

# HW4 - Gene expression - Clustering and Enrichment

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## The Biology

The data for this lesson comes from:

> Saigi *et al.* "MET-Oncogenic and JAK2-Inactivating Alterations Are Independent Factors That Affect Regulation of PD-L1 Expression in Lung Cancer" *PLoS ONE*. 2018 Jun 13;9(6):e99625. PMID: 24926665.

Purpose: The blockade of immune checkpoints such as PDL1 and PD-1 is being exploited therapeutically in several types of malignancies. Here, we aimed to understand the contribution of the genetics of lung cancer to the ability of tumor cells to escape immunosurveillance checkpoints. Experimental Design: More than 150 primary non-small cell lung cancers, including pulmonary sarcomatoid carcinomas, were tested for levels of the HLA-I complex, PD-L1, tumor-infiltrating CD8 $\beta$  lymphocytes, and alterations in main lung cancer genes. Correlations were validated in cancer cell lines using appropriate treatments to activate or inhibit selected pathways. We also performed RNA sequencing to assess changes in gene expression after these treatments. Results: MET-oncogenic activation tended to associate with positive PD-L1 immunostaining, whereas STK11 mutations were correlated with negative immunostaining. In MET-altered cancer cells, MET triggered a transcriptional increase of PD-L1 that was independent of the IFN $\gamma$ -mediated JAK/STAT pathway. The activation of MET also upregulated other immunosuppressive genes (PDCD1LG2 and SOCS1) and transcripts involved in angiogenesis (VEGFA and NRP1) and in cell proliferation. We also report recurrent inactivating mutations in JAK2 that co-occur with alterations in MET and STK11, which prevented the induction of immunoresponse-related genes following treatment with IFN $\gamma$ . Conclusions: We show that MET activation promotes the expression of several negative checkpoint regulators of the immunoresponse, including PD-L1. In addition, we report inactivation of JAK2 in lung cancer cells that prevented the response to IFN $\gamma$ . These alterations are likely to facilitate tumor growth by enabling immune tolerance and may affect the response to immune checkpoint inhibitors

## Data

This data was downloaded from GEO (GSE:GSE109720)

### Import count data and metadata

```
library(readr)
library(dplyr)
library(ggplot2)

rawcounts <- read_csv("data/lung_counts.csv")
metadata <- read_csv("data/lung_metadata.csv")

rawcounts
```

```
## # A tibble: 58,347 x 34
```

```
##      ensgene AE1148 AE1149 AE1150 AE1151 AE1152 AE1153 AE1154 AE1155 AE1156
##      <chr>    <dbl> <dbl> <dbl> <dbl> <dbl> <dbl> <dbl> <dbl> <dbl>
## 1 ENSG00~    0      0      5      0      7      0      0      0      5
## 2 ENSG00~   374    529    304    366    466    519    319    379    326
## 3 ENSG00~    0      0      0      0      0      0      0      0      0
## 4 ENSG00~    0      0      0      0      0      0      0      0      0
## 5 ENSG00~    0      0      0      0      0      0      0      0      0
## 6 ENSG00~    0      0      0      0      0      0      0      0      0
## 7 ENSG00~    0      0      0      0      0      0      0      0      0
## 8 ENSG00~    0      0      0      0      0      0      0      0      0
## 9 ENSG00~    0      0      0      0      0      0      0      0      0
## 10 ENSG00~   11      1      8     24     18     21     25     17     17
## # ... with 58,337 more rows, and 24 more variables: AE1157 <dbl>,
## #   AE1158 <dbl>, AE1159 <dbl>, AE1160 <dbl>, AE1161 <dbl>, AE1162 <dbl>,
## #   AE1163 <dbl>, AE1164 <dbl>, AE1165 <dbl>, AE1166 <dbl>, AE1167 <dbl>,
## #   AE1168 <dbl>, AE1169 <dbl>, AE1170 <dbl>, AE1171 <dbl>, AE1172 <dbl>,
## #   AE1173 <dbl>, AE1174 <dbl>, AE1175 <dbl>, AE1176 <dbl>, AE1177 <dbl>,
## #   AE1178 <dbl>, AE1179 <dbl>, AE1180 <dbl>
```

metadata

```
## # A tibble: 33 x 4
##   id      celltype dex      geo_id
##   <chr>   <chr>    <chr>    <chr>
## 1 AE1160 EBC1      No treatment GSM2949380
## 2 AE1163 EBC1      No treatment GSM2949381
## 3 AE1166 EBC1      No treatment GSM2949382
## 4 AE1162 EBC1      Crizotinib  GSM2949383
## 5 AE1165 EBC1      Crizotinib  GSM2949384
## 6 AE1168 EBC1      Crizotinib  GSM2949385
## 7 AE1161 EBC1      Interferon <U+03B3> GSM2949386
## 8 AE1164 EBC1      Interferon <U+03B3> GSM2949387
## 9 AE1167 EBC1      Interferon <U+03B3> GSM2949388
## 10 AE1169 H1573    No treatment GSM2949389
## # ... with 23 more rows
```

## Clustering - k-means

```
library(tibble)
library(tidyr)
# a plotting function
plot_heatmap <- function(km) {
  centers <- km$centers %>%
    tbl_df() %>%
    rownames_to_column('Cluster') %>%
    gather(Sample, value, -Cluster) %>%
    mutate(
      Cluster = factor(Cluster),
      Sample = factor(Sample)
    )
  ggplot(centers, aes(Sample, Cluster)) + geom_tile(aes(fill=value)) + geom_text(aes(label = round(value
})
```

```
# cluster the genes
```

```
rawcounts_RN<- data.frame(rawcounts, row.names=1)
km <- kmeans(rawcounts_RN,5)
km$centers
```

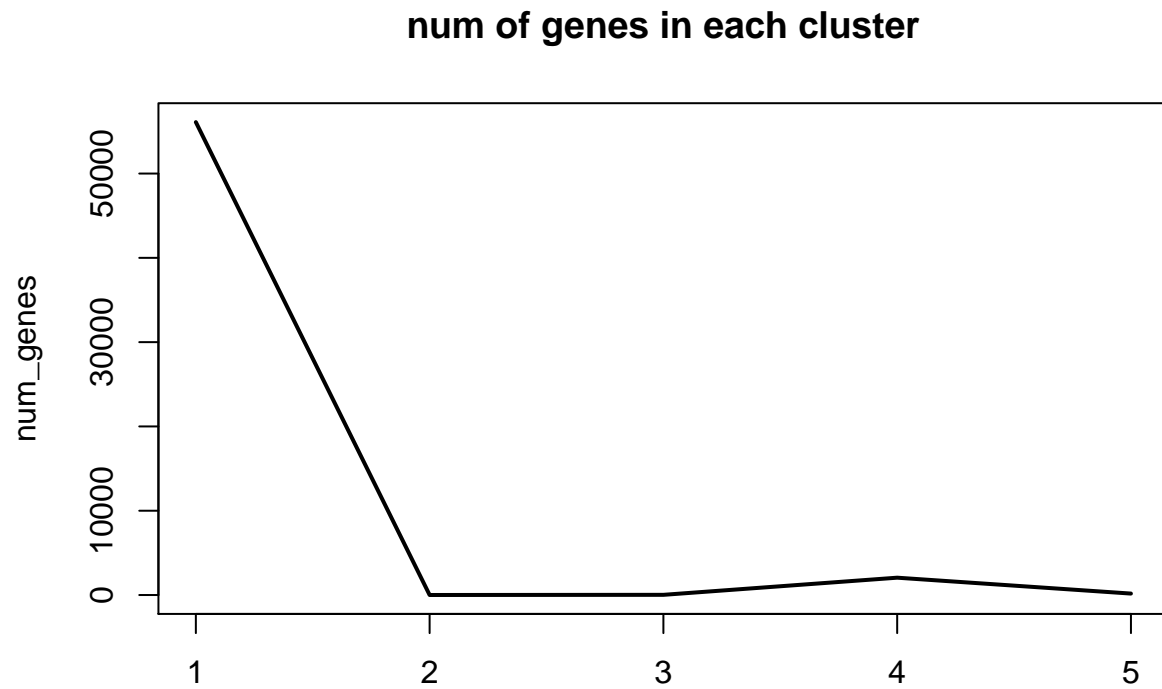
```
##      AE1148      AE1149      AE1150      AE1151      AE1152      AE1153
## 1      204.3794      260.8659      184.8346      225.175      226.5053      263.4797
## 2 261075.5000 355397.5000 249188.5000 298081.500 312029.0000 330232.5000
## 3 101059.4762 147734.7143  89713.5238 117618.429 120754.0000 151602.5238
## 4   6589.0833   8841.2332   6127.4839   7358.684   7346.2171   8890.0477
## 5   31052.4260  42212.6686  27571.1893  34696.260  32887.3373  41325.6095
##      AE1154      AE1155      AE1156      AE1157      AE1158      AE1159
## 1      177.6818      222.9686      196.5011      232.7849      189.8281      212.521
## 2 222399.5000 302143.5000 258949.5000 295158.0000 257717.0000 284918.500
## 3  90998.6190 124462.2857 104368.4286 134149.9048 106395.0952 117189.476
## 4   5868.3233   7272.5940   6356.2941   8001.1913   6352.8116   7040.299
## 5  26505.6568  33457.2663  29596.4379  37330.6331  30042.5799  32418.935
##      AE1160      AE1161      AE1162      AE1163      AE1164      AE1165
## 1      242.584      180.0566      253.0504      219.7236      191.4948      214.1561
## 2 413308.500 281465.5000 419312.0000 335008.5000 264320.5000 344465.0000
## 3 143324.762 104374.0952 132841.0000 122184.8571 107568.4286 112637.7143
## 4   7666.075   5913.3574   7290.1723   6847.2006   6203.9289   6185.2936
## 5  35868.917 27201.2367  34315.4142  31814.4260  29037.9941  29120.4734
##      AE1166      AE1167      AE1168      AE1169      AE1170      AE1171
## 1      241.4646      198.3505      238.9257      253.7209      189.6234      218.4082
## 2 384117.5000 304171.0000 421639.5000 605629.0000 430845.0000 507135.5000
## 3 135000.0476 111633.0476 126291.2857 169666.4762 124785.2381 141406.8095
## 4   7557.6928   6488.0501   6934.8676   7469.2434   5595.4722   6493.8734
## 5  34757.7219 29575.0533  32625.8402  36791.6982  27468.7456  31881.7219
##      AE1172      AE1173      AE1174      AE1175      AE1176      AE1177
## 1      185.7504      226.5967      210.5955      222.858      183.9239      211.8739
## 2 422003.5000 530275.0000 492796.5000 382136.500 308577.5000 364062.5000
## 3 117254.7619 146959.6667 137927.1429 127871.333 104154.3333 123308.0952
## 4   5550.7936   6760.0141   6311.3476   6946.255   5771.8062   6654.9367
## 5  27203.1006  33370.0118  31380.9112  29166.260  24118.6923  28170.8698
##      AE1178      AE1179      AE1180
## 1      165.6252      178.0214      225.1644
## 2 280669.0000 308508.0000 400801.0000
## 3  94938.3333 104582.6190 133835.4286
## 4   5181.0136   5586.3374   7055.9640
## 5  21687.6036  23821.4024  29880.8935
```

```
num_genes <- table(km$cluster)
```

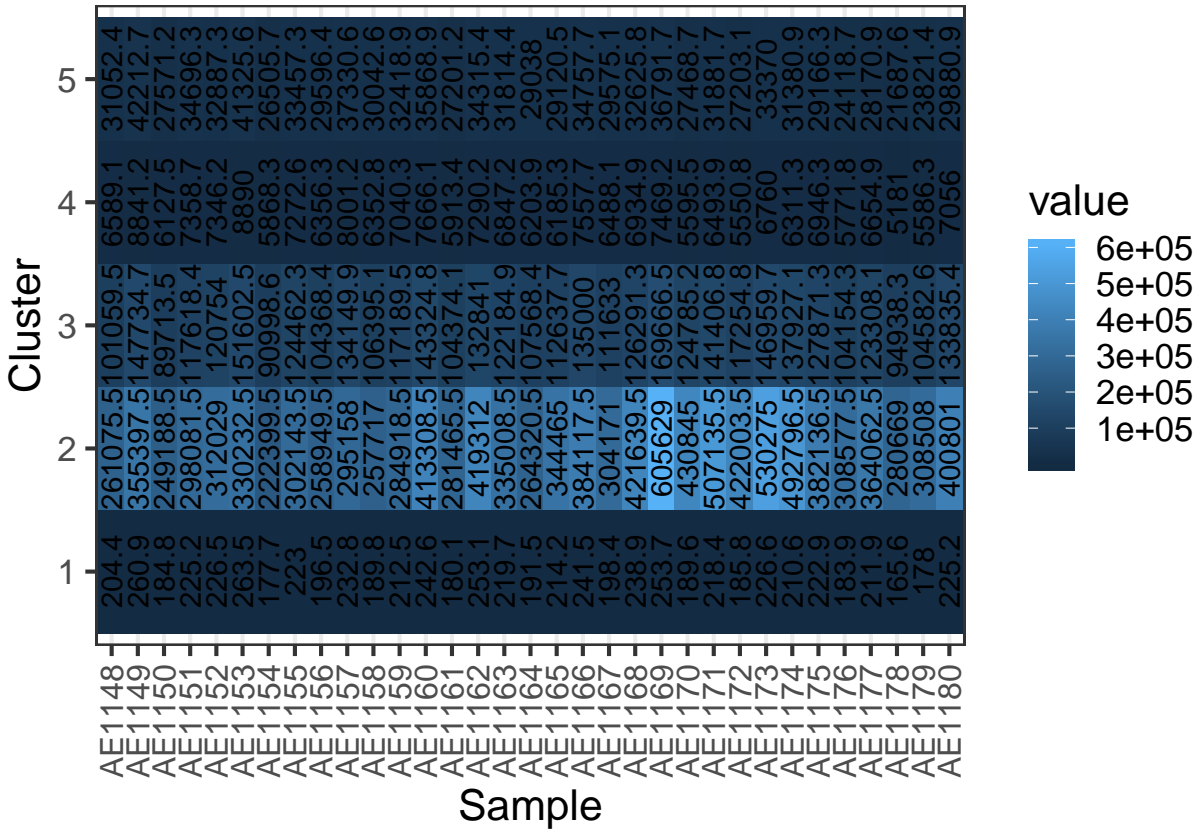
```
num_genes
```

```
##
##      1      2      3      4      5
## 56101      2     21   2054   169
```

```
plot(num_genes, type="l", main='num of genes in each cluster')
```



```
plot_heatmap(km)
```



```
# remove rows of zeroes
rawcounts_RN$sum <- rowSums(rawcounts_RN)
rawcounts_clean <- rawcounts_RN[ rawcounts_RN$sum >= 10, ]
rawcounts_clean$sum <- NULL

# apply log scaling
rawcounts_log = log10(1+rawcounts_clean)
km <- kmeans(rawcounts_log,5)
km$centers
```

```
##      AE1148      AE1149      AE1150      AE1151      AE1152      AE1153      AE1154
## 1 2.8768341 2.9629047 2.8159122 2.9162445 2.9151470 2.9548990 2.7748572
## 2 0.2224750 0.2595178 0.2365299 0.2465905 0.2664494 0.2856338 0.2270980
## 3 0.9926849 1.0658002 0.9937652 1.0406866 1.0859241 1.1021633 0.9782394
## 4 2.0053516 2.0718609 1.9683162 2.0457680 2.0753388 2.0995963 1.9408546
## 5 3.5738212 3.6867595 3.5233650 3.6183988 3.6159370 3.6748730 3.4829931
##      AE1155      AE1156      AE1157      AE1158      AE1159      AE1160      AE1161
## 1 2.9035514 2.8555548 2.9088671 2.8171388 2.8838187 2.9247148 2.7762690
## 2 0.2611225 0.2488747 0.2554619 0.2473489 0.2562278 0.2735666 0.2295744
## 3 1.0667283 1.0603057 1.0567061 1.0293423 1.0579908 1.0154241 0.8954655
## 4 2.0608478 2.0370740 2.0484974 1.9979362 2.0467039 1.8938259 1.7459993
## 5 3.6039529 3.5517147 3.6395846 3.5207057 3.5946933 3.6473609 3.5152520
##      AE1162      AE1163      AE1164      AE1165      AE1166      AE1167      AE1168
## 1 2.9481320 2.8845318 2.8080246 2.8748433 2.9221639 2.8221034 2.9211485
## 2 0.3069437 0.2660096 0.2308454 0.2634969 0.2683428 0.2351569 0.2865346
## 3 1.0746557 0.9825569 0.9117918 0.9844687 1.0037558 0.9144569 1.0278350
```

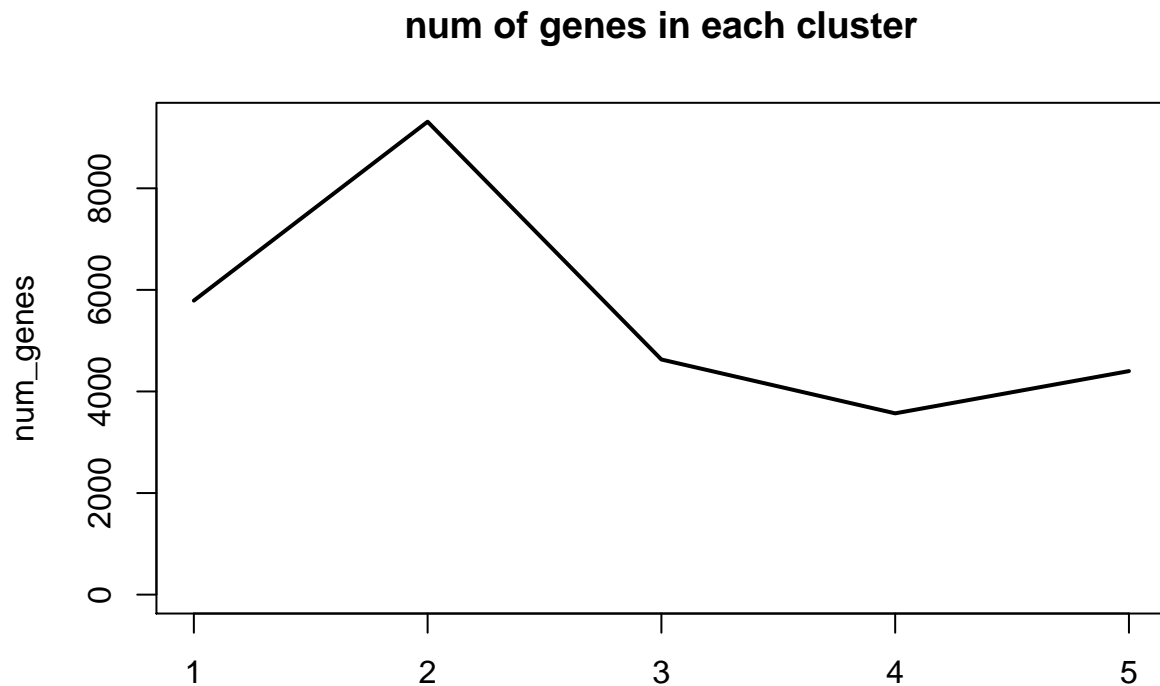
```
## 4 1.9523912 1.8613406 1.7745325 1.8706251 1.8907524 1.7858088 1.9209397
## 5 3.6335486 3.5997041 3.5385613 3.5622239 3.6428058 3.5588146 3.6114502
##      AE1169  AE1170  AE1171  AE1172  AE1173  AE1174  AE1175
## 1 2.9333166 2.806446 2.8682297 2.7963220 2.882997 2.8517416 2.8476059
## 2 0.4410393 0.373576 0.4068394 0.3665689 0.409207 0.3943318 0.2684219
## 3 1.1728400 1.052466 1.1166752 1.0400485 1.123027 1.1024374 0.9364739
## 4 2.0208283 1.892123 1.9587703 1.8819145 1.968288 1.9379473 1.8364030
## 5 3.6191436 3.491511 3.5584802 3.4891624 3.575307 3.5434462 3.5860752
##      AE1176  AE1177  AE1178  AE1179  AE1180
## 1 2.7616280 2.8245047 2.7176708 2.7502298 2.8514261
## 2 0.2289570 0.2646176 0.2237919 0.2291777 0.2697421
## 3 0.8611594 0.9207595 0.8351714 0.8435144 0.9420726
## 4 1.7454409 1.8129884 1.7102739 1.7326465 1.8332838
## 5 3.5038501 3.5662006 3.4583779 3.4908875 3.5926027
```

```
num_genes <- table(km$cluster)
```

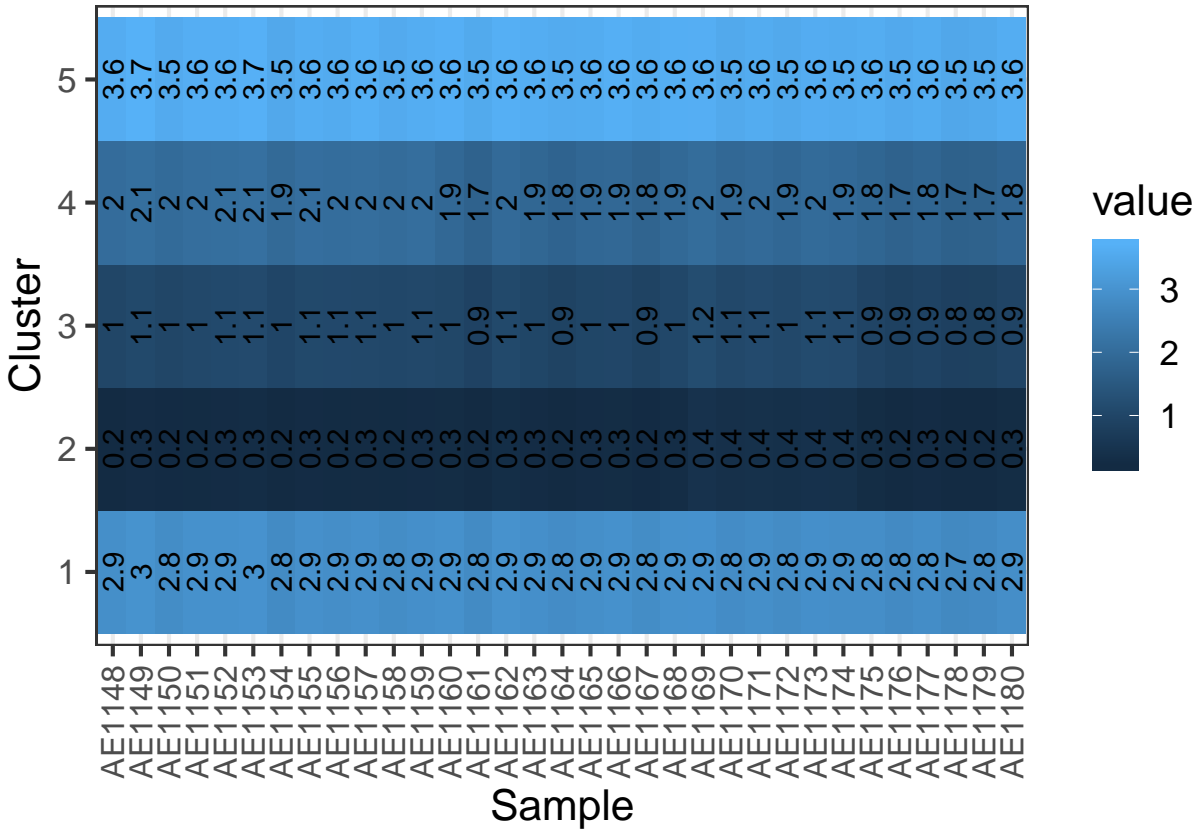
```
num_genes
```

```
##
##      1      2      3      4      5
## 5789 9311 4629 3569 4401
```

```
plot(num_genes, type="l", main='num of genes in each cluster')
```



```
plot_heatmap(km)
```



```
# Apply standardization
rawcounts_scaled <- scale(rawcounts_log)
km <- kmeans(rawcounts_scaled,5)
km$centers
```

```
##      AE1148      AE1149      AE1150      AE1151      AE1152      AE1153
## 1 -1.0693269 -1.0688768 -1.0633402 -1.0722110 -1.0782449 -1.0693484
## 2 -0.5008578 -0.4873795 -0.4943596 -0.4901676 -0.4734303 -0.4761762
## 3  0.8964867  0.8893151  0.8828349  0.8935212  0.8863283  0.8799729
## 4  0.2528079  0.2451968  0.2458377  0.2538863  0.2649167  0.2567951
## 5  1.4114811  1.4119526  1.4156985  1.4092971  1.4048429  1.4039390
##      AE1154      AE1155      AE1156      AE1157      AE1158      AE1159
## 1 -1.0586070 -1.0739779 -1.0785432 -1.0690393 -1.0662110 -1.0741021
## 2 -0.4916615 -0.4791412 -0.4702332 -0.4830918 -0.4779076 -0.4795858
## 3  0.8760129  0.8860388  0.8821716  0.8804559  0.8744648  0.8826296
## 4  0.2447623  0.2625378  0.2683054  0.2498533  0.2583316  0.2608463
## 5  1.4127032  1.4041744  1.4048258  1.4157064  1.4051733  1.4108421
##      AE1160      AE1161      AE1162      AE1163      AE1164      AE1165
## 1 -1.0365985 -1.0180156 -1.0427133 -1.0321912 -1.0220277 -1.0340571
## 2 -0.4976051 -0.5213826 -0.4809970 -0.5061895 -0.5183713 -0.5024793
## 3  0.8973837  0.8894981  0.8979515  0.8978315  0.8936499  0.9007449
## 4  0.1468150  0.1198996  0.1673943  0.1452124  0.1274776  0.1580055
## 5  1.4240540  1.4423877  1.4017358  1.4245303  1.4359796  1.4101721
```

```
##      AE1166      AE1167      AE1168      AE1169      AE1170      AE1171
## 1 -1.0346146 -1.0215992 -1.0380963 -1.0333268 -1.0232746 -1.0306172
## 2 -0.5015744 -0.5215339 -0.4957272 -0.4671921 -0.4872764 -0.4752458
## 3  0.8979212  0.8933030  0.8994738  0.8715029  0.8722969  0.8709739
## 4  0.1481989  0.1284032  0.1652151  0.1754403  0.1611897  0.1696153
## 5  1.4222051  1.4381401  1.4073590  1.3956382  1.4062621  1.4039338
##      AE1172      AE1173      AE1174      AE1175      AE1176      AE1177
## 1 -1.0237152 -1.0311316 -1.0302534 -1.0126447 -1.0052797 -1.0095451
## 2 -0.4907044 -0.4749960 -0.4750045 -0.5235211 -0.5360008 -0.5268240
## 3  0.8720849  0.8718143  0.8711739  0.8850181  0.8846325  0.8835753
## 4  0.1597162  0.1691050  0.1655199  0.1384030  0.1238105  0.1326525
## 5  1.4123471  1.4040635  1.4059962  1.4240011  1.4340972  1.4275570
##      AE1178      AE1179      AE1180
## 1 -1.0017273 -1.0019488 -1.0131401
## 2 -0.5422155 -0.5441582 -0.5208928
## 3  0.8831780  0.8858309  0.8852047
## 4  0.1194956  0.1214050  0.1338500
## 5  1.4386298  1.4360721  1.4257434
```

```
num_genes <- table(km$cluster)
```

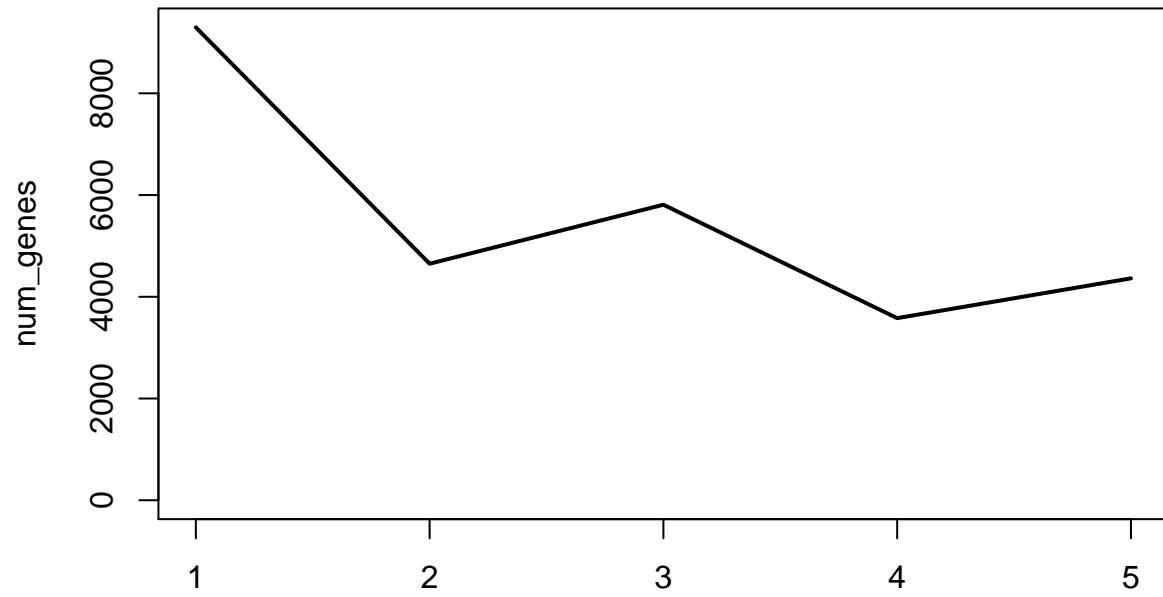
```
num_genes
```

```
##
##      1      2      3      4      5
## 9297 4650 5810 3580 4362
```

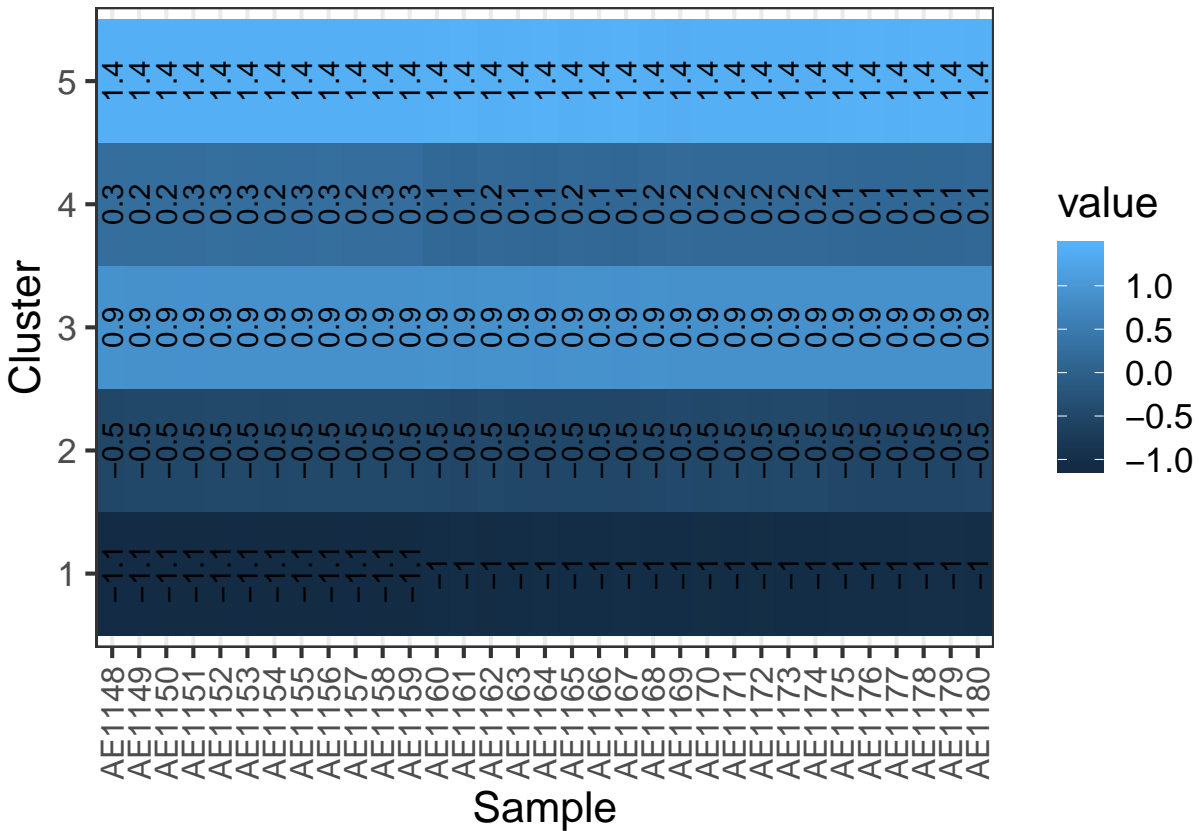
```
plot(num_genes, type="l", main='num of genes in each cluster')
```



**num of genes in each cluster**



```
plot_heatmap(km)
```



```
# load DE gene list
DE_res <- read_csv("data/sigresults.csv")
DE_genes <- DE_res$row
```

```
# example of filtering a data frame
dat_filtered = rawcounts_clean[DE_genes,]
rawcounts_log = log10(1+dat_filtered)
rawcounts_scaled <- scale(rawcounts_log)
km <- kmeans(rawcounts_scaled,5)
km$centers
```

```
##      AE1148      AE1149      AE1150      AE1151      AE1152      AE1153
## 1  0.42403366  0.40593559  0.41257406  0.41343195  0.378408740  0.32836900
## 2 -1.57036467 -1.53889857 -1.54205665 -1.55319030 -1.450632219 -1.39290277
## 3 -0.09426217 -0.05868337 -0.05744064 -0.07496552  0.005271765  0.08664749
## 4  1.32824453  1.34815737  1.35139359  1.34733238  1.382576790  1.40244856
## 5 -0.56425902 -0.58404432 -0.59448221 -0.57965220 -0.644722541 -0.65125022
##      AE1154      AE1155      AE1156      AE1157      AE1158      AE1159
## 1  0.33578948  0.34837334  0.35360157  0.36186704  0.34171469  0.36054754
## 2 -1.36066628 -1.39234203 -1.38216899 -1.45594594 -1.36793485 -1.41289991
## 3  0.06416777  0.04142446  0.03334408  0.03713265  0.01772979  0.04332771
## 4  1.38982555  1.39478217  1.39122149  1.38779412  1.38078743  1.38229875
## 5 -0.66385891 -0.65805263 -0.66612505 -0.63334140 -0.64423798 -0.65553963
##      AE1160      AE1161      AE1162      AE1163      AE1164      AE1165
## 1  0.4773720  0.4799396  0.47498824  0.4770977  0.4788714  0.4817270
## 2 -1.2482030 -1.2939910 -1.23358972 -1.2646579 -1.2663800 -1.2364346
```

```
## 3 -1.6214115 -1.5660180 -1.63189301 -1.6018460 -1.5875136 -1.6368159
## 4  1.1223981  1.1281802  1.09330082  1.1218241  1.1347879  1.0963944
## 5 -0.1107556 -0.1123038 -0.09187267 -0.1076159 -0.1235682 -0.1002032
##      AE1166      AE1167      AE1168      AE1169      AE1170      AE1171
## 1  0.4877514  0.4893930  0.4883743  0.4742062  0.4760763  0.4704565
## 2 -1.2599205 -1.2718339 -1.2509781 -1.3490956 -1.3586094 -1.3423383
## 3 -1.6219404 -1.6018767 -1.6234543 -1.3027711 -1.2760303 -1.2699420
## 4  1.1093457  1.1251012  1.0964178  1.1211957  1.1165703  1.1442426
## 5 -0.1095790 -0.1233956 -0.1062694 -0.1663521 -0.1703549 -0.1933458
##      AE1172      AE1173      AE1174      AE1175      AE1176      AE1177
## 1  0.4729096  0.4680305  0.4681295  0.4510429  0.4564274  0.4520655
## 2 -1.3416282 -1.3337278 -1.3261636 -1.5324000 -1.5477186 -1.5325070
## 3 -1.2875782 -1.2733156 -1.2828794 -0.9697346 -0.9441060 -0.9532551
## 4  1.1438410  1.1372523  1.1470611  1.1190033  1.1226214  1.1195577
## 5 -0.1903454 -0.1888319 -0.1966247 -0.1488695 -0.1597912 -0.1569221
##      AE1178      AE1179      AE1180
## 1  0.4545237  0.4518388  0.4539705
## 2 -1.5486074 -1.5390727 -1.5253211
## 3 -0.9526021 -0.9620322 -0.9742974
## 4  1.1219108  1.1278541  1.1172293
## 5 -0.1527941 -0.1551061 -0.1543482
```

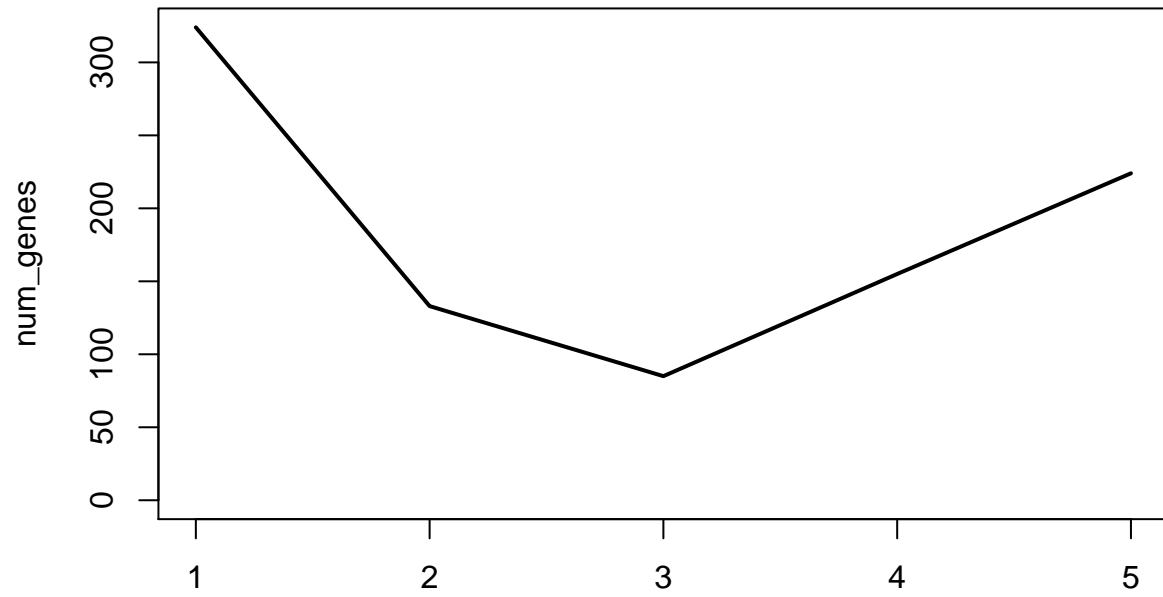
```
num_genes <- table(km$cluster)
```

```
num_genes
```

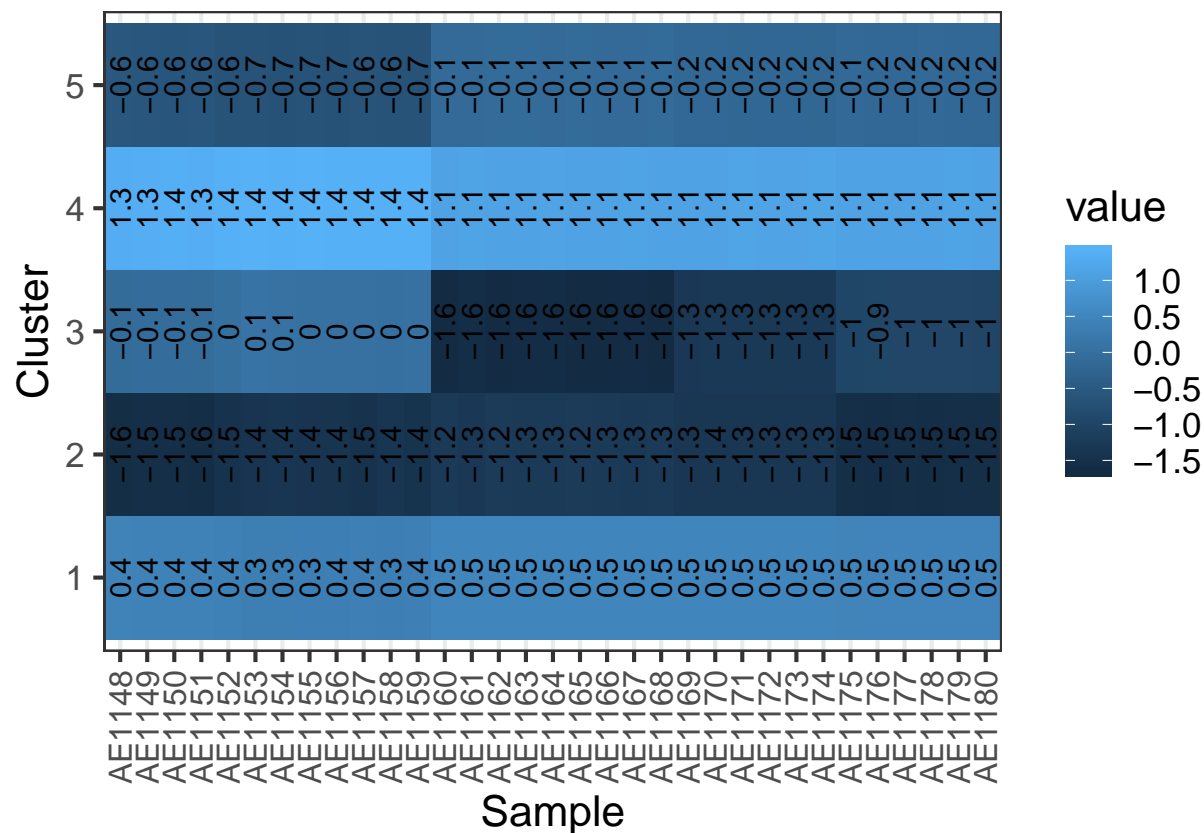
```
##
##  1  2  3  4  5
## 324 133 85 155 224
```

```
plot(num_genes, type="l", main='num of genes in each cluster')
```

**num of genes in each cluster**



```
plot_heatmap(km)
```



```
# try again with less clusters
km <- kmeans(rawcounts_scaled,3)
km$centers
```

```
##      AE1148      AE1149      AE1150      AE1151      AE1152      AE1153
## 1  0.9421614  0.9443945  0.9513452  0.9479200  0.9544944  0.9367624
## 2 -1.0897526 -1.0578491 -1.0588992 -1.0754732 -0.9956799 -0.9289602
## 3 -0.2359628 -0.2569866 -0.2628749 -0.2498311 -0.3033400 -0.3263202
##      AE1154      AE1155      AE1156      AE1157      AE1158      AE1159
## 1  0.9349843  0.9437989  0.9441724  0.9456099  0.9302650  0.9452465
## 2 -0.9224395 -0.9499582 -0.9432554 -0.9797709 -0.9325391 -0.9582931
## 3 -0.3285240 -0.3204519 -0.3247792 -0.3044573 -0.3181097 -0.3168620
##      AE1160      AE1161      AE1162      AE1163      AE1164      AE1165
## 1  0.82571167  0.8338699  0.80900981  0.82507007  0.83521581  0.81472352
## 2 -1.36041448 -1.3633053 -1.35171193 -1.36449421 -1.35355507 -1.35932245
## 3  0.03374135  0.0278140  0.04422371  0.03676334  0.02076732  0.04338717
##      AE1166      AE1167      AE1168      AE1169      AE1170      AE1171
## 1  0.82544492  0.83656652  0.82002231  0.84714767  0.84572955  0.85731922
## 2 -1.37172915 -1.36239212 -1.36760089 -1.31968529 -1.31536911 -1.30887236
## 3  0.04070543  0.02474592  0.04333567 -0.01050916 -0.01174189 -0.02645446
##      AE1172      AE1173      AE1174      AE1175      AE1176      AE1177
## 1  0.85917638  0.85284727  0.85763275  0.83012936  0.83458808  0.83105863
## 2 -1.31136582 -1.30026943 -1.29824918 -1.35161032 -1.35404301 -1.34482053
## 3 -0.02671463 -0.02737011 -0.03305205  0.02437834  0.02164494  0.01947869
##      AE1178      AE1179      AE1180
## 1  0.83425955  0.83615931  0.8312262
```

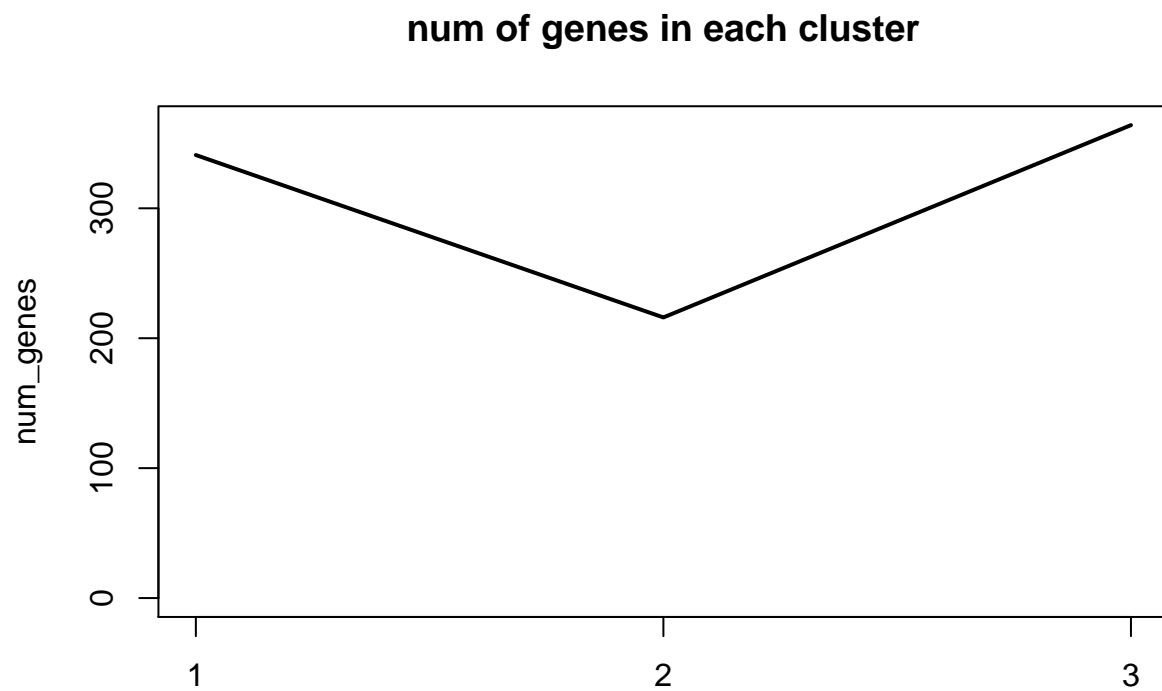
```
## 2 -1.35443662 -1.35491449 -1.3511697  
## 3  0.02218627  0.02069012  0.0230893
```

```
num_genes <- table(km$cluster)
```

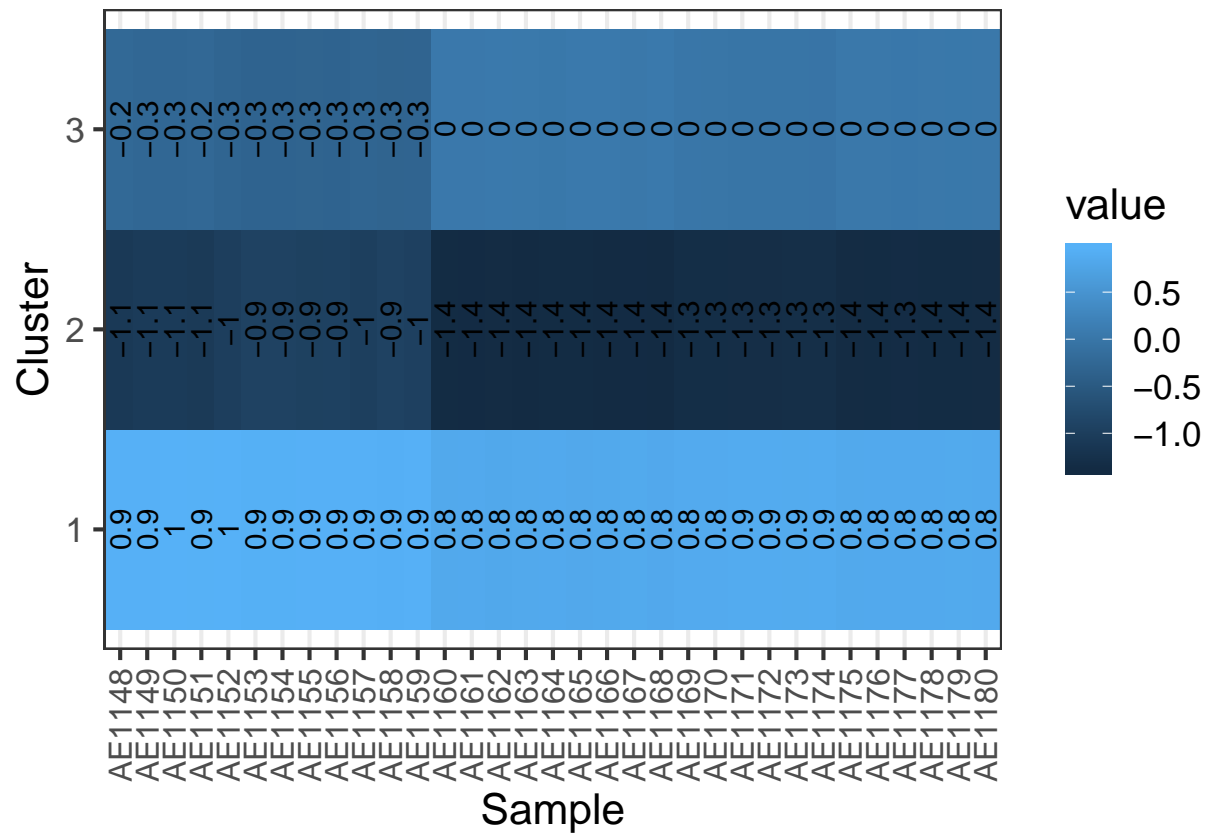
```
num_genes
```

```
##  
##  1  2  3  
## 341 216 364
```

```
plot(num_genes, type="l", main='num of genes in each cluster')
```



```
plot_heatmap(km)
```

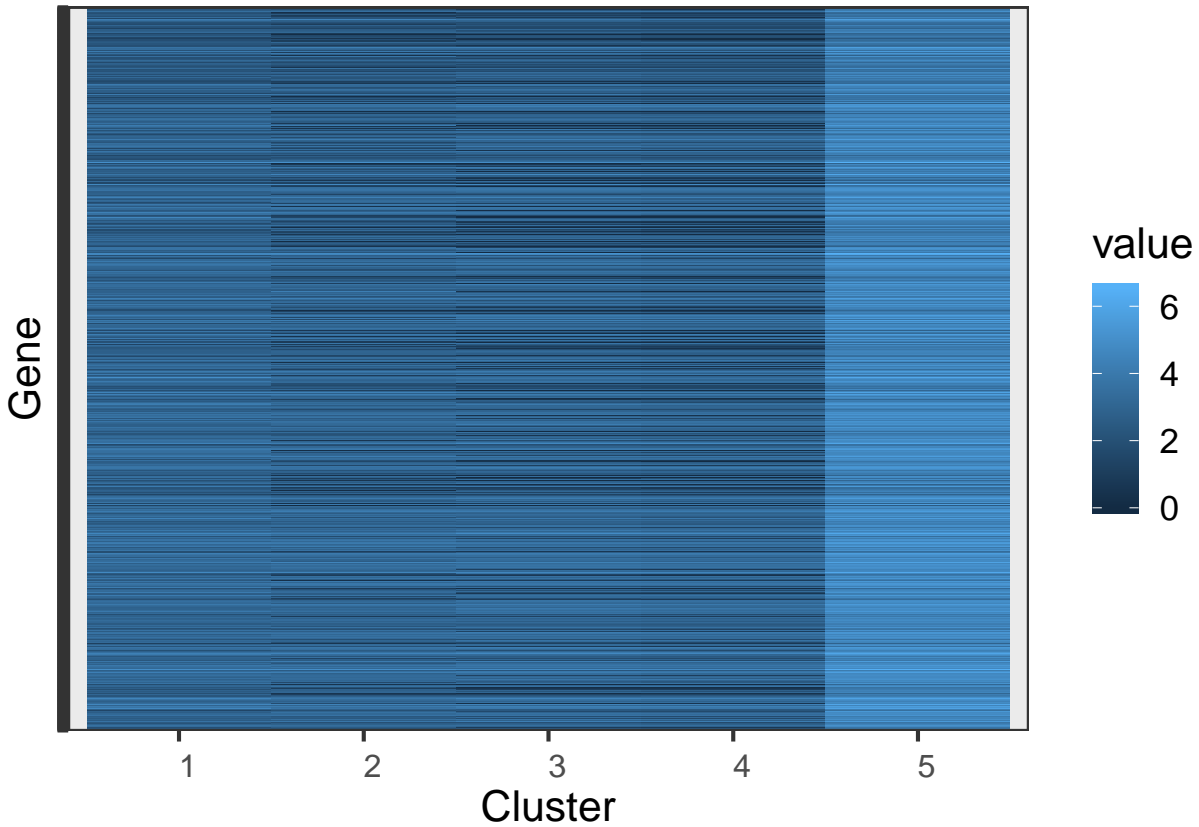


```
# a plotting function
plot_heatmap <- function(km) {
  centers <- km$centers %>%
    tbl_df() %>%
    rownames_to_column('Cluster') %>%
    gather(Gene, value, -Cluster) %>%
    mutate(
      Cluster = factor(Cluster),
      Gene = factor(Gene)
    )
  ggplot(centers, aes(Cluster, Gene)) + geom_tile(aes(fill=value)) + theme(axis.text.x=element_text(angle=90))
}

# cluster the samples
rawcounts_clean <- rawcounts_RN[ rawcounts_RN$sum >= 10, ]
rawcounts_log = log10(1+rawcounts_clean)
DE_res <- read_csv("data/sigresults.csv")
DE_genes <- DE_res$row
dat_filtered = rawcounts_log[DE_genes,]
km <- kmeans(t(dat_filtered), 5)
#km$centers
table(km$cluster)

##
## 1 2 3 4 5
## 12 12 6 3 1
```

```
plot_heatmap(km)
```



## Record `sessionInfo()`

The `sessionInfo()` prints version information about R and any attached packages. It's a good practice to always run this command at the end of your R session and record it for the sake of reproducibility in the future.

```
sessionInfo()
```

```
## R version 3.5.1 (2018-07-02)
## Platform: x86_64-w64-mingw32/x64 (64-bit)
## Running under: Windows 10 x64 (build 17134)
##
## Matrix products: default
##
## locale:
## [1] LC_COLLATE=English_Israel.1252  LC_CTYPE=English_Israel.1252
## [3] LC_MONETARY=English_Israel.1252 LC_NUMERIC=C
## [5] LC_TIME=English_Israel.1252
##
## attached base packages:
## [1] stats      graphics  grDevices  utils      datasets  methods   base
```



```
##
## other attached packages:
## [1] bindrcpp_0.2.2 tidyr_0.8.2    tibble_1.4.2    dplyr_0.7.8
## [5] readr_1.2.1    ggplot2_3.1.0  knitr_1.20
##
## loaded via a namespace (and not attached):
## [1] Rcpp_1.0.0      pillar_1.3.0    compiler_3.5.1  plyr_1.8.4
## [5] bindr_0.1.1     tools_3.5.1     digest_0.6.18   evaluate_0.12
## [9] gtable_0.2.0    pkgconfig_2.0.2 rlang_0.3.0.1   cli_1.0.1
## [13] yaml_2.2.0      withr_2.1.2     stringr_1.3.1   hms_0.4.2
## [17] grid_3.5.1      tidyselect_0.2.5 glue_1.3.0      R6_2.3.0
## [21] fansi_0.4.0     rmarkdown_1.11  purrr_0.2.5     magrittr_1.5
## [25] scales_1.0.0    codetools_0.2-15 htmltools_0.3.6 assertthat_0.2.0
## [29] colorspace_1.3-2 labeling_0.3     utf8_1.1.4      stringi_1.2.4
## [33] lazyeval_0.2.1  munsell_0.5.0   crayon_1.3.4
```