



Electroporation of *L. rhamnosus* GG.

(Estimated time: 4 hours for procedure, 3 days for confirmation of transformants)

Materials

- ❖ Pre-cooled electroporation cuvette (Eurogentec) with a 0.2-cm electrode gap
- ❖ Cool competent cell suspension
- ❖ DNA in a concentration sufficient to provide 400 ng in 4 - 5 μg
- ❖ MRS medium with 2 mM CaCl_2 and 20 mM MgCl_2

- ❖ MRS agar with appropriate antibiotic
- ❖ Parafilm

Equipment

- Electroporation equipment Gene Pulser; Bio-Rad Laboratories
- Incubator at 37°C

Methodology

1. Pre-cool electroporation cuvette (Eurogentec) with a 0.2-cm electrode gap
2. Take 100 μl of a cooled competent cell suspension and 400 ng of DNA (maximum volume, 4 to 5 μl)
3. Transfer into the pre-cooled electroporation cuvette (Eurogentec) with a 0.2-cm electrode gap and immediately electroporate (Gene Pulser; Bio-Rad Laboratories) by using the following settings: peak voltage, 1.7 kV; capacitance, 25 μF ; and parallel resistance, 200 Ω .
4. Immediately dilute with 5 ml of MRS medium containing 2 mM CaCl_2 and 20 mM MgCl_2
5. Incubate (without agitation) at 37°C for 3 h
6. Plate onto MRS agar containing the appropriate antibiotic.
7. Incubate anaerobically (with parafilm) or with a BBL GasPak system, at 37°C for 48-72 hours
8. Assess transformation efficiency
9. Select colonies and culture in MRS medium with antibiotic for miniprep or other procedures
10. Culture the colony into another MRS+ antibiotic agar plate for 48-72 hours for a stock of transformants and keep in fridge, lyophilize if possible