Having high quality RNA is the MOST important thing you can do to insure the success of your experiment!
Introduction to RNAseq

- Sample Requirements
- Applications
- Methods

TREX
Why do RNASeq?

- Gene Expression Profiling
  - Reference (Annotated)
  - De Novo (discovery)
- Variant Analysis or Discovery
- Pathogen ID
TREX

Applications

Introduction to RNAseq

Sample Requirements

Methods
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- What are my research goals?
- What is my RNA quality?
- How many samples do I have?
- How much RNA do I have?
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Why Choose Lexogen 3'RNA Seq?

- High Throughput: BRC Service requires >32 samples
- Experimental design tolerant of dropouts
- The information you are interested in is at the 3' end of the RNA strand
- Tolerant of:
  - Input concentration diversity
  - RNA quality diversity
3' method (LEXO)

**Step 1:** 1st strand synthesis of polyA tailed RNA from total RNA using oligo dT primers

```
5' mRNA
  ^  ^
  3'   AAAAAA 3'
```

**Step 2:** Degradation of the RNA template

```
5' 3'
  5' 3'
```

**Step 3:** 2nd strand synthesis with random primers containing 5' Illumina-compatible linker sequences

```
3' 3'
  5' Random primer
  3'
```

**Step 4:** Amplification using random primers that add barcodes and cluster generation sequences

```
  3' 3'
  5'
```

**Step 5:** Sequencing
Why Choose Truseq RNA

Flexible:
- Input type:
  - Total RNA
  - Ribosomal Depleted RNA
  - Poly A Selected RNA
  - Input Concentration: 1000-100ng
  - RNA Integrity: Intact OR Degraded
  - Sample Number: <384

Directional Or Non-Directional

Highly Supported
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Fully Supported

Directional Or Non-Directional

Modular
What is Poly A Selection?

Keep any RNA fragment with a Poly A stretch in it

Discard everything else
   Ribosomal RNA
   Some lncRNA
   Other Housekeeping RNAs
   Degraded RNA - only keep the pieces with Poly A tails
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  - Bacteria don't have a Poly A tail
  - If you are looking for RNA's that don't have a Poly A tail, ex: LNC RNA
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• If your RNA is intact:
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  - RQN is >7
- If you don't care about 3' bias

RQN 10
What is Ribosomal Depletion

- Total RNA contains greater than 90% rRNA (red).
- Binding of ssDNA Probes
- Single-stranded DNA probes hybridize specifically to rRNA molecules.
- rRNA Degradation by Ribonuclease H (RNase H) Enzyme
- RNase H degrades the hybridized rRNA (rRNA).
- Probe Degradation by DNase I Enzyme & Clean Up
- DNase I degrades the DNA probes.
- rRNA-depleted RNA
- Non-rRNA species (blue) are enriched.
Total RNA contains greater than 80% rRNA (red).

Binding of ssDNA Probes

Single-stranded DNA probes hybridize specifically to rRNA molecules.

rRNA Degradation by Ribonuclease H (RNase H) Enzyme

RNase H degrades the hybridized RNA (rRNA).

Probe Degradation by DNase I Enzyme & Clean Up

DNase I degrades the DNA probes.

rRNA-depleted RNA

Non-rRNA species (blue) are enriched.
ssDNA probes are organism specific.
ssDNA probes are organism specific

ONLY the hybridized RNA is degraded
ssDNA probes are organism specific

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ONLY the hybridized RNA is degraded

Housekeeping RNAs
Why Choose Ribosomal Depletion?
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If your RNA is degraded: RQN <7
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If your RNA is degraded: RQN <7
If your organism is not compatible with Poly A
Why Choose Ribosomal Depletion?

If your RNA is degraded: RQN <7
If your organism is not compatible with Poly A
If you are looking for RNA's that don't have a poly A tail
What is the difference between Directional and Nondirectional?
Prior to PCR amplification, the dUTP-marked strand is selectively degraded by Uracil-DNA-Glycosylase (UDG). The remaining strand is amplified to generate a cDNA library suitable for sequencing.
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Why choose Directional?
- More information
- Which strand your RNA is being transcribed from
- More accurate count of genes in differential expression analysis
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RNAseq Decision Tree

**RNA Quality**  
RQN ≥ 7

- **polyA⁺ OK**
  - ≥ 10 ng total RNA  
  - Directional (stranded) RNAseq  
  - ≥ 10 ng

- ≤ 10 ng total RNA  
  - Non-stranded or amplified RNAseq  
  - <10 ng

- ≥ 100 ng total RNA   
  - ≥ 100 ng total RNA
  - HMR rRNA⁻ (stranded) RNAseq
  - eukaryote

- ≤ 100 ng total RNA
  - Other rRNA⁻ (stranded) RNAseq
  -  
  - eukaryote
  - bacteria/mix

**PolyA⁺ RNAseq**  
RQN ≥ 7 and polyA⁺ OK

**rRNA⁻ RNAseq**  
RQN < 7 or polyA⁺ not OK
Ribo Zero from illumina has been discontinued!
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Why choose Small RNA?

Analyzing microRNAs, siRNAs, piRNAs
- Selecting for 20-30nt small RNAs
- Minimum input of 100ng of cellular Total RNA
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* For RNA Extraction: make sure you use a method that keeps small RNA’s *
What is small RNA

- Type of ncRNA
- Small, 25-250NT's
- Involved in regulating translation of target RNA's
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Small RNA Seq

- 3' Adaptor ligation, primer hyb
- 5' Adaptor ligation
- cDNA synthesis
- PCR
- Index
- P7 Adaptor
- small RNA insert
- P5 Adaptor
Small RNA Seq

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Hydroxyl Group
Introduction to RNAseq
Transcriptional Regulation and Gene Expression

**Mission:** Develop and provide high quality, project-oriented genomics services to the Cornell research community.

**Goal:** Enable successful research, from funding to publication