Pediococcus spp.

**Microorganism**
*Pediococcus* spp.

**Cell type**
Bacteria, gram positive

**Molecules injected**
Plasmid DNA (pGK12 and pPN-1)

**Growth medium**
MRS-G with 0.5 M sorbitol, 3 % glycine and 40 mM DL-threonine

**Washing solution**
0.5 M sorbitol, 10 % glycerol

**Electroporation solution**
0.5 M sorbitol, 1 mM K2HPO4, 1 mM MgCl2, pH 7.0

**Outgrowth medium**
MRS with 0.5 M sorbitol, 20 mM MgCl2 and 2 mM CaCl2

**Cuvette**
1 mm gap width

**Reference**
Caldwell, S. L. et al • 1996 • Applied and Environmental Microbiology 62, No. 3 • 936-941

**Making electrocompetent cells:**
1. Cultivate cells by adding 12 ml overnight preculture (grown in MRS-G with 0.5 M sorbitol) to 800 ml growth medium. Incubate for 2 to 4 hours at 37 °C to a cell density of O.D.600 of 0.4-0.6.
2. Harvest by centrifugation.
3. Wash twice in 25 ml washing solution.
4. Resuspend in 1 ml electroporation solution.

**Electroporation of cells:**
1. Add 400-600 ng plasmid DNA to 80 µl of electrocompetent cells. Homogenize by gently mixing with pipette several times. Transfer mixture into cuvette.
2. Wipe moisture from the cuvette and insert the cuvette into the device.
3. Electroporation:
   - **Mode**
     - Prokaryotes “O”
   - **Voltage (V)**
     - 1,800 V
   - **Time constant (τ)**
     - 5 ms
4. Immediately add 2 ml outgrowth medium and keep on ice for approx. 5 min. Incubate for 2 h at 37 °C.
5. Plate aliquots onto selective agar plates; incubate 2-5 days at 37 °C.

**Expected results:**
Transformation efficiency up to $4.6 \times 10^3$ transformants/µg of DNA.