

LAB ____: CLONING PAPER PLASMID

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 (puc18 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 puc18 plasmid DNA in a long strip.

AAATCGTTTGC

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



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

GGATCGAAAGC

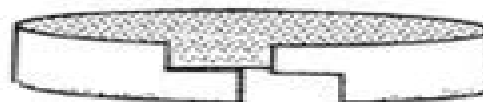
The start and stop sequences for transcribing the Jellyfish GFP or Glo gene are highlighted. These are needed to transcribe the gene properly when it is read.

In addition, the HindIII & 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')
<i>Hind</i> III	A ↓AGCT T
	T TCGA↑ A
<i>Eco</i> RI	G ↓AATT C
	C TTAA↑ G

The six letter sequence represents the nitrogen base sequence that the enzyme recognizes, and ↓ represents the place where the DNA will be cut by the enzyme. For example, HindIII cuts between A and A whenever it encounters the six base sequence AAGCTT.

4. Cut the green Jellyfish DNA as if you have used the a restriction enzyme, HindIII. Be sure to leave "sticky ends."
5. Also, cut the white puc18 plasmid DNA as if you have performed a restriction enzyme digest with HindIII. Be sure to leave "sticky ends."



Lab Cloning Paper Plasmid Answers

Jaishree Soni



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Table of Contents Lab Cloning Paper Plasmid Answers

1. Understanding the eBook Lab Cloning Paper Plasmid Answers
 - The Rise of Digital Reading Lab Cloning Paper Plasmid Answers
 - Advantages of eBooks Over Traditional Books
2. Identifying Lab Cloning Paper Plasmid Answers
 - 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 Lab Cloning Paper Plasmid Answers
 - User-Friendly Interface
4. Exploring eBook Recommendations from Lab Cloning Paper Plasmid Answers
 - Personalized Recommendations
 - Lab Cloning Paper Plasmid Answers User Reviews and Ratings
 - Lab Cloning Paper Plasmid Answers and Bestseller Lists
5. Accessing Lab Cloning Paper Plasmid Answers Free and Paid eBooks
 - Lab Cloning Paper Plasmid Answers Public Domain eBooks
 - Lab Cloning Paper Plasmid Answers eBook Subscription Services
 - Lab Cloning Paper Plasmid Answers Budget-Friendly Options
6. Navigating Lab Cloning Paper Plasmid Answers eBook Formats
 - ePub, PDF, MOBI, and More
 - Lab Cloning Paper Plasmid Answers Compatibility with Devices
 - Lab Cloning Paper Plasmid Answers Enhanced eBook Features
7. Enhancing Your Reading Experience
 - Adjustable Fonts and Text Sizes of Lab Cloning Paper Plasmid Answers
 - Highlighting and Note-Taking Lab Cloning Paper Plasmid Answers
 - Interactive Elements Lab Cloning Paper Plasmid Answers
8. Staying Engaged with Lab Cloning Paper Plasmid Answers

- Joining Online Reading Communities
 - Participating in Virtual Book Clubs
 - Following Authors and Publishers Lab Cloning Paper Plasmid Answers
9. Balancing eBooks and Physical Books Lab Cloning Paper Plasmid Answers
- Benefits of a Digital Library
 - Creating a Diverse Reading Collection Lab Cloning Paper Plasmid Answers
10. Overcoming Reading Challenges
- Dealing with Digital Eye Strain
 - Minimizing Distractions
 - Managing Screen Time
11. Cultivating a Reading Routine Lab Cloning Paper Plasmid Answers
- Setting Reading Goals Lab Cloning Paper Plasmid Answers
 - Carving Out Dedicated Reading Time
12. Sourcing Reliable Information of Lab Cloning Paper Plasmid Answers
- Fact-Checking eBook Content of Lab Cloning Paper Plasmid Answers
 - 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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