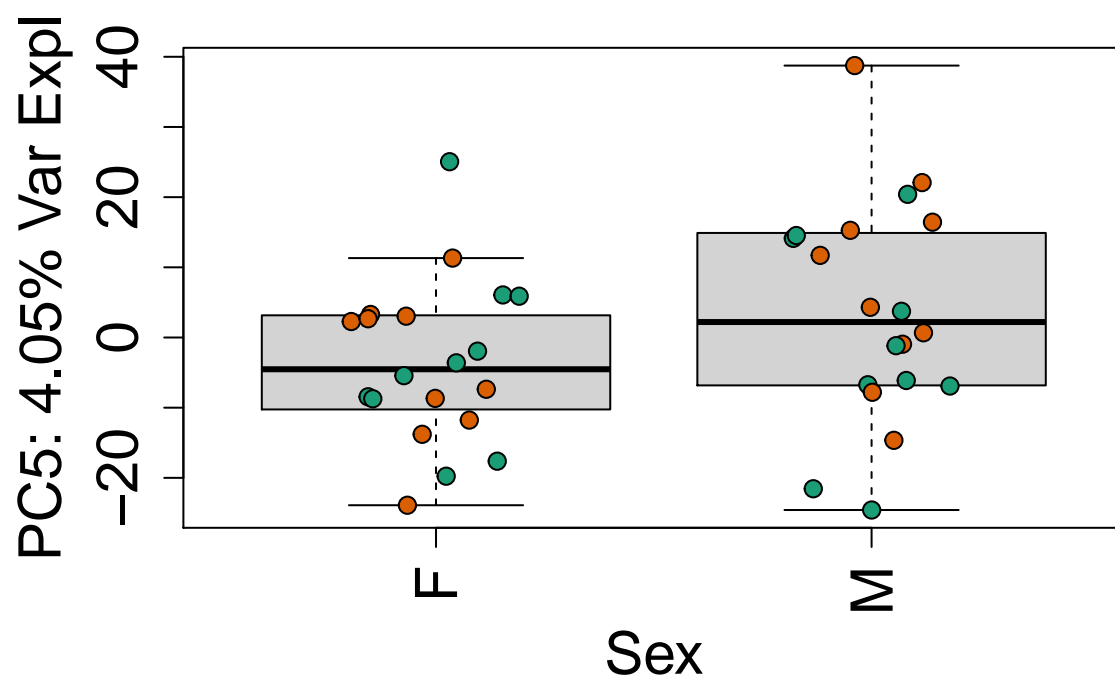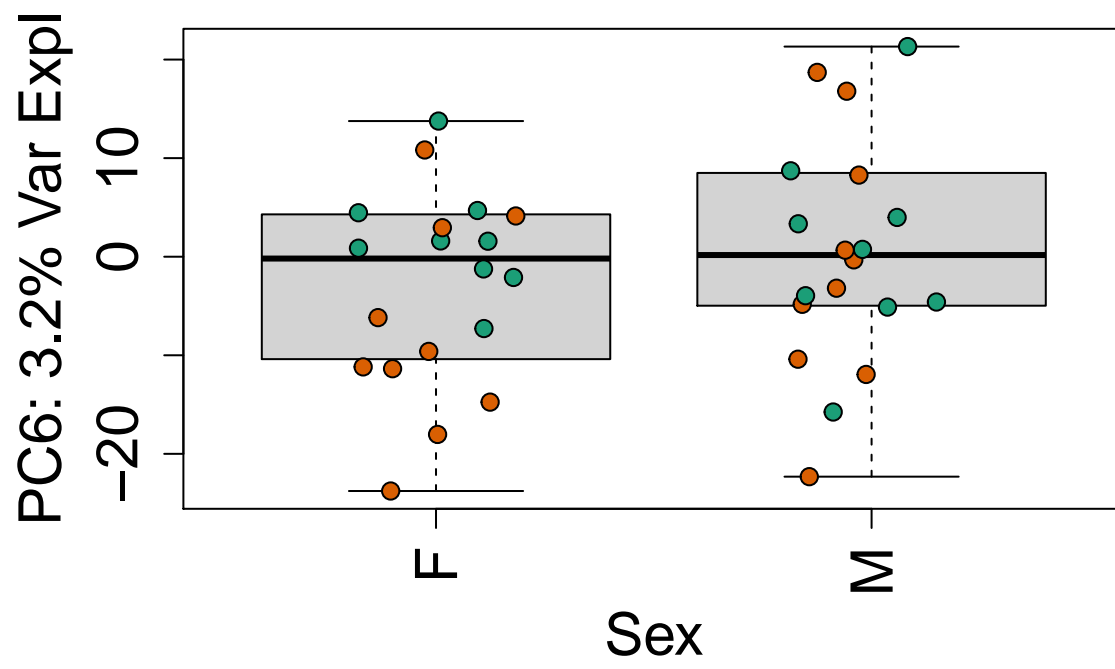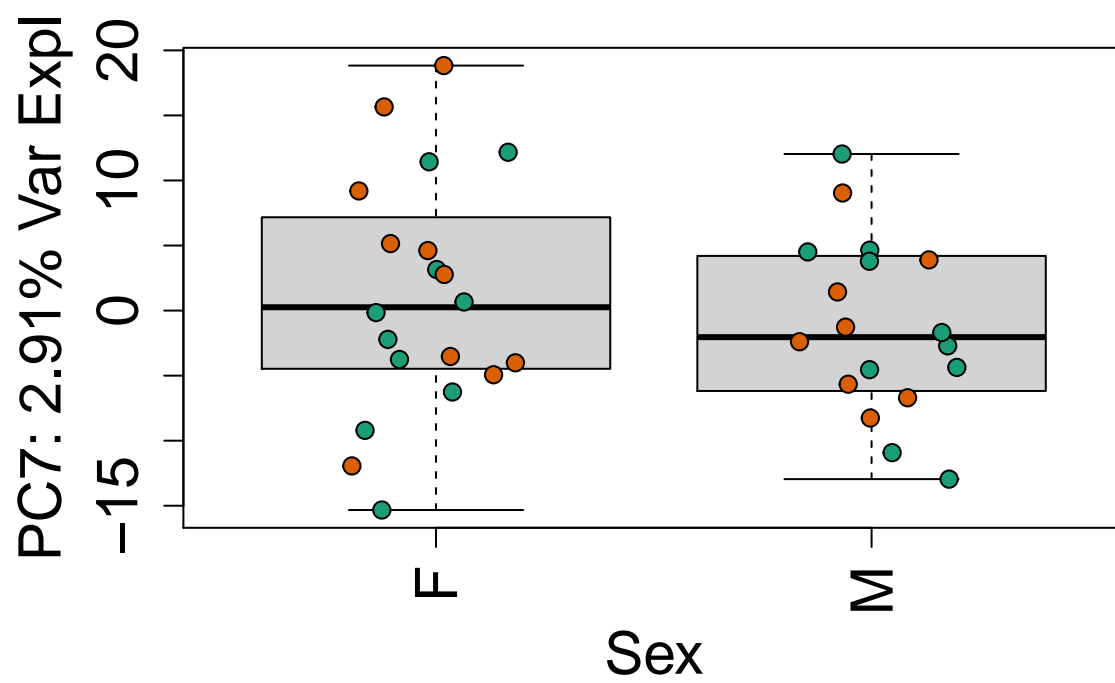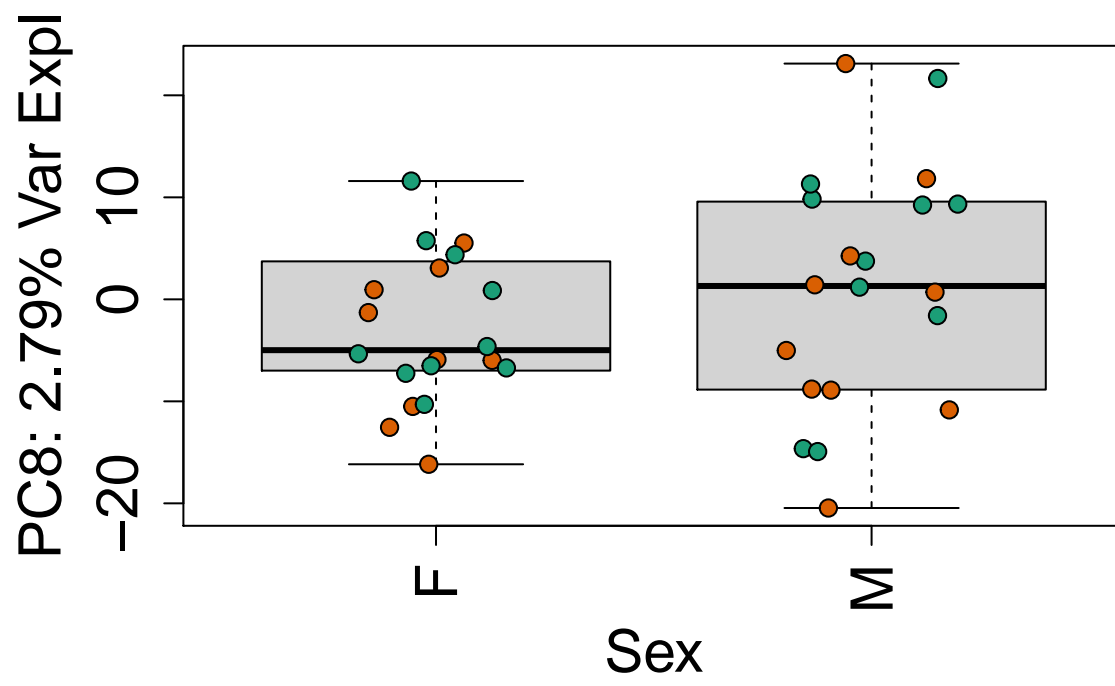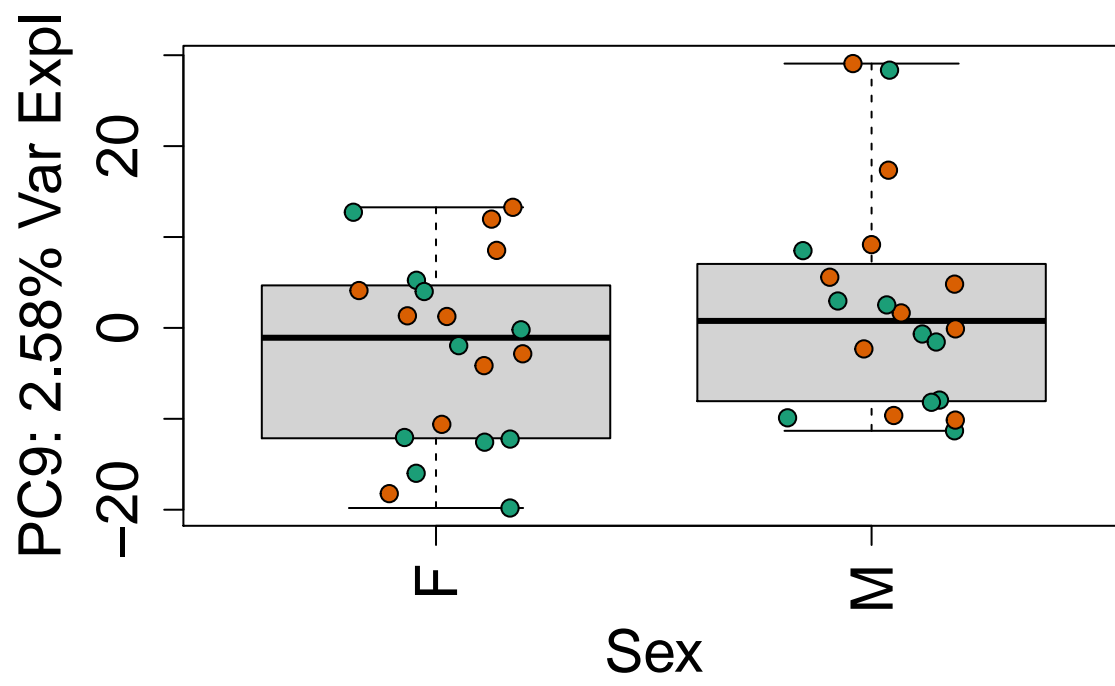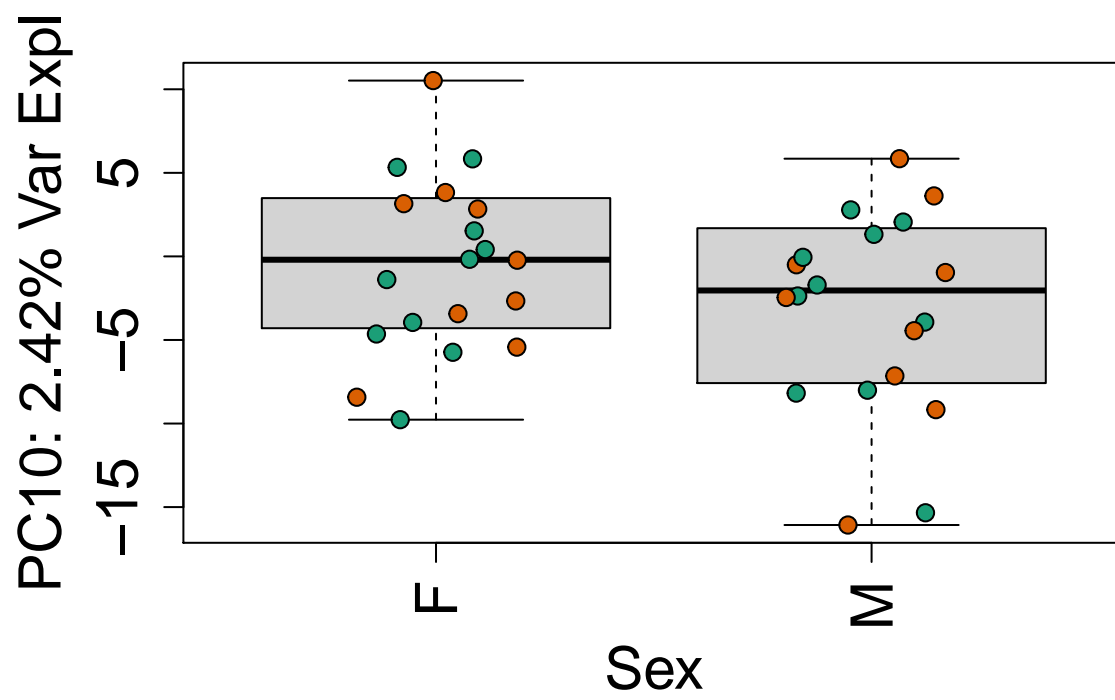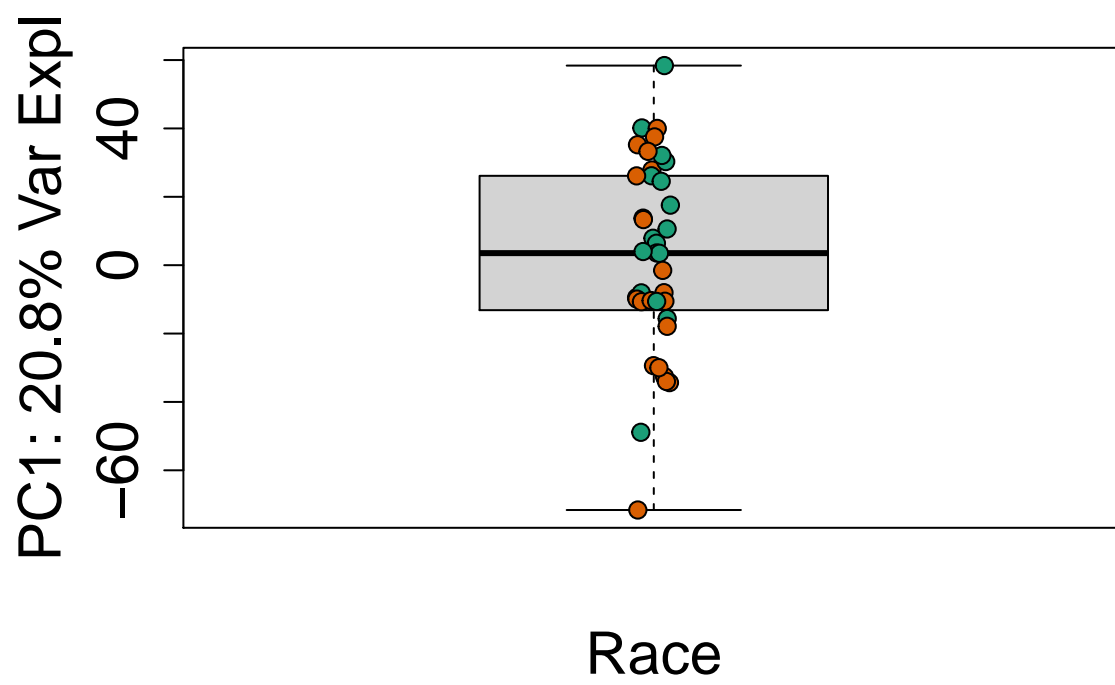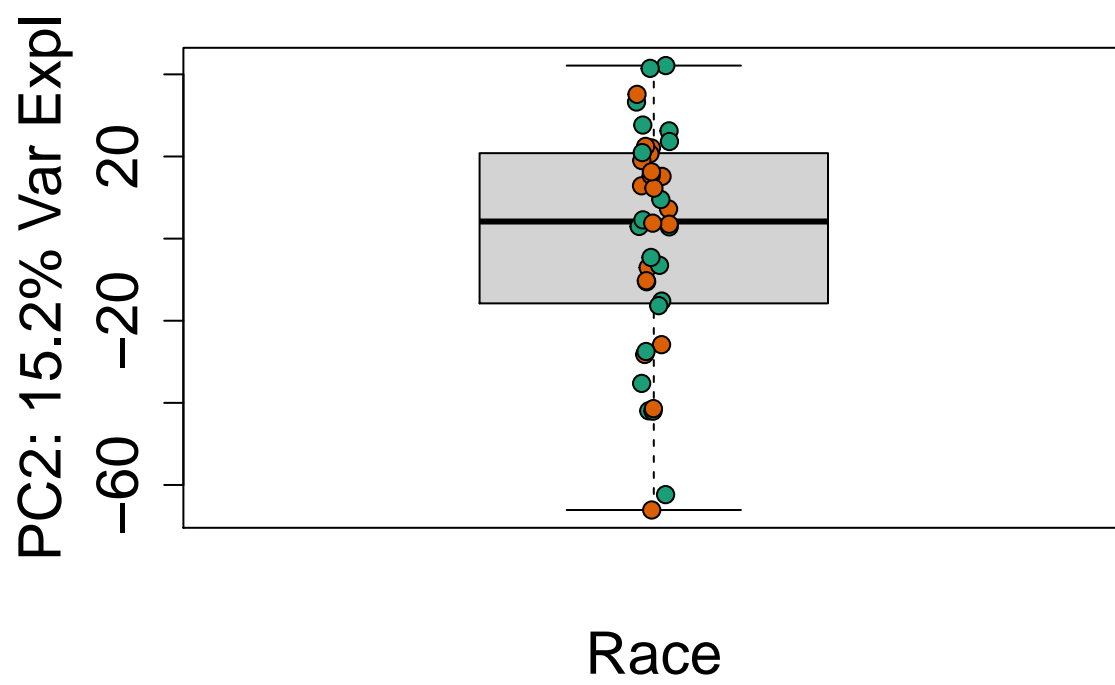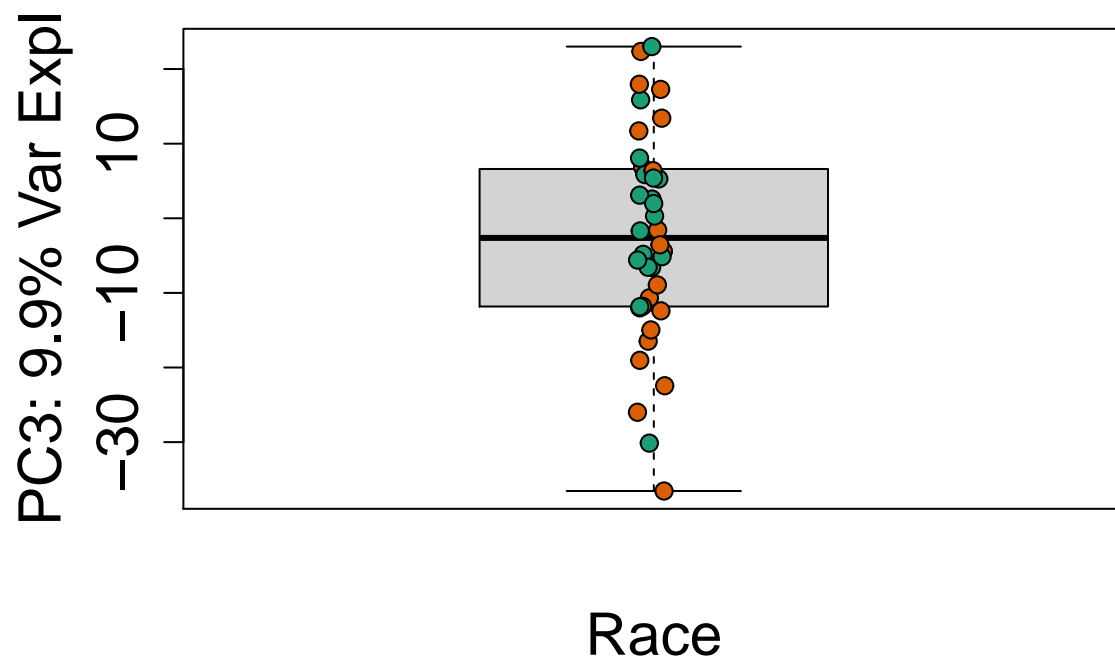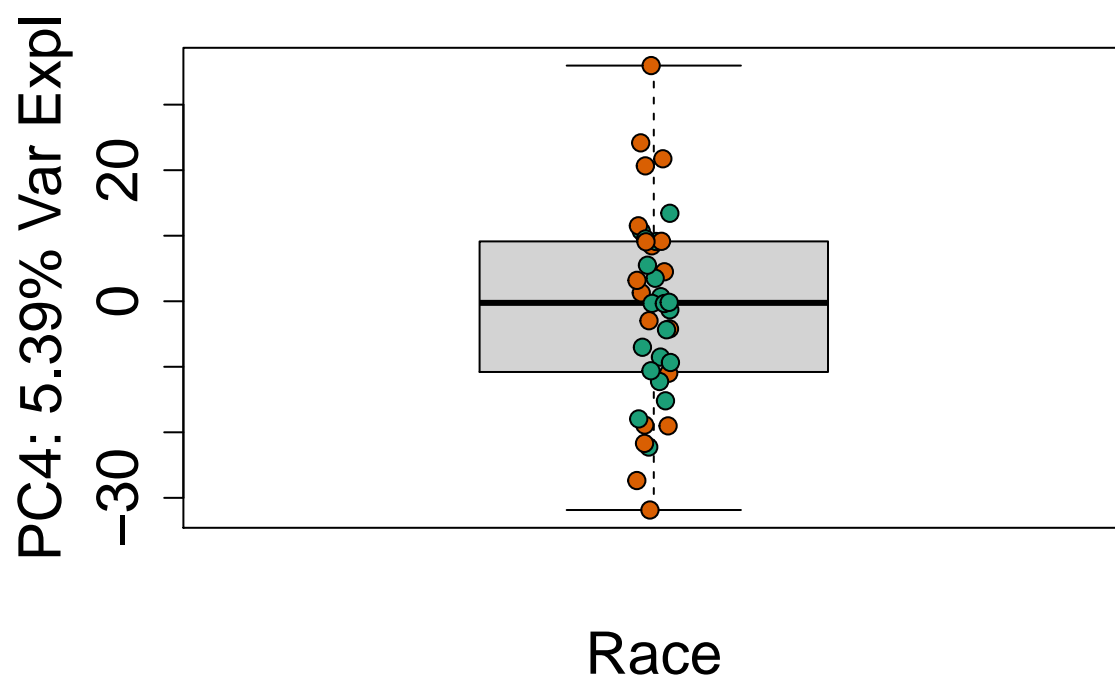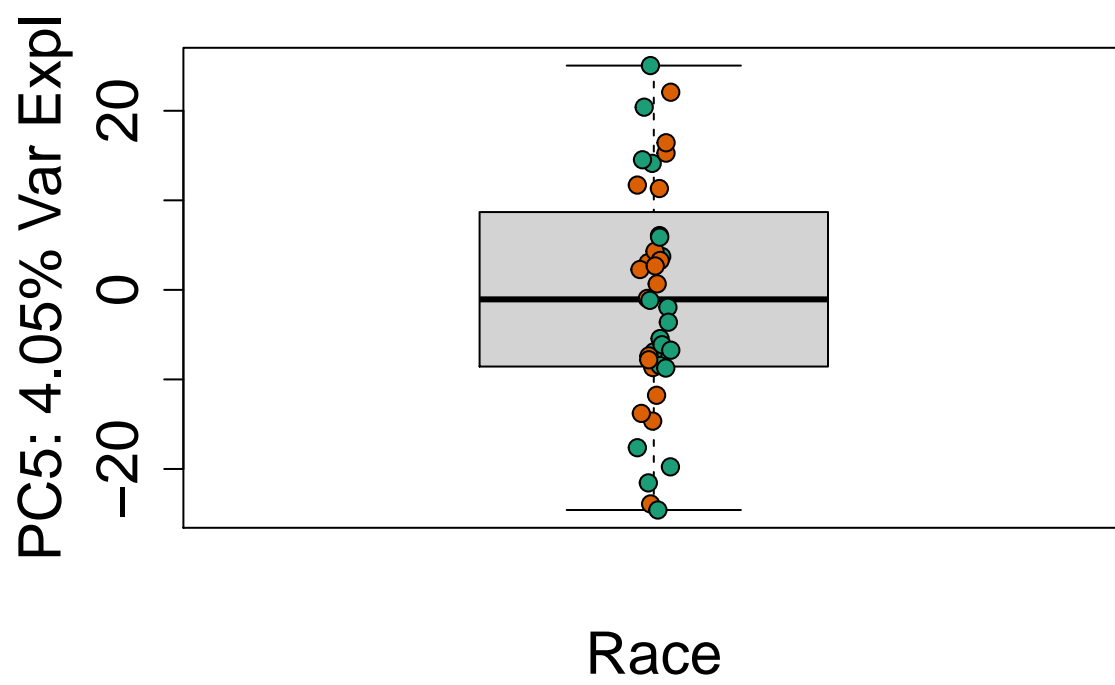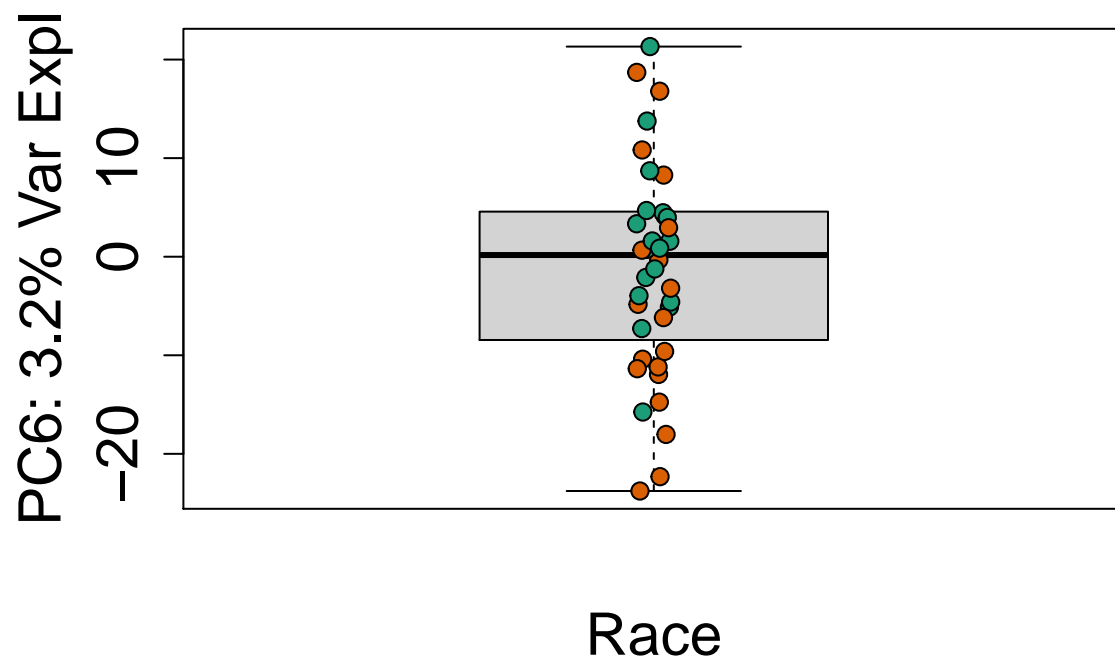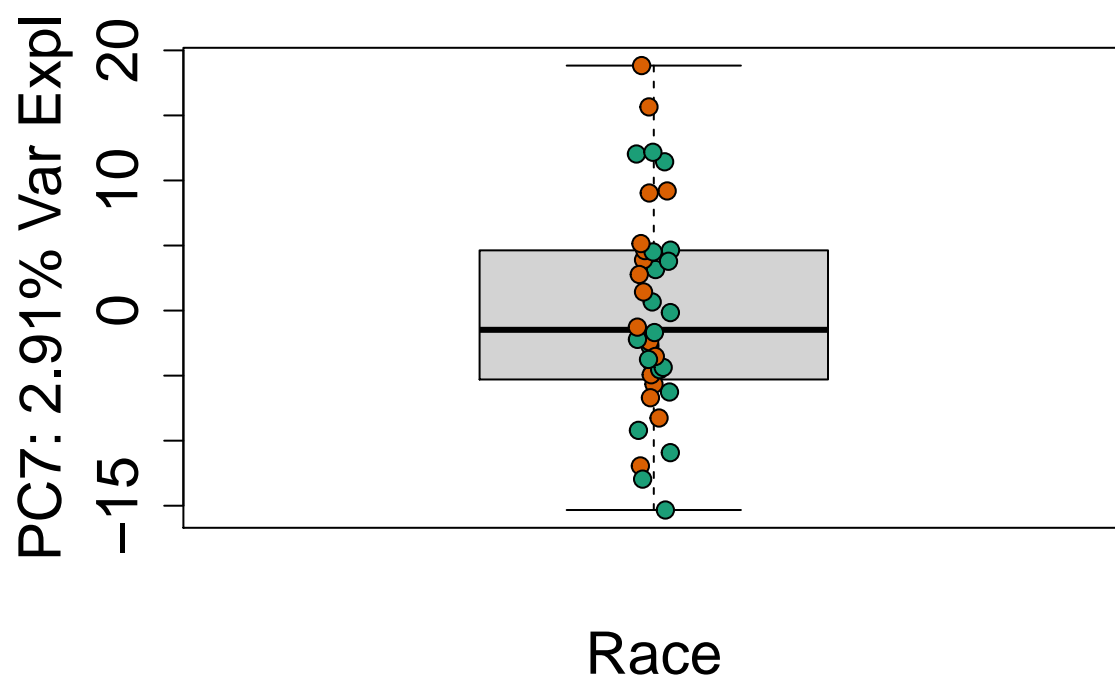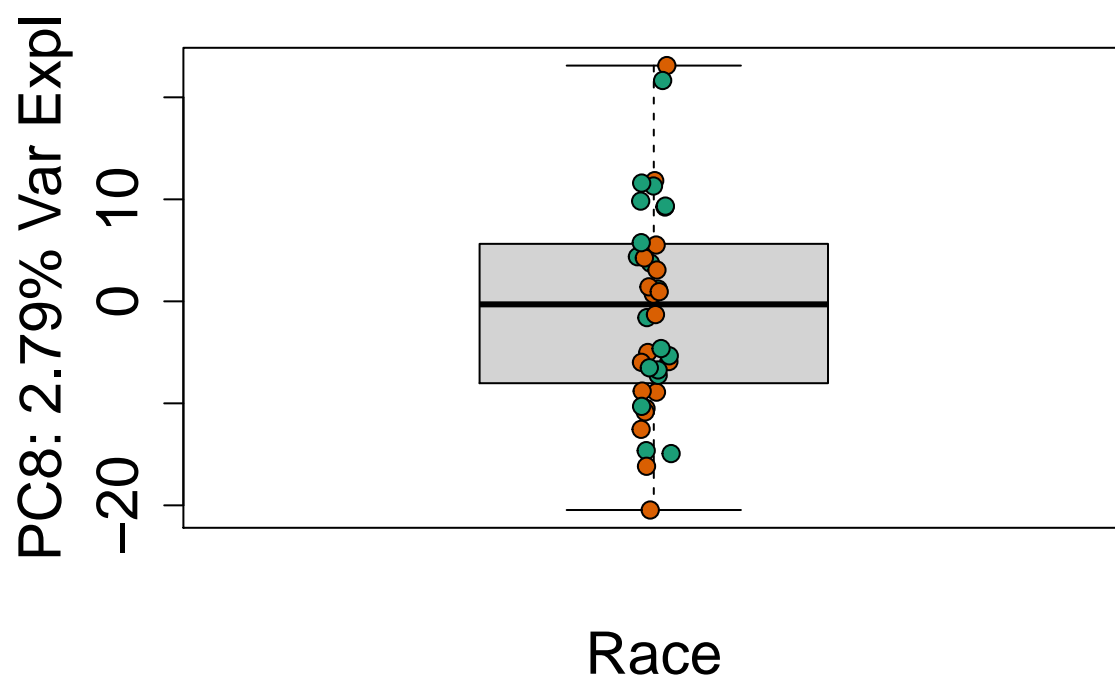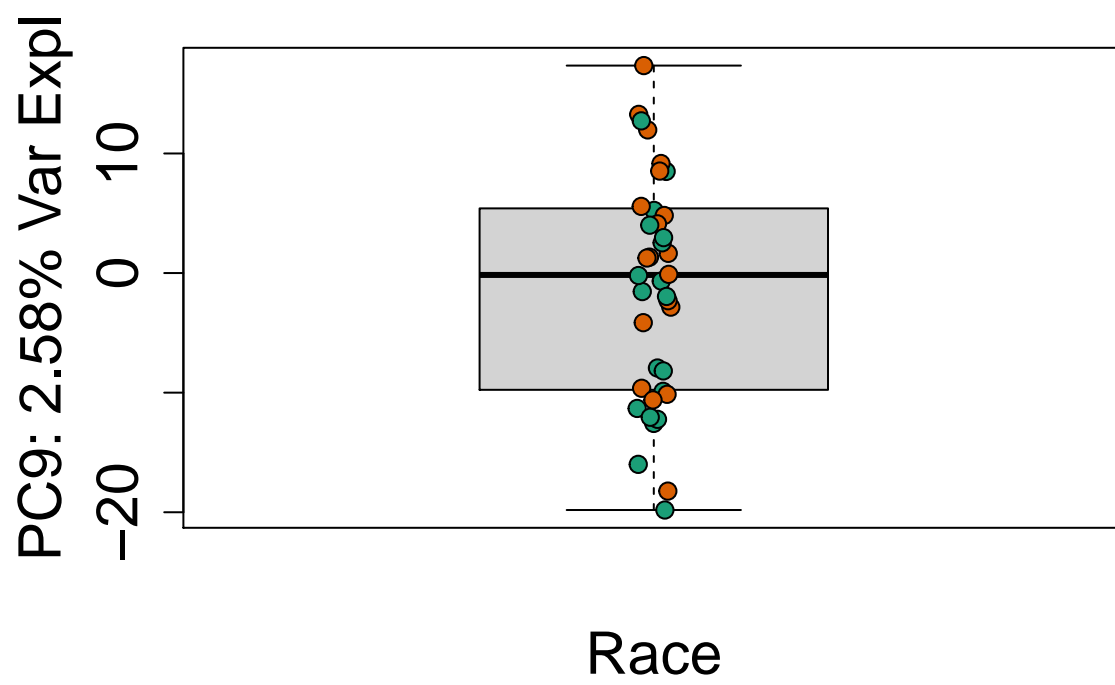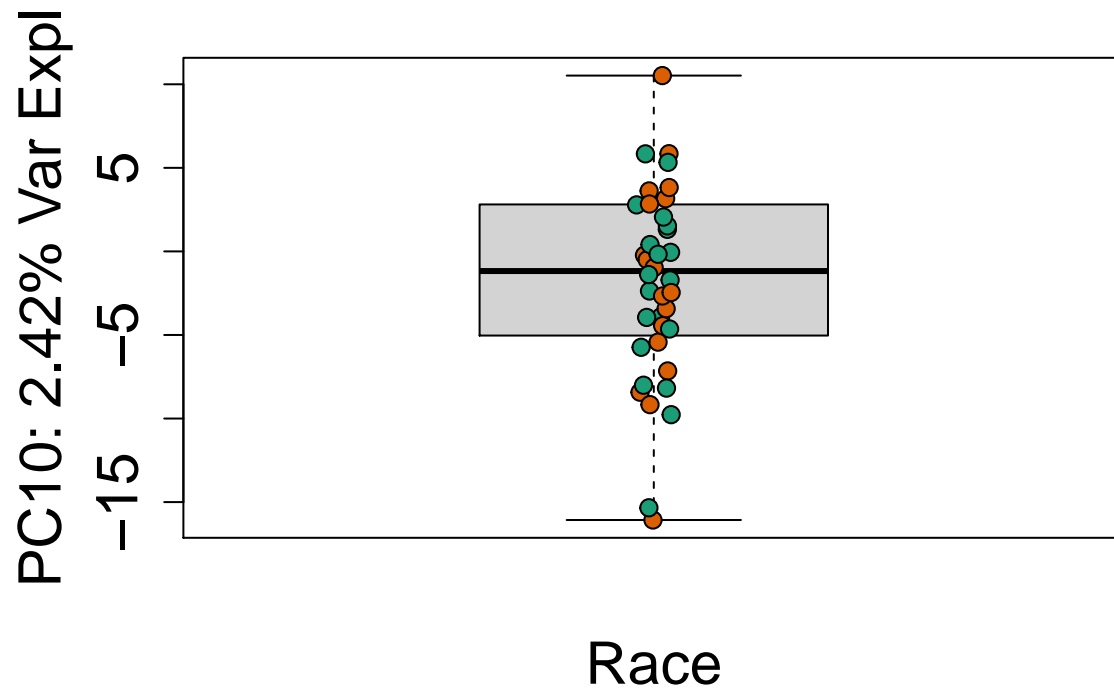
```r
# Write plots to PDF
pdf(here("DE_analysis", "pdfs", "PCA_plotsExprs.pdf"), w=9)
generate_plots()
dev.off()
```

```
## pdf
##   2
```

1.6 Modeling

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

# Mean-variance
mod = model.matrix(~PrimaryDx + PC + BrainRegion,
    data=colData(rse_gene))

vGene = invisible(voom(dge,mod,plot=TRUE))
```

```
## Coefficients not estimable: PrimaryDxOther
```

```
## Warning: Partial NA coefficients for 26708 probe(s)
```

voom: Mean–variance trend
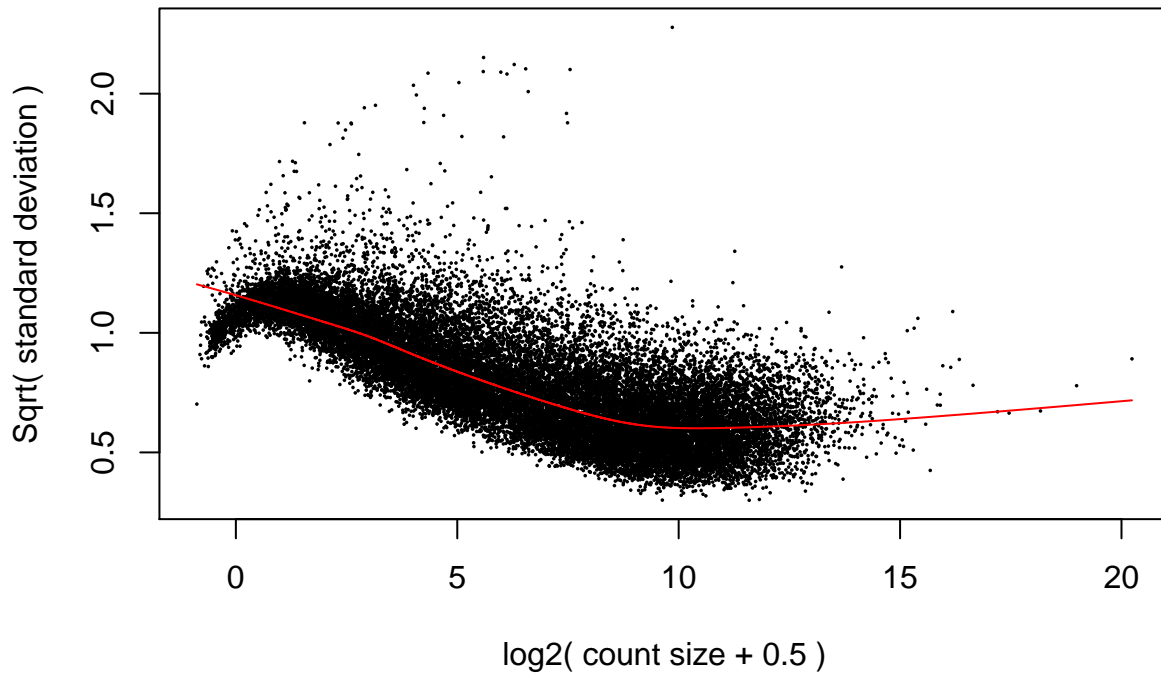

```
# Also write mean-variance plot to PDF
pdf(file = "DE_analysis/pdfs/vGene.pdf")
invisible(voom(dge,mod,plot=TRUE))
```

```
## Coefficients not estimable: PrimaryDxOther
```

```
## Warning: Partial NA coefficients for 26708 probe(s)
```

```
dev.off()
```

```
## pdf
##   2
```

```
# Get duplicate correlation
gene_dupCorr = duplicateCorrelation(vGene$E, mod,
    block=colData(rse_gene)$SAMPLE_ID)
```

```
## Coefficients not estimable: PrimaryDxOther
```

```
save(gene_dupCorr, file = here("DE_analysis", "rdas", "gene_dupCorr_neurons.rda"))
```

```
# Fit linear model
fitGeneDupl = lmFit(vGene,
    correlation=gene_dupCorr$consensus.correlation,
    block=colData(rse_gene)$SAMPLEID)
```

```
## Coefficients not estimable: PrimaryDxOther

## Warning: Partial NA coefficients for 26708 probe(s)

# Here we perform an empirical Bayesian calculation to obtain our significant genes
ebGeneDupl = eBayes(fitGeneDupl)

## Warning in .ebayes(fit = fit, proportion = proportion, stdev.coef.lim = stdev.coef.lim, : Estimation
## - set to default value

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()

## pdf
##   2

hist(outGeneDupl$P.Value)
```


Histogram of outGeneDupl$P.Value
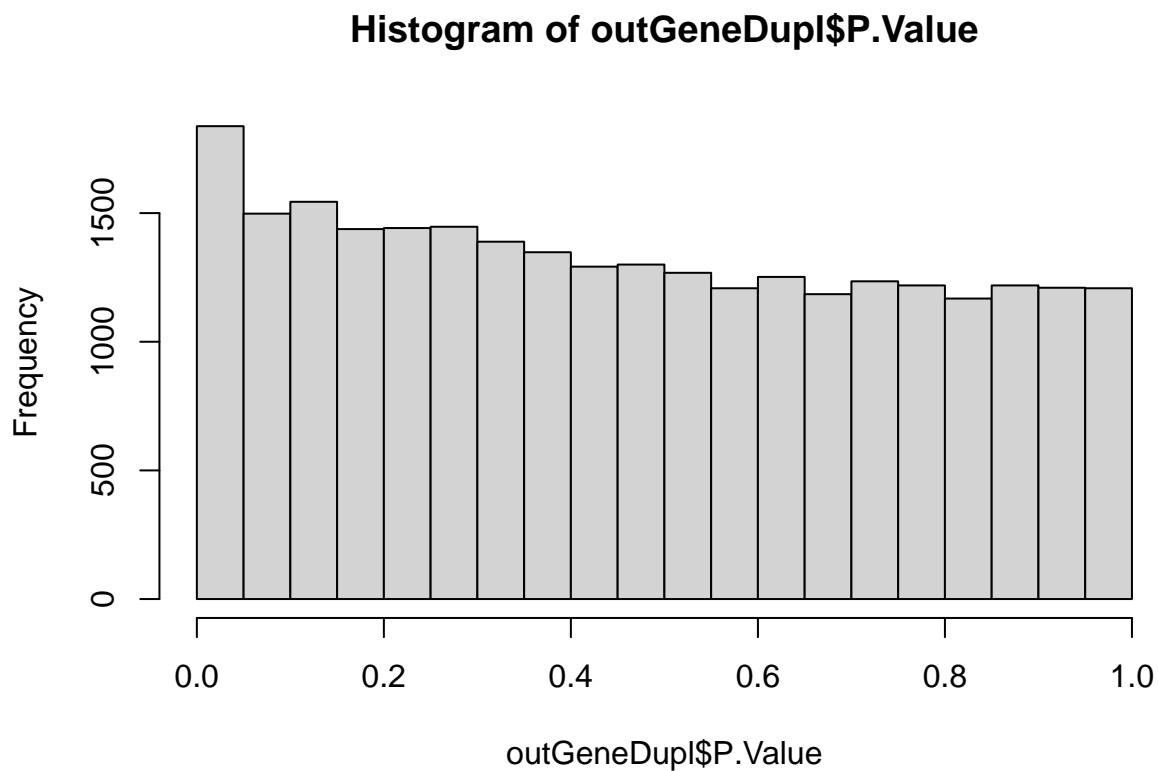
```r
table(outGeneDupl$adj.P.Val < 0.05)
```

```
##
## FALSE   TRUE
## 26707      1
```

```r
table(outGeneDupl$adj.P.Val < 0.1)
```

```
##
## FALSE   TRUE
## 26706      2
```

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

sigGeneDupl[,c("Symbol","logFC", "P.Value","AveExpr")]
```

```
##                    Symbol      logFC      P.Value   AveExpr
## ENSG00000269699.6    ZIM2 -1.1238665 1.448916e-06 0.8970203
## ENSG00000126368.6    NR1D1 -0.7637626 6.833687e-06 4.6128957
```

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

```
## [1] Symbol  logFC   P.Value
## <0 rows> (or 0-length row.names)
```

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

```
##                    Symbol      logFC      P.Value
## ENSG00000269699.6    ZIM2 -1.1238665 1.448916e-06
## ENSG00000126368.6    NR1D1 -0.7637626 6.833687e-06
```

```r
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"))
```

1.7 Check plots

```r
exprs = vGene$E[rownames(sigGeneDupl),]

# Group together code for displaying boxplots
generate_plots = function() {
    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_gene$PrimaryDx, outline=FALSE,
```

```
        ylim=range(yy), ylab="Normalized log2 Exprs", xlab="",
      main = paste(sigGeneDupl$Symbol[i], "-", sigGeneDupl$gencodeID[i]))
      points(yy ~ jitter(as.numeric(rse_gene$PrimaryDx)),
          pch = 21, bg= rse_gene$PrimaryDx,cex=1.3)
      ll = ifelse(sigGeneDupl$logFC[i] > 0, "topleft", "topright")
      legend(ll, paste0("p=", signif(sigGeneDupl$P.Value[i],3)), cex=1.3)
   }
}

# Show boxplots
generate_plots()
```

NR1D1 – ENSG00000126368.6
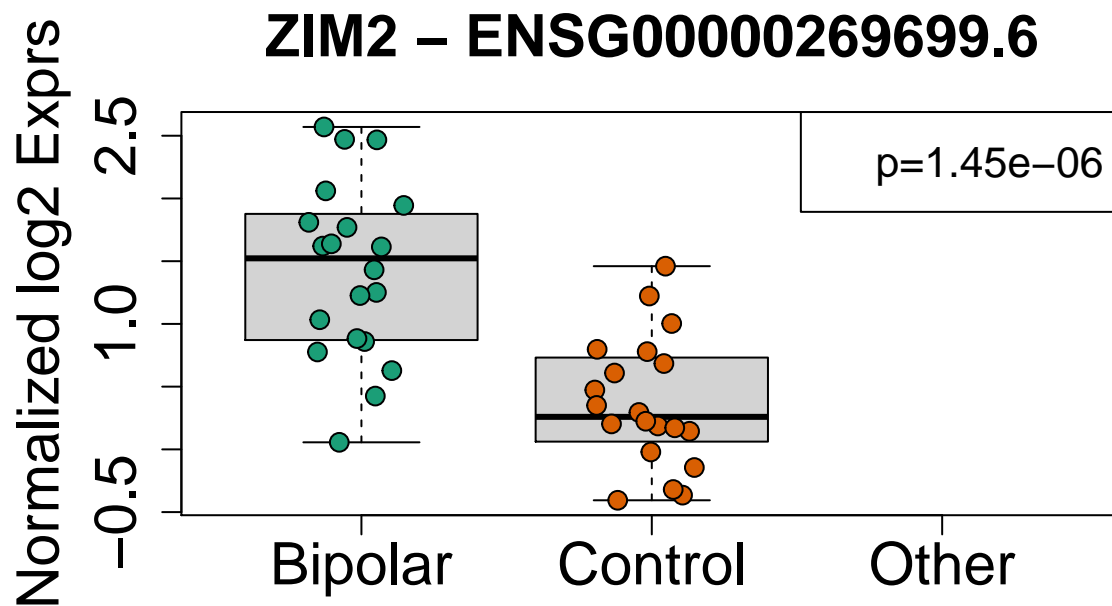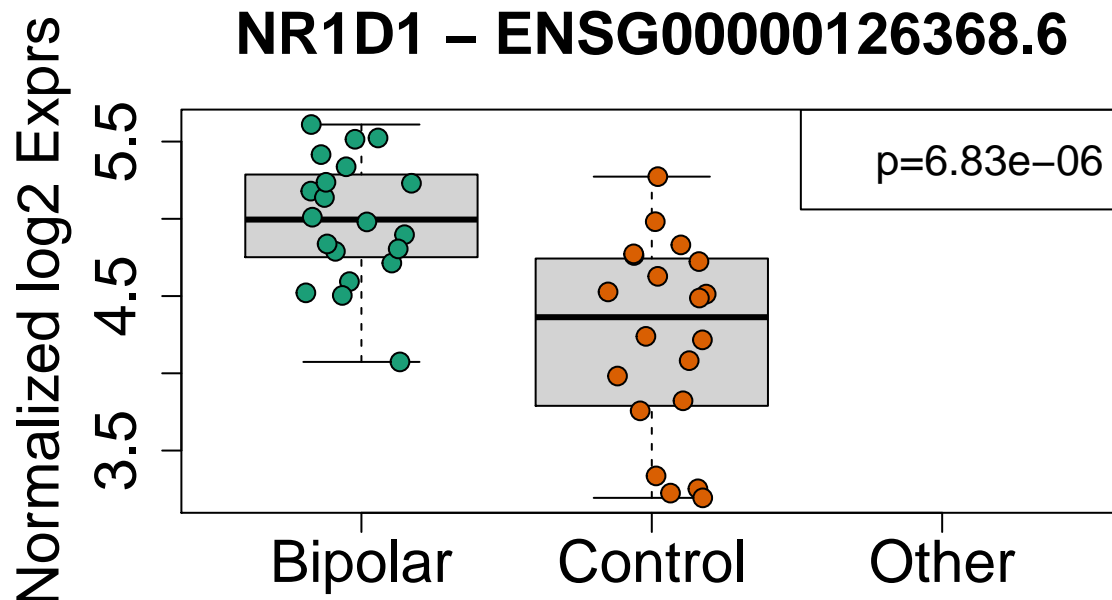

```
# Write plots to PDF
pdf(here("DE_analysis", "pdfs", "DE_boxplots_byDiagnosis.pdf"), w=10)
generate_plots()
dev.off()
```

```
## pdf
##   2
```

```
e = geneExprs[rownames(sigGeneDupl),]

generate_plots = function() {
    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_gene$PrimaryDx, las=3,outline=FALSE,
                ylim=range(yy), ylab="log2(RPKM+1)", xlab="",
        main = paste(sigGeneDupl$Symbol[i], "-", sigGeneDupl$gencodeID[i]))
        points(yy ~ jitter(as.numeric(rse_gene$PrimaryDx)),
            pch = 21, bg= rse_gene$PrimaryDx,cex=1.3)
        ll = ifelse(sigGeneDupl$logFC[i] > 0, "topleft", "topright")
        legend(ll, paste0("p=", signif(sigGeneDupl$P.Value[i],3)), cex=1.3)
    }
}
```

```
# Display plots
generate_plots()
```

ZIM2 – ENSG00000269699.6
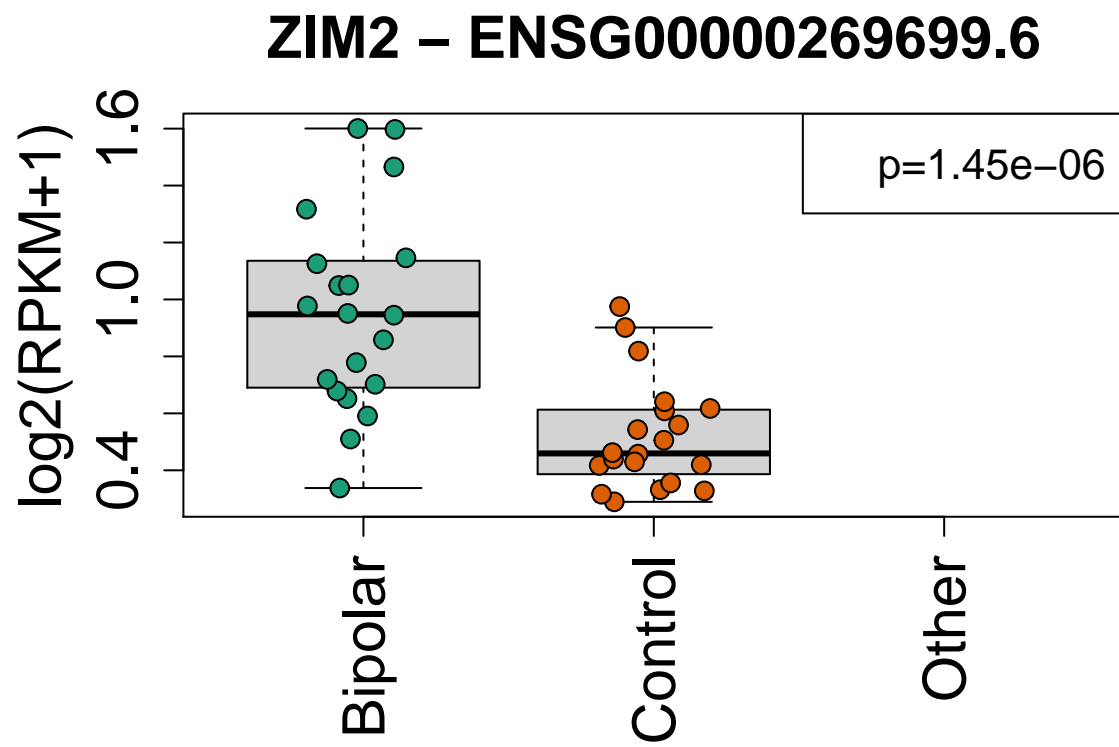
NR1D1 – ENSG00000126368.6


```r
# Write the same plots to PDF
pdf(here("DE_analysis", "pdfs", "DE_boxplots_byGenome_log2RPKM.pdf"), w=10)
generate_plots()
dev.off()
```

```
## pdf
##   2
```

1.8   Gene ontology

clusterProfiler is a gene ontology package we will use to see if our genes are specifically differentially expressed in certain pathways.

```r
# Get significant genes by sign
sigGene = outGeneDupl[outGeneDupl$P.Value < 0.005,]
sigGeneList = split(as.character(sigGene$EntrezID), sign(sigGene$logFC))
sigGeneList = lapply(sigGeneList, function(x) x[!is.na(x)])
geneUniverse = as.character(outGeneDupl$EntrezID)
geneUniverse = geneUniverse[!is.na(geneUniverse)]

# 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)]
```

```
##      Ontology Cluster      ID                                                                      Des
## 1311      BP       1 GO:0032640                                               tumor necrosis factor p
## 1312      BP       1 GO:0071706                    tumor necrosis factor superfamily cytokine p
## 1313      BP       1 GO:0050865                                                 regulation of cell ac
## 1314      BP       1 GO:0032611                                                   interleukin-1 beta p
## 1315      BP       1 GO:0032612                                                        interleukin-1 p
## 1316      BP       1 GO:0032680                                  regulation of tumor necrosis factor p
## 1317      BP       1 GO:1903555       regulation of tumor necrosis factor superfamily cytokine p
## 1318      BP       1 GO:0032651                               regulation of interleukin-1 beta p
## 1319      BP       1 GO:0050867                                  positive regulation of cell ac
## 1320      BP       1 GO:0002521                                                      leukocyte differe
## 1321      BP       1 GO:0002694                                      regulation of leukocyte ac
## 1322      BP       1 GO:0032652                                 regulation of interleukin-1 p
## 1323      BP       1 GO:0009617                                                         response to l
## 1324      BP       1 GO:0042742                                            defense response to l
## 1325      BP       1 GO:0071346                               cellular response to interfe
## 1326      BP       1 GO:0050900                                                      leukocyte m
## 3316      MF       1 GO:0045028           G protein-coupled purinergic nucleotide receptor
## 1327      BP       1 GO:0002687                             positive regulation of leukocyte m
## 1328      BP       1 GO:0034341                                              response to interfe
## 3317      MF       1 GO:0046935               1-phosphatidylinositol-3-kinase regulator
## 1329      BP       1 GO:0031347                                           regulation of defense
## 1330      BP       1 GO:0002685                                   regulation of leukocyte m
## 1331      BP       1 GO:0071219                       cellular response to molecule of bacteria
## 1332      BP       1 GO:0032760                   positive regulation of tumor necrosis factor p
```

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```
## 1333       BP       1 GO:1903975                                                                regulation of glial cell n
## 1334       BP       1 GO:1903557 positive regulation of tumor necrosis factor superfamily cytokine p
## 1335       BP       1 GO:0032635                                                                interleukin-6 p
## 1336       BP       1 GO:0006909                                                                          phag
## 1337       BP       1 GO:0032609                                                           interferon-gamma p
## 1338       BP       1 GO:0042116                                                              macrophage a
## 3318       MF       1 GO:0035014                           phosphatidylinositol 3-kinase regulator
## 1339       BP       1 GO:0002696                                          positive regulation of leukocyte a
## 1340       BP       1 GO:0050702                                                         interleukin-1 beta s
## 1341       BP       1 GO:0060216                                                              definitive her
## 3319       MF       1 GO:0001614                                          purinergic nucleotide receptor
## 3320       MF       1 GO:0016502                                                       nucleotide receptor
## 1342       BP       1 GO:0071216                                             cellular response to biotic
## 3321       MF       1 GO:0004930                                                 G protein-coupled receptor
## 1343       BP       1 GO:0050701                                                              interleukin-1 s
## 1344       BP       1 GO:0043032                                          positive regulation of macrophage a
## 1345       BP       1 GO:0050901                                                          leukocyte tethering o
## 1346       BP       1 GO:0032729                             positive regulation of interferon-gamma p
## 1347       BP       1 GO:0006911                                                              phagocytosis, e
## 1348       BP       1 GO:0070098                                                    chemokine-mediated signaling
## 1349       BP       1 GO:0007599                                                                          he
## 1350       BP       1 GO:0032691                             negative regulation of interleukin-1 beta p
## 3137       MF      -1 GO:0070888                                                                          E-bo
## 1351       BP       1 GO:0032692                                   negative regulation of interleukin-1 p
## 1352       BP       1 GO:0001819                                          positive regulation of cytokine p
## 1353       BP       1 GO:0031349                                             positive regulation of defense
## 1354       BP       1 GO:0050766                                                positive regulation of phag
## 3871       KEGG     1    hsa04061         Viral protein interaction with cytokine and cytokine
## 1355       BP       1 GO:0043410                                                  positive regulation of MAPI
## 1356       BP       1 GO:0071222                                             cellular response to lipopolys
## 1357       BP       1 GO:0099024                                                       plasma membrane inv
## 1358       BP       1 GO:1990868                                                                response to
## 1359       BP       1 GO:1990869                                                       cellular response to
## 1360       BP       1 GO:0032637                                                              interleukin-8 p
## 1361       BP       1 GO:0050729                                          positive regulation of inflammatory
## 1362       BP       1 GO:0050718                                positive regulation of interleukin-1 beta s
## 1363       BP       1 GO:0032675                                                   regulation of interleukin-6 p
## 1364       BP       1 GO:0035590                                   purinergic nucleotide receptor signaling
## 1365       BP       1 GO:0050716                                     positive regulation of interleukin-1 s
## 1366       BP       1 GO:0032103                             positive regulation of response to external
## 1367       BP       1 GO:0002831                                          regulation of response to biotic
## 1368       BP       1 GO:0002275                          myeloid cell activation involved in immune
## 1369       BP       1 GO:0030851                                                              granulocyte differ
## 1370       BP       1 GO:2000403                                          positive regulation of lymphocyte r
## 1371       BP       1 GO:0002573                                                  myeloid leukocyte differ
## 1372       BP       1 GO:0010324                                                                membrane inv
## 1373       BP       1 GO:0061756                              leukocyte adhesion to vascular endothel
## 1374       BP       1 GO:0032649                                          regulation of interferon-gamma p
## 3872       KEGG     1    hsa04060                               Cytokine-cytokine receptor in
## 3322       MF       1 GO:0005126                                                              cytokine recepto
## 1375       BP       1 GO:0032496                                                       response to lipopolys
## 1376       BP       1 GO:1903532                                          positive regulation of secretion
## 1377       BP       1 GO:0072676                                                              lymphocyte r
## 1378       BP       1 GO:0007159                                                  leukocyte cell-cell
```

```
## 1379      BP       1 GO:0032755                                          positive regulation of interleukin-6 pr
## 1380      BP       1 GO:0002237                                               response to molecule of bacteria
## 1381      BP       1 GO:0002683                                         negative regulation of immune system
## 1382      BP       1 GO:1905521                                              regulation of macrophage m
## 1383      BP       1 GO:0007596                                                            blood coa
## 1384      BP       1 GO:0050817                                                                  coa
## 1385      BP       1 GO:0050706                                        regulation of interleukin-1 beta s
## 1386      BP       1 GO:0051047                                               positive regulation of s
## 1387      BP       1 GO:0042035                                       regulation of cytokine biosynthetic
## 1388      BP       1 GO:0050764                                                  regulation of phag
## 1389      BP       1 GO:0001774                                                   microglial cell ac
## 1390      BP       1 GO:0002269               leukocyte activation involved in inflammatory
## 1391      BP       1 GO:0030225                                                  macrophage differe
## 1392      BP       1 GO:0050704                                        regulation of interleukin-1 s
## 3323      MF       1 GO:0000978    RNA polymerase II cis-regulatory region sequence-specific DNA
## 1393      BP       1 GO:0030335                                           positive regulation of cell m
## 3324      MF       1 GO:0005525                                                                   GT
## 1394      BP       1 GO:0030099                                              myeloid cell differe
## 1395      BP       1 GO:0072604                                                    interleukin-6 s
## 3325      MF       1 GO:0032550                                            purine ribonucleosid
## 3326      MF       1 GO:0001883                                               purine nucleosid
## 3327      MF       1 GO:0032549                                                  ribonucleosid
## 1396      BP       1 GO:0008217                                              regulation of blood
## 1397      BP       1 GO:0042089                                               cytokine biosynthetic
## 1398      BP       1 GO:0042107                                                 cytokine metabolic
## 3328      MF       1 GO:0000987             cis-regulatory region sequence-specific DNA
## 1399      BP       1 GO:1902105                                        regulation of leukocyte differe
## 1400      BP       1 GO:0045766                                           positive regulation of ang
## 1401      BP       1 GO:2000147                                           positive regulation of cell
## 1402      BP       1 GO:0002363                                          alpha-beta T cell lineage co
## 1403      BP       1 GO:0150078                            positive regulation of neuroinflammatory
## 1404      BP       1 GO:0033003                                           regulation of mast cell ac
## 3329      MF       1 GO:0001882                                                           nucleosid
## 1405      BP       1 GO:0032731                         positive regulation of interleukin-1 beta pr
## 3330      MF       1 GO:0019001                                               guanyl nucleotide
## 3331      MF       1 GO:0032561                                          guanyl ribonucleotide
## 1406      BP       1 GO:0001818                                     negative regulation of cytokine pr
## 1407      BP       1 GO:0048863                                                      stem cell differe
## 1408      BP       1 GO:0043030                                          regulation of macrophage ac
## 1409      BP       1 GO:0002697                                         regulation of immune effector
## 1410      BP       1 GO:0040017                                             positive regulation of le
## 1411      BP       1 GO:0043369      CD4-positive or CD8-positive, alpha-beta T cell lineage co
## 1412      BP       1 GO:0098883                                                                synaps
## 1413      BP       1 GO:0051272                              positive regulation of cellular component
## 3332      MF       1 GO:0070888                                                                  E-bo
## 1414      BP       1 GO:0097529                                                  myeloid leukocyte m
## 1415      BP       1 GO:0043300                                          regulation of leukocyte degra
## 1416      BP       1 GO:0002244                                 hematopoietic progenitor cell differe
## 1417      BP       1 GO:0002703                                       regulation of leukocyte mediated
## 1418      BP       1 GO:0070374                                     positive regulation of ERK1 and ERK
## 3333      MF       1 GO:0048020                                                 CCR chemokine recepto
## 1419      BP       1 GO:1903708                                             positive regulation of her
## 1420      BP       1 GO:0032732                          positive regulation of interleukin-1 pr
## 1421      BP       1 GO:1905517                                                      macrophage m
```

```
## 1422          BP          1 GO:0070372                                      regulation of ERK1 and ERK2
## 1423          BP          1 GO:1903037                              regulation of leukocyte cell-cell
## 1424          BP          1 GO:0071692                              protein localization to extracellula
## 1425          BP          1 GO:0045088                                   regulation of innate immune
## 1426          BP          1 GO:0061900                                                  glial cell ac
## 1427          BP          1 GO:0010919           regulation of inositol phosphate biosynthetic
## 1428          BP          1 GO:0033004                            negative regulation of mast cell ac
## 1429          BP          1 GO:0060100                         positive regulation of phagocytosis, er
## 1430          BP          1 GO:1905155                          positive regulation of membrane inva
## 1431          BP          1 GO:0050878                                         regulation of body flu
## 1432          BP          1 GO:0002761                          regulation of myeloid leukocyte differe
## 1433          BP          1 GO:1904018                       positive regulation of vasculature dev
## 1434          BP          1 GO:0008347                                                   glial cell m
## 1435          BP          1 GO:0043551            regulation of phosphatidylinositol 3-kinase
## 1436          BP          1 GO:0072678                                                       T cell m
## 3334          MF          1 GO:0019955                                                        cytokine
## 1437          BP          1 GO:0001562                                                  response to p
## 1438          BP          1 GO:0042832                                          defense response to p
## 1439          BP          1 GO:0060099                          regulation of phagocytosis, er
## 1440          BP          1 GO:1903978                            regulation of microglial cell ac
## 1441          BP          1 GO:2000401                               regulation of lymphocyte m
## 1442          BP          1 GO:0043270                            positive regulation of ion t
## 1443          BP          1 GO:0042108            positive regulation of cytokine biosynthetic
## 1444          BP          1 GO:0070371                                              ERK1 and ERK
## 1445          BP          1 GO:0002790                                                     peptide s
## 1446          BP          1 GO:0002360                                          T cell lineage co
## 1447          BP          1 GO:0042104           positive regulation of activated T cell proli
## 1448          BP          1 GO:1905153                              regulation of membrane inva
## 1449          BP          1 GO:0002886          regulation of myeloid leukocyte mediated
## 1450          BP          1 GO:0045765                                      regulation of ang
## 1451          BP          1 GO:0032677                        regulation of interleukin-8 pr
## 1452          BP          1 GO:0045123                                          cellular extra
## 1453          BP          1 GO:0050727                             regulation of inflammatory
## 1454          BP          1 GO:0002577          regulation of antigen processing and pres
## 1455          BP          1 GO:0150146                                      cell junction dis
## 1456          BP          1 GO:0006954                                              inflammatory
## 1457          BP          1 GO:0031663              lipopolysaccharide-mediated signaling
##             pvalue
## 1311 4.442846e-08
## 1312 5.955807e-08
## 1313 2.042035e-07
## 1314 2.762117e-07
## 1315 4.846558e-07
## 1316 5.291141e-07
## 1317 6.457953e-07
## 1318 2.297132e-06
## 1319 2.349005e-06
## 1320 3.605350e-06
## 1321 3.605350e-06
## 1322 3.637541e-06
## 1323 6.604984e-06
## 1324 9.663583e-06
## 1325 2.188166e-05
## 1326 2.555242e-05
```

```
## 3316 3.086309e-05
## 1327 3.901699e-05
## 1328 3.938836e-05
## 3317 4.223712e-05
## 1329 4.302992e-05
## 1330 4.665008e-05
## 1331 4.862740e-05
## 1332 5.573978e-05
## 1333 6.417224e-05
## 1334 6.438378e-05
## 1335 6.633435e-05
## 1336 7.418961e-05
## 1337 7.924753e-05
## 1338 8.474696e-05
## 3318 9.186960e-05
## 1339 9.207168e-05
## 1340 9.843140e-05
## 1341 1.011745e-04
## 3319 1.142974e-04
## 3320 1.142974e-04
## 1342 1.169372e-04
## 3321 1.277876e-04
## 1343 1.320556e-04
## 1344 1.498475e-04
## 1345 1.498475e-04
## 1346 1.586476e-04
## 1347 1.733017e-04
## 1348 1.733017e-04
## 1349 2.032086e-04
## 1350 2.116152e-04
## 3137 2.187705e-04
## 1351 2.478391e-04
## 1352 2.514522e-04
## 1353 2.564692e-04
## 1354 2.825185e-04
## 3871 2.923628e-04
## 1355 2.986591e-04
## 1356 3.212669e-04
## 1357 3.279249e-04
## 1358 3.279249e-04
## 1359 3.279249e-04
## 1360 3.783360e-04
## 1361 3.798024e-04
## 1362 4.321639e-04
## 1363 4.766011e-04
## 1364 4.888890e-04
## 1365 4.888890e-04
## 1366 4.971077e-04
## 1367 5.110894e-04
## 1368 5.390682e-04
## 1369 5.501749e-04
## 1370 5.501749e-04
## 1371 5.551129e-04
## 1372 5.626928e-04
```

```
## 1373 6.161675e-04
## 1374 6.754461e-04
## 3872 7.205888e-04
## 3322 7.359591e-04
## 1375 7.424577e-04
## 1376 7.846042e-04
## 1377 8.033448e-04
## 1378 8.182442e-04
## 1379 8.495298e-04
## 1380 9.655090e-04
## 1381 9.791509e-04
## 1382 1.021620e-03
## 1383 1.034691e-03
## 1384 1.082942e-03
## 1385 1.118743e-03
## 1386 1.143604e-03
## 1387 1.166745e-03
## 1388 1.166745e-03
## 1389 1.330039e-03
## 1390 1.330039e-03
## 1391 1.330039e-03
## 1392 1.330039e-03
## 3323 1.337238e-03
## 1393 1.367487e-03
## 3324 1.384455e-03
## 1394 1.431051e-03
## 1395 1.444451e-03
## 3325 1.493956e-03
## 3326 1.580520e-03
## 3327 1.580520e-03
## 1396 1.596835e-03
## 1397 1.632240e-03
## 1398 1.632240e-03
## 3328 1.632513e-03
## 1399 1.635117e-03
## 1400 1.649303e-03
## 1401 1.654766e-03
## 1402 1.677685e-03
## 1403 1.677685e-03
## 1404 1.691385e-03
## 3329 1.798052e-03
## 1405 1.824131e-03
## 3330 1.830975e-03
## 3331 1.830975e-03
## 1406 1.942791e-03
## 1407 1.942791e-03
## 1408 1.963208e-03
## 1409 2.003158e-03
## 1410 2.018912e-03
## 1411 2.042202e-03
## 1412 2.042202e-03
## 1413 2.049387e-03
## 3332 2.051128e-03
## 1414 2.052809e-03
```

```
## 1415 2.108721e-03
## 1416 2.115847e-03
## 1417 2.180288e-03
## 1418 2.180288e-03
## 3333 2.213958e-03
## 1419 2.246151e-03
## 1420 2.260772e-03
## 1421 2.260772e-03
## 1422 2.292524e-03
## 1423 2.346125e-03
## 1424 2.374893e-03
## 1425 2.400666e-03
## 1426 2.419462e-03
## 1427 2.440725e-03
## 1428 2.440725e-03
## 1429 2.440725e-03
## 1430 2.440725e-03
## 1431 2.485301e-03
## 1432 2.602808e-03
## 1433 2.748562e-03
## 1434 2.757143e-03
## 1435 2.757143e-03
## 1436 2.757143e-03
## 3334 2.788894e-03
## 1437 2.872826e-03
## 1438 2.872826e-03
## 1439 2.872826e-03
## 1440 2.872826e-03
## 1441 2.936322e-03
## 1442 3.000153e-03
## 1443 3.122514e-03
## 1444 3.132401e-03
## 1445 3.149398e-03
## 1446 3.338079e-03
## 1447 3.338079e-03
## 1448 3.338079e-03
## 1449 3.516285e-03
## 1450 3.629843e-03
## 1451 3.724031e-03
## 1452 3.724031e-03
## 1453 3.782205e-03
## 1454 3.836064e-03
## 1455 3.836064e-03
## 1456 3.902893e-03
## 1457 3.939125e-03
```

1.9 Visualize differentially expressed genes

Here we visualize DEGs with a heatmap.

```
exprs_heatmap = vGene$E[rownames(sigGene),]

df <- as.data.frame(colData(rse_gene)[,c("PrimaryDx")])
```
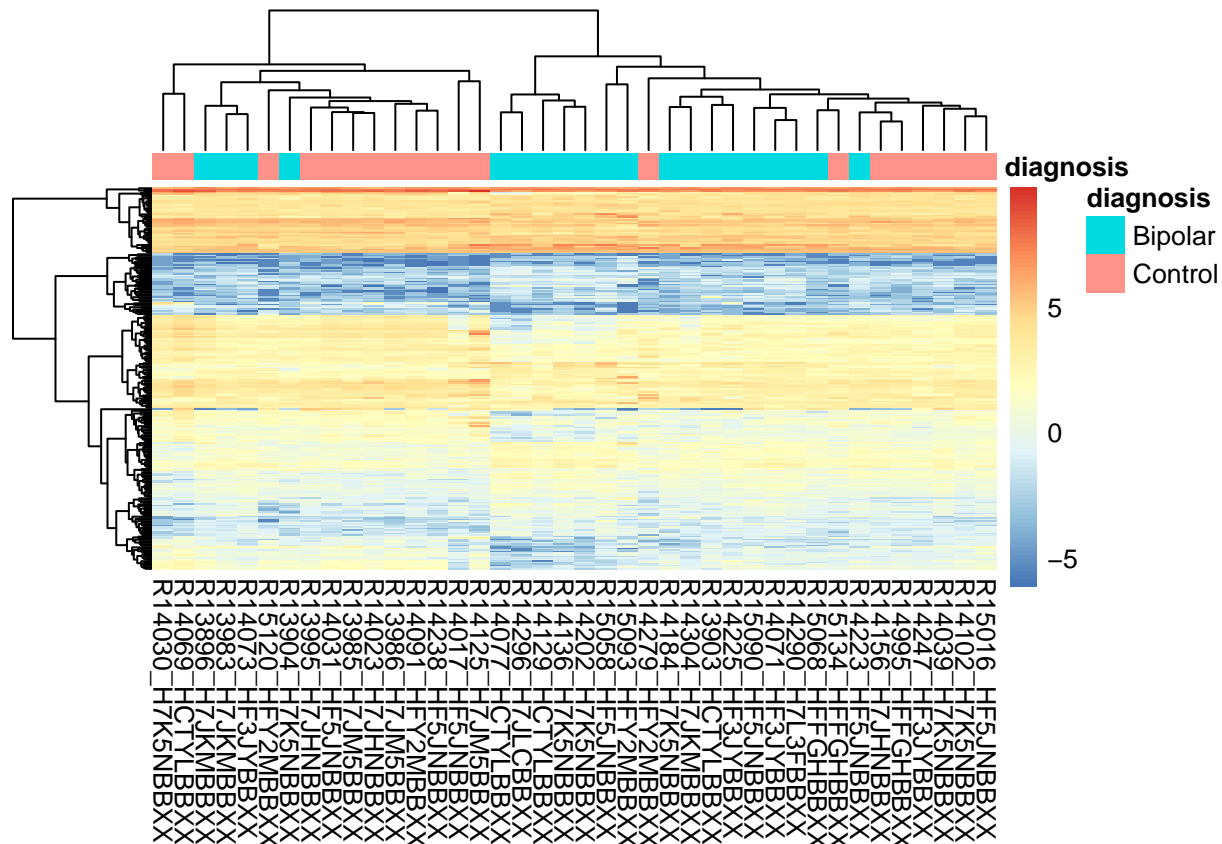
```r
rownames(df) <- colnames(exprs_heatmap)
colnames(df)<-"diagnosis"

# Display heatmap
pheatmap(exprs_heatmap, cluster_rows=TRUE, show_rownames=FALSE,
         cluster_cols=TRUE, annotation_col=df)
```



```r
# Write heatmap to PDF
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()
```

```
## pdf
##   3
```

2 Reproducibility

This analysis report was made possible thanks to:

- R (Kolde, 2019)
- *BiocStyle* (R Core Team, 2020)
- *clusterProfiler* (Allaire, Xie, McPherson, Luraschi, et al., 2020)

- *devtools* (Boettiger, 2019)
- *edgeR*
- *here* (Collado-Torres, Nellore, Kammers, Ellis, et al., 2017)
- *jaffelab* (Collado-Torres, Jaffe, and Burke, 2019)
- *limma* (Ellis, Collado-Torres, Jaffe, and Leek, 2018)
- *knitcitations* (Collado-Torres, Nellore, Kammers, Ellis, et al., 2020)
- *org.Hs.eg.db* (Frazee, Langmead, and Leek, 2011)
- *pheatmap* (Imada, Sanchez, Collado-Torres, Wilks, et al., 2020)
- *RColorBrewer* (McCarthy, Chen, and Smyth, 2012)
- *recount*
- *rmarkdown*
- *SummarizedExperiment* (Xie, Allaire, and Grolemund, 2018)
- *voom* (Yu, Wang, Han, and He, 2012)

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```
# Time spent creating this report:
diff(c(timestart, Sys.time()))
```

Time difference of 1.15595 mins

```
# Date this report was generated
message(Sys.time())
```

2020-09-18 17:01:57

```
# Reproducibility info
options(width = 120)
devtools::session_info()
```

```
## - Session info ---------------------------------------------------------------------------------------
##   setting  value
##   version  R version 4.0.2 Patched (2020-09-10 r79182)
##   os       Ubuntu 20.04.1 LTS
##   system   x86_64, linux-gnu
##   ui       X11
##   language (EN)
##   collate  en_US.UTF-8
##   ctype    en_US.UTF-8
##   tz       UTC
##   date     2020-09-18
##
## - Packages -------------------------------------------------------------------------------------------
##   package              * version date       lib source
##   AnnotationDbi        * 1.51.3  2020-07-25 [1] Bioconductor
##   askpass                1.1     2019-01-13 [2] RSPM (R 4.0.0)
##   assertthat             0.2.1   2019-03-21 [2] RSPM (R 4.0.0)
##   backports              1.1.9   2020-08-24 [2] RSPM (R 4.0.2)
##   base64enc              0.1-3   2015-07-28 [2] RSPM (R 4.0.0)
##   bibtex                 0.4.2.2 2020-01-02 [1] RSPM (R 4.0.0)
##   Biobase              * 2.49.1  2020-09-03 [1] Bioconductor
##   BiocFileCache          1.13.1  2020-08-04 [1] Bioconductor
##   BiocGenerics         * 0.35.4  2020-06-04 [1] Bioconductor
##   BiocManager          * 1.30.10 2019-11-16 [2] CRAN (R 4.0.2)
##   BiocParallel           1.23.2  2020-07-06 [1] Bioconductor
##   BiocStyle            * 2.17.0  2020-04-27 [1] Bioconductor
##   biomaRt                2.45.2  2020-07-05 [1] Bioconductor
##   Biostrings             2.57.2  2020-06-09 [1] Bioconductor
##   bit                    4.0.4   2020-08-04 [1] RSPM (R 4.0.2)
##   bit64                  4.0.5   2020-08-30 [1] RSPM (R 4.0.2)
##   bitops                 1.0-6   2013-08-17 [1] RSPM (R 4.0.0)
##   blob                   1.2.1   2020-01-20 [1] RSPM (R 4.0.0)
##   bookdown               0.20    2020-06-23 [1] RSPM (R 4.0.2)
##   BSgenome               1.57.6  2020-09-02 [1] Bioconductor
##   bumphunter             1.31.0  2020-04-28 [1] Bioconductor
##   callr                  3.4.4   2020-09-07 [2] RSPM (R 4.0.2)
##   checkmate              2.0.0   2020-02-06 [1] RSPM (R 4.0.0)
##   cli                    2.0.2   2020-02-28 [2] RSPM (R 4.0.0)
```

```
##   cluster            2.1.0    2019-06-19 [3] CRAN (R 4.0.2)
##   clusterProfiler  * 3.17.3   2020-09-10 [1] Bioconductor
##   codetools          0.2-16   2018-12-24 [3] CRAN (R 4.0.2)
##   colorspace         1.4-1    2019-03-18 [1] RSPM (R 4.0.0)
##   cowplot            1.1.0    2020-09-08 [1] RSPM (R 4.0.2)
##   crayon             1.3.4    2017-09-16 [2] RSPM (R 4.0.0)
##   curl               4.3      2019-12-02 [2] RSPM (R 4.0.0)
##   data.table         1.13.0   2020-07-24 [1] RSPM (R 4.0.2)
##   DBI                1.1.0    2019-12-15 [1] RSPM (R 4.0.0)
##   dbplyr             1.4.4    2020-05-27 [1] RSPM (R 4.0.0)
##   DelayedArray     * 0.15.7   2020-07-14 [1] Bioconductor
##   derfinder          1.23.1   2020-05-10 [1] Bioconductor
##   derfinderHelper    1.23.1   2020-05-10 [1] Bioconductor
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##   devtools         * 2.3.1    2020-07-21 [2] RSPM (R 4.0.2)
##   digest             0.6.25   2020-02-23 [2] RSPM (R 4.0.0)
##   DO.db              2.9      2020-09-10 [1] Bioconductor
##   doRNG              1.8.2    2020-01-27 [1] RSPM (R 4.0.0)
##   DOSE               3.15.0   2020-04-27 [1] Bioconductor
##   downloader         0.4      2015-07-09 [1] RSPM (R 4.0.0)
##   dplyr              1.0.2    2020-08-18 [1] RSPM (R 4.0.2)
##   edgeR            * 3.31.4   2020-06-10 [1] Bioconductor
##   ellipsis           0.3.1    2020-05-15 [2] RSPM (R 4.0.0)
##   enrichplot         1.9.1    2020-04-29 [1] Bioconductor
##   europepmc          0.4      2020-05-31 [1] RSPM (R 4.0.0)
##   evaluate           0.14     2019-05-28 [2] RSPM (R 4.0.0)
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##   fgsea              1.15.0   2020-04-27 [1] Bioconductor
##   foreach            1.5.0    2020-03-30 [1] RSPM (R 4.0.0)
##   foreign            0.8-80   2020-05-24 [3] CRAN (R 4.0.2)
##   Formula            1.2-3    2018-05-03 [1] RSPM (R 4.0.0)
##   fs                 1.5.0    2020-07-31 [2] RSPM (R 4.0.2)
##   generics           0.0.2    2018-11-29 [1] RSPM (R 4.0.0)
##   GenomeInfoDb     * 1.25.11  2020-09-03 [1] Bioconductor
##   GenomeInfoDbData   1.2.3    2020-09-10 [1] Bioconductor
##   GenomicAlignments  1.25.3   2020-06-09 [1] Bioconductor
##   GenomicFeatures    1.41.3   2020-09-03 [1] Bioconductor
##   GenomicFiles       1.25.0   2020-04-27 [1] Bioconductor
##   GenomicRanges    * 1.41.6   2020-08-12 [1] Bioconductor
##   GEOquery           2.57.0   2020-04-27 [1] Bioconductor
##   ggforce            0.3.2    2020-06-23 [1] RSPM (R 4.0.2)
##   ggplot2            3.3.2    2020-06-19 [1] RSPM (R 4.0.1)
##   ggplotify          0.0.5    2020-03-12 [1] RSPM (R 4.0.0)
##   ggraph             2.0.3    2020-05-20 [1] RSPM (R 4.0.2)
##   ggrepel            0.8.2    2020-03-08 [1] RSPM (R 4.0.2)
##   ggridges           0.5.2    2020-01-12 [1] RSPM (R 4.0.0)
##   glue               1.4.2    2020-08-27 [2] RSPM (R 4.0.2)
##   GO.db              3.11.4   2020-09-10 [1] Bioconductor
##   googledrive        1.0.1    2020-05-05 [1] RSPM (R 4.0.0)
##   GOSemSim           2.15.2   2020-09-04 [1] Bioconductor
##   graphlayouts       0.7.0    2020-04-25 [1] RSPM (R 4.0.2)
##   gridExtra          2.3      2017-09-09 [1] RSPM (R 4.0.0)
```

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##  gridGraphics      0.5-0    2020-02-25 [1] RSPM (R 4.0.0)
##  gtable           0.3.0    2019-03-25 [1] RSPM (R 4.0.0)
##  here           * 0.1      2017-05-28 [1] RSPM (R 4.0.0)
##  Hmisc            4.4-1    2020-08-10 [1] RSPM (R 4.0.2)
##  hms              0.5.3    2020-01-08 [1] RSPM (R 4.0.0)
##  htmlTable        2.0.1    2020-07-05 [1] RSPM (R 4.0.2)
##  htmltools        0.5.0    2020-06-16 [2] RSPM (R 4.0.1)
##  htmlwidgets      1.5.1    2019-10-08 [2] RSPM (R 4.0.0)
##  httr             1.4.2    2020-07-20 [2] RSPM (R 4.0.2)
##  igraph           1.2.5    2020-03-19 [1] RSPM (R 4.0.0)
##  IRanges        * 2.23.10  2020-06-13 [1] Bioconductor
##  iterators        1.0.12   2019-07-26 [1] RSPM (R 4.0.0)
##  jaffelab       * 0.99.30  2020-09-10 [1] Github (LieberInstitute/jaffelab@42637ff)
##  jpeg             0.1-8.1  2019-10-24 [1] RSPM (R 4.0.0)
##  jsonlite         1.7.1    2020-09-07 [1] RSPM (R 4.0.2)
##  knitcitations  * 1.0.10   2019-09-15 [1] RSPM (R 4.0.0)
##  knitr            1.29     2020-06-23 [2] RSPM (R 4.0.2)
##  lattice          0.20-41  2020-04-02 [3] CRAN (R 4.0.2)
##  latticeExtra     0.6-29   2019-12-19 [1] RSPM (R 4.0.0)
##  lifecycle        0.2.0    2020-03-06 [2] RSPM (R 4.0.0)
##  limma          * 3.45.14  2020-09-12 [1] Bioconductor
##  locfit           1.5-9.4  2020-03-25 [1] RSPM (R 4.0.0)
##  lubridate        1.7.9    2020-06-08 [1] RSPM (R 4.0.2)
##  magrittr         1.5      2014-11-22 [2] RSPM (R 4.0.0)
##  MASS             7.3-53   2020-09-09 [3] CRAN (R 4.0.2)
##  Matrix         * 1.2-18   2019-11-27 [3] CRAN (R 4.0.2)
##  matrixStats    * 0.56.0   2020-03-13 [1] RSPM (R 4.0.0)
##  memoise          1.1.0    2017-04-21 [2] RSPM (R 4.0.0)
##  munsell          0.5.0    2018-06-12 [1] RSPM (R 4.0.0)
##  nnet             7.3-14   2020-04-26 [3] CRAN (R 4.0.2)
##  openssl          1.4.2    2020-06-27 [2] RSPM (R 4.0.2)
##  org.Hs.eg.db   * 3.11.4   2020-09-18 [1] Bioconductor
##  pheatmap       * 1.0.12   2019-01-04 [1] RSPM (R 4.0.0)
##  pillar           1.4.6    2020-07-10 [2] RSPM (R 4.0.2)
##  pkgbuild         1.1.0    2020-07-13 [2] RSPM (R 4.0.2)
##  pkgconfig        2.0.3    2019-09-22 [2] RSPM (R 4.0.0)
##  pkgload          1.1.0    2020-05-29 [2] RSPM (R 4.0.0)
##  plyr             1.8.6    2020-03-03 [1] RSPM (R 4.0.2)
##  png              0.1-7    2013-12-03 [1] RSPM (R 4.0.0)
##  polyclip         1.10-0   2019-03-14 [1] RSPM (R 4.0.0)
##  prettyunits      1.1.1    2020-01-24 [2] RSPM (R 4.0.0)
##  processx         3.4.4    2020-09-03 [2] RSPM (R 4.0.2)
##  progress         1.2.2    2019-05-16 [1] RSPM (R 4.0.0)
##  ps               1.3.4    2020-08-11 [2] RSPM (R 4.0.2)
##  purrr            0.3.4    2020-04-17 [2] RSPM (R 4.0.0)
##  qvalue           2.21.0   2020-04-27 [1] Bioconductor
##  R6               2.4.1    2019-11-12 [2] RSPM (R 4.0.0)
##  rafalib        * 1.0.0    2015-08-09 [1] RSPM (R 4.0.0)
##  rappdirs         0.3.1    2016-03-28 [1] RSPM (R 4.0.0)
##  RColorBrewer   * 1.1-2    2014-12-07 [1] RSPM (R 4.0.0)
##  Rcpp             1.0.5    2020-07-06 [2] RSPM (R 4.0.2)
##  RCurl            1.98-1.2 2020-04-18 [1] RSPM (R 4.0.0)
##  readr            1.3.1    2018-12-21 [1] RSPM (R 4.0.2)
##  recount        * 1.15.1   2020-05-12 [1] Bioconductor
```

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## RefManageR            1.2.12    2019-04-03 [1] RSPM (R 4.0.0)
## remotes              2.2.0     2020-07-21 [1] RSPM (R 4.0.2)
## rentrez              1.2.2     2019-05-02 [1] RSPM (R 4.0.0)
## reshape2             1.4.4     2020-04-09 [1] RSPM (R 4.0.2)
## rlang                0.4.7     2020-07-09 [2] RSPM (R 4.0.2)
## rmarkdown            2.3       2020-06-18 [1] RSPM (R 4.0.1)
## rngtools             1.5       2020-01-23 [1] RSPM (R 4.0.0)
## rpart                4.1-15    2019-04-12 [3] CRAN (R 4.0.2)
## rprojroot            1.3-2     2018-01-03 [2] RSPM (R 4.0.0)
## Rsamtools            2.5.3     2020-06-26 [1] Bioconductor
## RSQLite              2.2.0     2020-01-07 [1] RSPM (R 4.0.2)
## rstudioapi           0.11      2020-02-07 [2] RSPM (R 4.0.0)
## rtracklayer          1.49.5    2020-08-07 [1] Bioconductor
## rvcheck              0.1.8     2020-03-01 [1] RSPM (R 4.0.0)
## S4Vectors          * 0.27.12   2020-06-09 [1] Bioconductor
## scales               1.1.1     2020-05-11 [1] RSPM (R 4.0.0)
## scatterpie           0.1.4     2019-11-08 [1] RSPM (R 4.0.0)
## segmented            1.2-0     2020-06-23 [1] RSPM (R 4.0.2)
## sessioninfo          1.1.1     2018-11-05 [1] RSPM (R 4.0.0)
## statmod              1.4.34    2020-02-17 [1] RSPM (R 4.0.0)
## stringi              1.5.3     2020-09-09 [2] RSPM (R 4.0.2)
## stringr              1.4.0     2019-02-10 [2] RSPM (R 4.0.0)
## SummarizedExperiment * 1.19.6  2020-07-09 [1] Bioconductor
## survival             3.2-3     2020-06-13 [3] CRAN (R 4.0.2)
## testthat             2.3.2     2020-03-02 [2] RSPM (R 4.0.0)
## tibble               3.0.3     2020-07-10 [2] RSPM (R 4.0.2)
## tidygraph            1.2.0     2020-05-12 [1] RSPM (R 4.0.2)
## tidyr                1.1.2     2020-08-27 [1] RSPM (R 4.0.2)
## tidyselect           1.1.0     2020-05-11 [1] RSPM (R 4.0.0)
## triebeard            0.3.0     2016-08-04 [1] RSPM (R 4.0.2)
## tweenr               1.0.1     2018-12-14 [1] RSPM (R 4.0.2)
## urltools             1.7.3     2019-04-14 [1] RSPM (R 4.0.2)
## usethis            * 1.6.1     2020-04-29 [2] RSPM (R 4.0.0)
## VariantAnnotation    1.35.3    2020-06-26 [1] Bioconductor
## vctrs                0.3.4     2020-08-29 [2] RSPM (R 4.0.2)
## viridis              0.5.1     2018-03-29 [1] RSPM (R 4.0.0)
## viridisLite          0.3.0     2018-02-01 [1] RSPM (R 4.0.0)
## withr                2.2.0     2020-04-20 [2] RSPM (R 4.0.0)
## xfun                 0.17      2020-09-09 [2] RSPM (R 4.0.2)
## XML                  3.99-0.5  2020-07-23 [1] RSPM (R 4.0.2)
## xml2                 1.3.2     2020-04-23 [2] RSPM (R 4.0.0)
## XVector              0.29.3    2020-06-25 [1] Bioconductor
## yaml                 2.2.1     2020-02-01 [2] RSPM (R 4.0.0)
## zlibbioc             1.35.0    2020-04-27 [1] Bioconductor
##
## [1] /usr/local/lib/R/host-site-library
## [2] /usr/local/lib/R/site-library
## [3] /usr/local/lib/R/library
```