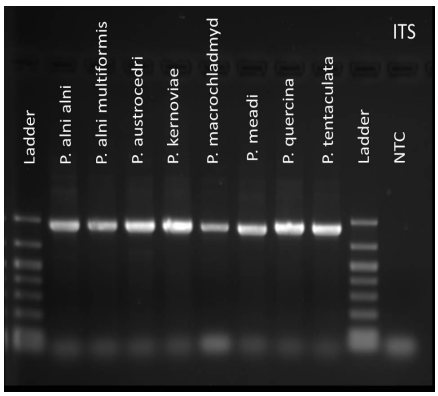
Gels: electrophoresis and photos

1. Set up a gel box with appropriate combs (i.e. 1.0 mm, 20 well)
2. Measure the amounts of agarose and 1x TAE buffer and add to an Erlenmeyer flask to make a 1.5% gel.
3. Heat the mixture in the microwave.
    a. Heat for 30 seconds on the highest power setting (i.e. 100%), then mix the solution thoroughly.
    b. Heat for 2 min at half power (i.e. 50%), then mix the solution carefully and thoroughly. Use a protective mitt/glove to protect your hands when handling the flask.
4. Allow the agarose to cool (approx. 5-10 min) prior to pouring the gel to avoid damaging the gel tray. The flask can be placed in a water bath programmed at 42°C to cool the agarose without it solidifying.
5. Pour the liquid into the gel tray and check for the presence of bubbles. If any are present, then a pipet tip can be used to pop them or drag them to the side of the gel. Rinse out the flask with water to avoid the agarose residue from sticking to the flask.
6. Allow the gel to sit for 30 min to solidify and set up. During this time, prepare the mix for the ladder and samples with dye as described in steps 9 and 10 below.
7. After 30 min, carefully transfer the gel tray into the electrophoresis apparatus, with the first row of wells in the gel closest to the black node.
8. Pour 1x TAE buffer into the apparatus until it covers the gel. Carefully remove the combs from the gel.
9. **Samples:** Mix 12 µL of the 25 µL total PCR reaction with 3 µL of 6X loading buffer. Mix by pipetting up and down and load mixture into the gel.
10. **Ladder:** Prepare DNA ladder samples as follows: Mix 3µL of 100 bp DNA ladder (i.e. BioVentures) with 9 µL of dH_2O and 3 µL of 6X loading buffer per ladder sample. Load ladders on both sides of the PCR samples (flanking), as shown in the figure to the right.
11. After all samples and ladders have been loaded, run the electrophoresis apparatus at 100 V for 60 min.
12. Transfer the gel from the gel tray to a staining tray containing ethidium bromide solution (300 mL water + 35 µL ethidium bromide (10 µg/mL) and place on a rotary shaker for 10-15 min.



13. Transfer the gel from the staining tray to a second tray containing distilled water to destain the gel and place back on the rotary shaker for 10-15 min.
14. Remove the gel from the water and document the assay results using a digital imaging system.
15. Dispose of the gel, and any other contaminated materials into the hazardous waste receptacle.

*Note: Ethidium bromide may be a carcinogen, a mutagen, or a teratogen and is considered **hazardous**. Do NOT use latex gloves to handle any ethidium bromide waste.* ***Please read the ethidium bromide SDS prior to handling and use.***

Citation
Abad Z.G. and Bienapfl J.C. 2019. Gels: electrophoresis and photos. Document control number: SOP-PID-04.01 *from* Abad, Z.G., Burgess T., Bienapfl J.C., Redford A.J., Coffey M., and Knight L. 2019. IDphy: Molecular and morphological identification of *Phytophthora* based on the types. USDA APHIS PPQ S&T Beltsville Lab, USDA APHIS PPQ S&T ITP, Centre for *Phytophthora* Science and Management, and World *Phytophthora* Collection. http://idtools.org/id/phytophthora/index.php, <date you accessed site>

Prepared by: Z. Gloria Abad, Ph.D. ZOILA ABAD Digitally signed by ZOILA ABAD Date: 2019.08.23 13:36:19 -04'00'

Submitted by:
KURT ZELLER Digitally signed by KURT ZELLER Date: 2019.08.27 15:16:37 -04'00'
Kurt Zeller, Ph.D.
Mycology Section Supervisor, Beltsville Lab

Approved by:
ASHLEE BARTH Digitally signed by ASHLEE BARTH Date: 2019.09.04 13:13:04 -04'00'
Ashlee Barth
Quality Manager, Beltsville Lab

MARK NAKHLA Digitally signed by MARK NAKHLA Date: 2019.09.05 10:18:39 -04'00'
Mark Nakhla, Ph.D
Laboratory Director, Beltsville Lab