M2D8: Mod2 data analysis

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

Extra Help for Mod2:

- Today we will give you code for Ex 3
- Thursday we will help you through all Ex4
- Outline of exact figures you should include on wiki

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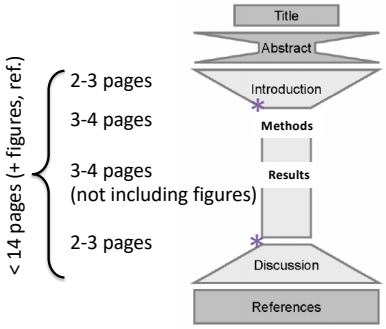
Mod2 Research Article



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

-Top 5 Up GO terms, top 5 Down GO terms (Ex2)
- qPCR
-Plots from Ex3: PCA and heatmap
-Plots from Ex4: GO heatmaps and scatterplots

- Discussion (10%)
- References (not included in page count)

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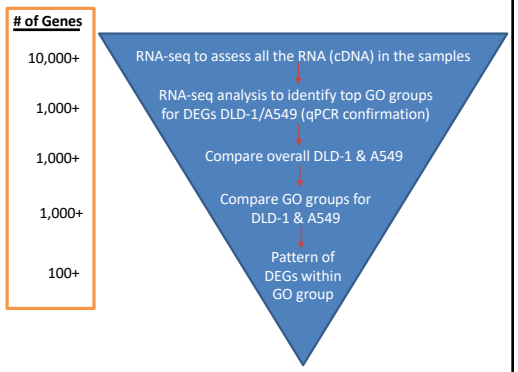
Big data figures deserve a large scope introduction

- 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
- Introduction should cover more than what etoposide does to a single cell type or a subset of DNA damage genes



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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: histogram 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

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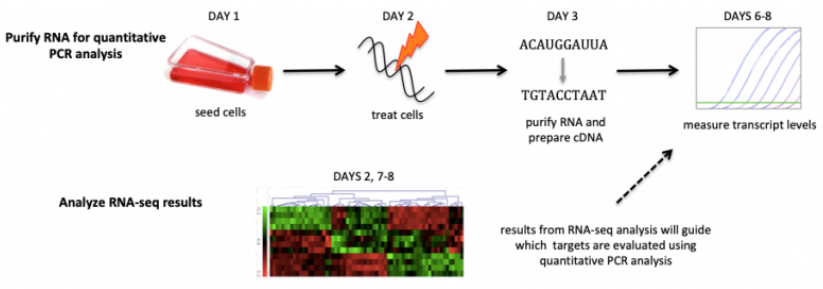
Mod2: Experimental overview



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Overview of the purpose of RNA sequencing

- Understanding the sum of mRNA in a cell or organism (called transcriptome) is key if we are to connect the information about our genes with protein expression
- RNA-seq can suggest which genes are turned on or off in a cell by their level of expression
- This allows scientists 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
- These results could further highlight more effective prevention, diagnostics, and therapy
- RNA-Seq data can provide a unique snapshot of the transcriptomic status of a disease and look at an **unbiased** population of transcripts that allows the identification of novel transcripts that would not be detected through other technologies
- How would you determine the effect of an unknown drug?

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

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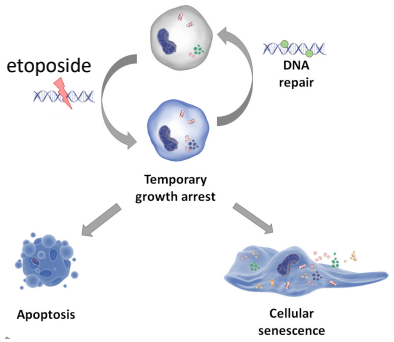
How does gene expression change upon etoposide treatment in DLD-1?



Top up and down gene ontology terms:

- response to stimulus
- cell communication
- Signaling
- Signal transduction
- cellular response to stimulus
- chromosome organization
- RNA splicing
- biogenesis/cellular component organization
- RNA splicing
- mRNA splicing

Soto Gamez et al. Regulation of Survival Networks in Senescent Cells: From Mechanisms to Interventions. JMB July 2019

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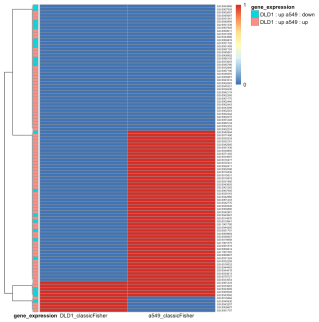
Top 100 enriched GO terms across DLD-1 and A549



- 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**

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

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

Note: GO terms on M2D6 gene list

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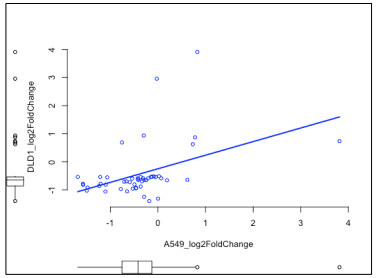
Notes on Interpreting Scatterplots



- 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. Black circles on the axis are the points which fall outside the quartiles (25-75%).
- 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, and some genes may even be expressed in the opposite direction.

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M2D8 "Lab" Checklist

1. Work through the thought questions on the wiki introduction in your benchling notebook.
2. Ask questions and understand the RNA-seq analysis for Ex3 and start Ex4
3. Complete qPCR analysis with confidence interval and Student's t-test statistical analysis
4. Ask questions!!

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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

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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).

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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

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