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## Metagenome 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. Please contact your Project Manager or Erika Lindquist ([ealindquist@lbl.gov](mailto:ealindquist@lbl.gov)) with any questions or concerns.

Note: If a metagenome reference sequence is not currently available and determined to be required for the success of the metatranscriptome project, one must be generated by the JGI before RNA projects can commence.

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

### RNA sample requirements:

Most projects begin with total RNA. The JGI requires 5 ug of nondegraded, noncontaminated, high quality total RNA for each RNA-seq library.

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. **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.: <http://probes.invitrogen.com/media/pis/mp11490.pdf>) or other fluorescent dye-based assay. *Samples submitted that are quantified using a spectrophotometer or NanoDrop will not be accepted.*
4. Samples must be in RNase-free water

Volume: 50-100 uL

Concentration: 50-100 ng/uL (preferably ~100 ng/uL)

Mass: >5 ug

5. If samples will be submitted in 96-well plate format, please consult the document entitled "Plate-Based Sample Requirements"

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.

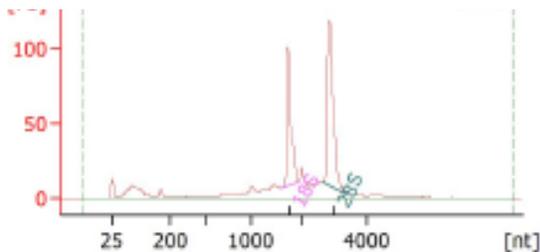
**Shipping:**

Prior to shipping samples to the JGI, the Sample Information spreadsheet provided to you by your Project Manager must be completed in its entirety. If you have questions about required information on this form, 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**. The tube should be legibly labeled with the sample ID that will be provided after the sample has been approved for shipping. 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.

**Examples of Bioanalyzer results of RNA:**

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



*Last Updated 9/26/2012*