

**Student Exercise: FGFB Inquiry**  
**Biology 131, Principles of Biology**

1. In the first week of lab you exposed yeast cells to ultraviolet light. Below is a data set that came from one lab pair; the numbers indicate the number of observed colonies. Below make a rough sketch of the number of colonies observed versus the UV dose.

<b>Group</b>	<b>Controls</b>	<b>Ultraviolet Light Dose (mJ)</b>			
		<b>3000</b>	<b>6000</b>	<b>12,000</b>	<b>24,000</b>
EP	10,180 6,240	1128	1204	320	208

2. The lab manual states that one known effect of ultraviolet light is that it can change DNA. Using your understanding of the central dogma and the information provided on page 30, provide a specific scenario in which a change in DNA could lead to the accumulation of red color in the cell. (Your instructor will pass judgment on whether the scenario is specific enough). In this scenario, provide the name of the red molecule that accumulates and an explanation of why it is accumulating. The explanation should have its roots in the UV treatment.

3. Given the specific scenario, would you expect the red cell to be able to make adenine? Explain.

4. Given the specific scenario, would you expect the red cell to be able to grow and divide (and thus form a colony) if adenine were present in the diet? Explain.

5. Given the specific scenario, would you expect the red cell to be able to grow and divide (and thus form a colony) if adenine were not present in the diet? Explain.

6. Do the predictions of growth in the presence or absence of adenine agree with your observations of red types of yeast made in lab today? Explain.
7. Assuming that you could deduce the base sequence of any gene from either the red type of yeast and the white type of yeast, what data would prove that your hypothesis is the basis for the red type of yeast?
8. It so happens that the base sequence of all 5726 genes in the white yeast are already known. Why would purifying the DNA from the red version of yeast be a good first step in testing whether your scenario (hypothesis) explains the basis for the red color?
9. The data shown in question 1 demonstrates that the most obvious impact of the UV light is not to turn cells red but rather to kill them (ie, most of the cells spread do not grow and divide to form colonies). Use information in question 8 and your understanding of the important role that some proteins have in cells (just one example is DNA helicase) to explain why it is more common for cells to be killed by UV light than to become red.



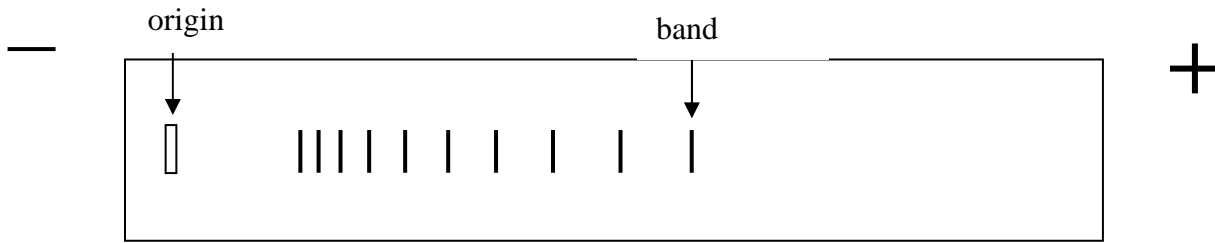
7. Examine each of the products that you described in question 6. Assume that each of the four ddNTPs (but only the ddNTPs) that were added in the reaction had a color (ddATP=green, ddCTP=blue, ddGTP=black, ddTTP=red). For each of the products of question 6, note what color the molecule would have based on which ddNTP(s) were present.

8. When the chemical urea is added to DNA it is able to disrupt the H bonding between complementary base pairs. This results in single-stranded DNA. When the products of the reaction that you listed in question 6 are mixed with urea there should be a mixture of single stranded DNA. What single stranded DNA molecule is common to each of the products that you drew in question 6?

9. In what way does each of the newly made DNA strands differ from each other?

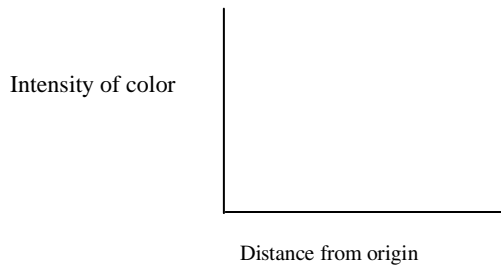
10. We have stated that gel electrophoresis can be used to detect DNA and to separate DNA of different sizes. Place a number by each of the products listed in question 6. The number should indicate the rank order of the speed with which the DNA molecule would move through agarose when in an electric field. Assume that urea is present and thus all DNA will be present as single stranded molecules.

11. Below is a sketch from the overhead view of an agarose gel after electrophoresis. There are ten molecules of DNA that have been separated. Assume that the mixture of DNA molecules that have been separated were the products of the reaction described in question 6. Note (from question 7) that each of these molecules may have color. Based on your knowledge of electrophoresis and your answer to question 10, write what color each of the DNA molecules would have on the sketch below:



12. If one closely examines a band of DNA (think of a band as a pile of the exact same DNA molecules---they are present in the same spot because they have the same size) the following is observed:

Make a graph that illustrates the relationship between the intensity of color present as a function of the distance from the origin for a single band of DNA.

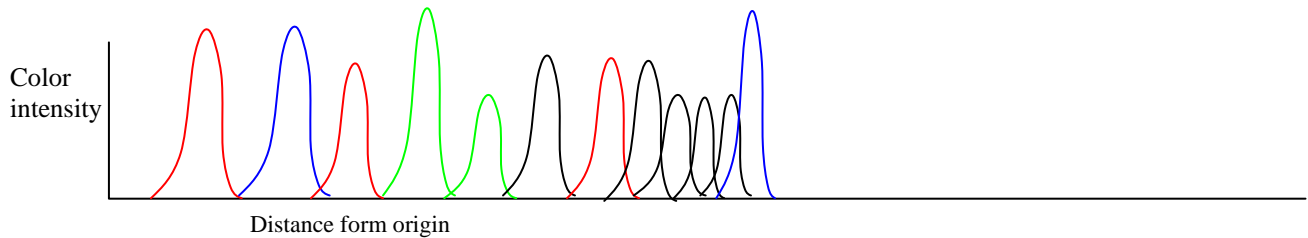


13. Now draw a plot that shows the intensity of color present as a function of the distance from the origin that includes each of the bands shown in question 10.



14. For each of the ten bands that you have plotted in question 12, indicate which of the four colors would be present at that distance from the origin.

15. Note that the information for the sequence of bases in a DNA molecule is present within the plot that you drew and labeled in question 12 and 13. In that case, the base sequence of DNA was already given to you (in question 2). Below is a sketch that shows data from an experiment that was aimed at determining the sequence of a molecule of DNA:



Given what you have learned about DNA sequencing and the information in question 7 (regarding the color associated with each ddNTP), write the sequence of bases that is present in the DNA molecule that resulted in the generation of the above data.

16. From the preceding exercise, you should recognize that in order to determine the sequence of bases in a DNA molecule the following is needed:

- many copies of the DNA molecule whose sequence of bases is to be determined
- a primer that will bind to the DNA molecule and enable DNA polymerase to start replication
- a mixture of dNTPs and color-labeled ddNTPs
- DNA polymerase
- Gel electrophoresis
- A detector that can detect the intensity and color at different locations on the gel.

The information that is generated by the detector is displayed in a way similar to what you see in question 14. You will now be given a real example of sequencing data from ADE1 (or ADE2) from a red strain of yeast. You will use the information to determine the sequence of bases and then to compare that sequence to the sequence of bases found in the white strain of yeast.

## **Student Exercise: FirstGlance**

This file contains two handouts explaining how to look at the impact of the *ade* mutations on the structure of the encoded enzymes. The first handout is for use with the dataset that contains the *ade1* missense mutation. The second handout is for use with the *ade2* nonsense mutation dataset.

In our labs we originally used Protein Explorer ([http://www.umass.edu/microbio/chime/pe\\_beta/pe/protexpl/](http://www.umass.edu/microbio/chime/pe_beta/pe/protexpl/)) to do these exercises. However, Protein Explorer requires a Chime plug-in, and on a Mac, an older operating system (OS9) and browser. In addition, several of the operations involved clicking through many windows. Thus, the handouts have been re-written to use FirstGlance, which accomplishes the same ends, but in a more easily accessible and useable interface.

## Using FirstGlance to look at the Ade1 Protein

Last week you found that in our representative red mutant that the *ADE1* gene contained a mutation that created a missense codon somewhere in the gene. But how serious is this mutation? It would be nice to be able to look at the protein and see where the change occurs in our mutant. Fortunately, someone has determined the three dimensional structure of the Ade1 protein. To view the structure we are going to use FirstGlance, which accesses a public protein structure database (PDB).

### To display structure:

Open Firefox (or other browser).

Go to FirstGlance at <http://molvis.sdsc.edu/fgj/>

Find the box that says “Enter a PDB Identification Code here.” Type in 1a48 and hit Go. This is the “catalog number” for the Ade1 protein.

The program will load and if all goes well you will see (eventually) a rotating molecule.

You are looking at the Ade1 protein backbone (and a couple of sulfate groups -one yellow atom and 4 red atoms –that was associated with the protein). If at any point you see there are LOTS of other red balls covering the molecule, hit the square button (upper left) that says “water” to eliminate all the red things, which are water molecules. You probably will not see them at this point. You can get rid of the sulfates by clicking the box next to “Ligands” to turn them off. Save memory by hitting the “Spin” button to turn off the spinning. Now you can put your mouse on a section of the molecule and click and hold – moving the mouse now rotates the molecule in whatever direction you desire. Observe the secondary structures that are present – lots of alpha helices are readily seen. Rotate the structure and see if you can find a cleft that might serve as the active site (place where the enzyme binds the intermediates to help them react with each other).

Near the bottom of the box at the upper right, click the box that says PDB. This takes you to a page that tells you more about the protein, including that it is called SAICAR Synthase (title, near top). This might be moderately reassuring if you remember that this was the name of the protein encoded by the *ADE1* gene. You can also see that the title of the paper describing this structure notes that the enzyme is involved in purine biosynthesis.

Return to the original window. What we want to do is to highlight the part of Ade1 that was altered in our mutant. Then we will be able to see if the alteration is in a critical part of the protein, such as the active site. Below is an alignment of part of the mutant and wild type Ade1 sequences to remind you what the mutation looked like:

```
Ade1 wild type: QFLRDWLTANKLNG  
Ade1 mutant:   QFLRDWPTANKLNG
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In the top box on the left, click the “find” link. This will open a text box in the lower left window. Type LEU267 since the amino acid that is altered in the mutant is L or leucine and it is the 267<sup>th</sup> amino acid in the chain. Hit return to start the search for this amino acid.



On the structure you can now see the position of the mutated amino acid – the atoms are shown in yellow. Remember that the ribbon structure represents the only backbone part of the amino acids, so the yellow leucine atoms that are in the side chain will not overlies the ribbon part of the structure, but rather project from it.

Rotate the structure around by positioning the mouse over it and dragging while holding the mouse button down. Does this amino acid participate in a structure close to the active site? Does this amino acid normally participate in an alpha helix? The latter question is relevant because proline (P) is an amino acid known as a “helix breaker.” A proline, because of its looped over side chain creates kinks in the chain making it incompatible with a helix structure. Thus, if the L (leucine) normally participated in an alpha-helix, this helix would be shortened or destroyed by a change to a P (proline).

**Given the position of the altered amino acid, would you expect the mutated protein to be active? Does your prediction agree with the result that these mutants cannot live without adenine? You can include this information in the final draft of your lab report.**

A few more explorations, just for fun: Hit the Composition link near the top of the left top window. This converts the ribbon structure to a beige spacefilling model that more accurately shows the position of the atoms and the space they occupy. You will need to turn off the display of the water (shown in blue) and the ligands (shown in green) by clicking the Water and Ligand boxes. It is hard to see the highlighted leucine in this view. Now chose the N->C Rainbow link. This colors the backbone in a rainbow so you can trace the chain from its start at the N terminus (blue) to the C terminus (red). Hit the Secondary Structure link. This will color the protein so that the alpha helices are pinkish “rockets” and the beta sheets are flat gold arrows. When you rotate the protein by dragging on it, you can see that the active site in SAICAR synthetase is a cradle made mostly of beta sheet. It is rather pretty, isn't it? The altered leucine is clearly part of an alpha helix.

## Using FirstGlance to look at an Ade2 Protein Homologue

Last week you found that in our representative red mutant that the *ADE2* gene contained a mutation that created a stop codon somewhere in the gene. But how serious is this mutation? It would be nice to be able to look at the protein and see how much of it remains in our mutant. Unfortunately, no one has determined the three dimensional structure of the Ade2 protein. What researchers normally do in this situation is to work with structures of related proteins, as it is very laborious to determine the structure of your particular protein if you don't have to. Luckily, we can view the structure of an Ade2-related protein, PurK, which is the bacterial version of the Ade2 protein. To view the structure we are going to use FirstGlance, which accesses a public protein structure database (PDB).

### To display structure:

Open Firefox (or other browser).

Go to FirstGlance at <http://molvis.sdsc.edu/fgij/>.

Find the box that says "Enter PDB Identification Code here." Type in 1b6r and hit the Submit button. This is the "catalog number" for PurK, the bacterial protein that is homologous to Ade2.

The program will load and if all goes well you will see (eventually) a rotating molecule.

You are looking at the PurK protein backbone (and a sulfate group -one yellow atom and 4 red atoms – that was associated with the protein). If at any point you see LOTS of other red balls covering the molecule, hit the square button (upper left) that says "water" to eliminate all the red things, which are water molecules. You probably will not see them at this point. Save memory by hitting the "Spin" button to turn off the spinning. Now you can put your mouse on a section of the molecule and click and hold – moving the mouse now rotates the molecule in whatever direction you desire. Observe the secondary structures that are present – lots of alpha helices are readily seen. Rotate the structure and see if you can find a cleft that might serve as the active site (place where the enzyme binds the intermediates to help them react with each other). The place where the sulfate is bound is believed to be part of the active site. Once you see the cleft you can get rid of the sulfate by clicking the box next to "Ligands" to turn the display of non-protein atoms off.

Near the bottom of the box at the upper left, click the box that says PDB. This takes you to a page that tells you more about the protein, including that it is called N5-CARBOXYAMINOIMIDAZOLE RIBONUCLEOTIDE SYNTHETASE, which might be moderately reassuring if you remember that some of these words (aminoimidazole) were present in the Ade2 protein's name – phosphoribosylaminoimidazole carboxylase or AIR carboxylase. This is yet another name for this enzyme. You can also see the title of the paper that described this protein's structure. In the box summarizing the paper it says that the enzyme "catalyzes the conversion of 5-aminoimidazole ribonucleotide (AIR), ATP, and bicarbonate to N5-CAIR, ADP, and Pi," showing that this enzyme is involved in converting AIR (the second red intermediate) to the next compound in the adenine biosynthesis pathway.

Returning to the original window. What we want to do is to highlight just the part of Ade2 that remains in our mutant. We know that the first part of the protein will be made, but how can we find out where it stops? We must compare the PurK protein sequence to our mutant Ade2 sequence. Below is an alignment of a small section of Ade2 with two other protein sequences. One is a general

“consensus” sequence of this region that is collated from looking at many species and determining the most common amino acid for each position. The other is from the bacterial PurK protein that is in the protein structure database we are viewing. The sequence in this region obviously varies quite a bit across species, but we are able to see that there are several amino acids in common (shown in bold). Lining these up shows us that the “E” that was changed to a stop in Ade2 (top line, boxed) lines up with an “F” in the bacterial version of this enzyme, PurK.

Ade2 :	<span style="border: 1px solid black; padding: 2px;">EKCDVLT</span> <b>IEIEHVDVPTL</b>
consensus :	AKCDV <b>ITYEFEN</b> VP <b>A</b> EAL
PurK	FQ <b>QSVITAEIER</b> WP <b>E</b> TAL

This F is the 41<sup>st</sup> amino acid in PurK. Thus, if the gene that encodes PurK had a nonsense mutation equivalent to what we saw in our *ade2* gene, the part of the protein that would still be made would be amino acids 1 through 40. The rest of the protein would not be made because the codon encoding this “F” amino acid would now be a stop codon.

To see how much of the protein the first 40 amino acids represents, click on the “Find” link in the top box on the left. This will open a text box in the lower left window. Type 1-40 to highlight just the part of the chain that would remain in our nonsense mutant. Hit return to start the search for these amino acids.

On the structure you can now see the positions of the amino acids that would still be produced in a mutant with a stop codon at position 41 – the atoms are shown in yellow. Remember that the ribbon structure represents the only backbone part of the amino acids, so the yellow atoms that are in the side chains will not overlie the ribbon part of the structure, but rather project from it. EVERYTHING except for this yellow region would all be absent due to the stop codon.

Rotate the protein around to get a sense of how much is gone in our mutant. **Does it seem like the active site cleft is intact? Would you expect this truncated protein to be active? Does your interpretation of the structure agree with the result that these mutants cannot live without adenine?**

A few more explorations, just for fun: Hit the Composition link near the top of the left top window. This converts the ribbon structure to a beige spacefilling model that more accurately shows the position of the atoms and the space they occupy. You will need to turn off the display of the water (shown in blue) and the ligands (shown in green) by clicking the Water and Ligand boxes. This view lets us clearly see most of the protein would be missing in the nonsense mutant. Now chose the N->C Rainbow link. This colors the backbone in a rainbow so you can trace the chain from its start at the N-terminus (blue) to the C-terminus (red). It’s rather pretty, isn’t it? As expected, the part of the protein that would still be made is the dark blue N-terminus.