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Rapid Simple Sequence Repeat Genotyping using FTA Cards and High Resolution Melt Analysis

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ABSTRACT

Molecular biology tools are often designed to be applicable in the lab to provide answers to specific research questions. However, many of the techniques cannot be readily applied as high throughput low cost approaches that would have utility for commercial industries or certification agencies. In genetic marker analysis two areas that increase costs and slow throughput are DNA extraction and fragment analysis. In an effort to address these problems we have tested the utility of rapid DNA extraction utilizing FTA cards and rapid marker scoring utilizing High Resolution Melt (HRM) analysis in two turfgrass genera.
In this study two grass species (*Agrostis stolonifera,*and *Danthonia spicata)* were tested. DNA was extracted and stored using FTA cards followed by primer specific PCR and high resolution melt analysis. Concentrations of DNA extracted via FTA card were quantified by a Nano Drop and found to be at satisfactory concentrations for successful amplification using PCR. The FTA cards containing transferred plant material were stored in a dry (humidity controlled) area where they can last several months without deterioration. Results show that FTA cards can provide researchers with an effective and efficient tool for collecting storing and extracting grass DNA. The results indicate that FTA card DNA extraction combined with high resolution melt analysis after PCR can be used for rapid SSR genotyping in turfgrass species.

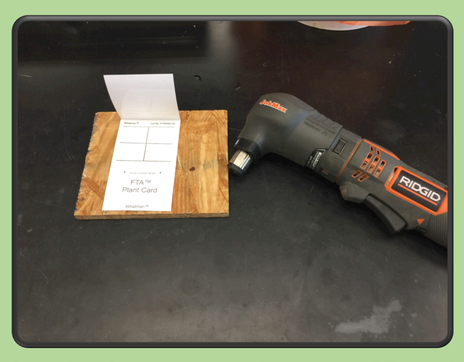
Danthonia spicata

Agrostis stolonifera

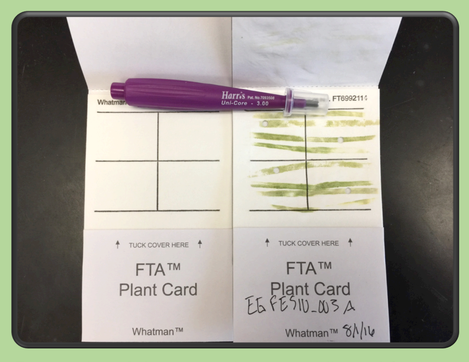
Danthonia spicata is a native cool-season grass commonly known as poverty oatgrass that is highly drought tolerant and able to thrive in low nutrient soils. *D. spicata* is likely apomictic and we are developing molecular marker methods to rapidly identify novel sources of variation.

Agrostis stolonifera is a cool-season grass commonly grown on golf courses. Enhanced biotic and abiotic stress tolerance are important breeding objectives for *A. stolonifera*. The *Agrostis* genus is large with numerous species that exhibit enhanced biotic and abiotic stress tolerance. The utilization of molecular markers to rapidly identify interspecific hybrids is being explored.

FTA-Card DNA Extraction



Collection: Samples are crushed onto an FTA-card using an auto-hammer and a wood support.



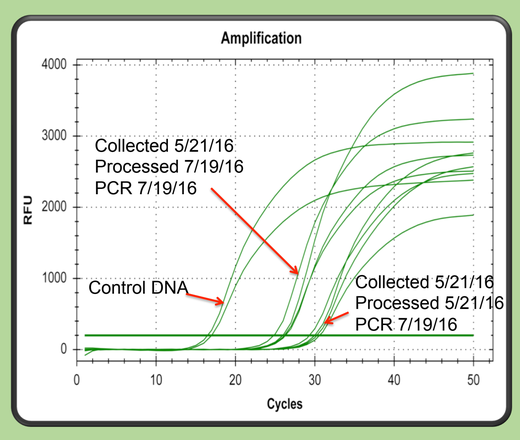
Processing: 3.0mm punches are collected from FTA-cards using a Harris hole punch then washed 2x with FTA-card solution and 2x with TE. 50 µl of TE is added and DNA is released from the disk after a 30 min 98° C incubation and 60 pulses on a vortexer. The disk is discarded and 2 µl is used in each 10 µl PCR reaction.

PCR: Bio-Rad CFX-96 machine was used for RT-PCR with Eva-green as the detection dye.

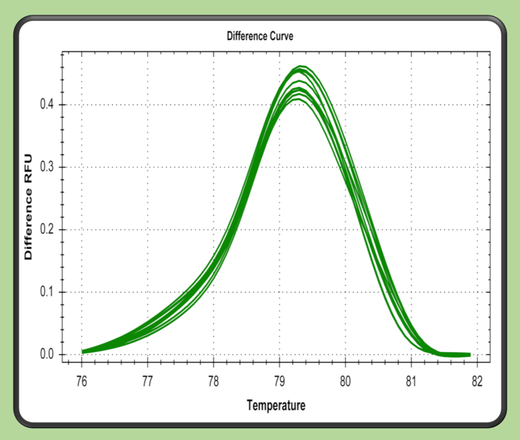
Benefits of FTA-card DNA extraction:

- Samples can be collected when tissue is in optimum condition and processed several months later.
- Multiple samples can be collected per card lowering the per sample extraction costs well below $1.
- Sixteen leaves per card would cost about 50¢ per sample, conventional kits are about $4/sample.
- The process is reasonably fast, 96 samples can be processed by one person in a couple hours.
- Liquid handling could increase the speed considerably.

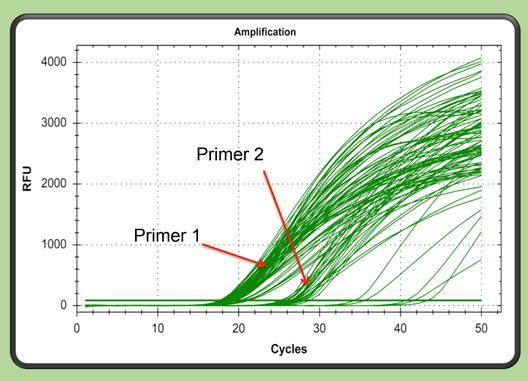
High Resolution Melt Analysis



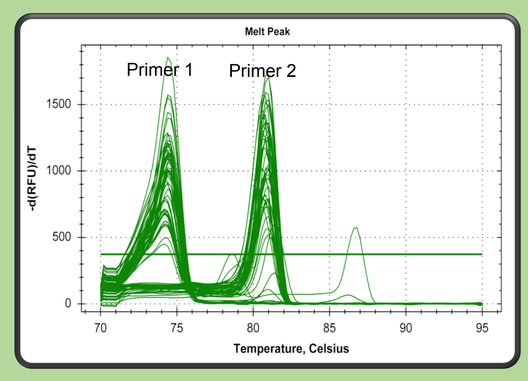
D. spicata DNA amplified using the Bio-Rad CFX96 thermocycler. Control kit extracted DNA begins amplification earlier than FTA-card extracted DNA. DNA stored on the FTA-card for 2 months amplified earlier than DNA collected on FTA-cards processed the same day and stored for 2 months.



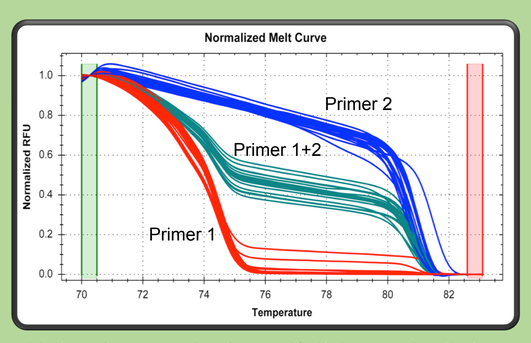
All *D. spicata* DNA samples produced the same melt profile when scored using Bio-Rad Precision Melt Analysis™ Software



Thirty two *A. stolonifera* FTA-card extracted DNA samples were amplified with one of two SSR primer pairs or a multiplex reaction with both primer pairs included. All thirty two samples produced PCR products.



The use of species specific primer pairs that have different melt temperature peaks can allow for hybrid detection.



Melt analysis can clearly identify DNA samples that are amplified with both primer pairs and therefore putative hybrids.

Conclusions:

- FTA-card extracted DNA and High Resolution Melt analysis can be reliably used for molecular marker application in *D. spicata* and *A. stolonifera*.
- The overall cost per sample in this analysis was about 85¢ compared to potentially 10x that amount for kit extration and fragment analysis.
- A considerable savings in time is also expected as extraction and analysis can easily be preformed in one day.