JGI JOINT GENOME INSTITUTE
UNITED STATES DEPARTMENT OF ENERGY

Microbial Sequencing Program DNA Sample Submission Guidelines

The JGI has sequenced over 3000 prokaryotic, eukaryotic and metagenomic projects and finished over 300 genomes. DNA quality (molecular weight and purity) and quantity have always been the two critical factors in the success of the sequencing projects. If you cannot meet the JGI specifications for DNA submission have 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. **Samples not meeting minimum submission requirements with regard to quantity and quality will result in a delay to project initiation.**

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

JGI Starting Material Specifications: Quality (purity)

For prokaryotes, the starting culture should be axenic, strain pure, and started from a single colony or from a culture diluted to extinction. It may not be possible to assemble a genome that is not derived from an axenic and strain-pure culture. If an axenic and strain-pure culture is not possible, a description and justification is required.

All samples must meet the following criteria:

1. Appropriate mass for project initiation as indicated by fluorometric measurement (PicoGreen) A protocol for measurement of DNA mass by PicoGreen can be found here: http://probes.invitrogen.com/media/pis/mp07581.pdf

2. $A_{260/280}$ between 1.6 and 2.0 (spectrophotometer/NanoDrop)

3. We assess the mass of dsDNA in a sample using fluorometry (Qubit). NanoDrop measurements are generally only reliable indicators of DNA contamination with RNA, carbohydrate, organic solvents and other impurities that may interfere with sequencing. Obtaining DNA & RNA of suitable quantity and quality has been the rate-limiting step for many projects at the JGI. The quality of the starting material is one of the greatest predictors of a successful sequencing project. These documents (posted with permission) demonstrate the importance of utilizing this platform for nucleic acid quantification in our NextGen sequencing workflow:

- Importance of Sample QC.pdf : http://my.jgi.doe.gov/general/sampleprep/Importance-of-Sample-QC.pdf
- Sample Quality and Contamination.pdf : http://my.jgi.doe.gov/general/sampleprep/Sample-Quality-and-Contamination.pdf

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4. Although the JGI does not require submission of a gel photo, investigators are **strongly advised** to assess the quality of materials to be submitted by gel electrophoresis. Heavily degraded samples cannot be used for construction of long mate pair libraries.

5. RNA blobs and discrete bands require clean-up. Please refer to the recommended RNAse I clean-up protocol:

http://my.jgi.doe.gov/index.html

6. In some cases, the presence of some impurities (polysaccharides or proteins) can be predicted by examining the way DNA fragments migrate (or do not migrate) in an agarose gel. If most of the DNA fragments form a streaky pattern or get stuck on top of the wells in the gel, the degree of impurity or contamination may cause problems in the shearing of the DNA. Also, if you notice that your samples are too viscous (samples sticking to the outside of the pipette tips), which can lead to an incorrect quantitation of the samples, we request that you further purify your samples or dilute to the recommended concentration prior to shipping to the JGI.

JGI Starting Material Specifications: Quality (molecular weight)

Molecular weight of the DNA sample determines the insert size of the library we can construct. Most DNA preparation protocols should be able to generate DNA fragments of approximately 100kb in size, appropriate for Illumina and/or PacBio library construction. In general, we will not accept samples exhibiting a majority of DNA fragments smaller than 23Kb in size or heavily degraded DNA samples.

JGI Starting Material Specifications: Mass

The table below should be used as a guide for preparation of DNA samples required to complete the most common JGI product types:

	Microbial Minimal Draft	Microbial Improved Draft	Microbial Single-Cell Sequencing	Microbial Resequencing	Methylation Detection Only
Volume per sample	50-100 uL	100-200 uL	50-100 uL	50-100 uL	50-100 uL
Concentration	5-10 ng/uL	60-120 ng/uL	5-10 ng/uL	5-10 ng/uL	30-60 ng/uL
Mass (minimum)	0.5 ug	12 ug	0.5 ug	0.5 ug	3 ug **Note: MDA products cannot be used for methylation detection*

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Please consult with your project manager if you may not be able to provide adequate material. Projects will have the greatest odds of success with greater quantities of material. In some cases, JGI may be able to amplify the DNA.

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.

Preparation of DNA samples for shipping:

1. DNA should be completely dissolved in $^{1}/_{10}$ TE DNA Suspension Buffer (10 mM Tris, pH 7.5-8.0, 0.1 mM EDTA) and shipped on dry ice.

2. **You must ship all samples in the barcoded tubes (or plates) provided by the JGI.**

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