**ABSTRACT**

Human epithelial growth factor receptor 2 (HER2) gene amplification (increased copy number), not only is a strong indicator for tumor progression and poor prognosis but also presents on an effective drug target. Accurate measurement of HER2 copy number (CN) is so critical that it determines patient qualification for HER2-targeted therapy with Trastuzumab (Herceptin) for early or advanced breast cancer. Currently, in situ hybridization-based and microarray-based technologies are commonly used, but they can be subjective, lengthy, labor intensive and less quantitative, especially for samples whose HER2/CEP17 ratio fall into the equivocal range (1.8-2.2). Life Technologies offers TaqMan® Copy Number Assays for copy number analysis on real-time PCR platform, but PCRs also become less quantitative for CN calls reach higher than 4, due to its relative quantification (RQ) methodology. Recently launched QuantStudio™ 3D (QSD) Digital PCR System provides a new platform with absolute quantification capability to address these challenges. We have developed a copy number application on the QSD PCR system and can calculate copy number based on absolute quantitation exported from QSD Analysis Suite. We demonstrate that the system not only can quantify gene copy numbers from zero to eight with high accuracy and precision but also is able to differentially detect heterogeneous samples with 5% resolution. We also present our technical assessment for quantifying HER2 copy number using breast cancer FFPE samples and our results show high concordance with results from SISH method. As a robust platform, the dPCR system not only leverages our existing TaqMan® CN assays but also allows customers to measure higher CN with absolute accuracy, simple workflow and fast turnaround time.

**INTRODUCTION**

Gene amplification is a common genetic abnormality in many types of cancers and has been implicated in playing important roles in cancer development. HER2 gene amplification occurring in about 10-30% of breast cancer cases, is strongly associated with aggressive tumor progression and poor prognosis, and also an effective therapeutic target. Breast cancer patients with HER2+ are eligible for Herceptin treatment. Therefore, accurately determining HER2 gene amplification is essential for challenging for cancer researchers and clinicians. Currently, IHC (immunohistochemical analysis), FISH (fluorescence in situ hybridization) or SISH (in situ hybridization) are routinely used. However, the challenge is that these methods are laborious and less quantitative, especially when CN falls into equivocal range. In the study, we use PCRs to determine HER2 copy number. The results show high concordance with results from SISH method, but they can be subjective, lengthy, labor intensive and less quantitative, especially for samples whose HER2/CEP17 ratio fall into the equivocal range (1.8-2.2). Life Technologies offers TaqMan® Copy Number Assays for copy number analysis on real-time qPCR platform, but qPCRs also become less quantitative for CN calls reach higher than 4, due to its relative quantification (RQ) methodology. Recently launched QuantStudio™ 3D (QSD) Digital PCR System provides a new platform with absolute quantification capability to address these challenges. We have developed a copy number application on the QSD PCR system and can calculate copy number based on absolute quantitation exported from QSD Analysis Suite. We demonstrate that the system not only can quantify gene copy numbers from zero to eight with high accuracy and precision but also is able to differentially detect heterogeneous samples with 5% resolution. We also present our technical assessment for quantifying HER2 copy number using breast cancer FFPE samples and our results show high concordance with results from SISH method. As a robust platform, the dPCR system not only leverages our existing TaqMan® CN assays but also allows customers to measure higher CN with absolute accuracy, simple workflow and fast turnaround time.

**RESULTS**

Fig. 1. Workflow of QuantStudio™ 3D Digital PCR System

- Mix
- Load
- Amplify
- Read

B. QuantStudio™ 3D Digital PCR 20K Chip

Fig. 2. Quantitative 3D Digital PCR Can Accurately Quantify Higher Copy Numbers

- High accuracy of copy number determination is illustrated in 3D isometric bar chart. The y-axis shows the relative number of FAM-labeled HER2 target and the x-axis shows the standard deviation from mean. The green line is drawn roughly CN=4.4 corresponding to the cutoff of HER2/C17 ratio (2.2) for calling positive in SISH.

**DISCUSSION**

The limitation of qPCR for CNV detection is that it measurement lose accuracy when copy number is above 4 due to its relative quantification (RQ) method. We demonstrate that the QuantStudio™ 3D Digital PCR System provides a new platform with absolute quantification capability to address these challenges. We have developed a copy number application on the QSD PCR system and can calculate copy number based on absolute quantitation exported from QSD Analysis Suite. We demonstrate that the system not only can quantify gene copy numbers from zero to eight with high accuracy and precision but also is able to differentially detect heterogeneous samples with 5% resolution. We also present our technical assessment for quantifying HER2 copy number using breast cancer FFPE samples and our results show high concordance with results from SISH method. As a robust platform, the dPCR system not only leverages our existing TaqMan® CN assays but also allows customers to measure higher CN with absolute accuracy, simple workflow and fast turnaround time.

**CONCLUSIONS**

- The QuantStudio™ 3D Digital PCR System provides a sensitive, accurate, robust technology with a simple workflow for copy number analysis. Copy number can be calculated based on the absolute quantitation exported from QSD3D Analyze Software. It is a reliable platform for measuring gene amplification and copy number variation (CNV).
- We can accurately measure copy number from 0 to 8 with high precision (with CV of 1.0-2.5%) using CCL51 as a model gene and the samples with known copy number for the gene.
- We also demonstrate the ability of QSD to differentially detect copy number differences in heterogeneous samples with high sensitivity of 5% or even lower.
- We have assessed HER2 gene amplification in 43 breast cancer FFPE samples and high concordance is achieved compared to SISH results from the same set of samples.

**ACKNOWLEDGEMENTS**

We thank Bruno Ping, Molecular Diagnostics Department at Royal Surrey County Hospital, Guildford, United Kingdom for sharing the breast cancer FFPE samples and their SISH data.

**TRADEMARKS/LICENSES**

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