APPLICATION NOTE

Operating HydroShear Plus. How to Choose the Proper Shearing Assembly Unit.

Figure 1. HydroShear Plus (HSP00000)

INTRODUCTION

HydroShear Plus represents the second generation of hydrodynamic DNA shearing systems performing semi-automated reliable and highly reproducible fragmentation of a long DNA into fragments larger than 0.8kB. Recently, it was successfully used for shearing mRNA as well (for additional information, see the corresponding Application Note). 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 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. Currently, Digilab can supply customers with three types of shearing assemblies. There are certain rules the customer needs to follow while choosing the appropriate shearing assembly. This technical note describes how to choose the proper shearing assembly unit and calibrate it for use with your HydroShear Plus system.

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PROCEDURE

1. The size of DNA fragments, generated by HydroShear Plus system, depends on the type of shearing assembly. Currently, Digilab manufactures three types of shearing assemblies:

Small assembly	(HSP20002):	Fragment size 850bp to 3kb – orifice size 0.0016"
Standard assembly	(HSP20001):	Fragment size 1.4kb to 8 kb – orifice size 0.0025"
Large assembly	(HSP20003):	Fragment size 4kb to >50kb – orifice size 0.0050"

- 2. Because of the minor variations in the manufacturing process, shearing assemblies of the same type 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 gel-electrophoresis (regular system for 1-10kB, or PFGE system for larger fragments), or Bioanalyzer module. 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. The obtained graph can be used to choose the speed code, suitable for generation of DNA fragments of desired size (see examples in the Figures 2 and 3).

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

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Figure 3. Typical sizes of DNA fragments, generated by HydroShear Plus with large shearing assembly.

4. When the same shearing assembly is used, reproducibility of the size distribution patterns and the average DNA fragment sizes is generally very high, as illustrated by Figure 4. Here, DNA of phage Lambda was sheared with a standard shearing assembly (speed codes 13 and 15, marked as SC13 and SC15 respectively), or a large shearing assembly (speed codes 7 and 9, marked as LG-SC7 and LG-SC9 respectively) for 20 cycles and resulting fragments were analyzed by a Bioanalyzer module.



Figure 4. Reproducibility of DNA shearing results with standard or large shearing assemblies.

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