ONLINE RESOURCES

Cross-species amplification of human microsatellite markers in pig-tailed and stump-tailed macaques

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Introduction

Microsatellite markers characterized in one species have been tested in other related species with varying degree of success (Chambers *et al.* 2004). Stump-tailed macaque (*Macaca arctoides*) and pig-tailed macaque (*Macaca nemestrina*) are biomedically important species but are becoming endangered fast. In the present study, 20 human tetranucleotide microsatellite markers were used to test their cross-species amplification in captive stump-tailed macaques and pig-tailed macaques. Out of 20 markers screened, 11 were polymorphic and 9 showed no amplification, non-specific amplification or were nonpolymorphic. These 11 microsatellite markers showed an average of 6.6 and 6.4 alleles per locus, and mean H_o values of 0.84 and 0.78 in pig-tailed and stump-tailed macaques, respectively. These markers were found to be potentially useful in range of genetic studies including paternity testing, pedigree analysis, population genetic studies etc., in captive populations of pig-tailed and stump-tailed macaques.

Nonhuman primates are the most relevant model organisms to understand the human biology and are extensively used in biomedical research. Genus *Macaca* belongs to family Cercopithecidae, comprises of 19 species (Fooden 1976). Pig-tailed macaque (*M. nemestrina*) and stump-tailed macaque (*M. arctoides*) belongs to this genus and are excellent models for studying diseases like AIDS (Lowenstine 1992; Haigwood 2004), reproduction (Fraser *et al.* 2005; Muehlenbein *et al.* 2005), animal behaviour (Santillan-Doherty *et al.* 1991; Weed and Hienz 2006) etc. Pig-tailed macaque is a relevant model for viral infection studies (Mansfield *et al.* 1999) and stump-tailed macaque for studying androgenetic alopecia (Uno 1986). However, these species are quickly becoming endangered due to habitat loss, poaching in the wild and inbreeding in captivity. Therefore they require immediate attention towards their conservation *in situ*, as well as in captivity; through selection of genetically healthy individuals and their maintenance as founder stocks for future conservation breeding.

Genetic markers are the basic tools to assess genetic variation in any population and are also helpful for subsequent management of populations in the wild as well as in captivity. Microsatellite markers are being extensively used for genetic analysis, population genetics, conservation assessments, paternity testing and in understanding male and female reproductive strategies in several species (Bruford and Wayne 1993; Griffith *et al.* 2002; Di Fiore 2003). Microsatellites are 2–6 nucleotide long, tandemly repetitive units present in the genome, which are often flanked by sequences that are evolutionarily conserved. Microsatellites show Mendelian inheritance and have high rate of mutation. Recently a large number of microsatellite markers have been characterized from human genome. The close relationship of humans and other primates and highly conserved nature of the flanking sequences of these microsatellite loci facilitates cross-species amplification in primates eliminating the laborious process of developing new markers. Human microsatellites have been successfully used in nonhuman primates earlier too (Nair *et al.* 2000; Chambers *et al.* 2004; Rogers *et al.* 2005).

The present study was planned with an objective to generate a set of common microsatellite markers, polymorphic in more number of species of genus *Macaca* including pig-tailed and stump-tailed macaques. The presence of common polymorphic markers for different species not only cuts down the cost of multiple sets of primers for each species but also saves time. Earlier reports of Nair *et al.* (2000) and Roeder *et al.* (2009) lacked sufficient information about cross-species amplification and polymorphism in rhesus macaques as well as in pig-tailed and stump-tailed macaques.

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Therefore, we selected human-derived microsatellite markers, which were already known to be polymorphic in rhesus macaques (Rogers *et al.* 2005, 2006) and are likely to be polymorphic in pig-tailed and stump-tailed macaques.

Materials and methods

In the present study, blood samples of 14 pig-tailed macaque and 20 stump-tailed macaque were collected from Aizawl Zoological Park (Mizoram), Assam State Zoological Park (Assam) and Sepahijala Zoological Park (Tripura), under conservation breeding programme. DNA was isolated from these samples using standard phenol–chloroform method (Sambrook *et al.* 1989) and was then quantified using Nanodrop spectrophotometer (Thermo Scientific, Wilmington, USA). PCR amplification was carried out using primer sets for 20 tetranucleotide microsatellite loci (table 1). We have selected only those microsatellite loci (Rogers *et al.* 2005, 2006) which were reported to be polymorphic, unlinked to each other and have been mapped well in rhesus macaque. The primer sequences were obtained from UNISTS (unified, nonredundant view of sequence tagged sites) database of NCBI (National Center for Biotechnology Information, http://www.ncbi.nlm.nih.gov). All the forward primers were fluorescently labelled. PCR amplification was carried out in DNA engine (BioRad, Hercules, USA) in 15 μL reaction mixture containing 20 ng DNA, 1× buffer, 1.0 mM dNTPs, 2.0 mM $MgCl_2$, 2.0 pmol of each primer and 0.5 U of Amplitaq gold DNA polymerase (Applied Biosystems, Foster City, USA). PCR amplification was carried out with initial denaturation at 94°C for 10 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing temperature between 48–50°C for 15 s and extension at 72°C for 30 s. The final extension was at 72°C for 10 min followed by final hold at 4°C. The PCR was repeated atleast thrice for all the markers and samples.

The PCR products were loaded on 2.5% agarose gel to check for amplification. The amplified products were visualized on ABI 3730 DNA analyser, using LIZ500 (Applied Biosystems, USA) size standard. The genotypes of all the samples were analysed using GENEMAPPER 4.0 software (Applied Biosystems, Foster City, USA). Genetic variation in terms of number of alleles (K), observed heterozygosity (H_o) and expected heterozygosity (H_e) in both pig-tailed and stump-tailed macaques were calculated using Arlequin 3.1 software (Laurent *et al.* 2006) and tests for deviation from Hardy–Weinberg equilibrium were done using exact tests based on Markov chain algorithm. Polymophism information content (PIC) was calculated using CERVUS 3.0.3 software (Kalinowski *et al.* 2007).

Results and discussion

Of the 20 microsatellite loci (table 1) tested in stump-tailed macaques and pig-tailed macaques, 11 were found to be highly polymorphic (table 2) while, remaining 9 loci (D1S1594, D6S1056, D10S2327, D12S297, D17S1290, D13S797, D18S537, D19S559 and D16S750) showed no amplification or were monomorphic and were not investigated further. In pig-tailed macaques, the number of alleles (K) for different loci ranged from 5–8. Observed heterozygosity (H_o) ranged from 0.71–1.0 with an average of 0.84 while expected heterozygosity (H_e) ranged from 0.73–0.88

Table 1. List of 20 microsatellite loci tested in pig-tailed macaques (PTM) and stump-tailed macaques (STM).

Locus	GenBank accession no.	Repeat motif	Rhesus chromosome	Result (STM)	Result (PTM)
D1S1594	G07796	Tetra	1	No amplification	No amplification
D3S3045	G08279	Tetra	2	Polymorphic	Polymorphic
D21S1246	L16366	Tetra	3	Polymorphic	Polymorphic
D6S2419	G10474	Tetra	4	Polymorphic	Polymorphic
D6S1056	G08558	Tetra	4	Monomorphic	Monomorphic
D4S1645	G08333	Tetra	5	Polymorphic	Polymorphic
D5S1466	G08454	Tetra	6	Polymorphic	Polymorphic
D15S823	G07912	Tetra	7	Polymorphic	Polymorphic
D8S1466	G09586	Tetra	8	Polymorphic	Polymorphic
D10S2327	G08826	Tetra	9	Monomorphic	No amplification
D12S372	G08946	Tetra	11	Polymorphic	Polymorphic
D12S297	L16426	Tetra	11	Monomorphic	No amplification
D2S1333	G08155	Tetra	12	Polymorphic	Polymorphic
D9S934	G08751	Tetra	15	Polymorphic	Polymorphic
D17S1290	G07956	Tetra	16	No amplification	Monomorphic
D13S797	G09011	Tetra	17	Monomorphic	Monomorphic
D18S537	G07990	Tetra	18	No amplification	No amplification
D18S869	G09618	Tetra	18	Polymorphic	Polymorphic
D19S559	L30499	Tetra	19	No amplification	No amplification
D16S750	G07924	Tetra	20	Monomorphic	Monomorphic

Table 2. Characteristics of 11 polymorphic microsatellite loci used in pig-tailed and stump-tailed macaques.

	Pig-tailed macaque					Stump-tailed macaque				
Locus	Allelic range (bp)	K	H_o	H_e	PIC	Allelic range (bp)	K	H_o	H_e	PIC
D18S869	228–272	8	1.00	0.87	0.82	179–195	5	0.72	0.76	0.70
D21S1246	410–446	8	0.71	0.80	0.74	406–438	8	0.89	0.86	0.81
D15S823	322–366	8	1.00	0.87	0.82	321–365	7	0.78	0.82	0.78
D6S2419	160–184	7	0.79	0.83	0.77	192–238	12	0.78	0.90	0.86
D3S3045	190–206	5	0.86	0.74	0.67	178–198	6	0.83	0.85	0.80
D2S1333	296–316	5	0.71	0.73	0.65	288–304	5	0.78	0.78	0.72
D5S1466	280–312	7	0.86	0.88	0.83	276–312	7	0.83	0.80	0.75
D8S1466	108–132	8	0.93	0.81	0.75	116–128	5	0.78	0.71	0.64
D4S1645	272–288	5	0.79	0.78	0.72	274–290	5	0.72	0.71	0.63
D9S934	190–210	6	0.79	0.84	0.78	186–202	5	0.72	0.78	0.72
D12S372	181–209	6	0.86	0.78	0.72	162–178	5	0.73	0.67	0.59

K is the number of alleles, H_o is observed heterozygosity, H_e is expected heterozygosity and PIC is polymorphism information content.

with an average of 0.81. Locus D6S2419 showed significant deviation from Hardy–Weinberg equilibrium ($P < 0.05$). In stump-tailed macaques, the number of alleles (K) for different loci ranged from 5–12. H_o ranged from 0.72–0.89 with an average of 0.78 and H_e from 0.67–0.90 with an average of 0.78. Two loci (D6S2419 and D8S1466) showed significant deviations from Hardy–Weinberg equilibrium ($P < 0.05$) in stump-tailed macaques. These deviations from Hardy–Weinberg equilibrium may be due to the reason that the samples have been collected from zoological parks/captive population and not from a random mating population.

An important aspect of this study is the use of tetranucleotide markers. The advantage of using tetranucleotide markers is that they can be reliably scored. Several other studies have also shown that trinucleotide and tetranucleotide markers can reduce amplification artifacts-like stutters leading to exact allele identification. Further, these 11 microsatellite loci have already been mapped in rhesus macaque (Rogers *et al.* 2006) and were found to be located on different chromosomes and unlinked. The polymorphism in these loci (which have genome wide distribution) would reflect the neutral variation at the whole genome level.

Roeder *et al.* (2009) have reported a few tetranucleotide microsatellite markers earlier, which amplified in stump-tailed macaques but did not show any significant level of polymorphism. Further, they have used a very small sample size ($n < 5$). However, in the present study a comparatively larger sample size ($n = 20$) was used. Similarly, tetranucleotide microsatellite markers reported by Nair *et al.* (2000) failed to show polymorphism in our initial screening of stump-tailed macaques, though they were polymorphic in pig-tailed macaques.

We conclude that our study can serve as a good pilot study and provides markers useful to test polymorphism and paternity in these two species of macaques on a large scale. These markers have a greater potential in genetic testing of stump-tailed and pig-tailed macaque individuals and can thus help in distinguishing outbred and inbred individuals. This would greatly contribute to conservation of these two vulnerable species as well as several other species of nonhuman primates in captivity as well as in the wild.

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