Cell-Free RNA Urogenital Cancer Biomarker Detection from Urine

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Abstract

Urogenital cancers encompass a variety of tumor types including kidney, bladder and prostate cancer, the most prevalent cancer in men. Current early detection methods rely on blood screening of prostate-specific antigen (PSA), however this method is associated with overdiagnosis and overtreatment, has a high rate of false positives, and the subsequent procedures carry risks. As a result, the search for alternative biomarkers is of much interest. Urine is an ultra-non-invasive analyte ideal for urogenital cancer detection, including prostate cancer. Use of cell free RNA (cfRNA) is ideal for biomarker identification for use in diagnostics, treatment monitoring, and tumor tissue of origin prediction.

In this study, we evaluated urine cfRNA extraction and RNA sequencing (RNA-Seq) from samples derived from patients with urogenital cancers and healthy controls. Multiple extraction methods were assessed, including a novel cfRNA isolation approach designed for high-efficiency recovery across a wide range of input volumes (1–50 ml) in a single extraction. Extracted cfRNA underwent both quantitative and qualitative quality control, followed by functional evaluation using RNA-Seq to assess yield, transcriptome complexity, and detection of known cancer-associated biomarkers.

Preliminary testing of the cfRNA isolation methods showed that traditional extraction methods did not achieve the yield required for detection of low-abundance biomarkers. In contrast, the novel extraction method demonstrated high efficiency cfRNA recovery. Along with traditional quantitative and qualitative QC of the extracted cfRNA, functional testing was performed using RNA-Seq. RNA-Seq results demonstrated detection of rare transcripts and cancer-associated biomarkers, confirming this is a reliable method for biomarker detection.

As these results show, cfRNA can be extracted from urine, with the detection of rare biomarkers associated with disease. The high-efficiency extraction method enables sensitive detection of low-frequency transcripts, addressing limitations of conventional techniques. This method is not limited to urine and cfRNA, with the potential for application in other biofluids including plasma and other nucleic acid types such as mitochondrial DNA.

In collaboration with: nRichDX POWERING LIQUID BIOPSY

URINE SAMPLES

Pooled healthy donor urine with LNCaP spike-in or negative control

5ml = 50 Total LNCaP Cells

10ml = 100 Total LNCaP Cells

20ml = 200 Total LNCaP Cells

40 ml = 400 Total LNCaP Cells

Extraction & QC

CELL FREE RNA EXTRACTION KIT

✓ nRichDX® Revolution Plus System

Library Prep & QC

3 LIBRARY PREP KITS

✓ ThermoFisher ION AmpliSeq™ RNA (target ~25k genes in transcriptome)

✓ Twist RNA Exome (target ~20k genes in transcriptome)

✓ Takara SMARTer® Stranded Total RNA-Seq Kit v3 – Pico Mammalian (unbiased, whole transcriptome)

Sequencing & Analysis

ILLUMINA® SEQUENCING & ANALYSIS

✓ ~20M reads/sample

✓ Read quality & mapping

✓ Functional analysis of known prostate cancer markers and variants

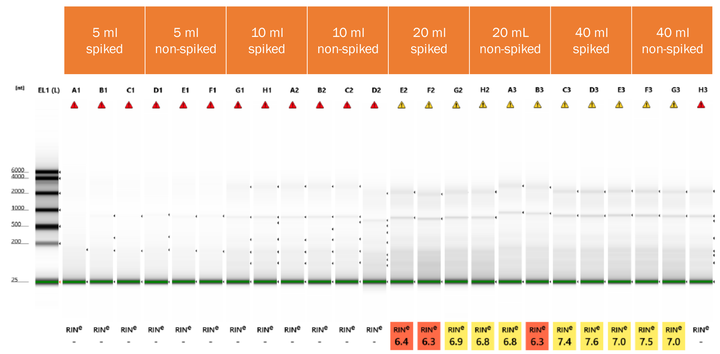
Figure 1. Workflow for cell-free RNA sequencing from urine. A series of 24 samples (4 positive conditions in triplicate, plus 12 matched negative controls) were subjected to the nRichDX extraction method, which previously proved best for cfRNA extraction from plasma. The nRichDX extracted samples, were then subjected to three library prep methods: Takara whole transcriptome, ThermoFisher AmpliSeq RNA Transcriptome Panel, and Twist RNA Exome. All samples were sequenced to approximately 20M reads and analyzed for functional QC of prostate cancer associated transcripts.

EXTRACTION CHARACTERIZATION

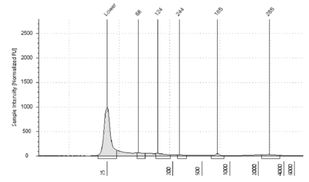
RNA Qubit

Sample	Condition	Conc. (ng/ul)	Mean Conc. (ng/ul)	Total Yield (ng)	Mean Total Yield (ng)
1	5 ml spiked	Too Low	Too Low	Too Low	Too Low
2		Too Low		Too Low	
3		Too Low		Too Low	
4	5 ml non-spiked	Too Low	Too Low	Too Low	Too Low
5		Too Low		Too Low	
6		Too Low		Too Low	
7	10 ml spiked	.736	.70	36.8	34.93
8		.712		35.6	
9		.648		32.4	
10	10 ml non-spiked	.680	.65	34	32.33
11		.612		30.6	
12		.648		32.4	
13	20 ml spiked	1.23	1.28	61.5	63.83
14		1.45		72.5	
15		1.15		57.5	
16	20 mL non-spiked	1.33	1.18	66.5	59.00
17		1.15		57.5	
18		1.06		53	
19	40 ml spiked	1.72	1.75	86	87.50
20		1.87		93.5	
21		1.66		83	
22	40 ml non-spiked	1.68	1.68	84	84.17
23		1.79		89.5	
24		1.58		79	

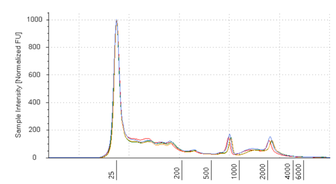
TapeStation



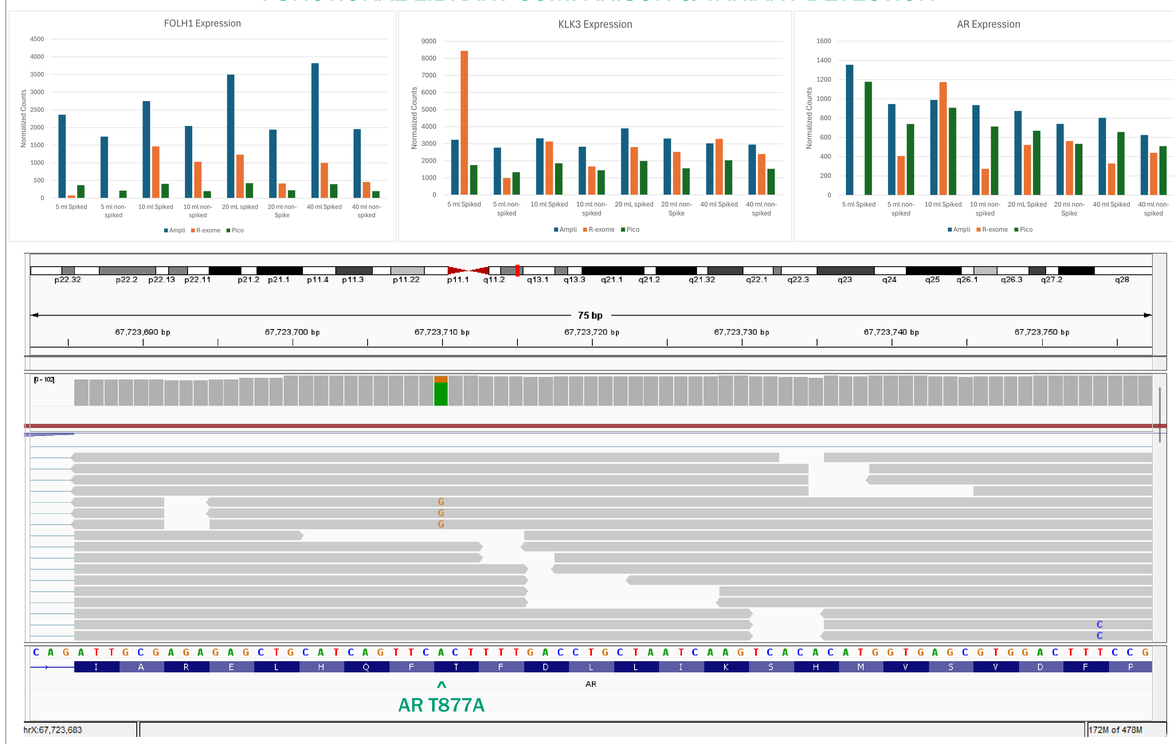
10 ml spiked = no RNA detected



40 ml spiked = RNA detected



FUNCTIONAL LIBRARY COMPARISON & VARIANT DETECTION



Conclusions

- nRichDX technology demonstrates efficient cfRNA recovery from urine, with yield scaling proportionally with input volume; clear linear increase in total yield was observed from 10–40 ml inputs. Low-input samples (5 ml) were below the limit of detection by Qubit and TapeStation, while higher input volumes (≥10 ml) showed robust and reproducible recovery. Regardless of detection, all samples proceeded to library preparation.
- Library preparation was successful for all samples, even those that were below the limit of detection for Qubit and TapeStation.
- All libraries were successfully sequenced, and post-sequencing QC metrics were consistent across all samples, including 5 ml samples below Qubit/TapeStation detection limits. Functional QC indicates increased expression in key prostate cancer genes (average expression of FOLH1/PSMA, KLK3/PSA, and AR). Functional analysis also confirmed expression of mutant AR T877A in spike-in samples 10 ml and above.