Demonstration of FlashGel™ Recovery with Sheared DNA

More and more molecular biologists are working with sheared DNA. Sheared DNA is critical for constructing genomic libraries, and is utilized in DNA reassociation and hybridization analysis. Published methods for DNA fragmentation can be classified into four categories: sonication, enzymatic digestion, hydrodynamic shearing, and nebulization. Regardless of the fractionation method, gel electrophoresis is one of the best ways to estimate mean size and distribution of the DNA material[1].

The FlashGel System for Recovery is suitable for separating and recovering DNA, including fragmented DNA[2]. With the recent addition of a double-tier 2.2% concentration cassette format, it is possible to separate and recover a narrow window within a sheared DNA sample. DNA may be recovered in as little as five minutes, without the need for UV light or downstream purification.

CAPABILITIES OF THE FLASHGEL SYSTEM FOR RECOVERY OF SHEARED DNA

The sensitivity of the FlashGel System allows for a wide range of starting material concentrations. As with most gel recovery systems, the higher the concentration of starting sample, the higher the recovery yield. Intensity of recovered DNA from FlashGel Cassettes scales well with load levels of starting DNA, (Figures 1A and 1B). The FlashGel Recovery System is capable of recovering various size ranges and various size range windows within the sheared DNA (Figure 2).

Recovery steps may be performed in parallel lanes to recover samples from multiple lanes, and multiple samples may be recovered from a single lane. Simply run the smallest of the desired size range in to the recovery well, stop the voltage, add FlashGel Recovery Buffer, and collect the sample. Then continue running the unwanted DNA through the recovery well, until the next desired size range reaches the well. Then stop the voltage, add more recovery buffer, and collect the next sample.

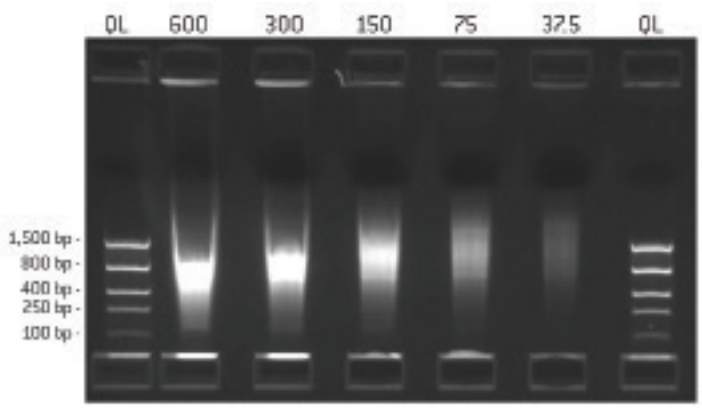

Figure 1A: A dilution series of sheared DNA 600-37.5ng in amount and approximately 200-4,000bp in size were run on a double-tier 1.2% FlashGel Recovery Cassette at 275V for four minutes. Lanes QL contain FlashGel QuantLadder.

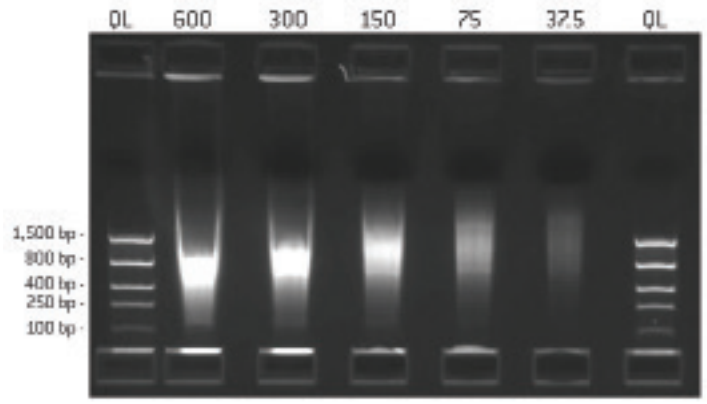

Figure 1B: 4µL aliquot of Figure 1A samples post recovery were run on a single-tier 1.2% FlashGel Cassette at 275V for five minutes. Lanes QL contain the FlashGel QuantLadder. Lane C contains 50ng of the original sheared DNA sample as a reference. Numeric lane labels indicate amount of starting material correlated from Figure 1A.

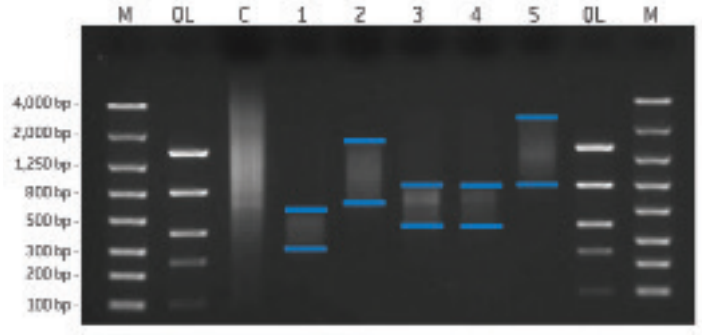

Figure 2: Recovered samples of various size selections from fragmented genomic lambda DNA. Lanes M contain the FlashGel 100-4,000bp Marker, Lanes QL contain the FlashGel QuantLadder, Lane C contains 50ng of Sheared DNA, Lanes 1-5 contain DNA extractions from several experiments taken from 1.2% FlashGel Recovery Cassettes. Samples were run on a single-tier 1.2% FlashGel Cassette at 275V for seven minutes.

Lane 1: 280-550bp; Lane 2: 650-1,190bp; Lane 3: 425-800bp; *Lane 4:* 425-800bp (smaller load volume of lane 3); Lane 5: 800-3,000bp

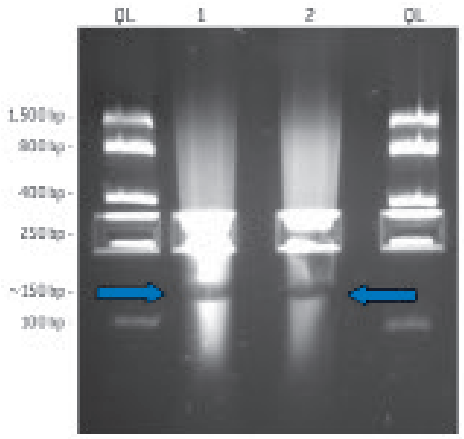

Figure 3: Lower MW fragments from two sheared DNA samples were recovered from a double-tier 2.2% FlashGel Recovery Cassette. Several seconds of 275V current were applied after the selected areas were removed from the samples. The images show the area where the recovered samples were removed. Samples of higher molecular weight are ready to be recovered. Lanes QL contain FlashGel QuantLadder.

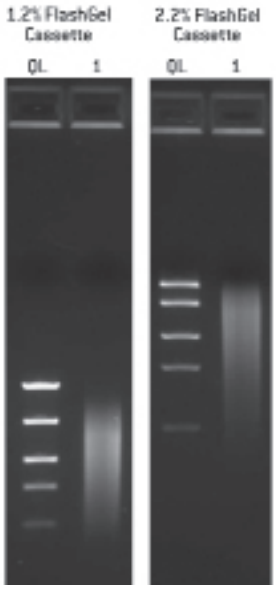

Figure 4: Sheared DNA consisting of fragments 50-3,000bp run on 1.2% and 2.2% FlashGel Cassettes. Lane QL is FlashGel Quant Ladder, lane 1 is the DNA.

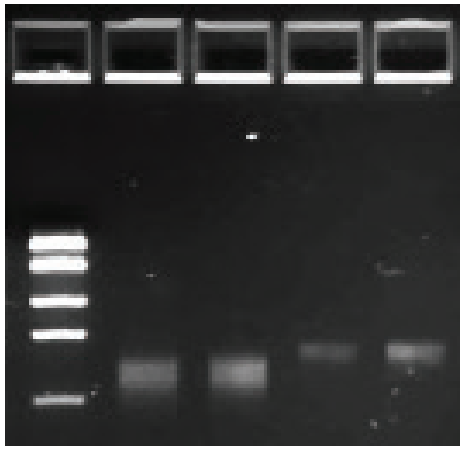

Figure 5: Lanes labeled 1 and 3 are extraction of samples through recovery well window from 1.2% and 2.2% cassettes. Lanes 2 and 4 are slightly higher load volumes of Lanes 1 and 3 respectively. Lane QL contains the FlashGel QuantLadder.

As long as current is applied, DNA that is not recovered will migrate through the well and leave no trace contamination (Figure 3). Only when the current is stopped, and the FlashGel Recovery Buffer is added, will DNA remain in the recovery well. Recovering DNA using the FlashGel System is highly efficient. Figure 3 shows clear voids where samples were removed during recovery.

CAPABILITIES OF 1.2% & 2.2% FLASHGEL CASSETTES

Extraction range windows are predefined by selecting and using the 1.2% or the 2.2% Recovery Cassettes. Optimal separation range for the 1.2% FlashGel Recovery Cassettes is 50-4,000bp, and optimal separation range for the 2.2% Recovery Cassettes is 10-1,000bp.

The 2.2% cassettes provide a larger spread of bands in the 10-400bp range, while the 1.2% cassettes show a wider spread in the larger size range (Figure 4). The 2.2% cassettes will provide a tighter and more defined range than the 1.2% cassettes within the same size range (Figure 5). The 1.2% cassettes may be better suited for a wider size selection range in a single extraction cycle.

SUMMARY

The FlashGel System is an efficient tool for fragmented DNA size selection. The two recovery cassette concentrations (1.2% and 2.2%) are optimized to provide narrow separation over a wide size range. With the FlashGel System, DNA fragments may be separated, recovered, and photographed in as little as five minutes, without the use of UV light. Recovered samples are immediately ready for downstream applications–eliminating agarose gel preparation, band excision, and purification.

REFERENCES

1. *Ordahl CP, Johnson TR, Caplan AI. (1976). Sheared DNA Fragment Sizing: Comparison of Techniques.* Nucleic Acids Res. *3(11):2985-99.*
2. *Mary Riley and Hugh White. (2009). FlashGel System for DNA Recovery.* ResourceNotes. *6(1): 17-20. White paper available on line: www.lonza.com/go/literature/2212.*
3. *(2009). FlashGel™ System - Protocol. Document # 00521123-0209-1. Available online: www.lonza.com/go/literature/1657.*

Description	Cat. No.
FlashGel Recovery Starter Kit contains: • FlashGel Recovery Cassettes, 1.2%, 8+1 (18-well), Double Tier (Pk. 9) • FlashGel Loading Dye (5x1mL, 5X Concentrate) • FlashGel Recovery Buffer, Ready-to-Use (2x500µL) • FlashGel QuantLadder (100bp (3ng) -1.5kb (30ng) (50 apps, 250µL) • Visualization Glasses • Control Fragment	**95053-314**
FlashGel Recovery Cassette, 1.2%, 8+1 (18-well), Double Tier	**95053-310**
FlashGel Recovery Cassette, 2.2%, 8+1 (18-well), Double Tier	**89135-718**
FlashGel Recovery Buffer (2x500µL)	**95053-312**
FlashGel System contains: • FlashGel Dock • FlashGel Camera • FlashGel DNA Cassettes, 1.2%, 12+1 well, Single Tier (Pk. 9) • FlashGel Loading Dye and DNA Marker	**95045-604**