Northern blot

Manoj Nath, Post Graduate Teacher
Department of Human Physiology, Women's College
Agartala, Tripura (West), India, PIN-799001

The northern blot is a technique used in molecular biology research to study gene expression by detection of RNA (or isolated mRNA) in a sample. With northern blotting it is possible to observe cellular control over structure and function by determining the particular gene expression levels during differentiation, morphogenesis, as well as abnormal or diseased conditions.

The northern blot technique was developed in 1977 by James Alwine, David Kemp, and George Stark at Stanford University. Northern blotting takes its name from its similarity to the first blotting technique, the Southern blot, named for biologist Edwin Southern.

Procedure

The first step in a northern blot is to denature, or separate, the RNA within the sample into single strands. The RNA molecules are then separated according to their sizes by gel electrophoresis. Following separation, the RNA is transferred from the gel onto a blotting membrane. Next, the membrane is treated with a small piece of DNA or RNA called a probe, which has been designed to have a sequence that is complementary to a particular RNA sequence in the sample; this allows the probe to hybridize, or bind, to a specific RNA fragment on the membrane. In addition, the probe has a label, which is a radioactive atom or a fluorescent dye. Thus, following hybridization, the probe permits the RNA molecule of interest to be detected from among the many different RNA molecules on the membrane.

Northern blot analysis reveals information about RNA identity, size, and abundance, allowing a deeper understanding of gene expression levels.

Steps of Northern Blotting may be summarized as follows

RNA Isolation-

Obtaining high-quality, intact RNA is a critical step in performing northern blot analysis.

Probe Generation-

RNA probes can be produced by *in vitro* transcription reactions.

Denaturing-

Once RNA samples are isolated, the next step in northern blot analysis is denaturing agarose gel electrophoresis.

Transfer to Solid Support and Immobilization-

Once separated by denaturing agarose gel electrophoresis, the RNA is transferred to a positively charged nylon membrane and immobilized for subsequent hybridization.

Hybridization with Probe-

Although double-stranded DNA probes must be denatured prior to use, RNA probes and single-stranded DNA probes can be diluted and then added to the prehybridized blot. As few as 10,000 molecules can be detected.

Washing-

After hybridization, unhybridized probe is removed by washing in buffer. Low stringency washes remove

the hybridization solution and unhybridized probe. High stringency washes remove partially hybridized molecules.

Detection-

The hybrid signals are then detected by X-ray film and can be quantified by densitometry.

Applications

- Northern blotting allows us to observe a particular gene's expression pattern between tissues, organs, developmental stages, environmental stress levels, pathogen infection, and over the course of treatment.
- The technique has been used to show over expression of oncogenes and downregulation of tumor-suppressor genes in cancerous cells when compared to 'normal' tissue, as well as the gene expression in the rejection of transplanted organs.
- If an upregulated gene is observed by an abundance of mRNA on the northern blot the sample can then be sequenced to determine if the gene is known to researchers or if it is a novel finding.
- The variance in size of a gene product can also indicate deletions or errors in transcript processing. By altering the probe target used along the known sequence it is possible to determine which region of the RNA is missing.