LSOP Title	Protoplast Isolation
LSOP No.	LSOP04
Version	1.1
Location	UQ Node/Centre-wide
Policy/Procedure Link	*UQ- Equipment* *UQ -waste* *OGTR*
Risk Assessment	
Approved by	*Milos Tanurdzic*
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1.0 Scope

This procedure outlines how to isolate Arabidopsis protoplasts including how to create protoplast isolation solutions.

This SOP does not cover how to analyse or use protoplasts in assays.

2.0 Definitions

Col-0 – Columbia-0 *Arabidopsis*

Ler – Landsberg erecta *Arabidopsis*

KCl – Potassium Chloride

$CaCl_2$ – Calcium Chloride

NaOH – Sodium Hydroxide

MES – a type of selective medium (basal salt)

$MgCl_2$ – Magnesium Chloride

ppm – plant preservative mixture

3.0 Materials and Equipment

1. Tweezers
2. Red Electrical Tape
3. Scalpel
4. Petri Dish
5. Platform Shaker
6. Microscope
7. Falcon Tubes
8. Centrifuge
9. Yellow 50µm CellTrics filter
10. Pipette and Pipette Tips

4.0 Prescribed Actions

1. Using a tweezer, take three-five leaves from Col-0 and three-five leaves from Ler (3-5 weeks old)
2. Stabilise upper epidermis to tape (red electric tape)
3. Cut around the leaf as close to the tissue as possible
4. Make very light incisions around vasculature near the petiole: one horizontal incision where the leaf meets the petiole and two horizontal incisions either side of the primary vein – this prevents the petiole being taken up with the tape
5. Place another piece of tape onto the lower epidermis, lightly press down on to the tape to ensure the entire surface of the lower epidermis is fixed to the tape
6. Pull the tape fixed to the lower epidermis away from the leaf – holding the petiole for support (peeling away the lower epidermis)

7. Transfer the peeled leaves to a petri dish containing 10ml of enzyme solution and 10µl of plant preservative mixture (ppm).
8. Shake for 60 minutes on a platform shaker at 40rpm until protoplasts are released.

9. Check presence of protoplasts with microscope (if solution is green you probably don't need to do this)
10. Filter solution using yellow 50µm CellTrics filter into 15ml falcon tubes

    NB: Blow first part of solution through – then the remainder of the solution will flow through

11. Centrifuge protoplasts for 5 minutes at 100RCP (room temperature, acceleration & deceleration: 3)

12. Remove supernatant and wash with 10ml of enzyme buffer solution (count cells before next step)

13. Centrifuge protoplasts for 10 minutes at 100RCP (room temperature, acceleration & deceleration: 3)

14. Remove supernatant and resuspend pellet in MMg solution to a final concentration of 2 to the $5x10^5$ cells/ml^{-1}
15. Store cells in 15ml falcon tubes at -4°c, store horizontally to prevent cells sinking to the bottom of the tube and prevent hypoxia

See appendix for solutions

5.0 Appendix

Solutions:

Enzyme buffer solution (500ml):

1. 400 mM mannitol (36.5 g)
2. 20 mM KCl (0.76 g)
3. 10 mM $CaCl_2$ (0.74 g)
4. 20 mM MES (1.95 g)
5. pH 5.7 (adjusted with 1 M NaOH)
6. Autoclave
7. 0.5% ppm (2.5ml)

Enzyme cocktail (100ml):

8. Cellulase 1% (1 g)
9. Macerozyme 0.25% (0.25 g)
10. Adjust to 100 ml with enzyme buffer solution
11. Filter the solution (0.45 µm)
12. Aliquot by 12 ml into 15ml falcon tubes and store at -20°C

MMg Solution (500ml):

13. 15 mM $MgCl_2$ (1.52 g) （0.09%）
14. 4 mM MES (0.4 g)
15. 400 mM mannitol (36.5 g)
16. pH 5.7 (adjusted with 1 M NaOH)
17. Autoclave
18. 0.5% ppm (2.5ml)