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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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- 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.