Feb 21, 2023

Identification of a Plasmid: Transformation

DOI
dx.doi.org/10.17504/protocols.io.8epv5jmj6l1b/v1

SGD[1]

[1]na

SGD

OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.8epv5jmj6l1b/v1

Protocol Citation: SGD 2023. Identification of a Plasmid: Transformation. **protocols.io** https://dx.doi.org/10.17504/protocols.io.8epv5jmj6l1b/v1

Protocol status: Working
We use this protocol and it's working

Created: February 20, 2023

Last Modified: February 21, 2023

Protocol Integer ID: 77305

Keywords: plasmid, transformation protocol for the identification, transformation protocol, transformation, using transformation

Abstract

Protocol for the identification of a plasmid by using transformation of E. Coli

Troubleshooting

Transformation

30s

1 Transfer 1 mL E. Coli to each of two 1.5 mL Eppendorf tube

2 Centrifuge at full speed for 00:00:30 , then remove the supernatant

30s

3 Add 450 µL of TFB1 to each tube, then resuspend gently by pipetting up and down (do not vortex)
Keep everything On ice
Incubate on ice for 00:10:00

10m

4 Pellet the bacteria by centrifugation at full speed for 00:00:30
Remove the supernatant
Resuspend the pellets in 100 µL of TFB2
Incubate on On ice for 00:10:00

10m 30s

5 Add 100 ng of plasmid DNA to the competent cells, and mix gently

6 Heat shock the cells by transferring directly from the ice to the 37 °C hot block for 00:05:00
Then incubate on ice for 00:05:00 , and add 1 mL LB broth to each tube

10m

7 Incubate again at 37 °C , for 00:30:00 , and allow the cells to recover

30m

8 Pellet the bacteria again by centrifuging at full speed for 00:00:30
Discard the supernatant

30s

9 Resuspend the pellet in 400 µL of LB broth

10 Spread 100 µL of cells per plate, onto the appropriate antibiotic plates

11 Incubate the plates Overnight at 37 °C 30s