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Rapid Preparation of Limited Biological Samples for Small-volume PCR

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The ability to analyze the DNA of a biological sample has revolutionized the fields of genetic disease diagnosis and forensic medicine. Small-volume PCR with extremely rapid cycling parameters, known as capillary PCR, has enabled complete DNA analysis of multiple loci in a few hours.⁽¹⁾ Traditional organic extraction of DNA from a chorionic villus sample (CVS) for prenatal diagnosis takes several hours and small biopsies yield little or no amplification template. Similarly, fresh or dried blood needs to be acquired in relatively large quantities and treated extensively for extraction of enough viable material in a DNA study.⁽²⁾ Thus, the small-volume reactions, apart from being rapid and economically attractive, provide increased specificity to DNA amplification. This is a direct result of the decreased times at temperatures between those programmed for the cycling procedure.

We describe a 10-min protocol for the partial extraction of genetic material from microgram amounts of tissue, including whole blood and fetal products, to complement the speed and sensitivity of capillary PCR. Although these methods, as outlined in this report, are used in a purely diagnostic manner, the advantages of specificity and speed are useful for the rapid optimization of new primer and target combinations in research.

METHODS

Fresh, thawed, or dried (Guthrie spot) whole blood was initially and crudely hemolyzed to remove inhibitors of DNA amplification reactions, such as hemoglobin.⁽³⁾ Aliquots of 10 μ l of blood or approximately 8 mm² of blood-stained material were resuspended in 200 μ l of 10 mM Tris-HCl (pH 7.8), 1 mM EDTA, and 0.5% NP-40 (TED). Samples were then centrifuged to form a pellet of cellular material and the procedure was repeated after resuspending the pellet in 200 μ l of TED. The matrix of a dried blood spot was left in the tube during this procedure. Samples from a CVS (1 mg; equivalent to a single villus strand) or amniotic fluid cells (1 mg) were centrifuged to a pellet and the supernatant was decanted. All samples, regardless of origin, were then treated identically. Routinely, 40 μ l of 0.1% Triton X-100 was added to resuspend the pelleted cells. Partial digestion of cell membranes

and denaturation of proteins in the sample was achieved by addition of 10 μ l of 0.4 M NaOH and heating to 95°C for 5 min. The solution was neutralized with 10 μ l of 1 M Tris (pH 7.5). Sufficient DNA template for capillary PCR was obtained from 2 μ l of this crude extract.

PCR amplification was carried out in a buffer containing 67 mM Tris-HCl (pH 8.8) 16 mM (NH₄)₂SO₄, 0.45% Triton X-100, 200 μ g/ml gelatin, 2–3 mM MgCl₂, 150 μ M dNTPs, and 10 pmoles of each oligonucleotide primer. Nondetergent buffers were also used and provided successful amplification. Relatively high levels of *Taq* DNA polymerase (0.25–0.5 units) (Biotech Int., Australia) and magnesium ions were required in these 10- μ l reactions, probably due to the increased surface area of the reaction vessel compared to tube PCR. Reactions were carried out in heat-sealed positive displacement pipette tips or in ordinary microliter pipette tips overlaid with paraffin oil. Reaction vessels were incubated in the FTS-1S capillary thermocycler (Corbett Research, Australia). Cycle conditions varied for each set of primers, although denaturation at 95°C for 5 sec, annealing at 60°C for 10 sec and 72°C for 30 sec to extend represents typical parameters for microsatellite sequence analysis (Figs. 1 and 2). Amplification of adequate quantities of DNA for electrophoretic analysis was achieved in less than 30 cycles for all applications, total amplification time being less than 40 min.

RESULTS AND DISCUSSION

The analysis of dinucleotide repeats for use as genetic markers has been achieved using the described techniques with electrophoretic analysis of products on polyacrylamide gels (Figs. 1 and 2). Routinely, we have applied rapid extraction of DNA and capillary PCR to the determination of fetal sex, detection of exonic deletions, and genetic linkage via polymorphic restriction fragments or microsatellite sequences for prenatal diagnosis of Duchenne and Becker muscular dystrophy. Multiplex PCR for the detection of up to nine commonly deleted regions in the dystrophin gene⁽⁴⁾ has been successfully adapted to both capillary PCR and the use of dried blood spots as amplification template (data not shown). The combination of these methods has also been the basis for genetic studies on

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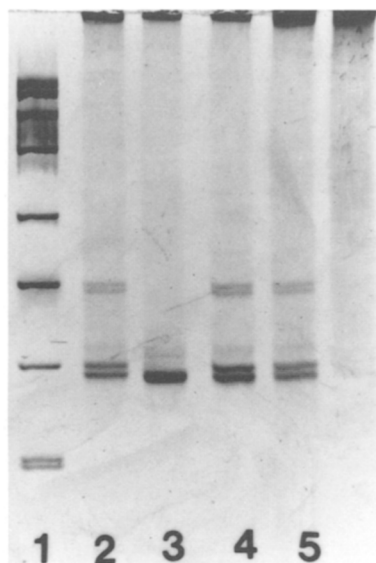


FIGURE 1 Silver-stained 8% polyacrylamide gel showing dinucleotide repeat alleles at D5S125⁽⁷⁾ amplified by capillary PCR for prenatal diagnosis of acute spinal muscular atrophy. (Lane 1) pUC19/*Hpa*II; (lanes 2–5) results for father, proband, and mother's CVS and genomic DNA, respectively. CVS was analyzed directly. Note the heteroduplex formation in heterozygote samples. The alleles are 147 bp and 143 bp.

limited samples derived from terminally ill or deceased patients.

These extraction procedures combined with the technology of capillary PCR make analysis of DNA possible in approximately 1 hr. The method of sample treatment described is significantly simpler, faster, and more applicable to a wide variety of samples than rapid methods currently in use.^(5,6) Time-consuming proteinase K digestions and successive phenol and chloroform extractions, which may further inhibit PCR, are no longer necessary. Sample preparation by simply boiling in water or nonionic detergents alone has not proven suitable for the applications mentioned. Similarly, microwave treatment of biological samples has not been successful as a fast alternative to PCR template extraction in our laboratory.

The use of the described method has hastened the process of providing information on prenatal diagnoses for carriers of genetic disease, including muscular dystrophy and spinal muscular atrophy, thereby reducing the waiting time and worry for patients. It is now possible to identify genetic mutations much faster and efficiently from archival

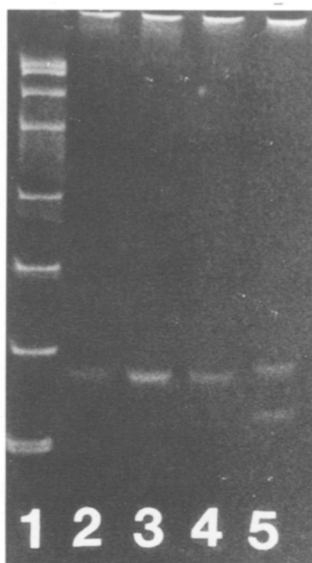


FIGURE 2 Ethidium bromide-stained capillary PCR amplification products from 10- μ l blood samples. The locus studied is at the distal end of the dystrophin gene.⁽⁸⁾ (Lane 1) pUC19/*Hpa*II; (lanes 2 and 3) hemizygote allele 2; (lane 4) homozygote allele 2; (lane 5) heterozygote showing alleles 1 and 4.

blood deposits or extremely small biological specimens.

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