### Transformation Protocol

**Microorganism**  
*Nocardia asteroides*

**Cell type**  
Bacteria, gram positive

**Molecules injected**  
Plasmid DNA (in TE buffer)

**Growth medium**  
N2 medium (20 g/l glucose, 2 g/l yeast extract, 4 g/l beef extract, 6 g/l tryptone, 2 g/l NaCl, 10 g/l glycine)

**Washing solution**  
Distilled water

**Electroporation solution**  
0.3 M sucrose, 15% glycerol

**Outgrowth medium**  
N2 medium (without antibiotics)

**Cuvette**  
2 mm gap width

**Reference**  
Yao W., et al. 1994 • Current Microbiology 29 • 223-227

### Making electrocompeotent cells:

1. Inoculate a fresh overnight culture of bacteria into N2 medium. Grow cells at 28 °C with shaking to an O.D.\textsubscript{600} of 0.7.
2. Harvest by centrifugation.
3. Wash twice with ½ volume of distilled water.
4. Resuspend in a final volume of 1/20 of the original volume of electroporation solution.

### Electroporation of cells:

1. Add 2 µl (0.1 µg) plasmid DNA to 50 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:

<table>
<thead>
<tr>
<th>Mode</th>
<th>Prokaryotes &quot;O&quot;</th>
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<tbody>
<tr>
<td>Voltage (V)</td>
<td>2,500 V</td>
</tr>
<tr>
<td>Time constant (τ)</td>
<td>5 ms</td>
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4. Add 1 ml N2 medium and incubate for 2 hours at 30 °C.
5. Plate cells on selective YEME medium with a 3 ml YEME soft agar (0.4%) overlayment.

### Expected results:

Transformation efficiency up to 8 x 10⁴ transformants/µg of DNA.