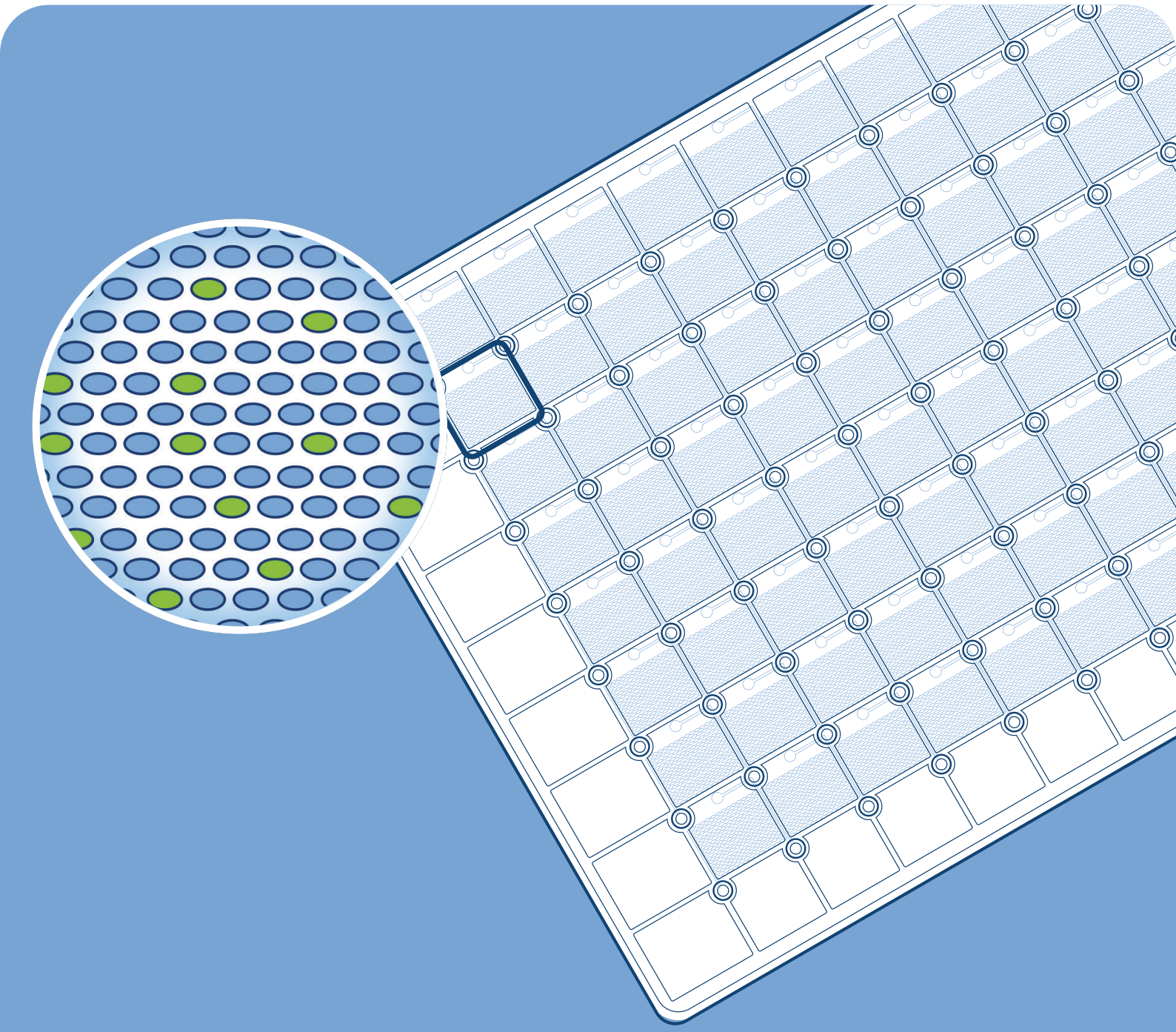
Digital PCR Applications eBooklet



Introduction

Digital PCR (dPCR) has become increasingly applicable to various research topics over the past decade. The method offers application-specific advantages, such as precision, robustness, speed and multiplexing capabilities. In this booklet, discover why and how to adapt dPCR to your area of research. Benefit from experimental data and discover complete workflows for optimal dPCR performance in cell & gene therapy, mutation detection, gene expression analysis, microbial detection and many more.

What can you do with digital PCR?

Adeno-associated virus (AAV) titration

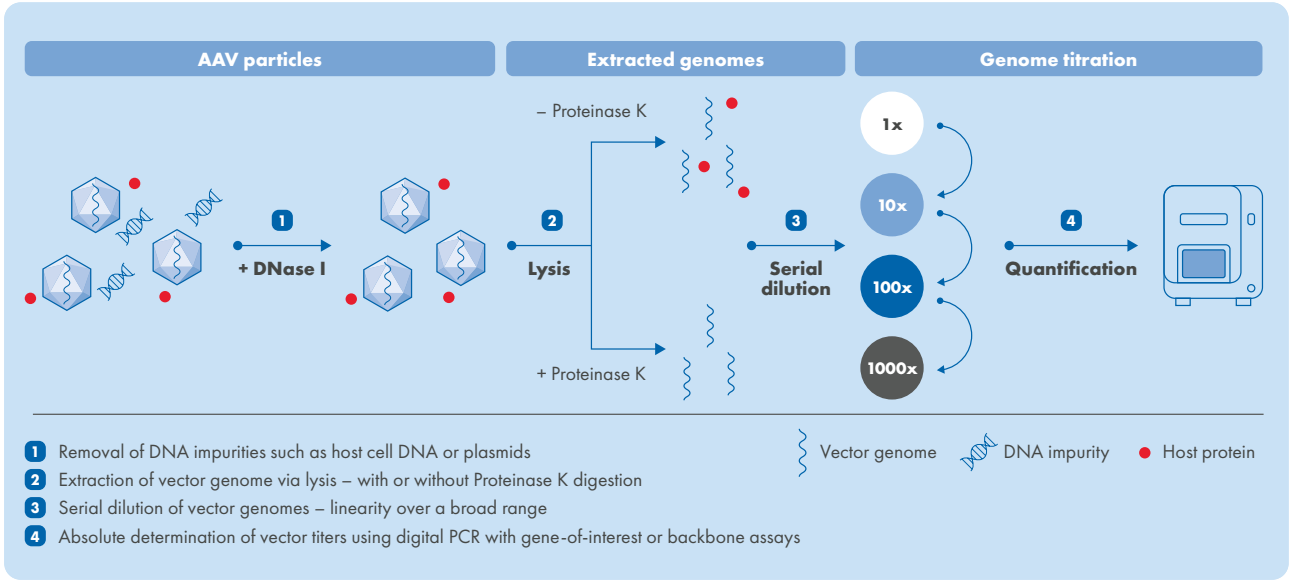
Background

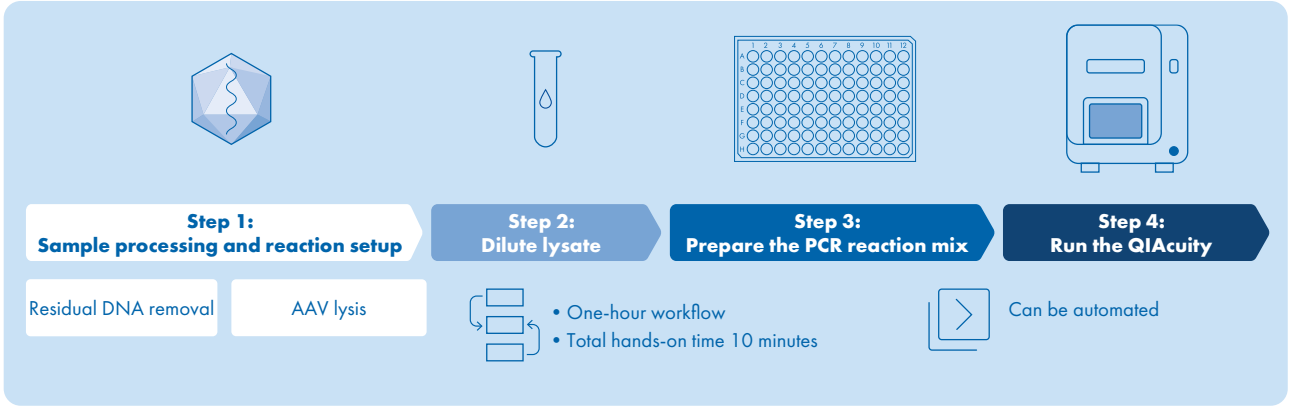
Whenever you generate and purify viral vectors, you need precise quality control to achieve safe and reliable dosing during clinical studies or patient care. The new workflow for viral vector genome quantification using the QIAcuity Digital PCR System enables vector genome titration with outstanding accuracy, reproducibility, speed and ease of use.

Benefits

- Consistent, robust and accurate determination of final titer thanks to the efficient capsid lysis and residual DNA removal offered by the improved formula of the CGT Viral Vector Lysis Kit
- Reduced manual steps with only 10 min hands-on time
- Easy implementation with one protocol for both standardization and quality control
- Higher accuracy and efficiency thanks to multiplexing with as many as 10 single target assays with different dye combinations
- Customization and flexibility with option to extend 3-plex capacity to 5-plex with user-chosen genes of interest (GOIs)

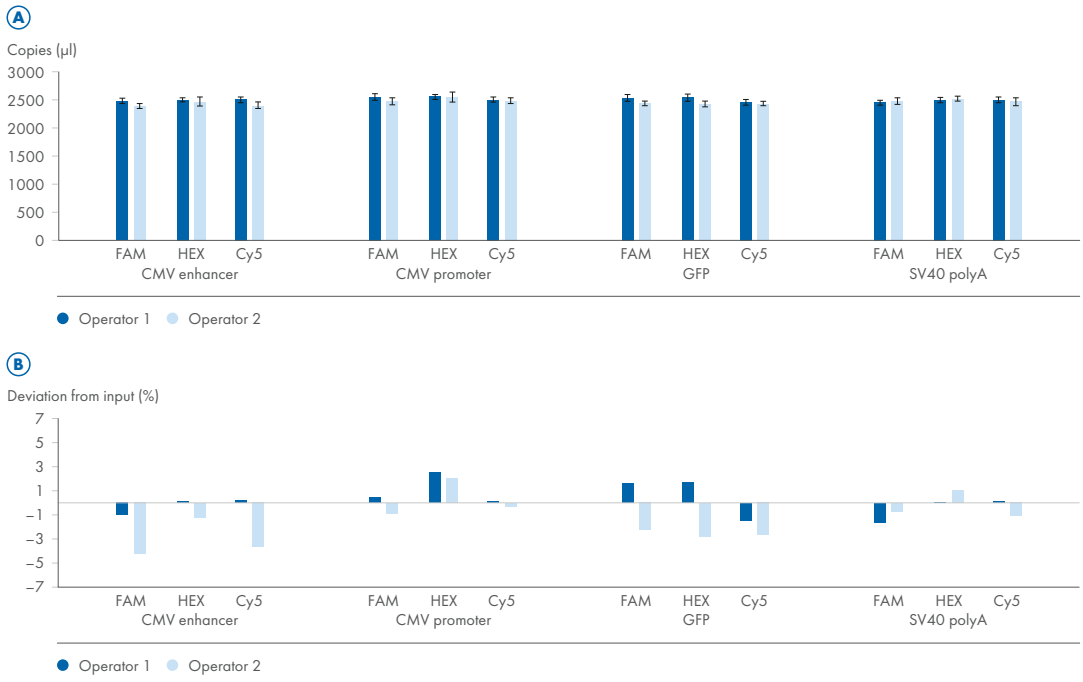
Workflow





Data

CGT Viral Vector Lysis Kit from QIAGEN vs. currently used heat lysis protocols



High accuracy between operators using the QIAcuity CGT dPCR Assays Mean CV among all assays and operators is ~2%

Publications

- Martorana D et al. Determination of adeno-associated virus (AAV) titers using the QIAcuity® Digital PCR System. QIAGEN, 2023.
- Liu H-W et al. Optimized in-process recombinant adeno-associated virus (rAAV) vector genome titer protocol using the QIAcuity® Digital PCR System. QIAGEN and Resilience, 2022.
- Martorana D et al. Quantification and qualification of adeno-associated virus (AAV) using dedicated CGT assays and the QIAcuity Digital PCR System. QIAGEN, 2023.

Ordering Information

Product	Contents	Cat. no.
CGT Viral Vector Lysis Kit	CGT Sample Stabilizer, DNase I, Nuclease-Free Water, CGT Lysis buffer, CGT DNase I buffer and CGT Dilution Buffer enough to process 100 AAV samples	250272
QIAcuity Cell & Gene Therapy (CGT) dPCR Assays	For 500x12 µL reactions (20x): QIAGEN Cell and Gene Therapy assay for GFP; ITR2/5, Sv40 promoter, AMP resistance or others to be used with the QIAcuity dPCR system	250255
QIAcuity Probe PCR Kit	1 mL 4x concentrated QIAcuity Probe Mastermix, 2 x 1.9 mL Water	250101

Copy number variation (CNV) analysis

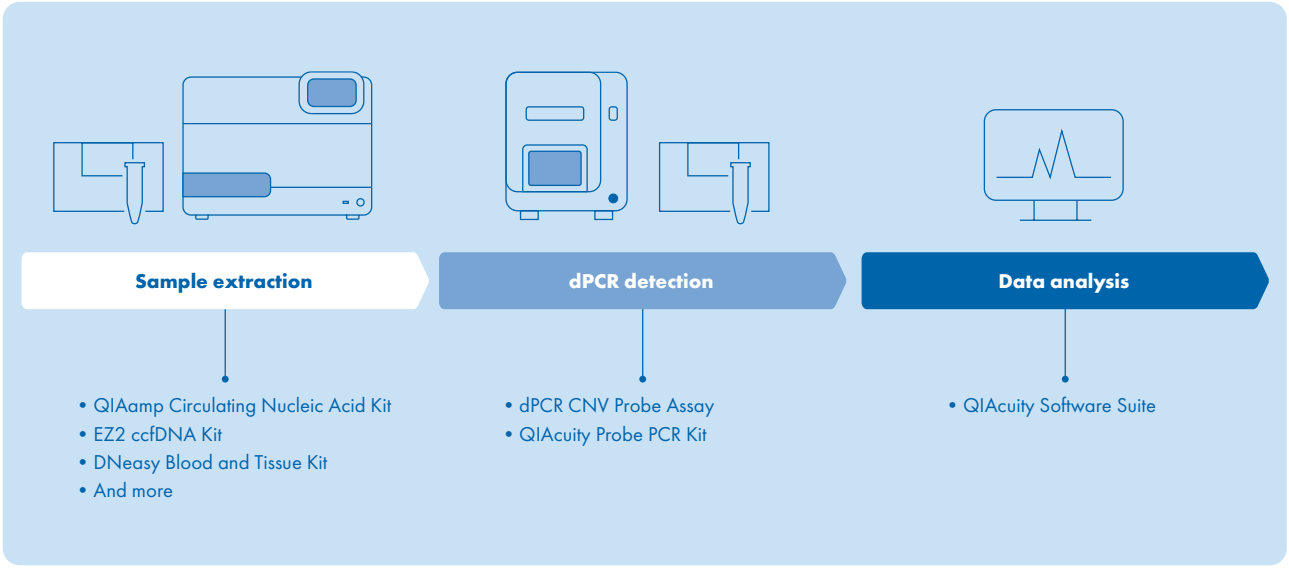
Background

Copy number variations (CNVs) are structural changes that alter the diploid state in the human genome. CNVs are linked to common and complex traits and diseases, such as cancer.

Benefits

- Detect precise copy number changes
- Complete workflow in two hours or less
- Improve accuracy and efficiency by multiplexing up to 5 targets in one reaction

Workflow



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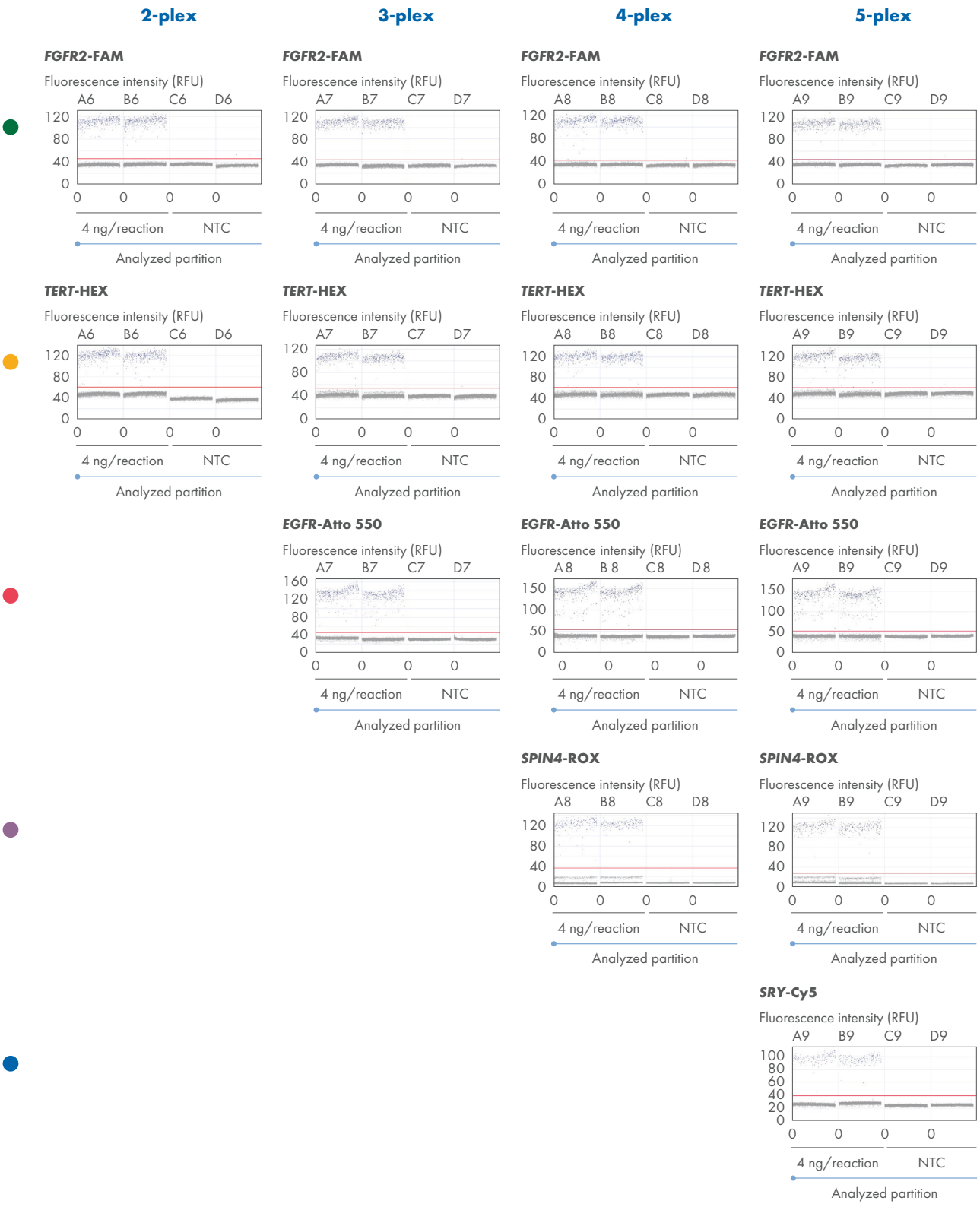


Five dPCR Probe CNV Assays were multiplexed in a single reaction using 4 ng of gDNA per reaction.

Publications

- Toffoli M et al. Comprehensive short and long read sequencing analysis for the Gaucher and Parkinson's disease-associated GBA gene. *Communications Biology*. 2022; 5:670.
- Tergemina E et al. A two-step adaptive walk rewires nutrient transport in a challenging edaphic environment. *Science Advances*. 2022; 8(20).
- Shoop WK et al. Precise and simultaneous quantification of mitochondrial DNA heteroplasmy and copy number by digital PCR. *Journal of Biological Chemistry*. 2022; 298(11):102574.

Ordering Information

Product	Contents	Cat. no.
dPCR Copy Number Assay	Single tube containing ready-to-use 25x-concentrated assay; sufficient for 200 dPCR reactions of 12 µL each	250205
QIAcuity EG PCR Kit	1 mL 3x concentrated QIAcuity EvaGreen Mastermix, 1 x 1.9 mL Water	250111
dPCR CNV Probe Assay	Single tube containing ready-to-use, 20x-concentrated reference assay; sufficient for 300/500/1000 dPCR reactions of 12 µL each	250213
QIAcuity Probe PCR Kit	1 mL 4x concentrated QIAcuity Probe Mastermix, 2 x 1.9 mL Water	250101

Gene expression quantification

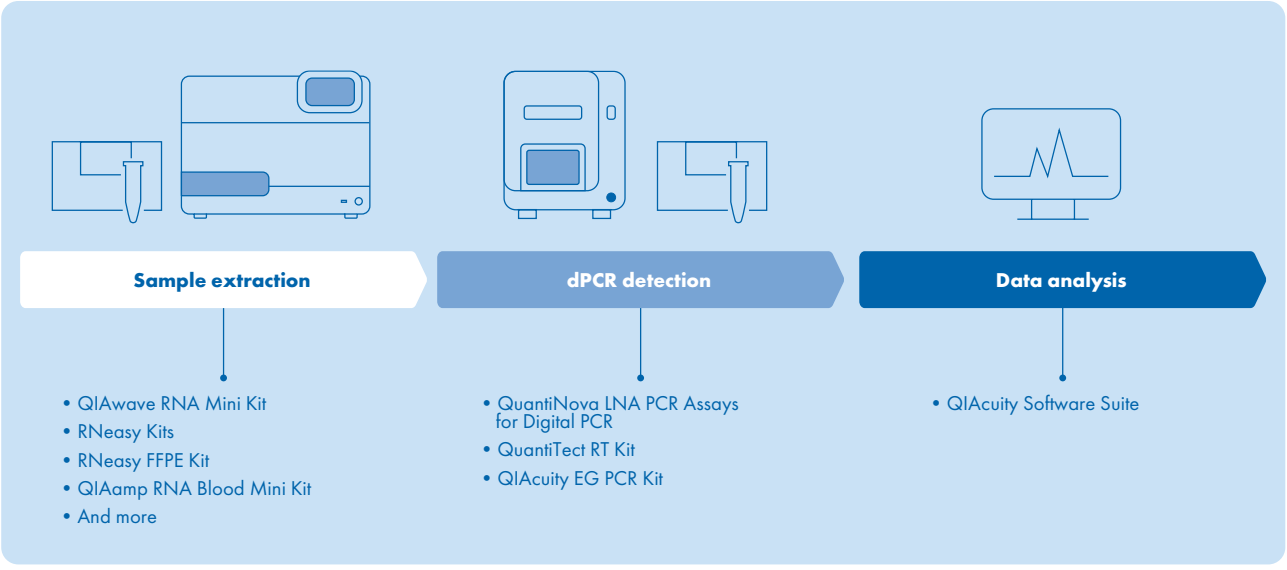
Background

With dPCR, you can quantify gene expression and detect small fold changes of RNA levels. The high precision of dPCR is beneficial to gene expression profiling, single gene expression studies, investigating gene regulated pathways or developing diagnostic biomarkers.

Benefits

- In-depth, accurate and robust detection of very small fold changes of mRNA and lncRNA using EvaGreen chemistry
- Superior sensitivity and specificity, thanks to LNA – enhanced primers
- More than 1.3 million pre-designed assays covering all human, mouse, rat mRNA and lncRNA transcripts

Workflow



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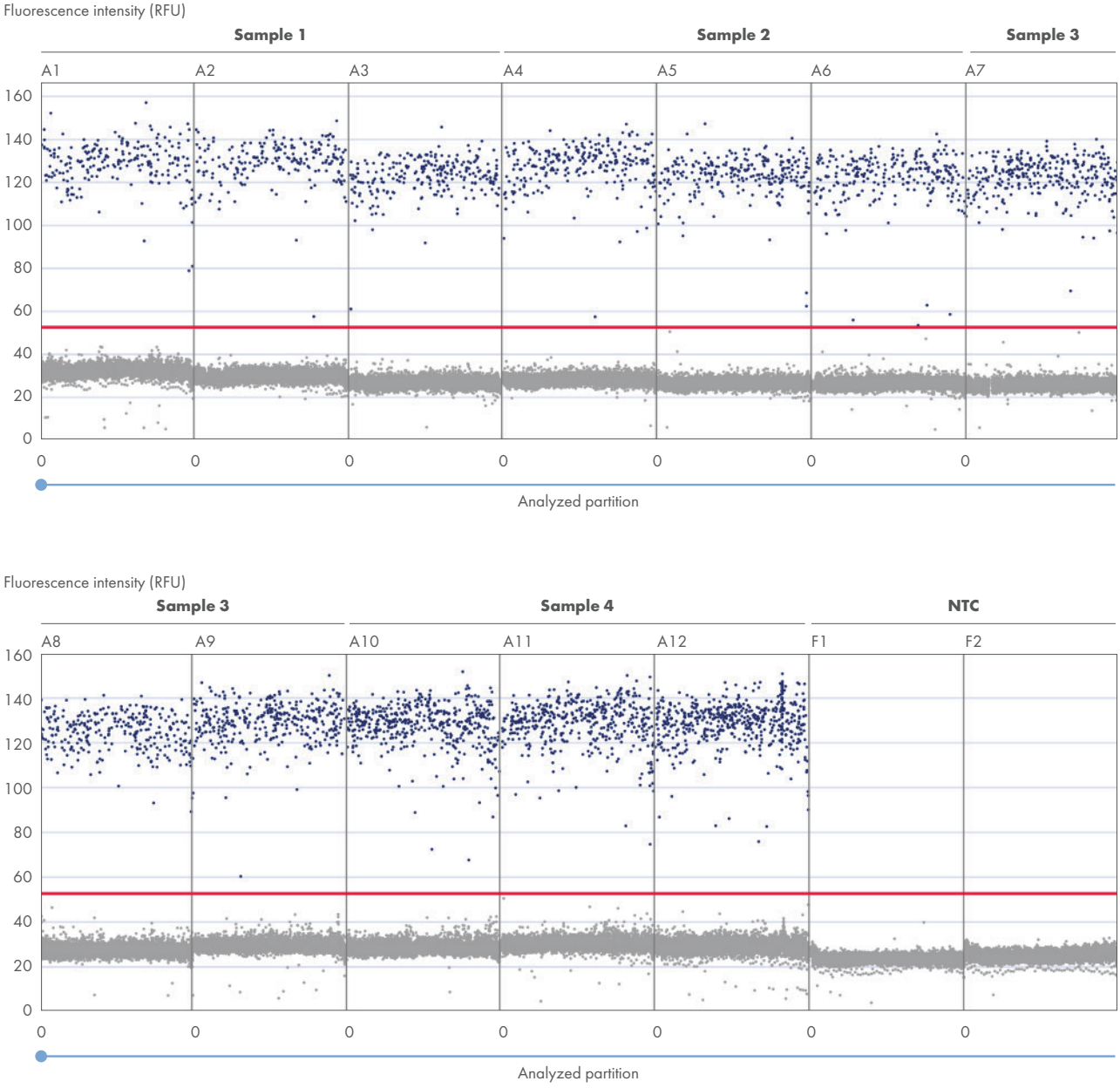


Precise and robust detection of small fold changes in gene expression. 1D Scatter Plot of IL4 target assay (QuantiNova LNA PCR Assays) and sample 1 (decreased amount of spiked-in synthetic IL4 RNA sample), sample 2 (reference sample with spiked synthetic IL4 RNA sample), sample 3 and 4 (increased amount of spiked-in synthetic IL-4 RNA sample), each with 3 technical replicates, plus NTC

Publications

- Jurek B et al. Oxytocin accelerates tight junction formation and impairs cellular migration in 3D spheroids: evidence from Gapmer-induced exon skipping. *Front. Cell. Neurosci.* 2022: 16.
- Feliciello I et al. Regulation of ssb Gene Expression in Escherichia coli. *International Journal of Molecular Sciences.* 2022; 23(18):10917.
- Kirchhof L et al. G3BP1-UPF1-Associated Long Non-Coding RNA CALA Regulates RNA Turnover in the Cytoplasm. Non-Coding RNA. 2022; 8(4):49.

Ordering Information

Product	Contents	Cat. no.
QuantiNova LNA PCR Assays for Digital PCR	Predesigned mRNA/lncRNA-specific primer mixture in a single tube; for 200 qPCR reactions or 400 dPCR reactions	249990
QuantiTect RT Kit	100 µL 7x gDNA Wipeout Buffer, 50 µL Quantiscript Reverse Transcriptase, 200 µL 5x Quantiscript RT Buffer, 50 µL RT Primer Mix, 1.9 mL RNase-Free Water: for 50 x 20 µL reactions	205311
QIAcuity EG PCR Kit	1 mL 3x concentrated QIAcuity EvaGreen Mastermix, 1 x 1.9 mL Water	250111

Microbial testing in wastewater

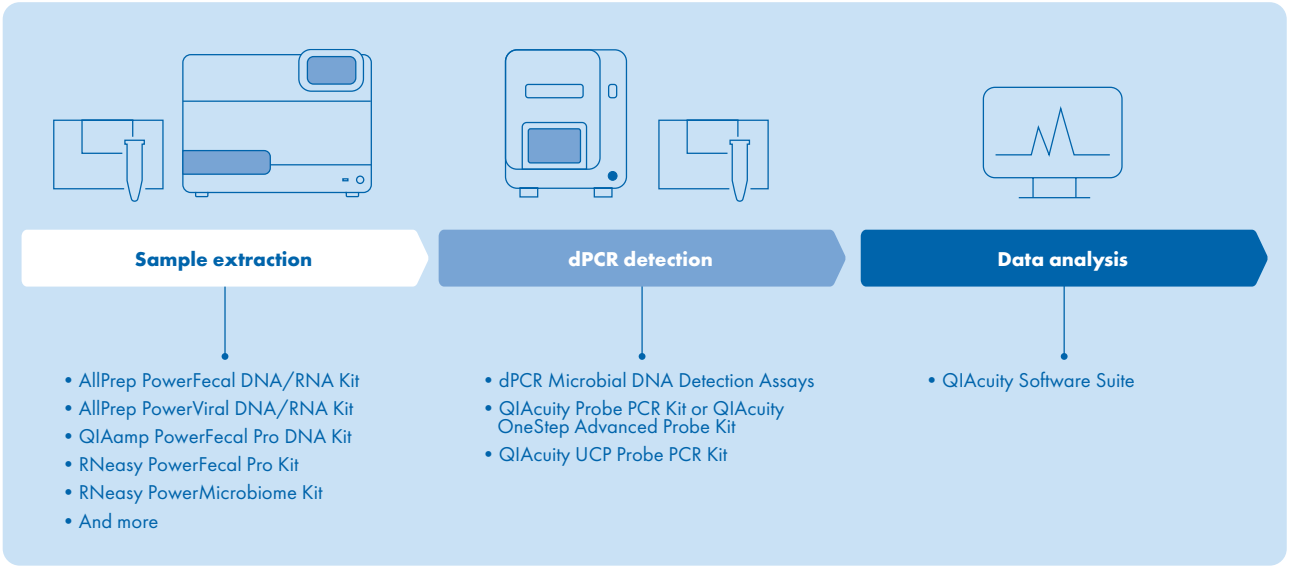
Background

Detection of microbial material related to human viruses in wastewater and natural sewage serves as a powerful surveillance tool for epidemiological outbreaks.

Benefits

- Precise and absolute quantification of microbial material, including potential epidemiologic viruses
- High robustness for viral detection from complex samples
- High tolerance to inhibitors
- Multiplexing up to 5 assays possible with selection from custom designed assays or nearly 700 catalogue assays for microbial targets (bacterial, viral, virulence factors, AMG, etc.) including SARS-CoV-2 and MPXV

Workflow



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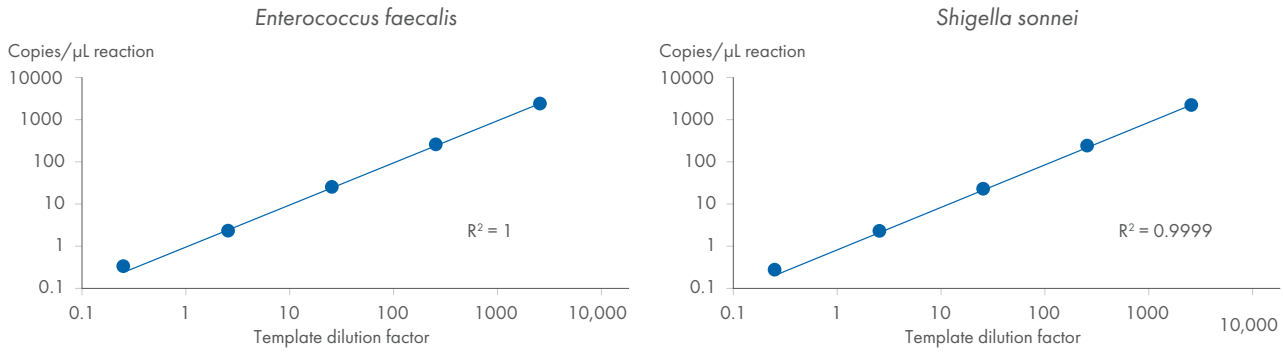
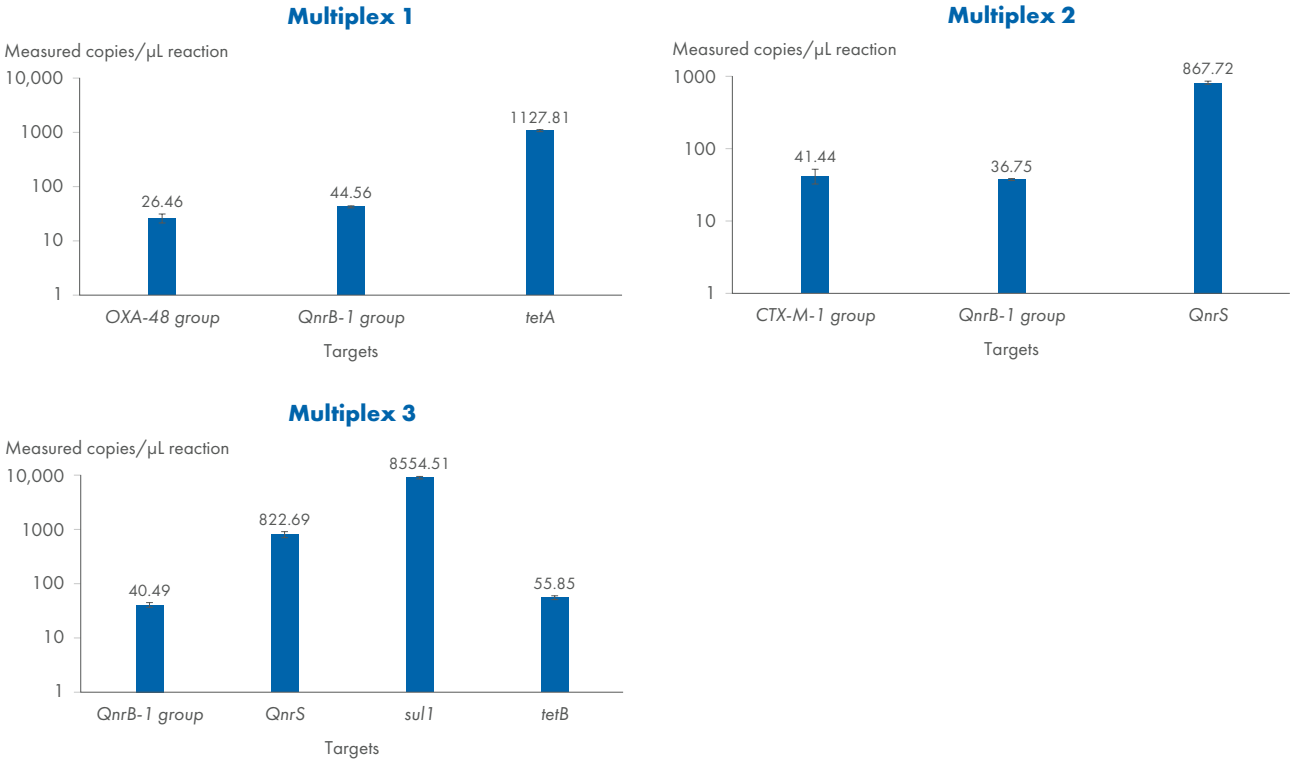


Accurate detection of microbial targets over a broad dynamic range. Four-log dynamic range of linear quantification of two different bacterial strains. Plots depict mean measures copies/µL values against a dilution factor of the input template.



Multiplex and mix-and-match capabilities for parallel quantification by QIAcuity dPCR of AMR targets in wastewater. Reaction setup of 3 multiplex combinations. Error bars depict standard deviation values from 3 replicates each.

Publications

- Donohoe et al. Wastewater-based epidemiology workflows with QIAcuity® digital PCR. QIAGEN, 2023.
- Amman F et al. Viral Variant-Resolved Wastewater Surveillance of SARS-CoV-2 at National Scale. *Nature Biotechnology.* 2022; 40:1814–1822.
- Tiwari A et al. Application of Digital PCR for Public Health-Related Water Quality Monitoring. *Science of The Total Environment.* 2022; 837:155663.
- Thakali O et al. Pilot study on wastewater surveillance of dengue virus RNA: Lessons, challenges, and implications for future research. *Environmental Challenges.* 2022; 9: 100614.
- Wurtzer S et al. First Detection of Monkeypox Virus Genome in Sewersheds in France: The Potential of Wastewater-Based Epidemiology for Monitoring Emerging Disease. *Environmental Science & Technology Letters.* 2022; 9(11): 991–996.
- Wilhelm A et al. Early Detection of SARS-CoV-2 Omicron BA.4 and BA.5 in German Wastewater. *Viruses.* 2022; 14(9):1876.
- Li J et al. Impact of sewer biofilms on fate of SARS-CoV-2 RNA and wastewater surveillance. *Nature Water.* 2023; 1: 272–280.
- Ahmed W et al. Comparison of RT-qPCR and RT-dPCR Platforms for the Trace Detection of SARS-CoV-2 RNA in Wastewater. *ACS EST Water.* 2022; 2(11): 1871–1880.

Ordering Information

Product	Contents	Cat. no.
dPCR Microbial DNA Detection Assays	One tube with lyophilized assay, dye (fluorophore) configurable, 200 reactions (40 µL reaction in Nanoplate 26k)	250207
QIAcuity Probe PCR Kit	1 mL 4x concentrated QIAcuity Probe Mastermix, 2 x 1.9 mL Water	250101

miRNA detection

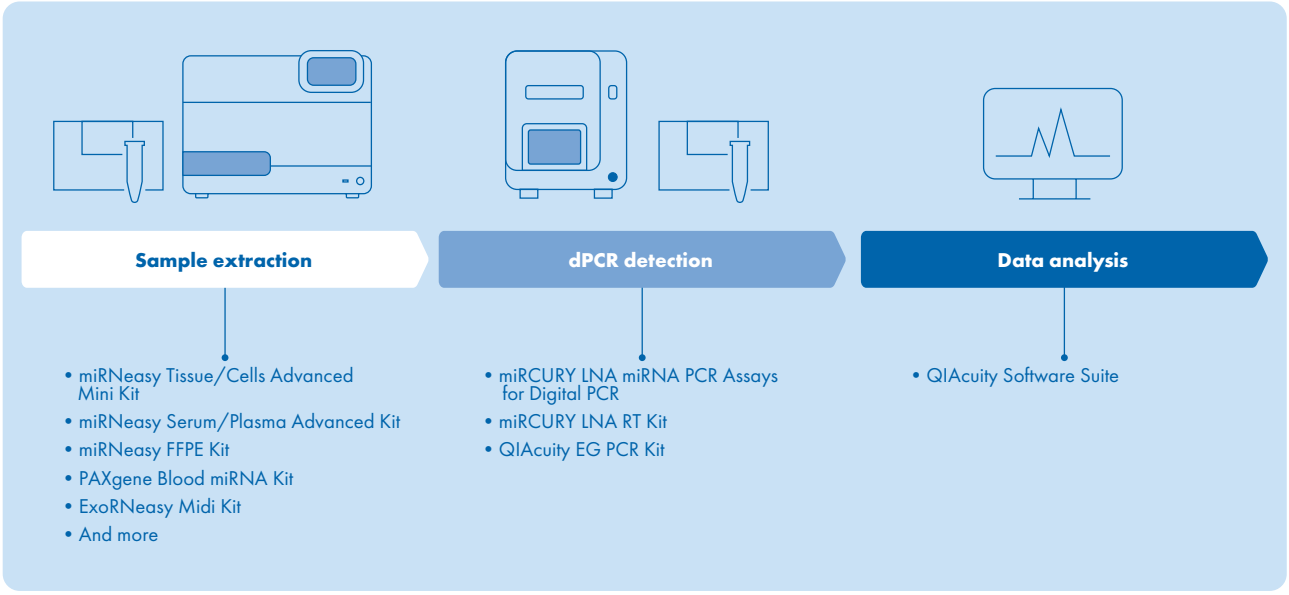
Background

MicroRNAs (miRNAs) are small non-coding RNAs (~21 nucleotides) which play a key role in gene regulation of important molecular pathways. Many diseases, such as cancer, have altered miRNA expression profiles. miRNAs show great potential as diagnostic and prognostic biomarkers.

Benefits

- Precise and robust detection of miRNA, even at low expression levels
- Discrimination of single nucleotide differences in closely sequence-related miRNAs, thanks to high specificity of dPCR
- Absolute quantification of subtle miRNA expression changes

Workflow



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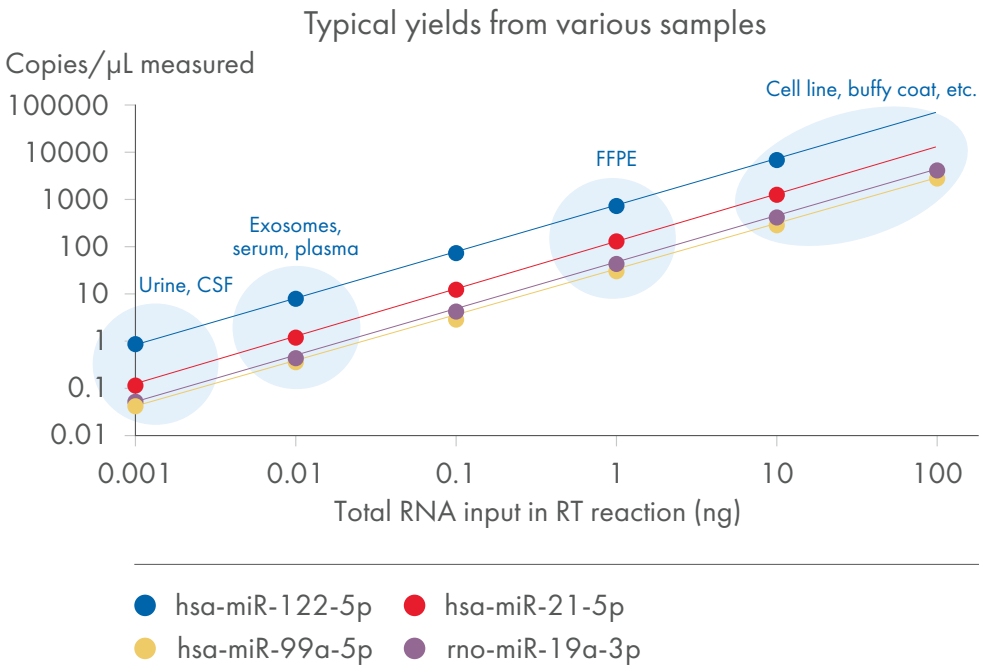


Superior sensitivity with reliable miRNA detection at 1 pg RNA input for a wide range of sample types

Publications

- Matorana D et al. Absolute quantification of miRNAs with high accuracy and precision using digital PCR. QIAGEN, 2023.
- Chithanathan K et al. Paradoxical attenuation of neuroinflammatory response upon LPS challenge in miR-146b deficient mice. *Frontiers in Immunology*. 2022; 13.

Ordering Information

Product	Contents	Cat. no.
miRCURY LNA PCR Assays for Digital PCR	Forward and reverse primers for 200 SYBR® Green-based, real-time qPCR reactions, 166 EvaGreen-based digital PCR reactions for Nanoplate 8.5k or 50 EvaGreen-based digital PCR reactions for Nanoplate 26k	339306
miRCURY LNA RT Kit	5x RT SYBR Green Reaction Buffer, 5x RT Probe Reaction Buffer, 10x RT Enzyme Mix, UniSp6, RNA Spike-in template, RNase-Free Water: for 8 – 64 cDNA synthesis reactions	339340
QIAcuity EG PCR Kit	1 mL 3x concentrated QIAcuity EvaGreen Mastermix, 1 x 1.9 mL Water	250111

Mutation detection in oncology

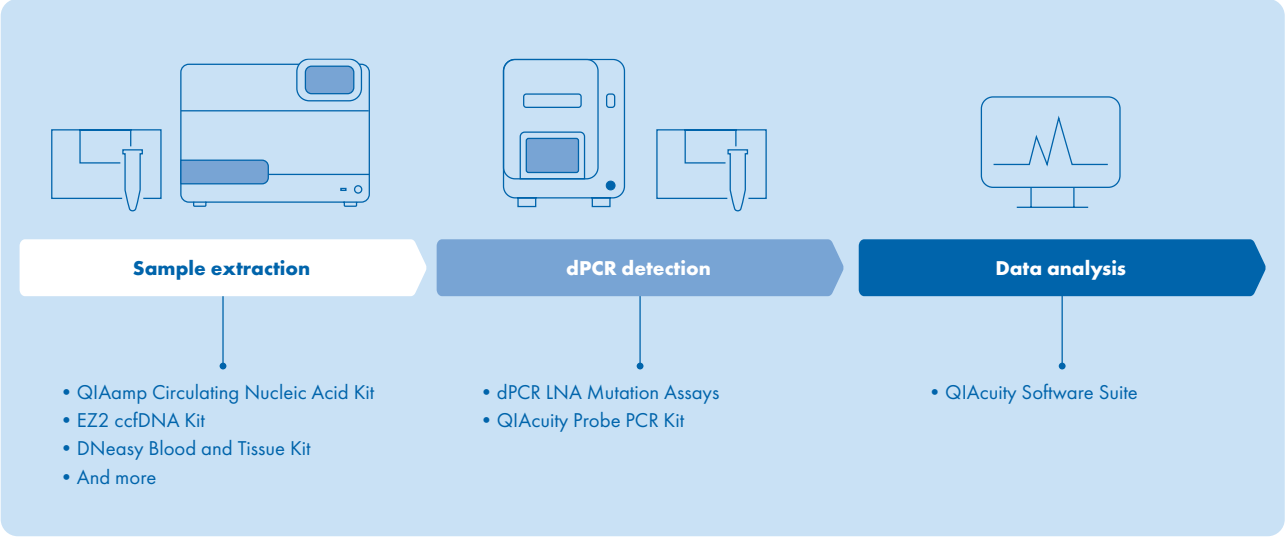
Background

Liquid biopsies provide a non-invasive way to retrieve genetic material from tumors and to investigate biomarkers and tumor heterogeneity.

Benefits

- High sensitivity in detection and absolute quantification of rare events and sequence variants
- Detect the presence of a mutation at levels as low as 0.1% in a complex background of wild-type genomic DNA

Workflow



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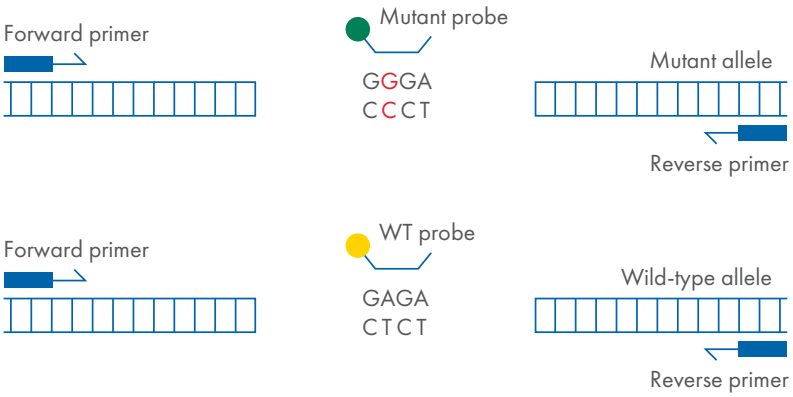
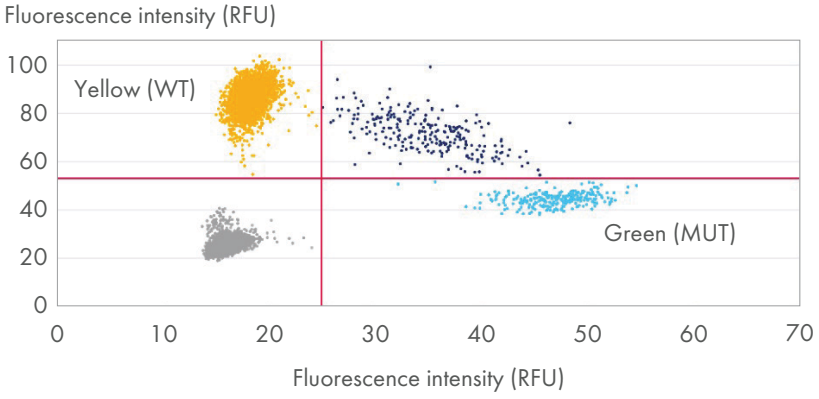
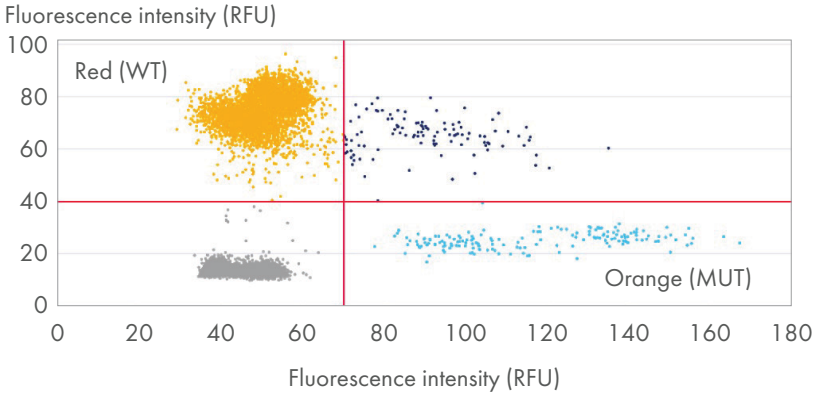


The assay contains a primer pair and two probes, a mutant probe and a wild-type (WT) probe, for detecting both mutant and wild-type alleles in the same reaction.

EGFR T790M (FAM/HEX)



BRAF V600E (Atto550/ROX)



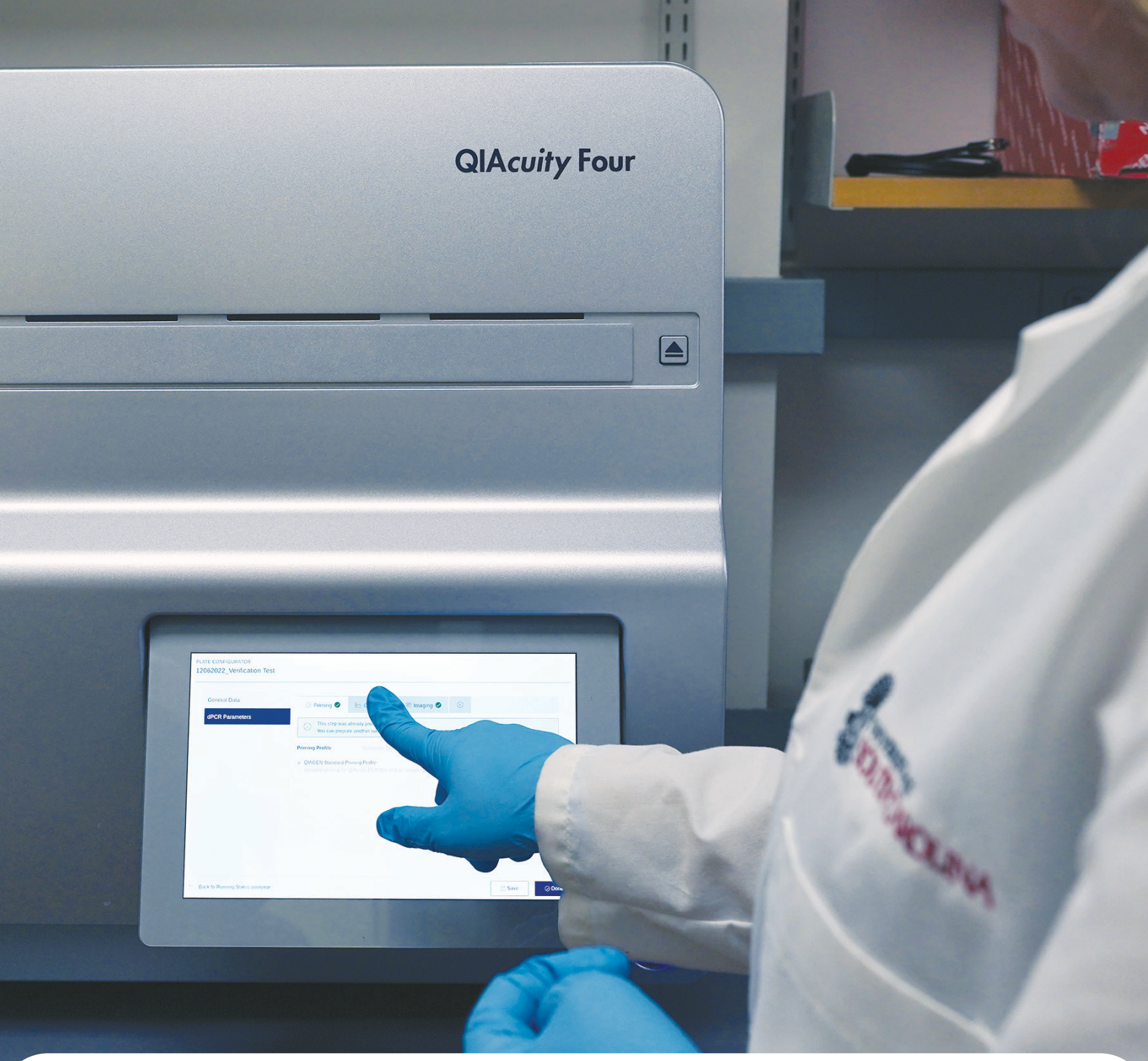
A four colors experiment for detecting both EGFR T790M and BRAF V600E in the same well.

Publications

- Andric F et al. Immune Microenvironment in Sporadic Early-Onset Versus Average-Onset Colorectal Cancer. *Cancers*. 2023; 15(5):1457.
- Hélias-Rodzewicz Z et al.Molecular and clinicopathologic characterization of pediatric histiocytosis. *American Journal of Hematology*. 2023; DOI:10.1002/ajh.26938.
- Crucitta S et al. Comparison of digital PCR systems for the analysis of liquid biopsy samples of patients affected by lung and colorectal cancer. *Clinica Chimica Acta*. 2023; 541:117239.

Ordering Information

Product	Contents	Cat. no.
dPCR LNA Mutation Assay	Single tube containing ready-to-use 30x-concentrated assay with choice of FAM + HEX or Atto 550 + ROX detection dyes; sufficient for 200 dPCR reactions of 40 µL each	250200
QIAcuity Probe PCR Kit	1 mL 4x concentrated QIAcuity Probe Mastermix, 2 x 1.9 mL Water	250101



Protein detection

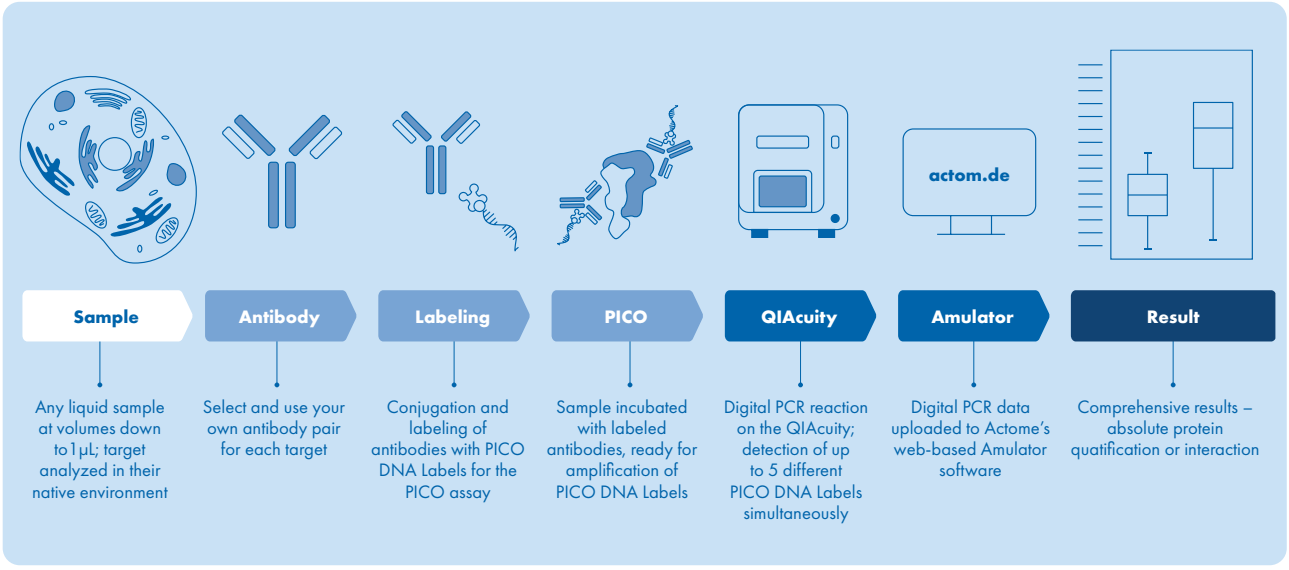
Background

Protein analysis is an up-and-coming application of digital PCR. For its solution for protein detection, QIAGEN collaborates with Actome to adapt their proprietary Protein Interaction Coupling (PICO) technology to our installed base.

Benefits

- The only technology on the market for quantifying proteins using dPCR
- Detect proteins, protein-protein interaction and post translational modifications
- Absolute and relative quantification is possible

Workflow



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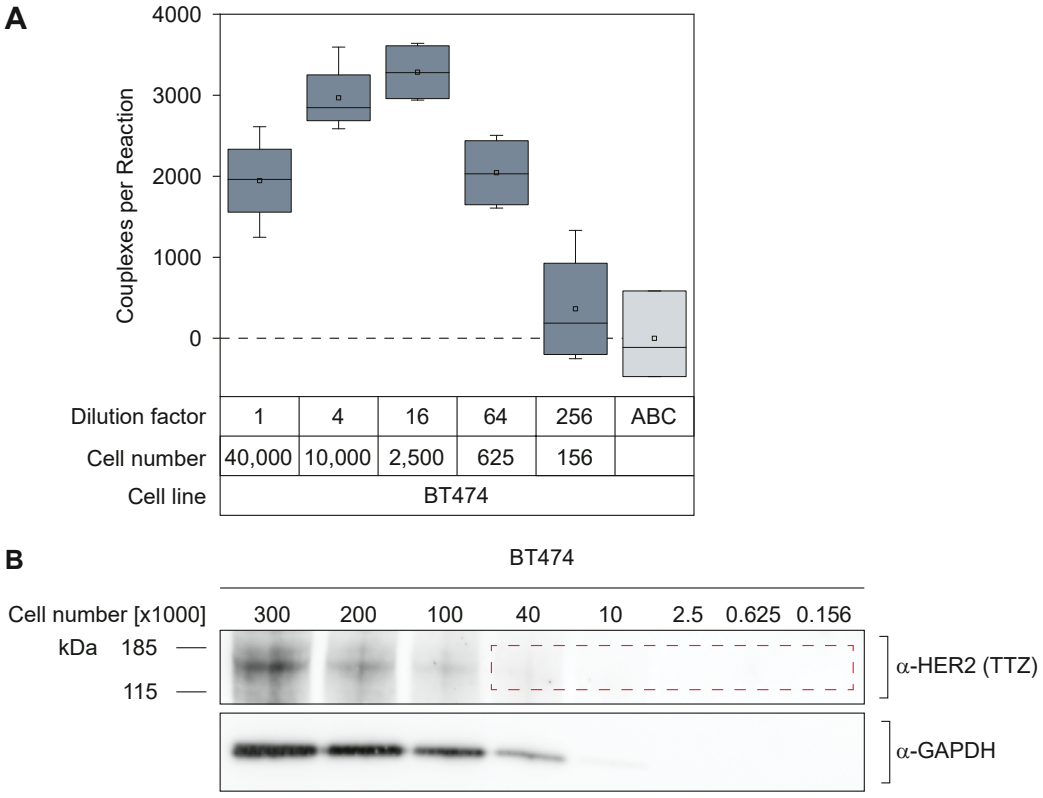


HER2 detection in BT474 cell line. A) Couplexes per reaction from different amount of BT474 cells that were detected in the PICO assay, using the HER2-specificantibodies PTZ and TTZ. B) Western blot of whole cell lysate dilution series of BT474cells, probing done with HER2-specific antibody TTZ and GAPDH-specific antibody. The red box reflects the cell amounts that were used in the PICO assay.

Publications

- Gross T et al. Highly sensitive HER2 detection in BT474 and MCF7 cells using the PICO technology. Actome, 2022.

Ordering Information

Product	Contents	Cat. no.
QIAcuity Probe PCR Kit	1 mL 4x concentrated QIAcuity Probe Mastermix, 2 x 1.9 mL Water	250101

Quantification of next-generation sequencing (NGS) libraries

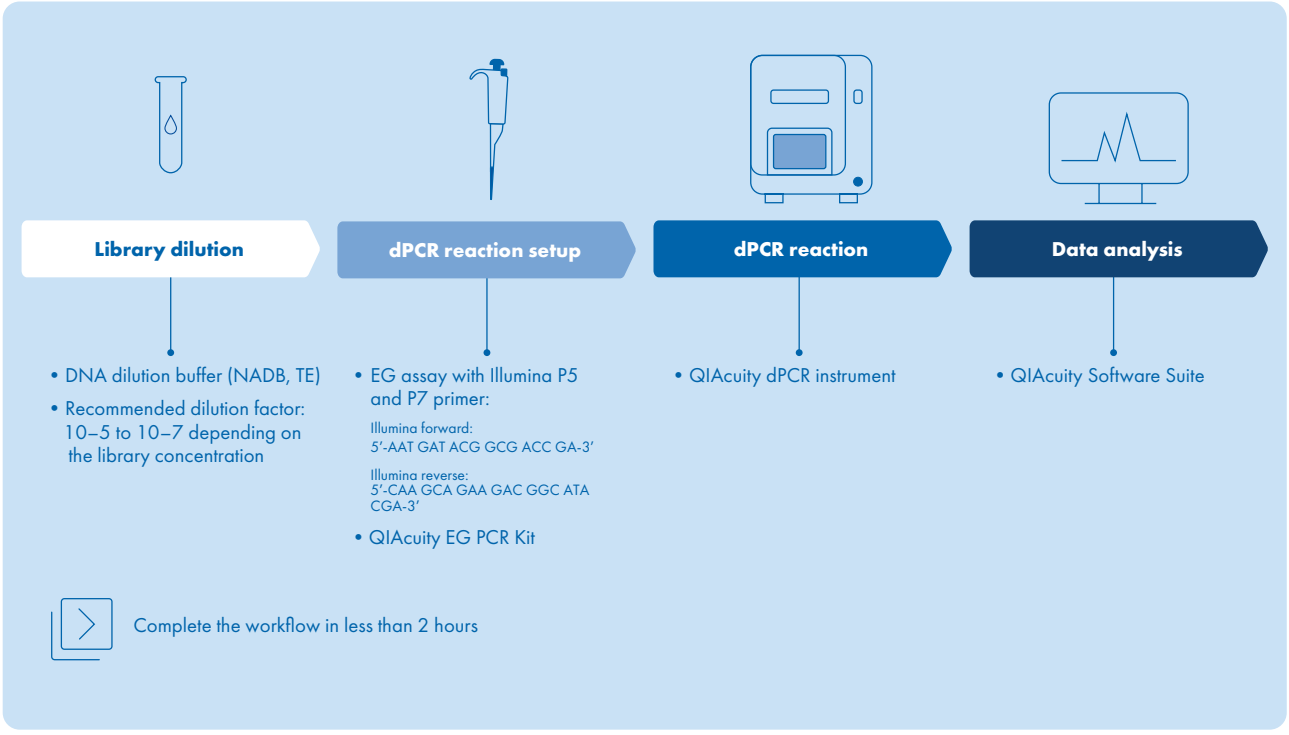
Background

Determining the absolute concentration of an NGS library pool is crucial to obtaining optimal yield and reducing cost per sample.

Benefits

- Absolute quantification of amplifiable library fragments without amplification bias, without standard bias
- Quantification in 2 hours and suitable for routine testing
- High reproducibility and superior uniformity for library pooling
- Coverage of all Illumina library types with one assay

Workflow



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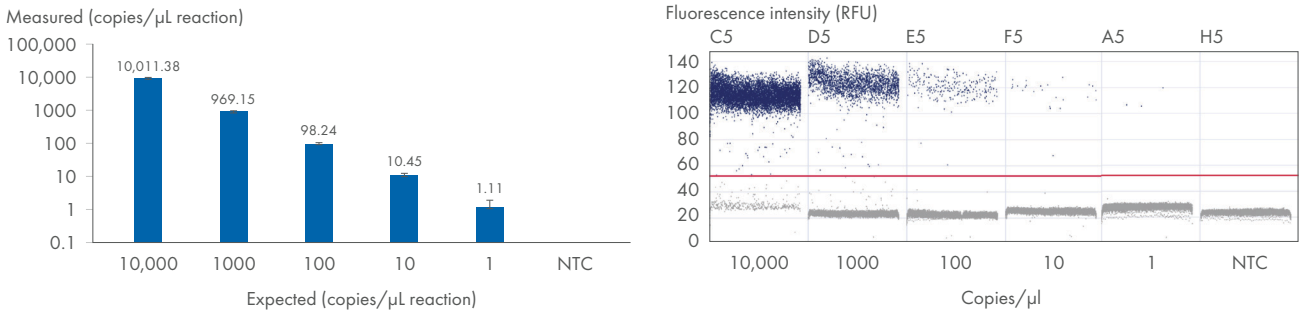
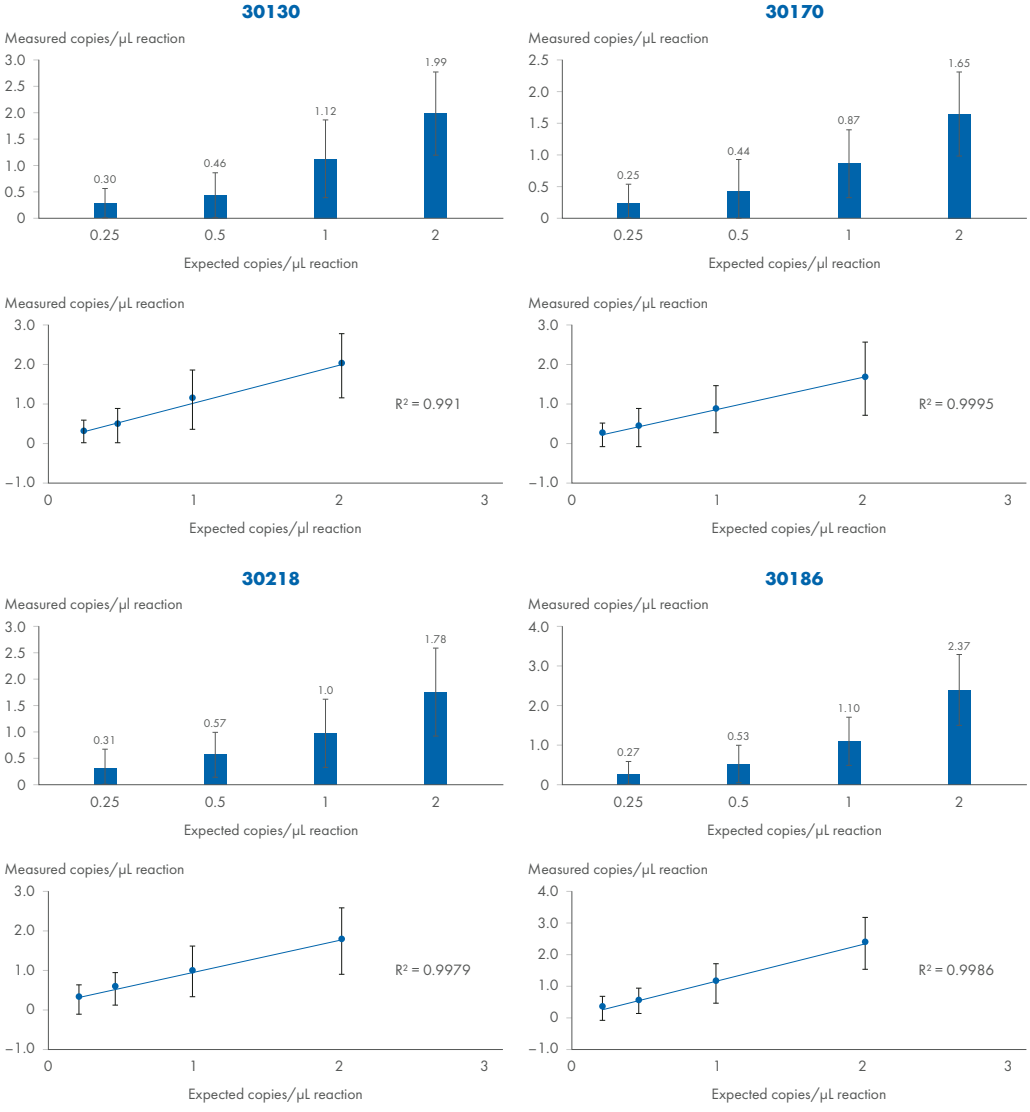


Precise linear quantification across a 4-log dynamic range and strong signal-to-noise separation. Quantification of a QIAseq 16S/ITS library with a fragment length of 795 bp. 10-fold dilution series with 12 replicates. QIAcuity dPCR with Illumina primers and the QIAcuity EG PCR kit in a 96-well nanoplate.



Linear quantification below the lower end of the dynamic range supports an LLOQ of 1 copy/µL for the 96-well nanoplate Quantification of four test libraries. Four dilutions at low concentrations with 48 replicates each. QIAcuity dPCR with Illumina primers and the QIAcuity EG PCR Kit in a 96-well nanoplate.

Publications

- Kellner R et al. Accurate NGS library quantification using nanoplate digital PCR. QIAGEN, 2023.

Ordering Information

Product	Contents	Cat. no.
QIAcuity EG PCR Kit	1 mL 3x concentrated QIAcuity EvaGreen Mastermix, 1 x 1.9 mL Water	250111

Residual DNA quantification

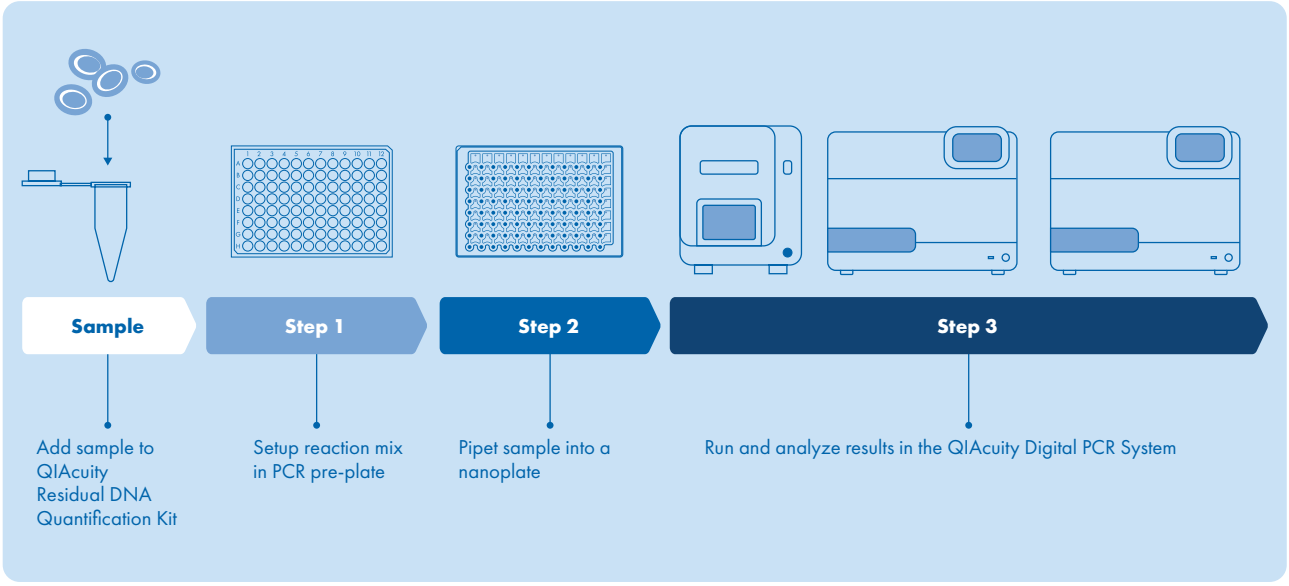
Background

Residual DNA (rDNA) is defined as DNA fragments from a host organism that may be present in the final product of recombinant biological processes. These impurities represent a safety concern due to their association with higher oncogenicity, infectivity and immunogenicity. Therefore, it is a regulatory requirement (from WHO, EU, FDA) to limit the amount of rDNA in these products before product release.

Benefits

- Easy set up and detection of host cell DNA thanks to premixed master mix with controls
- Accurate detection of rDNA from *Escherichia coli (E.coli),* Chinese Hamster Ovary cells (CHO) and Human Embryonic Kidney 293 cells (HEK293) down to low femtograms of host cell DNA
- Quantification of highly fragmented host cell DNA with a multi copy target assay

Workflow



Data

Loading amount per reaction (fg/rxn)	*E.coli* Standard (copies/µL)	Internal Control (copies/µL)*
50,000	2949.7	99.9
5,000	291.6	91.7
500	27.6	91.4
50	2.8	93.2
25	1.46	92.5
5	0.45	90.6
NTC	0	98.9

QIAcuity *E. coli* Residual DNA Quantification Kit DNA at amounts as low as 5 fg in a single reaction

Loading amount per reaction (fg/rxn)	*E.coli* Standard (copies/µL)	Internal Control (copies/µL)*
50,000	4939,3	108.7
5,000	508.3	104.9
500	50.2	97.6
50	4.7	99.5
25	2.6	101.3
5	0.6	100.4
NTC	0	104.7

QIAcuity CHO Residual DNA Quantification Kit detects residual DNA as low as 5 fg in a single reaction

Loading amount per reaction (fg/rxn)	*E.coli* Standard (copies/µL)	Internal Control (copies/µL)*
50,000	1104.9	96.6
5,000	104.5	92.5
500	8.83	92.4
50	0.99	94.4
NTC	0	96.2

QIAcuity HEK293 Residual DNA Quantification Kit detects residual DNA as low as 5 fg in a single reaction

Publications

- Karaly O et al. Direct quantification of residual host cell DNA using the QIAcuity® Digital PCR Platform. QIAGEN, 2023.

Ordering Information

Product	Contents	Cat. no.
QIAcuity Residual DNA Quantification Kits	*E. coli*/CHO/HEK293 resDNA Quant Standard (1x), Rehydration Buffer	250220 – 250225

Virulence genes detection

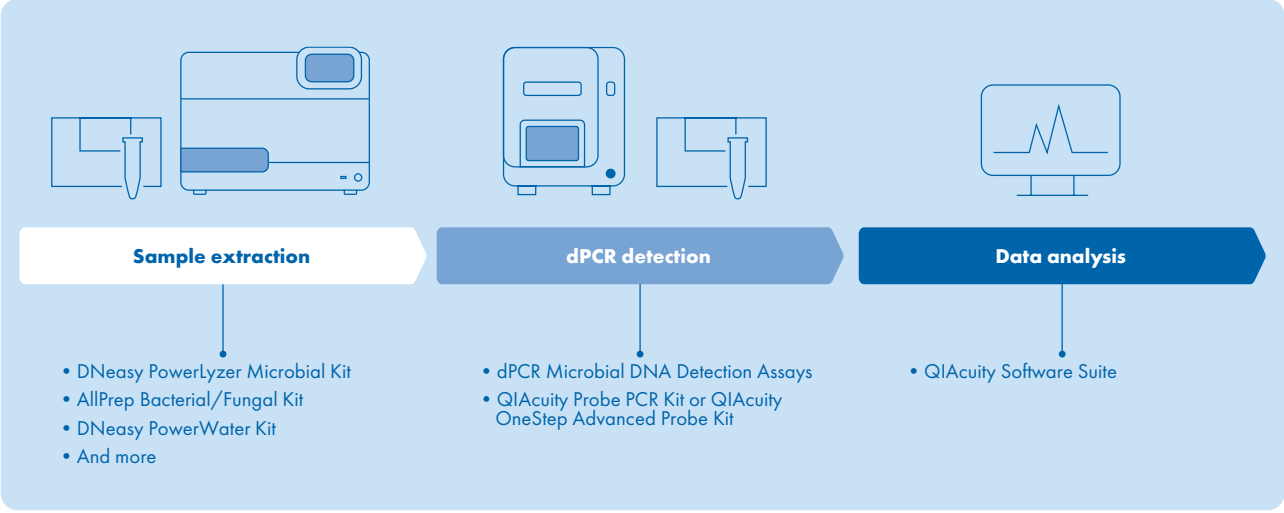
Background

Investigating virulence and resistance genes furthers our understanding of colonization and invasion of host cells. This knowledge ultimately improves health care and therapy.

Benefits

- Accurate and efficient analysis by multiplexing up to five targets in one reaction
- Analyze both microbial and viral targets with the new QIAcuity OneStep Advanced Probe Kit
- Highly specific detection of only the sequence of interest

Workflow



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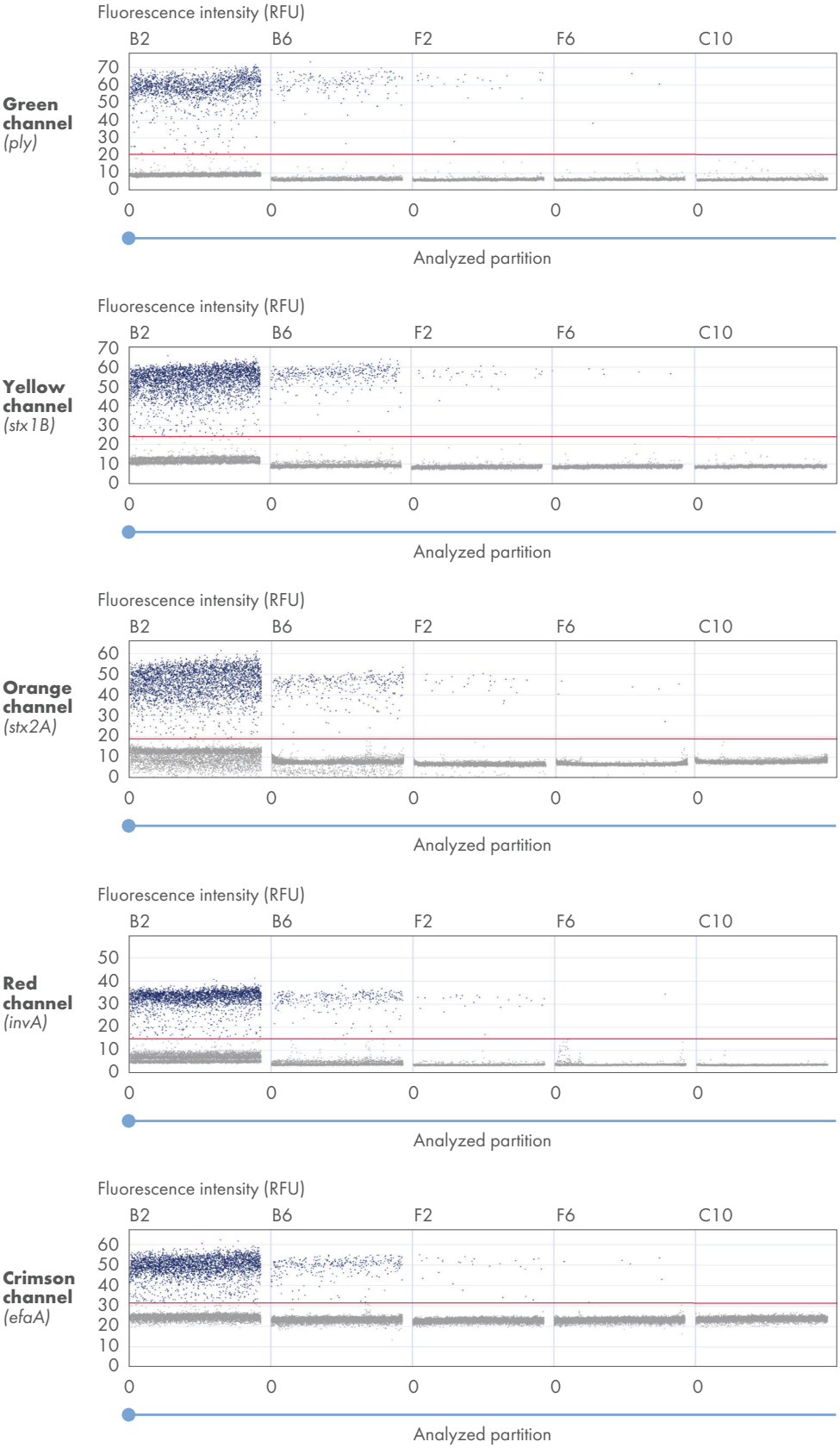

Five bacterial virulence gene targets run in multiplex using the 96-well 8.5k nanoplate. Four dilutions of PMCv2 input template starting with 1 µL PMC input and diluted down to 0.001 µL. 1-D scatterplots with the auto threshold set by the QIAcuity Software Suite.

Publications

- Assis AB et al. A Secreted Chorismate Mutase from *Xanthomonas arboricola pv. juglandis* Attenuates Virulence and Walnut Blight Symptoms. International Journal of Molecular Sciences. 2021; 22(19):10374.
- Rattanachak N et al. Hydroquinine Possesses Antibacterial Activity, and at Half the MIC, Induces the Overexpression of RND-Type Efflux Pumps Using Multiplex Digital PCR in *Pseudomonas aeruginosa. Tropical Medicine and Infectious Disease.* 2022; *7*(8):156.
- Walas N et al. Phylodynamics Uncovers the Transmission of Antibiotic-Resistant *Escherichia coli* between Canines and Humans in an Urban Environment *bioRxiv.* 2023; 2023.06.01.543064.

Ordering Information

Product	Contents	Cat. no.
dPCR Microbial DNA Detection Assays	One tube with lyophilized assay, dye (fluorophore) configurable, 200 reactions (40 µL reaction in Nanoplate 26k)	250111
QIAcuity Probe PCR Kit	1 mL 4x concentrated QIAcuity Probe Mastermix, 2 x 1.9 mL Water	250101
QIAcuity OneStep Advanced Probe Kit	1 mL OneStep Advanced Probe Master Mix (4x), 45 µL OneStep RT Mix (100x), 1 mL Enhancer GC, 20 µL QN Internal Control RNA, 2 x 1.9 mL RNase-free water; for 100 reactions in Nanoplate 26K and 333 reactions in Nanoplate 8.5K	250131

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