FRALIN LIFE SCIENCES INSTITUTE
VIRGINIA TECH.

Genomics Sequencing Center Contact Information

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General Instructions for VT Researchers

1. **Submission Forms:** This file has **2 sheets** to fill, ① **Customer & Project Info (Red Tab)** **and** ② **RNA Sample Info (Green Tab)**.
   Researcher must fill both sheets completely and email to flsi-illuminaseq-g@vt.edu
   before submitting the samples. Please also include a hard copy when you drop off samples.
2. **Two Steps for Submitting Samples:** Researchers must first submit QC aliquot and get approved, before submitting aliquots for RNA-Seq.
   Step 1-Aliquot for RNA-QC: We will perform NanoDrop and Agilent BioAnalyzer/TapeStation analysis. If the samples fail QC, we will discuss options how to move forward.
   Step 2-Aliquot for RNA-Seq: Based on our NanoDrop concentrations the stock samples must be diluted to the requirments listed in the table below and submit for library preparation and sequencing
3. **Specific Instructions for Specific Sample Types:** Please read the instructions carefully. We have specific instructions for Eukaryotic and Prokaryotic organisms, standard input and low input RNA, small/miRNA, poly(A) or rRNA depleted RNA.
4. **Sequencing Turnover Times: Please contact the GSC for current turnaround time estimates.**
5. **Data Analysis and Data Delivery Times: Data analysis and data delivery is performed by VT Data Analysis Core (DAC) managed by Dr. Robert Settlage. Please contact Bob Settlage directly for exact turnaround times. For most standard NGS data generated at GSC, DAC will perform de-multiplexing and quality check. For researchers doing their own analysis, DAC will provide a link to download the data in 3-5 business days. For researchers who have paid for data analysis, please contact Bob directly for the status of your analysis. For all questions related to data analysis and data delivery please contact Dr. Robert Settlage at 540-231-2777 or email at rsettlag@vt.edu.**

RNA-QC Submission Instructions for VT Researchers

RNA-Seq Services:	① **mRNA Stranded Seq Library Prep**	② **Small/miRNA Profiling Library Prep**
Sample Type:	1. Eukaryotic Total RNA: GSC will perform poly(A) enrichment followed by library preps.	

	2. Prokaryotic Total RNA: Investigator must provide Epicentre's RiboZero Mag kit that works best for their specific organism. We will setup Robotics for rRNA depletion and library preps. We require a minimum of 2-3 RiboZero reactions (2-3 ug of total RNA) per sample (to reduce the number of PCR duplicates). Click here for Epicentre's RiboZero Mag Kits compatible with our robotics. **3. polyA (Eukaryotic) or rRNA depleted RNA (Prokaryotic):** Investigators can do their own poly(A) enrichment or rRNA depletion and provide the necessary amounts for QC and library prep (see below) **4. Small/miRNA-Seq: We do not need pre-enriched small/miRNA. Investigator submits total RNA and GSC will perform small/miRNA enrichment during library prep.**
RNA Extraction Requirements:	• **DNAse Treatment Required:** Investigators must perform DNAse treatment. Presence of excess DNA overestimates RNA concentrations by UV-Spec methods, and interferes with library preparation protocols. • **Elution / Resuspension Buffer:** Use Nuclease-Free Water (Ambion). Do not use DEPC water ot TE. You can use nuclease free 10 mM Tris pH 7-7.5. EDTA is not recommended in the elution/resuspension buffer.
Recommended Kits/Protocols :	**1.** Follow the kits/protocols that work the best for your organism (bacteria, plants etc.) and tissue. **2. Recommended Kits for RNA Extraction:** . • **Qiagen miRNeasy Mini Kit (Cat# 217004):** This kit with the DNAse step incorporated works for most species and tissues. This kit isolates all RNA including small RNA, and can be used for both RNA-Seq and small/miRNA-Seq. • **Zymo Research DNA-Free RNA Kit (Cat# R2050) :** This kit comes with DNAse I, and isolates all RNA including small RNAs, and can be used for both RNA-Seq and small/miRNA-Seq. • **From Lysates in TriReagent or Trizol:** Zymo Research Direct-Zol RNA MiniPrep (Cat# R2050) kit comes with DNAse I and isolates all RNA including small RNA, and can be used for both RNA-Seq and small/miRNA-Seq. **3. Previously extracted RNA fom TriReagent or Trizol based methods: :** . • If your input sample is not limiting: we require re-extraction of RNA using a column. We recommend Zymo Research RNA Clean & Concentrator-5 (Cat# R1015), which enables up to 10 ug of DNAse treated RNA to be eluted in 6-10 ul. • If your input sample is limiting: You may lose RNA passing through a column. Instead of column purification, wash the RNA pellet with cold 70% EtOH at least 3 times to remove phenol and salt contamination.
Step 1 - Aliquot for RNA-QC: RNA must first be approved before submission for RNA-Seq	**Tube Requirements and Sample/Tube Naming** **1. Tubes:** Submit in 1.5 ml DNAse-RNAse free centrifuge tubes. Please call when you have 48 samples or more. **2. Naming Samples and Tubes:** • The best names are unique alpha-numeric names. Do not use spaces or dashes or other characters. • Do not name samples 1, 2, 3 etc. One suggestion is to use your first and last intials followed by numbers. • Mark the tubes clearly for **QC** and name the tubes with your sample names/IDs. • Make sure to enter the same names on the RNA Sample Info sheet. **Submission Amounts for QC for mRNA-Seq** **1. Total RNA -** For Non-Limiting RNA Amounts**:** 5-10 ul at 100 ng/ul (in nuclease free water).

2. **Total RNA -** Low input RNA Amounts**:** Submit separate aliquots of 5-10 ul at 1-4 ng/ul in nuclease free water.
3. **Poly(A) or rRNA Depleted mRNA:** Submit 5-10 ul at 25 ng/ul.

Submission Amounts for QC for Small/miRNA-Seq

1. **Total RNA:** We prefer the investigator to submit total RNA for both QC and Sequencing. We will enrich for smallRNA during library prep.
   - 4-10 ul at 200 ng/ul in nuclease free water.
2. **Enriched miRNA:** It is the investigator's responsibility to make sure miRNA is enriched from intact RNA with RIN ≥ 8. If the original total RNA is degraded the resulting sequencing data will be contaminated with reads from mRNA.
   - 4-10 ul at 2 ng/ul in nuclease free water.

RNA QC Analysis

NanoDrop Analysis: We will provide you concentrations (ng/ul) and OD 260/280 and OD 260/230.

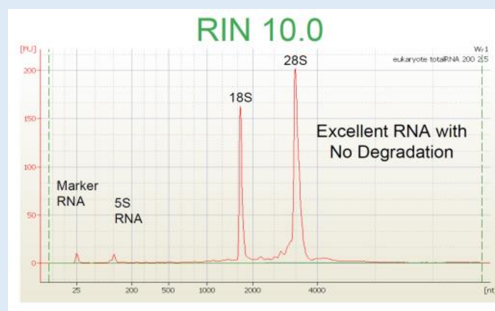
NanoDrop Requirements for Approval:

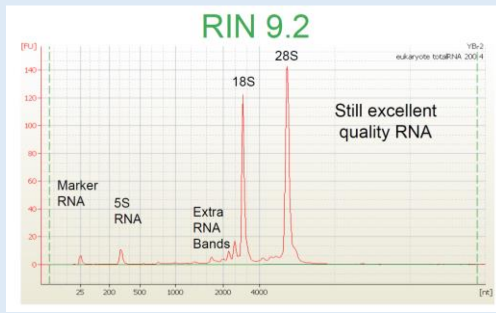
- RNA with OD 260/280 and OD 260/230 ≥ 1.8 are approved and are optimal for RNA-Seq library preps.

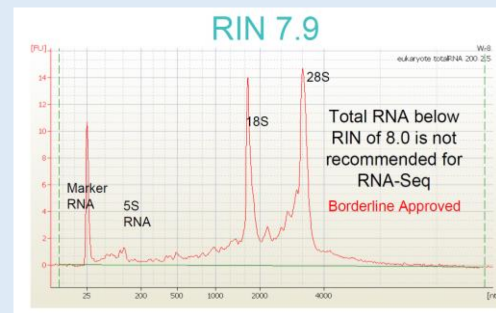
Agilent/TapeStation Analysis: We will provide RNA traces and RNA Integration Number (RIN) values. RIN values vary from 1 to 10. RIN 1 represents completeley degraded RNA, and 10 represents no degradation of RNA.

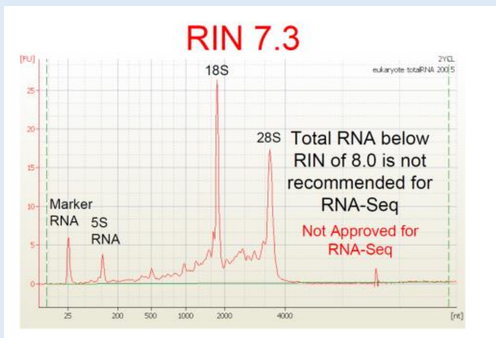
Requirements for Approval:

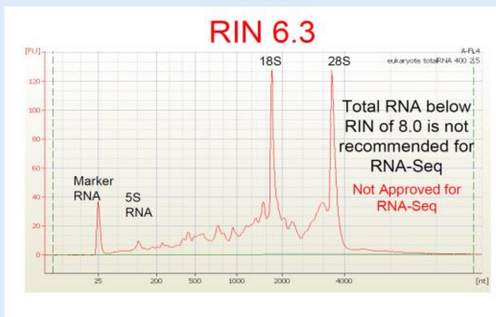
- Total RNA with RIN ≥ 8 are approved and are optimal RNA-Seq library preps.
- For poly(A) and rRNA depleted RNA, the majority of the transcripts should be between 1-4 Kb and the RIN values are not used.
- Degraded RNA Samples: Options are available for prepping partially degraded RNA samples.

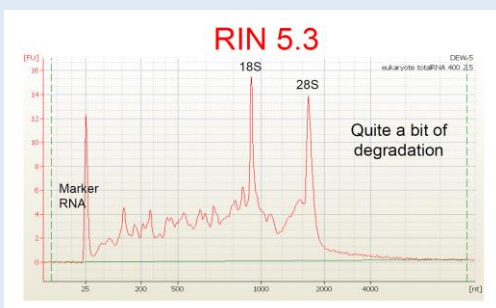
RIN Number	RNA Traces of RIN 2 to10
The Agilent Expert software assigns a RIN number to each trace. It assigns a number according to how much signal is found between the 5S and 18S band, between the 18S and 28S bands, and after the 28S band. A RIN number of 10 is perfect score. The software does not always call RIN numbers for prokaryotic RNA and the RIN can be misleading for samples containing additional RNA bands such as those from chloroplasts or a symbiotic RNA. The following slides show some examples of total RNA run on the bioanalyzer.	

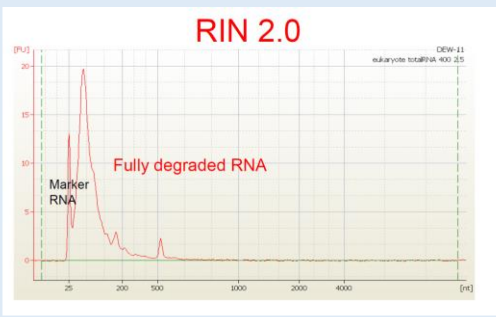

RIN 9.2
18S
28S
Still excellent quality RNA
Marker RNA
5S RNA
Extra RNA Bands


RIN 7.9
28S
18S
Total RNA below RIN of 8.0 is not recommended for RNA-Seq
Borderline Approved
Marker RNA
5S RNA


RIN 7.3
18S
28S
Total RNA below RIN of 8.0 is not recommended for RNA-Seq
Not Approved for RNA-Seq
Marker RNA
5S RNA


RIN 6.3
18S
28S
Total RNA below RIN of 8.0 is not recommended for RNA-Seq
Not Approved for RNA-Seq
Marker RNA
5S RNA


RIN 5.3
18S
28S
Quite a bit of degradation
Marker RNA


RIN 2.0
Fully degraded RNA
Marker RNA

Step 2-Aliquot for RNA-Seq: Dilute your stocks based on our NanoDrop concentrations	**Tube Requirements and Sample/Tube Naming** 1. **Tubes:** Submit in 1.5 ml DNAse-RNAse free centrifuge tubes. 2. **Naming Samples and Tubes:** ● Mark the tubes clearly for **Seq**, and enter sample names same as on the QC tubes. ● Make sure to enter the same names on the RNA Sample Info sheet. **Submission Amounts for mRNA-Seq:** We can prepare RNA-Seq libraries from 100 ng - 500 ng of total RNA. ● Dilution of the stock for RNA-Seq submission: Dilute your stock based on GSC NanoDrop concentrations in nuclease free water. 1. **Total RNA -** For Non-Limiting RNA Amounts**:** Submit 25 ul at 50 ng/ul (1.25 ug) . Please call 540-231-1229 if you do not have 1.25 ug. 2. **Total RNA -** Low input RNA Amounts**:** Submit 100 ng in 25 ul. Please call 540-231-1229 if you do not have 100 ng. 3. **Poly(A) or rRNA Depleted mRNA:** Submit 5-10 ul at 25 ng/ul. **Submission Amounts for Small/miRNA-Seq:** Dilute your RNA stocks based on our NanoDrop to 200 ng/ul and submit: 1. **Total RNA:** Submit 11ul at 200 ng/ul (2.2 ug) in nuclease free water.