

Transformation of Chemically Competent Cells

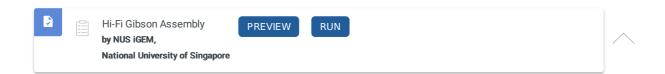
NUS iGEM1

¹National University of Singapore

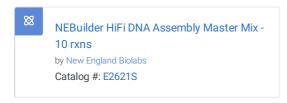




1 Add $\boxed{1}$ μ I of miniprep plasmid or $\boxed{5}$ μ I of Gibson product into competent cell

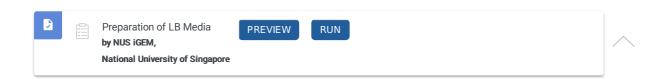


- 1.1 Calculate for volumes of respective fragments to assemble based on their length and concentration
- 1.2 Add calculated volume of each fragment (maximum volume: $\frac{1}{2}$ 5 μ I)
- 1.3 Add in 35 µl of 2x Hi-Fi DNA Assembly Master Mix into the PCR tube

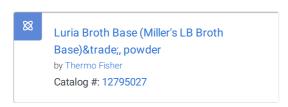


- 1.4 Vortex to mix
- 1.5 Spin down PCR tube
- I.6 Incubate samples at § 50 °C for © 00:45:00

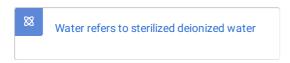
- 2 Incubate cells in 8 42 °C water bath for © 00:00:45
 - Incubate cells on ice for (00:02:00
- 4 Add 11 ml of LB media into cell sample



4.1 Weigh 25 g of Luria Broth Base powder.

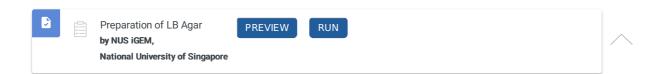


4.2 Add the powder into 11 L of water.



- 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



- 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 11 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

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