Operating HydroShear Plus.

Generating large DNA fragments for library construction or sequencing.

INTRODUCTION

Figure 1. *HydroShear Plus (HSP00000)*

HydroShear Plus represents the second generation of DNA shearing systems utilizing hydrodynamic force for semi-automated reliable and reproducible fragmentation of a genomic DNA into fragments larger than 0.8kB. 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 [1]. The system (Figure 1) consists of four major parts: the basic mechanical unit, interchangeable shearing assembly, software and consumables. All parts are available for purchase through either Digilab (www.digilabglobal.com) or its distributors both individually and in combination. The DNA fragment size depends on the type of shearing assembly and the speed at which the DNA passes through it. For generation of large fragments, a large shearing assembly suits the best. This technical note describes how to generate large DNA fragments using Digilab's HydroShear Plus system.

PROCEDURE

1. The size of DNA fragments, generated by HydroShear Plus system and a large shearing assembly, (HSP20003) depends on the speed code the customer can easily regulate and remains within the range from 4kB to100kB (Figure 2).

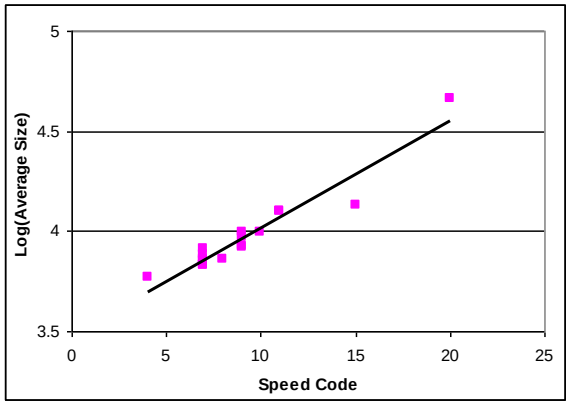

Figure 2. *Typical sizes of DNA fragments, generated by HydroShear Plus with a large shearing assembly.*

2. Because of the minor variations in the manufacturing process, large shearing assemblies may vary by performance. Digilab recommends calibrating every new shearing assembly separately. Calibration should be performed by running a high molecular weight DNA sample through the new shearing assembly using three-four different speed codes (use 20 cycles for each run).

3. The resulting fragments can be analyzed by PFGE gel-electrophoresis. A size marker should be run to allow calculation of the fragment sizes. The average size of the obtained fragments should be plotted in coordinates: log of the molecular weight of the fragment versus the particular speed code (see Figure 2 as an example). The obtained graph can be used to choose the speed code, suitable for generation of DNA fragments of desired size (see examples of DNA fragments made with a large shearing assembly by HydroShear Plus after separation by gel-electrophoresis in the Figures 3-4).

4. When the same shearing assembly is used, reproducibility of the size distribution patterns and the average DNA fragment sizes is generally very high with maximal length being less than twice the size of the smallest ones for 90% of the resulting fragments in the mixture. Hydrodynamic shearing neither generates heat, nor chemically alters DNA molecule, which results in production of DNA fragments with minimal damage. In agreement with that, previously published data [2] showed that about 60% of the resulting DNA fragments can be ligated and cloned even without additional end repair.

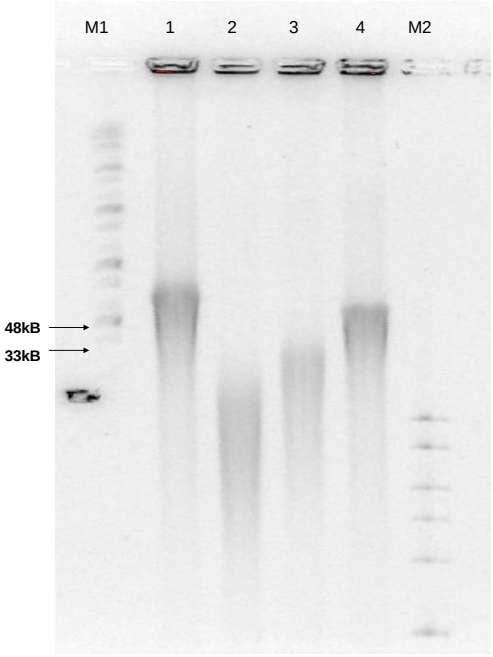

Figure 3.
Lane 1: Unsheared microcrustacean genomic DNA
Lane 2: Speed code 10; 20 cycles (average fragment size 7kB)
Lane 3: Speed code 15; 20 cycles (average fragment size 12kB)
Lane 4: Speed code 20; 20 cycles (average fragment size 38kB)
M1: Midrange I PFG Marker
M2: 1 kb DNA ladder

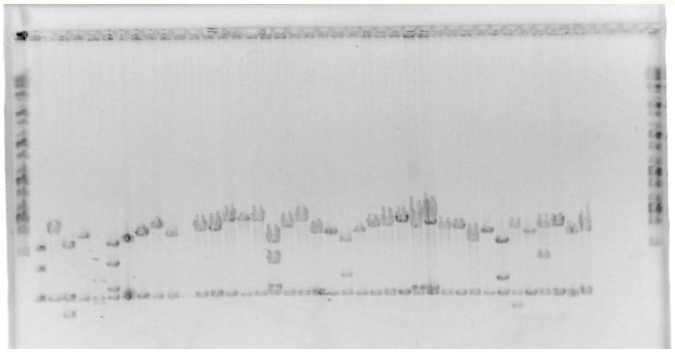

Figure 4. *A genomic fosmid library was constructed by shearing 20ug of genomic DNA using HydroShear Plus with a large shearing assembly with speed code 20, for 20 cycles. Sheared DNA was subject to end-repair and directly cloned into the pCC2FOS fosmid vector from Epicentre. Inserts ranged from 36-42kB with an average size of 38kB. Using of HydroShear Plus eliminated the need for time-consuming and tedious agarose gel size-selection process.*

REFERENCES:

1. Sten Linnarsson "Recent advances in DNA sequencing methods – general principles of sample preparation", *Experimental Cell Research*, 316(8): 1339-1343 (2010)

2. Yvonne R. Thorstenson, Scott P. Hunicke-Smith, Peter J. Oefner, and Ronald W. Davis "An Automated Hydrodynamic Process for Controlled, Unbiased DNA Shearing", *Genome Research*, 8(8): 848-855 (1998)

Acknowledgement: This Application Note is partially based on data, kindly provided by Dr. Eric Olivares (Pacific Biosciences), and Dr. Christopher Saski (Clemson University).

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