

## Computer Analysis of Proteins

In this lab exercise you will get some hands-on experience with computer resources for the analysis of proteins.

### Exercise 1 - Using the Protein Data Bank (PDB) to analyze the structure of proteins.

Launch your web browser and go to the following URL to access the PDB:

<http://www.rcsb.org/pdb/home/home.do>

In the “PDB ID or keyword” search box enter the following accession numbers for Ras-GTP and Ras-GDP respectively (do each in separate windows)

1LF0

1LF5

Click on **View in Jmol** to see the tertiary structure of Ras.

1. How many alpha helices are in Ras-GTP and Ras-GDP?
2. How many Beta strands are in Ras-GTP and Ras-GDP?
3. G-proteins like Ras act as molecular switches that are “on” when bound to GTP and “off” when bound to GDP. Try to describe any differences in tertiary structure between Ras-GTP and Ras-GDP. Hypothesize how the differences might contribute to the function of Ras?

### Exercise 2 - Using the Protein Calculator v3.3 to analyze physical properties of proteins.

Retrieve the one-letter amino acid sequence for human Ras by clicking on **Sequence** on the navigation bar. On the next page, click on **fasta**. You should get a simple text download of the human Ras sequence.

Highlight and copy the entire amino acid sequence.

Open a new tab and set the browser to:

<http://www.scripps.edu/~cdputnam/protcalc.html>.

Paste the amino acid sequence into the search box.

1. Use this site to find the following parameters of Ras:

Molecular weight

Isoelectric point (pI)

Charge at pH = 4

Charge at pH = 7

Charge at pH = 10

Number of amino acids

Most abundant amino acid

Least abundant amino acid

### Exercise 3 - Using BLAST to find homologous proteins

In this exercise you will determine whether there are any Ras orthologues encoded in the yeast genome.

Set your web browser to: <http://www.yeastgenome.org/>

Click on **BLAST** on the navigation bar.

The page that appears will be entitled: **S. cerevisiae WU-BLAST2 Search**

Paste the human Ras sequence in the query box.

Below the query box is a “pop down” menu for choosing the appropriate BLAST program.

From the pop down menu, click on: **BLASTP – protein query to protein db**

Under the header “**Choose one or more Sequence Datasets**”

Click on: **Open Reading Frames (DNA or protein)**

Click on the button: **Run WU-BLAST**

This begins the search. It will only take a few seconds to search the ~6000 genes in the yeast genome with your Ras query sequence. When the search is complete, you will automatically be taken to a page that displays your BLAST results. This page will give you the following information:

List of the yeast proteins that are similar to your query protein, and E (Expect) values.

The alignments of the human Ras protein (query) with the yeast proteins.

Percent Identities, Positives and Gaps for each alignment.

1. Record the following for the best “hit” from the BLAST search:

Expect value

Percent identities

Percent Positives

2. Is there a Ras protein in non-human organisms?

3. How might you experimentally test whether human Ras is a functional orthologue of the yeast protein discovered by BLAST.

**Assignment: On a separate sheet of paper, type the answers to all questions and turn in to me at the beginning of the next lab.**