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Review Article

Random Amplified Polymorphic DNA (RAPD) Markers

Fevzi BARDAKCI
Cumhuriyet University, Faculty of Arts and Science, Department of Biology, 58140, Sivas-TURKEY

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Abstract : Due to advances in molecular biology techniques, large numbers of highly informative DNA markers have been developed for the identification of genetic polymorphism. In the last decade, the random amplified polymorphic DNA (RAPD) technique based on the polymerase chain reaction (PCR) has been one of the most commonly used molecular techniques to develop DNA markers. RAPD markers are amplification products of anonymous DNA sequences using single, short and arbitrary oligonucleotide primers, and thus do not require prior knowledge of a DNA sequence. Low expense, efficiency in developinga large number of DNA markers in a short time and requirement for less sophisticated equipment has made the RAPD technique valuable although reproducibility of the RAPD profile is still the centre of debate.

Key Words : RAPD, PCR, DNA, polymorphism

Rastgele Artırılmış Polimorfik DNA (RAPD) İşaretleyicileri

Özet : Moleküler biyoloji tekniklerindeki ilerlemeler sayesinde, genetik polimorfizmi saptamak için oldukça yararlı çok sayıda DNA işaretleyicileri geliştirilmiştir. Son on yılda, DNA işaretleyicileri geliştirmek için en sık kullanılan moleküler teknik, Polimeraz Zincir Reaksiyonuna (PZR) dayalı olan Rastgele Artırılmış Polimorfik DNA (RAPD) tekniğidir. RAPD işaretleyicileri, tek bir kısa ve rastgele oligonükleotit primer kullanılarak, bilinmeyen DNA dizilerinin çoğaltılmış ürünleri olduklarından, önceden DNA baz dizisinin bilinmesi gerekmez. Her ne kadar, RAPD profilinin tekrarlanabilirliği halen tartışmanın odak noktası ise de, RAPD tekniği ucuz, kısa sürede çok sayıda DNA işaretleyicisi geliştirmedeki etkinliği ve çok gelişmiş aletlere daha az gereksinim göstermesi nedeniyle önemlidir.

Anahtar Sözcükler : RAPD, PCR, DNA, polimorfizim

Introduction

Advances in molecular biology techniques have provided the basis for uncovering virtually unlimited numbers of DNA markers. The utility of DNA-based markers is generally determined by the technology that is used to reveal DNA-based polymorphism. Currently, the restriction fragment length polymorphism (RFLP) assay (1) has been the choice for many species to measure genetic diversity and construct a genetic linkage map. However, an RFLP assay which detects DNA polymorphism through restriction enzyme digestion, coupled with DNA hybridisation, is, in general, time consuming and laborious. Over the last decade, polymerase chain reaction (PCR) technology has become a widespread research technique and has led to

the development of several novel genetic assays based on selective amplification of DNA (2). This popularity of PCR is primarily due to its apparent simplicity and high probability of success. Unfortunately, because of the need for DNA sequence information, PCR assays are limited in their application. The discovery that PCR with random primers can be used to amplify a set of randomly distributed loci in any genome facilitated the development of genetic markers for a variety of purposes (3,4). The simplicity and applicability of the RAPD technique have captivated many scientistsí interests. Perhaps the main reason for the success of RAPD analysis is the gain of a large number of genetic markers that require small amounts of DNA without the requirement for cloning, sequencing or any other form of the molecular characterisation of the genome of the species in question.

In this paper the principles and several of the most common applications of RAPD markers in biology are reviewed.

Principle of the RAPD Technique

The standard RAPD technology (3) utilises short synthetic oligonucleotides (10 bases long) of random sequences as primers to amplify nanogram amounts of total genomic DNA under low annealing temperatures by PCR. Amplification products are generally separated on agarose gels and stained with ethidium bromide. Decamer primers are commercially available from various sources (e.g., Operon Technologies Inc., Alameda, California). Welsh and McClelland (4) independently developed a similar methodology using primers about 15 nucleotides long and different amplification and electrophoretic conditions from RAPD and called it the arbitrarily primed polymerase chain reaction (AP-PCR) technique. PCR amplification with primers shorter than 10 nucleotides [DNA amplification fingerprinting (DAF)] has also been used producing more complex DNA fingerprinting profiles (5). Although these approaches are different with respect to the length of the random primers, amplification conditions and visualisation methods, they all differ from the standard PCR condition (2) in that only a single oligonucleotide of random sequence is employed and no prior knowledge of the genome subjected to analysis is required.

At an appropriate annealing temperature during the thermal cycle, oligonucleotide primers of random sequence bind several priming sites on the complementary sequences in the template genomic DNA and produce discrete DNA products if these priming sites are within an amplifiable distance of each other (Figure 1). The profile of amplified DNA primarily depends on nucleotide sequence homology between the template DNA and oligonucleotide primer at the end of each amplified product. Nucleotide variation between different sets of template DNAs will result in the presence or absence of bands because of changes in the priming sites (Figure 2). Recently, sequence characterised amplified regions (SCARs) analysis of RAPD polymorphisms (6, 7) showed that one cause of RAPD polymorphisms is chromosomal rearrangements such as insertions/deletions. Therefore, amplification products from the same alleles in a heterozygote differ in length and will be detected as presence and absence of bands in the RAPD profile. The

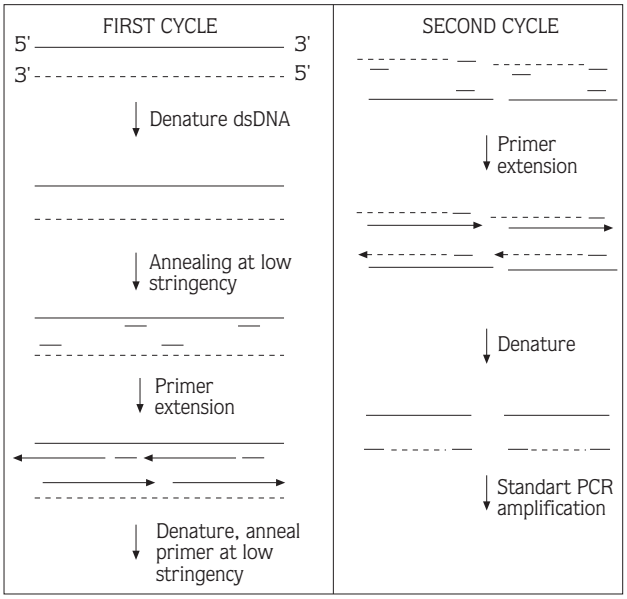

Figure 1. Schematic diagram of RAPD reaction for 2 loci (derived from Welsh and McClelland, 1994).

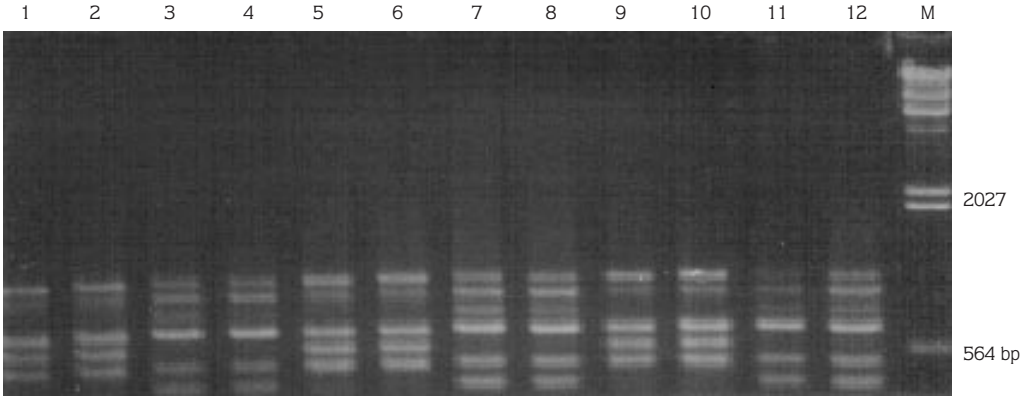

Figure 2. A typical RAPD banding pattern amplified with primer OPA 07 (Operon Tech. Inc.) that resolved in 1.5% agarose gel stained with ethidium bromide. Template DNA was from *Oreochromis aureus* (Pisces: Cichlidae) (lanes 1, 2, 5, 6, 9, 10) and *O. mossambicus* (3, 4, 7, 8, 11, 12). M is a *Hind*III DNA size marker.

profile of RAPD bands (Figure 2) is similar to that of low stringency minisatellite DNA fingerprinting patterns and is therefore also termed RAPD fingerprinting. On average, each primer directs amplification of several discrete loci in the genome so that allelism is not distinguishable in RAPD patterns. In other words, it is not possible to distinguish whether a

DNA segment is amplified from a locus that is heterozygous or homozygous. RAPD markers are therefore dominant.

Applications of RAPD Analysis

Because of the simplicity and low cost of the RAPD technique, it has found a wide range of applications in many areas of biology. Some of the areas where the technique is used are described below:

Genetic Mapping

Restriction fragment length polymorphisms (RFLPs) have been commonly used to map genes (1). This approach involves hybridisation of a probe (usually a cloned fragment) to Southern blotted genomic DNA digested with restriction endonucleases. A useful probe will detect differences in restriction fragment lengths arising from loss or gain of recognition sites or from deletions or insertions of stretches of DNA between sites (8, 9). Because the Southern blot approach of RFLP analysis is time consuming and the identification and isolation of clones is often tedious, there is increasing interest in PCR technology, which allows amplification of any sequence of interest from nanogram amounts of DNA, and direct visualisation of the amplified product. Although PCR seems promising in the amplification of minisatellites, macrosatellites and sequence tagged site (STS) loci to make genetic mapping easy, the prior sequence information needed to design specific primers is a limiting factor in developing large numbers of genetic markers for any organisms (10).

The necessity for sequence information for PCR was circumvented using short primers of arbitrary sequences to amplify DNA segments, namely RAPD. The speed and efficiency of RAPD analysis encouraged scientists to perform high-density genetic mapping in many plant species such as alfalfa (11), faba bean (12) and apple (13) in a relatively short time. Over 250 RAPD markers in *Arabidopsis* were mapped in 8 person-months (14) and 191 markers were constructed in only 6 person-months for loblolly pine (15). The RAPD approach has also been used to create saturated genetic maps in fish species. Postlethwait et al. (16) mapped 401 polymorphic DNA markers in zebrafish (*Danio rerio*). RAPD markers are also being used to construct genetic maps of tilapia species, *O. niloticus* and *O. aureus* (17) and rainbow trout, *Oncorhynchus mykiss* (18). One disadvantage of RAPD markers is that they are dominant, hence the statistical information generated is less per marker in F_2 populations. Therefore, when mapping with dominant markers, it is necessary to use backcross or recombinant inbred populations, haploid or gametophytic tissue, or alternatively an F_2 population where only RAPD markers amplified from a single parent are mapped (19).

Alternatively, the RAPD technique could provide a ready source of hybridisation probes for standard Southern blot analysis simply by isolating bands from gels to detect RFLPs. However, some polymorphic RAPD bands are not suitable as RFLP probes because of hybridisation to repetitive DNA sequences (3, 19).

Developing Genetic Markers Linked to a Trait in Question

One of the most widely used applications of the RAPD technique is the identification of markers linked to traits of interest without the necessity for mapping the entire genome. Martin et al. (20) have described an efficient method based on the RAPD technique to isolate DNA segments linked to certain traits. This approach based on near-isogenic lines (NILs) is accomplished by repeatedly backcrossing a line carrying a gene of interest (donor parent) to a cultivated line having otherwise desirable characteristics (recurrent parent). The introgression of the target gene produces a line with a small segment of donor parent genome in a genetic backround, which is almost exclusively from the recurrent parent. Thus, markers that show polymorphisms between these 2 lines are likely to be linked to the gene of interest. RAPD analysis of NILs has been successful in identifying markers linked to disease resistance genes in tomato (*Lycopersicon sp.*) (20), in lettuce (*Lactuca sp.*) (21) and in common bean (*Phaseolus vulgaris*) (22). Klein-Lankhorst et al. (23) identified chromosome specific RAPD markers in tomato by screening a *Lycopersicon esculentum* substitution line.

The main limitation of NILs is the availability of a pair of NILs and the long generation time for many species. In addition, several regions of the donor genome are likely to be co-introgressed into the near-isogenic line. This results in the identification of some polymorphic markers that are unlinked to the trait of interest (24).

Another RAPD-based approach is bulked segregant analysis (BSA) described by Michelmore et al. (25). Arnheim et al. (26) previously outlined a genome pooling strategy for RFLP markers that are not in linkage equilibrium to the targeted region of the genome. BSA involves bulking DNA samples of individuals segregating in a single population. Bulked DNA samples from individuals that have the target trait or gene are compared to bulked DNA samples of individuals lacking the trait. Markers that are polymorphic between the pools will be genetically linked to loci determining the trait used to construct the pool. The technique has been successfully used to identify RAPD markers linked to disease resistance genes in lettuce (25) and barley (27). The pooling strategy has also been useful in the identification of sex-linked RAPD markers in pistachio (*Pistachio vera*) (28) and a dioecious angiosperm *Silene latifola* (29). RAPD markers that are linked to a chromosome (30) or specific region of a chromosome (31) were developed using the pooled DNA method. RAPD analysis of pooled DNA samples of NILs has further increased the efficiency of gene tracking (27).

Population and Evolutionary Genetics

The advances in DNA techniques have had a great impact in addressing problems in many aspects of biology. Application of DNA-based approaches to population genetic studies has been limited, probably due to the need for large samples of individuals from each population to provide an accurate estimate of allele and genotype frequencies. The relatively high cost, the requirement for sophisticated equipment and well-trained personnel, and low speed are other limiting factors in population genetic studies. The RAPD technique has received a great deal of

attention from population geneticists (32) because of its simplicity and rapidity in revealing DNA-level genetic variation, and therefore has been praised as the DNA equivalent of allozyme electrophoresis (33). A major drawback of RAPD markers in population genetic studies of outbreeding organisms is that they are dominant. Thus gene frequency estimates for such loci are necessarily less accurate than those obtained with codominant markers such as allozymes and RFLPs. Lynch and Milligan (34) suggested that 2 to 10 times more individuals need to be sampled for dominant markers to achieve the same degree of statistical power as codominant markers such as allozymes and RFLPs. The assumption of homology between bands of apparently the same molecular weight from the same primer is potentially another problem for RAPD surveys. Homology between comigrating bands in different individuals is a good assumption when individuals are from the same population. This may not be true when individuals belong to different species or widely divergent populations (35-37). Because the chance of comigrating bands being homologous becomes less as populations diverge, it was suggested (19, 35) that RAPD analysis gives more accurate estimates between closely related populations and less accurate estimates for distantly related populations. RAPD data has been used for phlogenetic studies and generally supported existing taxonomies based on morphology, isozymes and RFLPs. Some contradictions were found, especially among more distantly related plant species (35).

The most recent studies on the utility of RAPD markers in the phylogeny of cichlid fishes (38) and the genus *Xiphophorus* (39) gave support to classical hypotheses of their phylogenetic relationships. RAPD markers have been successfully used to detect genetic variability in *Gliricidia* (40), mosquito species and populations (41), closely related species of black *Aspergilli* (42), cocoa (43), medfly (44) and parasitic protozoa (45). The technique has also been used to study genetic variation in several fish species. Bardakci and Skibinski (46) and Naish et al. (47) used RAPD markers to discriminate between commercially important tilapia species, subspecies and strains of tilapia. RAPD markers were also generated for several tropical fish species representing 7 families (48). Furthermore, RAPD analysis revealed high levels of genetic variation among individuals from the same broodstock of sea bass (*Dicentrarchus labrax*) (37). Finally, 721 strain-specific RAPD markers were identified in 2 laboratory strains of zebrafish (49).

RAPD markers are more suitable for clonal organisms than sexually reproducing organisms. As they breed asexually, a polymorphic fragment among individuals can be used to determine clonal identity (3, 33). Species-specific markers were developed in species and strains of micro-organism (4, 33, 50). Clone-specific markers have been identified in hydroids (51) and in fungal mycelia (52).

Although the value of RAPD markers in taxonomic and phylogenetic studies is not very clear, there is no doubt that these markers can be used for diagnostic purposes. RAPD markers unique to individuals from 1 species within a genus will be species-specific (inter-specific).

Similarly, genus-specific markers can be generated if the fragment is a unique polymorphism to individuals belonging to a certain genus.

Species-specific markers can be used in inter-specific gene flow and hybrid identification. Similarly, population-specific markers will be useful in identification of hybrid populations (51). Inter-specific gene flow was shown between 2 iris species, *Iris hexagona* and *I. vulva* using species-specific RAPD markers (53). F_1 hybrids from different inbred lines of maize were identified using AP-PCR (54).

RAPD polymorphism detected among individuals within a given species has been used to determine paternity and kinship relationships in large progenies of dragonfly (51). In this study, *Anax parthenope* males guard ovipositing females. It was suggested that the male might guard a female in order to assure a subsequent mating rather than immediate fertilisation. RAPD analysis of several unrelated males, the guarding male, the guarded female and offspring clutches demonstrated that the guarding male was the father. A specific RAPD marker was found in the guarding male and in the offspring but was rare in the population as a whole. Parentage analyses with RAPD markers are based on the presence of diagnostic markers (present in only 1 of the putative parents) in the offspring. A high frequency of non-parental RAPD bands has been reported in primate pedigrees (55). However, Scott et al. (56) found much lower frequencies of non-parental RAPD bands in beetles (*Nicrophorus tomentosus*) and strawberries (*Fragaria vesca*).

Plant and Animal Breeding

Genetic improvement of animals is limited by the fact that most traits of economic importance are polygenic in nature and are influenced by a variety of external (environmental) and internal factors. Such traits are termed quantitative traits and polygenic loci involved in their expression are termed quantitative trait loci (QTL). To date, RFLP markers have been used as genetic markers to monitor the transmission of useful QTL alleles from generation to generation in the course of breeding programs (57, 58). As stated above, the RAPD technique enabled the development of large numbers of genetic markers more efficiently than RFLP-based methods that have been used to construct saturated mapping of complex genomes. These markers can be used in monitoring these loci during introgression and selection programs. Markers linked to simple commercially important genetic traits such as disease resistance genes can also be identified from natural resources and introgressed into domestic strains or varieties.

The ability of the RAPD technique to reveal intra-specific variation can be used in screening for the degree of inbreeding in commercial plant and animal species to prevent an increase in the frequency of deleterious recessive alleles in populations.

Polymorphic RAPD markers transformed to SCAR markers can be more advantageous in commercial breeding programs if a quick plus/minus assay can be developed to detect the presence /absence of the product (6).

Reproducibility of RAPD Markers

Although the RAPD method is relatively fast, cheap and easy to perform in comparison with other methods that have been used as DNA markers, the issue of reproducibility has been of much concern since the publication of the technique. In fact, ordinary PCR is also sensitive to changes in reaction conditions, but the RAPD reaction is far more sensitive than conventional PCR because of the length of a single and arbitrary primer used to amplify anonymous regions of a given genome. This reproducibility problem is usually the case for bands with lower intensity. The reason for bands with high or lower intensity is still not known. Perhaps some primers do not perfectly match the priming sequence, amplification in some cycles might not occur, and therefore bands remain fainter. The chance of these kinds of bands being sensitive to reaction conditions of course would be higher than those with higher intensity amplified with primers perfectly matching the priming sites. The most important factor for reproducibility of the RAPD profile has been found to be the result of inadequately prepared template DNA (59). Differences between the template DNA concentration of 2 individuals' DNA samples result in the loss or gain of some bands (60).

Since RAPD amplification is directed with a single, arbitrary and short oligonucleotide primer, DNA from virtually from all sources is amenable to amplification. Therefore, DNA from the genome in question may include contaminant DNA from infections and parasites in the material from which the DNA has been isolated. Special care is needed for keeping out the DNA to be amplified from other sources of DNA.

Concluding remarks

RAPD markers have found a wide range of applications in gene mapping, population genetics, molecular evolutionary genetics and plant and animal breeding. This is mainly due to the speed, cost and efficiency of the RAPD technique to generate large numbers of markers in a short period compared with previous methods. Therefore, RAPD technique can be performed in a moderate laboratory for most of its applications. Despite the reproducibility problem, the RAPD method will probably be important as long as other DNA-based techniques remain unavailable in terms of cost, time and labour.

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