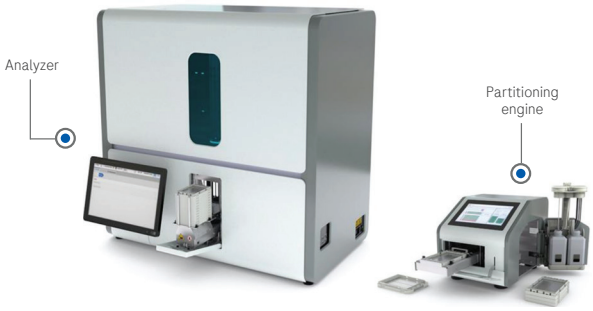
Tech Spotlight
dPCR System

Digital PCR (dPCR) for CNV analysis:

Discriminate small changes with great accuracy.

The Digital LightCycler® dPCR System



Digital PCR (dPCR) is a highly sensitive, precise method that's well-suited for detecting copy number variations (CNVs) (also known as copy number alterations, or CNAs).

CNVs/CNAs describe genomic regions where sequences have been deleted or amplified, and are associated with many different health conditions—including cancer and many neurodevelopmental disorders. Detecting even small changes in copy number variation can be critical for identifying risk factors and developing treatments.

Detect copy number changes as small as **10%** with the 100,000-partition High-Resolution Plate.

Assay overview (simplified)

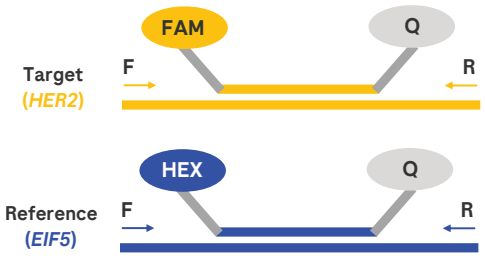


***Figure 1. Simplified assay overview**. Here, two targets are detected at the same time (multiplexing). Target-specific primers (arrows) extend during amplification, dislodging labeled probes from target sequences. The probes release a dye-specific signal (FAM or HEX) that is detected by the instrument. The Qs represent quencher molecules, which prevent dye activation until the probe is released.

1D scatter plot

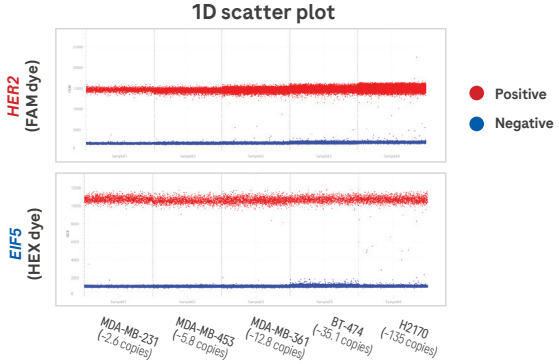


***Figure 2. Qualitative 1D scatter plot showing distinct clustering and minimal rain**. The results show increased *HER2* signal relative to *EIF5* in proportion to copy number. Each section of the chart represents one sample, and each dot represents one partition. **The distinct clustering and minimal rain demonstrate optimal assay performance and high signal-to-noise ratio**. 8 ng of cell line DNA was included per filling reaction on the 100,000-well High-Resolution Plate (cell lines are labeled above with expected *HER2* copy number).

***HER2* copy number across cell lines**

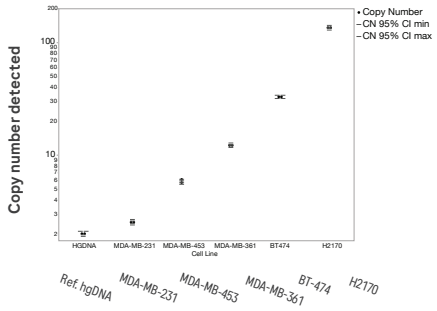


***Figure 3: Quantitative analysis of dPCR results across a wide range of copy numbers**. Here, wild-type hgDNA (copy number = 2) is used as a reference. For this analysis, the High-Resolution Plate (with 100,000 partitions) was used, as it enables clear discrimination over a wide range of HER2 copy numbers, ~2 to ~135.

High-resolution detection of small (~10%) changes in *HER2* gene dosage

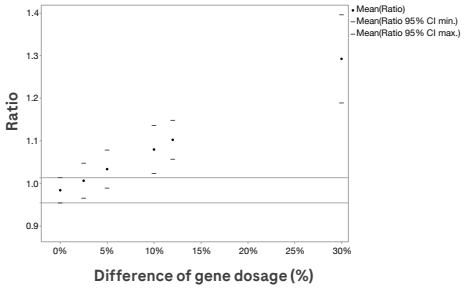


***Figure 4. Digital LightCycler dPCR System detects copy number changes as small as 10%**. Samples with varied copy numbers were created by spiking human gDNA with MD-MB-231 cell line DNA in specific ratios. See Figure 6 for an overview of how copy number is calculated.

*Data on file

Digital LightCycler® Workflow

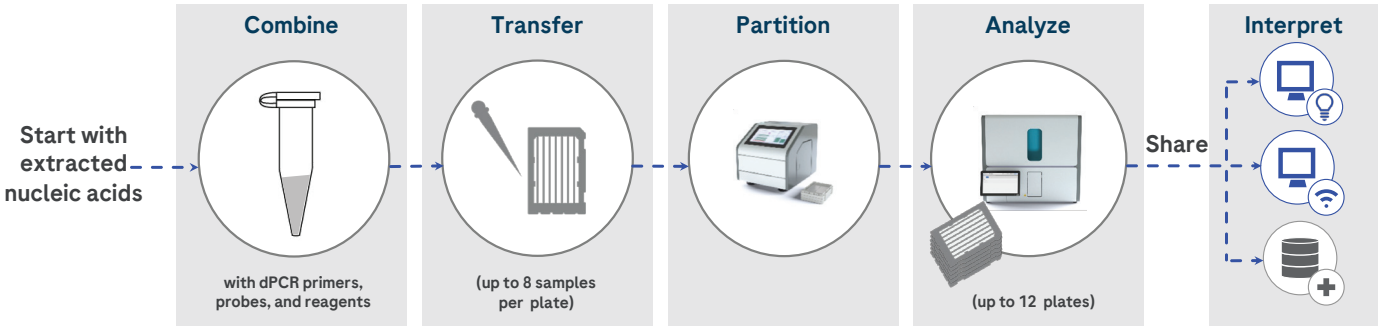
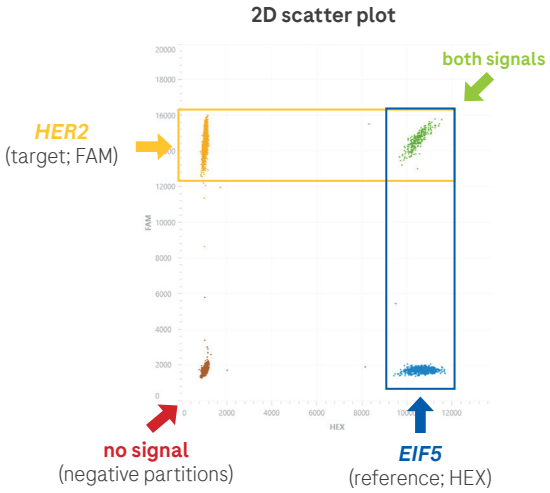


Figure 5. An overview of the Digital LightCycler dPCR System workflow. Extracted nucleic acids are combined with PCR reagents, including primers and probes. The reaction mixture is transferred to partitioning plates and partitioned on the Partitioning Engine. Plates are transferred to the Analyzer and processed with the appropriate cycles. The collected data is then analyzed with Digital LightCycler® software and web applications, and can be shared via LIMS.

How copy number is calculated



Following signal detection on the Analyzer, the total number of incidents of each signal is determined by Poisson distribution calculations.

Then, a ratio of target/reference is used to determine copy number.

For example: $\frac{410 \text{ (copies/ul)}}{142 \text{ (copies/ul)}}$ X 2 = ~ 5.8 copies of *HER2* in MDA-MB-453 cells

Figure 6. An example of how copy number is calculated using a 2-plex assay. In the 2D scatter plot (one way of visualizing the data from the Analyzer), each dot represents a detection event. The analysis software calculates the absolute number of incidents of each signal, and then a ratio is calculated to provide the copy number of the target.

Learn more

about the **Digital LightCycler® dPCR System** and how it can help you in your preclinical or clinical research at **go.roche.com/dpcr** or by scanning the code below.

*Data on file
The Digital LightCycler® is a Class II US IVD instrument.

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