AP® INVESTIGATION #8

TRANSFORMATION – TEACHER'S GUIDE

Kit #36W7408

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ABSTRACT

Students transform common bacteria, normally killed by antibiotics, with a plasmid containing DNA that will confer antibiotic resistance. Students calculate efficiencies of transformations and investigate factors that might alter the effectiveness of transformation. This lab illustrates the basic processes of genetic transformation, gene expression, and natural selection.

GENERAL OVERVIEW

The College Board has revised the AP Biology curriculum to begin implementation in the fall of 2012. Advanced Placement (AP) is a registered trademark of the College Entrance Examination Board. The revisions were designed to reduce the range of topics covered, to allow more depth of study and increased conceptual understanding for students. There is a shift in laboratory emphasis from instructor-designed demonstrations to student-designed investigations. While students may be introduced to concepts and methods as before, it is expected that they will develop more independent inquiry skills. Lab investigations now incorporate more student-questioning and experiment design. To accomplish this, the College Board has decreased the minimum number of required labs from 12 to 8 while keeping the same time requirement (25% of instruction time devoted to laboratory study). The College Board has defined seven science practices that students must learn to apply over the course of laboratory study. In brief, students must:

1. Use models
2. Use mathematics (quantitative skills)
3. Formulate questions
4. Plan and execute data collection strategies
5. Analyze and evaluate data
6. Explain results
7. Generalize data across domains

The College Board published 13 recommended laboratories in the spring of 2011. They can be found at: http://advancesinap.collegeboard.org/science/biology/lab

Many of these laboratories are extensions of those described in the 12 classic labs that the College Board has used in the past. The materials provided in this lab activity have been prepared by Ward's to adapt to the specifications outlined in AP Biology Investigative Labs: An Inquiry-Based Approach (2012, The College Board). Ward's has provided instructions and materials in the AP Biology Investigation series that complement the descriptions in this College Board publication. We recommend that all teachers review the College Board material as well as the instructions here to get the best understanding of what the learning goals are. Ward's has structured each new AP investigation to have at least three parts: Structured, Guided, and Open Inquiry. Depending on a teacher's syllabus, s/he may choose to do all or only parts of the investigations in scheduled lab periods.

The College Board requires that a syllabus describe how students communicate their experiment designs and results. It is up to the teacher to define how this requirement will be met. Having students keep a laboratory notebook is one straightforward way to do this.

RECORDING DATA IN A LABORATORY NOTEBOOK

All of the Ward's Investigations assume that students will keep a laboratory notebook for student-directed investigations. A brief outline of recommended practices to set up a notebook, and one possible format, are provided below.

1. A composition book with bound pages is highly recommended. These can be found in most stationary stores. Ward's offers several options with pre-numbered pages (for instance, item numbers 32-8040 and 15-8332). This prevents pages from being lost or mixed up over the course of an experiment.
2. The title page should contain, at the minimum, the student's name. Pages should be numbered in succession.
3. After the title page, two to six pages should be reserved for a table of contents to be updated as experiments are done so they are easily found.
4. All entries should be made in permanent ink. Mistakes should be crossed out with a single line and should be initialed and dated. This clearly documents the actual sequence of events and methods of calculation. When in doubt, over-explain. In research labs, clear documentation may be required to audit and repeat results or obtain a patent.
5. It is good practice to permanently adhere a laboratory safety contract to the front cover of the notebook as a constant reminder to be safe.
6. It is professional lab practice to sign and date the bottom of every page. The instructor or lab partner can also sign and date as a witness to the veracity of the recording.
7. Any photos, data print-outs, or other type of documentation should be firmly adhered in the notebook. It is professional practice to draw a line from the notebook page over the inserted material to indicate that there has been no tampering with the records.

For student-directed investigations, it is expected that the student will provide an experimental plan for the teacher to approve before beginning any experiment. The general plan format follows that of writing a grant to fund a research project.

1. Define the question or testable hypothesis.
2. Describe the background information. Include previous experiments.
3. Describe the experiment design with controls, variables, and observations.
4. Describe the possible results and how they would be interpreted.
5. List the materials and methods to be used.
6. Note potential safety issues.

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RECORDING DATA IN A LABORATORY NOTEBOOK (CONTINUED)

After the plan is approved:

7. The step-by-step procedure should be documented in the lab notebook. This includes recording the calculations of concentrations, etc., as well as the weights and volumes used.
8. The results should be recorded (including drawings, photos, data print-outs, etc.).
9. The analysis of results should be recorded.
10. Draw conclusions based on how the results compared to the predictions.
11. Limitations of the conclusions should be discussed, including thoughts about improving the experiment design, statistical significance, and uncontrolled variables.
12. Further study direction should be considered.

The College Board encourages peer review of student investigations through both formal and informal presentation with feedback and discussion. Assessment questions similar to those on the AP exam might resemble the following questions, which also might arise in peer review:

- Explain the purpose of a procedural step.
- Identify the independent variables and the dependent variables in an experiment.
- What results would you expect to see in the control group? The experimental group?
- How does a specific concept (XXXX) account for the described findings (YYYY)?
- Describe a method that could be used to determine a given concept/observation (XXXX).

MATERIALS CHECKLIST

MATERIALS INCLUDED IN KIT

Units per kit	Description
50	Disposable inoculating loops
1	* Coupon for 2 tubes of plasmid, pUC8 1 µg, (100 µL); 1 tube of *E. coli* JM101; 40 Luria plates; 40 Luria plates w/ampicillin
1	Biohazard bag
120	Sterile graduated pipets, 6"
1 pkg./30	Microfuge tubes, 1.5 mL
8	Sterile tryptic soy broth tubes
8	50 mM calcium chloride tubes
80	Disposable bacti-spreaders
1	Instructions (this booklet)

**** - It is recommended that you redeem your coupon for live/ perishable materials as soon as possible and specify your preferred delivery date. Generally, for timely delivery, at least two week's advance notice is preferred.***

For a list of replacement items, visit: www.wardsci.com, and click on the AP Biology tab for this kit/item #.

* At least two weeks in advance of your lab, redeem the coupon provided for the perishable components, *E. coli*, pUC8 plasmid.

- Store the vial of plasmid pUC8 frozen until needed.
- Store the *E. coli* culture refrigerated; use or subculture within two weeks.
- All other components may be stored at room temperature.

MATERIALS NEEDED BUT NOT PROVIDED

Incubator oven or seedling heat mat
Microfuge tube racks
Timer
Hot plate or temp-controlled water bath
Thermometer
Crushed ice in container
10% solution of bleach
Lab notebook
Gloves, safety goggles, lab aprons

OPTIONAL MATERIALS (NOT PROVIDED)

Water
Luria agar, 200 mL bottle
Incubator oven or seedling heat mat
UV light source
Penicillin discs to look at antibiotic effects/resistance
Streptomycin discs to look at antibiotic resistance
Ampicillin, x-gal, IPTG to utilize the lac Z system
Capillary micropipets to substitute for sterile pipets
H_2O_2 as a stress or a potential mutagen
Caffeine as a potential mutagen
Potassium nitrate
N,N-Dimethylformamide, 2 mL
Other plasmids: pBR322, pUC18, pGLO, etc.
Glass marking pens
Masking tape
Micropipettors with sterile tips 2–20 µL
Ruler
Heat protective gloves

This lab activity is aligned with the 2012 AP Biology Curriculum (registered trademark of the College Board). Listed below are the aligned Content Areas (Big Ideas and Enduring Understandings), the Science Practices, and the Learning Objectives of the lab as described in AP Biology Investigative Labs: An Inquiry-Based Approach (2012). This is a publication of the College Board that can be found at http://advancesinap.collegeboard.org/science/biology/lab.

CURRICULUM ALIGNMENT

Big Ideas

- **Big Idea 3:** Living systems store, retrieve, transmit, and respond to information essential to life processes.
- With connections to:
  Big Idea 1: The process of evolution drives the diversity and unity of life.

Enduring Understandings

- 1A2: Natural selection acts on phenotypic variations in populations.
- 1C3: Populations of organisms continue to evolve.
- 3A1: DNA, and in some cases RNA, is the primary source of heritable information.
- 3A3: The chromosomal basis of inheritance provides an understanding of the pattern of passage (transmission) of genes from parent to offspring.
- 3B1: Gene regulation results in differential gene expression.
- 3C1: Changes in genotype can result in changes in phenotype.
- 3C2: Biological systems have multiple processes that increase genetic variation.

Science Practices

- 1.4 The student can use representations and models to analyze situations or solve problems qualitatively and quantitatively.
- 3.1 The student can pose scientific questions.
- 5.3 The student can evaluate the evidence provided by data sets in relation to a particular scientific question.
- 6.2 The student can construct explanations of phenomena based on evidence produced through scientific practices.
- 6.4 The student can make claims and predictions about natural phenomena based on scientific theories and models.
- 7.1 The student can connect phenomena and models across spatial and temporal scales.
- 7.2 The student can connect concepts in and across domain(s) to generalize or extrapolate in and/or across enduring understandings and/or big ideas.

LEARNING OBJECTIVES

- The student is able to connect evolutionary changes in a population over time to a change in the environment (1A2 & SP 7.1).
- The student is able to evaluate given data sets that illustrate evolution as an ongoing process (1C3 & SP 5.3).
- The student can justify the claim that humans can manipulate heritable information by identifying at least two commonly used technologies (3A1 & SP 6.4).
- The student can predict how a change in a specific DNA or RNA sequence can result in changes in gene expression (3A1 & SP 6.4).
- The student is able to pose questions about ethical, social, or medical issues surrounding human genetic disorders (an application of genetic engineering) (3A3 & SP 3.1).
- The student can use representations to describe how gene regulation influences cell products and function (3B1 & SP 1.4).
- The student is able to predict how a change in genotype, when expressed as a phenotype, provides a variation that can be subject to natural selection (3C1 & SP 6.4, SP 7.2).
- The student is able to construct an explanation of the multiple processes that increase variation within a population (3C2 & SP 6.2).

TIME REQUIREMENTS

Part 1: Structured—Transformation	Day 1	30–45 minutes to transform and plate
	Day 2	15–45 minutes to collect and analyze data
Part 2: Guided—Transformation Optional— can be done concurrently with Part 1	Day 1	30 minutes— could be same day as Part 1 Day 2
	Day 2	15 minutes
Part 3: Open		Varies by student

SAFETY PRECAUTIONS

Lab Specific Safety

- The *E. coli* bacteria should be handled with care. Any items that come into contact with the culture should be treated with 10% bleach and disposed of in the biohazard waste. Biohazardous waste should be either autoclaved at 121 °C for 20 minutes or soaked in 10% bleach for 20 minutes. Check your school regulations before discarding in general trash or having biohazardous waste picked up for specialized disposal.
- Ampicillin may cause allergic reactions so wear protective clothing at all times and wipe down bench tops and equipment daily. If contact occurs, rinse well with water and seek medical advice.

General Safety

- The teacher should 1) be familiar with safety practices and regulations in his/her school (district and state) and 2) know what needs to be treated as hazardous waste and how to properly dispose of non-hazardous chemicals or biological material.
- Consider establishing a safety contract that students and their parents must read and sign. This is a good way to identify students with allergies (e.g., latex) so that you (and they) will be reminded of specific lab materials that may pose risks to individuals. A good practice is to include a copy of this contract in the student lab book (glued to the inside cover).
- Students should know where all emergency equipment (safety shower, eyewash station, fire extinguisher, fire blanket, first aid kit etc.) is located.
- Require students to remove all dangling jewelry and tie back long hair before they begin.
- Remind students to read all instructions, Material Data Safety Sheets (MSDSs) and live care sheets before starting the lab activities, and to ask questions about safety and safe laboratory procedures. Appropriate MSDSs and live care sheets can be found on the last pages of this booklet. Additionally, the most updated versions of these resources can be found at www.wardsci.com. The most updated version of most MSDSs can usually be found on the chemical manufacturer's website.
- In student directed investigations, make sure that collecting safety information (like MSDSs) is part of the experimental proposal.
- As general laboratory practice, it is recommended that students wear proper protective equipment, such as gloves, safety goggles, and a lab apron.

At the end of the lab:

- All laboratory bench tops should be wiped down with a 10% bleach solution or disinfectant to ensure cleanliness.
- Remind students to wash their hands thoroughly with soap and water before leaving the laboratory.

NOTES

PRE-LABORATORY PREPARATION

Prepare a starter plate of *E. coli* JM101 bacteria from the stock received through coupon redemption.

1. Subculture *E. coli:* The day before the experiment, obtain two (plain) Luria agar plates.
2. Using sterile technique and a sterile loop, streak bacteria on two plain Luria agar plates.
3. Invert the plates and place in a 37 °C incubator overnight. If an incubator is not available, invert and allow to incubate at room temperature 24–48 hours. These plates will be used as starter plates.
4. Efficient transformation requires cells in an active growth phase.

Make copies of Student Guide.

Make copies of the Student Guide (copymaster pages) prior to starting class.