

Direct Colony Identification by PCR-Miniprep

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Abstract

We have developed this PCR-based technique to directly identify transformed bacterial colonies before performing any DNA miniprep. Both specific as well as standard vector's primers can be used in this technique to analyze a large number of colonies in a relatively short time.

Results and Discussion

Miniprep of plasmid DNA is one of the routinely used methods in almost all laboratories of molecular biology. In general, the conventional methods of miniprep involve the growth of a single colony of transformed bacteria to log phase, lysis of the bacteria by boiling (Sambrook *et al.*, 1989; Birnboim and Doly, 1979) or alkaline treatment (Sambrook *et al.*, 1989; Homes and Qingley, 1981), precipitation of plasmid DNA, followed by analysis of purified DNA with restriction enzymes. These steps can be very cumbersome and time consuming especially when large numbers of colonies are to be analyzed. A PCR-based technique to directly analyze the bacterial colonies was first developed by Zon *et al.* (1989). This technique is much easier and faster than the conventional methods of miniprep, but its relatively high costs prevented it from becoming popular as the conventional methods.

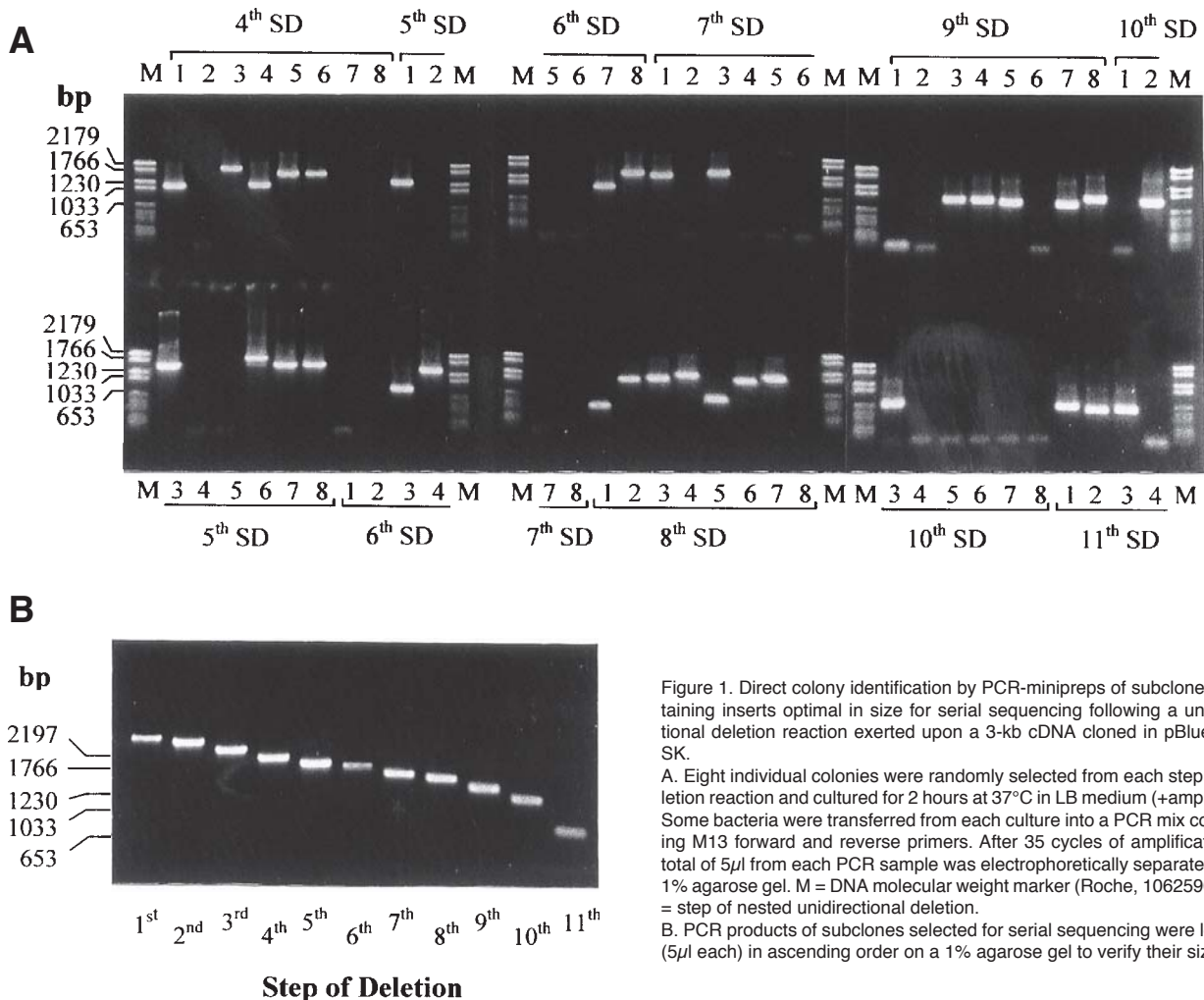


Figure 1. Direct colony identification by PCR-minipreps of subclones containing inserts optimal in size for serial sequencing following a unidirectional deletion reaction exerted upon a 3-kb cDNA cloned in pBluescript SK.

A. Eight individual colonies were randomly selected from each step of deletion reaction and cultured for 2 hours at 37°C in LB medium (+ampicillin). Some bacteria were transferred from each culture into a PCR mix containing M13 forward and reverse primers. After 35 cycles of amplification, a total of 5µl from each PCR sample was electrophoretically separated on a 1% agarose gel. M = DNA molecular weight marker (Roche, 1062590). SD = step of nested unidirectional deletion.

B. PCR products of subclones selected for serial sequencing were loaded (5µl each) in ascending order on a 1% agarose gel to verify their sizes.

In this study, we have modified the PCR-based procedure so that large numbers of bacterial colonies can be easily analyzed in a shorter time and with considerably lower costs than the conventional methods. In this technique, single colonies of transformed bacteria were picked up from agar plates using sterile toothpicks. Each colony was used to inoculate 100 μ l of LB medium (containing the appropriate antibiotic). The culture was carried out in 96-well microplates by incubation at 37°C for 2-3 h with moderate shaking of about 160 rpm. By the end of the incubation period, a PCR master mix was prepared containing the following constituents; 1.1x PCR reaction buffer [10 mM Tris-HCl (pH 8.8), 50 mM KCl, 0.08% Nonidet P40], 2.5 mM MgCl₂, 0.22 mM dNTPs, 2.5 μ M of each PCR primer, and 25 U of Taq DNA polymerase in a total volume of 0.9 ml. Aliquots of 9 μ l PCR master mix were distributed into each PCR tube and a small amount of transformed bacteria were directly transferred from the 100 μ l cultures into the PCR reaction tubes using toothpicks. The reaction solutions were overlaid each with a drop of mineral oil (also in the presence of a heated block lid) and then subjected to amplification reactions. At the beginning, the samples were incubated at 95°C for 1 min and then subjected to amplification using the following program: 40 sec at 95°C, 1 min at 55°C (or at a temperature suitable for the selected pair of primers), and 1 min at 72°C for inserts < 1 kb, or longer for oversized templates (n min per n kb), for 35 cycles. A total volume of 5 μ l from each PCR reaction was subjected to electrophoresis on agarose gels and amplified DNA products were visualized under UV light after staining with ethidium bromide. Once a colony was identified, a larger culture was started from the corresponding micro-culture for DNA mini-, midi- or maxiprep.

Figure 1 shows an application of this technique to screen subclones generated from a 3-kb cDNA insert by nested unidirectional deletions. Recombinant colonies were identified among non-recombinants by the presence of a DNA-band on the agarose gel bigger in size than the DNA sequence between the PCR primers in the non-recombinant cloning vector (Figure 1A). Plasmid subclones containing inserts of appropriate sizes in each deletion step were selected (Figure 1B). The primers used in this analysis were the same ones used later in sequencing reactions. This ensures the presence of intact complementary sequences to the sequencing primers in all the selected subclones.

We have tested six Taq DNA polymerases from different producers in direct colony identification by PCR. All the tested enzymes [MBI Fermentas, EP0404; GIBCO BRL, 18038-018; Pharmacia, 27-0799-01; Promega, M1861; SAWADY, pQLAB, 01-1010; Qiagen, 201203] proved to be optimal (not shown).

We are now using this technique routinely in our laboratory to analyze the size of each cloned DNA as well as to determine the orientation of inserts in cloning vectors before extracting plasmid DNA from any targeted clone.

References

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