Application Note

DNAble® Assay for Detecting Adventitious Presence of KK179 and *CP4 EPSPS* in non-GMO Alfalfa Seed Lots

DNAble

ENVIROLOGIX

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ABSTRACT

Nucleic acid amplification methods such as PCR are widely used to detect adventitious presence of GMOs in commercial seed lots. In this study, a novel isothermal nucleic acid amplification technology (DNAble) was validated for the low-level detection of GMO traits in alfalfa seed lots. Crude sample extractions were prepared from bulk grinds containing 750 seeds per grind. Crude extract was assayed using DNAble master mix for the detection of a *cp4 epsps* gene coding sequence and the KK179 GMO event using an Axxin T-16 ISO instrument. The entire testing process, from grinding seed to assay results, was completed in 65 minutes. Results of the validation support a test accuracy of >97.0% at the 95% confidence interval. In conclusion, DNAble was proven to have excellent test accuracy while drastically reducing the time to result compared to the standard PCR method (a 385% gain in workflow efficiency compared to PCR was realized).

INTRODUCTION

DNAble® is a rapid, isothermal amplification technology that uses molecular beacons to specifically detect amplified nucleic acid sequences. The DNAble system is tolerant of crude sample extracts and therefore no DNA purification is required, removing costly and time-consuming steps from the testing workflow. Since DNAble amplifies and detects target DNA sequences at a constant temperature, assay results are generated in as little as 5 minutes with excellent analytical sensitivity and specificity. The simple sample preparation combined with the rapid assay run time makes DNAble effective in low to high throughput testing environments where time to result and testing efficiency are critical for success.

In this study, DNAble assays were utilized to screen alfalfa seed lots for GMO adventitious presence (AP). AP is defined as the undesirable, low level contamination of a seed lot with seed of an undesirable genotype. In this case, the seed producer needed to certify that an alfalfa seed lot had <0.1% GMO contamination to market their seed as Non-GMO. The previous AP test was PCR-based and had an unacceptably long time to result (5.3 hours). The DNAble based AP test was able to deliver equivalent test accuracy as the PCR method while decreasing the time to result from 5.3 hours to 1.1 hours.

MATERIALS AND METHODS

1 | Instrumentation & Workflow

A Geno Grinder was used to perform a 3x2.5-minute grind. This was followed by using a Benchmark Scientific MyBlock™Mini for a 6-minute, 95°C sample extraction. These samples are diluted then spun down using a centrifuge capable of 10,000 x g. These were diluted a second time and the final samples were then used in the reaction. To maintain reaction temperature and capture fluorescence data resulting from amplification on an Axxin T16 ISO® Isothermal Fluorometer that was set to 56°C for 15 minutes.

2 | DNAble® Assay Reagents

The qualitative assay reagents for KK179 and CP4 epsps were prepared as lyophilized material in a clear PCR 8 well strip requiring only the addition of a Reaction Buffer to resuspend active reagents. This strip was formatted as a palindrome with 3 replicates of KK179 and 1 replicate of RR2 to be used per sample extract in the following format:

Figure 1. Assay master mix strip setup.

3 | Samples for Testing

Ground alfalfa seed samples were prepared in house by first placing seeds overnight in a 45°C incubator. Samples were then processed as 750-seed batches, contained in 15 mL tubes, and sealed with silicon padded lids. Each tube containing two 7/16" steel grinding balls and samples were placed in the Geno Grinder for 3 rounds of grinding (2.5 minutes @ 2000 SPM). Additional rounds of grinding were added to any sample where whole seeds were still visible following initial processing. Multiple 750-seed batches of ground conventional seed were pooled to make enough bulk conventional seed sample for the validation. Positive samples were created by mixing 1/3 ground conventional seed with 1/3 ground KK179 seed stock and 1/3 ground RR2 seed stock, by weight, that were on average 3 copies of trait per genome prior to mixing. The resulting mixture is a mock 100% KK179 and 100% RR grind containing 1 copy per genome of both traits. The percent GMO samples to be tested according to the validation plan were then made by diluting from the 100% stock with bulk ground conventional seed. Samples were then extracted by heating 2, 15 cc scoops worth of ground material at 95°C with extraction buffer for 6 minutes, adding a dilution buffer and centrifuging at 10,000 x g for 3 minutes. Finally, 25uL of sample supernatant was diluted in 25uL reaction buffer to be run in the DNAble assay.

RESULTS

4 | DNAble® Validation

The samples and master mix were manually pipetted by two users. The resulting validation of this low throughput method showed that the accuracy of the KK179 and CP4 epsps in alfalfa seed assay, using crude (un-purified) bulk seed samples, was ≥97.0% with a 95% confidence interval.

Trait/Sample Condition	Biological Replicates	Technical Replicates	Total # of Rxns	Deviant Calls	Invalid Calls	KK179 Accuracy (95% CI)	*CP4 EPSPS* Accuracy (95% CI)
0.0% KK179	156	3	468	0	0	98.1%	N/A
0.13% KK179	156	3	468	0	0	98.1%	N/A
0.0% *CP4 EPSPS*	156	1	156	0	0	N/A	98.1%
0.13% *CP4 EPSPS*	156	1	156	1	0	N/A	97.0%

Table 1. Validation results for the KK179 and CP4 epsps in bulk alfalfa seed assay. A deviant denotes a false call while an invalid is determined by a failure of the internal control to achieve amplification.

DISCUSSION

In conclusion, the KK179 and CP4 epsps in alfalfa seed workflow and validation provides a quick time to result in a crude sample matrix resulting in less hands-on user time and less wait time. When direct labor and lead time was compared to the current PCR standard testing method, DNAble provided an overall 385% gain in labor productivity while maintaining a high level of accuracy.

Technology	Direct Labor or Lead Time	Grinding	Sampling	Extraction	Detection	Total Time
DNAble	Direct Labor	20 min	5 min	12 min	7 min	44 min
DNAble	Lead Time	20 min	5 min	20 min	20 min	65 min
Standard PCR	Direct Labor	20 min	120 min	45 min	20 min	205 min
Standard PCR	Lead Time	20 min	120 min	120 min	60 min	320 min

Figure 2. DNAble Method vs Standard PCR for Alfalfa Seed AP Testing. Direct labor indicates hands on time whereas lead time indicates total time required to complete a process step. This exemplifies the ability of the DNAble solution to be beneficial and accurate in markets where samples need to be run in quick succession while maintaining a simple workflow process.

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