UNIT IV - FOUNDATIONS OF GENETIC ENGINEERING

Lesson 3: Genetic Modification

Objective/Competence: Describe the processes of genetic modification.

Study Questions

1. **What are gene mapping and gene sequencing?**
2. **How is DNA extracted?**
3. **What is restriction digestion?**
4. **What is gel electrophoresis?**
5. **What is gene splicing, and how is it accomplished?**

References

1. *Biotechnology: Applications in Agriculture (Student Reference)*. University of Missouri-Columbia: Instructional Materials Laboratory, 1998, Unit IV.
2. Transparency Master
   a) TM 3.1: Gel Electrophoresis
3. Activity Sheet
   a) AS 3.1: DNA Extraction

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TEACHING PROCEDURES

A. Review

Lesson 2 discussed how cells reproduce and pass on genetic information. A spontaneous change in the genetic code of a cell or organism is called a mutation. Recombinant DNA technology provides researchers with the tools necessary to intentionally alter specific parts of the genetic code of a cell. This lesson will describe some of these tools.

B. Motivation

Gel electrophoresis can be dramatized by the use of an obstacle course. Configure student chairs or desks to form three obstacle courses. Divide the students into three groups, with two to four students in the first group, four to eight in the second group, and eight to twelve in the third group. All groups should start the obstacle course at the same time and should be instructed not to touch any chair or desk. When the winning team reaches the finish line, all teams must freeze. Explain that the obstacle course represents the agarose gel through which the DNA fragments must pass in gel electrophoresis. The longer the fragment, the slower it will move across the gel.

C. Assignment

D. Supervised Study

E. Discussions

1. Ask students to describe gene mapping and gene sequencing.

What are gene mapping and gene sequencing?

a) Gene mapping is the process of finding the location of genes for specific traits on the chromosomes of an organism. Researchers use gene mapping to select specific genes for modification.
b) Gene sequencing is a process that shows the order of all the base units (A, T, C, G) as they line up on a particular gene. It allows scientists to recognize how to cut out a particular gene or gene fragment.

2. Ask students if DNA is a chemical substance. Because it is a chemical substance, it can be extracted chemically. Use AS 3.1 to allow students to perform a DNA extraction.

How is DNA extracted?

a) The cell membrane or cell wall must be broken down to release the cytoplasm, and the nuclear membrane must also be broken to release the chromosomes.
    1) This can be accomplished with a surfactant, which is a fatty acid compound like detergent.
    2) Heat accelerates this process.
b) Next, a protease must be used to split the protein contents of the cell, including protein molecules called Histones, around which the DNA strands are wrapped.
c) The last step involves separating the DNA from the other cell components. Cold alcohol is added to the cellular solution; the DNA clumps together and rises to the top of the alcohol.

3. Ask students what scissors are used for. Discuss how restriction digestion works.

What is restriction digestion?

a) Restriction digestion is the process of cutting DNA into smaller fragments with restriction enzymes.
b) Restriction enzymes are essentially biochemical scissors; each restriction enzyme cuts DNA at a different sequence of base pairs.
c) Researchers use restriction enzymes to cut genes or DNA fragments out of extracted DNA strands.

4. Ask students to recall the lesson in which DNA fingerprinting was discussed. Explain that the bands that make up the DNA fingerprint are a result of gel electrophoresis. Discuss the process of gel electrophoresis. Use TM 3.1 to illustrate how gel electrophoresis works.

What is gel electrophoresis?

a) Gel electrophoresis is a process in which an electric current is applied to a gel to separate different lengths of DNA fragments into groups; researchers can then recover a desired gene or gene fragment.
b) It requires an electrophoresis box, a buffer solution, a special power supply, and a gel made from agarose or another agent.
c) One end of the electrophoresis box has a positive pole and the other a negative pole.
d) DNA fragments to which a dye has been added are placed in small wells or pockets at the end of gel nearest the negative pole, and an electric current is applied to the gel.
e) Since DNA fragments are negatively charged, they will be repelled away from the negative pole and attracted to the positive pole.
f) Short lengths of DNA will move through the gel faster than long lengths.
g) When the electric current is removed, the fragments of DNA of the same size will be grouped at a one spot on the gel, which is called a band.

5. Ask students to define the term splicing. Explain that gene splicing is like splicing two pieces of rope together using a third piece of rope.

What is gene-splicing, and how is it accomplished?

a) Gene-splicing is the process of inserting a piece of DNA into a chromosome of a cell.
b) Gene-splicing is also called ligation because the enzyme ligase is the biochemical glue that joins the pieces of DNA.
c) Gene-splicing requires several steps.
    1) The researcher cuts out a piece of DNA with a restriction enzyme; the correct enzyme must be used so that the DNA contains complementary bases.
    2) Gel electrophoresis must be performed to separate the DNA fragments by size and isolate the appropriate fragment.
    3) The researcher joins the ends of the selected fragment to the DNA being transformed through a chemical reaction called a ligase reaction.
    4) The result is a cell containing DNA from two different sources, which is therefore called recombinant DNA.

F. Other Activities

1. Use pop beads to show how restriction digestion works.

2. If possible, use restriction analysis and gel electrophoresis experiments such as those found in the curriculum guide *An Introduction to Biotechnology: A Unit for High School Students (Book Three)* published by Kendall/Hunt.

G. Conclusion

This lesson describes some of the most common techniques used by molecular biologists involved in biotechnology research. Knowledge of these techniques will provide a basic understanding of exactly how DNA is manipulated.

H. Answers to Activity Sheet

AS 4.1

1. The soap helped break down the cell membranes and release the chromosomes.

2. Heating the solution accelerated the breakdown of the membranes. Most of the lipids and proteins precipitated out of the solution. However, the solution needed to be cooled after heating to avoid the soap breaking down the DNA.

3. The meat tenderizer contains the enzyme papain, which breaks down proteins.

4. DNA is the only component of the cell that is not soluble in alcohol and therefore precipitates out.

5. (Students should speculate on this question but may not be able to come up with this answer.) The DNA was not pure because some of the other cellular components could have clung to the DNA. It must be washed in alcohol several times to be pure.

I. Answers to the Evaluation

1. d
2. b
3. a
4. c
5. d

6. a) The cell membrane or cell wall and nuclear membranes are broken down with the use of a surfactant.
   b) Protease is used to break down the protein contents of the cell, including histones, around which the DNA is wrapped.
   c) DNA is separated from the other cell components by adding cold alcohol to the cellular solution, which causes the DNA to clump together and rise to the top of the alcohol.

7. Gene sequencing is a process that shows the order of all the base units (A, T, C, G) as they line up on a particular gene.

8. Restriction digestion is the process of cutting DNA into smaller fragments with restriction enzymes.

EVALUATION

Circle the letter that corresponds to the best answer.

1. Which of the following is considered to be a type of biochemical scissors?

   a. Agarose gel
   b. Histones
   c. Ligase
   d. Restriction enzymes

2. When a researcher does gel electrophoresis, what causes the DNA fragments to move across the gel?

   a. Buffer solution flows across the gel, moving the DNA fragments.
   b. Negatively charged DNA is repelled away from the negative pole and attracted toward the positive pole of the electrophoresis box.
   c. Positively charged DNA is repelled away from the positive pole and attracted toward the negative pole of the electrophoresis box.
   d. A chemical reaction involving ligase causes the movement of the DNA.

3. Ligase is an enzyme that:

   a. Chemically joins two DNA fragments.
   b. Acts as a restriction enzyme.
   c. Is used in the gel electrophoresis buffer solution.
   d. Is used in DNA extractions to break down the lipid components of the cell.

4. Surfactants, which are similar to detergents:

   a. Break down the protein contents of the cell.
   b. Cause DNA to float to the top of a cellular solution.
   c. Break down the cellular membranes.
   d. Speed up the process of DNA extraction.

5. Gene mapping can be defined as the:

   a. Process that allows researchers to separate different lengths of DNA fragments into groups.
   b. Process of inserting a piece of DNA into a chromosome of a cell.
   c. Process that finds the order of all the base units as they line up on a particular gene.
   d. Process of finding the location of genes on the chromosomes of an organism.

Complete the following short answer questions.

6. What are the three major steps of the DNA extraction process?

   a.

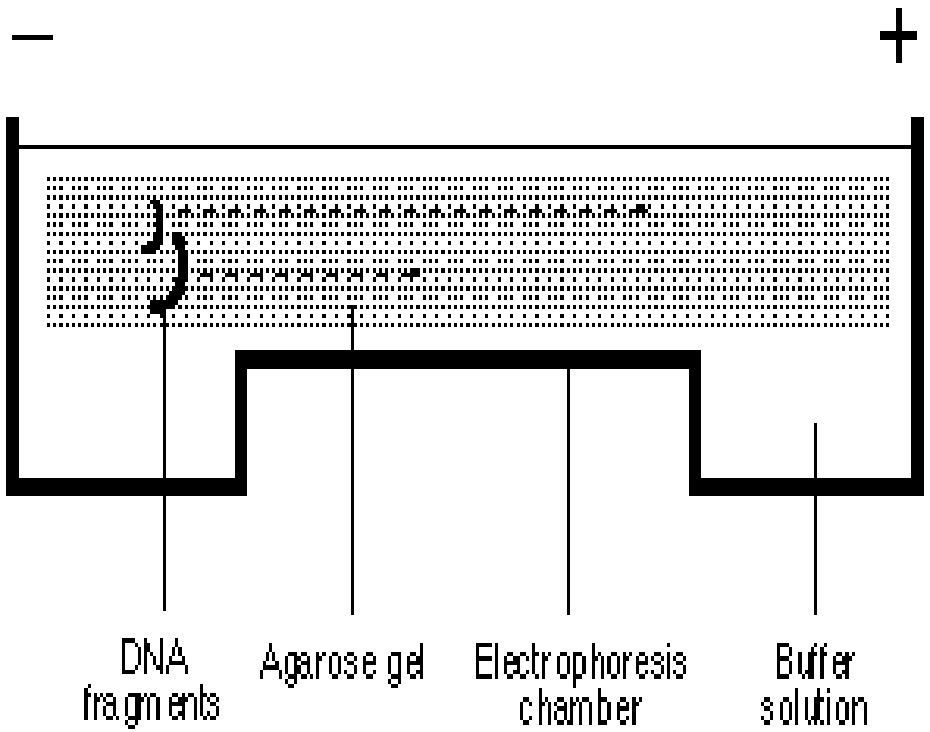
   b.

   c.

7. What is gene sequencing?

8. What is restriction digestion?

Gel Electrophoresis



Lesson 3: Genetic Modification Name _____

DNA Extraction

Objective: Perform DNA extraction.

Materials and Equipment:

1 hot water bath or hot plate set at 55 to 60 degrees Celsius
1 candy or laboratory thermometer (to regulate the hot water bath)
1 cold water bath (ice water in a large bowl)

Materials needed for each group of students:

1 glass or plastic graduated cylinder (100 ml)
1 plastic teaspoon
1 Pyrex glass or heat-resistant plastic beaker or flask (500 ml or larger)
1 piece of glass tubing bent to a 90 degree angle at the end
1 funnel or strainer with a coffee filter
2 flat toothpicks
1 medicine dropper or pipette
1 container of liquid soap (Woolite, laundry soap, or dish soap)
1 test tube for each student
½ medium onion, chopped (should not be finely chopped)
Refrigerated alcohol
Meat tenderizer

Procedure:

1. Observe the instructor as he or she performs steps 2-6.

2. Measure out 5 ml of soap and leave it in the graduated cylinder. Add ¼ teaspoon of salt to the soap. Next, add water to the soap until a total volume of 50 ml is reached. Set the cylinder aside. Place the onion in the beaker or flask. Pour the soap and salt water solution over the chopped onion.

3. Put the beaker or flask containing the onion solution into the hot water bath or onto the hot plate. Check to make sure that the temperature is approximately 55 to 60 degrees Celsius. The onion solution should be heated for 10 to 12 minutes. If the solution is heated for more than 15 minutes, the DNA will break down. Slowly stir the mixture periodically but not so much as to create foam. Make sure to record the time you placed the beaker or flask in the heating environment.

4. After 10 to 12 minutes, remove the solution from the hot water bath or hot plate and place it in the ice water bath for five minutes. Stir the solution slowly while it is cooling.

5. Filter the solution into a plastic cup using the funnel or strainer and coffee filter. Try to avoid getting any foam into the filtered mixture. This filtering procedure may be a slow process; if needed, it can be left to filter overnight in a refrigerator.

6. Divide the filtered solution into the test tubes, placing about one teaspoon in each. Stir the filtered solution while dividing it. The test tubes can be stored in the refrigerator overnight.

7. Add two toothpicks full of meat tenderizer to the solution in the test tubes and **gently** mix. Next, add **cold** alcohol to the solution until a 1 cm layer of alcohol forms on top of the solution. This can be done by using a medicine dropper or pipette. **Do not mix the solution!** Watch as the DNA begins to precipitate out into the alcohol. The bent piece of glass tubing can be used to spool the DNA. The DNA will look like white mucus.

Key Questions:

1. What did the soap do to aid in the extraction of the DNA?

2. Why was the onion solution heated and then cooled?

3. Why was meat tenderizer used? (Hint: Look at the ingredients in the meat tenderizer.)

4. Why did the DNA precipitate out in the alcohol?

5. Do you think the DNA obtained was pure? Why or why not?