

Video Article

Efficient Polyethylene Glycol (PEG) Mediated Transformation of the Moss *Physcomitrella patens*

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Abstract

A simple and efficient method to transform *Physcomitrella patens* protoplasts is described. This method is adapted from protocols for *Physcomitrella* protonemal protoplast and *Arabidopsis mesophyll* protoplast transformation¹. Due to its capacity to undergo efficient mitotic homologous recombination, *Physcomitrella patens* has emerged as an important model system in recent years². This capacity allows high frequencies of gene targeting³⁻⁹, which is not seen in other model plants such as *Arabidopsis*. To take full advantage of this system, we need an effective and easy method to deliver DNA into moss cells. The most common ways to transform this moss are particle bombardment¹⁰ and PEG-mediated DNA uptake¹¹. Although particle bombardment can produce a high transformation efficiency¹², gene guns are not readily available to many laboratories and the protocol is difficult to standardize. On the other hand, PEG mediated transformation does not require specialized equipments, and can be performed in any laboratory with a sterile hood. Here, we show a simple and highly efficient method for transformation of moss protoplasts. This method can generate more than 120 transient transformants per microgram of DNA, which is an improvement from the most efficient protocol previously reported¹³. Because of its simplicity, efficiency, and reproducibility, this method can be applied to projects requiring large number of transformants as well as for routine transformation.

Video Link

The video component of this article can be found at <https://www.jove.com/video/2560/>

Protocol

1. Making the Protoplasts

1. Add 9 mL of 8% mannitol to a Petri dish.
2. Using a spatula, put 7 day old moss from 2-3 PPNH4 plates of 5- in the Petri dish containing mannitol.
3. Add 3 mL of 2% Driselase (Sigma D9515-25G) to the Petri dish.
4. Incubate the Petri dish at room temperature with gentle shaking for 1 hour.
5. Filter the protoplast suspension through a 100 µm mesh (BD Falcon 352350).
6. Spin the filtered suspension at 250 g for 5 minutes. Remove the supernatant.
7. Re-suspend the protoplasts very gently with 500 µL of 8% mannitol.
8. Add 9.5 mL 8% mannitol in the culture tube. Make sure protoplasts are fully suspended.
9. Repeat the filtration and re-suspension steps (1.6, 1.7 and 1.8) two more times.
10. Take 10 µL of the protoplast solution and count the protoplasts in a hemocytometer.
11. Multiply the number of protoplast in a 16 square area by 10,000 to obtain the number of protoplasts per mL (See Figure 1).

2. Transformation

1. Spin the protoplast suspension at 250 g for 5 minutes. Remove the supernatant.
2. Re-suspend protoplasts in MMg solution at the concentration of 1.6 million protoplasts / mL.
3. Incubate the protoplast suspension at room temperature for 20 minutes.
4. Add 600 µL of protoplast suspension into a culture tube containing 60 µg DNA. Swirl the tube gently.
5. Add 700 µL of PEG/Ca solution into the protoplast / DNA mixture. Swirl the tube gently until whole mixture is homogeneous.
6. Incubate the mixture at room temperature for 30 minutes.
7. During this waiting period, cover PRM-B plates with cellophane (80 mm diameter circles, A.A. Packaging Limited, UK); allow cellophane to hydrate on the plate surface for at least 5 min.
8. With a spatula, remove any air bubbles trapped between the cellophane and the plate.
9. Dilute the mixture with 3 mL of W5 solution.
10. Spin the mixture at 250 g for 5 minutes. Remove the supernatant.

11. Re-suspend protoplasts in melted 2 mL of PRM-T (before adding it to the protoplasts this solution can be kept liquid at 42°C). Plate 1 mL of re-suspend protoplasts per PRM-B plate covered by cellophane. Wrap the plates with Micropore (3M, Health Care). Keep the plates at 25°C in a growth chamber.
12. Alternatively, protoplasts can be re-suspended in 1 mL of liquid plating medium and plated 0.5 mL on a PRM-B plate covered with cellophane. This allows faster regeneration, but the number of regenerating plants is lower.
13. Growth chamber is set for a 16 hr. light and 8 hr. dark cycle.
14. Move the cellophane onto to a fresh selection plate 4 days after transformation.

3. Representative Results:

An example of transformation is shown in Figure 2. The DNA used in this example was a supercoiled 7.8 kb plasmid carrying a hygromycin resistant cassette. The amount of protoplasts plated was reduced to 50% for photography. The pictures were taken two weeks after the plants were moved to the selection plates. The transformation efficiency is not affected by the DNA concentration up to 60 μ g. Higher concentrations of DNA are detrimental to the efficiency of transformation. The data showed that this method delivers consistent result over a wide range of DNA amounts. This method can also generate stable transformants effectively as shown in Table 1; we can get about 4 stable transformants per microgram of linearized DNA, which is much higher than what was reported previously¹⁴.

We also tested the effect of heat shock on transformation efficiency by transforming a supercoiled plasmid carrying a gene for a fluorescent protein as a marker. The heat shock was carried out 10 minutes after room temperature incubation (within Step 2.6) by incubating the mixture in a 45°C water bath for 3 minutes. The mixture was incubated in a room temperature water bath immediately after heat shock for 17 min. Results were recorded 22 hours after transformation. The ratios of transformants obtained with and without heat shock were very similar (~25%), but we observed an adverse effect of heat shock on the viability of the protoplasts (data not shown).

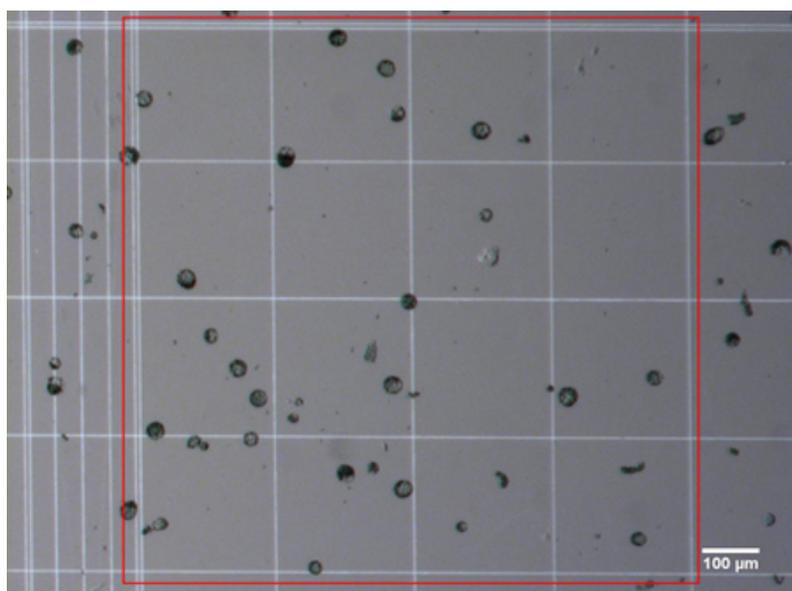


Figure 1. View of protoplasts in hemocytometer. The section needed for protoplast counting is indicated by a red square. Note the 16 squares required for counting protoplasts (see 1.11); the image shows a count of 24 protoplasts (240,000 cells/ mL).

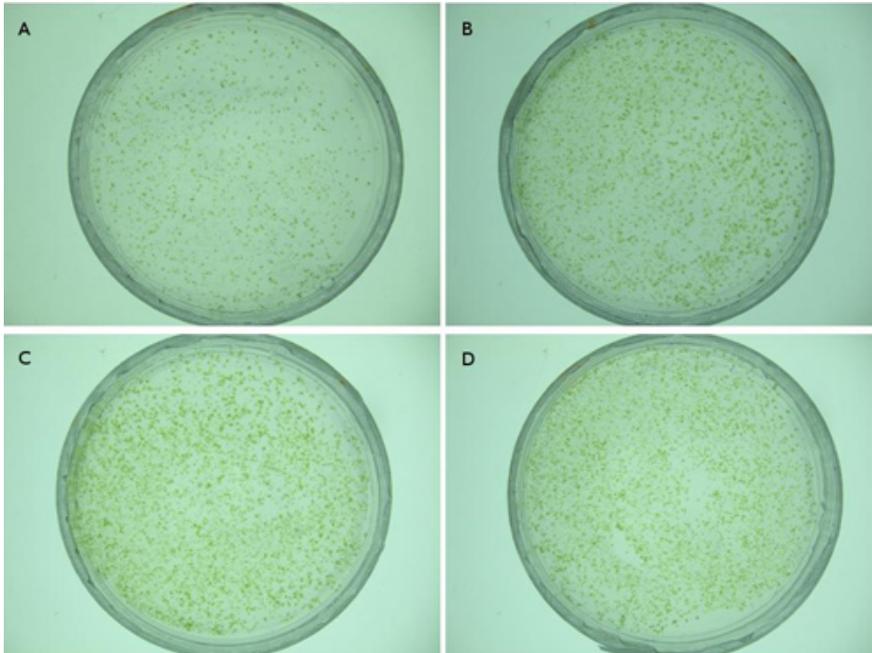


Figure 2. Pictures of 18 day old transient transformants. Protoplasts were transformed with the indicated amount of supercoiled plasmid. A: 15µg; B: 30µg; C: 60µg; D: 120µg.

Amount of DNA (µg)	Efficiency (transformants/µg DNA)
15, supercoiled plasmid	120±24
30, supercoiled plasmid	145±54
60, supercoiled plasmid	121±60
120, supercoiled plasmid	71±38
60, linearized plasmid	4±1

Table 1. Transformation efficiency at various DNA amounts.

Discussion

The method presented here enables a straightforward and consistent DNA transformation into *Physcomitrella patens* protoplasts. This method was originally developed for *Arabidopsis*¹, but here we demonstrated that it performs well with protoplasts of *Physcomitrella patens*, a simpler and different plant. Therefore, this method can be applied to other plant species with minor modifications. Possible modifications for optimization include protoplast concentration in MMg, incubation time in MMg and PEG/Ca solution. We choose to use 60 µg DNA in our routine experiments because the number of transformants increases linearly with the DNA concentration until this point (Table 1). We could get approximately 7000 transient transformants or 200 stable transformants on a single 60 µg DNA transformation, which allows for further screening and analysis of a large population of plants.

The transformed protoplasts can be either spread with liquid plating medium or PRM-T. Although the regeneration efficiency is lower when liquid plating medium is used, we choose this method when the protoplasts were transformed with supercoiled plasmids. As showed in Table 1, transformations done with supercoiled plasmid DNA generate many more transformants and thus, we can still obtain large numbers of plants with liquid plating medium. Furthermore, spreading protoplasts with liquid plating medium allows faster regeneration, which is important for experiments involving transient phenotypes, such as RNAi knock down experiments^{9,15}. In addition the absence of top agar allows for easy plant isolation for immunostaining and RT-PCR^{9,15}.

Disclosures

No conflicts of interest declared.

Acknowledgements

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