

Functional Annotation & Beyond with Integrated Microbial Genomes (IMG)

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Abstract

Explore and compare two strains of *Dehalococcoides mccartyi* to discover putative gene(s) responsible for complete dechlorination of PCE to nontoxic end product, ethylene.

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Guidelines

Background:

Organohalides (e.g., tetrachloroethylene, polychlorinated biphenyls, dioxins) constitute a large proportion of environmental pollutants, and reductive dehalogenases encoded by obligate organohalide-respiring bacteria (OHRB), catalyze their dehalogenation to non-toxic metabolites. Corrinoid cobalamin (B12) is an essential cofactor of reductive dehalogenases. De novo biosynthesis of corrinoid is one of the most complicated metabolic pathways in nature, and OHRB have developed different strategies to cope with their need for this cofactor.

Case Study:

Explore and compare two strains of *Dehalococcoides mccartyi* to discover putative gene(s) responsible for complete dechlorination of PCE to nontoxic end product, ethylene.

| Strain 195¹ | Strain CBDB1 ² |
|------------------------|---------------------------|
| 1.467 Mbp. | 1.395 Mbp |
| Chloroethylenes, etc | Chlorobenzenes, etc |
| PCE->TCE->DCE->VC->Eth | PCE->TCE->DCE |
| 17(+2) RDs | 32 RDs |

REFERENCES

- 1. Genome sequence of the PCE-dechlorinating bacterium Dehalococcoides ethenogenes. Seshadri R, et al. Science, 2005 Jan 7. PMID 15637277
- 2. Genome sequence of the chlorinated compound-respiring bacterium Dehalococcoides species strain CBDB1. Kube M et. Al. Nat Biotechnol. 2005 Oct;23(10):1269-73.
- 3. Microbial species delineation using whole genome sequences, Varghese, N. et. al. Nucl. Acids Res. (2015)
- 4. Trichloroethene reductive dehalogenase from Dehalococcoides ethenogenes: sequence of tceA and substrate range characterization. Magnuson JK, et al. Appl Environ Microbiol, 2000 Dec. PMID 11097881
- 5. The Genomes OnLine Database (GOLD) v.5: a metadata management system based on a four level (meta)genome project classification. Reddy TBK, et. al., Nucl. Acids Res. (2014)
- 6. Discovery of Novel Plant Interaction Determinants from the Genomes of 163 Root Nodule Bacteria. Seshadri, R. et.al. Nat. Sci. Rep. 2015. 5:16825.

Before start

If you do not have an existing JGI SSO account, make your request here: https://contacts.igi.doe.gov/registration/new

Protocol

Question 1

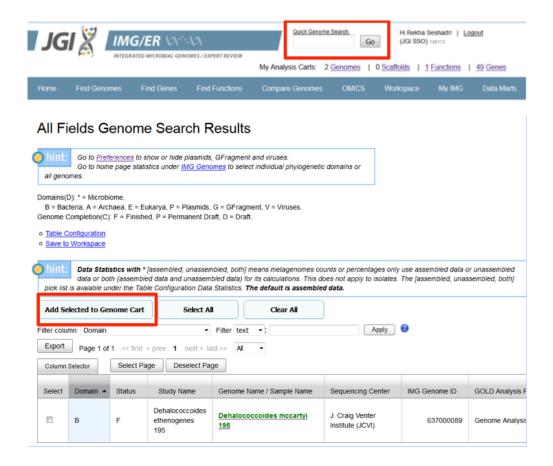
Step 1.

Question 1: Given that these are designated as strains of the same species based on 16S rRNA identity, what is the extent of conservation of gene order or "synteny"?

| Strain 195 ¹ | Strain CBDB1 ² |
|-------------------------|---------------------------|
| 1.467 Mbp | 1.395 Mbp |
| Chloroethylenes, etc | Chlorobenzenes, etc |
| PCE->TCE->DCE->VC->Eth | PCE->TCE->DCE |

Step 2.

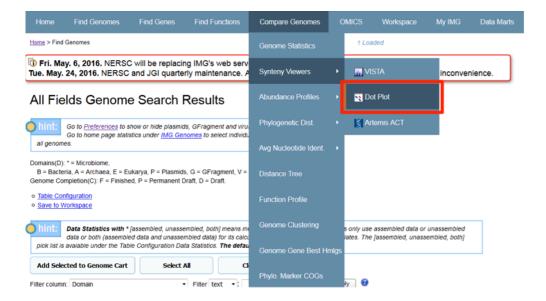
Add the following 2 genomes to your genome cart using the 'Quick genome Search' box at the top of the page: <u>Dehalococcoides mccartyi CBDB1</u> and <u>Dehalococcoides mccartyi 195</u>



Question 1

Step 3.

Use Compare Genomes > Synteny Viewers > Dot Plot



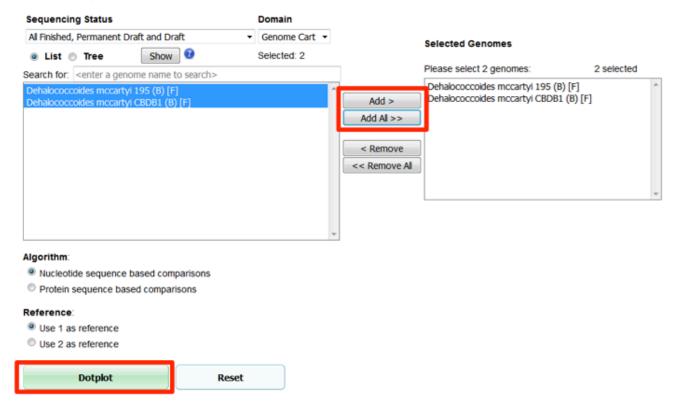
Step 4.

Select and Add both genomes and click "Dotplot"



Dot Plot employs <u>Mummer</u> to generate dotplot diagrams between two genomes. It uses input DNA sequences directly for comparing genomes with similar seque uses the six frame amino acid translation of the DNA input sequences (<u>PROmer</u>) for comparing genomes with dissimilar sequences (because the DNA sequence conserved as the amino acid translation).

Please select 2 genomes



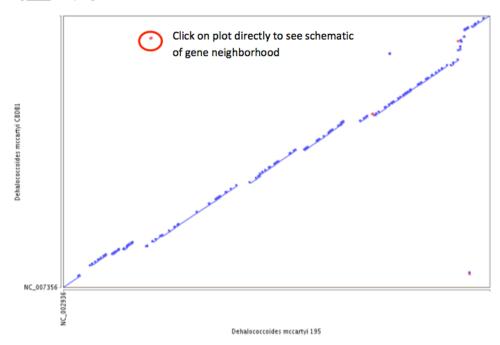
Question 1

Step 5.

Click on plot directly to see schematic of gene neighborhood

Dehalococcoides mccartyi 195 vs. Dehalococcoides mccartyi CBDB1

Using nucmer to compare genomes:



Question 1

Step 6.

Answer 1: Dot plot reveals extensive synteny with some gaps, rearrangement, transposition or inversion appears to involve reductive dehalogenase genes, key enzymes for their hallmark property.

Question 2

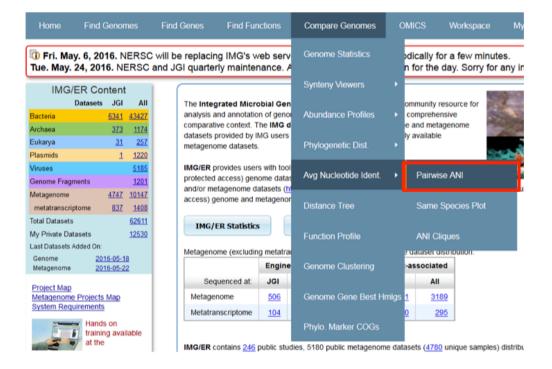
Step 7.

Question 2: What proportion of genes or proteins is conserved?

Question 2

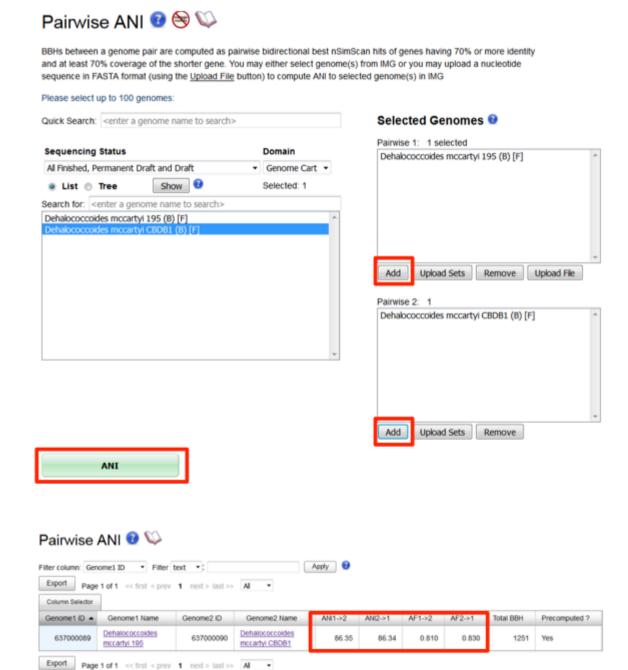
Step 8.

Use Compare Genomes > Average Nucleotide Identity > Pairwise ANI



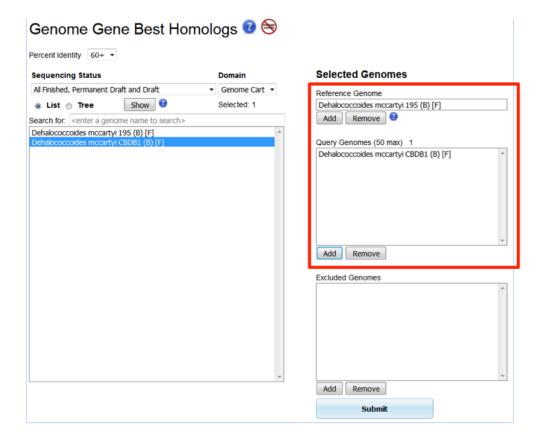
Step 9.

The Pairwise ANI page will look like this:



Step 10.

To compare proteins, use Compare Genomes > Genome Genes Best Hmlgs



Step 11.

Answer 2: Both nucleotide and amino acid sequence based comparisons suggest about 70-80% of the genes and proteins are conserved based on best reciprocal hits.

Question 3

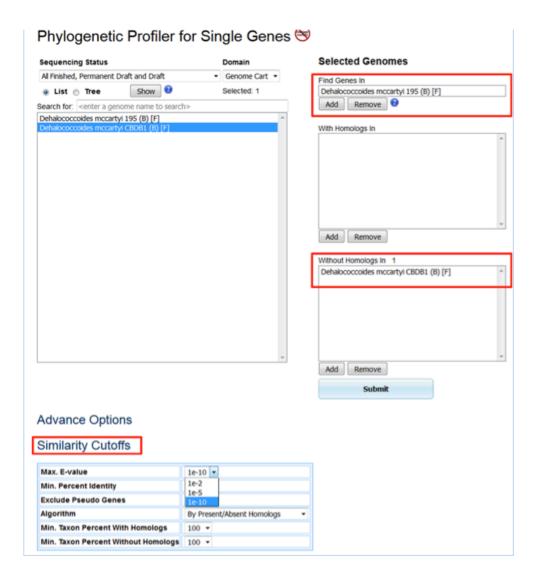
Step 12.

Question 3: How many genes or what functions appear to be unique to Strain 195?

Question 3

Step 13.

Use Find Genes > Phylogenetic Profiles > Single Genes



Step 14.

Results table filtered by Gene Name for "nitrogenase"

| Select | Result . | Gene Object ID | Locus Tag | Gene Name | Length | KEGG Map Name | KEGG Module Name |
|----------|----------|----------------------|--------------|---|--------|--|--|
| | 231 | 637120711 | DET1148 | nitrogenase cofactor biosynthesis protein NifB, putative | 276 | | |
| | 234 | 637120714 | DET1151 | dinitrogenase iron-molybdenum cofactor NifB/Y/X family protein | 134 | - | |
| = | 235 | 637120715 | DET1152 | nitrogenase molybdenum-iron protein, beta subunit, putative | 451 | | |
| | 236 | 637120716 | DET1153 | nitrogenase MoFe cofactor biosynthesis protein NifE | 454 | | |
| | 237 | <u>537120717</u> | DET1154 | Mo-nitrogenase MoFe protein subunit NifK (EC 1.18.6.1) | 461 | Microbial metabolism in diverse environments Metabolic pathways Chloroalkane and chloroalkene degradation Nitrogen metabolism | Nitrogen fixation, nitrogen => ammonia Nitrogen fixation, nitrogen => ammonia Nitrogen fixation, nitrogen => ammonia Nitrogen fixation, nitrogen => ammonia |
| | 238 | 637120718 | DET1155 | nitrogenase molybdenum-iron protein alpha chain | 539 | Microbial metabolism in diverse environments Chloroalkane and chloroalkene degradation Nitrogen metabolism Metabolic pathways | Nitrogen fixation, nitrogen => ammonia Nitrogen fixation, nitrogen => ammonia Nitrogen fixation, nitrogen => ammonia Nitrogen fixation, nitrogen => ammonia |
| | 239 | 637120721 | DET1158 | Mo-nitrogenase iron protein subunit Niff-I (EC 1.18.6.1) | 274 | Nitrogen metabolism Metabolic pathways Chloroalkane and chloroalkene degradation Microbial metabolism in diverse environments | Nitrogen fixation, nitrogen => ammonia |

Step 15.

Answer 3: About 300 genes appear to be unique to Strain 195 – these include a nitrogenase operon for nitrogen fixation, and a small number of reductive dehalogenases, that might correspond to observed differences in organohalide substrate specificities.

Question 4

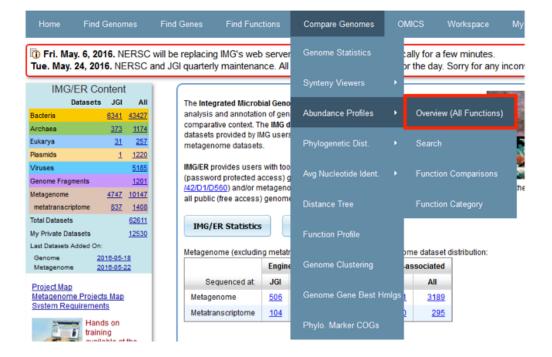
Step 16.

Question 4: The two strains have different dehalogenation profiles (i.e., preferences in halogenated substrate specificities), in particular, *D. mccartyi* strain195 is capable of COMPLETE dechlorination of PCE to ethylene, unlike Strain CBDB1. Can we identify the reductive dehalogenase(s) responsible for the terminal steps?

Question 4

Step 17.

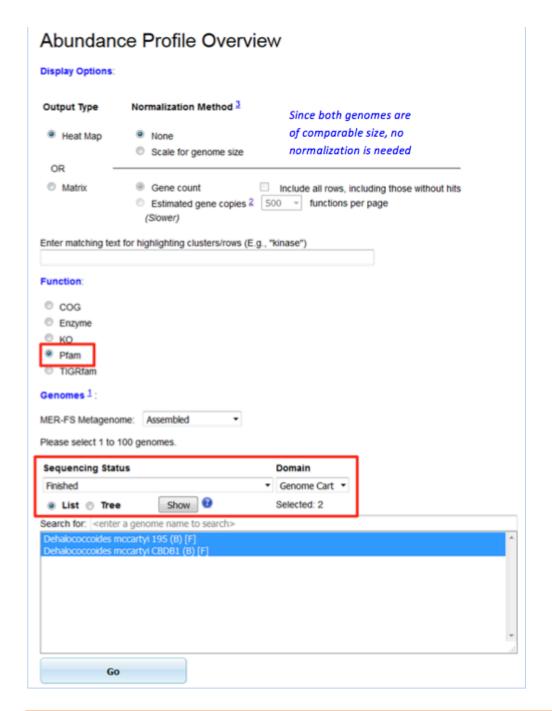
Use Compare Genomes > Abundance Profile Tools > Overview (All Functions) > Select Pfam (for function type)



Step 18.

Selecting Pfam page:

11



Step 19.

Add genes corresponding to reductive dehalogenases (pfam13486) from both genomes to your gene cart

Abundance Profile Overview Results (Gene Count)

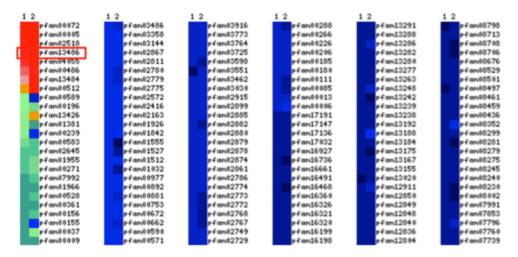
Mouse over labels to see additional information.

Clicking on the column number will sort rows for that column in descending gene count order.

Clicking on row cluster ID will add the cluster to the appropriate analysis cart (if cart is supported).

Mouse over heat map to see gene counts. Clicking in the heat map will take you to the gene list.

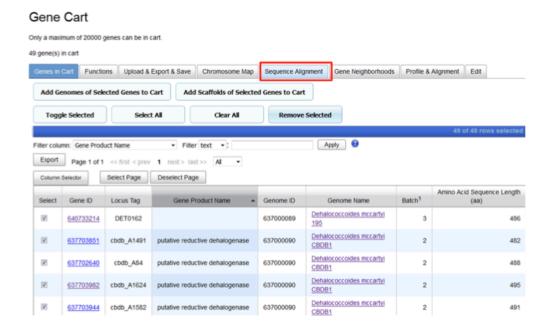
- 1 Dehalococcoides mccartyi 195
- 2 Dehalococcoides mccartyi CBDB1



Question 4

Step 20.

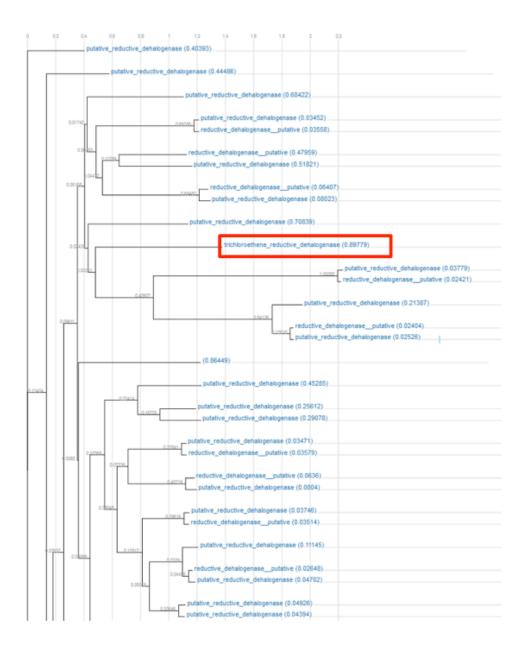
From Gene Cart page, use the Sequence Alignment Tab and "Do alignment"



Question 4

Step 21.

From the alignment results page, select "Rectangular Phylogram" tab to view tree



Step 22.

Answer 4: From the tree, it is possible to discern a handful of divergent candidates in strain 195 relative to CBDB1- the gene described as "trichloroethene reductive dehalogenase" is experimentally validated to dehalogenate DCE and VC to ethylene

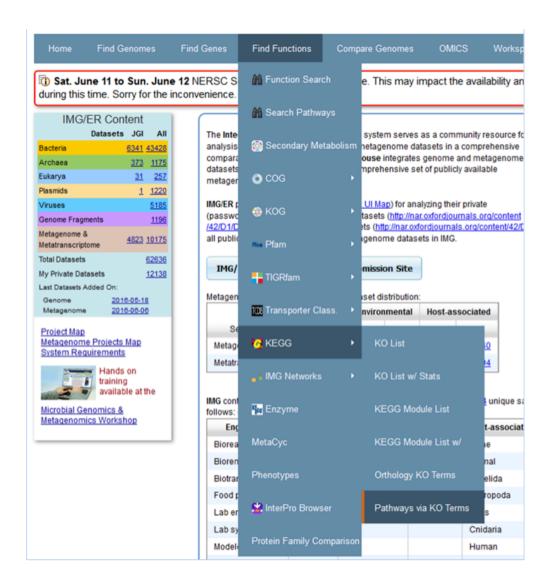
Question 5

Step 23.

Question 5. Corrinoid cobalamin (B12) is an essential cofactor of reductive dehalogenases. *De novo* biosynthesis of corrinoid is one of the most complicated metabolic pathways in nature (involving >30 enzymatic steps.), and dehalorespiring bacteria have developed different strategies to cope with their need for this cofactor. Are *D. mccartyii* strains capable of de novo cobalamin biosynthesis? Do they have partial pathways? Are they all auxotrophs? What is your conjecture based on your findings?

Step 24.

Hint: Find Functions > KEGG > Pathways via KO terms > Select Porphyrin and chlorophyll metabolism under Metabolism of cofactors and vitamins > Switch Tab to "view map for selected genomes> View maps individually for each genome> On the KEGG map, focus on the anaerobic portion of the pathway leading from precorrin to Vitamin B12



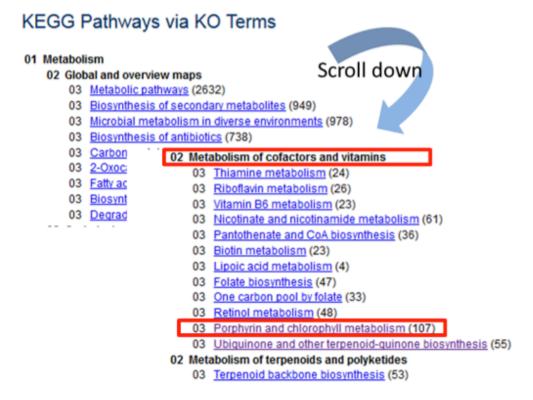
Question 5

Step 25.

KEGG Pathways Page:

KEGG Orthology (KO) Terms and Pathways

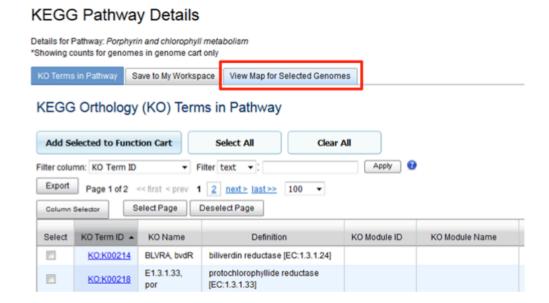
KEGG Orthology (KO) Terms Based on BRITE Hierarchy KEGG Pathways via KO Terms



Question 5

Step 26.

Tab over to "View Map for Selected Genomes" from the KEGG Pathway details page:



Question 5

Step 27.

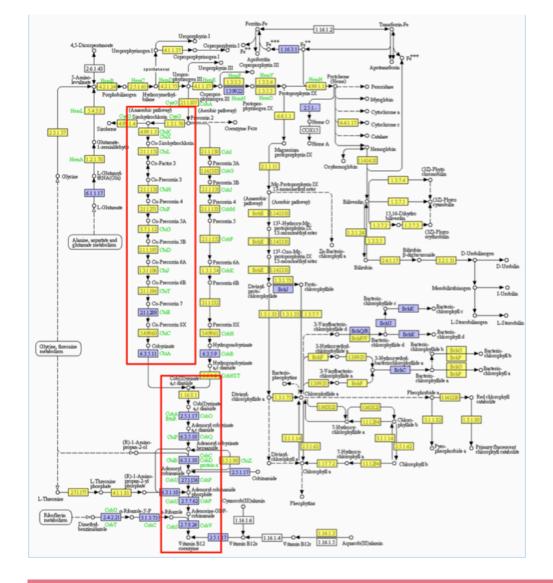
View map for all 5 genomes INDIVIDUALLY:

KEGG Pathway Details Details for Pathway: Porphyrin and chlorophyll metabolism *Showing counts for genomes in genome cart only KO Terms in Pathway Save to My Workspace **KEGG Map for Selected Genomes** Sequencing Status All Finished, Permanent Draft and Draft ▼ Genome Cart ▼ Selected Genomes Show List Tree Selected: 1 Please select genomes to highlight on KEGG Search for: <enter a genome name to search> Pathway Map: Dehalobacter restrictus DSM 9455 (B) [F] Dehalobacter sp. 11DCA (B) [F] Dehalobacter sp. CF (B) [F] Add > Add All >> Dehalogenimonas lykanthroporepellens BL-DC-9 (B) [F] < Remove << Remove All MER-FS Metagenome: Assembled Genes in selected genome Find missing enzymes View Map Reset

Question 5

Step 28.

On the KEGG map displayed for "Porphyrin and chlorophyll metabolism," examine the "Anaerobic Pathway" leading from Precorrin to Vitamin B12:



Step 29.

Answer 5: *D. mccartyi* strains may salvage B12 from a cobinamide intermediate based on the presence of the lower half of the pathway. However, it is wise to be skeptical – investigate whether CDS were missed, incorrectly identified or annotated before any conclusions are drawn. Given the results as is, neither strain is capable of de novo B12 synthesis.