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Identification of a Plasmid: Transformation

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ABSTRACT

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

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

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PROTOCOL integer ID:
77305

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) 10m
Keep everything On ice
Incubate on ice for 00:10:00

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

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 10m
00:05:00
Then incubate on ice for 00:05:00, and add 1 mL LB broth to each tube

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 30s
Discard the supernatant

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