Protocol for Electrocompetent *Lactobacillus casei* ATCC 334™ Cells Transformation
Protocole code: Trans_Lc

Materials:

- Laminar flow hood or sterile environment.
- Micropipettes.
- Tips for micropipettes.
- 1.5 mL microcentrifuge tubes.
- Microcentrifuge.
- Cold electroporation cuvettes of 2mm.
- Multiporator.
- *L.casei* ATCC 334™ competent cells.
- MRS media supplemented (MRS + 0.5M de sucrose) [Recuperation media]].
- Plasmid to transform.
- $_dH_20$ cold and sterile.
- PEG-8000 cold and sterile at 30%.
- MRS agar plates with the respective antibiotic.

Procedure:

Water pretreatment:

1. Unfreeze the cells to room temperature.
2. Add to each tube with 600µL of competent cells, 900µL of $_dH_20$ cold and sterile.
3. Incubate during 30 min at room temperature.
4. Centrifuge at 8500 rcf by 2 min at room temperature.
5. Discard the supernatant and resuspend in 1mL of PEG-8000 cold and sterile at 30%.
6. Centrifuge at 8500 rcf by 2 min at room temperature.
7. Discard the supernatant and resuspend in 500µL of PEG-8000 cold and sterile at 30%.
8. Aliquot 100µL of cells in 1.5mL microcentrifuge tubes.

Electroporation:

1. Add 200 ng of the desired plasmid to the 100µL of competent cells.
2. Pass the entire volume to the cold electroporation cuvettes of 2mm, pour the liquid through the walls of the cuvette.
3. Electroporate immediately at 25µF with a resistance of 200Ω and an electric field of 12.5 $kVcm^{-1}$ (approximately the same as 2500 V by 5ms).
4. Right after the shock, add 900µL of recuperation media to the cuvette and pour it gently to a sterile microcentrifuge tube and incubate at 37°C by 4h without shaking.
5. Scratch the plates with MRS and the respective antibiotic.
    i. Erythromycin --- 2.5 $\mu g/mL^{-1}$.
    ii. Ampicillin
    iii. Chloramphenicol.
    iv. Tetracycline.
    v. Kanamycin.
    vi. Gentamycin.
6. Wait until the agar to absorb the inoculum with cells, incubate the plates at 37°C at aerobic conditions.
7. Check for the colonies 2-4 days after the incubation.