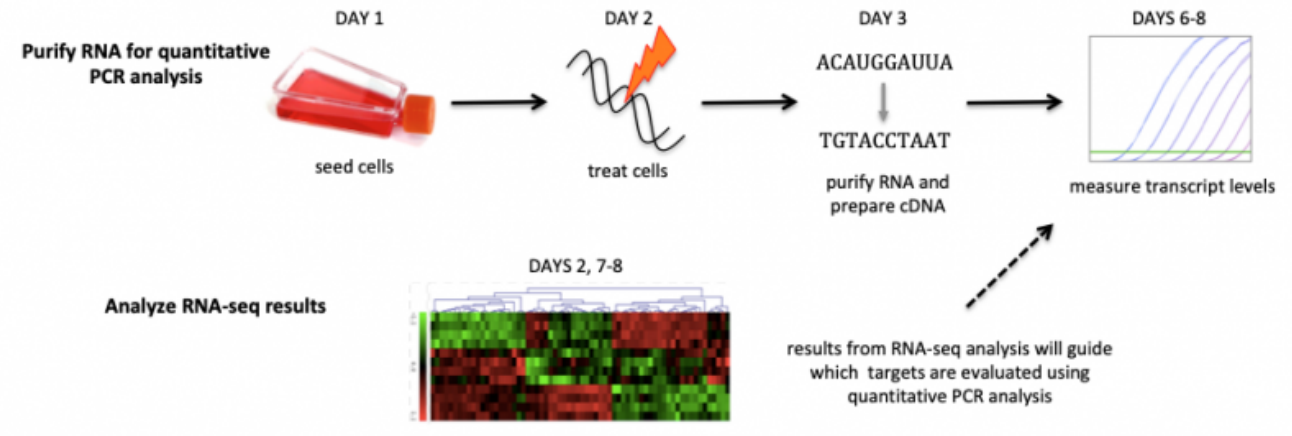
M2D8: Continue RNA-seq data analysis

1. Start R.studio.cloud exercise 4
2. Complete Ex.3 and qPCR analysis

Extra Help for Mod2:

- Sent code for Ex3 & first part of Ex4
- Friday we will help you through all Ex4
- Outline of exact figures you should include on wiki

Mod2: Experimental overview



How does gene expression change following etoposide treatment in DLD-1 and A549 cells?

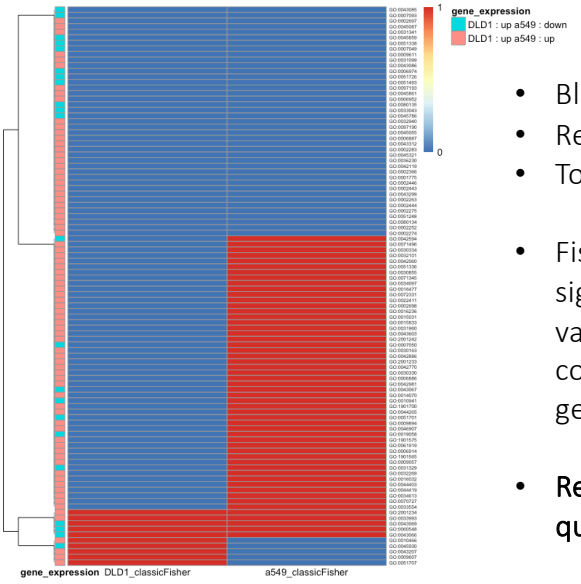
Purpose of RNA sequencing

- Understanding the sum of mRNA in a cell or organism (transcriptome) is key to connect genetic information with protein expression
- RNA-seq can suggest which genes are turned on or off in a cell by their level of expression
- Allows us to more deeply understand the biology of a cell and assess changes that may indicate disease
  - RNA-Seq has the potential to identify new disease biology
- RNA-Seq data can provide a unique snapshot of the transcriptomic status of a disease
  - Looks at an **unbiased** population of transcripts
  - Allows the identification of novel transcripts that would not be detected through other technologies
- These results could further highlight more effective prevention, diagnostics, and therapy

How would you design an experiment to determine the effect of an unknown drug?

technologynetworks.com, RNA-seq: Basics, Applications and Protocol, by Ruairi J Mackenzie

Top 100 enriched GO terms for DLD-1 & A549



- Blue= significant by Fisher statistic
- Red= not significant
- Top 100 was a value we chose for analysis

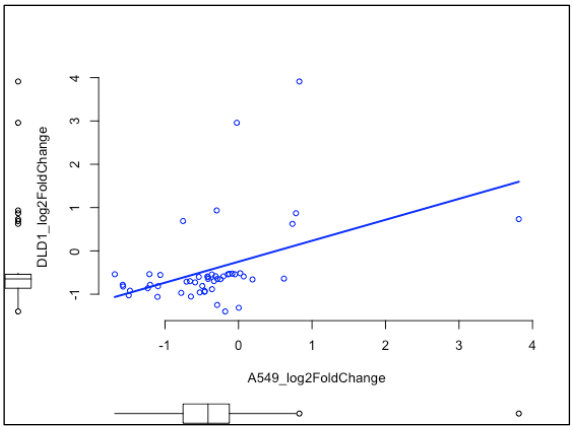
- Fisher exact test looks at the observed number of significantly differentially expressed genes (p-value cutoff 0.1) assigned to each GO term and compares it to the expected number of significant genes at random

- Read prompts on the wiki carefully and address questions in your Benchling notebook

GO terms associated with qPCR gene choices and Ex. 4 analysis

Gene Ontology term	Abbreviation for R
RNA splicing	RNA_spl_genes
Cell adhesion	cell_adhesion_genes
Cell proliferation	cell_pro_genes
Regulation of mitotic cell cycle	Reg_mcc_genes

Notes on Interpreting Scatterplots



- Comparing DLD-1 and A549 L2FC of genes in RNA splicing GO term
- Blue dots represent DEGs in this GO term
- Axis = box plot and black dots are DEG for one cell line. Box plot = quartiles (25-75%). Black circles on the axis are the points which fall outside the quartiles.
- The blue line is the correlation/regression line, and the slope tells us if it's a positive/negative correlation or if there is no correlation
- NOTE: just because one GO annotation/pathway is "upregulated/downregulated" doesn't mean that every gene is expressed in the same direction
- Some genes associated with the GO term are driving upregulation/downregulation of the pathway.
- Not all are similarly expressed--some genes may even be expressed in the opposite direction.

M2D8 “Lab” Checklist

1. Work through the thought questions on the wiki introduction in your Benchling notebook.
2. Work on RNA-seq analysis for Ex3 and Ex4
3. Complete qPCR analysis with confidence interval and Student’s t-test statistical analysis
   - (if you haven’t already)

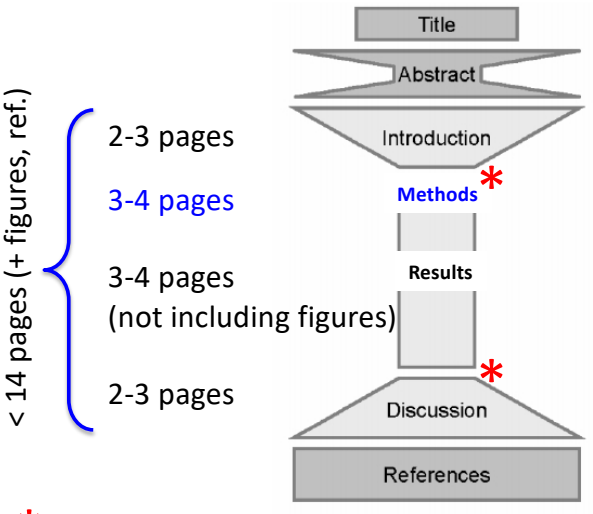
M2D9HW: Outline of figures

- you don’t need to draft actual figures
  - 1 sentence: describes the figure
  - 1 sentence: motivation
  - 1 sentence: transition
- Figure order can be found on under assignments tab -> Research Article

Assignments tab→ Research Article→ Results

1. Figure 1
   - experimental overview / schematic illustrating the work-flow (just the key steps!) used in your research project
2. Figure 2 (this figure should include three panels)
   - Panel A: tables with top 5 GO terms in DLD-1 and DLD-1 + etoposide
   - Panel B: bar graph containing the qPCR results for the genes of interest, including statistics
   - Panel C: heatmap comparing genes of interest across DLD-1 qPCR data, DLD-1 RNA-seq data, and A549 data
3. Figure 3 (this figure should include two panels)
   - Panel A: plot of PCA data showing DLD-1 + etoposide and A549 + etoposide
   - Panel B: heatmap comparing DLD-1 + etoposide and A549 + etoposide
4. Figure 4
   - heatmap comparing 4 GO terms
5. Figure 5
   - scatterplots generated from the GO terms used in Fig. 3

Mod2 Research Article



- Title, Abstract (10%)
- Introduction (10%)
- Methods (20%)
- Results, Figures and captions (50%)

-Top 5 Up GO terms, & Down GO terms (Ex2)

- qPCR

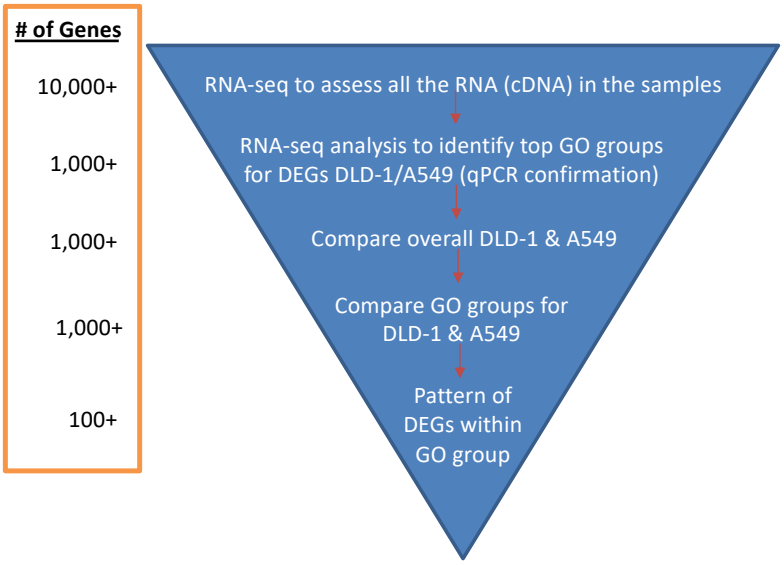
- RNA seq plots from Ex3 and Ex4

- Discussion (10%)
- References

* Last paragraph of introduction and first paragraph of discussion reiterate similar message

Introduction: Big data figures deserve a large scope introduction

- Introduction should cover more than what etoposide does to a single cell type or a subset of DNA damage genes
- Figures are documenting the analysis of thousands of genes
- Focused on identifying patterns of differential gene expression instead of identifying individual genes
  - Only looking at individual genes to confirm data set



Methods: Details for methods RNA-sequencing and analysis

- **Sequencing:** HiSeq 2000 sequencing at the Massachusetts Institute of Technology BioMicro Center.
- **Data analysis:** performed according to a workflow developed by Amanda Kedaigle, Anne Shen and Ernest Fraenkel at the Massachusetts Institute of Technology using Rstudio.cloud.
- DESeq2 (v. 1.26.0)
- Transcriptomic data for A549 cell line was obtained from the Gene Expression Omnibus (Wang 2017).

Results/Discussion: Notes from the wiki

- Results sub-sections titles with conclusion for each experiment
  - Each paragraph = one topic, but each sub-section may have more than one paragraph.
- Each results paragraph should start with an overview, or introductory, sentence that motivates and introduces the experiment.
  - What experiment did you do to get GO tables?
- Results section is a place to present all aspects of the data, even what doesn't match the hypothesis/narrative
- End each results paragraph with a concluding sentence that provides a transition to the next experimental step.
  - Lead into your next figure/topic

Mod2 major assignments

- **Research Article (20%)**
  - individual, submit on Stellar
  - due Monday April 20th at 10pm
  - format: word document, figures can be submitted separately
- **Journal Club Presentation (17.5%)**
  - presentation **slides** due on Stellar April 11th 10pm
  - Presentation **video** due to Dropbox April 11th 10pm
- Lab quizzes ~~M2D7~~, M2D9
- Homework and Notebook (10%)
- Blog (5%), 3 posts for full credit
  - ~~4/6 at 10 pm~~, 4/13 at 10 pm, 4/21 at 10 pm, 5/12 at 10pm