Enhanced cfDNA Fragmentation-Based Treatment Monitoring on the Ultima Genomics Platform

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#3863

BACKGROUND

- Whole-genome analysis of cell-free DNA (cfDNA) has emerged as a powerful tool for monitoring therapeutic response in patients with late-stage cancer
- The DELFI-Tumor Fraction (DELFI-TF) test uses fragmentation patterns from low coverage whole genome sequencing (WGS) of cfDNA to predict tumor fraction for longitudinal treatment monitoring[1]
- The Ultima Genomics sequencing platform provides reduced costs while enabling high quality variant calling through the ppmSeq technology[2,3]
- Using the Ultima platform as a foundation for the DELFI-TF assay may provide equal or improved performance at reduced cost
- In this study, we assessed the technical feasibility of the Ultima Genomics platform for the DELFI-TF test

METHODS

- Two cohorts were evaluated:
  - 48 pre- and on-treatment plasma samples collected from 16 stage IV lung cancer patients
  - 4 tumor, normal, and matched plasma samples of varying tumor types from treatment-naive stage IV patients
- cfDNA libraries were prepared and sequenced using the Illumina NovaSeq6000 and the Ultima UG 100 with the standard approach or ppmSeq
- Fragmentation features and DELFI-TF were computed, and DELFI-TF values were compared against somatic mutant allele frequencies (MAF) from a 500-gene targeted sequencing panel[4]
- Somatic variants from matched tumor/normal WGS were used to filter ppmSeq variant calls and categorize reads into two groups based on their likely origin: tumor or white blood cell (WBC). Fragmentation features were calculated per read group as well as for all reads combined

RESULTS

- Ultima sequences were high quality, with 88.3% of bases >Q30 base quality and 99.0% of reads aligned on average
- The distribution of frequencies per fragment length (FLDs) within the 100-220bp range and genome-wide fragmentation profiles were highly correlated between platforms
- DELFI-TF in the Ultima-sequenced libraries correlated strongly with MAF, showed high precision across replicates, and reflected longitudinal changes in tumor burden in response to treatment
- Detection of circulating tumor DNA (ctDNA) was similar between Ultima and Illumina platforms
- Classifying read origin by ppmSeq variant calls resulted in tumor-derived FLDs that displayed a higher proportion of short fragments than WBC-derived. When compared to a reference FLD derived from Ultima-sequenced samples with undetectable ctDNA, the tumor-derived FLD deviated more from the reference distribution than the WBC-derived and combined read FLDs
- The relative frequencies of NCGN fragment end motifs were consistently elevated in the tumor-derived fragments and associated with lower methylation at the corresponding CG site, supporting previous reports[5]

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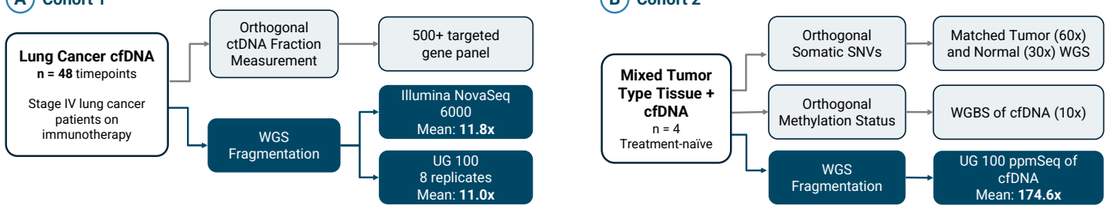
References:
[1] van't Erve et al, Nat Commun., 2024 Oct 21;15(1):8801 [2] Almogy et al, bioRxiv, 2022 Aug 10 [3] Cheng et al, bioRxiv, 2025 Aug 14 [4] Verner et al, J Mol Diagn, 2025 Mar;27(3) [5] Zhou et al, PNAS, 2022 Oct 26.

Figure 1:

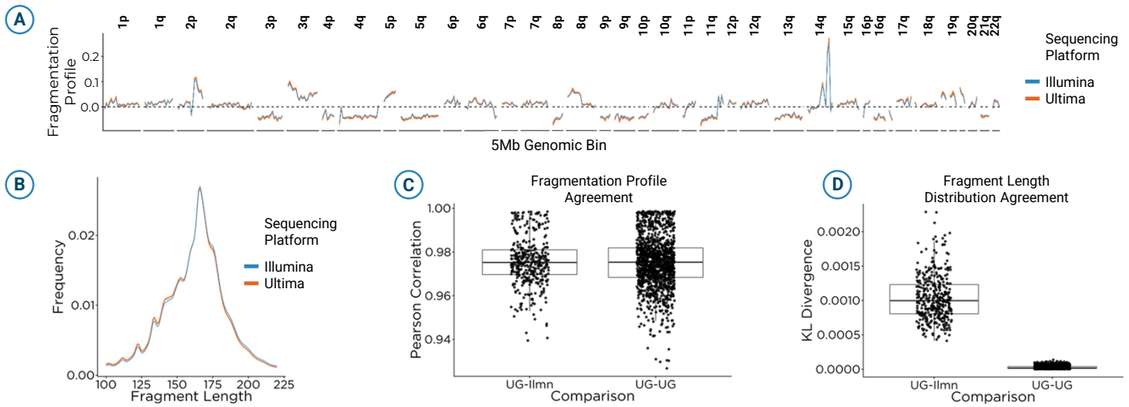
Study Design



(A) Plasma aliquots from 48 pre- and on-treatment timepoints were processed through a 500+ gene targeted panel as well as WGS library preparation. WGS libraries were sequenced on the NovaSeq 6000 (1 replicate) and the UG 100 instrument across 8 wafers to yield 8 replicates per timepoint. **(B)** Genomic DNA from four paired tumor and whole blood samples were processed with high coverage WGS to identify somatic SNVs. Donor-matched cfDNA was analyzed with whole genome bisulfite sequencing (WGBS) and the UG ppmSeq workflow.

Figure 2:

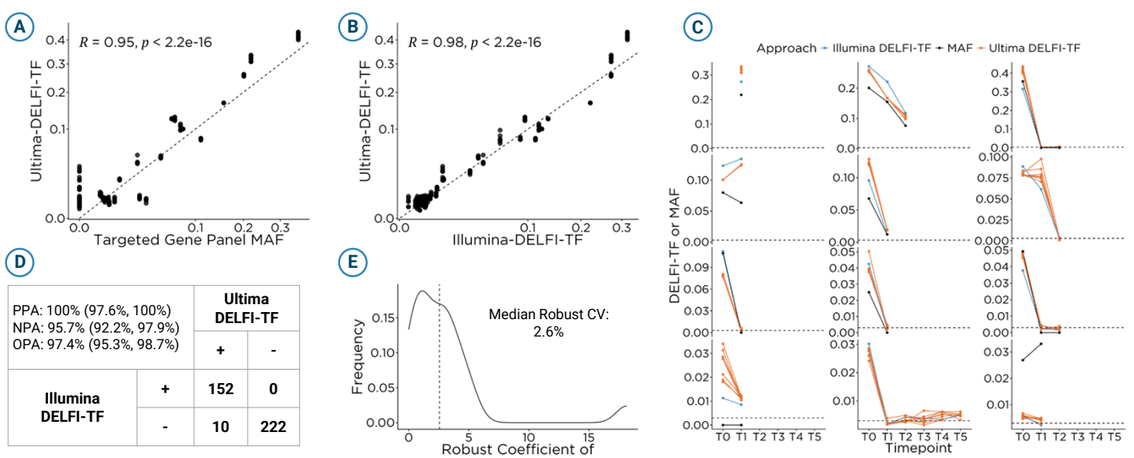
Fragmentation features are reproduced with high fidelity on the Ultima platform in Cohort 1



Fragmentation profile **(A)** and FLD **(B)** are aligned between Illumina (blue) and 8 replicates of UG 100 (orange). Pearson correlation between the fragmentation profile **(C)** and the Kullback-Leibler (KL) Divergence of the FLD **(D)** from sequencing replicates across Cohort 1. UG-Ilmn comparisons show correlations of 8 Ultima sequencing replicates to the Illumina replicate. UG-UG comparisons show pairwise correlations of the 8 Ultima sequencing replicates.

Figure 3:

Ultima-DELFI-TF shows high agreement with MAF and Illumina-DELFI-TF and is reproducible in Cohort 1

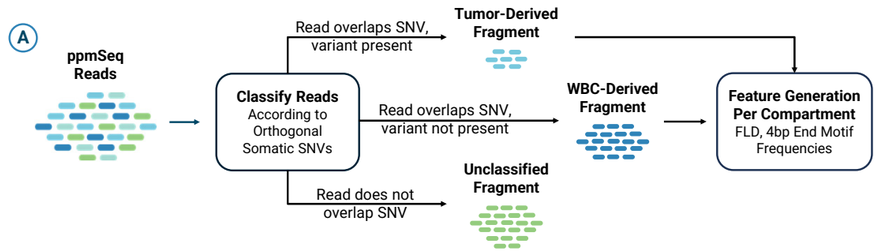


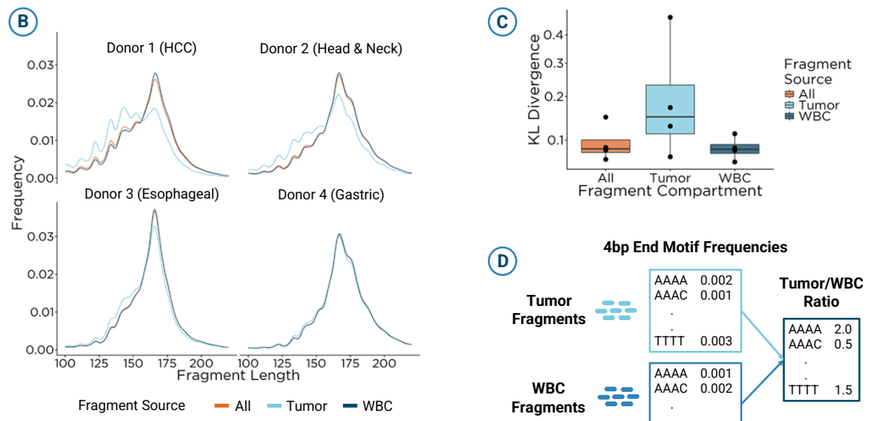
		Ultima DELFI-TF	
PPA: 100% (97.6%, 100%) NPA: 95.7% (92.2%, 97.9%) OPA: 97.4% (95.3%, 98.7%)		+	-
Illumina DELFI-TF	+	152	0
	-	10	222

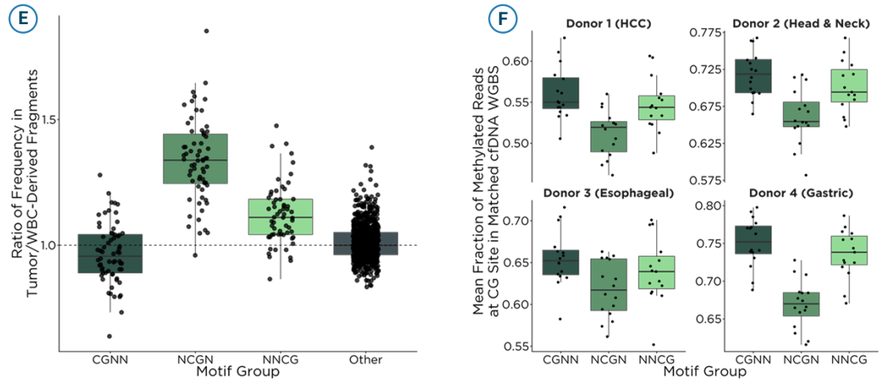
DELFI-TF from all Ultima replicates is highly correlated with MAF **(A)**, a proxy for the ctDNA fraction in the cfDNA, and with the DELFI-TF estimate from Illumina **(B)**. **(C)** Longitudinal tumor fraction changes are similarly captured between MAF, Ultima-DELFI-TF, and Illumina-DELFI-TF. Longitudinal trajectories are consistent across Ultima technical replicates. Dashed lines represent DELFI-TF LOB. **(D)** ctDNA detection status is similar between sequencing platforms. **(E)** Ultima-DELFI-TF shows high reproducibility, with median robust coefficient of variation (CV) of 2.6%.

Figure 4:

ppmSeq-driven discrimination of fragments reveals fragmentation patterns specific to the tumor in Cohort 2







(A) ppmSeq reads were filtered into compartments based on the presence of a somatic variant identified in tumor tissue. Reads that did not overlap a variant position remained unclassified. **(B)** FLDs from tumor-derived fragments differ from those from WBC-derived fragments. "All" fragments include tumor-derived, WBC-derived, and unclassified. **(C)** KL Divergence was calculated between the FLD of each fragment source and a reference FLD from Cohort 1 samples with undetectable ctDNA. **(D)** The frequencies of 4bp fragment end motifs were calculated in the tumor and WBC-derived fragments and a ratio between the two was taken. **(E)** NCGN motif frequencies are elevated in tumor-derived fragments. **(F)** Motif ratio in CG-containing motifs is related to methylation fraction.

- **Fragmentation features and DELFI-TF values are consistent across the Illumina and Ultima sequencing platforms, demonstrating flexibility of the fragmentomics approach.**
- **The Ultima ppmSeq assay enables segregation of fragments into compartments, providing opportunities to improve DELFI-TF sensitivity and enabling proxies for epigenomic features.**