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Electroporation

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

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1 Separate the bacterial liquid in shaking tube into 1.5 mL (2 tubes) per tube, centrifuge quickly for 90 s at 4℃ and 12000 rpm.

2 Add 1 mL of 300 mM sucrose solution to each tube of bacterial liquid, centrifuge for 2 min at 12000 rpm and 4℃, and pour it out.

3 Resuspend the competent cells (tube one) by adding 100 µl 300 mM sucrose solution, then transfer the liquid to tube two to resuspend the competent cells, and divide into 50 µl tubes (two tubes in total).

4 Add 1 µg plasmid into each tube, gently blow and mix.

5 Add 50 µl of suspension to the electroporation cuvettes.

Electroporation

6 Turn on electroporator and set to 3kv, 400 ohms and 25 µF

7 Take out the liquid culture medium of LB with 37℃ heat preservation for standby.

8 Transfer the DNA-cell mixture to the cold cuvette, tap on countertop, wipe water from exterior of cuvette and place in the electroporation module and press pulse.

9 Immediately add LB culture solution, mix by pipetting up and down once and transfer to a microcentrifuge tube, 5 ml culture tube.

10 Place in the shaker/incubator at 37°C incubator for 1 h-2 h

11 Incubate overnight at 37°C