Transformation of Protoplasts

Note: Use at least 0.5-1 x 10^7 protoplasts for each transformation and perform the transformation steps on ice.

1. Add 100 µL of protoplast suspension to 13 x 100 mm test tubes.

2. Add 25 µL of DNA (5-10 µg PCR product of 10-15 µg linearized plasmid DNA in STC or Tris-EDTA (TE, 10 mM Tris.HCL/1 mM EDTA, pH 8.0)). Mix gently by rolling the tube. Incubate for 2-10 min. For co-transformation use 5-10 µg PCR product or 10-15 µg linearized plasmid DNA and 2-3 µg of linearized plasmid carrying a second selectable marker.

3. Add freshly prepared PEG in three aliquots of 200, 200 and 800 µL each, the last at room temperature. Mix well after each addition by rolling the tube. Incubate the tube on ice for 2-10 min after the first two additions and at room temperature after the last addition. Incubate for 2-10 min.

4. Dilute with 1 mL STC and plate for viability determination and Hygromycin B, Geneticin, or PPT selection as described below.

5. Include a no DNA control.

For determination of protoplast viability

6. Remove a 10 µL aliquot from one of the samples. Determine the concentration of the protoplast suspension with a hemacytometer and record.

7. Prepare dilutions from the same sample of 10^2-10^6/mL in STC.

8. Plate 100 µL aliquots of each dilution in molten regeneration medium by pouring approximately 20 mL of medium into a Petri plate, then adding protoplasts and swirling with the pipet tip to mix. Allow the medium to solidify, then incubate at 30 °C. Estimate protoplast viability as the number of colonies observed/the number of protoplasts plated. Viability of 5-20% is typical.

For Hygromycin B, Geneticin/G418, or Phosphothricin (PPY)/Bialaphos selection:

6. Plate 200-500 µL aliquots in 20 mL molten regeneration medium as described above. After overnight incubation at 30 °C, overlay with 10 mL or 1% agar containing Hygromycin B at 150 µh/mL, Geneticin (1000 µ/mL), or PPT (150 µg/mL). The final concentration of Hygromycin B, Geneticin, or PPT in the plate will be 50, 333, and 50 µg/mL, respectively. Use one plate/transformation as no overlay control.

7. Incubate plates at 30 °C; transformants generally appear after 3-7 days. Verification that hygromycin B-resistant colonies are indeed transformants requires additional genetic and molecular analyses.