7841

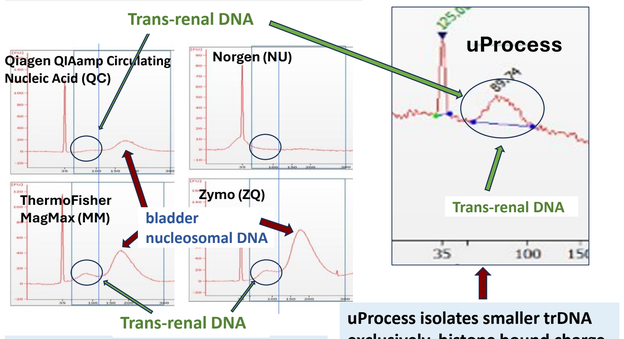
Evaluation of uProcess for detection of oncogene sequences in buffer and urine by qPCR following 3-minute capture and 5-minute purification procedure.

Floyd Taub[1], Charles Preston Neff[2], Alejandra Davila[1],
[1]aiGENE Inc., [2]Department of Medicine, University of Colorado, AMC, Aurora, CO

ABSTRACT

Cell-free DNA (cfDNA) and circulating tumor DNA (ctDNA), found in blood and urine, have firmly established themselves as key analytes for the diagnosis of various cancers and defining therapy. Primary sequence and epigenetic markers within cfDNA are key in screening and monitoring for cancer. However, both screening and long-term monitoring have two major limitations – test sensitivity and individual participation. Even as NGS, ddPCR, and other methods continue to improve, they require large amounts of cfDNA to reliably detect rare ctDNA fragments. For example, a single BioRad ddPCR reaction can analyze 1 µg of short cfDNA, but a single tube of blood might yield on the order of 10ng, thus cutting potential sensitivity 100x. Additionally, participation is typically a severe impediment to screening and long-term monitoring. Historically, this has been a major obstacle, especially for screening. Travel to clinics, pain/difficulty of phlebotomy, and patient aversion to it limit participation. Urine offers a far more accessible alternative, but ctDNA concentrations are lower and current urine-based cfDNA/ctDNA collection methods have yet to achieve yields adequate for highly sensitive testing. **A µg of cfDNA** is required for detection at the 0.001% level. Herein, we test and optimize a system (uProcess) designed for at-home urine filtration; it previously demonstrated to capture over 2 µg of cfDNA from 200 mL of urine on a mail-in filter cartridge. Compared to previous versions, we have **reduced the elution volume 5-fold from 2.5 to 0.5 mLs,** allowing concentrated samples for downstream processing. We demonstrated that the **improved device can efficiently concentrate oncogene sequences, in buffer or urine, into a small elution volume in under ten minutes** vs. typical procedures taking 90-120 minutes. We then detected **KRAS and TP53 oncogenes by qPCR,** underscoring that the method produces ctDNA suitable for downstream usage. Together, these findings support the potential of uProcess to significantly expand access to cfDNA/ctDNA testing, improve sensitivity, shorten cfDNA purification time, and overcome longstanding barriers to cancer screening or monitoring.

Four extraction methods using the same urine sample [3]



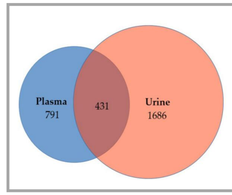
Market kits isolate predominantly bladder nucleosomal cfDNA (from Lee et al. 2020)

uProcess isolates smaller trDNA exclusively, histone bound charge neutralized bladder nucleosomes do not bind to the chitosan membrane.

Floyd Taub M.D. CEO (303) 249-9174
FloydTaub@a-i-GENE.com www.a-i-GENE.com

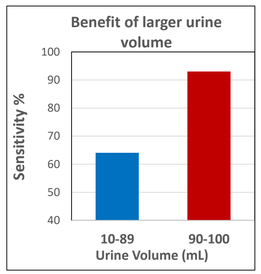
Is urine complementary or better than blood?

Urine ctDNA contains variants not detected in plasma



Herzog et al. isolated cfDNA from 4 mL of plasma and 10 mL of matching urine supernatant for each patient using the QIAamp MinElute ccfDNA Midi Kit (QIAGEN, Hilden, Germany) [2]

Sensitivity at various volumes:



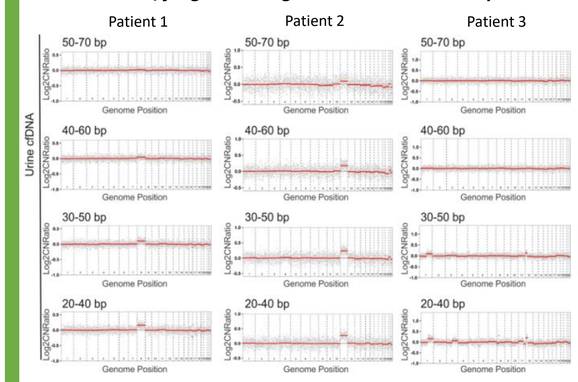
> 90 mls of urine needed for optimal T790M detection [Reckamp, et al., [1]

EGFR MUTATION SENSITIVITY vs. VOLUME

	5mL	10mL	40mL	80mL	150mL	1L
1%	100%	100%	100%	100%	100%	100%
0.1%	75%	75%	75%	100%	100%	100%
0.01%	28%	28%	28%	75%	75%	100%
0.001% (10 ppm)	0%	0%	0%	28%	28%	75%

Adapted from Ruppert, et al. [4] up-scaling model, This includes adjustment for actual recovery observed.

What DNA size/fragment length contains cancer sequences?



Bhambihani et al 2024, by low pass sequencing, studied urine cfDNA and, as illustrated by the larger hop ups in the red lines, found the most cancer in the smallest fragments, **indicating the need to isolate these small trDNA pieces.** [5]

Trans-renal DNA (trDNA) Collection

Blood ctDNA in both histone bound (overall neutral charge) nucleosomes, and free/transcription factor bound active ctDNA.

Only smaller NAKED DNA with negative charge enter urine space, blood nucleosomes do not.

The size limit is ~100bp.

Kidney and bladder cells add urothelial nucleosomes.

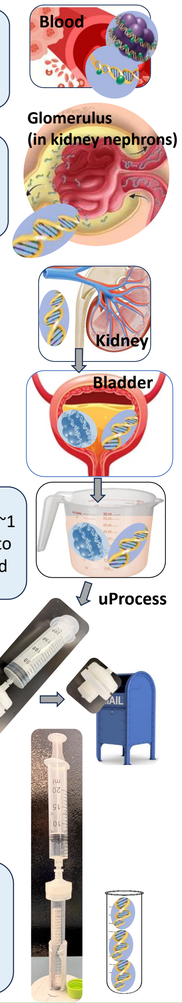
Urine collected **AT HOME** in cup pre-loaded with rapid dissolve preservative and acidification buffer.

Subject pours urine into syringe and pushes through filters cartridge (~1 min). At home electrostatic binding to chitosan. trDNA binds, histone bound bladder DNA does not.

Ambient shipping of cartridge to lab.

AT LAB
Save large pre-filter for cells/gDNA; Specific capture filter has trDNA .

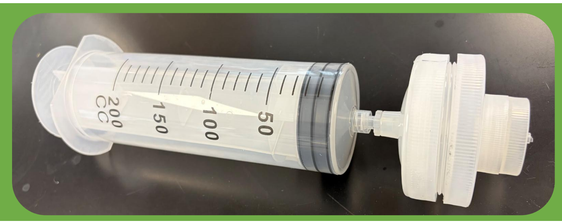
At lab use syringe to perform low pH wash (2 min). Elution of trDNA in high pH TE (2 min). Total lab time 4-5 mins.

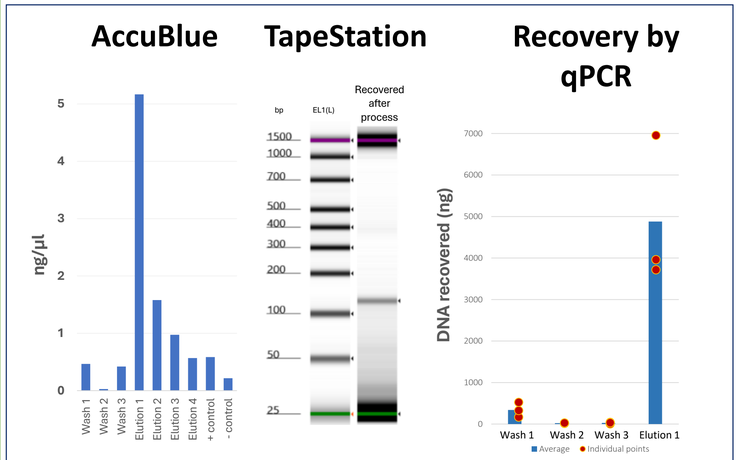


cfDNA Collection Comparison

	aiGENE uProcess	QIAamp Circulating Nucleic Acid Kit	Zymo Urine method (largest sample)
Lay user steps	• Collect urine in cup at home. • Push through syringe.	• Travel to site. • Wait with possibly infected patients. • Provide sample. • Travel home.	
Time	5 min. (plus ambient mailing)	1-4 hours	1-4 hours
Lab steps	• Push wash buffer through filter with syringe. • Push elution buffer through filter.	16 steps include some that require connection to their vacuum system, plus freezing and thawing. Must also transport to molecular Dx lab with dry ice and biological hazard shipping restrictions	Numerous, including 7-8 centrifuge steps.
Time	<10 min.	1.5 hours	1.5 hours
Cost	$100-250/Liter. Includes collection.	~$8580/L, Isolation kit only, plus collection costs.	~$270/L Isolation kit only, plus collection costs.

uProcess Device and Results





Methods: 5 µg of a 100bp oligo containing both KRAS and TP53 was spiked into 10 ml of urine and isolated with uProcess. Accublue (quantification of recovery), TapeStation (visualization of DNA band) and PCR (quantification of DNA sequences) illustrate efficient recovery of ctDNA sequence from urine matrix without prior centrifugation or sample preparation steps.

DISCUSSION & CONCLUSIONS

The new uProcess device, with a smaller chitosan DNA capture filter, allows near quantitative capture and elution (in 0.5 mls) of oncogene sequences from fresh, not previously centrifuged.

The eluted trDNA is without inhibitors and PCR ready, previous data on human urine samples document they are also NGS ready.

Capture, purification, and elution are routinely completed in less than the time it typically takes to prepare urine for cfDNA purification using centrifugation.

REFERENCES

[1] Reckamp KL, Melnikova VO, Karlovich C, et al. A Highly Sensitive and Quantitative Test Platform for Detection of NSCLC EGFR Mutations in Urine and Plasma. J Thorac Oncol. 2016 Oct;11(10):1690-700. doi: 10.1016/j.jtho.2016.05.035. Epub 2016 Jul 25. PMID: 27468937.

[2] Herzog H, et al. Targeted Sequencing of Plasma-Derived vs. Urinary cfDNA from Patients with Triple-Negative Breast Cancer. Cancers (Basel). 2022 Aug 24;14(17):4101. doi: 10.3390/cancers14174101. PMID: 36077638; PMCID: PMC9454533.

[3] Lee EY, et al. Comparison of Four Commercial Kits for Isolation of Urinary Cell-Free DNA and Sample Storage Conditions. Diagnostics (Basel). 2020 Apr 18;10(4):234. doi: 10.3390/diagnostics10040234. PMID: 32325682; PMCID: PMC7236016.

[4] Ruppert T, Roth A, Kollmeier J, Mairinger T, Frost N. Cell-free DNA extraction from urine of lung cancer patients and healthy individuals: Evaluation of a simple method using sample volume up-scaling. J Clin Lab Anal. 2023;37:e24984. doi:10.1002/jcla.24984

[5] Bhambhani C, Kang Q, Hovelson DH, et al. ctDNA transiting into urine is ultrashort and facilitates noninvasive liquid biopsy of HPV+ oropharyngeal cancer. JCI Insight. 2024 Feb 8;9(6):e177759. doi: 10.1172/jci.insight.177759. PMID: 38516891; PMCID: PMC11018327.

aiGENE appreciates the assistance of the Allergy and Clinical Immunology Flow Core at the Anschutz Medical Campus, University of Colorado.

Numerous aspects of uProcess are patent pending