

Drug-Resistant TB: A Genetic Analysis

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Suggestions for Teachers

Subject area: Biology, Genetics, AP Biology

Grade level: 9-12

Purpose: This lesson asks students to compare gene sequences between two *Mycobacterium tuberculosis* (TB) strains: one wild-type and one variant. Students will identify a mutation as a single-nucleotide polymorphism (SNP), use online bioinformatics tools to align gene and protein sequences, and then critically hypothesize whether their variant strain will be resistant to a TB drug or not. Individual/group work is then shared for comparison among the class.

Length of lesson: 90 minutes

Objectives

By the end of this activity the student will be able to:

- 1) identify and explain what a single-nucleotide polymorphism (SNP) is when comparing two gene sequences.
- 2) navigate online scientific tools to translate DNA into polypeptide sequences and to compare and contrast wild-type and variant polypeptide sequences.
- 3) determine whether your given SNP will result in 'sense' or 'missense' in the resulting amino acid sequence.
- 4) hypothesize whether a SNP will likely cause antibiotic resistance.

National Science Standards:

Content Standard A: Science as Inquiry

- Use technology and mathematics to improve investigations and communications.
- Formulate and revise scientific explanations and models using logic and evidence.
- Communicate and defend a scientific argument.

Content Standard C: Life Science

- The molecular basis of heredity

Colorado Science Standards:

I. Standard 2: Life Science, Grades 9-12—*Students know and understand the characteristics and structure of living things, the processes of life, and how living things interact with each other and their environment.*

A. Concept and skills students master—*Physical and behavioral characteristics of an organism are influenced to varying degrees by heritable genes, many of which encode instructions for the production of proteins.*

1. Evidence outcomes—Students can:

- a. Analyze and interpret data that genes are expressed portions of DNA.
- b. Analyze and interpret data on the processes of DNA replication, transcription, translation, and gene regulation, and show how these processes are the same in all organisms.
- c. Explain using examples how genetic mutations can benefit, harm, or have neutral effects on an organism.

Vocabulary

Gene: a segment of DNA that codes for a protein

Single-nucleotide polymorphism (SNP): a single base-pair difference observed when comparing gene sequences between two organisms or between homologous chromosomes

Synonymous polymorphism: a SNP that codes for the same amino acid as its homologue due to redundancy in the genetic code

Nonsynonymous polymorphism: a SNP that codes for a different amino acid than its homologue

Sense: An amino acid sequence with the same amino acids as its homologue, despite a SNP in the gene that codes for that amino acid sequence.

Missense: An amino acid sequence with one or more different amino acids than its homologue; one result of a nonsynonymous polymorphism

Nonsense: An amino acid sequence that has an early stop codon compared to its homologue; one result of a nonsynonymous polymorphism

Rifampin: An antibiotic that targets a binding site on the beta-subunit of the RNA polymerase protein. It is used to treat TB infections.

rpoB: An abbreviation for **R**NA **p**olymerase **b**eta subunit.

Materials

- Introductory Power Point presentation
- Electronic files: TB gene sequences, Sets A, B, C, D, E, F, G, H
- Copymaster 1: Genetic Code chart
- Copymaster 2: Properties of Amino Acids chart
- Class set of laptops (or reserve your school's computer lab)
- Copies of the accompanying Student Activity handout

Preparation and Procedure

1) Lesson Pacing and Planning:

- | | |
|--|------------|
| • Introductory Power Point (Background) | 30 minutes |
| • Align gene sequences, identify SNPs, translate sequences, align amino acid sequences, answer questions (Parts A-D) | 30 minutes |
| • Compare all variant genes in a class discussion (Part E) | 20 minutes |

Safety

Students should handle computers carefully.

Hints and Answers for Questions along the Way

Prepare: You will need to post the MTB Variant Files A-H on edmodo.com for students to access during class.

Background: Use the Introductory Power Point to provide the necessary background information on TB, SNPs, and antibiotic resistance to rifampin.

Part A, #1: There are 8 variant MTB gene sequences (A-H). Each one contains one SNP. A description of each sequence is provided for you in the Teacher Answer Key at the end of this document. The sequences are designed so that A and B are synonymous polymorphisms while C-H are nonsynonymous polymorphisms. Consider strategically differentiating your class by assigning variants A-B to lower performing students, and C-H to middle-to-higher performing students. Variants F-H likely require the highest level of analysis, so they could ideally be given to top-performing students.

Part A, #2: Consider offering a prize for the first group to find their SNP. Some students may try to cut and paste their sequences to align each row of the wild-type and variant sequences. Praise their ingenuity, but still keep the time to a minute. The important thing for students to realize is that visually scanning for SNPs is not very efficient.

Part A, #3: The common answer will be *no*. Students will likely report that it is difficult and may suggest that the process could be easier if each line of the two sequences were aligned right next to each other.

Part A, #5: Answers will vary. See the Teacher's Answer Key for specifics.

Part A, #6: Students will likely suggest that they would need to transcribe each sequence into RNA, identify codons, use a genetic code chart to identify amino acids, and then translate each RNA sequence into a polypeptide of amino acids.

Part A, #7: 282 bp / 3 bp per codon = 94 codon. The 94th codon will code for STOP, so technically there will only be 93 amino acids.

Part C, #12: Students with variants A and B should answer ‘identical.’ Students with C-H should answer ‘different.’

Part C, #13: The purpose of this chart is to have students identify the position of their amino acid change or loss of amino acids in the cases of Variants G & H (This information will be important in Part D.), to be familiar with 3 ways of representing amino acids (1-letter, 3-letters, full name), and to identify the chemical property categories of amino acids.

Part C, #14: Variants A-B: *sense*; Variants C-F: *missense*; Variants G-H: *nonsense*

Part C, #15: Variants A-B: *synonymous*; Variants C-H: *nonsynonymous*

Part D, Prediction & Rationale: Evaluate whether or not the student’s rationale matches her prediction. The Teacher Answer Key provides a break down of each variant’s likely susceptibility to rifampin.

Part E, Comparing All Variant rpoB MTB Genes in the Class

Fill in the table as results are displayed on the board in class. Consider leaving the last column blank and having students discuss their inferences in small groups before sharing out with the entire class.

MTB Variant	Sense, missense or nonsense in the amino acid sequence	Amino acid change between wild-type and variant (From ___ to ___ OR <i>no change</i>)	Synonymous or Nonsynonymous Polymorphism	Is the amino acid change in the binding site for rifampin? (Between 65 & 80 aa)	Inference: Will this variant be resistant to rifampin?
A	Sense	No change	Synonymous	N/A	No (susceptible)
B	Sense	No change	Synonymous	N/A	No (susceptible)
C	Missense	Asn → His	Nonsynonymous	No (10)	No (susceptible)
D	Missense	Iso → Met	Nonsynonymous	No (43)	No (susceptible)
E	Missense	Gln → Leu	Nonsynonymous	Yes (69)	Yes (resistant)
F	Missense	Ala → Val	Nonsynonymous	Yes (78)	No (susceptible)
G	Nonsense	Leu → stop	Nonsynonymous	No (86)	Likely resistant (8 aa missing at end)
H	Nonsense	Trp → stop	Nonsynonymous	No (91)	Maybe resistant (2 aa missing at end)

Suggestions for Assessment

Evaluate student answers on pages 7 & 8 for correctness. The rationale on page 8 should show evidence that the student understands that a mutation in DNA will not always result in a change in an amino acid sequence, and only if the mutation is in a binding site region (or an early stop codon) will there *likely* be a change in that protein's function (e.g. resistance to an antibiotic).

On the next exam, pose this question:

*You are a doctor and diagnose a female patient with tuberculosis. You are not sure if rifampin will be a useful antibiotic to treat her. So you send a sample of her sputum (spit up) to a diagnostics lab that CAN sequence the rpoB gene from the bacterial sample but CANNOT grow the bacteria in culture to test rifampin directly on the bacteria. The results come back and the lab says that the strain (variant) of TB that she has is **resistant to rifampin**.*

Explain how the lab could conclude that her strain is resistant. Use the following words in your answer: SNP (single-nucleotide polymorphism), binding site, sense/missense/nonsense, synonymous/nonsynonymous polymorphism.

Where to Go From Here

Ideally, this lesson would be delivered after students understand transcription and translation. If you are a Denver Public Schools teacher, this lesson is an excellent extension after the sickle cell poster activity (and that activity is referenced in the Introductory Power Point). A second variant sequence could be given to students who finish early.

References and Resources

I'd like to thank Rebecca Davidson, Ph.D., Michael Strong, Ph.D., Eve Farias-Hesson, Ph.D., Paul Reynolds, Ph.D., and Andrew Schaumberg of National Jewish Health for their evaluation of this lesson.

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Student Activity

Overview

This lesson asks students to compare gene sequences among one wild-type and a variety of mutant *Mycobacterium tuberculosis* (TB) strains. Students will identify mutations as single-nucleotide polymorphisms (SNPs), transcribe and translate their DNA sequences into RNA and amino acid sequences, respectively, and then critically hypothesize (evaluate?) whether their mutant strains will be resistant to a TB drug or not.

Objectives

By the end of this activity you will be able to:

- 1) identify and explain what a single-nucleotide polymorphism (SNP) is when comparing two gene sequences.
- 2) practice transcribing a DNA sequence into mRNA and translating mRNA into an amino acid sequence.
- 3) determine whether your given SNP will result in 'sense' or 'missense' in the resulting amino acid sequence.
- 4) navigate an online scientific tool, the TB Drug Resistance Database, to gather evidence to support a conclusion of drug-resistance or not.

Background

This section will serve as an introduction to the content to be covered in this activity. It should contain a brief overview of the basic concepts that students should know before beginning the activity. You may want to consider including some figures or illustrations to get your point across. It should be written in such a way that a student with a general science knowledge can read the information and know enough to perform and understand the activity.

Materials

Provide a list of the materials that each group of students will need. Provide quantities, concentrations and warnings as necessary. For example,

- triple-beam balance
- 5 - 50 mL beakers
- wax pencil or marker
- 150 mL of 1M NaCl solution
- dropper bottle of 1M HCl (caution: caustic, may cause burns, wear safety goggles and gloves)

Procedure

List, step by step, the procedure to be followed by students. Include safety information and warnings where applicable.

- 1) Put on safety goggles, gloves and apron before beginning. Keep these items on during the entire activity.
- 2) Using the graduated cylinder, measure out 100 mL of the NaCl solution. Carefully pour it into the beaker.
- 3) Carefully add 3 drops of the 1M HCl to the beaker. Be careful that you do not come in contact with the acid. If you do, rinse immediately with water and contact your teacher.
- 4) Make observations and record them in the data table.
- 5) Clean up your lab area and return items as instructed by your teacher.

Data

Provide adequate space for students to record data. You may organize a data table for the student or have them design one of their own. Include units and labels wherever possible. Try to use the table feature of MS Word for this component of the activity. Use the table below as a sample.

Data Table 1 – Heart rate of *Artemia* at three different temperatures.

Trial Number	10°C	20°C	30°C
1			
2			
3			
Mean			

You can also include less structured data tables if desired.

Calculate Q_{10} for heart rate in *Artemia* using the following formula. _____

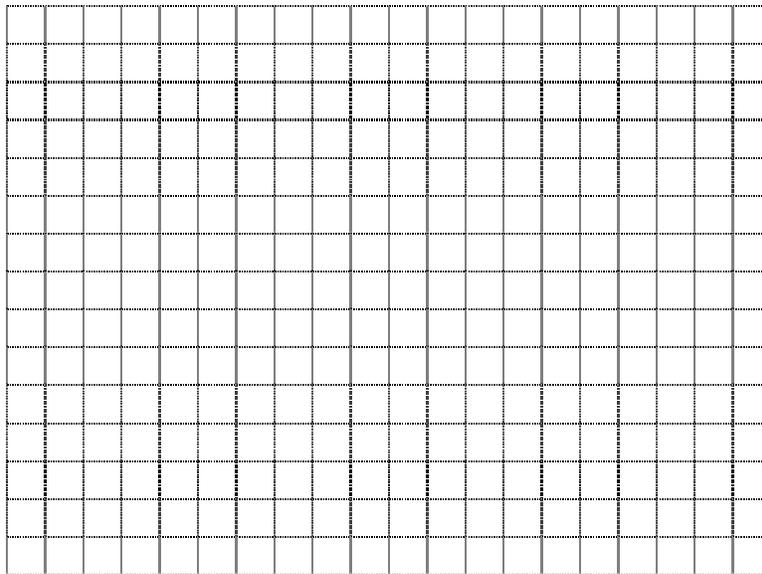
Show your work.

$$Q_{10} = \left[\frac{k_2}{k_1} \right]^{(10 / t_2 - t_1)}$$

Where:

t_2 = higher temperature
 t_1 = lower temperature
 k_2 = rate at t_2
 k_1 = rate at t_1

Graphs may also be included in this section. You can choose to label axes for students or leave it up to them. Be sure to include space to include legends or titles. You may produce graph paper in MS Word using the table feature as well. You may need to drastically increase the number of columns and rows as you create the table. Using a gray or fine line will be easier on the eyes. A sample graph is given below.



Analysis Questions

This section can be used to assess students' knowledge of content and determine their ability to make inferences and conclusions based upon the results of their activity. Feel free to make this portion as directed or as open-ended as the activity requires. Use the following format for your questions.

1. What is Q_{10} ? (Describe it in words, don't just give me the formula above.)
2. Why do you suppose that heart rate was chosen as the physiological parameter to measure in this activity?

3. What are some environmental factors (other than temperature) that may have influenced your results?
4. Why would the technique used in this activity NOT be effective if the organism studied was a human?

Going Further

In this section you can give students some additional activities that will extend the concepts covered. You may want to consider activities such as library research, classroom presentations or additional experiments.

