Characterisation of novel, patient-like cfDNA reference material for molecular diagnostic liquid biopsy assays

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Background & objectives

Molecular diagnostic analysis of cfDNA purified after liquid biopsy is rapidly gaining importance and acceptance for clinical diagnostics. The need for precise and reliable detection of actionable molecular alterations from cfDNA for precision medicine is therefore leading to an increased need in reference materials (RM). A common way for generation of cfDNA RM is the combination of sheared genomic DNA with spike-in variants in adjustable allele frequencies for molecular alterations. This approach has proven to be technically valid and useful and provide RM for ddPCR and Next Generation Sequencing (NGS) assays. However, both users and important regulatory bodies such as the Food and Drug Administration of the USA (FDA) are asking for truly patient-like material e.g. derived from cell cultures for use as contrived samples with rare molecular markers in clinical studies.

Methods

Genomic DNA is purified from lymphoblastoid or cancer cell lines. Lysis, purification of DNA and/or nucleosomes from cells, enzymatic treatment is based on standard and/or patent-pending methods. DNA is characterized by electrophoresis, ddPCR, and NGS followed by bioinformatic analysis.

Results

A proprietary method was developed for standardized enzymatic fragmentation of cell-line derived chromosomal DNA based on nucleosomal protection. The resulting DNA size profile is indistinguishable from patient profiles and can be used with different cell lines e.g. for generation of unmutated background cfDNA for blending with cfDNA from a mutated cell line.

Conclusion

- The size profile of the novel, patient-like nucleosomal cfDNA is a very close mimick to and virtually indistinguishable from natural patient-derived cfDNA

- The nucleosomal cfDNA is compatible with ddPCR and NGS assays
- n-cfDNA based RM should improve interlaboratory comparisons in proficiency studies
- n-cfDNA based material should increase acceptance of regulatory bodies such as the FDA for use as contrived samples in clinical studies