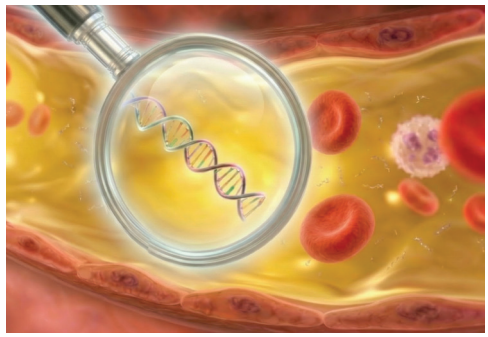
Tech Spotlight
Liquid Biopsy

KAPA EvoPrep Boost Kit for cfDNA research

Rapid and efficient library prep for high-confidence mutation analysis

Liquid biopsy is an important, minimally-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 cfDNA-specific rapid workflow that offers faster turnaround times without compromising performance.



[1] *Zalis 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

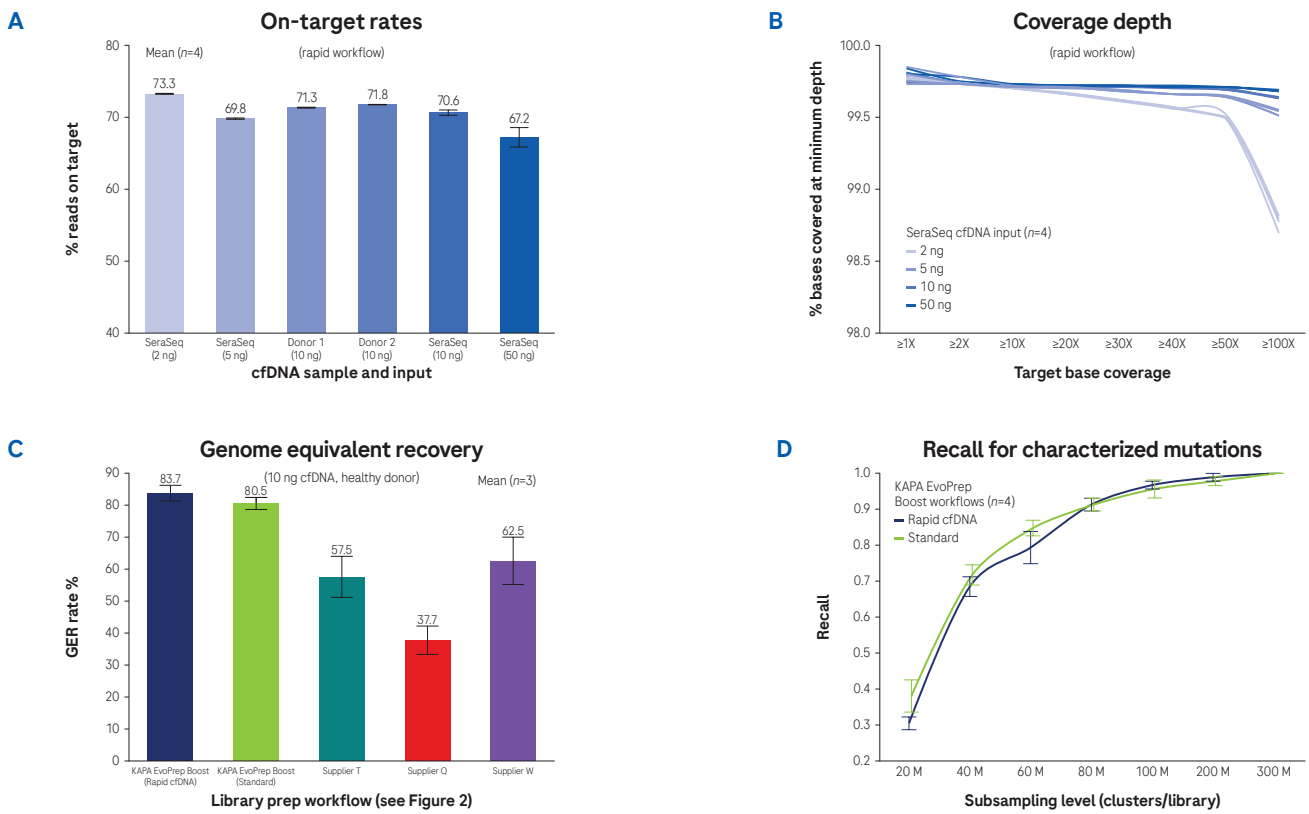


Figure 1. High-performance analysis of cfDNA using targeted sequencing and the KAPA EvoPrep Boost Kit rapid cfDNA workflow. The KAPA EvoPrep Boost Kit rapid cfDNA protocol (used here with the KAPA HyperCap Oncology Panel) enables: **(A)** consistently high on-target rates; **(B)** excellent coverage metrics across a wide range of cfDNA inputs; and **(C)** industry-leading recovery of unique molecules (genome equivalent recovery, GER), which translates to **(D)** accurate, high-confidence variant calling from cfDNA samples across a range of subsampling levels. **Method summary:** Libraries were generated with the KAPA EvoPrep Boost Kit using the standard workflow or rapid cfDNA workflow (where indicated in C and D; see workflow summaries in Figure 2), 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 (A). 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.

Data on file at Roche.

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High efficiency cfDNA-specific library prep in <2 hours

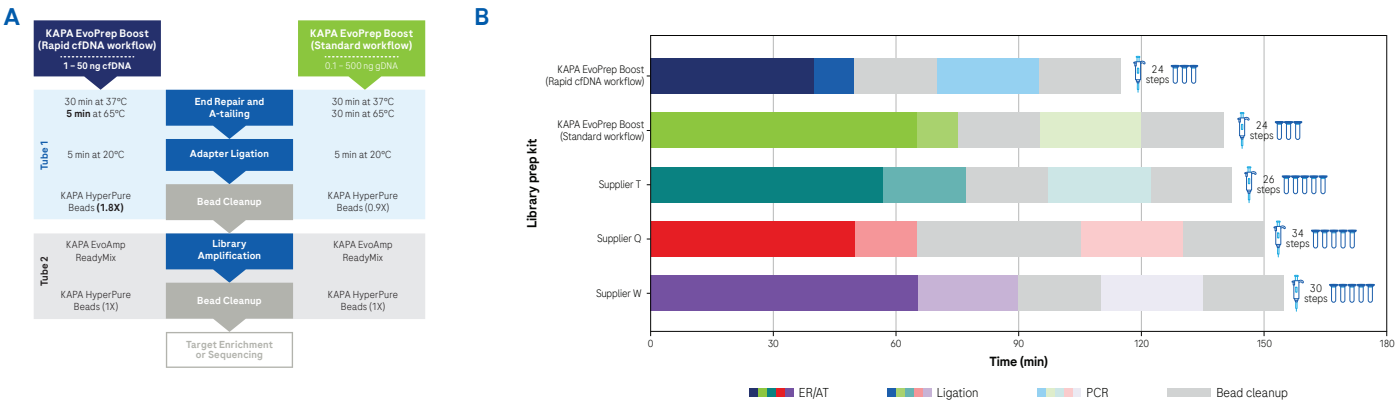

Figure 2. Workflows used in this study. (A) The KAPA EvoPrep Boost rapid cfDNA workflow (Panel A, left) was derived from the standard KAPA EvoPrep Boost workflow by reducing the End Repair/A-Tailing (ERAT) time, and increasing the stringency of the post-ligation cleanup to retain shorter fragments. **(B)** Turnaround times, number of pipetting steps, and number of reagents needed 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:

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MC-US-18851 04/26