Rare-target detection from single cells
Real-time digital PCR with the QuantStudio™ 12K Flex Real-Time PCR System.

Digital PCR (dPCR) is a relatively new approach to nucleic acid detection that provides greater sensitivity and precision than standard real-time PCR. The QuantStudio™ 12K Flex Real-Time PCR System is an all-in-one quantitative PCR (qPCR) instrument that offers multiple configurations for both real-time dPCR and standard real-time PCR. Using this instrument, Celula, Inc.—a life sciences company in San Diego, California—has developed a sensitive rare-allele detection assay. Compared with conventional PCR assays, their dPCR workflow requires much less sample and reagents, while incorporating quality-control runs using real-time PCR.

Detecting rare sequences with digital PCR

dPCR is a valuable tool for detecting target alleles in quantities as low as one copy per cell population. dPCR enables researchers to “count” individual DNA sequences of interest by isolating single molecules prior to the PCR process. Compared with conventional real-time PCR, dPCR offers increased sensitivity and precision, as well as absolute quantification of nucleic acids in samples.

The isolation of single DNA molecules requires partitioning the sample into many independent reaction vessels so that each reaction receives, on average, either one or zero copies of a target sequence. Once individual DNA molecules have been isolated, standard PCR cycling parameters are applied and wells containing DNA are amplified and detected (Figure 1).

Using the QuantStudio™ 12K Flex system and QuantStudio™ Digital PCR Plates, one user can generate more than 49,000 digital PCR data points per day, without the use of robotics. Each QuantStudio™ Digital PCR Plate is pretreated to accept TaqMan® Assays, TaqMan® OpenArray® Digital PCR Master Mix, and your samples—simply mix, load, cycle, and analyze.

Developing single-cell assays for rare alleles

Celula develops molecular diagnostic assays for single cells with rare genetic variants. These assays are based on a process that integrates the enrichment of cell populations from primary tissues with the molecular analysis of target cells from aliquots containing 10,000 or fewer cells from these enriched mixtures. The assays are designed to target every cell in a limiting mixture. By reducing background noise that typically obscures limited target signals, they are extremely useful for identifying and quantifying markers that exist in only a few cells in a population.

At each step during cell enrichment, the researchers must analyze aliquots of the cell population, both to count how many
cells containing the rare genetic variant are still present and to
determine how many cells were lost. Throughout this process,
the methods must maximize cell retention in order to maintain
enough cells to complete the entire analysis.

The rare-allele detection assay developed by Celula includes
the use of both TaqMan® Assays and custom SNP genotyping
assays, followed by allelic discrimination analysis. To evaluate
whether the QuantStudio™ 12K Flex system generates genotyping
data comparable to a standard 384-well real-time PCR system, the
researchers compared cluster distribution on the QuantStudio™
12K Flex system with that on the 7900 Real-Time PCR System.
As shown in Figure 2, although data quality was similar for both
systems, the 7900 instrument required 10 ng human DNA per
reaction, compared with only 0.03 ng for the QuantStudio™ 12K
Flex system. Because it can generate genotyping data compa-
rable to a standard real-time PCR instrument but with much less
DNA sample and reagent, the QuantStudio™ 12K Flex system can
provide significant savings in both sample and reagent costs for
rare-allele detection assays.

Distinguishing positive signals from noise
To accurately detect and quantify rare genetic variants in single
cells, it is essential to determine whether the signals in positive
wells actually reflect the presence of a target allele or locus.
Celula runs real-time PCR amplification curves in parallel to the
dPCR reaction to help distinguish true signals from background
noise—which can result from PCR artifacts or probe degradation. A
viable amplification curve indicates a true-positive signal (Figure 3).

Optimizing rare-allele detection in single cells
Based on their research, Celula concluded that the QuantStudio™
12K Flex Real-Time PCR System, when incorporated into their rare-
allele detection workflow, offers several advantages over standard
real-time PCR for the detection and absolute quantification of rare
alleles in a cell population. Importantly, the QuantStudio™ 12K
Flex system requires very small amounts of sample and reagents
relative to standard real-time PCR, resulting in significant cost
savings. Also, because the system can rapidly switch from dPCR
to real-time PCR, it can be easily integrated into a quality control
workflow to distinguish positive signals from noise.

Learn more about the QuantStudio™ 12K Flex system and our
other products for PCR at lifetechnologies.com/bp68.