
Microbial Sequencing Program DNA Sample Submission Guidelines

The JGI has sequenced over 650 prokaryotic, eukaryotic and metagenomic projects and finished over 250 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, please contact your project manager or email jgi_project_management@lanl.gov.

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, 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 2.2 (spectrophotometer/NanoDrop)

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.

Notes on contamination:

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. You can request that JGI ship you a set of mass standards for this purpose by contacting your project manager.

Please refer to http://my.jgi.doe.gov/general/protocols/Genomic_DNA_QC_Guides.pdf for detailed instructions on running the QC gel.

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>.

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, the degree of impurity or contamination may cause problems in the shearing of the DNA.

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. For projects in need of a fosmid library we request that the majority of the DNA fragments be greater than 40Kb in size.

For other library types, high molecular weight DNA will give us a better chance of constructing a high quality library. In general, we will not accept samples exhibiting a majority of DNA fragments smaller than 20Kb in size or heavily degraded DNA samples.

JGI Starting Material Specifications: Mass

For Illumina sequencing, a minimum of 2 ug is needed for each standard shotgun library for paired-end sequencing and between 12-62 ug for each large-insert paired-end library, depending on the insert size.

This translates into the following estimates of total DNA quantity required to complete some of the common JGI product types:

	Microbial standard draft	Microbial improved standard draft	Microbial single-cell sequencing	Resequencing projects
Volume per sample	50-500 uL	50-500 uL	50-500 uL	50-500 uL
Concentration	10-500 ng/uL	100-500 ng/uL	10-500 ng/uL	10-500 ng/uL
Mass	2 ug	54 ug	1 ug	2 ug

JGI Starting Material Specifications: 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.

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. Individual DNA samples should be shipped to the JGI in **one tube** (preps suitable for pooling should be pooled into a **single tube** for shipping to JGI). The tube should be legibly labeled with the sample ID that will be provided after the sample has been approved for shipping.

Last Updated 8/20/2012