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The IMG/M data management and analysis system v.7: content updates and new features

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ABSTRACT

The Integrated Microbial Genomes & Microbiomes system (IMG/M: https://img.jgi.doe.gov/m/) at the Department of Energy (DOE) Joint Genome Institute (JGI) continues to provide support for users to perform comparative analysis of isolate and single cell genomes, metagenomes, and metatranscriptomes. In addition to datasets produced by the JGI, IMG v.7 also includes datasets imported from public sources such as NCBI Genbank, SRA, and the DOE National Microbiome Data Collaborative (NMDC), or submitted by external users. In the past couple years, we have continued our effort to help the user community by improving the annotation pipeline, upgrading the contents with new reference database versions, and adding new analysis functionalities such as advanced scaffold search, Average Nucleotide Identity (ANI) for high-quality metagenome bins, new cassette search, improved gene neighborhood display, and improvements to metatranscriptome data display and analysis. We also extended the collaboration and integration efforts with other DOE-funded projects such as NMDC and DOE Biology Knowledgebase (KBase).

INTRODUCTION

The Integrated Microbial Genomes & Microbiomes (IMG/M: https://img.jgi.doe.gov/m/) is a public data resource that includes isolate and single cell genomes (archaea, bacteria, eukarya, plasmids, viruses), metagenomes and metatranscriptomes. In addition to datasets sequenced at the DOE's Joint Genome Institute (JGI), IMG also includes public datasets downloaded from GenBank (1) and the Sequence Read Archive (SRA) (2), and datasets submitted by external users through the IMG submission system (https://img.jgi.doe.gov/submit/). Each dataset is associated with a large number of curated metadata information from the Genomes OnLine Database (GOLD) (3).

Starting with an input FASTA sequence file, IMG performs gene calling and functional annotation as described previously (4,5). A recently released new feature allows the submission of genomes in the General Feature Format (GFF3) (http://gmod.org/wiki/GFF3) to bypass the IMG gene calling step. Since the IMG native pipeline can only reliably predict prokaryotic genes, this new feature is particularly useful for eukaryotes and viruses.

Another important enhancement in IMG is the addition of the computationally predicted GTDB-Tk (6) taxonomic information to isolate genomes, enabling the users to view and to compare the default NCBI taxonomy to GTDB's genome sequence-based assignment.

Most of the software packages and reference databases used in IMG v.7 remain the same as in v.6 (4), including CRT (7) for CRISPR element detection, tRNAscan-SE 2.0.8 (8) for tRNAs prediction, Rfam covariance models and Infernal tools (9–11) for RNA features prediction, and Prodigal v2.6.3 (12) and GeneMarkS-2 v1.14_1.25 (13) for CDS (protein-coding sequence) prediction. Functional annotation of CDSs is performed using a thread-optimized hmmsearch from the HMMER v3.1b2 (14,15) package based on the following databases:

- updated 2014 version of COGs (16)
- version 15.0 of TIGRFAM (17)
- version 1.75 of SUPERFAMILY (18)
- version 01_06_2016 of SMART (19)
- version 4.2.0 of CATH-FunFam (20)

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- SignalP v4.1 (21) and TMHMM 2.0c (22) (for the prediction of signal peptides and transmembrane regions)

Two reference database sources have been updated in IMG v.7. We now use a new version of Pfam v34.0 (23) released in March 2021, and a new version of KEGG v98.0 (24) released in April 2021. MetaCyc v24.5 (25) pathways are recomputed using the new Enzyme Commission (EC) derived from KO terms. Our plan is to update reference databases every year subject to resource availability. As part of this reference database update process, Pfam, KO and EC annotations for all isolate genomes in IMG have been refreshed. Due to resource limitations, we were unable to reannotate all existing metagenomes in IMG with the updated reference databases. From the microbiome detail page, the version of the IMG annotation pipeline used to process this metagenome is reported under 'IMG Release/Pipeline Version.' Annotation pipeline change log can be found in the Help section (https://img.jgi.doe.gov/help.html).

Even though the gene calling and annotation methods of the IMG pipeline remain largely the same (with additional automated genetic code detection improvements), we have modified the implementation method to use the Workflow Description Language (WDL) (https://openwdl.org/). This allows us to provide an open version of the pipeline available through NMDC (https://microbiomedata.org/) that can run on different platforms and computational resources. As part of our efforts to scale up and improve throughput for the metagenome annotation process, several hundreds of metagenomes have been annotated using the WDL-version of the pipeline on Amazon Web Service (AWS) (https://aws.amazon.com/) over the past several months, highlighting cloud-based services as a possible avenue for running large-scale annotation of metagenomes using the IMG pipeline.

IMG currently has around 8000 active users per month based on the IP addresses. There are >24 000 registered users who can access additional password-protected features of IMG such as private genome submissions and private workspace datasets. Based on the user registration information, many academic institutions used IMG for research or teaching purposes.

DATA CONTENT

Genomes, Metagenomes and Metatranscriptomes

As of August 2022, IMG v.7 includes 451 million genes from genomes (24% growth since August 2020) and a total of 75.11 billion genes from metagenomes and metatranscriptomes (16% growth since August 2020). Table 1 shows the current IMG content, as compared with its content in August 2020.

External users continued submitting their datasets to IMG for annotation and analysis, with submissions of 4349 genomes and 1891 metagenomes over the past two years. In addition, we continued importing reference genomes and metagenomes from NCBI Genbank and SRA, respectively. These datasets are publicly available to all IMG users for comparative analysis. JGI recently updated its Data Policy as described on the JGI website (https://jgi.doe.gov/user-program-info/pmo-overview/policies/). While this new policy states that sequencing data is now subject to a one-year embargo, some public JGI datasets have legacy use restrictions, as indicated under 'JGI Data Utilization Status' on the dataset overview page. Data users are required to verify any use restrictions and contact the listed PI(s) if/as needed.

Table 1. IMG dataset content comparison (unit: dataset)

	Total (8/2022)	Public (8/2022)	Total (8/2020)	Public (8/2020)
Archaea	3190	2258	3011	1967
Bacteria	116 439	99 477	99 004	83 768
Eukaryota	1069	724	746	710
Virus	17 315	15 802	9804	8392
Plasmid	1208	1188	1208	1188
Metagenome	34 196	29 932	26 488	21 813
Metatranscriptome	8499	7372	6371	6174
Cell enrichment	2948	2430	2357	2110
Single Particle sort	8153	6608	5806	5378
Metagenome bin	197 851	176 089	85 565	83 287

DATA ANALYSIS

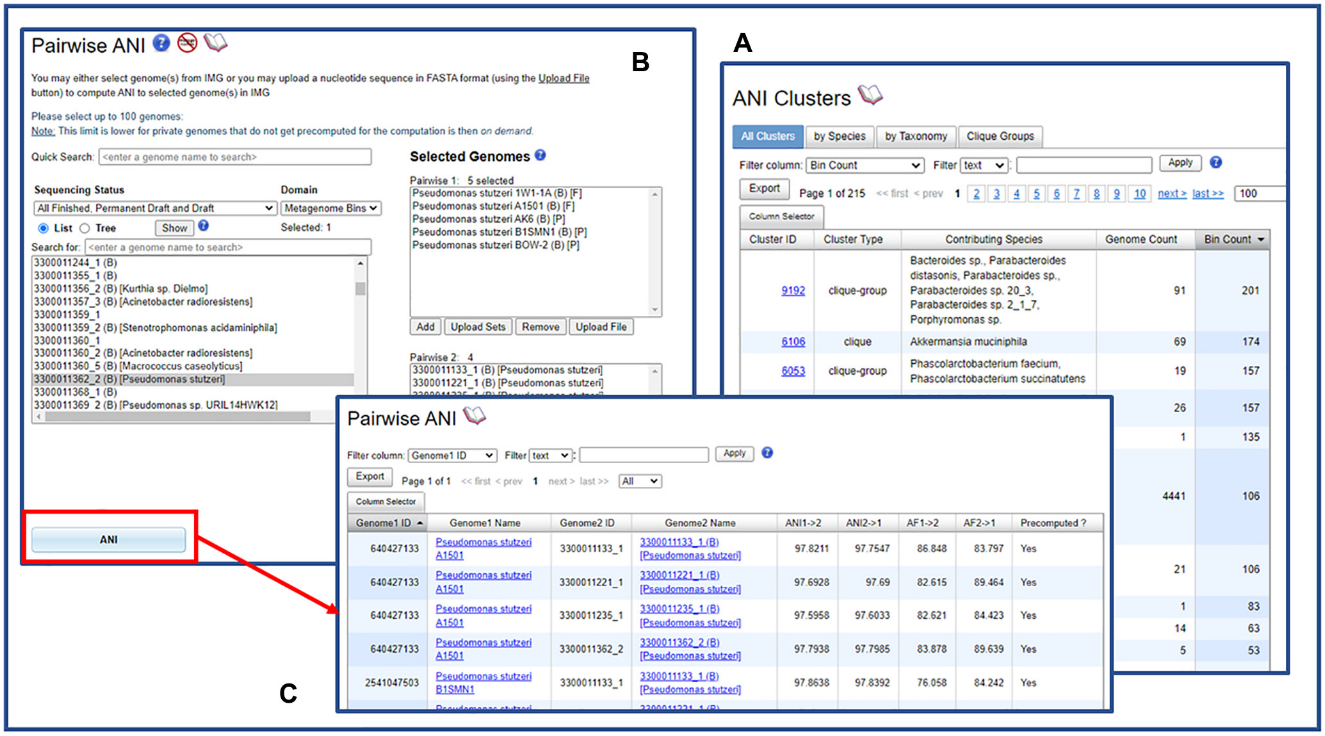
Several new features have been added to the User Interface (UI) (https://img.jgi.doe.gov/m/) to expand the IMG comparative analysis capabilities and to improve user experience. We describe the new improvements of the IMG UI below.

An updated scaffold search and list display with configuration

In IMG v.5 (26) and IMG v.6 (4), respectively, we revamped and improved the genome and gene search capabilities. In IMG v.7 we have made similar improvements to scaffold search. The new Scaffold Search under Find Genomes menu provides two search modes: Quick Search allows querying of scaffolds in IMG using scaffold IDs, scaffold external accessions, genome IDs or scaffold names. This search mode includes metagenome scaffold IDs, which may not be globally unique. Advanced Search Builder provides the capability to construct more complicated queries combining conditions on scaffold lineage, scaffold statistics (length, GC content) and functional content, similar to the existing genome and gene advanced search features. The default search results in IMG include a few mandatory fields, such as IMG-specific identifiers, as well as fields included in the query conditions. Similar to the genome and gene search results, scaffold search result lists are configurable and can include additional user-specified fields. Currently four categories are available to choose from: Scaffold Taxonomy, Function IDs, Function Names, and Scaffold Statistics.

Scaffold search results can be saved to the Scaffold Analysis Cart. Seven categories of scaffold metadata types are there for selection in the cart:

- IDs and Names – Scaffold ID (*), Scaffold Name, IMG Genome/Taxon ID (*), Genome Name (*)
- Scaffold Information – Scaffold Topology (linear or circular), NCBI Molecular Type (chromosome, plasmid, etc.)
- Scaffold Statistics – Scaffold Gene Count (*), Scaffold Nucleotide Length (*), Scaffold GC Content, Scaffold Read Depth



Pairwise ANI

You may either select genome(s) from IMG or you may upload a nucleotide sequence in FASTA format (using the Upload File button) to compute ANI to selected genome(s) in IMG

Please select up to 100 genomes:
Note: This limit is lower for private genomes that do not get precomputed for the computation is then *on demand*.

Quick Search: <enter a genome name to search>

Sequencing Status: All Finished, Permanent Draft and Draft
Domain: Metagenome Bins
● List ○ Tree Show
Selected: 1
Search for: <enter a genome name to search>

3300011244_1 (B)
3300011355_1 (B)
3300011356_2 (B) [Kurthia sp. Dielmo]
3300011357_3 (B) [Acinetobacter radioresistens]
3300011359_1
3300011359_2 (B) [Stenotrophomonas acidaminiphila]
3300011360_1
3300011360_2 (B) [Acinetobacter radioresistens]
3300011360_5 (B) [Macrococcus caseolyticus]
3300011362_2 (B) [Pseudomonas stutzeri]
3300011368_1 (B)
3300011369_2 (B) [Pseudomonas sp. URIL14HWK12]

Selected Genomes

Pairwise 1: 5 selected
Pseudomonas stutzeri 1W1-1A (B) [F]
Pseudomonas stutzeri A1501 (B) [F]
Pseudomonas stutzeri AK6 (B) [P]
Pseudomonas stutzeri B1SMN1 (B) [P]
Pseudomonas stutzeri BOW-2 (B) [P]

Add | Upload Sets | Remove | Upload File

Pairwise 2: 4
3300011133_1 (B) [Pseudomonas stutzeri]
3300011221_1 (B) [Pseudomonas stutzeri]

ANI Clusters

All Clusters | by Species | by Taxonomy | Clique Groups

Filter column: Bin Count Filter text Apply
Export Page 1 of 215 << first < prev 1 2 3 4 5 6 7 8 9 10 next > last >> 100
Column Selector

Cluster ID	Cluster Type	Contributing Species	Genome Count	Bin Count
9192	clique-group	Bacteroides sp., Parabacteroides distasonis, Parabacteroides sp., Parabacteroides sp. 20_3, Parabacteroides sp. 2_1_7, Porphyromonas sp.	91	201
6106	clique	Akkermansia muciniphila	69	174
6053	clique-group	Phascolarctobacterium faecium, Phascolarctobacterium succinatutens	19	157
			26	157
			1	135
			4441	106
			21	106
			1	83
			14	63
			5	53

Pairwise ANI

Filter column: Genome1 ID Filter text Apply
Export Page 1 of 1 << first < prev 1 next > last >> All
Column Selector

Genome1 ID	Genome1 Name	Genome2 ID	Genome2 Name	ANI1->2	ANI2->1	AF1->2	AF2->1	Precomputed ?
640427133	Pseudomonas stutzeri A1501	3300011133_1	3300011133_1 (B) [Pseudomonas stutzeri]	97.8211	97.7547	86.848	83.797	Yes
640427133	Pseudomonas stutzeri A1501	3300011221_1	3300011221_1 (B) [Pseudomonas stutzeri]	97.6928	97.69	82.615	89.464	Yes
640427133	Pseudomonas stutzeri A1501	3300011235_1	3300011235_1 (B) [Pseudomonas stutzeri]	97.5958	97.6033	82.621	84.423	Yes
640427133	Pseudomonas stutzeri A1501	3300011362_2	3300011362_2 (B) [Pseudomonas stutzeri]	97.7938	97.7985	83.878	89.639	Yes
2541047503	Pseudomonas stutzeri B1SMN1	3300011133_1	3300011133_1 (B) [Pseudomonas stutzeri]	97.8638	97.8392	76.058	84.242	Yes

Figure 1. IMG now also assigns Average Nucleotide Identity (ANI) to high-quality metagenome bins. (**A**) ANI Cluster list shows both genome count and bin count. (**B**) Users can select both genomes and high-quality metagenome bins to perform pairwise ANI analysis. (**C**) The screen shows some Pairwise ANI analysis results.

- Scaffold Lineage – Lineage, Lineage Percentage
- Bin Information – In Bins
- Viral Information – Perc VPFs (Viral Protein Families), Viral Cluster, Viral Sequences
- Additional Metadata – Ecosystem, Ecosystem Category, Ecosystem Type, Ecosystem Subtype, Specific Ecosystem, Habitat, Host Name

Default display fields are marked with (*) in the above list. Selected scaffolds with additional user-specified fields can be exported as tab-delimited text files.

Average Nucleotide Identity for high-quality bins

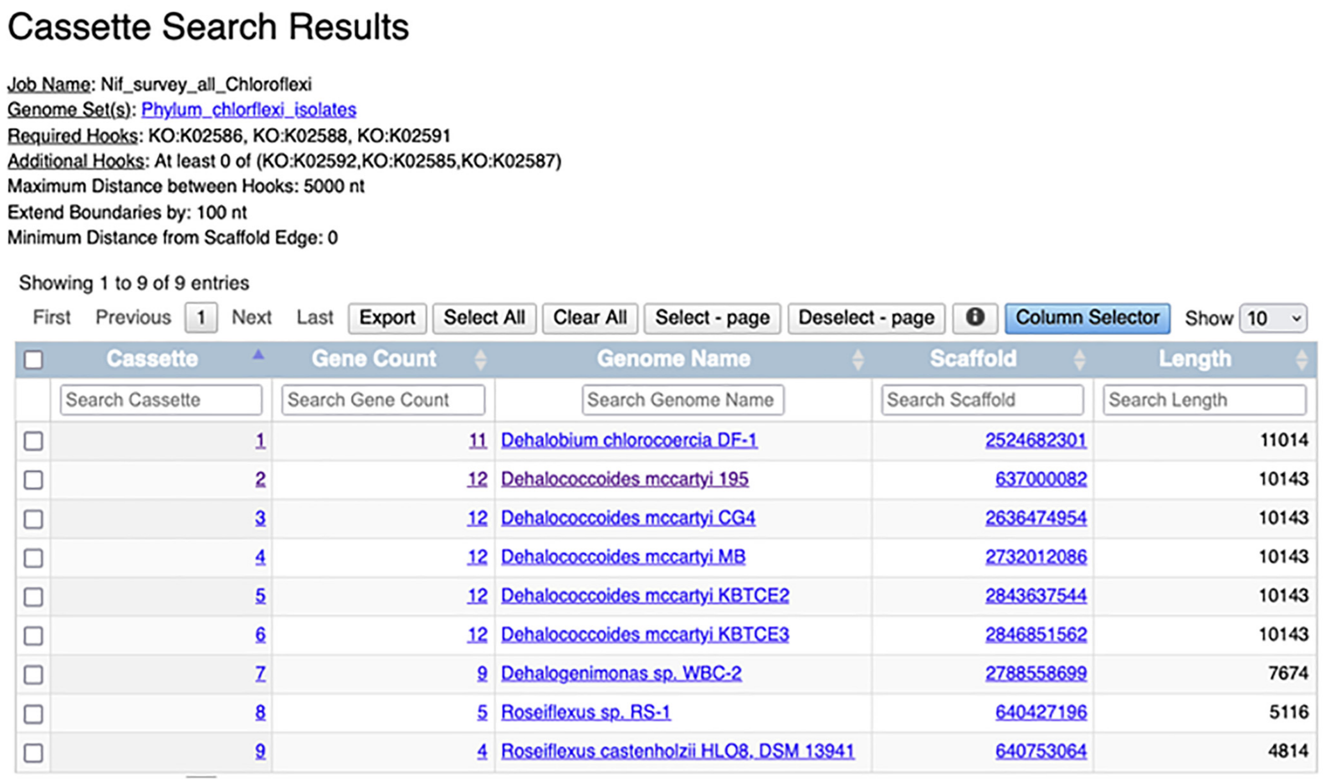
In addition to isolate genomes, IMG now also calculates Average Nucleotide Identity (ANI) (27) to and between high-quality metagenome bins. Briefly, to compute the ANI we use the MiSI method (27). In this method, for each genome pair, the CDS are compared to one another using the LAST sequence similarity search tool. Next, from the results, bi-directional best hits (BBHs) are identified as pairwise bidirectional best hits of genes having 70% or more identity and at least 70% coverage of the shorter gene. These BBHs are then used to compute the Average Nucleotide Identity (ANI) and the Alignment Fraction (AF). **ANI Clusters** in the **Compare Genomes ANI** menu item now lists both genome count and bin count (Figure 1A). Users can click on a cluster ID to view the genomes and bins belonging to this ANI cluster. They can also save the genomes to Genome Analysis Cart or Workspace Genome Set. Metagenome bins can be saved as Workspace Scaffold Sets.

Pairwise ANI in **Compare Genomes ANI** allows pairwise analysis of both genomes and high-quality metagenome bins (Figure 1B). Figure 1C shows some pairwise ANI comparison results. In the login version of IMG/MER (https://img.jgi.doe.gov/mer/) a 'submit computation' option exists for users to compare large amounts of data in the background to avoid UI timeouts.

This newly expanded ANI feature allows users to compare high-quality metagenome bins to similar metagenome bins, single-amplified genomes (SAGs), and isolate genomes without the need to submit bins as metagenome-assembled genomes (MAGs) into IMG.

Cassette Search and Cassette Workspace

We have extended gene search capabilities in IMG by introducing an improved version of the **ClusterScout** tool from IMG/ABC (28), allowing users to select genes based on their conserved chromosomal neighborhood and find 'guilt-by-association' connections between protein families. Similar to ClusterScout, a user can specify a set of 'hooks' - *i.e.* protein families expected to be found in close proximity to each other on the chromosome, within conserved chromosomal neighborhoods termed 'cassettes.' Unlike ClusterScout, which only works with Pfams as 'hooks,' Cassette Search allows users to select COG, Pfam, TIGRfam, KO terms or enzymes and to specify that some protein families must be associated with the same gene (e.g. Pfams corresponding to the N- and C-terminal domains of the same



Cassette Search Results

Job Name: Nif_survey_all_Chloroflexi
Genome Set(s): Phylum_chlorflexi_isolates
Required Hooks: KO:K02586, KO:K02588, KO:K02591
Additional Hooks: At least 0 of (KO:K02592,KO:K02585,KO:K02587)
Maximum Distance between Hooks: 5000 nt
Extend Boundaries by: 100 nt
Minimum Distance from Scaffold Edge: 0

Showing 1 to 9 of 9 entries

☐	Cassette	Gene Count	Genome Name	Scaffold	Length
☐	1	11	Dehalobium chlorocoercia DF-1	2524682301	11014
☐	2	12	Dehalococcoides mccartyi 195	637000082	10143
☐	3	12	Dehalococcoides mccartyi CG4	2636474954	10143
☐	4	12	Dehalococcoides mccartyi MB	2732012086	10143
☐	5	12	Dehalococcoides mccartyi KBTCE2	2843637544	10143
☐	6	12	Dehalococcoides mccartyi KBTCE3	2846851562	10143
☐	7	9	Dehalogenimonas sp. WBC-2	2788558699	7674
☐	8	5	Roseiflexus sp. RS-1	640427196	5116
☐	9	4	Roseiflexus castenholzii HLO8, DSM 13941	640753064	4814

Figure 2. New Cassette Search is an extension and improvement of the ClusterScout in IMG/ABC. Users can use any COG, Pfam, TIGRfam, KO terms or enzymes as 'hooks,' and can specify whether certain functions must be on the same genes. This example shows the use of the cassette search tool by surveying the genomes of *Chloroflexi* for the presence of a nitrogenase operon.

protein). Since Cassette Search is time consuming, this function is only provided in IMG/MER (log-in required) which allows submission of a background computation job. Users will then receive an email notification when their job completes.

We will illustrate the use of the cassette search tool by surveying the genomes of *Chloroflexi* for the presence of a nitrogenase operon (Figure 2). Nitrogen fixation is an important but energetically expensive process (16 ATP consumed per N2 molecule reduced to NH4+) catalyzed by a multi-subunit enzyme called nitrogenase. Nitrogenase (*nif*) genes show limited and uneven distribution across prokaryotic genomes (29). Somewhat unexpectedly, a full nitrogenase operon (*nifHIDKENB*) was observed in the genome of *Dehalococcoides mccartyi* 195, the first sequenced member of the phylum *Chloroflexi* (30), an anaerobic dehalorespirer growing in oligotrophic contaminated sites with limited means of energy production. Strain 195 has been experimentally shown to fix N2 to sustain growth (31); however, due to high energy demand of nitrogen fixation, this is accompanied by decreased cell density and dechlorination activity (with implications for *in situ* bioremediation using such microbes).

KO terms used as hooks to retrieve nitrogenase operons in *Chloroflexi* include:

- (Required) KO:K02586 – nitrogenase molybdenum-iron protein alpha chain (nifD)
- (Required) KO:K02588 – nitrogenase iron protein (nifH)
- (Required) KO:K02591 – nitrogenase molybdenum-iron protein beta chain (nifK)
- (Optional) KO:K02585 – nitrogen fixation protein (nifB)
- (Optional) KO:K02587 – nitrogenase molybdenum-cofactor synthesis protein (nifE)
- (Optional) KO:K02592 – nitrogenase molybdenum-iron protein (nifN)

The distance between 'hooks' is set to 5000 nt, which allows for the presence of additional genes between them. Boundary extension (set to 100) may be increased to show the regions up- and downstream of the requested hooks allowing the user to identify any additional protein families potentially associated with nitrogenase operons. The minimum distance from scaffold edge is set to '0,' so that operons close to the scaffold/contig edge of scaffold in possibly highly fragmented draft genome assemblies are not overlooked. The query is run on a set of 133 public isolate genomes of *Chloroflexi*, which were previously saved as a Workspace Genome set. The user is notified by email when the search result is ready.

The query retrieves nine cassettes from nine genomes, which include several species of *Dehalococcoides, Dehalobium* and *Dehalogenimonas*, but also two *Roseiflexus* species, which are quite distant from dehalorespirers and belong to a different class. In order to handle the results of Cassette Search, IMG Workspace has been extended to support the new 'cassette sets,' whereby each cassette represents a region on a contig or scaffold, delineated by the presence

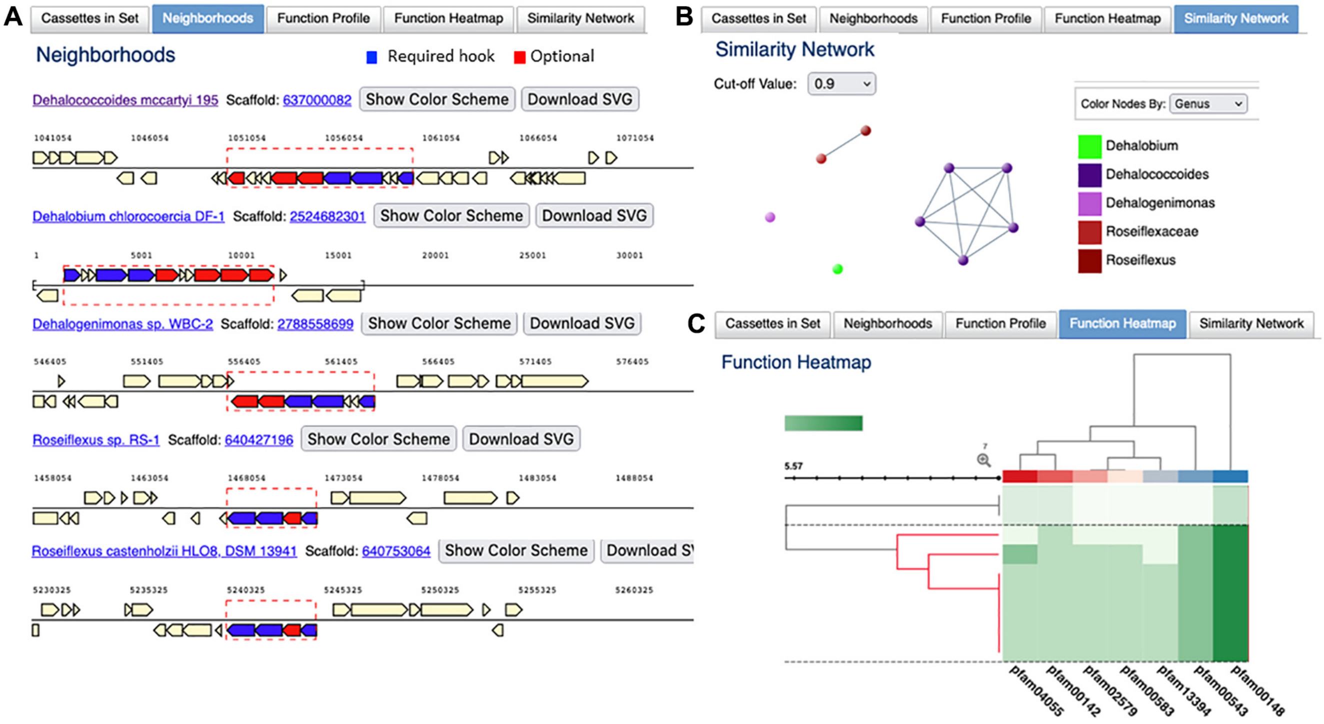
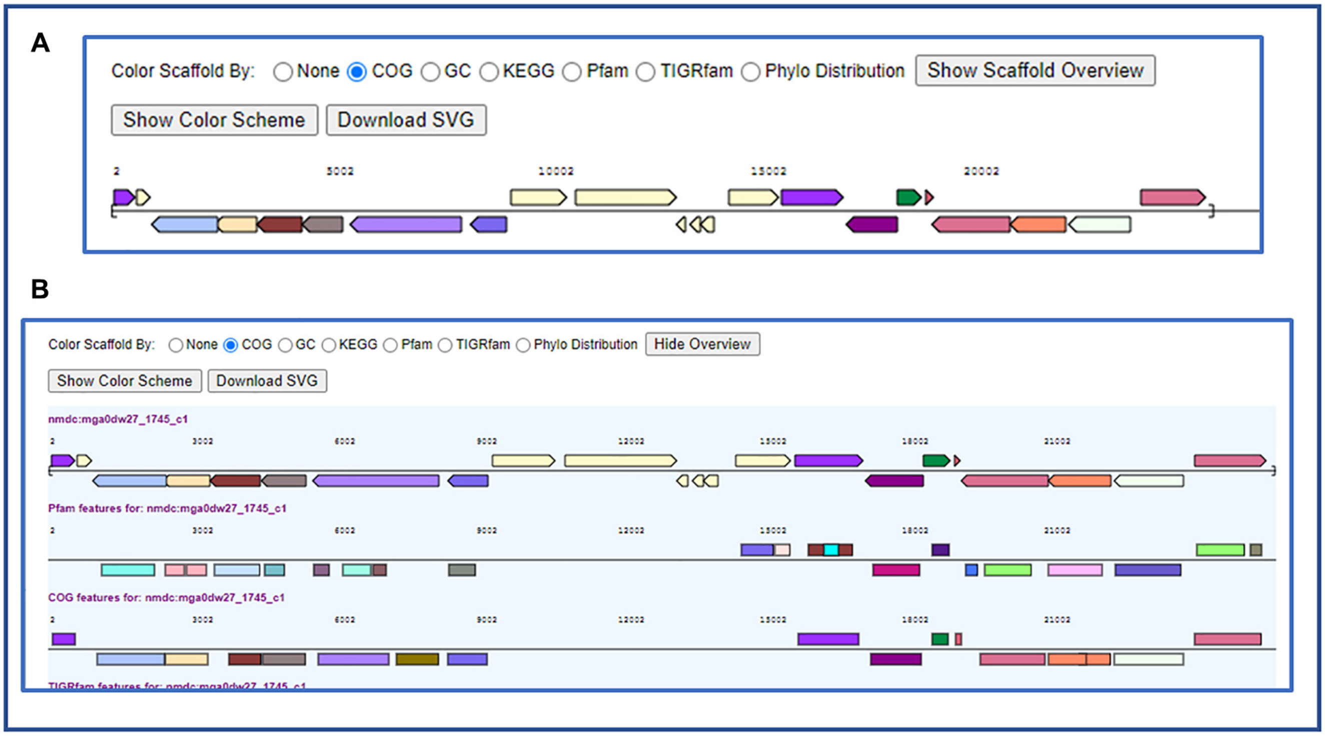


Figure 3. Cassette Search results can be saved as Workspace Cassette Sets. Workspace provides additional functions to analyze cassettes. (**A**) Genome neighborhood of cassette search results show required hooks (colored blue) and optional additional hooks (colored red). The specific boundaries of the requested cassettes as specified are outlined. Single cassette for *D. mccartyi* str 195 is shown (four other identical cassettes from the other strains are omitted for ease of display). (**B**) Similarity network graph to summarize the data showing 5 D. mccartyi cassettes forming a distinct group (purple) and separated from the two 4-gene *Roseiflexus* cassettes and singletons. Nodes can be recolored by taxon level (phylum to species). (**C**) Function heatmap can be used to visualize the Pfam content of cassettes. Cells are colored with hues of green based on the number of copies of the selected Pfam in the cassette (darker signifies a higher copy number). Rows are individual cassettes while columns are Pfams that occur in all cassettes and define the core functions of the cassette. Metadata cells are hidden in the current display but hovering over these metadata cells provides row and column details. The cassettes from the 7 dehalorespiring members cluster together (branches colored in red) based on their Pfam profiles.

of 'hooks' within specified distance (in bp) from each other. A user can select a number of cassettes from the Cassette Search and save them to a named Workspace Cassette set. From a Workspace Cassette set, a subset of cassettes can be selected to perform four types of analysis. The **Function Profile** tab displays the counts of different types of protein families found in the cassettes (COG, Pfam, TIGRfam, EC, KO). This option allows users to find which protein families are most commonly associated with their 'hooks' of interest. **Neighborhoods** tab provides a graphical display of gene neighborhoods for all selected cassettes with multiple coloring options (highlighting the 'hook' proteins only or color-coding genes by different protein families). A visual inspection of neighborhoods confirms all 9 genomes have a minimal Nif gene set, but the number of optional hooks is clearly different (Figure 3A). While two *Roseiflexus* spp. have just the minimal set, dehalorespiring species have an extended version of Nif operon with 6 or more Nif genes including regulators. The differences in protein content between Nif cassettes can be visualized using the **Similarity Network** tab with Jaccard index cutoff of 0.9 where the 5 *D. mccartyi* cassettes form a distinct cluster from two *Roseiflexus* cassettes, with *Dehalogenimonas* and *Dehalobium* appearing as singletons (Figure 3B). The **Function Heatmap** tab, which provides an alternative way of visualizing the differences in protein content, also shows that *Roseiflexus* cassettes are distinct from those found in dehalorespiring species (Figure 3C). The data is hierarchically clustered using InCHlib Javascript library (32) with the Jaccard distance based on Pfam composition of the cassettes and Ward linkage options. Hovering the mouse above rows, columns, and cells displays the details of Pfams, cassettes (including taxonomy) and the counts, respectively. It is also interesting to note that out of 30 *D. mccartyi* strains in the *Chloroflexi* genome set, only these five highly similar strains (based on ANI) encode a Nif cassette, suggesting recent horizontal gene transfer in the last common ancestor of this lineage. With this result, one may conclude that N2 fixation is not widespread among dehalorespiring taxa. Many other IMG tools and features can be used to explore other correlations or the potential origin of this operon in these strains.

Improved gene neighborhood display

Gene neighborhood display has always been a popular feature of the IMG UI. It provides a graphical overview of the functional content of contigs and scaffolds by color-coding the genes according to their COG, Pfam, etc. (Figure 4A). Two major limitations of the existing viewer are that: (i) only one functional distribution is shown at a time, and (ii) the distribution of Pfams along the length of a single gene reflecting its domain composition is not shown.



A

Color Scaffold By: ○ None ● COG ○ GC ○ KEGG ○ Pfam ○ TIGRfam ○ Phylo Distribution [Show Scaffold Overview]

[Show Color Scheme] [Download SVG]

B

Color Scaffold By: ○ None ● COG ○ GC ○ KEGG ○ Pfam ○ TIGRfam ○ Phylo Distribution [Hide Overview]

[Show Color Scheme] [Download SVG]

2
5002
10002
15002
20002
nmdc:mga0dw27_1745_c1
2
3002
6002
9002
12002
15002
18002
21002
Pfam features for: nmdc:mga0dw27_1745_c1
COG features for: nmdc:mga0dw27_1745_c1
TIGRfam features for: nmdc:mga0dw27_1745_c1

Figure 4. Improved gene neighborhood display. (**A**) Original gene neighborhood display shows only a single function category. (**B**) The new scaffold overview shows multiple functional categories and the exact location of each function.

The new gene neighborhood display overcomes these limitations by now providing a viewer that displays all protein family classifications at once and shows the exact positions of the protein family hits on the genes. For example, by clicking the 'Show Scaffold Overview' button, the Pfam, COG, TIGRfam, KO and EC distributions are displayed in a single viewer (Figure 4B). Moreover, when a gene has multiple Pfam hits, the exact location of each Pfam hit is shown. In addition, a new 'Download SVG' button allows the download of the gene neighborhood graph in publication quality SVG (Scalable Vector Graphics) format. This new display also provides expanded features for the representation of expression data, as described below.

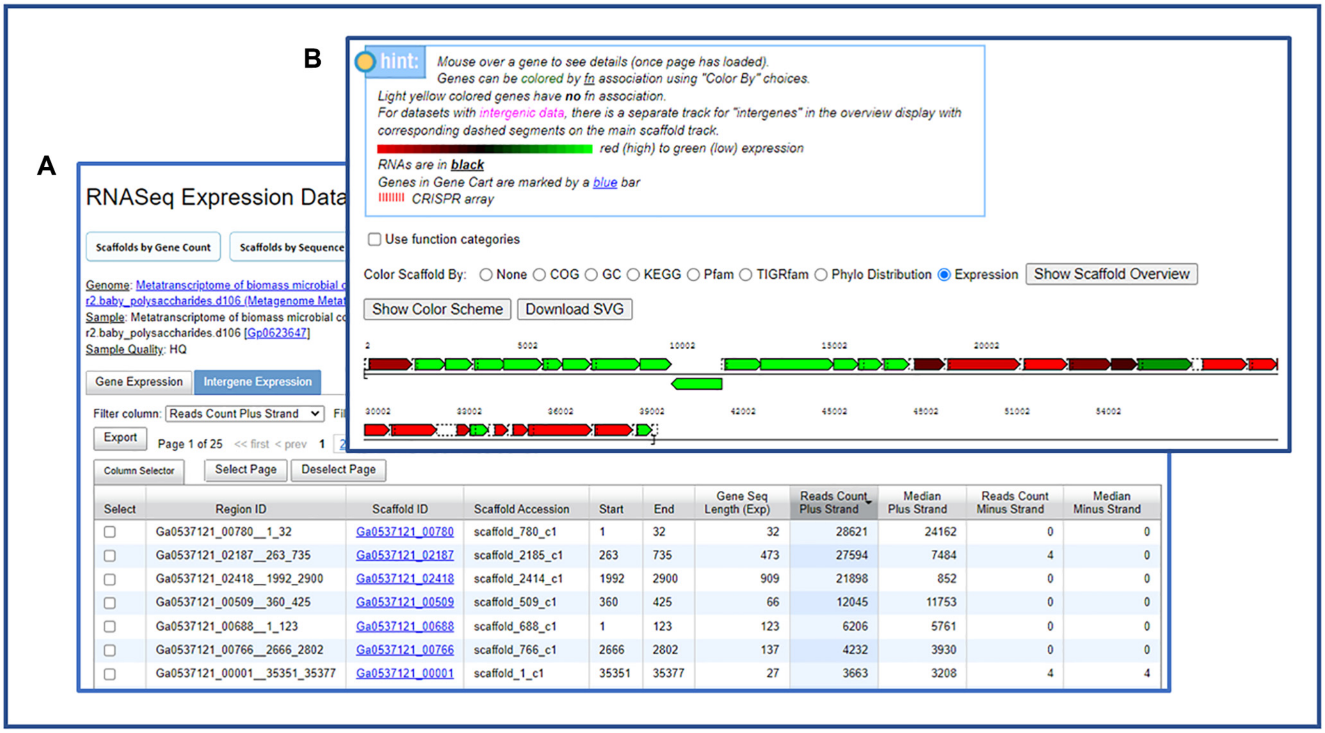
Improvements to metatranscriptome data display and analysis

IMG has improved both the quality and the display of metatranscriptome data over the past two years. Important RNASeq library details are now shown in the list of all RNA experiments. As the default JGI protocol creates stranded RNASeq libraries, the majority of reads (>85%) in a metatranscriptome dataset are expected to map to the sense strand. However, some libraries are generated using a non-stranded protocol; such samples can be distinguished by signifying 'non-stranded' in the 'Quality' field. On the other hand, some stranded samples end up having high levels of antisense strand coverage due to DNA contamination and/or amplification artifacts; such samples are labeled 'LQ' for 'low quality.'

In addition to the sense-strand expression data for coding sequences (defined as the strand on which the coding sequence is predicted), metatranscriptome datasets now include antisense-strand expression information for coding sequences and **Intergenic Region Expression** data (Figure 5A). These new features allow users to find the coding sequences with unusual expression patterns, such as high levels of expression on antisense strand, which may be indicative of the presence of antisense regulatory RNAs or, alternatively, caused by various artifacts, such as DNA contamination and erroneous gene prediction. Several filters based on gene lengths and expression values can help users focus their analysis on specific genes and contigs of interest, and links to scaffold distributions by the number of genes and scaffold length allow users to view a graphical summary of gene expression for scaffolds of interest in the context of their functional annotation. The **Intergene Expression** data tab lists the intergenic regions and their expression values on the forward and reverse strand, with strand direction corresponding to that of the scaffold/contig. Sorting and filtering the data table allows users to identify intergenic regions with specific expression patterns and by clicking on the scaffold ID link, users can compare the intergenic region expression to the neighboring genes in the new gene neighborhood display (Figure 5B). The neighborhood can be downloaded using the same 'Download SVG' button as described above.

COLLABORATION EFFORTS

As proposed in the previous IMG paper (4), we extended our collaborative efforts with two other DOE funded projects: the DOE Biology Knowledgebase (KBase) (33) and the National Microbiome Data Collaborative



Mouse over a gene to see details (once page has loaded).
Genes can be colored by fn association using "Color By" choices.
Light yellow colored genes have **no** fn association.
For datasets with intergenic data, there is a separate track for "intergenes" in the overview display with corresponding dashed segments on the main scaffold track.
red (high) to green (low) expression
RNAs are in **black**
Genes in Gene Cart are marked by a blue bar
CRISPR array

□ Use function categories

Color Scaffold By: ○ None ○ COG ○ GC ○ KEGG ○ Pfam ○ TIGRfam ○ Phylo Distribution ● Expression

Genome: Metatranscriptome of biomass microbial c
r2.baby_polysaccharides.d106 (Metagenome Metat
Sample: Metatranscriptome of biomass microbial co
r2.baby_polysaccharides.d106 [Gp0623647]
Sample Quality: HQ

Filter column: Reads Count Plus Strand Fil

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Select	Region ID	Scaffold ID	Scaffold Accession	Start	End	Gene Seq Length (Exp)	Reads Count Plus Strand	Median Plus Strand	Reads Count Minus Strand	Median Minus Strand
□	Ga0537121_00780__1_32	Ga0537121_00780	scaffold_780_c1	1	32	32	28621	24162	0	0
□	Ga0537121_02187__263_735	Ga0537121_02187	scaffold_2185_c1	263	735	473	27594	7484	4	0
□	Ga0537121_02418__1992_2900	Ga0537121_02418	scaffold_2414_c1	1992	2900	909	21898	852	0	0
□	Ga0537121_00509__360_425	Ga0537121_00509	scaffold_509_c1	360	425	66	12045	11753	0	0
□	Ga0537121_00688__1_123	Ga0537121_00688	scaffold_688_c1	1	123	123	6206	5761	0	0
□	Ga0537121_00766__2666_2802	Ga0537121_00766	scaffold_766_c1	2666	2802	137	4232	3930	0	0
□	Ga0537121_00001__35351_35377	Ga0537121_00001	scaffold_1_c1	35351	35377	27	3663	3208	4	4

Figure 5. Metatranscriptome data in IMG now includes both Gene Expression and Intergene Expression. (**A**) The Intergene Expression tab includes expression data on both forward and reverse strands. (**B**) Users can view intergenic expression data in the new gene neighborhood viewer.

(NMDC) (34). In the IMG UI, the two new submenu items **KBase** and **NMDC** under **Collaborations** describe these collaborative efforts. The main goal of the collaboration is to share software and datasets to leverage the resources available across all these systems. Additional capabilities have been added to the IMG UI as the results of the collaborations.

KBase collaboration

IMG used to include InterPro (35) and Gene Ontology (GO) (36) annotations through its isolate genome annotation pipeline. These two functions were removed due to resource limitation caused by the lengthened annotation cycle time. As InterPro and GO annotations provide valuable information for the research, however, users have been requesting them.

IMG collaborated with the KBase team to develop a new UniProt (37) Search function, which allows the identification of sequencing similarity via LAST (38) against a UniRef90 reference database (released in February 2020) (39). From a gene details page (Figure 6A), clicking on the **Search** button next to **UniProt Search** leads to the UniRef90 hits with specified e-value and number of hits. Following the corresponding UniProt links on the UniProt search result (Figure 6B), allows accessing more detailed information from the UniProt website (https://www.uniprot.org/) (Figure 6C). InterPro and Gene Ontology hits (Figure 6D) are also accessible from the same gene detail page.

InterPro and Gene Ontology search functions are part of an ID mapping service co-developed by JGI and KBase. The ID mapping service creates gene mappings in the NCBI NR (non-redundant) database, UniRef100 (39), IMG NR database and KBase NR database. Identical sequences (those having 100% identical sequences) are bidirectionally mapped to one another using UniRef100 as the canonical sequences. UniRef100 ID is then mapped to InterPro (using protein2ipr.dat file) and Gene Ontology (using interpro2go file), respectively. This allows us to derive InterPro and GO ontology of an IMG gene.

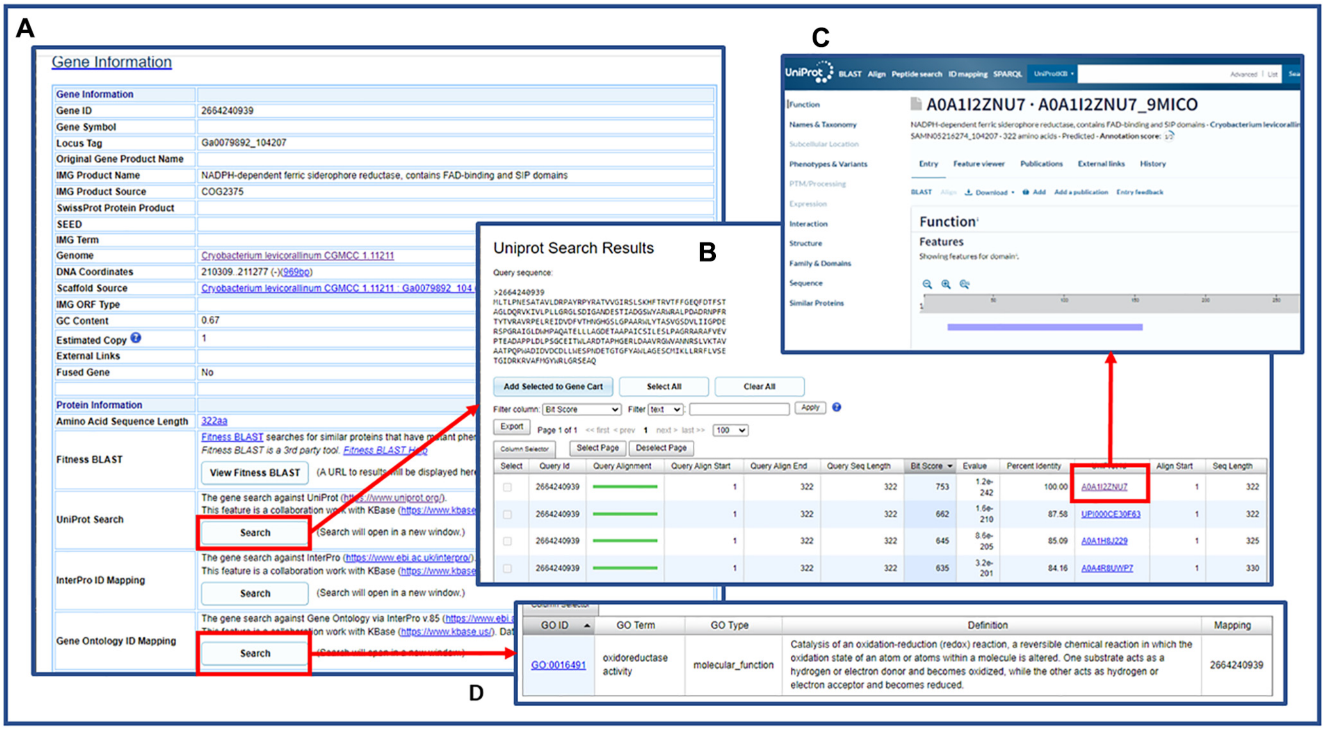
NMDC collaboration

IMG shares its metagenome gene calling and annotation pipeline code with NMDC. Since metagenome datasets in IMG and NMDC are annotated using the same annotation pipeline, it's possible to share datasets with each other. From the IMG UI NMDC Collaboration page, all the metagenome datasets with corresponding data in NMDC are available (Figure 7A).

For all NMDC datasets in IMG, there is a new NMDC ID field in the genome detail page (Figure 7B). Users can also view NMDC read-based taxonomic composition analysis in a Krona display (40) (Figure 7C). There are three different types of analysis: Centrifuge (41), Gottcha2 (42) and Kraken2 (43), and the interactive Krona diagram allows a user to easily navigate between and compare the results of these different analyses for a given metagenome.

CONCLUDING REMARKS AND FUTURE PLANS

IMG's main mission remains the support of JGI users, as well as the broader user community focused on microbiome data science. To achieve this goal, we continuously grow and



Gene Information

Gene Information	
Gene ID	2664240939
Gene Symbol	
Locus Tag	Ga0079892_104207
Original Gene Product Name	
IMG Product Name	NADPH-dependent ferric siderophore reductase, contains FAD-binding and SIP domains
IMG Product Source	COG2375
SwissProt Protein Product	
SEED	
IMG Term	
Genome	Cryobacterium levicorallinum CGMCC 1.11211
DNA Coordinates	210309..211277 (-)(969bp)
Scaffold Source	Cryobacterium levicorallinum CGMCC 1.11211 : Ga0079892_104
IMG ORF Type	
GC Content	0.67
Estimated Copy	1
External Links	
Fused Gene	No
Protein Information	
Amino Acid Sequence Length	322aa
Fitness BLAST	Fitness BLAST searches for similar proteins that have mutant phe Fitness BLAST is a 3rd party tool. Fitness BLAST Help View Fitness BLAST (A URL to results will be displayed here
UniProt Search	The gene search against UniProt (https://www.uniprot.org/). This feature is a collaboration work with KBase (https://www.kbase Search (Search will open in a new window.)
InterPro ID Mapping	The gene search against InterPro (https://www.ebi.ac.uk/interpro/). This feature is a collaboration work with KBase (https://www.kbase Search (Search will open in a new window.)
Gene Ontology ID Mapping	The gene search against Gene Ontology via InterPro v.85 (https://www.ebi.a This feature is a collaboration work with KBase (https://www.kbase.us/). Dat Search (Search will open in a new window.)

Uniprot Search Results

Query sequence:

```
>2664240939
MLTLPNESATAVLDRPAYRPYRATVVGIRSLSKHFTRVTFFGEQFDTFST
AGLDQRVKIVLPLLGRGLSDIGANDESTIADGSWYARWRALPDADRNPFR
TYTVRAVRPELREIDVDFVTHNGHGSLGPAARWLYTASVGSDVLIIGPDE
RSPGRAIGLDWHPAQATELLLAGDETAAPAICSILESLPAGRRARAFVEV
PTEADAPPLDLPSGCEITWLARDTAPHGERLDAAVRGWVANNRSLVKTAV
AATPQPWADIDVDCDLLWESPNDETGTGFYAWLAGESCMIKLLRRFLVSE
TGIDRKRVAFMGYWRLGRSEAQ
```

Add Selected to Gene Cart | Select All | Clear All

Filter column: Bit Score Filter text Apply

Export Page 1 of 1 << first < prev 1 next > last >> 100

Column Selector | Select Page | Deselect Page

Select	Query Id	Query Alignment	Query Align Start	Query Align End	Query Seq Length	Bit Score	Evalue	Percent Identity		Align Start	Seq Length
□	2664240939		1	322	322	753	1.2e-242	100.00	A0A1I2ZNU7	1	322
□	2664240939		1	322	322	662	1.6e-210	87.58	UPI000CE30F63	1	322
□	2664240939		1	322	322	645	8.6e-205	85.09	A0A1H8J229	1	325
□	2664240939		1	322	322	635	3.2e-201	84.16	A0A4R8UWP7	1	330

UniProt BLAST Align Peptide search ID mapping SPARQL UniProtKB Advanced | List Sea

A0A1I2ZNU7 · A0A1I2ZNU7_9MICO

NADPH-dependent ferric siderophore reductase, contains FAD-binding and SIP domains · Cryobacterium levicorallin
SAMN05216274_104207 · 322 amino acids · Predicted · Annotation score: 1/5

Function | Names & Taxonomy | Subcellular Location | Phenotypes & Variants | PTM/Processing | Expression | Interaction | Structure | Family & Domains | Sequence | Similar Proteins

Entry | Feature viewer | Publications | External links | History

BLAST Align Download Add Add a publication Entry feedback

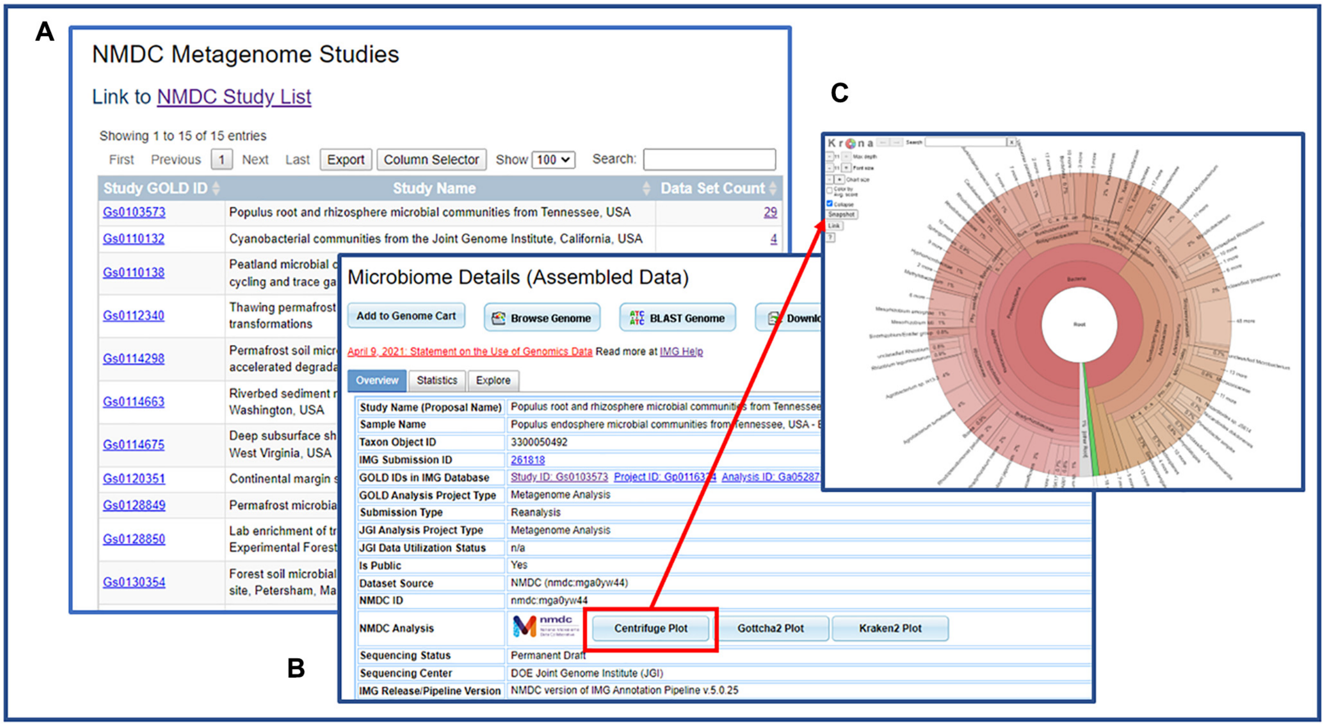
Function

Features

Showing features for domain.

GO ID	GO Term	GO Type	Definition	Mapping
GO:0016491	oxidoreductase activity	molecular_function	Catalysis of an oxidation-reduction (redox) reaction, a reversible chemical reaction in which the oxidation state of an atom or atoms within a molecule is altered. One substrate acts as a hydrogen or electron donor and becomes oxidized, while the other acts as hydrogen or electron acceptor and becomes reduced.	2664240939

Figure 6. IMG gene detail page shows additional functions co-developed by JGI and KBase. (**A**) UniProt Search, InterPro ID Mapping and Gene Ontology ID Mapping are 3 new functions. (**B**) Uniprot Search results show top UniRef90 hits of this gene. (**C**) Users can follow the UniProt links to find more information from the UniProt website. (**D**) Gene Ontology (GO) mapping result shows the GO ID(s) associated with this gene.



NMDC Metagenome Studies

Link to NMDC Study List

Showing 1 to 15 of 15 entries

First Previous 1 Next Last Export Column Selector Show 100 Search:

Study GOLD ID	Study Name	Data Set Count
Gs0103573	Populus root and rhizosphere microbial communities from Tennessee, USA	29
Gs0110132	Cyanobacterial communities from the Joint Genome Institute, California, USA	4
Gs0110138	Peatland microbial c cycling and trace ga	
Gs0112340	Thawing permafrost transformations	
Gs0114298	Permafrost soil micr accelerated degrada	
Gs0114663	Riverbed sediment r Washington, USA	
Gs0114675	Deep subsurface sh West Virginia, USA	
Gs0120351	Continental margin s	
Gs0128849	Permafrost microbia	
Gs0128850	Lab enrichment of tr Experimental Forest	
Gs0130354	Forest soil microbial site, Petersham, Ma	

Microbiome Details (Assembled Data)

Add to Genome Cart | Browse Genome | BLAST Genome | Downlo

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Overview | Statistics | Explore

Study Name (Proposal Name)	Populus root and rhizosphere microbial communities from Tennessee
Sample Name	Populus endosphere microbial communities from Tennessee, USA - E
Taxon Object ID	3300050492
IMG Submission ID	261818
GOLD IDs in IMG Database	Study ID: Gs0103573 Project ID: Gp0116374 Analysis ID: Ga05287
GOLD Analysis Project Type	Metagenome Analysis
Submission Type	Reanalysis
JGI Analysis Project Type	Metagenome Analysis
JGI Data Utilization Status	n/a
Is Public	Yes
Dataset Source	NMDC (nmdc:mga0yw44)
NMDC ID	nmdc:mga0yw44
NMDC Analysis	nmdc Centrifuge Plot Gottcha2 Plot Kraken2 Plot
Sequencing Status	Permanent Draft
Sequencing Center	DOE Joint Genome Institute (JGI)
IMG Release/Pipeline Version	NMDC version of IMG Annotation Pipeline v.5.0.25

Figure 7. IMG UI shows NMDC studies and datasets. (**A**) The NMDC Metagenome Studies list shows all GOLD studies that have NMDC data. (**B**) The metagenome detail page shows NMDC ID and provides links to NMDC read-based analysis plots. (**C**) NMDC read-based analysis Centrifuge Plot shows the inferred taxonomic distribution within this metagenome.

enrich the IMG database contents, and improve the user experiences through additional new features in the IMG UI. In order to support data growth, we re-implement and improve the IMG annotation pipeline using WDL, allowing us to use computational resources outside of NERSC (https://www.nersc.gov/). We also collaborate with NMDC so IMG can import public metagenomic datasets annotated by NMDC and provide them to the users for comparative analysis. A next step of NMDC collaboration is to share metatranscriptomic datasets. Since IMG can currently only process metatranscriptomic data generated by the JGI, this will provide the infrastructure to expand the external metatranscriptomic data collection in IMG.

Original IMG analysis tools were limited to genomes and metagenomes. With the recent introduction of metagenome bins, there is an increasing desire for functionalities allowing the analysis of metagenome bins with genomes. We are currently systematically expanding all previously existing analysis tools to be inclusive of metagenome bins. The Pairwise ANI analysis described in this paper is one such example. Since IMG has many analysis tools, this will be an ongoing process. We have increased our collaboration effort with KBase, adding more analysis functions to the IMG UI, and will continue this effort in the foreseeable future.

The complexity of the IMG system continues to increase not only in terms of data size and types, but also in terms of the available analysis tools and overall functionalities. It is thus critical for us to both keep the system user-friendly and train scientists in the most efficient use of IMG. Many users have participated in IMG workshops and webinars in order to learn to use the system for their research needs. We will continue to provide hands-on IMG training workshops at the JGI, as well as in other locations, upon user request.

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