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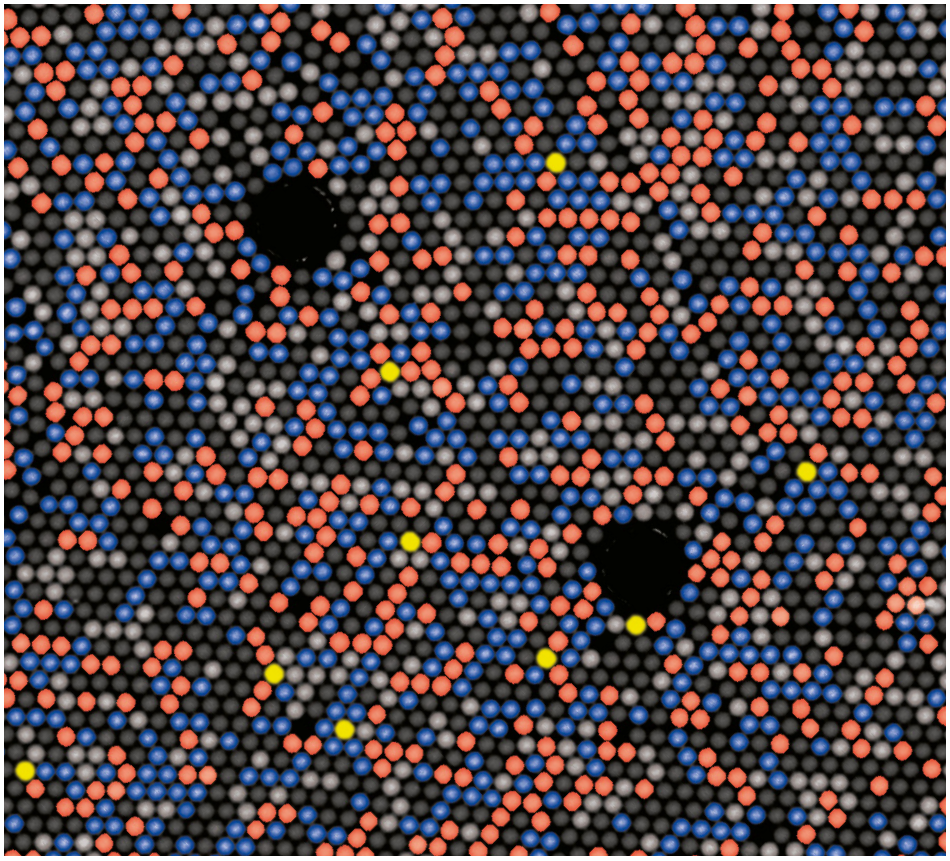
Multiplex Crystal Digital™ PCR

Quantifying *EGFR* mutations

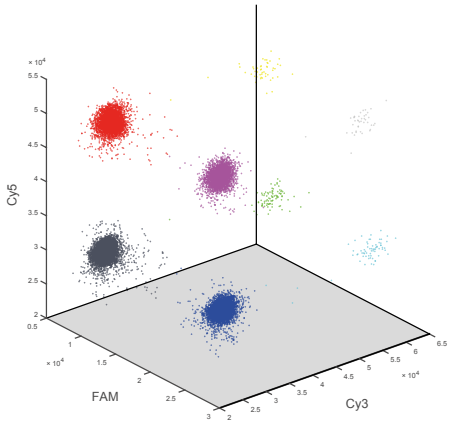


Multiplex Crystal Digital PCR

When confronted with limited sample availability, as is often the case for biological samples, it is critical for researchers and clinicians to obtain as much information as possible from a single assay.

In this context, Crystal Digital PCR is the ideal approach to detect and quantify multiple biomarkers in a single test without sacrificing the precision and reliability of the results.

The Naica System™ for Crystal Digital PCR unambiguously distinguishes between targets through the use of 3 different fluorescence channels. This fast and easy-to-use solution is compatible with various combinations of fluorophores, greatly expanding possibilities for assay design.

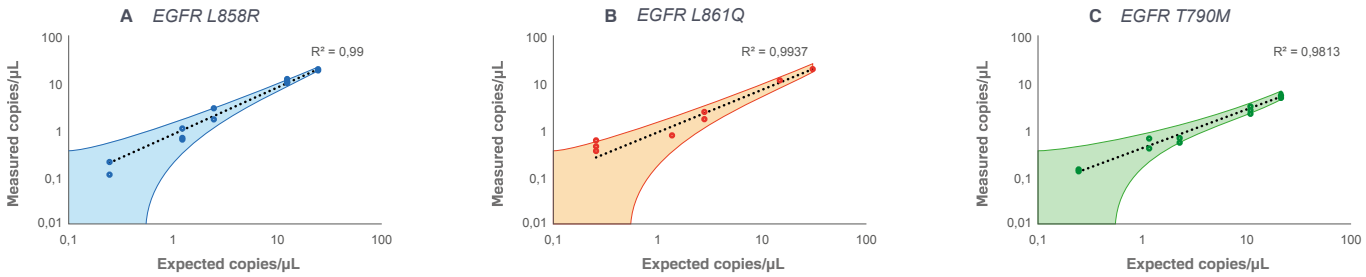


Three-dimensional representation of droplet fluorescence intensities from a triplex Crystal Digital PCR experiment using the Naica System.

In Focus: Detecting activating and resistance *EGFR* mutations in a single assay

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

To monitor patient response to TKIs treatment with a simple assay, a multiplex digital PCR assay targeting *EGFR L858R*, *L861Q* and *T790M*, as well as wild-type *EGFR* was developed. These mutations were successfully and reliably detected in a background of wild-type DNA at concentrations down to 0.25 copies per microliter (final concentration), representing a mutant allele frequency of 0.05% (Figure A, B and C).



Evaluation of multiplex Crystal Digital PCR performance for the *EGFR* quadruplex assay. Titration (25-0.25 cp/µl) of *EGFR L858R* (A), *EGFR L861Q* (B) and *EGFR T790M* (C), in a background of 500 cp/µl of WT *EGFR*. The theoretical 95% confidence intervals are represented as shaded areas.

Contact

STILLA Technologies

Villejuif Biopark
1 mail du professeur Georges Mathé
94 800 Villejuif // France

info@stilla.fr
www.stilla.fr

+33 9 82 29 50 50

STILLA