

Northern Blot Analysis with Digoxigenin in PCR-labeled Probes in Research Samples from Myocardial Biopsies

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Abstract

To establish a simple non-radioactive approach for identifying mRNAs on Northern blots, PCR digoxigenin (DIG)-labeled cDNAs are used as probes in combination with chemiluminescence-based detection. cDNAs are designed as probes for several mRNAs in research samples derived from small myocardial biopsies [1]. Total RNA (5–10 µg) was applied to the gel and blotted onto the positively charged nylon membranes (Roche Molecular Biochemicals). The membranes are hybridized with 25–200 ng/ml DIG-labeled probes. Following hybridization, hybrids were detected using the CSPD chemiluminescent alkaline phosphatase chemiluminescence substrate (Roche Molecular Biochemicals). The quantification analysis was performed using the NIH Image 1.6 software. The 28S gene expression was used to normalize our results.

Introduction

Identification of a particular mRNA is normally achieved through Northern blot analysis by hybridization with a cDNA labeled with ³²P. Several non-radioactive detection methods have been developed for nucleic acid blotting as alternatives to techniques employing radioactivity [1]. The aim of this study is to develop a sensitive, non-radioactive, semiquantitative procedure for analyzing gene expression in small myocardial biopsies.

We report here our procedure of labeling cDNA probes with DIG-dUTP during PCR, followed by Northern blot analysis of atrial natriuretic factor (ANF), brain natriuretic peptide (BNP) and heat shock protein (HSP) genes. The 28S gene expression was used to normalize our results.

Materials and Methods

RNA Isolation

Total RNA was isolated from research samples derived from myocardial biopsies according to the TriPure Isolation Reagent (Roche Molecular Biochemicals)

procedure, which is an improvement on the single-step RNA isolation method developed by Chomczynski and Sacchi [2]. Total RNA extracted was quantified by spectrophotometric measurements and diluted to 200 ng/µl. Dilutions from different RNA samples were put together in a pool.

For the Northern blot analysis, 5–10 µg of total RNA was separated by 1.5% agarose gel electrophoresis under denaturing conditions and blotted onto positively charged nylon membranes (Roche Molecular Biochemicals). UV cross-links were performed, and completeness of the transfer was verified.

Probe Labeling

The 200 ng/µl RNA pool was first transcribed to the cDNA and then amplified by Taq Polymerase (Roche Molecular Biochemicals). Reverse transcription was performed at 42°C for 1 hour using specific primer according to the target sequence. The PCR conditions were adapted to the different target genes. During PCR, cDNA probes were labeled by incorporation of DIG-11-dUTP (Roche Molecular Biochemicals). The PCR fragments were characterized by 6% acrylamide gel electrophoresis and stained by silver nitrate. They were eluted from the acrylamide gel left overnight at 37°C in 0.5 M ammonium acetate (pH 8) and 1 mM EDTA buffer [3]. Sodium acetate (3 M, pH 5.2) and ultrapure ethanol precipitation was performed [3].

Estimating the yield of DIG-cDNA

The DIG-cDNA was diluted 1:200 in a DNA dilution buffer (Roche Molecular Biochemicals). Next, 1 µl of the sample and 1 µl of the labeled control DNA (Roche Molecular Biochemicals) was spotted onto a nylon membrane. The membrane was then detected using the CSPD chemiluminescent alkaline phosphatase substrate (Roche Molecular Biochemicals). The spots were quantified by densitometric analysis (NIH Image 1.6 software): the ratio between samples and labeled control allowed the labeling yield to be estimated.

Hybridization and detection

Following prehybridization for 2 hours at 42°C in DIG Easy Hyb buffer (Roche Molecular Biochemicals) or standard buffer containing 50% formamide, hybridization was carried out using 6 ml per 100 cm² DIG Easy

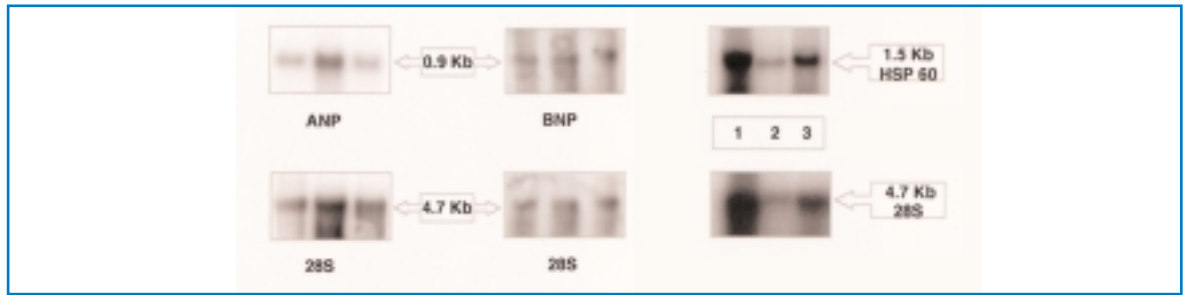


Figure 1: Detection of human mRNAs with DIG-labeled probes. ANP and BNP mRNA: 10 µg from human right atrium. Lane 1: 30 µg from human right atrium; lane 2: 5 µg from human right ventricle; lane 3: 10 µg from human right ventricle.

Hyb buffer (Roche Molecular Biochemicals) or standard buffer containing 50% formamide overnight at 42°C. The DIG-labeled cDNA probes were used at concentrations ranging from 25 ng/ml to 200 ng/ml. Following hybridization, filters were detected using the CSPD chemiluminescent alkaline phosphatase substrate. The quantification analysis was performed using the NIH Image 1.6 software.

Conclusions

DIG-11-dUTP incorporation during PCR is a very simple procedure for labeling specific cDNAs [4]. The resulting probes are very sensitive, and the yield from the labeling reaction is quite high. Northern blot analysis of low copy RNAs in research samples derived from small myocardial biopsies is shown to be feasible without radioactive labeling.

References

- [1] Trayhurn, P., *et al.* (1994) *Analytical Biochemistry* **222**: 224-230.
- [2] Chomczynski, P., and Sacchi, N., (1987) *Analytical Biochemistry* **162**: 156-159.
- [3] Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*. Second edition, Cold Spring Harbor Laboratory Press.
- [4] *The DIG System User's Guide for Filter Hybridization* (1995), Boehringer Mannheim.

For further information about this field of research, please order the "The DIG System User's Guide for Filter Hybridization" from our local representative.

Product	Cat. No.	Pack Size
TriPure Isolation Reagent	1 667 157	50 ml
	1 667 165	200 ml
Nylon membranes, positively charged	1 209 299	20 sheets
	1 209 272	10 sheets
	1 417 240	1 roll
Taq DNA Polymerase, 5 units/µl	1 146 165	100 units
	1 145 173	500 units
	1 418 432	4 x 250 units
	1 596 594	10 x 250 units
	1 435 094	20 x 250 units
Taq DNA Polymerase, 1 unit/µl	1 647 679	250 units
	1 547 687	4 x 250 units
Anti-Digoxigenin AP	1093274	150 U (200 µl)
Digoxigenin-11-dUTP, alkali-labile	1 573 152	25 nmol
	1 573 179	125 nmol
Digoxigenin-11-dUTP, alkali-stable	1 093 088	25 nmol
	1 558 706	125 nmol
	1 570 013	5 x 125 nm
DIG DNA Labeling Mix	1 277 065	50 µl (25 reactions)
DIG-labeled Control DNA	1 585 738	50 µl
CSPD	1 655 884	1 ml
DIG Easy Hyb	1 603 558	500 ml
DIG Easy Hyb Granules	1 796 895	1 set

