# Diaporthe perjuncta

Multiporator/Eppendorf Eporator®

Transformation Protocol

**Protocol No. 4308 915.544 –09/2003**

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| **Microorganism** | *Diaporthe perjuncta* |
| **Cell type** | Phytopathogenic fungus |
| **Molecules injected** | Virus RNA (*in vitro*-transcribed) from Diaporthe RNA virus |
| **Growth medium** | 2 % potato dextrose agar (PDA) |
| **Spheroplast solution** | Chitinase-cellulase mixture, dissolved in 1 M $MgSO_4$ |
| **Washing solution** | 1 M sorbitol; STC (1 M sorbitol, 50 mM Tris HCl (pH 8.0), 50 mM $CaCl_2$ |
| **Storage solution** | Mixture of: STC, PTC, DMSO (80:20:1) |
| | [PTC: 40 % PEG 6000, 50 mM Tris HCl (pH 8), 50 mM $CaCl_2$] |
| **Electroporation solution** | 1 M sorbitol (ice-cold) |
| **Outgrowth medium** | 1 g/l casein hydrolysate, 1 g/l yeast extract, 342 g/l sucrose, 16 g/l agar |
| **Cuvette** | 1 mm gap width |

**Reference** Moleleki, N. et al • 2003 • Applied and Environmental Microbiology 69 • 3952-3956

**Preparation of spheroplasts:**

1. Suspend ten-day-old mycelium in a chitinase/cellulase solution overnight at room temperature.
2. Gravity filter the mycelium through a 120 µm pore size nylon mesh.
3. Add an equal volume of 1 M sorbitol (ice-cold) and centrifuge at 2,700 x g for 5 min at 4 °C.
4. Wash again with 1 M sorbitol, then resuspend pellet in 500 µl STC and centrifuge as above.
5. Resuspend spheroplasts in storage solution and use immediately or store at – 80 °C.

**Electroporation of spheroplasts:**

1. Resuspend spheroplasts in 85 µl of ice-cold 1 M sorbitol and place on ice.
2. Add RNase inhibitor (12 U) to a 15 µl transcription reaction mixture containing the in vitro-produced viral RNA transcripts.
3. Mix the viral transcripts with the spheroplast suspension and place on ice for 5 min.
4. Transfer mixture to a pre-chilled curette.
5. Wipe moisture from the cuvette and insert the cuvette into the device.
6. Electroporation:

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| **Mode** | Prokaryotes “O“ |
| **Voltage (V)** | 1,800 V |
| **Time constant (τ)** | 5 ms |
| **No. of pulses (n)** | 3 (manually) |

7. Add 750 µl 1 M sorbitol (ice-cold) to the cuvette and place on ice for 10 min.
8. Pipette portions of 200 µl into 90-mm diameter petri dishes.
9. Add outgrowth medium at 48 °C to each petri dish and place plates on a laminar flow bench until the agar solidifies. Seal the petri dishes with parafilm and incubate overnight on a bench top at room temperature.
10. Incubate at 25 °C for 48 h, then for 5-10 days on a bench top at room temperature (20-25 °C).

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