



Plasmid transduction using competent cell V.2

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Version 2 ▼

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1 Works for me

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ABSTRACT

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

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MATERIALS TEXT

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

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- 1 Take competent cell out from -80°C fridge and thaw on ice.
- 2 When the cells are completely thawed, pipette  **2 µL** plasmid DNA solution into  **100 µL**^{30m} competent cell.
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 ^{3m 30s}
 **00:01:30** .
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**^{45m} for

🕒 00:45:00

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

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.

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.

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