L. REUTERI ELECTROPORATION (AUKRUST)

Alternative Lactobacillus reuteri transformation protocol, as described By Aukrust et al.[1]

MATERIALS:

- MRS Broth
- MRS plates (with low antibiotic concentrations)
- 20% Glycine solution
    - 20 g glycine, dH_2O (MiliQ) to 100 mL
- MRS-SM
    - MRS, 0.5*M* sucrose, 0.1*M* $MgCl_2$
    - (concentrated MRS, 17.1 g sucrose, 2.0 g $MgCl_2 \cdot 6H_2O$, dH_2O to 100 mL)
- SM (sucrose medium i.e electroporation buffer)
    - 326 g sucrose (952 m*M*), 0.71 g $MgCl_2 \cdot 6H_2O$ (3.5 m*M*), dH_2O to 1 L. Filter-sterilize as above

PREARATION OF ELECTROCOMPETENT CELLS – PROCEDURE 1 (PREFERRED)

1. 25ml preculture of *Lactobacilli* in exponential growth phase to inoculate 100ml of MRS (or MRS supplemented with glycine). (Inoculate to A_{600}=0.25 and incubate the culture at 30C until A_{600}=0.6 (Usually, 2-4 hours)
2. Pellet the cells by centrifugation and decant the supernatant. USE THE MINIMUM SPEED AND TIME SUFFICIENT TO PELLET THE CELLS. (About 1500g for 5 min).
3. FIRST WASH -- Resuspend cells carefully (by pipetting the cells) in 100ml of SM
4. Pellet the cells by centrifugation as in step 2
5. SECOND WASH -- Resuspend cells carefully (by pipetting the cells) in 100ml of SM
6. Incubate for 15 min on ice.
7. Pellet the cells by centrifugation.
8. Resuspend cels gently in 1 mL of SM
9. Make 100uL aliquots of the cells into separate microcentrifuge tubes.
10. Store on ice until needed for electroporation or at -80C

PREARATION OF ELECTROCOMPETENT CELLS – PROCEDURE 2

1. Inoculate 25ml Lactobacillus overnight culture in 100ml of MRS (or MRS supplemented with glycine). Grow the culture at 30ºC to OD_{600}=0.6 (Usually, 2-4 hours)
2. Pellet the cells by centrifugation and decant the supernatant. USE THE MINIMUM SPEED AND TIME SUFFICIENT TO PELLET THE CELLS. (About 1500g for 5 min).
3. FIRST WASH -- Resuspend cells carefully (by pipetting the cells) in 100ml of 1mM MgCl2
4. Pellet the cells by centrifugation as in step 2
5. SECOND WASH -- Resuspend cells carefully (by pipetting the cells) in 100ml of 30% PEG
6. Pellet the cells by centrifugation.
7. Resuspend cells gently in 1 mL of 30% PEG
8. Make 40 µL aliquots of the cells into separate microcentrifuge tubes.

[1] Aukrust, T. W., M. B. Brurberg, and I. F. Nes. 1995. Transformation of *Lactobacillus* by electroporation. Methods Mol. Biol. 47:201-208

9. Store on ice until needed for electroporation or at -80C

Electroporation:

1. Immediately before electroporation, add 5uL DNA. Mix with the pipet tip and transfer carefully to an ice col electroporation cuvette. Avoid trapping air bubbles and make sure that the cell suspension is distributed evenly on the bottom of the cuvette
2. Voltage: 1.5 kV, Capacitance: 25 µF, Resistance 800 Ohm, Time: 10ms
3. Immediately following the discharge, add 1 mL MRSSM and transfer the cell suspension to a microcentrifuge tube.
4. Incubate at 30C for 2 hours.
5. Spread undiluted and serial dilutions of the cell suspension on MRS plate containing appropriate antibiotics.
6. Incubate plates at 30C for up to 3 days.

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