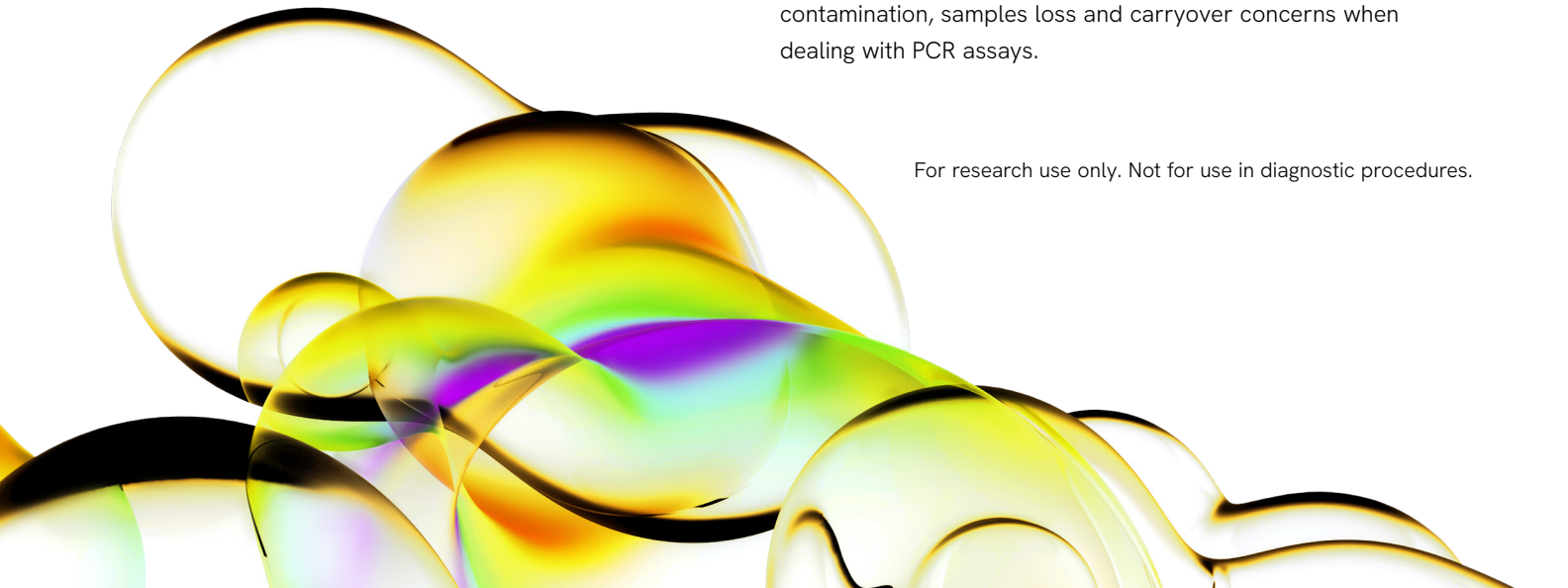
Detection and cross-contamination evaluation of roundup ready soy via mechanical shear homogenization using the Omni GLH 850.

Introduction

Genetically Modified (GM) crops are food products that have had their DNA modified to introduce new traits such as resistance to pests, diseases or environmental conditions. One popular GM crop is Roundup Ready® soy by Monsanto. Roundup Ready® soy is genetically engineered soybeans designed to be tolerant of the herbicide Roundup®. The Roundup® herbicide is a glyphosate that inhibits the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) (1). EPSPS catalyzes an essential step in the biosynthesis of the amino acids phenylalanine, tyrosine and tryptophan in plants (2). When sprayed Roundup® quickly inhibits the growth of plants by interfering with the synthesis of these essential amino acids. Roundup® is typically used to inhibit the growth of weeds but usually at the cost of reduced crop yields. Roundup Ready® soybeans have been genetically engineered to express a version of the EPSPS gene that confers resistance to glyphosates therefore allowing it to survive the doses required to kill weeds that infect farmers fields (1).

In order to determine the detection of Roundup Ready® soy, the soy products are homogenized and the DNA is extracted using a variety of techniques. Following extraction, the genetically modified EPSPS gene is detected via Polymerase Chain Reaction (PCR). Rotor stator homogenizers are one of the preferred methods for processing a variety of samples from soybeans to soy powder. The Omni GLH 850 is a programmable rotor stator homogenizer that can quickly process samples at speeds of up to 25,000 RPM and sample volumes up to 10 liters. Particularly, the Omni GLH 850 can process samples using a variety of stainless steel and Omni Tip™ plastic homogenizing probes. Omni Tip plastic homogenizing probes can process a range of sample matrices and can significanty reduce cross-contamination, samples loss and carryover concerns when dealing with PCR assays.



Herein, we evaluate the Omni GLH 850's capability of processing a variety of soy sample matrices for the detection of Roundup Ready® soy. In conjunction, the potential for sample cross contamination from the use of a standard stainless steel generator probes was evaluated.

Materials and methods

Equipment

- **Omni General Laboratory Homogenizer (GLH) 850** (Cat # 20-010)
- **10 mm Stainless Steel Wide Window Generator** (Cat # G10-95W)
- **7 mm Hard Tissue Omni Tip** (Cat # 30750H)

7 mm Hard Tissue Plastic Omni Tip (Cat # 30750H)

10 mm Stainless Steel Wide Window Generator
(Cat # G10-95W)

Sample preparation

0.5 g of organic soybeans and 0.5 g of soy powder was processed in 2.5 mL of commercially available plant tissue lysis buffer with the Omni GLH 850 fitted with a 10 mm stainless steel wide window generator probe at 20,000 RPM for approximately 15-30 seconds until a complete homogenate was achieved. The generator probe was disassembled and rinsed under a stream of ddH_2O between samples. An additional 0.5 g of organic soybeans and 0.5 g of soy powder was processed in 2.5 mL of commercially available plant tissue lysis buffer with the Omni GLH 850 fitted with a 7 mm Hard Tissue Omni Tip. The samples were processed at 20,000 RPM for up to 60 seconds until a complete homogenate was achieved. Each sample was processed with its own individual plastic generator probe.

DNA isolation, quantification, and separation

500 µL of each homogenate was added to 10 µL of 2-mercaptoethanol, vortexed and incubated at 65 °C for up to 30 minutes (15 minutes for soybean). After incubation, the DNA was purified using a commercially available plant DNA extraction kit following manufacturer's instructions. 1 µL of each eluant was analyzed for DNA quantification on the NanoDrop Spectrophotometer (Thermo Fisher Scientific).

The size and purity of the isolated DNA was determined by agarose gel electrophoresis. Approximately 300 ng of DNA was mixed with 10 µL of TBE/Urea loading dye (Bio-Rad) and separated on a 1 % agarose gel in TBE running buffer (Bio-Rad) . Electrophoresis was carried out at 140V for 45 minutes. The gel was stained in ethidium bromide for 15 minutes and visualized on a GelDoc EZ System (images/data not show).

PCR assay and fragment analysis

Primers for the amplification of the 5′-enolpyruvylshikimate-3-phosphate synthase (EPSPS) gene where chosen using published sequences. Primers were synthesized by Integrated DNA Technologies (Iowa, USA).

Table 1. PCR Primers

Name	Sequence (5′-3′)	Reference
RRO1	TGGCGCCCAAAGCTTGCATGGC	3
RRO4	CCCCAAGTTCCTAAATCTTCAAGT	3

Each amplification reaction was established by adding 1x reaction buffer; 10 mM Tris-HCl, 50 mM KCl, 1.5 mM MgCl2, 0.2 mM dNTPs, 5% Glycerol, 25 U Taq (New England Biolabs), 0.5 µM of each primer and 20 ng of template. Amplification was carried out using the T100 Thermal Cycler by Bio-Rad per the settings in table 2 (4).

Table 2. PCR Program

Name	Temperature	Time
Hot start/Denaturation	95 °C	3 minutes
50 cycles	95 °C	45 seconds
	60 °C	45 seconds
	72 °C	25 seconds
Final extension	72 °C	10 minutes

PCR products were analyzed on a 2% agarose gel and stained using ethidium bromide. Fragments were visualized and analyzed via Bio-Rad's Gel Doc EZ system.

Table 3. Soybean and soy powder DNA concentrations

Sample	Stainless steel generator probe	Omni plastic generator probe
Soybean	174.9 ng/µL	114.0 ng/µL
Soy powder	284.9 ng/µL	217.0 ng/µL

Results

In this study, we investigated the Omni GLH 850's capability of homogenizing a variety of soy matrices for the detection of Roundup Ready® resistance genes. In conjunction, we observed the potential for cross-contamination when using stainless steel generator probes. When performing assays such as PCR, cross-contamination can lead to erroneous and false positive results. In this particular assay, soybeans and soy powder was processed using the Omni GLH 850 using both stainless steel and plastic generator probes. Genomic DNA was extracted from each sample and the glyphosate resistance EPSPS gene was detected via end-point PCR.

Genomic DNA quantification

The isolated genomic DNA was quantified by spectrophotometry. DNA yields averaged 144.5 ng/µL and 250.9 ng/µL of soybean and soy powder respectively (Table 3).

PCR detection

The fragment length of each amplicon from each sample type was analyzed on a 2 % agarose gel. The expected size of the amplified EPSPS gene is shown to be 356 bp. As shown, the soy powder samples contained bands at the expected base pair length for the EPSPS gene (Figure 1).

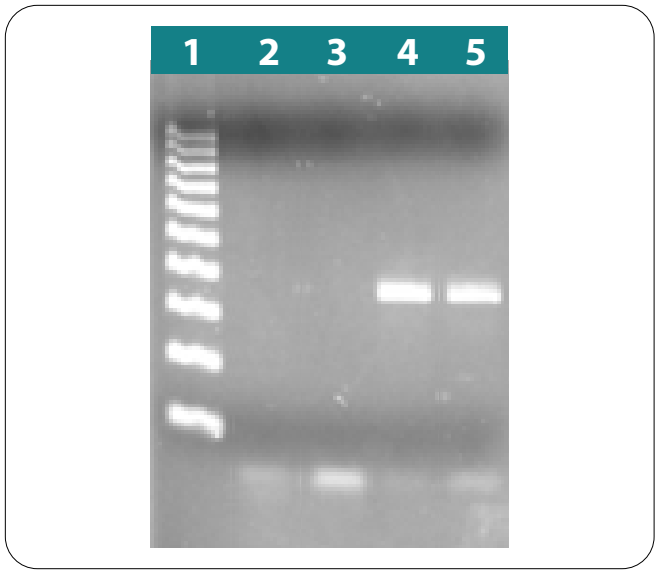


Figure 1. Roundup Ready Detection from Disposable Omni Tip. Lane 1: 100 bp Ladder, 2-3: Soybean, 4-5: soy powder

The Roundup Ready® resistance gene was not detected for the organic soybean samples while a single fragment at the desired length was detected from the soy powder samples.

However, when processing with a single stainless steel generator probe cross-contamination was evident in the soybean sample. Despite disassembling and rinsing the probe, trace amounts of soy powder DNA remained on the probe and tainted the soybean sample creating a false positive (Figure 2). In order to significantly minimize the risk of cross-contamination when using a stainless steel generator probe, it is imperative that the probe, is disassembled, thoroughly cleaned and autoclaved.

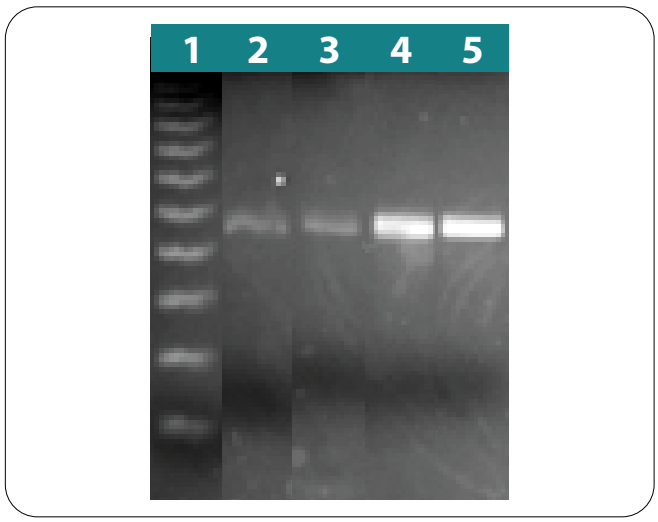


Figure 2. Roundup Ready Detection from Stainless Steel Probe. Lane 1: 100 bp Ladder, 2-3: Soybean, 4-5: Soy Powder

Conclusion

Rotor stator homogenizers such as the Omni GLH 850 are capable of disrupting a variety of sample matrices for DNA extraction with subsequent PCR analysis. The Omni GLH 850 robustly processed soy samples for the extraction of genomic DNA in excess of 110 ng/µL. Using either the stainless steel or plastic generator probes, the extracted DNA resulted in reliable results when subjected to PCR analysis. However, it is noteworthy that when processing multiple samples with a stainless steel generator probe, carryover is very likely without the use of thorough cleaning techniques. In cases where very sensitive assays are performed, the Omni Tip homogenizing probes are highly recommend since they can be disposed of after each sample.

References

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