OCT 16, 2019

Transformation of Chemically Competent Cells

Nus Igem¹

¹National University of Singapore

Nus Igem

National University of Singapore

OPEN ACCESS

DOI:

dx.doi.org/10.17504/protocols.io.8achsaw

Protocol Citation: Nus Igem 2019. Transformation of Chemically Competent Cells.

protocols.io

<https://dx.doi.org/10.17504/protocols.io.8achsaw>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: Oct 16, 2019

Last Modified: Oct 16, 2019

PROTOCOL integer ID:

28708

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

Protocol

NAME

Hi-Fi Gibson Assembly

CREATED BY

Nus Igem

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

Protocol

NAME

Preparation of LB Media

CREATED BY

Nus Igem

PREVIEW

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 **Contributed by users**

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

NAME

Preparation of LB Agar

CREATED BY

Nus Igem

PREVIEW

- 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 of deionized water.

9.3 Autoclave entire bottle of LB media.

10 Spread the cells evenly

11 Incubate at 37 °C overnight