Higher molecular weight proteins and high acrylamide concentrations require longer transfer times. Transfer efficiency can be checked via stain in Ponceau S solution before preparing the membrane for detection of proteins.

Following transfer, the membrane is prepared for antibody staining by incubation with a blocking solution which prevents nonspecific binding. Typically 5% nonfat dry milk or 3% bovine serum albumin (BSA) are used for blocking and are in solution with a detergent. This is then followed by incubation of the membrane with primary antibody to target of interest. This facilitates the direct detection of the protein of interest. A secondary antibody targets the primary antibody's Fc region (species specific) and is linked to an enzyme that catalyzes a chromogenic, fluorescent, or chemluminescent signal. This provides the signal amplification to visualize very small quantities of protein. Secondary antibodies are conjugated to biotin or enzymes such as alkaline phosphatase (AP) or horseradish peroxidase (HRP). When the enzyme substrate is added, either a colored precipitate (colorimetric detection), or a chemiluminescent or fluorescent product is formed and the light signal is captured.

Table 1. Comparisons between Northern, Southern and Western Blots.

	Southern Blot	Northern Blot	Western Blot
Target for Detection	DNA	RNA	Protein
Sample Prep	DNA extraction, enzymatic digestion by restriction enzymes	RNA isolation, denaturation w. formaldehyde	Protein extraction, protein denatured with SDS
Separation	Agarose Gel Electrophoresis	Agarose Gel Electrophoresis	SDS-PAGE
Membrane	Nylon	Nylon	Nitrocellulose or PVDF
Probe	Nucleic acid probe w/ single stranded sequence homologous to target DNA	RNA, DNA or oligodeoxynucleotide	Primary antibody(Direct), Primary + Secondary (Indirect) conjugated to fluorophore, reporter enzyme
Detection Methods	X-ray film, Chemiluminescence	X-ray film, Chemiluminescence	CCD Camera, LED or Infrared imaging