



# Making the invisible visible

Decode the microbial universe – from microbiome to microbes – with our application-specific workflows



# Legal disclaimer



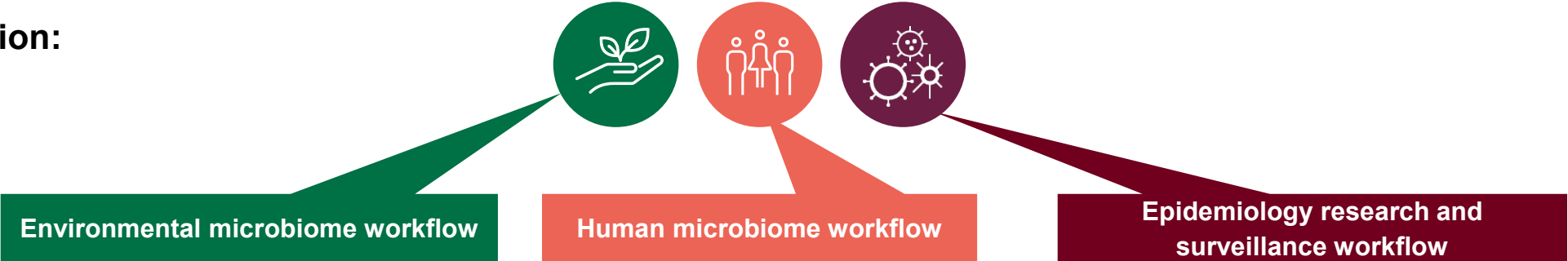
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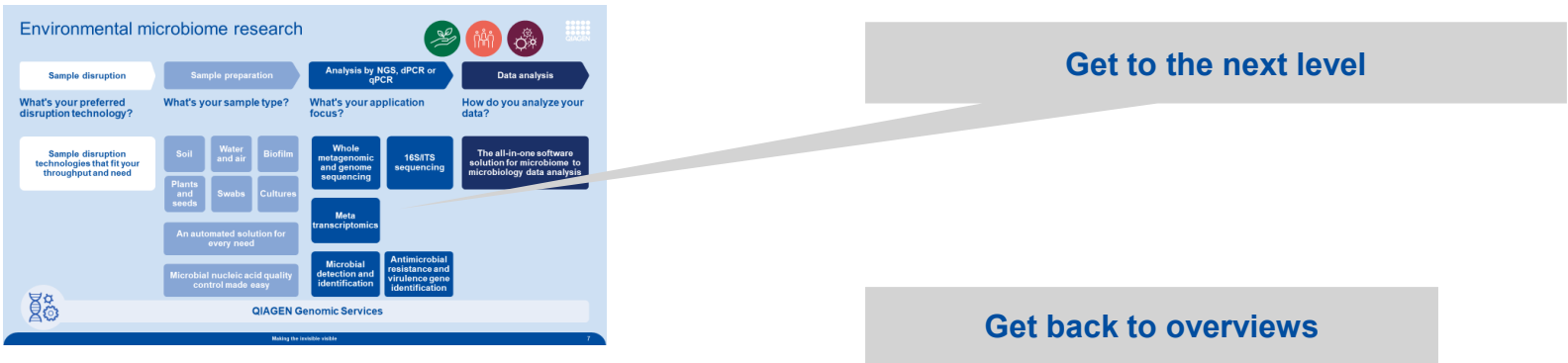
# Navigation elements in this booklet



Top navigation:



Workflow navigation:



Further navigation:



# From microbiomes to microbes



- Microbes are ubiquitous, playing varied roles across environments, including the health and well-being of humans
- Microbial communities are highly diverse, as are their compositions, interactions and functions
- Microbiome research investigates the impact that these complex communities have on our health and the environment
- Besides microbiome research, the advent of new technologies for testing, rapid detection and systematic surveillance of various microbes is helping us identify and possibly prevent crises related to health and safety

# Decoding the microbial universe from research to routine testing and surveillance



QIAGEN sample technologies and workflow solutions, including dedicated next-generation sequencing (NGS), digital PCR (dPCR) and real-time PCR assays, support various microbiome research applications:



**Microbiome research in human applications**



**Microbiome research in environmental applications**



**Functional microbiome analysis – metatranscriptomics**



**Identification of antimicrobial resistance genes**



**Strain typing and outbreak analysis**



**Bacterial species identification and detection of virulence genes**

# Let's discuss our capabilities based on your focus

## Environmental microbiome research

- The environmental microbiome adapts to external changes and is central to the earth's systems and cycles
- Microbes also play an essential role in agricultural practices and – through applied microbiology – provide solutions to our everyday needs



## Human microbiome research

Understanding the human microbiome and its interactions offer:

- New routes for personalized healthcare strategies
- New therapeutic strategies for improving well-being or multiple diseases



## Epidemiology research and surveillance

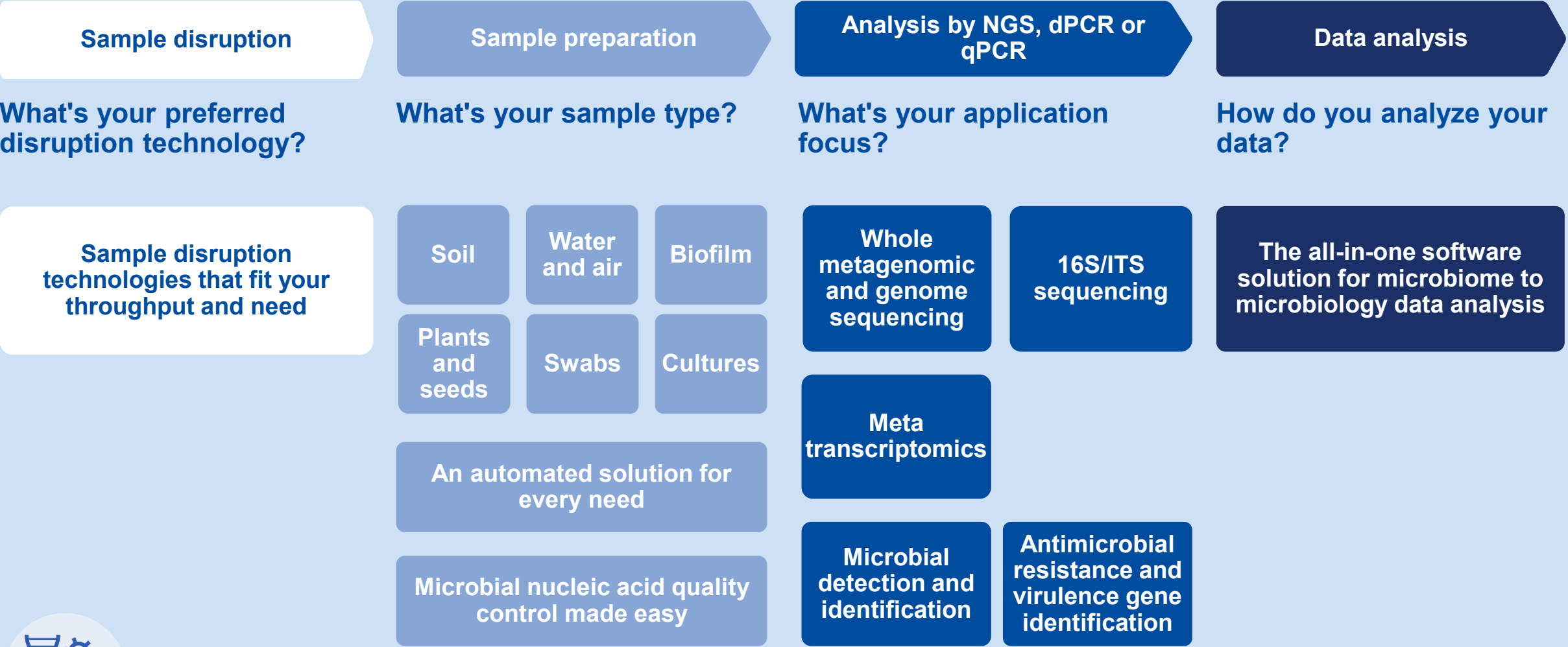
Response to public health scenarios demands systematic collection, analysis, and rapid detection and identification of:

- Pathogenic organisms
- Virulence genes
- Antimicrobial resistance (AMR) genes



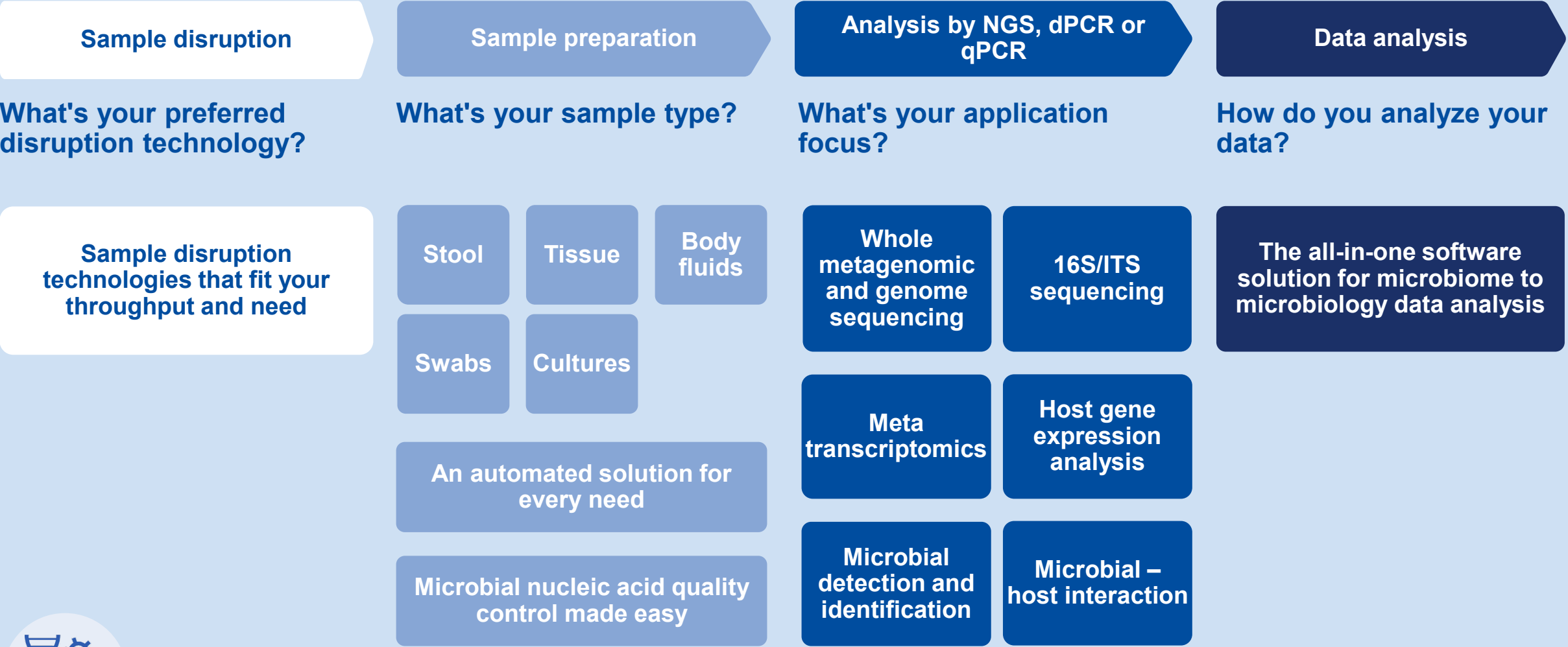
Want to first get an overview of our complete workflow solutions? Go to the [portfolio overview](#)

# Environmental microbiome research



QIAGEN Genomic Services

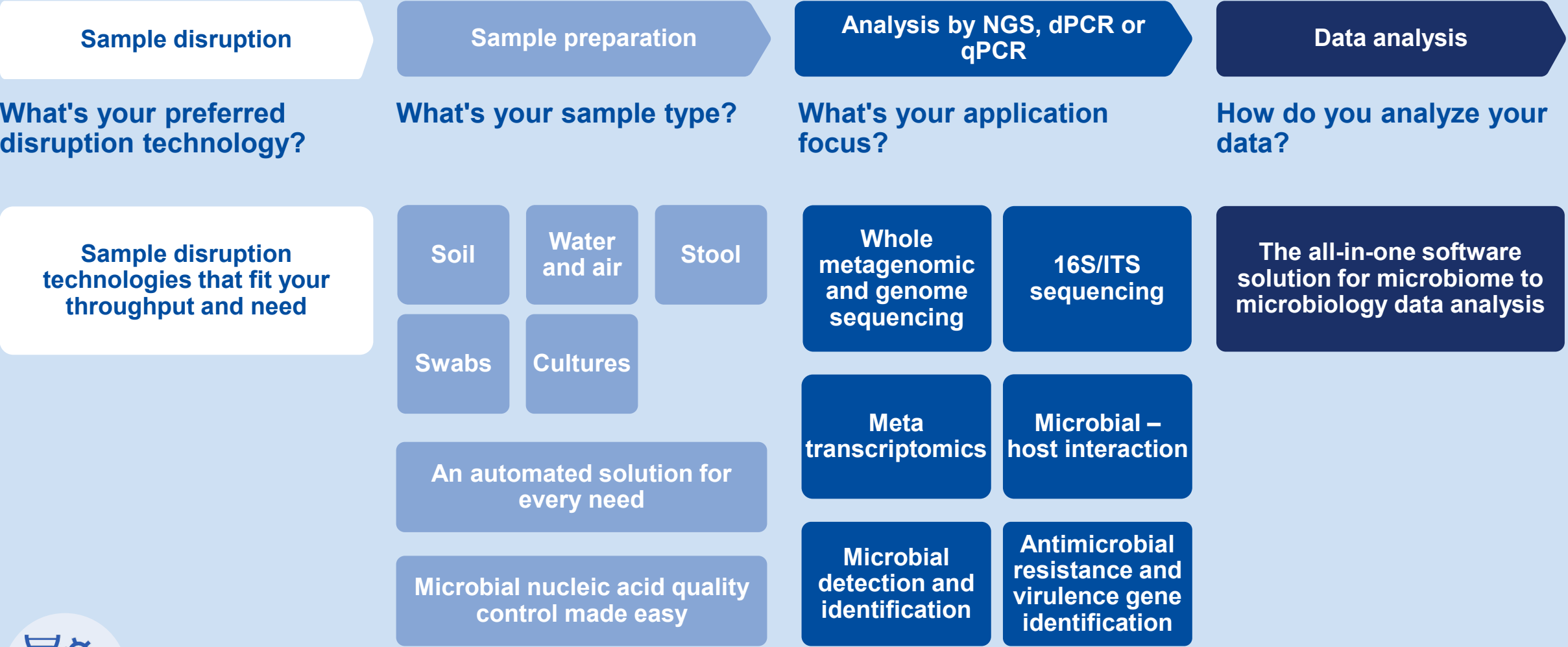
# Human microbiome research



QIAGEN Genomic Services



# Epidemiology research and surveillance



QIAGEN Genomic Services

# Sample disruption – various options to meet your needs



## TissueLyser III

### Up to 192 samples at the same time

- Throughput of up to 192 samples in 96-well plates
- Tube volumes of 2, 5 mL, 96-well, 50 mL to be added soon
- User-friendly touchscreen and rotary knob
- Seven pre-programmed protocols and five customizable protocols
- Custom program cycles for added convenience



## Vortex adapters

### Connects directly to the Vortex-Genie 2 Vortex for hands-free disruption

- Maximizes vortexing by shaking multiple tubes at the same time, ideal for bead beating
- Adapters are available for 1.5–2 mL, 5 mL, 15 mL and 50 mL tubes



## QIAGEN PowerBead Pro Tubes and Plates

### Pre-filled tubes and plates for optimized lysis

- The PowerBead Pro Tubes and Plates use the latest bead-beating technology from QIAGEN
- Filled with zirconia beads
- Combined with the lysis buffer, it allows a better disruption of gram-positive bacteria, archaea and fungi



Target	Process	Format/ samples per run	Instrument	Recommended kit	Good to know
Microbial DNA	Manual or automated	Spin column	Automatable on <a href="#">QIAcube Connect</a>	<a href="#">DNeasy PowerSoil Pro</a>	High purity to achieve amplifiable DNA for unbiased results
		Silica spin filter tubes	N.A.	DNeasy PowerMax Soil	Up to 10 g for low biomass (e.g., sandy and clayey soils)
		96-well plate	N.A.	<a href="#">DNeasy 96 PowerSoil Pro</a>	Manual processing on plates
	Automated	Up to 12	<a href="#">QIAcube Connect</a>	<a href="#">DNeasy PowerSoil Pro</a> <a href="#">QIAcube</a>	For your convenience spin columns and tubes already loaded onto the rotor
		96	<a href="#">QIAcube HT</a>	<a href="#">DNeasy 96 PowerSoil Pro</a> <a href="#">QIAcube HT</a>	High throughput isolation of DNA
		96	KingFisher Flex Eppendorf epMotion	<a href="#">MagAttract PowerSoil Pro</a> <a href="#">DNA</a>	Improved yield, improved inhibitor removal
	Automation compatible	Spin column + seq kit	Extraction automatable <a href="#">QIAcube Connect</a>	<a href="#">DNeasy PowerSoil Pro</a> <a href="#">DNA WGS SeqSet</a>	All-in-one microbial sequencing set
Microbial RNA	Manual	Maxi spin column	N.A.	<a href="#">RNeasy PowerMax Soil</a> <a href="#">Pro</a>	Starting amount up to 15 g for all & low biomass soil samples



Soil can contain high amounts of humic substances, which are large water-soluble anionic molecules that interfere with amplification reactions during analysis

The DNeasy and RNeasy Power Pro Kits provide a novel bead tube and optimized chemistry for efficient lysis and the QIAGEN Inhibitor Removal Technology (IRT)\* to successfully remove those inhibitors during nucleic acid preparation

\*Patent pending



Target	Process	Format/ samples per run	Instrument	Recommended kit	Good to know
Microbial DNA	Manual or automated	Spin column	Automatable on QIAcube Connect	DNeasy PowerWater	<ul style="list-style-type: none"> <li>Optimized for use with most filter membrane types</li> <li>Optimized to increase DNA yields from low biomass samples</li> <li>Easily removes blocks to downstream PCR with IRT</li> </ul>
	Manual	Spin column	N.A.	DNeasy PowerWater Sterivex	Optimized for use with Sterivex filter units
RNA	Manual or automated	Spin column	Automatable on QIAcube Connect	RNeasy PowerFecal Pro	Recommended for most efficient detection of SARS-CoV-2 in wastewater samples
		Spin column	N.A.	RNeasy PowerWater	For use with most filter membrane types
DNA and RNA	Manual or automated	Spin column	N.A.	AllPrep PowerFecal Pro DNA/RNA	Recommended for detection of SARS-CoV-2 in wastewater samples
			Automatable on QIAcube Connect	AllPrep PowerViral DNA/RNA	Isolate viral or bacterial DNA and RNA from wastewater, stool, biosolids and gut material



## Our recommendations for the most efficient detection of SARS-CoV-2 in wastewater samples:

- Centrifuge to create a solid pellet
- Extract nucleic acids with RNeasy PowerFecal Pro (preferred) or AllPrep PowerViral DNA/ RNA
- Elute in 50 µL
- Bead beating is necessary when processing pellets, but not supernatant
- Use of phenol-chloroform-isoamyl (PCI) may be beneficial but is not critical for the detection
- Absolute quantification with nanoplate-based dPCR on the [QIAcuity dPCR System](#)



Target	Process	Format	Instrument	Recommended kit	Good to know
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Microbial DNA	Manual or automated	Spin column	Automatable on QIAcube Connect	DNeasy PowerBiofilm	<ul style="list-style-type: none"><li>Efficient cell lysis by pairing chemical and mechanical lysis techniques</li><li>IRT removes concentrated inhibitors such as humic acid, metals, salts and pesticides, typically present in biofilms</li><li>High yields of inhibitor-free DNA from all types of biofilms, including microbial mats</li></ul>
RNA	Manual or automated	Spin column	N.A.	RNeasy PowerBiofilm	<ul style="list-style-type: none"><li>High-quality RNA isolation from all biofilms, challenging dental plaques and microbial mats</li><li>High RNA yields with pre-treatment, mechanical and chemical lysis</li><li>Efficient elimination of PCR inhibitors with IRT</li></ul>



Depending on the microbial species producing the biofilm, its structure, chemistry, enzymatic and water compositions and thickness can vary widely

To build biofilms, microbes produce extracellular polymeric substances (EPS), which comprise polysaccharides, proteins, extracellular DNA (eDNA), humic substances and lipids in various amounts

# Plants and seeds



Target	Process	Format	Instrument	Recommended kit	Good to know
Microbial DNA	Manual or automated	Spin column	Automatable on QIAcube Connect	DNeasy Plant Pro	<ul style="list-style-type: none"> <li>Highly efficient lysis and release of DNA from tough plant materials and associated plant pathogens</li> <li>Superior PCR performance with IRT</li> <li>Rapid extraction of ready-to-use DNA</li> <li>No organic extraction or ethanol precipitation</li> </ul>
		96-well plate	N.A.	DNeasy 96 Plant	<ul style="list-style-type: none"> <li>Uses bead-beating technology, replacing cumbersome DNA isolation procedures such as CTAB, phenol or chloroform extraction</li> <li>Recovery of high-quality DNA from the toughest sample types</li> <li>IRT to remove PCR inhibitors, including polysaccharides, polyphenolics and other secondary metabolites</li> </ul>
RNA	Manual or automated	Spin column	N.A.	RNeasy PowerPlant	<ul style="list-style-type: none"> <li>Optimized to isolate RNA from the most difficult plant types</li> <li>IRT removes 100% of polyphenolics, polysaccharides and other PCR inhibitors</li> <li>Rapid protocol enables isolation of RNA in 30 minutes without the use of harsh chemicals</li> </ul>



Detecting plant pathogens, such as viruses, bacteria, fungi and yeast is essential for monitoring agricultural ecosystems

Plant pathogen detection starts with high-quality nucleic acid extraction from various sample types

Bead-beating technology, IRT and removal of high polyphenolic content ensure efficient and reproducible results for any downstream application, like PCR and NGS



Target	Process	Format	Instrument	Recommended kit	Good to know
Microbial DNA	Manual or automated	Spin column	Automatable on QIAcube Connect	<u><a href="#">QIAamp DNA Microbiome</a></u>	With the effective depletion of host DNA this kit is recommended for whole genome sequencing applications
		Spin column	Automatable on QIAcube Connect	<u><a href="#">QIAamp PowerFecal Pro DNA</a></u>	Our recommendation when performing 16S/ITS sequencing



**Want to discuss your research project or any tissue-specific needs?**

**Get in touch!**

**[ask.a.microbiome.scientist@qiagen.com](mailto:ask.a.microbiome.scientist@qiagen.com)**



Target	Process	Format/ samples per run	Instrument	Recommended kit	Good to know
Microbial DNA	Manual or automated	Spin column	Automatable on QIAcube Connect	<a href="#">QIAamp PowerFecal Pro DNA</a>	Yields high alpha diversity in sequencing
		Spin column	Automatable on QIAcube Connect	<a href="#">QIAamp DNA Microbiome</a>	Effective depletion of host DNA
		96-well plate	N.A.	<a href="#">DNeasy 96 PowerSoil Pro</a>	Manual processing on plates
	Automated	Up to 12	QIAcube Connect	<a href="#">QIAamp PowerFecal Pro DNA</a> <a href="#">QIAcube</a>	Spin columns and tubes are already loaded onto the rotor for convenience
		24 or 96	QIASymphony SP	<a href="#">QIASymphony PowerFecal Pro DNA</a>	Mid- to high-throughput automated sample preparation
		96	QIAcube HT	<a href="#">DNeasy 96 PowerSoil Pro QIAcube HT</a>	96 well format for scaling up your sample throughput
		96	KingFisher Flex Eppendorf epMotion	<a href="#">MagAttract PowerSoil Pro DNA</a>	Improved yield, improved inhibitor removal
Host gDNA	Manual or automated	Spin column	Automatable on QIAcube Connect	QIAamp Fast DNA Stool Mini Kit	Isolate human DNA as well as microbial DNA



In the Power Pro kits, the swabs are put directly in the bead tube or plate





Target	Process	Format/ samples per run	Instrument	Recommended kit	Good to know
Microbial RNA	Manual or automated	Spin column	Automatable on QIAcube Connect	<u>RNeasy PowerFecal Pro</u>	Novel bead tube and optimized chemistry and IRT
Microbial DNA and RNA	Manual or automated	Spin column	N.A.	<u>AllPrep PowerFecal Pro DNA/RNA</u>	Metagenomic and metatranscriptomic analysis from the same sample
	Automated	96	KingFisher Flex, Eppendorf epMotion	MagAttract PowerMicrobiome DNA/RNA	Optimized for automated, hands-free purification  Improved downstream applications with IRT



The Power Pro kits provide a novel bead tube and optimized chemistry for efficient lysis and QIAGEN IRT to successfully remove those inhibitors during nucleic acid preparation

# Stool

DNA from stool

RNA and total NA from stool



Target	Process	Format/ samples per run	Instrument	Recommended kit	Good to know
Microbial DNA	Manual or automated	Spin column	Automatable on <a href="#">QIAcube Connect</a>	<a href="#">QIAamp PowerFecal Pro DNA</a>	Yields high alpha diversity in sequencing
		96-well plate	N.A.	<a href="#">DNeasy 96 PowerSoil Pro</a>	Manual processing on plates
	Automated	Up to 12	<a href="#">QIAcube Connect</a>	<a href="#">QIAamp PowerFecal Pro DNA QIAcube</a>	Spin columns and tubes are already loaded onto the rotor for convenience
		24 or 96	<a href="#">QIASymphony SP</a>	<a href="#">QIASymphony PowerFecal Pro DNA</a>	Mid- to high-throughput automated sample preparation
		96	<a href="#">QIAcube HT</a>	<a href="#">DNeasy 96 PowerSoil Pro QIAcube HT</a>	96-well format for scaling up your sample throughput
		96	KingFisher Flex, Eppendorf epMotion	<a href="#">MagAttract PowerSoil Pro DNA</a>	Improved yield, improved inhibitor removal
	Automation compatible	Spin column + seq kit	Extraction automatable on <a href="#">QIAcube Connect</a>	<a href="#">QIAamp PowerFecal Pro WGS SeqSet</a>	All-in-one microbial sequencing set
Host gDNA	Manual or automated	Spin column	Automatable on <a href="#">QIAcube Connect</a>	<a href="#">QIAamp Fast DNA Stool Mini Kit</a>	Isolate human DNA as well as microbial DNA



Stool samples should be preserved as soon as possible after collection to protect the integrity of the nucleic acids

[PowerProtect DNA/RNA](#) is a bulk stabilization solution for stool samples that ensures the integrity of the microbial community and functional profile before nucleic acid extraction



Target	Process	Format/ samples per run	Instrument	Recommended kit	Good to know
Microbial RNA	Manual or automated	Spin column	Automatable on QIAcube Connect	<u>RNeasy PowerFecal Pro</u>	Novel bead tube and optimized chemistry and IRT
Microbial DNA and RNA	Manual or automated	Spin column	N.A.	<u>AllPrep PowerFecal Pro DNA/RNA<sup>1</sup></u>	Metagenomic and metatranscriptomic analysis from the same sample
		Spin column	Automatable on QIAcube Connect	AllPrep PowerViral DNA/RNA	Optimized to isolate viral or bacterial DNA and RNA from wastewater, stool, biosolids and gut material
	Automated	96	KingFisher Flex, Eppendorf epMotion	MagAttract PowerMicrobiome DNA/RNA	Optimized for automated, hands-free purification  Improved downstream applications with IRT



Stool samples should be preserved as soon as possible after collection to protect the integrity of the nucleic acids

[PowerProtect DNA/RNA](#) is a bulk stabilization solution for stool samples that ensures the integrity of the microbial community and functional profile before nucleic acid extraction

(1) Custom protocol available

# Cultured bacteria



Target	Process	Format	Instrument	Recommended kit	Good to know
Microbial DNA	Manual or automated	Spin column	Automatable on QIAcube Connect	<u>DNeasy PowerSoil Pro</u>	Our latest generation solution for efficient isolation of microbial DNA
		Spin column	Automatable on QIAcube Connect	DNeasy PowerLyzer Microbial	Bead-based isolation of high-quality DNA from microbial culture
		Silica spin filter	Automatable on QIAcube Connect	DNeasy UltraClean Microbial	Fast and easy DNA purification with spin filter extraction
		Spin column	N.A.	QIAamp BiOstic Bacteremia DNA Kit	Isolation of bacterial DNA from cultured blood, fecal and cervical swabs
Microbial RNA	Manual or automated	Spin column	Automatable on QIAcube Connect	<u>RNeasy PowerFecal Pro</u>	Our latest generation solution for efficient isolation of microbial RNA
Microbial DNA and RNA	Manual or automated	Spin column	N.A.	AllPrep PowerFecal Pro	Our latest generation solution for efficient isolation of microbial DNA and RNA
		Spin column	Automatable on QIAcube Connect	AllPrep Bacterial/Fungal DNA/RNA/Protein Kits	Extraction of protein and nucleic acids from bacterial cell cultures



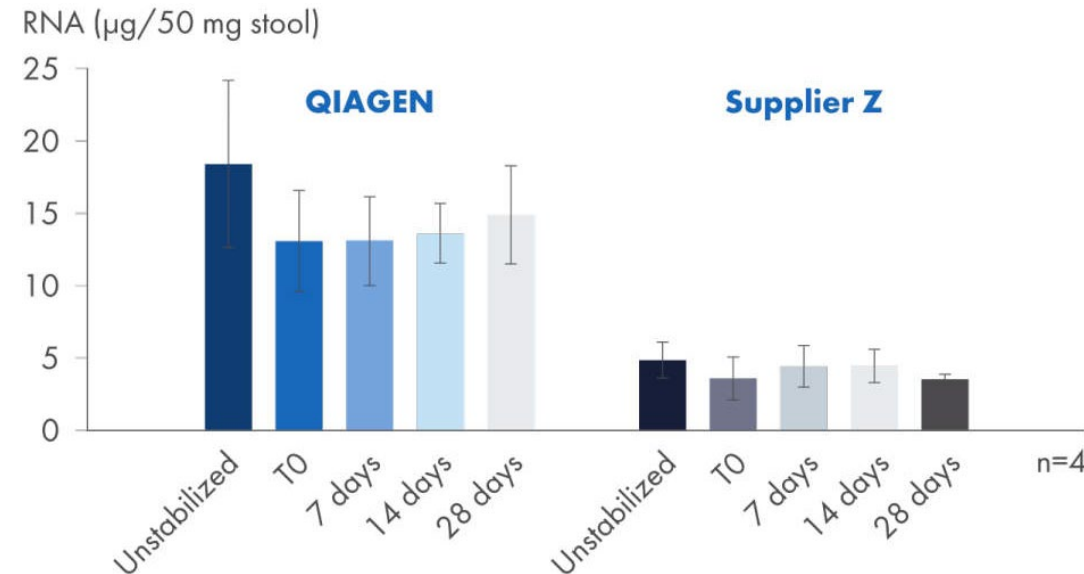
The Power Pro kits provide a novel bead tube and optimized chemistry for efficient lysis and QIAGEN IRT to successfully remove inhibitors during nucleic acid preparation



## Stabilization and preservation of the microbial community profile and expression profile in stool samples

- Preserves bacterial DNA and RNA in stool samples after collection
- Penetrates cells and halts biological activity
- Maintains the microbial community and functional profile during transport and storage
- Stabilizes nucleic acids at room temperature or frozen for an extended period
- Stabilizes nucleic acids for a shorter duration at 35°C

## The QIAGEN workflow with PowerProtect DNA/RNA delivers consistent yields for up to 28 days and higher DNA concentration



- Four replicates of one stool sample were processed using the QIAGEN workflow or the Supplier Z workflow
- The samples were stored in PowerProtect DNA/RNA or in Supplier Z at room temperature before they were extracted with RNeasy PowerFecal Pro or Supplier Z at time points 0, 7, 14 and 28 days
- The unstabilized sample was used as reference



To achieve the greatest purity and most accurate representation of microbial diversity with the Power Pro kits, your starting material should be stabilized in PowerProtect DNA/RNA instead of another commercial solution

If your sample is stabilized in Zymo's DNA/RNA Shield solution, contact QIAGEN Technical Service for the pre-treatment protocol

# DNeasy PowerSoil Pro Kit

## DNeasy PowerSoil Pro QIAcube Kit

Soil

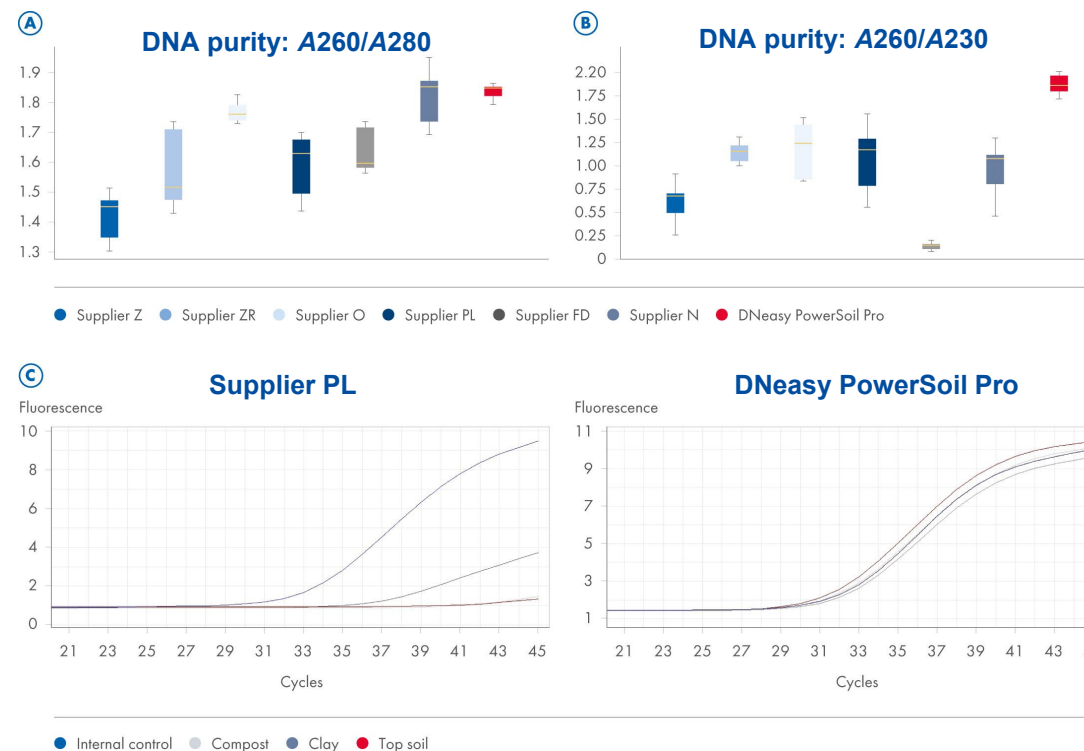


### Efficient isolation of microbial genomic DNA from all soil types

- Efficient lysis of bacteria and fungi in all soil types
- Up to 8-fold higher DNA yields compared to alternative methods
- Recovery of inhibitor-free DNA for direct use in NGS applications
- Unbiased results accurately represent sample alpha diversity
- Manual processing or automated workflow on the QIAcube Connect

Data obtained using experiments conducted by QIAGEN R&D in Hilden, Germany

### DNA extracted with the DNeasy PowerSoil Pro Kit with IRT shows an increased purity with no inhibition of PCR



- Soil samples (250 mg each) were prepared using commercially available sample preparation solutions and compared to the DNeasy PowerSoil Pro Kit and DNA purity depicted via UV measurements
- Inhibitor removal was visualized with an inhibitor-sensitive PCR with an internal control (eluate-free reactions) spiked with eluates from Supplier PL and the DNeasy kit



The kit features a novel bead tube and optimized chemistry for more efficient lysis of soil bacteria and fungi

The kit also contains streamlined IRT to rapidly eliminate the challenging inhibitors commonly found in soil and environmental samples

# RNeasy PowerMax Soil Pro Kit

Soil

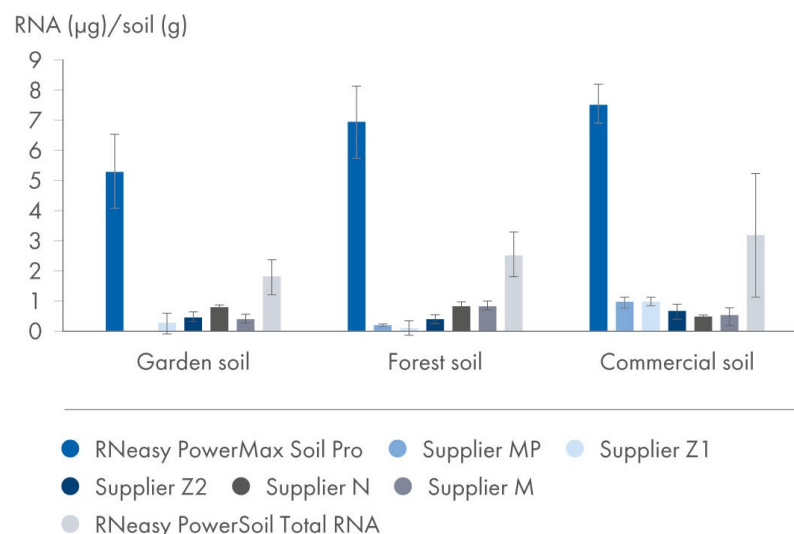


**Maximal isolation of microbial RNA from large volumes of soil samples including compost, sediment, and manure**

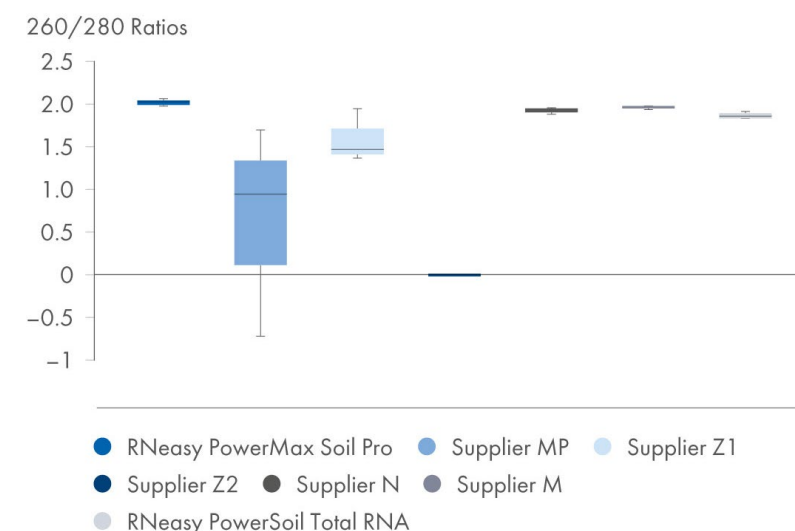
- Uses second-generation IRT
- Efficient bead beating and lysis chemistry ensures high RNA yields from large sample quantities
- Suitable for soil types rich in humic acid, including compost, sediment, and manure
- Ready-to-use RNA suitable for downstream applications such as RT-PCR, digital PCR, qPCR, and next-gen sequencing

**Extractions using the RNeasy PowerMax Soil Pro Kit result in high RNA yield with excellent purity**

**RNA yield – extraction efficiency**



**RNA purity: A260/A280**



- RNA was isolated from soil of three different sources in triplicate according to the respective manufacturer's protocol. RNA yield was quantified by a fluorometric measurement and the extraction efficiency was calculated using the soil amount applied for each preparation.
- RNA purity from all sources was determined by a UV-Vis measurement. Error bars represent standard deviation.

Data obtained using experiments conducted by  
QIAGEN R&D in Hilden, Germany



# QIAamp PowerFecal Pro DNA Kit

## QIAamp PowerFecal Pro DNA QIAcube Kit

DNA from stool

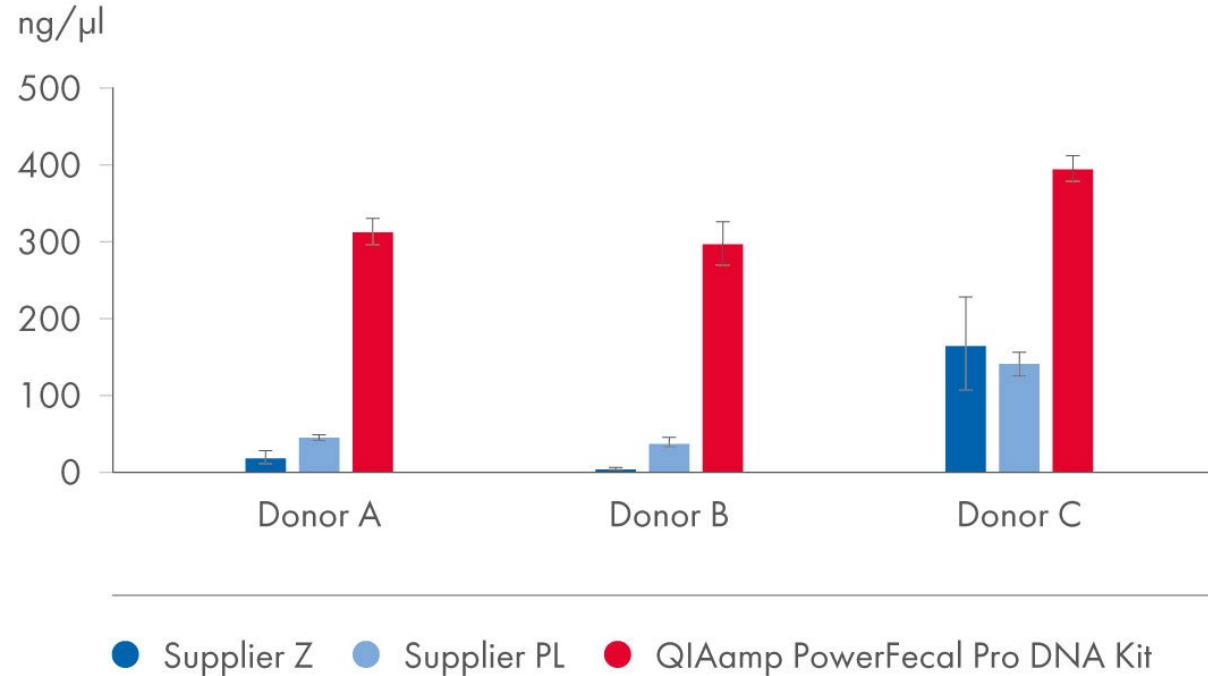
DNA from swabs



### Efficient isolation of bacterial and fungal DNA from stool and gut samples

- Up to 20-fold more DNA compared to alternative methods
- Yields higher alpha diversity in sequencing compared to other methods
- Recovers inhibitor-free DNA for direct use in downstream applications
- Manual processing or automated workflow on the QIAcube Connect

### Higher yields of DNA with the QIAamp PowerFecal Pro DNA Kit



- DNA was isolated from stool samples (200 mg) from three donors using commercially available sample preparation solutions and the new QIAamp PowerFecal Pro DNA Kit and compared
- Yields were measured by fluorometric quantification (Qubit)



[Li S. et al. Salt-Sensitive Ileal Microbiota Plays a Role in Atrial Natriuretic Peptide Deficiency-Induced Cardiac Injury. \*Nutrients\*. 2022;14\(15\):3129](#)

[Juckel G, Manitz MP, Freund N, Gatermann S. Impact of Poly I:C induced maternal immune activation on offspring's gut microbiome diversity - Implications for schizophrenia. \*Prog Neuropsychopharmacol Biol Psychiatry\*. 2021;110:110306](#)



# DNeasy 96 PowerSoil Pro Kit

## DNeasy 96 PowerSoil Pro QIAcube HT Kit

DNA from stool

Soil

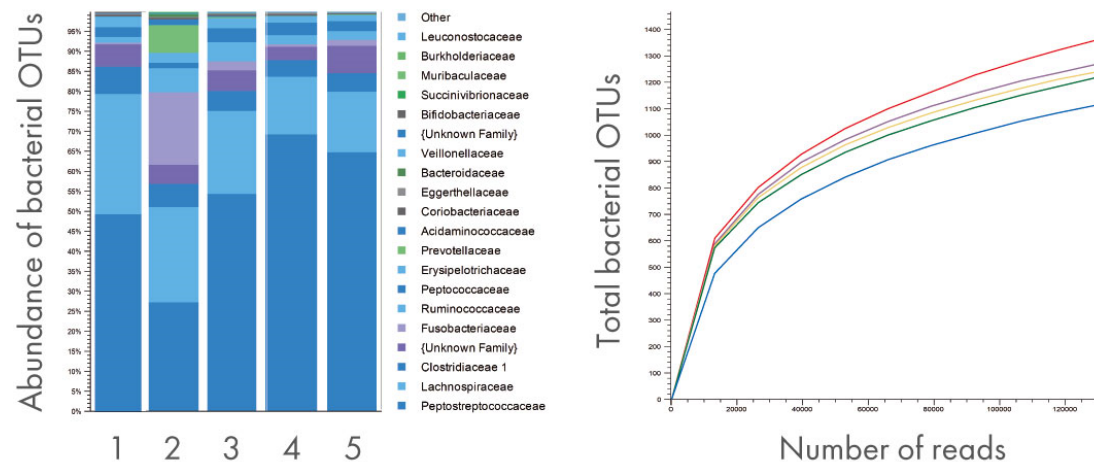
DNA from swabs



High-throughput isolation (manual or automated on QIAcube HT) of bacterial and fungal DNA from all soil and stool types, including difficult samples such as compost, sediment and manure

- Efficient lysis of bacteria and fungi efficiently from soil, stool and gut samples
- 96-well format for scaling up with the same proven technology of the DNeasy PowerSoil Pro Kit
- Recovers inhibitor-free DNA, ready to use directly in downstream applications

The DNeasy 96 PowerSoil Pro shows increased bacterial representation compared to other technologies



1 and ● DNeasy 96 PowerSoil Pro 2 and ● Supplier N  
3 and ● Supplier Z 4 and ● Supplier MN1 5 and ● Supplier MN2

- DNA prepared from dog stool samples was isolated with DNeasy 96 PowerSoil Pro and other technologies
- 16S libraries of the 16S gene (V4 region) were prepared using barcoded 515fB and 806rB primers and the QIAGEN UCP Multiplex PCR Kit
- Data analysis was performed with the QIAGEN CLC Genomics Workbench using the SILVA database, and each result represents an aggregation of two datasets per sample



[Antoine D, Singh PK, Tao J, Roy S. Neonatal morphine results in long-lasting alterations to the gut microbiome in adolescence and adulthood in a murine model. \*Pharmaceutics\*. 2022;14\(9\):1879](#)

[Charles Jacob HK, et al. Identification of novel early pancreatic cancer biomarkers KIF5B and SFRP2 from "first contact" interactions in the tumor microenvironment. \*J Exp Clin Cancer Res\*. 2022;41\(1\):258](#)

[L.Tian et al. Rhizosphere bacterial community structure of three minor grain crops: A case-study from paired field sites in Northern China. \*Land Degrad. Dev\*. 2022;33\(1\),104–116](#)

# QIASymphony PowerFecal Pro DNA

DNA from stool

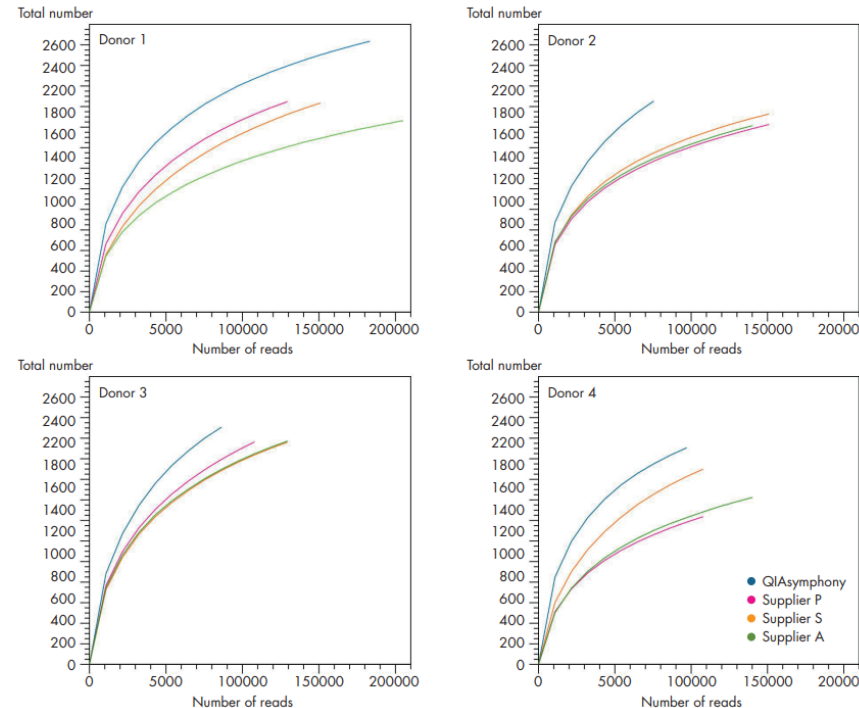
DNA from swabs



## Efficient lysis of bacteria and fungi in all stool and soil samples

- Automated workflow with medium throughput on QIASymphony SP
- Standardized DNA extraction
- Unbiased results accurately represent sample alpha diversity
- The kit is designed for 192 samples with continuous loading of 24-sample batches

## Highest microbial diversity with QIASymphony PowerFecal Pro DNA



- DNA from four different stool samples was isolated using the QIASymphony PowerFecal Pro DNA Kit and three other commercially available kits, with each sample processed in quadruplicate
- DNA was then used for library construction with the QIAGEN UCP Multiplex PCR Kit using adapter-modified 515F-806R 16S primer, sequenced on a MiSeq system and analyzed using QIAGEN CLC Genomics Workbench



Automated viral RNA extraction on QIASymphony SP – a use case:

[Wurtzer. S. et al. Several forms of SARS-CoV-2 RNA can be detected in wastewaters: Implication for epidemiology and risk assessment. \*Water Res.\* 2021;198:117183](#)

# MagAttract PowerSoil Pro DNA

DNA from swabs

Soil

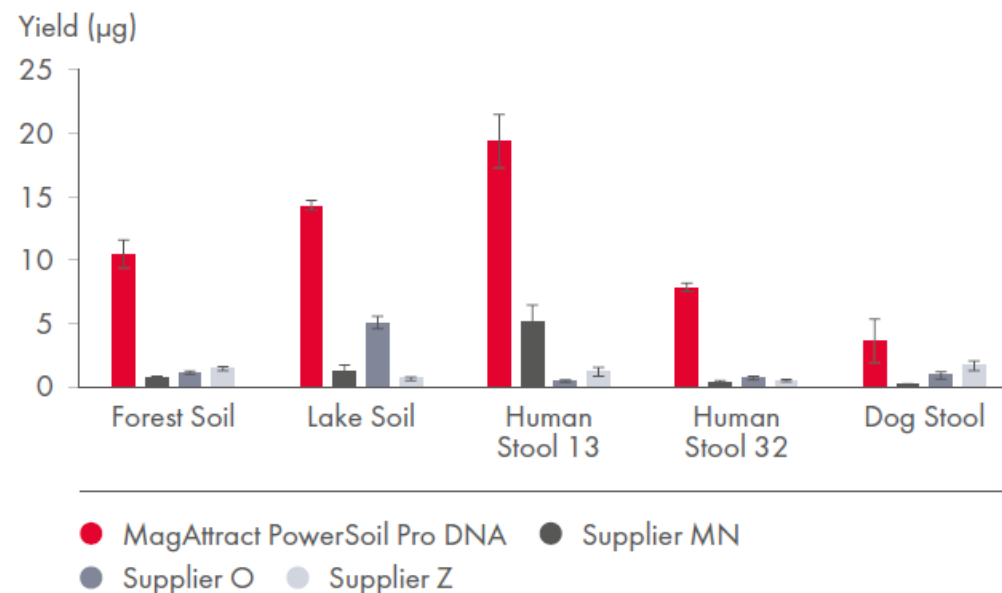
DNA from stool



## High quality DNA isolation from tough soil, stool and other environmental samples

- Improved yield through more efficient PowerBead Pro Plates and Tubes, and lysis chemistry
- Improved inhibitor removal eliminates more PCR-inhibiting compounds for greater nucleic acid purity

## Higher yields were obtained with the MagAttract PowerSoil Pro DNA Kit than all competitor kits



- DNA was isolated from soil (forest, lake) and stool (human and dog) according to the appropriate protocol
- The DNA yields were measured with a Qubit Fluorometer



The MagAttract PowerSoil Pro DNA Kit allows automated high-throughput isolation of DNA from up to 384 soil samples in less than one day and is optimized for use with the ThermoFisher KingFisher Flex platform and Eppendorf epMotion

# RNeasy PowerFecal Pro

RNA and total NA from stool

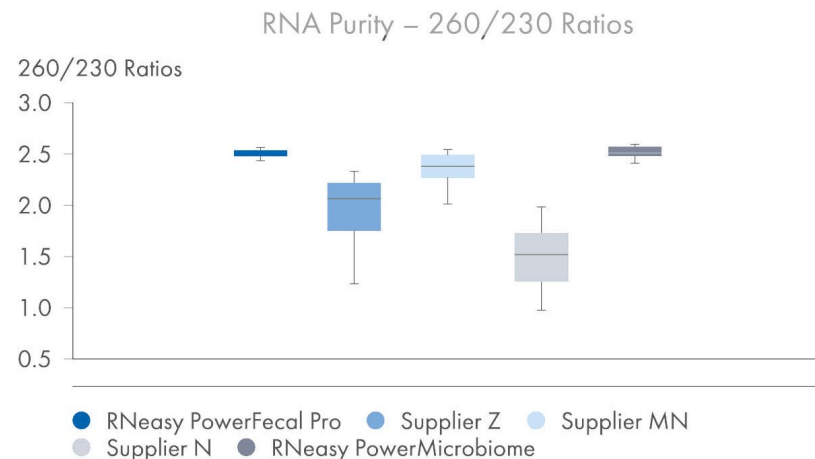
RNA and total NA from swabs



## Isolation of microbial RNA from stool and gut samples, sludge, or wastewater or swabs

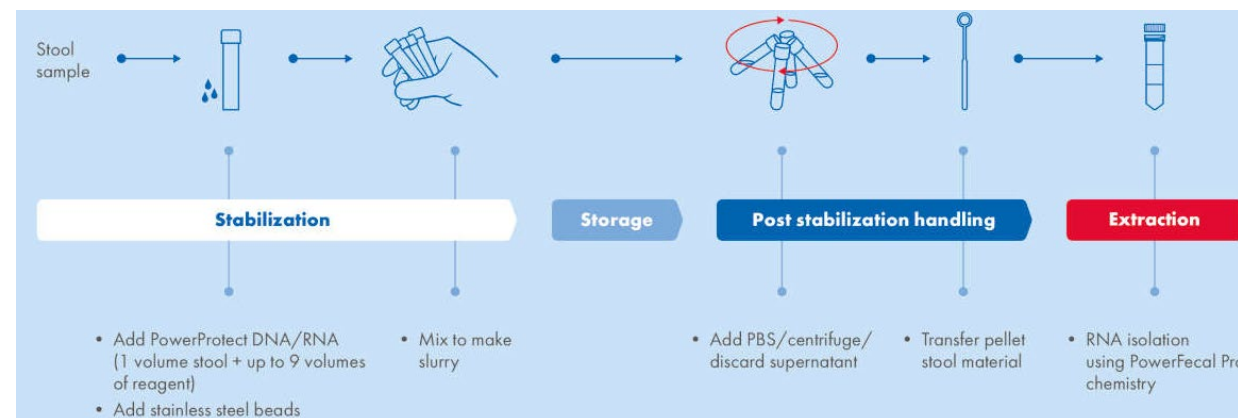
- Efficient isolation of high-quality microbial RNA from difficult microbiome samples
- Easy-to-use protocol
- The kit features a novel bead tube and optimized chemistry for more efficient lysis of stool bacteria and fungi
- The kit also contains streamlined IRT to rapidly eliminate the challenging inhibitors commonly found in stool and gut samples
- Automatable on QIAcube Connect

## Pure RNA isolated from stool with RNeasy PowerFecal Pro



- Three commercially available kits and the RNeasy PowerMicrobiome kits were used for the comparison with RNeasy PowerFecal Pro Kit
- Four frozen stool samples in triplicates were tested

## Workflow including sample stabilization with PowerProtect DNA/RNA Kit



Data obtained using experiments conducted by QIAGEN R&D in Hilden, Germany

# AllPrep PowerFecal Pro DNA/RNA

RNA and total NA from stool

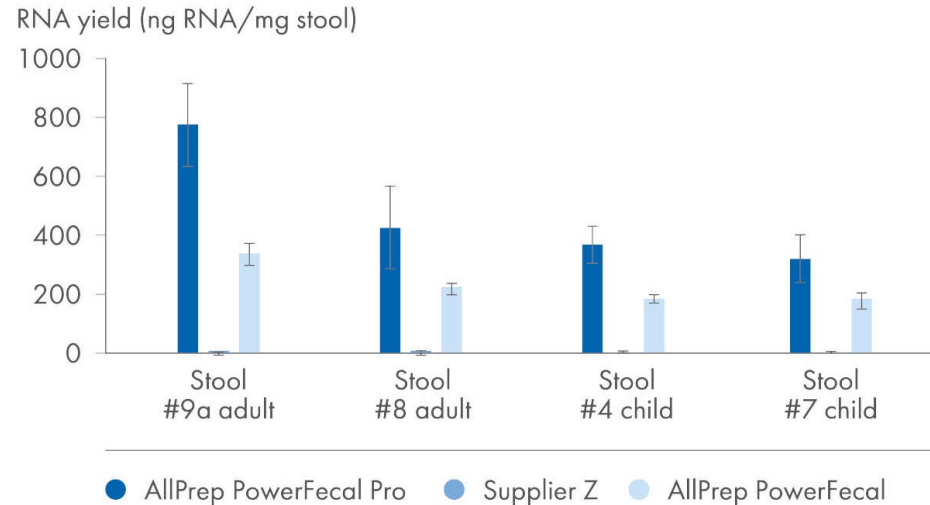
RNA and total NA from swabs



## Simultaneous purification of microbial DNA and total RNA from the same stool sample

- Improved, second generation IRT
- Separates RNA and DNA from the same sample into different eluates in one procedure
- Optimized lysis for increased DNA and RNA yields
- RNA and DNA for metagenomic and metatranscriptomic analysis

## AllPrep PowerFecal Pro DNA/RNA Kit shows the highest RNA yields in all four stool samples



- Four frozen stool samples were compared in triplicates; two samples from adults and two from children
- The samples were extracted using one commercially available kit and the legacy AllPrep PowerFecal DNA/RNA Kit
- The RNA yield was quantified by QIAxpert Instrument (high-speed spectrophotometer)



**Did you know the “AllPrep” kits come in red QIAGEN boxes?**



The AllPrep PowerFecal Pro DNA/RNA, launched in May 2022, is the upgraded version of the AllPrep PowerFecal DNA/RNA

Data obtained using experiments conducted by QIAGEN R&D in Hilden, Germany

# QIAamp DNA Microbiome Kit

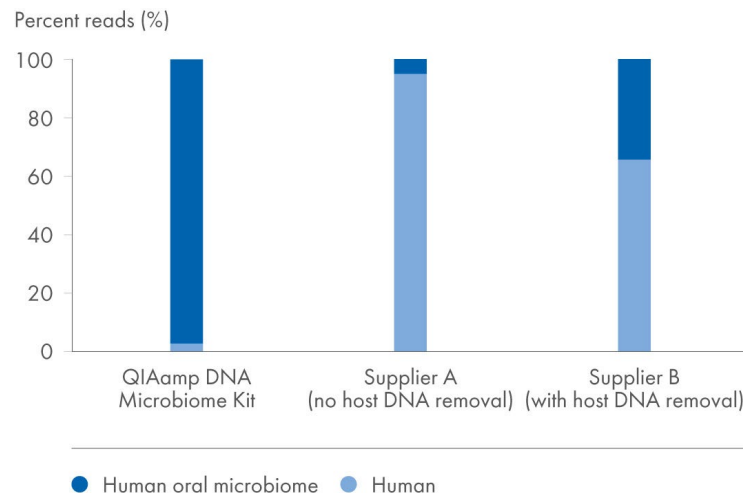
DNA from swabs



## Isolation of bacterial DNA from swabs and body fluids

- Effective depletion of host DNA
- Optimized mechanical and chemical cell lysis

## Effective host DNA removal by the QIAamp DNA Microbiome Kit enhances whole metagenome shotgun sequencing results



DNA isolated from buccal swabs using the QIAamp DNA Microbiome Kit or solutions from alternative suppliers was analyzed by whole metagenome shotgun sequencing on the Illumina MiSeq platform



Removal of host DNA increases the coverage of microbial reads in sequencing experiments

The QIAamp DNA Microbiome Kit efficiently removes host DNA by differential lysis of host cells and subsequent enzymatic digestion of host DNA

Then, using a combination of mechanical and chemical lysis, intact cells are efficiently lysed, and the released bacterial DNA is purified using proven QIAamp chemistry

***"Human DNA in undepleted CF sputum accounted for 94.3% of the total DNA. Saponin, the NEBNext kit and the QIAamp kit reduced human DNA levels by an average of 38.7%, 61.8% and 94.8%, respectively. None of the depletion methods reduced total bacterial DNA concentrations. QIAamp depletion did not influence taxa richness or alpha diversity however, alterations to the core genera were noted following depletion."***

***While all methods reduced human DNA in the CF sputum samples, the QIAamp DNA microbiome kit reduced Human DNA levels significantly while leaving bacterial DNA levels unchanged.."***

– [Klosinka, K. et al. Reducing human DNA bias in cystic fibrosis airway specimens for microbiome analysis, J Microbiol Methods. 2022;200:106540.](#)

Data obtained using experiments conducted by QIAGEN R&D in Hilden, Germany



# Select your automated solution based on your needs



## QIAcube Connect with QIASphere

### Technology

Spin-column-  
based

### Samples per run

Up to 12

### Features

- Automates over 80 QIAGEN kits with over 140 standard protocols for DNA, RNA and protein sample processing
- Optional protocol customization expands the use to over 3000 protocols



## QIAcube HT

Silica  
membrane  
technology

Up to 96

- Mid- to high-throughput solution with dedicated kits for microbial DNA preparation
- User-friendly software allows for easy data management and documentation



## QIASymphony SP

QIAGEN  
magnetic-  
particles  
chemistry

1–96 samples in 4  
batches

- Flexible loading of new samples during a run
- Protocols customizable (protocol steps, labware, etc.) on demand
- Traceability and LIMS compatibility



The QIASphere remote instrument-monitoring app gives full connectivity so you can step out of the lab to ponder on your research while keeping an eye on your instrument run status

Stay alert to important instrument events with QIASphere's push notifications or check maintenance status and manage run reports from anywhere or store them locally at any time

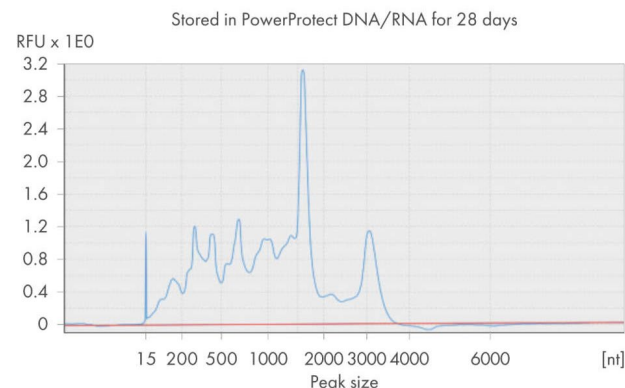
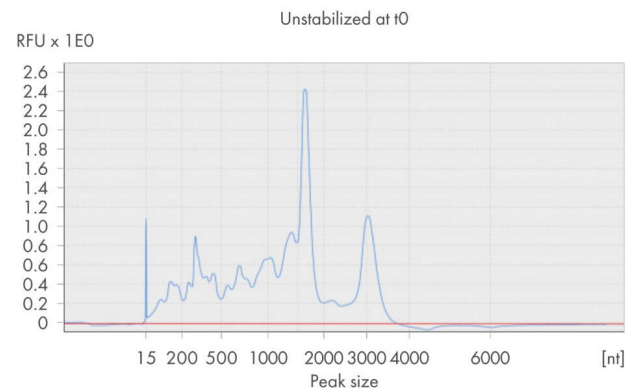
# Size and integrity assessment made easy



## QIAxcel Connect Capillary gel electrophoresis system

- Convenient run setup and cost efficiency with ready-to-use gel cartridges
- Fast turnaround with 12 samples in ~10 minutes and up to 96 samples in a single run
- Sensitive detection of total RNA to 50 pg/ $\mu$ L and DNA fragments down to 5 pg/ $\mu$ L
- User-independent nucleic acid integrity assessment with metrics like RIS (RNA integrity score) or DV200
- NGS library assessment, including the setting of pass/fail criteria based on respective kit specifications

## QIAxcel ScreenGel electropherograms confirms RNA quality in terms of the integrity of total RNA samples



- Stool sample (unstabilized at t0 and stored in PowerProtect DNA/RNA for 28 days) and extracted with RNeasy PowerFecal Pro
- The two distinct ribosomal peaks correspond to 16S and 23S





# Metagenomics or whole genome sequencing



## QIAseq FX DNA Library Kit

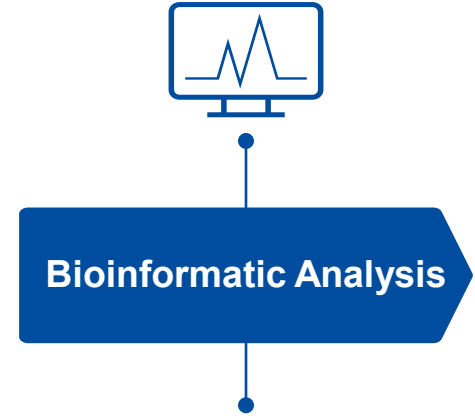
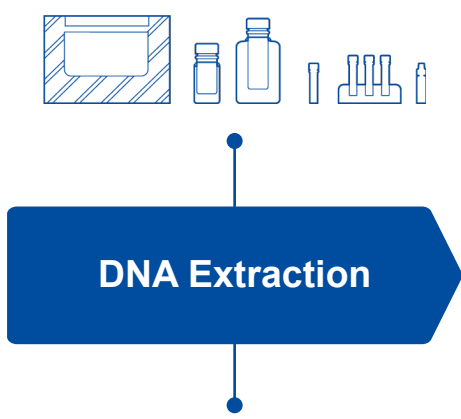
- All-enzymatic library prep
- Streamlined 2.5-hour workflow from purified DNA to NGS library
- High library complexity and uniform coverage maximizes interpretable data

## Microbiome WGS SeqSets

- Sets tailored for microbiome research
- All-in-one sets, including all that you need for DNA extraction, library preparation and Microbial Analysis Platform (MAP) digital analysis
- Available for stool and soil samples

# All-in-one sets tailored for microbiome research

## Microbiome WGS SeqSets



**QIAamp PowerFecal Pro WGS SeqSet**

QIAamp PowerFecal Pro DNA

- Stool

OR

**DNeasy PowerSoil Pro WGS SeqSet**

DNeasy PowerSoil Pro

- Soil

QIAseq FX DNA Library Kit

- All-enzymatic library prep for WGS

QIAseq Normalizer Kit

- Rapid library normalization for high-throughput sequencing

QIAseq Beads

Microbial Analysis Portal (MAP)

- Comprehensive metagenomic analysis

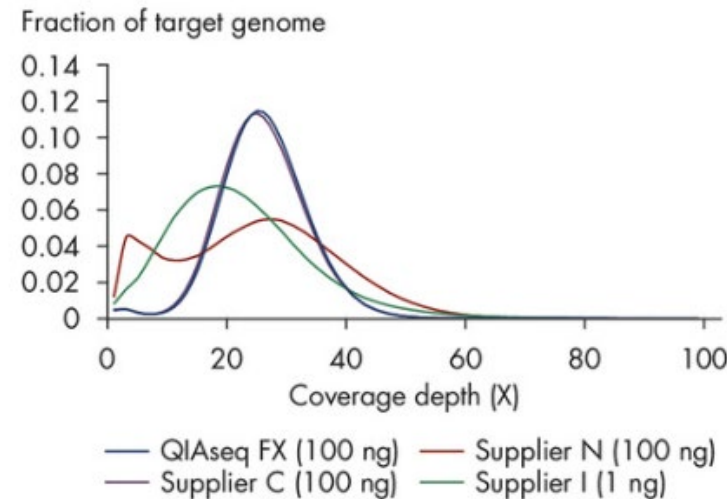
# Metagenomics or whole genome sequencing with an all-enzymatic library prep



## QIAseq FX DNA Library Kit

- Streamlined 2.5-hour workflow from purified DNA to NGS library
- High library complexity and uniform coverage maximizes interpretable data
- Unique dual index (UDI) adapters available for multiplexing up to 384 samples
- Fragment size, input amount and batch size customizable to any sequencing experiment
- Flexible DNA inputs of 20 pg – 1 µg

## Better coverage distribution



***"More specifically, we recommend protocol B (QIAseq FX DNA Library Kit) for taxonomic profiling based on its high accuracy of quantification of the DNA mock community, low GC bias, and excellent transferability across laboratories owing to the use of enzymatic DNA fragmentation"***

— Tourlousse DM et al. Validation and standardization of DNA extraction and library construction methods for metagenomics-based human fecal microbiome measurements. *Microbiome*; 9 (1):95 2021

- The input was 100 ng samples of an equimolar mixture of gDNA from three bacterial species with vastly different GC contents: *Fusobacterium nucleatum* with 27% GC; *Escherichia coli* with 50% GC; and *Bordetella pertussis* with 67% GC (except the sample undergoing tagmentation, as only specific input amounts are accepted).
- The single, narrow peak for the library created with QIAseq FX DNA Library Kit shows that most genomic targets have very similar total coverage depth, comparable to DNA fragmented by mechanical shearing



## Are you dealing with highly fragmented DNA?

The QIAseq Ultralow Input Library Kit is highly suited for fragmented DNA <1000 bp and/or a DIN of <3

Processes low input quantities of 10 pg – 100 ng while providing similar features and benefits as QIAseq FX DNA Library Kit

## Performing multiplexed sequencing?

The [QIAseq Normalizer Kit](#) quickly normalizes libraries without library quantification

# 16S/ITS sequencing with a phased primer approach



- Interrogate all bacterial 16S rRNA gene variable regions and fungal ITS regions
- The use of "phased primers" increases base diversity, leading to an increase in quality scores
- Low-bioburden reagents minimize background contamination
- DNA input can be as low as 1 pg to profile low-biomass samples

For robust profiling of bacterial and fungal communities:

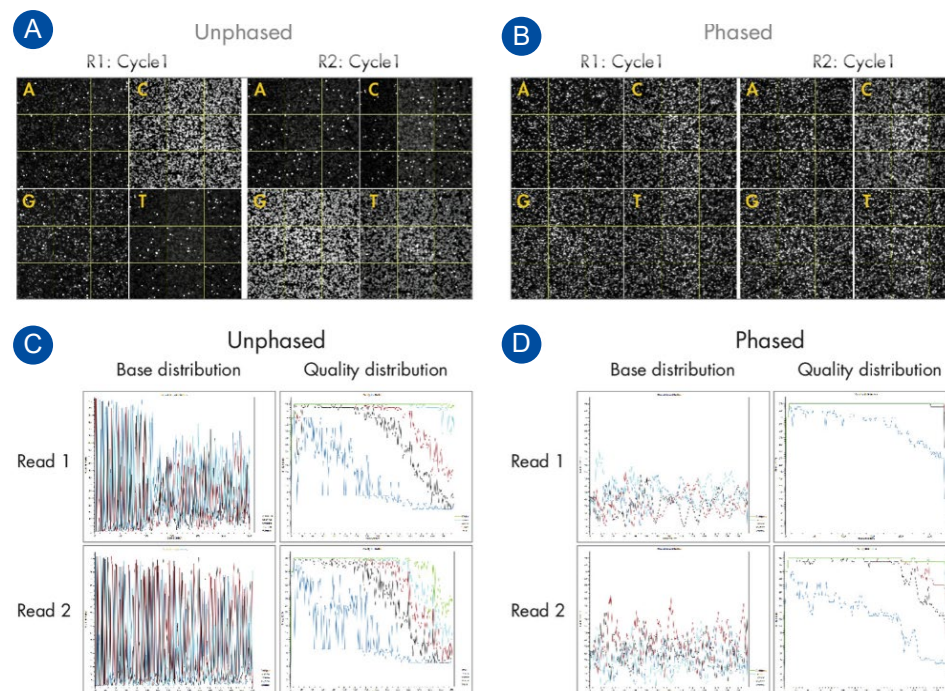
## QIAseq 16S/ITS Region Panels

Select your target regions and configure on: [GeneGlobe Design & Analysis Hub](#)

For screening a panel of variable regions and more robust bacterial profiling:

## QIAseq 16S/ITS Screening Panel

## Phased primers increase base diversity and quality scores



- Fluorescent images (top row) taken from the first cycle of read 1 (R1) showing uneven base distribution in an unphased primer run (A) and more even base distribution in a phased primer run (B)
- Low base diversity and read quality scores (bottom row) are apparent in a run using unphased V3V4 primers (C) and significantly improved using phased V3V4 primers (D)



After sequencing, data is analyzed using the [QIAGEN Genomic Workbench Premium](#)

A custom workflow, which can be further edited as needed, is available for automating FASTQ file import, sample library demultiplexing, quality-controlled filtering and trimming, OUT clustering and secondary bioinformatics analysis

**QIAseq 16S/ITS Smart Control** is a synthetic DNA that can be used as a positive control for library construction steps



# Efficient bacterial rRNA removal for improved read allocation

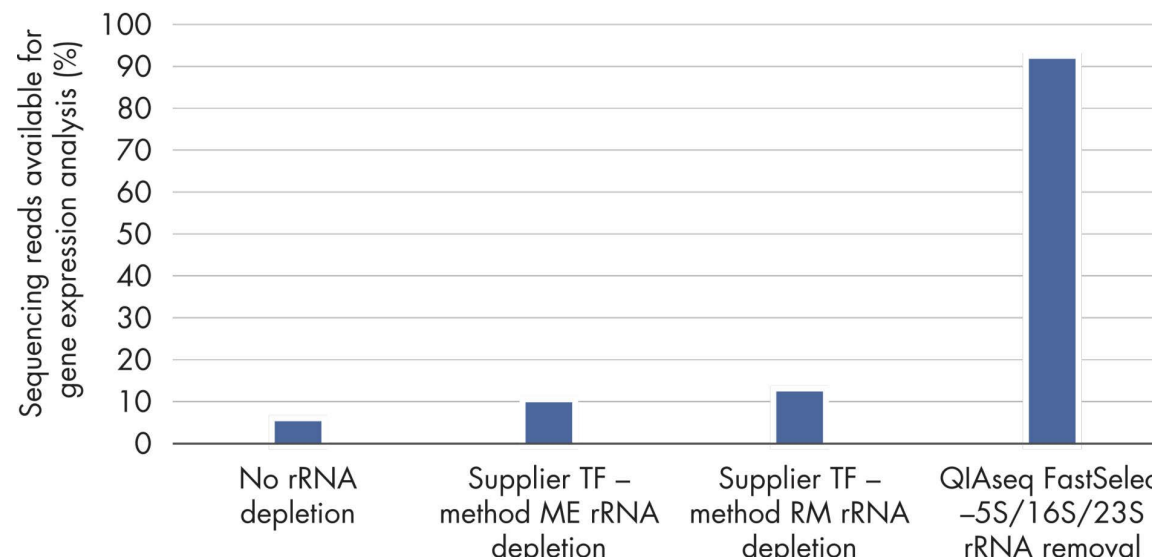
Library prep methods



## QIAseq FastSelect – 5S/16S/23S

- A fast 20-minute workflow for pan-bacterial 5S/16S/23S rRNA removal from fragmented or full-length RNA
- Novel RNA removal mechanism that doesn't involve hybrid capture or enzymatic digestion
- Integrated into **QIAseq FastSelect RNA Library Kit** and **QIAseq UPXome RNA Library Kit** or combine FastSelect with **QIAseq Stranded RNA Library Kit** for whole transcript sequencing
- Compatible with Illumina, NEB and KAPA stranded RNA-seq library preparation kits
- In-silico design predicts blocking of >95% of all 5S, 16S and 23S rRNA database sequences

## QIAseq FastSelect method frees up a substantial read budget for gene expression analysis



- Total RNA was isolated from Gut Microbiome Whole cell Mix (ATCC) using the RNeasy PowerMicrobiome Kit (QIAGEN)
- Stranded transcriptome libraries were then prepared from 1 µg aliquots of the RNA using the QIAseq Stranded Total RNA Lib Kit
- For rRNA depletion, three methods were used, as shown
- Sequencing was performed on a NextSeq 550, and data analysis was performed using QIAGEN CLC Genomics Workbench



### Working with soil, water, stool or sludge samples?

QIAseq FastSelect – 5S/16S/23S was specifically designed for complex community microbial sample types with numerous and diverse bacterial populations

### Want to get rid of Human rRNA for host-microbe studies or analysis of viruses?

Use QIAseq FastSelect Epidemiology Kit for both ribosomal RNA removal from bacteria and human, mouse and rat samples

Data obtained using experiments conducted by QIAGEN R&D in Hilden, Germany

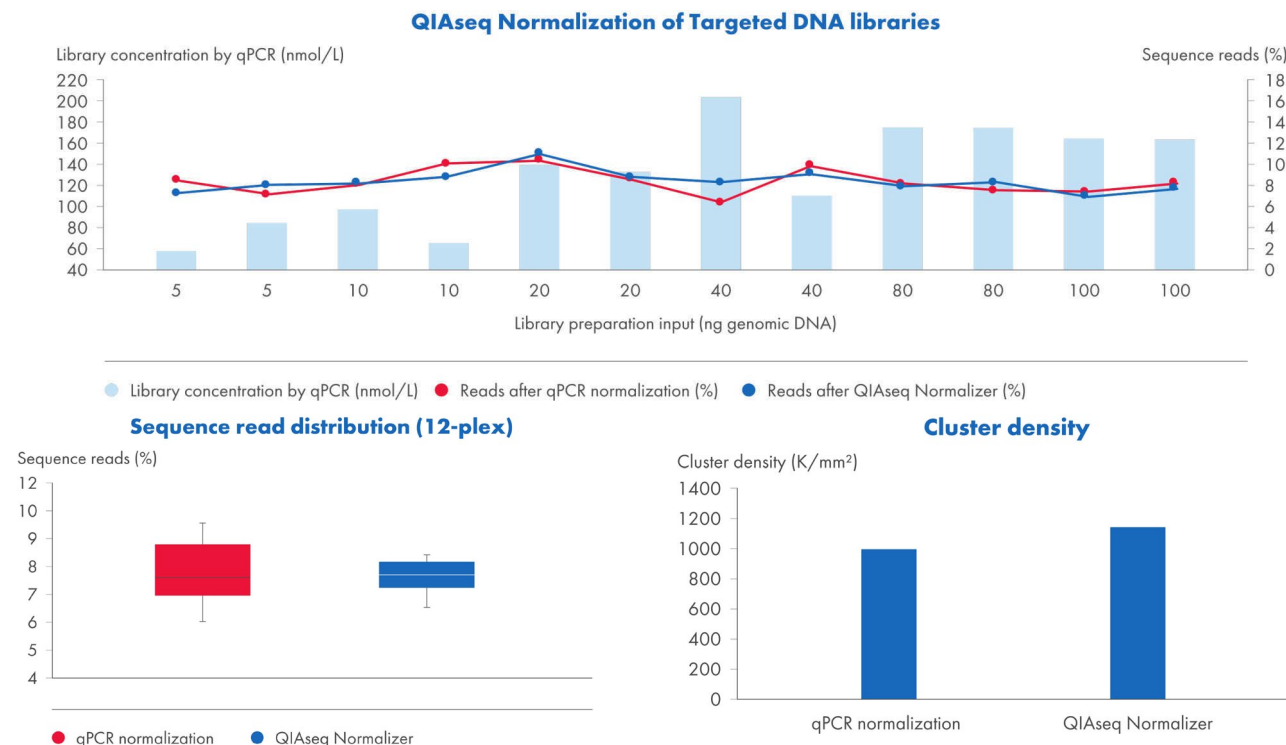
# Rapid normalization for multiplexed sequencing



## QIAseq Normalizer Kit

- Streamlined, 30-min benchtop protocol
- Achieve qPCR-level quality without the long workflow
- Works with a broad range of concentrations from 15 to over 300 nmol/L
- Libraries normalized libraries to 4 nmol/L for optimal clustering on Illumina flow cells
- Compatible with most Illumina libraries
- Automation-friendly workflow

## Achieve library normalization without tedious library quantification



- Using 5–100 ng of gDNA, 12 QIAseq Targeted DNA libraries were prepared.
- All libraries were subjected to qPCR and diluted to 4 nmol/L before sequencing on the Illumina MiSeq (V2 reagents, 2x150 bp paired-end)
- For comparison, the same libraries were normalized using the QIAseq Universal Normalizer Kit and sequenced on the Illumina MiSeq



## Compatible QIAseq kits with QIAseq **Library** Normalizer

- QIAseq FX DNA Library kit
- QIAseq 1-Step Amplicon Library Kit
- QIAseq Ultralow Input Kit

## Compatible QIAseq kits with QIAseq **Universal** Normalizer

- QIAseq FastSelect RNA Library Kits
- QIAseq UPXome RNA Library Kits
- QIAseq 16S/ITS Screening Panels and Index Kits

# Flexible library preparation methods for metatranscriptome sequencing



## QIAseq FastSelect rRNA removal

- Efficient bacterial rRNA removal for improved read allocation in metatranscriptomic studies
- Stand-alone kit for bacterial rRNA removal: [QIAseq FastSelect –5S/16S/23S](#)
- Integrated into QIAseq FastSelect RNA Library Kit and QIAseq UPXome RNA Library Kit or combine FastSelect with QIAseq Stranded RNA Library Kit for whole transcript sequencing

## QIAseq FastSelect RNA Lib Bacteria Kit

For meta-transcriptomic studies of bacteria; includes RNA library preparation reagents; QIAseq FastSelect –rRNA Bacteria for removal of ribosomal RNA and QIAseq Beads

- Simple and fast workflow; <5 hours
- Use with [QIAseq Normalizer](#) for rapid library normalization
- Up to 768 samples per flow cell lane

## QIAseq UPXome RNA Lib Kit Bacteria

For meta-transcriptomic studies of bacteria; includes RNA library preparation reagents; QIAseq FastSelect –rRNA Bacteria for removal of ribosomal RNA and QIAseq Beads.

- Workflow for ultra-low input samples
- Suitable for high throughput analysis with a large number of samples where 8 to 24 libraries can be pooled
- Up to 18,432 samples possible per flow cell lane

## RNA input range per sample

0.5 to 100 ng

1 to 1000 ng

More than 1000 ng

## QIAseq products for RNA Library Prep

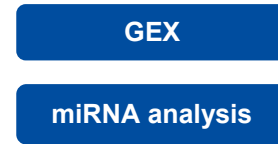
QIAseq UPXome RNA Library Kit



QIAseq FastSelect RNA Library Kit

QIAseq FastSelect -5S/16S/23S Kit

QIAseq Stranded RNA library Kit UDI

# Host gene expression analysis – choose from a comprehensive portfolio



Application		Recommended solution	Good to know	Configuration and customization	Data analysis
NGS	rRNA removal for RNA-seq library preparation from human, mouse, and rat samples	QIAseq FastSelect –rRNA HMR Kit	Efficiently removes human, mouse and rat rRNA to maximize read allocation	N.A.	<ul style="list-style-type: none"> <li>• RAP</li> <li>• CLC</li> </ul>
	Rapid rRNA removal and optimized Stranded RNA-seq library construction for complete transcriptome coverage	QIAseq FastSelect RNA Lib HMR Kit	<ul style="list-style-type: none"> <li>• QIAseq FastSelect included</li> <li>• Simple and fast workflow; &lt;5 hours</li> <li>• Up to 768 samples per flow cell lane</li> <li>• For 1ng to 1000ng Total RNA input</li> </ul>		
dPCR		QuantiNova LNA PCR Assays for Digital PCR	Absolute quantification of expression changes using LNA-enhanced, EvaGreen digital PCR		QIAcuity Software Suite
qPCR		QuantiNova LNA PCR Assays QuantiNova LNA Probe PCR Assays	LNA-enhanced SYBR Green-based or hydrolysis probe-based real-time PCR		







# miRNA analysis – choose based on your research focus

GEX  
miRNA analysis



Application	Recommended library preparation kit	Good to know	Configuration and customization	Data analysis
NGS	QIAseq miRNA Library Kit	Gel-free miRNA library preparation, integrated unique molecular indices (UMIs), available with 768 unique dual indices for high-throughput sequencing on Illumina NovaSeq instruments	N.A.	RAP CLC
dPCR	miRCURY LNA miRNA PCR Assays for Digital PCR	Extremely sensitive and specific miRNA quantification using QIAcuity EvaGreen-based dPCR with a simple and rapid plate-based workflow		QIAcuity Software Suite
qPCR	miRCURY LNA miRNA PCR Assays and Panels miRCURY LNA miRNA Probe PCR Assays and Panels	Extremely sensitive and specific miRNA quantification using LNA-optimized, SYBR Green-based or hydrolysis probe-based real-time PCR		



[GeneGlobe Design & Analysis Hub](#)

RAP: Complimentary access to [RNA-seq Analysis Portal](#)

CLC [QIAGEN CLC Genomics Workbench](#)

# Microbial – host interaction research



## Application

## Recommended solution

## Good to know

## Data analysis




NGS	rRNA removal for RNA-seq library preparation from human, mouse, rat and bacteria samples	QIAseq FastSelect Epidemiology Kit	Efficiently removes bacterial & HMR (human, rat & mouse) rRNA to maximize read allocation	N.A.
	Rapid rRNA removal and optimized Stranded RNA-seq library construction for complete transcriptome coverage	QIAseq FastSelect RNA Lib Epi. Kit	<ul style="list-style-type: none"><li>• QIAseq FastSelect Epidemiology Kit included</li><li>• Simple and fast workflow; &lt;5 hours</li><li>• Use with QIAseq Normalizer for rapid library normalization</li><li>• Up to 768 samples per flow cell lane</li><li>• For 10ng to 1000ng total RNA input</li></ul>	<ul style="list-style-type: none"><li>• RAP</li><li>• CLC</li></ul>

RAP: Complimentary access to [RNA-seq Analysis Portal](#)

CLC [QIAGEN CLC Genomics Workbench](#)

# Identify species target, AMR or virulence genes for profiling and surveillance






Application		Platform	Recommended assay	Configuration and customization	Data analysis	Good to know
NGS	Targeted sequencing	Illumina sequencer	<a href="#">QIAseq xHYB Viral and Bacterial Panels</a>	N.A.		Hybrid capture-based targeted sequencing enables high-sensitivity sequence analysis of focus regions at a low cost and with faster turnaround times
	Whole genome sequencing	Illumina sequencer	<a href="#">QIAseq FX DNA Library Kit &amp; QIAseq Normalizer Kit</a>	N.A.	 CLC	All-enzymatic DNA library kit to go from purified DNA to NGS library in just 2.5 hours and fast normalization with library quantification
<a href="#">dPCR</a>		<a href="#">QIAcuity</a>	<a href="#">dPCR Microbial DNA Detection Assays</a> QIAcuity UCP Probe PCR Kit QIAcuity OneStep Advanced Probe Kit		QIAcuity Software Suite	Nanoplate-based dPCR enables highest precision and absolute quantification with a robust workflow
qPCR		QIAquant Rotor-Gene Q	<a href="#">qPCR Microbial DNA Assays &amp; Arrays</a>		N.A.	Application-focused microbial target assays and arrays for detection and quantitation using lab-verified assays and for screening or LDTs in research and surveillance using user-developed assays
			<a href="#">QuantiNova Pathogen +IC Kit</a>		N.A.	
PCR		N.A.	UCP Multiplex PCR	N.A.		Ultra-clean produced multiplex hot-start PCR for detection or library amplification.



# Profiling and detection of antimicrobial resistance and virulence genes



Application	Platform	Recommended assay	Configuration and customization	Data analysis	Good to know
NGS	Targeted sequencing	Illumina sequencer  <u>QIAseq xHYB Viral and Bacterial Panels</u>	N.A.		Hybrid capture-based targeted sequencing enables high-sensitivity sequence analysis of focus regions at a low cost and faster turnaround times
	Whole genome sequencing	Illumina sequencer  <u>QIAseq FX DNA Library Kit</u> & <u>QIAseq Normalizer Kit</u>	N.A.	CLC, 	An all-enzymatic library preparation kit proven in multiple microbial research studies and fast normalization with library quantification
<u>dPCR</u>	<u>QIAcuity</u>	dPCR Microbial DNA Detection assays: • <u>dPCR AMR Assays</u> • <u>dPCR Virulence Gene Assays</u>		QIAcuity Software Suite	Nanoplate-based dPCR enables highest precision and absolute quantification with a robust workflow
<u>qPCR</u>	QIAquant RotorGene Q	<u>qPCR Microbial DNA Assays &amp; Arrays</u>		N.A.	Application-focused microbial target assay and arrays with guaranteed detection and quantitation using lab-verified assays



[GeneGlobe Design & Analysis Hub](#)

CLC: [QIAGEN CLC Genomics Workbench](#)

# Targeted viral and bacterial sequencing

Microbial ID

AMR, VG



## QIAseq xHYB Viral and Bacterial Panels

- Target only your genes of interest – no need to “waste” reads on non-relevant genome segments
- Detects low-abundance genes with high-level enrichment – guaranteed to capture your region of interest
- Multiplex up to 384 samples when paired with the QIAseq FX DNA Library Kit

## QIAseq xHYB AMR Panel

Detect 2786 antimicrobial resistance genes (6200+ AMR markers) in bacteria and consists of targets pulled from the QIAGEN CLC QMI-AR database

## QIAseq xHYB Viral Respiratory Panel

Detect and characterize 89 separate viral targets, including SARS-CoV-2, influenza, rhinovirus, enterovirus and more

## QIAseq xHYB Adventitious Agent Panel

Detect 132 separate viral targets, including human adenovirus, norovirus, rotavirus, influenza, SARS-CoV-2, HPV, Epstein-Barr, HIV, hepatitis A/B viruses and more

## QIAseq xHYB Viral STI Panel

Detect common STI viruses: HBV, HIV-1 and nineteen types of high-risk HPV

## QIAseq xHYB Microbial Target List

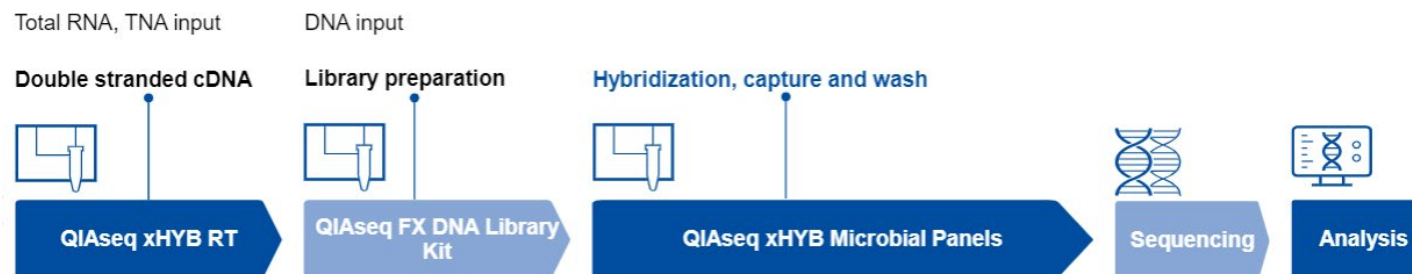


Resulting FASTQ can be analyzed via the analysis portal on GeneGlobe or via the QIAGEN CLC Genomics Workbench

GeneGlobe Analysis Portal offers a simplified overview of the sequencing results for identifying and quantifying either viral species or antimicrobial resistance genes detected in any samples

QIAGEN CLC Genomic Workbench is also available for deeper analysis for taxonomic identification, variant analysis, phylogenetic trees and viral integration sites

**A flexible sample-to-sequencing workflow:**





# Digital PCR

Microbial ID

AMR, VG



**QIAcuity – a nanoplate-based digital PCR system**

**dPCR Microbial DNA Detection Assays:**

1

**Accuracy and precision**

2

**Parallel quantification of RNA and DNA targets**

3

**Parallel quantification of up to 5 virulence target genes**

4

**Parallel quantification of up to 5 AMR target genes**



# QIAcuity – a nanoplate-based digital PCR system

Digital PCR

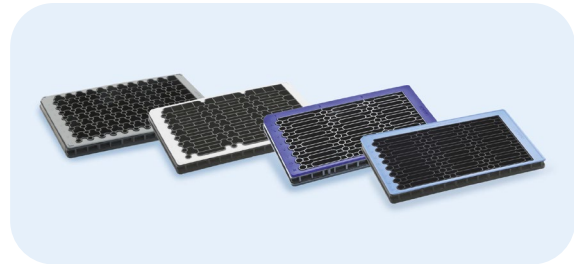


## QIAcuity dPCR Instruments

Fully integrated instrument design; One instrument for partitioning, cycling and imaging

Multiplexing – up to 5 channels

Fast workflow – up to 96 samples in ~2 hours



## QIAcuity Nanoplates

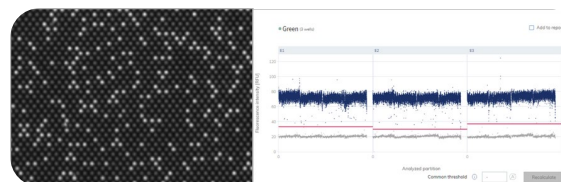
Nanoplate 26k 8-well/24-well (26,000 partitions)

Nanoplate 8.5k 24-well/ 96-well (8500 partitions)



## QIAcuity EvaGreen, Probe PCR, UCP Probe PCR and OneStep Advanced Probe Kits

Nanoplate-optimized chemistry



## QIAcuity dPCR Software Suite

Data visualization

Primary and secondary analyses

### Top benefits:

#### Absolute target quantification

- No need for references/standard curves

#### Higher tolerance to inhibitors

- Due to partitioning and endpoint measurement

#### High precision

- Detect minute fold changes (<2 fold)

#### High analytical sensitivity

- Detection of <10 copies/reaction
- Analysis of up to 27  $\mu$ L template/reaction

#### High reproducibility

- Eliminate amplification efficiency bias

#### Large dynamic range

- Quantification of up to 200,000 copies/reaction



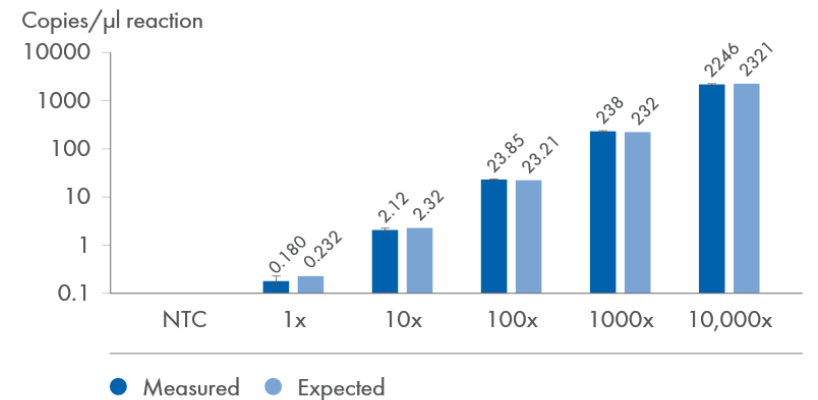
# Accuracy and precision with dPCR Microbial DNA Detection Assays

Digital PCR



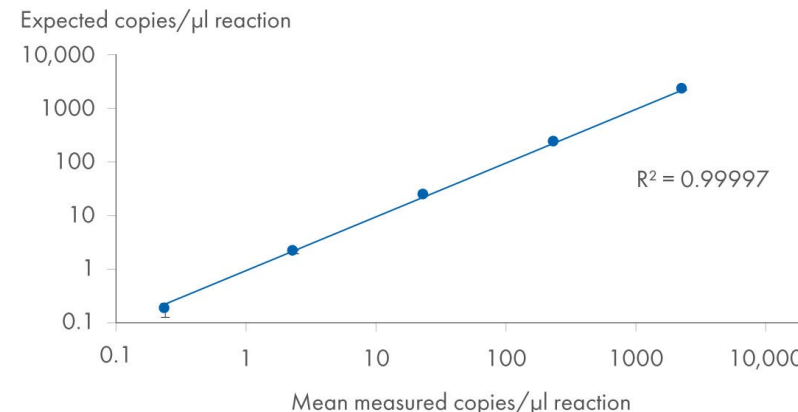
- Detect bacteria, fungi, viruses, parasites, virulence genes or antibiotic-resistance genes
- 685 probe-based assays each premixed and lyophilized in one tube, including over 200 wet-lab tested assays
- Selectable fluorophore (FAM, HEX, ROX, TAMRA, Cy5) enables multiplexing using a mix-and-match approach
- Over 35 wet-lab tested bundles of up to 5 assays combining common targets of interest in multiplex dPCR on the QIAcuity
- Compatible with OneStep Advanced Probe PCR Kit for detection of RNA viruses.
- Select your assays and bundles on [GeneGlobe Design & Analysis Hub](#)

## Accurate quantification of the NIST gDNA reference standard 8376 from the bacterium *Shigella sonnei* as input



Single-copy gene assay from the dPCR Microbial DNA Detection Assay portfolio to detect one target molecule per genome. Bar chart shows the mean measured concentrations (copies/μL) of three replicates

## Precise quantification across a 4-log dynamic range of the NIST gDNA reference standard 8376 from the bacterium *Shigella sonnei*



Individual datapoints represent mean values of 3 replicates each

Data obtained using experiments conducted by QIAGEN R&D in Hilden, Germany

# Parallel quantification of RNA and DNA targets

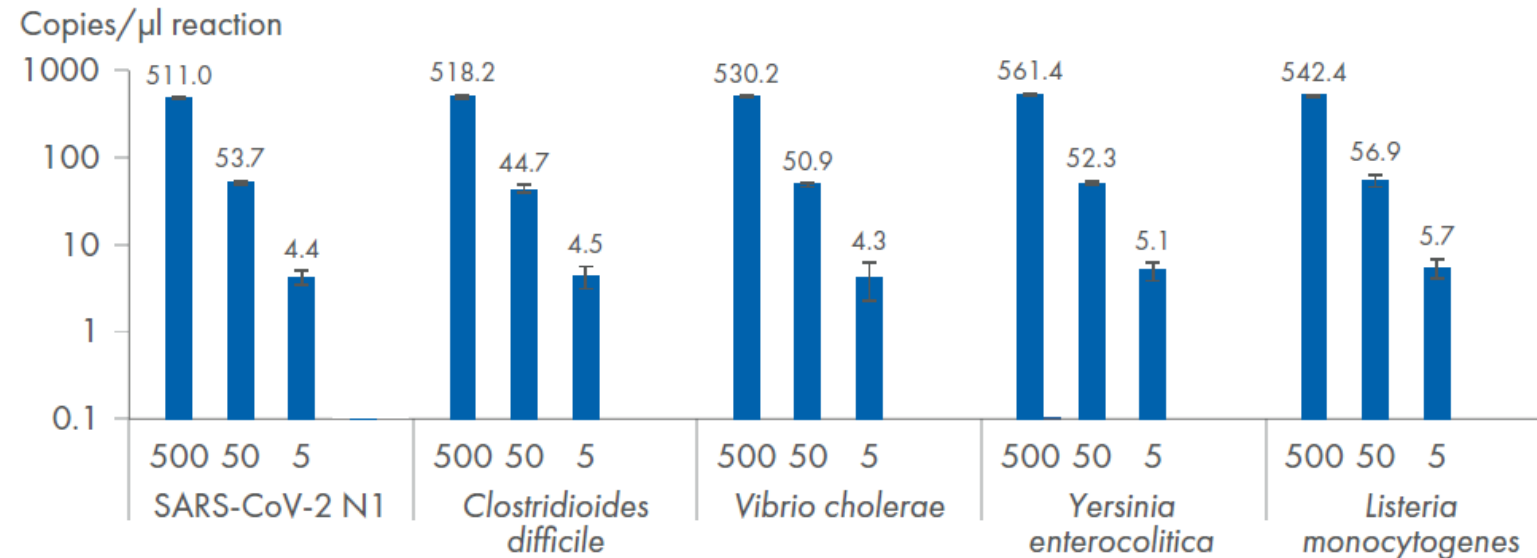
Digital PCR



The combination of the OneStep Advanced Probe PCR Kit with the dPCR Microbial DNA Detection Assays enables:

- Multiplex dPCR quantification of DNA and RNA targets
- Simple and fast one-step RT dPCR workflow on the QIAcuity in less than 3 hours
- Suitability for detection of RNA viruses in e.g., wastewater surveillance approaches
- Improved tolerance towards inhibitors contained in the extracted DNA and/or RNA sample
- Improved performance in multiplex dPCR reactions

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



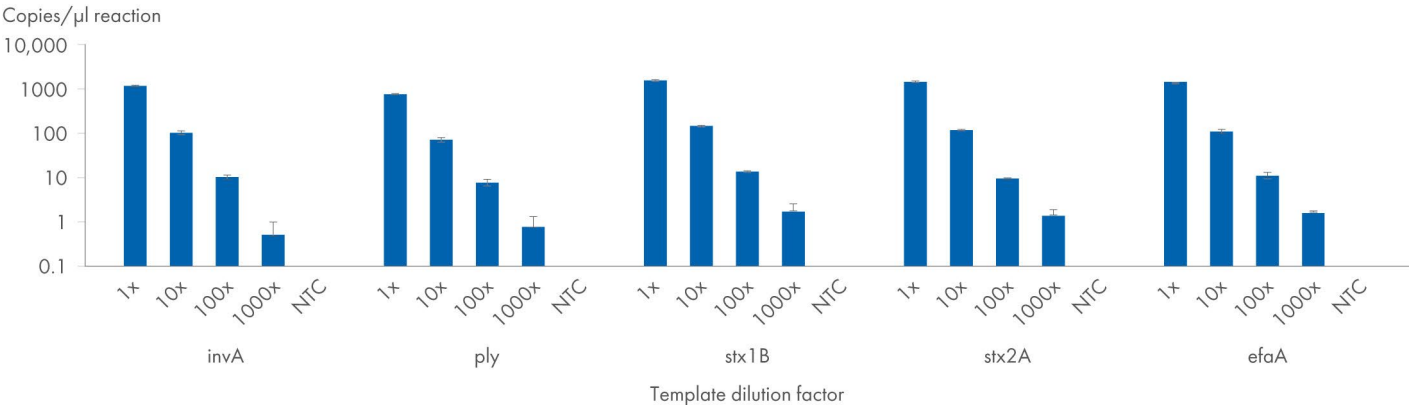
- A mixture of four bacterial gDNAs (*Clostridioides difficile*, *Vibrio cholerae*, *Yersinia enterocolitica*, *Listeria monocytogenes*) and SARS-CoV-2 RNA was used as input
- dPCR, with three replicates per condition, was run using 8.5k 96-well Nanoplates and the QIAcuity OneStep Advanced Probe Kit 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 for each of the five targets

Data obtained using experiments conducted by QIAGEN R&D in Hilden, Germany

# Parallel quantification of up to five virulence target genes

- Six virulence gene assay bundles were wet-lab tested
- Individual bundles can be configured on the [GeneGlobe Design & Analysis Hub](#)

## Parallel quantification of five virulence target genes – virulence genes 6 bundle



- Four dilutions of positive control input template (PMC) starting with 1 μL input and diluting down to 0.001 μL and no template controls (NTC)
- Three replicates per condition were tested

Data obtained using experiments conducted by QIAGEN R&D in Hilden, Germany

Digital PCR

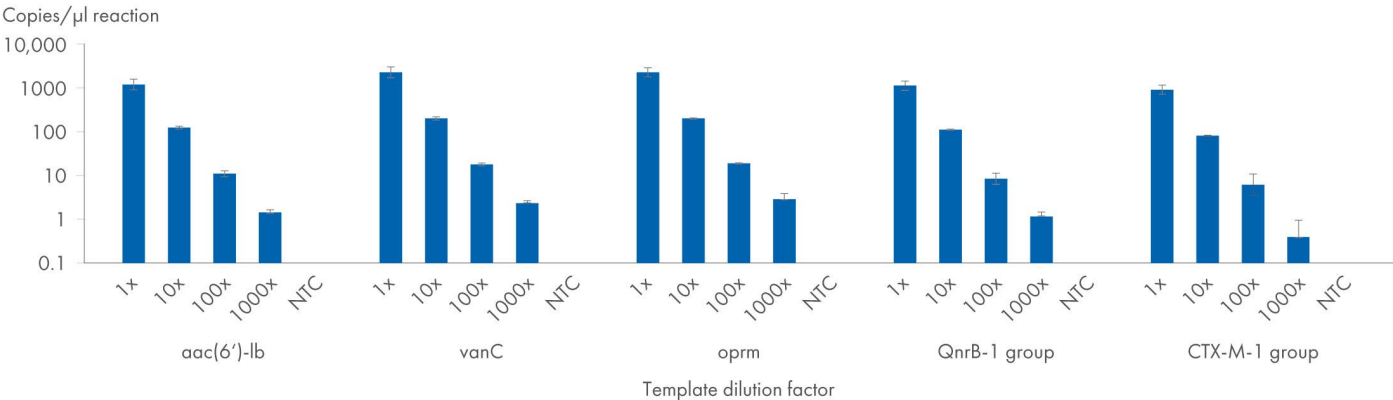


Bundle name	Assay name
Virulence genes 1	Minor fimbrial subunit (fimH)
	Gamma-hemolysin component B (hlgB)
	Shiga-like toxin 1 subunit B encoded within prophage CP-933V
	Shiga toxin subunit B; receptor binding subunit
	Accessory cholera enterotoxin (ace)
Virulence genes 2	ply
	stx1B
	stx2A
	invA
	stxB
Virulence genes 3	wbkA
	ptxA
	ace (E. faecalis)
	ace (V. cholerae)
	efaA
Virulence genes 4	glycosyltransferase wbkA
	pertussis toxin subunit 1 precursor
	collagen adhesin protein ace
	accessory cholera enterotoxin ace
	endocarditis specific antigen
Virulence genes 5	Minor fimbrial subunit
	shiga-like toxin 1 subunit B encoded within prophage CP-933V
	gamma-hemolysin component B
	invasion protein invA
	endocarditis specific antigen
Virulence genes 6	invA
	ply
	stx1B
	stx2A
	efaA

# Parallel quantification of up to five AMR target genes

- Five resistance gene assay bundles were wet-lab tested
- Individual bundles can be configured on the [GeneGlobe Design & Analysis Hub](#)

## Parallel quantification of five AMR target genes – resistance genes 3 bundle



- Four dilutions of positive control input template (PMC) starting with 1 µL input and diluting down to 0.001 µL and no template controls (NTC)
- Three replicates per condition were tested

Data obtained using experiments conducted by QIAGEN R&D in Hilden, Germany

Digital PCR					
Bundle name	Assay name				
Resistance genes 1	Fluoroquinolone resistance gene QnrS				
	Class D beta-lactamase OXA-10 group				
	Vancomycin resistance gene vanB				
	Tetracycline efflux pump gene tetA				
	Tetracycline efflux pump gene tetB				
Resistance genes 2	Fluoroquinolone resistance geneQepA				
	Class B beta-lactamase blaVIM-1 group				
	Class D beta-lactamase OXA-48 group				
	Sulfonamide resistance gene sul1				
	Class D beta-lactamase OXA-58 group				
Resistance genes 3	<i>aac(6')-Ib</i>				
	<i>vanC</i>				
	<i>oprM</i>				
	QnrB-1 group				
	CTX-M-1 Group				
Resistance genes 4	Class C beta-lactamase ACC-1 Group				
	Class C beta-lactamase CFE-1				
	VanC				
	Class C beta-lactamase dha				
	Class C beta-lactamase fox				
Resistance genes 5	Multidrug efflux pump protein OqxA ( <i>E. coli</i> , <i>Klebsiella sp.</i> , <i>Salmonella sp.</i> )				
	<i>fosA</i> ( <i>Klebsiella sp.</i> )				
	Fluoroquinolone resistance QepA				
	Class D beta-lactamase OXA-23 Group				
	Class A beta-lactamase Per-1 Group				

# Microbial DNA qPCR Assays for characterization of microbes and traits

Microbial ID

AMR, VG



## Real-time qPCR for microbial identification and profiling with high sensitivity and specificity

- Uniform qPCR efficiency and amplification conditions
- Experimentally verified microbial DNA qPCR assays with high specificity and sensitivity
- 5' hydrolysis probe detection for high sensitivity and specificity

## A broad range of assays and panels to detect microbial species, virulence genes or antibiotic resistance genes

### Microbial DNA qPCR Assays

Experimentally verified assays with high sensitivity and specificity

### Microbial DNA qPCR Assay Kits

Complete kit for qPCR-based microbial detection, with assay, controls, and reagents

### Microbial DNA qPCR Multi-Assay Kits

Includes 3–6 assay tubes in one box

### Microbial DNA qPCR Panels

Collections of assays to identify and profile microbial species, virulence factor genes or antibiotic resistance genes

Each panel includes controls for host DNA to verify the presence of bacterial DNA and the success of the PCR reaction



## AMR gene profiling using Microbial DNA qPCR panels – a use case

[Young, C.C.W. et al \(2022\) Antibiotic resistance genes of public health importance in livestock and humans in an informal urban community in Nepal, Nature Scientific Reports](#)



**Configure at GeneGlobe – find or custom design the right target-specific assays and panels to research your biological targets of interest**

# Multiplex qPCR detection – QuantiNova Pathogen kits with Internal Control

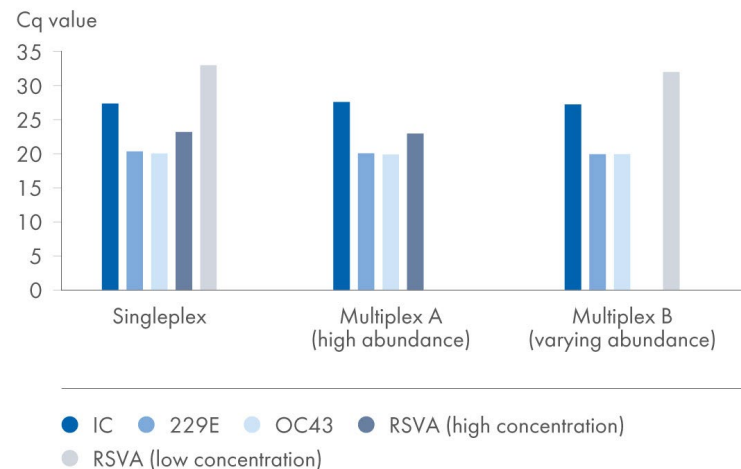
Microbial ID



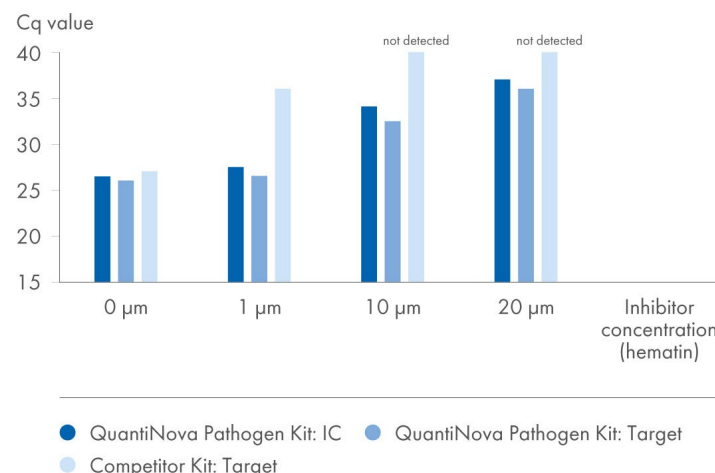
**For ultrafast, simultaneous detection of viral RNA/DNA and bacterial DNA, including in-process safety measures**

- Pre-optimized internal controls for extraction and amplification at no extra cost
- Four-plex (RT)-PCR for detection of both pathogen targets and internal control
- Unique two-phase hot-start procedure for convenient room-temperature setup (fully automatable)
- Stable RT included in the master mix, providing one easy-to-use protocol
- Visual pipetting control to minimize error

**Comparison of singleplex to multiplex results**



**Performance in presence of PCR inhibitor**



**Reliable multiplex detection of low and high abundant targets equivalent to singleplex results**

- Coronavirus 229E, OC43, human Respiratory Syncytial Virus A and QuantiNova Internal Control RNA were amplified in singleplex and multiplex PCR showing highly consistent results.
- In multiplex B reactions, no competitive effects or sensitivity loss for low-concentrated RSVA was observed in the presence of various high-abundant targets with Delta Cq of more than 10

**Robust performance and IC alert in presence of PCR inhibitors**

- Increasing concentrations of a common inhibitor (hematin) were spiked into PCR reactions using QuantiNova Pathogen Kit and a commonly used Competitor Kit
- While QuantiNova chemistry showed excellent inhibitor robustness, the QuantiNova Internal Control clearly indicated the presence of inhibitors by Cq shifts of more than 2 Cq's, providing additional process safety

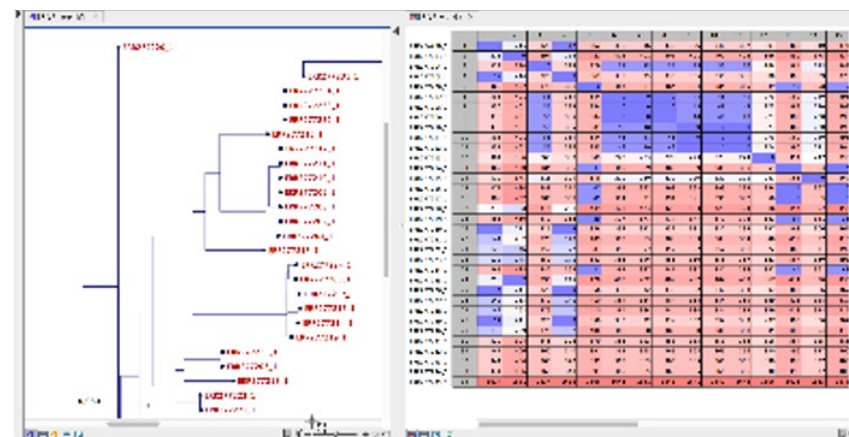
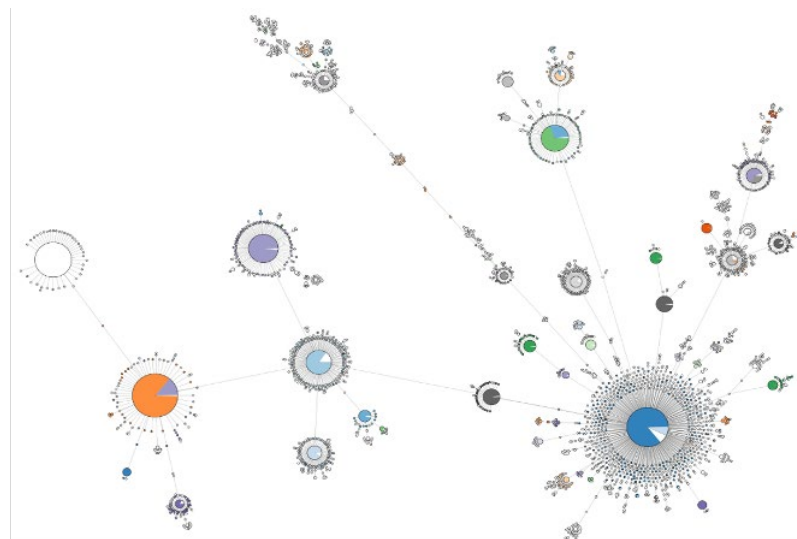
Data obtained using experiments conducted by QIAGEN R&D in Hilden, Germany



# QIAGEN CLC Genomics Workbench Premium – a platform for microbial bioinformatic analysis



- NGS analysis of virus and bacterial outbreak
- Antimicrobial resistance analysis
- Easy access to relevant reference data
- Workflows for the analysis of data from targeted panels, WGS and whole metagenome samples
- Supports QIAseq and other panels with workflows bundled with reference data
- Tools for sequence alignment and phylogenetic analysis
- Tools for wg/cgMLST and k-mer and SNP-trees
- Rich, interactive visualizations



Graphical user interface

Easy onboarding with tutorials

***“CLC Genomics Workbench [...] does not require previous knowledge of Unix-based tools; it is arguably the most user-friendly [of the tools tested] and delivers reliable results for microbial identification and antimicrobial resistance gene detection.”***

— Couto N. et al. [Critical steps in clinical shotgun metagenomics for the concomitant detection and typing of microbial pathogens](#) [published correction appears in *Sci Rep.* 2019 Apr 17;9(1):6406]. *Sci Rep.* 2018;8(1):13767



# GeneGlobe Design and Analysis Hub



The GeneGlobe Design and Analysis Hub streamlines the complete research cycle of target exploration, product configuration, data analysis and follow-up experiment planning – with just one tool: <https://geneglobe.qiagen.com>

## GeneGlobe Design

- Supports customization and configuration of real-time PCR and NGS-based products
- Assays and panels can be configured to contain different fluorophores and chemical modifications or to fit specific instruments
- PCR customization tools can be used to design assays and panels that interrogate specific targets of interest
- NGS customization tools can be used to create panels that contain the specific targets most pertinent to the researcher

Visit: <https://geneglobe.qiagen.com/customize>

## GeneGlobe Data Analysis Center

- Includes various web-based tools to streamline and simplify your data analysis
- Access is complimentary when analyzing data acquired with QIAGEN kits
- The real-time PCR modules transform threshold cycle (Cq) values to calculated results for gene and miRNA expression, somatic mutation detection and copy number measurements from QIAGEN PCR assays and panels
- The NGS modules support primary and secondary analysis of QIAseq NGS library prep kits and target enrichment panels.
- The new RNA-seq Analysis Portal takes your NGS analyses further and provides a fully integrated analysis and biomarker discovery workflow

Visit: <https://geneglobe.qiagen.com/analyze>

# Microbial Analysis Portal (MAP)



## Comprehensive

- Advanced taxonomic and antimicrobial resistance
- Powered by QIAGEN CLC Genomics workbench

## Easy

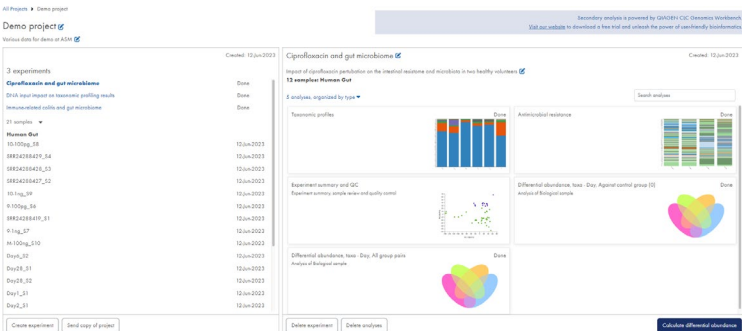
- Standardized analysis accessible to non-bioinformaticians
- Quickly get the breakdown of the microbial species in a sample

## Online

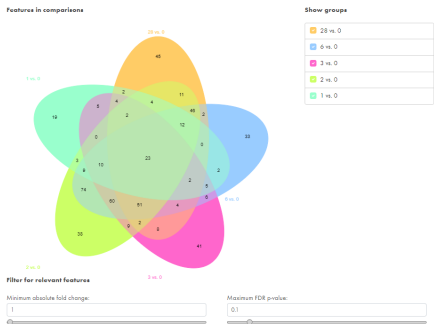
- Web-based platform
- Access is included as standard with QIAamp PowerFecal Pro WGS Seqset or DNeasy PowerSoil Pro WGS SeqSet

## Features include:

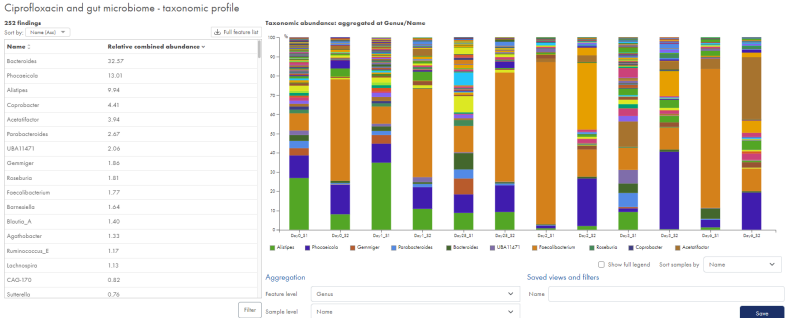
### CLC genomics workbench



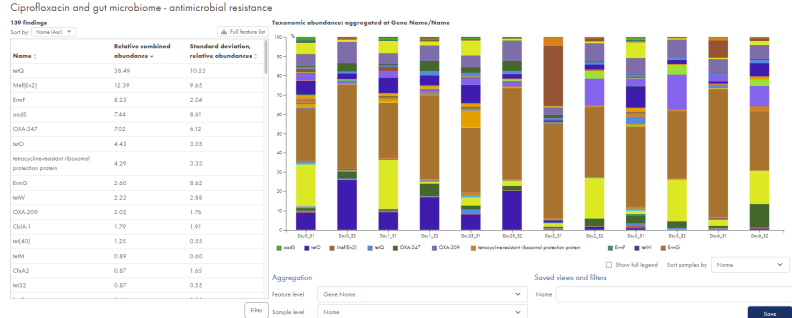
### Differential abundance calculation



### Taxonomic ID and abundance



### AMR abundance and profiling



# RNA-seq Analysis Portal – easy data analysis



## Comprehensive

- Analyzes data from 18 different species using proven published algorithms
- Analyzes data from QIAseq and other major RNA Library Kits

## Easy

- Takes the stress out of data analysis
- Fast-tracks path to gene expression insights
- Integrates results with dPCR and qPCR verification

## Fast

- Goes from FASTQ files to pathway analysis insights in hours instead of days

## Online

- Performs analyses anytime, anywhere

## Demo version

- Allows software evaluation using demo data without registering or logging in

## Free trial

- Allows software evaluation using real data

## Graphics tell your story



1. Features table listing differentially expressed targets
2. Volcano plot graphs fold-change versus p-value
3. Heatmap clusters targets and samples
4. Biological insights lists related biological information
5. Filter narrow down the target list
6. What's next helps design follow-up experiments

Access is included as standard with QIAseq RNA and miRNA library kits

Analysis credits can be purchased separately for analyzing data from the commonly used third-party kits



## Sample disruption

## Sample preparation

## Analysis by NGS, dPCR or qPCR

## Data analysis

### Genomic Services bacterial and fungal communities profiling:

- **DNA isolation** based on optimized bead-beating and IRT

- **End-to-end service:** We take care of every step, from sample preparation to data analysis
- **Improved sequencing output:** We help increase read and base quality
- **Guidance and flexibility:** We aid in designing your project and help you make the right decisions
- **Ready-to-publish data:** We deliver comprehensive reports and data packages and provide guidance on the next steps

### Microbial profiling

- Microbiome analysis 16S/ITS
- Metagenomic sequencing

### Viral genotyping

- SARS-CoV-2 whole genome sequencing

### NGS

- Whole transcriptome-seq
- Targeted 3'-mRNA
- Targeted RNA-seq panels
- miRNA-seq
- Whole genome sequencing

### Digital PCR services



Visit:  
[www.qiagen.com/applications/genomic-services](https://www.qiagen.com/applications/genomic-services)

# A comprehensive portfolio for microbiome and microbial identification applications



[Back to intro slide](#)

- TissueLyser III
- Vortex adapters

- QIAcube Connect
- QIAcube HT
- QIASymphony SP
- QIAxcel Connect for QC

- QIAcuity
- QIAquant
- Rotor-Gene Q
- QIAgility

## Sample disruption

## Nucleic acid isolation

## NGS

## dPCR, qPCR and PCR

## Data analysis

- PowerBead Pro Tubes
  - PowerBead Pro Plates
- Stabilization (stool)**
- PowerProtect DNA/RNA

- DNA**
- QIAamp PowerFecal Pro DNA; QIASymphony PowerFecal Pro DNA Kit; QIAamp DNA Microbiome
  - DNeasy PowerSoil Pro DNeasy PowerMax Soil; MagAttract PowerSoil Pro
  - DNeasy PowerWater; DNeasy PowerWater Sterivex; DNeasy PowerBiofilm
- RNA**
- RNeasy PowerFecal Pro
  - RNeasy PowerSoil Total RNA; RNeasy PowerMax Soil Pro
  - RNeasy PowerWater; RNeasy PowerBiofilm
- DNA and RNA**
- AllPrep PowerFecal Pro DNA/RNA; AllPrep PowerViral DNA/RNA; MagAttract PowerMicrobiome DNA/RNA
- ... and more

- Microbial detection**
- QIAseq xHYB Viral and Bacterial Panels
- 16S rRNA and ITS profiling**
- QIAseq 16S/ITS Panels
- Metagenomic shotgun sequencing**
- QIAseq FX DNA Library Prep
- Metatranscriptomics**
- QIAseq FastSelect -5S/16S/23S; QIAseq FastSelect RNA Lib Bacteria Kit; QIAseq UPXome RNA Lib Kit Bacteria
- Host gene expression analysis**
- QIAseq FastSelect RNA Library Kit; QIAseq UPXome RNA Library Kit; ... and more
- Microbial-host interaction**
- QIAseq FastSelect RNA Lib Epidemiology Kit; QIAseq UPXome Dual-seq RNA Lib Kit
- Normalization**
- QIAseq Normalizer Kits

- dPCR**
- dPCR Microbial DNA Detection Assays
  - QIAcuity UCP Probe PCR Kit
  - QIAcuity OneStep Advanced Probe Kit
- qPCR/PCR**
- Microbial DNA qPCR Assays and Arrays
  - UCP Multiplex PCR
  - QuantiNova Pathogen +IC Kit

- QIAGEN CLC Workbench Premium
- GeneGlobe Design & Analysis Hub
- RNA-seq Analysis Portal
- Microbial Analysis Portal
- QIAcuity Software Suite

## Nucleic acid isolation, NGS & data analysis

- QIAamp PowerFecal Pro WGS SeqSet; DNeasy PowerSoil Pro WGS SeqSet



**QIAGEN Genomic Services**



Discover more at [www.qiagen.com/applications/microbiome](https://www.qiagen.com/applications/microbiome)



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