A Student's Guide to:

next vivo

LETHBRIDGE iGEM 2017

Transcription and Translation Education Kit:

&

SIGHT

SMELL

Table Of Contents

Definitions

Bacteria: a class of single cell organisms that does 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 that is able to convert a protein to a different form

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

Gene: a unit of genetic information encoded within DNA

Methyl salicylate: a chemical produced from salicylic acid, smells of wintergreen

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

Ribosome: the central 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

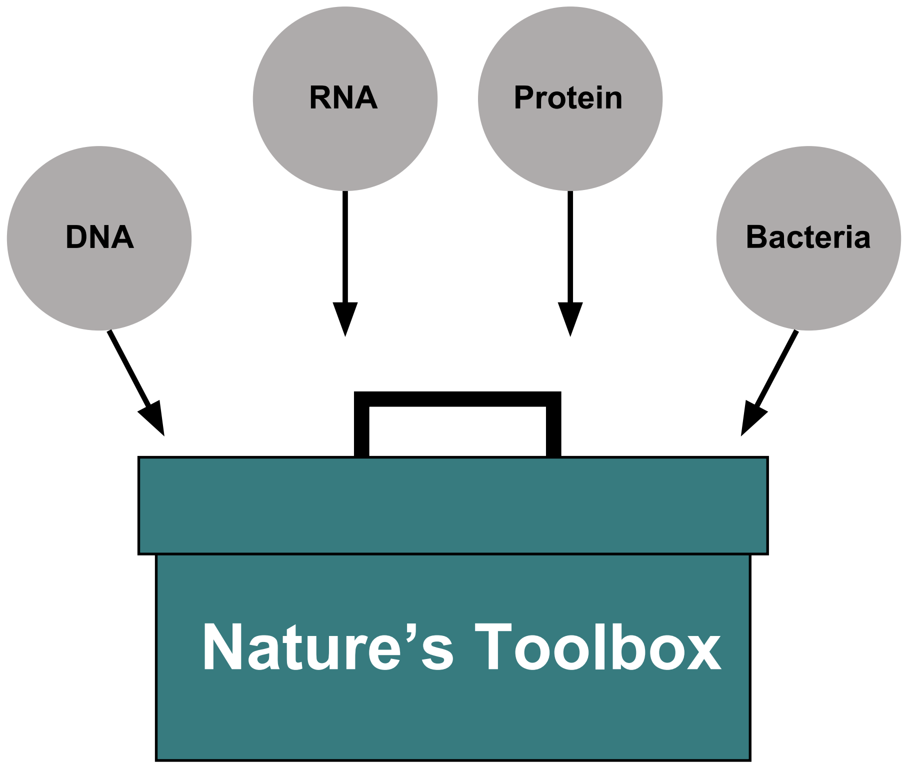
Translation: converting the RNA message into a protein

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

Nature's Toolbox

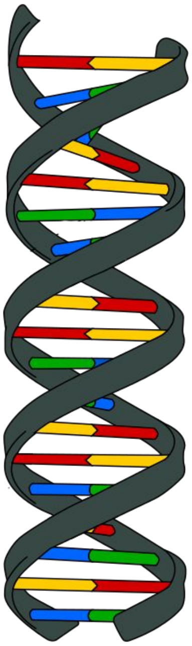
Naturally occurring biological systems meet the needs of their hosts, resulting in a collection described as "nature's toolbox."

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



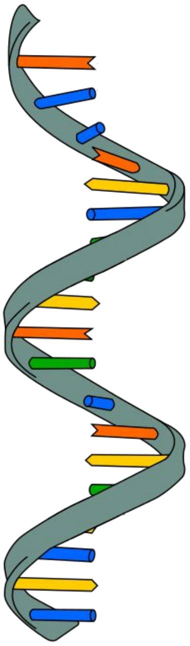
Nucleic Acids

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.

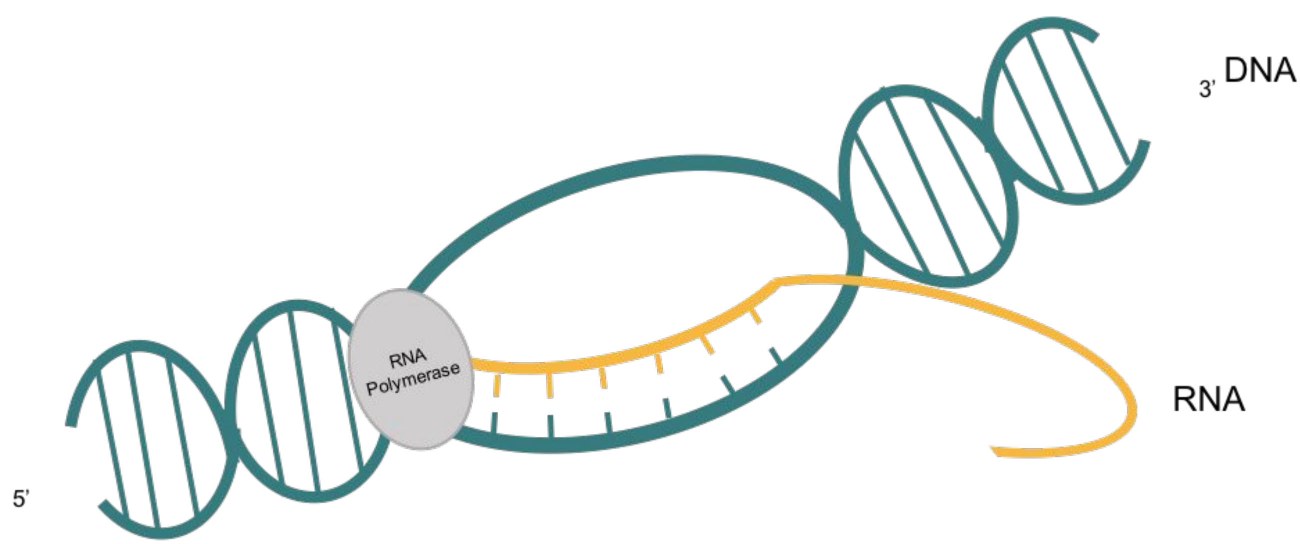
RNA



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 the thymine base is replaced with uracil (U). RNA contains the message of the DNA in a way that the cell can interpret it and produce essential proteins.

Transcription

DNA contains the 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 from the beginning (5’) to the end (3’) of the strand into RNA, until the RNA transcript is complete.

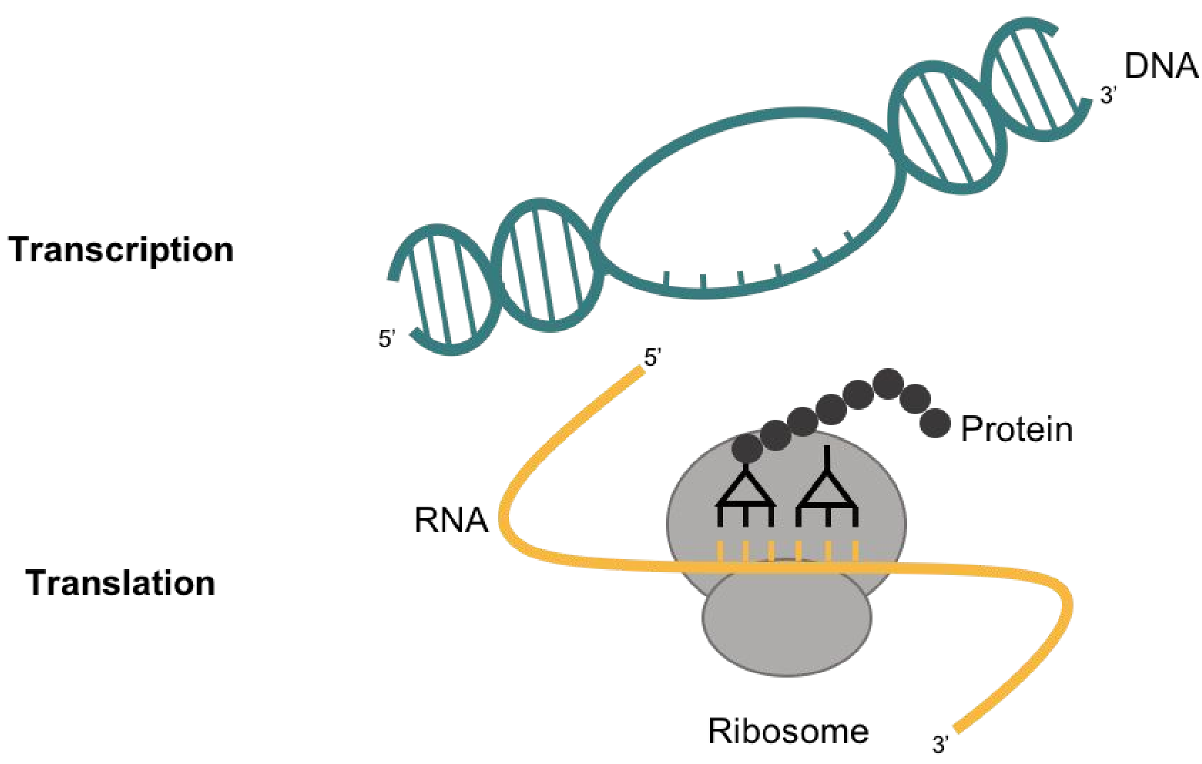


Translation

Translation

RNA → Protein

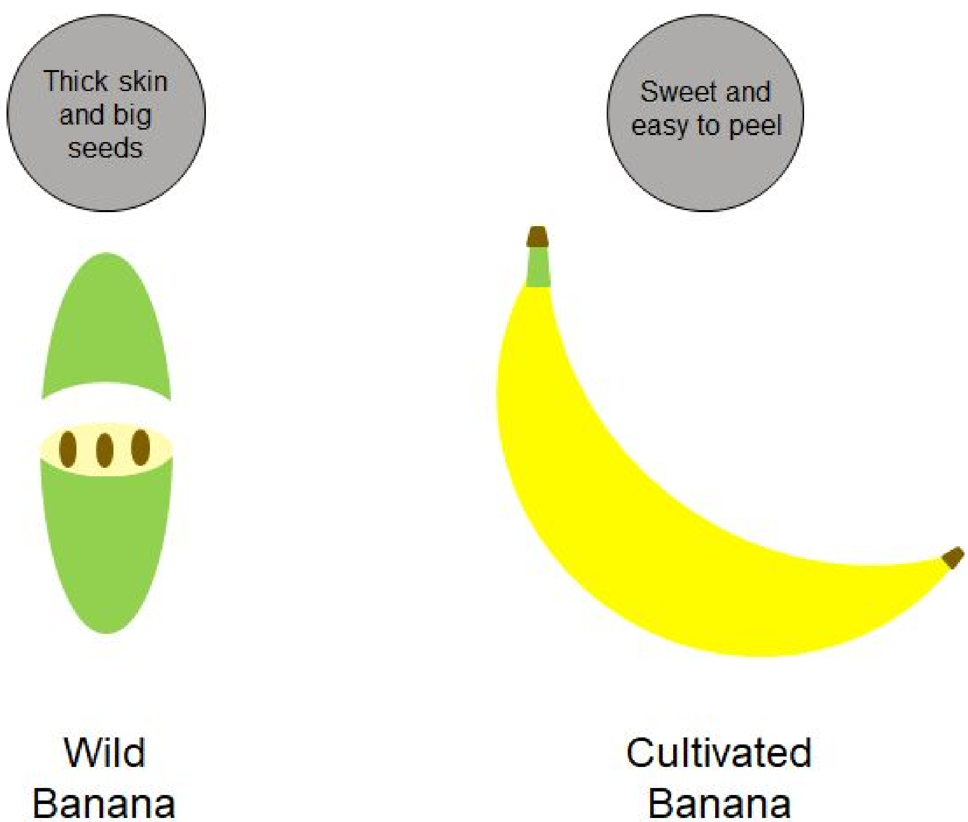
The RNA will take this genetic code to a structure in the cell called a ribosome that will takeover; producing the protein from the RNA. The ribosome binds the RNA and then decodes the information contained within the sequences of nucleotides. Three nucleotides of the genetic code are read together as 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

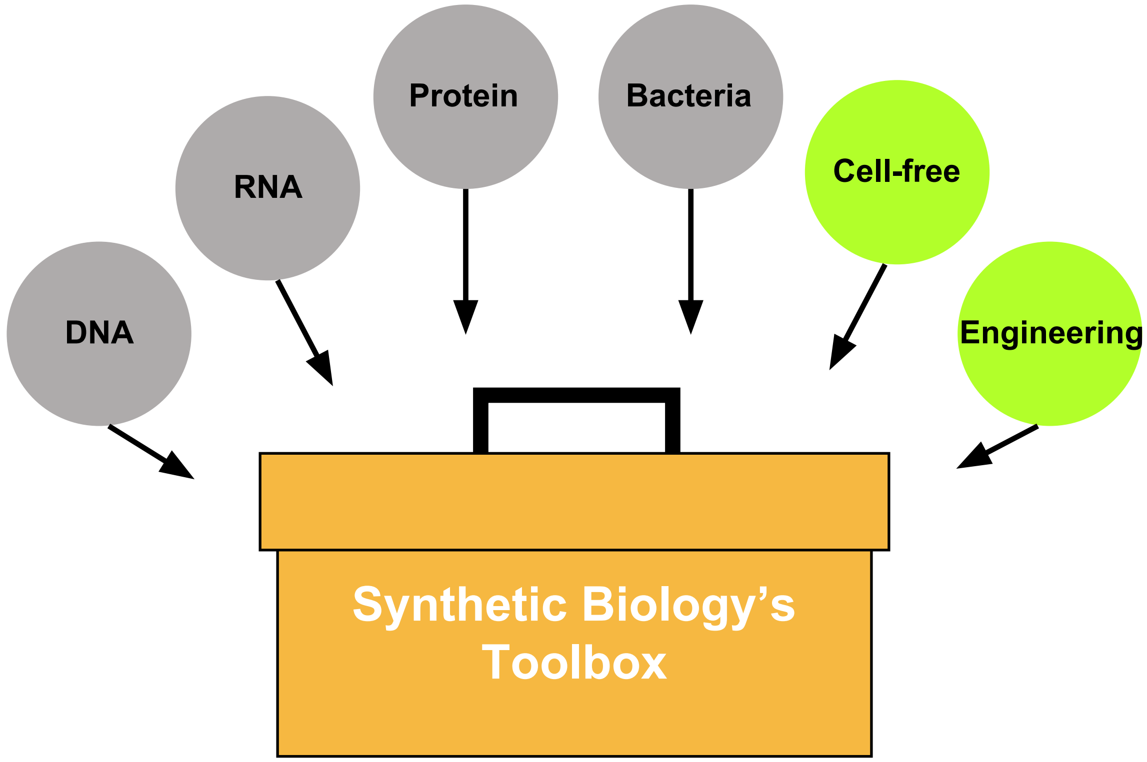
Genetic engineering has been happening since humans began to cultivate plants and domesticate animals. Incremental genetic changes caused by selective breeding have accumulated over time and manifest in new characteristics. For example, the bananas we eat have been modified over the years to appear like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

The development of molecular biology tools has allowed us to greatly speed up that rate at which genetic changes can be made in organisms.



Synthetic Biology

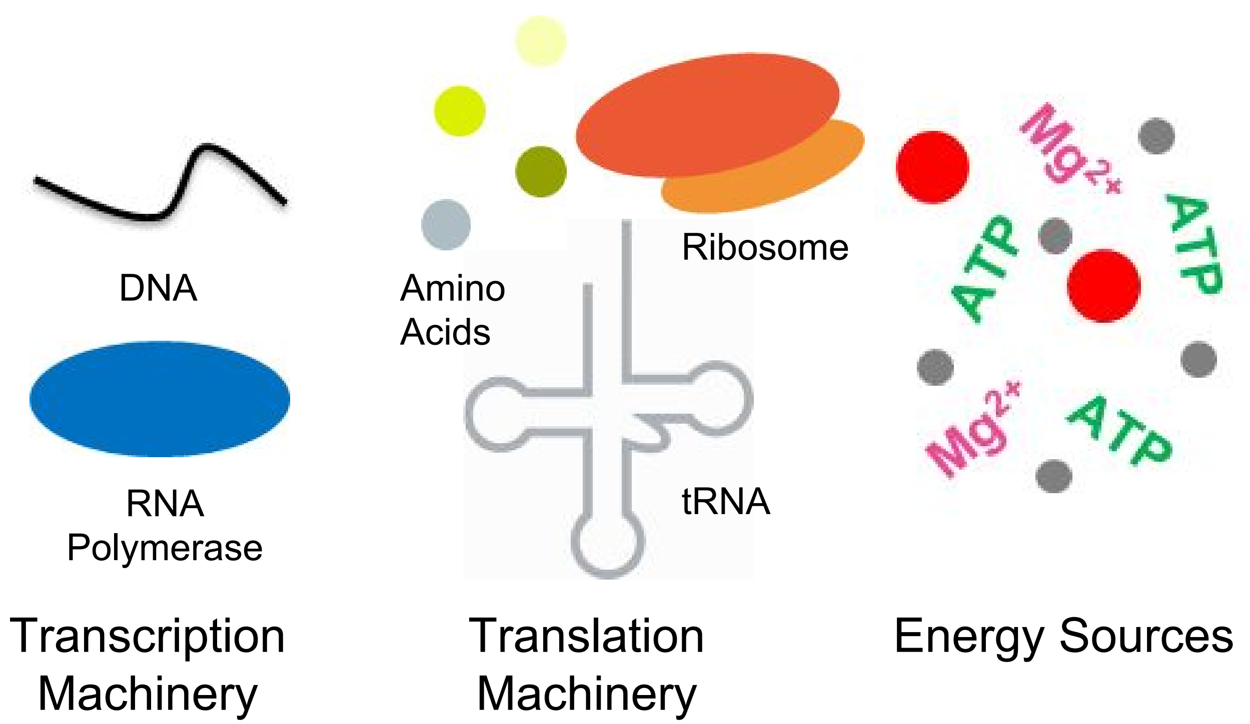
Synthetic biology is a relatively new area of research, combining both biology and engineering. In recent years, the emerging field of synthetic biology has attempted to engineer biological systems using primarily parts from nature's toolbox to create their own toolbox. Synthetic biologists can "program" biological systems, using 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.



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 keep the cells able to grow, divide, and perform a variety of other functions.

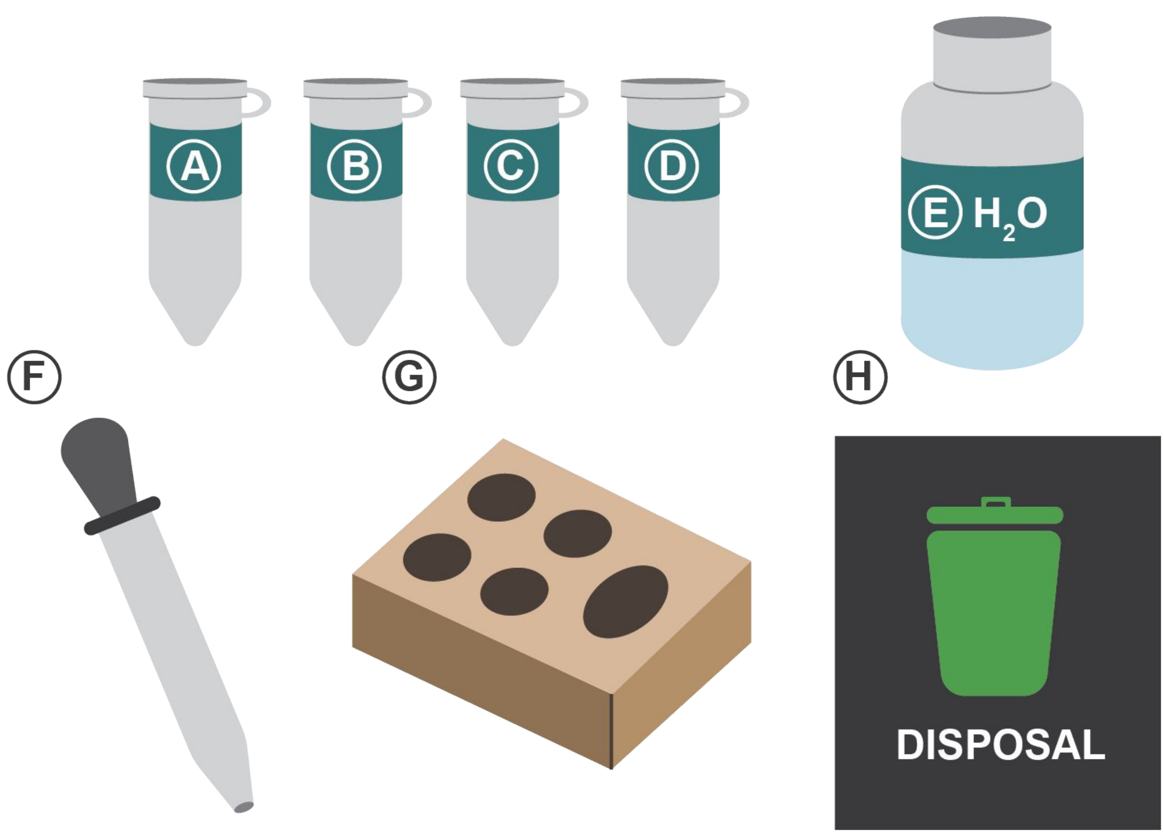
Most of these other functions are inessential for the purposes of synthetic biology, and many of the mechanisms that keep the cell in balance can make controlling the cell difficult. Accordingly, taking the essential transcription and translation machinery out of the cell and putting it into a tube retains 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.



Kit Contents

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:

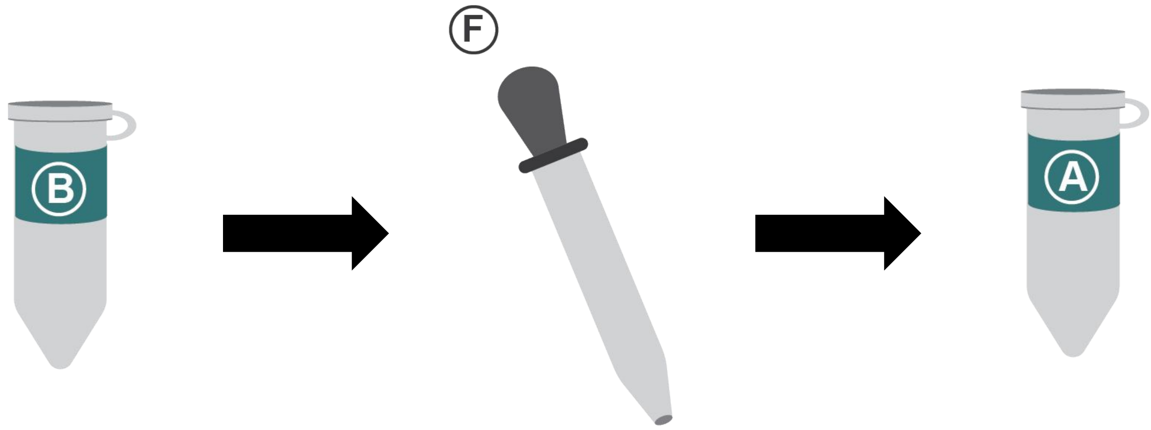
- Wear gloves when handling all contents of this kit. – Although everything is safe to handle, it is important to practice good lab techniques.
- Clean your workspace before and after the use of this kit.
- When smelling the wintergreen scent, make sure to waft the scent towards you to observe the scent at a lesser concentration.
- Disposal: All components can be disposed of in the provided disposal bag. As good practice, waste materials that have been exposed to living material are autoclaved (exposed to very high temperature and pressure) to ensure degradation and containment of the waste material.

Instructions

Transcription

1 **Using an eyedropper, add 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!)



What is Happening?

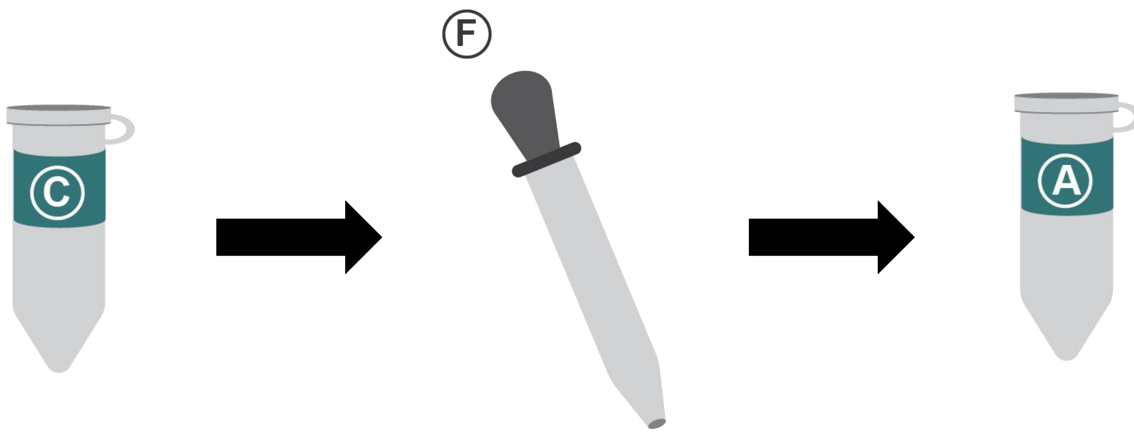
Note: RNA Mango and BMST1 are encoded for on the same RNA strand. They are separated to show that both are being produced.

Instructions

Transcription

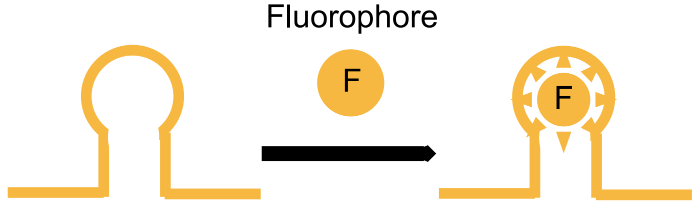
2 **Add one drop from tube C, containing thiazole orange, to tube A.**

- Rinse eyedropper with water (bottle E) and dry by tapping on paper towel before this step.



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.

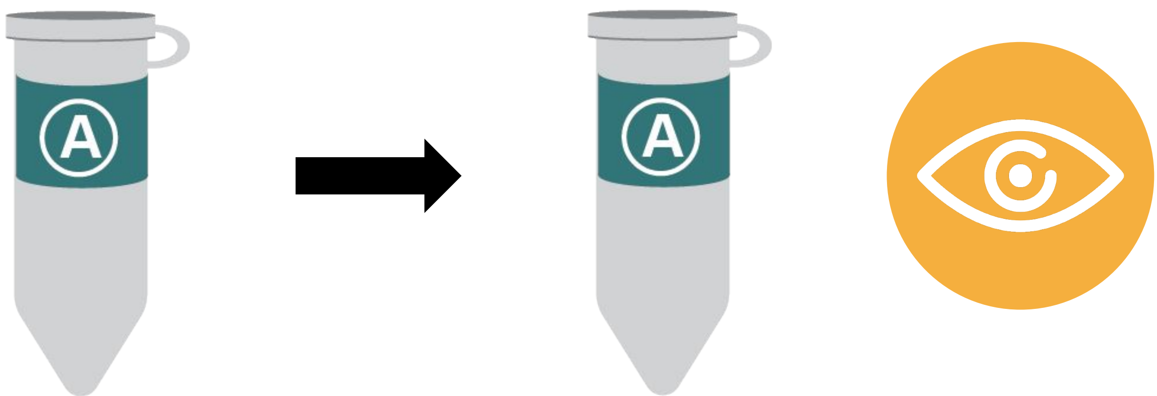


Instructions

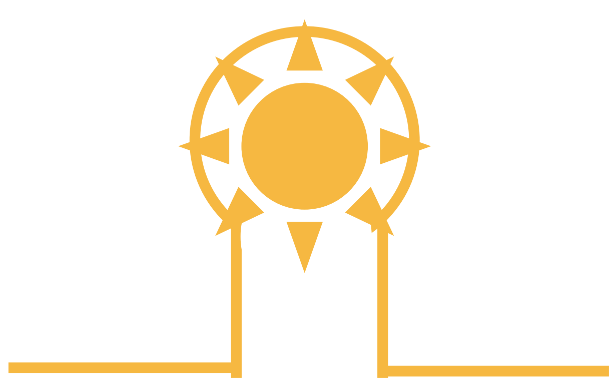
Transcription

3 **Warm tube A in hand until fluorescence is observed**

- Turn off lights to enhance observed fluorescence
- If no color is observed, continue to mix tube A



What is Happening?



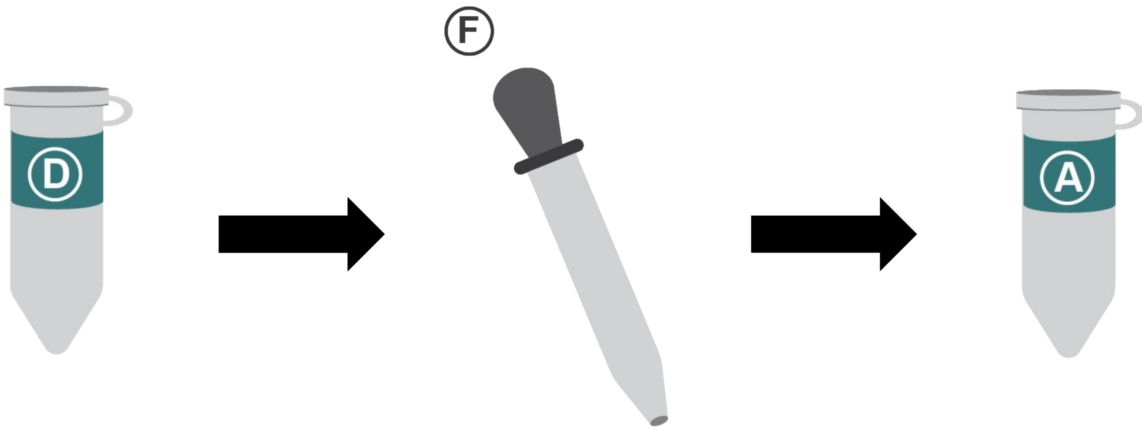
The binding of thiazole orange to the RNA aptamer causes fluorescence. This indicates that transcription has finished and that translation is about to begin.

Instructions

Translation

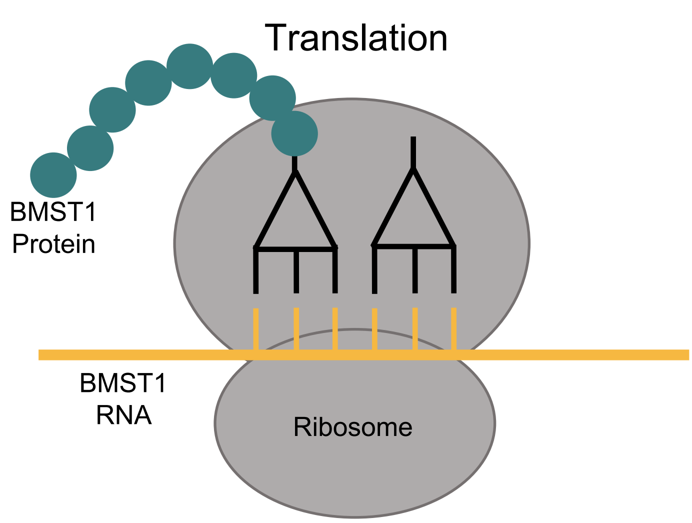
4 **Add one drop from tube D (salicylic acid) to tube A**

- Mix well by flicking tube, ensuring the lid is closed again



What is Happening?

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

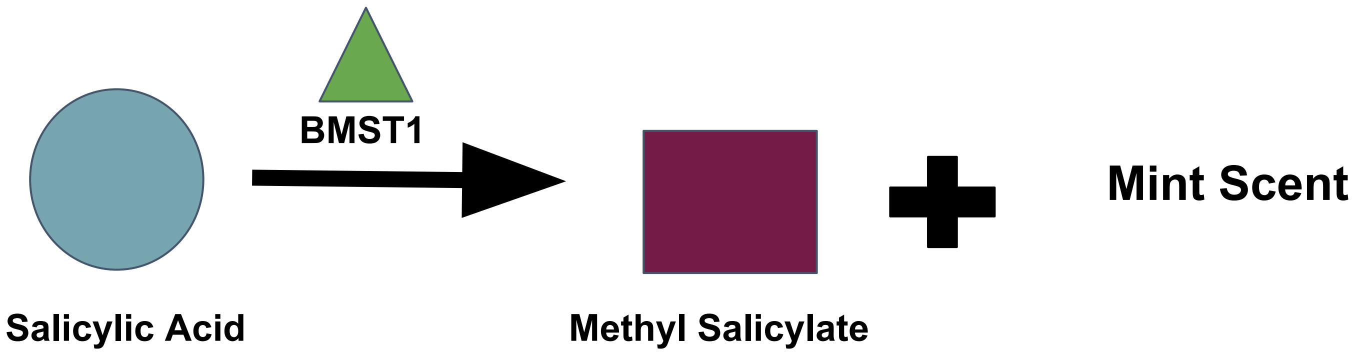


Translation

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

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.



Wintergreen Plant

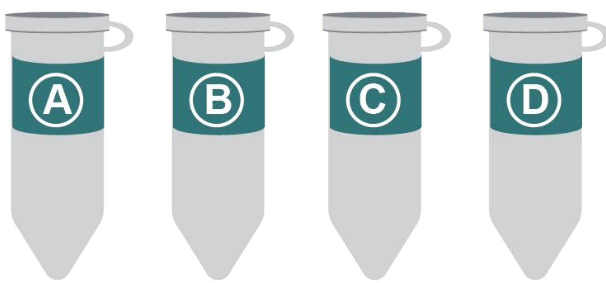
Instructions

Clean-up

6 **Disposal:**

Place all tubes into the provided disposal bag and give the bag for your teacher to dispose of.

Clear and clean your workspace!



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?
6) Activity: research and create your own organism that can produce a protein that is used for a medical purpose.