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

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Abstract

Background:

Liquid biopsy is promising for detecting low-frequency variants in cancer and mosaic diseases, but interpreting Next-generation sequencing (NGS) data remains challenging due to genome complexity and technical failures in sample preparation. To address these challenges, an increasing number of clinical laboratories are adopting reference standard materials for calibrating NGS measurements and validating assays. However, current approaches have their own strengths and limitations; the absence of reference materials that mimic native cfDNA is a major obstacle in standardizing liquid biopsy NGS assays across laboratories . Typically, cfDNA has a length correlating to nucleosome protection, whereas existing reference materials are only composed by naked DNA primarily derived from cell lines.

Objectives:

To develop reference materials for controlling the whole NGS workflow, from blood collection to bioinformatic analysis. Results of a proof of concept evaluation.

Methods:

Recombinant nucleosomes were assembled in vitro using a 147bp DNA sequences bearing EGFR (T790M), BRAF (V600E) or KRAS (G12D) mutations and the histone core proteins. A mixture of these recombinant nucleosomes were spiked, either in whole blood or in EDTA plasma samples. Then, DNA were extracted and analyzed by Digital droplet PCR (dPCR) or Sequencing.

Results:

Recombinant nucleosomes spiked in whole blood were only detected in the plasma fraction, and recombinant nucleosomes mixture accurately represented the fragment length distribution of cfDNA from patient samples. Interestingly, a linear correlation in Mutated Allelic Fraction (MAF) was observed by sequencing, when recombinant nucleosomes were spiked, not only in plasma but also in whole blood. Consistently, a linear correlation between MAF and copies/µl was observed when recombinant nucleosomes was quantified by dPCR with no effect on the recovery of endogenous cfDNA upon recombinant nucleosomes spiking. Additionally, reproducible production of recombinant nucleosomes mixtures was achieved (n=2), further confirming the reliability of the system.

Conclusion:

Our findings highlight the potential of recombinant nucleosomes with specific DNA mutations as key reference materials for sequencing or dPCR experiments, processed alongside patient samples, from DNA extraction to final results. These recombinant nucleosome mixtures are adaptable to any MAF, making them versatile tools for Liquid Biopsy NGS studies.

Do you have any conflicts of interest?

Yes, I have a conflict of interest.

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