470134-776

AP® Biology Investigation #9:

Genetics and Information Transfer: Restriction Enzyme

Meets Revised College Board AP Biology Standards

ward's science

table of contents

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WACP470132-880 – Rev. 12/14

abstract

This lab starts with a scenario that illustrates how students might use agarose gel electrophoresis technology to solve a problem. The students use hands-on modeling of complex molecular processes to understand the necessary steps and underlying logic in common biotechnology methods. After modeling the processes, the students compare the DNA samples found at a crime scene to DNA isolated from fictional "suspects" using hands-on gel electrophoresis. The students compare fragments of known size to fragments of an unknown size to calculate relative molecular weights, and match fragment patterns from unknown samples to known samples. Students are encouraged to consider the benefits, risks and ethics that come with the availability of such powerful techniques.

required prior knowledge

Students should:

- be able to make and record good observations.
- be able to graph and analyze data.
- be proficient in using lab equipment ie, pipettors.

activity learning objective

In this investigation, students will practice loading a gel and perform gel electrophoresis to help them answer the following question:

How can an individual's genetic makeup be used to convict them of a crime?

materials checklist

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

materials included in kit:

- 1 coupon, perishable material (*see below)
- 480 red pop beads, 10 mm
- 480 white pop beads, 10 mm
- 120 blue pop beads, 10 mm
- 120 yellow pop beads, 10 mm
- 120 orange pop beads, 10 mm
- 120 green pop beads, 10 mm
- 240 clear connectors
- 1 orange felt tip marker
- 1 blue felt tip marker
- 1 yellow felt tip marker
- 1 green felt tip marker
- 200 mL prepared agarose, 0.8%
- 32 microfuge tubes (1.5 mL)
- 8 agarose gel staining trays
- 500 mL Tris-Borate-Edta, 5x
- 1 Ward's QUIKView DNA Stain
- 8 zip plastic bags
- 1 instructions (this document & student guide)

materials needed but not provided:

- Lab notebook
- Electrophoresis chambers
- Aluminum foil
- Graduated cylinder, 1 L
- Erlenmeyer flask, 1 L or larger
- Microfuge tube racks
- Hot plate
- Gel casting trays with 16 well combs
- Power supplies
- Hot water bath or microwave
- 10 µL micropipets
- Metric ruler
- Calculators
- Safety goggles
- Chemical-resistant lab aprons
- Chemical-resistant gloves
- Heat-resistant gloves/mitts
- Semi-log graph paper
- Distilled water
- Scissors

optional materials (not provided)

- White light box
- Lambda DNA (uncut)
- Restriction enzymes: BamHI, EcoRI, HindIII, etc.
- Masking tape (if needed for sealing ends of gel casting trays)
- Microcentrifuge

*** At least two weeks in advance of your lab, redeem the coupon provided for the perishable components. Store DNA samples frozen until use. All other reagents may be stored at room temperature.**

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.
	With connections to:	
	1	The process of evolution drives the diversity and unity of life.
Enduring Understandings	**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.
Science Practices	**3.1**	The student can pose scientific questions.
	6.4	The student can make claims and predictions about natural phenomena based on scientific theories and models.
Learning Objectives	**3.5**	The student can justify the claim that humans can manipulate heritable information by identifying at least two commonly used technologies.
	3.13	The student is able to pose questions about ethical, social, or medical issues surrounding human genetic disorders [an application of genetic engineering].

time requirements

	TIME FRAME	TEACHER TASK(S)	STUDENT TASK(S)
pre-lab prep	45 minutes, at least 1 hour before lab	See page 11.	Read background and answer pre-lab questions.
activity 1	45 minutes		Assemble DNA molecule and simulate restriction enzyme digest and electrophoresis.
activity 2	60 minutes (assuming teacher pre-pours gel), plus analysis time.	Agarose gels can be poured ahead of time and stored in 1XTBE until use.	Perform electrophoresis.
activity 3	Varies, depending on students' experiment designs.		

safety precautions

lab specific safety

- Tris-Borate-EDTA is slightly toxic if ingested.
- Ward's QuickView DNA stain will stain clothing and skin.
- CAUTION: The power supply produces a high voltage that can cause severe electrical shock if handled improperly. For safe operation, follow all directions and precautions.
- **Examine all components of the electrophoresis apparatus prior to each use**, including all cords, plugs, jacks, the electrophoresis chamber itself, and the power supply.
- Do not operate the electrophoresis apparatus in a damp or humid environment. Any condensed moisture may short out electrical components.
- You may wish to designate one area of the laboratory specifically for electrophoresis equipment, where cells and power supplies are connected. Ensure that power cords and patch cords are free from moisture, and that any wall outlet is properly wired; i.e., that correct polarity exists (use a circuit tester).
- Be sure that students are well acquainted with the correct procedure for making electrical connections. **Students should be supervised at all times when performing this investigation.**
- Do not come in personal contact with or allow metal or any conductive material to come in contact with the reservoir buffer or the electrophoretic cell while the power supply is on.

(continued on next page)

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**. Updated 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.

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.

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