## Differentiation of closely related deer meats by PCR analysis

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### SUMMARY

In order to identify deer meat, we have determined an entire sequence of the cytochrome b gene from red deer (*Cervus elaphus*). The red deer cytochrome b gene showed 94.1% similarity to the Japanese deer (*Cervus nippon*) gene. A pair of PCR primers (RD1/RD2), based on the red deer cytochrome b gene, was designed to amplify deer specific fragments. The PCR amplified 194 bp fragments from the red deer and Japanese deer and no fragment from cattle, pig, chicken, sheep, goats, horse and rabbits. The red deer was differentiated from the Japanese deer by the PCR-RFLP analysis using the restriction enzymes *EcoR*I, *BamH*I or *Sca*I.

### INTRODUCTION

Two kinds of deer meats appear on the market in Japan. One is Japanese deer obtained in Japan, and the other is red deer imported from New Zealand and other countries. These meats, especially Japanese deer, are very expensive in Japan. Protein analyses may be inadequate for differentiation of the two deer meats because they are closely related species belonging in the same genus. DNA analyses were useful for the identification among closely related animals (Chikuni *et al.*1994a,b), thus we determined a sequence of the red deer cytochrome b gene and then designed the deer specific PCR primers.

### MATERIALS AND METHODS

Mitochondrial DNAs were obtained from the meat of red deer, Japanese deer, cattle, pig, chicken, sheep, goats, horse and rabbits. The red deer cytochrome b gene was amplified by the PCR using the common primers (Chikuni *et al.*1995) and then determined through the direct cycle-sequencing procedures. Sequences of the deer specific primers were 5'-TCATCGCAGCACTCGCTATAGTACACT-3' and 5'-ATCTCCAAGTAGGTCTGGTGCGAATAA-3' for the RD1 and RD2, respectively (Fig.1). The PCR was run as follows: each cycle of denaturation for 0.5 min at 94℃, annealing for 0.5 min at 60℃ and extension for 0.5 min at 72℃ for 35 cycles.

### RESULTS AND DISCUSSIONS

The red deer cytochrome b gene was coded in 1140 bp length and showed 94.1 % similarity to the Japanese deer sequence. On the basis of the deer sequences, the primers RD1 and RD2 were designed at the deer specific regions (Fig.1). The sequences of the primers were the same to the red deer cytochrome b gene and one substitution each primer on the Japanese deer gene. The PCR using RD1/RD2 amplified 194 bp fragments corresponding to the region 563-756 of the deer cytochrome b gene and nothing from the other animals (Fig.2). These results showed that the deer meats would be identified by the PCR amplification. Some recognition sites of restriction enzymes existed on the PCR fragments and differed among the two deer species (Fig.1). Figure 3 shows the digestion pattern of the PCR-RFLP analysis. The *EcoR*I digestion of the PCR products resulted in 67/127 bp from the red deer and 194 bp fragments from the Japanese deer. The red deer gene has no recognition site for *BamH*I and *Sca*I on the amplified region, therefore these digestion resulted in 194 bp fragments from the red deer. The Japanese deer gene was resulted in 48/146 bp and 49/145 bp fragments by *BamH*I and *Sca*I digestion, respectively. The red deer meat would be differentiated from the Japanese deer one by using the restriction enzymes *EcoR*I, *BamH*I or *Sca*I.

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          RD1 →      580                 600                 620                 640                 660
R.deer    TCATCGCAGCACTCGCTATAGTACACTTACTCTTCCTTCACGAAACAGGATCTAATAACCCAATAGGAATTCCATCAGACGCAGACAAAATCCCCTTTCA
J.deer    ·····T·····G·····C··C·····C·····C·····G·····C··
Bovine    ·····AT····A·T··C·····C···C····A·····C·····C··C··C·····C·····T·C·····T·····A··C··
Pig       ····TA·C··C·····AGCC·····TC·CA·A·····G·····C·····C··C·····T·CC·····CT·····AT·····T··A·····
Chicken   CA·····GTA·TA····CA·C···C·CAC·····A·····T····C··A··C·····CC····C··CT····C···T·T·····T··A·····
Sheep     ·····C·····C·····T···C·····C·····C··C·····C·C·····G···A····T·····T·····C··
Goat      ·····A····C·····C·····C···C·····C·····C··C·····C·C·····A····T·····A·····
Horse     ·····A····C··G·TAG·C·····T·····A··T·····C·····CTC·····C·····C··TATG·····A··C··
Rabbit    ····T···A·TT·A·TCT··A·T···C·C·····T··A··T·····T··C··C··C·····C·C·····T···A··T····T·····T··C··

          680                 700                 720                 740          ← RD2
R.deer    TCCTTATTATACCATTAAAGATATCTTAGGCATCCTACTTCTTGTACTCTTCTTAATATTACTAGTATTATTCGCACCAGACCTACTTGGAGAT
J.deer    ·····C··C·····T·····A·····C·····G·····
Bovine    C··C··C·····G··C·····GGC···CT·A··AA·T··AGCTC·····C·····C·····C·····C··C·····C
Pig       C··A··C··C··T·····C··TC····AGC·T··T··A·AA····AA··C··C··A·C··T···C·····T·····A·····C
Chicken   C··A··C··CT··T·C·····C··TC·G···T·AACT··CA·AC·CAC·CCA··CC··AC·····CCC·····T·C··CA····C··A·····C
Sheep     C·····C·····C···C····TGCTA·C··A··AA·C···A··C·C··GC·····C·····A····T···T····C·····C
Goat      C·····C··C·····GC·A·G··A··AA·T··TG··C·····C·····C·····A····C·····T··C·····C
Horse     C··A·····A·····C···C····AC····C··C··GA·CT·GC··C··C··ACT·····T·C··C·····C··A·····C
Rabbit    C··C··C··C··A··C·····C·C·C····TT····TG·AGCCA·T··TC··C·CC·TA·TT····CC····TT·····T·A·····C
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Fig.1. Partial sequences of the cytochrome b genes.
Open boxes indicate the primer regions. Double-underlines indicate *EcoR* I, *BamH* I or *Sca* I sites on the red deer and Japanese deer sequences. The entire sequence of the red deer cytochrome b gene have been submitted to the DDBJ with the accession number AB001612. The other accession numbers are D32192(Japanese deer; Chikuni *et al.*1995), V00654(Bovine; Anderson *et al.*1982), X56295(Pig; Irwin *et al.*1991), X52392(Chicken; Desjardins *et al.*1990), X56284(Sheep; Irwin *et al.*1991), X56289 (Goat; Irwin *et al.*1991), D32190(Horse; Chikuni *et al.*1994b) and D32168(Rabbit; Chikuni *et al.*1994b).

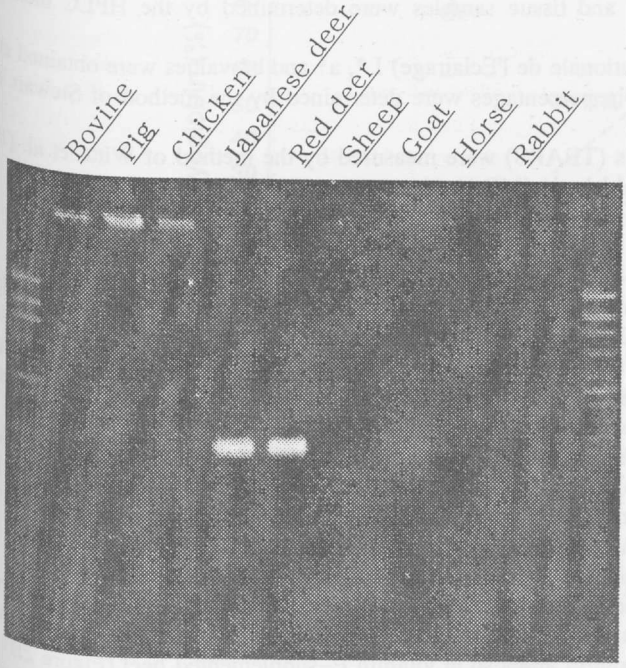


**Fig.2. Agarose gel electorophoresis of the PCR products amplified with the RD1/RD2 primers. The molecular marker is $\phi$ x174/*Hinc* II digest.**

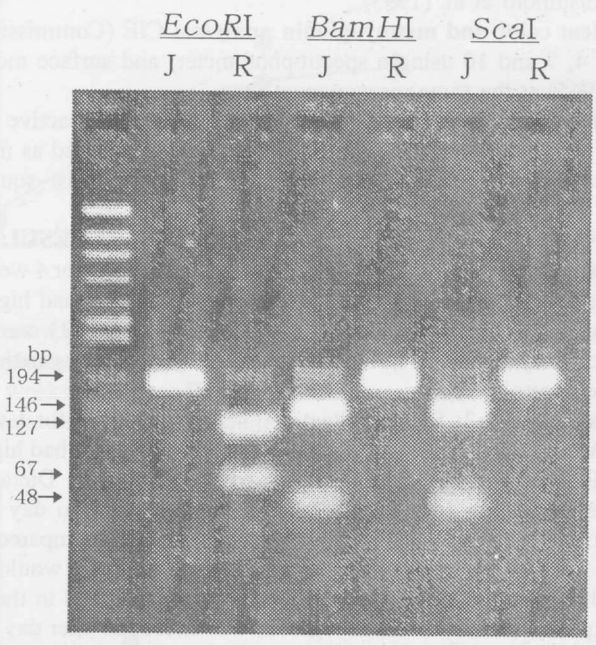


**Fig.3. Agarose gel electrophoresis of the PCR products digested with restriction enzymes. J,Japanese deer; R,red deer.**