ThermoFisher SCIENTIFIC

Comparing real-time and digital PCR technologies

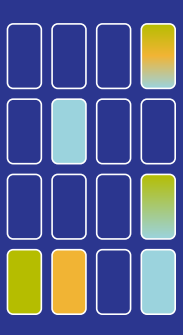
Both digital PCR (dPCR) and real-time or quantitative PCR (qPCR) can be used to quantify nucleic acids in a sample. This is performed by amplifying a target nucleic acid molecule with a DNA polymerase enzyme.

dPCR vs. qPCR

TECHNOLOGY

dPCR

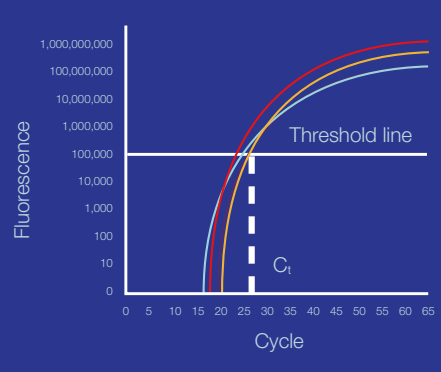
Distribute reaction, amplify, and count at endpoint



Absolute measurement—counts target of interest via single-molecule amplification across a large number of PCR replicates. Run at limiting dilution to ensure at least one reaction does not contain target DNA.

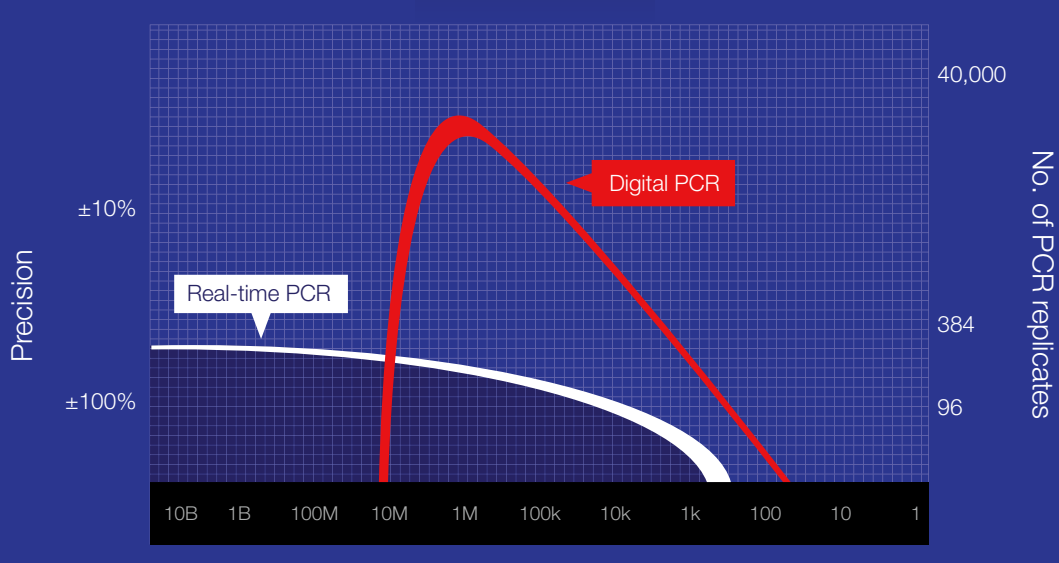
qPCR

Measure bulk reaction fluorescence at each cycle until plateau phase

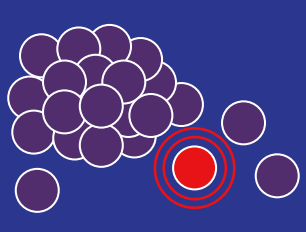


Measures PCR amplification against a reference as it occurs. Data are collected during the exponential (log) phase of PCR.

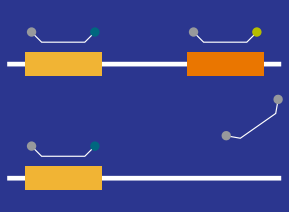
QUANTITATIVE



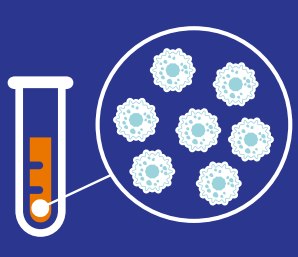
APPLICATIONS



Rare-target detection

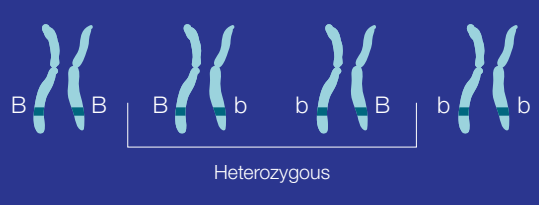


Single molecule characterization and quantification



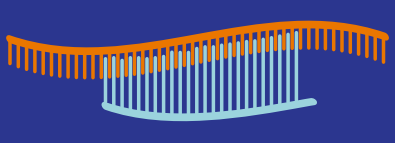
Absolute quantification of viral load

Somatic copy number variation or low fold changes

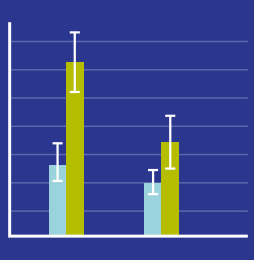


SNP genotyping

Relative gene expression analysis



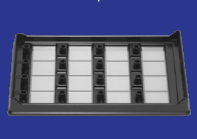
MicroRNA analysis



Standard copy number variation

WORKFLOW

Mix PCR reagents: nucleic acid, master mix, primers, and probes

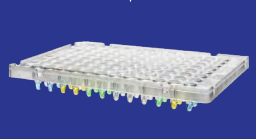


Load wells of plate

Place on instrument for dPCR

Watch the video

Mix PCR reagents: nucleic acid, master mix, primers, and probes



Load individual strip tubes or plate

Place on instrument for qPCR

ADVANTAGES

- Quantitative data output—no reliance on references or standards for conversion of data points
- Capable of analyzing rare targets against wild-type or non-target background
- Unlike traditional qPCR, digital PCR provides a linear response to the number of copies present to allow for small fold-change differences to be detected
- Single molecule resolution interrogation enables identification and quantification of molecules containing multiple targets (e.g., phased targets or engineered plasmids)
- Improved tolerance to some PCR inhibitors

- Broadly accepted, well-established protocols and assays
- Increased dynamic range of detection
- Detection is capable down to a 2-fold change
- Higher sample throughput with lower cost
- Collects data in the exponential phase of PCR, providing a permanent record of amplicon amplification

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