JGI JOINT GENOME INSTITUTE
A DOE OFFICE OF SCIENCE USER FACILITY

CSP FY2025 Functional Genomics Call (status: OPEN)

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 JGI staff 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 gamma-proteobacteria hosts. **We currently do not offer domestication of new strains to users.**

The current list of preferred microbial species offered through this call include:

Pseudomonas putida KT2440

Pantoea agglomerans ATCC 13460 (Eh1087 (ICMP 13301))

Dickeya solani DSM 28711

Yersinia aldovae DSM 18303

Aeromonas piscicola LMG 24783

Photorhabdus luminescens laumondii TTO1

Shewanella oneidensis MR-1

Photobacterium halotolerans DSM 18316

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 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 between 70-92 transcription factors. DNA/gene synthesis should also be requested for construction of affinity-tagged transcription factor clones used in the assay.

8) RNA-seq. 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.

Proposal Schedule

CSP Functional Genomics proposals are accepted on a continuous basis and will be reviewed twice a year. Submission deadline for reach review process is listed below. Letters of intent are not required.

Submission deadlines:

FY25: January 30, 2025 (review March 2025)
FY26: January 29, 2026 (review March 2026)

Proposal Review Process

All proposals undergo scientific review as described at https://jgi.doe.gov/user-programs/program-info/csp-review-process-and-contract-documents/. In addition, proposals requesting DNA synthesis that are tentatively approved will undergo an additional review of potential impacts as described below prior to project initiation.

Proposals requesting DNA synthesis are evaluated by at least three external reviewers in a process known as Synthetic Biology Internal Review (SBIR). SBIR encourages investigators to extensively consider broader aspects of their research (e.g., biosafety, biosecurity,

bio-containment and environmental issues) to evaluate both positive and negative impacts and to propose strategies to mitigate concerns. If issues are not sufficiently addressed, users will be asked to modify their proposal. If issues are not resolved, the proposal may be rejected. SBIR generally takes three weeks.

Investigators should not merely write “None” or “All research will be conducted in a safe manner according to Federal regulations” in the broader implications statement, as this will lead to requests for proposal modifications, incurring delays of three weeks or longer.

Investigators must explicitly state whether their proposed research would:

Demonstrate how to make a vaccine ineffective
Confer resistance to antibiotics or antiviral agents
Enhance a pathogen’s virulence or make a non-virulent microbe virulent
Increase transmissibility of a pathogen
Alter the host range of a pathogen
Enable a pathogen’s ability to evade diagnostic or detection modalities
Enable the weaponization of a biological agent or toxin