RNA Sequencing Best Practices and Jigsaw

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- Each item is graded for 0-5 points, rubric (will be) on the git repo

Overview

1. Goals
2. Experimental Design
   - Number of Replicates
3. Data Analysis
   - Handling Reads
   - Transcripts
   - Differential Expression
   - Functional Profiling

Today's Goals

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- Jigsaw Activity

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- Good overview of current “best practices”

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- Compare and contrast during the activity

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  - Have to use with bacterial samples (lack polyA)

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- Use a “Saturation Curve” to assess results at given depth

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  3. Desired Statistical Power
- Minimum of 3 replicates per group
- Conduct a power analysis

Number of Replicates

Table 1 Statistical power to detect differential expression varies with effect size, sequencing depth and number of replicates

	Replicates per group		
	3	5	10
Effect size (fold change)			
1.25	17 %	25 %	44 %
1.5	43 %	64 %	91 %
2	87 %	98 %	100 %
Sequencing depth (millions of reads)			
3	19 %	29 %	52 %
10	33 %	51 %	80 %
15	38 %	57 %	85 %

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- Reads accumulating at the 3' end of transcripts could be a sign of poor RNA quality (polyA enrichment only)

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- Montebello - quantification AND isoforms! (project idea?)

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- Is necessary for correctly ranking gene expression levels

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- Not necessaily memory intensive software (DESeq runs in R)

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- GOseq, Blast2GO, Gene Set Variation Analysis, SeqGSEA

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4. Share your results and discuss the differences – **15-20 mins**

Jigsaw Activity

- **Mouse Olf** - Sisi, Kevin, Alan
- **Lemurs** - Alvin, Rahul, Helena
- **AD** - Austin, Othmane, Jenn
- **Obese Chickens** - Hank, Nayib, Jake

1. Number (and type) of replicates
2. Software used
3. What functional analysis was done?
4. What conclusions were drawn?

The End