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, and it is labor-intensive and irreproducible. Covaris and Sage Science have developed two new products, the Covaris g-TUBE and the Sage BluePippin system, that provide a simplified and complementary workflow for optimizing high molecular weight DNA fragmentation and size-selection.

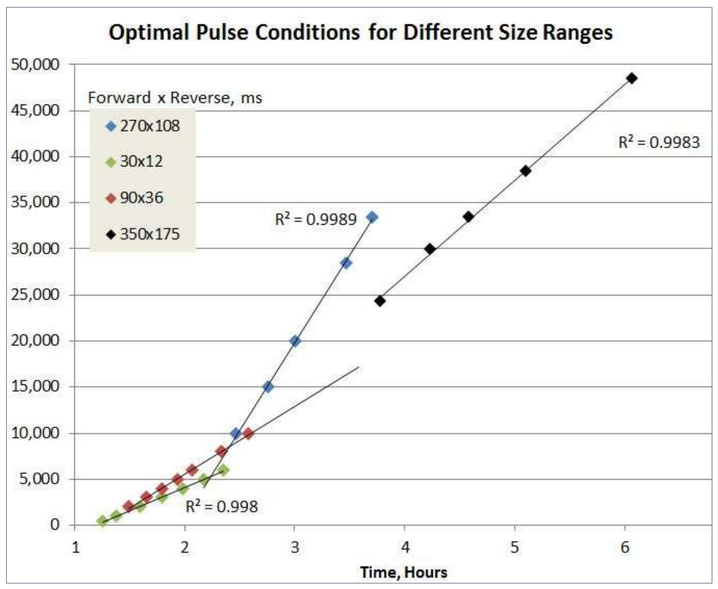
The Covaris g-TUBE

The Covaris g-TUBE uses centrifugal force to pass the DNA sample through a finely engineered shearing orifice, and then captures the fragmented DNA sample in an integrated collection chamber. By adjusting the centrifugation time, fragment sizes between 7 kb - 20 kb can be generated in 2 minutes or less, with sample recovery > 90%.

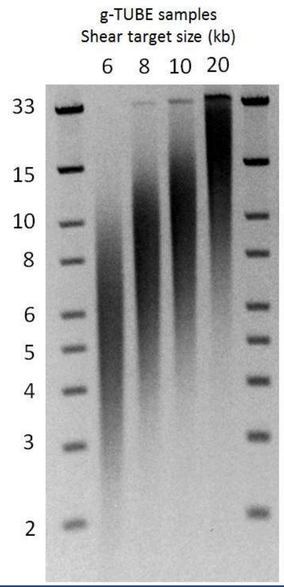
The Sage BluePippin

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 50kb. Selected DNA fractions are automatically eluted into a small volume of liquid buffer, ready for enzymatic library construction reactions.

By varying the duration and ratio of forward and reverse voltage pulses, the BluePippin can resolve and purify DNAs between 1 and 50kb. The BluePippin can also be used in constant field DC mode like the original Pippin Prep for DNA fractionations between 50 and 1500bp.



g-TUBE Samples after shearing



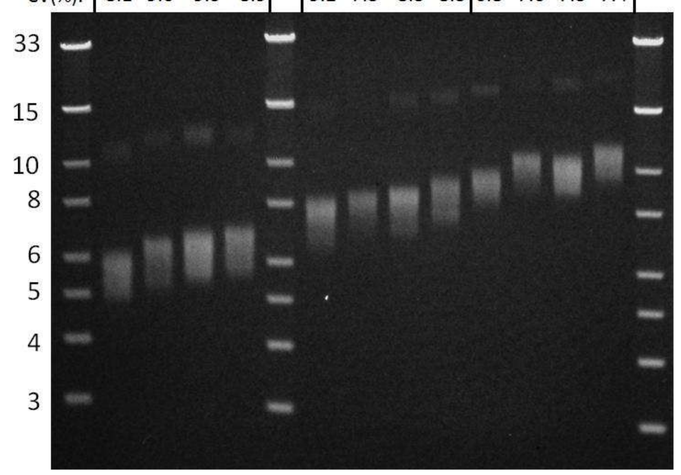
g-TUBE conditions	Measured ave. size(kb):
1 – 6 kbp / 14500 rpm	5.9
2 – 8 kbp / 9400 rpm	8.4
3 – 10 kbp / 8000 rpm	10.6
4 – 20 kbp / 6500 rpm	16.9

Phage lambda DNA was diluted to 50μg/ml in TE buffer and 8 μg samples were sheared in g-TUBES at 4 different speeds. 500 ng samples were analyzed on pulsed field analytical agarose gels and post-stained with EtBr. The average size of the sheared product was determined from the gel images.

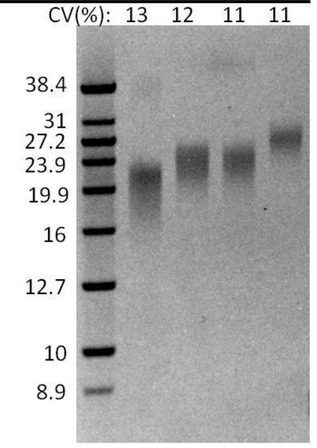
High-resolution size selection using the BluePippin

1.5 μg aliquots of the sheared DNA samples shown above were fractionated in the BluePippin using the settings indicated above the gel images. The right panel used the 20kb g-TUBE sheared samples. In general, accuracy was around 10% or better out to 10kb, and ranged 15-22% at 20kb. CV's ranged between 7-13%.
(The trace HMW bands are dimers caused by hybridization of cos-containing fragments after the Pippin fractionation.)

g-TUBE shear size:	6				8				10			
Pippin setting(kb):	5.4	6	6.3	6.6	7.2	8.0	8.4	8.8	9.0	10.0	10.5	11.0
Actual(kb):	**5.5**	**6**	**6.2**	**6.4**	**7.5**	**8.2**	**8.0**	**8.6**	**9.2**	**10.1**	**9.8**	**10.4**
CV(%):	8.1	9.0	9.5	8.9	9.2	7.3	8.3	8.3	6.8	7.6	7.3	7.4



Programmed(kb):	19	20	21	22
Actual(kb):	**21**	**23**	**24**	**27**
CV(%):	13	12	11	11



Conclusions

A combination protocol using Covaris g-TUBE shearing followed by high resolution size selection on the Sage BluePippin instrument is a convenient and effective method to prepare tightly-sized HMW libraries over the range of 6-20kb. The sheared g-TUBE product can be reliably targeted to specific size ranges by control of centrifugation speed. The g-TUBE product distributions are approximately 3-7 kb wide. Subsequent use of the BluePippin can refine the target size of the product DNA, and reproducibly generate samples with CV's around 10%.

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