

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 G/o 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 G/o 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 G/o 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>HindIII</i>	A ↓AGCT T
	T TCGA↑ A
<i>EcoRI</i>	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."



Biology Lab Cloning Paper Plasmid Answers

Richard Bailey



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Table of Contents Biology Lab Cloning Paper Plasmid Answers

1. Understanding the eBook Biology Lab Cloning Paper Plasmid Answers
 - The Rise of Digital Reading Biology Lab Cloning Paper Plasmid Answers
 - Advantages of eBooks Over Traditional Books
2. Identifying Biology 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 Biology Lab Cloning Paper Plasmid Answers
 - User-Friendly Interface
4. Exploring eBook Recommendations from Biology Lab Cloning Paper Plasmid Answers
 - Personalized Recommendations
 - Biology Lab Cloning Paper Plasmid Answers User Reviews and Ratings
 - Biology Lab Cloning Paper Plasmid Answers and Bestseller Lists
5. Accessing Biology Lab Cloning Paper Plasmid Answers Free and Paid eBooks
 - Biology Lab Cloning Paper Plasmid Answers Public Domain eBooks
 - Biology Lab Cloning Paper Plasmid Answers eBook Subscription Services
 - Biology Lab Cloning Paper Plasmid Answers Budget-Friendly Options
6. Navigating Biology Lab Cloning Paper Plasmid Answers eBook Formats

- ePub, PDF, MOBI, and More
 - Biology Lab Cloning Paper Plasmid Answers Compatibility with Devices
 - Biology Lab Cloning Paper Plasmid Answers Enhanced eBook Features
7. Enhancing Your Reading Experience
 - Adjustable Fonts and Text Sizes of Biology Lab Cloning Paper Plasmid Answers
 - Highlighting and Note-Taking Biology Lab Cloning Paper Plasmid Answers
 - Interactive Elements Biology Lab Cloning Paper Plasmid Answers
 8. Staying Engaged with Biology Lab Cloning Paper Plasmid Answers
 - Joining Online Reading Communities
 - Participating in Virtual Book Clubs
 - Following Authors and Publishers Biology Lab Cloning Paper Plasmid Answers
 9. Balancing eBooks and Physical Books Biology Lab Cloning Paper Plasmid Answers
 - Benefits of a Digital Library
 - Creating a Diverse Reading Collection Biology Lab Cloning Paper Plasmid Answers
 10. Overcoming Reading Challenges
 - Dealing with Digital Eye Strain
 - Minimizing Distractions
 - Managing Screen Time
 11. Cultivating a Reading Routine Biology Lab Cloning Paper Plasmid Answers
 - Setting Reading Goals Biology Lab Cloning Paper Plasmid Answers
 - Carving Out Dedicated Reading Time
 12. Sourcing Reliable Information of Biology Lab Cloning Paper Plasmid Answers
 - Fact-Checking eBook Content of Biology 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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