Plasmid transduction using competent cell V.1

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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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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
00:45:00

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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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