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## Transformation of Chemically Competent Cells

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Protocol status: Working

We use this protocol and it's working

Created: October 15, 2019



Last Modified: September 11, 2023

**Protocol Integer ID: 28708** 

Keywords: cell

## Troubleshooting

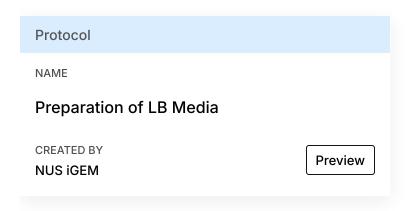


1 Add  $\perp 1 \mu L$  of miniprep plasmid or  $\perp 5 \mu L$  of Gibson product into competent cell



- 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:  $\Delta 5 \mu$ )
- 1.3 Add in 4 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
- 2 Incubate cells in 42 °C water bath for 00:00:45

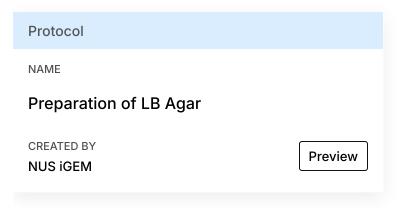
- 3 Incubate cells on ice for 00:02:00
- 4 Add 4 1 mL of LB media into cell sample



- 4.1 Weigh 4 25 q of Luria Broth Base powder.
  - Luria Broth Base (Miller's LB Broth Base)™, powder Thermo Fisher Catalog #12795027
- 4.2 Add the powder into 4 1 L of water.
  - Water refers to sterilized deionized water
- 4.3 Autoclave entire bottle of LB media.
- 5 Incubate cells at \$\mathbb{8}\$ 37 °C for \( \bigotimes \) 01:00:00
- 6 Spin down the cells at \text{ \cdots } 6000 \text{ x g} for \text{ \cdots } 00:01:00



- 7 Remove 4 700 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 q of LB agar powder.
  - 🔀 LB Agar, powder (Lennox L agar) Thermo Fisher Catalog #22700025
- 9.2 Add the powder into  $\bot$  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

