# Lactobacillus plantarum

## Multiporator/ Eppendorf Eporator ®

**Transformation Protocol**

**Protocol No. 4308 915 546-02-2012**

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| **Microorganism** | *Lactobacillus plantarum* CECT 220 |
| **Cell type** | Bacteria, gram positive |
| **Molecules injected** | Plasmid DNA pRS4C1, 7.8 kb |

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| **Growth medium** | MRS medium (0.4 M sucrose, 1 mM $MgCl_2$, 5 mM $Kh_2PO4$; pH 6, Biolife) |
| **Washing solution I** | 10 mM $MgCl_2$ |
| **Washing solution II** | 1:1 sucrose (0.5 M) and glycerol (10 % w/v) |
| **Outgrowth medium** | MRS medium with chloramphenicol (7.5 µg/mL) agar plates |
| **Cuvette** | 1 mm gap width |
| **Reference** | M. Teresa Alegre, M. Carmen Rodriguez, Juan M. Mesas. 2004. FEMS Microbiology Letters 241. 73-77 |

**Making electrocompetent cells:**

1. Dilute 10 mL of an overnight culture of *L. plantarum* into fresh media (1:10) MRS. Grow at 30 °C for approx. 3-4 h with shaking until reaching an $O.D._{600}$ of 0.85.
2. Pellet cells by centrifugation (4 °C), wash twice with 10 mL chilled $MgCl_2$ (10 mM) and once with 10 mL of a chilled solution of sucrose (0.5 M) and glycerol (10 % w/v).
3. Resuspend in 2-3 mL of the same solution and store until use in an ice bath, but no longer than 4 h. The electrocompetence of frozen cells decreases in 1-2 logs.

**Electroporation of cells:**

1. Add 100 ng plasmid DNA diluted in 5 µL TE buffer to 50 µL ($3x10^8$) freshly prepared competent cells. Homogenize by gently mixing with pipette several times. Transfer mixture into a pre-chilled cuvette.
2. Wipe moisture from the cuvette and insert the cuvette into the device.
3. Electroporation:

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| Voltage (V) | 1,300 V |
| Time constant (t) | 5 ms |

4. Immediately add 1 mL of pre-warmed MRS medium and incubate at 30 ºC for 3 h with shaking.
5. Plate on selective MRS agar plates and incubate al 30 ºC for 48 h.

**Expected results:**

Transformation efficiency up to $5.8x10^5$ +/- $2.2x10^5$ transformants/µg of DNA.

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