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Geno/Grinder®

Tissue
Homogenization
Cell Lysis

APPLICATION NOTE

Comparison of Methods for the Isolation of DNA from Soybeans

With kind permission David Burden, Ph.D., President of BT&C Incorporated

The isolation of nucleic acids from intact seeds requires mechanically disrupting the seed followed by the extraction and subsequent purification of the nucleic acid. The mechanical disruption is often performed manually with a mortar and pestle, an approach that is not practical for high throughput screening of seeds as manual grinding is slow and reuse of mortar and pestles may lead to cross-contamination. Alternatively, nucleic acids can be isolated from seeds in a microwell plate format using a ball mill that mechanically disrupts the seeds. Conventional isolation methodologies can then be used to purify the nucleic acids from the seed homogenates.

The efficiency of seed disruption is dependant upon the type of ball mill used in the grinding process. Standard bead mills adapted to microwell plates are modeled after "paint shakers" and move the plates in a "figure-eight" motion. This motion does not lead to uniform seed disruption. The Geno/Grinder® is designed to effectively disrupt cellular materials by oscillating the plate vertically. This motion allows balls to impact the seeds more directly than standard mills where balls impact the well walls in addition to the seed. Soybeans that are soaked overnight in water are effectively and uniformly homogenized in less than 3 minutes. The resulting pulp can then be used as a source of DNA for genetic analysis.

Materials and Methods

Soybeans were placed in each 2.0 mL deep-well of a 96-well plate containing a 4 mm stainless steel grinding ball (Cat. No. 2150) and soaked for 12 hours in distilled water prior to processing. Using a grinding ball dispenser a grinding ball was also placed on top of each seed. The plate was sealed with a fitted Teflon®/silicone mat and placed in the Geno/Grinder. A piece of adsorbent paper was placed on top of the plate and the plate was locked into the grinder. The seeds were disrupted for 2.5 minutes at 1500 rpm. The deep well plate with lid was then centrifuged at 1500 rpm to pellet lysate and condense liquid from the rim and walls of the well. Without centrifugation, the probability of well to well cross contamination of genetic material is greatly increased. Once centrifuged, the lid is carefully removed.

Four different DNA methods were compared for the isolation of DNA from the soybean homogenates, namely the CTAB, Wizard Genomic, Qiagen Plant DNeasy, and Qiagen Genomic methods. Each of these methods is summarized briefly below.

DNA Purification Using the Qiagen DNeasy™ Plant Kit

After grinding, the soy was mixed with 400 µL Buffer AP1 and 4 µL RNase stock solution. The mixture was incubated for 10 minutes at 65° C. Buffer AP2 (130 µL) was then added to the lysate, mixed, and incubated for 5 minutes on ice. The precipitates were removed with the QIAshredder™ spin column. The eluent was mixed with 0.5 volume of Buffer AP3 and 1 volume of ethanol (96-100%). After mixing, the lysate was applied to the DNeasy mini spin column and centrifuged at 9000 rpm for 1 minute. During this step, the DNA binds to the column. The flow-through is

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discarded. The column was washed with Buffer AW, and then centrifuged at maximum speed in order to dry the membrane. The DNA was eluted with 50 µL Buffer AE by incubating for 5 minutes at room temperature and then centrifuging for 1 minute at 9000 rpm.

DNA Purification with the Promega Wizard® Genomic DNA Purification Kit

After grinding, the soy was mixed with 600 µL Nuclei Lysis Solution and incubated for 15 minutes at 65° C. RNase was added followed by a15 minute incubation at 37° C and a 5 minute cooling to room temperature. Protein Precipitation Solution was added followed by centrifugation to pellet precipitated proteins. The supernatant containing the DNA was transferred to another microfuge tube containing isopropanol. The sample was mixed gently by inversion and then centrifuged at 9000 rpm. The supernatant was removed and the pellet was washed with 70% ethanol. The tube was centrifuged and the ethanol was decanted. Excess ethanol was removed by inverting the tube onto absorbent paper and air-drying for 15 minutes. The DNA was resuspended in 50 µL DNA Rehydration Solution during incubation for 1 hour at 65° C.

CTAB Method for DNA Purification

The ground soy was diluted in 500 µL CTAB buffer (20 g CTAB/L, 1.4 M NaCl, 0.1 M Tris/HCl, 20 mM EDTA). This mixture was incubated for 30 minutes at 65° C. It was then centrifuged for 10 minutes at 9000 rpm. The upper layer was extracted with an equal volume of chloroform. After mixing for 30 seconds, the mixture was centrifuged for 10 minutes at 9000 rpm. The supernatant was transferred to a new tube, and two volumes of CTAB precipitation solution (5 g CTAB/L, 0.04 M NaCl) were added. The mixture was incubated for 60 minutes at room temperature and then centrifuged for 5 minutes at 9000 rpm. The supernatant was removed, and the precipitate was dissolved in 1.2 M NaCl. This solution was chloroform extracted. The upper layer (aqueous phase) was transferred to a new tube and 0.7 volumes of isopropanol was added. The solution was then frozen for 20 minutes at -80° C. The sample was thawed at room temperature and centrifuged. The resulting DNA pellet was washed with ice-cold 70% ethanol and centrifuged. The ethanol was discarded, and residual ethanol was removed with a DNA SpeedVac. The DNA pellet was resuspended in TE buffer.

DNA Purification Using a Qiagen Genomic Column

After grinding, the soy was suspended in 10 mL of Buffer G2. A Qiagen genomic tip 100 column was equilibrated with Buffer QBT. Following equilibration, the soy sample was applied to the column, and the column was washed two times with Buffer QC. Elution of the DNA was done with 5 mL Buffer QF. The DNA was precipitated with 0.7 volumes of isopropanol and freezing at -80° C for 20 minutes. The sample was thawed at room temperature and centrifuged to pellet the DNA. The pellet was washed with 70% ice-cold ethanol and then centrifuged. The ethanol was removed, and any residual ethanol was removed by centrifuging in the DNA Speed Vac. The DNA was then resuspended in 50 µL Buffer TE.

A comparison of the DNA yields for the isolation procedures was made by agarose gel electrophoresis (0.7% agarose in 1X TAE buffer) which is illustrated in Figure 1.

The genomic DNA was tested for genetically modified sequences, i.e., GMO analysis, using PCR. Samples were analyzed for the soy lectin gene and Cauliflower Mosaic Virus 35S promoter/Petunia transcription sequence marker found in genetically modified soybean (Roundup ReadySoya). Analysis of the reactions was done electrophoretically (1X TAE with 3% agarose) and is illustrated in Figure 2.

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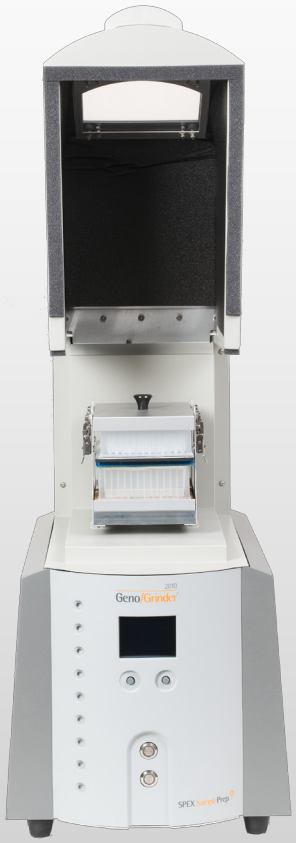

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Results and Discussion

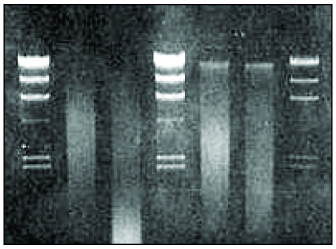

Figure 1. Comparison of methods for DNA isolation from Soybean. Lanes 1, 4 and 7 – Lambda HindIII digest, Lane 2 – Qiagen Genomic DNA Kit, Lane 3 – CTAB Methood, Lane 5 – Promgea Wizard Kit, Lane 6 – Qiagen DNeasy Kit.

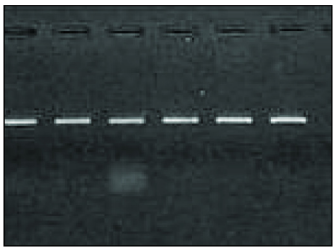

Figure 2. PCR analysis of isolated DNA from soybean

The isolation method used to extract DNA from soybean homogenate affects the quantity and quality of the genomic DNA isolated. Agarose gel electrophoresis of the genomic DNA (Fig. 2) illustrates a significant difference in yield and fragment size of the DNA. Based on the fluorescence of the DNA smear in the ethidium bromide stained gel, the CTAB method produced the greatest yield, but fragment size is extremely small as compared to the other DNA preparations and measured against the molecular weight markers. Depending upon the application of the DNA, lower yields of large sized fragments (e.g., the Wizard method or Genomic Column method) may be preferred.

PCR analysis of the samples was positive for the soy lectin gene and negative for the 35 S promoter/Petunia TS sequence. This analysis demonstrates that soybean homogenates generated from the Geno/Grinder are suitable for genetic analysis. This is particularly useful for high throughput PCR analysis in agricultural biotechnology laboratories or laboratories performing GMO analysis.

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