Sep 22, 2019 Version 2

Mix and go competent cells V.2

DOI
dx.doi.org/10.17504/protocols.io.7jnhkme

Ben Kuipers[1]

[1]Wageningen University

iGEM Wageningen 2019

Ben Kuipers

DOI: https://dx.doi.org/10.17504/protocols.io.7jnhkme

Protocol Citation: Ben Kuipers 2019. Mix and go competent cells. **protocols.io https://dx.doi.org/10.17504/protocols.io.7jnhkme**

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

Created: September 22, 2019

Last Modified: September 22, 2019

Protocol Integer ID: 27982

Keywords: protocol for the preparation, competent cell, preparation, protocol, zymo research mix, cell

Abstract

Mix and Go protocol for the preparation of competent E. coli cells. (This protocol is according to Zymo Research mix and Go protocol).

Troubleshooting

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.