# Dictyostelium discoideum

Multiporator/Eppendorf Eporator®

Transformation Protocol

**Protocol No. 4308 915.543 –08/2003**

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| **Microorganism** | *Dictyostelium discoideum* Ax-2 |
| **Cell type** | Cellular slime mould |
| **Molecules injected** | Plasmid DNA |
| **Growth medium** | Axenic medium supplemented with 1 mM folate |
| **Washing solution** | 10 mM $K_2HPO_4/KH_2PO_4$, 50 mM sucrose, pH 6.2 (ice-cold, sterile) |
| **Electroporation solution** | 10 mM $K_2HPO_4/KH_2PO_4$, 50 mM sucrose, pH 6.2 (ice-cold, sterile) |
| **Outgrowth medium** | Axenic medium supplemented with 1 mM folate |
| **Cuvette** | 4 mm gap width |
| **Reference** | Weidenhaupt, M. et al ♦ 2000 ♦ European Journal of Biochemistry 267 ♦ 2062-2070 |

**Making electrocompetent cells:**

1. Grow *Dictyostelium discoideum* in shaken suspensions (170 rpm) or in Petri dishes at 21 °C in axenic medium.
2. Typically 5 x $10^7$ amoebae were harvested by centrifugation (1000 x g, 4 min, 4 °C).
3. Wash twice in 50 ml ice-cold sterile electroporation buffer.
4. Resuspend in electroporation buffer at 1 x $10^7$ cells/ml.

**Electroporation of cells:**

1. Mix 4 x $10^6$ cells on ice with DNA. Homogenize by gently mixing with pipette several times. Transfer mixture into a prechilled, sterile cuvette and incubate for 2 min on ice.
2. Wipe moisture from the cuvette and insert the cuvette into the device.
3. Electroporation:

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| **Mode** | Prokaryotes “O“ |
| **Voltage (V)** | 480 V |
| **Time constant (τ)** | 5 ms |

4. Incubate cells for 10 min on ice before suspend in 24 ml cold outgrowth medium.
5. Distribute in 24-well dishes and incubate at 21 °C in the dark. After 8 h, G418 was added at 20 µg/ml.

**Expected results:**

Groups of 10-50 amoebae issued from a transformed clone appear approx. 7 days after electroporation.

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