JGI JOINT GENOME INSTITUTE
UNITED STATES DEPARTMENT OF ENERGY

Plant Sequencing Program RNA Sample Submission Guidelines

The success of an RNA project is primarily dependent on the quality of the material received from the collaborator. JGI currently uses the Illumina platform (RNA-seq) for all RNA-based projects, including genome annotation and gene expression profiling projects. If you have any questions or concerns, contact your **Project Manager** (pmo_jgi_project_management@lists.jgi-psf.org) or Mansi Chovatia (mrchovatia@lbl.gov) regarding sample preparation and/or QC or Chew Yee Ngan (cyngan@lbl.gov) regarding library construction.

Note: For RNA projects that will be used to annotate a reference genome, a draft DNA reference sequence must be generated by the JGI ***before*** RNA projects can commence. Please see the document entitled "Plant DNA Sample Requirements" for information on materials required to produce a DNA reference.

Procedures for isolating RNA are available here: http://my.jgi.doe.gov/index.html

RNA sample requirements:

Different types of samples may require specific RNA isolation techniques. JGI has successfully used standard methods involving Trizol, Rneasy and for many samples further purified using LiCl. Contaminants in the preparation will often cause an overestimate of RNA quantity by OD measurement. It is advisable to submit RNA of the highest quality possible. Degraded/fragmented RNA tends to overlap with the region/size selected for small RNA and introduces noise into the sequencing data. **Samples not meeting minimum submission requirements with regard to quantity and quality will result in a delay to project initiation.**

All samples must meet the following criteria:

1. All samples **must** be treated with RNase-free DNase prior to submission and free of DNA contamination.

2. $A_{260/280}$ greater than 1.8 (spectrophotometer/NanoDrop)

3. Quantification must be performed using a RiboGreen/Qubit system (Life Technologies, Inc.:) or other fluorescent dye-based assay. *Samples submitted that are quantified using a spectrophotometer or NanoDrop will not be accepted.*

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4. Samples must be in RNase-free water. General guidelines are:

	Standard RNAseq poly-A (annotation or counting)	Small RNA projects	Laser-Capture Microdissection (LCM)
Volume	25-50 uL	60-120 uL	25-30 uL
Concentration	60-120 ng/uL	100-200 ng/uL	1-2 ng/uL
Total Mass	3 ug	12 ug	30-50 ng

5. For small RNA, any commercially available kit can be used for isolation of total RNA containing miRNA or small RNA (<200bp). A silica column-based prep is typically used to filter out RNA <200bp. In order to retain RNA <200bp, a modified protocol is often needed. Please refer to the manufacturer's instructions.

6. Place sample into JGI-supplied barcoded tube or plate. If samples will be submitted in 96-well plate format, please consult the document entitled "Plate-Based Sample Requirements" on our website.

Notes on RNA sample preparation:
-As stated above, total RNA must have an OD260/280 ratio greater than 1.8. A lower ratio is often indicative of protein/DNA contamination; a ratio higher than 2.1 may indicate residual guanidine thiocyanate or beta-mercaptoethanol. Protein contaminants should be re-extracted using phenol:chloroform:isoamyl alcohol; other contaminants by EtOH ppt. In general, JGI recommends LiCl extraction for cleaning up RNA.

-RNA must not show signs of degradation as measured by distinct bands of ribosomal peaks of relative intensities (gel electrophoresis or Bioanalyzer results). Please see example at the end of the document. The JGI does not require submission of either a gel photo or Bioanalyzer trace with samples. However, Investigators are **strongly advised** to assess the quality of materials to be submitted by gel electrophoresis or Bioanalyzer.

-If pooled RNA samples are to be submitted, pool them prior to sample submission and shipment. It is recommended that each sample be checked for quality prior to including it in the pool.

-Gels should be run with a standard MW marker to assess the size of the ribosomal bands or a Bioanalyzer image generated. Please note that a Bioanalyzer image is preferred.

Shipping:

Prior to shipping samples to the JGI, all sample metatdata must be completed in its entirety using the JGI web interface. If you have questions about required information, consult with your Project Manager early in the submission process.

1. RNA should be completely dissolved in RNase-free water and shipped on dry ice.

2. Individual RNA samples should be shipped to the JGI in **one tube per sample**. If the RNA is prepared in multiple preps, each prep should be QC'ed separately, and good preps pooled into one tube for shipping to the JGI. **You must ship all samples in the barcoded tubes provided by the JGI.**

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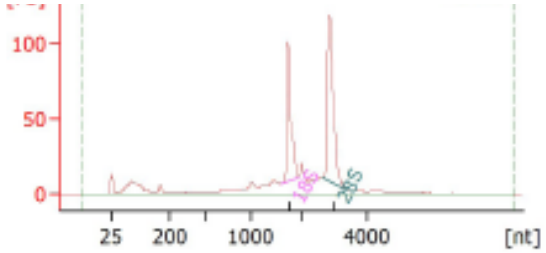
Example of Bioanalyzer results of RNA:

Total RNA: Clear 18s and 28s rRNA peaks; no degradation.



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