

Large-scale Gel Purification of DNA Fragments using Wizard™ PCR* Preps Resin

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*The polymerase chain reaction (PCR) process for amplifying nucleic acid is covered by U.S. Patent Nos. 4,683,195 and 4,683,202, owned by Hoffman-La Roche. Patents pending or issued in other countries.

Introduction

Many procedures in molecular biology require the purification of specific DNA fragments away from other DNA fragments, including vector DNA, nonspecific amplification products or other contaminating fragments generated in a restriction digestion. Moreover, a number of applications call for large amounts of specific DNA fragments. Gel purification of DNA is a convenient means of isolating high-quality DNA fragments in large amounts. While many investigators avoid large-scale gel purification, this technique can be much more rapid and cost-effective than multiple small-scale preparations.

Standard methods for preparing large amounts of specific DNA fragments typically require separation of the DNA fragments on an agarose gel, followed by purification of the DNA fragment of interest from the gel. Electrophoresis onto DEAE-cellulose membranes, electroelution and chromatography are widely used methods for recovering DNA fragments from agarose. These purification methods work well in certain situations but frequently are limited by low recovery and poor quality of the DNA recovered. Additionally, these methods can be time consuming and labor intensive and often require further purification or extraction steps (1). These limitations are effectively overcome using Wizard™ PCR* Preps DNA Purification Resin for large-scale purification of DNA fragments from high- or low-melting temperature agarose gels. A number of common methods of purifying DNA fragments from agarose gels are compared in [Table 1](#).

Methods

[Figure 1](#) depicts the use of Wizard PCR Preps DNA Purification Resin for large-scale gel purification of DNA fragments. A detailed protocol follows.

1. Separate the digested DNA fragments by electrophoresis on either a high-melting or low-melting temperature agarose gel until the DNA fragment of interest is well resolved.
2. Excise the DNA fragment in a minimal amount of agarose using a clean razor or scalpel.
3. For **high-melting temperature agarose**, transfer the agarose slice to a microcentrifuge or screw cap tube and add approximately 2.5 volumes of Wizard PCR Preps Resin. Incubate at approximately 65°C in a waterbath for 5 minutes or until the agarose is completely melted. Proceed directly to Step 4.

For **low-melting temperature agarose**, transfer the agarose slice to a microcentrifuge or screw cap tube and incubate at approximately 70°C in a

waterbath until the agarose is completely melted. Determine the volume of the melted sample and add 2.5 volumes of Wizard PCR Preps Resin. Mix thoroughly, but do not vortex.

4. Attach a Wizard Maxicolumn to a vacuum manifold (e.g., Vac-Man™ Laboratory Vacuum Manifold). Load the Resin/DNA mixture onto the column.
5. Apply a vacuum to draw the Resin/DNA mixture into the Maxicolumn, allowing the void volume to pass through the Maxicolumn.
6. To wash the Maxicolumn, turn off the vacuum, add 30ml of 80% isopropanol and re-apply the vacuum. Repeat the wash and allow the Maxicolumn to dry under continuous vacuum for 15-20 minutes.
7. Remove the Maxicolumn and transfer it to a Wizard Reservoir or a 50ml disposable centrifuge tube. Centrifuge the Maxicolumn for 5 minutes at 2,500rpm (1,300 x g) in a swinging bucket rotor to remove residual isopropanol.
8. Transfer the Maxicolumn to a new Reservoir or centrifuge tube and apply 2-5ml of pre-warmed TE buffer (65°C). Centrifuge the Maxicolumn at 2,500rpm (1,300 x g) in a swinging bucket rotor to elute the DNA.
9. Remove and discard the Maxicolumn. Transfer the eluate to several microcentrifuge tubes and spin at top speed to pellet any Resin fines. Transfer the eluate to a new tube, carefully avoiding any Resin fines that may be present.

Table 1. Comparison of Common Purification Methods for the Large-Scale Preparation of Specific DNA Fragments from Agarose Gels

Method	Time Required	Comments
Wizard PCR Preps DNA Purification Resin	1-1.5 hours	Rapidly purifies specific DNA fragments from low- or high-melting temperature agarose; no organic extraction required; efficient purification of fragments 172bp-23kb in size.
Electrophoresis onto DEAE-cellulose	1.5-2 hours	Purifies DNA fragments from high-melting temperature agarose; no organic extraction required; ethanol precipitation required.
Electroelution into dialysis bags	2-3 hours	Organic extraction or column chromatography required; ethanol precipitation required.
Electroelution into troughs	2-3 hours	Purifies DNA fragments from high-melting temperature agarose; organic extraction or column chromatography required; ethanol precipitation required.
		Purifies DNA fragments from

Organic extraction from low-melting temperature agarose

2-3 hours

low-melting temperature agarose; organic extraction required; ethanol precipitation required.

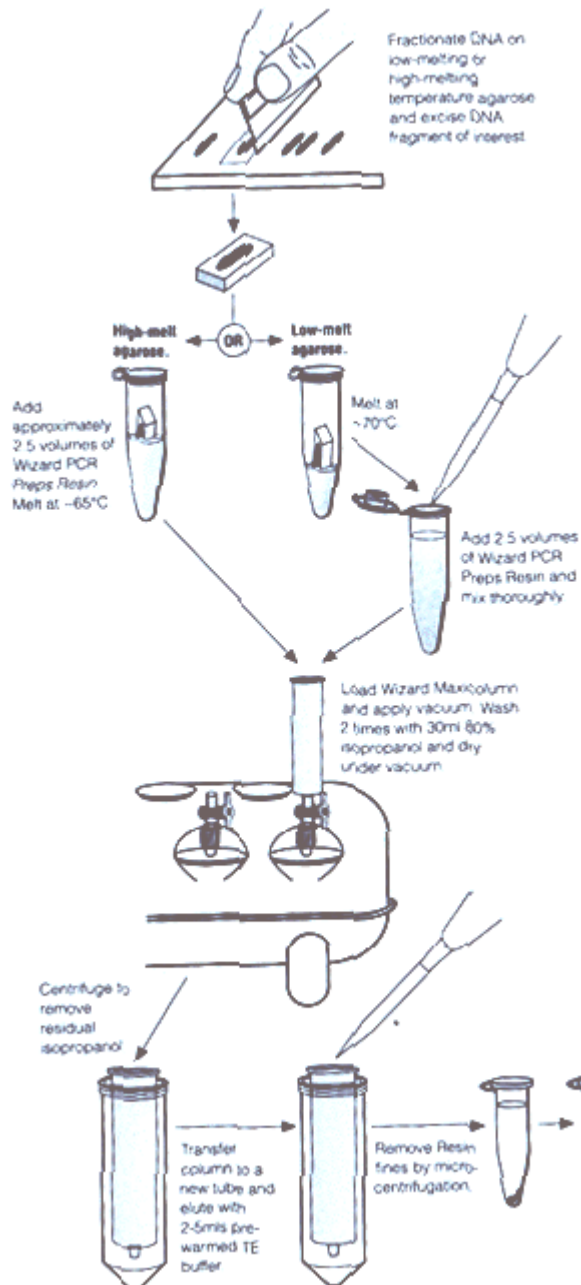


Figure 1. Schematic diagram of the protocol for large-scale gel purification of DNA fragments using Wizard PCR Preps Resin.

Results

Isolation of high-quality DNA fragments with Wizard PCR Preps Resin

The 3.8kb plasmid, pMK11, was double-digested with *EcoR* I and *BamH* I, and the resulting DNA fragments were separated on low-melting or high-melting temperature agarose gels. The Cytomegalovirus (CMV) Immediate Early Gene promoter DNA fragment was isolated as a 1.2kb *EcoR* I-*BamH* I fragment from the gels using Wizard PCR Preps Resin as described above. (This 1.2kb fragment is the control DNA for the HeLa Nuclear Extract *in vitro* Transcription System. [Figure 2](#) depicts the structure of this fragment.) The average percent recovery from both gel systems was 57%. Using this method, up to 600µg of the CMV fragment have been processed with comparable recovery between preparations. [Figure 3A](#) compares CMV promoter fragments purified using organic extraction and the Wizard PCR Resin method for DNA purification from low-melting and high-melting temperature agarose gels.

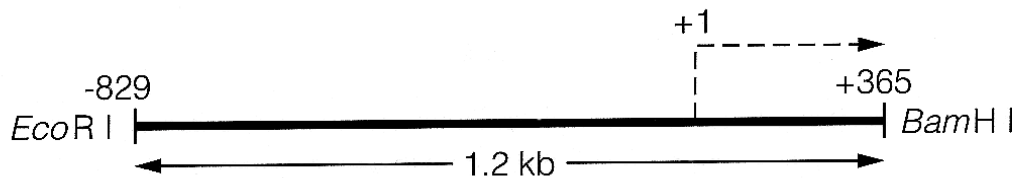


Figure 2. Structure of the 1.2kb CMV Immediate Early Gene promoter fragment isolated from pMK11.

Functional activity of DNA fragments purified using Wizard PCR Preps Resin

To evaluate the functional activity of the Wizard PCR Preps Resin-purified DNA, samples of the 1.2kb CMV promoter fragments were assayed for their ability to serve as templates for *in vitro* transcription. The Resin-purified fragments, isolated from either low-melting or high-melting temperature agarose, supported high-level production of the full-length (363 base) CMV run-off transcript in the HeLaScribeTM Nuclear Extract *in vitro* Transcription System ([Figure 3B](#)).

To further examine the quality of the DNA purified with Wizard PCR Preps Resin, linearized pGEM[®]-3Zf(+) was isolated from both low-melting and high-melting temperature agarose. The quality of the DNA was assessed using Promega's Blue/White Cloning Assay, a rigorous quality control assay used to determine the integrity of restriction fragment termini and the ability of DNA fragments to be ligated and subsequently used for transformation. DNA isolated using Wizard PCR Preps Resin demonstrated efficient ligation and transformation: 1.0-1.3% white colonies, 6.36×10^5 CFU/µg DNA.

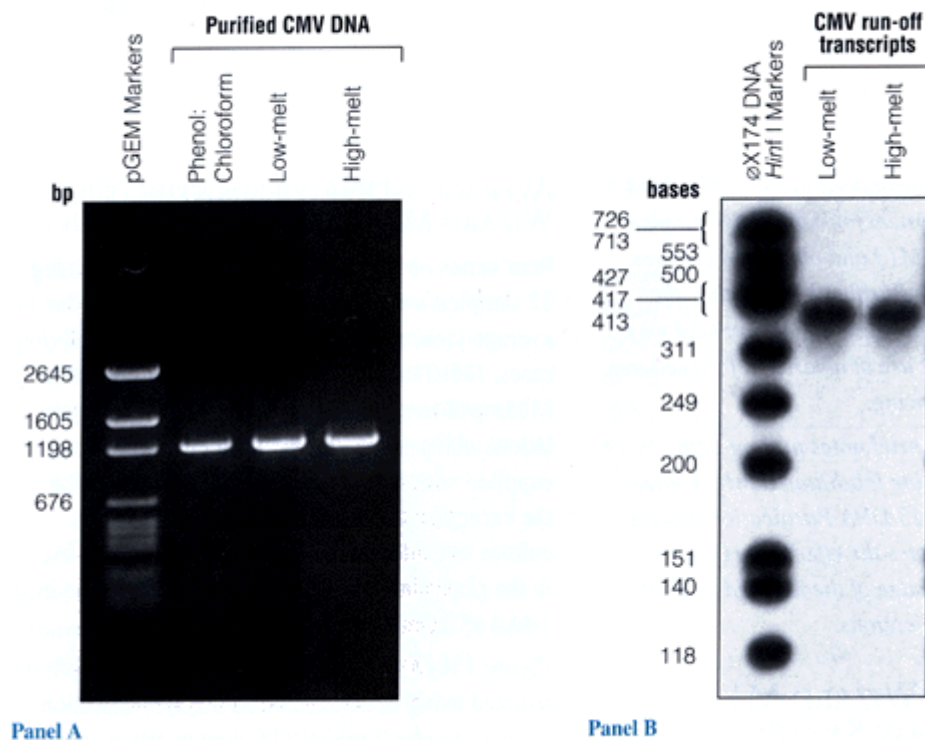


Figure 3. Quality of DNA isolated from low-melting and high-melting temperature agarose using Wizard PCR Preps Resin. Panel A: 500ng of the indicated DNA samples were resolved on a 1% agarose gel. The 1.2kb EcoR I-BamH I promoter fragment of CMV DNA was purified by phenol:chloroform extraction or using Wizard PCR Resin to isolate the fragment from low-melting or high-melting temperature agarose as described in the methods section. **Panel B:** ϕ X174 DNA/Hinf I Markers and run-off transcription products from the 1.2kb CMV promoter fragment isolated from low-melting or high-melting temperature agarose using Wizard PCR Preps Resin. Transcription reactions were performed using the HeLaScribe Nuclear Extract in vitro Transcription System.

Conclusions

Taken together, these results indicate that DNA fragments purified from high-melting or low-melting temperature agarose using Wizard PCR Preps Resin remain structurally intact and functionally active. Thus, Wizard PCR Resin provides the scientist with an effective alternative to less efficient standard methods of large-scale gel purification of DNA fragments.

Reference

1. Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual, 2nd Edition*. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Ordering Information

Product	Size	Cat.#
Wizard™ PCR Preps DNA Purification Resin	250ml	A7181
Wizard™ Maxicolumns with Reservoirs	50 each	A7421
Vac-Man™ Laboratory Vacuum Manifold		A7231
HeLaScribe™ Nuclear Extract Positive Control DNA	300ng	E3621
HeLaScribe™ Nuclear Extract <i>in vitro</i> Transcription System	40 reactions	E3110

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