Large DNA Size Selection with the ProNex® Size-Selective Purification System

Promega Corporation

Materials Required

- ProNex® Size-Selective Purification System (Cat.# NG2001, NG2002 or NG2003)
- magnetic stand or plate
- Benchtop pGEM® DNA Markers (Cat.# G7521)
- 1kb DNA Ladder (Cat.# G5711)
- Lambda DNA/HindIII Markers (Cat.# G1711)
- up to 9µg of sheared DNA

Introduction

Long-read DNA sequencing platforms, with read lengths >10kb, are working towards improving the assembly of complex genomes. These techniques require high-molecular weight DNA; however, purification of such long sequences is difficult to obtain. Even those purification methods specifically designed to purify high-molecular weight DNA often return a broad range of fragment sizes, requiring some form of post-extraction cleanup procedure. In this application note we describe the use of the ProNex® Size-Selective Purification System for effective, rapid and automatable purification of fragments >10kb.

Methods

DNA ladders of various sizes were used with the ProNex® Size-Selective Purification System in a single-sided cleanup mode. Ratios of ProNex® Chemistry:DNA were tested from 1–0.81X to expand the DNA size cutoff range in Table 1 to sizes above 1kb. Further information, including a detailed example protocol of this system, can be found in the *ProNex® Size-Selective Purification System Technical Manual* #TM508.

Note: We strongly recommend performing a pilot purification with a disposable sample, such as a DNA ladder, in the sample matrix before attempting to purify an important sample.

Table 1. Standard Ratios for Size-Selective Purification of DNA Fragments.[a]

ProNex® Chemistry Ratio (v/v)[a]	Approximate Size Cutoff (bp)
3X	100
2X	150
1.5X	250
1.3X	350
1.2X	475
1.15X	550
1.1X	650
1.05X	800
1X	1,000

[a]The presence of DNA precipitants, such as polyethylene glycol (PEG), in the sample can lead to the retention of lower molecular weight DNA fragments than intended. For proper size selection of samples with DNA precipitants present, the ratio required to achieve desired size cutoffs must be reduced.

Results

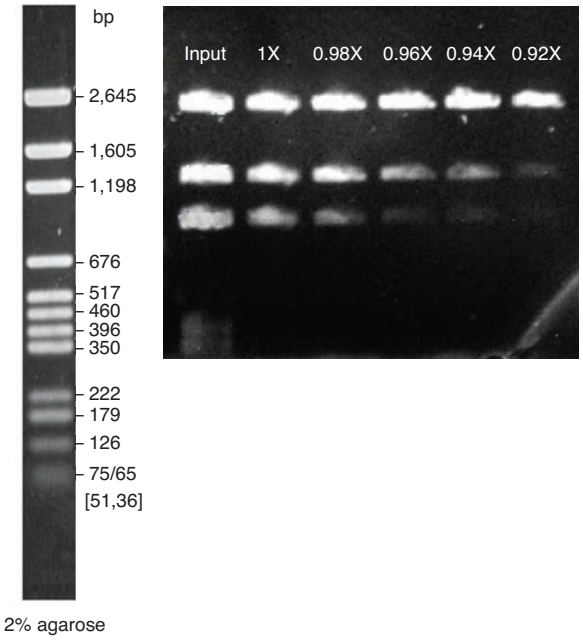

Figure 1. Agarose gel image of BenchTop pGEM® DNA Marker size-selected using the ProNex® Size-Selective Purification System. Size-selected BenchTop pGEM® DNA Markers (Cat.# G7521) were electrophoresed on a 0.7% agarose gel. To the left of the gel are the BenchTop pGEM® DNA Markers with their fragment sizes. The first lane of the gel is labeled Input and contains input DNA without size selection. Ratio in volume/volume of ProNex® Chemistry added to DNA is labeled above each well**.**

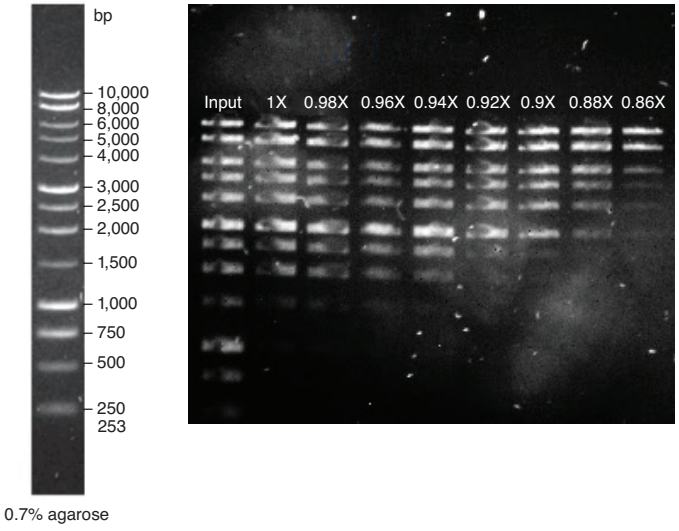

Figure 2. Agarose gel image of 1kb DNA Ladder size-selected using the ProNex® Size-Selective Purification System. Size-selected 1kb DNA Ladder (Cat.# G5711) was run on a 0.7% agarose gel. To the left of the gel is the 1kb DNA Ladder with its fragment sizes. The first lane of the gel is labeled input and contains the 1kb DNA Ladder without size selection. Ratio in volume/volume of ProNex® Chemistry added to DNA is labeled above each well.

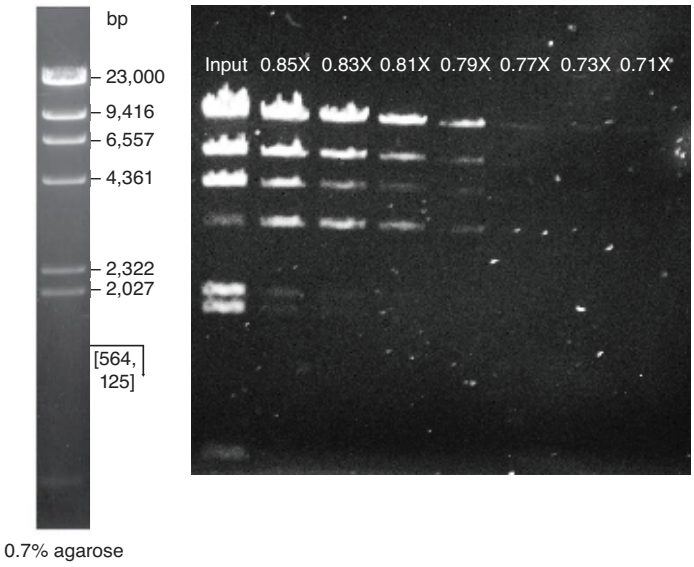

Figure 3. Agarose gel image of Lambda DNA/HindIII Markers size-selected using the ProNex® Size-Selective Purification System. Size-selected Lambda DNA/HindIII Markers (Cat.# G1711) were run on a 0.7% agarose gel. To the left of the gel are Lambda DNA/HindIII Markers with their fragment sizes. The first lane of the gel is labeled input and contains Lambda DNA/HindIII Markers without size-selection. Ratio in volume/volume of ProNex® Chemistry added to DNA is labeled above each well.

Conclusion

Third-generation sequencing platforms such as those from PacBio or Oxford NanoPore are making steady progress towards overcoming many of the pitfalls of short-read sequencing. Using pure, high-molecular weight DNA is even more critical for these systems to ensure optimal results. Here we show that the ProNex® Size-Selective Purification System can be used for the purification of large DNA fragments >10kb with an effective and rapid method.