miniPCR bio™

miniPCR bio™ Learning Lab

BioBits™: Central Dogma

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Student's Guide

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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.

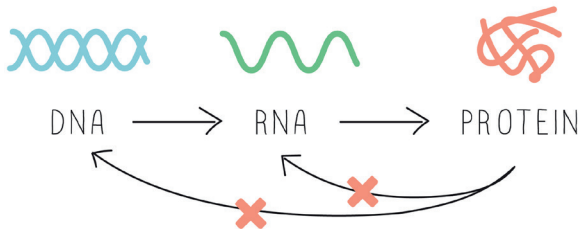


Figure 1: One schematic representation of the central dogma of molecular biology. DNA information can be transferred to RNA and used to make proteins, but proteins cannot store the information needed to make DNA, RNA or protein.

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.

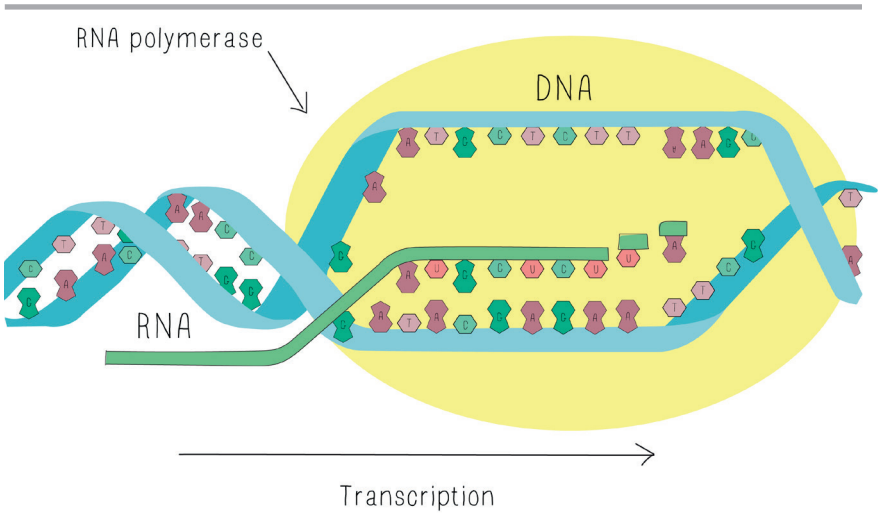


Figure 2: Transcription —the process of copying information from DNA to RNA. RNA polymerase moves down the DNA sequence and joins ribonucleotides together into mRNA.

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).

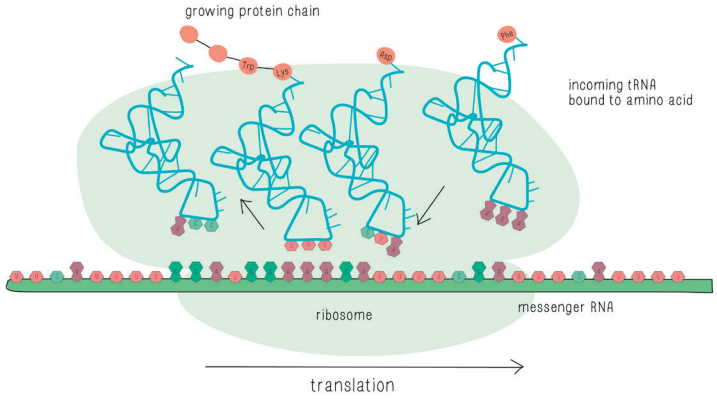


Figure 3: Translation —the process of making proteins from RNA information. At the ribosome, the mRNA is read by complementary tRNAs and each tRNA's corresponding amino acid is linked into the growing protein chain.

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.

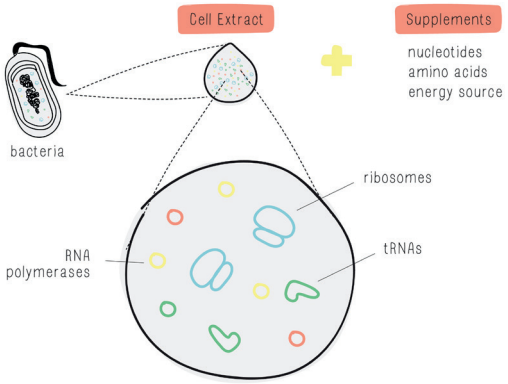


Figure 4: Essential cellular machinery can be extracted from cells and supplemented with molecular building blocks and energy to create a cell-free system that is still capable of carrying out transcription and translation.

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).

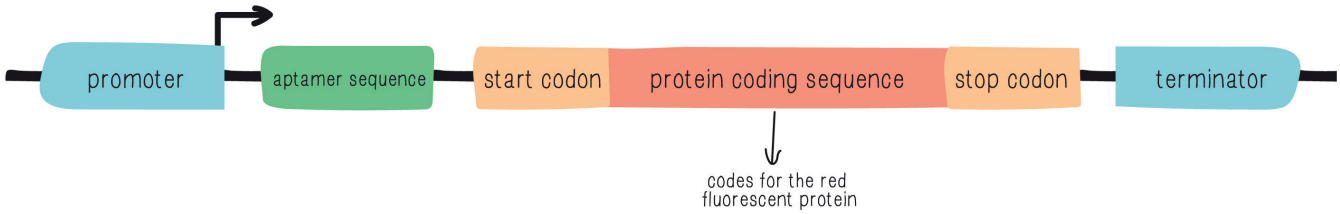


Figure 5: The structure of the DNA sequence you will be using in this lab, including the aptamer sequence for the green RNA signal and the protein coding sequence for the red fluorescent protein.

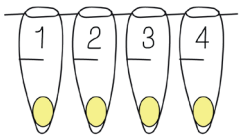
You will perform four reactions that allow you to investigate the central dogma. The first reaction will serve as a negative control, where you will add water instead of DNA. To your second reaction, you will add the DNA sample described and shown above. You will add this same DNA to your third reaction, 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

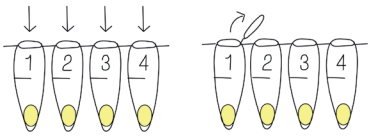
You will investigate three samples and one negative control using the BioBits™ cell-free system.

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



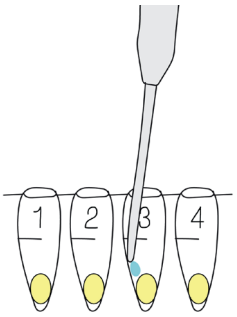
- Label the tubes 1 through 4
- Label a group name/symbol somewhere on the tubes
- Tube 1 will be for your negative control
- Tube 2 will be for your reference reaction
- Tube 3 and 4 will be for your experimental reactions
  - You will know what you added to tube 3 and will have to predict the result.
  - 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 to tubes 2 and 3.
  - Add 5 µl of DNA 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	5 µl DNA A	5 µl DNA B

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

- Add 7 µL of water to tube 1.
- Add 2 µL of kanamycin to tube 3.
- Add 2 µL of water 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	2 µl water	2 µl kan	2 µl water

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 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.
- During the 15 minutes of incubation, predict what you will see in **Table 1** (page 17) below. If you have not yet done so, also complete the pre-lab activity and questions in the **Study questions** section below (page 20).

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

- Make sure the blue light is filtered out with an orange filter.
- 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 may also continue incubating at 37°C, but this is not necessary.
- 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 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

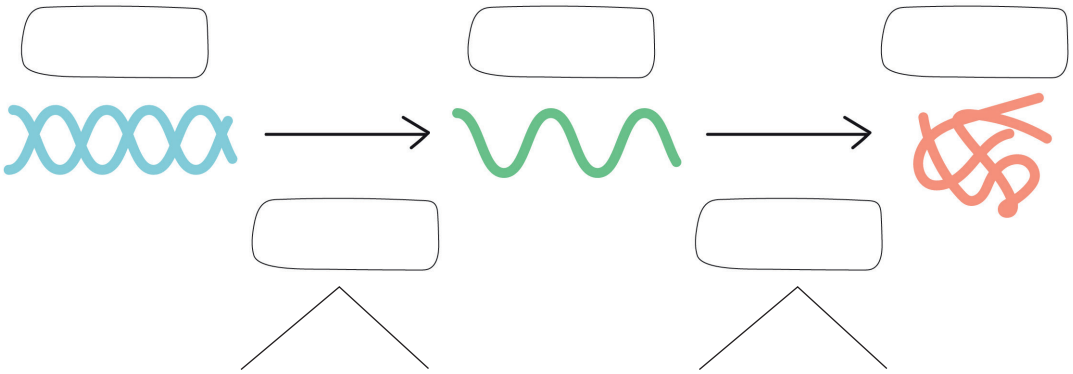
Explain clealry why the data you presented supports your claim. Include the underlying scientific principles that link your evidence to your claim

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

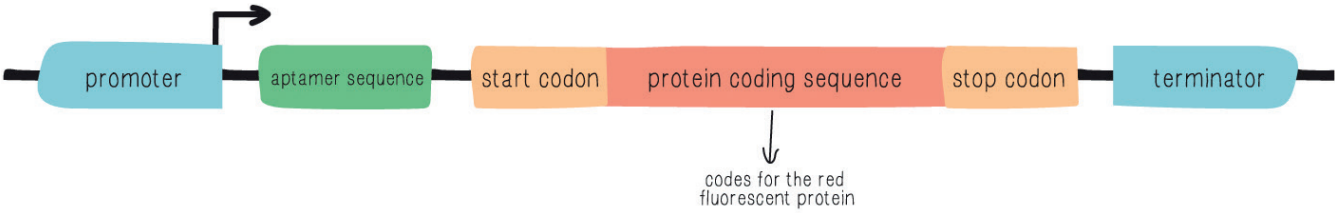
Component	Role

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, 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

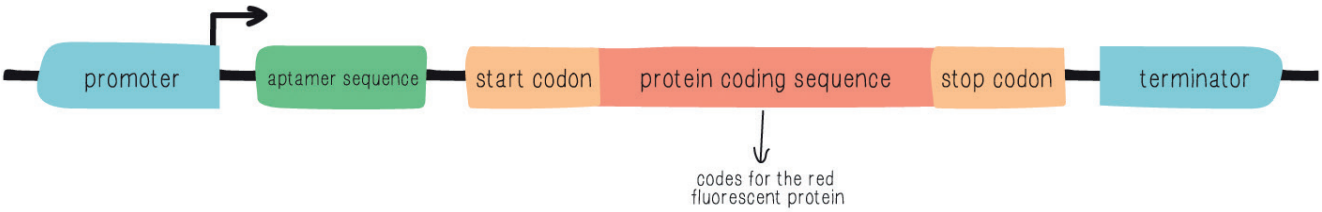
1. In this experiment, tube 1 was a negative control. Why is having a negative control important for your experiment?

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.

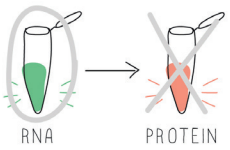
5. For each of the following statements, predict what you think might happen. For each statement circle the tube if you think it would produce RNA (and fluoresce green) and/or protein (and fluoresce red). If you do not think it will produce RNA and/or protein cross the tube out and then justify your 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.



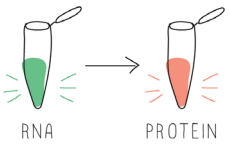
Example:

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

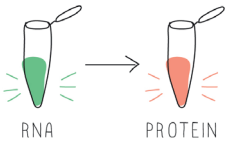
a. The presence of a promoter and terminator sequence means the mRNA would be produced correctly, but without a start codon the ribosome would never start translation properly.



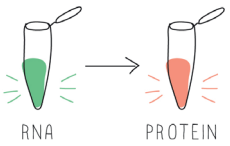
b) The coding sequence does not have promoter before it.



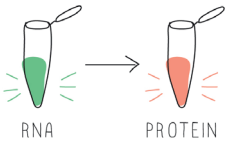
c) The coding sequence does not have a stop codon.



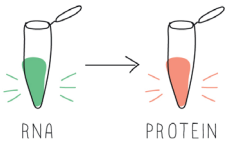
d) There is a promoter and a coding sequence but no terminator sequence.



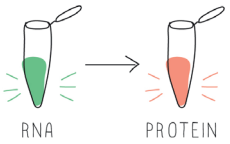
e) There is a promoter and a terminator sequence, but no coding sequence.



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?