Genomics | Application Note

Automated Liquid Biopsy Sample Preparation with the nRichDX Revolution® System on the Hamilton Microlab® STAR

Authors: Nafiseh J. Jafari[1], David Grove[2], Sha Liao[2]

1 nRichDX, Inc., Irvine, CA, USA. info@nrichdx.com | www.nrichdx.com
2 Hamilton Company, Reno, NV, USA. marketingrequest@hamiltoncompany.com | www.hamiltoncompany.com/robotics

Introduction

Liquid biopsy is transforming oncology by providing a non-invasive method to detect and monitor cancer biomarkers such as cfDNA, cfRNA, and circulating tumor cells (CTCs). As these applications expand into minimal residual disease (MRD) and early detection, laboratories face the challenge of processing larger sample volumes efficiently while maintaining high yield and reproducibility.

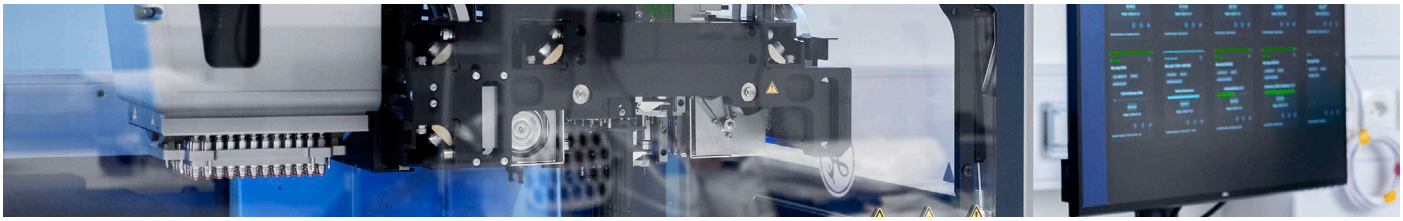
The nRichDX Revolution Plus Sample Prep System overcomes these challenges by enabling extraction from 1 to 50 mL of diverse biofluids, such as plasma, urine, whole blood, cerebrospinal fluid (CSF), and peritoneal fluid, using its proprietary nRicher cartridges. The system uniquely supports multi-analyte recovery (cfDNA, cfRNA, cfTNA, and CTCs) from a single sample, making it ideal for translational and clinical research where rare analyte detection is critical.

Through collaboration with Hamilton Company, nRichDX has automated the Revolution workflow on the Hamilton Microlab STAR Liquid Handling System. The integration enables full automation of wash and elution steps for cfDNA, cfRNA, cfTNA, and CTC workflows, delivering high-throughput, walk-away automation for liquid biopsy laboratories.

Methods & Workflow Overview

The nRichDX Revolution™ Plus Sample Prep System is a semi-automated workflow designed to maximize the recovery of rare biomarkers like cfDNA, cfRNA, cfTNA, and CTCs across a wide range of biofluids using proprietary nRicher™ cartridges. These cartridges integrate extraction, enrichment, and concentration steps into a single-use unit, eliminating inefficiencies like sample transfers, pooling, and re-elution, which often reduce yield in traditional methods. The system supports input volumes from 1 up to 50 mL (and up to 100 mL for CTC enrichment) in a single run, offering both “Kit Mode” (IVD-labeled) and user-programmable “Research Mode” for flexibility and regulatory compliance.

Hamilton Microlab STAR automates the post capture wash and elution steps to ensure consistent, high quality cfDNA recovery. The instrument executes two washes with Revolution cfDNA Wash Solution, followed by two 80% ethanol rinses to remove salt and inhibitors. Throughout these cycles, the on deck Revolution Magnetic Base Plate facilitates precise magnetic bead capture for efficient supernatant removal and optimal drying. Finally, the STAR dispenses Revolution cfDNA Elution Buffer—typically 50 µL (user selectable 25–100 µL), to release the purified cfDNA, producing a standardized, reproducible eluate ready for immediate NGS or PCR.



Revolution Plus Semi-Automated Workflow

Single Revolution Plus Processor

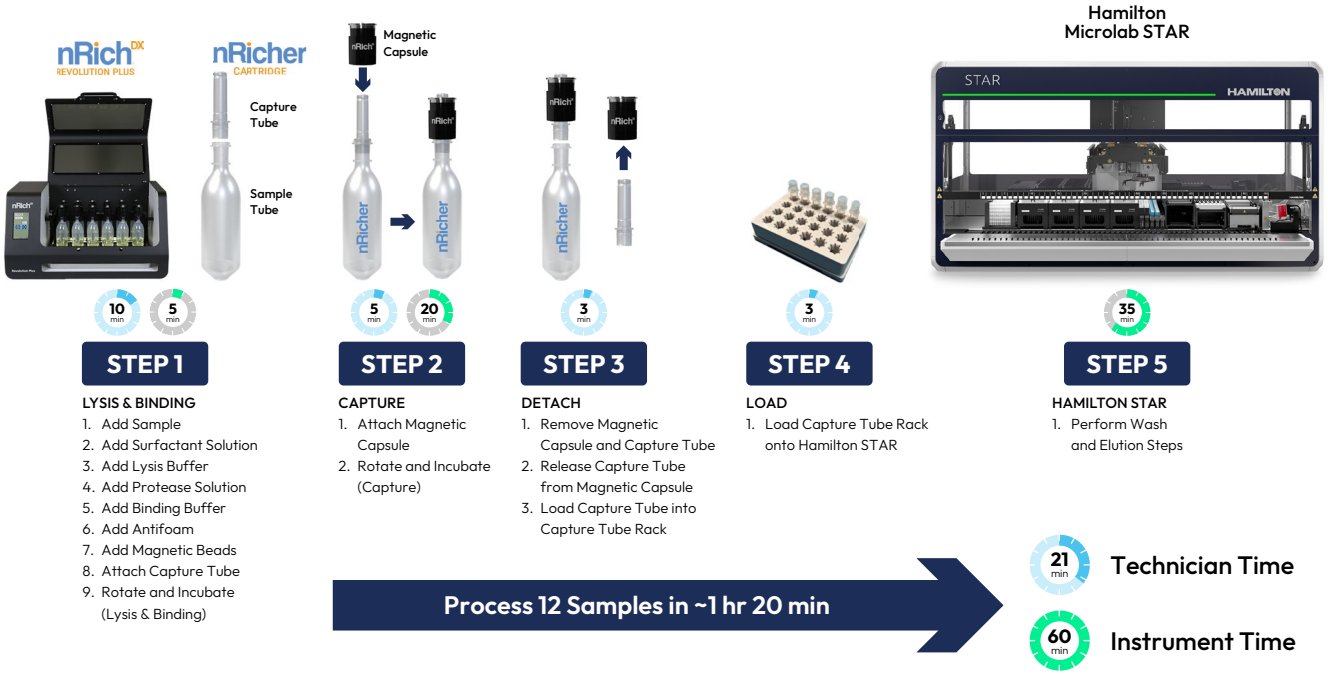
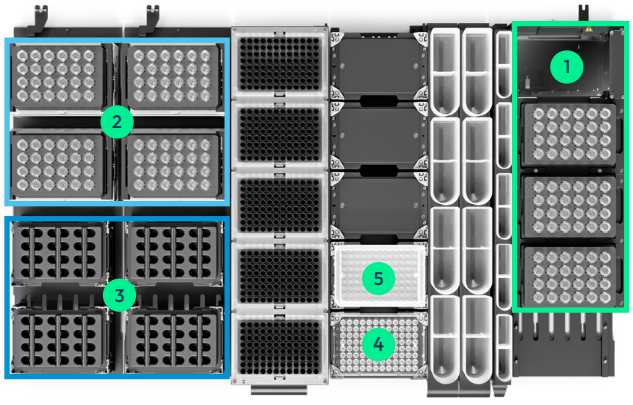


Figure 1: Revolution Kit Workflow for Semi-Automated cfDNA Extraction.

Workflow Description

1. Biofluid samples are loaded into nRicher cartridges for semi-automated extraction using the Revolution kits and Revolution Plus.
2. Automated Wash and Elution: The Hamilton Microlab STAR performs all wash and elution steps using pre-programmed scripts optimized for each analyte type.

Deck Configuration



1. 2D BC scanning of Capture Tube Rack
2. Capture Tube Racks on Hamilton Heater Shakers
3. Capture Tube Racks on nRichDX Magnetic Base Plate
4. Elution Plate
5. Liquid Waste Trough

Figure 2: Hamilton Microlab STAR Deck Layout.



Figure 3: Wash & Elution Workflow on Hamilton Microlab STAR.

Results & Discussion

Scalability and Extraction Efficiency

Modern liquid biopsy workflows must maintain high extraction efficiency across varying sample volumes. As clinical needs shift toward detecting rare, low-abundance mutations, laboratories often need to process more than 10 mL of plasma to ensure sufficient target recovery.

To test our platform's performance, we conducted a scalability study comparing cfDNA recovery from 1 mL to 20 mL of plasma. We aimed to determine if nucleic acid yield remains linear across this range or if manual processing hits a "ceiling" that limits recovery at higher volumes. The following data reveals a significant performance gap between automated and manual workflows, particularly as sample volumes increase toward the upper limits of clinical standards.

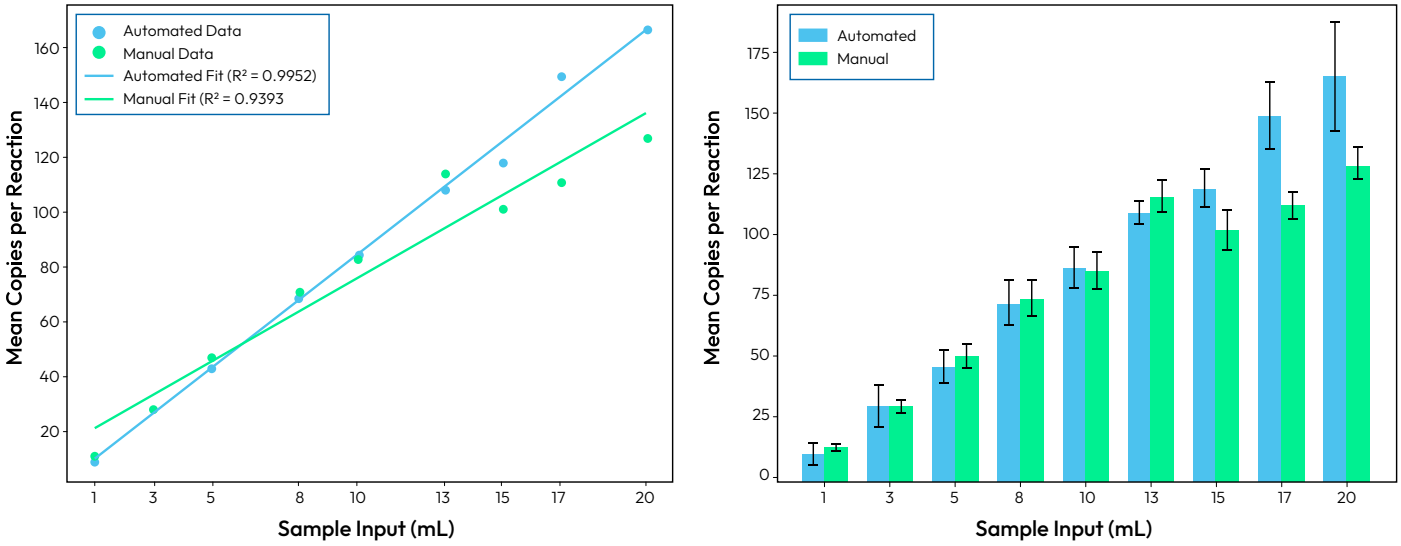


Figure 4: Correlation of Sample Input Volume to cfDNA Recovery via Linear Regression and Mean Copy Yield Analysis.

The data demonstrates that the automated protocol on the Hamilton STAR significantly enhances extraction efficiency, linearity, and scalability compared to manual workflows. While manual extraction exhibits plateauing results and reduced predictability ($R^2 = 0.9393$) at larger sample volumes, the automated system maintains high precision with a superior ($R^2 = 0.9952$). By delivering higher recovery yields and consistent Mean Copies per Reaction across the full 15–20 mL range, the automated protocol effectively scales the nRichDX kit's capabilities and eliminates the volume-dependent deviations inherent in manual processing.

Cross-Contamination and Carryover Validation

To ensure the integrity of the automated workflow, we performed a rigorous Checkerboard Carryover Validation (Table 1). This study utilized a high-concentration "Spiked/Positive" sample alternating with a "Non-Spiked/Negative" control in a 24-well plate format.

This alternating pattern is the industry standard for detecting potential cross-contamination resulting from aerosolization, liquid splashing, or pipette tip carryover during the automated extraction process.

Following the automated extraction, each sample was analyzed for the presence of the target nucleic acid. The results demonstrated 100% concordance with the plate map:

- Positive Wells: All Spiked/Positive wells (Green) yielded expected copy numbers, confirming consistent extraction efficiency across the plate.
- Negative Wells: All Non-Spiked/Negative wells (Light Green) remained consistently below the limit of detection (LOD). No detectable nucleic acid was found in the negative wells, even those immediately adjacent to high-titer positive samples in the X, Y, or diagonal planes.

Table 1: Checkerboard Plate Layout for Cross-Contamination and Carryover Validation.

	1	2	3	4	5	6
A	Spiked/Positive	Non-Spiked/ Negative	Spiked/Positive	Non-Spiked/ Negative	Spiked/Positive	Non-Spiked/ Negative
B	Non-Spiked/ Negative	Spiked/Positive	Non-Spiked/ Negative	Spiked/Positive	Non-Spiked/ Negative	Spiked/Positive
C	Spiked/Positive	Non-Spiked/ Negative	Spiked/Positive	Non-Spiked/ Negative	Spiked/Positive	Non-Spiked/ Negative
D	Non-Spiked/ Negative	Spiked/Positive	Non-Spiked/ Negative	Spiked/Positive	Non-Spiked/ Negative	Spiked/Positive

Table 2: Reproducibility of cfDNA Extraction Across Variable Batch Sizes.

Input Volume	Sample Size	Mean Ct	St Dev Ct	%CV Ct
5 mL	6	32.4	0.39	1.20%
	12	32.7	0.26	0.80%
	24	32.3	0.45	1.40%
	48	32.4	0.31	1.00%
	72	32.2	0.33	1.00%
	96	32	0.44	1.40%
Total	**258**	**32.3**	**0.36**	**1.10%**

Reproducibility

To evaluate the consistency of the automated platform across different throughput demands, a precision study was conducted using a fixed 5 mL sample input volume. Replicates were processed in three distinct batch sizes: n=6, n=12, n=24, n=48, n=72, and n=96. This design was intended to verify that the accuracy of the Hamilton pipetting system remains stable regardless of sample count or position within the run. Cycle threshold (Ct) values were recorded for each sample to calculate the mean, standard deviation (St Dev), and coefficient of variation (%CV).

The analysis of Cycle Threshold (Ct) values demonstrates exceptional reproducibility across all tested batches. As shown in Table 2, the mean Ct values remained remarkably stable, ranging from 32.0 to 32.7. This minimal variance (< 1% shift in mean Ct) indicates that the extraction efficiency is not compromised when scaling from a low-throughput run to a full-capacity batch.

Discussion

Automating nRichDX workflows on the Hamilton Microlab STAR provides a robust, high-throughput solution for liquid biopsy applications by delivering scalable performance and walk-away automation for cfDNA, cfRNA, cfTNA, and CTC extraction. This integration maintains exceptional precision and extraction efficiency across sample volumes ranging from 1 to 50 mL, achieving superior linearity (R^2 = 0.9952) compared to manual methods that often plateau at higher volumes. The system's precision is further evidenced by a low coefficient of variation (~1.1% CV) and stable mean Ct values across variable batch sizes up to 96 samples. Furthermore, the workflow ensures high-quality results through a contamination-free process, validated by checker-board testing that shows 100% concordance and no detectable carryover between spiked and negative wells. Ultimately, this synergy between advanced sample-prep chemistry and precision robotics offers the flexibility of both IVD-labeled and research modes, meeting the rigorous demands of modern clinical and translational oncology.

Conclusion

The combination of the nRichDX Revolution® System and the Hamilton Microlab STAR offers a fully automated, high-yield solution for extracting critical cancer biomarkers while maintaining a contamination-free environment. By uniting innovative sample-prep chemistry with precision robotics, the platform provides the consistent and scalable performance essential for modern oncology and molecular diagnostic workflows.

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HAMILTON

Hamilton Americas & Pacific Rim
Hamilton Company Inc.
4970 Energy Way
Reno, Nevada 89502 USA
Tel: +1-775-858-3000
Fax: +1-775-856-7259

Hamilton Europe, Asia & Africa
Hamilton AG
Via Crusch 8
CH-7402 Bonaduz, Switzerland
Tel: +41-58-610-10-10

Web: www.hamiltoncompany.com

Connect with our team at: www.hamiltoncompany.com/contact