Microfluidic-based nanoplate technology to overcome challenges in digital PCR

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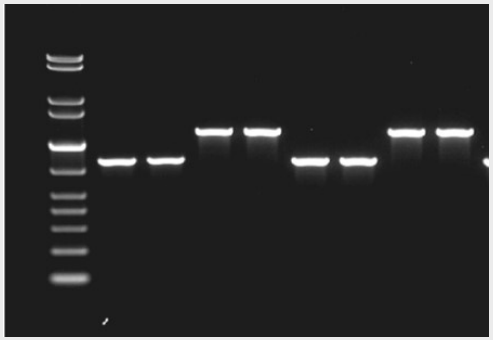
dPCR Instruments

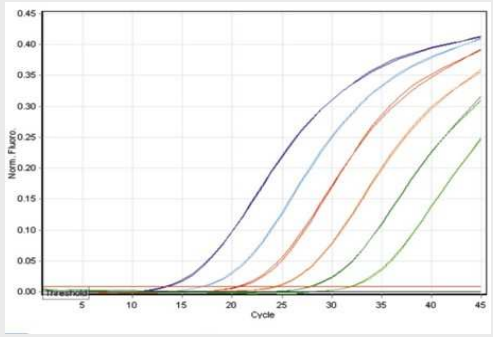
Comparison of PCR techniques at a glance

1st generation
Conventional PCR



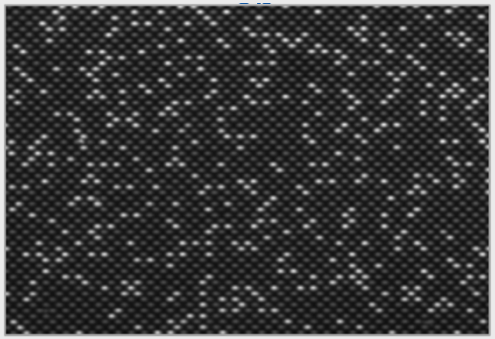
Qualitative
- Technically simple
- Multiplexing capabilities
- End-point detection
- Low cost

2nd generation
Quantitative RT-PCR (qPCR)



Relative quantification
- High accuracy, sensitivity and specificity
- Rapid cycling and throughput
- Non-specific amplification
- Real-time detection

3rd generation
Digital PCR (dPCR)



Absolute quantification
- No standard curves
- Higher precision and reproducibility
- Less sensitive to inhibitors
- End-point detection

Digital PCR – principles

Absolute quantification in four steps

Step 1: Sample dilution and PCR reaction mix setup

- Over concentrated sample – all positives partitions
- A number of negative partitions are required for accuracy
- Intercalating dye or hydrolysis probe-based reactions

Step 2: Partitioning of PCR reaction

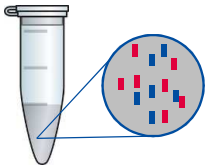
- Droplet generation
- Partition generation

Step 3: Amplification of partitioned PCR reaction

- End-point thermocycling

Step 4: Readout and quantification

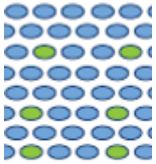
- Positive and negative partitions are counted per reaction
- Data analysis



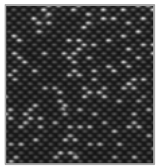
Target

Background (gDNA, cDNA; primers/probes; master mix)

PCR reaction partitioning into thousands individual reactions



Positive reactions
Negative reactions



Absolute quantification

Digital PCR – principles

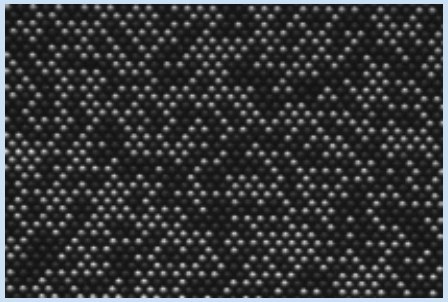
Poisson statistics at 95% confidence intervals – sample concentration estimation

No target

All partitions are negative

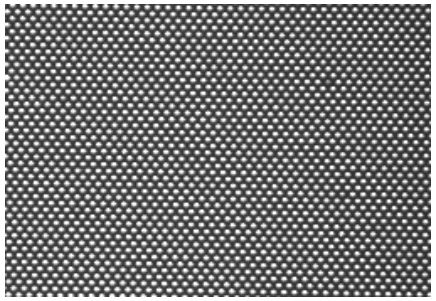
Lower end

Positive and negative partitions



Higher end

Positive and negative partitions



Over saturated

All partitions are positive

Target copies per partition = -ln(1-p)

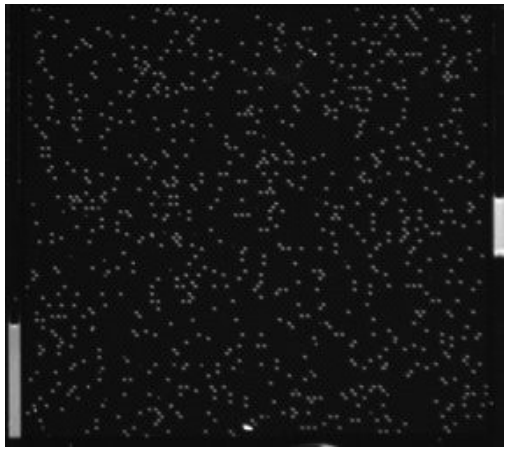
p = fraction of positive partitions

Copies of DNA target/microliter

QIAcuity Nanoplates – reference and detection channels

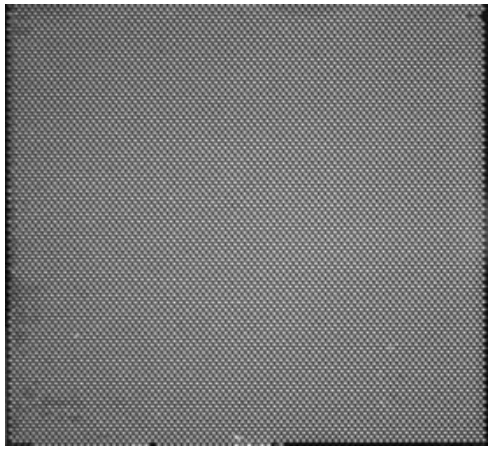
Green channel

- Counts the number of positive partitions
- Calculates the number of copies using Poisson statistics



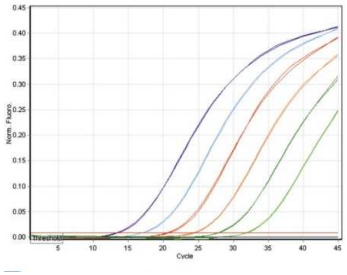
Reference channel

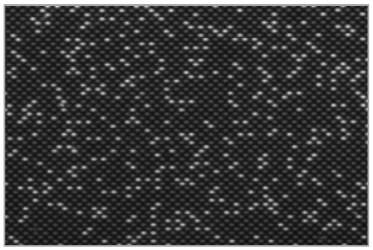
- Counts the number of filled partitions
- Determines the analyzable volume (µl)



Calculates copies/µl

Evaluating strengths of dPCR over real-time PCR

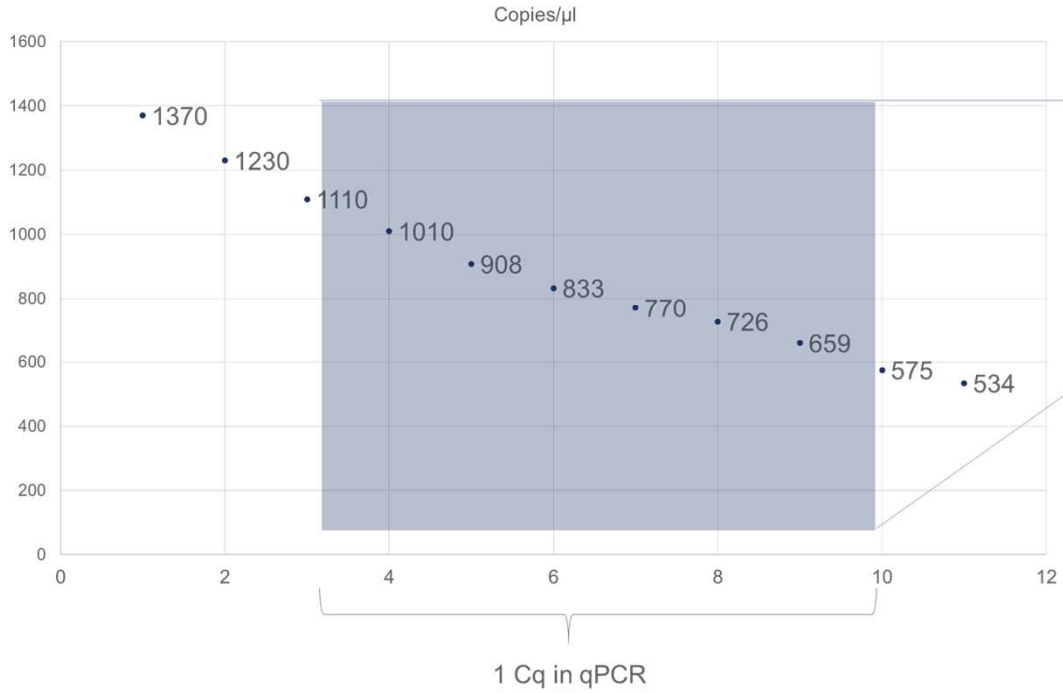


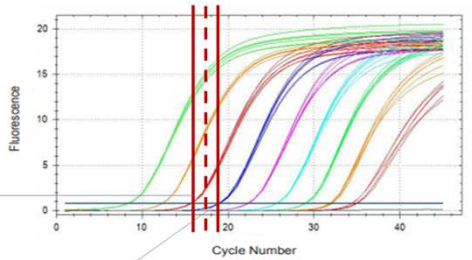


	Real-time PCR	dPCR
Precision	Low; detects mutation rate at >1%	High; detects mutation rate at ≥0.001%
Reaction	Bulk reaction; flexible volumes	Fixed reaction volume in partitions; higher inhibitor tolerance and statistical power
Detection	Broad dynamic range	Detect small fold changes and rare targets; +/- 10% precision
Standard curve	Yes; relative quantification	No; absolute quantification
Tolerance to PCR inhibitors	Lower	Higher; robust quantification
Reproducibility	Lower	Higher

Key advantage of dPCR in comparison to qPCR

Unparalleled precision – +/- 10% precision





Benefits

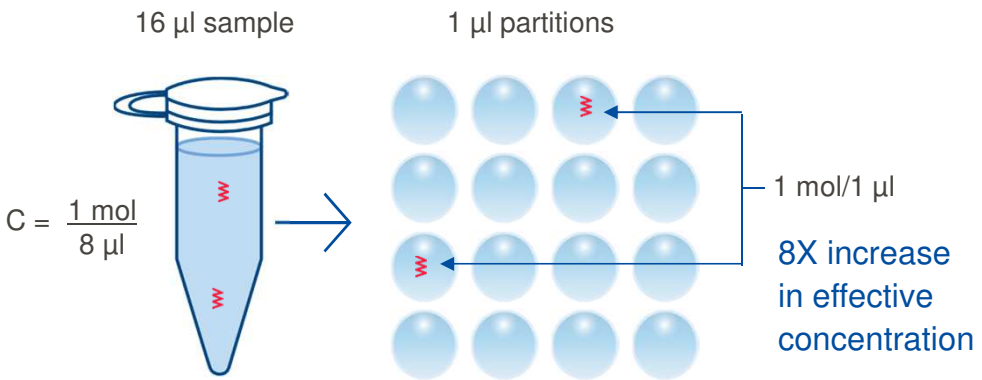
- Detect small differences in expression
- High resolution and precision

*10% dilution of a sample

Benefits of partitioning

Random distribution of molecules into partitions creates an increase in effective concentration and an ‘enrichment effect’

Increase of effective concentration



Decrease of interfering molecules that creates an ‘enrichment effect’

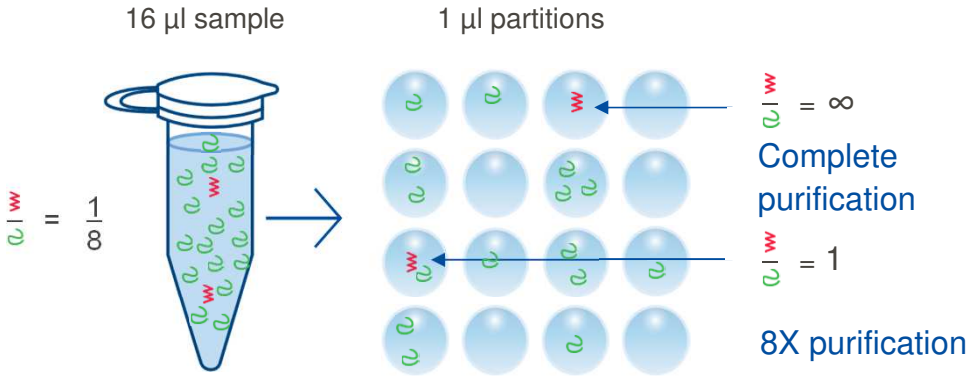
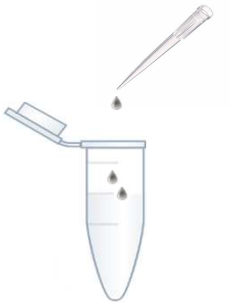


Image adapted from: Basu, A. S. (2017). Digital assays part I: partitioning statistics and digital PCR. SLAS TECHNOLOGY: Translating Life Sciences Innovation, 22(4), 369–386.

dPCR versus qPCR

Workflow comparison

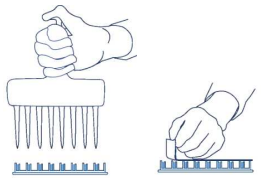
Prepare PCR reaction



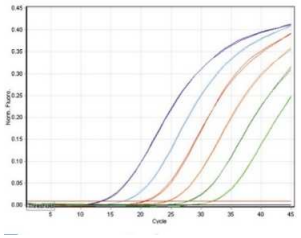
Intercalating dye or probes

Real-time PCR

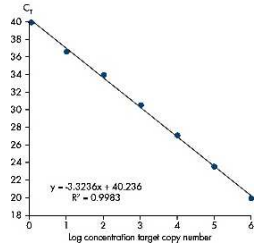
Load reaction mix to PCR plate; add DNA/RNA samples



Real-time PCR amplification

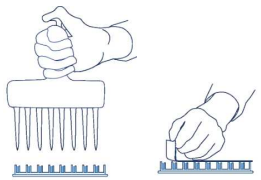


~1.5–2 h to results

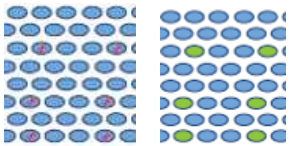


Digital PCR

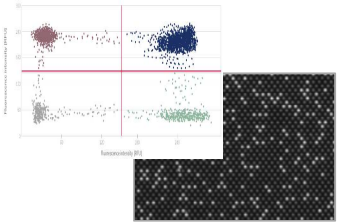
Load reaction mix containing DNA/RNA to nanoplates



Partitioning, end-point PCR amplification, readout



Under 2 h to results



Challenges and opportunities for digital PCR

Absolute quantification

- Ability to resolve rare events and access targets at low template concentrations without the need to compare to standard curves. Potential to not only improve routine quantitative nucleic acid analysis but also to be used as a primary reference measurement procedure.

Sensitivity and precision

- Sample partitioning means more precision in the analysis of even small concentration changes as well as high sensitivity in detecting rare mutations in a background of wild-type and PCR inhibitors.

Complex workflow

- Laborious workflow over disintegrated instruments means a longer time to result and increasing numbers of samples waiting to be processed. Moreover, laboratories have limited space and struggle with instrument running costs.

Limited multiplexing

- More and more applications require accurate quantification of multiple targets in the same reaction. Multiplexing can enable the development of assays with reduced cost and sample consumption, increased throughput and the potential for built-in assay controls.

QIAcuity digital PCR system

Fully integrated nanoplate-based dPCR instruments

QIAcuity One

QIAcuity Four

QIAcuity Eight

QIAcuity – Nanoplate-based digital PCR system

A fully integrated solution

QIAcuity – Nanoplate-based digital PCR system

Features and specifications

Scalability

- Low to high throughput (1-, 4- and 8-plate)
- 1–2 thermocyclers

Throughput

- Under 2 h – the first plate 96 or 24 samples
- Every ~30/60 mins – the following plate (depending on the instrument configuration used)
- ~5 h – up to 768 samples/8 plates

Multiplexing

6 channels

- 5 channels – target detection
- 1 channel – reference

Nanoplates

3 types

- 96-well × ~8,500 partitions
- 24-well × ~8,500 partitions
- 24-well × ~26,000 partitions

QIAcuity – Nanoplate-based digital PCR system

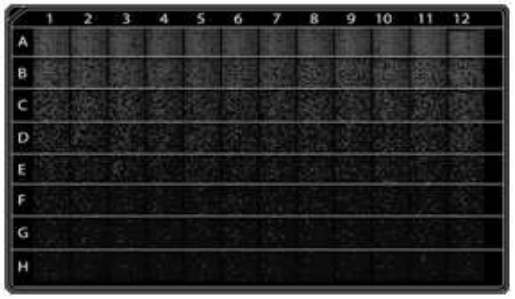
Detection channels and fluorophores

Detection channels	Recommended dyes*
Green	FAM, EvaGreen
Yellow	VIC, HEX
Orange	TAMRA
Red	ROX
Crimson	Cy5

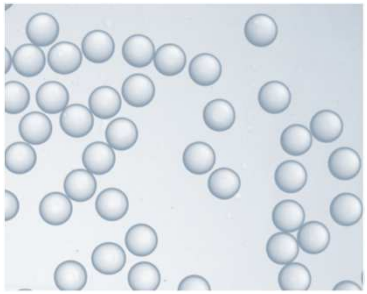
*Alternative dyes are being tested

Digital PCR nanoplates

Why nanoplates and not droplet?



Nanoplate

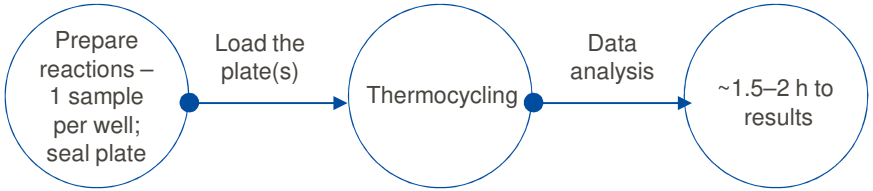


Droplet

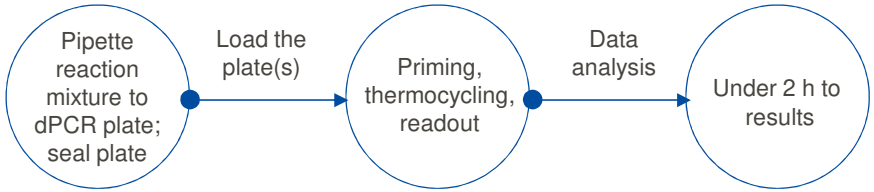
- Fixed and sealed partitions prevent variation in size and coalescence
- Sealed nanoplates prevent well-to-well contamination
- Faster readout possible due to simultaneous reading of all partitions of a sample
- Simple workflow and user-friendly handling, just like for qPCR
- Plates are amenable to front-end automation

Workflow comparison

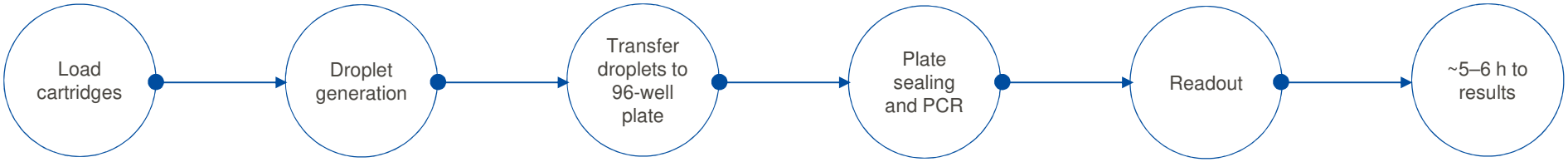
Real-time PCR



Nanoplate dPCR

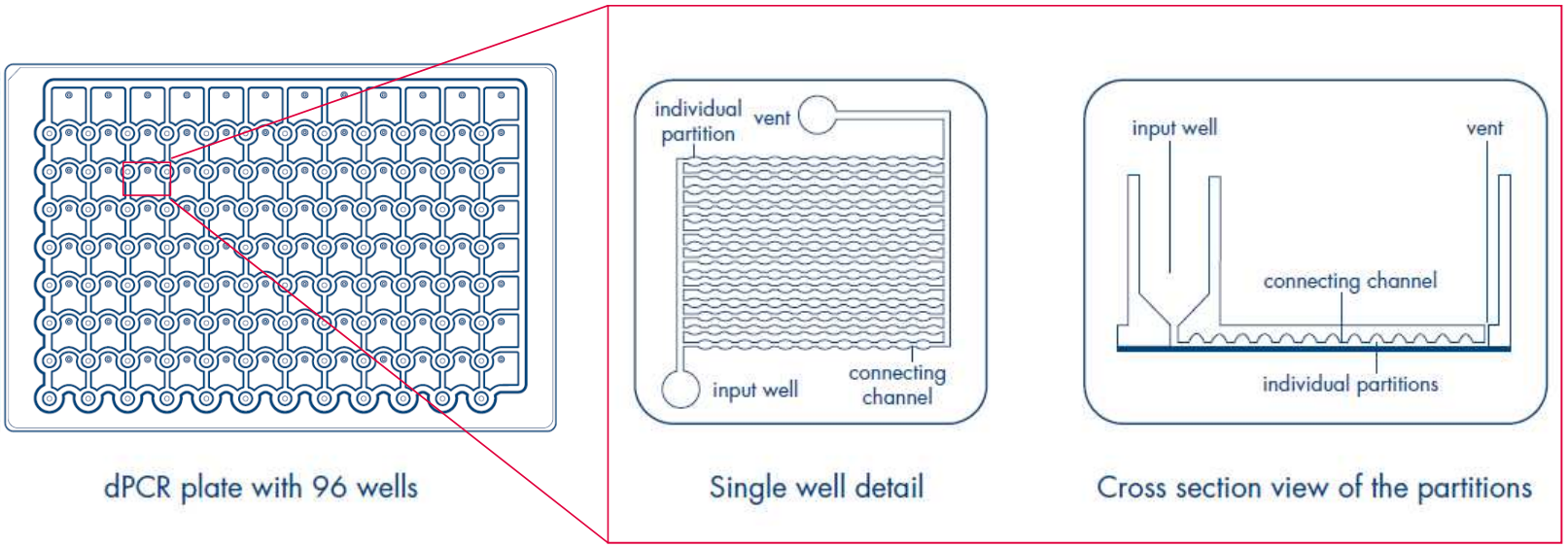


Droplet dPCR



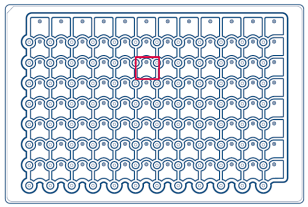
Digital PCR nanoplates

Well structure

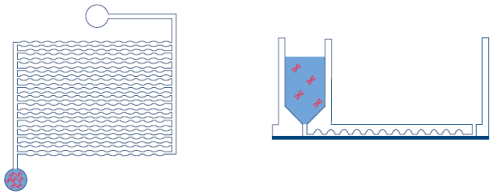


Digital PCR nanoplates

Well function and workflow

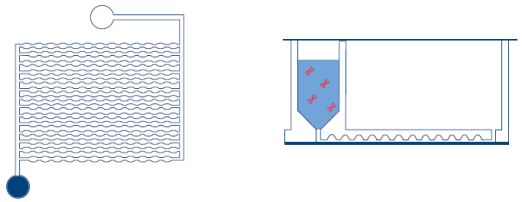


1



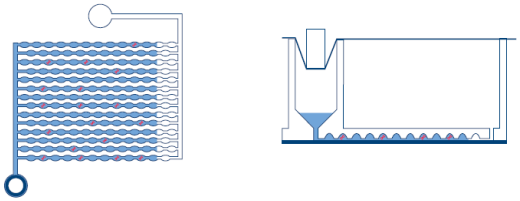
The PCR reaction mixture is pipetted into the input wells.

2



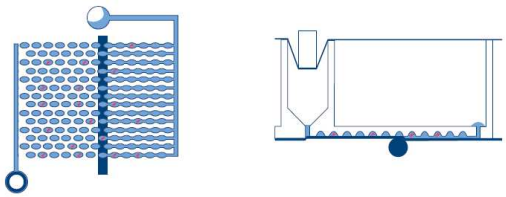
A rubber seal is applied to the top of the plate to seal it.

3



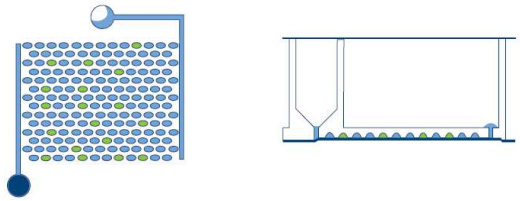
The pistons in the dPCR system push PCR reaction mixture through partitions automatically.

4



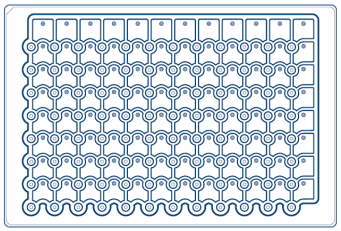
A roller compresses the bottom seal and seals partitions individually, distributing individual copies of target DNA throughout the partitions.

5



During thermocycling, the target DNA is doubled with each cycle, and partitions containing the target DNA fluoresces.

6



The plate is imaged to count the number of positive/fluorescent partitions.

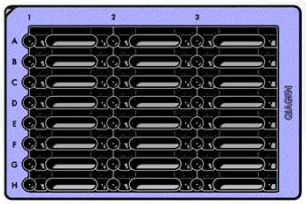
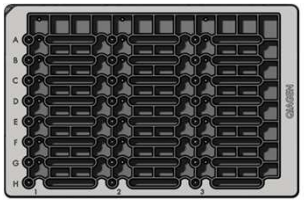
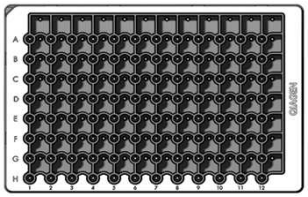
QIAcuity in action – a simple and rapid workflow

Fully automated partitioning, thermocycling and imaging of the QIAcuity Nanoplates

www.qiagen.com/applications/digital-pcr/workflow/qiacuity-demo#workflow-partitioning_video

QIAcuity – Nanoplates

Features and specifications

	Nanoplate	Specification	Application
	Nanoplate 26K 24-well	24-well × ~26,000 partitions	Rare mutation detection, liquid biopsy, pathogen detection, etc.
	Nanoplate 8.5K 24-well	24-well × ~8,500 partitions	CNV determination, NGS library quantification, genome edit detection, etc.
	Nanoplate 8.5K 96-well	96-well × ~8,500 partitions	CNV determination, NGS library quantification, genome edit detection, etc.

QIAcuity – Reagents

Features and specifications

	Specification	Description
 QIAcuity Probe PCR Kit	For single and multiplex use (1- to 5-plex)	• Optimized for best performance in nanoplate microfluidic • Includes special reference dye needed for dPCR analysis and counting analyzable partitions
 QIAcuity EG PCR Kit	EvaGreen-based master mix	• Optimized for best performance in nanoplate microfluidic • Includes special reference dye needed for dPCR analysis and counting analyzable partitions

For RNA samples and cDNA synthesis, the QuantiNova Reverse Transcription Kit is recommended upfront.

Remote analysis

Software concept

Embedded instrument software

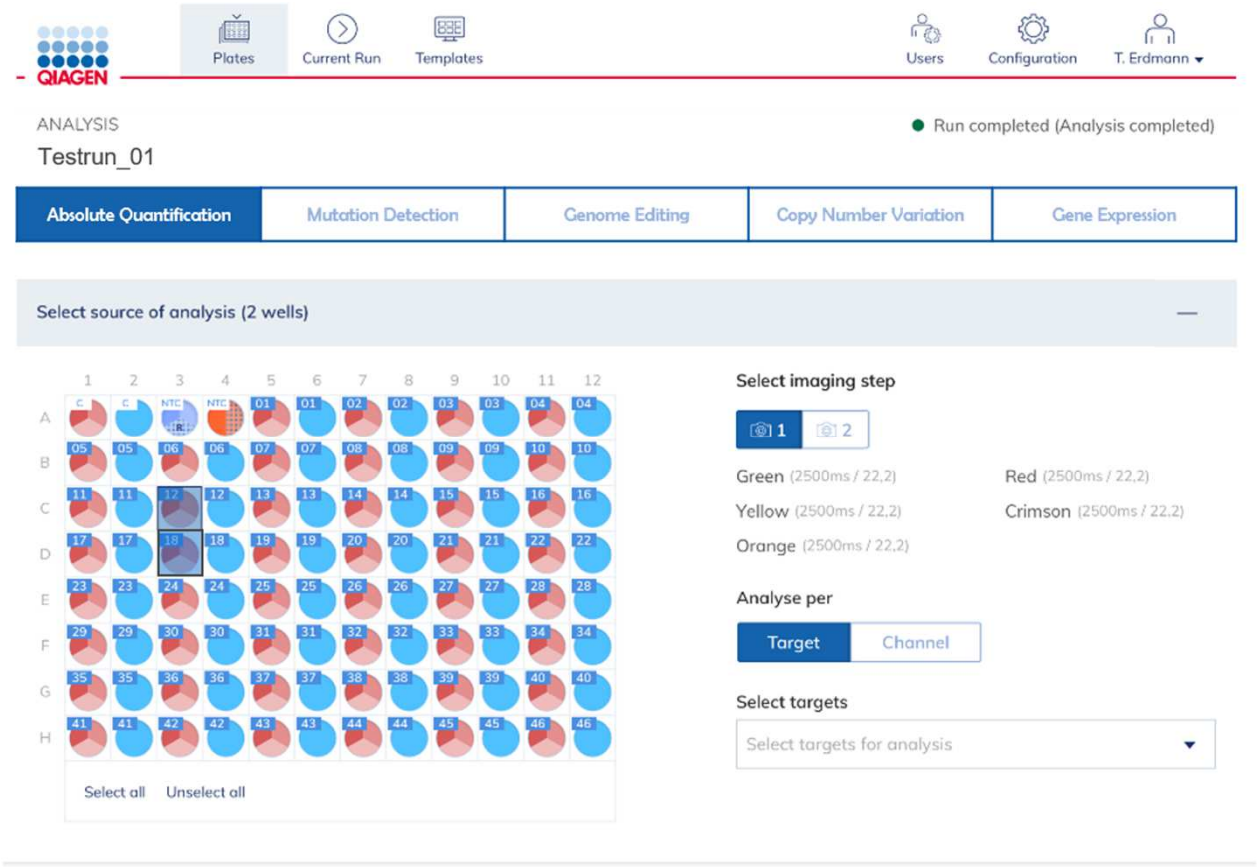
dPCR Software Suite installed on a computer or server

dPCR Software Suite (web application)

Access, design and analyze from anywhere

QIAcuity dPCR Software Suite

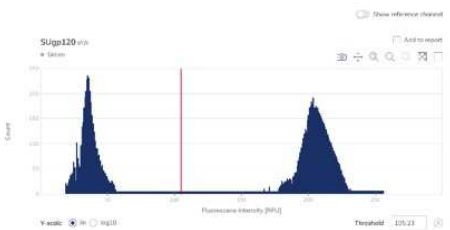
Analysis



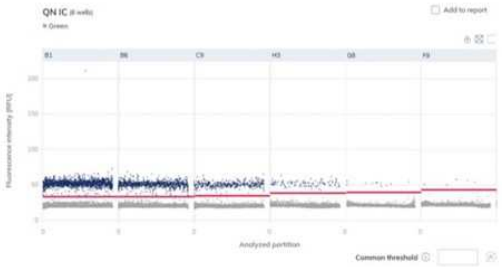
QIAcuity dPCR Software Suite

Run and analysis views – examples

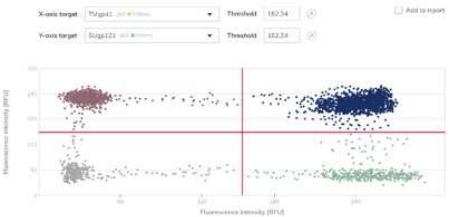
Plate image



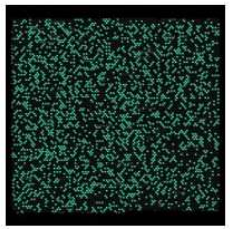
Histogram



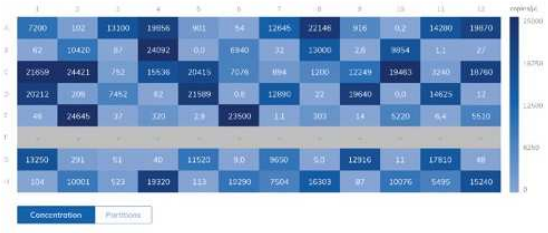
1D Scatterplot



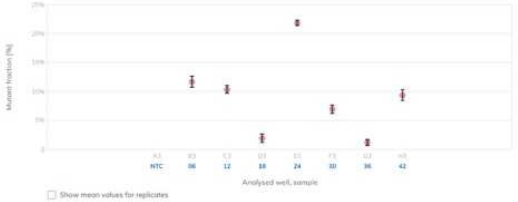
2D Scatterplot



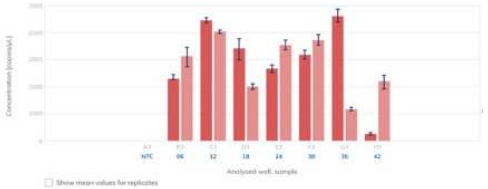
Signal map



Heatmap (Concentration/Partition)



Point diagram



Concentration diagram

Applications

Applications covered by new QIAGEN dPCR assays

- Mutation detection
- Copy number variation
- Gene expression, miRNA detection
- Gene/cell therapy
- Gene editing detection (CRISPR/Cas9)
- SNP genotyping
- Microbiome analysis and pathogen detection
- GMO detection
- NGS validation
- Characterization of reference standards

Applications

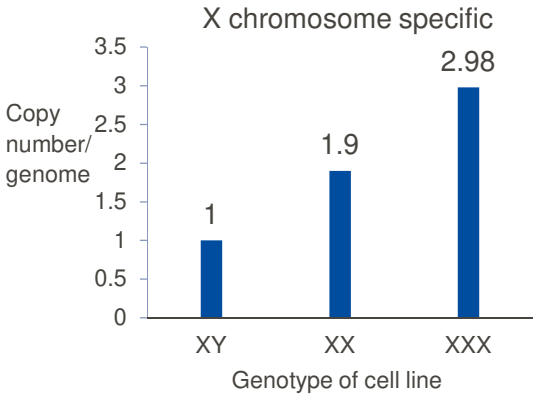
Copy number variation – aneuploidy testing concentrations

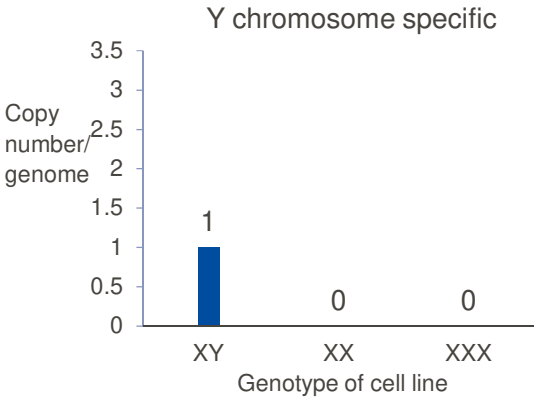
Setup:

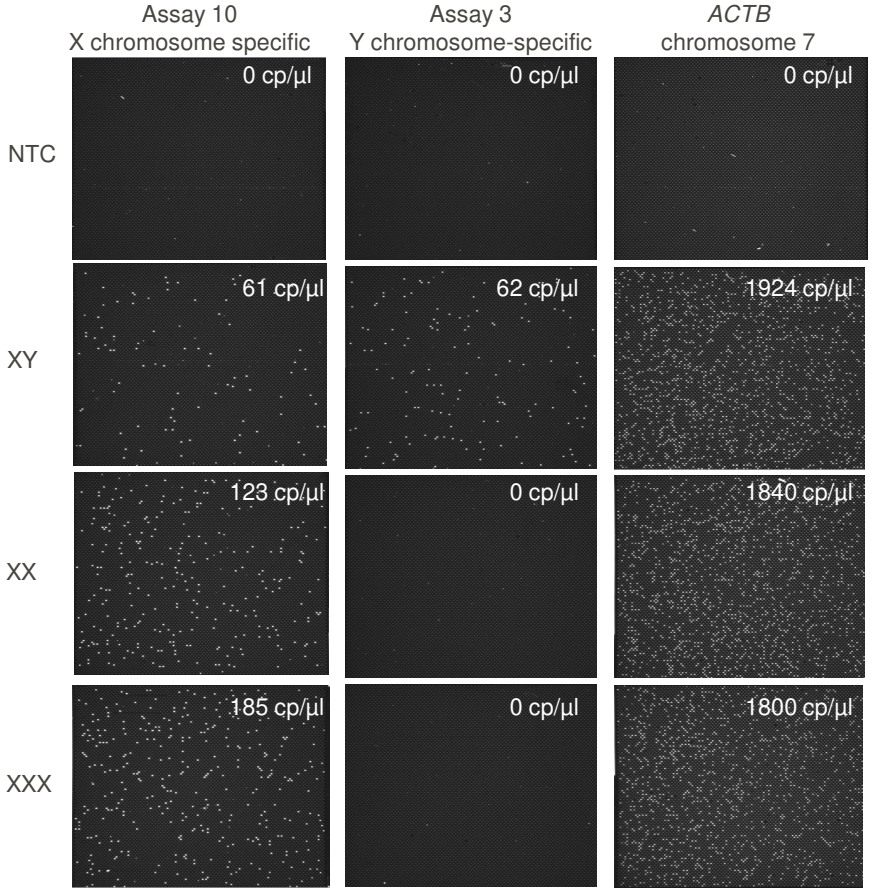
- QIAcuity Nanoplate 8.5K 96-well
- QIAcuity EG PCR Kit/dPCR Copy Number Assays EvaGreen
- Template – 4 ng/rxn human cell lines

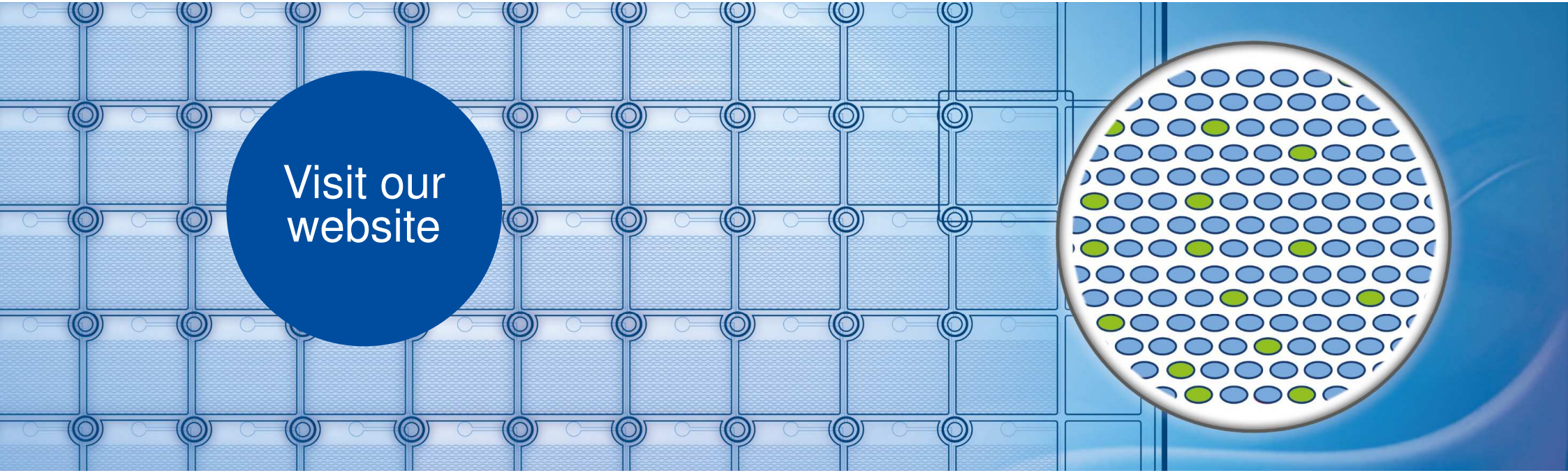
Known CNVs contain 1 copy (XY), 2 copies (XX) or 3 copies (XXX) of the X-chromosome

Human *ACTB* gene (20 copies/diploid genome) was used as a reference to normalize copy numbers









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