Day 8: Advanced DESeq2 Experimental Designs Homework

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**The homework data and metadata are located in**

**GITHUB????**

**The homework will be completed on your local computer using R. Download all files before you start your homework.**

Question 1. Load in the files labeled h8\_data1.txt and homework\_day8\_q1\_metadata1.csv as data frames in R.

1. How many different batches are there? **4**
2. How many different values are in the treatment column? **2**
3. Create a DESeq2-compatible matrix from the count data. Run DESeq2. Use a design that includes a batch effect correction and the treatment. **~batch + treatment**
4. Which gene is the top hit in arsenic treatment? What is the adjusted p-value? **Gene267, padj=2.18746283181935e-23**

Question 2: Load in the counts file labeled h8\_data2.txt

Uh-oh! You accidentally deleted your metadata file! You’ll have to re-create it before running DESeq2. Luckily, you named all of your columns in your counts file using the same pattern: sex\_treatment\_replicate.

You used three different treatments: control, LPS, and PolyI:C (PIC)

1. Rebuild the metadata file. You can use any program of your choice (R, Excel, vim, etc.)

**Answer in folder**

1. You decide to group the samples by treatments only (ignoring sex). Write the design formula that investigates both treatment effects but not differences between the sexes. **~treatment**
2. Run DESeq2. What is the top hit (lowest padj) for LPS treatment? How about PIC? Hint: Use a contrast

**LPS:gene72, padj=1.23932791648368e-12**

**PIC:gene120, padj=7.69129153516872e-15**

1. Reviewer 2 thinks you weren’t thorough enough in your analysis above- after all, you didn’t correct for baseline variations (not interactions) due to sex differences. What design formula would you use to satisfy their request? **~sex + treatment**
2. Did the top gene in PIC treatment change using the new design formula? **Yes, in PIC: gene81**
3. How would you adjust the design formula to instead look at sex-specific effects in the treatment? **~sex + treatment + sex:treatment**
4. What is the top hit for the interaction effect of sex and LPS treatment?