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Polymerase Chain Reaction (PCR) Amplification and Human Leukocyte Antigen (HLA)-DQα Oligonucleotide Typing on Biological Evidence Samples: Casework Experience

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ABSTRACT: The polymerase chain reaction (PCR) method of specific gene amplification was used in casework to synthesize millions of copies of the polymorphic second exon of the human leukocyte antigen (HLA)-DQα (or DQA1) locus from a variety of evidence samples. The HLA-DQα allelic variants in the amplified deoxyribonucleic acid (DNA) were determined in a rapid non-radioactive test by hybridization to sequence-specific oligonucleotide probes in both the dot-blot and reverse dot-blot formats. This genetic typing system has been subjected to blind proficiency testing; the performance of this test in the analysis of experimentally mixed samples was also evaluated. As of August 1990, over 250 cases have been tested and more than 2000 individual evidence (bloodstains, semen stains, individual hairs, bone fragments, and tissue sections) and reference samples have been analyzed. The first 198 of these cases are summarized in this paper; in 65% of the cases with conclusive results a suspect was included, and in 35%, all suspects were excluded. Individual cases as well as some of the general issues relating to forensic science analysis and this genetic typing system are discussed. The high rate of exclusion reported here combined with the ability of PCR to type old evidence samples suggests the relevance of this genetic test for postconviction review; two cases in which the convicted suspect was excluded are discussed.

KEYWORDS: pathology and biology, polymerase chain reaction (PCR), deoxyribonucleic acid (DNA), human leukocyte antigen

The ability of the polymerase chain reaction (PCR) [*1–3*] to amplify specific segments of deoxyribonucleic acid (DNA) has made possible the analysis of genetic variation in samples whose DNA is too degraded or present in insufficient amounts for restriction fragment length polymorphism (RFLP) typing [*4,5*]. Moreover, PCR has allowed the use of simple and rapid methods of detecting sequence variation in genomic DNA, such as non-radioactive oligonucleotide probes and dot-blot hybridization [*4,6,7,11*]. These properties make PCR useful in genetic typing of biological evidence samples.

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PCR is an in vitro method that uses two oligonucleotide primers and a heat-stable DNA polymerase (*Taq* polymerase) [*3,8*] to synthesize millions of copies of a particular DNA segment. Repeated cycles of strand separation by heating the double-stranded template DNA (denaturation), primer annealing to specific target sequences in the separated strands, and elongation by DNA polymerase of the primers annealed to the template stand, result in the exponential accumulation of a discrete DNA fragment whose termini are defined by the 5′ end of the primers. Because the products of one cycle can, following denaturation, serve as templates in the next cycle, the number of specific DNA segments approximately doubles with each cycle until the reaction reaches a "plateau" level [*3*]. With the availability of the heat-stable *Taq* polymerase, all of the reaction components could be introduced into a single tube and the amplification reaction carried out by simply varying the reaction temperature with an automated thermal cycling instrument.

PCR-based genetic typing involves the analysis of polymorphism in the DNA amplified from the sample. Length polymorphism in the PCR products as a result of variable-number tandem repeats (VNTRs) in the region flanked by the primers can be detected by gel electrophoresis [*9,10*] while sequence polymorphism can be detected by a variety of methods. The most complete description is the determination of the nucleotide sequence; however, once the spectrum of allelic sequence diversity at a locus has been identified, much simpler and rapid methods can be used for genetic typing of biological evidence samples. One such approach uses PCR primers complementary to conserved regions which flank a polymorphic DNA segment of a given locus; sequence-specific oligonucleotide hybridization probes can then be used either in the dot-blot format [*6,7*] or in the recently developed and simpler reverse dot-blot format [*11*] to determine the alleles present in the sample. The human leukocyte antigen (HLA)-DQα (now DQA1, see Nomenclature subsection in **Materials and Methods**) locus was the first polymorphic locus analyzed using this oligonucleotide probe approach [*6,12*]. Four major allelic types (DQA1, A2, A3, and A4) have been identified; DQA1 and DQA4 have three subtypes (DQA1.1, 1.2, 1.3 and DQA4.1, 4.2, and 4.3) for a total of eight alleles defined by sequencing the second exon of the DQα gene [*13,14*]. A panel of eight probes distinguishes six alleles; the subtypes of the DQA4 (previously known as 4.1, 4.2, and 4.3—now *0501, 0401, 0601) are not distinguished by this current panel of probes. The dot-blot method involves the immobilization of the amplified PCR product from an individual sample to several different nylon membranes and the hybridization of each membrane to one of a panel of labeled oligonucleotide probes. The reverse dot-blot method is based on the immobilization of unlabeled oligonucleotide probes in a defined array on a single nylon membrane. In this procedure, the PCR product is labeled during amplification and then hybridized in one reaction to *all* of the typing probes.

The first U.S. criminal case involving PCR analysis was *Pennsylvania v. Pestinikis, 1986*, in which the HLA-DQA1 locus was amplified from formaldehyde-treated autopsy tissue samples and typed with oligonucleotide hybridization probes [*4*]. This report describes the analysis of ~200 cases and ~2000 evidence samples (bloodstains, semen stains, individual hairs, tissue samples, and bone fragments) and reference samples and discusses some of the issues related to forensic science use of this PCR-based genetic test.

Materials and Methods

DNA Extraction

DNA was isolated from the samples following digestion in proteinase K (Boehringer Mannheim, Indianapolis, Indiana), extraction by the phenol/chloroform method, and Centricon 100 (Amicon, Danvers, Massachusetts) microdialysis for DNA concentration

[5]. A differential lysis procedure was used for sexual assault samples, in which epithelial cells were preferentially lysed in a digestion buffer containing 2% sodium dodecyl sulfate ($NaDodSO_4$) and proteinase K [15]. The sperm, which are resistant to digestion in the absence of reducing agent, were pelleted by centrifugation, repeatedly washed, and resuspended in digestion buffer to which dithiothreitol (DTT) and proteinase K had been added [5].

Amplification

Extracted sample DNA was added to 100 μL of PCR mix, containing 50m*M* KCl, 10m*M* Tris-HCl (pH 8.3), 2.5 or 4m*M* magnesium chloride ($MgCl_2$), 187μ*M* of each dATP, dCTP, dGTP, and dTTP, 20 pmoles each biotinylated primer GH26 and GH27 [13], and 2.5 or 2.9 units of *Taq* DNA polymerase (Perkin-Elmer/Cetus, Norwalk, Connecticut). The cycling reaction was done in a programmable heat block (DNA Thermal Cycler; Perkin-Elmer/Cetus) set to heat at 94°C for 30 or 60 s (denature), incubate at 60°C for 30 s (anneal), and incubate at 72°C for 30 s (extend) by the "step-cycle" program. After 35 or 40 cycles,[4] the samples were incubated an additional 10 min at 72°C.

Typing

Each DNA probe strip containing immobilized single-stranded HLA-DQα sequence specific probes [11] was placed in 3 mL of hybridization solution containing ×5 SSPE (×1 SSPE is 180m*M* sodium chloride (NaCl), 10m*M* sodium phosphate, monobasic, monohydrate (NaH_2PO_4), 1m*M* EDTA, pH 7.4), 0.5% $NaDodSO_4$, and 300 ng of streptavidin-horseradish peroxidase conjugate (Cetus Corp., Emeryville, California). PCR-amplified DNA (35 or 50 μL) was denatured by heating at 95°C for several minutes, and then added immediately to the hybridization solution, which was then incubated at 55°C for 20 min. The probe strips were briefly rinsed once in ×2 or ×2.5 SSPE/0.1% $NaDodSO_4$ at room temperature, and then washed once in ×2 or ×2.5 SSPE/0.1% $NaDodSO_4$ at 55°C for 10 to 14 min. The probe strips were then washed once in ×2 or ×2.5 SSPE/0.1% $NaDodSO_4$ and once in 0.1*M* sodium citrate, pH 5.0, at room temperature. Color development was performed by incubating the Probe Strips in 0.1*M* sodium citrate, pH 5.0, containing 0.1-mg/mL 3, 3', 5, 5' tetramethylbenzidine (Fluka Chemical Corp., Ronkonkoma, New York, and 0.003% hydrogen peroxide. After 2/1990, all samples were analyzed with the Amplitype™ HLA-DQα Forensic Kit (Perkin-Elmer/Cetus).

Nomenclature

According to the most recent system of nomenclature adopted by the World Health Organization HLA Nomenclature Committee [16], the DQα locus is now known as DQA1, and the linked and homologous locus, previously known as DXα, is now termed DQA2. The allelic designations are DQA1*0101 (previously A1.1), *0102 (previously A1.2), *0103 (previously A1.3), DQA1*0201 (previously A2), *0301 (previously A3), DQA1*0401 (previously A4.2), DQA1*0501 (previously A4.1), and DQA1*0601 (previously A4.3). Since the new locus designation (DQA1) is the same as our previous allele designation, DQA1, in the present paper we have retained the older nomenclature to avoid confusion.

[4]In general, the sensitivity of the system increased as it was developed because of increases in the efficiency of the amplification and product detection. Accordingly, the number of cycles used was reduced to minimize stochastic fluctuation in the detection of alleles in heterozygous samples.

Results

Test System Development

The initial development of PCR-based DQα typing involved the characterization of the DQα primer pair GH26 and GH27 [*13*] and the oligonucleotide probes and hybridization conditions required to determine correctly the DQα genotype of homozygous typing cells (HTCs) and other cell lines whose DQα alleles had been previously determined by sequencing. The DQα primers, GH26 and GH27, amplify all known DQα alleles in the human population [*14,15,17*] as well as DQα alleles from all primate species examined [*19*]. These primers do not amplify a specific DNA fragment from DNA from dogs, cows, deer, pigs, and cats [*20*]. The oligonucleotide typing probes used were first shown to identify correctly the sequences upon which they were based. Under the appropriate amplification and hybridization conditions ([*18*]; also see **Materials and Methods**), these DQα oligonucleotide probe hybridizations were highly specific and the typing of homozygous cell lines whose DQα DNA had been sequenced and seven individuals whose DQα alleles had also been sequenced all gave the expected DQα types. Some HLA serologic specificities are highly correlated with specific DQα alleles, (for example, HLA-DR1 with DQα1.1, HLA-DR7 with DQα2, HLA-DQw1 with DQα1.1, DQα1.2, and DQα1.3, HLA-DQw3 with DQα3). A detailed discussion of the relationship of HLA serologic specificities and DQα alleles will be reported elsewhere.

DNA typing using this approach was found to be highly reproducible. As part of the study to determine allele and genotype frequencies and inheritance patterns [*18*], nearly 1000 different DNA samples were typed in duplicate with 10% of them done in triplicate. Using the dot-blot format, there was one discordant typing due to a weak dot intensity for one sample.[5] This potential source of typing error is eliminated in the reverse dot-blot format where no type is "called" unless the C dot positive control is visible (the C dot probe is designed to be the weakest dot on the DNA Probe Strip). A total of 1916 samples typed from different ethnic groups were used in the determination of allele and genotype frequencies; many samples were also typed in the reverse dot-blot format. A total of 11 different human populations were examined [*18*].

In these population studies, the power of discrimination—the chance two persons chosen at random from a population will have different genotypes [*21*]—for the DQα marker varied from 0.83 (in one Mexican population) to 0.94 (in Caucasians) [*18*]. Thus, inclusion of a suspect as being a possible donor of biological evidence by this one test is not as discriminating as a combination of RFLP markers, which can claim a power >0.999 99. However, most of the cases reported here involve evidence in which the DNA is too degraded or in too small amount for RFLP analysis. Frequently, the number of possible suspects in a given case is limited by other evidence (for example, see Casework, Case 2). Additional informative PCR markers are being developed that will increase the available discrimination power.

In none of the populations surveyed did the observed distribution of genotypes deviate significantly from the expected distribution based on Hardy-Weinberg equilibrium assumptions [*18*]. For some RFLP markers, significant differences between observed and

[5]One sample was typed (in the dot-blot format) twice as a DQα1.1,4 and once, with weak dot intensities, as a DQα1.2,4. This latter incorrect typing was due to a weak DQα1.1 dot which was scored as negative. One of the subtyping probes reacts with the DQα1.2, the DQα1.3, and the DQα4 alleles, and was positive for this sample. The reactivity of the 1.2/1.3/4 probe for this sample is due to the presence of the DQα4 allele. The only difference in dot pattern between the DQα1.1,4 genotype and the DQα1.2,4 genotype is the reactivity of the DQα1.1 probe dot. With the current reverse dot-blot test (utilized in the AmpliType™ HLA-DQα Forensic kit) and interpretation guidelines based on the control C dot intensity, the overall weak dot pattern observed for this sample would be scored as "inconclusive."

expected genotype frequencies have been reported [*22*]. These differences have been attributed to population substructure [*22*] and, more recently, to systematic overestimates of the frequency of homozygotes due to typing problems, such as the inability to resolve alleles with similar electrophoretic mobilities or to detect all alleles [*23*]. For DQα, the agreement of observed and expected genotype frequencies does not necessarily imply that all the assumptions of Hardy-Weinberg equilibrium (random mating, no selection, and so forth) obtain but does show that there is no fundamental, systematic error with the typing method. For Caucasian populations, similar frequency distributions have been obtained by others [*24*].

The reliability of the typing method is also indicated by the Mendelian segregation pattern of the DQα PCR/oligonucleotide defined types in 39 Centre d'Etude du Polymorphisme Humaine (CEPH) pedigrees containing 502 samples [*25*]. In these families, the genotypes of the parents restrict the possible types of the 312 offspring. In no case is the type of an offspring inconsistent with the parental types. Also, in the cases where parents are of a homozygous type, there are no apparent failures to transmit that allele, a result consistent with the absence of "blank" or undetected alleles in this study.

Immobilizing the PCR product and hybridizing it to a series of oligonucleotide probes (dot blot) has been shown to give equivalent typings to those obtained by immobilizing the probes and hybridizing all the probes simultaneously to the PCR product (the "reverse dot-blot" [*11*]). This has been done in multiple blind tests involving all 21 DQα genotypes [*26,27*]. Also, in several cases, for example, *Texas v. Fuller* (see Casework, Case 2), the casework samples were analyzed in both formats with no contradictory typings. The detection principle for the dot-blot and the reverse dot-blot is the same. Both typing methods are based on the sequence specific hybridization reaction between the PCR product and an oligonucleotide probe of defined sequence. The specificity of hybridization is controlled, in part, by maintaining a temperature of between 54 and 56°C for the hybridization and wash reactions. Below 54°C, cross-hybridization can be observed. Above 56°C, some of the probes can react weakly, giving weak dot intensities. In general, the results obtained by both methods are identical, although in the non-isotopic formats tested, the reverse dot-blot was two to four times more sensitive. Since reverse-dot membranes can be premade and tested, the method is much more convenient and not subject to handling errors in immobilizing PCR product that may occur in the traditional dot blot.

Several studies on samples treated under conditions relevant to potential crime scenes have also been carried out with this test. Aged blood, semen stains, and hairs have been examined and have shown either no change in type as a function of age [*34*] or an inability to be typed. Environmental exposure (10% SDS, bleach, gasoline, motor oil, 1*N* sodium hydroxide (NaOH) 1*M* glacial acetic acid, 0.1*M* potassium phosphate buffer, pH 6.8, soil) has been examined and also found to cause no change in type obtained [*35,32*]. However, samples exposed to soil in these studies failed to amplify as a result of degradation of the DNA, inability to extract the DNA, or inhibition of amplification. Other samples exposed to soil have amplified and typed successfully [*33*].

Validation

Proficiency Testing

For any new genetic marker, it is important to examine the typing method on forensic science evidence samples [*36*]. Two blind trials involving DQα typing of simulated evidentiary samples (blood stains, semen stains, and hair) organized by the California Association of Crime Laboratory Directors (CACLD) were carried out as well as a blind trial involving hair specimens and blood samples, organized by the trial court as part of

the *Texas v. Fuller* case (see below). In the first CACLD blind trial carried out in 1987 using the DQα dot-blot format, all 50 samples gave typing results, in contrast to the 2 RFLP typing approaches which reported results on 73 and 90% [*37*] of the samples. However, 1 sample out of 50 was incorrectly[6] typed as DQA4. Subsequently, this sample was retyped correctly in the reverse dot-blot format as DQα1.2,4. One of two different explanations could account for the one incorrect typing observed in this blind trial. This mistake may have been due to the failure to immobilize the PCR product from the appropriate sample on the membrane which was subsequently hybridized to the DQA1 probe. This potential source of operator error is eliminated in the reverse dot-blot format in which the probes are pre-immobilized on a single membrane and only one hybridization reaction is required for complete typing.

Alternatively, the incorrect typing of this sample could have been due to preferential amplification of the DQA4 allele in one amplification reaction. The phenomenon of preferential amplification was originally observed with a DQα1.1,4 sample of denatured genomic DNA that occasionally typed as DQα4,4 [*31*]. This observation was attributed to differential thermal stability of the two DQα alleles and to inadequate heating during the denaturation step of the PCR thermal cycle. The critical role of the denaturation temperature in preferential amplification was identified by systematic experiments with denatured genomic DNA which revealed that at measured denaturation temperatures of 88 to 89°C, the DQA4 allele was amplified but not the DQA1 allele [*38*]. Below this temperature, neither allele was amplified and at temperatures between 90 and 96°C, both alleles were amplified. This differential denaturation can be attributed to the higher GC content (hence, higher thermal stability) of the DQα1 alleles (1.1, 1.2, and 1.3) DNA duplexes relative to the other DQα alleles. To avoid the potential of differential denaturation in wells of the thermal cycler which failed to reach adequate temperatures, the thermal cycling profile has been adjusted so that the denaturation temperature is programmed for 60 s at 94°C. It is also recommended that the thermal cycler temperatures be checked routinely.

In the second CACLD blind trail carried out in 1989, 50 samples (bloodstains and semen stains) were analyzed by the reverse dot blot typing method [*39*]. All 50 samples were correctly typed. In the *Texas v. Fuller* blind trial, all 10 specimens (hair, blood, and zero DNA blank samples) were correctly typed using the reverse dot-blot method. A blind trial was conducted in association with another case (*California v. Vargas*) where DQα typing was carried out on 9 hair samples. Extracts from 2 of the hairs did not amplify, and so no DQα typing results were obtained. However, the correct DQα types were obtained for the remaining 7 hairs.

Another blind trial was conducted by Cetus (1990) in which 5 forensic science laboratories used the AmpliType™ HLA-DQα kit to obtain the DQα types of purified DNA, bloodstains, plucked hairs, a semen stain, and postcoital samples. The test sites had little or no experience with either PCR or the AmpliType HLA-DQα Kit and were given only limited training (analysis of four samples in a three-day training course) before the evaluation. Of the 180 DNA-containing samples analyzed, results were reported for 178. Of the 178 samples with results, all were correctly typed [*33*]. These data, combined with other blind trials, give a total of 299 forensic science-type samples subjected to blind testing.

Both the dot-blot and the reverse dot-blot methods have been successfully transferred to other laboratories. The DQα reverse dot-blot test has been used in studying autopsy pathology specimens [*25*], prenatal HLA [*26*], and in population studies (P. Gill, unpublished), and has been extensively studied by the FBI [*27,28,29*].

[6]Since types of the samples are not known a priori, an "incorrect" type is one inconsistent with that of other samples known to be from the same individual.

Analysis of Mixed Samples

Biological evidence found at the crime scene can consist of a mixture of tissues or bodily fluids or both from more than one donor. Such mixtures may complicate the interpretation of genetic analysis of the evidence samples. The most frequent example of a mixture is in vaginal swabs or semen stains taken from victims of sexual assault; these samples can be demonstrated microscopically to contain both male (sperm) and female (epithelial cell) components. For these samples, there are effective procedures for the separation of sperm DNA from other DNA [*5,15,40*].

Two important elements are involved in the analysis of mixed samples: (1) the *detection* of mixtures, that is, detecting the presence of more than one genotype in a sample and, more importantly, (2) the *interpretation* of mixtures, that is, identifying the different genotypes that are present in a mixed sample.

The detection of mixtures depends, in part, on the fact that any one individual can have at most two alleles of a given gene; the presence of more than two alleles in a sample indicates a mixture. Even if only two alleles are present, a mixture can still be detected if the two samples are mixed in different proportions. In this case, a mixture is suspected if the relative dot intensities corresponding to the two alleles are very different. A mixture cannot necessarily be detected, however, if the two contributors to a mixture contribute no more than two alleles in total and contribute approximately equivalent amounts of DNA. In this case, the typing pattern is indistinguishable from that of a heterozygous sample. One then depends on other evidence—such as having obviously mixed body fluids or tissues or both from different sources (for example, blood on a hair), or the circumstances of deposition of the evidence, or testimony—to predict a mixed sample.

The interpretation of mixtures involves identifying the different genotypes that are present in the mixture. One interpretation that is frequently made is that, even when the separation of sperm from female DNA in a sexual assault sample is incomplete, the relative enrichment of some alleles in the differential lysis procedure indicates the sperm origin of those alleles. As the quantity of DNA corresponding to the minor component genotype is decreased relative to the major component genotype, the resulting dot intensity for the minor component decreases relative to the major component. Thus, mixtures can be identified and interpreted based on relative dot intensities, where dots corresponding to the minor component genotype are clearly lighter than dots corresponding to the major component genotype.

As an example, we have mixed two purified DNA samples of different genotypes in various ratios (mixed before amplification) and typed these samples using the reverse dot-blot DQα system. The results of these experiments are shown in Figs. 1 and 2. Figure 1 shows typings in which the total amount of the combined genomic DNAs amplified was relatively low with respect to the sensitivity of the typing system (2 ng). Figure 2 shows typings in which the combined amount was higher (50 ng). In these mixtures containing DQα1,2,3 and 4 alleles, the question is, at what point is it possible to infer which two alleles "go together" as a genotype as judged by relative dot intensities? One convention is to use the C dot positive control as an internal standard to judge relative dot intensities. In Figs. 1 and 2 the dots corresponding to the minor component are less intense than the C dot when that component is approximately less than 1 part in 16.

These studies of experimental mixtures of different DNA samples in known proportions indicate that mixtures in which the concentration of the two components is sufficiently different can often be interpreted, and the contributing genotypes identified. These data are also consistent with our experience in typing mixed sexual assault samples, in which the relative female and male contributions could be assessed by microscopic examination of the samples for epithelial and sperm cell types.

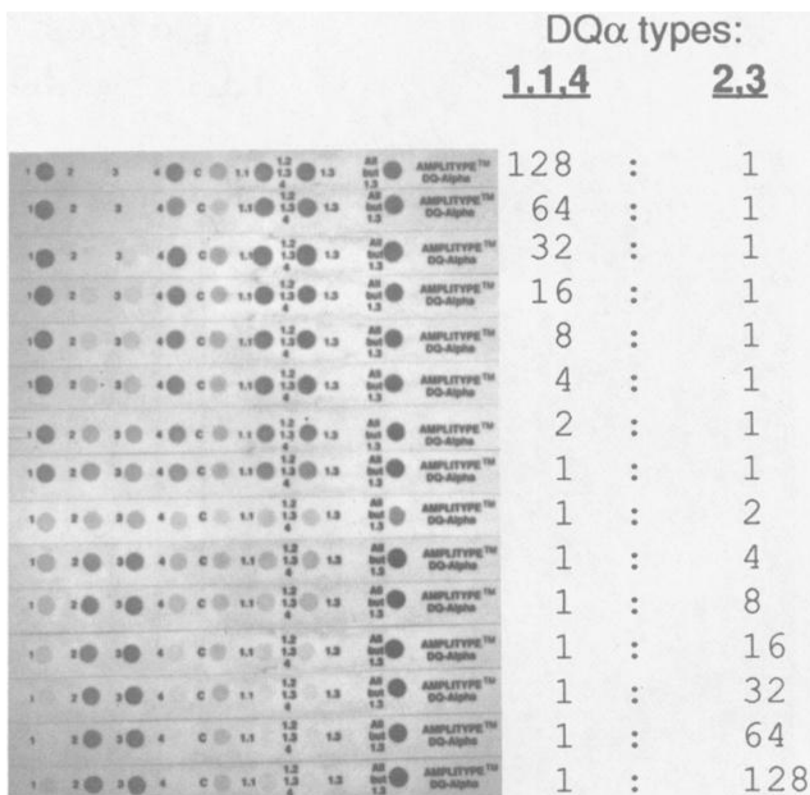


FIG. 1—*A 2-ng mixture: DQα1.1,4 DNA and DQα2,3 DNA were mixed in the proportions indicated above. For each sample, a total of 2 ng of this DNA mixture was added to the PCR mix. The samples were amplified for 32 cycles, and DQα typing was performed as described in* **Materials and Methods**. *As the quantity of DNA corresponding to the minor component genotype is decreased relative to the major component genotype, the resulting dot intensity for the minor component decreases relative to the major component.*

Casework

Case 1. Pennsylvania v. Pestinikis

The first use of PCR in a criminal case illustrates dramatically the value of PCR amplification in the analysis of degraded DNA samples. This case has been described briefly in an article on the use of nonradioactive oligonucleotide probes [*4*]. In this case, formaldehyde-preserved tissue samples (kidney, liver, lung) from two different autopsies were HLA-DQα typed by PCR at the request of the prosecution. These autopsy samples were all allegedly derived from a single individual, the possible victim in a wrongful death suit. The typing was carried out to test the hypothesis that the samples, in fact, came from different individuals. The DNA extracted from these tissue samples was extremely degraded with the mean molecular weight, based on electrophoretic mobility in agarose gels, of ~100 bp, making RFLP analysis impossible. In DNA extracted from these tissue samples, which would not support amplification of the 242-bp fragment, primers (GH26 and GH84; [*4*]) that amplified a smaller DQα fragment (166 bp) allowed genetic typing to be carried out. The samples from both autopsies all had the same DQα type (DQα1.1,1.1)

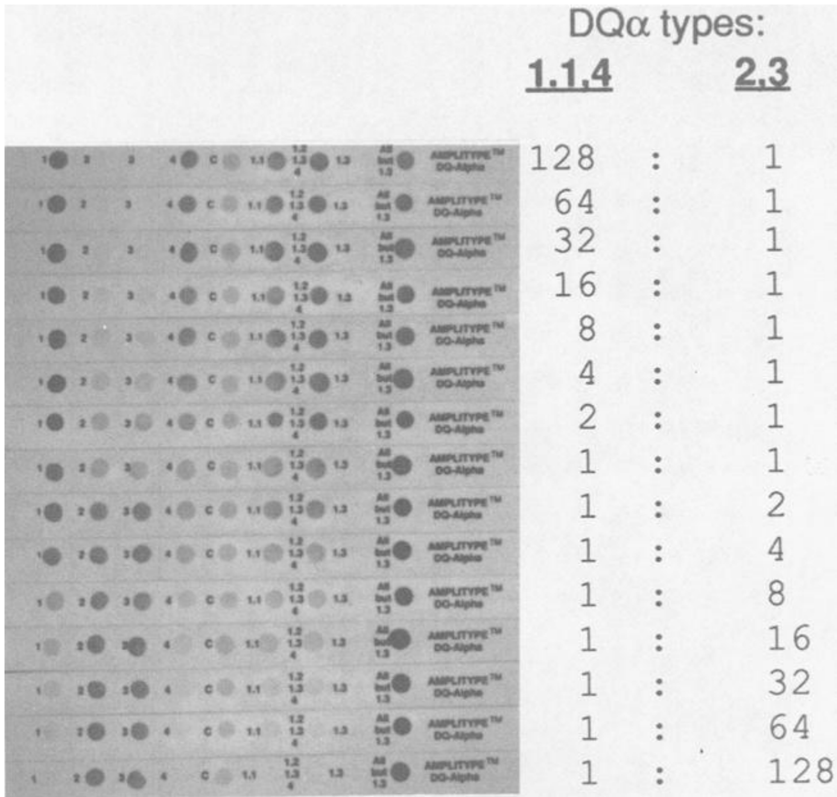


FIG. 2—*A 50-ng mixture: DQα1.1,4 DNA and DQα2,3 DNA were mixed in the proportions indicated above. For each sample, a total of 50 ng of this DNA mixture was added to the PCR mix. The samples were amplified for 32 cycles, and DQα typing was performed as described in* **Materials and Methods**. *As the quantity of DNA corresponding to the minor component genotype is decreased relative to the major component genotype, the resulting dot intensity for the minor component decreases relative to the major component.*

[*4*], consistent with the notion that the two autopsies were from the same individual. At the time of the case, before the determination of the HLA-DQα population frequencies reported by Helmuth et al. [*18*], the probability that a random individual would have this DQα type was estimated at about 1%, based on the published estimates of the serologic specificities HLA-DR1 (correlated with DQA1.1) and HLA-DQw1 [*4*]. Based on the PCR/oligonucleotide DQα typing of more than 400 Caucasian individuals [*18*], the point estimate of the DQα1.1,1.1 genotype frequency is 2% (with a 95% confidence interval of 1 to 4%), consistent with the earlier estimate derived from the frequencies of HLA serologic specificities.

Case 2. Texas v. Fuller

The *Texas v. Fuller* case illustrates the use of PCR DQα typing in the analysis of individual hair samples as well as semen stains in a rape/homicide. Following the analysis of the evidence samples, a blind trial involving ten samples was carried out. All ten of these samples were correctly typed. In this case, three men were known to be involved in the crime, but the actual role of the three assailants was in question and could not be determined by conventional protein marker or blood group typing. This case is described

in more detail in Reynolds et al. [*41*]. One of the three men confessed to the rape and murder (Suspect 1), although it was considered possible that he was attempting to protect Suspect 2; a second was held on the basis of a bloody footprint at the scene (Suspect 2); and the third was linked by association with the other two suspects (Suspect 3). Individual hair samples, semen stains and reference samples from the victim, her boyfriend and the three suspects were analyzed to determine if any of the suspects could be eliminated as donors of the evidence specimens. The victim and the three suspects are distinguishable from one another, but the victim's boyfriend and Suspect 3 have the same type (DQA1.1,1.1).

For the initial analysis, 18 hairs microscopically distinguishable from the victim's hair were selected; 3 of these were contaminated with blood. DNA was isolated from the hairs and amplified for DQα typing. Thirteen hairs typed as DQα 2,3, three hairs gave no result and the remaining two hairs had a mixed type of 1.2,2,3,4. This result is consistent with a mixture of the victim's type (1.2,4) with a type 2,3. Additional hairs, including some that could have come from the victim, were subsequently typed. From this set, eight had the victim's type (1.2,4), three were determined to be DQα 2,3, and two hairs had the mixed type seen with the other set of hairs tested. Experience gained from this case indicated the value of having a control for cellular contamination of the surface of the hairs. Following this and similar cases, a procedure was adopted where the root and an equal length of hair shaft were processed concurrently. The shaft portion served as a negative control for contaminating cellular material.

Sperm-containing semen samples were differentially extracted from a vaginal swab and a stain on a sheet. Intact sperm cells were separated from epithelial cell debris in the sample prior to extraction and the two fractions were analyzed independently. The sperm DNA from both the swab and the stain samples typed as DQα 2,3, consistent with the type of the hair donor(s). The DQα type of the epithelial cell fraction was DQα1.2,4, consistent with the victim.

The man who confessed to the rape and murder (Suspect 1) was eliminated as the donor of the hairs and semen found at the scene; his type is DQα 1.1,1.2. Suspect 3 and the victim's boyfriend (DQα1.1,1.1) were also eliminated as donors of these specimens. The man who matched the bloody footprint at the scene (Suspect 2) has a DQα 2,3 type and therefore cannot be eliminated as the source of the hair and the semen. The DQα 2,3 type occurs in 3% of the U.S. black population (all three suspects were black). The jury for this case convicted this suspect (Suspect 2) of rape and murder while the man who confessed to the rape and murder was convicted as a coconspirator.

Case 3. Missing Persons

HLA-DQα typing was also informative in the analysis of a bone fragment in a case involving a missing child [*41*]. A three-year-old girl was reported as a missing person in 1984. No body was ever found, but in 1986 a portion of a small skull was discovered within two miles of the parents' residence. Anthropologic examination of the skull cap suggested that it was from a two-to-five-year-old human child. Material for DNA analysis was obtained by scraping the external and internal surfaces of the skull and by chipping off a piece of bone. The skull scrapings and bone chip were digested in the presence of sodium dodecyl sulfate, dithiothreitol and proteinase K and extracted with phenol/chloroform. DQα typing was carried out on this sample and on DNA from the two parental samples.

The mother and father were determined to be DQα types 3,4 and 4,4, respectively. Any child of these two people would be either a DQα type 3,4 or 4,4 with equal probability. The combined frequency of DQα types 3,4 and 4,4 is about 19% in the Caucasian population [*18*] so there is an 81% chance of excluding an unrelated individual. Amplification of the scrapings did not produce a typeable product: the scrapings therefore serve

as a control for surface contamination on the skull bone. In contrast, the bone chip material did amplify, and it typed as DQα 3,4. This typing is consistent with the skull cap coming from the missing child, but it does not allow an absolute identification.

Recently, a polymorphic mitochondrial DNA fragment was amplified from the DNA from the bone chip and was analyzed by oligonucleotide probe hybridization and by sequencing [*42*]. Mitochondrial DNA is inherited matrilineally so that a child's mitochondrial sequences are shared with all siblings and with the mother. The mitochondrial DNA sequences of the bone chip and of the mother of the missing child were identical. The frequency of this mitochondrial DNA type within the Caucasian population is estimated at $<0.7\%$ [*42*]. Thus, the results of the HLA-DQα and mitochondrial DNA typing indicate that the source of the bone fragments discovered in the desert is very likely to be the missing child.

Case 4.

One alleged rape case involved the analysis of vaginal aspirate material as well as bloodstains and semen stains from various hospital pieces of linen. A woman was injured in a car accident and brought to a hospital emergency room and allegedly raped by the suspect, an orderly. Two sheets and a blanket containing bloodstains as well as semen stains were retrieved from the laundry. The evidence was initially submitted to Lifecodes Corporation (Valhalla, New York) but their report states that there was insufficient high-molecular-weight human DNA isolated from the evidence for RFLP analysis. PCR based HLA-DQα typing of the various evidence samples was carried out to determine whether the suspect could be eliminated as the sperm donor from the victim's vaginal specimen or semen stains on the hospital bedding or both. In addition, since the history of the stained hospital linen was unclear, the typing was necessary to determine whether the victim was, in fact, the source of the bloodstains or semen bearing vaginal drainage stains.

As shown in Fig. 3, the DQα type of the DNA isolated from the sperm fraction of the vaginal aspirate and the semen stains was DQα 4,4. The victim's reference sample typed as DQα 1.1,2. The epithelial cell (E-cell) bearing stain from the flannel sheet was typed as DQα 1.1,2, compatible with the stain originating from the victim. The E-cell fraction from some semen stains showed a mixed genotype (DQα1.1,2 with a trace of DQα4). The E-cell fraction of the vaginal aspirate also typed as DQα4,4, as a result presumably of the large amount of sperm (see Table 3). The sperm fraction from some semen stains also had a mixed genotype (DQα4,4 with a trace of DQα1.1,2). All of the bloodstains from both sheets typed as DQα 1.1,2, a genotype whose frequency is $\sim3\%$ in the Caucasian population [*18*]. The DQα type of the sperm fractions did not match the husband (DQα 1.1,3) but did match the suspect (DQα 4,4). Reference samples from the two other male hospital employees on duty when the victim was allegedly assaulted were also tested and determined to be DQα 1.1, 1.2 and DQα 1.1,4. Thus, DQα typing was important in identifying that particular evidence stains (bloodstains and vaginal epithelial cells) were derived from the victim associating the sheets with her as well as in excluding some individuals and including the suspect by analyzing the sperm fraction.

Case 5. California vs. Quintinilla

DQα typing proved valuable in another sexual assault case (Fall 1987) in which the initial suspect was *excluded* and, about a year later, a second suspect was identified and *included* by this test. The rape victim identified Suspect 1 (a Hispanic) as her assailant and DNA samples from the vaginal vault, posterior fornix, and rectal swabs were amplified and typed for DQα polymorphism. The sperm typed as DQα 1.1,4 and the victim

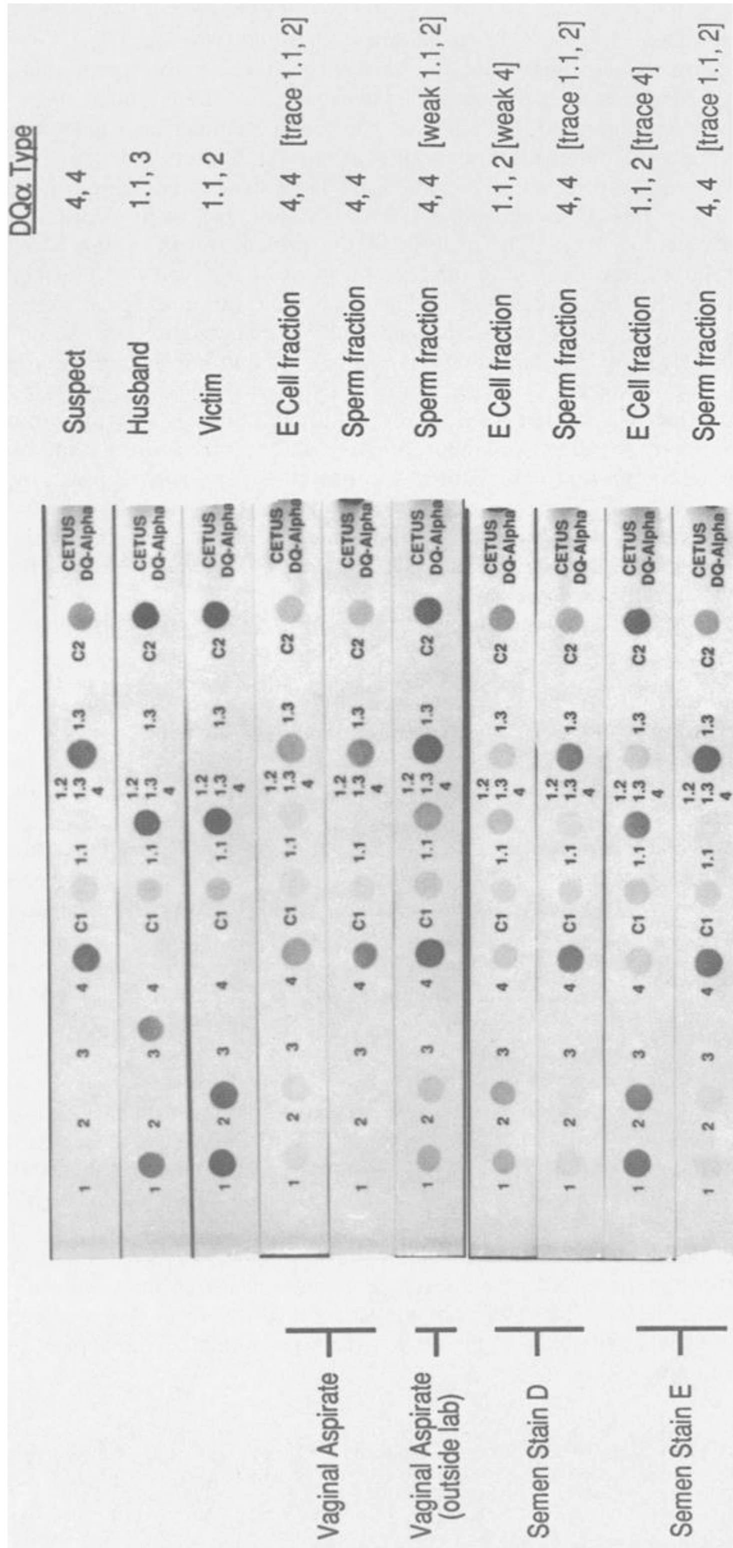
was DQα 3,4. Because the victim's boyfriend type was DQα 1.3,4, the source of the sperm was assumed to be the assailant. Suspect 1 was typed as DQα 1.2, 1.3, or 1.3, 1.3[7] and therefore this suspect was excluded as the donor of the sperm typed in these samples. Based on this evidence, the prosecution declined to continue the case and the suspect was released. About one year later, a different individual was being investigated in several rape cases in the same area. He matched the original victim's physical description, possessed jewelry stolen from this victim, and his fingerprints matched those found earlier in her car. The analysis of the evidence in these rape cases that investigators believed were related to the rape of Victim 1 revealed that the sperm donor was DQα 1.1,4. By this time, however, no swabs from the initial rape victim were available. A shoe belonging to the victim and that was part of the crime scene contained a semen stain (Fig. 4). To confirm the typing of a sperm sample from the original sexual assault, this stain was analyzed. The sperm fraction was typed as DQα1.1,4 (Fig. 5). The E-cell fraction of one area of the stain (A) also typed as 1.1,4, due presumably to the large amount of sperm (see Table 3). Thus, this second suspect could not be excluded as a potential sperm donor in this case; this DQα genotype is present in ~7% of Caucasian and Mexican-American populations and 9% of the black population [*18*]. Therefore, in this case, DQα typing was instrumental in both excluding the original suspect and, one year later, including another suspect. In this case, the DNA evidence was admitted and the second suspect was ultimately convicted.

Case 6. Virginia v. Spencer

In *Virginia v. Spencer*, a 15-year-old female had been found strangled in her bed and a sexual assault was suspected. DQα typing was carried out on bloody semen stains found on the sheet and on the vaginal slide smear prepared by the medical examiner. In both forms of evidence, the sperm DNA was adequately separated from the female's epithelial cell DNA and typed as DQα1.2,2 (Fig. 6). This type is present in ~5% of the Caucasian and black populations, and 3.5% of the Southeast Asian population and less than 1% of the Japanese [*18*]. The defendant, who was a suspect in other similar crimes, was determined to be type DQα1.2,2 and could, therefore, not be excluded as the sperm donor in this case.

During the course of analyzing the reference samples for the victim, which consisted of eleven pieces of bloodstained cloth, it was found that three of these samples could not have originated from the victim based on the following observations. The DQα type of eight of these specimens was determined to be DQα3,4. The other three specimens were typed as DQα1.1,2; the conclusion that these three specimens were distinct from the other eight specimens was supported by typing with the ACP [*43*], and PGM [*43*] genetic marker systems. Furthermore, the DQα type of the epithelial cell fraction determined from the victim's vaginal smear and the bloody semen stains was DQα3,4. In addition, the analysis of the victim's mother (DQα2,3) and father (DQα3,4) revealed that the DQα3,4 type but not the DQα 1.1,2 type is compatible with potential children of these parents. Thus, it is likely that these three incorrect reference stains were inadvertently packaged with the reference materials from the victim since the genetic types match neither the victim nor the suspect. The suspect was convicted of rape/homicide and the admissibility of the DQα typing was upheld, on an appeal, by the Virginia State Supreme Court.

[7]This ambiguity was the result of not using the probe GH76 (12) to distinguish these two genotypes. This probe has been used routinely since 1988. The sample has subsequently been determined to be DQα1.2,1.3.


DQα Type
Suspect 4, 4
Husband 1.1, 3
Victim 1.1, 2
Vaginal Aspirate
E Cell fraction 4, 4 [trace 1.1, 2]
Sperm fraction 4, 4
Vaginal Aspirate (outside lab)
Sperm fraction 4, 4 [weak 1.1, 2]
Semen Stain D
E Cell fraction 1.1, 2 [weak 4]
Sperm fraction 4, 4 [trace 1.1, 2]
Semen Stain E
E Cell fraction 1.1, 2 [trace 4]
Sperm fraction 4, 4 [trace 1.1, 2]
1 2 3 4 C1 1.1 1.2 1.3 4 1.3 C2
CETUS DQ-Alpha

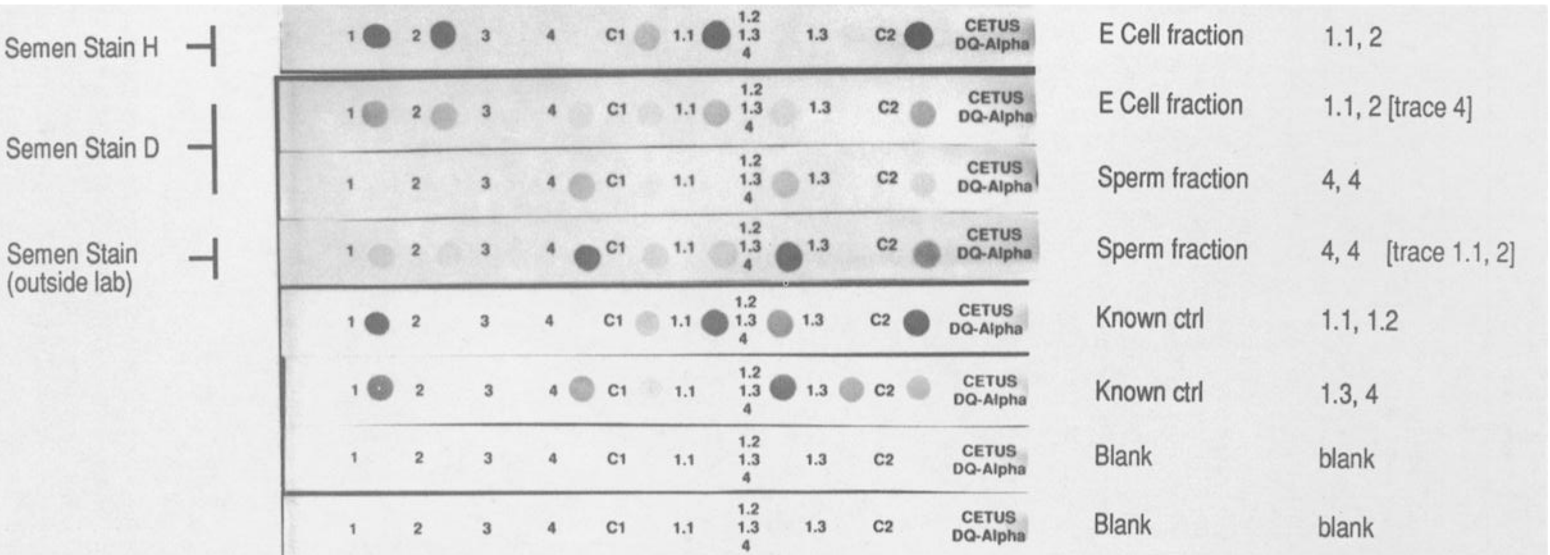


FIG. 3—*Case 4: The evidence in this sexual assault case included semen stains on hospital bedding and vaginal aspirate fluid. The sperm DNA was determined to be DQα4,4 and was adequately separated from the victim's epithelial cell DNA in most of the samples. The suspect was also determined to be DQα4,4 and therefore could not be eliminated as the source of the sperm. DNA extracted in the E cell fraction of the stained bedding samples was the same as the DQα type of the victim (DQα1.1,2), thereby confirming the association of the hospital sheets with the victim. No sperm were observed microscopically in the sperm fraction of stain H, so no typing was done. Note that for two of the samples above, the DNA was extracted by an outside lab that had failed to obtain RFLP typing results for these samples.*

TABLE 1—*DQα casework experience.*

Total No. of Cases: 198
No. of inclusions 101 (51%)[a]
No. of exclusions 56 (28%)[b]
No. of no result 37 (19%)[c]
No of inconclusive 4 (2%)[d]

[a]A case is scored as an inclusion if the DQα types obtained in the case associate any suspect(s) with the evidence.
[b]A case is an exclusion if the DQα types obtained do not associate any suspect(s) with the evidence.
[c]"No result" refers to cases where no DQα typing result was obtained for evidence samples.
[d]"Inconclusive" refers to cases where the DQα types obtained or circumstances of the case or both were such that no clear conclusion could be reached regarding the inclusion or exclusion of suspects. For example, in one case DNA extracted from an alleged saliva stain on the victim's panties was the same as the DQα type of the victim, but not of the suspect. This case was scored as inconclusive because of the possibility that the saliva stain was nonprobative.

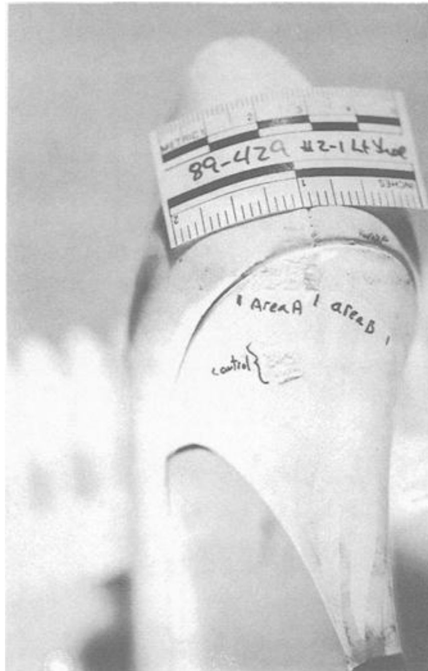


FIG. 4—*Shoes in Case 5: Photograph of the victim's shoe with semen stains. Semen stains from area A and area B were analyzed. An unstained portion of the shoe was also analyzed as a control.*

Case 7. Washington vs. Gentry

This case illustrates some of the technical complexities of PCR typing from bloodstains and hairs and some approaches to overcome these difficulties. It also illustrates the complexities of interpreting genetic typing of evidence samples. On 15 June 1988, the body of a 12-year-old Caucasian female was discovered in a wooded area in Kitsap County, Washington. A black male was identified as a suspect. Shoes recovered from the suspect

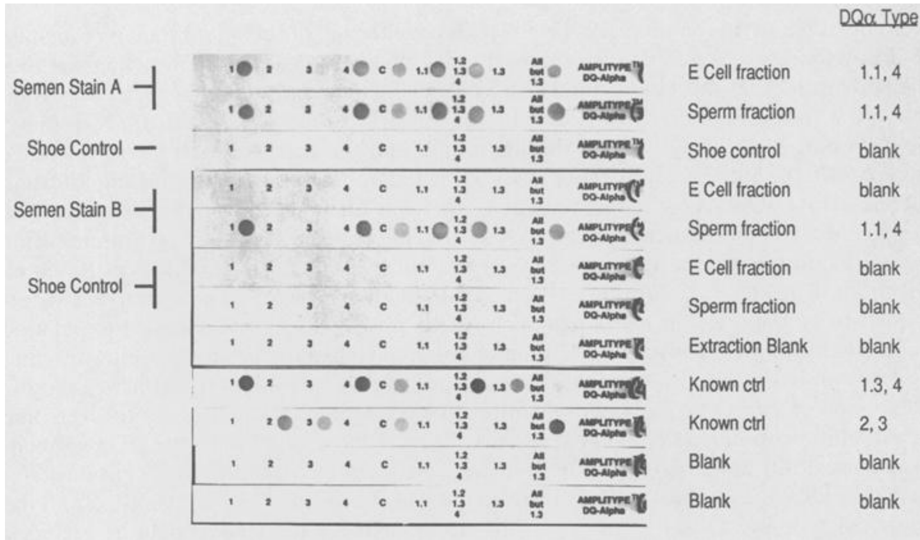


FIG. 5—*Case 5: DNA was extracted from semen stains found on the victim's shoes. A photograph of this shoe is shown in Fig. 4. The results above indicate that the sperm DNA was DQα1.1,4 for both stains A and B. The suspect had previously been determined to be DQα1.1,4 (data not shown), and therefore could not be eliminated as the source of the sperm. Note that the E cell fraction for stain A also types as DQα1.1,4; the E cell fraction can reflect the sperm type when there are so many sperm or so few epithelial cells that sperm DNA predominates in the epithelial cell fraction, even after differential extraction (see also Table 3).*

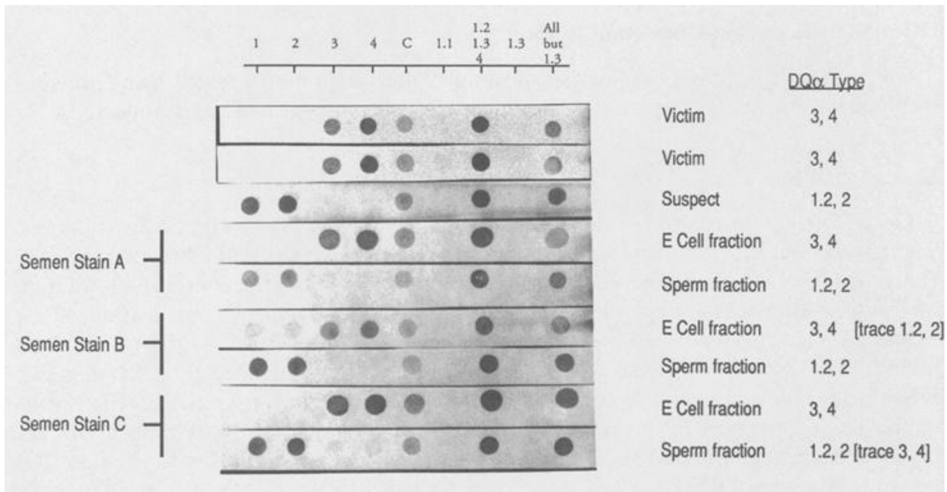


FIG. 6—*Case 6,* Virginia v. Spencer: *A 15-year-old female was found strangled in her bed, and a sexual assault was suspected. The figure above shows DQα typing results from bloody semen stains found on the bed sheets. The sperm DNA was adequately separated from the female's epithelial cell (and presumably, blood cell) DNA, and typed as DQα1.2,2. The suspect was determined to be DQα1.2,2 and therefore could not be eliminated as the source of the sperm.*

contained blood on the shoelaces (Fig. 7). Genetic testing of the blood stain by the State Patrol Laboratory in Seattle using conventional blood group and protein typing methods demonstrated that the blood could not have originated from the suspect but was compatible with the victim. A few Negroid hairs were recovered from the victim's clothing. DQα typing was carried out on the bloodstain and the hairs, as well as on reference samples. In the initial analysis of the DNA extracted from both shoelaces, no amplification of the HLA-DQα segment was obtained. This was attributed to inhibition of the *Taq* polymerase by bloodstain material removed from the shoelaces. Additional protein typing of the bloodstain on the left shoelace, carried out by B. Wraxall (Serological Research Institute, Richmond, California), revealed haptoglobin type 2-2. The victim was determined to be haptoglobin type 2-2, which occurs in ~30% of the Caucasian population [*43*], and the suspect was typed as haptoglobin 2-1. Several months after the initial attempt at DQα amplification, PCR amplification of the same sample was carried out again using 12.5 units of *Taq* DNA polymerase (instead of the usual 2.5 units) to try to overcome the inhibition observed in the initial reaction.[8] Under these conditions, the DQα segment was successfully amplified from the shoelace bloodstain DNA and the PCR product was typed as DQα1.2,3 (Fig. 8). The victim's type was determined to be DQα1.2,3 and the suspect's type was DQα1.2,1.3. Thus, the suspect but not the victim could be excluded as the source of the shoelace bloodstain. The frequency of the DQα1.2,3 genotype in the Caucasian population is ~7% [*18*]. The combined genetic analysis (DQα plus haptoglobin) of the shoelace bloodstain gave a probability that 2% of the Caucasian population would also match the observed genetic pattern.

The DQα typing of a bloodstain on the suspect's pants pockets as well as hairs found on the victim revealed that the genotype of these samples matched neither the suspect nor the victim. The Negroid hair typed as DQα1.2,1.2, distinct from the suspect's reference sample. However, the clothes worn by the suspect during the alleged assault belonged to his brother, who was typed as DQα1.2,1.2. Thus, with the exception of the shoelace bloodstain, the analysis of these evidence samples was not probative. In this case, the evidence was admitted and the suspect was ultimately convicted.

DQα Analysis in Post-Conviction Review

Given that PCR allows the analysis of samples that could not be tested using previous techniques, several cases were reviewed long after the suspect had been convicted.

Case 8. Dotson Case

Gary Dotson was accused of rape in 1977 and convicted in 1979 by the State of Illinois. The alleged victim, Cathleen Webb, recanted in 1985 saying she had fabricated the rape charge against Dotson, but her revised story was not accepted by the court. The resolution of this well-known case depended on the analysis of an 11-year-old semen stain. DNA extracted from this stain had previously been analyzed by Jeffreys [*44*] using an RFLP method but yielded inconclusive results. This result was not unexpected as the genomic DNA from old (> 3 years) semen stains is usually degraded (< 5-kb MW). The results of the DQα typing on DNA extracted from this stain and from the reference samples have been reported previously [*5*]. The reference samples from the alleged assailant (G. Dotson) type as a DQα1.1,4 and those of the alleged victim (C. Webb) as DQα1.1,3. The sperm fraction from several different semen stains (now about 11 years old) was

[8]The addition of more *Taq* polymerase (up to 24 units) has been shown not to affect DQα oligonucleotide typing results (Walsh, unpublished). Dilution of the sample DNA is also an approach to overcoming *Taq* polymerase inhibition.

FIG. 7—*Shoelaces in Case 7: Shown is the bloodstained portion of the left shoelace. The bloodstain is visible at about the 1-in. (2.5-cm) mark in the photograph.*

	DQα Type
VICTIM	1.2, 3
SUSPECT	1.2, 1.3
SHOELACE, RT BLOODSTAIN	1.2, 3
SHOELACE, CTRL	WEAK 1 *
SHOELACE, LT BLOODSTAIN	1.2, 3
SHOELACE, CTRL	BLANK
KNOWN CTRL	1.1, 1.2
KNOWN CTRL	1.3, 4
BLANK	BLANK
BLANK	BLANK

FIG. 8—*Case 7: DNA extracted from bloodstains on the suspect's shoelaces had the same DQα type as that of the murdered victim (DQα1.2,3). The shoelace control samples were cuttings adjacent to the bloodstains on the shoelaces. A very small unknown stain was present on the right shoelace "control" sample (denoted by an asterisk above). A very weak DQα type could be identified following amplification and typing of this sample. The other shoelace control sample gave no DQα type.*

typed as DQα2,3, consistent with the boyfriend's (D. Beirne) DQα type and with the Webb recantation. Also analyzed was a DNA sample prepared by Jeffreys from the evidence semen stain which yielded no RFLP pattern. This sample was typed as DQα2,3 but shows some contamination with Webb's vaginal epithelial cells, which were not separated from the sperm in this preparation. About a year after these results were obtained, Gary Dotson's conviction was overturned.

Case 9. West Virginia v. Woodall

This case involves two separate sexual assaults against two women in West Virginia on 22 Jan. 1987 and on 16 Feb. 1987. A suspect was convicted of both incidents and has been incarcerated since 6 March 1987. The decision was based, in part, on serological typing which failed to exclude the suspect as the sperm donor in both cases. For the first victim, only ABO typing was informative. For the second victim, ABO and PGM typing were informative; for these types there was a probability of ~2% that the suspect would have the same types by chance [*43*], although in the original trial it was represented that the chance was 0.06% [*45*]. The decision was appealed [*45*] and, in response to a request for DNA testing, vaginal swabs from both victims as well as semen stains were sent to Cellmark Corporation (Germantown, Maryland); they reported "insufficient high molecular weight DNA" was obtained for RFLP testing. It was then requested that PCR-based typing be applied to the appropriate specimens to determine whether or not the suspect could be eliminated as a potential sperm donor in these two cases. The sperm DNA from vaginal swabs and from five different semen stain areas on the panties of the first victim was separated from the epithelial cell DNA and determined to be DQα3,4. The victim's reference sample typed as DQα1.2,3. The sperm DNA from the vaginal swabs and from three separate semen stains on the second victim's skirt typed as DQα3,4. The second victim's reference sample typed as DQα1.2,4. Thus, the DQα type of the sperm donor in both cases is DQα3,4, a genotype which occurs in ~11% of the Caucasian population [*18*]. The suspect was determined to be DQα2,3 and is, therefore, eliminated as the source of the sperm for either set of samples. In this case, the conviction was overturned.

Casework Summary Data

The PCR-based DQα oligonucleotide typing method was used to analyze biological evidence in the 198 cases tabulated here, as well as in over 50 more recent cases not included in this analysis. Thus far, over 2000 evidence samples (bloodstain, hairs, semen stains, bone fragments, and tissue) and reference samples have been analyzed in these >250 cases. As shown in Table 1, 28% of the cases resulted in an exclusion of the suspect, 51% in an inclusion, and the rest of the cases either gave no result for the evidence samples (19%), or were inconclusive (2%).[9] This ratio of inclusions to exclusions of about 2 to 1 is similar to the inclusion/exclusion rates obtained by other laboratories practicing RFLP DNA typing [*46,47*]. (The DQα test is expected to give only a slightly lower exclusion rate than the more discriminating multi-locus RFLP analysis.) Many of the cases described here involved the exclusion of some suspects and the inclusion of one as the potential donor of the relevant biological evidence. In this circumstance, the case is scored as an inclusion for this summary.

Table 2 shows the typing success rates for the three most commonly encountered types of case work samples; single hairs, sperm and bloodstains. Bloodstains submitted as reference samples were not included. Most cases discussed here involve evidence specimens for which RFLP results are either negative or unlikely. Table 2 shows the success rates for cases done before 1 Jan. 1990 and after this date for comparison (more efficient amplification and extraction protocols were incorporated after approximately 1/90). The success rate for single hairs (34 to 38%) is relatively unchanged before and after 1/90. Many of these hairs were shed hairs (telogen phase hairs), which have previously been shown to contain much less DNA than forcibly pulled hairs [*48*]. The best typing success rates were sperm typings from sexual assault samples; the success rate for these samples

[9] "Inconclusive" refers to cases where the DQα types obtained or circumstances of the case or both were such that no clear conclusion could be reached regarding the inclusion or exclusion of suspects. See Table 1.

TABLE 2—*Typing success rate for single hairs, sperm, and bloodstain samples submitted in casework at FSA.*[a]

	Before 1/90		After 1/90	
	Total No.	No. Clearly Typed	Total No.	No. Clearly Typed
Single hairs	183	69 (38%)	85	29 (34%)
Sperm	233	166 (71%)	80	74 (93%)
Evidence bloodstains	76	23 (30%)	42	26 (62%)

[a]The success rates in the table were determined by counting the number of casework samples analyzed for which DQα typing results were obtained. Amplification of some samples (for example, bloodstains containing inhibitors of *Taq* polymerase) was attempted more than once. Shown are success rates for casework samples analyzed before 1 Jan. 1990 and samples analyzed after 1 Jan. 1990.

improved from 71% before 1/90 to 93% after 1/90. Samples which were microscopically found to contain fewer than 100 sperm total were generally not attempted. A dramatic improvement in typing success rate was achieved for evidence bloodstains for samples analyzed after 1/90, from 30 to 62%. This improvement is attributed to new extraction protocols designed to remove more efficiently porphyrin compounds thought to inhibit the PCR reaction (specifically *Taq* polymerase) [*49*]. This improvement is also attributable to the generally successful practice of adding a dilution of the DNA extract to the PCR mix if no result is obtained on the first amplification attempt. The purpose of diluting the DNA extract is to reduce the quantity of inhibitor added to the PCR reaction. Another approach is to use additional *Taq* polymerase (see Casework, Case 7).

In general, any study which involves the analysis of samples for which a particular type is expected can serve to validate the test. A valuable source of evidentiary stains for such comparisons are the vaginal (or rectal) epithelial cells differentially extracted from semen stains in sexual assault evidence. The genotype of this material would be expected to match that of the victim's reference sample. A comparison of victim reference type with evidence epithelial cell fractions is shown in Table 3. In each of the 180 cases where a typing result was obtained, the E-cell type could be accounted for as originating from the victim (152 cases), the sperm donor, or a mixture of both. There were no samples where the type obtained for the epithelial cell fraction could not be accounted for by either the victim's reference samples (Category I in Table 3), the sperm DNA type (Category III), or a mixture of the victim's reference type and the sperm DNA type (Category II). Thus, in the comparisons of the DQα type of the epithelial cell fraction with that of the victim's reference sample, no exceptions in the 180 samples tested were observed.[10]

In another comparison, the allele frequencies were calculated for casework sperm samples assumed to be from Caucasian donors, (that is, those samples with the same DQα type as a suspect known to be Caucasian). The allele frequencies for these casework sperm samples do not differ significantly from the allele frequencies found in a Caucasian population [*18*]. These results are expected, assuming that Caucasian sperm donors in

[10]There were two apparent exceptions that were explained by sample mixups. In one exception, as was confirmed by a variety of genetic tests, it turned out that the victim's reference sample initially provided was not from the victim (see discussion of *Virginia v. Spencer* above). The correct reference sample proved to have the same DQα type as the typing of the vaginal epithelial cells. This DQα genotype was also compatible with the genotype of the victim's parents. In the other exception the victim's blood sample was labeled (by the lab that collected it) with the suspect's name and the suspect's blood sample was labeled with the victim's name. The mixup was shown by typing saliva stains from both individuals.

TABLE 3—*DQα typing concordance study: comparison of victim reference type with evidence epithelial cell fractions of sexual assault samples.*

	No. of Samples
Category I[a]	152
E-cell type same as victim	
Category II[b]	16
E-cell type a mixture; subtraction of sperm fraction type gives victim's type	
Category III[b]	12
E-cell type same as sperm fraction type, not victim's type	
Category IV[c]	0
E-cell type differs from both victim and sperm fraction type	

[a]Category I describes the expected result in which the DQα type obtained from the epithelial cell fraction of a sexual assault sample is the same as the victim's DQα type.

[b]Categories II and III may be explained as instances of incomplete separation of cell types in which sperm DNA type is represented in the epithelial cell fraction. For Category III, there may be so many sperm or so few epithelial cells that even after the cell separation, sperm predominate in the epithelial cell fraction.

[c]Category IV represents the unexpected result in which the DQα type for the epithelial cell fraction cannot be accounted for by either the victim or the sperm DNA.

sexual assault cases have the same distribution of DQα alleles as the general Caucasian population, and indicates, to the extent possible with this small sampling, the absence of any systematic typing problem with the casework evidence.

Data on the genotypes (homozygote or heterozygote) of evidence samples was used to test the assumption that the distribution of genotypes found in these samples are independent of whether or not the evidence DQα type leads to an inclusion or an exclusion for that case. Because a typing error is more likely to result in a false exclusion rather than an inclusion, mistypings as a result of some systematic typing error would be expected to distort either the number of heterozygotes or homozygotes for cases of exclusion. Table 4, however, shows that the percent heterozygotes found in cases of inclusion (80%) is not significantly different from the percent heterozygotes found in cases of exclusion (73%).

Approximately 70% of the cases were done at the request of the prosecution, and the other 30% were done for the defense. Table 5 shows that the proportion of inclusions (65%) and exclusions (35%) is the same for cases done at the request of the prosecution and the defense.

As of September 1991, the HLA-DQα test has been introduced as courtroom evidence into 44 cases and has been evaluated in 25 admissibility hearings in 20 different states,

TABLE 4—*Comparison of percent heterozygote and percent homozygote genotypes for cases of inclusion versus exclusion.*[a]

Inclusions:	
No. of heterozygous	82 (81%)
No. of homozygous	19 (19%)
Exclusions:	
No. of heterozygous	41 (73%)
No. of homozygous	15 (27%)

[a]Shown are the number of cases (and %) of each category (see Table 1) in which the genotype of the evidence sample was heterozygous or homozygous for DQα.

TABLE 5—*Comparison of number of cases of inclusion and exclusions (and %) for cases done at the request of the prosecution versus defense.*[a]

Prosecution	107
Inclusion	70 (65%)
Exclusion	37 (35%)
Defense	48
Inclusion	31 (65%)
Exclusion	17 (35%)

[a]Not shown are cases giving either no result, or an inconclusive result. Prosecution includes cases dealt with at the request of a district attorney, investigator, or crime laboratory personnel. Two cases not included could not be categorized as prosecution or defense.

as shown in Table 6. In 23 hearings, it has been admitted and, in the case of *Virginia v. Spencer*, this ruling was upheld by the Virginia Supreme Court. In two hearings in California, the test was ruled inadmissible. In one of these two cases (*California v. Martinez*, 1989), in which the test was opposed by the prosecution, the defense called only a single expert witness. In the other case (*California v. Mack*), the test, which "included" the suspect, was opposed by the defense. After the test was ruled inadmissible, the suspect admitted during sworn testimony before the jury to having consensual sex with the victim; he was convicted of rape and murder.

Discussion

The PCR-based DQα oligonucleotide typing method has been used to analyze biological evidence in over 250 cases thus far. Approximately 70% of these analyses were carried out at the request of the prosecution and 30% for the defense (Table 5). Of the first 198 of those cases giving conclusive results, 35% resulted in an exclusion of the suspect and 65% in an inclusion (Table 1). Cases that involved the exclusion of some suspects and the inclusion of one as the potential donor of the relevant biological evidence were considered inclusions. The percent inclusions were the same (65%) for cases done at the request of either the prosecution or the defense. This rate of exclusion is similar to that reported by other labs using RFLP DNA analysis, both in this country and in England [*46,47*]. Many explanations may account for this high rate, for example, lack of relevance of the evidence to the crime [*50*], or, in sexual assault cases, failure of the rapist to ejaculate and recent sexual activity of the victim [*51*]. However, the possibility must be considered that, in the absence of DNA typing, some innocent people are being convicted. In addition, given that it is possible to do PCR analysis on samples that are years (if not centuries) old, PCR analysis will have great impact upon the post-conviction review of cases. Two case examples, both of which exclude the suspect, are given in this paper. As of September 1991, the DQα test has been introduced as courtroom evidence into 44 cases and has been evaluated in 25 admissibility hearings in 20 different states (Table 6). In 23 hearings, it has been admitted and, in the case of *Virginia v. Spencer*, this ruling was upheld by the Virginia Supreme Court. Standards for the legal admissibility of scientific evidence differ from state to state [*52*].

One issue that has been frequently raised in these hearings is that DNA used in forensic science analyses is inherently different from DNA from clinical samples. It has been claimed that, unlike DNA from clinical samples, DNA isolated from biological evidence samples is often degraded, modified, present in small amounts, and sometimes present as a mixture of two genotypes (for example, semen stain with cells from the female and sperm from the male). None of these properties, however, are unique to forensic science samples. Impraim et al. used PCR to analyze the β-globin genotype of degraded and probably modified DNA from paraffin pathology sections [*53*] and Shibata et al. used

the DQα typing system to examine the identity of several histologic sections [28]. Prenatal HLA-DQα typing of amniocytes has also been used to determine whether the fetus could serve as an HLA-matched bone marrow donor for a sibling with Franconi's anemia [29], and chorionic villus biopsies have been used to screen first trimester fetuses for cystic fibrosis [54,55]. Amniotic fluid samples typically contain very few cells (usually insufficient for RFLP analysis) and are often contaminated with maternal cells, as are some chorionic villus biopsies. HIV status has also been determined from months-old, discarded needles [56].

Another issue that has arisen in some cases is the ability to analyze mixed genotypes in biological evidence. As would be expected, heterozygotes (for example, 2,4) cannot be distinguished from a 50-50 mixture of two homozygotes (for example, 2,2 and 4,4) because the intensities of the 2 and 4 dot would be equivalent. This problem is, of course, not unique to PCR and would be true for any genetic test, including RFLP analysis. However, a sufficiently unequal mixture of these homozygotes could be distinguished from a true heterozygote because the 2 dot would be significantly lighter or darker than the 4 dot. The analysis of mixed genotypes in known proportions has shown (Figs. 1 and 2) that, although not strictly quantitative, the DQα test can frequently distinguish genotypes mixed in unequal proportions. In any case, an investigator should always be alert to the possibility of a mixture. For some kinds of biological evidence samples, such as seminal stains or vaginal swab samples, a genotypic mixture is not unexpected. In these, the source of the cells "contaminating" the sperm can usually be assumed to be the victim. Casework analysis of individual hairs has also shown that a contaminating genotype

TABLE 6—*PCR DNA court cases. HLA DQα forensic DNA amplification and typing.*

Case	Outcome
Alabama v. Don Curry Alexander	PCR analysis admitted without objection/grand-jury testimony.
Alabama v. William Slagle	PCR analysis admitted without objection.
Alabama v. Emmitt Wright, Jr.	PCR analysis admitted without objection.
California v. Julian Covington	PCR analysis admitted without objection.
California v. Paul Mack	DNA evidence opposed by the defense. Evidence not admitted after Frye hearing.
California v. Martinez	DNA evidence was successfully opposed by the Los Angeles District Attorney's Office. A single expert witness was called during the Frye hearing (1989).
California v. William Mello	DNA evidence opposed by the prosecution, admitted after a Frye hearing.
California v. Moffett	PCR analysis admitted after a Frye hearing.
California v. LaPeer Moore	PCR analysis admitted after a Kelly/Frye hearing based on *CA v. Mack and Moffett* transcripts.
California v. Quintinilla	PCR analysis admitted after a Frye hearing.
California v. Theodore Scott	RFLP elimination (Cellmark) for prosecution; PCR elimination for defense. No Frye hearing; defendant acquitted.
Colorado v. Vincent Groves	Testimony regarding PCR analysis. Defendant waived Frye hearing, although the Frye issues were addressed during the trial.
Florida v. Benjamin Hankerson	PCR analysis admitted without objection.
Florida v. Timothy Ray Perry	DNA evidence admitted after stipulation.
Florida v. Robert Beeler Power	DNA evidence admitted without objection.
Kansas v. Jimmy L. Searles	DNA evidence admitted after a Frye hearing.
Louisiana v. Robert Houghtion	PCR analysis admitted without objection.
Massachusetts v. Douglas	PCR and RFLP analysis admitted after a Frye hearing.
Michigan v. Albert Lee	PCR analysis admitted after a Davis/Frye hearing.
Michigan v. Lamont Marshall	PCR analysis admitted after a Davis/Frye hearing.
Mississippi v. Wilson Young	PCR analysis admitted without objection.

TABLE 6—*Continued*

Case	Outcome
New Jersey v. Richard C. Williams	PCR analysis admitted after a Frye hearing.
New York v. Jeffrey Williams	DNA evidence admitted, not opposed by the defense.
Ohio v. Steve Durbin	DNA evidence admitted after an admissibility hearing.
Ohio v. David Penton	PCR analysis admitted after a relevancy hearing.
Ohio v. Harvey Stafford	Suspect eliminated as sperm contributor. Case dismissed by State.
Oregon v. Robert Lyons	DNA evidence admitted after an admissibility hearing.
Pennsylvania v. Lloyd James	DNA testing performed for the public defender; results were subpoenaed by the prosecutor.
Pennsylvania v. Pestinikas	First United States criminal case in which DNA evidence was used (1986).
South Dakota v. Carl Stevens	Typing compatible with victim's husband.
Texas v. Jeffrey Balawajder	DNA evidence admitted after a Frye hearing.
Texas v. Matthew Clarke	DNA evidence admitted after a Frye hearing.
Texas v. Richard Danziger	DNA evidence admitted, not opposed by the defense.
Texas v. Tyrone Fuller	DNA evidence admitted after an extensive Frye hearing.
Texas v. Ector Garza	PCR analysis admitted after a Frye/relevancy hearing.
Texas v. Hunt	PCR analysis admitted without objection.
Texas v. David Lopez	DNA evidence admitted after a Frye hearing.
Texas v. Frank B. McFarland	DNA evidence admitted after a Frye hearing.
Texas v. Ronald S. Trimboli	DNA evidence admitted after a Frye hearing.
Virginia v. Timothy Spencer	DNA evidence admitted after a Frye hearing. **Decision upheld in Virginia Supreme Court (Record 900001 & 900002 6/8/90).**
Virginia v. Yeager	PCR analysis admitted without objection.
Washington v. Jonathon Gentry	PCR analysis admitted after an extensive Frye hearing.
Wisconsin v. Robert Wirth	PCR analysis admitted after a Frye/relevancy hearing.
West Virginia v. Glen Dale Woodall	Conviction and sentence vacated after a *Frye/Habeas Corpus* hearing.
Total cases to date: 44 (as of September 1991)	

is occasionally found. Often, this DQα type corresponds to that of the victim, as in the Fuller case (see above) and in several others.

Another issue of concern to forensic scientists is the effect of contaminating DNA of human origin on the genetic type obtained from a forensic science specimen. (In some cases, the crime scene specimen consists of a mixture of body fluids, like blood and saliva, from different individuals. The analysis of mixed samples is discussed above.) Contaminating material could, in principle, be introduced by handling the specimens, from another specimen sample, or from the PCR products of a previous reaction (PCR product carryover). The procedures and protocols for minimizing cellular contamination and PCR product carryover have been detailed elsewhere [*57,58*]. Experiments designed to detect potential contamination from specimen handling have revealed no contribution of the genotype of the individual handling the specimen [*32*]. In the case of sperm samples, contaminating nonsperm cellular material from handling would be expected to be found either in the E-cell fraction or to be washed from the sperm pellet in the differential extraction procedure. In general, contamination can be revealed by the presence of more than two alleles and is monitored by running negative controls. Our casework experience indicates that the above-mentioned procedural precautions and controls have proved effective.

In conclusion, we have found PCR-based DQα testing to be a very useful tool for the analysis of biological evidence. Most of the more than 250 cases that have used PCR-based DQα typing have involved samples that could not be analyzed by either RFLP typing or by conventional genetic marker tests. The availability of this simple test makes

possible the genetic analysis of virtually all forms of biological evidence and promises to have a major impact in forensic science.

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