Agrobacteria Meditated Transformation Protocol

Transforming *Agrobacteria* -

1. Add 1 µg of DNA to the frozen agrobacteria stock cells

2. Thaw the cells by incubating the tubes in a 37 °C water bath for 5 min.

3. Add 1ml of LB medium to the tube and incubate at 28 °C for 2-4 h with gentle shaking. This period allows the bacteria to express the antibiotic resistance genes.

4. Spread the cells on a LB agar plate containing appropriate antibiotic selection. Incubate the plate at 28 °C. Transformed colonies appear in 2-3 days.

Transforming *Ganoderma* –

1. Grow *Agrobacteria* cells overnight in MM.

2. Pellet the bacterial cells by centrifugation. Discard the LB media. Resuspend in 5 ml IM with antibiotics. Transfer 200 to 400 µl of the suspension into 5 ml IM with AS (the OD_{600} of the dilution must be 0.1-0.15). Grow overnight at 28°C (OD_{600} ~ 0.5-0.8). Note: the use of AS at this step increases the probability of multiple insertions in the genome, but can increase transformation efficiency.

3. At the same time as beginning Step 2, inoculate blended *Ganoderma* in 100 ml liquid CYM at room temp (RT) in the dark for 24 hours.

4. In a 50 ml sterile conical tube, mix 5 ml *Ganoderma* culture with 5 ml *Agrobacteria* culture and leave at RT for 20-30 min at natural light, dark might be better.

5. Place cellulose nitrate (CN) membrane on IM plates.

6. Plate 500 µl of the *Agrobacteria* and *Ganoderma* mixture onto CN discs and spread with a pipette tip covering the entire membrane disc.

7. Incubate the CN membrane plates at 20-22°C in the dark for 48 h. (time is crucial for efficiency). Lift off the CN membranes and place on CYM selection plates.

8. Incubate at 22-25°C until colonies appear.

9. Transfer the transformants onto the same CYM selection plates

Minimal Media (MM) -

10 mM K2HPO4, 10 mM KH2PO4, 2.5 mM NaCl, 2 mM MgSO4*7H2O, 0.7 mM CaCl2, 9 µM FeSO4*7H2O, 4 mM (NH4)2SO4, 10 mM glucose, pH 7.0

To make 1L of Minimal Media:

1.75g K2HPO4
1.35g KH2PO4
0.15g NaCl
0.50g MgSO4*7H20
0.08g CaCl2
0.0025g FeSO4*7H2O
0.50g (NH4)2SO4
1.80g glucose

Fill to 1L, bring to pH 7.0 if necessary

Induction Media (IM) -

MM containing 0.5% (w/v) glycerol, 200 µM aceto-syringone (AS), 40 mM 2-(N morpholino) ethanesulfonicacid (MES), pH 5.3

To make 1L of Induction Media:

Follow steps to make minimal media and add 5 mL glycerol before autoclaving and the following after cooling from autoclave.

0.04g aceto-syringone (AS)
7.80g 2-(N morpholino) ethanesulfonicacid (MES)

Bring to pH 5.3 using HCl