

# Differential Expression Analyses

## Differential expression analysis on gene expression data.

### Setup and read in data

```
# load libraries
library(easypackages)
libraries("limma","qvalue","here")
options(stringsAsFactors = FALSE)

# create directory to save results in
dir.create(here("DEresults"))

# Read in data
load(here("data","processed","exprDataAdj.Rdata"))
```

### DE analysis ASD subtypes vs TD

```
fdr_thresh = 0.05

# construct model
full_model = model.matrix(~0+as.factor(subgrp2), data=labelData)
colnames(full_model) <- c("Good","Poor", "TD")

# make contrast matrix
contrast.matrix <- makeContrasts(TD-Poor,TD-Good,Good-Poor,
                                levels=full_model)

# fit DE limma model
fit = lmFit(exprDataAdj,full_model)

# fit contrasts
fitContrasts = contrasts.fit(fit,contrast.matrix)

# use empirical bayes
eb = eBayes(fitContrasts)
pvals_pairwise_comps = eb$p.value

# get table of DE results
DEresults = topTable(eb, number = dim(geneInfo)[1], adjust.method = "fdr")
pvals_pairwise_comps = pvals_pairwise_comps[rownames(DEresults),]
geneInfo2 = geneInfo[rownames(DEresults),]
DEresults = cbind(GeneSymbols = geneInfo2$geneSymbol, DEresults, pvals_pairwise_comps)
colnames(DEresults)[2:4] = c("TD_vs_ASDPoor.tstat",
                            "TD_vs_ASDBGood.tstat",
                            "ASDBGood_vs_ASDBPoor.tstat")
colnames(DEresults)[9:11] = c("TD_vs_ASDPoor.pval",
```

```

"TD_vs_ASDBGood.pval",
"ASDBGood_vs_ASDBPoor.pval")

# compute FDR on pairwise group comparisons
DEresults$TD_vs_ASDBPoor.fdr = p.adjust(DEresults$TD_vs_ASDBPoor.pval,
method = "fdr")
DEresults$TD_vs_ASDBGood.fdr = p.adjust(DEresults$TD_vs_ASDBGood.pval,
method = "fdr")
DEresults$ASDBGood_vs_ASDBPoor.fdr = p.adjust(DEresults$ASDBGood_vs_ASDBPoor.pval,
method = "fdr")

# save results to csv file
write.csv(DEresults, file = here("DEresults", "DEresults_gene.csv"))

# show top 20 genes
knitr::kable(head(DEresults[,c("GeneSymbols", "F", "P.Value", "adj.P.Val")], 20), digits = 4)

```

	GeneSymbols	F	P.Value	adj.P.Val
ILMN_1805636	PGAP3	12.4505	0e+00	0.0968
ILMN_1673275	TRAPPC2	12.3410	0e+00	0.0968
ILMN_1740903	C7orf49	11.3902	0e+00	0.1222
ILMN_2073012	TMEM203	11.1560	0e+00	0.1222
ILMN_1661888	MEF2A	10.9644	0e+00	0.1222
ILMN_1765332	TIMM10	10.7507	1e-04	0.1222
ILMN_1668752	FLJ20850	10.4846	1e-04	0.1224
ILMN_1748529	RLN2	10.2888	1e-04	0.1224
ILMN_1769783	ZDHHC2	10.2710	1e-04	0.1224
ILMN_1764380	GLTP	9.7212	1e-04	0.1764
ILMN_1761058	ACAD11	9.4124	2e-04	0.1848
ILMN_1775743	BTG1	9.3052	2e-04	0.1848
ILMN_1738229	NDRG3	9.2666	2e-04	0.1848
ILMN_2154566	RPL10A	9.1465	2e-04	0.1848
ILMN_1689189	ZBTB3	9.0809	2e-04	0.1848
ILMN_1701914	CD274	9.0166	2e-04	0.1848
ILMN_1706645	C6orf150	8.9635	2e-04	0.1848
ILMN_1656682	AZIN1	8.9334	2e-04	0.1848
ILMN_2047599	TMEM50B	8.9133	2e-04	0.1848
ILMN_1812250	LOC644642	8.8201	3e-04	0.1848