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# Restriction Enzyme & Bacterial Transformation Activity Guide

**Exploring Restriction Enzymes:**

This activity guide will help you to consider various ways that you may use these materials as you explore the restriction enzymes process and the technical applications that they can be used in. We encourage you to modify these lessons and activities to meet the needs of your students and the learning objectives present in your curriculum.

**Targeted Subject(s):**

- Introductory level biology - CP/Honors
- AP Biology
- Biotechnology

**Intended Grade Level(s):**

This activity has been written at the high school level. However, this activity can be tailored to your student population and differentiated as needed.

**Prior Student Knowledge Required:**

It is expected that students understand the following concepts prior to working through this model:

- Hydrogen and covalent bonding
- DNA structure
- Deoxyribose structure
- Enzymes (structure, active sites, and generalized function)
- Base pairing rules
- DNA replication

**Key Words:**

- DNA
- Enzyme
- Endonuclease
- Base pairing
- Complementarity
- Hydrogen bonds
- Covalent bonds
- Phosphodiester bonds
- Restriction site
- Molecular recognition
- Restriction fragment
- Palindromic
- Sticky ends
- Recombinant DNA
- Plasmid
- Molecular cloning
- Vector
- Restriction Mapping

**Learning Objectives:**

Students will

- Explain the function of restriction enzymes and the role of restriction sites along the DNA.
- Demonstrate the location of endonuclease cuts based upon a given sequence.
- Model how a restriction enzyme will make cuts in a segment of DNA.
- Describe how the resulting restriction fragments will appear on an agarose gel.
- Investigate how sticky ends can be used in a biotechnological setting.
- Model and describe the process used by engineers to modify the genome of bacteria.
- Explain why bacteria are genetically modified more often than other organisms.
- Discuss possible applications for genetically modified bacteria.

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**Materials Needed:**

For introductory lesson:

- Colored Nucleotides (80/ Nucleotide base)

For the extension lesson

- Colored Nucleotides (20/Nucleotide base)
- Grey Nucleotides (20/Nucleotide base)
- 4 Yellow Cell Membrane Pieces

**Other Materials Suggested:**

- Dynamic DNA Kit© (available from 3D Molecular Designs)
- Flow of Genetic Information Kit© (available from 3D Molecular Designs)

**Possible Extensions:**

- Connection to agarose gel electrophoresis
- Use within molecular cloning
- Use with disease identification
  - For example: Restriction enzymes can be used within the diagnosis of sickle cell anemia. The enzyme DdeI is utilized for this reaction. DdeI has a recognition sequence of 5' - CTNAG - 3'. The presence of the sickle cell mutation removes the recognition sequence of the endonuclease site. Electrophoretic resolution of the fragment pattern reveals the presence or absence of the mutation. Clear genotyping of normal, carrier and homozygous DNA is achieved.

**Instructions for the Activity:**

***Pre-Lesson Set Up***

Prior to conducting the lesson, you should set up the student workstations in the following manner.

**Lesson 1: Examining Restriction Enzymes**

Part 1: Identifying Restriction Enzyme Cuts

- See Teacher Tips for ideas on how to structure this component of the lesson.

Part 2: Modeling a Restriction Digest

- The three restriction enzymes that will cut a section of DNA are: HindIII, EcoRI, and HaeIII.
- Prior to this lesson, the teacher should make cards of restriction enzymes. The cards can have one or more of the above restriction enzymes, or a different one (which will result in a cut not being able to be made).
  - Choice of the enzyme used is dependent on class/student grouping. Adds a layer of differentiation that the teacher can elect to explore.

**Lesson 2: Bacterial Transformation**

Part 1: What are we transforming?

- See Teacher Tips for ideas on how to structure this component of the lesson.

Part 2: Modeling the Process

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**Teacher Tips or Notes for Successful Implementation:**

### Lesson 1: Examining Restriction Enzymes

- Depending on the number of student stations and the availability of nucleotides, you could elect to pre-assemble the short segments of DNA that the students will be simulating various cuts in.
    - This activity could also be set up as a station activity. Each station would have one type of restriction enzyme represented in the given sequence. The students would then progress through the stations as they investigated the various restriction enzymes.
    - This activity can also be laid out like a memory matching game. You could put the recognition sequence and resulting cleave site on one card, and a picture of the cleave site modeled with the nucleotides as another. Students would then have to identify matches and enhance short term recall for positioning. This could be done either as a whole class or small (lab groups and can additionally be presented as a "game".
- The final example of a restriction digest is a nice summative activity where there are three possible cuts within the DNA segment.
    - Student groups should all be given the same starting sequence of DNA.
    - Student groups could be given task cards that illustrate different types of restriction enzymes.

  The students can be given a variety of restriction enzymes that may or may not be found in the sample of DNA. Task cards may have up to three restriction enzymes listed.
        - The restriction enzymes cards provided to the students can either be one or more of the enzymes listed below.
        - Additionally, the cards could have other restriction enzymes that are not present within the strand. This would produce a digest that would be the full size of the original target DNA strand.

| BamHI | EcoRI | HaeIII |
|---|---|---|
| 5'-CGTACCTAG | GAGCTTAA | GCCTCCTACC GGACC-3' |
| 3'-GCATG | GATCCTCG | AATTCGGAGGATGG CCTGG-5' |

    - Student groups can be organized to form a "lane" in the gel. They should be asked to lay out their band fragments as they would appear on an agarose gel.
        - You can elect to either draw/model a ladder in "lane 1" so that the students can reinforce judging band size based upon the ladder.

### Lesson 2: Extending to Bacterial Transformations

- Depending on the level of your classroom, you may or may not elect to conduct this component of the activity.
- This is designed to front-load a typical bacterial transformation wet lab and provide students with the background knowledge to understand the workflow of the lab and the implications associated with the protocol.
    - The first section of the handout could potentially be flipped out to the students. Depending on their command of the background knowledge, they could work through the beginning information of restriction enzyme recall and plasmid structure/function basics outside of class. This would enable them to come to class ready to model.

- The location of the restriction site spans across the linear chromosome. It is only visible once the students turn the linear strip of dsDNA into a circular plasmid. An option for differentiation would be to change the location of the EcoRI site in your own format of this activity. Placing it in the middle of the linear reading frame would enable students to visualize it on the paper as well as the model.
- During modeling:
    - Students can be encouraged to get creative when they are modeling components like heat, ice, CaCl2, and LB Broth. Ideas for how to model those components, drawing on a piece of paper with markers or on the lab tabletops with neon window markers or chalk.
    - Students can also be challenged to create stop-motion-animation like videos where they move the foam pieces. Voice overs can be added in to enhance the description of the process.

## Resources:


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