Transformation of Chemically Competent (Smart) Cells

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Working

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ABSTRACT

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

GUIDELINES

A video explaining the technique: http://www.jove.com/science-education/5059/bacterial-transformation-the-heat-shock-method?utm_campaign=website&utm_source=sendgrid.com&utm_medium=email

SAFETY WARNINGS

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.

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