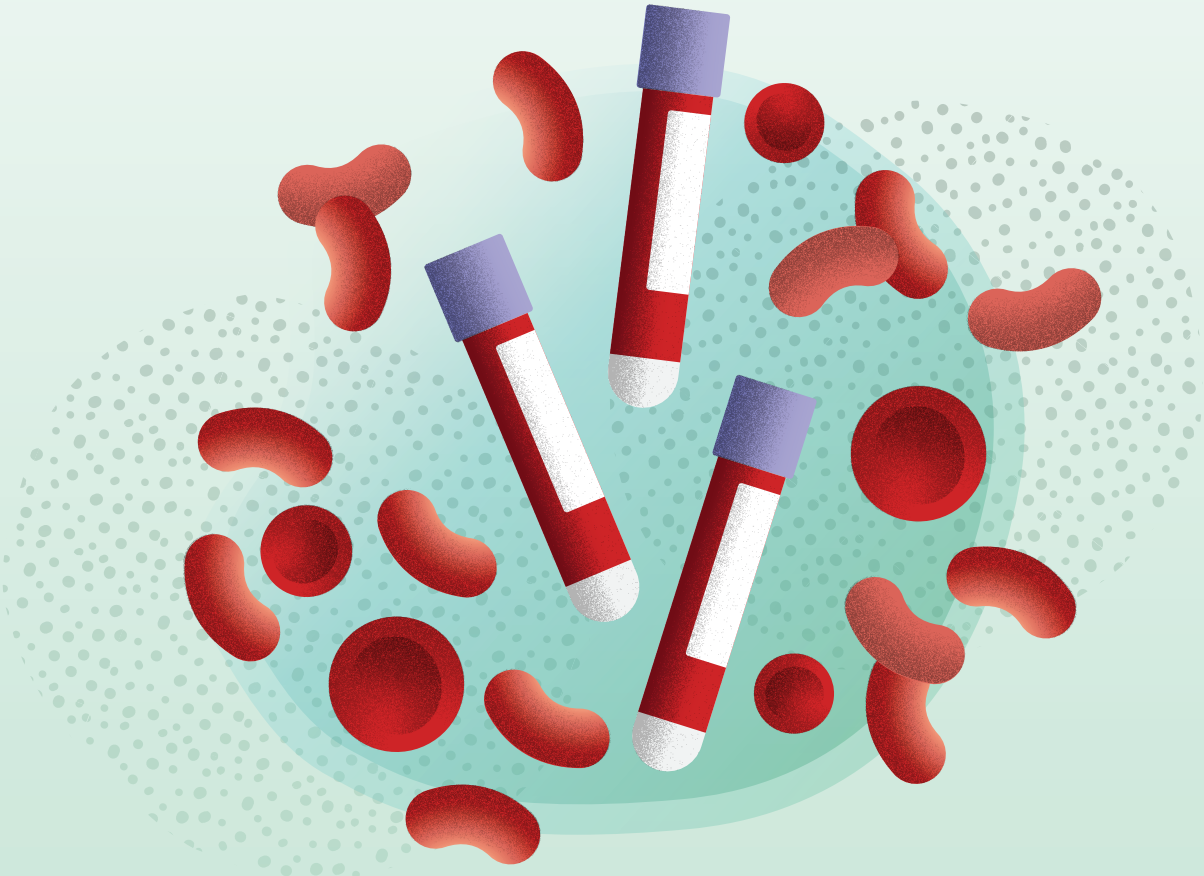
CASE STUDY

SEPTEMBER 2021

TWIST BIOSCIENCE

Detecting Cancer's Methylation Fingerprint in Blood Plasma

Since 2012, Universal Diagnostics has been working to revolutionize early cancer detection. To make their high-throughput, NGS-based assay a reality, they needed a reputable, trustworthy source of custom panels for hybrid capture. They found the source they were looking for — as well as an enthusiastic research partner — in Twist Bioscience.



Cancer Detection, Cancer Prevention

Universal Diagnostics (Universal DX), a biotech company with HQ based in Sevilla, Spain, was founded in 2012 with a single mission: to build a future where cancer is curable. Since then, the company has expanded to include offices in Madrid, Spain; Ljubljana, Slovenia; and Cambridge, Massachusetts, in the United States.

With recent advances in cancer detection and prevention, this mission is starting to feel within reach. One significant area of progress is early detection, which can go a long way toward eliminating cancer. Take colorectal cancer, for example. If detected at the pre-cancer stage (also known as advanced adenoma), the five-year survival rate is 100%. Breast, cervical, ovarian, and prostate cancers are other examples where early detection is critical for survival.

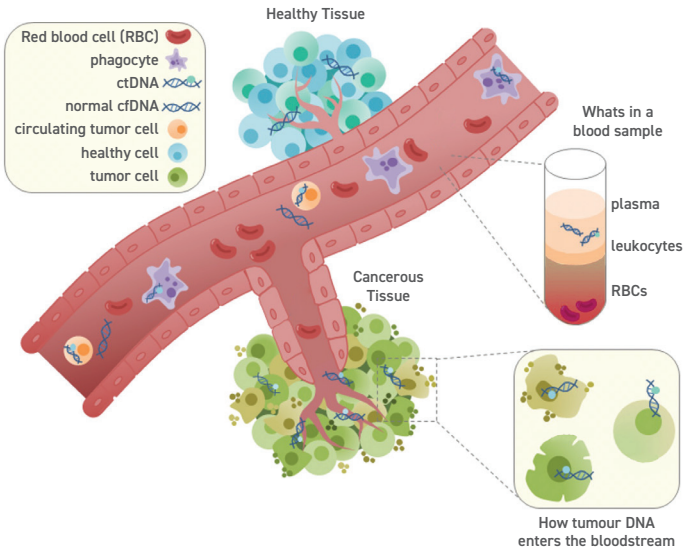
Most early detection methods, such as mammograms and colonoscopies, are unpleasant and, in some cases, invasive. Compliance rates for early screening tests are only about 50–60%, contributing to cancer going undetected until it's too late. Alternative, minimally invasive methods are needed to improve patient compliance and improve early detection rates. Universal DX is working to fulfill that need through liquid biopsy, where cancer can be detected from a single blood sample.

"Taking a blood sample is simply part of a routine doctor visit," says Kristi Kruusmaa, Head of Research at Universal DX. "We have really concentrated on this because we can see how much non-invasive procedures could change early-stage cancer detection. About 85% of colorectal cancers develop from pre-cancerous lesions like advanced adenomas, so if you can detect those with an accessible screening method, you can prevent the cancer. That's the key: early detection is not only early-stage cancer detection, it's prevention."

DNA Methylation: The Key to Detecting Cancer's Fingerprint in Blood

Initial research efforts at Universal DX focused on metabolites and proteins, says Kruusmaa. But eventually, the company pivoted to DNA methylation patterns. This is because these patterns are tissue-specific, highly sensitive, and more definitively differentiate between healthy patients and patients with cancer or even pre-cancer. Additionally, DNA methylation patterns are more disease- and tissue-specific than SNPs/mutations, with more abundant signals occurring with higher "allele frequency," making them an exceptionally good choice for early cancer detection.

Focusing on colorectal cancer, Universal DX characterized the full spectrum of DNA methylation patterns in pathological tissues from colorectal cancer and advanced adenoma patients. They then verified that they could detect cancer from tumor DNA circulating in the bloodstream by searching for those same DNA methylation patterns with Next Generation Sequencing.



NOT ALL CELL-FREE DNA COMES FROM TUMORS	
CELL-FREE DNA (cfDNA)	**CIRCULATING TUMOR DNA** (ctDNA)
Freely circulates in the bloodstream	Freely circulates in the bloodstream
Released by healthy cells, tumor cells, fetal cells, and transplanted cells	Released by tumor cells
Usually at a concentration of ~10 ng in a standard blood sample containing 4 ml blood plasma	Concentration in plasma varies by patient and cancer type, but less than 1% of total cfDNA
Can be easily isolated from plasma via commercially available kits	Low concentration and high fragmentation of ctDNA means highly sensitive and specific detection methods are necessary

Cancer tests are measured by two key metrics: sensitivity and specificity, denoting the proportion of tests that yield true positives or negatives respectively. Universal DX's assay, which is currently undergoing a 3,000-patient pre-FDA preclinical trial, boasts 55% sensitivity for pre-cancerous advanced adenoma, compared to the gold standard fecal-based test currently in use, which has only 42% sensitivity. The assay is also showing excellent results for colorectal cancer, with 92% sensitivity and 97% specificity compared to 92% sensitivity and 87% specificity with the fecal-based test[1].

Kruusmaa says these levels of sensitivity and specificity, which are crucial for early detection, are possible because of the custom hybrid capture probes from Twist Bioscience. The probes permit the complexity needed to quickly analyze thousands of different regions for their DNA methylation patterns.

"Cancer is a complex disease, and you cannot capture it with only one or two regions, says Kruusmaa. "For example, we see sub-categorization in patients depending on the cell of origin, leading to different profiles in the cancer itself."

[1] *J M Kinross et al. Accurate early-stage colorectal cancer detection through analysis of cell-free circulating tumor DNA (ctDNA) methylation patterns. Journal of Clinical Oncology 39, no. 15_suppl (May 20, 2021) 3606-3606. DOI: 10.1200/JCO.2021.39.15_suppl.3606*

How Methyl-seq Works

In experimental planning, researchers decide whether they will sequence the full genome and analyze only the regions of interest, or if they will extract just the parts of the genome they are interested in by using a target capture workflow.

In experimental planning, if researchers are using target capture, they also decide whether methylation conversion will be performed pre- or post-capture. Pre-capture conversion is typically the method of choice for low DNA-input, sensitive applications.

During the preparation of the methyl-seq library, a chemical or enzymatic method is used to convert unmethylated cytosines to uracil.

During the amplification step of library preparation, uracil is paired with adenine on the complementary strand, ultimately swapping unmethylated cytosines with thymines.

After sequencing, when analyzing the results, the only cytosines that show up should be those that were methylated.

Universal Dx's Workflow

1. **Blood Collection:** A patient aged 45 years or more is recommended for colorectal cancer screening. Instead of undergoing a colonoscopy, the patient has a single tube of blood (~9 mL) drawn at their doctor's office. Only one tube of blood (~9 mL) is needed. The blood is sent to Universal DX.
2. **DNA Extraction and Conversion:** The patient's blood is centrifuged to isolate and obtain the plasma. Cell-free DNA (cfDNA) is extracted from the plasma, which then undergoes chemical conversion to reveal methylated areas of DNA. As low as 9 ng of cfDNA are required for the assay to work, and according to Kruusmaa Universal DX is working to push to sensitivities as low as 5 ng.
3. **Library Preparation:** Extracted and converted DNA is prepared for sequencing. Illumina sequencing adapters are ligated to the DNA. A unique barcode is also added to allow multiple samples to be run simultaneously on a single sequencing flowcell.
4. **Enrich for Regions of Interest:** Converted and indexed DNA is then captured by hybridization with a custom panel of target enrichment probes. Universal DX collaborated with Twist to design the custom panel for accurate capture of their cancer-specific methylation patterns. This step increases sensitivity and specificity because it captures the DNA regions of interest and gets rid of the background noise. [reference box, see below]
5. **Sequencing and Quality Control:** Once the converted DNA is captured by Twist's custom probes, it is sequenced. Prior to analysis, the sequencing output must meet a certain level of quality, which ensures accurate classification and reduces the chance of false positives.
6. **Detect Cancer:** High-quality sequences are analyzed using Universal DX's proprietary bioinformatics pipeline. Each individual sequence is scored based on how well it matches cancer-associated methylation patterns determined from tissue. All reads and scores are analyzed statistically to produce the final cancer prediction. The clinician receives these results and shares them with their patient and recommends further steps.

Twist's technical support team has been so responsive in discussing our probe panel, and they helped us with its design. —KRISTI KRUUSMAA

Detecting Cancer Isn't Easy

One of the earliest challenges Universal DX faced when developing their liquid biopsy assay was meeting the required levels of sensitivity and specificity.

"The amount of circulating tumor DNA (ctDNA) compared to total DNA in the sample is really low, so you need to be as sensitive and as specific as possible," explains Kruusmaa. "You need to be able to identify patterns using just fragments of DNA, and it could be one read in a million that ends up being cancer-derived."

In the beginning, the team used a qPCR-based approach because amplicon-based NGS methods of that time did not allow for highly multiplexed targeted methylation detection from cfDNA. But it didn't take long for the team to realize that they *had* to use an NGS approach to move forward, as PCR simply isn't sensitive or high-throughput enough to detect the hundreds of different regions that comprise colorectal cancer's methylation signature. That's when the Universal DX R&D team partnered with Twist to develop their NGS liquid biopsy assay, centered around the hybrid capture of DNA fragments of interest.

"Twist's custom panel has enabled us to achieve the enrichment level and precision that we need," says Kruusmaa. "When we started with NGS for methylation detection, the maximum we could target was 30 regions, but now with our Custom Panel from Twist, we can capture thousands with better coverage."

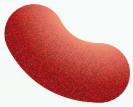
Twist has continued working with Universal DX to address other challenges. Kruusmaa says the traditional use of bisulfite conversion in methyl-seq is problematic because it is very harsh to cfDNA. Universal DX is currently testing whether a less damaging enzymatic conversion method offered through the Twist Methylation Detection System further improves their workflow.

"Twist's technical support team has been so responsive in discussing our probe panel, and they helped us with its design. It has never been off-the-shelf, take-it-or-leave-it with Twist. As they have been developing their methyl-seq product line further, we have been sharing information to get to the best result," says Kruusmaa "That's what I really like about working with Twist."

Together, Universal DX and Twist have made significant strides in transforming early cancer detection and prevention through liquid biopsy. Although Universal DX's initial focus has been on colorectal cancer, Kruusmaa says their knowledge can and will be expanded and applied to other cancers in the future.

Kruusmaa, looking toward a bright future, sums up their work:

"Our results show how promising liquid biopsy is and show that there is hope for early detection and cancer prevention." ■



WHAT CAN TWIST DO FOR YOU?

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#WeMakeDNA