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Introduction to CRISPR

Forked from Introduction to CRISPR

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DISCLAIMER:

Introduction to CRISPR

Goal

The intent of this lab is to teach you how to conduct genome editing using CRISPR/Cas-9. Not only should you learn the technique, but you should recognize the applications using this mechanism can have in the lab

Learning Objectives

Students will learn and perform:

- LB Agar production for petri dishes
- Bacterial culturing
- Mechanics of Gene editing with CRISPR

Safety

ALWAYS WEAR NECESSARY PPE

This safety sheet has been designed to provide you with the tips and guidelines to follow in order to maintain the utmost safety when doing this experiment. This lab will combine a DNA amplification method with the gene-editing tool CRISPR. Both of these techniques require you to be very careful about the reagents you are using and how you are using them.

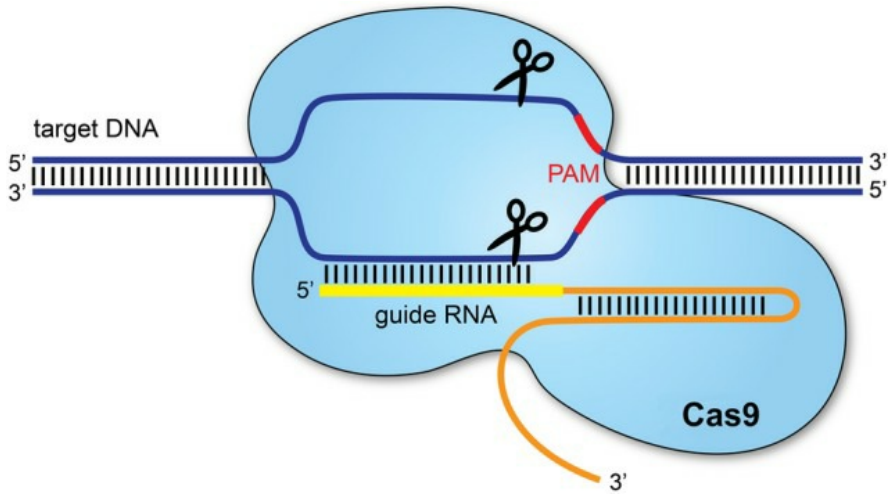
You will be utilizing chemicals and reagents that can definitely be troublesome if you do not properly use them in the correct ways. This is why it is essential that you know the type of reagents you will be working with and the potential harm they can cause you. With a sound understanding of your reagents and their specific uses in the protocol, you will not only gain a better understanding of how hazardous the reagent itself is but you will have a better understanding of the protocol as a whole.

Since you will be working with bacteria it is worth mentioning that you should **treat all microorganisms as if they're pathogenic.** Even though we will be working with E. Coli that isn't harmful to humans, you should take extra measures and precautions when working with it. When handling it make sure that it is properly contained and that you are wearing the appropriate attire when doing so. Refrigerators will be needed to store the consumables. In order to minimize the risk of cross-contamination, try not to store these items in the same places as food. Ideally, lab settings have entire refrigerators dedicated only to DNA, RNA, and other reagents. Since this course is being done inside your homes, we must try our best to minimize these contaminants as possible, make sure food and DNA that must be stored together are in good proximity apart and that the consumables are in another form of sealed containment(i.e plastic bag or box). Since you are working with bacteria, LB media will also be utilized to provide the bacteria with nutrients to replicate. This means that any spills should be attended to as soon as possible. This should already be common practice but should be especially enforced when using LB media, as a simple spill can cause undesired bacteria to grow in unwanted places.

Tips and Hazards

- Use a cloth, towel, or heat resistant gloves to take out Molten AGAR from the microwave; or wait for it cool where you can touch it comfortably.
- Don't pour Molten AGAR down the sink, this will clog pipes once it solidifies.
- An open plate may expose the agar to additional bacteria. This will cause unwanted colonies to form. Make sure you are plating and storing in a sanitary location.
- Keep all materials away from food.

Background



The CRISPR-CAS 9 system takes advantage of a naturally occurring phenomenon in bacteria's immune system. When an invading virus enters bacterial cells, the bacteria retain pieces of the virus's DNA to create CRISPR (clustered regularly interspaced short palindromic repeats) segments. This process is similar to antibody production in which it helps the host cell with viral recognition. If a viral invasion were to reoccur, the bacteria would be able to produce RNA from the CRISPR segment to target the virus' DNA. The CRISPR RNA (crRNA) is connected by a nucleotide linker to the trans-activating crRNA (tracrRNA). The tracrRNA is the link between the Cas9 protein and the crRNA. When the crRNA finds a complementary DNA strand, Cas9 cleaves the region of the viral DNA. This mechanism allows for bacteria to disrupt the DNA of invading viruses. The CRISPR/Cas9 complex has been applied to genetic engineering due to this ability to splice DNA in a specified region.

A double-stranded DNA splice caused by CRISPR can be repaired via two mechanisms: nonhomologous recombination repair (NHEJ) or homologous recombination repair (HRR). When NHEJ occurs, the overhanging DNA pieces are joined together by DNA ligase IV and any remaining spaces are filled in. This can induce a knockout DNA product and/ or cause a mutation. Alternatively,

HRR is the process in which the damaged DNA uses a homologous template from undamaged DNA. We will be using this same principle to knock in a gene by providing a template strand containing regions homologous to both sides of the double-stranded splice as well as the insert of the desired nucleotide between.

In this experiment, the goal is to genetically engineer E Coli to survive on streptomycin. To do so, we are targeting the rpsL gene which produces the small ribosomal subunit. Typically streptomycin binds to the rpsL gene and prevents protein formation, killing the bacteria. However, this edit prevents streptomycin from binding and inhibiting protein production. In other words, it becomes resistant to streptomycin. The template contains a mutation of the lysine amino acid, changing AAA to ACA. The mutation of the adenine to cytosine results in the codon change to a threonine. You will prepare the E coli for uptake of the provided template, let Cas-9 do its thing, and then test the E coli's survival on an agar plate containing streptomycin.

Resources

Read:

CRISPR/Cas9: what is gene editing (NIH article)

The ODIN protocol(ODIN document protocol) (refer to pages 2-5, 10-11, 14-15, 20)

Extra:

Papers supporting mutation effect (how the CRISPR edit makes the E coli antibiotic-resistant)

info on rpsL

Disclaimer:

The information provided on this document is intended for the educational purposes of the BME 22L laboratory course. It is worth noting that the information listed on this document is subject to change and is not finalized. Therefore, the information on this document should not be used outside of this course.