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Version 2

Agrobacterium-mediated transformation of the chytrid fungus *Spizellomyces punctatus* (Sp) V.2

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Sarah M Prostak¹, Edgar M Medina¹, Erik Kalinka¹, Lillian Fritz-Laylin¹

¹University of Massachusetts at Amherst



fritzlaylinlab.umass Fritz-Laylin

University of Massachusetts Amherst

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We use this collection and it's working

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Abstract

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

Attachments



[Spizellomyces_transf...](#)

912.1MB



[Spizellomyces_transo...](#)

79KB



[Transformation_proto...](#)

189KB

Guidelines

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



Materials

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

- [M] 5 millimolar (mM) KH_2PO_4 (340.2 mg)
 Potassium phosphate monobasic Merck MilliporeSigma (Sigma-Aldrich) Catalog #P0662-1KG
- [M] 5 millimolar (mM) KH_2PO_4 (435.5 mg)
 Potassium phosphate dibasic Merck MilliporeSigma (Sigma-Aldrich) Catalog #P3786-1KG
- [M] 5 millimolar (mM) $(\text{NH}_4)_2\text{HPO}_4$ (330.15 mg)
 Ammonium phosphate dibasic Merck MilliporeSigma (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] 0.5 millimolar (mM) MgCl_2 (11.9 mg)
 1 M Magnesium Chloride (MgCl_2) Merck MilliporeSigma (Sigma-Aldrich) Catalog #M8266
- [M] 0.5 millimolar (mM) CaCl_2 (13.87 mg)
 Calcium chloride Merck MilliporeSigma (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)

- 100 mL DS Stock Solution I
- 100 mL DS Stock Solution II
- 800 mL 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 **Merck MilliporeSigma (Sigma-Aldrich) 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 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #T7293**)
- 1% NaCl ( 10 g)  Sodium Chloride **Fisher BioReagents™ Fisher Scientific Catalog #BP358-1**
- 0.5% Yeast Extract ( 5 g) (w/v;
 Fisher BioReagents™ Microbiology Media Additives: Yeast Extract **Fisher Scientific Catalog #BP1422-2**)
- *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) **Merck MilliporeSigma (Sigma-Aldrich) 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) KH_2PO_4 (3.625 g)
 Potassium phosphate monobasic **Merck MilliporeSigma (Sigma-Aldrich) Catalog #P0662-1KG**
- [M] 29.4 millimolar (mM) KH_2PO_4 (5.125 g)
 Potassium phosphate dibasic **Merck MilliporeSigma (Sigma-Aldrich) Catalog #P3786-1KG**
- [M] 6.4 millimolar (mM) 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 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #2303915**
- [M] 1.1 millimolar (mM) $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (0.165 g)
 Calcium Chloride Dihydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #C7902-500G**
- [M] 22.3 micromolar (μM) $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (6.2 mg)
 Iron(II) sulfate heptahydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #F8263-1KG**
- [M] 9.5 millimolar (mM) $(\text{NH}_4)_2\text{SO}_4$ (1.250 g)
 Ammonium sulfate **Merck MilliporeSigma (Sigma-Aldrich) 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 (5.3) (7.7 g) (2-(N-morpholino)ethanesulfonic acid)
 MES hydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #M2933-500G**
- [M] 200 micromolar (μM) acetosyringone (0.0392 g)
 3'5'-Dimethoxy-4'-hydroxyacetophenone **Merck MilliporeSigma (Sigma-Aldrich) Catalog #D134406-5G**
- pH with KOH
- MES must be at (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 Merck MilliporeSigma (Sigma-Aldrich) 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

Troubleshooting

Attachments



Spizellomyces transformans
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Spizellomyces transformans
s_transo...
79KB



Transformation protocol
on_proto...
189KB

Files

 SEARCH

Protocol

NAME

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

VERSION 1

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University of Massachusetts Amherst

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Protocol

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

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

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Protocol 4: Creating depressions in induction media plates

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

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Protocol 6: Selecting for Spizellomyces punctatus transformants

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

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