In situ hybridization: The *in situ* hybridization method for DNA analysis can be performed on chromosomal preparations, frozen sections, cytologic specimens, or formalin-fixed paraffin-embedded sections. Its utility results from its ability to localize relevant DNA sequences within larger structures, thus linking biochemistry with cytogenetics or histochemistry. Genes can be localized to specific chromosomes by radioactive (autoradiography) or fluorochrome-labeled probes (fluorescence *in situ* hybridization, FISH). Important information about gene expression can be obtained in relation to the development of specific tissues by identifying the presence of mRNA in histological sections by autoradiography or fluorescence microscopy. Viral genomes can be specifically localized within tissues with this technique.

In situ hybridization is similar to blot methods, in that the probe is placed directly on the specimen that has the DNA sequences immobilized in a solid substrate. The tissue on the slide is treated with a protease to allow the probe access to the nuclear DNA, which is denatured by heating. The probe is added in an overlying hybridization buffer and hybridization occurs if the target DNA complementary to the probe is present. Extensive washing following the hybridization reaction removes unbound probe. The probe is then detected with a variety of reporting systems ranging from avidin-biotin enzyme reactions, fluorescence, or autoradiography. Once the duplex DNA strand forms between the target and the probe, the duplex can be quantitated or visualized on the basis of the label used. To date, these techniques have been primarily used to detect viral sequences in tissues. Alternatively, mRNA, which may be present in more copies than DNA, may be the target of the probe in tissue specimens.[9] In this way the DNA is detected and localized within individual chromosomes or in cells in tissues.

Restriction fragment length polymorphisms (RFLPs): In the early years of DNA diagnostics, the most frequently used technique to follow the transmission of genetic pathology was the use of RFLPs. The first step in this type of indirect gene analysis depends upon the identification of a fragment or piece of DNA that is so closely linked to the gene of interest that they are invariably transmitted together during meiosis. The tight linkage between this DNA fragment and the gene of interest makes the segregating fragment a clear signpost or road sign for the presence of the gene itself. This unique, close relationship could only be disrupted by a crossover between chromosomal homologues during meiosis that might transfer the marker or RFLP to the other homologue. In general, the smaller the physical distance between the gene of interest and RFLP the less likelihood that crossover or recombination might occur. In order to use this DNA marker to follow the gene, it is necessary that the restriction fragment that is generated be polymorphic or at least dimorphic in the family under consideration. This is critical since the RFLP enables you to follow the gene of interest only if it can distinguish one member of a chromosomal pair from the other. Polymorphism simply indicates that a given segment of DNA may have extensive sequence length variation without affecting the organism. This type of variation in the structure of DNA is seen particularly in regions that do not code for proteins, and regions which are not involved in important regulatory functions. Nevertheless, these differences or polymorphisms can affect any type of DNA sequence. A benign alteration in DNA producing an RFLP may occur within a non-coding sequence of a gene (intron), sequences between genes, repetitive DNA, and even within a coding sequence of a gene. These variations between sequences at the same loci on two homologous chromosomes can be detected by observing fragments of different size generated by cutting with a restriction enzyme. Pairs of variation or dimorphisms or alleles are inherited as traits and segregate in a Mendelian fashion. These inherited variations in the size of DNA fragments after restriction enzyme digestion are referred to as RFLPs. Figure 5 is a hypothetical or drawing-board example of the use of this technique for disease diagnosis. The theoretical family under study is from Kenya and had three female children. The oldest child is a 22-year-old female who has been diagnosed as Kallman's syndrome due to an absence of gonadotropin-releasing hormone (GnRH). The two younger children are 5 and 7 years, respectively. The parents would like to know if either or both of their younger girls will have Kallman's syndrome. The gene for GnRH has been isolated, cloned, and mapped to the short arm of the number 8 chromosome in man as indicated previously.[10] A reasonable approach to diagnosis would be to study the DNA of the affected child using standard Southern analysis with the GnRH gene as the probe. However, this approach reveals identical Southern blots in all family members, including the child affected with GnRH deficiency. Now one wonders whether the mutation in the GnRH gene is at a site that is not recognized by a restriction enzyme and requires allele specific oligonucleotide probing. The second approach is a possibility, especially since the entire nucleotide sequence encoding the normal human GnRH gene and its flanking sequences has recently been determined.[11] The synthesis of an allele specific oligonucleotide probe for the normal gene is technically easy. The different types and numbers of mutations affecting the mutated gene obfuscate the development of an ASO mutant probe. It might be difficult to synthesize and test a series of allele specific oligonucleo-