LSOP Title	Protoplast Transfection
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Version	1.1
Location	UQ Node/Centre-wide
Policy/Procedure Link	
Risk Assessments	
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1.0 Scope

This procedure covers protoplast transfection.

2.0 Definitions

DNA - Deoxyribonucleic acid

PEG – Polyethylene glycol

MES - 2-(N-morpholino)ethanesulfonic acid agar

NaCl – Sodium Chloride

$CaCl_2$ – Calcium Chloride

KCl – Potassium Chloride

3.0 Materials and Equipment

1. DNA
2. Protoplasts
3. PEG, W1 & W5 solutions (see appendix)
4. Centrifuge
5. Pipette & Pipette Tips
6. 1.5mL tubes

7. Aspirator
8. 96 well plate

4.0 Prescribed Actions

DNA-PEG–calcium transfection (Less than 1 h for 20 samples)

1. Add 10µl DNA (10–20µg of plasmid DNA of 5–10 kb in size) to 1.5ml tube
2. Add 100µl protoplasts (2x104 protoplasts) and mix gently.
3. Add 110µl of PEG solution (see appendix for solutions), and then mix completely by gently tapping and inverting the tube.
4. Incubate the transfection mixture at room temperature for up to 15 minutes.
5. Dilute the transfection mixture with 400–440 ml W5 solution at room temperature and mix well by gently rocking or inverting the tube to stop the transfection process.
6. Centrifuge at 100g for 2 min at room temperature using a bench-top centrifuge and remove supernatant with an aspirator.
7. Re-suspend protoplasts gently with 1 ml WI solution and transfer to the 6 well plate.
8. Keep cells in the dark for 12-24 hours before performing further experiments.

5.0 Appendix

Solutions:

PEG-Solution: (10ml)

- 20–40% (wt/vol) PEG4000 in ddH2O (3g = 30% - this is the concentration I use with best results)
- 0.2 M mannitol (0.36434g)
- 100 mM $CaCl_2$. (0.11098g)

CRITICAL Prepare PEG solution at least 1 h before transfection to completely dissolve PEG. The PEG solution can be stored at room temperature and used within 5 d. However, freshly prepared PEG solution gives much better protoplast transfection efficiency. Do not autoclave PEG solution.

<u>W5 Solution</u>

- 2 mM MES (pH 5.7)

- 154 mM NaCl

- 125 mM $CaCl_2$
- 5 mM KCl

<u>WI solution</u>

- 4 mM MES (pH 5.7)
- 0.5 M mannitol
- 20 mM KCl.