

# Deeper Sequencing Analysis

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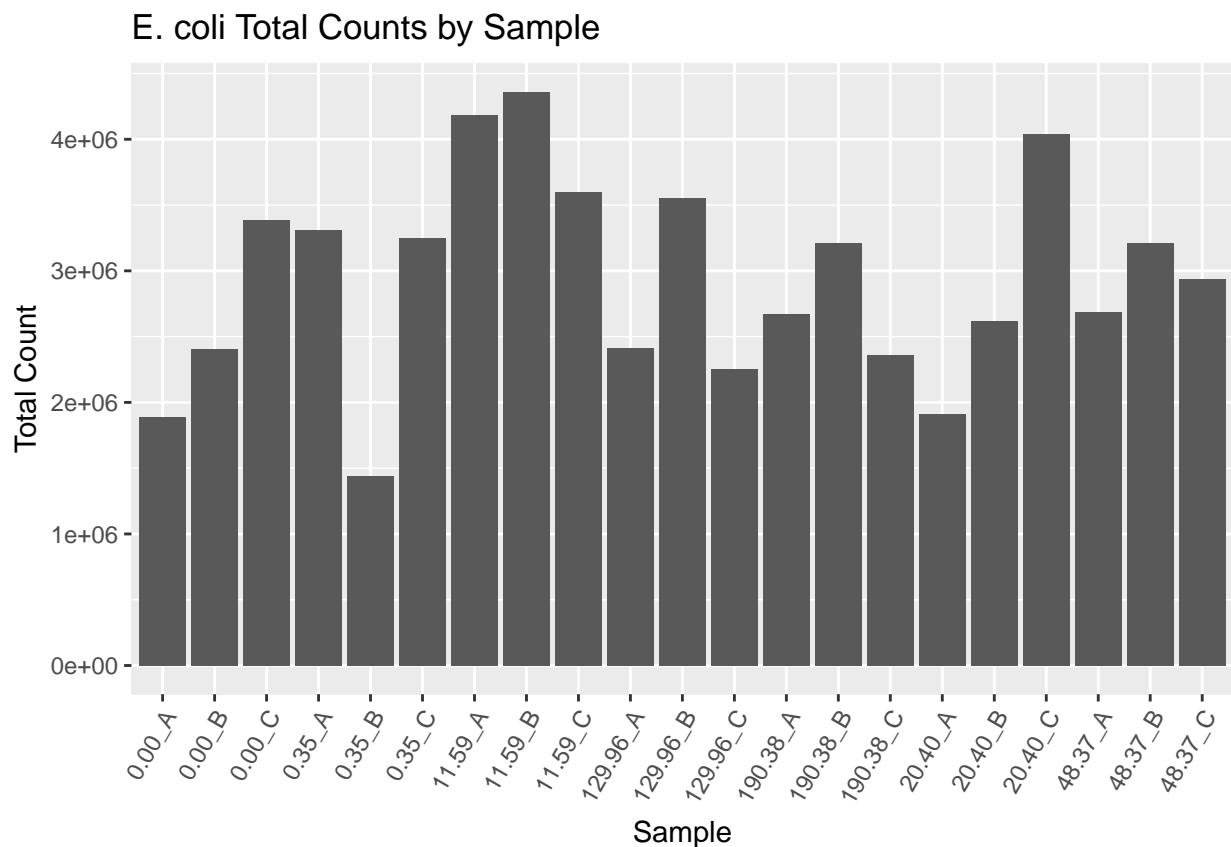
1/10/2018

Note: for ease, “deep” data refers to the data just sequenced more deeply while “shallow” data refers to the data we worked on this summer.

The following document compares some diagnostics between the shallow data and deep data, then visualizes the gene expression patterns of both datasets in a clustering setting.

## Total Counts and Size Factors

### Shallow Data



```
## # A tibble: 21 x 6
##   condition sample median    Q3 sizeFactor totalCount
##   <dbl> <chr>    <dbl> <dbl>      <dbl>      <dbl>
## 1     0     A        4     20      0.887    1889658
## 2     0     B        1      4      0.183    2409296
## 3     0     C        3     14      0.621    3386987
## 4    0.35 A       7     29      1.32    3308303
## 5    0.35 B       1      3      0.133    1436978
```

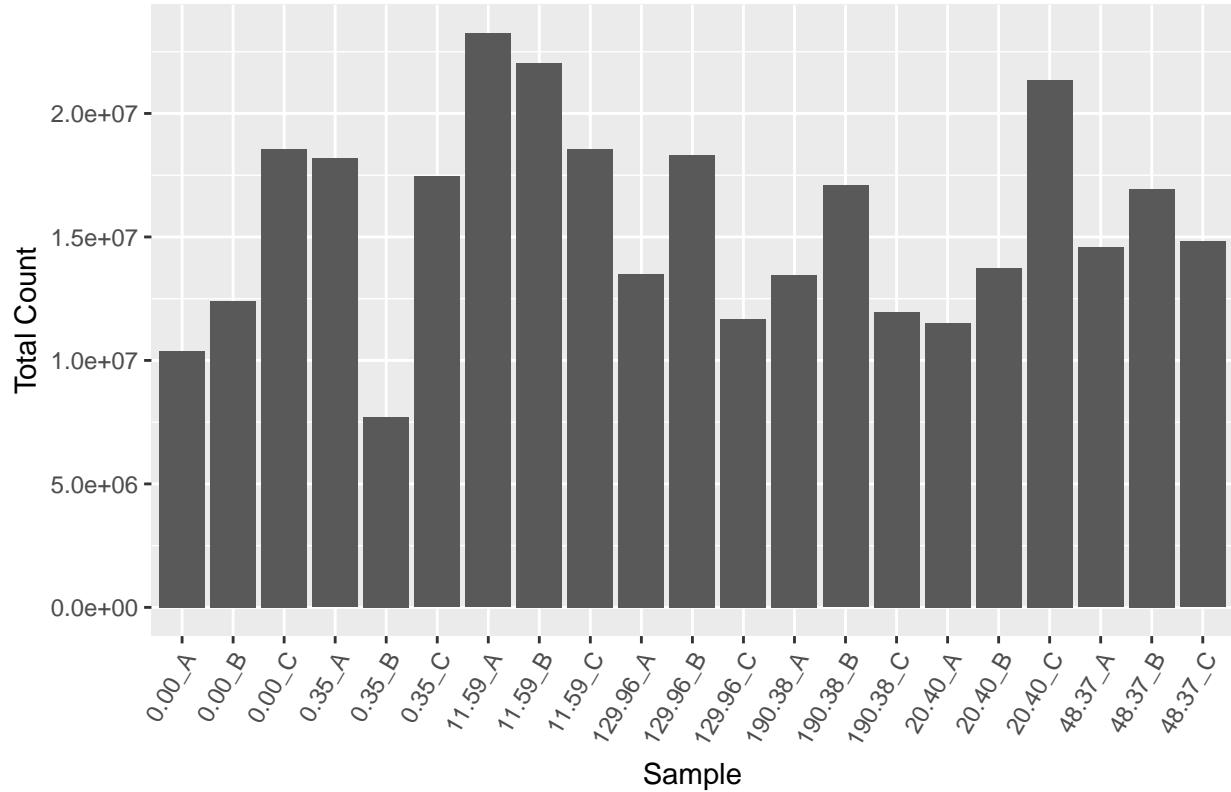
```

## 6      0.35 C      6      28      1.25    3246960
## 7     11.6 A      8      40      1.71    4187736
## 8     11.6 B      9      40      1.70    4362489
## 9     11.6 C      8      35      1.52    3600378
## 10    20.4 A      2      8      0.346   1915087
## # ... with 11 more rows

```

## Deep Data

### E. coli Total Counts by Sample



```

## # A tibble: 21 x 6
##       condition sample median    Q3 sizeFactor totalCount
##       <dbl> <chr>    <dbl> <dbl>      <dbl>      <dbl>
## 1        0      A        4    20      0.903    10395764
## 2        0      B        1     4      0.176    12391466
## 3        0      C        3    14      0.641    18554337
## 4      0.35  A        7    29      1.35    18179978
## 5      0.35  B        1     3      0.123    7703955
## 6      0.35  C        6    28      1.28    17446114
## 7     11.6   A        8    40      1.74    23240163
## 8     11.6   B        9    40      1.65    22053814
## 9     11.6   C        8    35      1.48    18570454
## 10    20.4   A        2     8      0.363    11522258
## # ... with 11 more rows

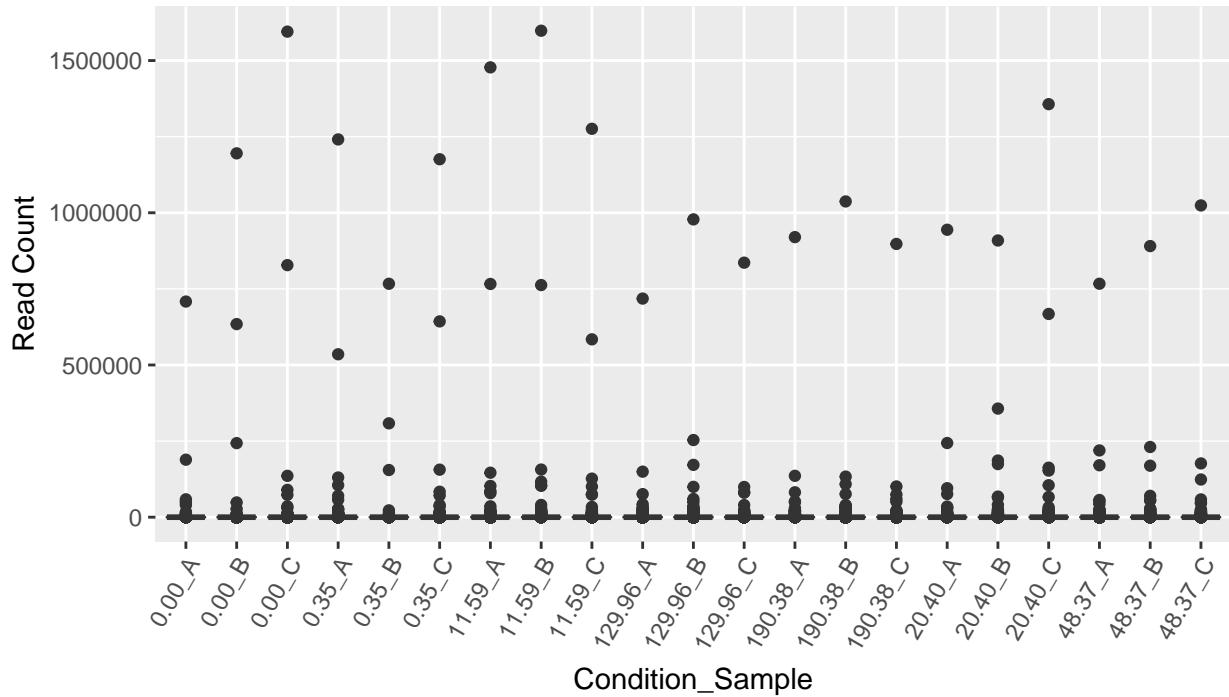
```

We see, unsurprisingly, that the total count per sample of the deeper sequenced data. is larger than that of the shallower sequenced data. However, the size facotors are similar because the distribution of total counts

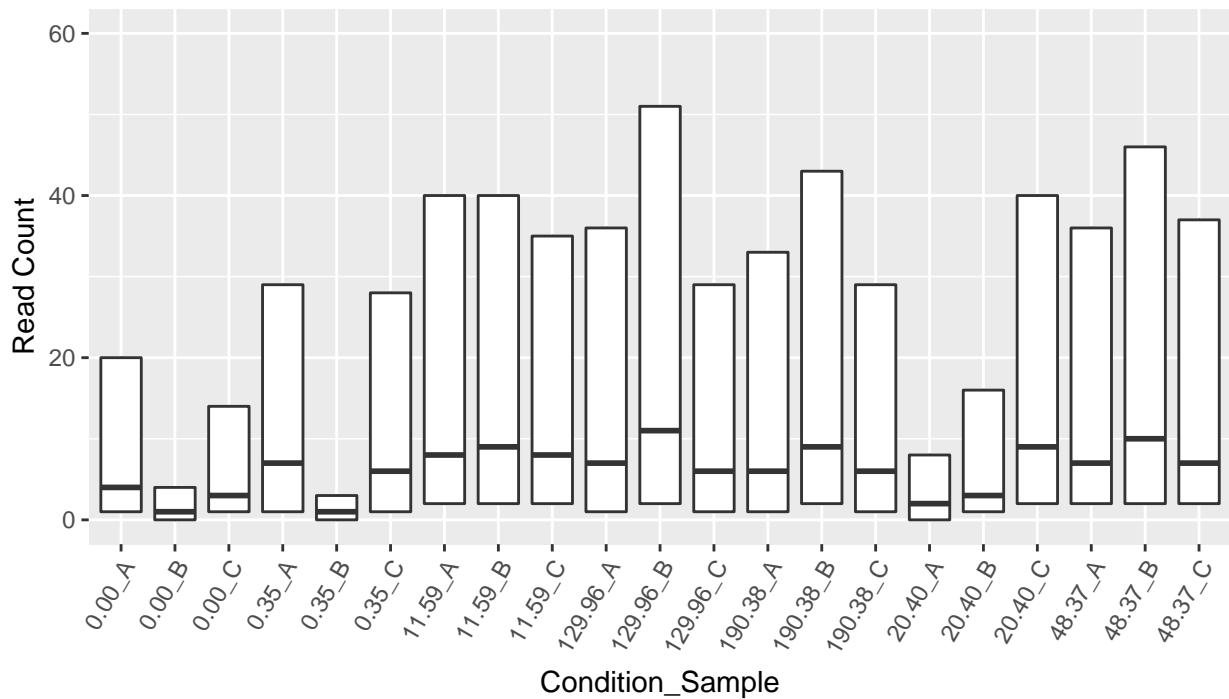
across samples is similar for both depths of sequencing.

### Shallow Data

E. coli Read Count Interquartile Range

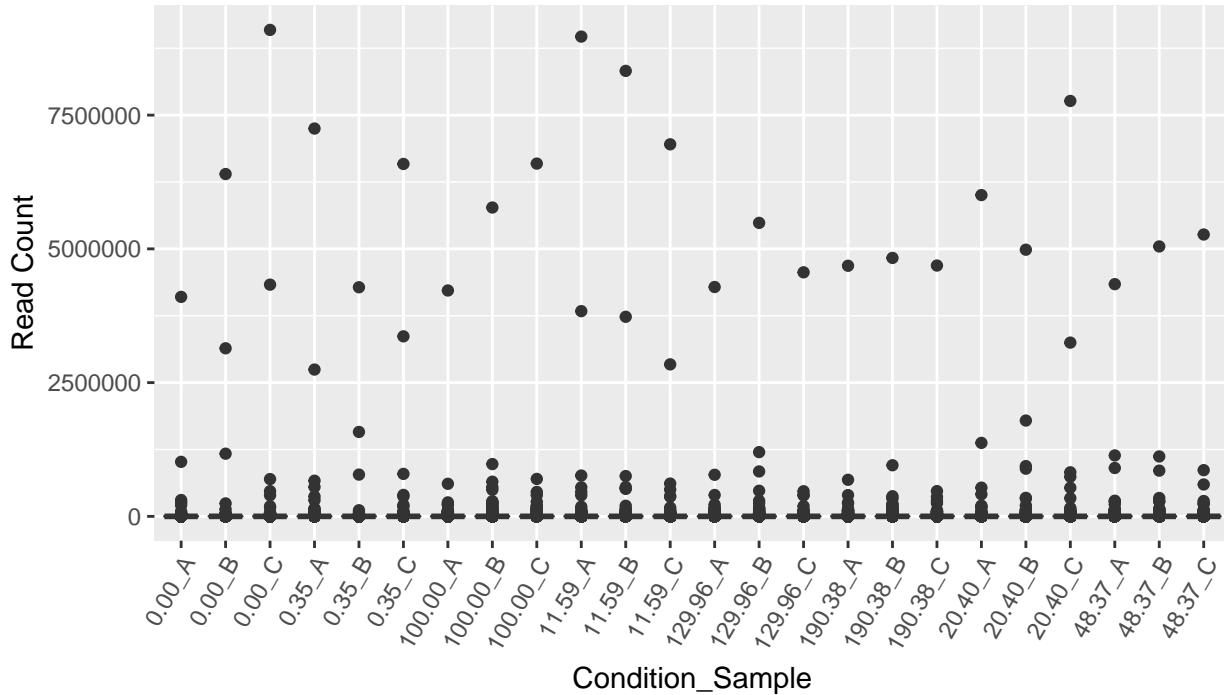


E. coli Read Count Interquartile Range

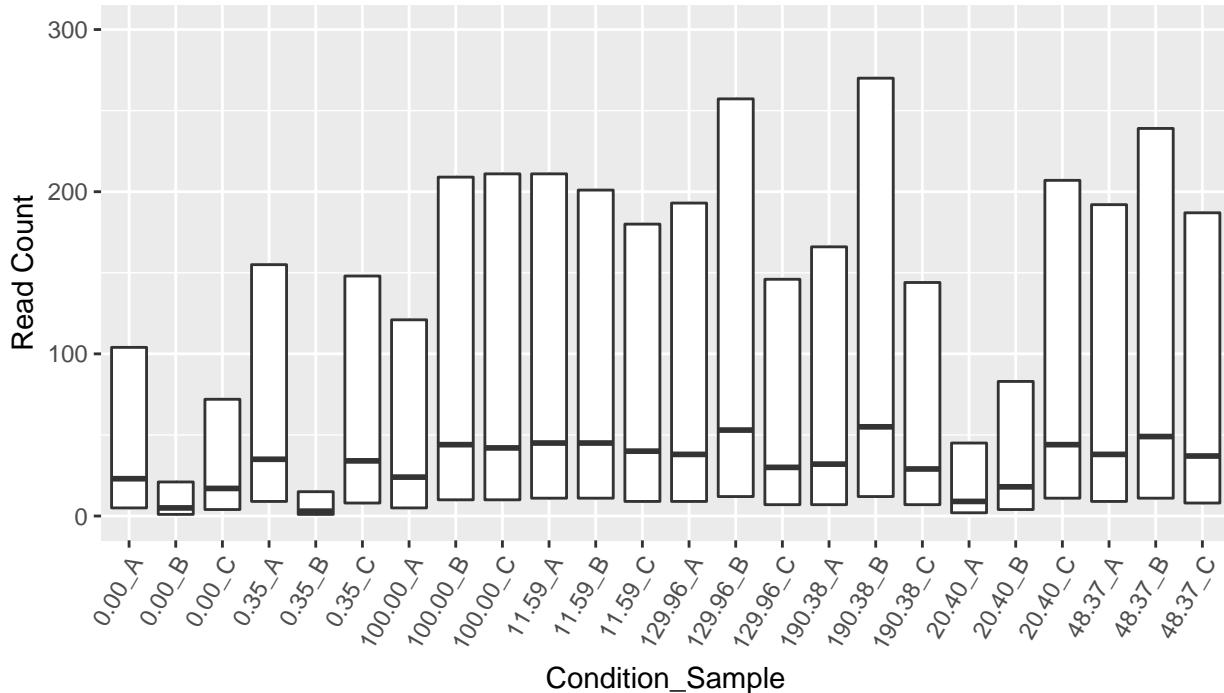


## Deep Data

E. coli Read Count Interquartile Range



E. coli Read Count Interquartile Range



Note that the y axes on both graphs have different scales, but the distribution of readcounts per sample look similar across the two sequencing depths. the samples with majority lower read count genes in the shallow data still display majority lower read count in the deep data. That is, the samples with the lowest Q3 read

count in the shallow data are the same as the samples with lowest read count in the deep data: namely, 0.00\_B, 0.35\_B, and 20.40\_A. The rest of the samples in the deep data follow a similar distribution to those in the shallow data.

This result could suggest that the deep data is confirming what the shallow data saw, but gene by gene plots are needed to see if this is true.

Before, we eliminated genes for which the maximum unnormalized count of any condition was less than 5. In the shallower sequenced data, there were 3,183 such genes. In the deeper data, we have 938 such genes. This makes sense because the deeper sequenced data is more deeply sequenced!

```
## [1] 938.0000000 0.5833333
```

## Dominating Genes

### Shallow Data

### Deep Data

Of the ten most expressed genes from each level of sequencing, eight are the same in both levels of sequencing. These are smpB-intA, rna69, rna106, rmf b0953, rna98, cspE, rna54, and rna71.

```
## [1] 844 3
## [1] 156 2
```

In fact, of the 1000 most expressed genes in each level of sequencing, 844/1000 are the same and 156/1000 are different. This shows that the genes which dominated the shallow data tend to be the same genes dominating the deep data.

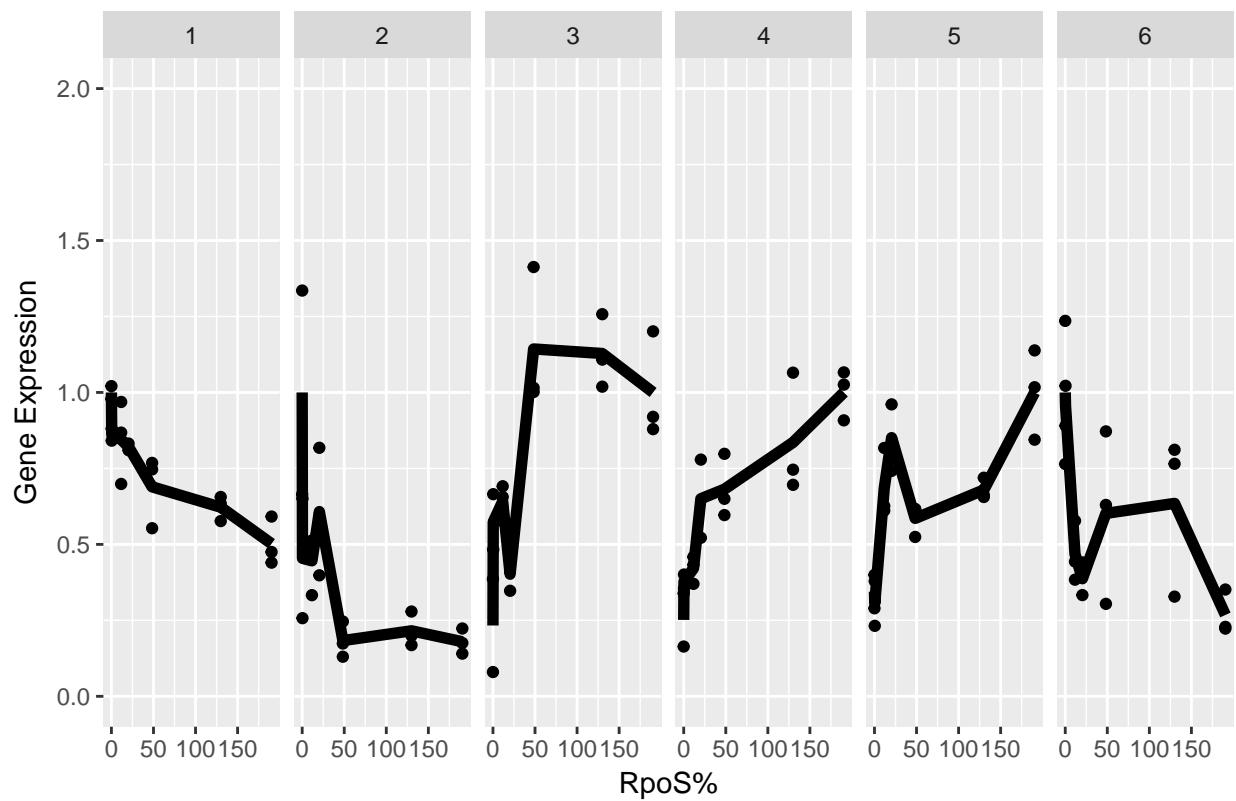
## Differential Expression, Gene Expression Shapes, and Clustering

We throw out the samples with much lower median read count (0.00\_B, 0.35\_B, and 20.40\_A) as well as the samples with 100% RpoS (100.00\_A, 100.00\_B, 100.00\_C) because it has a different strain of E. coli than the rest of the samples.

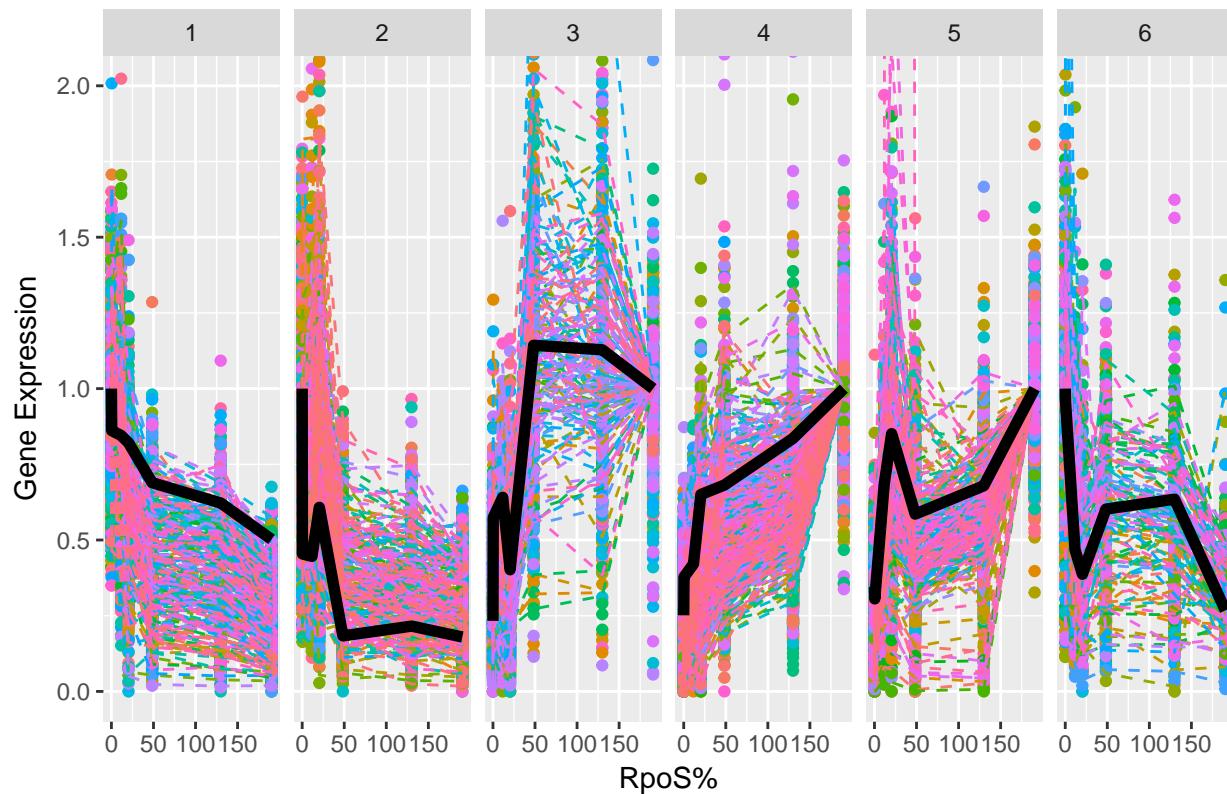
### Shallow Data

```
##
## out of 10374 with nonzero total read count
## adjusted p-value < 0.05
## LFC > 0 (up)      : 1040, 10%
## LFC < 0 (down)    : 790, 7.6%
## outliers [1]       : 0, 0%
## low counts [2]     : 3620, 35%
## (mean count < 7)
## [1] see 'cooksCutoff' argument of ?results
## [2] see 'independentFiltering' argument of ?results
```

PAM medoids, k=6



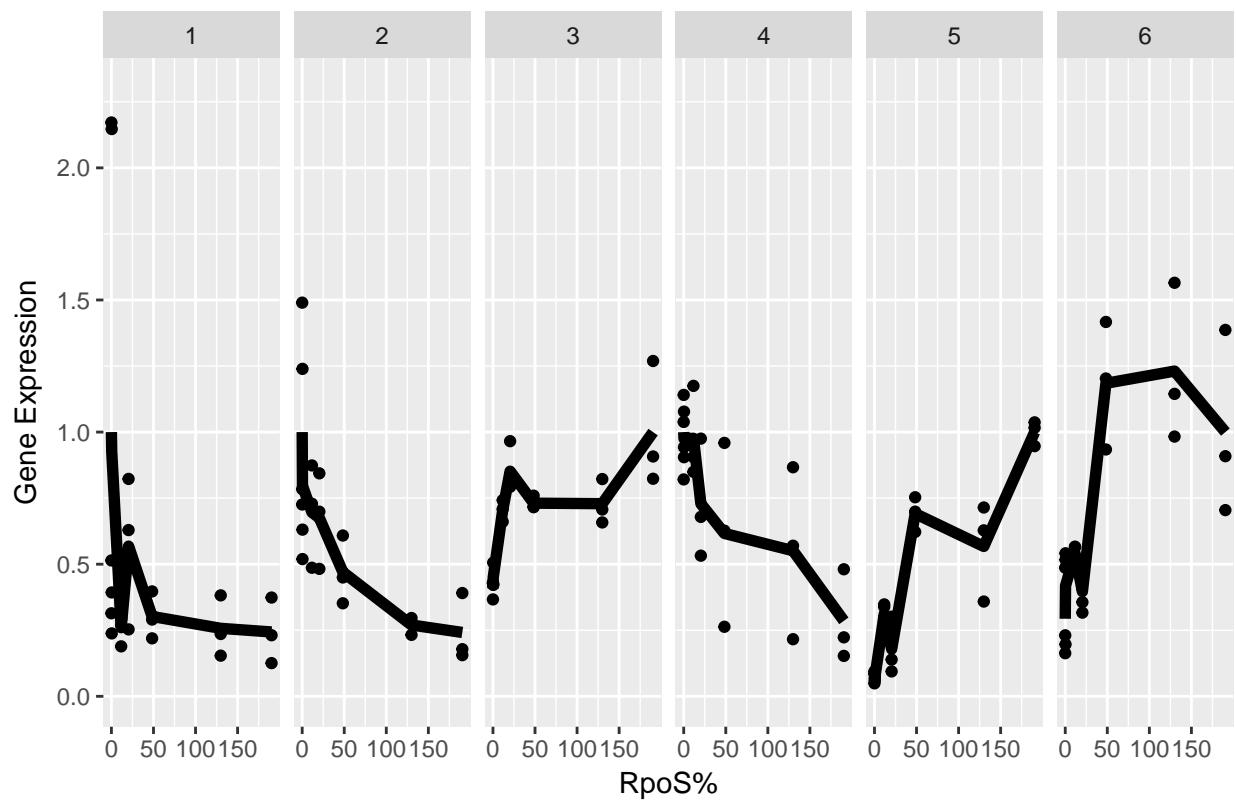
## Gene Expression Clustering, PAM k=6; Medoids Overlaid in black



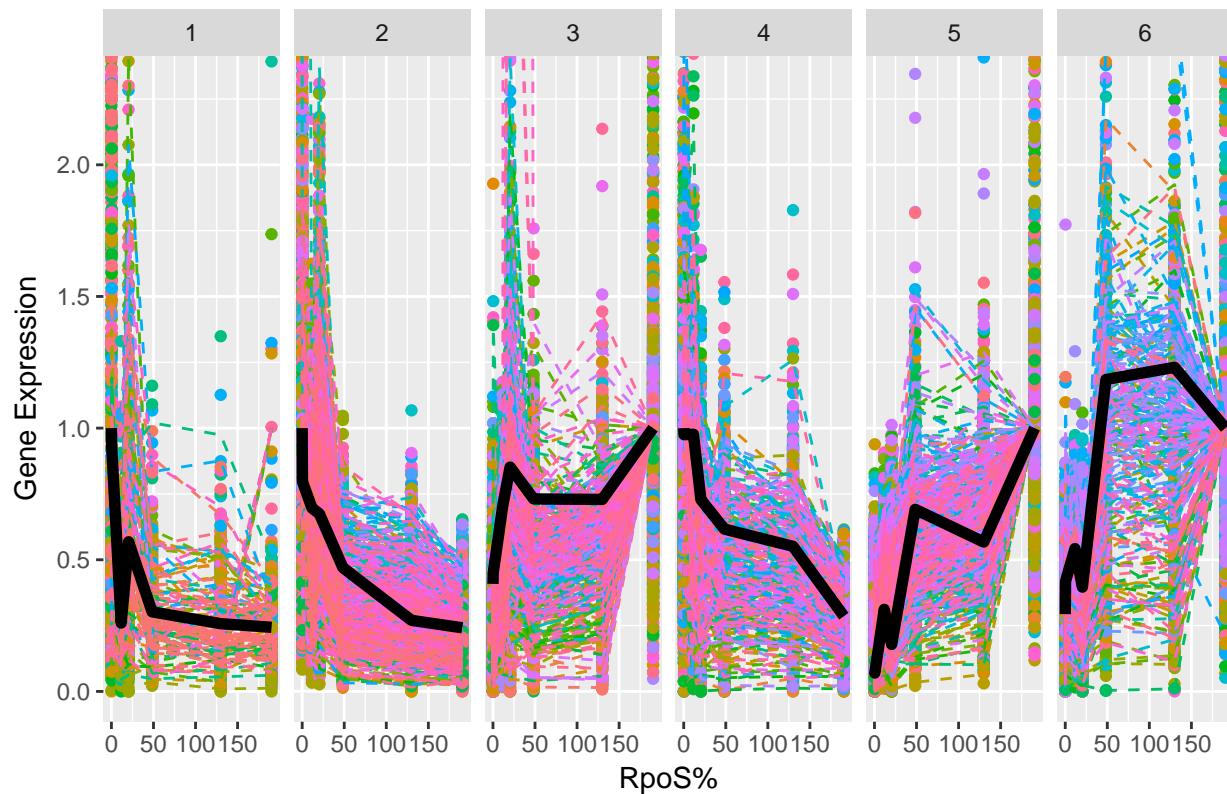
Deep Data, all samples except 100% RpoS

```
##
## out of 12989 with nonzero total read count
## adjusted p-value < 0.05
## LFC > 0 (up)      : 1510, 12%
## LFC < 0 (down)    : 1204, 9.3%
## outliers [1]       : 254, 2%
## low counts [2]     : 2768, 21%
## (mean count < 8)
## [1] see 'cooksCutoff' argument of ?results
## [2] see 'independentFiltering' argument of ?results
```

PAM medoids, k=6



## Gene Expression Clustering, PAM k=6; Medoids Overlaid in black

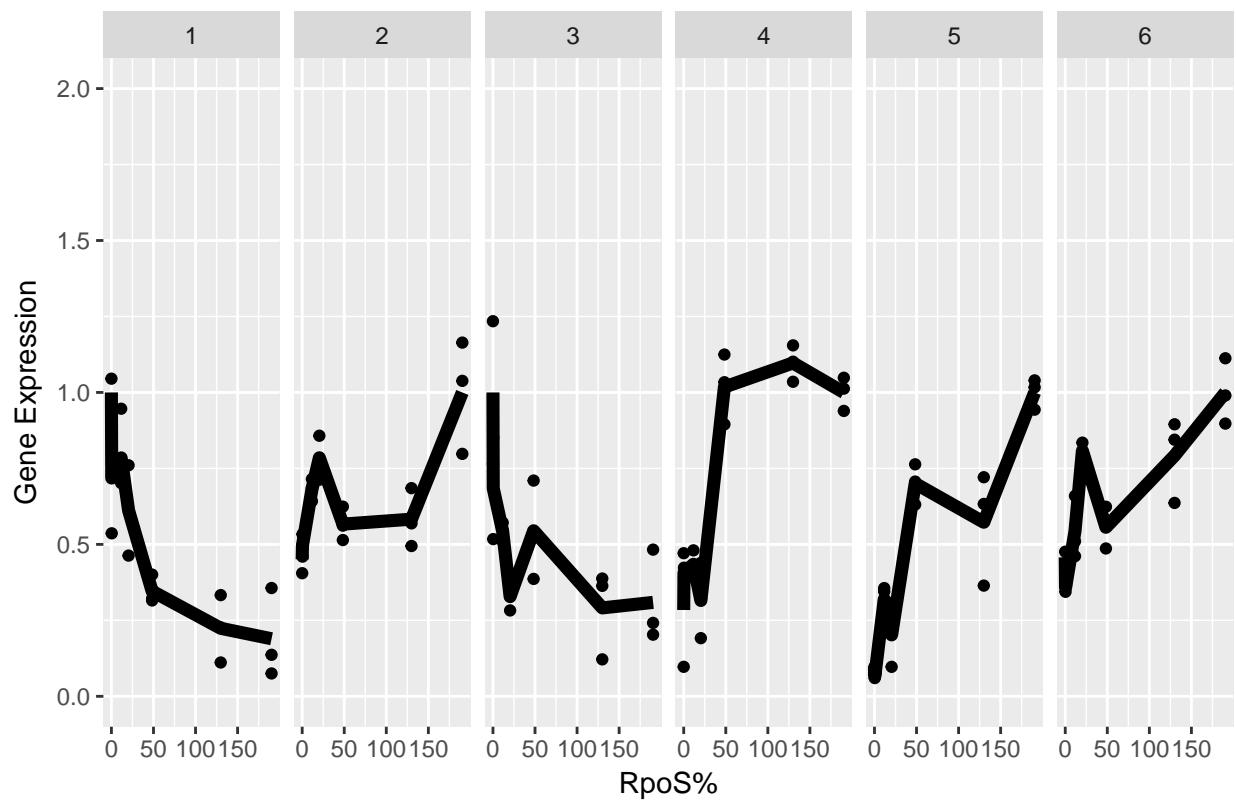


Deep Data, 0.00\_B, 0.35\_B, 20.40\_A and 100% RpoS removed

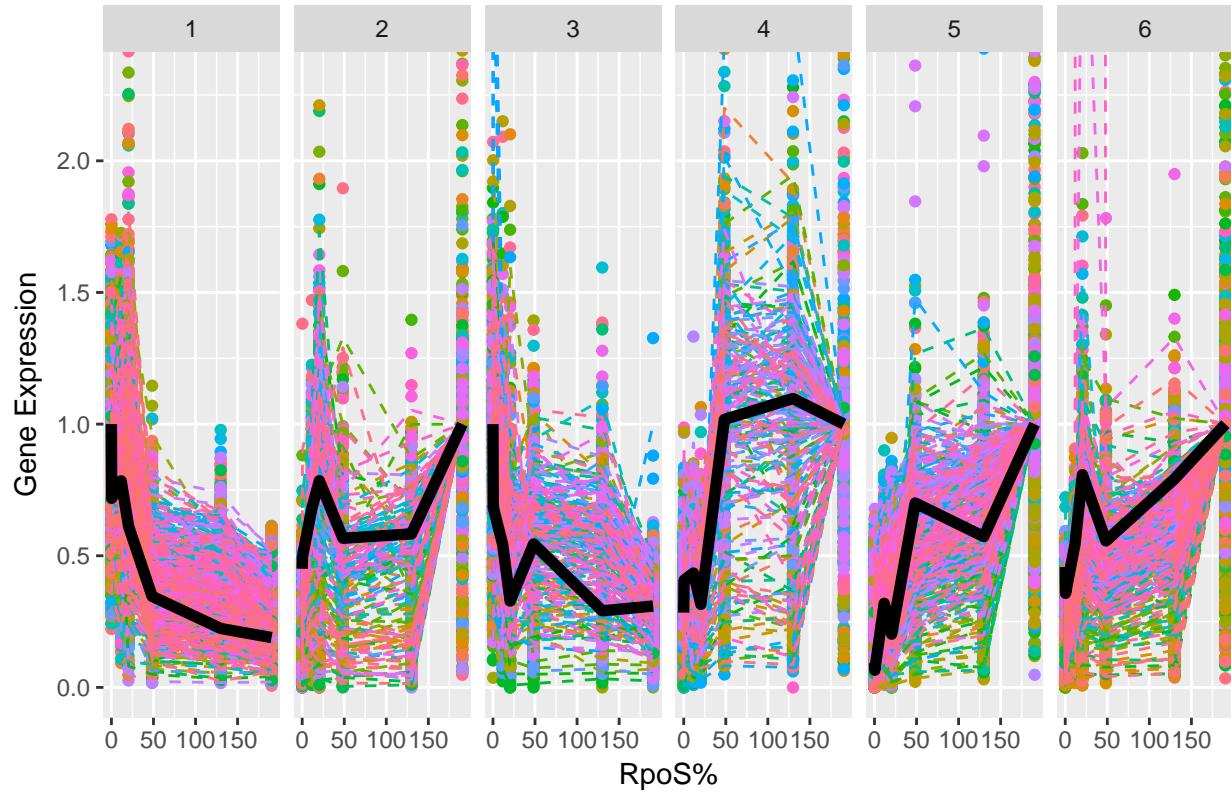
We remove the samples with 100% RpoS and 0.00\_B, 0.35\_B, 20.40\_A.

```
##
## out of 12989 with nonzero total read count
## adjusted p-value < 0.05
## LFC > 0 (up)      : 1361, 10%
## LFC < 0 (down)    : 988, 7.6%
## outliers [1]       : 67, 0.52%
## low counts [2]     : 2770, 21%
## (mean count < 10)
## [1] see 'cooksCutoff' argument of ?results
## [2] see 'independentFiltering' argument of ?results
```

PAM medoids, k=6



## Gene Expression Clustering, PAM k=6; Medoids Overlaid in black



In the shallow and deep data, a similar proportion of genes are found to be differentially expressed across the most extreme levels, 0% and 190.38% RpoS.

In both the shallow and deep data, we notice similar “common shapes” (medoids) and a considerable amount of nonmonotonicity in the gene expression across different levels of RpoS.

### How Similar are the Deep and Shallow Clusterings?

ARI: Deep (0.00\_B, 0.35\_B, 20.40\_A removed) vs. Shallow:

```
## [1] 0.505
```

The adjusted rand index suggests above that the clusterings for  $k = 6$  of the deep data (with 0.00\_B, 0.35\_B, 20.40\_A removed) and shallow datasets are more similar than dissimilar, but certainly don't agree wholeheartedly.

ARI: Deep (0.00\_B, 0.35\_B, 20.40\_A removed) vs. Deep (0.00\_B, 0.35\_B, 20.40\_A included)

```
## [1] 0.597
```

The clusterings for the deep data with and without 0.00\_B, 0.35\_B, and 20.40\_A are more similar than the deep vs. shallow, but certainly don't agree wholeheartedly either.