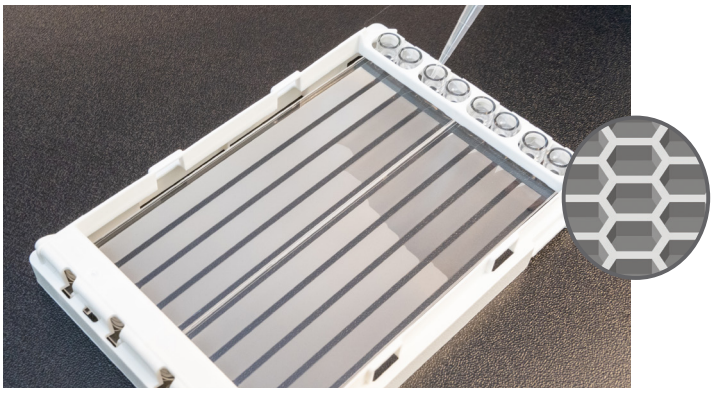
Tech Spotlight
dPCR System

Partitioning samples for dPCR

Physical partitioning (nanowells), or droplets?



The principle of digital PCR is the partitioning of a PCR reaction mixture into microreactions, followed by end-point amplification. Each partition is then assessed for whether it contained the target sequence (positive) or not (negative), and the absolute quantification of the target in the input sample is determined by Poisson calculations.

The earliest dPCR technologies used special oils to create tiny droplets to contain the microreactions. More recent developments—such as the Roche Digital LightCycler® dPCR System—use physical partitioning on nanowell plates, a method that offers several advantages over droplet-based dPCR.

Nanowell plates yield consistent numbers of equally sized partitions

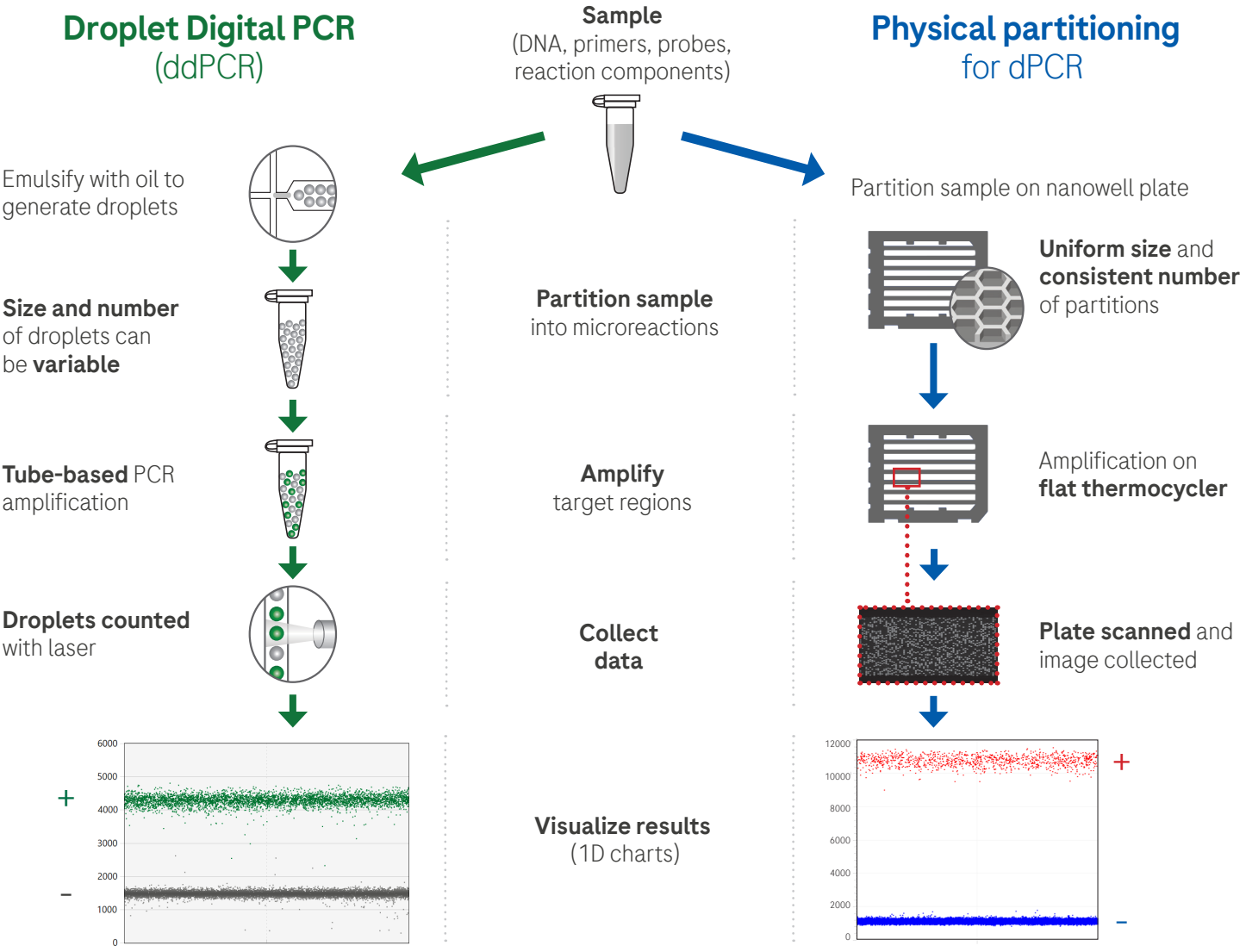
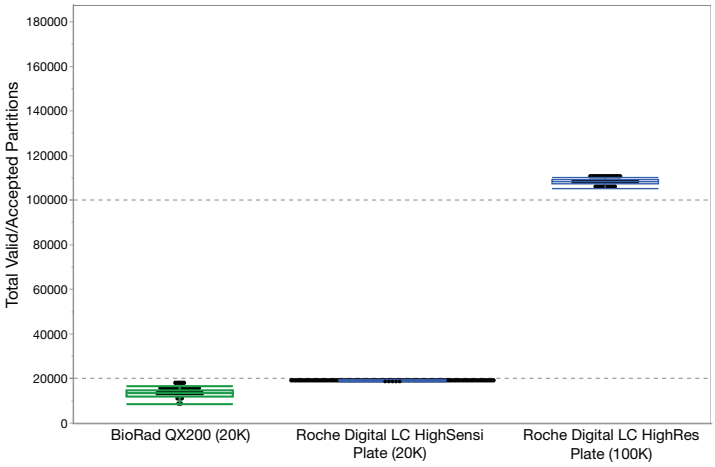


Figure 1. A comparison between sample partitioning workflows for droplet digital PCR (ddPCR) and nanowell-based physical partitioning. The Roche Digital LightCycler® dPCR instrument uses nanowell plates, and each run yields high numbers of valid partitions **(see Figure 2)**.



Digital LightCycler® dPCR System consistently yields high numbers of valid partitions.

Figure 2. A comparison of the number of valid partitions obtained with three different partitioning methods. Each column shows partitions obtained for several sample types (cfDNA, FFPE DNA, RNA), and each black dot represents the number of valid partitions from one sample. Partitioning was carried out on the BioRad QX200 (n=51) and on the Roche Digital LightCycler® dPCR System using two different plate configurations: High Sensitivity plate (n=95) and High Resolution plate (n=55).

Partitions are created on the Partitioning Engine instrument

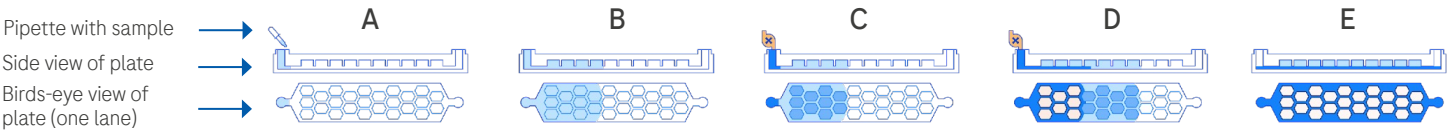
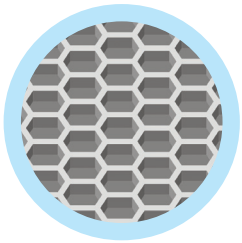
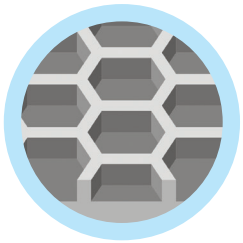


Figure 3. Illustration of how the partitioning fluid separates the sample on the nanowell plate. (A) The dPCR reaction mixture (template/sample and PCR components) is manually loaded into the inlet port of each lane; the dPCR reaction mixture is shown in light blue. **(B)** The sample then flows across the plate via passive filling, travelling across about one-third of the lane. **(C)** The plate is placed on the Partitioning Engine, and partitioning fluid (dark blue fluid) is injected into the inlet ports. **(D)** As the partitioning fluid moves across the lanes, the sample is pushed upwards and into the partitions (sealed partitions shown in gold). **(E)** At the end of the process, the partitioning fluid has traveled down the length of each lane and sealed each partition from the bottom, isolating it within the nanowell plate.

The Digital LightCycler® dPCR System offers 3 unique nanowell configurations



High Resolution
~15 µL sample volume
~100,000 partitions
Copy Number Variation
NIPT
Human Genetic Disease



Universal
~30 µL sample volume
~28,000 partitions
Gene Expression
Transplant Rejection

High Sensitivity
~45 µL sample volume
~20,000 partitions
Cell-free DNA
Oncology
Rare Mutation Detection

Learn more

about the **Digital LightCycler® dPCR System** and how it can help you at **go.roche.com/dpcr** or by scanning the **QR code**.

The Digital LightCycler® is a Class II US IVD instrument.

MC-US-13838 A726 10/23