1.2 Methodology

The Development and Evaluation of New Genetic Markers for the Application of PCR to Forensic Casework

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INTRODUCTION

The forensic group at Cetus Corporation is developing a variety of PCR-based genetic markers for the analysis of forensic evidence samples. We also are working on a system that enables you to assess the quality or "amplifiability" of extracted DNA samples.

SEQUENCE POLYMORPHISM MARKERS

PolyMarker Amplification and Typing System

The Amplitype HLA DQα system was the first PCR-based marker applied to forensic casework samples. This reverse dot blot typing system distinguishes six DQα alleles which result in 21 genotypes. The Pd for this single marker system is 0.94 for Caucasians.

To increase the power of discrimination obtained from a single sample test, we developed a system in which five genetic markers are coamplified with DQα. The six PCR products amplified by this PolyMarker system are listed below along with the sizes of the products, the number of alleles distinguished, and the chromosomal locations of the markers:

Markers	PCR product (bp)	Alleles	Chromosomes
LDL^r	254	2	19
DQα	239/242	6	6
gypA	190	2	4
$^G\gamma$-globin	172	3	11
D7S8	151	2	7
Gc	138	3	4

The combined Pd value for these six markers are greater than 0.999 for Caucasian, Black and Hispanic individuals in the United States.

The five markers containing two or three alleles are typed on a single strip using the reverse dot blot technology; the DQα type is determined on a separate strip. Both sets of strips are hybridized and processed under identical conditions.

We are completing our forensic validation studies which address each of the validation issues in the TWGDAM DNA Quality Assurance Guidelines.

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Gender Identification

We have developed a gender identification system in which a single primer pair is used to amplify a polymorphic region of a homologous zinc protein found on the X and Y chromosomes. This region contains a HaeIII site common to both the X and Y chromosome sequences and a polymorphic HaeIII site that is present only on the Y chromosome. Digestion of PCR products with HaeIII reveals the presence of X and Y chromosomes in the extracted DNA sample.

The advantages of this gender identification system over previously described systems are:

1. A product is generated for both the X and Y chromosomes such that the presence female DNA is signalled by a positive response rather than a lack of Y chromosome amplification.

2. Only one primer pair is utilized to generate X and Y specific products of the same size. Potential problems due to preferential priming between X and Y primer pairs and preferential amplification of smaller products are eliminated.

3. The polymorphic, homologous zinc finger protein is present in only one copy on each chromosome, allowing quantitation of X and Y chromosomes.

LENGTH POLYMORPHISM MARKERS

DIS80

We have developed and released a reagent set for the amplification and sizing of DIS80 alleles. We developed a DIS80 ladder containing 15 of the 29 alleles we have identified. Alleles containing 14, 16, and 18 repeat units have been sequenced in both directions, allowing the ladder to be registered and allele designations to be made according to the number of repeat units they contain.

We also are developing reagents sets for the YNZ22, apoB and col2AI loci. We have tested all of these AMP-FLP systems in the TC480 and TC9600 Perkin Elmer-Cetus thermal cyclers, and we have obtained comparable results from both instruments.

AMP-FLP Validation Studies

Frequently forensic DNA samples are degraded to some extent. It has been demonstrated previously that amplification of severely degraded DNA samples yields either the correct HLA DQα type or no type. More recent studies indicate that it is possible to obtain ambiguous and even incorrect DIS80 types from moderately degraded DNA samples.

Ambiguous types are obtained when the smaller of two observed alleles is amplified to a significantly greater extent than the other allele. This type of result can arise from amplification of a partially degraded DNA sample or from amplification of one of a variety of mixed samples.

Incorrect types are obtained when the degraded DNA sample can support amplification of products in the size range of the smaller allele but not in the size range of the larger allele. As a result, a sample from a heterozygous individual can be analyzed incorrectly as a homozygous type. More severely degraded DNA samples simply yield no DIS80 type.

Given some of the inherent properties of amplifying PCR products of significantly different lengths, it is likely that AMP-FLP markers will not be as robust as amplified sequence polymorphism markers. Consequently, great care will have to be taken to interpret AMP-FLP results.

DNA Quality Indicator

It is important to assess the quality of a DNA sample so that the most discriminating information can be obtained from AMP-FLP systems. However, it is not always possible to use agarose gel electrophoresis to analyze the quality of an extracted sample. For example, a portion of DNA extracted from hairs or small stains is too limited to visualize on an ethidium bromide stained gel. Also, single stranded DNA samples resulting from Chelex extraction cannot be visualized on ethidium bromide stained gels.

Clearly, another method for assessing quality is needed. While the term "quality" generally refers to the degradation state of DNA, it also must reflect the "amplifiability" of a DNA sample when PCR based systems are being employed. This aspect of a sample is important to consider because 10 ng of a high molecular weight sample will amplify more efficiently than 10 ng of a significantly degraded sample and 10 ng of a sample containing trace amounts of inhibitors.

We are developing a DNA Quality Indicator system designed to indicate how well a DNA sample will amplify HLA DQα and PCR products in the size range of the DIS80, YN722, apoB and col2AI AMP-FLP alleles. Our first generation indicator contains HLA DQα primers and two additional pairs of primers that yield 1 and 2 kilobase PCR products. The presence and absence of these three products on a gel will indicate which AMP-FLP systems can be amplified reliably from the DNA sample in question. We are modifying the sizes of the PCR products in the Quality Indicator to be more applicable to the AMP-FLP systems listed above.

Since the DNA Quality Indicator system contains HLA DQα primers, samples yielding a HLA-DQα product band on the gel can be typed directly on the HLA-DQα probe strips. This feature of the system allows discriminating information to be obtained from the sample in addition to the information regarding which AMP-FLP systems can be typed, and provides an advantage over agarose gel electrophoresis.