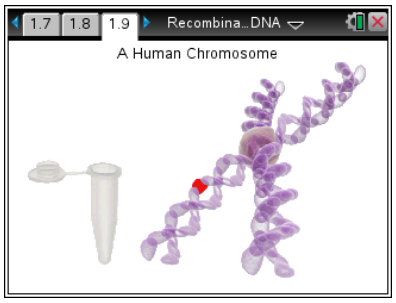
Science Objectives

- Students will explore how scientists are able to isolate and study single genes.
- Students will be introduced to recombinant DNA and the molecular biology of cloning.
- Students will become familiar with the process of bacterial transformation, as well as with important molecular biology tools, including restriction enzymes, plasmids and ligase.



Vocabulary

- cloning
- palindrome
- sticky ends
- recombinant DNA
- transformation
- restriction enzyme
- plasmid
- ligase
- model organism

About the Lesson

- Using simulations, students complete the steps required to clone a single gene and express it in a model organism. Assessments are embedded in the activity to engage discussion and gauge learning.
- As a result, students will:
  - Learn about the process of molecular genetics, including restriction enzymes, ligation, DNA transformation and the use of model organisms.
  - Learn how scientists isolate a gene, recombine DNA and express that DNA in bacteria.

TI-Nspire™ Navigator™

- Send out the *Recombinant_DNA.tns* file.
- Monitor student progress using Class Capture.
- Use Live Presenter to have students demonstrate how to negotiate the simulations and to spotlight student answers.
- Collect student responses from assessment items that are embedded throughout the document.

Activity Materials

- *Recombinant_DNA.tns* document
- TI-Nspire™ Technology

TI-Nspire™ Technology Skills:

- Download a TI-Nspire document
- Open a document
- Move between pages
- Explore Hot Spots
- Open Directions Box
- Answer assessment questions within a document

Tech Tips:

Make sure that students know how to move between pages by pressing ctrl ◀ (left arrow) and ctrl ▶ (right arrow).

Lesson Materials:

Student Activity

- Recombinant_DNA_Student.doc
- Recombinant_DNA_Student.pdf

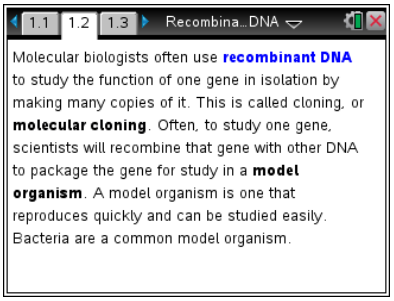
TI-Nspire document

- Recombinant_DNA.tns

Discussion Points and Possible Answers

Allow students to read the background information on the student activity sheet.

Part 1: Cloning



Move to pages 1.2–1.4.

Students should read the background information on pages 1.2. Following those pages, there are several questions that assess the students' background knowledge of cells. These questions would probably be best used for discussion after the students answer them.

Have students answer questions 1 and 2 on the handheld, the activity sheet, or both.

Q1. Why might a scientist want to isolate a gene by cloning it to study it?

Sample Answers: simpler conditions, over-expression

Isolating a gene is the first step to expressing the gene in a model organism, which is by definition easier to work with.

Q2. What other model organisms are you familiar with?

Sample Answers: yeast, plants, flies, mice, guinea pigs, rats

Generally model organisms are easy to study, reproduce quickly and share common features with the organisms of interest. Example, bacteria share the machinery required to replicate and express human DNA, in addition to replicating quickly.

Move to pages 1.5–1.6.

1. Students are to read the information on pages 1.5 and 1.6 about the use of restriction enzymes in molecular cloning.

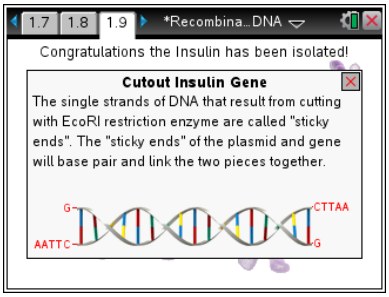
Have students answer question 3 on the handheld, the activity sheet, or both.

Q3. Which DNA sequence is a palindrome?

Answer: C. AAGCTT

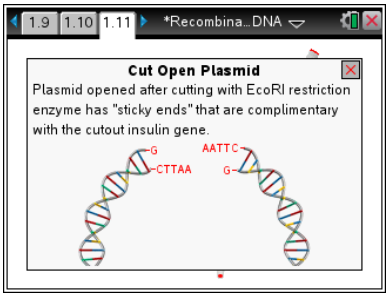
A palindrome sequence reads the same one both strands. Always read DNA 5' to 3'.

Move to pages 1.8–1.9.



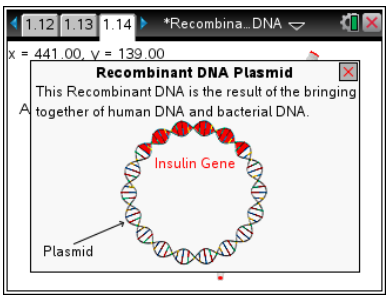
2. Students should read and follow the directions on page 1.9 to isolate the human insulin gene. They can click ☒ to close the directions and view the simulation. If needed at any time during the simulation, students can press menu if they would like to view the directions again. Once isolated, they should click on the gene in the test tube for more information.

Move to pages 1.10–1.11.



3. Students are to read the information on page 1.10 about the next step after isolating the insulin gene. They should follow the instructions on page 1.11 to prepare the plasmid. Once prepared, they should click on the cut plasmid DNA for more information.

Move to pages 1.12–1.14.



4. Students are to read the information on pages 1.12 and 1.13 about the next step in cloning the insulin gene. They should follow the instructions on page 1.14 to use the ligase enzyme. Once the ligase combines the insulin gene with the bacteria, they should click on the assembled plasmid vector for more information.

Q4. How do sticky ends of DNA help in cloning?

Sample Answers: can be repaired by ligase, can connect to other sticky ends

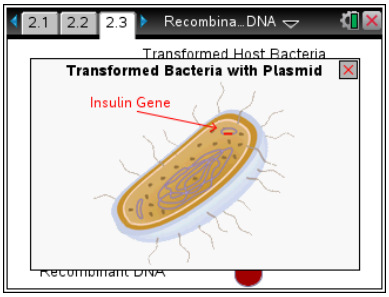
The phrase “sticky ends” refers to the unsatisfied hydrogen bonds on the single stranded segment of DNA. This sequence will seek out a sequence to bond to in solution, then the ligase can repair two single strand breaks.

Q5. Ligase is found in normal cells. What do you think it does normally?

Sample Answers: DNA repair, DNA replication (Okazaki fragments)

Ligase repairs single strand breaks, which occur in the life cycle of a cell from damage or during replication.

Part 2: Transforming and Culturing



Move to pages 2.1–2.3.

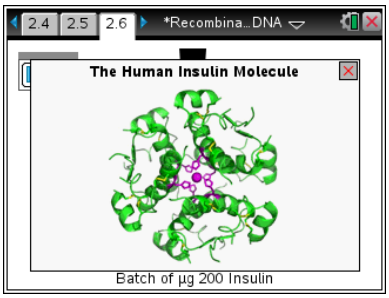
5. Students are to read the information on pages 2.1 and 2.2 about how the isolated insulin gene can now be expressed by bacteria. They should follow the directions on page 2.3 to transform recombinant DNA into bacteria. Once transformed, students should click on the test tube for more information.

Q6. Which of the following result from transformation? Select all that apply.

Answers: B. DNA is taken up by the host. C. Gene can be expressed by the host.

Transformation is the process that moves the DNA into the host cell so that the gene of interest can be expressed in the host.

Move to pages 2.5–2.6.



6. Students are to read the information on page 2.5 about why bacteria used to study a gene such as insulin. They should follow the directions on page 2.6 to grow insulin expressing bacteria. Once the bacteria have been grown, students should click on the test tube for more information.

Q7. As the bacteria population grows, what happens to the total amount of insulin?

Answer: increases

As the number of bacteria producing insulin increases, the total amount of insulin increases, which is one reason scientists choose to work with bacteria. This is a much cheaper, faster method to produce this life saving enzyme than purifying the enzyme form pig blood (the former method used to make clinical insulin).

Q8. Why do you think scientists use bacteria as a model organism?

Answer: Cheaper, faster, easier

This is a much cheaper, faster method to produce this life saving enzyme than purifying the enzyme form pig blood (the former method used to make clinical insulin). Ask students to imagine trying to get insulin from human blood.

Q9. Making recombinant DNA and transforming bacteria are both very inefficient. Which steps might slow down the process?

Sample Answer(s): transformation is stressful, incomplete ligation, wrong combinations of ligations, Incomplete cutting by enzyme

This inefficiency is why millions of DNA molecules are used in these protocols, this increases the chances of some DNA correctly assembling and transforming.

Q10. If a scientist wanted to make recombinant DNA using a different gene, which steps should be used? Select all that apply.

Answer: A. Isolate gene of interest, B. Ligate, C. cut plasmid

Transformation is not essential to create recombinant DNA, but the other steps are.

TI-Nspire Navigator Opportunities

Choose a student to be a Live Presenter to demonstrate each simulation. The questions in the activity may be distributed as Quick Polls or used as a formative or summative assessment

Wrap Up

When students are finished with the activity, retrieve the .tns file using TI-Nspire Navigator. Save grades to Portfolio. Discuss activity questions using Slide Show.

Assessment

- Formative assessment will consist of questions embedded in the .tns file. The questions will be graded when the .tns file is retrieved. The Slide Show will be utilized to give students immediate feedback on their assessment.