PATHWAY: Biotechnology Research & Development

COURSE: Applications of Biotechnology

UNIT 6: Advanced Lab Skills Molecular Biology



#### **Annotation:**

This unit introduces students to some common advanced biotechnology laboratory techniques such as DNA isolation, restriction digestion of DNA, and transformation of bacteria cultures.

## Grade(s):

9<sup>th</sup> 10<sup>th</sup> X 11<sup>th</sup> X 12<sup>th</sup>

### Time:

Twenty 50-minute class periods

#### **Author:**

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#### Students with Disabilities:

For students with disabilities, the instructor should refer to the student's IEP to be sure that the accommodations specified are being provided. Instructors should also familiarize themselves with the provisions of Behavior Intervention Plans that may be part of a student's IEP. Frequent consultation with a student's special education instructor will be beneficial in providing appropriate differentiation.



#### **GPS Focus Standards:**

#### **HS-ABT-7** Students will demonstrate proficiency in advanced biotechnology techniques.

- a) Describe the foundations for molecular analysis quantity, quality, and purity.
- b) Perform DNA isolation and restriction digests.
- c) Describe various methods of transformation including chemical, physical, and biological.

# **GPS Academic Standards:**

ademic Stai	ndards:
<u>SB1</u>	Students will analyze the nature of the relationships between structures and functions in
	living cells.
SB3	Students will derive the relationship between single-celled and multi-celled organisms and
<u> </u>	the increasing complexity of systems.
<u>SB4</u>	Students will assess the dependence of all organisms on one another and the flow of energy
	and matter within their ecosystems.
SCSh3	Students will identify and investigate problems scientifically.
SCSh4	Students use tools and instruments for observing, measuring, and manipulating scientific
	equipment and materials.
SCSh5	Students will demonstrate the computation and estimation skills necessary for analyzing
	data and developing reasonable scientific explanations.
SCSh6	Students will communicate scientific investigations and information clearly.
<u>SC7</u>	Students will characterize the properties that describe solutions and the nature of acids and
<u>307</u>	bases.
SCSh8	Students will understand important features of the process of scientific inquiry.
SPS10	Students will investigate the properties of electricity and magnetism.
	, ,
<u>MM1A1</u>	Students will explore and interpret the characteristics of functions, using graphs, tables, and
	simple algebraic techniques.
MM1D3	Students will relate samples to a population.

**MM1P1** Students will solve problems (using appropriate technology).

**MM1P4** Students will make connections among mathematical ideas and to other disciplines.

**MM1P5** Students will represent mathematics in multiple ways.

MM2D2 Students will determine an algebraic model to quantify the association between two quantitative variables.

# **UNDERSTANDINGS & GOALS**

## **Enduring Understandings:**

Certain biotechnology laboratory techniques must be learned because they build on each other.

## **Essential Questions:**

- How are small volumes measured accurately?
- How is DNA cut?
- What is gel electrophoresis?

- What is the name of the process in which bacteria receive and express recombinant plasmid DNA?
- Which two techniques are used to increase transformation efficiency?

## **Knowledge from this Unit:**

Students will be able to:

- Describe the protocol for growing, isolating and transforming bacteria
- Explain the procedure for measuring small volumes
- Explain the protocol for cutting DNA
- Describe the procedure for agarose gel electrophoresis
- Define sterile technique

#### Skills from this Unit:

Students will be able to:

- Use a micropipette
- Pour an agarose gel
- Perform a bacterial transformation
- Make dilutions
- Prepare LB agar and pour sterile LB agar Petri plates
- Streak bacterial plates and start broth cultures
- Cut DNA and load it onto a gel.



## **Assessment Method Type:**

Х	Pre-test Pre-test
Χ	Objective assessment - multiple-choice, true- false, etc.
·-	_X_ Quizzes/Tests
	Unit test
	Group project
Χ	Individual project
	Self-assessment - May include practice quizzes, games, simulations, checklists, etc.
	Self-check rubrics
	Self-check during writing/planning process
	Journal reflections on concepts, personal experiences and impact on one's life
	Reflect on evaluations of work from teachers, business partners, and competition judges
	Academic prompts
	Practice quizzes/tests
	Subjective assessment/Informal observations
	Essay tests
	Observe students working with partners
	Observe students role playing
	Peer-assessment
	Peer editing & commentary of products/projects/presentations using rubrics
	Peer editing and/or critiquing
Χ	Dialogue and Discussion
	Student/teacher conferences
	Partner and small group discussions

	Whole group discussions Interaction with/feedback from community members/speakers and business partners
	Constructed Responses
	Chart good reading/writing/listening/speaking habits
	Application of skills to real-life situations/scenarios
	Post-test

# **LESSON PLANS**

# • LESSON 1: QUANTITY, QUALITY, AND PURITY

1. Identify the standards. Standards should be posted in the classroom.

## **HS-ABT-7** Students will demonstrate proficiency in advanced biotechnology techniques.

- a) Describe the foundations for molecular analysis quantity, quality, and purity.
- b) Perform DNA isolation and restriction digests.
- c) Describe various methods of transformation including chemical, physical, and biological.
- 2. Review Essential Question(s). Post Essential Questions in the classroom.
  - How are small volumes measured accurately?
  - How is DNA cut?
  - What is gel electrophoresis?
  - What is the name of the process in which bacteria receive and express recombinant plasmid DNA?
  - Which two techniques are used to increase transformation efficiency?
- 3. Identify and review the unit vocabulary. Terms may be posted on word wall.

Agarose	LB Agar	Recumbent Plasmid DNA	
Broth	Restriction Digestion	Negative electrode	
E. coli	Restriction Enzymes	Positive electrode	
Electrophoresis	Transformed	Micropipette	
DNA Ligase	Vector	Buffer	
Plastic Frame	Reaction	Heat shock	
Gel	Electroporation		

- 4. Interest approach Mental set
  - Review micropipetting with students using the Georgia Bioscience Technology Institute's
     <u>Micropipette Exercise</u> and the <u>Micropipetting DNA Template</u> handouts.
    - See attached supplementary files
  - Explain the different micropipettes used in the lab and their range of operation.
  - Demonstrate how the micropipette works and should be handled.
  - Make sure students record all pertinent information in their lab notebooks.
- LESSON 2: DNA ISOLATION AND RESTRICTION DIGESTION OF DNA

- 1. Review Essential Questions. Post Essential Questions in the classroom.
  - How is DNA cut?
  - What is gel electrophoresis?

#### 2. Gel Electrophoresis

- Have students research the process of gel electrophoresis and record notes in their lab notebooks.
- Give each student a copy of the **Gel Rig Diagram** worksheet.
  - See attached supplementary files
- Instruct students to draw and label the main components on a gel rig and answer the associated questions, following the directions on the worksheet.

#### 3. Agarose Gels

- Give students the <u>Agarose Gel</u> worksheet and instruct them to research and answer the questions.
  - See attached supplementary files
- · Discuss answers and check for understanding.

#### 4. Cutting DNA

- Note: Before teaching this lesson, find a procedure for cutting DNA which you can share with the class.
- Display the DNA splicing procedure to the class, and ask students what materials they would need to do this procedure.
- Ask students, "Do you think any DNA can be spliced, or does it have to be a specific type of DNA?"
- Give students a copy of the <u>Restriction Enzymes</u> worksheet and have them access <u>www.neb.com</u> to answer the questions about a specific enzyme.
  - o See attached supplementary files
- Discuss and check for understanding.

#### 5. Making DNA Visible

- Write the following information on the board and have students record it in their lab notebooks:
  - O DNA can be seen by using different dyes, such as ethidium bromide and methylene blue.
  - Ethidium bromide is used in gel electrophoresis to detect DNA and RNA by glowing orange when it is placed under a fluorescent light.
  - Methylene blue also stains RNA and DNA but is less harmful than ethidium bromide.

#### 6. Putting it all Together

- Split the class into small lab groups.
- Have each group research and create a procedure for isolating DNA and then splicing it using the techniques discussed in class.
- Obtain the materials required for the experiments and have students perform the procedures they designed.
  - o **Note:** This may take several class periods.
- Make sure students record all relevant information in their lab notebooks.

#### LESSON 3: BACTERIA CELL TRANSFORMATION

- 1. Review Essential Questions. Post Essential Questions in the classroom.
  - What is the name of the process in which bacteria receive and express recombinant plasmid DNA?
  - Which two techniques are used to increase transformation efficiency?
- 2. Review sterile technique with the class.
- 3. Bacterial Transformation Experiment
  - Split the class into small lab groups and have students research and design a procedure for bacterial transformation.
  - Instruct students to research two different methods and describe them in their lab notebooks.
    - o **Example:** Heat shock vs. Electroporation
  - Instruct students to perform their procedure.
    - Note: Review the students' procedures prior to implementing them. This lesson might take more than one class period.
- 4. Growing Bacteria
  - Write the following techniques on the board and have students research the them and record their research in their lab notebooks:
    - o How to make a bacterial growth media
    - o How to pour a growth plate
    - How to streak a growth plate to obtain single colonies
    - How to store plates properly stacking and temperature
  - Instruct students to break into their lab groups again and demonstrate growing a single bacterial colony in a growth plate.
    - o Tell students to record all data in their lab notebooks.

#### ATTACHMENTS FOR LESSON PLANS

Micropipetting Exercise
Micropipetting DNA Template
Gel Rig Diagram
Agarose Gels
Restriction Enzymes
Vocabulary Glossary

### • NOTES & REFLECTION:

Students should become comfortable with recording and implementing procedures in their lab notebooks. It may be interesting to have them exchange lab books and see if they can follow each other's notes. This can show them the importance of writing down clear directions, as mistakes are often caused by unclear notes in lab books. Scientists constantly struggle with poorly written protocols in lab manuals and even journal articles.

# CULMINATING PERFORMANCE TASK

## **Culminating Unit Performance Task:**

Students will perform a bacterial transformation and a lab in which they use restriction enzymes to cut DNA and then run it on a gel to separate the fractions.

# **Culminating Unit Performance Task Description/Directions/Differentiated Instruction:**

Protocols for the above labs can be found on the internet. They will require specific equipment which can be purchased or loaned.

## **Attachments for Culminating Performance Task:**



#### Web Resources:

www.firstmarket.com/cutter/cut2 www.protocol-online.org www.hudsonalpha.org/education/outreach/basics www.neb.com

## **Materials & Equipment:**

- New England Bio Labs will give free restriction enzymes to teachers
- To perform the tasks, you will need:
  - Submarine gel rigs
  - o Pipettes: p20, a p200 and a p1000
  - o A water bath
  - o Incubator oven
  - o Micro centrifuge
  - o Glassware and hot plate
  - o Power supply
- Equipment can be loaned in Georgia for free from GBTI lending lab
  - o Contact alatimer@athenstech.edu

# 21<sup>st</sup> Century Technology Used:

Slide Show Software	Χ	Graphing Software		Audio File(s)
Interactive Whiteboard	Χ	Calculator	Χ	Graphic Organizer
Student Response System		Desktop Publishing		Image File(s)
Web Design Software		Blog		Video
Animation Software		Wiki		Electronic Game or Puzzle Maker
Email	Χ	Website		•