TECHNICAL NOTE

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Amplification of a Variable Number of Tandem Repeats (VNTR) Locus (pMCT118) by the Polymerase Chain Reaction (PCR) and Its Application to Forensic Science

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ABSTRACT: A genetic locus (D1S58, defined by DNA probe pMCT118) that contains a variable number of tandem repeats (VNTR) has been successfully amplified from a very small amount of genomic deoxyribonucleic acid (DNA) by the polymerase chain reaction (PCR). The DNA sequence of the locus was determined and was found to consist of a 16-base consensus sequence and flanking sequences. Oligonucleotide primers complementary to the flanking sequences were synthesized to serve as primers for amplification of MCT118 by the PCR method. Human genomic DNA isolated from blood (2 ng from each sample) was successfully amplified at the MCT118 locus, and polymorphic bands were detectable by ethidium bromide staining after electrophoresis on polyacrylamide gels. Determination of genotypes at this VNTR locus can now be routinely achieved within 24 h, without the need for Southern blots or radioactive materials. Furthermore, the small size (387 to 723 base pairs) of the DNA fragments produced in the PCR amplification permits good resolution of individual alleles that differ by only one repeat unit. The precise specification of the number of tandem repeats present in each allelic fragment is reproducible from one analysis to another.

KEYWORDS: forensic science, genetic typing, deoxyribonucleic acid (DNA)

Some variable-number-tandem-repeat (VNTR) deoxyribonucleic acid (DNA) markers show so much variation within the population that they are coming into wide use for paternity testing and individual identification. The usual technique for determining gen-

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otypes at VNTR loci, the Southern blot assay, requires several days' time, the use of radioactive materials, and several micrograms of the DNA being tested. The recent development of the polymerase chain reaction (PCR) for amplifying DNA sequences [*1*] has raised the possibility that VNTR polymorphisms might be analyzed without these difficulties.

Jeffreys et al. [*2*] used PCR to amplify minisatellite loci from 1 to 10 ng of human DNA, but their protocol required an isotopically labeled probe and Southern blots to detect the alleles. Horn et al. [*3*] amplified the VNTR locus YNZ22 (D17S30) and analyzed it using ethidium bromide staining of electrophoretic gels, but that procedure required a 1-μg test sample.

To avoid these technical disadvantages, we chose to study a VNTR locus, D1S58 (defined by the probe pMCT118), because its allelic fragments revealed by the restriction enzyme *Alu* I are very small. Southern blot analysis had revealed a heterozygosity of 78%, and more than ten alleles, at this locus among 100 Caucasians [*4*].

We digested a plasmid MCT118 insert with *Alu* I and subcloned the fragments into M13 mp18 [*5*]. The nucleotide sequence of the locus defined by pMCT118 was determined by the dideoxy chain termination method [*6*]. The sequence, shown in Fig. 1, reveals a

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AGGCT CCCAG CTCAG GGTGC TCGCT TGGAG CAAAC AGGCC AGAGG GGAGC
GAACA GAGTG ?TGAG GGGCC GATGG ACGCT TCCAC TGGCC TCATG GACCG
GCCCC TCACG GTGCC AAGGA AACAG CCCCA CCATG AGGCG CTGAG AGAAA
CTGGC CTCCA AACAC TGCCC GCCGT CCACG GCCGG CCGGT CCTGC GTGTG
AATGA CCCAG GAGCG TAT?C CCCAC GCGCC A?ACT GCATT CAGAT AAGCG
CTGGC TCAGT G
GCAGC CCA-A GG-AA G
ACAGA CCACA GGCAA G
GAGGA CCACC GGAAA G
GAAGA CCACC GGAAA G
GAAGA CCACC GGAAA G
GAAGA CCACA GGCAA G
GAGGA CCACC GGAAA G
GAAGA CCACC GGCAA G
GAGGA CCACC GGCAA G
GAGGA CCACC AGGAA G
GAGGA ----- ----- -
----- ----- -GGAA G
GAGGA CCACT GGCAA G
GAAGA CCACC GGCAA G
CCTGC AAGGG GCACG TGCAT CTCCA ACAAG ACAAA ATAAA CAAGC CAGAG
AGGGC TTGTG ACCAG TGTGG CATTT GTCAC
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FIG. 1—*Nucleotide sequence of MCT118. The tandem repeats are aligned in a vertical column. Primer sequences are underlined.*

16-base pair repeating unit, which contains the core sequence (GNNGTGGG) that is characteristic of this class of VNTR repeats [7].

Initially, several sets of oligonucleotide primers were synthesized and used to prime PCR amplification, but all failed to show sufficient specificity. Finally, a pair of oligonucleotide primers, 28 and 29 base pairs in length, respectively (identified by underlining in Fig. 1), were synthesized; these gave a high degree of specificity to the PCR reaction. The tandemly repeated region of MCT118 was amplified by a slightly modified version of the PCR method.

Two nanograms of each human DNA sample, obtained from peripheral lymphocytes of random individuals, were amplified in a reaction mixture containing 25 μL of 67m*M* Tris/hydrochloric acid (HCl) (pH 8.3); 6.7m*M* magnesium chloride ($MgCl_2$); 16.6m*M* ammonium sulfate [$(NH_4)_2SO_4$]; 10m*M* 2-mercaptoethanol; 170 μg/mL bovine serum albumin; 10% dimethyl sulfoxide (DMSO); and 2.5m*M* each of the deoxy forms of adenosine triphosphate (dATP), cytidine triphosphate (dCTP), guanosine triphosphate (dGTP), and thymidine triphosphate (dTTP); 1.25 units of *Taq* polymerase; and 2 μ*M* of each primer. After denaturation of the DNA at 95°C for 1 min, annealing was done at 65°C for 1 min, with an extension at 70°C for 8 min, and repeated for 35 cycles.

Polymorphic bands were detectable by ethidium bromide staining after electrophoresis on polyacrylamide gels; the bands were separated clearly according to the number of repeats in the corresponding allele (Fig. 2). We obtained the best results using 2 ng of template, with 30 to 35 cycles of amplification; more than 35 cycles of amplification resulted in the appearance of extra bands, and using more than 10 ng of template resulted in "ladder" bands.

At a concentration of 2 μ*M*, the 28- or 29-base primers usually gave almost identical intensities in the allelic bands from a heterozygote, regardless of allele size. This is in contrast to the results reported by Horn et al. [3], in which bands of higher molecular size disappeared or were significantly less intense when the primer concentrations were <1 μ*M*. However, even in our own experiments, an annealing temperature of 60°C resulted in the appearance of a high-molecular-weight extra band or bands; furthermore,

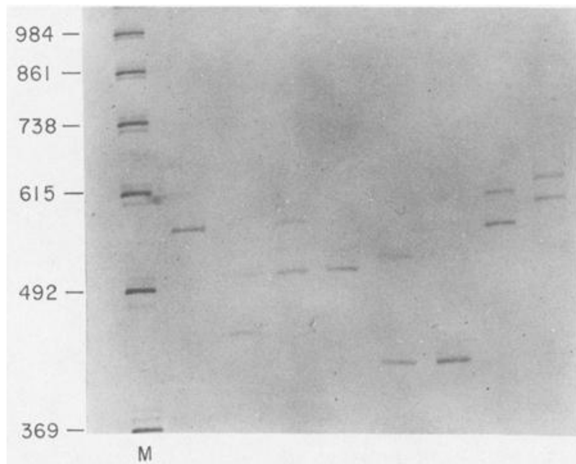

FIG. 2—*Ethidium-bromide-stained polyacrylamide gel after PCR amplification of MCT118 in DNA from eight unrelated individuals. The alleles in test lane 4 (a homozygote) and the heavier of the two alleles in test lane 5 differ by only one repeating unit. M = the marker lane; the molecular sizes (in base pairs) are indicated to the left of the markers.*

when we used shorter primers (20-base length), the appearance of multiple artifactual bands, or "ladders," hid the appropriate bands (data not shown).

In order to gain an impression of the number of alleles that would be revealed by this system in several populations, we amplified MCT118 in DNA isolated from 67 unrelated Caucasian and Japanese subjects. Twenty-one alleles, ranging in size from 387 to 723 base pairs, were seen within this population sample; these fragments reflected alleles containing 15 to 36 repeating units of the size present in MCT118 (Fig. 3). We also amplified DNA from 16 members of a Japanese family and demonstrated a Mendelian pattern of inheritance of alleles at the MCT118 locus.

The small size of the allelic fragments derived from MCT118 provides several advantages for forensic analysis. Perhaps most important, small fragments permit good resolution of individual alleles that differ in length by only one repeating unit. (Thus far, all alleles at VNTR loci that have been examined by sequencing differ by an integral number of repeating units.) This degree of resolution makes it possible to assign a specific number of repeating units to the observed alleles.

Furthermore, the ability to amplify a locus by PCR means that one can quickly identify the alleles present in a sample by ethidium bromide staining of the amplified fragments, without the need for Southern transfers or hybridization with isotopically labeled probes, even when the samples contain as little as 2 ng of DNA.

Finally, the small digestion fragments one obtains from MCT118 should make it possible to generate good signals from forensic samples whose DNA has been substantially degraded. Conventional VNTR systems require DNA of high molecular weight.

These data show that pMCT118 will be a valuable addition to the arsenal of reagents available for paternity testing and for forensic identification of individuals.

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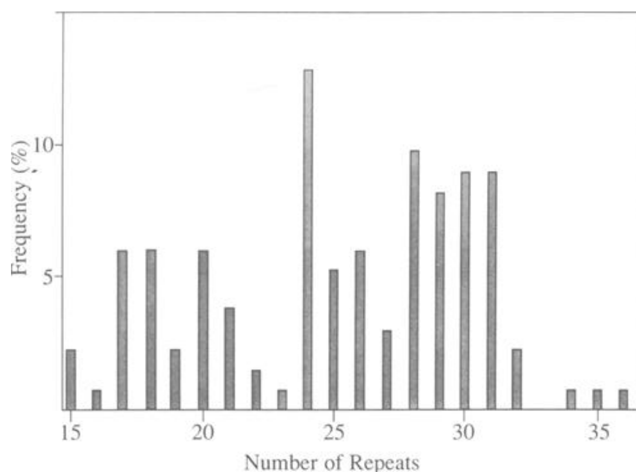

FIG. 3—*Distribution of MCT118 alleles among 67 unrelated Caucasian and Japanese individuals.*

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