Further Optimization of Random Amplified Polymorphic DNA (RAPD) Analysis in Common Bean

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Introduction

The recent development of the random amplified polymorphic DNA (RAPD) marker system (Williams et al., 1990) has spawned considerable interest within the plant breeding community. The simplicity of the assay itself, as compared to restriction fragment length polymorphism (RFLP) analysis, potentially affords the opportunity to apply this marker system to the benefit of both basic and applied plant breeding problems.

Since early 1991, the bean breeding and genetics program at Michigan State University has been working with the RAPD system as a means of providing molecular markers for several major disease resistance genes. Early work in our laboratory demonstrated the rapidity with which RAPD markers could be identified in common bean (Miklas et al., 1993) and also provided useful information regarding the choice of thermostable enzyme (*Stoffel Fragment* DNA Polymerase) for use in the polymerase chain reaction (PCR) (Miklas and Kelly, 1992). In this report we outline several modifications that we have since made to our PCR set-up and cycling profile. We believe that these modifications have not only improved our capacity for data acquisition but have improved the overall efficiency of the RAPD system in our laboratory.

Original Cycling Protocol

94 °C/1 min, 35 °C/1 min, 72 °C/2 min; 47 cycles
1 sec "Auto-Segment Extension" (for Extension phase of PCR)

The total cycling time for this protocol (in our Perkin Elmer DNA Thermocycler 480) is 5 hours and 20 minutes. This cycling profile proved useful in identifying a RAPD marker tightly-linked to an important rust resistance gene (Up_2; Miklas et al., 1993).

Bi-Phasic Annealing Temperatures

94 °C/1 min, 35 °C/1 min, 72 °C/2 min; 3 cycles
94 °C/1 min, 40 °C/1 min, 72 °C/2 min; 44 cycles
1 sec "Auto-Segment Extension" (for Extension phase of 44-cycle portion of PCR)

This cycling profile proved useful in identifying two RAPD markers tightly-linked to the B-190 rust resistance gene block (Haley et al., 1993c). Although those well-versed in PCR suggest the use of greater annealing stringency during initial PCR cycles (Innis and Gelfand, 1990), our results suggest that this may not be entirely the case for amplification of bean genomic DNA using short, arbitrary primers (random decamers).

Reduction in Cycle Number, Addition of "Terminal" Extension Cycle

94 °C/1 min, 35 °C/1 min, 72 °C/2 min; 3 cycles
94 °C/1 min, 40 °C/1 min, 72 °C/2 min; 34 cycles
72 °C/5 min; 1 cycle
1 sec "Auto-Segment Extension" (for Extension phase of 34-cycle portion of PCR)

The total cycling time for this protocol (Haley et al., 1993b) is 4 hours and 23 minutes, one hour less than the original protocol. The reduction in total cycle number (from 47 to 37) reportedly serves to avoid the generation of various nonspecific background products (observed as a "DNA smear") due to the "Plateau Effect" (Innis and Gelfand, 1990). Our experience suggests that this may be true, although perhaps not with each and every DNA – primer combination tested.

Reduction in Reaction Volume

19.8 µl reaction volume (75% of original volume; all reagents at *same concentration* as 25 µl reaction)
12.5 µl reaction volume (50% of original volume; all reagents at *same concentration* as 25 µl reaction)

The main impetus for this modification was the desire to reduce overall DNA polymerase costs, yet attempts at simply reducing the amount of polymerase in a 25 µl reaction volume were unsuccessful.

We have adopted the 19.8 µl reaction volume (a 25% cost savings) for most of our work yet feel that the 12.5 µl reaction volume (a 50% cost savings) is amenable to further modification and is still generally useful for specific "primary" (brighter or more intensely-stained) bands.

Preferential Amplification of Small, Unrepeatable Fragment

94 °C/3 min, 35 °C/1 min, 72 °C/90 sec; 1 cycle
94 °C/1 min, 35 °C/1 min, 72 °C/90 sec; 2 cycles
94 °C/10 sec, 40 °C/20 sec, 72 °C/90 sec; 34 cycles
72 °C/5 min; 1 cycle
No "Auto-Segment Extension"

This protocol proved extremely useful in improving the repeatability of amplification of a small DNA fragment tightly-linked to an important rust resistance gene (*Ur-3*; Haley et al., 1993a). The reduction in the extension phase of the PCR appears to preferentially inhibit the amplification of larger (> 1 kbp) DNA fragments, which served to enhance the appearance of the fragment linked to *Ur-3*.

Reduction in Denaturation and Annealing Times

94 °C/1 min, 35 °C/1 min, 72 °C/2 min; 3 cycles
94 °C/10 sec, 40 °C/20 sec, 72 °C/2 min; 31 cycles
72 °C/5 min; 1 cycle
1 sec "Auto-Segment Extension" (for Extension phase of 31-cycle portion of PCR)

The total cycling time for this protocol is 3 hours and 14 minutes, two hours less than the original protocol. This protocol appears to result in a greater degree of amplification (observed as brighter and sharper bands) as also reported by Yu and Pauls (1992). We have adopted this protocol for the majority of the work in our laboratory.

Conclusions

We believe that our modifications have demonstrated the inherent flexibility of the PCR for use in common bean, in spite of the highly complex nature of both the PCR and RAPD technologies. Informal trial experiments during the development of these protocols indicated that there was generally no effect, compared to the original protocol, on either the number of DNA fragments amplified or the presence or absence of specific DNA fragments amplified with previously characterized DNA – primer combinations. These modifications have proven extremely useful in our laboratory and, with carefully controlled optimization, should find application by other researchers working with the RAPD system in common bean.

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