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Application Note

SCIENCE IMAGING SYSTEMS

Application Note No.21

Gel Shift Assay Using Fluorescent Probe

Foreword

Gene expression analysis is an increasingly active area of research.

One method useful for analyzing gene expression is the gel shift (electrophoretic mobility shift) assay, which detects complexes of DNA and proteins that bind with specific base sequences. Almost all conventional methods use RI for detection. Although attempts have been made to switch to fluorescent detection, the low sensitivity and poor band definition of the fluorescent method have proved difficult obstacles to practical utilization.

Now, however, a group at the Biological Resources Utilization Laboratory of the Horticulture Department at Chiba University reports successful detection by the gel shift assay using fluorescence. They graciously offered to outline their findings in this Application Note No. 21.

Contents

Summary

- Gel shift assay can be conducted in an ordinary laboratory without use of RI.
- The procedures are simpler and safety is markedly better than when using RI.

1 Introduction

Gene expression is regulated by interaction of certain types of protein (transcription factors) with characteristic gene arrangements (cis-arrangements) present in the gene control region. In transcription control factor studies, a need often arises to determine what kind of transcription factors bind with what kind of cis-arrangements. The gel shift assay is frequently used in such situations because it offers a simple method for *in vitro* analysis of binding capability between transcription-regulating cis-arrangements and transcription factors. The gel shift assay utilizes the fact that when oligonucleotides contained in a gene arrangement of interest are labeled and then reacted for a given period of time with a nucleoprotein prepared beforehand, the complex of the labeled DNA and the nucleic protein migrates a shorter distance when electrophoresed through a non-denaturing polyacrylamide gel than the same DNA not bound with the protein (Fig. 1-1). Because of the need to label the oligonucleotides with a radioactive substance (e.g., [γ-^{32}P]ATP or [α-^{32}P]dNTP), however, the method tended to be avoided out of concern about the dangers of radioactive substances to the human body, limitations on place of use and difficulty of disposal.

At our laboratory, we frequently use the gel shift assay in research related to transcription control of oxygen-regulated plant genes reduced with nitrate. We therefore developed a method that uses a nucleic acid fluorescent labeling agent in combination with a Fuji Photo Film fluorescence imaging system to perform gel shift assays without using radioactive substances. The method overcomes the problems encountered when using RI and also eliminates various precautions involved in handling radioactive substances. Another advantage is that fluorescently labeled oligonucleotides can be stored over long periods (several months).

Cis-arrangement
DNA base sequence that governs gene promoter activity

Transcription factor
DNA-binding protein that regulates transcription by acting on cis-arrangement

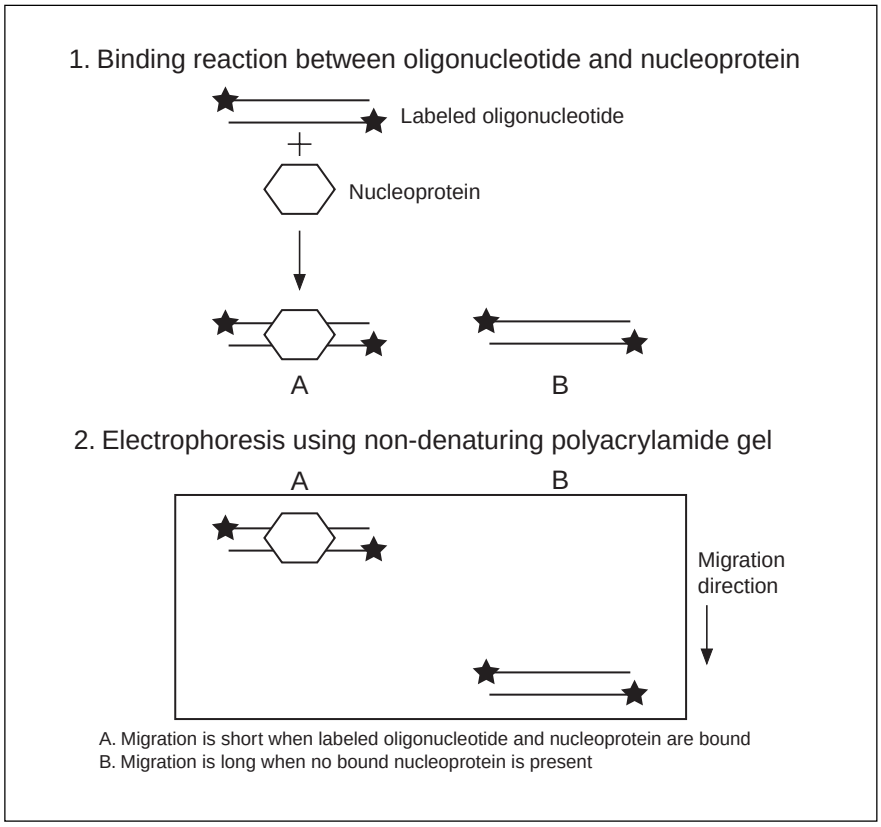

Fig. 1-1

Fig. 1-1 Conceptual rendering of the gel shift assay

2 Test Procedure

Preparation of nucleoprotein

Nucleoprotein was prepared from cultured spinach cells. [1)]

Preparation of fluorescently labeled probe

Complementary synthetic oligonucleotide (20μM) was annealed by heating to 70°C in the presence of 50mM NaCl followed by cooling to 25°C over 30 min. The 5' terminal was then fluorescently labeled using 5'-Oligolabelling Kit for Fluorescence [*1] (Amersham Bioscience) according to the manufacturer's directions. The unreacted fluorescent substance was removed using MicroSpin ™ G-25 Column (Amersham Bioscience). The fluorescently labeled oligonucleotide obtained was used as the fluorescent probe in the gel shift assay method.

[*1)] The fluorescent labeling agent contained in this kit is 5-fluorescein iodoacetamide.

Gel shift assay

Binding reaction between oligonucleotide and nucleoprotein

The nucleoprotein and the probe were reacted under the following conditions.

Preservative solution	Concentration	Composition [2)]	
1 x Binding Buffer	4**X**	80mM HEPES-KOH (pH7.9), 200mM KCl, 20mM DTT 0.8mM EDTA (pH8.0), 2mM PMSF, 40%(v/v)glycerol	5.5 μl
Poly(dI-dC)	1mg/ml		2 μl
Fluorescent probe			1~2 μl
Nucleoprotein			5 μl
DW			7.5~8.5 μl
			22 μl

The reaction was continued for 30 min at room temperature, followed by addition of 3μl of 20 x GS Dye (5% (v/v) glycerol, 0.05M EDTA, BPB, XC) containing no dye solution [*2].

Electrophoresis

(1) 4.5% non-denaturing polyacrylamide gel having the following composition was prepared.

Preservative solution	Concentration	Composition [2)]	
4.5% (w/v)Acrylamide	30%(w/v)		1.5 ml
1**X**TGE Buffer	5**X**(pH8.5)	500mM Tris-HCl, 2M Glycine, 10mM EDTA	2 ml
3%(v/v) Glycerol	50%(v/v)		0.6 ml
0.1%(w/v)APS	10%(w/v)		100 μl
TEMED			5 μl
DW			5.8 ml
			ca. 10 ml

HEPES
N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid
DTT
Dithiothreitol
EDTA
Ethylenediaminetetraacetic acid
PMSF
Phenylmethylsulfonyl fluoride
DW
Distilled water
BPB
Bromophenol blue
XC
Xylene cyanole FF

[*2)] To avoid detection of a dye signal, no dye is added during development of the specimen.

APS
Ammonium persulfate
TEMED
N,N,N',N'-Tetramethylethylenediamine

(2) The electrophoresis apparatus was set up and a pre-run conducted at 100 V for 10 min using 1 **X** TGE Buffer (pH 8.5) as the electrophoretic buffer solution. The power was turned off and the well was washed with the electrophoretic buffer solution. The specimen obtained in (1) was then applied to the well. A specimen containing dye (BPB or XC) was simultaneously developed in an empty edge lane as an index of electrophoresis time. Electrophoresis was conducted for 45 min at 15 mA under low-temperature condition (4°C).
(When using a 100 mm **X** 105 mm gel plate, the electrophoresis is stopped when the BPB dye has migrated to around the middle of the plate.)

Signal detection

The gel plate was removed from the electrophoresis apparatus and the glass surfaces cleaned. Fluorescence was detected with the gel still sandwiched between the glass plates.

3 Test results

Sufficient detection was possible using 1 µl (equivalent to 20 ng) of the fluorescent probe. The same electrophoresis pattern was obtained irrespective of the amount of the fluorescent probe (1 µl or 2 µl). The signal intensity of the shifted band depended on the probe amount.

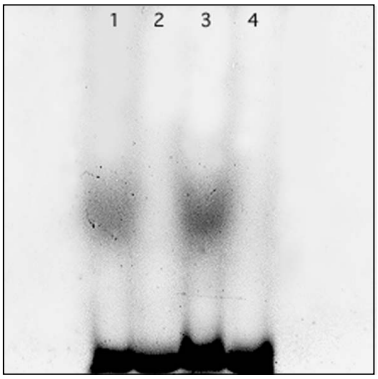

Fig. 3-1

Fig. 3-1 Gel shift analysis of NR gene 5' upstream region sequence. Nucleoprotein extracted from cultured spinach cells was used.
Lane 1: Containing nucleoprotein.
Probe: 1 µl
Lane 2: Not containing nucleoprotein.
Probe: 1 µl
Lane 3: Containing nucleoprotein.
Probe: 2 µl
Lane 4: Not containing nucleoprotein.
Probe: 2 µl
NR gene 5' upstream region sequence used:
5'-TTAAACAAGTCATTCACCA-3'

4 References

1) Hattori M., Tugores A., Veloz L., Karin M. and Brenner DA., A simplified method for the preparation of transcriptionally active liver nuclear extracts, *DNA Cell Biol*.,9,777-781 (1990).
2) Liu Z., Thompson K.S., and Towle H.C., Carbohydrate regulation of the Rat L-type pyruvate kinase gene requires two nuclear factors, LF-A1 and a member of the c-myc family, *J. Biol. Chem*., 268,12787-12795(1993).

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