KIT 470134-774

AP® Biology Investigation #8:

Genetics and Information Transfer: Transformation

Meets Revised College Board AP Biology Standards

Teacher's Guide

WARD470140-116

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Abstract

In this lab, students will transform common bacteria, normally killed by antibiotics, with a plasmid containing DNA that will confer antibiotic resistance. Students will also 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.

Required Prior Knowledge

Students should:

- be able to make and record good observations.
- have a strong understanding of the structure and function of DNA.

Activity Learning Objectives

In this investigation, students will perform activities to help them answer the following question:

Aside from mutations, how does the genetic makeup of an organism get altered?

Materials Checklist

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

Included in Kit:

- 50 disposable inoculating loops
- 1 coupon for:
  - 2 tubes of plasmid, pUC19 (1 µg in 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 calcium chloride tubes (50 mm)
- 80 disposable bacti-spreaders
- 1 instructions (this booklet & student guide)

At least two weeks in advance of your lab, redeem the coupon provided for the perishable components.

- Store the vial of plasmid pUC19 frozen until needed.
- Store the *E. coli* culture refrigerated; use or subculture within two weeks.
- All other components may be stored at room temperature.

Also Needed:

- Incubator oven or seedling heat mat
- Microfuge tube racks
- Timer
- Hot plate or temp-controlled water bath
- Thermometers
- 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
- 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
- Micropipettes with sterile tips 2–20 µL
- Ruler
- Heat protective gloves

Standards Alignment

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://media.collegeboard.com/digitalServices/pdf/ap/APBioTeacherLabManual2012_2ndPrt_lkd.pdf*

Big Ideas	**3**	Living systems store, retrieve, transmit, and respond to information essential to life processes.
	1	The process of evolution drives the diversity and unity of life.
Enduring Understandings	**1.A.2**	Natural selection acts on phenotypic variations in populations.
	1.C.3	Populations of organisms continue to evolve.
	3.A.1	DNA, and in some cases RNA, is the primary source of heritable information.
	3.A.3	The chromosomal basis of inheritance provides an understanding of the pattern of passage (transmission) of genes from parent to offspring.
	3.B.1	Gene regulation results in differential gene expression, leading to cell specialization.
	3.C.1	Changes in genotype can result in changes in phenotype.
	3.C.2	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.

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Standards Alignment *continued*

Learning Objectives	**1.5**	The student is able to connect evolutionary changes in a population over time to a change in the environment.
	1.26	The student is able to evaluate given data sets that illustrate evolution as an ongoing process.
	3.5	The student can justify the claim that humans can manipulate heritable information by identifying at least two commonly used technologies.
	3.6	The student can predict how a change in a specific DNA or RNA sequence can result in changes in gene expression.
	3.13	The student is able to pose questions about ethical, social, or medical issues surrounding human genetic disorders (and application of genetic engineering).
	3.21	The student can use representations to describe how gene regulation influences cell products and function.
	3.24	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.
	3.28	The student is able to construct an explanation of the multiple processes that increase variation within a population.

Time Requirements

	TIME FRAME	TEACHER TASK(S)	STUDENT TASK(S)
PRE-LAB PREP	At least 2 weeks in advance	Redeem Coupon.	Read background and complete pre-lab questions.
	12-48 hours in advance	See pre-lab prep to subculture.	
Activity 1	Day 1: 30–45 minutes	Assist with procedure.	Transform and plate.
	Day 2: 15–45 minutes		Collect and analyze data. Calculate transformation efficiency.
Activity 2	Day 1: 30 minutes - could be same day as Activity 1 Day 2		Vary time, temperature and quantity parameters.
	Day 2: 15 minutes		
Activity 3	Varies by student	Review student designs.	

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.
- 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, SDSs and live care sheets** before starting the lab activities, and to ask questions about safety and safe laboratory procedures. The SDSs and the most updated versions of live care sheets can be found at www.**wardsci.com**. The SDSs can also usually be found on each chemical manufacturer's website.
- In student directed investigations, make sure that collecting safety information (like SDSs) is part of the experiment procedure.
- As general laboratory practice, it is recommended that students **wear proper protective equipment**, such as gloves, safety goggles, and a lab apron.
- For more additional safety concerns, the NSTA offers a great collection of resources on its website. Visit http://www.nsta.org/safety/

At the End of the Lab:

- Before disposing of any chemicals in the trash or down the drain, review local regulations or consult with local authorities.
- 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.