AMPLIFIED FRAGMENT LENGTH POLYMORPHISMS (AFLP's)

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Resumen. AFLP es una técnica que combina PCR y análisis de fragmentos de restricción para detectar polimorfismos debidos a cambios en, o cerca de sitios de enzimas de restricción. La técnica detecta múltiples loci polimórficos a través del genoma. Son útiles para generar huellas genéticas o para mapeo. Las ventajas que ofrece el método de AFLP's son confiabilidad y consistencia, debido a las condiciones de alta estringencia bajo las cuales se llevan a cabo las reacciones de PCR y la habilidad de detectar rápidamente muchos loci polimórficos.

Palabras claves: AFLP, PCR, marcador molecular.

Summary. AFLP is a combination restriction fragment/PCR molecular marker technique which detects polymorphisms due to changes at or in the vicinity of restriction enzyme sites. The technique detects multiple polymorphic loci throughout the genome and may be used for fingerprinting and mapping purposes. The main advantages of the method are the consistency and reliability of the technique due to stringent PCR conditions and the ability to rapidly detect many polymorphic loci.

Key words: AFLP, PCR, molecular marker.

One of the newest molecular marker techniques is that of AFLP or Amplified Fragment Length Polymorphism. This methodology was developed by Dr. Marc Zabeau at the Dutch company Keygene and first published in 1995 by Vos *et al.* (1995). The method is based on selective restriction fragment amplification using the polymerase chain reaction (PCR). Polymorphisms detected are in fact restriction fragment length polymorphisms (RFLP's).

In the normal RFLP method, specific probes are used in a hybridization reaction in order to detect sequences homologous to the probe amongst the thousands of fragments produced when genomic DNA is subjected to digestion with a restriction enzyme. Polymorphisms occur when mutations affect the position of a restriction enzyme site in one individual as compared to another. The resulting restriction fragments will be of different sizes and bands migrating differently during electrophoresis will be observed following the hybridization analysis. In the case of AFLP's, a 'selection' or 'subset' of restriction fragments from a ge-

nomic digest, which can be easily analyzed, are obtained.

The technique consists in digesting to completion genomic DNA using two restriction enzymes: a hexacutter recognizing a 6 base pair sequence and a tetracutter recognizing a 4 base pair sequence. The enzymes most often used are EcoRI and MseI. Adapter molecules specific for the cohesive ends of the restriction sites are then ligated to the digested fragments to produce fragments of genomic DNA flanked by specific adapter sequences. Subsequently, two PCR amplification reactions are carried out. The oligonucleotides used for the 1st amplification reaction correspond to the sequences of the adapter molecules plus one extra nucleotide internal to the fragment. This is diagrammed in figure 1a. The result of this amplification reaction is that only fragments with the extra nucleotide, (A in the figure), directly following the restriction sites will be amplified, *i.e.* 25% of all possible fragments assuming that there is an equal probability that A, C, T and G follow each site. An aliquot

of this first amplification reaction is taken to undergo the second amplification reaction. In the second reaction, three selective nucleotides (ANN) are used as shown in figure 1b. This means that only fragments with this particular combination of 6 nucleotides adjacent to the restriction sites will be amplified. One of the oligonucleotides in the second amplification reaction (normally that homologous to the hexacutter), is radioactively or fluorescently labeled. Therefore following separation of the amplified fragments on a polyacrylamide sequencing gel, bands can be detected by autoradiography or detection of fluorescence. Normally in the case of plant genomic DNA under these amplification conditions, 50-100 bands are observed per sample. A typical result of an AFLP gel is shown in figure 2. Selection occurs on different levels, in the original restriction digest three types of fragments are produced: EcoRI-EcoRI, EcoRI-MseI and MseI-MseI. In general, EcoRI-EcoRI fragments will be longer than EcoRI-MseI or MseI-MseI and many will either be unable to enter the sequencing gel or outwith the capacity of the PCR amplification reactions. In addition Vos *et al.* (1995) have suggested that MseI-MseI fragments are poorly amplified due to the formation of hairpin like structures. The MseI-MseI fragments will also be undetected in the final analysis since the EcoRI specific oligonucleotide is labeled, therefore only short EcoRI-EcoRI or EcoRI-MseI frag-

ments will be detected. This size fragment selection is combined with selection using specific additional bases as described above to produce a number of bands which are easily separated and analyzed on a sequencing gel. The possibility of using different combinations of selective oligonucleotides at each restriction site and also of using different restriction enzymes makes the AFLP technique extremely powerful in detecting polymorphisms throughout the genome.

The bands observed on the AFLP gels are classified as present or absent in each individual and the method is most commonly used as a dominant/recessive system. However, van Eck *et al.* (1995) have successfully been able to use it as a codominant system by comparing band intensities in order to determine heterozygotes. AFLP's although having features in common with randomly amplified polymorphic DNA markers, (RAPD's) are much more reproducible due to the use of long oligonucleotide primers which are 100% homologous to the target site, thus allowing the use of stringent amplification conditions. When adequate molecular weight markers are used, AFLP's should also be reproducible between laboratories. The main advantage of AFLP's is the capacity to analyze many loci simultaneously from the whole genome and rapidly score large numbers of markers.

Although currently sophisticated equipment and/or radioactivity are necessary in order to carry out AFLP

Figure 1a. First selective amplification. 1 and 2 represent the two strands of the DNA molecule. The black boxes represent the adapter molecules ligated to the ends of the restriction fragments, hatched boxes represent the oligonucleotides used for the amplification reaction. A represents the selective nucleotide and the arrows indicate the direction of synthesis.

Figure 1b. Second selective amplification reaction. Description of symbols as above.

Figure 2. Results of a typical AFLP analysis.

analysis, the method may be easily automated for high throughput of samples. Fluorescent labels are replacing radioactivity and the number of data points produced in a short time outweighs the costs.

Since the AFLP technique was first published in 1995 a flood of articles involving the technique have been published. Several articles have demonstrated the efficiency of AFLP's for germplasm characterization and phylogenetic studies in various organisms including plants, bacteria (Lin, J.-J. and Kuo, J. 1995) and fungi (Majer, D. *et al.* 1996). This methodology has also been used in population genetic studies to characterize natural populations of the bean anthracnose pathogen in Mexico where a strong correlation between clonal population structure and geographical distribution was observed, (Gonzalez and Simpson in preparation).

Many articles on the use of AFLP's for mapping in different plant species including potato, barley and Arabidopsis of both simple and quantitative characters have been published (Becker, J. *et al.* 1995; Cervera, M.T. *et al.* 1996; Lin, J.-J. and Kuo, J. 1995; van Eck, H. *et al.* 1995). It has been proposed that AFLP's could be used to saturate the region around a gene of interest producing very closely linked markers (Kiscum, M. and Oeller, P. 1996; Tanksley, S.D. *et al.* 1995).

AFLP technology is being used not only for mapping and fingerprinting but being adapted for use in other types of investigation. One important innovation is the use of AFLP to carry out RNA fingerprinting and differential display analysis (Bachem, C.B. *et al.* 1996). Another important application is the use of AFLP to study changes in methylation patterns during the different developmental stages of given organism by incorporating a methylation sensitive enzyme into the AFLP protocol. In this case the differences in banding pattern observed between two samples is due to the ability of the restriction enzyme to cut when the recognition site is methylated or unmethylated. By using this technique specific patterns of methylation during the development of dimorphic fungi could be determined (Reyna-Lopez, G.E. *et al.* 1997). This method of methylation pattern analysis could be very useful to study the effects of methylation in plants, for example in relation to imprinting in maize.

The AFLP technique has been rapidly adopted by many groups working in the fields of molecular map-

ping and phylogenetics, commercial kits are widely available and use of the method should lead to rapid advances in these as well as in associated areas.

References

- Bachem, C.B., Van der Hoeven, R.S., de Bruijn, S.F., Vreugdenhil, D., Zabeau, M., and Visser, R.G.F. 1996. Visualisation of differential gene expression using a novel method of RNA fingerprinting based on AFLP: Analysis of gene expression during potato tuber development. *the Plant Journal* **9**(5):745-753.
- Becker, J., Vos, P., Kuiper, M., Salamini, F., and Heun, M. 1995. Combined mapping of AFLP and RFLP markers in barley. *Molecular and General Genetics* **249**:65-71.
- Cervera, M.T., Gusmão, J., Steenackers, M., Peleman, J., Storme, V., Vanden Broeck, A., Van Montagu, M., and Boerjan, W. 1996. Identification of AFLP molecular markers for resistance against *Melampsora larici-populina* in *Populus*. *Theoretical and Applied Genetics* **93**:733-737.
- Kiscum, M., and Oeller, P. 1996. AFLP not only for fingerprinting but for positional cloning. Internet: <http://carnegiedpb.stanford.edu/methods/AFLP.html>
- Lin, J.-J., and Kuo, J. 1995. AFLP: A novel PCR-based technique for plant and bacterial DNA fingerprinting. *Focus* **17**(2):66-70.
- Majer, D., Mithen, R., Lewis, B.G., Vos, P., and Oliver, R.P. 1996. The use of AFLP fingerprinting for the detection of genetic variation in fungi. *Mycology Research* **100**:1107-1111.
- Reyna-López, G.E., Simpson, J., and Ruiz-Herrera, J. 1997. Differences in DNA methylation patterns are detectable during the dimorphic transition of fungi by amplification of restriction polymorphisms. *Molecular and General Genetics*, en prensa.
- Tanksley, S.D., Ganai, M.W., and Martin, G.B. 1995. Chromosome landing a paradigm for map-based cloning in plants with large genomes. *Trends in Genetics* **11**(2):63-68.
- van Eck, H., Rouppe van der Voort, J., Draaistra, J., van Zandvoort, P., van Enckevort, E., Segers, B., Peleman, J., Jacobsen, E., Helder, J., and Bakker, J. 1995. The inheritance and chromosomal location of AFLP markers in a non-inbred potato offspring. *Molecular Breeding* **1**:397-410.
- Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M., and Zabeau, M. 1995. AFLP: A new technique for DNA fingerprinting. *Nucleic Acids Research* **23**:4407-4414.