Tutorial Assignment 4

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(a) Import Libraries

```
#!/usr/bin/env Python3
# coding=utf-8
from math import log
import numpy as np
import pandas as pd
import seaborn as sns
import matplotlib
import matplotlib.pyplot as plt
from scipy import stats
```

(b) Dataset Input

- Read the data from local file as excel: smokers.xls
- Rmoker is the dataset we apply analysis on, which for convenience only contain the ID_REF as primary key and drop the columns: Species Scientific Name and Gene Label
- Smoker_cp is the copy containing all the info of original file, in the case user want to find corresponding gene label

```
smoker_cp = pd.read_excel("./smokers.xls", sep="\t", index_col=0)
smoker = pd.read_excel("./smokers.xls", sep="\t", index_col=0)

smoker = smoker.drop("Gene Label", axis = 1)
smoker = smoker.drop("Species Scientific Name", axis =1)

print(smoker.head())
print("Data contains %d normalized probsets, %d samples." % smoker.shape)
```

```
            GSM101095    GSM101096    GSM101097    GSM101098    GSM101099  \
ID_REF
1007_s_at  3884.318400  1657.214200  2237.643600  1474.739300  2231.866000
1053_at      82.294170    74.921800    76.623764    54.349518    72.081345
117_at       37.470535    77.169880    27.224297    29.231043    30.802940
121_at      254.769700   173.070400   177.904650   135.697620   228.584460
1255_g_at     9.972142     9.346519    11.320443     8.536531    10.041258
```

```
             GSM101100    GSM101101    GSM101102    GSM101103    GSM101104  \
ID_REF
1007_s_at  2535.999500  1956.101300  2298.428000  2410.525400  2495.134300
1053_at      78.715040   101.283134   110.999010   114.482040    80.897100
117_at       26.526035    24.233334    24.874979    26.564205    32.034077
121_at      151.093810   168.721050   170.702000   149.034820   209.503080
1255_g_at     9.035363     9.285970    10.272593    10.256649     9.776113

            ...    GSM101107    GSM101108    GSM101109    GSM101110  \
ID_REF      ...
1007_s_at   ...  1862.330400  1844.315700  2097.944000  2466.514200
1053_at     ...    65.823784    58.283974    80.165850    89.279990
117_at      ...    39.444940    51.292150    32.644653    43.022903
121_at      ...   229.414550   245.255550   156.447740   168.562560
1255_g_at   ...     9.948459     9.965394    10.015328    11.016840

             GSM101111    GSM101112    GSM101113    GSM101114    GSM101115  \
ID_REF
1007_s_at  1992.740700  2232.849400  3326.898000  2238.369400  2615.229700
1053_at      83.198265    60.594906    76.577590    76.259480    68.664340
117_at       33.190834    26.173641    28.923296    26.477106    23.935286
121_at      135.459460   177.648970   190.692440   188.691470   171.054470
1255_g_at     9.567991    10.641581     9.388623     8.894799     9.506586

             GSM101116
ID_REF
1007_s_at  2130.132600
1053_at      60.522540
117_at       27.868433
121_at      129.480180
1255_g_at     9.235668

[5 rows x 22 columns]
Data contains 54613 normalized probsets, 22 samples.
```

(c) Data Reorganization

- Observe the data, it is easy to notice that the number magnitude is diversed: from thousand to unit, which is not good for further analysis and visualization.
- Apply Log 2 transformation to reorder the number magnitude.
- **Print the smoker dataset head again, notice the data has already been transformed.**

```
dataset =
["GSM101095","GSM101096","GSM101097","GSM101098","GSM101099","GSM101100","GSM1
01101",

"GSM101102","GSM101103","GSM101104","GSM101105","GSM101106","GSM101107","GSM10
1108",

"GSM101109","GSM101110","GSM101111","GSM101112","GSM101113","GSM101114","GSM10
1115",
           "GSM101116"]
for target_col in dataset:
    smoker[target_col] = np.log2(smoker[target_col])
print(smoker.head())
```

```
           GSM101095  GSM101096  GSM101097  GSM101098  GSM101099  GSM101100  \
ID_REF
1007_s_at  11.923446  10.694544  11.127765  10.526244  11.124035  11.308339
1053_at     6.362718   6.227314   6.259720   5.764195   6.171554   6.298567
117_at      5.227685   6.269966   4.766823   4.869429   4.944996   4.729337
121_at      7.993050   7.435215   7.474960   7.084252   7.836584   7.239301
1255_g_at   3.317903   3.224429   3.500859   3.093650   3.327868   3.175583

           GSM101101  GSM101102  GSM101103  GSM101104  ...  GSM101107  \
ID_REF                                                 ...
1007_s_at  10.933765  11.166432  11.235132  11.284902  ...  10.862893
1053_at     6.662250   6.794403   6.838977   6.338016  ...   6.040537
117_at      4.598921   4.636623   4.731412   5.001536  ...   5.301768
121_at      7.398496   7.415336   7.219506   7.710828  ...   7.841813
1255_g_at   3.215053   3.360728   3.358488   3.289261  ...   3.314473

           GSM101108  GSM101109  GSM101110  GSM101111  GSM101112  GSM101113  \
ID_REF
1007_s_at  10.848870  11.034760  11.268258  10.960538  11.124670  11.699962
1053_at     5.865027   6.324916   6.480265   6.378482   5.921125   6.258850
117_at      5.680666   5.028775   5.427033   5.052713   4.710043   4.854160
121_at      7.938142   7.289537   7.397140   7.081717   7.472886   7.575104
1255_g_at   3.316927   3.324138   3.461639   3.258216   3.411641   3.230914

           GSM101114  GSM101115  GSM101116
ID_REF
1007_s_at  11.128232  11.352722  11.056728
1053_at     6.252845   6.101489   5.919401
117_at      4.726674   4.581067   4.800560
121_at      7.559885   7.418312   7.016587
1255_g_at   3.152962   3.248927   3.207216

[5 rows x 22 columns]
```

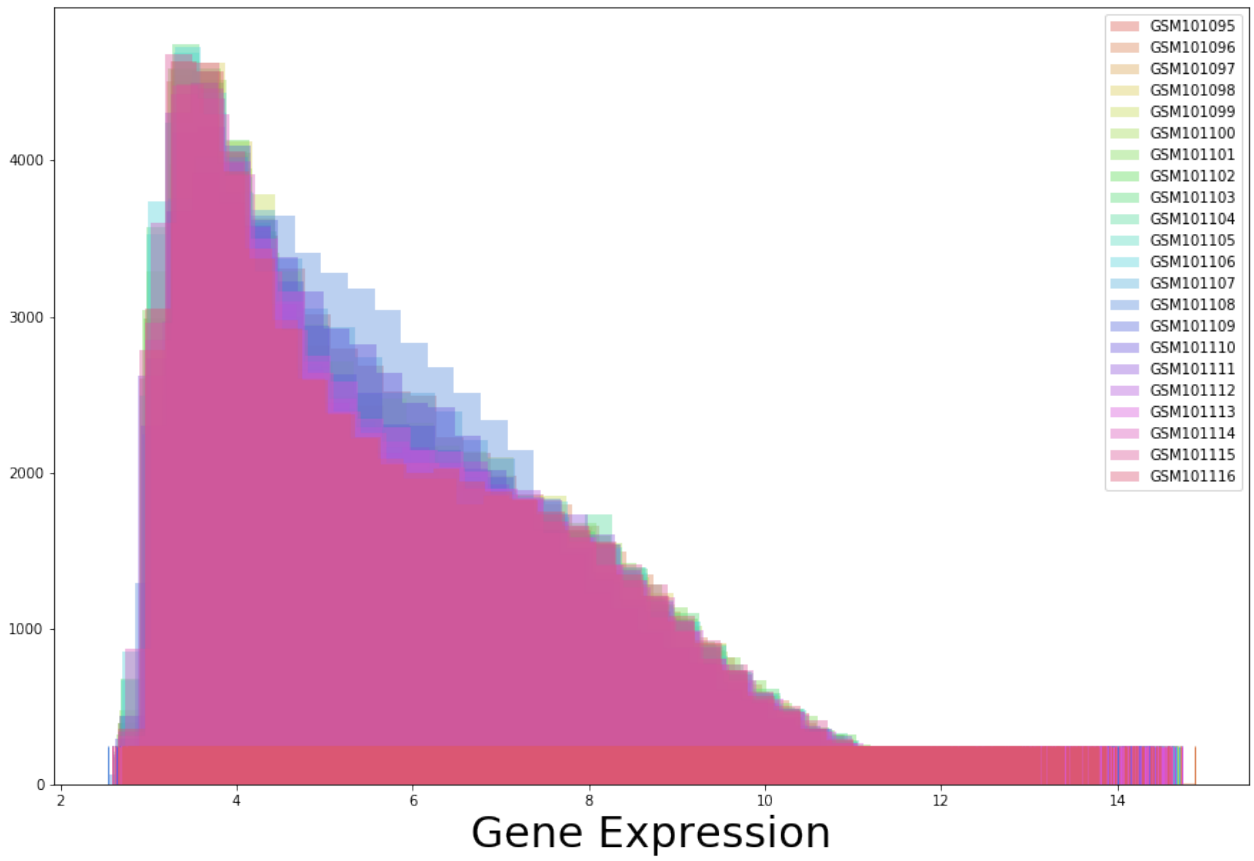
(d) Question 1

Plot the gene expression value distribution among 22 samples

- One Histogram Graph
- Separated Histogram Graph
- One Histogram Graph with Kernel Density Estimation
- Separated Histogram Graph with Kernel Density Estimation
- Box Plot

Plot a histogram of the subject group of 22 samples

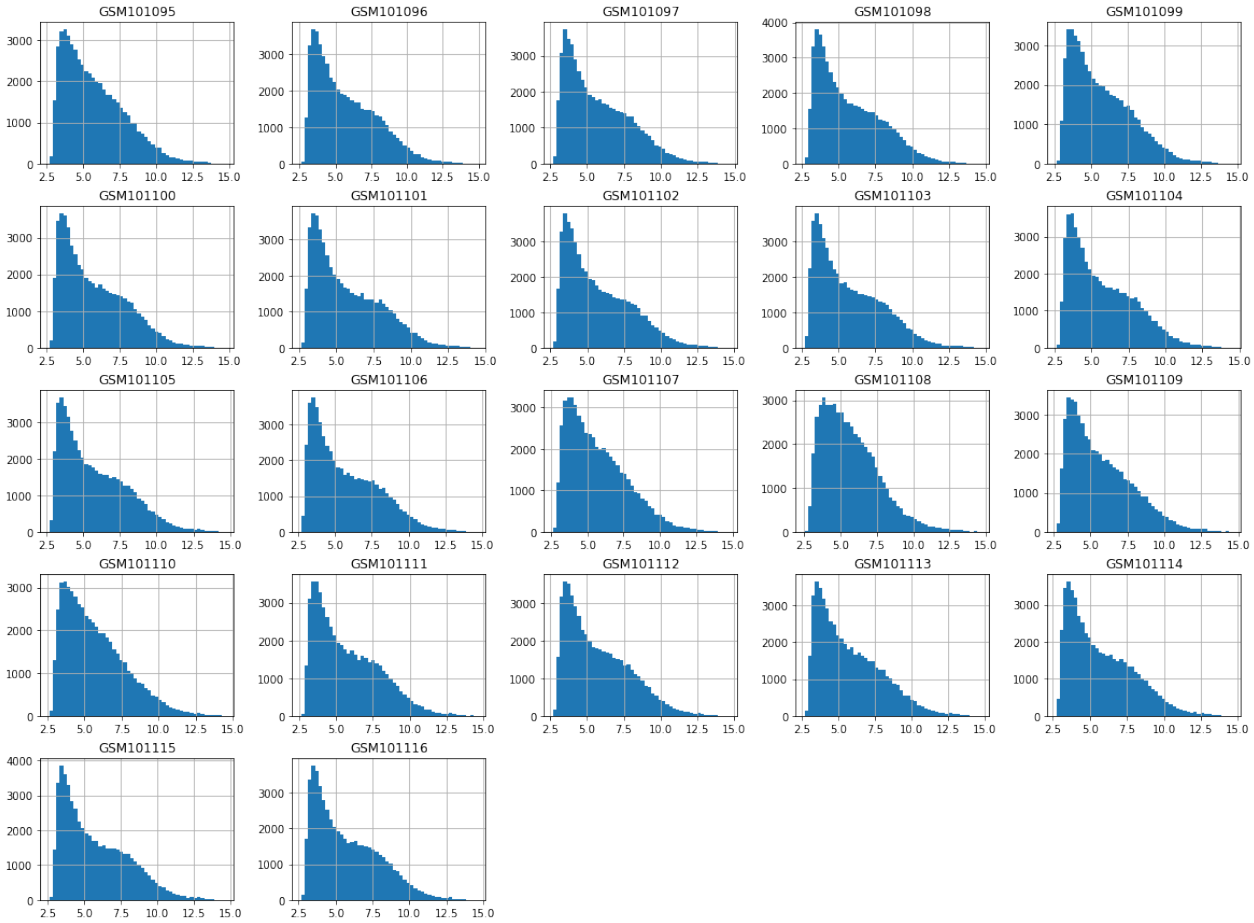
```
plt.figure(figsize=[15,10])
with sns.color_palette('hls',22):
    for target in dataset:
        ax = sns.distplot(smoker[target], bins=40, label=target, rug=True,
kde=False)
plt.xlabel("Gene Expression", size = 30)
lgd = plt.legend()
```



Plot a histogram for each sample respectively

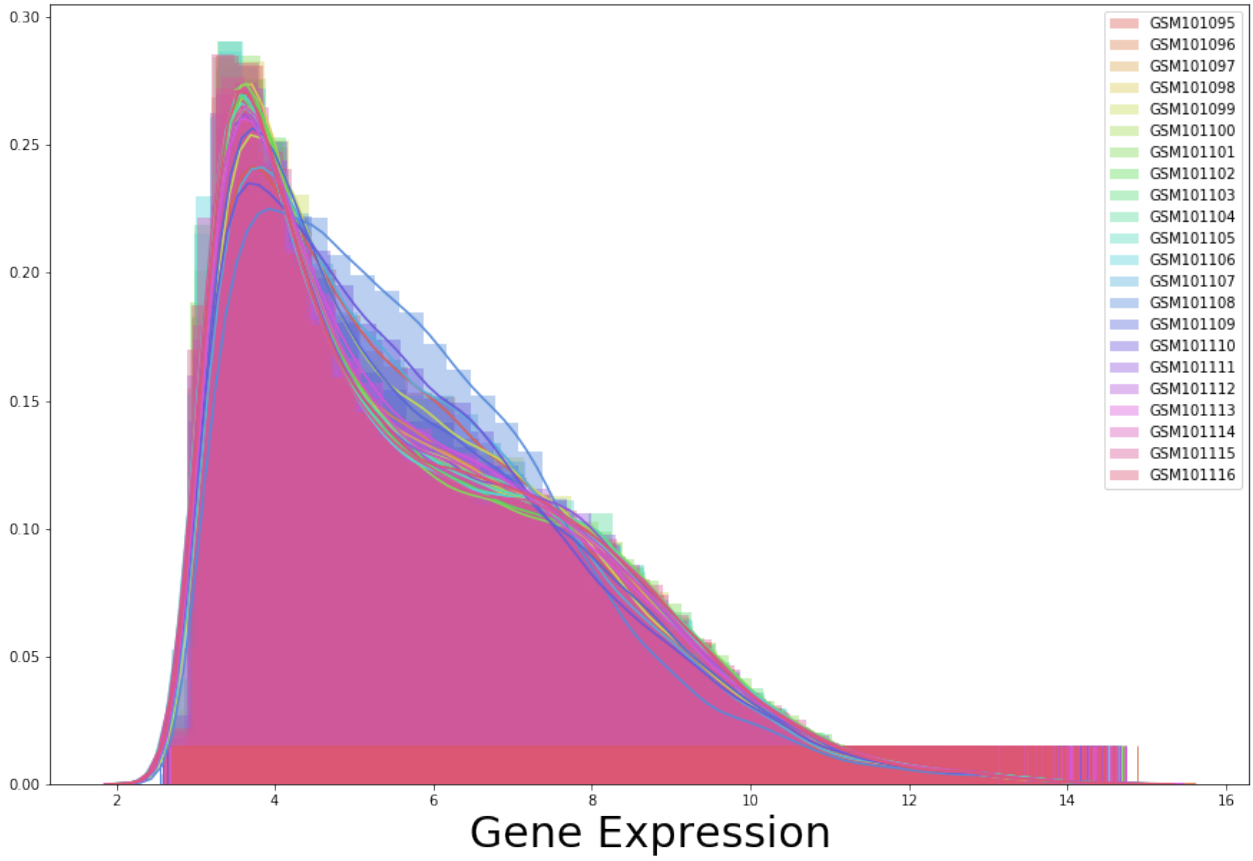
Notice the y axis range in these figures are almost the same, therefore it is still easy for us to compare the data.

```
smoker.hist(bins=50, figsize=(20,15))
plt.show()
```



Plot a histogram of the subject group of 22 samples with kde

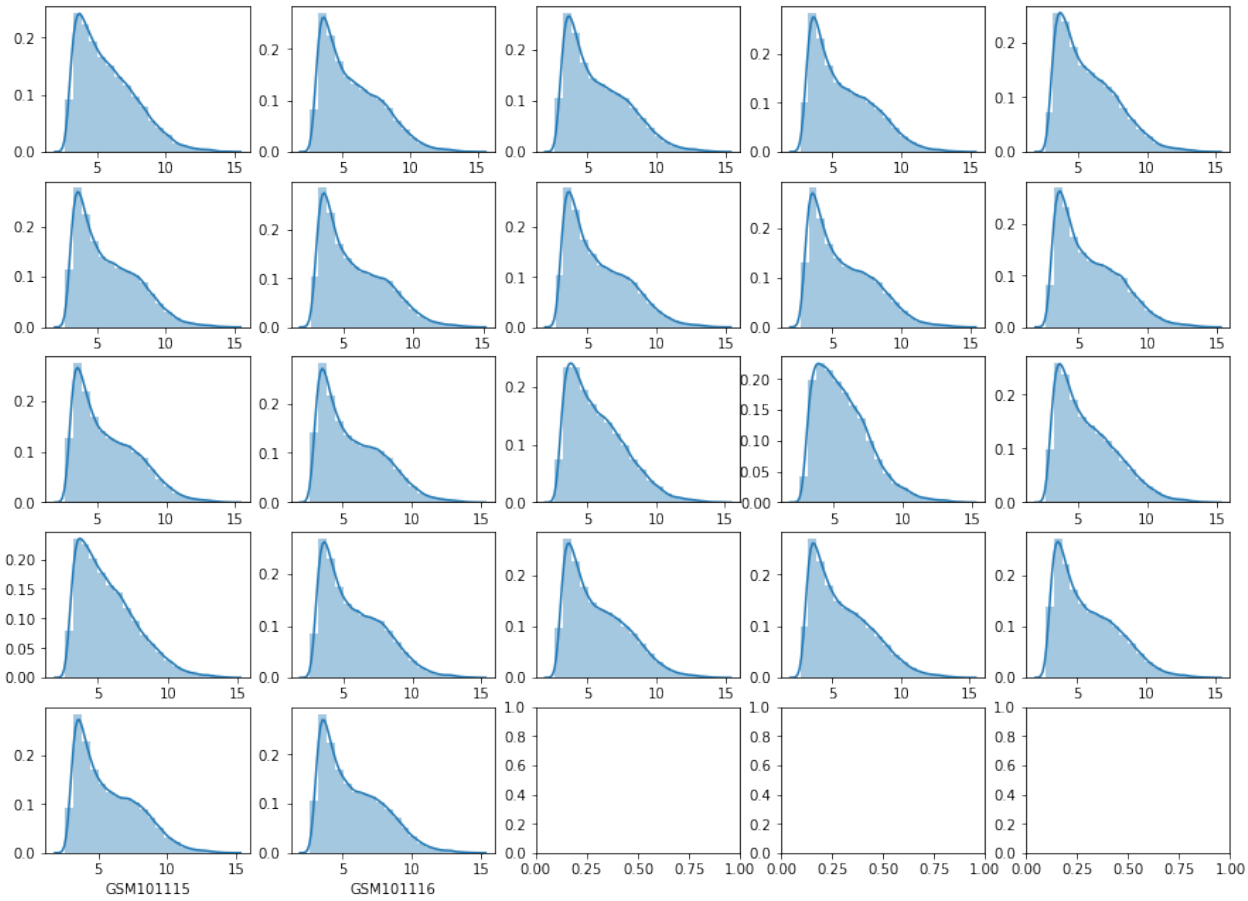
```
plt.figure(figsize=[15,10])
with sns.color_palette('hls',22):
    for target in dataset:
        ax = sns.distplot(smoker[target], bins=40, label=target, rug=True,
kde=True)
plt.xlabel("Gene Expression", size = 30)
lgd = plt.legend()
```



Plot a histogram with kde for each sample respectively

Notice the y axis range in these figures are almost the same, therefore it is still easy for us to compare the data

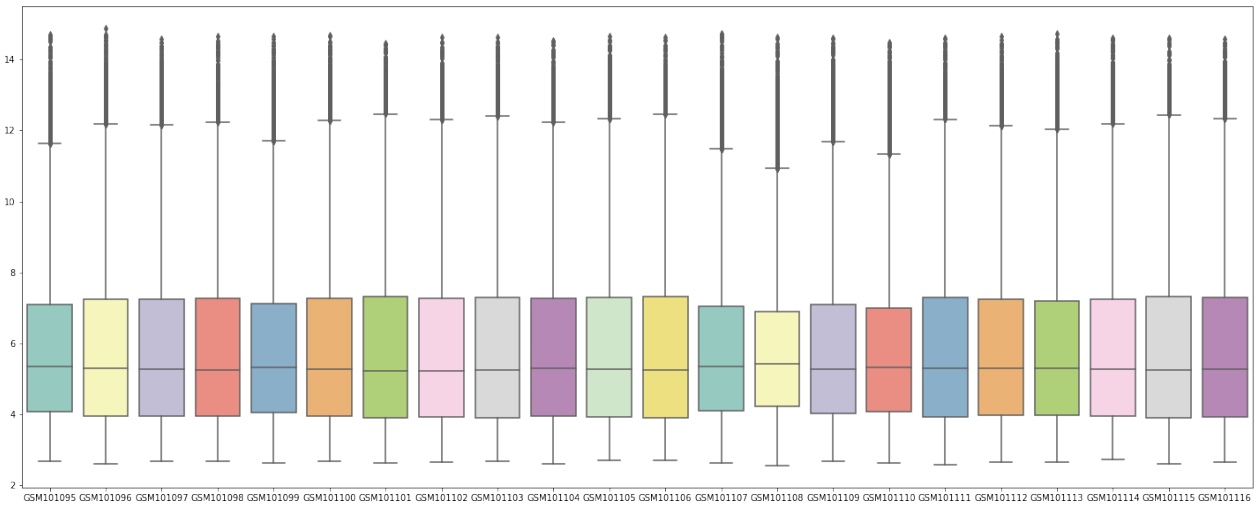
```
fig, axes = plt.subplots(5,5,figsize = (15,11))
count = 0
with sns.color_palette('hls',22):
    for target in dataset:
        ax.set_ylim(0,0.3)
        sns.distplot(smoker[target], bins=20, label=target, rug=False,
kde=True, ax = axes[count//5][count%5])
        count = count+1
```



Considering the histogram for 22 samples does hard to see the details, choose box plot to vitualize the data

```
plt.figure(figsize=[25,10])
sns.boxplot(data=smoker,palette="Set3")
```

```
<matplotlib.axes._subplots.AxesSubplot at 0x1703845f8>
```



Question 1 Quiz: Why normalization is required for microarray data?

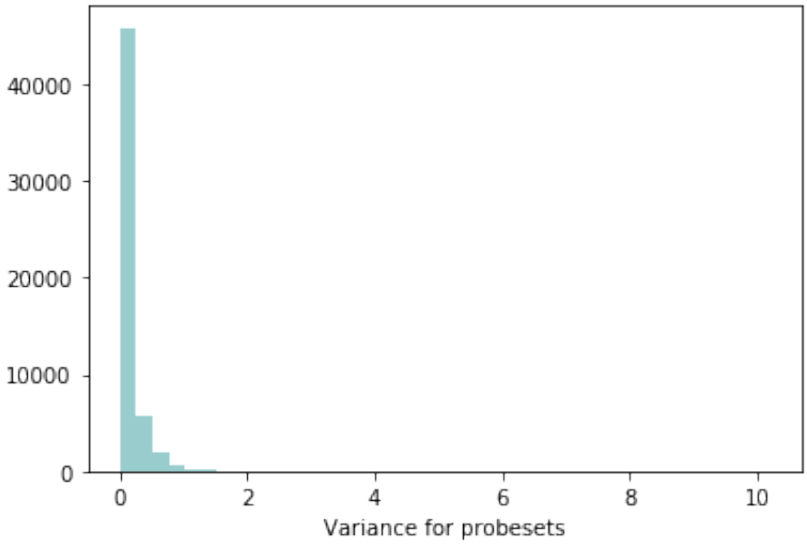
Anwser

1. Normalization is a process of eliminating the variations caused by differential labelling efficiency of teh two fluorescent dyes or different amounts of starting mRNA material in two samples. This process is suitable for this dataset: firstly, the dataset is consisted by different samples from different people sets, existing large possibility to have variation; secondly, as the reason we apply log2 normalization, it is obvious that the data varies large quantile in magnitude amount: some are thousands, some are units, which is abnormal, even after log transformation.
2. For such a large, random dataset, the result of the distribution should be closed to **Normal Distribution**.
   However, as we can observed from all the above figures that firstly, the central axis of the distribution **deviates from the central** from the range 0 to 16, most of the samples (global maximum value) accumulate at 3 to 5; secondly, the figure is **asystemitric**. This phenomenon is actually abnormal for a large dataset, which indicates there exists noise points or something else need to be refined by normalization.
3. The differences between two sets of samples(smokers and non-smokers) are hard to tell directly from the original figure, since the base number of the dataset is quite large, therefore even if the differences between samples seems small in some places, it could exist larger differential expression than we thought. In this perspective, normalization is also needed.

(e) Filter Genes

Before we perform unsupervised learning to explore the gene expression data, a common procedure is to filter out genes with low variance as they may simply represent noise. We can rank genes based on the variances across the dataset and select the 80% most variant probsets for use in the incoming analysis.

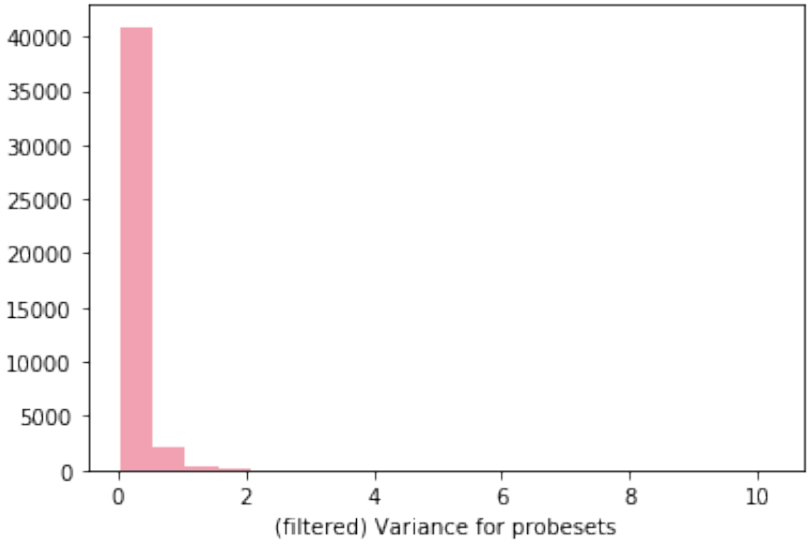
```
var_smoker = smoker.var(axis=1)
sns.distplot(var_smoker, bins=40, kde=False, color="#008080")
lab = plt.xlabel("Variance for probesets")
#plt.xlim([0, 25])
```




```
smoker_filt = smoker[pd.qcut(var_smoker, q=5, labels=False)  > 0]
print("Futher filtered dataset: %d filtered probsets, %d samples." %
smoker_filt.shape)
```

```
Futher filtered dataset: 43690 filtered probsets, 22 samples.
```

```
varsmoker_filt = smoker_filt.var(axis=1)
sns.distplot(varsmoker_filt, bins=20, kde=False, color="#dc143c")
lab = plt.xlabel("(filtered) Variance for probesets")
#plt.xlim(0, 25)
```



(f) Question 2 + 3: Wilcoxon Signed-Rank test, Paired Sample T-Test and Visualization

- Wilcoxon Signed-Rank Test
- Wilcoxon Signed-Rank Test Visualization
- Paired Sample T-Test
- Paired Sample T-Test Visualization

Divide two sets of samples according to labele.txt.
Notice that in order to make the balance to apply paired analysis, delete the final two samples in non_smoker_list.
The current result is 10 samples for each list.

```
non_smoker_list =
["GSM101095","GSM101096","GSM101097","GSM101098","GSM101099",

"GSM101100","GSM101101","GSM101102","GSM101103","GSM101104"]
smoker_list = ["GSM101107", "GSM101108", "GSM101109", "GSM101110",
"GSM101111",
               "GSM101112", "GSM101113", "GSM101114", "GSM101115",
"GSM101116"]
```

(a) Wilcoxon sign-rank test

- Setting P-Value is actually essential and here, we set it to be 0.006 instead of 0.05.
- Actually p-value = 0.05 is suitable for the analysis according to the original article: ***Pauwels RA, Buist AS, Calverley PM, Jenkins CR, Hurd SS (2001) Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease. NHLBI/WHO Global Initiative for Chronic Obstructive Lung Disease (GOLD) workshop summary. Am J Respir Crit Care Med 163:1256–1276***.
- However, we choose this p-value to generate a more strict result for the further dendrogram and clustering, since if the left data is too large, the dendrogram is hard to visualize without using *fastcluster* library.
- The p-value = 0.006 finally gives us **404 differential expressions**, which is acceptable.

```
significant_inds_wilcox, significant_pvals_wilcox = [], []
for ii in smoker_filt.index:
    pval = stats.wilcoxon(smoker_filt[non_smoker_list].loc[ii],
smoker_filt[smoker_list].loc[ii], alternative="two-sided").pvalue
    if pval < 0.006:
        significant_inds_wilcox.append(ii)
        significant_pvals_wilcox.append(pval)
        print("%s is significant probeset, with pvalue=%4f" % (ii, pval))
print(len(significant_inds_wilcox))
```

```
1552307_a_at is significant probeset, with pvalue=0.005062
1552497_a_at is significant probeset, with pvalue=0.005062
```

```
/Library/Frameworks/Python.framework/Versions/3.7/lib/python3.7/site-packages/scipy/stats/morestats.py:2863: UserWarning: Sample size too small for normal approximation.
  warnings.warn("Sample size too small for normal approximation.")
```

```
1552834_at is significant probeset, with pvalue=0.005062
1553172_at is significant probeset, with pvalue=0.005062
1553602_at is significant probeset, with pvalue=0.005062
1553709_a_at is significant probeset, with pvalue=0.005062
1553994_at is significant probeset, with pvalue=0.005062
1553995_a_at is significant probeset, with pvalue=0.005062
1555824_a_at is significant probeset, with pvalue=0.005062
1556467_at is significant probeset, with pvalue=0.005062
1556616_a_at is significant probeset, with pvalue=0.005062
1557038_s_at is significant probeset, with pvalue=0.005062
1557117_at is significant probeset, with pvalue=0.005062
1557158_s_at is significant probeset, with pvalue=0.005062
1557632_at is significant probeset, with pvalue=0.005062
1557719_at is significant probeset, with pvalue=0.005062
1557797_a_at is significant probeset, with pvalue=0.005062
1557965_at is significant probeset, with pvalue=0.005062
1558019_at is significant probeset, with pvalue=0.005062
1558703_at is significant probeset, with pvalue=0.005062
1558738_at is significant probeset, with pvalue=0.005062
1558868_a_at is significant probeset, with pvalue=0.005062
1559280_a_at is significant probeset, with pvalue=0.005062
1559814_at is significant probeset, with pvalue=0.005062
1560599_a_at is significant probeset, with pvalue=0.005062
1561002_at is significant probeset, with pvalue=0.005062
1562914_a_at is significant probeset, with pvalue=0.005062
1563189_at is significant probeset, with pvalue=0.005062
1563478_at is significant probeset, with pvalue=0.005062
1566123_at is significant probeset, with pvalue=0.005062
1566163_at is significant probeset, with pvalue=0.005062
1568699_at is significant probeset, with pvalue=0.005062
1568780_at is significant probeset, with pvalue=0.005062
1569917_at is significant probeset, with pvalue=0.005062
1570299_at is significant probeset, with pvalue=0.005062
200644_at is significant probeset, with pvalue=0.005062
200810_s_at is significant probeset, with pvalue=0.005062
200878_at is significant probeset, with pvalue=0.005062
201250_s_at is significant probeset, with pvalue=0.005062
201266_at is significant probeset, with pvalue=0.005062
201377_at is significant probeset, with pvalue=0.005062
201387_s_at is significant probeset, with pvalue=0.005062
201463_s_at is significant probeset, with pvalue=0.005062
201467_s_at is significant probeset, with pvalue=0.005062
201468_s_at is significant probeset, with pvalue=0.005062
```

```
201572_x_at is significant probeset, with pvalue=0.005062
201669_s_at is significant probeset, with pvalue=0.005062
201681_s_at is significant probeset, with pvalue=0.005062
201718_s_at is significant probeset, with pvalue=0.005062
201802_at is significant probeset, with pvalue=0.005062
201884_at is significant probeset, with pvalue=0.005062
201939_at is significant probeset, with pvalue=0.005062
201976_s_at is significant probeset, with pvalue=0.005062
202193_at is significant probeset, with pvalue=0.005062
202360_at is significant probeset, with pvalue=0.005062
202425_x_at is significant probeset, with pvalue=0.005062
202435_s_at is significant probeset, with pvalue=0.005062
202436_s_at is significant probeset, with pvalue=0.005062
202437_s_at is significant probeset, with pvalue=0.005062
202472_at is significant probeset, with pvalue=0.005062
202831_at is significant probeset, with pvalue=0.005062
202925_s_at is significant probeset, with pvalue=0.005062
203037_s_at is significant probeset, with pvalue=0.005062
203060_s_at is significant probeset, with pvalue=0.005062
203233_at is significant probeset, with pvalue=0.005062
203249_at is significant probeset, with pvalue=0.005062
203600_s_at is significant probeset, with pvalue=0.005062
203687_at is significant probeset, with pvalue=0.005062
203691_at is significant probeset, with pvalue=0.005062
203703_s_at is significant probeset, with pvalue=0.005062
203707_at is significant probeset, with pvalue=0.005062
203757_s_at is significant probeset, with pvalue=0.005062
203787_at is significant probeset, with pvalue=0.005062
203894_at is significant probeset, with pvalue=0.005062
203939_at is significant probeset, with pvalue=0.005062
204041_at is significant probeset, with pvalue=0.005062
204058_at is significant probeset, with pvalue=0.005062
204059_s_at is significant probeset, with pvalue=0.005062
204066_s_at is significant probeset, with pvalue=0.005062
204083_s_at is significant probeset, with pvalue=0.005062
204098_at is significant probeset, with pvalue=0.005062
204151_x_at is significant probeset, with pvalue=0.005062
204179_at is significant probeset, with pvalue=0.005062
204287_at is significant probeset, with pvalue=0.005062
204372_s_at is significant probeset, with pvalue=0.005062
204379_s_at is significant probeset, with pvalue=0.005062
204434_at is significant probeset, with pvalue=0.005062
204497_at is significant probeset, with pvalue=0.005062
204532_x_at is significant probeset, with pvalue=0.005062
204546_at is significant probeset, with pvalue=0.005062
204547_at is significant probeset, with pvalue=0.005062
204653_at is significant probeset, with pvalue=0.005062
204967_at is significant probeset, with pvalue=0.005062
205076_s_at is significant probeset, with pvalue=0.005062
```

```
205324_s_at is significant probeset, with pvalue=0.005062
205328_at is significant probeset, with pvalue=0.005062
205383_s_at is significant probeset, with pvalue=0.005062
205429_s_at is significant probeset, with pvalue=0.005062
205499_at is significant probeset, with pvalue=0.005062
205535_s_at is significant probeset, with pvalue=0.005062
205609_at is significant probeset, with pvalue=0.005062
205621_at is significant probeset, with pvalue=0.005062
205623_at is significant probeset, with pvalue=0.005062
205632_s_at is significant probeset, with pvalue=0.005062
205821_at is significant probeset, with pvalue=0.005062
206094_x_at is significant probeset, with pvalue=0.005062
206153_at is significant probeset, with pvalue=0.005062
206460_at is significant probeset, with pvalue=0.005062
206561_s_at is significant probeset, with pvalue=0.005062
206818_s_at is significant probeset, with pvalue=0.005062
206932_at is significant probeset, with pvalue=0.005062
207096_at is significant probeset, with pvalue=0.005062
207126_x_at is significant probeset, with pvalue=0.005062
207180_s_at is significant probeset, with pvalue=0.005062
207367_at is significant probeset, with pvalue=0.005062
207414_s_at is significant probeset, with pvalue=0.005062
207469_s_at is significant probeset, with pvalue=0.005062
207541_s_at is significant probeset, with pvalue=0.005062
207574_s_at is significant probeset, with pvalue=0.005062
207830_s_at is significant probeset, with pvalue=0.005062
208091_s_at is significant probeset, with pvalue=0.005062
208596_s_at is significant probeset, with pvalue=0.005062
208680_at is significant probeset, with pvalue=0.005062
208700_s_at is significant probeset, with pvalue=0.005062
209043_at is significant probeset, with pvalue=0.005062
209160_at is significant probeset, with pvalue=0.005062
209205_s_at is significant probeset, with pvalue=0.005062
209213_at is significant probeset, with pvalue=0.005062
209270_at is significant probeset, with pvalue=0.005062
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91617_at is significant probeset, with pvalue=0.005062
404
```
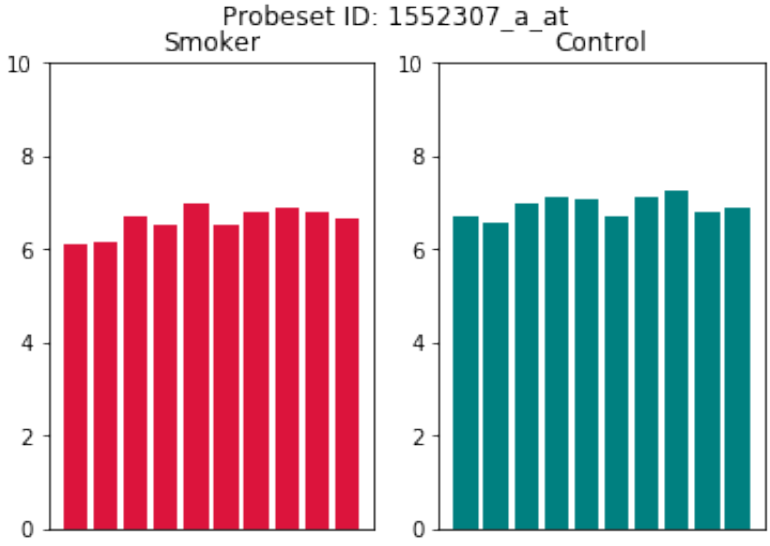
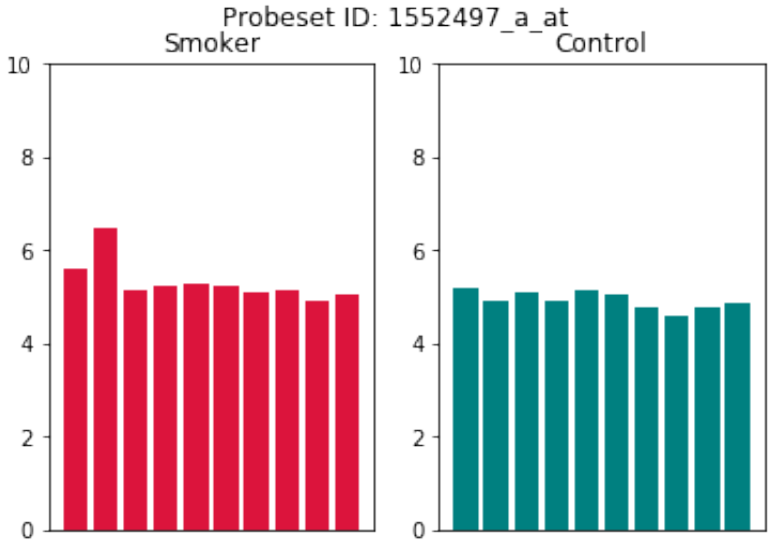

(b) Wilcoxon sign-rank test Vistualization

select Gene: '1552307_a_at','1552497_a_at','1552834_at'

- Bar Plot
- Scatter Plot

```
select_w_list= ['1552307_a_at','1552497_a_at','1552834_at']
smoker_wilcox = smoker_filt[non_smoker_list + smoker_list].loc[select_w_list]
for ii in smoker_wilcox.index:
    fig = plt.figure()
    ax1 = fig.add_subplot(121)
    ax1.bar(smoker_list, smoker_wilcox[smoker_list].loc[ii], color="#dc143c")
    plt.xticks(''); plt.title("Smoker"); plt.ylim(0, 10)
    ax2 = fig.add_subplot(122)
    ax2.bar(smoker_list, smoker_wilcox[non_smoker_list].loc[ii],
color="#008080")
    plt.xticks(''); plt.title("Control"); plt.ylim(0, 10)
    fig.suptitle("Probeset ID: %s" % ii)
```


Probeset ID: 1552307_a_at
Smoker
Control
10
8
6
4
2
0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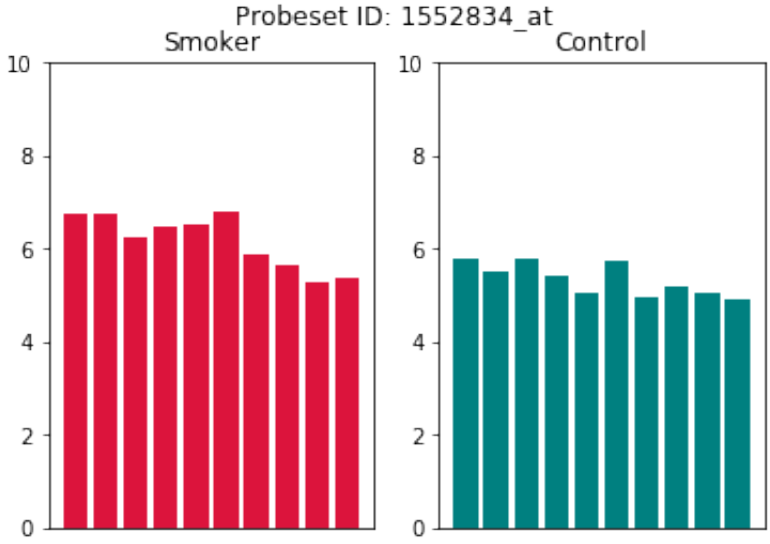
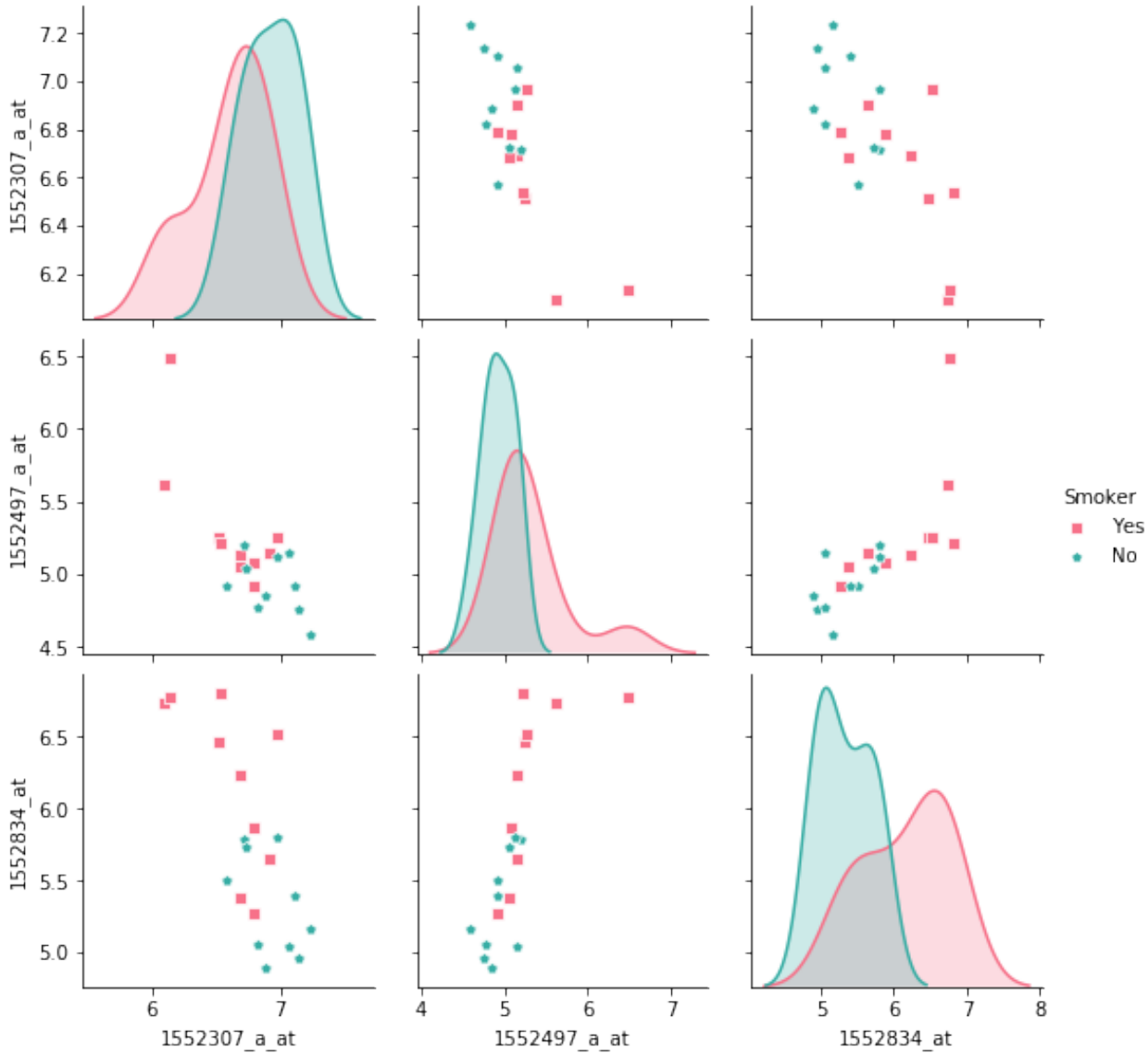
Control
10
8
6
4
2
0


Probeset ID: 1552834_at
Smoker
Control
10
8
6
4
2
0

```
# Scatter plots combining the probesets
df_scatter_s = smoker_wilcox[smoker_list].T
df_scatter_s['Smoker'] = 'Yes'
df_scatter_c = smoker_wilcox[non_smoker_list].T
df_scatter_c['Smoker'] = 'No'
df_scatter = pd.concat([df_scatter_s, df_scatter_c], axis=0)
# print(df_scatter)
# print(df_scatter.index)
# Pairplot
pp = sns.pairplot(df_scatter, hue='Smoker', diag_kind='auto', markers=['s',
'p'], palette="husl")
```



(c) T-test

- Setting P-Value is actually essential and here, we set it to be 0.003 instead of 0.05.
- Actually p-value = 0.05 is suitable for the analysis according to the original article: ***Pauwels RA, Buist AS, Calverley PM, Jenkins CR, Hurd SS (2001) Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease. NHLBI/WHO Global Initiative for Chronic Obstructive Lung Disease (GOLD) workshop summary. Am J Respir Crit Care Med 163:1256–1276***.

- However, we choose this p-value to generate a more strict result for the further dendrogram and clustering, since if the left data is too large, the dendrogram is hard to visualize without using *fastcluster* library.
- The p-value = 0.006 finally gives us **642 differential expressions**, which is acceptable but it would be better to use *fastcluster*.
- Notice the p-value compared to the Wilcoxon sign-rank test is even smaller with more differential expressions, which reflects Wilcoxon sign-rank test might be a more strict method in this problem

```
significant_inds_ttest, significant_pvals_ttest = [], []
for ii in smoker_filt.index:
    pval = stats.ttest_rel(smoker_filt[non_smoker_list].loc[ii],
smoker_filt[smoker_list].loc[ii]).pvalue
    if pval < 0.003:
        significant_inds_ttest.append(ii)
        significant_pvals_ttest.append(pval)
        print("%s is significant probeset, with pvalue=%4f" % (ii, pval))
print(len(significant_inds_ttest))
```

```
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1553709_a_at is significant probeset, with pvalue=0.000588
1553729_s_at is significant probeset, with pvalue=0.000685
1553961_s_at is significant probeset, with pvalue=0.002265
1553994_at is significant probeset, with pvalue=0.000737
1553995_a_at is significant probeset, with pvalue=0.000100
1554085_at is significant probeset, with pvalue=0.002918
1554168_a_at is significant probeset, with pvalue=0.001449
1554182_at is significant probeset, with pvalue=0.002013
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1555095_at is significant probeset, with pvalue=0.002860
1555824_a_at is significant probeset, with pvalue=0.000334
1555854_at is significant probeset, with pvalue=0.001114
1555886_at is significant probeset, with pvalue=0.000580
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1557681_s_at is significant probeset, with pvalue=0.002270
1557965_at is significant probeset, with pvalue=0.000615
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```

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229852_at is significant probeset, with pvalue=0.001469
229964_at is significant probeset, with pvalue=0.000064
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235647_at is significant probeset, with pvalue=0.002374
```

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```

```
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55872_at is significant probeset, with pvalue=0.000368
65630_at is significant probeset, with pvalue=0.001782
823_at is significant probeset, with pvalue=0.000334
91617_at is significant probeset, with pvalue=0.000163
642
```
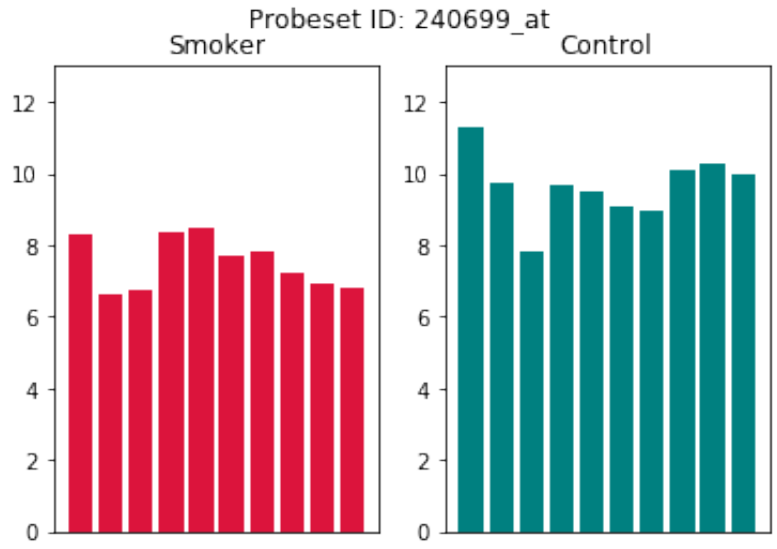
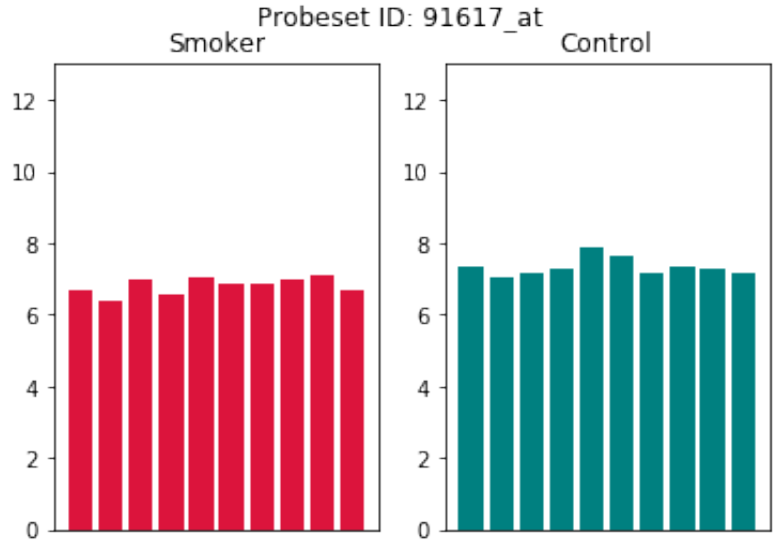

(d) T-test Vistualization

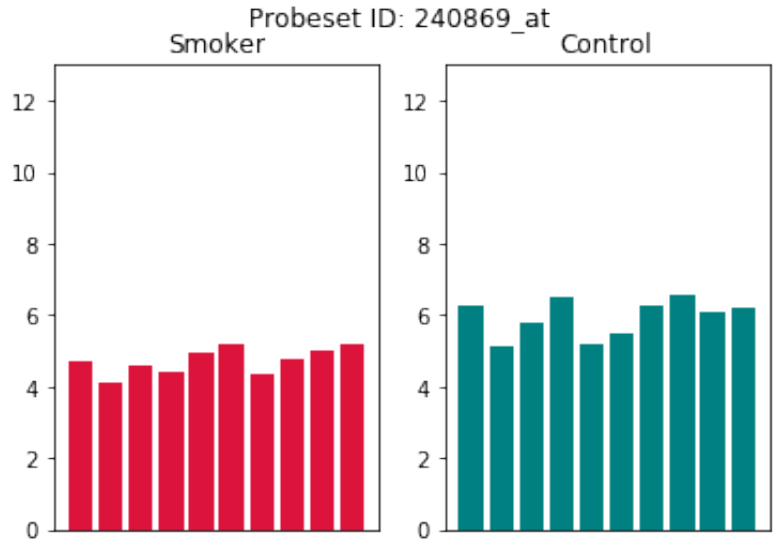
select Gene: '40699_at, 91617_at, 240869_at'

- Bar Plot
- Scatter Plot

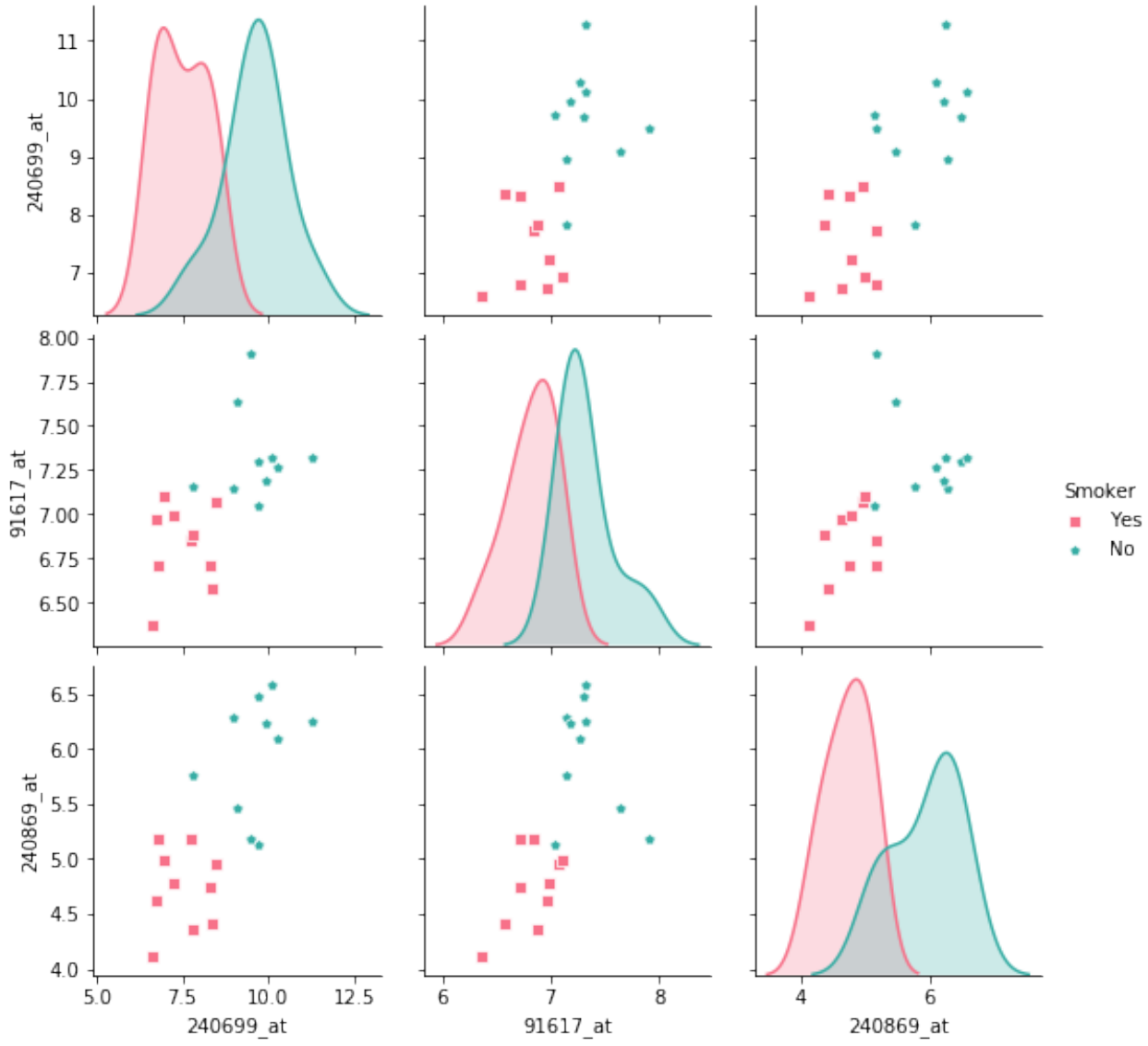
```
select_w_list= ['240699_at', '91617_at', '240869_at']
smoker_ttest = smoker_filt[non_smoker_list + smoker_list].loc[select_w_list]
for ii in smoker_ttest.index:
    fig = plt.figure()
    ax1 = fig.add_subplot(121)
    ax1.bar(smoker_list, smoker_ttest[smoker_list].loc[ii], color="#dc143c")
    plt.xticks(''); plt.title("Smoker"); plt.ylim(0, 13)
    ax2 = fig.add_subplot(122)
    ax2.bar(smoker_list, smoker_ttest[non_smoker_list].loc[ii],
color="#008080")
    plt.xticks(''); plt.title("Control"); plt.ylim(0, 13)
    fig.suptitle("Probeset ID: %s" % ii)
```


Probeset ID: 240699_at
Smoker
Control
0
2
4
6
8
10
12


Probeset ID: 91617_at
Smoker
Control
0
2
4
6
8
10
12


Probeset ID: 240869_at
Smoker
Control
0
2
4
6
8
10
12

```
# Scatter plots combining the probesets
df_scatter_s = smoker_ttest[smoker_list].T
df_scatter_s['Smoker'] = 'Yes'
df_scatter_c = smoker_ttest[non_smoker_list].T
df_scatter_c['Smoker'] = 'No'
df_scatter = pd.concat([df_scatter_s, df_scatter_c], axis=0)
# print(df_scatter)
# print(df_scatter.index)
# Pairplot
pp = sns.pairplot(df_scatter, hue='Smoker', diag_kind='auto', markers=['s',
'p'], palette="husl")
```



Question 3 Quiz: Comment the results based on your observations

Anwser

Firstly, up/down regulation in 6 selected genes: observing the bar plot, reader can notice the expression ratio in test(smoke) and control(non-smoke) samples are up/down regulated. compare the reference group for each samples:

- 1552307_a_at: down regulation
- 1552497_a_at: up regulation
- 1552834_at: up regulation
- 240699_at: down regulation
- 91617_at: down regulation
- 240869_at: down regulation

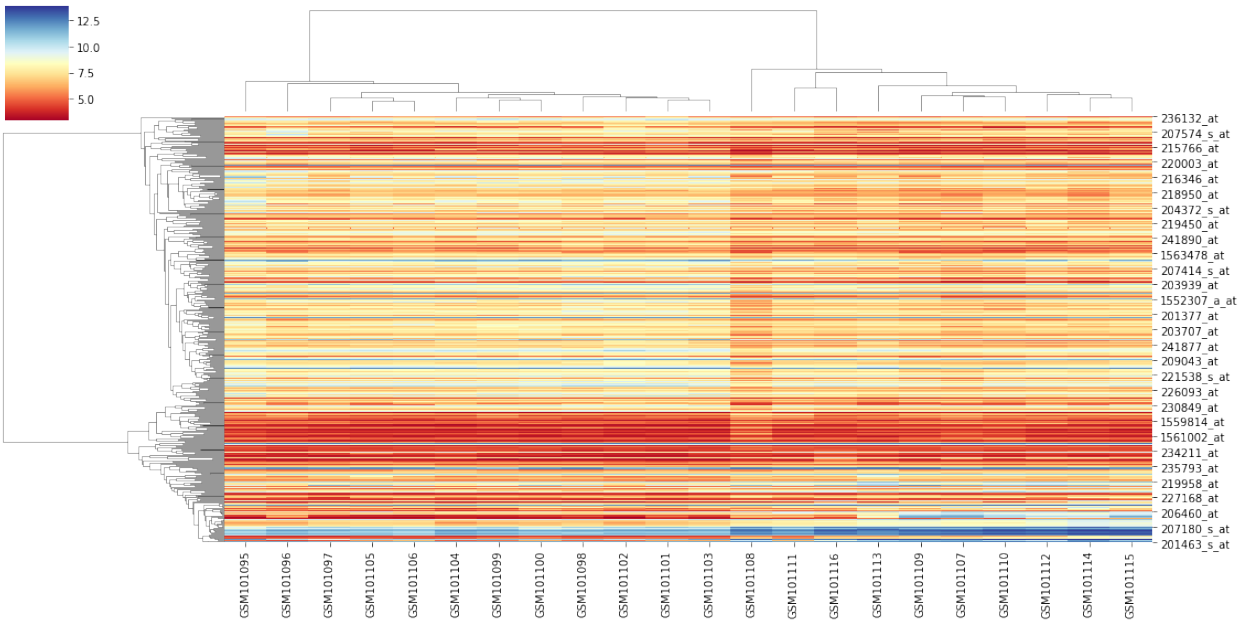
Secondly, for a specific gene to compare, though basically exists up or down regulation, the level of up or down regulation is different in different samples. For example, take reference ID: 1552497_a_at as an example, the second sample in the smoker group is relatively in high expression ratio compared to others in the same group.

Thirdly, the scatter plot indicates for the correlations/similarity between two selected genes: some are not correlated as the points can be divided into two clusters easily; some are correlated as the points are mixed together. The "correlation" might be the evidence for the genes control the same functional categories, which would be mentioned in the part (h).

(g) Question 4: Hierarchical Clustering and Dendrogram

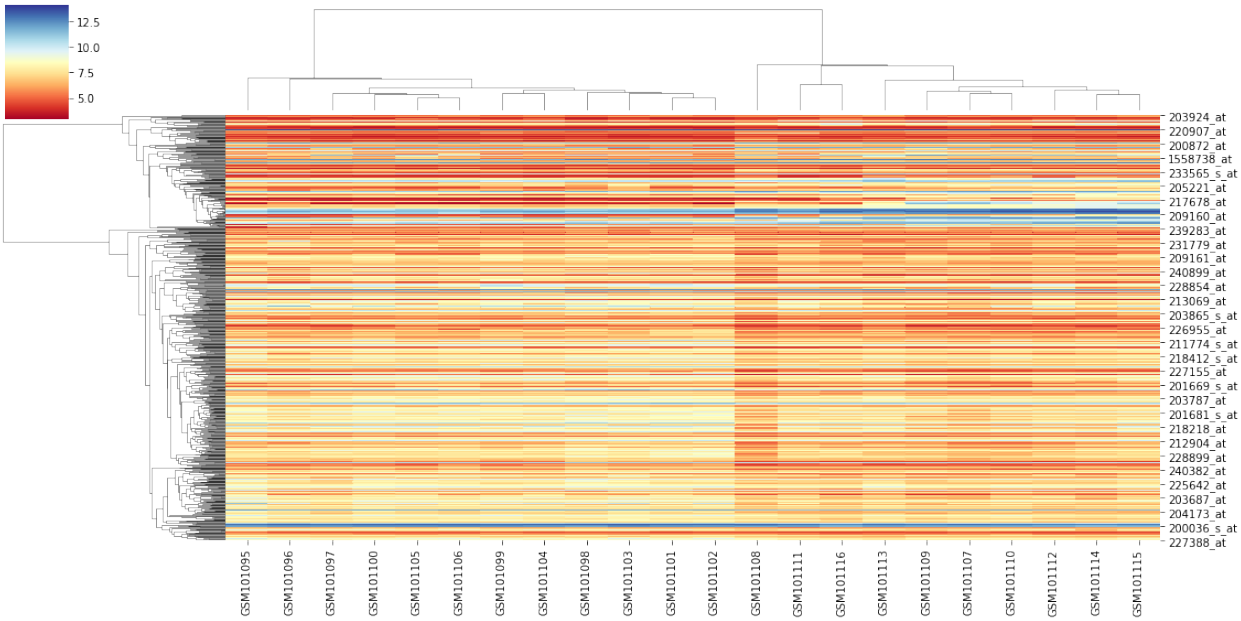
- Wilcoxon Signed-Rank Test
- T-test

```
# Wilcoxon Signed-Rank Test
w_target_df = pd.DataFrame(smoker_filt, index=significant_inds_wilcox)
w_hc = sns.clustermap(w_target_df, method='average', metric='correlation',
cmap='RdYlBu', figsize=(16, 8))
```



```
# T-test
t_target_df = pd.DataFrame(smoker_filt, index=significant_inds_ttest)
t_hc = sns.clustermap(t_target_df, method='average', metric='correlation',
cmap='RdYlBu', figsize=(16, 8))
```

```
/Library/Frameworks/Python.framework/Versions/3.7/lib/python3.7/site-
packages/seaborn/matrix.py:624: UserWarning: Clustering large matrix with
scipy. Installing `fastcluster` may give better performance.
  warnings.warn(msg)
```



Question 4 Quiz: Give a brief interpretation for the results.

Anwser

- From Gene: most of the selected genes are up regulated or normal regulated, only an extremely part is down regulated (e.g. 207180_s_at in Wilcoxon Signed-Rank Test).
- From Sample: it can be observed that the heatmap is splited into two "different" parts, which implies different expression patterns for control and test sample sets; in the same set(i.e. control set or test set), the expression patterns are quite similar.
- From Dendrogram:

1. the relationships among different samples in one set(i.e. control set or test set) are quite random, however, the relationship among different sets is absolute the most estranged one.
2. the genes have most different expression pattern, have the most estranged relation; vice versa.
3. the genes have close relationship or be clustered into the same group should be highlighted,

since they might control the similar/same functions.

- Although two test selected different differential expressed genes, for the same genes in two tests, the absolute expression value might be different, but the expression pattern rule remains similar.

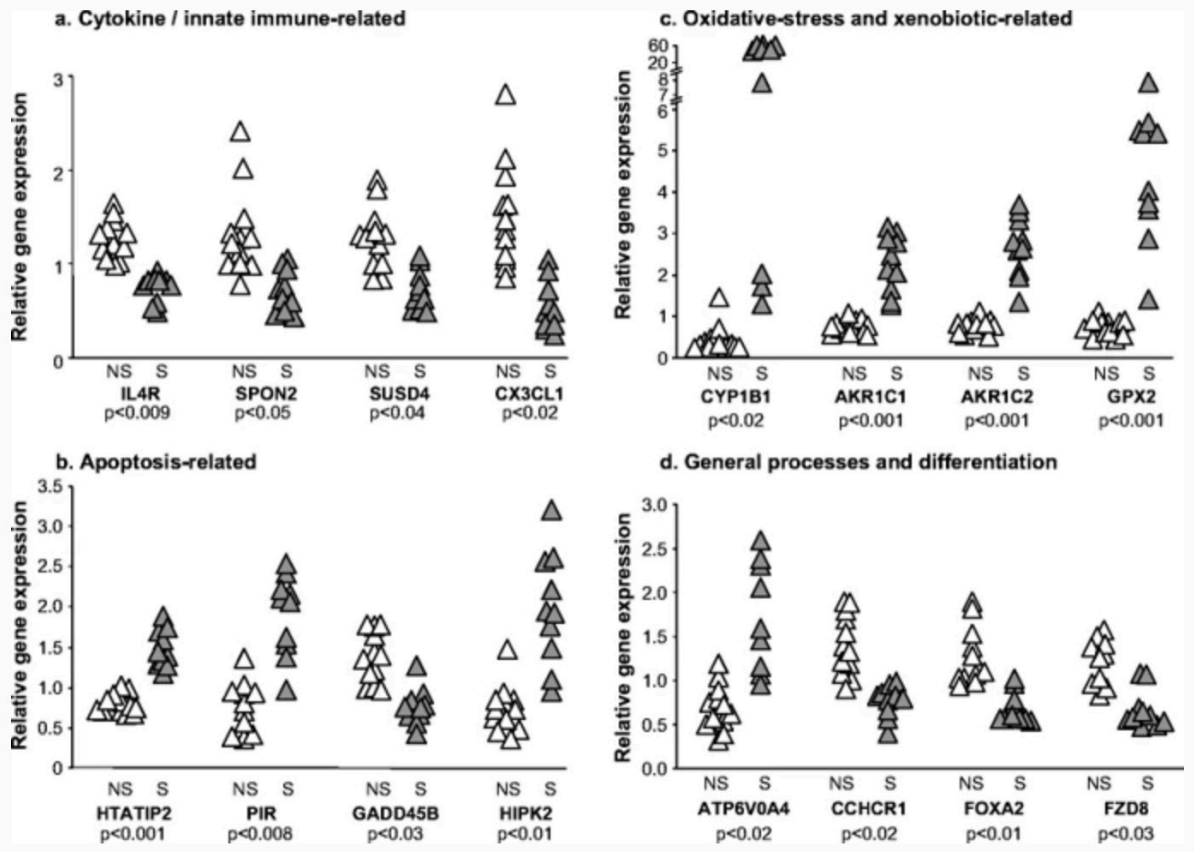
(h) Additional Part: Further Comments

Introduction for Dataset

- This experiment aims to understand how smoking modifies small airway structure and function. The dataset provided in the assignemnt is the second dataset in this project. - Although the smokers were phenotypically normal, microarray analysis of gene expression of the small airway epithelium of the smokers compared to the nonsmokers demonstrated up- and down regulation of genes in multiple categories relevant to the pathogenesis of chronic obstructive lung disease (COPD), including genes coding for cytokines/innate immunity, apoptosis, mucin, response to oxidants and xenobiotics, and general cellular processes.

Optimize Method for The Analysis of Assignment 4

- Apply different p-value thresholds for different genes
  The Wilcoxon Signed-Rank test and Paired Sample T-Test here we applied only set one p-value for all the genes, however, the differential level for different genes diverse a lot. For example, the interleukin-4 (IL4) receptor gene with $p < 0.002$, the chemokine (C-X3-C motif) ligand 1 with $p < 0.02$, the spondin 2 with $p < 0.04$.



- Check the differential differences between the samples for constant variables.
  If we want to compare different samples, we need to make sure the differences brought by sample itself will not cause large impact, that is the pvalue for factors like age, gender and etc. should reject the test: no differences in age with $p > 0.2$, sex with $p > 0.6$, or race with $p > 0.7$ among the smokers and nonsmokers.
- Cluster the genes according to functional category.
  It would be more signifcant if we cluster the gene to identify the gene function.