

CSP FY27 Functional Genomics Call

The current call for proposals offers multiple capabilities, as described below. Applicants are invited to request one or more capabilities within a single proposal. Sequence requests should not exceed 3 Tb in total.

1) Synthesis of genes and pathways for functional characterization. A single proposal can request a total of 100 to 500 kb of DNA synthesis capacity per proposal. A consortium (with co-PIs from at least 3 different institutions) can request up to 1,500 kb. All constructs are synthesized and assembled into user-defined plasmids, sequence validated, and transformed into an *E. coli* strain before shipment to users. The products are delivered to users as glycerol stocks. Projects requiring specific nucleotide sequences (such as those required for homology-based recombination) may experience lower successful assemblies due to difficulties in synthesizing precise DNA sequences in the absence of refactoring. Therefore, we may have to adjust the scope of the project depending on the complexity of the sequence constraints. Prospective users are encouraged to contact [Ian Blaby](#) to discuss.

2) Synthesis of combinatorial pathway libraries for fast-track metabolic engineering. Each proposal may request from 100 to 500 kb of DNA *de novo* synthesis capacity to produce millions of basepairs of combinatorial variants. A consortium (with co-PIs from at least 3 different institutions) can request up to 1,500 kb. The JGI will also help identify a panel of each pathway component and design final constructs. All constructs are assembled using type II restriction-enzyme-based technologies (e.g., golden gate assembly) into user-defined plasmids and are transformed into *E. coli* strains before shipment to users; no sequencing validations will be performed for the constructs. The products are delivered to users as glycerol stocks.

3) Synthesis of sgRNA libraries. Each proposal may request up to 50,000 gRNA sequences. The JGI can help design sgRNA sequences based on the genome sequences of targeted microbes. All sgRNA constructs are synthesized, cloned into user-defined plasmids, and transformed into an *E. coli* strain as pools. The quality of these libraries is evaluated with sequencing-based analysis using MiSeq before shipment to users. The JGI will deliver the libraries to users as glycerol stocks. The subsequent transformation into the targeted microbes and functional screenings will be performed by users. The JGI can further evaluate enriched sgRNA libraries with sequencing-based analysis using MiSeq.

4) Strain Engineering: Genomic Integration of Synthetic Constructs into a Set of Bacterial Strains. JGI is offering a limited capacity of Chassis-independent recombinase-assisted genome engineering (CRAGE) to users. This technology enables integration of large, complex genetic constructs directly into the chromosomes of diverse gamma-proteobacteria with high accuracy and efficiency. Proposals may request up to 96 constructs to be cloned into a CRAGE compatible vector under the control of a T7 promoter and conjugated into a maximum of 5 host strains. The current list of preferred microbial species offered this call is [here](#). **We currently do not offer domestication of new strains to users.**

Reference: *CRAGE enables rapid activation of biosynthetic gene clusters in undomesticated bacteria* <https://www.nature.com/articles/s41564-019-0573-8>

For additional information (literature citations, video), see this CRAGE [blog post](#).

5) Sequence data mining. The JGI's genome portals IMG, MycoCosm and Phytozome contain a wealth of genomic data from microbes, fungi, plants and microbiomes. Proposals may request assistance with database searches for the selection of target genes and pathways for synthesis. However, capacity for analyzing search results and aiding in target selection is very limited; users needing assistance with these tasks should contact JGI in advance to discuss feasibility.

6) Metabolomics based functional analyses. Metabolomic technologies at JGI enable users to examine diverse polar and non-polar metabolites from plants, microbes, and environments. In addition, users may request targeted analysis of stable isotope labeling for specific metabolites. Proposals should clearly indicate how the data obtained will be linked to gene function, and may request up to 50 polar metabolite sample analyses or 150 non-polar metabolite sample analyses.

7) Mapping of transcription factor binding sites (DAP-seq). High-throughput mapping of putative transcription factor binding sites enables large-scale characterization of gene regulatory networks in a selected species. Proposals can request *in vitro* transcription factor binding site mapping by DNA affinity purification sequencing (DAP-seq) for 92 transcription factors. This is a 96-well plate based protocol where 4 wells in each plate are reserved for negative controls. All transcription factors within a plate will be assayed against either a single genomic DNA library or a single pool of genomic DNA libraries. See the [multiDAP method paper](#) for more details on pooled libraries. DNA/gene synthesis should also be requested for construction of affinity-tagged transcription factor clones used in the assay.

8) RNA-seq and metatranscriptomes. Transcriptional profiling can aid in characterizing gene regulatory pathways activated in response to perturbations or environmental stimuli. Proposals may request RNA sequencing of between 22 and 92 samples (including replicates) from plants, algae, fungi, or microbes, including microbial communities, for the purpose of testing gene function or elucidating regulatory networks. Limited numbers of annotated reference genomes to be used for analysis of the RNA-seq dataset may also be

requested (allowable sample numbers are specified in the proposal form). Requests for draft genomes or metagenomes outside the context of 'omics analysis are not allowed as part of this call.

9) EcoFAB pilot projects. The JGI can provide up to 50 EcoFAB devices (<https://eco-fab.org/>) to study plant-microbiome interactions. These devices allow for non-destructive root imaging and sampling of the growth media while maintaining a sterile environment. In addition, the JGI can provide a standardized defined microbial community that colonizes plant roots and Brachypodium germplasm, if desired. Users would conduct experiments using these resources and return samples to the JGI for analysis by existing JGI capabilities e.g. metabolomics and transcriptomics. Contact: [Trent Northen](#)

DOE Mission Areas

The following topics are not within scope for this call for proposals: human health, pharmaceuticals, and cosmetics; food crops, feed, animal agriculture or aquaculture, and related production systems; invasive plant species; and first-generation biofuel targets (i.e. corn, sugarcane, rapeseed/canola, palm oil). Also excluded are projects focused on environmental treatment, mitigation, or pollution, including biogas, greenhouse gases, climate change, wastewater treatment, sewage, solid or liquid waste, bioremediation of organic contaminants, per- and polyfluoroalkyl substances (PFAS), and harmful algal blooms. Projects focused on the fundamental science of insect and other animal microbiomes, or on plant pathogens not associated with bioenergy- or bioproduction-relevant host organisms, are out of scope, as are marine-focused projects (more than 185 km from land).

Please refer to the [BER Mission Areas](#) and reach out to [Tanja Woyke](#) with any questions about the suitability of your proposal.

Proposal Schedule

Proposals are accepted on a continuous basis and will be reviewed annually.

Upcoming submission deadlines:

FY27: January 29, 2027 (review in March 2027)

FY28: January 31, 2028 (review in March 2028)