Digital PCR is a new approach to nucleic acid detection and quantification that offers an alternate method to conventional real-time quantitative PCR for absolute quantification and rare allele detection. Digital PCR works by partitioning a sample of DNA or cDNA into many individual, parallel PCR reactions; some of these reactions contain the target molecule (positive) while others do not (negative). A single molecule can be amplified a million-fold or more. During amplification, TaqMan® chemistry with dye-labeled probes is used to detect sequence-specific targets. When no target sequence is present, no signal accumulates. Following PCR analysis, the fraction of negative reactions is used to generate an absolute count of the number of target molecules in the sample, without the need for standards or endogenous controls.

The use of a nanofluidic chip provides a convenient and straightforward mechanism to run thousands of PCR reactions in parallel. Each well is loaded with a mixture of sample, master mix, and TaqMan® Assay reagents, and individually analyzed to detect the presence (positive) or absence (negative) of an endpoint signal. To account for wells that may have received more than one molecule of the target sequence, a correction factor is applied using the Poisson model.

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