Poster Number 5042

Quantitative Comparison of cfRNA and cDNA Recovery From Plasma and Cell Culture Media Using Two Commercial Extraction Platforms

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INTRODUCTION

The comprehensive capture and isolation of circulating cell-free RNA (cfRNA) from plasma and conditioned media are critical for the success of transcriptomic applications in oncology. Variability in extraction methods can impact the yield, size distribution, and ultimately, downstream cDNA synthesis from extracted cfRNA. This study compares the recovery of cfRNA and cDNA using two commercially available extraction platforms: the nRichDX Revolution cfTNA Max 20 kit and the Qiagen exoRNeasy kit.

MATERIALS & METHODS

H358 non-small cell lung cancer cells were cultured, and 40 mL of conditioned media was harvested and evenly divided for processing. Similarly, 3 mL of human plasma was evenly split for side-by-side extraction. Use of both the nRichDX kit and the Qiagen kit followed manufacturer protocols. cfRNA quantification was performed on the Qubit 4 fluorometer. RNA integrity and size profiles were assessed using the Agilent 2100 Bioanalyzer with the RNA Pico chip. Reverse transcription was performed using the Takara SMART-Seq HT Plus kit, and cDNA quantification was conducted using Qubit DNA HS assay and Bioanalyzer DNA Pico chip. RNA input was normalized to 1 ng/µL before cDNA conversion.

RESULTS

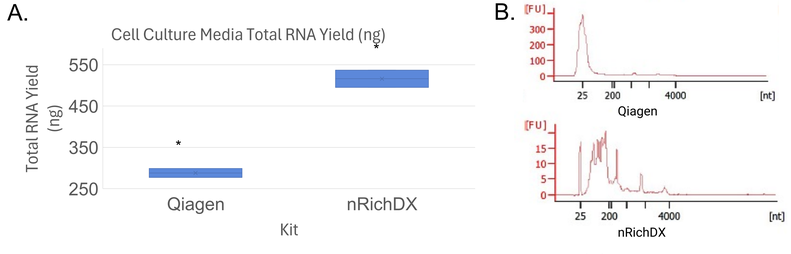

Figure 1. cfRNA recovery from H358-conditioned medium. (A) Qubit 4 fluorometric quantification of total cfRNA (ng); box-and-whisker plots. Two-tailed paired t-test, P < 0.05. (B) Agilent 2100 Bioanalyzer RNA Pico electropherograms: the nRichDX kit shows defined cfRNA peaks, whereas the Qiagen kit show low recovery and loss of defined peaks consistent with degradation.

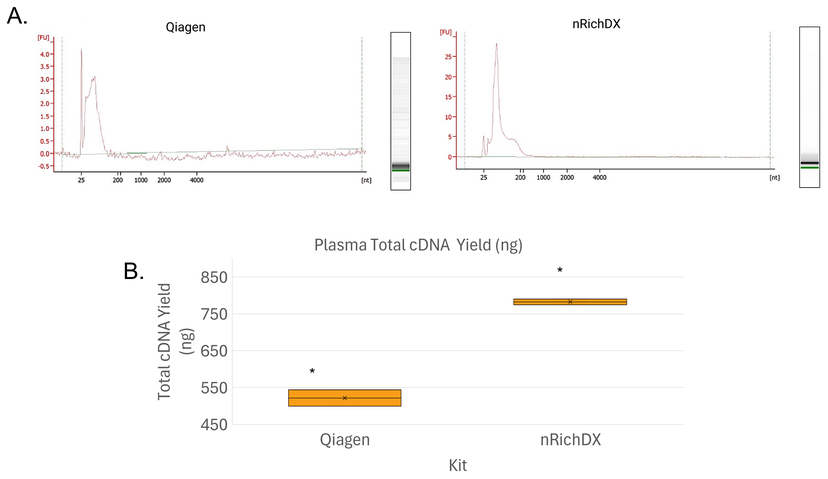

Figure 2. Plasma cfRNA profiles and cDNA yield after library prep. (A) Agilent 2100 Bioanalyzer RNA Pico electropherograms showing defined cfRNA peaks from plasma. (B) cDNA yield after reverse transcription with the Takara SMART-Seq HT Plus kit; box-and-whisker plots, two-tailed paired t-test, P < 0.05. nRichDX extractions generated significantly more RNA, translating to higher cDNA concentrations—consistent with higher-quality cfRNA for downstream analyses.

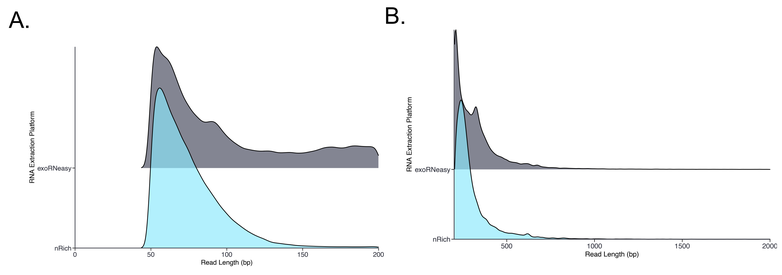

Figure 3. Plasma cfRNA fragment-size distribution after ONT sequencing. Patient plasma cfRNA libraries sequenced on the Oxford Nanopore PromethION show read-length histograms indicating recovery of both (A) short cfRNA species and (B) long RNA fragments.

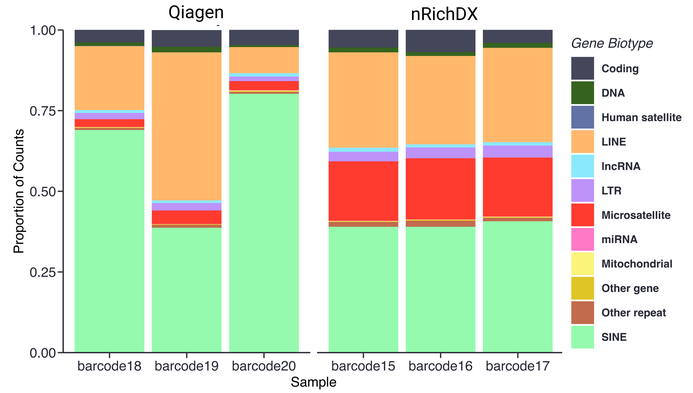

Figure 4. Plasma cfRNA biotype composition. Patient samples sequenced on the Oxford Nanopore PromethION. nRichDX-extracted replicates show low variance in RNA biotype proportions, enabling robust within-group analyses. The nRichDX kit captures a more complete and consistent total RNA population while the Qiagen kit captures more variable RNA populations from exosomal RNA. Demonstrating that the nRichDX kit is better for broad transcriptome coverage and multi-analyte biomarker discovery through the extraction of total cfRNA.

CONCLUSION

Across all experiments, the nRichDX Revolution cfTNA Max 20 kit outperformed the Qiagen exoRNeasy kit. In H358 conditioned media, the nRichDX kit yielded significantly more cfRNA (Qubit; P<0.05) and produced Bioanalyzer traces with clear peaks, whereas the Qiagen kit showed low recovery and loss of defined peaks. In plasma, the nRichDX kit extractions led to higher cDNA output after reverse transcription (P<0.05). ONT sequencing demonstrated recovery of both short cfRNA species and long RNA fragments, and the nRichDX kit replicates exhibited low variance in RNA biotype composition, supporting robust within-group analysis. Taken together, these results show that extraction platform choice decisively shapes cfRNA outcomes. A high-volume, total-cfRNA approach with the nRichDX kit delivers higher yield, better integrity, broader fragment coverage, and more consistent biotype representation than exosome-enrichment methods—providing a more complete and reliable input for multi-analyte biomarker discovery in oncology.