

Example exploratory workflow through the Ocean Protein Portal:

You are interested in understanding how different metals are used by microorganisms in the marine environment. Your favorite metal is Nickel.

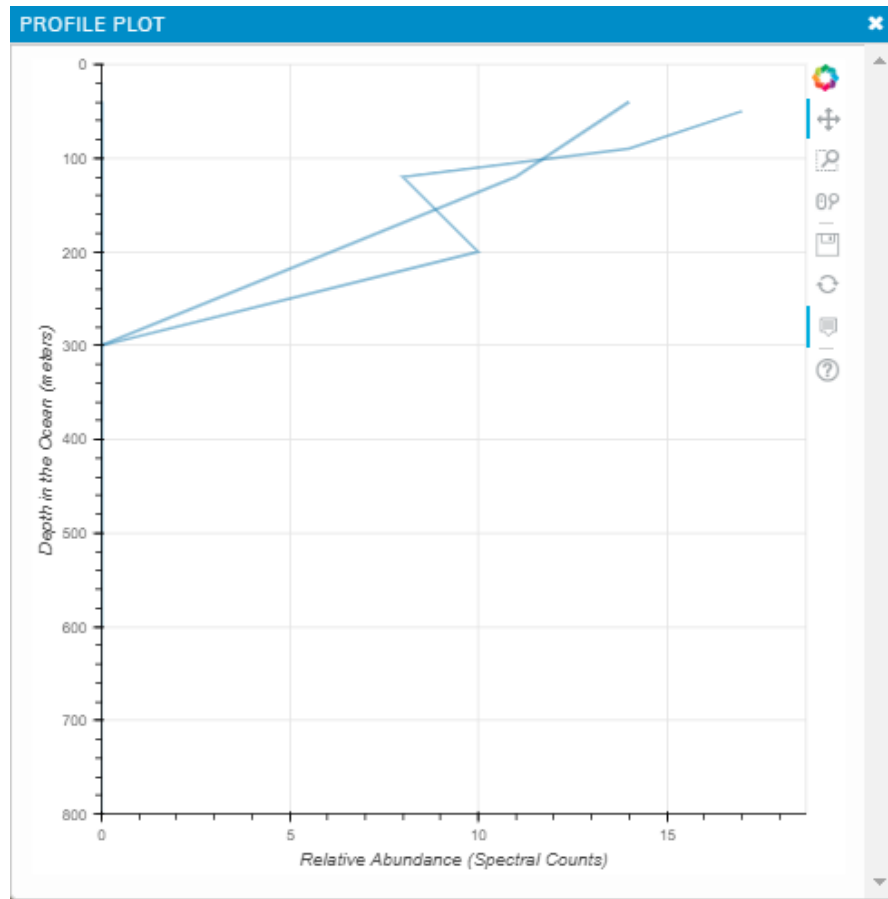
- Enter "Nickel" into the search field
- The following screen will show up with proteins that have "nickel" in their descriptions

The screenshot shows the Ocean Protein Portal interface. On the left is a search sidebar with filters for Product Name, Search Value (nickel), Concentration (0-300), Depth Range (0-6000), Filter Size, Cruise, and Date Range. The main area features a world map with blue dots indicating protein locations. Below the map is a table of search results.

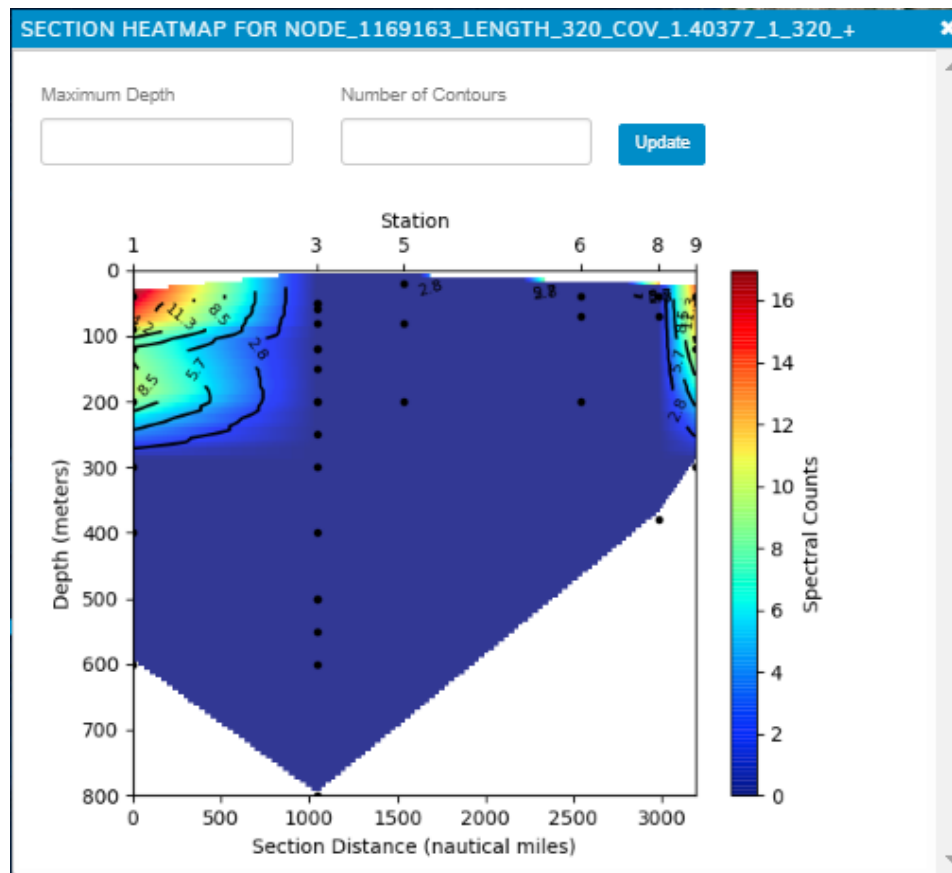
| Product Name | Protein ID | NCBI Taxon | UniProt IDs | pfams | Peptides | View Sequence | View Section |
|---|--|---|-------------|------------------|-------------------|---------------|--------------|
| peptide ABC transporter, K02035 peptide/nickel transport ... | NODE_1351973_length_300_cov_1.1551_1_300_... | Agrobacterium radiobacter K34 | B9JL82 | PF00496 | No peptides found | View Sequence | View Section |
| oligopeptide/dipeptide ABC transporter, ATPase subunit, ... | NODE_13038_length_3425_cov_4.73234_1326_... | Ketoglulonicigenium vulgare Y25 | E3F4T4 | PF00005, PF08352 | No peptides found | View Sequence | View Section |
| appA: oligopeptide-binding protein AppA, K02035 peptide/... | NODE_13919_length_3802_cov_4.11369_1964_... | Roseobacter littoralis Och 149 | | PF00496 | 12 peptides | View Sequence | View Section |
| ABC-type dipeptide transport system, periplasmic compon... | NODE_1164342_length_342_cov_1.68641_1_34_... | Sinorhizobium fredii NGR234 | Q6W118 | PF00496 | 1 peptide | View Sequence | View Section |
| ABC-type dipeptide/oligopeptide/nickel transport system ... | NODE_1176535_length_342_cov_1.5261_1_304_... | Dehalococcoides VS | D2BH66 | PF00496 | No peptides found | View Sequence | View Section |
| putative nickel-containing superoxide dismutase precursor ... | NODE_1169163_length_320_cov_1.40377_1_32_... | Prochlorococcus marinus str. MIT 9301 | A3PEC7 | PF09055 | 2 peptides | View Sequence | View Section |
| 4-phylase (EC:3.1.3.26), K02035 peptide/nickel transport ... | NODE_1211_length_16640_cov_10.5162_1_161_... | Methylobacterium radiotolerans JCM 2831 | B1LV35 | PF00496 | 3 peptides | View Sequence | View Section |
| extracellular solute-binding protein family 5, K02035 pep... | NODE_1285261_length_327_cov_1.97426_1_32_... | Variovorax paradoxus S110 | | PF00496 | 2 peptides | View Sequence | View Section |
| peptide ABC transporter, periplasmic substrate-binding pr... | NODE_12400_length_3693_cov_21.0454_946_2... | Roseobacter denitrificans Och 114 | Q163W0 | PF00496 | 16 peptides | View Sequence | View Section |

Showing Proteins 1 to 100 of 143

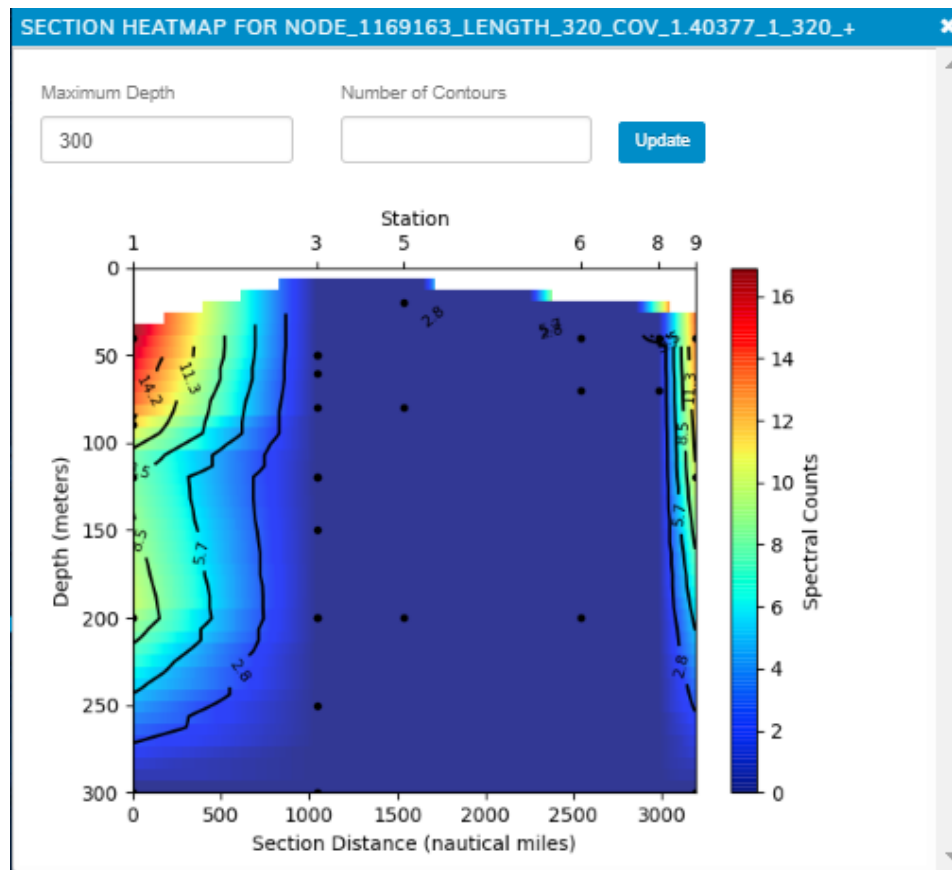
- You know that *Prochlorococcus* is the most abundant photosynthetic organism on the planet! (fun fact) and so pretty much anything *Prochlorococcus* does is important because of their sheer abundance and contribution to primary production. You select the first *Prochlorococcus* protein that comes up to investigate further.
 - putative nickel-containing superoxide dismutase precursor (NISOD) (EC:1.15.1.1); K00518 superoxide dismutase [EC:1.15.1.1] NODE_1169163_length_320_cov_1.40377_1_320_+ Prochlorococcus marinus str. MIT 9301
- The first thing you do is see that the protein is a super oxide dismutase. But you're not sure what exactly that means, so you use the tools available to find out more.
 - Click on the "PF09055" link -- it will take you to the Pfam website which tells you that superoxide dismutase is used to clean up free superoxide radicals and convert them to more stable products (O₂, H₂O₂)... This is super important in an oxygenic photosynthesizing organism as these superoxide radicals can cause cell damage if not regulated.
- Now that you know what the nickel superoxide dismutase is, you want to see what the sequence is for this organism. You click on "View Sequence" and it shows you the sequence of the protein. You can click on "blast" button to send this sequence to NCBI blast tools.
- Great! Now you know what this protein is, you want to know where it is in this transect. So you click on the radio button and click "Profile Plot"
 -



- You can see that this protein is more abundant in the surface than at depth
- Click on "View Section"



- Interesting! Appears that this protein is only found in certain locations. Since all the spectral counts are found in the surface, re-focus the section plot on the top 300m [set max depth to 300 & hit enter]

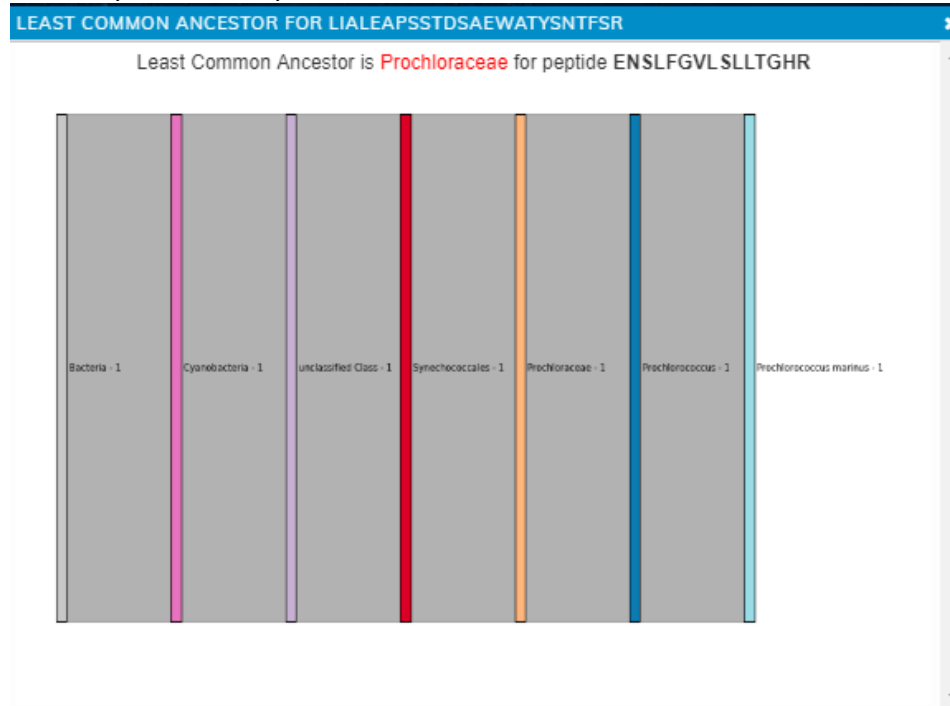


- You know that whole proteins cannot be detected by these analyses... The molecules are just too big for our mass spectrometers! So the proteins are broken up into peptides and it's the peptides which are actually getting measured, with informatics used to estimate protein abundance. You want to look at the peptide information that resulted in this protein information. **[click on "2 peptides" link]** which will redirect you to a new tab "Peptides Found"

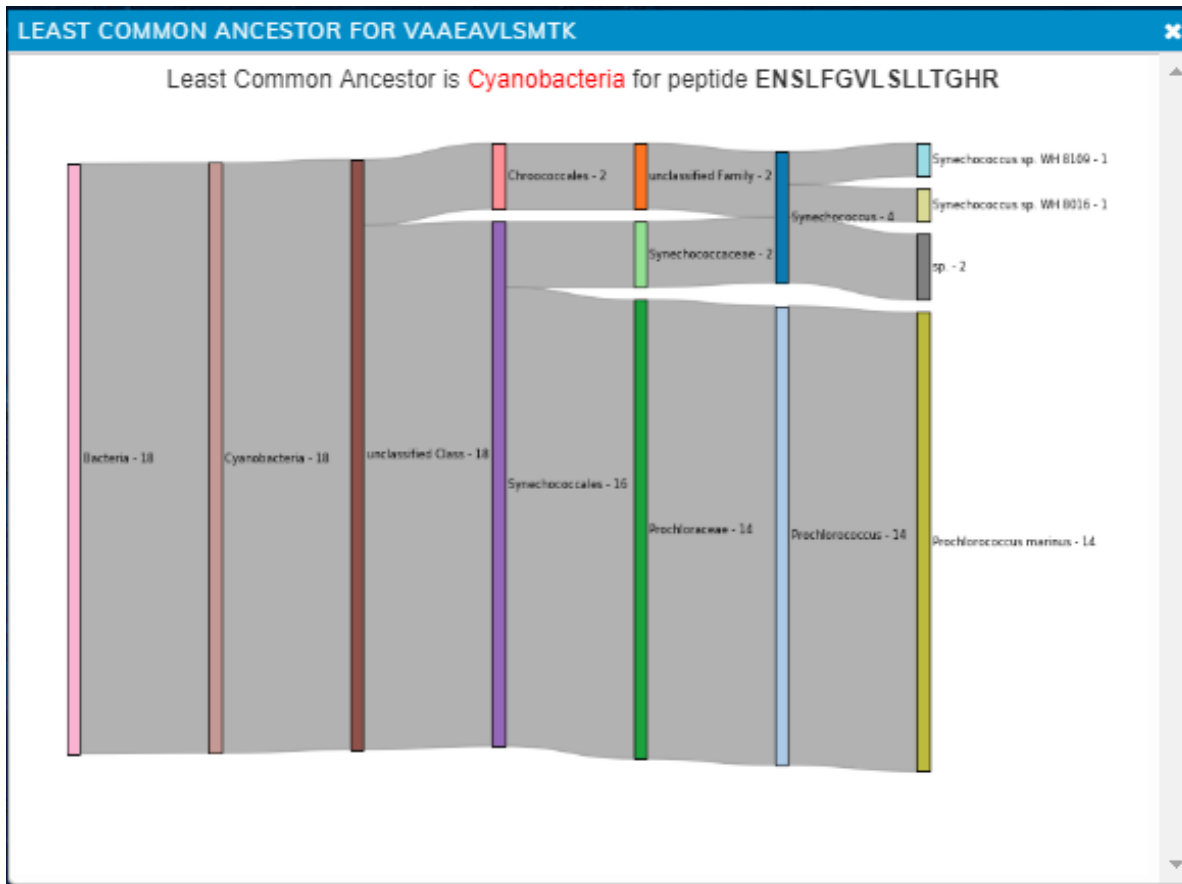
Peptides for NODE_1169163_length_320_cov_1.40377_1_320_+

| Peptide Sequence | |
|--|---------------------------------------|
| LIALEAPSSTDSAEWATYSNTFSR | Least Common Ancestor |
| VAAEAVLSMTK | Least Common Ancestor |

- These are the two peptides that were identified from the Nickel Superoxide Dismutase.
- Since peptides are very small... These can be highly conserved and found in many different organisms. You'd like to know what different organisms these peptides may have been from (aside from *Prochlorococcus* MIT9301 which you saw in the "NCBI Taxon" description of the protein. You click on "Least Common Ancestor" for LIALEAPSSTDAEWATYSNTFSR.



- You see that this peptide is particular to *Prochlorococcus*. This information is being gathered from METATRYP. METATRYP searches for the peptide against a curated database focused on marine microorganisms. ****note: yes, there is a bug in the heading of this Sankey/tree. It is fixed in the portal test server.**
- You go back and click on the "Least Common Ancestor" link for the other peptide.



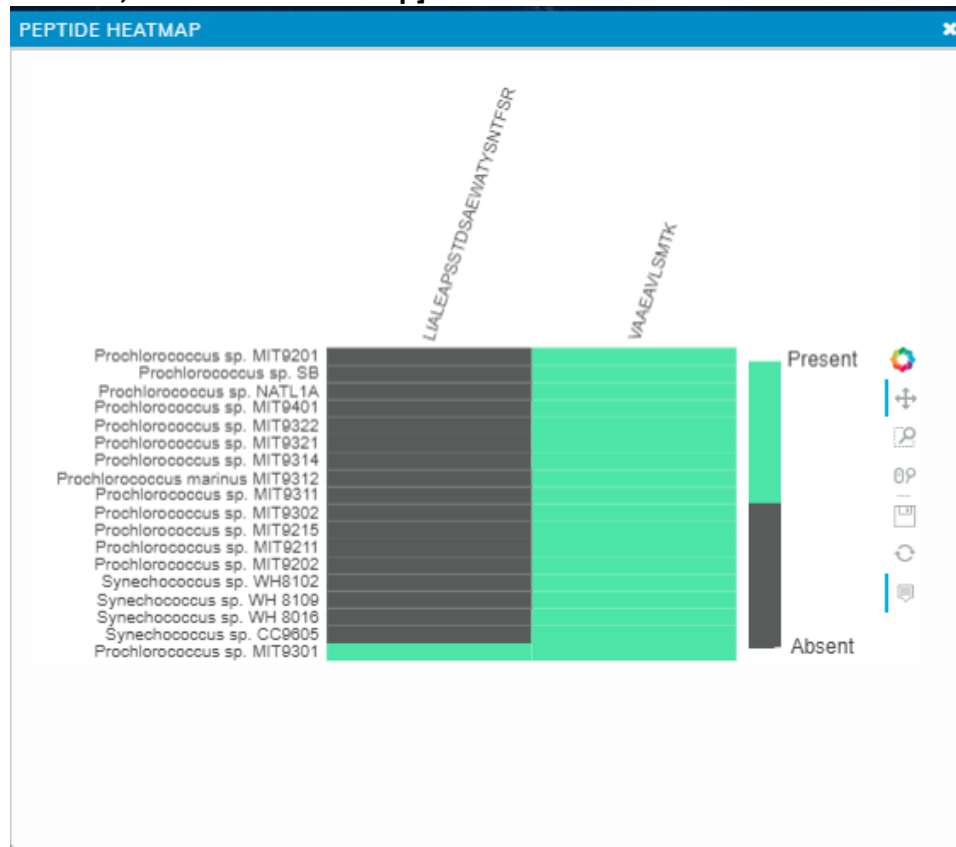
- That's a little more interesting. You see that this is found in more than just *Prochlorococcus*. You want to find more about what these other organisms are, so you explore the METATryp output more directly. **[Go back to the previous screen and select the hyperlink of the peptide itself VAAEAVLSMTK].**

Proteins Found Protein Data Peptides Found Peptide Data METATryp Metagenome Search METATryp Genome Search Export Data

Least Common Ancestor is **Synechococcales** for peptide VAAEAVLSMTK

| Contig Taxon ID | Contig Taxon | Contig Taxon Level | Annotation | ORF Taxon ID | ORF Taxon | ORF Taxon Level |
|-----------------|-------------------------|--------------------|-----------------------------|------------------|-------------------------|-----------------|
| 1218 | Prochlorococcus | genus | superoxide dismutase, Ni | scf7180008710917 | | |
| 1218 | Prochlorococcus | genus | superoxide dismutase, Ni | scf7180008710818 | | |
| 1129 | Synechococcus | genus | superoxide dismutase, Ni | scf7180008743347 | | |
| 1129 | Synechococcus | genus | superoxide dismutase, Ni | scf7180008827203 | | |
| 1129 | Synechococcus | genus | superoxide dismutase, Ni | scf7180008884065 | | |
| 1219 | Prochlorococcus marinus | species | superoxide dismutase, Ni | scf7180009003011 | | |
| 1218 | Prochlorococcus | genus | superoxide dismutase, Ni | scf7180009062071 | | |
| 1218 | Prochlorococcus | genus | superoxide dismutase, Ni | scf7180009258987 | | |
| 1218 | Prochlorococcus | genus | nifH-like nickel-containing | scf7180009293168 | Prochlorococcus marinus | species |

- The METATryp output for Metagenomes is displayed. What this shows is that from the marine metagenome assemblies in the curated marine microorganism search database, this peptide can be found in these organisms. You know that Synechococcales is the taxonomic group which consists of *Prochlorococcus* and its sister genus *Synechococcus*
- **[Click on the Peptides Found tab]** Your goal is to look for a specific peptide which can act as a biomarker target for Nickel Superoxide Dismutase in both *Prochlorococcus* and *Synechococcus*. You use the heatmap tool to check which organisms in the METATryp genome database have these peptides. **[With both peptides selected, click on the Heatmap]**



- Looks like VAAEAVLSMTK would be a much better target for catching Pro & Syn Superoxide dismutase. However, you check the METATRYP database to see just what genomes are in it:
<https://metatryp.who.edu/database/>
- You could then use this information to make a peptide standard of VAAEAVLSMTK and directly quantify how much of this protein is in a sample with targeted proteomics (if you are a proteomics researcher).