Cfdna Reference Material: Mimicking Human-Like Profiles

Abstract Submitter: Renu Datta, United Kingdom*

Co-Authors: Daniel Williams, Anup Chugani, Gemma Halliday

*Revvity

Abstract

Background: Cell-free DNA (cfDNA) has emerged as a powerful non-invasive biomarker in clinical oncology. Its applications are diverse, and it might be a promising tool for enhancing cancer diagnosis and treatment, potentially leading to improved outcomes and personalized care for individuals. One way to detect cancer-associated mutations in cfDNA is next-generation sequencing (NGS) of liquid biopsies. Reference materials containing cancer-causing mutations are essential for cfDNA-NGS workflows, as they allow comparison of human samples with an undefined genotype to a known reference set. Most common methods to generate cfDNA reference materials include mechanical shearing of genomic DNA from cells by sonication and spiking synthetic oligos bearing mutations of interest into cfDNA isolated and amplified from plasma. While these reference materials are useful for NGS workflows, they do not fully represent the human cfDNA profile. They primarily match with the average cfDNA fragment size, which is approximately 166 base pairs. As a result, important information like fragmentation and methylation pattern etc imprinted onto cfDNA may be lost. Objective + Methods: To improve the current cfDNA reference materials, we aimed to develop a method that mimics the natural process of cfDNA generation in the human body. For this purpose, we treated DNA isolated from cell lines which contain common cancer-associated mutations, with an enzymatic blend that generates cfDNA reference materials mimicking human like profile. Results: Compared to cfDNA generated by sonication, cfDNA reference materials produced through enzymatic fragmentation exhibits a laddering effect characteristic of cfDNA found in human plasma. Conclusion: Generating cfDNA reference standards via enzymatic fragmentation represents an improvement over sonicated cfDNA and can enhance advancement of research and development of testing by providing a more accurate representation of human like cfDNA, capturing details like fragmentation and methylation patterns that are often lost with traditional methods.

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Do you have any conflicts of interest?

No, I do not have a conflict of interest.