@home
by miniPCR bio™

BioBits® @home

CENTRAL DOGMA LAB

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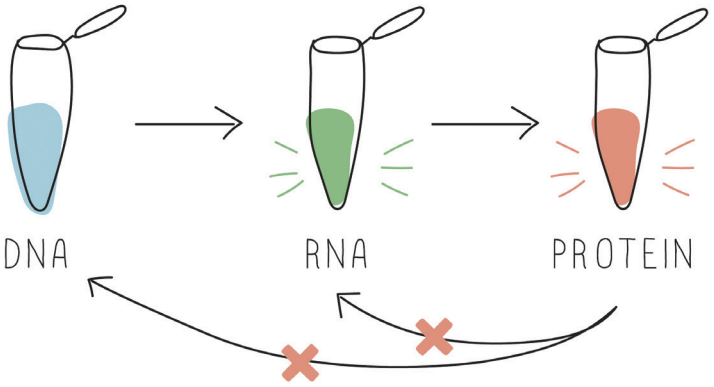

Welcome to BioBits®

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 in distance learning settings.

Overview

Today, starting with DNA, you will make RNA and protein. Using fluorescence, you will be able to watch in real time as these important biological molecules are made!

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

At a glance

DAY 1

A. Setup of BioBits® reactions

10 min.

B. Incubation and initial observations

20 min.

DAY 2

C. Final observation*

10 min.

*Final observation can be done anytime between 8 to 72 hours after setting up the BioBits® reactions (typically 24 hours).

Materials

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Storage notes:

- The reagents supplied in the kit are sufficient to perform the activity twice.
- Reagents are viable for up to 6 months when stored in the freezer from the date of receipt.
- Store any unused pellets in an airtight bag in the freezer with the supplied orange desiccant card.
- If freezer storage is not available, the reagents may be stored in the refrigerator. Reagents are viable for up to 3 months when stored in the refrigerator from the date of receipt.

Included in the kit

Reagents and supplies	Amount provided	Storage
BioBits® pellets in strip tubes • Keep BioBits® in the vacuum-sealed pouch in the freezer as long as possible	One 8-tube strip to be broken into two strips of 4 tubes	Freezer
Nuclease-Free Water	50 µl	Freezer
DNA A	40 µl	Freezer
DNA B	25 µl	Freezer
Kanamycin	20 µl	Freezer
P51™ molecular fluorescence viewer with the orange filter	1 unit	
4 µl fixed volume micropipette (minipette)	1 unit	
Disposable micropipette tips	20 tips	

All reagents and materials provided in this kit are non-hazardous and may be disposed of in the regular trash or according to local regulations. Safety data sheets may be found at https://www.minipcr.com/product/biobits-central-dogma/

Lab background

The central dogma of molecular biology

The *central dogma of molecular biology* explains that the information stored in DNA can be passed through RNA to make protein. This simple idea has been a guiding principle of molecular biology for over 50 years.

Today, we usually 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.

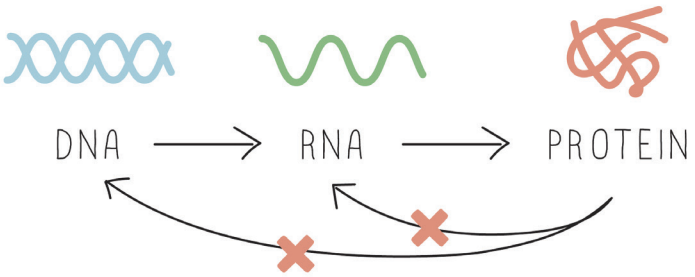

Figure 1: DNA information can be transferred to RNA and used to make proteins, but proteins cannot store the information needed to make DNA, RNA or protein.

In this activity, you will observe this flow of information from DNA to RNA to protein. But before you do, it's important to review the molecules and the processes that you will observe.

The flow of genetic information starts with DNA. DNA consists of building blocks called nucleotides arranged in a long chain. These nucleotides are the A's, C's, T's, and G's that you are probably familiar with. It is the specific order of these A's, C's, T's, and G's in the long chain of DNA that contains the information needed to make different proteins.

Transcription

Transcription is the process of transferring the information in DNA into a temporary copy called messenger RNA or *mRNA*. When transcribing DNA into mRNA, there are three important sequences of nucleotides in the DNA that help to control the process.

- The *promoter* indicates where transcription should start.
- The *protein coding sequence* is a part of the nucleotide sequence that is transcribed to the mRNA and determines the order of the amino acids in the resulting protein.
- The *terminator* signals the end of transcription.

The process starts when an enzyme known as *RNA polymerase* binds to a promoter sequence. RNA polymerase then moves down the DNA, reading one strand of the DNA and building a complementary strand of RNA (Figure 2). The four building blocks of RNA are called ribonucleotides and are structurally similar to DNA nucleotides. RNA polymerase links together free ribonucleotides to build a chain of RNA. Transcription continues until RNA polymerase reaches a terminator sequence.

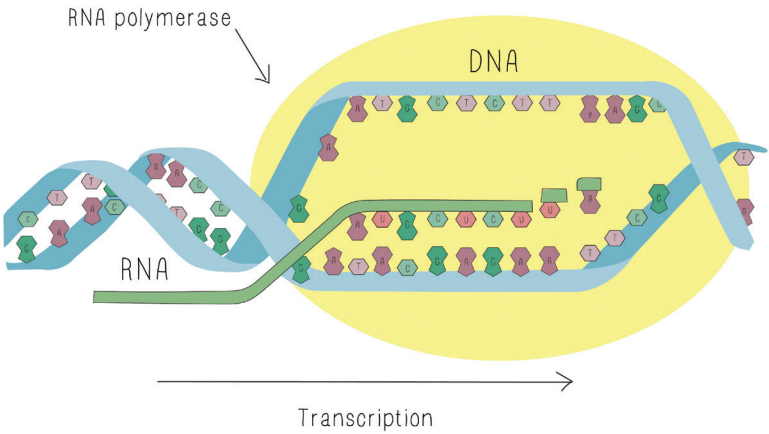

Figure 2: Transcription is the process of copying information from DNA to RNA.

Translation

The information stored in the mRNA sequence is read by the ribosome to make a protein. Proteins are made of building blocks called amino acids. Translation is the process of converting the information stored in mRNA into the specific sequence of amino acids needed for the protein being built. In translation, the mRNA nucleotides are read in groups of three known as codons, each different codon corresponding to a specific amino acid. Translation starts at a *start codon* that marks the start of the protein coding sequence.

At the ribosome, a different kind of RNA, called the transfer RNA or tRNA, is used to read the mRNA. Individual tRNAs deliver the appropriate amino acids based on the codons in the mRNA. On one end of the tRNA are three nucleotides that pair with the codon on the mRNA by rules of base pairing. On the other end of the tRNA is the amino acid. When a tRNA that matches the mRNA codon enters the ribosome, the ribosome links the amino acid onto a growing chain, building the protein. Translation continues until the ribosome reaches a stop codon, which marks the end of the protein coding sequence. A stop codon triggers the ribosome to release the newly formed amino acid chain.

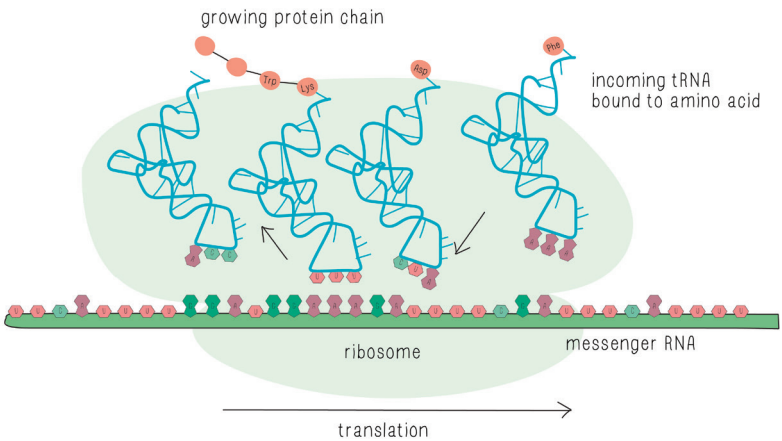

Figure 3: Translation is the process of making proteins from RNA information.

Today's lab

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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 ribonucleotides 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 living organisms.

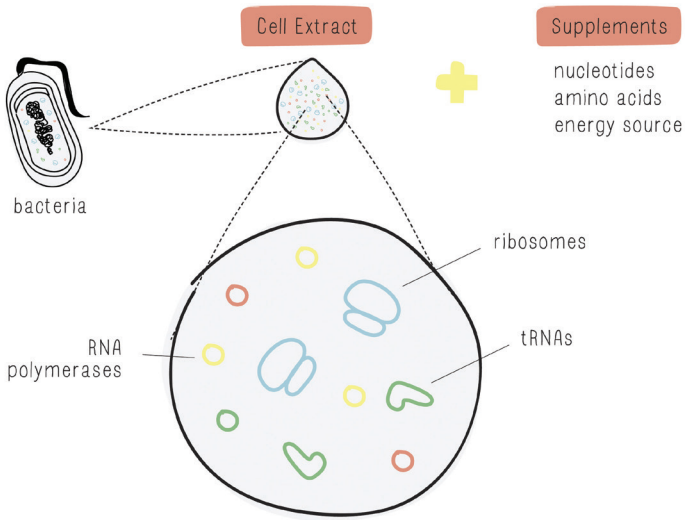


Figure 4: Essential cellular machinery can be extracted from cells and supplemented with molecular building blocks and energy to create a cell-free system that is still capable of carrying out transcription and translation.

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. 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. Observing the first step in the flow of genetic information, the transcription of DNA into mRNA, is usually difficult because mRNA is not typically visible to the naked eye. However, in this lab you will be able to visualize mRNA as it is being made! This is possible through a unique feature engineered into this gene called an *aptamer* (Figure 5). This aptamer is encoded just upstream of the protein coding sequence and it has been designed such that, when transcribed into mRNA, the aptamer will bind to a small molecule present in the BioBits® pellets. This small molecule, when combined with the aptamer, emits green fluorescence. So in this activity, the mRNA will fluoresce green, indicating that transcription has occurred! Then you will see this fluorescence turn red, which is confirmation that mRNA has been successfully translated into red fluorescent protein. The flow of genetic information from DNA to protein, by way of mRNA, is then complete (Figure 6).

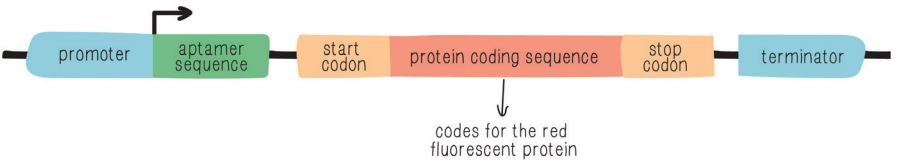


Figure 5: The structure of the DNA sequence you will be using in this lab, including the aptamer sequence for the green RNA signal and the protein coding sequence for the red fluorescent protein.

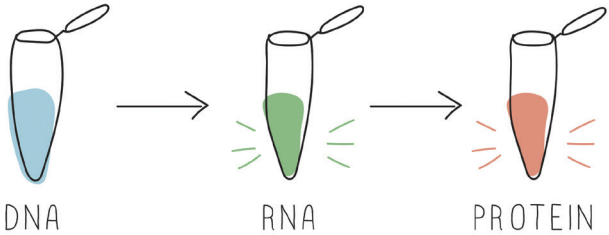
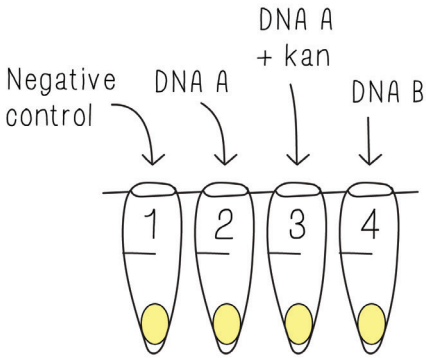


Figure 6: Green florescence signals transcription and red fluorescence signals translation.

You will perform four reactions that allow you to investigate the flow of genetic information. 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.

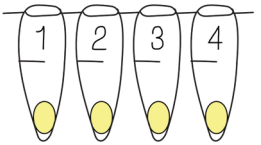


Lab Guide

A. Setup of BioBits® reactions

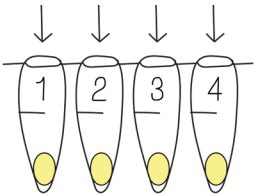
You will investigate three samples and one negative control using the BioBits® cell-free system.

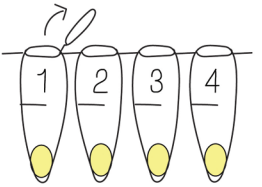
1. **Label the tubes 1 through 4**



2. **Uncap the 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.



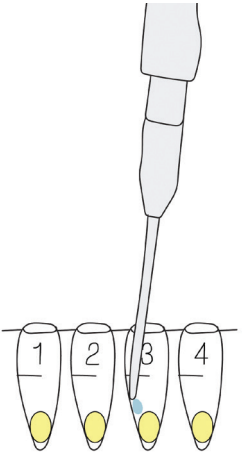


3. **Add 4 µl of DNA A to tubes 2 and 3. <u>Use a new tip for each sample</u>**

- Do not use the second stop on the pipette.

4. **Add 4 µl Of DNA B to tube 4. <u>Use a new tip for each sample</u>**

- Do not use the second stop on the pipette.
- Do not add any liquid to tube 1.



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. After step 4, your tubes should contain the following volumes:

	Tube 1	Tube 2	Tube 3	Tube 4
DNA	none	4 µl DNA A	4 µl DNA A	4 µl DNA B

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

- Add volumes according to the table below.
- To add 8 µl to Tube 1, add 4 µl twice.

	Tube 1	Tube 2	Tube 3	Tube 4
Reagent	8 µl water	4 µl water	4 µl kan	4 µl water

6. 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.

7. Immediately observe your tubes in the P51™ viewer

- Make sure the blue light is on and that the 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**
- Body temperature is about 37°C. Hold the tubes in your closed fist, under the arm, or in your pocket to warm the tubes.
- Predict what you will see in Table 1 (page 17) below during the 15 minutes of incubation.

2. **After 15 minutes, observe your tubes in the P51™ viewer**
- Make sure the orange filter is in place.
- Dim ambient lights as needed for proper observation.
- Record 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 the P51™ or laying flat on the lab bench or table.
- You may continue observing your tubes at additional time points and recording your observations.

C. Final observations. Day 2

1. **Observe your tubes in the P51™ viewer**
- Day 2 observations can be done any time 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

Additional study questions to be completed before and after the lab can be found at www.minipcr.com/product/biobits-home-central-dogma/

Table 1: Predictions

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

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 a few words. Tube 4 will be analyzed separately after you make your observations. Use the Background section of this lab and the study questions linked above for help.

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 min				
Day 2				

Table 3: What processes occured?

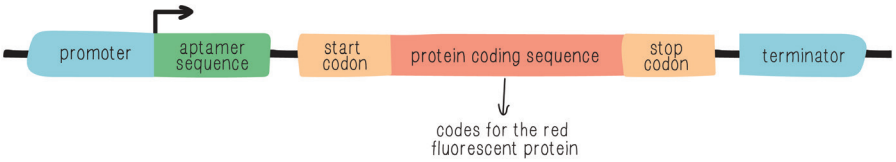
Based on the colors you observed, state whether or not you think transcription and/or translation happened in each of the tubes 1-4. Then explain whether this conclusion agrees with your initial predictions. If it does not, can you think of a reason that it may not?

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

Putting it all together

In this activity, you saw DNA be transcribed to RNA and then translated to protein—right in front of your eyes! Now that you have all your observations, let's analyze what happened in this experiment—and specifically, what happened in Tube 4.

Using the Lab Background, Tables 1-3, and the image below, answer the Claim Evidence Reasoning table below.



Claim:
What do you think is the most likely explanation for your observations in tube 4? *Hint: Focus on what might make this tube different from the other tubes. Note: There is more than one way to get the result that you observed in tube 4. You should explain just one.*

Evidence:
What evidence or data from the lab supports your claim?

Reasoning:
Why does this data support your claim?

For more activities, readings, and videos on this topic and others, check out minipcr.com/products/biobit