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Plasmid transduction using competent cell V.2

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Protocol status: Working
We use this protocol and it's working

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Disclaimer

Abstract

Plasmid can be transduced into bacteria at competent state using heat shock. This protocol helps transduce plasmid into competent cells.

Materials

Competent cell, DNA plasmid solution, LB broth medium, LB agar plate (with antibiotics)

Troubleshooting

1 Take competent cell out from -80℃ fridge and thaw on ice.

2 When the cells are completely thawed, pipette 2 µL plasmid DNA solution into 100 µL competent cell. 30m
Put the cell in ice for 00:30:00

3 Conduct heat shock on the competent cell by placing the cell in 42 °C water bath for 00:01:30 . 3m 30s
Put the cells back into ice for 00:02:00

4 Add 900 µL LB broth medium into competent cell mixture. Shake at 180 rpm, 37°C for 00:45:00 45m

5 Centrifuge at 6000 rpm, Room temperature, 00:05:00 . 5m

Note

Centrifuge radius = 6 cm.

6 Discard 900 µL supernatant and resuspend the pellet in the rest 100 µL supernatant.

7 Spread the cells onto LB agar plates.

Note

LB agar plates may contain antibiotics, which is determined by the transduced plasmid.

8 Place the plate with lid on upside for 01:00:00 . 1h

9 Invert the plate and culture at 37 °C in a biomedical incubator overnight.

Note

If the bacteria turn out to be too concentrated, dilute the cell before spreading on the plate next time.