Optimizing CRISPR-Cas9 for *Rhizopus delemar*: sgRNA Screening via Bacterial On-Targeting Assays

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Introduction

The 2013 National Health Interview Survey (NHIS) estimated that 2.7 percent of U.S. adults were immunosuppressed. Recently in 2021, a NHIS study estimated that the prevalence of immunosuppressed adults in the U.S. has more than doubled, to 6.6 percent [1]. This may be due to increased used of immunosuppressive medications. The problem that arises from increases immunosuppression is severe infections caused by opportunistic fungi. A focus of this study is *Rhizopus delemar*, a zygomycetous fungus that causes mucormycosis, a rare, but aggressive and life-threatening disease. Mucormycosis presents flu-like symptoms, especially if it infects the sinuses or lungs [2]. Despite the serious infections, there is a lack of knowledge about the pathobiology of *R. delemar* due to its recalcitrant to genetic studies that could reveal molecular underpinnings of pathogenesis [3].

CRISPR-Cas9, which is a short for clustered regularly interspaced short palindromic repeats and CRISPR-associated protein 9, was adapted from the bacteria's defense system against bacteriophages, and allowed for researchers to edit the genomes of an organism's DNA [4]. We sought to use CRISPR-Cas9 gene editing as a method to analyze pathobiology at the molecular level with the goal of helping treatments for patients affected with mucormycosis.

Overall, this study tests a tool to quickly identify sgRNAs with high on-target efficiency that facilitates CRISPR-Cas9-mediated gene editing in *R. delemar*, using an all-in-one noodle construct, prior to their usage in gene edit of the fungus.

Methods

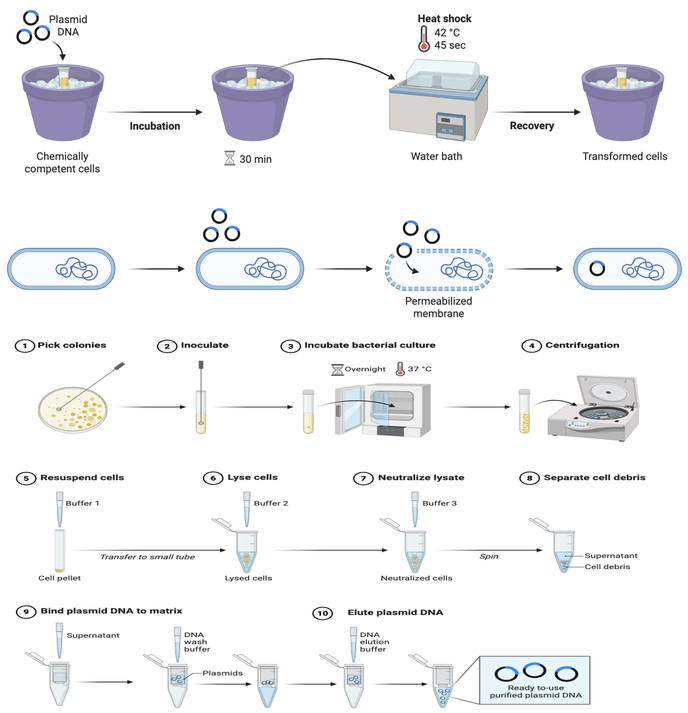

Figure 1. Cellular transformation and purification of plasmid DNA was performed to set-up a double digest. The double digest was visualized on agarose gel. Refer to figure 3.

Noodle Constructs

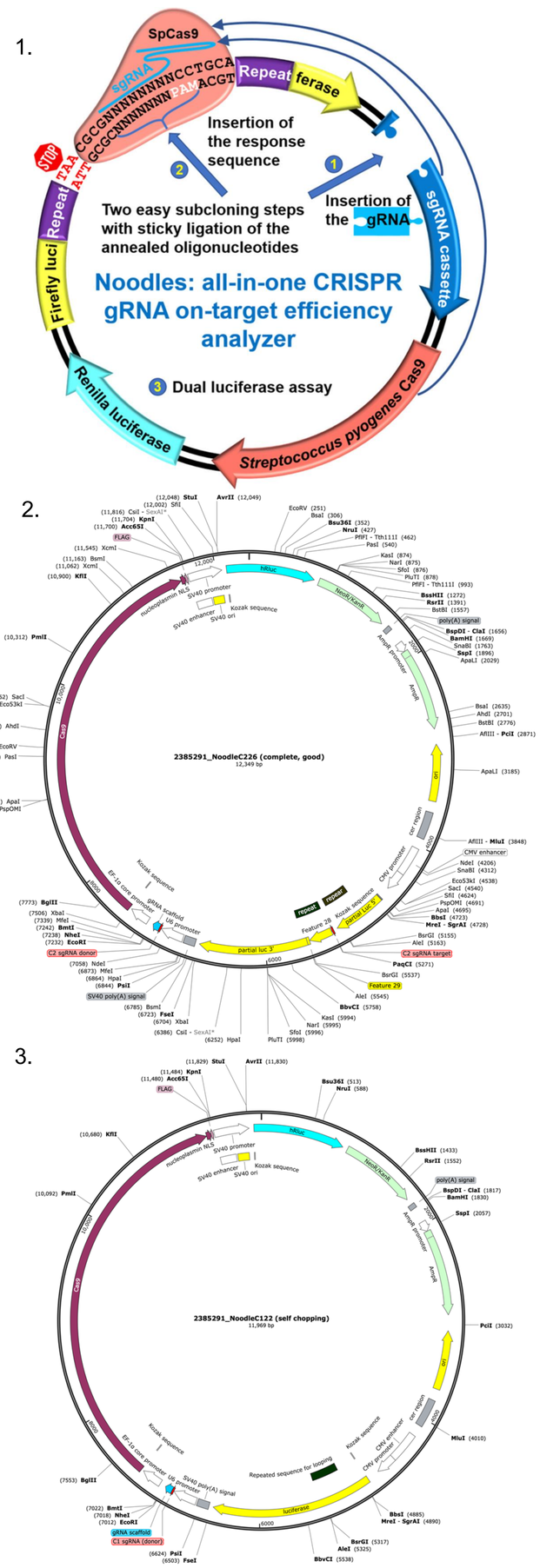

Figure 2. The noodle construct has sgRNA expression and targeting, Cas9, and two fluorescence markers (1). A finished construct containing sgRNA C226 and sgRNA C226 target (sgRNA + PAM) (2). A construct with self-targeting in bacteria resulting in reconstitution of the luciferase reporter (3).

Results

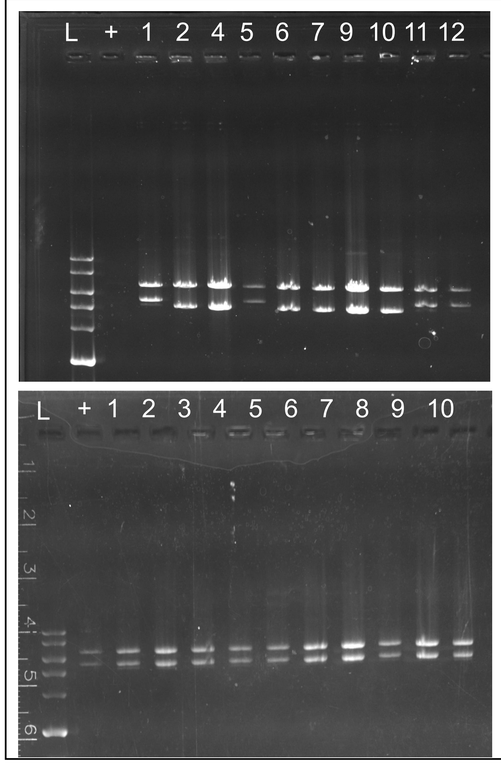

Figure 3. Top row: self-targeting occurring in 6 out of 10 C122 bacterial transformants. A smaller fragment (5.2 kb) indicates the excision of the repeater sequences resulting in reconstitution of the luciferase genes for transformants #2, #4, #6, #7, #9, and #10. Bottom row: none of the 10 C226 transformants undergo self-targeting in bacteria.

Conclusion & Future Work

The noodle construct is designed for estimating sgRNA expression and on-target efficiency through a mammalian expression system following cell culture transformation. Here we showed that the construct can also allow sgRNA expression and on-targeting during the vector cloning process in bacteria. The difference in ratios of those underwent self-targeting provide a means to quickly select sgRNAs with high on-target efficiency. Overall, this study is expected to facilitate CRISPR-Cas9-mediated gene editing and pathogenesis understanding in *R. delemar*.

In the future, the all-in-one constructs will be tested in a mammalian cell line.

Acknowledgement

I would like to thank LSUHSC for the opportunity at their summer program and assigning me to Dr. Wang, as I am grateful for his guidance. Additionally, the NSF helped make this possible with their funding.

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This research project was supported by Award Number: DBI-2349224 through the National Science Foundation (NSF), Research Experiences for Undergraduates (REU) Program