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

Sarah M Prostak[1], Edgar M Medina[1], Erik Kalinka[1], Lillian Fritz-Laylin[1]

[1]University of Massachusetts at Amherst

Fritz-Laylin Laboratory

fritzlaylinlab.umass

VERSION 2

FEB 09, 2024

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.x54v9dd1pg3e/v2

External link:
https://doi.org/10.1099/acmi.0.000566.v3

Collection Citation: Sarah M Prostak, Edgar M Medina, Erik Kalinka, Lillian Fritz-Laylin 2024. Agrobacterium-mediated transformation of the chytrid fungus Spizellomyces punctatus (Sp). **protocols.io** https://dx.doi.org/10.17504/protocols.io.x54v9dd1pg3e/v2Version created by fritzlaylinlab.umass

ABSTRACT

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

ATTACHMENTS

Spizellomyces_transformation_video.1.mp4

Spizellomyces_transorfmation_video_transcript.pdf

Transformation_protocol_outline_v2.pdf

GUIDELINES

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

MANUSCRIPT CITATION:
Edgar M Medina Kristyn A Robinson Kimberly Bellingham-Johnstun Giuseppe Ianiri Caroline Laplante Lillian K Fritz-Laylin Nicolas E Buchler (2020) Genetic transformation of Spizellomyces punctatus, a resource for studying chytrid biology and evolutionary cell biology eLife 9:e52741. https://doi.org/10.7554/eLife.52741

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

Created: Feb 09, 2024

Last Modified: Feb 09, 2024

COLLECTION integer ID: 94975

Keywords: Electroporation, Agrobacterium tumefaciens, Spizellomyces punctatus, chytrid fungi, chytrids

Funders Acknowledgement:
Gordon and Betty Moore Foundation
Grant ID: #9337
National Science Foundation
Grant ID: IOS-1827257
Pew Charitable Trusts
Grant ID: Pew Scholar award

MATERIALS

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

- [M] 5 millimolar (mM) KH_2PO_4 (340.2 mg)
  Potassium phosphate monobasic **Sigma Aldrich Catalog #P0662-1KG**
- [M] 5 millimolar (mM) KH_2PO_4 (435.5 mg)
  Potassium phosphate dibasic **Sigma Aldrich Catalog #P3786-1KG**
- [M] 5 millimolar (mM) $(NH_4)_2HPO_4$ (330.15 mg)
  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] 0.5 millimolar (mM) $MgCl_2$ (11.9 mg)
  1 M Magnesium Chloride (MgCl2) **Sigma Aldrich Catalog #M8266**
- [M] 0.5 millimolar (mM) $CaCl_2$ (13.87 mg)
  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)

- 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 **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;
  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) **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) KH_2PO_4 (3.625 g)
  Potassium phosphate monobasic **Sigma Aldrich Catalog #P0662-1KG**
- [M] 29.4 millimolar (mM) KH_2PO_4 (5.125 g)
  Potassium phosphate dibasic **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) $MgSO_4 \cdot 7H_2O$ (1.250 g)
  Magnesium sulfate heptahydrate **Millipore Sigma Catalog #2303915**
- [M] 1.1 millimolar (mM) $CaCl_2 \cdot 2H_2O$ (0.165 g)
  Calcium Chloride Dihydrate **Sigma Catalog #C7902-500G**
- [M] 22.3 micromolar (µM) $FeSO_4 \cdot 7H_2O$ (6.2 mg)
  Iron(II) sulfate heptahydrate **Millipore Sigma Catalog #F8263-1KG**
- [M] 9.5 millimolar (mM) $(NH_4)_2SO_4$ (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 (µM) 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_transf...

Spizellomyces_transo...

Transformation_proto...

FILES

SEARCH

Protocol

NAME

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

VERSION 1

CREATED BY

fritzlaylinlab.umass

OPEN →

Protocol

NAME

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

VERSION 1

CREATED BY

fritzlaylinlab.umass

OPEN →

Protocol

NAME

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

VERSION 1

CREATED BY

fritzlaylinlab.umass

OPEN →

Protocol

NAME

Protocol 4: Creating depressions in induction media plates

VERSION 1

CREATED BY

fritzlaylinlab.umass

OPEN →

Protocol

NAME

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

VERSION 1

CREATED BY

fritzlaylinlab.umass

OPEN →

Protocol

NAME

Protocol 6: Selecting for Spizellomyces punctatus transformants

VERSION 1

CREATED BY

fritzlaylinlab.umass

OPEN →

Protocol

NAME

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

VERSION 1

CREATED BY

fritzlaylinlab.umass

OPEN →