Tip-Top Cleanup: NiX Tips for Dynamic PCR Product Cleanup

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HIGHLIGHT: Automated method with no additional hardware required

INTRODUCTION

This method demonstrates NiX tips for PCR cleanup produces comparable results to bead-based cleanup in both amount of recovered DNA product and speed of the cleanup protocol. However, NiX tips are superior for high throughput walk-away solutions as they are shelf stable and require no extra handling considerations nor hardware requirements. Magnetic beads are the gold standard for PCR cleanup in NGS processes. However, they require specific handling that can make high throughput automation difficult.

Table 1: *Comparison of current bead-based cleanup to new NiX Tips workflow.*

Bead-based Cleanup	NiX Cleanup
Storage at 4 ° C	Storage at room temperature
Difficult to keep uniform suspension	Handle like other tip consumables
Bead carryover can affect further PCT performance	No carryover of binding substrate
Magnet required to pellet beads	No additional hardware required

NiX tips were tested as an alternative to the current method to understand the following:

- **Efficacy**: Can NiX tips remove residual primers?
- **Speed**: How quick was a protocol that yields sufficient target product?
- **Adaptability**: How could we adjust the protocol to allow for size selection?

METHODS

PCR product was made using primers targeting the β2M locus and HEK cell whole-genome DNA template. PCR reactions were amplified for 35 cycles, pooled, and analyzed on a TapeStation prior to input into cleanups. For NiX tip cleanups, 25 µl of PCR product was mixed with DPX binding buffer at a 1x ratio, and mixed for variable binding cycles with NiX tips (NiX Tips, DPX Technologies, Columbia SC). NiX tips were then washed twice in 80% ethanol, and eluted with 25 µL of water. Bead-based cleanups were performed with a 1.2x ratio of beads to PCR product, mixed, and incubated before pelleting the beads on the magnet. The supernatant was then removed, and beads were washed twice with 80% ethanol. The pellets were airdried for a minute, and then eluted in water. Analysis of the cleaned product was performed using TapeStation D1000 assay. Percent recovery was calculated by comparing an un-cleaned control aliquot to the cleaned product concentration. Data for binding cycles versus percent recovery was generated using 16 replicates each. To get size selection data, a DNA ladder was cleaned up using different buffer ratios, and analyzed on a Bioanalyzer.

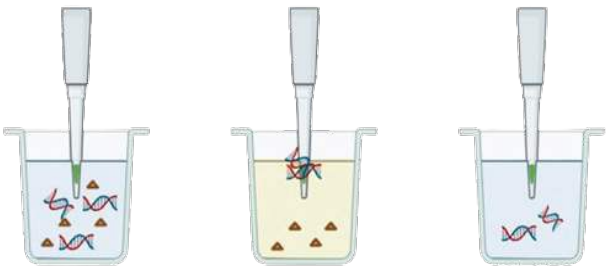

Figure 1 : *PCR Cleanup*

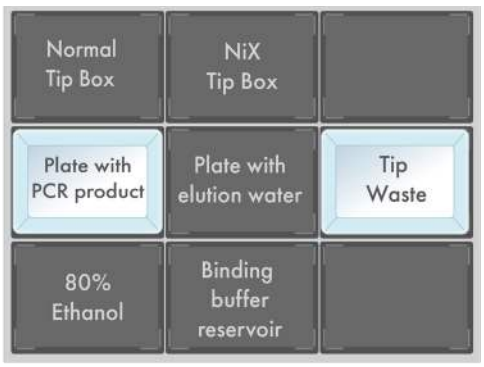

Figure 2: *Bravo Deck Layout*

RESULTS

1. Efficacy

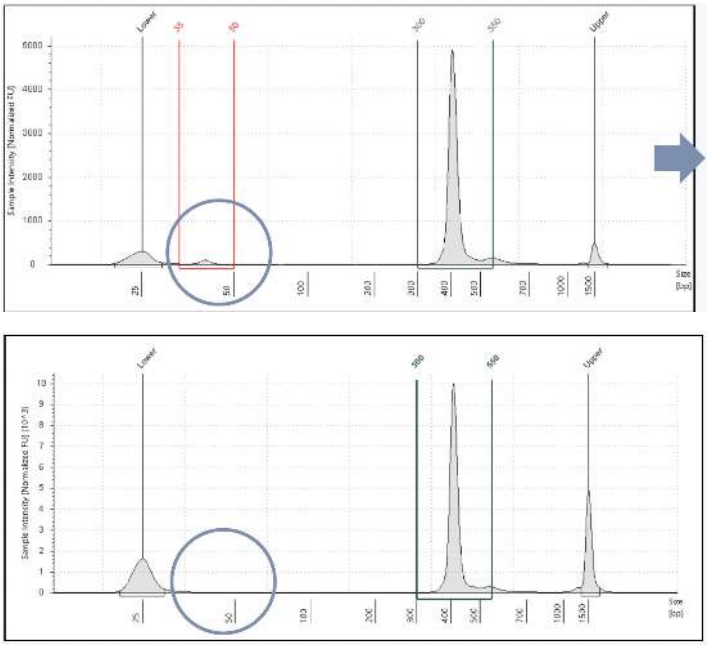

Figure 3: *Cleanup after 50 binding cycles. Results using a 1x binding buffer ratio and 50 binding cycles with NiX tips showed removal of PCR primer contaminants at ~40bp.*

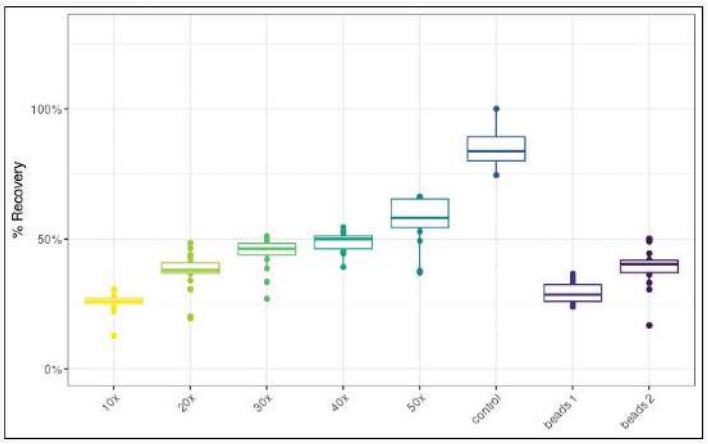

Figure 4: *Box plot of percent recovery for different binding cycle counts, compared with bead-based cleanups. Increasing the number of binding cycles performed with NiX tips increased the percent recovery of the PCR product. Two types of magnetic beads were used to compare a fast bead-based recovery; the amount of product recovered from beads was well within the range recoverable using NiX tips.*

2. Speed

Table 2: *Percent recovery and cleanup duration per binding cycle or bead cleanup. Increasing the number of NiX binding cycles will increase recovery, but also increase the duration of the cleanup. Using 30 cycles with NiX tips will result in more recovery than a bead cleanup that takes a similar amount of time.*

Binding cycles	1 cycle	10 cycles	20 cycles	30 cycles	40 cycles	50 cycles	Avg. Bead Cleanup
Avg % recovery	5.0%	25.5%	37.3%	44.5%	48.8%	57.1%	29%
Cleanup time (min:sec)	7:05	10:13	13:25	16:42	19:54	23:07	17:00

3. Adaptability

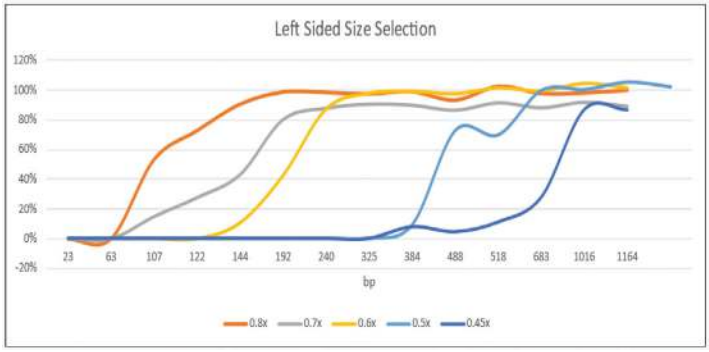

Figure 5: *Binding buffer ratios and recovery product length. Decreasing the binding buffer ratio will lead to retention of larger PCR product lengths.*

CONCLUSIONS

PCR cleanups with NiX tips consistently resulted in primer-free PCR product, regardless of the number of binding cycles completed. When compared to a bead-based cleanup, NiX tips showed comparable percent recovery when optimized for speed. Binding buffer ratios can be adjusted for left-handed size selection. NiX tips are automation-friendly, eliminating difficulties with magnetic beads.

REFERENCES

"Tip-Top Cleanup..." Poster Presentation, SLAS, Boston 2024, Julia Portocarrero[1], John Russell[1], Michael Gonzalez[1], Matt Fitts[2], Paul Meeh[2], Willam E. Brewer[2] [1]Life Edit Therapeutics, [2]DPX Technologies"