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Manuela Tripepi

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From Gene to Function: A Safe Laboratory Exercise Promoting the Learning of the Central Dogma of Biology for the Undergraduate Biology Curriculum†

Manuela Tripepi^{1*}, Morgan Clear^{2‡}, Jack Lyons Bondi¹, Elvira Brunelli³, and Arianna Berardi²

¹Thomas Jefferson University, Philadelphia, PA 19144, USA;

²Arcadia University, Glenside, PA 19038, USA;

³University of Calabria, Rende, Italy

INTRODUCTION

A key concept in undergraduate biology classes around the world is the central dogma of biology, which explains how the information in genes flows into proteins. This fundamental idea describing the flow of genetic material within an organism can be difficult for students to grasp (1). Many students associate DNA and genotype directly with an organism's phenotype without understanding or considering the importance of gene products, such as RNA and proteins, as being responsible for these observable features (2). Listening to a lecture or reading a textbook on a subject has proven to be less effective for students' learning than supplemental laboratory instruction (3). By contrast, providing a hands-on method of study through laboratory work promotes active learning and critical thinking (3, 4).

To improve students' comprehension of the central dogma of biology, we designed a practical laboratory activity to complement classroom learning. The chosen model organism for this project is the haloarchaea *Haloflexax volcanii*. This is a nonpathogenic organism that reproduces quickly and is easy and inexpensive to grow and maintain. During this activity, students run PCR, gel electrophoresis, and an agar assay.

Further, students investigate the genotype of a strain of *H. volcanii*, using PCR and primers that target the flagellin gene *flgA1*. The absence of this gene renders *H. volcanii* nonmotile. The flagellar operon is responsible for swimming motility in *H. volcanii*; the flagellin genes present in its genome are *flgA1* and *flgA2* (4). Since *flgA1* is understood

to be a major flagellin, it is necessary for motility (5). Furthermore, in mutant strains lacking *flgA1*, no motility is observed (5). Modified agar plates (motility assays) are used to visualize this movement, or lack thereof. Motility in agar is characterized by halo formation around the initial placement of the organism, with a nonmotile strain therefore lacking halo formation and appearing as only a dot at the point of inoculation.

The active learning in this activity was designed to increase student learning and understanding of the central dogma of biology (4). With this lab activity, the instructor can show the students that the flagellin gene, after transcription and translation into the flagellin protein, renders the cell motile. By using PCR and electrophoresis to verify the presence of the gene and by correlating it with motility using the motility assay, students observe motility if the strain contains the flagellin gene and will see that the lack of the flagellin gene corresponds to a nonmotile strain because of a lack of protein production (Fig. 1).

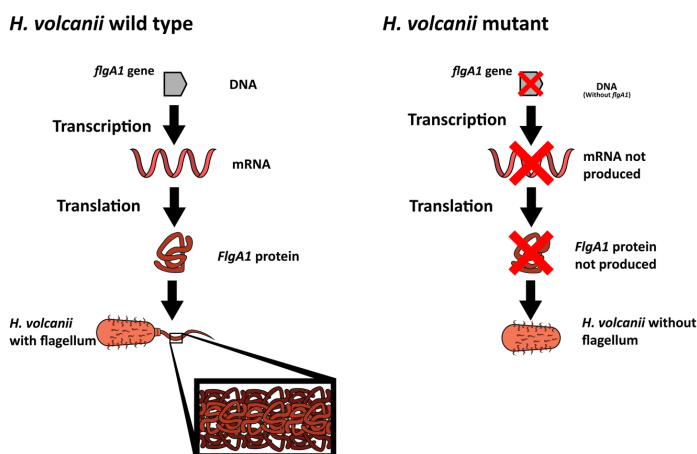


FIGURE 1. Information flow from gene to protein integrated in the lab exercise.

* Corresponding author. Mailing address: Thomas Jefferson University, 4201 Henry Ave., Philadelphia, PA 19144. Phone: 215-951-0424. E-mail: manuela.tripepi@jefferson.edu.

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†Supplemental materials available at <http://asmscience.org/jmbe>

‡Present address: Perritt Laboratories, Highstown, NJ 08520.

Learning objectives

Second-year undergraduate biology students will be able to:

1. Understand and explain how information flows from gene to protein.
2. Learn the concept of a target gene and identify the target gene in the current experiment.
3. Understand the function of the primers and select primer sequences to be used in the experiment.
4. Run and analyze the PCR reactions and correlate them with the protein function results of the motility assay.

PROCEDURE

Safety issues

The organism used for this project, the haloarchaea *H. volcanii*, is a nonpathogenic organism that reproduces quickly and is easy and inexpensive to grow and maintain. There is no risk of contamination from unwanted bacteria, as *H. volcanii* medium has a very high salt concentration that inhibits the growth of bacteria. All experiments follow the ASM Guidelines for Biosafety in Teaching Laboratories. Strains can be handled at biosafety level I (BSL1), with appropriate personal protective equipment. Students should be trained in BSL1 procedures prior to conducting this laboratory activity.

Materials

The list of media ingredients and instructions is available in Appendix 1. The materials needed are available from commercial suppliers, and the two strains of *H. volcanii* are available for shipping, upon request, from Dr. Tripepi's Lab. An alternative to the regular lab medium has been published and offers a way to grow *H. volcanii* using ingredients available at grocery stores (6). Sterile technique is not required due to the nature of this halophile organism.

Experiments

Students are provided with two strains of *H. volcanii*, a wild-type strain (H53), which is the motile control, and an unknown strain (either a motile or nonmotile strain) (Fig. 2). Students need to characterize this unknown strain as motile or nonmotile based on the PCR and motility assay results. Students start the experiment by extracting the DNA of the two strains (Appendix 1). They use this product to run a PCR, using primers that are specific for the *flgA1* gene, which is responsible for motility in the strain. After the PCR is set, the students can test the motility phenotype of

both strains using a motility plate assay. They stab-inoculate a motility plate with one or two colonies of the unknown strain and the wild type. The plates are incubated at 45°C, and colonies can be visualized within 2 to 5 days. In the next class or lab period, students analyze their results. At the end of these activities, students are able to compare the results of the PCR (genotype), visualized by electrophoresis, to the motility assay (phenotype) results and discuss the implication of the presence of the gene and the respective phenotype seen in the plate.

This laboratory activity can be modified based on instructor preferences; a prelab activity can be added to provide an opportunity for students to design custom primers to target part of the *flgA1* gene. Moreover, it could be expanded to add protein analysis to show students the presence or absence of the protein product by using protein gel and Coomassie stain.

CONCLUSION

This laboratory has been implemented as a second-year undergraduate lab activity. Reactions were largely positive: the students liked the problem-solving approach and the hands-on nature of the activity. They enjoyed solving the unknown question and determining the genotype of the unknown strain. We provide an activity for students to work on during and after the lab in Appendix 2.

To allow students to visualize their PCR results in real time, we used MiniOne electrophoresis boxes, which provide a real-time safe visualization of the gel for the students. A dedicated laboratory iPad or phone can be used to take pictures of the gel and share them easily with the class. This was a positive aspect of the lab, as it provided a quick way for students to gather their results in an easily accessible format.

In summary, this inquiry-based lab is easy and safe to perform, allows students to follow the information flow from a gene to a protein product, and facilitates teaching the central dogma of biology.

SUPPLEMENTAL MATERIALS

- Appendix 1: Materials and methods
- Appendix 2: Student worksheet

ACKNOWLEDGMENTS

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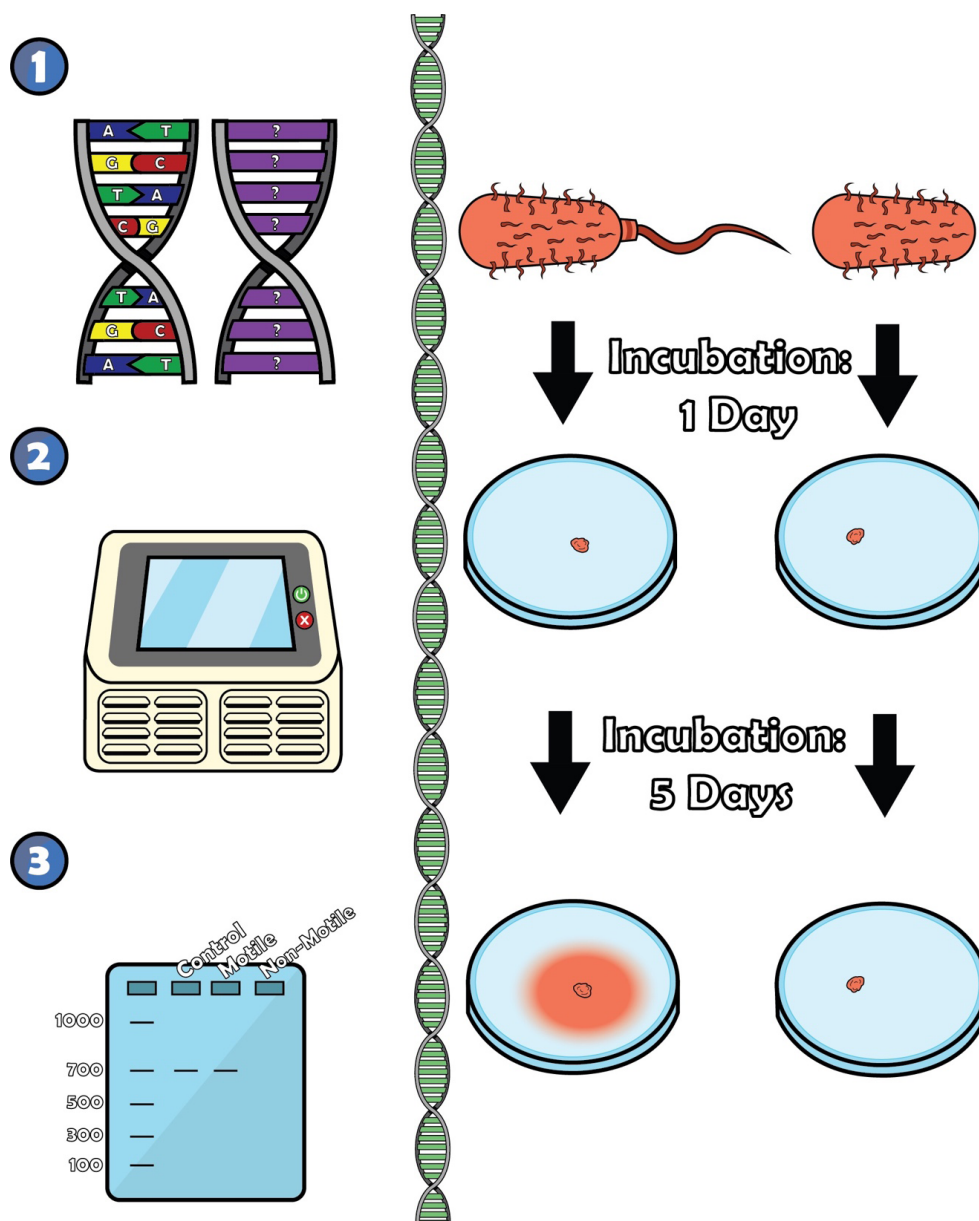


FIGURE 2. Experimental design and components. The left part of the figure indicates the process used to identify the presence of the flagella gene. 1) DNA extraction; 2) PCR; 3) electrophoresis and data analysis. The right side of the figure highlights the difference in phenotype the students observe depending on which strain they work with.

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