Chemically competent cells transformation

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[1]In-house protocols

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1 Works for me This protocol is published without a DOI.

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ABSTRACT

Chemically competent cells transformation protocol

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GUIDELINES

1. Preparation of competent cells
2. Transformation

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ABSTRACT

Chemically competent cells transformation protocol

Preparation for competent cells

1 Inoculate a single colony into 5 mL appropriate media containing appropriate antibiotics

2 Incubate overnight using appropriate temperature and shaking conditions

3 Dilute overnight culture to an optical density of 0.01 in 10 mL of appropriate media containing appropriate antibiotics

4 Incubate at appropriate temperature and shaking conditions until optical density reaches 0.4 to 0.6. Do not allow to grow past this point.

5 Spin down cells at 3500 RPM at 4°C for 10 min

6 Resuspend pellet in 10 mL ice-cold 0.1M $CaCl_2$

7 Place cells on ice for 20 min (do not go under this time; can go longer)

8 Spin down cells as in step 5.

9 Resuspend pellet in 1 mL ice-cold 0.1M $CaCl_2$ + 13% glycerol (for a higher concentration of cells, use less)

10 Place on ice for 20 min

11 Use immediately or store at -80°C (0.25 mL/tube)

Transformation

12 Add 50 µL competent cells into a sterile Eppendorf tube

13 Add 1 µL plasmid DNA or ligation reaction (can vary this)

14 Place on ice for 30 min

15 Heat shock at 42°C for 1 min

16 Place on ice 2 min

17 Add 0.5 mL recovery media (SOC, LB, etc.)

18 Incubate for 1 hour at appropriate growth conditions.

19 Plate 200 µL on appropriate plate with appropriate antibiotics.

20 Incubate at 37°C overnight