Digital PCR with QIAcuity

Maria Rifé
Product Manager - Biotecnología

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Agenda

PCR digital

QIACuity – Solución integrada

Aplicaciones

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PCR digital

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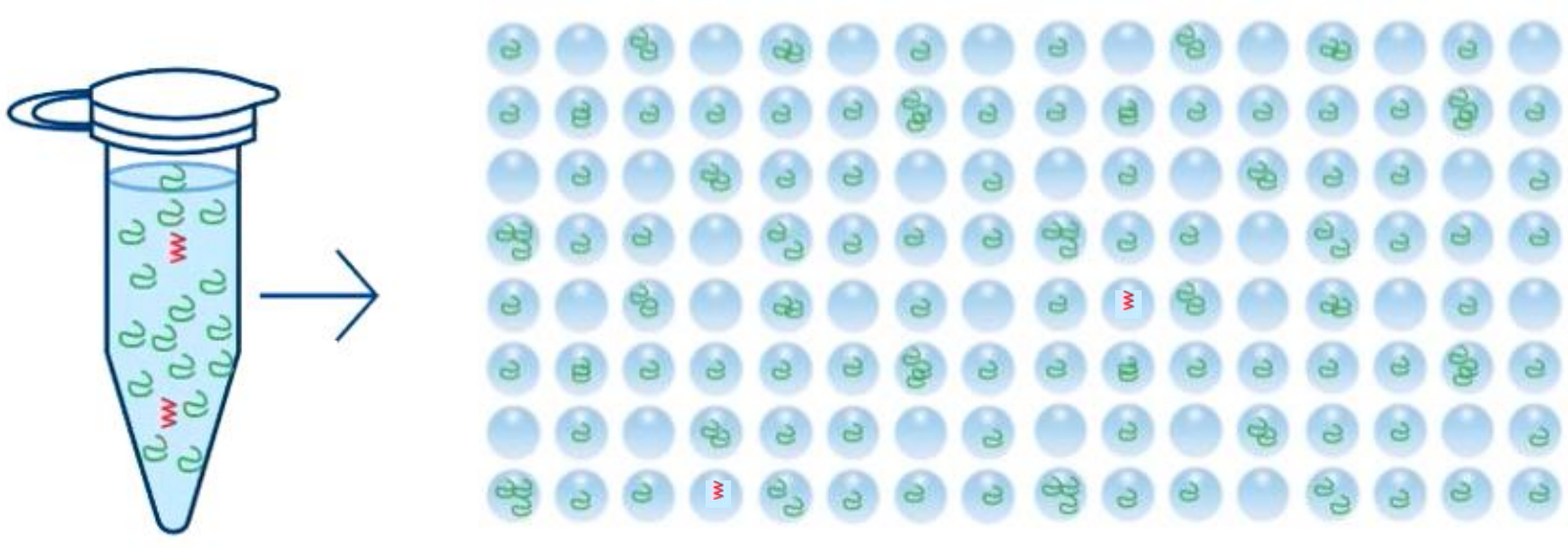
PCR digital

Principio de la PCR digital: PARTICIÓN



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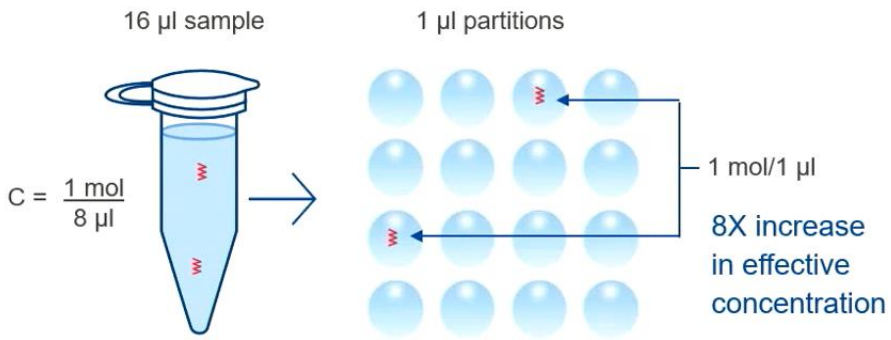
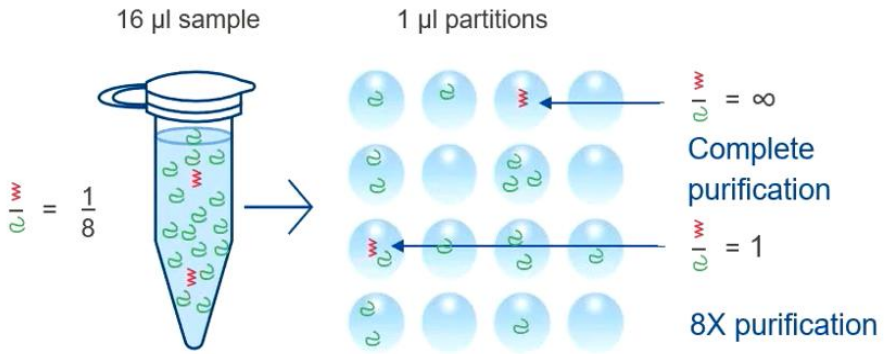
PCR digital

Principio de la PCR digital: PARTICIÓN

La distribución de moléculas al azar en particiones, crea e incrementa la concentración efectiva y tiene un efecto de enriquecimiento.





Molécula diana está muy diluida

Moléculas interferentes

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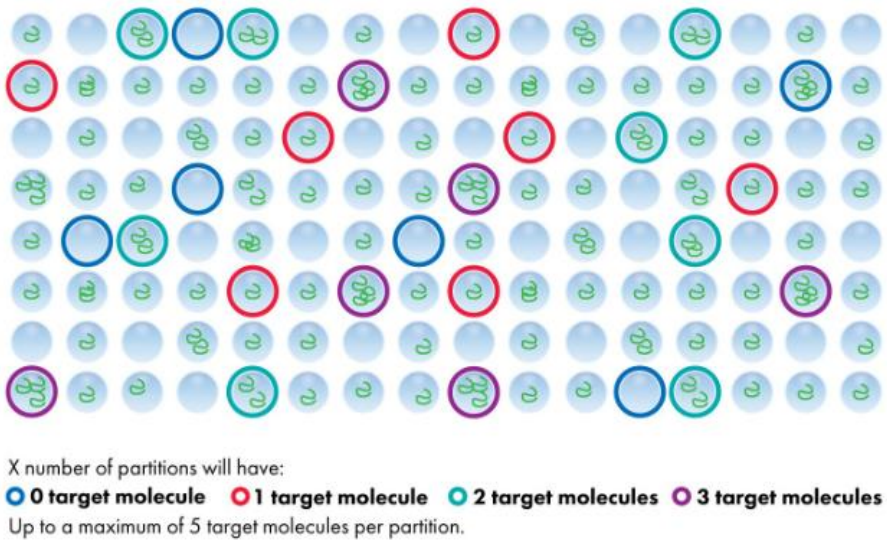
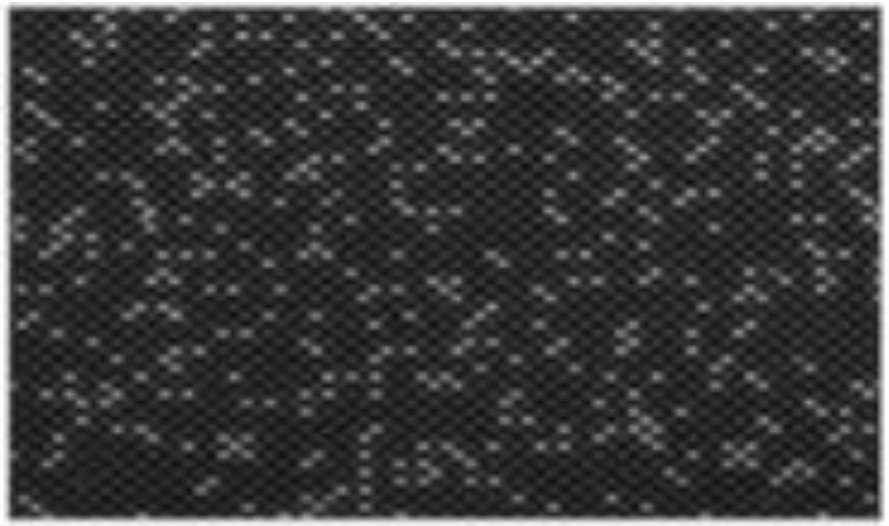
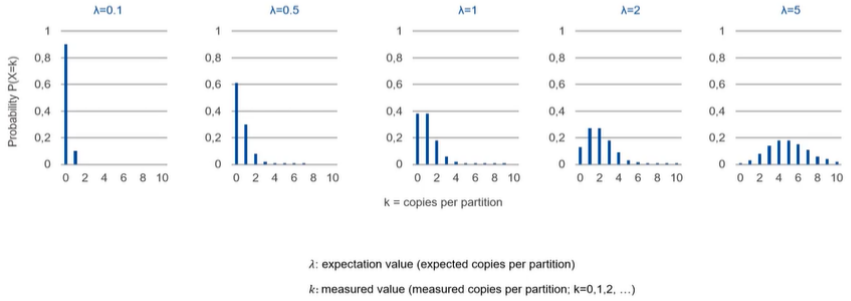
Cuantificación

La distribución de moléculas al azar en particiones permite la cuantificación según la distribución de Poisson.







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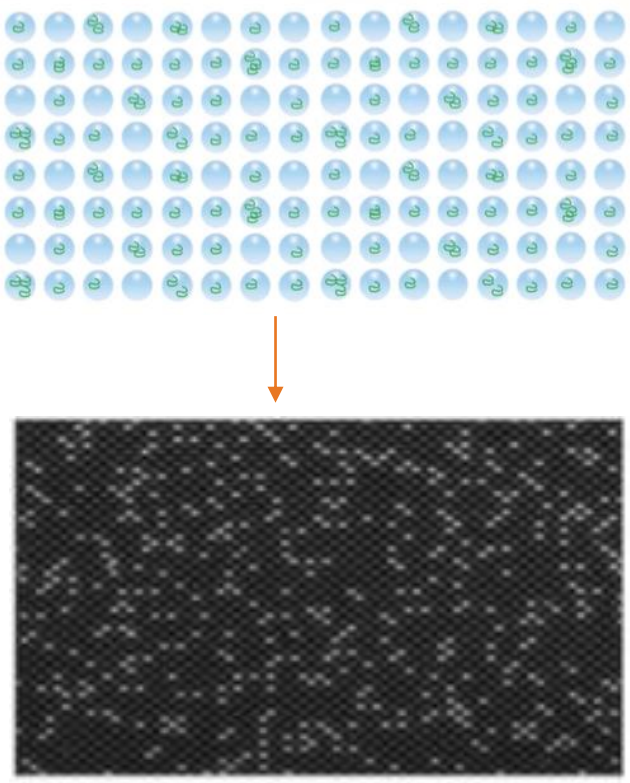
PCR digital

PCR digital

Variación de la PCR que permite la **cuantificación absoluta** mediante la partición de la muestra en miles de particiones, donde se realizará la reacción de PCR.



- No requiere curvas de calibración > cuantificación absoluta
- Mayor sensibilidad, precisión y reproducibilidad
- Mayor robustez en presencia de inhibidores

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QIAcuity
Solución integrada

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QIAcuity – Solución integrada

- ✓ Equipo compacto
- ✓ Flujo de trabajo integrado
- ✓ Resultados en ~2h
- ✓ Prodecimiento similar a qPCR
- ✓ Multiplexado hasta 5-plex

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QIAcuity – Solución integrada

	QIAcuity One	QIAcuity Four	QIAcuity Eight
Placas procesadas	1	4	8
Canales detección	2 o 5	5	5
Termociclador	1	1	2
Tiempo hasta resultado	< 2h	1ª placa < 2 h Siguientes, cada 30 min	1ª placa < 2 h Siguientes, cada 30 min
Muestras por día	480 (96-well) 120 (24-well)	960 (96-well) 240 (24-well)	1344 (96-well) 336 (24-well)

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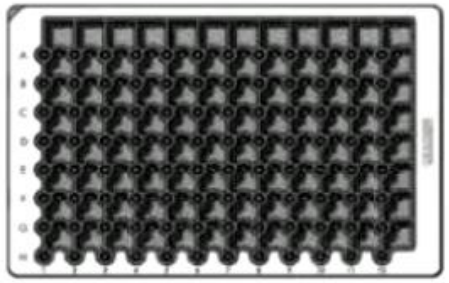
QIAcuity – Nanoplacas

24-well; 26.000 particiones/pocillo

Mutaciones raras, biopsia líquida, detección patógenos, etc.

8-well;
26.000 particiones/pocillo

24-well;
8,500 particiones/pocillo



96-well;
8.500 particiones/pocillo

CNV, cuantificación librerías NGS, detección edición genómica, etc.

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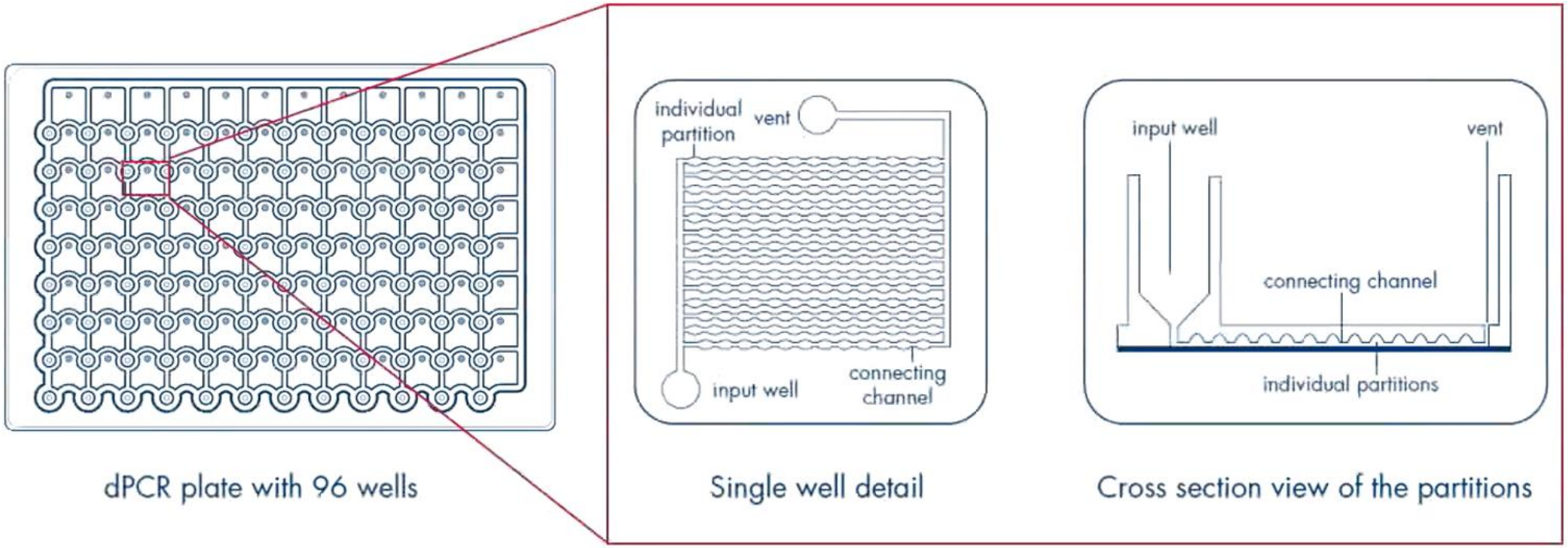
QIAcuity – Nanoplacas



dPCR plate with 96 wells

Single well detail

Cross section view of the partitions

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QIAcuity – PCR mix

dPCR Master Mix

- QIACuity Probe PCR Kit: con sonda
- QIAcuity EvaGreen PCR Kit: con agente intercalante
- QIAcuity Advanced One-Step RT-PCR

- QuantiNova Taq Polimerasa
- Máxima distribución en la nanoplaca
- Fluoróforo de referencia

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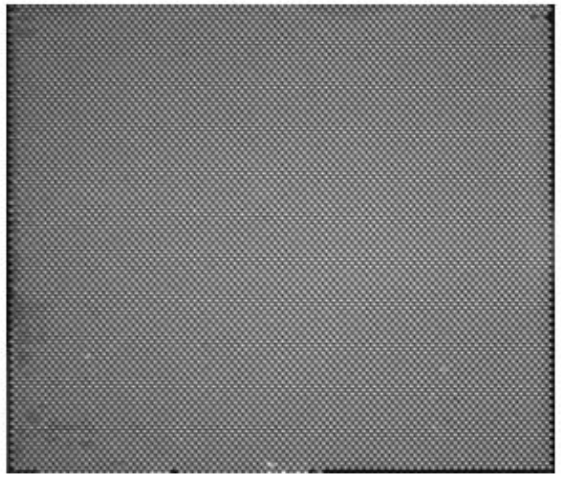
QIAcuity – PCR mix

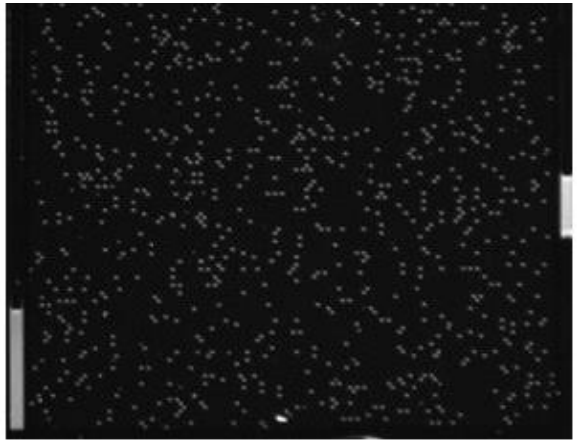
dPCR Master Mix – Canal de Referencia



Canal referencia

Número particiones con mezcla inicial.

Particiones válidas.



Canal Green

Número particiones positivas para el fluoróforo correspondiente.

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

Cálculo copias/ul

QIAcuity

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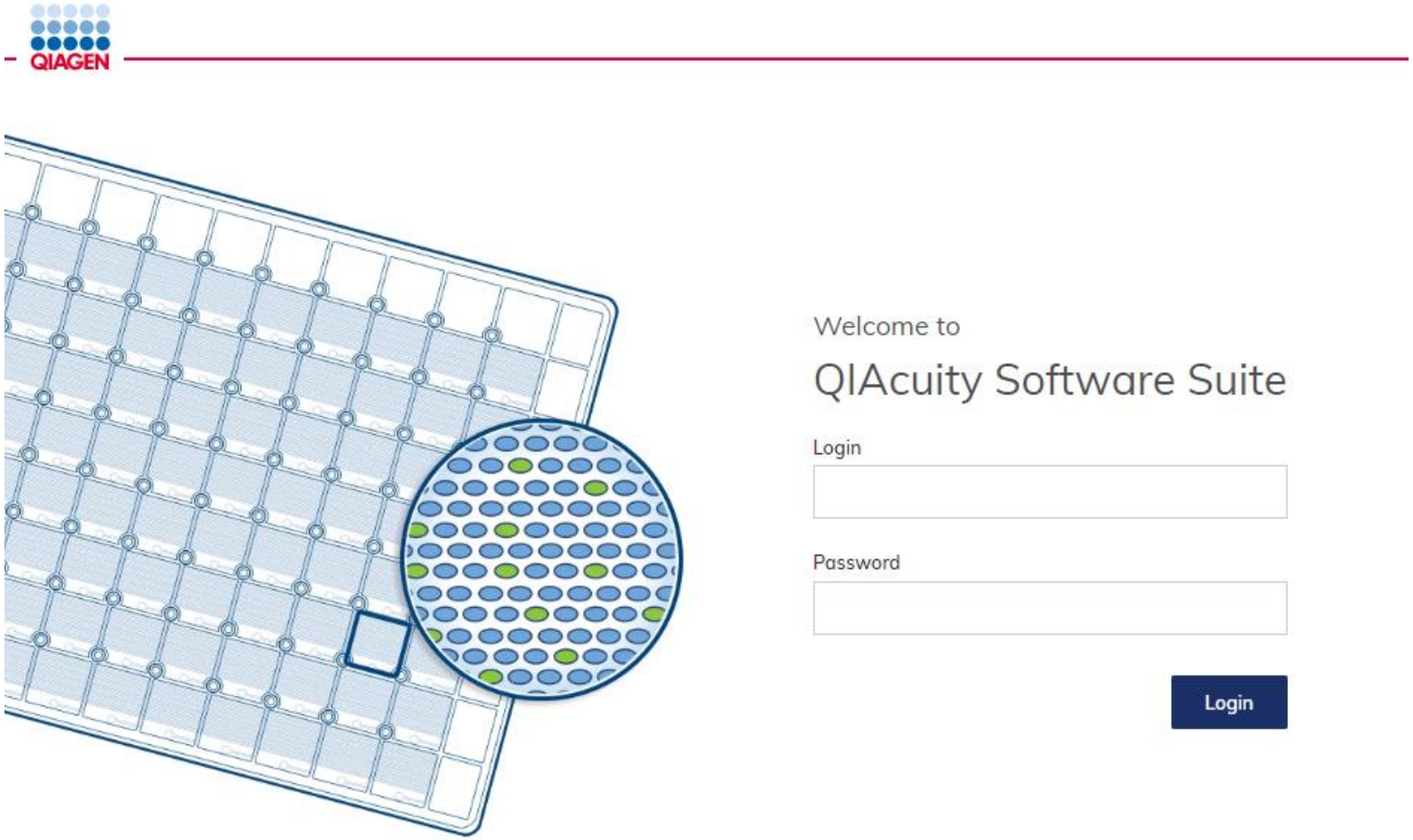
QIAcuity - Software

dPCR Software Suite



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QIAcuity – Software Suite

Add to report | Show mean values for replicates | Export to CSV...

	Sample/NTC/Control	Reaction Mix	Target	IC	Control type	Concentration * copies/µL	CI (95%)	Partitions valid	positive	negative	Threshold
A1	01 1.1	Elf-α1	elfa1	-	-	384.7	6.1%	8150	967	7183	110.89
A2	02 1.2	Elf-α1	elfa1	-	-	25.27	24.3%	8185	66	8119	110.89
A3	03 1.3	Elf-α1	elfa1	-	-	6.906	47.4%	8110	18	8092	110.89
B1	04 1.4	Elf-α1	elfa1	-	-	0.000	-	8181	0	8181	110.89
B2	05 2.1	Elf-α1	elfa1	-	-	489.0	5.6%	7991	1122	6869	110.89
B3	06 2.2	Elf-α1	elfa1	-	-	28.84	22.9%	8269	74	8195	110.89
C1	07 2.3	Elf-α1	elfa1	-	-	9.074	40.8%	8257	24	8233	110.89
C2	08 2.4	Elf-α1	elfa1	-	-	0.792	168.6%	8253	2	8251	110.89
C3	09 3.1	Elf-α1	elfa1	-	-	0.390	274.4%	8260	1	8259	110.89
D1	10 3.2	Elf-α1	elfa1	-	-	0.756	168.6%	8258	2	8256	110.89
D2	11 3.3	Elf-α1	elfa1	-	-	0.396	274.4%	8245	1	8244	110.89

← Back to plate configurator | 0/70 items selected ▲ | Create report

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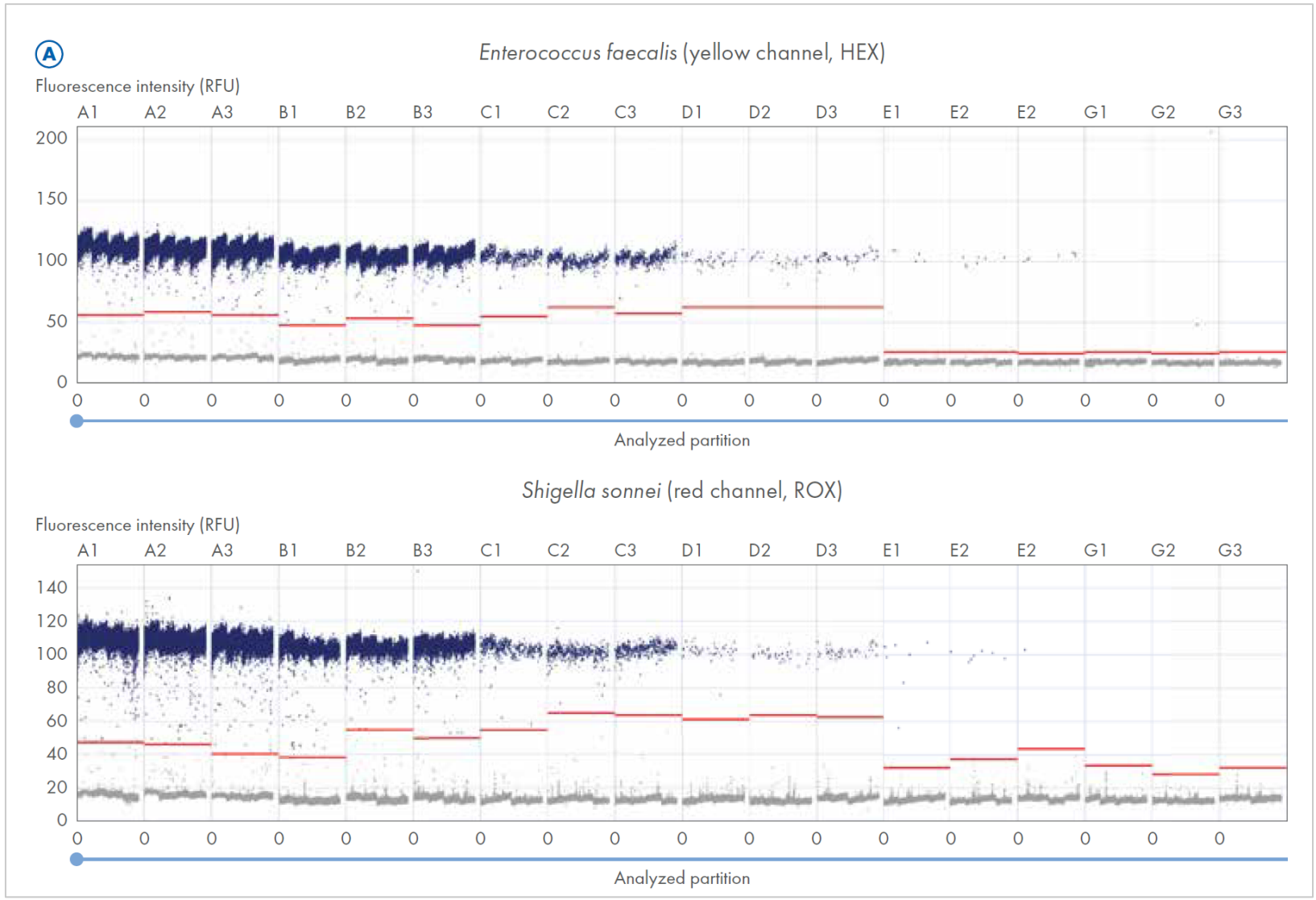
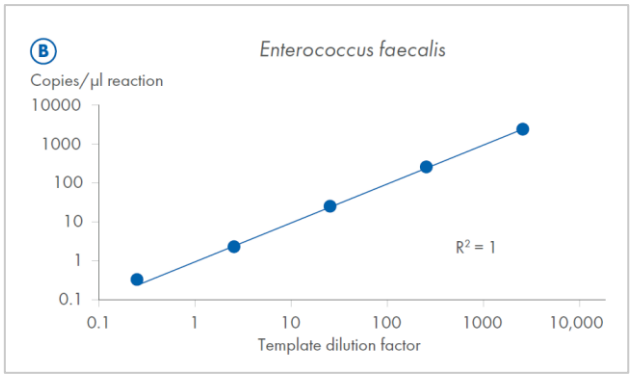
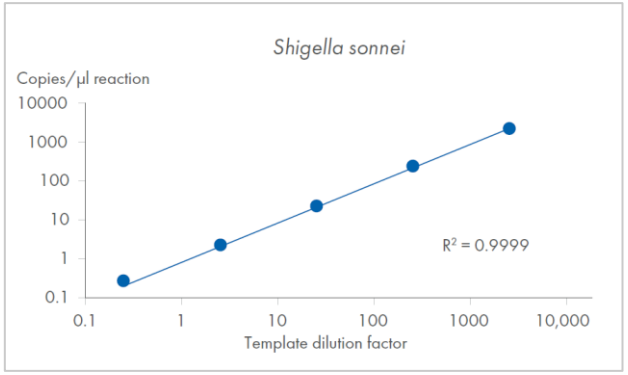
QIAcuity – Software Suite







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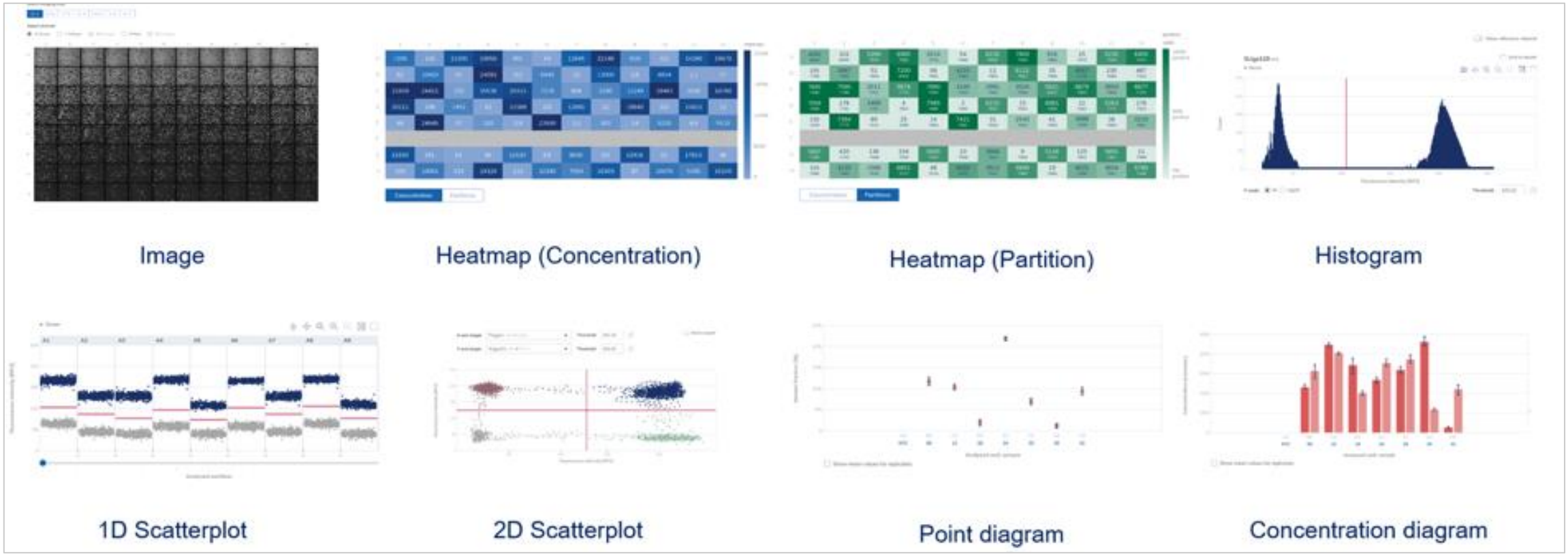

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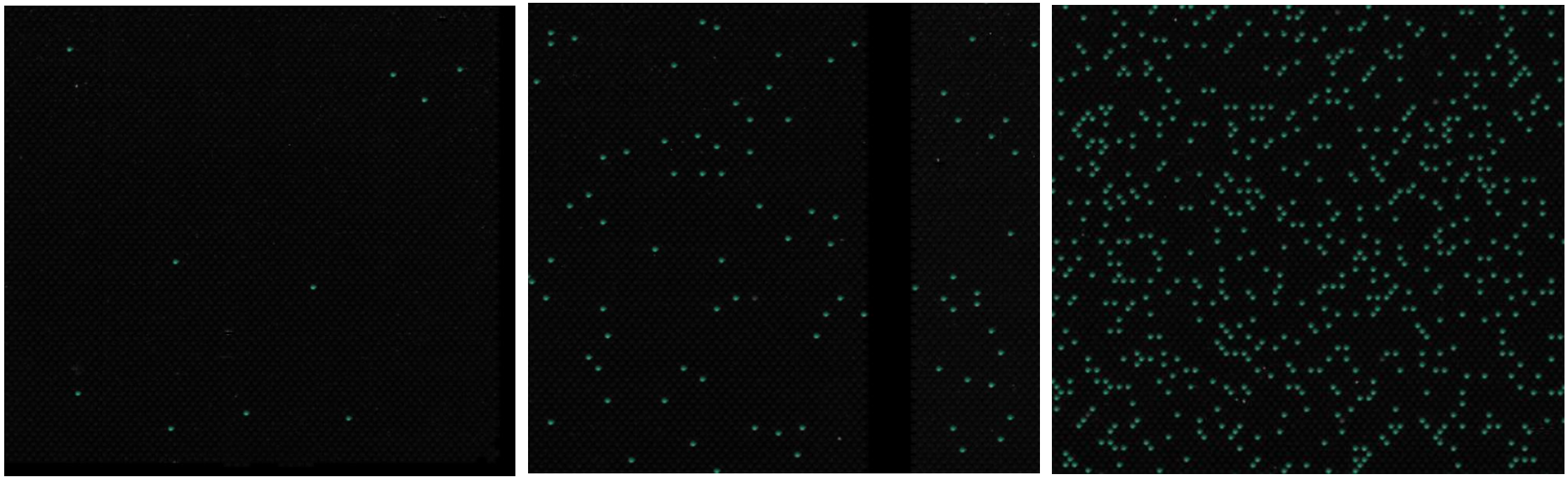

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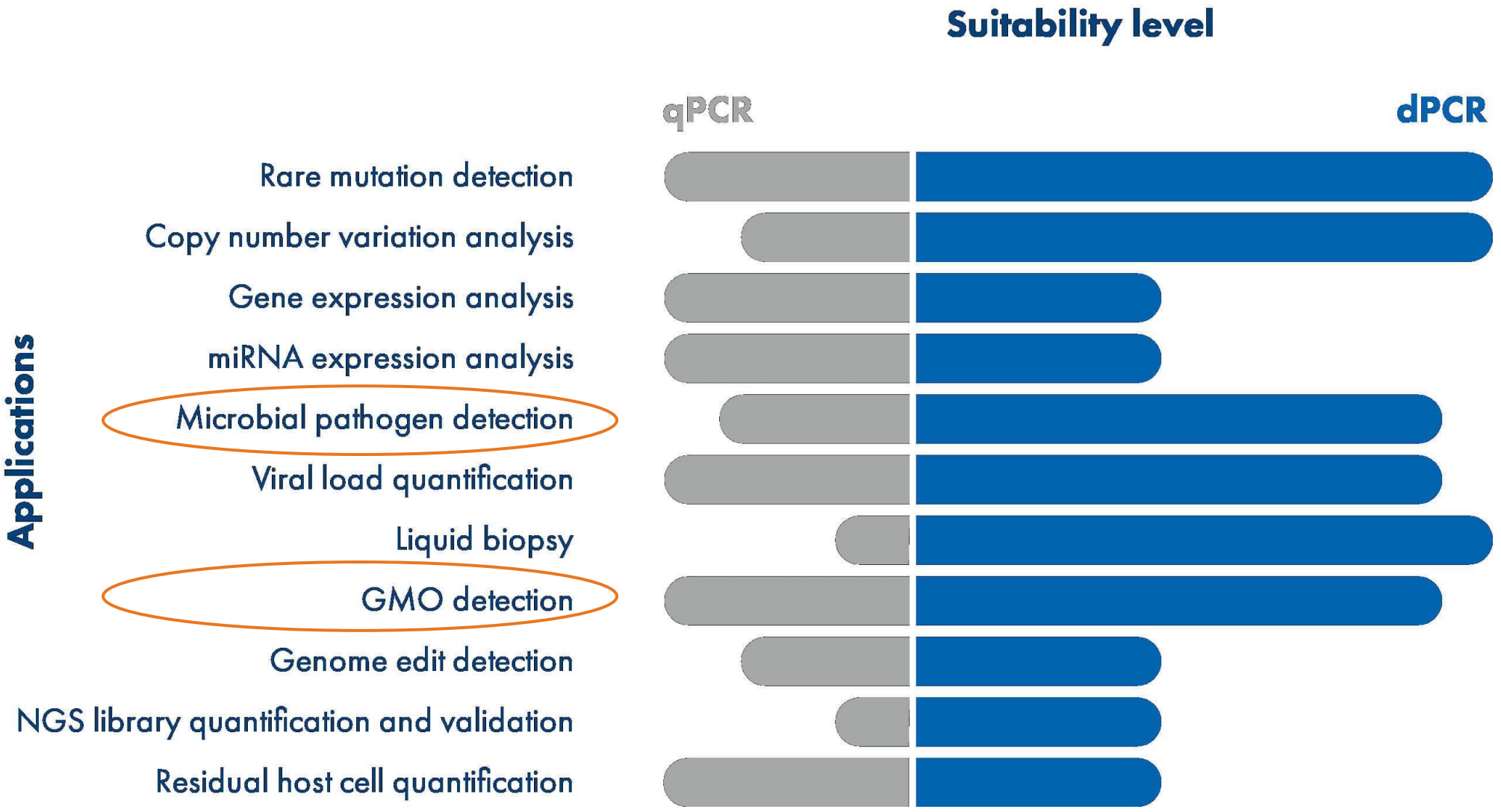

QIAcuity

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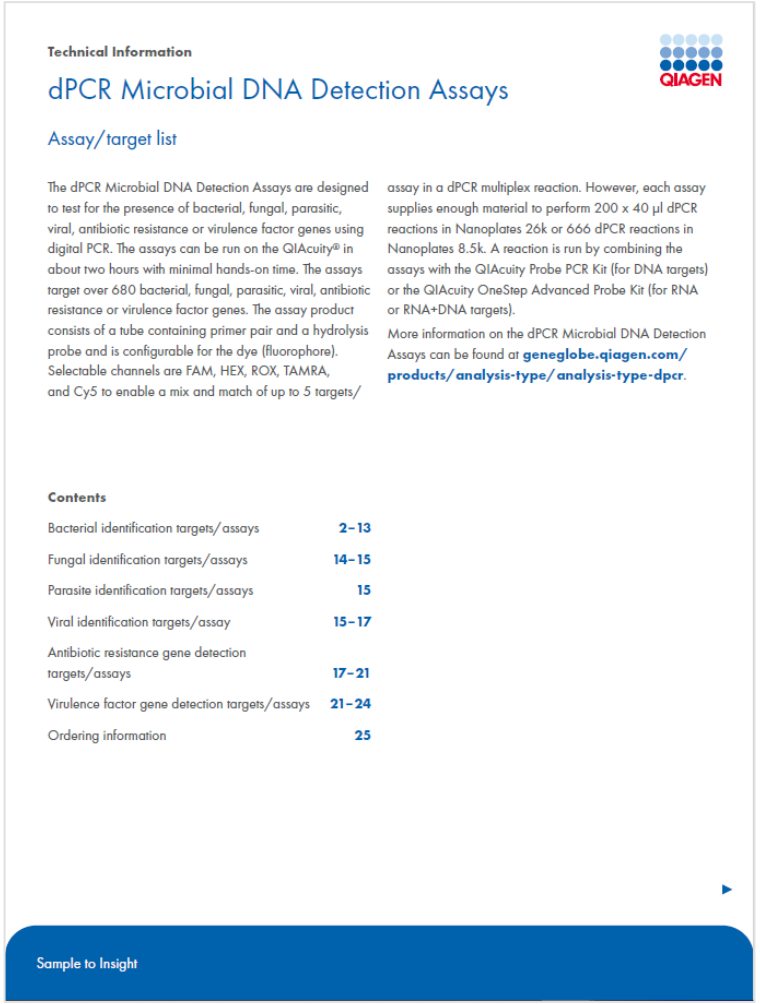
QIAcuity – dPCR Microbial DNA Detection Assays

- Detección de:
  - bacterias,
  - hongos,
  - parásitos,
  - virus,
  - genes de resitencia a antibiótios o virulencias
- Más de 680 ensayos
- Posibilidad de multiplexar hasta 5 dianas distinta por reacción
- Detección de dianas DNA y RNA en una misma reacción, utilizando el QIAcuity OneStep Advanced Probe Kit.



Technical Information

dPCR Microbial DNA Detection Assays

Assay/target list

The dPCR Microbial DNA Detection Assays are designed to test for the presence of bacterial, fungal, parasitic, viral, antibiotic resistance or virulence factor genes using digital PCR. The assays can be run on the QIAcuity® in about two hours with minimal hands-on time. The assays target over 680 bacterial, fungal, parasitic, viral, antibiotic resistance or virulence factor genes. The assay product consists of a tube containing primer pair and a hydrolysis probe and is configurable for the dye (fluorophore). Selectable channels are FAM, HEX, ROX, TAMRA, and Cy5 to enable a mix and match of up to 5 targets/assay in a dPCR multiplex reaction. However, each assay supplies enough material to perform 200 x 40 µl dPCR reactions in Nanoplates 26k or 666 dPCR reactions in Nanoplates 8.5k. A reaction is run by combining the assays with the QIAcuity Probe PCR Kit (for DNA targets) or the QIAcuity OneStep Advanced Probe Kit (for RNA or RNA+DNA targets).

More information on the dPCR Microbial DNA Detection Assays can be found at **geneglobe.qiagen.com/products/analysis-type/analysis-type-dpcr**.

Contents

Bacterial identification targets/assays	2–13
Fungal identification targets/assays	14–15
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Sample to Insight

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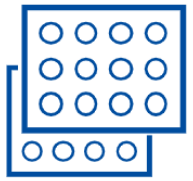
QIAcuity

Scalability

Low- to high-throughput instrument configurations

- 1-, 4- and 8-plate

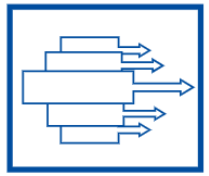
Up to two integrated thermocyclers



Throughput

Samples processed in a workday can increase from 96 to 1248 depending on the configuration used:

- Plate: 24- and 96-well
- Instrument: 1-, 4- and 8-plate



Multiplexing

Up to 5 detection channels, exclusive of reference

- 2-plex
- 5-plex

Time to result

Simple and rapid plate-based workflow takes users from sample to result in under 2 hours

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QIAcuity

Top 5 benefits

Absolute target quantification
No need for references or standard curves

High tolerance to inhibitors
Due to partitioning and endpoint measurement

Superior precision
Detect very small fold change differences

Increased sensitivity
Detect rare mutations and low abundance targets

High reproducibility
Eliminate amplification efficiency bias

Top 5 applications

Copy number variation

Rare mutation detection

NGS library quantification

Viral load detection

Gene expression analysis

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