Colletotrichum Transformation (Slower Method)
From the Hanau Lab

This protocol is based on one developed by the Hanau lab, which was later modified by Thon. The Thon modification makes the protocol much faster and easier to do, but it MAY reduce the efficiency of the transformation. The original protocol may give better results in some cases, especially if the number of transformants must be maximized. The main difference is that there is a non-selective overlay in this protocol, and that may allow more transformants to survive.
References: Panaccione et al., 1988, MPMI 1(3): 113-120; Rasmussen et al., 1992, MGG 235: 74-80

1. If protoplasts are frozen, thaw them gently on ice.
2. Mix your transforming DNA (1-3 micrograms)[1] in 5 microliters of water with 2 microliters of 50 mM spermidine and 5 microliters of 2.5 mg/ml stock of heparin.[2] Leave on ice for 30 minutes.
3. Add protoplasts (100 microliters) to the DNA mixture, mix gently, and incubate on ice for 30 minutes.
4. Add 1 ml of PEG solution (40% PEG 4000, 0.6M KCl, 50 mM Tris-HCl pH 8.0, 50 mM $CaCl_2$) slowly[3], drop by drop with mixing, to the protoplasts. Leave at room temperature for 20 minutes.
5. Suspend the mixture in 3 ml of molten regeneration medium (below) containing 0.6 % agar. Overlay onto a bottom agar plate containing 1.5% agar.

FOOTNOTES

1. Linearized DNA usually gives better transformation efficiencies than circular.
2. These may be expendable, it has been suggested that they stabilize the DNA and thus increase the transformation efficiency.

3. PEG is very toxic to protoplasts, thus the suggestion to add very slowly. In the original protocol of Pananccione, the PEG was removed by aspiration and replaced with 300 microliters of STC before adding the agar, but this may not be necessary since the PEG becomes quite diluted by the agar.

Regeneration Media BOTTOM AGAR (from protocol of Bob Hanau)

Ingredient	Amount	Final Conc.
Water (NANO pure)	160 ml	
Sucrose	68.4 g	1 M
Yeast Extract 0.1%	0.2 g	0.10%
Casein Hydrolysate (enzymatic)	0.1 g	0.05%
Casein Hydrolysate (acid)	0.1 g	0.05%
Bacto Agar	3.0 g	1.50%

To avoid boilovers in the autoclave, prepare in a 1 Liter Erlenmeyer flask and completely dissolve the sucrose before autoclaving.
Keep molten at 45°C-48°C before use.

Add Hygromycin just to the bottom agar just before using the media (final conc. of Hyg B is 250 mg/ml)