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Examination of Parameters Affecting Polymerase Chain Reaction in Studying RAPD

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PCR에 의한 RAPD marker들의 증폭에 영향을 주는 조건들에 대한 고찰

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ABSTRACT: The effects of several parameters on PCR amplification in using RAPD were studied. The results of this study suggest that approximately 15 ng of genomic DNA in 20 μl of reaction mixture results in discrete and reproducible PCR products. In addition, the results indicate that concentration or amounts of reaction components studied are highly inter-dependent in their effects, and RNA can interfere severely with PCR amplification. Suitable concentrations or amounts of reaction components were found to be 30 ng of 10-mer primer, 200 μM of dNTP, 0.001% gelatin 1.5 mM $MgCl_2$, 10 mM Tris-Cl (pH 8.8), 50 mM KCl, 0.1% Triton X-100, 2 units of *Taq* DNA polymerase, and 15 ng of RNase-treated genomic DNA in 25 μl of reaction mixture.

KEYWORDS: RAPD, PCR, population genetics, molecular systematics.

The polymerase chain reaction (PCR) has facilitated rapid advances in molecular biology. This technique amplifies small quantities of target DNA sequences to a millionfold through repeated cycles of DNA synthesis in short periods of time (Mullis and Faloona, 1987; Saiki *et al.*, 1985). Recently, Williams *et al.* (1990) developed a procedure using PCR-based "Random Amplified Polymorphic DNA (RAPD)" for genetic studies. This approach uses arbitrarily chosen 10-mer primers in conjunction with the PCR, providing a means of comparing overall genetic similarity among different individuals.

As Williams *et al.* (1990) noted, RAPD markers have several advantages over other genetic markers, such as restriction fragment length polymorphisms (RFLPs). In using RAPD, information is not required about flanking sequences of regions to be amplified. Because each 10-mer primer screens 20 nucleotides for each amplified product, polymorphisms are efficiently recognized. Using RAPD is faster than using RFLPs because cloning is not required, nor are restriction endonulease digestions, southern blotting, hybridizations, or procedures for detecting bands on membranes. Thus, among the significant advantages of this approach are its speed and economy, making it particularly well-suited for studies on populations of large numbers of individuals.

Because of these advantages, this technique has received much attention for studies involving fungal molecular systematics and population genetic Guthrie and Magill, 1992; Leung *et al.*, 1991; Yoon and Glawe, 1992). However, suitable amplification reaction conditions for different organisms vary because each organism differs in genome size and cellular chemistry. Thus, suitable reaction conditions for efficient PCR amplification should be determined before a particular organism is investigated.

Herein, suitable reaction conditions for studying RAPD markers are described, using *Hypoxylon truncatum* (Schw. ex. Fr.) Mill. The effects of several parameters on amplification of DNA were studied. These parameters included different concentrations or amounts of genomic DNA, $MgCl_2$, dNTP, gelatin, primer, and *Taq* DNA polymerase, different temperature profiles, and the presence or absence of RNA in the reaction mixture. In addition, different treatments were investigated to reduce the effects of possible contaminants on PCR amplification. These treatments included adding Tween 20, increasing the volume of the reaction mixture, and incubating the reaction mixture at 95℃ prior to addition of *Taq* DNA polymerase. The results of this study should provide useful information to researchers regarding parameters that can affect PCR amplification of DNA in studies using RAPD markers.

Materials and Methods

Isolates used and DNA isolation from fungal mycelium: Isolates of *H. truncatum*, derived from 4 collections at 3 different areas, were used: one isolate (isolate number 1-7) from Lake of the Woods Country Park, Champaign Co., Illinois, two isolates (isolate number 3-1 and 3-2) from Lake Murphysboro State Park, Jackson Co., Illinois, and the other isolate (isolate number 4-9) from Starved Rock State Park, Lasalle Co., Illinois. To produce mycelia for DNA isolation, agar plugs carrying mycelium were transferred to flasks containing 5g Difco yeast extract and 20g glucose/L of tap water. Mycelia were grown in shake cultures at room temperature for approximately a week, harvested by filtration, and blotted on paper towels.

DNA isolation was performed as described previously (Yoon *et al.*, 1991). Concentration of DNA in samples were estimated prior to PCR amplification by comparing the intensity of DNA bands in 0.8% agarose gels with a series of λ DNA dilutions, and by viewing them under UV light (310 nm) after staining with ethidium bromide.

Effects of different amounts of genomic DNA: Different amounts (0.1, 1, 15, and 25 ng) of *H. truncatum* (isolate number 3-1) genomic DNA were examined in PCR amplification to determine the suitable concentration. Herein, the term suitable concentration or amount is defined as a concentration or amount which results in discrete and reporducible DNA bands after resolution by agarose-gel electrophoresis.

Amplification reaction mixtures were 25 μl in volume, and contained 10 mM Tris-Cl (pH 8.8), 50 mM KCl, 0.1% Triton X-100, 1.5 mM $MgCl_2$, 0.001% gelatin (Sigma), 100 μM of dNTP (Perkin-Elmer-Cetus), 15 ng of primer A-11 (CAATCGCCGT) (Operon Technologies), 0.7 unit of *Taq* DNA polymerase (Promega), and different amounts (0.1, 1, 15, and 25 ng) of genomic DNA. For each amount of genomic DNA, five reaction mixtures were prepared, using the same conditions. The reaction mixtures were overlaid with mineral oil (Sigma) and placed in an MJ Research DNA thermal cycler (Model PTC-100-60), in which the heating/cooling block was preheated to 70℃. Subseguent amplification reactions were performed after 2 min of preincubation at 94℃ to enhance denaturation of genomic DNA. The amplification protocol consisted of 40 cycles of 1 min of denaturation at 94℃, 1 min of annealing at 36℃, and 2 min of extension at 72℃. Amplified DNA fragments were resolved by electrophoresis (5 V/cm for 1 h) in a gel composed of 0.7% NuSieve GTG agarose (FMC Bioproducts) and 0.7% ultra pure agarose (Sigma), and detected by staining with ethidium bromide and viewing with UV transillumination. Photographs were taken of each gel over a UV transilluminator, using a Polaroid camera (Model DS-34) and black and white film (Type 667, Polaroid Corp.).

Investigation of possible causes when amplification failed to occur: When PCR products were not observed, different treatments were performed on the reaction mixture in each experiment in order to determine whether interference with PCR amplification results from the inhibitory effect of possible contaminants such as acidic polysaccharides (Table 1). For these experiments, primer A-13 (CAGCACCCAC) was used because it

Table 1. Examination of different treatments to reduce the effects of possible contaminants on PCR amplification, using primer A-13.

Experiment #	Treatment	Reaction condition	Tials	PCR products
1	0.5% Tween 20 added to the reaction mixtures	.7 U of *Taq* DNA polymerase, 1.5 mM $MgCl_2$, 100 μM of dNTP, 15 ng of primer, 0.001% gelatin, 10 mM Tris-Cl (pH 8.8), 50 mM KCl, 0.1% Triton X-100, 15 ng of DNA	2	None
2	volume of reaction mixture increased to 50 μl	1 U of *Taq* DNA polymerase, 1.5 mM $MgCl_2$, 100 μM of dNTP, 15 ng of primer, 0.001% gelatin, 10 mM Tri-Cl (pH 8.8), 50 mM KCl, 0.1% Triton X-100, 15 ng of DNA	3	None
3	incubation of reaction mixture at 95℃ for 5 min prior to addition of *Taq* DNA polymerase	0.7 U of *Taq* DNA polymerase, 1.5 mM $MgCl_2$, 100 μM of dNTP, 15 ng of primer, 0.001% gelatin, 10 mM Tri-Cl (pH 8.8), 50 mM KCl, 0.1% Triton X-100, 15 ng of DNA	3	None

Table 2. Examination of the effects of differing temperature profilles on PCR amplification, using primer A-13.

Experiment #	Temperature profile	Reaction condition	Tials	PCR products
1	92℃ for 1 min, 36℃ for 1 min, 71℃ for 1 min in each of 40 cycles	0.7 U of enzyme, 15 ng of DNA, 100 μM of dNTP, 15 ng of primer, 0.001% gelatin, 10 mM Tris-Cl (pH 8.8), 50 mM KCl, 1.5 mM $MgCl_2$, 0.1% Triton X-100	2	None
2	92℃ for 30 sec, 50℃ for 30 sec, 71℃ for 1 1/2 min in each of 40 cycles	0.7 U of enzyme, 15 ng of DNA, 100 μM of dNTP, 15 ng of primer, 0.001% gelatin, 10 mM Tris-Cl (pH 8.8), 50 mM KCl, 1.5 mM $MgCl_2$, 0.1% Triton X-100	2	None
3	90℃ or 92℃ for 1 min, 36℃ for 1 min, 72℃ for 2 min in each of 40 cycles	0.7 U of enzyme, 15 ng of DNA, 100 μM of dNTP, 15 ng of primer, 0.001% gelatin, 10 mM Tris-Cl (pH 8.8), 50 mM KCl, 1.5 mM $MgCl_2$, 0.1% Triton X-100	2	None
4	94℃ for 1 min, 36℃ for 1 min, 72℃ for 2 min in each of 30 or 35 cycles	0.7 U of enzyme, 15 ng of DNA, 100 μM of dNTP, 15 ng of primer, 0.001% gelatin, 10 mM Tris-Cl (pH 8.8), 50 mM KCl, 1.5 mM $MgCl_2$, 0.1% Triton X-100	4	None
5	94℃ for 1 min, 45, 50 or 55℃ for 1 min, 72℃ for 2 min in each of 40 cycles	0.7 U of enzyme, 15 ng of DNA, 100 μM of dNTP, 15 ng of primer, 0.001% gelatin, 10 mM Tris-Cl (pH 8.8), 50 mM KCl, 1.5 mM $MgCl_2$, 0.1% Triton X-100	2	None

Table 3. Examination of the effects of variables in reaction mixtures on PCR amplification, using primer A-13.

Experiment #	Variables of reaction component tested	Reaction condition	Tials	PCR products
1	0.7, 1, 1.5, 2 U of *Taq* DNA polymerase	15 ng of DNA, 100 μM of dNTP, 15 ng of primer, 0.001% gelatin, 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-Cl (pH 8.8), 0.1% Triton X-100	3	None
2	0.1, 0.5, 1, 1.5, 2, 2.5, 3 mM $MgCl_2$	0.7 U of *Taq* DNA polymerase, 15 ng of DNA, 100 μM of dNTP, 15 ng of primer, 0.001% gelatin, 50 mM KCl, 10 mM Tris-Cl (pH 8.8), 0.1% Triton X-100	2	None
3	100, 200 μM of dNTP	0.7 U of *Taq* DNA polymerase, 15 ng of DNA, 15 ng of primer, 0.001% gelatin, 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-Cl (pH 8.8), 0.1% Triton X-100	4	None
4	15, 30 ng of primer	0.7 U of *Taq* DNA polymerase, 15 ng of DNA, 100 μM of dNTP, 0.001% gelatin, 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-Cl (pH 8.8), 0.1% Triton X-100	2	None
5	0.0001, 0.002, 0.01% gelatin	0.7 U of *Taq* DNA polymerase, 15 ng of DNA, 100 μM of dNTP, 15 ng of primer, 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-Cl (pH 8.8), 0.1% Triton X-100	3	None
6	0.1, 1, 15, 25 ng of DNA	0.7 U of *Taq* DNA polymerase, 100 μM of dNTP, 15 ng of primer, 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-Cl (pH 8.8), 0.1% Triton X-100	3	None

usually resulted in reproducible PCR products. To counteract the possible inhibitory effect of acidic polysaccharides, 0.5% Tween 20 was added to the reaction mixture (Table 1). In addition, 50 μl rather than 25 μl of reaction mixtures was used to dilute the effects of possible inhibitors (Table 1). In order to denature possible contaminants, reaction mixtures were incubated at 95℃ for 5 min prior to addition of *Taq* DNA polymerase (Table 1).

Effects of differing temperature profiles on PCR amplification were investigated, using primer A-13 (Table 2). As shown in experiments 1 and 2 in Table 2, various temperatures and lengths of each programmed step were examined: 92℃ for 1 min, 36℃ for 1 min, and 71℃ for 1 min; and 92℃ for 30 sec, 50℃ for 30 sec, and 71℃ for 1/2 min. Experiments 3 and 4 in Table 2 were performed

Table 4. Examination of the effects of increased concentration or amount of reaction components on PCR amplification, using primer A-13.

Concentration or amounts of reaction components examined	Trials	PCR products
2 U of *Taq* DNA polymerase, 1.5 mM $MgCl_2$, 30 ng of primer A-02, 200 μM of dNTP, 0.001% gelatin, 15 ng of DNA, 10 mM Tris-Cl (ph 8.8), 50 mM KCl, 0.1% Triton X-100	3	no or faint bands resolved on an agarose gel

Table 5. Examination of the effects of RNA on PCR amplification, using primer A-13.

Experiment #	Reaction condition	Trials	PCR products
1	2 U of *Taq* DNA polymerase, 1.5 mM $MgCl_2$, 200 μM of dNTP, 30 ng of primer, 0.001% gelatin, 15 ng of DNA without RNase treatment, 10 mM Tris-Cl (pH 8.8), 50 mM KCl, 0.1% Triton X-100	3	no or faint bands resolved on an agarose gel
2	2 U of *Taq* DNA polymerase, 1.5 mM $MgCl_2$, 200 μM of dNTP, 30 ng of primer, 0.001% gelatin, 15 ng of RNase-treated DNA 10 mM Tris-Cl (pH 8.8), 50 mM KCl, 0.1% Triton X-100	3	discret and reproducible bands resolved on an agrose gel

to determine whether failure of PCR amplification is associated with loss of *Taq* DNA polymerase activity due to high temperatures or fluctuation through cycles. In these experiments, the denaturation temperature was reduced from 94℃ to 90℃ or 92℃. The number of amplification cycles was shortened from 40 cycles to 30 or 35 cycles. Experiment 5 in Table 2 was performed to determine whether smeared PCR products resulted from non-specific binding of primer to its template: annealing temperature was increased to 40, 45, 50, and 55℃.

Several experiments (Table 3) were performed in order to determine the effect of each component (*Taq* DNA polymerase, $MgCl_2$, dNTP, primer A-13, gelatin, or genomic DNA).

Another experiment was done to test the effect of increasing concentrations or amounts of *taq* DNA polymerase, dNTP, and primer A-13 in the same reaction mixture (Table 4).

The possibility that RNA interfered with PCR amplification was investigated by testing the effects of treating DNA solutions with RNase. For this purpose, DNA solutions containing 100 μg of RNase A/*ml* final concentration were incubated at 37℃ for 1 h to digest RNA, and the results of using this DNA in 25 *μl* of amplification mixtures were compared with those of using untreated-DNA solutions (Table 5). For this experiment, primer A-13 was used.

Re-amplification: When the amplified reaction mixtures did not produce any PCR products at various conditions described above, the amplified reaction mixture was reamplified to determine whether re-amplified products are useful for data analysis. Re-amplification was performed, using 25 *μl* of reaction mixture containing 2-4 *μl* of previously amplified reaction mixture, 10 mM Tris-Cl (pH 8.8), 50 mM KCl, 0.1% Triton X-100, 1.5 mM $MgCl_2$, 0.001% gelatin, 200 μM of dNTP, 30 ng of primer A-11, and different amounts of *Tag* DNA polymerase (0.5, 1, 1.5, and 2 units).

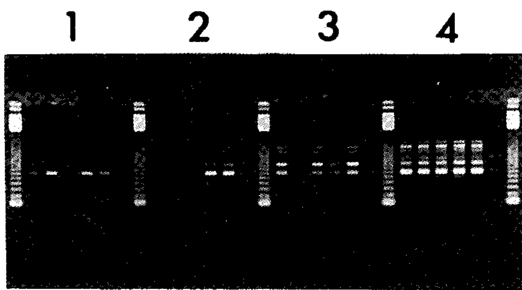

Fig. 1. Amplification products of *Hypoxylon truncatum* (isolate number 3-1) at various amounts of genomic DNA. First five lanes in number 1: PCR products from using 0.1 ng of genomic DNA in amplification; first five lanes in number 2: PCR products from using 1 ng of genomic DNA in amplification; first five lanes in number 3: PCR products from using 15 ng of genomic DNA in amplification; first five lanes in number 4: PCR products from using 25 ng of genomic DNA in amplification. The last lanes in each number were used as controls which lacked genomic DNA. Each treatment was replicated five times.

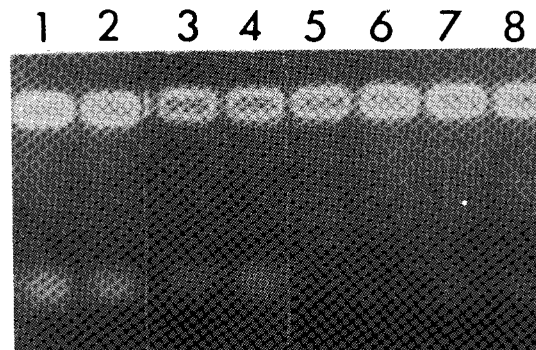

Fig. 2. Amplification products after increasing the amount of *Taq* DNA polymerase to 1 unit. Lanes 1 and 2: PCR products from DNA of *H. turncatum* (isolate number 3-2); Lanes 3 and 4: PCR products from DNA of *H. truncatum* (isolate number 1-7); Lanes 5 and 6: PCR products from DNA of *H. truncatum* (isolate number 3-1); Lanes 7 and 8: PCR products from DNA of *H. truncatum* (isolate number 4-9).

Results

PCR products resulting from different amounts of genomic DNA: Fig. 1 shows the effects of different amounts (0.1, 1, 15, and 25 ng) of *H. truncatum* (isolate number 3-1) genomic DNA on amplification. All DNA bands reflecting amplified DNA fragments were sufficiently discrete and reproducible to be useful when 15 or 25 ng of genomic DNA was used. Using 15 ng of genomic DNA for amplification resulted in clearer DNA bands with less background smears than using 25 ng. However, some products did not result when 0.1 or 1 ng of genomic DNA was used.

Effects of different parameters on interference with PCR amplification: As shown in Table 1, neither addition of 0.5% Tween 20 to reaction mixtures, nor incubation of reaction mixtures at 95℃ for 5 min prior to addition of *Taq* DNA polymerase resulted in PCR products. In addition, increasing the volume of reaction mixtures to 50 μl did not reverse inhibition of PCR amplification. Failure of PCR amplification was not reversed by changes in amplification protocols, such as changing length of each step, the denaturation and annealing temperatures, and numbers of cycles (Table 2). These results suggest that interference with PCR amplification was not the result of inhibitory effects of contaminants, loss of *Taq* DNA polymerase activity because of high temperature of fluctuation through many cycles. Increasing annealing temperatures up to 55℃ did not reduce smearing of bands after electrophoretic separation.

As shown in Table 3, changing each component individually did not improve amplification (Table 3). For example, increasing the amount of *Taq* DNA polymerase to 1 unit, without changing concentrations or amounts of other reaction components, did not improve amplification (Fig. 2).

Possible causes preventing PCR amplification: Some of PCR products did not result when concentrations or amounts of *Taq* DNA polymerase, dNTP, and primer were increased in the same reaction mixture (Table 4 and Fig. 3). As shown in Fig. 3, in lanes where no or faintly staining DNA bands were resolved, RNA-templated PCR products were recognized based on their characteristic band patterns such as the size and smears (Innis *et al.*, 1990). In order to determine whether RNA could inhibit PCR amplification, RNase-treated DNA solutions were amplified as described in Table 5. After such treatment, RNA-templated PCR products were not observed but discrete DNA-templated PCR products were resolved by

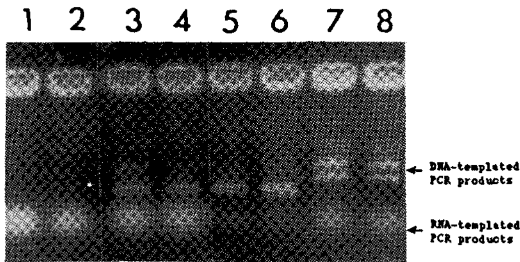

Fig. 3. Amplification products after increasing concentrations or amounts of *Taq* DNA polymerase, dNTP, and primer in the same reaction mixture. Lanes 1 and 2: PCR products from DNA of *H. truncatum* (isolate number 3-2); Lanes 3 and 4: PCR products from DNA of *H. truncatum* (isolate number 1-7); Lanes 5 and 6: PCR products from DNA of *H. truncatum* (isolate number 3-1); Lanes 7 and 8: PCR products from DNA of *H. truncatum* (isolate number 4-9).

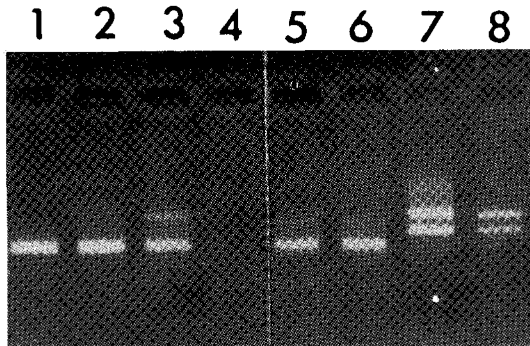

Fig. 4. Amplification products after RNase treatment on DNA solution. Lanes 1 and 2: PCR products from DNA of *H. truncatum* (isolate number 3-2); Lanes 3 and 4: PCR products from DNA of *H. truncatum* (isolate number 1-7); Lanes 5 and 6: PCR products from DNA of *H. truncatum (isolate number 3-1); Lanes 7 and 8: PCR products from DNA of H. truncatum* (isolate number 4-9).

agarosegel electrophoresis (Fig. 4). These results suggest that RNA inhibited PCR amplification, and that in the presence of abundant RNA, amplification from RNA templates is favored over amplification from DNA templates.

PCR products from re-amplification: When no PCR products resulted even after treating DNA solutions with RNase and concentrations or amounts of all reaction components had been varied, re-amplification of the amplified reaction mixture resulted in PCR products (Fig. 5). In three repeated trials for each of different amounts (0.5, 1, 1.5, and 2 units) of *Taq* DNA polymerase, approximately 2 units of *Taq* DNA polymerase consistently produced PCR products. However, results depended on the amount of *Taq* DNA polymerase used. When less than 1 unit of enzyme was used, not all of the same PCR products were observed as when 1.5 and 2 units were used.

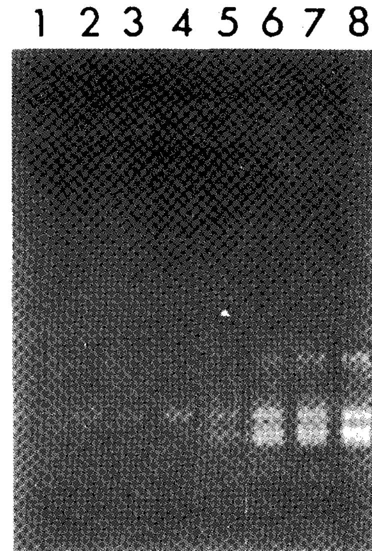

Fig. 5. Re-amplification products at various amounts of *Taq* DNA polymerase, using *Hypoxylon truncatum* (isolate number 3-1). Lanes 1 and 2: PCR products from using 0.5 unit of enzyme in amplification; Lanes 3 and 4: PCR products from using 1 unit of enzyme in amplification; Lanes 5 and 6: PCR products from using 1.5 units of enzyme in amplification; Lanes 7 and 8: PCR products from using 2 units of enzyme in amplification.

Discussion

Considerations in determining suitable amplification reaction conditions in using RAPD: Amount of genomic DNA is a major factor affecting PCR amplification. As long as the amount of genomic DNA was between 15 and 25 ng in 25 μl of reaction mixtures, results were consistent. Thus, amounts of genomic DNA in this range appear suitable for reproducible PCR products.

When interference with PCR amplification was encountered, this interference was not reversed

by varying individual reaction components in the reaction mixture, by changing temperature profiles, by adding 0.5% Tween 20 to reaction mixtures, by incubating reaction mixtures at 95℃ prior to addition of *Taq* DNA polymerase, or by increasing the volume of the reaction mixture. However, when concentrations or amounts of *Taq* DNA polymerase, dNTP, and primer were increased to 2 units, 200 μM, and 30 ng, respectively in the same reaction mixture, amplification tended to occur more frequently. As shown in Fig. 3, even though these changes resulted in some samples exhibiting DNA bands, these bands stained faintly. Other samples did not exhibit any DNA bands. This result indicated that some sources of interference remained even after changing reaction conditions. This remaining interference with PCR amplification was almost completely reversed by treating DNA solutions with RNase prior to amplification. Resulting DNA bands after treating DNA solutions with RNase prior to amplification were more distinct and stained more intensely (Fig. 4) than those resulting from DNA solutions not treated with RNase (Fig. 3). This result indicates that in the presence of abundant RNA, RNA-templated amplification appears to be favored over DNA-templated amplification. This finding is consistent with the report of Deragon and Landry (1992), who inferred the possibility of RNA interference with PCR amplification in using RAPD.

It is possible that primers can bind to complementary sequences in single-stranded RNA during preparation of reaction mixtures, or during the time before the temperature of a heating block reaches 94℃ when denaturation occurs. In addition, because primers used are composed of 10 nucleotides, there is a higher probability that sequences of the primers are complementary to RNA than when longer priming sequences are used, as when specific sequences are amplified (Innis *et al.*, 1990). For these reasons, subsequent amplification reactions may result in RNA-templated PCR products. Results of this situation may be seen in Fig. 2, in which RNA-templated PCR products are more intense than DNA-templated PCR products. However, when excess amounts of primer, dNTP, and *Taq* DNA polymerase were used in amplification reactions, PCR products resulted from DNA templates as well as RNA templates (Fig. 3). When RNase-treated DNA was amplified, only DNA-templated products were resolved on an agarose gel (Fig. 4). These results suggest that RNA can have inhibitory effects on PCR amplification, and that amplification depends on proper concentrations or amounts of *Taq* DNA polymerase, primer, and dNTP. Thus, to study RAPD, suitable reaction conditions would include 1.5 mM $MgCl_2$, 0.001% gelatin, 10 mM Tris-Cl (pH 8.8), 50 mM KCl, 0.1% Triton X-100, 30 ng of 10-mer primer, 200 μM of dNTP, 2 units of *Taq* DNA polymerase, and 15 ng of RNase-treated genomic DNA.

Usefulness of re-amplification: When 2 units of *Taq* DNA polymerase were used, re-amplification provided consistent PCR products (Fig. 5). Because the DNA fragments from previously amplified reaction mixtures are less than 4 Kb in length (based on migration rates relative to standards), they are denatured more easily than the longer template DNA in the original amplification mixtures. In addition, because the ends of all amplified fragments consist of the sequences complementary to the primer, annealing of primer to these fragments takes place more efficiently than for original template DNA. For these reasons, reamplification results in reproducible PCR products efficiently. Thus, re-amplification appears to be an alternative solution to the problem of interference with PCR amplification, and can be tried when failure of PCR amplification is not reversed by changes of reaction conditions. However, in order to avoid biased results, it is necessary for all samples to be re-amplified because sometimes re-amplification results in PCR products which were not produced in the first amplification.

적 요

재현성 있는 RAPD marker들의 증폭을 위해서 PCR에 영향을 주는 조건들에 대해 조사를 하였다. 그 결과 약 15 ng의 DNA가 효과적인 PCR에 적합한 양이었으며 PCR에 사용되는 성분(reaction compo-

nent)들의 농도가 PCR 결과에 있어서 상호의존관계에 있었고 DNA 용액에 포함되어 있는 RNA가 DNA 증폭을 방해하는 작용을 하였다. 25 μl의 PCR 반응용액에 30 ng의 10-mer primer, 200 μM의 dNTP, 0.001% gelatin, 1.5 mM $MgCl_2$, 10 mM Tris-Cl(pH 8.8), 50 mM KCl, 0.1% Triton X-100, 2 units의 *Taq* DNA polymerase, 그리고 RNA를 제거한 15 ng의 DNA를 사용한 결과 가장 재현성있는 RAPD marker들이 증폭되었다.

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