

Group 1: RNA-seq (coding) analysis AHTA

Luca Visser

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Contents

General info	1
Pipeline	1
Import gene counts and metadata	1
Differential expression analysis	14
Gene set analysis	17

```
suppressPackageStartupMessages({  
  library(ggplot2)  
  library(htmltools)  
  library(biomaRt)  
  library(tximport)  
  library(dplyr)  
  library(GEOquery)  
  library(annotation)  
  library(edgeR)  
  library(limma)  
  library(SummarizedExperiment)  
})
```

General info

Platform type: Illumina NextSeq 500

The GEO dataset with accession ID GSE159699 (expression profiling by high-throughput sequencing sequencing of human postmortal hippocampal brain tissues) will be analysed in a case versus control framework for differential gene expression. The sequencing was done with single ends.

Pipeline

Import gene counts and metadata

```
annot <- pData(gse)
```

```
unlink(id, recursive = T)
filePath <- rownames(getGEOSuppFiles(id))

for (file in list.files(id, full.names = T)){
  gunzip(file)
}
```

```
counts <- read.delim(list.files(id, full.names = T))

library('org.Hs.eg.db')
ENSIDs <- mapIds(org.Hs.eg.db, counts$refGene, 'ENSEMBL', 'SYMBOL')
counts <- counts[!(duplicated(ENSIDs) | is.na(ENSIDs)),]
rownames(counts) <- ENSIDs[!(duplicated(ENSIDs) | is.na(ENSIDs))]
counts <- dplyr::select(counts, -refGene)
```

```
annot$disease <- "CT"
annot$disease[grep("AD", annot$title)] <- "AD"
annot$disease <- as.factor(annot$disease)

print(annot)
```

##		title	geo_accession							status
##	GSM4837814	10-8A-Old [RNA-seq]	GSM4837814	Public	on	Oct	21	2020		
##	GSM4837815	11-10T-Old [RNA-seq]	GSM4837815	Public	on	Oct	21	2020		
##	GSM4837816	12-6A-Old [RNA-seq]	GSM4837816	Public	on	Oct	21	2020		
##	GSM4837817	13-11T-Old [RNA-seq]	GSM4837817	Public	on	Oct	21	2020		
##	GSM4837818	14-7A-Old [RNA-seq]	GSM4837818	Public	on	Oct	21	2020		
##	GSM4837819	15-13T-Old [RNA-seq]	GSM4837819	Public	on	Oct	21	2020		
##	GSM4837820	16-14T-Old [RNA-seq]	GSM4837820	Public	on	Oct	21	2020		
##	GSM4837821	17-9A-Old [RNA-seq]	GSM4837821	Public	on	Oct	21	2020		
##	GSM4837822	18-10A-Old [RNA-seq]	GSM4837822	Public	on	Oct	21	2020		
##	GSM4837823	19-11A-Old [RNA-seq]	GSM4837823	Public	on	Oct	21	2020		
##	GSM4837824	20-1T-AD [RNA-seq]	GSM4837824	Public	on	Oct	21	2020		
##	GSM4837825	21-1A-AD [RNA-seq]	GSM4837825	Public	on	Oct	21	2020		
##	GSM4837826	2-12A-Young [RNA-seq]	GSM4837826	Public	on	Oct	21	2020		
##	GSM4837827	22-2T-AD [RNA-seq]	GSM4837827	Public	on	Oct	21	2020		
##	GSM4837828	23-2A-AD [RNA-seq]	GSM4837828	Public	on	Oct	21	2020		
##	GSM4837829	24-3T-AD [RNA-seq]	GSM4837829	Public	on	Oct	21	2020		
##	GSM4837830	25-5T-AD [RNA-seq]	GSM4837830	Public	on	Oct	21	2020		
##	GSM4837831	26-3A-AD [RNA-seq]	GSM4837831	Public	on	Oct	21	2020		
##	GSM4837832	27-5A-AD [RNA-seq]	GSM4837832	Public	on	Oct	21	2020		
##	GSM4837833	28-8T-AD [RNA-seq]	GSM4837833	Public	on	Oct	21	2020		
##	GSM4837834	29-6T-AD [RNA-seq]	GSM4837834	Public	on	Oct	21	2020		
##	GSM4837835	30-9T-AD [RNA-seq]	GSM4837835	Public	on	Oct	21	2020		
##	GSM4837836	31-7T-AD [RNA-seq]	GSM4837836	Public	on	Oct	21	2020		

##	GSM4837837	3-17T-Young	[RNA-seq]	GSM4837837	Public	on	Oct	21	2020
##	GSM4837838	4-13A-Young	[RNA-seq]	GSM4837838	Public	on	Oct	21	2020
##	GSM4837839	5-18T-Young	[RNA-seq]	GSM4837839	Public	on	Oct	21	2020
##	GSM4837840	6-14A-Young	[RNA-seq]	GSM4837840	Public	on	Oct	21	2020
##	GSM4837841	7-19T-Young	[RNA-seq]	GSM4837841	Public	on	Oct	21	2020
##	GSM4837842	8-15A-Young	[RNA-seq]	GSM4837842	Public	on	Oct	21	2020
##	GSM4837843	9-16A-Young	[RNA-seq]	GSM4837843	Public	on	Oct	21	2020
##		submission_date	last_update_date	type	channel_count				
##	GSM4837814	Oct 20 2020	Oct 21 2020	SRA	1				
##	GSM4837815	Oct 20 2020	Oct 21 2020	SRA	1				
##	GSM4837816	Oct 20 2020	Oct 21 2020	SRA	1				
##	GSM4837817	Oct 20 2020	Oct 21 2020	SRA	1				
##	GSM4837818	Oct 20 2020	Oct 21 2020	SRA	1				
##	GSM4837819	Oct 20 2020	Oct 21 2020	SRA	1				
##	GSM4837820	Oct 20 2020	Oct 21 2020	SRA	1				
##	GSM4837821	Oct 20 2020	Oct 21 2020	SRA	1				
##	GSM4837822	Oct 20 2020	Oct 21 2020	SRA	1				
##	GSM4837823	Oct 20 2020	Oct 21 2020	SRA	1				
##	GSM4837824	Oct 20 2020	Oct 21 2020	SRA	1				
##	GSM4837825	Oct 20 2020	Oct 21 2020	SRA	1				
##	GSM4837826	Oct 20 2020	Oct 21 2020	SRA	1				
##	GSM4837827	Oct 20 2020	Oct 21 2020	SRA	1				
##	GSM4837828	Oct 20 2020	Oct 21 2020	SRA	1				
##	GSM4837829	Oct 20 2020	Oct 21 2020	SRA	1				
##	GSM4837830	Oct 20 2020	Oct 21 2020	SRA	1				
##	GSM4837831	Oct 20 2020	Oct 21 2020	SRA	1				
##	GSM4837832	Oct 20 2020	Oct 21 2020	SRA	1				
##	GSM4837833	Oct 20 2020	Oct 21 2020	SRA	1				
##	GSM4837834	Oct 20 2020	Oct 21 2020	SRA	1				
##	GSM4837835	Oct 20 2020	Oct 21 2020	SRA	1				
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##	GSM4837838	Oct 20 2020	Oct 21 2020	SRA	1				
##	GSM4837839	Oct 20 2020	Oct 21 2020	SRA	1				
##	GSM4837840	Oct 20 2020	Oct 21 2020	SRA	1				
##	GSM4837841	Oct 20 2020	Oct 21 2020	SRA	1				
##	GSM4837842	Oct 20 2020	Oct 21 2020	SRA	1				
##	GSM4837843	Oct 20 2020	Oct 21 2020	SRA	1				
##		source_name_ch1	organism_ch1						
##	GSM4837814	CNDR brain bank (Penn)	Homo sapiens						
##	GSM4837815	CNDR brain bank (Penn)	Homo sapiens						
##	GSM4837816	CNDR brain bank (Penn)	Homo sapiens						
##	GSM4837817	CNDR brain bank (Penn)	Homo sapiens						
##	GSM4837818	CNDR brain bank (Penn)	Homo sapiens						
##	GSM4837819	CNDR brain bank (Penn)	Homo sapiens						
##	GSM4837820	CNDR brain bank (Penn)	Homo sapiens						
##	GSM4837821	CNDR brain bank (Penn)	Homo sapiens						
##	GSM4837822	CNDR brain bank (Penn)	Homo sapiens						
##	GSM4837823	CNDR brain bank (Penn)	Homo sapiens						
##	GSM4837824	CNDR brain bank (Penn)	Homo sapiens						
##	GSM4837825	CNDR brain bank (Penn)	Homo sapiens						
##	GSM4837826	CNDR brain bank (Penn)	Homo sapiens						
##	GSM4837827	CNDR brain bank (Penn)	Homo sapiens						
##	GSM4837828	CNDR brain bank (Penn)	Homo sapiens						

[illegible]

##	GSM4837821	age: old	total RNA
##	GSM4837822	age: old	total RNA
##	GSM4837823	age: old	total RNA
##	GSM4837824		total RNA
##	GSM4837825		total RNA
##	GSM4837826	age: young	total RNA
##	GSM4837827		total RNA
##	GSM4837828		total RNA
##	GSM4837829		total RNA
##	GSM4837830		total RNA
##	GSM4837831		total RNA
##	GSM4837832		total RNA
##	GSM4837833		total RNA
##	GSM4837834		total RNA
##	GSM4837835		total RNA
##	GSM4837836		total RNA
##	GSM4837837	age: young	total RNA
##	GSM4837838	age: young	total RNA
##	GSM4837839	age: young	total RNA
##	GSM4837840	age: young	total RNA
##	GSM4837841	age: young	total RNA
##	GSM4837842	age: young	total RNA
##	GSM4837843	age: young	total RNA

[illegible]

[illegible]

[illegible]

```

## GSM4837828 FeatureCounts was used to generate a matrix of mapped fragments per RefSeq annotated gene
## GSM4837829 FeatureCounts was used to generate a matrix of mapped fragments per RefSeq annotated gene
## GSM4837830 FeatureCounts was used to generate a matrix of mapped fragments per RefSeq annotated gene
## GSM4837831 FeatureCounts was used to generate a matrix of mapped fragments per RefSeq annotated gene
## GSM4837832 FeatureCounts was used to generate a matrix of mapped fragments per RefSeq annotated gene
## GSM4837833 FeatureCounts was used to generate a matrix of mapped fragments per RefSeq annotated gene
## GSM4837834 FeatureCounts was used to generate a matrix of mapped fragments per RefSeq annotated gene
## GSM4837835 FeatureCounts was used to generate a matrix of mapped fragments per RefSeq annotated gene
## GSM4837836 FeatureCounts was used to generate a matrix of mapped fragments per RefSeq annotated gene
## GSM4837837 FeatureCounts was used to generate a matrix of mapped fragments per RefSeq annotated gene
## GSM4837838 FeatureCounts was used to generate a matrix of mapped fragments per RefSeq annotated gene
## GSM4837839 FeatureCounts was used to generate a matrix of mapped fragments per RefSeq annotated gene
## GSM4837840 FeatureCounts was used to generate a matrix of mapped fragments per RefSeq annotated gene
## GSM4837841 FeatureCounts was used to generate a matrix of mapped fragments per RefSeq annotated gene
## GSM4837842 FeatureCounts was used to generate a matrix of mapped fragments per RefSeq annotated gene
## GSM4837843 FeatureCounts was used to generate a matrix of mapped fragments per RefSeq annotated gene
##
##           data_processing.2
## GSM4837814 Genome_build: hg19
## GSM4837815 Genome_build: hg19
## GSM4837816 Genome_build: hg19
## GSM4837817 Genome_build: hg19
## GSM4837818 Genome_build: hg19
## GSM4837819 Genome_build: hg19
## GSM4837820 Genome_build: hg19
## GSM4837821 Genome_build: hg19
## GSM4837822 Genome_build: hg19
## GSM4837823 Genome_build: hg19
## GSM4837824 Genome_build: hg19
## GSM4837825 Genome_build: hg19
## GSM4837826 Genome_build: hg19
## GSM4837827 Genome_build: hg19
## GSM4837828 Genome_build: hg19
## GSM4837829 Genome_build: hg19
## GSM4837830 Genome_build: hg19
## GSM4837831 Genome_build: hg19
## GSM4837832 Genome_build: hg19
## GSM4837833 Genome_build: hg19
## GSM4837834 Genome_build: hg19
## GSM4837835 Genome_build: hg19
## GSM4837836 Genome_build: hg19
## GSM4837837 Genome_build: hg19
## GSM4837838 Genome_build: hg19
## GSM4837839 Genome_build: hg19
## GSM4837840 Genome_build: hg19
## GSM4837841 Genome_build: hg19
## GSM4837842 Genome_build: hg19
## GSM4837843 Genome_build: hg19
##
##           data_processing.3
## GSM4837814 Supplementary_files_format_and_content: read count per gene
## GSM4837815 Supplementary_files_format_and_content: read count per gene
## GSM4837816 Supplementary_files_format_and_content: read count per gene
## GSM4837817 Supplementary_files_format_and_content: read count per gene
## GSM4837818 Supplementary_files_format_and_content: read count per gene
## GSM4837819 Supplementary_files_format_and_content: read count per gene

```


[illegible]

```

## GSM4837843      GPL18573      Yemin,,Lan University of Pennsylvania
##               contact_address contact_city contact_state
## GSM4837814 3400 Civic Center Blvd Philadelphia      PA
## GSM4837815 3400 Civic Center Blvd Philadelphia      PA
## GSM4837816 3400 Civic Center Blvd Philadelphia      PA
## GSM4837817 3400 Civic Center Blvd Philadelphia      PA
## GSM4837818 3400 Civic Center Blvd Philadelphia      PA
## GSM4837819 3400 Civic Center Blvd Philadelphia      PA
## GSM4837820 3400 Civic Center Blvd Philadelphia      PA
## GSM4837821 3400 Civic Center Blvd Philadelphia      PA
## GSM4837822 3400 Civic Center Blvd Philadelphia      PA
## GSM4837823 3400 Civic Center Blvd Philadelphia      PA
## GSM4837824 3400 Civic Center Blvd Philadelphia      PA
## GSM4837825 3400 Civic Center Blvd Philadelphia      PA
## GSM4837826 3400 Civic Center Blvd Philadelphia      PA
## GSM4837827 3400 Civic Center Blvd Philadelphia      PA
## GSM4837828 3400 Civic Center Blvd Philadelphia      PA
## GSM4837829 3400 Civic Center Blvd Philadelphia      PA
## GSM4837830 3400 Civic Center Blvd Philadelphia      PA
## GSM4837831 3400 Civic Center Blvd Philadelphia      PA
## GSM4837832 3400 Civic Center Blvd Philadelphia      PA
## GSM4837833 3400 Civic Center Blvd Philadelphia      PA
## GSM4837834 3400 Civic Center Blvd Philadelphia      PA
## GSM4837835 3400 Civic Center Blvd Philadelphia      PA
## GSM4837836 3400 Civic Center Blvd Philadelphia      PA
## GSM4837837 3400 Civic Center Blvd Philadelphia      PA
## GSM4837838 3400 Civic Center Blvd Philadelphia      PA
## GSM4837839 3400 Civic Center Blvd Philadelphia      PA
## GSM4837840 3400 Civic Center Blvd Philadelphia      PA
## GSM4837841 3400 Civic Center Blvd Philadelphia      PA
## GSM4837842 3400 Civic Center Blvd Philadelphia      PA
## GSM4837843 3400 Civic Center Blvd Philadelphia      PA
##               contact_zip/postal_code contact_country data_row_count
## GSM4837814                19104                USA                0
## GSM4837815                19104                USA                0
## GSM4837816                19104                USA                0
## GSM4837817                19104                USA                0
## GSM4837818                19104                USA                0
## GSM4837819                19104                USA                0
## GSM4837820                19104                USA                0
## GSM4837821                19104                USA                0
## GSM4837822                19104                USA                0
## GSM4837823                19104                USA                0
## GSM4837824                19104                USA                0
## GSM4837825                19104                USA                0
## GSM4837826                19104                USA                0
## GSM4837827                19104                USA                0
## GSM4837828                19104                USA                0
## GSM4837829                19104                USA                0
## GSM4837830                19104                USA                0
## GSM4837831                19104                USA                0
## GSM4837832                19104                USA                0
## GSM4837833                19104                USA                0
## GSM4837834                19104                USA                0

```

##	GSM4837835	19104	USA	0
##	GSM4837836	19104	USA	0
##	GSM4837837	19104	USA	0
##	GSM4837838	19104	USA	0
##	GSM4837839	19104	USA	0
##	GSM4837840	19104	USA	0
##	GSM4837841	19104	USA	0
##	GSM4837842	19104	USA	0
##	GSM4837843	19104	USA	0
##	instrument_model library_selection library_source			
##	GSM4837814	Illumina NextSeq 500	cDNA transcriptomic	
##	GSM4837815	Illumina NextSeq 500	cDNA transcriptomic	
##	GSM4837816	Illumina NextSeq 500	cDNA transcriptomic	
##	GSM4837817	Illumina NextSeq 500	cDNA transcriptomic	
##	GSM4837818	Illumina NextSeq 500	cDNA transcriptomic	
##	GSM4837819	Illumina NextSeq 500	cDNA transcriptomic	
##	GSM4837820	Illumina NextSeq 500	cDNA transcriptomic	
##	GSM4837821	Illumina NextSeq 500	cDNA transcriptomic	
##	GSM4837822	Illumina NextSeq 500	cDNA transcriptomic	
##	GSM4837823	Illumina NextSeq 500	cDNA transcriptomic	
##	GSM4837824	Illumina NextSeq 500	cDNA transcriptomic	
##	GSM4837825	Illumina NextSeq 500	cDNA transcriptomic	
##	GSM4837826	Illumina NextSeq 500	cDNA transcriptomic	
##	GSM4837827	Illumina NextSeq 500	cDNA transcriptomic	
##	GSM4837828	Illumina NextSeq 500	cDNA transcriptomic	
##	GSM4837829	Illumina NextSeq 500	cDNA transcriptomic	
##	GSM4837830	Illumina NextSeq 500	cDNA transcriptomic	
##	GSM4837831	Illumina NextSeq 500	cDNA transcriptomic	
##	GSM4837832	Illumina NextSeq 500	cDNA transcriptomic	
##	GSM4837833	Illumina NextSeq 500	cDNA transcriptomic	
##	GSM4837834	Illumina NextSeq 500	cDNA transcriptomic	
##	GSM4837835	Illumina NextSeq 500	cDNA transcriptomic	
##	GSM4837836	Illumina NextSeq 500	cDNA transcriptomic	
##	GSM4837837	Illumina NextSeq 500	cDNA transcriptomic	
##	GSM4837838	Illumina NextSeq 500	cDNA transcriptomic	
##	GSM4837839	Illumina NextSeq 500	cDNA transcriptomic	
##	GSM4837840	Illumina NextSeq 500	cDNA transcriptomic	
##	GSM4837841	Illumina NextSeq 500	cDNA transcriptomic	
##	GSM4837842	Illumina NextSeq 500	cDNA transcriptomic	
##	GSM4837843	Illumina NextSeq 500	cDNA transcriptomic	
##	library_strategy			
##	GSM4837814	RNA-Seq		
##	GSM4837815	RNA-Seq		
##	GSM4837816	RNA-Seq		
##	GSM4837817	RNA-Seq		
##	GSM4837818	RNA-Seq		
##	GSM4837819	RNA-Seq		
##	GSM4837820	RNA-Seq		
##	GSM4837821	RNA-Seq		
##	GSM4837822	RNA-Seq		
##	GSM4837823	RNA-Seq		
##	GSM4837824	RNA-Seq		
##	GSM4837825	RNA-Seq		
##	GSM4837826	RNA-Seq		

```

## GSM4837827      RNA-Seq
## GSM4837828      RNA-Seq
## GSM4837829      RNA-Seq
## GSM4837830      RNA-Seq
## GSM4837831      RNA-Seq
## GSM4837832      RNA-Seq
## GSM4837833      RNA-Seq
## GSM4837834      RNA-Seq
## GSM4837835      RNA-Seq
## GSM4837836      RNA-Seq
## GSM4837837      RNA-Seq
## GSM4837838      RNA-Seq
## GSM4837839      RNA-Seq
## GSM4837840      RNA-Seq
## GSM4837841      RNA-Seq
## GSM4837842      RNA-Seq
## GSM4837843      RNA-Seq
##
##                                     relation
## GSM4837814 BioSample: https://www.ncbi.nlm.nih.gov/biosample/SAMN16486067
## GSM4837815 BioSample: https://www.ncbi.nlm.nih.gov/biosample/SAMN16486065
## GSM4837816 BioSample: https://www.ncbi.nlm.nih.gov/biosample/SAMN16486064
## GSM4837817 BioSample: https://www.ncbi.nlm.nih.gov/biosample/SAMN16486063
## GSM4837818 BioSample: https://www.ncbi.nlm.nih.gov/biosample/SAMN16486062
## GSM4837819 BioSample: https://www.ncbi.nlm.nih.gov/biosample/SAMN16486061
## GSM4837820 BioSample: https://www.ncbi.nlm.nih.gov/biosample/SAMN16486060
## GSM4837821 BioSample: https://www.ncbi.nlm.nih.gov/biosample/SAMN16486059
## GSM4837822 BioSample: https://www.ncbi.nlm.nih.gov/biosample/SAMN16486058
## GSM4837823 BioSample: https://www.ncbi.nlm.nih.gov/biosample/SAMN16486057
## GSM4837824 BioSample: https://www.ncbi.nlm.nih.gov/biosample/SAMN16486056
## GSM4837825 BioSample: https://www.ncbi.nlm.nih.gov/biosample/SAMN16486055
## GSM4837826 BioSample: https://www.ncbi.nlm.nih.gov/biosample/SAMN16486054
## GSM4837827 BioSample: https://www.ncbi.nlm.nih.gov/biosample/SAMN16486053
## GSM4837828 BioSample: https://www.ncbi.nlm.nih.gov/biosample/SAMN16486052
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## GSM4837830 BioSample: https://www.ncbi.nlm.nih.gov/biosample/SAMN16486081
## GSM4837831 BioSample: https://www.ncbi.nlm.nih.gov/biosample/SAMN16486080
## GSM4837832 BioSample: https://www.ncbi.nlm.nih.gov/biosample/SAMN16486079
## GSM4837833 BioSample: https://www.ncbi.nlm.nih.gov/biosample/SAMN16486078
## GSM4837834 BioSample: https://www.ncbi.nlm.nih.gov/biosample/SAMN16486077
## GSM4837835 BioSample: https://www.ncbi.nlm.nih.gov/biosample/SAMN16486076
## GSM4837836 BioSample: https://www.ncbi.nlm.nih.gov/biosample/SAMN16486075
## GSM4837837 BioSample: https://www.ncbi.nlm.nih.gov/biosample/SAMN16486074
## GSM4837838 BioSample: https://www.ncbi.nlm.nih.gov/biosample/SAMN16486073
## GSM4837839 BioSample: https://www.ncbi.nlm.nih.gov/biosample/SAMN16486072
## GSM4837840 BioSample: https://www.ncbi.nlm.nih.gov/biosample/SAMN16486071
## GSM4837841 BioSample: https://www.ncbi.nlm.nih.gov/biosample/SAMN16486070
## GSM4837842 BioSample: https://www.ncbi.nlm.nih.gov/biosample/SAMN16486069
## GSM4837843 BioSample: https://www.ncbi.nlm.nih.gov/biosample/SAMN16486068
##
##                                     relation.1
## GSM4837814 SRA: https://www.ncbi.nlm.nih.gov/sra?term=SRX9317798
## GSM4837815 SRA: https://www.ncbi.nlm.nih.gov/sra?term=SRX9317799
## GSM4837816 SRA: https://www.ncbi.nlm.nih.gov/sra?term=SRX9317800
## GSM4837817 SRA: https://www.ncbi.nlm.nih.gov/sra?term=SRX9317801
## GSM4837818 SRA: https://www.ncbi.nlm.nih.gov/sra?term=SRX9317802

```

```

## GSM4837819 SRA: https://www.ncbi.nlm.nih.gov/sra?term=SRX9317803
## GSM4837820 SRA: https://www.ncbi.nlm.nih.gov/sra?term=SRX9317804
## GSM4837821 SRA: https://www.ncbi.nlm.nih.gov/sra?term=SRX9317805
## GSM4837822 SRA: https://www.ncbi.nlm.nih.gov/sra?term=SRX9317806
## GSM4837823 SRA: https://www.ncbi.nlm.nih.gov/sra?term=SRX9317807
## GSM4837824 SRA: https://www.ncbi.nlm.nih.gov/sra?term=SRX9317808
## GSM4837825 SRA: https://www.ncbi.nlm.nih.gov/sra?term=SRX9317809
## GSM4837826 SRA: https://www.ncbi.nlm.nih.gov/sra?term=SRX9317810
## GSM4837827 SRA: https://www.ncbi.nlm.nih.gov/sra?term=SRX9317811
## GSM4837828 SRA: https://www.ncbi.nlm.nih.gov/sra?term=SRX9317812
## GSM4837829 SRA: https://www.ncbi.nlm.nih.gov/sra?term=SRX9317813
## GSM4837830 SRA: https://www.ncbi.nlm.nih.gov/sra?term=SRX9317814
## GSM4837831 SRA: https://www.ncbi.nlm.nih.gov/sra?term=SRX9317815
## GSM4837832 SRA: https://www.ncbi.nlm.nih.gov/sra?term=SRX9317816
## GSM4837833 SRA: https://www.ncbi.nlm.nih.gov/sra?term=SRX9317817
## GSM4837834 SRA: https://www.ncbi.nlm.nih.gov/sra?term=SRX9317818
## GSM4837835 SRA: https://www.ncbi.nlm.nih.gov/sra?term=SRX9317819
## GSM4837836 SRA: https://www.ncbi.nlm.nih.gov/sra?term=SRX9317820
## GSM4837837 SRA: https://www.ncbi.nlm.nih.gov/sra?term=SRX9317821
## GSM4837838 SRA: https://www.ncbi.nlm.nih.gov/sra?term=SRX9317822
## GSM4837839 SRA: https://www.ncbi.nlm.nih.gov/sra?term=SRX9317823
## GSM4837840 SRA: https://www.ncbi.nlm.nih.gov/sra?term=SRX9317824
## GSM4837841 SRA: https://www.ncbi.nlm.nih.gov/sra?term=SRX9317825
## GSM4837842 SRA: https://www.ncbi.nlm.nih.gov/sra?term=SRX9317826
## GSM4837843 SRA: https://www.ncbi.nlm.nih.gov/sra?term=SRX9317827
## supplementary_file_1 age:ch1
## GSM4837814 NONE old
## GSM4837815 NONE old
## GSM4837816 NONE old
## GSM4837817 NONE old
## GSM4837818 NONE old
## GSM4837819 NONE old
## GSM4837820 NONE old
## GSM4837821 NONE old
## GSM4837822 NONE old
## GSM4837823 NONE old
## GSM4837824 NONE <NA>
## GSM4837825 NONE <NA>
## GSM4837826 NONE young
## GSM4837827 NONE <NA>
## GSM4837828 NONE <NA>
## GSM4837829 NONE <NA>
## GSM4837830 NONE <NA>
## GSM4837831 NONE <NA>
## GSM4837832 NONE <NA>
## GSM4837833 NONE <NA>
## GSM4837834 NONE <NA>
## GSM4837835 NONE <NA>
## GSM4837836 NONE <NA>
## GSM4837837 NONE young
## GSM4837838 NONE young
## GSM4837839 NONE young
## GSM4837840 NONE young
## GSM4837841 NONE young

```

```

## GSM4837842          NONE    young
## GSM4837843          NONE    young
##                                     tissue:ch1 disease
## GSM4837814 Lateral temporal lobe from Frozen postmortem brain tissue      CT
## GSM4837815 Lateral temporal lobe from Frozen postmortem brain tissue      CT
## GSM4837816 Lateral temporal lobe from Frozen postmortem brain tissue      CT
## GSM4837817 Lateral temporal lobe from Frozen postmortem brain tissue      CT
## GSM4837818 Lateral temporal lobe from Frozen postmortem brain tissue      CT
## GSM4837819 Lateral temporal lobe from Frozen postmortem brain tissue      CT
## GSM4837820 Lateral temporal lobe from Frozen postmortem brain tissue      CT
## GSM4837821 Lateral temporal lobe from Frozen postmortem brain tissue      CT
## GSM4837822 Lateral temporal lobe from Frozen postmortem brain tissue      CT
## GSM4837823 Lateral temporal lobe from Frozen postmortem brain tissue      CT
## GSM4837824 Lateral temporal lobe from Frozen postmortem brain tissue      AD
## GSM4837825 Lateral temporal lobe from Frozen postmortem brain tissue      AD
## GSM4837826 Lateral temporal lobe from Frozen postmortem brain tissue      CT
## GSM4837827 Lateral temporal lobe from Frozen postmortem brain tissue      AD
## GSM4837828 Lateral temporal lobe from Frozen postmortem brain tissue      AD
## GSM4837829 Lateral temporal lobe from Frozen postmortem brain tissue      AD
## GSM4837830 Lateral temporal lobe from Frozen postmortem brain tissue      AD
## GSM4837831 Lateral temporal lobe from Frozen postmortem brain tissue      AD
## GSM4837832 Lateral temporal lobe from Frozen postmortem brain tissue      AD
## GSM4837833 Lateral temporal lobe from Frozen postmortem brain tissue      AD
## GSM4837834 Lateral temporal lobe from Frozen postmortem brain tissue      AD
## GSM4837835 Lateral temporal lobe from Frozen postmortem brain tissue      AD
## GSM4837836 Lateral temporal lobe from Frozen postmortem brain tissue      AD
## GSM4837837 Lateral temporal lobe from Frozen postmortem brain tissue      CT
## GSM4837838 Lateral temporal lobe from Frozen postmortem brain tissue      CT
## GSM4837839 Lateral temporal lobe from Frozen postmortem brain tissue      CT
## GSM4837840 Lateral temporal lobe from Frozen postmortem brain tissue      CT
## GSM4837841 Lateral temporal lobe from Frozen postmortem brain tissue      CT
## GSM4837842 Lateral temporal lobe from Frozen postmortem brain tissue      CT
## GSM4837843 Lateral temporal lobe from Frozen postmortem brain tissue      CT

```

Differential expression analysis

```

dge <- DGEList(counts)
colnames(dge) <- substring(gsub('\\.', '-', colnames(dge)), 2)
annot <- annot[order(annot$disease), ]

design <- model.matrix(~disease, annot)
keep <- filterByExpr(dge, design)
table(keep)

```

```

## keep
## FALSE TRUE
## 7280 15991

```

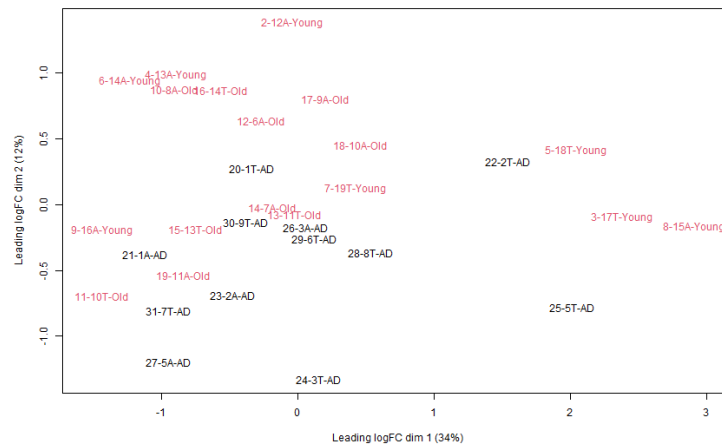
```

dge <- dge[keep, ,keep.lib.sizes=FALSE]

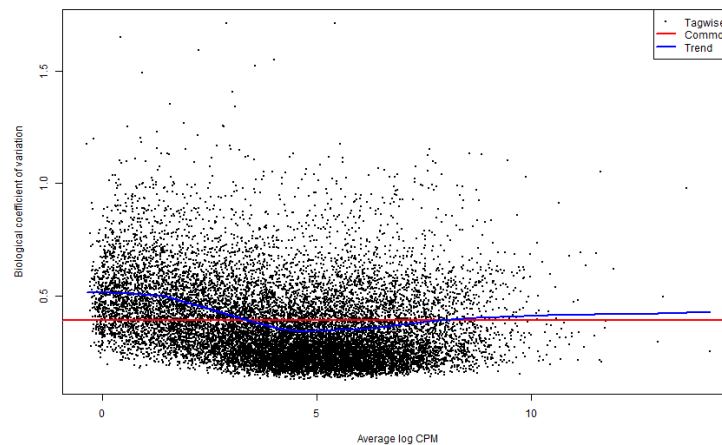
dge <- calcNormFactors(dge)

```

```
plotMDS(dge, top = 500, col=as.double(annot$disease))
```



```
dge <- estimateDisp(dge, design, robust=TRUE)
plotBCV(dge)
```



```
fit = glmQLFit(dge, design)
LRT <- glmQLFTest(fit)
topTags(LRT)
```

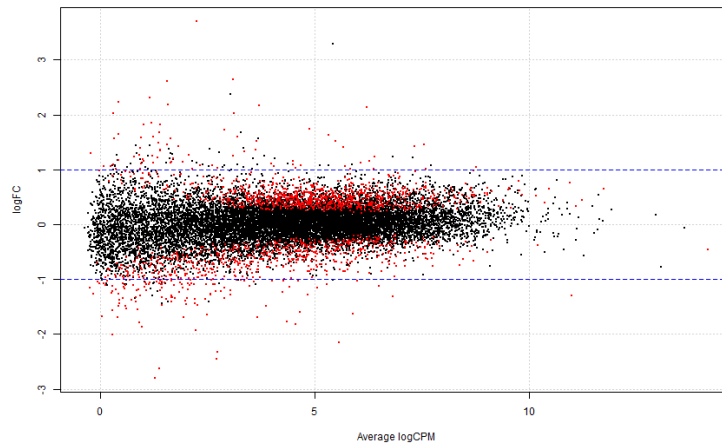
```
## Coefficient: diseaseCT
##          logFC  logCPM      F      PValue      FDR
## ENSG00000136933  0.5893419  3.701181 44.27497 1.780250e-07 0.00123596
## ENSG00000105877 -1.2620152  2.564962 43.31467 2.185691e-07 0.00123596
## ENSG00000109016  0.5762048  3.430957 41.20348 3.465148e-07 0.00123596
## ENSG00000002016 -0.7626227  4.869323 41.10910 3.538413e-07 0.00123596
## ENSG00000100348  0.3944258  5.353248 38.70849 6.083446e-07 0.00123596
## ENSG00000170043  0.5775731  4.890728 38.18764 6.859832e-07 0.00123596
## ENSG00000063322  0.6167025  6.522888 38.06898 7.051046e-07 0.00123596
```

```
## ENSG00000231889 -0.5807385 4.691997 37.83752 7.440575e-07 0.00123596
## ENSG00000092850 -1.0310797 1.390662 37.34583 8.346117e-07 0.00123596
## ENSG00000239306 -0.8849709 6.265442 37.34170 8.354204e-07 0.00123596
```

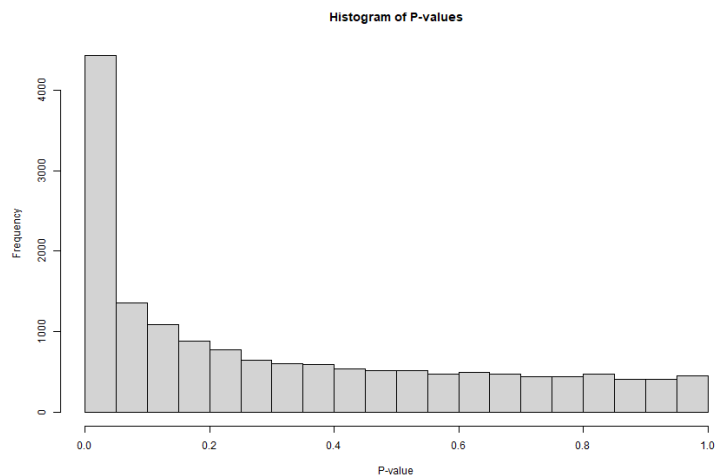
```
summary(dt <- decideTestsDGE(LRT))
```

```
##          diseaseCT
## Down          747
## NotSig       14263
## Up            981
```

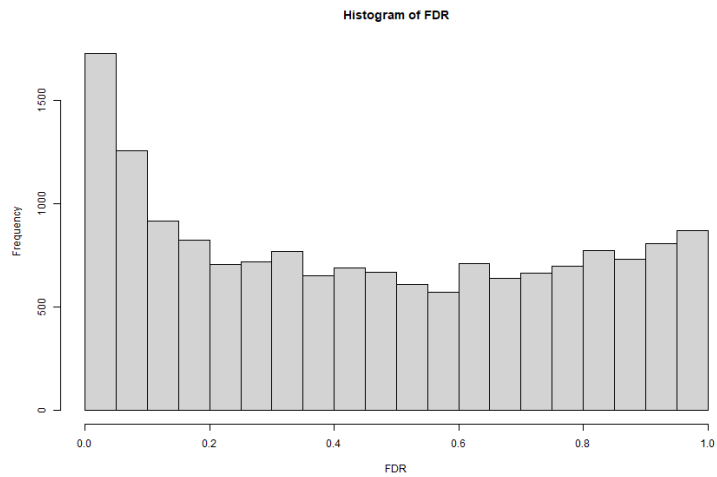
```
plotSmear(LRT,de.tags=rownames(dge)[as.logical(dt)])
abline(h=c(1,-1), col=c("blue", "blue"), lty=c(2,2))
```



```
hist(LRT$table$PValue, xlab="P-value", main="Histogram of P-values")
```




```
FDR <- p.adjust(LRT$table$PValue, "fdr")
hist(FDR)
```



```
LRT$table$FDR <- FDR
sign_genes <- LRT$table[FDR<0.05,]
sign_genes <- sign_genes[order(sign_genes$FDR),]

topHits <- LRT$table[order(LRT$table$FDR, decreasing=F),]
GeneSymbols <- mapIds(org.Hs.eg.db, rownames(topHits), 'SYMBOL', 'ENSEMBL')

topHits <- topHits[1:10,c("logFC", "FDR")]
topHits
```

```
##           logFC      FDR
## ENSG00000092850 -1.0310797 0.00123596
## ENSG00000174132  0.7477023 0.00123596
## ENSG00000231889 -0.5807385 0.00123596
## ENSG00000105877 -1.2620152 0.00123596
## ENSG00000136933  0.5893419 0.00123596
## ENSG00000166295 -0.5812867 0.00123596
## ENSG00000239306 -0.8849709 0.00123596
## ENSG00000002016 -0.7626227 0.00123596
## ENSG00000170043  0.5775731 0.00123596
## ENSG00000109016  0.5762048 0.00123596
```

Gene set analysis

```
EntrezIDs <- mapIds(org.Hs.eg.db, rownames(sign_genes), 'ENTREZID', 'ENSEMBL')

#subset for non duplicated and mapped genes
sign_genes_entrez <- sign_genes[!(duplicated(EntrezIDs) | is.na(EntrezIDs)),]
#make rownames the Entrez gene ID
rownames(sign_genes_entrez) <- EntrezIDs[!(duplicated(EntrezIDs) | is.na(EntrezIDs))]
```

```

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

```

##		Term	Ont	N
##	G0:0044237	cellular metabolic process	BP	10271
##	G0:0006996	organelle organization	BP	3556
##	G0:0006807	nitrogen compound metabolic process	BP	10310
##	G0:0044238	primary metabolic process	BP	10825
##	G0:0009060	aerobic respiration	BP	194
##	G0:0045333	cellular respiration	BP	240
##	G0:0034641	cellular nitrogen compound metabolic process	BP	7053
##	G0:0006091	generation of precursor metabolites and energy	BP	505
##	G0:0009199	ribonucleoside triphosphate metabolic process	BP	249
##	G0:0009205	purine ribonucleoside triphosphate metabolic process	BP	242
##	G0:0007005	mitochondrion organization	BP	542
##	G0:0009144	purine nucleoside triphosphate metabolic process	BP	247
##	G0:0009141	nucleoside triphosphate metabolic process	BP	267
##	G0:0046034	ATP metabolic process	BP	217
##	G0:0042773	ATP synthesis coupled electron transport	BP	101
##	G0:0042775	mitochondrial ATP synthesis coupled electron transport	BP	101
##	G0:0046483	heterocycle metabolic process	BP	6336
##	G0:0015980	energy derivation by oxidation of organic compounds	BP	333
##	G0:1901360	organic cyclic compound metabolic process	BP	6632
##	G0:0006119	oxidative phosphorylation	BP	147
##	G0:0006139	nucleobase-containing compound metabolic process	BP	6165
##	G0:0008152	metabolic process	BP	12433
##	G0:0006725	cellular aromatic compound metabolic process	BP	6392
##	G0:0009987	cellular process	BP	17266
##	G0:1901575	organic substance catabolic process	BP	2120
##	G0:1901564	organonitrogen compound metabolic process	BP	6535
##	G0:0019693	ribose phosphate metabolic process	BP	475
##	G0:0019646	aerobic electron transport chain	BP	93
##	G0:0009150	purine ribonucleotide metabolic process	BP	447
##	G0:0009056	catabolic process	BP	2579
##	G0:0009259	ribonucleotide metabolic process	BP	466
##	G0:0046907	intracellular transport	BP	1587
##	G0:0022904	respiratory electron transport chain	BP	122
##	G0:0044260	cellular macromolecule metabolic process	BP	3266
##	G0:0033108	mitochondrial respiratory chain complex assembly	BP	99
##	G0:1901576	organic substance biosynthetic process	BP	5877
##	G0:0009117	nucleotide metabolic process	BP	597
##	G0:0009058	biosynthetic process	BP	5962
##	G0:0044085	cellular component biogenesis	BP	3273
##	G0:0006163	purine nucleotide metabolic process	BP	476
##	G0:0071840	cellular component organization or biogenesis	BP	6601
##	G0:0006753	nucleoside phosphate metabolic process	BP	605
##	G0:0043933	protein-containing complex organization	BP	1845
##	G0:0072521	purine-containing compound metabolic process	BP	507
##	G0:0044271	cellular nitrogen compound biosynthetic process	BP	4865

##	G0:0009057		macromolecule catabolic process	BP	1385
##	G0:0009201		ribonucleoside triphosphate biosynthetic process	BP	123
##	G0:0022607		cellular component assembly	BP	3011
##	G0:0009206		purine ribonucleoside triphosphate biosynthetic process	BP	117
##	G0:0032543		mitochondrial translation	BP	131
##		DE	P.DE	FDR.DE	
##	G0:0044237	965	4.271072e-20	6.538442e-17	
##	G0:0006996	397	2.397043e-16	2.117050e-13	
##	G0:0006807	947	6.055583e-16	5.039696e-13	
##	G0:0044238	986	6.145168e-16	5.039696e-13	
##	G0:0009060	51	3.098510e-15	2.371703e-12	
##	G0:0045333	56	3.671179e-14	2.634415e-11	
##	G0:0034641	681	4.234797e-14	2.946777e-11	
##	G0:0006091	89	1.060211e-13	7.160480e-11	
##	G0:0009199	56	1.912262e-13	1.254608e-10	
##	G0:0009205	55	1.985360e-13	1.266384e-10	
##	G0:0007005	92	3.908133e-13	2.361643e-10	
##	G0:0009144	55	4.836249e-13	2.847559e-10	
##	G0:0009141	57	1.175566e-12	6.748629e-10	
##	G0:0046034	50	1.423809e-12	7.974370e-10	
##	G0:0042773	32	1.894553e-12	9.855029e-10	
##	G0:0042775	32	1.894553e-12	9.855029e-10	
##	G0:0046483	613	1.974182e-12	9.855029e-10	
##	G0:0015980	65	2.317697e-12	1.132367e-09	
##	G0:1901360	636	3.032329e-12	1.421048e-09	
##	G0:0006119	39	4.071429e-12	1.869844e-09	
##	G0:0006139	596	6.255495e-12	2.782741e-09	
##	G0:0008152	1083	6.301552e-12	2.782741e-09	
##	G0:0006725	614	7.700635e-12	3.274624e-09	
##	G0:0009987	1421	7.990243e-12	3.335999e-09	
##	G0:1901575	245	1.832012e-11	7.130254e-09	
##	G0:1901564	623	2.049681e-11	7.757276e-09	
##	G0:0019693	80	2.060680e-11	7.757276e-09	
##	G0:0019646	29	3.185009e-11	1.179635e-08	
##	G0:0009150	76	4.145639e-11	1.420840e-08	
##	G0:0009056	285	4.676394e-11	1.579177e-08	
##	G0:0009259	78	5.019944e-11	1.670623e-08	
##	G0:0046907	193	5.259017e-11	1.725183e-08	
##	G0:0022904	33	1.029992e-10	3.331226e-08	
##	G0:0044260	344	1.127885e-10	3.597170e-08	
##	G0:0033108	29	1.728907e-10	5.293453e-08	
##	G0:1901576	563	1.995961e-10	5.952370e-08	
##	G0:0009117	91	2.197180e-10	6.386563e-08	
##	G0:0009058	569	2.809006e-10	8.062899e-08	
##	G0:0044085	342	3.424318e-10	9.707731e-08	
##	G0:0006163	77	3.488002e-10	9.767681e-08	
##	G0:0071840	620	3.954915e-10	1.094177e-07	
##	G0:0006753	91	4.440621e-10	1.213928e-07	
##	G0:0043933	213	5.339682e-10	1.442531e-07	
##	G0:0072521	79	1.240783e-09	3.094531e-07	
##	G0:0044271	473	1.909195e-09	4.566755e-07	
##	G0:0009057	167	2.299665e-09	5.444041e-07	
##	G0:0009201	31	2.500253e-09	5.858502e-07	
##	G0:0022607	314	2.884279e-09	6.660888e-07	

```
## GO:0009206    30 2.900705e-09 6.660888e-07
## GO:0032543    32 3.215364e-09 7.225843e-07
```

```
goanaOut_BP <- goanaOut[goanaOut$Ont == "BP",]
print(paste("Amount of significant GO Biological Process terms:",
as.character(sum(goanaOut_BP$FDR.DE < 0.05))))
```

```
## [1] "Amount of significant GO Biological Process terms: 242"
```

```
topGORNA[order(topGORNA$N),]
```

##		Term	Ont	N
## GO:0019646		aerobic electron transport chain	BP	93
## GO:0033108		mitochondrial respiratory chain complex assembly	BP	99
## GO:0042773		ATP synthesis coupled electron transport	BP	101
## GO:0042775	mitochondrial	ATP synthesis coupled electron transport	BP	101
## GO:0009206	purine ribonucleoside triphosphate	biosynthetic process	BP	117
## GO:0022904		respiratory electron transport chain	BP	122
## GO:0009201	ribonucleoside triphosphate	biosynthetic process	BP	123
## GO:0032543		mitochondrial translation	BP	131
## GO:0006119		oxidative phosphorylation	BP	147
## GO:0009060		aerobic respiration	BP	194
## GO:0046034		ATP metabolic process	BP	217
## GO:0045333		cellular respiration	BP	240
## GO:0009205	purine ribonucleoside triphosphate	metabolic process	BP	242
## GO:0009144	purine nucleoside triphosphate	metabolic process	BP	247
## GO:0009199	ribonucleoside triphosphate	metabolic process	BP	249
## GO:0009141	nucleoside triphosphate	metabolic process	BP	267
## GO:0015980	energy derivation by oxidation of organic compounds		BP	333
## GO:0009150	purine ribonucleotide	metabolic process	BP	447
## GO:0009259	ribonucleotide	metabolic process	BP	466
## GO:0019693	ribose phosphate	metabolic process	BP	475
## GO:0006163	purine nucleotide	metabolic process	BP	476
## GO:0006091	generation of precursor metabolites and energy		BP	505
## GO:0072521	purine-containing compound	metabolic process	BP	507
## GO:0007005		mitochondrion organization	BP	542
## GO:0009117	nucleotide	metabolic process	BP	597
## GO:0006753	nucleoside phosphate	metabolic process	BP	605
## GO:0009057	macromolecule	catabolic process	BP	1385
## GO:0046907		intracellular transport	BP	1587
## GO:0043933	protein-containing complex	organization	BP	1845
## GO:1901575	organic substance	catabolic process	BP	2120
## GO:0009056		catabolic process	BP	2579
## GO:0022607		cellular component assembly	BP	3011
## GO:0044260	cellular macromolecule	metabolic process	BP	3266
## GO:0044085		cellular component biogenesis	BP	3273
## GO:0006996		organelle organization	BP	3556
## GO:0044271	cellular nitrogen compound	biosynthetic process	BP	4865
## GO:1901576	organic substance	biosynthetic process	BP	5877
## GO:0009058		biosynthetic process	BP	5962
## GO:0006139	nucleobase-containing compound	metabolic process	BP	6165
## GO:0046483		heterocycle metabolic process	BP	6336
## GO:0006725	cellular aromatic compound	metabolic process	BP	6392

##	G0:1901564		organonitrogen compound metabolic process	BP	6535
##	G0:0071840		cellular component organization or biogenesis	BP	6601
##	G0:1901360		organic cyclic compound metabolic process	BP	6632
##	G0:0034641		cellular nitrogen compound metabolic process	BP	7053
##	G0:0044237		cellular metabolic process	BP	10271
##	G0:0006807		nitrogen compound metabolic process	BP	10310
##	G0:0044238		primary metabolic process	BP	10825
##	G0:0008152		metabolic process	BP	12433
##	G0:0009987		cellular process	BP	17266
##		DE	P.DE	FDR.DE	
##	G0:0019646	29	3.185009e-11	1.179635e-08	
##	G0:0033108	29	1.728907e-10	5.293453e-08	
##	G0:0042773	32	1.894553e-12	9.855029e-10	
##	G0:0042775	32	1.894553e-12	9.855029e-10	
##	G0:0009206	30	2.900705e-09	6.660888e-07	
##	G0:0022904	33	1.029992e-10	3.331226e-08	
##	G0:0009201	31	2.500253e-09	5.858502e-07	
##	G0:0032543	32	3.215364e-09	7.225843e-07	
##	G0:0006119	39	4.071429e-12	1.869844e-09	
##	G0:0009060	51	3.098510e-15	2.371703e-12	
##	G0:0046034	50	1.423809e-12	7.974370e-10	
##	G0:0045333	56	3.671179e-14	2.634415e-11	
##	G0:0009205	55	1.985360e-13	1.266384e-10	
##	G0:0009144	55	4.836249e-13	2.847559e-10	
##	G0:0009199	56	1.912262e-13	1.254608e-10	
##	G0:0009141	57	1.175566e-12	6.748629e-10	
##	G0:0015980	65	2.317697e-12	1.132367e-09	
##	G0:0009150	76	4.145639e-11	1.420840e-08	
##	G0:0009259	78	5.019944e-11	1.670623e-08	
##	G0:0019693	80	2.060680e-11	7.757276e-09	
##	G0:0006163	77	3.488002e-10	9.767681e-08	
##	G0:0006091	89	1.060211e-13	7.160480e-11	
##	G0:0072521	79	1.240783e-09	3.094531e-07	
##	G0:0007005	92	3.908133e-13	2.361643e-10	
##	G0:0009117	91	2.197180e-10	6.386563e-08	
##	G0:0006753	91	4.440621e-10	1.213928e-07	
##	G0:0009057	167	2.299665e-09	5.444041e-07	
##	G0:0046907	193	5.259017e-11	1.725183e-08	
##	G0:0043933	213	5.339682e-10	1.442531e-07	
##	G0:1901575	245	1.832012e-11	7.130254e-09	
##	G0:0009056	285	4.676394e-11	1.579177e-08	
##	G0:0022607	314	2.884279e-09	6.660888e-07	
##	G0:0044260	344	1.127885e-10	3.597170e-08	
##	G0:0044085	342	3.424318e-10	9.707731e-08	
##	G0:0006996	397	2.397043e-16	2.117050e-13	
##	G0:0044271	473	1.909195e-09	4.566755e-07	
##	G0:1901576	563	1.995961e-10	5.952370e-08	
##	G0:0009058	569	2.809006e-10	8.062899e-08	
##	G0:0006139	596	6.255495e-12	2.782741e-09	
##	G0:0046483	613	1.974182e-12	9.855029e-10	
##	G0:0006725	614	7.700635e-12	3.274624e-09	
##	G0:1901564	623	2.049681e-11	7.757276e-09	
##	G0:0071840	620	3.954915e-10	1.094177e-07	
##	G0:1901360	636	3.032329e-12	1.421048e-09	

```
## G0:0034641 681 4.234797e-14 2.946777e-11
## G0:0044237 965 4.271072e-20 6.538442e-17
## G0:0006807 947 6.055583e-16 5.039696e-13
## G0:0044238 986 6.145168e-16 5.039696e-13
## G0:0008152 1083 6.301552e-12 2.782741e-09
## G0:0009987 1421 7.990243e-12 3.335999e-09
```

```
edgeR_res <- LRT$table[order(LRT$table$FDR, decreasing=F),]
write.table(edgeR_res, sep= "\t", file="C:/Users/Luca Visser/Documents/1st master/AHTA/Practica/Project,
RNAseq_GSA_res <- topGO(goanaOut, ontology="BP", number=100)
write.table(RNAseq_GSA_res, sep= "\t", file="C:/Users/Luca Visser/Documents/1st master/AHTA/Practica/Pr
```