

Extraction of Genomic DNA from *Phytophthora infestans*

This method has been modified (to work with the equipment in the Kamoun Lab) from a method found online (adapted by Judelson from other methods).

Growing the mycelia

- Cultures were grown in either Pea Broth or Plich media in the tall 82 mm plates.
- Two to three plugs of mycelia were used to inoculate each plate and allowed to grow until the plate was ~80% full. This took ~2 weeks for the Plich media and ~a week for the Pea Broth.
- The mycelia was harvested on filter paper in a Buchner funnel and washed with H₂O.
- The Mycelia was frozen at -80°C and then freeze dried.

Extraction of DNA

Extraction Buffer 0.2 M Tris pH8.5
 0.25 M NaCl
 25 mM EDTA
 0.5% SDS

Tris equilibrated phenol (7 mL/extraction)

Chloroform

Isopropanol (2-propanol)

70% Ethanol

50 mL Centrifuge tubes (purple lid VWR 20171-030)

Mortar and pestle

Liquid N₂

1. Pre-cool the mortar with liquid N₂ (~1/3 full).
2. Grind the mycelia with a mortar and pestle in liquid N₂ until it has the consistency of a fine powder.
3. Add 10 mL of extraction buffer (per 100 mg – 200 mg of freeze dried mycelia) mix well with the pestle and transfer into a centrifuge tube. This can be added as 2 x 5 mL aliquots where the second aliquot is used to

- rinse the mortar. Don't worry if the extraction buffer freezes in the mortar, just wait for it to thaw.
4. Add 7 mL of phenol and 3 mL chloroform.
 5. Mix and leave at room temperature for one hour.
 6. Spin 15 min at 6000g (7500 rpm)
 7. Transfer the supernatant to a fresh tube.
 8. Add an equal volume of chloroform and mix gently.
 9. Spin 5 min at 6000g (7500 rpm)
 10. Transfer the supernatant to a fresh tube.
 11. Add 0.6 volumes of isopropanol and mix gently
 12. Allow the DNA to precipitate on ice for ~30 min.
 13. Spin 15 min at 6000g
 14. Wash the DNA pellet with 20 mL 70% ethanol. If the pellet doesn't stick to the tube then the DNA can be respun as in step 13.
 15. Air dry (don't over dry).
 16. Resuspend in 500 μ L of H₂O (use a smaller volume if the pellet looks small)
 17. Rinse the tube with another 200 μ L (pooled both samples)

Check the DNA for quality and quantity

The quantity is checked on the spec using a 260/280 reading or with the Nanodrop. NB the Nanodrop gave readings that were ~10 fold higher than they should be. It may pay to compare the sample visually against the mass standard for confirmation.

The quality of the DNA is checked by running undigested DNA on a 0.7% gel. The DNA may require dilution if the concentration is high. The DNA can also be digested and checked on a gel.

Notes

The first time I tried this prep (Carolyn Folder 1 22nd November 2004) I used mycelia that had been grown in Pea broth. The DNA was ~100-200 ng/ μ L final concentration (700 μ L final volume).

A 1/50 dilution worked well for PCR.