Detection of microsatellite polymorphisms without cloning

Kun-sheng Wu, Ronny Jones, Leslie Danneberger and Pablo A. Scolnik*
Central Research and Development, E.I. Du Pont de Nemours & Co., PO Box 80402, Wilmington, DE 19880-0402, USA

Received April 21, 1994; Revised and Accepted June 29, 1994

Microsatellites or simple sequence repeats provide an attractive source of genetic polymorphisms for both mammals and plants (1). Microsatellites can be detected by PCR using minute amounts of starting material and they provide codominant markers with a high degree of allelic polymorphism. However, these markers are obtained through a difficult and labor-intensive procedure. In contrast, RAPD markers are easy to develop and less expensive to assay but they are usually genetically dominant (2) and the degree of polymorphism is low. To compensate for the weaknesses of these two approaches, we have combined microsatellites and RAPDs into a new method to detect and map co-dominant polymorphisms without cloning and sequencing. Our method is based on the random distribution of nucleotide sequences immediately flanking the simple sequence repeats. A primer consisting of a 5' anchor and 3' repeats is end-labeled sequences immediately flanking the simple sequence repeats. A primer consisting of a 5' anchor and 3' repeats is end-labeled with γ32P-dATP and used to amplify genomic DNA in the presence or absence of decamers of arbitrary sequences (RAPD primers). The resulting products are resolved in denaturing polyacrylamide gels and, since only the repeat primer is labeled, only the amplification products derived from the anchored primer is detected. To facilitate the annealing of two primers with different Tm and to reduce the amplification mediated by the random 10mer alone, we employed a modified termally asymmetric PCR profile (3). The program was designed to switch between high and low annealing temperatures during the PCR reaction. Since the Tm's of the anchored primers are usually 10–15°C higher than those of the RAPD primers, in the PCR cycles with higher annealing temperature only the anchored primer should anneal efficiently, whereas at low annealing temperature cycles both anchored microsatellite and RAPD primers should anneal. Thus, DNA sequences from microsatellite loci are preferentially amplified. We named these polymorphisms RAMPs (random amplified microsatellite polymorphisms) to differentiate them from specific microsatellites with unique primers. Figure 1 shows an example of RAMP amplifications from two Arabidopsis lines using primer K7 (Table 1) and different RAPD primers. Typical banding patterns of microsatellite polymorphisms were detected in a number of fragments (lane A, 260 bp, lane C, 225 bp, lane D, 190 bp, 350 bp, lane G, 350 bp etc.). Some of these fragments showed co-dominant allelic relation and Mendelian segregation in an Arabidopsis mapping population (4). So far, we have synthesized a number of anchored primers (Table 1) and tested the potential for detection of microsatellite polymorphisms. All the anchored primers listed led to amplification of codominant loci when combined with appropriate RAPD primers. Extensive studies with K7 indicated that, in combination with different random 10-mers, this primer led to the amplification of 104 polymorphic loci, of which 67 appear to be codominant although segregation tests were not completed at all the loci. A majority of the random 10-mers (24 out of 37) used in conjunction with K7 gave rise to at least one novel codominant polymorphic marker. We have also tested the degree of polymorphism using 11 Arabidopsis ecotypes and found the number of alleles among 18 co-dominant loci ranging from 2 to 7. DNA sequencing of a randomly amplified PCR product with K7 primer revealed a 10 perfect CT repeats, further confirming that PCR products generated by this method indeed contain microsatellite repeats. We have mapped 37 RAMP markers in Arabidopsis and the genomic distribution appears to be random. Markers obtained with the same RAMP primer and different 10mer primers map to different genomic loci, indicating that they represent new loci rather than new alleles from the same locus.

Amplifications with single RAMP primers result in 10–30 fragments useful as genetic markers. Theoretically, there are 256 sequence combinations in a four base anchor. Further diversity can be obtained by including different microsatellite repeats. Although preliminary experiments show that in some cases similar patterns are obtained with different anchors, inclusion of RAPD primers results in a significant alteration of the banding pattern. Most fragments obtained with RAMP primers alone disappear when RAPD primers are included, and different patterns are obtained with the same RAMP primer and different RAPDs. Thus, these RAMP primers can successfully compete with the RAMP primers in the amplification reaction, probably during

* To whom correspondence should be addressed
the low stringency stage of the cycle. (A)n repeats are abundant in many genomes, including plants. However, microsatellite markers containing (A)n, (AT)n, (AAAN)n, and (AAN)n are difficult to identify because these repeats are also present in the bacterial hosts used for cloning. Except for (AT)n repeats, which failed to amplify because of self-complementarity, we were able to amplify (A)n and (T)n repeats with the method described here. AP-PCR amplification using simple repeats (5), a recently described fingerprinting procedure (6), and the work described here, extend the usefulness of the PCR for the analysis of complex genomes.

ACKNOWLEDGEMENTS

The authors would like to thank Glenn E.Bartley, Antoni Rafalski, Robert S.Reiter, Sandra H.Russell, and Julie Vogel (DuPont) for critical discussions.

REFERENCES


Figure 1. Polymorphisms detected by randomly anchored microsatellite primers. End-labeled anchor primer K7 was used to amplify Arabidopsis genomic DNA from WS (1) and W100 (2) in the presence of RAPD primers SP3A1 (A), SP3C1 (B), SP3G1 (C), SP3A2 (D), SP3C2 (E), SP3E2 (F), SP3A3 (G), SP3C3 (H), SP3B1 (I), and SP3F1 (J). PCR reaction mixtures contained 10 mM Tris-HCl pH 8.3, 10 mM KCl, 2 mM MgCl₂, 0.2 µM γ³²P-DATP end-labeled anchor microsatellite primer, 0.2 µM RAPD primer, 200 µM each of dGTP, dATP, dTTP and dCTP, 1 U AmpliTaq® polymerase Stoffel fragment (Perkin Elmer), and 20 ng genomic DNA in a final 20 µl volume. The asymmetric PCR cycling consisted of: 94°C for 2 min; 5 cycles of 94°C for 30 sec, 50°C for 30 sec and 72°C for 30 sec; 15 cycles of 94°C for 30 sec, 50°C for 30 sec, 72°C for 30 sec, 94°C for 30 sec, 50°C for 30 sec, 72°C for 45 sec, 94°C for 30 sec, 30°C for 30 sec, 72°C for 45 sec, and an additional extension period at 72°C for 5 min.