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Plasmid transduction using competent cell V.1

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Protocol status: Working

We use this protocol and it's working

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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.

Troubleshooting



- 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.
Put the cell in ice for  00:30:00 30m
- 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.