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TESTING RELIABILITY OF SERUM SAMPLES AS A DNA SOURCE ON CAPTIVE BREEDING LONG-TAILED MACAQUES (*Macaca fascicularis*)

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ABSTRACT

The serum reliability was tested in captive breeding conditions on the long-tailed macaques (*Macaca fascicularis*). Serum and buffy coat were applied to standard protocol of DNA extraction following the amplification of microsatellite DNA locus of D2S1777 by Polymerase Chain Reaction (PCR) and visualized PCR products by means of Polyacrylamide gel electrophoresis (PAGE) with silver staining. The amount of genomic DNA extracted from serum was sufficient for genotyping individuals at DNA microsatellite locus of D2S1777 with allele size of approximately 160 bp. The study showed that serum could provide reliable alternative for obtaining DNA when taking blood using anticoagulant was impracticable.

Keywords: serum, DNA source, captive breeding, monkeys

INTRODUCTION

Primates play an essential role in biomedical research. The study of primates has also contributed to our understanding of basic biological phenomena such as reproduction and several diseases, to the development of drugs, and vaccines (Nathanson & Maithieson 2000; Sibal & Samson 2001).

As in other model organisms, genetic background in primates such as long-tailed (*Macaca fascicularis*) and rhesus macaques (*M. mulatta*) is an experimental variable that affects the response of other study variables (Stevison & Kohn 2008; Hernandez *et al.* 2007). A standardized system for genetic testing would be beneficial for the management of breeding colonies and also facilitate the characterization of other important traits, including MHC type, resistant to Simian Immunodeficiency Virus (SIV) and the susceptibility to many human pathogens (Satkoski *et al.* 2008). Moreover, data on reference of hematologic and serum parameters are essential for

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evaluating results obtained from laboratory animals and this baseline biological data are needed before they are used for scientific purposes (Bonfanti *et al.* 2009). In fact, to fulfill the entire criteria mentioned, the analyses relied on the availability of blood samples.

Upon the purposes of studies, ordinarily blood samples are taken from the animals without anticoagulant or with anticoagulant, such as heparine and sodium citrate (especially for genetic characterization). However, when obtaining blood samples with anticoagulant is impracticable for some reasons, available samples particularly serum or clot samples will be an alternative samples to be utilized.

The lack of published description for detailed protocol on serum application, however, facilitated the remarkably approach for genetic analyses of captive breeding monkeys. As a result, our study aimed to examine the applicability and reliability of serum samples as a DNA source.

MATERIALS AND METHODS

Blood samples were collected in conjunction with routine health physical examinations of individuals in captive breeding population of long-tailed macaques (*Macaca fascicularis*) housed at Primate Research Center, Bogor Agricultural University. Two kinds of blood samples were available for analyses: one using heparin as anticoagulant and the other without heparin. Blood with anticoagulant were centrifuged at 3,000 rpm for 15 minutes. Erythrocyte and plasma were stored at -20 °C for other purposes, and buffy coat was directly applied to DNA extraction protocol. Along with processing blood with anticoagulant, serum was obtained after samples were allowed to clot.

In total 38 sera and buffy coat were used as DNA sources. DNA extraction was carried out following the method of Kan *et al.* (1977) with minor modification for serum and buffy coat separately to avoid potential contamination. Serum or buffy coat was washed with 0.9% NaCl- 1mM EDTA. Centrifugation was performed at 2,000 rpm for 10 minutes. Supernatant was discarded and pellet was suspended in 2.5 mL STE buffer (salted Tris-EDTA), 200 µL 10% SDS and digested with 40 microL proteinase K (5 mg/mL) for a minimum of 2 hours at 37 °C. Then genomic DNA was extracted with standard phenol-chloroform method.

As DNA microsatellites are the most common neutral nuclear marker applied in genetic diversity studies, we tested microsatellite amplification to validate amplification success from the tested sources. The test was based on amplification of D2S1777 microsatellite marker, a monomorphic DNA microsatellite locus on genetic study of long-tailed and pig-tailed macaques (Perwitasari-Farajallah *et al.* 2007; Perwitasari-Farajallah *et al.* 2010). Allele size in contemporary long-tailed macaques was $\pm$ 160 bp.

PCR was conducted in a PTC 100 MJ Research Inc. in 12.5 µL reaction volumes using PCR buffer (containing 25 mM $MgCl_2$), 1.0 U/µL Taq polimerase (PROMEGA), 10xPCR buffer, 2.5 mM dNTP. Each reaction contained 25 pM of each primer flanking the microsatellite region (f:5'-TCCCCAAGTAAAGCATTGAG-3';r:5'-

GTATGTAGGTAGGGAGGCAGG-3') and approximately 10-100 ng of DNA. After 3 minutes initial denaturation at 94 °C, PCR was performed in a total of 30 cycles using the following conditions: 30 seconds denaturation at 94 °C, 60 seconds annealing at 48 °C, and 60 seconds elongation at 70 °C (Perwitasari-Farajallah *et al.* 2004). With a final elongation of 5 minutes at 72 °C, PCR was terminated. Strict precautions against PCR contamination were performed. Separate area were utilized for the preparation of amplification reactions, the addition of DNA template, and the carrying out of amplification reactions. Negative water PCR blanks were included in every analysis.

Two microliters of each PCR products were electrophoresed on 6% polyacrylamide gels and visualized by silver-staining following the technique described by Tegelström (1986). Allele sizes were verified using DNA size standard of 100 bp ladder (BioRad).

RESULTS AND DISCUSSION

DNA was successfully extracted from almost all the samples tested. Seventy four percent of the serum samples (n = 15) and 100% of the buffy coat (n = 19) were successfully amplified and gave strong amplification products (Fig.1 & 2).

It revealed that DNA derived from buffy coat samples have sufficient DNA for repeats genotyping attempts. In contrast, insufficient DNA yielded in serum samples may in part be attributed to inadequate amount of nucleated cell inside the samples.

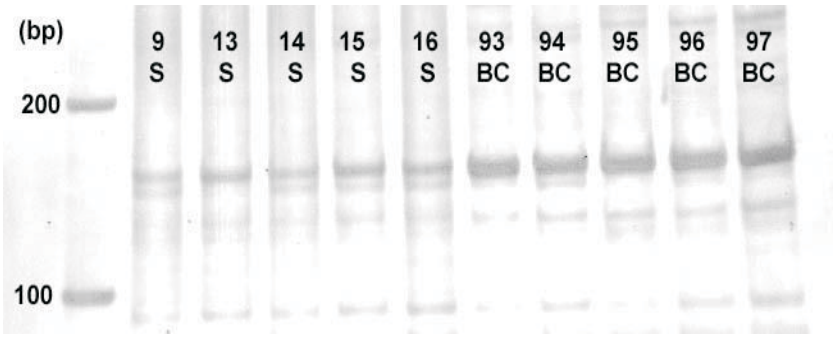

Figure 1. Amplification of the D2S1777 microsatellite locus from ten long-tailed macaques serum and buffy coat. M: size standard, 100-bp DNA ladder; Lanes 1-5, DNA extracted from serum; Lanes 6-10, DNA extracted from buffy coat.

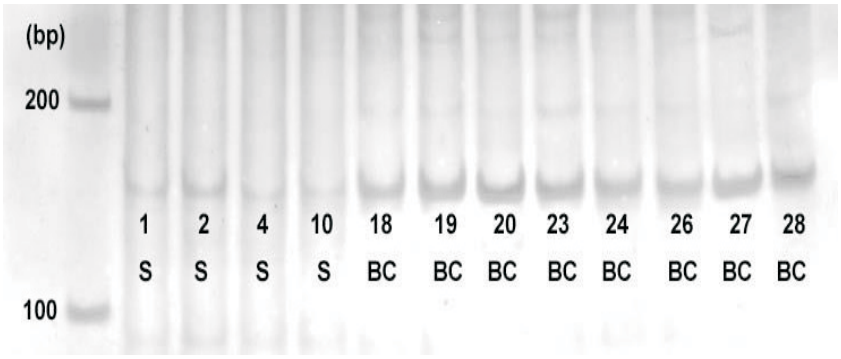

Figure 2. Amplification of the D2S1777 microsatellite locus from ten long-tailed macaques serum and buffy coat. Size standard, 100-bp DNA ladder; Lanes 1-4, DNA extracted from serum; Lanes 5-12, DNA extracted from buffy coat. S = serum; BC = Buffy coat

DNA size standard of 100 bp (BioRad) and PCR products on 6% PAGE stained with silver demonstrated that the concentration of the PCR products of isolated DNA from serum samples and from buffy coat were lower and higher compared to the DNA size standard, respectively. Although the DNA extracted from serum samples yielded low concentration of PCR product, we conceived that it revealed reliable results for further analyses.

Present study demonstrated that D2S1777 locus revealed to be monomorphic (Fig.1&2) as detected in our previous research with the allele size of approximately 160 bp. Although false alleles or amplification artefact are typically not only of low frequency, but also sporadic in occurrence, it cannot be disregarded as a potential source of error in microsatellite genotyping (Taberlet *et al.* 1999). Despite report by Fernandes *et al.* (2007) that the use of species-specific primers reduces the risk of false species assignment; the results obtained in this study consisted of apparent allele repeatedly amplified from several samples. Moreover, the D2S1777 is a human-derived microsatellite locus used in our laboratory for the routine genotyping of long-tailed macaques (*M. fascicularis*) using buffy coat as DNA source (Perwitasari-Farajallah *et al.* 2010; Perwitasari-Farajallah *et al.* 2007). Many genotyping studies depend on cross-species amplification, that is, the application of primers derived in one species for characterization of individuals in another, usually closely related species (Bradley *et al.* 2008). Therefore, a variety of strategies to determine DNA quality and detect or reduce genotyping errors should be determined (Paetkau 2003).

Utilization of serum samples was described previously by Jiminez and Tarantal (2003). They showed that fetal gender can be reliably determined in the early first trimester from maternal serum samples of rhesus macaques (*M. mulatta*) by real-time PCR. They adapted and applied the idea of some researchers (Lo *et al.* 1997, 1998; Costa *et al.* 2001; Honda *et al.* 2002) regarding fetal gender determination in human by

real-time PCR analysis of maternal serum samples. Even though they used serum for different purposes if compared to what we performed in our study, it appeared to be all of them obtained satisfactory results.

CONCLUSIONS

Our study provided, probably (to our knowledge), the first description of serum applicability as a source of DNA for future genetic studies of long-tailed macaques captive breeding population when obtaining blood samples using anticoagulant is impracticable.

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