Customer spotlight

Epigenetics Opens the Door to Faster Cancer Detection

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Liquid biopsy tools based on studying DNA methylation patterns could revolutionize cancer detection and monitoring, but to reach the clinic they will need to make the best use of limited samples.

With liquid biopsies, detecting cancer and tracking treatment progress can be as easy as taking a blood test. This is an increasingly popular way of monitoring cancer, because it's much less invasive than solid tumour biopsies. And liquid biopsies can become even more sensitive if they capture methylation information as well as genetic data.

Usually, liquid biopsies for cancer rely on the detection of small amounts of DNA that are shed from a tumour into the bloodstream. But especially in the disease's early stages, circulating tumour DNA (ctDNA) levels are very low and point mutations linked to cancer can be easy to miss.

"If we want to develop assays to detect cancer earlier, we need very sensitive detection of these rare tumour fragments," says Charlotte Proudhon, PhD, group leader at the Research Institute for Environmental and Occupational Health in Rennes, France, whose team are among those now developing liquid biopsy methods that include epigenetic markers, such as methylation.

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Detecting cancer from methylation patterns

"Alterations in DNA methylation are a hallmark of cancer," says Proudhon. And unlike single point mutations in ctDNA, methylation sites are abundant throughout the genome, so they're easier to spot in a plasma sample. Proudhon's lab is now developing a method that can detect multiple forms of cancer from methylation patterns found in a single liquid biopsy sample.[1]

Methylation markers can also show how cancers progress or respond to treatment. Professor Gerhardt Attard, MD, PhD, FRCP, is a clinician-scientist and team leader at University College London Cancer Institute, where he studies prostate cancer's progression. Liquid biopsies are a key tool in monitoring this cancer, because the samples are much easier to collect than invasive tumour biopsies.

"Prostate cancer is mostly a disease of copy number change," says Attard. That means that following disease progression from liquid biopsy samples is not as straightforward as tracking point mutations in ctDNA. "Over the years, we developed a number of assays for measuring copy number change and are now also looking at methylation changes."[2]

Attard's methylation assays currently require him to split the sample in two: one half is used for DNA sequencing and the other for methylation analysis. This puts even more pressure on an already scarce resource. If there was a way to avoid this split, he would be able to get more information from precious liquid biopsy samples.

Getting more from liquid biopsies

Attard gets liquid biopsy samples for his research from prostate cancer patients in his clinic. "They opted in to donate an extra sample for research when their routine blood is taken," says Attard. "And they rarely miss a donation."

Even with these generous blood sample donations from his patients, there isn't a lot of material to work with. A standard sample includes only a few milliliters of plasma, which contains about 10 to 20 ng of DNA. From this minuscule amount, Attard needs to gather both genetic and epigenetic information. To make matters worse, the standard protocols for these analyses are not compatible.

"Current targeted enrichment ctDNA assays require the DNA to be amplified to efficiently complete the target enrichment workflow, erasing methylation markers in the process," explains Bram Herman, PhD, director of emerging technologies at life sciences and diagnostics company Agilent Technologies. "To overcome this, DNA is converted at the beginning of this process, through bisulfite conversion. This changes the base composition significantly, which can create bias in target enrichment efficiency, especially for partially methylated fragments. Converted DNA fragments are also not suitable for DNA variant analysis."

Attard has been splitting his samples to safely gather both genetic and methylation data, but he is looking for alternatives. Attard recently tried the Agilent Avida Duo target enrichment system. The technology creates a pre-capture library without using PCR, leaving the DNA with its methyl groups intact. "That gives us the opportunity to obtain both bits of information from the same DNA," he says. Because this effectively doubles the size of the sample, it increases the sensitivity of the assay.

"The technology optimizes sample recovery, which enables DNA and methylation analysis without sample splitting, maximizing sample insights without compromise," Herman says. "It's taking complexity out of the workflow."

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– **Bram Herman,**
Director, Emerging Technologies Team,
Agilent Technologies

From lab to clinic

Proudhon has another challenge to solve. Her group has been able to detect multiple cancers at once from plasma samples by studying methylation patterns at retrotransposons. However, she says, "the protocol we use in the lab is difficult to adapt to a clinic." Their go-to method has been amplicon targeting with bisulfite treatment. But although this worked well at a small scale, it's too slow and involved to scale up.

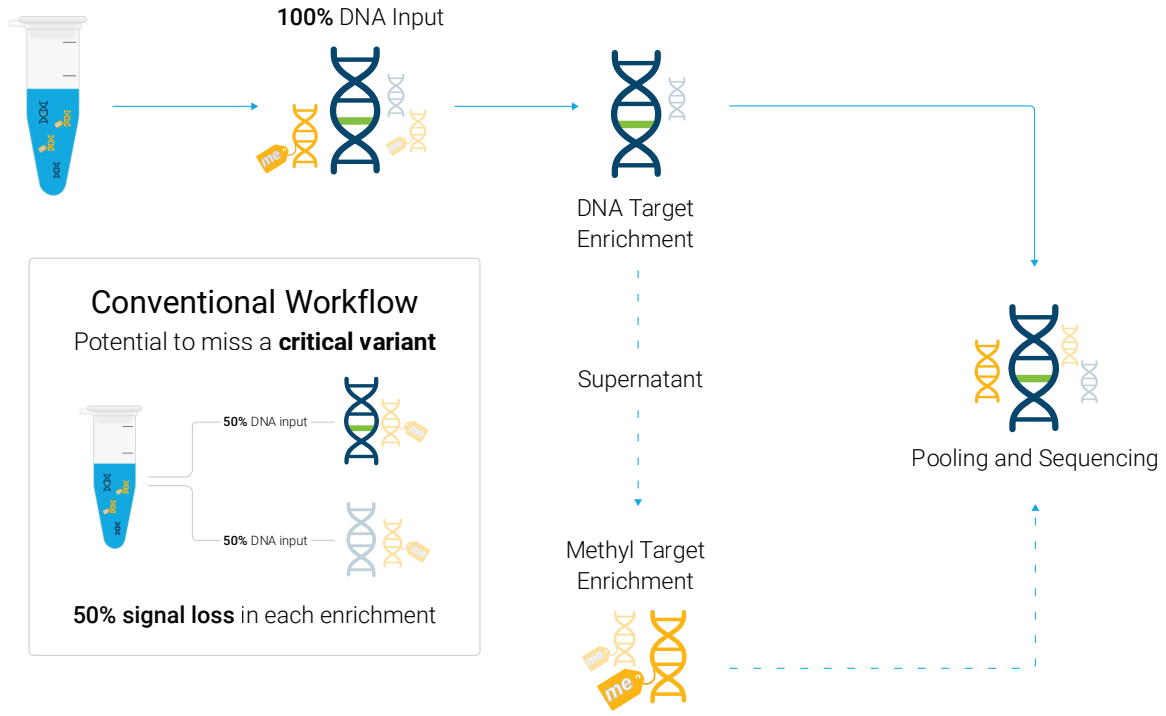
Proudhon's group is now exploring Avida Duo to use in her research. "The main challenge in our study is that we are interested in repetitive elements, and usually when you do capture, you avoid capturing these regions that tend to generate off-target reads," she says. But if the reagents can be validated in her lab, Proudhon's team is well on the way to adapting the method as a potential clinical test. "It's fast and straightforward," she says. "It could be a great tool in the clinic."

Methylation-based assays such as the ones Proudhon and Attard are working on have the potential to change cancer diagnostics. New epigenetic biomarkers can be used in personalized medicine, for example, while more sensitive assays could detect cancers earlier. "Many studies have shown how tumour DNA from blood can be used and what it means," says Attard. "We now need much better assays to bring this into practice."

Using DNA methylation to monitor cancer from liquid biopsies also makes it possible to find the source of ctDNA. Since DNA methylation profiles are specific to cell identities, the methylation patterns on tumour DNA hint at the origin of the cancer. "If liquid biopsy reaches such a sensitivity that you can use it for screening, you would need to know where to look for the tumour," says Proudhon. "With a capture method profiling DNA methylation patterns, we could do that."

Designed to leave no fragment behind: **Avida Duo**

The Agilent Avida Duo workflow is purpose-built to capture both genetic variants and differentially methylated targets from one input of your precious sample.



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To learn more about how the Agilent Avida Duo target enrichment system helps to maximize the potential of every sample, scan the QR code.

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