

## 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<sup>r</sup>).

# Lab Cloning Paper Plasmid

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## **Table of Contents Lab Cloning Paper Plasmid**

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

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