

Group 1: Expression profiling by array analysis

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```
suppressPackageStartupMessages({  
  library(affy)  
  library(arrayQualityMetrics)  
  library(ArrayExpress)  
  library(limma)  
  library(oligo)  
  library(siggenes)  
  library(htmltools)  
  library(biomaRt)  
  library(dplyr)  
  library(GEOquery)  
  library(annotation)  
  library(annotate)  
  library(affycoretools)  
  library(knitr)  
})
```

General info

Platform type: Affymetrix Human Gene 1.0 ST Array [transcript (gene) version].

The GEO dataset with accession ID GSE36980 (expression profiling by array of human postmortal brain tissues including frontal cortex, lateral cortex and hippocampus) will be analysed in a case versus control framework for differential gene expression.

Pipeline

Import intensity values and metadata

The corresponding GEO SOFT file is parsed into an R data structure.

```
# Retrieve GEO accession
id <- "GSE36980"
gse <- getGEO(id, GSEMatrix = TRUE)
gse <- gse[[1]]
```

The supplementary files contain the raw intensity values in the form of CEL files. These must first be downloaded and subsequently unzipped, which this chunk will perform if not done manually from the browser page.

```
# Download and unzip CEL files
unlink(id, recursive = T, force = T)
filePath <- rownames(getGEOSuppFiles(id))
untar(filePath, exdir = paste0(id, "/data"))
for (file in list.files(paste0(id, "/data"), full.names = T)){
  gunzip(file)
}
```

Within the parsed data the experiment annotations can be found. Seeing as the RNA-seq was performed exclusively on hippocampus tissue, we wish to only read in CEL files associated with this tissue type.

```
# Store annotation and filter to only keep hippocampus samples
annot <- pData(gse)
head(annot)
```

```
##               title geo_accession                status
## GSM907792 AD_FC, biological rep1      GSM907792 Public on Apr 17 2013
## GSM907793 AD_FC, biological rep2      GSM907793 Public on Apr 17 2013
## GSM907794 AD_FC, biological rep3      GSM907794 Public on Apr 17 2013
## GSM907795 AD_FC, biological rep4      GSM907795 Public on Apr 17 2013
## GSM907796 AD_FC, biological rep5      GSM907796 Public on Apr 17 2013
## GSM907797 AD_FC, biological rep6      GSM907797 Public on Apr 17 2013
##      submission_date last_update_date type channel_count
## GSM907792      Apr 02 2012      Apr 17 2013  RNA             1
## GSM907793      Apr 02 2012      Apr 17 2013  RNA             1
## GSM907794      Apr 02 2012      Apr 17 2013  RNA             1
## GSM907795      Apr 02 2012      Apr 17 2013  RNA             1
## GSM907796      Apr 02 2012      Apr 17 2013  RNA             1
## GSM907797      Apr 02 2012      Apr 17 2013  RNA             1
##      source_name_ch1 organism_ch1  characteristics_ch1
## GSM907792 Frontal cortex of AD brain Homo sapiens tissue: Frontal cortex
## GSM907793 Frontal cortex of AD brain Homo sapiens tissue: Frontal cortex
## GSM907794 Frontal cortex of AD brain Homo sapiens tissue: Frontal cortex
## GSM907795 Frontal cortex of AD brain Homo sapiens tissue: Frontal cortex
## GSM907796 Frontal cortex of AD brain Homo sapiens tissue: Frontal cortex
## GSM907797 Frontal cortex of AD brain Homo sapiens tissue: Frontal cortex
##      characteristics_ch1.1 characteristics_ch1.2
```

```

## GSM907792          age: 84          Sex: M
## GSM907793          age: 105         Sex: F
## GSM907794          age: 88          Sex: F
## GSM907795          age: 88          Sex: M
## GSM907796          age: 91          Sex: F
## GSM907797          age: 95          Sex: F
##
## GSM907792 During autopsy dissection, parts of the frontal cortex, temporal cortex and hippocampus we
## GSM907793 During autopsy dissection, parts of the frontal cortex, temporal cortex and hippocampus we
## GSM907794 During autopsy dissection, parts of the frontal cortex, temporal cortex and hippocampus we
## GSM907795 During autopsy dissection, parts of the frontal cortex, temporal cortex and hippocampus we
## GSM907796 During autopsy dissection, parts of the frontal cortex, temporal cortex and hippocampus we
## GSM907797 During autopsy dissection, parts of the frontal cortex, temporal cortex and hippocampus we
##
## GSM907792 We examined postmortem human brains donated for the Hisayama study.
## GSM907793 We examined postmortem human brains donated for the Hisayama study.
## GSM907794 We examined postmortem human brains donated for the Hisayama study.
## GSM907795 We examined postmortem human brains donated for the Hisayama study.
## GSM907796 We examined postmortem human brains donated for the Hisayama study.
## GSM907797 We examined postmortem human brains donated for the Hisayama study.
##
## GSM907792          growth_protocol_ch1
## GSM907792          total RNA
## GSM907793          total RNA
## GSM907794          total RNA
## GSM907795          total RNA
## GSM907796          total RNA
## GSM907797          total RNA
##
## GSM907792 Total RNA was isolated using a combination of Isogen (Nippon Gene) and the RNeasy Mini Kit
## GSM907793 Total RNA was isolated using a combination of Isogen (Nippon Gene) and the RNeasy Mini Kit
## GSM907794 Total RNA was isolated using a combination of Isogen (Nippon Gene) and the RNeasy Mini Kit
## GSM907795 Total RNA was isolated using a combination of Isogen (Nippon Gene) and the RNeasy Mini Kit
## GSM907796 Total RNA was isolated using a combination of Isogen (Nippon Gene) and the RNeasy Mini Kit
## GSM907797 Total RNA was isolated using a combination of Isogen (Nippon Gene) and the RNeasy Mini Kit
##
## GSM907792          label_ch1
## GSM907792          biotin
## GSM907793          biotin
## GSM907794          biotin
## GSM907795          biotin
## GSM907796          biotin
## GSM907797          biotin
##
## GSM907792 The Ambion WT Expression Kit and the GeneChip WT Terminal Labeling and Controls Kit (Affym
## GSM907793 The Ambion WT Expression Kit and the GeneChip WT Terminal Labeling and Controls Kit (Affym
## GSM907794 The Ambion WT Expression Kit and the GeneChip WT Terminal Labeling and Controls Kit (Affym
## GSM907795 The Ambion WT Expression Kit and the GeneChip WT Terminal Labeling and Controls Kit (Affym
## GSM907796 The Ambion WT Expression Kit and the GeneChip WT Terminal Labeling and Controls Kit (Affym
## GSM907797 The Ambion WT Expression Kit and the GeneChip WT Terminal Labeling and Controls Kit (Affym
##
## GSM907792          taxid_ch1
## GSM907792          9606
## GSM907793          9606
## GSM907794          9606
## GSM907795          9606
## GSM907796          9606

```

```

## GSM907797      9606
##
## GSM907792 Following fragmentation, 5.5 µg of cDNA were hybridized for 17 hr at 45°C on Affymetrix Human Genome Chip 2.0
## GSM907793 Following fragmentation, 5.5 µg of cDNA were hybridized for 17 hr at 45°C on Affymetrix Human Genome Chip 2.0
## GSM907794 Following fragmentation, 5.5 µg of cDNA were hybridized for 17 hr at 45°C on Affymetrix Human Genome Chip 2.0
## GSM907795 Following fragmentation, 5.5 µg of cDNA were hybridized for 17 hr at 45°C on Affymetrix Human Genome Chip 2.0
## GSM907796 Following fragmentation, 5.5 µg of cDNA were hybridized for 17 hr at 45°C on Affymetrix Human Genome Chip 2.0
## GSM907797 Following fragmentation, 5.5 µg of cDNA were hybridized for 17 hr at 45°C on Affymetrix Human Genome Chip 2.0
##
##                                     scan_protocol
## GSM907792 GeneChips were scanned using the Affymetrix GeneChip® Scanner 3000 7G.
## GSM907793 GeneChips were scanned using the Affymetrix GeneChip® Scanner 3000 7G.
## GSM907794 GeneChips were scanned using the Affymetrix GeneChip® Scanner 3000 7G.
## GSM907795 GeneChips were scanned using the Affymetrix GeneChip® Scanner 3000 7G.
## GSM907796 GeneChips were scanned using the Affymetrix GeneChip® Scanner 3000 7G.
## GSM907797 GeneChips were scanned using the Affymetrix GeneChip® Scanner 3000 7G.
##
##      description
## GSM907792
## GSM907793
## GSM907794
## GSM907795
## GSM907796
## GSM907797
##
##                                     data_processing
## GSM907792 CEL files were imported into Affymetrix Expression Console Software, and CHP files were generated
## GSM907793 CEL files were imported into Affymetrix Expression Console Software, and CHP files were generated
## GSM907794 CEL files were imported into Affymetrix Expression Console Software, and CHP files were generated
## GSM907795 CEL files were imported into Affymetrix Expression Console Software, and CHP files were generated
## GSM907796 CEL files were imported into Affymetrix Expression Console Software, and CHP files were generated
## GSM907797 CEL files were imported into Affymetrix Expression Console Software, and CHP files were generated
##
##      platform_id      contact_name      contact_email
## GSM907792      GPL6244 Yusaku,,Nakabeppu yusaku@bioreg.kyushu-u.ac.jp
## GSM907793      GPL6244 Yusaku,,Nakabeppu yusaku@bioreg.kyushu-u.ac.jp
## GSM907794      GPL6244 Yusaku,,Nakabeppu yusaku@bioreg.kyushu-u.ac.jp
## GSM907795      GPL6244 Yusaku,,Nakabeppu yusaku@bioreg.kyushu-u.ac.jp
## GSM907796      GPL6244 Yusaku,,Nakabeppu yusaku@bioreg.kyushu-u.ac.jp
## GSM907797      GPL6244 Yusaku,,Nakabeppu yusaku@bioreg.kyushu-u.ac.jp
##
##      contact_phone      contact_laboratory
## GSM907792 81-92-642-6800 Division of Neurofunctional Genomics
## GSM907793 81-92-642-6800 Division of Neurofunctional Genomics
## GSM907794 81-92-642-6800 Division of Neurofunctional Genomics
## GSM907795 81-92-642-6800 Division of Neurofunctional Genomics
## GSM907796 81-92-642-6800 Division of Neurofunctional Genomics
## GSM907797 81-92-642-6800 Division of Neurofunctional Genomics
##
##      contact_department
## GSM907792 Department of Immunobiology and Neuroscience
## GSM907793 Department of Immunobiology and Neuroscience
## GSM907794 Department of Immunobiology and Neuroscience
## GSM907795 Department of Immunobiology and Neuroscience
## GSM907796 Department of Immunobiology and Neuroscience
## GSM907797 Department of Immunobiology and Neuroscience
##
##      contact_institute
## GSM907792 Medical Institute of Bioregulation, Kyushu University
## GSM907793 Medical Institute of Bioregulation, Kyushu University
## GSM907794 Medical Institute of Bioregulation, Kyushu University

```

```

## GSM907795 Medical Institute of Bioregulation, Kyushu University
## GSM907796 Medical Institute of Bioregulation, Kyushu University
## GSM907797 Medical Institute of Bioregulation, Kyushu University
##           contact_address contact_city contact_state
## GSM907792 3-1-1 Maidashi Higashi-ku      Fukuoka      Fukuoka
## GSM907793 3-1-1 Maidashi Higashi-ku      Fukuoka      Fukuoka
## GSM907794 3-1-1 Maidashi Higashi-ku      Fukuoka      Fukuoka
## GSM907795 3-1-1 Maidashi Higashi-ku      Fukuoka      Fukuoka
## GSM907796 3-1-1 Maidashi Higashi-ku      Fukuoka      Fukuoka
## GSM907797 3-1-1 Maidashi Higashi-ku      Fukuoka      Fukuoka
##           contact_zip/postal_code contact_country
## GSM907792           812-8582           Japan
## GSM907793           812-8582           Japan
## GSM907794           812-8582           Japan
## GSM907795           812-8582           Japan
## GSM907796           812-8582           Japan
## GSM907797           812-8582           Japan
##
##                                           supplementary_file
## GSM907792 ftp://ftp.ncbi.nlm.nih.gov/geo/samples/GSM907nnn/GSM907792/suppl/GSM907792.CEL.gz
## GSM907793 ftp://ftp.ncbi.nlm.nih.gov/geo/samples/GSM907nnn/GSM907793/suppl/GSM907793.CEL.gz
## GSM907794 ftp://ftp.ncbi.nlm.nih.gov/geo/samples/GSM907nnn/GSM907794/suppl/GSM907794.CEL.gz
## GSM907795 ftp://ftp.ncbi.nlm.nih.gov/geo/samples/GSM907nnn/GSM907795/suppl/GSM907795.CEL.gz
## GSM907796 ftp://ftp.ncbi.nlm.nih.gov/geo/samples/GSM907nnn/GSM907796/suppl/GSM907796.CEL.gz
## GSM907797 ftp://ftp.ncbi.nlm.nih.gov/geo/samples/GSM907nnn/GSM907797/suppl/GSM907797.CEL.gz
##
##                                           supplementary_file.1
## GSM907792 ftp://ftp.ncbi.nlm.nih.gov/geo/samples/GSM907nnn/GSM907792/suppl/GSM907792.chp.gz
## GSM907793 ftp://ftp.ncbi.nlm.nih.gov/geo/samples/GSM907nnn/GSM907793/suppl/GSM907793.chp.gz
## GSM907794 ftp://ftp.ncbi.nlm.nih.gov/geo/samples/GSM907nnn/GSM907794/suppl/GSM907794.chp.gz
## GSM907795 ftp://ftp.ncbi.nlm.nih.gov/geo/samples/GSM907nnn/GSM907795/suppl/GSM907795.chp.gz
## GSM907796 ftp://ftp.ncbi.nlm.nih.gov/geo/samples/GSM907nnn/GSM907796/suppl/GSM907796.chp.gz
## GSM907797 ftp://ftp.ncbi.nlm.nih.gov/geo/samples/GSM907nnn/GSM907797/suppl/GSM907797.chp.gz
##
##           data_row_count age:ch1 Sex:ch1      tissue:ch1
## GSM907792           33297      84      M Frontal cortex
## GSM907793           33297     105      F Frontal cortex
## GSM907794           33297      88      F Frontal cortex
## GSM907795           33297      88      M Frontal cortex
## GSM907796           33297      91      F Frontal cortex
## GSM907797           33297      95      F Frontal cortex

```

```

# Filter to only keep annotation of hippocampus samples
temp <- annot[annot$characteristics_ch1 == "tissue: Hippocampus",]
annot.filt <- temp[order(temp$title),] %>%
  mutate(disease = c(rep("AD", 8), rep("CT", 10))) %>%
  AnnotatedDataFrame()

```

```

# Only read in CEL files associated with hippocampus
acc <- annot.filt$geo_accession
celfiles <- list.celfiles(paste0(id, "/data"), full.names = T)
cels.hippo <- vector()
for (i in acc){
  cels.hippo <- append(cels.hippo, grep(i, celfiles, value = T))
}

data.raw <- read.celfiles(cels.hippo, verbose=T, phenoData=annot.filt)

```

```
## Reading in : GSE36980/data/GSM907854.CEL
## Reading in : GSE36980/data/GSM907855.CEL
## Reading in : GSE36980/data/GSM907856.CEL
## Reading in : GSE36980/data/GSM907857.CEL
## Reading in : GSE36980/data/GSM907858.CEL
## Reading in : GSE36980/data/GSM907859.CEL
## Reading in : GSE36980/data/GSM907860.CEL
## Reading in : GSE36980/data/GSM4764672_AD_17_HI_HuGene-1_0-st-v1_.CEL
## Reading in : GSE36980/data/GSM907861.CEL
## Reading in : GSE36980/data/GSM907870.CEL
## Reading in : GSE36980/data/GSM907862.CEL
## Reading in : GSE36980/data/GSM907863.CEL
## Reading in : GSE36980/data/GSM907864.CEL
## Reading in : GSE36980/data/GSM907865.CEL
## Reading in : GSE36980/data/GSM907866.CEL
## Reading in : GSE36980/data/GSM907867.CEL
## Reading in : GSE36980/data/GSM907868.CEL
## Reading in : GSE36980/data/GSM907869.CEL
```

```
head(exprs(data.raw))
```

```
##      GSM907854 GSM907855 GSM907856 GSM907857 GSM907858 GSM907859 GSM907860
## 1          7862          8639          10936          6124          8415          8700          7956
## 2           132           146            111            65           129           112            57
## 3          9083          9101          10215          6122          8398          9006          8065
## 4           137           206            95            58            89            57            74
## 5           198           221            94            79           122            46           106
## 6            95           150            79            44            72            84            43
##      GSM4764672 GSM907861 GSM907870 GSM907862 GSM907863 GSM907864 GSM907865
## 1          11041          9676          7924          8313          6879          8569          8712
## 2            61           132            96           141           120           128            82
## 3          11667          9703          7844          8839          7946          9412          8898
## 4            74            92            97           102           140           123           134
## 5            54            62            66           148           136           134            79
## 6            54            76            40            87           105            99           113
##      GSM907866 GSM907867 GSM907868 GSM907869
## 1          9326          9082          6632          8651
## 2           105            87            59           102
## 3          9830          8803          6362          8722
## 4            61            63            47            56
## 5           151            63           123            85
## 6           111           137            51            43
```

Quality Control on raw data

We perform quality control on both the remaining pure raw data and log-transformed raw data. A summary of each report can be assessed by opening the “index.html”-file.

```
arrayQualityMetrics(data.raw, outdir="raw", force=T)
arrayQualityMetrics(data.raw, outdir="rawlog", force=T, do.logtransform=T)
```

Preprocessing of raw data

Currently, there are 10 control samples versus 8 cases, which is an acceptable ratio. We still choose to remove sample ‘GSM4764672’ as this was shown in the QC to be a severe outlier, most likely due to this sample being taken at a much later date than the rest.

Background correction, normalization and summarization are subsequently performed with the ‘oligo’ package.

```
# Remove outliers based on QC
r <- c("GSM4764672")
cels.hippo.filt <- cels.hippo[!grepl(paste(r,collapse="|"), cels.hippo)]

annot.filt <- pData(data.raw)

annot.filt <- annot.filt %>%
  filter(!(geo_accession %in% r)) %>%
  AnnotatedDataFrame()

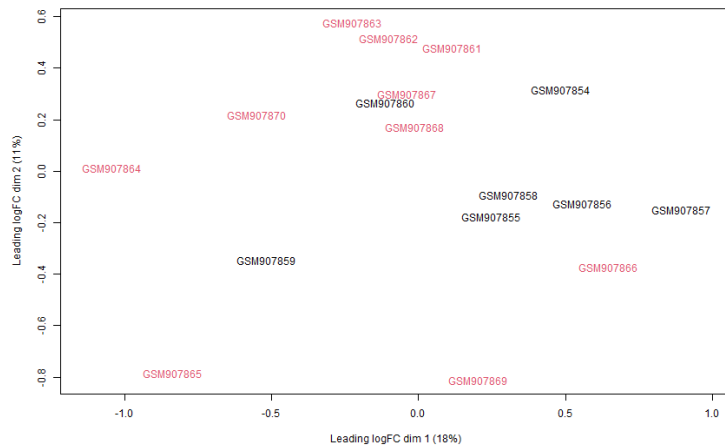
data.raw <- read.celfiles(cels.hippo.filt, verbose=T, phenoData=annot.filt)
```

```
## Reading in : GSE36980/data/GSM907854.CEL
## Reading in : GSE36980/data/GSM907855.CEL
## Reading in : GSE36980/data/GSM907856.CEL
## Reading in : GSE36980/data/GSM907857.CEL
## Reading in : GSE36980/data/GSM907858.CEL
## Reading in : GSE36980/data/GSM907859.CEL
## Reading in : GSE36980/data/GSM907860.CEL
## Reading in : GSE36980/data/GSM907861.CEL
## Reading in : GSE36980/data/GSM907870.CEL
## Reading in : GSE36980/data/GSM907862.CEL
## Reading in : GSE36980/data/GSM907863.CEL
## Reading in : GSE36980/data/GSM907864.CEL
## Reading in : GSE36980/data/GSM907865.CEL
## Reading in : GSE36980/data/GSM907866.CEL
## Reading in : GSE36980/data/GSM907867.CEL
## Reading in : GSE36980/data/GSM907868.CEL
## Reading in : GSE36980/data/GSM907869.CEL
```

```
# RMA with oligo
data.norm <- oligo::rma(data.raw,target = "core")
```

```
## Background correcting
## Normalizing
## Calculating Expression
```

```
annot.filt <- pData(data.norm) %>%
  rename(sex = "Sex:ch1") %>%
  mutate(sex = factor(sex)) %>%
  mutate(disease = factor(disease)) %>%
  mutate(patient_id = factor(paste0(rep("p", 17), 1:17)))
plotMDS(data.norm, top = 500, col=as.double(annot.filt$disease))
```

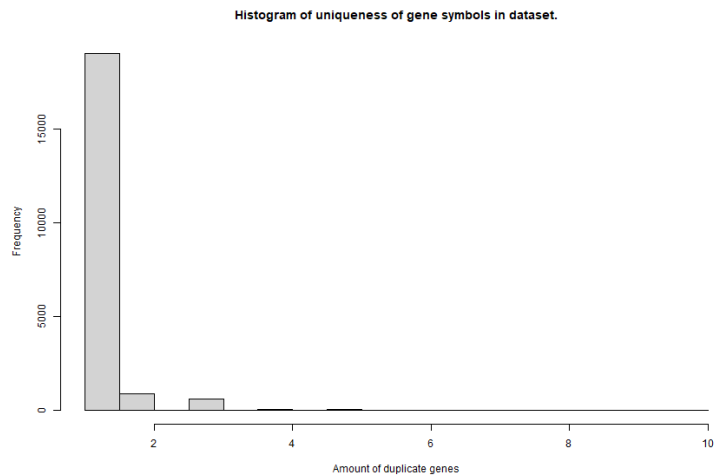


The probeIDs must be converted into gene IDs with a library before they can be used for differential gene expression analysis.

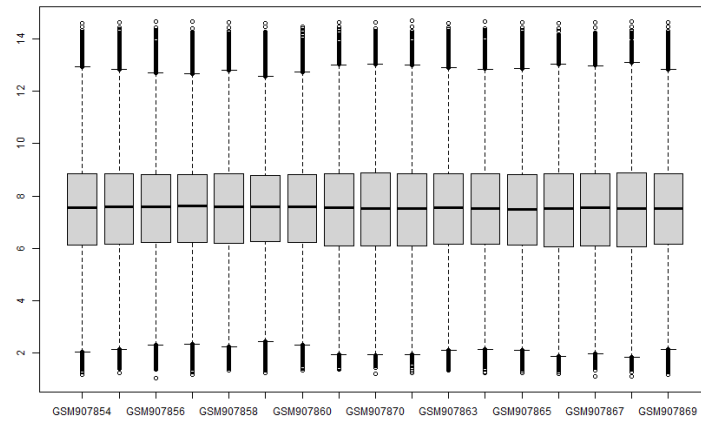
```
library(hugene10sttranscriptcluster.db)
ID <- featureNames(data.norm)

geneAcc <- hugene10sttranscriptclusterENSEMBL[ID]
rownames(data.norm) <- paste(as.character(c(1:nrow(data.norm)))), geneAcc, sep=".")

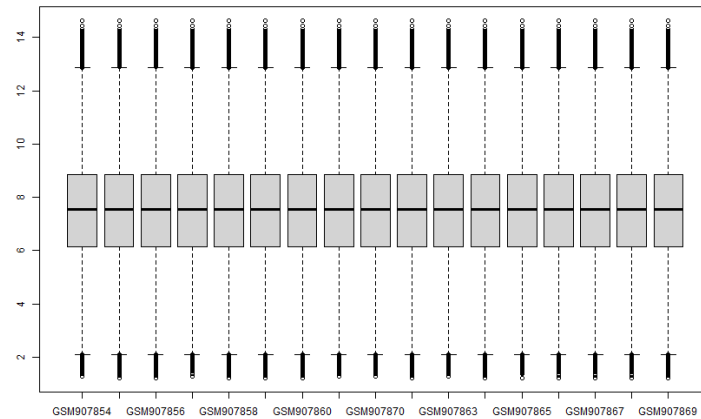
hist(table(as.factor(as.character(geneAcc))), xlab = "Amount of duplicate genes", main="Histogram of un
```



```
d <- exprs(data.norm)
boxplot(d)
```

```
d2 <- normalizeQuantiles(d)
boxplot(d2)
```



```
humanRMA <- d
```

Quality control on preprocessed data

```
QC <- arrayQualityMetrics(data.norm, outdir="rma", force=TRUE, intgroup = c("age:ch1")) #RMA produces l
```

Differential expression analysis with RMA preprocessed data

```
head(pData(data.norm))
```

```
##               title geo_accession          status
## GSM907854 AD_HI, biological rep1    GSM907854 Public on Apr 17 2013
```

```

## GSM907855 AD_HI, biological rep2      GSM907855 Public on Apr 17 2013
## GSM907856 AD_HI, biological rep3      GSM907856 Public on Apr 17 2013
## GSM907857 AD_HI, biological rep4      GSM907857 Public on Apr 17 2013
## GSM907858 AD_HI, biological rep5      GSM907858 Public on Apr 17 2013
## GSM907859 AD_HI, biological rep6      GSM907859 Public on Apr 17 2013
##      submission_date last_update_date type channel_count
## GSM907854      Apr 02 2012      Apr 17 2013  RNA              1
## GSM907855      Apr 02 2012      Apr 17 2013  RNA              1
## GSM907856      Apr 02 2012      Apr 17 2013  RNA              1
## GSM907857      Apr 02 2012      Apr 17 2013  RNA              1
## GSM907858      Apr 02 2012      Apr 17 2013  RNA              1
## GSM907859      Apr 02 2012      Apr 17 2013  RNA              1
##      source_name_ch1 organism_ch1 characteristics_ch1
## GSM907854 Hippocampus of of AD brain Homo sapiens tissue: Hippocampus
## GSM907855 Hippocampus of of AD brain Homo sapiens tissue: Hippocampus
## GSM907856 Hippocampus of of AD brain Homo sapiens tissue: Hippocampus
## GSM907857 Hippocampus of of AD brain Homo sapiens tissue: Hippocampus
## GSM907858 Hippocampus of of AD brain Homo sapiens tissue: Hippocampus
## GSM907859 Hippocampus of of AD brain Homo sapiens tissue: Hippocampus
##      characteristics_ch1.1 characteristics_ch1.2
## GSM907854      age: 88      Sex: F
## GSM907855      age: 95      Sex: F
## GSM907856      age: 95      Sex: F
## GSM907857      age: 100     Sex: F
## GSM907858      age: 99      Sex: M
## GSM907859      age: 83      Sex: M
##
## GSM907854 During autopsy dissection, parts of the frontal cortex, temporal cortex and hippocampus we
## GSM907855 During autopsy dissection, parts of the frontal cortex, temporal cortex and hippocampus we
## GSM907856 During autopsy dissection, parts of the frontal cortex, temporal cortex and hippocampus we
## GSM907857 During autopsy dissection, parts of the frontal cortex, temporal cortex and hippocampus we
## GSM907858 During autopsy dissection, parts of the frontal cortex, temporal cortex and hippocampus we
## GSM907859 During autopsy dissection, parts of the frontal cortex, temporal cortex and hippocampus we
##      growth_protocol_ch1
## GSM907854 We examined postmortem human brains donated for the Hisayama study.
## GSM907855 We examined postmortem human brains donated for the Hisayama study.
## GSM907856 We examined postmortem human brains donated for the Hisayama study.
## GSM907857 We examined postmortem human brains donated for the Hisayama study.
## GSM907858 We examined postmortem human brains donated for the Hisayama study.
## GSM907859 We examined postmortem human brains donated for the Hisayama study.
##      molecule_ch1
## GSM907854      total RNA
## GSM907855      total RNA
## GSM907856      total RNA
## GSM907857      total RNA
## GSM907858      total RNA
## GSM907859      total RNA
##
## GSM907854 Total RNA was isolated using a combination of Isogen (Nippon Gene) and the RNeasy Mini Kit
## GSM907855 Total RNA was isolated using a combination of Isogen (Nippon Gene) and the RNeasy Mini Kit
## GSM907856 Total RNA was isolated using a combination of Isogen (Nippon Gene) and the RNeasy Mini Kit
## GSM907857 Total RNA was isolated using a combination of Isogen (Nippon Gene) and the RNeasy Mini Kit
## GSM907858 Total RNA was isolated using a combination of Isogen (Nippon Gene) and the RNeasy Mini Kit
## GSM907859 Total RNA was isolated using a combination of Isogen (Nippon Gene) and the RNeasy Mini Kit

```

```

##          label_ch1
## GSM907854      biotin
## GSM907855      biotin
## GSM907856      biotin
## GSM907857      biotin
## GSM907858      biotin
## GSM907859      biotin
##
## GSM907854 The Ambion WT Expression Kit and the GeneChip WT Terminal Labeling and Controls Kit (Affymetrix)
## GSM907855 The Ambion WT Expression Kit and the GeneChip WT Terminal Labeling and Controls Kit (Affymetrix)
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## GSM907859 The Ambion WT Expression Kit and the GeneChip WT Terminal Labeling and Controls Kit (Affymetrix)
##          taxid_ch1
## GSM907854      9606
## GSM907855      9606
## GSM907856      9606
## GSM907857      9606
## GSM907858      9606
## GSM907859      9606
##
## GSM907854 Following fragmentation, 5.5 µg of cDNA were hybridized for 17 hr at 45°C on Affymetrix Human Genome U133A Array
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## GSM907859 Following fragmentation, 5.5 µg of cDNA were hybridized for 17 hr at 45°C on Affymetrix Human Genome U133A Array
##          scan_protocol
## GSM907854 GeneChips were scanned using the Affymetrix GeneChip® Scanner 3000 7G.
## GSM907855 GeneChips were scanned using the Affymetrix GeneChip® Scanner 3000 7G.
## GSM907856 GeneChips were scanned using the Affymetrix GeneChip® Scanner 3000 7G.
## GSM907857 GeneChips were scanned using the Affymetrix GeneChip® Scanner 3000 7G.
## GSM907858 GeneChips were scanned using the Affymetrix GeneChip® Scanner 3000 7G.
## GSM907859 GeneChips were scanned using the Affymetrix GeneChip® Scanner 3000 7G.
##          description
## GSM907854
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##
##          data_processing
## GSM907854 CEL files were imported into Affymetrix Expression Console Software, and CHP files were generated
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##          platform_id      contact_name      contact_email
## GSM907854      GPL6244 Yusaku,,Nakabeppu yusaku@bioreg.kyushu-u.ac.jp
## GSM907855      GPL6244 Yusaku,,Nakabeppu yusaku@bioreg.kyushu-u.ac.jp
## GSM907856      GPL6244 Yusaku,,Nakabeppu yusaku@bioreg.kyushu-u.ac.jp
## GSM907857      GPL6244 Yusaku,,Nakabeppu yusaku@bioreg.kyushu-u.ac.jp

```

```

## GSM907858      GPL6244 Yusaku,,Nakabeppu yusaku@bioreg.kyushu-u.ac.jp
## GSM907859      GPL6244 Yusaku,,Nakabeppu yusaku@bioreg.kyushu-u.ac.jp
##               contact_phone               contact_laboratory
## GSM907854 81-92-642-6800 Division of Neurofunctional Genomics
## GSM907855 81-92-642-6800 Division of Neurofunctional Genomics
## GSM907856 81-92-642-6800 Division of Neurofunctional Genomics
## GSM907857 81-92-642-6800 Division of Neurofunctional Genomics
## GSM907858 81-92-642-6800 Division of Neurofunctional Genomics
## GSM907859 81-92-642-6800 Division of Neurofunctional Genomics
##               contact_department
## GSM907854 Department of Immunobiology and Neuroscience
## GSM907855 Department of Immunobiology and Neuroscience
## GSM907856 Department of Immunobiology and Neuroscience
## GSM907857 Department of Immunobiology and Neuroscience
## GSM907858 Department of Immunobiology and Neuroscience
## GSM907859 Department of Immunobiology and Neuroscience
##               contact_institute
## GSM907854 Medical Institute of Bioregulation, Kyushu University
## GSM907855 Medical Institute of Bioregulation, Kyushu University
## GSM907856 Medical Institute of Bioregulation, Kyushu University
## GSM907857 Medical Institute of Bioregulation, Kyushu University
## GSM907858 Medical Institute of Bioregulation, Kyushu University
## GSM907859 Medical Institute of Bioregulation, Kyushu University
##               contact_address contact_city contact_state
## GSM907854 3-1-1 Maidashi Higashi-ku      Fukuoka      Fukuoka
## GSM907855 3-1-1 Maidashi Higashi-ku      Fukuoka      Fukuoka
## GSM907856 3-1-1 Maidashi Higashi-ku      Fukuoka      Fukuoka
## GSM907857 3-1-1 Maidashi Higashi-ku      Fukuoka      Fukuoka
## GSM907858 3-1-1 Maidashi Higashi-ku      Fukuoka      Fukuoka
## GSM907859 3-1-1 Maidashi Higashi-ku      Fukuoka      Fukuoka
##               contact_zip/postal_code contact_country
## GSM907854      812-8582      Japan
## GSM907855      812-8582      Japan
## GSM907856      812-8582      Japan
## GSM907857      812-8582      Japan
## GSM907858      812-8582      Japan
## GSM907859      812-8582      Japan
##               supplementary_file
## GSM907854 ftp://ftp.ncbi.nlm.nih.gov/geo/samples/GSM907nnn/GSM907854/suppl/GSM907854.CEL.gz
## GSM907855 ftp://ftp.ncbi.nlm.nih.gov/geo/samples/GSM907nnn/GSM907855/suppl/GSM907855.CEL.gz
## GSM907856 ftp://ftp.ncbi.nlm.nih.gov/geo/samples/GSM907nnn/GSM907856/suppl/GSM907856.CEL.gz
## GSM907857 ftp://ftp.ncbi.nlm.nih.gov/geo/samples/GSM907nnn/GSM907857/suppl/GSM907857.CEL.gz
## GSM907858 ftp://ftp.ncbi.nlm.nih.gov/geo/samples/GSM907nnn/GSM907858/suppl/GSM907858.CEL.gz
## GSM907859 ftp://ftp.ncbi.nlm.nih.gov/geo/samples/GSM907nnn/GSM907859/suppl/GSM907859.CEL.gz
##               supplementary_file.1
## GSM907854 ftp://ftp.ncbi.nlm.nih.gov/geo/samples/GSM907nnn/GSM907854/suppl/GSM907854.chp.gz
## GSM907855 ftp://ftp.ncbi.nlm.nih.gov/geo/samples/GSM907nnn/GSM907855/suppl/GSM907855.chp.gz
## GSM907856 ftp://ftp.ncbi.nlm.nih.gov/geo/samples/GSM907nnn/GSM907856/suppl/GSM907856.chp.gz
## GSM907857 ftp://ftp.ncbi.nlm.nih.gov/geo/samples/GSM907nnn/GSM907857/suppl/GSM907857.chp.gz
## GSM907858 ftp://ftp.ncbi.nlm.nih.gov/geo/samples/GSM907nnn/GSM907858/suppl/GSM907858.chp.gz
## GSM907859 ftp://ftp.ncbi.nlm.nih.gov/geo/samples/GSM907nnn/GSM907859/suppl/GSM907859.chp.gz
##               data_row_count age:ch1 Sex:ch1 tissue:ch1 disease
## GSM907854      33297      88      F Hippocampus      AD
## GSM907855      33297      95      F Hippocampus      AD

```

```
## GSM907856      33297      95      F Hippocampus      AD
## GSM907857      33297     100      F Hippocampus      AD
## GSM907858      33297      99      M Hippocampus      AD
## GSM907859      33297      83      M Hippocampus      AD
```

```
head(humanRMA)
```

```
##
## GSM907854 GSM907855 GSM907856 GSM907857 GSM907858 GSM907859
## 1.ENSG00000225972 5.373545 2.472182 3.635325 2.838655 4.508038 2.395272
## 2.ENSG00000228794 5.704015 4.245984 5.281775 5.026610 4.895309 4.609310
## 3.ENSG00000187634 4.161747 4.806782 3.907872 5.184441 4.805272 5.159105
## 4.ENSG00000187961 10.077611 10.418894 10.235572 10.336492 10.188078 10.095389
## 5.ENSG00000187583 3.486729 4.390324 3.879165 4.682291 3.926791 3.463152
## 6.ENSG00000187608 4.831227 4.432991 5.462134 5.198932 5.561691 4.466113
##
## GSM907860 GSM907861 GSM907870 GSM907862 GSM907863 GSM907864
## 1.ENSG00000225972 4.713447 4.918408 5.102253 4.868611 3.445955 3.425331
## 2.ENSG00000228794 6.256081 5.246101 5.677444 5.130609 5.595823 4.552713
## 3.ENSG00000187634 5.017246 5.173290 4.975246 4.097729 4.928699 4.266874
## 4.ENSG00000187961 10.204295 10.165844 9.723007 10.009661 9.668018 10.067589
## 5.ENSG00000187583 3.625267 4.447447 5.444197 4.116339 4.726618 3.587965
## 6.ENSG00000187608 5.313742 4.824642 5.187788 5.271999 4.109134 5.088669
##
## GSM907865 GSM907866 GSM907867 GSM907868 GSM907869
## 1.ENSG00000225972 5.260321 4.338701 3.251536 3.000522 3.685577
## 2.ENSG00000228794 3.871669 5.001110 5.302094 4.913317 4.806092
## 3.ENSG00000187634 4.773060 4.995980 4.635103 4.769816 5.182605
## 4.ENSG00000187961 10.012460 10.188266 9.943303 10.316292 10.503210
## 5.ENSG00000187583 4.722337 3.802165 3.485902 4.496879 5.062478
## 6.ENSG00000187608 3.883368 4.827659 5.691601 4.842977 4.337667
```

```
## Differential expression by LIMMA
design <- model.matrix(~sex+disease, data = annot.filt)
fit <- lmFit(data.norm, design)

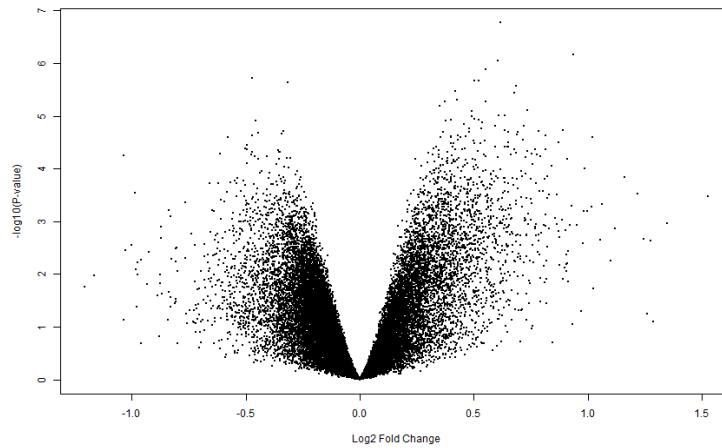
cont.matrix <- makeContrasts(ADvsCT="diseaseCT",levels=design)
rownames(cont.matrix)[1] <- "(Intercept)"

fit2 <- contrasts.fit(fit, cont.matrix)
fit2 <- eBayes(fit2)

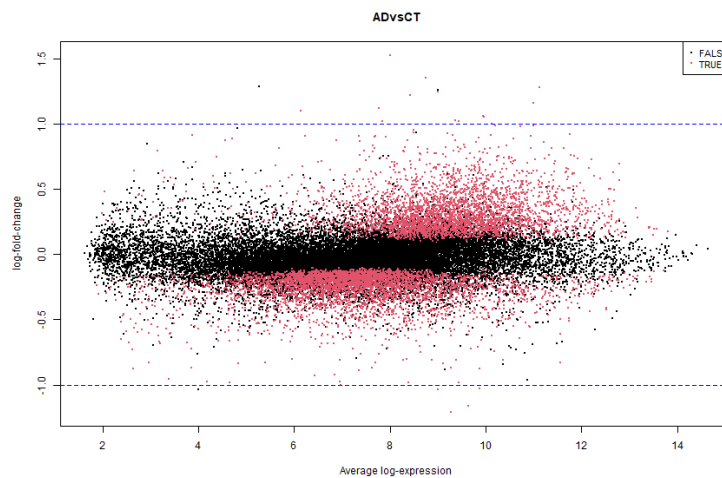
results <- decideTests(fit2)
summary(results)
```

```
## ADvsCT
## Down 420
## NotSig 32078
## Up 799
```

```
# Volcano plot
volcanoplot(fit2)
```



```
# MA plot
p <- fit2$p.value < 0.05
limma::plotMA(fit2, status = p, hl.cex = 0.1)
abline(h=c(1,-1), col=c("blue", "blue"), lty=c(2,2))
```



```
# DE results
LIMMAout_RMA <- topTable(fit2, adjust="BH", number=nrow(data.norm))
head(LIMMAout_RMA, n=10)
```

##		logFC	AveExpr	t	P.Value	adj.P.Val
##	33250.ENSEG000000242779	0.6161607	11.814335	8.428341	1.675760e-07	0.005579778
##	14868.ENSEG000000087008	0.9353745	8.488672	7.613016	6.759165e-07	0.009510968
##	20726.ENSEG000000156313	0.6073496	7.709785	7.456015	8.930147e-07	0.009510968
##	9948.ENSEG000000160472	0.5512948	9.784034	7.246011	1.302871e-06	0.009510968
##	20772.ENSEG000000274588	-0.4732591	7.186132	-7.046497	1.875616e-06	0.009510968
##	18268.ENSEG000000127399	0.5039090	10.022263	6.974057	2.143775e-06	0.009510968
##	17345.ENSEG000000234280	0.5200456	11.898881	6.972256	2.150928e-06	0.009510968
##	21474.ENSEG000000196299	-0.3162402	8.348068	-6.939580	2.285123e-06	0.009510968
##	4762.ENSEG000000064102	0.6870980	10.096402	6.851689	2.691034e-06	0.009955930

```
## 15185.ENSG00000177300 0.4186793 10.393287 6.732802 3.362787e-06 0.011081186
## B
## 33250.ENSG00000242779 7.174066
## 14868.ENSG00000087008 5.980536
## 20726.ENSG00000156313 5.738539
## 9948.ENSG00000160472 5.408578
## 20772.ENSG00000274588 5.088416
## 18268.ENSG00000127399 4.970554
## 17345.ENSG00000234280 4.967613
## 21474.ENSG00000196299 4.914156
## 4762.ENSG00000064102 4.769498
## 15185.ENSG00000177300 4.571803
```

Gene set analysis

```
LIMMAout_filtered <- LIMMAout_RMA[LIMMAout_RMA$adj.P.Val < 0.1, ]
EntrezIDs <- mapIds(hugene10sttranscriptcluster.db, gsub('.*\\.', '', rownames(LIMMAout_filtered)), 'ENTREZID')

# Subset for non duplicated and mapped genes
IDs_unique <- EntrezIDs[!(duplicated(EntrezIDs) | is.na(EntrezIDs))]

goanaOut <- goana(de=IDs_unique, species="Hs", trend=T)
goanaOut <- goanaOut[order(goanaOut$P.DE, decreasing=FALSE), ]
goanaOut$FDR.DE <- p.adjust(goanaOut$P.DE, method="BH")
topGOarray <- topGO(goanaOut, ontology="BP", number=50)
topGOarray
```

##	Term
## GO:0009987	cellular process
## GO:1901564	organonitrogen compound metabolic process
## GO:0044237	cellular metabolic process
## GO:0043507	positive regulation of JUN kinase activity
## GO:0002396	MHC protein complex assembly
## GO:0002501	peptide antigen assembly with MHC protein complex
## GO:0006807	nitrogen compound metabolic process
## GO:0071396	cellular response to lipid
## GO:0051716	cellular response to stimulus
## GO:0072077	renal vesicle morphogenesis
## GO:0050896	response to stimulus
## GO:0051259	protein complex oligomerization
## GO:0072283	metanephric renal vesicle morphogenesis
## GO:0071248	cellular response to metal ion
## GO:0044238	primary metabolic process
## GO:0008150	biological process
## GO:0050794	regulation of cellular process
## GO:0019538	protein metabolic process
## GO:0072087	renal vesicle development
## GO:0044260	cellular macromolecule metabolic process
## GO:0035556	intracellular signal transduction
## GO:0048545	response to steroid hormone
## GO:0002399	MHC class II protein complex assembly
## GO:0002503	peptide antigen assembly with MHC class II protein complex

G0:0045600 positive regulation of fat cell differentiation
 ## G0:0071383 cellular response to steroid hormone stimulus
 ## G0:0061900 glial cell activation
 ## G0:0009058 biosynthetic process
 ## G0:0071241 cellular response to inorganic substance
 ## G0:0051260 protein homooligomerization
 ## G0:0031323 regulation of cellular metabolic process
 ## G0:0033993 response to lipid
 ## G0:1901576 organic substance biosynthetic process
 ## G0:0033962 P-body assembly
 ## G0:0051171 regulation of nitrogen compound metabolic process
 ## G0:0044249 cellular biosynthetic process
 ## G0:0071840 cellular component organization or biogenesis
 ## G0:0034660 ncRNA metabolic process
 ## G0:0001774 microglial cell activation
 ## G0:0042273 ribosomal large subunit biogenesis
 ## G0:0002495 antigen processing and presentation of peptide antigen via MHC class II
 ## G0:0061355 Wnt protein secretion
 ## G0:0019886 antigen processing and presentation of exogenous peptide antigen via MHC class II
 ## G0:0043506 regulation of JUN kinase activity
 ## G0:0007154 cell communication
 ## G0:0006950 response to stress
 ## G0:0010038 response to metal ion
 ## G0:0150076 neuroinflammatory response
 ## G0:0045598 regulation of fat cell differentiation
 ## G0:0035641 locomotory exploration behavior
 ##

	Ont	N	DE	P.DE	FDR.DE
## G0:0009987	BP	17266	2557	7.884158e-09	4.252359e-05
## G0:1901564	BP	6535	1019	6.408401e-05	1.051115e-01
## G0:0044237	BP	10271	1554	8.867290e-05	1.357464e-01
## G0:0043507	BP	43	16	1.538807e-04	1.980686e-01
## G0:0002396	BP	20	10	1.552600e-04	1.980686e-01
## G0:0002501	BP	20	10	1.552600e-04	1.980686e-01
## G0:0006807	BP	10310	1555	1.794420e-04	2.168698e-01
## G0:0071396	BP	608	118	2.102327e-04	2.413787e-01
## G0:0051716	BP	7653	1172	2.732974e-04	2.988442e-01
## G0:0072077	BP	18	9	3.351098e-04	3.497785e-01
## G0:0050896	BP	9216	1394	3.862034e-04	3.634716e-01
## G0:0051259	BP	247	55	3.917251e-04	3.634716e-01
## G0:0072283	BP	15	8	4.151627e-04	3.634716e-01
## G0:0071248	BP	198	46	4.273715e-04	3.634716e-01
## G0:0044238	BP	10825	1621	5.032971e-04	3.873451e-01
## G0:0008150	BP	18903	2732	5.088538e-04	3.873451e-01
## G0:0050794	BP	11335	1693	5.120033e-04	3.873451e-01
## G0:0019538	BP	5494	854	5.502308e-04	3.873451e-01
## G0:0072087	BP	19	9	5.566515e-04	3.873451e-01
## G0:0044260	BP	3266	524	6.695712e-04	4.393718e-01
## G0:0035556	BP	2673	435	6.835287e-04	4.393718e-01
## G0:0048545	BP	339	70	7.044401e-04	4.393718e-01
## G0:0002399	BP	16	8	7.270883e-04	4.393718e-01
## G0:0002503	BP	16	8	7.270883e-04	4.393718e-01
## G0:0045600	BP	72	21	7.737223e-04	4.458274e-01
## G0:0071383	BP	209	47	7.960164e-04	4.458274e-01
## G0:0061900	BP	54	17	9.329003e-04	5.100521e-01


```
## G0:0009058 BP 5962 918 1.044661e-03 5.578733e-01
## G0:0071241 BP 229 50 1.108461e-03 5.783705e-01
## G0:0051260 BP 190 43 1.133418e-03 5.783705e-01
## G0:0031323 BP 5737 884 1.252331e-03 6.044936e-01
## G0:0033993 BP 918 163 1.263585e-03 6.044936e-01
## G0:1901576 BP 5877 904 1.322674e-03 6.074514e-01
## G0:0033962 BP 21 9 1.354811e-03 6.100102e-01
## G0:0051171 BP 5792 891 1.447683e-03 6.392911e-01
## G0:0044249 BP 5807 893 1.485936e-03 6.428746e-01
## G0:0071840 BP 6601 1008 1.537623e-03 6.428746e-01
## G0:0034660 BP 634 117 1.548471e-03 6.428746e-01
## G0:0001774 BP 47 15 1.567782e-03 6.428746e-01
## G0:0042273 BP 76 21 1.653515e-03 6.468964e-01
## G0:0002495 BP 34 12 1.727934e-03 6.468964e-01
## G0:0061355 BP 5 4 1.802960e-03 6.468964e-01
## G0:0019886 BP 30 11 1.876126e-03 6.487121e-01
## G0:0043506 BP 62 18 1.892771e-03 6.487121e-01
## G0:0007154 BP 6682 1017 2.218768e-03 7.387211e-01
## G0:0006950 BP 4044 632 2.302365e-03 7.471901e-01
## G0:0010038 BP 360 71 2.310260e-03 7.471901e-01
## G0:0150076 BP 73 20 2.355114e-03 7.511179e-01
## G0:0045598 BP 147 34 2.423438e-03 7.612893e-01
## G0:0035641 BP 15 7 2.614249e-03 7.612893e-01
```

```
goanaOut_BP <- goanaOut[goanaOut$Ont == "BP",]
print(paste("Amount of significant GO Biological Process terms:", as.character(sum(goanaOut_BP$FDR.DE <
```

```
## [1] "Amount of significant GO Biological Process terms: 1"
```

```
topGOarray[order(topGOarray$FDR.DE), ]
```

##	Term
## G0:0009987	cellular process
## G0:1901564	organonitrogen compound metabolic process
## G0:0044237	cellular metabolic process
## G0:0043507	positive regulation of JUN kinase activity
## G0:0002396	MHC protein complex assembly
## G0:0002501	peptide antigen assembly with MHC protein complex
## G0:0006807	nitrogen compound metabolic process
## G0:0071396	cellular response to lipid
## G0:0051716	cellular response to stimulus
## G0:0072077	renal vesicle morphogenesis
## G0:0050896	response to stimulus
## G0:0051259	protein complex oligomerization
## G0:0072283	metanephric renal vesicle morphogenesis
## G0:0071248	cellular response to metal ion
## G0:0044238	primary metabolic process
## G0:0008150	biological process
## G0:0050794	regulation of cellular process
## G0:0019538	protein metabolic process
## G0:0072087	renal vesicle development
## G0:0044260	cellular macromolecule metabolic process
## G0:0035556	intracellular signal transduction

G0:0048545 response to steroid hormone
 ## G0:0002399 MHC class II protein complex assembly
 ## G0:0002503 peptide antigen assembly with MHC class II protein complex
 ## G0:0045600 positive regulation of fat cell differentiation
 ## G0:0071383 cellular response to steroid hormone stimulus
 ## G0:0061900 glial cell activation
 ## G0:0009058 biosynthetic process
 ## G0:0071241 cellular response to inorganic substance
 ## G0:0051260 protein homooligomerization
 ## G0:0031323 regulation of cellular metabolic process
 ## G0:0033993 response to lipid
 ## G0:1901576 organic substance biosynthetic process
 ## G0:0033962 P-body assembly
 ## G0:0051171 regulation of nitrogen compound metabolic process
 ## G0:0044249 cellular biosynthetic process
 ## G0:0071840 cellular component organization or biogenesis
 ## G0:0034660 ncRNA metabolic process
 ## G0:0001774 microglial cell activation
 ## G0:0042273 ribosomal large subunit biogenesis
 ## G0:0002495 antigen processing and presentation of peptide antigen via MHC class II
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 ## G0:0007154 cell communication
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 ## G0:0010038 response to metal ion
 ## G0:0150076 neuroinflammatory response
 ## G0:0045598 regulation of fat cell differentiation
 ## G0:0035641 locomotory exploration behavior

##	Ont	N	DE	P.DE	FDR.DE
## G0:0009987	BP	17266	2557	7.884158e-09	4.252359e-05
## G0:1901564	BP	6535	1019	6.408401e-05	1.051115e-01
## G0:0044237	BP	10271	1554	8.867290e-05	1.357464e-01
## G0:0043507	BP	43	16	1.538807e-04	1.980686e-01
## G0:0002396	BP	20	10	1.552600e-04	1.980686e-01
## G0:0002501	BP	20	10	1.552600e-04	1.980686e-01
## G0:0006807	BP	10310	1555	1.794420e-04	2.168698e-01
## G0:0071396	BP	608	118	2.102327e-04	2.413787e-01
## G0:0051716	BP	7653	1172	2.732974e-04	2.988442e-01
## G0:0072077	BP	18	9	3.351098e-04	3.497785e-01
## G0:0050896	BP	9216	1394	3.862034e-04	3.634716e-01
## G0:0051259	BP	247	55	3.917251e-04	3.634716e-01
## G0:0072283	BP	15	8	4.151627e-04	3.634716e-01
## G0:0071248	BP	198	46	4.273715e-04	3.634716e-01
## G0:0044238	BP	10825	1621	5.032971e-04	3.873451e-01
## G0:0008150	BP	18903	2732	5.088538e-04	3.873451e-01
## G0:0050794	BP	11335	1693	5.120033e-04	3.873451e-01
## G0:0019538	BP	5494	854	5.502308e-04	3.873451e-01
## G0:0072087	BP	19	9	5.566515e-04	3.873451e-01
## G0:0044260	BP	3266	524	6.695712e-04	4.393718e-01
## G0:0035556	BP	2673	435	6.835287e-04	4.393718e-01
## G0:0048545	BP	339	70	7.044401e-04	4.393718e-01
## G0:0002399	BP	16	8	7.270883e-04	4.393718e-01
## G0:0002503	BP	16	8	7.270883e-04	4.393718e-01

```
## G0:0045600 BP 72 21 7.737223e-04 4.458274e-01
## G0:0071383 BP 209 47 7.960164e-04 4.458274e-01
## G0:0061900 BP 54 17 9.329003e-04 5.100521e-01
## G0:0009058 BP 5962 918 1.044661e-03 5.578733e-01
## G0:0071241 BP 229 50 1.108461e-03 5.783705e-01
## G0:0051260 BP 190 43 1.133418e-03 5.783705e-01
## G0:0031323 BP 5737 884 1.252331e-03 6.044936e-01
## G0:0033993 BP 918 163 1.263585e-03 6.044936e-01
## G0:1901576 BP 5877 904 1.322674e-03 6.074514e-01
## G0:0033962 BP 21 9 1.354811e-03 6.100102e-01
## G0:0051171 BP 5792 891 1.447683e-03 6.392911e-01
## G0:0044249 BP 5807 893 1.485936e-03 6.428746e-01
## G0:0071840 BP 6601 1008 1.537623e-03 6.428746e-01
## G0:0034660 BP 634 117 1.548471e-03 6.428746e-01
## G0:0001774 BP 47 15 1.567782e-03 6.428746e-01
## G0:0042273 BP 76 21 1.653515e-03 6.468964e-01
## G0:0002495 BP 34 12 1.727934e-03 6.468964e-01
## G0:0061355 BP 5 4 1.802960e-03 6.468964e-01
## G0:0019886 BP 30 11 1.876126e-03 6.487121e-01
## G0:0043506 BP 62 18 1.892771e-03 6.487121e-01
## G0:0007154 BP 6682 1017 2.218768e-03 7.387211e-01
## G0:0006950 BP 4044 632 2.302365e-03 7.471901e-01
## G0:0010038 BP 360 71 2.310260e-03 7.471901e-01
## G0:0150076 BP 73 20 2.355114e-03 7.511179e-01
## G0:0045598 BP 147 34 2.423438e-03 7.612893e-01
## G0:0035641 BP 15 7 2.614249e-03 7.612893e-01
```

```
write.table(LIMMAout_RMA, sep= "\t", file="C:/Users/Luca Visser/Documents/1st master/AHTA/Practica/Proj
array_GSA_res <- topGO(goanaOut, ontology="BP", number=100)
write.table(array_GSA_res, sep= "\t", file="C:/Users/Luca Visser/Documents/1st master/AHTA/Practica/Proj
```