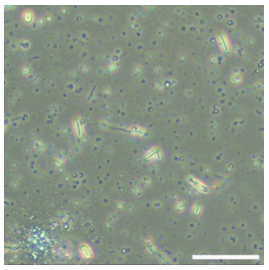
Sep 24, 2024

A small scale of *Fusarium oxysporum* protoplast generation



DOI
dx.doi.org/10.17504/protocols.io.4r3l226w3l1y/v1

Jun-Ze Zheng[1], Tao-Ho Chang[1]

[1]Program in Plant Health Care, Academy of Circular Economy, National Chung Hsing University

Tao-Ho Chang
Program in Plant Health Care, Academy of Circular Economy, N...

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Protocol Citation: Jun-Ze Zheng, Tao-Ho Chang 2024. A small scale of Fusarium oxysporum protoplast generation. **protocols.io https://dx.doi.org/10.17504/protocols.io.4r3l226w3l1y/v1**

Manuscript citation:

Protocol status: In development
We are still developing and optimizing this protocol

Created: October 02, 2023

Last Modified: September 24, 2024

Protocol Integer ID: 88643

Keywords: Fungus protoplast, Small scale, Protoplast generation

Funders Acknowledgement:
National Science and Technology Council, Taiwan
Grant ID: NSTC-112-2313-B-005-008-

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Abstract

The generation of fungal protoplasts is crucial for advancing fungal gene editing methods. We aim to implement the ribonucleoprotein (RNP) method for gene editing (Wang et al., 2018). Generating protoplasts is a demanding task that requires substantial resources to obtain an adequate amount for a single transformation reaction. The cost of cell wall degrading enzymes is the most significant expense in the protoplast generation process. In this study, we have developed a process for small-scale protoplast generation that not only reduces resource usage but also significantly cuts down the amount of cell wall degrading enzyme required.

Materials

Biomaterials: different species of *Fusarium oxysporum*
Growth medium: half-strength potato dextrose broth
Digestion buffer: 10 mg/mL Driselase and 15 mg/mL ß-glocanase dissolved in 0.8 M NaCl solution
SuTC stabiliser buffer: 20% sucrose, 10 mM $CaCl_2$,10 mM Tris-HCl, pH 7.5

Before start

The protocol for generating protoplasts on a small scale is for *Fusarium oxysporum* and is in development. We are open to other fungal species attempting the task and providing a report on their success or any modifications to the methods.

Fungus single spore isolation

1 Rinse *Fusarium oxysporum* fungal spores from the two-week-old culture plate with 7 mL distilled water, and filter the spore suspension with sterilized filter paper or 4 layers of Miracloth. 5m

2 Count the number of spores in spore suspension with hemocytometer. 5m

3 The 10^6 / mL spore suspenstion were added into the culture medium 50 mL half-strength PDB, and grow the culture in 28 ºC for 20-24 hours. 20h

Mycelium collection

4 Assemble sterilized collection tube and tissue filter column (Lot.: CDC25049A, FAVORGEN®Biotech Corp., Pingtung, Taiwan).

5 The 700 µL of germinated spores were added into the filter column.

6 1000 x g, 28°C 30s
Centrifuge to remove the ungerminated spores

7 Wash the mycelium with 700 µL of distilled water, and continue pipetting until the mycelium is suspended in the distilled water.

8 Centrifuge again to remove water and change new collection tubes.

Enzymes digestion

9 Add the digestion buffer (10 mg/mL Driselase and 15 mg/mL ß-glocanase dissolved in 0.8 M NaCl solution) 700 µL

10 Mix 10-20 rpm 03:00:00 in room temperature 3h

Protoplast isolation

11 Change collection tube to 2 mL tube for collecting protoplast.

12 5000 x g, 4°C 10m
Centrifuge the mixture, pelleting the protoplast to the centrifuge tubes.

13 Remaining the pellet of protoplast, remove the digestion buffer carefully.

14 Add 1000 µL of SuTC stabilizer buffer (20% sucrose, 10 mM $CaCl_2$,10 mM Tris-HCl, pH 7.5) for washing pellet.

15 centrifuge at 1000 xg for 5 min, and repeat wash process twice. 5m

16 dissolve pellet carefully in 500 µL SuTC buffer, and count protoplast concentration with hemocytometer.

17 Generally, the concentration of protoplast per column will be fall around 10^5 to 10^6 / mL.

Observation and following applications

18 The protoplasts are able to apply in CRISPR/Cas gene editing system or subject to cell staining for microscopy.

Protocol references

Wang, Q., Cobine, P. A. & Coleman, J. J. Efficient genome editing in Fusarium oxysporum based on CRISPR/Cas9 ribonucleoprotein complexes. *Fungal Genetics and Biology* **117**, 21–29 (2018).