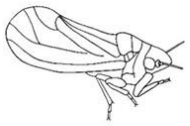
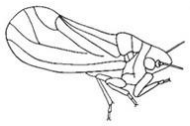
WEEK 15. PRACTICE

Differential expression analysis using DESeq2

Differentially expressed gene(DEG) analysis



27℃ 15 min

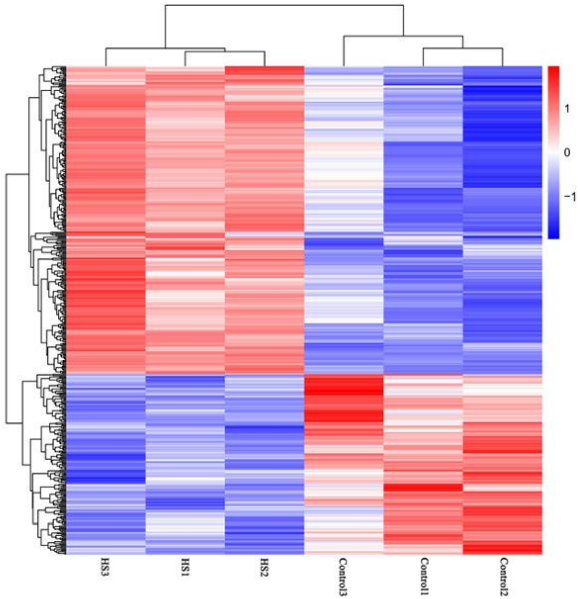


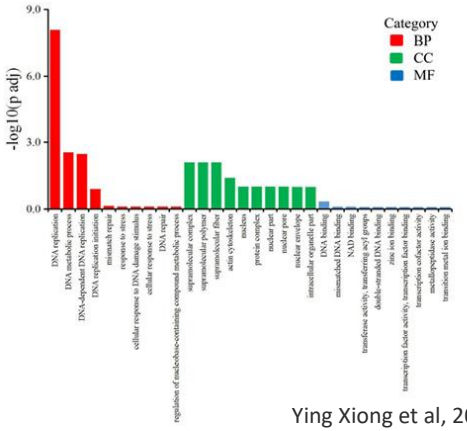
40℃ 15 min

RNA-seq

Differentially expressed genes

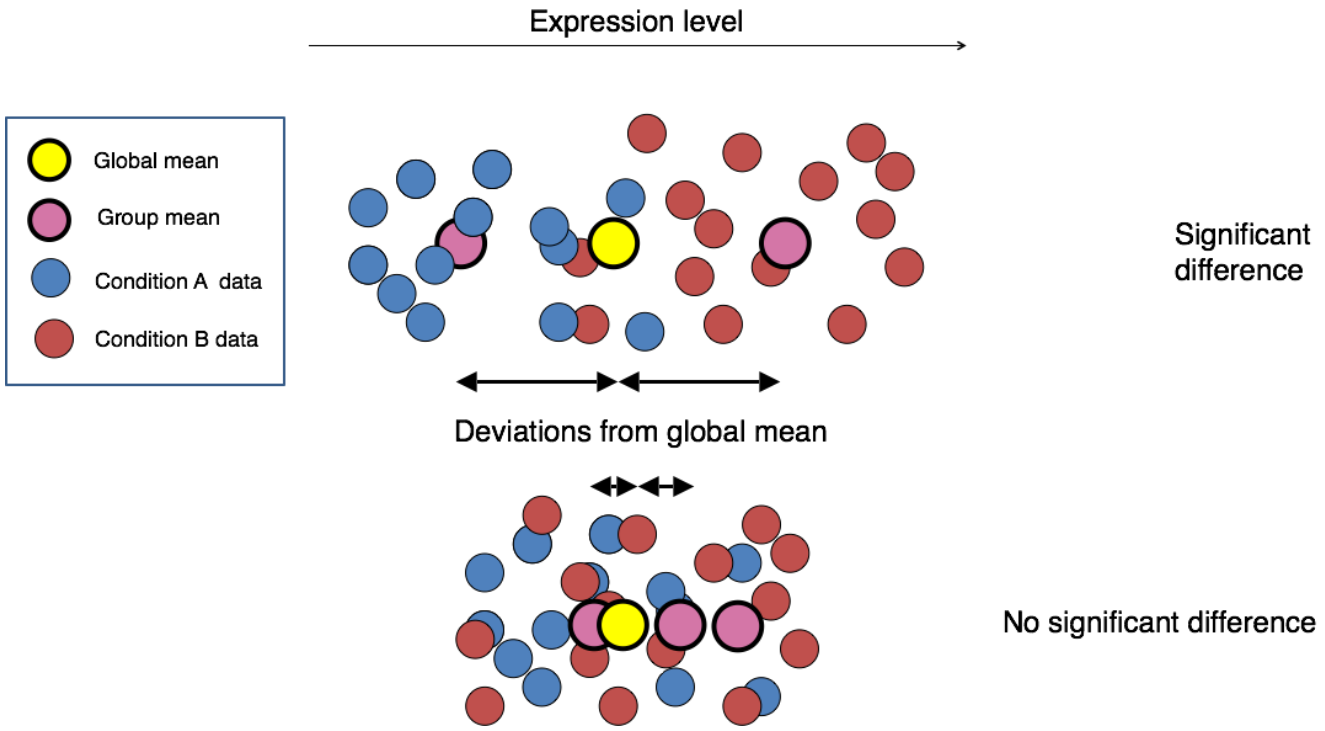
Gene ontology analysis





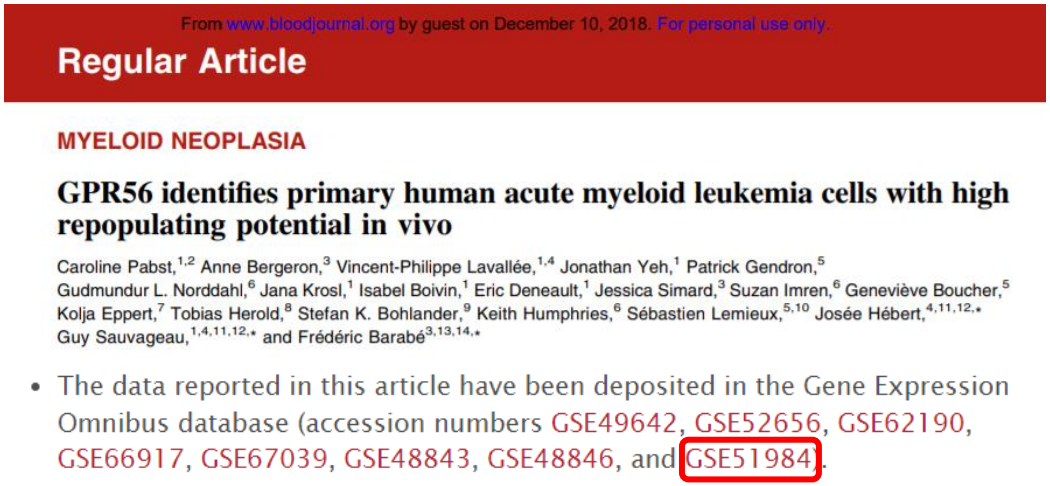
Ying Xiong et al, 2019

Differential expression analysis with DESeq2



https://hbctraining.github.io/DGE_workshop/lessons/04_DGE_DESeq2_analysis.html

Input data for this week



Regular Article

MYELOID NEOPLASIA

GPR56 identifies primary human acute myeloid leukemia cells with high repopulating potential in vivo

Caroline Pabst,[1,2] Anne Bergeron,[3] Vincent-Philippe Lavallée,[1,4] Jonathan Yeh,[1] Patrick Gendron,[5] Gudmundur L. Norddahl,[6] Jana Krosl,[1] Isabel Boivin,[1] Eric Deneault,[1] Jessica Simard,[3] Suzan Imren,[6] Geneviève Boucher,[5] Kolja Eppert,[7] Tobias Herold,[8] Stefan K. Bohlander,[9] Keith Humphries,[6] Sébastien Lemieux,[5,10] Josée Hébert,[4,11,12,*] Guy Sauvageau,[1,4,11,12,*] and Frédéric Barabé[3,13,14,*]

- The data reported in this article have been deposited in the Gene Expression Omnibus database (accession numbers GSE49642, GSE52656, GSE62190, GSE66917, GSE67039, GSE48843, GSE48846, and GSE51984).

- RNA-seq of T-cells and B-cells (5 replicates for each cell types)
- The genes whose variances of RNA-seq expression values (FPKM) among the samples are high were selected (501 genes)

https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE51984

Identification of DEGs using DESeq2

- DESeq2

  - test for differential expression based on a model using the negative binomial distribution

- Usage (in R):

```
dds <- DESeqDataSetFromMatrix(countData = <read count table>,
                              colData = <label table>,
                              design = ~ <group attribute in label>)
dds <- DESeq(dds)
```

Input files for the DESeq2

cp /home/biguser/tutor/Session15/* .

```
-rw-r--r-- 1 biguser biguser  3593 Dec  8 15:24 DESeq2_update.R
-rw-r--r-- 1 biguser biguser   311 Dec  8 15:24 label_table.txt
-rw-r--r-- 1 biguser biguser 39129 Dec  8 15:24 readcount.txt
```

Sample	group	paired
B_cells_01	Bcell	paired-end
B_cells_02	Bcell	paired-end
B_cells_03	Bcell	paired-end
B_cells_04	Bcell	paired-end
B_cells_05	Bcell	paired-end
T_cells_01	Tcell	paired-end
T_cells_02	Tcell	paired-end
T_cells_03	Tcell	paired-end
T_cells_04	Tcell	paired-end
T_cells_05	Tcell	paired-end

ID	Symbol	B_cells_01	B_cells_02	B_cells_03		B_cells_04		B_cells_05		T_cells_01		T_cells_02
ENSG00000019582.10	CD74	164084	393381	335146	377965	179726	7248	9580	11369	11884	9673	
ENSG00000204287.9	HLA-DRA	78780	135217	105086	126555	60643	836	462	412	1231	637	
ENSG00000198712.1	MT-CO2	155975	411920	263438	152005	87205	129234	111245	141479	90318	77609	
ENSG00000112306.7	RPS12	30589	78362	47051	58940	29070	67696	69411	85748	62374	53368	
ENSG00000198804.2	MT-CO1	380232	911234	651133	468509	252012	310912	279098	372894	264151	243672	
ENSG00000177954.7	RPS27	51124	109201	70998	88332	43779	80835	76318	101795	75735	69858	
ENSG00000156508.13	EEF1A1	172420	417900	239409	379887	200479	403599	419327	527892	379702	368594	
ENSG00000198840.2	MT-ND3	47436	94410	67487	37505	26247	38020	28714	35585	20148	21662	
ENSG00000166710.13	B2M	65091	170656	80238	129395	69703	157766	164030	204816	159849	192607	
ENSG00000212907.2	MT-ND4L	28302	69691	58661	37725	19451	24929	23878	31947	23615	16736	
ENSG00000196126.6	HLA-DRB1		19952	51776	34618	42725	25455	838	625	544	2097	1370

R code to run DESeq2

```
-rw-r--r-- 1 biguser biguser  3593 Dec  8 15:24 DESeq2_update.R
-rw-r--r-- 1 biguser biguser   311 Dec  8 15:24 label_table.txt
-rw-r--r-- 1 biguser biguser 39129 Dec  8 15:24 readcount.txt
```

```r
##install the DESeq2 package##
#source("https://bioconductor.org/biocLite.R")
#biocLite("DESeq2")

##get a DESeq2 package##

DESeq2 <- function(readCountTable, tableInfoFile, outputFile, gradeIn, gradeBk)   {
    library('DESeq2', verbose = F)
    library(ggplot2, verbose = F)
    library(reshape2, verbose = F)

##read count matrix table and information table##
    avgReadCount <- read.delim(readCountTable, header = TRUE, sep = '\t', row.names = 1, check.names = FALSE)
    avgReadCount <- avgReadCount[c(-1)]    #remove gene symbol column
    avgReadCountInfo <- read.table(tableInfoFile, header = TRUE, sep = '\t', row.names = 1, check.names = FALSE)

##DEG run##
    print ('Running DESeq2')
    dds <- DESeqDataSetFromMatrix(countData = avgReadCount, colData = avgReadCountInfo, design = ~ group)
        dds <- dds[ rowSums(counts(dds)) > 20, ]
    dds <- DESeq(dds)
    result_05 <- results(dds, alpha = 0.05, contrast=c("group", gradeIn, gradeBk))

    outputPdf <- unlist(strsplit(outputFile, split = '.txt', fixed = TRUE))[1]
    outputMaPdf <- paste(c(outputPdf, '.maplot.pdf'), collapse = '')
    pdf(outputMaPdf, width = 4, height = 4)
    plotMA(result_05, main = paste(c(gradeIn, 'vs', gradeBk), collapse = ' '),
           alpha = 0.05)#, ylim = c(-max(abs(result_05$log2FoldChange)), max(abs(result_05$log2FoldChange))))
    abline(h=c(-2,2), col = 'dodgerblue', lwd = 2)
    dev.off()
```

R code to run DESeq2

```
##whole table##
    newColumn <- c('gene','baseMean','log2FoldChange','lfcSE','stat','pvalue','padj')
    writeTable <- data.frame(result_05)
    writeTable <- data.frame(row.names(writeTable), writeTable)
    colnames(writeTable) <- newColumn

    outputFile <- strsplit(outputFile, split = 'txt')
    allOutFile <- paste(c(outputFile, 'all.txt'), collapse = '')
    write.table(writeTable, file = allOutFile, quote = FALSE, sep = '\t', col.names = TRUE, row.names = FALSE)

##adjusted P-value cutoff##
    sig_result_05 <- subset(result_05, padj < 0.05)
    df_sig_result_05 <- data.frame(sig_result_05)
    df_sig_result_05 <- data.frame(row.names(df_sig_result_05), df_sig_result_05)
    colnames(df_sig_result_05) <- newColumn

    sigOutFile <- paste(c(outputFile, 'sig.txt'), collapse = '')
    write.table(df_sig_result_05, file = sigOutFile, quote = FALSE, sep = '\t', col.names = TRUE, row.names = FALSE)
    print ('DESeq done')

##volcano plot
    writeTable <- na.omit(writeTable)
    sigDeg <- as.factor(abs(writeTable$log2FoldChange) >= 2 & writeTable$padj <= 0.05)
    lgfcMax <- max(c(abs(min(writeTable$log2FoldChange)-0.5), max(writeTable$log2FoldChange)+0.5))
    plt <- ggplot(writeTable, aes(log2FoldChange, -log10(padj), colour=sigDeg)) +
        geom_point(alpha=0.4, size=1) + labs(x="log fold change", y="-log10 adjusted P-value", title=paste(c(gradeIn, 'vs', gradeBk), collapse = ' ')) +
        theme_bw() +
        theme(panel.grid.major = element_blank(), panel.grid.minor = element_blank(), plot.title = element_text(hjust = 0.5),
            text = element_text(size = 16, colour = 'black'), legend.position="none") +
        scale_color_manual(values=c("#999999", "red2"))
    outputVolPdf <- paste(c(outputPdf, '.volcanoplot.pdf'), collapse = '')
    ggsave(outputVolPdf, units = 'cm', height = 10, width = 10)
}

args <- commandArgs(trailingOnly = TRUE)
if (args[1] == '-h' | args[1] == '--help'){
    print ('Rscript DESeq2_update.R <read count> <label> <output> <group of interest> <background group>')
} else {
    DESeq2(args[1], args[2], args[3], args[4], args[5])
```

Running DESeq2

$Rscript DESeq2_update.R .readcount.txt label_table.txt Bcell_background_vs_Tcell Tcell Bcell

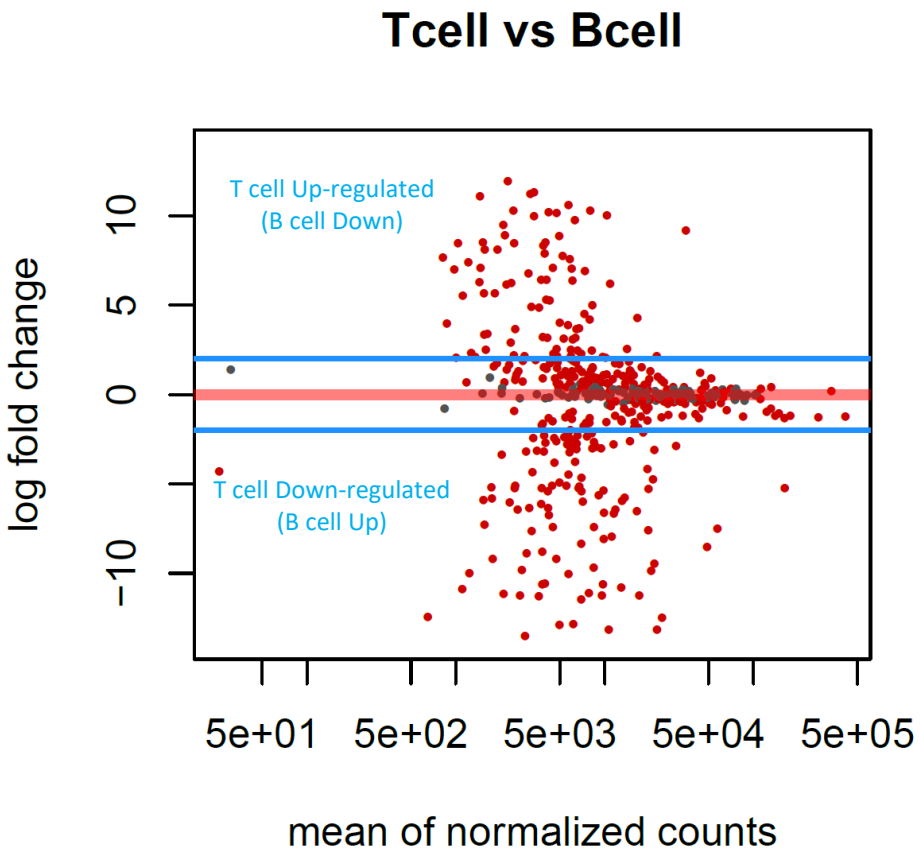
```
[1] "Running DESeq2"
estimating size factors
estimating dispersions
gene-wise dispersion estimates
mean-dispersion relationship
final dispersion estimates
fitting model and testing
[1] "DESeq done"
```

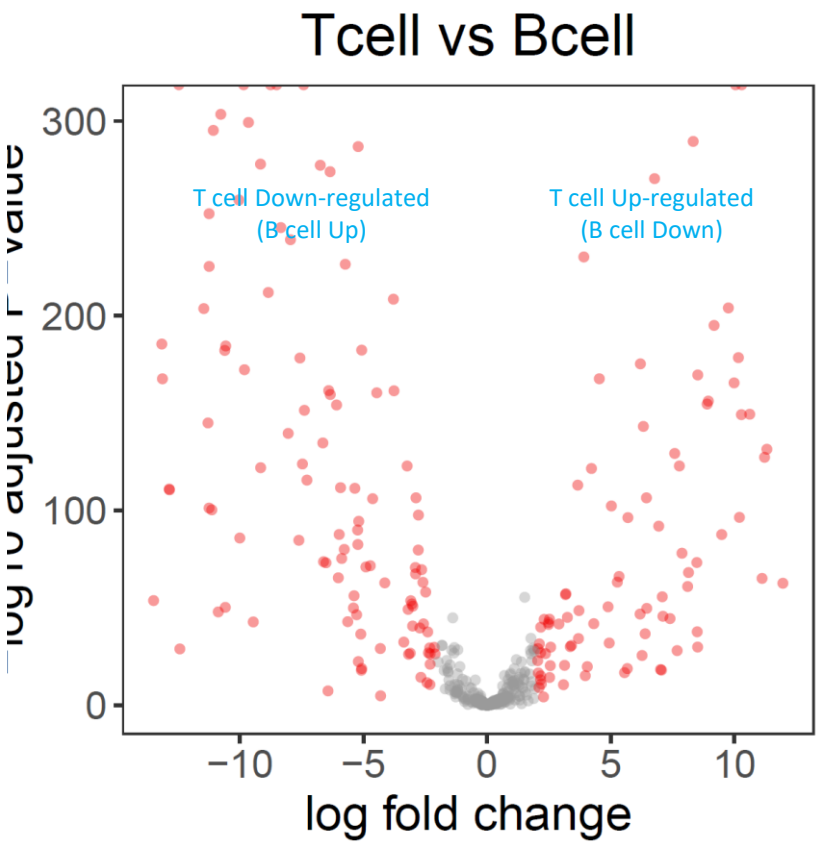
```
-rw-rw-r-- 1 biguser biguser 64380 Dec  8 16:29 Bcell_background_vs_Tcellall.txt
-rw-rw-r-- 1 biguser biguser  8928 Dec  8 16:29 Bcell_background_vs_Tcell.maplot.pdf
-rw-rw-r-- 1 biguser biguser 49492 Dec  8 16:29 Bcell_background_vs_Tcellsig.txt
-rw-rw-r-- 1 biguser biguser 33708 Dec  8 16:29 Bcell_background_vs_Tcell.volcanoplot.pdf
```

all 501 genes

significant DEGs

Output plots for DESeq2

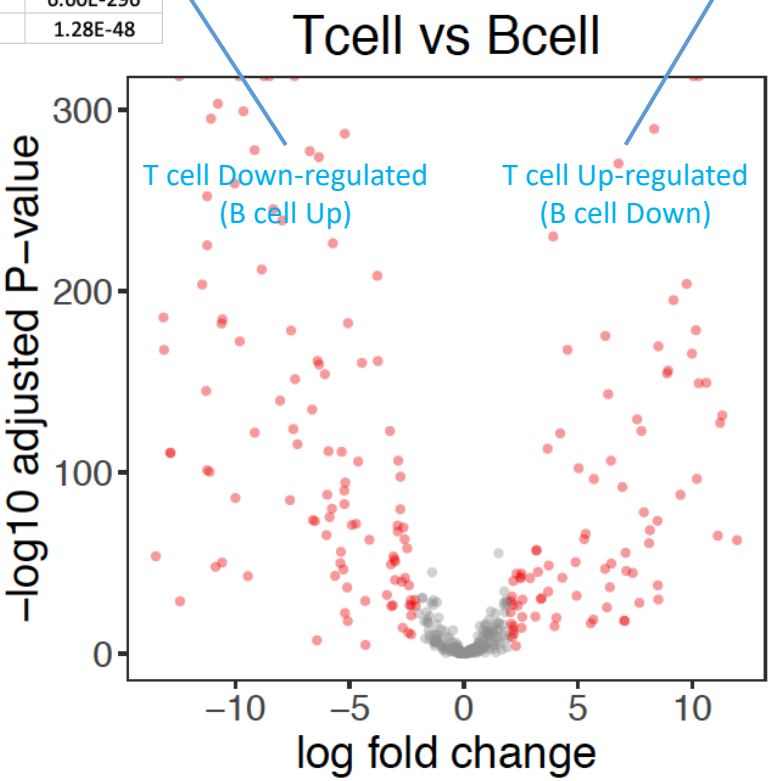




Output files for DESeq2

gene	baseMean	log2FoldChange	padj
BLNK	2918.700756	-13.48608045	1.98E-54
IGJ	22518.04467	-13.15386721	3.49E-186
PAX5	10692.86908	-13.12825157	2.51E-168
FCRLA	4976.028019	-12.85521385	1.04E-111
TCL1A	6164.88851	-12.84404967	2.79E-111
CD22	24352.29797	-12.4651748	0
TNFRSF17	649.0131117	-12.42537173	1.34E-29
FCER2	6978.040165	-11.45555395	2.20E-204
SPIB	3614.323174	-11.28601261	1.17E-145
CD180	2702.923748	-11.24383308	6.01E-102
BANK1	17125.89172	-11.24194382	4.12E-253
ADAM28	9602.185366	-11.23896948	4.92E-226
CLEC17A	2095.91957	-11.13254712	5.20E-101
CD19	7848.139949	-11.07137013	6.60E-296
CD1C	1106.907196	-10.87108855	1.28E-48

gene	baseMean	log2FoldChange	padj
CD8B	2234.852487	11.96167336	2.51E-63
CD3D	3356.313502	11.31389779	3.84E-132
CD28	3174.132576	11.22003263	5.19E-128
TRAT1	1454.112144	11.11966502	8.11E-66
CD8A	5733.489469	10.62088863	3.94E-150
ITK	8005.9051	10.29467119	0
CD3G	2435.870636	10.2838521	6.27E-150
CD2	4216.003782	10.20424873	3.64E-97
GIMAP5	4753.338392	10.16280914	3.40E-179
CD3E	10366.87008	10.04740398	0



Python script to extract significant DEGs

```
#!/usr/bin/env python

import sys

deg_result= "Bcell_background_vs_Tcellall.txt"

deg_open= open(deg_result, "r")
deg_lineL= deg_open.readlines()
deg_open.close()

sig_up= list()
sig_down= list()

for i_line in deg_lineL[1:]: ## First line is header so skip it
        infoL= i_line.strip().split("\t")
        geneid= infoL[0]
        try:
                l2fc= float(infoL[2])
                padj= float(infoL[6])
        except :
                continue

        if padj > 0.05: ## padj cutoff is 0.05
                continue
        if l2fc > 2.0:
                sig_up.append(geneid)
        elif l2fc < -2.0:
                sig_down.append(geneid)
        else:
                pass

print "Number of genes highly expressed in T-cell :", len(sig_up)
print "Number of genes highly expressed in B-cell :", len(sig_down)
```

Number of genes highly expressed in T-cell : 85
Number of genes highly expressed in B-cell : 106

Python script to extract significant DEGs

```
countfile= "readcount.txt"

count_open= open(countfile, "r")
count_lineL= count_open.readlines()
count_open.close()

gene_idsymbolD= dict()
for i_line in count_lineL[1:]: ## first line is header
        infoL= i_line.strip().split("\t")
        geneid= infoL[0]
        genesymbol= infoL[1]
        gene_idsymbolD[geneid]= genesymbol

## Function to write output gene list (gene symbol)
def writeOutput(outputname, genelist, gene_idsymbolD):
        fileopen= open(outputname, "w")
        for i_gene in genelist:
#               geneid= i_gene[0]
                genesymbol= gene_idsymbolD[i_gene]
                outputline= genesymbol+ "\n"
                fileopen.write(outputline)
        fileopen.close()

## T-cell specific genes
tcell_output= "tcell_specific_gene_symbols.txt"
writeOutput(tcell_output, sig_up, gene_idsymbolD)

## B-cell specific genes
bcell_output= "bcell_specific_gene_symbols.txt"
writeOutput(bcell_output, sig_down, gene_idsymbolD)
```

List of genes (symbols) that are significantly, differentially expressed

```
-rw-rw-r-- 1 biguser biguser   678 Dec  8 16:46 bcell_specific_gene_symbols.txt
-rw-r--r-- 1 biguser biguser  1517 Dec  8 16:47 getSigGenes.py
drwxr-xr-x 2 biguser biguser   116 Dec  8 16:39 inputTables
-rw-rw-r-- 1 biguser biguser   479 Dec  8 16:46 tcell_specific_gene_symbols.txt
```

```
CD74
HLA-DRA
HLA-DRB1
CD79A
IGJ
MS4A1
CD79B
FCRL1
HLA-DPB1
HLA-DRB5
CD37
CD22
HLA-DMA
HLA-DPA1
FAM129C
FCER2
CD19
BANK1
TCL1A
IL4R
HLA-DQA2
HLA-DMB
BLK
IRF8
FCRL2
VPREB3
HLA-DQB1
CTSZ
TNFRSF13C
RALGPS2
SYK
ALOX5
HLA-DOA
CYBB
MZB1
P2RX5
FCRLA
STAP1
HLA-DQA1
RNASE6
HSH2D
BTK
BIRC3
HLA-DOB
bcell_specific_gene_symbols.txt
```

```
IL7R
CCL5
NKG7
S100A4
CD3E
CD3D
GNLY
CD2
SPOCK2
IL32
LCK
HCST
GZMH
SELPLG
ZAP70
IFITM1
PRF1
GZMA
CST7
GIMAP4
GIMAP7
CD8A
CTSW
TCF7
KLRB1
GZMM
ANXA1
PIM1
ARL4C
CD8B
CD5
LEF1
CD6
GZMB
GIMAP6
SAMHD1
TNFRSF25
S100A11
MAL
CD247
FGFBP2
PLCG1
CD7
ATP8B2
tcell_specific_gene_symbols.txt
```

Biological signatures associated with list of genes