

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

David Baud



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