

White Paper:

FlashGel® System for DNA Recovery

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Introduction

Direct DNA recovery using the FlashGel® Recovery System eliminates agarose gel preparation, band excision, and purification, and delivers highly efficient recovery, free from inhibitors and UV-induced damage, in a simple 5–10 minute protocol.

DNA recovery post-agarose gel separation is a fundamental tool of molecular biology research. Basic techniques for band excision and spin column DNA purification have evolved very little in the past several decades and present several areas of concern for the researcher. First, band excision requires careful removal of agarose material to avoid loss of DNA and minimize agarose in the sample. This process requires DNA damaging UV light to visualize the DNA bands to be removed. Second, column purification requires careful and precise placement of elution buffer in the membrane, monitoring of pH sensitive binding and elution, and user caution in carrying over residual ethanol from wash steps. Finally, a minimum of one hour is required for the entire process of separating a DNA sample on an agarose gel, excising it from the gel, and purifying it on a spin column.

We have developed the FlashGel® Recovery System, which eliminates both the preparation and wait time associated with gel electrophoresis, and the band excision and purification steps associated with recovery. The entire process is reduced to 10 minutes or less, with greater than 80% recovery efficiencies. The recovered sample is free of inhibitors and UV-induced mutations, and subsequent re-amplification, cloning and ligation is equivalent or superior to column-recovered DNA.



The FlashGel® System



Product Overview

The FlashGel® System revolutionized DNA separation by combining electrophoresis speed and visible light illumination, such that separation is completed in just 5 minutes and band migration can be viewed in real-time. The FlashGel® Recovery System enables recovery of DNA samples directly from the gel, in a simple procedure that takes just 4–10 minutes, depending upon sample type. The system eliminates the need to cut away and then purify bands, maximizes the efficiency of recovered DNA, and minimizes the amount of handling post-recovery.

As DNA migrates to the second tier of wells, it is free from the agarose matrix and easily extracted via pipette, with the aid of the FlashGel® Recovery Buffer. Visible light from the compact FlashGel® Dock illuminates the recovery wells without damage to the DNA or hazard to the user. Samples are recovered at 80–100% efficiency, are free of inhibitors, and ready for subsequent re-amplification, cloning, or other techniques. The proprietary stain in the FlashGel® Cassettes enables separation and recovery of very small quantities of DNA, and minimizes user exposure to potential mutagens. Depending upon initial separation time in the recovery step, the same cassette may be used for analysis verification of the recovered sample.

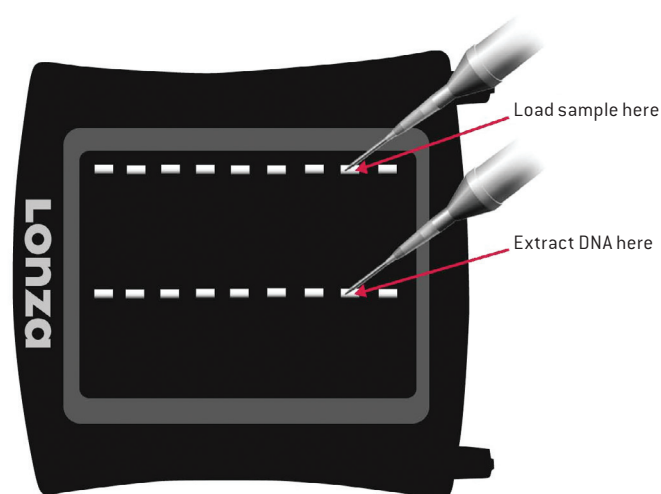
Methodology

Several experiments were conducted to demonstrate performance in typical preparative applications.

FlashGel® recovery capabilities (Fig. 1)

To demonstrate general recovery efficiency, serial doubling dilutions (18.25–600 ng) of 1000 bp BioMarkers® Purified DNA Fragments (BioVentures, Inc.) were separated and recovered using a FlashGel® Recovery Cassette (Figs. 1A: 1 & 2). Briefly, the samples were loaded in to the top tier of wells and run to the top edge of the second tier of wells. The run was stopped and 20 µl FlashGel® Recovery Buffer was added to the appropriate wells in the second tier. The run was recommenced and the fragment run fully in to the wells of the second tier. The DNA was recovered by reverse pipetting from the well. 5% of each recovered sample was subsequently analyzed using a 1.2% FlashGel® DNA Cassette (Fig. 1A: 3). Comparison to the FlashGel® QuantLadder run on the gel showed that recoveries appeared to range from 80–90%.

To demonstrate recovery over a broad fragment size range, 100 ng samples of fragments ranging in size from 50 bp to 4000 bp were separated and recovered using a FlashGel® Recovery Cassette. 3 µl aliquots of the recovered samples were analyzed on a 1.2% FlashGel® DNA Cassette. (Fig. 1B).

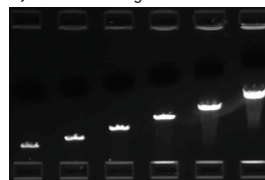


Procedure

1. Load samples in top tier of wells.
2. Run until samples reach the second tier of wells.
3. Stop the run and add FlashGel® Recovery Buffer.
4. Extract DNA from wells by pipette

Panel A – DNA Concentration Range

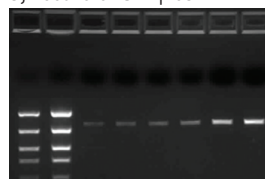
1) Pre-Recovery



2) Post-Recovery



3) Recovered Samples



Panel B – DNA Size Range

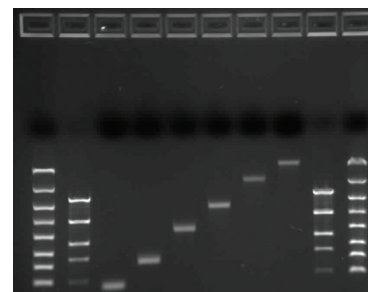


Figure 1. FlashGel® System recovery capabilities. A: Images 1 & 2 show the FlashGel® Recovery Cassette before and after recovery of serial dilutions of a 1000 bp fragment. Image 3 shows analysis of 5% of each recovered sample using a 1.2% FlashGel® DNA Cassette. B: 100 ng samples of fragments ranging in size from 50 bp to 4000 bp were recovered, and 3 µl aliquots of the recovered DNA were separated on a 1.2% FlashGel® DNA Cassette, along with the FlashGel® QuantLadder and FlashGel® DNA Marker (100 bp to 4 kb).

Spin column comparison (Fig. 2)

300 ng of 1000 bp BioMarkers® Purified DNA Fragments (BioVentures, Inc.) were recovered using The FlashGel® Recovery System (FG) or spin column type recovery products (C1 and C2) according to the manufacturer's instructions. The samples recovered with the spin column products were separated on a 1% Reliant® Gel (Lonza) prior to band excision and recovery. Fig. 2 shows comparison of 10% of the recovered samples separated on a 1.2% FlashGel® DNA Cassette. Time required to complete the process from gel electrophoresis to recovered sample was 8 minutes for the FlashGel® System method, as compared to 90 minutes for the Reliant® Gel and column systems methods.

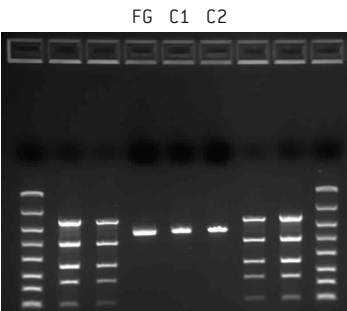
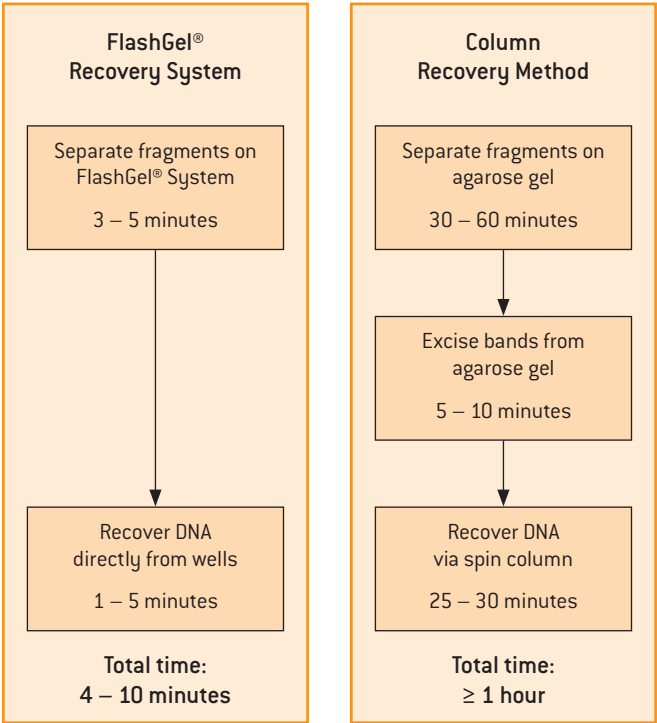


Figure 2. Spin column comparison. 300 ng of 1000 bp DNA fragment were recovered using The FlashGel® Recovery System (FG) or spin column type recovery products (C1 and C2). This image shows comparison of 10% of the recovered samples separated on a 1.2% FlashGel® DNA Cassette, along with the FlashGel® QuantLadder and FlashGel® DNA Marker (100 bp to 4 kb).

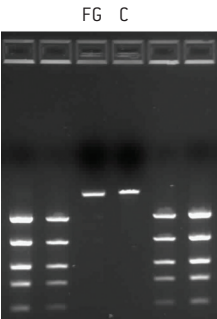
Time comparison. FlashGel® System vs. Column Recovery.



DNA recovery and cloning (Fig. 3)

Plasmid DNA (pBr322; New England BioLabs) was subjected to restriction enzyme double digestion using *Pst*I and *Bam*HI (New England BioLabs). Samples of the restricted DNA were separated and 3.2 kb DNA fragments were recovered using the FlashGel® Recovery System (FG) or a spin column kit (C). 5% of each recovered DNA sample was analyzed on a 1.2% FlashGel® DNA Cassette (Fig. 3A). Aliquots of the recovered DNA samples were ligated into *Pst*I/*Bam*HI double digested pUC19 vector using the Rapid DNA Ligation Kit (Fermentas, Inc.) and transformed into NEB 5-alpha *E. coli* Competent Cells (New England BioLabs). The number of colonies obtained with both samples were very similar. Plasmid samples from two colonies from each sample were digested with *Pst*I/*Bam*HI and analyzed on a 1.2% FlashGel® DNA Cassette along with a restricted sample of vector with no insert (Fig. 3B). The data clearly shows appropriate excision of the expected insert.

Panel A – Recovered Samples



Panel B – Plasmid Restriction Digests

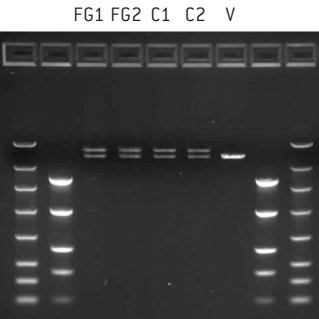


Figure 3. Recovery and cloning comparison. Samples of *Pst*I/*Bam*HI cut pBr322 were separated and 3.2 kb DNA fragments were recovered using the FlashGel® Recovery System (FG) or spin column kits (C1 and C2). A: shows the comparison between 5% of each recovered DNA sample separated on a 1.2% FlashGel® DNA Cassette, along with the FlashGel® QuantLadder. B: *Pst*I/*Bam*HI cut plasmid samples from colonies transformed with recovered DNA samples; lane "V" is digest of vector with no insert. Other lanes contain the FlashGel® QuantLadder and FlashGel® DNA Marker (100 bp to 4 kb).

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FlashGel® Dock and Cassettes.

Cloning of recovered PCR amplicons (Fig. 4)

A 500 bp amplicon was amplified using control reagents supplied in the GeneAmp® Gold Kit (Applied Biosystems). An aliquot of this reaction was separated and recovered using the FlashGel® Recovery System. An 8 µl aliquot of the recovered PCR sample was ligated into pJET1.2 using reagents supplied in the CloneJET™ PCR Cloning Kit (Fermentas, Inc.). Aliquots of these ligation reactions were then transformed into NEB 5-alpha *E. coli* Competent Cells (New England BioLabs). Fig. 4 shows the results of colony PCR amplifications run on several randomly selected colonies. The results show amplification products of the expected size, indicative of successful cloning of the original PCR products.

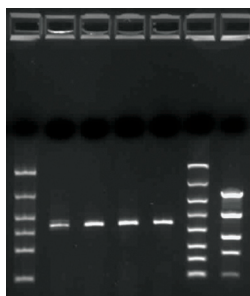
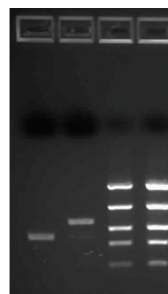


Figure 4. Cloning of recovered PCR product. A 500 bp amplicon was amplified and recovered using the FlashGel® Recovery System. 8 µl of recovered PCR sample was ligated into the pJET1.2 and transformed into *E. coli* Competent Cells.

PCR amplification of recovered DNA (Fig. 5)

Reagents from the GeneAmp® Gold Kit (Applied Biosystems) were used to amplify 300 and 500 bp fragments in a multiplex PCR amplification reaction. A 6 µl aliquot of the PCR reaction was separated on the FlashGel® Recovery System and the PCR product bands were recovered. 4 µl of both recovered PCR fragments were analyzed on a 1.2% FlashGel® DNA Cassette (Fig. 5A). Aliquots of the recovered fragments were also used as templates for new PCR amplifications. Fig. 5B shows 0.5 µl aliquots of these amplification reactions separated on a FlashGel® DNA Cassette. The results show that the recovered DNA could be used successfully as a template for amplification.

Panel A – Recovered PCR Products



Panel B – Reamplified PCR Products

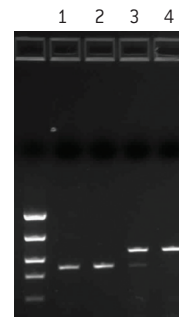


Figure 5. PCR amplification of recovered DNA. Reagents from the GeneAmp® Gold Kit were used to amplify 300 and 500 bp fragments in a multiplex PCR amplification reaction. 6 µl of the PCR reaction was separated on the FlashGel® Recovery System and the PCR product bands were recovered. A: 4 µl of both recovered PCR fragments. The recovered fragments were used as templates for new PCR amplifications. B: 0.5 µl aliquots of these amplification reactions. The resulting amplification products were observed for all reactions by gel analysis on a 1.2% FlashGel® DNA Cassette, along with the FlashGel® QuantLadder. Reactions 1 & 3 used primers for 300 and 500 bp fragments while reactions 2 & 4 used only single primer set.

Summary

The FlashGel® Recovery System is a fast and effective tool for most preparative applications, providing users with an alternative method that both maximizes recovery efficiency and minimizes opportunity for damage to precious DNA. When used as a combined preparative and analytical tool, the FlashGel® Recovery System is a highly economical method to transform a laboratory DNA workflow.

www.flashgel.com

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