Oct 13, 2020

Protocol 2: CRISPR Transformation

Forked from Protocol 2: CRISPR Transformation

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1 *Works for me* This protocol is published without a DOI.

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PROTOCOL CITATION

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MATERIALS TEXT

Perishables
55µL of 100ng/µL - Cas9 Plasmid Kanr
55µL of 100ng/µL - gRNA Plasmid Ampr
55µL of 1mM- Template DNA
1 - Sterile Water Tube

Non-perishables
1 - 1 mL Bacterial Transformation Buffer 25mM CaCl2, 10% PEG 8000
1 - Bag of Microcentrifuge Tubes
1 - Microcentrifuge Tube Rack
1 - 10-100 µL Variable Volume Adjustable Pipette
1 - Box 1-200 µL Pipette Tips
5 - Inoculation Loops
5 - 1.5mL Microcentrifuge Tubes containing LB Broth
BentoLab

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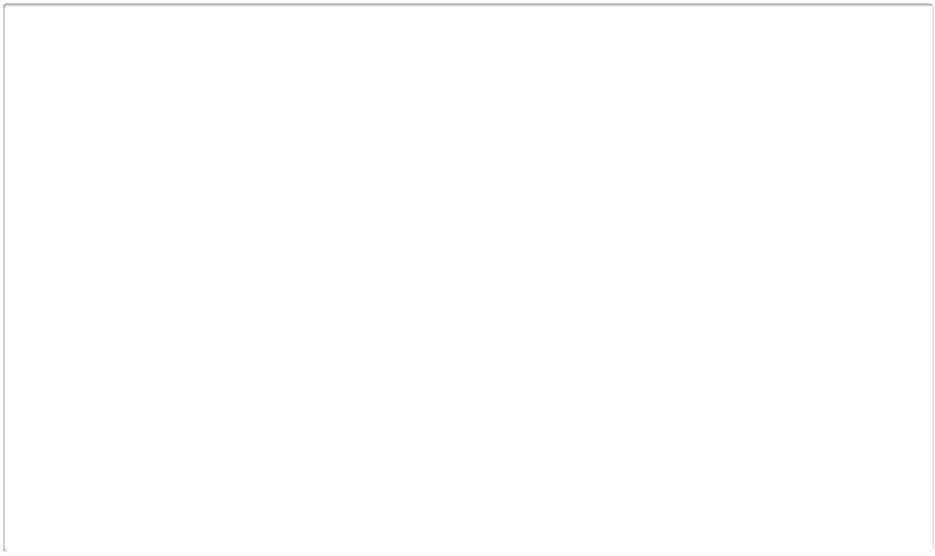
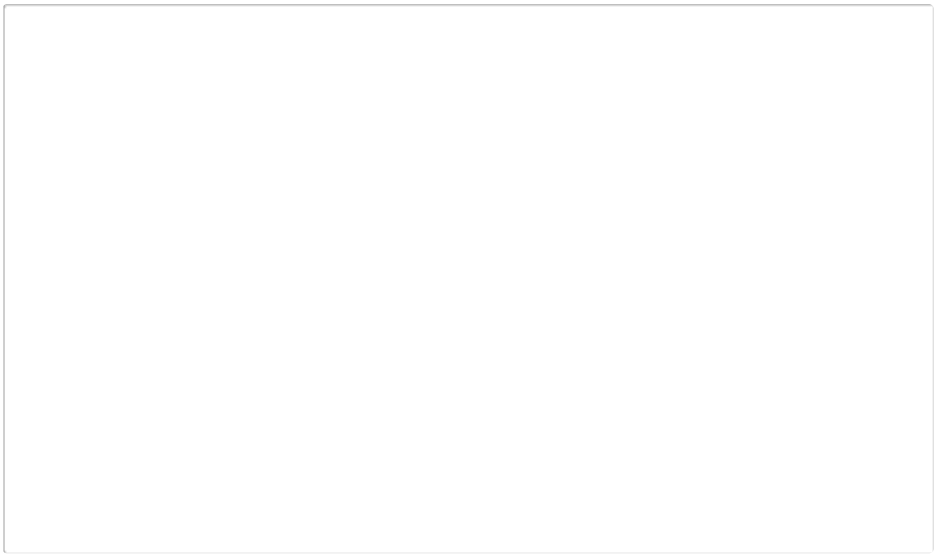
BEFORE STARTING

The following video will demonstrate techniques used in E. Coli transformation. Keep in mind this is not the exact same protocol, but a general guide showing good lab practice.



The following video shows the necessity of making competent bacterials cells for transformation.



1 **Mix Bacterial transformation solution with cultured E coli.** Pipette 100uL of Bacterial Transformation mix into a microcentrifuge tube. Use an inoculation loop to lightly scrape E coli and transfer to the microcentrifuge tube. Use pipetting technique of aspirating and dispensing to homogenize the mixture.

2 **Add CRISPR reagents.** Retrieve tubes of Cas9 Plasmid, gRNA Plasmid, and Template DNA. Add 55uL of sterile water to each. Don't forget to change the pipette tip. Make sure they are mixed well. In this order, pipette 10 uL from Cas9 Plasmid, gRNA Plasmid, and Template DNA into a centrifuge tube. Add each to the bottom of the competent cell mixture tube and follow by flicking your wrist or tapping the tube to the table to mix after each reagent addition.

3 **Place the mixture into a PCR tube.**

4 **Incubate the tube in the refrigerator for 30 minutes. While you are waiting for the prepare the following:**

4.1 **LB media.** Add 1.5 mL of room temperature water to the 1.5mL microcentrifuge tubes containing LB broth. Flick or tap the tube to mix. Once the mixture is completely dissolved, add 250 uL of the LB media into a new microcentrifuge tube.

4.2 On the bento lab, **create a custom heating with PCR option**. Make a setting where the PCR rack heats up to 42°C for 30 seconds.

5 **Take the tube out of the refrigerator and place in the PCR rack for 30 seconds.**

6 **Place contents of the PCR tube into the microcentrifuge tube containing 250 uL of LB Broth.**

7 **Incubate for 6-12 hours.** If room temperature is cool, let the tube incubate for a longer period of time. If room temperature is warm, let the tube incubate for a shorter period of time.