DNA CAPTURING METHODS

Valentine Nakato, Idd Ramathani and Fen Beed, IITA-Uganda

Plant pathology Unit

FAO project: January 2010

OUTLINE

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Introduction

- Deoxyribonucleic acid (DNA) is a nucleic acid that contains the genetic instructions used in the development and functioning of all known living organisms and some viruses.

- The main role of DNA molecules is the long-term storage of information.

Methods for DNA capturing in the field

a) FTA Whatman Cards
b) Phytopass
c) 2 minute nucleic acid extraction Dip stick
d) Silica gel in vials

Advantages for using these methods

– Require very few chemicals from treatment to PCR preparation
– Samples are stored at room temperature
– Maintain the integrity of DNA for a very long time (5 years and more)
– Avoids transfer of pathogens across borders
– Easier to transport from field to any lab without any restrictions
– Cheap in the long run
– Doesn't require a lot of skills to collect
– Time duration is limited /short .

FTA Whatman card

- ✓ FTA cards is a chemically treated filter paper that allows for the rapid isolation of pure DNA
- ✓ Whatman FTA cards have been designed to obtain both DNA and RNA. They are impregnated with a blend of chemicals that lyses cell membranes and denatures proteins on contact, leading to trapping, immobilization, and stabilization of nucleic acids (Subrungruang *et al*. (2004)).

- ✓ Why use FTA cards – Easy Field collection
  - – No need to hire skilled personnel to collect samples
  - – Room temperature storage means you will always have access to your organism genetic information. After they are dry they can be kept at room temperature for decades and will still provide excellent DNA
  - – FTA Cards rapidly inactivate organisms, and prevent the growth of bacteria and other microorganisms.

- Capture nucleic acid in one easy step.
- Captured nucleic acid is ready for downstream applications in less than 30 minutes.
- FTA Cards are stored at room temperature before and after sample application, reducing the need for laboratory freezers.
- Suitable for virtually any cell type.
- Indicating FTA Cards change color upon sample application to facilitate handling of colorless samples
- FTA Cards are available in a variety of configurations to meet application requirements.

Use FTA for a wide range of applications

Forensics
Transfusion medicine
Food and agriculture testing
Genomics
Animal identification
Whole genome amplification
Transgenic identification
Plasmid screening
Drug discovery
STR analysis
Molecular diagnostics
Molecular biology

Phytopass

The system allows

1. Field sampling
    - Fast, easy and no need of additional tools
    - No risk of cross contamination
    - No risk of sample confusion
2. Transport and storage of samples
    - Stable at room temperature for several weeks
    - Easily dispatches by regular post mail

3. Preparation of crude extract
   - Extract is ready in less than 5 minutes
   - At least 4-6x faster than classic protocol
   - No freezing or grinding

2 minute nucleic acid extraction dipstick

These kits have been developed by CSL and are based on the (serological) Lateral Flow Device for plant pest identification.

Procedure for 2 minute dipstick

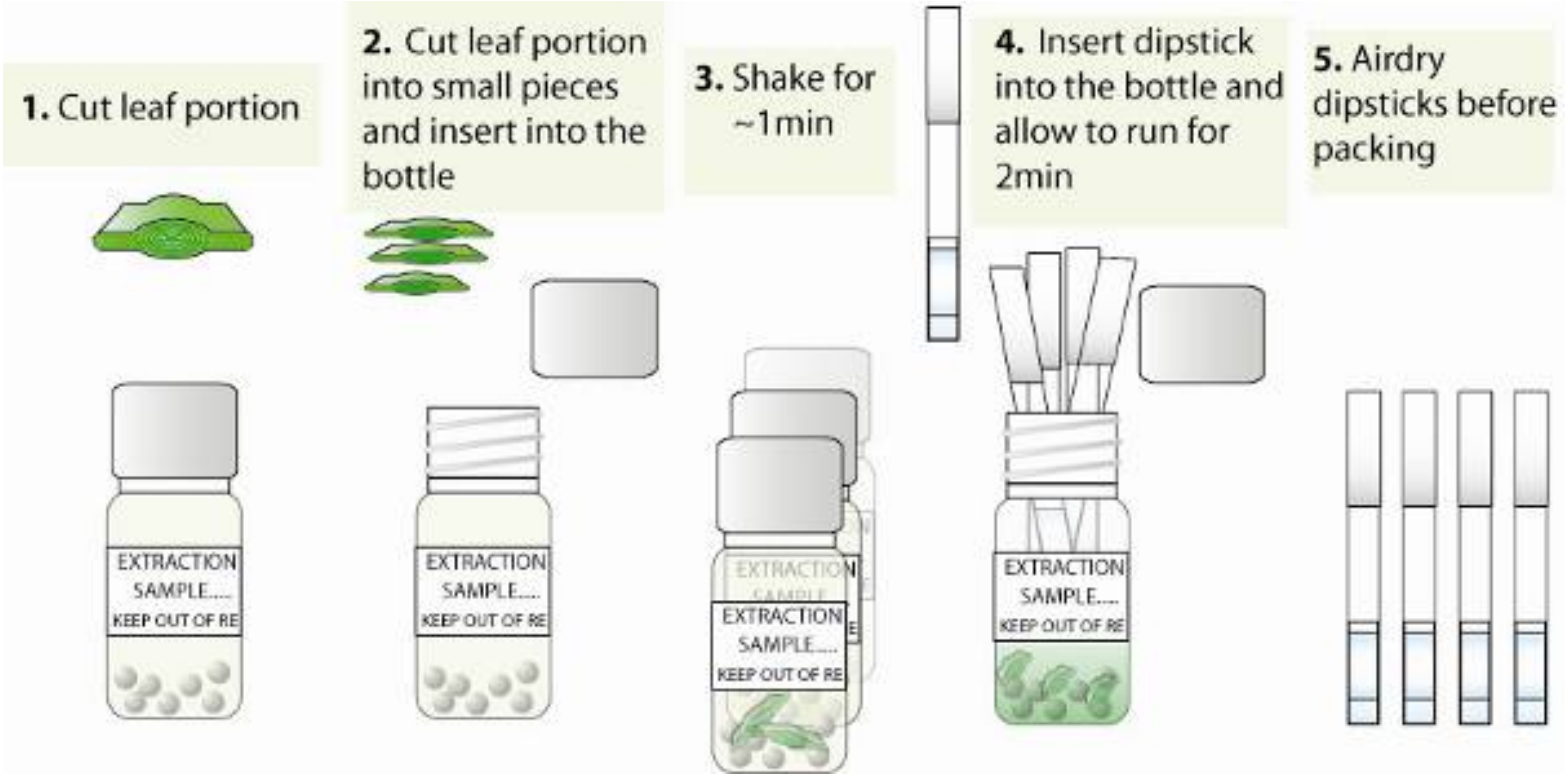
- Disrupt sample by placing <0.5g or 4cm^2 of leaf material into the bottle provided.

- Replace the lid and shake the bottle with the sample for between 15s seconds and 2 minutes[1]. It is necessary to break down the tissue, but not completely homogenise it; ‘soft’ tissue needs less shaking than ‘hard’ tissue.

- For a new sample type it is strongly recommended that the length of shaking required be optimized in a pilot experiment before testing large numbers of samples.

- Transfer ≈ 100μl of sample to a tube and insert the glassfibre release pad into the sample (a).

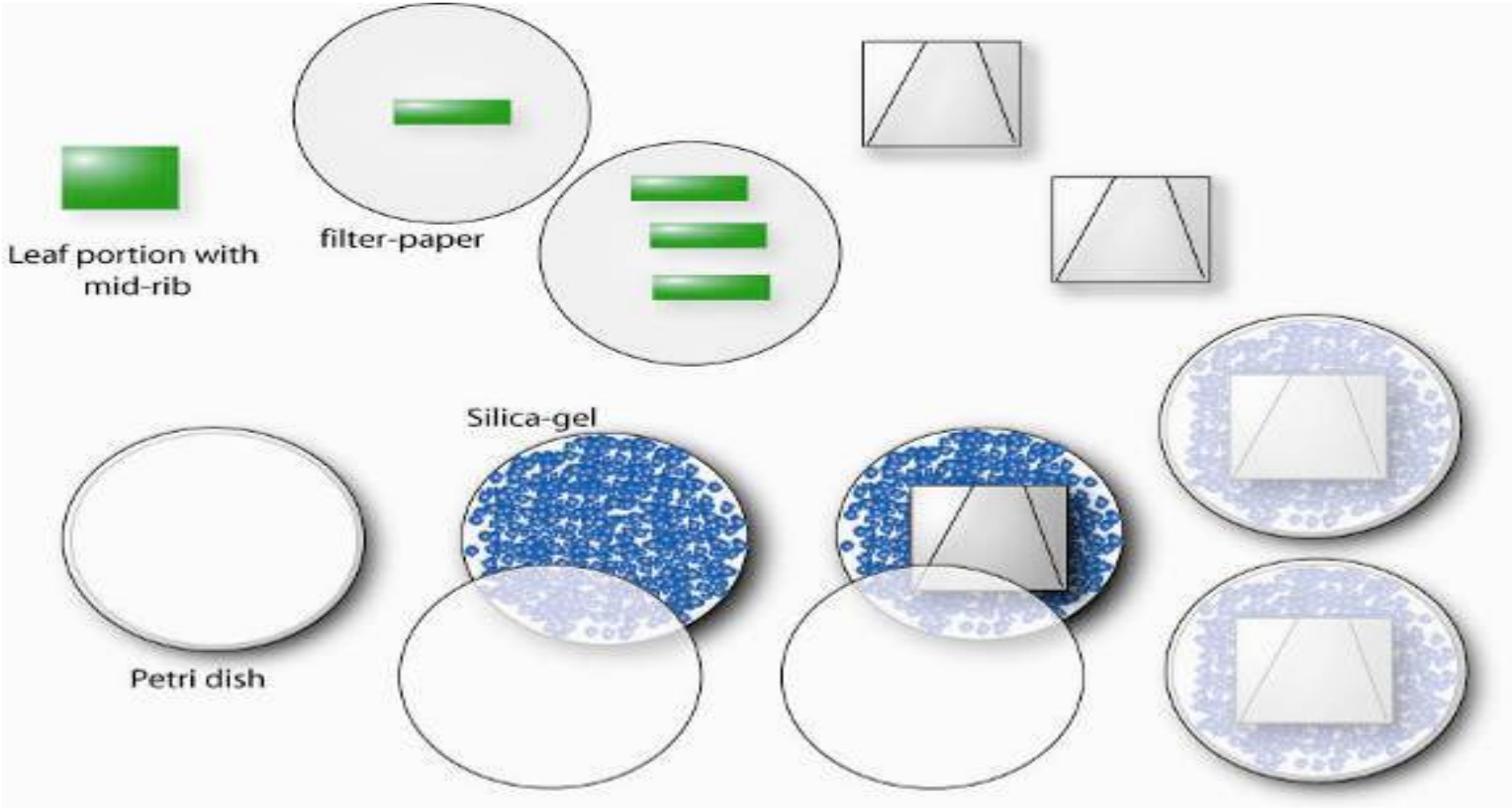
- Allow the dipstick to run for ≈ 2 minutes.
- Cut a section of membrane (≈1x2mm) from centre of device (b).
- Place membrane directly into PCR master mix (c) – cycle as usual.



Silica gel

- Desiccated silica gel placed in air tight sealed glass vials can be used to house diseased plant samples (~0.5 cm2). The purpose of the silica gel is to remove water from the plant sample thus preserving plant and pathogen DNA by preventing further metabolism and degradation from saprophytes

Silica-gel sampling scheme



- This is used for storage in country and not for dispatch across borders as too high risk of disease spread

THANK YOU..... QUESTIONS???????