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Teaching Central Dogma of Biology Using Cell Free Environment

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Teaching Central Dogma of Biology Using Cell Free Environment

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Abstract: In this lesson plan, students will learn and visualize how genetic information coded in DNA is transcribed into RNA which is further translated into protein. This entire process, DNA->RNA-> Protein, is known as the Central Dogma of Molecular Biology. This hands-on module is designed for 9th through 12th grade students. To adjust the difficulty level of the modules, some aspects can be modified or removed based on the grade. This module includes an instructional slide show, video tutorial, assessment options, and links to additional resources.

Instructional Video (6 minutes): <https://youtu.be/DSsHZACUPC4>

Supplementary File: PowerPoint Presentation

Lesson Description

- Grade Level: Grade 9-12 (can be modified for Grade 6-8)

Estimated Time for Completing Activity

- One 45-minute class period for day 1, and one 20-minute class period for day 2.

Learning Outcomes

Students will be able to:

- Define Central Dogma, transcription, and translation.
- Develop a model which shows how the information is carried from DNA to protein via RNA
- Carry out an experiment to show how the use of antibiotic kanamycin affects protein translation.

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- Analyze the data and predict what would occur if there was an error or blockage in any step of the Central Dogma
 - Apply the knowledge of Central Dogma in genetic engineering to design techniques to answer the global health problem questions
-

South Dakota Career and Technical Education (CTE) Standards:

- *INDICATOR #STEM 2:* Understand how Science relates to STEM in CTE
 - *SUB-INDICATOR 1.1 (Webb Level 2: Skill/Concept):* Understand the components of STEM in CTE and the impact of STEM on society
 - *INDICATOR #STEM 6:* Understand how technical and soft skills apply to STEM and CTE careers
 - *SUB-INDICATOR 6.1 (Webb Level 2: Skill/Concept):* Explore health standards and safety skills in relation to STEM in CTE careers
-

South Dakota State Science Standards:

- **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. (SEP: 6; DCI: LS1.A; CCC: Structure/Function)
- **HS-LS1-2:** Develop and use a model to illustrate the hierarchical organization of interacting systems that provide specific functions within multicellular organisms. (SEP: 2; DCI: LS1.A; CCC: Systems)
- **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. (SEP: 1; DCI: LS1.A, LS3.A; CCC: Cause/Effect)
- **MS-LS3-1:** Develop and use a model to describe why structural changes to genes (mutations) located on chromosomes may affect proteins and may result in harmful, beneficial, or neutral effects to the structure and function of the organism. (SEP:2; DCI: LS3.A, LS3.B; CCC: Structure/Function)
- **MS-LS1-2:** Develop and use a model to describe the function of a cell as a whole and ways parts of cells contribute to the function. (SEP: 2; DCI: LS1.A; CCC: Structure/Function)

<https://doe.sd.gov/contentstandards/documents/sdSciStd.pdf>

National Standards

Next Generation Science Standards (NGSS: MS-LS1-2, MS-LS3-1, HS-LS1-1; www.nextgenscience.org; definitions on the last page)

- **MS-LS1-2:** Develop and use a model to describe the function of a cell as a whole and ways the parts cells contribute to the function.
- **MS-LS3-1:** Develop and use a model to describe why structural changes to genes (mutations) located on chromosomes may affect proteins and may result in harmful, beneficial, or neutral effects to the structure and function of the organism.
- **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.

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Pre-requisites

- A general level of pipetting skill.

Video Tutorial on Pipetting

- <https://www.youtube.com/watch?v=QGx490kuKjg>
- Instructional Video: <https://www.youtube.com/watch?v=MazftfKNltE>

Materials/Supplies

BioBits Kit¹

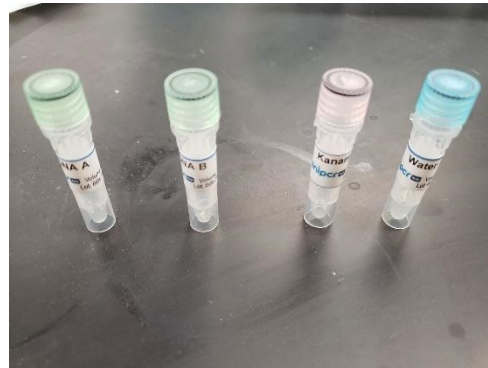
BioBits Tubes



<https://www.minipcr.com/product-category/biobits/>

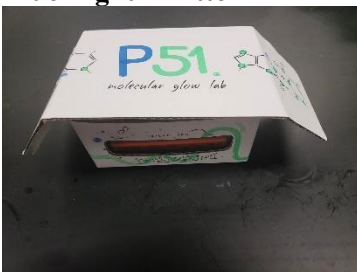
Reagents

(DNA A, DNA B, Water, Kanamycin)



<https://www.minipcr.com/product-category/biobits/>

Blue Light Emitter



Micropipette + Tips



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https://www.minipcr.com/product/p51-molecular-glow-lab/	https://www.minipcr.com/product/4-%c2%b51-minipette-fixed-volume-micropipette/ https://www.minipcr.com/product/200-%C2%B51-micropipette-tips-2-racks-of-96/
Nitrile Gloves  https://www.fishersci.com/shop/products/powder-free-dark-blue-nitrile-gloves/19181609	Tube Rack  https://www.fishersci.com/shop/products/fisherbrand-microcentrifuge-tube-racks-19/0554144

- Strips of tubes containing BioBit pellets cut into sets of 4 tubes per group (Modified from BioBits Protocol1)
- Microtubes containing reagents DNA A, DNA B, Nuclease-Free water, and Kanamycin; 1 set per group
- 0.2-2 mL tubes to aliquot reagents
- Blue light Illuminator Box from kit, 1 per group
- Nitrile gloves, one pair per student
- Micropipettes with capacity for 4ul (2-20ul range)
- Tube rack
- Sterile Pipette tips, at least 10 per group
- Freezer that reaches -20° C for storing reagents
- Paper towels
- Small beaker for discarded pipette tips
- Writing utensils
- Post lab worksheet, one for each student
- Lab predictions/results tables
- Projector
- Computer with Central Dogma PowerPoint

Glossary of Terms

- Amino Acids: Building blocks of protein
- Antibiotic: chemical that kills or inhibits growth of living things, usually targeting some necessary function
- Aptamer sequence: RNA sequence that binds to another molecule

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- Central Dogma: DNA → RNA → Protein
- DNA: Double stranded molecule containing genetic information
- Green fluorescent protein (GFP): Protein that glows green when exposed to ultraviolet light
- mRNA: RNA that has genetic information to make proteins
- Nucleotides: Building blocks of DNA and RNA (ATGC and U)
- Promoter: Where transcription starts on DNA
- Protein: Complex molecule that has a variety of functions, made by central dogma
- Replication: DNA being duplicated to make identical copies of itself
- Ribosome: Structure where mRNA is translated into protein
- RNA: Single stranded molecule containing genetic information, made using DNA's template
- Start Codon: Where translation starts (AUG)
- Stop Codon: Where translation ends (UAA, UAG, UGA)
- Terminator: Where transcription ends on DNA
- Transcription: Information from DNA used to make RNA
- Translation: Information from RNA used to make protein
- tRNA: RNA that decodes mRNA to help build proteins during translation

Helpful Links and References

- <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5602739/>
- https://en.wikipedia.org/wiki/Central_dogma_of_molecular_biology
- <https://www.yourgenome.org/facts/what-is-the-central-dogma>
- <https://www.minipcr.com/product/biobits-central-dogma/>
- <https://www.youtube.com/watch?v=FZHs-TZtWKQ>
- How to use a micropipette: <https://www.youtube.com/watch?v=Jfqafjt4q6U>
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- <https://www.khanacademy.org/science/biology/gene-expression-central-dogma/translation-polypeptides/v/translation-mrna-to-protein?modal=1>
- Khan Academy. (n.d.). Overview of translation Retrieved October 31, 2021, from Khan Academy:
- <https://www.khanacademy.org/science/biology/gene-expression-central-dogma/translation-polypeptides/a/translation-overview?modal=1>
- BioBits supplementary resources: <https://www.minipcr.com/products/biobits/>

Background

The Central Dogma of Life (also called the Central Dogma of molecular biology or simply Central Dogma) is a set of principles explaining genetic information flow. The genetic information is encoded on DNA, and this information can be transcribed into RNA in a process called Transcription. RNA is used by the cell for specific functions, but some RNA (called mRNA) can be translated into functional

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proteins. The necessary information to make a functional protein is encoded onto the DNA and transcribed into messenger RNA to further carry the message to ribosomes which build the protein in a process known as translation.

In this experiment, students will visualize the processes of the Central Dogma using a cell-free experiment kit known as BioBits. Using pellets full of necessary cellular components and adding the required reagents (DNA, water, or antibiotic), students will activate the production of specific fluorescent protein depending on what stage of the Central Dogma they are on. The fluorescent protein can be visualized with the student's own eyes. This demonstrates how the DNA code can yield to RNA and then to a protein, in this case, a fluorescent protein.

Procedure:

The attached PowerPoint slides have notes for guidance through the lesson.

Day 1:

Ask introductory questions:

- Does anyone know what a “dogma” is?
- It is basically an idea or principle that is accepted by a group of people as true.
- Does anyone know what the Central Dogma of molecular biology is?
- Does anyone know what transcription is? Translation?
- Play Central Dogma Introduction Video (<https://youtu.be/FZHs-TZtWKQ>)

While the video is playing, hand out materials to your groups of students.

Materials per group

BioBit Kit

- 8 runs per kit
- Approximate cost: \$185 for new kit with blue light emitter, tubes, and reagents and micropipette with tips.
- \$99 for refill of reagents and tubes¹

Materials provided

- 4 BioBit Tubes
- 1 Blue light illuminator viewer
- 1 Tube of Sterile water (from -20°C freezer)
- 1 Tube of DNA A (from -20°C freezer)
- 1 Tube of DNA B (from -20°C freezer)
- 1 Tube of Kanamycin (from -20°C freezer)
- 1 Micropipette plus tips

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Pre-Lab Procedures:

1. Walk through introductory slides with students explaining the components of the lab (Discussion notes in slides)
 2. Make predictions for each tube for the experiment in **Table 1** of the worksheet
-

Experimental Procedure Safety:

1. Wear gloves when handling samples.
 2. Clean the workbench before and after use.
 3. Properly Dispose of all reagents upon completion.
-

Day 1 Activities

1. Label 4 tubes on the side of the cap 1, 2, 3 and 4.
 2. Gently uncap tubes. Be careful not to pop the pellets out of the tubes.
 3. Add 4ul DNA A to tubes 2 and 3 using the micropipette, directly onto the pellet (not all measurements need to be exact).
 4. Add 4 ul of DNA B to tube 4 using the micropipette, directly onto the pellet.
 5. Add 8 ul water to tube 1 using the micropipette, directly onto the pellet.
 6. Add 4 ul Kanamycin using the micropipette, directly onto the pellet.
 7. Close all the caps until they click shut.
 8. Centrifuge down or tap the pellets down so they sit and are dissolved in the solution.
 9. Immediately put the tubes into the blue light illuminator. Switch the illuminator on and observe the tubes. Turn the illuminator back off. To visualize the color of the tubes clearly, dim the lights of the room.
 10. Write down observations in **Table 2**. Under “Time 0” (There should be no change).
 11. Place tubes in 37°C incubators or hold in hands to warm it up by body heat.
 12. Observe the clock or start timer and wait 15 minutes.
 13. After 15 minutes put the tubes into the blue light illuminator, turn on, and dim lights and observe, turn the illuminator back off.
 14. Write down observations in **Table 2**. Under “15 minutes”
 15. Before the class period ends store the tubes in a dark place where they will not be disturbed until the next class period and return reagents into the -20°C freezer
-

Assessment for Day-1:

1. Diagnostic: Pre-lab prediction table
 2. Formative: Following discussion.
-

Day 2:

Review: Have students look at their prediction tables and discuss why they expected certain results for their Day 2 tubes as well as remind themselves of their results from Day 1.

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Day 2 Activities

1. Retrieve tubes and blue light viewers from previous day's activity (this observation can occur 8-72 hours after Day 1).
 2. Put the tubes into the blue light illuminator, turn on, and dim lights and observe, turn illuminator back off
 3. Write observations in **Table 2.** under "Day 2"
 4. Compare your predictions table and observations table and describe how they were the same or different from each other and why
-

Discussion

- Based on your observations, did transcription and/or translation occur in each of the 4 tubes?
 - Why did or why didn't transcription and/or translation occur in each of the tubes?
 - What evidence has led you to these conclusions?
 - Do your observations match your predictions?
-

Assessment for Day-2:

- Formative Assessment – Pre-lab and Post lab Discussions
 - Diagnostic Assessment – In lab worksheet **Post Lab Questions**
-

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Lab Worksheet

Table 1. Pre-Experiment Prediction on Central Dogma. A good scientist predicts (hypothesizes) the result of an experiment based on available data even when little data is available (like Tube 4). Predict your results and justify your prediction.

Time	Tube 1	Tube 2	Tube 3	Tube 4
0 minutes	<u>Prediction:</u> <u>Justification:</u>	<u>Prediction:</u> <u>Justification:</u>	<u>Prediction:</u> <u>Justification:</u>	<u>Prediction:</u> <u>Justification:</u>
15 minutes	<u>Prediction:</u> <u>Justification:</u>	<u>Prediction:</u> <u>Justification:</u>	<u>Prediction:</u> <u>Justification:</u>	<u>Prediction:</u> <u>Justification:</u>
Day 2 (about 24 hours later)	<u>Prediction:</u> <u>Justification:</u>	<u>Prediction:</u> <u>Justification:</u>	<u>Prediction:</u> <u>Justification:</u>	<u>Prediction:</u> <u>Justification:</u>

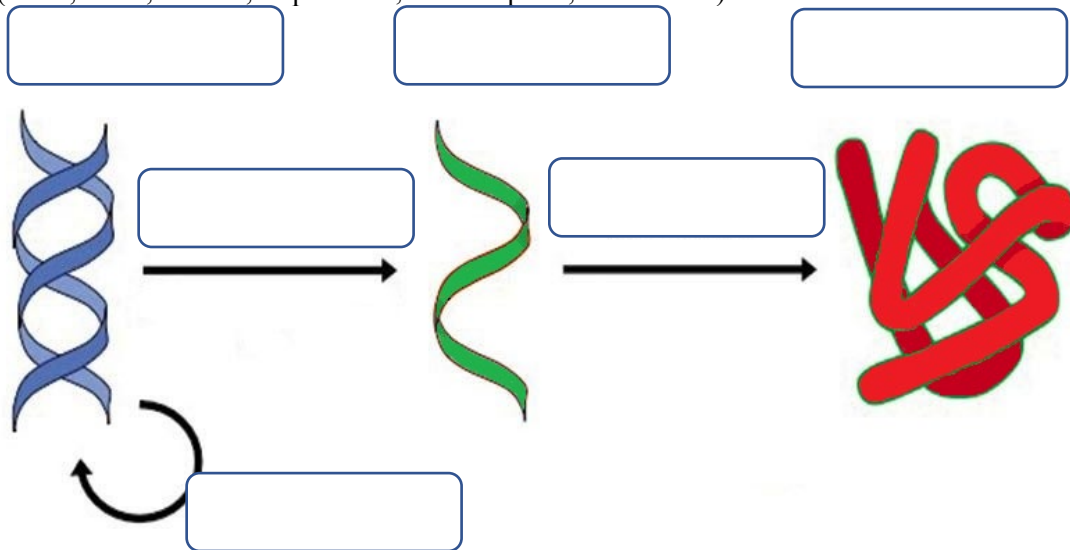
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Table 2. Experiment Observations Table. Describe your results and how they are similar or different from what you predicted before the experiment. A good scientist accepts the results of an experiment regardless of prior predictions.

Time	Tube 1	Tube 2	Tube 3	Tube 4
0 minutes				
15 minutes				
Day 2 (about 24 hours later)				

Post-Lab Questions

1. Show the flow of genetic information and the involved processes by filling in the missing terms (DNA, RNA, Protein, Replication, Transcription, Translation).



2. In your own words, what is the central dogma of biology?

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3. Tube 1 was a negative control for this experiment. What is a negative control, and why is it important to have?

4. In Tube 3 the antibiotic kanamycin was added. Kanamycin interferes with ribosomes and causes mistranslation of proteins. What results do you expect to see? What did you see? Why?

5. Match the vocab term with the definition, each letter is only used once:

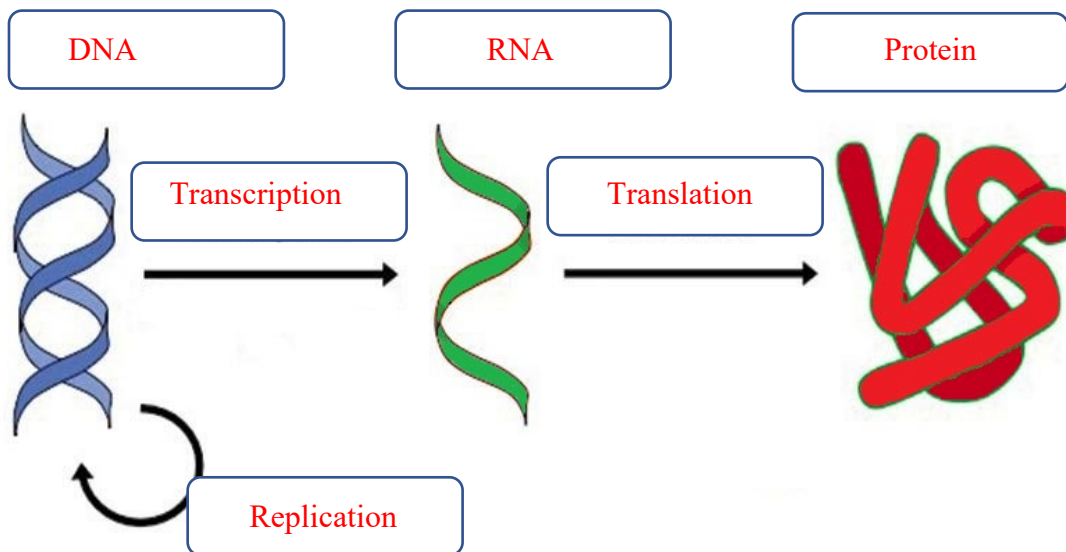
Term	Answer	Match
Start Codon:	_____	A. The production of protein using RNA as a template.
Stop Codon:	_____	B. The production of DNA using DNA as a template.
Promoter:	_____	C. The place on the RNA where the ribosome binds to initiate translation.
Transcription:	_____	D. A sequence of DNA where DNA polymerase binds to initiate transcription.
Translation:	_____	E. The place where translation is terminated.
Replication:	_____	F. Production of RNA using DNA as a template.

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Answer Key

Post Lab Questions

1. Show the flow of genetic information and the involved processes by filling in the missing terms.



2. In your own words, what is the central dogma of biology?

DNA->RNA->Protein

3. Tube 1 was a negative control for this experiment. What is a negative control, and why are they important to have?

A group that is not expected to change during the experiment because it did not receive treatment. It is important because it proves that any changes that occur in the experiment are due to the treatment of the experimental groups, not any other factor

4. In Tube 3 the antibiotic kanamycin was added. Kanamycin interferes with ribosomes and causes mistranslation of proteins. What results do you expect to see? What did you see? Why?

Transcription should still occur (green fluorescence on day 1) but translation should not occur (no red on day 2). The ribosome is used for translation to occur, and if the ribosome doesn't work, translation cannot occur.

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5. Match the vocab term with the definition:

Term	Answer	Match
Start Codon:	<u> C </u>	A. The production of protein using RNA as a template.
Stop Codon:	<u> E </u>	B. The production of DNA using DNA as a template.
Promoter:	<u> D </u>	C. The place on the RNA where the ribosome binds to initiate translation.
Transcription:	<u> F </u>	D. A sequence of DNA where DNA polymerase binds to initiate transcription.
Translation:	<u> A </u>	E. The place where translation is terminated.
Replication:	<u> B </u>	F. Production of RNA using DNA as a template.

Multiple choice questions

- 1) What is the use of Kanamycin in the central dogma experiment?
 - a) Blocks DNA replication
 - b) Blocks the formation of RNA from DNA
 - c) Blocks protein synthesis
 - d) Blocks DNA synthesis
- 2) Where does protein synthesis occur in bacterial cell?
 - a) In the cell membrane
 - b) In the endoplasmic reticulum
 - c) In the nucleus
 - d) In the cytoplasm
- 3) What will happen if we block the process of translation?
 - a) mRNA will not be produced
 - b) Protein will not be produced
 - c) Proteins will be degraded
 - d) DNA will be degraded
- 4) What will happen if protein mistranslation occurs?
 - a) Protein will not be produced
 - b) Protein will be changed to DNA

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- c) Incorrect protein production
- d) Protein will be exported out of the cell
- 5) What is needed the most for protein synthesis in bacteria?
 - a) Nucleus
 - b) Golgi apparatus
 - c) Ribosomes
 - d) Endoplasmic Reticulum

Supplementary File includes PowerPoint presentation, Student Observation Table, and Worksheet, Guided Lesson video

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