nRichDX POWERING LIQUID BIOPSY | APPLICATION NOTE

From Sample to Result:

Combining Scalable cfDNA Extraction with the nRichDX Revolution Sample Prep System and cfDNA Detection using Bio-Rad ddPCR

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Abstract

This study evaluated an integrated workflow that combines the nRichDX Revolution Sample Prep System for cell-free DNA (cfDNA) extraction with Bio-Rad's QX600™ Droplet Digital™ PCR (ddPCR™) System for downstream cfDNA detection. The objective was to assess yield, linearity, and mutation detection sensitivity across plasma input volumes representative of liquid biopsy research workflows. The nRichDX workflow maintained linear recovery from 1–15 mL plasma and is uniquely scalable to 50 mL inputs, which enables efficient processing of large-volume samples. Contrived plasma samples were used to compare the performance of the nRichDX Sample Prep System to the Qiagen QIAamp Circulating Nucleic Acid Kit. Results showed that cfDNA purified using the nRichDX system exhibited higher fractional abundance of actionable EGFR E746_A750delELREA and KRAS G12C mutations compared with Qiagen under the tested conditions, indicating improved recovery of low-frequency mutant molecules essential for precision oncology applications. Qiagen extractions produced higher Qubit readings primarily due to carrier RNA contribution, reflecting total nucleic acid rather than true cfDNA content. Collectively, these data highlight the superior analytical performance, accuracy, and scalability of the nRichDX–Bio-Rad ddPCR workflow, providing a robust end-to-end solution for high-volume cfDNA analysis and mutation quantification in liquid biopsy research.

Introduction

Accurate and efficient extraction of cell-free DNA (cfDNA) is a critical prerequisite for downstream molecular analyses such as mutation detection and liquid biopsy assays. As cfDNA is typically present at low abundance in biofluids such as plasma or urine, maximizing recovery and maintaining integrity are essential to ensure sensitivity in downstream applications such as Droplet Digital PCR (ddPCR) and next-generation sequencing (NGS). Traditional column-based purification methods, such as the Qiagen QIAamp Circulating Nucleic Acid Kit, are widely used but are limited by a 5 mL maximum plasma volume, multi-step manual workflows, and the use of carrier RNA that can inflate apparent nucleic acid yields without contributing to actionable cfDNA recovery.

The nRichDX Revolution Sample Prep System is a scalable, semi-automated solution specifically engineered for high-volume cfDNA extraction. The proprietary cartridge design and magnetic capture technology enable efficient recovery of nucleic acids from up to 20 mL per run—and scalable to 50 mL—without the need for multiple parallel extractions or reconcentration steps. This innovation minimizes hands-on time, reduces sample loss, and ensures high reproducibility, making it particularly suited for liquid biopsy and rare variant detection applications.

Figure 1 compares the nRichDX and the Qiagen cfDNA extraction workflows. The nRichDX workflow (top) simplifies the process through one-step reagent/sample addition, automated binding and capture, magnetic washing, and elution into a single clean fraction. In contrast, the Qiagen workflow (bottom) requires multiple manual steps—lysis, binding, washing, and elution—performed under vacuum using the QIAvac 24 Plus manifold and is constrained to low sample volumes.

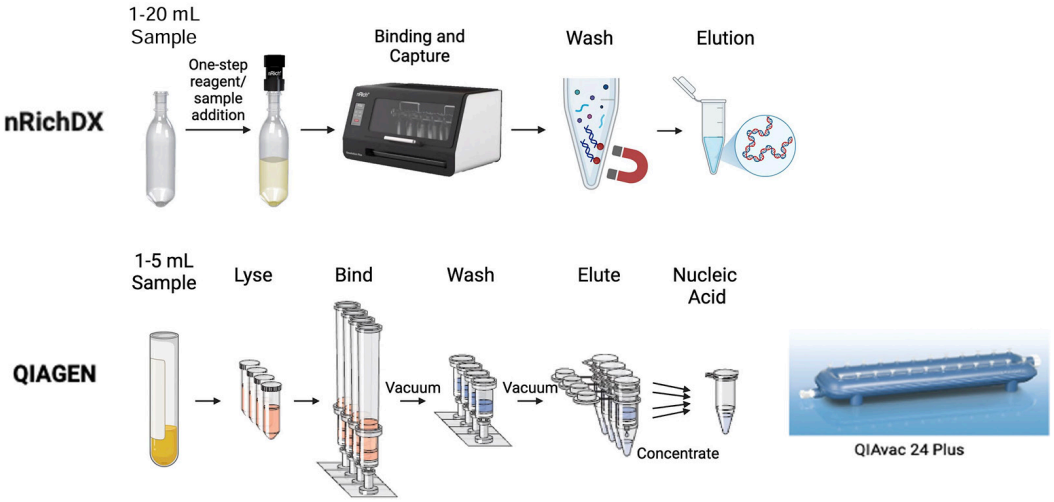


Fig. 1: Comparison of cfDNA extraction workflows for the nRichDX Revolution Sample Prep System and the Qiagen QIAamp Circulating Nucleic Acid Kit. The nRichDX workflow enables streamlined, high-volume extraction with minimal manual intervention. The Qiagen workflow relies on multiple manual steps and lower sample capacity.

Materials and Methods

Templates and ddPCR assays

Whole blood derived pooled human plasma anticoagulated with K2 EDTA (IPLAWBK2E) was purchased from Innovative Research (Novi, MI). Human female DNA (G1521) was purchased from Promega (Madison, WI) and sheared to an average size of 200 bp using the Covaris Laboratory DNA shearing service (Woburn, MA). Two gBlock double-stranded DNA fragments containing the mutant EGFR E746_A750delELREA and KRAS G12C target sequences were synthesized by Integrated DNA Technologies (Coralville, IA). The lyophilized gBlocks were resuspended at 1 ng/µL with polyA buffer (10 mM Tris, pH 8, 0.1 mM EDTA, 100 ng/µL polyadenylic acid) that is formulated by adding polyadenylic acid (Sigma Aldrich, #10108626001) to DNA suspension buffer (Teknova, T0223). The two gBlock solutions were serially diluted using polyA buffer. Serial dilutions and ddPCR reactions were prepared using Eppendorf DNA LoBind microcentrifuge tubes (VWR, 0.5 mL: 80077-236 or 1.5 mL: 80077-230). The MET (FAM, dHsaCP2500321), RPP30 (HEX, dHsaCP2500350), EGFR E746_A750delELREA (FAM/HEX, dHsaMDV2010039), and KRAS G12C (FAM/HEX, dHsaMDV2510584) assays are from Bio-Rad. The FGFR1 (Cy5), SOD1 (Cy5.5), MYCN (ROX), and PIK3CA (Atto 590) assays are IDT custom orders of the corresponding Bio-Rad FAM assays.

Plasma Ladder Experiment

Healthy human plasma was thawed at room temperature and centrifuged at 16,000 × g for 10 min at 4 °C. The supernatant was combined and thoroughly mixed prior to aliquoting. Cell-free DNA (cfDNA) was extracted from plasma volumes of 1, 5, 10, and 15 mL (each in triplicate) using the Revolution cfDNA Max20 Kit (408000) from nRichDX (Irvine, CA). All cfDNA samples were eluted in 50 µL of the supplied elution buffer. DNA concentration of the eluates (5 µL) was fluorometrically quantified using the Qubit 1x dsDNA High Sensitivity Assay Kit (Thermo Fisher Scientific, Q33230). Total DNA yields for each sample were calculated by multiplying the Qubit concentrations (ng/µL) by the elution volume to obtain the mass of DNA (ng). The total DNA yield of the three replicates was used to calculate the mean and standard deviation.

Contrived Mutant Allele Plasma Samples

Healthy human plasma was thawed at room temperature and centrifuged at 16,000 × g for 10 min at 4 °C. The supernatant was combined and thoroughly mixed prior to aliquoting. Contrived cfDNA samples were prepared using human plasma spiked with sheared wild-type DNA (200 bp) and mutant gBlocks (EGFR E746_A750delELREA or KRAS G12C, 500 bp) at a nominal variant allele frequency (VAF) of 1% (30,000 wild-type and 300 mutant copies/mL plasma). The wild-type ddPCR copy number of the sheared DNA was based on the average concentration of six different target gene sequences (MET, RPP30, FGFR1, SOD1, MYCN, and PIK3CA), which were determined using a 6-plex ddPCR experiment. cfDNA was extracted from triplicate 1 mL contrived plasma samples using both the nRichDX Revolution cfDNA Max20 Kit and QIAamp Circulating Nucleic Acid Kit (55114) from Qiagen (Germantown, MD). Each Qiagen extraction included 1 µg carrier RNA as recommended in the instructions to enhance binding of nucleic acids to the QIAamp columns. All cfDNA was eluted in 50 µL using the provided elution buffer. Eluate aliquots of either 5 µL or 10 µL were used to prepare 24 µL ddPCR reactions.

Qubit Quantitation of cfDNA Yield

Healthy human plasma was thawed at room temperature and centrifuged at 16,000 × g for 10 min at 4 °C. The supernatant was combined and thoroughly mixed prior to aliquoting. For endogenous cfDNA analysis, three 1 mL replicates were extracted using the Revolution cfDNA Max20 Kit and three 1 mL replicates were extracted using the QIAamp Circulating Nucleic Acid Kit. A similar set of samples was prepared for spike cfDNA analysis, but 100 ng of sheared DNA per mL plasma was added and thoroughly mixed prior to aliquoting the 1 mL samples. All cfDNA samples were eluted in 50 µL of the supplied elution buffer. DNA concentration of the eluates (5 µL) was fluorometrically quantified using the Qubit 1x dsDNA High Sensitivity Assay Kit. Data from the three replicates was used to calculate the mean and standard deviation.

ddPCR and Data Analysis

Master mixes were prepared and aliquoted whenever possible to minimize differences in sample preparation. ddPCR Supermix for Probes (no dUTP) (Bio-Rad: 1863027) was used in EGFR E746_A750delELREA and KRAS G12C experiments and ddPCR Multiplex Supermix (Bio-Rad: 12005909) was used for the 6-plex experiments to determine the concentration of the sheared DNA stocks. Samples were pipet-mixed, thoroughly vortexed, pulsed on a microcentrifuge and emulsified using a QX200 manual droplet generator (Bio-Rad: 1864002). Droplets were transferred to semi-skirted, 96-well plates (Bio-Rad: 12001925), sealed with pierceable metal foil (Bio-Rad: 1814040) for 5 s at 180°C using a PX1 PCR plate sealer (Bio-Rad: 1814000), and thermal cycled using a C1000 Touch Thermal Cycler with a 96-deep well reaction module (Bio-Rad: 1851197). The ramp rate was 2°C/s for all temperature steps using the following parameters: 95°C for 10 min, then 40 cycles of 94°C for 30 s and annealing/extension for 60 s, followed by incubation at 98°C for 10 min and a final hold at 4°C. The annealing/extension temperature was 60°C for the 6-plex experiment using sheared female genomic DNA as a template and was 55°C for the other templates. Droplet fluorescence was determined using a QX600 Droplet Reader (12013328). Data from two technical replicates for each sample were merged prior to data analysis. The fractional abundance is calculated using QX Manager software. The reported results are determined by Poisson analysis of the positive and negative droplets and the error bars represent the 95% confidence interval of the total error.

Results

Linear Recovery Across Input Volumes

There was a linear correlation (R^2 = 0.9874) between plasma input volume (1, 5, 10, and 15 mL) and cfDNA yield using the nRichDX Revolution cfDNA Max20 Kit (Fig. 2). The results demonstrate consistent recovery of cfDNA for the tested plasma volumes.

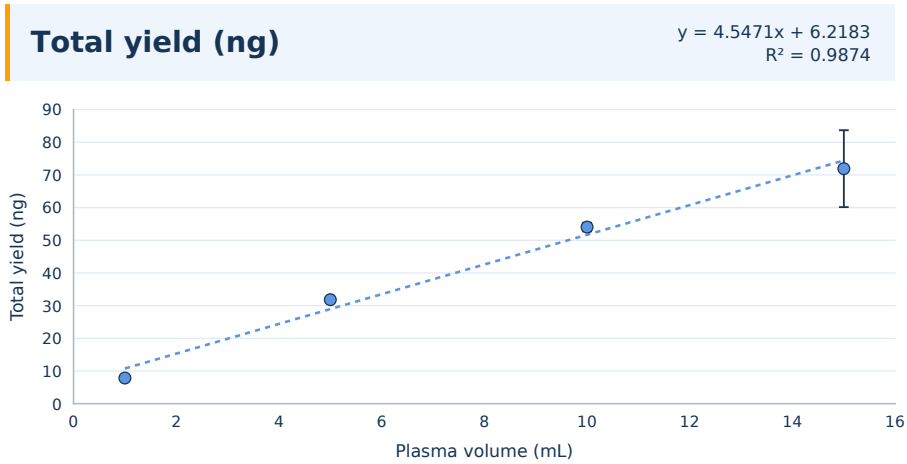


Fig. 2: cfDNA recovery. The total yield (ng) of cfDNA extracted using the nRichDX Revolution cfDNA Max20 Kit at different plasma input volumes (1, 5, 10, and 15 mL) are based on Qubit analysis of cfDNA. Data and error bars represent the mean and standard deviation of triplicate extractions at each plasma volume.

Improved Mutation Detection Sensitivity

Contrived plasma samples containing EGFR E746_A750delELREA (Fig. 3A) and KRAS G12C (Fig. 3B) mutant gBlocks at a nominal fractional abundance of 1% were analyzed using Bio-Rad ddPCR technology. For both targets, the cfDNA extracted using the nRichDX system tended to exhibit fractional abundances closer to the nominal value of 1% and have smaller error bars compared to cfDNA extracted using the Qiagen system. The increased fractional abundance for nRichDX extracted cfDNA suggests a more selective recovery of short cfDNA fragments that harbor clinically relevant variants, which is a critical parameter for sensitive liquid biopsy applications.

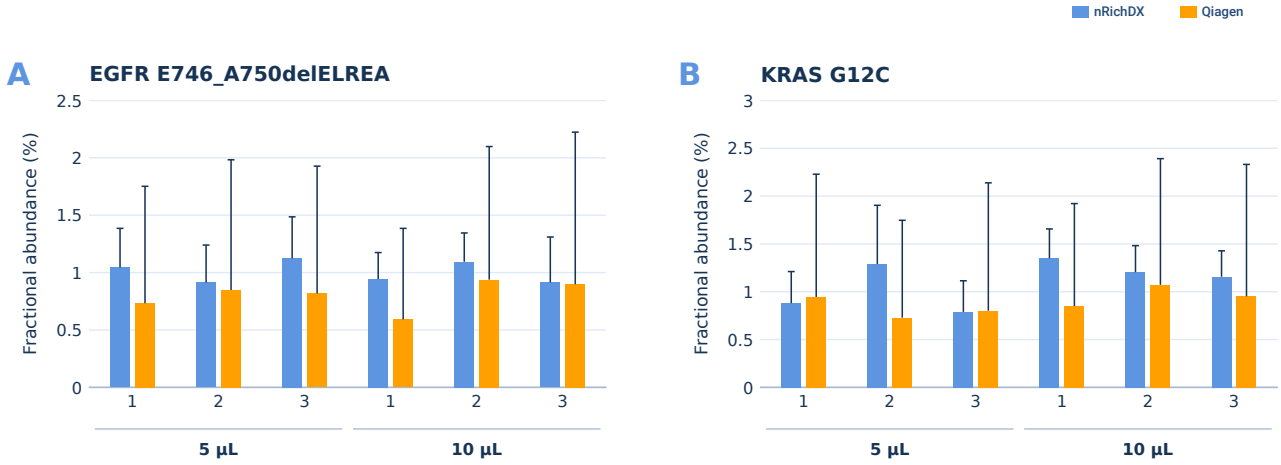
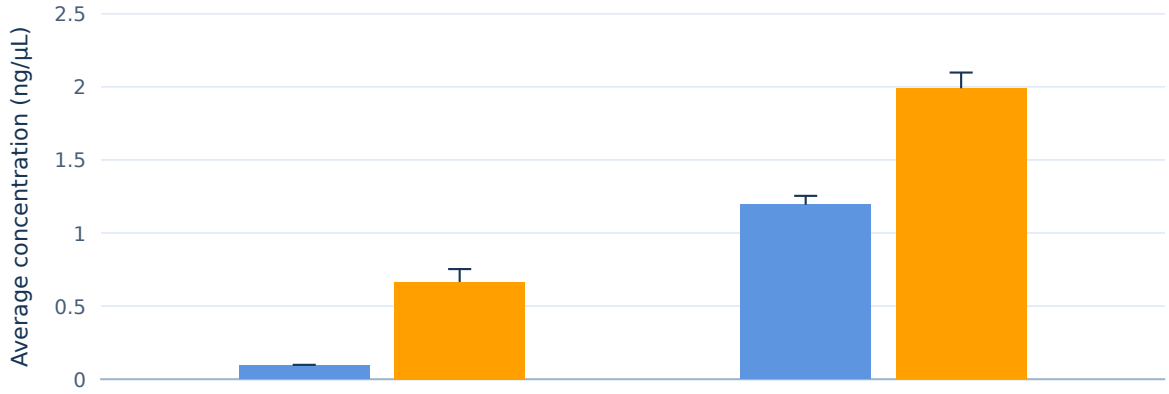


Fig. 3: EGFR E746_A750delELREA and **KRAS G12C fractional abundance.** ddPCR results for (A) EGFR E746_A750delELREA and (B) KRAS G12C using either 5 µL or 10 µL of eluate from extractions performed in triplicate. nRichDX tended to show a fractional abundance closer to the nominal value of 1% and smaller error bars.

Fluorometric overestimation of cfDNA concentration due to carrier RNA

Qubit fluorometric quantitation revealed higher overall nucleic acid concentrations in Qiagen eluates than in nRichDX eluates (Fig. 4). In these experiments, the recommended carrier RNA concentration used in the Qiagen workflow is in large excess relative to the cfDNA. As a result, the actual cfDNA concentration is overestimated because of the background fluorescence from the carrier RNA.



	Endogenous	Spike
nRichDX	0.093733333	1.193333333
Qiagen	0.665333333	1.99

Fig. 4: Qubit Quantitation of cfDNA Yield. The mean nucleic acid concentrations (± SD, n = 3) from nRichDX and Qiagen extraction methods. The Qiagen yields were higher due to inclusion of carrier RNA in the cfDNA extraction protocol.

Conclusion

A workflow that combines the individual benefits of the nRichDX Revolution Sample Prep System and the Bio-Rad Droplet Digital PCR (ddPCR) platform delivers a powerful, scalable, and highly reproducible solution for cfDNA extraction and mutation detection in liquid biopsy applications. Across multiple plasma volumes, the nRichDX system demonstrated linear cfDNA recovery, enhanced detection sensitivity of mutations in contrived plasma samples, and accurate representation of actionable cfDNA molecules compared with a Qiagen workflow. The simplified one-step reagent addition and automated magnetic capture technology minimized manual handling while enabling processing of more than 5 mL of plasma in a single cartridge—eliminating the need for multiple parallel extractions or reconcentration steps. Overall, the nRichDX workflow consistently provided cfDNA that translated into more reliable ddPCR quantification. These results establish an integrated nRichDX–Bio-Rad ddPCR workflow as an advanced, end-to-end solution that improves analytical sensitivity, scalability, and workflow efficiency for translational and clinical research in liquid biopsy.

Acknowledgment

Application Note was authored by Nafiseh Jafari, PhD (nRichDX, Inc) and ddPCR analysis was performed at Bio-Rad Laboratories.

Questions about this study?

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