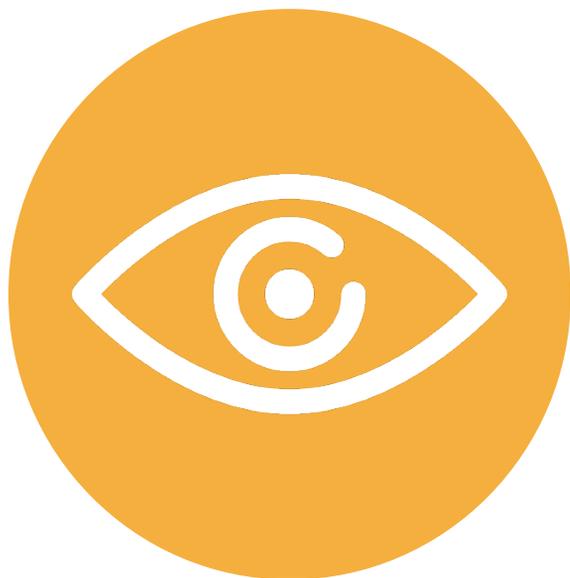


A Teacher's Guide to:



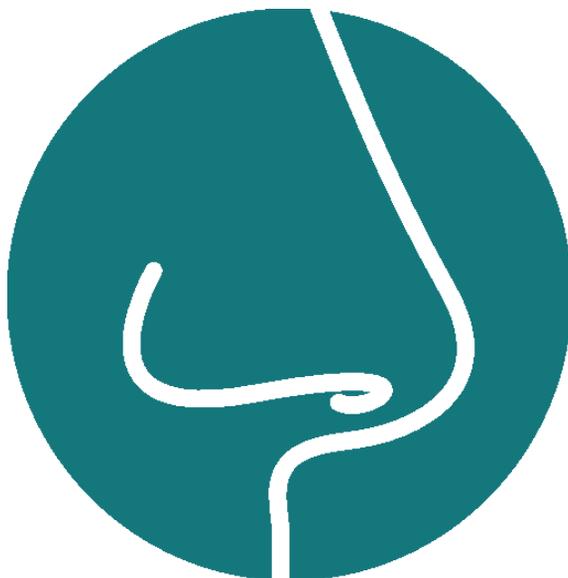
LETHBRIDGE iGEM 2017

Transcription and Translation Education Kit:



SIGHT

&



SMELL

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Learning Objectives

This connects to Unit C in the Biology 30 curriculum and addresses the concepts of transcription, translation, and genetic engineering.

Attitudes: develop positive interest in science

Knowledge: enhance student's abilities in understanding the central dogma in biology

Science, Technology, and Society: the implications that genetic engineering has within society and what further advancements will mean for everyday life

Skills: developing lab skills and good safety habits, data interpretation, experimental troubleshooting

Key Questions

- Why do we learn about transcription and translation?
 - It is a part of the Central Dogma in biology and is utilized by all living organisms
- Why do researchers manipulate organisms?
 - Manipulating organisms have led to significant scientific discoveries that have allowed for advancement in different areas, such as medicine, environmental control, etc.
- What are some concerns with using organisms to make genetically modified organisms?
 - There are concerns with environmental contamination, like uncontrolled growth. Additionally, some proteins cannot be produced using cells.

Definitions

Bacteria: a class of single cell organisms that do not contain a nucleus

BMST1: a protein enzyme that converts salicylic acid to methyl salicylate

Catalysis: helping the conversion of a reactant into a product

DNA (deoxyribonucleic acid): a series of linked nucleic acids (Adenine, Cytosine, Guanine, and Thymine) that contains the information to create your genetic make up

Enzyme: a functional molecular machine made of protein

Fluorophore: a molecule that glows when it becomes charged with energy

Gene: a unit of genetic information encoded within DNA

Methyl salicylate: produced from salicylic acid, smells of wintergreen

Protein: a functional molecule made by linking together smaller molecules called amino acids

Ribosome: the most important molecular machine that converts genetic information into proteins

Definitions

RNA (ribonucleic acid): a molecule produced from DNA that serves a variety of functions within a cell. Uracil is used instead of thymines in strands of RNA

RNA aptamer: a small, functional RNA molecule that binds a specific substrate

Salicylic acid: a component of aspirin obtained from the willow tree

Synthetic: from a chemical process not necessarily occurring in nature

Thiazole orange: a fluorophore that binds to RNA Mango aptamer

Transcription: converting DNA into an RNA message

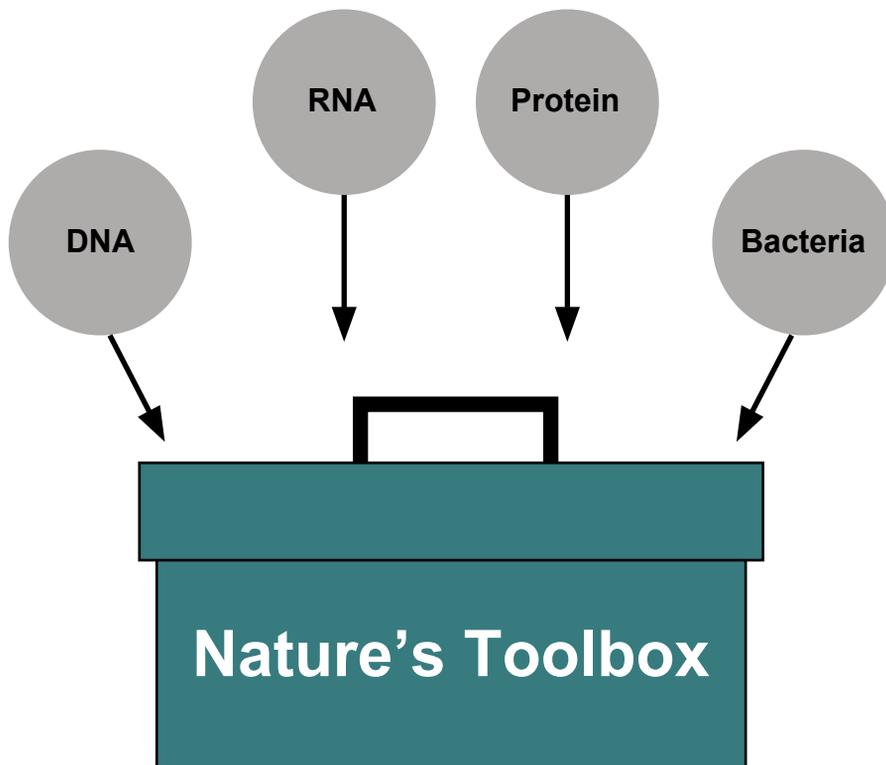
Translation: converting the RNA message into a protein

Wafting: a technique to observe the smell of a mixture at a lesser concentration

Nature's Toolbox

Naturally occurring biological systems meet the needs of their hosts, resulting in a collection described as “nature’s toolbox.”

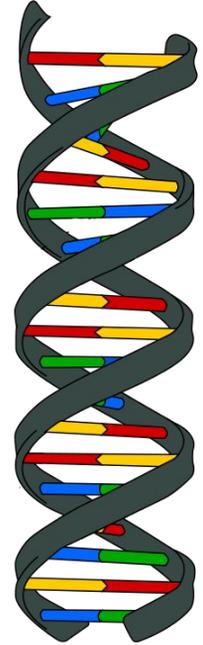
This includes DNA, RNA, protein, and the host organism (e.g. bacteria)



Nucleic Acids

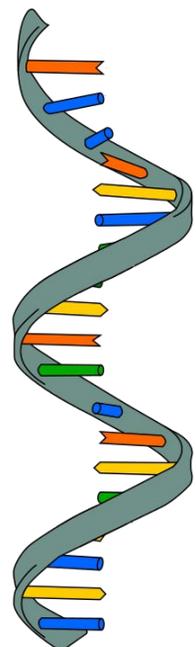
DNA

DNA is composed of four nucleic acid bases linked together in long strings. These bases are: adenine (A), thymine (T), cytosine (C), and guanine (G). Two of these DNA strings are wound together in the well-known double-stranded helix for stability and storage. The order of these nucleic acids determines the information found within a gene.



RNA

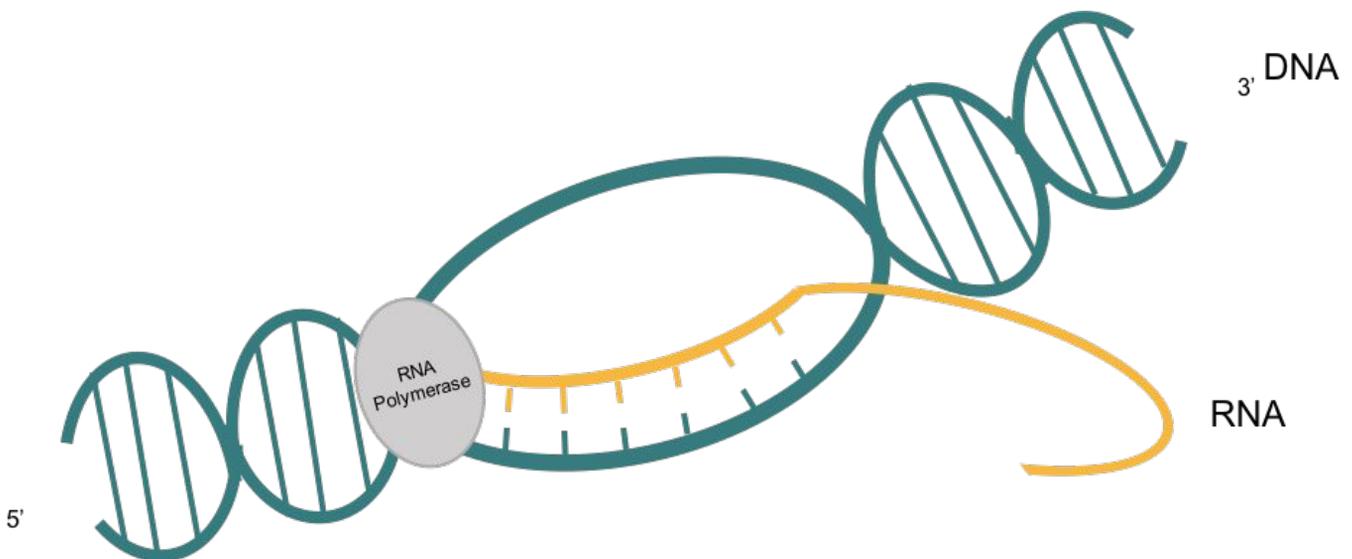
In order for the information of DNA to be used, an intermediate molecule called RNA is required. RNA is similar to DNA, except it is single-stranded and uses the bases adenine, cytosine, guanine, and uracil (U) instead of thymine. RNA contains the message of the DNA in a way that the cell can interpret it and produce essential proteins.



Transcription



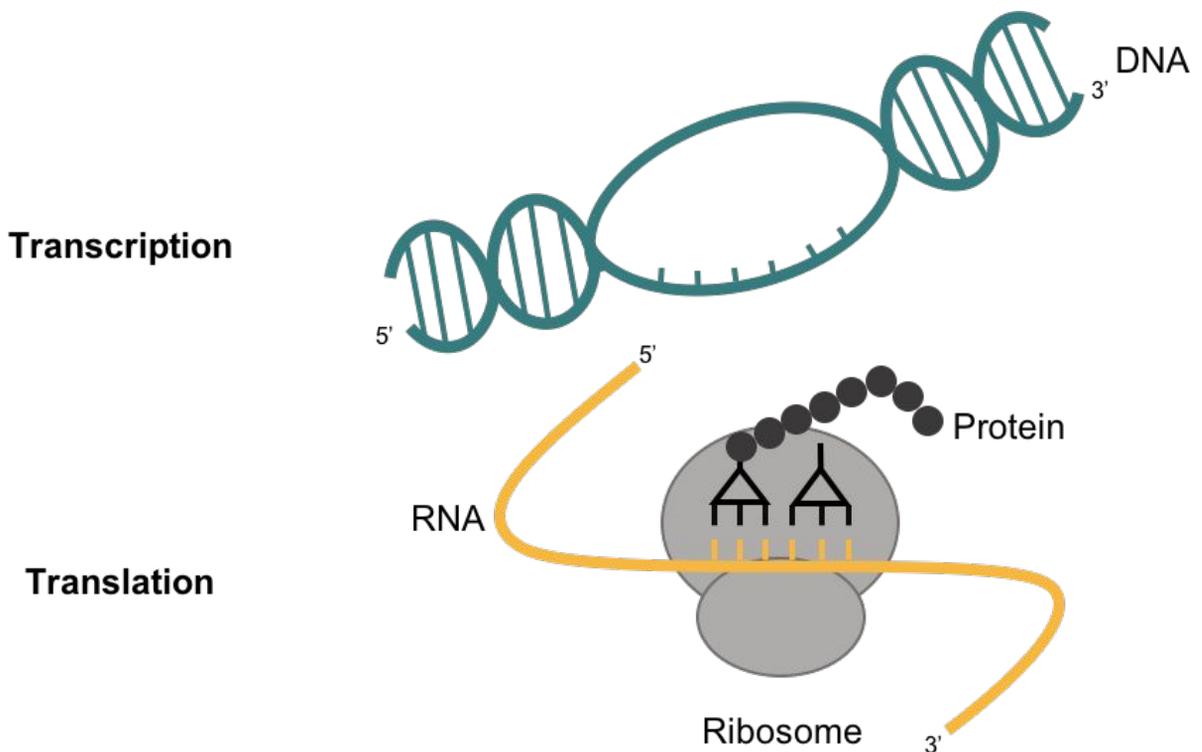
DNA contains the starting genetic material for a cell to produce specific proteins. For example, DNA can instruct the cell to produce proteins that determine hair colour or eye colour. In order to make a protein, the genetic material in DNA must be converted into RNA by a process known as transcription. This means that the DNA is used as a template to make a copy of this message called RNA. In order to do this, an enzyme called RNA polymerase, binds to the DNA and unwinds it. Then the DNA template is transcribed into RNA from the 5' to 3' ends until the RNA transcript is complete.



Translation



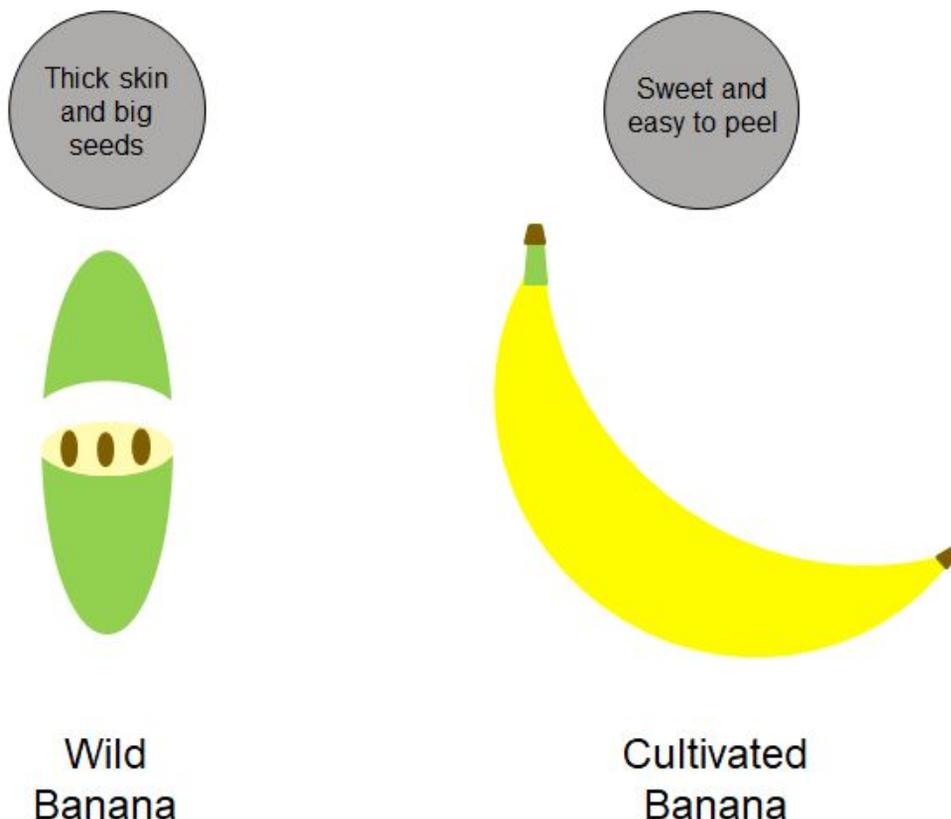
The RNA will take this genetic code to a structure in the cell called a ribosome that will takeover; producing the protein from the RNA. The ribosome binds the RNA and then decodes the information contained within the sequences of nucleotides. Three nucleotides of the genetic code are read together as a “codon.” When the ribosome reads a codon, the appropriate amino acid is incorporated into a growing chain. This sequence of amino acids is a called a peptide, which will mature into a functional protein. Proteins perform a variety of complex functions within the cell, and can be repurposed for human benefits.



Genetic Engineering

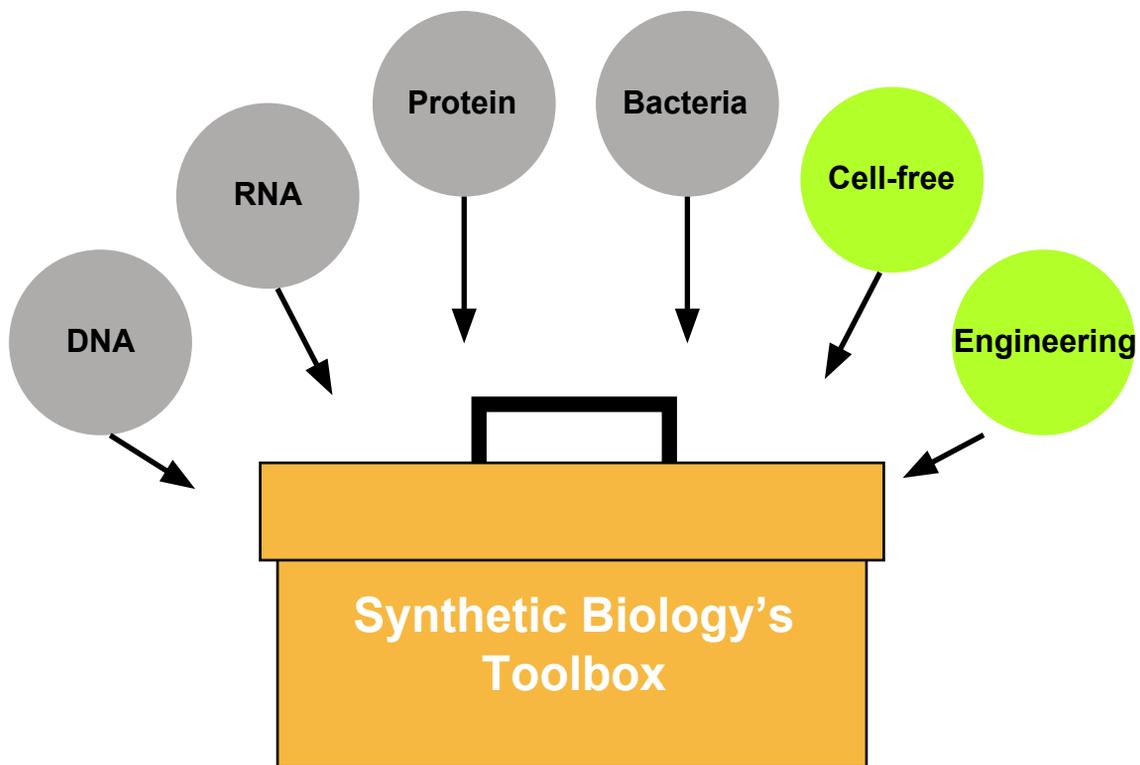
Genetic engineering has been happening since humans began to cultivate plants and domesticate animals. Incremental genetic changes caused by selective breeding have accumulated over time and manifest in new characteristics. For example, the bananas we eat have been modified over the years to appear like they do now.

The development of molecular biology tools has allowed us to greatly speed up the rate at which genetic changes can be made in organisms. Synthetic Biology is the application of engineering standards to the practice of genetic engineering in order to maintain a consistent set of molecular tools that everyone can use.



Synthetic Biology

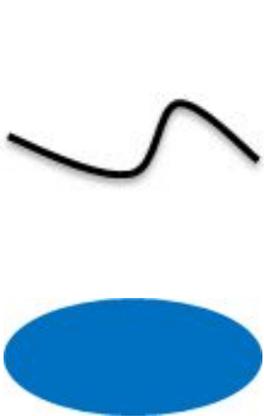
Synthetic biology is a relatively new area of research, combining both biology and engineering. In recent years, the emerging field of synthetic biology has attempted to engineer biological systems using primarily parts from nature's toolbox to create their own toolbox. Synthetic biologists can "program" biological systems and use these parts to perform a useful function. Synthetic biology can also be used to develop new biological systems and parts that are not limited by the availability of parts in nature. This further increases the possibilities of what can be achieved with synthetic biology.



Cell-free

Cells contain a large amount of DNA within their chromosomes in addition to a large amount of proteins, various nucleic acids, carbohydrates, and other small molecules that allow cells to grow, divide, and perform a variety of other functions.

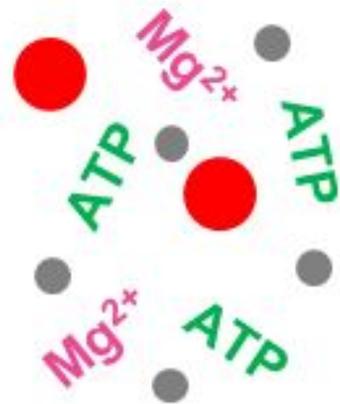
Most of these other functions are inessential for the purposes of synthetic biology, and many of the mechanisms that keep the cell in balance can make controlling the cell difficult. Accordingly, taking the essential transcription and translation machinery out of the cell and putting it into a tube retains the basic functionality of the system. This is a safe and easy way to understand the basics of molecular biology, without the difficulty of maintaining a population of living cells.



Transcription Machinery



Translation Machinery



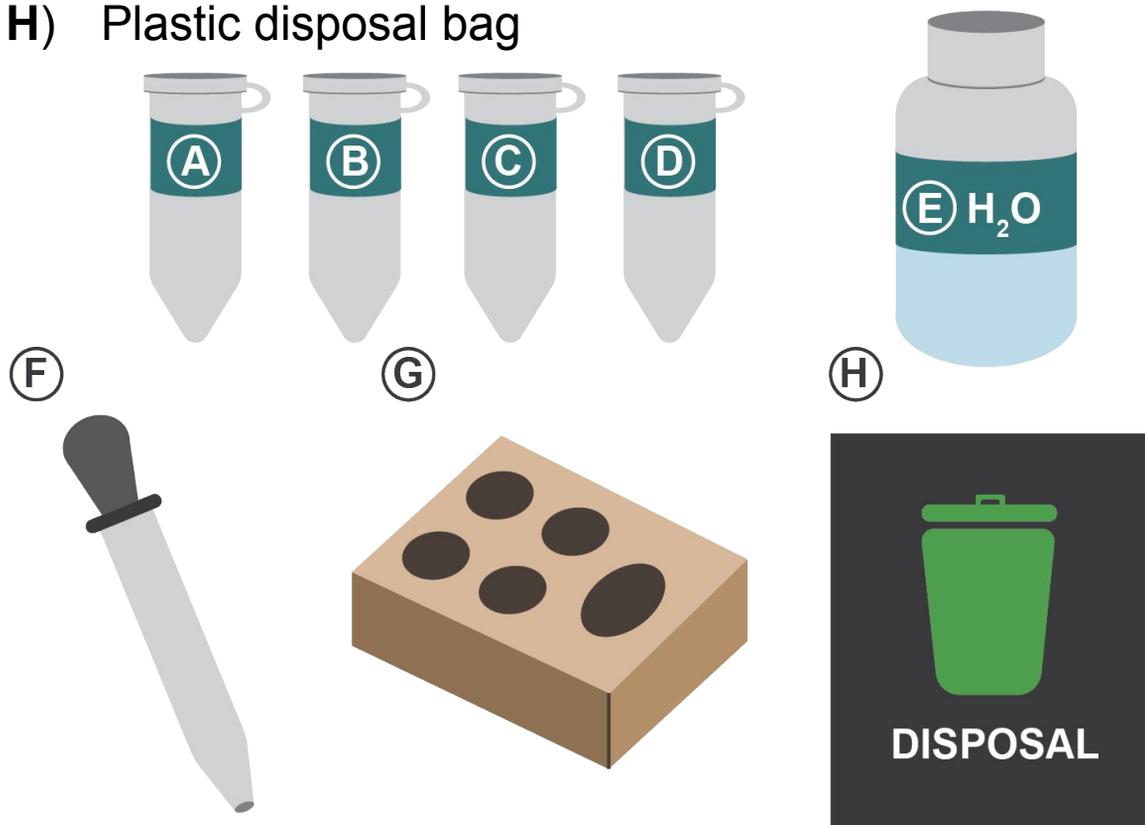
Energy Sources

Kit Contents

Each tube contains enough material for 30 reactions.

For one reaction:

- A)** Freeze-dried cellular components necessary for transcription and translation to occur
- B)** DNA coding for the BMST1 protein
- C)** Thiazole orange
- D)** Salicylic acid
- E)** Water (H_2O)
- F)** Eyedropper
- G)** Cardboard tube rack
- H)** Plastic disposal bag



Safety

Biosafety is defined as the measures that are put in place to prevent incidents from occurring in a laboratory setting. Some important safety precautions for this activity are:

- Wear gloves when handling all contents of this kit.
 - Although everything is safe to handle, it is important to practice good lab techniques.
- Clean your workspace before and after the use of this kit.
- When smelling the wintergreen scent, make sure to waft the scent towards you to observe the scent at a lesser concentration. Make sure that students don't hold the tube too close to their face when trying to detect the scent.
- Disposal: All components can be disposed of in the provided disposal bag that will go through further processing before actual disposal.

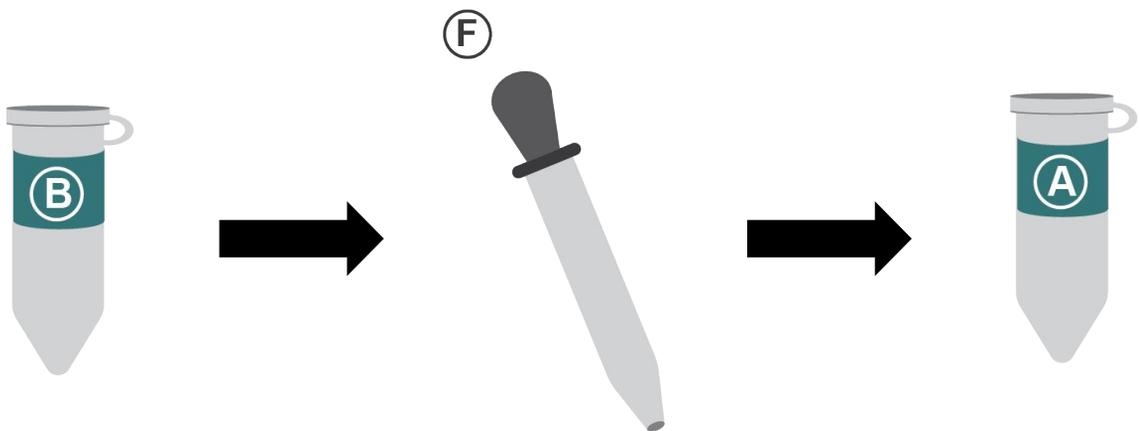
Instructions

Transcription

1

Using an eyedropper, add at least one drop of DNA from tube B to tube A, which contains freeze-dried transcription and translation components.

- Ensure freeze-dried components are resuspended by flicking the bottom of tube A (make sure lid is closed!)
- Ensure that students fully mix the contents in the tube to make sure the reaction will occur
- Students can tap tube gently on counter to collect liquid droplets in the bottom of the tube



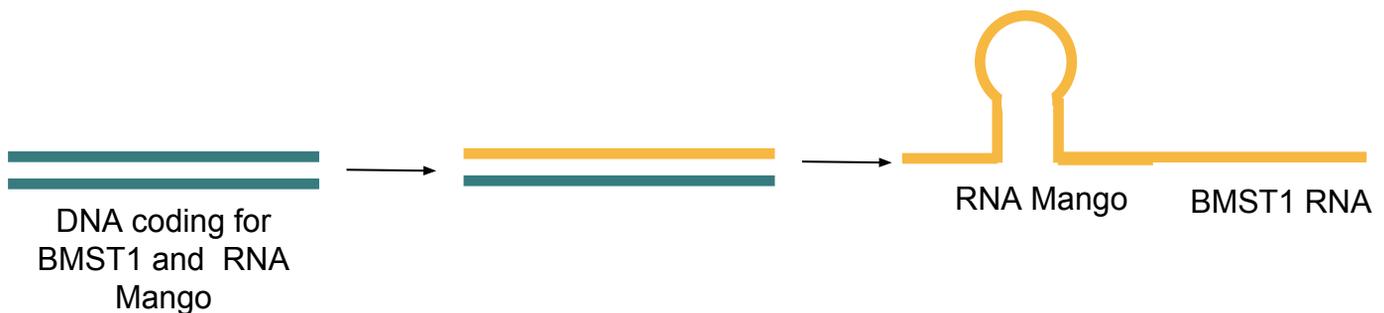
Instructions

Transcription

1

Using an eyedropper, add at least one drop of DNA from tube B to tube A, which contains freeze-dried transcription and translation components.

What is Happening?



Note: RNA Mango and BMST1 are encoded for on the same RNA strand. They are separated to show that both are being produced.

- Ensure that students make the connection that the orange strand encodes for the RNA Mango aptamer AND the BMST1 protein
- You can also explain that since RNA is single stranded, it is able to change its shape

Instructions

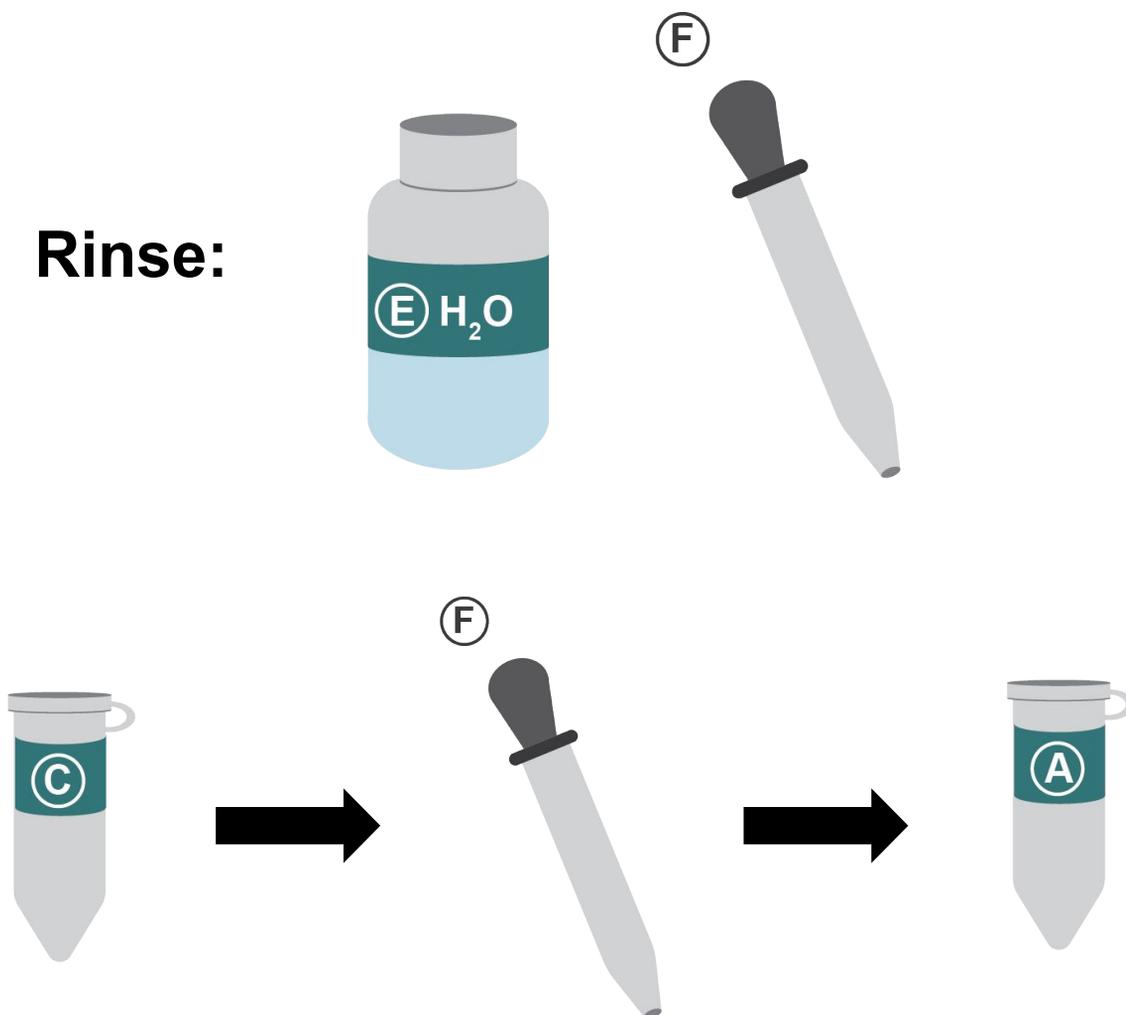
Transcription

2

Add at least one drop from tube C, containing thiazole orange, to tube A.

- Rinse eyedropper with tap water before this step. Dry by tapping on paper towel.
- Please make sure that students understand that if they don't rinse the eyedropper, this can lead to contamination of the tubes!

Rinse:



Instructions

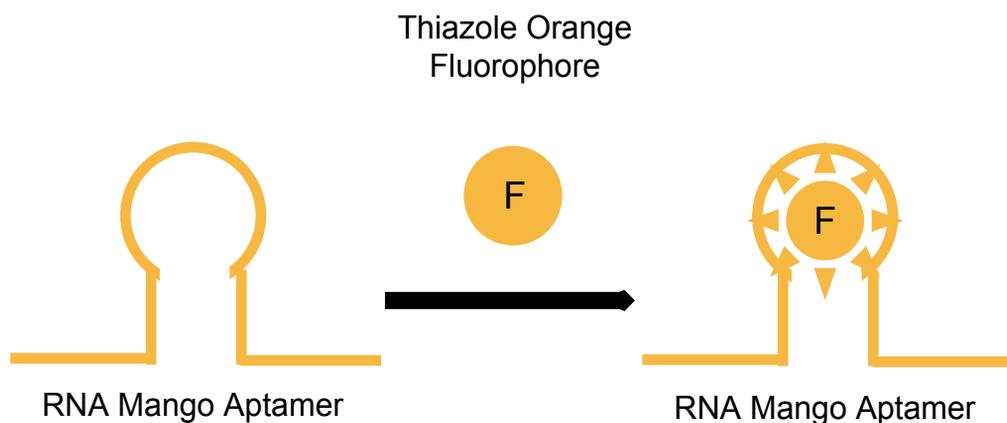
Transcription

2

What is Happening?

Thiazole orange is a fluorophore. A fluorophore emits a fluorescent signal when it is bound to an aptamer. If DNA is transcribed into RNA, then RNA Mango will be available for the thiazole orange to bind to it. The binding of thiazole orange to RNA Mango will cause it to fluoresce a bright orange colour. The production of an orange colour therefore indicates that transcription has occurred.

Research has shown that RNA Mango is capable of being seen with the naked eye so students should be able to see a colour produced. If not, they can try adding more thiazole orange to the tube.



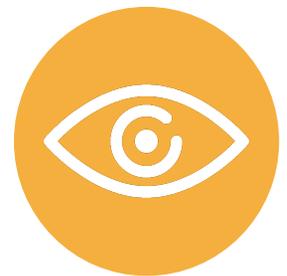
Instructions

Transcription

3

Warm tube in hand until fluorescence is observed

- Turn off lights to enhance observed fluorescence. If no color is observed, continue to mix tube A.
- The human body has a temperature of 37 °C so the heat from their hands should be warm enough to result in successful transcription and binding of thiazole orange to RNA Mango.



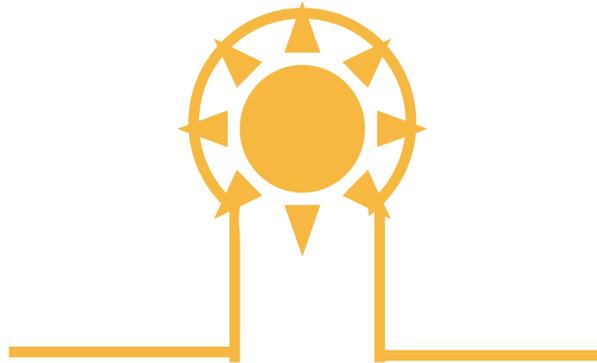
Instructions

Transcription

3

Warm tube in hand until fluorescence is observed

What is Happening?



The binding of thiazole orange to the RNA aptamer causes fluorescence. This indicates that transcription has finished and that translation is about to begin.

Instructions

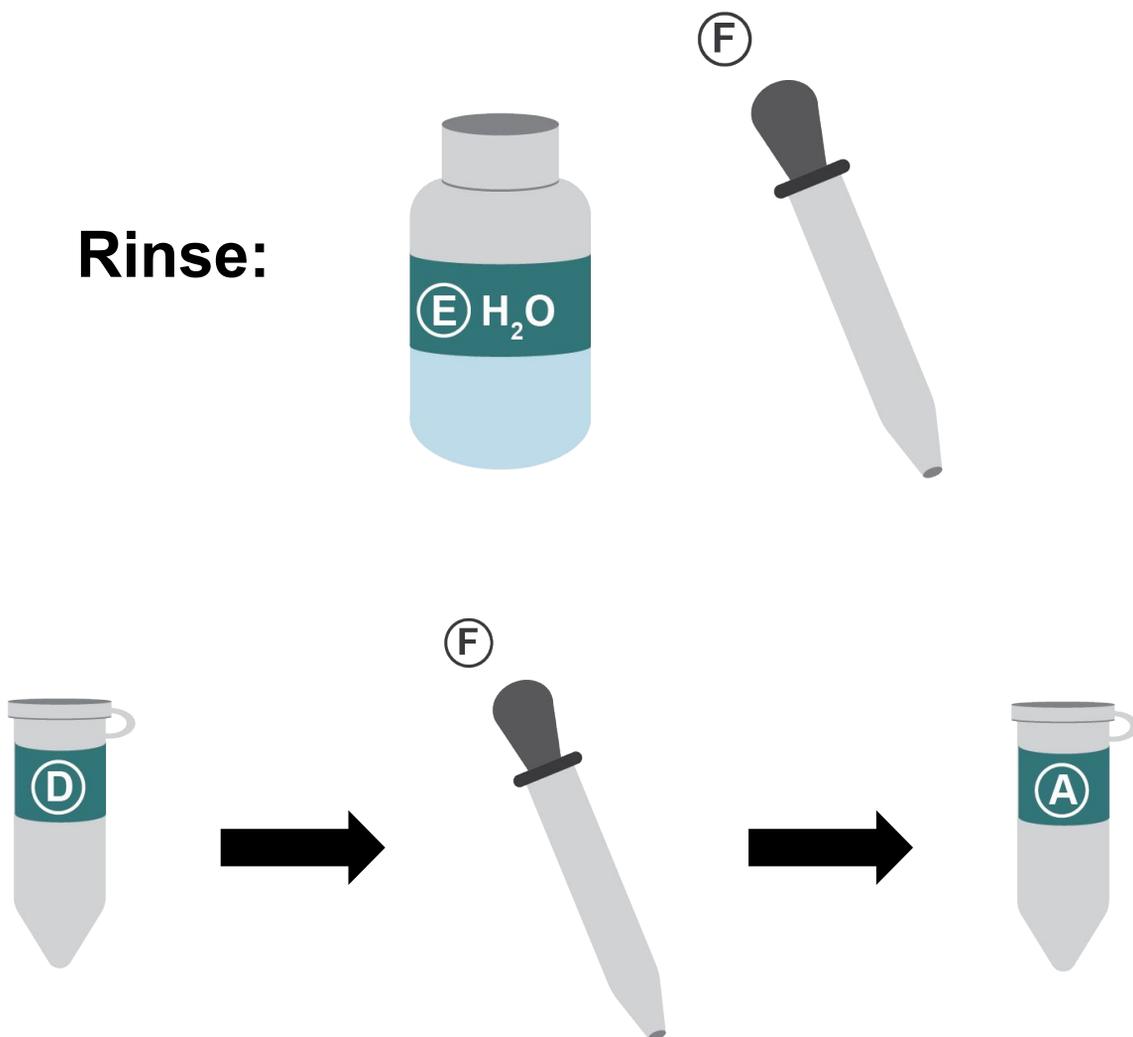
Translation

4

Add at least one drop from tube D (salicylic acid) to tube A

- Mix well by flicking tube
- Again, ensure that students are continuously cleaning the eye dropper

Rinse:



Instructions

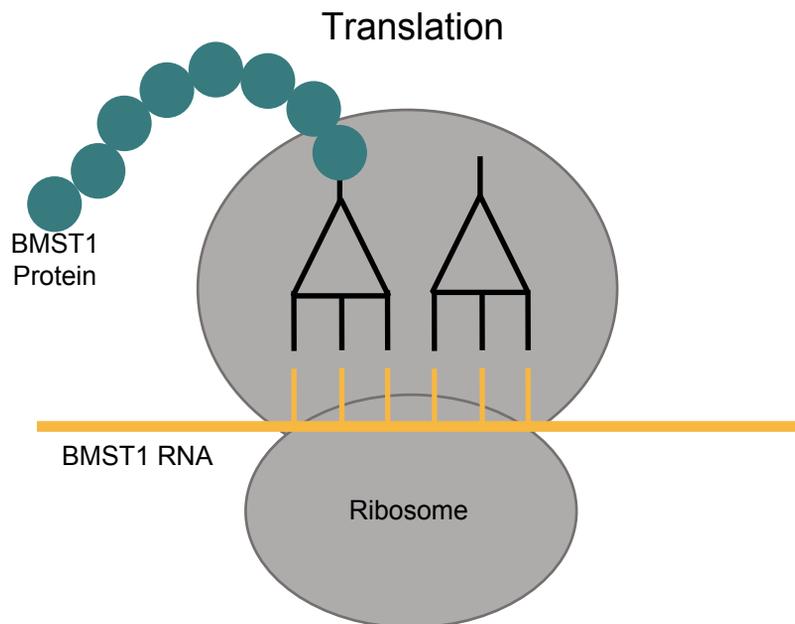
Translation

4

Add at least one drop from tube D (salicylic acid) to tube A

What is Happening?

The BMST1 RNA will be translated, to create the BMST1 protein. Once salicylic acid is added, the BMST1 protein will convert the salicylic acid into a mint (wintergreen) scented product called methyl salicylate.



Instructions

Translation

5

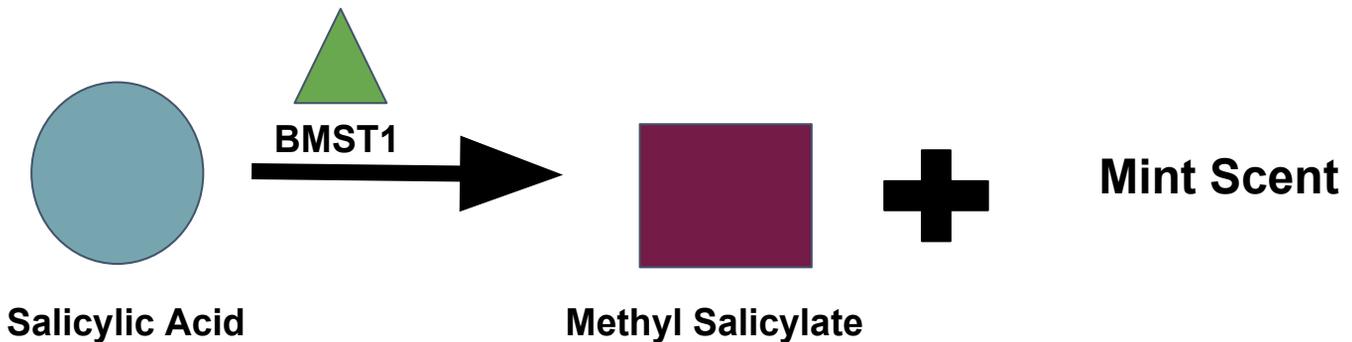
By wafting the tube, a mint scent should be observed.

- Ensure that students do not hold the tube too close to their nose



What is Happening?

Methyl salicylate is the compound in wintergreen plants that causes the mint scent. The production of a mint scent therefore indicates that translation, and thus production of the protein, has occurred.



Instructions

Clean-up

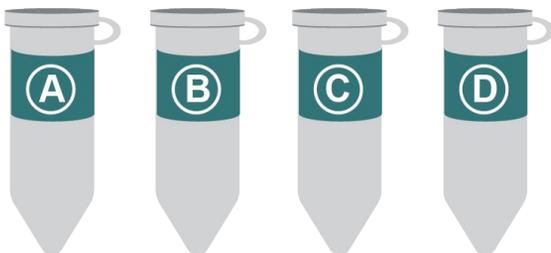
6

Disposal:

Please place all tubes and materials that came into contact with any solution into the provided biohazard bag and give the bag for further disposal and clean up workspace after everything else is cleaned up.

Please note that all materials can be disposed of down the sink. However, we have included them disposing of materials using similar bags to those found in an actual lab setting to encourage proper safety habits. The bags can be disposed of in a waste bin.

You can talk about how normal biohazard disposal bags go through a process called autoclaving, which exposes the contents in them to a high temperature and steam at a high pressure to sterilize the contents. This process effectively should kill any microorganisms present. Cell-free systems do not need to go through this process as they have been purified of any bacterial parts.



- 1** Add one drop of DNA from tube B to tube A, which contains transcription/translation components.
- 2** Add one drop from tube C, containing thiazole orange, to tube A.
- 3** A fluorescent orange colour should be observed, indicating that transcription has occurred.
- 4** Add one drop from tube D, containing Salicylic acid, to tube A.
- 5** By wafting the tube, a mint scent should be observed, indicating that translation has occurred.
- 6** Disposal: All components in the provided disposal bags can be placed in a waste bin.



Questions/Activity

- 1) What is being produced after transcription? Be specific to this experiment.
- 2) What is being produced after translation? Be specific to this experiment.
- 3) Were you able to see fluorescence after transcription? If not, can you come up with any reasons why it didn't work?
- 4) Were you able to smell a mint scent? If not, can you come up with any solutions why it didn't work?
- 5) Can you think of any other types of functional proteins that you could produce with a cell-free system?

Answers to Questions

- 1) An RNA transcript of the BMST1 protein and RNA Mango is being produced
- 2) The protein BMST1
- 3) Multiple reasons can exist. More of the fluorophore may need to be added to interact with the mixture and cause fluorescence. The mixture may also need to be incubated for a longer period
- 4) Multiple reasons can exist. More salicylic acid may need to be added to interact with the mixture. The protein may not be functional due to issues with the conversion from a peptide to a protein, which would prevent the reaction from occurring
- 5) Various, examples include insulin, hemoglobin, lactase

Activity

Activity: research and create your own organism that can produce a protein that is used for a medical purpose. The focus of this activity is at the teacher's discretion.