

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).

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**Martina Newell-McGloughlin, Edward
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The Top Books of the Year Lab Cloning Paper Plasmid The year 2023 has witnessed a remarkable surge in literary brilliance, with numerous compelling novels captivating the hearts of readers worldwide. Lets delve into the realm of bestselling books, exploring the captivating narratives that have charmed audiences this year. Lab Cloning Paper Plasmid : Colleen Hoover's "It Ends with Us" This heartfelt tale of love, loss, and resilience has captivated readers with its raw and emotional exploration of domestic abuse. Hoover skillfully weaves a story of hope and healing, reminding us that even in the darkest of times, the human spirit can prevail. Uncover the Best : Taylor Jenkins Reids "The Seven Husbands of Evelyn Hugo" This captivating historical fiction novel unravels the life of Evelyn Hugo, a Hollywood icon who defies expectations and societal norms to pursue her dreams. Reids absorbing storytelling and compelling characters transport readers to a bygone era, immersing them in a world of glamour, ambition, and self-discovery. Discover the Magic : Delia Owens "Where the Crawdads Sing" This evocative coming-of-age story follows Kya Clark, a young woman who grows up alone in the marshes of North Carolina. Owens crafts a tale of resilience, survival, and the transformative power of nature, captivating readers with its evocative prose and mesmerizing setting. These popular novels represent just a fraction of the literary treasures that have emerged in 2023. Whether you seek tales of romance, adventure, or personal growth, the world of literature offers an abundance of compelling stories waiting to be discovered. The novel begins with Richard Papen, a bright but troubled young man, arriving at Hampden College. Richard is immediately drawn to the group of students who call themselves the Classics Club. The club is led by Henry Winter, a brilliant and charismatic young man. Henry is obsessed with Greek mythology and philosophy, and he quickly draws Richard into his world. The other members of the Classics Club are equally as fascinating. Bunny Corcoran is a wealthy and spoiled young man who is always looking for a good time. Charles Tavis is a quiet and reserved young man who is deeply in love with Henry. Camilla Macaulay is a beautiful and intelligent young woman who is drawn to the power and danger of the Classics Club. The students are all deeply in love with Morrow, and they are willing to do anything to please him. Morrow is a complex and mysterious figure, and he seems to be manipulating the students for his own purposes. As the students become more involved with Morrow, they begin to commit increasingly dangerous acts. The Secret History is a masterful and suspenseful novel that will keep you speculating until the very end. The novel is a warning tale about the dangers of obsession and the power of evil.

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