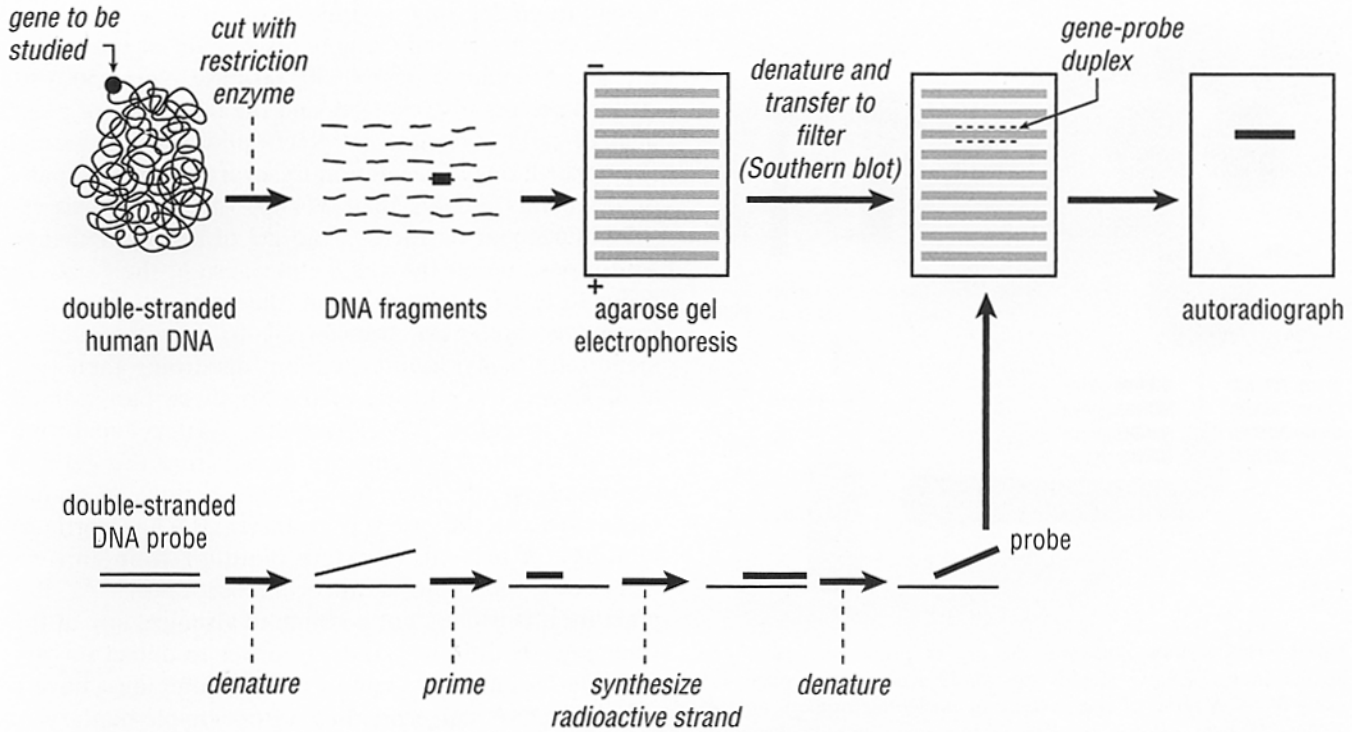

Fig. 3 Technique of Southern blotting. Extracted DNA is exposed to an appropriate restriction enzyme and the resultant fragments subjected to electrophoresis on an agarose gel. The denatured DNA fragments are transferred and permanently fixed to a nitrocellulose filter. The filter is placed in a solution of an appropriate probe of labeled DNA or RNA for hybridization to occur. Hybridization with the radioactively labelled probe is detected by autoradiography. (ref. 13. Reprinted by permission from McDonough PG. Molecular Biology in Reproductive Endocrinology. In Yen SSC, Jaffe RB, eds: *Reproductive Endocrinology*, third edition. Philadelphia: WB Saunders 1991 p 59.)

DNA probes can be prepared or obtained in many different ways. For example, a target sequence can be amplified by PCR, labeled, and used as a DNA probe. Other DNA probes can be obtained from portions of human DNA that are cloned into bacteriophages or plasmids and propagated in bacterial culture systems. Many DNA probes are the product of the direct chemical synthesis of DNA sequences from the nucleotides one by one in the correct order. Synthesis of oligonucleotides (about 20 nucleotides long) is routine and can be contracted through various service laboratories. Another example is the preparation of probes such as for the Y chromosome. One can isolate the Y chromosome by chromosome sorting, digest it into small fragments and put each of the Y fragments into a vector DNA such as a plasmid. The plasmids containing these Y fragments or inserts can be amplified or cloned in bacteria. After cloning, the individual Y inserts can be cut out of the plasmid vector and used as probes for Y DNA. Once a probe is constructed and refined, it can be used in a variety of ways. The DNA probe corresponding to the gene or sequence of interest must first be labeled or tagged so that its capture or hybridization with the target DNA can be easily detected. The probe DNA is first made single-stranded by boiling and is radiolabeled by replacing the phosphorus atoms with ^{32}P. Two of the procedures to radiolabel probes to a high degree of specific activity are "nick translation" and "random primers." The nitrocellulose or nylon membrane is soaked in a solution containing the radioactive probe at appropriate temperature and washed to eliminate the unbound probe. The presence of hybridized probe is detected by autoradiography. The probe or gene of interest will hybridize to its complementary strand that is immobilized on the nitrocellulose or nylon membrane. This hybridization must take place under controlled conditions of temperature and salt concentration (stringency). After baking the membrane and exposing it to X-ray, the autoradiograph will reveal the precise fragment(s) of DNA corresponding to the gene probe being utilized. An analysis of these fragments will reveal information concerning gene presence and structure.

Allele-specific oligonucleotide probes (ASO): In most instances, the mutation in the gene does not ablate or