Streamlined *P. infestans* electroporation method

Judelson lab, 2019

1. Inoculate about five 15-cm rye-sucrose (rye B) agar plates by spreading ~1x10^4 sporangia on each with a sterile glass rod. Grow for 8-9 days in the dark at 18°C.
   Incubation times for growing hyphae and releasing zoospores are species and strain-specific. Most P. infestans strains should not be grown for more than 10 days, otherwise zoospore release may be slower and less synchronous. Most zoospores are typically released by 2 hr, and longer incubations may reduce transformation rates. Zoospore release is more synchronous if the plates are uniformly inoculated by spreading with sporangia, compared to plug-inoculation.

2. Pour 20 ml of cold Petri's solution on each plate. Use a glass spreader to rub off the sporangia, decanting what should be a milky solution into a 150 mm plate or beaker.
   Modified Petri's is 0.25 mM $CaCl_2$, 1mM $MgSO_4$, 1mM KH_2PO_4, 0.8 mM KCl.

3. Induce zoosporogenesis by incubation at 10°C for about 2 hr
   Normally this entails filling a plastic tub with ice, placing a plastic sheet above the ice and then covering the tub. After about 10 minutes, the chamber is about 10°C.

4. Harvest the zoospores by pouring the suspension through 15 µm nylon mesh into a 400-ml beaker. This and the following steps should be done on ice.
   A convenient holder for nylon mesh is sold by "Biodesign of New York".

5. Divide the zoospores into two 50 ml tubes. Add one-fiftieth the volume of 5M LiCl to each, and mix by gentle inversion (about 4 times). Remove an aliquot and count the zoospore concentration using a hemocytometer.
   The LiCl prevents encystment.

6. Pellet the zoospores by spin at 400 ×*g* for 5 min at 4°C in a swinging bucket rotor.

7. Decant most of the supernatant and resuspend the zoospore pellet by gently pipetting up and down with a wide-bore pipette (avoid using a P20 tip, it might be too narrow). Add enough Petri's solution to set the concentration between 6.3 x 10^6 and 2.5 x 10^7 zoospores per ml.

8. Gently mix 800 µl of zoospores with 30 µg of DNA in a prechilled tube. If your plan is to co-express two plasmids, use 15 µg of each.

9. Pipette the zoospores into a cooled 4 mm-gap cuvette. Cap the cuvette and wipe dry with a tissue. Electroporate at 550 V, 50 µF and 1575 Ω. The time constant will usually be 1.8 to 2.6 ms (keep track of this value).

10. Immediately after electroporation, place the cuvette on ice and add 800 µl of regeneration media. Mix gently and pipette into a 15 ml tube containing an additional 9 ml of regeneration media. This is Rye B (rye-sucrose) broth clarified by centrifugation plus 100 mM mannitol, 1 mM KCl, 2.5 mM $CaCl_2$.

11. Lay the tube on its side and incubate at 18°C for 20 hr. Don't go longer, since the hyphae will mat together and be difficult to spread on selection plates.

12. After 20 hr, count the concentration of germinated cysts. Multiply by the volume, and divide by the original number of zoospores to determine the regeneration rate. This is normally between 5 and 40%. This serves as a quality control step, in case you do not get many transformants (normally 5-15 are expected).

13. Concentrate the tissue by spinning the cultures at 1000 ×*g* for 5 min at room temperature. Decant all but about 1.6 ml of the liquid. Gently resuspend the zoospores in the residual liquid by pipetting up and down.

14. Spread 0.2 ml of the tissue on each of eight rye-sucrose agar plates containing the appropriate antibiotics. Incubate at 18°C. Colonies will appear after 8 to 12 days.
Normally G418 is used at 8 µg/ml. Nystatin (4 ml of commercial suspension per liter) and penicillin G (25 µg/ml) are often added to reduce the likelihood of contamination.