Species identification by polymerase chain reaction and direct sequencing

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INTRODUCTION

Blood stain gives many informations on forensic science. Human identification of blood stains is essential for forensic practice. Immunological and biochemical methods have been used for the identification of human blood stains. However, it is difficult to determine the species of blood stains except human. In this study, we present the method for human identification by Polymerase chain reaction(PCR) and direct sequencing. This method is useful for not only human identification but also the determination of the species of forensic samples.

MATERIALS AND METHODS

Human and animal blood stains were prepared with 10 µl of whole blood on filter paper. Dried blood specimens were stored at room temperature. DNA samples were extracted from whole blood and blood stains. Oligonucleotide primers were designated based on a common conserved sequence in a region of cytochrome b of mitochondrial DNAs which were already published. Used primers were L14839: 5′-CCATCCAACATCTCAGGCATGATGA-3′ and H15155:5′-TGTGGCCCCTCAGAATGATATTTG-3′. The L or H in the primer name refers to the light or heavy strand, respectively, and number identifies the base at the 3′ end according to the numbering of the published sequence in human. A region of cytochrome b in mitochondrial DNA was amplified by PCR using DNA samples and primers. PCR products were separated and purified by agarose gel electrophoresis. Subsequently, single-stranded DNA was generated by an asymmetric PCR and its nucleotide sequences were determined by the dideoxy-chain termination method. DNA sequences were compared for the identification of the sample species.

RESULTS AND DISCUSSION

The fragments of cytochrome b were amplified by PCR using the DNA samples extracted from whole blood of human and several animals and primers based on a common region of cytochrome b in mitochondrial DNA. DNA sequence of 155 nucleotides in the amplified fragment of cytochrome b region was compared among human and five animal species as shown in Figure 1. The amplified DNA fragments from human and several animals showed the characteristic DNA sequences on each species. The DNA sequences from two Japanese were differed from the published data of a complete human mitochondrial DNA sequence by Anderson et al(1981). A mutation which was a transition from G to A was observed at the position of 15043 which referred to the light strand of the published sequence. However, the change did not affect amino acid sequence. Partly DNA sequences of cytochrome b in cow and mouse accorded with those in published data perfectly. There are moderate differences in the DNA sequences of PCR products among species. The mean number and frequency of the

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difference in nucleotides were 39.7 and 25.6%, respectively. The number and frequency of common nucleotides in 155 nucleotides of cytochrome b were 82 and 52.9%, respectively.

Using DNA samples extracted from blood stains, the fragments of cytochrome b region were also amplified by PCR in human and several animal species. The DNA sequences of amplified fragments were also determined by direct sequencing. The determined DNA sequences were in accordance with those using whole blood samples. From the blood stain of cow, only 49 nucleotides(15059-15107 region which refers to the strand of the published sequence of human) were sequenced clearly. However, the difference of nucleotides was enough to distinguish the species of blood stain from other species. The analysis of DNA sequence in the region of cytochrome b in mitochondrial DNA by PCR and direct sequencing are useful method for not only making distinction between human and animals but also the determination of the species of blood stains.

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            14980                                        15019
Human*          CATCCGCTACCTTCACGCCAATGGCGCCTCAATATTCTTT
Human**         CATCCGCTACCTTCACGCCAATGGCGCCTCAATATTCTTT
Horse           TATCCACCATCTCCATGCTAACGGAGCGTCCATATTCTTC
Cow             CATCCGATACATACACGCAAACGGAGCTTCAATGTTTTTT
Pig             TATTCGCTATCTACATGCAAACGGAGCATCCATATTCTTT
Rabbit          CTATCGATATCTCCATGCCAATGGAGCATCGATATTTTTT
Mouse           AATCCGATATATACACGCAAACGGAGCCTCAATATTTTTT

            15020                                        15059
Human*          ATCTGCCTCTTCCTACACATCGGGCGAGGCCTATATTACG
Human**         ATCTGCCTCTTCCTACACATCGGACGAGGCCTATATTACG
Horse           ATCTGCCTCTTTATTCACGTAGGACAAGGCCTTTACTACA
Cow             ATCTGCTTATATATGCACGTAGGACGAGGCTTATATTACG
Pig             ATTTGCCTATTCATCCACGTAGGCCGAGGTCTATACTACG
Rabbit          ATTTGCCTCTATATACACGTAGGCCATGGAATCTATTATG
Mouse           ATTTGCTTATTCCTTCATGTCGGACGAGGCTTATATTATG

            15060                                        15099
Human*          GATCATTTCTCTACTCAGAAACCTGAAACATCGGCATTAT
Human**         GATCATTTCTCTACTCAGAAACCTGAAACATCGGCATTAT
Horse           GCTCCTACACCTTCCTAGAAACATGAAATATTGGGATTCT
Cow             GGTCTTACACTTTTCTAGAAACATGAAATATTGGAGTAAT
Pig             GATCCTATATATTCCTAGAAACATGAAACATTGGAGTAGT
Rabbit          GCTCATATACATATCTAGAAACCTGAAACATCGGCATTAT
Mouse           GATCATATACATTTATAGAAACCTGAAACATTGGAGTACT

            15100                                    15134
Human*          CCTCCTGCTTGCAACTATAGCAACAGCCTTCATAG
Human**         CCTCCTGCTTGCAACTATAGCAACAGCCTTCATAG
Horse           CCTATTTCTTCCAGTAATAGCTACAGCATTCATGG
Cow             CCTTCTGCTCACAGTAATAGCCACAGCATTTATAG
Pig             CCTACTATTTACCGTTATAGCAACAGCCTTCATAG
Rabbit          TCTATTATTCTAAGAAATAGCAACAGCATTCATAG
Mouse           TCTACTGTTCGCAGTCATAGCCACAGCATTTATAG
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Fig. 1 Comparison of partial DNA sequences in cytochrome b among human and five animal species

*: The sequences from the published data of a complete mtDNA sequence for one individual by Anderson et al(1981).

**: Two Japanese mtDNA sequences

Table 1 The number and frequencies(%) of different nucleotides in the 155 nucleotide of cytochrome b region (14980-15134)* among human and five animals

	Human[b]	Horse	Cow	Pig	Rabbit	Mouse
Human[a]	1 (0.65)	46 (29.7)	45 (29.0)	39 (25.2)	44 (28.4)	41 (26.5)
Human[b]		45 (29.0)	44 (28.4)	39 (25.2)	44 (28.4)	40 (25.8)
Horse			39 (25.2)	34 (21.9)	43 (27.7)	46 (29.7)
Cow				35 (22.6)	44 (28.4)	25 (16.1)
Pig					40 (25.8)	33 (21.3)
Rabbit						41 (26.5)

*: The number shows the nucleotide position which refers to the light strand of the published sequence by Anderson et al(1981).

a: The sequences from the published data of a complete mtDNA sequence by Anderson et al (1981).

b: Japanese mtDNA sequence

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2 DNA (RFLP, VNTR) Systems