identify losses of heterozygosity (2) and a silver staining procedure is applied to the detection of nucleic acids in the gel (1). This protocol represents a nonradioactive detection assay that is particularly suited for screening procedures.

Following separation of the DNA, the sequencing apparatus is disassembled and the glass plate with the adhering polyacrylamide gel is placed on the lower unit of the staining device. The glass plate carrying the gel is sandwiched between the lower unit and the staining frame (Figure 1). A watertight seal is made by drawing the lower unit snugly to the staining frame with four wing nuts. Solutions can now be poured into the frame and decanted. The construction of this container allows for a fluid-proof seal without leakage. This apparatus results in a reliable and uniform fixation of the polyacrylamide gel at the gel's four margins.

Our staining device was constructed to fit a standard size sequencing gel. However, alternative models can be built for any gel dimension. The outer margins of the staining frame match the actual size of the gel. The linings of the frame are approximately 20 mm wide and therefore overlap the gel by this margin. The height of the frame's lining is approximately 40 mm. A gasket is attached to the bottom of the frame with grease. The lower unit is approximately 5 cm longer and wider than the upper frame.

We have extensively used this device for studies on tumor-associated loci in human brain tumors and for SSCP analysis of selected exons of the tumor suppressor genes p53 and NF1. Silver staining was carried out according to a published protocol (1) and PCR-based analysis of CA-dinucleotide repeats was performed as previously described (2). A representative example for a microsatellite assay is provided in Figure 2. This simple device will be particularly useful for large-scale microsatellite and SSCP screening of biological samples.

REFERENCES

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**Generation of Bacteriophage λ Lysogens by Electroporation**

The genetic regulatory system involved in the decision of the two propagative states of the bacteriophage λ, lysis and lysogeny, is well understood as recently reviewed (2.8). The potential of λ as an expression vector for the production of recombinant proteins has been evaluated by several groups (4,5,7). Using a temperature-sensitive mutant of λ in cl gene, switching from the lysogenic state (where the product gene is stably maintained) to the lytic state (where the copy number of λ is amplified to yield high-cloned gene products) can be easily accomplished by shifting the temperature. To obtain lysogens, it has been necessary to package λ DNA (or recombinant λ DNA) before infecting a suitable host. In this report a simple and rapid method of obtaining lysogens by electroporation without packaging is described.

Electroporation of competent cells of Escherichia coli XL-1 Blue (Stratagene, La Jolla, CA, USA) was prepared by the method of Dower et al. (1). The efficiency of transformation using pUC19 was 8.4 × 10⁸ transformants per µg DNA. The bacteriophage DNA used was Lambda ZAP II (Stratagene). Electroporation was carried out using the Gene Pulsar apparatus (Bio-Rad, Hercules, CA, USA) with 0.2 cm electrode-gap cuvettes. Electroporation conditions were field strength of 12.5 kV/cm, capacitance of 25 µF and parallel resistance of 200 Ohm, which typically resulted in the time constant of 4.3–4.5 ms. Cells were transformed with 0.05, 0.1 and 0.2 µg DNA, outgrown for 1 h at 37°C in Luria Broth (LB), and plated on LB, 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside, isopropyl β-D-thiogalactopyranoside (LB-X-gal-IPTG) medium containing ampicillin (Ap) (50 µg/ml) after appropriate dilution. Plates were incubated either at 30°C or at 37°C. Lysogens appeared as blue colonies after 18 h at 37°C or after 30 h at 30°C. The number of lysogens obtained at different conditions is summarized in Table 1. As a comparison, Lambda ZAP II was packaged using the Gigapack II packaging extract (Stratagene) and used to obtain lysogens after infection. Lambda ZAP II (0.2 µg) was packaged and used to infect XL-1 Blue following the protocols recommended by Stratagene. Packaging efficiency was 1.4 × 10⁸ plaque-forming units (pfu) per µg DNA

<table>
<thead>
<tr>
<th>Amount of DNA Used in Electroporation (µg)</th>
<th>Number of Lysogens per µg DNA</th>
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</thead>
<tbody>
<tr>
<td>0.05</td>
<td>2.6 × 10⁴</td>
</tr>
<tr>
<td>0.1</td>
<td>1.2 × 10⁴</td>
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<tr>
<td>0.2</td>
<td>1.1 × 10⁴</td>
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Table 1. The Number of Lambda ZAP II Lysogens Obtained by Electroporation Using Undigested DNA at Different Conditions

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Lysogens were obtained by infecting XL1-Blue with packaged λ at a multiplicity of approximately 0.1 followed by plating on LB-X-gal-IPTG+Ap plate. The number of lysogens per μg DNA (based on the amount of DNA before packaging) obtained by incubating at 30°C was 4.6 ± 0.5 x 10^2, which was lower by a factor of 100 than that obtained by electroporation. We also investigated the possibility of lysogen formation by the calcium chloride transformation protocol (6). The efficiency of transformation using pUC19 was 6.4 x 10^9 transformants per μg DNA. The number of lysogens per μg Lambda ZAP II was 1.1 ± 0.4 x 10^2 after 30 h at 30°C. It was not examined if another more efficient transformation method, such as that of Hanahan (3), could result in a higher number of lysogens.

We next tested if λ DNA from the ligation reaction could be directly used to obtain lysogens by electroporation. Lambda ZAP II (2.5 μg) was digested with EcoRI and ligated with 5.6 kb EcoRI fragment of Alcaligenes eutrophus genomic DNA (0.5 μg) in a 12 μL reaction using T4 DNA ligase (New England Biolabs, Beverly, MA, USA) for 16 h at 16°C. Lysogens of Lambda ZAP II containing the insert would appear as white colonies on the plates containing X-gal and IPTG. The results of the electroporation using the ligated DNA are shown in Table 2. Total number of lysogens obtained was approximately 5 x 10^2 per μg ligated DNA. The number of lysogens that appeared as blue and white colonies was similar. We checked a number of white colonies for the presence of the right insert by agarose gel electrophoresis of phage DNA prepared using the standard protocol (6). About 70% of the white colonies had the right insert, and there was one lysogen that had two tandem inserts (data not shown).

In this article we demonstrated that λ lysogens could be conveniently obtained without packaging by electroporation. It was shown that the ligation mixture could also be directly used to obtain recombinant lysogens. It was necessary to incubate plates at 30°C (lower than 32°C) to obtain a good number of λ lysogens possessing temperature-sensitive cI gene. For the λ derivatives carrying no mutation on the cI gene, however, it was not necessary to incubate the electroporated cells at 30°C (data not shown). We have also used this method to obtain lysogens of other bacteriophage λ derivatives without any difficulty.

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