Elchrom Scientific
Excellence in Electrophoresis

RAPD Fingerprinting of *Bradyrhizobium japonicum*

Application Note
Poly(NAT)® Gels
SEA 2000® Electrophoresis System

Introduction

The randomly amplified polymorphic DNA (RAPD) method (1, 2) has been successfully applied for the identification of many bacterial strains (3-8). This fast and sensitive method is able to provide reproducible and characteristic fingerprints of complex genomes without prior sequence information. The use of short primers of arbitrary sequence during PCR results in amplification of different segments of genomic DNA, which after gel electrophoresis give rise to characteristic band patterns. Most informative DNA bands are usually in the 300-3,000 bp range. Agarose gels are typically used for separating RAPD bands because polyacrylamide gels are not suitable for resolving DNA fragments that are longer than about 1 kbp. Resolution of 1-4 kbp DNA fragments is excellent on Poly(NAT)® gels (9).

Bradyrhizobium japonicum is a nitrogen-fixing microorganism, frequently used for seed inoculation of soybean to increase the crop yield. Precast 6% Poly(NAT)® gels have been used to determine the effectiveness of the inoculation (10). The 6% Poly(NAT)® gels have also been used for identification of Legionella species by amplification of intergenic 16S-23S rDNA spacer regions (11).

Advantages of the method include

- More information, because precast 6% Poly(NAT)® gels resolve better RAPD bands than do agarose or polyacrylamide gels
- Full reproducibility due to precise control of electrophoresis conditions
- Convenience and ease of submarine electrophoresis and precast gels
- High throughput, as up to 100 samples can be analyzed on one gel
- Easy and inexpensive staining with common fluorescent dyes

Results

Electrophoresis of RAPD fragments on a 6% precast Poly(NAT)® gel produces a number of well separated sharp bands up to about 3,000 bp. The appearance of a new band, or absence of an existing band, is an indication that the samples are different. The results show clear difference between *B. japonicum* strains which were used for inoculation of soybean seeds (lanes 13 and 14) and the strains isolated from the soil (other lanes). The use of precast gels and the SEA 2000® apparatus allows for reproducible and precise estimation of molecular sizes, with standard deviation of less than 1% between gels (11).

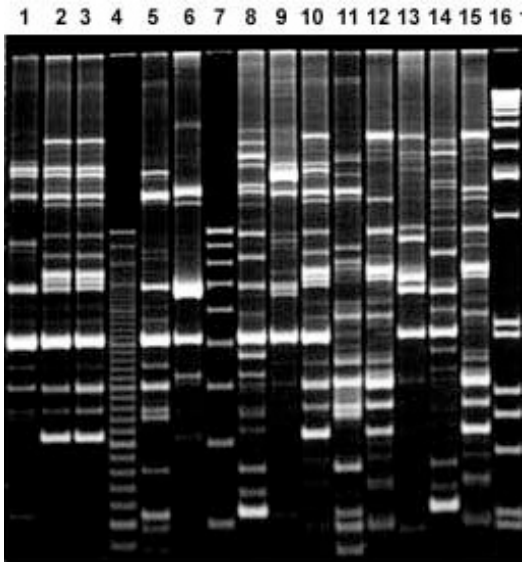

Fig. 1
Detection of genetic diversity of *B. japonicum* strains by electrophoresis of RAPD fragments on a precast 6% Poly(NAT)® Wide Mini S-50 gel. One third of the gel is shown. Lanes 13 and 14 represent the strains used for inoculation, whereas other lanes represent various strains isolated from the soil. The markers are 20 bp ladder (lane 4), 100 bp ladder (lane 7) and 1 kb (lane 16). The 20 and 100 bp ladders end with a 1,000 bp fragment.

Experimental Procedures

Equipment

The electrophoretic analysis were performed in the SEA 2000® submarine electrophoresis apparatus. Temperature of the running buffer was kept constant at 20°C by connecting the SEA 2000® to a circulating water bath.

Sample preparation

PCR samples were generated according to standard protocols as described (10). The samples shown in Figure 1 were obtained with a 10 base long primer 5í-GATCGGAGCG. Typically, 4µl of each sample were mixed with 1µl of loading buffer prior to loading to the gel.

Running Conditions

The 6% Poly(NAT) ® Wide Mini S-50 gel was run at 7 V/cm and 20°C for 2 h and 45 min.

Detection

The gel was stained with Ethidium Bromide (0.5 µg/ml) in Elchrom Scientific AG's Easy Stain Tray, destained in water and then photographed.

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Acknowledgement

We thank Dr. S. Sikora, Faculty of Agriculture, University of Zagreb, Croatia, for providing the photograph showing RAPD samples.

Order Information

Product	P/N
ORIGINS by Elchrom Scientific, 230 V	2100E
ORIGINS by Elchrom Scientific, 115 V	2100U
SEA 2000® Apparatus, 230V	2001E
SEA 2000® Apparatus, 115V	2001U
6% Poly(NAT) Mini Gels (10 gels)	3006
6% Poly(NAT) Wide Mini S-2x13 Gels (12 gels)	3026
6% Poly(NAT) Wide Mini S-2x25 Gels (12 gels)	3048
6% Poly(NAT) Wide Mini S-4x13 Gels (24 gels)	3052
6% Poly(NAT) Wide Mini S-4x25 Gels (24 gels)	3096
40 x TAE Running buffer (20 tubes a 50 ml)	3031
Easy Stain Tray	2344
Power Supply, 200 V/2000 mA, incl. timer	2029E
PeelIT™	2355

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PCR process is covered by US patents 4,683,195 and 4,683,202 owned by Hoffman-La Roche. Use of the process may require a license.

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