Innovative NiXTip™ Technology for Efficient Size Selection in Sequencing Library Preparation

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HIGHLIGHTS: Fully automated, fast (< 30 min), customizable

NIX

INTRODUCTION

Solid Phase Reversible Immobilization (SPRI) with magnetic beads has been the gold standard for DNA clean up and size selection in Next Generation Sequencing (NGS) protocols. The use of magnetic beads eliminates the need for centrifugation but has its own inherent draw backs such as bead capture time, bead carryover, bead resuspension and the requirement for magnets. DPX Technologies has developed NiXTips™ that eliminate magnetic beads and magnets for DNA clean up and size selection in NGS library preparations. NiXTips incorporate a proprietary silica media within a pipette tip to utilize aspirate and dispense steps, which streamline DNA recovery while maximizing efficiency. Applications include DNA capture from PCR and enzymatic reaction cleanup, as well as double-sided size selection for sequencing libraries. Results demonstrate almost 100% recovery of DNA depending on target fragment size and buffer composition of the sample. The data presented herein shows that NiXTips simplify workflows while providing high yield of DNA targets.

MATERIALS AND METHODS

Figure 1. *NiXTips shown on a Hamilton® Robotics platform.*

NiXTips were utilized with the accompanying proprietary room-temperature binding buffer for optimal performance (DPX Technologies, Columbia, SC). Wash buffer (80% ethanol) and elution buffer (molecular biology grade water) were also utilized but are not provided in the NiXTips kit. DNA samples prepared using an in-house PCR product with an amplicon size of 205 bp were used for the cleanup and Sanger sequencing experiments. DNA molecular weight marker (MWM) VIII with a size range of 19 to 1114 bp (Millipore Sigma, Burlington, MA) was used for the size selection and binding cycle studies. PCR reactions were performed on a QuantStudio™ 3 (Thermo Fisher Scientific, Waltham, MA) with the TaqMan™ Genotyping MM from Thermo Fisher Scientific. For these studies, 25 µL of DNA sample was used. All samples, reagents, and tips were loaded onto a Hamilton® NIMBUS automated liquid handler (ALH). Multiple iterations were performed with various binding buffer ratios and binding cycles to perform size selection and optimize yield, respectively.

1 Sample Prep	ALH picks up transfer tips and aspirates binding buffer to achieve the desired binding buffer to sample ratio. Binding buffer is added to the sample and mixed by aspirating and dispensing 20 times.
2 Bind	NiXTips aspirate and dispense sample up to 40 times to bind DNA.
3 Wash	Wash with 80% ethanol by aspirating and dispensing 50 µL of wash buffer with the NiXTip, 1 time. Dry the proprietary silica media using an air blowout to eliminate any residual ethanol.
4 Elute	NiXTips aspirate and dispense 27 µL of elution buffer at 56°C, 2 times.

Table 1. *NiXTip Method. For elution, 27 µL is used to achieve a final eluent volume of 25 µL due to a 2 µL loss to the media.*

The eluted DNA was analyzed to assess purity and yield using a Thermo Fisher Scientific NanoDrop One Microvolume UV-Vis Spectrophotometer and an Agilent 2100 Bioanalyzer (Santa Clara, CA). To confirm size selection, agarose gel electrophoresis was performed. Compatibility for downstream applications was verified by Sanger sequencing of the GAPDH gene.

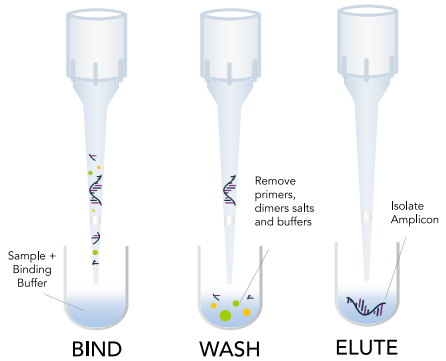

Figure 2. *Schematic of NiXTips workflow.*

RESULTS

To optimize speed and efficiency, a study was conducted to determine the impact of number of binding cycles on DNA yield and overall method time. DNA MWM VIII (25 µL) was combined with binding buffer (20 µL) to achieve a 0.8X binding buffer ratio. The method outlined in **Table 1** was then performed using 5, 10, 15, 20, 25, and 30 aspirate and dispense cycles for binding (**Figure 3**). Results show that binding plateaus around 20 cycles (31 ng/µL) with no significant increase occurring at 25 cycles (33 ng/µL) and 30 cycles (33 ng/µL).

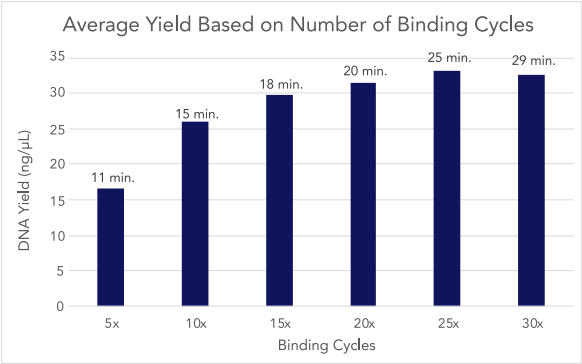

Figure 3. *Recovery of DNA MWM VIII in ng/µL with different (5, 10, 15, 20, 25, 30) aspirate/dispense cycles (binding cycles).*

For PCR cleanup of primers and primer-dimers, binding buffer was added to the sample to achieve a 0.8X ratio and the method was run with 30 binding cycles on the NiXTip. Purified samples were run on a 1% agarose gel with an unpurified PCR sample loaded as a control (**Figure 4**). The gel shows the effective removal of primers and primer-dimers by NiXTips. The purified amplicons were also sent out for Sanger sequencing of the GAPDH gene which was successful as shown in **Figure 5**.

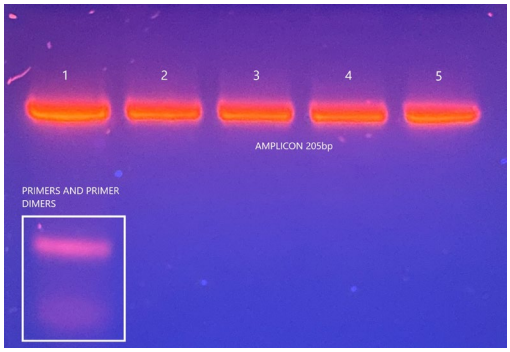

Figure 4. *Gel electrophoresis of PCR products before (lane 1) and after NiXTip cleanup (lanes 2-5).*

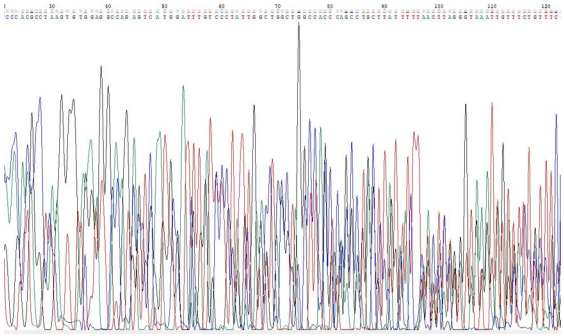

Figure 5. *Results of Sanger sequencing a PCR product purified using NiXTip technology.*

DNA fragment size capture was determined using DNA MWM VIII with different ratios of binding buffer and 30 binding cycles. The recovery of DNA fragments at binding buffer ratios of 0.4X to 1X was determined using the bioanalyzer. This data was used to generate the size selection graph shown in **Figure 6**. The results displayed in **Figure 6** can be used to determine the optimal binding buffer to sample ratio based on the size of the desired amplicon or fragment of interest.

A double-sided size selection (n=4) was performed to demonstrate the capability of NiXTips. For this study, the targeted fragment size was between 110 and 400 bp and the range of interest is bracketed by the 0.5X and 0.8X ratio curves. **Figure 7** shows the captured fragment sizes from each tip at a 0.5X and 0.8X binding buffer ratio. Lanes 3, 5, 7, and 9 show the final product from double-sided size selection that would be utilized for downstream applications.

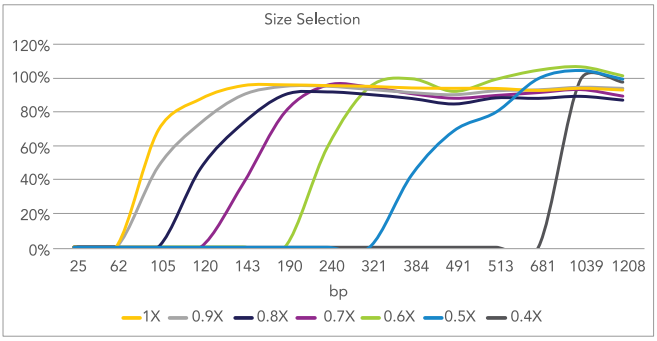

Figure 6. *Percent recovery of DNA fragments at different binding buffer to sample ratios.*

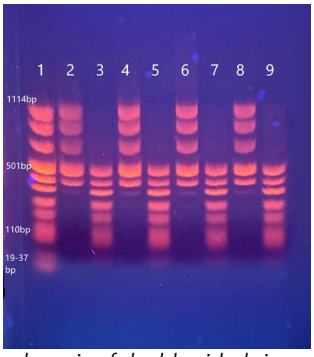

Figure 7. *Gel electrophoresis of double-sided size selection using NiXTips. Lane 1 is DNA MWM VIII. Lanes 2, 4, 6, and 8 show DNA fragments bound to the NiXTip at 0.5X binding buffer to sample ratio. Lanes 3, 5, 7, and 9 show DNA fragments bound to a second NiXTip when the same samples are brought up to 0.8X binding buffer to sample ratio.*

DISCUSSION

NiXTips offer a promising alternative to magnetic beads, providing efficient and high-yield DNA amplicon recovery from PCR reactions as well as precise double-sided size selection for DNA fragments in sequencing library preparations. The fully automated bind-wash-elute protocol reduces hands-on time, eliminates the need for magnets and magnetic beads, and makes it ideal for high-throughput applications. The flexibility to adjust the number of binding cycles offers customizable workflows based on the user's desired yield and time constraints.