Detection of Genomic DNA from Seeds

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INTRODUCTION

DNA isolation from seeds is a common practice for both research and agricultural industries. The DNA can be used for research by genotyping (1), analysis of seed or plant chemical attributes (2), or DNA fingerprinting (3). For agricultural purposes, DNA isolated from seeds can be used to increase crop efficiency and yields (4). However, many common problems are affiliated with the extraction of DNA from seeds. Extractions often involve the grinding of seeds down to a fine powder which degrades the DNA quality and potentially shears the DNA. DNA in seeds can also degrade over time, especially for seeds that are dried and packaged. Seeds are also known to have a high content of polysaccharides and starch, which has many adverse effects on the isolation of DNA, particularly for column based methods. Polysaccharides and starch can also inhibit PCR, therefore restricting the use of purified seed DNA for downstream applications.

Purification of DNA from seeds is also important as it pertains to the detection of genetically modified crops. Today, genetically modified crops including canola, corn, and soya (5) are used to accommodate for the growing population. Genetically modified foods must be regulated under the Food and Drugs Act of Canada (6) and the only way to detect genetically modified strands is by DNA analysis by PCR. As noted above, isolating good quality DNA from seeds free of PCR inhibitors can be difficult.

Norgen Biotek's Plant/Fungi DNA Isolation Kit has already been proven to isolate high quality DNA from several plant sample types, such as roots, leaves, stem, fruit skin, and flour from various plant species. Furthermore, the DNA isolated using the kit is compatible with downstream applications such as qPCR, Southern blotting, SNP analysis and sequencing. This application note illustrates that DNA suitable for downstream PCR analysis can be isolated from corn, sunflower, olive, and soybean seeds.

MATERIALS AND METHODS

DNA Isolation - Seeds

One gram of store-bought corn, sunflower, olive, and soybean seeds were crushed down to a powder with a mortar and pestle containing liquid nitrogen. The crushed seeds were then mixed with 4 mL of Lysis Buffer L from Norgen's Plant/Fungi DNA Isolation Kit (Cat 26200). Next, 600 µL of lysate was transferred to a micro centrifuge tube then incubated at 65°C for 10 minutes with intermittent mixing to keep the lysate homogenous. To clean the supernatant, 100 µL of Binding Buffer I was added, followed by incubation at -20°C for 5 minutes. The lysate was spun through Norgen's filter column at 14,000 rpm for 2 minutes. Next, 650 µL of clean supernatant was mixed with an equal amount of 70% ethanol and was passed through Norgen's column to bind DNA. Following a series of wash steps the DNA was eluted in 75 µL of Elution Buffer B.

Gel electrophoresis

For visual analysis of the DNA concentration, 10 µL of the total 75 µL elution was loaded onto a 1.2% agarose TAE gel. The gel was run for 20 minutes at 170 V, with the use of Norgen's HighRanger 1 kb DNA Ladder (Cat. 11900). For analysis of the PCR amplification (see below) the entire volume of the PCR reaction was loaded onto a 1.4% agarose TAE gel. The gel was run for 25 minutes at 170 V with the use of Norgen's FastRunner DNA Ladder (Cat. 12800). Gel photos were taken using an AlphaImager™ IS-2200 (Alpha Innotech).

PCR Amplification

The purified DNA was used as a template in end-point PCR reactions. To amplify plant genomic DNA, 5 µL of isolated DNA from olive and soybean, and 1 µL of sunflower and corn DNA was added to 18 µL of reaction mixture. The mixture contained 0.1 µM Lec-294 (7) and Plant A2/C1 (8) primers. Reactions were run using the program; 95°C for 3 minutes for an initial denaturation, 45 cycles of 95°C for 15 seconds for denaturation, 60°C for 30 seconds for annealing and 72°C for 45 seconds for extension, and a

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final 72°C for 7 minutes. The reaction was run on an iCycler iQ thermocycler (Bio-Rad).

RESULTS AND DISCUSSION

DNA was isolated from corn, olive, sunflower, and soybean seeds using Norgen's Plant/ Fungi DNA Isolation Kit according to the provided protocol. The DNA was run on a 1.2% agarose TAE gel for visual analysis. DNA isolated from sunflower, and corn produced a large quantity of intact DNA. Olive and soybean samples provided much less DNA, to the point where it is not detectable on the gel **(Figure 1).**

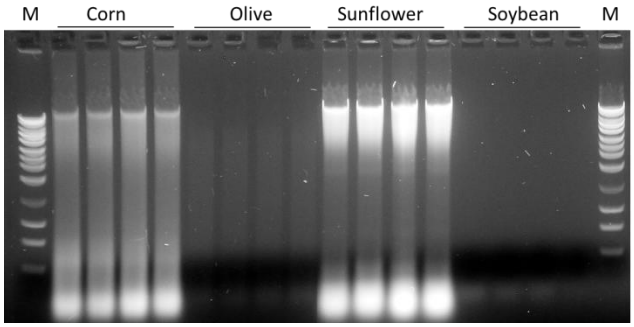

Figure 1. 10 µL of the 75 µL elution loaded onto a 1.2% agarose TAE gel. DNA was isolated using Norgen's Plant/Fungi DNA Isolation Kit. M= HighRanger 1 kb DNA Ladder.

The quality of the purified DNA was then tested by using it as a template in end-point PCR. The end-point PCR amplified the four seed samples using the Lec-294 and Plant A1/C2 as universal primers to detect plant DNA. The amplified DNA was run on 1.4% agarose TAE gels for visual analysis. The gels showed that both primers Lec-294 and Plant A1/C2 were successfully amplified in all four samples. The amplicons show consistent band intensity throughout all samples, even though different amounts of templates were used **(Figure 2 and Figure 3)**. Under such circumstances where DNA amounts present in seed samples are too low to detect by gel electrophoresis, DNA quality becomes more crucial than DNA yield to ensure its use in downstream PCR applications. Therefore, Norgen's Plant/Fungi DNA Isolation Kit is capable of isolating DNA from store bought packaged and dried seeds. The purified DNA was also found to be of a high quality, as evidenced by successful PCR amplification of all samples.

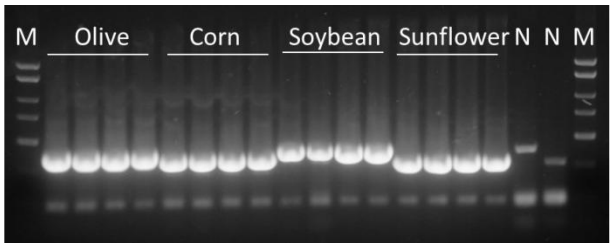

Figure 2. Plant-A1/C2 amplicons run on 1.4% agarose TAE gel following end-point PCR. DNA samples were isolated from store bought seeds using Norgen's Plant/Fungi DNA Isolation Kit. N = no template control. M= FastRunner DNA Ladder.

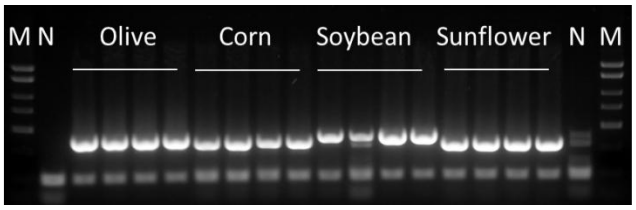

Figure 3, Lec-294 amplicons ran on 1.4% agarose TAE gel following end-point PCR. The DNA samples were isolated from store bought seed samples using Norgen's Plant/Fungi DNA isolation kit. N = no template control. M= FastRunner DNA Ladder.

CONCLUSION

Norgen's Plant/Fungi DNA Isolation Kit is versatile in terms of the large variety of plant samples from which it is able to isolate DNA, including from seeds. Furthermore, the kit is sensitive enough to isolate trace amounts of DNA not detectable by gel electrophoresis, and in good enough quality that can be used in downstream applications such as PCR. This makes the kit suitable for isolating high quality DNA to be used for testing of genetic authenticity in seeds, or for DNA fingerprinting.

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