

# Complementary DNA Shearing and Size-selection Tools for Mate-pair Library Construction



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## Introduction

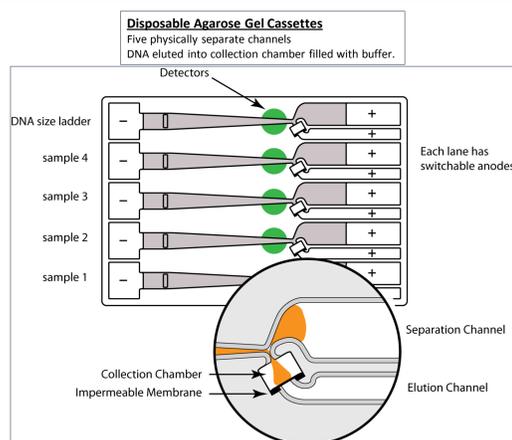
Mate - pair library sequencing is an essential technique for de novo genome sequencing, genome finishing, and analyses of genomic structural variation. Current methods for creating mate-pair libraries are extremely inefficient and time-consuming for two main reasons. First, it is difficult to develop reproducible DNA fragmentation methods that produce narrow size distributions in the range of 2 to 20kb. Second, manual preparative agarose gel electrophoresis is the only size-selection technique currently available for this size range. Digilab and Sage Science have developed two new products, the Digilab Hydroshear Plus and the Sage BluePippin system, that provide a simplified and complementary workflow for optimizing high molecular weight DNA fragmentation and size-selection. The Hydroshear Plus represents the second generation of DNA shearing systems utilizing hydrodynamic force for semi-automated, reliable, and reproducible fragmentation of genomic DNA into fragments greater than 0.8 Kb. It is the only system in the current market for reliable and consistent preparation of large DNA fragments necessary for genomic library construction and third-generation sequencing. The Sage BluePippin system is a new automated preparative electrophoresis system that features an alternating field power supply capable of size-fractionating DNA fragments as large as 50 kb. Selected DNA fractions are automatically eluted into a small volume of liquid buffer, ready for enzymatic library construction reactions. We will demonstrate the advantages of combining Hydroshear Plus and BluePippin for producing tight DNA fragment distributions in the 4 kb size range.

## Instrumentation

Hydroshear technology is based on the shearing of DNA as it is forced through a channel with an abrupt step-down in diameter. By varying the channel dimensions and flow rates, different size distributions can be obtained, ranging from around 1kb to around 40kb. Shearing is independent of sample DNA concentration, and can be carried out in volumes as low as 40 µl. Total processing time is approximately 10 min. Hydroshear Plus represents the second generation of hydrodynamic DNA shearing systems, featuring a multi-port valve, liquid reservoirs, and optimized wash reagents for hands-free, automated flow cell washing.



The BluePippin is an enhanced version of the Sage's original Pippin Prep instrument. The system includes disposable pre-cast agarose gel cassettes with five physically isolated lanes. Each lane has a tapered separation channel that branches into two channels with positive electrodes at their termini. Through independent control of the positive electrodes, DNA exiting the separation channel can be directed left or right at the branch point. DNA for library formation is collected in liquid buffer within a membrane-delimited elution module. As in the original, the BluePippin instrument contains a single board PC, a fluorescence detection unit, and an electrophoresis power supply.

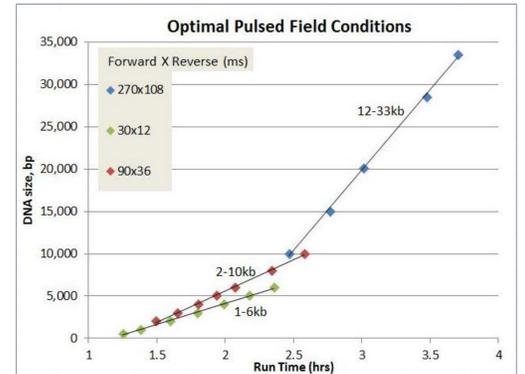


The BluePippin instrument has several improvements for fractionation of HMW DNA. The BluePippin features a **pulsed field power supply** for improved resolution of HMW genomic DNA. Pulsed field programs are available for DNA size ranges of 1-6 kb, 3-10kb, and 12-33 kb (using 0.75% agarose cassettes). The instrument can also operate in a low voltage mode (25V DC) for very high resolution fractionations in the 1-6 kb range. The optical system has been modified to detect fluorescein-labeled DNA markers. This allows the markers to be used as internal standards (mixed with sample DNA) for greater accuracy.

Blue Pippin can also be used for DNA fractionations in the 50bp - 1kb range, like the original Pippin Prep. In this range, **run speed has been improved so that a 500 bp fraction can be collected within 35 minutes.**

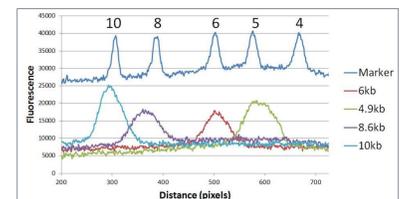
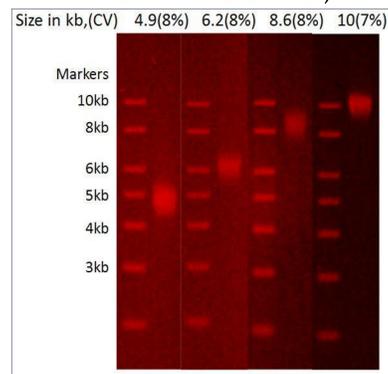
## Run Times for HMW DNA size fractionation on BluePippin

For accurate HMW size selections, the BluePippin uses pulsed field conditions that provide a linear relationship between run time and DNA size in the 0.75% cassette. The figure shows pulsed field conditions for three different size ranges, 1-6kb (green), 2-10kb (red), and 12-33kb (blue). Sizes out to 10kb can be selected in 2.5 hrs or less. Larger sizes out to 33kb can be collected in less than 4 hrs.



## Accurate, Tight Product Size Distributions on BluePippin

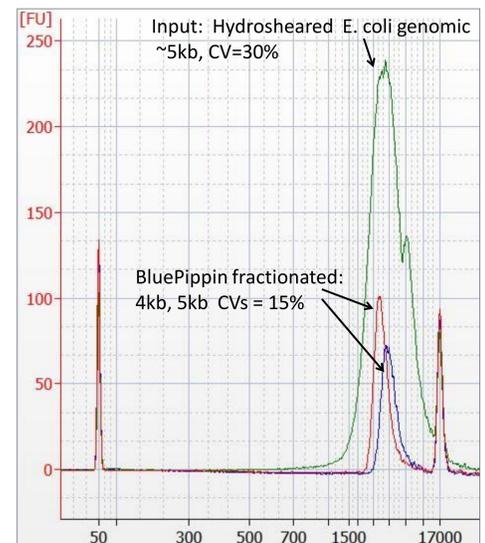
Size selection products produced from randomly sheared E.coli DNA using the BluePippin (2-10kb pulsed field conditions) are shown at right. The fractionations demonstrate the tight size distributions possible with the BluePippin --  $\leq 10\%$  CV -- even at 10kb. (Products were analyzed on 0.75% agarose analytical gels and poststained with ethidium bromide.)



## Complementary Hydroshear and BluePippin Protocols

The figure at right examines the benefits of using BluePippin and Hydroshear together. The Hydroshear Plus was used to shear a sample of HMW E. coli genomic DNA to an average size of approximately 5kb. Following shearing, two samples were size selected on the BluePippin to produce DNA fractions centered on 4 and 5 kb. The Hydroshear product was centered on 5kb, with a CV of approximately 30% (measured on the Agilent Bioanalyzer 12000 chip). After processing on the BluePippin, the size distribution was narrowed by at least 2-fold, with good yield (around 40-50% in this experiment).

Combining Hydroshear Plus and BluePippin is attractive to make efficient use of genomic sample DNA. The Hydroshear Plus shears small genomic samples to a relatively narrow size distribution. Using the Hydroshear output, the BluePippin can further refine the DNA size distribution and average size as needed.



(Methods: HMW E.coli DNA was diluted in H<sub>2</sub>O to 5µg/200µl. 200µl of this solution (5µg total gDNA) was sheared using the standard Hydroshear shearing assembly. The shearing protocol used 20 cycles at speed code 13. Two sheared DNA aliquots (1.5µg, 30µl) were processed in a 0.75% dye-free BluePippin cassette, using tight settings centered on 4 and 5 kb with the pulsed field program for 2-10kb range.)

## Contacts

**Come see the Hydroshear Plus and BluePippin at the Sage Science Booth, #202**



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