

Excellent sensitivity and wide detection range

To examine the sensitivity and detection range of the kit, a serial dilution of Lambda DNA ranging from 10^7 to 128 copies was produced. Each qPCR reaction included: 1X TAQXpedite™ GREEN Real-Time PCR MasterMix, 12.5 pmole of forward and reverse primers, and the appropriate amount of lambda DNA. Cycling conditions were 30 seconds at 98°C, followed by 35 cycles of 1 second at 92°C and 6 seconds at 70°C.

FIG 2 shows that, in as little as 30 minutes, the TAQXpedite GREEN MasterMix Kit produces excellent qPCR sensitivity and efficiency with a wide dynamic range. The R^2 values and PCR efficiency indicate that the TAQXpedite GREEN MasterMix Kit

accurately determines the copy number present over an extremely wide detection range, ensuring accurate measurement of even low abundance transcripts.

Conclusion

The TAQXpedite GREEN Real-Time PCR MasterMix Kit enables qPCR reactions to be performed in as little as 30 minutes using standard qPCR instrumentation. Even with faster reaction times, results show that the TAQXpedite GREEN MasterMix Kit produces excellent values for R^2 and PCR efficiency while maintaining high sensitivity. The same excellent qPCR data were obtained after incubating the TAQXpedite GREEN reaction mixtures at room temperature for one hour prior to thermocycling, demonstrating its applicability in high-throughput applications.

References

1. Henke, W. *et al.*, (1997) *Nucl. Acids Res.* **25**(19), 3957.
2. Abu Al-Soud, W. and Rådström, R. J. *Clin. Microbiol.* **38**(12), 4463.

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NEW! TAQXpedite™ GREEN Real-Time PCR MasterMix Kit

TXG70796 96-25 µl Reactions
TXG707400 400-25 µl Reactions
Contents: 2X MasterMix with Enzyme Blend, Passive ROX Reference Dye, Stabilizer, and Nuclease Free Water.

Use of Betaine for DNA Polymerase Reactions, including, but not limited to use for PCR or DNA Sequencing, is covered by, U.S. Patent No. 6,270,962, European Patent No. 0742838, German Patent No. DE4411588C1 and other issued or pending applications in the U.S. and other countries that are either assigned or exclusively licensed to EPICENTRE. These products are accompanied by a limited non-exclusive license for the purchaser to use the purchased products solely for life science research. Contact EPICENTRE for information on licenses for uses in diagnostics or other fields.



Easy and Rapid Extraction of Plant DNA Using the QuickExtract™ Plant DNA Extraction Solution

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The QuickExtract™ Plant Solution enables the inexpensive processing of one to hundreds of samples simultaneously without centrifugation, spin columns, or the use of toxic organic solvents.

Introduction

Currently, there is no simple effective solution for high throughput extractions of plant leaf DNA. Many methods require multiple steps and often more than one expensive microtiter dish. Traditional hexadecyltrimethylammonium bromide (CTAB) based methods are laborious, and kits based on spin columns are expensive and not designed for high throughput. These methods also produce DNA samples that are likely to contain polyphenolic compounds and polysaccharides, which can cause the DNA to be unsuitable for PCR amplification.

EPICENTRE's new QuickExtract™ Plant DNA Extraction Solution is a simple, rapid, and scalable plant leaf DNA extraction method for PCR-based applications, including Random Amplification of Polymorphic DNA (RAPD) and Short Tandem Repeat (STR) analyses end-point PCR, or quantitative PCR. The QuickExtract Plant Solution enables the

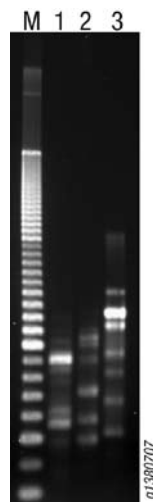


FIG 1. RAPD analysis using the QuickExtract™ Plant DNA Extraction Kit. One microliter of undiluted QuickExtract Plant DNA from the leaves of various species of plants were amplified with the RAPD primer UBC-888 (5'-BDB(CA)_n-3'), where B=G/T/C and D=G/A/T. The PCR products were separated on a 2% agarose gel and were visualized by staining with SYBR® Gold. **Lane M**, 100 bp ladder markers; **Lane 1**, pepper; **Lane 2**, soybean; **Lane 3**, spelt.

inexpensive processing of one to hundreds of samples simultaneously without centrifugation, spin columns, or the use of toxic organic solvents. It is also an excellent option for high throughput applications, and is completely compatible for use with robotic systems. The 8-minute, 1-tube protocol

requires no freezing, grinding, or bead beating and yields PCR-ready plant DNA after two short heating steps.

The QuickExtract Plant Solution has been successfully used to extract DNA from a wide range of plant species including corn, soybean, spelt, pepper, rosemary, grape, spinach, hopvine and *Arabidopsis*.

Methods

DNA was extracted from freshly cut 3-5 mm leaf discs of spelt, soybean, pepper, and spinach. Each leaf disc was placed in an individual 500 µl tube or microtiter plate, and 100 µl of the QuickExtract Plant Solution was added to immerse the leaf sample. Care was taken not to grind or otherwise damage the leaf tissue as this may release contaminating polyphenolic compounds. The samples were then incubated at 65°C for 6 minutes, 98°C for two minutes, and then placed on ice.

PCR amplification

End-Point PCR—PCR was carried out using EPICENTRE's FailSafe™ PCR PreMix Selection Kit. This kit contains 12 different PreMixes which enables the rapid optimization of PCR primers in a single PCR run. One microliter from each sample of undiluted QuickExtract Plant DNA was added to each of the 12 PCR FailSafe PreMixes along with

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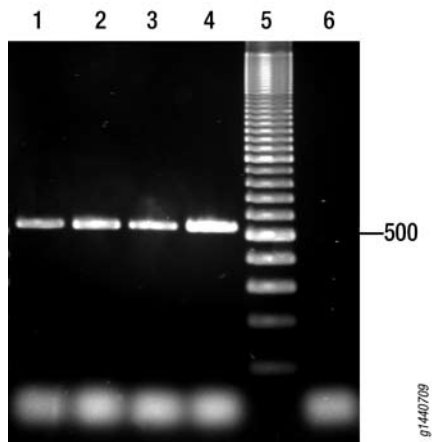


FIG 2. PCR of a single-copy gene using QuickExtract™ Plant DNA Extraction Kit template DNA. One microliter of spinach leaf DNA was obtained from four different spinach leaves using the QuickExtract Plant Solution. The PCR products were separated on a 2% agarose gel and visualized by staining with SYBR® Gold. **Lanes 1-4**, spinach DNA from four leaves; **Lane 5**, 100 bp ladder; **Lane 6**, no DNA negative control.

primers and the FailSafe™ PCR Enzyme Mix. The DNA was denatured at 95° for 2 min, and then PCR amplified for 40 cycles, each consisting of 95°C for 30 sec, 50°C for 10 sec, 72°C for 45 sec, followed by 72°C for 2 min. The PCR products were then separated on a 2% agarose gel and visualized by staining with SYBR® Gold.

Real-Time PCR—Leaf samples were processed in the same manner as for end-point PCR. The QuickExtract Plant DNA extracts were then serially diluted, and, along with negative control samples, amplified using the FailSafe™ GREEN Real-Time PCR System.

Results

DNA was extracted from spelt, soybean and pepper using the QuickExtract Plant Solution. DNA from all three samples was successfully used for RAPD analysis as shown in FIG 1. PCR on single-copy genes from four different samples of spinach DNA clearly demonstrated the high quality and uniformity of the extracted DNA (FIG 2).

The QuickExtract™ Plant DNA samples were also shown to work with real-time PCR techniques (FIG 3 & 4). The QuickExtract Plant Solution provided sufficient template DNA for good PCR results even when the DNA sample was diluted 1000-fold. With this efficient DNA extraction, even very small samples can provide enough template DNA for hundreds or thousands of qPCR reactions.

Conclusion

The QuickExtract Plant DNA Extraction Solution represents a rapid and efficient way to extract PCR-ready plant genomic DNA from most plant samples. The 8-minute, 1-tube protocol enables the inexpensive processing

of one to hundreds of samples simultaneously without centrifugation, spin columns, or toxic organic solvents. The procedure is fully compatible with robotic automation, and produces highly reproducible PCR results.

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NEW! QuickExtract™ Plant DNA Extraction Solution

QEP70750 50 ml
Bulk solution, sufficient to perform 500 100 µl extractions.

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FailSafe™ PCR PreMix Selection Kit

FS99060 60 Units
Contents: FailSafe™ PCR Enzyme Mix and all 12 FailSafe™ PCR 2X PreMixes.

www.EpiBio.com/failsafegreen.asp

FailSafe™ GREEN Real-Time PCR Optimization Kit

FSR360 96-25 µl Reactions
Contents: FailSafe™ PCR Enzyme Mix, 12 FailSafe™ GREEN Real-Time PCR 2X PreMixes, and Passive Reference Dye.

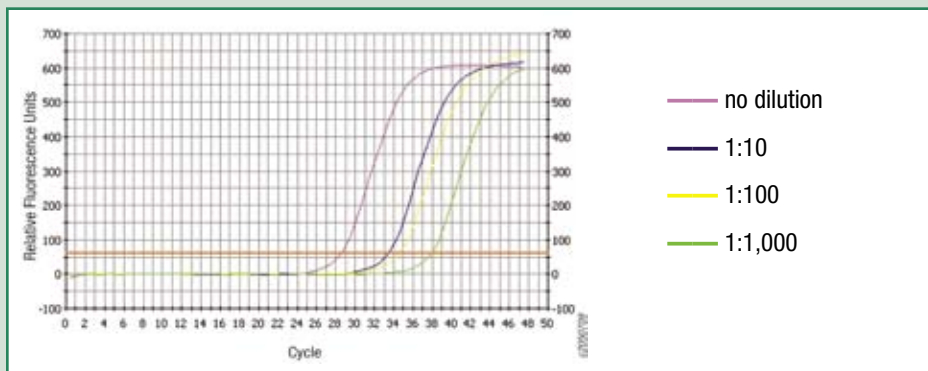


FIG 3. Real-time PCR using spinach seed DNA extracted using the QuickExtract™ Plant DNA Extraction Kit. The target amplicon is a 520 bp region of the single-copy chromosomal HSC70 gene. The results show clear amplification at a thousand-fold dilution of the QuickExtract Plant DNA sample. Negative controls showed no amplification (data not shown).

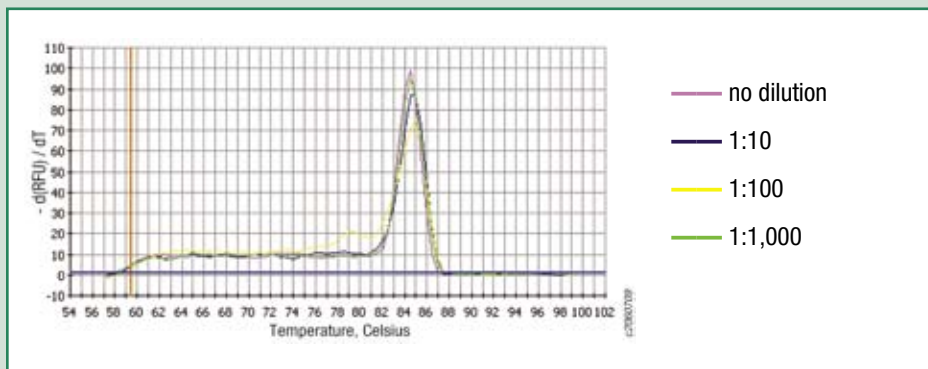


FIG 4. Melting point differential plot for real-time PCR of the spinach leaf HSC70 Gene. The congruence of the melting curves indicates that the real-time fluorescence was derived from the expected amplicon.