Detection of *HER2* Copy Number Variation with Crystal Digital PCR™

Triplex Crystal Digital PCR assay to detect *HER2* Copy Number amplification

Investigating *HER2* amplification status is crucial in breast cancer to determine the indication of anti-*HER2* targeted therapy. Assessing *HER2* copy number variation is challenging especially when tumor DNA is diluted in DNA from healthy cells. Here, we illustrate how Crystal Digital PCR is capable of reliably identifying *HER2* amplification in samples with low tumoral fraction.

An assay previously described for the detection of *HER2* (*ERBB2*) and MRM1 on chromosome 17, and *TSN* (reference gene) on chromosome 2[1], was optimized for Crystal Digital PCR. This assay enables the quantification of *HER2* copy number and the distinction between *HER2* amplification and chromosome 17 polysomy, a condition that could lead to *HER2* status misinterpretation.

Evaluation of the sensitivity of *HER2* amplification detection in a reconstruction experiment

Genomic DNA extracted from SKBR3 cell line (*HER2*/*TSN* ratio of 10:1) was spiked into non-amplified genomic DNA with mutant fractions from 0% to 12%, modeling a theoretical range of *HER2*/*TSN* ratios from 1 to 2.08

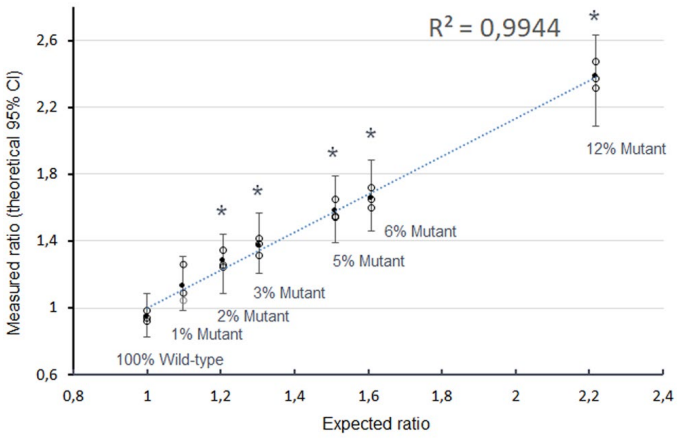

Figure 1 | Measure of different HER2/TSN ratios in reconstruction experiment (* : indicates that detection is statistically significant).

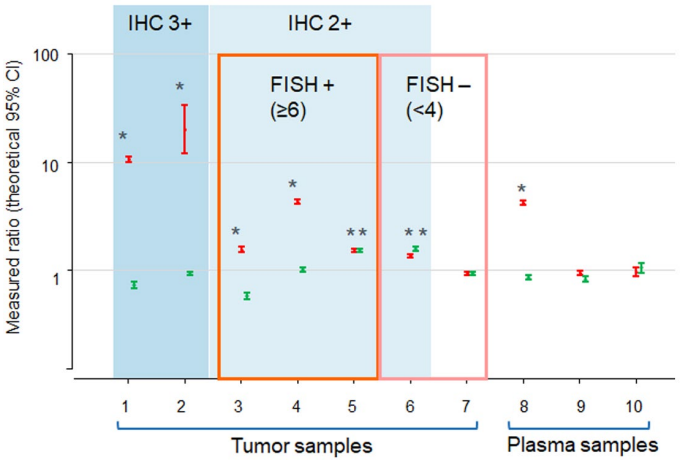

Figure 2 | HER2 amplification (red color) and chromosome 17 polysomy (green color) in plasma and tumor from breast cancer patients (* : indicates that detection is statistically significant)

Absolute concentrations for *TSN* and *HER2* were calculated using Poisson law and a Z-test was applied on the log-ratio *HER2*/*TSN* (approximated by a Normal distribution) to interrogate the presence of mutant DNA with statistical significance of α=β=5%[2]. Using this method, we were able to significantly detect the presence of mutant DNA for mutant fractions as low as 2%, corresponding to a *HER2*/*TSN* normalized ratio of 1.2.

HER2 amplification and chromosome 17 polysomy status in breast cancer patients

Current methods to evaluate *HER2* amplification in tumor samples rely on immunohistochemistry (IHC) to assess *HER2* overexpression, which, for equivocal IHC *HER2* expression (IHC2+), may be complemented by fluorescence in-situ hybridization (FISH) to detect *HER2* gene amplification. These methods are labor-intensive and require a high level of expertise for analysis. Molecular methods may provide simpler, faster and unbiased alternatives.

The present study was conducted using DNA extracted from tumors from 7 patients with breast cancer, provided by Institut Gustave Roussy (France)[§], and results obtained by Crystal Digital PCR were compared to those obtained using standard diagnostic procedure.

Results obtained with IHC/ FISH and Crystal Digital PCR were in agreement for patients 1-4 samples, deemed positive for *HER2* amplification by both methods, as well as for samples from patients 6 and 7 identified as negative for *HER2* amplification. Crystal Digital PCR additionally identified a case of chromosome 17 polysomy for patient 6. However, whereas *HER2* amplification was identified for patient 5 sample using standard diagnostic procedure, Crystal Digital PCR identified the elevated *HER2* signal as attributable to chromosome 17 polysomy.

To learn more about digital PCR, please visit Stilla Technologies' Learning Center at **stillatechnologies.com/digital-pcr**

[1] Jacquemier et al. "SISH/CISH or qPCR as alternative techniques to FISH for determination of HER2 amplification status on breast tumors core needle biopsies:a multicenter experience based on 840 cases". BMC Cancer. 2013 Jul 22;13:351. PubMed PMID: 23875536

[2] Whale et al. "Comparison of microfluidic digital PCR and conventional quantitative PCR for measuring copy number variation". Nucleic Acids Res. 2012 Jun;40(11): PubMed PMID: 22373922

§ Courtesy of Dr. Cécile Jovelet, Translational Research Laboratory, Institut Gustave Roussy, France