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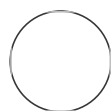
**Protocol status:** Working  
 We use this collection and it's working

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# Agrobacterium-mediated transformation of the chytrid fungus *Spizellomyces punctatus* (Sp)

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## ABSTRACT

This is a collection of protocols for *Agrobacterium*-mediated transformation of the chytrid fungus *Spizellomyces punctatus*.

## ATTACHMENTS

[Spizellomyces transformation steps.pdf](#) [Spizellomyces transformation video.1.mp4](#) [Spizellomyces transformation video transcript.pdf](#)

## GUIDELINES

Please thoroughly read through each protocol entry before starting, including the materials, guidelines, and warnings

## MATERIALS TEXT

### Section 1: Dilute Salts Stock Solution I (10x)

- [M] 0.5 millimolar (mM)  $\text{KH}_2\text{PO}_4$  ( 68.05 g )  
 Potassium phosphate monobasic **Sigma Aldrich Catalog #P0662-1KG**
- [M] 0.5 millimolar (mM)  $\text{KH}_2\text{PO}_4$  ( 87.09 g )  
 Potassium phosphate dibasic **Sigma Aldrich Catalog #P3786-1KG**
- [M] 0.5 millimolar (mM)  $(\text{NH}_4)_2\text{HPO}_4$  ( 66.04 g )  
 Ammonium phosphate dibasic **Sigma Aldrich Catalog #215996**
- 500 mL Water
- Sterilize by filtration
- Store at room temperature for up to 12 months

### Section 2: Dilute Salts Stock Solution II (10x)

- [M] 0.05 millimolar (mM)  $\text{MgCl}_2$  ( 25.42 g )  
 1 M Magnesium Chloride ( $\text{MgCl}_2$ ) **Sigma Aldrich Catalog #M8266**

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- [M] 0.05 millimolar (mM)  $\text{CaCl}_2$  ( 18.38 g )  
⊗ Calcium chloride **Sigma – Aldrich Catalog #C1016**
- 250 mL Water
- Sterilize by filtration
- Store at Room temperature for up to 12 months

### Section 3: Dilute Salts Solution (1x) (Machlis, 1958)

- 500  $\mu\text{L}$  DS Stock Solution I
- 100  $\mu\text{L}$  DS Stock Solution II
- 1 L sterile water
- Prepare solution in a sterile laminar flow hood with sterile supplies
- Store at Room temperature for up to 12 months

### Section 4: K1 Media (liquid and solid)

- 0.06% Bacto Peptone ( 0.6 g ) (w/v;  
⊗ Bacto™ Peptone **Thermo Fisher Scientific Catalog #211677** )
- 0.04% Yeast Extracts ( 0.4 g ) (w/v;  
⊗ Fisher BioReagents™ Microbiology Media Additives: Yeast Extract **Fisher Scientific Catalog #BP1422-2**
- 0.18% Glucose ( 1.8 g ) (w/v;  
⊗ D-(+)-Glucose **Millipore Sigma Catalog #G5767-5KG**
- *For solid media only:* 1.5% (w/v) agar ( 15 g )  
⊗ Agar **Fisher Scientific Catalog #BP1423-500**
- Water up to 1 L
- Sterilize by autoclaving
- Let cool to 60 °C before adding any selection antimicrobials
- Store at 4 °C for up to 6 months

### Section 5: LB media (liquid and solid)-- made from individual components

- 1% Tryptone ( 10 g ) (w/v,  
⊗ Tryptone **Millipore Sigma Catalog #T7293** )
- 1% NaCl ( 10 g )  
⊗ Sodium Chloride Fisher BioReagents™ **Fisher Scientific Catalog #BP358-1**
- 0.5% Yeast Extract ( 5 g ) (w/v;

- For solid media only: 1.5% (w/v) agar ( 15 g )

Agar **Fisher Scientific Catalog #BP1423-500**

- Water up to 1 L
- Sterilize by autoclaving
- Let cool to 60 °C before adding any selection antimicrobials
- Store at 4 °C for up to 6 months

## Section 6: LB media (liquid and solid)-- commercially available

- 25 g LB powder (

LB Broth (Miller) **Millipore Sigma Catalog #L3522-1KG** )

- For solid media only: 1.5% agar ( 15 g ) (w/v;

Agar **Fisher Scientific Catalog #BP1423-500** )

- Water up to 1 L
- Sterilize by autoclaving
- Let cool to 60 °C before adding any selection antimicrobials
- Store at 4 °C for up to 6 months

## Section 7: Minimal Salts Solution (2.5x)

- [M] 26.6 millimolar (mM)  $\text{KH}_2\text{PO}_4$  ( 3.625 g )

Potassium phosphate monobasic **Sigma Aldrich Catalog #P0662-1KG**

- [M] 29.4 millimolar (mM)  $\text{KH}_2\text{PO}_4$  ( 5.125 g )

Potassium phosphate dibasic **Sigma Aldrich Catalog #P3786-1KG**

- [M] 6.4 millimolar (mM)  $\text{NaCl}$  ( 0.375 g )

Sodium Chloride Fisher BioReagents™ **Fisher Scientific Catalog #BP358-1**

- [M] 5 millimolar (mM)  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  ( 1.250 g )

Magnesium sulfate heptahydrate **Millipore Sigma Catalog #2303915**

- [M] 1.1 millimolar (mM)  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  ( 0.165 g )

Calcium Chloride Dihydrate **Sigma Catalog #C7902-500G**

- [M] 22.3 micromolar ( $\mu\text{M}$ )  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  ( 6.2 mg )

Iron(II) sulfate heptahydrate **Millipore Sigma Catalog #F8263-1KG**

- [M] 9.5 millimolar (mM)  $(\text{NH}_4)_2\text{SO}_4$  ( 1.250 g )  
 ☒ Ammonium sulfate **Millipore Sigma Catalog #A2939-500g**
- Water up to 1 L
- No need to sterilize, precipitate is normal
- Store at Room temperature for up to 1 year

## Section 8: MES with acetosyringone

- [M] 40 Molarity (M) MES (pH 5.3) ( 7.7 g ) (2-(N-morpholino)ethanesulfonic acid)  
 ☒ MES hydrate **Millipore Sigma Catalog #M2933-500G**
- [M] 200 micromolar ( $\mu\text{M}$ ) acetosyringone ( 0.0392 g )  
 ☒ 3'5'-Dimethoxy-4'-hydroxyacetophenone **Sigma Aldrich Catalog #D134406-5G**
- pH with KOH
- MES must be at pH 5.3 before adding acetosyringone
- Water up to 50 mL after pHing
- Filter sterilize, DO NOT autoclave
- Add to IM recipe after other components are autoclaved and cooled

## Section 9: Induction Media (liquid and solid)

- 1x Minimal salts solution ( 400 mL of 2.5x stock solution, see recipe above)
- [M] 10 millimolar (mM) glucose ( 0.9 g ) (w/v;  
 ☒ D-(-)-Glucose **Millipore Sigma Catalog #G5767-5KG**)
- 0.5% glycerol (v/v; 5 mL )  
 ☒ Glycerol (Certified ACS) Fisher Chemical™ **Fisher Scientific Catalog #G33-1**
- For solid media only: 1.5% (w/v) agar ( 15 g )  
 ☒ Agar **Fisher Scientific Catalog #BP1423-500**
- Water up to 950 mL
- Sterilize by autoclaving BEFORE adding MES with acetosyringone
- 50 mL MES with acetosyringone (see recipe above; only add after autoclaving other components and cooling to 58 °C )
- DO NOT autoclave acetosyringone, this will degrade the hormone
- Store at 4 °C for up to 1 month



## ATTACHMENTS

[Spizellomyces transformation steps.pdf](#)

[Spizellomyces transformation video.1.mp4](#)

[Spizellomyces transformation transcript.pdf](#)

## FILES

### Protocol



NAME

Protocol 1: Electroporation of Agrobacterium tumefaciens with a plasmid of interest

**VERSION 1**

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## Protocol



NAME

Protocol 2: Culturing Spizellomyces punctatus (Sp) prior to transformation day

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## Protocol



NAME

Protocol 3: Growing liquid cultures of Agrobacterium prior to transformation day

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## Protocol



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Protocol 4: Creating depressions in induction media plates

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## Protocol



NAME

Protocol 5: Agrobacterium-mediated transformation of Spizellomyces punctatus (Sp)

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## Protocol



NAME

Protocol 6: Selecting for Spizellomyces punctatus transformants

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## Protocol



NAME

Protocol 7: Picking colonies of transformed *Spizellomyces punctatus* (Sp)

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