PCR Protocol for DNA Recovery from Spurr’s-embedded Muscle Biopsies

Francesca Capon,1 Stefania Lo Cicero,1 Giuseppe Novelli,2 and Bruno Dallapiccola1

1Department of Public Health and Cell Biology, Tor Vergata University of Rome and C.S.S. Hospital, San Giovanni Rotondo, Rome, Italy; 2Chair of Human Genetics, Catholic University of Rome, Rome, Italy

PCR has been used extensively for the retrospective molecular biological studies of a wide variety of pathological archival material, including air-dried or stained bone marrow smears, formalin-fixed tissues, fixed cytogenetic preparations, Guthrie spots, and autopsied and frozen tissues.1-6 Thus, PCR can be applied to the diagnosis of a variety of genetic disorders that are lethal in early childhood, including medium-chain acyl-coenzyme A dehydrogenase deficiency and spinal muscular atrophy, and to the detection of infectious disease pathogens and minimal residues of disease.7-8

Spinal muscular atrophies (SMAs; MIM* 253300) are neuromuscular disorders, characterized by proximal, symmetrical limb and trunk muscle weakness resulting from specific degeneration of anterior horn cells of the spinal cord. (8) SMA I (Werdnig–Hoffman disease), SMA II (an intermediate form), and SMA III (Wohlfart–Kugelberg–Weandler disease) are recognized on the basis of age onset, severity, and survival. These three disorders have proved to be a result of allelic mutations of a locus assigned to 5q12-q14.1-2

At present, the molecular diagnosis of SMA is performed by linkage analysis using microsatellite DNA markers.10 However, linkage analysis is often prevented by the early death of the patient, which in SMA I usually occurs by the age of 18 months. This difficulty can be circumvented by PCR analysis of DNA isolated from microscopic glass slides and/or frozen muscle tissue of the proband, even on samples stored for several years.11 A potential difficulty in recovering DNA comes from viscosity of the embedding media. The low-viscosity Spurr’s mixture12 is widely used in electron microscopy science, where it provides a high-quality and rapid infiltration. So far, no protocol has been developed for recovering DNA from Spurr’s blocks. Here, we report a reproducible and sensitive method for PCR amplification of microsatellite DNA from muscle biopsy sections on Spurr’s blocks that allows retrospective molecular characterization of deceased patients.

Spurr’s-embedded muscle biopsies of the gluteus or vastus of five SMA I patients were prepared 1 month to 5 yr before use with a commercial resin kit (Taab Laboratories, Berkshire, England). The mixture, which was composed of vinyl cyclohexane dioxide, diglycidyl ether of polypropylene glycol, and non-ethyl succinic anhydride, in a ratio of 10:60:26:0.4, respectively, was combined in a firm medium, according to the recommendations of the manufacturers. Dehydration, infiltration, and polymerization were carried out according to Spurr.12 Sections 3–5 mm thick were cut from the blocks using a razor and treated with tetrahydrofuran (THF) (Panreac, Barcelona, Spain) at room temperature for 5 min. Depolymerized material was washed in ether saturated with distilled water to remove THF and dried at room temperature. Fifty microliters of a lysis buffer containing 20 mm Tris-HCl (pH 8.0), 20 mm EDTA (pH 8.0), 10 mm NaCl, 2% Tween 20, 1 mm dithiothreitol, and 500 μg/ml proteinase K was added to each sample and incubated overnight at 37°C. The lysate was boiled for 8 min in a programmable heating block (Perkin-Elmer Roche, Branchburg, NJ) and centrifuged for 10 min at 10,000 rpm. Nucleic acids were extracted with phenol/chloroform/isoamyl alcohol solution and precipitated overnight with two volumes of cold absolute ethanol at −20°C. DNA was lyophilized and resuspended in 50 μl of a PCR mixture containing 25 pmol of oligonucleotide primers, 200 μM each dGTP, dATP, dTTP, and dCTP; 0.5 μCi of [α-32P]dCTP (Amersham International, Amersham, UK); 2.5 units of Taq polymerase (AmpliTaq, Perkin-Elmer Roche); 50 mm Tris-HCl (pH 8.5); and 1.5 mm MgCl2, and 0.1% (wt/vol) autoclaved gelatin. Each reaction was overlaid with two drops of mineral oil (Sigma Chemicals, St. Louis, MO). The reactions were performed in a DNA thermocycler (Perkin-Elmer Roche) at 95°C for 5 min for one cycle, 95°C for 30 sec, 62°C for 40 sec, 72°C for 1 min for 33 cycles, and 72°C for 7 min. On completion of temperature cycling, 5 μl of loading dye (10 mm NaOH, 95% formamide, 0.05% bromphenol blue, 0.05% xylene cyanol) was added to 4 μl of amplified product and electrophoresed on 8% acrylamide/7 M urea sequencing gels at 40 W. Gels were dried under vacuum and exposed to X-ray film at −80°C with intensifying screens. This protocol was also successfully applied to the study of six additional Spurr’s-embedded specimens stored for 1–8 yr (courtesy of G. Zelano, Catholic University of Rome, Rome, Italy), which were evaluated for the HLAQα locus using a commercial kit (AmpliTyper, Perkin-Elmer Roche).
As shown in Figure 1, DNA prepared from the five Spurr's block samples was amplified using primers specific to sequences containing (CT)n dinucleotide repeats on 5q12-q13 (D5S127 locus, 10 alleles, 96–114 bp). Specific amplified alleles were observed in all DNA samples recovered from the embedded tissues, irrespective of the duration of storage and amplification product lengths. Microsatellite alleles scored on the Spurr's sections always matched those in parents and in parents of the dead patients, and complete informativity was reached in the five families analyzed (data not shown). No misinterpretation occurred as a result of single-allele amplification, and no artifactual band was evident from specimens. Extracted DNA was sufficient for amplifying at least three microsatellites and allowing complete haplotyping of the 5q12-q14 region.

These experiments demonstrate that genetic analysis of DNA prepared from Spurr's-embedded tissue may be accomplished by PCR techniques. In particular, highly polymorphic DNA sequences can be amplified successfully from previously uninvestigated archival tissue, making reliable retrospective genotyping of SMA families.

ACKNOWLEDGMENTS

We are indebted to D. Cavallaro (University of Verona) and G. Zelano (Catholic University of Rome) for providing Spurr's-embedded tissues and Massimo Gennarelli (Tor Vergata University of Rome) for expert advice in preparing oligonucleotide primers. This work was supported by Telethon, Italy, and Associazione Italiana per lo Studio delle Malformazioni (Mila). S.L.C. is a fellow of Associazione Italiana Ricerca e Cura Handicaps (Italy).

REFERENCES


FIGURE 1 PCR analysis of DNA purified from Spurr's-embedded muscle biopsies. DNA isolation and PCR reactions were performed as described in the text from five Spurr's blocks (lanes 1–5). Microsatellite alleles of the D5S127 locus were visualized by gel electrophoresis followed by autoradiography. Amplicon length (in bp) is indicated at right.
PCR protocol for DNA recovery from Spurr's-embedded muscle biopsies.

F Capon, S L Cicero, G Novelli, et al.

*Genome Res.* 1993 3: 211-212