Direct inhibition of the NOTCH TF. Differenctial expression analysis

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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

```
Script 1.1.1 (python)

import rpy2.rinterface
%reload_ext rpy2.ipython

Script 1.1.2 (R)

%R
2 ##1. Load libraries
```

```
1 %%R
2 ##1. Load libraries
3 library("affy")
4 library("limma")
5 library("genefilter")
6 library(hgu133plus2.db)
7 wd = getwd()
8 wd
```

[1] "/Users/nandoide/misc_work/Desktop/uni/TRREP"

```
Script 1.1.3 (R)

1 %%R

2 #Normalization and differential expression for Affymetrix arrays

3 #2. Import .CEL files

4 setwd("GSE18198_data")

5 files_HPB <- list.files(pattern = "HPB*")

6 names_HPB <- gsub(".CEL.gz", "", files_HPB)

7 abatch_HPB <- ReadAffy(filenames = files_HPB, compress = TRUE, sampleNames = names_HPB)

8 setwd(wd)
```

```
1 %%R
2 #3. Normalize with RMA
# #qenerates object eset (class ExprSet),
4 #expresso function provides intensities in log scale
5 #pmonly = perfect match only method
6 eset_HPB <- expresso(abatch_HPB,</pre>
                    bg.correct = TRUE,
                    bgcorrect.method="rma",
                    normalize = TRUE,
9
                    normalize.method="quantiles",
                    pmcorrect.method="pmonly",
11
                    summary.method="medianpolish",
12
                    verbose = TRUE
13
14 )
```

Output

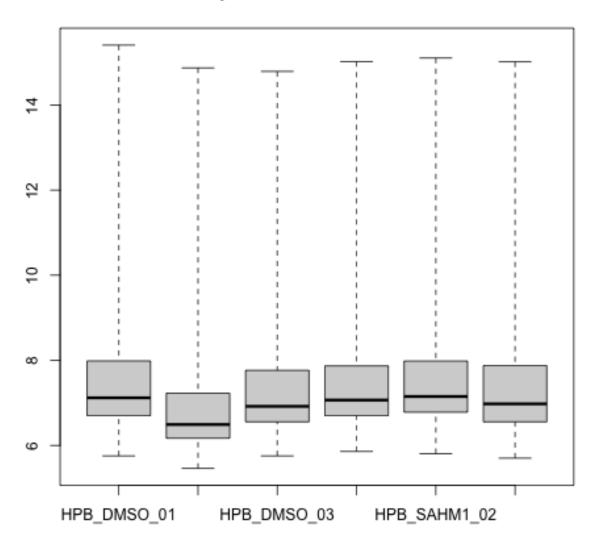
```
/Users/nandoide/miniconda3/lib/python3.7/site-packages/rpy2/rinterface/__init__.py:146:

→ RRuntimeWarning:

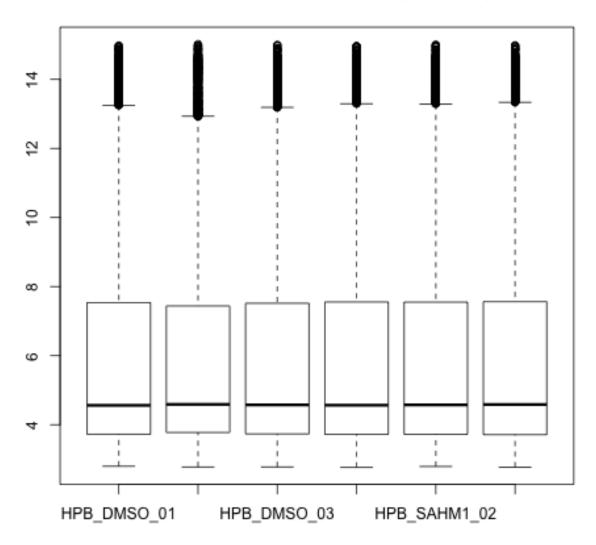
warnings.warn(x, RRuntimeWarning)
```

```
1 %%R
2 #4. Generate BOXPLOTS before and after normalization
3 #boxplot for raw data
4 boxplot(abatch_HPB,
5 main="Boxplot Before Normalization",
6 col = "lightgrey")
```

Boxplot Before Normalization



Boxplot After Normalization (log scale)



```
Script 1.1.7 (R)

1 %%R
2 #5.Data filtering using IQR.
3 esetIQR_HPB <- varFilter(eset_HPB, var.func=IQR, var.cutoff=0.5, filterByQuantile=TRUE)

4 ######Differential expression analysis.######

6 #6. Design matrix.

7 design_HPB <- cbind(DMSO=c(1,1,1,0,0,0), SAHM1=c(0,0,0,1,1,1))
```

Script 1.1.8 (R)

Script 1.1.9 (bash)

```
1 %%R
2 options(repr.matrix.max.rows=50, repr.matrix.max.cols=6)
3 toptableIQR_anot[c(1,2,6)]
```

	Symbol	logFC	adj.P.Val
201466_s_at	JUN	2.1333931	2.108830e-06
201464_x_at	JUN	1.5541848	7.688286e-06
229147_at	RASSF6	1.6458323	7.688286e-06
230710_at	MIR210HG	-1.9761492	7.688286e-06
201465_s_at	JUN	2.2743608	1.053822e-05
225342_at	AK4	-2.1078858	1.128982e-05
219270_at	CHAC1	1.3362724	3.651273e-05
202672_s_at	ATF3	1.3481402	3.651273e-05

```
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                              EFEMP1 -1.3678548 6.004820e-05
201842_s_at
235638_at
                              RASSF6 1.7804932 7.997079e-05
200953_s_at
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                                DTX1 -1.3841443 8.636324e-05
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                               FLRT3 1.3254635 1.138577e-04
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227347_x_at
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206744_s_at ZMYM5 0.7566891 9.024380e-03 213998_s_at DDX17 1.1177577 9.100637e-03 209383_at DDIT3 0.6302660 9.131640e-03 244502_at NA 0.8617368 9.153540e-03 243354_at NA 0.7398037 9.153540e-03 1556049_at RTN4 1.1124065 9.153540e-03 244620_at NA 0.6068910 9.153540e-03 244620_at NA 0.6068910 9.153540e-03 212422_at PDCD11 -0.6612868 9.153540e-03 223577_x_at MALAT1 1.4803259 9.153540e-03 235762_at TAS2R14 0.7112482 9.153540e-03 227082_at ZBTB20 0.6264831 9.153540e-03 21740_s_at CENPA 0.6035383 9.153540e-03 21747_a TAS2R14 0.7112482 9.153540e-03 20718_at CTSC -0.5959453 9.153540e-03 20718_at GPI -0.6175011 9.160738e-03 217025_s_	1557813_at	NA	0.6421955	9.024380e-03
213998_s_at DDX17 1.1177577 9.100637e-03 209383_at DDIT3 0.6302660 9.131640e-03 244502_at NA 0.8617368 9.153540e-03 243354_at NA 0.7398037 9.153540e-03 1556049_at RTN4 1.1124065 9.153540e-03 243031_at NA 0.6068910 9.153540e-03 244620_at NA 0.6068910 9.153540e-03 212422_at PDCD11 -0.6612868 9.153540e-03 223577_x_at MALAT1 1.4803259 9.153540e-03 235762_at TAS2R14 0.7112482 9.153540e-03 227082_at ZBTB20 0.6264831 9.153540e-03 217440_s_at CENPA 0.6035383 9.153540e-03 217140_s_at VDAC1 -0.6001627 9.153540e-03 201487_at CTSC -0.5959453 9.153540e-03 201487_at CTSC -0.5959453 9.153540e-03 201487_at GPI -0.6175011 9.160738e-03 21702	209569_x_at	NSG1	-0.6057988	9.024380e-03
209383_at DDIT3 0.6302660 9.131640e-03 244502_at NA 0.8617368 9.153540e-03 243354_at NA 0.7398037 9.153540e-03 1556049_at RTN4 1.1124065 9.153540e-03 243031_at NA 0.6945105 9.153540e-03 244620_at NA 0.6068910 9.153540e-03 212422_at PDCD11 -0.6612868 9.153540e-03 223577_x_at MALAT1 1.4803259 9.153540e-03 235762_at TAS2R14 0.7112482 9.153540e-03 227082_at ZBTB20 0.6264831 9.153540e-03 210821_x_at CENPA 0.6035383 9.153540e-03 217140_s_at VDAC1 -0.6001627 9.153540e-03 21748_at CTSC -0.5959453 9.153540e-03 201487_at CTSC -0.5959453 9.153540e-03 201487_at GTSC -0.5959453 9.153540e-03 201487_at GTSC -0.5959453 9.153540e-03 201487_	206744_s_at	ZMYM5	0.7566891	9.024380e-03
244502_at NA 0.8617368 9.153540e-03 243354_at NA 0.7398037 9.153540e-03 1556049_at RTN4 1.1124065 9.153540e-03 243031_at NA 0.6945105 9.153540e-03 244620_at NA 0.6068910 9.153540e-03 212422_at PDCD11 -0.6612868 9.153540e-03 223577_x_at MALAT1 1.4803259 9.153540e-03 235762_at TAS2R14 0.7112482 9.153540e-03 227082_at ZBTB20 0.6264831 9.153540e-03 217410_s_at CENPA 0.6035383 9.153540e-03 217410_s_at VDAC1 -0.6001627 9.153540e-03 201487_at CTSC -0.5959453 9.153540e-03 201487_at CTSC -0.5959453 9.153540e-03 204135_at GPI -0.6175011 9.160738e-03 204135_at FILIPIL 0.6305731 9.197263e-03 217025_s_at DBN1 -0.7229927 9.298988e-03 207	213998_s_at	DDX17	1.1177577	9.100637e-03
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