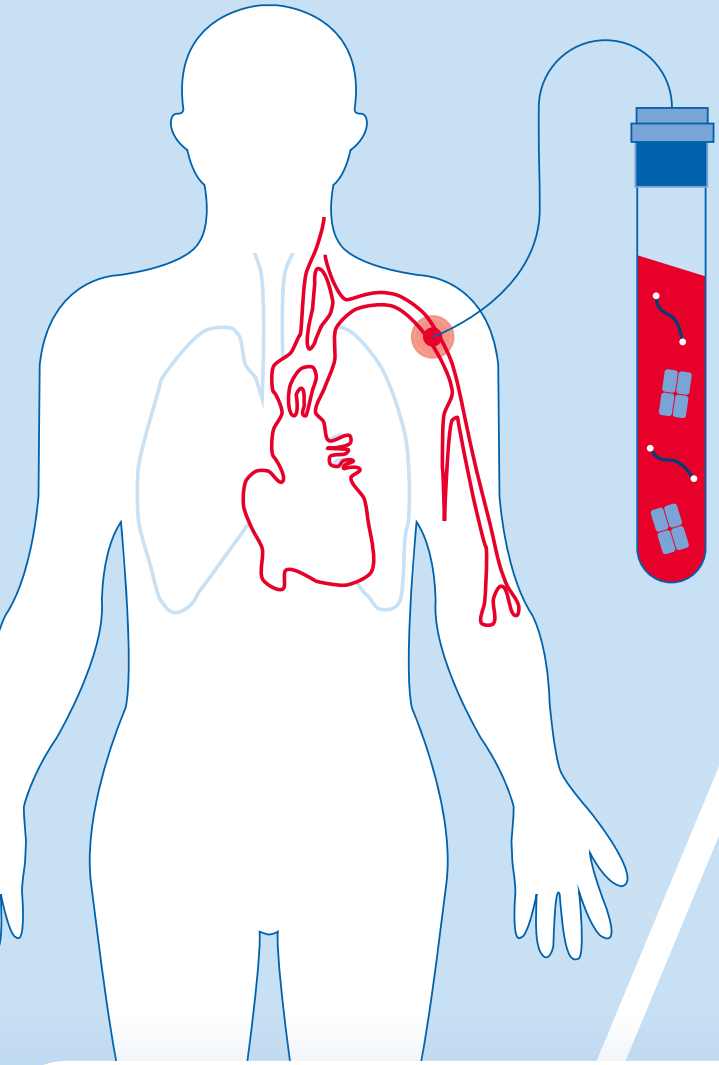


Preparation

NGS/dPCR

Interpretation

QIAGEN

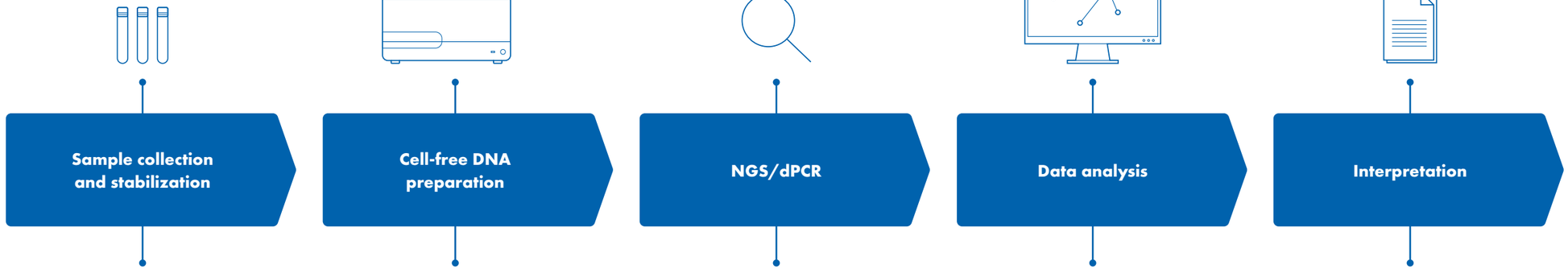
Liquid biopsy: your integrated cell-free DNA workflow solutions

Explore our integrated, complete cell-free DNA (cfDNA) workflow solutions for your needs:

- Analyze cfDNA from sample collection to receiving actionable insights
- Prepare cfDNA for varying application needs
- Analyze mutations or methylations for variant detection or screening applications
- Accelerate your research with next-generation sequencing (NGS) and digital PCR technologies
- Ensure quality control of your cfDNA samples or your generated NGS libraries

Sample to Insight® cell-free DNA analysis

Our comprehensive solutions for liquid biopsy start the process of molecular analysis with blood collection and stabilization – and cover quality control. This process includes dedicated cfDNA preparation to integrated data analysis solutions for various applications by NGS or digital PCR – and is scalable to your sample volume, throughput or outsourcing needs.



Collect the liquid biopsy sample in a single tube to stabilize, transport and store it for standardized preanalytical processing with our integrated solutions.

- PAXgene® Blood ccfDNA Tubes

Complete and optimize your portfolio with our manual and low-, medium- and high-throughput automation solutions for cfDNA preparation to achieve high sensitivity in downstream applications.

Manual cfDNA preparation

- QIAamp® MinElute® ccfDNA Kits
- QIAamp Circulating Nucleic Acid Kits

Automated cfDNA preparation

- EZ2® Connect
- QIAsymphony® SP
- QIAcube® Connect

Our NGS library preparation or digital PCR analysis analysis solutions offer you various applications: Mutation analysis (screening and low variant somatic or hereditary mutation analysis by targeted NGS or digital PCR), whole genome sequencing, CNV and methylation analysis.

NGS

- QIAseq® Targeted DNA Panels
- QIAseq Targeted Methyl Panels
- QIAseq Human Exome Kits
- QIAseq cfDNA Library Kits

Digital PCR

- QIAcuity® dPCR System
- dPCR LNA Mutation Assays
- dPCR Copy Number Assays

Complete your NGS and ´omics data analysis tools with flexible access. Our platform provides rapid, evidence-powered variant annotation for human exome, genome, and large cohort sequencing data.

- QIAGEN® CLC Genomics Workbench

Generate biological hypotheses. Identify and prioritize variants for your follow-up.

- COSMIC
- QCI® Interpret Translational for QIAseq
- QIAGEN Ingenuity Pathway Analysis

Analyze and perform fully automated quality control of your cfDNA samples or your generated NGS libraries.

- QIAxcel® Connect System

Genomic Services

- Extend your in-house resources quickly and conveniently
- Ensure your data is high quality using our expertise and tailored services
- Get support with just the time-consuming parts of your project or with your entire Sample to Insight journey with our exclusive spectrum of approved technologies
- Benefit from expert consultation and make the best decisions to achieve your goals

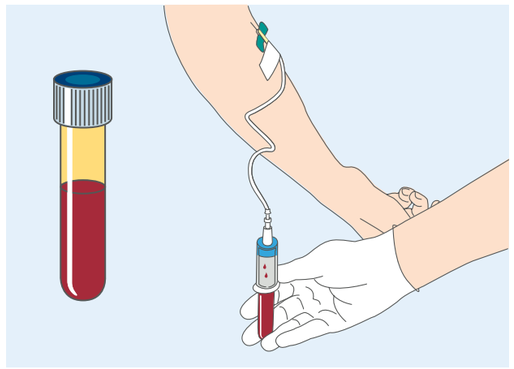
GeneGlobe® Design & Analysis Hub

- Quickly and easily explore targets in their scientific context
- Find and customize the right products to study those targets
- Analyze the data and plan your follow-up studies

Sample collection, stabilization and cfDNA isolation for your throughput

PAXgene Blood ccfDNA Tubes

- Minimize release of DNA into plasma and stabilize your sample during transport and storage (Figure 1)
- Benefit from a formaldehyde-free, non-crosslinking stabilization of the circulating, cfDNA in plasma and genomic DNA in the nuclear cellular fraction
- Choose the optimal duration for stabilization for your sample:
  - 2–25°C for up to 10 days
  - 2–30°C for up to 7 days
  - 2–37°C for up to 3 days



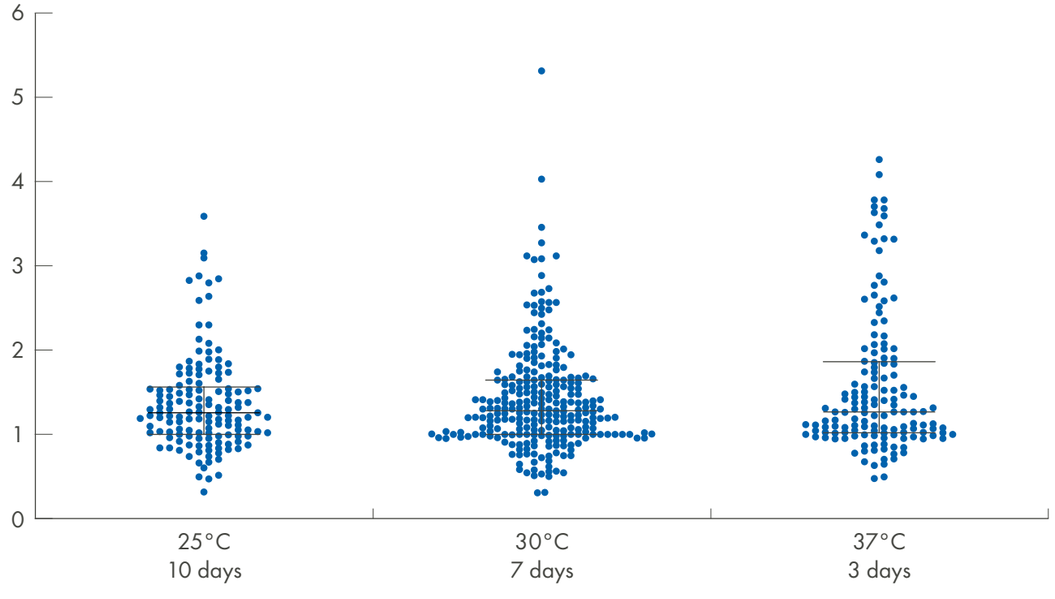
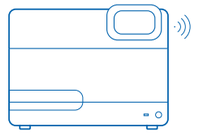


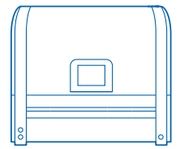
Figure 1. Minimization of DNA release into plasma from blood samples stored in the PAXgene Blood ccfDNA Tubes.
Change in cfDNA yield after blood storage in comparison to plasma separated within 2 hours of blood collection (Day 0). Blood was stored for 10 days at 25°C (n = 141 subjects), 7 days at 30°C (n = 261) or 3 days at 37°C (n = 144) followed by centrifugation and cfDNA purification using the automated QIAsymphony PAXgene Blood cfDNA Kit or the manual QIAamp DSP Circulating Nucleic Acid Kit. The relative cfDNA yield of 18S rDNA copy numbers is shown after sample storage compared to copy numbers at Day 0. Medians and the 25th and 75th percentiles are indicated (black lines).

Automated cell-free DNA preparation



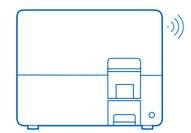
EZ2 Connect

- Purify cfDNA from 1–24 samples fully automated
- Boost reproducibility and convenience with prefilled reagent cartridges
- Achieve high sensitivity with fully automated large-volume processing of up to 8 ml
- Stay productive even outside the lab with QIAsphere® connectivity
- Minimize manual steps with onboard pipetting, heating and automated piercing of prefilled cartridges



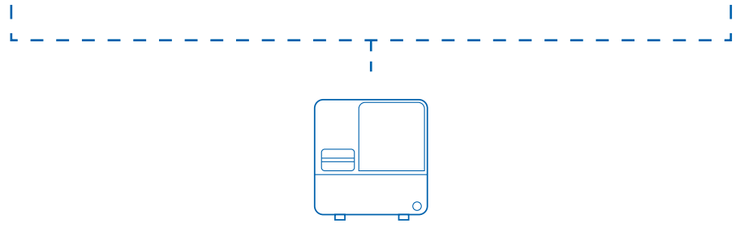
QIAsymphony SP

- Fully automate your cfDNA sample preparation of 1–96 samples
- Continuously load your samples, with bar code reading for sample tracking
- Load ready-to-run reagent cartridges prefilled with all reagents required for purification
- Use validated protocols for 2 ml and 4 ml, customized protocols for large volumes (up to 10 ml) for molecular biology applications
- Import your sample lists and export your sample sheets
- Directly process primary blood collection tube with a primary tube handling option



QIAcube Connect

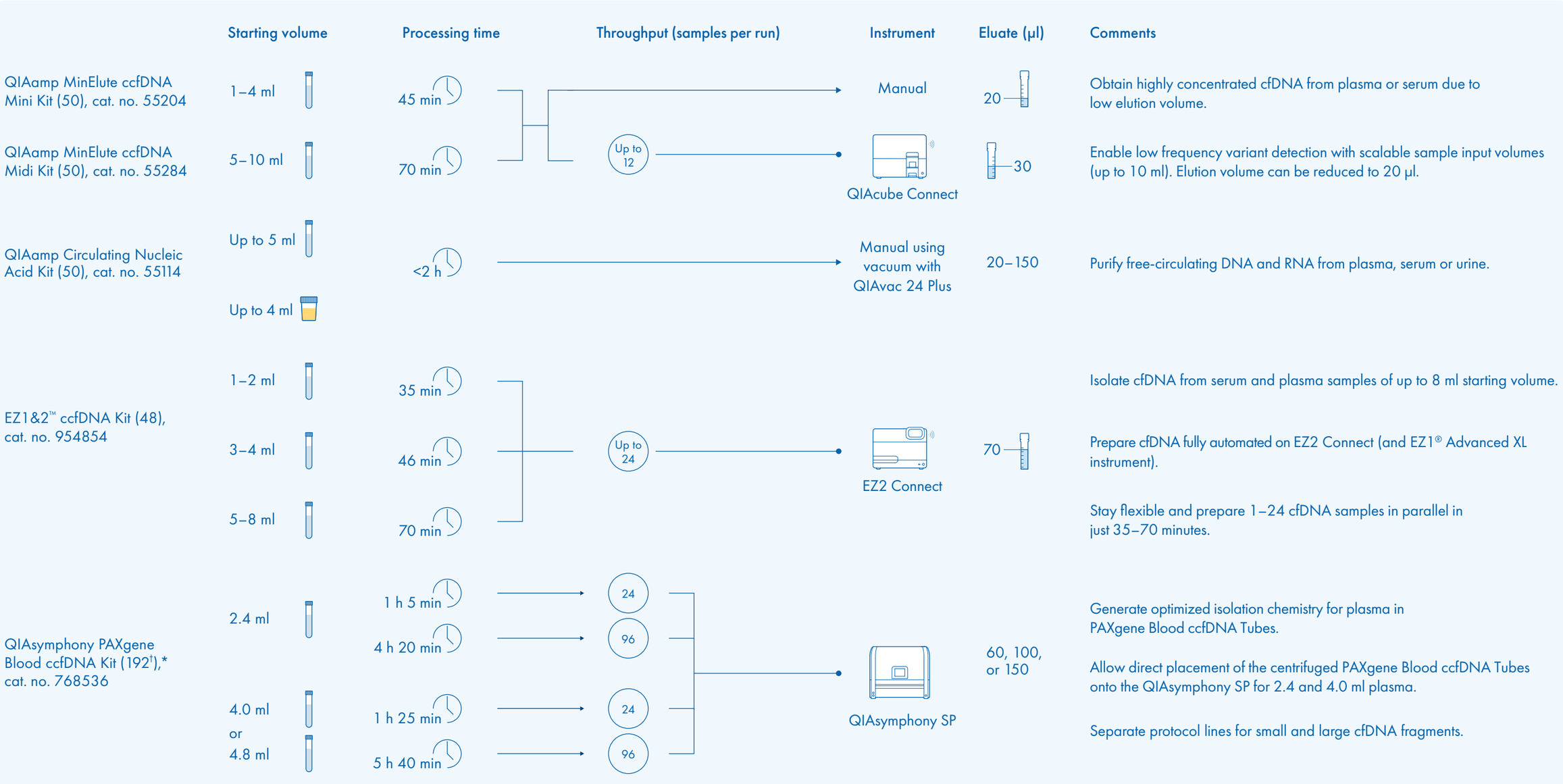
- Automate over 80 QIAGEN kits with over 140 standard protocols for DNA, RNA and protein sample processing
- Process up to 10 ml input volume
- Analyze high concentrations due to elution volumes down to 20 µl
- Increase your efficiency with QIAsphere and monitor your runs remotely by using the QIAsphere App



QIAxcel Connect System

- Fully automate quality control of up to 96 samples per run
- Perform quality control of your cfDNA samples and NGS libraries with a limit of detection of 5 pg/µl
- Collect high-resolution data by DNA capillary electrophoresis
- Receive your results in real time
- Eliminate tedious gel or consumable preparation by using ready-to-run gel cartridges
- Stay productive outside the lab with the option of the QIAsphere system

Our sample preparation solutions for your individual research applications



* Not available in all countries.

† Customized protocols for large volumes available (only for molecular biology applications).

Plasma/serum Urine

Our sample preparation solutions for your individual diagnostic applications

	Starting volume	Processing time	Throughput (samples per run)	Instrument	Eluate (µl)	Comments
QIAamp DSP Circulating NA Kit (50),* cat. no. 61504	Up to 5 ml	<2 h		Manual using vacuum with QIAvac 24 Plus	20–150	Purify cell-free DNA and RNA from plasma for in vitro diagnostic use.
QIAsymphony DSP Circulating DNA Kit (192†),* cat. no. 937556	2 ml or	70 min	24	QIAsymphony SP	60	Purify cfDNA from human plasma and urine using automated systems.
		4 h 40 min	96			
	4 ml or	90 min	24		60	Benefit from our workflow compatibility with a wide range of cfDNA stabilizing and non-stabilizing blood collection tubes (cat. no. 76815).
		6 h	96			
QIAsymphony PAXgene Blood ccfDNA Kit (CE-IVD)(192†),* cat. no. 768566	2.4 ml	1 h 5 min	24		60	Allow direct placement of the centrifuged PAXgene Blood ccfDNA Tubes onto the QIAsymphony SP by primary tube handling protocols for 2.4 and 4.0 ml plasma.
		4 h 20 min	96			
	4.0 ml or 4.8 ml	1 h 25 min	24		60	
		5 h 40 min	96			

Plasma/serum Urine

* Not available in all countries.

† Customized protocols available on demand for higher starting volumes and for lower and higher elution volumes (only for molecular biology applications).

‡ Intended for automated isolation and purification of cfDNA from plasma generated from human venous whole blood collected in the PAXgene Blood ccfDNA Tube.

cfDNA analysis by digital PCR

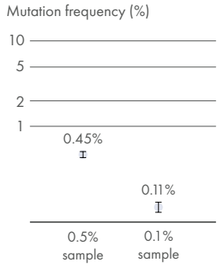


Figure 2. Highly sensitive detection at 0.1% mutation frequency.
To test the sensitivity of the dPCR LNA Mutation Assay for PIK3CA p.C420R, an artificial sample was used. The two samples were set up in a (mutant/WT) ratio of 0.5% and 0.1% mutant fragment and run using QIAcuity Nanoplate 26k 24-well.

Variant detection with highest sensitivity

- Detect mutated and wild-type sequences with the duplex assay (Figure 2 and 3)
- Choose between FAM and HEX or Atto 550 and ROX fluorescent dye combinations
- Increase your assay specificity and sensitivity by using LNA-enhanced primers and probes
- Benefit from our assays that are digital PCR wet-lab tested down to 0.1% VAF

dPCR LNA Mutation Assays, cat. no. 250200

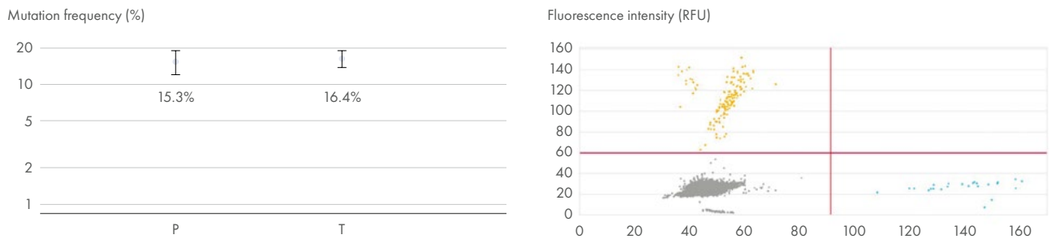


Figure 3. Precise mutation analysis from plasma samples.
PIK3CA p.C420R mutation detection using dPCR LNA Mutation Assays on QIAcuity. For the dPCR run on the QIAcuity, 2 µl DNA sample extracted from breast cancer patient samples (FPPE tissue as well as plasma sample) were loaded into two single wells of Nanoplate 26K 24-well. Genomic DNA was extracted from two FFPE sections using the QIAamp DSP DNA FFPE Tissue Kit (elution volume 20 µl) and ctDNA from 2 ml plasma using the QIAamp DSP Circulating Nucleic Acid Kit. **A**: Mutation frequencies showing strong concordance between plasma (P) and FFPE tissue (T) samples. **B**: 2D scatter plot of a single well from the Nanoplate 26k of one plasma sample. The plot shows clear separation of positives and negatives. Gray cluster: negative partitions; blue cluster: positive partitions for the mutated DNA only, yellow cluster: positive partitions for wild-type DNA only.

Copy number change analysis for an individual gene or region of interest

- Use our predesigned assays for all genes in the human genome
- Choose between 3 design locations per gene: 5', middle and 3'
- Analyze your data even more conveniently using the QIAcuity Software Suite

dPCR Copy Number Assays, cat. no. 250205

Nanoplate-based digital PCR

- Use microfluidic, nanoplate based, fully integrated digital PCR systems
- Choose from a scalable format (1-, 4- and 8-plate instruments) with advanced multiplexing capabilities (up to 5plex)
- Achieve comprehensive results in under two hours with workflow and hands on as in qPCR
- Use nanoplates with 26K or 8.5K partitions

QIAcuity dPCR systems, cat. no. varies

Impactful discoveries powered by NGS

DNA sequencing of your region of interest

- Choose between a wide range of cataloged, extended, booster or custom DNA panels
- Detect low frequency variants with unique molecular indices (UMIs) and single primer extension (SPE)
- Reduce index hopping and read misassignment using Unique Dual Indices

QIAseq Targeted DNA Pro Panels, cat. no. 333651

Targeted DNA methylation analysis by NGS

- Start targeting methylation status of DNA from as low as 10 ng cfDNA input using our liquid biopsy-compatible solutions
- Receive PCR error correction with UMIs
- Increase sensitivity with the SPE approach

QIAseq Targeted Methyl Panels, cat. no. 335501

Whole genome sequencing of cfDNA

- Perform whole genome sequencing specially optimized for your cfDNA analysis
- Go directly from eluant to library prep without quantification using 1–100 ng range of cfDNA input
- Minimize PCR bias with high-fidelity amplification reagents or generate PCR-free libraries from just 10 ng of cfDNA

QIAseq cfDNA Library Kits, cat. no. 180015

Exome library preparation

- Enjoy a single-day, automation-compatible sample to sequencing workflow
- Get exceptional coverage uniformity with variable hybridization times (even from FFPE and cfDNA samples) (Figure 4)

QIAseq Human Exome Kits, cat. no. 333937

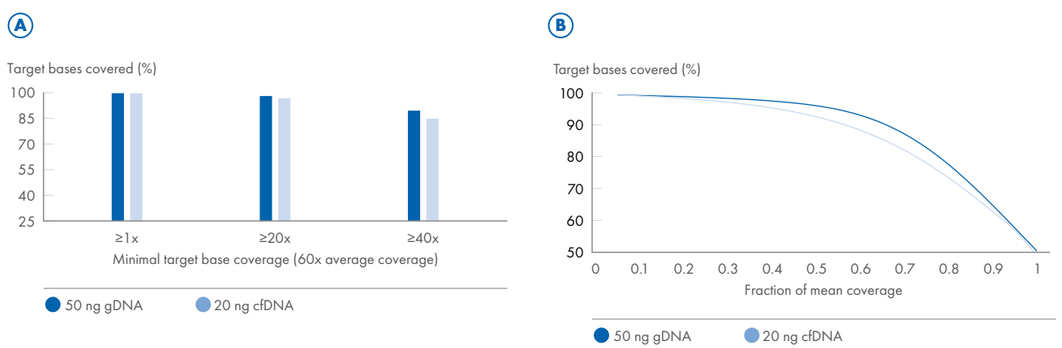


Figure 4. Analysis of whole exome libraries from cfDNA samples.
Plasma-derived cfDNA was isolated using the QIAamp MinElute ccfDNA Mini Kit. After whole genome library preparation from 20 ng cfDNA using the QIAseq cfDNA Library Kit, the QIAseq Human Exome Kit was used for whole exome enrichment of the samples. **A**: Minimal base coverage. **B**: Uniformity plot. Despite the reduced input amount and the challenging nature of cfDNA, the resulting data were of adequate quality and comparable to data obtained from gDNA.

Get expert curated genomic and clinical knowledge, together with bioinformatics software and services, for actionable insights

QIAGEN CLC Genomics

Complete NGS and 'omics data analysis tools with flexible access, from a laptop to a local server to the cloud. Powerful analysis easy to learn for non-bioinformaticians.

COSMIC

World's largest source of expert manually curated somatic mutation information relating to human cancers the database attempts to collate all known (published) genetic mutations responsible for human cancers.

QIAGEN Ingenuity Pathway Analysis (IPA®)

Quickly visualize and understand complex 'omics data and perform insightful data analysis and interpretation by placing your experimental results within the context of biological systems.

QCI Interpret Translational

Rapid, evidence-powered NGS variant annotation, filtering and triage.

Learn how to integrate your cfDNA workflows: **qiagen.com/cfDNA-workflow**

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