TREx
Transcriptional Regulation and Gene Expression Facility

Our Team

Workshops

New Pricing!
Our Team

Director: Jen Grenier
Lab Manager: Chrissy Butler
Data Analyst: Faraz Ahmed
Project Manager: Ann Tate
Upcoming Workshops

1. RNA Extraction - October 24th
2. From Library Prep to Analysis - 4 weeks, Wednesdays from October 30th - November 20th
3. Data Analysis Tools for Biological Discovery - December

Sign up
# New Pricing

## RNA Seq

| OPTIONS                | CORNELL PRICING 
<table>
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<tr>
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<th>SAMPLES 1-24</th>
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<tr>
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<td></td>
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## Small RNA

| OPTIONS                | CORNELL PRICING 
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TREx Project Roadmap
Experimental Design

- You:
  - Bringing Biological Question

- TREx:
  - Bringing Technological Expertise
Project Planning

- You: Biological Question

- TREx: How to use the technology to answer that question
  - How many Replicates do you need?
  - How are you going to extract your samples?
  - How much RNA will you have?
  - What will the quality of that RNA be?
  - What is your budget?
  - What type of library do you need?
Training

- You: I've never done that before and I want to try it

- T Rex: Come watch us!
  - RNA Handling
  - RNA Extraction
  - Library Prep
  - Bead Wash
  - QC Help
Emerging Technologies

- You: Biological Question

- TREx: Try this new technology, it may help answer your question in a better way
  - ATAC-Seq
  - Pro-Seq
  - Single Cell Sequencing
Sample Prep: RNA Extraction

- You:
  - Select your samples
  - Extract the RNA

- TREx:
  - Provide help with extraction process

Trizol

Column
Trizol Prep

Phase separation

- Aqueous phase
- Interphase
- Organic phase
- RNA pellet

Isopropanol precipitation
Library Prep

- You: Nothing :)
- TREx:
  - Make libraries
NEB Next Ultra II RNA
NEB SmRNA
TREx Project Roadmap

1. Collect Samples
2. QC
3. Sample Form
4. QC
5. Data Analysis
6. Biological Analysis

Experimental Design
Sample Prep
Library Prep
Pooling & Sequencing
Publish
Sequencing

- You: Nothing
- TREx
  - Pooling libraries
  - Submitting for Sequencing
  - Re-pooling
  - Re-sequencing as necessary

Pooling

Sequencing
Sequencing

• Standard Read length 75bp
• Standard depth
  • RNAseq - 20Million raw reads
  • Small RNAseq- 10million raw reads

• Special Projects
  • Transcriptome assembly
    • longer paired end reads
    • deeper sequencing
  • Pathogen ID
    • deeper sequencing
Nextseq 500

• BRC Specifications:
  • 333-400 Million Reads/Flowcell
    • 16-RNAseq libraries
    • 32-smRNA libraries

• TREx Benefit:
  • We will fill the flowcell
    • Select barcodes
    • Combine Projects
    • Guaranteed Balancing
Pooling Libraries

- Barcode/Index: Unique identifier
- Pool: Combine different libraries into one tube
TREx Project Roadmap
Data QC

- NEXTSEQ 500
- BCL2FASTQ2
- FASTQ
- TrimGalore
- STAR*/HISAT2/BWA/Bowtie2
- RNA
  - HTSEQ-Counts
  - DESeq2
  - CUFFDIFF
Data QC

Primary QC

NEXTSEQ 500

BCL2FASTQ2

FASTQ

FastQC

TrimGalore

STAR*/HISAT2/
BWA/Bowtie2

RNA

HTSEQ-Counts

DESeq2

CUFFDIFF
Data QC

Primary QC

Secondary QC

NextSeq 500 → BCL2FASTQ2 → FASTQ → FastQC → TrimGalore → STAR*/HISAT2/BWA/Bowtie2 → RNA → HTSeq-Counts

Deseq2 → Cuffdiff
Data QC

Primary QC
Secondary QC
Tertiary QC
TREx Project Roadmap
Preliminary Data Analysis:

You:
- Review desired outcomes
- Make space for data

TREx:
- Review QC data
- Generate reports
- Hand off data
Data

- Excel report with differentially expressed genes
- HTML reports
  - Mapping Summary
  - Analysis QC Plots
  - Clustering
- FASTQ files on request

- Excel report: Analysis of Annotated miRNAs
  - mirdeep2 quantifications
  - read count summary
  - Log2 fold change calculation
- FASTQ files on request
TREx Project Roadmap
RNA QC

- You:
  - Complete QC

- TREx:
  - Help with QC interpretation
  - Advise on how QC affects technology
Nanodrop:

- Chemical Purity
- Concentration: >20ng/μL
Bad 260/230: Salty RNA

- **Cause**
  - Touching the interface
  - Not drying the pellet enough
  - Column clean-up
- **Solution**
  - Repeat final clean-up step
  - Be careful to avoid the interface
  - Ethanol Wash
    - Move the pellet
    - Dry the pellet
    - Repeat 2-3x
Fragment Analyzer: RNA Quality

(A3) Rice Root Total RNA

RNA Property Summary

RNA Concentration (ng/μL)
205.6057
25S/18S
1
RNA Quality Number
7.8

(B3) Degraded Yeast Total RNA

RNA Property Summary

RNA Concentration (ng/μL)
96.3007
28S/18S
0
RNA Quality Number
1.7
Fragment Analyzer: RNA Quality

High Quality
Intact
RQN>7
Fragment Analyzer: RNA Quality

High Quality
Intact
RQN>7
Poly A Selection
Fragment Analyzer: RNA Quality

- High Quality
- Intact
- RQN>7
- Poly A Selection
- Degraded
- RQN<7
Fragment Analyzer: RNA Quality

High Quality
Intact
RQN>7
Poly A Selection

Degraded
RQN<7
Discuss
Fragment Analyzer: RNA Quality

- High Quality
- Intact
- RQN>7
- Poly A Selection

- Degraded
- RQN<7
- Ribosomal Depletion

Discuss
Fragment Analyzer: RNA Quality

High Quality
Intact
RQN>7
Poly A
Selection

Degraded
RQN<7
Ribosomal
Depletion
Discuss
Re-Extract
Qubit:

Concentration for <20ng/uL
TREx Project Roadmap
Library QC

- Qubit: Concentration
- Fragment Analyzer:
  - Size Distribution
Library QC

- Qubit: Concentration
- Fragment Analyzer:
  - Size Distribution
Library QC

- Qubit: Concentration
- Fragment Analyzer:
  - Size Distribution
Library QC

- Qubit: Concentration

- Fragment Analyzer:
  - Size Distribution
  - Primer: <10%
  - Adapter Dimer <10%
Library QC

- Qubit: Concentration
- Fragment Analyzer:
  - Size Distribution
  - Primer: <10%
  - Adapter Dimer <10%
Sample Submission Form:

- You:
  - Fill out form
  - Submit form via e-mail
  - Drop off samples
    - VRT 4th floor -80
    - Biotech 333

- TREx:
  - Double check everything looks good
  - Start Library Prep
Biological Analysis

- You: You Got This!
- TREx:
  - Advise on programs/tools to use
  - Suggest further steps
Biological Analysis Tools

- **iDEP** bioinformatics.sdstate.edu/idep web interface for PCA, clustering, DEgenes (R tools)
- **Heatmapper** www2.heatmapter.ca/expression heatmap with clustering
- **Panther** pantherdb.org GO term, pathway enrichment in DE genes
- **DAVID** david.ncifcrf.gov GO term, pathway enrichment in DE genes
- **Reactome** reactome.org pathway enrichment in DE genes, expression overlay
- **GSEA/MSig** software.broadinstitute.org/gsea gene set enrichment across full log2FC
- **Ingenuity**† www.qiagenbioinformatics.com/products/ingenuity-pathway-analysis/