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Mix and go competent cells V.1

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We use this protocol and it's working

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Abstract

Mix and Go protocol for the preparation of competent E. coli cells

Attachments

MixANDGo.pdf

741KB

1 Use 0.5 ml of fresh, overnight E. coli culture grown in LB to inoculate 50 ml ZymoBroth™ or SOB medium in a 500 ml culture flask. Shake culture vigorously (150 - 250 rpm) at the appropriate temperature* until the OD600nm is 0.4 - 0.6. (Buffer Preparation Prior to Harvesting the Cells: The Wash and Competent Buffers are provided as 2X stock solutions. They need to be diluted to 1X by adding an equal amount of Dilution Buffer. To prepare 5 ml of 1X Wash Buffer: Add 2.5 ml Dilution Buffer and 2.5 ml of 2X Stock Wash Buffer. To prepare 5 ml of 1X Competent Buffer: Add 2.5 ml Dilution Buffer and 2.5 ml of 2X Stock Competent Buffer. Please keep these freshly prepared 1X Buffers ice cold. These 1X Buffers are good for 2 days at 0-25°C. It is important that each step of the following procedure should be done on ice or at 0-4°C.)

2 Transfer the culture from Step 1 to ice. After 10 minutes, pellet the cells by centrifugation at 3,000 - 3,700 rpm (i.e., 1,600 - 2,500 x g) for 10 minutes at 0 - 4°C.

3 Remove the supernatant and resuspend the cells gently in 5 ml ice-cold 1X Wash Buffer. Re-pellet the cells as in Step 2.

4 Completely remove the supernatant and gently resuspend the cells in 5 ml ice-cold 1X Competent Buffer.

5 Aliquot (on ice) 0.1-0.2 ml of the cell suspension into sterile microcentrifuge tubes. Cells are now ready for transformation with DNA or can be stored below -70°C for transformation at a later time.