Synthesis of genes and pathways for functional characterization. A single proposal can request a total of 100 to 500 kbp of DNA synthesis capacity per proposal. A consortium (with co-PIs from at least 3 different institutions) can request up to 1,500 kbp. 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.

Synthesis of combinatorial pathway libraries for fast-track metabolic engineering. Each proposal may request up to 500 kbp of DNA *de novo* synthesis capacity to produce millions of basepairs of combinatorial variants. 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.

Synthesis of sgRNA libraries. Each proposal may request up to six libraries comprising up to 12,000 sgRNA sequences per library, or more than six libraries with less degree of variants per library. 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.