

# A quick reference guide to metatranscriptome sequencing at JGI

Alicia Clum and Emiley Eloe-Fadrosh

January 2022

The U.S. Department of Energy Joint Genome Institute is a DOE Office of Science User Facility that provides sequencing and analysis to scientists from all over the world studying carbon cycling, bioenergy and biogeochemistry. These services are primarily provided through several competitive grant calls: the annual Community Science Program (CSP) call, a twice annual CSP New Investigator call and the "Facilities Integrating Collaborations for User Science" (FICUS) calls. Further details on each can be found on JGI's [User Program Info page](#). Each of these calls places caps on the total amount of sequencing that can be requested. The purpose of this guide is to describe the standard products the Metagenome Program offers for metatranscriptome samples. Recommendations for metagenome samples are covered in a separate guide.

We offer two standard product types for metatranscriptome samples detailed in Table 1. In addition to our standard products, we have experimental sequencing products that we offer on a provisional basis. It is best to consult with JGI staff during the proposal writing process if you are interested in using experimental products. The standard products are sequenced using Illumina NovaSeq sequencers. We encourage you to focus on sample quality and take advantage of [JGI guidance and suggestions on sample prep](#) as sample quality is a key factor in project success. In our experience working with tens of thousands of samples, the largest user controllable factor contributing to a successful project delivered on time, is high quality starting material. By contrast, samples which are low quality, contaminated, or low concentration typically produce inferior results and result in project delays.

**Table 1: JGI Metagenome Program product offerings**

Product	Sequencing mode	Read length (bp)	Library creation (amount)	Target depth (M reads)	Example sample types
Metagenome Metatranscriptome	Illumina NovaSeq S4 paired end	150	Qiagen FastSelect (100 ng - ribo-depletion)	100	Primarily targeting prokaryotes
Eukaryote Community Metatranscriptome	Illumina NovaSeq S4 paired end	150	Illumina Truseq Stranded mRNA Library Prep (1000 ng - polyA selection)	100	Primarily targeting eukaryotes

Table 1: The modes and typical yields of standard sequencing products from the Metagenome Program. Some common environments are also listed in the table. The exact allocation of samples is done collaboratively with JGI staff after proposals have been accepted. This guide should help you propose a reasonable sample allocation for your project based on our normal workflows. More detailed information on submitting samples is available on at JGI's [sample submission page](#).

In addition to the above standard products offered, the JGI provides analysis support for combined metatranscriptome assemblies that computationally combine sequencing data from multiple samples where it makes sense biologically. For metatranscriptome samples we can also accommodate requests to map metatranscriptome reads for up to 10 metagenome datasets in IMG per metatranscriptome sample. Please contact your JGI Project Manager if you are interested in these analysis offering.

## Frequently Asked Questions

***Should I deplete my metatranscriptome samples?*** We offer rRNA depletion or polyA selection as a standard service on metatranscriptome samples depending on the sample type. We have achieved good success in many environments with our protocol, and users are strongly encouraged to send total RNA. Consultation with JGI staff is required for any other sample type.

***What if I am expecting prokaryotes and eukaryotes in my metatranscriptome samples?*** We can split the samples and generate one library using polyA selection to target the eukaryotes and one library using ribosomal depletion to target prokaryotes.

***What if I have synthetic spike-ins I want to use?*** The JGI strongly discourages the submission of samples that include internal standards, either synthetic or known template material, to avoid unwanted propagation of these sequences in public repositories. While the JGI has implemented robust quality-control measures to ensure the metagenome and metatranscriptome sequence data produced is high quality, we do not currently have processes in place to detect and remove an expanded set of synthetic or known template material that can be applied across the diverse set of environmental samples the JGI handles. If your study design or planned sample submission requires the use of custom internal standards, please be sure to discuss with your JGI Project Manager to determine a suitable solution.