Analysis of PCR Products (pMCT118) by Polyacrylamide Gel Electrophoresis

B. Budowle[1], A. M. Giusti[1], and R. C. Allen[2]
1) FSRTC, FBI Academy, Laboratory Division, Quantico, Virginia 22135
2) Department of Pathology, MUSC, Charleston, South Carolina 29425

Introduction

Identity tests rely on the genetic differences among individuals. The most informative genetic markers for the genetic characterization of people are variable number of tandem repeat (VNTR) loci (Nakamura, et al 1987). The detection of VNTRs is made possible by restriction fragment length polymorphism (RFLP) analysis via Southern Blotting (Southern, 1975). The RFLP procedure, although extremely effective for VNTR analyses, is time consuming and requires isotopic detection to type VNTRs in 10-50 ng of human genomic DNA samples (Budowle and Baechtel, 1989). Additionally, due to the inability of the technology to resolve discrete alleles of the VNTR loci that provide the greatest sensitivity of detection, more elaborate statistical analyses are required than for traditional genetic marker systems.

The polymerase chain reaction (PCR) (Saiki, et al 1985) offers a viable alternative to RFLP analysis of VNTRs, particularly for analyzing limited quantities of DNA, as it obviates the need for isotopic detection, and reduces assay time and costs. With appropriate VNTR loci and a high resolution electrophoretic system, amplification of specified DNA sequences by PCR could prove useful for identity testing, population genetics, and disease susceptibility studies. This paper describes a simple, high resolution discontinuous gel electrophoresis technique for the analysis of the VNTR locus pMCT 118 (D1S80) (Nakamura, et al 1988). The procedure resolves alleles of D1S80 into discrete entities, uses an inexpensive silver stain for detection, and provides a permanent record of the electrophoretically separated DNA fragments.

Materials and Methods

Whole blood was obtained in EDTA vacutainer tubes by venipuncture from donors at the FBI Academy. The DNA was extracted as previously described (Budowle and Baechtel, 1989).

Amplification of pMCT118 (D1S80) was achieved using the primers described by Kasai, et al (1989). The primers were 5'-GAAACTGGCCTCCAAACACTGCCCGCCG-3' and 5'GTCTTGTTGGAGATGCACGTGCCCCTTGC-3'. Each sample amplified contained 100ng DNA, 10 mM Tris-Cl, pH 8.3, 50 mM KCl, 1.5mM $MgCl_2$, 0.01% gelatin, 2.5 units of AmpliTaq™ DNA Polymerase (Perkin-Elmer-Cetus), 1uM of each primer, and 200 uM of each dNTP. The volume of each sample was 50 ul. The PCR was carried out in a Perkin-Elmer Thermocycler for 25 cycles. Each cycle was one minute at 95C for denaturation, one minute at 65C for primer annealing, and eight minutes at 70C for primer extension.

Ultrathin-layer polyacrylamide gels (5%T, 3%C; 400 um thick) were cast onto Gelbond (FMC, Rockland, Maine) using the flap technique (Allen, 1980). The gels were cross-linked with either BIS or piperazine di-acrylamide (PDA) (Hochstrasser, et al 1988). All gels contained 7.1% glycerol and 33 mM Tris-Sulfate buffer, pH 9.0. The electrode buffer contained 0.52 M Tris-Borate and 0.01% bromophenol blue. Electrophoresis was carried out horizontally on an ICE apparatus (EC Corporation,

Advances in Forensic Haemogenetics 3
Edited by H. F. Polesky and W. R. Mayr

Germany). Alternatively, the samples were absorbed onto LKB applicator tabs (2.5 × 5 mm), lightly blotted, and applied to the gel surface one cm from the cathode. The interelectrode wick distance was ten cm. Electrophoretic conditions were as previously described (Allen, et al 1989). Following electrophoresis, the gels were stained with silver (Allen, et al 1989).

Results and Discussion

Figure 1 shows that amplified fragment length polymorphism (AMP-FLP) typing of D1S80 can be performed using PCR and this simple polyacrylamide gel electrophoretic technique. Each band (or allele) is completely separated, and thus could be specifically designated without determining base pair size. The D1S80 alleles temporarily have been designated 1 through 13.

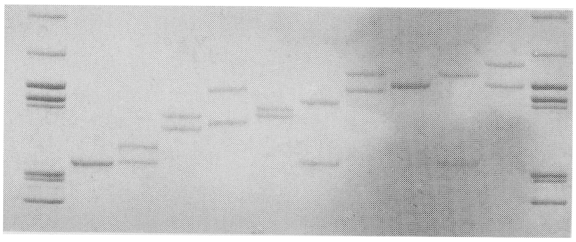

Figure 1. D1S80 profiles. The alleles have been temporarily named 1-13 (from smallest to largest in size). The genotypes of the samples from left to right are: 1-3-1, 7-5, 11-6, 8-7, 9-1, 12-10, 11-11, 12-1, and 13-11.

An advantage of analyzing D1S80 is that all alleles examined to date are less than 600 base pairs in length. The efficiency of amplification or yield of PCR products is related to the length of the target site between the primers; as observed for D17S30 (Horn, et al 1989), larger alleles could be less intense than smaller ones. Thus, unless proper quantities of amplified product were applied to the gel, incorrect genotype assignments could be made. However, with the D1S80 locus, there is no apparent difference in band intensity between the alleles of any heterozygote.

BIS is traditionally used as a cross-linker in polyacrylamide gels. However, PDA can be substituted on a gram-for-gram basis for BIS. It appears that polyacrylamide gels containing PDA have less background staining from silver than BIS gels. Thus, the sensitivity of detection of AMP-FLPs in PDA gels is enhanced and it is then possible to maintain silver-stained PDA gels permanently.

In conclusion, discontinuous gel electrophoresis can be used to type effective AMP-FLPs. AMP-FLP analysis offers these advantages over RFLP analysis of VNTRs: the ability to resolve all alleles at a particular locus, non-isotopic silver staining which will facilitate technology transfer, and relatively rapid assays. Compared with other PCR-related techniques the cost is relatively low, there are no requirements for probes, nylon or nitrocellulose membranes, and expensive assay chemicals. Additionally, the greater the number of samples analyzed at one time, the lower the cost of the assay will become. At present, we are generating population data on D1S80.

This is publication number 90-1 of the Laboratory Division of the Federal Bureau of Investigation. Names of commerical manufacturers are provided for identification only and inclusion does not imply endorsement by the Federal Bureau of Investigation.

References

Allen, R. C. (1980) Rapid isoelectric focusing and detection of nanogram amounts of roteins from body tissues and fluids. Electrophoresis 1:32-37.

Allen, R. C., Graves, G., Budowle, E. (1989) Polymerase chain reaction amplification products separated on rehydratable polyacrylamide gels and stained with sifver. BioTechniques 7:736-744.

Budowle, B., Baechtel, F. S. (1989) Modification to improve the effectiveness of restriction fragment length polymorphism typing. Appl. Theor. Electrol (in press).

Hochstrasser, D., Patchornik, A., Merril, C. (1988) Development of polyacrylamide gels that improve the separation of proteins and their detection by silver staining. Anal. Biochem. 173: 412-423.

Horn, G. T., Richards, B., Klinger, K. W. (1989) Amplification of a highly polymorphic VNTR segment by the polymerase chain reaction. Nuc. Acids Res. 17: 2140.

Kasai, K., Nakamura, Y., White. R. (1989) Amplification of VNTR locus by the polymerase chain reaction (PCR). Proceedings of an International Symposium on the Forensic Aspects of DNA Analysis, Governmental Printing Office, Washington, D. C. (in press).

Nakamura, Y., Carlson, M., Krapcho, K., White, R. (1988) Isolation and mapping of a polymorphic DNA sequence (pMCT118) on Chromosome 1p (D1S80). Nuc. Acids Res. 16: 9364.

Nakamura, Y., Leppert, M., O'Connell, P., Wolff, R., Holm, T., Culver, M., Martin, C., Fujimoto, E., Hoff, M., Kumlin, E., White, R., (1987) Variable number of tandem repeat markers for human gene mapping. Science 235: 1616-1622.

Saiki, R. K., Scharf, S., Faloona, F., Mullis, K. B., Horn, G. T., Erlich, H. A., Arnheim, N. (1985) Enzymatic amplification of beta-globin genomic sequences and restriction analysis for diagnosis of sickle-cell anemia. Science 230: 1350-1354.

Southern E. M. (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. 98: 503-517.