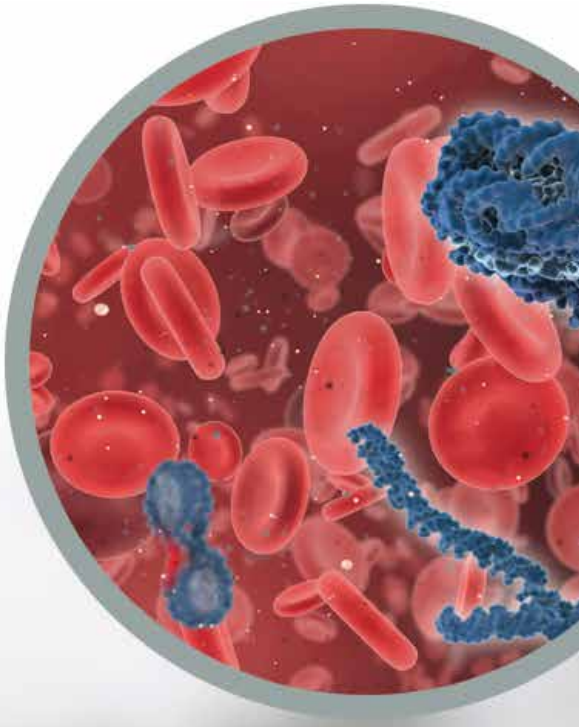
QIAGEN

# Liquid Biopsy

## Accelerate Your Circulating Biomarker Research




Sample to Insight

# Cross the bridge from discovery to insight

Liquid biopsy is a new non-invasive approach to help you detect disease biomolecules in blood, urine and other body fluids. It is a useful method when:

- Your tissue sample is limited
- Your sample contains insufficient tumor tissue
- Tumors are hard to reach
- Regular monitoring is needed

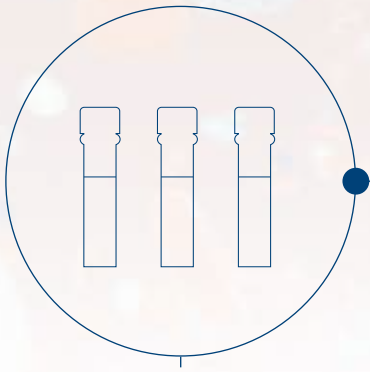
Liquid biopsy begins with sample processing and demands high efficiency for successful downstream detection. To unlock the molecular biomarkers in body fluids, QIAGEN® has sample processing solutions for all major liquid biopsy approaches – including automation.

A workflow with dedicated NGS library prep solutions ensures optimized conversion rates for either whole genome or targeted DNA sequencing and allows the detection of low-frequency variants with high confidence.

QIAGEN's Bioinformatics Advanced Testing Solution combines state-of-the-art data analysis and interpretation. Optimized, streamlined end-to-end workflows enable high sensitivity in detection, filtering and interpretation of variants.

ccfDNA/RNA = circulating cell-free DNA or RNA.

## Blood collection and stabilization

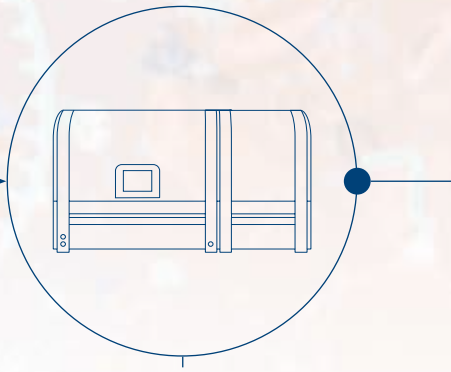


- Ensure long-term stability of markers
- Eliminate analysis errors caused by transportation and storage

### ccfDNA

- PAXgene® Blood ccfDNA Tubes

## Sample preparation



- Explore the biomarker universe with circulating nucleic acids, exosomes and CTCs
- Achieve highest yields and purity from precious samples

### ccfDNA

- QIAamp® MinElute® ccfDNA Kits
- EZ1® ccfDNA Kits
- QIAsymphony® PAXgene Blood ccfDNA Kit
- QIAcube®
- EZ1 Advanced XL
- QIAsymphony

### ccfRNA

- miRNeasy Serum/Plasma Advanced Kit
- exoRNeasy Serum/Plasma Kit

### ccfRNA & DNA (total nucleic acids)

- QIAamp ccfDNA/RNA Kit

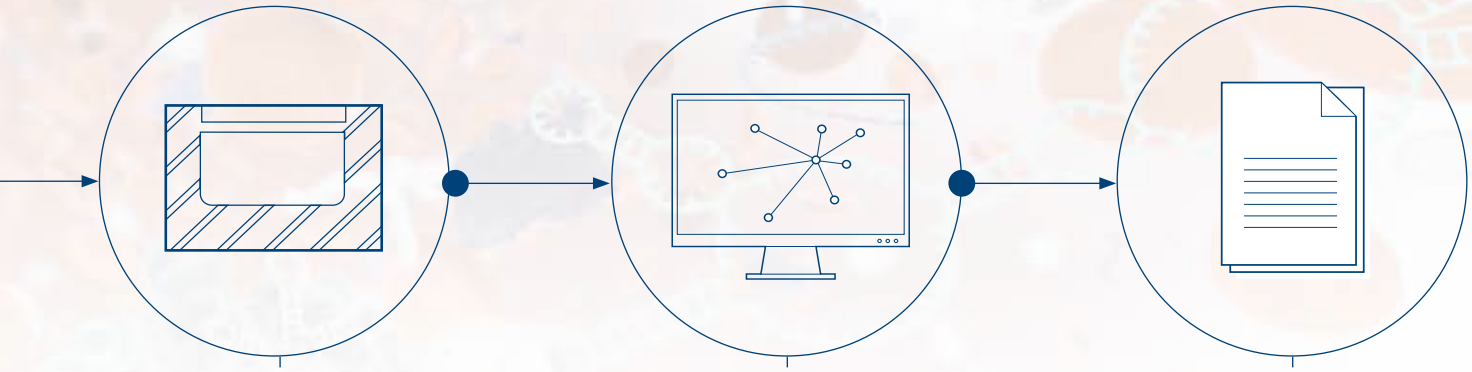
### Exosomes

- exoEasy Maxi Kit
- exoRNeasy Serum/Plasma Kit
- miRCURY Exosome Kits

### Circulating tumor cells

- AdnaTest CancerSelect

## NGS analysis



- Focus on relevant markers with targeted sequencing
- Discover the biomarker space with best-of-class platform-agnostic NGS solutions

### DNA sequencing

- QIAseq™ Targeted DNA Panels
- QIAseq cfDNA Library Kits

### miRNA sequencing

- QIAseq miRNA Library Kits

## Data analysis

- Identify variants and determine changes in expression with high accuracy
- Validate results with integrated visualization

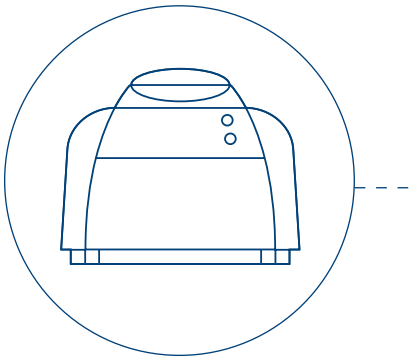
- Biomedical Genomics Workbench
- CLC Genomics Workbench

## Interpretation

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

- Ingenuity® Variant Analysis
- Ingenuity Pathway Analysis (IPA®)

## PCR-based analysis



- Identify disease-relevant gene expression signatures
- Achieve meaningful results with wet bench-validated assays

### RNA biomarker profiling

- miRCURY PCR Panels
- RT² Profiler Arrays
- RT² lncRNA Profiler Arrays
- QuantiNova® PCR Kits

### Circulating tumor cells

- AdnaTest CancerDetect

Collection and stabilization

# Ensure accurate preservation

## PAXgene Blood ccfDNA Tubes (RUO)

Avoid variation in the quality and integrity of circulating, cell-free DNA (ccfDNA) isolated from blood specimens. PAXgene Blood ccfDNA Tubes contain a reagent that prevents gDNA release to effectively stabilize ccfDNA levels in the plasma without impacting downstream assays. Pair seamlessly with the QIAamp MinElute ccfDNA and EZ1 ccfDNA kits, or benefit from optimized system performance and primary tube handling options when using the QIAsymphony PAXgene Blood ccfDNA Kit.

### Novel noncrosslinking stabilization reagent compatible with a wide spectrum of downstream applications.

- Accurately detect and quantify ccfDNA from blood samples stored in tubes for up to 7 days at room temperature (15–25°C)
- Test for methylated circulating tumor DNA even in challenging assays

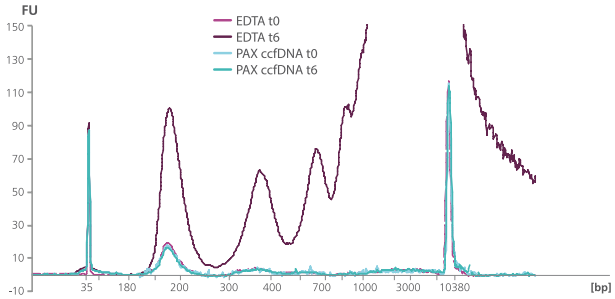



**Figure 1. PAXgene Blood ccfDNA stabilization helps prevent release of gDNA into plasma.** Whole blood was stored in EDTA tubes or PAXgene Blood ccfDNA Tubes. ccfDNA was purified from the plasma immediately following blood collection (t0) or after 6 days storage (t6) at room temperature. Eluate (1 µl) was analyzed using the Agilent High Sensitivity DNA Kit. Plasma from EDTA tubes showed an increase in apoptotic gDNA fragments, whereas plasma from PAXgene Blood ccfDNA Tubes showed a ccfDNA profile comparable to day 0.

### Unparalleled stabilization of red blood cells and minimized hemolysis during whole blood storage.

- Maximize plasma recovery
- Mitigate the risk of gDNA background
- Eliminate a second centrifugation step in plasma prep
- Allow possibilities for gDNA analysis from the buffy coat

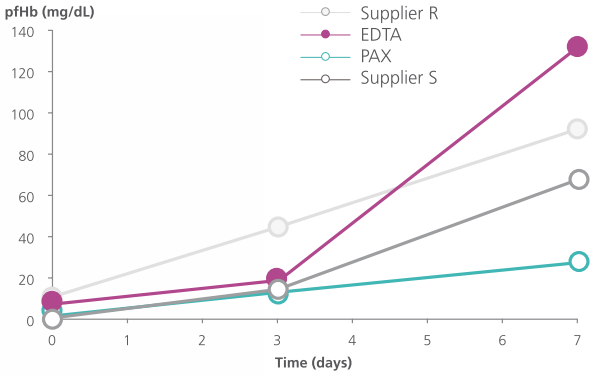



**Figure 2. PAXgene Blood ccfDNA stabilization minimizes hemolysis compared to alternative solutions.** Whole blood from 20 subjects was collected into PAXgene Blood ccfDNA Tubes, EDTA tubes and blood collection tubes designed for ccfDNA stabilization from two other suppliers. Plasma was separated directly after blood draw, after 3 days and after 7 days storage at room temperature (15–25°C). Hemolysis was assessed. Increase in sample hemolysis during sample storage at room temperature was minimized in PAXgene Blood ccfDNA Tubes compared to the other blood collection tubes.

# Get the most from your precious sample

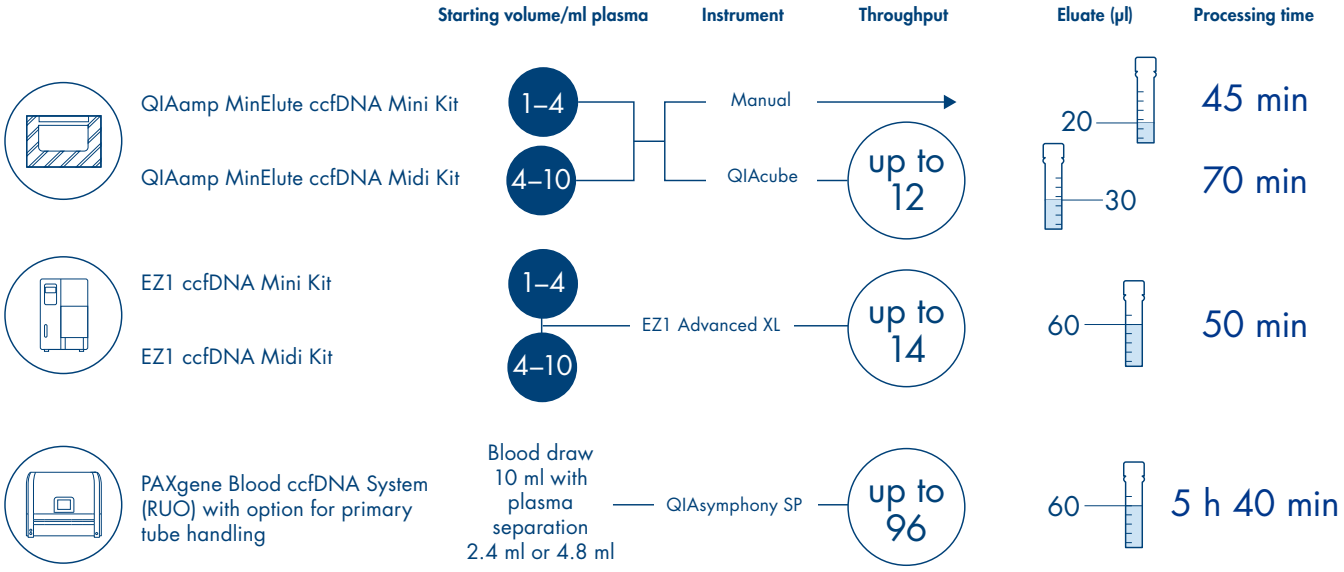
Manual and automated solutions

## ccfDNA preparation

Choose from our ccfDNA extraction portfolio with unmatched flexibility, for high yield, high concentration ccfDNA extraction: **QIAamp MinElute ccfDNA** and **EZ1 ccfDNA kits** with scalable input volumes and workflows that can be automated on QIAcube and EZ1 Advanced XL.

**QIAsymphony SP** automates your nucleic acid preparations and seamlessly integrates into your daily work. It standardizes your workflow and delivers reproducible, high-quality data. The QIAsymphony SP enables sample preparation of nucleic acids – including DNA, ccfDNA, RNA and bacterial or viral nucleic acids – from a wide range of starting materials, such as whole blood, buffy coat, serum, plasma, urine and tissue.

**ccfDNA purification for research applications**




**ccfDNA purification for diagnostic applications**

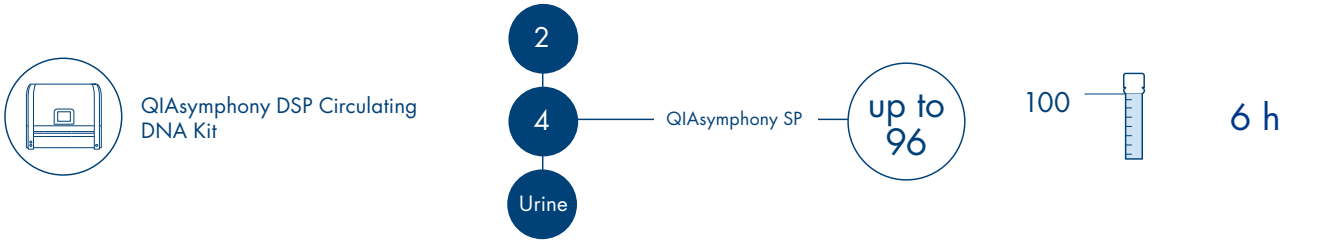



**Figure 3. ccfDNA purification selection guide.**

## Preparation of cell-free miRNA

The **miRNeasy Serum/Plasma Advanced Kit** efficiently purifies total RNA, including miRNA, from serum and plasma samples. The phenol-free protocol uses easy-to-automate MinElute spin column technology to allow for small elution volumes. Extracellular vesicles are lysed to allow analysis of RNA in and outside of vesicles.

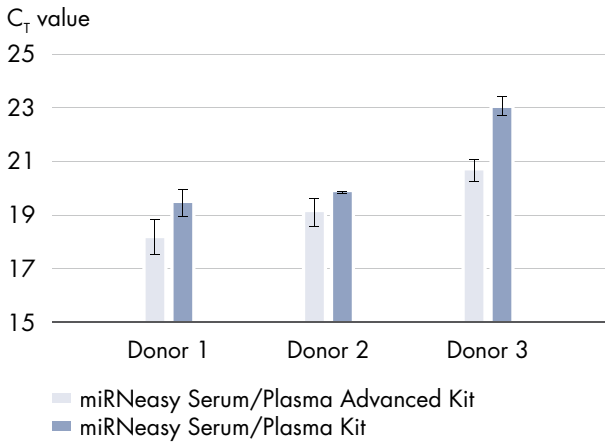



**Figure 4. High recovery of miRNA without the need for phenol.**

## You want them all?

The **QIAamp ccfDNA/RNA Kit** offers co-isolation of ccfDNA and ccfRNA from the same plasma sample, providing excellent yield and quality of both analytes in the same eluate. This allows you to analyze both DNA and RNA mutations in the same liquid biopsy sample or can boost detection sensitivity to discover low-frequency tumor mutations.

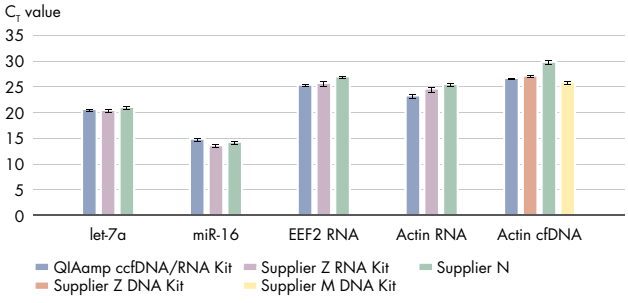



**Figure 5. Competitive performance without compromising on DNA or RNA yields.** Cell-free DNA/RNA was isolated in replicates from 1 ml EDTA plasma using the QIAamp ccfDNA/RNA Kit or kits from other suppliers.

## Exosome isolation kits

Explore the secrets hidden in exosomes. Enjoy spin column simplicity with ultracentrifugation quality – try the **exoRNeasy** and **exoEasy Kits**.

- Isolate extracellular vesicles in just 25 minutes
- Use high input volumes to get low abundance transcripts
- Separate vesiclar vs. non-vesicular miRNA

## The CTC AdnaTest

The **AdnaTest** is a highly specific immunomagnetic cell selection system that combines an optimized antibody mixture with highly sensitive RT-PCR technology.

- Detection and molecular characterization of CTCs
- Highest CTC specificity and sensitivity

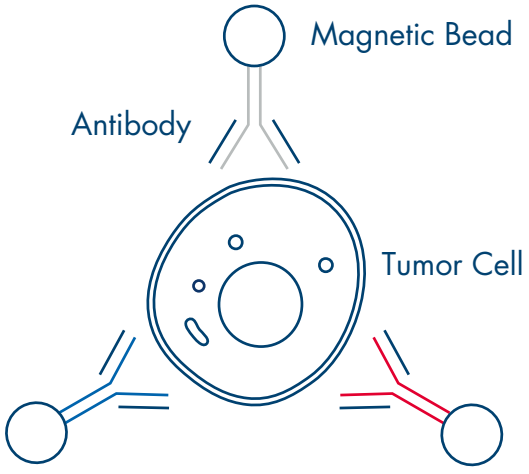



**Figure 6. Tumor cell captured by 3 different antibodies ensuring highly specific isolation and detection of CTCs.**

NGS analysis

# Explore biomarkers in any liquid biopsy sample with NGS

## QIAseq Targeted Panels

Through Digital NGS powered by unique molecular indices (UMIs), **QIAseq Targeted DNA panels** allow the detection of low-frequency variants with high confidence by overcoming the issues of PCR duplicates, false positives and library bias. Target design through single primer extension (SPE) removes conventional restrictions of two-primer amplicon designs – also reducing the amount of required primers.

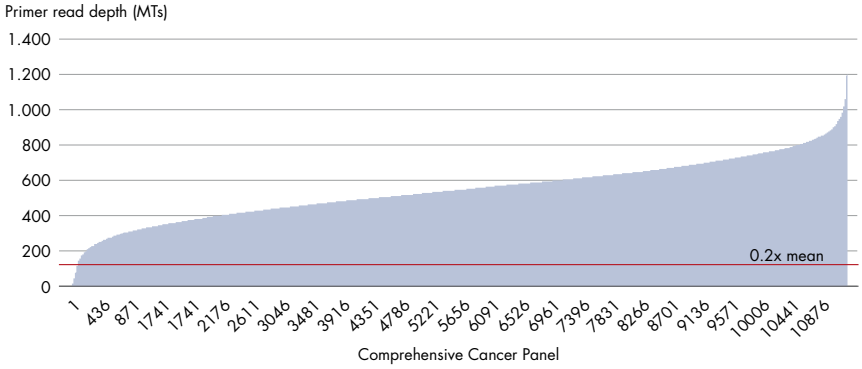



**Figure 7. Outstanding uniformity of target regions achieved by QIAseq Targeted DNA panels.** The panel achieved a uniformity of 99.5% at 0.2x of mean coverage.

## QIAseq library preparation

For new biomarker discoveries or to search for aneuploidies from any cell-free DNA sample, QIAGEN's **QIAseq cfDNA Library Kits** provide highly efficient library preparation to ensure maximum sample conversion and sensitive variant detection – for whole genome or exome sequencing applications.

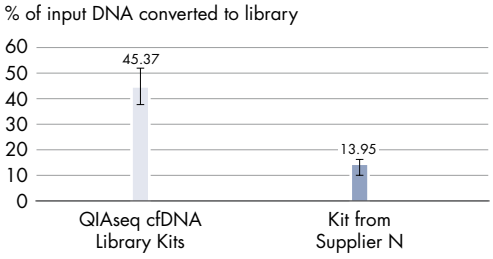



**Figure 8. Superior conversion rate of cfDNA to NGS library.** The average calculated conversion rate of the replicate samples is displayed. QIAseq cfDNA Library Kits shows significantly higher conversion rates.

## QIAseq miRNA library preparation

QIAseq miRNA sequencing kits deliver high-quality data by using unique molecular indices (UMIs) and a simple gel-free workflow. **The QIAseq miRNA Library Kit** achieves greater sequencing efficiency by eliminating adapter dimers and unwanted RNA species, resulting in the most efficient use of your sequencing instrument for miRNA, piRNA and small RNA discovery.

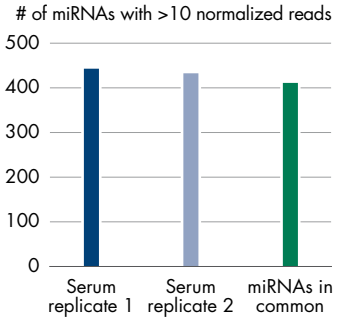



**Figure 9. The QIAseq miRNA Sequencing Kit has been designed to enhance yields from biofluids such as serum.** The graph shows robust detection of miRNA from serum samples.

PCR-based RNA biomarker profiling

# Discover disease-specific biomarker signatures

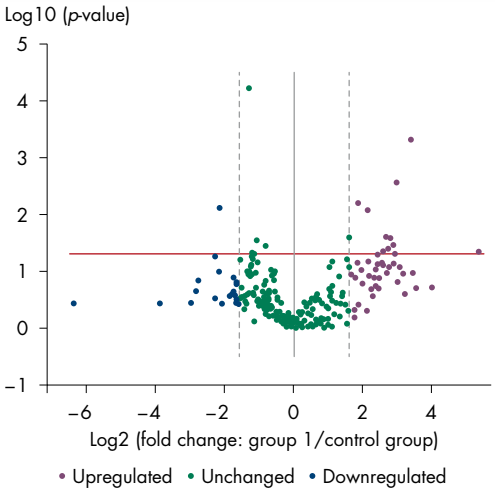



**Figure 10. Differential detection of miRNA expression using the miScript PCR System.** Volcano plot of miRNA expression changes in NSCLC patient serum samples, compared with healthy donor serum samples. Y: p-value; X: log 2 (fold change).

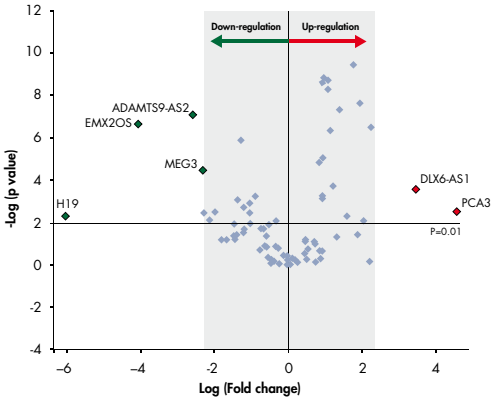



**Figure 11. Successful detection of lncRNAs in serum samples with preamplification.** Volcano plot of lncRNA gene expression changes in NSCLC serum samples compared with healthy donor serum samples. Y: p-value; X: Log2 (fold change).

## Simultaneously profile mRNA, miRNA and lncRNA

Total RNA discovery. The study of RNA has evolved from the simplicity of the central dogma of molecular biology. There are now multiple known noncoding RNA species that directly regulate gene expression. To truly understand gene expression, exploring regulatory RNA, such as miRNA and lncRNA, is key.

- miRCURY PCR Panels
- RT² PCR Arrays
- RT² lncRNA PCR Arrays

## Solutions for mRNA quantification

The QuantiNova family of qPCR kits combines top-notch performance with various in-process control features to ensure unbiased, reproducible results – even with minor changes in transcript levels.

Process and analysis errors are reduced with:

- Internal Control RNA
- Visual pipetting control
- gDNA removal
- Room temperature setup

While the **QuantiNova Multiplex PCR and RT-PCR Kits** provide the maximum information from a single PCR run, a complete kit portfolio for 1- or 2-step RT-PCR covers any qPCR need you may have for screening or confirmation – delivering instant success at the first attempt.

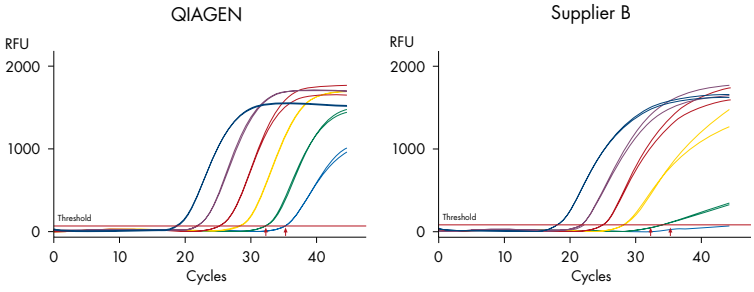



**Figure 12. Superior sensitivity of the QuantiNova Probe RT-PCR Kit.** The performance was compared to a probe RT PCR kit from supplier B using 10-fold dilutions of Hela total RNA from 100 ng to 10 pg. Sensitivity was clearly differing for 100 and 10 pg of total RNA (red arrows).

# Concentrate on insights, not data

Data analysis and interpretation

## Variant identification and interpretation of ccfDNA

There are many advantages to using cell-free DNA for identifying new variant biomarkers and for monitoring cancer patients after drug treatment. However, data analysis can be difficult, as it requires identifying low-frequency variants at levels of 1% and lower. In addition, data interpretation for detecting potential cancer driver variants comes with its own challenges, such as identifying variants having a functional impact.

QIAGEN Bioinformatics Advanced Testing Solution, featuring **Biomedical Genomics Workbench** and **Ingenuity Variant Analysis**, combines state-of the-art data analysis and data interpretation into a single offering. Optimized and streamlined end-to-end workflows enable high sensitivity in detection, filtering and interpretation of known and potential new cancer driver variants down to 1% (or lower) allelic fractions.

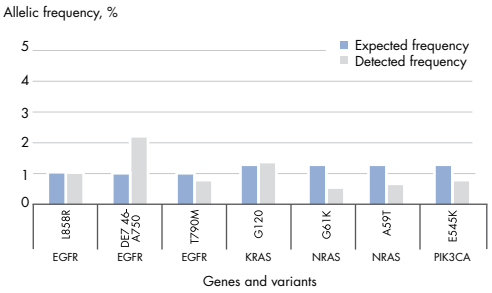



**Figure 13. Sensitive mutation detection from cfDNA.** Pathogenic variants at a ~1% allelic frequency were detected even at a moderate average read coverage of 500.

## RNA sequencing for exosomes

RNA and small RNA sequencing are powerful tools in gene and transcript expression profiling for determining underlying disease mechanisms and discovering disease-specific biomarker signatures. The RNA-Seq Explorer Solution enables you to start from FASTQ and align, analyze, QC, normalize and determine differential expression. Now you can identify the biology associated with differentially expressed genes and isoforms of varying expressions without being a bioinformatician.

## Understand complex 'omics data with IPA

**Ingenuity Pathway Analysis (IPA)** is a powerful analysis and search tool that uncovers the significance of 'omics data and identifies new targets or candidate biomarkers within the context of biological systems. IPA has broadly been adopted by the life science research community and is cited in thousands of articles for the analysis, integration and interpretation of data derived from 'omics experiments, such as RNA-seq, small RNA-seq, microarrays including miRNA and SNP, metabolomics, proteomics and small-scale experiments.

## Ordering Information

| Product | Contents | Cat. no. |
|---|---|---|
| **Blood collection and stabilization** | | |
| PAXgene Blood ccfDNA Tubes (100) | 100 blood collection tubes (10 ml) | 768115 |
| **Preparation** | | |
| QIAamp MinElute ccfDNA Mini or Midi Kit (50) | Purification of free-circulating DNA from 1–4 ml (Mini) or 4–10 ml (Midi) serum or plasma, automatable on QIAcube | 55204<br>55284 |
| EZ1 ccfDNA Mini or Midi Kit (48) | Automated purification of circulating cell-free DNA from 1–4 ml (Mini) or 4–10 ml (Midi) serum or plasma on the EZ1 Advanced XL System | 954134<br>954154 |
| QIAsymphony PAXgene Blood ccfDNA Kit (192) | Purification of ccfDNA from plasma using the QIAsymphony SP with optimized chemistry and input volumes for PAXgene Blood ccfDNA Tubes | 768536 |
| miRNeasy Serum/Plasma Advanced Kit (50) | Phenol-free isolation of total RNA including miRNA from serum and plasma samples | 217204 |
| QIAamp ccfDNA/RNA Kit (50) | Co-purification of cell-free DNA and RNA from plasma and serum | 55184 |
| exoEasy Maxi Kit (20) | Purification of exosomes and other extracellular vesicles (EVs) from plasma, serum and cell culture supernatant | 76064 |
| exoRNeasy Serum/Plasma Midi or Maxi Kit (50) | Purification of RNA from exosomes and other extracellular vesicles from up to 1 ml (Midi) or 4 ml (Maxi) serum or plasma | 77044<br>77064 |
| miRCURY Exosome Serum/Plasma Kit | Enrichment of exosomes and other extracellular vesicles from serum or plasma | 76603 |
| miRCURY Exosome Cell/Urine/CSF Kit | Enrichment of exosomes and other extracellular vesicles from biofluids such as cerebrospinal fluid, urine or cell culture media | 76743 |
| EZ1 Advanced XL, System | Robotic workstation for automated purification of nucleic acids from up to 14 samples using EZ1 Kits | 9001874 |
| QIAcube | Robotic workstation for automated purification of DNA, RNA, or proteins using QIAGEN spin-column kits | 9001293 |
| QIAsymphony SP | For fully integrated automated sample preparation of 1–96 samples | 9001297 |
| **NGS analysis** | | |
| QIAseq Targeted DNA Panels | For confident detection of low-frequency variants using unique molecular indices | Varies |
| QIAseq cfDNA Library T Kit | NGS library preparation from ccfDNA for whole genome or exome sequencing on Ion Torrent sequencers | 1102308 |
| QIAseq cfDNA Library Kit | NGS library preparation from ccfDNA for whole genome or exome sequencing on Illumina® sequencers | 180015 |

## Ordering Information

| Product | Contents | Cat. no. |
|---|---|---|
| **NGS analysis** | | |
| QIAseq miRNA Library Kits* | For gel-free miRNA sequencing library preparation | 331502 |
| **PCR-based analysis** | | |
| miRCURY PCR Panels | SYBR Green-based, real-time PCR profiling of miRNAs using the miScript® PCR System | Varies |
| RT² Profiler PCR Arrays | Pathway-focused gene expression analysis using laboratory-verified assays | Varies |
| RT² lncRNA Profiler Arrays | lncRNA expression analysis using laboratory-verified SYBR® Green qPCR assays | Varies |
| QuantiNova PCR Kits | Highly sensitive, specific, and ultrafast real-time PCR | Varies |
| **Data analysis** | | |
| Biomedical Genomics Workbench | For analyzing, comparing and visualizing human hereditary and cancer NGS data for biomarker discovery | 832070 |
| CLC Genomics Workbench | For analyzing, comparing, and visualizing NGS data | 832000 |
| **Biological interpretation** | | |
| Ingenuity Variant Analysis | For identifying disease-causing variants from human whole genome, exome and gene panel next-generation sequencing studies | Varies |
| Ingenuity Pathway Analysis (IPA) | For modeling, analyzing, and understanding complex ´omics data | Varies |

*Various options are available; please inquire.

| Product | Contents | Cat. no. |
|---|---|---|
| **ccfDNA preparation for diagnostic applications** | | |
| QIAsymphony DSP Circulating DNA Kit | For fully automated purification of human circulating cell-free DNA from human plasma and urine using the QIAsymphony SP instrument | 937556 |

**Visit qiagen.com today to find out more about Liquid Biopsy products!**

The QIAsymphony DSP Circulating DNA Kit is for in vitro diagnostic use. All other products listed in this brochure are intended for molecular biology applications. These products are not intended for the diagnosis, prevention or treatment of a disease.

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