Aquaculture Genome Technologies
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Chapter 4
Amplified Fragment Length Polymorphism (AFLP)

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Multilocus DNA fingerprinting technologies based on polymerase chain reaction (PCR) are of enormous value for the study of genetic variations. These fingerprinting technologies, such as random amplified polymorphic DNA (RAPD) (Chapter 3 of this book, as well as Williams et al. 1990, Welsh and McClelland 1990) and amplified fragment length polymorphism (AFLP) (Vos et al. 1995), allow rapid generation of large amounts of genetic data. Genetic fingerprinting using these technologies does not require prior knowledge, making them "ready to be used" technologies for any species without previous genetic information. The fingerprints may be used as a tool to identity a specific DNA sample or to assess the relatedness between samples. Conserved common bands define relatedness, while polymorphic bands define differentiation in phylogenetic and population genetic analyses.

AFLP technology combines the advantages of restriction enzyme fingerprinting using restriction fragment length polymorphism (RFLP) and those of PCR-based fingerprinting using RAPD. It is based on the selective amplification of a subset of genomic restriction fragments using PCR. DNA is digested with restriction enzymes, and double-stranded DNA adaptors are ligated to the ends of the DNA fragments to generate primer-binding sites for PCR amplification. The sequence of the adaptors and the adjacent restriction site serve as primer binding sites for subsequent amplification of the restriction fragments by PCR. Selective nucleotides extending into the restriction sites are added to the 3′ ends of the PCR primers in such a way that only a subset of the restriction fragments is recognized. Only restriction fragments in which the nucleotides flanking the restriction site match the selective nucleotides will be amplified. The subset of amplified fragments is then analyzed by denaturing polyacrylamide gel electrophoresis to generate the fingerprints.

To fully appreciate the advantages and applications of AFLP, this chapter is written to describe the course of the technology development in relation to several other existing technologies, the procedures and principles, the molecular basis of polymorphism, and the potential power for genetic analysis using AFLP. As detailed below, AFLP is a technology that provides robustness, reliability, and efficiency. Its simultaneous analysis of hundreds of loci using only a single primer combination offers a robust power of differentiation. AFLP is also advantageous because markers are inherited in Mendelian fashion; it does not require prior genetic information and is therefore adaptable to genetic analysis of any species. AFLP truly provides the multilocus coverage and potential for genome-wide coverage for analysis of genetic variations. For comparisons of AFLP with other marker systems, readers are referred to other chapters of this book and a review on applications of DNA markers in fisheries and aquaculture (Liu and Cordes 2004).

Background of AFLP Technology

Genomic DNA must first be cut into small pieces for molecular analysis. Geneticists have a limited capacity for making direct analysis of large segments of DNA. Although chromosomes or chromosome segments can be directly analyzed through a special gel electrophoresis known as pulse field electrophoresis, little genetic information can be obtained from such analysis concerning genetic variation. In contrast, resolution of differentiation can be drastically increased when DNA is cut into small segments.

Restriction enzymes are site-specific "molecular scissors" for DNA (for details, see Chapter 2). They recognize specific sequences 4–8 base pairs (bp) long. In a restriction digest reaction, a restriction enzyme is mixed with genomic DNA and incubated under specific buffer and temperature conditions as required by the restriction enzyme. Within usually 1 hour of incubation, a restriction enzyme "searches" through the entire DNA lengths for its specific 4–8 bp recognition sequences. The genomic DNA is then "cut" by the enzyme whenever the proper recognition sequences are found. The cutting frequency of any restriction enzyme is directly related to the length of its recognition sequences. On average, a restriction enzyme with 4-bp recognition sequences should cut DNA once every 256 bp ($1/4^4$); a restriction enzyme with 6-bp recognition sequences should cut DNA once every 4,096 bp ($1/4^6$); and a restriction enzyme with 8-bp recognition sequences should cut DNA once approximately every 65,000 bp ($1/4^8$). These cutting frequencies should be considered when choosing restriction enzymes. This is particularly true for AFLP, which will be discussed later in the chapter. Assuming a fish genome of one billion base pairs (10^9 bp), a 4-bp cutter will digest the genome into approximately 4 million segments; a 6-bp cutter will digest the genome into about a quarter-million segments; and a 8-bp cutter will digest the genome into just 15,000 segments. In addition to the length of recognition sequences, the genomic content also affects the cutting frequency of a restriction enzyme. For instance, AT-rich genomes are in favor of restriction enzymes with AT-rich recognition sequences, while GC-rich genomes are in favor of restriction enzymes with GC-rich recognition sequences.

Molecular Basis of Genetic Variation Detected in AFLP

In the long history of evolution, genomes have evolved in each species to have a fixed number of chromosomes whose shape and sizes are constant. The number of genes and gene locations on each chromosome are also relatively constant so that genetic linkage maps can be constructed. Such structural and organizational order is maintained by accurate inheritance of genes from generation to generation. However, just as constant as the inheritance of genes and traits from parents to progenies, mutations are also constant events. Mutations can happen spontaneously or under induction of adverse environmental cues such as radiation, UV light, or chemical mutagens. Spontaneous mutations occur at a very low rate of 1×10^{-5}–2×10^{-6} per gene per generation. Assuming an average gene size of 2,000 bp, this low spontaneous mutation rate translates into only 1 to 5 base mutations throughout the entire genome of one billion base pairs per generation. However, through the long process of evolution, many mutations have accumulated. The basic idea behind genetic analysis lies in using

the accumulation of different mutations in reproductive isolated populations and individuals.

Mutations are random events and can happen in any part of the genome, although mutation hot spots are often reported. As a result, mutations are accumulated in evolution more often in noncoding regions. First, because the nonprotein coding regions of the genome account for the vast majority of the entire genome, most mutations occur by chance in these regions; second, nature has placed great selection pressure for advantageous mutations and neutral mutations, but against deteriorating mutations inside the protein coding sequences. In the coding regions, silent mutations (single base substitutions that do not change the amino acid sequence) are the most predominant. Mutations can be categorized at the molecular level as having been caused by deletions, insertions, inversions, base substitutions, and rearrangements. Deletions are losses of, while insertions are additions of, DNA bases of variable sizes ranging from a single base to long stretches of DNA. Base substitutions are changes of a specific base to any other of the three bases. For instance, base A can be mutated to any of C, G, or T. Mutations from purines (A and G) to purines or from pyrimidines (C and T) to pyrimidines are called transitions; mutations from purines to pyrimidines or vice versa are called transversions. Transitions are the most frequent mutations because the chemical reactions involved in such mutations are more likely to occur. In relation to molecular analysis, deletions and insertions are expected to cause changes of fragment lengths of at least one base pair while base substitutions generally do not affect fragment sizes unless the base substitutions cause the gain or loss of restriction sites. A base substitution within the restriction enzyme recognition site causes loss of the restriction site, and therefore, leads to loss of the restriction fragment. In contrast, a single base change may lead to the generation of a new cutting site for the restriction enzyme. For instance, the recognition sequences for restriction enzyme *Eco* RI are GAATTC. If the original sequence was GgATTC, a single base change of the second G into A would generate a new restriction site for *Eco* RI and the production of an additional restriction fragment. Rearrangements do not affect fragment lengths unless the rearranged fragments contain restriction enzyme sites.

Other Genetic Variations at the Molecular Level Affecting AFLP Profiles

In addition to the mechanisms of mutations mentioned above, several other highly mutable sequences should also be noted because they may account for a significant portion of polymorphism as revealed by AFLP analysis. The first is microsatellite sequences. As detailed in Chapter 5, microsatellites are simple sequence repeats of 1–6 bp. High levels of mutation rates can happen at microsatellite loci. In some cases, mutation rates can be as high as 0.2% per locus per generation (Crawford and Cuthbertson 1996, Levinson and Gutman 1987). Such a high mutation rate is believed to be caused by slippage of DNA polymerase with the repeated microsatellite sequences, leading to microsatellite expansion or contraction. The differences in repeat numbers of microsatellite sequences cause changes in fragment lengths. In a sense, this type of mutation is a special form of insertions or deletions. Due to large numbers of microsatellite loci existing in fish and their high mutation rates, their contribution to the overall polymorphism of genomes should

not be neglected regardless of the approach used for genetic variation analysis. Secondly, unequal crossing over of minisatellite and satellite sequences may also contribute to a significant level of genetic variations among genomes.

Molecular Analysis Related to Development of AFLP Technology

AFLP methodology was developed by using and combining several of the technological advances that ushered in the genomics era; it was based on RFLP and PCR reactions resolved by sequencing gel electrophoresis.

The need for a sensitive, efficient approach to analyzing genetic variation on a genomic scale was evident early on during the genomics revolution. PCR appeared to offer the power necessary for such an approach and was used in the development of RAPD in 1990 (Williams et al. 1990, Welsh and McClelland 1990). However, RAPD's usefulness is limited by its low reproducibility because of low annealing temperatures necessitated by using short, arbitrary primers during PCR.

AFLP combines the strengths of RFLP and RAPD. It is a PCR-based approach requiring only a small amount of starting DNA. It does not require any prior genetic information or probes, and it overcomes the problem of low reproducibility inherent to RAPD. AFLP is capable of producing far greater numbers of polymorphic bands than RAPD in a single analysis, significantly reducing costs and making possible the genetic analysis of closely related populations. The use of AFLP markers in genetic linkage mapping (Meksem et al. 1995, Cho et al. 1996, Mackill et al. 1996) and analyses of genetic resource pools (Folkertsma et al. 1996, Keim et al. 1997) has facilitated progress that would otherwise take a much longer time using other technologies. It is particularly well adapted for stock identification because of the robust nature of its analysis. The other advantage of AFLP is its ability to reveal genetic conservation as well as genetic variation. In this regard, it is superior to microsatellites for applications in stock identification. Microsatellites often possess large numbers of alleles, too many to obtain a clear picture with small samples. Identification of stocks using microsatellites, therefore, would require large sample sizes. For instance, if 10 fish are analyzed, each of the 10 fish may exhibit distinct genotypes at a few microsatellite loci, making it difficult to determine relatedness without any commonly conserved genotypes. In closely related populations, AFLP can readily reveal commonly shared bands that define the common roots in a phylogenetic tree and polymorphic bands that define branches in the phylogenetic tree.

The Procedures and Principles of AFLP Analysis

Genetic variations are widely spread among genomes of even very closely related individuals. The problem is how to reveal the very minor differences among genomes. In principle, AFLP can be viewed as a multilocus or genome-wide RFLP analysis (Figure 4.1). The technique starts with restriction digestion of genomic DNA using two restriction enzymes, most often, *Eco* RI and *Mse* I. *Eco* RI recognizes a 6-bp sequence of GAATTC, and *Mse* I recognizes a 4-bp sequence of AATT. For a genome of one billion base pairs, *Eco* RI digestion should produce about 250,000 fragments,

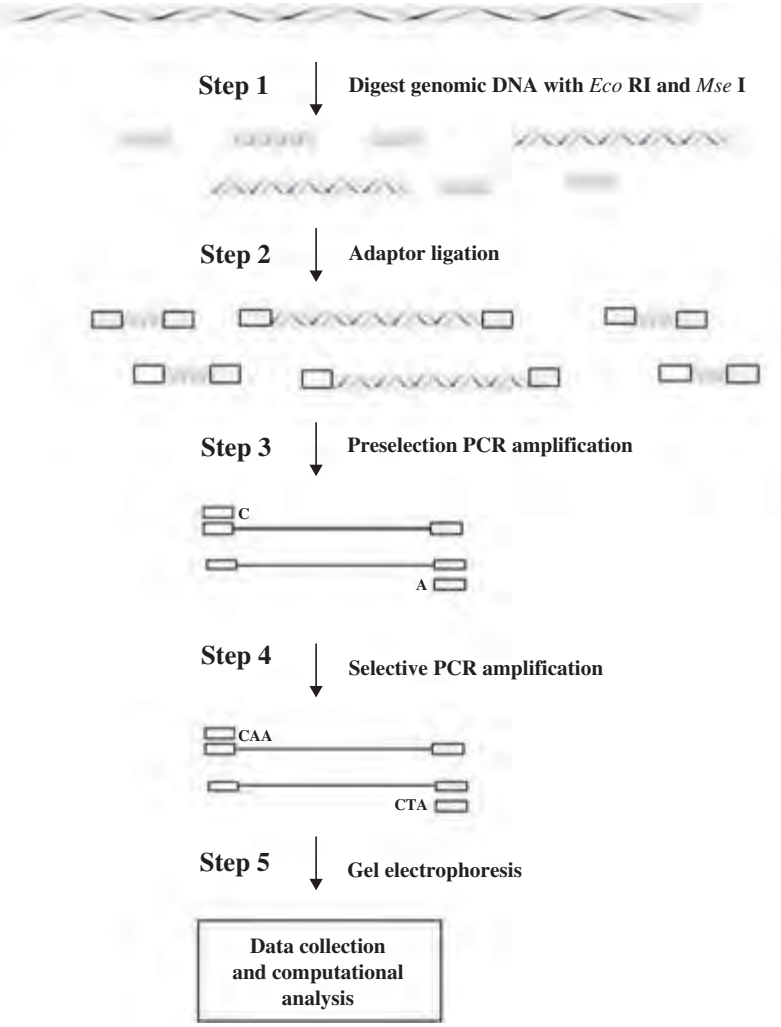

Figure 4.1. Schematic presentation of AFLP analysis. Step 1, genomic DNA is digested by *Eco*RI and *Mse*I into many segments of various sizes. For a genome of 10^9 bp, you expect ~250,000 *Eco*RI fragments (10^9/4,000) and 4 million *Mse*I fragments (10^9/256); step 2, adaptors are ligated to the ends of the DNA fragments. The majority of fragments should be *Mse*I-*Mse*I fragments, some *Eco*RI-*Mse*I fragments, few, if any, *Eco*RI-*Eco*RI fragments. This step is to add adaptors with known sequences to create PCR primer binding sites. For a genome of 10^9 bp, you expect 2× 250,000 *Eco*RI-*Mse*I fragments. Note that the sequence of *Eco*RI adaptor (open box) is different from that of the *Mse*I adaptor (dotted box); step 3, preselection amplification of a subset of the restriction fragments by adding an extra arbitrary base at the 3′ end of the PCR primers, which leads to 1/16 fragments to be amplified; step 4, selective amplification of a subset of the restriction fragments by adding three extra arbitrary bases at the 3′ end of the PCR primers, which leads to a subset of *Eco*RI-*Mse*I fragments (1/4,096) to be amplified; step 5, PCR products are resolved on a sequencing gel.

and *Mse* I digestion should produce 4 million fragments. Because the 4-bp cutter *Mse* I cuts DNA at a frequency 16 times greater than *Eco* RI, essentially all *Eco* RI fragments should be further digested by *Mse* I. The double enzyme digest would produce approximately 500,000 *Eco* RI-*Mse* I fragments (one original *Eco* RI fragment is now cleaved by many *Mse* I sites leaving both ends as *Eco* RI-*Mse* I fragments), and about 4 million *Mse* I-*Mse* I fragments.

The second step of AFLP analysis is to add adaptors on both ends of digested DNA fragments. The *Eco* RI-*Mse* I fragments must be amplified by PCR to be detected because they represent a small amount of DNA. However, there is no sequence information about these fragments. The first challenge is to "create" two stretches of known sequences in each of these fragments for PCR. This can be achieved simply by connecting a short segment of DNA with known sequences on the *Eco* RI end and a short segment of DNA with different known sequences on the *Mse* I end. These short segments of DNA with known sequences are called *Eco* RI adaptors and *Mse* I adaptors. They are called adaptors because they harbor specific end sequences allowing them each to be perfectly paired and ligated to the double digested *Eco* RI-*Mse* I fragments. After ligation, each *Eco* RI-*Mse* I fragment now harbors known sequences on both ends allowing PCR amplification of these segments by using primers with the same sequences as the adaptors.

The third step of AFLP is the preselection PCR amplification. In the 500,000 *Eco* RI-*Mse* I fragment pool, one can imagine that many of these *Eco* RI-*Mse* I fragments must exhibit size difference or length polymorphism even between two highly related individuals. However, 500,000 fragments are too many to be resolved in any kind of gel electrophoresis. This demands that somehow the 500,000 bands must be reduced approximately 2,000 times to reach the resolvable goal of a couple hundred bands. Vos and others (1995) intelligently met this challenge by adding additional arbitrary bases at the 3′ end of the PCR primers. As each extra arbitrary base is added, the PCR primer can match to only 1/4 subset of the fragments because at each base of DNA, and there are four possibilities: A, C, G, or T. When a given base is added to the 3′ end of the PCR primer, only 1/4 of the total fragments are amplifiable. When a single base is added to the 3′ end of both PCR primers, only a subset of 1/16 of the total fragments will be amplifiable. When two additional bases are added to each of the PCR primers, the reduction power is now 256 (16×16). When three additional bases are added to each 3′ end of the two PCR primers, the reduction power now is 4,096 (64×64). Now with a reduction power of 4,096, the original 500,000 fragments should become about 100 bands. These bands can then be visualized after electrophoresis. The preselective PCR first reduces the *Eco* RI-*Mse* I fragments to a subset containing 1/16 of the original fragments. The selective PCR further reduces the number of bands by amplifying only a subset of the preselective PCR products. AFLP chooses to analyze only the *Eco* RI-*Mse* I fragments. This is achieved by labeling only *Eco* RI primers. Since the *Mse* I primer is not labeled, none of the amplified *Mse* I-*Mse* I fragments are visible during electrophoresis.

The Power of AFLP Analysis

It is possible to scan the entire genome for examination of all 500,000 *Eco* RI-*Mse* I fragments by use of all possible combinations of the selective bases. That would take 64 *Eco* RI primers and 64 *Mse* I primers or 4,096 primer combinations. However, it is

probably never necessary to perform such an exhaustive analysis. Since more than 100 loci can be analyzed by a single primer combination, a few primer combinations should display thousands of fingerprints. For genetic resource analysis, the number of primer combinations required for construction of phylogenetic trees/dendrograms depends on the level of polymorphism in the populations, but probably will take no more than 10 primer combinations. However, dense genetic maps can be constructed by using a large number of primer combinations.

The potential power of AFLP in the study of genetic variation is enormous. In principle, any combination of a 6-bp cutter with a 4-bp cutter in the first step can be used to determine potential fragment length polymorphism. In the above tour through the procedures, *Eco* RI and *Mse* I were used as restriction enzymes to examine the 500,000 *Eco* RI-*Mse* I fragments. Theoretically, 4,096 primer combinations compose a complete genome-wide scan of the fragment length polymorphism using the two restriction enzymes. Because hundreds of restriction endonucleases are commercially available, the total power of AFLP for analysis of genetic variation can never be exhausted.

Molecular Basis of AFLP Polymorphism

AFLP analysis is an advanced form of RFLP. Therefore, the molecular basis for RFLP and AFLP are the same. First, any deletions and/or insertions between the two restriction enzymes (e.g., between *Eco* RI and *Mse* I in the above example) will cause shifts in fragment sizes. Second, base substitution at the restriction sites will lead to loss of restriction sites and thus a size change. However, only base substitutions in all *Eco* RI sites and 1/8 of *Mse* I sites are detected by AFLP since only the *Eco* RI primer is labeled and AFLP is designed to analyze only the *Eco* RI-*Mse* I fragments. Third, base substitutions leading to new restriction sites may also produce AFLP. Once again, gaining *Eco* RI sites always leads to production of AFLP, gaining *Mse* I sites must be within the *Eco* RI-*Mse* I fragments to produce new AFLP. In addition to the common mechanisms involved in polymorphism of RFLP and AFLP, AFLP also scans for any base substitutions at the first three bases immediately after the two restriction sites. Considering large numbers of restriction sites for the two enzymes (250,000 *Eco* RI sites and 500,000 *Mse* I sites immediately next to *Eco* RI sites), a complete AFLP scan would also examine more than 2 million bases immediately adjacent to the restriction sites.

Inheritance of AFLP Markers

AFLP markers are inherited in a Mendelian fashion as dominant markers. Similar to the traditional meaning of dominance in genetics, one dose is enough to determine phenotype (hence the band patterns). Dominant markers provide relatively less genetic information since homozygous and heterozygous individuals cannot be differentiated; they each produce a band at the locus though band intensities may vary depending on allele numbers. Although double alleles often produce double the amount of PCR products, homozygous alleles and heterozygous alleles cannot be distinguished

with certainty. As detailed below, caution must be exercised when scoring AFLP markers as codominant markers.

Major Strengths and Weaknesses of AFLP Markers

Several major strengths make AFLP the markers of choice in certain situations. The first strength is the procedure's requirement of no prior molecular information for application to the species of interest. This is particularly useful for aquaculture species where there is often no molecular information available. Second, AFLP is highly robust allowing the generation of a large number of polymorphic markers with limited efforts and resources. Third, when the robustness is coupled to the availability of many primer combinations, AFLP is a very powerful marker system for genomic differentiation. With such a technique, very minor genomic differences can be readily revealed. Fourth, as long as PCR primers are used, stringent annealing temperatures can be used for high reproducibility. This is definitely a significant improvement over the less reliable RAPD procedure (see Chapter 3). Finally, AFLP markers are relatively economical because each primer combination can often produce many polymorphic markers. Even though AFLP kits are relatively expensive, the cost on a per polymorphic marker basis is low. The major weakness of AFLP markers is their dominant nature of inheritance. Genetic information is limited with dominant markers because essentially only one allele is scored; at the same time, since the true alternative allele is scored as a different locus, AFLP also inflates the number of loci under study. As dominant markers, information transfer across laboratories is difficult. In addition, AFLP is more technically demanding, requiring special equipment such as automated DNA sequencers for optimal operations.

Genotyping AFLP Gels

AFLP markers are inherited as dominant markers. Because of the dominance nature of AFLP, they are scored as presence/absence type of markers in genotyping. Each band is treated as a locus (not an allele). Although the true alternative allele must be somewhere in the gel with a different fragment size, there is practically no way to know the exact location. In some cases, complementary phases of bands are observed, indicating they may be the alternative alleles of the locus, but in the absence of molecular evidence, each band is still scored as a separate locus. Therefore, the total number of AFLP loci under analysis is inflated about twofold because all of the alleles are treated as loci. Under this treatment, the presence of one band is treated as one allele at the locus, and the absence of the band is treated as the alternative allele.

In strictly controlled mating systems, it is possible to score AFLP markers as codominant markers. In such cases, the scoring is based not only on length polymorphism, but also on intensity polymorphism. The rationale is that two alleles in homozygotes should produce twice the amount of PCR products as that produced from a single allele in heterozygotes. As a matter of fact, computer software is available for quantitative scoring of intensity polymorphism. AFLP-Quantar™ Pro marketed by Keygene Products B.V. in the Netherlands is an example. In spite of its success, I would like to urge caution in

the use of intensity polymorphism, simply because of the nonlinear nature of PCR at high rounds of cycles. For identification of stocks and population analysis, use of intensity polymorphism should be discouraged because scoring may be extremely difficult with samples from random mating populations.

The term "informative AFLP" is used to indicate only polymorphic AFLP bands in genetic linkage mapping analysis. In the case of linkage mapping, only polymorphic bands are expected to segregate and thus provide genetic linkage information. Therefore, commonly shared nonpolymorphic bands are not scored. For population studies, all of the bands are actually informative. In fact, the commonly shared bands are extremely important since they define the common ancestor or roots for dendrogram grouping. The shared bands are used to calculate Nei's similarity F values (Nei and Li 1979). Of course, the polymorphic bands provide information about differentiation or branches for dendrogram grouping. Therefore, all AFLP bands need to be scored for population genetic analysis.

Application of AFLP for Aquaculture Genome Research

AFLP is well adapted for many types of genetic analysis such as:

- molecular systematics
- analysis of population structures
- migration
- hybrid identification
- strain identification
- parentage identification
- genetic resource analysis
- genetic diversity
- reproduction contribution
- endangered species protection
- molecular ecology
- marker-assisted selection
- genome mapping

Different authors discuss the applications of AFLP later in this book concerning analysis of data and choice of models and software for population genetic analysis, therefore, here I will only briefly discuss its application in fish population studies with an emphasis on genetic linkage analysis using AFLP.

Despite the advantages of AFLP, published literature on its application for the analysis of genetic variation of fish populations is still limited (Seki et al. 1999, Jorde et al. 1999, Sun et al. 1999, Cardoso et al. 2000, Chong et al. 2000, Kai et al. 2002, Mickett et al. 2003, Whitehead et al. 2003, Mock et al. 2004, Campbell and Bernatchez 2004, Simmons et al. 2006). Many AFLP analyses in fish so far have been limited to genetic linkage analysis (Liu et al. 1998, 1999; Kocher et al. 1998; Griffiths and Orr 1999; Agresti et al. 2000; Robison et al. 2001; Rogers et al. 2001; Liu et al. 2003; Li et al. 2003; William et al. 2005), and analysis of parental genetic contribution involving interspecific hybridization (Young et al. 2001) and meiogynogenesis (Felip et al. 2000). In a recent study of the black rockfish (*Sebastes inermis*), Kai and others (2002) used AFLP to distinguish three-color morphotypes, in which diagnostic AFLP loci were identified as well as loci with significant frequency differences. In such reproductive isolated populations, it is likely that "fixed markers" of AFLP can be identified to serve as diagnostic markers. Fixed markers are associated most often with relatively less migratory, reproductive

Table 4.1. Some examples of the use of AFLP markers for the construction of linkage maps in aquaculture or fish species.

Species	Common name	Reference
Oncorhynchus mykiss	Rainbow trout	Young et al. 1998
Salmo salar	Atlantic salmon	Moen et al. 2004
Salvelinus alpinus	Arctic char	Woram et al. 2004
Oreochromis sp.	Tilapia	Kocher et al. 1998 Agresti et al. 2000
Ictalurus punctatus	Channel catfish	Liu et al. 2003
Clarias macrocephalus	Walking catfish	Poompuang and Na-Nakorn 2005
Paralichthys olivaceus	Japanese flounder	Coimbra et al. 2003
Plecoglossus altivelis	Ayu	Watanabe et al. 2004
Penaeus monodon	Black tiger shrimp	Wilson et al. 2002
Penaeus japonicus	Kuruma prawn	Li et al. 2003
Penaeus vannamei	White shrimp	Pérez et al. 2004
Penaeus chinensis	Chinese shrimp	Li et al. in press
Crassostrea virginica	Eastern oyster	Yu and Guo 2003
Chlamys farreri	Zhikong scallop	Li et al. 2005
Haliotis discus hannae	Pacific abalone	Liu et al. 2006

isolated populations (Kucuktas et al. 2002). With highly migratory fish species, fixed markers may not be available. However, distinct populations are readily differentiated by difference in allele frequencies. For instance, Chong and others (2000) used AFLP for the analysis of five geographical populations of Malaysian river catfish (*Mystus nemurus*) and found that AFLP was more efficient for the differentiation of subpopulations and for the identification of genotypes within the populations than RAPD, although similar clusters of the populations were concluded with either analysis.

AFLP can be used effectively for genetic linkage mapping. As a matter of fact, many genetic linkage maps have been constructed using AFLP markers among aquaculture species, as summarized in Table 4.1. However, AFLP as a dominant marker, lacks the ability to be transferred across species borders, and it is difficult to transfer data among laboratories. As a result of its high efficiency, it is well suited for association analysis of traits with markers. However, after initial identification of associated AFLPs, it is highly recommended that such AFLPs be converted to sequence characterized amplified region (SCAR) markers.

Conclusion

AFLP analysis is a robust, multilocus PCR-based DNA fingerprinting technique that provides the most efficient, reliable, and economical analysis of population genetics. AFLPs are nuclear DNA markers inherited in Mendelian fashion, in contrast to environmental markers and mitochondrial DNA markers. As compared to other nuclear markers such as RFLP and RAPD, AFLPs provide a much greater level of polymorphism and a much wider genomic coverage. AFLP is probably also superior to microsatellites for population genetic studies because of its ability to display hundreds

of loci simultaneously. However, AFLP markers are inherited as dominant markers. Caution should be exercised for transfer of information across laboratories. The need for special equipment such as sequencers may limit its wide application. These disadvantages can be compensated for by the robustness of the multilocus AFLP analysis, which not only provides high levels of polymorphism, but also provides a great level of band sharing, which is required to establish relatedness among populations. Most importantly, AFLP (and also RAPD) analysis does not require any previous knowledge and thus is suitable to population genetic analysis of any species. Because of these advantages, the application of AFLP in fish population genetic studies is increasing. As time goes on, its application in the studies of fish population genetics is likely to widen. For genome research, use of AFLP markers may provide a rapid shortcut for the assessment of markers linked to certain traits, but its coupled uses with codominant markers such as microsatellites should be beneficial. In a well-defined closed mating system involving limited number of founders, genetic mapping using AFLP can add much greater resolution to framework linkage maps made with microsatellites.

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