# BIMS 8382 Exercises

# bioconnector.org/bims 8382

# Spring 2016

# Contents

R Basics		<b>2</b>
Exercise 1		. 2
Exercise 2		. 2
Data Frames		2
Advanced Data Manipulation		3
Exercise 1		. 3
Exercise 2		. 3
Exercise 3		. 3
Exercise 4		. 4
Data Visualization		10
Exercise 1		. 10
Exercise 2		. 10
Exercise 3		. 11
Exercise 4		. 11
RNA-seq		13
Exercise 1		. 13
Exercise 2		. 14
Exercise 3		. 14
Exercise 4		. 18
Evereise 5		20

### R Basics

### Exercise 1

What are the values after each statement in the following?

### Exercise 2

See ?abs and calculate the square root of the log-base-10 of the absolute value of -4\*(2550-50). Answer should be 2.

## **Data Frames**

- 1. What's the standard deviation expression (hint: get help on the sd function with ?sd).
- 2. What's the range of rate represented in the data? (hint: range()).

## Advanced Data Manipulation

### Exercise 1

- 1. Display the data where the gene ontology biological process (the bp variable) is "leucine biosynthesis" (case-sensitive) and the limiting nutrient was Leucine. (Answer should return a 24-by-7 data frame 4 genes × 6 growth rates).
- 2. Gene/rate combinations had high expression (in the top 1% of expressed genes)? Hint: see ?quantile and try quantile(ydat\$expression, probs=.99) to see the expression value which is higher than 99% of all the data, then filter() based on that. Try wrapping your answer with a View() function so you can see the whole thing. What does it look like those genes are doing? Answer should return a 1971-by-7 data frame.

### Exercise 2

- 1. First, re-run the command you used above to filter the data for genes involved in the "leucine biosynthesis" biological process and where the limiting nutrient is Leucine.
- 2. Wrap this entire filtered result with a call to arrange() where you'll arrange the result of #1 by the gene symbol.
- 3. Wrap this entire result in a View() statement so you can see the entire result.

### Exercise 3

Here's a warm-up round. Try the following.

Show the limiting nutrient and expression values for the gene ADH2 when the growth rate is restricted to 0.05. *Hint:* 2 pipes: filter and select.

```
## Source: local data frame [6 x 2]
##
##
      nutrient expression
##
          (chr)
                     (dbl)
## 1
       Glucose
                      6.28
## 2
       Ammonia
                      0.55
## 3 Phosphate
                     -4.60
## 4
       Sulfate
                     -1.18
## 5
       Leucine
                      4.15
## 6
        Uracil
                      0.63
```

What are the four most highly expressed genes when the growth rate is restricted to 0.05 by restricting glucose? Show only the symbol, expression value, and GO terms. *Hint:* 4 pipes: filter, arrange, head, and select.

```
## Source: local data frame [4 x 4]
##
##
     symbol expression
                                          bp
                                                                          mf
      (chr)
##
                  (dbl)
                                       (chr)
                                                                        (chr)
       ADH2
                   6.28
## 1
                              fermentation* alcohol dehydrogenase activity
## 2
      HSP26
                  5.86 response to stress*
                                                   unfolded protein binding
## 3
       MLS1
                   5.64
                           glyoxylate cycle
                                                   malate synthase activity
## 4
                   5.56
       HXT5
                           hexose transport glucose transporter activity*
```

When the growth rate is restricted to 0.05, what is the average expression level across all genes in the "response to stress" biological process, separately for each limiting nutrient? What about genes in the "protein biosynthesis" biological process? *Hint:* 3 pipes: filter, group\_by, summarize.

```
## Source: local data frame [6 x 2]
##
##
      nutrient
                  meanexp
##
         (chr)
                    (dbl)
       Ammonia 0.9426667
## 1
## 2
       Glucose 0.7426667
## 3
       Leucine 0.8106667
## 4 Phosphate 0.9806667
## 5
       Sulfate 0.7430769
        Uracil 0.7313333
## 6
## Source: local data frame [6 x 2]
##
##
      nutrient
                   meanexp
##
         (chr)
                     (dbl)
       Ammonia -1.6133514
## 1
## 2
       Glucose -0.6911351
## 3
       Leucine -0.5735676
## 4 Phosphate -0.7496216
## 5
       Sulfate -0.9134807
## 6
        Uracil -0.8799454
```

#### Exercise 4

That was easy, right? How about some tougher ones.

First, some review. How do we see the number of distinct values of a variable? Use n\_distinct() within a summarize() call.

```
ydat %>% summarize(n_distinct(mf))
```

Which 10 biological process annotations have the most genes associated with them? What about molecular functions? *Hint:* 4 pipes: group\_by, summarize with n\_distinct, arrange, head.

```
## Source: local data frame [10 x 2]
##
##
                                                                    bp
                                                                           n
                                                                 (chr) (int)
##
## 1
                                          biological process unknown
                                                                         269
## 2
                                                                         182
                                                 protein biosynthesis
## 3
                                 protein amino acid phosphorylation*
                                                                          78
## 4
                                                                          73
                                                protein biosynthesis*
## 5
                             cell wall organization and biogenesis*
                                                                          64
##
   6
      regulation of transcription from RNA polymerase II promoter*
                                                                          49
## 7
                             nuclear mRNA splicing, via spliceosome
                                                                          47
## 8
                                                          DNA repair*
                                                                          44
                                                                          42
## 9
                                                 aerobic respiration*
## 10
                                               ER to Golgi transport*
                                                                          42
## Source: local data frame [10 x 2]
##
##
                                            mf
                                                    n
##
                                         (chr)
                                                (int)
## 1
                   molecular function unknown
                                                  886
## 2
          structural constituent of ribosome
                                                  185
## 3
                              protein binding
                                                  107
## 4
                                   RNA binding
                                                   63
## 5
                             protein binding*
                                                   53
## 6
                                  DNA binding*
                                                   44
## 7
                 structural molecule activity
                                                   43
## 8
                                                   40
                              GTPase activity
## 9
      structural constituent of cytoskeleton
                                                   39
## 10
               transcription factor activity
                                                   38
```

How many distinct genes are there where we know what process the gene is involved in but we don't know what it does? *Hint*: 3 pipes; filter where bp!="biological process unknown" & mf=="molecular function unknown", and after selecting columns of interest, pipe the output to distinct(). The answer should be 737, and here are a few:

```
## Source: local data frame [737 x 3]
##
##
      symbol
                                                                             bp
##
       (chr)
                                                                          (chr)
        SFB2
## 1
                                                         ER to Golgi transport
## 2
        EDC3
                                         deadenylylation-independent decapping
## 3
        PER1
                                                 response to unfolded protein*
## 4
       PEX25
                                      peroxisome organization and biogenesis*
## 5
        BNT5
                                                                   cytokinesis*
## 6
       CSN12 adaptation to pheromone during conjugation with cellular fusion
## 7
       SEC39
                                                              secretory pathway
        ABC1
## 8
                                                       ubiquinone biosynthesis
## 9
       PRP46
                                       nuclear mRNA splicing, via spliceosome
## 10
        EMAM
                                   mitochondrion organization and biogenesis*
## ..
## Variables not shown: mf (chr)
```

When the growth rate is restricted to 0.05 by limiting Glucose, which biological processes are the most upregulated? Show a sorted list with the most upregulated BPs on top, displaying the biological process and the average expression of all genes in that process rounded to two digits. *Hint:* 5 pipes: filter, group by, summarize, mutate, arrange.

```
## Source: local data frame [881 x 2]
##
##
                                                     bp meanexp
##
                                                  (chr)
                                                           (dbl)
## 1
                                         fermentation*
                                                           6.28
## 2
                                                           5.29
                                      glyoxylate cycle
## 3
      oxygen and reactive oxygen species metabolism
                                                           5.04
## 4
                                  fumarate transport*
                                                           5.03
                                                           4.32
## 5
                             acetyl-CoA biosynthesis*
## 6
                                       gluconeogenesis
                                                           3.64
## 7
                            fatty acid beta-oxidation
                                                           3.57
## 8
                                    lactate transport
                                                           3.48
## 9
                                 carnitine metabolism
                                                           3.30
## 10
                                  alcohol metabolism*
                                                           3.25
## ..
                                                             . . .
```

Group the data by limiting nutrient (primarily) then by biological process. Get the average expression for all genes annotated with each process, separately for each limiting nutrient, where the growth rate is restricted to 0.05. Arrange the result to show the most upregulated processes on top. The initial result will look like the result below. Pipe this output to a View() statement. What's going on? Why didn't the arrange() work? *Hint:* 5 pipes: filter, group\_by, summarize, arrange, View.

```
## Source: local data frame [5,257 x 3]
  Groups: nutrient [6]
##
##
      nutrient
                                      bp meanexp
##
          (chr)
                                   (chr)
                                           (dbl)
## 1
       Ammonia
                  allantoate transport
                                          6.6400
## 2
       Ammonia
                 amino acid transport*
                                          6.6400
## 3
       Ammonia
                   allantoin transport
                                          5.5600
## 4
       Ammonia
                   proline catabolism*
                                          5.1400
## 5
       Ammonia
                        urea transport
                                          5.1400
## 6
       Ammonia asparagine catabolism*
                                          4.7325
## 7
       Ammonia
                 allantoin catabolism*
                                          4.4400
## 8
       Ammonia
                     peptide transport
                                          3.9200
## 9
       Ammonia
                      glyoxylate cycle
                                          3.9100
## 10
       Ammonia
                  sodium ion transport
                                          3.2650
## ..
                                     . . .
                                             . . .
```

Let's try to further process that result to get only the top three most upregulated biolgocal processes for each limiting nutrient. Google search "dplyr first result within group." You'll need a filter(row\_number()....) in there somewhere. *Hint:* 5 pipes: filter, group\_by, summarize, arrange, filter(row\_number().... *Note:* dplyr's pipe syntax used to be %.% before it changed to %>%. So when looking around, you might still see some people use the old syntax. Now if you try to use the old syntax, you'll get a deprecation warning.

```
## Source: local data frame [18 x 3]
## Groups: nutrient [6]
##
##
       nutrient
                                                               bp meanexp
##
          (chr)
                                                            (chr)
                                                                    (dbl)
## 1
        Ammonia
                                           allantoate transport
                                                                    6.640
## 2
        Ammonia
                                          amino acid transport*
                                                                    6.640
## 3
        Ammonia
                                            allantoin transport
                                                                    5.560
## 4
        Glucose
                                                   fermentation*
                                                                    6.280
## 5
        Glucose
                                                glyoxylate cycle
                                                                    5.285
## 6
        Glucose oxygen and reactive oxygen species metabolism
                                                                    5.040
## 7
        Leucine
                                                   fermentation*
                                                                    4.150
## 8
        Leucine
                                                                    3.720
                                            fumarate transport*
## 9
        Leucine
                                                glyoxylate cycle
                                                                    3.650
## 10 Phosphate
                                glycerophosphodiester transport
                                                                    6.640
                                 vacuole fusion, non-autophagic
## 11 Phosphate
                                                                    4.195
                         regulation of cell redox homeostasis*
## 12 Phosphate
                                                                    4.030
## 13
        Sulfate
                                                                    3.400
                                         protein ubiquitination
## 14
        Sulfate
                                                                    3.270
                                            fumarate transport*
## 15
        Sulfate
                                  sulfur amino acid metabolism*
                                                                    2.690
```

There's a slight problem with the examples above. We're getting the average expression of all the biological processes separately by each nutrient. But some of these biological processes only have a single gene in them! If we tried to do the same thing to get the correlation between rate and expression, the calculation would work, but we'd get a warning about a standard deviation being zero. The correlation coefficient value that results is NA, i.e., missing. While we're summarizing the correlation between rate and expression, let's also show the number of distinct genes within each grouping.

```
ydat %>%
      group_by(nutrient, bp) %>%
      summarize(r=cor(rate, expression), ngenes=n_distinct(symbol))
## Warning in cor(c(0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.05, 0.
## 0.05, : the standard deviation is zero
## Source: local data frame [5,286 x 4]
## Groups: nutrient [?]
##
##
                    nutrient
                                                                                                                                                                               bp
                                                                                                                                                                                                                           r ngenes
##
                               (chr)
                                                                                                                                                                     (chr)
                                                                                                                                                                                                              (dbl)
                                                                                                                                                                                                                                     (int)
                                                                                           'de novo' IMP biosynthesis*
## 1
                       Ammonia
                                                                                                                                                                                            0.31247162
                                                                                                                                                                                                                                                  8
                                                                                                                                                                                                                                                  3
## 2
                       Ammonia
                                                       'de novo' pyrimidine base biosynthesis -0.04817745
## 3
                       Ammonia 'de novo' pyrimidine base biosynthesis*
                                                                                                                                                                                                                                                  4
                                                                                                                                                                                            0.16699596
## 4
                       Ammonia
                                                                       35S primary transcript processing
                                                                                                                                                                                            0.50795855
                                                                                                                                                                                                                                               13
## 5
                       Ammonia
                                                                   35S primary transcript processing*
                                                                                                                                                                                            0.42397321
                                                                                                                                                                                                                                               30
## 6
                       Ammonia
                                                                                                                  acetate biosynthesis
                                                                                                                                                                                            0.46768319
                                                                                                                                                                                                                                                  1
## 7
                       Ammonia
                                                                                                                         acetate metabolism
                                                                                                                                                                                            0.92909260
                                                                                                                                                                                                                                                  1
## 8
                                                                                                                      acetate metabolism* -0.68551933
                                                                                                                                                                                                                                                  1
                       Ammonia
## 9
                       Ammonia
                                                                                                        acetyl-CoA biosynthesis -0.85122895
                                                                                                                                                                                                                                                  1
## 10
                       Ammonia
                                                         acetyl-CoA biosynthesis from pyruvate
                                                                                                                                                                                                                                                  1
## ..
```

Take the above code and continue to process the result to show only results where the process has at least 5 genes. Add a column corresponding to the absolute value of the correlation coefficient, and show for each nutrient the singular process with the highest correlation between rate and expression, regardless of direction. *Hint:* 4 more pipes: filter, mutate, arrange, and filter again with row\_number()==1. Ignore the warning.

```
## Source: local data frame [6 x 5]
```

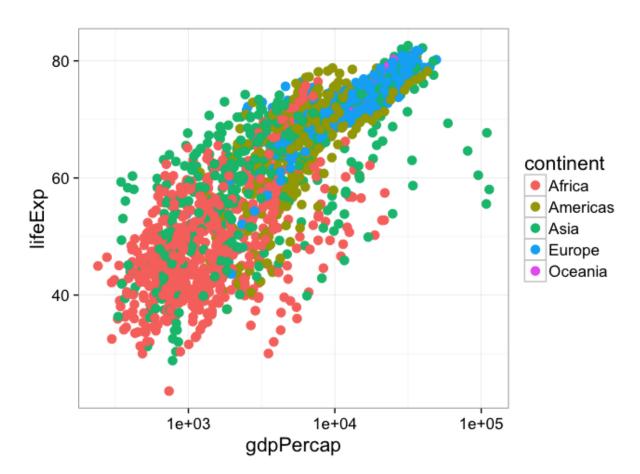
```
## Groups: nutrient [6]
##
##
      nutrient
                                                         bp
                                                                r ngenes
                                                                          absr
##
         (chr)
                                                      (chr) (dbl)
                                                                   (int) (dbl)
       Ammonia telomerase-independent telomere maintenance -0.91
## 1
                                                                          0.91
## 2
       Glucose telomerase-independent telomere maintenance -0.95
                                                                          0.95
## 3
                                                                          0.90
       Leucine telomerase-independent telomere maintenance -0.90
                                                                       7
## 4 Phosphate telomerase-independent telomere maintenance -0.90
                                                                          0.90
                                                                       7
## 5
       Sulfate
                                 translational elongation* 0.79
                                                                          0.79
## 6
        Uracil telomerase-independent telomere maintenance -0.81
                                                                          0.81
```

## **Data Visualization**

### Exercise 1

Re-create this same plot from scratch without saving anything to a variable. That is, start from the ggplot call.

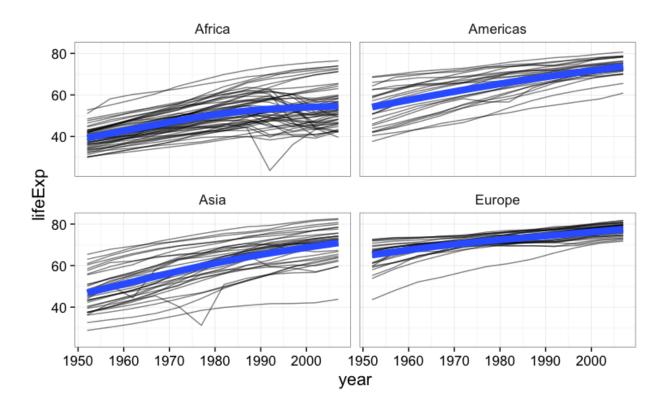
- Start with the ggplot() function.
- Use the gm data.
- Map gdpPercap to the x-axis and lifeExp to the y-axis.
- Add points to the plot
- Make the points size 3
- Map continent onto the aesthetics of the point
- Use a log10 scale for the x-axis.



### Exercise 2

1. Make a scatter plot of lifeExp on the y-axis against year on the x.

- 2. Make a series of small multiples faceting on continent.
- 3. Add a fitted curve, smooth or lm, with and without facets.
- 4. **Bonus**: using <code>geom\_line()</code> and and aesthetic mapping <code>country</code> to <code>group=</code>, make a "spaghetti plot", showing <code>semitransparent</code> lines connected for each country, faceted by continent. Add a smoothed loess curve with a thick (lwd=3) line with no standard error stripe. Reduce the opacity (alpha=) of the individual black lines. <code>Don't</code> show Oceania countries (that is, filter() the data where <code>continent!="Oceania"</code> before you plot it).



### Exercise 3

- 1. Make a jittered strip plot of GDP per capita against continent.
- 2. Make a box plot of GDP per capita against continent.
- 3. Using a log10 y-axis scale, overlay semitransparent jittered points on top of box plots, where outlying points are colored.
- 4. **BONUS**: Try to reorder the continents on the x-axis by GDP per capita. Why isn't this working as expected? See **?reorder** for clues.

### Exercise 4

1. Plot a histogram of GDP Per Capita.

- 2. Do the same but use a log10 x-axis.
- 3. Still on the log10 x-axis scale, try a density plot mapping continent to the fill of each density distribution, and reduce the opacity.
- 4. Still on the log10 x-axis scale, make a histogram faceted by continent *and* filled by continent. Facet with a single column (see ?facet\_wrap for help).
- 5. Save this figure to a 6x10 PDF file.

## RNA-seq

### Exercise 1

If we look at our metadata, we see that the control samples are SRR1039508, SRR1039512, SRR1039516, and SRR1039520. This bit of code will take the rawcounts data, mutate it to add a column called **controlmean**, then select only the gene name and this newly created column, and assigning the result to a new object called **meancounts**.

```
meancounts <- rawcounts %>%
 mutate(controlmean = SRR1039508+SRR1039512+SRR1039516+SRR1039520) %>%
 select(ensgene, controlmean)
meancounts
## Source: local data frame [64,102 x 2]
##
##
              ensgene controlmean
##
                (chr)
                            (int)
     ENSG0000000003
                             3460
## 1
     ENSG00000000005
## 3 ENSG0000000419
                             2092
## 4 ENSG00000000457
                             1001
## 5
                              254
     ENSG00000000460
## 6 ENSG00000000938
                                3
## 7 ENSG0000000971
                            21325
## 8 ENSG0000001036
                             5949
## 9 ENSG0000001084
                             2630
## 10 ENSG0000001167
                             1876
## ..
```

1. Build off of this code, mutate it once more (prior to the select()) function, to add another column called treatedmean that takes the mean of the expression values of the treated samples. Then select only the ensgene, controlmean and treatedmean columns, assigning it to a new object called meancounts. It should look like this.

```
## Source: local data frame [64,102 x 3]
##
##
              ensgene controlmean treatedmean
##
                 (chr)
                             (int)
                                          (int)
## 1
      ENSG00000000003
                              3460
                                           2475
##
      ENSG0000000005
                                  0
                                              0
  3 ENSG00000000419
                              2092
                                           2187
## 4 ENSG00000000457
                              1001
                                            935
```

```
## 5
     ENSG00000000460
                               254
                                            213
## 6
     ENSG00000000938
                                  3
                                              0
## 7
      ENSG00000000971
                             21325
                                          26953
## 8 ENSG0000001036
                              5949
                                           4491
## 9 ENSG0000001084
                                           2291
                              2630
## 10 ENSG0000001167
                              1876
                                           1264
## ..
                                . . .
```

2. Directly comparing the raw counts is going to be problematic if we just happened to sequence one group at a higher depth than another. Later on we'll do this analysis properly, normalizing by sequencing depth. But for now, summarize() the data to show the sum of the mean counts across all genes for each group. Your answer should look like this:

```
## Source: local data frame [1 x 2]
##
## sum(controlmean) sum(treatedmean)
## (int) (int)
## 1 89561179 85955244
```

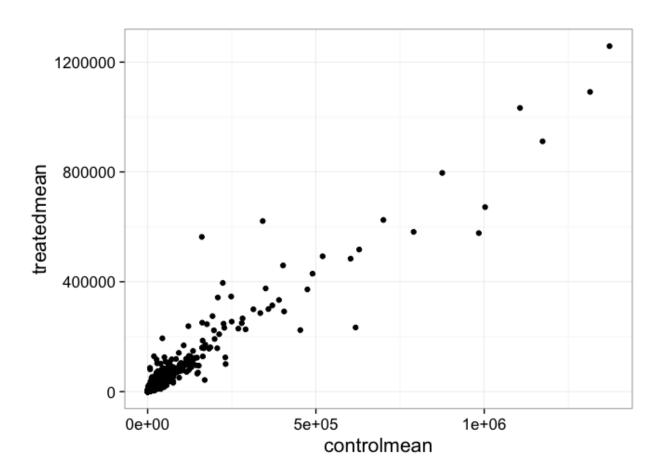
#### Exercise 2

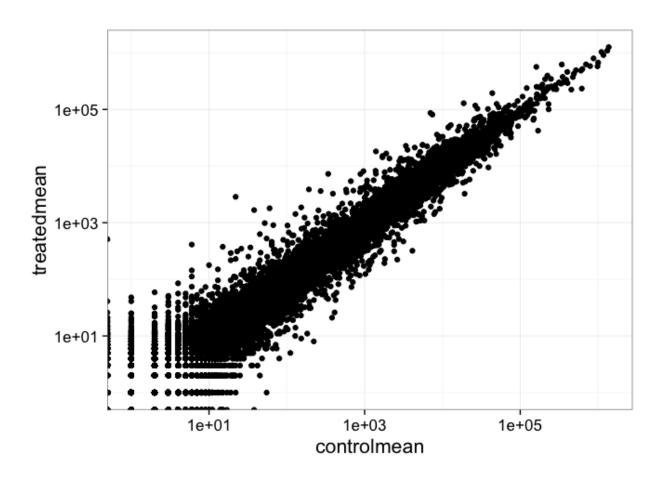
- 1. Create a scatter plot showing the mean of the treated samples against the mean of the control samples.
- Wait a sec. There are 60,000-some rows in this data, but I'm only seeing a few dozen dots at most outside of the big clump around the origin. Try plotting both axes on a log scale (hint: . . . + scale\_...\_log10())

### Exercise 3

Go back and refresh your memory on using <code>inner\_join()</code> to join two tables by a common <code>column/key</code>. You previously downloaded <code>annotables\_grch37.csv</code> from <code>bioconnector.org/data</code>. Load this data with <code>read\_csv()</code> into an object called <code>anno</code>. Pipe it to <code>View</code> or click on the object in the Environment pane to view the entire dataset. This table links the unambiguous Ensembl gene ID to things like the gene symbol, full gene name, location, Entrez gene ID, etc.

```
anno <- read_csv("data/annotables_grch37.csv")
anno</pre>
```





```
## Source: local data frame [67,416 x 9]
##
##
               ensgene entrez
                                 symbol
                                                                 end strand
                                           chr
                                                    start
##
                 (chr)
                         (int)
                                  (chr) (chr)
                                                    (int)
                                                               (int)
                                                                      (int)
      ENSG0000000003
## 1
                          7105
                                 TSPAN6
                                             Χ
                                                99883667
                                                           99894988
                                                                         -1
## 2
                                             Χ
      ENSG00000000005
                        64102
                                   TNMD
                                                99839799
                                                           99854882
                                                                          1
##
  3
      ENSG00000000419
                         8813
                                   DPM1
                                            20
                                                49551404
                                                           49575092
                                                                         -1
## 4
                        57147
                                  SCYL3
                                             1 169818772 169863408
                                                                         -1
     ENSG00000000457
## 5
                        55732 Clorf112
      ENSG00000000460
                                             1 169631245 169823221
                                                                          1
## 6
     ENSG00000000938
                          2268
                                    FGR
                                                27938575
                                                           27961788
                                                                         -1
## 7
      ENSG00000000971
                          3075
                                    CFH
                                             1 196621008 196716634
                                                                          1
## 8
      ENSG0000001036
                          2519
                                  FUCA2
                                             6 143815948 143832827
                                                                         -1
      ENSG0000001084
                          2729
                                   GCLC
                                                                         -1
                                                53362139
                                                           53481768
## 10 ENSG0000001167
                          4800
                                   NFYA
                                                41040684
                                                           41067715
                                                                          1
##
                           . . .
                                     . . .
## Variables not shown: biotype (chr), description (chr)
```

1. Take our newly created meancounts object, and arrange it descending by the absolute value (abs()) of the log2fc column. The results should look like this:

```
## Source: local data frame [27,450 x 4]
##
##
               ensgene controlmean treatedmean
                                                     log2fc
##
                 (chr)
                              (int)
                                           (int)
                                                      (dbl)
## 1
      ENSG0000109906
                                 22
                                            2862
                                                   7.023376
## 2
      ENSG00000250978
                                  6
                                             411
                                                   6.098032
##
  3
     ENSG00000128285
                                 55
                                                1 - 5.781360
## 4
      ENSG00000260802
                                  1
                                               48
                                                   5.584963
## 5
                                 38
                                            1670
                                                   5.457705
      ENSG00000171819
##
      ENSG00000137673
                                  1
                                                   5.357552
## 7
      ENSG00000127954
                                 60
                                            1797
                                                   4.904484
      ENSG00000249364
                                  2
                                              59
                                                   4.882643
      ENSG00000267339
                                222
                                                8 -4.794416
## 10 ENSG0000100033
                                             375
                                                   4.643856
                                 15
## ..
```

2. Continue on that pipeline, and inner\_join() it to the anno data by the ensgene column. Either assign it to a temporary object or pipe the whole thing to View to take a look. What do you notice? Would you trust these results? Why or why not?

```
## Source: local data frame [29,034 x 12]
##
## ensgene controlmean treatedmean log2fc entrez
## (chr) (int) (int) (dbl) (int)
```

```
## 1
      ENSG0000109906
                                22
                                           2862
                                                 7.023376
                                                                7704
## 2 ENSG00000250978
                                 6
                                            411
                                                                  NA
                                                 6.098032
## 3 ENSG00000128285
                                55
                                              1 - 5.781360
                                                                2847
## 4 ENSG00000260802
                                             48
                                                 5.584963
                                 1
                                                              401613
## 5
     ENSG00000171819
                                38
                                           1670
                                                 5.457705
                                                               10218
## 6 ENSG00000137673
                                 1
                                             41
                                                 5.357552
                                                                4316
## 7 ENSG00000127954
                                60
                                           1797
                                                 4.904484
                                                               79689
## 8 ENSG00000249364
                                 2
                                             59
                                                 4.882643 101928858
                                              8 -4.794416
## 9 ENSG00000267339
                               222
                                                              148145
## 10 ENSG0000100033
                                15
                                            375
                                                 4.643856
                                                                5625
## ..
## Variables not shown: symbol (chr), chr (chr), start (int), end (int),
     strand (int), biotype (chr), description (chr)
```

#### Exercise 4

1. Using a %>%, arrange the results by the adjusted p-value.

```
## Source: local data frame [64,102 x 7]
##
##
                         baseMean log2FoldChange
                  row
                                                       lfcSE
                                                                  stat
##
                            (dbl)
                                            (dbl)
                (chr)
                                                       (dbl)
                                                                  (dbl)
                         997.4398
                                        4.285847 0.19605831
## 1
      ENSG00000152583
                                                              21.86006
                                        1.434388 0.08411178
                                                              17.05336
      ENSG00000148175 11193.7188
## 3 ENSG00000179094
                         776.5967
                                        2.984244 0.18864120
                                                              15.81968
## 4 ENSG00000109906
                         385.0710
                                        5.137007 0.33077733
                                                              15.53010
## 5 ENSG00000134686
                       2737.9820
                                        1.368176 0.09059205
                                                              15.10261
## 6
     ENSG00000125148
                       3656.2528
                                        2.127162 0.14255648
                                                              14.92154
## 7
     ENSG00000120129
                       3409.0294
                                        2.763614 0.18915513
                                                              14.61030
## 8
      ENSG00000189221
                       2341.7673
                                        3.043757 0.21020671
                                                              14.47983
## 9 ENSG0000178695
                        2649.8501
                                       -2.374629 0.17015595 -13.95560
## 10 ENSG00000101347 12703.3871
                                        3.414854 0.24787488
                                                              13.77652
## ..
## Variables not shown: pvalue (dbl), padj (dbl)
```

2. Continue piping to inner\_join(), joining the results to the anno object. See the help for ?inner\_join, specifically the by= argument. You'll have to do something like ... %>% inner\_join(anno, by=c("row"="ensgene")). Once you're happy with this result, reassign the result back to res. It'll look like this.

```
## row baseMean log2FoldChange lfcSE stat
## 1 ENSG00000152583 997.4398 4.285847 0.19605831 21.86006
## 2 ENSG00000148175 11193.7188 1.434388 0.08411178 17.05336
```

```
## 3 ENSG00000179094
                        776.5967
                                        2.984244 0.18864120 15.81968
## 4 ENSG00000179094
                        776.5967
                                        2.984244 0.18864120 15.81968
## 5 ENSG0000109906
                        385.0710
                                        5.137007 0.33077733 15.53010
   6 ENSG00000134686
                       2737.9820
                                        1.368176 0.09059205 15.10261
##
            pvalue
                                     entrez
                                              symbol chr
                                                                           end
                             padj
                                                              start
                                        8404 SPARCL1
  1 6.235872e-106 1.119464e-101
                                                        4
                                                           88394487
                                                                     88452213
##
  2
     3.300017e-65
                     2.962096e-61
                                        2040
                                                STOM
                                                        9 124101355 124132531
## 3
     2.276377e-56
                     1.362184e-52 102465532
                                                PER1
                                                       17
                                                            8043790
                                                                      8059824
    2.276377e-56
                                                PER1
## 4
                     1.362184e-52
                                        5187
                                                       17
                                                            8043790
                                                                      8059824
                                              ZBTB16
## 5
     2.170243e-54
                     9.740052e-51
                                        7704
                                                       11 113930315 114121398
##
     1.556576e-51
                     5.588730e-48
                                        1912
                                                PHC2
                                                           33789224
                                                                     33896653
##
     strand
                    biotype
## 1
         -1 protein coding
## 2
         -1 protein coding
         -1 protein_coding
## 3
         -1 protein coding
## 4
          1 protein coding
## 5
## 6
         -1 protein coding
##
                                                                    description
## 1
                          SPARC-like 1 (hevin) [Source: HGNC Symbol; Acc: 11220]
                                        stomatin [Source:HGNC Symbol;Acc:3383]
## 2
## 3
                       period circadian clock 1 [Source: HGNC Symbol; Acc: 8845]
## 4
                       period circadian clock 1 [Source: HGNC Symbol; Acc: 8845]
## 5 zinc finger and BTB domain containing 16 [Source: HGNC Symbol; Acc: 12930]
           polyhomeotic homolog 2 (Drosophila) [Source: HGNC Symbol; Acc: 3183]
## 6
```

3. How many are significant with an adjusted p-value <0.05? (Pipe to filter()).

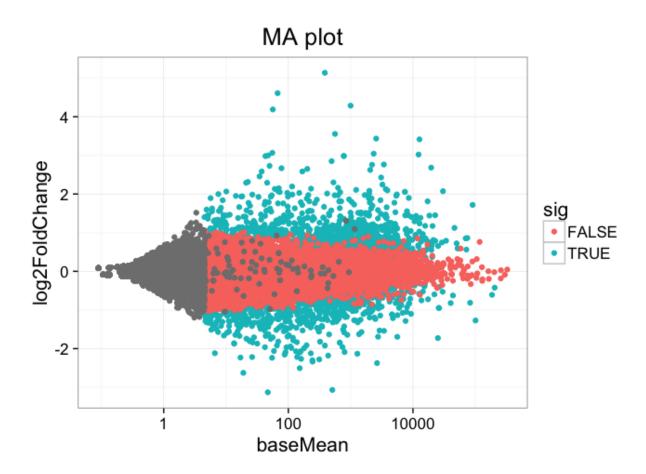
```
## Source: local data frame [2,852 x 15]
##
                         baseMean log2FoldChange
##
                                                        lfcSE
                                                                   stat
                   row
                                                                  (dbl)
##
                 (chr)
                            (dbl)
                                            (dbl)
                                                        (dbl)
##
      ENSG00000152583
                         997.4398
                                         4.285847 0.19605831
                                                               21.86006
##
     ENSG00000148175 11193.7188
                                         1.434388 0.08411178
                                                               17.05336
##
     ENSG00000179094
                         776.5967
                                         2.984244 0.18864120
                                                               15.81968
## 4
     ENSG00000179094
                         776.5967
                                         2.984244 0.18864120
                                                               15.81968
##
   5
     ENSG00000109906
                         385.0710
                                         5.137007 0.33077733
                                                               15.53010
## 6
     ENSG00000134686
                        2737.9820
                                         1.368176 0.09059205
                                                               15.10261
## 7
      ENSG00000125148
                        3656.2528
                                         2.127162 0.14255648
                                                               14.92154
## 8
      ENSG00000120129
                        3409.0294
                                         2.763614 0.18915513
                                                               14.61030
## 9
      ENSG00000189221
                        2341.7673
                                         3.043757 0.21020671
                                                               14.47983
## 10 ENSG00000178695
                        2649.8501
                                        -2.374629 0.17015595 -13.95560
##
## Variables not shown: pvalue (dbl), padj (dbl), entrez (int), symbol (chr),
```

```
## chr (chr), start (int), end (int), strand (int), biotype (chr),
## description (chr)
```

### Exercise 5

Look up the Wikipedia articles on MA plots and volcano plots. An MA plot shows the average expression on the X-axis and the log fold change on the y-axis. A volcano plot shows the log fold change on the X-axis, and the  $-log_{10}$  of the p-value on the Y-axis (the more significant the p-value, the larger the  $-log_{10}$  of that value will be).

1. Make an MA plot. Use a  $log_{10}$ -scaled x-axis, color-code by whether the gene is significant, and give your plot a title. It should look like this. What's the deal with the gray points?



2. Make a volcano plot. Similarly, color-code by whether it's significant or not.

