Droplet Digital™ PCR Detection of HER2 Expression in FFPE Breast Tissue Samples

Nicholas J Heredia PhD,1 Phillip Belgrader PhD 2, Shenglong Wang PhD,3 Angela M Cosman2, Serge Saxonov PhD1, Benjamin Hindson PhD1,
1 Bio-Rad Laboratories, Inc., 7068 Koll Center Pkwy, Ste. 401, Pleasanton, CA 94566; 2 University of Mississippi Medical Center, Department of Pathology, 2500 N. State St., Jackson, MS 39216

Abstract
The human epidermal growth factor receptor 2 (HER2), also known as ERBB2 gene is involved in signal transduction for cell growth and differentiation. It is a cell surface receptor tyrosine kinase and a proto-oncogene. Overexpression of ERBB2 is of clinical relevance in breast cancer due to its prognostic value in correlating elevated expression with worsening clinical outcome. ERBB2 assessment is also of importance because successful anti-tumor treatment with Herceptin is strongly correlated with ERBB2 overexpression in the tumor (approximately 30% of all breast tumors overexpress ERBB2). In a comprehensive national study by Wolff et al. (JCO 2007), "Approximately 20% of current ERBB2 testing may be inaccurate," which underscores the importance of developing more accurate methods to determine ERBB2 status. Droplet Digital™ PCR (ddPCR®) has the potential to improve upon ERBB2 measurement because of its ability to quantify DNA and RNA targets with high precision and accuracy. Here we present a study which investigates whether Droplet Digital PCR can be used to assess expression levels of HER2 transcripts in FFPE human breast tissues samples that were concurrently assessed by pathologists using FISH and IHC. We also determined copy number of ERBB2 compared with reference genes.

Methods
ddPCR was performed using the Bio-Rad QX100™ Droplet Digital™ PCR system, dPCR supermix, and Bio-Rad standard reagents for droplet generation and reading. Assays were purchased from Applied Biosystems (ABI) at 20x concentration (see list below) and cDNA was generated with MultiScribe high capacity kit (without RNase inhibitor). Biosystems (ABI) at 20x concentration (see list below) and cDNA was generated with MultiScribe high capacity kit (without RNase inhibitor). Samples were either from Origene fresh-frozen RNA, Ambion, or 50 clinical FFPE human breast tumor samples from the University of Mississippi, which had IHC and FISH analyses performed, of which a subset of 12 was assessed by ddPCR for gene expression levels.

TaqMan assays used in this study include:

Gene Expression Assays
- Hs01001595_m1 (ERBB2)
- Hs02758991_g1 (GAPDH)
- Hs01001556_m1 (ERBB2 5')
- Hs01001580_m1 (ERBB2 best coverage)
- Hs99999005_mH (ERBB2 3')
- Hu GAPDH PL

Copy Number Assays
- Hs28503918_c1 (ERBB2)
- CEP7

Samples used in this study include:
- Origene CR562124
- Origene CR560258
- Origene CR561507
- Origene CR560259
- Ambion normal breast RNA
- 50 FFPE derived breast tissue sections from the University of Mississippi Medical Center Department of Pathology

Thermal Gradient Optimization
Example of thermal gradient optimization for duplex assay: ERBB2/GAPDH. In this case Applied Biosystems gene expression assays were used (Hs01001595_m1 ERBB2 and Hu GAPDH PL). The optimum temperature for both assays in duplex was 60°C.

Conclusions
- Droplet Digital PCR is a new and powerful tool to further elucidate clinically relevant materials at genomic and transcriptional levels
- ddPCR provides absolute quantitation of nucleic acids in samples with varying extents of degradation and can be paired with reference assays for DNA copy number assessments or normalized transcript concentrations
- In the example of ERBB2, ddPCR results are well correlated with IHC and FISH results
- Better discrimination of ERBB2+ and ERBB2– samples and better predictors may be enabled by more sophisticated reference normalizations using multiple reference assays