Transformation of Chemically Competent (Smart) Cells

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GUIDELINES


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

Created: Aug 21, 2018
1. Remove cells from the −80 °C freezer and place directly into your ice bucket. Thaw on ice for 15 minutes.

2. Add DNA from plasmid to be transformed. For ligations/PCR products use 10uL of ligation reaction per 50-100uL of cells. For purified plasmid (miniprep), use 1 uL of plasmid per aliquot of cells. Do not mix or stir the cells they are very fragile.

3. Incubate cells on ice with the DNA for 30 minutes, in the meantime make sure there is a 42°C water bath/block that has water in it.

4. Bring ice bucket with cells and a timer over to the water bath/heat block. Immerse tube of cells in 42°C bath for 45-60 seconds.

5. Remove tube from waterbath and place directly on ice to recover for 2 minutes.

6. Add 900 uL of sterile LB (no antibiotics) and grow with shaking for 1 hour at 37 °C.

7. Plate out 50-200 uL on antibiotic selective plate of choice OR if cloning (small amounts of DNA for transformation) proceed to next step.

8. Spin cells in tabletop centrifuge for 1 minute at ~2,000 rpm, remove 700uL of media and resuspend the cell pellet in the remaining media in the tube. Plate this onto the correct antibiotic
LB plate.