METHYLATION SEQUENCING IN CANCER DETECTION

Custom Publishing From

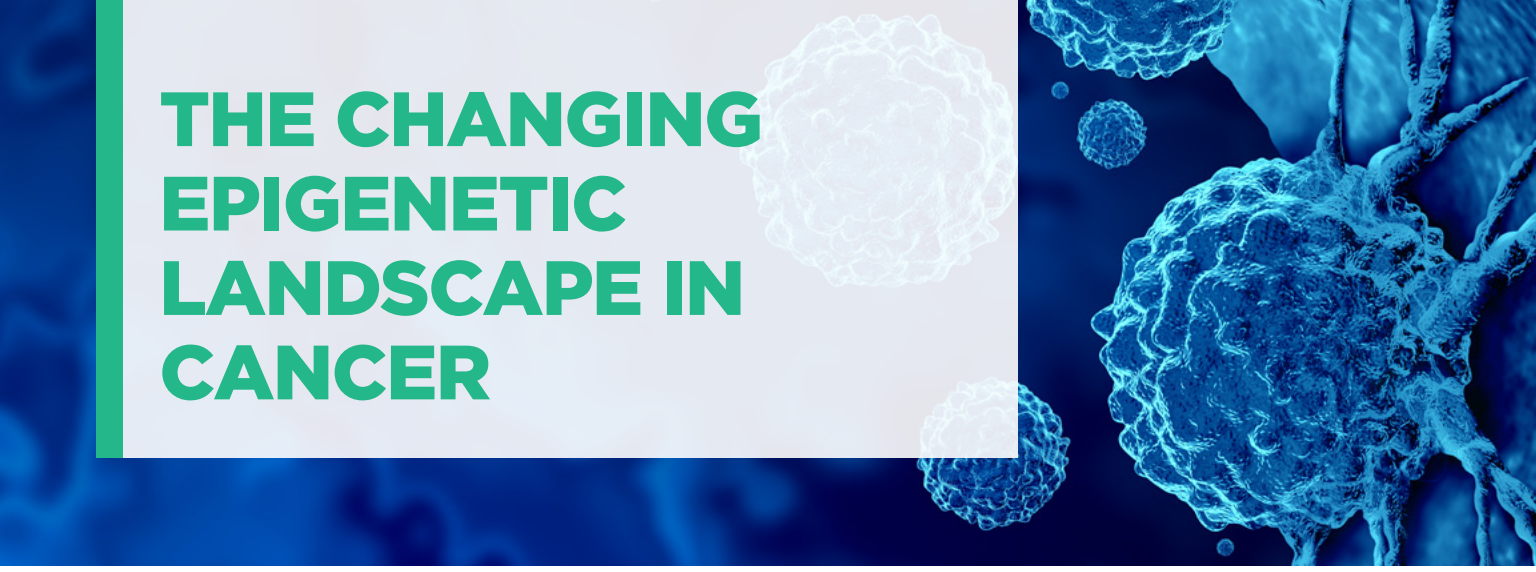
TheScientist
EXPLORING LIFE, INSPIRING INNOVATION

Sponsored By

TWIST BIOSCIENCE


THE GOOD FIGHT DESERVES GREAT TOOLS
As you aggressively pursue innovative solutions toward the personal treatment of cancer, count on Twist for:
Efficient elucidation of cancer genetics
High-resolution mapping of cancer's epigenetic hallmarks
Cost-effective mutant and oncogene validation
Rapid biologic therapeutic innovation and development
Learn more at twistbioscience.com/stronger-together
TWIST BIOSCIENCE

THE CHANGING EPIGENETIC LANDSCAPE IN CANCER



Alterations in DNA methylation patterns are among the earliest and most widespread genome changes seen in cancer.[1] Every type of cancer has its own signature pattern of methylation, known as its methylome, which can be used to identify the cancer type and the extent of disease progression.[2] Technological advances in methylation sequencing (methyl-seq) now allow researchers to quickly investigate methylation in regions of interest, making methylation patterns exciting potential biomarkers for cancer screening.

"Technological advances in methylation sequencing (methyl-seq) now allow researchers to quickly investigate methylation in regions of interest, making methylation patterns exciting potential biomarkers for cancer screening."

Consequences of Hypo- and Hypermethylation

DNA methylation status can shed light on disease pathology. For example, broad hypomethylation of DNA associates with transcriptional activity. In tumors, hypomethylation destabilizes the genome by de-repressing repetitive sequences and increasing the likelihood of detrimental mitotic recombination.[1,2] A particularly fascinating instance of the effects of hypomethylation in carcinogenesis is that of viral genome reactivation. Cells repress expression from integrated human papillomavirus genomes via methylation, keeping the viral genome in a latent state. The process of demethylation that occurs in cancer can de-repress the HPV genome and trigger re-expression, potentially driving cervical cancer progression.[2]

DNA hypermethylation also appears in cancer,[3] typically at CpG islands—500-1000 base pair stretches of DNA with a high frequency of cytosine-guanine pairs. CpG islands occur mainly in the first exon and promoter region of human genes, and they tend to be unmethylated and thereby transcriptionally active in normal somatic cells.[2] In cancer, however, hypermethylation of key promoter regions promotes tumorigenesis by silencing a variety of genes, including those involved in tumor suppression and DNA repair. This phenomenon was first observed in retinoblastoma, a pediatric cancer associated with very few genetic mutations.[4] Retinoblastoma patients showed dramatic hypermethylation in the retinoblastoma tumor-suppressor gene (*RB1*) promoter.[5] Hypermethylation and repression of *BRCA1*, a gene important for double-strand break DNA repair, occurs in breast and ovarian cancers.[3]

Focus on miRNA

A great deal of research has also focused on the importance of cancer-associated DNA methylation changes in microRNAs (miRNAs). These 18-25 nucleotide non-coding RNAs inhibit gene translation by silencing or degrading messenger RNA molecules.[2,6] miRNA expression is broadly dysregulated in cancer, with an overall downregulation observed in the majority of tumors.[7] CpG hypermethylation occurs in some cases, including for genes with key roles in tumor suppression. Hypermethylation of the miRNA miR-124a in colon cancer de-represses its target, cell division protein kinase 6 (*CDK6*), an oncogene that phosphorylates and inactivates the tumor suppressor Retinoblastoma (RB) protein.[2] Another instance of this type of miRNA silencing is found in bladder cancer, where the hypermethylation of miRNA-127 drives dysregulation of B-cell lymphoma 6 (*BCL-6*), an oncogenic factor.[2]

Researchers are only now beginning to understand the importance of dynamic DNA methylation in cancer. Next-generation technologies that enable doctors and scientists to quickly and accurately assess epigenetic modulations in individual patients have the potential to drive advances for earlier diagnosis, new therapies, and personalized treatments.

METHYLATION SEQUENCING: CONVERSION AND CAPTURE

Early in cancer development, DNA methylation changes occur. These altered methylation patterns show up in circulating cell-free DNA, making them attractive potential biomarkers for diagnosis and disease monitoring.[1,2] Liquid biopsies allow researchers and clinicians to analyze cells or nucleic acids from tumors by sampling blood or other bodily fluids. This noninvasive approach reveals altered methylation patterns and can be repeated in the same patient, circumventing bias associated with a biopsy from a small area of a heterogenous tumor.[1] However, the amount of cell-free DNA in a typical plasma sample is small, so accurate analysis of these specimens presents technical challenges.

"New enzymatic conversion technologies solve many of the technical problems associated with traditional bisulfite sequencing."

The Benefits of Enzymatic Conversion

Researchers commonly perform DNA methylation detection using bisulfite sequencing. When they expose DNA to bisulfite sodium, unmethylated cytosines chemically convert to uracil, while methylated cytosines are protected from this chemical modification. After conversion, researchers amplify their samples using PCR and sequence the amplicons with next-generation sequencing technologies. This method has a number of advantages, including single base resolution and a relatively straightforward library preparation method.[3] Nevertheless, bisulfite treatment requires high temperatures and a harsh pH that can damage DNA by causing strand breaks and depyrimidination.[3] Unmethylated cytosines are especially vulnerable to degradation in this system, leading to lower coverage in GC-rich areas. This is problematic given that CpG island methylation is an area of special interest for cancer researchers.[2]

New enzymatic conversion technologies solve many of the technical problems associated with traditional bisulfite sequencing. These technologies swap out the bisulfite conversion step for enzymes that modify unmethylated cytosines. In one example of enzymatic conversion, a TET enzyme first oxidizes methylated cytosines (5-methylcytosine and 5-hydroxymethylcytosine) to protect them from deamination, which occurs in the following step when an APOBEC enzyme converts unmethylated, unprotected cytosines to uracils.[4] This two-step process yields a better quality, more accurate readout.[5] Enzymatic conversion causes less DNA damage than bisulfite sequencing because the reactions do not require a harsh temperature or pH, an advantage that is reflected in greater library yields after a lower number of cycles at the amplification step. Running fewer PCR cycles during amplification generates higher library complexity and fewer duplicates during sequencing.[5] Another important benefit of switching to enzymatic conversion is the evenness of genome coverage; the degradation of GC-rich areas observed with bisulfite conversion is eliminated, resulting in uniform coverage across all DNA regions, as well as detection of up to 15% more methylated cytosines.[6]

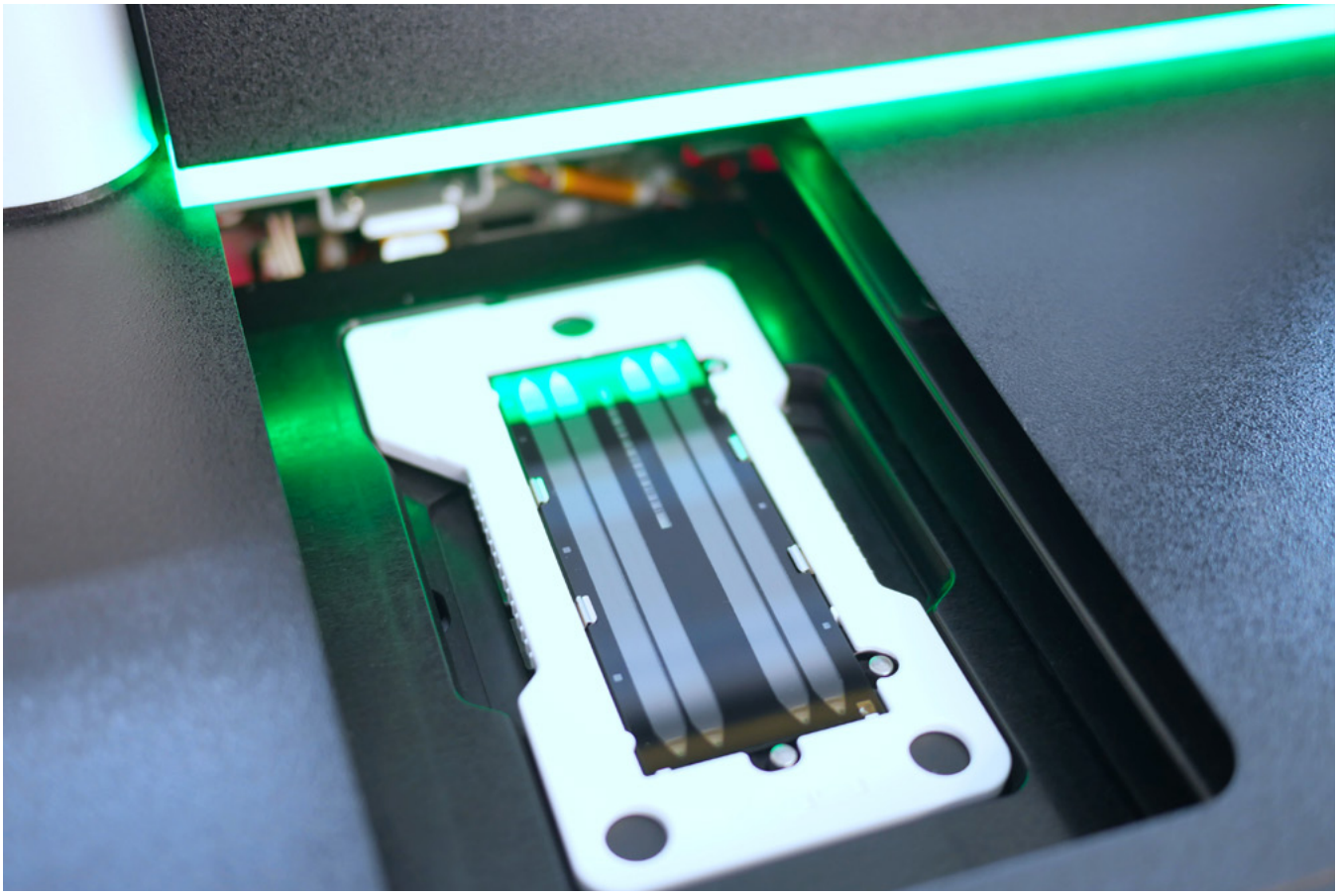
Getting Specific with Target Capture

Although scientists sometimes assess methylation patterns across the entire genome using whole-genome methyl-seq, it saves time and money to perform target enrichment. This strategy allows greater depth of sequencing for areas of interest by utilizing probes that hybridize with and isolate target sequences, removing unwanted sequences in the process.[7] With probe panels, researchers can enrich their samples for related sets of sequences such as oncogenes. In the methyl-seq workflow, target enrichment can be performed before conversion when sample DNA is plentiful. Alternatively, target enrichment after conversion is preferred for low-input, sensitive assays because the PCR step after conversion

"Kits with custom target capture libraries are built using the latest oligo synthesis technologies, which produce highly uniform panels that evenly capture sequences. These improvements lead to fewer wasted reads and improved read depth for rare variants."

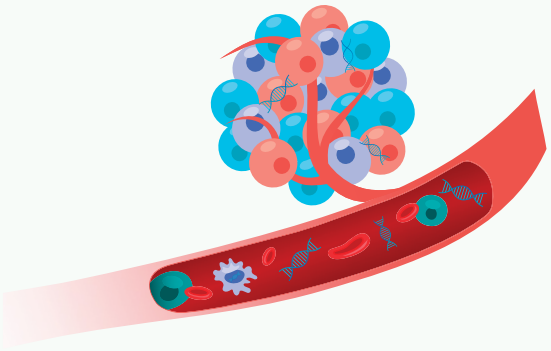
increases the yield of desired sequences. However, carrying out target enrichment after conversion presents additional challenges for panel design because the unique DNA sequences generated during conversion must be considered. Because unmethylated cytosines are sequenced as thymines after conversion and PCR amplification, some converted DNA sequences become less complex and therefore more difficult to capture, driving high off-target rates. Fortunately, new commercially available custom methylation panels have increased sensitivity due to an innovative design that enables them to capture all four potential types of DNA at a target site following conversion: methylated, unmethylated, sense, and antisense. Finally, researchers can use highly optimized, commercially available methyl-seq workflows to cut down on off-target capture and save time in the lab. The custom target capture panels within these wokflows are built using the latest oligo synthesis technologies, which produce highly uniform panels that evenly capture sequences. These improvements lead to fewer wasted reads and improved read depth for rare variants.

Taken together, these new technologies make it possible for scientists to quickly and accurately assess the unique methylomes of even trace amounts of cell-free DNA across multiple patient samples, representing a step forward for cancer researchers.



Understanding the Methylation Sequencing Workflow

Methylation is one of the most-studied epigenetic modifications. DNA methyltransferases add methyl groups to cytosine residues of cytosine-phosphoguanine (CpG) dinucleotides, which can silence transcription or promote genomic instability.



Sample Prep

Isolate and purify DNA

Library Prep

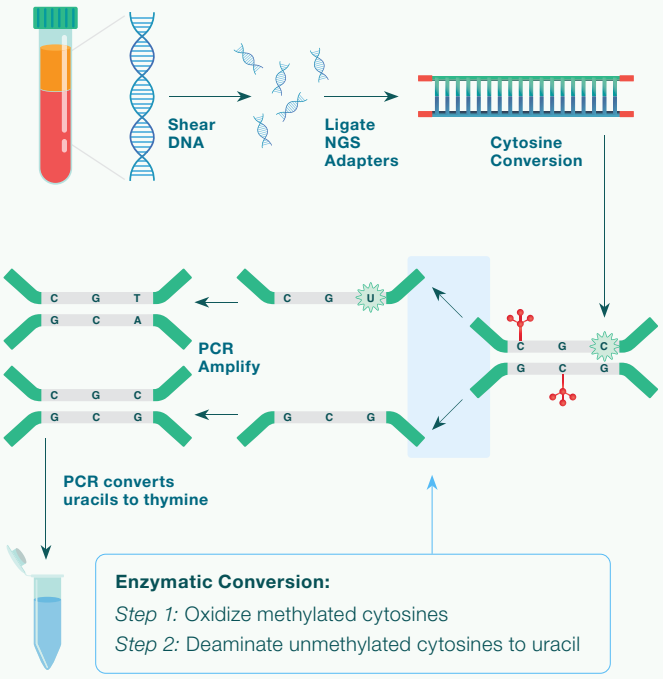
Perform conversion, target capture, and DNA amplification

Methyl-seq for Cancer Detection

Aberrant DNA methylation occurs very early during cancer progression; thus, methylated sequences are promising diagnostic biomarkers for early cancer screening.

Tumor cells release DNA fragments into the blood stream. Scientists collect liquid biopsies from patients and analyze the circulating tumor DNA (ctDNA) for unusual methylation patterns via methyl-seq experiments.

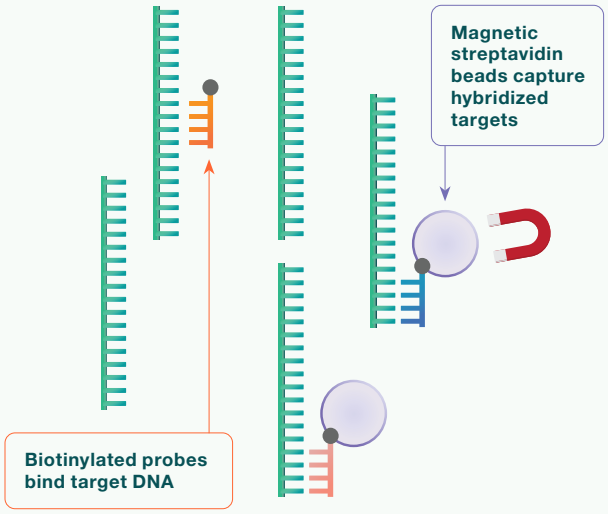
Scientists can use methyl-seq of ctDNA for cancer screening, to understand the prognosis of those with cancer, and to find methylation signatures common in various types of cancer to provide a better treatment plan.



Library Prep: Conversion

Bisulfite versus enzymatic conversion

- Both convert unmethylated cytosines to uracil.
- Bisulfite treatment degrades DNA, especially unmethylated cytosines, decreasing the sequencing sensitivity in low-concentration samples.
- Enzymatic conversion does not damage DNA and detects more CpG sequences than bisulfite.



Library Prep: Target Acquisition

- Researchers perform target capture before or after conversion to enrich for sequences of interest.
- Custom probe panels capture methylated, unmethylated, sense, and antisense strands.
- Specialized blocking reagents enhance detection of methylated DNA and reduce off-target capture events.

Sequencing / Analysis

Align data with reference sequences and identify methylation

TAKING METHYL-SEQ TO THE CLINIC

Novel techniques for detecting and diagnosing cancer are urgently needed in the clinic. Conventional methods for cancer diagnosis, many of which rely on biopsies or imaging, are prone to sampling bias, have limited resolution, or may expose patients to ionizing radiation, which can enrich the population of cancer stem cells.[1,2] Moreover, prompt detection is essential for patients; starting therapy at an early stage prior to metastasis increases the chance of survival and likelihood of successful treatment.[3]

Distinct cancer types have their own reproducible methylation signatures. These epigenetic modifications correlate with tumor stage and type in many cases, making them ideal biomarkers for early detection and diagnosis, non-invasive screening, and disease monitoring.[2] For example, hypermethylation of several known cancer-related genes can reliably, sensitively, and specifically differentiate cancerous from benign tissue in prostate cancer.[4]

The Role of Methyl-Seq

While microarray and PCR-based methods of methylation biomarker identification have utility in the clinic, methylation sequencing (methyl-seq) provides greater coverage of the methylome, allowing researchers to better appreciate global methylation pattern changes in cancer and discover new biomarkers.[5] Performing methyl-seq on liquid biopsies of biofluid samples containing circulating tumor DNA (ctDNA), the DNA fragments released from broken-down tumor cells, provides a non-invasive option to detect methylation patterns in the clinic.[6]

Methyl-seq can be particularly useful in pediatric cancers, which often have a low rate of genetic mutations, but show profound alterations in DNA methylation.[7,8] In a recent publication, researchers reported that by analyzing epigenetic changes in ctDNA in the cerebrospinal fluid, they detected medulloblastoma onset and identified the cancer subtype. They suggested that this technique could be used to monitor treatment response and disease recurrence.[7]

Precision Medicine Potential

Beyond early detection, epigenetic testing holds great promise for precision medicine. Cancer is heterogeneous, even at the level of an individual tumor, which can differ both spatially and over time. Unfortunately, most standard cancer care, including surgery, chemotherapy and radiation, does not account for this heterogeneity. Screening patient-specific methylomes is a key part of understanding and categorizing cancer subtypes and has already proved helpful for predicting a patient's response to treatment.[2] Breast cancer patients with *BRCA1* mutations are more sensitive to platinum-based chemotherapy, while those with *GSTP1* methylation likely respond well to doxorubicin treatment.[9,10] In glioblastoma, patients with O^6-methylguanine-DNA methyltransferase (*MGMT*) promoter hypermethylation have better responses to alkylating neoplastic agents.[11]

The Future of Cancer Therapy

In addition to yielding powerful information about cancer onset, progression, and treatment susceptibility, research into epigentic markers harbored by distinct cancers may allow researchers to develop medicines targeting the cancer methylome. Several of these therapies, known as epidrugs, are already in use; multiple DNA methylation inhibitors that trigger re-activation of tumor suppressor genes by reversing their hypermethylation have been approved by the FDA.[2] These drugs may prove doubly effective in combination with new immunotherapies. Cancer cells evade the immune system by epigenetically silencing the expression of cell surface molecules that render them vulnerable to immune targeting. Derepression of tumor-associated antigens with epidrugs may render cancer cells and tumors susceptible to elimination by the immune system. Indeed, combinations of epidrugs and immunotherapies for a variety of cancer types are currently in clinical trials.[2]

As new methylation analysis technologies such as enzymatic methyl-seq empower scientists to more deeply understand epigenetic modifications across cancer subtypes and stages, patients will reap the benefits of early diagnosis, individually tailored therapeutics and cutting-edge epitherapies.

References

1 | The Changing Epigenetic Landscape in Cancer

References

1) W.J. Locke et al., "DNA methylation cancer biomarkers: translation to the clinic," *Front Genet*, 10:1-22, 2019.
2) M. Kulis, M. Esteller, "DNA methylation and cancer," *Adv Genet*, 70:27-56, 2010.
3) K. Skvortsova et al., "The DNA methylation landscape in cancer," *Essays Biochem*, 63:797-811, 2019.
4) A.P. Feinberg et al., "Epigenetic modulators, modifiers and mediators in cancer aetiology and progression," *Nat Rev Genet*, 17:284-99, 2016.
5) V. Greger et al., "Epigenetic changes may contribute to the formation and spontaneous regression of retinoblastoma," *Hum Genet*, 83:155-58, 1989.
6) C. Moutinho, M. Esteller, MicroRNAs and epigenetics," *Adv Cancer Res*, 135:189-200, 2017.
7) B.T. Joyce et al., "MiRNA-processing gene methylation and cancer risk," *Cancer Epidemiol Biomarkers Prev*, 27(5): 550-57, 2018.

2 | Methylation Sequencing: Conversion and Capture

References

1) H. Luo et al., "Liquid biopsy of methylation biomarkers in cell-free DNA," *Trends Mol Med*, 27(5):482-500, 2021.
2) W.J. Locke et al., "DNA methylation cancer biomarkers: translation to the clinic," *Front in Genet*, 10:1-22, 2019.
3) Y. Li, T.O. Tollefsbol, "DNA methylation detection: bisulfite genomic sequencing analysis," *Methods Mol Biol*, 791, 11-21, 2011.
4) E.K. Schutsky et al., "APOBEC3A efficiently deaminates methylated, but not TET-oxidized, cytosine bases in DNA," *Nucleic Acids Res*, 45:7655-65, 2017.
5) A. Hoppers, et al., "Enzymatic methyl-seq: next generation methylomes," *J Biomol Tech*, 31, S15, 2020.
6) L. Williams et al., "Enzymatic methyl-seq: the next generation of methylome analysis," *New England Biolabs*, 2019.
7) I. Kozarewa et al., "Overview of target enrichment strategies," *Curr Protoc Mol Biol*, 112:7.21.1-7.21.23, 2015.

3 | Taking Methyl-Seq to the Clinic

References

1) J.C.M. Wan et al., "Liquid biopsies come of age: towards implementation of circulating tumor DNA," *Nat Rev Cancer*, 17:223-38, 2017.
2) A. Roberti et al., "Epigenetics in cancer therapy and nanomedicine," *Clin Epigenetics*, 81(11), 2019.
3) "Why is early diagnosis important?," Cancer Research UK, January 11, 2021, https://www.cancerresearchuk.org/about-cancer/cancer-symptoms/why-is-early-diagnosis-important.
4) S. Yegnasubramanian et al., "Hypermethylation of CpG Islands in primary and metastatic human prostate cancer," *Cancer Res*, 64:1975-86, 2004.
5) C. Shu et al., "Comparison of methylation capture sequencing and Infinium MethylationEPIC array in peripheral blood mononuclear cells," *Epigenetics Chromatin*, 13, 51, 2020.
6) M. Berdasco, M. Esteller, "Clinical epigenetics: seizing opportunities for translation," *Nat Rev Genet*, 20:109-27, 2019.
7) J. Li et al., "Reliable tumor detection by whole-genome methylation sequencing of cell-free DNA in cerebrospinal fluid of pediatric medulloblastoma," *Sci Adv*, 6(42):eabb5427, 2020.
8) A.P. Feinberg et al., "Epigenetic modulators, modifiers and mediators in cancer aetiology and progression," *Nat Rev Genet*, 17:284-99, 2016.
9) O.A. Stefansson et al., "BRCA1 epigenetic inactivation predicts sensitivity to platinum-based chemotherapy in breast and ovarian cancer," *Epigenetics*. 7(11):1225-29, 2012.
10) E. Dejeux et al., "DNA methylation profiling in doxorubicin treated primary locally advanced breast tumours identifies novel genes associated with survival and treatment response," *Mol Cancer*, 9:68, 2010.
11) M. Esteller et al., "Inactivation of the DNA-repair gene MGMT and the clinical response of gliomas to alkylating agents," *NEJM*, 343(19):1350-54, 2000.