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Protocol 6: Selecting for Spizellomyces punctatus transformants

In 1 collection

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ABSTRACT

Once the co-culture IM plates have grown for four days, it is time to select Sp transformants. Selecting these transformants requires harvesting the cells from the co-culture plates, dislodging and removing any remaining Agro from the culture, and plating Sp onto K1 selection media. In our laboratory, we use 300 mg/L Hygromycin B (Gibco #10687010) as our selection marker. Additionally, we add 50 mg/L Carbenicillin (Carb) and 50 mg/l Tetracycline (Tet) to our K1 plates to kill Agrobacterium. All steps here, with the exception of centrifugation steps, must be carried out in a sterile environment, ideally in the sterile area around an open flame.

ATTACHMENTS

Spizellomyces_transformation_steps.pdf

MATERIALS TEXT

Materials

Co-culture of Agro and Sp after 4 days of growth (see *Protocol 5: Agrobacterium-mediated transformation of Spizellomyces punctatus*)
Single edge razor blades, sterile (such as Personna .009 RD #94-120-71)
K1 plates with and without selection antimicrobials
Forceps (such as AESCULAP BD232)
DS solution, sterile (see recipe)
50 mL conical tubes, sterile (such as

Centrifuge Tubes-Bag 50mL Centrifuge Tube - Bag Sterile **Cell Treat Scientific Products Catalog #229421**

)
100-1,000 µL micropipette such as

Eppendorf Research Plus Single Channel pipette 100-1000 uL blue operating button for use wit 100 **pipette.com Catalog #3123000063 ES-1000**

20-200 µL micropipette such as

Eppendorf Research Plus single channel pipette 2-20 uL yellow operating button for use with 2 uL **pipette.com Catalog #3123000039**

Filter tips for the micropipettes, sterile such as

TIPONE® FILTER TIPS **USA Scientific Catalog #1122-1830**

Vortexer such as

Equipment

Variable Speed Mini Vortex Mixer	NAME
Vortexer	TYPE
Fisherbrand™	BRAND
14-955-163	SKU
https://www.fishersci.com/shop/products/fisher-scientific-variable-speed-mini-vortex-mixer/14955163	LINK

Centrifuge capable of holding 50 mL conicals (such

Equipment

Eppendorf™ 5810R Centrifuge	NAME
Centrifuge	TYPE
Eppendorf	BRAND
02-262-8187	SKU
https://www.fishersci.com/shop/products/eppendorf-5810r-centrifuge-rotor-packages-16/022628187	LINK

)
Plastic storage containers, or equivalent (e.x. plastic shoe containers)
Open flame source

SAFETY WARNINGS

This protocol involves the use of single-edge razor blades. It is extremely important that proper safety protocols regarding sharps be taken– protective eye equipment and a sharps container must be used while conducting this protocol and disposing of razors.

ATTACHMENTS

Spizellomyces_transformation_steps.pdf

15m

Steps

1 For each plasmid transformed, prepare a 50 mL conical with 30 mL of DS solution.

2 From the appropriate conical for the plate to be harvested, remove 1 mL of DS.

3 Distribute the DS among the four quadrants of the corresponding IM plate.

5m

4 Incubate at Room temperature for 00:05:00.

5 Using forceps, sterilize both sides of a single edge razor blade with the flame.

6 Tilt the rehydrated IM plate and gently scrape the surface of the agar and collect the growth into the DS pooled at the bottom.

7 Rotate the plate and scrape along the agar until most of the opaque areas on the plate are gone.

8 Use a 1000 µL pipette tip to scrap any remaining growth from the razor blade and resuspend the growth to the appropriate conical tube filled with DS.

Note

Once the growth is taken off the razor blade, the blade should be thoroughly sterilized using the flame and then discarded into a sharps container.

9 Aspirate all of the liquid from the scraped plate and slowly dispense the liquid back into the appropriate conical tube.

Note

A density gradient between the heavier cell suspension and the lighter DS solution will form. Maintaining this gradient at this pointby placing retrieved co-culture in the bottom of the tube. This can help you continuously retrieve “clean” DS to continue harvesting the plate more easily.

10 From the top of the density gradient, remove 1 mL of DS.

11 Wash the surface of the scraped IM plate several times with this fresh DS.

12 Return the volume back to the conical.

13 Invert the conical 3 times.

14 Vortex the conical for 1-2 seconds to dislodge any remaining *Agro* from *Sp* cells.

15 Repeat steps 2-14 for each plasmid transformed (i.e, for each IM plate you have)

16 Centrifuge the conical(s) for 2000 rcf, Room temperature, 00:10:00 . 10m

17 Check for the presence of a pinkish pellet, this is the chytrid Sp.

18 Gently pour off the supernatant with the high side of the pellet facing up.

Note

This step can result in a great loss of *Sp* cells, thus pouring with this orientation will reduce the amount of cells lost.

19 Gently resuspend the pellet into 500 µL of fresh DS.

20 Pipette 200 µL of the resuspended *Sp* onto a K1 plate with selection antibiotics.

Note

We use 300 undetermined Hygromycin B (Hygromycin B (50 mg/mL) **Thermo Fisher Catalog #10687010**) and 50 undetermined Carb and 50 undetermined Tet to prevent further *Agro* growth.

21 Spread the cells using 4-5 sterile glass beads.

22 Once the plate is dry, remove the glass beads.

23 Seal and incubate plates in a humidity chamber at Room temperature for 4 days or until colonies appear on the plate.