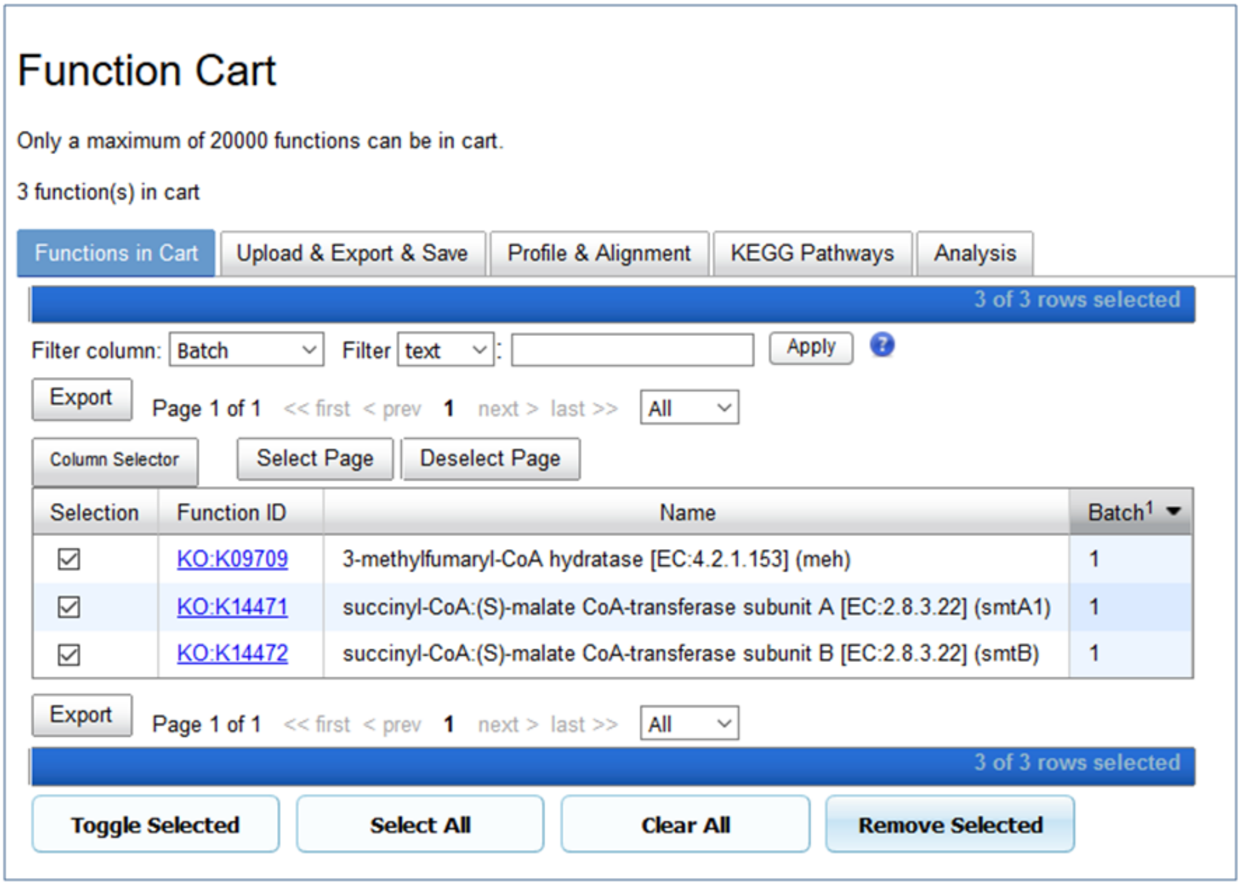
Function Cart

IMG users can save functions of interest to **Function Cart** for further studies. There are many ways to save functions into Function Cart; for example,

- Use **Function Search** to find functions of interests to add to **Function Cart**;
- Find functions of interest during browsing;
- Find functions of interest in some gene or functional analysis results;
- Load previously functions of interest saved in a **Workspace Function Set**.

After a user adds some functions into Function Cart, there will be many analysis options as shown in Figure 1:



Function Cart

Only a maximum of 20000 functions can be in cart.

3 function(s) in cart

3 of 3 rows selected

Filter column: Batch Filter text :

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Selection	Function ID	Name	Batch¹
☑	KO:K09709	3-methylfumaryl-CoA hydratase [EC:4.2.1.153] (meh)	1
☑	KO:K14471	succinyl-CoA:(S)-malate CoA-transferase subunit A [EC:2.8.3.22] (smtA1)	1
☑	KO:K14472	succinyl-CoA:(S)-malate CoA-transferase subunit B [EC:2.8.3.22] (smtB)	1

Page 1 of 1

3 of 3 rows selected

Figure 1. Function Cart.

Functions in Cart

The first tab **Functions in Cart** shows all functions that have been added to the Function Cart. Users can select up to 20,000 functions to add to the cart. The function list shows the following fields:

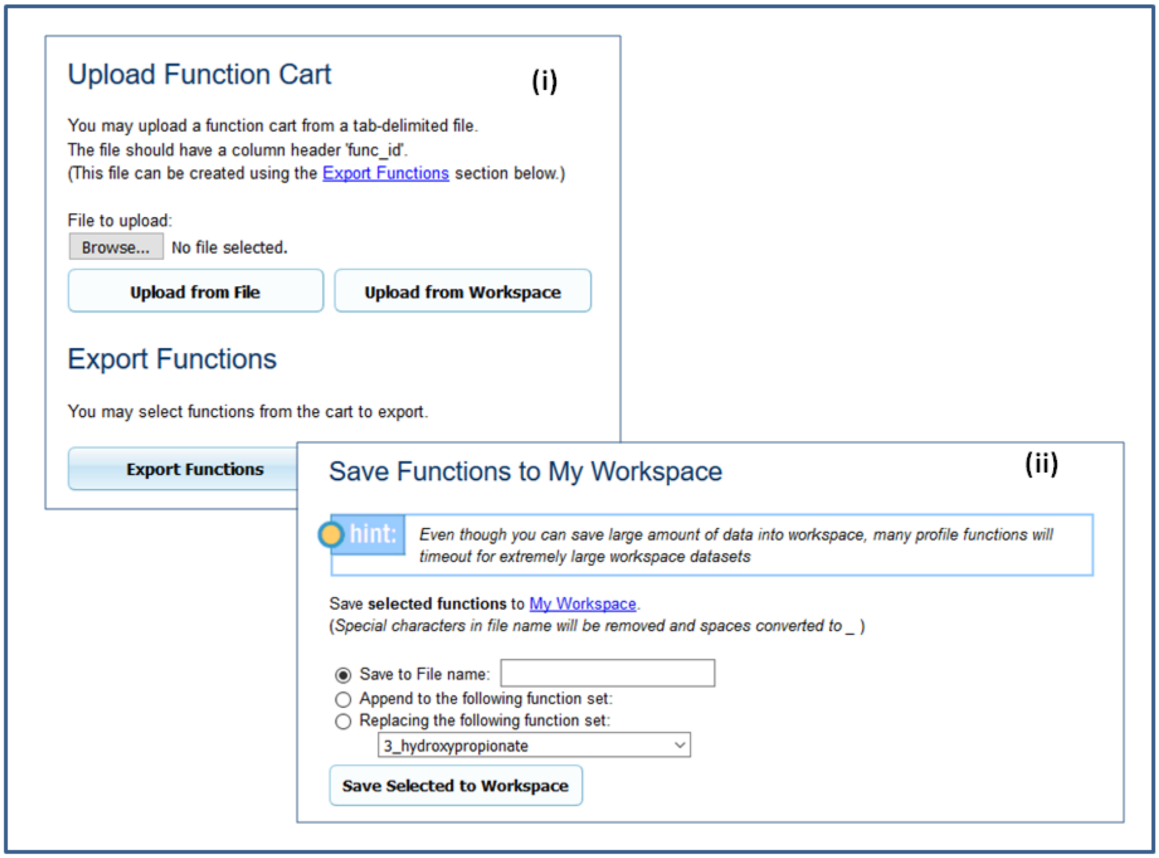
- Function ID: IMG Function ID;
- Name: function name;
- Batch: An integer to distinguish functions added in different batches.

There are 4 additional buttons under the table display:

- **Toggle Selected**: Un-select any preselected functions and select all functions that have not been selected previously.
- **Select All**: Select all functions in the cart.
- **Clear All**: Un-select all functions in the cart.
- **Remove Selected**: Remove all selected functions from the cart.

Upload & Export & Save

This tab includes 3 functions: **Upload Function Cart**, **Export Functions** and **Save Functions to My Workspace**.



Upload Function Cart

You may upload a function cart from a tab-delimited file.
The file should have a column header 'func_id'.
(This file can be created using the Export Functions section below.)

File to upload:
No file selected.

Export Functions

You may select functions from the cart to export.

Save Functions to My Workspace

Even though you can save large amount of data into workspace, many profile functions will timeout for extremely large workspace datasets

Save **selected functions** to My Workspace.
(Special characters in file name will be removed and spaces converted to _)

● Save to File name:
○ Append to the following function set:
○ Replacing the following function set:
3_hydroxypropionate

Figure 2. Function Cart: Upload & Export & Save.

Upload Function Cart

Users can either upload functions from a tab-delimited file or from Workspace Function Set (Figure 2(i)). If a user wishes to select the **Upload from File** option, then the selected file must have a column header 'func_id'. If the user is not sure what the file format should be, he/she can use the **Export Functions** function described below to obtain an example file.

Clicking the **Upload from Workspace** button will lead to the Workspace Function Set page for function set selection.

Export Functions

Users can export basic function information in tab-delimited format (see Figure 2(i)).

Save Functions to My Workspace

Users can also save selected functions in the cart to a Workspace Function Set. The result can be saved to a new function set, appended to an existing function set, or replacing an existing function set (see Figure 2(ii)).

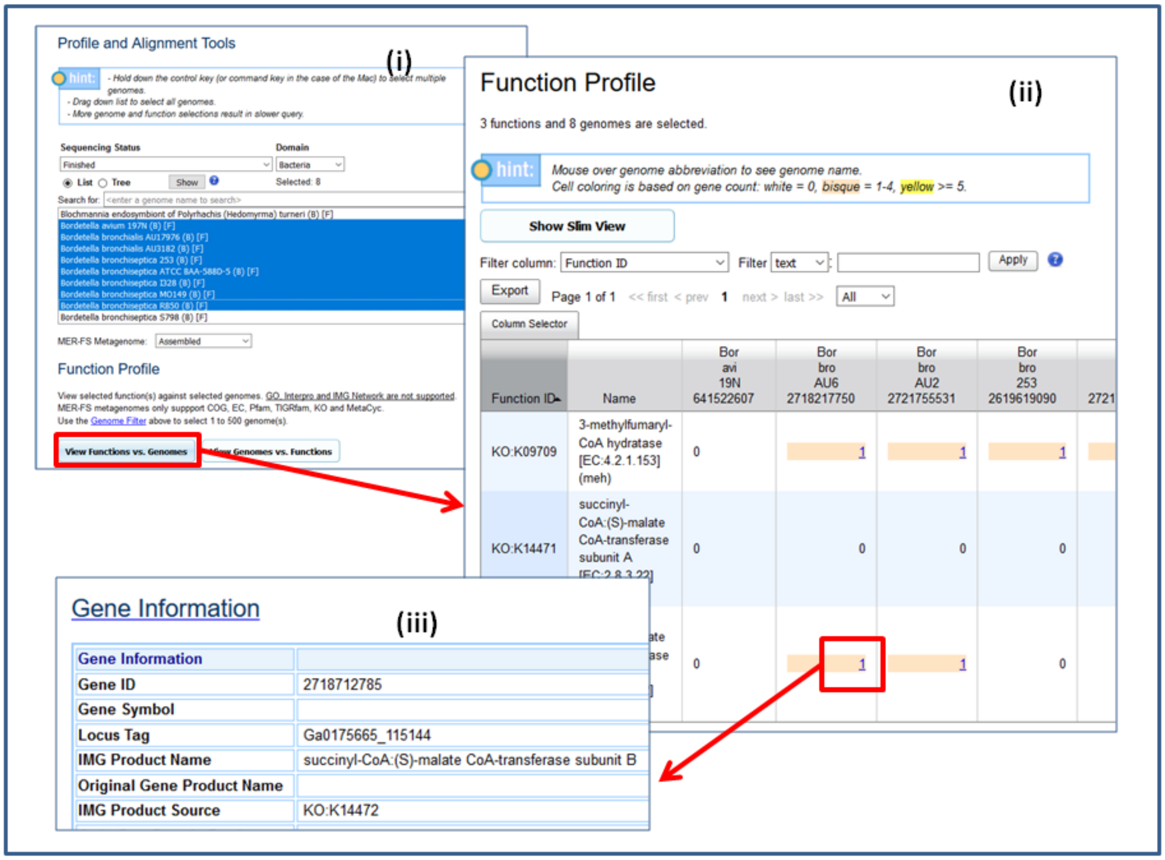
Profile & Alignment

The **Profile & Alignment** tab provides 5 functions: **Function Profile**, **IMG Pathway Profile**, **Occurrence Profile**, **Function Alignment**, and **Gene Cart**.

Function Profile

Function Profile function allows users to view selected functions against selected genomes through gene-function association. GO, Interpro and IMG Network are not supported. (To view IMG Network profile, use the **IMG Pathway Profile** function described below.) Metagenomes only suppport COG, EC, Pfam, TIGRfam, KO and MetaCyc.

Users will have to select 1 to 500 genomes first, and then click either **View Functions vs. Genomes** or **View Genomes vs. Functions** button (see Figure 3(i)). The result table (in Figure 3(ii)) shows the number of genes in each genome annotated with a specific function. Users can click on any non-zero count to view gene list or gene detail (Figure 3(iii)).



Profile and Alignment Tools

- Hold down the control key (or command key in the case of the Mac) to select multiple genomes.
- Drag down list to select all genomes.
- More genome and function selections result in slower query.

Sequencing Status: Finished
Domain: Bacteria
● List ○ Tree
Selected: 8
Search for: <enter a genome name to search>

Blochmannia endosymbiont of Polyrhachis (Hedomyrma) turneri (B) [F]
Bordetella avium 197N (B) [F]
Bordetella bronchialis AU17976 (B) [F]
Bordetella bronchialis AU3182 (B) [F]
Bordetella bronchiseptica 253 (B) [F]
Bordetella bronchiseptica ATCC BAA-588D-5 (B) [F]
Bordetella bronchiseptica I328 (B) [F]
Bordetella bronchiseptica MO149 (B) [F]
Bordetella bronchiseptica RB50 (B) [F]
Bordetella bronchiseptica S798 (B) [F]

MER-FS Metagenome: Assembled

Function Profile

View selected function(s) against selected genomes. GO, Interpro and IMG Network are not supported. MER-FS metagenomes only suppport COG, EC, Pfam, TIGRfam, KO and MetaCyc. Use the Genome Filter above to select 1 to 500 genome(s).

Function Profile

3 functions and 8 genomes are selected.

Mouse over genome abbreviation to see genome name.
Cell coloring is based on gene count: white = 0, bisque = 1-4, yellow >= 5.

Filter column: Function ID Filter text :

Page 1 of 1 << first < prev 1 next > last >> All

Function ID	Name	Bor avi 19N 641522607	Bor bro AU6 2718217750	Bor bro AU2 2721755531	Bor bro 253 2619619090	2721
KO:K09709	3-methylfumaryl-CoA hydratase [EC:4.2.1.153] (meh)	0	1	1	1	
KO:K14471	succinyl-CoA:(S)-malate CoA-transferase subunit A [EC:2.8.3.22]	0	0	0	0	
	ate ase]	0	1	1	0	

Gene Information

Gene Information	
Gene ID	2718712785
Gene Symbol	
Locus Tag	Ga0175665_115144
IMG Product Name	succinyl-CoA:(S)-malate CoA-transferase subunit B
Original Gene Product Name	
IMG Product Source	KO:K14472

Figure 3. Function Cart: Function Profile.

IMG Pathway Profile

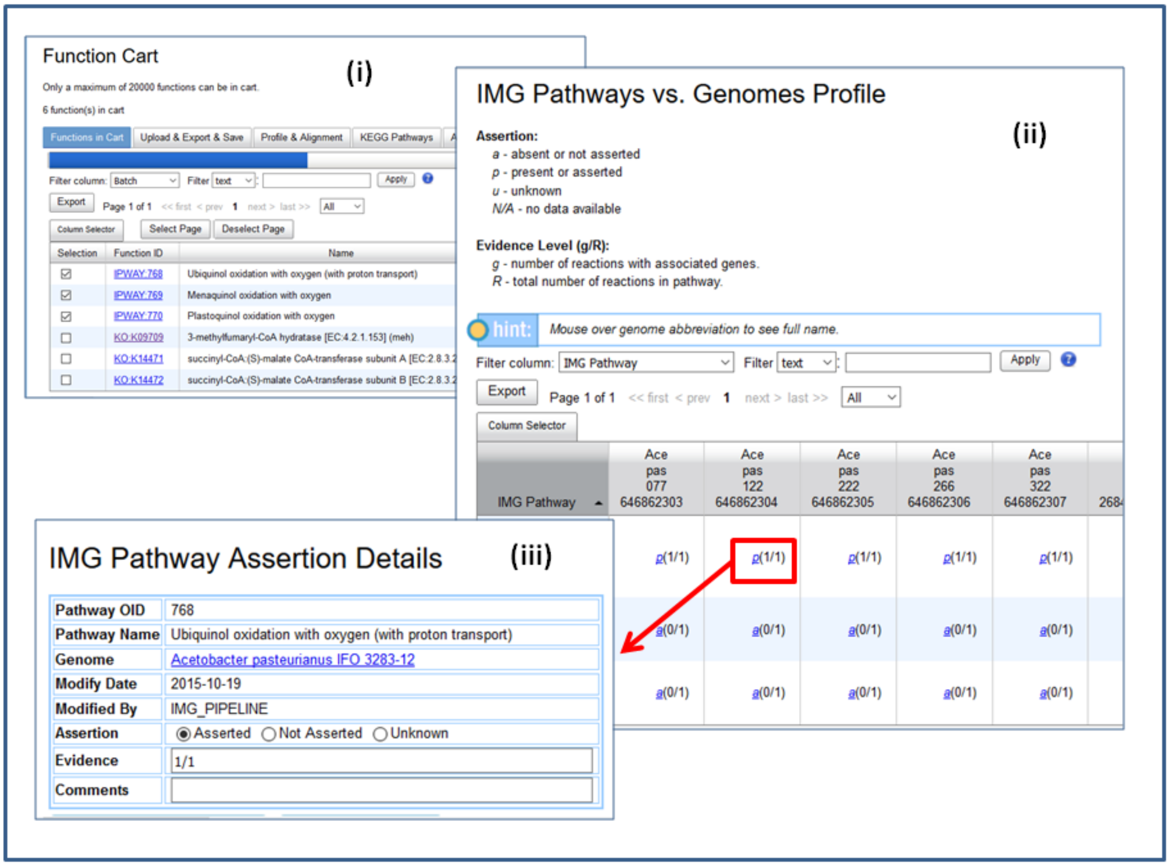
This function shows a profile of selected IMG pathways vs. selected genomes. The result can be viewed as pathways vs. genomes, or genomes vs. pathways. In order to use this function, there must be some IMG pathways selected in the Function Cart (Figure 4(i)). Click on the **View IMG Pathway vs. Genomes** button in the **IMG Pathway Profile** section to view the pathway profile as shown in Figure 4(ii)).

There can be 4 kinds of assertion results:

- *a* - absent or not asserted
- *p* - present or asserted
- *u* - unknown
- *N/A* - no data available

Users can click on the result to see pathway assertion detail (Figure 4(iii)).

For more information on IMG pathway assertion, please refer to: PLoS ONE 8(2): e54859. doi:10.1371/journal.pone.0054859



Function Cart

Only a maximum of 20000 functions can be in cart.

6 function(s) in cart

Selection	Function ID	Name
☑	IPWAY:768	Ubiquinol oxidation with oxygen (with proton transport)
☑	IPWAY:769	Menaquinol oxidation with oxygen
☑	IPWAY:770	Plastoquinol oxidation with oxygen
☐	KO:K09709	3-methylfumaryl-CoA hydratase [EC:4.2.1.153] (meh)
☐	KO:K14471	succinyl-CoA:(S)-malate CoA-transferase subunit A [EC:2.8.3.2
☐	KO:K14472	succinyl-CoA:(S)-malate CoA-transferase subunit B [EC:2.8.3.2

IMG Pathways vs. Genomes Profile

Assertion:
a - absent or not asserted
p - present or asserted
u - unknown
N/A - no data available

Evidence Level (g/R):
g - number of reactions with associated genes.
R - total number of reactions in pathway.

hint: *Mouse over genome abbreviation to see full name.*

IMG Pathway	Ace pas 077 646862303	Ace pas 122 646862304	Ace pas 222 646862305	Ace pas 266 646862306	Ace pas 322 646862307	268
	p(1/1)	p(1/1)	p(1/1)	p(1/1)	p(1/1)	
	a(0/1)	a(0/1)	a(0/1)	a(0/1)	a(0/1)	
	a(0/1)	a(0/1)	a(0/1)	a(0/1)	a(0/1)	

IMG Pathway Assertion Details

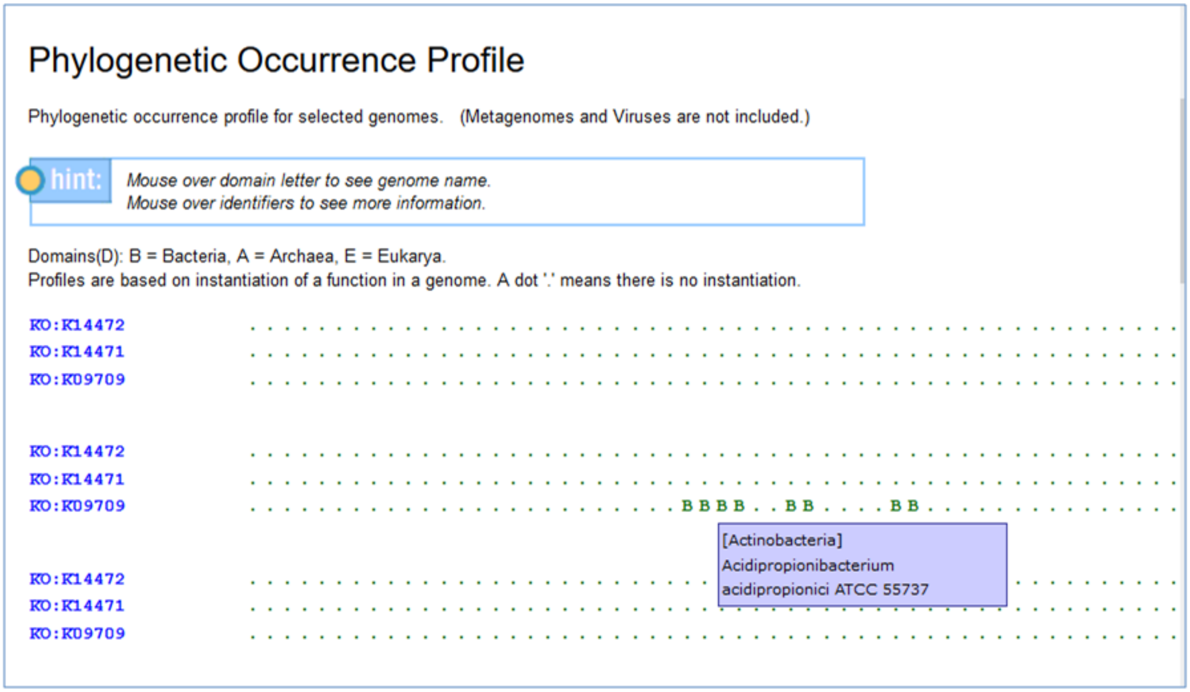
Pathway OID	768
Pathway Name	Ubiquinol oxidation with oxygen (with proton transport)
Genome	Acetobacter pasteurianus IFO 3283-12
Modify Date	2015-10-19
Modified By	IMG_PIPELINE
Assertion	● Asserted ○ Not Asserted ○ Unknown
Evidence	1/1
Comments	

Figure 4. Function Cart: IMG Pathway Profile.

Occurrence Profile

This function shows phylogenetic occurrence profile of selected functions and genomes (through gene-function association). GO, Interpro and IMG Network are not supported. Metagenomes are not supported either.

To view occurrence profile, first select some functions in the Function Cart, and select genomes of interest, then click the **View Phylogenetic Occurrence Profiles** button in the **Occurrence Profile** section. The result shows which genomes have genes annotated with the selected functions. Mouse over to view the genome name (see Figure 5).



Phylogenetic Occurrence Profile

Phylogenetic occurrence profile for selected genomes. (Metagenomes and Viruses are not included.)

Mouse over domain letter to see genome name.
Mouse over identifiers to see more information.

Domains(D): B = Bacteria, A = Archaea, E = Eukarya.
Profiles are based on instantiation of a function in a genome. A dot '.' means there is no instantiation.

KO:K14472
KO:K14471
KO:K09709

KO:K14472
KO:K14471
KO:K09709

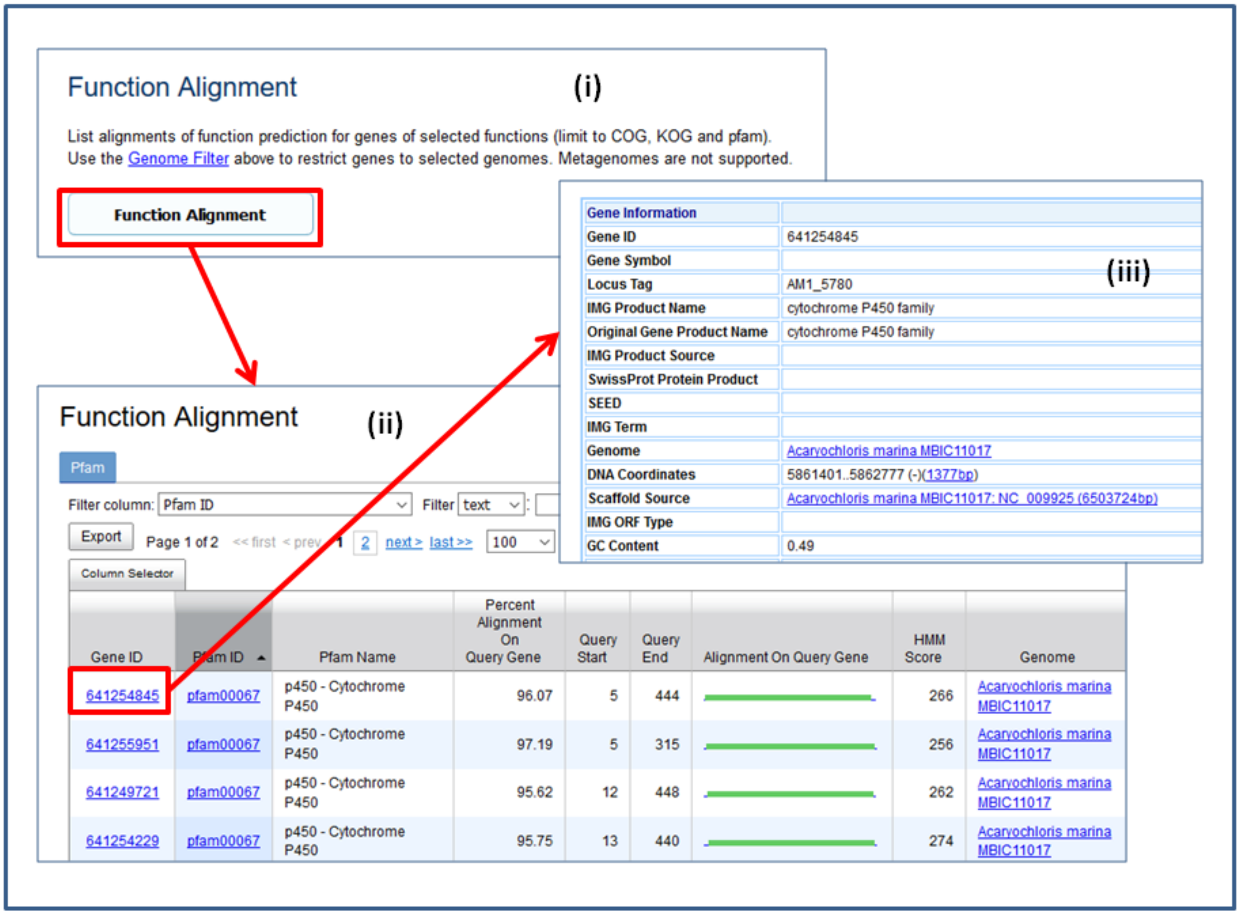
[Actinobacteria]
Acidipropionibacterium
acidipropionici ATCC 55737

KO:K14472
KO:K14471
KO:K09709

Figure 5. Function Cart: Occurrence Profile.

Function Alignment

This function lists alignments of function prediction for selected genes (limited to COG, KOG and pfam). Metagenome Genes are not supported. In order to perform this analysis, first select some COG, KOG or pfam functions in the Function Cart, make proper genome selection, and then click the **Function Alignment** button (see Figure 6(i)). The result shows genes in the selected genomes annotated with selected functions. Each gene is shown with an alignment with coordinates, percent alignment and score (see Figure 6(ii)). Users can click on a gene ID to view gene detail information (Figure 6(iii)).



Function Alignment

List alignments of function prediction for genes of selected functions (limit to COG, KOG and pfam).
Use the Genome Filter above to restrict genes to selected genomes. Metagenomes are not supported.

Gene Information	
Gene ID	641254845
Gene Symbol	
Locus Tag	AM1_5780
IMG Product Name	cytochrome P450 family
Original Gene Product Name	cytochrome P450 family
IMG Product Source	
SwissProt Protein Product	
SEED	
IMG Term	
Genome	Acaryochloris marina MBIC11017
DNA Coordinates	5861401..5862777 (-)(1377bp)
Scaffold Source	Acaryochloris marina MBIC11017: NC_009925 (6503724bp)
IMG ORF Type	
GC Content	0.49

Function Alignment

Filter column: Pfam ID Filter text

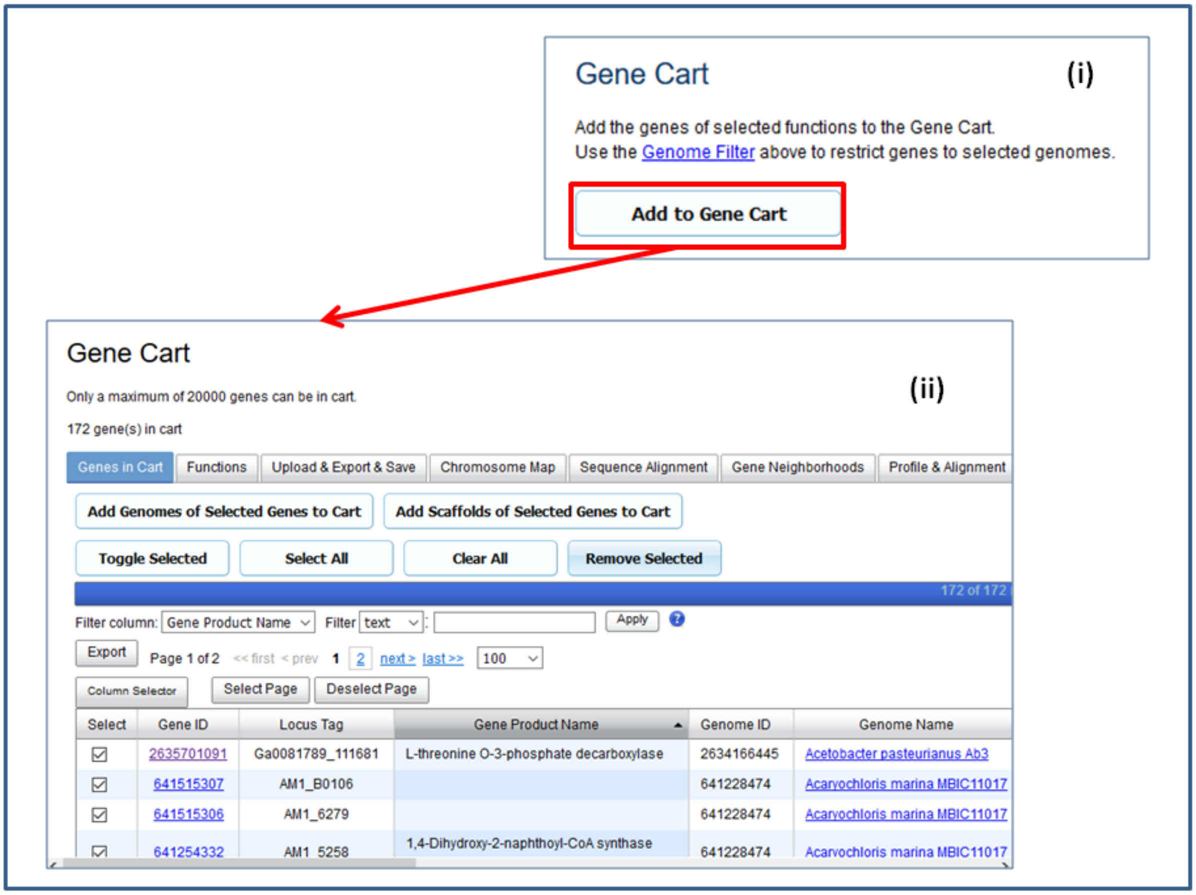
Page 1 of 2 << first < prev 1 2 next > last >> 100

Gene ID	Pfam ID	Pfam Name	Percent Alignment On Query Gene	Query Start	Query End	Alignment On Query Gene	HMM Score	Genome
641254845	pfam00067	p450 - Cytochrome P450	96.07	5	444		266	Acaryochloris marina MBIC11017
641255951	pfam00067	p450 - Cytochrome P450	97.19	5	315		256	Acaryochloris marina MBIC11017
641249721	pfam00067	p450 - Cytochrome P450	95.62	12	448		262	Acaryochloris marina MBIC11017
641254229	pfam00067	p450 - Cytochrome P450	95.75	13	440		274	Acaryochloris marina MBIC11017

Figure 6. Function Cart: Function Alignment.

Gene Cart

This function adds the genes of selected genomes with selected functions to the Gene Cart. In order to perform this analysis, first select some functions in the Function Cart, make proper genome selection, and then click the **Add to Gene Cart** button (see Figure 7(i)). Genes of selected genomes with selected functions will be added to Gene Cart as shown in Figure 7(ii). Please refer to the Gene Cart User Guide for functions in the Gene Cart.



Gene Cart (i)

Add the genes of selected functions to the Gene Cart.
Use the Genome Filter above to restrict genes to selected genomes.

Gene Cart (ii)

Only a maximum of 20000 genes can be in cart.

172 gene(s) in cart

Filter column: Gene Product Name Filter text

Page 1 of 2 << first < prev 1 2 next > last >> 100

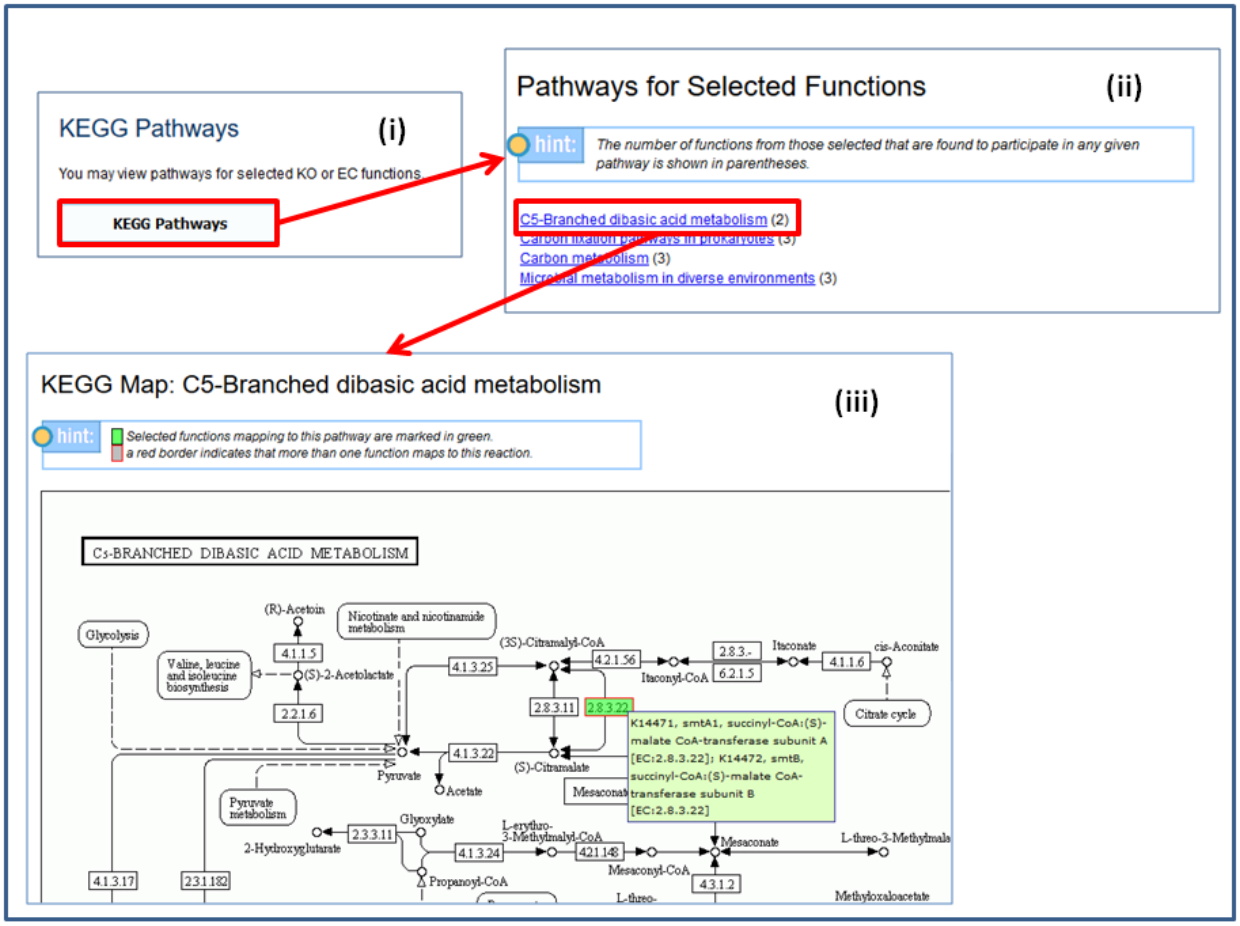
Select	Gene ID	Locus Tag	Gene Product Name	Genome ID	Genome Name
☑	2635701091	Ga0081789_111681	L-threonine O-3-phosphate decarboxylase	2634166445	Acetobacter pasteurianus Ab3
☑	641515307	AM1_B0106		641228474	Acaryochloris marina MBIC11017
☑	641515306	AM1_6279		641228474	Acaryochloris marina MBIC11017
☑	641254332	AM1_5258	1,4-Dihydroxy-2-naphthoyl-CoA synthase	641228474	Acaryochloris marina MBIC11017

Figure 7. Function Cart: Add to Gene Cart.

KEGG Pathways

This function allows users to view all KEGG pathways associated with selected KO terms and/or enzymes in the Function Cart. First select some KO terms and/or enzymes in the cart, then click the **KEGG Pathways** button in the **KEGG Pathways** tab (Figure 8(i)). All KEGG pathways associated with selected functions will be displayed (Figure 8(ii)). Users can click on a pathway name to view the KEGG pathway image with selected function(s) highlighted. Mouse over to see the detailed function description (Figure 8(iii)).

Please refer to the Find Functions User Guide for more information on KEGG pathways.



You may view pathways for selected KO or EC functions

The number of functions from those selected that are found to participate in any given pathway is shown in parentheses.

C5-Branched dibasic acid metabolism (2)
Carbon fixation pathways in prokaryotes (3)
Carbon metabolism (3)
Microbial metabolism in diverse environments (3)

🟩 Selected functions mapping to this pathway are marked in green.
🟥 a red border indicates that more than one function maps to this reaction.

K14471, smtA1, succinyl-CoA:(S)-malate CoA-transferase subunit A [EC:2.8.3.22]; K14472, smtB, succinyl-CoA:(S)-malate CoA-transferase subunit B [EC:2.8.3.22]

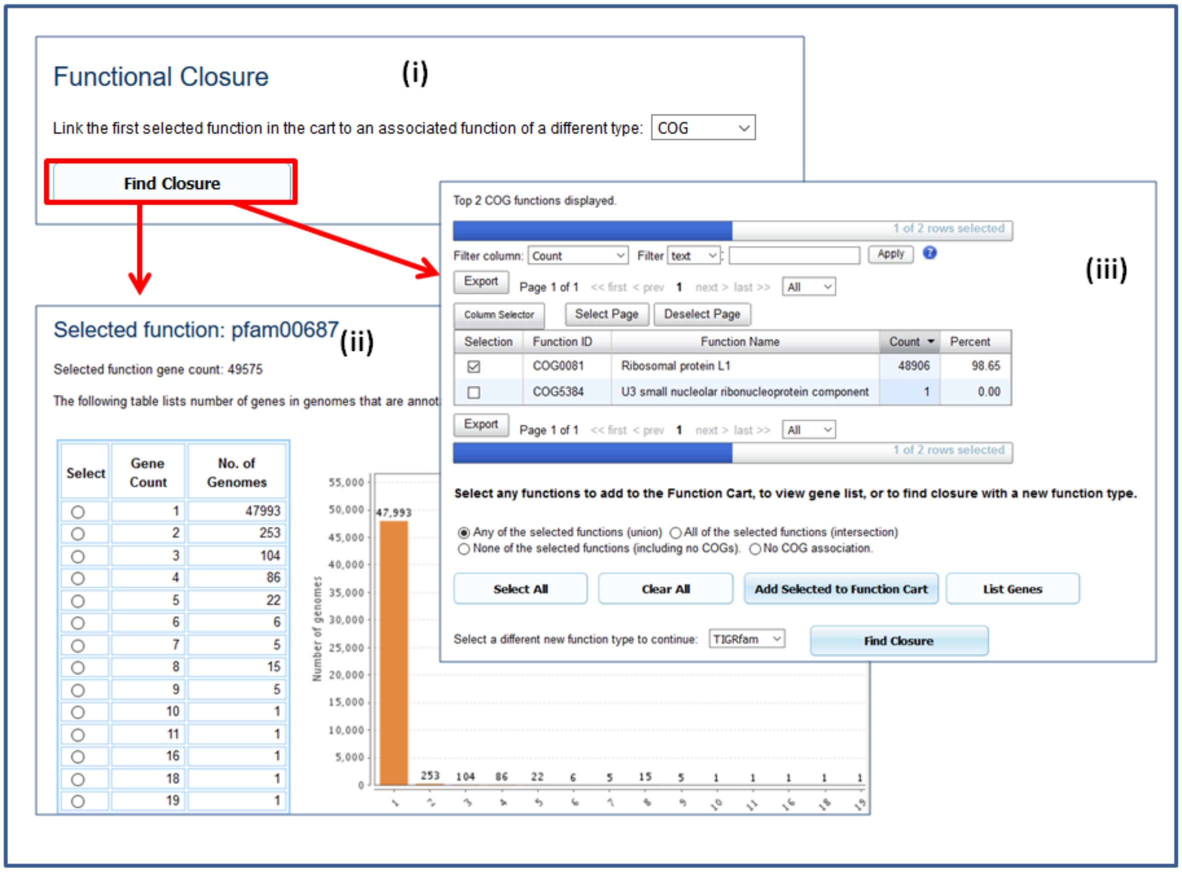
Figure 8. Function Cart: KEGG Pathways.

Analysis

The **Analysis** tab provides a function for users to check Functional Closure of a selected function in the Function Cart.

For example, a user selects *pfam00687: Ribosomal protein L1p/L10e family* and selects *COG* to perform functional closure analysis (Figure 9(i)). The analysis result shows that most genomes only have a gene associated with this *pfam* function (Figure 9(ii)), and among these genes most of them are also annotated with COG function *COG0081: Ribosomal protein L1* (Figure 9(iii)).

The user can click on **List Genes** button to view the gene list. It is also possible to continue the analysis by adding another function category, say *TIGRfam*. To do so, select *COG0081* in the checkbox, and select *TIGRfam* in the dropdown list, then click the **Find Closure** button near the bottom of the page in Figure 9(iii).



Functional Closure

Link the first selected function in the cart to an associated function of a different type: COG

Selected function: pfam00687

Selected function gene count: 49575

The following table lists number of genes in genomes that are annot

Select	Gene Count	No. of Genomes
○	1	47993
○	2	253
○	3	104
○	4	86
○	5	22
○	6	6
○	7	5
○	8	15
○	9	5
○	10	1
○	11	1
○	16	1
○	18	1
○	19	1

Top 2 COG functions displayed.

1 of 2 rows selected

Filter column: Count Filter text:

Page 1 of 1 << first < prev 1 next > last >> All

Selection	Function ID	Function Name	Count	Percent
☑	COG0081	Ribosomal protein L1	48906	98.65
☐	COG5384	U3 small nucleolar ribonucleoprotein component	1	0.00

Page 1 of 1 << first < prev 1 next > last >> All

1 of 2 rows selected

Select any functions to add to the Function Cart, to view gene list, or to find closure with a new function type.

● Any of the selected functions (union) ○ All of the selected functions (intersection)
○ None of the selected functions (including no COGs). ○ No COG association.

Select a different new function type to continue: TIGRfam

Figure 9. Function Cart: Functional Closure.

Please note that the analysis is based on public, high-quality, isolate genomes only for better result.

(**Known problem**: The analysis can time out when the database is very busy.)

For more information on Functional Closure, please refer to: PLoS ONE 8(2): e54859. doi:10.1371/journal.pone.0054859