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

In 1 collection

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

[1]University of Massachusetts at Amherst

fritzlaylinlab.umass

ABSTRACT

If transformation was successful, antibiotic-resistant Spizellomyces colonies should appear after 4-6 days of growth on selection media. These colonies should be rough, white, and opaque. When viewed under a microscope, there should be clustering of sporangia and some movement of zoospores. Picking these colonies and culturing them until there are enough cells for cryopreservation and to use in downstream molecular analysis is the last protocol in the entire transformation process. All steps here, with the exception of centrifugation steps, must be carried out in a sterile environment, either in a laminar flow hood or in the sterile area around an open flame.

ATTACHMENTS

Spizellomyces_transformation_steps.pdf

MATERIALS TEXT

Materials

- Active plate of colonies of Sp transformants (see *Protocol 6: Selecting for Spizellomyces punctatus transformants*)
- DS Solution, sterile (see recipe)
- 18G needle, sterile 18G needle **BD Biosciences Catalog #305196**
- 1.5 mL microcentrifuge tubes, sterile such as
  Fisherbrand™ Low-Retention Microcentrifuge Tubes **Fisher Scientific Catalog #0 681-331**
- K1 agar plates (1.5% w/v) with selection antimicrobials, sterile (see recipe)
- 20-200 µL micropipette such as
  Eppendorf Research Plus single channel pipette 2-20 uL yellow operating button for use with 20 uL **pipette.com Catalog #3123000039**
- Filter tips for the micropipettes, sterile such as
  TIPONE® FILTER TIPS **USA Scientific Catalog #1122-1830**
- Laminar flow hood and/or open flame, for maintaining sterility
- 70% (v/v) ethanol for maintaining sterility (if using laminar flow hood)

SAFETY WARNINGS

Proper handling of 18G needles must be observed to avoid personal injury. All sharps should be disposed of into a proper sharps container.

ATTACHMENTS

Spizellomyces_transformation_steps.pdf

Steps

1 Divide one K1 plate with selection antimicrobials into four sections using a marker on the bottom of the plate.

Note

One plate can be used to plate up to 4 colonies for one plasmid transformation.
This reduces materials used in the initial amplification stage.

2 Aliquot 50 µL of DS into one microcentrifuge tube per colony to be picked.

3 Using an 18G needle, gently lift the colony of interest from the agar.

Note

- Be careful not to poke too deeply into the agar. The goal is to lift as little agar as possible from the plate, while picking up most of the colony.
- To prevent cross contamination between colonies, either flame the needle between picking colonies, or use a new, sterile needle for each colony.

4 Resuspend the colony into the appropriate tube pre-filled with DS.

Note

Swirl the needle gently, but with enough force to dislodge the colony from the needle.

5 Pipette gently to mix and break up the pellet.

6 Pipette 50 µL of the resuspended colony onto one quadrant of the K1 plate prepared in step 1.

7 Repeat steps 3-6 for each colony to be picked.

8 Let the cells grow for 2-3 days before rehydrating the quadrants with 100 µL of DS and transferring each colony to its own quadrant of a new K1 selection plate.

> **Note**
>
> Only a small portion of the quadrant is needed for colony expansion.

9 After 2-4 rounds of colony expansion there is enough inoculum to transfer each isolated colony into its own plate.

10 Continuing subculturing each colony until enough culture exists to freeze and perform downstream analyses.