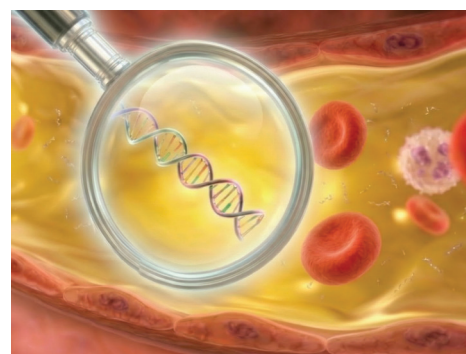


Liquid biopsy is an important, non-invasive approach for detecting cell-free, circulating tumor DNA (cf/ctDNA) for cancer research applications.¹ Next-generation sequencing (NGS) of cfDNA samples is **inherently challenging** due to low nanogram input amounts. Library preparation efficiency is critical to enable high-confidence analysis of low-frequency variants.

KAPA EvoPrep Boost Kits offer evolved reagents and limited sample handling to maximize genome equivalent recovery (GER). Here, we demonstrate mutation analysis of cfDNA using targeted sequencing with a rapid workflow that offers faster turnaround times without compromising performance.

¹Zalis M, et al. Next-generation sequencing impact on cancer care: applications, challenges, and future directions. [Front. Genet. 2024](#).



Rapid, high-efficiency library prep offers faster turnaround times for cfDNA analysis without compromising performance

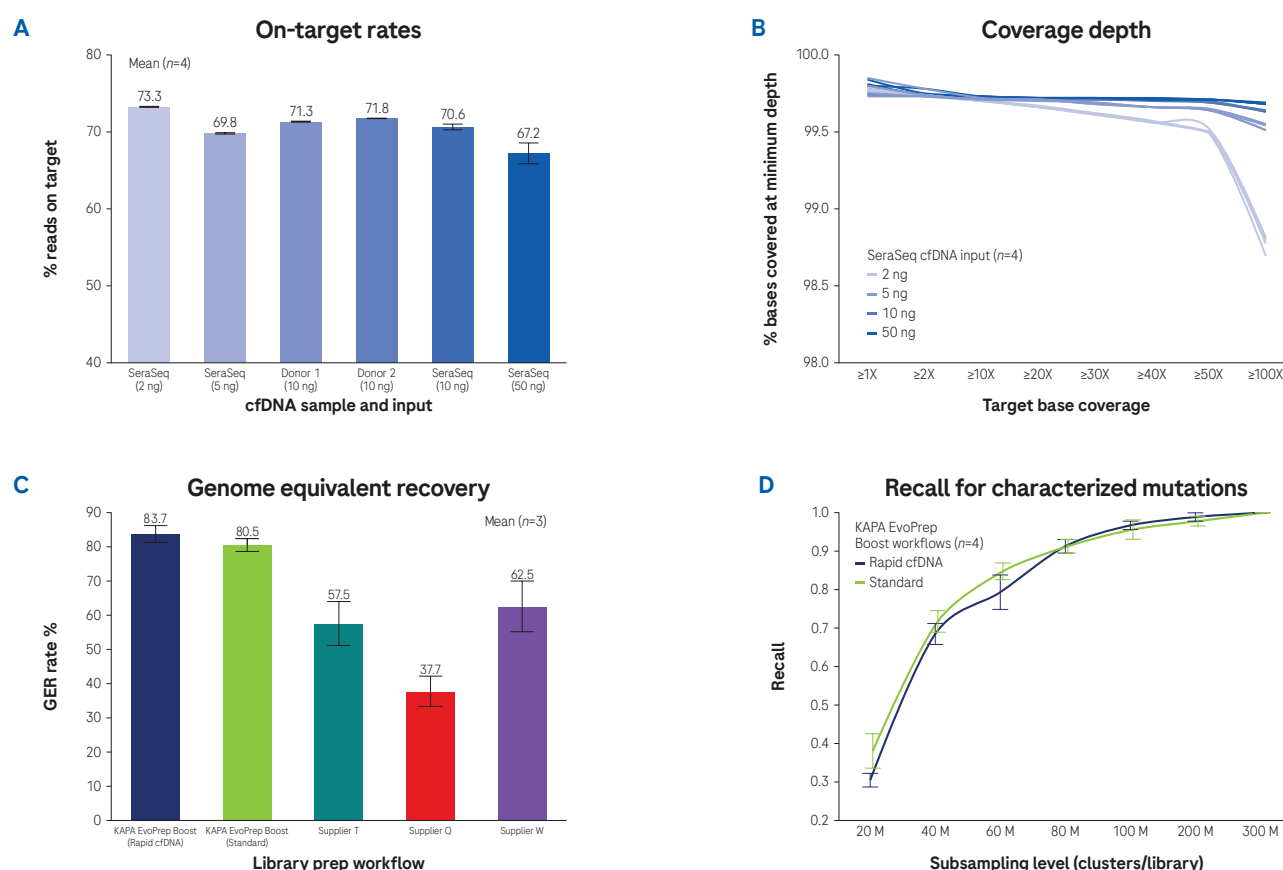


Figure 1. Highly efficient library preparation with the KAPA EvoPrep Boost Kit rapid cfDNA workflow enables high performance analysis of cfDNA using targeted sequencing. With advanced reagent formulations, the evolved KAPA EvoT4 DNA Ligase, the highly optimized KAPA EvoAmp ReadyMix, and an optimized post-ligation cleanup (see Figure 2A), sequencing-ready cfDNA libraries may be prepared in less than two hours. The KAPA EvoPrep Boost Kit rapid cfDNA protocol supports (A) consistently high on-target rates, and (B) excellent coverage metrics across a wide range of cfDNA inputs. (C) Industry-leading recovery of unique molecules ([genome equivalent recovery](#), GER) is maintained with the rapid protocol, and translates to (D) accurate, high-confidence variant calling from cfDNA samples. Library prep was performed with the [KAPA EvoPrep Boost Kit](#), using the standard or rapid cfDNA workflows, or with kits from other suppliers (according to recommended protocols). Replicate libraries were prepared from varying amounts (2, 5, 10, or 50 ng) of a contrived, commercial cfDNA sample ([SeraSeq® ctDNA Mutation Mix v2 AF 0.125%](#)) or 10 ng inputs of two cfDNA samples from healthy donors. Target enrichment was performed as described in the [KAPA HyperCap cfDNA Evolved Workflow v3.0](#), using the [KAPA HyperCap Oncology Panel](#) (214 kb) which covers 24 SNVs and 12 indels of the characterized variants in the SeraSeq sample. Sequencing (2 x 150 bp) was performed on an Illumina® NovaSeq™ instrument (S4 flow cells). Unless indicated otherwise, data were downsampled to 50 million clusters per library. Data analysis was performed using an internal RUO analysis pipeline.

High efficiency cfDNA-specific library prep in <2 hours

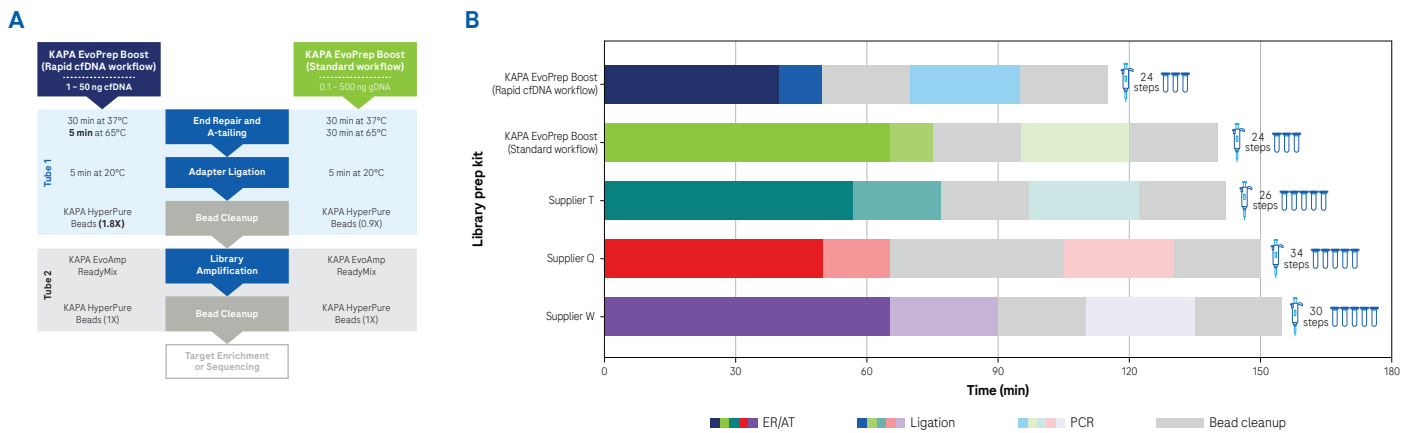


Figure 2. Workflows used in this study. (A) The cfDNA-specific rapid KAPA EvoPrep Boost workflow (right) was derived from the standard KAPA EvoPrep Boost workflow by reducing the End Repair/A-Tailing (ERAT) time, and increasing the stringency of the post-ligation cleanup to retain shorter fragments. **(B)** Turnaround times and number of pipetting steps for the five workflows compared in this study. The standard KAPA EvoPrep Boost workflow is already a highly streamlined, single-tube protocol that employs engineered enzymes, ready-to-use reagent mixes, and minimal pipetting steps to preserve unique input molecules and reduce the potential for error, as well as plastic waste. Further optimization for cfDNA inputs enables the preparation of libraries for direct sequencing or target enrichment in less than 2 hours with minimal hands-on time.

Summary



KAPA EvoPrep Boost Kits utilize the **evolved KAPA T4 DNA Ligase**, **ReadyMix formats**, a **streamlined protocol with minimal handling**, and the highly optimized, high-fidelity [KAPA EvoAmp ReadyMix](#) to achieve exceptional recovery of unique cfDNA molecules (genome equivalents).



Highly efficient library preparation translates to **high on-target rates** and **coverage depth (excellent sequencing economy)**, and **high-confidence variant calling** from cfDNA samples.



Specific workflow modifications for rapid processing of cfDNA samples enable **industry-leading turnaround times** for liquid biopsies (for WGS or targeted sequencing) **without compromising performance**.

Learn more...

about the **KAPA EvoPrep Boost Kit** and how it can enable you to prepare the highest quality libraries for methylation analysis.

Click [here](#) or scan the code below:

