DNA Preparation Guidelines

The JGI has sequenced over 9000 prokaryotic and eukaryotic drafts and nearly 6000 metagenome projects. DNA quality (molecular weight and purity) and quantity have always been the two critical factors in the success of the sequencing projects. **Samples not meeting minimum submission requirements with regard to quantity and quality will result in a delay to project initiation.** If you have any questions or concerns, contact your Project Manager or the Project Management Office.

View protocols for isolating DNA, provided by the JGI user community.

DNA Sample Requirements

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 accurate nucleic acid quantification in both our Illumina and PacBio sequencing workflows:

Importance of Sample QC
Sample Quality and Contamination
DNA Extraction 101 – tips, kits, and protocols (from PacBio)
Guidance for sample collection and storage (from PacBio)
Best practices for High Molecular Weight DNA extraction and quality control (from PacBio)
JGI's Genomic DNA Sample QC Protocol

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.

For eukaryotes, the starting DNA should be free of contaminants and/or symbionts, should have <5% organellar DNA, and <1% polymorphism wherever possible. If a haploid form for the organism exists, this is preferable. It may not be possible to assemble a genome that is derived from a contaminated or polymorphic sample.

***All samples must meet the following criteria**:*

1. Appropriate mass for project initiation as indicated by fluorometric measurement (for example, Qubit Fluorometer – Life Technologies, Inc.). NanoDrop measurements are generally only

reliable indicators of DNA contamination with RNA, carbohydrate, organic solvents and other impurities that may interfere with sequencing, and should not be used for quantification.

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

Product Type	Requested mass (ng)	Tube volume (ul)	Plate volume (ul)	Concentration range (ng/ul)
Microbial Minimal Draft (Illumina)	500	25-400	25-150	10-1000
Microbial Improved Draft (PacBio) (including Methylation Analysis)	3000	25-400	–	25-1000
Microbial Methylation Analysis	1500	25-400	–	10-1000
Metagenome Minimal and Standard Drafts	500	25-400	25-150	10-1000
Fungal Minimal Draft (Illumina)	500	25-400	25-150	10-1000
Fungal Standard Draft (PacBio)	5000	25-400	–	25-1000
Plant/Algal Standard Draft (NOTE: Arizona Genomics Institute will provide HMW DNA for these projects.)	100,000	25-400	–	80-1000
Resequencing	500	25-400	25-150	10-1000
DAP-Seq	12,000 per 92 TFs	25	–	100-1000

The actual amounts of material needed may vary by individual project. Please consult with your Project Manager if you may not be able to provide adequate material. Projects have the greatest odds of success with greater quantities of material. In some cases, JGI may be able to amplify the DNA; however the JGI does not recommend using MDA material for metagenome projects or methylation detection.

2. Absorbance measurements. $A_{260/280}$ ratio should be between 1.6 and 2.0 (spectrophotometer/NanoDrop). A lower ratio usually indicates contamination by protein or

residual phenol or other reagents associated with the DNA extraction protocol. $A_{260/230}$ ratio should be between 2.0 and 2.2. A lower ratio usually indicates contamination with salt, humic acids, guanidine, peptides or other chemicals.

3. 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 or another method. Heavily degraded samples cannot be used for construction of large insert libraries.
4. RNA blobs and discrete bands require clean-up. Please refer to the recommended RNAse A clean-up protocol.
5. 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), we request that you further purify your samples or dilute to the recommended concentration prior to shipping to the JGI since high viscosity can lead to incorrect quantitation and processing of the samples.
6. Molecular weight of the DNA sample determines the insert size of the library we can construct. In general, we will not accept samples exhibiting a majority of DNA fragments smaller than 23Kb in size or heavily degraded DNA samples. Most DNA preparation protocols should be able to generate DNA fragments of approximately 100kb in size, appropriate for Illumina and/or PacBio library construction.

Shipping:

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

1. DNA should be completely dissolved (recommended: 1x Low EDTA TE [10 mM Tris, pH 7.5-8.0, 0.1 mM EDTA]) and shipped on dry ice. (Low-EDTA buffer should be molecular biology grade, and RNase, DNase and protease-free. One supplier is VWR.)
2. Individual DNA samples should be shipped to the JGI in a single tube (or well of a plate). Preps suitable for pooling should be pooled prior to shipping to the JGI. **You must ship all samples in the barcoded tubes or plates provided by the JGI.**
3. **Do not ship samples until you have received a shipping approval email from the JGI.** Samples that have not been approved will be returned to sender.

Last update: 8/8/23