

Biology 3210 – Over-Expression Worksheet

To answer the following questions, locate the GenBank entries for RNase II from *E. coli* using the NCBI Reference Sequence number of NP_415802. See below for instructions on searching GenBank.

- (1) Go to the GenBank website.
<http://www.ncbi.nlm.nih.gov/Genbank/index.html>
- (2) Click on the 'GenBank' tab and select the 'Search' option.
- (3) The default search option is for the 'Nucleotide' database but the NCBI Reference Sequence numbers are for the proteins themselves (not the genes or a specific nucleotide sequence). Click on the 'Search' drop down menu currently displaying 'Nucleotide' and select 'Protein' instead.
- (4) Enter the Reference Sequence number provided and perform the search. Enter the the number provided *exactly* as it appears, including the underscore.
- (5) Currently displayed is the NCBI 'flatfile', use this information to aid in completion of this worksheet. There are a variety of links, you may need to explore some of these links in order to obtain all the information necessary.

1. What is the function of RNase II?
2. What is the name of the gene encoding RNase II from *E. coli*?
3. How many nucleotides make up the coding sequence of this gene?
4. How many amino acids make up RNase II?
5. Use ProtParam (Protein Parameters) to determine the native molecular weight for the enzyme.
<http://web.expasy.org/protparam>
6. If you were to use the values calculated from ProtParam in a lab report (or publication), you would need to provide a reference. What is the reference to be used for ProtParam?
7. What is the predicted molecular weight for the expressed product produced in lab, if the gene was successfully cloned into the *Nhe* I site of pET28a? Note: the gene retains its native stop codon.

The information you have gathered will play a key role in properly analyzing some of the experimental results obtained in lab.