Double-Sided Size Selection in Short-Read Library Preparation Using 300 µL NiXTips

HIGHLIGHTS: Efficient, Reproducible, Scalable, Reduced Consumable Wasted

NIX

PURPOSE AND OBJECTIVE

- DNA fragment size selection is a critical step in sequencing library preparation, directly influencing read quality and downstream performance.
- Conventional bead-based double-sided methods can introduce variability through multiple transfers and increased handling.
- 300 µL NiXTips streamline this process by performing binding, washing, and elution entirely within the pipette tip, minimizing loss and cross-contamination.
- On the Hamilton STARlet, the automated double-sided workflow enables consistent enrichment of DNA fragments within the desired range.

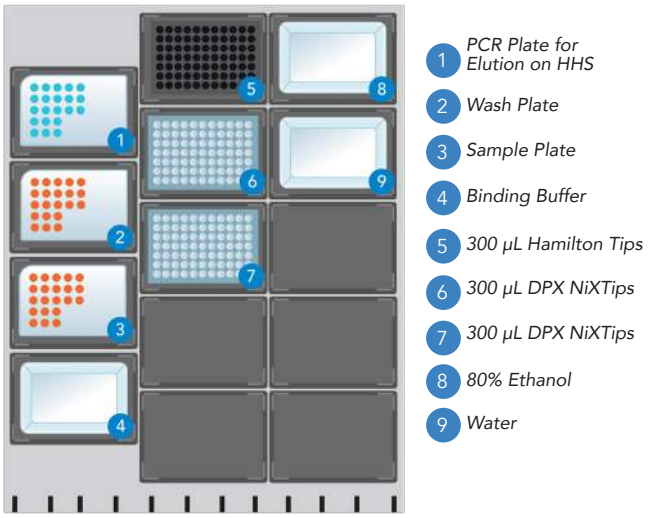


Figure 1. *Hamilton STARlet deck layout for double-sided size selection. Deck layout can be customized.*

METHOD

Genomic DNA was obtained from Oncorhynchus (salmon testes DNA, G-Biosciences, catalog no. 50-111-8027). Sixteen 3 µg aliquots were digested using NEB dsDNA Fragmentase (New England Biolabs, catalog no. M0348L) for 16 minutes at 37°C in a Quant 3 Applied Biosystems thermocycler to generate a distribution of DNA fragments suitable for short-read library construction. Double-sided size selection of the fragmented DNA was performed on a Hamilton STARlet using a commercially available, DPX-authored Hamilton script. The double-sided workflow enables the removal of both oversized and undersized fragments through sequential binding with functionalized NiXTips (DPX Technologies, *Catalog # NIX-HM300-96*; including proprietary Binding Buffer). Right-sided size selection was accomplished by the addition of 25 µL (0.5X) of binding buffer to the 50 µL fragmented DNA reaction. A 300 µL NiXTip was utilized to selectively bind and remove high molecular weight fragments. Binding was performed via 30 aspiration/dispense cycles to ensure maximum capture efficiency. Left-sided size selection was accomplished by subsequent addition of 4 µL (0.58X) of binding buffer to the 75 µL fragmented DNA reaction. A new 300 µL NiXTip was used to capture fragments within the desired size distribution. The bound DNA was washed by two cycles of aspiration/dispense of 80% ethanol. DNA was subsequently eluted in 50 µL of Molecular Biology Grade water pre-heated to 56°C.

RESULTS

Electrophoretic analysis confirmed that the double-sided size selection produced fragments enriched in the expected size range suitable for short-read sequencing. The profiles shown in **Figure 2** demonstrate reproducible fragment recovery and consistent size distribution across varying input amounts. This chart highlights the enrichment of fragments within the desired range and the removal of both oversized and undersized DNA.

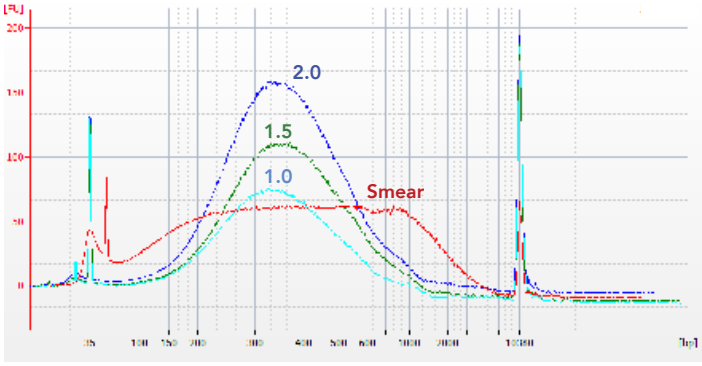
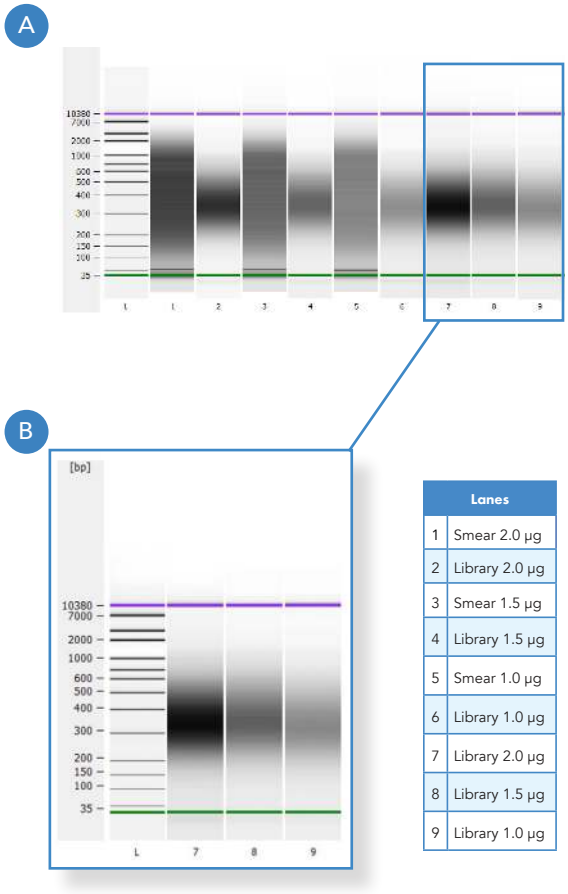


Figure 2. *Double-sided size selection of salmon genomic DNA using 300 µL NiXTips. Agilent 2100 Bioanalyzer High Sensitivity DNA traces showing overlaid electropherograms of double-sided size-selected fragments from 2 µg, 1.5 µg, and 1 µg input DNA.*



Lanes	
1	Smear 2.0 µg
2	Library 2.0 µg
3	Smear 1.5 µg
4	Library 1.5 µg
5	Smear 1.0 µg
6	Library 1.0 µg
7	Library 2.0 µg
8	Library 1.5 µg
9	Library 1.0 µg

Figure 3. *Bioanalyzer gel-like image of salmon genomic DNA before and after double-sided size selection with 300 µL NiXTips at 2.0, 1.5 and 1.0 µg of input DNA.*

Figure 3A shows the transition from broad smears to discrete bands which demonstrates efficient removal of undersized and oversized DNA fragments. **Figure 3B** displays only the size-selected products from 2 µg,1.5 µg, and 1 µg inputs (left to right), illustrating the consistency and reproducibility of the double-sided selection process.

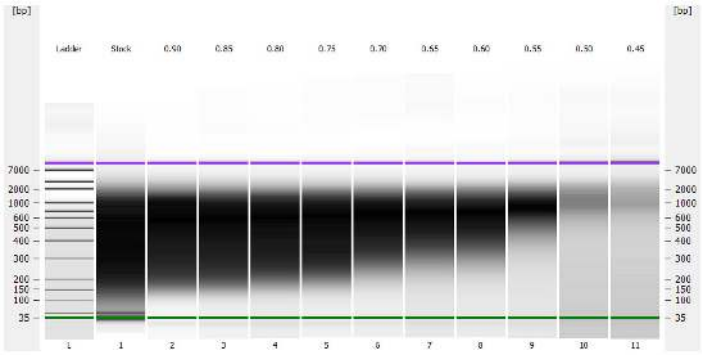


Figure 4. *Left sided size selection chart using varying ratios of Binding Buffer with salmon genomic DNA.*

The NiXTip product line can provide efficient size selection across a range of fragment sizes, from 100 to 2000 bp, with a capacity of up to 140 µg. **Figure 4** shows distinct size selection efficiency with a range of Binding Buffer ratios, making it an ideal product for various short-read sequencing applications.

DISCUSSION

Electrophoretic analysis demonstrated that the DPX 300 µL NiXTip workflow produced consistent enrichment of DNA fragments within the desired size range for short-read sequencing. The removal of both oversized and undersized fragments was efficient across input amounts ranging from 1–2 µg, resulting in reproducible profiles and well-defined bands on Bioanalyzer traces. These results confirm that the double-sided size selection process is robust to variations in DNA input and consistently yields fragment distributions compatible with downstream sequencing applications.

A key advantage of the NiXTip approach lies in its fully integrated, tip-based format, which eliminates the need for beads or magnetic separation. By conducting both binding and washing directly within the pipette tip, the workflow minimizes sample transfers that can lead to loss, cross-contamination, or variability. This design enhances reproducibility while streamlining library preparation on automated platforms such as the Hamilton STARlet.

Furthermore, the programmable binding parameters in the provided Hamilton script offer flexibility in adapting the workflow to user-specific input amounts or size-selection requirements. This enables laboratories to fine-tune fragment recovery without significant alterations to protocol structure. Collectively, these results establish the DPX 300 µL NiXTips as a reliable and high-performance alternative to conventional bead-based double-sided size selection methods.

Available NiXTips Formats:

Automations	Volume		Amount	
Hamilton	50 µL	300 µL	96 tips/rack	
Integra	125 µL	300 µL*	384 tips/box	96 tips/rack*
Agilent	125 µL		384 tips/box	
Tecan	200 µL*		96 tips/rack	

*Coming soon