Product Profile

QIAseq cfDNA All-in-One Kit

For generating libraries from cfDNA in plasma samples for analysis on Illumina® and Ion Torrent™ instruments

Next-generation sequencing (NGS) is a powerful method for high-throughput, high-accuracy analysis of circulating cell-free DNA (cfDNA). Starting with a simple blood sample, a successful NGS-based workflow can reveal valuable, previously hard-to-access insights into genomic drivers of cancer development or chromosome aneuploidies in fetal development research.

The analysis of circulating cell-free DNA in peripheral blood, known as a "liquid biopsy", presents a low-cost, low-risk way to access valuable genomic information without invasive surgical procedures. However, analyses of cfDNA can still be more challenging than starting with tissue specimens due to the limited and highly variable levels of cfDNA in blood plasma. Since available plasma sample volumes can also be limited, researchers may have to look for mutations in only a few nanograms of cfDNA. Signal-to-noise ratios in a cfDNA sample are typically low, so relevant mutations can be present at very low allelic frequencies. Therefore, it is essential to use an appropriately high-quality, high-efficiency cfDNA extraction and NGS library prep method to enable sensitive mutation detection.

The QIAseq cfDNA All-in-One Kit is a dedicated solution for creating NGS libraries from liquid biopsy samples. Combining highly efficient QIAamp technology for cfDNA extraction, high library prep reaction volumes optimized for dilute samples and a superior conversion rate of cfDNA to NGS library, the kit ensures optimal performance and sensitive detection of even the rarest mutations.

Benefits of the QIAseq cfDNA All-in-One Kit:

- Dedicated protocol combining extraction and library preparation from any plasma sample for any cfDNA NGS application
- Optimal conversion of cfDNA to NGS library thanks to highly efficient library preparation chemistries
- PCR-free NGS libraries from as little as 10 ng cfDNA
- Optional HiFi library amplification to minimize PCR bias, ensuring low error rates and accurate results
- Uniform coverage distribution and highly sensitive variant detection thanks to high-efficiency adapter ligation enzyme formulations

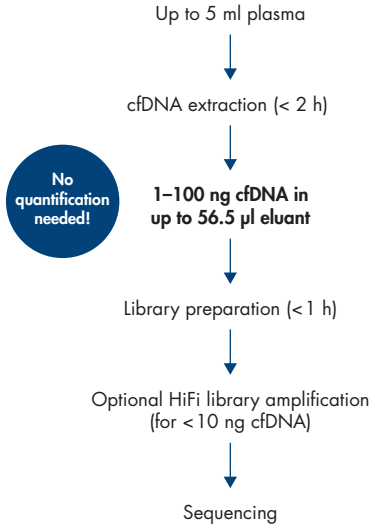

Figure 1. The QIAseq cfDNA All-in-One Kit workflow. Up to 5 ml plasma can be used for circulating cfDNA extraction using QIAamp Mini Columns. 24 samples are processed in less than 2 h. For library preparation, 1–100 ng of cfDNA can be directly used from up to 56.5 µl eluant volume. Quantifcation of cfDNA prior to library prep is not necessary. The library prep includes end-polishing and adapter ligation and takes < 1 h. Optional HiFi amplification can be performed for < 10 ng cfDNA before samples undergo sequencing.

A fully optimized solution for any cfDNA NGS application

Optimizing individual cfDNA extraction and library preparation kits from different vendors is a costly, time-consuming process that can lead to inefficient workflows that do not yield maximum conversion of cfDNA.

By combining highly efficient cfDNA extraction and library preparation chemistries in one dedicated kit, QIAseq cfDNA All-in-One technology ensures optimal sample conversion at every step, with no further optimization needed. The protocol takes less than 3 hours from cfDNA-containing plasma to NGS libraries ready for use on Illumina and Ion Torrent instruments (Figure 1). The Ion Torrent-compatible library preparation combines end-polishing and adapter ligation in a new all-in-one enzyme mix, reducing the time needed for library preparation to only 35 minutes.

Extraction of circulating cfDNA from up to 5 ml plasma is facilitated with QIAamp® Mini Spin Column technology. Parts of the extraction protocol can be automated on the QIAcube® or cfDNA can be extracted using a QIAsymphony® SP or any other automated platform with equivalent results (Figure 2). Since quantification is unnecessary due to the protocol supporting the widest range of cfDNA input (1–100 ng), the workflow goes directly from eluant to library preparation. The QIAseq cfDNA All-in-One Kit contains single-use barcoded sequencing adapters pre-dispended to a convenient 96-well plate, enabling multiplex sequencing of up to 24 or 96 samples per run. This significantly reduces the risk of adapter cross-contamination and makes the sample handling process much easier.

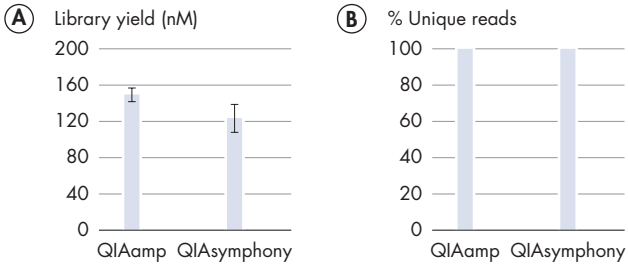

Figure 2. Equivalent results with manual and automated extraction. As part of the QIAseq cfDNA All-in-One workflow, circulating cfDNA was isolated using the manual QIAamp or automated QIAsymphony protocols. Then 1 ng isolated cfDNA underwent library prep (in 4 replicates) and was sequenced. **A** The QIAseq cfDNA library prep yields very comparable library concentrations for cfDNA isolated with the QIAamp and QIAsymphony protocols and shows very low sample-to-sample variation. **B** Sequencing quality metrics (represented here by the percentage of unique reads) are very good for libraries prepped from cfDNA isolated with the QIAamp and QIAsymphony protocols as part of the QIAseq cfDNA All-in-One workflow.

Optimal conversion of cfDNA to NGS library

To detect even the rarest variants in a sample with a low signal-to-noise ratio, maximum conversion of cfDNA molecules to an adapter-ligated NGS library is crucial. The ultra-efficient ligation enzymes and buffers in the QIAseq cfDNA All-in-One Kit ensure significantly superior conversion compared to results obtained with the next leading library preparation kit (Figure 3). Thus, even very dilute samples or low cfDNA input amounts can be reliably analyzed with NGS using the QIAseq cfDNA All-in-One Kit. Conversion rates for samples with varying cfDNA input amounts are also very reproducible confirming the robustness of the adapter ligation chemistry.

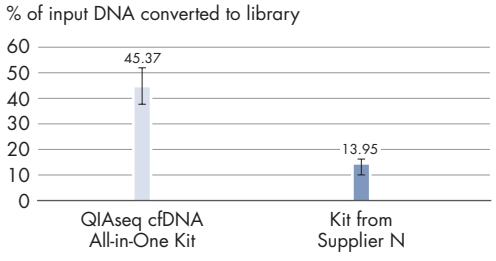

Figure 3. Superior conversion rate of cfDNA molecules to NGS library. 8 cfDNA samples (input 20–37 ng in a total volume of 43 µl) were used for library construction using either the QIAseq cfDNA All-in-One Kit or a kit from supplier N. The average calculated conversion rate of the replicate samples is displayed. The QIAseq cfDNA All-in-One Kit shows significantly higher conversion rates.

PCR-free library prep with the option of high-fidelity PCR amplification

PCR amplification is a potential source of bias and errors in NGS workflows. Eliminating the need for library amplification or minimizing the error rates introduced during library amplification is essential to maintain high confidence in variant detection. This is especially relevant for cfDNA samples because variants may be present in only low allelic fractions. The QIAseq cfDNA library prep chemistry consistently generates the 2 nM library concentration needed for sequencing on an Illumina MiSeq or NextSeq 500 sequencer from as little as 10 ng of cfDNA input with no need for PCR (Figure 4A).

Furthermore, the QIAseq cfDNA All-in-One Kit contains high-fidelity library amplification reagents to give researchers the option to perform PCR enrichment if the library yield needs to be increased. However, no significant differences in genomic coverage are detected across the genome when the workflow is run with or without PCR amplification (Figure 4B).

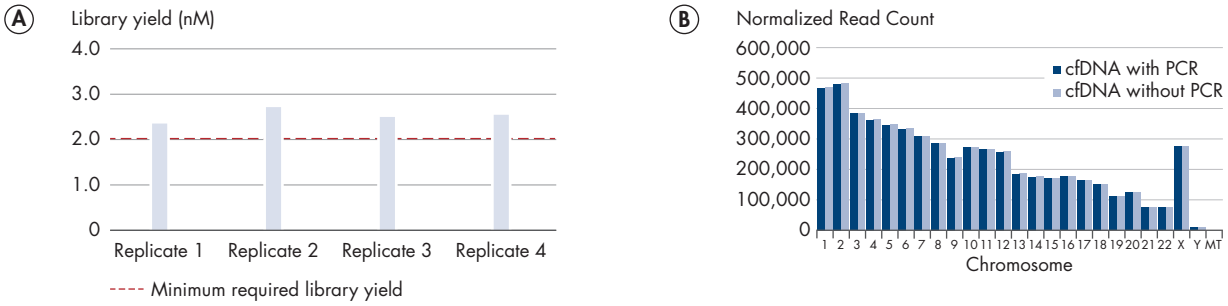

Figure 4. Low error rates and high accuracy in workflows with and without PCR. A 4 independant replicates of the same circulating cfDNA sample with an input amount of 9.35 ng underwent PCR-free library preparation with the QIAseq cfDNA reagents. Even with inputs of less than 10 ng, the QIAseq cfDNA library prep reagents achieved consistent library yields higher than 2 nM. **B** A comparison of normalized coverage distribution for libraries generated from cfDNA samples using the QIAseq cfDNA All-in-One Kit shows no significant differences in coverage for the workflow with or without PCR.

High coverage uniformity and mutation detection sensitivity

The uniformity of read coverage is a key indicator for the quality of an NGS workflow. High coverage uniformity means an even representation of the sample and higher mutation detection sensitivity. The QIAseq cfDNA All-in-One Kit yields reproducibly uniform coverage for varying amounts of cfDNA input (Figure 5A). The highly efficient extraction and library preparation reagents maximize the yield per sequencing run so that the complexity of the cfDNA sample is optimally represented in the data. This enables very sensitive mutation detection down to 1% allelic fractions (Figure 5B). ▶

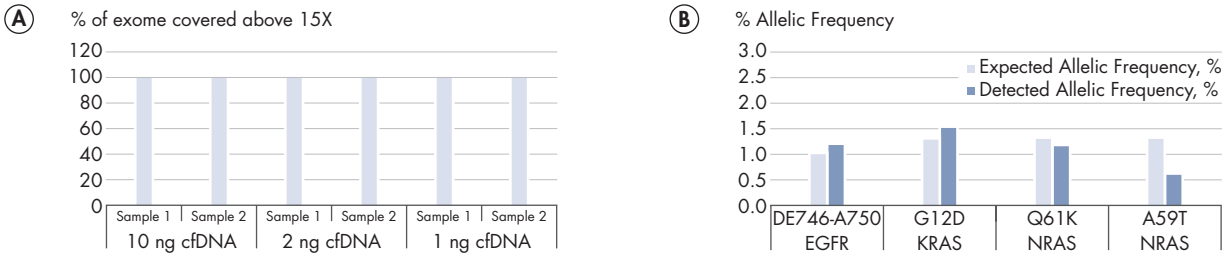

Figure 5. Reliable detection of very rare variants. A The QIAseq cfDNA All-in-One Kit gives highly uniform exome coverage distributions for cfDNA samples independent of the input amount. Libraries produced from duplicate 10 ng, 2 ng and 1 ng cfDNA samples were tested. **B** Reference samples of cfDNA with known mutations underwent the QIAseq cfDNA workflow. Sensitive detection of variants down to even 1% allelic frequency was found. The expected allelic frequencies from Horizon Discovery were used as the standard.

The QIAseq cfDNA All-in-One Kit is a dedicated solution for creating next-generation sequencing libraries for Illumina and Ion Torrent instruments from plasma samples containing circulating cell-free DNA (cfDNA). The highly efficient reagents for cfDNA extraction and library preparation ensure optimal sample conversion at every step and minimize the need for PCR library amplification, although the kit does contain reagents to support that option. The QIAseq cfDNA All-in-One Kit has been shown to maximize NGS library yields from liquid biopsy or NIPT samples, making it suitable for a broad range of cfDNA applications.

Ordering Information

Sequencer	Product	Contents	Cat. no.
Any Illumina sequencer	QIAseq cfDNA All-in-One Kit (24)	For 24 reactions on Illumina sequencers: QIAamp Mini Columns, tubes, reagents and buffers for cfDNA extraction for NGS. Enzymes and buffers for cfDNA library prep, Illumina Adapter Plate 24-plex, Illumina Library Amplification Primer and PCR Master Mix	180023
	QIAseq cfDNA All-in-One Kit (96)	For 96 reactions on Illumina sequencers: QIAamp Mini Columns, tubes, reagents and buffers for cfDNA extraction for NGS. Enzymes and buffers for cfDNA library prep, Illumina Adapter Plate 96-plex, Illumina Library Amplification Primer and PCR Master Mix	180025
	QIAseq cfDNA Library Kit (96)	Compatible with automated cfDNA extraction protocols, for 96 reactions on Illumina sequencers: enzymes and buffers for cfDNA library prep, Illumina Adapter Plate 96-plex, Illumina Library Amplification Primer and PCR Master Mix	180015
Any Ion Torrent sequencer	QIAseq cfDNA All-in-One T Kit (24)	For 24 reactions on Ion Torrent sequencers: QIAamp Mini Columns, tubes, reagents and buffers for cfDNA extraction for NGS. Enzymes and buffers for cfDNA library prep, Ion Torrent Adapter Plate 24-plex, Ion Torrent Library Amplification Primer and PCR Master Mix	180043

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