3-COLOR CRYSTAL DIGITAL PCR™ ASSAYS FOR *EGFR*-MUTATION DETECTION

TWO MULTIPLEX DIGITAL PCR ASSAYS DETECT *EGFR* ACTIVATING AND RESISTANCE MUTATIONS

In non-small cell lung cancer, the epidermal growth factor receptor (*EGFR*) is a common therapeutic target. *EGFR* activating mutations, such as L858R, L861Q, and exon 19 deletions are predictive of disease responsiveness to targeted therapy using tyrosine kinase inhibitors (TKIs)[1]. On the contrary, the presence of *EGFR* T790M mutation is associated with tumor resistance to TKIs[2].

To detect and quantify these mutations in single tests without sacrificing the precision and reliability of the results, two multiplex assays were developed. Primers and probes using three different fluorescence channels of the naica® system enable detection of *EGFR* L858R/L861Q and T790M in panel 1, and *EGFR* exon 19 deletions (as a drop-off assay) and T790M in panel 2. Both panels also detect wild-type (WT) *EGFR*.

Panel 1: *EGFR* L858R / L861Q and T790M

Primers/Probes	5' Fluorophore	Sequence	3' Quencher
L858R-Forward	-	GCAGCATGTCAAGATCACAGATT	-
L858R-Reverse	-	CCTCCTTCTGCATGGTATTCTTTCT	-
L858 WT Probe	**FAM**	**AGTTTGG{C}{C}A{G}CCCAA**	**BHQ-1**
L858R-Probe	**Cy5**	**AGTTTGGC{C}C{G}CCCAA**	**BHQ-3**
L861Q-Probe	**Cy5**	**ACCCAG{C}T{G}TTTGGCCA**	**BHQ-3**
T790M-Forward	-	GCAGGTACTGGGAGCCAAT	-
T790M-Reverse	-	GCATCTGCCTCACCTCCA	-
T790M-Probe	**Yakima Yellow**	**TGAGCT{G}{C}A{T}GATG**	**BHQ-1**

Panel 2: *EGFR* exon 19 deletions and T790M

Primers/Probes	5' Fluorophore	Sequence	3' Quencher
Del19-Forward	-	GTGAGAAAGTTAAAATTCCCG	-
Del19-Reverse	-	CACACAGCAAAGCAGAAAC	-
Del19 ref Probe	**FAM**	**CACATCGAGGATTTCCTTGTTGGC**	**BHQ-1**
Del19 WT probe	**Cy5**	**AGGAATTA{A}GA{G}AAG{C}AACATC**	**BHQ-3**
T790M-Forward	-	GCAGGTACTGGGAGCCAAT	-
T790M-Reverse	-	GCATCTGCCTCACCTCCA	-
T790M-Probe	**Yakima Yellow**	**TGAGCT{G}{C}A{T}GATG**	**BHQ-1**

Bases between {} are Locked Nucleic Acid (LNA) bases

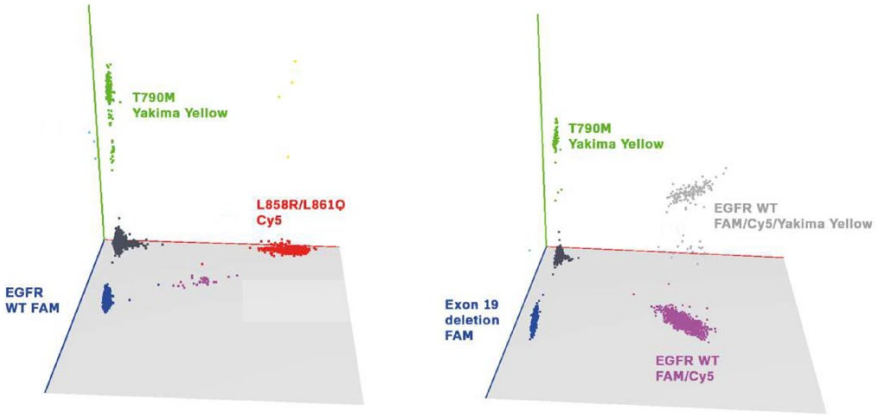

Figure 1: 3D dot plot generated by Crystal Miner™ software for mutant and WT *EGFR* positive controls using **A**: *EGFR* panel 1 and **B**: *EGFR* panel 2.

THREE-COLOR ASSAYS ROBUSTLY AND RELIABLY DETECT *EGFR* MUTATIONS

The targeted *EGFR* mutations were detected with a 95% confidence interval at final concentrations down to 1 and 0.4 copies per microliter (cp/µl), depending on the detected mutation. All assays were performed in a back ground of 400 copies per microliter of wild-type DNA, and thus the *EGFR* concentrations represent a mutant allele frequency of 0.25% and 0.1%, respectively (**Figure 2**).

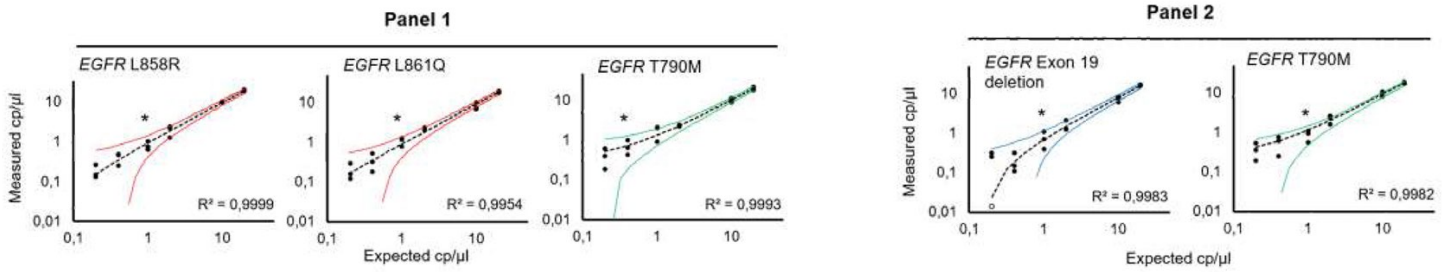

Figure 2: Evaluation of multiplex Crystal Digital PCR™ performance for the *EGFR* multiplex assays. Titration (20 - 0.20 cp/µl) of *EGFR* L858R, *EGFR* L861Q, *EGFR* T790M, and *EGFR* exon 19 deletion, in a background of 400 cp/µl of WT *EGFR* (10,000 copies per 25µl reaction). The theoretical 95% confidence intervals are represented as colored curves. *Indicates the theoretical limit of detection at a 95% confidence level interval, derived from the limit of blank measured on 30 replicates containing 10,000 copies of wild-type DNA per 25µl reaction. To learn more about how to determine a limit of blank and a limit of detection, please visit our learning center at stillatechnologies.com/digital-pcr

EGFR MUTATION DETECTION IN NON-SMALL CELL LUNG CANCER PATIENT SAMPLES

The two *EGFR* 3-color digital PCR panels were evaluated on mutant DNA extracted from non-small cell lung cancer patients and compared to next generation sequencing (NGS) measurements. The measured mutant allelic *EGFR* fractions displayed a strong correlation (**Figure 3**).

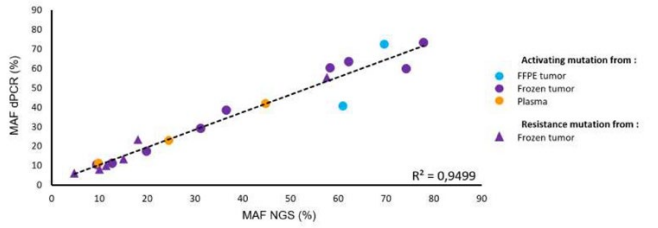

Figure 3: Mutant allele fraction (MAF) of activating (●) and resistance (▲) *EGFR* mutations in DNA extracted from frozen (green) and FFPE (blue) tumor and plasma (red) measured by digital PCR using *EGFR* panels 1 and 2 and by NGS. Compared samples displayed a good correlation with a significant Pearson coefficient (R = 0.9746; P < 0.05).

REFERENCES

1 Nan X, Xie C, Yu X, Liu J. *EGFR* TKI as first-line treatment for patients with advanced *EGFR* mutation-positive non-small-cell lung cancer. Oncotarget. 2017 Aug 9;8(43):75712-75726

2 Yu HA, Arcila ME, Rekhtman N, Sima CS, Zakowski MF, Pao W, Kris MG, Miller VA,Ladanyi M, Riely GJ. Analysis of tumor speci mens at the time of acquired resistance to *EGFR*-TKI therapy in 155 patients with *EGFR*-mutant lung cancers. Clin Cancer Res. 2013 Apr 15;19(8):2240-7.

APPLICATION NOTE HIGHLIGHTS

- The naica® 3-color Crystal Digital PCR™ system robustly and reliably quantifies *EGFR* resistant and activating mutations.
- *EGFR* mutations were detected in a background of wild-type DNA with a 95% confidence level at 0.25% to 0.1% mutant allele frequencies depending on the detected *EGFR* mutation.
- An excellent correlation was observed between the expected and measured *EGFR* target concentrations in serial dilution experiments.
- Mutant allelic *EGFR* fractions measured by 3-color digital PCR and Next Gene ration Sequencing in tumor and plasma from non-small cell lung cancer patients displayed a strong correlation with a significant Pearson coefficient R of 0.97 (P < 0.05).

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