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Techniques and technologies for detection of genomic abnormalities

ABSTRACT

Identification of both genetic and epigenetic alterations on DNA is a major interest of molecular biology and medicine. New generation FISH techniques such as comparative genome hybridization (CGH), matrix-based CGH, and Spectral Karyotyping enable researchers to identify either partial or whole loss/gain of chromosomes or more complex rearrangements in a more specific way, and thus improve and contribute to the elucidation of gross genomic abnormalities. Detection of relatively small DNA alterations such as micro deletions, insertions, duplications, and substitutions is also highly valuable for identification of causative mutations, mapping studies, and determination of population profiles. In addition to the conventional Southern Blot technique, a growing number of Polymerase Chain Reaction (PCR)-based approaches are applied to detect quantitative and qualitative differences in the DNA sequences. Moreover, application of highly sophisticated technologies allows simultaneous examination of large numbers of test samples for multiple sequence alterations. In addition to these changes along the DNA sequence, the existence and the degree of DNA methylation, the epigenetic mechanism associated with selective gene silencing, can be explored by using a variety of techniques. In this review, some of the techniques and technologies applied for detection of especially small genomic abnormalities will be summarized with emphasis on the relative advantages and disadvantages of each, along with recent improvements.

Key words: *DNA variation; Molecular and cytogenetic techniques; DNA methylation*

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INTRODUCTION

Any type of alteration in the DNA sequence, either in quantity and/or quality, resulting in a distinguishable phenotypic malformation can be defined as a genomic abnormality. Mutations such as translocations, duplications or deletions involving either a part of, or the whole chromosome(s) are a common cause of several developmental, mental and neoplasmic abnormalities and even lethality. Micro alterations on DNA, on the other hand, constitute relatively smaller lengths of DNA (ranging from a single base pair (bp) to several kilobases (kb)), but they are also associated with serious phenotypic abnormalities. Cytogeneticists and molecular geneticists have introduced and/or applied several techniques for detection of abnormalities in the genome. In fact, each technique may have advantages and disadvantages over another in terms of reliability, cost-effectiveness, and sensitivity (summarized in Table 1). Clearly, elaborate modifications of the already existing systems, and introduction of new and more powerful techniques are required, and underway, to further improve the efficiency, sensitivity, reproducibility, and broad-range applicability. The advance in the technology and the information derived from scientific research will ultimately let us analyze and interpret the data in a more effective way to better understand the structure and function of the genome. Moreover, hopefully preventive and therapeutic approaches for genetic conditions will not be so far from being a reality.

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CYTOGENETICS

Chromosomal rearrangements associated with an aberrant number of individual chromosome (aneuploidies), loss or gain of part of a chromosome (deletions, duplications), and breakage of a part of a chromosome followed by re-localization on a non-native chromosome (translocations), are among the most frequent mutational events observed in genetic conditions. Identification of these structural abnormalities is essential to elucidate the exact nature of the condition and to identify the gene(s) involved in the development of the associated phenotype. Several high-resolution cytogenetic techniques are available to identify these gross alterations in the genome. Initial karyotype analyses, like G-banding and *In Situ* Hybridization (ISH), allowed detection of most of the chromosomal imbalances. The introduction of fluorochromes and other technical advances led to the development of the high resolution "fluorescence" ISH (FISH) technique, one of the most powerful and widely applied tools to detect structural and numerical chromosomal abnormalities in the genome. Recently, multi color FISH and spectral karyotyping techniques, which utilize 24 different probes each specific for a particular chromosome, have been developed (1, 2). These techniques allow the simultaneous detection of the whole set of chromosomes in a single experiment and thus improve the efficiency of data collection. However, the need for fresh samples for metaphase spreads has been a limitation for the applicability of these techniques. This restriction on the karyotype analyses has been overcome by the development of a highly exciting technique called Comparative Genome Hybridization (CGH) (3). CGH requires a minute amount of genetic material that can even

be obtained from paraffin embedded tissue sections. In short, this technique involves the labeling of the test and the reference samples with different fluorochromes by using either nick-translation whenever high molecular DNA is available, or degenerate oligonucleotide PCR (DOP) for the whole genome amplification when the DNA sample is from a micro-dissected tissue (4-7). Those labeled molecules are then used as hybridization probes on the metaphase spreads derived from a karyotypically normal sample. Comparison of the band intensities of the test and the control samples provides an unbiased indication of partially or wholly gained or lost chromosome(s) (3, 5-7). Therefore, CGH enables screening of the whole genome in a single step for deletions, duplication or amplifications. In addition, the whole genome amplification by DOP allows the analysis of a limited quantity of genetic material for chromosomal abnormalities, which makes CGH an important tool for prenatal and pre-implantation diagnosis purposes (7). Unfortunately, CGH is not able to give any idea about the presence of balanced and unbalanced translocations. Moreover, CGH cannot screen the regions containing repetitive elements like centromeres and heterochromatic regions. In addition, the sensitivity of the technique is mainly dependent on the interpretation of visual images (technical details and the limitations of the CGH have been reviewed in ref.5). Therefore, other cytogenetic or molecular techniques are required to complete the scanning of the test sample's genome. Recently, CGH has been applied on target DNA arrays (matrix-based CGH) (5, 8, 9). This application allows the routine analysis of large numbers of test samples easily in an automated fashion and also overcomes some of the technical problems related to interpretation of the results.

MOLECULAR GENETICS

Micro alterations of the DNA sequence such as single base substitutions, and small insertions or deletions are among the most frequent sequence variations observed in the genome. Those present in either the control or the coding regions of genes, which cause altered expression pattern or "sense" of the encoded protein, are among the most common mutational events associated with genetic abnormalities. Therefore, analysis of these mutations is critical for determination of the exact structure and function of the genes, as well as for medical purposes like molecular diagnosis. Phenotypically silent changes in both coding and non-coding parts of the genome (polymorphisms) are also valuable for gene mapping and haplotype analyses.

Micro alterations can be classified as either qualitative (e.g., base substitutions) or quantitative (e.g., deletions, duplications or amplification). A variety of molecular techniques are available to identify these variations. In some cases, the search is a blind one, consisting of scanning a part of the genome for the possible existence of sequence variation (scanning). In other cases, specific known mutations may be of interest, and more efficient techniques can be applied for their detection in DNA samples.

A. Detection of qualitative alterations in DNA

Scanning for variations

Depending on the base composition, a DNA segment may adopt a specific conformation and, therefore can be differentiated from other DNA sequences upon non-denaturing polyacrylamide gel electrophoresis (PAGE). Single Strand Conformation Polymorphism (SSCP, or better termed as analysis, SSCA) is based on that principle, and is commonly used for mutation screening (10). In this technique, the PCR-amplified fragments are denatured and loaded onto a non-denaturing gel. Comparison of the band pattern with a reference sequence allows the identification of test samples that contain sequence variation. Effects of certain electrophoretic parameters, such as gel composition, denaturants, and temperature, on the sensitivity of SSCA, have been well documented (11-14). One of the major limitations for this analysis is the size of the fragment under investigation; the sensitivity of SSCA substantially decreases as the size of the fragment becomes larger than 200 bp. However, whenever possible, restriction digestion of the large fragments followed by SSCA analysis may overcome that limitation of the technique (15). Sometimes, it may be possible for more than one fragment to be analyzed on the same gel, allowing multiplex screening which reduces the time and material required for analysis (16). Unfortunately, a single running condition that is optimal for all DNA fragments can not be established for this technique. Thus, extensive optimization of electrophoresis conditions (e.g., temperature and gel composition) may be required for each sequence investigated.

Heteroduplex Analysis (HA) depends on the creation and the detection of heteroduplex molecules formed between variant and wild type sequences present in an environment that allows hybridization (17). PCR amplified fragments are first denatured and then cooled down for re-annealing, and fractionated on PAGE. The mismatch formed between the variant and the wild type complementary sequences results in the retardation of the migration of the heteroduplex molecule relative to the wild type and variant homoduplexes, and thus can be detected on gels. The greater the mismatch, the more easily it can be detected on gels (our own observation). However, while the heterozygote samples for a particular variant will be identified, homozygous variants will not. To detect homozygotes, the test samples are run in two forms; a) alone, b) mixed with the wild type sequence. By comparing the patterns obtained for these two runs, homozygous or heterozygous status of the test sample can be determined. Size limitation in HA is not as strict as with SSCA, but mismatches close to primer sites (approximately 50 bp) may not be detected by this technique. Powerful multiplex HA screening protocols that reduce time and effort have also been reported (18).

Denaturing Gradient Gel Electrophoresis (DGGE) is a highly sensitive technique for detecting sequence variation. It relies on different melting characteristics of DNA fragments. The samples are run in a gradient of denaturing conditions such as increasing temperature or increasing concentration of denaturants (e.g., urea). Since the melting profile of a DNA sequence is mainly dependent on its nucleotide sequence, any change in the sequence may be reflected as a different melting pattern. When the gradient level of the denaturant matches the melting properties of the DNA fragment, the fragment starts denaturing, which results in the formation of bubbles on the molecule, and retardation of migration on the gel. Mixing of the test samples with the wild type sequence helps to detect homozygotes where the variation may not necessarily change the melting characteristics of the fragment (19). This technique, however, requires usage of a specific primer with approximately 40 bp long GC-clamps at its 5′ end to ensure controlled melting of DNA fragments containing more than one melting domain, meaning an additional cost (20). Up to one kb long DNA fragments can be screened for variation by using DGGE. However, although broad-range applications using a single running condition effective for screening several different DNA sequences are reported (19), optimization of the gel and the running conditions may be required for each DNA fragment to be analyzed.

The above techniques are among the most widely used assays in laboratories to scan DNA fragments for the possible existence of variation. However, sensitivity of each technique is questionable. Combination of any two of the assays or co-analysis by the same technique using different experimental conditions may help to perform a more complete analysis, but some variation may still be undetected. Broad range and multiplex screening applications are especially helpful to reduce the time and the material used, however, extensive optimization steps are required, which is time consuming. Therefore, the need for application of technologies providing better sensitivity, speed and reproducibility for simultaneous screening of large numbers of samples is apparent. Capillary Electrophoresis (CE) and Denaturing High Performance Liquid Chromatography (DHPLC) are two examples of technologies full-filling these criteria. Approaches like SSCA can be performed in combination with CE technology (21-23). The fluorescence used to label the fragments under investigation is detected as peaks upon software

analysis and any deviation of peak patterns from that of the normal sample indicates the potential variant sample. Multiplex screening approaches making use of different fluorophores with different peak values can be successfully applied for multiplex screening purpose (22). The automated nature as well as the speed, sensitivity and the requirement for a smaller amount of test samples make CE one of the most popular technologies for routine analysis. DHPLC is another highly informative mutation detection system recently adopted. It is based on the detection of heteroduplexes in a semi-automated fashion (24). One hundred per cent sensitivity and a considerable speed for this analysis have been reported, making it a highly promising alternative for analysis of sequence variations in large number of samples (24-27). Some authors were able to observe sequence-specific patterns in the test samples upon comparison with the samples of known sequence, and therefore were able to predict the nature of the variation prior to DNA sequencing (25). However, this seems to depend on the conditions applied and the region/variation to be analyzed because other reports showed no sequence-specific correlation of the pattern (27).

Melting curve analysis based on the LightCycler™ technology (28), is another highly sensitive, specific, cost and labor effective system for routine screening of DNA variation. This assay involves the automated detection of the differences in the melting characteristics of the DNA fragments immediately after PCR amplification. The reaction mixture contains the standard reagents for PCR amplification as well as two fluorescently labeled oligonucleotide probes. One of these probes is complementary to the sequence harboring the variation of interest, and is coupled to a donor fluorophor at its 3′ end. The other probe is complementary to a nearby sequence, and is labeled with an acceptor fluorophor at the 5′ end. Whenever the two fluorophors are in close vicinity, the energy from the donor dye is transferred to the acceptor dye (fluorescence resonance energy transfer, FRET). After the completion of the PCR amplification, the reaction mix is gradually heated to establish a melting curve. Since the dissociation of the probe from the target is dependent on the complementary nature of the target sequence, any mismatches between them may lead to a decrease in the melting temperature. Liberation of the donor fluorophor upon dissociation of the probe from the template leads to the termination of FRET, which can easily be detected by monitoring the fluorescence. A growing number of reports making use of that technology to detect mutations are available in literature (29-32).

Such techniques are suitable for detection of patterns different from normal, however, most of them are far from giving an idea about either the nature or the location of the sequence variation. Therefore, DNA sequencing analysis is required to explore the exact nature of the sequence variation observed in the test samples. Once it has been characterized, several other strategies can be adopted/developed to specifically detect its existence.

Screening for characterized variations:

Restriction fragment (RF) analysis is one of the most widely used approaches to distinguish between DNA sequences in which differences result in either creation or abolishment of the recognition site of a certain restriction enzyme. Several mutations and polymorphisms (RFLP's) have been successfully detected and analyzed by this approach. In cases where the sequence difference does not create or abolish a recognition site, a forced RF analysis may be applied by using a specific primer (33). In short, this assay requires designing a primer which mismatches a particular nucleotide that is close to the variant base. The position and the nature of this mismatch nucleotide are chosen so as to create/abolish a restriction enzyme site when present on the same strand as the variant base. Thus, upon PCR amplification and subsequent enzymatic digestion, the variant base can be detected, and therefore, the genotype of the sample can be determined. As long as the sequence is recognized by a particular enzyme, either naturally or in forced form, the RF analysis is one of the simplest and most specific approaches.

Another system, Allele Refractory Mutation System (ARMS), makes use of the ultimate 3′ end base of one of the PCR primers as an amplification criteria (34-37). If this base complements with the base on the target DNA, then amplification takes place. However, if a mismatch occurs, the resulting structure at the primer-target DNA junction may prevent amplification. Thus, by designing primers complementary for each of the variant bases and using them in two different PCR reactions, both homozygous and heterozygous samples can be genotyped. Alternatively, a single PCR amplification can be performed to genotype the samples by using two allele specific primers. In that approach, one of the allele specific primers contains an extra sequence at the 5′ end, and therefore yields a longer PCR product relatively to the other allele specific primer (38). The amplicons are fractionated on gels, and presence or absence of a particular band implies the genotype of the sample. Extensive optimization of the amplification conditions is generally essential for proper ARMS analysis (39, 40). Another approach relying on a similar principle is called Mismatch Amplification Mutation Assay (MAMA) (41). In this approach, the base just before the last base at the 3′ end of the primer is changed so as to create a mismatch with both of the alleles. Presence of two successive mismatches at the polymerization site of the primer ensures the inhibition of amplification. On the other hand, if the last base matches, amplification occurs. Therefore, biallelic discrimination is possible for all single base pair substitutions by using MAMA. Both MAMA and ARMS can be applied in combination with the TaqMan® system (28) (see below) in a more rapid and automated format (42, 43).

Single nucleotide primer extension (SNuPE) is a highly specific application for detection of the presence of a particular nucleotide (44, 45). This system is based on the PCR amplification of the sequence of interest and subsequent hybridization with a primer ending just prior to the variant base. This complex is then incubated with DNA polymerase in the presence of labeled individual dNTP's. If the dNTP in the environment is complementary to the base on the template, then this dNTP is incorporated to the 3′ end of the primer by the polymerase enzyme. Subsequent gel analysis and detection of the signal then reveals which dNTP is incorporated and thus allows determination of the nature of the base at the variant site.

Oligonucleotide Ligation Assay (OLA), on the other hand, depends on whether two oligonucleotide probes are perfectly complementary to the adjacent target sequences (46). If so, DNA ligase enzyme covalently links the two probes. However, if the match is not perfect due to the presence of a nucleotide change in one of the target sequences, the ligation reaction fails. Differential labeling of each of the two allele-specific probes with different fluorophores and ELISA based-detection of the products enables to genotype samples in a single reaction, therefore reducing the time and the material required for this type of analysis (47).

Allele Specific Oligonucleotide (ASO) hybridization is similar to those approaches where allele specific oligonucleotide probes are used in hybridization reactions under high stringency conditions (48). Detection of the signal from either or both of the allele specific probes indicates the genotype of the test samples. Application of large numbers of samples on a membrane (dot-blot) followed by hybridization with the probes provides a relatively high throughput screening.

A highly sophisticated and automated screening application similar to dot-blot is called DNA microchip technology. This technology can be used for several different purposes including exploration of gene expression, and detection of polymorphisms and mutations (reviewed in ref. 49-51). In short, the system is based on the hybridization of complementary strands of highly similar nucleic acids. Depending on the purpose, oligonucleotide probes, PCR products or cDNA molecules are arrayed at defined locations on a supportive matrix. Fluorescently labeled molecules are then hybridized with the molecules on the array, and the resulting fluorescence on the arrays is processed by image analysis. Mutation/polymorphism detection can be achieved by using differentially labeled test and reference samples in the same hybridization reaction. Gene expression can be analyzed by using samples derived from different tissues. Increased speed, automated analysis as well as ability to analyze large numbers of

sequences/samples at the same time, make DNA chip technology a major advance in development of high throughput analysis systems.

B. Detection of quantitative alterations in DNA

Homozygous deletion of a particular region of DNA can easily be detected by a variety of techniques such as Southern blotting or duplex PCR. On the other hand, detection of heterozygosity or determination of exact copy number of a sequence requires quantitative approaches to these assays.

Initial PCR-dependent quantitative analyses took advantage of the fact that the final quantity of amplification product can be used to calculate the amount of the starting template. Comparison of the quantities of amplicons derived from test and reference samples added into the reactions in equal amounts enabled estimation of the initial copy number of the sequence of interest. However, this approach is somewhat cumbersome and is not always reproducible because of the fact that tube-to-tube amplification efficiency may change. Several approaches to overcome this problem have been developed. In differential PCR, for example, the reference sequence and the test sequence are co-amplified in the same PCR reaction (52). Then, dosage analysis of the amplified products is used to estimate the relative quantity of the test sequence. However, different amplification efficiencies for the two sequences can reduce the accuracy of the result. Competitive PCR, on the other hand, relies on the simultaneous amplification of a "mimic" sequence along with the test sequence (53). This mimic sequence is slightly different from the target sequence allowing them to be distinguished after PCR by a variety of analyses, yet they can both be amplified by the same primer set. Thus, amplification efficiencies of the two primer sets are no longer of concern in competitive PCR.

The approaches mentioned so far can successfully detect quantitative alterations in both DNA and cDNA level. However, the requirements for; a) extensive optimization of the reaction conditions, and b) the extra steps after the PCR amplification, indicated the need for more practical techniques.

Real-time PCR is a powerful approach developed to quantify the nucleic acids during the amplification reaction. Therefore, no post-PCR procedure is required. LightCycler™ and TaqMan® technologies are two of the highly powerful and popular approaches used for the real time quantification purposes (28, 54-57). Both of these systems make use of sequence-specific and fluorescently labeled probes. For example, in the LightCycler™ system (28), whenever the two probes hybridize to the same target sequence during PCR cycles, the energy transferred from the donor to acceptor fluorophor reflects the original template quantity. The TaqMan® assay, on the other hand, relies on the 5′ nuclease activity of Taq DNA Polymerase (28, 55-57). An oligonucleotide probe complementary to an internal part of the sequence to be amplified is labeled at both 5′ and 3′ ends with two different fluorescence dyes (reporter and quencher dyes, respectively). During the PCR cycles, the probe hybridized with the target sequence is degraded by the 5′ nuclease activity of Taq Polymerase. Degradation of the probe results in the liberation of the reporter dye, which is detected as an increase in the reporter fluorescence. The 5′ nuclease activity ensures the quantification of only those templates for which the polymerization is underway by Taq Polymerase. A number of studies based on this assay have now been reported in literature (58-60).

Table 1. Summary of main advantages and disadvantages of techniques used for detection of genomic abnormalities

Technique/ Methodology	Application	Main advantages	Main disadvantages
CGH	Screening of chromosomal abnormalities	No need for fresh samples	Not every chromosomal region can be effectively analyzed
Matrix-based CGH	Screening of chromosomal abnormalities	Automated CGH analysis	Cost and labor effective?
SSCP, HA, DGGE	Variation scanning	Easy, and informative in most of the cases	No single optimum conditions could be established, not 100% sensitive
CE, DHPLC	Variation scanning	Automated, fast, informative	Variation specific-pattern may not be obtained in all cases
Melting analysis	Variation scanning	Automated, fast, sensitive	?
RE	Screening of a specific variation	Easy, cheap, 100% informative	Not applicable in all sequence variations
ARMS, MAMA	Screening of a specific variation	Easy, informative	Extensive optimization may be required
SNuPE, OLA, ASO	Screening of a specific variation	Highly specific	Labor effective?
Microchip technology	Screening of a specific variation	Automated, labor-effective	Optimization may be required
Real-time PCR	Quantitative analysis	Automated, fast, sensitive	?
Methylation-specific PCR	Methylation analysis	Fast and easy	Not quantitative
COBRA	Methylation analysis	Specific	Not applicable in all sequences
Ms-SNuPE	Methylation analysis	Specific and quantitative	Comparatively laborious

MOLECULAR EPIGENETICS

Alterations of the nucleotide sequence itself are not the only cause of phenotypic change. In fact, the epigenetic mechanism, DNA methylation, can result in selective gene silencing without any nucleotide change in the gene structure (reviewed in ref. 61). Nearly 50% of the genes in mammals contain CG-rich sequences (CpG islands) at their 5′ end. The chemical attachment of a methyl group to the C-5 position of cytosine residues at CpG dinucleotides has been shown to inhibit initiation of transcription at the promoter region of several genes. Imprinted genes, X-chromosome inactivation and certain types of tumor suppressor genes involved in development of neoplasm are extensively studied examples. The exact mechanisms underlying de novo and inherited DNA methylation are not well known yet. However, alterations in the affinities of transcription factors for the methylated regulatory DNA sequences have been well-documented (61). Moreover, another effect of methylation on gene expression is the recruitment of histone deacetylase to the methylated strand. This results in an increase in the affinities of histones for one another, and the formation of a more compact chromatin structure on the methylated promoters (61).

Initial studies for detection of methylated sequences were mainly dependent on digestion with a methylation specific restriction endonuclease followed by for Southern blot analysis. Increased sensitivity, specificity, and requirement for a considerably lower amount of genomic DNA have been achieved by the introduction of several PCR-based approaches. For instance, one PCR application makes use of the digestion of genomic DNA with a methylation specific restriction enzyme (e.g., HpaII) prior to PCR amplification (62). If methylation is present in the original template, then no amplification can be obtained from this digested template. However, the need for several controls to monitor complete digestion complicates this analysis. In addition, the requirement for the presence of a specific restriction site within the region of interest makes it far from a universal application. On the other hand, chemical (sodium bisulfite) treatment of genomic DNA bypasses the requirement for such sites, and thus provides a more practical alternative (63, 64). In short, on single stranded DNA sequences sodium bisulfite deaminates cytosine residues and converts them into uracil. The methylated cytosines, however, are resistant to this deamination reaction, and therefore remain unchanged. During PCR amplification, uracil and methylated cytosine residues are replaced by thymine and cytosine residues in the reaction mix, respectively. The location and the number of cytosine residues methylated on a sequence then can be determined by using several approaches. One of these applications involves the cloning of the PCR products followed by direct sequencing (65). Although somewhat laborious, direct sequencing provides the most accurate information about the location and the quantity of the methylated cytosine residues along a DNA segment. Alternatively, any mutation detection system like SSCA can also be coupled to dosage analysis to quantitatively elucidate DNA methylation (66). An allele specific PCR amplification (methylation specific PCR) can also be used for screening of methylation

status of a particular cytosine residue (67). In short, this application involves designing primers for specific amplification of either cytosine and thymine residues after the bisulfite treatment. Although it can be specifically used for detection of methylation, this approach is not suitable for quantitative purposes. Another highly specific approach, COBRA, relies on the creation of a restriction site for a restriction endonuclease after bisulfite treatment (68). Although specific, this approach can not be used for all DNA sequences. A relatively more laborious but highly specific approach is called as methylation specific SNuPE (Ms-SNuPE) (69). Ms-SNuPE depends on the detection of methylated or unmethylated cytosine residues at a specific location. The PCR amplified fragments are incubated with labeled dCTP or dTTP (or dGTP and dATP for the other strand), in separate reactions, in the presence of DNA polymerase and a specific primer ending just prior to the base of interest. If the nucleotide in the reaction tube is complementary to the nucleotide on the template immediately after the primer, it is incorporated into the 3′ end of the primer. Subsequent gel electrophoresis and detection of the signal indicate which nucleotide is incorporated and thus the methylated or unmethylated nature of the cytosine residue at that position. Dosage analysis of the signal derived can then be used to quantify the degree of methylation.

CONCLUSION

Variation in DNA sequences is a common molecular event. Characterization of the alterations leading to manifestation of a certain phenotypical abnormality is of great importance and essential for; a) establishing the diagnosis, b) better understanding of the structure, and therefore, the function of the genome and, c) the development of preventive and therapeutical strategies. Identification and analysis of polymorphisms is also of major interest; the information derived from them enables us to map genes, trace inheritance of genetic material, and evaluate the genetic structures of different populations, therefore enhancing understanding of the genome. Several techniques and methodologies to detect and identify these variations are available. DNA chip technology and CE allow high throughput and sensitive automated analyses though, not all laboratories may have the opportunity to establish such systems at the present time. Scientific advances that are having a major impact on medical research, are leading to the development and application of high speed, cost effective technologies.

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