BioBits®

Protein Structure and Function

BioBits®
Protein Structure and Function
KT-1910-03

Version: 1.2
Release: March 2026

Contents

At a glance

Lab overview

Today, your students get to be protein engineers looking to improve the function of fluorescent proteins!

Students investigate the relationship between protein structure and function through a hands-on lab using fluorescent proteins. They analyze protein sequences and structures, apply basic protein engineering concepts, and express fluorescent proteins to test their predictions, gaining insight into protein folding, function, and engineering applications.

TECHNIQUES

Micropipetting
Cell-free protein expression
Fluorescence visualization

TOPICS

Protein structure and function

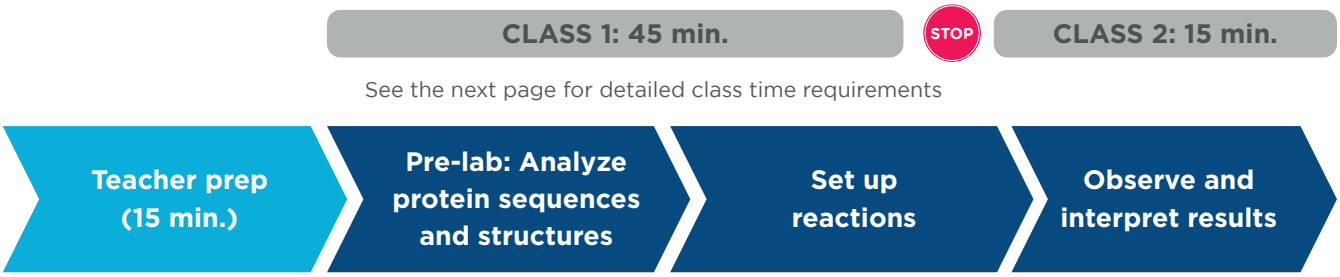
GRADE LEVEL

General high school
Advanced high school
College

Required lab skills

- Students must be proficient in accurately pipetting 5 µl of liquid.
- Instructional videos, worksheets, and free activities to help students build micropipetting skills can be found at https://www.minipcr.com/micropipetting/

Planning your time



See the next page for detailed class time requirements

See page 6 for details

Technical support

If you have any questions about implementing this activity, contact support@minipcr.com

Class time requirements

		Time required
Pre-lab	Analyze protein sequences and structures	30 minutes (can be completed outside of class)
Hand-on day 1	Set up BioBits reactions	15 minutes
Hand-on day 2*	Observe and interpret results	15 minutes

* Protein fluorescence becomes visible approximately 8 hours after Day 1 setup, and remains observable for at least 1 week.

Materials needed

Supplied in kit (KT-1910-03)

- Kit contains reagents for eight lab groups.
- If kept in the freezer, reagents can be stored for 6 months after receipt. If kept in the refrigerator, reagents can be stored for 1 month after receipt.
- Plastic tubes for distributing reagents to individual groups and pipette tips are sold separately.

Contents	Provided	Required per group	Storage
BioBits pellets in PCR strip tubes	Four 8-tube strips	One 4-tube strip	Freezer
DNA samples for protein expression • DNA FP1 • DNA FP2 • DNA FP3	100 µl each	10 µl each	Freezer
Nuclease-free water	100 µl	10 µl	Freezer
Handheld UV flashlight	8	1	Room temp.

Required equipment

Item	Recommended quantity
P51™ Molecular Fluorescence Viewer or other blue light illuminator with an orange filter	1 per group
2-20 µl adjustable micropipette and tips	1 pipette per group

Other materials supplied by user

- Plastic tubes for dispensing reagents (1.5 or 0.2 ml tubes can be used)
- Disposable laboratory gloves
- Protective eyewear
- Fine-tipped permanent marker

Teacher prep

Protective gloves and eyewear should be worn for the entirety of this experiment.

Overview

The table below provides an overview of the teacher prep, and the subsequent pages provide detailed instructions.

Prep	Time required	Timeline
Dispense reagents	10 minutes	Can be completed up to one week before use
Split BioBits strip tubes	< 5 minutes	Best if performed just before use

Dispense reagents

- Reagents can be dispensed up to one week in advance and stored in the refrigerator until use.
- This kit provides sufficient reagents for eight lab groups.

Materials needed

From the lab kit (stored in the freezer):

- DNA FP1
- DNA FP2
- DNA FP3
- Nuclease Free Water

Note: leave the BioBits pellets in the sealed bag until just before use.

Supplied by user:

- Plastic tubes for dispensing reagents (1.5 ml or 0.2 ml tubes can be used)
- 2-20 µl micropipette and tips
- Fine-tipped permanent marker

1. Thaw reagents by placing tubes at room temperature.

2. Collect the liquid at the bottom of each tube. Either spin briefly in a microcentrifuge or shake the liquid down with a flick of the wrist.

3. When you open each tube, check for liquid stuck inside the cap. If necessary, put the cap back on and repeat step 2.

4. For each lab group, dispense reagents into labeled plastic tubes as shown in the table below. 1.5 ml or 0.2 ml plastic tubes can be used.

Reagent	Volume per group	Label tube as
DNA FP1	10 µl	1
DNA FP2	10 µl	2
DNA FP3	10 µl	3
Nuclease Free Water	10 µl	W

5. If you are dispensing the reagents more than 24 hours before class, store the tubes in the refrigerator until use. Dispensed reagents can be stored in the refrigerator for up to one week.

Split BioBits strip tubes

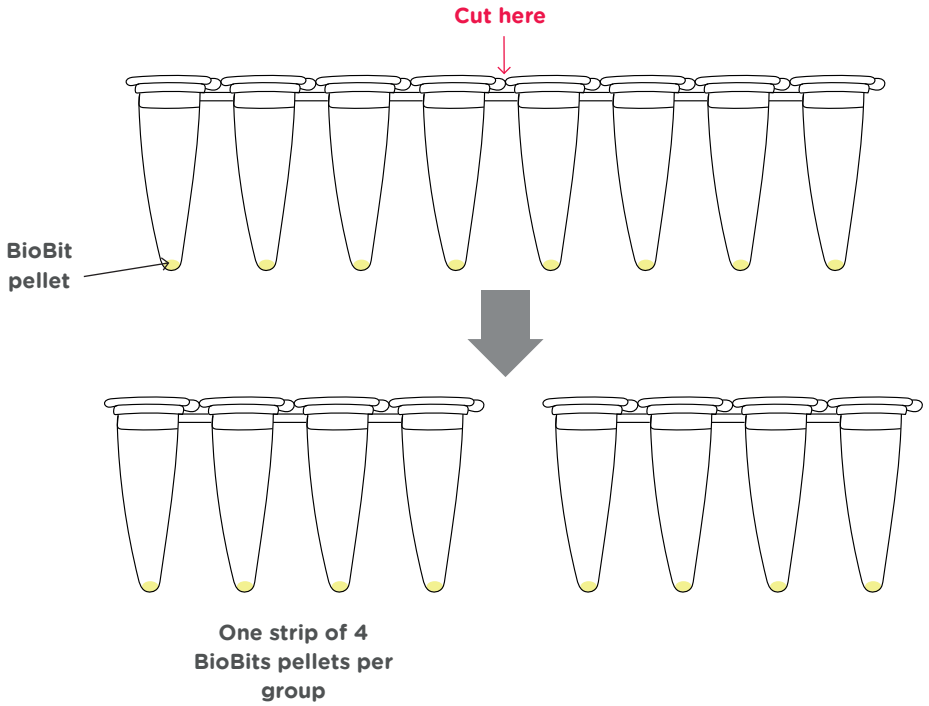
Materials needed

From the lab kit (stored in the freezer):
- BioBits pellets in strip tubes

Supplied by user:
- Razor or scissors

1. Remove the BioBits pellets from the airtight bag. Do this as close to the start of the lab as possible.

2. Use a razor or scissors to separate each strip of 8 BioBits pellets into two strips of 4 tubes.



3. Place any unused BioBits pellets in an airtight bag with the included desiccant card and store them in the freezer. Properly stored BioBits pellets will be stable for six months from the time of shipment.

Student workstation setup

Every lab group should have:	
BioBits pellets	1 half-strip (4 tubes)
DNA FP1 (tube 1)	10 µl
DNA FP2 (tube 2)	10 µl
DNA FP3 (tube 3)	10 µl
Water (tube W)	10 µl
2-20 µl variable volume micropipette and tips	
P51 Molecular Fluorescence Viewer or other blue light illuminator with an orange filter	
Handheld UV flashlight (provided in lab kit)	

Student guide

Background information

Proteins: function follows form

Proteins are essential tools for life. They perform functions as varied as generating movement, transmitting signals, providing structural support, and catalyzing enzymatic reactions. Every protein has a specific function, which is dependent on its shape, or structure—much the same way a hammer has a blunt end for hitting nails, and a screwdriver has a tip shaped to fit specific screws. For example, the structure of hemoglobin **(Figure 1)** provides four specific sites, shown in dark red, that bind to oxygen to transport it in blood. On the other hand, actin's long fibrous-like shape allows it to provide scaffolding support to give cells structural strength. And the glucose transporter is shaped like a tube to allow glucose molecules to move in and out of cells.

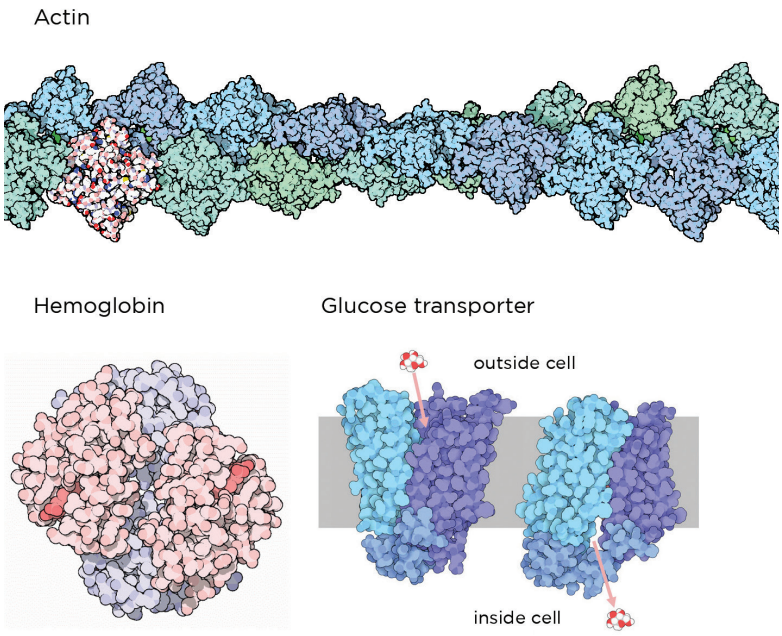


Figure 1: Examples of proteins. Top: actin, bottom left: hemoglobin, bottom right: glucose transporter. Images from David S. Goodsell and RCSB PDB.

No matter a protein's size or shape, almost all organisms make proteins with a combination of just the same 20 amino acids. These basic building blocks are connected in a long chain that can then be arranged into all sorts of intricate three-dimensional structures. The DNA sequence of a given gene determines the order of amino acids in that long chain. Through the process of transcription, DNA gets copied into RNA. Then, during the process known as translation, this genetic information is translated from RNA into a string of amino acids. As a protein is made, this string of amino acids will fold into a specific three-dimensional shape. Generally, an amino acid chain will always fold into the same shape—the most stable three-dimensional structure possible for that amino acid chain.

This three-dimensional shape is determined by the amino acids and their properties. Every one of the 20 amino acids has a different chemical structure, which gives it a unique set of characteristics, such as size, charge, and hydrophobicity. For example, amino acids with a charge will be attracted to other amino acids of the opposite charge. In the cytoplasm, amino acids that are hydrophobic, or tend to repel water, will be more likely to be found buried inside of a folded protein, while amino acids that are hydrophilic, or tend to mix with water, will be more likely to be found on the outside

of the folded protein. The size of an amino acid will also play into how it fits and interacts with other amino acids in the protein.

When scientists talk about how proteins twist and fold, they tend to consider four levels of structure (**Figure 2**):

- Primary structure: during translation, the protein is assembled one amino acid at a time, like beads on a thread, to form a linear chain. The order of the amino acids in this chain is referred to as a protein's primary structure.
- Secondary structure: hydrogen and oxygen atoms in the backbone of the amino acids will interact with those in nearby amino acids by forming hydrogen bonds, stabilizing the chain into local three-dimensional structures. The two most common secondary structures are:
    - alpha helix, where the amino acids twist into a spiral, and
    - beta-pleated sheet, where the amino acid chain winds back and forth in relatively straight lines to form parallel strands. These parallel strands together are known as a pleated sheet.
- Tertiary structure: as the amino acid chain continues to fold, more distant amino acids will come into contact to form various interactions, including hydrogen bonds, covalent bonds, and ionic interactions. This three-dimensional shape, consisting of all the amino acids in the chain, is referred to as a protein's tertiary structure. For many proteins, this is their final structure.
- Quaternary structure: for some proteins, multiple tertiary structures will further interact to form a larger, multi-unit structure. For example, hemoglobin **(Figure 1)** is made of four independent amino acid chains (shown as the two red and the two blue regions), each folded into a tertiary structure, that come together to form the final functional protein. This final structure, made of multiple tertiary structures, is bound together through hydrogen bonding and other electrostatic interactions to form the final functional protein.

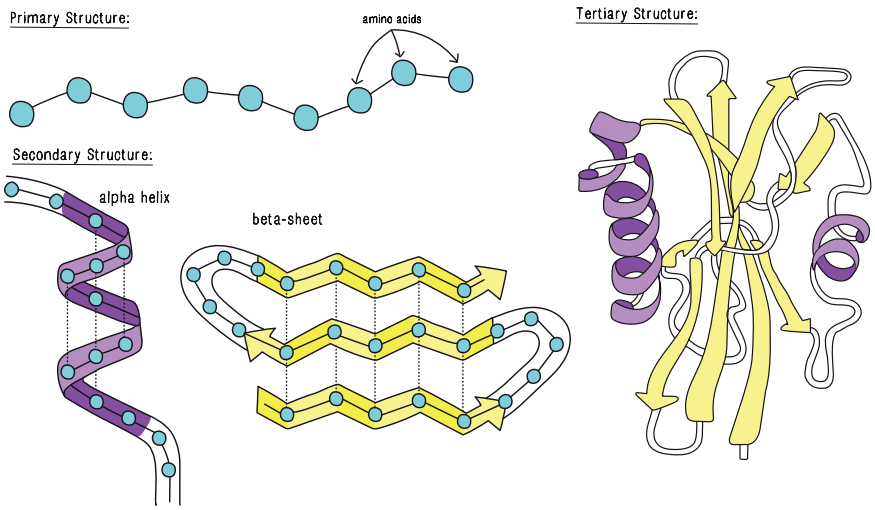


Figure 2: A protein may be analyzed at four levels of structure. Examples of primary, secondary, and tertiary protein structure are shown here.

The vast majority of what we know about protein structures and what they look like comes from the field of X-ray crystallography. In X-ray crystallography, scientists crystallize millions or even trillions of copies of a specific protein into a lattice-like framework. They then shoot this crystal structure with an x-ray beam. By recording the resulting pattern of how the X-rays scatter on a detector,

scientists can deduce the precise atomic structure of the protein. Essentially, the X-rays are used to make very fine shadows of the protein, so that by analyzing these fine shadows, we can deduce the architecture of the protein—down to the level of single atoms. All the structures shown in this guide are based on data collected using X-ray crystallography.

Using what we know to design new proteins

Proteins perform all sorts of important functions, and many of these functions have been harnessed by scientists for applied uses in industry. For example, certain enzymes have been used for decades in laundry detergents to make the detergent effective even in cold water. Yet, for all their varied shapes and functions, proteins that already exist in nature can only perform a limited set of functions. Now, imagine a world where scientists could design a protein to do exactly what they wanted—an enzyme that could catalyze chemical reactions to produce biofuels or one that could capture the excess of carbon dioxide in the atmosphere to combat climate change. Imagine designing an antibody that specifically targeted cancerous cells or an antigen that could be used in a vaccine against an emerging new virus. You've just entered the field of protein engineering, where the eventual goal is to improve existing protein structures to carry out specific functions more efficiently or new structures that are not currently found in nature.

To create these new or improved functions, scientists will either 1) change some of the amino acids in a protein of known structure or, 2) select new structures with the desired function from a pool of randomly generated ones. We'll focus on the first approach, termed 'rational design'.

Protein engineers often select a protein that functions similarly to their target function, giving them a significant head start in designing a protein that meets their criteria. They can then make educated guesses on how to modify it. This is similar to how a chef may use an old recipe as a starting point when creating a new dish. The key is to remember that function follows form. For example, if we want to create an enzyme to bind and interact with a specific substrate, we will need to design the enzyme's shape such that its three-dimensional structure perfectly fits the substrate. The catch is that we cannot directly modify a protein's tertiary structure. We can only directly modify the primary structure—that is, the amino acid sequence.

When substituting amino acids to design a new protein, it is important to consider the chemical properties of amino acids. Changing an amino acid into one that has different properties can drastically change the way the protein folds and functions. Because there are many different possible interactions between the amino acids within a protein sequence, correctly predicting a protein's structure is extremely complicated. To help determine what kind of impact amino acid substitutions may actually have, scientists can create computational models that aim to predict how a newly designed protein sequence will fold and what its final three-dimensional shape will be.

Starting in the 1990s, as computers became more and more powerful, protein engineers began to make progress in analyzing amino acid interactions and predicting how proteins will fold. Recent advancements in artificial intelligence have made remarkable strides in this field, demonstrating the ability to predict many protein structures and opening up exciting possibilities in protein engineering that weren't achievable before. But still, even with the current computing power, not every protein structure can be modeled. And even if the structure can be predicted, sometimes small changes in structure can have unexpectedly large effects on a protein's precise function. Actual experiments are still needed to confirm the predictions made by computers.

At the end of the day, a protein engineer's job often involves at least as much tinkering as it does rational design—once we find out where the functionally important amino acids reside within a protein's structure, we can modify them through trial and error to obtain new and exciting functions.

Today's lab

Your goal today is to help a group of protein engineers design a new green fluorescent protein (GFP) that they can use in their research. Fluorescent proteins are naturally occurring in many marine organisms like jellyfish, sea anemones, and corals. Scientists often use them because when illuminated with certain wavelengths of light these proteins fluoresce, or glow in bright colors. Scientists can use fluorescent proteins to visually track cell components in live cells and animals. Unfortunately, many GFPs fluoresce best when exposed to the high energy rays of a UV light source, but exposure to UV light can cause DNA mutations. That is why today you will help scientists engineer new fluorescent proteins that can be optimally excited by safe blue light.

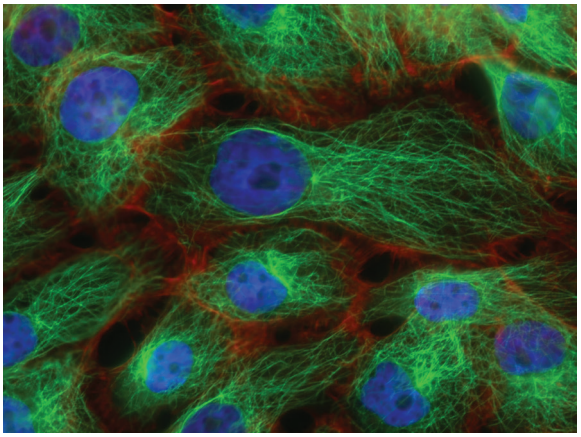


Figure 3: Scientists have harnessed the function of fluorescent proteins to visualize structures and components in cells.

When designing the proteins you will work with, the protein engineers took two different approaches. The first was to analyze an existing GFP and identify potential modifications to make it fluoresce optimally under blue light instead of UV. The second was to examine other fluorescent proteins found in nature that are already excited by blue light. Using these as a starting point, the engineers designed a new protein that may work in a research setting.

Each of these two approaches resulted in a new protein. The protein engineers would like you to determine which new sequence will best suit their research needs described above. First, you will use protein structure prediction models to examine what these novel sequences might look like once folded into a three-dimensional protein. Next, you will analyze the sequences in detail and compare them to other known sequences to help make your own predictions about how these proteins will function and fluoresce under different types of light. Finally, you will actually make these fluorescent proteins and experimentally test their functions!

Typically, protein engineers take the DNA sequences they have designed and insert them into living cells where they will undergo transcription and translation to express the corresponding protein. Today, you will instead be using a cutting-edge cell-free technology called BioBits® that allows you to quickly express the proteins from DNA without having to use living cells. Once you have made your proteins, you will expose them to both UV and blue light and observe how the proteins fluoresce under each type of light. You will also note the color of the resulting emitted fluorescence. From your analysis and experimental results, you will report back to the researchers whether they have succeeded in producing a green fluorescent protein that fluoresces under blue light.

Pre-lab: Analyze protein sequences and structures

Most known fluorescent proteins share a very similar tertiary structure **(Figure 4)**. They are shaped like a barrel, with the outside cylinder of the barrel made of beta-pleated sheets. Through the middle of the barrel is an alpha helix. Near the center of that alpha helix, three amino acids form a structure known as the chromophore. The chromophore is the portion of the protein most directly responsible for fluorescence, but the protein's entire structure is necessary for a fluorescent protein to glow.

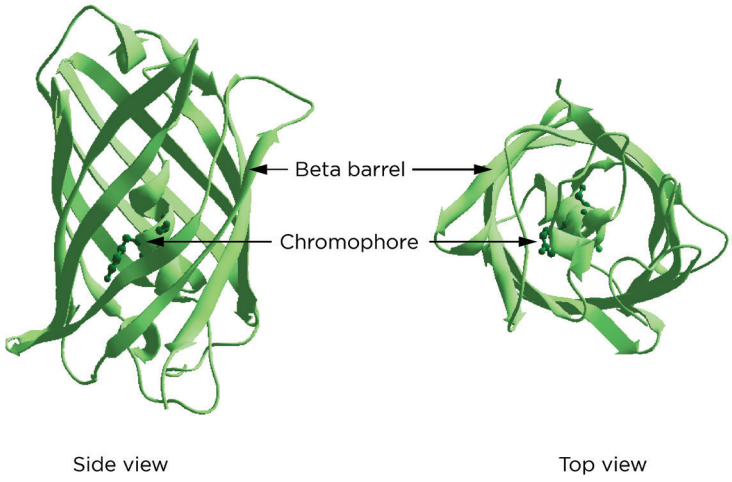


Figure 4: Example outline of a green fluorescent protein from both the side and top view. The barrel made of beta-pleated sheets and the chromophore near the central alpha helix are labeled.

To fluoresce, a protein must first absorb energy from light through the chromophore. The excited chromophore then releases that energy back out as light of a longer wavelength. But not all fluorescent proteins are the same. Some absorb mostly UV light, others blue, green, or yellow light. The type of light that the protein fluoresces best under is known as its excitation wavelength. And the color of fluorescence emitted also differs across proteins. Some fluoresce green, others blue, yellow, orange, or red. The type or fluorescent color of light that the protein gives off or emits is known as its emission wavelength. The different colors that proteins fluoresce (emission) and wavelengths of light that they respond to (excitation) are due to small differences in the proteins' structures.

Remember that your goal is to obtain a green fluorescent protein (GFP) that fluoresces optimally under blue light instead of UV light. Your engineering team started with a known GFP called FP1 that fluoresces best under UV light. You must analyze two candidate protein sequences, FP2 and FP3, for their potential to replace the original FP1 (see **Table 1**).

Table 1: Protein sequences for the lab

Name	Description
FP1	Original GFP sequence (fluoresces green best under UV light)
FP2	Candidate GFP sequence (modified from FP1)
FP3	Candidate GFP sequence (engineered from fluorescent proteins that fluoresce best under blue light)

You will analyze the proteins' primary, secondary and tertiary structures, and then, you will perform a detailed analysis of the amino acids in the chromophore region. From your analysis, you must predict which protein will best meet the engineering team's goal. You will then test your predictions experimentally by producing these proteins in BioBits® cell-free reactions.

Analyzing primary structure

Listed below are the entire amino acid sequences for the three proteins, written out using one-letter amino acid abbreviations. Here, we have lined up the amino acids of FP1 with both FP2 and FP3 so that you may better compare their primary structures. The entire primary structures are between 227 and 240 amino acids long, so sequences are broken up over several lines. In all the primary structures, the three amino acids that make up the chromophore are marked in bold. If two amino acids are identical between the primary structures, they are highlighted in yellow.

```
FP1: MVSSGEDIFSGLVPILIELEGDVNGHRFSVRGEGYGDASNGKLEIKFICT
FP2: MVSSGEDIFSGLVPILIELEGDVNGHRFSVRGEGYGDASNGKLEIKFICT

FP1: TGRLPVPWPTLVTTLSYGVQCFAKYPEHMRQNDFFKSAMPDGYVQERTIS
FP2: TGRLPVPWPTLVTTLTYGVQCFAKYPEHMRQNDFFKSAMPDGYVQERTIS

FP1: FKEDGTYKTRAEVKFEGEALVNRIDLKGLEFKEDGNILGHKLEYSFNSHY
FP2: FKEDGTYKTRAEVKFEGEALVNRIDLKGLEFKEDGNILGHKLEYSFNSHY

FP1: VYITADKNRNGLEAQFRIRHNVDDGSVQLADHYQQNTPIGEGPVLLPEQH
FP2: VYITADKNRNGLEAQFRIRHNVDDGSVQLADHYQQNTPIGEGPVLLPEQH

FP1: YLTTNSVLSKDPQERRDHMVLVEFVTAAGLSLGMDELYKS
FP2: YLTTNSVLSKDPQERRDHMVLVEFVTAAGLSLGMDELYKS
```

FP1 and FP2

As you can see, FP1 and FP2 are nearly identical. There is only a single amino acid difference engineered into the protein. Specifically, the serine (S) in the chromophore of FP1 was changed to a threonine (T) in FP2.

1. The chromophore is the portion of the protein most directly responsible for fluorescence. Would you expect a change in the chromophore to be more likely or less likely to affect the protein's function than a change to an amino acid outside the chromophore?

2. FP1 and FP2 are 240 amino acids long. If only a single amino acid is different, what percentage of amino acids are the same between the two proteins? Show your work.

FP1 and FP3

```
FP1: MVSSGEDIFSGLVPILIELEGDVNGHRFSVRGEGYGDASNGKLEIKFICT
FP3: -----MSVIKQVMKTKLHLEGTVNGHDFTIEGKGEGKPYEGLQHMKMTVT

FP1: T-GRLPVPWPTLVTTLSYGVQCFAKYPEHMRQNDFFKSAMPDGYVQERTI
FP3: KGAPLPFSVHILTPSHMYGSKPFNKYPADIP--DYHKQSFPEGMSWERSM

FP1: SFKEDG--TYKTRAEVKFEGEALVNRIDLKGLEFKEDGNILGHKL---EY
FP3: IFEDGGVCTASNHSSINLQENCFIYDVKFKGVNLPPDGPVMQKTIAGWEP

FP1: SFNSHYVYITADKNRNG-LEAQFRIRHNVDDGSVQLADHYQQNTPIGEGP
FP3: SVETLYV-------RDGMLKSDTAMVFKLKGGGHHRVDF--KYTYKAKKP

FP1: VLLPEQHYLTTNSVLSKDPQERRDHMVLVEFVTAAGLSLGMDELYKS
FP3: VKLPEFHFVEHRLELTKHDKDTTWDWWEAAEGHFSPLPKALP
```

When comparing FP3 and FP1, you will notice that there are far more differences. You will also notice that the primary structures are different lengths. FP3 is only 227 amino acids, whereas FP1 is 240 amino acids. A computer program has optimally aligned these sequences. To align sequences that are different lengths, spaces that don't exist in the actual protein need to be introduced. When you see a "-", it means that a space has been added so that the two amino acid sequences can be aligned properly.

We know that a protein's primary structure determines its secondary and tertiary structures. Looking at the FP1 and FP3 alignment, it is clear that the primary structure of FP3 is very different from that of FP1. In fact, only 55 of the 227 amino acids in FP3 seem to match an amino acid in FP1.

3. By comparing these primary structures, do you think you would be able to say how those differences affect the structure and function of FP3? Or do you think it would be very hard for you to predict? Explain your answer.

4. FP3 is 227 amino acids long. If 55 amino acids are the same between FP1 and FP3, what percentage of amino acids in FP3 match corresponding amino acids in FP1? Show your work.

Analyzing secondary and tertiary structure

To understand how a protein will function however, we need to know something about its structure. Predicting secondary structures based on primary structure alone can now be done quite reliably using computers. But predicting tertiary structure from primary structure or secondary structure alone is quite difficult and generally unreliable even with the best models.

Luckily, computer algorithms are quite good at recognizing protein sequences that are similar to other proteins with a known structure. The computer can then predict the structure of the new protein through comparison. This is called homology modeling.

Below, we have done just that. All three protein amino acid sequences were put through homology modeling software and the resulting structures are shown below. The computer recognized these proteins sequences as similar to another known proteins and predicted their structures accordingly. You will first compare the predicted secondary and tertiary structures of FP1 and FP2. Then you will compare FP1 and FP3.

In **Figure 5**, you can view FP1 and FP2 from two angles. The top row shows a "side view" of the protein, then below, the protein is turned 90° for a "top-down" view of the protein. In each case, you can observe both secondary and tertiary structure.

Observing secondary structure: In these diagrams, yellow arrows represent beta-sheets, while purple spirals

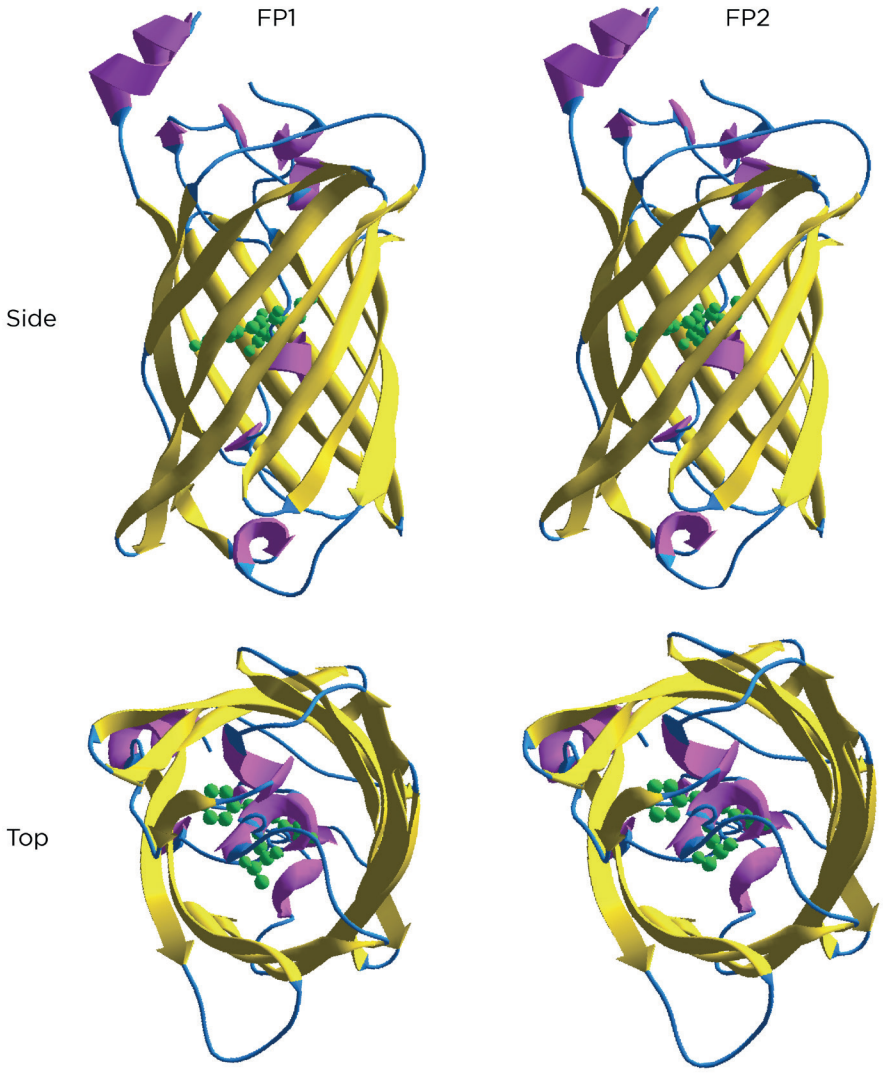


Figure 5: Predicted three-dimensional structures of FP1 and FP2 from the side and top views. Yellow arrows represent beta-sheets. Purple spirals represent alpha helices. The chromophore region is colored green.

depict alpha helices. Areas with no regular predicted secondary structure are depicted as blue lines.

Observing tertiary structure: The beta-sheets form the outer barrel of the protein. The alpha helices are found through the center of the barrel and also at the top and bottom of the barrel. (Again, regions that do not form a known secondary structure are colored blue.) Finally, the predicted chromophore is colored green and is shown as a ball and stick model in the center of the barrel. Ball and stick models show the arrangement of all the atoms in an amino acid.

From the similarities between other known proteins and the predicted structures, the computer algorithm identified all three proteins as having tertiary structures consistent with fluorescent proteins.

FP1 and FP2 show no obvious differences in their predicted secondary or tertiary structures. If you look very closely, you may be able to see the single amino acid difference in the ball and stick model of the chromophore.

5. Look closely at the "top-down" view. Can you identify the difference in the chromophore region? Put a star (*) next to any differences you see between the two models.

6. Review the two proteins' primary structures once again (page 17). After reviewing the primary structures, does it surprise you that the tertiary structures are so similar? Why or why not?

FP1 and FP3

FP1 and FP3 showed a lot of differences in their primary structures. Now see how those differences relate to their secondary and tertiary structures in **Figure 6**.

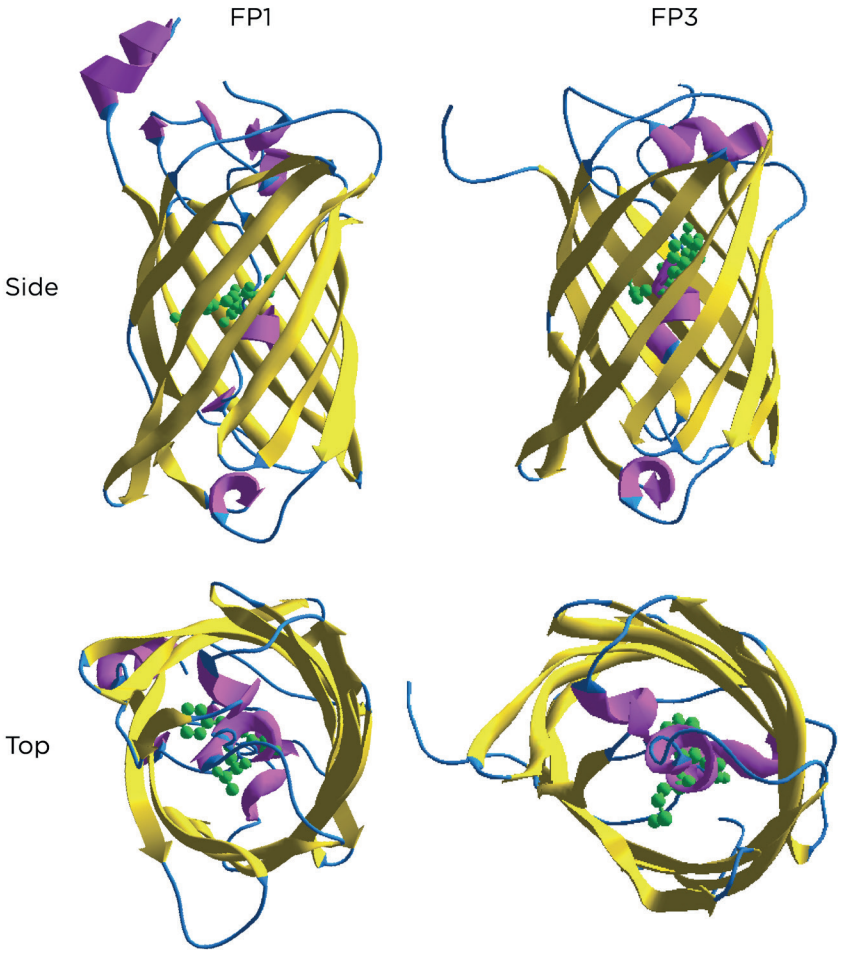


Figure 6: Predicted three-dimensional structures of FP1 and FP3 from the side and top views. Yellow arrows represent beta-sheets. Purple spirals represent alpha helices. The chromophore region is colored green.

7. Can you identify differences between the secondary structures of FP1 and FP3, even if minor? See if you can draw a circle around at least two places where the secondary structures of FP1 and FP3 are different. As a reminder, these are places where the yellow arrows or purple spirals don't match. Note what the differences are on your paper.

8. Can you find any differences between their tertiary structures, even if minor? Try to draw arrows pointing to at least three differences between the models of FP1 and FP3 and note what they are next to your arrows.

9. Circle the bolded word that completes the sentence the best.

   (Primary/Secondary/Tertiary) structure determines both **(primary/secondary/tertiary)** and **(primary/secondary/tertiary)** structures. But sometimes **(similar/different)** primary structures can lead to very **(similar/different)** secondary and tertiary structures.

Making final predictions using homology

Looking at the predicted secondary and tertiary structures of our proteins, it is clear that we are working with fluorescent proteins. The computer program we used to model their structures recognized them to be most similar to other fluorescent proteins whose structures are already known. From that, we can see that the three proteins we are analyzing have the barrel shape with a central alpha helix common to fluorescent proteins.

To more specifically predict how each protein will function, we will focus on the region surrounding the chromophore for clues. We will compare this 25-amino-acid section to several other known fluorescent proteins.

Table 2 lists the three proteins, FP1, FP2, and FP3, along with the corresponding partial amino acid sequences for four other fluorescent proteins, the organism they are derived from, the type of light that corresponds to their excitation wavelength (blue light or UV light), and color of the fluorescence that is emitted in response. This table is reproduced on page 25 in a format you may cut out to more easily perform your analysis. In both tables, the chromophore for each protein is marked in bold.

We'll use this information to make predictions of how the different proteins might fluoresce.

Table 2: Known protein sequences

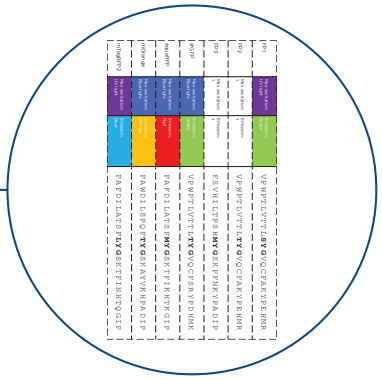
Protein Name	Organism	Excitation wavelength	Color of emitted fluorescence	Protein sequence (chromophore marked in bold)
FP1		UV light	Green	VPWPTLVTTL**SYG**VQCFAKYPEHMR
FP2		?	?	VPWPTLVTTL**TYG**VQCFAKYPEHMR
FP3		?	?	FSVHILTPSH**MYG**SKPFNKYPADIP
1. sfGFP	Aequorea victoria (jellyfish)	Blue light	Green	VPWPTLVTTL**TYG**VQCFSRYPDHMK
2. equaRFP	Entacmaea quadricolor (sea anemone)	Blue light	Red	FAFDILATSF**MYG**SKTFIKHTKGIP
3. mOrange	Discosoma sp. (coral anemones)	Blue light	Orange	FAWDILSPQF**TYG**SKAYVKHPADIP
4. mTagBFP2	Entacmaea quadricolor (sea anemone)	UV light	Blue	FAFDILATSF**LYG**SKTFINHTQGIP

10. Draw a box around the amino acids that are the same across all seven proteins.

11. As we've seen from the previous analysis, there can be differences in primary structure that do not lead to changes in three-dimensional structure. But certain amino acids seem to remain the same across different proteins. This suggests those particular amino acids are critical for the correct function of the protein.
    a. Knowing this, compare how similar the chromophore region is compared to the other amino acids in the sequence. Are there any other amino acids that are the same across all the proteins?

    b. If an amino acid is the same across all proteins, what might that suggest about its importance for the proper function of fluorescent proteins?

For the following questions it may be easier to use the cutout activity provided on page 25. If you have not cut out the activity, do so now.



See page 25

We've mentioned before that the chromophore region (written in bold) is the region mainly responsible for fluorescence. Let's look at the chromophore region in these four known proteins (sfGFP, equaRFP, mOrange, and mTagBFP2).

12. Let's look at chromophore regions. Which known protein sequence(s) has/have the same three amino acids in their chromophore as our candidate proteins? Record your answers in the "Proteins with identical chromophore(s)" column of **Table 3**.

13. Amino acids outside the chromophore can also have an important role in the function of the protein. For each of our unknown proteins, identify the known protein sequence(s) that seem the most similar to it. Record this in the "Most similar known sequences(s)" column in **Table 3**.

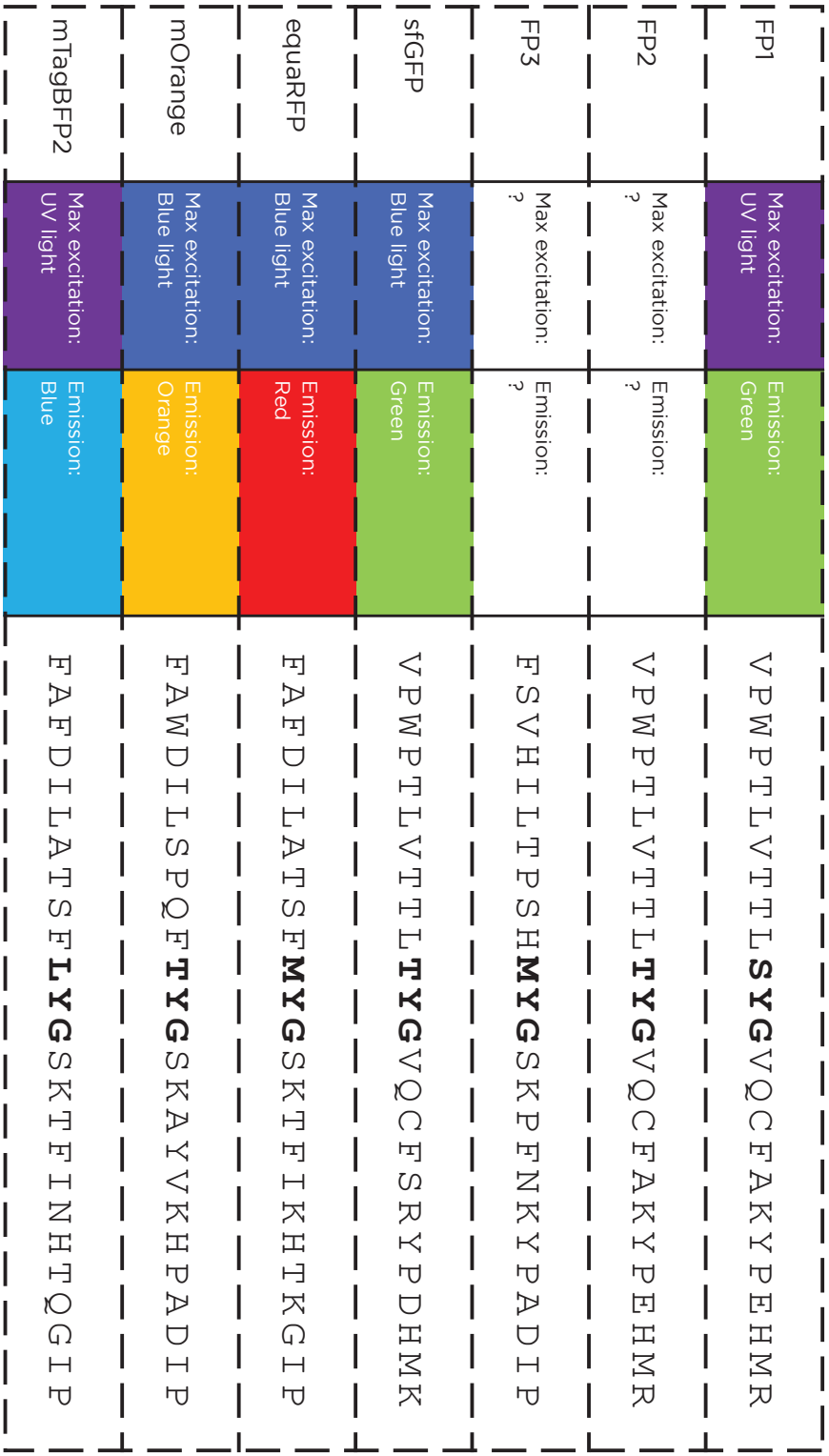
14. Based on your answers to both questions 3 and 4, what do you think the excitation light and the emitted color will be for each of the unknown proteins in the experiment? Fill out **Table 3** with your predictions and justify your answers. FP1 has already been filled out, as we already know its fluorescent properties.

Table 3: Comparisons and Predictions

Protein	**Protein(s) with identical chromophores**	**Most similar known sequence(s)**	**Predicted excitation light**	**Predicted color of emitted fluorescence**
FP1	none	sfGFP	UV or blue light: UV	Color: Green
FP2			UV or blue light:	Color: Justification:
FP3			UV or blue light: Justification:	Color: Justification:

15. As a reminder, the goal of the researchers is to identify a new fluorescent protein that glows green and is best excited by blue light. This is difficult to do with just sequence analysis, and we will have to test our predictions to know for sure. But looking at your predictions in **Table 3**, which protein, FP2 or FP3, do you think is most likely to suit these requirements? Justify your answer.

Homology modeling paper cutout



Printed here are the three fluorescent proteins from this experiment plus four other fluorescent proteins for comparison, as described on page 22. The 25 amino acids listed here come from the region surrounding the chromophore. As before, the three amino acids that make up the chromophore are written in bold.

Cut out the seven proteins along the dotted lines of the table so that you may better compare them individually.

Student lab protocol

Protective gloves and eyewear should be worn for the entirety of this experiment.

Day 1

1. Number your BioBits tubes FP1, FP2, FP3, C.

2. Gently tap tubes on the table to collect pellets at the bottom.

3. Carefully remove the strip of lids. We recommend pushing the strip of lids from the front side of the tubes in a rolling motion. Watch a short video demonstrating this technique here: https://links.minipcr.com/open_biobits.

How to pipette when working with BioBits

- Touch your pipette tip to the side of the tube and dispense the liquid, then tap the tube on the tabletop to collect the liquid at the bottom of the tube.
- Do not touch your pipette tip to the BioBits pellet.
- Do not pipette up and down to mix.

4. Using a fresh pipette tip for each addition, add reagents to each BioBits pellet according to the table below:

	FP1	FP2	FP3	C (control)
Reagent	5 µl DNA FP1 (tube 1)	5 µl DNA FP2 (tube 2)	5 µl DNA FP3 (tube 3)	5 µl water (tube W)

5. Close the caps on the tubes. When closed correctly, you should feel the caps "click" into place.

6. Make sure all the liquid is at the bottom of the tube. If liquid is stuck on the sides of the tubes, shake it down with a flick of the wrist or spin it down briefly in a microcentrifuge.

7. Observe the BioBits reactions in a P51 Viewer or other blue light illuminator with an orange filter. Dimming the lights in the room can make it easier to observe the fluorescence.

8. Record your **blue light** observations in the "Time 0" section of Table 4 on page 33.

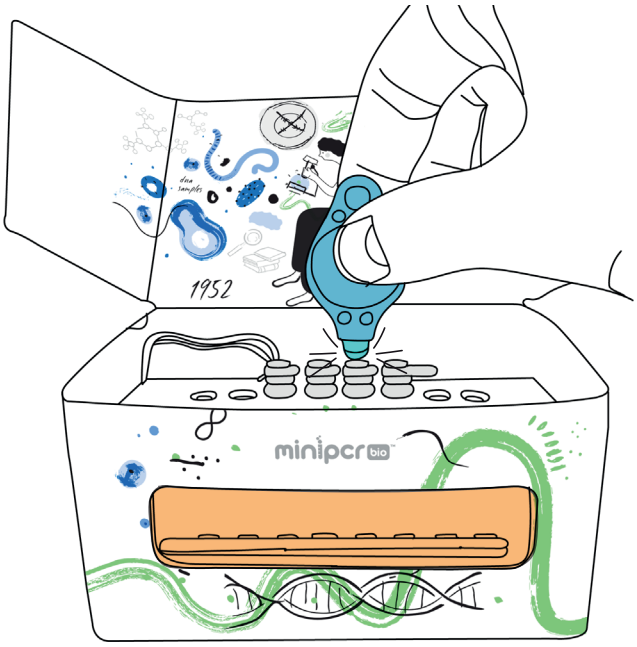
9. Turn off the P51's blue light.

10. Shine the UV flashlight straight down the cap of each tube in the P51 and observe each sample through the orange viewing window.
Caution: Be careful not to shine the UV light into anyone's eyes.

11. Record your **UV light** observations in the "Time 0" section of Table 4 on page 33.

12. Leave your samples at room temperature overnight.

    Optional stopping point: The BioBits reactions are stable at room temperature for several days.



Day 2

13. Observe the BioBits reactions again in a P51 Viewer or other blue light illuminator with an orange filter. Dimming the lights in the room can make it easier to observe the fluorescence.

14. Record your **blue light** observations in the "Day 2" section of Table 4 on page 33.

15. Turn off the P51's blue light.

16. Shine the UV flashlight straight down the cap of each tube in the P51 and observe each sample through the orange viewing window.
Caution: Be careful not to shine the UV light into anyone's eyes.

17. Record your **UV light** observations in the "Day 2" section of Table 4 on page 33.

Pre-lab questions

Review

1. The _____ structure of the protein plays a major role in determining its function.

2. The background section talked a lot about the structure and function of proteins. Ultimately, what contains the information for how a protein is built?

3. Name one similarity and one difference between an alpha helix and a beta-sheet secondary structure.

   a. Similarity:

   b. Difference:

4. If you are told that the amino acids in a protein are ordered: isoleucine, proline, alanine, threonine, cysteine, valine. What level of structure did we just describe to you? Explain your answer.

5. If you are told that two amino acids from different regions of the protein form a covalent bond, what level of structure does that describe? Explain your answer.

6. Name one potential benefit of being able to design a new protein from scratch.

7. Which level of structure is it easiest for protein engineers to change directly? Explain why.

8. What properties of an amino acid will determine how that amino acid interacts with other amino acids?

9. What is the advantage of starting with an existing protein to modify, instead of designing one from scratch?

Critical thinking

10. Look back at the amino acids listed in question 4: isoleucine (I), proline (P), alanine (A), threonine (T), cysteine (C), valine (V).
    a. Using those same six amino acids, design what you think is a different primary structure than the one listed there. Use the one-letter amino acid abbreviations to make things easier.

    b. Explain why your answer represents a new primary structure.

    c. You just changed the primary structure of the protein. Could this change also affect other levels of structure? If so, which ones? Explain your answer.

11. Imagine that you are redesigning a protein that is an enzyme. Consider the following three amino acids from this hypothetical protein:

    - An arginine (R) (a large positively charged amino acid) located in the active site of the enzyme (the place on the enzyme that interacts with the substrate).
    - A tyrosine (Y) (a large hydrophobic amino acid) located near the center of the three-dimensional structure of the protein.
    - A threonine (T) (a small hydrophilic amino acid) located on the outside of the enzyme far away from the active site.

    a. Propose changing just one of the three amino acids above to a new amino acid in a way that would very likely have a large effect on the function of the enzyme. You may describe the change generally (e.g., bigger to smaller, keep the charge the same, etc.) or suggest a specific amino acid if you are able.

    b. Explain why you think the change you suggested would have a large effect on the function of the enzyme.

    c. Propose changing just one of the three amino acids above to a new amino acid in a way that you think would be likely to have very little effect on the function of the enzyme. You may describe the change generally (e.g., bigger to smaller, keep the charge the same, etc.) or suggest a specific amino acid if you are able.

    d. Explain why you think the change you suggested would have little effect on the function of the enzyme.

Using computational thinking

Using the 20 standard amino acids, the total possible primary structures that could exist is equal to 20^n, where n is the number of amino acids in the sequence. For example, if you have an amino acid sequence that is just two amino acids long, there would be 20^2 possible amino acid sequences. Because $20^2 = 400$, there are 400 unique sequences that are two amino acids long.

12. How many possible proteins can you make that are just five amino acids long? You should use a calculator, but show your work.

13. The FP1 used in this lab is 240 amino acids long. How many other 240 amino acid proteins could theoretically be made? You should use a calculator, but show your work.

14. Beta-galactosidase, a common enzyme that breaks down sugars, is composed of about 1,020 amino acids. How many other 1,020 amino acid proteins could theoretically be made? You should use a calculator, but show your work.

15. What do the answers to the previous three questions tell you about how many different proteins could possibly exist?

Advanced discussion questions

16. Your sequence analysis looked at 25 of the more than 200 amino acids in this lab activity's fluorescent proteins. This subset was chosen to include the chromophore region and surrounding amino acids. Why do you think this region was chosen for analysis?

17. Look back at the primary structure alignments (pages 17 and 18). Based on this information, are there other regions that you think may be especially important for maintaining the structure and function of fluorescent proteins? If so, how did you recognize those regions?

18. Refer back to the homology exercise (**Table 2**, page 22). Imagine you wanted to make a red fluorescent protein that would fluoresce under UV light. Select a protein from this table to start with and suggest which amino acid modifications you would try first. (Hint: Use the other proteins in the table to help guide your answer.)

    It's OK if you are unsure about your answer. Remember, a lot of protein engineering is making educated guesses. It is more important that you justify your choices.

    a. Starter protein:

    b. Amino acid(s) modifications:

    c. Justify your answer:

Post-lab questions

Interpreting results

1. Record your observations in the table below by describing the fluorescence in each tube at each observation time point.
    - You should compare the experimental tubes FP1, FP2, and FP3 to the control tube. You only added water to the control tube, so any fluorescence you see in this sample is baseline fluorescence from the BioBIts pellets themselves.
    - Record observations on:
        - The color of the fluorescence
        - The brightness of the fluorescence (bright vs dim): To help decide how bright the fluorescence is, you can compare the brightness of each tube to the brightness of the other tubes. You can also compare how bright a tube looks under blue vs. UV light.

Table 4: results

Time 0 observations				
	Tube FP1	Tube FP2	Tube FP3	Control (water only)
	Blue light excitation			
Color				
Brightness				
	UV excitation			
Color				
Brightness				
Day 2 observations				
	Tube FP1	Tube FP2	Tube FP3	Control (water only)
	Blue light excitation			
Color				
Brightness				
	UV excitation			
Color				
Brightness				

2. Review the predictions you made in **Table 3** (page 24) and summarize them in one sentence in the "Predictions" column below. Review the observations you made in **Table 4** (page 33) and summarize them in one or two sentences in the "Observations" column below.

Table 5: Predictions vs. Observations

Sequence	Predictions	Observations
FP2		
FP3		

3. How well did your predictions match your observations for each protein?
   a. FP2:

   b. FP3:

4. Why was the control tube important in this experiment? If it were omitted, what incorrect conclusions could have been made?

Critical thinking

5. In making your predictions for this lab, you compared FP2 and FP3 to the known protein FP1 and several other fluorescent proteins. Overall, how helpful do you think primary, secondary and tertiary structure analysis were for predicting the function of FP2 and FP3?
   a. Primary:

   b. Secondary:

   c. Tertiary:

6. If I gave you a protein you knew nothing about (not a fluorescent protein) and asked you to predict its function, which do you think would be most helpful to analyze, primary, secondary or tertiary structure? Justify your answer using evidence from the lab.

CER table

Fill in the table based on your results from the lab. Use the rubric on the next page to help your answers.

Question:

Some people argue that tertiary structure is most important for a protein's function. Some people argue primary structure is most important. What do you think: is primary or tertiary structure most important for the function of a protein?

(People are likely to disagree with you, so be sure to back up your answer with evidence from the lab and to explain your reasoning.)

Claim Make a clear statement that answers the above question.	
Evidence Provide data from the lab that supports your claim (both from the pre-lab analysis activity and the lab).	
Reasoning Explain clearly why the data you presented supports your claim. Include the underlying scientific principles that link your evidence to your claim.	

Score	4	3	2	1
CLAIM A statement that answers the original question/ problem.	Makes a clear, accurate, and complete claim.	Makes an accurate and complete claim.	Makes an accurate but incomplete or vague claim.	Makes a claim that is inaccurate.
EVIDENCE Data from the experiment that supports the claim. Data must be relevant and sufficient to support the claim.	All of the evidence presented is highly relevant and clearly sufficient to support the claim.	Provides evidence that is relevant and sufficient to support the claim.	Provides relevant but insufficient evidence to support the claim. May include some non-relevant evidence.	Only provides evidence that does not support claim.
REASONING Explain why your evidence supports your claim. This must include scientific principles/ knowledge that you have about the topic to show why the data counts as evidence.	Provides reasoning that clearly links the evidence to the claim. Relevant scientific principles are well integrated in the reasoning.	Provides reasoning that links the evidence to the claim. Relevant scientific principles are discussed.	Provides reasoning that links the evidence to the claim, but does not include relevant scientific principles or uses them incorrectly.	Provides reasoning that does not link the evidence to the claim. Does not include relevant scientific principles or uses them incorrectly.

We recommend that teachers use the following scale when assessing this assignment using the rubric. Teachers should feel free to adjust this scale to their expectations.

Rubric score	3	4	5	6	7	8	9	10	11	12
Equivalent Grade	55	60	65	70	75	80	85	90	95	100

Extension: Making biology brighter

A Nobel Prize winning discovery

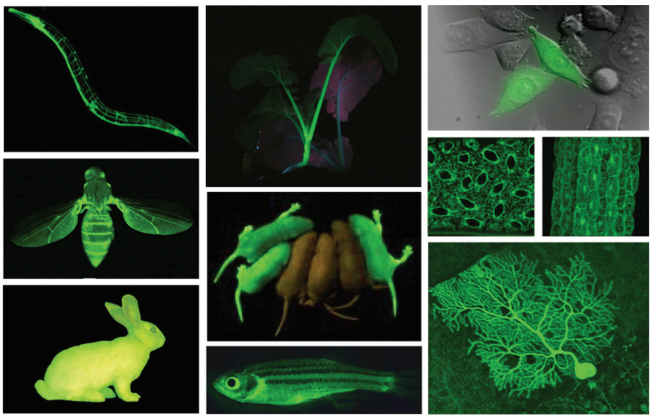


Figure A: Examples of different live organisms with the gene sequence that codes for GFP inserted into their DNA. When the DNA undergoes transcription and translation, the GFP is expressed and fluoresces green.

Perhaps some of the most iconic images of modern biotechnology are ones of green glowing organisms—bacteria, worms, flies, plants, and even mice, monkeys, and other animals. The bright green fluorescence seen in many of these images can be traced back to a single protein: green fluorescent protein (GFP). You used fluorescent proteins like this in today's lab activity as a visual representation of the relationship between a protein's structure and its function. Similarly, scientists also use GFP and other fluorescent proteins to illuminate diverse phenomena, from tracking how proteins are made to visualizing how cancer spreads within an organism. But GFP wasn't something that was invented from scratch in the lab. Like many other biotechnology tools, it was first observed in nature, then isolated to allow scientists to study it further, and finally modified and engineered so it could be widely used in biological research.

The story of GFP starts in the Pacific Ocean, in a type of jellyfish called the crystal jelly (*Aequorea victoria*). In 1961, biochemist Osamu Shimomura first discovered and isolated GFP from this jellyfish and noted its green fluorescent properties. Three decades later, another biochemist, Martin Chalfie, came up with the idea of using GFP as a tool in research. To do so, he took the gene sequence for GFP and inserted it at the end of a gene for another protein. By doing this, when the organism expressed that other gene, GFP was also expressed and the two proteins were fused together. This technique allowed proteins to be visually tracked within a live organism through the fluorescence of the GFP fused to them.

To make it the robust and versatile tool it is today, that original GFP needed to be modified. Biochemist Roger Tsien made changes to the DNA sequence of GFP so it would fluoresce best under blue light rather than UV light—very similar to today's lab activity. In 2008, the Nobel Prize in Chemistry was awarded to Shimomura, Chalfie, and Tsien for the discovery and development of GFP as a biological tool. Others have subsequently made additional modifications to further improve GFP. Today, there are dozens of fluorescent proteins that researchers use in their experiments, ranging in different brightnesses, colors, and other characteristics.

Designing a free-use GFP

There are many reasons why scientists have modified GFP for use in different experiments, such as to improve the stability of GFP, the temperature at which it best functions, or the brightness of the fluorescence that's emitted. This case study is about another kind of motivation—one that's rooted in a legal reason rather than a biological one: patents.

Patents ensure that inventors are recognized for their innovations. The holder of a patent for a given protein has the right to define the terms under which the protein can be used for research or commercial purposes, including monetary compensation.

Since most fluorescent proteins are protected by patents, an Australian research group led by Dr. Nicholas Coleman decided to create new fluorescent proteins that could be freely accessible to anyone and not infringe on existing patent rights so that anyone in the world could use them. Specifically, they wanted to create versions of one of the commonly used GFP variants, called superfolder GFP (sfGFP), which is easy to use in biological experiments because it folds quickly and fluoresces very brightly. Under the legal terms of the patent for sfGFP, their new free-use GFP had to have an amino acid sequence that was at least 20% different than the sfGFP sequence to be considered a different protein. This means that one in five of the amino acids in the sfGFP sequence had to be replaced, all while still maintaining the protein's overall three-dimensional structure and bright green fluorescent function.

One of the researchers in Coleman's group, Mark Somerville, took on this engineering challenge. First, he analyzed the sequences of many different fluorescent proteins that also glow green like sfGFP to identify regions that had the same amino acids across these different sequences. These regions would be considered highly conserved across different green fluorescent proteins and were likely to be more important for green fluorescent function. From there, he designed four new potential GFP sequences, keeping the conserved positions untouched but swapping out amino acids in non-conserved spots for others. Even though the non-conserved spots were less likely to be important for fluorescent function, they still contribute to the overall shape and structure of the protein, so he made sure the new substituted amino acids had similar chemical properties to the original ones.

Somerville's initial approach worked—sort of. The proteins he made fluoresced, but unfortunately, they were not as brightly fluorescent as the original sfGFP. It also wasn't clear what changes could make them better. The next step was to try a protein engineering technique called gene shuffling. Instead of trying to make directed changes, amino acid by amino acid, gene shuffling allowed Somerville to make hundreds of new proteins very quickly, all based on the four proteins he already designed.

He did so by taking copies of the DNA that coded for his four GFP sequences and chopping the DNA up into randomly sized fragments using an enzyme. Then, these chopped-up pieces were mixed together and reassembled. The new genes had all the DNA needed to make a fluorescent protein, but they might have the beginning from one DNA sequence, the end of another, with the middle made of a third. And because there were many copies of the DNA sequences being shuffled, all chopped up into different fragments of random sizes that reassembled in many different ways, Somerville ended up with hundreds of new gene combinations to test [1].

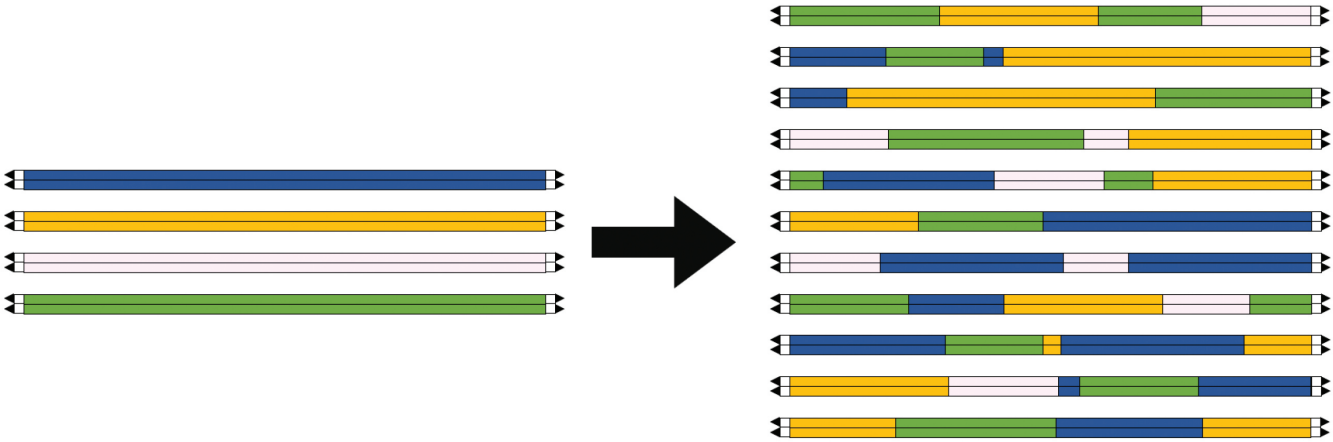


Figure B: In gene shuffling, pieces from different copies of the same gene are rearranged in a random way. This quickly creates many new unique genetic sequences that can each be tested independently.

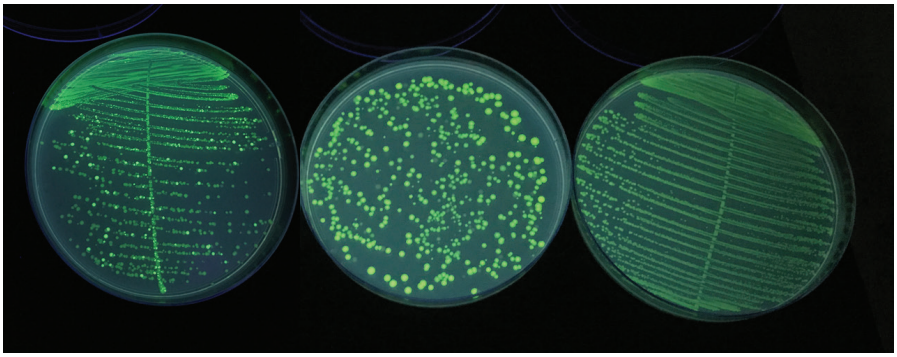


Figure C: All of the possible GFP sequences obtained from DNA shuffling were transformed into these bacterial cells to be tested. The cells expressed the corresponding GFPs and the brightest GFPs were selected.

To test which, if any, of these new gene sequences would produce a protein with bright green fluorescence, he put each new sequence into bacterial cells, which then expressed the genes into proteins. Some sequences were changed in a way that caused them to no longer fluoresce. Other sequences, however, resulted in very bright green fluorescent proteins. He identified the proteins that were the brightest green and determined the corresponding gene sequences. This artificial selection of the proteins after DNA shuffling allowed him to quickly find the sequences that gave the needed structures and functions in a matter of days—much faster than typical protein engineering strategies.

[1] For more details on how this technique works, see Meyer, Adam J, Ellefson, Jared W., and Ellington, Andrew D. "Library Generation by Gene Shuffling." Current Protocols in Molecular Biology, January 2015.

In the end, this shuffling technique resulted in some new DNA sequences that produced proteins just as bright as sfGFP. After a few more small tweaks to make sure that a full 20% of amino acids were different from the original sfGFP (as required by patent law), they had a few new free-use GFPs that functioned just as well as the patented version. In fact, these are the very same green fluorescent proteins you used in today's lab. Not only did this project result in fluorescent proteins that can be freely used by anyone, but it also explored a whole range of amino acid sequences that can all produce functional GFPs. In this way, the process of protein engineering can shed even more light on the protein structure and function relationship.

Review

1. Was the original GFP discovered or invented by scientists?

2. How can GFP be attached to other proteins?

3. Why might you want to do this in a cell?

4. Name one modification that was made to GFP to improve it for use as a tool in experiments.

5. What is one reason a researcher may wish to patent a protein they engineer?

6. Mark Somerville's first approach to engineering a new protein consisted of switching amino acids for ones with similar chemical properties.
   a. How well did this work?

   b. Does this surprise you or is this what you would expect? Explain your answer.

7. Using the process of gene shuffling:
   a. Roughly how many new proteins was Somerville able to generate?

   b. How long did it take him?

8. When selecting the proteins to use after gene shuffling, did Somerville know the amino acid sequence of the proteins before or after he knew which ones functioned the best?

9. In your own words, explain how it is possible to change more than 20% of the amino acid sequence of a protein and still result in a protein with nearly identical tertiary structure and function.

Critical thinking

10. Where would you predict the conserved positions in GFP to be more likely found: in the chromophore region or in the barrel region? Justify your answer.

11. Gene shuffling is sometimes used as a part of a protein engineering technique called "directed evolution." In directed evolution, scientists introduce random genetic changes in ways that are similar to natural processes, like mutation and recombination. From these new variants, the scientist selects the proteins that function the best and then repeats the process. In each round of random change and selection, the protein's function improves.

    Is gene shuffling more similar to the natural process of mutation or recombination? Explain your answer in as much detail as you are able.

References

Figure A: Chalfie, Martin. "GFP: Lighting up life." Proceedings of the National Academy of Sciences, June 2009.

Figure C: Somerville, Mark. Nicholas Coleman Lab at the University of Sydney. https://coleman-lab.org/

Instructor guide

For technical support, contact support@minipcr.com

For answers to the student questions, email answers@minipcr.com
Please include in the body of the email:
- The name of the lab
- Your name, school, and job title

Expected results

Listed below are the expected results for each reaction, accompanied by a brief explanation. You may observe small differences in brightness across different groups, which can arise from variability in micropipetting and sample handling.

FP1: DNA FP1 encodes a green fluorescent protein. The peak absorption for this protein is in the UV spectrum, meaning it will be brightest when viewed under UV light.

FP2: DNA FP2 also encodes a green fluorescent protein, but the peak absorption for this protein is in the blue light spectrum, meaning it will be brightest when viewed under blue light.

FP3: DNA FP3 encodes a red fluorescent protein. The peak absorption for this protein is in the blue light spectrum. However, since the intensity of the UV light used may be stronger than the blue light used, the protein may appear bright under UV light as well.

Control (water only): No fluorescence is expected throughout the course of the experiment. Students may detect low levels of autofluorescence from the BioBits pellets.

Notes on lab design

We graciously acknowledge and thank Dr. Nicholas Coleman and Mark Somerville from the University of Sydney (https://coleman-lab.org/) for engineering the green fluorescent proteins (FP1 and FP2) and sharing these proteins for this lab activity. To read more about the development of these proteins, visit https://schaechter.asmblog.org/schaechter/2019/05/the-story-of-free-use-gfp-fugfp.html or use the extension activity found on page 38.

The design of this lab has simplified certain elements to achieve its goals. Some of these elements include:

- Students analyzed protein sequences and structures on paper to properly illustrate the concepts of protein structure/function relationships. Researchers typically carry out this analysis computationally. For a more realistic (but more challenging) look at how this is done, students may use the bioinformatics extension (refer to the next page) in parallel with the basic pre-lab activity.
- In this lab, students tested two different candidate protein sequences to find one that suited the stated research objective. Protein engineers typically screen anywhere from dozens to hundreds of potential protein sequences. They also often will do multiple rounds of engineering and screening to optimize the protein to the desired function or role. For a further explanation of how this directed evolution technique works, students may reference the reading listed in the extension activities section on page 43.
- Homology modeling is typically done with the entire protein sequence. In this lab, students only look at a section of the sequence around the chromophore for simplicity. To have your students perform homology modeling with the entire sequence, students may use the bioinformatics extension (refer to the next page) in parallel with the basic pre-lab activity.
- Students may ask for an explanation of why a single amino acid change from FP1 to FP2 caused FP2 to fluoresce better under blue light instead of UV light. The full explanation involves advanced biochemical and biophysics concepts out of the scope of this lab. Remind students that exactly how specific amino acids will affect protein structure and function can be difficult to predict.

Additional student supports

E-worksheets: The student questions accompanying this lab are available for download here as editable text documents you can customize and upload to your LMS. E-worksheets can also be accessed from the Curriculum Downloads tab at https://www.minipcr.com/product/biobits-structure-function/

Cell-free protein synthesis DNAdots: Learn more about how cell-free protein synthesis systems like BioBits work and explore real-world applications. DNAdots include background reading and study questions. https://dnadots.minipcr.com/dnadots/cell-free-technology

miniPCR tutorials: Access an extensive set of free resources to help your students succeed in molecular biology techniques. Visit https://www.minipcr.com/tutorials/. The resources most relevant to this lab are listed below.

- **Micropipetting:** Video, worksheet, and hands-on activity resources to train students in the basic use of a micropipette.

miniPCR Digital: Interactive tools for experiment-based learning with or without hands-on lab kits. Visit https://digital.minipcr.com/

Extension activities

The following optional extension activities are provided for students to explore topics more deeply.

Making Biology Brighter (page 38): Fluorescent proteins play a very important role in molecular biology research. In this activity, students will explore the history of fluorescent proteins and the actual engineering process that led to some of the proteins used in this lab.

Bioinformatics extension: If you wish for your students to use real-world online bioinformatics tools to analyze the protein sequences from the lab, this activity may be completed in addition to the Pre-lab activity. The worksheet is available for download here and can also be accessed from the Curriculum Downloads tab at https://www.minipcr.com/product/biobits-structure-function/

Directed evolution: This lab gives a simplified overview of the protein engineering techniques used by researchers. For a more in-depth explanation of one of these techniques, students can read more about directed evolution and its applications in protein engineering. DNAdots include articles and study questions. Find this article at: https://dnadots.minipcr.com/dnadots/directed-evolution

Protein gel electrophoresis extension: After completing the BioBits Protein Structure and Function lab, students will have produced three fluorescent proteins. This extension activity allows you to characterize the overall charge of each fluorescent protein using agarose gel electrophoresis. This activity can be accessed from the Curriculum Downloads tab at https://www.minipcr.com/product/biobits-structure-function/

Learning goals and skills developed

Student learning goals

- Predict how structural changes may affect protein function.
- Identify how amino acid sequences determine the structure and function of the resulting protein.
- Use comparative sequence data to identify functional regions of proteins.
- Identify functional differences between similar proteins and relate these differences to changes in amino acid sequence

Scientific inquiry skills:

- Identify or pose a testable question
- Formulate hypotheses
- Identify dependent and independent variables and appropriate experimental controls
- Follow detailed experimental protocols
- Create tables or graphs to present their results
- Interpret data presented in a chart or table
- Use data to evaluate a hypothesis
- Make a claim based in scientific evidence
- Use reasoning to justify a scientific claim

Molecular biology skills:

- Micropipetting
- Cell-free protein expression
- Fluorescence visualization

Standards alignment

The standards alignment document for this activity is available for download here. This document can also be accessed from the Curriculum Downloads tab at https://www.minipcr.com/product/biobits-structure-function/

This activity is aligned to the following standards:
• Next Generation Science Standards (NGSS): High School Life Science
• Advanced Placement Biology
• Texas Essential Knowledge and Skills (TEKS): Biology
• Texas Essential Knowledge and Skills (TEKS): Biotechnology
• Biotechnician Assistant Credentialing Exam (BACE)
• Common Core ELA/Literacy Standards (9-10)

For additional information on alignment to state standards, please contact support@minipcr.com

