Again with the Automation? Yes.

Automated Isolation of Genomic DNA Using Promega's DNA Binding Plates on the Beckman Biomek® 2000

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Abstract

Genomics applications often require genomic DNA isolation from a large number of samples. Therefore, high-throughput processing of these samples is required. This article demonstrates the use of Promega's Wizard® SV 96 Binding Plate on the Beckman Biomek® 2000 for high-throughput genomic DNA isolation from multiple sample types.

We have developed a single, robust procedure to isolate genomic DNA from many different sample types in a 96 well format using a Beckman Biomek® 2000.

Introduction

A high-throughput genomic DNA isolation procedure must provide for the isolation of high-quality DNA quickly. We have developed a robust procedure to isolate genomic DNA from many different sample types in a 96 well format using a Beckman Biomek® 2000. The procedure takes only one hour using Wizard® SV 96 DNA Binding Plates (Cat.# A2271) to purify genomic DNA from lysates of mouse tail clippings, plant tissue, tissue culture cells, whole blood or cheek swabs. A single automated procedure can be used regardless of the sample type. The genomic DNA isolated is of high-quality and serves as an excellent template for PCR[(a)] analysis. Moreover, in these studies, no sample-to-sample contamination from the DNA purification procedure was detected.

Figure 1. Initial deck configuration of the Biomek® 2000. The tools required include Wash 8, MP200 and Gripper, all placed at position A1. Positions A2 and B1–B4 remain empty. Boxes of P250 tips are placed at positions A3 and A4. Position A5 contains a reservoir with 10ml of Nuclease-Free Water (Cat.# P1193). The 96 well plate containing lysates is placed at position B5. The Wizard® SV 96 DNA Binding Plate is placed at position B6. Two vacuum manifolds are assembled at positions A6 and B6: DNA elution is performed at position A6 and DNA binding and washing at B6.

Automated Isolation of Genomic DNA

Sample lysate preparation will differ depending on the sample type for isolating genomic DNA. Table 1 briefly describes the lysate preparation for each sample type tested for this report. Once the lysate is prepared, genomic DNA isolation is performed on the Beckman Biomek® 2000, using a single protocol for all sample types.

Table 1. Protocols for Lysate Preparation from Indicated Sample Types.

	Procedure
Mouse Tail	1. Digest each 0.5cm mouse tail snip with 200µl Nuclei Lysis Solution, 48µl EDTA (0.5M) and 17.5µl Proteinase K (20mg/ml) solution. Vortex to mix. 2. Incubate for 3 hours to overnight at 55°C, mixing periodically. 3. After digestion, add 250µl SV RNA Lysis Buffer and vortex to mix. **Note:** Lysates can be stored at –70°C.
Plant Tissue (Tomato Leaf)	1. Crush 100mg of plant tissue to a fine powder in liquid nitrogen with a mortar and pestle. 2. Add 1.5ml SV RNA Lysis Buffer to the tissue powder in the mortar after the liquid nitrogen has evaporated. 3. Crush the tissue further in SV RNA Lysis Buffer until tissue particles and fibers are no longer seen. 4. Transfer lysate to new 1.5ml microcentrifuge tube. **Note:** Lysates can be stored at –70°C.
CHO Cells	1. Grow cells in a 96 well tissue culture plate at a density of 1×10^6 cells per well. 2. Wash cells once with 1X PBS. 3. Add 150µl SV RNA Lysis Buffer to each well. Mix by pipetting. **Note:** Lysates can be stored at –70°C.
Whole Blood	1. Add 400µl of Lysis Buffer (SV RNA Lysis Buffer plus 1% Triton® X-100) to 200µl of whole blood. Vortex to mix. **Note:** Lysate **cannot** be stored; use immediately after preparing.
Cheek Swab	1. Scrape the inside of the cheek with a clean pipette tip. 2. Swish the pipette tip in 150µl SV RNA Lysis Buffer plus 0.97% β-mercaptoethanol. Vortex to mix. **Note:** Do not use a cotton swab to collect cheek sample. Cotton fibers from the swab will clog the Wizard® SV 96 DNA Binding Plate.

Table 2. Biomek® 2000 Program for Isolation of Genomic DNA.

Step	Process
1.	Biomek® 2000 transfers 150µl of sample lysate from the 96 well sample plate to the Wizard® SV 96 DNA Binding Plate. The sample lysate is pulled through the plate by vacuum and the DNA binds.
2.	The samples are washed twice with 500µl of Wizard® SV 96 Wash Solution containing 60% ethanol, using the Wash 8 tool.
3.	The Binding Plate is dried briefly by vacuum.
4.	The Binding Plate is transferred from the B6 to the A6 vacuum manifold position where it is placed on top of an elution plate fitted with a vacuum manifold collar.
5.	DNA is recovered in the elution plate by addition of Nuclease-Free Water (75µl) to the Binding Plate.

For genomic DNA, 150µl of prepared lysate is placed into the well of a 96 well plate and the plate is placed on the deck of the Beckman Biomek® 2000. As shown in Figure 1, the remainder of the Biomek® 2000 deck is assembled according to the program's initial deck configuration. Once configured, the Biomek® program is initiated and takes approximately one hour per 96 well plate (up to 96 samples) (see Table 2).

Analysis

Purified genomic DNA is eluted in 75µl of Nuclease-Free Water (Cat.# P1193). For mouse tail, plant tissue and tissue culture cell samples, a 20µl aliquot was analyzed by agarose gel electrophoresis (Figure 2).

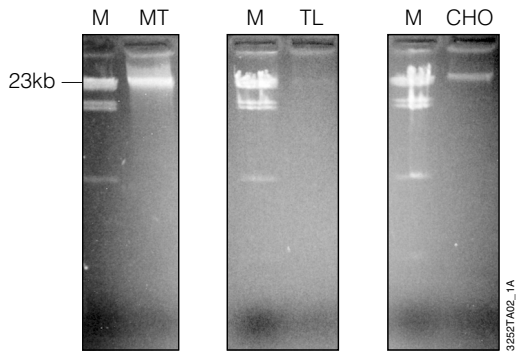

Figure 2. Agarose gel electrophoresis of purified genomic DNA from mouse tail (MT), tomato leaf (TL) and CHO cells. Twenty-microliter aliquots of a 75µl prep were resolved on a 1% agarose gel and visualized by ethidium bromide staining. Lane M, Lambda DNA/*Hind* III Markers (Cat.# G1711).

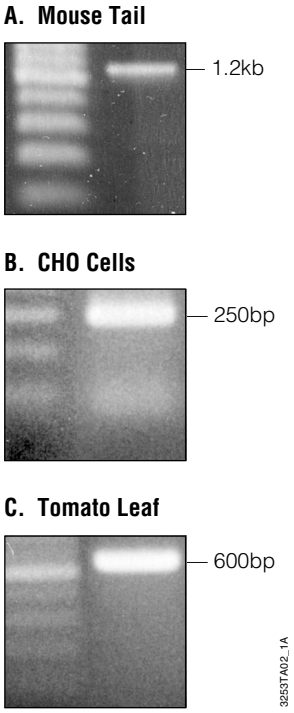

Figure 3. Agarose gel electrophoresis of PCR products amplified from 1µl of mouse tail, CHO cells and tomato leaf samples. Ten microliters of PCR product are visualized on a 1.5% agarose gel, stained with ethidium bromide. **Panel A**: IL-1β (1.2kb) amplified from mouse tail. **Panel B:** β-actin (250bp) amplified from CHO cells. **Panel C:** Chloroplast DNA (600bp) amplified from tomato leaf. Lane M, 1kb DNA Ladder.

Quality of the genomic DNA was assessed by PCR. One microliter of the genomic DNA was used for PCR amplification in a 50µl reaction. Specific oligonucleotide primers were used to target genomic DNA sequences: IL-1β gene for mouse tail, β-actin for CHO cells, and a noncoding region of chloroplast DNA (1) for tomato leaf samples (Figure 3).

Sample cross-contamination was investigated by assaying an array of blood and cheek swab samples, alternating with water controls, in a 96 well plate. A prothrombin transcript was targeted by PCR in samples from all wells. As evident in Figure 4, no sample-to-sample contamination was observed in this experiment.

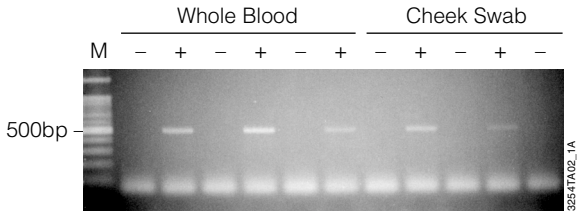

Figure 4. Cross-contamination analysis. Agarose gel electrophoresis of PCR products amplified from whole blood and cheek swab samples. Ten microliters of PCR product containing a 600bp fragment of the prothrombin gene were resolved on a 1.5% agarose gel and visualized by ethidium bromide staining. Wells containing water only were also used in PCR and samples were visualized on the same gel. (+), tissue lysate; (–), water blank. Lane M, 1kb DNA Ladder.

Discussion

An advantage of this automated genomic DNA isolation system is the flexibility of the procedure. In this study we isolated genomic DNA from a good representation of some common sample types. Types varied from those easily processed (e.g., tissue culture cells), to more difficult ones (e.g., whole blood and plant tissue).

Purified genomic DNA isolated using this automated procedure consists of largely intact genomic DNA of greater than 23kb. The isolated DNA contains trace amounts of RNA, which can be removed easily by adding 1µl of RNase ONE™ Ribonuclease (Cat.# M4261) directly to the eluted DNA or by adding 100µl per 10ml Nuclease-Free Water used to elute the DNA. The purified genomic DNA can be amplified by PCR and is suitable for use in many downstream applications. The processing time for isolation of genomic DNA is approximately one hour per 96 well plate, with no manual intervention. Multiple samples can be processed in one run with no cross-contamination.

A potential limitation of this automated genomic DNA isolation system is in processing viscous lysates. Sample lysates that contain excessive amounts of cellular debris will be viscous and will not move easily through the Wizard® SV 96 DNA Binding Plate. These samples may even clog the plate wells. For the samples processed in this study, the lysate preparation and solution volumes were optimized. Optimization should be considered for other samples to achieve successful genomic DNA isolation.

Conclusions

Genomic DNA can be isolated in a high-throughput format using Promega's Wizard® SV 96 DNA Binding Plate on the Beckman Biomek® 2000. The automated procedure described here can be used to isolate quality genomic DNA >23kb that is ready for downstream applications. We demonstrated the flexibility of the procedure by isolating genomic DNA from multiple sample types. Importantly, no cross-contamination was detected using this automated procedure.

Reference

1. Taberlet, P. *et al.* (1991) *Plant Mol. Biol.* **17**, 1105–1109.

Protocols

- *Wizard® SV 96 Plasmid DNA Purification System Technical Bulletin* #TB272, Promega Corporation. (www.promega.com/tbs/tb272/tb272.html)
- *Wizard® Genomic DNA Purification Kit Technical Manual* #TM050, Promega Corporation. (www.promega.com/tbs/tm050/tm050.html)

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Ordering Information

Product	Size	Cat.#	Price ($)
Wizard® SV 96 DNA Binding Plates*	10 pack	A2271	517
Wizard® SV 96 Wash Solution*	185ml	A1311	50
SV RNA Lysis Buffer*	50ml	Z3051	30
RNase ONE™ Ribonuclease*	1,000 units	M4261	77
Proteinase K*	100mg	V3021	77
Nuclei Lysis Solution*	100ml	A7941	18

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Technical Questions?
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