Arabidopsis protoplast isolation and transformation (from Niyogi lab Sept 2005)

<Reagents>

100mM MES (3.9g in 100ml pH to 5.7)
10%w/v BSA (1g in 10 ml)
0.8M Mannitol (29.14g in 200ml)
5M NaCl
1M KCl, $CaCl_2$, $MgCl_2$

W5 solution (250ml)

7.7ml	5M NaCl
31.25ml	1M $CaCl_2$
1.25ml	1M KCl
5ml	100mM MES
204.8ml	dH_2O

MMg (100ml)

50ml	0.8M Mannitol
1.5ml	1M $MgCl_2$
4ml	100mM MES

Enzyme Solution (50ml)

0.5g	Cellulase (Karlan)
130mg	Macerozyme (Karlan)
25ml	0.8M Mannitol
1ml	1M KCl
10ml	100mM MES
13.5ml	dH_2O

Dissolve completely, cover with parafilm, heat at 55°C for 10 minutes, cool to RT.
When cool add:

0.5ml	1M $CaCl_2$
50mg	BSA

40% PEG (10ml)

4g	PEG 4000 (Fluka 81240)**important that the brand be correct
3ml	dH_2O
2.5ml	0.8M Mannitol
1ml	1M $CaCl_2$

<Preparation>

Autoclave:
funnels
50 or 75 μm nylon filter (large enough to fit in funnel)
small beaker
MMg
W5--then chill to 4°C

<Protoplast preparation>

1. Cut leaves into ~1mm wide strips. Use a very sharp scalpel, cut leaves in sterile water and blot lightly before placing in enzyme solution.

2. Place leaves into 15-100ml enzyme solution. Cut leaves until enzyme solution is relatively full.
Volumes are scalable depending on how many protoplasts you need).

3. Vacuum infiltrate leaves by exposing beaker to vacuum until bubbling appears and repeat.

4. Shake at low (~80 rpm) speed in dark on shaker for 2-3 hours.

5. Increase speed slightly (~140 rpm) for 1 minute.

6. Filter digested leaves through mesh in funnel into falcon tube.
Treat very gently from here on out.

7. Centrifuge at 200xg for 2 min.

8. Carefully remove supernatant being sure not to uncover the pellet.

9. Resuspend in 10-20ml cold W5 solution (depending on starting volume).
Add W5 slowly down the side of the tube and very gently resuspend with a pasteur pipet.

10. Repeat steps 9-11.

11. Incubate on ice for 30 min.

12. Repeat steps 9 and 10.

13. Resuspend in 1ml MMg.

14. Count cells and dilute to $2x10^5$ cells/ml.
Protoplasts will last several days at 4°C.

<Transformation>

1. To 100µl protoplasts, in a microfuge tube, add 10µl plasmid DNA (~1µg/µl) and 110µl 40% PEG solution.
If using 35s::gene::GFP, be sure to include 35s::GFP and water only controls. Add and mix gently.

2. Incubate for 30min at RT.

3. Dilute with 2xvol (440µl) W5 solution.
Add dropwise, slowly. W5 should now be RT. Mix gently.

4. Centrifuge 200xg 2 min.

5. Remove supernatant.

6. Resuspend in 1ml W5.

7. Incubate O/N in the light.

8. Observe by microscopy.