High-throughput Genomic Analysis of Urine Samples Using Pixelated-Ultrasound to Improve Urine Biopsy Workflow

Martha Evans-Holm[1], Nicolai Lumbres[1], Rwik Sen[2], Patricia Montilla-Perez[2], Marc A. Paradise[2], Peter Lentz[1]
[1]**Convergent Genomics**, South San Francisco, CA, 94080, USA [2]**Active Motif, Inc.**, Carlsbad, CA 92008, USA

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Enabling Epigenetics Research

Abstract

The challenge addressed here is an improvement to sample preparation of liquid biopsy from cancer patients for sequencing. We present the advantages of a novel pixelated ultrasound technology which significantly improves sample-preparation in a very fast, economical, and high-throughput manner with very consistent output between samples.

The technology works consistently for both low and high cell numbers which is a critical factor for heterogeneous samples from patients with no cross-contamination. In conclusion, we present and improvement in the workflow of non-invasive liquid biopsy using urine samples. This improvement will greatly enable unique fingerprinting of an individual's cancer or cancer risk across large cohorts, with the potential for integration in automation workflows.

PIXUL® Sonicator

Cells | FFPE | Virus | Tissues | Chromatin

Plant Samples | Genomic DNA | Microbial Cells

Workflows

Genomics | Proteomics | Transcriptomics | Pharmaceutical

Metabolomics | Chemistry | Epigenetics | Mesoscale Discovery

Epigenetics:
- ChIP-Seq
- Hi-C
- DNA & RNA Methylation

Mesoscale Discovery:
- Cytokine Profiling

Introduction

The most common malignancy of the urinary tract is bladder cancer, ranking as the 9th most common cancer worldwide and 2nd most common cause of death due to genitourinary tumors in the United States [1]. Considering the statistics, recurrence and costs associated with treatment bladder cancer and other urological cancers, it is important to develop efficient methods to screen patients for cancer risk assessment and prevention. Several factors contribute to risk factors including age, ethnicity, gender, genetics and environmental carcinogenic exposure. A crucial step for the success of NGS-based experiments is the preparation of good quality libraries which will undergo sequencing and subsequent analysis. Generating a good quality library is a major bottleneck because DNA fragments in a good quality library consistently need to belong to a specific size range which is difficult to obtain using traditional library preparation methods. Further, reproducibility, consistency, and rigor across high-throughput samples are required for screening and risk assessments to prevent cancers. Overall, it is very challenging to consistently fragment DNA to a specific size range with good reproducibility across high-throughput samples in a fast and economical manner requiring low input material, all at the same time. Hence, we present pixelated ultrasound (PIXUL®) technology [2, 3] which helps to obtain DNA fragments with sizes that qualify for good libraries consistently across high-throughput samples, *i.e.* 96-well plate in a fast and economical manner.

Schematic of Workflow for Urine Genomic DNA Analysis Using PIXUL® Sonicator

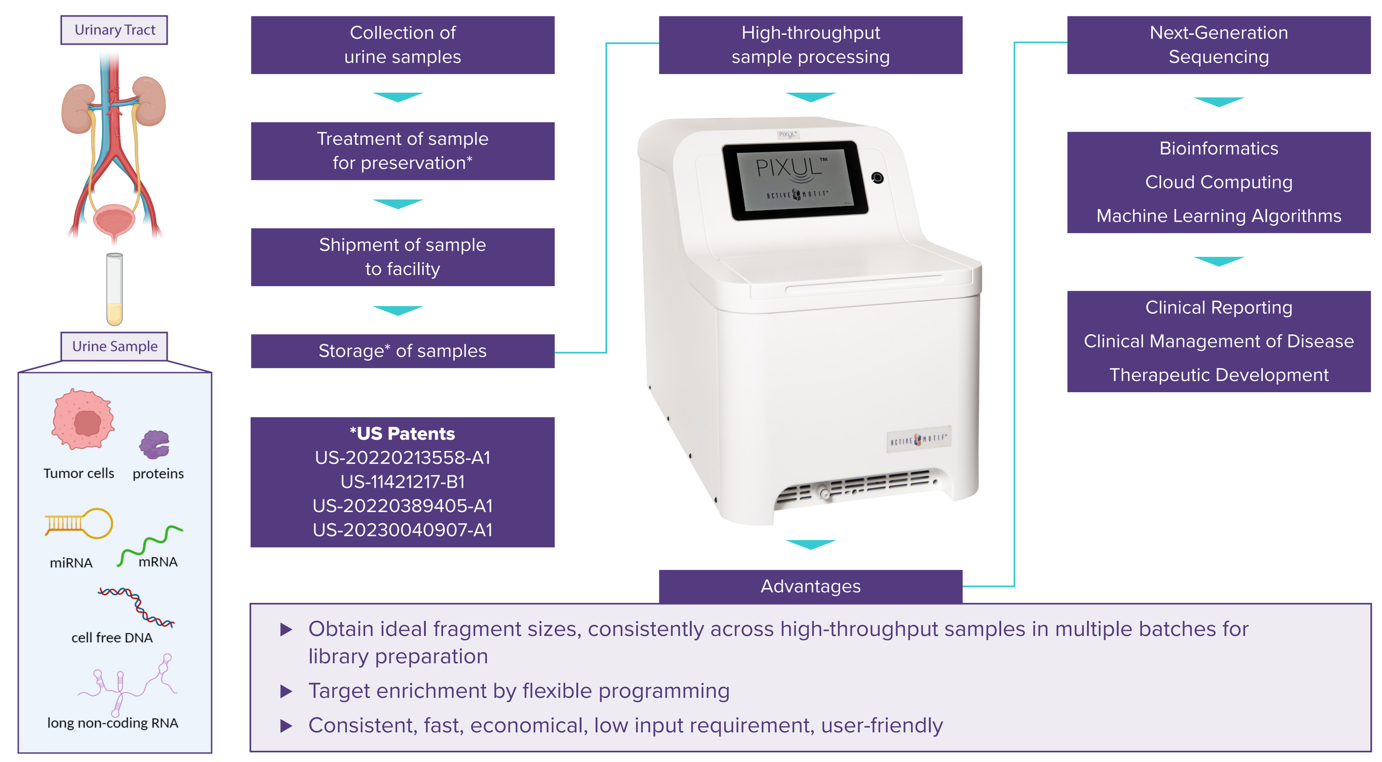


Figure 1. Schematic for processing high-throughput samples derived from urine and integration of PIXUL in the protocol leading to overall improvement of workflow, and hence clinical management of disease. Proprietary steps of sample treatment in the protocol are covered by US patents as indicated.

Materials and Methods

Sample collection and processing: Healthy human donor urine sample collection is a manual process where the donor collects their sample in a classic urine collection cup. It is then transferred to Vacutainer tubes. Sample processing is centered around a genomic DNA extraction method. The urine samples undergo extraction and nucleic acids are stored at 4°C or at -20°C for downstream analysis.

PIXUL Sonication: PIXUL® Multi-Sample Sonicator (Active Motif Catalog No. 53130) uses a transducer-lens assembly capable of focusing ultrasound, without auditory hazard. Individual samples are pipetted into specific wells of commercially available 96-well U-bottom polypropylene microplates (Corning Catalog No. 3799) which is then sealed and loaded into PIXUL® for processing. A high-power amplifier drives the transducer array, while an in-built Peltier cooling system is used to reduce heating of the samples. The built-in computer controls the ultrasound pulse parameters (number of cycles, treatment configurations and processing time). The device is capable of processing 96 samples at one time. It also allows the user to assign different sonication settings in each column (total 12) of a 96-well plate using the touch-screen user interface. The following recommended default settings were used:

Sonication Parameter	Setting
Pulse [N]	50
PRF [kHz]	1.00
Process Time [min]	30:00 or 36:00 (for ChIP) / 2:00 or 5:00 (for protein-based assays)
Burst Rate [Hz]	20.00

We compared the PIXUL sonicator with an alternative non-PIXUL high-throughput sonicator, samples were loaded into the manufacturer's proprietary consumable labware and processed. The manufacturer's recommend protocol was followed.

DNA Fragmentation Analysis: Fragment Analyzer from Agilent Technologies and its compatible kit for DNA fragment analysis were used to test the fragmented DNA samples using the manufacturers recommended protocol. The included ProSize software was used for data analysis.

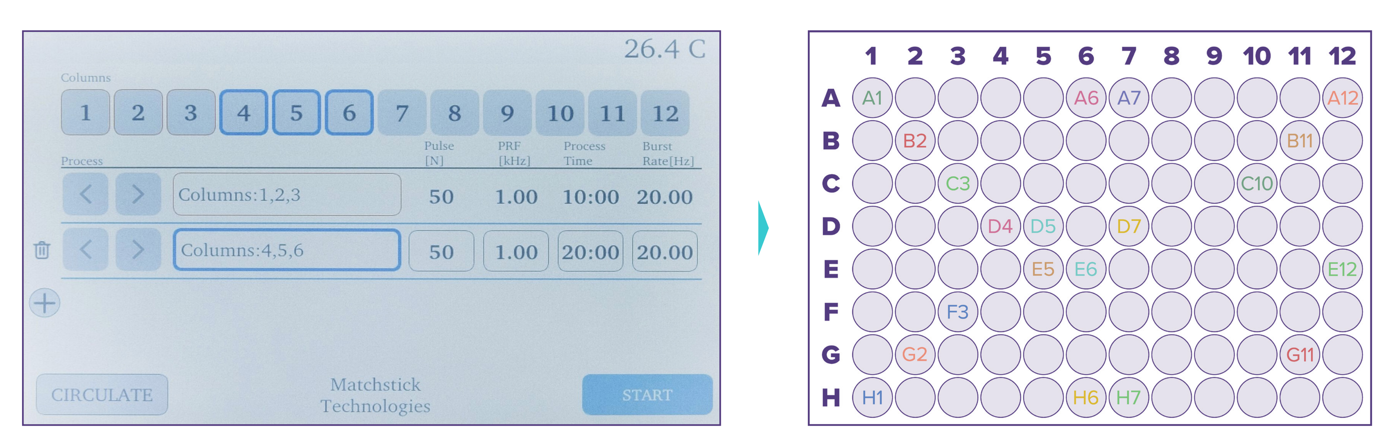
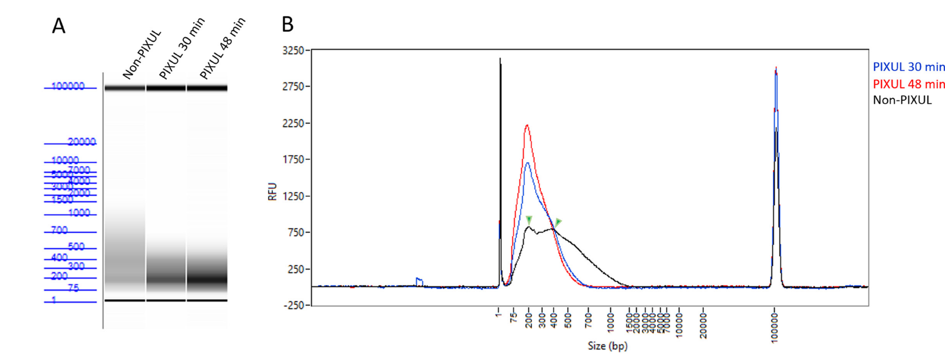


Figure 2. Left, PIXUL easy set up. Right, donor samples loaded across the 96-well, emphasizing the edges, corners, and center of the plate so that sonication in wells at diverse locations on the plate can be compared to test well to well consistency.

Results: Superior Consistency in Output using PIXUL® sonicator



Equipment	Average Size (base pairs)
PIXUL 30 min	248
PIXUL 48 min	224
Non-PIXUL	168, 491 (2 peaks)

Figure 3. Comparison between PIXUL® and non-PIXUL sonicator

Samples processed with PIXUL® consistently exhibited more thorough fragmentation, with a single major peak, and lesser variation whereas the samples processed by the non-PIXUL alternative showed multiple major peaks with broader variation. Additionally, PIXUL® produced a narrower overall range of DNA fragment sizes than the non-PIXUL alternative.

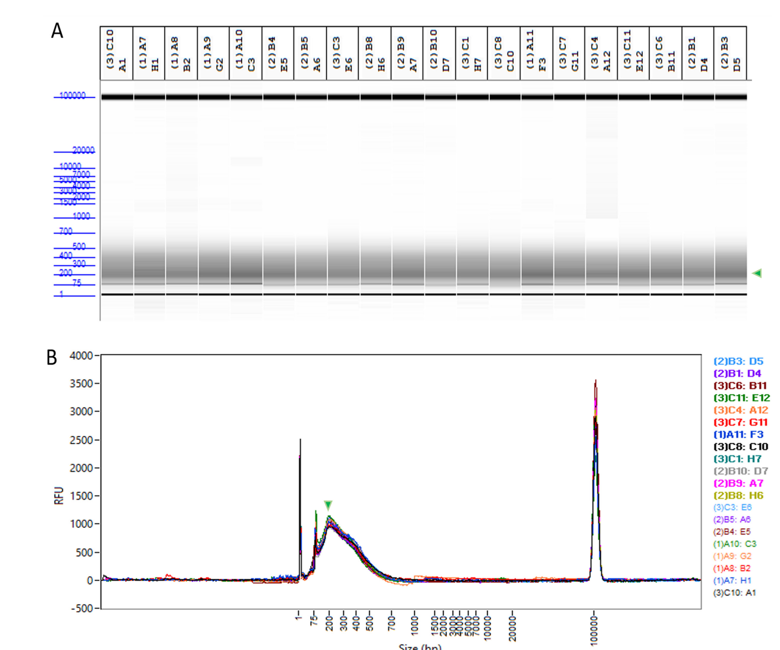


Figure 4. High Consistency between wells of PIXUL® sonicator

PIXUL®'s consistency through fragmentation across a 96-well plate. Instruments often exhibit variability in performance across a plate due to engineering limitations, such as edge-effect, making this an essential consideration. Once again, PIXUL® performed exceedingly well in this regard.

Acknowledgments and References

1. Crocetto F., *et al.* 2022 Liquid biopsy in bladder cancer: State of the art and future perspectives Critical Reviews in Oncology/Hematology 170:103577
2. Bomsztyk K., *et al.* 2019 PIXUL-ChIP: integrated high-throughput sample preparation and analytical platform for epigenetic studies Nucleic Acids Research 47(12):e69
3. Mar D., *et al.* 2024 A High-Throughput PIXUL-Matrix-Based Toolbox to Profile Frozen and Formalin-Fixed Paraffin-Embedded Tissues Multiomes Laboratory Investigation 104(1):100282
4. PIXUL® is sold under an exclusive license to patents owned by Matchstick Technologies Inc. and University of Washington, specifically Matula, T.J.; Bomsztyk, K.; Darlington, G.P., Maxwell, A.D.; MacConaghy, B.E., Reed, J. "Ultrasound system for shearing cellular material". US Patent US10809166. European Patent EP3169451

Summary

1. The results prove the efficacy of the technology in providing precise DNA fragments from limited input samples in a fast, consistent, and economical manner on a high-throughput platform.
2. PIXUL® provides the flexibility of assigning 12 different sonication programs to the 12 columns of the 96-well plate.
3. PIXUL® is a novel technology which provides a consistent, high-throughput, fast and economical method of enabling urinary liquid biopsy.