2021.7.16

using GEO data to explore DEGs between autistic spectrum disorder patients (neural diversity people) and normal people (neural typical people)

GSE42133

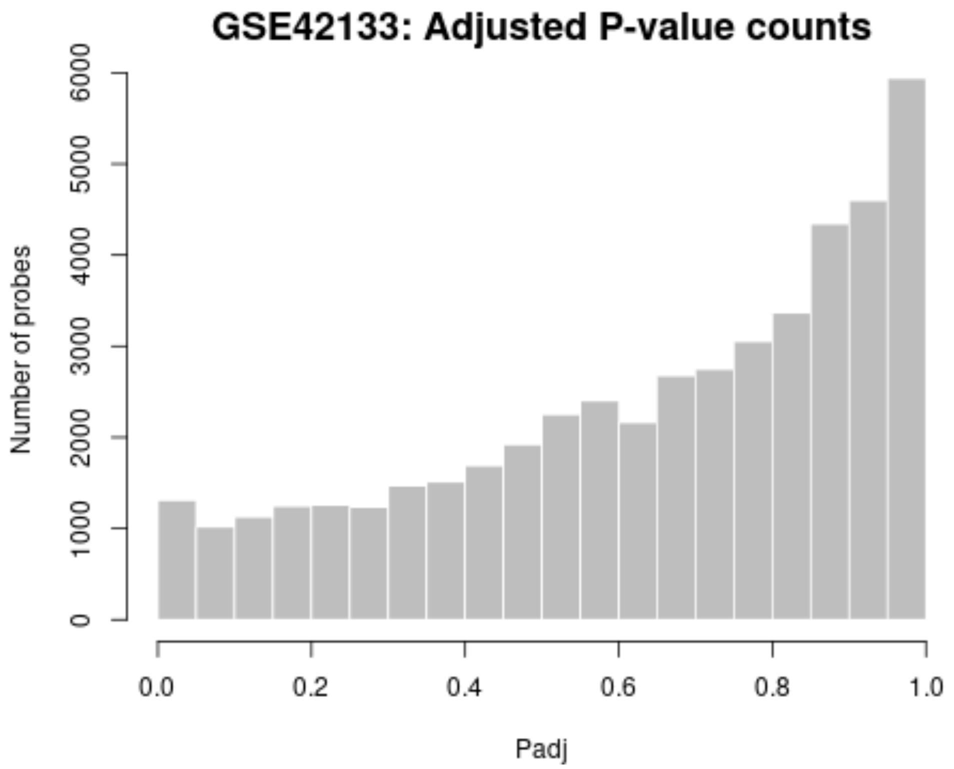
data source	GSE42133
title	Disrupted functional neworks in autism underlie early brain maldevelopment and provide accurate classification
Organism	Homo sapiens
Experiment type	Expression profiling by array
Status	Public on Mar 24, 2015

use GEO2R to analyze

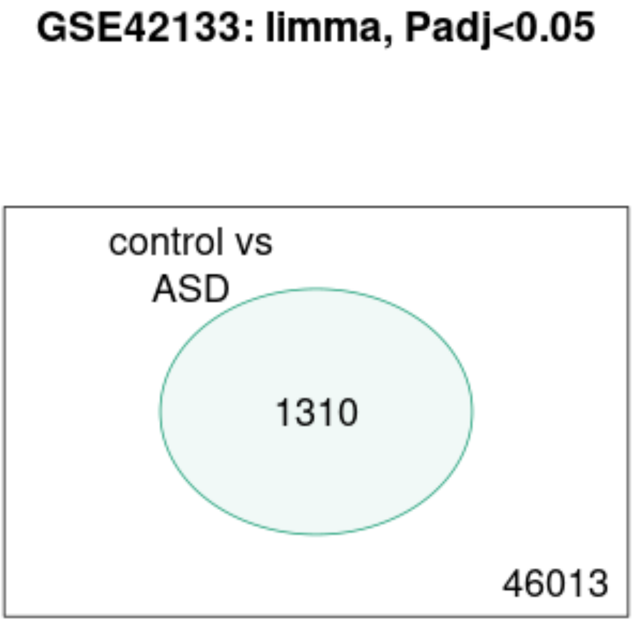
```
# Version info: R 3.2.3, Biobase 2.30.0, GEOquery 2.40.0, limma 3.26.8
################################################################
#   Differential expression analysis with limma
library(GEOquery)
library(limma)
library(umap)

# load series and platform data from GEO

gset <- getGEO("GSE42133", GSEMatrix =TRUE, AnnotGPL=TRUE)
if (length(gset) > 1) idx <- grep("GPL10558", attr(gset, "names")) else idx <-
1
gset <- gset[[idx]]

# make proper column names to match toptable
fvarLabels(gset) <- make.names(fvarLabels(gset))

# group membership for all samples
gsms <- paste0("11110010101111000100110111000000101000000001100000",
        "11010011100101010110001011111110111111011111111001",
        "11001011111011110111111111101111110111001111110")
sml <- strsplit(gsms, split="")[[1]]
```

```r
# log2 transformation
ex <- exprs(gset)
qx <- as.numeric(quantile(ex, c(0., 0.25, 0.5, 0.75, 0.99, 1.0), na.rm=T))
LogC <- (qx[5] > 100) ||
          (qx[6]-qx[1] > 50 && qx[2] > 0)
if (LogC) { ex[which(ex <= 0)] <- NaN
  exprs(gset) <- log2(ex) }

# assign samples to groups and set up design matrix
gs <- factor(sml)
groups <- make.names(c("control","ASD"))
levels(gs) <- groups
gset$group <- gs
design <- model.matrix(~group + 0, gset)
colnames(design) <- levels(gs)

fit <- lmFit(gset, design)  # fit linear model

# set up contrasts of interest and recalculate model coefficients
cts <- paste(groups[1], groups[2], sep="-")
cont.matrix <- makeContrasts(contrasts=cts, levels=design)
fit2 <- contrasts.fit(fit, cont.matrix)

# compute statistics and table of top significant genes
fit2 <- eBayes(fit2, 0.01)
tT <- topTable(fit2, adjust="fdr", sort.by="B", number=250)

tT <- subset(tT,
select=c("ID","adj.P.Val","P.Value","t","B","logFC","Gene.symbol","Gene.title")
)
write.table(tT, file=stdout(), row.names=F, sep="\t")
```

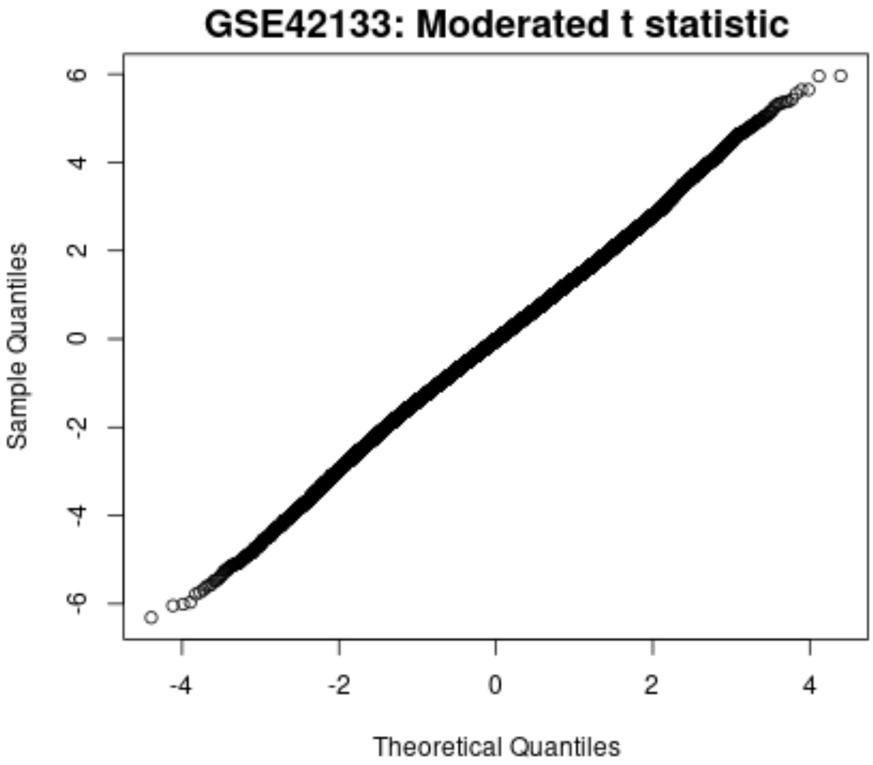
```r
# Visualize and quality control test results.
# Build histogram of P-values for all genes. Normal test
# assumption is that most genes are not differentially expressed.
tT2 <- topTable(fit2, adjust="fdr", sort.by="B", number=Inf)
hist(tT2$adj.P.Val, col = "grey", border = "white", xlab = "P-adj",
  ylab = "Number of genes", main = "P-adj value distribution")
```



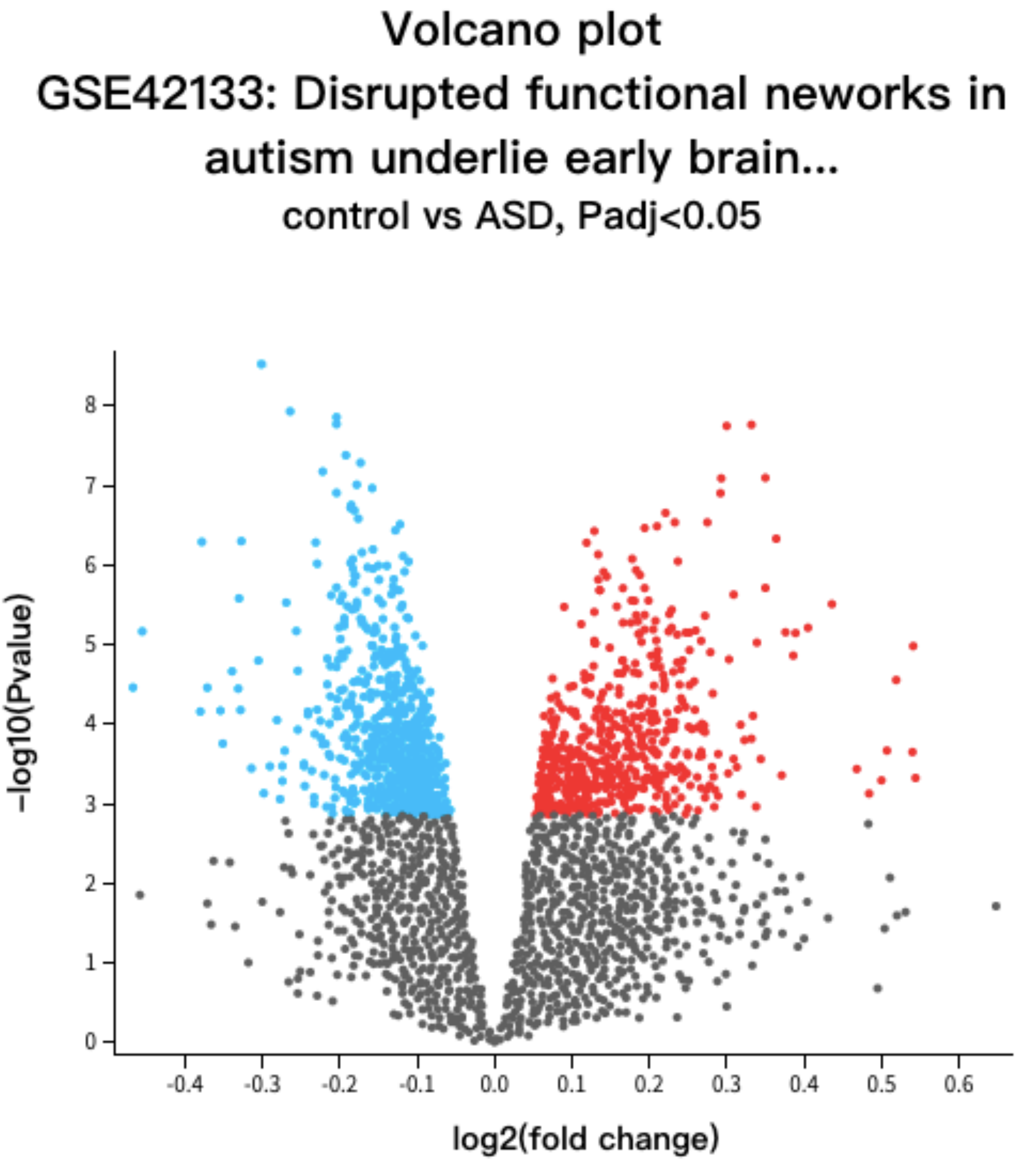
```r
# summarize test results as "up", "down" or "not expressed"
dT <- decideTests(fit2, adjust.method="fdr", p.value=0.05)

# Venn diagram of results
vennDiagram(dT, circle.col=palette())
# download significant genes in genes.tsv
```



```
# create Q-Q plot for t-statistic
t.good <- which(!is.na(fit2$F)) # filter out bad probes
qqt(fit2$t[t.good], fit2$df.total[t.good], main="Moderated t statistic")
```

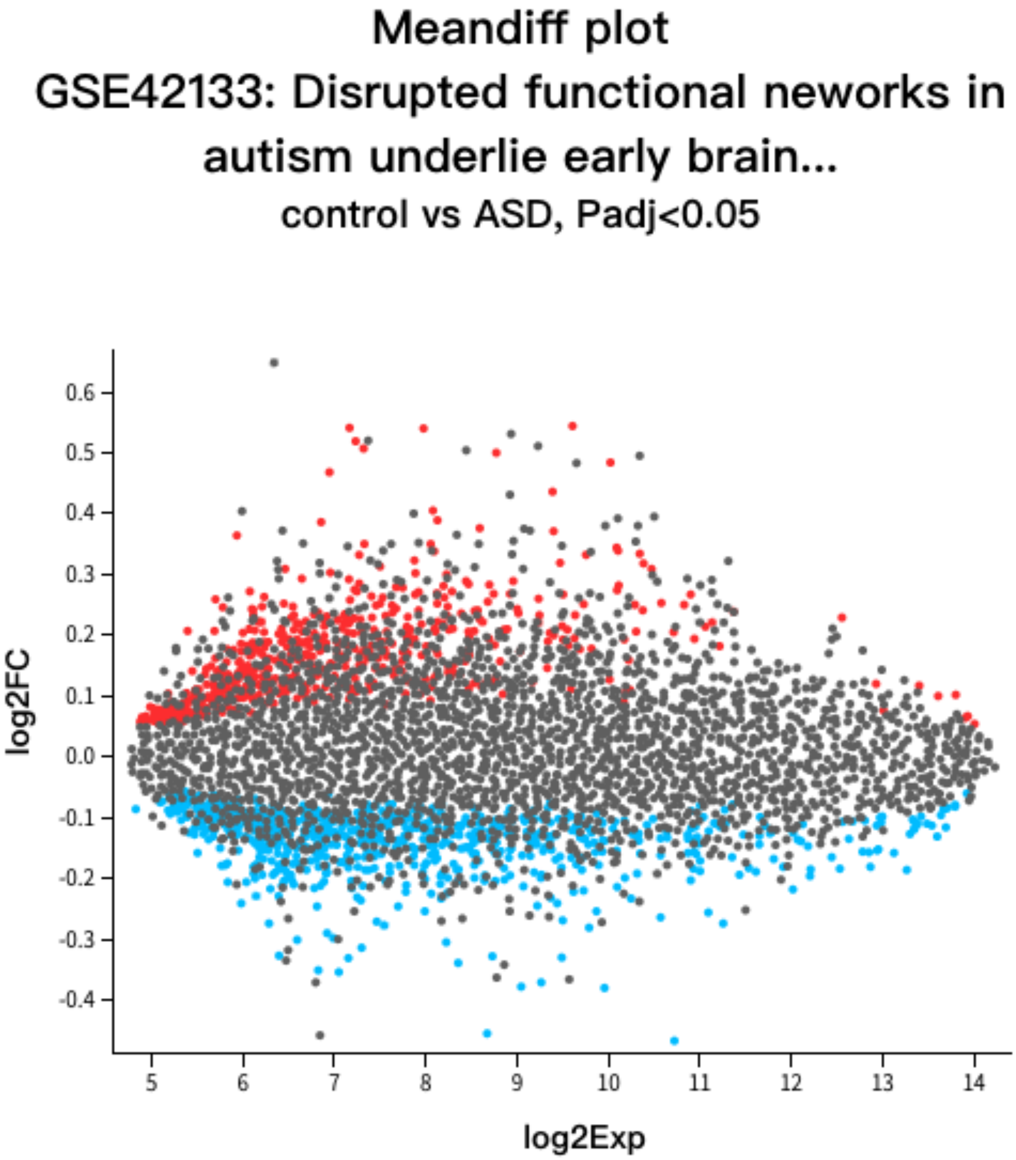


```
# volcano plot (log P-value vs log fold change)
colnames(fit2) # list contrast names
ct <- 1        # choose contrast of interest
volcanoplot(fit2, coef=ct, main=colnames(fit2)[ct], pch=20,
  highlight=length(which(dT[,ct]!=0)), names=rep('+', nrow(fit2)))
```

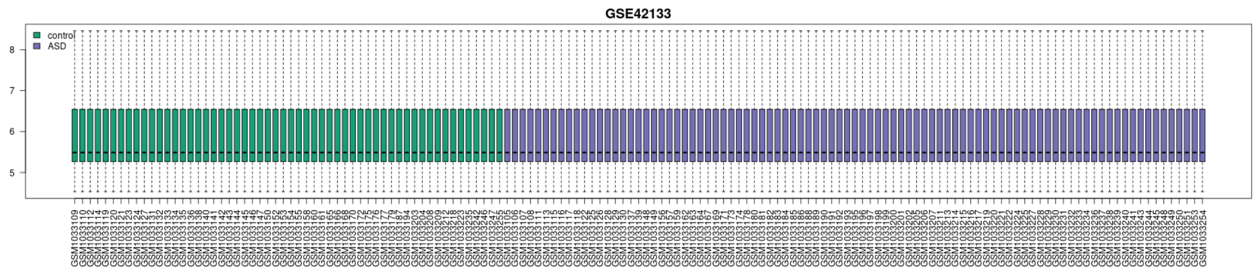


Volcano plot
GSE42133: Disrupted functional neworks in autism underlie early brain...
control vs ASD, Padj<0.05

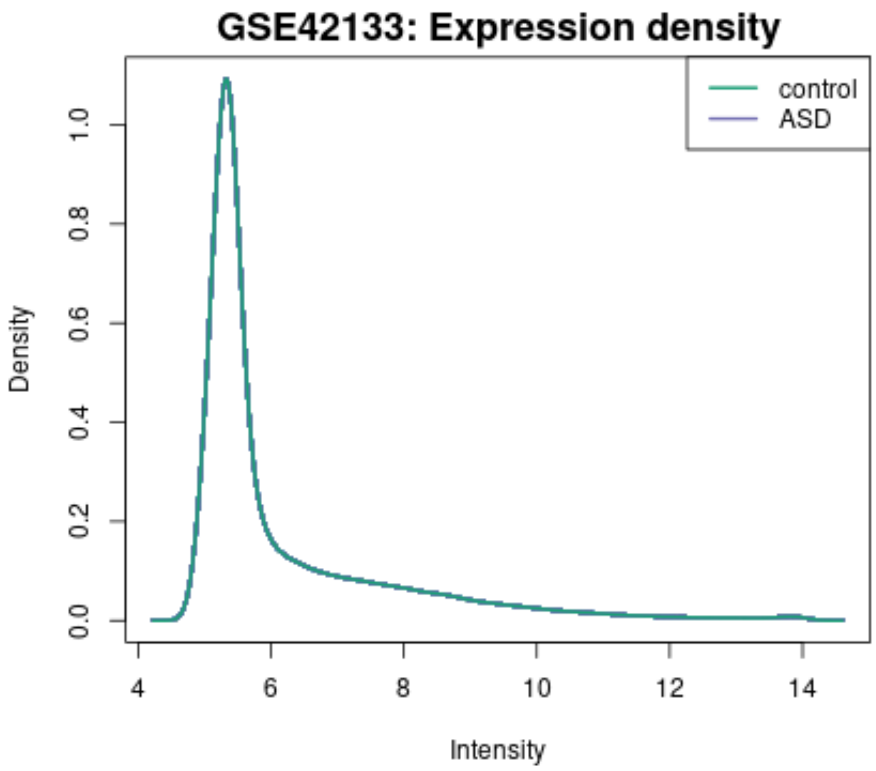
```
# MD plot (log fold change vs mean log expression)
# highlight statistically significant (p-adj < 0.05) probes
plotMD(fit2, column=ct, status=dT[,ct], legend=F, pch=20, cex=1)
abline(h=0)
```



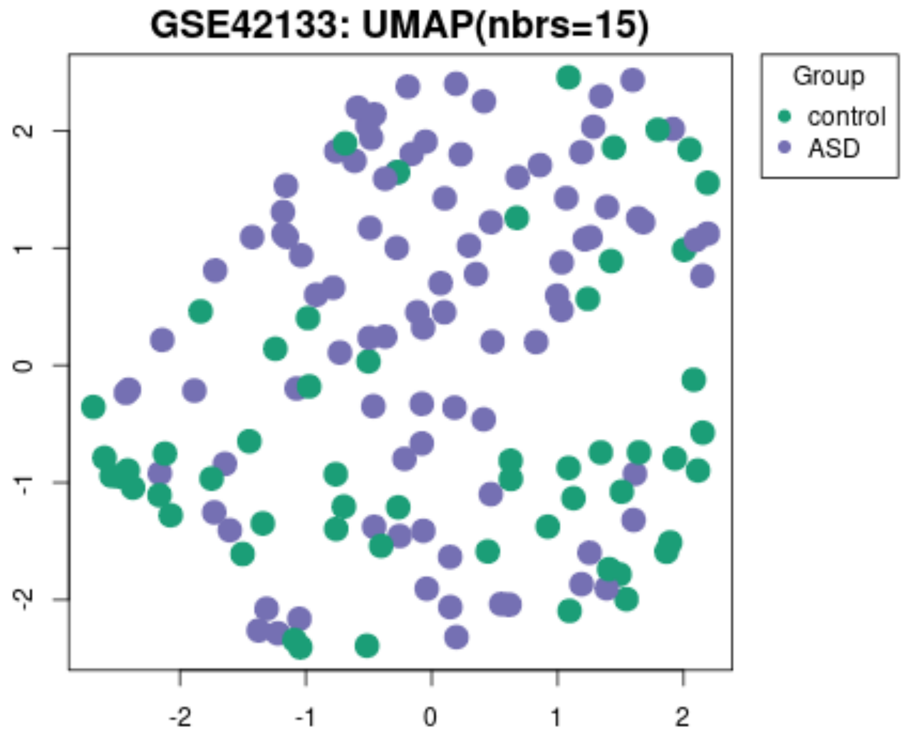
```
################################################################
# General expression data analysis
ex <- exprs(gset)

# box-and-whisker plot
dev.new(width=3+ncol(gset)/6, height=5)
ord <- order(gs)  # order samples by group
palette(c("#1B9E77", "#7570B3", "#E7298A", "#E6AB02", "#D95F02",
          "#66A61E", "#A6761D", "#B32424", "#B324B3", "#666666"))
par(mar=c(7,4,2,1))
title <- paste ("GSE42133", "/", annotation(gset), sep ="")
boxplot(ex[,ord], boxwex=0.6, notch=T, main=title, outline=FALSE, las=2,
col=gs[ord])
legend("topleft", groups, fill=palette(), bty="n")
dev.off()
```



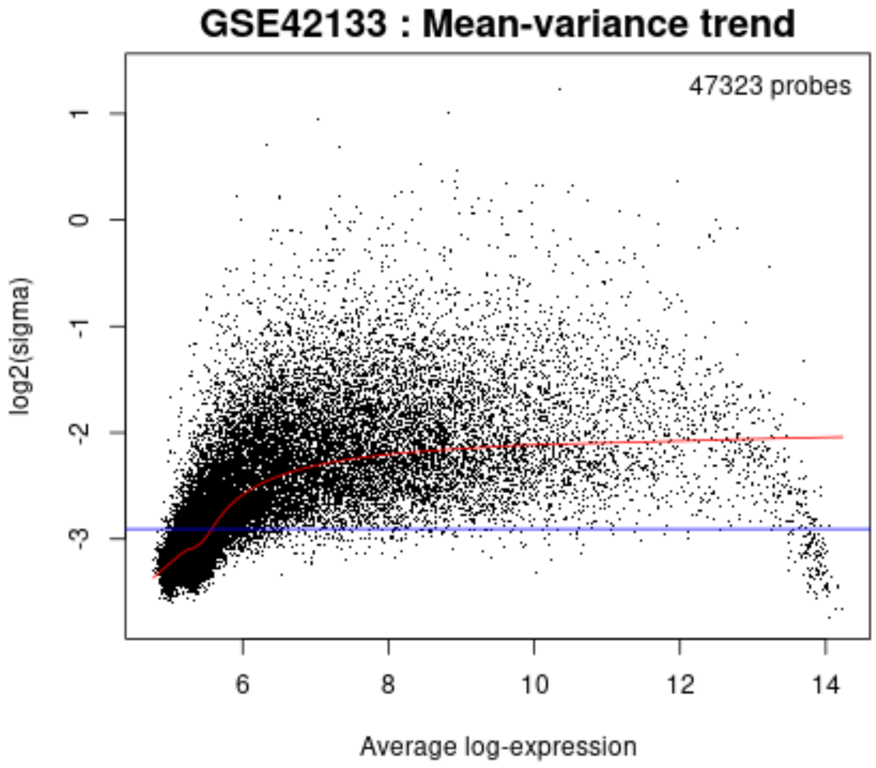
```
# expression value distribution
par(mar=c(4,4,2,1))
title <- paste ("GSE42133", "/", annotation(gset), " value distribution", sep
="")
plotDensities(ex, group=gs, main=title, legend ="topright")
```



```
# UMAP plot (dimensionality reduction)
ex <- na.omit(ex) # eliminate rows with NAs
ex <- ex[!duplicated(ex), ]  # remove duplicates
ump <- umap(t(ex), n_neighbors = 15, random_state = 123)
par(mar=c(3,3,2,6), xpd=TRUE)
plot(ump$layout, main="UMAP plot, nbrs=15", xlab="", ylab="", col=gs, pch=20,
cex=1.5)
legend("topright", inset=c(-0.15,0), legend=levels(gs), pch=20,
col=1:nlevels(gs), title="Group", pt.cex=1.5)
library("maptools")  # point labels without overlaps
pointLabel(ump$layout, labels = rownames(ump$layout), method="SANN", cex=0.6)
```



```
# mean-variance trend, helps to see if precision weights are needed
plotSA(fit2, main="Mean variance trend, GSE42133")
```


GSE42133 : Mean-variance trend
47323 probes
log2(sigma)
Average log-expression