Direct amplification of samples associated with strawberry leaf punches

Abstract

Biological extractions are essential for collection of nucleic acids and removal of PCR inhibitors prior to quantitative PCR (qPCR). In this document, we will introduce you to IDT's PrimeTime™ One-Step 4X Broad-Range Master Mix as an alternative to the costly and time-consuming extraction steps. Here, demonstrated extraction free amplification can efficiently amplify leaf punch samples when compared to extracted samples. Furthermore, the data suggest that amplification still occurs when leaf particulate is present within the reaction but is more pronounced in reactions where the leaf particulate is removed.

Introduction

Susceptibility testing in agriculture products has become mainstream in today's culture due to changes in climate, increased pressure from disease, and increased demand from consumers for higher quality food. The market has undergone similar pressures as consumers are pressing for larger, juicer berries with longer shelf life and more flavor. The advancement of the genomic age has introduced numerous advancements to our identification of markers that can improve the overall quality of our food including the addition of qPCR and next generation sequencing (NGS). While NGS provides a wealth of information, like culturing, the turnaround time associated with sequencing can be days to weeks and costs considerably more than qPCR-based tests.

qPCR is gaining popularity within the testing community as the go-to method for novel tests. qPCR offers distinct advantages over NGS and cell culture including a reduction in cost and turnaround time. In addition to the above advantages, qPCR also allows for increased accuracy in discriminating between pathogens and availability to query multiple targets in a single reaction through multiplexing assays. Traditionally, however, steps were needed to extract nucleic acid from the sample of interest, and this would slow down the time from sample collection to results by 1-2 hours. The advent of IDT's PrimeTime One-Step 4x Broad-Range Master Mix is changing how researchers approach their set-up. The Broad-Range master mix allows researchers to skip the timely extraction step and go directly from sample collection to amplification without the loss in activity, a basic outline of the testing workflow is shown in Figure 1. Furthermore, eliminating the extraction step will save researchers $1-2 per sample per extraction. Below we will explore direct amplification of strawberry leaf punches and how the data compares to the same samples following traditional extraction protocols.

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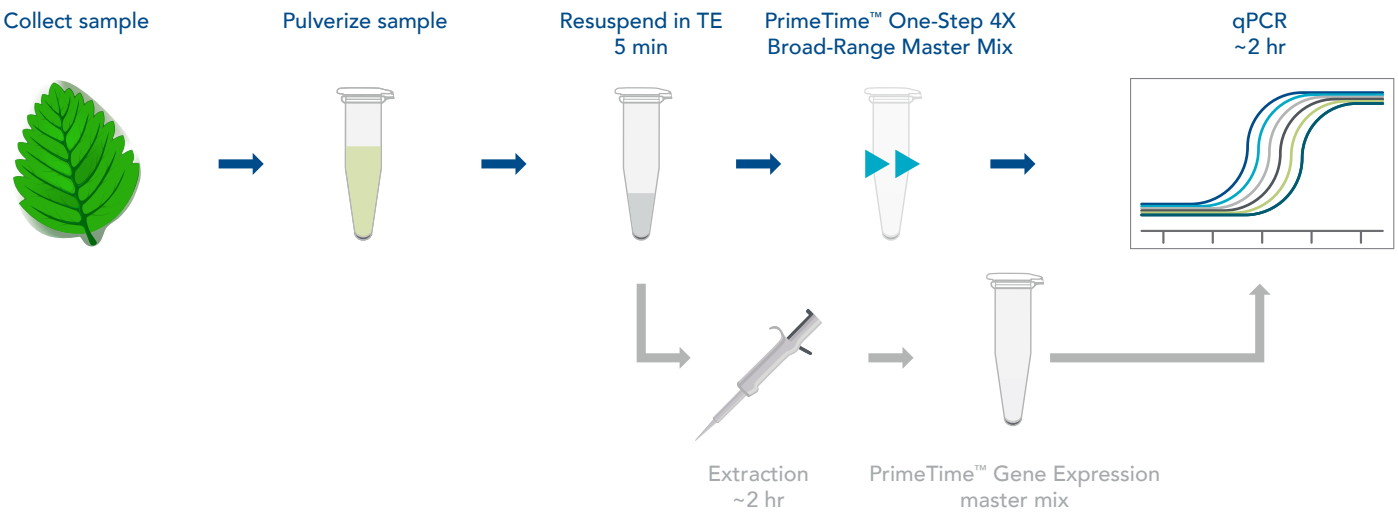

Figure 1. Basic workflow of susceptibility testing in strawberry leaf punches. Utilizing the PrimeTime One-Step Broad-Range Master Mix gives the researcher the opportunity to shorten the workflow by skipping the lengthy and costly extraction step (2 hours and $1-2/sample for extraction).

Materials and Methods

Quantitative PCR

qPCR was performed using PrimeTime One-Step 4X Broad-Range Master Mix with enhancer solution (Integrated DNA Technologies) with custom DNA oligos for primers (500 nM final concentration; from Integrated DNA Technologies) and Affinity Plus probes (250 nM final concentration; from Integrated DNA Technologies). For the plant samples, 100 µl of TE buffer was added to each pulverized sample then extracted or added directly to the PCR reaction. After addition of the buffer or extraction, 5.91 µl of the samples were added to 10 µl reactions. Cycling conditions were: 50° C for 15 min, 95° C for 3 min followed by 40 cycles at 95° C for 15 sec, then 60° C for 1 min.

Results

Amplification of extracted vs unextracted samples

Normal laboratory workflow requires crushing of the leaf sample followed by extraction prior to PCR to look for the presence of resistance markers. To establish a baseline for direct PCR amplification, validated laboratory workflows were followed, and 96 samples were checked for the presence or absence of a targeted resistance marker (Figure 2A). Amplification of the extracted samples proved mostly unreliable as there was no discernable clustering of the resistant samples nor was there discernable clustering of the susceptibility marker (Figure 2A, left panel). Utilizing the same samples and protocol, extraction was skipped in favor of direct addition of the sample following a centrifugation step to remove the remaining leaf particulate. The data generated from the direct amplification provided clean clustering of resistant or susceptible samples (Figure 2A, right panel).

Amplification of samples with or without leaf particulate

Direct amplification of leaf samples showed strong results so it was postulated that keeping the leaf samples within the reaction may improve amplification through additional release of DNA during the heat lysis step. For a comparison, the above leaf sample was used with a portion of the sample undergoing centrifugation while a second portion of the sample went directly into the PCR reaction with leaf particulate present (Figure 2B). While the sample containing the leaf particulate performed very well, the centrifugation step prior to PCR resulted in a cleaner amplification plot and tighter clustering.

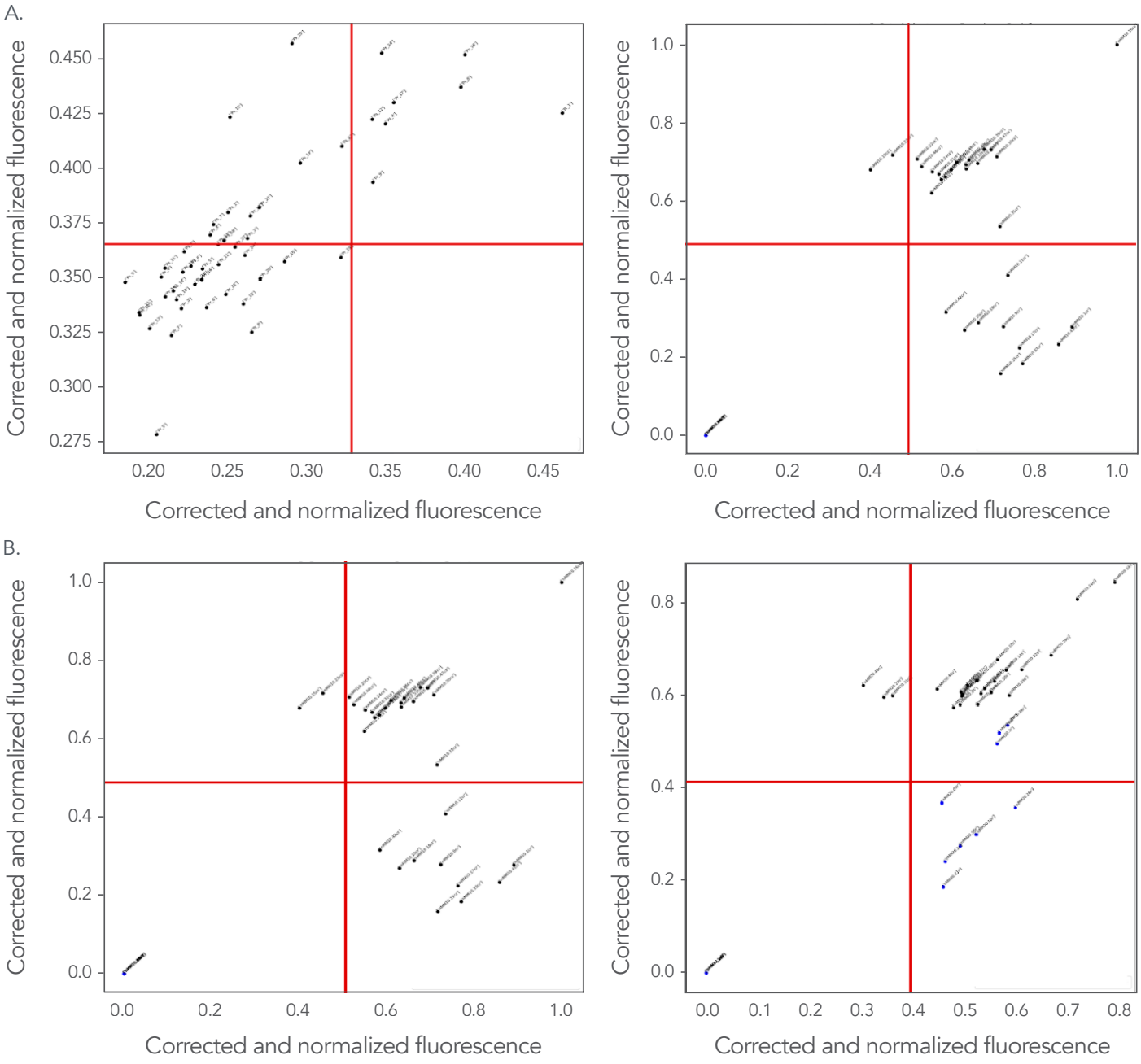

Figure 2. Amplification data for susceptibility markers in strawberry leaf punches. (**A**) Comparison of susceptibility markers when samples were extracted using PrimeTimeTm Gene Expression Master Mix (left panel) or direct amplification of pulverized leaf punch samples using PrimeTime™ 1-step Broad Range Master Mix (right panel). (**B**) Comparison of susceptibility markers in samples where leaf particulate was removed by centrifugation (left panel) or left in the PCR reaction amplified with PrimeTime 1-step Broad Range Master Mix (right panel).

Conclusion

The results demonstrated here show that the use of IDT's PrimeTime One-Step 4X Broad-Range Master Mix is an effective solution for direct amplification for identification of resistance markers in strawberry leaf samples. Furthermore, samples containing leaf particulate performed nearly as well as samples where the leaf particulate was removed by a centrifugation step prior to the PCR step. The implementation of direct amplification results in faster times from sample collection to analysis of data and can reduce overall experimental costs by removing expensive extraction steps.

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