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Bacterial Transformation

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

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Troubleshooting

1 Thaw a tube of DH5a Competent E. coli cells on ice for 5-10 minutes

2 Add 1µl of plasmid DNA to per 100µl cell mixture. Flick the tube 4-5 times to mix cells and DNA but do not vortex.

3 Place the mixture on ice for 30 minutes. Do not mix.

4 Heat shock at exactly 42°C for exactly 90 seconds. Immediately place on ice for 2-3 minutes. Do not mix.

5 Pipette 800 µl of 42°C LB liquid medium into the mixture.

6 Place at 37°C, 180-220 rpm for 45-60 minutes.

7 Centrifuge the bacterium at 3000r for 3 minutes.

8 Discard 300 µl supernatant and resuspend the sediment.

9 Spread 50-100 µl of each dilution onto a selection plate and incubate for 12 hours at 37°C.