



Protocol for sampling *Phytophthora infestans* DNA using FTA cards

Despite this long text, **it's really simple!** Watch the YouTube videos for demo!

1. Use **1 card** with 2 sampling areas (circles) **per field**.
2. **Sample 2 lesions per infected field**, 1 lesion for each sample area (circle), preferably from the same plant.
3. **Label the FTA card** with a reference number (if not already done)
Provide the rest of the information on the sample form or XL sheet.
4. **Take the sample (instructions below)**
Do not touch the sampling area except with the *Phytophthora* sample!
5. **Fill out the sampling form or the XL sheet with the sample details**
Please use decimal lat/long codes
6. **Air-dry the card**, store and **return cards + XL sheet** to your local coordinator.



Sampling

Experience has shown that **sampling is the most critical process**.
Time spent sampling correctly is well spent!

Select:

Select leaflets with a single, fresh, nicely sporulating lesion for each sample (Figure 1). If the lesions are not sporulating they can be stored in separate moist plastic bags overnight to encourage sporulation.



Figure 1. Select a leaflet with a single lesion.



Figure 2. Select a fresh, sporulating area of the lesion and cut a sample 1-2 cm² from the area indicated.

Avoid:

- Dead leaves, old or dry lesions, leaflets with many lesions
- Wet, water soaked (bacterially infected) looking leaves
- Small restricted lesions



Collect sample on FTA card:

- Cut a 1-2 cm² piece of **sporulating lesion (Figure 2)**
- **Place the lesion sample inside a clean circular sampling area on the FTA matrix. Sporulating side facing down.**
- **Replace the cover sheet.**
- **Apply moderate pounding/pressure to the leaf sample through the cover sheet with a blunt object such as a small hammer, spoon, screw driver handle, car key or pestle. Take care not to damage the matrix.**
- When the **green leaf extract is visible on the back** of the FTA matrix the collection process is complete.
- **Remove plant residue from card**, ensure that no large pieces of plant tissue remain adhered to the FTA card (Figure 3).
- **Fill out the sampling form/XL sample sheet**
- **Allow the FTA card to air dry** for a minimum of one hour at room temperature.
- Store **dry** FTA cards separately in a paper envelope or plastic zip-lock bag.
- Collect cards + Sampling Forms and return to local coordinator.
- Figure 3 shows a card after processing in the laboratory so you can see what the next stage will be

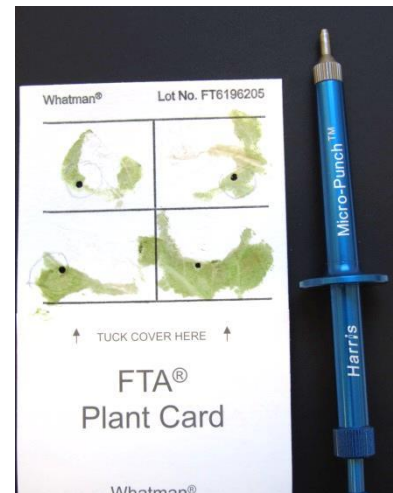


Figure 3. FTA card containing samples for SSR analysis. DNA has been extracted from the 2mm disks punched from each sample.

Materials needed:

- Knife/Scissors for sampling the lesion (clean after use!)
- Whatman FTA plant-card
- Pen/Pencil
- **Blunt** object such as a small pestle, small hammer etc
- Zip lock bag to store used and air-dried FTA cards

Related You Tube videos:

1. <https://www.youtube.com/watch?v=BQLe0G7vdHY>

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