miniPCR bio™ Learning Lab

BioBits®: Central Dogma

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BioBits®: Central Dogma
Instructor's and Student's Guide

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Instructor's Guide Contents

Overview

With minimal equipment requirements and a quick and straightforward protocol, students will use BioBits® reactions to visualize the flow of genetic information and monitor transcription and translation in real-time through fluorescence. This activity serves as an excellent interactive tool for learning the central dogma of molecular biology and exposes students to cutting-edge synthetic biology.

BioBits® cell-free reactions are tiny molecular factories that can create a variety of proteins, from brightly colored fluorescent proteins to functional enzymes, without the need for cell culture. When dry, BioBits® pellets are dormant, but they can be activated by simply adding water. Researchers have been using cell-free reactions in their laboratories for years, with applications ranging from novel therapeutic discovery to field diagnostics. Now the BioBits® cell-free system makes this cutting-edge technology accessible anywhere to anyone interested in learning molecular biology and is an excellent teaching tool to enhance biology education both within and beyond the classroom.

TIME

30 MIN. + 10 MIN.

Two class periods:
A. Initial set up and observations: 30 minutes.
B. Final observations: 10 minutes. Period B should be conducted between 8 and 72 hours after A.

TECHNIQUES

Micropipetting
Cell-free protein synthesis

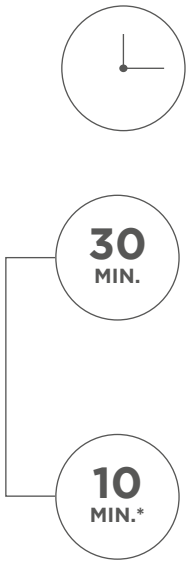
TOPICS

Central dogma of molecular biology
Protein synthesis
Transcription and translation
Gene expression
Fluorescence.

LEVEL

- ✓ Advanced
- ✓ Intermediate
- ✓ Introductory

At a glance



Preparatory activities (Instructor)	Class-time activities (Students)
Portion DNA, water, kanamycin and BioBits® pellets for each group 10 min.	**A. Setup of BioBits® reactions** 10 min. **B. Incubation and initial observations** 20 min.
	C. Final observation 10 min.

*Final observation can be done anytime between 8 to 72 hours after the first class period (typically 24 hours).

Materials needed

The reagents supplied in the kit are sufficient for 8 lab groups (recommended lab group size: 2-4).

Storage notes: Pellets can be stored for up to 6 months and DNA and kanamycin for 12 months from date of receipt when stored at -20 °C. Store any unused pellets in an airtight bag with the supplied orange desiccant card. If freezer storage is not available, the reagents may be stored in the fridge (approx. 4˚C). Pellets are viable for up to three months from the date of receipt when stored in the fridge. DNA and kanamycin can be stored for up to six months when kept in the fridge.

	Reagents and supplies	Amount provided in kit	Amount needed per lab group	Storage	Teacher's checklist
Supplied in kit	**BioBits® pellets in PCR strip tubes** • Keep BioBits® in the vacuum-sealed pouch at -20˚C as long as possible	Four 8-tube strips to be broken into strips of 4 tubes	**1 strip of four tubes**	-20˚C	
Supplied in kit	**Nuclease-Free Water**	**200 µl**	**20 µl**	-20˚C	
Supplied in kit	**DNA A**	**150 µl**	**15 µl**	-20˚C	
Supplied in kit	**DNA B**	**100 µl**	**10 µl**	-20˚C	
Supplied in kit	**Kanamycin**	**100 µl**	**10 µl**	-20˚C	
Supplied by teacher	**Plastic tubes:** to aliquot reagents • Any size 0.2 – 1.7 mL		**4**		
Supplied by teacher	**37°C heat source** • e.g., miniPCR™ machine, incubator, water bath • Body heat works as well		**1** (equipment can also be shared between groups)		
Supplied by teacher	**P51™ molecular fluorescence viewer or other blue light Illuminator:** e.g., blueGel™ or blueBox™. The illuminator needs to have an orange (not yellow) filter.		**1** (equipment can also be shared between groups)		
Supplied by teacher	**Micropipettes:** 2-20 µl are recommended		**1**		
Supplied by teacher	**Disposable micropipette tips**		**10+**		
Supplied by teacher	**Other supplies:** • Disposable laboratory gloves • Protective eyewear • Permanent marker • Optional: tube rack				

Preparatory activities

The following activities should be carried out by the instructor ahead of class.

10 MIN.

Distributing reagents and supplies

• For each lab group, label and dispense in separate microtubes:

- DNA A	Label as "A"	15 µL
- DNA B	Label as "B"	10 µL
- Water (Nuclease-Free)	Label as "W"	20 µL
- Kanamycin	Label as "K"	10 µL

• Each lab group will additionally need the following supplies:

- 1 strip of 4 tubes of BioBits® pellets. Separate each strip of 8-tubes in half to create 4-tube strips.
    • Recommended method is by razor blade to cleanly cut the strip in half. Scissors will also work.
- Micropipettes, one per group (2-20 µL range is recommended).
- Disposable micropipette tips (at least 10 per group) and a small beaker/cup to dispose of them.
- Permanent marker (ideally fine-tipped).
- Access to a P51™ molecular fluorescence viewer or other blue light illuminator (with an orange —not yellow— filter).
- Access to a 37°C heat source (body heat works well if other heat source not available).

Student's Guide Contents

Background and significance

Overview

Today, you will be using cutting-edge BioBits® cell-free technology to explore the world of synthetic biology. Each BioBits® pellet contains all the necessary reagents and cellular components required to perform transcription and translation without cells —all you need to do is simply add water and your DNA of interest. Researchers use these kinds of cell-free reactions to develop new therapeutics, medical diagnostics, and more. The BioBits® cell-system allows anyone interested in tinkering with biology to make proteins anywhere.

In today's activity you will use the BioBits® cell-free system to visualize the flow of genetic information and monitor the processes of transcription and translation in real time.

Protein synthesis is usually carried out inside living cells, but BioBits® pellets allow this process to be carried out without cells. Using DNA that encodes for green and red fluorescent markers, you will be able to observe both the production of RNA and of protein as they occur in real time. You will also explore ways to interrupt specific steps in the molecular flow of information from DNA to protein.

The central dogma of molecular biology

In 1957, Francis Crick, one of the discoverers of the structure of DNA, gave a lecture that profoundly influenced how biologists think about genetic information and molecular biology in general. It was only four years after the discovery of DNA structure, but it was already well accepted that DNA was the molecule of heredity. Yet the details of how DNA actually encoded genetic information and what that information encoded for were still largely uncertain. This 1957 lecture proposed a conceptual framework for how the system most likely functions based on the little data that was available then. Crick called this framework the *central dogma of molecular biology.*

Dogma is a term typically used to convey an idea that is so fundamental to a field that its truth is undebatable. Crick chose this word because he felt so strongly that his central idea must be correct, even though the evidence available at the time was scant. When we use the term central dogma today, we do not mean to imply that this framework must be accepted unquestioningly. Instead, dogma conveys that this idea is so fundamental to understanding life and heredity that, in order to understand molecular biology, one must first comprehend this central principle.

What was this realization that is now so central to the understanding of all molecular biology? Crick outlined the process by which the instructions contained in DNA are transformed into cellular function. Simply stated, the central dogma of molecular biology explains the flow of genetic information whereby DNA is able to code for RNA and RNA is able to code for protein. More specifically, it says that DNA and RNA can both store and transfer genetic information needed to make proteins, whereas protein cannot store the information needed to make DNA or RNA (Figure 1). In Crick's words, "the main function of the genetic material is to control … the synthesis of proteins". And "once information has got into a protein, it can't get out again." These predictions were strikingly accurate —to the point that his lecture even accurately predicted the existence of yet undiscovered molecules necessary to turn genetic information into functional products.

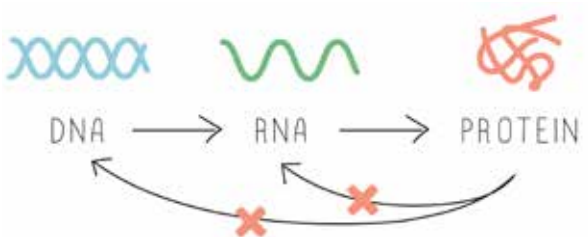


Figure 1: One schematic representation of the central dogma of molecular biology. DNA information can be transferred to RNA and used to make proteins, but proteins cannot store the information needed to make DNA, RNA or protein.

Scientists have since discovered the molecules involved in the flow of genetic information, and have worked out in great detail how the information in DNA is processed by the cell. But still, more than sixty years later, as modern research continues to shed new light on the flow of genetic information within a cell, the central dogma remains the foundation for understanding the relationships between DNA, RNA and protein.

Proteins: the tools of life

A large number of the molecules carrying out most of life's essential functions are proteins. As Crick argued, the major role of DNA is to provide the instructions on how to produce these proteins. Proteins are made by linking smaller building blocks called *amino acids* together in a long chain. That chain then folds into a unique three-dimensional structure. What makes one protein different from another —whether it breaks down the starches that you eat like amylase does, provides structure to your cells like tubulin, or moves your muscles like actin and myosin —is the order of the amino acids in that chain. The main information stored by DNA is simply the order of those amino acids for each unique protein. The cellular machinery has the ability to read DNA, transfer DNA information into RNA, and to build the correct amino acid sequences, but cannot reverse the process to read protein to produce a DNA or RNA sequence.

How to make a protein

We now think of the flow of information from DNA to protein as a two-step process: *transcription* —the production of RNA from a DNA sequence, and *translation* — the production of protein from an RNA sequence.

Like protein, DNA consists of building blocks arranged in a long chain. The four building blocks of DNA are called nucleotides and named adenine (A), thymine (T), guanine (G), and cytosine (C). These nucleotides are arranged in a specific order and connected into a long double helix. Within this double helix are sequences of nucleotides that contain the information to make different proteins. The exact structure of this information can vary across classes of organisms, but there are some commonalities throughout: a *promoter* sequence that tells the cellular machinery where the information begins, a *protein coding sequence* that contains the information that determines the order of the amino acids, and finally a signal that marks where the transcribed information ends. In prokaryotic systems, like the one you will be working with in this lab, we call this signal the *terminator* sequence.

To start the process, the genetic information stored in DNA is first transferred into a temporary copy called messenger RNA or *mRNA*. We call the process of copying DNA to RNA transcription. Transcription starts when the RNA polymerase recognizes a promoter. The *RNA polymerase* binds to the promoter sequence and begins moving down the DNA, unwinding the double helix as it goes. As the RNA polymerase travels, it joins the building blocks of RNA into a long strand (Figure 2). The four building blocks of RNA are called ribonucleotides and named adenine (A), uracil (U), guanine (G) and cytosine (C). The *ribonucleotides* are structurally similar to DNA nucleotides and their correct order is determined by pairing the ribonucleotides to their complementary DNA sequence. RNA polymerase links together the unbound ribonucleotides to the growing single strand of RNA with energy from *ATP* driving the reaction.

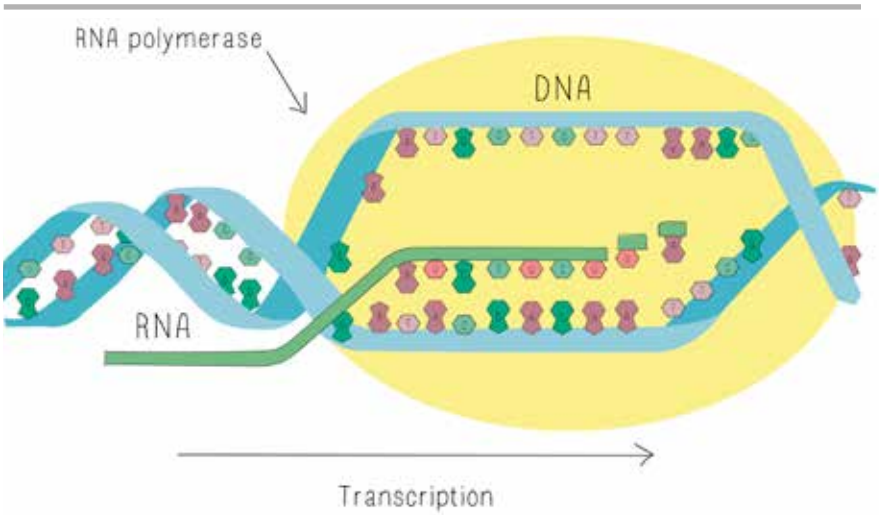


Figure 2: Transcription —the process of copying information from DNA to RNA. RNA polymerase moves down the DNA sequence and joins ribonucleotides together into mRNA.

The information now stored in the mRNA will next be read to make a protein in a process called *translation*. Reading the information encoded in the mRNA takes place at the ribosome. The mRNA binds to and starts being fed through the ribosome. As the mRNA moves through the ribosome, the order of nucleotides is read in groups of three known as *codons* —you can think of the nucleotides as letters, and the codons as words made up of three nucleotide letters. The start of the protein coding sequence is marked by a special order of three nucleotides known as the *start codon*. To read the coding sequence in the mRNA, a different kind of RNA called transfer RNA or tRNA, binds to the mRNA. On one end of the tRNA are three nucleotides that pair with the codon on the mRNA by the rules of base pairing. On the other end of the tRNA is the amino acid that is specific to the codon on that *tRNA*. As the tRNAs bind to and translate the information in the mRNA, the ribosome links the corresponding amino acid to the one before it, creating the chain that will become the protein. Just like in transcription, translation is fueled by ATP as an energy source (Figure 3).

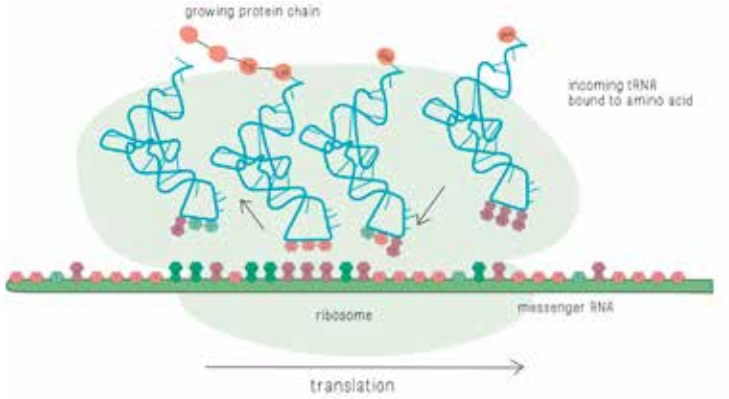


Figure 3: Translation —the process of making proteins from RNA information. At the ribosome, the mRNA is read by complementary tRNAs and each tRNA's corresponding amino acid is linked into the growing protein chain.

Transcription and translation analogy

• When you are copying something word for word, like writing down an exact quote from an interview, you are transcribing that information. The process of transcribing is known as transcription. When RNA is produced, the order of the bases in the RNA will resemble an exact copy of one of the DNA strands the RNA was copied from. For this reason, scientists call this process transcription. The main difference between the RNA transcript and the original DNA is that RNA uses ribonucleotides, where the base uracil is substituted for the structurally similar thymine.

• When you are copying something in one language into another, say from English to Spanish, you say you are translating that information. The process of translating is known as translation. At the ribosome, mRNA nucleotides are read to make a sequence of amino acids, the building blocks of protein. You can think of it as the language of nucleotides being translated into the language of amino acids. For this reason, scientists call this process translation.

The end of the protein coding sequence is marked by a *stop codon*; upon reaching this codon, the ribosome will release the newly formed amino acid chain, which will continue to fold into its final three-dimensional structure. This new protein may do any of countless functions depending on its sequence of amino acids. But regardless of what protein is made or what organism the process occurs in, whether it be a bacterium or a whale, an amoeba or an oak tree, the same basic process is followed. DNA is transcribed into RNA by RNA polymerase using ribonucleotides as building blocks and ATP as an energy source. RNA is then translated at the ribosome, with tRNAs deciphering the genetic code, bringing amino acids that are joined together into a chain, again with ATP fueling the reaction. Despite all the complexities that have been discovered in modern molecular biology there is no evidence that the process can start with protein and go the other way, just as Crick predicted.

Today's lab

Transcription and translation without cells

Transcription and translation typically happen inside the cells of living organisms. But it is possible to perform these processes in a synthetic system without cells. The BioBits® system you will be using today is an example of such a system. BioBits® pellets contain all of the necessary cellular components, such as RNA polymerases for transcription and ribosomes for translation. They also contain the required building blocks —the nucleotides to build mRNA and the amino acids to build proteins. Furthermore, they contain ATP, the energy source that powers the reactions (Figure 4). Any DNA carrying a properly structured protein-coding gene that is added to the system will result in the synthesis of the protein encoded by the DNA. In this way, we can make proteins quickly and easily without any of the difficulties of culturing live organisms.

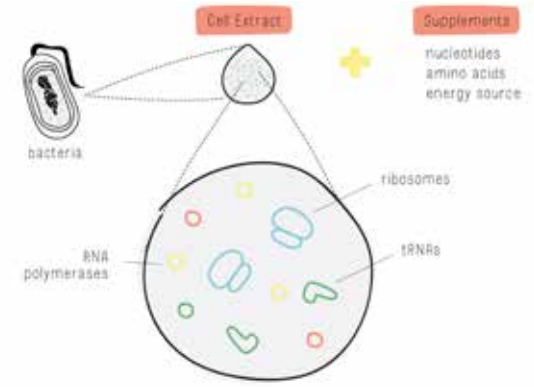


Figure 4: Essential cellular machinery can be extracted from cells and supplemented with molecular building blocks and energy to create a cell-free system that is still capable of carrying out transcription and translation.

Lab setup

In this lab, you will use DNA that encodes the information for making a fluorescent protein. Fluorescent proteins can be found in organisms such as jellyfish or coral and will light up (fluoresce) when exposed to a specific wavelength of light, usually blue or UV light. In nature, it is hypothesized that organisms use fluorescent proteins to ward off predators or attract prey. In research, scientists use these same fluorescent proteins as visual markers or signals that illuminate what is happening in their experiments. Similarly, today you will be using fluorescence to track the flow of genetic information as the DNA is transcribed to RNA, and the RNA is translated to protein.

You will be given a sample of DNA containing a gene with the information to make a red fluorescent protein. When the gene is transcribed and then translated, you will be able to visually confirm the presence of the protein by observing red fluorescence. However, observing the presence of mRNA after transcription is usually more difficult because mRNA is not typically visible to the naked eye. For this lab, we will be able to visualize mRNA with a unique genetic feature built into this gene. Just upstream of the coding sequence the gene encodes an *aptamer,* a specially designed sequence of nucleotides that has the ability to selectively bind to other molecules when transcribed. BioBits® pellets contain a specific chemical that this aptamer will bind to, and when it does, it will emit

green fluorescence. Hence, green fluorescence will signal transcription, the synthesis of mRNA from DNA. In this way, using both the green RNA aptamer and the red fluorescent protein as visual readouts, you will know that you have successfully transcribed DNA to RNA when you see the green fluorescence and that you have successfully translated RNA to protein when you see the red fluorescence (Figure 5).

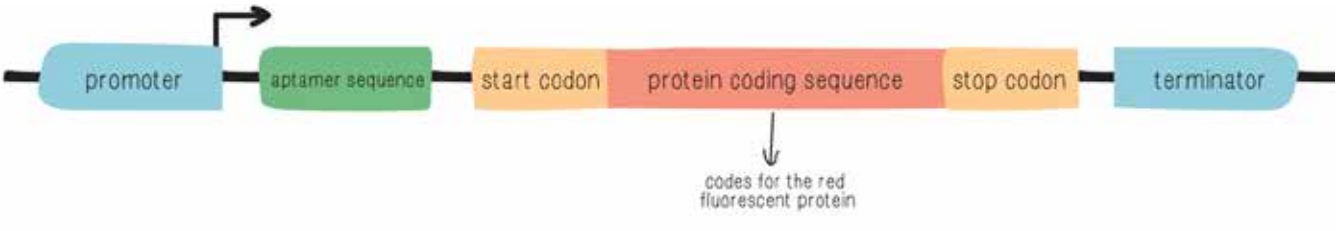


Figure 5: The structure of the DNA sequence you will be using in this lab, including the aptamer sequence for the green RNA signal and the protein coding sequence for the red fluorescent protein.

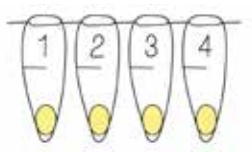
You will perform four reactions that allow you to investigate the central dogma. The first reaction will serve as a negative control, where you will add water instead of DNA. To your second reaction, you will add the DNA sample described and shown above. You will add this same DNA to your third reaction, but you will also add kanamycin, an antibiotic drug that interferes with ribosome function. In your fourth reaction, you will add a different DNA sample. Your job, based on your knowledge of the central dogma, is to predict what you will observe in the first three reactions and then deduce what occurred in the fourth reaction based on your observations.

Laboratory guide

A. Setup of BioBits® reactions

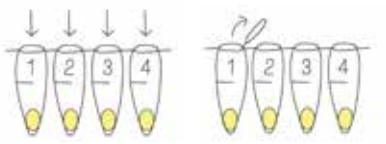
You will investigate three samples and one negative control using the BioBits® cell-free system.

1. Label each tube in your strip of four BioBits® pellets on the side, not cap, of the tube



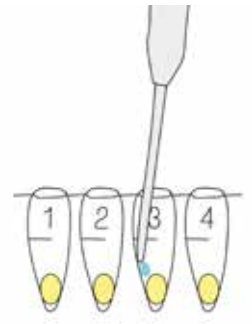
- Label the tubes 1 through 4
- Label a group name/symbol somewhere on the tubes
- Tube 1 will be for your negative control
- Tube 2 will be for your reference reaction
- Tube 3 and 4 will be for your experimental reactions
  - You will know what you added to tube 3 and will have to predict the result.
  - You will not know what you added to tube 4 and will have to deduce what you added based on the result.

2. Uncapping BioBits® strip tubes



- Gently tap tubes on the table to collect pellets at the bottom.
- To open tubes, CAREFULLY remove each cap in the strip one at a time, taking care not to dislodge BioBits® pellets.

3. Add DNA to each BioBits® pellet in the strip. Use a new tip for each sample.



- Use a micropipette to add the DNA solution to dissolve the pellet.
  - Do not use the second stop on the pipette.
  - Do not yet add liquid to tube 1.
  - Add 5 µl of DNA A (A) to tubes 2 and 3.
  - Add 5 µl of DNA B (B) to tube 4.

Do not touch your pipette tip to the pellet or the pellet may get stuck inside the tip. Instead, it may help to touch the pipette tip to the side of the tube so the DNA is added down the side of the tube, and then to tap the tube so the liquid collects at the bottom of the tube and dissolves the pellet.

Because the reaction volumes are so small, you want to avoid bubble formation. We advise against using the second stop on your micropipette, and also against pipetting up and down to mix.

	Tube 1	Tube 2	Tube 3	Tube 4
DNA	none	5 µl DNA A (A)	5 µl DNA A (A)	5 µl DNA B (B)

4. Pipette the additional reagents to each tube. Use a new tip for each sample.

- Add 7 µL of water (W) to tube 1.
- Add 2 µL of kanamycin (K) to tube 3.
- Add 2 µL of water (W) to tubes 2 and 4.
- Use a micropipette to add the reagents. Do not use the second stop on the pipette.

	Tube 1	Tube 2	Tube 3	Tube 4
Reagent	7 µl water (W)	2 µl water (W)	2 µl kan (K)	2 µl water (W)

5. Close the caps on the tubes.

- You should feel the caps "click" into place if they are closed correctly.
- Make sure all the liquid volume has dissolved the pellet and collects at the bottom of the tube.
- If necessary, shake down with a flick of the wrist or spin briefly in a microcentrifuge.

6. Immediately observe your tubes in the P51™ viewer or other blue light illuminator.

- Make sure the blue light is on and that an orange filter is in place.
- Dim ambient lights as needed for proper observation.
- Record your observations in **Table 2** (page 18) in the "Time 0" row.

B. Incubation and initial observations

1. Place the tubes at 37°C.

- Use a miniPCR™ machine set to heat block mode or a 37°C incubator.
- If you don't have a miniPCR™ machine or other heat source, you can use body heat (i.e., your hands, under the arm, in your pocket) to warm the tubes.
- If you have not yet done so, predict what you will see in **Table 1** (page 17) below during the 15 minutes of incubation. You can also complete the pre-lab activity and questions in the **Study questions** section below (page 20).

2. After 15 minutes, observe your tubes in the P51™ viewer or other blue light illuminator.

- Make sure an orange filter is in place.
- Dim ambient lights as needed for proper observation.
- Recording your observations in **Table 2** (page 18) in the "15 minutes" row.

3. Store tubes at room temperature.

- The rest of the reaction will occur overnight at room temperature.
- You can leave the tubes in a tube rack or laying flat on the lab bench or table.
- If you have a longer class period, you can continue observing your tubes at additional time points and record your observations.

C. Final observation

1. The next class time, observe your tubes in the P51™ viewer or other blue light illuminator.

- Day 2 observation is usually done at approximately 24 hours after Day 1, but can be done anytime between 8 hours to 72 hours after Day 1.
- Make sure the blue light is filtered out with an orange filter.
- Dim ambient lights as needed for proper observation.
- Record your observations in **Table 2** (page 18) in the "Day 2" row.
- In **Table 3** (page 18), compare your Table 1 predictions and Table 2 observations.

Observation tables and questions

While you are waiting for your tubes to incubate, predict the colors of the reaction in tubes 1 through 3 and explain your thinking in one or two sentences. Tube 4 will be analyzed separately after you make your observations. Use the Background section of this lab and your **concept map** for help (page 20).

Table 1: Predictions

	Tube 1	Tube 2	Tube 3
Time 0	Prediction:	Prediction:	Prediction:
	Justification:	Justification:	Justification:
15 minutes	Prediction:	Prediction:	Prediction:
	Justification:	Justification:	Justification:
Day 2	Prediction:	Prediction:	Prediction:
	Justification:	Justification:	Justification:

Table 2: Observations

Note the color of the reaction in each tube at each observation time point.

	Tube 1	Tube 2	Tube 3	Tube 4
Time 0				
15 minutes				
Day 2				

Table 3: What processes occured?

Based on the colors you observed, state whether or not you think transcription and/or translation happened in each of the tubes 1-4. Then explain whether this conclusion agrees with your initial predictions. If it does not, can you think of a reason that it may not?

	Transcription?	Translation?	Do your conclusions match your predictions from Table 1?
1			
2			
3			
4			

CER Table

Fill in the table based on your results from the lab.

What do you think is the most likely explanation for your observations in tube 4?

Claim

Make a clear statement that answers the above question (Hint: Focus on what you did to make this tube different from the other tubes.)

Evidence

Provide data from the lab that supports your claim

Reasoning

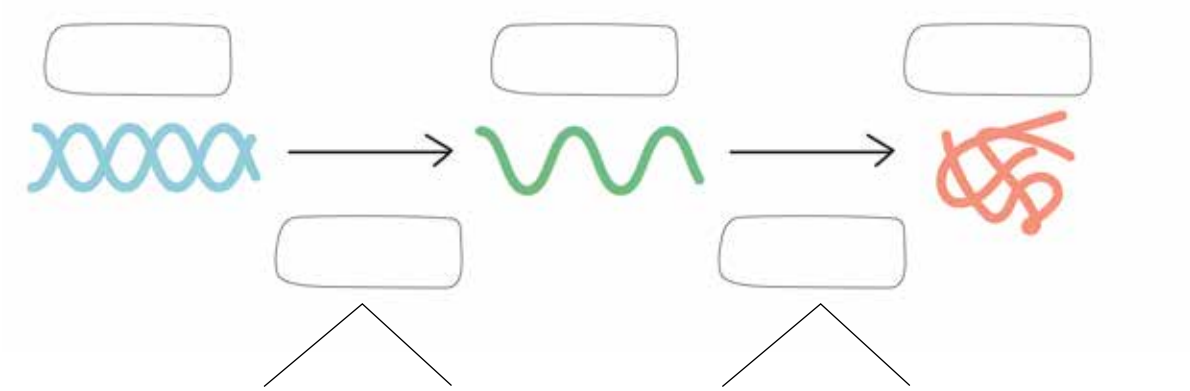
Explain clealry why the data you presented supports your claim. Include the underlying scientific principles that link your evidence to your claim

Study questions

Questions before experiment

Concept map: Processes and components involved in the central dogma

Using the provided word bank, fill in the blanks on this concept map to show the correct flow of genetic information and the processes involved. Then, use the table below to list the molecular components involved in each process and describe their role/function. You do not need to fill in every row of each table. Some words may be used more than once.



Component	Role

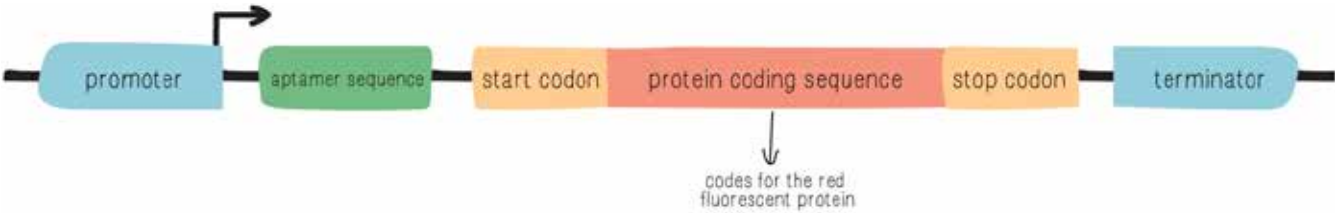
Component	Role

Word bank:

DNA	Transcription	Translation	RNA Polymerase	Ribonucleotides	mRNA
Ribosome	Amino acids	Protein	ATP	tRNAs	

Structure of the gene used in today's lab

Think about the processes of transcription and translation and how they relate to the gene structure shown below.



1. Look at the diagram above. If your only goal were to design a sequence that could be transcribed (regardless of whether it could be translated), which parts of this diagram would be most important? Which parts could you get rid of and still have transcription occur? Justify your answer.

Most important

Could get rid of

Justification

2. Refer to the diagram again. Draw a similar diagram here showing what the mRNA would look like. Only include those features that you think would be transcribed to the mRNA.

Which features of the original diagram did you not include in your diagram of mRNA?

Explain why you did not include them.

3. In today's lab, we have visual markers to know if transcription and translation have occurred.

a. mRNA is not usually fluorescent. Explain how in this lab green fluorescence indicates transcription has occurred. Include a small drawing or diagram in your answer.

b. Explain how red fluorescence indicates that translation has occurred. Include a small drawing or diagram in your answer.

Questions for after the experiment

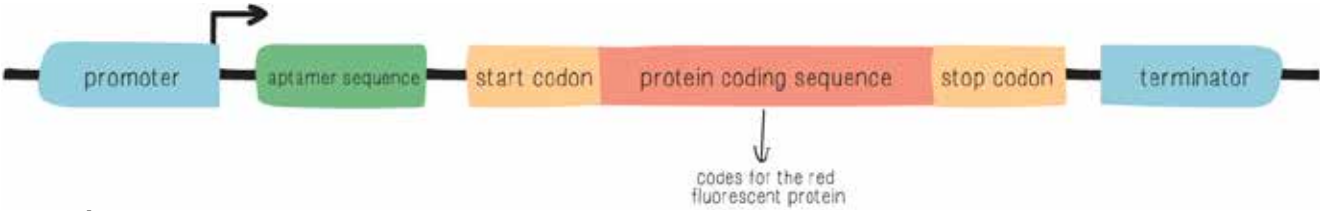
1. In this experiment, tube 1 was a negative control. Why is having a negative control important for your experiment?

2. If tube 1 was not included in this lab, what incorrect conclusions could a person make?

3. The central dogma states that genetic information can be passed from DNA to protein, but not in the other direction. Which reaction (tube number 1, 2, 3, or 4) most clearly demonstrates that information was passed from DNA to protein? Explain your answer using evidence from the lab.

4. You added kanamycin, an antibiotic, to tube 3. In bacteria, kanamycin interferes with the ribosomes and causes mistranslation of proteins. Do your results support this? Explain why or why not.

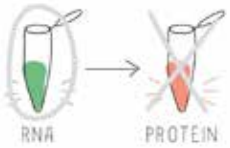
5. For each of the following statements, predict what you think might happen. For each statement circle the tube if you think it would produce RNA (and fluoresce green) and/or protein (and fluoresce red). If you do not think it will produce RNA and/or protein cross the tube out and then justify your answer in 1-2 sentences. Refer back to the gene structure below to help guide your answers. You may not know for sure what will happen in each situation, but give the answer you think is most likely. The first one has been done for you.



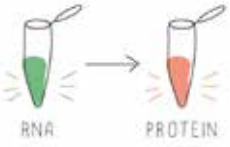
Example:

a) The coding sequence does not have a start codon.

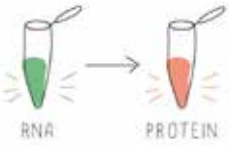
a. The presence of a promoter and terminator sequence means the mRNA would be produced correctly, but without a start codon the ribosome would never start translation properly.



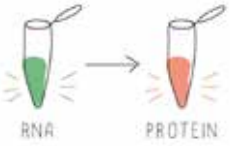
b) The coding sequence does not have promoter before it.



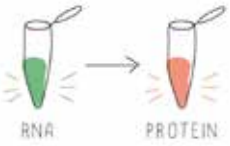
c) The coding sequence does not have a stop codon.



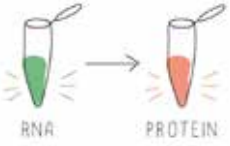
d) There is a promoter and a coding sequence but no terminator sequence.



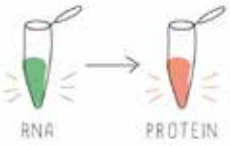
e) There is a promoter and a terminator sequence, but no coding sequence.



f) No ATP is added to the BioBits® pellet.



g) There is no aptamer sequence, but nothing else is changed.



Advanced questions

1. This lab was performed in a cell-free BioBits® reaction. Both aptamers and fluorescent proteins can be used in living cells as well. What parts of this activity do you think would be much more difficult in a live cell system?

2. Can you think of anything that could be easier in a live cell system that may be more difficult or is not possible in this cell-free system?

3. mRNA as an intermediary between DNA and protein.

   a. What might be the advantages of using mRNA as an intermediary between DNA and protein?

   b. Imagine a system in which DNA could be translated directly into protein. Can you think of any advantages of a system that did not use an intermediate molecule? If so, what might they be?

4. Scientists believe the earliest forms of life did not have both DNA and RNA, but they were able to make proteins. Based on the processes described here, which do you think evolved first, DNA or RNA? In other words, which molecule could you more easily imagine a cell living without and still being able to make protein?

Expected Results

Expected results

Listed below are expected results for each reaction accompanied by a brief explanation. Note that exact timing of results may differ based on incubation temperature or other factors, but the overall trends should not change. You may also observe small differences in brightness across different groups which can arise from variability in micropipetting and sample handling.

Tube 1 (Negative control):

Students added only water with no DNA to tube 1, so no green or red fluorescence is expected throughout the course of the experiment. Students may detect low levels of autofluorescence, as the components in BioBits® pellets will show low levels of fluorescence on their own. This low level of autofluorescence can be used to demonstrate the importance of including negative controls in experiments. Absent a negative control, baseline levels of autofluorescence may be mistaken for a true reaction product.

Tube 2 (Reference reaction):

Students added DNA A, encoding both the RNA aptamer sequence and the coding sequence for a red fluorescent protein. Students can expect observable green fluorescence after ~15-30 minutes, arising from the RNA aptamer and indicating the presence of messenger RNA. Red fluorescence, indicating the presence of red fluorescent protein, should become visible after three to four hours and reach full fluorescence after approximately eight hours. The red fluorescent protein will remain visible for at least 1-2 weeks.

Tube 3 (Experimental reaction - students predict the result):

Students added DNA A, encoding both the RNA aptamer sequence and the coding sequence for a red fluorescent protein. They then added the translational inhibitor kanamycin. Students can expect to observe green fluorescence after ~15-30 minutes, indicating the presence of messenger RNA (as in tube 2). This green fluorescence arising from the RNA aptamer will fade after three to four hours and will not be visible the following day. This is because RNA is typically transient and degrades over time (for a more detailed explanation, see “Additional observations and explanations” below). Students are not expected to observe any red fluorescence. This is because kanamycin will interfere with translation by binding to the ribosome, causing misalignment of the mRNA during translation. This will result in non-functional mistranslated protein, or no protein at all.

Tube 4 (Experimental reaction)

Students added DNA B to tube 4. DNA B contains a promoter sequence, the RNA aptamer sequence, and a transcription termination sequence. DNA B does not contain a protein coding sequence. Students should observe green fluorescence after ~15-30 minutes, indicating the presence

of RNA (as in tube 2 and 3). This green fluorescence will fade after five to six hours and will not be visible when viewed the following day. This is because RNA is typically not stable in solution and is expected to degrade over time (for a more detailed explanation, see "Additional observations and explanations" below). Since DNA B is missing a protein coding sequence, students will not observe any red fluorescence as no protein is being translated.

Students should be able to reach the conclusion that there has been some change to the coding sequence of the DNA, but they will not have enough information to definitively conclude that the coding sequence is missing entirely. Other reasonable explanations that students may suggest for what occurred in tube 4 include but are not limited to:

- DNA B has a mutation in the protein coding sequence that makes the protein non-functional.
- DNA B is missing either start codon or a stop codon.
- DNA B has a mutation that introduces a premature stop codon.

Additional observations and explanations

Observations concerning green fluorescence:
In tubes 2, 3, and 4, the green fluorescence (indicating messenger RNA) will be visible after ~15-30 minutes, but will begin to fade after a few hours and will no longer be visible the next day. This is because messenger RNA is not stable and will begin to break down in solution over time, likely due to the action of RNases present in the BioBits® pellet. Furthermore, the fluorescent RNA aptamer is selected for optimal function at 37°C. This is why the BioBits® reactions must be incubated at 37°C before initially viewing the green fluorescence. Changing the temperature of the reaction may affect the function of the RNA aptamer, thus reducing fluorescence, independent of RNA degradation.

Students may additionally observe that the amount of green fluorescence varies over time across the three different tubes. This is true even though all three tubes are producing mRNA.

For tube 2, fading of the green fluorescence may not be observed, as the green fluorescence fades around the same time as the red fluorescence starts becoming visible (three to four hours after the start of the reaction), masking the loss of green fluorescence. Students may notice a shift from green fluorescence to yellow/orange fluorescence before observing the expected red fluorescence. Yellow/orange fluorescence will occur when both the green fluorescent aptamer and the red fluorescent protein are expressed simultaneously. Eventually, the green fluorescence from the aptamer will fade, as the red fluorescent protein reaches its peak brightness, leaving only the red fluorescence visible at around 8 hours.

For tube 3, fading of the green fluorescence is typically observed after three to four hours.

For tube 4, the green fluorescence may appear brighter than tubes 2 or 3 and the fading of the

green fluorescence is not observed until around hour five to six. This brighter, longer lasting green fluorescence may be due to the fact that DNA B does not contain a protein coding sequence or ribosomal binding site, thus extending the effective life of the RNA aptamer. Using this fact, you may invite your students to offer plausible hypotheses that would explain this observation.

Observations concerning red fluorescence:
In tube 2, it typically takes approximately 8 hours for full red fluorescence to be observed. This may seem longer than would typically be expected for most proteins to be expressed; theoretically, a protein of the size made in this lab should be able to be fully translated and folded within just a few minutes of the start of transcription. However, fluorescent proteins undergo a post-translational process called maturation. Maturation involves the spontaneous rearrangement of amino acids at the center of the protein to form the mature chromophore (the part of the protein responsible for the fluorescent color). Depending on the specific fluorescent protein and the system in which it is expressed, this process can take as little as under ten minutes or as long as several hours. The red fluorescent protein used in this lab has a long maturation time. This means that even though the first mRNAs are likely translated within minutes, it takes several hours for enough fluorescent proteins to complete maturation to be visible.

Optional extension questions
If you would like to explore these ideas and explanation with your students, here are optional relevant questions that can be used to inspire classroom discussions.

1) You may notice that the green fluorescence begins to fade in tubes 2-4 after a few hours and is the same as the baseline negative control the next day. Propose an explanation of why you believe this green fluorescence disappears.

2) If you were able to observe your tubes after 3-4 hours, you may have noticed orange or yellow fluorescence in tube 2, even though no orange or yellow fluorescent molecules or proteins are present in this reaction. Propose an explanation of where the orange/yellow fluorescence came from and why they “disappeared” over time.

3) If you were able to observe your tubes after 3-4 hours, you may have noticed that while tube 3 no longer had visible green fluorescence, tube 4 still had green fluorescence, which would not have faded until around hour 5-6. Since the DNA used in tube 4 (DNA B) does not contain a protein coding sequence or ribosomal binding site, the resulting mRNA will 1) be shorter than the mRNA resulting from DNA A and 2) not bind to the ribosome. Propose an explanation of why DNA B may result in a longer visible green fluorescence than DNA A.

4) While green fluorescence is visible within minutes, red fluorescence is only visible after 3-4 hours and does not reach full fluorescence until around hour 8. Research typical rates of transcription and translation. Do the times observed here match typical protein expression rates? Investigate the steps involved in the expression of fluorescent proteins specifically. Using this information, can you propose an explanation for the fluorescence times you observed in this reaction?

Additional Student Supports and Extension Activities

Overview

The activities in this section are provided as optional activities to do in conjunction with this BioBits® lab. These activities are not included in the Student's Guide.

Additional supports

The following optional supports are to help provide additional scaffolding for students who need it.

Micropipetting 101 – Students performing this lab should be familiar with proper micropipette technique and be able accurately to pipette volumes in the 2-5 µL range. This introductory pipetting activity will introduce students to proper pipetting technique and have them practice pipetting a variety of volumes. (http://www.minipcr.com/micropipetting/)

Alternatively, have students practice pipetting small volumes of food coloring beforehand.

Extension activities

This BioBits® lab is based on cutting-edge freeze-dried, cell-free (FD-CF) technology. This central dogma lab is just one example of many uses that FD-CF has in education and in scientific research. For students curious about the different applications of FD-CF technology, we encourage them to read through these extension activities and answer the study questions to learn more about how FD-CF reactions work and how they are used by scientists in real-world scenarios.

1. Cell-free protein synthesis: This BioBits® lab uses cell-free protein synthesis technology to create a low-cost and easy-to-use hands-on activity. Read more about how cell-free reactions work and its different real-world applications. DNAdots include articles and study questions. Browse free articles at: dnadots.minipcr.com

DNAdots™ Simple explanations of modern genetic technologies

2. RNA aptamers: The green signal associated with RNA and transcription in this lab is made possible with a structure called an RNA aptamer. If students are curious about how RNA aptamers work and the role they played in the lab, this activity will have the students read more about RNA aptamers and answer study questions about them.

3. Crick and the central dogma: Read more about about the history of Francis Crick's lecture in this 60th anniversary article from PLoS Biology. Article is available for free download at the link below, or search using the following citation: Cobb M (2017) 60 years ago, Francis Crick changed the logic of biology. PLoS Biol 15(9): e2003243. https://doi.org/10.1371/journal.pbio.2003243

Extension: RNA aptamers

What they are and how they work

In this lab, BioBits® reactions use fluorescence as a tool to observe the production of both RNA and protein. Without the use of these fluorescent markers, a BioBits® reaction would look like a clear liquid before, during, and after transcription and translation. This is because DNA and RNA are transparent in solution and proteins frequently are as well. In this lab, however, we were able to observe RNA as fluorescent green and protein as fluorescent red.

To visualize protein production, this lab used a red fluorescent protein. Fluorescent proteins come in many different colors (red and green are most common), and are common laboratory tools for visualizing cellular structures and processes. The fluorescent proteins used in laboratories are derived from those found in nature, with modifications made by scientists to make these proteins brighter and more stable. Through genetic engineering, scientists have adapted these naturally occurring proteins for biotechnology uses.

To visualize RNA, this lab used an RNA aptamer. An aptamer is a sequence of DNA or RNA that is capable of binding to a specific molecule. While there are some aptamers that occur in natural systems, most aptamers used in biotechnology, unlike fluorescent proteins, are purely synthetic—that is, they are designed from scratch by scientists for a specific purpose.

We normally think of DNA and RNA as long linear molecules. But in solution, these molecules actually twist and fold to make complex three-dimensional structures. This is because the nucleotides that make up DNA (A, T, C, and G) and RNA (A, U, C, and G) readily form hydrogen bonds and other electrostatic interactions when in solution. These nucleotides bind to each other according to the rules of base pairing — C to G and A to T or U, which is why DNA forms a double helix and how RNA is transcribed from DNA. But this binding potential also means that long RNA molecules are typically more stable as twisted, folded structures, binding to themselves and even other molecules for stability. If you are familiar with the structure of tRNAs or with the fact that the ribosome contains both RNA and protein bound to each other, you are already familiar with this phenomenon.

Synthetic biologists have taken advantage of this property of nucleic acids and have designed specific nucleotide sequences that will fold into three-dimensional structures which then fit and bind to a specific molecule of interest. They call these sequences aptamers from the Latin root *aptus,* which means “to fit”.

Aptamers function very similarly to antibodies (proteins used by the immune system to specifically target pathogens) and can be used in many of the same scenarios that antibodies are used. The key difference is that antibodies are made of protein, while aptamers are comprised of nucleic acids. This gives aptamers some unique properties. They can be added on to longer nucleic acid sequences much like protein tags can be added to protein sequences. They also tend to be significantly smaller than antibodies, and so in some cases, can better infiltrate tissues and cells for scientists to visualize these components. The bonds formed by aptamers tend to be weaker and more susceptible to changes in temperature, which is the main reason why you incubated the BioBits® reactions at or close to 37°C when waiting to view the green fluorescent signal. RNA also tends to be degraded relatively quickly in cells, and so RNA aptamers often have a much shorter lifespan once introduced to the cellular environment.

In this lab, a sequence was added to the DNA so that when it is transcribed, the RNA will form an aptamer. This aptamer binds to a fluorescent molecule very similar to the chromophore found in green fluorescent protein (GFP). At the heart of any fluorescent protein is the *chromophore,* a small molecular structure that gives the protein its color. In the case of GFP, three amino acids in the center of the protein are modified after translation to make the chromophore. The rest of the protein structure stabilizes the chromophore and creates the correct molecular environment for it to fluoresce.

Scientists have made a synthetic version of the GFP chromophore (Figure 1), and it is this molecule that is in the BioBits® pellet. The chromophore alone however is not fluorescent in solution; to fluoresce, it requires a stabilizing structure that will provide the correct molecular environment. (This is why the BioBits® reaction does not fluoresce green immediately when water is added.) Instead of using a protein, scientists developed an RNA aptamer to do just that. When this aptamer is transcribed from DNA to RNA, it will fold up on itself and make a specific three-dimensional structure that holds the chromophore in just the right way to allow it to fluoresce brightly.

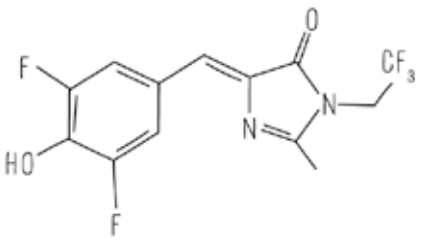


Figure 1. The chromophore used in this lab closely resembles the chromophore found in GFP proteins.

Because the chromophore that this aptamer binds to will fluoresce green, the aptamer was given the name Broccoli. By inserting the Broccoli sequence between the promoter and the start codon of a coding sequence, when that particular mRNA is transcribed, the aptamer sequence will fold up and bind to the chromophore if it is present. The rest of the RNA is unchanged and will function normally. In the case of this lab it will go on to be translated by the ribosome, producing the red fluorescent protein you observed. To make the RNA fluoresce even brighter, two broccoli aptamers in a row are present in the RNA sequence.

The Broccoli aptamer was developed as a tool for scientists to image RNA in living cells. By adding the Broccoli aptamer to a DNA sequence of interest, researchers can visually establish exactly when that DNA sequence is transcribed, and even trace where in the cell the RNA goes. In this way, the use of the Broccoli aptamer in BioBits® reactions mirrors the way researchers are using it in the lab, but put in your hands using simple tools.

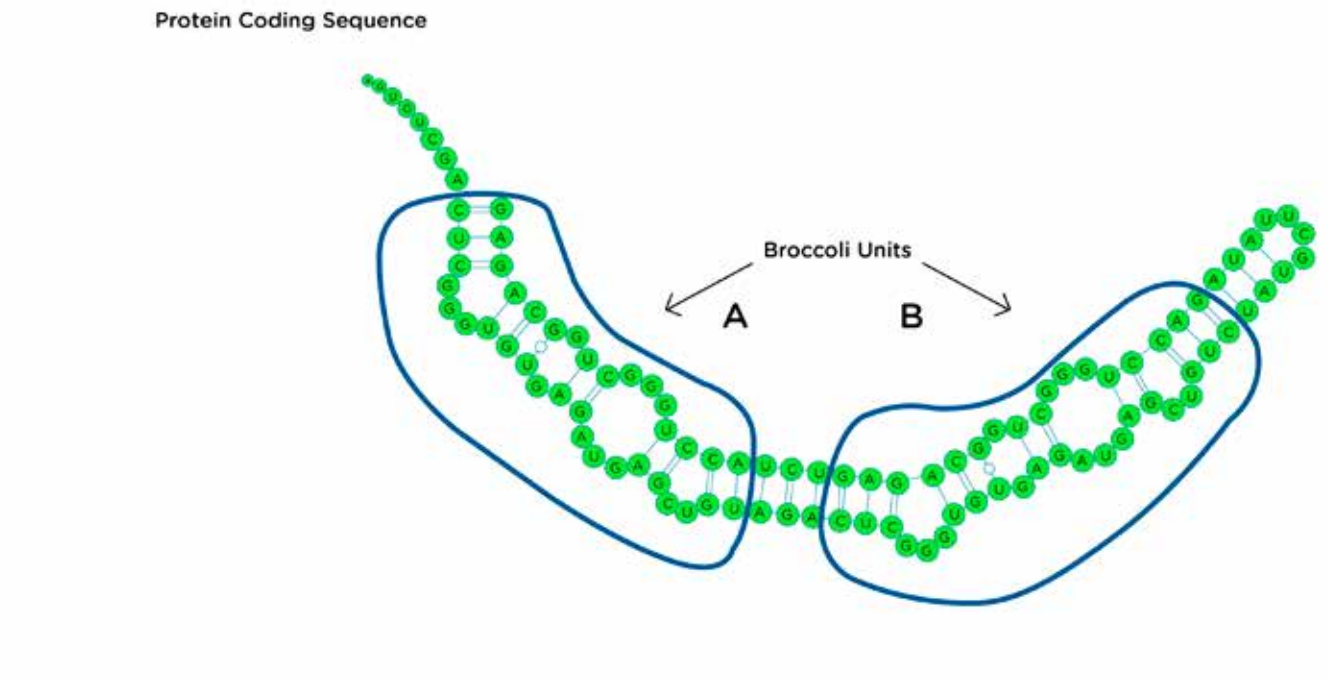


Figure 2. A single RNA strand folds into a double stranded structure. The circled regions will each bind to a chromophore in solution.

Adapted from: Filonov, G.S., Moon, J.D, Svenson, N. and Jaffrey, S.R. (1994). Broccoli: Rapid Selection of an RNA Mimic of Green Fluorescent Protein by Fluorescence-Based Selection and Directed Evolution. Journal of the American Chemical Society, 136 (46), pp. 16299-16308.

Study questions

1. How is an RNA aptamer similar to a protein? How is it different?

2. Why do you think it would be important for scientists to track the RNA in cells, instead of tracking DNA?

3. If you looked at your freeze-dried BioBits® pellets (before adding any liquid) under the blue light, you would see the green signal. Why do you think this might be?

4. The green fluorescence from the RNA aptamer was likely gone when you viewed your sample the next day. But the fluorescent protein that was made will last for weeks? Can you suggest why that may be using information from the text?

5. RNA is generally considered a single-stranded molecule. How would you describe the structure in Figure 2?

The sequence below is the aptamer sequence as it exists in the DNA that you added to your tubes. Use the sequence and the diagram of the aptamer sequence from the text to answer the questions below.

```
GAGACGGTCGGGTCCATCTGAGACGGTCGGGTCCAGATATTCGTATCTGTCGAGTAGAGTGTGGGCTCAGATGTCGAGTAGAGTGTGGGCTC
····|····|····|····|····|····|····|····|····|····|····|····|····|····|····|····|····|····|··
CTCTGCCAGCCCAGGTAGACTCTGCCAGCCCAGGTCTATAAGCATAGACAGCTCATCTCACACCCGAGTCTACAGCTCATCTCACACCCGAG
```

6. Which strand of the DNA (top or bottom) was the aptamer transcribed off. In other words, which strand does the RNA polymerase use as a template? Add a 5' and 3' label to each strand of DNA if you are able.

7. Find the broccoli units in the DNA template sequence (the strand you indicated in Question 6). Circle the parts of the sequence that are part of a broccoli unit. Label each circle either A or B to indicate which Broccoli unit it belongs to.

8. This structure can be described as a stem-loop, also sometimes called a hairpin. Draw a vertical line in your sequence indicating where the sequence folds over to bind to itself.

9. Is the line in your sequence in the exact middle of the sequence? If not, explain how the point where the sequence folds to bind to itself is not in the middle. Mark on the sequence with an asterisk(*) the regions that cause this asymmetry.

Teacher's Notes

Teacher's notes and placement in unit

Teacher's notes

Before performing this lab students should have the following skills/background knowledge:

- Basic competence using a micropipette
- Basic understanding of DNA, RNA and protein
- Understanding of proteins and their synthesis

See the additional supports section of this lab for ways to scaffold this assignment for students who may be less comfortable with the above skills.

This lab is designed to be performed in classes ranging from introductory life science to advanced biotechnology classes. For more introductory classes, focus most strongly on the order of colors that you will observe and what those colors represent. For more advanced classes, dive deeper into the molecular processes and specific molecules that are active in each step of the reaction, as well the cell-free technology used in the activity.

Placement in unit

Transcription and translation

This lab is designed for students to experiment with transcription and translation in an authentic and meaningful way. Use this lab to reinforce concepts at the end of your transcription and translation unit or at the beginning of a unit as a way to introduce the concepts and important biological molecules involved.

Biotechnology and synthetic biology

This lab uses cutting-edge freeze-dried cell-free technology. Use this lab to introduce students to the field of synthetic biology, focusing on the potential uses of cell-free technology. As part of this approach follow this lab up by reading more about cell-free technology in DNAdots™, a free resource from miniPCR bio™ (dnadots.minipcr.com). Also, have your students learn more about the aptamer technology used in this lab in the included extension.

Learning goals and skills developed

Student Learning Goals – students will:

- Distinguish between individual steps of the central dogma of molecular biology
- Describe the function of each step of the central dogma and their relationship to each other
- Predict how manipulating different steps in the central dogma will affect observations
- Analyze and construct an explanation based on the results of an unknown reaction

Scientific Inquiry Skills – students should be able to:

- Formulate hypotheses and predict results
- Compare results to their predictions and draw conclusions based on hypotheses
- Identify potential sources of experimental error and their impact

Molecular Biology Skills:

- Micropipetting
- Use of fluorescence to identify nucleic acids and proteins
- Cell-free protein synthesis

Standards alignment

Next Generation Science Standards alignment

Students who demonstrate understanding can:

HS-LS1-1.	Construct an explanation based on evidence for how the structure of DNA determines the structure of proteins which carry out the essential functions of life through systems of specialized cells.
HS-LS3-1.	Ask questions to clarify relationships about the role of DNA and chromosomes in coding the instructions for characteristic traits passed from parents to offspring.

Science and Engineering Practice	Disciplinary Core Ideas	Crosscutting Concepts
• Asking Questions and Defining Problems • Developing and Using Models • Planning and Carrying Out Investigations • Analyzing and Interpreting Data • Constructing Explanations and Designing Solutions • Engaging in Argument from Evidence • Obtaining, Evaluating, and Communicating Information	**LS1.A: From Molecules to Organisms: Structures and Processes** •All cells contain genetic information in the form of DNA molecules. Genes are regions in the DNA that contain the instructions that code for the formation of proteins, which carry out most of the work of cells. (HS-LS1-1) **LS3.A: Inheritance of Traits** • Each chromosome consists of a single very long DNA molecule, and each gene on the chromosome is a particular segment of that DNA. The instructions for forming species' characteristics are carried in DNA. All cells in an organism have the same genetic content, but the genes used (expressed) by the cell may be regulated in different ways. Not all DNA codes for a protein; some segments of DNA are involved in regulatory or structural functions, and some have no as-yet known function. (HS-LS3-1)	• Patterns • Cause and Effect • Scale, Proportion, And Quantity • Systems and System Models • Energy and Matter • Structure and Function • Stability and Change • Interdependence of Science, Engineering, and Technology • Influence of Engineering, Technology, and Science on Society and the Natural World

Common Core ELA/Literacy Standards

RST.9-10.1	Cite specific textual evidence to support analysis of science and technical texts, attending to the precise details of explanations or descriptions.
RST.9-10.3	Follow precisely a complex multistep procedure when carrying out experiments, taking measurements, or performing technical tasks, attending to special cases or exceptions defined in the text.
RST.9-10.4	Determine the meaning of symbols, key terms, and other domain-specific words and phrases as they are used in a specific scientific or technical context relevant to grades 9-10 texts and topics
RST.9-10.5	Analyze the structure of the relationships among concepts in a text, including relationships among key terms (e.g., force, friction, reaction force, energy).
RST.9-10.9	Compare and contrast findings presented in a text to those from other sources (including their own experiments), noting when the findings support or contradict previous explanations or accounts.
WHST.9-10.1	Write arguments focused on discipline-specific content.
WHST.9-10.2	Write informative/explanatory texts, including the narration of historical events, scientific procedures/ experiments, or technical processes.
WHST.9-10.9	Draw evidence from informational texts to support analysis, reflection, and research.

*For simplicity, this activity has been aligned to high school NGSS and grades 9-10 Common Core standards. This lab is easily aligned to other grade levels as well.

Ordering information

To request miniPCR bio™ Learning Labs reagent kits, you can:

Call (781)-990-8PCR

email us at orders@minipcr.com

visit www.minipcr.com

BioBits® Central Dogma kit (catalog no. KT-1910-01) contains the following reagents:

- 32 BioBits® pellets in 8-tube PCR strips
- DNA Samples
- Nuclease-Free Water
- Kanamycin

Materials are sufficient for 8 lab groups, or 32 students.
All components should be stored as indicated in the storage notes (see below).

Storage notes

* Pellets are viable for up to six months from the date of receipt when properly stored in the freezer (approx. -20°C).
* Once opened, store unused pellets in the freezer in an airtight bag with the supplied orange desiccant card.
* DNA and kanamycin can be stored for up to 12 months when kept in the freezer (approx. -20°C).
* If freezer storage is not available, the reagents may be stored in the fridge (approx. 4°C). Pellets are viable for up to three months from the date of receipt when stored in the fridge. DNA and kanamycin can be stored for up to six months when kept in the fridge.

About miniPCR bio™ Learning Labs and the BioBits® system

This Learning Lab was developed by the miniPCR bio™ team in an effort to help more students understand concepts in molecular biology and to gain hands-on experience in real biology and biotechnology experimentation.

We believe, based on our direct involvement working in educational settings, that it is possible for these experiences to have a real impact in students' lives. Our goal is to increase everyone's love of DNA science, scientific inquiry, and STEM. We develop miniPCR bio™ Learning Labs to help achieve these goals, working closely with educators, students, academic researchers, and others committed to science education.

The BioBits® cell-free system was developed after realizing that the advantages of freeze-dried, cell-free technology, such as simplicity of use, minimal equipment needs, and low cost, would be ideal for a classroom setting to illustrate biomolecular concepts to students and teach basic laboratory techniques. Researchers have been using cell-free reactions in their laboratories for years, and the BioBits® system now makes this cutting-edge technology accessible anywhere to anyone interested in learning molecular biology as an excellent teaching tool to enhance biology education both within and beyond the classroom.

The guiding premise for this lab is that a simple freeze-dried, cell-free-based experiment can visually recapitulate a fundamental biological concept using a real-life biotechnology application (BioBits®) and provide the right balance between intellectual engagement, inquiry, and discussion. Starting on a modest scale working with Massachusetts public schools, miniPCR bio™ Learning Labs have been well received, and their use is growing rapidly through academic and outreach collaborations across the world.