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Bacteria Transformation

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Protocol status: Working
We use this protocol and it's working

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Abstract

General protocol for bacteria transformation

Materials

Reagents:

- Competent E. coli cells (NEB, C3040H)
- LB plates with necessary antibiotic
- 50% Glycerol

Bacteria Transformation:

1h 24m

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1. Pipette 2 µL of product to 50 µL of E. coli competent cells and pipette slowly up and down 5 times to mix. For plasmids, use 2 µL ul of [M] 0.005 µg/µL plasmid solution. For ligation reactions, DNA concentrations will vary so use 2 µL ul of reaction.
2. Incubate for 00:20:00 on ice.
3. Heat shock bacteria in 42 °C waterbath for 00:01:00 .
4. Incubate on Ice for 00:03:00 .
5. Add 100 µL of SOC media and shake in warm room for 01:00:00 .
6. Pipette 100 µL of bacteria onto LB-Antibiotic Plate and spread cells out with plate spreader to get individual colonies.
7. Incubate overnight in 37 °C warm room.
8. Pick colonies for miniprep growth and sequencing.

Glycerol Bacteria Stocks:

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1. In screw top 2 mL tube. Mix 500 µL of Bacteria Solution (Either from Miniprep or Maxiprep before centrifugation) and 500 µL of 50% (v/v) Glycerol Solution.
2. Store at -80 °C .
3. To pull out bacteria, use bacteria loop to scrap some of still frozen glycerol stock and streak bacteria onto LB-Antibiotic plate. Then pick colonies for growth.