TABLE 83e-1 COMPARISON OF CYTOGENETIC AND CYTOGENOMIC TECHNIQUES

Method	Requires Growing Cells	Detects Deletions and Duplications	Detects Balanced Structural Rearrangements	Detects Uniparental Disomy	Detection Limits (Lower Limit)
G-banding	Yes	Yes	Yes	No	5–10 million bases
Metaphase FISH	Yes	Yes	Yes	No	40–250 thousand bases
Interphase FISH	No	Yes	Some	No	40–250 thousand bases
CGH Array	No	Yes	No	No	Single exon or single gene
SNP Array	No	Yes	No	Some	Single exon or single gene

test is a function of the number of probes or DNA sequences present on the array. Arrays may use probes of different sizes (ranging from 50 to 200,000 base pairs of DNA) and different probe densities depending on the requirements of the application. Low-resolution platforms can have hundreds of probes, targeted to known disease regions, whereas high-resolution platforms can have millions of probes spread across the entire genome. Depending on the size of the probes and the probe placement across the genome, array-based testing may be able to detect single exon deletions or duplications.

Comparative Genomic Hybridization (CGH) and Single Nucleotide Polymorphism (SNP) Analysis CGH and SNP-based genotyping arrays can both be used for the analysis of genomic deletions and duplications. For both techniques, oligonucleotide probes are placed onto a slide or chip in a grid format. Each of these probes is specific for a particular genomic region. In array CGH, the amount of DNA from a patient is compared to that in a clinically normal control, or pool of controls, for each of the probes present on the array. DNA from a patient is fluorescently labeled with a dye of one color, and DNA from a control individual is labeled with another color. These DNA samples are then hybridized at the same time to the array. The resulting fluorescent signal will vary depending on whether both the control and patient DNA are present in equal amounts or if one has a different copy number than the other. SNP platforms use arrays targeting SNPs that are distributed across the genome. SNP arrays vary in density of markers and in the technology used for genotyping, depending on the manufacturer of the array. SNP arrays were initially designed to determine genotypes at a biallelic, polymorphic base (e.g., CC, CT, or TT) and have been increasingly used in genome-wide association studies to identify disease susceptibility genes. SNP arrays were subsequently adapted to identify genomic deletions and duplications (Fig. 83e-2). SNP arrays, in addition to identifying copy number changes, can also detect regions of the genome that have an excess of homozygous genotypes and absence of heterozygous genotypes (e.g., CC and TT genotypes only, with no CT genotypes). Absence of heterozygosity is sometimes associated with uniparental disomy (discussed later in this chapter) but is also observed when an individual's parents are related to one another (identity by descent). Regions of homozygosity have been used to help identify genes in which homozygous mutations result in disease phenotypes in families with known consanguinity.

Array-based techniques (which we will now refer to as cytogenomic analysis) have proven superior to chromosome analysis in the identification of clinically significant deletions or duplications. It is estimated that for a deletion or duplication to be visualized by standard cytogenetics it must be minimally between 5 and 10 million base pairs in size. In almost all cases, deletions and duplications of this size contain multiple genes, and these deletions and duplications are disease causing. However, utilization of array-based cytogenomic testing, which can routinely identify deletions and duplications smaller than 50,000 base pairs, reveals that clinically normal individuals all have some deletions and duplications. This presents a dilemma for the analyst to discern which smaller copy number variations (CNVs) are disease causing (pathogenic) and which are likely benign polymorphisms. Although initially burdensome, the cytogenomics community has been curating these CNVs for almost a decade, and databases have been created reporting CNVs routinely seen in clinically normal individuals and those routinely seen in individuals with clinical abnormalities. Nevertheless, each copy number variant that is identified in an individual undergoing genomic testing must be evaluated for gene content and overlap with CNVs in other patients and in controls.

Array technologies are DNA based, unlike cytogenetic technologies, which are cell based. Although resolution of gains and losses are greatly increased with array technology, this technique cannot identify structural changes. When DNA is extracted for array studies, chromosomal structure is lost because the DNA is fragmented for better hybridization to the slides. As an example, the array may be able to detect a duplication of a small region of a chromosome, but no information on the location of this extra material can be determined from this test. The location of this extra copy in the genome may be critical, as the chromosomal material may be involved in a translocation, insertion, marker, or other complex rearrangement. Depending on the chromosomal position of this extra material, the patient may have different clinical outcomes, and recurrence risks for the family can be significantly different. Often, combinations of array-based and cytogenetic-based techniques are required to fully characterize chromosomal abnormalities (see Table 83e-1 for comparison of these technologies).

NEXT-GENERATION SEQUENCING—BASED METHODOLOGIES

Recent advances in genomic sequencing, known as next-generation sequencing (NGS), have vastly increased the speed and throughput of DNA sequence analysis. NGS is rapidly finding its way into the diagnostic lab for detection of clinically relevant intragenic mutations, and new bioinformatic tools for analysis of genomic deletions and duplications are being developed. It is anticipated that NGS will soon allow the complete analysis of a patient's genome, with identification of intragenic mutations as well as chromosome abnormalities resulting in gain or loss of genetic material. Identification of completely balanced translocations is the most challenging for NGS, but recent reports of successes in this area suggest that in a matter of time, sequencing will be used for all types of genomic analysis.

INDICATIONS FOR CHROMOSOME/CYTOGENOMIC ANALYSIS

Cytogenetic analysis is most commonly used for (1) examination of the fetal chromosomes or genome during pregnancy (prenatal diagnosis) or in the event of a spontaneous miscarriage; (2) examination of chromosomes in the neonatal or pediatric population to look for an underlying diagnosis in the case of congenital or developmental anomalies, including short stature and abnormalities of sexual differentiation or progression; (3) chromosome analysis in adults who are facing fertility problems; or (4) examination of cancer cells to look for alterations that aid in establishing a diagnosis or contributing to the prognosis of a tumor (Table 83e-2).

PRENATAL DIAGNOSIS

Prenatal diagnosis is carried out by analysis of samples obtained by four techniques: amniocentesis, chorionic villous sampling, fetal blood sampling, and analysis of cell free DNA from maternal serum. Amniocentesis, which has been the most commonly used test to date, is usually performed between 15 and 17 weeks of gestational age and carries a small but significant risk for miscarriage. Amniocentesis can be performed as early as 12 weeks, but because there is a lower volume of fluid, the risks for fetal injury or miscarriage are greater. Chorionic villous sampling (CVS) or placental biopsy is routinely carried out earlier than amniocentesis, between 10 and 12 weeks, but a reported increase in limb defects when the procedure is carried out earlier than