Direct inhibition of the NOTCH TF. Differenctial expression analysis

Fernando Freire

May 26, 2019

Contents

1 Array expression profiling

Summary

NOTCH proteins regulate signaling pathways involved in cellular differentiation, proliferation and death. Overactive Notch signaling as been observed in numerous cancers and has been extensively studied in the context of T-cell acute lymphoblastic leukemia (T-ALL) where more than 50% of pateints harbour mutant NOTCH1. Small molecule modulators of these proteins would be important for understanding the role of NOTCH proteins in malignant and normal biological processes.

We were interested to measure the global changes in gene expression upon treatment of the human T-ALL cell lines HPB-ALL and KOPT-K1 with either vehicle alone (DMSO) or SAHM1, an alpha-helical hydrocarbon stapled peptide derived from the MAML1 co-activator protein.

Overall design Triplicate cultures of KOPT-K1 or HPB-ALL cells were treated with either DMSO alone or SAHM1 (20 uM) for 24 hours. Total RNA was extracted and hybridized to Affymetrix human U133 plus 2.0 microarrays (three arrays per treatment per cell line for a total of 12 arrays).

1.1 Pipeline

1.2 Python imports

Script 1.2.1 (python)

```python
import rpy2.rinterface
%reload_ext rpy2.ipython
```

1.3 R imports

Script 1.3.1 (R)

```R
%%R
##1. Load libraries
library("affy")
library("limma")
library("genefilter")
library(simpleaffy)
library(hgu133plus2.db)
wd <- "/Users/nandoide/misc_work/Desktop/uni/TRREP"
setwd(wd)
```

1.4 Functions

Script 1.4.1 (R)

```R
%%R

import_CEL <- function(pattern) {
    # Import CEL files into affiBatch object
    files <- list.files(pattern = pattern)
```

```r
6      names <- gsub(".CEL.gz", "", files)
7      abatch <- ReadAffy(filenames = files,  compress = TRUE, sampleNames = names)
8      return(abatch)
9  }
10
11 create_eset <- function(affyBatch) {
12     # Generates object eset (class ExprSet),
13     # expresso function provides intensities in log scale
14     return(expresso(affyBatch,
15             bg.correct = TRUE,
16             bgcorrect.method="rma",
17             normalize = TRUE,
18             normalize.method="quantiles",
19             pmcorrect.method="pmonly",
20             summary.method="medianpolish",
21             verbose = TRUE))
22 }
23
24 boxplots <- function(affyBatch, eset, title) {
25     # Generate BOXPLOTS before and after normalization
26     boxplot(affyBatch,
27         main=paste0("Boxplot Before Normalization ", title),
28         col = "lightgrey")
29     df_eset <- as.data.frame(exprs(eset))
30
31     boxplot(data.frame(df_eset),
32         main=paste0("Boxplot After Normalization (log scale) ", title), col = "white")
33 }
34
35 create_TopTable <- function(eset, control_samples=c(1,1,1,0,0,0)) {
36     # Generate Toptable with limma
37
38     # Data filtering using IQR.
39     esetIQR <- varFilter(eset, var.func=IQR, var.cutoff=0.5, filterByQuantile=TRUE)
40
41     # Differential expression analysis.#######
42     r_control_samples <- 1 - control_samples
43     design <- cbind(DMSO=control_samples, SAHM1=r_control_samples)
44
45     rownames(design) <- colnames(eset)
46
47     #7. Contrasts matrix.
48     cont.matrix <- makeContrasts(DMSO_SAHM1 = SAHM1 - DMSO, levels = design)
49
50     #8. Obtaining differentially expressed genes (DEGs)
51     #Linear model and eBayes
52     fit <- lmFit(esetIQR, design)  ##getting DEGs from IQR
53     fit2 <- contrasts.fit(fit, cont.matrix)
54     fit2 <- eBayes(fit2)
55
56     #Table with DEGs results
```

```
57     toptableIQR <- topTable(fit2, number=dim(exprs(esetIQR))[1], adjust.method="BH",
   →   sort.by="p")
58     return(toptableIQR)
59 }
60
61 anotate_TopTable <- function(toptable) {
62     # Obtain gene names from probe names and chip symbol dataset
63     probenames_toptable <- as.character(rownames(toptable ))
64     genesymbols_toptable <- as.character(mget(probenames_toptable, hgu133plus2SYMBOL))
65     # Annotated gene table
66     toptable_anot <- cbind(Symbol = genesymbols_toptable, toptable)
67     return(toptable_anot)
68 }
69
70 generank_table <- function(toptable, rnk.file) {
71     # Generate rank of table top 50 upregulared and top 50 downregulated from 250 better
72     # adjustes p-values
73     more_significant = toptable[order(toptable$adj.P.Val, decreasing = FALSE),][1:250,]
74     up_50 = more_significant[which(toptable$logFC > 0), ] [1:50,] # up reg top 50
75     down_50 = more_significant[which(toptable$logFC < 0), ] [1:50,] # down reg top 50
76
77     print("Down-regulated genes")
78     print(down_50[order(down_50$logFC), c(1,2,6)])
79
80     print("Up-regulated genes")
81     print(up_50[order(up_50$logFC), c(1,2,6)])
82
83     d <- rbind(down_50[order(down_50$logFC), c(1,2,6)], up_50[order(up_50$logFC), c(1,2,6)])
84
85     #df <- data.frame(paste0(dSymbol,'|',row.names(d)),dlogFC)
86     df <- data.frame(row.names(d), d$logFC)
87     write.table(df,row.names=FALSE,col.names=FALSE,
88             quote=FALSE,sep="\t",file=paste0(rnk.file, ".rnk"))
89 }
```

1.5   Quality control

```
1 %%R
2
3 setwd("GSE18198_data")
4 affyBatch = import_CEL("*")
5 setwd(wd)
6 affyBatch_MAS5 <- call.exprs(affyBatch,"mas5")
7 qcs <- qc(affyBatch, affyBatch_MAS5)
8 plot(qcs)
9 qcs
```

Script 1.5.1 (R)

```
An object of class "QCStats"
```

4

```
Slot "scale.factors":
 [1] 0.4624769 0.9923886 0.5749658 0.5299016 0.4725059 0.4445994 1.4066966
 [8] 1.2441075 1.3089160 1.9466663 2.1150710 2.3084671


Slot "target":
[1] 100


Slot "percent.present":
  HPB_DMSO_01.present   HPB_DMSO_02.present   HPB_DMSO_03.present
             45.75583             41.61500              44.97485
 HPB_SAHM1_01.present  HPB_SAHM1_02.present  HPB_SAHM1_03.present
             45.39369             45.37906              46.93004
  KOPT_DMSO_01.present  KOPT_DMSO_02.present  KOPT_DMSO_03.present
             39.18793             39.72016              40.17558
KOPT_SAHM1_01.present KOPT_SAHM1_02.present KOPT_SAHM1_03.present
             38.46182             38.39049              38.57888


Slot "average.background":
  HPB_DMSO_01    HPB_DMSO_02    HPB_DMSO_03   HPB_SAHM1_01   HPB_SAHM1_02
     71.40948       51.82766       67.15371       73.25355       78.06755
 HPB_SAHM1_03   KOPT_DMSO_01   KOPT_DMSO_02   KOPT_DMSO_03  KOPT_SAHM1_01
     64.75102       58.67351       58.61665       56.23122       53.46125
KOPT_SAHM1_02 KOPT_SAHM1_03
     44.98804       45.99328


Slot "minimum.background":
  HPB_DMSO_01    HPB_DMSO_02    HPB_DMSO_03   HPB_SAHM1_01   HPB_SAHM1_02
     67.78476       49.90441       64.95388       68.98329       74.59893
 HPB_SAHM1_03   KOPT_DMSO_01   KOPT_DMSO_02   KOPT_DMSO_03  KOPT_SAHM1_01
     61.72838       56.40174       54.42714       53.83356       50.28008
KOPT_SAHM1_02 KOPT_SAHM1_03
     42.42914       43.87834


Slot "maximum.background":
  HPB_DMSO_01    HPB_DMSO_02    HPB_DMSO_03   HPB_SAHM1_01   HPB_SAHM1_02
     73.33486       52.82101       69.57489       75.70203       79.83810
 HPB_SAHM1_03   KOPT_DMSO_01   KOPT_DMSO_02   KOPT_DMSO_03  KOPT_SAHM1_01
     66.69588       60.24642       60.27544       57.78016       54.91569
KOPT_SAHM1_02 KOPT_SAHM1_03
     45.78716       46.82307


Slot "spikes":
            AFFX-r2-Ec-bioB-3_at AFFX-r2-Ec-bioC-3_at AFFX-r2-Ec-bioD-3_at
HPB_DMSO_01             8.248482             9.704242             12.15884
HPB_DMSO_02             9.488241            11.021774             13.39970
HPB_DMSO_03             8.174298             9.589539             12.00550
HPB_SAHM1_01            8.381753             9.772156             12.21394
HPB_SAHM1_02            8.159541             9.712199             12.13458
```

```
HPB_SAHM1_03            7.898193          9.390437           11.90994
KOPT_DMSO_01            8.920814          8.512096           12.74309
KOPT_DMSO_02            9.007404          8.715061           12.82763
KOPT_DMSO_03            8.978651          8.593016           12.75616
KOPT_SAHM1_01           9.710773          9.355043           13.32068
KOPT_SAHM1_02          10.167828          9.646911           13.70326
KOPT_SAHM1_03          10.414865          9.875183           13.89010
                   AFFX-r2-P1-cre-3_at
HPB_DMSO_01            13.20270
HPB_DMSO_02            14.44458
HPB_DMSO_03            13.18265
HPB_SAHM1_01           13.31337
HPB_SAHM1_02           13.19213
HPB_SAHM1_03           12.97417
KOPT_DMSO_01           14.04051
KOPT_DMSO_02           14.10125
KOPT_DMSO_03           14.00066
KOPT_SAHM1_01          14.63294
KOPT_SAHM1_02          14.82687
KOPT_SAHM1_03          15.01834


Slot "qc.probes":
          AFFX-HSAC07/X00351_3_at AFFX-HSAC07/X00351_5_at
HPB_DMSO_01            12.73093                11.92493
HPB_DMSO_02            13.63221                12.27401
HPB_DMSO_03            12.77779                11.90235
HPB_SAHM1_01           12.79605                11.63738
HPB_SAHM1_02           12.66274                11.31439
HPB_SAHM1_03           12.56844                11.59679
KOPT_DMSO_01           13.53358                13.02196
KOPT_DMSO_02           13.49021                13.00410
KOPT_DMSO_03           13.50476                12.99276
KOPT_SAHM1_01          13.78372                13.04395
KOPT_SAHM1_02          13.85151                12.82967
KOPT_SAHM1_03          13.88275                12.84090
          AFFX-HSAC07/X00351_M_at AFFX-HUMGAPDH/M33197_3_at
HPB_DMSO_01            12.19455                12.87855
HPB_DMSO_02            12.84310                13.87735
HPB_DMSO_03            12.24595                12.89411
HPB_SAHM1_01           12.17076                12.98237
HPB_SAHM1_02           11.91872                12.87606
HPB_SAHM1_03           11.97960                12.69856
KOPT_DMSO_01           13.21759                13.80409
KOPT_DMSO_02           13.20014                13.72550
KOPT_DMSO_03           13.16413                13.78359
KOPT_SAHM1_01          13.33575                14.13775
KOPT_SAHM1_02          13.31856                14.20714
KOPT_SAHM1_03          13.29794                14.34094
```

```
                AFFX-HUMGAPDH/M33197_5_at AFFX-HUMGAPDH/M33197_M_at
HPB_DMSO_01                      12.86110                  12.72697
HPB_DMSO_02                      13.80521                  13.68863
HPB_DMSO_03                      12.86872                  12.70080
HPB_SAHM1_01                     12.84679                  12.78306
HPB_SAHM1_02                     12.80222                  12.66854
HPB_SAHM1_03                     12.67308                  12.53523
KOPT_DMSO_01                     13.68232                  13.59723
KOPT_DMSO_02                     13.74016                  13.55294
KOPT_DMSO_03                     13.71389                  13.56799
KOPT_SAHM1_01                    14.11439                  13.93807
KOPT_SAHM1_02                    14.20615                  13.97154
KOPT_SAHM1_03                    14.29181                  14.05688

Slot "bioBCalls":
  HPB_DMSO_01.present    HPB_DMSO_02.present    HPB_DMSO_03.present
               "P"                    "P"                    "P"
 HPB_SAHM1_01.present   HPB_SAHM1_02.present   HPB_SAHM1_03.present
               "P"                    "P"                    "P"
 KOPT_DMSO_01.present   KOPT_DMSO_02.present   KOPT_DMSO_03.present
               "P"                    "P"                    "P"
KOPT_SAHM1_01.present  KOPT_SAHM1_02.present  KOPT_SAHM1_03.present
               "P"                    "P"                    "P"

Slot "arraytype":
[1] "hgu133plus2cdf"
```

QC Stats

	actin3/actin5 (△)	gapdh3/gapdh5 (○)
KOPT_SAHM1_03	38.58%	45.99
KOPT_SAHM1_02	38.39%	44.99
KOPT_SAHM1_01	38.46%	53.46
KOPT_DMSO_03	40.18%	56.23
KOPT_DMSO_02	39.72%	58.62
KOPT_DMSO_01	39.19%	58.67
HPB_SAHM1_03	46.93%	64.75
HPB_SAHM1_02	45.38%	78.07
HPB_SAHM1_01	45.39%	73.25
HPB_DMSO_03	44.97%	67.15
HPB_DMSO_02	41.61%	51.83
HPB_DMSO_01	45.76%	71.41
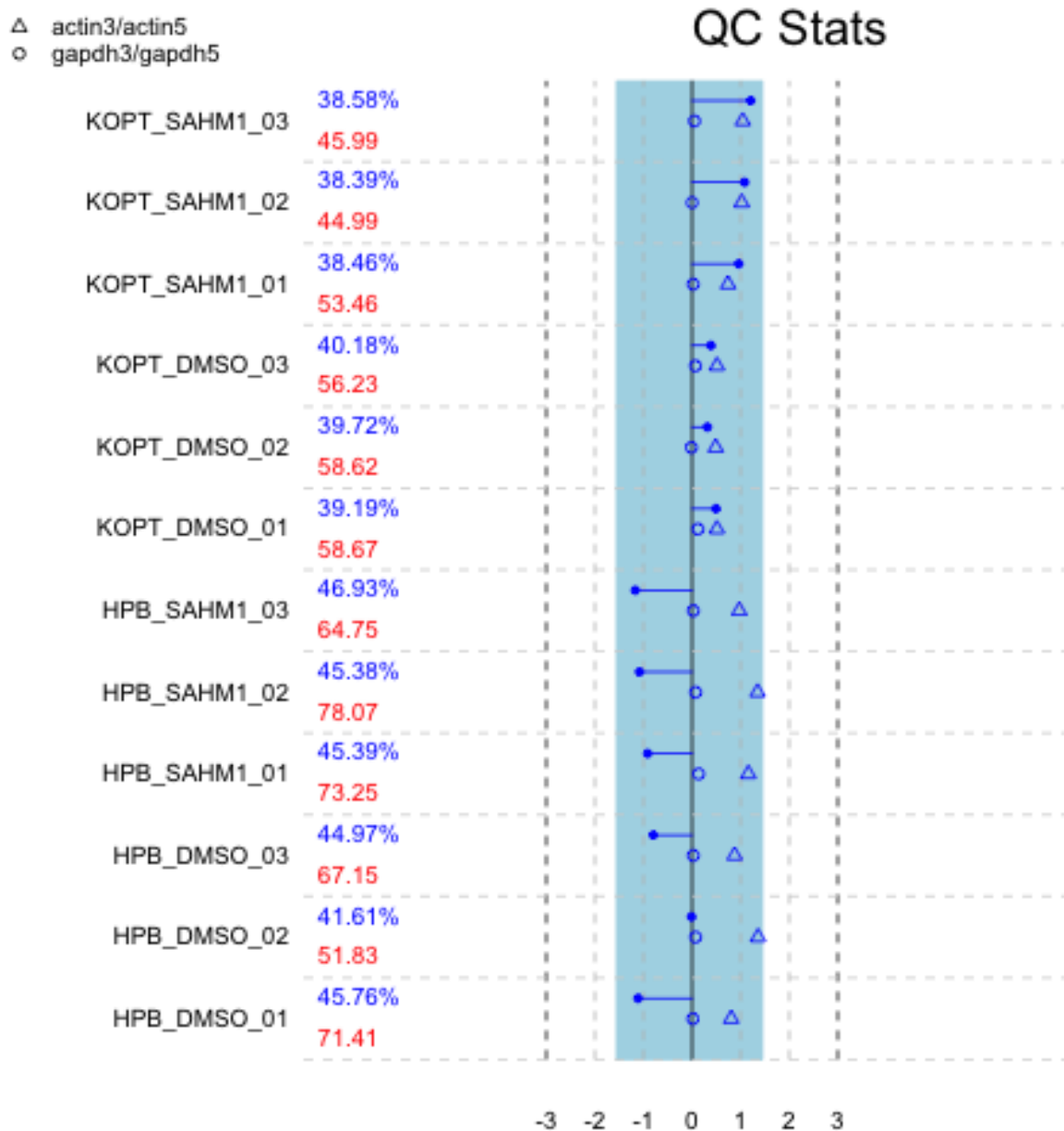
1.6 Load raw data

Script 1.6.1 (R)

```R
%%R
setwd("GSE18198_data")
affyBatch_HPB = import_CEL("HPB*")
affyBatch_KOPT = import_CEL("KOPT*")
setwd(wd)
```

8

1.7 Create expression sets

Script 1.7.1 (R)

```r
%%R
eset_HPB <- create_eset(affyBatch_HPB)
eset_KOPT <- create_eset(affyBatch_KOPT)
```

```
background correction: rma
normalization: quantiles
PM/MM correction : pmonly
expression values: medianpolish
background correcting...done.
normalizing...done.
54675 ids to be processed
|                    |
|####################|
background correction: rma
normalization: quantiles
PM/MM correction : pmonly
expression values: medianpolish
background correcting...done.
normalizing...done.
54675 ids to be processed
|                    |
|####################|
```

Script 1.7.2 (R)

```r
%%R
save(eset_HPB, file="eset_HPB.RData")
save(eset_KOPT, file="eset_KOPT.RData")
```

1.8 Quality plots

Script 1.8.1 (R)

```r
%%R
boxplots(affyBatch_HPB, eset, "HPB Cell Line")
boxplots(affyBatch_KOPT, eset, "KOPT Cell Line")
```

Boxplot Before Normalization HPB Cell Line

HPB_DMSO_01   HPB_DMSO_03   HPB_SAHM1_02
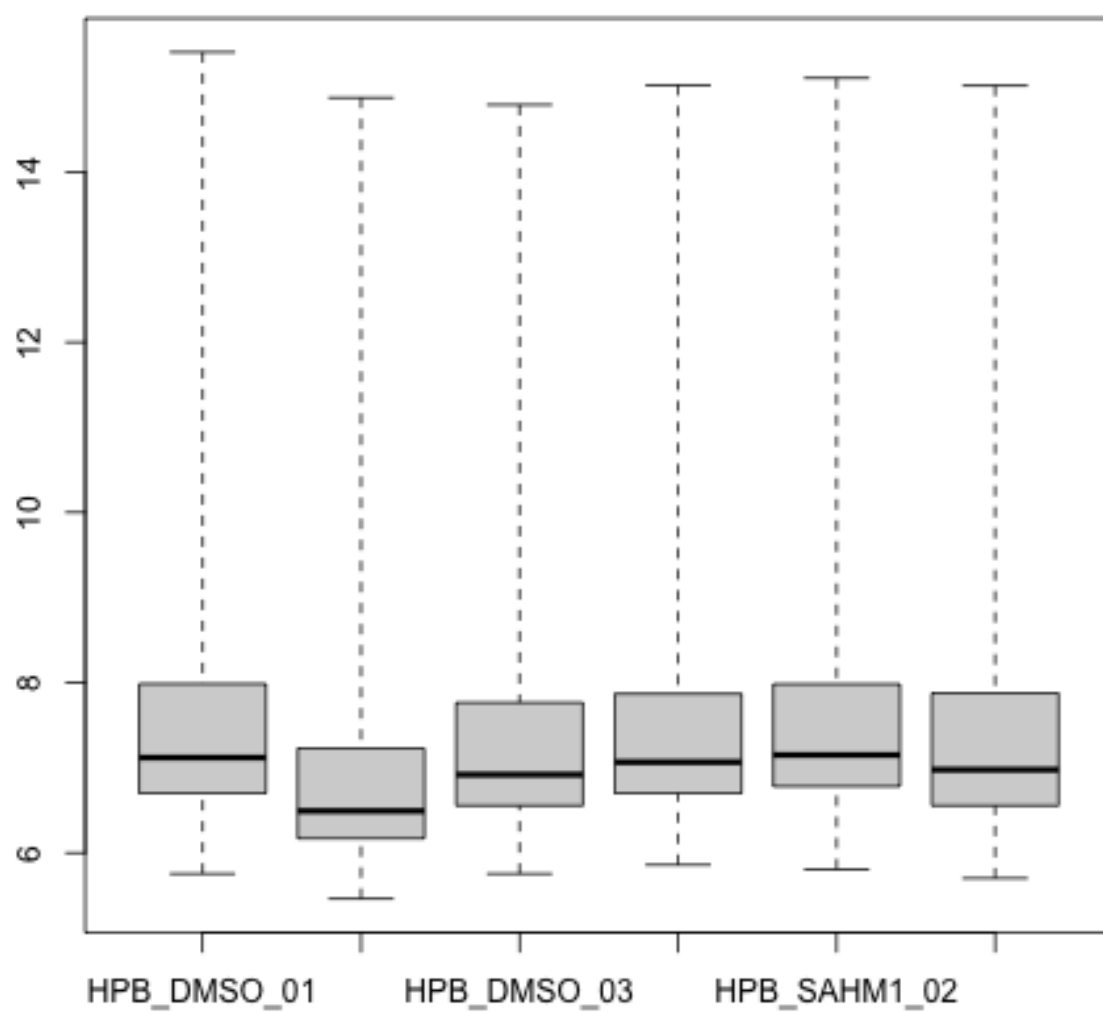
Boxplot After Normalization (log scale) HPB Cell Line
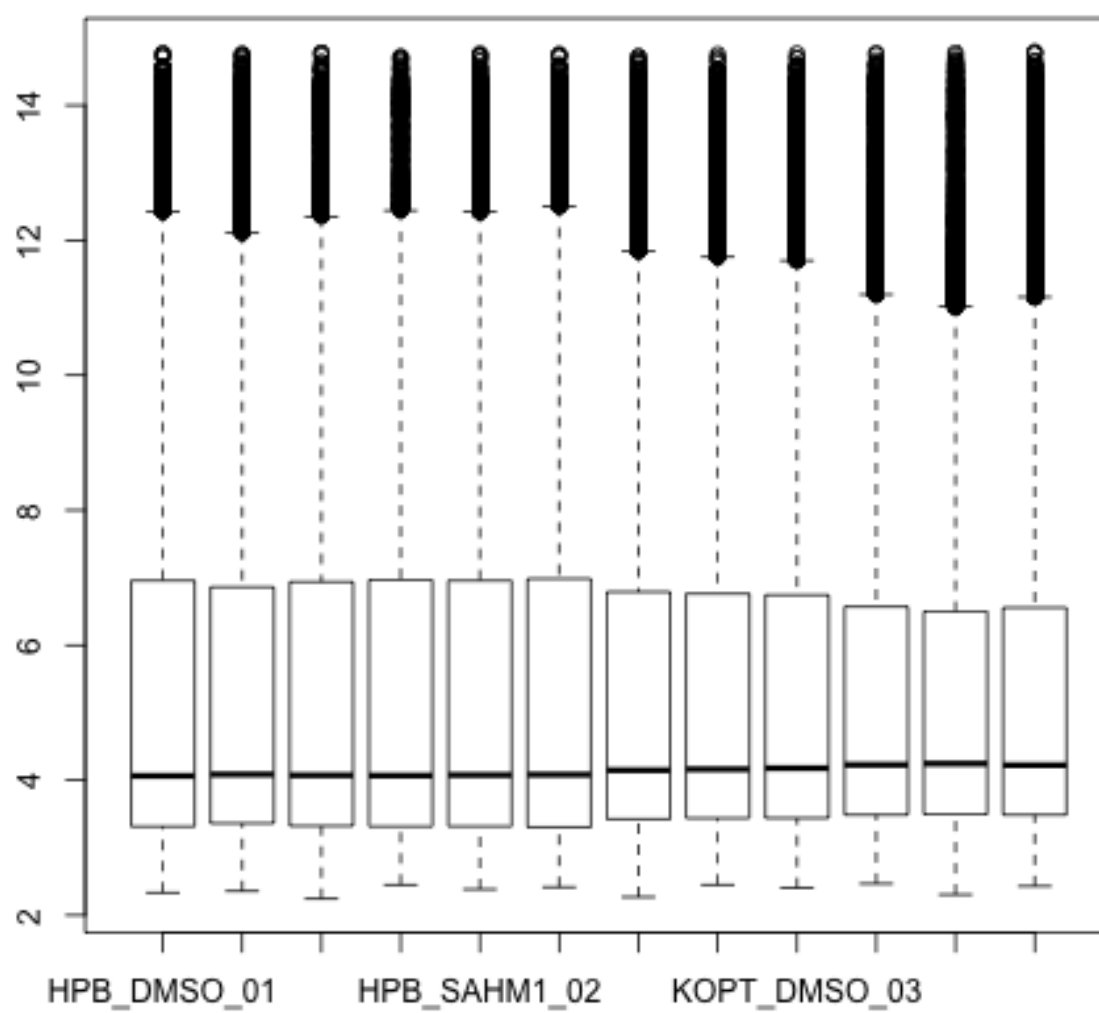
Boxplot Before Normalization KOPT Cell Line
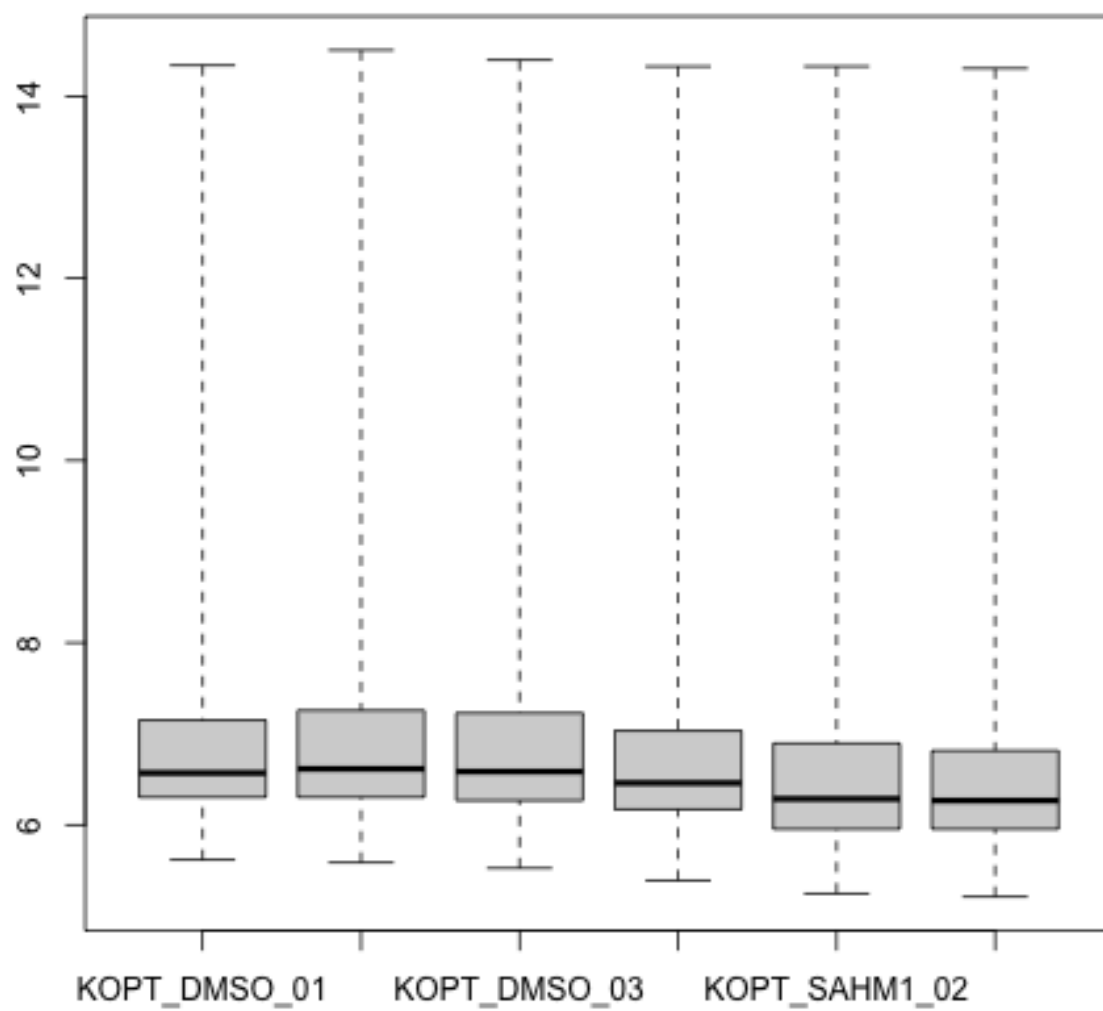
Boxplot After Normalization (log scale) KOPT Cell Line
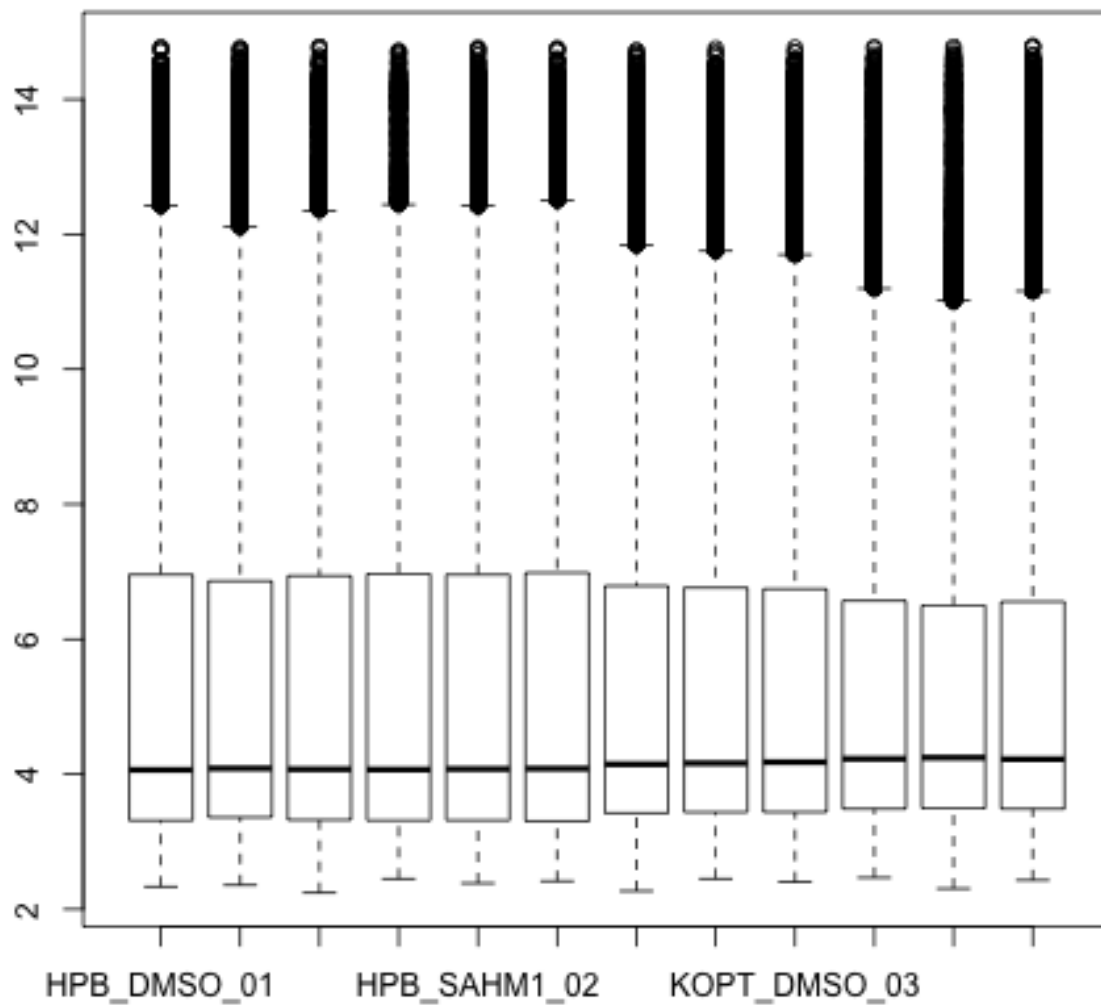
1.9 Differential expressed genes

Script 1.9.1 (R)

```R
%%R
toptable_HPB <- create_TopTable(eset_HPB)
toptable_anot_HPB <- anotate_TopTable(toptable_HPB)
generank_table(toptable_anot_HPB, "generank_HPB")
```

```
[1] "Down-regulated genes"
              Symbol      logFC    adj.P.Val
225342_at        AK4 -2.1078858 1.128982e-05
230710_at   MIR210HG -1.9761492 7.688286e-06
227336_at       DTX1 -1.3841443 8.636324e-05
201842_s_at   EFEMP1 -1.3678548 6.004820e-05
204348_s_at      AK4 -1.3491612 3.350727e-04
227347_x_at     HES4 -1.2640712 1.228528e-04
200953_s_at     CCND2 -1.2357909 7.997079e-05
202464_s_at    PFKFB3 -1.1937490 1.228528e-04
202022_at      ALDOC -1.1753644 1.248092e-04
240546_at   LINC01120 -1.1131135 1.552628e-04
227337_at     ANKRD37 -1.1079168 2.110727e-04
200894_s_at    FKBP4 -1.0737202 5.300330e-04
217078_s_at   CD300A -1.0719092 8.239876e-04
201170_s_at   BHLHE40 -1.0402130 2.549012e-04
202934_at        HK2 -1.0377714 2.104071e-04
201848_s_at    BNIP3 -1.0052154 6.744018e-04
218051_s_at   NT5DC2 -1.0049990 2.851346e-03
203394_s_at     HES1 -0.9993973 7.294799e-04
219371_s_at     KLF2 -0.9800918 3.918155e-04
201251_at        PKM -0.9787897 4.328581e-03
201849_at      BNIP3 -0.9724936 6.167814e-04
213746_s_at     FLNA -0.9705676 1.430899e-03
201516_at        SRM -0.9428084 1.627743e-03
203523_at       LSP1 -0.9360851 2.263449e-03
225944_at        NLN -0.9317256 1.421369e-03
214183_s_at    TKTL1 -0.9290362 2.452591e-03
236180_at         NA -0.9287828 3.049302e-03
201194_at     SELENOW -0.9285463 6.167814e-04
231922_at     ZNF276 -0.9269927 4.098501e-03
209933_s_at   CD300A -0.9049911 8.239876e-04
214752_x_at     FLNA -0.9018607 1.636952e-03
226348_at      FUT11 -0.8976507 1.075839e-03
201212_at       LGMN -0.8972886 1.381530e-03
218305_at        IPO4 -0.8783786 1.923264e-03
205544_s_at      CR2 -0.8502424 1.206402e-03
202145_at        LY6E -0.8406636 2.534145e-03
200859_x_at     FLNA -0.8368746 2.851346e-03
202887_s_at     DDIT4 -0.8271607 1.813467e-03
200965_s_at    ABLIM1 -0.8229490 1.522219e-03
203504_s_at    ABCA1 -0.8211483 1.430899e-03
208116_s_at   MAN1A1 -0.7931451 4.037398e-03
202472_at        MPI -0.7831169 2.929531e-03
207543_s_at    P4HA1 -0.7783781 2.263449e-03
201563_at       SORD -0.7663457 4.281959e-03
222150_s_at     GSAP -0.7532244 4.098501e-03
207539_s_at      IL4 -0.7404704 4.098501e-03
```

```
205895_s_at        NOLC1 -0.7357033 4.328581e-03
219389_at          SUSD4 -0.7347418 3.852969e-03
201562_s_at         SORD -0.7343969 4.098501e-03
218984_at           PUS7 -0.7076878 4.281959e-03
[1] "Up-regulated genes"
                      Symbol      logFC    adj.P.Val
204962_s_at               NA 0.8016614 1.927646e-03
222670_s_at             MAFB 0.8314472 1.414249e-03
244075_at                 NA 0.8555977 1.927646e-03
205047_s_at             ASNS 0.8566562 1.373571e-03
236153_at                 NA 0.8615325 1.373571e-03
228999_at               CHD2 0.8640573 1.731300e-03
202847_at               PCK2 0.8839066 1.373571e-03
242388_x_at            TAGAP 0.8933876 1.116736e-03
243368_at                 NA 0.9077303 1.731300e-03
1558212_at                NA 0.9381646 1.381530e-03
212907_at            SLC30A1 0.9572462 5.305252e-04
241505_at                 NA 0.9630797 1.430899e-03
230659_at                 NA 0.9742286 8.892420e-04
203279_at              EDEM1 0.9785129 3.944123e-04
218923_at               CTBS 0.9839137 1.116736e-03
1558920_at         SLC8A1-AS1 0.9839933 1.272470e-03
215071_s_at         HIST1H2AC 0.9867407 1.634677e-03
217678_at            SLC7A11 1.0002602 6.787587e-04
235795_at               PAX6 1.0066337 6.744018e-04
1556294_at             FXYD2 1.0219109 1.381530e-03
229538_s_at            IQGAP3 1.0315469 5.922793e-04
206864_s_at              HRK 1.0324569 1.969355e-03
243495_s_at           ZNF652 1.0513033 1.522219e-03
218145_at              TRIB3 1.0673820 2.149263e-04
219892_at             TM6SF1 1.0684116 7.475097e-04
244377_at             SLC1A4 1.0880295 2.042060e-04
201010_s_at            TXNIP 1.1062690 1.552628e-04
209921_at            SLC7A11 1.1333321 1.248092e-04
209822_s_at            VLDLR 1.1350680 2.104071e-04
230795_at                 NA 1.1699268 2.104071e-04
213931_at                 NA 1.1702639 3.804126e-04
201009_s_at            TXNIP 1.2328092 1.663743e-04
244042_x_at               NA 1.2501748 1.080676e-03
218559_s_at             MAFB 1.2553755 5.305252e-04
225957_at              CREBRF 1.2981952 4.382754e-04
222853_at              FLRT3 1.3254635 1.138577e-04
219270_at              CHAC1 1.3362724 3.651273e-05
202672_s_at             ATF3 1.3481402 3.651273e-05
218280_x_at               NA 1.3495011 1.272470e-03
207076_s_at             ASS1 1.4918960 3.651273e-05
201008_s_at            TXNIP 1.4920023 1.663743e-04
243871_at        LOC100130476 1.5055489 4.060560e-04
```

```
201464_x_at          JUN 1.5541848 7.688286e-06
236962_at             NA 1.5966520 1.522219e-03
235412_at        ARHGEF7 1.5978442 2.999663e-04
229541_at             NA 1.6143542 2.042060e-04
229147_at         RASSF6 1.6458323 7.688286e-06
235638_at         RASSF6 1.7804932 7.997079e-05
201466_s_at          JUN 2.1333931 2.108830e-06
201465_s_at          JUN 2.2743608 1.053822e-05
```

<div style="background:green">Script 1.9.2 (R)</div>

```R
1  %%R
2  toptable_KOPT <- create_TopTable(eset_KOPT)
3  toptable_anot_KOPT <- anotate_TopTable(toptable_KOPT)
4  generank_table(toptable_anot_KOPT, "generank_KOPT")
```

```
[1] "Down-regulated genes"
              Symbol      logFC    adj.P.Val
209921_at     SLC7A11 -2.916468 2.066610e-11
205047_s_at      ASNS -2.757747 2.764706e-11
209369_at       ANXA3 -2.661459 2.066610e-11
219270_at       CHAC1 -2.264373 7.569090e-10
226517_at       BCAT1 -2.216157 2.899776e-10
214452_at       BCAT1 -2.166267 3.402665e-09
225285_at       BCAT1 -2.072317 2.630231e-10
217678_at     SLC7A11 -2.058710 7.569090e-10
230748_at     SLC16A6 -2.004791 6.729951e-09
219892_at      TM6SF1 -1.957664 1.012031e-09
220892_s_at     PSAT1 -1.938100 4.136414e-10
204351_at       S100P -1.902944 5.924315e-09
223195_s_at     SESN2 -1.889417 7.569090e-10
214079_at       DHRS2 -1.873374 5.924315e-09
209822_s_at      VLDLR -1.863734 4.214321e-09
212290_at      SLC7A1 -1.860735 2.786337e-08
202847_at        PCK2 -1.828534 1.164949e-09
225520_at          NA -1.790127 1.147212e-09
223062_s_at     PSAT1 -1.786577 1.164949e-09
223196_s_at     SESN2 -1.730910 3.463629e-08
200924_s_at    SLC3A2 -1.698282 2.186020e-08
1553972_a_at      CBS -1.682036 3.595785e-09
207076_s_at      ASS1 -1.675254 2.113541e-08
229787_s_at       OGT -1.662192 1.743772e-07
222632_s_at    LZTFL1 -1.643945 9.106004e-08
212816_s_at       CBS -1.599989 1.068633e-07
224839_s_at      GPT2 -1.553796 1.734549e-08
```

```
240983_s_at        CARS -1.552793 5.615021e-08
215411_s_at    TRAF3IP2 -1.540251 2.113541e-08
221539_at     EIF4EBP1 -1.533442 1.149078e-08
201195_s_at      SLC7A5 -1.511384 1.336464e-08
214390_s_at       BCAT1 -1.459915 2.424870e-07
204999_s_at        ATF5 -1.451634 2.113541e-08
210512_s_at       VEGFA -1.448871 5.654616e-08
200878_at        EPAS1 -1.435966 3.072051e-07
212501_at        CEBPB -1.428290 2.844253e-08
224580_at      SLC38A1 -1.428164 1.068633e-07
204744_s_at        IARS -1.419418 2.844253e-08
208693_s_at        GARS -1.406661 4.090508e-08
223059_s_at     FAM107B -1.392809 5.070038e-08
218437_s_at      LZTFL1 -1.332751 1.769993e-07
1558212_at          NA -1.308392 1.982077e-07
205653_at        CTSG -1.279331 2.502696e-07
217078_s_at      CD300A -1.278830 2.424870e-07
203627_at        IGF1R -1.275358 3.751987e-07
221933_at       NLGN4X -1.272235 4.113388e-07
231894_at        SARS -1.263321 2.424870e-07
214095_at        SHMT2 -1.235111 1.982077e-07
201263_at        TARS -1.197960 3.751987e-07
226181_at        TUBE1 -1.189891 3.344154e-07
[1] "Up-regulated genes"
                          Symbol     logFC   adj.P.Val
224429_x_at                   NA 0.9259213 6.376426e-06
220725_x_at                DNAH3 0.9423323 6.447180e-06
210686_x_at             SLC25A16 0.9631964 4.491576e-06
213605_s_at                   NA 0.9632616 6.833714e-06
1556206_at            LINC00408 0.9653901 4.353339e-06
244114_x_at                   NA 0.9679377 6.750428e-06
241632_x_at                   NA 0.9686426 4.885508e-06
239017_at                    NA 0.9834736 7.316357e-06
1558496_at            LINC02053 0.9853247 5.091816e-06
211585_at                  NPAT 0.9926606 6.429684e-06
236389_x_at                   NA 1.0027588 4.491576e-06
208120_x_at               FKSG49 1.0184438 2.815681e-06
206323_x_at                OPHN1 1.0212889 5.601191e-06
224284_x_at               FKSG49 1.0251308 3.950099e-06
201464_x_at                  JUN 1.0348832 3.490668e-06
220828_s_at           LINC01949 1.0397500 4.491576e-06
81737_at           LOC100505915 1.0462560 5.662707e-06
210800_at                TIMM8A 1.0479516 4.730744e-06
215182_x_at                   NA 1.0559763 4.491576e-06
243489_at                    NA 1.0626878 4.070717e-06
240988_x_at                   NA 1.0629756 4.779299e-06
224288_x_at               FKSG49 1.0813463 1.113017e-06
AFFX-r2-Ec-bioB-5_at          NA 1.1020958 5.322529e-06
```

```
242862_x_at              NA 1.1113007 1.113017e-06
1563674_at           FCRL2 1.1127799 6.945728e-06
AFFX-BioC-5_at           NA 1.1199525 6.430788e-06
210718_s_at              NA 1.1396478 6.670158e-06
1562755_at               NA 1.1425399 2.235176e-06
232964_at                NA 1.1460961 1.656041e-06
220232_at              SCD5 1.1545960 1.183928e-06
1569940_at          SLC6A16 1.1781696 8.464513e-07
211454_x_at          FKSG49 1.1800267 5.834686e-07
AFFX-BioB-M_at           NA 1.1849198 6.376426e-06
209700_x_at         PDE4DIP 1.1871538 8.406263e-07
1566145_s_at             NA 1.1918754 3.003518e-07
234949_at           FRG1BP 1.1922760 1.231065e-06
1560144_at               NA 1.2077566 1.790306e-06
1553185_at           RASEF 1.2129105 6.160739e-07
231597_x_at              NA 1.2169048 1.477715e-06
242619_x_at              NA 1.2257718 4.302148e-07
1553186_x_at         RASEF 1.2330332 6.881976e-07
227952_at           ZNF595 1.2408024 5.034675e-07
1561754_at               NA 1.2457988 9.800827e-07
224159_x_at          TRIM4 1.2471076 7.904835e-07
243689_s_at         FRG1BP 1.3748167 6.160739e-07
231598_x_at              NA 1.4418759 1.469974e-07
228919_at                NA 1.4589605 1.682330e-06
1562527_at        LOC441666 1.4936054 7.062932e-08
1558048_x_at             NA 1.5005510 2.186020e-08
211565_at           SH3GL3 1.5671795 2.844253e-08
```

1.10   Generate GSEA gct, cls files

Script 1.10.1 (R)

```R
%%R
exprs2_HPB <- cbind(c(" "), exprs(eset_HPB))
write.table(exprs2_HPB,row.names=TRUE,col.names=FALSE,quote=FALSE,file="eset_HPB.tsv", sep =
   "\t")
exprs2_KOPT <- cbind(c(" "), exprs(eset_KOPT))
write.table(exprs2_KOPT,row.names=TRUE,col.names=FALSE,quote=FALSE,file="eset_KOPT.tsv", sep
   = "\t")
```

Script 1.10.2 (bash)

```bash
%%bash
echo "#1.2" > gct.head.HPB
echo "$(cat eset_HPB.tsv | wc -l) 6" >> gct.head.HPB
```

```
4  echo "GID       NAME       HPB_DMSO_01       HPB_DMSO_02        HPB_DMSO_03        HPB_SA⌐
   ↪ HM1_01        HPB_SAHM1_02       HPB_SAHM1_03" >>
   ↪ gct.head.HPB
5
6  echo "#1.2" > gct.head.KOPT
7  echo "$(cat eset_KOPT.tsv | wc -l) 6" >> gct.head.KOPT
8  echo "GID       NAME       KOPT_DMSO_01       KOPT_DMSO_02       KOPT_DMSO_03       KOP⌐
   ↪ T_SAHM1_01       KOPT_SAHM1_02       KOPT_SAHM1_03" >>
   ↪ gct.head.KOPT
9
10 cat gct.head.HPB eset_HPB.tsv > eset_HPB.gct
11 cat gct.head.KOPT eset_KOPT.tsv > eset_KOPT.gct
12
13 echo "6        2        1" > phenotypes.cls
14 echo "#DMSO SAHM1" >> phenotypes.cls
15 echo "0        0        0        1        1        1" >> phenotypes.cls
```

1.11 Processing all samples

Script 1.11.1 (R)

```
1  %%R
2  setwd("GSE18198_data")
3  affyBatch = import_CEL("*")
4  setwd(wd)
5  eset <- create_eset(affyBatch)
6  toptable <- create_TopTable(eset, control_samples=c(1,1,1,0,0,0,1,1,1,0,0,0))
7  toptable_anot <- anotate_TopTable(toptable)
8  generank_table(toptable_anot, "generank")
9  save(eset, file="eset.RData")
```

```
background correction: rma
normalization: quantiles
PM/MM correction : pmonly
expression values: medianpolish
background correcting...done.
normalizing...done.
54675 ids to be processed
|                    |
|####################|
[1] "Down-regulated genes"
              Symbol      logFC      P.Value
227347_x_at      HES4 -1.2391846 4.541483e-05
227336_at        DTX1 -1.0787341 1.772044e-03
230263_s_at     DOCK5 -1.0589452 2.174812e-04
218051_s_at    NT5DC2 -0.9507155 1.126824e-03
205544_s_at       CR2 -0.9438572 5.961088e-05
202464_s_at    PFKFB3 -0.9408542 1.077045e-04
```

```
226452_at           PDK1 -0.8325181 2.019305e-05
223364_s_at        DHX37 -0.8207408 1.605856e-03
206686_at           PDK1 -0.8172945 9.936732e-04
203627_at          IGF1R -0.8050123 1.277490e-03
203867_s_at         NLE1 -0.7963720 7.746991e-04
204513_s_at        ELMO1 -0.7885410 1.839908e-04
207543_s_at        P4HA1 -0.7663495 3.287902e-05
212063_at           CD44 -0.7621344 1.769410e-03
227337_at        ANKRD37 -0.7620441 3.022164e-04
239410_at            HK2 -0.7615742 2.557385e-03
1554918_a_at       ABCC4 -0.7263246 1.912084e-03
231094_s_at           NA -0.7232606 9.131629e-04
200965_s_at       ABLIM1 -0.7085777 6.009393e-04
210625_s_at        AKAP1 -0.6987526 1.183409e-04
231310_at         TRIM71 -0.6679915 6.024183e-04
219253_at       TMEM185B -0.6494920 1.313180e-03
215195_at          PRKCA -0.6258312 2.255091e-04
228205_at            TKT -0.6126859 8.915350e-04
218806_s_at         VAV3 -0.5936019 2.050231e-03
206923_at          PRKCA -0.5924819 2.694018e-04
201367_s_at      ZFP36L2 -0.5879670 1.331411e-03
236180_at             NA -0.5535885 1.051495e-03
221989_at             NA -0.5531493 1.744579e-04
223058_at        FAM107B -0.5499447 2.198129e-03
208858_s_at        ESYT1 -0.5411513 2.844208e-04
1555434_a_at     SLC39A14 -0.5382917 2.440482e-03
1553138_a_at      ANKLE1 -0.5306391 5.104033e-04
227099_s_at      C11orf96 -0.5289498 2.627354e-03
203612_at           BYSL -0.5257364 1.818973e-04
226938_at          DCAF4 -0.5161954 1.874088e-03
214484_s_at       SIGMAR1 -0.5153579 1.465927e-03
206653_at         POLR3G -0.5067532 2.177288e-03
226498_at           FLT1 -0.5041981 2.319967e-03
208758_at           ATIC -0.4943349 1.053895e-03
208997_s_at         UCP2 -0.4905291 2.479831e-03
209461_x_at        WDR18 -0.4862286 8.241993e-04
201161_s_at        YBX3 -0.4839871 9.730003e-04
225883_at        ATG16L2 -0.4745402 2.113101e-03
201692_at        SIGMAR1 -0.4727785 1.380736e-03
217139_at          VDAC1 -0.4553310 2.588750e-03
229236_s_at        SFXN4 -0.4251656 1.858039e-03
224824_at          COX20 -0.4173264 2.375617e-03
204027_s_at       METTL1 -0.4116541 2.046282e-03
201250_s_at        SLC2A1 -0.3947427 2.225655e-03
[1] "Up-regulated genes"
                 Symbol    logFC      P.Value
232059_at       DSCAML1 0.3346000 0.0068497056
209392_at          ENPP2 0.3540105 0.0052561029
```

```
205381_at            LRRC17 0.3645748 0.0064690771
214710_s_at           CCNB1 0.3671989 0.0073863284
1569680_at               NA 0.3814353 0.0033810217
1559023_a_at        EFCAB14 0.3845018 0.0052968042
236353_at                NA 0.3952903 0.0082999985
206448_at            ZNF365 0.4055256 0.0032538740
226936_at             CENPW 0.4121128 0.0055882829
243469_at                NA 0.4133233 0.0048561664
201896_s_at           PSRC1 0.4219794 0.0084642203
1568596_a_at          TROAP 0.4261581 0.0017179111
238875_at                NA 0.4288226 0.0078367183
242966_x_at            RFX2 0.4321379 0.0027616154
236253_at            ZNF546 0.4657924 0.0075436998
1557290_at               NA 0.4742477 0.0041564711
243992_at                NA 0.4761079 0.0040639088
204641_at              NEK2 0.4770373 0.0034259514
220167_s_at              NA 0.4845848 0.0045882849
241685_x_at            PURA 0.4879466 0.0058069390
239735_at                NA 0.4897705 0.0030891034
202644_s_at         TNFAIP3 0.4934166 0.0004891236
242476_at                NA 0.5030038 0.0056605223
238595_at                NA 0.5058669 0.0059647137
213605_s_at              NA 0.5068902 0.0050211914
244427_at             KIF23 0.5209074 0.0015710943
242637_at                NA 0.5222779 0.0047282688
232953_at        LINC00266-1 0.5259613 0.0041673250
1559156_at               NA 0.5325258 0.0057922093
228390_at             RAB30 0.5332846 0.0071758418
238407_at                NA 0.5390014 0.0066182068
216756_at                NA 0.5406353 0.0019487381
239248_at         SDCBP2-AS1 0.5441412 0.0010999281
213544_at              ING2 0.5513826 0.0046805162
239531_at                NA 0.5536821 0.0026133238
243709_at            SLC38A9 0.5569113 0.0009694726
241745_at        LOC100507557 0.5600510 0.0027569686
1557813_at               NA 0.5691647 0.0029037060
215599_at                NA 0.5931984 0.0012579337
244114_x_at              NA 0.6411913 0.0034400322
213281_at               JUN 0.6419108 0.0061333797
228834_at              TOB1 0.6427144 0.0006729806
244532_x_at              NA 0.6470301 0.0046383310
230795_at                NA 0.6509000 0.0039752814
216094_at                NA 0.6647132 0.0040965858
234759_at        LOC100287497 0.6758224 0.0008985553
244075_at                NA 0.6914067 0.0071383024
215071_s_at        HIST1H2AC 0.7357238 0.0038095135
210718_s_at              NA 0.7879121 0.0013751119
201465_s_at             JUN 1.5137055 0.0061879051
```

Script 1.11.2 (R)

```
1  %%R
2  # Create files for GSEA
3  # Add description column for gct
4  exprs2 <- cbind(c(" "), exprs(eset))
5  write.table(exprs2,row.names=TRUE,col.names=FALSE,quote=FALSE,file="eset.tsv", sep = "\t")
```

Script 1.11.3 (bash)

```
1  %%bash
2  echo "#1.2" > gct.head
3  echo "$(cat eset.tsv | wc -l) 12" >> gct.head
4  echo "GID       NAME       HPB_DMSO_01       HPB_DMSO_02       HPB_DMSO_03       HPB_SA↵
   ↪ HM1_01       HPB_SAHM1_02       HPB_SAHM1_03       KOPT_DMSO_01       KOPT_DMSO_02↵
   ↪        KOPT_DMSO_03       KOPT_SAHM1_01       KOPT_SAHM1_02       KOPT_SAHM1_03" >>
   ↪ gct.head
5  #head gct.head
6  cat gct.head eset.tsv > eset.gct
7
8  echo "12       2       1" > phenotypes_all.cls
9  echo "#DMSO SAHM1" >> phenotypes_all.cls
10 echo "0       0       0       1       1       1       0       0       0       1↵
   ↪        1       1" >>
   ↪ phenotypes_all.cls
```

1.12   GSEA results

1.13   Conclusions

22
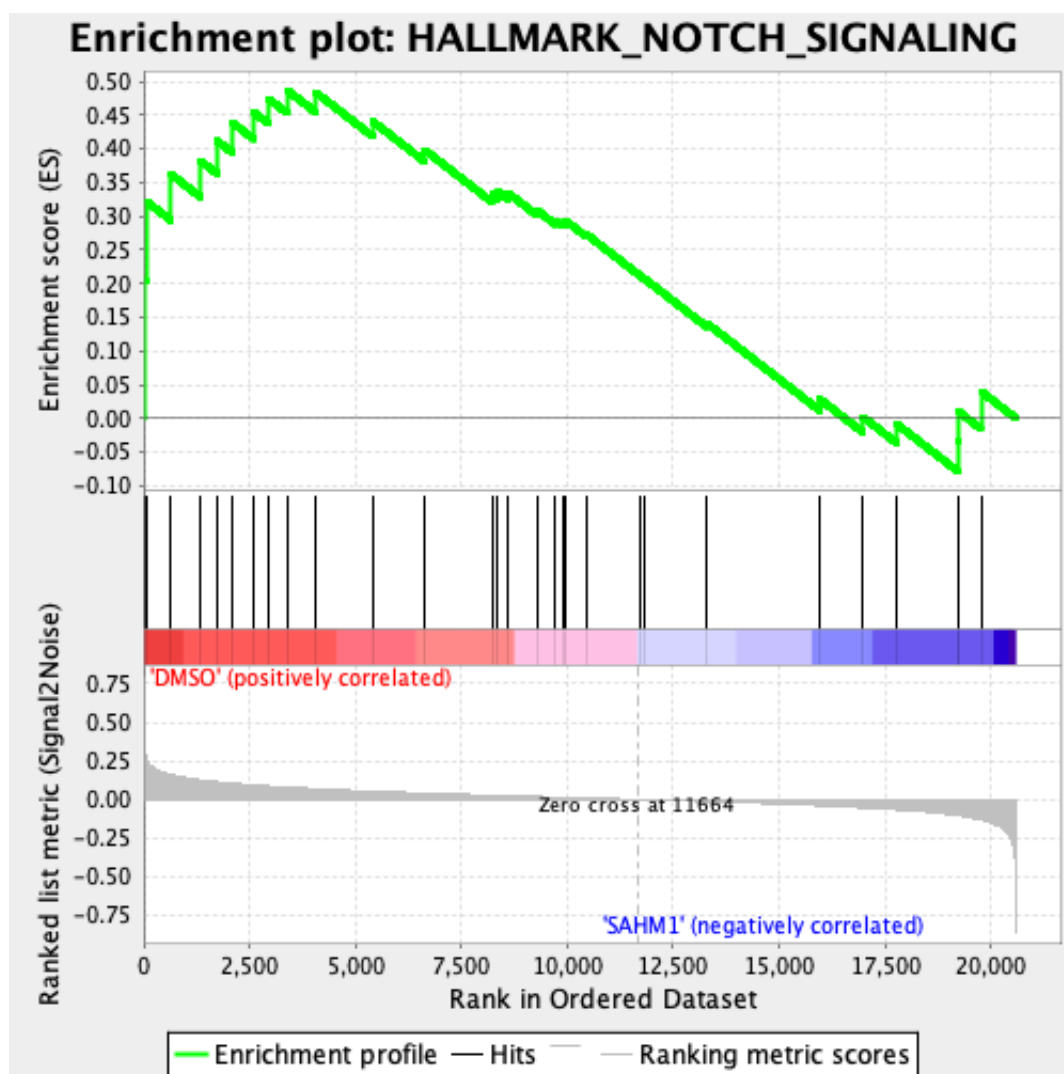
Figure 1: Enrichment plot Notch Signalling Pathway

NAME	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val	RANK AT MAX	LEADING EDGE
HALLMARK_WNT_BETA_CATENIN_SIGNALING	40	0.49850842	1.3996072	0.0	0.9853856	277	2947	tags=23%, list=14%, signal=26%
HALLMARK_NOTCH_SIGNALING	29	0.48723552	1.3520839	0.09484536	0.7166934	374	3383	tags=31%, list=16%, signal=37%
HALLMARK_GLYCOLYSIS	186	0.42253992	1.3206861	0.0	0.64004976	473	4630	tags=35%, list=22%, signal=45%
HALLMARK_INTERFERON_ALPHA_RESPONSE	88	0.34315822	1.279187	0.096114516	0.6929842	527	4447	tags=27%, list=22%, signal=35%
HALLMARK_REACTIVE_OXIGEN_SPECIES_PATHWAY	46	0.3981605	1.2701172	0.09168444	0.5729008	527	7669	tags=57%, list=37%, signal=90%
HALLMARK_INTERFERON_GAMMA_RESPONSE	187	0.3087745	1.2654618	0.08958333	0.49679276	527	5351	tags=32%, list=26%, signal=43%
HALLMARK_ADIPOGENESIS	184	0.2826048	1.2613991	0.0911017	0.43812412	527	7541	tags=42%, list=37%, signal=65%
HALLMARK_OXIDATIVE_PHOSPHORYLATION	188	0.35985056	1.2546434	0.10103093	0.4135756	577	9489	tags=56%, list=46%, signal=104%
HALLMARK_MTORC1_SIGNALING	183	0.26467624	1.2359246	0.19502075	0.4407044	0.69	5436	tags=35%, list=26%, signal=47%
HALLMARK_IL2_STAT5_SIGNALING	182	0.31952778	1.2251521	0.0	0.44391555	0.69	2989	tags=23%, list=15%, signal=27%
HALLMARK_FATTY_ACID_METABOLISM	150	0.27257207	1.2249079	0.09663866	0.40792337	0.69	4963	tags=24%, list=24%, signal=31%
HALLMARK_PEROXISOME	98	0.30541733	1.2229942	0.091649696	0.38731882	0.69	4429	tags=26%, list=21%, signal=32%
HALLMARK_HYPOXIA	188	0.38151017	1.2137458	0.0	0.3664674	0.69	4409	tags=35%, list=21%, signal=44%
HALLMARK_UV_RESPONSE_UP	150	0.26630923	1.2116135	0.18930042	0.3437194	0.69	5540	tags=34%, list=27%, signal=46%
HALLMARK_ALLOGRAFT_REJECTION	191	0.3419296	1.1964117	0.09033614	0.3680125	739	4439	tags=28%, list=22%, signal=36%
HALLMARK_MYC_TARGETS_V2	47	0.65528375	1.1840152	0.0	0.3599941	739	4315	tags=66%, list=21%, signal=83%
HALLMARK_INFLAMMATORY_RESPONSE	189	0.34822693	1.1837027	0.097363085	0.34164155	739	4313	tags=33%, list=21%, signal=41%
HALLMARK_MYC_TARGETS_V1	173	0.4086298	1.1757741	0.19381443	0.3362698	788	7640	tags=44%, list=37%, signal=69%
HALLMARK_APICAL_JUNCTION	193	0.31684956	1.1519539	0.28781512	0.3974147	835	4296	tags=26%, list=21%, signal=33%
HALLMARK_ESTROGEN_RESPONSE_EARLY	183	0.29886743	1.119735	0.2801636	0.43835357	888	4844	tags=32%, list=24%, signal=42%
HALLMARK_ANGIOGENESIS	34	0.43509555	1.1002749	0.29411766	0.47263375	888	3335	tags=26%, list=16%, signal=32%
HALLMARK_ESTROGEN_RESPONSE_LATE	194	0.29403758	1.0978482	0.28305784	0.45791104	888	4138	tags=30%, list=20%, signal=38%
HALLMARK_MYOGENESIS	196	0.37049508	1.0864803	0.18958333	0.4909446	888	4781	tags=37%, list=23%, signal=47%
HALLMARK_CHOLESTEROL_HOMEOSTASIS	71	0.2772793	1.0851066	0.28661087	0.47539067	888	5068	tags=35%, list=25%, signal=47%
HALLMARK_COMPLEMENT	192	0.26265764	1.0589281	0.31237322	0.4991516	888	3189	tags=18%, list=15%, signal=21%
HALLMARK_PROTEIN_SECRETION	88	0.20329766	1.0475459	0.38381743	0.49959674	888	6061	tags=24%, list=29%, signal=34%
HALLMARK_ANDROGEN_RESPONSE	94	0.2609074	1.039261	0.2897959	0.52019876	0.95	5980	tags=31%, list=29%, signal=43%
HALLMARK_DNA_REPAIR	145	0.17039137	1.031202	0.2790224	0.526112	0.95	7845	tags=36%, list=38%, signal=58%
HALLMARK_KRAS_SIGNALING_DN	188	0.33348864	1.0201786	0.38655463	0.5383922	0.95	5627	tags=38%, list=27%, signal=52%
HALLMARK_UV_RESPONSE_DN	135	0.19810459	0.95020545	0.77867204	0.70491356	1.0	2015	tags=11%, list=10%, signal=12%
HALLMARK_KRAS_SIGNALING_UP	192	0.26940593	0.9457102	0.70416665	0.6904847	1.0	4576	tags=28%, list=22%, signal=35%
HALLMARK_HEDGEHOG_SIGNALING	35	0.30286688	0.94172853	0.58906883	0.6778487	1.0	3103	tags=26%, list=15%, signal=30%
HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION	192	0.2777393	0.9384078	0.4926004	0.6730365	1.0	4711	tags=30%, list=23%, signal=38%
HALLMARK_IL6_JAK_STAT3_SIGNALING	85	0.2657967	0.93745136	0.5821501	0.6546528	1.0	4439	tags=28%, list=22%, signal=36%
HALLMARK_XENOBIOTIC_METABOLISM	194	0.2366412	0.93364125	0.68541664	0.6441654	1.0	5724	tags=29%, list=28%, signal=40%
HALLMARK_PI3K_AKT_MTOR_SIGNALING	98	0.17684619	0.9051278	0.4888438	0.69095284	1.0	2157	tags=12%, list=10%, signal=14%
HALLMARK_SPERMATOGENESIS	124	0.25800297	0.8618422	0.7	0.7451759	1.0	2215	tags=15%, list=11%, signal=17%
HALLMARK_APICAL_SURFACE	42	0.21887365	0.7824094	0.8102767	0.90781873	1.0	7749	tags=45%, list=38%, signal=72%
HALLMARK_COAGULATION	133	0.23493658	0.76809555	0.9	0.89633375	1.0	7086	tags=38%, list=34%, signal=58%

Figure 2: Gene sets enriched in phenotype DMSO(Cell Line HPB-ALL)

Columns: HPB_DMSO_01, HPB_DMSO_02, HPB_DMSO_03, HPB_SAHM1_01, HPB_SAHM1_02, HPB_SAHM1_03

SampleName

AK3L1	AK3L1	adenylate kinase 3-like 1
EFEMP1	EFEMP1	EGF-containing fibulin-like extracellular matrix protein 1
DTX1	DTX1	deltex homolog 1 (Drosophila)
TKTL1	TKTL1	transketolase-like 1
CCND2	CCND2	cyclin D2
LOC54103	LOC54103	–
BHLHB2	BHLHB2	basic helix-loop-helix domain containing, class B, 2
HES4	HES4	hairy and enhancer of split 4 (Drosophila)
NKG7	NKG7	natural killer cell group 7 sequence
ALDOC	ALDOC	aldolase C, fructose-bisphosphate
NT5DC2	NT5DC2	5'-nucleotidase domain containing 2
FUT11	FUT11	fucosyltransferase 11 (alpha (1,3) fucosyltransferase)
HTR2B	HTR2B	5-hydroxytryptamine (serotonin) receptor 2B
MLC1	MLC1	megalencephalic leukoencephalopathy with subcortical cysts 1
C14ORF148	C14ORF148	chromosome 14 open reading frame 148
LSP1 /// LOC649377		
F7	F7	coagulation factor VII (serum prothrombin conversion accelerator)
ANKRD37	ANKRD37	ankyrin repeat domain 37
LOC283999	LOC283999	–
TUBB4	TUBB4	tubulin, beta 4
PFKFB3	PFKFB3	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3
GLOXD1	GLOXD1	glyoxalase domain containing 1
LOC653127		
PNOC	PNOC	prepronociceptin
HK2	HK2	hexokinase 2
BGN	BGN	biglycan
VARS	VARS	valyl-tRNA synthetase
CNTN5	CNTN5	contactin 5
TPO	TPO	thyroid peroxidase
ANKRD47	ANKRD47	ankyrin repeat domain 47
ANKRD13B	ANKRD13B	ankyrin repeat domain 13B
LOC440455		
LOC389043	LOC389043	–
MAP3K8	MAP3K8	mitogen-activated protein kinase kinase kinase 8
LOC51252	LOC51252	–
HDC	HDC	histidine decarboxylase
TRAF4	TRAF4	TNF receptor-associated factor 4
TNS4	TNS4	tensin 4
KLF2	KLF2	Kruppel-like factor 2 (lung)
PLEKHB1	PLEKHB1	pleckstrin homology domain containing, family B (evectins) member 1
CYP17A1	CYP17A1	cytochrome P450, family 17, subfamily A, polypeptide 1
LDHC	LDHC	lactate dehydrogenase C
DOCK5	DOCK5	dedicator of cytokinesis 5
NAV3 /// LOC652725		
EFNA3	EFNA3	ephrin-A3
ADM	ADM	adrenomedullin
CD40LG	CD40LG	CD40 ligand (TNF superfamily, member 5, hyper-IgM syndrome)
FCRL4	FCRL4	Fc receptor-like 4
KLRF1	KLRF1	killer cell lectin-like receptor subfamily F, member 1
PLOD1	PLOD1	procollagen-lysine 1, 2-oxoglutarate 5-dioxygenase 1
RASSF6	RASSF6	Ras association (RalGDS/AF-6) domain family 6
HIST1H1B	HIST1H1B	histone cluster 1, H1b
HIST1H1E	HIST1H1E	histone cluster 1, H1e
HIST1H1D	HIST1H1D	histone cluster 1, H1d
LOC644241		
FLRT3	FLRT3	fibronectin leucine rich transmembrane protein 3
CHAC1	CHAC1	ChaC, cation transport regulator homolog 1 (E. coli)
ASS1	ASS1	argininosuccinate synthetase 1
LOC284952	LOC284952	–
JUN	JUN	jun oncogene
LRRC2	LRRC2	leucine rich repeat containing 2
DDX6	DDX6	DEAD (Asp-Glu-Ala-Asp) box polypeptide 6
PAR5 /// SNORD108 //		
LOC648832		
FLJ35024	FLJ35024	–
HIST1H2AJ	HIST1H2AJ	histone cluster 1, H2aj
HIST2H2AA3	HIST2H2AA3	histone cluster 2, H2aa3
C3ORF20	C3ORF20	chromosome 3 open reading frame 20
HRK	HRK	harakiri, BCL2 interacting protein (contains only BH3 domain)
ZMAT1	ZMAT1	zinc finger, matrin type 1
SLC8A1	SLC8A1	solute carrier family 8 (sodium/calcium exchanger), member 1
IQGAP3	IQGAP3	IQ motif containing GTPase activating protein 3
HIST1H3D	HIST1H3D	histone cluster 1, H3d
TEX9	TEX9	testis expressed sequence 9
ATF3	ATF3	activating transcription factor 3
HIST1H1C	HIST1H1C	histone cluster 1, H1c
CCDC18	CCDC18	coiled-coil domain containing 18
EGR1	EGR1	early growth response 1
STYX /// LOC653890		
ZNF257	ZNF257	zinc finger protein 257
VLDLR	VLDLR	very low density lipoprotein receptor
LOC57400	LOC57400	–
MAFB	MAFB	v-maf musculoaponeurotic fibrosarcoma oncogene homolog B (avian)
LOC400723 /// LOC400		
SLC35E4	SLC35E4	solute carrier family 35, member E4
LOC158160		
PLEKHH2	PLEKHH2	pleckstrin homology domain containing, family H (with MyTH4 domain) member 2
RBAK	RBAK	RB-associated KRAB zinc finger
HIST1H2AM	HIST1H2AM	histone cluster 1, H2am
SERPINI1	SERPINI1	serpin peptidase inhibitor, clade I (neuroserpin), member 1
HIST1H2AC	HIST1H2AC	histone cluster 1, H2ac
TNFSF13B	TNFSF13B	tumor necrosis factor (ligand) superfamily, member 13b
DPY19L2 /// FLJ36166		
EMB	EMB	embigin homolog (mouse)
BLNK	BLNK	B-cell linker
SLC7A11	SLC7A11	solute carrier family 7, (cationic amino acid transporter, y+ system) member 11
AQP12B	AQP12B	aquaporin 12B
CXORF42	CXORF42	chromosome X open reading frame 42
C9ORF126	C9ORF126	chromosome 9 open reading frame 126
HIST1H3I	HIST1H3I	histone cluster 1, H3i

Figure 3: Heatmap(Cell Line HPB-ALL)

25

NAME	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val	RANK AT MAX	LEADING EDGE
HALLMARK_ANDROGEN_RESPONSE	94	0.293221	1.4200062	0.0	0.4326947	263	3888	tags=30%, list=19%, signal=37%
HALLMARK_MITOTIC_SPINDLE	185	0.386933	1.3821146	0.0	0.23884742	308	4208	tags=38%, list=20%, signal=47%
HALLMARK_MTORC1_SIGNALING	183	0.5215603	1.3441344	0.0	0.26536438	403	2776	tags=27%, list=13%, signal=31%
HALLMARK_G2M_CHECKPOINT	178	0.3373856	1.3382939	0.0	0.21027331	403	6753	tags=45%, list=33%, signal=66%
HALLMARK_PROTEIN_SECRETION	88	0.3678097	1.3335762	0.10330579	0.19757529	403	6221	tags=52%, list=30%, signal=75%
HALLMARK_MYC_TARGETS_V1	173	0.40847418	1.3236173	0.0	0.20407657	454	6363	tags=35%, list=31%, signal=51%
HALLMARK_E2F_TARGETS	173	0.33912787	1.3138137	0.0	0.20767564	454	7519	tags=48%, list=36%, signal=75%
HALLMARK_DNA_REPAIR	145	0.2616415	1.3059548	0.20245399	0.18734114	454	5134	tags=26%, list=25%, signal=34%
HALLMARK_INTERFERON_ALPHA_RESPONSE	88	0.29699197	1.3052112	0.1002004	0.17152534	454	3260	tags=26%, list=16%, signal=31%
HALLMARK_MYC_TARGETS_V2	47	0.66136664	1.2859757	0.0	0.18164015	0.5	5065	tags=62%, list=25%, signal=82%
HALLMARK_CHOLESTEROL_HOMEOSTASIS	71	0.33102265	1.2468052	0.21237114	0.22184616	604	1292	tags=15%, list=6%, signal=16%
HALLMARK_IL2_STAT5_SIGNALING	182	0.3490652	1.2443198	0.0	0.20710902	604	3186	tags=30%, list=15%, signal=35%
HALLMARK_UNFOLDED_PROTEIN_RESPONSE	104	0.6079186	1.227846	0.0	0.23289144	604	4903	tags=48%, list=24%, signal=63%
HALLMARK_ALLOGRAFT_REJECTION	191	0.26240367	1.2218562	0.0	0.23272453	604	3260	tags=23%, list=16%, signal=26%
HALLMARK_P53_PATHWAY	182	0.2778233	1.2039973	0.0	0.24092272	604	3999	tags=23%, list=19%, signal=28%
HALLMARK_HEME_METABOLISM	184	0.25559813	1.1812468	0.0	0.26794052	646	2746	tags=14%, list=13%, signal=16%
HALLMARK_PI3K_AKT_MTOR_SIGNALING	98	0.29646376	1.1782368	0.11434511	0.26249218	646	5469	tags=32%, list=27%, signal=43%
HALLMARK_GLYCOLYSIS	186	0.29053313	1.1596437	0.0	0.2764851	646	5803	tags=38%, list=28%, signal=52%
HALLMARK_HYPOXIA	188	0.26141116	1.1396848	0.2238193	0.32033673	898	3547	tags=27%, list=17%, signal=32%
HALLMARK_TGF_BETA_SIGNALING	50	0.36157095	1.1116209	0.20977597	0.39753282	945	2675	tags=24%, list=13%, signal=28%
HALLMARK_ADIPOGENESIS	184	0.2005336	1.0934803	0.22154471	0.4228268	945	5096	tags=27%, list=25%, signal=35%
HALLMARK_COMPLEMENT	192	0.25042418	1.0874768	0.18383838	0.43187666	1.0	2949	tags=19%, list=14%, signal=22%
HALLMARK_INTERFERON_GAMMA_RESPONSE	187	0.22316597	1.0854584	0.106177606	0.4260444	1.0	3581	tags=24%, list=17%, signal=29%
HALLMARK_NOTCH_SIGNALING	29	0.38857377	1.0616399	0.28716904	0.45897862	1.0	2954	tags=34%, list=14%, signal=40%
HALLMARK_FATTY_ACID_METABOLISM	150	0.21143477	1.0521207	0.28947368	0.4667693	1.0	5347	tags=33%, list=26%, signal=44%
HALLMARK_UV_RESPONSE_UP	150	0.23067562	1.0252469	0.2275574	0.5528972	1.0	2525	tags=13%, list=12%, signal=15%
HALLMARK_HEDGEHOG_SIGNALING	35	0.3210423	1.0227196	0.49588478	0.5402487	1.0	3048	tags=26%, list=15%, signal=30%
HALLMARK_TNFA_SIGNALING_VIA_NFKB	185	0.2405712	1.005899	0.40368852	0.5560466	1.0	2882	tags=20%, list=14%, signal=23%
HALLMARK_UV_RESPONSE_DN	135	0.32195312	0.99710524	0.51934826	0.5491897	1.0	3304	tags=26%, list=16%, signal=31%
HALLMARK_APOPTOSIS	159	0.20274885	0.9836564	0.39130434	0.5896945	1.0	2882	tags=18%, list=14%, signal=20%
HALLMARK_ESTROGEN_RESPONSE_EARLY	183	0.2312291	0.9552753	0.58943087	0.67311716	1.0	2938	tags=18%, list=14%, signal=21%
HALLMARK_OXIDATIVE_PHOSPHORYLATION	188	0.16545603	0.92549586	0.5323887	0.7178825	1.0	16491	tags=99%, list=80%, signal=491%
HALLMARK_PEROXISOME	98	0.16470067	0.85989714	0.7217742	0.8381856	1.0	5944	tags=28%, list=29%, signal=39%
HALLMARK_APICAL_SURFACE	42	0.24479878	0.7153503	0.81287724	0.98692274	1.0	2692	tags=21%, list=13%, signal=25%
HALLMARK_REACTIVE_OXIGEN_SPECIES_PATHWAY	46	0.101305366	0.43637094	0.9117647	1.0	1.0	4676	tags=17%, list=23%, signal=22%

Figure 4: Gene sets enriched in phenotype DMSO(Cell Line KOPT-K1)

NAME	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val	RANK AT MAX	LEADING EDGE
HALLMARK_GLYCOLYSIS	186	0.43311334	1.6781958	0.004192872	0.036362655	25	5630	tags=47%, list=27%, signal=64%
HALLMARK_PI3K_AKT_MTOR_SIGNALING	98	0.34280387	1.6242404	0.010121457	0.030869437	45	5884	tags=36%, list=29%, signal=50%
HALLMARK_MTORC1_SIGNALING	183	0.4759005	1.6139847	0.004158004	0.023340473	51	4779	tags=42%, list=23%, signal=54%
HALLMARK_UNFOLDED_PROTEIN_RESPONSE	104	0.548177	1.5873568	0.03992016	0.024172837	64	4153	tags=38%, list=20%, signal=48%
HALLMARK_NOTCH_SIGNALING	29	0.5714514	1.5091325	0.016597511	0.04010106	116	4882	tags=55%, list=24%, signal=72%
HALLMARK_UV_RESPONSE_UP	150	0.2950838	1.4034421	0.0041237115	0.124392934	301	2906	tags=22%, list=14%, signal=25%
HALLMARK_REACTIVE_OXIGEN_SPECIES_PATHWAY	46	0.40161857	1.3443292	0.07068607	0.21853316	471	7374	tags=57%, list=36%, signal=88%
HALLMARK_MYC_TARGETS_V2	47	0.71475863	1.3281219	0.06759443	0.23772013	531	3914	tags=74%, list=19%, signal=92%
HALLMARK_UV_RESPONSE_DN	135	0.3603946	1.306755	0.05679513	0.2657795	587	2828	tags=26%, list=14%, signal=30%
HALLMARK_HYPOXIA	188	0.35863873	1.2884322	0.043392505	0.295965	651	5635	tags=44%, list=27%, signal=60%
HALLMARK_FATTY_ACID_METABOLISM	150	0.2589689	1.2816921	0.037113402	0.2849363	661	5652	tags=33%, list=27%, signal=46%
HALLMARK_INTERFERON_ALPHA_RESPONSE	88	0.3972688	1.2601771	0.18774703	0.31406602	717	4741	tags=38%, list=23%, signal=48%
HALLMARK_ADIPOGENESIS	184	0.2585026	1.2498535	0.06625259	0.31768975	754	6432	tags=38%, list=31%, signal=54%
HALLMARK_DNA_REPAIR	145	0.27045995	1.220323	0.25440314	0.37851238	844	6445	tags=34%, list=31%, signal=49%
HALLMARK_ESTROGEN_RESPONSE_EARLY	183	0.29162228	1.2086581	0.057494868	0.39013368	877	1687	tags=14%, list=8%, signal=15%
HALLMARK_PEROXISOME	98	0.26575372	1.2073532	0.115384616	0.36860234	881	6877	tags=38%, list=33%, signal=56%
HALLMARK_MYC_TARGETS_V1	173	0.481558	1.1877586	0.38492063	0.40412134	915	7998	tags=57%, list=39%, signal=92%
HALLMARK_ANDROGEN_RESPONSE	94	0.2428143	1.1861122	0.102713175	0.38849032	915	4883	tags=31%, list=24%, signal=40%
HALLMARK_WNT_BETA_CATENIN_SIGNALING	40	0.34349075	1.1845423	0.15767635	0.37267235	916	4889	tags=38%, list=24%, signal=49%
HALLMARK_HEDGEHOG_SIGNALING	35	0.3436916	1.183618	0.18837675	0.35571563	916	1072	tags=17%, list=5%, signal=18%
HALLMARK_CHOLESTEROL_HOMEOSTASIS	71	0.33077985	1.1479423	0.17760618	0.42038524	0.95	2256	tags=18%, list=11%, signal=20%
HALLMARK_PROTEIN_SECRETION	88	0.35464782	1.1016811	0.42352942	0.5150438	972	6980	tags=42%, list=34%, signal=63%
HALLMARK_OXIDATIVE_PHOSPHORYLATION	188	0.32158187	1.0274464	0.5450902	0.69437057	986	7107	tags=34%, list=34%, signal=51%
HALLMARK_IL2_STAT5_SIGNALING	182	0.3837396	0.99643314	0.5445545	0.75440615	989	2494	tags=24%, list=12%, signal=27%
HALLMARK_ALLOGRAFT_REJECTION	191	0.3501197	0.9865181	0.5752577	0.7505662	989	4113	tags=28%, list=20%, signal=34%
HALLMARK_APICAL_SURFACE	42	0.30094463	0.95951355	0.57715434	0.7958004	993	4367	tags=31%, list=21%, signal=39%
HALLMARK_TGF_BETA_SIGNALING	50	0.286461	0.8809257	0.72745097	0.96194637	995	4256	tags=30%, list=21%, signal=38%
HALLMARK_TNFA_SIGNALING_VIA_NFKB	185	0.25158665	0.8724303	0.70178926	0.9456293	995	3627	tags=23%, list=18%, signal=27%
HALLMARK_COMPLEMENT	192	0.25019673	0.86665404	0.68937874	0.9242008	995	3369	tags=20%, list=16%, signal=24%
HALLMARK_HEME_METABOLISM	184	0.19858962	0.85600936	0.8579882	0.91439253	999	6128	tags=30%, list=30%, signal=43%
HALLMARK_INTERFERON_GAMMA_RESPONSE	187	0.29006875	0.8416879	0.63842976	0.90728647	1.0	4763	tags=33%, list=23%, signal=42%
HALLMARK_APICAL_JUNCTION	193	0.21188706	0.8365217	0.87649405	0.8868689	1.0	6030	tags=33%, list=29%, signal=46%
HALLMARK_MITOTIC_SPINDLE	185	0.31453067	0.8285555	0.678501	0.8730112	1.0	4322	tags=28%, list=21%, signal=35%
HALLMARK_MYOGENESIS	196	0.20663904	0.7016324	0.9665971	0.9819069	1.0	2350	tags=15%, list=11%, signal=17%
HALLMARK_INFLAMMATORY_RESPONSE	189	0.25482258	0.6774285	0.79761904	0.969574	1.0	1679	tags=15%, list=8%, signal=17%
HALLMARK_G2M_CHECKPOINT	178	0.19088042	0.601792	0.8170974	0.9818409	1.0	4846	tags=24%, list=24%, signal=31%
HALLMARK_E2F_TARGETS	173	0.17057835	0.59205115	0.85265225	0.9589176	1.0	7214	tags=34%, list=35%, signal=52%

Figure 5: Gene sets enriched in phenotype DMSO(Cell Lines: HPB-ALL KOPT-K1)

SampleName

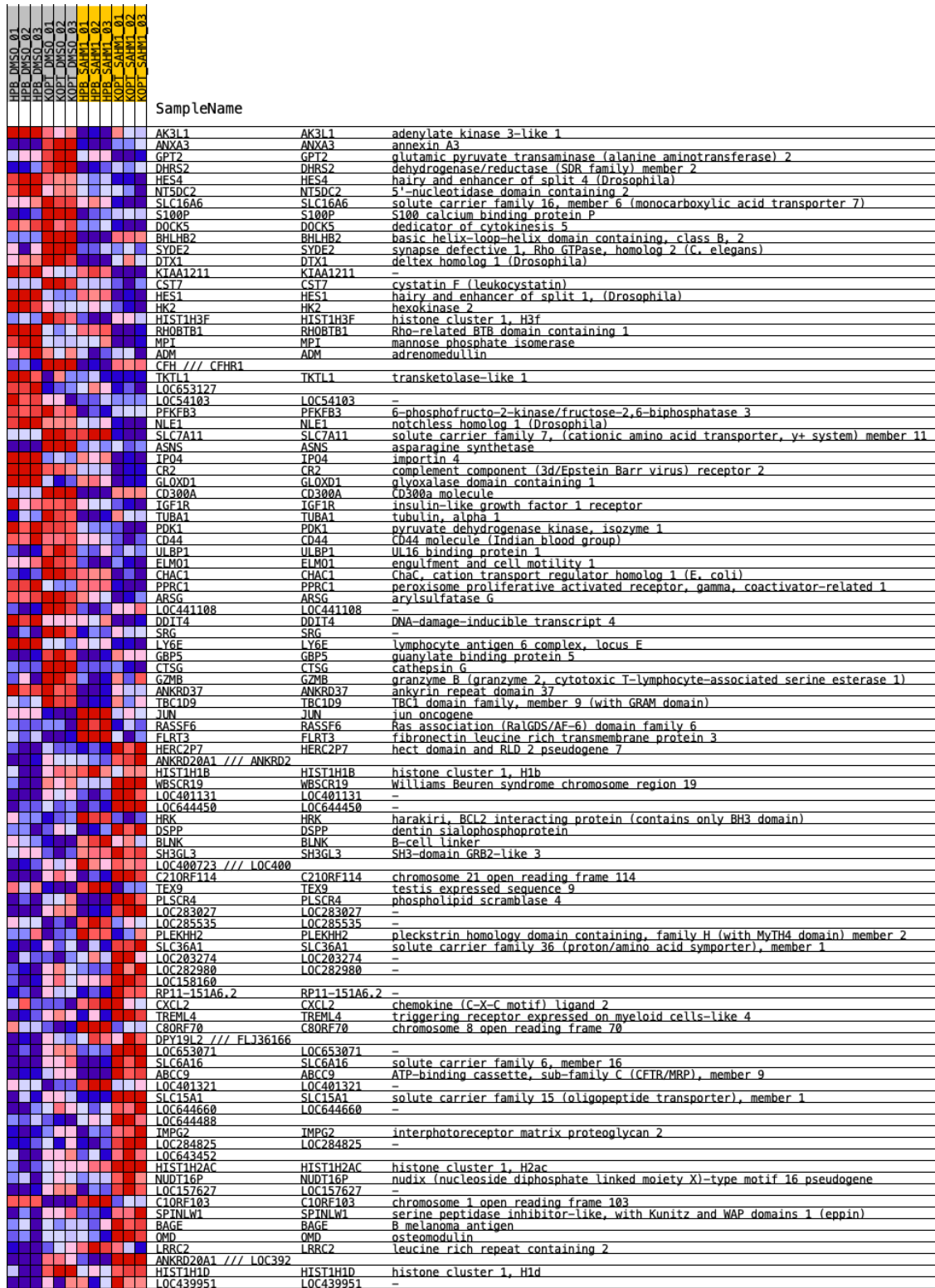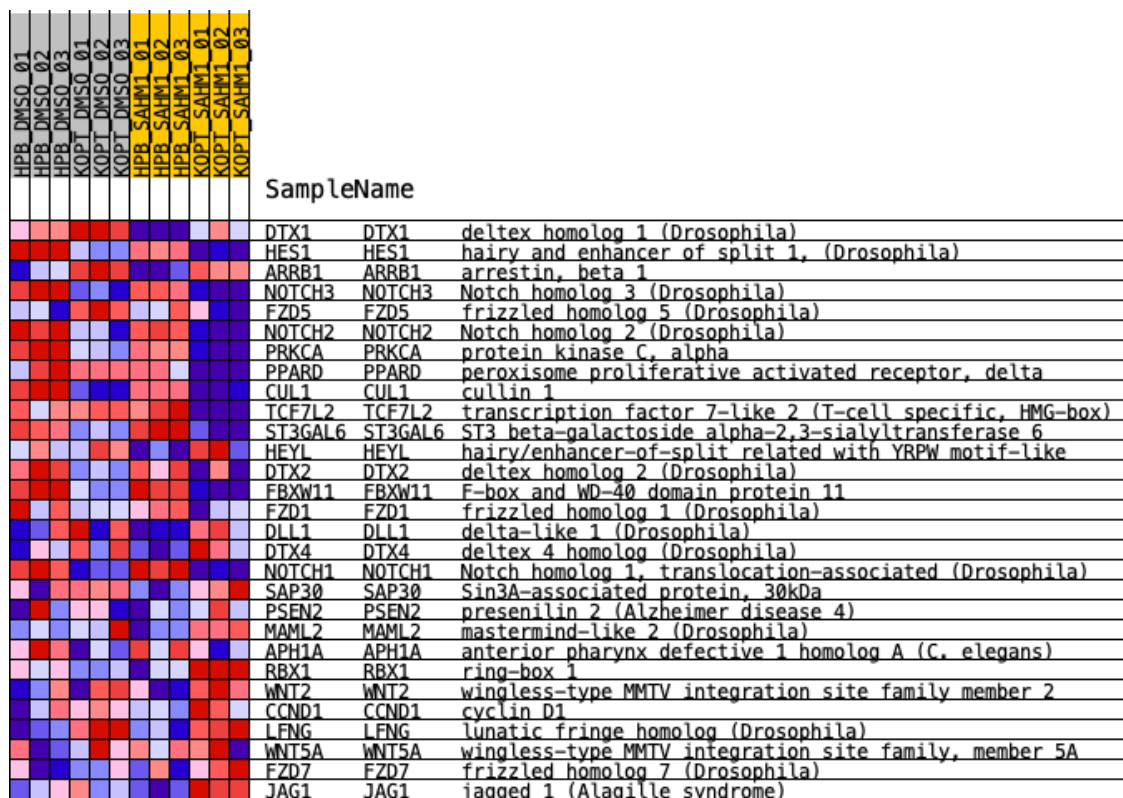| Symbol	Gene	Description
AK3L1	AK3L1	adenylate kinase 3-like 1
ANXA3	ANXA3	annexin A3
GPT2	GPT2	glutamic pyruvate transaminase (alanine aminotransferase) 2
DHRS2	DHRS2	dehydrogenase/reductase (SDR family) member 2
HES4	HES4	hairy and enhancer of split 4 (Drosophila)
NT5DC2	NT5DC2	5'-nucleotidase domain containing 2
SLC16A6	SLC16A6	solute carrier family 16, member 6 (monocarboxylic acid transporter 7)
S100P	S100P	S100 calcium binding protein P
DOCK5	DOCK5	dedicator of cytokinesis 5
BHLHB2	BHLHB2	basic helix-loop-helix domain containing, class B, 2
SYDE2	SYDE2	synapse defective 1, Rho GTPase, homolog 2 (C. elegans)
DTX1	DTX1	deltex homolog 1 (Drosophila)
KIAA1211	KIAA1211	-
CST7	CST7	cystatin F (leukocystatin)
HES1	HES1	hairy and enhancer of split 1, (Drosophila)
HK2	HK2	hexokinase 2
HIST1H3F	HIST1H3F	histone cluster 1, H3f
RHOBTB1	RHOBTB1	Rho-related BTB domain containing 1
MPI	MPI	mannose phosphate isomerase
ADM	ADM	adrenomedullin
CFH /// CFHR1		
TKTL1	TKTL1	transketolase-like 1
LOC653127		
LOC54103	LOC54103	-
PFKFB3	PFKFB3	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3
NLE1	NLE1	notchless homolog 1 (Drosophila)
SLC7A11	SLC7A11	solute carrier family 7, (cationic amino acid transporter, y+ system) member 11
ASNS	ASNS	asparagine synthetase
IPO4	IPO4	importin 4
CR2	CR2	complement component (3d/Epstein Barr virus) receptor 2
GLOXD1	GLOXD1	glyoxalase domain containing 1
CD300A	CD300A	CD300a molecule
IGF1R	IGF1R	insulin-like growth factor 1 receptor
TUBA1	TUBA1	tubulin, alpha 1
PDK1	PDK1	pyruvate dehydrogenase kinase, isozyme 1
CD44	CD44	CD44 molecule (Indian blood group)
ULBP1	ULBP1	UL16 binding protein 1
ELMO1	ELMO1	engulfment and cell motility 1
CHAC1	CHAC1	ChaC, cation transport regulator homolog 1 (E. coli)
PPRC1	PPRC1	peroxisome proliferative activated receptor, gamma, coactivator-related 1
ARSG	ARSG	arylsulfatase G
LOC441108	LOC441108	-
DDIT4	DDIT4	DNA-damage-inducible transcript 4
SRG	SRG	-
LY6E	LY6E	lymphocyte antigen 6 complex, locus E
GBP5	GBP5	guanylate binding protein 5
CTSG	CTSG	cathepsin G
GZMB	GZMB	granzyme B (granzyme 2, cytotoxic T-lymphocyte-associated serine esterase 1)
ANKRD37	ANKRD37	ankyrin repeat domain 37
TBC1D9	TBC1D9	TBC1 domain family, member 9 (with GRAM domain)
JUN	JUN	jun oncogene
RASSF6	RASSF6	Ras association (RalGDS/AF-6) domain family 6
FLRT3	FLRT3	fibronectin leucine rich transmembrane protein 3
HERC2P7	HERC2P7	hect domain and RLD 2 pseudogene 7
ANKRD20A1 /// ANKRD2		
HIST1H1B	HIST1H1B	histone cluster 1, H1b
WBSCR19	WBSCR19	Williams Beuren syndrome chromosome region 19
LOC401131	LOC401131	-
LOC644450	LOC644450	-
HRK	HRK	harakiri, BCL2 interacting protein (contains only BH3 domain)
DSPP	DSPP	dentin sialophosphoprotein
BLNK	BLNK	B-cell linker
SH3GL3	SH3GL3	SH3-domain GRB2-like 3
LOC400723 /// LOC400		
C21ORF114	C21ORF114	chromosome 21 open reading frame 114
TEX9	TEX9	testis expressed sequence 9
PLSCR4	PLSCR4	phospholipid scramblase 4
LOC283027	LOC283027	-
LOC285535	LOC285535	-
PLEKHH2	PLEKHH2	pleckstrin homology domain containing, family H (with MyTH4 domain) member 2
SLC36A1	SLC36A1	solute carrier family 36 (proton/amino acid symporter), member 1
LOC203274	LOC203274	-
LOC282980	LOC282980	-
LOC158160		
RP11-151A6.2	RP11-151A6.2	-
CXCL2	CXCL2	chemokine (C-X-C motif) ligand 2
TREML4	TREML4	triggering receptor expressed on myeloid cells-like 4
C8ORF70	C8ORF70	chromosome 8 open reading frame 70
DPY19L2 /// FLJ36166		
LOC653071	LOC653071	-
SLC6A16	SLC6A16	solute carrier family 6, member 16
ABCC9	ABCC9	ATP-binding cassette, sub-family C (CFTR/MRP), member 9
LOC401321	LOC401321	-
SLC15A1	SLC15A1	solute carrier family 15 (oligopeptide transporter), member 1
LOC644660	LOC644660	-
LOC644488		
IMPG2	IMPG2	interphotoreceptor matrix proteoglycan 2
LOC284825	LOC284825	-
LOC643452		
HIST1H2AC	HIST1H2AC	histone cluster 1, H2ac
NUDT16P	NUDT16P	nudix (nucleoside diphosphate linked moiety X)-type motif 16 pseudogene
LOC157627	LOC157627	-
C1ORF103	C1ORF103	chromosome 1 open reading frame 103
SPINLW1	SPINLW1	serine peptidase inhibitor-like, with Kunitz and WAP domains 1 (eppin)
BAGE	BAGE	B melanoma antigen
OMD	OMD	osteomodulin
LRRC2	LRRC2	leucine rich repeat containing 2
ANKRD20A1 /// LOC392		
HIST1H1D	HIST1H1D	histone cluster 1, H1d
LOC439951	LOC439951	-

Figure 6: Heatmap(Cell Lines: HPB-ALL KOPT-K1)

Figure 7: Heatmap(NOTCH signaling pathway)