A silver staining procedure for nucleic acids in polyacrylamide gels without fixation and pretreatment

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ABSTRACT

Silver staining of nucleic acid has been widely used in molecular marker analysis such as simple sequence repeat (SSR), single-strand conformation polymorphism (SSCP), and amplified fragment-length polymorphism (AFLP). Many alternatives to silver staining methods have been described, but these methods are not efficient or cost-effective. Here we report a silver staining method that requires less than 10 min for one gel and can save chemicals as well. It has a detection limit of approximately 5.6 pg of DNA/mm\textsuperscript{2} in nondenaturing polyacrylamide gels and 12.8 pg/mm\textsuperscript{2} in denaturing polyacrylamide gels.

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Keywords: Silver staining; Nucleic acids; Polyacrylamide gels; Detection limit; Efficiency.

Notes & Tips

Silver staining is a sensitive method to detect proteins and nucleic acids in polyacrylamide gels. It originally was described for ultrasensitive detection of polypeptides separated by polyacrylamide gel electrophoresis [1]. Recently, it has been widely applied to nucleic acid detection in molecular marker analysis such as simple sequence repeat (SSR),\textsuperscript{1} single-strand conformation polymorphism (SSCP), and amplified fragment-length polymorphism (AFLP). Many alternatives to silver staining methods for DNA detection have been described. However, many of them are not rapid enough because they include a number of time-consuming steps and involve the repeated changing of solutions. It is difficult to establish a high-throughput staining system. Although a number of simplified methods have been reported [4–8], they cannot display high sensitivity and/or efficiency.

Here we report a silver staining method for DNA in polyacrylamide gels that is efficient, sensitive, and cost-effective. After electrophoresis, gels were fixed and stained in a solution containing 5% ethanol, 1% nitric acid, and 0.1% AgNO\textsubscript{3} for 5 min, and the solution was reclaimed. The gels were briefly rinsed with redistilled water for 10 s and then were developed with a solution of 1.3% NaOH, 0.65% Na\textsubscript{2}CO\textsubscript{3}, and 0.4% HCOH for 2 min. Development was then stopped with a solution containing 5% ethanol and 1% nitric acid for 1 min, and the stopping solution was reclaimed.

This method simplified the involved techniques, such as prefixing steps and multiple washes, described in other procedures [2,3], and gels were fixed and stained simultaneously. This protocol requires less than 10 min. In a comparison between our method and other methods (Table 1), the procedures of Bassam and coworkers [2] and Creste and coworkers [3] were time-consuming and had more steps. Although many methods have simplified the number of steps [4–8], some of them cannot display high sensitivity [4–7] and the method of Byun and coworkers requires prearming developing solution to 55 °C [8]. This suggested that our method was efficient and cost-effective because it consumed little time and staining solution and stopping solution can be reused at least eight times without loss of sensitivity.

To estimate the sensitivity of our silver staining method, we used a pUC19/MspI marker (MBI Fermentas) to quantify the sensitivity in 6% nondenaturing and denaturing polyacrylamide gels (Fig. 1). A serial dilution of the marker revealed a detection limit of 5.6 pg/mm\textsuperscript{2} in nondenaturing polyacrylamide gels and 12.8 pg/mm\textsuperscript{2} in denaturing polyacrylamide gels. We have used the improved method for analyses of SSRs in Hevea brasiliensis (rubber tree), and it gives satisfactory results.

Acknowledgments

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Appendix A. Supplementary data

Fig. 1. Sensitivity of our silver staining method using a pUC19/\(Mtr\)I marker in 6% polyacrylamide gel. In lane 1, the DNA was loaded so as to give 4675 pg in the 501-bp band. Lanes 3, 5, 7, 9, 11, and 13 were twofold serial dilutions of this standard such that there was nominally 73 pg of DNA in the 501-bp band in lane 13. In lane 2, the DNA was loaded so as to give 3117 pg in the 501-bp band. Lanes 4, 6, 8, 10, 12, and 14 were twofold serial dilutions of this standard such that there was nominally 48.7 pg of DNA in the 501-bp band in lane 14. The area of each well was 5 mm\(^2\).

### Table 1
Comparison of silver staining protocols from our method and other methods.

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</thead>
<tbody>
<tr>
<td>Used time</td>
<td>~65 min</td>
<td>~45 min</td>
<td>~20 min</td>
<td>~20 min</td>
<td>~15 min</td>
<td>~10 min</td>
<td>~10 min</td>
<td>~8 min</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>1–1.5 pg/mm(^2)(^a)</td>
<td>3 ng(^a)</td>
<td>0.44 ng(^a)</td>
<td>3.5 ng(^b)</td>
<td>0.44 ng(^b)</td>
<td>1.75 ng(^b)</td>
<td>12.8 pg/mm(^2)(^a)</td>
<td>5.5 pg/mm(^2)(^b)</td>
</tr>
<tr>
<td>Fix</td>
<td>10% acetic acid; 20 min</td>
<td>10% ethanol; 1% acetic acid; 10 min</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Pretreat</td>
<td>–</td>
<td>1.5% nitric acid; 3 min</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<td>–</td>
</tr>
<tr>
<td>Wash</td>
<td>H(_2)O; 2 min; three times</td>
<td>H(_2)O; 1 min</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Stain</td>
<td>0.1% Ag(_2)O(_3); 30 min</td>
<td>0.2% Ag(_2)O(_3); 20 min</td>
<td>25% ethanol, 1% nitric acid, 0.2% Ag(_2)O(_3); 5–10 min</td>
<td>0.1% Ag(_2)O(_3); 10–15 min</td>
<td>1% nitric acid, 0.1% Ag(_2)O(_3); 5 min</td>
<td>10% ethanol, 0.5% acetic acid, 0.2% Ag(_2)O(_3); 5 min</td>
<td>5% ethanol, 1% nitric acid, 0.1% Ag(_2)O(_3); 5 min</td>
<td></td>
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<tr>
<td>Wash</td>
<td>H(_2)O; 20 s (optional)</td>
<td>H(_2)O; 30 s; two times</td>
<td>H(_2)O; 5 s; two times</td>
<td>H(_2)O; 5 s; two times</td>
<td>H(_2)O; 5 s; two times</td>
<td>–</td>
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<tr>
<td>Develop</td>
<td>3% Na(_2)CO(_3), 0.15% HCOH, 0.002% Na(_2)SO(_3), 0.05% HCOH; 4–7 min</td>
<td>3% Na(_2)CO(_3), 0.2% HCOH; 2–5 min</td>
<td>0.04% Na(_2)CO(_3), 0.2% HCOH; 5 min</td>
<td>2% NaOH, 0.01% HCOH, 10 s</td>
<td>2% NaOH, 0.01% HCOH; 2–3 min</td>
<td>2% NaOH, 0.04% NaCO(_3), 2–3 min</td>
<td>1.3% NaOH, 0.65% HCOH; 2–3 min</td>
<td></td>
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<tr>
<td>Stop</td>
<td>10% acetic acid; 5 min</td>
<td>10% acetic acid; 5 min</td>
<td>10% acetic acid; 5 min</td>
<td>5% acetic acid; 2–5 min</td>
<td>2.5% Amp; 5 s; two times</td>
<td>10% ethanol, 0.5% acetic acid; 1 min</td>
<td>5% ethanol, 1% nitric acid; 1 min</td>
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</table>

\(^a\) Detection sensitivity of silver staining in denaturing polyacrylamide gel. 
\(^b\) Detection sensitivity of silver staining in nondenaturing polyacrylamide gel.
References


