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Aspergillus nidulans protoplast isolation for transfections

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1 Works for me dx.doi.org/10.17504/protocols.io.bhxrj7m6

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ABSTRACT

Modified *Aspergillus nidulans* protoplast isolation using Novozyme VinoTaste Pro as an enzyme source. Protocol from previously established method adapted to produce viable protoplasts for transfections from an inexpensive and commercially available cellulase/chitinase source.

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Szewczyk E, Nayak T, Oakley CE, Edgerton H, Xiong Y, Taheri-Talesh N, Osmani SA, Oakley BR. Fusion PCR and gene targeting in *Aspergillus nidulans*. *Nat Protoc*. 2006;1(6):3111-3120. doi:10.1038/nprot.2006.405

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IMAGE ATTRIBUTION

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MATERIALS

NAME	CATALOG #	VENDOR
VinoTaste Pro		
1.1M Potassium Hydroxide		
2M Potassium Chloride		
Citric Acid (Anhydrous)		

NAME	CATALOG #	VENDOR
Aspergillus Media (MM & CM)		
1.2M Sucrose Solution (filter sterile)		

Grow Overnight Culutre 15m

1 **Late afternoon-evening the day before**, grow scraped condispores (two arms from MM complete agar plate, ~ 1 x 10^8 total spores) in 30ml liquid CM, supplemented with pyridoxine and riboflavin, overnight at 25°C 18-20 hrs or 30°C 11-12 hrs on orbital shaker at 150rpm. Growth can be arrested at 4°C for an hour or two prior to protoplasting if needed.

Hyphae formation after 12 hours shaking and incubation at 30°C.

Prepare Protoplast Solution 50m

2 **An hour before protoplast isolation**, prepare 25ml fresh 2x PP Solution in 50ml conical tube as follows: 10m

- 13.7ml 2M KCL
- 480mg Citric Acid (Anhydrous)
- 6.4ml 1.1 M KOH
- 3.2g VinoTaste Pro (Novozymes)
- ~3.0ml ddH_20 (final volume 25ml)

3 Shake vigorously in 50ml conical tube. Filter sterilize through 125 or 250ml single use 0.22µm low-binding SFCA filter unit (or rinse CA filter with liquid CM prior to remove surfactant). Allow 30-40min to filter using house vacuum. During this time, harvest hyphae. 40m

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Harvest Hyphae & Cell Wall Digestion 2h 15m

4 Collect hypha over a fine mesh or sterile filter paper (or single use polyester tea-bag sprayed with 70% Ethanol). 5m

5 Using a heat sterilized spatula, wash with 10ml CM, gather hyphae and place into 3ml of CM in a 15ml conical tube; when completed, note volume. 5m

6 Add an equal volume of room temp 2x PP solution. 2m

7 Digest cell wall in shaking incubator set at 30°C 80rpm for approximately 2 hours (28°C; 2-3 hours), checking the progress under a microscope. 2h

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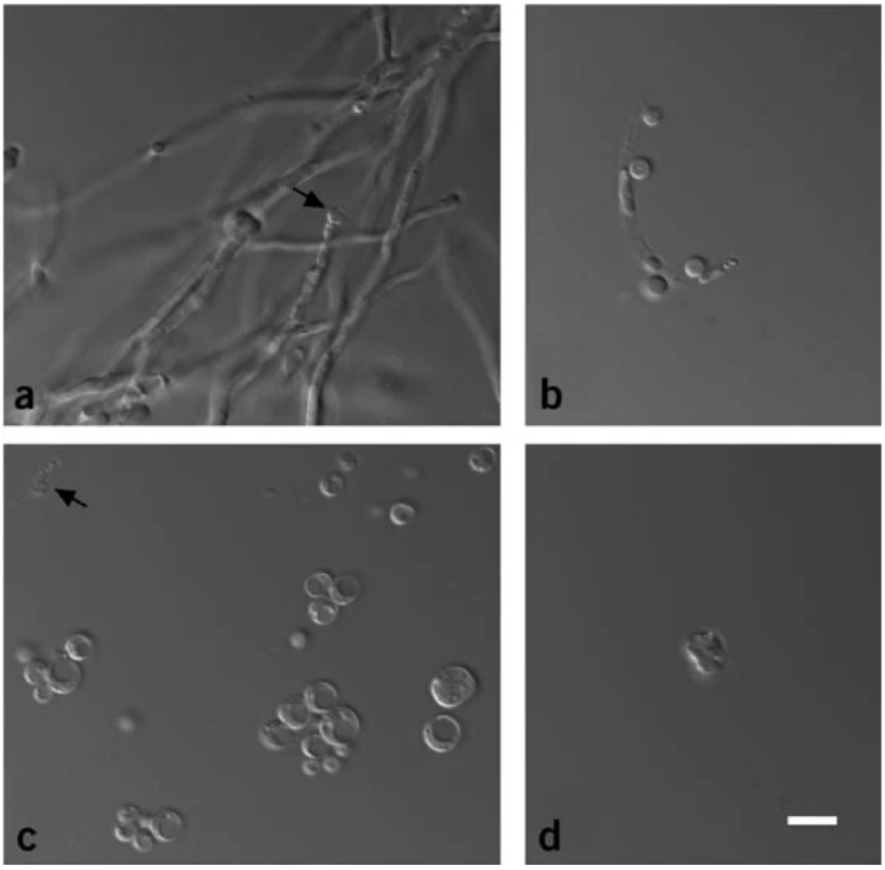

Hyphal mat, pre-digestion (a); protoplasts and undigested hypha (b); collected protoplasts after running digested prep over sucrose cushion, vacuole formation is typical at this step, arrow: hyphal remnant (c); fused protoplasts after PEG treatment (d). Bar:10µm. From Szewczyk *et al.* 2006 Nat. Protoc.1(6):3111-3120.

Isolate Protoplasts 45m

8 When satisfactorily digested, filter undigested material and hyphal clusters with 70µm MACS Smart Strainer (catalog number 130-110-916) into 15ml tube, rinse mesh and remaining residue with 2ml CM. 5m

9 Slowly underlay 1-2ml filter sterile 1.2M sucrose solution using a sterile 9-inch Pasteur pipette. 2m

10 Spin at 1800 x g for 10 min at 4°C. 12m

11 Collect free protoplasts into 1.5ml Eppendorf tube at the interface with sterile Pasteur pipette. Place on Ice in 2.0ml Eppendorf tube. 5m

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12 Add cold STC (1.2M sorbitol, 10mM Tris pH7.5, 10mM $CaCl_2$) to 1.5ml and mix by very gentle inversion 3 times, spin at 1800 x g for 10min at 4°C. 12m

13 Carefully remove supernatant and resuspend cell pellet in cold STC 300-500µl STC for 3-5 transfections. 2m

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