Hyun-Joon Yang

BISC 478

yanghyun@usc.edu

HW4

In [1]:
```
from IPython.display import Image
from IPython.core.display import HTML

HTML("""
<style>
.output_png {
    display: table-cell;
    text-align: center;
    vertical-align: middle;
}
.output_jpeg {
    display: table-cell;
    text-align: center;
    vertical-align: middle;
}
</style>
""")
```

Out[1]:

In [2]:
```
import numpy as np
import pandas as pd
```

Q1-4

Below are the un-normalized counts for a fictional RNA-seq experiment with only four genes.

In [3]:
```
df = pd.read_csv('data.csv')
df.index = ['A', 'B', 'C', 'D']
df
```

Out[3]:

	Size	Condition 1-1	Condition 1-2	Condition 2-1	Condition 2-2
A	1	1000	2000	4000	3000
B	20	5000	10000	20000	15000
C	5	1500	3000	12000	9000
D	5	1000	2000	4000	3000

1. (10 pts) Compute RPKM.

In [4]:
```
def RPKM(df):
    df = df.copy()
    for i in range(df.shape[1]-1):
        i += 1
        total = df.iloc[:,i].sum()
        factor = total / 1000000
        df.iloc[:,i] = df.iloc[:,i] / factor
#     print(df)
    for i in range(df.shape[0]):
        df.iloc[i,1:] = df.iloc[i,1:] / df.iloc[i,0]
    return df
```

In [5]:
```
# print(df)
RPKM(df)
```

Out[5]:

	Size	Condition 1-1	Condition 1-2	Condition 2-1	Condition 2-2
A	1	117647.058824	117647.058824	100000.0	100000.0
B	20	29411.764706	29411.764706	25000.0	25000.0
C	5	35294.117647	35294.117647	60000.0	60000.0
D	5	23529.411765	23529.411765	20000.0	20000.0

2. (10 pts) Compute TPM.

In [6]:
```
def TPM(df):
    df = df.copy()
    for i in range(df.shape[0]):
        df.iloc[i,1:] = df.iloc[i,1:] / df.iloc[i,0]
#     print(df)
    for i in range(df.shape[1]-1):
        i += 1
        total = df.iloc[:,i].sum()
        factor = total / 1000000
        df.iloc[:,i] = df.iloc[:,i] / factor
    return df
```

In [7]:
```
# print(df)
TPM(df)
```

Out[7]:

	Size	Condition 1-1	Condition 1-2	Condition 2-1	Condition 2-2
A	1	571428.571429	571428.571429	487804.878049	487804.878049
B	20	142857.142857	142857.142857	121951.219512	121951.219512
C	5	171428.571429	171428.571429	292682.926829	292682.926829
D	5	114285.714286	114285.714286	97560.975610	97560.975610

3. (2 pts) Which (if any) of the four genes are expressed more under condition 2 than condition 1? (To answer this question, you do not need to do a statistical test.)

Gene C

4. (2 pts) Which of the four genes has the highest expression for condition 1? (To answer this question, you do not need to do a statistical test.)

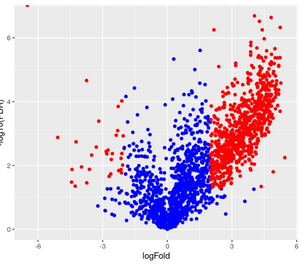
Gene A

Q5

5. (2 pts) Below is a Volcano Plot for a real RNA-seq experiment with many more genes than the example above. "Fold" is the gene expression under condition 1 divided by the gene expression under condition 2. Let N1 be the number of genes that are expressed significantly more under condition 1 than condition 2, and let N2 be the number of genes that are expressed significantly more under condition 2 than condition 1. Based on this plot, are N1 and N2 about equal, is N1 > N2, is N1 < N2, or is there not enough information to know?

In [8]:
```
Image('img5-1.jpg')
```

Out[8]:



Since there are more points where logFold is positive, N1 > N2.

Q6-7

Below are ten un-adjusted p-values.

0.2, 0.6, 0.35, 0.33, 0.0001, 0.008, 0.88, 0.9, 0. 7, 0.62

In [9]:
```
pvs = np.array([0.2, 0.6, 0.35, 0.33, 0.0001, 0.008, 0.88, 0.9, 0.7, 0.62], dtype='float')
pvs
```

Out[9]:
```
array([2.0e-01, 6.0e-01, 3.5e-01, 3.3e-01, 1.0e-04, 8.0e-03, 8.8e-01,
       9.0e-01, 7.0e-01, 6.2e-01])
```

6. (10 pts) Compute the ten adjusted p-values by the False Discovery Rate (Benjamini-Hochberg) procedure.

In [10]:
```
def FDR(pvs):
    pvs = pd.Series(np.sort(pvs), name='p-value')
    rank = pd.Series(np.arange(1,len(pvs)+1), name='rank')
    df = pd.concat([pvs, rank], axis=1)
    df['pre'] = df['p-value'] * df.shape[0] / df['rank']
    adjusted = np.empty(df.shape[0])
    adjusted[:] = np.nan
    adjusted = pd.Series(adjusted, name='adjusted')
    adjusted.iloc[-1] = df['pre'].iloc[-1]
    for i in range(df.shape[0], 1, -1):
        i -= 2
        adjusted.iloc[i] = min(adjusted.iloc[i+1], df['pre'].iloc[i])
    df = pd.concat([df, adjusted], axis=1)
    return df
```

In [11]:
```
FDR(pvs)
```

Out[11]:

	p-value	rank	pre	adjusted
0	0.0001	1	0.001000	0.001000
1	0.0080	2	0.040000	0.040000
2	0.2000	3	0.666667	0.666667
3	0.3300	4	0.825000	0.700000
4	0.3500	5	0.700000	0.700000
5	0.6000	6	1.000000	0.875000
6	0.6200	7	0.885714	0.875000
7	0.7000	8	0.875000	0.875000
8	0.8800	9	0.977778	0.900000
9	0.9000	10	0.900000	0.900000

7. (2 pts) If instead of the FDR we had used the Bonferroni correction, how many of these ten p-values would be less than significance level $\alpha = 0.05$ after doing the Bonferroni correction?

$$\alpha^* = \frac{\alpha}{n} = \frac{0.05}{10} = 0.005$$

Only 1

Q8-9

An RNA-seq experiment is done to test for genes that are differentially expressed between conditions 1 and 2. The False Discovery Rate is set at 0.05.

8. (2 pts) After doing the FDR adjustment, 800 genes are significantly differentially expressed. About how many of these do we expect to be false positives? (You can give a numeric answer or say there is not enough information to know).

$$FDR = V/R$$
$$V = 0.05 \times 800 = 40$$

9. (2 pts) After doing the FDR adjustment, 11,200 genes are not significantly differentially expressed. About how many of these do we expect to be false negatives? (You can give a numeric answer or say there is not enough information to know).

There is not enough information to know

Q10

10. (3 pts) Imagine you have done an RNA-seq experiment similar to the example at the beginning of this HW, except you have data for all ~20,000 genes. Similar to this example, you find there is one gene that clearly has much higher expression than all the other genes under condition 1.

A scientist disputes your finding. This scientist argues that there is a bias in RNA-seq experiments that is not taken into account by RPKM or TPM. This scientist claims that in addition to gene expression, the read count in an RNA-seq experiment also depends on the GC-content of the reads. In particular, reads with GC-content near 50% are counted more relative to reads with much lower or much higher GC-content.

Describe a method to test this scientist's claim. Assume you have data for many RNA-seq experiments, the DNA sequence of the reference genome, and the ability to write computer code. (Note: there is more than one correct response to this question.)

You could run RNA-seq on a sample with known levels of expression and GC-content. You can then normalize the result also accounting for the relative expression of the genes. Then you can plot the counts according to the GC-content of the gene. If the scientist's claim is true, you would expect to find a normal distribution with the mean at GC-content=0.5 whereas if it is false, you would see a uniform distribution.