Strategy Using Pooled DNA to Identify 56 Short Tandem Repeat Polymorphisms for the Bolivian Squirrel Monkey


Short tandem repeat polymorphisms (STRP) have been extraordinarily useful for mapping disease genes because they are numerous and highly polymorphic genetic markers that are randomly distributed throughout the genome. STRP markers have played an increasingly important role in gene mapping strategies and are being applied to a wide variety of genetic problems in many species (22). STRP markers have usually been developed by screening DNA libraries with short tandem repeat probes (2,10,19,20) or by performing computer searches of DNA databases (13,14). However, hybridization screening with short tandem repeat probes (while frequently necessary) is labor-intensive, and for most species (including the squirrel monkey), there is very limited DNA sequence information in the available databases. A third strategy is to use microsatellite primers designed for one species to amplify polymorphic repeated sequences in a related species (10,14). However, since the efficiency of such an approach may be relatively poor, techniques that facilitate screening a population of animals with a large number of markers would be very useful.

We report here a rapid and efficient adaptation of the third strategy that requires neither database information nor complicated screening experiments. It allowed us to rapidly identify 56 STRP for the Bolivian squirrel monkey. This strategy is based on the availability of thousands of human STRP markers and takes advantage of the fact that, in spite of divergence from the common ancestor of humans and squirrel monkeys about 55 million years ago (11), the two species share many conserved sequences (8,9,23).

Historically, the squirrel monkey has been and continues to be the most common New World primate used in biomedical research. As potential experimental subjects, New World monkeys such as the Bolivian squirrel monkey (Saimiri boliviensis boliviensis) have several advantages over Old World nonhuman primates. Squirrel monkeys are smaller, easier to handle and less expensive to maintain than most Old World monkeys. Also, they appear less likely to carry zoonotic diseases, such as Herpes B and tuberculosis, which are transmissible to humans and are a constant threat to individuals who work with Old World primates (1). As research animals, New World monkeys are less well-characterized than Old World primates. The value of the squirrel monkey for research will be greatly enhanced by the availability of a large set of polymorphic markers for this species.
enhanced by improved knowledge of the genetics of these animals. Although we are unaware of any published New World Monkey linkage map or of any comparison with humans, it is likely that many squirrel monkey and human chromosome regions are syntenic. A recent comparison of baboons and humans with regard to a long region of human chromosome 1 revealed remarkable conservation of marker order and map distances (18). Even such distantly related mammals as cats, cows and mice have numerous chromosomal regions with similar linkage relationships to those of humans (15).

Blood samples were obtained from 125 squirrel monkeys by femoral venipuncture using a protocol approved by the University of South Alabama Animal Care and Use Committee. Leukocyte DNA was extracted from 200 µL of blood using a QIAamp® bloodkit (No. 29104; Qiagen, Chatsworth, CA, USA). Ten microliters of each of the monkey DNA samples were mixed to form a single pooled DNA specimen. We used the methods of Weber and May (21) to amplify and identify the STRP. Aliquots of the pooled DNA were amplified using 501 different pairs of primers of human STRP markers (MapPairs™; Research Genetics, Huntsville, AL, USA) in 96-well plates. The final reaction conditions were 10 mM Tris-HCl, pH 8.3, 2.5 mM MgCl₂, 0.01% gelatin, 200 mM each dGTP, dTTP and dCTP, 2.5 mM dATP, 0.70 µCi of [³³P]dATP, 2.0 pmol of each PCR primer, 30 ng of genomic pooled DNA and 0.30 U AmpliTaq® DNA Polymerase (Perkin-Elmer, Norwalk, CT, USA) in a final volume of 10 µL. Samples were subjected to 30 cycles consisting of 30 s at 95°C, 75 s at 55°C, 50°C or 45°C and 30 s at 72°C using a Perkin-Elmer GeneAmp® System 9600. One microliter of the PCR product was loaded onto 6.5% polyacrylamide sequencing gels with a 12-channel syringe (No. 0155220; Hamilton, Reno, NV, USA). After electrophoresis, the gels were dried and then exposed to X-OMAT X-ray films (Eastman Kodak, Rochester, NY, USA) overnight and for 20 days at room temperature. Statistical analyses were performed using the paired t test and multiple linear regression with the Abstat™ software package (Anderson Bell, Parker, CO, USA).

Seventy-two of the 501 markers amplified a pattern of signals (4 or more bands) from the pooled DNA that was considered to be highly likely to reflect a polymorphic STRP. Five examples are shown (Figure 1). Each of these 72 markers was then used to amplify the DNA of 12 individual monkeys from three multigeneration families. Fifty-six squirrel monkey STRP having at least three (and usually more) alleles were confirmed. Codominant segregation of the 56 STRP was observed in all of the families. Autoradiographs (Figure 2) of two of the markers for one family (Figure 3) are shown.

The STRP included 40 dinucleotide and 16 tetranucleotide repeats. Information about the median size of human STRP alleles for each marker was included in the Abstat™ software package.
obtained by anonymous file transfer protocol (FTP) from Dr. James Weber’s public database at the Marshfield Research Foundation, Marshfield, WI or from the Genome Database at Johns Hopkins University (7). The means of the median size of the monkey and human dinucleotide STRP were comparable (163 ± 42 vs. 165 ± 40 bp, P = 0.67), whereas the monkey tetranucleotide STRP were smaller on average (178 ± 39 vs. 198 ± 36, P = 0.0016).

There was a highly significant correlation between the median size of the monkey and human STRP (r = 0.791, t = 9.37, P < 0.00001) suggesting that homologous STRP are amplified in both species. In addition, we have directly sequenced the squirrel monkey PCR product of one set of human primers. The 5′ flanking region was 84% identical to the human sequence over a 105-bp stretch of DNA. Both the human and monkey STRP were CA dinucleotide repeats (data not shown).

An optimal strategy to identify squirrel monkey STRP with pooled DNA samples is to first screen for amplification using an annealing temperature of 55°C. At this temperature, it is easy to identify the polymorphic markers because the occurrence of artifactual background signals is minimized. However, some of the potentially useful primers do not amplify at this temperature, and a second screen using an annealing temperature of 50°C identifies additional polymorphic markers. The results using a 45°C annealing temperature are the same as those using 50°C, and no additional polymorphic markers are identified. For a more general application of the method, it would be reasonable to initially use the optimal annealing temperature reported for the marker when it is used to amplify DNA from the original species. If that condition does not work, then repeating the amplification using a 5°C lower annealing temperature would be worthwhile.

We have previously used pooled DNA as a base pair “ladder” in our STRP studies of humans and have observed that the number and intensity of specific bands amplified from a pooled specimen usually corresponds well with the reported population allele frequencies for that marker. The use of a DNA pool of many squirrel monkeys enabled us to screen a human STRP marker with a single PCR for the capacity to amplify monkey DNA and provide, at the same time, an estimate of the number and frequency of polymorphic alleles. However, it should be stressed that interpretation of the pattern of bands derived from an STRP reaction (especially for dinucleotide repeats) is complicated by the occurrence of strand-slipage artifacts. Such “laddering” may be more frequent with STRP near repetitive elements such as those associated with CA repeats in sheep (4). After a potentially useful STRP is identified using our strategy, the precise number and population frequency of the alleles must be determined in experiments using individual animals. Sixteen of the 72 markers with a pattern of 4 bands on the initial screen of pooled DNA did not appear usefully polymorphic in the family studies. Some of these might prove useful if larger numbers of animals were studied. This represents a significant “false-positive” rate, but it is a minor problem when large numbers of markers can be rapidly screened.

The 56 potentially useful genetic markers that we have identified, along with others that may result from a similar study of the additional thousands of human markers that are available, should contribute significantly to improving the usefulness of the squirrel monkey as a model of primate metabolism and disease. In addition, this approach should be of more general utility. Since New World monkeys such as the squirrel monkey diverged from its common ancestor with man well before Old World monkeys such as the Rhesus, we would expect an even greater yield of potentially useful markers if DNA from Old World primates including monkeys and apes were pooled and screened with human-derived STRP primer pairs. The substantial number of tetranucleotide STRP that we have identified is worth emphasizing, since only a few isolated tetranucleotide STRP have been reported for non-primate mammals (3,6,17). Heterologous markers are useful, as well, for studies of feline (12) and artdactyl species (2,14). With regard to the latter, approximately 40% of bovine markers may be useful for sheep (5,14) or goats (16).

REFERENCES
Addition of a Competitive Primer Can Dramatically Improve the Specificity of PCR Amplification of Specific Alleles


Polymerase chain reaction (PCR) amplification of specific alleles (PASA) is a rapid nonradioactive approach to diagnose single-base changes in the DNA templates (5). It is also known as allele-specific amplification or ASA (3), allele-specific PCR or ASPCR (8) and amplification refractory mutation system or ARMS (2). The technique is based on the principle that the desired allele is readily amplified by the perfectly matched primer set, but it is only poorly amplified if at all by a mismatched primer set, especially when the mismatch occurs at the 3′-terminal base of the primer (4).

However, studies have demonstrated that the degree of the specificity observed with mismatched primers depends on the mismatch type (1,2). The only primer/template mismatches that

![Diagram of PCR amplification]

Figure 1. (A) Strategy for PCR amplification of specific alleles (PASA). The SS and AZ-R allele-specific forward primers were designed to match the SS and AZ-R cDNA templates, respectively. The addition of a competitive, allele-nonspecific forward primer in the PASA creates a competition for AmpliTaq DNA polymerase between the allele-nonspecific and allele-specific primers (i.e., SS or AZ-R specific primer). If there is a mismatch to the DNA template at the 3′-terminal base of the allele-specific primer, the allele-nonspecific primer will have a competitive advantage over the mismatched allele-specific primer and result in the predominance of the allele-nonspecific PCR product. If there is no mismatch between the allele-specific primer and the DNA template, both forward primers along with the reverse primer will generate both the allele-nonspecific and allele-specific products. (B) Agarose gel electrophoretic analysis of the PASA products amplified in the presence of the competitive primer. Lane M, PCR markers, 50 ng/band (Promega, Madison, WI, USA). Fragment size in base pairs from top to bottom: 163, 80, 500, 300, 150 and 50. The 2.5% agarose gel was run in 1× TBE buffer at a constant voltage of 100 V for 45 min. The allele-specific product (i.e., 80-bp fragment) was generated only when there was no mismatch between the allele-specific primer and the DNA template in the presence of the competitive, allele-nonspecific forward primer.