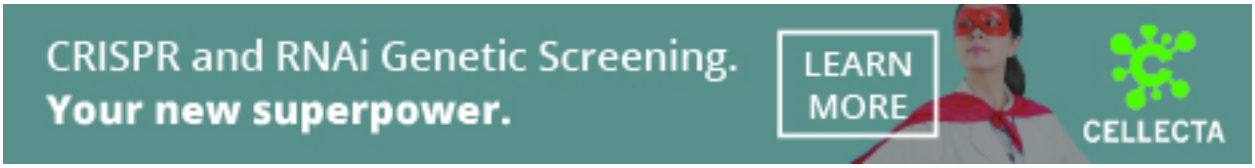
Rapid method for separation of microsatellite alleles by the PhastSystem.

Z Buzás and L Varga

Genome Res. 1995 4: 380-381



Rapid Method for Separation of Microsatellite Alleles by the PhastSystem

Zsuzsa Buzás[1] and László Varga[2]

[1]Institute for Biochemistry and Protein Research, [2]Institute for Molecular Genetics, Agricultural Biotechnology Center, H-2101, Gödöllö, Hungary

Microsatellites are tandemly repeated short (1–6 bp) simple sequences that are highly abundant in most eukaryotic genomes.[1–4] Because of the variation in the number of repeats, these microsatellites show extensive length polymorphism. Thus, they are very effective as genetic markers for linkage analysis, paternity testing, or population analysis.

Microsatellites are detected by using PCR, and the resulting products are separated by agarose or polyacrylamide gel electrophoresis.[5] As alleles often differ in length by only 2 bp, the most accurate detection can be achieved by radioactive PCR and denaturing sequencing gels.

In this paper we report an alternative nonisotopic, high-resolution method for the separation of microsatellite alleles on a partially automated electrophoretic system, the PhastSystem (Pharmacia).

MATERIALS AND METHODS

Amplification of a murine microsatellite D4Mit27[6] was carried out in 10-μl volume containing 50 ng of genomic DNA, 120 nM of each primer, 2 mM $MgCl_2$, 200 μM of each dNTP, and 0.5 units of *Taq* polymerase (Promega). A first denaturation step at 94°C for 3 min was followed by 32 cycles of 1 min denaturation at 94°C, 1 min annealing at 55°C, and 1 min primer extension at 72°C and a final extension for 3 min at 72°C.

For separation of alleles we used a discontinuous buffer system originally introduced for the separation of SDS proteins,[7] modified in its stacking limits to make it applicable for gel electrophoresis of nucleic acids.[8] This electrophoretic system markedly improves the resolution of bands by taking advantage of the different electrochemical properties of buffers and allowing for the determination of relative mobility (R_f) values for fragment identification; R_f values are expressed as the ratio of the migration distance of any fragment to that of the moving boundary.

The homemade nondenaturing polyacrylamide gels (50×43×0.5 mm) were cast on GelBond PAG film (FMC) using the Pharmacia gel cassette. The length of the stacking gel (5% T 3% C_{Bis}; 0.4 M Bis-tris, 0.1 M H_2SO_4, 2.2 mM AMPS, 8.6 mM TEMED at pH 6.7) was 15 mm, whereas the resolving gel (8% T 5% C_{Bis}; 0.4 M Tris, 0.045 M H_2SO_4, 1.75 mM AMPS, 6 mM TEMED at pH 8.6) was 35 mm long. Gel buffer strips were made from 2.8% agarose IsoGel (FMC). The agarose was melted in the appropriate buffer (cathode buffer, 0.2 M bicine, 0.1 M NaOH at pH 8.2; anode buffer, 0.2 M Tris, 0.05 M H_2SO_4 at pH 8.1) and then poured in the molds. The gels were run with constant current (10 mA) at 15°C for 45 Vh (~20 min) and silver stained (~40 min).

RESULTS AND DISCUSSION

Two D4Mit27 microsatellite genotypes with three alleles were separated on a 6% denaturing polyacrylamide sequencing gel (Fig. 1). The same DNA samples were separated on an 8% nondenaturing polyacrylamide gel by the PhastSystem (Fig. 2). The 2 bp difference between alleles (116 bp and 118) was clearly demonstrated. This system gives the best separation in and under this molecular weight range. Microsatellites have a multiband appearance that is resolved by denaturing sequencing gels.[9] We have a similar phenomenon in our system; every allele has a main band and a trailing shadow band. The homozygote 116/116 genotype has two additional faint bands at the 150-bp region, that are not seen on the sequencing gel. These bands must be PCR artifacts, which do not complicate the genotypic interpretation.

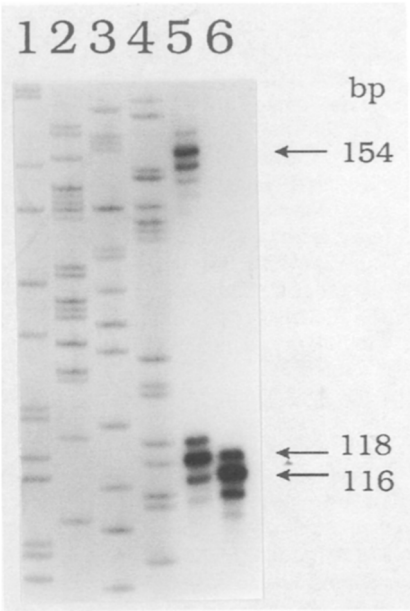


FIGURE 1 Separation of microsatellite alleles on a 6% denaturing polyacrylamide sequencing gel. (Lanes *1–4*) DNA sequencing ladders prepared from a known sequence; (lane *5*) genotype 118/154; (lane *6*) genotype 116/116.

4:380–381©1995 by Cold Spring Harbor Laboratory Press ISSN 1054-9803/95 $5.00

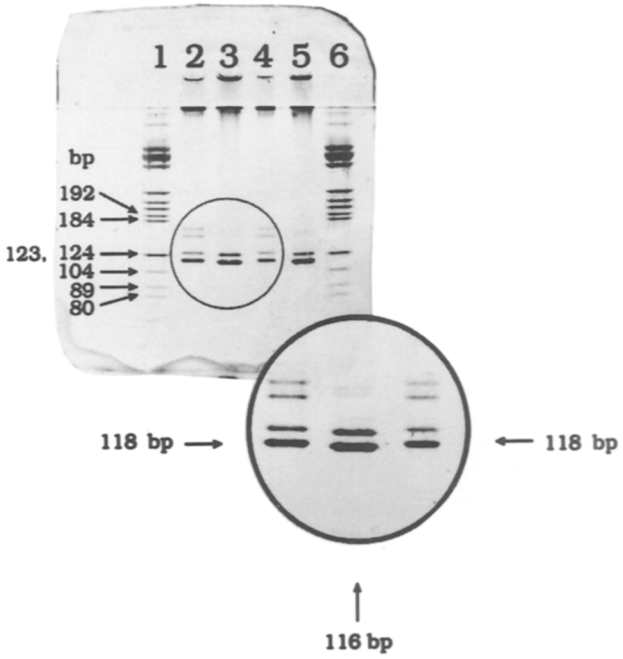


FIGURE 2 Separation of microsatellite alleles on 8% nondenaturing polyacrylamide gel by the PhastSystem. (Lanes *1,6*) DNA molecular weight marker V (Boehringer); (lanes *2,4*) genotype 118/154; (lanes *3,5*) genotype 116/116.

Using this high-resolution technique, 20 min after finishing the PCR reaction the gels were developed, without the need of radioactive compounds and the time-consuming electrophoresis of sequencing gels.

ACKNOWLEDGMENTS

We are grateful to Drs. L. Orbán for his valuable suggestions, Professor L. Orosz for his support, and A. Chrambach and M. Garner (National Institutes of Health) for critical reading of the manuscript. We also thank Anikó Illés for her skilled technical assistance.

REFERENCES

1. Hamada, H., M. Petrino, and T. Kakunaga. 1982. A novel repeated element with Z-DNA-forming potential is widely found in evolutionarily diverse eukaryotic genomes. *Proc. Natl Acad. Sci.* **79:** 6456–6459.
2. Weber, J.L. and P.E. May. 1989. Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. *Am. J. Hum. Genet.* **44:** 388–396.
3. Litt, M. and J.A. Luty. 1989. A hypervariable microsatellite revealed by in vitro amplification of a dinucleotide repeat within the cardiac muscle actin gene. *Am. J. Hum. Genet.* **44:** 397–401.
4. Tautz, D. 1989. Hypervariability of simple sequences as a general source for polymorphic DNA markers. *Nucleic Acids Res.* **17:** 6463–6471.
5. Love, J.M., A.M. Knight, M.A. McAleer, and J.A. Todd. 1990. Towards construction of a high resolution map of the mouse genome using PCR-analysed microsatellites. *Nucleic Acids Res.* **18:** 4123–4130.
6. Dietrich, W., H. Katz, S.E. Lincoln, H.S. Shin, J. Friedman, N.C. Dracopoli, and E.S. Lander. 1992. A genetic map of the mouse suitable for typing intraspecific crosses. *Genetics* **131:** 423–447.
7. Wiltfang, J., N. Arold, and V. Neuhoff. 1991. A new multiphasic buffer system for sodium dodecyl sulfate-polyacrylamide gel electrophoresis for proteins and peptides with molecular masses 100,000–1,000, and their detection with picomolar sensitivity. *Electrophoresis* **12:** 352–366.
8. Zsolnai, A., L. Orbán, and A. Chrambach. 1993. Agarose electrophoresis of DNA in discontinuous buffers, using a horizontal slab apparatus and a buffer system with improved properties. *Electrophoresis* **14:** 179–184.
9. Litt, M., X. Hauge, and V. Sharma. 1993. Shadow bands seen when typing polymorphic dinucleotid repeats: some causes and cures. *BioTechniques* **15:** 280–284.

Received January 26, 1995; accepted in revised form March 29, 1995.