Selective Roles for CBP and p300 as Coregulators for Androgen-Regulated Gene Expression in Advanced Prostate Cancer Cells*

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SUPPLEMENTARY INFORMATION

SUPPLEMENTARY TABLE 1. **qRT-PCR mRNA and pre-mRNA primer sequences**

Primer Name	Sequence 5' to 3'
p300_mRNA_F	TACCCAGTCATCTCCGGCTCCA
p300_mRNA_R	AAAGATCCATGGGGCTCTTC
CBP_mRNA_F	GACGACCCTTCACAGCCCCAG
CBP_mRNA_R	TTCAAGCAGTTGTCGCACAC
18S_F	GAGGATGAGGTGGAACGTGT
18S_R	TCTTCAGTCGCTCCAGGTCT
PSA_F premRNA (1)	GTTTTTGCCTGGCCCGTAG
PSA_F mature mRNA (1)	GGCAGCATTGAACCAGAGGAG
PSA reverse mRNA (1)	GCATGAACTTGGTCACCTTCTG
KLK2 F (1)	GCTGCCCATTGCCTAAAGAAG
KLK2 R (1)	TGGGAAGCTGTGGCTGACA
TMPRSS2 Forward	CCTGCAAGGACATGGGCTATA
TMPRSS2 Reverse	CCGGCACTTGTGTTCAGTTTC
TMPRSS2 Forward premRNA	TTCAACTGTTTAGGGGTCACCACC
TMPRSS2 Reverse premRNA	CGGATGCACCTCGTAGACAGTG
FKBP5 Forward (2)	AGGCTGCAAGACTGCAGATC
FKBP5 Reverse (2)	CTTGCCCATTGCTTTATTGG
FKBP5 premRNA For	AGCCACTGTTGCTGAGCAGG
FKBP5 premRNA Rev	ACATTATCCACCCCAGCCCC

SUPPLEMENTARY TABLE 2. **ChIP primer sequences**

Primer Name	Sequence 5' to 3'
TMPRSS2 14kb ARE V + (3)	TGGTCCTGGATGATAAAAAAAGTTT
TMPRSS2 14kb ARE V - (3)	GACATACGCCCCACAACAGA
TMPRSS2 promoter (-0.1kb) Forward	CTACAGGAGCTCGTGAGGTAGCA
TMPRSS2 promoter (-0.1kb) Reverse	AGGAAGGGGATTCTGGGGAG
TMPRSS2 TSS +363 forward	CTGCGAGTCCCTAGCCAGTT
TMPRSS2 TSS +485 reverse	CTCCCCAAAGAGAAAAGGCG
FKBP5 TSS forward (4)	CTTTTGGGGGCGGACTGAC
FKBP5 TSS reverse (4)	CAGGACCCGCCTTCCATAG

FKBP5 ARE VIII/IX forward	GCATGGTTTAGGGGTTCTTGC
FKBP5 ARE VIII/IX reverse	AACACCCTGTTCTGAATGTGGC

Please see attached Excel File for the following tables:

SUPPLEMENTARY TABLE 3. **Genes Significantly Regulated by DHT**
The table list all genes for which expression was significantly (q-value ≤ 0.05) different for siNS DHT versus siNS vehicle treated samples. Column E represents $\log_2$ fold change in expression.

SUPPLEMENTARY TABLE 4. **Genes Affected Significantly by p300 Depletion**
The table list all genes for which expression was significantly (q-value ≤ 0.05) different for sip300 DHT versus siNS DHT treated samples. Column E represents $\log_2$ fold change in expression. Column G indicates whether the gene was also found (TRUE) in Supplementary Table 3, hormone regulated genes.

SUPPLEMENTARY TABLE 5. **Genes Affected Significantly by CBP Depletion**
The table list all genes for which expression was significantly (q-value ≤ 0.1) different for siCBP DHT versus siNS DHT treated samples. Column E represents $\log_2$ fold change in expression. Column G indicates whether the gene was also found (TRUE) in Supplementary Table 3, hormone regulated genes.

SUPPLEMENTARY REFERENCES

1. Jia, L., Kim, J., Shen, H., Clark, P. E., Tilley, W. D., and Coetzee, G. A. (2003) *Mol Cancer Res* **1**, 385-392
2. Bolton, E. C., So, A. Y., Chaivorapol, C., Haqq, C. M., Li, H., and Yamamoto, K. R. (2007) *Genes Dev* **21**, 2005-2017
3. Wang, Q., Li, W., Liu, X. S., Carroll, J. S., Janne, O. A., Keeton, E. K., Chinnaiyan, A. M., Pienta, K. J., and Brown, M. (2007) *Mol Cell* **27**, 380-392
4. Makkonen, H., Kauhanen, M., Paakinaho, V., Jaaskelainen, T., and Palvimo, J. J. (2009) *Nucleic Acids Res*

Code for *Selective roles for CBP and p300 as coregulators for androgen-regulated gene expression in advanced prostate cancer cells.*

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1 Preface

In the interests of reproducible research (http://reproducibleresearch.net) I have included the code I used to process the data and get the results for this paper.

We ran 24 samples on 2 Illumina HT12v4 microarrays at The Southern California Genotyping Consortium. These samples were processed at the facility with default outlier removal and did not include TIFF images. The resulting data files (idats) were read into Genome Studio and exported without normalization or background correction using the export 'standard probe profile' and export 'control probe profile' feature using the default number of significant digits. Standard error and number of probes were also included in the export (but not used) as were 9 probes with some imputed values (not significant in comparisons of interest).

These two probe files, which can be reconstructed from the 'raw' data on GEO, are the bead summerized datasets that are used for further analysis in R/bioconductor.

2 Read in and Quality Check

Read in bead summerized probes and target file, target file can be extracted from GEO data but I have also included it at the end of this document.

```
> library(limma)
> library(qvalue)
> #x is eset that holds raw values
> #y is eset that holds log2 transformed normalized values
> #z is eset that holds batch corrected values
> x = read.ilmn(files="irina-spp.txt", ctrlfiles="irina-cpp.txt")

Reading file irina-spp.txt ... ...
Reading file irina-cpp.txt ... ...

> targets = read.table("sample description.txt", header=T, row.names=1)
> targets = cbind(targets, Type=paste(targets[,1], targets[,2], sep="_"))
> x$targets = targets = targets[x$targets$SampleNames,]
```

2.1 Raw expression boxplots + MDS clustering

```
> boxplot(log2(x$E[x$genes$Status=="regular",]),range=0,
+ xlab="Arrays",ylab="log2 intensities", main="Regular probes")


> boxplot(log2(x$E[x$genes$Status=="NEGATIVE",]),range=0,
+ xlab="Arrays",ylab="log2 intensities", main="Control probes")
```

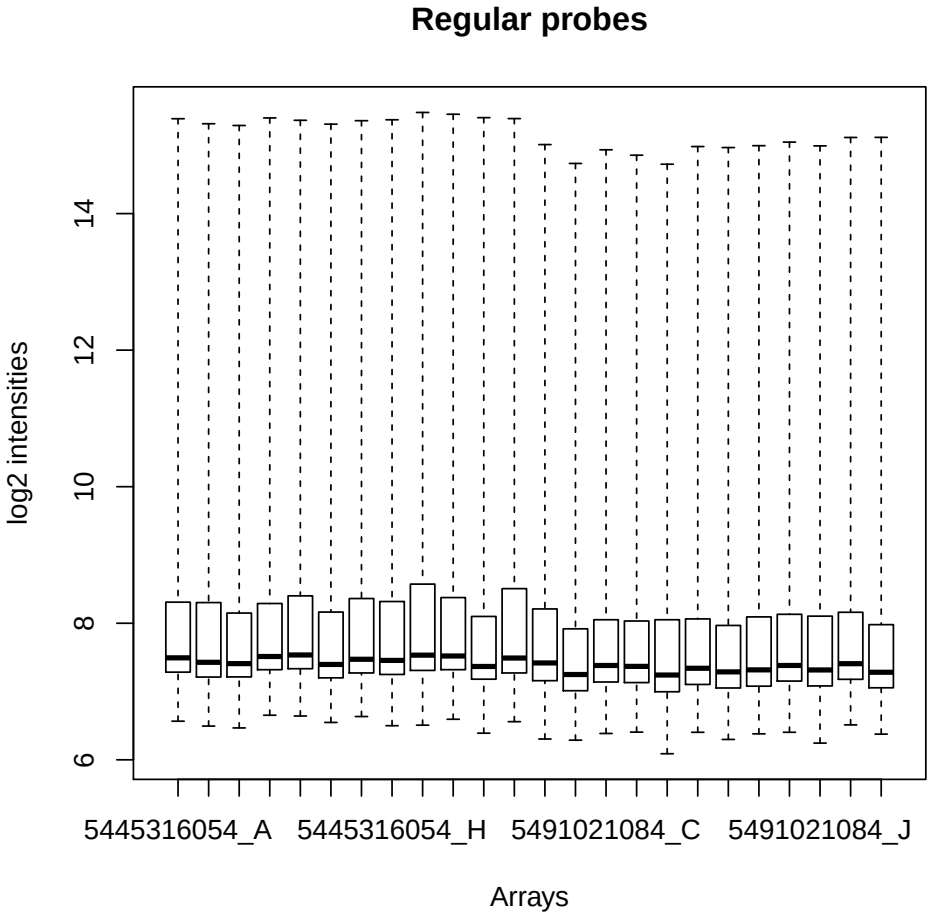


Figure 1: Boxplot of raw expression intensitiy of regular probes

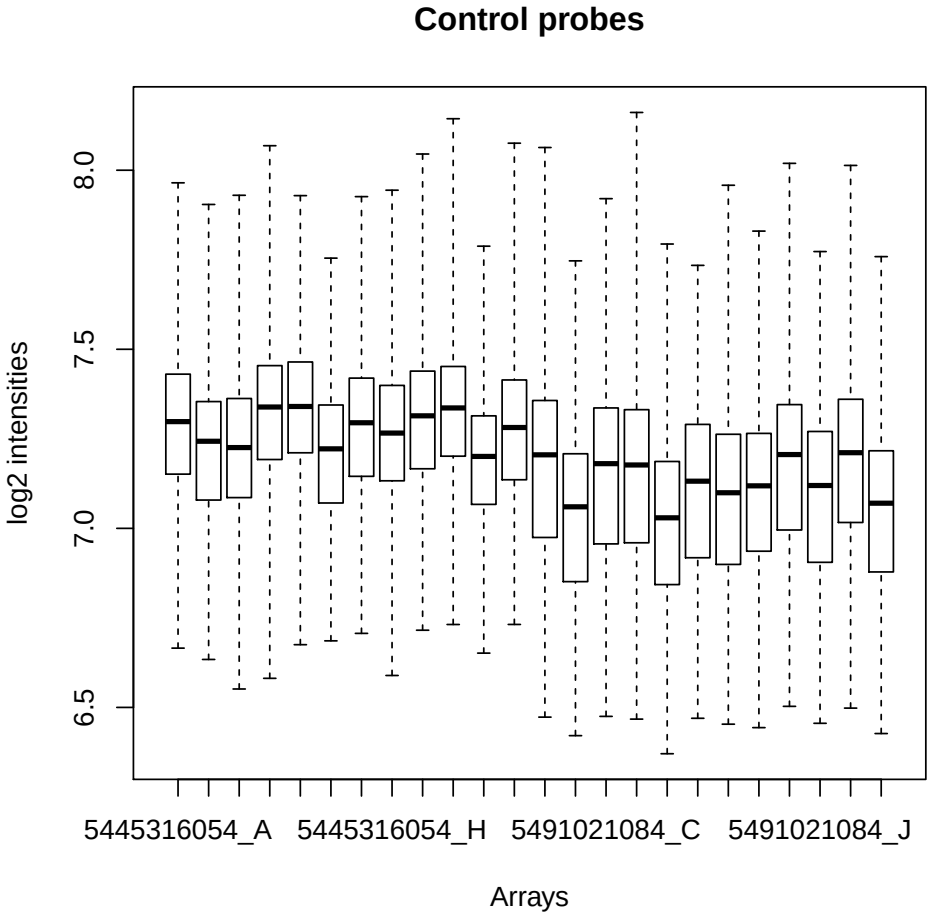


Figure 2: Boxplot of raw expression intensitiy of control probes

```
> y = neqc(x) #log2 transform + normalize
> plotMDS(y,labels=paste(targets[,1], targets[,2], unclass(targets[,3]), sep="_"),
+  col=unclass(x$targets$Type),xlim = c(-1.5,1.5), ylim=c(-1,1)) #color by type
```

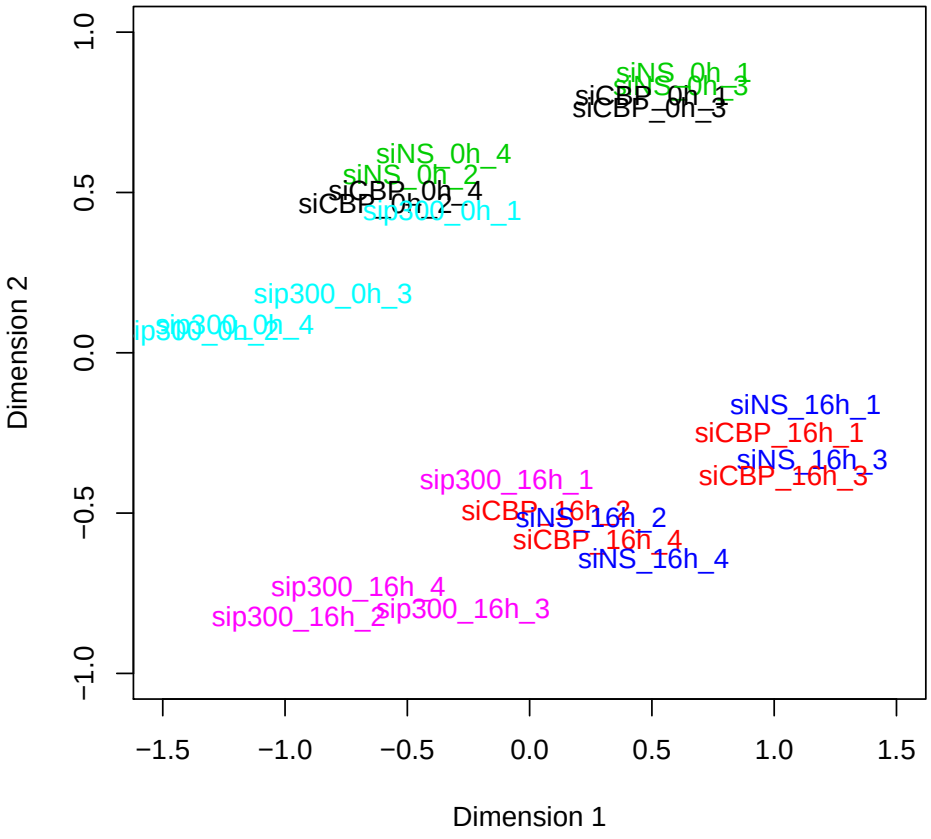


Figure 3: MDS plot of normalized arrays colored by experiment

```
> plotMDS(y,labels=paste(targets[,1], targets[,2], unclass(targets[,3]), sep="_"),
+   col=unclass(x$targets$batch),xlim = c(-1.5,1.5), ylim=c(-1,1)) #color by batch
```

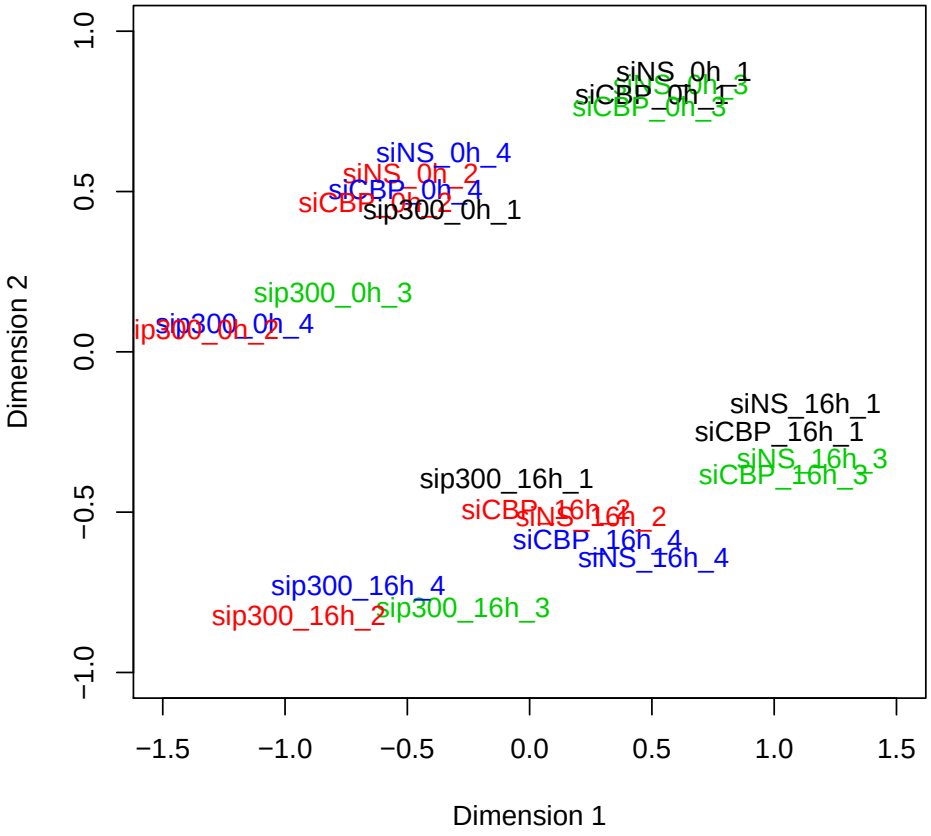


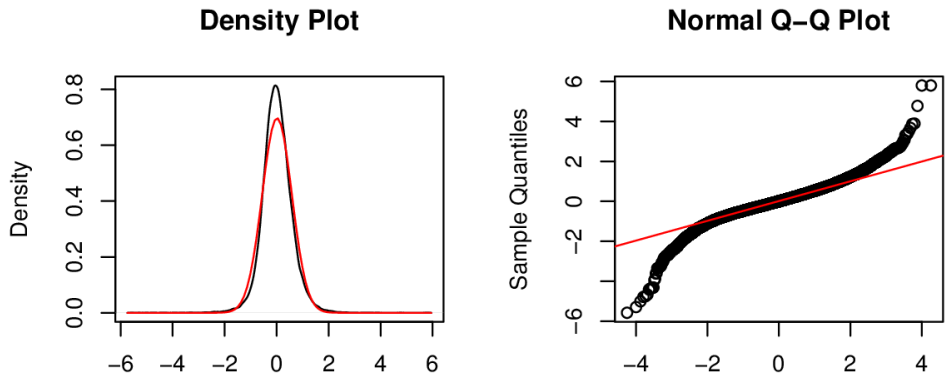
Figure 4: MDS plot of normalized arrays colored by batch

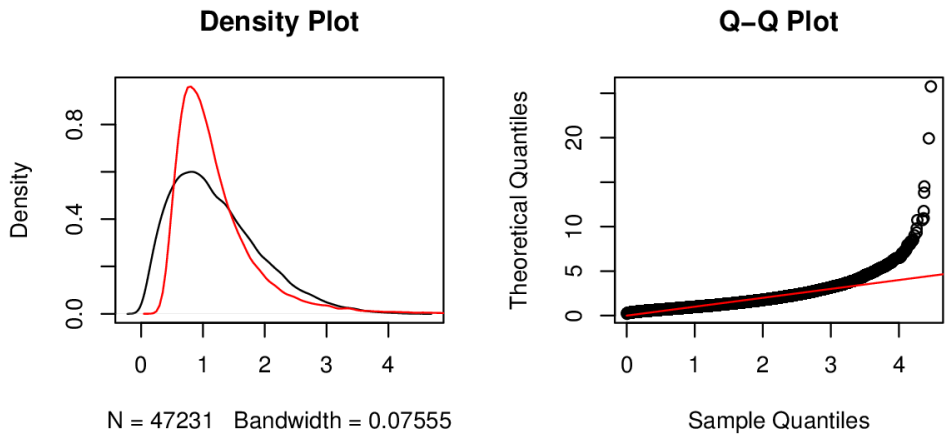
From the above plots, there might be some batch effects that keep the CBP and NS groups together (1+3, 2+4) `Combat.R` is run to remove these effects

```
> source("../ComBat_mod.R") #slightly modified to accept different input
> cb_targets = cbind("Array_Name"=colnames(y$E),
+  "Sample_Name"=as.character(y$targets$Type), "Batch"=unclass(y$targets$batch))
> cb_adj = ComBat_mod(y$E, cb_targets, write=F, skip=1) #takes a while

Reading Sample Information File
Reading Expression Data File
Found 4 batches
Found 0 covariate(s)
Standardizing Data across genes
Fitting L/S model and finding priors
Finding parametric adjustments
Adjusting the Data

> colnames(cb_adj) = colnames(y$E) #combat chops up 1st col name
> z = y
> z$E = cb_adj
```





```
> plotMDS(z,labels=paste(targets[,1], targets[,2], unclass(targets[,3]), sep="_"),
+  col=unclass(x$targets$Type),xlim = c(-1.5,1.5), ylim=c(-1,1)) #color by type
```

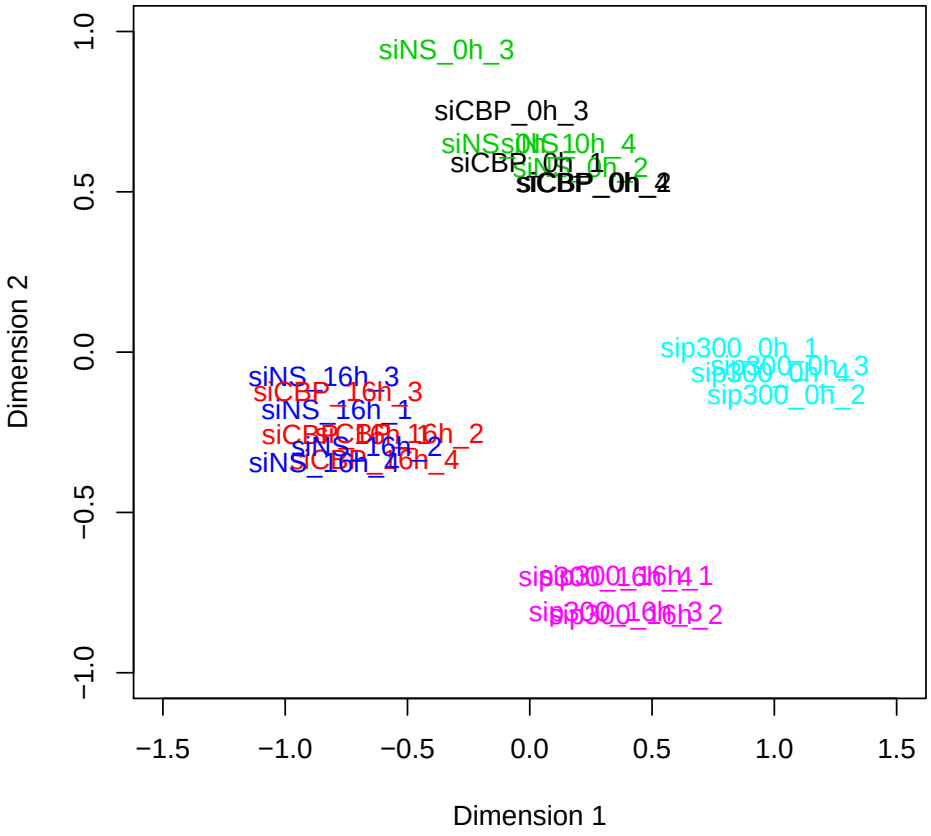


Figure 5: MDS plot of normalized, batch corrected arrays colored by experiment

3 Identify differentially regulated genes

Use eBayes from limma package to find CBP regualted genes, p300 regulated genes and DHT-regulated genes. (see paper for details)

3.1 CBP regulated

```
> sel = z$targets[,1] != "sip300" & z$targets[,2] == "16h" #cbp regulated
> lumisub = z$E[,sel]
> pd = z$targets[colnames(lumisub),] #phenotype data
> des = matrix(0, ncol(lumisub), length(levels(factor(pd$treat))))
> for(i in 1:length(levels(factor(pd$Type)))){
+ des[pd$Type==levels(factor(pd$Type))[i],i]=1
+ }
> colnames(des) = levels(factor(pd$treat))
> des
```

```
     siCBP siNS
[1,]     1    0
[2,]     0    1
[3,]     0    1
[4,]     1    0
[5,]     1    0
[6,]     0    1
[7,]     1    0
[8,]     0    1
```

```
> cm = rbind(1,-1) #assume col2 is NS
> if(!grepl(colnames(des)[2], "siNS")) { cm = -cm }
> cm
```

```
     [,1]
[1,]    1
[2,]   -1
```

```
> fit = lmFit(lumisub,des)
> fit2 = contrasts.fit(fit,cm)
> efit = eBayes(fit2)
> cbp_efit = efit
> cbp_efit$qv = qvalue(efit$p.value)$qvalues
> sig_fdr = which(cbp_efit$qv<0.1)
> sig16cbp = rownames(efit[sig_fdr,][order(efit$p.value[sig_fdr]),]) #illumina IDs
> length(sig16cbp) #7
```

```
[1] 7
```

```
> z$genes[match(sig16cbp, z$genes[,1]), 2]
```

```
[1] "CREBBP"   "SERPINE2" "MAPK9"    "ANXA9"    "C19orf4"  "SCO1"     "TMEM20"
```

3.2 p300 regulated

```
> sel = z$targets[,1] != "siCBP" & z$targets[,2] == "16h" #p300 regulated
> lumisub = z$E[,sel]
> pd = z$targets[colnames(lumisub),]
```

```
> des = matrix(0, ncol(lumisub), length(levels(factor(pd$treat))))
> for(i in 1:length(levels(factor(pd$Type)))){
+ des[pd$Type==levels(factor(pd$Type))[i],i]=1
+ }
> colnames(des) = levels(factor(pd$treat))
> des

     siNS sip300
[1,]    1      0
[2,]    0      1
[3,]    1      0
[4,]    0      1
[5,]    0      1
[6,]    1      0
[7,]    0      1
[8,]    1      0

> cm = rbind(1,-1) #assume col2 is NS
> if(!grepl(colnames(des)[2], "siNS")) { cm = -cm }
> cm

     [,1]
[1,]   -1
[2,]    1

> fit = lmFit(lumisub,des)
> fit2 = contrasts.fit(fit,cm)
> efit = eBayes(fit2)
> p300_efit = efit
> p300_efit$qv = qvalue(efit$p.value)$qvalues
> sig_fdr = which(p300_efit$qv<0.05)
> sig16p300 = rownames(efit[sig_fdr,][order(efit$p.value[sig_fdr]),])
> length(sig16p300) #4582

[1] 4582

> head(z$genes[match(sig16p300, z$genes[,1]), 2])

[1] "PCDHB2" "NTNG1"  "PHLDA2" "CAB39L" "CAB39L" "CBR3"
```

3.3 DHT regulated

```
> sel = z$targets[,1] == "siNS" #hormone regulated
> lumisub = z$E[,sel]
> pd = z$targets[colnames(lumisub),]
> des = matrix(0, ncol(lumisub), length(levels(factor(pd$hour))))
> for(i in 1:length(levels(factor(pd$Type)))){
+ des[pd$Type==levels(factor(pd$Type))[i],i]=1
+ }
> colnames(des) = levels(factor(pd$hour))
> des

     0h 16h
[1,]  1   0
[2,]  1   0
```

```
[3,]  0   1
[4,]  0   1
[5,]  0   1
[6,]  1   0
[7,]  0   1
[8,]  1   0
```

```
> cm = rbind(1,-1)
> fit = lmFit(lumisub,des)
> fit2 = contrasts.fit(fit,cm)
> efit = eBayes(fit2)
> hr_efit = efit
> hr_efit$qv = qvalue(efit$p.value)$qvalues
> sig_fdr = which(hr_efit$qv<0.05)
> hor_reg = rownames(efit[sig_fdr,][order(efit$p.value[sig_fdr]),])
> length(hor_reg) #676
```

```
[1] 676
```

```
> head(z$genes[match(hor_reg, z$genes[,1]), 2])
```

```
[1] "KLK2"   "RHOU"   "SNAI2"  "ACSL3"  "MICAL1" "SGK1"
```

```
> table(efit[sig_fdr,]$coefficients>0) #up and down regulated genes
```

```
FALSE  TRUE
  416   260
```

```
> table(is.element(hor_reg, sig16p300)) #DHT regulated AND p300 regulated
```

```
FALSE  TRUE
  357   319
```

4 Output

4.1 GEO spreadsheet

GEO output for Illumina expression excel template

```
> out = matrix(0, ncol=ncol(z$E)*2, nrow=nrow(z$E))
> colnames(out) = as.character(1:(ncol(z$E)*2))
> for(i in 1:ncol(z$E)) {
+   out[,(2*(i-1)+1)] = z$E[,i]
+   out[,(2*(i-1)+2)] = z$other[[1]][,i]
+   colnames(out)[(2*(i-1)+1)] = colnames(z$E)[i]
+   colnames(out)[(2*(i-1)+2)] = "Detection Pval"
+ }
> rownames(out) = rownames(z$E)
> head(out[,1:8])
```

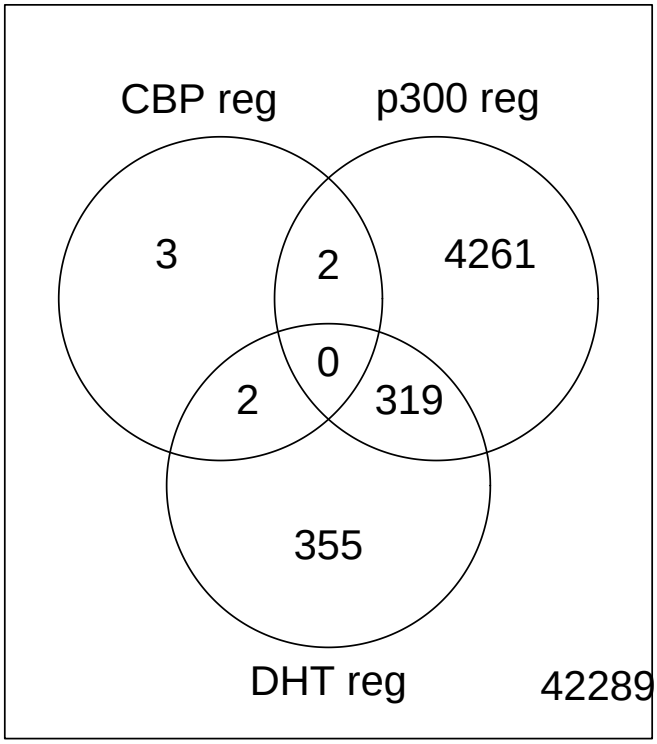
```
            5445316054_A Detection Pval 5445316054_B Detection Pval
ILMN_1762337     5.347191      0.2272727     5.430834     0.16753250
ILMN_2055271     5.349342      0.2259740     5.940997     0.01688312
ILMN_1736007     5.567351      0.1155844     5.317307     0.26623380
ILMN_2383229     5.065763      0.5051948     4.978239     0.71818180
ILMN_1806310     5.178655      0.3779221     5.815979     0.02467532
```

```
ILMN_1779670     4.755641      0.8662338     4.763895     0.85584410
             5445316054_C Detection Pval 5445316054_D Detection Pval
ILMN_1762337     5.118083      0.4363636     5.155345     0.46363640
ILMN_2055271     5.478488      0.2298701     5.911265     0.02597403
ILMN_1736007     5.040926      0.5792208     5.431196     0.19220780
ILMN_2383229     5.152320      0.4480520     5.525455     0.12857140
ILMN_1806310     5.095255      0.4532467     5.155710     0.41428570
ILMN_1779670     4.838303      0.8077922     4.778316     0.82727270
```

```
> #write.table(out, file="GEO_norm.txt",sep="\t", quote=F) #rerun w/x for raw
```

4.2 Venn Diagram

```
> a = vennCounts(cbind(CBPreg=cbp_efit$qv<0.1,
+  p300reg=p300_efit$qv<0.05, hormreg=hr_efit$qv<0.05))
> vennDiagram(a, names=c("CBP reg", "p300 reg", "DHT reg"))
> #figure 1c is based on this, figure in paper is generated using Vennerable library
> # properly weighted venn digram looked terrible due to low number of CBP regulated genes
```



5 Other

5.1 Targets file

```
> read.table("sample description.txt", header=T, row.names=1) #targets file
```

```
             treatments hour    batch
5445316054_A       siNS   0h 8.25.10A
5445316054_B     sip300   0h 8.25.10B
5445316054_C       siNS   0h  8.20.10
5445316054_D      siCBP  16h 8.25.10B
5445316054_E      siCBP   0h  8.18.10
5445316054_F       siNS  16h 8.25.10B
5445316054_G     sip300  16h 8.25.10A
5445316054_H       siNS  16h  8.18.10
5445316054_I     sip300   0h  8.20.10
5445316054_J      siCBP  16h  8.18.10
5445316054_K      siCBP   0h 8.25.10A
5445316054_L     sip300  16h  8.18.10
5491021084_A     sip300   0h 8.25.10A
5491021084_B      siCBP  16h  8.20.10
5491021084_C     sip300  16h 8.25.10B
5491021084_D       siNS  16h 8.25.10A
5491021084_E     sip300  16h  8.20.10
5491021084_F      siCBP   0h 8.25.10B
5491021084_G      siCBP  16h 8.25.10A
5491021084_H      siCBP   0h  8.20.10
5491021084_I       siNS   0h  8.18.10
5491021084_J     sip300   0h  8.18.10
5491021084_K       siNS  16h  8.20.10
5491021084_L       siNS   0h 8.25.10B
```

5.2 R/bioconductor version

```
> sessionInfo()
```

```
R version 2.13.0 (2011-04-13)
Platform: x86_64-pc-linux-gnu (64-bit)

locale:
 [1] LC_CTYPE=en_US.UTF-8       LC_NUMERIC=C
 [3] LC_TIME=en_US.UTF-8        LC_COLLATE=en_US.UTF-8
 [5] LC_MONETARY=C              LC_MESSAGES=en_US.UTF-8
 [7] LC_PAPER=en_US.UTF-8       LC_NAME=C
 [9] LC_ADDRESS=C               LC_TELEPHONE=C
[11] LC_MEASUREMENT=en_US.UTF-8 LC_IDENTIFICATION=C

attached base packages:
[1] stats     graphics  grDevices utils     datasets  methods   base

other attached packages:
[1] qvalue_1.26.0 limma_3.8.2

loaded via a namespace (and not attached):
[1] tcltk_2.13.0
```

5.3 Modifications to Combat.R

`Combat.R` can be found here: http://jlab.byu.edu/ComBat/Download.html
Header comments were removed before the `diff` command. These modifications were made so I did not have to write out and read in a file everytime I wanted to rerun `Combat.R` with different parameters. Function arguments were changed to allow for passing in matrix of expression values.

```
$ diff ComBat.R ComBat_mod.R
1c1,2
< ComBat <- function(expression_xls, sample_info_file, type='txt', write=T, covariates='all', par.prior=T, filter=F, skip=0, prior.plots=T){
---
> ComBat_mod <- function(expression_xls, sample_info_file, type='txt', write=T,
>  covariates='all', par.prior=T, filter=F, skip=0, prior.plots=T){
4c5,6
<  saminfo <- read.table(sample_info_file, header=T, sep='\t',comment.char='')
---
>  saminfo = sample_info_file #alternate loading, not fully done yet
>  #saminfo <- read.table(sample_info_file, header=T, sep='\t',comment.char='')
8,9c10,11
<  if(type=='csv'){
<  dat <- read.csv(expression_xls,header=T,as.is=T)
---
>  #if(type=='csv'){
>  # dat <- read.csv(expression_xls,header=T,as.is=T)
13c15
<  colnames(dat)=scan(expression_xls,what='character',nlines=1,sep=',',quiet=T)[1:ncol(dat)]
---
>  # colnames(dat)=scan(expression_xls,what='character',nlines=1,sep=',',quiet=T)[1:ncol(dat)]
15,20c17,23
<  }
<           else{
<  dat <- read.table(expression_xls,header=T,comment.char='',fill=T,sep='\t', as.is=T)
<  dat <- dat[,trim.dat(dat)]
<  colnames(dat)=scan(expression_xls,what='character',nlines=1,sep='\t',quiet=T)[1:ncol(dat)]
<  }
---
>  # }
>      #else{
>  # dat <- read.table(expression_xls,header=T,comment.char='',fill=T,sep='\t', as.is=T)
>  # dat <- dat[,trim.dat(dat)]
>  # colnames(dat)=scan(expression_xls,what='character',nlines=1,sep='\t',quiet=T)[1:ncol(dat)]
>  # }
>  dat = expression_xls
23,24c26,27
<                dat <- dat[,-c(1:skip)]}
<          else{geneinfo=NULL}
---
>                dat <- dat[,-c(1:skip)]
>  }else{geneinfo=NULL}
137c140
<  output_file <- paste('Adjusted',expression_xls,'.xls',sep='_')
---
>  output_file <- paste('Adjusted expression.xls',sep='_')
```