Genomics Sequencing Center Contact Information

Next-Gen Seq Specialist:	Megan Frair	540-231-1229	mfrair@vt.edu
Next-Gen Seq Specialist:	Jennifer Jenrette	540-231-1229	jenrette@vt.edu
Sanger Seq Specialist:	Kris Lee	540-231-1229	leek1@vt.edu
Business Admin:	Becky Morgan	540-231-4044	b0287253@vt.edu
GSC Address:	Fralin Life Sciences Institute at Virginia Tech 1015 Life Sciences Circle Blacksburg, VA 24061-0477 540-231-1229 Lab		

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 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 will be diluted to the requirments listed 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:** Depends on the samples that are in queue. In general it takes 1 week for RNA-QC. There would be delays if the samples do not pass QC. Library prep and QC takes 1-2 weeks. Sequencing takes 2-14 days depending on the type and length of sequencing. Please contact the GSC at 540-231-1229 or flsi-illuminaseq-g@vt.edu for more up-to-date turnover times.

RNA-Seq 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 your specific organism. 3. **polyA (Eukaryotic) or rRNA depleted RNA (Prokaryotic):** Investigator can do his 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 DNA 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 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. 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 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:

1. **Total RNA:** We prefer the investigator to submit total RNA for Sequencing. We will enrich
   - Submit 23 ul at 100 ng/ul (2.3 ug) 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.
   - Submit 13 ul at 10 ng/ul (23 ng) in nuclease free water.

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Customer Information

Investigator's Name & Address:			
Email:		**Phone:**	
Contact Person's Name:			
Email:		**Phone:**	
Business Admin's Name:			
Email:		**Phone:**	
Quote Number:		**ISR Number** (Email a copy):	

RNA Preparation Information

Species:		**Type of RNA:**	
RNA Extraction Protocol used:			
Whether DNAse Treatment Done*:		**RNA Eluted in:**	Nuclease free water preferred, No DEPC water, No TE

Comments

GSC Use Only

Date Received:	
By:	
Completion Date:	

External RNA Sample Information for Agilent RNA-Seq

Investigator's Name:					
Quote Number:			**ISR Number:**		
Treatment Groups (as many as as in your experimental design)	**Sample IDs** (as many replicates as you have)	**(Submit 50ng/ul) NanoDrop Conc (ng/ul)**	**GSC's 260/280**	**GSC's 260/230**	**(Submit 25ul) Volume Submitting for Seq**
Experiment Group 1	N1				
	N2				
	N3				
	N4				
	N5				
Experiment Group 2	N1				
	N2				
	N3				
	N4				
	N5				
Experiment Group 3	N1				
	N2				
	N3				
	N4				
	N5				
Experiment Group 4	N1				
	N2				
	N3				
	N4				
	N5				
Experiment Group 5	N1				
	N2				
	N3				
	N4				
	N5				
Experiment Group 6	N1				
	N2				
	N3				
	N4				
	N5				
Experiment Group 7	N1				
	N2				
	N3				
	N4				
	N5				
Experiment Group 8	N1				
	N2				
	N3				
	N4				
	N5				
Experiment Group 9	N1				
	N2				
	N3				
	N4				
	N5				
Experiment Group 10	N1				
	N2				

	N3				
	N4				
	N5				
Experiment Group 11	N1				
	N2				
	N3				
	N4				
	N5				
Experiment Group 12	N1				
	N2				
	N3				
	N4				
	N5				
Experiment Group 13	N1				
	N2				
	N3				
	N4				
	N5				
Experiment Group 14	N1				
	N2				
	N3				
	N4				
	N5				
Experiment Group 15	N1				
	N2				
	N3				
	N4				
	N5				
Experiment Group 16	N1				
	N2				
	N3				
	N4				
	N5				
Experiment Group 17	N1				
	N2				
	N3				
	N4				
	N5				