RNA Recovery from 1-20mL Inputs Enables Sensitive KRAS G12V Detection in a Liquid Biopsy Model

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INTRODUCTION

Liquid biopsy applications increasingly require concurrent access to tumor-derived DNA and RNA, high sensitivity at low variant allele frequencies, and the ability to process larger plasma input volumes for robust detection in early-stage disease and minimal residual disease settings. Reliable recovery of circulating RNA across inputs ranging from 1 to 20 mL is therefore critical for sensitive and scalable assay workflows.

These workflows must preserve RNA while minimizing inhibitor carryover that could affect downstream enzymatic performance. This study evaluates the analytical performance, scalability, and downstream compatibility of a circulating RNA workflow for high-sensitivity liquid biopsy applications.

MATERIALS & METHODS

Conditioned media from KRAS-G12V–positive NCI-H441 cells were pooled and clarified to form a uniform tumor-conditioned matrix.

Experiment I: Replicate extractions (n = 3 per condition) were performed at 1, 5, 10, and 20 mL using the Revolution cfTNA Max 20 kit. Metrics included total yield, KRAS-G12V total copies, and coefficient of variation (CV).

Experiment II: Rare-event performance was tested using a dilution series (100%, 20%, 5%, 1%, 0.2% KRAS-G12V) extracted at 20 mL (n = 2 per level). Total yield (ng) and total copies of target mutation were quantified to determine linearity and sensitivity between dilution series.

Figure 1. nRichDX Revolution cfTNA Max 20 Kit Workflow.

RESULTS

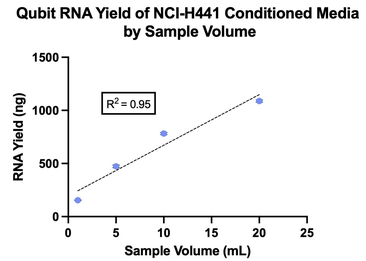

Table 1. RNA Qubit analysis of NCI-H441 conditioned media demonstrated a positive relationship between total RNA yield and sample input volume. Total RNA yield (ng) increased with increasing sample volume, indicating improved RNA recovery sensitivity at higher input volumes.

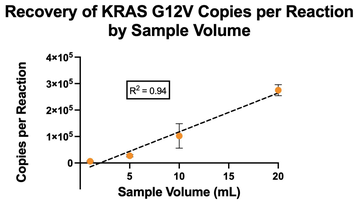

Figure 2. KRAS G12V copies per reaction increased with sample input volume, demonstrating improved detection sensitivity at higher volumes. A strong linear relationship was observed between KRAS G12V copies per reaction and sample volume ($R^2 > 0.95$), indicating that larger input volumes enabled more efficient recovery and detection of the target.

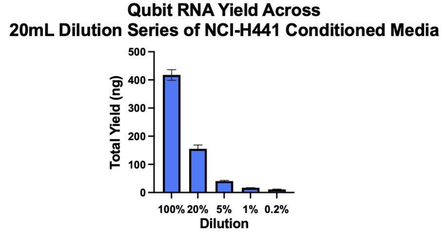

Figure 3. Qubit-measured RNA yield from 20 mL plasma input across the NCI-H441 conditioned media dilution series. Quantifiable RNA recovery was demonstrated down to 0.6 ng/mL at the lowest dilution tested (0.2%), supporting the sensitivity of the extraction workflow for low-abundance circulating RNA targets.

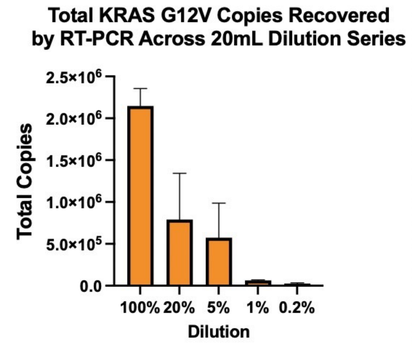

Figure 4. Total KRAS G12V copies recovered from 20 mL plasma input across the dilution series. Successful target detection was achieved down to 1,657 copies/mL at the lowest dilution tested (0.2%), demonstrating that the extraction workflow concentrates circulating RNA sufficiently to enable low-level mutant transcript detection from large- volume inputs.

CONCLUSION

Recovery of circulating RNA was demonstrated across a 20-fold range of input volumes (1–20 mL) using NCI-H441 cell line- conditioned media, with yields exhibiting linearity proportional to input volume ($R^2 = 0.95$) down to 0.6 ng/mL. Functional recovery of the KRAS G12V mutant transcript was demonstrated down to 1,657 copies/mL, confirming the ability of the workflow to concentrate low- abundance circulating RNA into a quantifiable eluate suitable for downstream mutation detection.

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