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

In 1 collection

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ABSTRACT

In order to increase transformation efficiency, a more synchronous culture of Sp is needed. This process involves subculturing active Sp plates in the days leading up to transformation day. At least two generations of Sp should be grown on antibiotic free K1 medium to ensure no trace antibiotics are present in the culture for transformation day. Proper sterile technique should be followed at all times when maintaining cultures of Sp; use either a laminar flow hood or work in the sterile area around an open flame.

ATTACHMENTS

Spizellomyces_transformation_steps.pdf

MATERIALS TEXT

Materials

- Active, healthy culture of wild-type *Spizellomyces punctatus* (
  Spizellomyces punctatus (Koch) Barr **ATCC Catalog #48900**
)
- K1 agar plates (1.5% w/v) without any antimicrobial (see recipe)
- DS solution (see recipe)
- 50 mL conicals, 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 f use with 100 **pipette.com Catalog #3123000063 ES-1000**
- Filter tips for the micropipette, sterile such as
  TIPONE® FILTER TIPS **USA Scientific Catalog #1122-1830**
- 20 mL luer-lock syringes (such as
  BD General Use Syringes **Fisher Scientific Catalog #22-124-967**
)
- 25 mm syringe filter holders (
  Cole-Parmer Advantec 43303010 Polypropylene Filter Holder for 25 mm Membranes **Fisher Scientific Catalog #NC9972954**
  ), preloaded with Grade 1 Whatman paper (
  Cytiva Whatman™ Qualitative Filter Paper: Grade 1 Circles **Fisher Scientific Catalo #09-927-223**
  ), sterile
- Laminar flow hood, or open flame to maintain sterility
- 70% (v/v) ethanol for maintaining sterility (if using laminar flow hood)
- Incubator at 28 °C

BEFORE START INSTRUCTIONS

In order to prepare enough plates to provide an adequate amount of zoospores for transformation, subculture one plate of Sp per plasmid to be transformed. One active plate is enough to subculture 2-3 new plates. Complete this protocol roughly 36 hours and then again roughly 18 hours prior to the intended transformation time.

ATTACHMENTS

Spizellomyces_transformation_steps.pdf

Steps

1 Flood enough active Sp plates each with 1 mL of DS to fit your needs.

Note

One active plate is enough to seed 2-3 new plates and you will need one plate of active Sp per plasmid to be transformed on transformation day.

2 Holding the plate at an angle, gently wash the DS over the agar.

2.1 Wash the 1 mL of DS over the agar. (1/3)

2.2 wash the 1 mL of DS over the agar. (2/3)

2.3 Wash the 1 mL of DS over the agar. (3/3)

3 Collect all zoospores into a 50 mL conical.

4 Filter all zoospores into a new 50 mL conical using the sterile 25 mm syringe filter preloaded with Whatman Grade 1 filter paper.

5 Inoculate the proper amount of new K1 plates for your needs with 250 µL to 500 µL of filtered zoospores.

Note

The amount of spores transferred to new plates depends on the density of the plate being harvested, be careful not to overcrowd the plates, as zoospore release will decrease.

6 Add 500 µL of fresh DS to the newly inoculated plates.

7 Gently tilt the plates to spread the liquid across the entire surface of the agar.

8 Incubate at 30 °C in a humidity chamber.

Note

Using a humidity chamber is standard practice, as it prevents the agar from drying out. Placing the plates into a plastic bin with a beaker of ~ 20 mL of water should be enough.

Complete this protocol in its entirety 36 and 18 hours prior to intended transformation time.