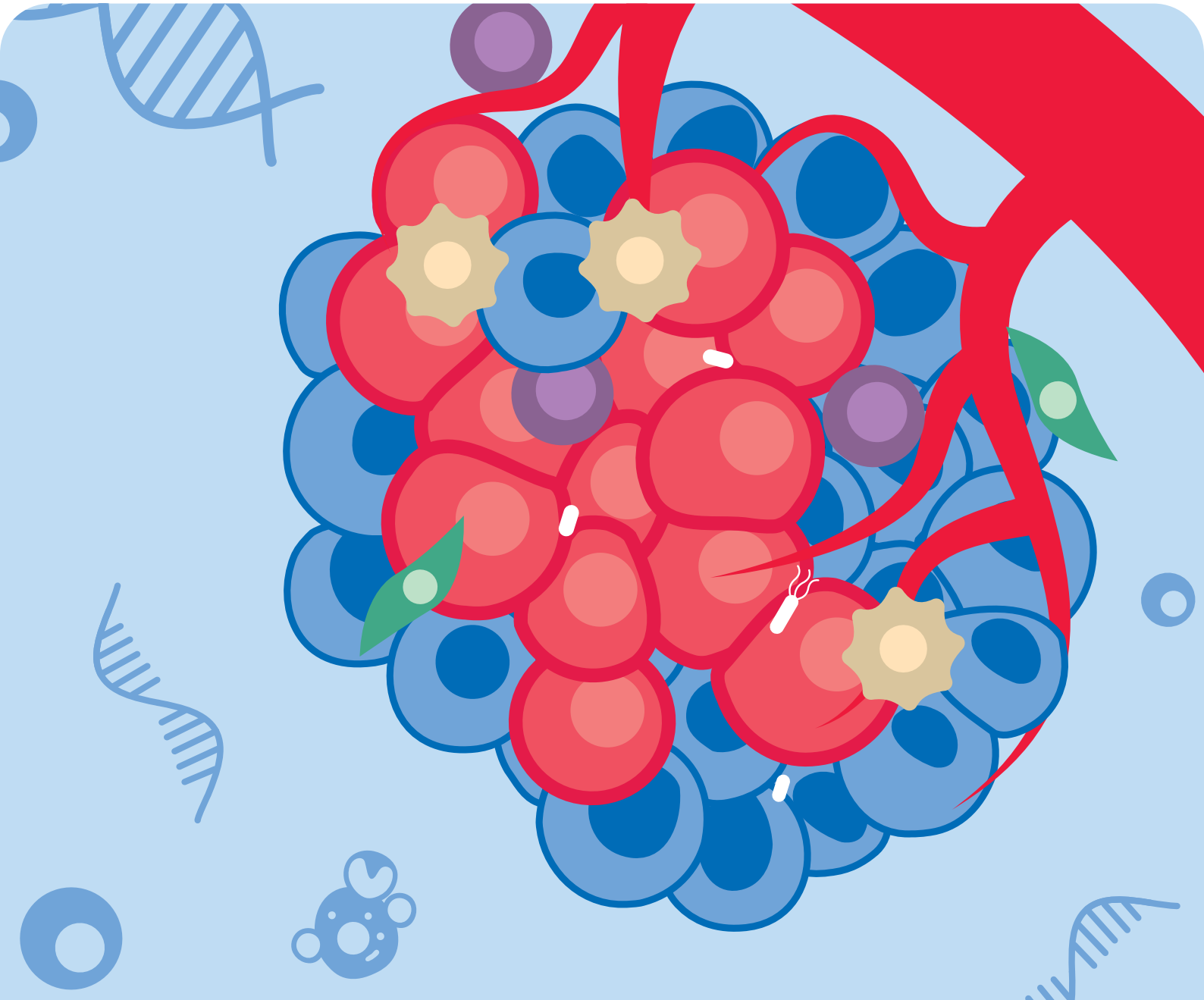
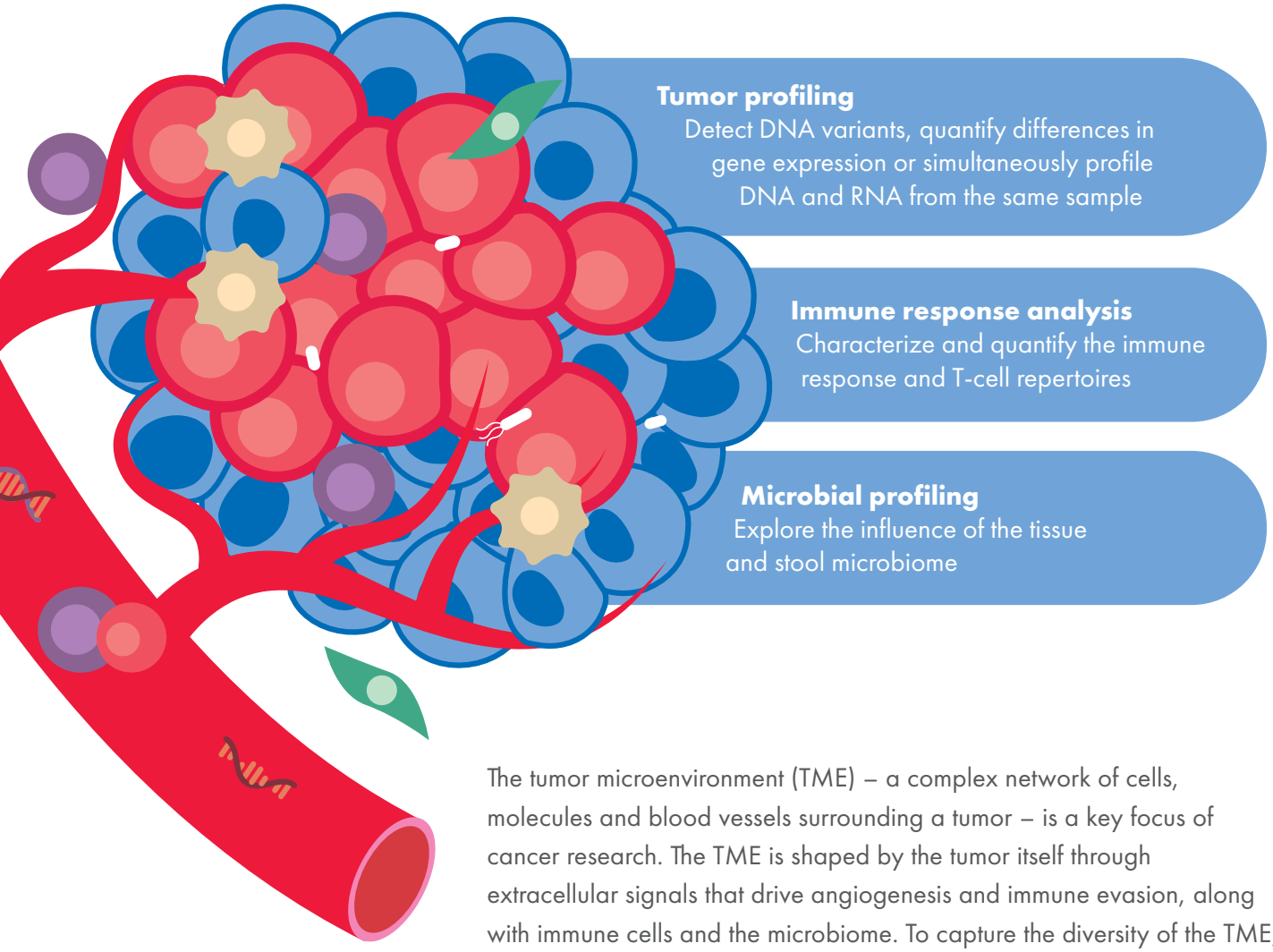
Hot, Cold and Everything in Between

Decode the Tumor Microenvironment With High-Resolution Molecular Tools



Profile tumor, immune and microbiome dynamics in the TME



Tumor profiling
Detect DNA variants, quantify differences in gene expression or simultaneously profile DNA and RNA from the same sample

Immune response analysis
Characterize and quantify the immune response and T-cell repertoires

Microbial profiling
Explore the influence of the tissue and stool microbiome

The tumor microenvironment (TME) – a complex network of cells, molecules and blood vessels surrounding a tumor – is a key focus of cancer research. The TME is shaped by the tumor itself through extracellular signals that drive angiogenesis and immune evasion, along with immune cells and the microbiome. To capture the diversity of the TME, which encompasses the immune-responsive 'hot' TME, the immunosuppressive 'cold' TME and everything in between, researchers must adopt molecular tools that enable high-resolution analysis.

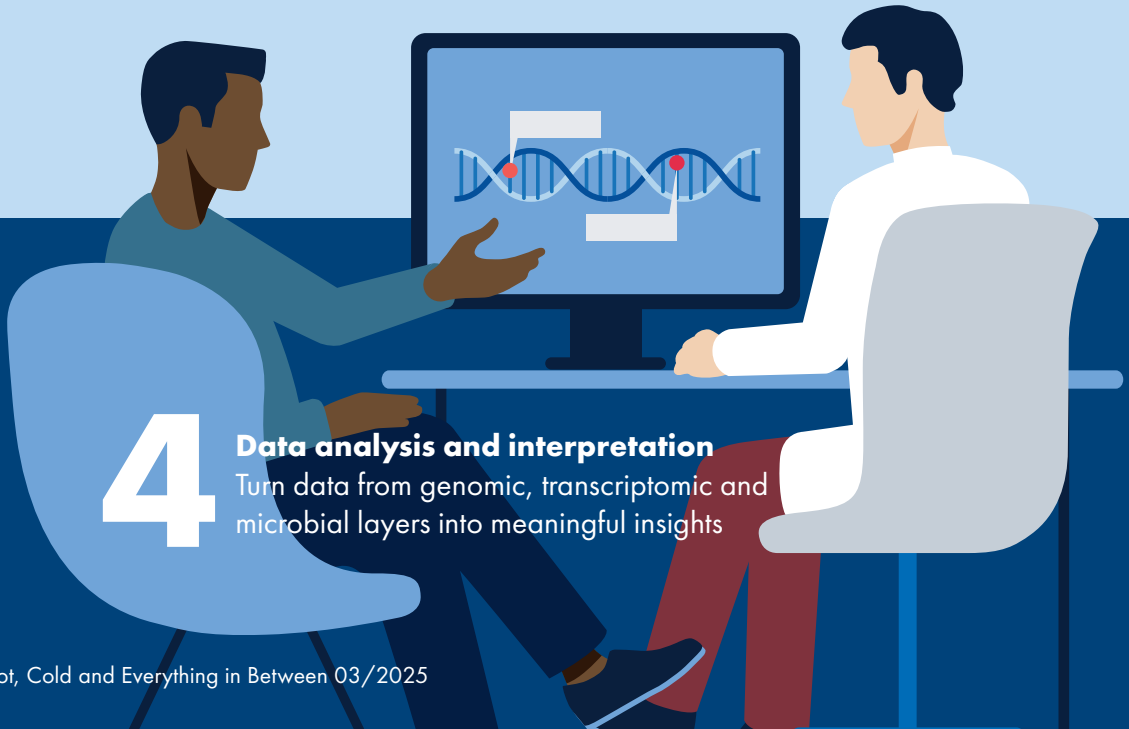
Explore QIAGEN® Sample to Insight® workflows – from reliable nucleic acid isolation to highly sensitive next-generation sequencing (NGS) and digital PCR (dPCR) detection and intuitive data analysis – to gain multiomic insights from the TME.

A comprehensive workflow to study the TME

1 **Nucleic acid isolation**
Extract nucleic acids from any sample type, including challenging materials

2 **Discovery with NGS**
Discover variants in DNA, RNA – or both – and explore the microbiome's role in cancer

3 **Focused analysis with dPCR and qPCR**
Quantify targets or confirm findings to ensure the reliability and accuracy of your conclusions



4 **Data analysis and interpretation**
Turn data from genomic, transcriptomic and microbial layers into meaningful insights

Nucleic acid isolation

High-quality nucleic acid isolation from any sample type

Deciphering the molecular details that shape the TME requires nucleic acids of the highest quality. Cancer samples in the form of fresh/frozen tissue, formalin-fixed, paraffin-embedded (FFPE) tissue sections or blood are often the starting point. However, retrieving usable nucleic acids (NA) from small sample quantities, FFPE sections and other challenging samples remains a key bottleneck. Explore how our comprehensive sample technologies allow you to efficiently extract DNA, RNA – or both – from challenging samples for your TME research.

Table 1. Nucleic acid isolation from cancer research samples

Key sample types	FFPE tissue	Fresh/frozen tissue & blood	Microbiome from tissue & stool
Common challenges	• Cross-linking • Cytosine deamination • NA fragmentation • Lengthy procedures	• Low sample input • Variable tissue matrix, cellular debris and protein contamination • RNA instability • Labor-intensive phenol-based methods for RNA isolation and loss of small RNAs	• Low bacterial biomass • High host DNA background • Degradation of bacterial cell envelopes • Inhomogeneous distribution of bacterial cells in tissue matrix
Solutions	• Remove paraffin without xylene or similar solvents • Use kits that integrate uracil-N-glycosylase (UNG) treatment • Use fully-automatable kits	• Use kits that remove contaminants and inhibitors efficiently • Use micro kits and MinElute® columns for small sample inputs • Isolate DNA & RNA from the same sample • Use phenol-free kits • Use kits that ensure isolation of RNA smaller than 200 nt • Use stabilization reagents	• Perform unbiased lysis • Use kits that remove host DNA effectively • Use kits that break down tissue matrix • Use kits that remove inhibitors in stool samples
Recommended products	• QIAamp® DNA FFPE Advanced UNG Kit • AllPrep® DNA/RNA FFPE Kit • EZ2® FFPE Kits	• QIAamp Fast DNA Tissue Kit • DNeasy® Blood & Tissue Kits (for animal samples) • RNeasy® and miRNeasy Advanced Kits • AllPrep DNA/RNA Kits • QIAamp Blood Mini Kit • DNeasy® Blood & Tissue Kits (for animal samples) • PAXgene® Blood Tubes and Kits	• QIAamp DNA Host-Free Microbiome Kit • QIAamp PowerFecal® Pro DNA Kit • RNeasy PowerFecal Pro Kit • AllPrep PowerFecal Pro DNA/RNA Kit
Automation friendly	Explore options for automation on QIAcube® Connect or EZ2 Connect: www.qiagen.com/dna-rna-purify		

Struggling with low-input samples?

QIAGEN MinElute spin-columns ensure high and consistent nucleic acids even from:

- Less than 10 mg tissue or 5 x 10^6 cells for DNA extraction using QIAamp DNA Micro Kit
- Less than 5 mg tissue or 5 x 10^5 cells for RNA and combined DNA/RNA workflows using RNeasy, miRNeasy Advanced and AllPrep DNA/RNA Micro Kits

An easy start for FFPE tissues with EZ2 Connect

FFPE samples are a treasure trove of information for TME researchers, offering genomic and transcriptomic insights. However, due to challenges such as cross-linking, cytosine deamination, nucleic acid fragmentation and labor-intensive nucleic acid isolation methods, they are notoriously tricky to work with.

The EZ2 Connect system simplifies the extraction of DNA, RNA – or both – from the same FFPE tissue sample while ensuring consistent, high-quality nucleic acid yields (see Figure 1). Every step, except deparaffinization, is automated on the EZ2 Connect system, thereby drastically reducing hands-on time and minimizing errors.

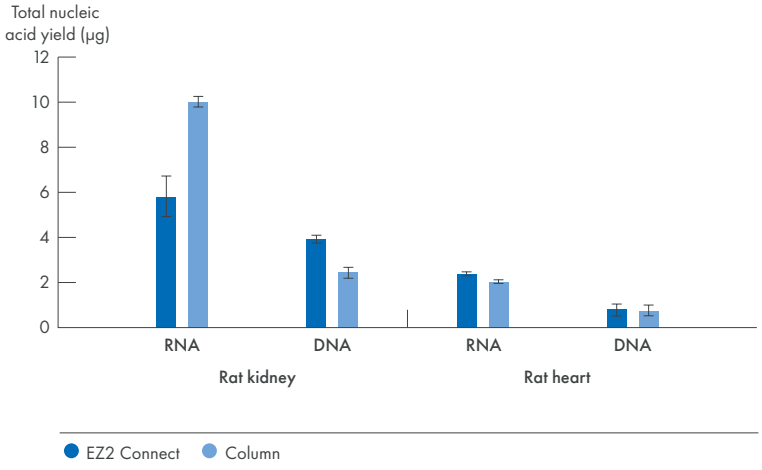


Figure 1. Higher yields of DNA and RNA with EZ2 Connect. DNA and RNA were extracted from FFPE rat kidney and FFPE rat heart tissues, either separately using the standard column procedure, or simultaneously with the EZ2 AllPrep DNA/RNA FFPE Kit on an EZ2 Connect instrument. RNA yields were measured via fluorometer. Except for RNA from rat kidney, EZ2 Connect delivered higher yields compared to the column procedure, demonstrating efficient extraction of RNA and DNA from the same cell population.

For tips & tricks on FFPE sample prep, download the tech guide: **www.qiagen.com/clp/ffpe-tech-guide.**

Assess FFPE sample quality using cartridge-based capillary gel electrophoresis

Explore QIAxcel Connect **www.qiagen.com/qiaxcel-connect**

Benefit from host DNA removal and unbiased lysis for tissue microbiome profiling

Tissue microbiome studies demand sample preparation solutions that can address challenges unique to this sample type, such as high host DNA background and uneven bacterial cell distribution. The QIAamp DNA Host-Free Microbiome Kit integrates a host DNA depletion step with a refined protocol for tissue dissociation,ensuring efficient access to microbial DNA and delivering reliable, high-quality data (see Figure 2).

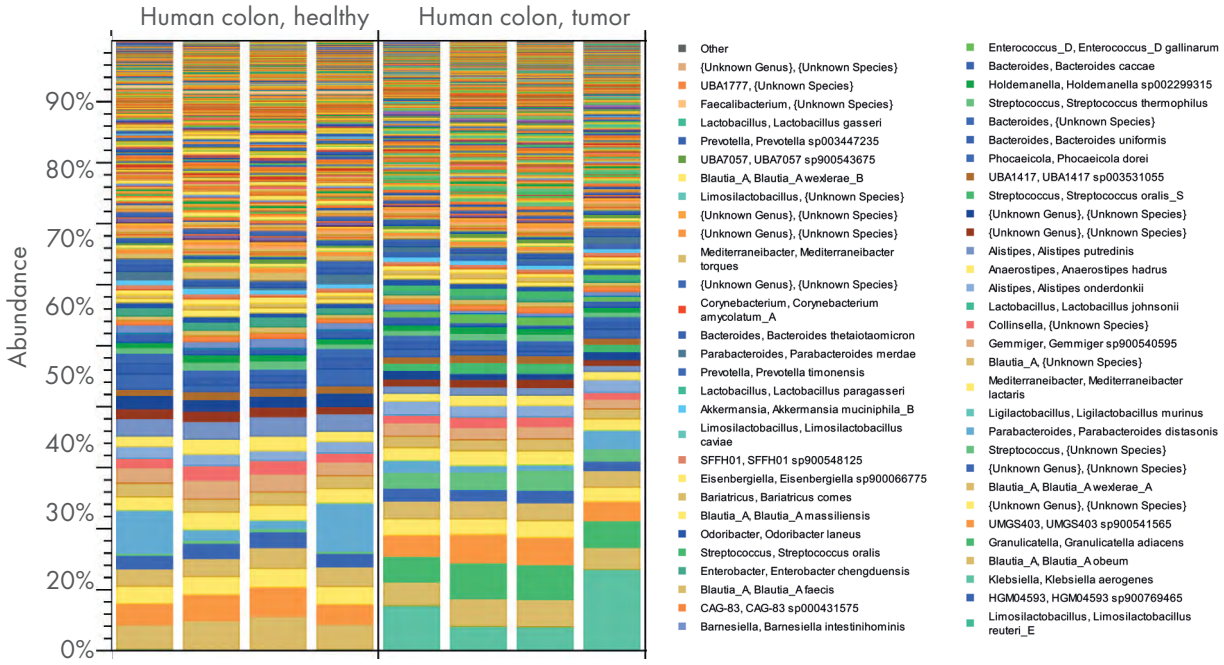


Figure 2. High taxa discrimination between largely similar samples: Colon tumor and healthy tissue. Microbial DNA was extracted from human colon tumors and adjacent healthy tissues using the QIAamp DNA Host-Free Microbiome Kit and analyzed via whole genome sequencing. Taxonomic profiles were assigned with the UHGG database using QIAGEN CLC Genomics Workbench and CLC Microbial Genomics Module. The workflow enabled recovery of high-abundance taxa (e.g., Lactobacillaceae) and diverse low-abundance Gram-positive and Gram-negative bacteria, allowing clear differentiation between colon adenocarcinoma and adjacent healthy tissue samples.

Explore microbiome-specific workflow solutions

Whether its sample collection, nucleic acid extraction, dPCR, NGS or digital data analysis, find the products designed specifically for your human microbiome studies.

Download interactive booklet: **www.qiagen.com/microbiome-solutions**

High-resolution NGS for tumor profiling and immune response analysis

NGS empowers cancer researchers with high-resolution insights into the TME by enabling precise DNA variant detection, gene expression profiling and multimodal DNA-RNA analysis. By providing a comprehensive, data-rich approach, NGS helps profile variants and analyze tumor-immune dynamics and the role of the microbiome on tumor progression. Discover how our QIAseq® NGS technology enables highly sensitive analysis of DNA and RNA to study the TME.

Tumor profiling with NGS

Find DNA variants with confidence

Uncovering the genetic drivers of cancer requires precise detection of even the smallest changes. Tumor samples, including FFPE tissues, fresh biopsies and complex cancer models, often pose challenges due to small sample sizes, degradation or high heterogeneity. Identifying low-frequency SNVs, Indels and CNVs can be particularly difficult. Benefit from fast, cost-efficient NGS with integrated bioinformatics to reach the extensive scope of genomic discovery that your research merits.

Table 2. Selection guide for QIAseq Kits

Application	Kit	Analysis
Whole genome and metagenome sequencing	QIAseq FX DNA Library Kit	QIAGEN CLC Genomics Workbench
Whole exome and targeted exome sequencing	QIAseq Human Exome Kits	
Targeted sequencing	QIAseq Targeted DNA Pro Panels*	
	QIAseq Targeted Methyl Panels*	
Single-cell or nucleus sequencing	QIAseq UPX Single Cell DNA Library Kits	

* QIAseq Targeted Methyl Panels can be analyzed with GeneGlobe® as well as the QIAGEN CLC Genomics Workbench.

Cancer research panels for targeted DNA sequencing

Benefit from unprecedented ease-of-use and biological insights with our wide range of catalog panels for your cancer research. Custom panel design is also available.

Learn more: **www.qiagen.com/qiaseq-targeted-pro**

Ultraplex sequencing of single cells and single nuclei

Single-cell and -nuclei analysis helps understand tumor heterogeneity at a deeper level but is often hindered by limited DNA input, uneven amplification and high costs. Single-cell and single-nuclei sequencing with the QIAseq UPX Single Cell DNA Library Kit ensures uniform amplification and reproducible results, while saving precious time and costs (see Figure 3).

- Optimized multiple displacement amplification (MDA) and library preparation
- High multiplexing (up to 576 samples) reduces library numbers through pooling
- Compatible with QIAseq xHyb Panels for targeted sequencing of regions of interest

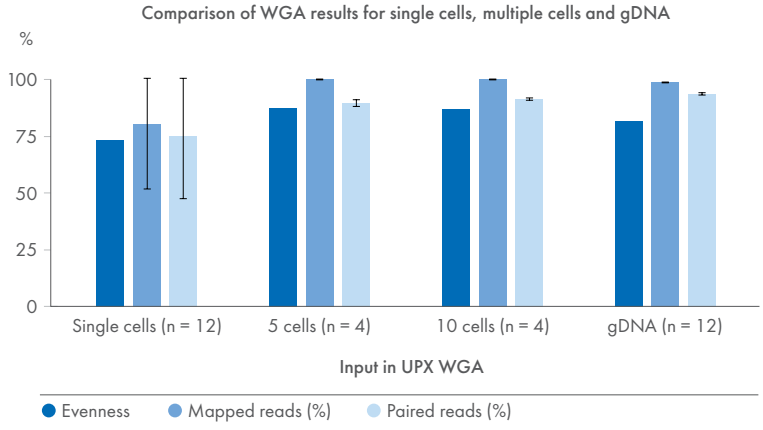


Figure 3. Comparison of whole genome amplification (WGA) results single cells, multiple cells and genomic DNA (gDNA) show high and similar barcode evenness, independent of the number of cells. MCF7 cells were detached from the culture plates and washed with PBS. Serial dilution was used to generate 1-, 5- and 10-cell dilutions. Then, 12 WGAs were performed using QIAseq UPX Single Cell DNA Library Kit from single-cell dilutions, 4 from each of the 5- or 10-cell dilutions, and 12 from 1 ng gDNA purified from the same cell type. The barcoded WGAs for each cell input were pooled and libraries were generated for each pool. Libraries were sequenced using a MiSeq® benchtop sequencer with ~1 million reads per library. Analysis was performed with QIAGEN CLC Genomics Workbench.

Unravel the secrets of the tumor transcriptome

Understanding the TME requires comprehensive insights into gene expression and the role of miRNA, piRNA and other small RNAs in regulating cell signaling. Obtaining reliable data from these unstable molecules demands robust RNA-seq workflows that address issues such as rRNA contamination and low RNA input. Our high-sensitive library kits offer unique advantages for RNA-seq analysis, such as integrated unique molecular indices (UMIs) for high-precision, ultraplex (UPX) library construction for low-input and single-cell analysis and streamlined workflows for speed and reproducibility.

Table 3. Selection guide for QIAseq Kits

Application	Kit	Analysis
rRNA and globin RNA removal	QIAseq FastSelect® rRNA and Globin mRNA Removal Kits	GeneGlobe or RNA-seq Analysis Portal on GeneGlobe
Whole transcriptome sequencing	QIAseq FastSelect RNA Library Kits	
miRNA sequencing	QIAseq miRNA Library Kit	RNA-seq Analysis Portal on Gene Globe
Single-cell or single-nuclei gene expression	QIAseq Single Cell RNA Library Kits UDI	QIAGEN CLC Genomics Workbench
Targeted gene expression	QIAseq Targeted RNA Panels	GeneGlobe
Fusion and exon-skipping detection	QIAseq RNA Fusion XP Panels	GeneGlobe and QIAGEN CLC Genomics Workbench
T-cell receptor sequencing	QIAseq Targeted RNA-seq Panel for T-cell Receptor	GeneGlobe

Overcome challenges in miRNA profiling

miRNA expression is often deregulated in cancer and miRNAs are known to be involved in remodeling the TME. Understanding the role of miRNAs in the TME is therefore crucial for fully deciphering the molecular mechanisms underlying tumor progression.

Our high-performance QIAseq miRNA Library Kit enables accurate identification of miRNA signatures and reliable differential expression analysis from varied and challenging sample types (see Figure 4).

- Gel-free miRNA sequencing library prep speeds up library construction
- Elimination of adapter dimers and hY4 RNA results in reproducible data from low-input serum, plasma and exosome samples
- Integrated UMIs enable quantification of individual miRNA molecules

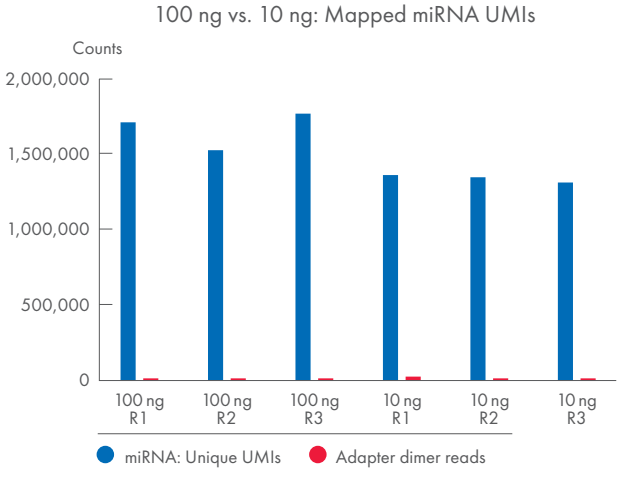


Figure 4. UMIs improve miRNA quantification. UMIs are attached early in the workflow, ensuring that bias from library amplification and sequencing is minimized. In addition, adapter dimers account for only a small percentage of reads in comparison to mapped reads, even with the gel-free, bead-based purification.

Perform bias-free RNA-seq with catalog panels

QIAseq Targeted RNA Panels enable accurate and bias-free gene expression profiling using RNA sequencing. With integrated UMIs and a streamlined PCR-based workflow, these panels ensure high specificity, sensitivity and reproducibility across human and mouse samples.

- Reliable quantification down to ~100 copies of an RNA target
- Sample input as low as 25 ng total RNA
- Custom panel design available via GeneGlobe

Table 4. Selection guide for QIAseq Targeted RNA Panels*

Cancer Transcriptome	Apoptosis & Cell Death	Inflammation & Immunity Transcriptome	Extracellular Matrix & Cell Adhesion Molecules
Signal Transduction PathwayFinder	Molecular Toxicology Transcriptome	Immuno-Oncology	Stem Cell & Differentiation Markers
Angiogenesis & Endothelial Cell Biology			

* Panels are available for human, mouse and rat and can also be customized with GeneGlobe. The QIAGEN Enterprise Genomic Solutions team also offers additional custom solutions.

Maximize gene expression reads by removing >95% rRNA

Degraded RNA samples, such as those resulting from the FFPE process, can impact RNA-seq success. The challenge is exacerbated by the presence of unwanted RNA species. The efficient removal of rRNA is therefore a critical step for RNA-seq optimization. Integrate QIAseq FastSelect technology into your RNA-seq workflow for reliable rRNA removal from a range of samples in as little as 14 minutes.

Seeking expert genomic services for your lab?

QIAGEN Genomic Services offers tailored solutions for TME studies, from tumor and immune repertoire profiling to dPCR analysis

Visit: **www.qiagen.com/applications/genomic-services**

Profile DNA and RNA simultaneously for multiomic studies

Due to TME complexity, analyzing both genomic and transcriptomic details from the same sample can help build a more accurate picture. However, separate DNA and RNA workflows can be time-consuming, resource-intensive and ultimately inefficient.

Multimodal sequencing with QIAseq Multimodal solutions allow you to profile both DNA and RNA simultaneously from the same total nucleic acid sample using a consolidated workflow (see Figure 5).

- Work with input as low as 10 ng DNA or 20 ng RNA
- Amplicon- and hybrid capture-based enrichment options
- Lower costs, faster turnaround and compatible with challenging samples

Table 5. Selection guide for QIAseq Kits

Application	Kit
Amplicon-based multimodal sequencing	QIAseq Multimodal Panels (Pan-Cancer, Sarcoma, Leukemia, Lung and Custom)*
Hybrid capture-based multimodal sequencing	QIAseq Multimodal DNA/RNA Library Kit QIAseq xHYB Human Panels

* Panels can be customized with GeneGlobe, our browser-based design and analysis hub. The QIAGEN Enterprise Genomic Solutions team also offers additional custom solutions.

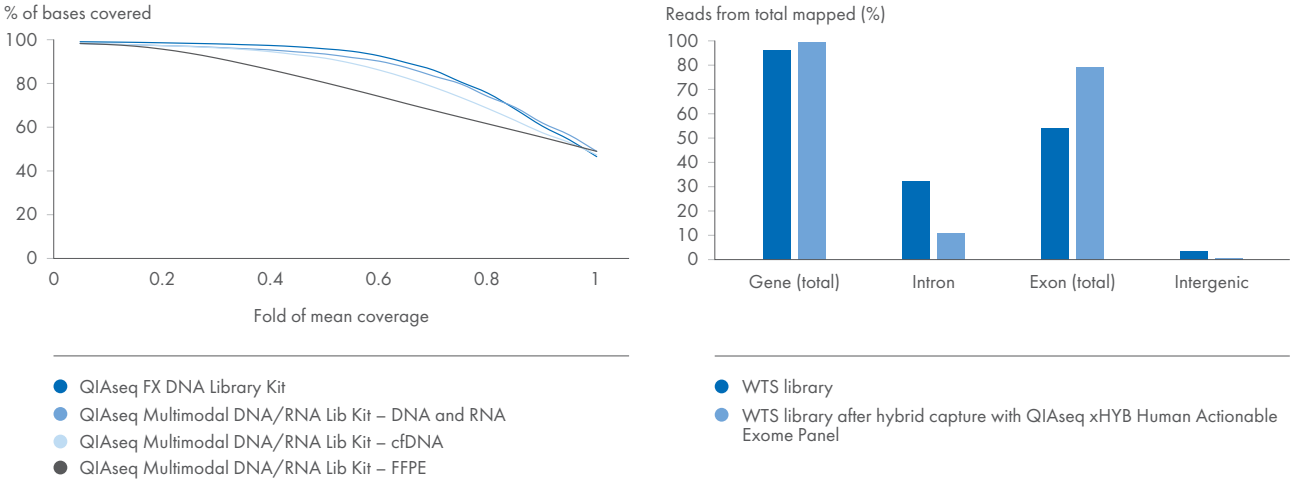


Figure 5. Hybrid capture-based target enrichment for whole exome and transcriptome sequencing (WES and WTS) libraries. WES libraries generated with the QIAseq Multimodal DNA/RNA Library Kit or QIAseq FX DNA Library Kit, enriched using the QIAseq Human Exome Kit, showed comparable performance. Despite decreased coverage uniformity in FFPE samples, 96.6% base coverage was achieved at 20% of the mean. Similarly, QIAseq Multimodal whole WTS libraries after hybrid capture show a higher percentage of reads mapped to coding regions.

Immune response analysis

Explore immune dynamics in the TME

Tumor infiltrating lymphocytes, such as T- and B-cells, play an important role in the TME. Traditional multiplex PCR-based methods for monitoring T- and B-cells often introduce PCR amplification bias, or are not sensitive enough for low-input samples. RNA-seq overcomes this limitation by providing an unbiased, high resolution view of the immune repertoire.

The QIAseq Targeted RNA-seq Panel for TCR enables highly accurate, bias-free NGS of TCR-alpha, beta, gamma and delta genes (see Figure 6).

- Profile single TCR receptors or perform a combined analysis in a single tube
- UMI technology removes amplification, duplication and sequencing artifacts to increase accuracy
- Includes access to GeneGlobe Analyze for data analysis, including read alignment and clonotype report

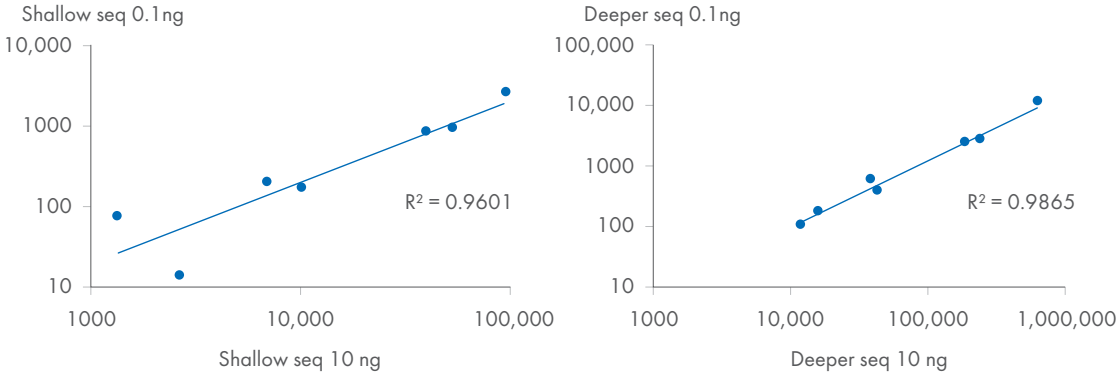
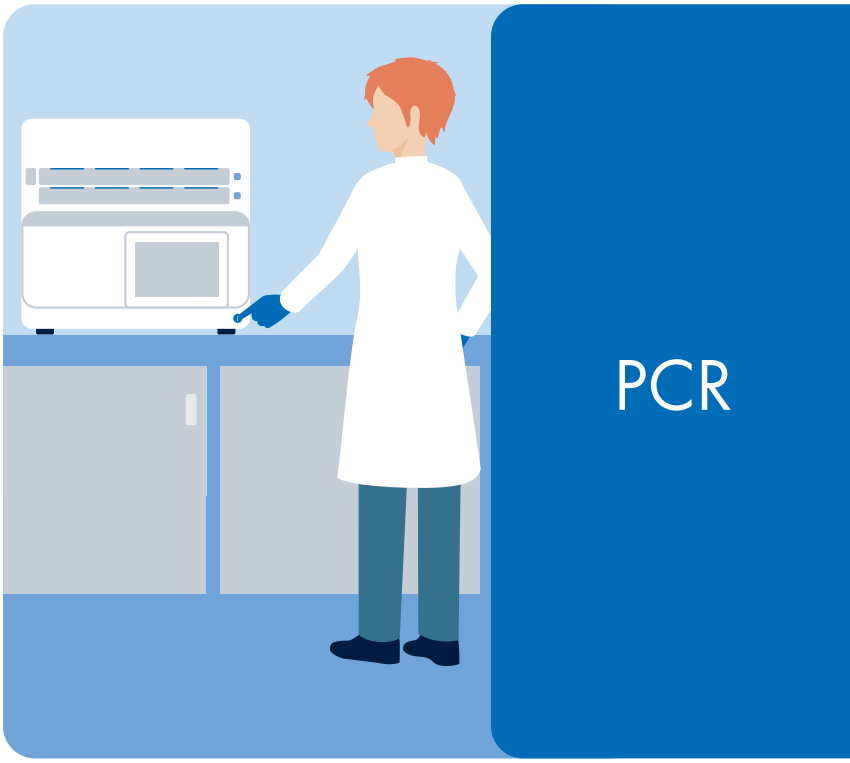


Figure 6. Consistent TCR expression across different input levels. Correlation of TCR clonotype detection between different input amounts (10 ng vs. 0.1 ng) with known clonotypes and relative expression levels. Both shallow sequencing (reads per UMI = 1.5–1.9) and deeper sequencing (reads per UMI = 2.8–4.7) results show highly correlated relative TCR clonotype levels, despite a 100-fold difference in input.

Focused analysis of target genes using dPCR and qPCR



Robust quantification and validation of genomic and transcriptomic markers is essential to confirm insights gathered through NGS. Detecting rare mutations, low-expression biomarkers and gene expression changes within the TME requires tools that provide both precision and sensitivity.

QIAcuity® dPCR enables absolute and precise quantification of rare targets in a nanoplate containing thousands of stable partitions, enabling a user-friendly two-hour workflow for precise biomarker characterization. More specifically, the QIAcuity Nanoplate 26k maximizes resolution, supports multiplex detection and eliminates the need for replicates. In addition, dPCR is more resistant to inhibitors, which makes it suitable for DNA isolated from FFPE samples. Complementing this, qPCR provides a broad dynamic range for detecting large gene expression changes and accommodates flexible sample volumes.

Together, dPCR and qPCR enable robust and reliable quantification of TME-related targets, supporting applications such as tracking tumor heterogeneity and immune escape, monitoring therapy response and resistance, identifying metabolic and stromal reprogramming events and assessing intra- and inter-tumoral heterogeneity.

Table 6. Selection guide for dPCR and qPCR kits and assays

Application	dPCR	qPCR
Mutation analysis	dPCR LNA® Mutation Assays dPCR PanCancer Kits	
CNV analysis	dPCR CNV Probe Assays*	
miRNA biomarker profiling	miRCURY® LNA miRNA PCR Assays for Digital PCR*	miRCURY LNA miRNA PCR Assays* and Panels
Gene expression analysis	QuantiNova® LNA PCR Assays for Digital PCR*	QuantiNova LNA PCR Assays* and Panels
Microbial analysis	dPCR Microbial DNA Detection Assays *	Microbial DNA qPCR Assays and Arrays*

* Custom design tool for dPCR assays available on GeneGlobe.

Detect rare variants down to 0.1% mutation frequency using dPCR

Detecting rare mutations is a major challenge due to low variant frequency and limited DNA availability. dPCR LNA Mutation Assays, with their duplex assay design, enable precise discrimination between mutated and wild-type sequences. LNA-enhanced primers and probes increase assay specificity and sensitivity, making them ideal for low-input and precious samples.

- Suitable for use with DNA from FFPE samples, tissues and liquid biopsy samples
- LNA-enhanced primers and probes enable detection down to 0.1% mutation frequency
- Wet-lab tested dPCR assays available for more than 200 targets, including common oncogenes

Detect up to 12 CNVs in a single, high-multiplex dPCR reaction

The dPCR CNV Probe Assays enables locus-specific analysis of copy number variations and alterations by singleplex or multiplex dPCR. For multiplex detection, combine the dPCR CNV Probe Assays with the QIAcuity High Multiplex Probe PCR Kit and QIAcuity Software 3.1. You can confidently quantify 10 genes of interest and two reference gene targets in a single reaction to capture detailed biological phenomena, such as genome instability resulting from cancer (see Figure 7).

- Assays for more than 200 wet-lab-validated targets
- Custom design tool is available on GeneGlobe
- Higher-order multiplexing on existing QIAcuity hardware

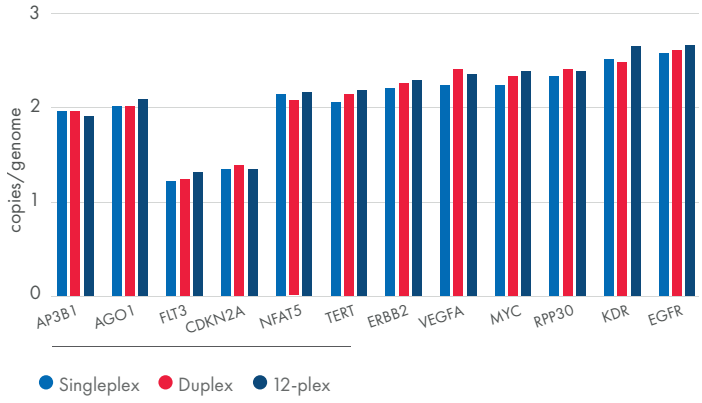


Figure 7. dPCR quantifications using single, duplex or 12-plex reactions are highly concordant. The copy number of ten genes of interest in U-2 OS sarcoma cell line were analyzed by 12-plex dPCR reactions. The average copy number of the reference genes AGO1 and AP3B1 was used for normalization. All ten gene targets were also quantified in singleplex and duplex for comparison. The results from both the singleplex, duplex and 12-plex reactions revealed genome instability in the sarcoma cell line, with the gene of interest copy numbers varying from 1.25 to 2.7.

Customize dPCR assays for CNVs

With the GeneGlobe custom design tool, customize CNV assays with advanced algorithms, multiplex up to 12 targets and choose from flexible dye options for optimal specificity and sensitivity.

Explore the tool: **https://geneglobe.qiagen.com/customize/dpcr**

Quantify gene expression using LNA-enhanced PCR assays

Detecting low-expressed genes while minimizing background noise is critical in gaining key insights into tumor progression and response to therapy. Our QuantiNova LNA assays offer an extensive catalog of predesigned assays for qPCR and dPCR, along with customization options.

- LNA technology for exceptional sensitivity and specificity
- Accurate detection over a wide dynamic range starting at 1 RNA copy
- Includes assays that cover common cancer signaling and immune response pathways

Quantify miRNAs with high-resolution dPCR assays

Precise quantification of miRNAs helps understand their roles in various biological processes within the TME, including cell growth, apoptosis and tissue development. The miRCURY LNA PCR Assays for Digital PCR offer ultrasensitive and specific profiling of immune and tumor-derived miRNAs from FFPE tissues, cells and other sample types (see Figure 8).

- miRNA quantification from just 1 pg of total RNA
- High specificity discriminates closely related miRNAs and mature miRNA from precursors
- Fast and simple two-step dPCR protocol takes less than 3 hours

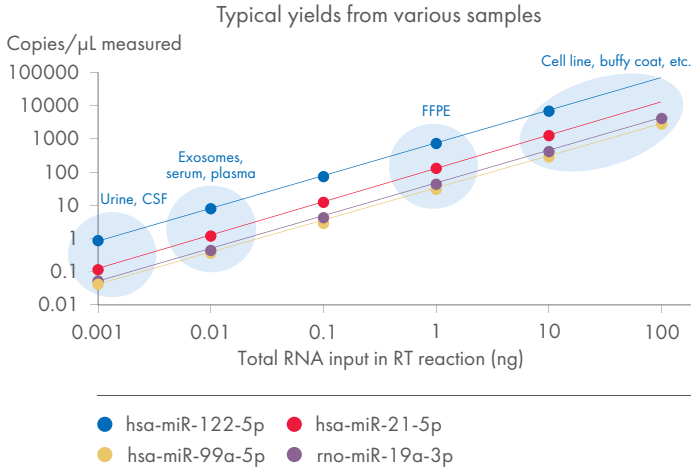


Figure 8. Accurate and precise detection of miRNAs across a broad RNA input range and sample types. miRNAs were quantified from serial dilutions of AM6000 total RNA, using 0.001–100 ng of input RNA and miRCURY LNA miRNA PCR Assays for Digital PCR. All miRNA assays demonstrated linear readout ($R^2 > 0.99$), enabling robust quantification, including low-expressed and AT-rich miRNAs.

Data analysis

Expert-curated genomic knowledge with bioinformatics tools and services

Complete workflow design

- GeneGlobe Design & Analysis Hub

Create tailored qPCR and NGS assays for specific instruments and targets, and analyze data with web-based tools for gene and miRNA expression, mutation detection and copy number variation.

- RNA-seq Analysis Portal

Align reads, explore differential expression and uncover pathway insights from your RNA-seq data – all in a few hours. This fast, accessible and comprehensive web-based platform integrates seamlessly with dPCR and qPCR validation tools.

Bioinformatic solutions

- QIAGEN CLC Genomics Workbench Premium

Analyze NGS and 'omics data using user-friendly, ultra-fast pipelines that are accessible to biologists. The workbench helps you annotate variants, detect low-frequency mutations and handle UMIs with custom tools designed for targeted panels, WGS and other 'omics data.

- QIAGEN Ingenuity® Pathway Analysis (IPA®)

Discover activation and inhibition relationships, master regulators and molecular pathways in minutes. With the largest curated knowledge base available, IPA provides context for your data that supports your publications.

Explore solutions for cancer reserach:
www.qiagen.com/applications/cancer-research