GENETIC ANALYSIS OF DNA IN BIOLOGICAL EVIDENCE
NIJ Grant 86-IJ-CX-0044

FINAL REPORT

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OVERVIEW

SUMMARY OF RESEARCH FINDINGS

A. Investigation of PCR for DNA Amplification
   1. Sample Preparation
   2. Differential Extraction
   3. Fidelity of Amplification
   4. Mixing Experiments
   5. Effects of Primer Mismatch
   6. Direct DNA Sequencing

B. Studies on Special Categories of Evidence
   1. Hair
   2. Postmortem Tissues
   3. Saliva Traces in Bitemarks, Envelope Lickings
   4. Urine
   5. Insect Bloodmeals

C. DNA Damage Studies
   1. Experimental Approach
   2. Degradation
   3. Damage by Ultraviolet Radiation
   4. Template Jumping in PCR
   5. Band Shifting in RFLP Analysis

D. Development of Typing Systems
   1. General Comment
   2. Group Specific Component (Gc)
   3. Cytoplasmic Acid Phosphatase (ACP1)
   4. Y Chromosome Detection
   5. Short Tandem Repeat (STR) Polymorphisms

APPENDICES

I. Publications
II. Presentations
III. Post-Doctoral and Student Research Supported
IV. Visiting Scientists

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OVERVIEW

The broad objective of this grant project was to advance the introduction of DNA technology into forensic science. At the time of the beginning of the grant period (1986), the dominant approach to genetic analysis at the DNA level was detection of restriction fragment length polymorphism (RFLP). Leading forensic laboratories in the U.S. and abroad were initiating efforts to bring RFLP analysis into forensic practice. We projected that the second generation of DNA analysis methods would be centered on the use of the polymerase chain reaction (PCR), a technique for selectively replicating short segments of DNA sequence. PCR offered a number of potential benefits for the analysis of biological evidence:

- genetic typing could be done on samples containing too little DNA for RFLP analysis
- genetic typing could be done on samples containing DNA too degraded for RFLP analysis
- PCR based genetic typing can be done by methods not requiring the use of radioactive isotopes
- PCR can be used to amplify any genetically informative sequence segment, thus making accessible for analysis the whole variability of the human genome
- PCR is an automated process and can be coupled to automated detection systems
- PCR based genetic typing can be done in a short time frame, often 24-48 hours

Accordingly, we focused most of our research effort on the use of PCR and PCR based technology.

The research effort was divided into four areas. The first involved investigations relevant to the application of PCR in the forensic context. The second area included studies on categories of evidence for which PCR might be particularly advantageous, i.e. evidence typically containing very small amounts of DNA and/or degraded DNA. The third addressed a particular potential problem for forensic DNA analysis, the consequences of chemical damage to DNA on the reliability of genetic typing. The last research area centered on the development of PCR based genetic typing methods. The research findings in each of these areas are summarized in the following sections.

Much of the work described here has been published at least in summary form if not in detail (see appendix I) and most has been presented at professional meetings (see appendix II). A series of text chapters (numbers 9, 29, and 34 in the publications list, appendix I) and a review article (number 30, appendix I) provide summaries of our research in the context of the broader picture of the application of PCR to biological evidence analysis;

these, particularly the last (#34), serve to some extent as parallel reports to this final report. Additional publications based on this grant supported research will be forthcoming.

A significant portion of the research described herein was undertaken by two postdoctoral fellows and by graduate students in our degree program; many have gone on to careers in forensic science (see appendix III) and continue to make contributions to the field. This, as much as the research findings, are a legacy of this grant project.

In addition to the research effort, a specific aim of the grant project was to help introduce DNA analysis into practicing forensic laboratories. This aim was addressed at three levels. First, the principal investigator, postdoctoral fellows, and students participated in workshops introducing DNA analysis methods to forensic practitioners (see appendix II); some of these were hands-on workshops. Second, arrangements were made with three local forensic laboratories - the Oakland Police Dept. Criminalistics Laboratory, the Contra Costa County Sheriff's Office Forensic Laboratory, and the California Dept. of Justice DNA Laboratory - to have some of their personnel work for varying periods of time in this laboratory. Forensic biologists from two of these laboratories and one from another local forensic laboratory entered our program as students and participated in various research projects. Finally, we were visited for periods of a week to several months by forensic scientists from the U.S. and abroad (appendix IV); short term visitors gained exposure to the technology and longer term visitors engaged in short research projects.

SUMMARY OF RESEARCH FINDINGS

A. Investigation of PCR for DNA Amplification

1. Sample Preparation

In working with samples with very small amounts of DNA, it is important to minimize DNA loss during purification steps. Prior to the beginning of the project, we had found that the use of centrifugation microdialysis cartridges (e.g., Centricon tubes) provided better and more consistent yields than the traditional ethanol precipitation method. These cartridges are expensive, however, and so an alternative approach using a solid phase extraction system (Geneclean) was investigated. In general, we found the Geneclean method to be faster than the Centricon method and to concentrate the DNA in smaller volumes. However, we encountered lot to lot variation with Geneclean resulting in inconsistent yields with occasional DNA loss. Accordingly, we continued with the Centricon method whenever we worked with samples with low DNA levels.

2. Differential Extraction

The development of the differential extraction procedure for separating sperm DNA from epithelial cell DNA is one of the corollary advances associated with DNA typing methods. We have undertook a study to investigate parameters of this procedure with the following findings. (1) The DNA in sperm heads stripped of protecting membrane by detergent and/or protease treatment is not available to attack by DNAses. This indicates that the protein packing around the sperm DNA is very protective. (2) Most bacterial and yeast DNA fractionates with the epithelial cell DNA in the differential extraction. (3) In case material, the efficiency of the differential extraction is good. Sperm DNA contaminates the epithelial cell fraction between 10 and 30% of the time, depending on the analyst. Epithelial cell DNA contaminates the sperm DNA only about 10% of the time. (4) In test tube experiments, prolonged first step digestion of sperm does not release sperm DNA. This is in apparent contrast with evidence samples, suggesting the exposure to the vaginal fluid environment somehow "softens" the sperm; the biochemical basis of this "softening" was not identified.

3. Fidelity of Amplification

An initial concern about PCR was the possibility that errors introduced during the replication process might result in PCR products with incorrect sequences. This would be a problem for genetic typing if and only if particular sequence errors occurred with such frequency that one genetic type might be converted to another. The misincorporation rate for Taq polymerase has been determined to be about 2×10^{-4} per nucleotide per cycle, i.e., one misincorporation per 5000 nucleotides. It has been demonstrated by calculation that this misincorporation rate, coupled with the random location of any misincorporated base, would not produce deviant amplification products leading to erroneous typing. Nevertheless, we attempted to test whether we could force errors to occur by selectively amplifying deviant products; our logic was that if we could not force amplification errors by strong selection for error, then we can discount the possibility (however remote) of naturally occurring error.

The design of the error selection experiment was as follows. Human hemoglobin A sequence was amplified through 50 cycles. For the first 30 cycles, the PCR product was treated every 5th cycle with a restriction enzyme that would cut products containing correct sequence thus disabling these products as templates for subsequent amplification. PCR products containing misincorporations at the restriction site, however, would escape cleavage and remain as templates for subsequent amplification. One of the 12 possible error sequences is the sequence for hemoglobin S; specific probing for this sequence was used to test for the production of errors. We were not able to detect any hemoglobin S sequence, indicating that base misincorporation by polymerase in PCR, even under these forced conditions, did not lead to genetic typing error.

4. Mixing Experiments

Simple mixing experiments using homozygote DNA samples mixed in differing proportions; these were amplified, and typed for DQA using the direct dot blot system. The typing results reflected the initial proportions, e.g., samples mixed 1:1 yielded spots of equal intensity, 1:4 mixes gave spots in 1:4 proportion, and so on. These results show that the final product yield is roughly proportional to the proportions of starting templates.

5. Effects of Primer Mismatch

The specificity of PCR resides in the specificity of the primers for the target sequence. It was thus of interest to investigate the consequences of primer mismatch. We designed a series of primers differing from the template sequence at one or two bases in various positions of the primer sequence and tested each under conditions of varying cycle number and annealing temperature. Our results indicate: (1) As a general rule, the closer the mismatch is to the 3' end of the primer, the greater the effect on amplification; a mismatch at the 3' end of the primer blocks amplification. (2) At a standard annealing temperature of 55°, multiple mismatches decrease PCR efficiency. At lower annealing temperatures, the effects of mismatches may be diminished, depending on their position. (3) Increasing the number of cycles can bring mismatch products up to the same level as control. (4) Reduced annealing temperatures diminishes the effects of mismatches but often with the trade off that there is increased nonspecific amplification. Overall, there was no indication that primer mismatches can confound genetic typing provided standard PCR conditions are used.

6. Direct DNA Sequencing

Many research groups were investigating approaches that would couple PCR to sequence determination. We tried an approach that substituted phosphorothioate nucleotide analogs for the standard deoxynucleotides in PCR; it had been previously demonstrated by Gish and Eckstein (Science 240:1520, 1988) that sequences containing phosphorothioate nucleotide analogs could be used for a one step sequence analysis. We had limited success in working out conditions for the process. Eckstein's group also worked on this approach, also with limited success. In the end, the method required almost as much work as conventional sequencing from PCR products and the results were not as clean. We did not further pursue this approach.

B. Studies on Special Categories of Evidence

1. Hair

Prior to the initiation of the grant project, the principal investigator had initiated collaborative work with Cetus on the use of PCR to amplify DNA in hair. The rationale for this work was that conventional genetic typing of hair (i.e., testing for blood group

and protein markers) was problematic at best; a demonstration that hair could be routinely typed at the DNA level would significantly improve the value of hair as evidence. Much of our work in the first two years of the project focused on various aspects of hair analysis.

a. General studies. Over 250 hairs from more than 20 donors were collected, classified according to morphology, and extracted for DNA. About 10% of the hairs were photographed to document hair root morphology and size; the root areas of the remainder were measured under the microscope. The hair collection contained hairs from different parts of the body, plucked and fallen out hairs, and fallen out hairs of different ages (i.e., hairs removed from brushes and clothing). The samples were typed for DQA using direct dot blotting. These studies provided a core of basic information about DNA typing in hair; some of these findings led to more detailed studies described in following sections.

1. DNA extraction experience counts: our least experienced person got typable DNA about 30% of the time whereas the success rate of our most experienced person was over 60%.
2. The two methods used for DNA purification, spin dialysis using Centricon tubes and solid phase isolation using Geneclean, generally yielded comparable results. (See section A.1 above for more detailed discussion.)
3. Anagen (growing) phase hairs gave a higher success rate than telogen (resting) phase hairs. This is not unexpected since the hair root in the latter is keratinized and would contain less DNA.
4. No significant differences were noted for hairs from different parts of the body.
5. In some cases, samples which contain enough DNA for amplification do seem to amplify; this appears to result from an inhibition of amplification, possibly by hair pigments.

These studies also revealed the potential of sample mixup and/or mislabeling when samples were processed in large batches; we had to discount 10-20% of our results due to obvious mixups, e.g., when one set of samples labeled to originate from one individual typed to a second and the samples labeled to the second typed to the first. These studies also marked our first encounter with contaminated equipment; a contaminated pipeter resulted in a run of samples giving a common background type. Both of these experiences occurred in the first year of the project and resulted in procedural changes to minimize the risk of their reoccurrence.

b. Quantitation. DNA in hair root material was quantitated by a modification of the fluorometric method described by Brunk, et al., Anal. Biochem. 92:497-500 (1979). In our hands, the sensitivity limit was 20 ng DNA per assay; samples containing lesser amounts of DNA were combined for measurement. Multiple hairs from multiple individuals were measured; hair root morphology and size was documented either by sketch or photography so that DNA levels could be correlated with physical dimensions. The overall results for hairs in different states are indicated in the table below:

Hair Type & Region	DNA Quantity (Avg.)	Range
Plucked, roots w/ sheaths	375 ng/hair	1 - 784
Plucked, roots w/o sheaths	54 ng/hair	43 - 64
Shed, roots w/o sheaths	3 ng/hair	0.8 - 12
Shafts	9 pg/cm shaft	0.2 - 40

These findings provide guidance to a strategy for DNA typing in hair evidence. Only hairs with ample sheath material have sufficient DNA for RFLP analysis, given the current sensitivity limits of RFLP analysis (50-100 ng DNA); about 75% of plucked hairs with sheaths contain enough DNA for RFLP analysis. For hairs with small or no sheaths, PCR offers the only viable approach. Hair shafts generally contain too little DNA for routine analysis. Given that a single cell contains about 5 pg DNA, a cm of hair contains on average somewhat less than 2 cell genome equilivents. Amplification of such a small amount of DNA introduces the risk of confounding by contaminant DNA. We offer the suggestion that hair shafts be used be used as a contaminant control when amplifying hair root DNA.

c. Hair shaft DNA characterization. Hair cuttings from several individuals (absent roots) have been extracted in bulk and the nucleic acid therein characterized for quantity and quality; quantities are reported in the table above. All samples contained a mixture of high molecular weight and degraded nucleic acid although in some cases, the nucleic acid fraction had to be concentrated to see the high MW complement. The extracted nucleic acids were found to be a mixture of DNA and RNA; the high MW fraction was DNA and the low MW "degraded" fraction was found to be predominantly RNA.

Hair shaft DNA was characterized as to nuclear and mitochondrial origin by Southern blot analysis using probes against whole nuclear DNA, against the Alu repeat of nuclear DNA, and against the D-loop of mitochondrial DNA. All three probes show signals on the Southern blots in the high and degraded DNA zones. However, the patterns do not match exactly the patterns seen with ethidium bromide staining. The shaft DNA samples have also been subjected to PCR analysis for two nuclear genes and one mitochondrial gene. Amplification was seen with some samples but not with all. The refractory samples tend to be those which contain dense pigment material; this is consistent with other observations that some pigments inhibit the Taq polymerase used in the PCR process.

d. Stability. Plucked hairs from two individuals (20 from each) were incubated for one month at room temperature (18-22°C) at 5 relative humidities (12-93%) in controlled humidity chambers. Quantitative analysis of the recovered DNA showed no significant differences between the different humidity treatments. All the samples amplified and typed without difficulty.

e. Tests on paired case samples. Head and pubic hair samples from retired cases were obtained from the Oakland Police Dept.; most dated from 1980-1983 and were at least 5 years old at the time of analysis. Many of the samples contained cut hairs and hence were of no immediate value. Samples collected from 26 individuals contained head

and pubic hairs with roots and were used for this experiment. Preliminary analysis showed that most of the hairs (ca. 75%) were anagen phase. All but one of the hairs amplified; 29 gave moderate to strong products and 22 amplified only weakly. This showed that DNA from stored hairs could be amplified. DNA typing of the hairs using a prototypic direct dot blot DQA typing system showed three patterns of results. No discordancies were seen in 13 of the 25 typable pairs. In 4 pairs, one or the other of the hairs yielded a dot blot pattern containing weakly staining dots in addition to a stronger set of dots; these weak signals were interpreted as background and the typings given by the strong patterns were concordant for each pair. The remaining eight pairs showed a discordancy due extra alleles appearing on the pubic hair sample; six of these included samples with low levels of amplification product. We believe that these pubic hair samples were contaminated by semen; in some cases, foreign material appeared to be on the surface of the hairs. This finding points out the importance of controlling for contamination in hair analysis. It is also possible that some of the hairs presumed to be from an individual in fact came from different individuals.

2. Postmortem Tissues

Postmortem tissues degrade at different rates. A small study was undertaken to determine which tissues would be best for the extraction of reference DNA from cadavers. Blood, heart, muscle, liver, spleen, hair, and bone were collected from 20 cadavers. Tissue samples were prepared by grinding in liquid nitrogen; DNA was extracted by a standard phenol/chloroform technique. Generally, the DNA extracted from liver was the most degraded and that from heart, spleen, and bone were the least. In a subsequent small study, teeth were determined to be a good source of DNA; DNA was recovered from teeth aged more than 100 years.

3. Saliva Traces in Bitemarks, Envelope Lickings

We demonstrated in the course of developing a sex identification test (see section D.4. below) that DNA could be recovered from saliva traces swabbed from bitemarks and envelope flaps.

4. Urine

Urine typically contains a small amount of cellular material and thus should be amenable to DNA typing. The practical questions are (a) how much DNA is present typically, (b) how much DNA can be recovered from stored urine samples, and (c) what state is the DNA in. We found, as have others, that centrifugation or filtration of the cellular material from fresh urine yields sufficient DNA for DNA typing by PCR based methods in most cases and for RFLP methods in many cases. However, we found the recovery of DNA from stored urine samples to be problematic. We terminated the project without a solid solution to these problems.

5. Insect Bloodmeals

The California Department of Health consulted us about a death that appeared to have been caused by an insect bite; they sought to determine whether the insect in question could be shown to have bitten (a) a human, and (b) the decedent. Analysis of the insect showed no human blood and we were unable to provide useful information. We continued with the problem to test the circumstances for species testing and genetic typing of blood meals from insects. We have demonstrated that mitochondrial DNA from blood meals can be detected in mosquitos up to three days after the bite. The blood meal DNA is degraded over this period and progressively smaller target sequences have to be used. Further, we have demonstrated that useful mtDNA cytochrome b gene sequence data can be derived from bloodmeals extracted from mosquitos which fed over 30 years before. This further demonstrates the power of PCR based genetic typing.

C. DNA Damage Studies

1. Experimental Approach

Evidence samples may experience a diverse range of environmental conditions, e.g., exposure to extremes of temperature, light, chemical contamination, and microbial contamination. Such exposure may cause damage to the DNA in the evidence material and this damage may affect typing results. Our objective in investigating DNA damage was (a) to identify the nature and extent of damage rendering the DNA untypable, and (b) to determine if any kind of damage might lead to errors in genetic typing.

Basic chemical considerations differentiate DNA damage into three general categories: strand breakage (degradation), chemical modification of nucleotides, and strand cross-linking. (Strictly speaking, strand cross-linking involves chemical modification of nucleotides; the differentiation is that cross-linking prevents strand separation whereas mere chemical modification does not.) The relationship of environmental insult to category of chemical damage is discussed in detail in two review chapters (numbers 29 and 34, appendix I). We focused on the two kinds of DNA damage judged to be of major significance in evidence: DNA degradation and nucleotide modification resulting from exposure to ultraviolet (UV) radiation.

For both kinds of damage, our general approach was to introduce damage quantitatively and assess the dose relationship on amplification and typability. Wherever possible, we made use of model systems - segments of DNA of known sequence - to measure as precisely as possible the impact of the introduced damage. Damage effects in the model systems were correlated with damage to purified whole genomic DNA ("naked DNA") and to damage in typical evidence samples, i.e., bloodstains, semen stains, etc.

2. Degradation

DNA recovered from evidence samples ranges in quality from the virtually intact to the badly degraded. Strand breakage may be caused by shearing, by chemical action, and by enzymatic action; the latter may result from digestion by endogenous or microbial nucleases. Enzymatic degradation may result in essentially random strand breaks (mediated by non-specific nucleases) or in sequence specific strand breaks (mediated by restriction nucleases). We found enzyme digestion to be the most easily controlled and used it in the majority of our experiments.

To evaluate quantitatively the relationship between degree of degradation and amplification potential, whole genomic DNA was degraded in a controlled fashion by enzymatic digestion to produce a set of samples degraded to different degrees; these were characterized with regard to the average size and distribution of fragments. Each sample was then tested for amplification for fragments of different sizes over the size range 75-2000 bp. As expected, the size of the fragment that could be amplified depended on the distribution of sizes in the template DNA.

The effects of specific nucleases, i.e., restriction enzymes, were also tested. Digestion with restriction enzymes that cut outside the region of amplification do not inhibit amplification. Digestion with restriction enzymes cutting inside the amplification region inhibit amplification. Cumulatively, these experiments indicate that target molecules containing strand breaks do not amplify.

This work is relevant to the use of PCR for the typing of the VNTR polymorphisms. In partially degraded samples, alleles of small size may amplify more efficiently than large alleles, leading to the possibility that heterozygote samples containing a small and large allele might be mistyped. This points out the importance of assessing the degradation state of DNA in evidence samples prior to genetic typing. Further, it suggests the use of a large fragment amplification control at the time of typing.

3. Damage by Ultraviolet Radiation

Damage to DNA caused by exposure to UV radiation has been extensively studied for its connection to DNA repair mechanisms and cancer. the major product of UV exposure is dimerization of adjacent pyrimidines. Dimer formation is roughly proportional to UV exposure up to a saturation dose; beyond this dose, the pyrimidine dimer level remains approximately constant. Despite much effort, we were unable to develop a reliable assay to measure dimer formation at specific sites using a dimer excision enzyme. Accordingly, we used UV exposure dose as our reference point for assessing damage.

Preliminary experiments indicated that DNA damaged by UV irradiation does not amplify. Dose effects on genomic naked DNA irradiated by short wave UV in solution showed that PCR product begins to drop off at doses above about 500 joule/m^2 and PCR product is lost above 1400-2500 joules/m^2. Typing intensity decreased proportionately. Irradiation

with long wave UV had no significant effect, demonstrating that the effect with short wave irradiation is specific.

To verify the nature of the effect, we developed a sequencing assay that allows sites of PCR chain termination to be identified. This assay shows that the PCR polymerase does not pass sites containing adjacent pyrimidines, presumably as a result of pyrimidine dimer formation. This assay has been used both with ß-globin sequences and with DQα sequences. We have also compared several polymerases with regard to UV damage effects. Taq polymerase (the one usually used with PCR) and Klenow polymerase appear to stop at the damage site whereas T_7 polymerase (Sequenase) appears to stop one base short. This reflects a difference either in the mechanism of the different enzymes or in their proof reading activity.

The effects of UV exposure on whole body fluids and dried stains show a different dose effect scale. Whole blood and semen in liquid and stain forms was exposed to measured doses of short wave (DNA damaging) UV far exceeding the doses (>10x) that render naked DNA unusable for PCR. DNA was extracted from the exposed materials, assessed for quantity and quality, and subjected to amplification by PCR. Liquid blood, liquid semen, and blood stains at all exposure levels yielded high molecular weight DNA that amplified normally. Since the highest UV exposures were more than adequate to fatally damage naked DNA, these results suggest that the protein matrix in the whole fluid materials protects the DNA from UV exposure. Semen stains exposed to damaging UV yielded reduced amounts of DNA; the reduction in yield was in rough proportion to the UV exposure level. Analysis of the extraction process showed that the semen stain DNA was being retained at the interface of the phenol-aqueous extract. This in turn suggests that the DNA is not completely stripped of protein; possibly the DNA-protein matrix is covalently cross-linked. We have run parallel experiments using long wave UV (365 nm); as with naked DNA, no effect were observed.

To further investigate the effects of UV damage, we designed two short oligonucleotide templates that contain single UV damage sites. The templates were exposed to UV and the damaged templates were separated from undamaged template. The damaged templates were subjected to a series of primer extensions under a variety of conditions. With short extension times (1 min. or less), there is no extension past the damage sites. However, with longer extension times, the primers extend past the damage sites; this is likely due to strand dissociation-association effects. The two templates behave somewhat differently; with one extension stops at the damage site whereas with the other extension stops one base short of the damage site. This difference may be accounted for by sequence differences 3' to the damage sites in the two templates.

The effects of UV damage on typing at the DQα locus was investigated as a model system. Naked DNA from six heterozygous individuals was UV irradiated at three dosage levels (30, 300, and 3000 J/M^2). The samples receiving the lowest dose showed good amplification and typed correctly; this is consistent with our previous studies which indicated that there should be little DNA damage at this dose. Moderate DNA damage is

expected at the second dose level and was observed; the samples amplified weakly and two of the samples were untypable. The third dose level gives severe damage; no PCR products were observed with any of the samples. To investigate whether the samples were irrevocably damaged, some of the amplified samples were reamplified for an additional 30 cycles. Some of these samples now showed a product; one sample (a type 3,4) showed signal loss for the 4 allele. Control experiments indicated that this could be accounted for by a stochastic effect, that is, random preferential amplification from a small number of templates. These results point out that problems might arise when the starting template number is very small (< 10 templates) and the number of amplification cycles is greatly extended past the normal number.

Parallel experiments using DQA heterozygote samples were conducted on whole semen and semen stains. Exposures up to 30,000 J/M^2 followed by amplification through the usual 30 cycles gave good levels of PCR products. All typed correctly. This further illustrates the protective effects of protein matrix in liquid and stain samples.

The observed dose effects were correlated to sunlight exposure using defined stain samples as a calibrator; our UV exposure meter could not accurately measure short wave UV in direct sunlight. Stains exposed up to 17 hrs showed little drop-off in DNA yield. At 40 hrs exposure, the yield was reduced and there was some degradation. The 110 hr samples showed only degraded DNA. Correlation of the UV lamp and sunlight exposures indicates about 12 hrs direct sunlight corresponds to about 30 J/m^2.

4. Template Jumping in PCR

It has been reported that PCR amplification of degraded and damaged DNA can result in hybrid amplification products due to template strand jumping (J. Biol. Chem. 265:4718, 1990). We designed a test system to determine (a) the conditions which allows this to occur, and (b) whether it is a practical problem in the presence of undegraded DNA. The test system required template jumping as an obligate condition for production of a PCR product. This system was evaluated directly and in competition with an intact template. The results show that the occurrence of strand jumping requires relatively high template concentrations. In the presence of intact template, the damaged template must be present at 1000x levels for the hybrids to be observed. Thus, on typical samples and under usual amplification conditions, strand jumping does not appear to be a problem.

5. Band Shifting in RFLP Analysis

Work reported by McNally et al (Appl. Theoret. Electrophoresis 1:267, 1990) indicated that band shifting in RFLP analysis was in some instances a consequence of alterations to the DNA in evidence samples. We designed a model system using lambda DNA to test whether several forms of DNA damage could give rise to band shifting. Included treatments were pyrimidine dimer formation from UV exposure, depurination by mild acid treatment, and single strand nicking. None of these treatments produced band shifting.

D. Development of Typing Systems

1. General Comment

During the first years of the grant project, the only highly informative PCR based typing system was the system developed by Cetus Corp. for the detection of allelic variants at the DQA locus in the human major histocompatibility complex. DQA has a discrimination index of 85-93% in major population groups; that is, DQA typing will distinguish two unrelated individuals 85-93% of the time. The development of the DQA system went through several stages: a direct dot blot system with radioactively labeled probes, a direct dot blot system with enzyme labeled probes, and finally the reverse dot blot system that is now commercially available. We employed DQA typing as a model system to represent generalized sequence based typing systems; most of our work with DQA employed the direct dot blot systems since that was what was available at the time.

It was evident that additional typing systems would be needed if the potential for PCR based genetic typing was to be realized. Since much was known about the many protein and blood group markers, specifically with regard to allele frequencies in different populations, we opted to pursue the development of DNA level typing systems for these markers. We selected two markers, Group Specific Component (Gc) and Red Cell Acid Phosphatase (ACP1), for initial study because both have relatively good discrimination indices. Having committed to these markers, we investigated only in a cursory way many of the other PCR based marker systems, notably, the VNTR (AMP-FLP) markers, that other research groups were looking at. We did begin to investigate in more detail, however, two of the short tandem repeat (STR) loci reported by Edwards et al (Amer. J. Hum. Genet. 49:746, 1991) since it was evident that this group of markers was likely to represent the future in forensic identity testing.

1. Group Specific Component (Gc)

Gc is well studied as a protein polymorphism. Three common alleles are present in most human populations; a number of rare variants are also recognized. Two cDNA sequences for Gc were known; they differed in sequence at multiple sites. We exploited the homology between Gc and albumin to identify potential intron positions so that primers spanning variable exons could be designed. We were then able to determine by sequencing the variable sites associated with each allele; we also determined that some of the previously reported variation was incorrect. The sequencing has been done on over 40 samples representing the common types and to a few of the rare types. The base substitutions for the three common types (1F, 1S, and 2) are at amino acid residue positions 416 and 420; the rare variant type 1A1 exhibits variation at position 429. There is restriction site variation at each of these positions, allowing the development of a simple typing system. A blind trial study has been completed in which a number of blood stain samples were successfully typed at the DNA level with no errors.

3. Cytoplasmic Acid Phosphatase (ACP1)

Over 2kb of genomic sequence containing over 90% of the coding sequence has been determined at the ACP1 locus. The gene consists of at least 6 exons spanning at least 6kb. Three sites of variation have been identified which allow the three common alleles - *A, *B, and *C - to be distinguished by a simple amplification/restriction assay.

4. Y Chromosome Detection

Detection of Y chromosome DNA in a sample indicates that the sample contains DNA from a male; failure to detect Y chromosome DNA coupled with a positive control for DNA is indicative that the sample contains DNA from a female. We exploited this approach to sex identification using a short (149 bp) repeat sequence specific to the Y chromosome (DYZ1) as the Y chromosome marker. Specific amplification of the 149 bp repeat was demonstrated by Southern blotting of the PCR product using a locus specific probe. To distinguish between negative PCR products resulting from female samples and from male samples that don't amplify, the assay includes co-amplification with DQα; the DQα amplification serves as a positive control. This assay was applied successfully to DNA extracted from bite marks and licked envelopes as well as from more conventional samples. At the time we reported this work, other groups described better sex typing systems; accordingly, we did not pursue this system further.

5. Short Tandem Repeat (STR) Polymorphisms

The extensive polymorphic variation at loci containing short tandem repeats promises to be the direction for future forensic identity testing. A key feature of STR typing systems is their ready translation to sequencing based technology; as the Human Genome Project advances, sequencing based technology is certain to become less expensive and capable of sustaining higher through-put. Sequencing based technology also allows genetic variants to be typed with single base resolution, greater resolution than is needed to distinguish the tri- and tetra-nucleotide repeat variants.

We focused our attention on two of the STR loci described by Edwards et al, HUMFABP and HUMTHO1; both these loci possess adjacent pyrimidines in their repeat unit and accordingly were good model systems for studying the effect of UV damage. we employed a sequencing system for typing; an M13 sequencing ladder was used to provide absolute allele sizing. Preliminary UV damage studies show results comparable to the results previously obtained with DQA; no spurious typings were observed. In collaboration with a visiting worker, Dr. M. Savill, population genetic data were generated for New Zealand Caucasians, Maoris, and Samoans; each population showed high heterozygosity and no deviations from Hardy-Weinberg equilibrium expectations were observed.

APPENDIX I: PUBLICATIONS

1. FORENSIC DNA ANALYSIS. C.H. von Beroldingen and G.F. Sensabaugh, California Department of Justice, Bureau of Forensic Services Tieline 12:27-44 (1987).
2. HLA TYPING OF SINGLE HUMAN HAIRS WITH ALLELE-SPECIFIC DNA PROBES. C.H. von Beroldingen, R. Higuchi, G.F. Sensabaugh, and H.A. Erlich. J. Canadian Soc. Forens. Sci. 20: 31 (1987) (abstract)
3. ANALYSIS OF ENZYMATICALLY AMPLIFIED HLA-DQa DNA FROM SINGLE HUMAN HAIRS. C.H. von Beroldingen, R.G. Higuchi, G.F. Sensabaugh, and H.A. Erlich. Amer. J. Human Genet. 41:A244 (1987). (abstract)
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In Preparation

DNA IN HAIR. R. Roby, S. Walsh, C. vonBeroldingen, and G.F. Sensabaugh.

DNA DEGRADATION AND STRAND JUMPING IN THE POLYMERASE CHAIN REACTION. M. Buoncristiani, R. Reynolds, and G.F. Sensabaugh.

DNA REPLICATION BY TAQ POLYMERASE BLOCKED AT SITES OF UV PHOTODAMAGE. M. Buoncristiani, L. Barcellos, and G.F. Sensabaugh.

MOLECULAR CHARACTERIZATION OF VARIANTS AT THE GROUP SPECIFIC COMPONENT (Gc) LOCUS. R. Reynolds and G.F. Sensabaugh.

APPENDIX II: PRESENTATIONS
(Presentations by G.F. Sensabaugh unless indicated;
presenting author underlined)

1. International Forensic Sciences meeting, Vancouver, August, 1987. PCR hair work presented.

2. International Forensic Haemogenetics meeting, Vienna, Austria, August, 1987. PCR hair work presented.

3. "DNA Research", Symposium on the Management of Forensic DNA Analysis sponsored by the Attorney General, State of California, 7 Jan. 1988.

4. "DNA Analysis: Plans for Future Work" S.T.E.P. seminar, State of California Bureau of Forensic Services, Asilomar, CA, 6 Jan. 1988.

5. DNA Workshop - New York State Crime Laboratory Advisory Committee, 21 Jan. 1988.

6. "Analysis of enzymatically amplified DNA from single human hairs" Annual meeting, American Academy of Forensic Sciences, Philadelphia, 17-20 Feb. 1988.

7. "DNA Research: Current Status" California Association of Crime Laboratory Directors, 31 March 1988.

8. "Scientific Background on DNA Identification." Career Criminal Apprehension Program Conference, Long Beach, CA 16-17 May, 1988.

9. "Effects of DNA damage on PCR amplification" M. Buonchristiani, C. vonBeroldingen, G. Sensabaugh. Presentation at California Association of Criminalists Semi-annual Meeting, Berkeley, CA, 19-21 May, 1988.

10. "DNA in Hair" S. Walsh, G. Sensabaugh. Presentation at California Association of Criminalists Semi-annual Meeting, Berkeley, CA, 19-21 May, 1988.

11. DNA Preparation Workshop - cotaught with C. vonBeroldingen, R. Higuchi, E. Blake. Presentation at California Association of Criminalists Semi-annual Meeting, Berkeley, CA, 19-21 May, 1988.

12. Speaker at symposium session "Criminal Justice Applications of Genetic Fingerprinting." Joint meeting of the Idaho Criminal Justice Conference and the National Criminal Custice Association, Boise, ID, 24-27 May, 1988.

13. Invited participant, FBI Laboratory Division Seminar on DNA Technology in Forensic Science, Quantico, VA, 31 May-2 June, 1988.

14. DNA typing: Theory and applications. California Association of Toxicologists Quarterly meeting, Redwood City, Aug 6, 1988.

15. The power of PCR in forensic science. Cetus Polymerase Chain Reaction Workshop, Emeryville, Aug 25, 1988.

16. Gene amplification: application to the analysis of biological evidence. American Chemical Society National Meeting, Los Angeles, Sept. 26, 1988.

17. The application of forensic DNA technology and the Frye standard: panel discussion. American Chemical Society National Meeting, Los Angeles, Sept. 26, 1988.

18. Proficiency testing: what can be learned? American Chemical Society National Meeting, Los Angeles, Sept. 26, 1988.

19. DNA Workshop. Fall meeting, Midwest Association of Forensic Scientists, Minneapolis, Oct. 3-7, 1988.

20. DNA technology applications in forensic science: PCR and RFLP analysis. Biotechnology symposium on protein and drug design and therapeutic targeting, Biotechnology research and education program, UCSF, Oct 6-7, 1988.

21. American Society of Human Genetics annual meeting, San Diego, CA, 7-10 October, 1988. Poster presentation on our hair work.

22. Application of DNA technology in the analysis of biological evidence. Scientific evidence training program, California Public Defenders Association, San Diego, Nov 12, 1988.

23. California Association of Crime Laboratory Directors - Seminar on DNA; Oakland, CA, 18 Nov. and Santa Anna, CA, 19 Nov. 1988. Lecture given: Detection of genetic variation at the DNA level.

24. Current status of PCR - its application to forensic science. Technical working group on DNA analysis methods, Quantico, VA, Nov. 20, 1988.

25. Introduction of Issues. Banbury conference on the Forensic Application of DNA Technology, Cold Spring Harbor Laboratory, NY, Nov. 29-Dec. 1, 1988.

26. DNA Technology in Forensic Science. California District Attorneys Association Seminar, Newport Beach, CA, Dec. 9, 1988.

27. The Use of the Polymerase Chain Reaction in Forensic Science. Banbury Conference on the Polymerase Chain Reaction, Cold Spring Harbor Laboratory, NY, Dec. 12-14, 1988.

28. DNA Technology: Application in Forensic Science. G.F. Sensabaugh. Ground Rounds, Dept. of Laboratory Medicine, Univ. California Medical Center, San Francisco, Jan. 9, 1989.

29. DNA in Hair. S. Walsh, R. Roby, C. VonBeroldingen, and G.F. Sensabaugh. Annual Meeting, American Academy of Forensic Sciences, Las Vegas, Feb. 13-18, 1989.

30. Effects of Dna Damage on DNA Amplification by the Polymerase Chain Reaction. M. Buoncristiani, C. von Beroldingen, and G.F. Sensabaugh. Annual Meeting, American Academy of Forensic Sciences, Las Vegas, Feb. 13-18, 1989.

31. Application of the polymerase chain reaction in forensic science. G.F. Sensabaugh. UCLA Symposium on the Polymerase Chain Reaction, Keystone, CO, April 3-7, 1989.

32. Amplification of Y chromosome specific sequences in biological evidence. L. von Beroldingen, C. von Beroldingen, and G.F. Sensabaugh. Spring meeting, Northwest Association of Forensic Scientists, Ashland, Oregon, April 3-7, 1989.

33. DNA in Hair: quantity and quality. R. Roby, S. Walsh, C. von Beroldingen, and G.F. Sensabaugh. Spring meeting, Northwest Association of Forensic Scientists, Ashland, Oregon, April 3-7, 1989.

34. The Polymerase Chain Reaction: Principles and Application to Paternity and Forensic Testing. C. von Beroldingen, G.F. Sensabaugh, and H. Erlich. Meeting on DNA for Parentage Testing sponsored by the American Association of Blood Banks, April 17-18, Leesburg, VA.

35. Use of the Polymerase Chain Reaction for Typing Gc Variants. R. Reynolds and G.F. Sensabaugh. Spring seminar, California Association of Criminalists, Sacramento, May 18-20, 1989.

36. Analysis of the Quantity and Quality of DNA from Hair. R.K. Roby, S. Walsh, C. von Beroldingen, and G.F. Sensabaugh. Spring seminar, California Association of Criminalists, Sacramento, May 18-20, 1989.

37. Effects of UV Damage on DNA Amplification by the Polymerase Chain Reaction. M. Buoncristiani, C. von Beroldingen, and G.F. Sensabaugh. Spring seminar, California Association of Criminalists, Sacramento, May 18-20, 1989.

38. Amplification of Y-Chromosome Specific Sequences in Biological Evidence. C. von Beroldingen, G.F. Sensabaugh, L. von Beroldingen, R. Higuchi, and H. Erlich. Spring seminar, California Association of Criminalists, Sacramento, May 18-20, 1989.

39. Effects of Enzymatic Degradation of DNA on RFLP Analysis. K. Konzak, R. Reynolds, c. von Beroldingen, M. Buoncristiani, and G. F. Sensabaugh. Spring seminar, California Association of Criminalists, Sacramento, May 18-20, 1989.

40. Forensic Applications of DNA Analysis: Future Directions. G.F. Sensabaugh. Invited talk at Applied Biosystems Inc., Foster City, June 7, 1989.

41. Consequences of Nucleotide Misincorporation during the Polymerase Chain Reaction. G.F. Sensabaugh. FBI International Symposium on the Forensic Aspects of DNA Analysis, Quantico, VA, June 19-23, 1989.

42. Effects of DNA Damage and Degradation on RFLP Analysis. K. Konzak, R. Reynolds, c. von Beroldingen, M. Buoncristiani, and G. F. Sensabaugh. FBI International Symposium on the Forensic Aspects of DNA Analysis, Quantico, VA, June 19-23, 1989.

43. Effects of DNA Degradation on Amplification by the Polymerase Chain Reaction. R. Reynolds, C. von Beroldingen, and G.F. Sensabaugh. FBI International Symposium on the Forensic Aspects of DNA Analysis, Quantico, VA, June 19-23, 1989.

44. Effects of UV Damage on DNA Amplification by the Polymerase Chain Reaction. M. Buoncristiani, C. von Beroldingen, and G.F. Sensabaugh. FBI International Symposium on the Forensic Aspects of DNA Analysis, Quantico, VA, June 19-23, 1989.

45. A Comparative Study of DNA Extracted from Seven Postmortem Tissues. S. Swarner and G.F. Sensabaugh. FBI International Symposium on the Forensic Aspects of DNA Analysis, Quantico, VA, June 19-23, 1989.

46. DNA in Hair. S. Walsh, R.K. Roby, C. von Beroldingen, and G.F. Sensabaugh. FBI International Symposium on the Forensic Aspects of DNA Analysis, Quantico, VA, June 19-23, 1989.

47. Invited moderator of workshop "Forensic Science, Genetic Screening, and Ethics" at the annual meeting of the Genetics Society of America, Atlanta, June 30-July 2, 1989.

48. Forensic Applications of DNA Analysis. Invited paper, annual meeting, Genetics Society of America, Atlanta, June 30-July 2, 1989.

49. Status Report on Forensic DNA Research at Berkeley. Invited presentation to Calif. Association of Crime Laboratory Directors, Concord, CA, July 20, 1989.

50. Evaluation of RFLP Patterns from Forensic Samples. Lecture for UC Extension course "Forensic DNA Analysis", July 28, 1989.

51. The Use of the Polymerase Chain Reaction in the Analysis of Biological Evidence. GF Sensabaugh, C vonBeroldingen, and R Reynolds. Invited presentation at annual meeting, Eastern Analytical Symposium, New York, NY, Sept. 26, 1989.

52. Workshop on the Polymerase Chain Reaction sponsored by the Northwest Association of Forensic Scientists, Concord, CA, Oct. 17-18, 1989.

53. Amplification of Y-Chromosome specific Sequences in Biological Evidence. C vonBeroldingen, GF Sensabaugh, L vonBeroldingen, R Higuchi, and HA Erlich. Presentation at 13th Congress, International Society for Forensic Haemogenetics, New Orleans, LA, Oct. 19-21, 1989.

54. Effects of UV Damage on DNA Amplification by the Polymerase Chain Reaction. M. Buoncristiani, C. vonBeroldingen, and GF Sensabaugh. Presentation at 13th Congress, International Society for Forensic Haemogenetics, New Orleans, LA, Oct. 19-21, 1989.

55. DNA Damage and RFLP Analysis. K. Konzak, R Reynolds, C vonBeroldingen, M Buoncristiani, and GF Sensabaugh. Presentation at 13th Congress, International Society for Forensic Haemogenetics, New Orleans, LA, Oct. 19-21, 1989.

56. Use of the Polymerase Chain Reaction for Typing GC Variants. R Reynolds and GF Sensabaugh. Presentation at 13th Congress, International Society for Forensic Haemogenetics, New Orleans, LA, Oct. 19-21, 1989.

57. Moderator, Session on Human DNA Markers, 13th Congress, International Society for Forensic Haemogenetics, New Orleans, LA, Oct. 19-21, 1989.

58. The Polymerase Chain Reaction: Principles and Applications. Presentation at Technical Workshop on "DNA Probe Technology", Annual Meeting, American Association of Blood Banks, New Orleans, LA, Oct. 22, 1989.

59. Forensic Concerns about the Polymerase Chain Reaction. Invited presentations to Forensic PCR Training Course, Cetus Corp., Emeryville, CA, Oct. 25 and Nov. 1, 1989.

60. DNA Research in Forensic Science. Invited presentation at workshop, "The Impact of DNA Technology on the Criminal Justice System", Annual meeting, American Society of Criminology, Reno NV, Nov. 11, 1989.

61. Current Directions in Forensic DNA Analysis. Invited seminar, Institute of Forensic Genetics, University of Copenhagen, Copenhagen, Denmark, Dec. 1, 1989.

62. Forensic application of the polymerase chain reaction, and Problems in the analysis and interpretation of DNA evidence. Invited Lecture Series, Department of Pure and Applied Chemistry, University of Strathclyde, Glasgow, Scotland, Dec. 4-8, 1989.

63. Forensic Application of the Polymerase Chain Reaction. Invited seminar, Virology Institute, University of Glasgow, Glasgow, Scotland, Dec. 4, 1989.

64. Genetic typing of DNA in Biological Evidence. Presentation at NIJ Research Review meeting, Cincinnati OH, Feb. 19, 1990.

65. Forensic Application of the Polymerase Chain Reaction, Invited Seminar, Chemistry Dept., Georgia Tech., Atlanta, GA, May 28, 1990.

66. The DNA Revolution in Forensic Biochemistry, Peter B. Sherry Memorial Lecture, Georgia Tech., Atlanta, GA, May 29, 1990.

67. Genetic Typing of Biological Evidence using the Polymerase Chain Reaction, Invited presentation, German Society of Forensic Medicine meeting, Cologne, Sept 13, 1990.

68. The Application of the Polymerase Chain Reaction in Forensic Science, invited presentation, DSIR Forensic Science Section seminar, Auckland, New Zealand, October 15, 1990.

69. The Application of the Polymerase Chain Reaction in Forensic Science, invited presentation, DSIR Forensic Science Section seminar, Wellington, New Zealand, October 17, 1990.

70. The Application of the Polymerase Chain Reaction in Forensic Science, invited presentation, DSIR Forensic Science Section seminar, Christchurch, New Zealand, October 18, 1990.

71. Workshop on PCR. Co-organized with Dr. Craig Fowler, Adelaide, Australia, October 22-23, 1990.

72. Forensic Application of the Polymerase Chain Reaction. Plenary presentation, International Association of Forensic Sciences Meeting, Adelaide, Australia, October 25, 1990.

73. Detection and Identification of Sequence Differences in Gc Variants using the Polymerase Chain Reaction. R. Reynolds (presenter) and G.F. Sensabaugh, Poster presentation at the International Association of Forensic Sciences Meeting, Adelaide, Australia, October 25, 1990.

74. The DNA revolution in Forensic Biology. Biotechnology Seminar, Department of Biology, University of California, Santa Cruz, March, 1991.

75. Case Consideration in the Analysis of DNA Evidence. Presentation at Biotechnology meeting on DNA Evidence in Forensics (sic), University of California, Riverside, CA, March, 1991.

76. Forensic DNA Analysis. Biology Seminar, Department of Biology, California State College, Stanislas, CA, May 1991.

77. Use of DNA Analysis in Forensic Science. Presentation to Industry Initiatives for Science and Math Education, San Jose, CA, March 1992.

78. Identification of Mosquito Bloodmeals. California Mosquito Research Meeting, Riverside CA, April 1992.

79. Forensic Implication of the Human Genome Project. South-North Human Genome Conference, Caxambu, Brazil, May, 1992.

80. Advances in the Analysis of Sexual Assault Evidence. Seminar to Law and Science Faculties, University of Mogi das Cruzes, Mogi das Cruzes, Brazil, May, 1992.

81. Gene Structure and Genetics of ACP1. Workshop on Acid Phosphatase (ACP1), Rome, Italy, Sept. 1992.

82. Developments on PCR in Forensic Science. Seminar, Facolta di Medicina e Chirurgia, Univ. Cattolica del Sacro Cuore, Rome, Italy, Sept. 1992.

83. A STS at the ACP1 Locus (2p25). Second International Workshop on Human Chromosome 2. Half Moon Bay, CA, Nov. 1992.

84. Genetic Structure of the Human Red Cell Acid Phosphatase (ACP1) Locus: Genetic Typing of the *A, *B, and *C Types at the DNA Level. K. A. Lazaruk, G.F. Sensabaugh, and J. Dissing. 81st Semi-annual Seminar of the California Association of Criminalists, Berkeley CA, May, 1993.

85. Species Identification from mitochondrial Cytochrome b Sequences. C. Cook and G.F. Sensabaugh. 81st Semi-annual Seminar of the California Association of Criminalists, Berkeley CA, May, 1993.

86. DNA from Ancient Teeth. D. DeGusta, C. Cook, and G.F. Sensabaugh. 81st Semi-annual Seminar of the California Association of Criminalists, Berkeley CA, May, 1993.

87. Studies on the Polymorphism at the HUM-FABP and HUM-THO1 Loci. M. Savill and G.F. Sensabaugh. 81st Semi-annual Seminar of the California Association of Criminalists, Berkeley CA, May, 1993.

88. Human Red Cell Acid Phosphatase: Genetic Typing of the *A, *B, and *C Alleles at the DNA Level. K. A. Lazaruk and G.F. Sensabaugh. 15th Congress of the International Society for Forensic Hemogenetics, Venice, Italy, Oct. 1993.

89. Studies on the Polymorphism at the THO1 and FABP Loci. M. Savill and G.F. Sensabaugh. 15th Congress of the International Society for Forensic Hemogenetics, Venice, Italy, Oct. 1993.

90. A New Look at Old Friends: The Molecular Biology of the Classical Markers. Plenary Presentation, 15th Congress of the International Society for Forensic Hemogenetics, Venice, Italy, Oct. 1993.

91. Advances in Forensic PCR Technology. Invited Seminar, Free University of Berlin, Oct. 1993.

APPENDIX III: POSTDOCTORAL AND STUDENT RESEARCH
(Listing includes research project and current position)

Post-Doctoral Researchers

Dr. Cecilia vonBeroldingen (1987-1989) Hair studies, DNA degradation, XY markers
Currently: DNA Section, Crime Detection Laboratory, Oregon State Police, Portland OR.

Dr. Rebecca Reynolds (1988-1990) DNA degradation, Gc typing
Currently: Group Leader, Forensic Research and Development, Roche Molecular Systems., Alameda, CA

Graduate Students

Martin Buoncristiani - DNA degradation, template jumping, UV damage
Currently: California Dept. of Justice DNA Laboratory, Berkeley CA

Lisa Calandro - Dinucleotide repeat polymorphisms
Currently: Graduate student, University of California, Berkeley CA

Elizabeth Chasin - Differential extraction
Currently: Unknown

Charles Cook - Insect bloodmeals
Currently: Graduate student, University of California, Berkeley CA

Deborah Fisher - RFLP band shifting
Currently: Armed Forces DNA Identification Laboratory, Washington DC

Kenneth Konzak - DNA degradation
Currently: California Dept. of Justice DNA Laboratory, Berkeley CA

Allison Lancaster - Semen detection in child sexual abuse
Currently: San Francisco Police Crime Detection Laboratory, San Francisco CA

Katherine Lazaruk - ACP1 typing
Currently: Graduate student, University of California, Berkeley CA

Huy Le - CYP450 typing
Currently: Unknown

Ma Maosheng - Differential extraction
Currently: Graduate student, University of California, Berkeley CA

Rhonda Roby - DNA in Hair
Currently: Armed Forces DNA Identification Laboratory, Washington DC

Jill Shirokawa - DNA in urine
Currently: Graduate student, University of California at Los Angeles, Los Angeles CA Berkeley CA

Susan Swarner - DNA in post mortem samples
Currently: Contra Costa County Criminalistics Laboratory, Martinez CA

Sara Tishkoff - DNA in Hair
Currently: Graduate Student, Yale University, New Haven CT

P. Sean Walsh - DNA in hair
Currently: Forensic DNA research and development group, Roche Molecular Systems, Alameda CA

APPENDIX IV: VISITING SCIENTISTS

Dr. John Bowen (July 1988) RCMP Laboratory, Edmonton, Alberta, Canada

Ms Patrica Wojtowicz (Feb-Mar. 1989) Minnesota Bureau of Criminal Apprehension, St. Paul, Minn.

Dr. Malcolm McGinnis (June-Aug 1990) Genetype Molecular Systems, Berkeley CA

Dr. Odo Feenstra (Feb. 1991) Amt der Karntner Landesregierung, Klagenfurt, Austria

Dr. Joseph Day (Jan-Mar 1991) Univ. of Washington, Seattle WA

Dr. Ate Kloosterman (Nov 1991) Dutch Forensic Science Institute, Rijswijk, Netherlands

Dr. Marion Savill (Nov 1991-Feb 1992) Institute of Environmental Health and Forensic Sciences, Christchurch, New Zealand.

Dr. Jose Lorente (May-July 1992) Dept. of Legal Medicine, Univ. of Grenada, Grenada, Spain.

Dr Hubert Poche (May 1992) Institute for Legal Medicine, Frie University, Berlin, Germany