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Transformation of Chemically Competent Cells

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1 Works for me dx.doi.org/10.17504/protocols.io.8achsaw

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

Hi-Fi Gibson Assembly
by NUS iGEM,
National University of Singapore
PREVIEW RUN

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
by 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

Preparation of LB Media
by NUS iGEM,
National University of Singapore
PREVIEW RUN

4.1 Weigh **25 g** of Luria Broth Base powder.

Luria Broth Base (Miller's LB Broth Base)™, powder
by 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

Preparation of LB Agar
by NUS iGEM,
National University of Singapore
PREVIEW RUN

9.1 Weigh 32 g of LB agar powder.

LB Agar, powder (Lennox L agar)
by 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