### Pseudomonas aeruginosa

**Microorganism**: *Pseudomonas aeruginosa*

**Cell type**: Bacteria, gram negative

**Molecules injected**: Plasmid DNA (pUC 18 with 1.8 kb insert in water)

**Growth medium**: LB medium

**Washing solution**: 300 mM sucrose

**Electroporation solution**: 300 mM sucrose

**Outgrowth medium**: LB medium

**Cuvette**: 2 mm gap width

**Reference**: Smith A.W. and Iglewski B.H. 1989 • Nucleic Acids Research 17, No. 24 • 10509

---

### Transformation Protocol

**Making electrocompetent cells:**

1. Grow cells in LB medium at 37 °C with shaking up to an O.D. 540 of 0.3-0.5.
2. Harvest by centrifugation (7,000 x g, 10 min, at 4 °C).
3. Wash pellet in the original volume with sucrose, centrifuge and wash again in ½ volume of washing solution.
4. Resuspend in 300 mM sucrose to a final concentration of 10¹¹ cells/ml, chill on ice for 30 minutes.

**Electroporation of cells:**

1. Add up to 5 µg plasmid DNA (1 µg/µl) to 40 µl of electrocompetent cells. Homogenize by gently mixing with pipette several times. Transfer mixture into a prechilled cuvette.
2. Wipe moisture from the cuvette and insert the cuvette into the device.
3. Electroporation:
   - **Mode**: Prokaryotes “O”
   - **Voltage (V)**: 1,600 V
   - **Time constant (τ)**: 5 ms
4. Add 1 ml LB medium, transfer to a sterile tube containing additional 2 ml of LB. Incubate 2 hours at 37 °C with shaking.
5. Plate on selective LB plates.

**Expected results:**
Transformation efficiency up to 10⁷ transformants/µg of DNA.