

Lab Report 2

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April 18, 2014

Dr. Muller supplied us with contigs generated from a metagenomic assembly of DNA from a sample of marine water. The contigs are thought to be an undescribed lineage within *Verrucomicrobia*. We used BLAST, GLIMMER, and web-based tools to identify ORFs within one of the contigs and predict possible functions. Using an interpolated markov model supplied by the instructors, we used GLIMMER to predict ORFs. We used a custom perl script `parse_Glimmer.pl` to extract the amino acid sequence of the predicted ORFs. We then made a BLAST database, using sequences supplied by the instructors, and used the database to identify the ORFs. 4 ORFs were found, 2 of which return significant BLAST hits using an -e threshold of 0.01. Each of the 2 ORFs with significant hits had one identical hit and two very weak hits. We then used a custom perl script `grab_seq.pl` to extract one of the ORFs with an identical hit to the reference database.

The extracted sequence was searched against the genbank nr database using blastp with default settings 1. All of the hits were hypothetical proteins and only one was full length. Next, a PSI blast search with 3 iterations, using default parameters, was used in order to discover more distally related putative homologs. This revealed many more full length matches, but all were still hypothetical proteins 2. We used the COG and CCD databases to search for conserved domains using default parameters. The COG database search returned a hit to a chromosome segregation ATPase 3, but the CCD database search did not return any hits with known function 4. Finally, we searched pfam in order to detect any protein families that the unknown sequence may be a part of, but this returned no significant result with known function. The 2 hits that were found were of unknown function 5. From all of the analyses overall, it appears that this ORF is either a novel protein that has not been assigned a function yet, or a conserved sequence susceptible to misassignment by automatic *ab initio* gene annotation program, since there are many hits to multiple databases, but little functional information. The one significant hit with functional information was a chromosome segregation ATPase. but this alone is not sufficient evidence to predict the function of this unknown sequence.

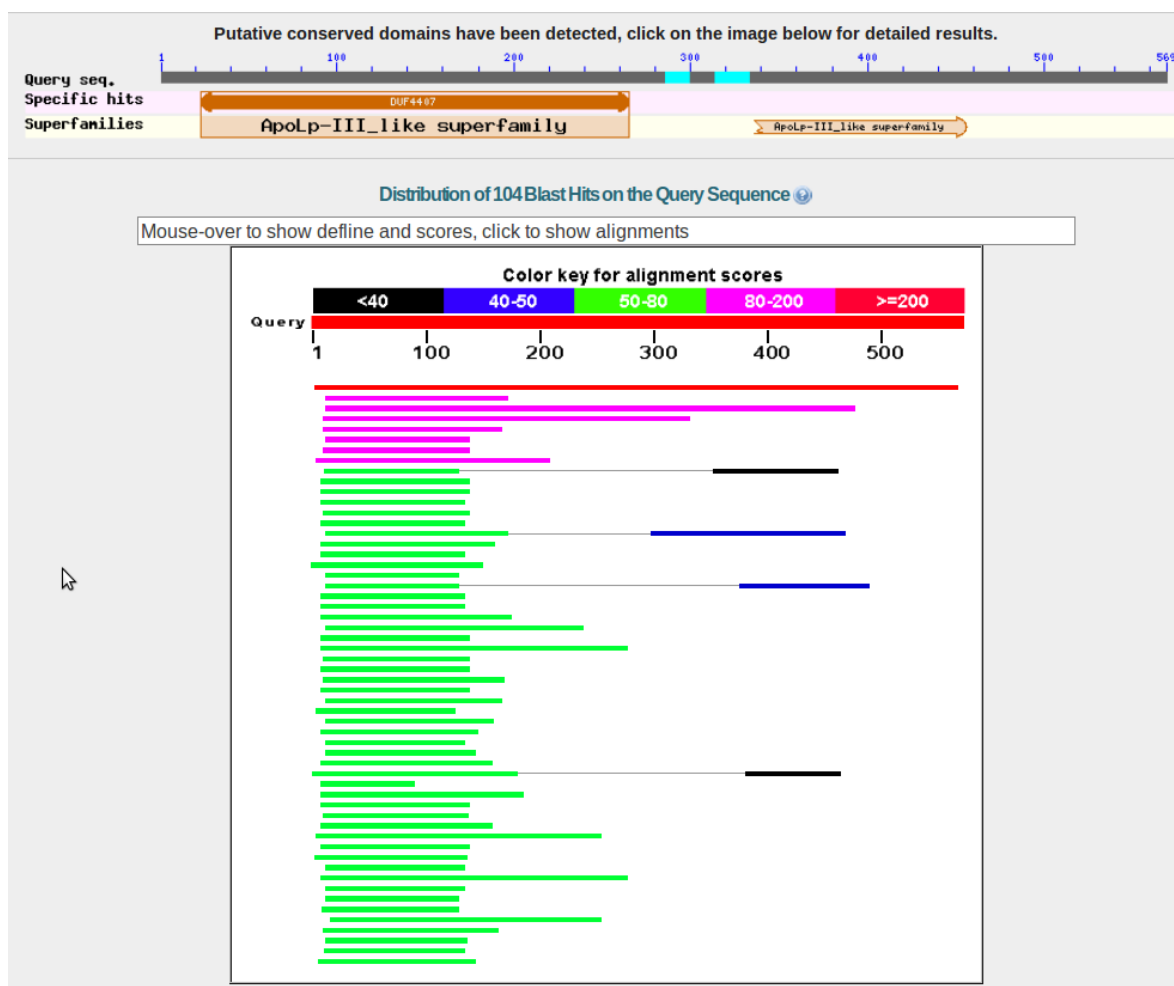


Figure 1: Screenshot of blastp results

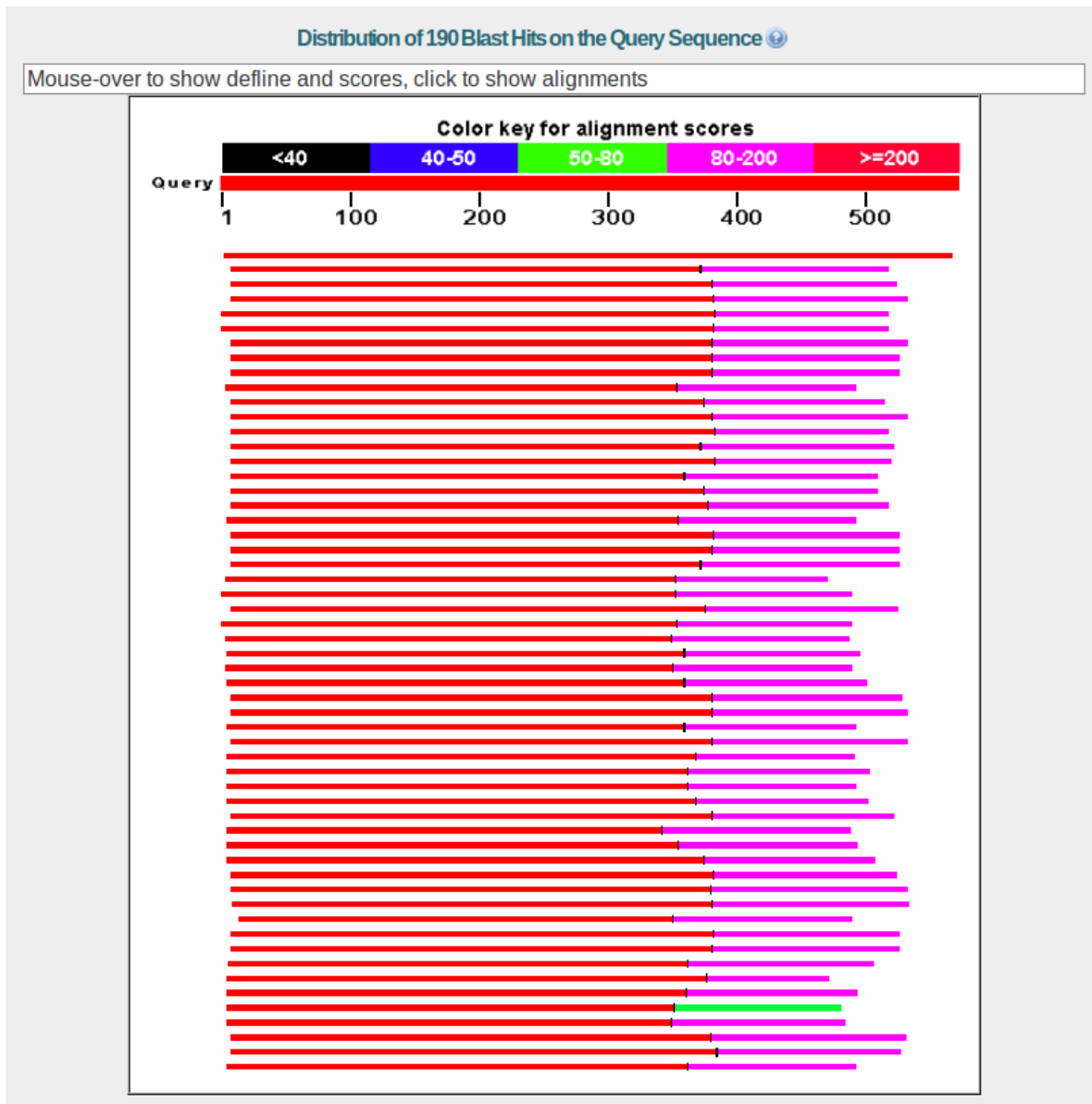


Figure 2: Screenshot of PSI-BLAST results

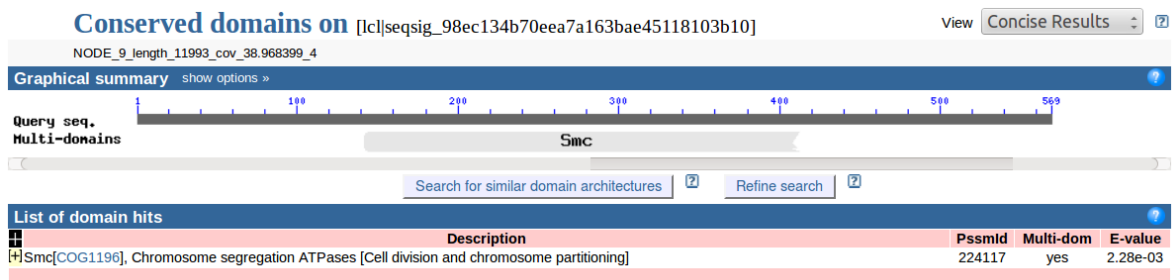


Figure 3: Screenshot of COG search results.

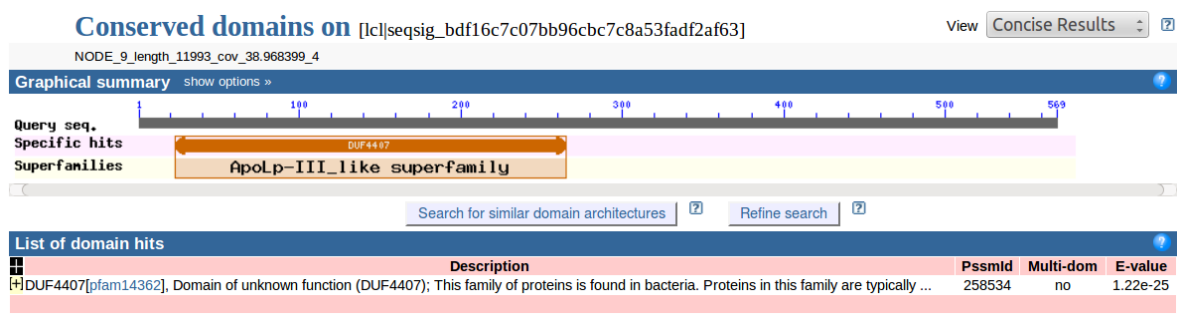


Figure 4: Screenshot of CCD search results

Sequence search results

[Show](#) the detailed description of this results page.

We found **3** Pfam-A matches to your search sequence (**2** significant and **1** insignificant). You did not choose to search for Pfam-B matches.



[Show](#) the search options and sequence that you submitted.

[Return](#) to the search form to look for Pfam domains on a new sequence.

Significant Pfam-A Matches

[Show](#) or [hide](#) all alignments.

Family	Description	Entry type	Clan	Envelope		Alignment		HMM		HMM length	Bit score	E-value	Predicted active sites
				Start	End	Start	End	From	To				
DUF4407	Domain of unknown function (DUF4407)	Family	n/a	22	332	24	322	4	228	301	99.3	1.9e-28	n/a
DUF4407	Domain of unknown function (DUF4407)	Family	n/a	277	458	285	456	127	299	301	40.6	1.4e-10	n/a

Insignificant Pfam-A Matches

[Show](#) or [hide](#) all alignments.

Family	Description	Entry type	Clan	Envelope		Alignment		HMM		HMM length	Bit score	E-value	Predicted active sites
				Start	End	Start	End	From	To				
V-SNARE	Vesicle transport v-SNARE protein N-term ...	Family	CL0147	328	399	331	397	5	70	79	12.8	0.099	n/a

Figure 5: Screenshot of pfam results