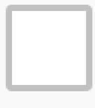
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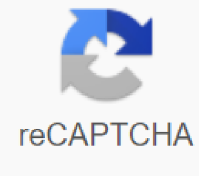

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Southern and northern blotting pdf

Hybridization of the southern blot refers to the detection of specific FRAGMENTs of DNA that have been separated by electrophoresis gel (Figure 1). After electrophoresis separated DNA fragments are denatured and transferred to nitrocellulose (or nylon) membrane leaf by blotting. The blotting gel is supported on a sponge in the alkaline bath, and the buffer is absorbed through the gel and sheet with paper towels stacked on top of the sheet of nitrocellulose. The buffer denatures DNA and transmits single stranded fragments of the gel to the surface of the sheet, where they stick firmly. A sheet of nitrocellulose containing related single-purpose DNA fragments is inserted out of the gel and placed in a sealed plastic bag or box along with a buffer containing a marked DNA probe specific to the DNA sequence of the target. The sheet is exposed to the probe in conditions conducive to hybridization. After hybridization, the sheet is removed from the bag, thoroughly washed to remove non-hybrid probes and viewed with autoradiography or ultraviolet light, depending on the labels used (radioactive fluorescent). Adaptation of the southern blotting North blotting, in which RNA molecules are electroforated through the gel instead of the DNA. Figure 1. The principle of the South blotting. A flow diagram describing the overall process of detecting RNA using north blotting. Northern blot, or RNA spot, is a method used in molecular biology research to study gene expression by the detection of RNA (or isolated mRNA) in a sample. With north blotting it is possible to observe cellular control over structure and function by identifying particular rates of gene expression during differentiation and morphogenesis, as well as in abnormal or sick conditions. The northern blot involves the use of electrophoresis to separate RNA samples by size and detection by hybridizing the probe, complementing part or the entire target sequence. The term northern blot actually refers specifically to capillary transmission of RNA from electrophoresis gel to blotting membrane. However, the whole process is commonly referred to as northern blotting. The Northern Blot technique was developed in 1977 by James Alvin, David Kemp and George Stark of Stanford University with the participation of Gerhard Heinrich. Northern blotting takes its name from its resemblance to the first blotting technique, a southern spot named after biologist Edwin Southern. The main difference is that RNA, not DNA, is analyzed in the northern blot. The General Blotting Procedure begins with the extraction of common RNA from a homogeneous tissue sample or from cells. Eukaryotic mRNA can be isolated by oligo (DT) cellulose chromatography to isolate only those RNA with poly(A) tail. RNA samples separated by gel electrophoresis. Because the gels are fragile and the probes cannot enter the matrix, RNA samples, now separated in size, are transferred to the nylon membrane through a capillary or vacuum blotting system. Installation of a capillary system to transfer RNA from electrophoresis gel to membrane. The positive-charged nylon membrane is most effective for use in northern blotting since negatively charged nucleic acids have a high affinity for them. The transmission buffer used for blotting usually contains forms because it lowers the annalization temperature of the probe-RNA interaction, thereby eliminating the need for high temperatures that can lead to RNA degradation. After RNA has been transferred to the membrane, it is immobilized through a covalent connection with the membrane by ultraviolet light or heat. Once the probe has been tagged, it is hybridized to RNA on the membrane. Experimental conditions that can affect the effectiveness and specifics of hybridization include ionian strength, viscosity, duplex length, incompatible base pairs and base composition. The membrane is washed to make sure that the probe is bound specifically and to prevent the appearance of background signals. Hybrid signals are then detected by X-ray film and can be quantified by densitometry. To create controls for comparison in the northern blot, samples that do not display a gene product of interest can be used after micro-array or RT-PCR have been identified. Gels RNA work on the agaroz gel formaldehyde to highlight 28S (top range) and 18S (lower band) ribosomes units. RNA samples are most often divided into agarose gels containing formaldehyde as a denature agent for RNA to limit the secondary structure. The gels can be colored by etBr bromide and viewed under ultraviolet light to observe the quality and quantity of RNA before blotting. Polyacri dominance gel electrophoresis with urea can also be used in the RNA department, but is most commonly used for fragmented RNA or microRNAs. The RNA ladder often passes next to the samples on the electrophoresis gel to observe the size of the resulting fragments, but in total RNA samples ribosomes can act as markers of size. Since the large ribosomes subunin area 28S (approximately 5kb) and the small ribosomes division 18S (approximately 2kb) two prominent bands appear on the gel, the greater the almost half the intensity is less. Probes for northern blots consist of nucleic acids with additional consistency for all or part of RNA of interest, they may be DNA, RNA or oliginocleotides with a minimum of 25 additional bases to target sequence. RNA probes (riboprotests) that in vitro, are able to withstand stricter washing steps, preventing some background noise. Usually kDNA with tagged primers for the RNA sequence of interests to act as a probe in the northern slick. Probes must be labeled either with radioactive isotopes (32P) or by chemiluminescence, in which alkaline phosphate or horseradish peroxidease (HRP) is broken down by chemical substrates that produce detectable light radiation. Chemistry can occur in two ways: either the probe is attached to an enzyme or the probe is tagged with ligand (e.g. biotin), for which ligand (e.g. avidine or streptavidine) is attached to an enzyme (e.g. HRP). X-ray film can detect both radioactive and chemiluminescent signals, and many researchers prefer hemiluminescent signals because they are faster, more sensitive and reduce the health hazard that is associated with radioactive labels. The same membrane can be examined up to five times without significant loss of target RNA. The use of Northern blotting allows to observe the expression of a particular gene between tissues, organs, stages of development, levels of environmental stress, pathogenic infection, and during treatment. The technique was used to show overexertion of oncogenes and downregulation of tumor suppressor genes in cancer cells compared to normal tissue, as well as gene expression when organ transplants were not transplanted. If the regulated gene is observed by an abundance of mRNA on the northern blot, the sample can be sequenced to determine whether the gene is known to researchers or a new find. Expression patterns obtained under these conditions can give an idea of the function of this gene. Since RNA is first separated by size, if only one type of variance probe is used at the level of each band on the membrane can provide an understanding of the size of the product by offering alternative splicing products of the same gene or repetitive sequence motives. The difference in the size of the gene product may also indicate removals or errors in the processing of the transcript. By changing the purpose of the probe used along a known sequence, it is possible to determine which area of RNA is missing. The pros and cons of Gene Expression Analysis can be performed by several different methods, including RT-PCR, RNase protection analyses, micro-array, RNA-sec, serial gene expression analysis (SAGE), and northern blotting. Micro-array is quite commonly used and is generally consistent with data from northern spots; however, at times northern blotting is able to detect small changes in the expression of a gene that microarrays cannot. The advantage of micro-array over northern spots is that thousands of genes can be visualized at the same time, while northern spots typically look at one or a small number of genes. The problem in the northern part of the blotting is degradation of RNases samples (both endogenous to sample and through pollution) that can be avoided by proper sterilization of glassware and the use of RNase inhibitors such as DEPC (ditilpirocarbonate). Chemicals used in most northern spots may be a risk to the researcher, as formaldehyde, radioactive material, etidia bromide, DEPC and UV radiation are harmful under certain effects. Compared to RT-PCR, the northern blot has low sensitivity, but it also has a high specificity, which is important for reducing false-post results. Benefits of using northern blotting include detection of RNA size, observation of alternative splicing products, use of probes with partial homologation, quality and quantity of RNA can be measured on the gel before blotting, and membranes can be stored and reprobed for years after blotting. For the northern blotting to detect acetylcholinesterase mRNA non-radioactive method has been compared to radioactive technology and found sensitive as radioactive, but does not require protection from radiation and less time. Reverse Northern Blot Researchers sometimes use a variant of the procedure known as reverse northern blot. In this procedure, the nucleic acid substrate (which is attached to the membrane) is a set of isolated DNA fragments, and the probe is RNA extracted from tissue and radioactively labeled. The use of DNA microarrhes, which were widely used in the late 1990s and early 2000s, is more like a reverse procedure because they involve the use of isolated DNA fragments attached to the substrate and hybridization using a probe made from cellular RNA. Thus, the reverse procedure, although initially unusual, allowed the northern analysis to turn into profiling of gene expression, in which many (perhaps all) genes in the body can control their expression. 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