



THE BIOTECHNOLOGY
EDUCATION COMPANY®

Edvo-Kit #

S-20

Edvo-Kit #S-20

How Do You Clone a Gene?

Experiment Objective:

The objective of this experiment is to gain an understanding of the structure of DNA, a genetically engineered clone, and how genes are cloned in plasmids.

See page 3 for storage instructions.

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Experiment Components

Contents

- A Plastic EDVO-Links™
- B Mini zip bags

Check (✓)

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-

Experiment #S-20 is designed for 10 groups.

There are many ways in which EDVO-Links™ can be used to demonstrate various concepts in biotechnology. Several suggested activities and demonstrations are described.

Requirements

There are no special requirements.

All experiment components are intended for educational research only. They are not to be used for diagnostic or drug purposes, nor administered to or consumed by humans or animals.

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Experiment Overview

EXPERIMENT OBJECTIVE:

- Students will gain an understanding of the structure of chromosomal and plasmid DNA.
- Students will learn how genes are cloned into plasmids.
- Students will understand how cloned genes are useful in pharmaceutical production.
- Students will learn about the structures of proteins.

LABORATORY SAFETY

This experiment contains small parts which can be a choking hazard. Not for children under three years old.

WORKING HYPOTHESIS

If a gene has been successfully cloned into a plasmid, then a useful gene product will be produced.

LABORATORY NOTEBOOKS:

Scientists document everything that happens during an experiment, including experimental conditions, thoughts and observations while conducting the experiment, and, of course, any data collected. Today, you'll be documenting your experiment in a laboratory notebook or on a separate worksheet.

Before starting the Experiment:

- Carefully read the introduction and the protocol. Use this information to form a hypothesis for this experiment.
- Predict the results of your experiment.

During the Experiment:

- Record your observations.

After the Experiment:

- Interpret the results – does your data support or contradict your hypothesis?
- If you repeated this experiment, what would you change? Revise your hypothesis to reflect this change.



Suggestions for Activities with EDVO-Links™

ACTIVITY ONE - STRUCTION OF DOUBLE STRANDED DNA

DNA is the genetic information of a cell and is responsible for cell function, cell division, and inheritance of physical traits. DNA is a molecule held together with base-pairs forming a double-stranded helical structure. Using two colors of the EDVO-Links™, the double-stranded alpha-helical form of DNA can be demonstrated (see Figure 1). Each of the two colors represent one of the two strands.

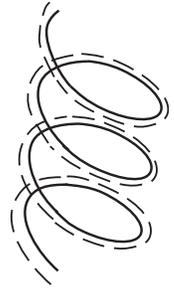


Figure 1: DNA Double Helix

ACTIVITY TWO - RESTRICTION ENZYME DIGESTION OF CIRCULAR DNA

Bacteria contain enzymes known as restriction enzymes which cut double-stranded DNA at specific sites. Using the EDVO-Links™ to form a circular plasmid DNA, cut the DNA at one site. How many pieces (fragments) of DNA result from this cut? Now, cut the circular plasmid at two or three sites. How many pieces of DNA does that produce?

ACTIVITY THREE - HOW TOPOISOMERASES CHANGE THE SUPERSTRUCTION OF DNA

DNA is a very long molecule, much longer than the cell in which it is located. DNA compacts into structures known as chromosomes that make it possible to fit inside the nuclei of cells. Plasmids are double-stranded circular superhelical forms of DNA found in bacteria and are not attached to the cell's DNA. Plasmids replicate independently of cell division.

Plasmid DNA molecules demonstrate the compactability of DNA. Special proteins called topoisomerases have the ability to wind and unwind DNA into the superhelical form (Figure 2). The circular double-stranded form of plasmid DNA is known as the relaxed form. Topoisomerases also wind and unwind cellular DNA. To demonstrate the action of topoisomerases, take your open circle of plasmid DNA and twist it to a more compact form, like twisting a rubber band.

Try placing the different DNA forms into the mini zip bags. Which form fits into the "cell" (bag)?

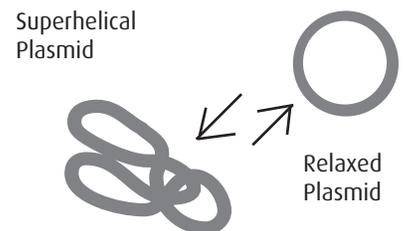


Figure 2: Differnt DNA Conformations

Suggestions for Activities with EDVO-Links™, continued

ACTIVITY FOUR - DEMONSTRATING GENES IN PLASMIDS

Genetic engineering is a term that refers to changing the genes found in DNA. The process of inserting a new gene into a DNA molecule is called cloning. The newly-constructed DNA molecule and the cell in which it is found is called a clone. The plasmid molecule in the cell replicates, making many more like itself. The cell will also divide many times and a large number of copies of the cloned gene will also be produced.

Plasmids are used in biotechnology as a cloning vector, that is, a vehicle to move genes from one cell to another. For example, a plasmid can be used to clone a gene such as one coding for insulin (see plasmid schematic, Figure 3). Insulin is a protein produced by the pancreas and is important in glucose metabolism. Diabetic individuals do not produce enough insulin and, therefore, have to take insulin injections. A bacterial cell that contains the plasmid with the cloned gene for insulin will now produce insulin. The insulin can be purified and used by diabetic individuals.

Plasmids can be isolated from bacterial cells that contain the plasmid. It can then be genetically engineered, and reintroduced into bacteria that do not carry the plasmid. The plasmid in Figure 4 carries two genes for antibiotic resistance, one for ampicillin and one for kanamycin. EDVO-Links™ can be used to make a model of this plasmid.

ACTIVITY FIVE - CLONING OF A GENE

Using our EDVO-Link™ plasmid model as our vector, we can clone a gene. A plasmid that is genetically engineered must be processed through several steps. Figure 5 outlines these steps.

First, the DNA molecule is cut open using a restriction enzyme. A restriction enzyme acts like a molecular “scissors” and will break the DNA molecule. A restriction enzyme will recognize a specific sequence in DNA.

Next, the gene is inserted into the plasmid DNA molecule. The ends of each piece of DNA are “sticky” and will come together. Finally, the DNA molecule is pasted back together using an enzyme called DNA ligase. The new clone is put into bacterial cells that do not contain the plasmid. Because the gene was inserted into it, the bacteria will now make the desire protein.

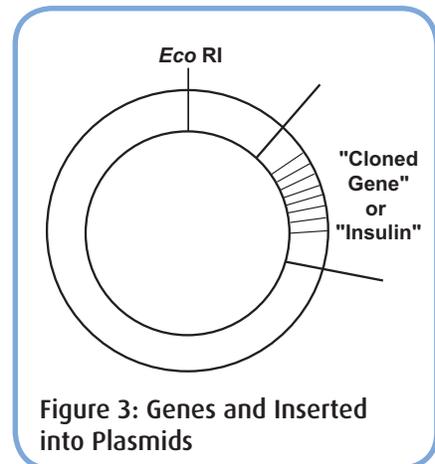


Figure 3: Genes and Inserted into Plasmids

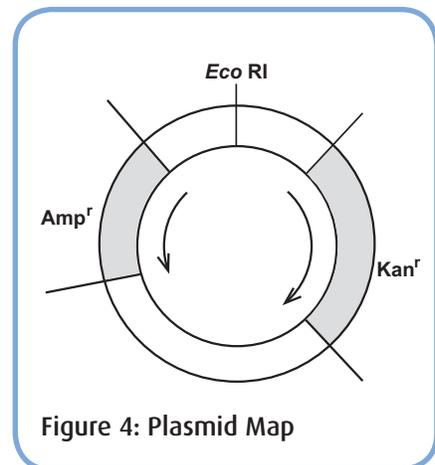


Figure 4: Plasmid Map

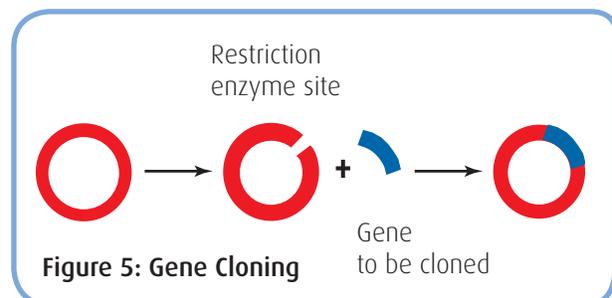


Figure 5: Gene Cloning

Suggestions for Activities with EDVO-Links™, continued

ACTIVITY SIX - DEMONSTRATING PROTEIN STRUCTURE

Proteins are gene products and are important molecules in cells. Proteins do all the work that cells need to have done, such as carrying oxygen to cells, metabolism, and reproduction. Proteins also can be structural, such as the parts of cells and body structures. Proteins have specific shapes called its conformation. In order for the proteins to work properly, they must have the correct conformation, which is unique to each protein. If a protein does not have the proper conformation, its biological activity may be decreased or totally lost. EDVO-Links™ can also be used to simulate proteins, where the links represent one or more amino acids in a polypeptide chain.

Proteins have primary, secondary, tertiary, and quaternary structures. The primary structure is the sequence of amino acids that make up the protein. The secondary structure is the result of covalent amino acid interactions such as disulfide bonds and ionic interactions between positive and negative charged amino acids within the protein structure. Tertiary structure, the protein's interaction with water molecules in its environment, provides each protein its specific three-dimensional structural shape. The quaternary structure is the interaction between protein chains. These interactions can be demonstrated by folding up the EDVO-Link™ model.



Figure 6: Protein Structure

ACTIVITY SEVEN - THE GREEN FLOURESCENT PROTEIN

Due to its bright-green, glowing color, the green fluorescent protein (GFP), isolated from the jellyfish *Aequorea victoria*, is a valuable research and education tool that is ideal for use as a gene marker in genetic research. Like all genes, the gene that codes for *gfp* is "turned on" by DNA sequences called promoters. A promoter typically is in front (upstream) or near the DNA sequence that codes for mRNA that in turn directs synthesis of GFP. In order to turn on GFP expression, an inducer molecule binds and releases the inhibitor that blocks the promoter. The release of the inhibitor allows RNA Polymerase (the enzyme that synthesizes RNA) to turn on production of GFP.

EDVO-Links™ provide the opportunity to demonstrate the following:

DNA and RNA-based Demonstrations

1. Use a pair of solid color EDVO-Links™ to demonstrate host genomic DNA.
2. Use of different colors to demonstrate the promoter in a recombinant plasmid that contains the *gfp*.
3. Other colors can be used as the inhibitor, inducer, and RNA polymerase to demonstrate the sequence of events necessary for the expression of the GFP.

continued

Suggestions for Activities with EDVO-Links™, continued

Protein-based Demonstration

1. Use a green EDVO-Link™ to demonstrate the GFP primary structure.
2. Folding the backbone to interact in different parts of GFP can represent the secondary structure of GFP.
3. Further folding of the green EDVO-Link™ in a ball like structure represents the native tertiary structure of GFP.
4. Unfolding of the tertiary protein structure represent denaturation of a protein, which is a transition form, a biologically native state to a denatured inactive state. Examples of conditions of denaturation are exposure to high temperatures, acidic and basic conditions.

Quick Reference Abbreviations

GFP	Green Fluorescent protein
pGFP	Plasmid for GFP expression
gfp	Gene for green fluorescent protein



Study Questions

1. How can a very long piece of DNA fit into a very small cell?
2. What is a topoisomerase?
3. What is genetic engineering?
4. What is a plasmid?
5. What steps are involved in cloning a gene into a plasmid?
6. Why are cloned genes important in pharmaceutical production?
7. Why is the shape or conformation of a protein important?

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**Please refer to the kit
insert for the Answers to
Study Questions**