

Southern, Northern, and Western Blots

Southern blotting: The Southern blot (named for its inventor) uses gel electrophoresis together with hybridization probes to characterize DNA restriction fragments. Genomic DNA or DNA from a specific source, such as a lambda phage or cosmid clone, is digested, usually to completion, with a restriction endonuclease (or sometimes with two or more restriction endonucleases). Electrophoresis is then used to separate the fragments by size. The fragments are then blotted from the electrophoretic gel onto a sheet of nitrocellulose or similar support material, and fixed onto it by heating or other treatments. The attached DNA fragments are denatured to separate the strands and annealed with a radioactive probe that is single stranded or also denatured. The nitrocellulose sheet is then washed, removing all unbound probe, and leaving radioactivity only where the probe has hybridized to the original DNA bound to the membrane. A sheet of X-ray film is then laid over the nitrocellulose for a time period long enough for the radioactivity to "expose" the film. When the film is developed, dark bands appear wherever there were DNA fragments capable of hybridizing with the radioactive probe. Size standards run on the same electrophoretic gel allow the sizes of the fragments identified by the probe to be determined (figure 9.31).

Interpreting Southern blots: Matching the positions of the radioactive spots with those of the size standards identifies the sizes of the digestion fragments that hybridize with the probe. For example, a cDNA probe for a gene that contains two internal cut sites for the restriction enzyme will generate three fragments (which will usually have enough size difference so that all three can be detected). More complex patterns generated by repetitive sequences form the basis for DNA fingerprinting, which will be discussed in a future lecture. Note that it is not necessary for the entire length of the probe to hybridize with the entire length of the DNA fragment. A relatively short complementary sequence (less than 100 bp) is usually enough to obtain a strong hybridization signal. In addition, modification of the annealing conditions can alter the stringency of hybridization (the precision of base-pair matching needed for hybridization). By using reduced stringency, it is often possible to obtain hybridization between slightly mismatched sequences, such as the coding sequences for the same protein from different species.

Alternative sources of multiple bands: If a probe hybridizes with only a single band, one can conclude that only one size class of fragments contains the probe sequence. However, if two or more bands hybridize, two very different interpretations are possible: 1) that there is a restriction endonuclease cut site within the sequence that hybridizes to the probe, causing the hybridizing sequence to be cleaved into two different restriction fragments that can both hybridize with parts of the probe; or 2) that more than one copy of the target sequence was present in the original DNA sample, with each copy emerging in a different sized restriction fragment. It is often desirable to use a relatively small probe to minimize the chance of a single target sequence being cleaved into two halves during digestion of the original sample. On the other hand, there are times when a much larger probe is more effective, for example to be certain that all of the genomic restriction fragments that contain any part of a protein coding sequence have been identified.

Dot blotting: In cases where the goal is simply to test for the presence of a specific sequence, such as whether or not an attempt to clone a particular gene has been successful, size separation can be bypassed altogether and a bit of DNA from each putative clone can be transferred to a nitrocellulose membrane as a "dot", followed by hybridization to a probe and autoradiography. Only those dots that contain the desired sequence will hybridize and become labeled. This procedure is similar to the colony and plaque hybridization techniques discussed in previous lectures on gene cloning.

Northern blotting: In a Northern blot (named because it is the opposite of a Southern blot), RNA molecules of varying lengths (often naturally occurring mRNAs) are separated by size and blotted onto nitrocellulose. A DNA probe (often a cDNA) is then used to identify bands that contain particular sequences. Northern blots are particularly useful for determining the conditions under which specific genes are being expressed, including which tissues in a complex organism express which of its genes at the mRNA level.

Western blotting: In a Western blot, proteins are separated by electrophoresis and blotted onto an appropriate support matrix. The matrix is then exposed to an antibody to the desired protein and all unbound antibody is washed off. The bands (or spots in a dot blot) where the antibody has bound are then detected by various means, such as binding of a second antibody that is radioactively labeled and specific for the first antibody