TRANSFORMATION INTO AGROBACTERIUM

Making competent cells

1. Set up culture in 5 ml LB with 10 μl Rifampicin (50 $mgml^{-1}$) – final conc. 100 μg/ml – at 28°C for 2 days.

2. Sub-culture into 500 ml LB with 1 ml Rifampicin (50 $mgml^{-1}$) and incubate at 28°C overnight.

3. Chill rotor, 2L 1 mM HEPES (pH 7.0) and 500 ml 10% glycerol in 1 mM HEPES (pH 7.0) overnight.

4. Measure OD_{600} of overnight culture and when value = 0.5, place on ice for 30'.

5. Spin at 4000 g for 20' in a cold rotor.

6. Resuspend cells in equal volume (500 ml) ice cold 1 mM HEPES (pH 7.0)

7. Spin at 4000 g for 20' and resuspend in 250 ml ice cold 1 mM HEPES (pH 7.0).

8. Spin as above. Wash pellet in 50 ml 10% glycerol in 1 mM HEPES (pH 7.0).

9. Spin again and resuspend pellet in 2.5 ml 10% glycerol in 1 mM HEPES (pH 7.0).

10. Aliquot in 200 μl lots, freeze in liquid nitrogen and store at –80°C.

Electroporation into Agrobacterium

1. Prepare electroporation equipment as per routine transformation.

2. Set machine as follows :

Capacitance 25μF, resistance 600 Ω, field strength 2.5$KVcm^{-1}$, vol – MAX

3. Add 1-5 μl of binary vector to competent cells, mix gently and add to cuvette.

4. Apply current and immediately add 1 ml LB (NOT SOC). Leave to recover at 28°C for 3 h with gentle shaking (150rpm).

5. Spin cells down at 5K for 5' and resuspend in 100 μl LB.

6. Plate out on Rifampicin (50 $\mu g ml^{-1}$) and Kanamycin (50 $\mu g ml^{-1}$) plates and leave at 28°C for 3 days. Any colonies that grow should contain the binary vector conferring the Kan resistance.