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DNA recommendations – 1992 report concerning recommendations of the DNA Commission of the International Society for Forensic Haemogenetics relating to the use of PCR-based polymorphisms

I. Introduction

The International Society of Forensic Haemogenetics (ISFH) published a reporten titled "1991 Report Concerning Recommendations of the DNA Commission of the International Society of Forensic Haemogenetics relating to the use of DNA polymorphisms (Forensic Sci Int, 1992, 52:125–130 and Int J Leg Med, 1992, 104:361–364). While this initial report contained recommendations applicable to DNA polymorphisms in general, it addressed primarily DNA polymorphisms detected by restriction fragment length polymorphisms (RFLP) analysis. With the advent of the polymerase chain reaction (PCR) and its anticipated impact on identity testing, the DNA Commission of the ISFH met in December 1991 to produce an addendum guideline concerning the application of PCR-based technologies. The recommendations of the "1991 Report" apply not only to RFLP analyses but are also compatible with PCR-based approaches. Laboratory protocols for genetic marker analyses for paternity and forensics should be adhered to for PCR assays, as well. The following guidelines are additional points to better define PCR-based DNA identity tests.

II. Nomenclature

Due to the fact that some DNA sites are coding and some are noncoding regions, the polymorphic segments of DNA (e.g. D1S80, D17S5, HLA-DQ alpha) have been designated a "genetic locus". The term "allele" will still be used to describe alternative forms of a genetic locus. When possible, the D-designation (Human Gene Mapping designation) should be used to describe the genetic locus. However, the common name, if desired, can be included in brackets or parentheses with the genetic locus designation (e.g. D1S80 (pMCT 118) and D17S5 (YNZ22).

To define the alleles at a particular genetic locus a numerical designation is desirable. A simple form of allelic designation for identifying VNTR alleles is the classification of alleles based on the number of repeat sequences. If this is not possible, an arbitrary nomenclature is acceptable, provided it follows the guidelines described below.

Standard allelic ladders are desirable for identifying alleles, particularly for VNTR systems. If possible, the ladder should be composed of alleles of human origin for human comparisons of paternity and forensic samples. While some alleles may correspond directly with the steps of an allelic ladder, others may not align perfectly. One suggestion is to use a system similar to that used for group-specific component. Bands residing above or below the step of a ladder should be designated C (for cathodal), A (for anodal), and M (for midway between two steps of a ladder). An alternative approach is to use a nomenclature comparable to that used for HLA. Digits after a decimal point will refer to alleles out of register with the standard alleles of a ladder (e.g. alleles between 2.00 and 3.00 will be designated 2.10, 2.20, ...).

To achieve a degree of standardization, it is recommended that the ISFH form a workshop committee through the DNA Commission to ratify the nomenclature of new DNA polymorphisms that will be used for identity testing purposes. The author or developer of a genetic marker system will provide the DNA Commission with the appropriate details for evaluation. These should include: formal genetics, PCR conditions, primer sequences, an allelic ladder (if appropriate), electrophoresis, dot blot, or sequencing parameters, ten control (or defined) samples, and a suggested working nomenclature. The DNA Commission, on a timely basis, would evaluate the system and recommend and publish an acceptable nomenclature in an appropriate forensic journal.

For VNTR systems, standard allelic ladders are desirable, and therefore, standard primers will be necessary. This recommendation should not preclude the development and/or use of alternative primers and allelic ladders for a genetic locus. If alternative primers are used, a comparable allelic ladder will be required. It is incumbent upon the researcher who suggests an alternative set of primers to establish a corresponding nomenclature with the generally accepted approach and provide known controls for comparison purposes.

For sex chromosome typing, the investigation of X and Y sequences should be carried out in parallel or

simultaneously. The distinction of male and female DNA cannot be made based solely on the absence of a band.

III. Measurement error

Although it can be anticipated that alleles of a genetic locus will be resolved more effectively with PCR-based approaches compared with RFLP analysis, measurement error will still be present. The measurement error for each system should be addressed and defined based upon operational limits. For example, co-electrophoresis of two samples can be used to determine whether or not the alleles in question are operationally similar.

IV. Contamination

Due to the sensitivity of detection that PCR can provide, sample contamination with foreign human DNA will have to be addressed in laboratory protocols. Proper controls and appropriate procedures should be established to minimize the potential of sample contamination to affect interpretations. One of the most critical means to minimize contamination is the physical separation of the sample extraction and PCR preparation area (i.e., pre-amplified DNA area) from that area of the laboratory where typing of amplified DNA takes place. The intent of this physical separation of portions of a PCR-based analysis is to limit the potential of amplified DNA from contaminating non-amplified DNA. Additionally, sample-to-sample contamination can be reduced and/or monitored by the use of dedicated pipettes, by the use of positive and negative controls, by the technician wearing a mask during the sample preparation stage of the analysis, and by performing, when feasible, duplicate analyses.

V. DNA quantification

Quantification of DNA is important for improving the quality of PCR-based DNA typing results. Specific working ranges of human DNA to serve as a PCR template are recommended. Therefore, initial quantification of human DNA should be performed. One method for quantification is through the use of a slot blot analysis protocol with a human specific alphoid sequence probe (D17S1) (Waye et al. 1989, Biotechniques 7:852–855).

VI. Quality control framework

Recommendations suggested in the "1991 Report" also apply to PCR-based assays. A laboratory protocol with proper controls should be established for PCR-based assays, which include electrophoretic, dot blot, and sequencing methods. At least one defined control should be included on every analytical gel or on one dot blot strip per set of analyses. As discussed above, protocols for limiting contamination and quantifying human DNA should be established. Also, a standard nomenclature is necessary for comparison of data between laboratories. Standard allelic ladders, or appropriate information for converting and comparing data from different allelic ladders, can make inter-laboratory comparisons more effective.

VII. Biostatistics

Genetic loci defined by PCR-based systems essentially are the same as all other single locus genetic markers. Therefore, interpretation and evaluation of PCR-based systems should be carried out according to standards established for traditional genetic marker systems.

The DNA Commission consisted of the Executive Committee of the International Society for Forensic Haemogenetics (W. Bär, B. Brinkmann, P. Lincoln, W. R. Mayr, U. Rossi) and coopted external experts (B. Budowle, A. Eisenberg, R. Fourney, P. Gill, S. Rand).