MULTIPLEX AMPLIFICATION OF SHORT TANDEM REPEAT LOCI

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of U.S. patent application Ser. No. 08/632,575, filed Apr. 15, 1996, now U.S. Pat. No. 5,843,660, issued Dec. 1, 1998, which is a continuation-in-part of U.S. patent application Ser. No. 08/316,544, filed Sep. 30, 1994. The entire disclosure of those applications is incorporated by reference herein.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

Not applicable.

FIELD OF THE INVENTION

The present invention is generally directed to the detection of genetic markers in a genomic system. The present invention is more specifically directed to the simultaneous amplification of multiple distinct polymorphic genetic loci using the polymerase chain reaction or other amplification systems to determine, in one reaction, the alleles of each locus contained within the multiplex system.

BACKGROUND OF THE INVENTION

DNA typing is commonly used to identify the parentage of human children, and to confirm the lineage of horses, dogs, other animals, and agricultural crops. DNA typing is also commonly employed to identify the source of blood, saliva, semen, and other tissue found at a crime scenes or other sites requiring identification of human remains. DNA typing is also employed in clinical settings to determine success or failure of bone marrow transplantation and presence of particular cancerous tissues. DNA typing involves the analysis of alleles of genomic DNA with characteristics of interest, commonly referred to as "markers". Most typing methods in use today are specifically designed to detect and analyze differences in the length and/or sequence of one or more regions of DNA markers known to appear in at least two different forms in a population. Such length and/or sequence variation is referred to as "polymorphism." Any region (i.e. "locus") of DNA in which such a variation occurs is referred to as a "polymorphic locus." The methods and materials of the present invention are designed for use in the detection of multiple loci of DNA, some or all of which are polymorphic loci.

Genetic markers which are sufficiently polymorphic with respect to length or sequence have long been sought for use in identity applications, such as paternity testing and identification of tissue samples collected for forensic analysis. The discovery and development of such markers and methods for analyzing such markers have gone through several phases of development over the last several years.

The first identified DNA variant markers were simple base substitutions, i.e. simple sequence polymorphisms, which were most often detected by Southern hybridization assays. For examples of references describing the identification of such markers, designed to be used to analyze restriction endonuclease-digested DNA with radioactive probes, see: Southern, E. M. (1975), *J. Mol. Biol.* 98(3):503–507; Schumm, et al. (1988), *American Journal of Human Genetics* 42:143–159; and Wyman, A. and White, R. (1980) *Proc. Natl. Acad. Sci, U.S.A.* 77:6754–6758.

The next generation of markers were size variants, i.e. length polymorphisms, specifically "variable number of tandem repeat" (VNTR) markers (Nakamura Y., et al. (1987), *Science* 235: 1616–1622; and U.S. Pat. No. 4,963,663 issued to White et al. (1990); U.S. Pat. No. 5,411,859 continuation of 4,963,663 issued to White et al. (1995)) and "minisatellite" markers (Jeffreys et al. (1985a), *Nature* 314:67–73; Jeffreys et al. (1985b) *Nature* 316:76–79., U.S. Pat. No. 5,175,082 for an invention by Jeffreys). Both VNTR and minisatellite markers, contain regions of nearly identical sequences repeated in tandem fashion. The core repeat sequence is 10 to 70 bases in length, with shorter core repeat sequences referred to as "minisatellite" repeats and longer repeats referred to as VNTRs. Different individuals in a human population contain different numbers of the repeats. The VNTR markers are generally more highly polymorphic than base substitution polymorphisms, sometimes displaying up to forty or more alleles at a single genetic locus. However, the tedious process of restriction enzyme digestion and subsequent Southern hybridization analysis are still required to detect and analyze most such markers.

The next advance involved the joining of the polymerase chain reaction (PCR) (U.S. Pat. No. 4,683,202 by Mullis, K. B.) technology with the analysis of VNTR loci (Kasai, K. et al. (1990) *Journal Forensic Science* 35(5):1196–1200). Amplifiable VNTR loci were discovered, which could be detected without the need for Southern transfer. The amplified products are separated through agarose or polyacrylamide gels and detected by incorporation of radioactivity during the amplification or by post-staining with silver or ethidium bromide. However, PCR can only be used to amplify relatively small DNA segments reliably, i.e. only reliably amplifying DNA segments under 3,000 bases in length Ponce, M & Micol, L. (1992) NAR 20(3):623; Decorte R, et al. (1990) *DNA Cell Biol.* 9(6):461–469). Consequently, very few amplifiable VNTRs have been developed.

In recent years, the discovery and development of polymorphic short tandem repeats (STRs) as genetic markers has stimulated progress in the development of linkage maps, the identification and characterization of diseased genes, and the simplification and precision of DNA typing. Specifically, with the discovery and development of polymorphic markers containing dinucleotide repeats (Litt and Luty (1989) *Am J. Hum Genet* 3(4):599–605; Tautz, D (1989) *NAR* 17:6463–6471; Weber and May (1989) *Am J Hum Genet* 44:388–396; German Pat. No. DE 38 34 636 C2, inventor Tautz, D; U.S. Pat. No. 5,582,979 filed by Weber, L.), STRs with repeat units of three to four nucleotides (Edwards, A., et al. (1991) *Am. J. Hum. Genet.* 49: 746–756.; Hammond, H. A., et al. (1994) *Am. J. Hum. Genet.* 55: 175–189; Fregeau, C. J.; and Fourney, R. M. (1993) *BioTechniques* 15(1): 100–119.; Schumm, J. W. et al. (1994) in *The Fourth International Symposium on Human Identification* 1993, pp. 177–187 (pub. by Promega Corp., 1994); and U.S. Pat. No. 5,364,759 by Caskey et al.; German Pat. No. DE 38 34 636 C2 by Tautz, D.) and STRs with repeat units of five to seven bases (See, e.g. Edwards et al. (1991) *Nucleic Acids Res.* 19:4791; Chen et al. (1993) *Genomics* 15(3): 621–5; Harada et al. (1994) *Am. J. Hum. Genet.* 55: 175–189; Comings et al. (1995), *Genomics* 29(2):390–6; and Utah Marker Development Group (1995), *Am. J. Genet.* 57:619–628; and Jurka and Pethiyagoda (1995) *J. Mol. Evol.* 40:120–126)), many of the deficiencies of previous methods have been overcome. STR markers are generally shorter than VNTR markers, making them better substrates for amplification than most VNTR markers.

STR loci are similar to amplifiable VNTR loci in that the amplified alleles at each such locus may be differentiated