Determination of Copy Number Variation using the Constellation Digital PCR System

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Copy number variation, or the structural variation of a DNA genome, is an important source of genetic variation for an array of medically- and agriculturally- relevant traits (1-5). In order to understand the biological basis for a number of important variations, it is critical to discriminate between the different number of genomic copies of specific DNA sequences. Digital PCR is a highly accurate technique for the absolute quantification of nucleic acid targets, and can be used to determine copy number variations between individuals in a population (6), between normal and tumor cell populations (7), and within somatic mosaics (8).

The number of copies of human genomic loci CCL3L1 present in an individual gene is currently being investigated as a protective adaptation against susceptibility to HIV (6). In the following experiment, the Formulatrix Constellation Digital PCR system is used to determine the number of copies present at two human genomic loci, CCL3L1 and RPPH1. The results are then compared against the known values of copy number variants in CEPH DNA samples ranging from 0-8 CCL3L1 copies/diploid genome.

About the Formulatrix Constellation

The Constellation is an intuitive, user-friendly, low-cost, high-throughput dPCR device. Based on a proprietary microtiter plate that conforms to SBS standards, the Constellation enables sample loading with conventional manual or robotic pipetting tools. The initial version of this plate contains 96 individual sample wells, each of which is processed by the Constellation through priming and sealing steps into 496 separate partitions. After thermal cycling on conventional equipment, the plates are read by the Constellation using up to four separate optical channels. Simple, clean software and a touchscreen interface make the entire process seamless - from priming through sealing, imaging, and data analysis.

Methods and Materials

Samples were run in duplicate wells on a single Constellation plate (Fig. 1, step 1). Partitions were sealed and primed using the Constellation (Fig. 1, steps 2 and 3), and the entire plate was thermally cycled for 40 cycles

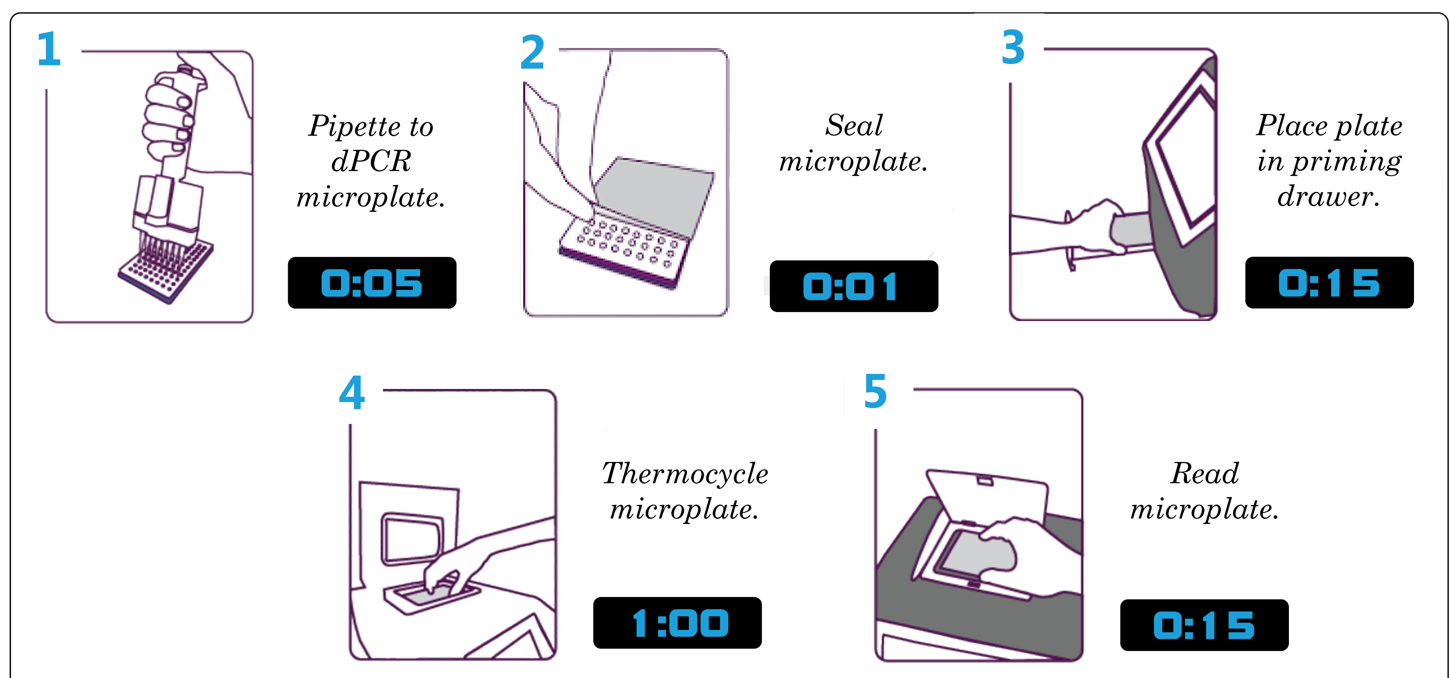

Figure 1. *Schematic outline for running a Constellation digital PCR plate, and estimated time required to complete each step.*

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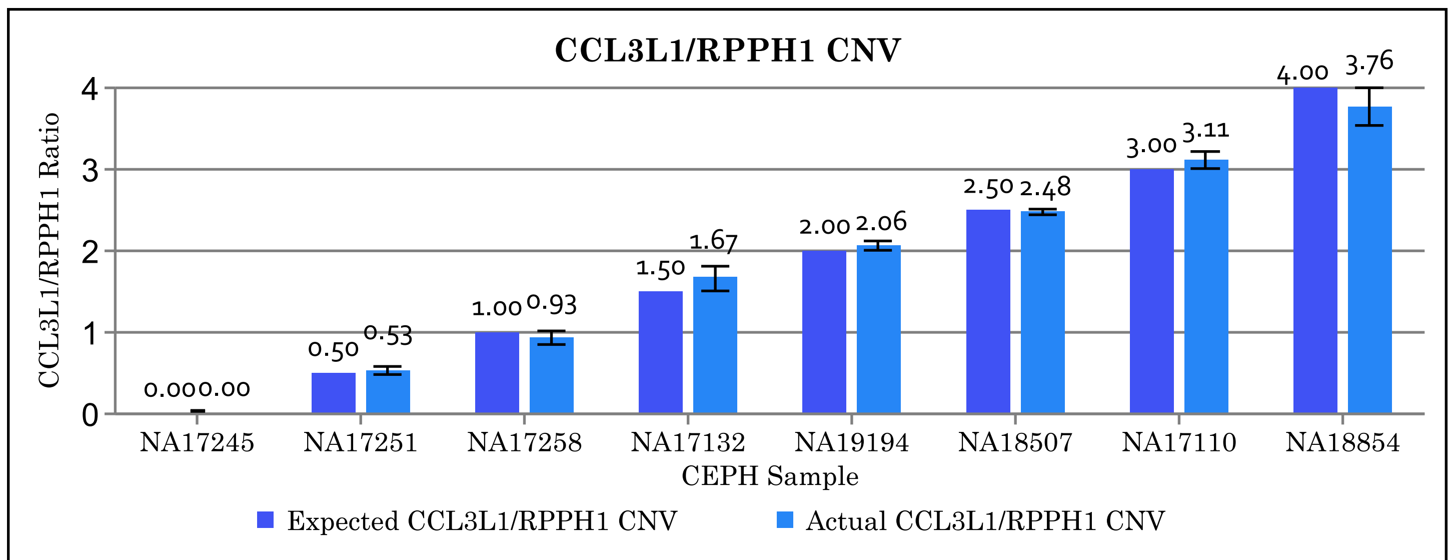

Figure 2. *Constellation digital PCR determination of CCL3L1/RPPH1 ratios for CEPH genomic DNA samples.*

(Fig. 1, step 4). The plate was imaged by the Constellation for ROX, FAM and VIC (Fig. 1, step 5), with a total time from sample loading to data of approximately 1.5 hours. Copy number quantities for each sample were determined by the Constellation software for individual wells, and the average and standard deviation of duplicate reaction pairs was reported.

Results

Using the Constellation digital PCR system, samples of known copy number were assayed for the quantity of CCL3L1 and RPPH1 targets present. Each sample was tested with 2 replicate wells and 992 partitions. The results are shown in Figure 2, with the normalized CCL3L1/RPPH1.

The seven CEPH DNA samples range in CCL3L1 copy number from 0 - 8 copies per genome, while the RPPH1 target remains constant across all samples at two copies per genome.

The ratio of CCL3L1 to RPPH1 thus allows for the determination of copy number of CCL3L1 while correcting for concentration differences between samples. For each sample, the expected target copy number ratio is contained within the interval of +/- one standard deviation.

Conclusions

The Constellation dPCR system accurately and precisely determines the number of copy number variations in CCL3L1. As expected, up to eight CCL3L1 copies per genome were accurately resolved, representing a maximal discriminatory power of 1.2-fold (20% difference in target) between samples tested in this experiment. Further extension of this range of resolution, either for the same samples or for higher levels of genomic copies, could be determined by using larger numbers of replicate wells.

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