# Copy Number Variation Analysis using Clarity™ Digital PCR System

## I. Overview

Copy number variations (CNVs) refer to the increase or decrease of the copy number of a locus compared to a reference genome. CNVs are largely involved in various human diseases especially genetic diseases and cancers, where they can be potentially used as biomarkers. Several methods for detection of CNVs have been developed, among which digital PCR (dPCR)-based technologies provide the most precise measurements with simple workflows[1,2]. In this study, we demonstrate that dPCR using the Clarity™ system can quantify different copy numbers (CNs) with high accuracy and precision.

## II. Quantitation of gene CNs with high accuracy using the Clarity™ dPCR system

In this study, CNVs of the *DEFB4* gene was examined. *DEFB4* is a member of beta defensin genes, which vary greatly in CNs in human and are candidates for differences in susceptibility to inflammatory and autoimmune disorders[3]. DPCR was performed on four genomic DNA (gDNA) samples, which were obtained from the NIGMS Human Genetic Cell Repository at the Coriell Institute for Medical Research: NA18552, NA15324, NA12760 and NA18858 with known CNs of DEFB4 gene (2, 4, 6 and 8, respectively[4]). Samples were mixed at different ratios to simulate gDNA samples with predicted copy numbers 3, 5 and 7. Using a SYBR® Green dye-based assay, the primers 5'-CACCTGTGGTCTCCCTGGAA-3(F) and 5'-AGCTTCTTGGCCTCCTCATG-3'(R) were used to amplify the *DEFB4* target, whereas the primers 5'-AGATTTGGACCTGCGAGCG-3'(F) and 5'-GAGCGGCTGTCTCCACAAGT-3'(R) were used to amplify the *RNase P* reference gene. Prior to dPCR, the genomic DNA samples were first digested by MseI enzyme (New England Biolabs) at 37°C for 1 hour, and restriction enzyme was then heat-deactivated at 65°C for 20 minutes. Results showed that the measured CNs were very close to the expected values (with a difference between 0.14%-0.75%), and relative uncertainty (RU) was ≤ 5% among the three individual sets of experiments, indicating a high degree of reproducibility.

| Samples | Expected CN | Detected CN | Relative Uncertainty |
|---|---|---|---|
| NA18552 | 2 | 1.99 | 0.02 |
| NA18552+NA15324 | 3 | 2.96 | 0.03 |
| NA15324 | 4 | 3.97 | 0.05 |
| NA15324+NA12760 | 5 | 5.01 | 0.02 |
| NA12760 | 6 | 6.02 | 0.03 |
| NA12760+NA18858 | 7 | 7.01 | 0.02 |
| NA18858 | 8 | 7.96 | 0.04 |

**Table 1. CN analysis of DEFB4 gene with the Clarity™ dPCR system.** Results are representative of three sets of independent experiments performed in triplicates.

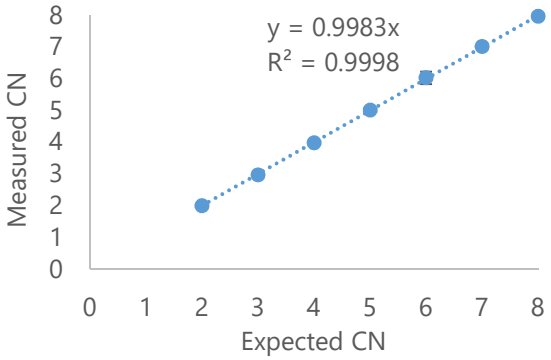


**Figure 1. Graphical representation of the results obtained in Table 1.** Error bars denote standard deviations among the mean of three independent experiments performed in triplicates.

## III. Conclusion

As CNVs have been associated with diverse disorders and disease susceptibility, identification of CNVs will refine genotype-phenotype relationships, understand disease pathogenesis and discover new drug targets. In this study, we showed that precise CNV determination can be achieved using the Clarity™ system with minimum number of replicates and easy workflow, which makes the system well suited for the detection of CNVs in academic and clinical research.

## References


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