Whatman FTA Protocol BC01

Processing Protocol for Downstream AFLP Analysis of Sample DNA on an FTA® Card

FTA® Technology

FTA Cards are impregnated with a patented chemical formula that lyses cell membranes and denatures proteins on contact. Nucleic acids are physically entrapped, immobilised and stabilised for storage at room temperature. FTA Cards protect nucleic acids from nucleases, oxidation, UV damage and microbial and fungal attack. Infectious pathogens in samples applied to FTA Cards are rendered inactive on contact. Samples collected on FTA Cards and enclosed in a multi-barrier pouch can be shipped through the post making them an extremely useful tool for field collection of blood, plants or other specimens.

Indicating FTA Cards turn from pink to white on sample application and are recommended for clear or colourless samples. CloneSaver™ Cards are optimised for the room temperature collection and storage of plasmid DNA.

Handling Instructions

- Always wear gloves when handling FTA or CloneSaver Cards to avoid contamination of the Cards.
- Store unused FTA or CloneSaver Cards in a cool, dry place (avoid light and excessive humidity).
- Follow universal precautions when working with biological samples.
- FTA or CloneSaver Cards are non-toxic to humans.

Materials Required

- Whatman FTA Card – Indicating FTA Cards are recommended for use with clear samples. If applied to non-Indicating Cards, circle the application spot with a ballpoint pen or pencil.
- Whatman FTA purification reagent (cat no. WB120204).
- TE^{-1} buffer (10mM Tris-HCl, 0.1mM EDTA, pH 8.0).
- 2.0mm diameter Harris micropunch or other paper punch.
- Proteinase K (10 mg/ml).
- Ligation buffer (ATP 1 µL + Adaptors EcoRI and MSe1 + 1 unit of T4 ligase).
- **Restriction enzymes of choice.**
- Restriction ligation buffer (BSA + Water).
- 2.0mL micro centrifuge tube with spin basket insert (e.g. Whatman VectaSpin™).
- Micro centrifuge capable of speeds up to 12,000 x g.
- 37°C and 60°C incubator.
- Whatman FTA Protocol BD09 "Removing a Sample Disc from an FTA or CloneSaver Card for Analysis".

The FTA Principle – Get it Right First Time, Every Time

FTA works by lysis of cells releasing the nucleic acid within the matrix of the Card, where the nucleic acid will be entrapped among the cellulose fibres. Therefore the key step to ensure success is getting DNA-containing cells into the FTA in the presence of moisture to activate the cell-lytic and DNA-protective chemicals.

Controls

It is recommended that internal standard controls are used during each analysis, these should include the following:

- Negative control.
- Positive control of a known DNA standard solution.

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Protocol

Preparing an FTA Disc for DNA Analysis

1. Take two sample discs from the dried spot following the instructions provided in the protocol entitled “Removing a Sample Disc from an FTA or CloneSaver Card for Analysis”, protocol number BD09. For plant samples a 2.0mm disc is recommended.
2. Place discs in the bottom of a spin basket insert.
3. Place the spin basket into a 2.0mL micro centrifuge tube.
4. Add 500µL FTA purification reagent to the basket.
5. Incubate for 1 minute at room temperature.
6. Centrifuge at 6000 x g for 30 seconds.
7. Remove the basket and decant used reagent.
8. Return the basket to the micro centrifuge tube and repeat Steps 4-7 twice for a total of 3 washes with the FTA purification reagent.
9. Add 495µL FTA purification reagent and 5µL Proteinase K (10mg/mL) to the FTA discs in the basket.
10. Incubate for 1 hour at 60°C to remove residual Histones.
11. Centrifuge at 6000 x g for 30 seconds.
12. Remove the basket and decant used reagent/buffer.
13. Return the basket to the micro centrifuge tube.
14. Add 500µl of TE^{-1} buffer to the tube.
15. Centrifuge at 6000 x g for 30 seconds.
16. Remove the basket and decant used buffer.
17. Return the basket to the micro centrifuge tube.
18. Repeat steps 14-17 twice for a total of 3 washes with TE^{-1} buffer.
19. Transfer discs to a new tube.
20. Ensure that all the liquid has been removed before performing analysis.

Restriction Step

21. Add 2 restriction enzymes to the two FTA washed discs, a frequent cutter and a rare cutter (e.g. EcoRI and MSe1).
22. Add 4 units of each in restriction ligation buffer (BSA + Water) for a final volume 30µL.
23. Incubate for 1 hour at 37°C.
24. Mix solution by pipette.

Adapter Ligation Step

25. Draw off 30µL of restriction buffer leaving the discs behind.
26. Add to a fresh tube and add 10µL of ligation buffer (ATP 1µL, Adaptors EcoRI, MSE1 and 1 unit of T4 ligase).
27. Incubate for 3 hours or overnight at 37°C.

Preamplification Step

28. As per your standard protocols.

Selective Amplification Step

29. As per your standard protocols.

Technical Help

If you experience any problems with this protocol or wish to obtain additional information please contact Whatman Technical Service Team on the following regional numbers. Alternatively, please visit www.whatman.com for additional product information and further contact details. This protocol was developed by a Whatman customer and represents a suggested use. This protocol has not been validated by Whatman directly and therefore, Whatman is limited in the amount of direct support available. However, Whatman will make every effort to assist and resolve any issues.

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