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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 (37°C).
- 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 parafilemed in the fridge. Can be stored for several weeks.