Plasmid transduction using competent cell V.2

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Version 2

Oct 06, 2022

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dx.doi.org/10.17504/protocols.io.j8nlkw4e5l5r/v2

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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.

DOI

dx.doi.org/10.17504/protocols.io.j8nlkw4e5l5r/v2

PROTOCOL CITATION

An.Huang 2022. Plasmid transduction using competent cell. **protocols.io** https://dx.doi.org/10.17504/protocols.io.j8nlkw4e5l5r/v2 Version created by An.Huang

CREATED

Oct 06, 2022

LAST MODIFIED

Oct 06, 2022

PROTOCOL INTEGER ID

70907

MATERIALS TEXT

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

DISCLAIMER:

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** 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 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** for 45m

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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.

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