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Molecular Approaches for the Detection of DNA Methylation

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ABSTRACT

The main goals of this review were to understand the main molecular approaches for the detection of different types of epigenetics modification such as chromatin modifications and DNA methylation .this review provide information about the most sensitive and reliable methods for the defection of epigenetics .generally there are two approaches for detection chromatin remodeling and five approaches for DNA methylation detection widely uses .the most of the useful techniques for chromatin remodeling was chromatin immunoprecipitation (ChIP) assay. Formaldehyde is used in this process to in vivo cross-link proteins to DNA, followed by chromatin extraction from cross-linked cells and tissues. Bisulfite conversion DNA methylation and Bisulfite sequencing methylation is used for DNA methylation. The bisulfite conversion mechanism is a key step in the identification and recognition of DNA methylation; the principles of bisulfite conversion rely on the presence or absence of unique cytosine methyl groups at the carbon-5 site. After treatment with sodium bisulfite, unmethylated cytosine residues are converted to uracil, while 5-methylcytosine (5mC) remains unchanged due to the block reaction of the methyl groups. However, the design of primers for converted and non-converted cytosine is necessary to avoid any errors.

Keywords: Epigenetics, DNA Methylation, Bisulfite Sequencing, CpG Island, Enzymatic Digestion

1. Introduction

Epigenetics is characterized as stable and inheritable gene expression changes without DNA sequence changes (Smail 2019, Pirola and Sookoian 2000). an Inactivation of X-chromosomes, and effects of telomere location. Such hereditary modifications do not include mutations but rather changes to DNA or related proteins, such as histones (Tollefsbol 2004). Methylation and acetylation of histones and regulatory factors, DNA methylation, and small non-coding RNAs are significant epigenetic events (Bayarsaihan 2011).

By coordinating cell proliferation, metastasis, and pluripotency, epigenetic-metabolomic interplay has a crucial role in tumorigenesis. Understanding the link between epigenetics and metabolism could unravel new molecular targets that may enhance cancer treatment (Wong *et al.,* 2017). For use in patients, small molecule inhibitors have been approved against two groups of these enzymes: DNA methyltransferase (DNMT) inhibitors and histone deacetylase inhibitors. Other epigenetic enzyme groups have been shown to correlate with diseases strongly and are currently being targeted for small molecule inhibition (Copeland *et al.,* 2010). In epigenetic regulation of gene expression, histone modification by acetylation plays a key role and is mediated by the balance between histone deacetylases (HDAC) and histone acetyltransferases (HAT). HDAC inhibitors provoke the arrest of cancer cell cycles (Eckschlager *et al.,* 2017).

A tool to identify how often a protein of interest binds to the DNA region is a chromatin immunoprecipitation (ChIP) assay. To research the mechanisms of how transcription factors or chromatin modifications control gene expression, this method is important (Kim and Lee 2020). Other modes of chromatin analysis, such as those that use endonuclease restriction accessibility, chromatin shift micrococcal nuclease analysis, and DNaseI hypersensitivity analysis, have continued to provide

important epigenome-related details (Marks et al.,2001).. There are now many different approaches to the analysis of the exact methylation content of DNA, such as Bisulfate conversion (Delpu *et al.,* 2013), CoBRA (combined bisulfite restriction analysis)(Suzuki and Bird 2008), Methylation-specific PCR (MSP) (Feng *et al.*,2007), Pyrosequencing (Hill 2011) and MethyLight™ (Olkhov *et al.*,2014).

2. Chromatin immunoprecipitation (ChIP) assay

Many essential nuclear functions are regulated by the interactions of proteins with DNA. A robust technique for researching protein-DNA interactions is Chromatin immunoprecipitation (ChIP). However, current ChIP assays either require huge numbers of cells that prohibit their application to unusual samples of cells or biopsies of small tissues or entail lengthy procedures. A 1-day micro ChIP (μChIP) protocol appropriate for up to eight parallel immunoprecipitations of histone and transcription factors from a single batch of 1,000 cells is described here (Dahl and Collas 2008). In addition, in recent years, the combination of ChIP assays with DNA microarrays and high-throughput sequencing technologies has allowed the profiling of histone modifications and transcription factor occupancy locations throughout the genome and in a high-resolution manner across a genomic region of interest. The chromatin immunoprecipitation (ChIP) assay, which allows the analysis of the interaction of regulatory molecules with particular promoters and histone changes in vivo, is one of the commonly used assays to study this. ChIP tests may provide insight into the regulatory mechanisms involved in in vivo gene expression, which is of tremendous importance (Gade and Kalvakolanu 2012). Allele-specific methylation status derived from BisChIP-seq data clearly showed that H3K27me3 histones could be correlated with methylated and unmethylated alleles simultaneously, highlighting that the DNA methylation status in these regions is not contingent on the status of Polycomb chromatin. BisChIP-seq is a new technique that can be widely used to specifically analyze the genomic relationship between methylation of allele-specific DNA, histone alteration, or other relevant epigenetic regulators (Statham *et al.,* 2012).

Among the most intensively researched areas of biology today are chromatin and transcriptional processes. An important development in this field is the invention of chromatin immunoprecipitations (ChIP) (Nelson *et al.,* 2006). The chIP can be used to assess if a transcription factor interacts with a target gene candidate and is used equally frequently to track the presence of histones at particular genomic locations with post-translational modifications (Carey *et al.,* 2009). However, chIP has been a tedious operation for a long time, requiring large cells (Collas 2010).In this procedure, formaldehyde is used to cross-link proteins to DNA in vivo, followed by chromatin extraction from cross-linked cells and tissues (DeCaprio and Khol 2020).

DNA and proteins are reversibly cross-linked in a classical ChIP assay to maintain the association of proteins with target DNA sequences. However, it is possible to omit cross-link when examining histone modifications (native ChIP) (O'Neill *et al.,* 2003). The completion of the ChIP assay takes several days, requires several-tube transfers, and uses either phenol-chloroform or spin columns to purify DNA. The conventional ChIP approach becomes a challenge (Nelson *et al.,* 2006). Without sample transfers, all steps are carried out in microplate wells. Matrix ChIP makes 96 histone and DNA-bound protein ChIPs in 1 day (Flanagin *et al.,* 2008). In addition, a technique was recently recorded for the whole genome mapping of histone modifications from as few as ~25,000 cells or 50 ng of ChIP DNA (Goren *et al.,* 2010).

3. Bisulfite conversion DNA methylation and Bisulfite sequencing methylation:

DNA methylation in the human genome is an

important epigenetic modification (Zhang *et al.,* 2009). For example, in sequences of a cytosine accompanied by guanine separated by a phosphate group (a 'CpG' site), DNA methylation occurs almost exclusively at the carbon-5 location of specific cytosines (then called 5-methylcytosine)(Perez and Capper 2020).In addition, DNA methylation patterns, such as diagnosis, prognosis, and treatment response, may be used as a biomarker for clinical management. Therefore, a range of high-throughput DNA methylation methods has been developed to evaluate the methylation status of many CpGs at once or even the entire genome (Pajares *et al.,* 2020).

Whole-genome bisulfite sequencing (WGBS), also referred to as BS-seq, has been commonly used to test whole-genome methylation at single-base resolution (Zhou *et al.,* 2020). Cytosine in vivo and in-vitro metabolic reaction pathways. Via the steps of sulfonation, hydrolytic deamination, and subsequent desulfonation with alkali, sodium bisulfite will convert cytosine into uracil. However, 5-Methylcytosine is shielded from this bisulfite reaction due to the presence of a methyl group that inhibits bisulfate sulfonation (Hayatsu 2008).

Principles of study of methylation using genomic sequencing of bisulfite. Unmethylated cytosine residues are converted to uracil after treatment with sodium bisulfite, whereas 5-methylcytosine (5mC) remains unchanged. The uracil residues are converted to thymine after PCR amplification. DNA methylation status may be decided by direct PCR sequencing or cloning sequencing (Leontiou *et al.,*2015). The bisulfite treatment method exploits the different susceptibility of cytosine and 5-MeC to bisulfite deamination in acidic environments, where cytosine is converted to uracil while 5-MeC remains unreactive (Warnecke *et al.,* 2002).

DNA methylation is commonly studied using bisulfite sequencing (BS-seq)-based designs, such as whole-genome bisulfite sequencing (Chung and Kang 2020). Single cytosine calculation and high precision have made it possible to become the gold standard in DNA methylation analysis for whole-genome bisulfite sequencing (WGBS) (Zhao *et al.,*2020). In addition, some special services, such as To map high-throughput bisulfite sequencing data into reference genomes, Bismark, BS Seeker, BSMAP, MethTools, QUMA, BISMA, and BiQ Analyzer HT have been developed (Su *et al.,*2013).

Responses of methylated and unmethylated cytosines to bisulfite therapy. (a) Bisulfite-treated unmethylated cytosines are either converted to uracil or are not converted and remain as cytosine. (b) The most common errors are 5-methylcytosines treated with bisulfite, either not converted or poorly converted to thymine (Genereux *et al.,*2008). Sequencing errors that change C to T and vice versa can lead to errors derived from the sequences in the methylation results. Therefore, against the genome sequence, BiQ Analyzer suggests removing all sequences that fall below a local sequence identity standard of 80 percent (Bock *et al.,* 2005).

A stable epigenetic change observed in many living organisms, from bacteria to higher eukaryotes, is the methylation of DNA at the fifth location in cytosine (5mC). During embryonic development, in processes such as genomic imprinting, transposon silencing, and X-chromosome inactivation, and during the differentiation of pluripotent cells, it is known to play a role in controlling transcriptional activity (Olova *et al.,* 2018). Mapping bisulfite reads one important step in naming a genome's methylation. As the non-methylated Cs are converted to Ts by bisulfite treatment and subsequent PCR, mapping bisulfite reads is different from that of ChIP-Seq and RNA-Seq results. Due to many mismatches between the transformed Ts and the initial Cs, bisulfite reads are hard to map to the reference genome (Lim *et al.,* 2012). Bisulfite genomic sequencing developed by Frommer and colleagues has been recognized as a revolution in

studying DNA methylation-based on genomic DNA conversion using sodium bisulfite. In addition to the different merits of the bisulfite genomic sequencing process, such as being highly qualitative and quantitative, many derived methods serve as a basic concept for better understanding the mystery of DNA methylation(Li and Tollefsbol 2011). However, the lack of software tools to process and analyze the large number of sequencing reads produced by this method is a major roadblock for the wider use of high-throughput bisulfite sequencing. Several software tools have been developed to process small-scale bisulfite sequencing data obtained by traditional Sanger sequencing (Lutsik *et al.,* 2011).

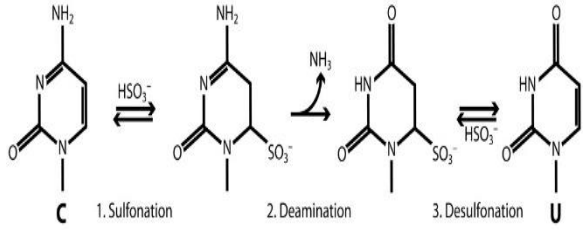

Figure 1: The response between cytosine and bisulfite (step 1) at acid pH leads to deamination (step 2). Afterward, uracil is formed by desulfonation at alkaline pH (step 3) (Darst et *al.*, 2010).

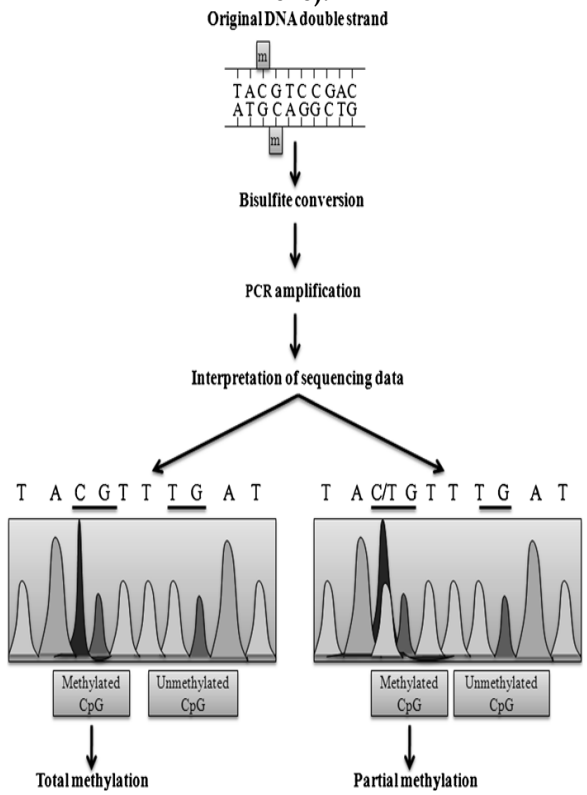

Figure 2: Interpretation of sequencing outcomes for methylation. Both unmethylated cytosines (C) transform to thymine (T) after bisulfite treatment, and the presence of a C-peak suggests the presence of 5mC in the genome. A single peak is seen by total methylation or full conversion of a single residue. Partial methylation or possibly incomplete bisulfite conversion suggests the presence of both C and T peaks (Li and Tollefsbol 2011).

4. Methylation-Specific PCR and Nested Methylation-Specific PCR :

Methylation-specific PCR (MSP) is a tool based on PCR for the study of CpG island methylation patterns (Ku *et al.,* 2011). Methylation-specific (MS)-PCR is a valuable tool with many advantages for qualitative DNA methylation analysis, including ease of design and execution, sensitivity to the detection of small amounts of methylated DNA, and the ability to quickly scan a large number of samples without the need for costly laboratory equipment to be purchased (Huang et al.,2013). Two pairs of primers are required for an MSP experiment. One pair is methylated DNA (M) specific and the other is unmethylated DNA (U) specific. One or more CpG sites are included in each primer (or at least one of the pair) sequence for discrimination of methylated and unmethylated DNA (Li *et al.,* 2002).

Apart from the use of cloning or methylation-sensitive restriction enzymes, MSP can rapidly determine the methylation status of virtually any cytosine from CpG sites within a CpG island. MSP requires very small amounts of DNA, is susceptible to 0.1 percent of a given CpG island locus methylated alleles, and can be carried out in paraffin-embedded DNA extracted samples (Herman *et al.,* 1996). Nested MSP can be conducted if an experiment could not amplify the product abundantly for analysis by direct MSP. An additional primer set is required for Nested MSP, which covers the amplified product sequence with two selected pairs of primers. A second PCR with two pairs of primers (each primer set for different methylation states) is carried out after the first PCR with nested MSP primers using the amplified products from the first PCR (Hanaei *et al.,* 2020).

The most frequently used technique to study DNA methylation of a locus of interest is possibly Methylation-Specific PCR (MSP). The methylation status of any group of CpG sites within a CpG island can be easily identified by MSP, not requiring methylation-sensitive restriction enzymes. It also requires minute quantities of DNA and is very sensitive as <0.1% of methylated alleles can be identified at a particular locus and used in various samples, including body fluids and paraffin-embedded samples (Ramalho *et al.*,2018). In addition, good agreements could not be established between quantitative methods and MSP for any of the loci investigated. The clinical validity of the quantitative DNA methylation evaluation was demonstrated by the strong association of the quantitative assessment but not of MSP-derived methylation data with clinically relevant characteristics in our patient cohort. Taken together, MSP is still the most widely used tool for evaluating DNA methylation (Claus *et al.*, 2012).

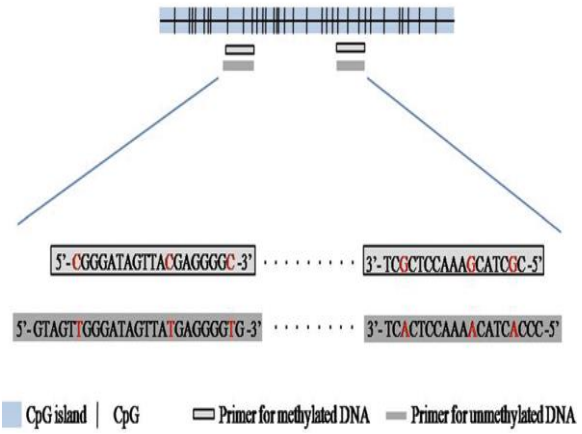

Figure 3: Option of primers for methylation-specific PCR. MSP primers are necessary for pairs that detect methylated DNA only (M primers) and unmethylated DNA only (U primers). Primers contain at least more than one CpG site, and the same CpG sites are used in two pairs of primers. However, two sets of primers, including the same CpC positions, may not have the same length and start point (Bayarsaihan 2011).

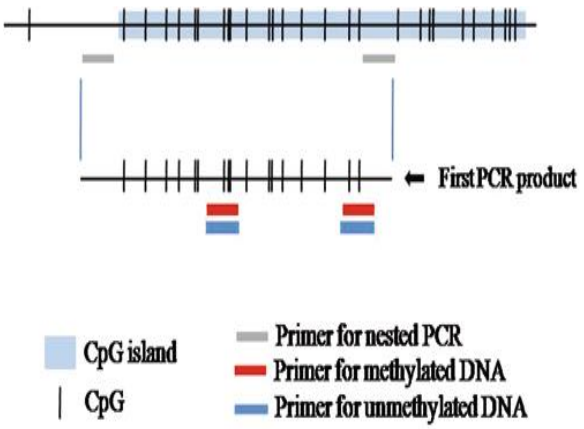

Figure 4: Diagram of nested PCR. When the products of direct MSP primers are not amplified, it needs another primer set surrounding the products of direct MSP primers (Licchesi and Herman 2009).

5. CoBRA (combined bisulfite restriction analysis) :

The bisulfite conversion of DNA relies on most techniques to test DNA methylation (Susan *et al.*, 1994). One such method, combined bisulfite restriction analysis (COBRA), involves amplifying bisulfite transformed DNA by PCR accompanied by enzymatic digestion (Xiong and Laird 1997). COBRA is theoretically easy and details on the methylation status of multiple CpG sites can be collected in a single reaction, depending on the area being investigated (Brena *et al.*, 2006). In the regulation of gene expression, DNA methylation plays a critical role. For example, an important mechanism for inactivating tumor suppressor genes in human cancers is abnormal promoter hypermethylation (Watanabe *et al.*, 2010). For input, PCR primers and file upload are available. Promoter sequences and restriction enzymes for recognition sites containing CpG- and GpC are retrieved. In the experimental work, four symbolic enzymes were successfully tested by COBRA. Therefore, the Methyl-Typing method provides a robust COBRA-restriction enzyme mining tool (Yang *et al.*, 2010).

The protocol consists of the following main steps: bisulfite conversion of non-methylated cytosines to uracils, locus-specific PCR amplification of converted DNA, digestion restriction, gel restriction pattern analysis, and ImageJ or related software

quantification of these restriction patterns (Bilichak and Kovalchuk 2017). First, Nested-PCR amplification and endonuclease (Taq I) digestion were performed after treating the genomic DNA with sodium bisulfite. Then, using 1.5% poly (ethylene) oxide (Mave, 8,000,000 g/mol) in the presence of electroosmotic flow, the digested DNA fragments were then separated by capillary electrophoresis (Chen *et al.,* 2012). The assay enables the study of different cytokines, including those theoretically targeted for symmetrical and nonsymmetrical methylation, due to different restriction enzymes that have cytosines in the restriction recognition sequence (Boyko and Kovalchuk 2010).

The two most commonly used enzymes are BstUI and TaqαI (Hill 2011 and Valente *et al.,* 2014). PCR products are obtained after enzyme digestion if the stated DNA region does not digest the methylated CpG sites. The quantitative study of DNA methylation allows for COBRA, Ms-SNuPE, and quantitative real-time MSP (Wong 2006). A forum for the rapid and quantitative evaluation of DNA methylation patterns in large sample sets is provided by Bio-COBRA. Its sensitivity and reproducibility make it an excellent method for clinical sample analysis of DNA methylation (Brena and Plass 2009). COBRA-seq applies to non-model organisms without a reference genome and is consistent with non-CpG methylation investigations using CpA, CpT, and CpC-containing restriction enzymes at their site of recognition (Varinli *et al.*,2015).

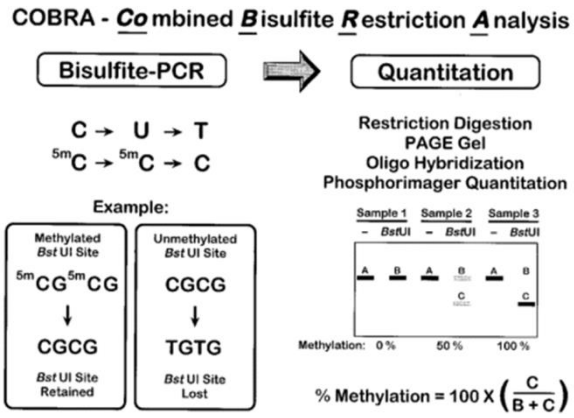

Figure 5: Outline of the treatment for COBRA. COBRA consists of a regular PCR sodium bisulfite treatment (Xiong and Laird 1997).

6. Analysis of DNA Methylation by Pyrosequencing

Pyrosequencing is a method of sequencing by synthesis that quantitatively tracks the incorporation of nucleotides in real-time through the enzymatic conversion of pyrophosphate released into a proportional light signal (Tost and Gut 2007). The basic pyrosequencing technique is based on the insertion of other dinucleotides into a template that is released in a predetermined order; it binds to the template strand when the correct nucleotide is released, releasing pyrophosphate (Hill 2011). The pyrosequencing reaction is swift, relatively inexpensive, and offers significant logistical benefits over previously mentioned validation methods (Shaw *et al.,* 2006).

For the simultaneous analysis and quantification of the methylation degree of several CpG positions close, the real-time luminometric detection of the release of pyrophosphate upon nucleotide integration in the Pyrosequencing ® technology is ideally suited(Tost and Gut 2007). (Mikeska *et al.,* 2011). DNA methylation ratios are determined strand-dependent from the light levels emitted from each nucleotide integrated at individual CpG positions (Delaney et al., 2015). Artificial development of C/T SNP through bisulfite modification allows real-time measurement of DNA methylation locally and globally (Candiloro *et al.,* 2011). Therefore, pyrosequencing has been used to research heterogeneously methylated loci

(Candiloro *et al.,* 2011). The length of the sequence read, and thus the number of CpGs that can be analyzed in one sequencing reaction and the relatively high cost that is shared equally between the step of PCR amplification, the reaction of pyrosequencing, and to a lesser extent, the step of purification, are reduced. Due to the low sequence complexity of bisulfite-treated genomic DNA, traditional multiplex detection using many sequencing primers is not feasible for DNA methylation analysis (Tost *et al.,* 2006).

An area of interest in Pyrosequencing is first amplified by polymerase chain reaction (PCR) from bisulfite-converted DNA before PCR amplicons are made single-stranded and annealed before sequencing with the Pyrosequencing primer (Tabish *et al.,*2015). The most critical move for a good study is to obtain a powerful amplicon in the pyrosequencing procedure (Šestáková *et al.,* 2011). DNA strand-specific is the design of the bisulfite Pyrosequencing methylation assay, and no CpG sites should be included in the design of the primer and should be free of high-frequency mutations. Also, Pyrosequencing assays must be tested during bisulfite PCR for preferential amplification to ensure the precision and reproducibility of the sequencing quantification (Poulin *et al.,* 2018).

Age prediction models based on DNA methylation were developed mainly for use with blood samples and are based either on a low number of CpGs (DNA methylation biomarkers), using locus-specific technologies such as pyrosequencing, or on a higher number of CpGs involving the use of epigenotype array technologies around the genome. These DNA methylation models outperform both the DNA-based age prediction models previously described and the RNA and protein-based age prediction models, rendering DNA methylation the most promising biomarker for molecular age prediction (Daunay *et al.,* 2019). Since DNA methylation detection relies on 5'-modified cytosine resistance to bisulfite-catalyzed conversion to uracil, parameters that affect the technical adequacy of mtDNA methylation analysis have been examined. Negative control amplicons (NCAs) devoid of cytosine methylation have been amplified to cover the entire human or mouse mtDNA by long-range PCR (Owa *et al.,* 2018). However, adequate quantitative approaches are needed to compare differences in DNA methylation among different tissue types. As a sequencing-by-synthesis technique, Pyrosequencing® enables such quantification with a single resolution of CpG and the ability to determine the threshold (Poulin *et al.,* 2018). Pyrosequencing is a sequencing technology for nonelectrophoretic nucleotide extension for various purposes, including single nucleotide polymorphism genotyping, tumor detection of bacterial strain typing mutation Island the methylation study of CpG, and quantitative CpG (Irahara *et al.,* 2010). Biotin-labeled polymerase chain reaction (PCR) products form the basis-pair nucleotide incorporation template that triggers a light-emitting cascade reaction that creates a program and the percentage methylation measurement for each site. It is necessary to bisulfite-convert the DNA sample before pyrosequencing and then perform locus-specific PCR for the region of interest. It is necessary to biotinylate one of the PCR primers and a separate sequencing primer is required for pyrosequencing itself (Colyer *et al.,* 2012). It must be remembered that by pyrosequencing, we calculated LINE-1 methylation as a proxy for global DNA methylation. It has been shown that the methylation of repetitive elements is a significant contributor to the overall methylation of genomic DNA in the human genome. Pyrosequencing has been widely used to test global DNA methylation and is a reproducible assay with a standard deviation of 2 percent.23 Although the absolute difference in LINE-1 methylation found in this study was small 3 percent), the risk of squamous c head and neck has previously

been associated with a modest and important difference in LINE-1 methylation (Zhang *et al.,* 2011).

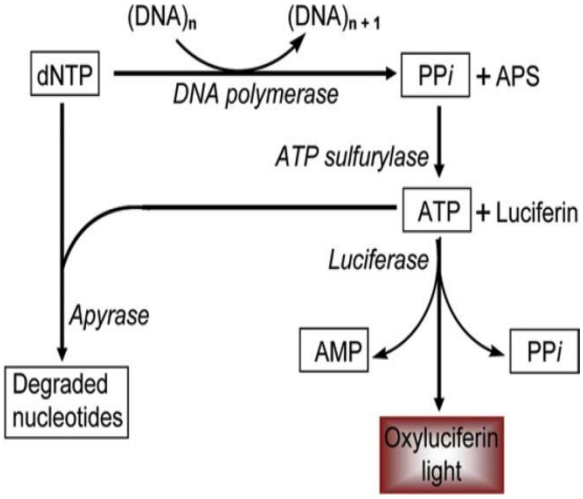

Figure 6: System of Enzyme Cascade in Pyrosequencing. The sequencing primer is first hybridized with an ssDNA template and combined with enzymes and two substrates (APS and luciferin). The released PPi reacts with APS in the presence of ATP sulfurylase, which gives rise to ATP after the effective incorporation of a nucleotide by DNA polymerase into growing DNA strands. In the presence of the luciferin substrate and the luciferase enzyme, ATP releases oxyluciferin, which generates visible light that an integrated CCD camera can observe. Before the next nucleotide dispensation, any unincorporated nucleotides and ATP are degraded by enzyme apyrase into their building blocks. For every dispensation, Cascade reactions repeat. ATP adenosine triphosphate, phosphosulfate of APS Adenosine 5, pyrophosphate of PPi(Delaney *et al.*,2015).

7. MethyLight™ to detect DNA methylation :

Cumbersome manual techniques that use gel electrophoresis, restriction enzyme digestion, radiolabeled dNTPs, or hybridization probes are needed by most techniques used to analyze cytosine-5 methylation patterns (Eads *et al.,* 2000). Real-time PCR assays have been identified to measure methylation of DNA (MethyLight) (Widschwendter *et al.,* 2004). In short, three sets of primers and probes specifically designed for bisulfite-converted DNA have been used (Ogino *et al.*, 2006). MethyLight depends on methylation-specific priming, combined with methylation-specific fluorescent testing (Campan *et al.,* 2009).

Using DNA oligonucleotides that anneal differentially to bisulfite-converted DNA according to their methylation status in the original genomic DNA, MethyLight assays quantify DNA methylation at a specific locus (Olkhov *et al.,* 2014). A quantitative evaluation of these low-frequency methylation events enables the quantitative precision of real-time PCR and the ability to design bisulfite-dependent, DNA methylation-independent control reactions together. The practical steps of the MethyLight analysis are listed in detail here (Campan *et al.,* 2018).

Mutational screens and methylation profiling provide the downstream study of such DNA. For PCR and cycle sequencing, screening for mutations requires a large amount of DNA. When the gene screened has many exons, this is self-inhibitory. DNA methylation profiling using MethyLight technology circumvents this problem and enables multiple DNA biomarkers derived from a single microscope slide of the tissue of interest to be mined (Dallol *et al.,* 2011). More sensitive than traditional MSP, MethyLight is. Changes in methylation in carcinogenesis are often heterogeneous, and so far, no single gene has been found methylated in any breast cancer specimen. Besides, the sensitivity was typically poor in most experiments examining methylation levels using single genes (Shan *et al.,* 2016). Although hundreds of PCR-based DNA methylation studies are published each year, it can be difficult for molecular genetics researchers not yet familiar with methylation analysis to select and implement suitable methods for these studies. Bisulfite sequencing PCR (BSP), methylation-specific PCR (MSP), MethyLight, and methylation-sensitive high resolution melting (MS-HRM) are the most widely used PCR-based DNA methylation analysis techniques (Hernández *et al.,* 2013).

The sequences of Sat2-M1, LINE-1-M1, and Alu-M2 probes and forward and reverse primers as defined in Weisenberger et al. In the MethyLight assay, these regions were selected because their methylation level was measured to be significantly correlated with HPLC-based global DNA methylation measurements

in a 10 ul reaction volume with 0.3 uM forward and reverse PCR primers, 0.1 ul probe, 3.5 uM MgCl 2, using the following PCR program: 95°C for 10min, then 55 cycles of 95°C for 15 s followed by 60°C for 1min. Assays were run on an ABI Prism 7900 Sequence Detection System (Perkin-Elmer, Foster City, CA) (Wu et al.,2012). Previous studies have shown that diet and the environment can alter DNA methylation profiles and that these profiles are particularly vulnerable during development. Thus the role of DNA methylation in developmental governance and subsequent progression of the disease is important to understand. Several molecular methods exist to test for global, gene-specific, and epigenome-wide methylation. We define these techniques here and address their relative strengths and constraints (Sant *et al.,* 2012).

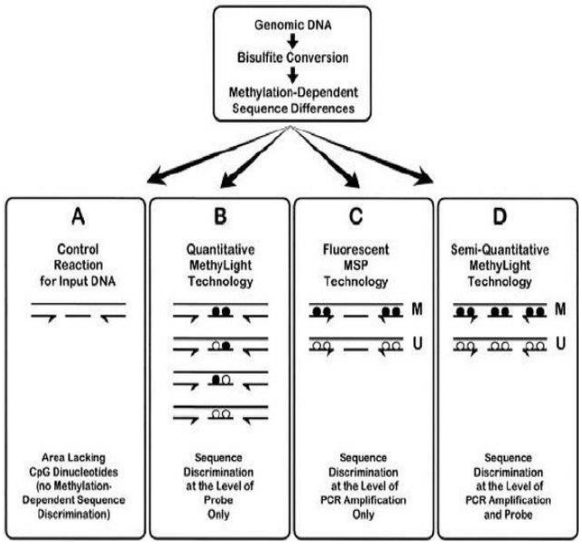

Figure 7: Schematic of the theoretical basis of MethyLight technology (Sulewska *et al.,* 2007).

8- Conclusions

From this review, I conducted the following:

- 1-there are many molecular approaches for the identification of different types of epigenetics modification
- 2-the most sensitive and reliable approaches are bisulfite conversions
- 3- Each of the approaches has many limitations
- 4-primer design is a crucial step for most of the approaches

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