LD12 genomes analysis pipeline

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All numbers are fake. This document describes a plan, it is not scientific work.

#	Name	Source	# Genes	Family	Partial
G1	AAA288-N07	Freshwater (LD12)	560	1	yes
G2	AAA028-D10	Freshwater (LD12)	478	1	yes
G3	AAA023-L09	Freshwater (LD12)	609	1	yes
G4	HIMB140	Marine (SAR11)	1091	2	yes
G5	HTCC9565	Marine (SAR11)	951	3	no
G6	HIMB083	Marine (SAR11)	980	3	no

1) Start: with N partial genomes and the FASTA files containing their genes (amino acid sequences). We also have metadata and annotations for each gene.

#	g1	g2	g3	g4	g5	g6	...
g1	–	1	89	0	112	1	...
g2	1	–	1	1	1	0	...
g3	45	1	–	1	44	1	...
g4	2	1	1	–	1	1	...
g5	87	0	12	1	–	1	...
g6	1	1	1	1	0	–	...
..	...	...	...	...	...	...	...

2) All against all BLAST: Perform a sequence similarity search of all genes against all genes. WIth a e-value cutoff of 0.001

#	L29	S17	Rib3	RIb
G1	1	1	1	1
G2	1	1	0	1
G3	1	0	1	1
G4	1	1	1	1
G5	1	1	1	1
G6	1	1	1	1

1B) Extract ribosomal genes: based on the annotations. This will act as the reference tree, these are supposed to be the less likely genes to be exchanged.

#	g1	g2	g3	g4	g5	g6	...
g1	–	1	89	0	112	1	...
g2	1	–	1	1	1	0	...
g3	45	1	–	1	44	1	...
g4	2	1	1	–	1	1	...
g5	87	0	12	1	–	1	...
g6	1	1	1	1	0	–	...
.	...	...	...	...	...	...	...

3) Filter the BLAST hits: Any hit with a minimum identity of 30% and a minimal coverage of 50%

Multiple sequence aligement

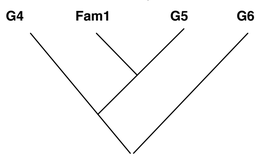
```
P1| KVFGRCELAAAMKRHGLDNYRGYSLGNWVC-----
P3| --------AAKFESNFNTQATNRNTDGSTDYGI--
P4| RWWCN--DGRTPGSRNLCN------IPCSAL---IT
P5| IWWCAK-----------KITPDGNGMNAWVAS--
G3| PRAA-----AAAERLWSNFF-------EGTFFIAA
G6| -----YVVWQEVFDNKVKVRP-------DTIIQVE
G8| ---------RLWPRAGAVAERLWSSNLTTNIDFRL
H1| ----------------------------AAAM--
H2| --KVFGRCELAAAM----------------------
H4| --EVD--------FTCW------KS-----NP---
H5| ----------------------AGFRALLSAPW--
H6| --QKT-------------------NRNTDGSTD--
H7| --------RWWCNDGRT-------------PGS---
```

Using all ribosomal genes

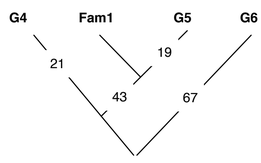
2B) Align each ribosomal protein cluster separately: run gblocks on the result, then concatenate each cluster together into one file, run that into RaxML

4) Compte synteny scores: Come up a matrix of synteny distances

5) Merge both matrices: Using a elitic-type weighing equation (if the elipse is a circle we are just doing 0.5*M1 + 0.5*M2)



3B) Build a seperate tree: the tips can be mixed, if we didn't find a specific ribosomal protein in one given genome, we can merge it with another genome of the same family and indicate the family on the tree leaf



4B) Build a seperate tree: Add the bootstrap. See dendropy.sumtrees

Clusters

C1 = g1+g3+g5
C2 = g2+g4
C3 = g6+g7+g8+g9

ETC...

6) Cluster: Using the MCL clustering, input the distance matrix and get a list of clusters of genes.

In a cluster do they all have the same annotations ?

#	C1	C2	C3	C4
G1	1	1	1	1
G2	1	1	2	1
G3	1	1	1	1
G4	1	2	0	2
G5	1	1	0	3
G6	2	0	0	3

7) Count within genomes: Given each cluster, how many of its members are associated with each genome

#	C1	C2
G1	1	1
G2	1	1
G3	1	1
G4	1	2
G5	1	1
G6	2	0

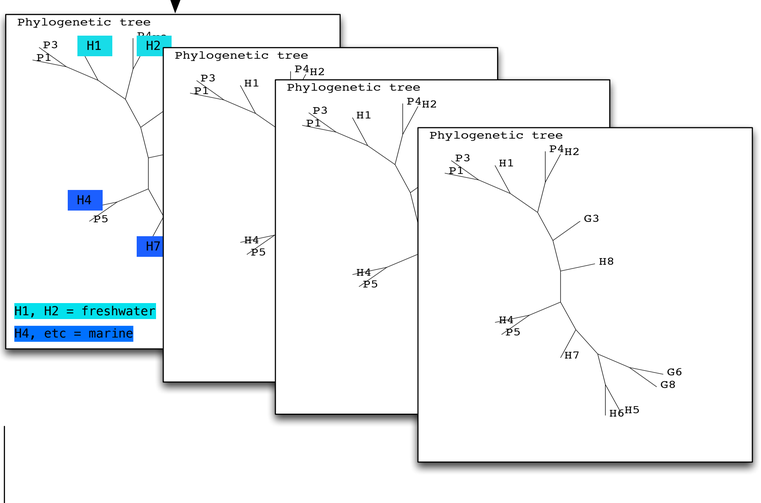
8) Choose some cluster that we like: Have at least one representative in each family and no more than 29 genes in the cluster.

#	C1	C2
G1	1	1
G2	1	1
G3	1	1
G4	1	1
G5	1	1
G6	1	0

9) Take only one gene per genome: Since we are allowing some cluster to contain two or more genes pertaining to the same genome, we filter out extraneous genes, keeping one randomly.

Current bottleneck

10) With the genes of a cluster: Align each protein with muscle, run gblocks on the result, run that into RaxML to make a tree.



Finally: For every tree (from clusters) compare with the ribosomal tree.

For every tree compare with the reference tree: How well does the structure correspond ? How often do we find the same split between the families ? When we don't fin the same split, which families are miss-spiting ?

1) Check that on the cluster trees the families are grouping together, either kick the ones that don't out, or report how many times a certain split is not conserved (uncollapsible)

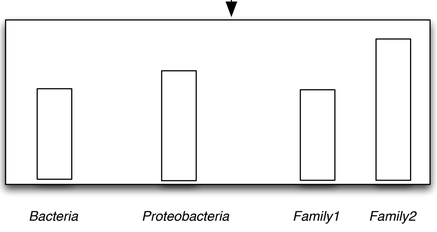
2) The trees that collapse nicely compare it with master tree setting the root at V and checking that the same branching patterns exists.

3) If there is a popular alternative branching pattern, draw it in the supplementary and add as numbers how many clusters had this exact alternative branching.

With the trees when considering how many conserve the split 3a-3b, only consider those that have bootstrap over 80%.

Make a table where we check using all trees if the successive splits of the reference tree are conserved

***Extra: Taking columns such as C3 where we have only freshwater genomes:**, BLAST against "refseq+marine" database. And check how many hits are to SAR11, alphaproteobactera, proteobacteria, archea ?*



Check that all the marine and freshwater genome are in the new refseq database. if some are missing add them to refseq.

-What is the top hit non fresh-water. Is it from marine or from something else in refseq. Make standard graph.

- Considering the list of top hits that are all from fresh-water. Stopping as soon as a top hit is not freshwater. Is there another hit within the same genome other than, obviously, the query itself. Get the list of the gene IDs.