

Recombinant nucleosomes as promising key reference materials for liquid biopsy next-generation sequencing.

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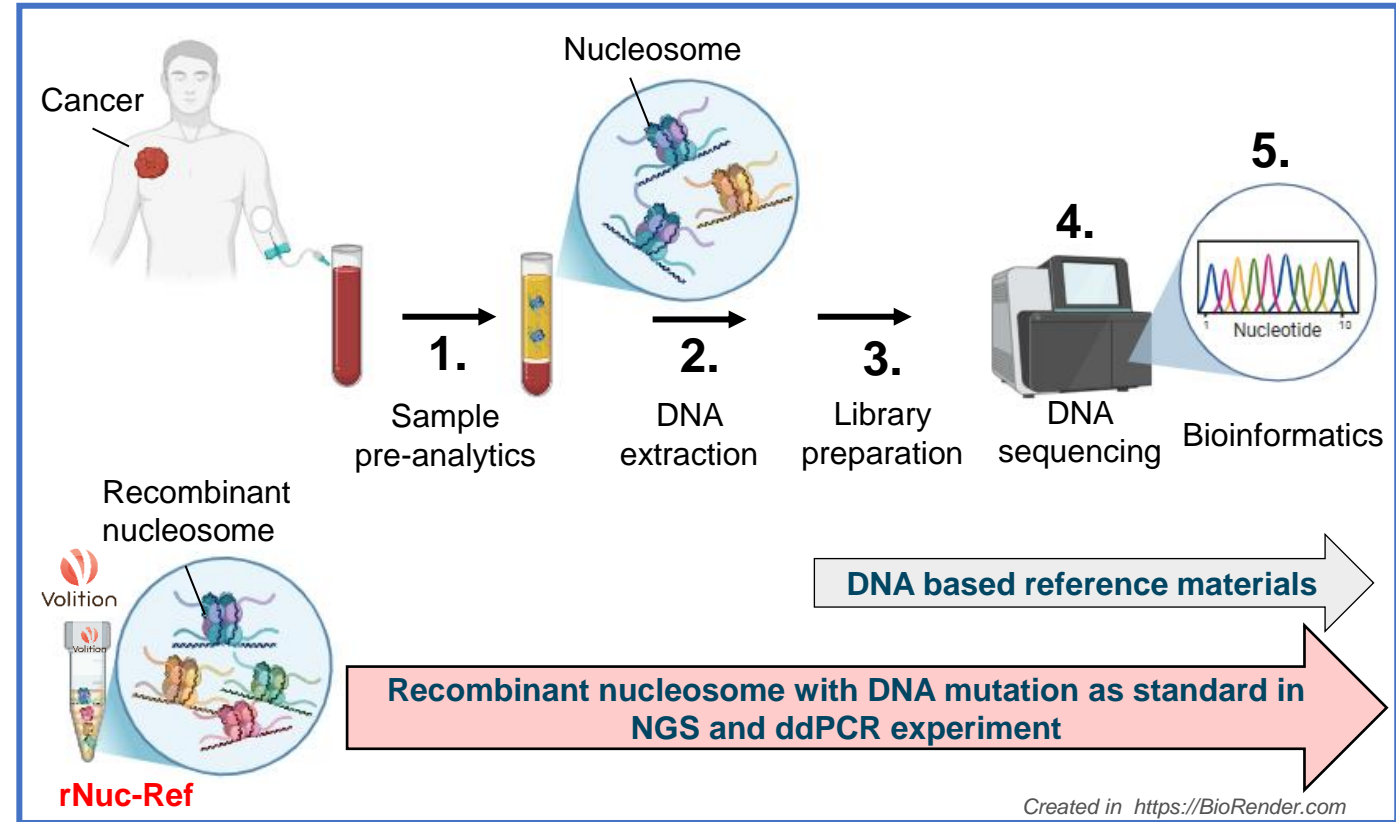
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Background and Introduction

Liquid biopsy is promising for detecting low-frequency variants in cancer, but interpreting Next-generation sequencing (NGS) data remains challenging due to genome complexity and technical failures in sample preparation. While reference standards are increasingly used to calibrate and validate NGS assays, current materials lack key features of native cfDNA, such as nucleosome association. Typically, most available standards rely on naked DNA from cell lines, which limits their relevance for liquid biopsy assay standardization, as native cfDNA is nucleosome-protected and exhibits a distinct fragmentation pattern.

Our Goal : develop a new reference material

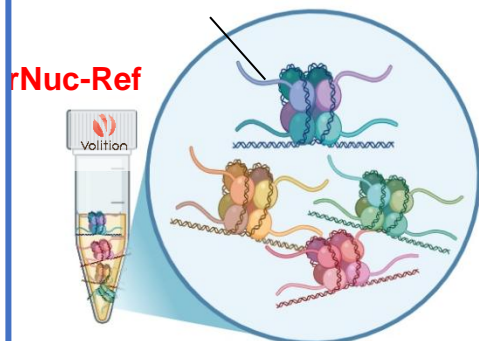
To provide new reference materials for full control of the entire NGS workflow – from blood collection to bioinformatic analysis – using recombinant nucleosomes (rNuc-Ref).



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Methods

Recombinant nucleosome

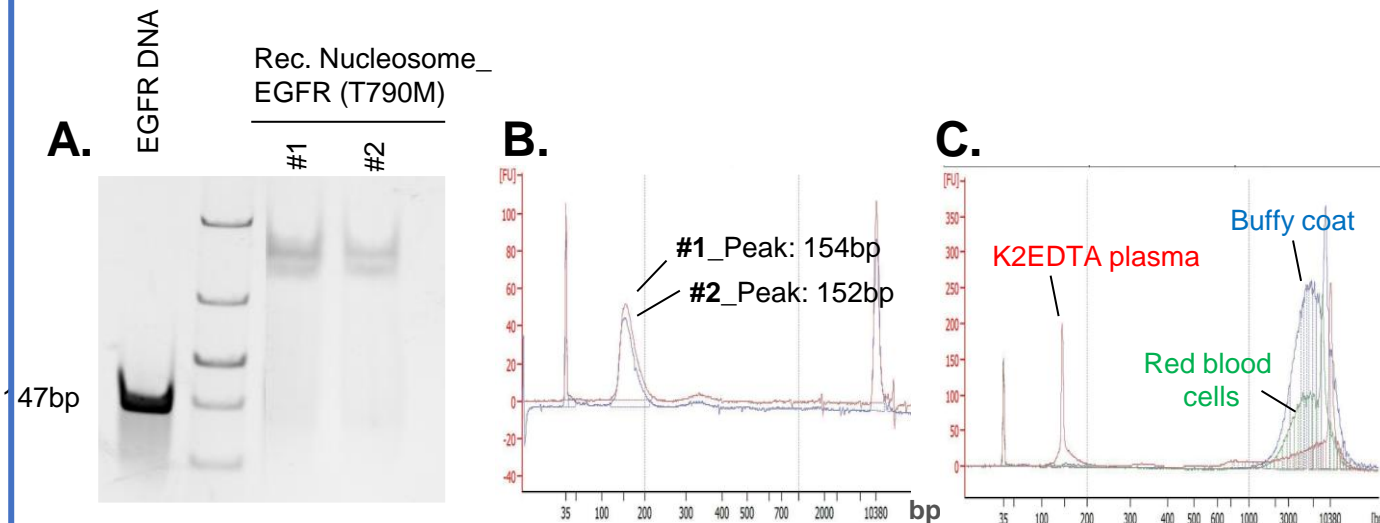


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Recombinant nucleosomes (rec. Nucleosome) were assembled *in vitro* using a 147bp DNA sequences bearing EGFR (T790M), BRAF (V600E) or KRAS (G12D) mutations and the histone core proteins (H3, H4, H2A, H2B).

A mixture of these recombinant nucleosomes (rNuc-Ref) were spiked, either in whole blood or in EDTA plasma samples.

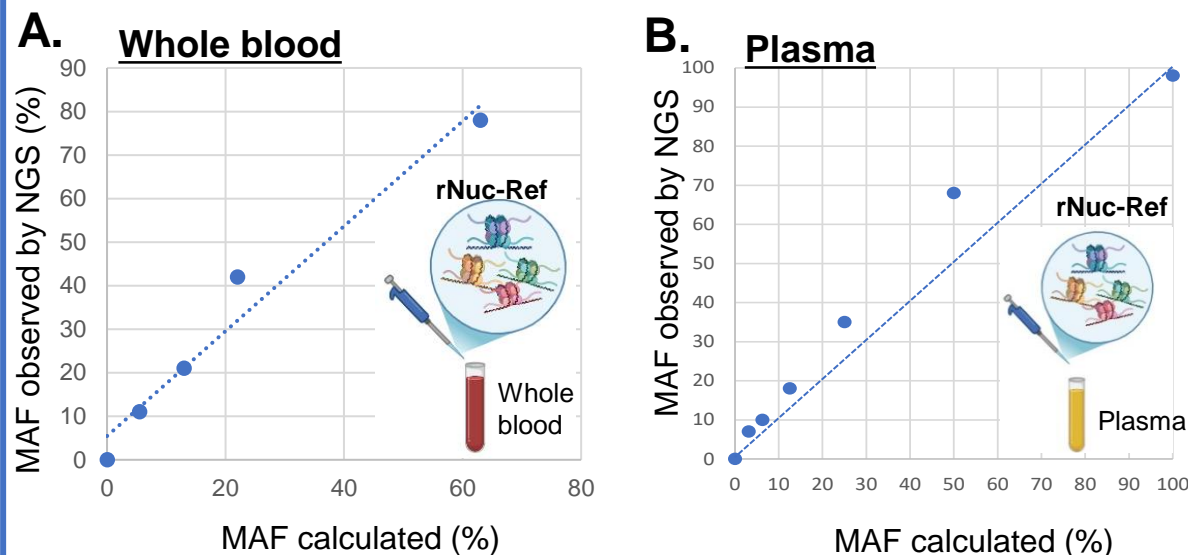
Then, DNA were extracted and analyzed by Digital droplet PCR (ddPCR) or Sequencing.



(A) DNA Electrophoresis showing DNA from two independent vials (#1 - 2) of recombinant EGFR nucleosomes carrying the T790M mutation, compared to free DNA bearing the same mutation. The distinct banding pattern confirms successful nucleosome assembly and integrity. (B) Bioanalyzer profiles of two independent recombinant nucleosome preparations (#1 and #2) show consistent fragment size distributions, closely resembling native cfDNA with a predominant peak around 150 bp. The high similarity between the two profiles demonstrates good reproducibility of the preparation method. (C) Bioanalyzer profile showing that the rNuc-Ref, spiked into whole blood, is fully recovered in the plasma fraction after centrifugation, with no detectable signal in the red blood cell or buffy coat fractions. This supports its suitability as a whole-process control for liquid biopsy workflows, from blood collection to cfDNA analysis.

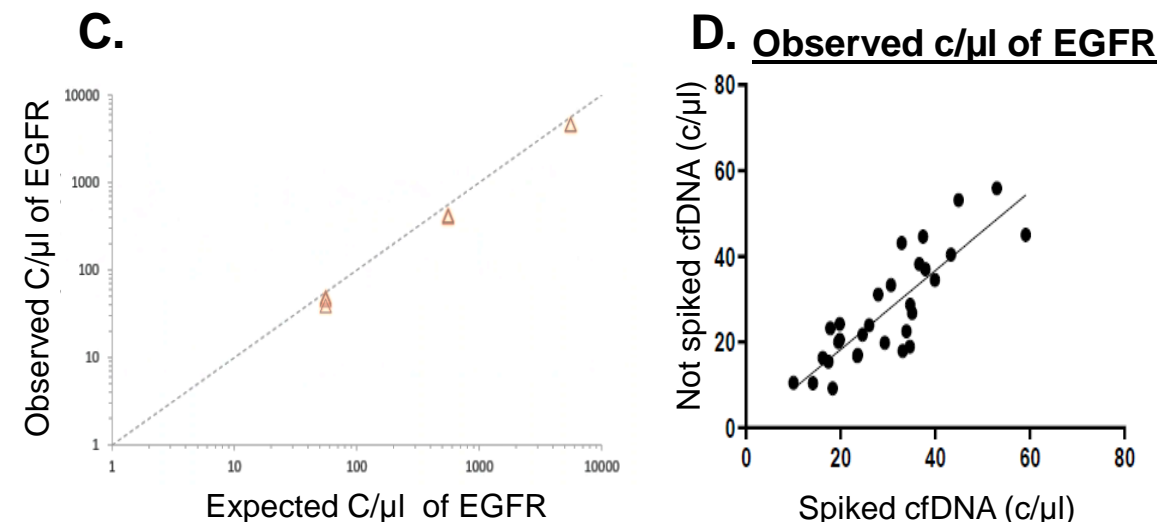
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Results



Spike-in of different proportions of recombinant nucleosome mixtures (rNuc-Ref) into whole blood (**A**) or artificial plasma matrix (SensID) (**B**), followed by sequencing using the AmpliSeq panel on IonTorrent (Seqalis), shows a linear correlation between the measured Mutant Allelic Fraction (MAF) and the theoretical expected percentage. This demonstrates the quantitative performance of the standard across matrices and input levels.

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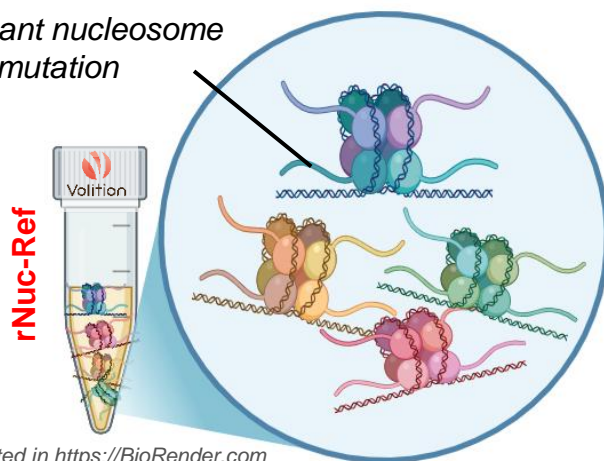


(**C**) Digital droplet PCR (ddPCR) analysis of spiked recombinant nucleosomes (EGFR with T790M mutation) shows a linear correlation between measured MAF and input copies/ μ L (c/ μ l). (**D**) The spiking of recombinant nucleosomes (EGFR with T790M mutation) in plasma K2EDTA had no impact on the recovery of endogenous cfDNA (unmutated EGFR), confirming compatibility with cfDNA quantification workflows (ddPCR) (n=10 patients, triplicate analysis).

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Conclusion - Discussion

Recombinant nucleosome
with DNA mutation



Our findings highlight the potential of recombinant nucleosomes carrying specific DNA mutations as robust reference materials for both sequencing and ddPCR-based assays. Their compatibility with the entire liquid biopsy workflow—from blood collection to cfDNA analysis—supports their use as whole-process controls processed alongside patient samples, ensuring reliability and standardization across platforms.

- DNA is protected by histone into the complex
- This complex can be lyophilised facilitating shipping
- The DNA can be synthesized according the need.

- ✓ Recombinant nucleosomes mixture represent the fragment length distribution of cfDNA from patient samples
- ✓ Recombinant nucleosome mixtures can be produced in a reproducible manner, reinforcing their potential as reliable reference materials for liquid biopsy applications
- ✓ Recombinant nucleosomes can be produced for any DNA sequence, offering a versatile platform to develop reference materials that closely mimic native cfDNA.