Liquid Biopsy Study Highlights Stilla's Tricolor Droplet PCR System as Firm Preps New 6-Plex Version

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Premium

NEW YORK (GenomeWeb) – French Firm Stilla Technologies, which last year launched a novel droplet-based digital PCR system with a singular three-color multiplexing capability, is now supplying its systems worldwide the company said this week.

According to the firm's President and Founder Rémi Dangla, Stilla is seeing two main types of customers for its Crystal Digital PCR: newcomers, who are new to digital PCR but are familiar with quantitative approaches and appreciate that Stilla's platform offers similar usability and time to results as other systems; and digital PCR experts, who have other systems in place already but have decided they need more multiplexing capability.

Applications of the technology vary, Dangla said, but one particularly exciting area for users has been in liquid biopsy, as demonstrated in a study published late last month in *PLoS One* by the company and a group of academic collaborators at France's Institut Gustave Roussy.

PCR approaches, especially digital methods, are becoming widely popular for detection of circulating cell-free DNA as a way of detecting or monitoring cancer without the need for a tissue biopsy.

For example, in lung cancer, clinicians have begun to embrace a variety of PCR technologies for detection of EGFR-sensitizing mutations and resistance mutations because of the potential benefit to patients of treatment with first-, second-, and third-generation EGFR tyrosine kinase inhibitors.

The Stilla and Gustave Roussy authors reported in *PLoS One* that recent recommendations in France have proposed that blood-based EGFR testing in NSCLC be considered a recommended alternative in patients who can't have tissue analysis and for assessment of resistance mutations in patients who have progressed on EGFR-TKI therapies.

In the US, Roche's Cobas PCR-based assay for EGFR mutations became the first liquid biopsy test approved by the US Food and Drug Administration last year.

But some groups have eschewed this FDA-approved platform for newer technologies like Bio-Rad's droplet-digital PCR, the Johns Hopkins-developed BEAMing technology, or targeted next-gen sequencing.

In their study, the Gustave Roussy investigators put Stilla's system (called Crystal Digital PCR because droplets self-arrange into a hexagonal, crystal-like pattern) up against targeted sequencing using Thermo Fisher Scientific's Ion Torrent platform, testing samples from 60 advanced non-small cell lung cancer patients for EGFR alterations.

Stilla's Crystal Digital PCR approach relies on a consumable called a Sapphire Chip, which is run on a droplet partitioning and amplification system called Naica. The Naica platform generates 2D arrays of monodispersed droplets, which are then thermocycled, and finally imaged using a fluorescent microscope in three distinct fluorescent channels.

The three colors allow users to simultaneously assay three different targets without some of the downsides that multiplexing poses to other PCR strategies, Dangla explained, including competition between different target reactions, and issues with varying reaction efficiencies.

For their application, the Gustave Roussy team designed multiplex assays for the parallel detection and quantification of a handful of EGFR-sensitizing and EGFR TKI resistance mutations, as well as the wild-type sequence.

Comparing the Naica readout with sequencing results, the researchers calculated that the three-color PCR method had about a 78 percent correlation with sequencing, though the discordance seemed to favor PCR over sequencing in terms of sensitivity. Seven samples with sensitizing mutations and four with resistance mutations were detected with Crystal Digital PCR, but not with sequencing.

Also, when the group monitored both types of mutations over time in a subgroup of six patients, they saw a clear correlation between circulating mutation levels and patients' clinical disease status.

Dangla called the publications a "really good proof of concept to show that this three-color capability is of value for clinical applications."

Like all PCR approaches, the Crystal Digital method is best suited to detection of known mutations, in contrast to the open-ended analysis offered by sequencing, the study authors wrote.

In the case of lung cancer, the Gustave Roussy authors wrote that it would be potentially be able to assay a few additional biomarkers in combination with EGFR mutation, for example, the HER2 and MET amplifications that can also contribute to acquired resistance to TKIs.

Fittingly, Dangla said this week that Stilla has turned its own R&D focus to increasing the multiplex capability of its technology — planning to increase the color channels incorporated from three to six.

"When you look at multiplexing challenges in digital PCR, it's not instrumentation that is the problem," Dangla said. "It's the molecular biology."

"You have to worry about competition of different reactions and about reaction efficiencies differing across analytes," he explained. "But what we have found with our three-color system in droplets is that you don't need to worry about efficiency … and competition is also not a problem because the majority of droplets won't have more than one reaction going on inside."

The major challenge for Stilla is in finding a way to make a six-dimensional color readout understandable and usable.

The company is currently building a prototype, which it is planning to make available for proof-of-concept studies with any interested groups by the first or second quarter of next year.

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