create a new restriction enzyme cut site. It becomes necessary to have alternative approaches to the detection of DNA mutations. As more genes are sequenced and mutations recognized, an even more direct approach to diagnosis can be utilized. Oligonucleotide probing depends upon the development of two DNA probes, one corresponding to the normal gene, and one complementary to the known mutant sequence. In practice, a sequence of 20 to 30 bp (oligomers) corresponding to the normal gene is manufactured. A similar series of 20 to 30 bp corresponding to the mutant gene is developed. Each of these oligomers can now be radiolabeled, and used to probe the DNAs of normal subjects, heterozygotes, and homozygotes for the mutation in question. An individual with two normal genes will hybridize to the normal probe, but not to its mutant counterpart. The DNA of an individual who is homozygous for the mutation will only hybridize to the mutant probe. A heterozygous carrier will reveal bands with both the normal and mutant probes (Fig. 4).

Oligonucleotide probing, in which there are two short pieces of DNA complementary to the normal and mutant allele, obviously requires sequence information on the normal and mutant genes. Genes that are known to have many different mutations are not so amenable to this approach. In such situations, one would need to know the spectrum of mutations and develop allele specific oligonucleotide probes for each of them. Fortunately, certain mutations tend to be more prevalent in specific ethnic groups or geographic areas, which can simplify the process. In addition, the hybridization conditions of allele-specific oligonucleotide probing must be carefully controlled and are usually quite stringent. In the technique of Reverse ASO screening, the various wild type and mutant DNAs are spotted to the filter and the labeled amplified DNA is used as a probe. ASO screening is more suitable for screening many patient samples for one or only a few possible missense mutations. Reverse ASO is better suited for screening one patient at a time for many different possible missense base pair substitutions. More recently the ability to amplify a target gene and sequence it directly has reduced some of the need for oligonucleotide probing. Nevertheless, by itself, or in adjunct with gene amplification, it is an important approach to DNA diagnosis at this time.

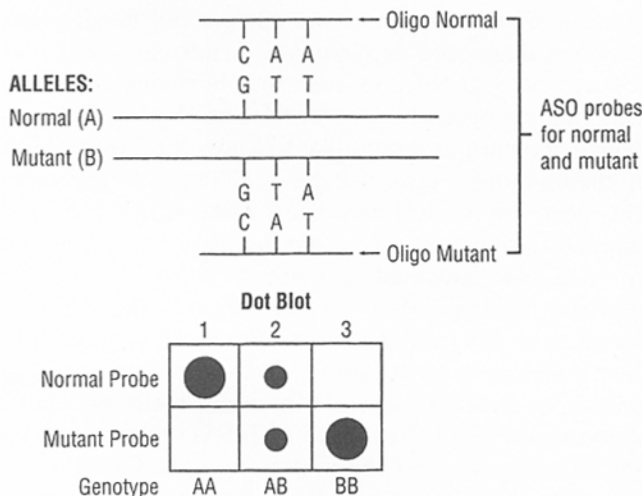

Fig. 4 This cartoon illustrates the use of allele specific oligonucleotide (ASO) probes with DOT BLOTS to identify the specific genotype of an individual's DNA. The ASO probes are synthesized to detect the normal (A) and mutant (B) alleles for a given gene. The DNA, obtained by cell lysis is denatured in high salt solution, fixed to a solid surface (nitrocellulose filters or blots) and exposed to the normal and mutant probes. DNAs that are homozygous normal (AA) will hybridize only with the normal probe (Lane 1) and DNAs from homozygous mutants (BB) only with the mutant probe (Lane 3). DNAs from individuals that are heterozygous (AB) will hybridize with both probes (Lane 2).

The final sequencing of the human genome will facilitate the continued development of synthetic oligonucleotides to probe for gene normalcy. Automated techniques for screening for mismatches between probe and target suggesting mutation will be part of the future technology of allele-specific oligonucleotide probing.

Dot blot (slot blot): The use of standard Southern blotting as described for gene analysis is specific, but requires restriction digestion of the DNA sample, electrophoresis and hybridization. A more rapid method used especially in microbiology is a technique called "slot" or "dot" blotting of DNA or RNA (Fig. 4). This variation of hybridization on a membrane eliminates the electrophoretic step and is used to detect the presence, absence, or amount of a genetic element. In this technique, isolated, unrestricted DNA or RNA, which is denatured in high salt concentration, is spotted onto nitrocellulose filters in a vacuum apparatus, baked, and hybridized to the labeled probe of interest. The technique is rapid and may be utilized to screen multiple samples. With radiolabeled probes of high specific activity, the resulting sensitivity (*i.e.* the smallest detectable amount) is about 0.2 to 0.5 pg. Although the technique is highly sensitive and rapid, it may not be as specific as the Southern blot. The signal generated from the hybridization in a slot blot may be nonspecific because appropriate band size is not determined. It has the weakness of not knowing the molecular size of the reacting nucleic acid target (equivalent to loss of specificity). Dot blotting must be standardized and controlled for each probe and appropriate stringency conditions determined. Nevertheless, it is a valuable technique for use with microgram quantities of samples and for the detection of specific DNA or RNA sequences in a sample.