# Twist Methylation Detection System and Custom Capture Panels Aid Research on Early Cancer Detection

As a rule of thumb, the sooner a cancer is detected, the easier it is to treat. Therefore, developing tools for early cancer detection can help save lives. In this effort, the capture and sequencing of cell-free DNA (cfDNA) has emerged as a promising source of diagnostic information. Researchers are using custom target enrichment panels to extract and analyze DNA fragments shed from cancerous cells and captured in blood samples—enabling the use of liquid biopsies for less invasive and more sensitive screening. Additionally, advances in methylation sequencing workflows are improving cfDNA analysis by exposing epigenetic clues about the cancer's subtype and tissue of origin. Together, custom target enrichment panels and methylation sequencing give researchers tools to develop more sensitive and specific early cancer detection assays.

TWIST METHYLATION DETECTION SYSTEM

Methylation is a repressive epigenetic mark on DNA that plays an important role in defining cell identity. Importantly, during transformation, cells undergo characteristic changes to their methylome. Methylation sequencing can thus be used to both identify malignant patterns and trace cfDNA to its tissue of origin.

Twist Bioscience has developed a new, highly sensitive method for detecting methylation patterns in cfDNA that uses enzymes—rather than bisulfite chemistry—to convert methylated cytosine to uracil. Relative to traditional methylation sequencing workflows, Twist's methylation detection system reduces damage to the DNA and enables up to 15% more methylation detection within promoter CpG islands. To further increase the efficiency of methylation sequencing, Twist also provides custom target enrichment panels.
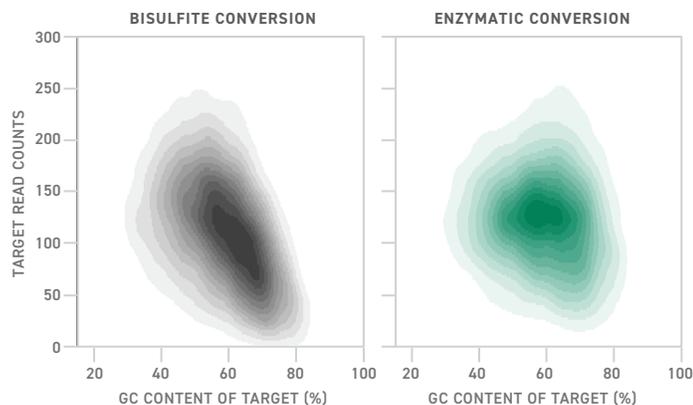
Sequencing coverage of target regions using bisulfite- and enzyme-converted libraries. Enzymatic conversion yields more even GC coverage than bisulfite conversion.

IMPROVING SENSITIVITY AND SPECIFICITY

In a blinded, multicenter clinical trial, Lin et al.,[2] evaluated methylation sequencing of cfDNA as a component in early detection of hepatocellular carcinoma (HCC). The researchers used a custom target enrichment panel (HelioLiver) synthesized by Twist Bioscience to enrich for HCC cfDNA fragments in liquid biopsies collected from 247 patients (122 with HCC). Enzymatic methylation sequencing was then used to identify cfDNA methylation patterns. This data was combined with demographic data and results from a conventional immunoassay (targeting HCC proteins) to determine if HCC was present.

Results show that the researchers' use of target enrichment and methylation sequencing in addition to conventional biomarker analyses improved HCC detection sensitivity. Specifically, they found sensitivity for stages I and II HCC to be 75.7%, well above the 64.9% sensitivity of the current gold standard diagnostic method (ultrasound using multiphasic MRI, GALAD). This is just one example of how Twist's custom target enrichment panels and methylation sequencing workflow can support the research and development of better diagnostic tools for early cancer detection.

Results from Lin et al.,[2] comparing early cancer detection tools. The HelioLiver test included a Twist custom target enrichment panel and methylation sequencing which led to more sensitive HCC detection at stages I and II. For comparison, the sensitivity of established HCC surveillance blood tests is shown. AFP = alpha fetal protein. GALAD = score that incorporates Demographic and AFP measures

CUSTOM TARGET CAPTURE

Rather than sequencing every bit of genetic material in a sample, custom target capture panels help researchers focus sequencing resources on just the targets of interest by hybridizing sample DNA with a pool of biotinylated oligonucleotide probes. After pulldown, the captured targets can then be sequenced.

Twist Bioscience synthesizes highly uniform, precise, and customizable capture panels that can be leveraged for early cancer detection research. According to Kristi Kruusmaa, Head of Research at Universal DX, "Twist's custom panel has enabled us to achieve the enrichment level and precision that we need. 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."[1]

When combined with methylation sequencing, custom panel design can be challenging. The reduced complexity of DNA fragments produced in methylation sequencing workflows can make it hard to predict which probes you need in your panel. In addition to panel synthesis, Twist has a team of panel design experts who are available to help ensure your panel's design is optimized to fit your needs.

Twist Methylation Detection system detects hypo- and hypermethylated DNA with high sensitivity. Shown here are results from an experiment using the Twist Methylation Detection system and libraries of varying methylation levels (0–100% methylation) in defined ratios.

REFERENCES

1. "Detecting Cancer's Methylation Fingerprint in Blood Plasma | Twist Bioscience." Www.twistbioscience.com, www.twistbioscience.com/resources/case-study/detecting-cancers-methylation-fingerprint-blood-plasma.

2. Lin, Nan, et al. "A Multi-Analyte Cell-Free DNA-Based Blood Test for Early Detection of Hepatocellular Carcinoma." Hepatology Communications, 3 Mar. 2022, 10.1002/hep4.1918. Accessed 5 Apr. 2022.