SOME CONSIDERATIONS FOR USE OF AMP-FLPS FOR IDENTITY TESTING

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INTRODUCTION

AMP-FLP (i.e., amplified fragment length polymorphism) technology can offer a high degree of discrimination among individuals when evaluating the potential origin of biological materials (Boerwinkle et al 1989, Budowle et al 1991, Horn et al 1989, Kasai et al 1990, Ludwig et al 1989). The technique combines the sensitivity of detection and specificity afforded by the polymerase chain reaction with the information content of variable number of tandem repeats (VNTR) loci to provide an efficacious approach to identity testing. A favorable attribute of the AMP-FLP approach to identity testing is that loci currently under study possess alleles that can be resolved readily by gel electrophoresis into discrete fragment bands (Budowle et al 1991, Allen et al 1989). Such resolution is not possible with restriction fragment length polymorphism (RFLP) analysis currently used for the examination of DNA recovered from biological materials.

Major considerations associated with the use of AMP-FLPs for identity testing include the approach to profile comparisons and the manner by which quantitative weight is placed upon profile occurrences. This short communication provides a brief discussion of possible approaches for these areas of AMP-FLP analysis. Validation and population genetic studies currently underway will determine whether or not any of these proposed approaches actually will come to fruition.

ALLELE DESIGNATIONS

With AMP-FLP systems, alleles can be evaluated without determining their base pair sizes. Unknown samples can be compared with an "allelic ladder" that is a composite of common alleles of a particular VNTR locus (Budowle et al 1991). Thus, phenotyping and designation of the various alleles are similar to the approach used for conventional protein genetic marker systems. Moreover, when possible, the allele classifications generally can be based upon the number of tandem repeats relative to an allelic ladder (Sajantila et al, in press).

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INTRA-LABORATORY COMPARISONS

Matching

While the resolution of AMP-FLP alleles is more readily possible than with RFLP systems, as with many genetic marker systems (including protein markers), the alleles are discrete only to a point. As shown in figures 1 and 2, microvariability in allele sizes can occur in a population. Such behavior is observed as apparently different sized allele which cluster around a step in the allele ladder. This microvariability that occurs among individuals could be due to sequence variation or slight differences in the size of alleles.

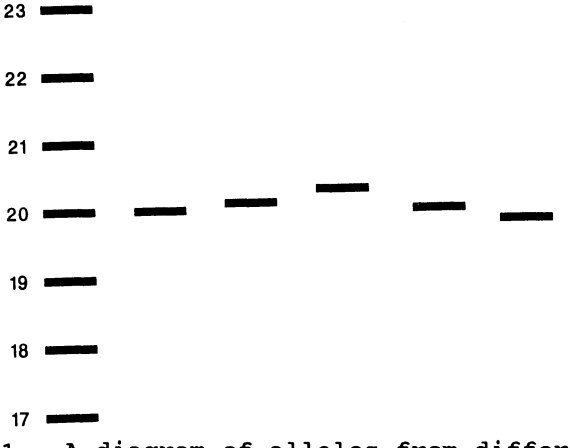

Figure 1. A diagram of alleles from different individuals displaying microvariability around allele 20.

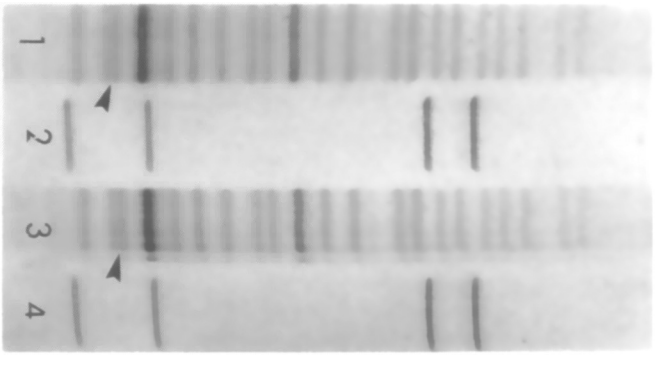

Figure 2. An AMP-FLP gel displaying a D1S80 allelic ladder in lanes 1, 3, and 5. The ladder is a composite of alleles from various individuals. Thus, the ladder serves as a mixing or co-electrophoresis experiment. The arrow points

to a doublet at allele 17. Both alleles of the doublet would be designated generically as a 17, but are clearly resolved in the mixing experiment. In lanes 2 and 4 are bands from the 1 Kb ladder (BRL, Gaithersburg, MD). From top to bottom the sizes of the 1 Kb ladder bands are 517, 506, 396, and 344 base pairs. It is evident that the 1 Kb ladder bands displayed do not migrate according to their true size.

However, this phenomenon should have little impact on determining whether or not two samples' profiles match (i.e., included or excluded as potentially originating from the same source). Although an allelic ladder is placed on the gel, it has little if any impact on the matching process. An inclusion or exclusion is determined by profile comparison and/or co-electrophoresis experiments (figures 3 and 4).

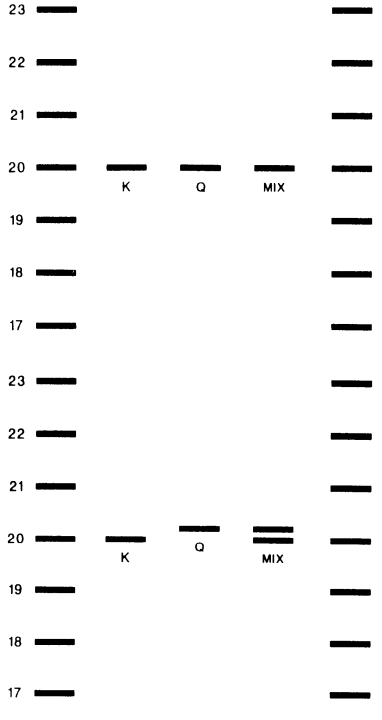

Figure 3. The diagram at the top displays an inclusion (or match), while the bottom diagram displays an exclusion. K is a known sample. Q refers to an unknown or questioned sample. MIX referes to a co-electrophoresis experiment of K and Q for resolving whether or not the two samples are considered similar by the analysis.

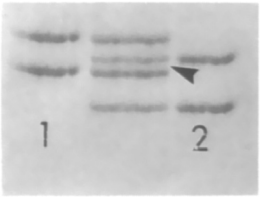

Figure 4. An AMP-FLP gel displaying a Collagen 2A1 VNTR marker. The center lane is a co-electrophoresis experiment of samples 1 and 2. Although samples 1 and 2 can be resolved visually without mixing and co-electrophoresis, the center lane demonstrates the effectiveness of mixing samples for comparison purposes. The arrow points to the two closest bands in the center lane which on the original gel are only 1 mm apart from each other.

WEIGHT CONSIDERATION

The allelic ladder is used after a match has been determined to assist in assigning proper quantitative weight to the occurrence of a profile. The common alleles which should be contained within an allelic ladder will have frequencies assigned to them based on empirical population studies. Gaps or areas in a ladder where there is no direct allele for comparison with the unknown sample, or a rare allele which lies outside the extremes of an allelic ladder, can be assigned an appropriate allele frequency.

Again, microvariability will not be a limitation for assigning the weight of a match. In the case of figure 1, where some of alleles in the sample population align with allele 20 and some are cathodal or anodal to allele 20, the frequencies of all these alleles can be totaled to provide a frequency for a generic allele 20. Thus, all alleles demonstrating microvariability around the step of an allelic ladder, although they could be resolved as different by a matching evaluation, will be given an overestimate of their true frequency in the sample population. Because generic allele frequencies will be used for estimating the frequency of occurrence of observed genotypes for highly polymorphic VNTR loci, classifications for population genetic data will be based on the generic allele classifications.

INTER-LABORATORY COMPARISONS

One of the advantages afforded by using AMP-FLP compared with RFLP technology is an increased ease of accomplishing inter-laboratory comparisons of DNA profiles. The nature of RFLP analysis is such that changes in some aspects of the technical procedure could result in slightly different size determinations of bands from the same DNA. This is of little concern for intra-laboratory comparisons, since the same protocol generally is used to compare sample profiles; however, it is not trivial for comparing DNA profiles developed in different laboratories. Thus, to make possible inter-laboratory comparisons of RFLP results there has been a concerted effort by a number of laboratories towards standardization of methodology. Requirements have included using the same restriction endonuclease, probes, and allelic controls. In fact, there are ongoing studies by the European DNA Profiling (EDNAP) Group and the Technical Working Group on DNA Analysis Methods (TWGDAM) to assess the inherent variation among laboratories using similar methodologies so that compatibility of data can be made possible (Schneider et al 1991, Hicks 1991).

In contrast, AMP-FLP techniques permit standardization based on the result instead of the method, as was possible with protein genetic marker systems. With an allelic ladder composed of alleles of the locus of interest and the ability to resolve DNA fragments discretely, the designation of alleles can be consistent among laboratories regardless of the analytical method used. For example, it would be anticipated that allele "18" of the D1S80 locus should be an "18" in all laboratories involved in the exchange of data. Of course the caveat that must be considered is the possibility of different resolving powers of the various electrophoretic systems employed by laboratories. A system of lower resolution will not be as effective for discriminating among individuals, but would be expected to provide higher frequencies for the alleles. However, the feasibility of standardizing on the AMP-FLP result has been demonstrated by Robertson et al (1991). They obtained similar AMP-FLP results for the D1S80 locus when using either a discontinuous polyacrylamide gel electrophoresis technique and silver staining or a continuous zone agarose gel electrophoresis approach in a gene scanner and fluorescently tagged primers.

CONCLUSION

This short communication is a brief statement of considerations for the effective use of AMP-FLP technology. It should be stressed that intra-laboratory comparisons of

AMP-FLPs will require standards within a laboratory but not standardization with other laboratories. These quality assurance standards are described elsewhere (Kearney et al 1991). While, methodological variation can be tolerated for situations such as identifying a potential serial rapist or the establishment of a DNA database identification system (Hicks 1991), a degree of standardization is essential. To ensure AMP-FLP compatibility between laboratories a nomenclature system for the alleles at each VNTR locus will have to be established. Moreover, the exchange or dissemination of allelic ladders (even if based on different primer sequences between laboratories) and/or known samples for corroborative testing should be encouraged.

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