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Polymorphic Microsatellite Loci for the Common Marmoset (*Callithrix jacchus*) Designed Using a Cost- and Time-Efficient Method

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Abstract

We describe a cost- and time-efficient method for designing new microsatellite markers in any species with substantial genomic DNA sequence data available. Using this technique, we report 14 new polymorphic dinucleotide microsatellite loci isolated from the common marmoset. The relative yield of new polymorphisms was higher with less labor than described in previous marmoset studies. Of 20 loci initially evaluated, 14 were polymorphic and amplified reliably (70% success rate). The number of alleles ranged from 3 to 9 with heterozygosity varying from 0.48 to 0.83.

Keywords

marmoset; microsatellite; markers; *Callithrix jacchus*; common marmoset

INTRODUCTION

The common marmoset (*Callithrix jacchus*) has its natural habitat in the swamps and Atlantic rainforest of northeastern Brazil. In addition to a number of studies of wild marmosets [Lazaro-Perea et al., 2004; Schiel and Huber, 2006], this species has been extensively studied in captivity. Marmosets have been used as animal models for biomedical research, including investigation of neuroscience [Jusuf et al., 2006; Przybyszewski et al., 2006], reproductive biology [Einspanier et al., 2006] and infectious disease. More specifically, this species has been used to model several chronic and infectious diseases such as Lassa fever, stroke, Parkinson's Disease, multiple sclerosis, Huntington's and Alzheimer's diseases [Brok et al., 2001; Carrion et al., 2007; Jacob et al., 2004; Kyuregyan et al., 2005; Lanford et al., 2003; Means, 2004]. Mansfield [2003] has provided an excellent review of the biomedical uses of *C. jacchus*. The significance of marmosets as a model species was recognized in the decision by the National Human Genome Research Institute to sequence the marmoset genome

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(http://www. genome.gov/Pages/Research/Sequencing/SeqProposals/MarmosetSeq.pdf), the first New World primate species to be selected for this investment of resources.

Given their importance in research, the development of tools for further genetic and genomic analyses of marmosets is desirable. One such tool that has proven to be quite useful for other nonhuman primate species is the identification of polymorphic microsatellite loci, also called short tandem repeats. Microsatellites are tandemly repeated motifs of 2 or more DNA bases that are found in all known prokaryotic and eukaryotic genomes. They have a high degree of length polymorphism. Because of their extensive variability among individuals within any given species, microsatellite markers have been widely employed in genetic analyses since their first description [Litt and Luty, 1989; Tautz, 1989; Weber and May, 1989]. The high heterozygosity of microsatellites makes them very powerful and informative genetic markers, and they are an extremely valuable tool for genome mapping in many organisms [Knapik et al., 1998; Kong et al., 2002; Rogers et al., 2000, 2006]. Applications of these polymorphisms span different areas ranging from ancient and forensic DNA studies to population genetics and conservation/management of biological resources. The identification of microsatellite markers in any species is quite useful for a variety of tasks, including colony management, pedigree verification and linkage analysis.

According to the inPRIMAT database (http://www.inprimat.org/Default.aspx?menu=5&submenu=2) only 30 microsatellite markers are currently reported for *C. jacchus*. Of these, 50% have no information about the allele numbers, heterozygosity and number of repeats in *C. jacchus*. Several of the microsatellites were first identified in other species including *Cebus apella* [Escobar-Paramo, 2000] and *Homo sapiens* [Coote and Bruford, 1996] and some of the microsatellite were tested in only one individual marmoset. Only 15 of the reported microsatellites have information on number of repeats, number of alleles and heterozygosity for marmosets [Nievergelt et al., 1998]. All these microsatellites reported to date for the common marmoset were identified by time-consuming processes such as cloning and sequencing directly from *Callithrix* DNA or screening polymerase chain reaction (PCR) primers first identified in other species. In our laboratory we screened 25 human markers and 15 rhesus-derived markers [Raveendran et al., 2006] using DNA from six marmosets. We were unable to find informative markers (i.e. markers that are polymorphic in marmosets). As a result, we decided to use the available marmoset whole genome DNA sequence to design new marmoset microsatellite markers. In this paper we describe this cost- and time-efficient method to identify new microsatellite polymorphisms in *C. jacchus*.

MATERIALS AND METHODS

Detection of Marmoset Microsatellites and Orthologous Human Loci

The marmoset genome assembly Callithrix_jacchus-2.0.2, produced by the Washington University Genome Sequencing Center and the Baylor College of Medicine Human Genome Sequencing Center, was downloaded from http://genome.wustl.edu/pub/organism/Primates/Callithrix_jacchus/assembly/Callithrix_jacchus-2.0.2/. This draft assembly consists of 49,724 supercontigs comprising ~2.92 Gbp of assembled sequence. On the basis of an average size of ~3 Gbp for the currently assembled primate genomes, the majority of the marmoset genome is present within this assembly. A large variety of software tools are available for the detection of microsatellites in DNA sequence. The Tandyman program version 2.6 (4/15/2003) (http://hemisphere.lanl.gov/tandyman/) was used to identify microsatellites in this study. Tandyman is a Perl program that can be run from a command line in any computing environment with at least Perl version 5.0 installed. The large amount of sequence data comprising the marmoset genome assembly made use of a local tool such as Tandyman more

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convenient than the use of a web-based tool. Additional microsatellite detection software that can be run locally includes the Perlbased Msatfinder (http://www.genomics.ceh.ac.uk/msatfinder/), the Python-based MSATCOMMANDER (http://code.google.com/p/msatcommander/) [Faircloth, 2008], and the MacPerl-based Ephemeris (http://www.uga.edu/srel/DNA_Lab/programs.htm) software. Links to a number of additional tools for the detection of microsatellites are included on the Msatfinder page. Some of these programs such as MSATCOMMANDER include additional functionality such as a graphical user interface and integration with Primer3 for primer design. For detection of microsatellites in relatively small DNA sequences, a web-based tool can be applied. Many of the above tools have web-based versions and additional tools such as CID (http://www.shrimp.ufscar.br/cid/index.php) [Freitas et al., 2008] are available exclusively online.

Microsatellites were defined as those sequences with ten or more perfect tandem copies of repeat units 2–10 basepairs long. To determine orthologous human loci, sequence spanning 300 bp upstream and downstream of the identified microsatellites was extracted from the assembled supercontigs and mapped onto the human genome (NCBI Build 36.1, UCSC Build hg18) using the Blat program [Kent, 2002]. A total of 122,977 marmoset microsatellites were mapped onto the human genome. Marmoset microsatellite mapping coordinates onto human and marmoset sequence data were stored in a database for visualization using the Genboree Discovery System (www.genboree.org).

PRIMER DESIGN AND PCR AMPLIFICATION

Sequences with more than ten tandem dinucleotide repeat units were selected and the Primer3 program (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) was used to design the PCR primers. In total 20 primer pairs were designed. Fluorescently labeled forward primers and unlabeled tailed reverse primers were obtained from commercial vendors, and initial PCR conditions were tested in six unrelated marmoset samples. Our sample set has 22 animals that include 14 unrelated marmosets and two families. One family has five members and the second family has three members. We included these two families to check that the marmoset-derived markers followed Mendelian patterns of inheritance. DNA was extracted from liver, blood or hair using standard phenol/chloroform methods. PCR amplification was carried out in 6 μL reaction using GeneAmp PCR system 9700 thermocycler (ABI, Foster city, CA) with 50 ng of genomic DNA as a template. PCR amplification conditions consisted of 0.05 μM of fluorescently labeled forward primer, 0.05 μM of unlabeled reverse primer (Table I), 1.5 mM $MgCl_2$, 0.8 mM dNTPs and 0.25 U of AmpliTaq Gold DNA polymerase (ABI). The thermal profile for PCR amplification was 95°C for 12 min, followed by 10 cycles at 94°C for 15 sec, a primer-specific annealing temperature (Table I) for 15 sec, 72°C for 30 sec, followed by 20 cycles at 89°C for 15 sec, a primer-specific annealing temperature (Table I) for 15 sec, 72°C for 30 sec and ending with a single extension of 72°C for 10 min. Allele sizes were determined by separation of amplification products on an ABI 3130XL DNA genetic analyzer and the fragment length was assigned by the Genemapper 3.7 program using Liz 500 (ABI) size standard.

Animal care, housing and management were performed in accordance with accepted veterinary practice, the ILAR *Guide for Care and Use of Laboratory Animals* and all relevant federal regulations. All animal procedures were approved by the Institutional Animal Care and Use Committee for the Southwest Foundation for Biomedical Research and adhered to the legal requirements of the USA. This study was carried out at Southwest Foundation for Biomedical Research, San Antonio, Texas from March 2006 to February 2008.

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RESULTS AND DISCUSSION

Of the 20 microsatellite primers we designed, 16 amplified in our marmoset samples. Fourteen were polymorphic and gave scoreable genotypes. Two loci were monomorphic. The other four markers did not amplify. We performed pedigree analysis for all of the polymorphic markers and they show appropriate results. Our success rate in identifying informative markers using whole genome sequence was thus 70%. When designing new markers using this approach, we can predict the allele size of PCR products for any given locus and set primers in various locations to manipulate the ultimate PCR product size (Table I). This is useful when trying to accommodate numerous markers in a single panel to be run together on the genotyping instrument. The observed number of alleles for this set of 14 polymorphisms varied from 3 to 9 with heterozygosity varying from 0.48 to 0.83. Methods such as cloning and sequencing that have been used by others [e.g. Nievergelt et al., 1998] to identify common marmoset microsatellite markers are time-consuming and expensive. Nievergelt et al. found 16 microsatellites that contained 8 or more tandem repeats by sequencing 120 positive clones. Out of those 16 microsatellites, seven were monomorphic or uninterpretable and only 9 markers gave scoreable products. In another study, Escobar-Paramo [2000] used microsatellites cloned from *C. apella* and then screened in various species including *C. jacchus*. Out of 11 markers identified in *C. apella* by cloning, only three amplified in *C. jacchus*. Coote and Bruford [1996] screened 85 published human markers in 5 unrelated baboons and 11 were informative in baboons. Out of these 11, only one amplified in *C. jacchus*.

In addition, we tested the search tools described above and the existing marmoset whole genome sequence by attempting to find 13 of the previously published marmoset markers using our method. First, we selected four previously published loci shown to exist in the marmoset genome, but originally derived from the human genome [Nievergelt et al., 1998]. We obtained the human DNA sequence for those loci, including the PCR primers and the intervening human DNA sequence. These sequences were mapped to the marmoset genome using Blat, and all four markers were identified in the available marmoset genomic sequence. Next, we used Blat to map nine marmoset-derived markers for which we only had primer sequences [Nievergelt et al., 1998]. We found four of these loci in the available marmoset genomic sequence.

Although our sample size of loci is still modest in terms of total loci tested, we did have a 70% success rate in finding informative useful genetic markers using this procedure. These preliminary data suggest that this approach can be used to design microsatellite markers rapidly at lower cost and with less effort in any species for which substantial amounts of genomic DNA sequences are available.

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REFERENCES

Brok HP, Bauer J, Jonker M, Blezer E, Amor S, Bontrop RE, Laman JD, ‘t Hart BA. Non-human primate models of multiple sclerosis. Immunol Rev 2001;183:173–185. [PubMed: 11782256]

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Carrion R Jr. Brasky K, Mansfield K, Johnson C, Gonzales M, Ticer A, Lukashevich I, Tardif S, Patterson J. Lassa virus infection in experimentally infected marmosets: liver pathology and immunophenotypic alterations in target tissues. J Virol 2007;81:6482–6490. [PubMed: 17409137]

Coote T, Bruford MW. Human microsatellites applicable for analysis of genetic variation in apes and Old World monkeys. J Hered 1996;87:406–410. [PubMed: 8904839]

Einspanier A, Lieder K, Bruns A, Husen B, Thole H, Simon C. Induction of endometriosis in the marmoset monkey (*Callithrix jacchus*). Mol Hum Reprod 2006;12:291–299. [PubMed: 16608906]

Escobar-Paramo P. Microsatellite primers for the wild brown capuchin monkey Cebus apella. Mol Ecol 2000;9:107–108. [PubMed: 10652080]

Faircloth BC. msatcommander: detection of microsatellite repeat arrays and automated, locus-specific primer design. Mol Ecol Res 2008;8:92–94.

Freitas PD, Martins DS, Galetti PM. cid: a rapid and efficient bioinformatic tool for the detection of SSRs from genomic libraries. Mol Ecol Res 2008;8:107–108.

Jacob JR, Lin KC, Tennant BC, Mansfield KG. GB virus B infection of the common marmoset (*Callithrix jacchus*) and associated liver pathology. J Gen Virol 2004;85:2525–2533. [PubMed: 15302946]

Jusuf PR, Martin PR, Grunert U. Random wiring in the midget pathway of primate retina. J Neurosci 2006;26:3908–3917. [PubMed: 16611806]

Kent WJ. BLAT—the BLAST-like alignment tool. Genome Res 2002;12:656–664. [PubMed: 11932250]

Knapik EW, Goodman A, Ekker M, Chevrette M, Delgado J, Neuhauss S, Shimoda N, Driever W, Fishman MC, Jacob HJ. A microsatellite genetic linkage map for zebrafish (*Danio rerio*). Nat Genet 1998;18:338–343. [PubMed: 9537415]

Kong A, Gudbjartsson DF, Sainz J, Jonsdottir GM, Gudjonsson SA, Richardsson B, Sigurdardottir S, Barnard J, Hallbeck B, Masson G, Shlien A, Palsson ST, Frigge ML, Thorgeirsson TE, Gulcher JR, Stefansson K. A high-resolution recombination map of the human genome. Nat Genet 2002;31:241–247. [PubMed: 12053178]

Kyuregyan KK, Poleschuk VF, Zamyatina NA, Isaeva OV, Michailov MI, Ross S, Bukh J, Roggendorf M, Viazov S. Acute GB virus B infection of marmosets is accompanied by mutations in the NS5A protein. Virus Res 2005;114:154–157. [PubMed: 16054723]

Lanford RE, Chavez D, Notvall L, Brasky KM. Comparison of tamarins and marmosets as hosts for GBV-B infections and the effect of immunosuppression on duration of viremia. Virology 2003;311:72–80. [PubMed: 12832204]

Lazaro-Perea C, Arruda MF, Snowdon CT. Grooming as a reward? Social function of grooming between females in cooperatively breeding marmosets. Anim Behav 2004;67:627–636. [PubMed: 17237884]

Litt M, Luty JA. A hypervariable microsatellite revealed by in vitro amplification of a dinucleotide repeat within the cardiac muscle actin gene. Am J Hum Genet 1989;44:397–401. [PubMed: 2563634]

Mansfield K. Marmoset models commonly used in biomedical research. Comp Med 2003;53:383–392. [PubMed: 14524414]

Means RE. Characterization of the Herpesvirus saimiri Orf51 protein. Virology 2004;326:67–78. [PubMed: 15262496]

Nievergelt CM, Mundy NI, Woodruff DS. Microsatellite primers for genotyping common marmosets (*Callithrix jacchus*) and other callitrichids. Mol Ecol 1998;7:1432–1434. [PubMed: 9787452]

Przybyszewski AW, Sosale S, Chaudhuri A. Quantification of three-dimensional exploration in the cylinder test by the common marmoset (*Callithrix jacchus*). Behav Brain Res 2006;170:62–70. [PubMed: 16530859]

Raveendran M, Harris RA, Milosavljevic A, Johnson Z, Shelledy W, Cameron J, Rogers J. Designing new microsatellite markers for linkage and population genetic analyses in rhesus macaques and other nonhuman primates. Genomics 2006;88:706–710. [PubMed: 17010566]

Rogers J, Mahaney MC, Witte SM, Nair S, Newman D, Wedel S, Rodriguez LA, Rice KS, Slifer SH, Perelygin A, et al. A genetic linkage map of the baboon (*Papio hamadryas*) genome based on human microsatellite polymorphisms. Genomics 2000;67:237–247. [PubMed: 10936045]

Rogers J, Garcia R, Shelledy W, Kaplan J, Arya A, Johnson Z, Bergstrom M, Novakowski L, Nair P, Vinson A, et al. An initial genetic linkage map of the rhesus macaque (*Macaca mulatta*) genome using human microsatellite loci. Genomics 2006;87:30–38. [PubMed: 16321502]

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Schiel N, Huber L. Social influences on the development of foraging behavior in free-living common marmosets (*Callithrix jacchus*). Am J Primatol 2006;68:1150–1160. [PubMed: 16900507]

Tautz D. Hypervariability of simple sequences as a general source for polymorphic DNA markers. Nucleic Acids Res 1989;17:6463–6471. [PubMed: 2780284]

Weber JL, May PE. Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. Am J Hum Genet 1989;44:388–396. [PubMed: 2916582]

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TABLE I

Primer Name, Primer Sequence, Repeat Motif (R), Annealing Temperature (T_a), Predicted Product Size (P_s), Actual Allele Size (A_s), Number of Alleles (N_a) and Heterozygosity (H_o)in *Callithrix jacchus*

Primers	Sequence (5′–3″)	R	T_a (°C)	P_s	A_s	N_a	H_o
CAJA1F	6FAM-GAAGACGGGGGCGTAAATA	$(CA)_{23}$	55	382	383, 385, 387, 389, 391	5	0.74
CAJA1R	TGTGGTGGCTCATACCTGAA						
CAJA5F	PET-GGCCCACAGCATTTAATTTG	$(GT)_{18}$	48	240	246, 248, 250	3	0.63
CAJA5R	CAACTTTACTGCCACCAGCA						
CAJA6F	NED-GAGCACCAAGATTGGCATTT	$(GT)_{15}$	48	220	220, 222, 224, 226, 228, 230	6	0.72
CAJA6R	CCAATACACATCGGCTTTGA						
CAJA9F	6FAM-GCTCCCAGACACAAGCTCAT	$(GT)_{13}$	55	182	188, 190, 200	3	0.51
CAJA9R	TGCTTTTCTTCTGCCCAATC						
CAJA10F	VIC-ACCCTACATTGCCAAATTGC	$(CA)_{22}$	55	190	178, 186, 188, 192, 194, 196, 198, 200, 203	9	0.82
CAJA10R	GCCTCTTCTGAGGGAAGTGA						
CAJA11F	6FAM-CGAAAGTGTGCTCAACAGGA	$(CA)_{13}$	48	243	243, 245, 251, 253, 256, 258	6	0.61
CAJA11R	AAGGTGGGATTCTGAAAGCA						
CAJA12F	VIC-GCCAACGAGAGGCTCTAATG	$(GT)_{17}$	48	228	229, 231, 233, 237, 240, 243, 245	7	0.83
CAJA12R	TTTTGCTTGTGGCAATGGTA						
CAJA13F	NED-TGAGCCAACGTACCTGGTTA	$(GT)_{17}$	48	358	365, 367, 369, 371, 375	5	0.64
CAJA13R	CTTTTCCAATGCGAGAGGAG						
CAJA14F	6FAM-AGCACATGAACACCCAGGTT	$(CA)_{16}$	57	213	201, 204, 215, 217, 219	5	0.57
CAJA14R	AGTGAAAACAGGCTGGGAGA						
CAJA15F	PET-ACCACATGATCCAGCAATCC	$(CA)_{23}$	57	153	133, 135, 137, 145, 147, 150, 153, 155, 157	9	0.80
CAJA15R	GATTCCGTGTCTTAGCCATCA						
CAJA16F	NED-AGGGCCTTCCACAGAGTGTA	$(GT)_{11}$	48	386	365, 367, 371, 388, 391, 396	6	0.48
CAJA16R	CCTCTGCACTCTTCCTTTGG						
CAJA17F	PET-GGGCACTCCAAGGTCAGTAA	$(CT)_{21}$	57	377	315, 325, 337, 348, 371, 373, 375, 380, 382	9	0.81
CAJA17R	TTGCCCCCTGCTTATTGTAG						
CAJA18F	VIC-ACTTGCAGGCCAGTGTTCTT	$(AC)_{17}$	48	303	288, 290, 296, 302, 306, 308, 317	7	0.68
CAJA18R	TGGACAGCTGAGGTTTCCTT						
CAJA19F	6FAM-AGTTCTCCGAGCTCCTCCTC	$(CA)_{13}$	48	356	329, 338, 346, 360, 367, 373	6	0.70

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Primers	Sequence (5′–3″)	R	T_a (°C)	P_s	A_s	N_a	H_o
CAJA19R	TGGGTGATTTTCATCCCTGT						

6FAM, PET, NED and VIC are florescent dyes attached to the primers.