

LAB: CLONING PAPER PLASMED

In this exercise you will use paper to simulate the cloning of a gene from one organism into a bacterial plasmid using a restriction enzyme digest. The plasmid (puc19 plasmid) can then be used to transform bacteria so that it now expresses a new gene and produces a new protein.

1. From the white paper, cut-out the puc19 plasmid (DNA) in a long strip.

GAATCTTTTC

2. Attach the ends together to make a loop to complete the circular DNA of a plasmid.



3. From the green paper, cut-out the Jellyfish-Glo gene (DNA) in a long strip. Leave this a straight strip. (This is a gene from a eukaryote not a bacterium, so it is not circular.)

TTGATTTTAAATC

The start and stop sequences for transcribing the Jellyfish-GFP or Glo gene are highlighted. These are needed to transcribe the gene properly when it is read. In addition, the **KpnI** & **EcoRI** restriction enzyme cutting sites (sequences of bases) are marked in **bold** on the Jellyfish-Glo gene (DNA). The two restriction enzymes and their respective restriction sites are listed below. These enzymes act as "molecular scissors" to cut the DNA at these sequences in the DNA.

Restriction enzyme	Recognition site (5' - 3')
KpnI	A AGCTT T
EcoRI	G AATTC A

The sequence represents the nitrogen base sequence that the enzyme recognizes, and T represents the place where the DNA will cut. For example, KpnI cuts between A and A whenever it encounters the sequence AAGCTT.

4. Examine the DNA sequence for the plasmid and the jellyfish gene. Which restriction enzyme should you use to cut the plasmid? The jellyfish gene? Remember, when you cut each gene, you need to retain the start and stop sequences. Should you use the same restriction enzyme to cut both the plasmid and the jellyfish gene? Why or why not?
5. Now you will incorporate the green Jellyfish-Glo gene into the plasmid. Attach the sticky ends of the Jellyfish-Glo gene to the sticky ends of the puc19 plasmid and seal with "molecular glue". (The enzyme ligase (which we will use later in our lab).
6. You have successfully cloned a gene! You now have a single plasmid with a new gene and can use that to transform a single bacterium. The bacterium will now make green Jellyfish glow protein and will glow under UV light.

Cloning Paper Plasmid Lab Answer Key

Xiang Xie



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Table of Contents Cloning Paper Plasmid Lab Answer Key

1. Understanding the eBook Cloning Paper Plasmid Lab Answer Key
 - The Rise of Digital Reading Cloning Paper Plasmid Lab Answer Key
 - Advantages of eBooks Over Traditional Books
2. Identifying Cloning Paper Plasmid Lab Answer Key
 - Exploring Different Genres
 - Considering Fiction vs. Non-Fiction
 - Determining Your Reading Goals
3. Choosing the Right eBook Platform
 - Popular eBook Platforms
 - Features to Look for in an Cloning Paper Plasmid Lab Answer Key
 - User-Friendly Interface
4. Exploring eBook Recommendations from Cloning Paper Plasmid Lab Answer Key
 - Personalized Recommendations
 - Cloning Paper Plasmid Lab Answer Key User Reviews and Ratings
 - Cloning Paper Plasmid Lab Answer Key and Bestseller Lists
5. Accessing Cloning Paper Plasmid Lab Answer Key Free and Paid eBooks

- Cloning Paper Plasmid Lab Answer Key Public Domain eBooks
 - Cloning Paper Plasmid Lab Answer Key eBook Subscription Services
 - Cloning Paper Plasmid Lab Answer Key Budget-Friendly Options
6. Navigating Cloning Paper Plasmid Lab Answer Key eBook Formats
 - ePub, PDF, MOBI, and More
 - Cloning Paper Plasmid Lab Answer Key Compatibility with Devices
 - Cloning Paper Plasmid Lab Answer Key Enhanced eBook Features
 7. Enhancing Your Reading Experience
 - Adjustable Fonts and Text Sizes of Cloning Paper Plasmid Lab Answer Key
 - Highlighting and Note-Taking Cloning Paper Plasmid Lab Answer Key
 - Interactive Elements Cloning Paper Plasmid Lab Answer Key
 8. Staying Engaged with Cloning Paper Plasmid Lab Answer Key
 - Joining Online Reading Communities
 - Participating in Virtual Book Clubs
 - Following Authors and Publishers Cloning Paper Plasmid Lab Answer Key
 9. Balancing eBooks and Physical Books Cloning Paper Plasmid Lab Answer Key
 - Benefits of a Digital Library
 - Creating a Diverse Reading Collection Cloning Paper Plasmid Lab Answer Key
 10. Overcoming Reading Challenges
 - Dealing with Digital Eye Strain
 - Minimizing Distractions
 - Managing Screen Time
 11. Cultivating a Reading Routine Cloning Paper Plasmid Lab Answer Key
 - Setting Reading Goals Cloning Paper Plasmid Lab Answer Key
 - Carving Out Dedicated Reading Time
 12. Sourcing Reliable Information of Cloning Paper Plasmid Lab Answer Key
 - Fact-Checking eBook Content of Cloning Paper Plasmid Lab Answer Key
 - Distinguishing Credible Sources
 13. Promoting Lifelong Learning
 - Utilizing eBooks for Skill Development

- Exploring Educational eBooks

14. Embracing eBook Trends

- Integration of Multimedia Elements
- Interactive and Gamified eBooks

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