

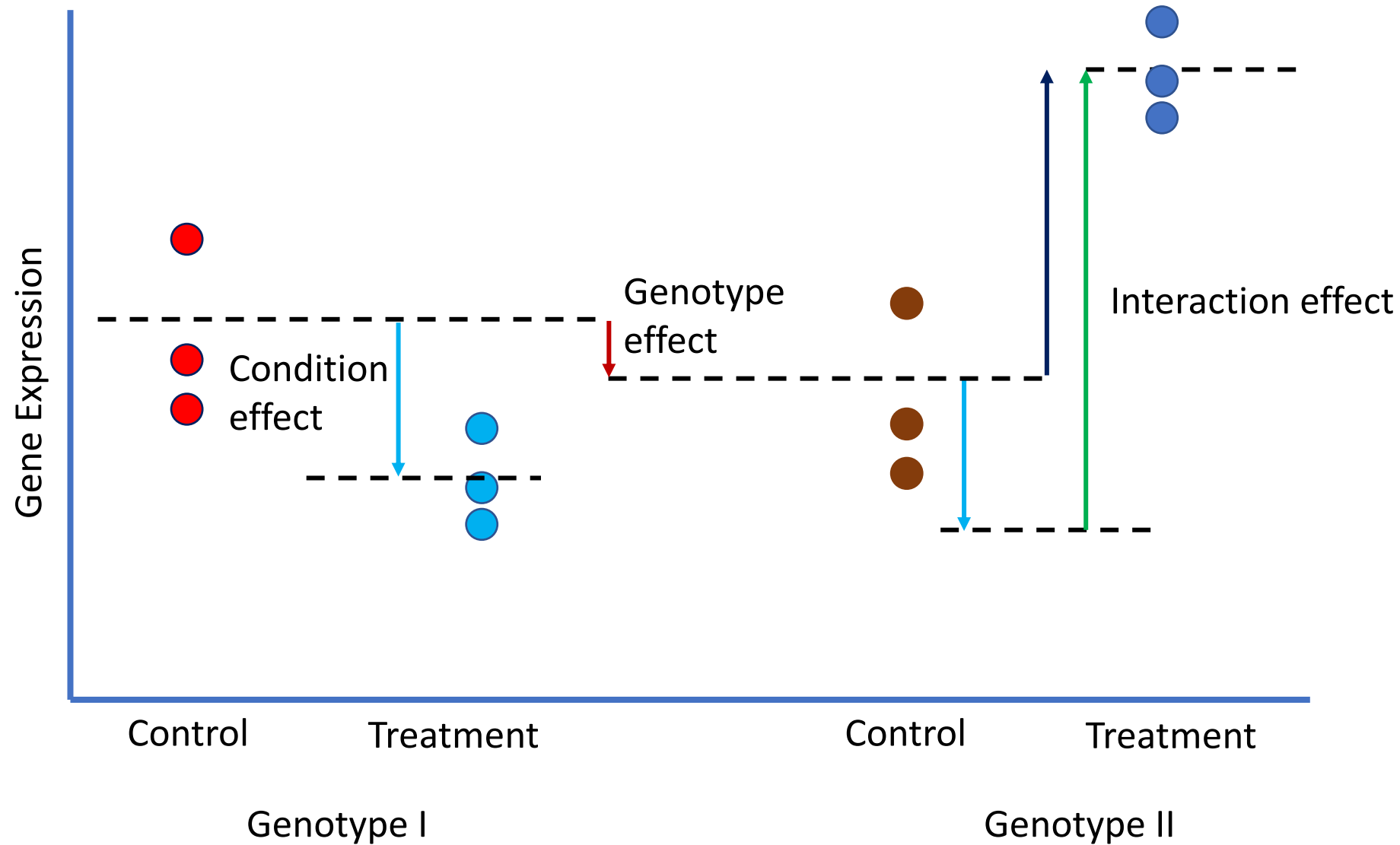
Day 8: Multifactor Designs in DESeq2

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Goals of the Day

1. Understand the principles of DESeq2 experimental designs
2. Interpret results of multifactor designs
3. Learn how to translate biological experimental setups into DESeq2 design matrices

With multifactor designs, we are interested in more than one comparison



Exercise (Follow along on your worksheet)

How to look up help:

Michael Love (author of original DESeq2 paper) is active on support.bioconductor.org:

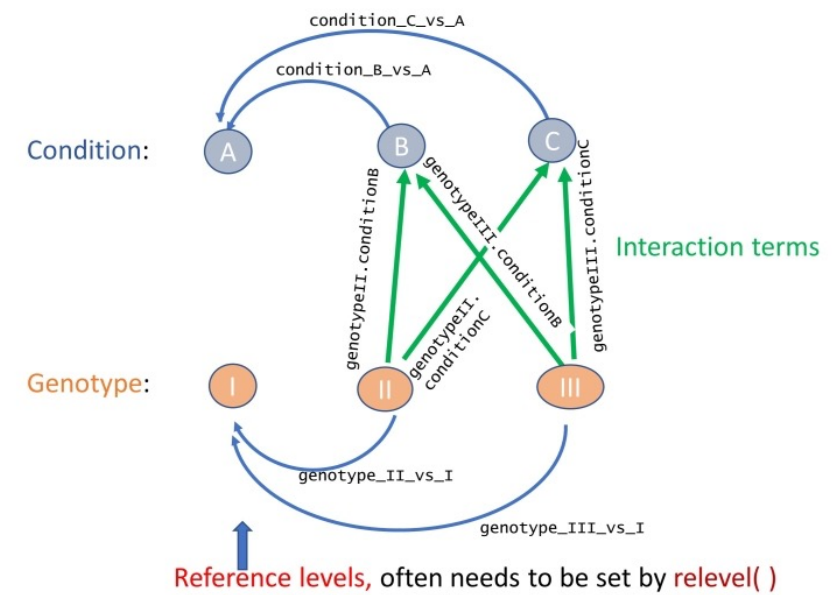
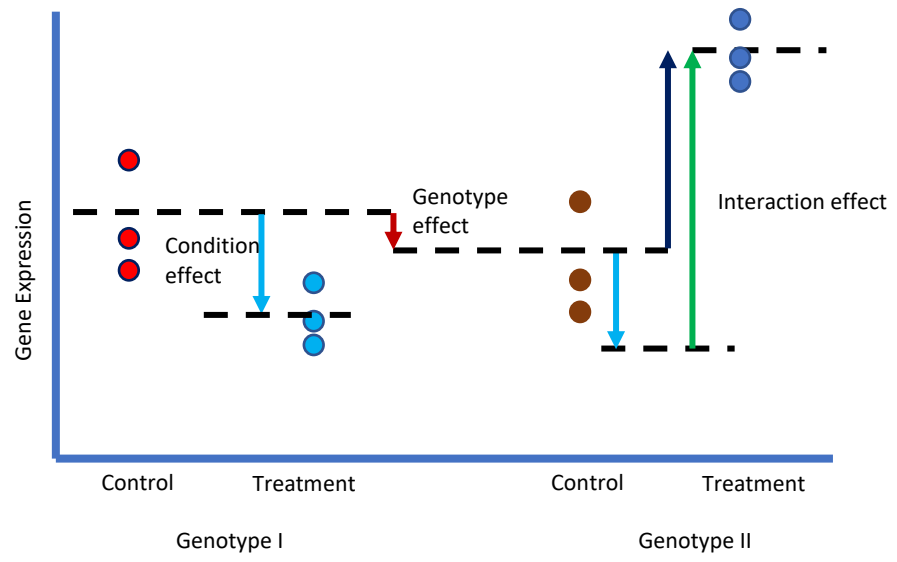
- Googling the phrase “DESeq2 multifactor design” will bring up years of forums with people asking for design help
- For a more comprehensive dive into DESeq2, GLMs, and the underlying statistics, take a look at the resources posted on the last slide

Independent Exercise

1. Load in the files labeled “independent_exercise_counts.txt” and “independent_exercise_metadata.csv”
2. Create a design matrix to account for batch, treatment1, treatment2, and the double treatment
3. Use contrasts to find the effects of treatment1, treatment2, and treatment1+2
4. Find summary statistics and generate MA plots for these results.

Remember, DESeq2 is just adding and subtracting fold change estimates

- Draw out a cartoon or a diagram to help you figure out which contrasts to make:



- Write out your question, contrast, and null hypothesis. Does your contrast fit your question?
Question: Does Gene X respond differently to treatment between Genotype I and Genotype II?
Contrast: Subtracting the treatment effect in Genotype I from the treatment effect in Genotype II
Null Hypothesis: Treatment effect in Genotype I minus treatment effect in Genotype II is zero

Resources: Bioconductor, DESeq2 Vignette, GLM Class by Michael Love

- <https://support.bioconductor.org/p/98628/>
- <http://bioconductor.org/packages/devel/bioc/vignettes/DESeq2/inst/doc/DESeq2.html>

Full course going over statistics, GLMs, and fitting process in DESeq2, with helpful video guides and example datasets

<http://rafalab.github.io/pages/harvardx.html>

Download day8_homework.pdf and follow the instructions in the document. All data are available in the day8/homework_files directory