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Transformation of Chemically Competent Cells

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

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Protocol Integer ID: 28708

Keywords: cell

Troubleshooting

1 Add 1 µL of miniprep plasmid or 5 µL of Gibson product into competent cell

Protocol

NAME

Hi-Fi Gibson Assembly

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Preview

1.1 Calculate for volumes of respective fragments to assemble based on their length and concentration

1.2 Add calculated volume of each fragment (maximum volume: 5 µL)

1.3 Add in 5 µL of 2x Hi-Fi DNA Assembly Master Mix into the PCR tube

NEBuilder HiFi DNA Assembly Master Mix - 10 rxns **New England Biolabs Catalog** #E2621S

1.4 Vortex to mix

1.5 Spin down PCR tube

1.6 Incubate samples at 50 °C for 00:45:00

2 Incubate cells in 42 °C water bath for 00:00:45

3 Incubate cells on ice for 00:02:00

4 Add 1 mL of LB media into cell sample

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Preparation of LB Media

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4.1 Weigh 25 g of Luria Broth Base powder.

Luria Broth Base (Miller's LB Broth Base)™, powder **Thermo Fisher Catalog #**12795027

4.2 Add the powder into 1 L of water.

Water refers to sterilized deionized water

4.3 Autoclave entire bottle of LB media.

5 Incubate cells at 37 °C for 01:00:00

6 Spin down the cells at 6000 x g for 00:01:00

7 Remove 700 µL of the supernatant

8 Re-suspend pellet in remaining media

9 Transfer 100 µL of the culture onto the agar plate containing appropriate antibiotic

Protocol

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Preparation of LB Agar

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9.1 Weigh 32 g of LB agar powder.

LB Agar, powder (Lennox L agar) **Thermo Fisher Catalog #**22700025

9.2 Add the powder into 1 L 1 of deionized water.

Water refers to sterilized deionized water

9.3 Autoclave entire bottle of LB media.

10 Spread the cells evenly

11 Incubate at 37 °C overnight