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Search for Improved Electrophoretic Conditions for PCR—Single-strand Conformation Polymorphism Analysis: Is an SDS Buffer Condition Useful?

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Single-strand conformation polymorphism (SSCP) is based on the relation between the electrophoretic mobility of single-stranded DNA (ssDNA) and its folded conformation. This conformation and its sequence are usually determined by intramolecular interaction. Because SSCP analysis coupled with PCR (PCR–SSCP) is a powerful technique that can be used to detect base substitutions,[1] it is an important nonradioisotopic detection method is important in clinical DNA diagnosis. Using ssDNA and double-stranded DNA (dsDNA) conformation polymorphism analyses, in combination with polyacrylamide gradient gel and silver staining detection,[2] we have reported some mutations in the human lactate dehydrogenase (LDH)-A and #LDH-B genes. The original PCR–SSCP analysis and the following modified methods have used mostly neutralizing (native) buffer conditions without SDS. Here, we show examples of PCR–SSCP using SDS buffer conditions as well as comparisons with native buffer conditions.

MATERIALS AND METHODS

Subjects for the present PCR–SSCP experiments were PCR-amplified DNA containing base substitutions in the human LDH-A or LDH-B gene. These mutations had been identified previously by either direct sequencing or DNA sequencing after M13 cloning of the respective exons.[2–5]

Two microliters of each amplified DNA was added to 2 μl each of 95% formamide, 20 mM EDTA, 0.05% bromphenol blue, and 0.05% xylene cyanol. The mixture was then heated to 80°C, and 0.3-μl aliquots were applied to a 7.5% or 12.5% homogeneous gel, or an 8–25% gradient gel (PhastGel, Pharmacia, Uppsala, Sweden), and electrophoresed by Phast System. Here, the gels (PhastGel) contain 0.112 M Tris-acetate buffer (pH 6.4). The electrode buffer strips were made of 3% agarose and contained 0.2 M Tris-tricine buffer (pH 8.1), with 0.55% SDS for SDS-PAGE and 0.88 M L-alanine 0.25 M Tris (pH 8.8) for native PAGE. Gels were prerun for 60 volt-hours (Vh) at 250 V (native buffer only) and electrophoresis was performed at 250 V. SSCP analysis was tentatively set at 15°C. The electrophoretic conditions (programs) are shown in Table 1. After electrophoresis, the gels were silver stained using the Phast System (Pharmacia, Uppsala, Sweden).

RESULTS AND DISCUSSION

By comparing the results depicted in Figure 1 and Table 2, both 12.5% homogeneous and 8–25% gradient gels were more useful for detecting the existence of point mutations than 7.5% homogeneous gels. Generally, the SDS buffer condition increased the resolution of each band and resulted in an improvement in detection sensitivity in most cases (K6E, A35E, S131R, R173H, Y147U).

TABLE 1 Program for Electrophoretic Conditions

Native buffer condition
apply sample down at 1.2, 0 Vh
apply sample up at 1.2, 2 Vh

(Sep)	(V)	(mA)	(W)	(°C)	(Vh)
1.1	250	10.0	3.5	15	60
1.2	250	1.0	3.5	15	2
1.3	250	10.0	3.5	15	no.[a]

SDS buffer condition
apply sample down at 1.2, 0 Vh
apply sample up at 1.2, 2 Vh

(Sep)	(V)	(mA)	(W)	(°C)	(Vh)
1.1	250	10.0	3.5	15	1
1.2	250	1.0	3.5	15	2
1.3	250	10.0	3.5	15	no.[a]

[a]Number (no.) Vh is dependent on kinds of supporting media and lengths of PCR-amplified products.

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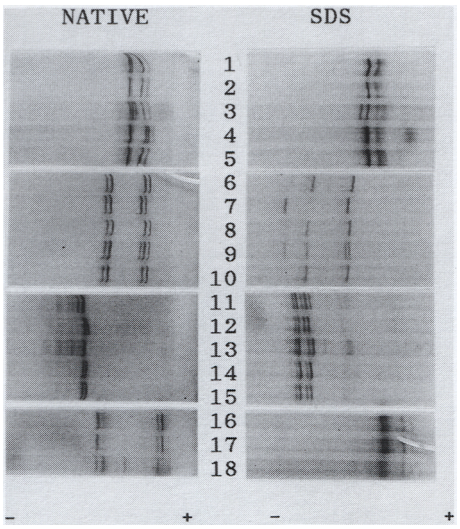

FIGURE 1 Effect of different buffer conditions of SSCP electrophoresis (12.5% homogeneous gel) on the mobility of ssDNA. Gels were prerun at 250 V for a total of 60 Vh (native buffer only), and electrophoresis was performed at 250 V for 360 Vh (lanes *1–18*) and for 450 Vh (lanes *19–23*) at 15°C. After electrophoresis, the gels were silver stained using the Phast System. (Lanes *1–5*) LDH-B, exon 1; (lanes *6–10*) LDH-B, exon 3; (lanes *11–15*) LDH-B, exon 4; (lanes *16–18*) LDH-A, ex. 7; (lanes *19–23*) LDH-B, ex. 4. (Lane *3*) Heterozygous mutation for K6E; (lane *4*) heterozygous for A35E; (lane *7*) homozygous for S131R; (lane *9*) heterozygous for S131R; (lane *11*) heterozygous for R173H; (lane *13*) homozygous for R173H; (lane *18*) heterozygous for E328U; (lane *19*) homozygous for Y147U; (lane *20*) heterozygous for Y174O; (lane *21*) homozygous for R173H; (lane *23*) heterozygous for F172V; other lanes are normal controls.

However, for certain mutated sequences such as E328U and Y174O, the native buffer condition moved to be superior at detecting base substitution mutations. In any new mutant sequences we cannot determine which is the better condition to detect mutations, SDS or native buffer; however, the SDS buffer condition has the possibility of obtaining a better detection sensitivity.

The greatest difference in mobility shift by point mutation was by electrophoresis of the 12.5% homogeneous gel. It will therefore be possible to sequence abnormal silver-stained bands by using the 12.5% homogeneous gel after cutting bands from the gel and amplifying by PCR. Moreover, polymorphisms can be also classified on the basis of subtle mobility shifts of ssDNA. In certain mutations (data not shown),[2] however, abnormalities in electrophoretic mobility do not exist in ssDNA but in dsDNA. Thus, the 8–25% gradient gel might be also useful.[2] Recently, Barros et al.[6] have reported that heteroduplexes and homoduplexes are detectable by using gradient gels and the SDS buffer condition.

Orita et al.[1] explained that the conformation of a single-stranded nucleic acid is presumably determined by the balance between thermal fluctuation and weak local stabilizing forces. Therefore, variations in environmental conditions, such as temperature or the presence of a denaturant such as glycerol, are likely to cause a change in conformation and thereby an alteration in electrophoretic mobility. Mobility shift is temperature dependent irrespective of native or SDS buffer conditions (data not shown). The several steps relating sample preparation, electrophoretic separation, and silver staining using the Phast System can be completed in <4 hr, achieving high resolution. These techniques should be used for the rapid screening of mutations and for genotypic detection or classification by use of the appropriate electrophoretic condition for targeted sequences. We conclude that it is important to perform PCR–SSCP under SDS buffer conditions as well as native buffer conditions in all cases.

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TABLE 2 Sensitivity in Detection of Point Mutations Using Different Electrophoretic Conditions for SSCP

Mutation	Amplified region	Native buffer 7.5[a] 110[b]	Native buffer 12.5[a] 360[b]	Native buffer 8–25[a] 140[b]	SDS buffer 7.5[a] 110[b]	SDS buffer 12.5[a] 360[b]	SDS buffer 8–25[a] 140[b]
K 6E (AAA→GAA)	LDH-B, ex. 1 (304 bp)	±	+	+	+	++	+
A 35E (GCG→GAG)	LDH-B, ex. 1 (304 bp)	−	+	−	++	++	+
S131R (AGT→CGT)	LDH-B, ex. 3 (360 bp)	−	+	±	++	++	++
R173H (CGG→CAC)	LDH-B, ex. 4 (380 bp)	±	±	±	+	+	+
Y147U (TAT→TAG)	LDH-B, ex. 4 (380 bp)	−	±[c]	−	+	+[c]	+
Y1740 (TAC→TAA)	LDH-B, ex. 4 (380 bp)	−	++[c]	+	−	+[c]	−
F172V (TTT→GTT)	LDH-B, ex. 4 (380 bp)	+	++[c]	+	+	++[c]	+
E328U (GAG→TAG)	LDH-A, ex. 7 (313 bp)	++	++	++	±	±	−
I115I[d] (ATA→ATC)	LDH-A, ex. 3 (297 bp)	−	−	+	−	−	+

(++, +, ±, or −) Whether or not the point mutation could be detected; (++) the presence of the distinct band that is easily isolated from gel.
[a]Electrophoretic media are 7.5% homogenous gel, 12.5% homogenous gel, and 8–25% gradient gel.
[b]Electrophoresis was performed for the programmed voltage-hours (Vh).
[c]Electrophoretic results for 450 Vh.
[d]The I115I mutation in the LDH-A gene is a silent substitution, which has been published.[2]

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