

Aipotu I: Genetics & Biochemistry

Objectives:

- To reinforce your understanding of Genetics, Biochemistry, and Molecular Biology
- To show the connections between these three disciplines
- To show how these three approaches can be combined to give a complete picture of a biological phenomenon
- To figure out a complete explanation of a biological phenomenon
- To experience how scientists develop and test hypotheses

Introduction:

Aipotu (pronounced “ā pō too” – the word is “utopia” spelled backwards) is a simulated world where users can explore a biological phenomenon in terms of genetics, biochemistry, molecular biology, and evolution.

The Biological Phenomenon Under Study

In this lab, you will explore the biological mechanisms behind the expression of flower color in a hypothetical plant. These flowers can be white, red, orange, yellow, green, blue, purple, or black.

Scenario:

You are the chief biologist for a breeder of fine flowers. Your company sells seeds that customers plant in their gardens. Since most of your customers expect that the flowers will grow each year from seeds produced the previous year, you try to produce true-breeding plants whenever you can.

You’ve found a new species of flower with an attractive shape. You’ve collected four plants from the wild: two green, one red, and one white. Your customers would really like to have purple flowers from this plant. You set out to create a true-breeding purple flower.

Hypothesis Testing

In the three Aipotu labs, you will use a process much like that used by practicing scientists as they conduct research. Although this process almost never follows a formula, it often proceeds as follows:

1. **Observe Patterns.** Observe the natural world and look for patterns, exceptional events, etc. For example, you might observe that red flowers sometimes have white offspring.
2. **Develop hypotheses.** From the observations, you define testable hypotheses – statements or questions that can be addressed experimentally. Continuing the example, you might reasonably hypothesize that red is dominant to white.
3. **Test hypotheses.** You then set up experiments or observations that will collect data that bear on your hypothesis. In the example, you might cross pure-breeding white with pure-breeding red. If your hypothesis is correct, all the offspring will be red. If you get another result, your hypothesis is incorrect.
4. **Revise hypotheses as necessary.** If your results do not match your prediction, you need to revise your hypothesis and go to Step (3) again until they do match.

You have already been doing this informally in the VGL labs. You should note that this process never really ends – typically there is more to investigate once you’ve reached a satisfactory conclusion.

The “answer” vs the “point”

Although there is an answer – the complete model of color formation in these plants – and you can find it with the tools in Aipotu, *having* the answer is not as important as *finding* the answer. We could just tell you the answer, but that would leave out the major learning goals of this lab:

- Experiencing how scientific hypothesis testing works.
- Experiencing how you can use the tools of modern biology to completely understand a phenomenon.

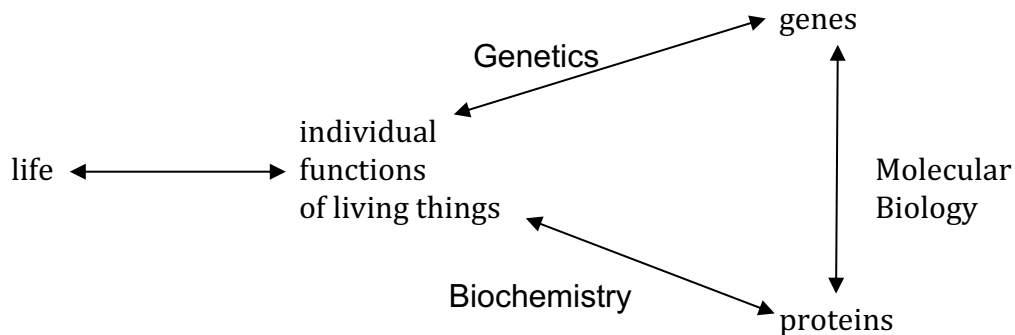
So, while we will work together to find ‘the answer’, the real *point* of the lab is the journey.

Unifying Three Parts of Modern Biology

The three major parts of this course are:

- **Genetics** = explaining biological phenomena in terms of genes. *How is flower color inherited?*
- **Biochemistry** = explaining biological phenomena in terms of proteins and other molecules. *How does protein sequence determine protein structure and color?*
- **Molecular Biology** = explaining the connection between genes (DNA) and protein. *How does the DNA sequence of the color gene lead to a particular color?*

The connections between these three fields of biology are shown below in a diagram credited to David Botstein (each of the arrows corresponds to “can be explained in terms of”, the words in this font are the different disciplines of biology):



Understanding any biological phenomenon requires information from all three of these disciplines. Throughout this course, you will re-visit this lab to create an increasingly complete picture of the phenomenon under study. Each time, you will add to the model of color production in these flowers.

Tools Available in Aipotu

For each of the three disciplines, there is a different tool in Aipotu that will allow you to explore the same set of creatures using a different set of techniques:

- **Genetics.** The flowers in this simulation are diploids. As with most flowers, they are all hermaphrodites (both male and female). With this tool, you can perform the following experiments:
 - *Cross any two organisms.* A new window will appear with the offspring of this cross.
 - *Self-cross any organism.* In this case, the single selected organism is both mother and father to the resulting offspring.
 - *Mutate any organism.* A new window will appear with a set of flowers that are mutant versions of the selected organism.
- **Biochemistry.** The color in these flowers results from the form(s) of pigment proteins present in an individual plant. With this tool, you can perform the following experiments:
 - *Examine the pigment proteins present in a plant.* The tool shows you the amino acid sequence and two-dimensional structure of the pigment proteins present in a given plant.
 - *Design your own proteins.* You can edit an existing protein sequence or type in an entirely new sequence. The program will then predict the two-dimensional structure of the resulting protein as well as its color. It will also predict the color resulting from the combination of any two proteins.
- **Molecular Biology.** The pigment proteins in these plants are produced by pigment protein genes. With this tool, you can perform the following experiments:
 - *Examine the pigment protein genes present in a plant.* The tool shows the DNA, pre-mRNA, mature mRNA, and protein sequences present in a given plant. You can explore the introns, exons, etc. of these genes.
 - *Design your own genes.* You can edit an existing DNA sequence or type in an entirely new sequence. The program will then predict the mRNA, protein sequence, two-dimensional structure of the resulting protein as well as its color. It will also predict the color resulting from the combination of any two proteins.
 - *Design your own plants.* You can save edited DNA sequences as new organisms for further study.

When you start Aipotu from the link on the On-Line Lab Manual (do not use the Aipotu app in the Dock), the web page will load the four starting flower types into the Greenhouse, you will then see a screen like this:

The screenshot shows the Aipotu web interface with several callout boxes explaining its components:

- Menus:** A box at the top left points to the 'Compare' and 'Greenhouse' buttons, stating: "These are the **Menus**; they apply to all the tools."
- Tabs:** A box at the top center points to the 'Genetics', 'Biochemistry', 'Molecular Biology', and 'Evolution' tabs, stating: "These **Tabs** select the tool you will be using."
- Greenhouse:** A box at the top right points to the 'Greenhouse' panel on the right side, stating: "The **Greenhouse** contains the starting set of organisms."
- Upper Workpanel:** A box in the center points to the upper workpanel, stating: "Upper Work Panel. Results of crosses, etc. will appear here." Below this text are buttons for 'Cross Two Organisms', 'Self-cross One Organism', and 'Mutate One Organism'.
- Lower Workpanel:** A box in the center points to the lower workpanel, stating: "Lower Work Panel. Results of crosses, etc. will appear here." Below this text are buttons for 'Cross Two Organisms', 'Self-cross One Organism', and 'Mutate One Organism'.
- History List:** A box at the bottom left points to the 'History List' panel, stating: "The **History List** stores the results of each of your experiments. These results can be sent to the upper or lower panes (see later)." The 'History List' panel contains buttons for 'Upper Window' and 'Lower Window'.

The interface also features a 'Greenhouse' panel on the right containing an 'Add...' button and four flower icons labeled 'Green-1', 'Green-2', 'Red', and 'White'.

Each organism is shown as a flower:



The color of the picture shows the color of the flower. These flowers can be white, red, orange, yellow, green, blue, purple, or black. When any organism is selected, the white background turns light blue to show that it has been selected.

The next sections of this manual will show you the various tools and the tasks that you will need to carry out.

First: Notebook Review

To be sure that you have all the information you need for this lab, with your lab partners, go over the notes you took while doing the SPOC and from lecture and be sure you have the information listed below. You should fill in any gaps in your notes so everyone in your group has all they need. You can check these items off as you go.

- How do each of the following bonds/interactions work to give proteins their shape?
 - Ionic bonds
 - Hydrogen bonds
 - Hydrophobic interaction

You will then discuss these with your TA as a class to clarify any issues that remain.

Part I: Genetics

Tasks: (specific questions can be found on page 8 and the Aipotu Worksheet at the end of this section of the manual)

- Determine how color is inherited in these flowers. **NOTE:** the color is controlled by one gene only.
 - Determine the colors of the alleles present in the original set of organisms.
 - Which alleles are dominant?
 - Which alleles are recessive?
 - How do the alleles combine to produce the overall color of the plant?
- Construct a purple organism to demonstrate your understanding of this process.

Using the tool:

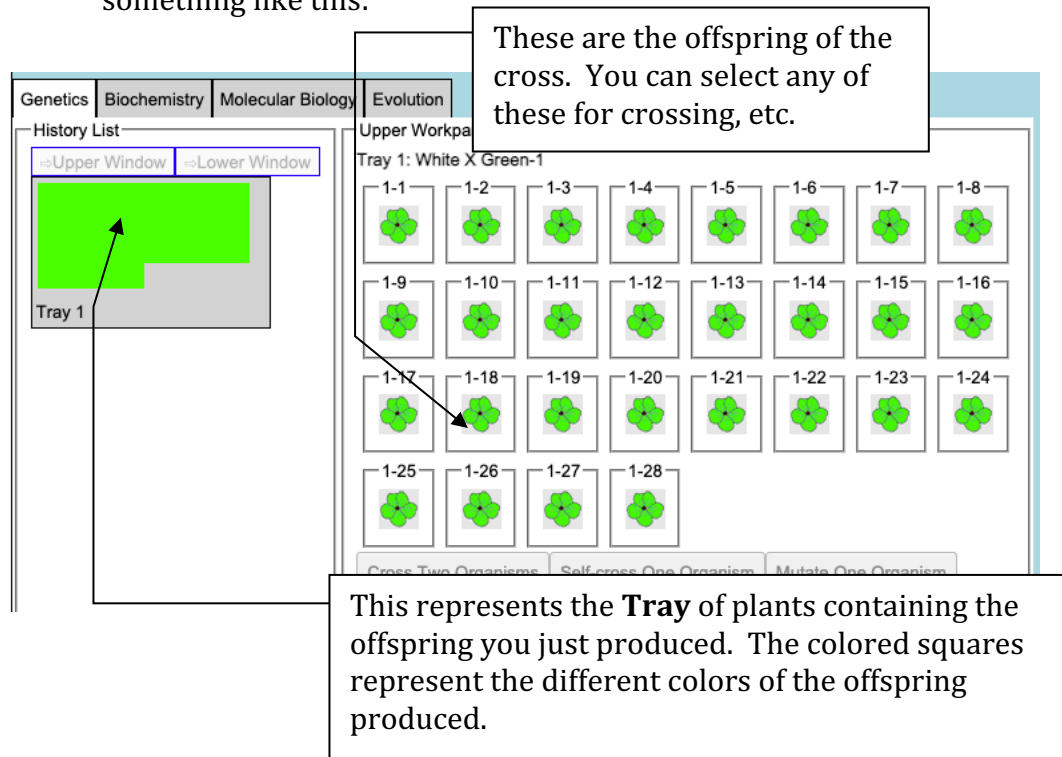
You can switch to this tool by clicking the “Genetics” tab near the top of the window.

There are three kinds of experiments you can perform with this tool. The following sections use examples to show you how to do each; you will need to devise your own experiments to carry out the tasks above.

1) Cross Two Organisms. Suppose that you wanted to cross Green-1 and White:

- 1) Click on Green-1 and then on White in the **Greenhouse**. The backgrounds of both should turn light blue to show that they have been selected. The “Cross Two Organisms” buttons in the **Upper** and **Lower Work Panels** should be activated.

2) Click the “Cross Two Organisms” button in the **Upper Work Panel**. You should see something like this:



If you click on the **Tray** in the **History List**, its border turns red and you can then:

- **Send to Upper Panel:** Sends this **Tray** to the **Upper Panel** so you can cross those organisms.
- **Send to Lower Panel:** Sends this **Tray** to the **Lower Panel** so you can cross those organisms.

II) *Self-cross a single Organism.* Suppose that you wanted to self-cross one of the offspring in Tray 1:

- 1) Select any one organism from Tray 1 in the **Upper Work Panel**. You can de-select an organism by clicking on it. When you have only one organism selected, the “Cross Two Organisms” buttons will be grayed out and the “Self-Cross One Organism” and “Mutate One Organism” buttons will be activated.
- 2) Click the “Self-Cross One Organism” button in the **Lower Work Panel**. You should see something like this (since offspring are generated by random choice of parental alleles, you will likely see slightly different numbers of red and white offspring):

You should see something like this:



The offspring of this self-cross are in the **Lower Work Panel**. Note the addition of Tray 2 to the **History List**.

At this point, there are several other things you can do:

A) If you find an interesting organism, you can save it to the **Greenhouse**:

- 1) Click the “Add...” button at the top of the **Greenhouse**.
- 2) You will be prompted to give the organism a name. Give it a descriptive name and click “OK”. You will see your new organism appear in the **Greenhouse**. You can now access it using the other tools in this program.
- 3) At this point, the organism is saved in your browser (so, if you re-start the browser, it will be there), but you cannot transfer it to another computer. To save the contents of the **Greenhouse** to a file you can e-mail to yourself or share with others, click on the **Greenhouse Menu** and select “Save Greenhouse to File...”.

B) You can cross or mutate any of the organisms visible in the **Greenhouse, Upper Work Panel, or Lower Work Panel**.

C) You can bring any **Tray** from the **History List** to a **Work Panel** by clicking the **Tray** and clicking the “=> Upper Window” or “=> Lower Window” button.

IMPORTANT NOTES:

(1) This software is under development. Please treat it gently and be patient. Please report any bugs to your TA.

(2) If you're using a lab computer, if you log out, you will lose all the organisms you've saved in the Greenhouse. If you want to save them, save the Greenhouse using the Greenhouse menu and e-mail yourself the resulting file.

Specific Tasks to do with this tool:

- a) How many different alleles are there? Which colors do they produce? It will be useful to use multiple-allele notation like this: C^R = red; C^G = green, etc.
- b) How do these alleles interact – what colors result when they're combined?
- c) Using the symbols you have developed, give the genotypes of the four starting organisms.
- d) Using this knowledge, construct a purple flower.
- e) Can you construct a pure-breeding purple flower with the starting set of organisms?

How to go about accomplishing these tasks

Follow the procedure from page (1) – look for patterns, make hypotheses, test hypotheses, revise hypotheses. Today, we will not write them out formally, but you should be thinking in these terms for the Aipotu II lab later in the semester.

Enter your data into the “Aipotu Worksheet” at the end of this section.

You will turn it in to your TA who will check it off and save it for the Aipotu II lab.

Part II: Biochemistry I

Tasks:

Work together as a class to:

- Explain, in terms of the proteins present, the interactions between the alleles you found in Part I.
 - Why is the color phenotype of some pigment proteins dominant while others are recessive?
 - How do the pigment proteins combine to produce the overall color of the plant?
- Determine the differences in amino acid sequence between the proteins produced by the alleles you found in Part I.
- Begin to determine how the amino acid sequence of a pigment protein determines its color – you will complete this task in the Aipotu II lab later in the semester.

As in real science, these tasks are too big to be solved by one group alone. If you think of real research as solving an enormous jigsaw puzzle, each researcher works on only one little corner of the puzzle. Scientists publish papers and present findings at conferences in order to connect the corners of the puzzle that each is working on.

Important Note: It is always important to keep in mind, the ‘scientist’s mantra’: always be asking yourself “How could I be being fooled by this?” To continue the example from the previous page, consider the following:

Suppose that the long thin protein were red, you might congratulate yourself that you had found the connection between shape and color. However, what if the real mechanism is that proteins containing arginine are red and your long thin protein just happened to be made with arginine. The red color would be fooling you into thinking you had it right.

How do you avoid this trap? Even the best scientists sometimes fall into traps like this. The answer is to *always be thinking of alternative explanations for your results*. In the case above, one long thin red protein does not mean that “long & thin = red”. You have to collect more data: proteins that aren’t long and thin; long and thin proteins with different amino acids; etc.

In this part of the lab, more than in the first part, it is the *process of science* rather than the *answer* that is most important. You will collaborate as a class to solve this scientific problem.

The data you collect and the conclusions you reach will be essential parts of Take Home Exam 7. Since this exam takes place later in the semester, **you should be sure to take good and thorough notes on this lab especially on the Worksheet.**

Using the tool:

Once you start Aipotu, you can switch to the tool for this section by clicking the “Biochemistry” tab near the top of the window. You will see something like this:

This is a reference for the names and properties of the amino acids.

- The single-letter code is shown below the three-letter code.
- White circles are hydrophilic; gray are intermediate; and black are hydrophobic.
- A red (-) indicates a negatively-charged side chain
- A blue (+) indicates a positively-charged side chain
- A green (*) indicates a side chain that can make a hydrogen bond.

This part of the program uses the one-letter code for the 20 amino acids:

<u>Amino Acid</u>	<u>3-letter code</u>	<u>1-letter code</u>	<u>Mnemonic</u>
Alanine	Ala	A	<u>A</u> lanine
Arginine	Arg	R	a <u>R</u> ginine
Asparagine	Asn	N	asparagi <u>N</u> e
Aspartic acid	Asp	D	aspar <u>D</u> ic acid
Cystine	Cys	C	<u>C</u> ystine
Glutamine	Gln	Q	<u>Q</u> -tamine
Glutamic Acid	Glu	E	glu-t <u>E</u> -amic acid
Glycine	Gly	G	<u>G</u> lycine
Histidine	His	H	<u>H</u> istidine
Isoleucine	Ile	I	<u>I</u> soleucine
Leucine	Leu	L	<u>L</u> eucine
Lysine	Lys	K	lysin <u>K</u>
Methionine	Met	M	<u>M</u> ethionine
Phenylalanine	Phe	F	<u>F</u> enylalanine
Proline	Pro	P	<u>P</u> roline
Serine	Ser	S	<u>S</u> erine
Threonine	Thr	T	<u>T</u> hreonine
Tryptophan	Trp	W	t <u>W</u> ptophan
Tyrosine	Tyr	Y	t <u>Y</u> rosine
Valine	Val	V	<u>V</u> aline

- Click in the **Amino Acid Sequence Box** at the top of the **Upper Folding Window**. Type a short sequence of letters and you will see a short amino acid sequence appear in the window. This tool converts the single-letter code to the three-letter code automatically.
- Click the “FOLD” button and a two-dimensional version of your amino acid sequence will appear in the **Folded Protein** window.

There are several important things to note about this folding process:

It is the same as you used in the Protein Investigator on the SPOC. This is a highly-simplified model of protein folding. It is not intended to predict the correct structures of any proteins; it is designed to illustrate the major principles involved in that process. The important features of proteins that this software retains are as follows:

- Amino acids have side-chains of varying hydrophobicity, charge, and hydrogen bonding capacity.
- The amino acids are connected in an un-branched chain that can bend.
- Hydrophobic amino acids will tend to avoid the water that surrounds the protein; hydrophilic amino acids will bind to the water.
- Amino acids that can form hydrogen bonds will tend to form hydrogen bonds if they can.
- Positively-charged amino acids will tend to form ionic bonds with negatively-charged amino acids if they can.
- Like-charged amino acids will repel each other if they can.
- Ionic interactions are stronger than hydrogen bonds, which are stronger than hydrophobic interactions.

Even though this software provides some important insights into protein folding, you should always keep in mind that this is an approximation. The most important "gotcha's" to be aware of are:

- This program folds proteins in 2-dimensions only.
- This program treats all amino acids as equal-sized circles.
- This program models an environment where disulfide bonds do not form.
- This program folds the protein based on the interactions between the side chains only.
- This program does not model secondary or quaternary structure.
- This program assumes that all side chains with hydrogen bonding capability can bond with each other.

These simplifications are necessary for two reasons. The first is technical: it turns out to be extremely difficult to predict the full 3-d folded structure of a protein given only its amino acid sequence. As of the writing of this lab manual, it takes a super-computer several days to predict the fully-folded shape of even a small protein like lysozyme. Even then, the predictions don't always match known structures. Given the computers we have in the Bio 111 labs, it might take years....

The second reason is educational. Proteins are complex 3-dimensional molecules; thus, it can be hard to find your way around when inside one. Likewise, it would be very difficult to visually compare two protein molecules to observe the effects of changes to their amino acid sequence. It would be easy to miss the forest (the forces that control protein structure) for the trees (the tiny details of the structures).

For these reasons, we will use this simplification. It retains the properties of amino acids that are important for this lab while being simple and fast.

There are three kinds of experiments you can perform with this tool. The following sections use examples to show you how to do each; you will need to devise your own experiments to carry out the tasks from the previous page.

1) Examine the Pigment Proteins Present in an Organism from the **Greenhouse**. This simulates extracting the pigment protein(s) produced by the two alleles of the pigment protein gene that an organism possesses, displaying their two-dimensional structures, and displaying their colors.

1) Click on the **Green-2** organism in the **Greenhouse**. You should see this:

The **Green** organism contains two alleles of the pigment protein gene. Each of these alleles produces a different protein. One of these proteins is shown in the **Upper Folding Window**; it is a blue-colored protein as shown by the blue square next to the “Color:” label. The other protein is shown in the **Lower Folding Window**; this is yellow-colored protein. The combined color of the two proteins is green as shown by the **Combined Color** in between the two **Folding Windows**.

II) Compare the amino acid sequences of two pigment proteins. This aligns the two amino acid sequences so that the highest number of matching amino acids is obtained and then finds the remaining differences.

- 1) Click on the **Green-2** organism in the **Greenhouse**. You should see that the **Upper Folding Window** shows a blue protein and the **Lower Folding Window** shows a yellow protein.
- 2) You can compare the amino acid sequence of these two proteins by clicking on the “Compare” menu and choosing “Upper vs. Lower”. A window will appear showing the differences between the two sequences. This is shown below:

```
Upper Sequence:  Met Ser Asn Arg His Ile Leu Leu Val Tyr Cys Arg Gln
Difference:      Met Ser Asn Arg His Ile Leu Leu Val Trp Cys Arg Gln
Lower Sequence:  Met Ser Asn Arg His Ile Leu Leu Val Trp Cys Arg Gln
Close
```

This shows that the only difference is that, in the upper (blue) protein, amino acid 10 is tyrosine, while in the lower (yellow) protein, amino acid 10 is tryptophan.

⇒ You can also copy the sequence of a particular protein as a “Probe” to compare to other sequences using the options in the **Compare** menu. You can then **Compare** a sequence to the one in the clipboard.

III) Edit a Protein Sequence or Create a New Protein Sequence and Determine its Two-Dimensional Structure and Color. You can edit the sequence in either of the **Amino Acid Sequence** boxes and click the “Fold” button to predict the two-dimensional structure and color of the protein. The tool will also give the color that results from the combination of the colors in the Upper and Lower windows.

For example, click anywhere in the “Tyr” corresponding to amino acid 10 in the **Upper Amino Acid Sequence** box. Click the “delete” key and that amino acid will disappear. Type an “L” (the one letter code for leucine) and the amino acid sequence should be:

Met Ser Asn Arg His Ile Leu Leu Val Val Cys Arg Gln

Click the “FOLD” button in the **Upper Folding Window** (or click the return key). You will see that the color of the new protein is white as shown by the “Color:” in the **Upper Folding Window**. You should also notice that:

- the “Combined Color” at the center of the window is now yellow.
- there is now an entry in the **History List** with your new protein. The background of **History List** entry is white to show the color of this protein.

You can also click the “Load Sample Protein” button. This will load a sample amino acid sequence that folds to a white-colored protein with a shape that is similar to many colored proteins.

If you click an entry in the **History List**, you can view it in either the upper or lower panel.

Specific Tasks for this section

Work as a class to:

- a) What are the differences in the amino acid sequences of the proteins produced by the alleles you define in Part I? Hint: use the **Compare** menu to find the difference(s) between the amino acid sequences.
- b) How do the colors combine to produce an overall color? How does this explain the genotype-phenotype rules you found in Part I?
- c) Start to work on these. You will finish this later in the semester:
 - What features of the amino acid sequence make a protein pigmented?
 - What features of the amino acid sequence make a protein a particular color?
 - Which proteins are found in each of the four starting organisms?
 - Using this knowledge, construct a purple protein.

Hints:

- It may be useful, before formulating any hypotheses, to look for *patterns* in the data. Which features do colored proteins have in common that uncolored proteins lack?
 - Try comparing the amino acid sequences of proteins with different colors.
 - Here are some additional interesting sequences to try:
 - FFFFFFFRRRRRR
 - RRRFFFFFFRRR
 - KKKKKKLLLLLLF
 - KKKKKKLLLLLL
 - SLQLNITMEVDFW
 - EEEWWWWWWEEE
- Remember that shape is also important – try altering proteins that are colored to see how the shape influences the color.
- Scientists, including yourselves, often find it useful to use mutation to study phenomena like this. Go to **Genetics** and make some mutants. Save any ones with interesting colors to the **Greenhouse**. Switch back to **Biochemistry** and look at the proteins they have.

Procedure:

1. Compare the proteins found in the starting strains to answer questions (a) and (b) on the following pages.
2. Your TA will assign your group one particular colored protein to study. Compare its sequence and shape to the “sample protein” that you get by clicking the **Load Sample Protein** button on one of the **Folding Windows** and then choosing from the **Compare** menu.
3. A representative from each group will come to the board to describe the sequence and shape difference(s) between their protein and the sample. Note that each subsequent group should relate their findings to the previously-presented data.
4. Based on these data, as a class, make several *specific* hypotheses that can be tested.
5. Each group should work on one or more of their hypotheses and take careful notes.
6. Your TA may stop for a mini-symposium to share data and design new hypotheses.
7. You will then be able to complete parts (d) through (f).

Enter your data in the Aipotu Worksheet.

Preparing for the Take Home Exam

Take Home Exam 7 will be, in part, based on this lab. You should look at the exam on Blackboard before you leave lab today. You may want to use some of the remaining time in lab to prepare for the exam.

Bio 111 Aipotu Worksheet

Name _____

Name _____

Name _____

This is a group effort for a group grade.

TA & Sect. _____

Score _____/30

This is due at the end of lab today.

1) Genetics

a) Give a complete genetic model for the color alleles you've found in the four starting Greenhouse strains using the table below. It will be useful to use multiple-allele notation like this: C^R = red; C^G = green, etc. Also, since the interaction is not easy to describe using terms like "co-dominance", make up a chart of all possible genotypes and the resulting phenotypes. (4 pts)

<u>allele</u>	<u>Color</u>	<u>Genotype</u>	<u>Phenotype</u>

(b) Using the symbols you gave in (a), give the genotypes of the four starting strains. (4 pts)

<u>Strain</u>	<u>Genotype</u>
Green-1	
Green-2	
Red	
White	

(c) Have your TA check off that you have constructed a purple flower. For full credit, you must be able to explain to your TA **why** the flower is purple. (2 pts)

2) Biochemistry

(a) Give the colors of the proteins found in each of the four starting organisms. (4 pts)

Upper Protein Color

Lower Protein Color

Green-1

Green-2

Red

White

(b) Using the "Sample" protein as a reference, give the amino acid differences between the "Sample" and each of the proteins found in the Greenhouse strains. (4 pts)

allele

color

amino acid sequence differences from "Sample"

(c) How is the color of the proteins determined by their structure? Specifically, what are the features of the protein's shape and amino acid sequence that cause a protein to have a particular color? (4 pts)

(d) How do the colors combine to produce an overall color? How does this explain the genotype-phenotype rules you found in Genetics? (4 pts)

(e) Show your TA that you have made a purple protein. In order to be prepared for the Take Home Exam, you need to explain to your TA *why* it is purple. (4 pts)



