

Cocaine_Gene_Expression.R

steve

2021-02-19

```
# Names: Steven Nguyen
#
# Title: Identifying differentially expressed genes between cocaine addict deaths and, non cocaine addi

# Importing libraries
library(affy)

## Warning: package 'affy' was built under R version 4.0.3
## Loading required package: BiocGenerics
## Warning: package 'BiocGenerics' was built under R version 4.0.3
## Loading required package: parallel
##
## Attaching package: 'BiocGenerics'
## The following objects are masked from 'package:parallel':
##
##   clusterApply, clusterApplyLB, clusterCall, clusterEvalQ,
##   clusterExport, clusterMap, parApply, parCapply, parLapply,
##   parLapplyLB, parRapply, parSapply, parSapplyLB
## The following objects are masked from 'package:stats':
##
##   IQR, mad, sd, var, xtabs
## The following objects are masked from 'package:base':
##
##   anyDuplicated, append, as.data.frame, basename, cbind, colnames,
##   dirname, do.call, duplicated, eval, evalq, Filter, Find, get, grep,
##   grepl, intersect, is.unsorted, lapply, Map, mapply, match, mget,
##   order, paste, pmax, pmax.int, pmin, pmin.int, Position, rank,
##   rbind, Reduce, rownames, sapply, setdiff, sort, table, tapply,
##   union, unique, unsplit, which.max, which.min
## Loading required package: Biobase
## Warning: package 'Biobase' was built under R version 4.0.3
## Welcome to Bioconductor
##
##   Vignettes contain introductory material; view with
##   'browseVignettes()'. To cite Bioconductor, see
##   'citation("Biobase)", and for packages 'citation("pkgname)".
```

```
library(dplyr)
```

```
##
## Attaching package: 'dplyr'
## The following object is masked from 'package:Biobase':
##
##   combine
## The following objects are masked from 'package:BiocGenerics':
##
##   combine, intersect, setdiff, union
## The following objects are masked from 'package:stats':
##
##   filter, lag
## The following objects are masked from 'package:base':
##
##   intersect, setdiff, setequal, union
```

```
library(ggplot2)
```

```
library(GEOquery)
```

```
## Warning: package 'GEOquery' was built under R version 4.0.3
## Setting options('download.file.method.GEOquery'='auto')
## Setting options('GEOquery.inmemory.gpl'=FALSE)
```

```
library(limma)
```

```
## Warning: package 'limma' was built under R version 4.0.3
##
## Attaching package: 'limma'
## The following object is masked from 'package:BiocGenerics':
##
##   plotMA
```

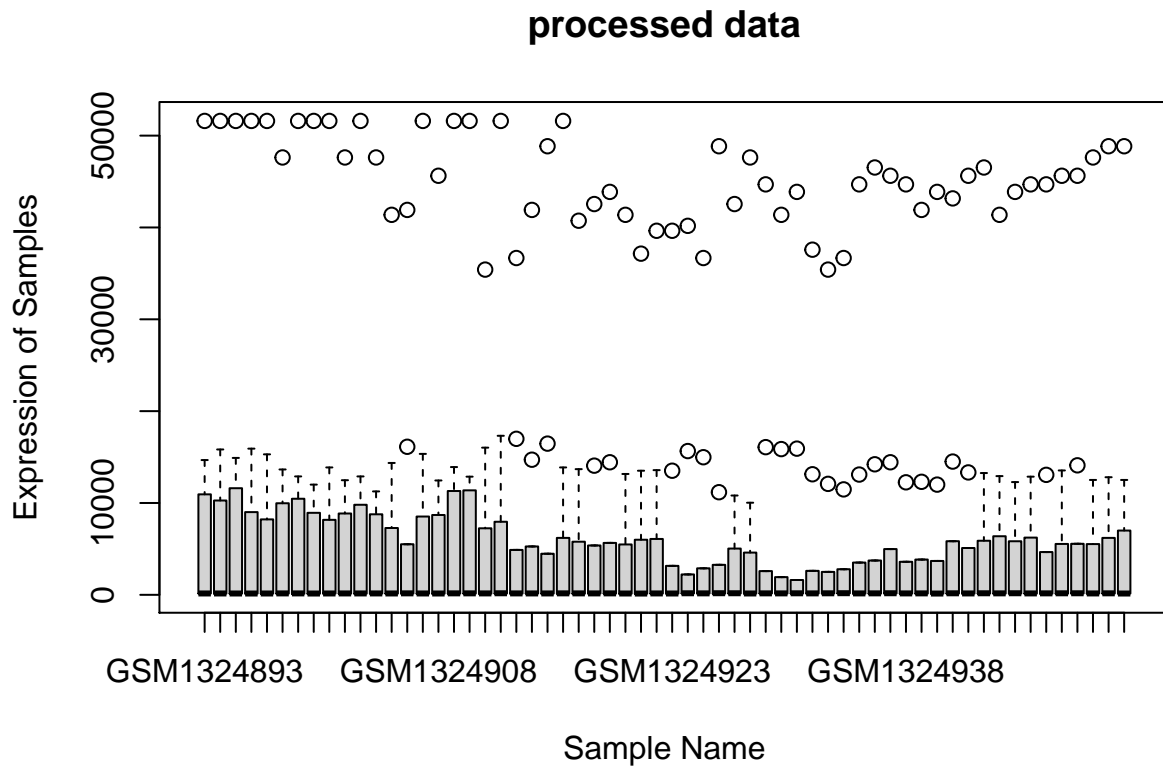
```
# 1 & 2: Importing the data from directory
GSE54839 <- getGEO("GSE54839")
```

```
## Found 1 file(s)
## GSE54839_series_matrix.txt.gz
##
## -- Column specification -----
## cols(
##   .default = col_double(),
##   ID_REF = col_character()
## )
## i Use 'spec()' for the full column specifications.
## File stored at:
## C:\Users\steve\AppData\Local\Temp\RtmpuWsqqn/GPL6947.soft
GSE54839.expr <- exprs(GSE54839[[1]])
GSE54839.p <- pData(GSE54839[[1]])
```

```
# 3: Generating a boxplot to see if processed data is already in log2 scale
```

```
GSE54839.exprTrunc <- head(GSE54839.expr, 10)
```

```
boxplot(GSE54839.exprTrunc, main = "processed data", xlab = "Sample Name", ylab = "Expression of Samples")
```

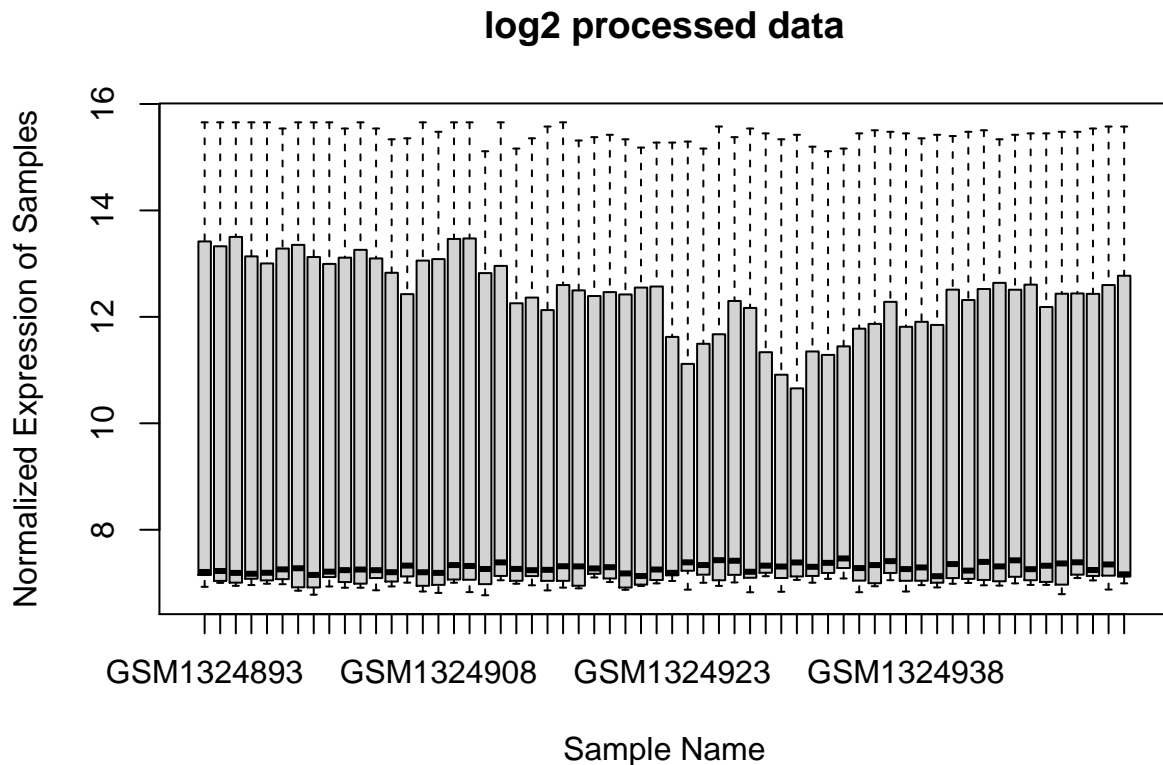


```
# Since not in log2 scale, normalizing data with log2 scale
```

```
GSE54839.exprLog <- log2(GSE54839.expr)
```

```
GSE54839.exprLogTrunc <- head(GSE54839.exprLog, 10)
```

```
boxplot(GSE54839.exprLogTrunc, main = "log2 processed data", xlab = "Sample Name", ylab = "Normalized Expression")
```



```
# 4: Finding the number of probes and samples
```

```
ncol(GSE54839.expr)
```

```
## [1] 60
```

```
nrow(GSE54839.expr)
```

```
## [1] 48761
```

```
# There are 60 samples and 48761 probes in the dataset
```

```
# 5: Extracting the column that contains the categories that we would like to compare
```

```
addition.status <- as.character(GSE54839.p$disease.state.ch1)
```

```
table(addition.status)
```

```
## addition.status
```

```
## cocaine addition      control
```

```
##           30           30
```

```
# There are 30 individuals that were part of the group with cocaine addiction, and there are 30 individuals in the control group
```

```
# 6: Finding the differentially expressed probes across the 2 groups
```

```
design <- model.matrix(~-1+addition.status)
```

```
colnames(design) <- c("control", "cocaine.addiction")
```

```
fit <- lmFit(GSE54839.exprLog, design)
```

```
contrast.matrix <- makeContrasts(control - cocaine.addiction, levels = design)
```

```
fit2 <- contrasts.fit(fit, contrast.matrix)
```

```

fit2 = eBayes(fit2)

tt20 <- topTable(fit2, sort.by = "p", p.value = 0.20, number = nrow(GSE54839.exprLog))

# 7: Constructing a boxplot for the probe with the lowest adjusted p-value

m <- match(rownames(tt20)[1], rownames(GSE54839.exprLog))
probe <- GSE54839.exprLog[m,]
df <- data.frame(expr = probe, addiction.status = addiction.status)

means <- df %>% group_by(addiction.status) %>% summarize(mean = mean(expr))

## 'summarise()' ungrouping output (override with '.groups' argument)
means

## # A tibble: 2 x 2
##   addiction.status    mean
##   <chr>              <dbl>
## 1 cocaine addiction  9.11
## 2 control           8.61

diff(means$mean)

## [1] -0.5029022

tt20$logFC[1]

## [1] 0.5029022

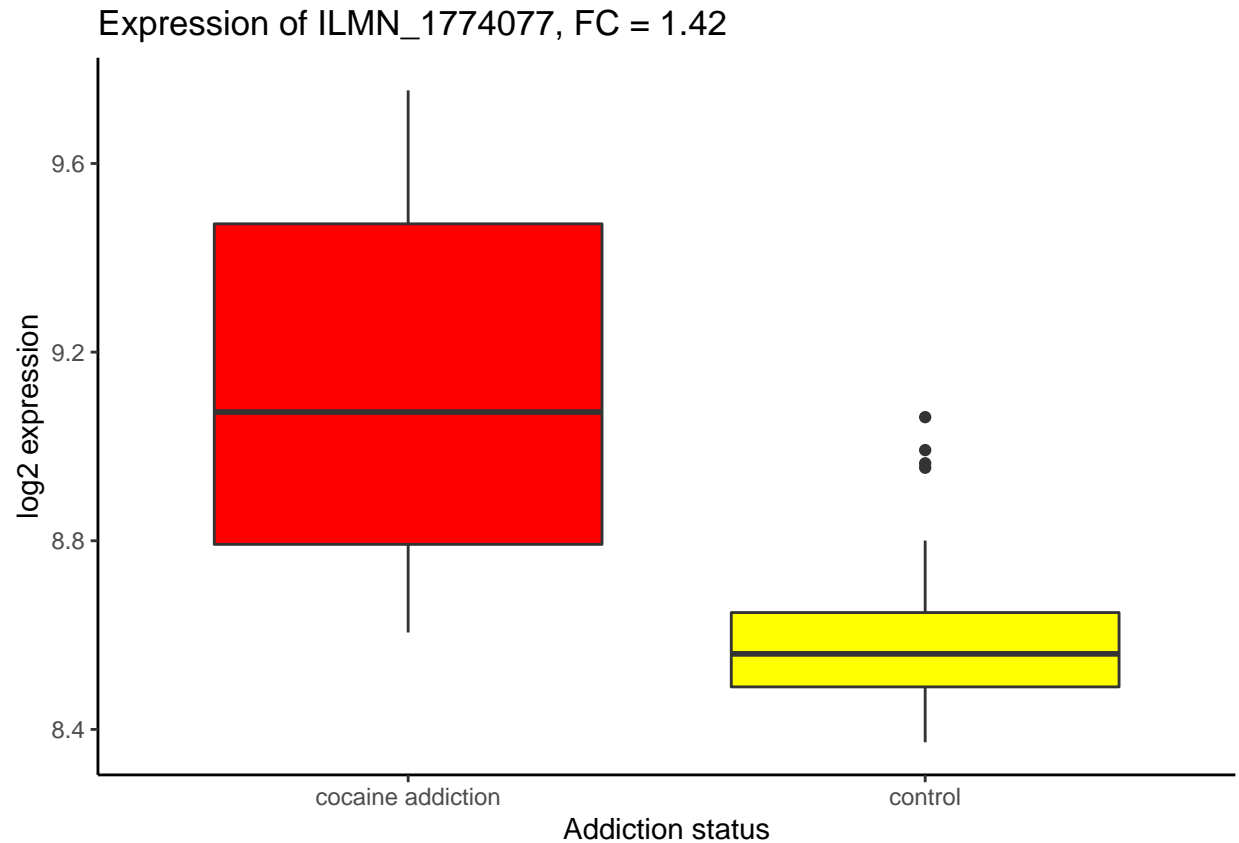
# convert to FC #
logFC <- tt20$logFC[1]
2**logFC

## [1] 1.417061

## visualize ##
FC <- paste0("FC = ", round(2**logFC, 2))
main <- paste0("Expression of ", rownames(tt20)[1], ", ", FC)

ggplot(df, aes(x = addiction.status, y = expr, fill = addiction.status)) + geom_boxplot() +
  ylab("log2 expression") + xlab("Addiction status") + ggtitle(main) +
  scale_fill_manual(values = c("red", "yellow")) +
  theme_classic() + theme(legend.position = "none")

```



```
# 8: Using R to output the annotation of the data
platform2 <- annotation(GSE54839[[1]])
```

```
# 9: Downloading the platform (GPL) for this data
pl2 <- getGEO(platform2)
```

```
## Using locally cached version of GPL6947 found here:
## C:\Users\steve\AppData\Local\Temp\RtmpuWsqqn/GPL6947.soft
pl2 <- Table(pl2)
```

```
probe <- rownames(tt20)[1]
m <- match(probe, pl2$ID)
pl2$ILMN_Gene[m]
```

```
## [1] "GBP2"
```

```
# 10: Finding the gene names corresponding to all probes, and creating a table
```

```
match <- match(rownames(tt20), rownames(GSE54839.exprLog))
ILMNGene <- pl2$ILMN_Gene[match]
```

```
df20 <- data.frame(tt20)
df220 <- df20 %>% select(logFC, adj.P.Val)
df220["ILMN_Gene"] <- ILMNGene
```

```
head(df220, 5)
```

```
##           logFC   adj.P.Val  ILMN_Gene
## ILMN_1774077  0.5029022 0.000346728    RGL4
## ILMN_2103919 -0.3447804 0.002191241  ITGB1BP1
## ILMN_1695706  0.4978619 0.004252562    COX4I2
## ILMN_1667825  0.4422986 0.004252562  HS.537387
## ILMN_1886493  0.3352531 0.004252562    BOLA2
```

11: Using DAVID to identify Gene Ontology and KEGG pathways that are associated with the differential

```
probe <- rownames(tt20)
m <- match(probe, p12$ID)
genes<-p12$ILMN_Gene[m]
keep <- genes!=" "
genes <- genes[keep]
genes <- strsplit(genes, " /// ")
genes <- unlist(genes)
genes <- unique(genes)
write.table(genes, row.names = FALSE, quote = FALSE)
```

```
## x
## GBP2
## LRFN3
## H3F3B
## MLKL
## HS.535028
## MCL1
## SHMT1
## SLC39A8
## NXT1
## KCNB1
## EMP1
## CCDC109B
## HIST2H2AA3
## C19ORF4
## VCAM1
## CHSY1
## TNFRSF10B
## TMEM191B
## GSTO1
## C12ORF52
## ADAM17
## CLIC1
## HS.562318
## CCL2
## RGS16
## GAPDH
## LRRFIP2
## CDKN1A
## C14ORF152
## ENTPD4
## ATP2A3
## C7ORF43
## CAPS
## SEZ6L
## PATL1
```

CERK
LOC730012
DNAJC15
LOC649999
COMTD1
AURKA
RDH12
FER1L3
COPS7A
SCARA3
HS.544112
RELL2
BTG3
AXUD1
BTG1
HIST2H2AC
PIB5PA
EVI5L
C12ORF10
CCNL1
NFKB1
C16ORF59
AP3D1
GFPT2
SDC1
LRRC32
HMG1
FAM69B
SLITRK1
PCDHB9
SAT1
CXCL10
TUBG1
RAB9B
RIPK2
C10RF142
MAST1
RAB3IP
SAP18
FOSB
TM4SF1
JUN
ECSIT
MED8
ATP5G1
LOC440359
LOC729378
OCIAD2
PORCN
F3
CSDA
FKBP2
LOC731835
C9ORF125

SPG3A
SERPINA3
COMMD5
APOL2
EVL
SLC1A1
UNC5A
RPL37
FLJ36070
MGST3
PSME3
SOCS2
LOC283514
SNX5
AP3S2
CAMK1G
HS.538770
CD44
LOC650128
C8ORFK32
CTHRC1
PTPN3
LOC653841
OVGPI
LOC728492
BTBD6
PTBP1
LOC643882
TAP1
DPYS
RNF165
ANGPT1
IRAK3
LOC649095
MESP1
FLJ44968
MDH2
CD93
PRMT8
SDHA
RRAD
NFKBIZ
NDUFV1
UBE3C
FIG4
SULT2B1
ATP6V0B
UQCR
ACTR1B
IRF7
STAG3L4
MXD1
PVALB
CSF2RA

TNFSF14
FRMPD1
RNF141
PNKD
CXXC4
EIF2B2
CACNA2D2
ZADH1
SPRYD5
CHCHD7
TFRC
TMEM25
C19ORF46
ANXA6
BDNF
ARAF
ITGB1BP1
ATF3
IER3
LOC728229
HEY1
GSTM1
PTTG2
FLJ41046
TIMM22
BMP7
STX11
LOC400451
COL4A1
EDG3
LOC145853
MAP2K3
FBXL15
TMEM141
ABCG4
ABCB9
C6ORF117
PPP2R5B
B4GALT4
POPDC3
DKFZP434N035
COL8A1
C8ORF70
DLK2
THAP1
CORO2A
GRK6
LOC653857
RBM23
KLF11
TMBIM4
PDE1A
RRAGA
PRKCG

LARGE
AP3B2
RBM17
SNCB
MAOB
MRPS15
BCCIP
LOC442117
SMPD1
SHD
ABCF3
SYT7
KIAA1754
RNASE1
TRIB1
C10RF102
UHMK1
SETD4
GSTM2
CHSY3
EIF4ENIF1
FSD1
CH25H
EXTL2
NPNT
NBPFF11
EHD4
EIF2S3
PSMB7
PELI1
TUBB6
LOC653199
UPF3A
SLC6A3
MSN
MYH9
GALNT9
ZNF385D
NUDT14
ANK1
LOC85389
TMEM103
CYC1
DNASE1L3
ATP5B
CDAN1
PLA1A
RABGGTA
MRRF
ICMT
C3ORF39
BAG3
APOC1
PI4KAP2

USP27X
LOC642755
RAB11FIP5
PABPC1
ST3GAL5
ARFGAP3
GPR4
DCAKD
ADM
CLEC4G
GADD45B
HLA-B
GBP1
CBLN2
HS.562947
SYNGR1
PLAUR
RET
PITPNM1
TUSC2
ARHGDIG
SCN3B
MAMDC2
ETS1
TMEM31
IL4R
HS.572444
GLTSCR2
UPP1
ANP32B
GARNL4
MARCH3
PRRT3
HS.561493
TMEM16F
SOD2
PKIB
PCDH8
ASPH
SH2B3
SMAD3
SMAD7
HERC5
TNRC4
TGIF1
C21ORF56
FOS
C10ORF10
ANKRD34A
PNOC
PRPF8
ROPN1
CACNG2
GALT

LOC730744
ZNF229
KCNJ6
SV2A
LECT1
C6ORF159
GEM
ZFP36
HS.544050
RASGRP2
KIAA0427
HS.554410
CEND1
RPL12
SMARCC1
CCDC136
SLC4A5
PDLIM3
RAXL1
PCDH21
DOLK
SLC24A6
HS.452398
SLITRK3
C6ORF168
RGS7BP
LOC653086
RPL4
CDK5
SEMA3E
PPAPDC3
DAPP1
VSTM2L
RNF215
ACCN1
ATP1A1
MAP4K4
CAMK2B
SRGN
ZC3HAV1
HS.434307
GSTTP2
PTDSS1
ASCC2
GPX4
IL17D
HIST1H2BD
LYSMD4
LOC220686
LPHN1
ITGA5
FOXQ1
CHRM5
CLEC2L

GNAI3
DTWD2
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PGAM5
POLR1E
SLIT2
LOC730051
MAGI1
FOXA2
C12ORF24
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CYP2S1
ME3
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DRD2
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HS.562122
TRA2A
DEPD6
ZFHX2
FAHD1
TNMD
MCOLN1
OR13C9
ICA1
CBLN1
HS.566010
HSPA6
ALG3
LOC644852

ATXN7L3
WDR1
SLC9A7
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TRIM3
CALD1
C20RF40
PKN0X1
CECR7
RNF26
GDAP1L1
ATP6V0D1
IRF1
C220RF39
TESK1
LGI2
VAV3
RNF5P1
PIP5K1B

EHD1
CNIH3
HS.146766
NTHL1
MGC39606
GBA
EPB49
PTPRR
RIMS3
SYNGR3
YIPF2
LIMS1
HS.584478
CD38
CLP1
CACNG7
ZNF179
CCDC24
HS.4290
LOC285307
PSMC2
NEDD9
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CCL8
HS.561963
C6ORF136
CPLX1
HS.387982
CADPS2
IL18R1
ALKBH6
TH
VCL
FLJ20581
ALDH2
HSPA1B
CAND2
KIAA0513
PPTC7
DENR
HS.568055
SLC22A18
PAPP
VARS
SFXN4
FAM71E1
GAL3ST3
HS.388347
CACNB3
PCDH10
BXDC1
NPHP3
EPHA8
CLTB

KIF3C
ATP2B2
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BLZF1
KEL
HS.531296
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HS.545303
TNFRSF6B
ADAMTS9
SERPINB3
STMN2
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DNAJB1
LOC345630
THY1
LOC647310
TTC8
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CTPS
CDK2
RPL5
PPM1H
COX10
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CHURC1
NR2F6
ADAM8
NFATC2IP
LRFN2
LRRN3
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MX2
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NECAB3
FAM43B

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TUSC1
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AHR
CRY1
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DIRAS2
HK1
SERPINB1
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ATP6V1F
SOCS3
SERTAD1
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POU4F1
PVRL1
CTSH
TSTA3
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C10RF54
SPRY2
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HS.444405
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RNF208
LOC648689
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PMS2L2

HS.184721
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CEP170
PSMD4
SERPINH1
TMEM55B
SOX12
CLIP3
LOC654103
HARS
PTPRCAP
RLTPR
PPP1R3C
ZNF219
SNX19
LOC643011
RTN3
EHD3
DHX30
CDK5R2
PRPH
EML3
PPP1R15A
CLPTM1L
FHOD1
CKAP5
ECE2
ZNF313
HS.543482
GBF1
DUSP26
DRAM
KPTN
PDZD7
LIMK1
KTI12
CEBPB
SNRPB
CLSTN3
HS.550452
MYBPH
CNNM1
C2ORF34
TMEM130
GNA01
HSDL2
HS.578993
P4HA1
GRIN1
LOC653489
UBE20
HS3ST2
PIN1
HAPLN4

LOC647509
MAP1LC3A
C12ORF11
LOC647834
HS.22689
LOC643453
ADAMTS1
IER5
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SERINC3
SLC25A37
C10RF83
CISD1
FBXW5
ISG20
BRSK1
NEFL
CCNA1
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TCP11L2
MAP4
LOC727922
PACRG
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FBL
LOC388275
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ARHGEF11
B3GALT4
NRD1
NRIP3
EXTL1
KATNAL2
TP53I13
IMPDH1
C6ORF154
DDX1
RNASE4
GZMK
MAFF
LDLR
RAB12
CBS
ANKRD46

LOC399947
CCDC51
SLC16A3
MYH7
NDRG4
ATM
FIBP
LOC647000
CACNG3
DOLPP1
Septin 6
HSPA1L
MAGI3
CYFIP2
ADAM29
MFSD5
BCL6
CMTM6
FBX044
OCIAD1
PITRM1
SORBS1
SLC10A4
STXBP1
HSPA1A
CTGLF3
LOC643968
SLC7A4
STAM
PARD6A
AES
COL7A1
RPL8
RFPL1S
GAS6
IGSF3
POLR3GL
TRAK2
LIN7B
SSTR1
ATP2B3
DYNLRB2
YARS
LRRTM1
ATP6V1B2
CCNK
RANBP3
TWIST1
TMEM51
NLRP8
MBTPS1
RAB6B
LOC644278
NPTN

DYM
LOC728729
APOL5
CCL20
KLHL13
LOC201140
SAE1
PNN
NEFM
RPUSD1
MED10
ZCCHC14
HS.577474
EN1
LOC645688
S100A10
ACOT7
ARRDC4
TNFAIP1
HPS6
TXNDC13
ITM2C
RBM9
DRD1IP
GLTPD1
ZNF329
FJX1
BBOX1
SVOP
CBARA1
RN7SK
TP53BP1
LOC388135
EIF3F
RHBDF1
FTH1
AJAP1
NME1
SLC35C1
C18ORF17
ST5
NFIL3
CEP27
CEP70
PIGA
HS.574124
SYT17
SLC25A22
LOC644596
PLEKHG1
SRD5A1
FXR2
HLA-E
STAT1

C210RF63
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RAB11FIP1
RASL10A
KIF1A
DET1
LOC286208
C10RF216
PRKCZ
KIAA0196
MKNK2
KIAA0040
NEK2
MTA3
CPLX2
EPHA2
ZNF224
GPR137
PTGS2
ZNF678
HOOK2
SSX4
DYDC1
HS.567036
C200RF100
INPP4B
HS.563926
LOC388610
LOC387856
PSEN2
FLJ45248
CLYBL
OR4K15
AKR1C3
NFATC3
KIAA0672
ACHE
CCRK
C10RF85
HS.156525
HIST2H2BF
HS.417262
LOC643286
STXBP2
FOXRED1
HS.566642
SERGEF
DNAH11
LRIG2
NP
ROD1
ATP13A2
ADFP
TMEM18

HS.134065
BFSP2
STOML1
CALM3
CPNE4
HIST1H3H
INSIG1
HS.541932
KIAA0406
SRI
KCNMB2
C10ORF84
GFRA1
ODC1
DIRAS1
FLJ20125
LOC646256
SNRPE
FLJ20160
DSCR1L1
VARS2
GRM1
ABHD14A
NOS3
SLC2A6
BARD1
GABRB3
FAM46C
SCYL1
CASP4
LOC651621
MSI2
MGST1
CAMK2A
LOC643075
PPP1R15B
CLN5
CNN3
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## HS.562356
## CREG2
## RALYL
```

```
# 12:
```

```
# We analyzed the GSE54839 dataset, where this dataset compares 2 groups, one group being the control (  
# have a cocaine addicton. In this dataset, there are 60 samples and 48761 probes included. Each group  
# we found there to be 1673 differentially expressed probes in GSE54839, and the names of the top 3 gen
```