PEG Mediated Transformation of *Arabidopsis thaliana (var. Columbia)* Cell Suspensions Protoplasts

1. Use cells 3 day after sub cultivation
2. Split the cells in 2 tubes (each 10 ml), Spin the cells at 400 g for 5 min.
3. Wash once with wall digestion buffer without enzymes (2 x 10 ml).
4. Re-suspend each pellet in digestion solution (2 x 7 ml) and dispense each into a Petri dish.
5. OPTIONs for digestion:
    a. Digest for 6 hrs in the dark, ~24°C, shaking 50 rpm. (This is what Caterina does).
    b. Incubate at 26°C in the dark overnight (~24 hrs) shaking at 50 rpm.
6. If using non-"turbo cells", filter protoplasts through a sterile 70 - 100 µM cell culture sieve onto a 10 Ø cm dish by gravity filtration (do not force cells through!). TURBO CELLS do not have to be filtered.
7. Collect protoplasts in 2 tubes by centrifugation at 100 g for 5 min at RT.
8. Wash once in wall digestion solution without enzymes (2 x 10 ml).
9. Centrifuge 100 g for 5 min.
10. Remove supernatant and dissolve pellet in remaining solution, add W5 solution up to 10 ml total volume, mix gently yet firmly.
11. Repeat step 9. **If you order protoplasts, your cells will be at this step, in W5, at 4°C in the dark.**
12. **Take an aliquot for counting (20 – 100 µl),** store the rest for 20-30 min or longer in the dark at 4°C (refrigerator). Protoplasts can be stored overnight at 4°C in W5 in the dark and be transfected the next day perfectly fine.
13. **Calculate the concentration you would like to have.** Count all cells that are spherical or near spherical or measure with a cytometer. Record the concentration and the total volume of cells. Standard 14 mL round bottom transfections use of ~3.5±0.5 x 10^6 pps mL^{-1}. **This means we *typically* resuspend the cells in 5mL MM** after decanting when in W5, and this ***usually* gives us the ~3.5±0.5 x 10^6 pps mL^{-1} concentration**.
14. For experiments using PEG 1500:
    a. **Centrifuge 100 g for 5 min at RT, decant W5 supernatant** and **resuspend pellet in MM** solution to obtain the desired density. As mentioned above, that **means usually 5mL. See step 10 above.**
    b. **Let protos sit for 20-30 min in MM (and in the residual W5 salts).**
    c. **Add 4 - 16 (max 40 µg) of plasmid DNA in volume of 40 µl** (this minimum volume is critical to superb transfection results). Usually, this is prepared in advance or during the MM incubation set above.
    d. **Distribute 125 µl of protoplasts into tubes**,
    e. **Wait 2 to 5 minutes.** Longer does not seem to severely affect the transfection process, but we usually don't wait longer than ~10min.
    f. **Add 125 µl PEG 1500 solution, mix IMMEDIATELY and well** and observe that the PEG is completely mixed in.

g. **Keep mixing every 2 min for a total incubation time of 6 min.** You should have mixed 3 to 4 times.

h. Add 125 µl of MM to each tube, mix thoroughly.

i. **Add 1.0mL** (to 2.0 mL) **K3 and done!** All facility transfections get 1mL K3. NOTE: 30 µL Base-transfections can be incubated in K3, i.e. 250 – 350 µL). The *cells do not need* to be centrifuged/washed, i.e. steps ii and iii). The recommended alternative incubation medium is WI.

ii. OPTIONAL ONLY. Alternatively, centrifuge 50 g for 5 min at RT. and resuspend pellet in 1 mL of K3 solution.

i. Incubate at 26°C in the dark for 6 to 20 hours. Free GFP is usually well expressed and fluorescent after 6 hrs.

Options and notes

The method is now so easy and straightforward that not doing your own transfections is a shame! We will train you and you can unleash your transfection power!

Base-Transfection (BTf)

Everything is based on a 30uL Base-Transfection (BTf), 2mL Eppis or 96-Well plates.

30µL protos (at 3.5-4,0 x10^6 cells/mL)
DNA in 9-10µL (max 10µg, optimal 1 - 4µg)
30µL PEG1500
30µL MM
350µL K3.
= 450µL

Recommended Transfections

Currently we recommend 60uL (2x BTf) in 2mL Eppis or 96-well Plates.

60µL protos (at 3.5-4,0 x10^6 cells/mL)
DNA in 20µL (max 20µg, optimal 2 - 8µg)
60µL PEG1500
60µL MM
700µL K3.
= 900µL

The "Large Scale" Tfs which Caterina does (~ 4x BTf), looks like this:

125µL protos (at 3.5-4,0 x10^6 cells/mL)
DNA in 40µL (max 40µg, optimal 4 - 16µg)
125µL PEG1500
125µL MM
1000µL K3.
= 1415µL

*this document should be error free. If you find any, please contact us right away.

References:

The protoplast transfection protocol beginning Feb. 2017 is currently published here:
2in1 Vectors Improve In Planta BiFC and FRET Analyses. Mehlhorn DG, Wallmeroth N, Berendzen KW, Grefen C., Methods Mol Biol. 2018;1691:139-158.
doi: 10.1007/978-1-4939-7389-7_11. PMID: 29043675

First publication with micro protocol and our cell culture:
Berendzen KW, Böhmer M, Wallmeroth N, Peter S, Vesić M, Zhou Y, Tiesler FK, Schleifenbaum F, Harter K. Screening for in planta protein-protein interactions combining bimolecular fluorescence complementation with flow cytometry. Plant Methods. 2012 Jul 12;8(1):25.

Transfections performed *before* Feb.2017 cite:
Schütze K, Harter K, Chaban C. Bimolecular fluorescence complementation (BiFC) to study protein-protein interactions in living plant cells. Methods Mol Biol. 2009;479:189-202. doi: 10.1007/978-1-59745-289-2_12.

Historical reference:
Negrutiu, I., Shillito,R.D., Potrykus, I., Biasini,G. and Sala,F. Hybrid genes in the analysis of transformation conditions I. Setting up a simple method for direct gene transfer in plant protoplasts. Plant Mol. Biol., 8, 363-373, 1987.

Solutions:

- **Wall digestion solution:**

for 15 ml:

1% Cellulase R10	0,150 g
0,25% Macerozyme R10	0,037 g
8 mM $CaCl_2$ (8 mM $CaCl_2 \cdot 2H_2O$)	0,013 g (0,018 g)
0,4 M Mannitol	1,093 g

pH = 5,5
sterile filtrate

- **Wall digestion solution without enzymes:**

for 40 ml:

8 mM $CaCl_2 \cdot 2H_2O$	0,048 g
0,4 M Mannitol	2,910 g

pH = 5,5
sterile filtrate

- **W5 solution**

for 1 liter:

154 mM NaCl	8,900 g
125 mM $CaCl_2$ (125 mM $CaCl_2 \cdot 2H_2O$)	13,873 g (= 18,377 g)
5 mM KCl	0,373 g
5 mM Glucose	0,990 g

pH = 5.8 - 6.0
autoclave

- **MM solution** for 1 liter:

0.4 M Mannitol	72.8688 g/l
5 mM MES	0.976 g/l
pH = 6,0	
autoclave or sterile filter	

- **PEG 1500 solution** makes ~26 mL:

Mannitol	1,275 g
$Ca(NO_3)_2 \times 4H_2O$	0,413 g
-> 17.5 ml ddH2O	
40% (w/v) PEG (1500) Roth	10g
pH ~6 with KOH (you may not have to adjust the pH, it should be around 6; maybe add 1mM MES, i.e. 0,051g for 26mL)	
sterile filter (0.22 µM)	

- **WI solution**

0,5 M Mannitol
4 mM MES
20 mM KCl
pH 5,7 with KOH or HCl
autoclave or sterile filter

********** * K3 Medium * **********

- **K3 solution**
  for 100 ml:
  10 ml macro stock

for 1 liter macro stock:
1,5 g $NaH_2PO_4 \bullet H_2O$
9,0 g $CaCl_2 \bullet 2H_2O$
25 g KNO_3
2,5 g NH_4NO_3
1,34 g $(NH_4)_2SO_4$
2,5 g $MgSO_4 \bullet 7H_2O$
add H_2O up 1 liter
autoclave

  0,1 ml micro stock

for 100 ml micro stock:
75 mg KI
300 mg H_3BO_3
1 g $MnSO_4 \bullet 7H_2O$ (0,6 g $MnSO_4 \bullet H_2O$)
200 mg $ZnSO_4 \bullet 7H_2O$
25 mg $Na_2MoO_4 \bullet 2H_2O$
2,5 mg $CuSO_4 \bullet 5H_2O$

2,5 mg $CoCl_2 \bullet 6H_2O$
add H_2O up 100 ml
sterile filter and freeze

0,1 ml vitamins stock

for 100 ml vitamins stock:
100 mg Nicotinic acid
100 mg Pyridoxin • HCl
1 g Thiamin • HCl
add H_2O up 100 ml
sterile filter and freeze

0,5 ml EDTA stock

for 1 liter EDTA stock:
7,46 g EDTA solve in 300 ml H_2O and cook
5,56 g $Fe(II)SO_4 \bullet 7H_2O$ solve in 300 ml H_2O and cook
mix and add H_2O up 1 liter
autoclave and keep in the dark

1 ml Ca-Phosphate stock

for 200 ml Ca-Phosphate stock:
1,26 g $CaHPO_4 \bullet 2H_2O$ solve in H_2O
add H_2O up 200 ml
pH = 3 with 25% HCl
autoclave and keep in the dark

10 mg Myo-Inositol
25 mg D(+)-Xylose
13,7 g Sucrose
pH = 5,6
sterile filter and freeze in aliquots

********** * END K3 Medium * **********