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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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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°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.