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Competent Transformation

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

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Abstract

Transform a Plasmid into previously obtained Competent E.coli Cells.

Safety warnings

Competent Cells are fragile. Please do not use Vortex and work with aseptic conditions

1 Melt in ice and aliquot of 100µL competent E-coli DH5α or BL21(DE3) .

2 Preheat 500 µL LB or SOC medium (37ºC).

3 Add 50-100µg DNA into competent cells and mix softly. This is usually 1-2µl from minipreps. I recommend using 10µl if the DNA comes from PCR and/or DpnI digestion.

4 Incubate 00:20:00 in ice. 20m

5 Incubate without shaking at 42 °C for 00:01:00 . 1m

6 Incubate 00:02:00 in ice. 2m

7 Add 500 µL LB or SOC pre-heated media. This must be done under Laminar Flux Cabin

8 Incubate 01:00:00 800 rpm, 37°C in shaker thermal block. 1h

9 Prepare Amp or Kan plates.

10 Add 100 µL competent cells into each plate and plate. Mix using sterile glass balls under laminar flux cabin.

11 Incubate 37 °C Overnight

12 Next day, store the plates parafilmed in the fridge. Can be stored for several weeks.