Bio314: Advanced Cell Biology

Working with Sequence databases and Protein Explorer Part II

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Introduction

In Part I of this lab, you learned the basics in working in the Student Interface of Biology Workbench and Protein Explorer. In this lab, you will expand and work with other features of these programs. In the Student Interface, databases containing lists of protein sequences found in other organisms are located in the BLASTP tool. Several GenBank databases are listed, which you will be searching in the GenBank Invertebrate database for fluorescent protein sequences of other invertebrates. Then you will compare them to the green fluorescent protein found in the jellyfish, *Aequorea victoria* using ClustalW and a denogram..

In Protein Explorer, using the Comparator tool of this program you will compare the blue fluorescent protein (BFP) to the green fluorescent protein at the same time. The BFP is a variant of the GFP, which biochemists have constructed many mutants using the GFP gene to improve the luminescence of the green fluorescent protein.

Objectives

The objectives of Part II of this experiment are to

- Search and compare fluorescent protein sequences found in other invertebrates to the green fluorescent protein found in the jellyfish using a BLASTP database.
- Compare the structures of the BFP and the GFP using the Comparator tool of Protein Explorer.

Procedure

Assignment
Answer questions 1-12
Log into the Student Interface to Biology Workbench.
-Select the GFP work session, and click the Resume button.
-Click on the Protein Tools button.
-Scroll or click to the bottom of the screen.

- Sequences you previously selected will be saved here.

-Select the 1GFL_A sequence.
-Scroll back up to **BLASTP**.

- **B**asic **L**ocal **A**lignment **S**earch **T**ool for **P**rotein.
- Compares a PS (protein sequence) to a PS DB (database).

-Highlight the database for
GenBank Invertebrate Sequences.
-Click the BLASTP button.

- The list will show protein sequences of fluorescent proteins found in other invertebrates.

-Select 5 or more protein sequences.

- Do not exceed 10.
- Try to choose sequences that are different.
- Read the descriptions carefully.

-Import sequences.

-Scroll down to the list and select the sequences that you just imported, starting at the top of the list.

- Every time you import sequences, they are added to the top of the list.

-Select the 1GFL_A sequence also.

-Scroll up to the CLUSTALW button and click on it.
-Observe and compare the sequences.
Answer the following questions.

1. Which invertebrate fluorescent protein sequences are most similar? Provide evolutionary evidence for why you chose those sequences. (Describe regions, compare descriptions and think about the phyla name.)

2. Which invertebrate fluorescent protein sequences are most different?   Provide evolutionary evidence for why you chose those sequences. (Describe regions, compare descriptions and think about the phyla name.)

-Copy and paste sequences into Microsoft word and save to print later.

-Go back to Workbench.
-Click on the Import Alignment button.
-Scroll down and select the box containing the sequences you just imported.
  - This time sequences will be added to the bottom of the list.
-Scroll up and click on the DRAWTREE button.
-Observe the phylogenetic tree and answer the following question.

3. Which invertebrate fluorescent protein sequences are most closely related?
> (Inquire about all relationships between all sequences.)

-Right click on the tree and select copy.
-Paste into the word document with the sequences.
-Print the word document that contains the Fasta and Workbench labels, sequences and the tree.
   (Optional; you can also save your work.)

-Close out the Student Interface window.
-Click on the Protein Explorer link from the Biology Workbench homepage.
Now you are going to compare 2 protein molecules at the same time.
-Scroll down. In the left hand column find and click on the Protein Comparator link.

-Click on the PE version 1.982 link.
-Scroll down. In the center column find and click on the blue Comparator link.
-Type 1gfl into the first small box and 1bfp into the second small box. Then click on the Protein Comparator button.

   You will now see a screen showing 2 protein molecules on the right side. The 1gfl protein should be in the top screen and 1bfp should be in the bottom screen. On the left side, the First Views control panel will be on top and the message box will be on the bottom.
   You can only make changes to one protein at a time. So, in the First Views control panel, the two proteins are listed with circles in between them that specify top or bottom. Click on these to switch back and forth, from one protein to the other when making changes. However, you can rotate each molecule individually without switching. As you switch back and forth the screen color will change. The protein you select will have a white of black background according to the color you select, while the unselected protein will have a gray background.

-Hide water, stop spinning for both proteins, and answer the following question.

4. Briefly describe the differences between both proteins. Include; Brookhaven codes, classification and molecule names, number of chains, disulfide bonds, DNA or RNA, and ligands (if ligands are present, what are they?) Much of this information is found by clicking on the Molecule information link.
  - Then click on Show counts.
  - You have to do this separately for each molecule.
  - Information will show up in the Message Panel at the bottom.
  - If you forget what something is, do not forget to click on the link related to what you want to know.

-Click on the <u>Explore More Views</u> link. Another button appears in this window, SYNCH. If you want to view the same region of both proteins and rotate them at the same time, first align them both up with each other then click the SYNCH button and rotate either one of the proteins.
-Click on the 2º button for each protein.

5. Describe the secondary structure of both proteins.

-SELECT; Chains, DISPLAY; Spacefill, COLOR; Polarity 2. Do this for both proteins. Answer the following questions.

6. What regions are mostly hydrophobic and hydrophilic in both proteins?

7. a. Based on your answers to 5 & 6, are these transmembrane proteins or are they soluble in water?

   b. What is the term used to describe these proteins?

-Now change to COLOR; Polarity 5 for both.
- To answer questions 8-10, use your book and click on the <u>charge at a given pH, or the isoelectric point</u> link.
- To calculate the isoelectric point (pI) follow the directions given in this link.
- When highlighting the sequence, make sure you only highlight the sequence and exclude the comment lines, and for the gfl protein, only highlight one chain.
- Shrink the Help/Index/Glossary page to the taskbar so that you can refer to it if necessary. Also so that you can go to the EMBL web page.

Answer the following questions.

8. a. What is the isoelectric point (pI) of any protein?

   b. Calculate the pI for both the gfl and bfp?

9. What is the quantitative net charge at pH 7 of each protein?

10. Why would this information be important to a biochemist?

   -Select the window containing the 1bfp molecule.
   -Click on Evolution under the DISPLAY menu. This is another way to get to ConSurf.
   -Type none in the Chain identifier box, then click on the Submit button.   Wait!
   -Click on the <u>Go to the results</u> link.
   -Click on the <u>View ConSurf Results with Protein Explorer</u> link.
Answer the following questions.

11. What regions in this protein are conserved in evolution?

12. If there is a ligand present in this protein, how conserved, are the residues closest to the ligand?

Resources

1. http://bsw-uiuc.net/tutorials.php
Accessed; 10-14-07
How to use Biology Workbench v.3.2

2. http://www.umass.edu/microbio/chime/pe_beta/pe/protexpl/qtour.htm
Accessed; 10-14-07
1-Hour Tour for Protein Explorer