

Lab 4: Clone simulation, clone gRNA into plasmid and transform plasmid into bacterial cells

Long-term goals of mismatch repair project

- Generate a specific mutation in yeast MSH2 gene that was previously identified in a human patient with Lynch Syndrome
- Determine if mutation to the yeast MSH2 gene impairs protein function and causes an increase in mutation rate to the yeast genome

Today's goal

After doing a paper simulation of cloning, the goal of this lab is to insert/clone the gRNA sequence into a plasmid that also has the Cas9 nuclease gene. After cloning, the recombinant plasmid is transformed into bacterial cells and plated on media with ampicillin to select for bacterial cells that have the plasmid.

Background

To perform gene editing with the CRISPR/Cas9 system, a cell must express both the Cas9 nuclease and a gRNA at the same time. During the last lab we designed a gRNA sequence that will initiate gene editing at the yeast MSH2 gene to generate your mutation of interest. In this lab, the gRNA sequence will be cloned into the pML104 plasmid that contains the Cas9 gene. In order for cloning to be successful, the pML104 plasmid has to be cut/digested so it can receive a new piece of DNA. The pML104 plasmid contains a "cloning site" for a 20 bp gRNA sequence, a gene sequence for the Cas9 nuclease, an ampicillin resistance gene, and a bacterial origin of replication (Figure 1).



Figure 1. pML104 plasmid. The pML104 plasmid contains the Cas9 gene and a cloning site for a gRNA sequence (gRNA) that is flanked by DNA sequences that are cut by the BclI and SmaI restriction enzymes. The pML104 also contains a bacterial origin of replication (Ori) and an ampicillin resistance gene (Amp^r).

Paper Plasmid Lab Answers

Yijin Wang



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Is there a more sinister reason behind Gina's involvement with the company? Is Gina's vaccine all it appears to be? And is Andy ready to acknowledge that there is more to life than work? BOOK JACKET Recombinant Molecules Roland F. Beers, Edward Graham Bassett, 1977 *Microbiology Abstracts*, 1988 **Recombinant DNA research. v. 3** |NV/SE publ **1978 SE, 1977/78**, 1978 Abstracts of the Annual Meeting of the American Society for Microbiology American Society for Microbiology. Annual Meeting, 1990

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Table of Contents Paper Plasmid Lab Answers

1. Understanding the eBook Paper Plasmid Lab Answers
 - The Rise of Digital Reading Paper Plasmid Lab Answers
 - Advantages of eBooks Over Traditional Books
2. Identifying Paper Plasmid Lab 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 Paper Plasmid Lab Answers
 - User-Friendly Interface
4. Exploring eBook Recommendations from Paper Plasmid Lab Answers
 - Personalized Recommendations
 - Paper Plasmid Lab Answers User Reviews and Ratings
 - Paper Plasmid Lab Answers and Bestseller Lists
5. Accessing Paper Plasmid Lab Answers Free and Paid eBooks

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