8.6: DNA Analysis: Blotting and Hybridization

Bands of DNA in an electrophoretic gel form only if most of the DNA molecules are of the same size, such as following a PCR reaction, or restriction digestion of a plasmid. In other situations, such as after restriction digestion of chromosomal (genomic) DNA, there will be a large number of variable size fragments in the digest and it will appear as a continuous smear of DNA, rather than distinct bands. In this case, it is necessary to use additional techniques to detect the presence of a specific DNA sequence within the smear of DNA separated on an electrophoretic gel. This can be done using a “Southern Blot”.

Southern Blots

A Southern blot (also called a Southern Transfer) is named after Ed Southern, its inventor. In the first step, DNA is digested with restriction enzymes and separated by gel electrophoresis (as discussed above). Then a sheet or membrane of nylon or similar material is laid under the gel and the DNA, in its separated position (bands or smear), is transferred to the membrane by drawing the liquid out of the gel, in a process called blotting (Figure 8.6.1). The blotted DNA is usually covalently attached to the nylon membrane by briefly exposing the blot to UV light. Transferring the DNA to the sturdy membrane is necessary because the fragile gel would fall apart during the next two steps in the process. Next, the membrane is bathed in a solution to denature (double stranded made single stranded) the attached DNA. Then a hybridization solution containing a small amount of single-stranded probe DNA that is complementary in sequence to a target molecule on the membrane. This probe DNA is labeled using fluorescent or radioactive molecules, and if the hybridization is performed properly, the probe DNA will form a stable duplex only with those DNA molecules on the membrane that are exactly complementary to it. Then, the unhybridized probe is washed off and remaining radioactive or fluorescent signal will appear in a distinct band when appropriately detected. The band represents the presence of a particular DNA sequence within the mixture of DNA fragments.
Genomic DNA that has been digested with a restriction enzyme is separated on an agarose gel, then the DNA is transferred from the gel to a nylon membrane (grey sheet) by blotting. The DNA is immobilized on the membrane, then probed with a radioactively labeled DNA fragment that is complementary to a target sequence. After stringent washing, the blot is exposed to X-ray film to detect what size fragment the probe is bound. In this case, the probe bound to different-sized fragments in lanes 1, 2, and 3. In the last image the orange represent the position of the digested DNA, but it is not actually present on the X-ray film. (Original-J. Locke-CC:AN)

The probe is sequence specific (requires complementarity). However, variation in hybridization temperature and washing solutions can alter the stringency of the probe. At maximum stringency (higher temperature) hybridization conditions, probes will only hybridize with the exact target sequences that are perfectly complementary (maximum number of hydrogen bonds). At lower temperatures, probes will be able to hybridize to targets to which they do not match exactly, but only are roughly complementary for part of the sequence.

Southern blotting is useful not only for detecting the presence of a DNA sequence within a mixture of DNA molecules, but also for determining the size of a restriction fragment in a DNA sample. Southern blots are useful for detecting fragments larger than those normally amplified by PCR, and when trying to detect fragments that may be only distantly related to a known sequence. Applications of Southern blotting will be discussed further in the context of molecular markers in a subsequent chapter. Southern blotting was invented before PCR, but PCR has replaced blotting in many applications because of its simplicity, speed, and convenience. Following the development of the Southern blot, other types of blotting techniques were invented.

**Northern Blots**

The Northern blot involves the size separation of RNA in gels like that of DNA. Because we wish to determine the native size of the RNA transcript (and because RNA is single stranded) no restriction enzymes are ever used. Because most RNA is single stranded and can fold into various conformations thorough intra-molecular base pairing, the electrophoresis separation is more haphazard and the bands are often less sharp, compared to that of double stranded DNA.
Western Blots

In a Western blot, protein is size separated on a gel (usually an acrylamide gel) before transferring to a membrane, which is then probed with an antibody that specifically binds to an antigenic site on the target protein. This antibody is then detected by other antibodies with some fluorescent or color production marker system. It will also give bands proportional to the amount and size of the target protein (Figure 8.6.2).

![Figure 8.6.2: Western Blot using a anti-lipoic acid primary antibody and an IR-dye labelled secondary antibody in Leishmania major extracts. (Wikipedia-TimVickers–CC:AS)](https://bio.libretexts.org/Bookshelves/Genetics/Book%3A_Online_Open_Genetics_(Nickle_and_Barrette-Ng)/08%3A_Techniq…)

A comparison of all three blotting methods is shown in Figure 8.6.3.
Figure 8.6.3: Comparison of Southern, Northern, and Western blots. Size and amount of DNA, RNA, and polypeptides can be determined using similar blotting methods. DNA is in blue, RNA in red, and polypeptides in green. A marker lane is shown in the left of each gel to determine size. A eukaryote cell is shown, but the same methods can be applied to prokaryotes, too. (Original-Locke-CC:AN)

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