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Protocol 4: Creating depressions in induction media plates

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

Transformation is carried out by co-culturing Agro and Spizellomyces on induction media (“IM”). Once its virulence genes are induced, Agro will infect Spizellomyces and the plasmid the transfer DAN is randomly incorporated into the Spizellomyces genome. The IM media and agar plates (see recipes) should be no more than 1 month old, as the hormone Acetosyringone is likely to degrade, decreasing its effectiveness. For each plasmid, four ratios of Agro and Sp will be made and plated onto one plate (See Protocol 5.3). Prepare IM plates with small depressions using a glass culture tube to keep the co-culture liquids from touching each other.

ATTACHMENTS

Spizellomyces_transformation_steps.pdf

MATERIALS TEXT

Materials

- Induction Media agar plates (1.5% agar w/v; see recipe)
- Large diameter glass culture tube (>10mm, such as
  PYREX™ Reusable Borosilicate Glass Tubes with Plain End **Fisher Scientific Catalo #14-957N**
)
- 50 mL conical (such as
  Centrifuge Tubes-Bag 50mL Centrifuge Tube - Bag Sterile **Cell Treat Scientific Products Catalog #229421**
)
- 70% ethanol to maintain sterility
- Open flame source to maintain sterility

SAFETY WARNINGS

Protective eye goggles must be worn during this procedure, as the potential for broken or cracked glass is a hazard.

BEFORE START INSTRUCTIONS

The protocol below outlines how to prepare the depressions and should be practiced until the agar is no longer scarred. Prepare these plates within a month of your intended Spizellomyces transformation time.

ATTACHMENTS

Spizellomyces_transformation_steps.pdf

Steps

1 On the bottom, divide an IM plate into quadrants.

2 Draw circles 2.5 mm in diameter in each quadrant.

> **Note**
>
> Ensure the circles do not overlap.

3 Place a 20-30 mm diameter glass culture tube into a 50 mL conical tube.

4 Add enough 70% ethanol to cover the first inch of the culture tube.

5 Burn off the ethanol on the tube using a flame, let the glass cool in the sterile area around the open flame.

6 Dip the culture tube back into the ethanol.

7 Repeat steps 3-5 a total of 3 times.

8 Working within the sterile area around the flame, gently press the warm (but not enough to melt the agar), sterile culture tube into the agar at the center of one circle drawn earlier.

9 Gently press on the agar and move the culture tube in circles until a depression is formed roughly the size of the circle drawn.

Note

- Ensure the four circles of one plate do not overlap and can hold about 200 µL of liquid.
- Depressions can be seen by holding the plate to light at an angle; the light should bend around the circle.

10 Repeat for all quadrants of all IM plates needed.