



Protocol

1. Prepare 17mm x 100mm sterile tubes (one for each conversion reaction) and restore the 2 * YT culture medium to room temperature.
2. Place the electric cup (0.1cm gap) and microcentrifuge tube on ice.
3. Remove the electropositive cells from the -80 °C freezer and place them in an ice box until completely melted (10-15 minutes).
4. After the cells have completely dissolved, gently pat and mix well. Take out 25 µl of cells into a pre cooled microcentrifuge tube placed on ice. (It is recommended to use one tube of competent cells at a time. If 25 µl is used at a time, please immediately put the remaining half back in the -80 °C freezer).
5. The volume of DNA added should not exceed 10% of the receptive state volume.
6. Gently transfer 25 µl of cell/DNA mixture into a pre cooled electric transfer cup, taking care to avoid the formation of bubbles. Quickly flick the test tube downwards with your fingers to allow the cells to deposit at the bottom of the well electrode rotating cup. Perform electric conversion according to the recommended conditions.
7. Add 975 µl of 2 * YT culture medium to an electric transfer cup after the pulse immediately, blow the resuspended cells up and down three times with gun, and then transfer the culture medium containing the cells to a sterile culture tube.
8. Place the culture tube on a shaker at 250 rpm and incubate at 37 °C for 1 hour.
9. Take 100 µl of transformed cells from the culture tube and coat them onto a 2 * YT agarose plate containing specific antibiotics.
10. Place the culture plate at 37 °C overnight for cultivation, and calculate the conversion efficiency the next day.

Culture medium formula

2 * YT Agar Plates (per 1L formula)

16g tryptone

10g yeast extract

5g NaCl

15g agar

Add all components to deionized water and adjust the pH to 7.0 with NaOH. Sterilize under high pressure and then cool to 55 °C.