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## 🌐 Identification of a Plasmid: Transformation

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**Protocol status:** Working

**We use this protocol and it's working**

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**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  $\mu$ L 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  $\mu$ L 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  00:05:00 10m  
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  $\mu$ L of LB broth
- 10 Spread  100  $\mu$ L of cells per plate, onto the appropriate antibiotic plates



11 Incubate the plates  Overnight at  37 °C

30s