Protocol for Genomic DNA Preparation from Fresh or Frozen Serum for PCR Amplification

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Blood is the primary human tissue for clinical diagnosis. Serum may be stored in banks after diagnosis, and most of the specimens may have available corresponding clinical records. These serum samples can be important sources of genomic DNA for human genetic studies in the identification of disease-causing genes or the study of mutations in prenatal diagnosis (10,16), in the detection of tumor DNA from patients with pancreatic (12), lung (3), colorectal (1) and breast cancer (15). However, DNA isolation from serum is difficult because of the low content of DNA in serum and the small amounts of serum that may be available.

Martin et al. (11) first described a procedure to isolate DNA for genotype analysis, and others have developed different methods for serum DNA isolation (6,14). Dixon et al. (4) have tested 13 currently available DNA extraction methods. We have also reported a method for DNA extraction from small amounts of serum (9). However, for DNA analysis on a larger scale, a simple, automation-amenable protocol is required. In the present study, we describe a simple protocol for DNA preparation from serum. It includes only two steps, alkaline lysis and neutralization, with two different solutions in one tube.

Blood was collected in a red top Vacutainer (no additives) (Becton Dickinson, Franklin Lakes, NJ, USA) with a 22G1 needle and was allowed to clot at room temperature for at least 2 h. The clot was detached from the wall of the tube by a wooden applicator stick and then kept at 4°C overnight. It was centrifuged at 700×g for 5 min; the serum (supernatant) was collected and used for DNA extraction.

For alkaline lysis, 10 μL serum were incubated with 1 μL 0.2 M KOH at 37°C for 20 min. During this treatment, various macromolecules in serum may be denatured or hydrolyzed (i.e., RNA) (7). To identify optimal conditions for the alkaline lysis, we tested different KOH concentrations (0.1 or 0.2 M) at different incubation temperatures (55°C, 45°C or 37°C). Ten microliters of serum were used, and the incubation time was 20 min. To neutralize the solution, 5 μL neutralization buffer (9 parts 1 M Tris, pH 8.3:1 part 2 M HCl) were added to the serum lysate and vortexed briefly. The final DNA solution volume was 16 μL, and 3 μL each condition were used for PCR amplification.

In this study, the target DNA se-
quences were from human surfactant protein A (SP-A) (primers 26 and 28A) (13) and B (SP-B) (primers 71A, 73, 94, 106, 114, 535 and 536) (8). The primer sequences (lowercase letters indicate a non-gene-specific sequence attached to the gene-specific sequence for other purposes) were: primer 26 (forward) 5’-GCTTATGACAAAAAGTT-GTCTA-3’; primer 28A (reverse) 5’-ACCACTAGCTAGGCCTACAT-3’; primer 71A (reverse) 5’-ggggaaaCTA TTAGCTGCTGAGAGATC-3’; primer 73 (reverse) 5’-ctcgaactCCAG CACCCCTCATTTCAAG-3’; primer 94 (forward) 5’-GAGGTGCATGCTGAGTCA-3’; primer 106 (reverse) 5’-GGGATCTGAGGGGATAAGC-3’; primer 114 (sense) 5’-TTGCTGCTCGGAGAGATC-3’; primer 535 (reverse) 5’-ggtctgctgacTCTATGACGCT-3’ and primer 536 (forward) 5’-gcgactagtCTATGACGTCTG-3’. Primers 535 and 536 were used for amplification in PCR 1 (PCR fragment is 1074 bp); and 26 (PCR fragment is 1656 bp); 73 and 94 in PCR 2 (PCR fragment is 3792 bp); 106 and 114 in PCR 3 (PCR fragment is 10741 bp); 71A and 536 were used for amplification in PCR 1 (PCR fragment is 1074 bp); and 26 and 28A in PCR 5 (PCR fragment is 220 bp). One hundred nanograms of genomic DNA prepared from whole blood (5) were used as control. The results from PCR amplification of two target DNA sequences (as shown in Figure 1) indicated that the optimal alkaline lysis condition was using 0.2 M KOH at 45°C for 20 min. An incubation temperature of 55°C did not enhance the result obtained with 45°C (data not shown). Comparison of 37°C and 45°C incubation temperatures yielded similar results when 0.2 M KOH was used; therefore, for convenience, an incubation temperature of 37°C can be used.

The size of DNA sequences from serum DNA was subsequently examined, using long PCR (8) for amplification of all size fragments. The target gene sequence for this set of experiments was the surfactant protein B. As shown in Figure 2, it was possible to amplify from fresh serum (as opposed to archived frozen serum), DNA fragments of 3792 bp and 1656 bp (PCR 2 and PCR 3, respectively) and a DNA fragment of 547 bp with quantities similar to that observed with 100 ng control DNA (PCR 4). However, it was not possible to amplify a 10741-bp fragment from serum DNA (PCR 1). Because genotype analyses usually require target sequences smaller than 1 kb (about 500 bp), the serum DNA preparation described here could be widely applied to other genetic studies.

Serum DNA stability was tested by storing the DNA at 4°C and -20°C. The stability of serum DNA was assessed by studying PCR products amplified from serum DNA stored under various conditions. The results indicate that serum DNA is stable for up to four months at -20°C, but a significant decline in stability is observed after one month if the DNA is stored at 4°C (data not shown).

Moreover, we have used serum collected several years ago (i.e., archived) to extract DNA and then perform genotype analysis as part of another study (data not shown). Using the protocol described here, we have analyzed genotypes at 9 single-nucleotide polymorphisms (SNPs) in SP-A, 4 SNPs in SP-B, 2 SNPs in SP-D and 4 microsatellite markers in SP-B flanking region from more than 10 fresh and more than 60 frozen archived serum samples. The genotyping method we used was PCR-based converted RFLP. The target DNA fragments were 51–354 bp. Using nested PCR, the target fragments, even from frozen samples, were successfully amplified in 96% of the cases. However,
we did observe PCR-generated loss of heterozygosity (LOH). This is usually caused by the low amount of DNA in serum, as suggested by Bluteau et al. (2). We also found that the PCR-generated LOH could be overcome through a marker-specific PCR optimization strategy. Therefore, we suggest that different PCR strategies or genotyping methods should be considered and tested before starting large-scale genotype analyses from serum DNA.

From the results described above, we conclude that (i) a simple protocol can be used for DNA extraction from serum. The entire procedure is carried out in two steps with two different solutions in one tube; (ii) the protocol can be easily automated; (iii) the quality of fresh serum DNA under the experimental conditions presented here is adequate to be used as a template to amplify a few kilobases of target DNA sequence. However, if nested PCR is used and the target PCR fragments are about 500 bp, the quantity of PCR products from fresh or archived serum samples is similar to that obtained from 100 ng control genomic DNA; and (iv) the protocol is suitable for microextraction. DNA can be prepared from as small a volume as 10 μL serum and can serve as PCR template in five reactions. Generally speaking, DNA quantities equivalent to 2 μL serum can be used as template in one reaction for either one SNP or one microsatellite marker.

In summary, serum can serve as a source of DNA for PCR. It is likely that serum is a more reliable source for small DNA target sequences (less than 500 bp) rather than larger target sequences.

REFERENCES

Rapid Mini-Scale Plasmid Isolation for DNA Sequencing and Restriction Mapping

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A frequently used step in most molecular biological investigations is screening large numbers of recombinant plasmids to examine the size, orientation and nucleotide sequence of cloned inserts. The screened clones often serve as templates in PCR to generate DNA fragments that are subsequently processed for any number of studies such as subcloning into specialized vectors, assaying for biological activity and detecting cognate binding sites for regulatory proteins. The currently available “miniprep” plasmid purification procedures use differential DNA precipitation with polyethylene glycol and/or phenol-chloroform extraction for the removal of many contaminants (4). These procedures are not entirely satisfactory because they are time consuming, tedious and require the use of expensive and undesirable chemicals.

Alternatively, it has been reported that plasmids released into the supernatant recovered from boiled bacterial suspensions can be used directly for restriction enzyme analysis (5). However, the quality of DNA is not always suitable for analyses such as DNA amplification and sequencing. Commercially available miniprep kits are costly and do not provide a simple enough isolation protocol for routine screening and analysis of large numbers of clones. We have developed a modified version of the alkaline lysis method (1) for plasmid isolation in high enough yield and purity so that, without further purification, the retrieved plasmids can be used in a variety of experimental manipulations such as ligation, bacterial transformation, restriction mapping, PCR amplification and DNA sequencing.

Our approach (Protocol 1) differs from the commonly followed alkaline lysis protocol in that ribonuclease A (RNase A) is added to the initial bacterial resuspension buffer. We find that including RNaseA from the beginning helps to keep the contaminants in the solution phase. RNaseA treatment simplifies the final step of DNA recovery because it prevents co-precipitation of contaminants that may interfere in subsequent enzymatic analyses. In addition, it allows the DNA to rapidly migrate into the solution phase. Our method has the additional advantages that (i) no phenol-chloroform extraction is needed, (ii) each centrifugation step is only 1 min or less and (iii) the entire procedure can be carried out at room temperature instead of 4°C.

Figure 1 shows comparative restriction enzyme digestion patterns of the same recombinant plasmid (pGEM3Z-HNF3B) prepared using three different isolation protocols: (i) a commercial miniprep DNA purification kit (Wizard® Plus SV miniprep system; Promega, Madison, WI, USA); (ii) our method as presented in Protocol 1; (iii) the conventional alkaline lysis method combined with phenol-chloroform extraction and DNA precipitation by polyethylene glycol. In all three cases, cleavage by different restriction enzymes generates very similar digestion patterns.