

This is a data report on the linear modeling analysis using affymetrix microarrays from RNA isolated from spleen tissue in CC mice at days 2, 4, 7, 12 post WNV infection. More information on linear modeling analysis in R using microarray data can be found here:

<http://www.bioconductor.org/packages/devel/bioc/vignettes/limma/inst/doc/usersguide.pdf>
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```
# display all load R and bioconductor packages along with their versions
library(limma)
```

```
## Warning: package 'limma' was built under R version 3.2.4
```

```
sessionInfo()
```

```
## R version 3.2.3 (2015-12-10)
## Platform: x86_64-w64-mingw32/x64 (64-bit)
## Running under: Windows 10 x64 (build 14393)
##
## locale:
## [1] LC_COLLATE=English_United States.1252
## [2] LC_CTYPE=English_United States.1252
## [3] LC_MONETARY=English_United States.1252
## [4] LC_NUMERIC=C
## [5] LC_TIME=English_United States.1252
##
## attached base packages:
## [1] stats      graphics  grDevices  utils      datasets  methods   base
##
## other attached packages:
## [1] limma_3.26.9
##
## loaded via a namespace (and not attached):
## [1] backports_1.0.4 magrittr_1.5      rprojroot_1.1    tools_3.2.3
## [5] htmltools_0.3.5 yaml_2.1.14      Rcpp_0.12.8      stringi_1.1.2
## [9] rmarkdown_1.3   knitr_1.15.1     stringr_1.1.0    digest_0.6.11
## [13] evaluate_0.10
```

```
library(limma)

# Load normalized expression matrix
norm_matrix <- read.csv(file="C:\\gale_lab\\oas1b_manuscript\\norm_matrix_geo.csv", row.names=1,
  header=T)

# Load target file
targets <- read.csv(file="C:\\gale_lab\\oas1b_manuscript\\target.csv", header=T)
```

```
f <- factor(targets$Treatment, levels = unique(targets$Treatment))
design <- model.matrix(~0 + f)
colnames(design) <- levels(f)

cont.matrix <- makeContrasts(x3032x16188_W_Sp_2 - x3032x16188_M_Sp_2,
x3032x16188_W_Sp_4 - x3032x16188_M_Sp_2,
x3032x16188_W_Sp_7 - x3032x16188_M_Sp_2,
x3032x16188_W_Sp_12 - x3032x16188_M_Sp_2,
x16513x16188_W_Sp_2- x16513x16188_M_Sp_12,
x16513x16188_W_Sp_4- x16513x16188_M_Sp_12,
x16513x16188_W_Sp_7- x16513x16188_M_Sp_12,
x16513x16188_W_Sp_12- x16513x16188_M_Sp_12,
x5489x16557_W_Sp_2 - x5489x16557_M_Sp_2,
x5489x16557_W_Sp_4 - x5489x16557_M_Sp_2,
x5489x16557_W_Sp_7 - x5489x16557_M_Sp_2,
x5489x16557_W_Sp_12 - x5489x16557_M_Sp_2,
x16750x13421_W_Sp_4 - x16750x13421_M_Sp_12,
x16750x13421_W_Sp_7 - x16750x13421_M_Sp_12,
x16750x13421_W_Sp_12 - x16750x13421_M_Sp_12,
x8034x8048_W_Sp_2 - x8034x8048_M_Sp_2,
x8034x8048_W_Sp_4 - x8034x8048_M_Sp_2,
x8034x8048_W_Sp_7 - x8034x8048_M_Sp_2,
x8034x8048_W_Sp_12 - x8034x8048_M_Sp_2,
x13067x5306_W_Sp_2 - x13067x5306_M_Sp_12,
x13067x5306_W_Sp_4 - x13067x5306_M_Sp_12,
x13067x5306_W_Sp_7 - x13067x5306_M_Sp_12,
x13067x5306_W_Sp_12 - x13067x5306_M_Sp_12,
x16680x8016_W_Sp_2 - x16680x8016_M_Sp_12,
x16680x8016_W_Sp_4 - x16680x8016_M_Sp_12,
x16680x8016_W_Sp_7 - x16680x8016_M_Sp_12,
x16680x8016_W_Sp_12 - x16680x8016_M_Sp_12, levels=design)
```

```
#Apply a linear model

fit <- lmFit(norm_matrix, design)

# apply our comparisons via a contrast matrix

fit2 <- contrasts.fit(fit,cont.matrix)

# Apply empirical bayes statistics for differential expression
fit2 <- eBayes(fit2)

# use a threshold of |1.5| fold change and an adjusted p-value of <=.05

results <- decideTests(fit2, lfc=(.58), method="separate", adjust.method="BH", p.value=0.05);

# write DE results to a file

write.fit(fit2, file="C:\\gale_lab\\oas1b_manuscript\\DE_oas1b.txt", digits=3,
method="separate", adjust="BH")

DE_results <- read.table(file="C:\\gale_lab\\oas1b_manuscript\\DE_oas1b.txt", header=T,
sep="\t")

#Add gene names to DE List

row.names(DE_results) <- row.names(norm_matrix)

write.csv(DE_results, file="C:\\gale_lab\\oas1b_manuscript\\DE_oas1b.csv")
```