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Protocol 1: Electroporation of Agrobacterium tumefaciens with a plasmid of interest

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

Electroporation is a widespread method of transforming competent *Agrobacterium tumefaciens* (*Agro*) cells with a plasmid containing a T-DNA of interest. The resulting Agro can be used to transform various plants and fungi, resulting in transformed cell lines. This protocol outlines the standard electroporation protocol we use to transform *Agro* in preparation for *Agrobacterium*-mediated transformation of the chytrid fungus *Spizellomyces punctatus*.

ATTACHMENTS

Spizellomyces_transformation_steps.pdf

GUIDELINES

Any binary plasmid that works in Agrobacterium tumefaciens strain EHA105 (GoldBio #CC-225-5x50) can be used for this procedure. We have had success using plasmids derived from pPZP201-BK (PMID: 7919218). It is imperative that all steps be carried out at 4°C up until electroporation. Ensure proper sterile technique throughout this protocol; perform all steps except centrifugation and electroporation charge delivery in a laminar flow hood or in the sterile area around an open flame.

MATERIALS TEXT

Materials:

- Use a fresh streak of Agrobacterium tumefaciens EHA105 (GoldBio #CC-225-5x50) to spread a lawn into a LB plate, (a new streak from a -80°C stock will take ~48 hours to display visible growth at 28°C , plan accordingly).
- LB agar plates (1.5% w/v) with and without selection antibiotics, sterile (see recipe)
- Purified plasmid(s) of interest resuspended in molecular biology grade water
- Molecular biology grade water, sterile such as MilliQ or equivalent
- Invitrogen™ S.O.C. Medium **Fisher Scientific Catalog #15-544-034**
- 10% (v/v) Glycerol, sterile (
  Glycerol (Certified ACS) Fisher Chemical™ **Fisher Scientific Catalog #G33-1**
)
- 1.5 mL centrifuge tubes, sterile (such as
  Fisherbrand™ Low-Retention Microcentrifuge Tubes **Fisher Scientific Catalog #0**
  681-331
)
- 0.5 mL centrifuge tubes, sterile (such as
  Fisherbrand™ Snap-Cap™ Flat-Top Graduated Microcentrifuge Tubes **Fisher Scientific Catalog #02-681-268**
)
- 2 mm electroporation cuvettes, sterile (
  Pkg of 50 individually wrapped 2mm electroporation cuvettes **Bulldog Bio Catalog #12358-346**
)
- Culture tubes, sterile (such as
  VWR® Culture Tubes Plastic with Dual-Position Caps **VWR Avantor Catalog #60818-703**
)
- 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**
- 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**
- 0.1-2.5 µL micropipette such as
  Eppendorf Research Plus single channel pipette 0.1-2.5 uL dark grey operating buttor
  for use with **pipette.com Catalog #3123000012**
- Filter tips for the micropipettes, sterile such as
  TIPONE® FILTER TIPS **USA Scientific Catalog #1122-1830**
- 5 mm Glass beads, sterile
- Ice bucket with ice
- Centrifuge capable of cooling to 4 °C
- Laminar flow hood and/or open flame, for maintaining sterility.
- 70% (v/v) ethanol for maintaining sterility (if using laminar flow hood)
- Exponential decay electroporator such as Gene Pulser Xcell (
  Gene Pulser Xcell Total System **Contributed by users Catalog ##1652660**
)
- Shaking incubator at 28 °C

BEFORE START INSTRUCTIONS

Electroporation should occur at least 4 days prior to the intended Spizellomyces transformation time to ensure that active, single colonies are available to be selected and transferred to liquid culture the night before transformation day (see Protocol "Growing liquid cultures of Agrobacterium prior to transformation day").

ATTACHMENTS

Spizellomyces_transformation_steps.pdf

4h 25m

Steps

20m

1 Cool the following materials On ice at least 00:20:00 prior to starting:

1. Plate with a lawn of wild-type Agrobacterium grown overnight at 28 °C.
2. Purified plasmid(s) of interest.
3. Molecular biology grade water, sterile.
4. 10% (v/v) glycerol, sterile.
5. 1.5 mL centrifuge tube(s), enough to hold the volume of Agro harvested.
6. 0.5 mL centrifuge tube(s), one per plasmid to be transformed, plus controls.
7. 2 mm electroporation cuvettes, one per plasmid to be transformed, plus controls.

2 Add 1 mL to 2 mL of ice-cold water to the plate of *Agrobacterium*. Hold the plate at ~45 degrees and run the water over the surface at least 3 times, gently scraping along the agar if necessary to recover the lawn of bacteria.

Note

Try to not drag too many big clumps.
The resulting harvest should have the consistency, color and density of whey.

3 Transfer the 1 mL of harvested cells to a 1.5 mL centrifuge tube, immediately place back On ice.

5m

4 Pellet the cells at 4000 rcf, 00:05:00 in a rotor prechilled to 4 °C.

Note

If in a pinch, a rotor for a centrifuge without cooling capabilities can be stored Overnight at 4 °C

or for 00:10:00 at -20 °C.

5 Remove the supernatant and gently resuspend the cells in 1 mL of water. Do not vortex.

Note

Keep cells On ice when not in use.

6 Repeat steps 4 and 5, 2 more times for a total of 3 washes.

7 Remove water and resuspend the cells in 800 µL of cold 10% (v/v) glycerol, place tubes back On ice.

8 Add 50 µL of cells to new 0.5 mL tubes, place back On ice.

Note

Use one 0.5 mL tube for each plasmid to be transformed, plus more for planned controls.

9 Add 1 µL of the plasmid of interest (200 ng/uL to 300 ng/uL; directly from a miniprep should work) to its appropriate tube of cells, place back On ice and mix gently by pipetting .

Note

Use 1 µL of water as a negative control.

10 Transfer the cells to cold 2 mm electroporation cuvettes, place back On ice.

Note

Again, use one cuvette for each control or plasmid to be transformed individually.

11 Prepare the recovery media in advance by placing 150 µL of Room temperature SOC medium to one 15 mL culture tube for each electroporation.

12 Turn on the electroporator and create an electroporation program with the following settings:

1. Voltage= 2400V
2. Capacitance = 25 µF
3. Resistance = 200 Ω
4. Cuvette size = 2 mm

13 Fully dry the cuvette before placing it into the electroporator chamber.

> **Note**
>
> Failure to fully dry the cuvette will lead to current arcing and improper electroporation.

14 Electroporate your cuvette.

15 As quickly but as gently as possible, remove a little less than 150 µL of SOC medium from the appropriate tube for the plasmid and pipette it into the cuvette. Remove the full volume from the cuvette and return it to the original culture tube.

> **Note**
>
> Do this by a flame and with good sterile technique.
>
> The best way to do this is to set a p200 to 150 µL, but to not pull the entire volume, leaving enough space left to fit the 50 µL that is in the cuvette.

4h

16 Incubate culture tubes at 28 °C, shaking at 225 rpm, 04:00:00.

> **Note**
>
> Meanwhile, place the appropriate number of LB plates with and without selection antibiotics to pre-warm

at 28 °C.

17 Add 4-6 sterile glass beads to each LB plate.

18 Add 10 µL cells to the appropriate plates.

Note

To make spreading this small volume easier, add 40 µL sterile water to the middle of the plate before adding the cells.

The electroporation efficiency for this protocol is very high: ≥1.6 x 10e8 cfu/µg pCAMBIA1391z DNA (GoldBio) or 1.25x10e5 cfu/µg plasmid (PMID: 29487777).

Do not add more than 10 µL of cells or you risk overgrowth and a lack of individual colonies.

19 Seal and invert the plates and incubate them at 28 °C for about 4 days.

Note

Colonies should appear within 4 days. If colonies of appreciable size (2-3 mm) appear earlier than that, continue on with Sp transformation.

Grow any colonies of interest in liquid media and freeze 25% glycerol stocks to avoid needing to re-electroporate Agro.