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

In 1 collection

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ABSTRACT

Overnight liquid cultures of *Agrobacterium* transformed with the plasmid(s) of interest must be prepared the night before *Spizellomyces* transformation day. Liquid cultures must be inoculated from active plates/colonie. If you would like to use 25% glycerol stocks of previously transformed *Agrobacterium*, streak the desired cells onto the appropriate LB+selection plate, let it grow for 2-4 days at 28°C, then use this plate to inoculate the liquid media at least 12 hours before the intended transformation time. Ensure proper sterile technique throughout the protocol, using either a laminar flow hood or working in the sterile area around an open flame

ATTACHMENTS

Spizellomyces_transformation_steps.pdf

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MATERIALS TEXT

Materials

- Active plate of wild-type *Agrobacterium tumefaciens* EHA105 (
  EHA105 Agrobacterium ElectroCompetent Cells **Gold Biotechnology Catalog # CC-225-5x50**
  ), grown at 28°C for at least 48 hours.
- Active plate(s) of Agrobacterium tumefaciens EHA105 (
  EHA105 Agrobacterium ElectroCompetent Cells **Gold Biotechnology Catalog # CC-225-5x50**
) transformed with your plasmid(s) of interest (see Protocol 1), grown at 28°C for at least 48 hours.
- LB liquid media with and without selection antibiotics, sterile (see recipe)
- Culture tubes, sterile (such as
  VWR® Culture Tubes Plastic with Dual-Position Caps **VWR Avantor Catalog #60818-703**
)
- Inoculation loops (such as
  Fisherbrand™ Disposable Inoculating Loops and Needles **Fisher Scientific Catalog #22-363-602**
  ; pipette tips are also an acceptable alternative)
- Laminar flow hood, or open flame to maintain sterility
- 70% (v/v) ethanol for maintaining sterility (if using laminar flow hood)
- Shaking incubator at 28 °C

BEFORE START INSTRUCTIONS

Perform this protocol the night before your intended *Spizellomyces* transformation time.

ATTACHMENTS

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12h

Steps

1 Fill the appropriate amount of culture tubes each with 5 mL of LB liquid media.

> **Note**
>
> Use one culture tube per plasmid or control. Double check you are using the proper selection antibiotic (or lack thereof) for the Agro strain to be grown.

2 Using an inoculation loop or pipette tip, gently scrape the surface of the appropriate Agro plate to collect the bacteria or pick the colony of interest.

3 Swirl the loop or tip to dislodge the bacteria into the media of the appropriate culture tube.

12h

4 Incubate at 28 °C, shaking at 225 rpm, 12:00:00, or Overnight.