Optimized library preparation kit and workflow for improving cfdna sequencing sensitivity

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Abstract

Background/Objectives: Next-Generation Sequencing (NGS) of cell-free DNA (cfDNA) has emerged as a promising strategy for early detection, diagnosis, and monitoring of cancer progression. In this process, cfDNA is extracted from a patient's blood and undergoes library preparation prior to NGS. The challenge with cfDNA library preparation is reliably capturing and converting all the fragmented DNA, especially when present in low concentrations within biological samples. Attaining low variant detection thresholds and variant calling confidence demands high-performance NGS libraries and targeted sequencing protocols.

Methods: Presented here is a workflow leveraging the Twist cfDNA Library Preparation Kit and an optimized target enrichment workflow to maximize the conversion of duplex, on-target, sequenceable sample molecules.

Results: It is demonstrated that by increasing target coverage we can increase detection sensitivity at low variant allele frequencies. In addition, this workflow improves detection of duplex-molecule families relative to comparable workflows due to more efficient four-point ligation and newly optimized target enrichment. It is also demonstrated that achieving such improvements in complexity does not necessitate compromising data fidelity by introducing artifacts or losing uniformity. This improved conversion and sensitivity is applicable to as low as 1ng input samples with both native cfDNA and control cfDNA.

Conclusion: In summary, our optimized library preparation kit and streamlined workflow emerges as a valuable asset for those deploying NGS-based liquid biopsy assays.

Do you have any conflicts of interest?

Yes, I have a conflict of interest.

Authors are full-time employees of Twist Bioscience