



# Making the invisible visible

Detect low-abundance microbes  
with a versatile workflow



Microbes are ubiquitous and touch all aspects of our lives, from health to food production. Effective techniques to detect and monitor microbes help decipher their biological functions, especially in infection and colonization of our body.

Conventional molecular techniques for sample enrichment and DNA-based quantification coupled with advances in sequencing technologies have expanded the boundaries of microbial and infectious disease research, public health and epidemiology. However, low microbe concentrations, complex sample matrices, lack of streamlined end-to-end workflows and limited throughput capabilities have hindered rapid profiling

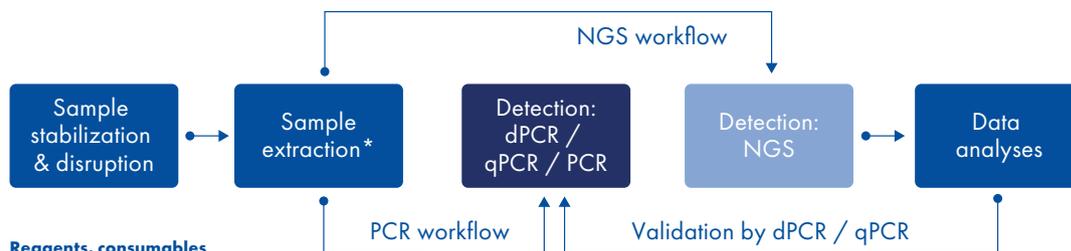
and identification of antibiotic resistance and virulence genes from various sample sources, such as wastewater, human microbiome, food production and the environment.

At QIAGEN, we are tackling these challenges to help you gain unbiased and reproducible results from your microbial samples.

# Detect and analyze your microbes of interest with a versatile workflow

## Instruments:

- **TissueLyser III**
- Vortex adapters
- **QIAcube® Connect**
- **QIAcube HT**
- EZ2® Connect
- QIAAsymphony®
- **QIAxcel® Connect**
- **QIAcuity® Digital PCR System**
- Rotor-Gene® Q
- QIAquant®
- **QIAxcel® Connect**



## Reagents, consumables and analysis tools:

- **PowerProtect DNA/RNA**
- PowerBead Pro Tubes & Plates
- **PowerFecal® Pro Kits**
- **QIAwave DNA Blood & Tissue Kit**
- AllPrep® Kits
- QIAamp® Kits
- DNeasy® PowerSoil® Pro Kit
- EZ1 & 2™ Kits
- QIAAsymphony DSP Virus/Pathogen Kit
- **QIAcuity OneStep Advanced Probe Kit**
- **dPCR Microbial DNA Detection Assays**
- Microbial DNA qPCR Assays and Arrays
- QuantiNova® Pathogen +IC Kit
- UCP Multiplex PCR Kit
- QIAcuity High Multiplex Probe PCR Kit
- **QIAseq® xHYB Viral and Bacterial Panels**
- QIAseq DIRECT SARS-CoV-2 Kit
- **QIAseq FX DNA Library Kit**
- QIAseq 16S/ITS Screening Panels
- **QIAGEN CLC Genomics Workbench Premium**
- **GeneGlobe® Design and Analysis Hub**

\* Our extraction kits cover wastewater, sludge, biofilm, stool, soil, swab samples and more

## Stabilization



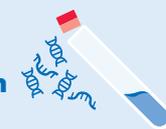
- Ensure the integrity of your microbial community and functional profile before nucleic acid extraction with PowerProtect DNA/RNA, a bulk stabilization solution
- Obtain consistent results with the stabilization solution designed for use with our extraction chemistry

## Disruption



- Achieve fast and efficient lysis and homogenization for various sample types and throughputs, thanks to robust sample disruption instruments
- Reduce risk of sample bias towards easy-to-lyse microbes by using PowerBead Pro Tubes and Plates combined with an optimized chemistry

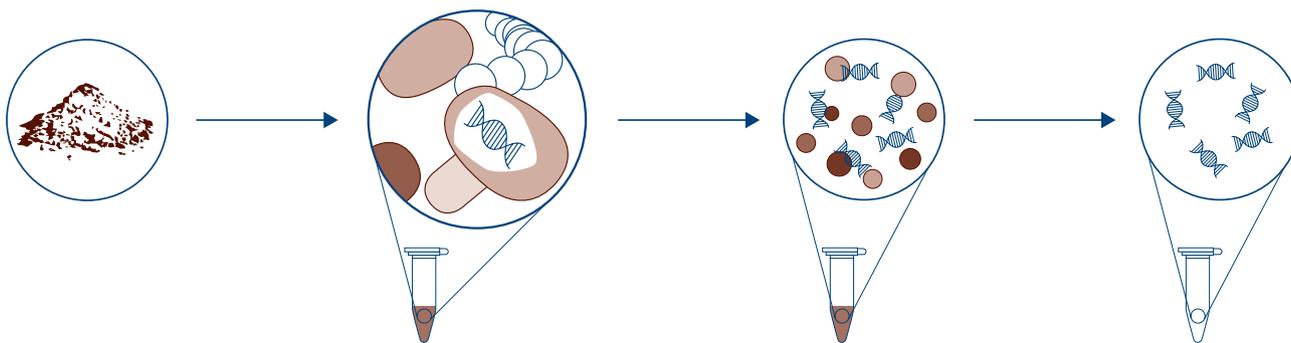
## Extraction



- Maximize yields with dedicated kits for simultaneous DNA and RNA extraction from the same sample
- Eliminate contaminants to get the best starting material for your downstream application, thanks to our patent-pending Inhibitor Removal Technology® (IRT) included in the Power kits
- Achieve higher reproducibility and reliability with automation

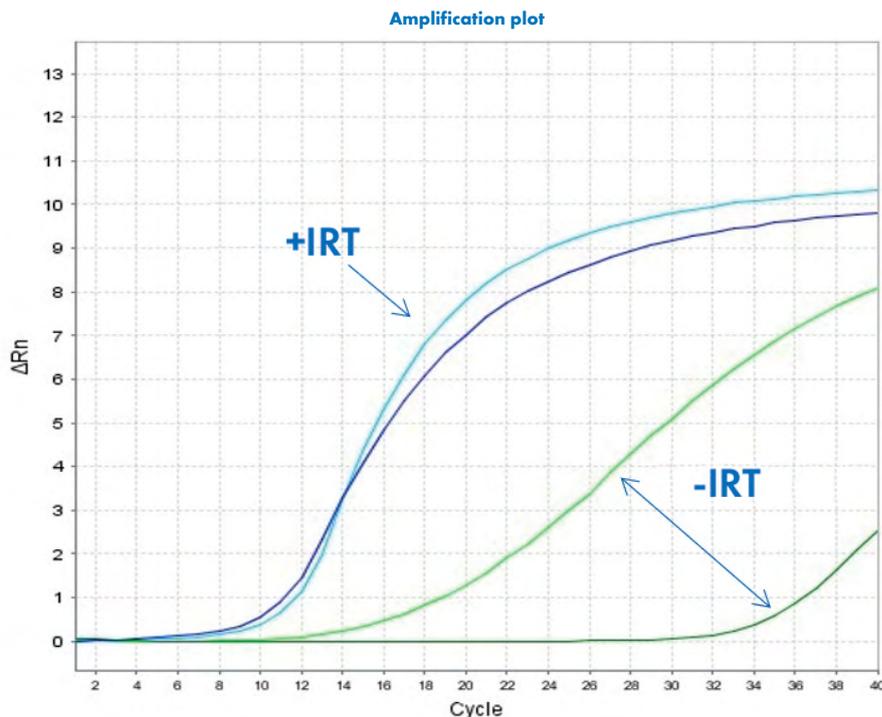
# Representative results despite PCR inhibitors

During cell lysis, PCR/RT-PCR inhibitors are released along with nucleic acids. These include humic/fulvic acids in soil, polysaccharides/polyphenolics in plants and bile, bilirubin and heme in stool.<sup>1</sup>



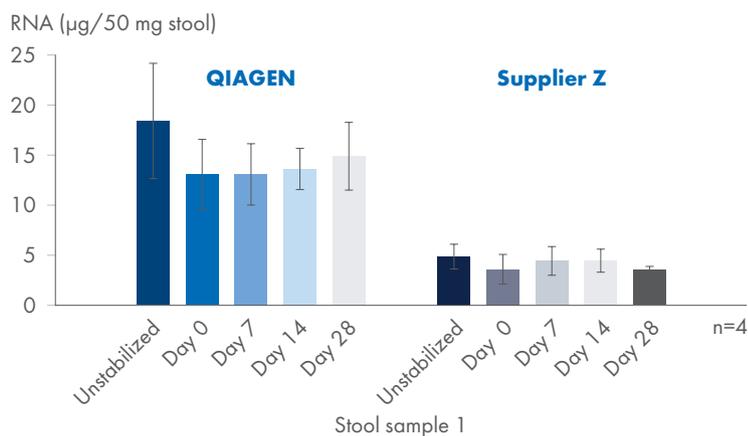
(1) Examples of additional matrices: Rådström P, Knutsson R, Wolffs P, Lövenklev M, Lofström C. Pre-PCR processing: strategies to generate PCR-compatible samples. *Mol Biotechnol.* 2004;26(2):133–46

Inhibitor Removal Technology (IRT) included in Power kits assures representative results by removing inhibitors. The amplification plot shows the influence of the IRT on the PCR/RT-PCR reaction. With IRT, amplification happens in the early cycles, but without IRT, there is a significant delay in amplification.



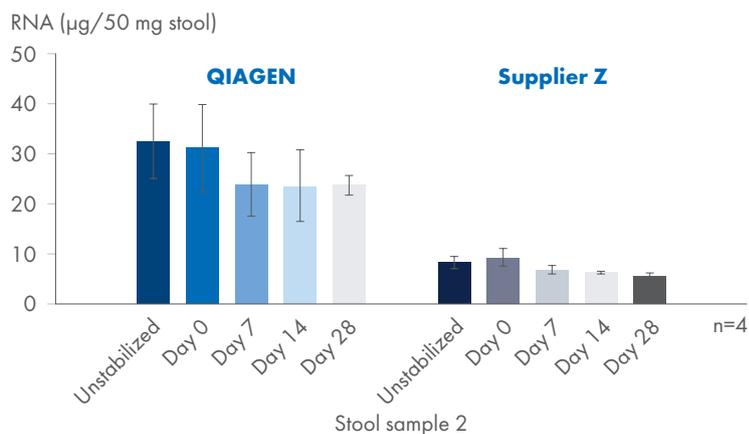
# Reliable fecal sample stabilization using PowerProtect DNA/RNA

## A RNA yield — room temperature storage



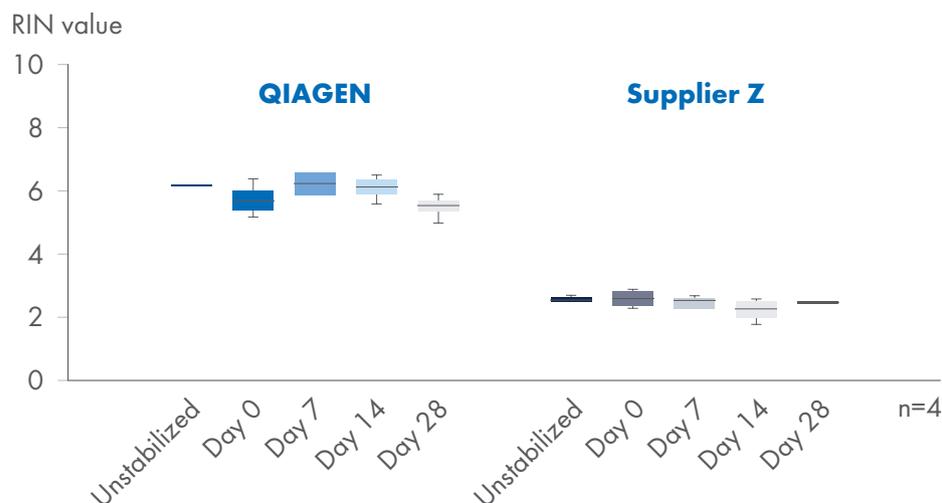
Two stool samples were collected, and four replicates of each were processed using the QIAGEN or the Supplier Z workflow. The unstabilized sample at day 0 was used as a reference. The other samples were stored in PowerProtect DNA/RNA or Supplier Z and extracted with RNeasy PowerFecal Pro or Supplier Z at 0, 7, 14 and 28 days.

(A) Whether unstabilized or stabilized for up to 28 days, the stool samples processed with the QIAGEN workflow delivered higher RNA yields than Supplier Z.

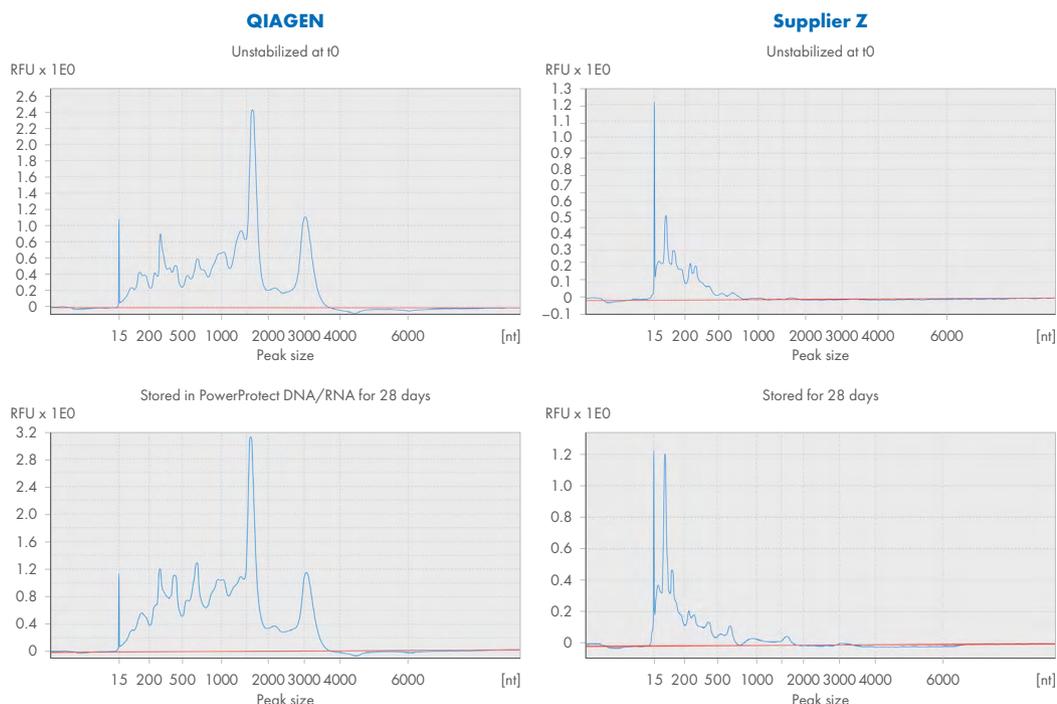


(B) The RIN value is used to estimate the integrity of total RNA samples. Higher RIN values indicate better quality of the extracted RNA. The average RIN values obtained by the QIAGEN workflow on samples stabilized over time are higher than Supplier Z.

## B RNA integrity — average RIN value (stool samples 1 and 2)



## C RNA quality



(C) QIAxcel ScreenGel® electropherograms confirm RNA quality in terms of the integrity of total RNA samples. Unlike Supplier Z electropherograms, QIAGEN electropherograms show two distinct ribosomal peaks corresponding to 16S and 23S, demonstrating no or little RNA degradation with RNA obtained using the QIAGEN workflow.

# Detecting and quantifying what goes unseen



As often in nature, the targets with low and rare abundance give you the most interesting insights. However, finding those targets is often a needle in a haystack problem. That's where digital PCR comes in handy with its high precision and sensitivity.

### Benefits using dPCR

- Absolute, precise and sensitive quantification of pathogens without the need for a standard curve
- Reduced reliance on PCR amplification efficiency and high tolerance to inhibitors found in complex sample matrices
- Enhanced analytical performance for rare target detection

### Benefits using nanoplate dPCR

- High-order multiplexing of up to 12 targets per well
- User-friendly workflow with rapid time-to-result (~2 h)
- Large sample volumes (up to 28  $\mu$ L) are possible for enhanced sensitivity

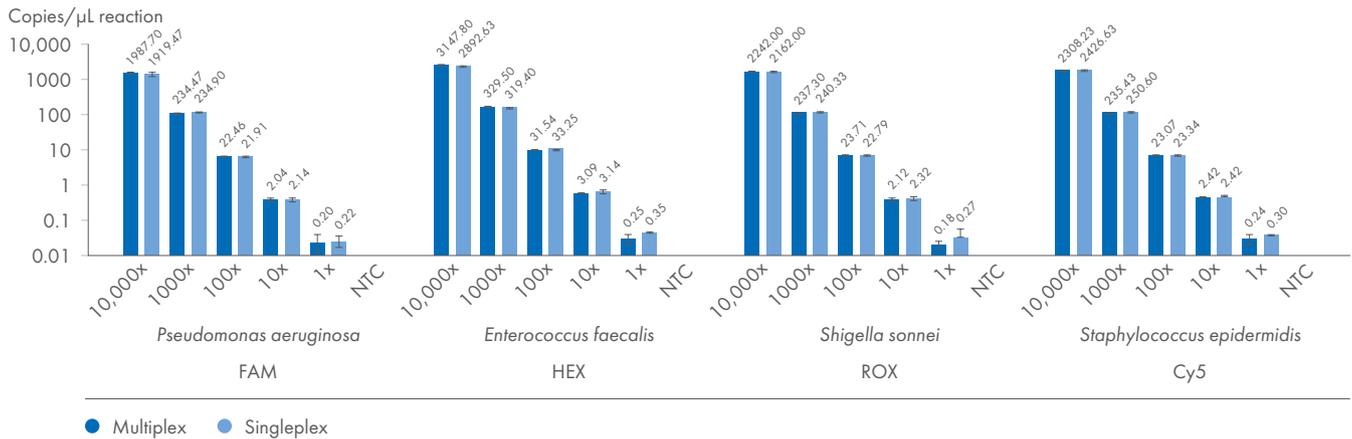
### dPCR Microbial DNA Detection Assays

[Visit GeneGlobe](#)

For use with the QIAcuity Probe PCR Kit (for DNA targets), the QIAcuity OneStep Advanced Probe Kit (for RNA or a mix of RNA and DNA targets) or the QIAcuity High Multiplex Probe PCR Kit (for DNA targets)

- Identify >700 targets including bacterial, fungal, parasitic, viral, antibiotic resistance and virulence factor genes
- Detect up to 12 targets per reaction
- Combine microbial DNA and viral RNA detection in one reaction

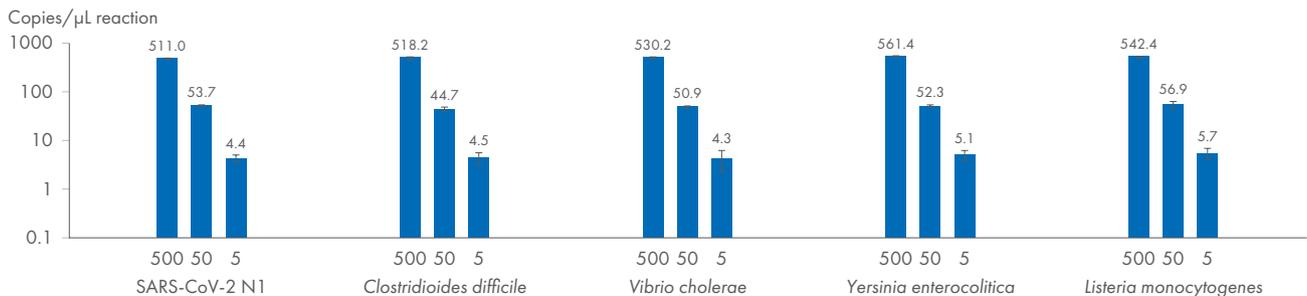
## Precise and specific quantification in singleplex and multiplex



### Singleplex versus multiplex setup quantifying four different bacterial targets.

Four assays were run in singleplex and 4-plex reactions using the same template genomic DNA material. In both setups, the same concentrations were observed for concentrations between 0.25 and 2500 copies/μL dPCR, with 3 replicates for each condition, was performed using 26K 24-well Nanoplates and the QIAcuity Probe PCR Kit on the QIAcuity Digital PCR System.

## Detect viral RNA and microbial DNA together in a multiplex reaction

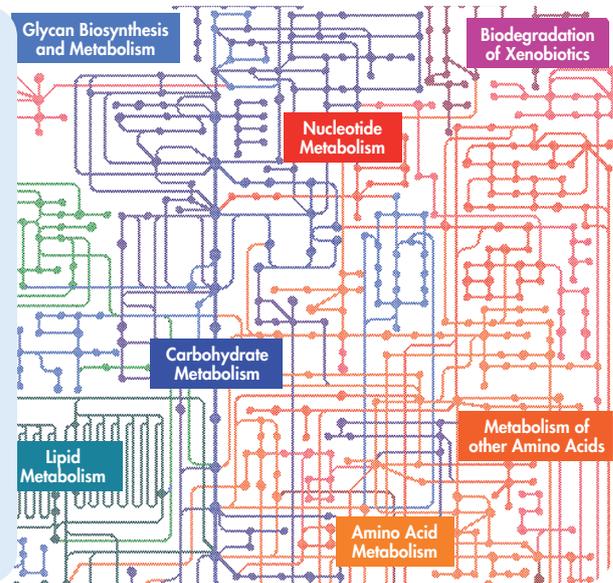


**Multiplex detection of RNA and DNA targets.** A mixture of four bacterial gDNAs (*Clostridioides difficile*, *Vibrio cholerae*, *Yersinia enterocolitica*, *Listeria monocytogenes*) and SARS-CoV-2 RNA was used as input. dPCR, with three replicates per condition, was run using 8.5k 96-well Nanoplates and the QIAcuity OneStep Advanced Probe Kit on the QIAcuity Digital PCR System. Three template dilutions with 500, 50 and 5 copies/μL were used. Bar chart shows the mean measured concentrations (copies/μL) of three replicates each for each of the five targets.



For more details on the assay performance, see the Product Profile:  
[www.qiagen.com/PP\\_dPCR-microbial-detection](http://www.qiagen.com/PP_dPCR-microbial-detection)

➔ Not ready for dPCR?  
**Try our Microbial DNA  
qPCR Assays and Arrays**



## Sequencing for deeper insights



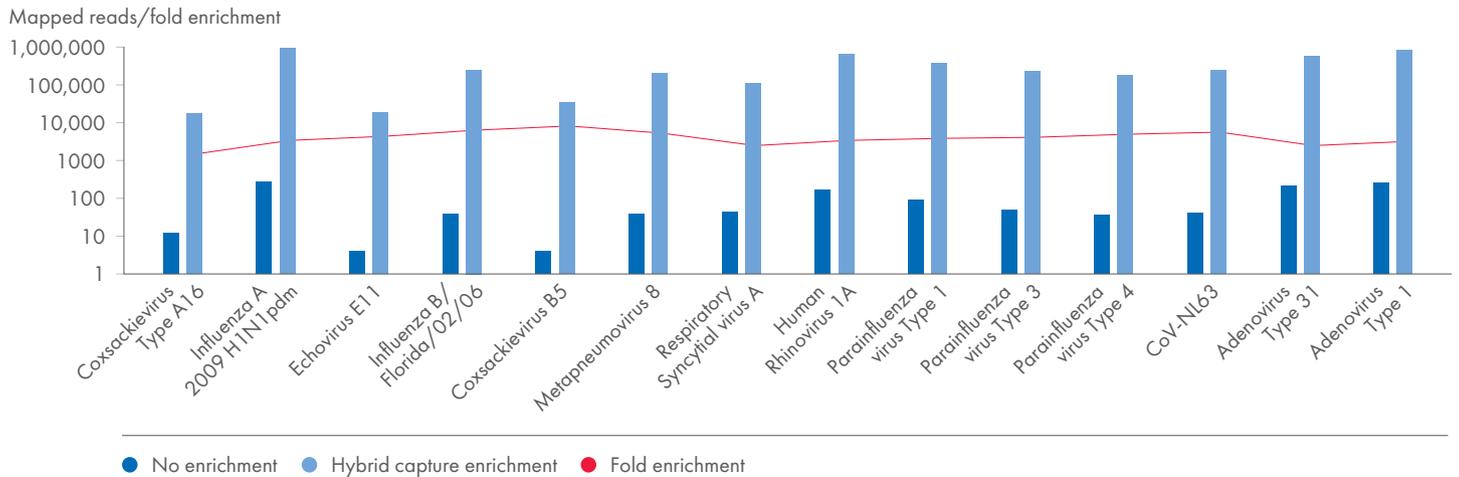
Next generation sequencing (NGS) determines the nucleic acid sequence that makes up a genome or targeted genomic regions of the pathogen without any previous knowledge of its existence. This allows the identification of novel pathogens. The unbiased nature of NGS also enables co-infections to be identified that are ignored by other assays. Moreover, NGS can be used for pathogen surveillance to detect genetic variants that may be evolving.

Tackle some of the challenges involved in implementing NGS for routine pathogen detection with QIAseq kits and panels (for example, pair the QIAseq xHYB Viral and Bacterial Panels with the QIAseq FX DNA Library UDI Kit):

- Unique Dual Index (UDI) adapters available for multiplexing up to 384 samples
- High library complexity and uniform coverage maximizes interpretable data
- Eliminate the data bottleneck and reduce overall turnaround time with rapid data analysis and variant interpretation



## High-quality enrichment of respiratory viral isolates



QIAseq xHYB gives an average of 2400x enrichment of viral targets vs. standard shotgun sequencing

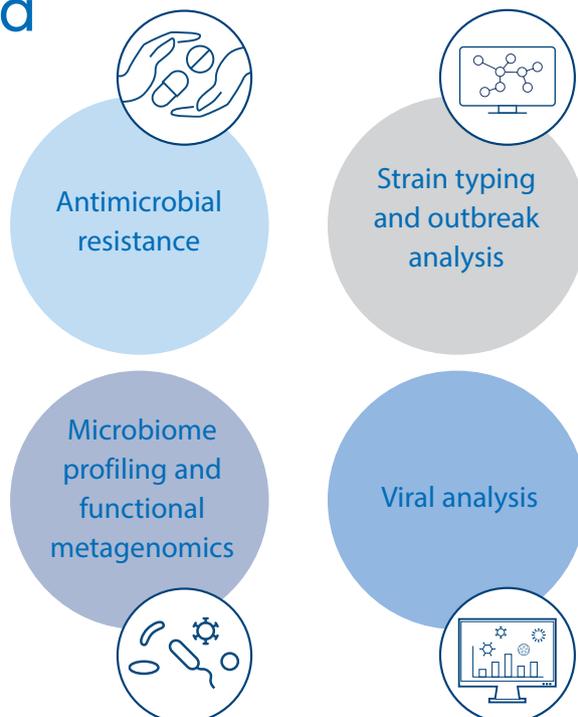


For more details on high-quality targeted viral and bacterial sequencing, visit: [www.qiagen.com/QIAseq-xHYB-Viral-and-Bacterial-Panels](http://www.qiagen.com/QIAseq-xHYB-Viral-and-Bacterial-Panels)

## Making sense of complex microbial genomic data

Only well-structured and clear data can lead you to actionable insights. However, the volume and complexity of the data can be quite overwhelming.

QIAGEN CLC Genomics Workbench Premium is just one of many bioinformatics platforms that is easy to use by bioinformaticians and non-bioinformaticians alike. It offers various tools and customizable workflows for diverse application areas, including microbial genomics and metagenomics.



## Ordering information

Product	Description	Cat. no.
TissueLyser III	For convenient and secure sample disruption for variable throughput, from tubes to 96-well plates	9003240
PowerProtect DNA/RNA	Bulk reagent for ambient temperature stabilization of nucleic acids in stool samples over time	14800 14810
QIAcube Connect*	For fully automated, spin-column-based nucleic acid extraction managed from outside the lab using QIAcube	9002864
QIAcube HT*	For automated mid- to high-throughput nucleic acid purification in a 96-well format	9001896
EZ2 Connect	For end-to-end automation of nucleic acid extraction, from reagent setup to elution	9003210
QIASymphony*	For automated sample preparation of DNA, RNA, and bacterial and viral nucleic acids from a wide range of starting materials	9001751
QIAxcel Connect	For effortless, cost-effective, high-resolution DNA or RNA gel electrophoresis – all in a single instrument	9003110
RNeasy PowerFecal Pro Kit	For the isolation of microbial RNA from stool and gut samples, sludge, or wastewater	78404
AllPrep PowerFecal Pro DNA/RNA Kit	For simultaneous purification of microbial DNA and total RNA from the same stool or wastewater sample.	80254
QIAwave DNA Blood & Tissue Kit	For a more eco-friendly alternative to our standard kit for extracting total DNA from animal blood and tissues, cells, yeast, or bacteria	69556
AllPrep PowerViral DNA/RNA Kit	For isolating viral or bacterial total nucleic acids from waste water and stool samples	28000-50
QIAamp DNA Micro Kit	For purification of genomic and mitochondrial DNA from small samples	56304
DNeasy PowerSoil Pro Kit	For the isolation of microbial genomic DNA from all soil types	47014 47016
QIAcuity Digital PCR System*	For absolute and accurate quantification of nucleic acid molecules in nanoplates	911035 911045
Rotor-Gene Q*	For outstanding performance in real-time PCR in a rotary format	9001620 9001640 9001660
QIAquant*	For fast and high performance real-time PCR in a 96- or 384-well format	9003010 9003011
QIAcuity OneStep Advanced Probe Kit	For one-step RT-PCR on the QIAcuity digital PCR instruments	250131 250132

## Ordering information

Product	Description	Cat. no.
dPCR Microbial DNA Detection Assays	For digital PCR detection of microbial targets, including bacterial, fungal, parasitic, viral, antibiotic resistance or virulence factor genes	250207
Microbial DNA qPCR Assays and Arrays	For real-time PCR detection of microbial species, virulence genes or antibiotic resistance genes	330025 33026
QuantiNova Pathogen +IC Kit	For ultrafast, simultaneous detection of viral RNA/DNA and bacterial DNA, including in-process safety measures	208654
UCP Multiplex PCR Kit	Ultra-Clean Production master mix for multiplex hot-start PCR and microbiome applications	206742 206744
QIAcuity High Multiplex Probe PCR Kit	Allows multiplexing of up to 12 assays in one reaction using the QIAcuity	250133 250134
QIAseq xHYB Viral Respiratory Panel*	For reverse transcription, hybrid capture and downstream post-hybrid capture amplification of viral respiratory targets; can be paired with QIAseq FX DNA Library Kit	333325 333322
QIAseq FX DNA Library UDI Kit	Buffers and reagents for DNA fragmentation (including end repair and A-addition), ligation and library amplification; for use with Illumina instruments	180477 180479
QIAseq DIRECT SARS-CoV-2 Kits*	For fast, targeted whole genome library preparation of SARS-CoV-2 for genomic surveillance and variant detection	333891
QIAseq 16S/ITS Screening Panels	For next-generation sequencing-based Sample to Insight profiling of bacterial and fungal communities	333812 333815
QIAGEN CLC Genomics Workbench Premium Desktop	For single cell analysis, microbial profiling, pathogen typing and outbreak analysis using state-of-the-art bioinformatics	832023

\* Additional instrument, kit, panel, assay and service bundles are available. For all systems, Installation and Training are included but are additionally available as separate service offerings. For specific catalog numbers and additional information, visit [www.qiagen.com](http://www.qiagen.com) or contact your local sales representative

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit instructions for use or user operator manual. QIAGEN kit instructions for use and user manuals are available at [www.qiagen.com](http://www.qiagen.com) or can be requested from QIAGEN Technical Services (or your local distributor).

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