The new normal: Lab

- Prelab and office hours via Zoom, links on the wiki
  - Instructors can also be reached via email
- Each prelab will have slides posted 1-2 hours prior to the beginning of class
- Instructors and Kevin will be available for entire class time to field questions
  - There will also be a Benchling notebook devoted to questions, especially for R
- Each prelab will be recorded and posted on the wiki for review purposes
  - I'd love to see you in video, but that is optional if you prefer privacy
- To ask or answer questions during class:
  - Use "raise hand" function
  - Can also type questions in the chat box rather than talk if preferred

1

The new normal: Homework/Quizzes

- Kevin will be checking benchling notebooks 24hrs following the beginning of lab to see your progress (i.e. Tues. class is checked at 1pm EDT on Wed.)
- Homework is due via Stellar by 10pm (EDT) the day of the lab session to be on time
- Homework will be returned via Stellar
  - See "comments" tab M1D7 and M2D2 for recent homework graded
- Quizzes will be emailed at the beginning of lab time and must be posted to Stellar by 10pm (EDT) on the same day to be on time

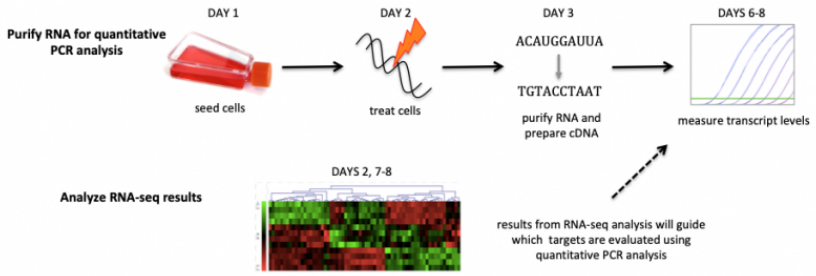
2

M2D6: Analyze RNA-seq data and prepare for qualitative PCR experiment

1. Prelab discussion
2. R.studio.cloud: clustering refresher
3. R.studio.cloud: a549 RNAseq analysis
4. Choose genes to further analyze by qPCR

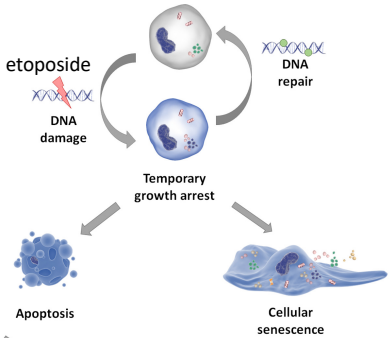
3

Mod2: Experimental overview



4

How does gene expression change upon etoposide treatment?



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

5

Review Ex2 : RNA-seq data was pre-processed

- Data from sequencer was reads: chopped up cDNA, example "ATTAGAGAACCA"
- Reads were aligned to human genome
- Aligned reads were counted
- RPKM corrects for differences in sequencing depth and gene length

Reads per kilobase million

$$\text{RPKM} = \frac{\text{number of reads mapped to gene sequence}}{\text{gene length (kb)} \times \text{total number of reads (millions)}}$$

- The counts of the aligned RNA-seq reads were loaded to DESeqDataset

6

Review Ex2: DESeqDataSet structure

Reads aligned to genes were loaded into data structure called "DESeqDataSet"

- colData: samples
- rowRanges: gene info
- assay: matrix of counts assigned to each gene for each sample

7

Review Ex2: Workflow RNA-seq analysis

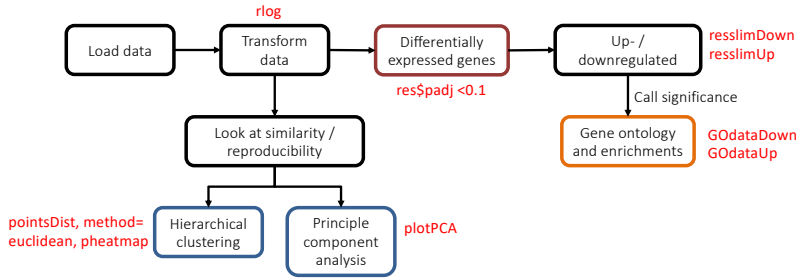
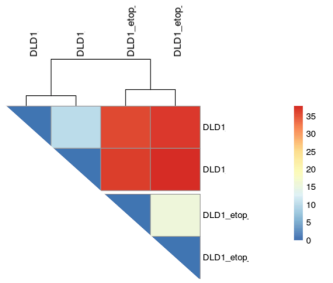


Image from Casper Enghuus, Sp17 TA 20.109

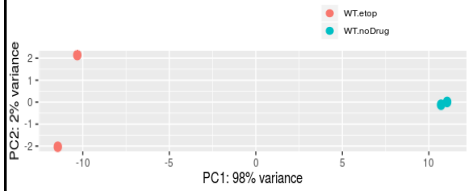
8

Review Ex2: Hierarchical clustering groups similar objects



9

Review Ex2: Principle component analysis (PCA) shows relatedness of objects



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Review Ex2: Gene ontology (GO) terms based on gene product properties

	GO.ID	Term	Annotated	Significant	Expected	Rank in classicFisher	classicFisher	classicKS
1	GO:0051301	cell division	145	16	21.52	952	0.97383	1.0e-07
2	GO:0031668	cellular response to extracellular stimu...	12	8	1.78	1	4.2e-05	0.00013
3	GO:0010389	regulation of G2/M transition of mitotic...	30	7	4.45	260	0.13535	0.00019

- GO table terms:
  - GO ID:
  - Term:
  - Annotated:
  - Significant:
  - Expected:
  - Classic Fisher:
  - Classic KS:

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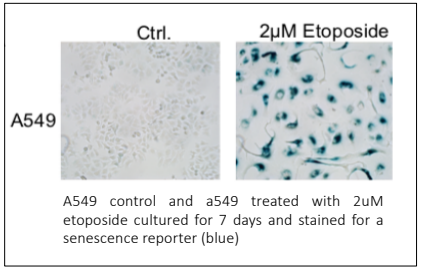
Apply R workflow from Ex2 to new RNA-seq dataset in Ex3



A549 control and a549 treated with 2uM etoposide cultured for 7 days and stained for a senescence reporter (blue)

- Authors studying senescence induction as an approach to cancer treatment
- A549, model cell line for lung cancer
- Treated with 2uM etoposide, harvested RNA for sequencing after 7 days
- RNA-seq read counts were made available as a public data set

Wang et al. High-Throughput Functional Genetic and Compound Screens Identify Targets for Senescence Induction in Cancer. Cell Reports 2017.

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Getting help with R:

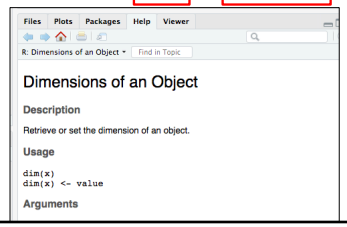
- Ask questions during lab. Anne will log into zoom from 3:30-4:30EST
- Review 20.109.Ex2.codeExplained.pdf under Ex2
- Ask questions on the Mod2 R.studio.cloud benchling page
- Make an appt with *new* BE data lab! mit.mywconline.net
- Use R help function

Method 1
`?function`

or

Method 2
`help(function)`

Example: Type `?dim` or `help(dim)`



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R Studio Cloud Ex3 Checklist

- ❑ Complete Exercise3_clustering_refresher.R
- ❑ Generate PCA plot of A549 data
- ❑ Generate GO tables of top upregulated and downregulated A549 genes in response to etoposide treatment, with statistical tests
- ❑ Generate PCA plot comparing etoposide treatment in DLD1 and A549 cells
- ❑ Create heatmap of DLD1 and A549 datasets

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M2D6 "Lab" Checklist

1. Ask questions and understand the RNA-seq data analysis
   – this analysis will translate to figures in your research article
2. You must choose genes for qPCR analysis, note this in your benchling notebook

- Homework due M2D7: Methods M2D1-M2D3 and draft Introduction

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Methods Reminders:

- Include enough information to replicate the experiment
  - list manufacturers name, like (Qiagen)
- Organize methods into subsections with descriptive titles
  - Put in logical order
  - Begin with topic sentence to introduce purpose
  - R subsection, include package and version, **DESeq2 (v. 1.26.0)**
- Use clear and concise full sentences
  - NO tables and lists
  - Passive voice and past tense
- Use the most flexible units
  - Write concentrations (when known) rather than volumes
- Eliminate 20.109 specific details
  - Example "labeled Row A, Row B…"
  - Do not include details about tubes and water!
  - Assume reader has some biology experience
  - Include steps teaching faculty carried out for you

M2D7 Methods HW should include experiments from M2D1-M2D3

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Tissue Culture:

TK6 cells were grown in a flask with 12ml RPMI supplemented with FBS. The cells were kept in an incubator at 37°C. A stain was used to assess if the cells were alive or dead.

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Improving the Methods paragraph

Maintaining lymphoblastoid cell line(s):

TK6 human lymphoblastoids (gift of the Engelward Lab, MIT, Cambridge MA) were cultured at 1-9 x 10^5 cells/mL, cell number calculated via hemocytometer and trypan blue stain. Cells were grown in RPMI medium 1640 (Invitrogen, Carlsbad CA) supplemented with 10% fetal bovine serum (Atlanta Biologicals, Atlanta, GA) and 100 units/mL penicillin-streptomycin (Invitrogen). Culture conditions were maintained at 37°C, 5% CO2 and 95% relative humidity.

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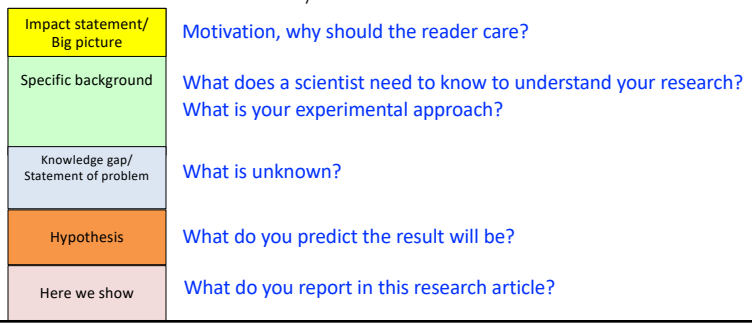
Mod2 Introduction Reminders

M2D7 homework should include:

- Draft the entire first big picture paragraph
- Topic sentence (first sentence) of each additional paragraph
- References in text and brief summary of each reference at the end



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M2D2HW feedback: for journal club presentations

- edit the figures / data you are presenting. Take time to describe one or two plots or images rather than list many
- identify color coding on slide in text if space allows
- Verbally transition to next experiment, what did the result motivate the authors to do next?

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