QIAGEN

Making the invisible visible

A versatile workflow for the detection of low-abundance microbes

Microbes are ubiquitous and touch all aspects of our lives, from health to food production. They can trigger various harmful or beneficial effects on us. And this makes the specific detection and monitoring of microbes important for understanding their biological function, especially in infection or colonization of our body.

Conventional molecular techniques for sample enrichment and DNA-based quantification coupled with advances in sequencing technologies have been able to push certain boundaries of microbial and infectious disease research, public health and epidemiology. However, the low concentrations of microbes, complex sample matrices, lack of simplified and fast end-to-end workflows and limited throughput capabilities of existing techniques have impeded the rapid profiling and identification of antibiotic resistance and virulence genes from diverse samples, including wastewater samples, the human microbiome, multiple drug resistance, sepsis, food production or environmental samples.

Microbial detection workflow

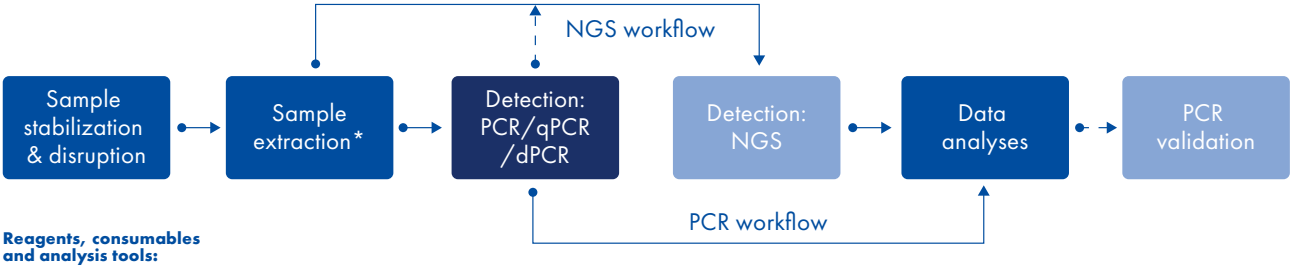
Instruments:

- **TissueLyser II**
- **PowerLyzer® 24 Homogenizer**
- Vortex Adapters

- **QIAcube® Connect**
- **QIAcube HT**
- EZ2® Connect
- QIAsymphony®
- **QIAxcel® Connect**

- **QIAcuity® Digital PCR System**
- Rotor-Gene® Q
- QIAquant®
- QIAamplifier® 96

- **QIAxcel® Connect**



Reagents, consumables and analysis tools:

- **PowerProtect DNA/RNA**
- RNAprotect®
- PowerBead Pro Tubes & Plates

- **PowerFecal® Pro Kits**
- **QIAwave DNA Blood & Tissue Kit**
- AllPrep® Kits
- QIAamp® Kits
- DNeasy® PowerSoil® Pro Kit
- EZ1&2™ Kits
- QIAsymphony DSP Virus/Pathogen Kit

- **QIAcuity OneStep Advanced Probe Kit**
- **dPCR Microbial DNA Detection Assays**
- GT-Digital SARS-CoV-2 Wastewater Surveillance Assay Kit for QIAcuity v1.0**
- Microbial DNA qPCR Assays and Arrays
- QuantiNova® PCR/RT-PCR Kits
- UCP Probe and SYBR® Green qPCR Kits
- UCP Multiplex 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 Analysis**

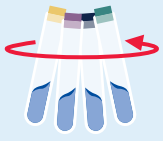
*Our extraction kits cover wastewater, sludge, biofilm, stool, soil, swab samples and more

** Customers from North America: Order from GT Molecular. Customers from other countries: Contact your QIAGEN sales representative.

Stabilization

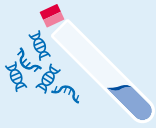
- PowerProtect DNA/RNA is a bulk stabilization solution for stool samples that ensures the integrity of your microbial community and functional profile before nucleic acid extraction
- Specially designed for use with our extraction chemistry
- For samples other than stool, choose the RNAprotect portfolio for the best results

Disruption



- Robust sample disruption instruments provide fast and efficient lyses and homogenization for various sample types and throughput
- PowerBead Pro Tubes and Plates combined with optimized chemistry provides efficient lysis, reducing the risk of sample bias towards easy-to-lyse microbes

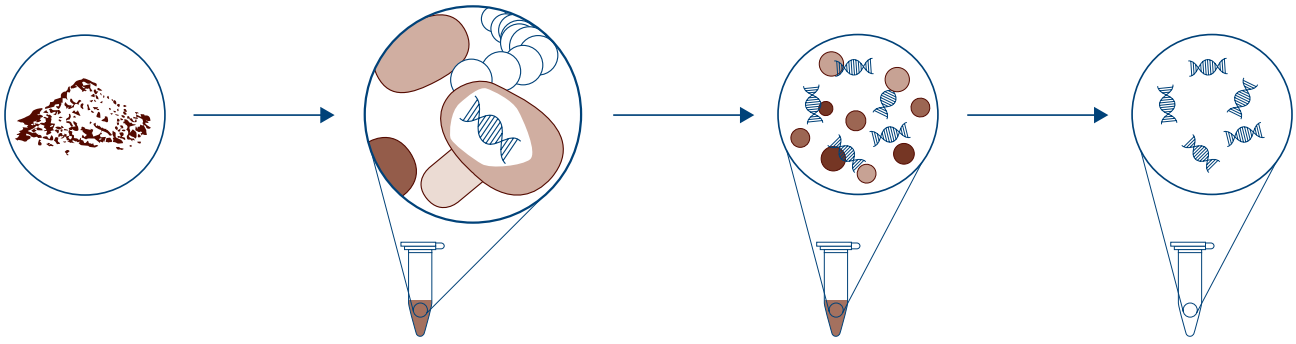
Extraction



- Dedicated kits for the simultaneous extraction of DNA and RNA from the same sample to maximize yields
- Our patented Inhibitor Removal Technology® (IRT) included in the Power kits eliminates contaminants, so you get the best starting material for your downstream application
- Achieve higher reproducibility and reliability with automation

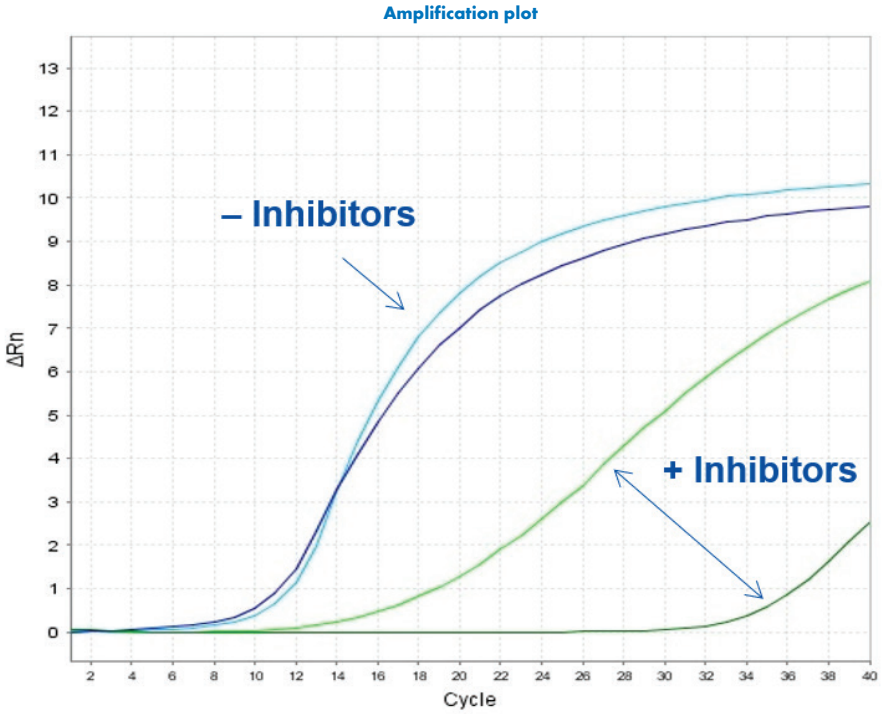
Sample-derived PCR/RT-PCR inhibitors

Other components, such as amplification inhibitors, are also released when breaking open cells to release nucleic acids. These include humic/fulvic acids in soil, polysaccharides/polyphenolics in plants and bile, bilirubin and heme in stool.[1]



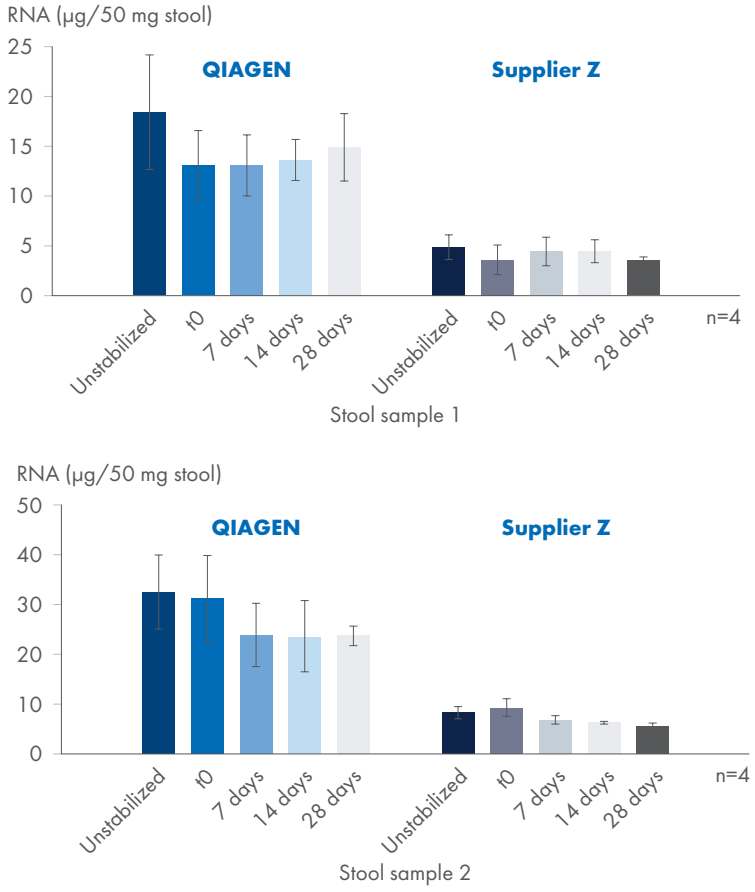
(1) Examples of additional matrices: Rådström, P. et al. (2004) Pre-PCR processing: Strategies to generate PCR-compatible samples. Mol. Biotechnol. 26, 133–46

The patented Inhibitor Removal Technology (IRT) assures representative results. The amplification plot shows the influence of the inhibitors on the PCR/RT-PCR reaction. In the absence of inhibitors, amplification shows in the early cycles, but when the DNA is co-isolated with inhibitors, there appears a significant delay in amplification.



Reliable fecal sample stabilization using PowerProtect DNA/RNA

(A) RNA Yield — room temperature storage

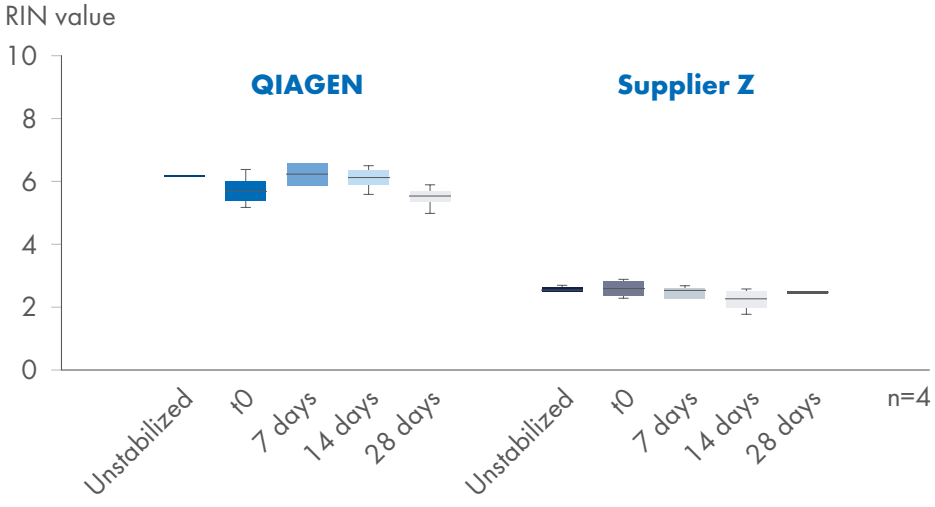


Two stool samples were collected, and four replicates of each were processed using the QIAGEN or the Supplier Z workflow. The unstabilized sample at t0 was used as a reference. The other samples were stored in PowerProtect DNA/RNA or Supplier Z and extracted with RNeasy PowerFecal Pro or Supplier Z at 0, 7, 14 and 28 days.

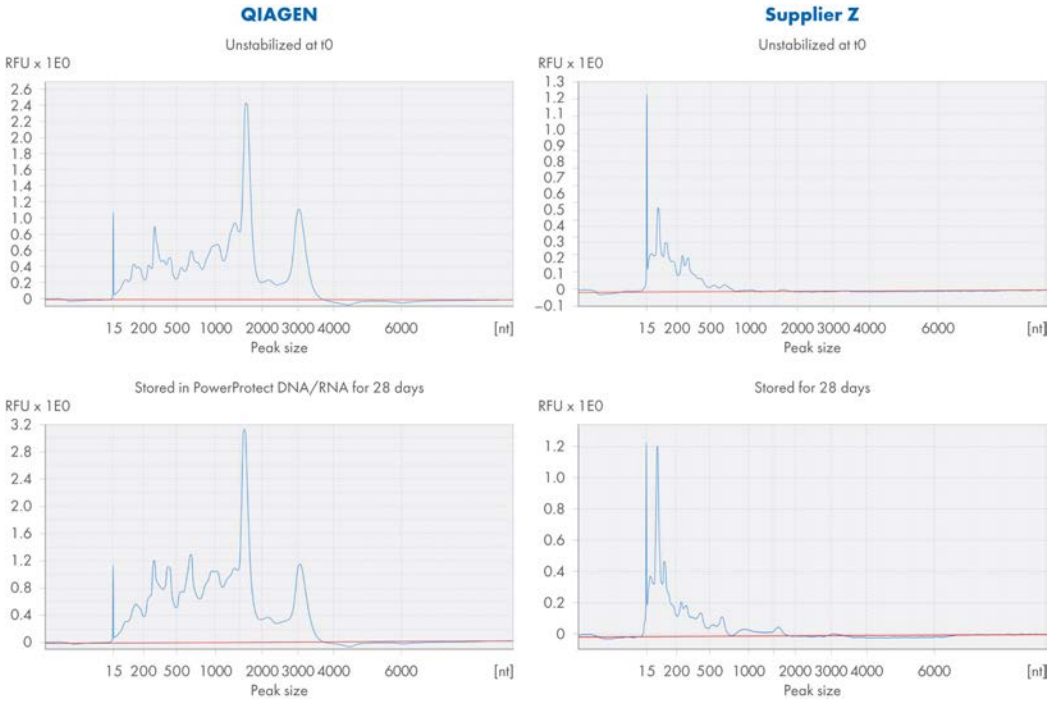
(A) Whether unstabilized or stabilized for up to 28 days, the stool samples processed with the QIAGEN workflow deliver greater RNA yields than Supplier Z.

(B) The RIN value is a tool designed to estimate the integrity of total RNA samples. The higher the RIN value, the greater the quality of the extracted RNA is. The average RIN values obtained by the QIAGEN workflow on samples stabilized over time are superior to Supplier Z.

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



© **RNA Quality**



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

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 5 targets per well
- User-friendly workflow with rapid time-to-result (~2 h)
- Large sample volumes (up to 28 µl) are possible for enhanced sensitivity

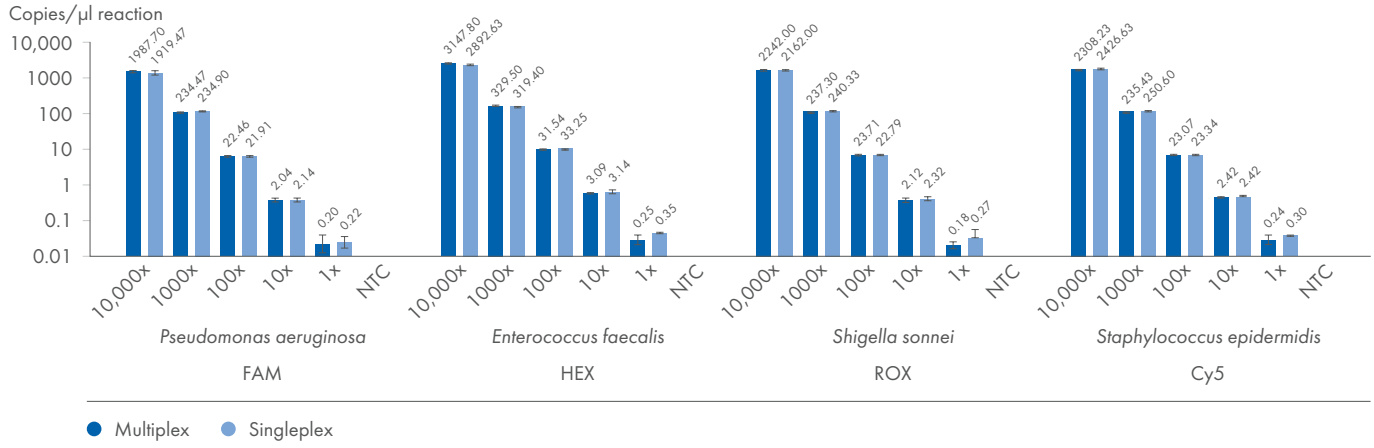
NEW!

dPCR Microbial DNA Detection Assays – now on GeneGlobe

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

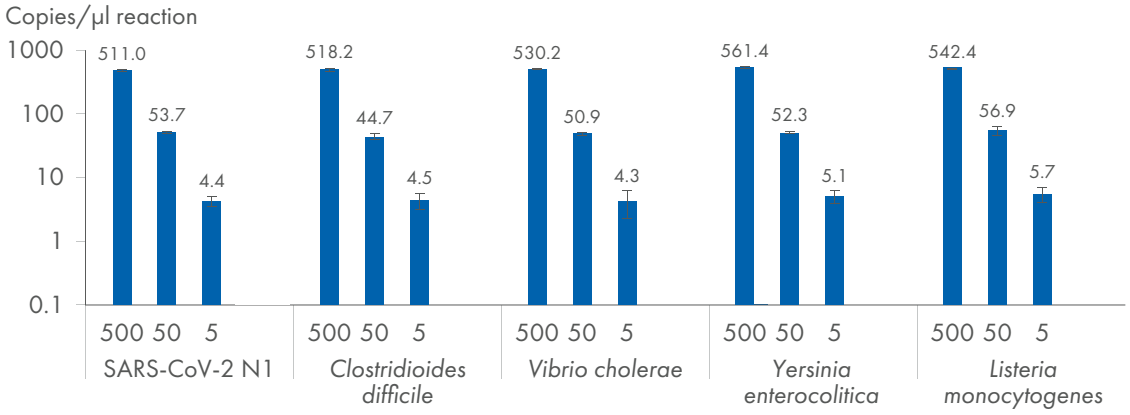
- Identify >680 targets including bacterial, fungal, parasitic, viral, antibiotic resistance and virulence factor genes
- Detect up to 5 targets per reaction
- Combine microbial DNA and viral RNA detection in one reaction

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

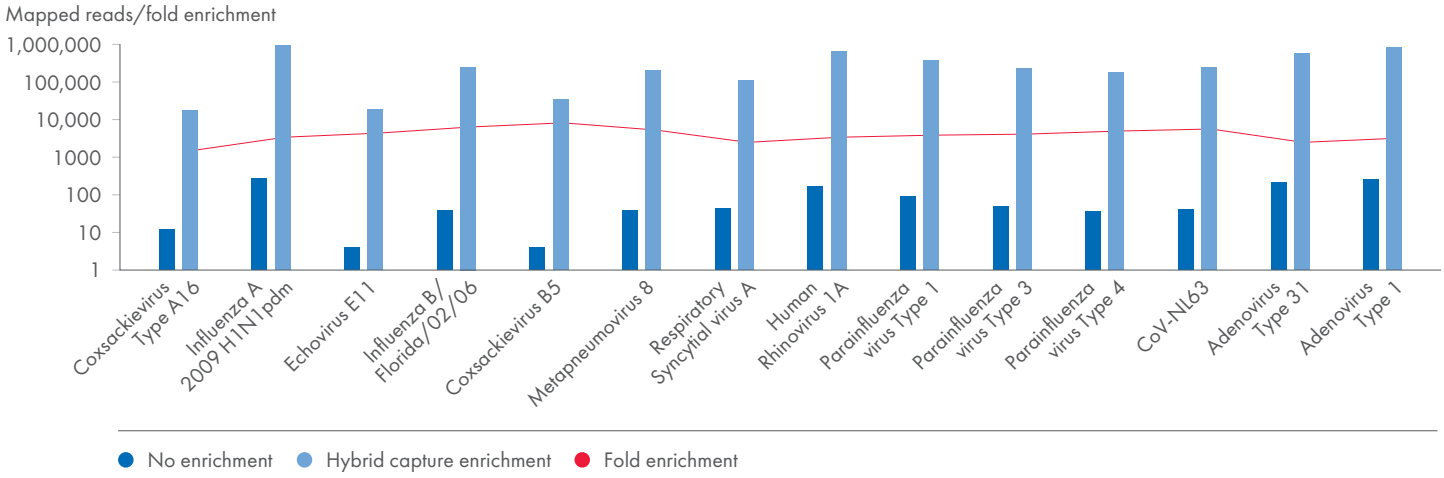
Sequencing for deeper insights

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 otherwise ignored by other assays. Moreover, it 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



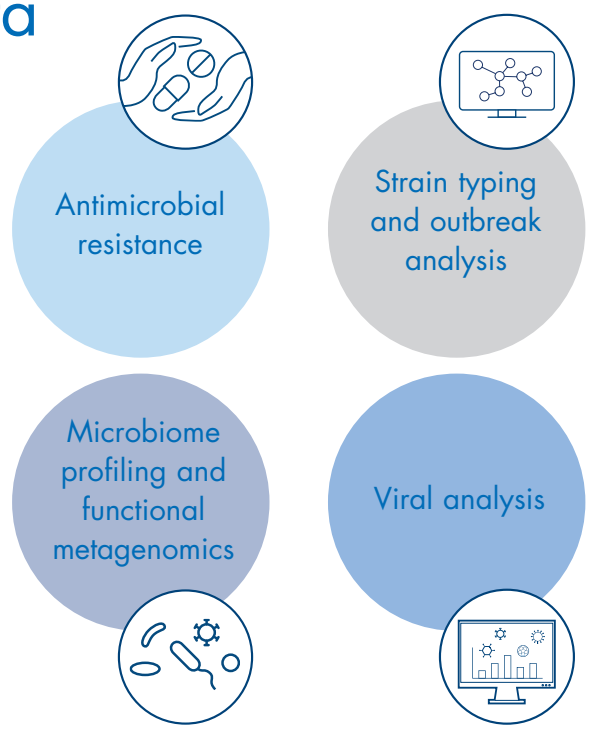
QIAseq xHYB gives an average of 2400x enrichment of viral targets vs. standard shotgun sequencing

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

Making sense of complex microbial genomic data

Only well-structured and clear data help you understand what is going on and allow you to take action. 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 and metagenomics.



Ordering information

Product	Description	Cat. no.
TissueLyser II	For convenient and secure sample disruption for variable throughput, from tubes to 96-well plates	85300
PowerLyzer 24 Homogenizer	For the fast homogenization of any biological sample in 2 ml tubes	13155
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 QIAsphere	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
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
QIAamplifier 96	For fast and high performance end-point PCR experiments in a 96-well format	9002991 9002990
QIAcuity OneStep Advanced Probe Kit	For one-step RT-PCR on the QIAcuity digital PCR instruments	250131 250132

* 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 or contact your local sales representative

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 PCR/ RT-PCR Kits*	For highly sensitive, specific and ultrafast probe-based real-time PCR/RT-PCR and multiplex PCR/RT-PCR, and unparalleled results using SYBR Green-based qPCR	208152 208352
UCP PCR Kits	For SYBR Green-based and probe-based, real-time quantitative PCR for microbiome or quality control applications using reagents from Ultra Clean Production	208012 208212
UCP Multiplex PCR Kit	Ultra-Clean Production master mix for multiplex hot-start PCR and microbiome applications	206742 206744
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

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 or can be requested from QIAGEN Technical Services (or your local distributor).

Trademarks: QIAGEN®, Sample to Insight®, QIAamp®, QIAamplifier®, QIAcube®, QIAcuity®, QIAseq®, QIAsymphony®, QIAquant®, AllPrep®, DNeasy®, Inhibitor Removal Technology®, PowerLyzer®, PowerFecal®, PowerSoil®, PowerViral®, QuantiNova® (QIAGEN Group); GT Molecular® (GT Molecular LLC). Registered names, trademarks, etc. used in this document, even when not specifically marked as such, may still be protected.

PROM-20709-003 1127765 06/2022 © 2022 QIAGEN, all rights reserved.

Ordering	**www.qiagen.com/shop**
Technical Support	**www.support.qiagen.com**
Website	**www.qiagen.com**