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Designing new microsatellite markers for linkage and population genetic analyses in rhesus macaques and other nonhuman primates

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Abstract

Identification of polymorphic microsatellite loci in nonhuman primates is useful for various biomedical and evolutionary studies of these species. Prior methods for identifying microsatellites in nonhuman primates are inefficient. We describe a new strategy for marker development that uses the available whole genome sequence for rhesus macaques. Fifty-four novel rhesus-derived microsatellites were genotyped in large pedigrees of rhesus monkeys. Linkage analysis was used to place 51 of these loci into the existing rhesus linkage map. In addition, we find that microsatellites identified this way are polymorphic in other Old World monkeys such as baboons. This approach to marker development is more efficient than previous methods and produces polymorphisms with known locations in the rhesus genome assembly. Finally, we propose a nomenclature system that can be used for rhesus-derived microsatellites genotyped in any species or for novel loci derived from the genome sequence of any nonhuman primate.

Keywords: Rhesus; Linkage map; Microsatellite markers; Nonhuman primates; Macaques

Nonhuman primates, and especially rhesus macaques, are important animal models for basic biological and biomedical research. Rhesus monkeys (*Macaca mulatta*) are used in studies of neuroscience [1,2], endocrinology [3], behavioral biology [4,5], pharmacology [6], virology [7], arthritis [8], cardiovascular diseases [9], and many other research areas. The development of resources and information for effective study of the rhesus monkey genome will facilitate progress in all these research areas. One critical type of genomic information is the characterization of whole genome sets of highly informative DNA polymorphisms. Microsatellite polymorphisms have become a standard tool for genomics in a wide variety of species. High-density whole genome sets of microsatellite polymorphisms for rhesus macaques would be valuable for whole genome linkage analysis, to locate the quantitative trait loci involved in determining risk for specific diseases, and for other types of genetic studies including colony management and pedigree testing. A high-resolution linkage map for the rhesus genome will also be useful in the analysis of whole genome DNA sequences for this species. Assembly of whole genome shotgun sequences depends primarily on physical mapping data such as contigs, scaffolds, and BAC end sequences. But at larger distances (e.g., several megabases), linkage information may be valuable for orienting large blocks of sequence or contigs that cannot be integrated together with high likelihoods based on physical mapping information alone.

Past efforts to identify and characterize highly polymorphic microsatellite loci in nonhuman primates have used two approaches. In some early studies of primates, researchers cloned novel microsatellites from nonhuman genomes [10]. In other studies investigators screened published human

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microsatellites and found particular loci that were also informative in nonhuman primates [11–13]. However, neither cloning novel microsatellites from nonhuman species nor screening human primer pairs to identify conserved microsatellites is an ideal method. Both approaches are time-consuming and inefficient. The reported success rate in identifying human markers that are polymorphic in rhesus macaques is 19% [14]. Therefore it is advantageous to develop a method to identify microsatellite polymorphisms in rhesus and other primates that is more efficient than past methods.

We have previously reported the construction of a genetic linkage map of the rhesus genome that includes 241 microsatellite loci that were all originally derived from and mapped in the human genome [15]. That set of 241 polymorphisms includes some loci first reported by other investigators [16,17] and a larger proportion of human microsatellites we found to be informative in rhesus monkeys. In this article, we describe a novel strategy that can identify and characterize new microsatellite markers derived from the rhesus genome directly. The loci described here were identified from a whole genome shotgun DNA sequence from the rhesus monkey genome that was generated by the Baylor College of Medicine Human Genome Sequencing Center, the Washington University Genome Sequencing Center, and other collaborators. These loci have not been previously studied in the rhesus genome. We also propose a nomenclature for the new rhesus-specific markers.

Results and discussion

We designed PCR primers to amplify potential polymorphic markers that map to large gaps (>8.0 cM) in the current rhesus linkage map (http://www.snprc.org/linkage/currentmaps/rhesusmaps.html). All of the products amplified show high fluorescence intensities, demonstrating the presence of ample PCR product. So far 54 of these loci have been genotyped in the same rhesus pedigrees ($n=1152$ animals) used for the full linkage map [15]. Initial linkage mapping has been completed for 51 rhesus-derived microsatellites that are located on 18 different rhesus chromosomes. Fig. 1 presents partial updated maps for the chromosomes with the novel rhesus-derived loci. Among these first 51 loci mapped, 48 were assigned by MultiMap to the locations we expected based on the initial alignment of rhesus shotgun sequences to the human genome and our human–rhesus comparisons. In the other three cases (MML1S13, MML2S2, and MML15S3), the loci did not map where initially predicted based on the initial alignment of rhesus sequences to the human genome. However, these three loci were predicted to occur adjacent to chromosomal inversions in rhesus that were previously identified using human-derived loci. The three loci were shown to map by linkage to plausible alternative locations that indicate that the inversions extend farther than previously recognized. In other words, these 3 loci fall into logically

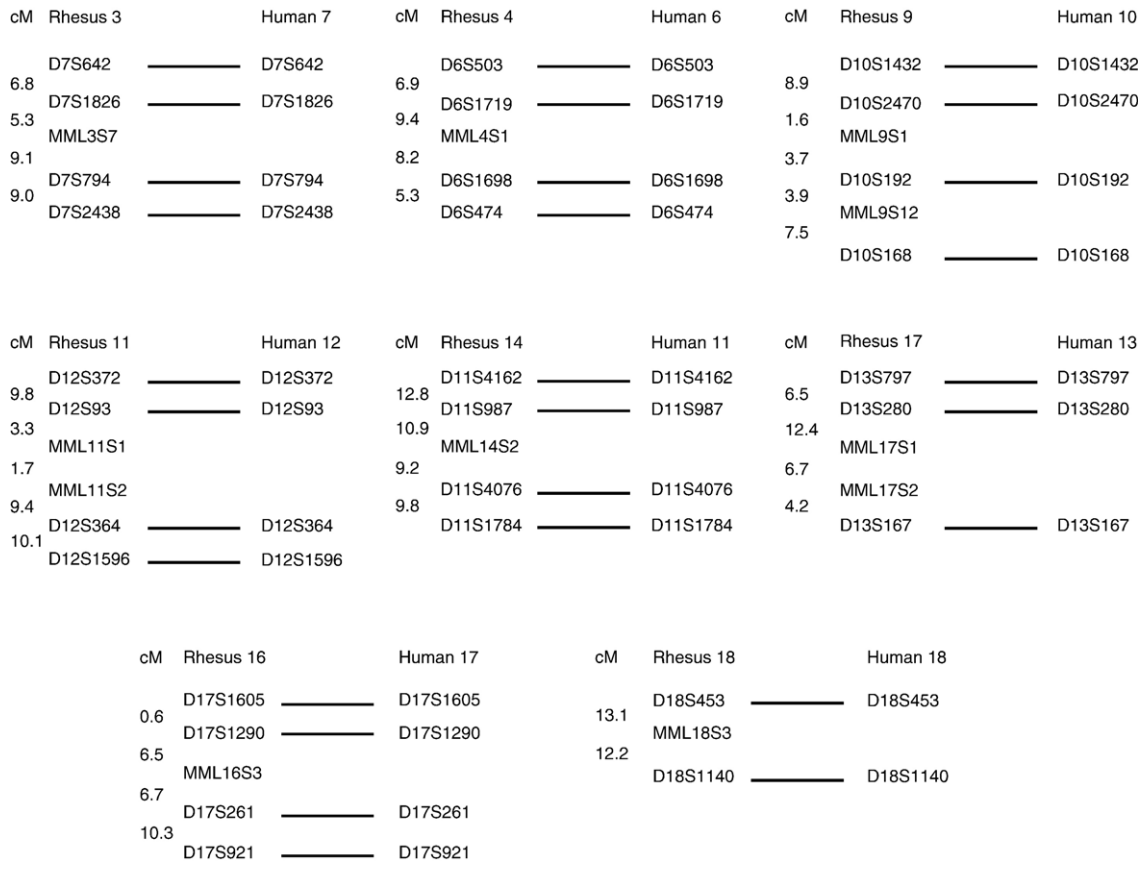

Fig. 1. Partial updated linkage maps of rhesus chromosomes with newly mapped rhesus-derived markers. cM indicates spacing in the rhesus linkage map in centimorgans. Numbers (Rhesus3 or Human7) indicate chromosome numbers.