

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).

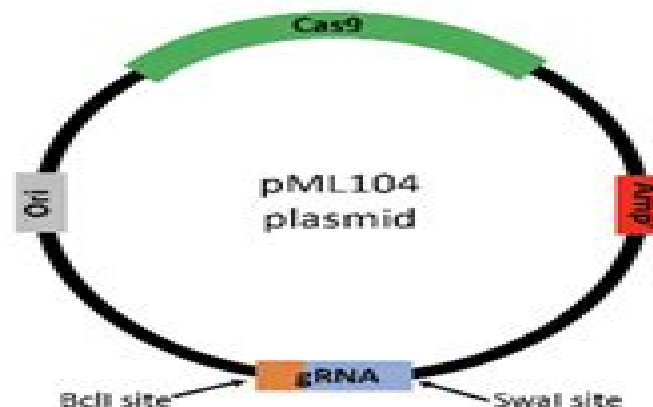


Figure 1. pML104 plasmid. The pML104 plasmid contains the Cas9 gene and a cloning site for a gRNA sequence (gRNA) that is flanked by DNA sequences that are cut by the BclI and SmaI restriction enzymes. The pML104 also contains a bacterial origin of replication (Ori) and an ampicillin resistance gene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

Lab Cloning Paper Plasmid

Gerry E. Hendershot, Karl E. Bauman



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The Molecular Mechanisms of Antibiotic Resistance in Aquatic Pathogens Xiangmin Lin, Patrícia Poeta, Bo Peng, Xiao-Peng Xiong, 2020-12-09 This eBook is a collection of articles from a Frontiers Research Topic Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series they are collections of at least ten articles all centered on a particular subject With their unique mix of varied contributions from Original Research to Review Articles Frontiers Research Topics unify the most influential researchers the latest key findings and historical advances in a hot research area Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office frontiersin.org about contact

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Molecular Genetics of Bacterial Pathogenesis Stanley Falkow,1994 **Addison-Wesley Biology** Addison Wesley,1996-04 **History of Chemoattractant Research** Bernhard Moser,2016-01-07 In the Research Topic History of Chemoattractant Research we will portray some of the key discoveries that helped to transform cell migration research into a global playing field within immunology and beyond Early progress had a profound effect on both academia and industry Today numerous academic laboratories are fully engaged in compiling a detailed road map describing the highly complex network of immune and tissue cells that respond to chemoattractants Industrial research on the other hand centers on drugs that interfere with immune cell traffic in inflammatory diseases and cancer The following series of short stories provide personal accounts on key discoveries The individual molecular discoveries enabled numerous research laboratories worldwide to unravel their significance in steady state or pathological immune processes Although ground breaking in their own right it is therefore worth emphasizing that rapid progress in chemoattractant research was made possible by many other laboratories who were not directly involved in the original discovery process Therefore the authors of this mini series are discussing their findings in the context of time place and subsequent progress enabled by their discoveries It is hoped that a wide readership will find these accounts entertaining as well as educational although those who wish to gain a more detailed knowledge are referred to the many outstanding reviews on chemokines and other chemoattractants **Soviet Genetics** ,1985 Conference Papers Index ,1987 Monthly Papers presented at recent meeting held all over the world by scientific technical engineering and medical groups Sources are meeting programs and abstract publications as well as questionnaires Arranged under 17 subject sections 7 of direct interest to the life scientist Full programs of meetings listed under sections Entry gives citation number paper title name mailing address and any ordering number assigned Quarterly and annual indexes to subjects authors and programs not available in monthly issues **Journal of Bacteriology** Charles-Edward Amory Winslow,James Morgan Sherman,John Roger Porter,1984 Biochemistry Abstracts ,1984 **Current Advances in Genetics** ,1978 Cancer Biochemistry Biophysics Harry Darrow Brown,1982 **Use of Services for Family Planning and Infertility, United States** Gerry E. Hendershot,Karl E. Bauman,1988 **Applied and Environmental Microbiology** ,2000 **Science in China** ,1991 **Biotechnology Research Abstracts** ,1991 Monthly Classified listing of references to worldwide articles dealing with all aspects of biotechnology Also includes books and conferences Each entry gives bibliographic information institutional address of author s and abstract Author and subject index **High School Molecular Biology Unit for Advanced Biology Students** Bruce Lee Buysse,1992

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