

HW 4

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Question 1

Importing the data

```
#setting working directory
setwd("/Users/muhtasim/Desktop/myRfolder/ling")
getwd()
```

```
## [1] "/Users/muhtasim/Desktop/myRfolder/ling"
```

```
# check content of working directory
dir()
```

```
## [1] "D1T1F1.CEL"      "D2T1F2.CEL"      "D3T1M1.CEL"      "D4T1M2.CEL"
## [5] "D5N1F1.CEL"      "D6N1F2.CEL"      "D7N1M1.CEL"      "D8N1M2.CEL"
## [9] "description.txt"  "miame.txt"        "targets.txt"
```

```
#loading the necessary libraries
library(limma)
library(affy)
#reading the data
abatch=ReadAffy()
```

Writing the design and contrast matrix

```
#design matrix for the given problem
#limma writes the design matrix columnwise
design=matrix(c(1,1,0,0,0,0,0,0,0,0,1,1,0,0,0,0,0,0,0,1,1,0,0,0,0,0,0,0,1,1),nrow=8)
design
```

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

```
#defining the contrast matrix for the given problem
contrastMatrix=matrix(c(
  + 1,1,-1,-1,
  + -1,1,-1,1,
  + 1,-1, -1,1),nrow=ncol(design))
contrastNames=c("DOM-WT", "M-F", "INT")
#INT=interaction,M-F=male-female,DOM-WT=domesticated/wild-type
colnames(contrastMatrix)=contrastNames
contrastMatrix
```

```
##      DOM-WT M-F INT
## [1,]      1 -1  1
## [2,]      1  1 -1
## [3,]     -1 -1 -1
## [4,]     -1  1  1
```

Analyzing the data

```
#normalizing the data
eset=rma(abatch)
```

```
## Background correcting
## Normalizing
## Calculating Expression
```

```
#using linear model and design matrix to fit the data
fit=lmFit(eset,design=design)
#calculating the contrasts to compare among trts
fit2=contrasts.fit(fit,contrastMatrix)
names(fit2)
```

```
## [1] "coefficients"      "rank"                "assign"              "qr"
## [5] "df.residual"         "sigma"               "cov.coefficients"    "stdev.unscaled"
## [9] "Amean"              "method"              "design"               "contrasts"
```

```
#empirical Bayes' fit
fit3=eBayes(fit2)
```

Plotting the top tables using FDR

```
#getting the toptable
topTable(fit3,coef="DOM-WT",adjust="fdr")
```

```
##          logFC AveExpr      t      P.Value    adj.P.Val
## Dr.8011.1.A1_at -4.708430 5.763724 -36.35335 4.049154e-10 4.654377e-06
## Dr.23469.1.S1_s_at -8.773875 5.564455 -34.61658 5.960655e-10 4.654377e-06
## Dr.19928.1.A1_at  4.603199 7.871002  27.96074 3.210048e-09 1.518120e-05
```

```
## Dr.3374.1.S1_at      -9.550506 6.766879 -26.72896 4.575826e-09 1.518120e-05
## Dr.18311.1.S1_at      5.360563 6.214703  26.52464 4.860473e-09 1.518120e-05
## Dr.20740.1.A1_at     -4.417324 4.364227 -25.77894 6.081882e-09 1.583013e-05
## AFFX-Dr-M62653-1_at  13.938849 6.621021  24.81064 8.215978e-09 1.809273e-05
## Dr.5122.1.S1_at     -4.008096 9.155867 -24.43272 9.268225e-09 1.809273e-05
## Dr.21035.1.S1_at      5.473244 4.866060  23.12673 1.426089e-08 2.304930e-05
## Dr.4198.1.S1_at     -8.086288 5.341381 -22.37271 1.849029e-08 2.304930e-05
##                               B
## Dr.8011.1.A1_at      12.76574
## Dr.23469.1.S1_s_at   12.53642
## Dr.19928.1.A1_at     11.41729
## Dr.3374.1.S1_at      11.15758
## Dr.18311.1.S1_at     11.11257
## Dr.20740.1.A1_at     10.94339
## AFFX-Dr-M62653-1_at  10.71155
## Dr.5122.1.S1_at      10.61713
## Dr.21035.1.S1_at     10.27255
## Dr.4198.1.S1_at      10.05978
```

```
topTable(fit3,coef="M-F",adjust="fdr")
```

```
##               logFC AveExpr      t      P.Value  adj.P.Val
## Dr.18392.1.A1_at -4.2306092 5.051867 -15.387892 3.398702e-07 0.005307753
## Dr.12949.1.S1_at  3.6599124 7.534308  13.002376 1.237031e-06 0.009659355
## Dr.16969.1.S1_at  1.7383152 9.914956   9.849927 9.985843e-06 0.051982971
## Dr.11557.1.A1_at  1.5111059 7.196302   8.966060 1.993404e-05 0.070731780
## Dr.7728.1.A1_at  -1.5413704 6.526586  -8.810549 2.264576e-05 0.070731780
## Dr.6751.1.S1_at   4.2055496 8.110849   6.985244 1.182608e-04 0.269556363
## Dr.18706.1.A1_at  0.9628411 4.442965   6.865983 1.332407e-04 0.269556363
## Dr.13698.1.S1_at  1.2975871 9.073959   6.743696 1.508156e-04 0.269556363
## Dr.9200.1.A1_at   0.8230549 8.867678   6.612324 1.726071e-04 0.269556363
## Dr.12402.1.S1_at  1.8257340 4.807467   6.542659 1.855601e-04 0.269556363
##                               B
## Dr.18392.1.A1_at  3.6266042
## Dr.12949.1.S1_at  3.2310115
## Dr.16969.1.S1_at  2.3786842
## Dr.11557.1.A1_at  2.0333850
## Dr.7728.1.A1_at   1.9661360
## Dr.6751.1.S1_at   0.9956832
## Dr.18706.1.A1_at  0.9187080
## Dr.13698.1.S1_at  0.8377833
## Dr.9200.1.A1_at   0.7485356
## Dr.12402.1.S1_at  0.7002163
```

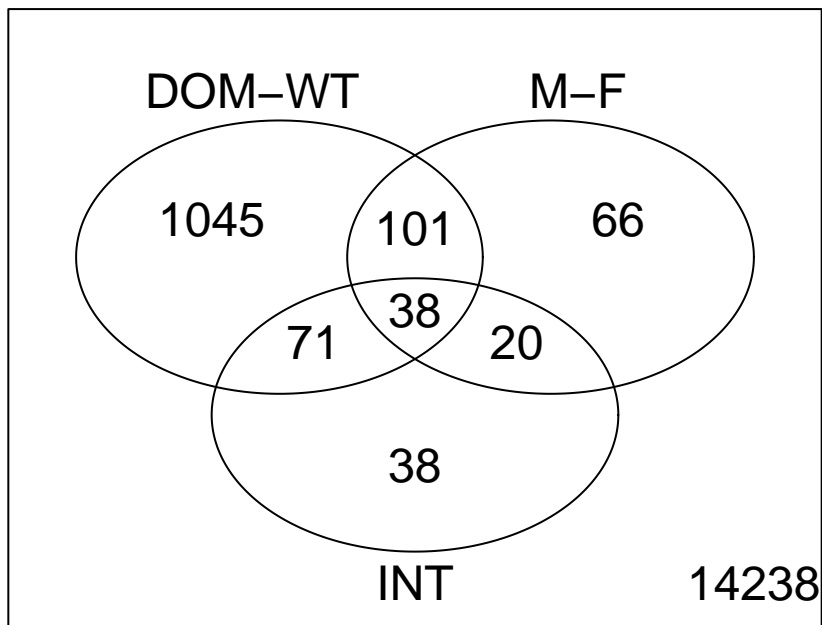
```
topTable(fit3,coef="INT",adjust="fdr")
```

```
##               logFC AveExpr      t      P.Value  adj.P.Val
## Dr.4878.1.S1_at    3.0970384 7.885147   7.569728 6.734229e-05 0.6809587
## Dr.14663.1.A1_at  -0.9053301 8.156115  -6.761652 1.480813e-04 0.6809587
## Dr.16727.1.A1_at  -0.7620132 5.320670  -6.318738 2.350454e-04 0.6809587
## Dr.15512.1.A1_at   0.7522141 5.637140   6.189195 2.702257e-04 0.6809587
## Dr.11393.1.S1_at   0.7888381 5.507129   5.921463 3.628721e-04 0.6809587
## Dr.13789.1.A1_at  -0.8413498 5.375193  -5.799373 4.163307e-04 0.6809587
```

```
## Dr.11052.2.A1_at -1.8534998 9.648527 -5.674363 4.801948e-04 0.6809587
## Dr.17145.1.S1_at -0.8129189 7.585872 -5.656442 4.902022e-04 0.6809587
## Dr.11978.1.A1_at -1.4349780 7.288957 -5.570832 5.412809e-04 0.6809587
## Dr.20279.2.S1_at 0.7830449 4.122770 5.562091 5.468171e-04 0.6809587
## B
## Dr.4878.1.S1_at -0.4284434
## Dr.14663.1.A1_at -0.6847867
## Dr.16727.1.A1_at -0.8515085
## Dr.15512.1.A1_at -0.9043021
## Dr.11393.1.S1_at -1.0197000
## Dr.13789.1.A1_at -1.0752828
## Dr.11052.2.A1_at -1.1342120
## Dr.17145.1.S1_at -1.1428313
## Dr.11978.1.A1_at -1.1846129
## Dr.20279.2.S1_at -1.1889362
```

Showing the Venn diagram to show the genes in common

```
F.stat=fit3$F
p.value=fit3$F.p.value
results=classifyTestsF(fit3,p.value=1.0E-2)
#Venn Diagram of results
vennDiagram(results,names=c("DOM-WT","M-F","INT"))
```



Question 2

| A1B1 | A2B1 | A1B2 | A2B2 | A1B3 | A2B3 | A1B4 | A2B4 |
|------|------|------|------|------|------|------|------|
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |

The Contrast matrix 1 is : (1, -1, 0, 0, 0, 0, 0, 0) The Contrast matrix 2 is : (-1/6, -1/6, -1/6, -1/6, 1/2, 1/2, -1/6, -1/6)

Question 3

| A1B1C1 | A1B1C2 | A1B2C1 | A1B2C2 | A2B1C1 | A2B1C2 | A2B2C1 | A2B2C2 |
|--------|--------|--------|--------|--------|--------|--------|--------|
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |