Application Note

DNAble® Assay for MON89788 Event ID/Zygosity in Soy Leaf

ENVIROLOGIX

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ABSTRACT

Determination of GMO trait zygosity using nucleic-acid based amplification is widely used in plant breeding and corn parental line maintenance programs. In this study, a novel isothermal nucleic acid amplification technology (DNAble) was validated for determining Event MON89788 zygosity in soy leaf. Crude soy leaf extracts were assayed using a duplexed DNAble reaction in a medium to high-throughput testing workflow. The entire process from sample extraction to result interpretation was completed in 40 minutes. All validation testing was completed using 5 µL reactions run on the Roche LightCycler480. Results indicated 95.6% or greater accuracy with a 95% Confidence Interval across homozygous MON89788 leaf, conventional leaf, and mock heterozygous leaf tissue. In conclusion, DNAble was proven to have excellent test accuracy while drastically reducing the time to result compared to the standard PCR method (>2-fold reduction in time to result).

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 duplexed assays were utilized to determine Event MON89788 zygosity in a soy leaf sample. Zygosity determination is required when performing trait fixation or parental line maintenance to ensure high purity breeder seed and therefore successful commercial seed production. The use of DNAble increases zygosity testing efficiency compared to PCR leading to at least a 2-fold gain in testing productivity.

MATERIALS AND METHODS

1 | Instrumentation & Workflow

A VWR 1500E incubator was used to perform a 30 minute, 95°C crude sample extraction followed by hand pipetting of the reagents using a multichannel pipette (both MM and sample) into Axygen 384 well PCR plates. To maintain reaction temperature and capture fluorescence data resulting from amplification, a Roche LightCycler® 480 II running LightCycler® 480 software v1.5.1.62 was set to 56°C for 15 minutes. Cold chain of the assay master mix and 384 plate was maintained prior to placing into the thermocycler.

2 | DNAble® Assay Reagents

The multiplex, qualitative assay for detection of the event ID MON89788 and a conventional/control target in soy leaf tissue was prepared as lyophilized material requiring only the addition of a Reaction Buffer to resuspend active reagents. This assay system amplifies crude sample nucleic acid sequences under isothermal conditions within fifteen minutes.

3 | Samples for Testing

Samples for this study included fresh and lyophilized soy leaf tissue from known homozygous MON89788 leaf or conventional leaf material. A mock hemizygous leaf sample was prepared by splitting known homozygous leaf samples in half and combining prior to processing. Leaf samples were processed by the addition of 200µL of MB15 extraction buffer followed by heating in a VWR 1500E incubator for 30 minutes @ 95°C. These samples were then allowed to cool on the benchtop for an additional 15 minutes. Extract was then taken directly from the leaf sample tubes and diluted 1:1 with DNAble master mix in a 384 well PCR plate (2.5 µL of sample + 2.5 µL of master mix).

RESULTS

4 | DNAble® Validation

The samples and master mix were manually pipetted by two users across the total 1,152 sample replicates run during the validation. The resulting validation of this medium throughput method showed that the accuracy of the multiplex MON89788 soy zygosity assay, including both fresh and lyophilized leaf samples, was ≥95% with a 95% confidence interval.

Trait/Sample Condition	Condition	#Pos/Total	Conventional Assay Accuracy	
			No CI	95% CI
Conventional Leaf	Fresh	192 / 192	100%	98.5%
	Lyophilized	192 / 192	100%	98.5%
	Combined	384 / 384	100%	99.2%
MON89788+ Leaf	Fresh	0 / 192	100%	98.5%
	Lyophilized	0 / 192	100%	98.5%
	Combined	0 / 384	100%	99.2%
Mock Het. Leaf	Fresh	185 / 192	96.4%	93.3%
	Lyophilized	189 / 192	98.4%	96.0%
	Combined	374 / 384	97.4%	95.6%
Trait/Sample Condition	**Condition**	**#Pos/Total**	**MON89788 Assay Accuracy**	
			No CI	**95% CI**
Conventional Leaf	Fresh	1 / 192	99.5%	97.6%
	Lyophilized	1 / 192	99.5%	97.6%
	Combined	2 / 384	99.5%	98.4%
MON89788+ Leaf	Fresh	192 / 192	100%	98.5%
	Lyophilized	192 / 192	100%	98.5%
	Combined	384 / 384	100%	99.2%
Mock Het. Leaf	Fresh	191 / 192	99.5%	97.6%
	Lyophilized	191 / 192	99.5%	97.6%
	Combined	382 / 384	99.5%	98.4%

Table 1. Validation results for Soy Zygosity assay

DISCUSSION

In conclusion, the DNAble soy zygosity screen provides a rapid time to while maintaining industry-standard accuracy rates (>95% sensitivity/specificity with 95% confidence interval). The operational advantage over standard PCR, along with the cost savings associated with crude sample preparation, leads to significant gains in a commercial testing environment where efficiency is key. Additionally, DNAble technology provides flexibility in point-of-detection for field-based applications; users are now able to survey fields in real-time, producing actionable data that can be leveraged within the same day as opposed to sending out samples for third party and/or laboratory-based testing.

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