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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

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

Glycerol Bacteria Stocks:

- 2
 1. In screw top $2\ \text{mL}$ tube. Mix $500\ \mu\text{L}$ of Bacteria Solution (Either from Miniprep or Maxiprep before centrifugation) and $500\ \mu\text{L}$ of 50% (v/v) Glycerol Solution.
 2. Store at $-80\ ^\circ\text{C}$.
 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.