Protocol for sampling *Phytophthora infestans* DNA using FTA cards 2013

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**.

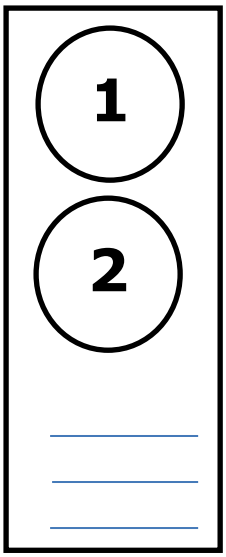
2. **Sample 2 lesions per infected field,** 1 lesion for each sample area (circle), preferably from the same plant.

3. **Label the FTA card** with a reference number (if not already done)
   Provide the rest of the information on the sample form.

4. **Take the sample (instructions below)**
   Do not touch the sampling area except with the *Phytophthora* sample!

5. **Fill out the sampling form**
   1 form per card, clearly state the reference number.
   Write clearly please!

6. **Air-dry the card,** store and **return card + sampling form** to your local coordinator.



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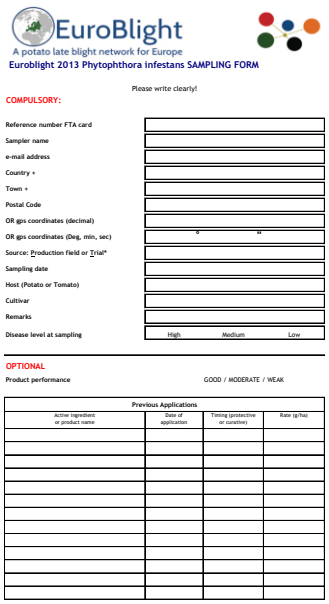

EuroBlight
A potato late blight network for Europe

Euroblight 2013 Phytophthora infestans SAMPLING FORM

Please write clearly!

COMPULSORY:

Reference number FTA card	
Sampler name	
e-mail address	
Country +	
Town +	
Postal Code	
OR gps coordinates (decimal)	
OR gps coordinates (Deg, min, sec)	° ''
Source: Production field or Trial*	
Sampling date	
Host (Potato or Tomato)	
Cultivar	
Remarks	
Disease level at sampling	High Medium Low

OPTIONAL

Product performance GOOD / MODERATE / WEAK

Previous Applications			
Active ingredient or product name	Date of application	Timing (protective or curative)	Rate (g/ha)

Sampling

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

Select:
Select a leaflet with a single, fresh, nicely sporulating lesion for each sample (Figure 1). Both samples may come from the same plant.

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 cm^2 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 cm^2 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.**
- **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. http://www.youtube.com/watch?v=Gir56iYspTE (Quick and dirty)
2. http://www.youtube.com/watch?v=BzQ-DEG9_aE (slow and for a lab environment)

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