

epigenomics

DNA Methylation Biomarkers

The SEPT9 DNA methylation based blood test is predicated on the observation that cancer specific DNA can be detected in the blood of individuals with disease (11). It is believed that DNA from tumor cells can enter the blood stream through necrosis or apoptosis of the malignant cells. Intuitively, the smaller or less vascularized the tumor is, the lower is the amount of DNA shed into the blood stream. Thus, tumor derived DNA in the blood stream or other body fluids is a formidable biomarker for the presence of a tumor. The challenge is the specific detection of this tumor DNA in a vast background of normal DNA. Epigenomics has solved this problem by the use of DNA methylation biomarkers. Regulation of gene expression by aberrant DNA methylation is well characterized in tumor biology (12,13) and extensively described for CRC (4,14).

DNA methylation is a natural and tightly controlled biological process that is involved in the regulation of genes and the stability of the human genome. Cytosine, one of the four bases in DNA, can be modified by the covalent addition of a methyl group. DNA methylation in gene regulatory regions (i. e. gene promoters) helps control gene activity. Every cell type has its unique DNA methylation “fingerprint” that changes in various normal biological processes and in many diseases, in particular cancer.

This offers the opportunity to identify and characterize tumor derived DNA based on a pattern of methylation in a particular genomic region that is tumor and organ specific. DNA methylation thus provides a rich source for highly specific biomarkers for organ-specific disease diagnosis, classification and prediction for therapeutic intervention.

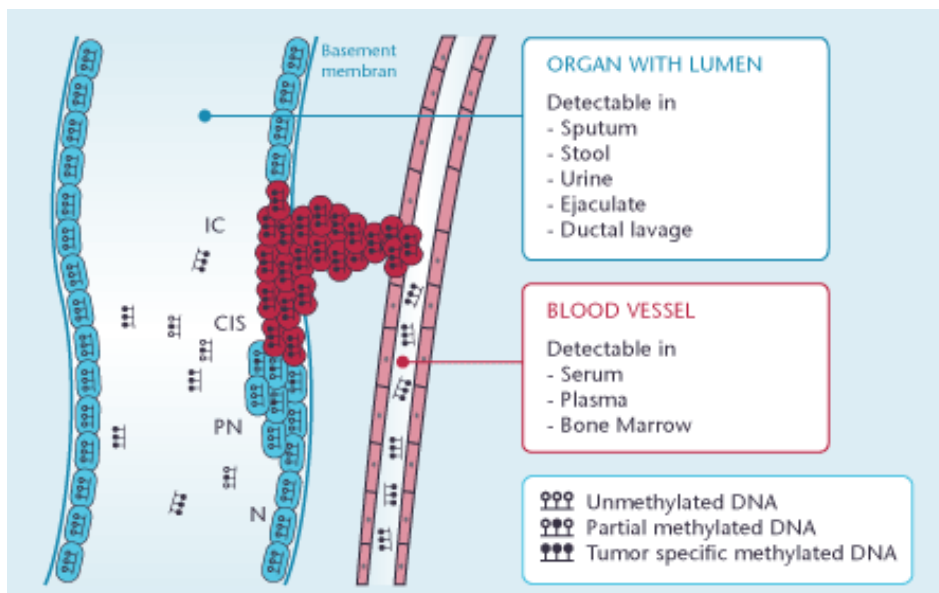


Figure 1: Illustration of various sources to detect tumor-specific methylated DNA. Normal epithelial cells (N) are unmethylated, whereas preneoplastic cells (PN) can develop some methylation. These methylation changes can progress towards carcinoma in situ (CIS) and invasive cancer (IC).

Epigenomics' biomarker discovery and validation process

At Epigenomics, a multi-step iterative process is used to identify and validate DNA methylation biomarkers (Figure 2). Candidates are initially identified using high-throughput technologies such as methylation-sensitive restriction enzyme based genome-wide discovery experiments on well-characterized patient tissue samples. DNA methylation arrays and/or quantitative (real-time) PCR are then used to quantify the extent of methylation of selected marker candidates in target versus control tissues with the goal to identify markers with maximum methylation differences.

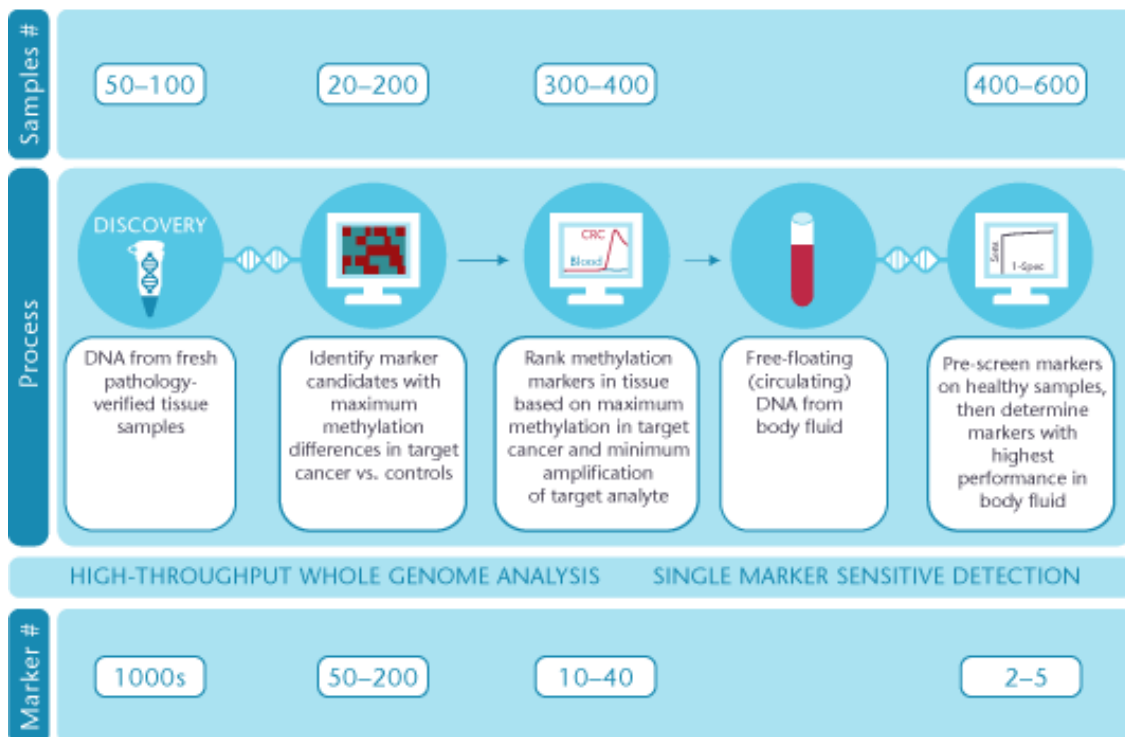


Figure 2. Multi-step marker discovery and validation process.

To evaluate specificity of marker candidates for the disease of interest, various tissue types are included at this step. In Epigenomics' CRC methylation marker discovery, for example, healthy and inflamed tissue from colon, large number of non-colon related cancers, other diseased tissues as well as healthy tissue from a variety of organs were analyzed. Finally, highly sensitive real-time PCR assays (MethyLight™ and Heavy MethyL™, HM) (15,16) are developed for the most discriminatory CpG positions of identified markers. Marker methylation, using these highly sensitive assays, is then assessed on independent tissue sample sets (17,18).

Plasma-Based DNA Methylation Tests

Once performance of a marker assay has been optimized in tissue samples, a series of studies in plasma are initiated. In the early stages of DNA methylation marker evaluation in plasma for a diagnostic application, several different marker assays are typically tested. These HM assays are screened against various model DNAs (i. e. peripheral blood lymphocyte (PBL) DNA, sperm DNA) and bulk healthy plasma samples to screen out unsuitable CpG positions and to determine specificity for the disease of interest before proceeding to the final analysis using clinical study plasma samples from cases and controls.

The principle of detection of methylated Sept9 used by Epigenomics in the feasibility studies is summarized in Figure 3. This test method is comprised of three steps, starting with a plasma sample that is prepared from whole blood using standard techniques. First free-floating DNA is extracted from patient plasma using magnetic bead-based nucleic acid extraction (Step 1). The extracted DNA is then chemically converted using sodium bisulfite (Step 2). This chemical treatment converts unmethylated cytosines to uracil whereas methylated cytosines are not changed. The conversion step essentially translates DNA methylation information into DNA sequence information that can be made 'visible' by standard molecular genetic techniques such as PCR.

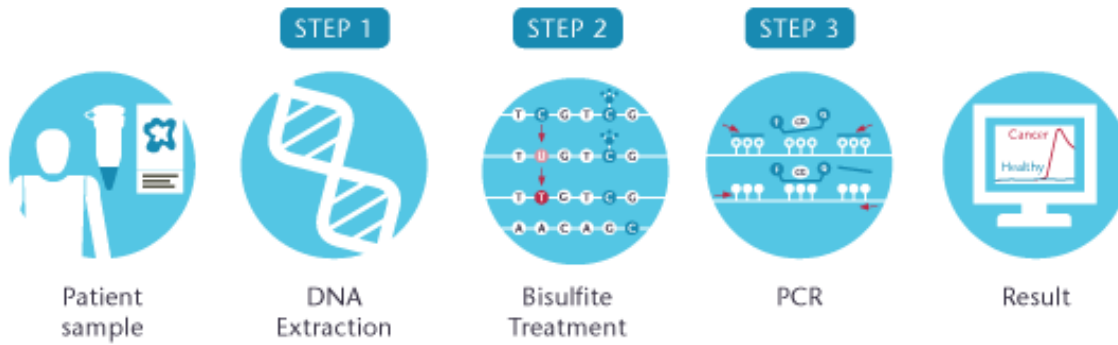


Figure 3. Principle of methylated DNA detection used by Epigenomics.

Lastly, a real-time PCR assay is used to measure the presence of methylated DNA in the bisulfite-treated DNA sample (Step 3). The real-time PCR technology used by Epigenomics in plasma is called the Heavy Methyl™ (HM) assay, a very sensitive assay that detects trace methylation in plasma. This assay consists of primers that are placed in regions without CpG dinucleotides. Blockers specific for unmethylated sequences within the region amplified by the primers are added to prevent amplification of these “healthy” sequences. In order to detect methylated sequences amplified during PCR, a methylation-specific fluorescent probe is included in the reaction.