limma

```
limma on v3-v1 (follow-up - baseline)
data <- read.csv("v3_v1_med_vs_he.csv",row.names=1)</pre>
dim(data)
## [1] 18594
              87
group <- read.csv("v3_v1_med_vs_he_group.csv")</pre>
library(limma)
## Warning: package 'limma' was built under R version 3.5.1
Group <- factor(group$Group, levels=c("Med","HE"))</pre>
Group
  ## [35] Med Med Med Med HE
                            ΗE
                                HE
                                       ΗE
                                              ΗE
                                                 ΗE
                                                         ΗE
                                                                ΗE
                                   HE
                                          ΗE
                                                     HE
                                                            HE
## [52] HE HE HE
                 HE
                     HE
                         ΗE
                            ΗE
                                HE
                                   HE
                                       HE
                                          HE
                                              HE
                                                 HE
                                                     HE
                                                         HE
                                                            HE
                                                                ΗE
                                      ΗE
## [69] HE HE
             HE HE
                        HE
                                                 ΗE
                                                                ΗE
                    HE
                           ΗE
                                HE HE
                                         HE
                                              HE
                                                     ΗE
                                                         ΗE
                                                            ΗE
## [86] HE HE
## Levels: Med HE
design <- model.matrix(~0+Group)</pre>
design
     GroupMed GroupHE
##
## 1
                  0
           1
## 2
                  0
           1
## 3
           1
                  0
## 4
           1
                  0
## 5
           1
                  0
## 6
           1
## 7
           1
                  0
## 8
           1
                  0
## 9
                  0
           1
## 10
           1
                  0
## 11
                  0
           1
## 12
                  0
           1
## 13
           1
                  0
## 14
           1
                  0
## 15
           1
                  0
## 16
           1
                  0
## 17
           1
                  0
## 18
           1
                  0
## 19
           1
                  0
## 20
           1
                  0
## 21
                  0
## 22
           1
                  0
## 23
           1
                  0
## 24
                  0
           1
## 25
                  0
```

##	26	1	0
##	27	1	0
##	28	1	0
##	29	1	0
##	30	1	0
##	31	1	0
##	32	1	0
##	33	1	0
##	34	1	0
##	35	1	0
##	36	1	0
##	37	1	0
##	38	1	0
##	39	1	0
##	40	0	1
##	41	0	1
##	42	0	1
##	43	0	1
##	44	0	1
##	45	0	1
##	46	0	1
##	47	0	1
##	48	0	1
##	49	0	1
##	50	0	1
##	51	0	1
##	52	0	1
##	53	0	1
##	54	0	1
##	55	0	1
##	56	0	1
##	57	0	1
##	58	0	1
##	59	0	1
##	60	0	1
##	61	0	1
##	62	0	1
##	63	0	1
##	64	0	1
##	65	0	1
##	66	0	1
##	67	0	1
##	68	0	1
##	69	0	1
##	70	0	1
##	71	0	1
##	72	0	1
##	73	0	1
##	74	0	1
##	75	0	1
##	76	0	1
##	77	0	1
##	78	0	1
##	79	0	1

```
0
## 83
                      1
## 84
             0
                      1
## 85
             0
                      1
## 86
             0
                      1
## 87
              0
                      1
## attr(,"assign")
## [1] 1 1
## attr(,"contrasts")
## attr(,"contrasts")$Group
## [1] "contr.treatment"
colnames(design) <- c("Med","HE")</pre>
fit <- lmFit(data,design)</pre>
fit <- eBayes(fit)</pre>
contrast.matrix <- makeContrasts( Med.vs.HE=Med-HE, levels=design)</pre>
fit2 <- contrasts.fit(fit, contrast.matrix)</pre>
fit2.eBayes <- eBayes(fit2)</pre>
tab2 <- as.data.frame(fit2.eBayes)</pre>
tt1 <- topTable(fit2.eBayes,number=Inf)</pre>
write.csv(tt1,file="v3_v1_med_vs_he_diff_exp_all.csv")
p <- tt1[tt1$P.Value < 0.05,]</pre>
write.csv(p,file="v3_v1_med_vs_he_diff_exp_p_0.05.csv")
p1 <- tt1[tt1$P.Value < 0.01,]</pre>
write.csv(p1,file="v3_v1_med_vs_he_diff_exp_p_0.01.csv")
HEATMAP OF DIFF EXP GENES
heat <- read.csv("v3_v1_med_vs_he_heatmap_input_p_0.05.csv",row.names = 1)
library(ggplot2)
library(gplots)
##
## Attaching package: 'gplots'
## The following object is masked from 'package:stats':
##
##
       lowess
groups <- c(rep("Med",38),rep("HE",48))
coloursSamples <- factor(groups, levels=c("Med","HE"))</pre>
coloursSamples <- colorRampPalette(c("royalblue", "orange"))(length(unique(coloursSamples)))[factor(col
```

80

81

82

0

0

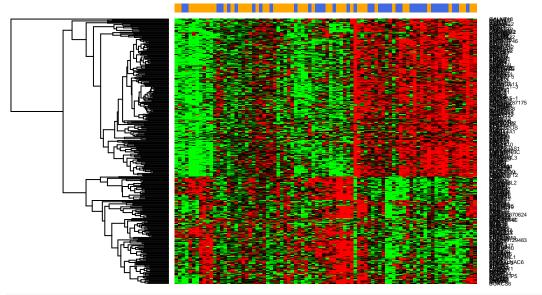
0

1

1

1

Warning in heatmap.2(as.matrix(heat), scale = "row", trace = "none", Colv
= T, : Using scale="row" or scale="column" when breaks are specified can
produce unpredictable results.Please consider using only one or the other.



#SECOND HEATMAP

heatmap.2(as.matrix(heat),scale="row",trace="none",Colv=F, ColSideColors=coloursSamples,dendrogram="row"

Warning in heatmap.2(as.matrix(heat), scale = "row", trace = "none", Colv
= F, : Using scale="row" or scale="column" when breaks are specified can
produce unpredictable results.Please consider using only one or the other.

