

AsiaBlight

Protocol for sampling *Phytophthora infestans* DNA using FTA cards 2022

The process takes longer to explain than to do - it's really simple!

1. Use **1 card** with 2 sampling areas (circles) **per site** (field).
 2. **Sample 2 lesions per infected site**. If only one plant is infected, collect 2 separate lesions from the same plant. If there are numerous infected plants, collect 1 lesion from each of 2 separate plants distributed across the site.
 3. **Correct sampling is critical**. **Select leaflets with single, fresh, nicely sporulating young lesions for each sample** (Figure 1), otherwise we won't get good results (maybe no results at all). Avoid older lesions, dead leaves, old or dry lesions, leaflets with many lesions, wet, water soaked (bacterially infected) looking leaves and those with small restricted lesions.
 4. **Label the FTA card** with a reference number. Provide the rest of the information on the sample form.
 5. **Cut out a piece of sporulating lesion approx. 1-2 cm square** at the edge of the lesion (Figure 2) using sterilised scissors or scalpel (use an alcohol wipe).
 6. **Place the lesion sample on one clean circular sampling area on the FTA card sporulating side down**. Do not touch the FTA card sampling area except with the lesion sample!
 7. Replace the FTA card's cover sheet over the lesion sample and **apply moderate rubbing pressure to the sample** through the cover sheet using a blunt object (e.g. the blunt end of a marker pen or pestle). Don't rub too hard or you will damage card. When green leaf extract is visible on the back of the FTA card, the collection process is complete.
 8. **Remove excess plant residue from the FTA card**, ensuring that no large pieces of plant tissue remain stuck to the card (Figure 3). It doesn't matter if some small bits remain.
- Air-dry the FTA cards for at least 1 hour at room temperature.**
9. **Store dry FTA cards separately in paper envelopes**. Do not place several cards in the same envelope as this can result in cross-contamination.

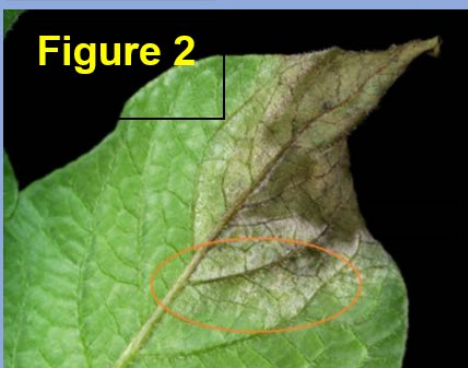


Figure 3

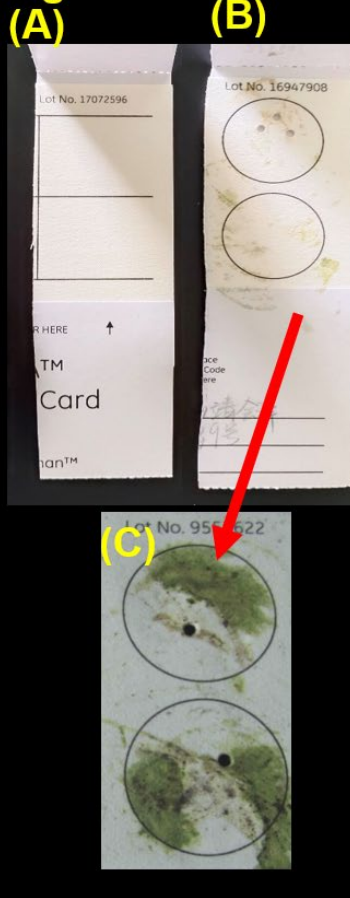


Figure 3. (A) An unused FTA card after arrival from AsiaBlight, (B) an FTA with samples and (C) and closeup of an FTA card after it has been used for SSR analysis (DNA has been extracted from the 2 mm disks punched out of each sample).