

PROFILES

IN DNA

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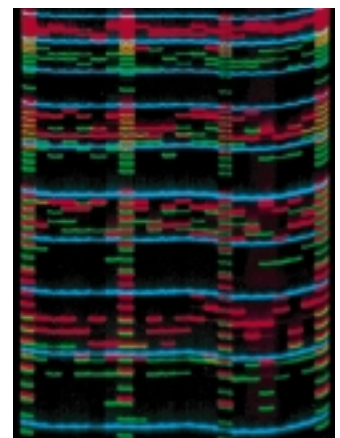
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The cover image shows a typical example of DNA profiles achieved using the *GenePrint™* PowerPlex™ Fluorescent STR System, the Fluorescent Ladder (CXR), 60-400 Bases and the Hitachi FMBIO® Fluorescent Scanner. See page 9 for further information.



Introducing *Profiles in DNA*

Dear Reader,

The use of DNA testing for both forensic and paternity applications is undergoing rapid growth. Local and national governments, the research community and private industry are all part of a combined effort to implement accurate, reproducible and reliable DNA testing methodologies. The objective of these efforts is the development of scientific tools that can be used as a routine resource in law enforcement, human and veterinary medicine, paternity testing and basic research. In order to accomplish these objectives, we in the DNA typing community need to communicate well with others and to train and educate those who may be new to the field. To facilitate this process, Promega sponsors symposia and provides training workshops. Both of these programs are continuing and expanding.

Our newsletter *Profiles in DNA* is a new service we are providing to the DNA typing community to create a forum for communication. *Profiles in DNA* will contain articles written largely by members of the community outside of Promega on subjects of general interest. You can expect to receive a copy of this publication four times a year. For regular updates, this newsletter will also be available on our web site at: www.promega.com/profiles/ or at: www.euro.promega.com/profiles/. By clicking on the *Profiles in DNA* icon, you will be able to read not only the latest edition of the newsletter, but also past articles and recent updates. We hope that our web site will become a useful resource for the community at large.

In our first issue of *Profiles in DNA* we provide readers an opportunity to keep up to date with current events in a variety of ways; the Human Identity Trade Association (HITA) presents their first contribution to *Profiles in DNA* in this issue. We also have a report on the proceedings of the 49th annual meeting of the American Academy of Forensic Sciences (AAFS). Information on upcoming meetings and training programs is also included.

The complex issues involved in the presentation of DNA evidence to a jury have been the focus of a great deal of public interest during the past year. In our feature article – *Presenting DNA Evidence at Trial: The “K.I.S.S. Principle”* – one prosecutor outlines his strategy for dealing with this type of evidence (page 3).

The National Institute of Justice Forensic DNA Laboratory Improvement Program provides funding for state and local forensic laboratories to enable them to conduct DNA testing. See our interview with Dr. Richard Rau (page 5) to obtain more information on the current status of this program.

Promega welcomes contributions from readers for inclusion in future newsletters. In addition, we ask you to submit ideas for information to be included on our web site and suggestions for subjects you would like to see covered in future issues. As well as providing information on current issues of relevance to the forensics and paternity communities, we hope that future newsletters will include more international news and a classified advertisement section detailing positions available and positions wanted. If you would like to make a contribution to this newsletter in any of these ways, please contact the editor at the address given, or by e-mail at profilesindna@promega.com.

We want to know what you think of *Profiles in DNA* so that we can tailor future issues to meet your needs. Please take the time to fill in and return the enclosed survey so that future newsletters can be of the most benefit to you.

Isobel Maciver
Editor, *Profiles in DNA*

Thomas J. Mozer
GenePrint™ Business Leader

Presenting DNA Evidence at Trial: The “K.I.S.S. Principle”

By James R. Wooley, JD

When a prosecutor seeks to use DNA evidence in a criminal case today, he or she generally has no concern regarding whether the judge will allow the evidence to be presented to the jury. This was not always the case, but a decade of battles over the admissibility of DNA evidence has yielded a legal landscape where juries, with few exceptions, are allowed to hear the results of DNA testing. The issue facing the prosecutor now is how to best present the evidence to the jury.

DOING IT THE HARD WAY

There are two basic approaches to presenting DNA evidence to juries. One approach involves a prosecutor using expert witness testimony to present a detailed explanation of the underlying scientific principles and techniques involved in DNA testing, in the hopes of creating a scientifically savvy jury which will be able to appreciate the scientific basis of the test results. The actual results are often presented to the jury hours (or even days) after the jury first was told about “A” always binding with “T”. We have all seen this approach on television in that California case involving several current television celebrities, authors and a star athlete.

KEEPING IT SIMPLE

A second approach, and the one advocated by this prosecutor, is to apply the “K.I.S.S. Principle”. The K.I.S.S. Principle advises trial lawyers who are presenting complex evidence to juries to “Keep it Simple, Stupid”. When the K.I.S.S. Principle is applied, DNA evidence is presented to the jury in about half an hour, at the conclusion of which the jury has a full appreciation of the power of the DNA test results in the case. When this approach is used, the jury will know that a reliable, powerful, widely accepted scientific procedure has demonstrated that a piece of biological evidence is

highly likely to have come from a suspect. The jury will also know that the suspect’s complaints regarding possible problems with the test result are without merit. The jury will not know that “A” binds to “T”, but I respectfully submit that they never needed or wanted to know that anyway.

When applied to the presentation of DNA evidence, the K.I.S.S. Principle works in the following way: The prosecutor calls the DNA expert who performed the tests and conducts a nontechnical direct examination. The examination focuses on the experience and integrity of the expert, the basic goal of DNA testing, the widespread usage and acceptance of DNA testing techniques, the test results and the fact that the evidence can be retested by anyone who claims the results are wrong. Breaking down the half-hour direct examination into separate lines of questioning, it goes something like this:

Who are you and what is it that you do?
(questions and answers to explain the basic goal of the test)

Why are you qualified to do it? *(the knowledge and experience of the expert)*

Do you and others perform DNA testing often? *(the widespread usage and acceptance of the test)*

What was the result of DNA testing in this case? *(the DNA test result showing that the suspect’s DNA has the same rare characteristics that were detected in the crime scene evidence)*

Have your DNA test results ever excluded a suspect? *(the integrity of the expert, who calls exclusions where appropriate)*

If someone disagrees with your result, is there a scientific way to check if you got the right answer? *(the defendant has the ability to retest the evidence if he really wants to challenge the accuracy of the test result)*

When the K.I.S.S. Principle is applied, DNA evidence is presented to the jury in about half an hour, at the conclusion of which the jury has a full appreciation of the power of the DNA test results in the case.

Lawyers who take a highly technical approach to arguing DNA evidence to juries are asking lay people to assess expert information in a way that is totally foreign to most of them.

DNA testing is a mature forensic science and it should be presented to the jury in the same manner as other mature forensic science evidence.

ADVANTAGES OF USING THE "K.I.S.S. PRINCIPLE"

The advantages of this simple approach are many. First, by not conducting long, technical, direct examinations, the prosecutor has made sure that the most important part of the DNA evidence – the result – is not obscured. In that certain California case, the results were powerfully presented, but only after days of articulate, yet totally mind-numbing, technical background was presented to the jury.

Second, by presenting DNA evidence in a nontechnical way which focuses on the power of the results and the ability to retest, the prosecutor has made it very difficult for the defense attorney to score points with the typical "something may have gone wrong with the testing procedure" attack which has been so prevalent in DNA litigation. Most defense attorneys decline the invitation to have the evidence retested and, instead, try to impress the jury with questions about possible technical problems with contamination, ethidium bromide, population substructure, etc.

A defense attorney will have a tough time making these possible technical problems clear to the jury if that attorney must first attempt to explain the technical background through cross-examination of the prosecution's DNA expert. Even if the defense attorney succeeds in making these possible tech-

Balanced against the advantages of the K.I.S.S. Principle approach is the idea that it is valuable to have a scientifically astute jury – one that really knows where to put its "A's, T's, G's and C's".

nical problems clear for the jury, he or she will have even more difficulty making the jury see the importance of the "something could have gone wrong" argument without presenting contradictory DNA test results.

Third, the simple presentation reflects the way in which lay jurors are presented with expert information in everyday life. When a juror meets a doctor who recommends a course of treatment for an ailment, the juror does not make the important decision whether to follow the doctor's advice by spending days trying to understand all of the scientific underpinnings of the problem. Instead the juror considers whether the doctor has experience and knowledge to render the advice and whether the advice is based on medical procedures that are widely used and accepted as reliable. Other factors he or she may take into consideration include whether the doctor's opinion is corroborated by other expert or nonexpert information and whether the advice is contradicted by a doctor who is more experienced and who may have used a more widely accepted diagnostic procedure. Lawyers who take a highly technical approach to arguing DNA evidence to juries are asking lay people to assess expert information in a way that is totally foreign to most of them.

Fourth, presenting DNA evidence in a nontechnical manner will mean that this type of forensic evidence will not be singled out for special scrutiny in the minds of the jury. In most cases involving DNA evidence, the prosecution will also present the results of other types of forensic science testing, including ballistics testing, serology, hair and fiber evidence, etc. The K.I.S.S. Principle is almost always invoked in the presentation of these other types of forensic evidence. Treating DNA evidence differently creates the erroneous impression that there is more reason for the jury to be concerned about the reliability of this particular type of forensic science. The fact is that DNA testing is a mature forensic science and it should be presented to the jury in the same manner as other mature forensic science evidence.

THE SCIENTIFICALLY ASTUTE JURY

Balanced against the advantages of the K.I.S.S. Principle approach is the idea that it is valuable to have a scientifically astute jury – one that really knows where to put its "A's, T's, G's and C's". Forgetting for the moment whether it really is valuable to have this type of jury, I question whether it is even remotely realistic to believe we can ever obtain such a jury in our system. I have spent significant portions of the last few years attempting, as a lay person, to understand the scientific theories, principles and techniques involved in DNA testing. I have been able to **truly understand** a small amount of the science involved only by talking to experts for extended periods of time, asking them to explain things to me over and over again, asking follow-up questions, reading and re-reading scientific publications, and then going back to the experts with even more questions. It has taken literally months of interactive study and I still place myself on the steep part of the learning curve. I accept and recognize that I may not be the best control for this type of study (I did once ask a human population geneticist who had written a paper on phylogenetic trees when he had first developed his interest in plants), but my experience makes me believe that twelve lay people cannot truly learn about DNA typing technology by silently sitting in a jury box for two days (or three, or four), certainly not to a level that would enable them to question the technical conclusions of any defense or prosecution expert in DNA testing. In other words, it is naïve to think that the only arguable benefit of the technical approach – the creation of a scientifically expert jury – will ever materialize in our system.

In closing, let me state that, while the K.I.S.S. Principle may not be as important to DNA testing as the ceiling principle approach for estimating DNA profile frequencies (although it is at least as scientific), proponents of DNA evidence in criminal cases would be well served to employ it when presenting this powerful evidence to juries.

The National Institute of Justice Forensic DNA Laboratory Improvement Program

An interview with Dr. Richard Rau

The National Institute of Justice Forensic DNA Laboratory Improvement Program is authorized by the "DNA Identification Act of 1994." This Act provides for the allocation of \$40 million dollars in funds over a five year period. The purpose of the program is "to increase the capabilities and capacity of state and local forensic laboratories to conduct DNA testing." The allocation of these funds supports the development of a comprehensive, nationwide criminal DNA database, compatible and cooperating with the FBI's Combined DNA Index System (CODIS).

The purpose of the program is "to increase the capabilities and capacity of state and local forensic laboratories to conduct DNA testing."

The 1996 solicitation for the NIJ Forensic DNA Laboratory Improvement Program (available on the Internet at: www.ncjrs.org:80/fedgrant.htm#NIJ) lists some of the specific objectives of this project as follows:

- To develop DNA testing in laboratories that currently do not conduct DNA testing.
- To improve forensic DNA testing in state and local laboratories that already conduct DNA testing, especially for sexual assaults having unknown assailants.
- To facilitate implementation of state laws requiring the establishment of databases of DNA records of convicted offenders.
- To foster compatibility and cooperation among forensic laboratories within and between states that are seeking to match and exchange DNA identification records for law enforcement purposes through FBI's CODIS.
- To provide the greatest overall improvement in the nation's DNA testing capabilities, recognizing current and projected DNA testing requirements and identifying current and foreseeable technological trends.

In an effort to obtain more information on the current status of this program, *Profiles in DNA* conducted an interview with Dr. Richard Rau. Dr. Rau is the Program Manager for the National Institute of Justice Forensic DNA Laboratory Improvement Program.

Q: Who is eligible to apply for funding under this program?

A: Eligible applicants must be state or local government laboratories. Awards may be used to purchase laboratory equipment and supplies, to make structural modifications to existing laboratory space to allow safe and accurate performance of DNA typing methodologies, to provide training for DNA analysts in accordance with TWGDAM (Technical Working Group on DNA Analysis Methods) guidelines, and to purchase hardware and software compatible with CODIS. In the short term, award money may also be used to pay for contractor-provided DNA typing services to expedite backlogs of collected but unanalyzed samples.

Generally speaking, proposals which demonstrate that statewide planning has taken place, and which provide justification of the need for DNA testing capabilities, will be most successful.

Q: In its first year (1995), the Forensic DNA Laboratory Improvement Program awarded 37 grants for a total amount of \$8,750,000, \$8 million of which was contributed by the FBI. Do you expect to continue to fund at this level this year?

A: This year we expect to allocate a further \$3 million. We have received 62 applications in response to the September 1996 solicitation, and are beginning the review process. A solicitation for applications for the third year of this program will be issued in the Summer of 1997.

Q: What constitutes your review process?

A: Applications for funding are reviewed by a panel of experts in the field of DNA typing. Given that we have 62 applications to review this year, the review process can be somewhat lengthy. Once completed, the panel's reviews are passed on to the Director of NIJ, who makes the final award decisions. Applicants receive a copy of the review when notified of the success or failure of their application.

Q: What are the characteristics of a successful application?

A: Successful awards emphasize the acquisition of equipment, supplies and training necessary to develop or improve DNA testing capabilities. Unsuccessful applications are typically poorly written and lack adequate descriptions of proposed DNA typing techniques. We recommend that labs that do not have DNA testing facilities currently in place

consult with an expert to aid in planning for future needs before completing their application. Our solicitations provide comprehensive instructions covering all aspects of the application process, including compilation of a detailed budget and budget narrative. Applicants who have questions are encouraged to contact me to discuss their proposal before submitting an application.

Q: Once an application is funded, how is the award administered?

A: Grants are awarded for a one year period. Funds awarded must be used during the life of the grant. Laboratories seeking continued funding to implement the next phase of a project begun under a previous award may submit an application for a continuation of funding at the time of the next year's solicitation.

Q: One of the stated aims of the program is to "foster compatibility and cooperation among forensic laboratories within and between states that are seeking to match and exchange DNA identification records for law enforcement purposes through the FBI CODIS program." Do you welcome combined applications from more than one laboratory?

A: Individual laboratories may submit applications in conjunction with their state, city or county, or on their own. We have received several consortium applications and actively encourage laboratories to work together to develop consistent techniques for statewide DNA typing programs. For small laboratories which have a low caseload, it may be difficult to justify a need for funding for all DNA testing methodologies, so it makes sense for these laboratories to be part of a larger consortium application. Generally speaking, proposals which demonstrate that statewide planning has taken place, and which provide justification of the need for DNA testing capabilities, will be most successful.

Q: Another stated aim of the grant program is to "provide the greatest overall improvement in the nation's DNA testing capabilities, recognizing current and projected DNA testing requirements and identifying current and foreseeable technological trends." What criteria are used to judge whether this improvement is being achieved? Is there a system in place to evaluate the success of the program?

A: The program seeks to provide funds to enable laboratories nationwide to perform DNA testing methodologies in accordance with the established TWGDAM guidelines. Adherence to these guidelines promotes nationwide uniformity in the field of DNA typing. Success of the program will also be indicated by the participation of increasing numbers of laboratories in the sharing and exchange of DNA identification records through CODIS. We do plan to have a formal evaluation of the program's success in the future, but the process by which this will occur has not yet been defined.

For further information on the DNA Laboratory Improvement Program contact:

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For a detailed explanation of how to put together an application for funds, the 1996 solicitation is available on the Internet at: www.ncjrs.org:80/fedgrant.htm#NIJ. A new solicitation for 1997 is expected to be published later in the Summer.

The Human Identity Trade Association



In 1992, representatives from private industry and private DNA testing laboratories organized the Human Identity Trade Association (HITA). The purpose of this association is to promote the general welfare of the human identity testing industry and to enhance contributions from this industry to the public welfare. To this end, HITA cooperates with federal, state and local agencies who have responsibilities in the area of human identity testing. The goals of HITA are to encourage public awareness, understanding, education and acceptance of the human identity testing industry's products and services, to develop and assist formulation of standards for human identity testing and to gather, as appropriate, statistics relating to the human identity testing industry.

The HITA board of directors is comprised of a President, a President-Elect, a Secretary, a Treasurer and up to three other Directors. Past Presidents include Daniel Garner, Cellmark Diagnostics and Kristine Garvin, Perkin Elmer/Applied Biosystems Division.

One of HITA's first major accomplishments was to have the language contained in the Omnibus Crime Bill, passed in October 1994, modified to state that one member of the DNA Advisory Board be from the private sector. As a result, the original membership of the DNA Advisory Board included Marsha Eisenberg from LabCorp of America. A second effort was made to have a HITA representative at the biannual TWGDAM (Technical Working Group on DNA Analysis Methods) meeting held at the FBI Academy. The current HITA representative to TWGDAM is David Bing, CBR Laboratories.

An ongoing activity of HITA is to support, as well as sponsor, open forums and meetings on both scientific and business issues. For example, in 1994 HITA organized an open panel discussion to address licensing issues with regard to key patents on PCR, chemiluminescence, STRs and AMPFLPs. The members of the panel included representatives from Roche Molecular Systems, Lifecodes Corporation, Promega Corporation, the Public Crime Laboratories and the FBI. In September 1996, HITA co-sponsored a technical workshop on new developments in the area of DNA-based paternity testing as well as DNA proficiency testing.

In the area of education, HITA has participated in a wet workshop on DNA testing for judges organized by the Einstein Institute and supported in part by the Human Genome Project. HITA is also exploring ways to support the educational programs of the National Forensic Science Training Center.

HITA welcomes membership from companies which manufacture and sell products or provide services in the area of human identity testing. HITA also encourages those wishing to join as individual members. For information on membership dues or for general information on HITA, please contact Richard Rubin at the address given on this page.

HITA is dedicated to support and help meet the needs of all members of the human identity testing scientific community, when feasible and appropriate. The HITA board encourages anyone in this field to contact any board member or director with questions, suggestions and requests for support. The board meets on a regular basis to carry out the business of HITA. It is, however, willing to consider requests for action or support at any time. Furthermore, if the matter warrants immediate action, HITA will bring the

issue to its entire membership as well as other concerned members of the human identity testing scientific and business community. The names and addresses of the current board members follow:

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Meeting Report

The American Academy of Forensic Sciences

*By Mary E. Hoenecke, Cynthia J. Sprecher
and Thomas J. Mozer*

The American Academy of Forensic Sciences (AAFS) 49th Annual Meeting was held in New York City at the Marriott Marquis Hotel, February 17-22, 1997. The Annual Meeting was comprised of workshops, scientific sessions, poster presentations and vendor exhibits representing various forensic disciplines, including DNA analysis. Over 1,500 scientists attended and 80 companies exhibited.

The "1997 Advanced DNA Technologies Workshop: Automation and Application" was held one day prior to the start of the Annual Meeting. The workshop was attended by over 250 scientists, a clear indication of the growing interest in the use of DNA testing in forensic science. The workshop, organized and chaired by Dr. Steven Lee of the California Department of Justice DNA Laboratory, was divided into three sessions: automation strategies, applications, and forensic DNA biotechnology company presentations. Topics covered under automation strategies included high throughput, automated instrumentation, microchip PCR and detection, and matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS). In addition, new genetic loci were also reviewed. The second session covered STR casework, data banking and STR standardization. The final session focused on recent developments from various biotechnology groups developing new STR multiplexes and automation strategies.

The General Session included topics such as DNA purification, STR multiplexes and new technologies. The presentations on DNA

purification covered the use of automation as well as DNA extraction from unusual sources such as drinking straws and toothbrushes. New STR loci and multiplexes were described in several talks. New technologies being developed included Genetic Bit Analysis and Sequence Specific Oligonucleotide Probe (SSOP) for analysis of mitochondrial DNA and for detection of polymorphisms for HLA class II antigens.

Among the high points of the meeting were several presentations by various members of the FBI study group that is now evaluating loci for inclusion in the CODIS database. This multi-laboratory group, led by Dr. Bruce Budowle of the FBI, will choose the loci to be used as the 'core' loci for this nationwide DNA database. Once this process is completed, the building of the CODIS database will see rapid growth in STR-analyzed samples. Eventually, that database will include DNA samples from millions of convicted felons. As such, it represents a watershed event in DNA testing in forensics and the dawn of a new age in criminal investigation. Specifically, Dr. Budowle discussed a current study which is evaluating STR loci and multiplexes. He showed how the study was progressing to choose loci and to develop protocols for their use. The study group has been working closely with manufacturers who are designing their products to meet this need. The study has clearly shown that the various STR multiplex reagents are the reagents of choice for inclusion in this database.

Upcoming Meetings

CALIFORNIA ASSOCIATION OF CRIMINALISTS

May 27-31, 1997

Sacramento, California

Contact: Ann Murphy
(916) 732 3840

SOUTHERN ASSOCIATION OF FORENSIC SCIENTISTS

September 4-6, 1997

Longboat Key, Florida

Contact: Michael Healy
(941) 747 3011
ext. 2280

GenePrint™ STR* Multiplexes: Reliability, Flexibility and Throughput in Database and Casework-Compatible STR Analysis

By James W. Schumm, Ph.D.



™ The era of databasing criminal populations to link suspects to crime scenes, and crime scenes to one another, through comparison of biological evidence is upon us. To achieve the ultimate benefit of this approach, legislation in the United Kingdom, Canada, the United States and many other countries has created centralized databases which will eventually include DNA profiles of hundreds of thousands to millions of individuals, primarily convicted criminals. To this end, all countries are moving to the use of short tandem repeat (STR) polymorphisms, which provide a rapid, reliable and inexpensive method of analyzing and unambiguously digitizing data for large numbers of samples. This article describes Promega's effort to develop and improve methods to fit the needs of the digital information age

while maintaining a flexible approach which allows large and small laboratories worldwide to apply the same genetic systems.

DNA markers which distinguish individuals from one another have been known since 1980. With the development of large numbers of VNTR (variable number tandem repeat) loci, a few of the most polymorphic markers became popular in forensic and paternity analyses. Despite the enormous discrimination power of VNTR systems, their use in databases is limited by their imprecision. Windows of standard deviation or the creation of bins is used to contend with this restriction. VNTRs are also limited in casework application because various markers require from 5 to 250ng of sample to assure reliable and reproducible success.

STR SELECTION CRITERIA

The use of properly selected STR loci can overcome the limitations of VNTR systems, producing digital results with 1ng or less of sample material. In our work, we have focused on STR selection criteria which provide highly discriminating markers without sacrificing quality of interpretation and digital storage. First, only STRs which demonstrate a high degree of variability within the population are selected. Second, the amplified products must be easily distinguished from one another. This means rejecting markers which contain frequent microvariants (i.e., alleles differing from one another by lengths shorter than the repeat length) as the closer and more random spacing of alleles is more difficult to interpret. Also, the prevalence of stutter bands (i.e., amplification artifacts which appear one or more repeat lengths above or below the true amplified allele) has led to the rejection of dinucleotide repeats as a class for these applications. In our work, only tetranucleotide repeats which display limited or no stutter have been selected.

The ability to amplify and detect very small amounts of DNA template (typically 1ng) is essential for forensic applications. In paternity analyses, the mutation rate of the markers chosen must be extremely low to avoid false exclusion of suspected fathers. In all cases, the reliability and reproducibility of the data must be irrefutable. Identification of the best markers for these applications is complicated by the fact that their desired traits are not fully compatible with one another. While it is possible to identify highly polymorphic markers with relatively low presence of stutter bands, there appears to be a correlation between a high degree of polymorphism, a tendency for microvariants and increased mutation frequency.

The amplification products of these multiplex systems may be detected using either the Hitachi FMBIO® or FMBIO® II Fluorescent Scanner, the ABI Model 373 or ABI PRISM™ 377 DNA Sequencer or the ABI Model 310 Capillary Electrophoresis Unit.

We have focused our attention on the development of tetranucleotide repeat loci which display few or no microvariants, minimal stutter bands, and have a relatively low mutation rate. The polymorphisms in our currently developed STR systems result almost exclusively from variation in the number of tetranucleotide repeats present at the locus, and not from insertion or deletion of one or two bases. This allows rapid and precise typing of easily amplified alleles ranging from 100 to 350 bases in length.

ALLELIC LADDERS

The discrete nature of alleles of these STR loci has also allowed the development of allelic ladders. Allelic ladders are composed of a collection of most or all of the amplified alleles found in the general population. These composites make ideal size markers because, in all STR loci we have developed to date except for vWA, the size markers and the amplified unknown alleles will contain not only the same size fragments, but the same sequence fragments. Thus, ladder components and unknowns co-migrate in gel electrophoresis regardless of the gel matrix or running buffer selected. In the same fashion, the addition of a fluorescein tag, often used as a reporter molecule in STR analysis, alters the ladder components and the amplified unknowns in an identical fashion. Thus, different laboratories using different separation techniques and different detection formats can compare their results with precision and reliability.

ADVANTAGES OF USING STR SYSTEMS

The fact that STR analysis is based on the PCR process offers several additional advantages. First, the detection of small amounts of template (e.g., 1ng of DNA) has become

routine. Second, there are a number of rapid purification methods which are compatible with PCR but which do not provide enough DNA of appropriate quality for use in Southern blot-based formats. Third, STR analysis is much faster than Southern blot-based protocols. Using STR analysis, results are obtained in 1-2 days while Southern-based typing methods may take 5-7 days to complete.

STR MULTIPLEXES

The major disadvantage in using STR systems is that those which display few or no microvariants and low mutation rates are not as polymorphic as the best of the VNTR markers. Thus, with STRs, there is a need to develop high throughput approaches to overcome this deficiency. The selection of individual STR loci, each with a limited size range of known alleles, means that several STR systems may be detected simultaneously in limited and well defined regions of the same lane on a gel. This offers the potential to develop STR multiplex systems. The multiplex systems we have developed for analysis using either semi-automated fluorescent detection methods or manual silver stain detection methods are listed in Table 1.

FLUORESCENT DETECTION

The PowerPlex™ System allows co-amplification of eight STR loci in a single reaction vessel, and the FFFL System allows co-amplification of four additional loci in a single reaction. Using the PowerPlex™ System in combination with the FFFL System allows the amplification of twelve STR loci in two multiplex reactions. The amplification products of these multiplex systems may be detected using either the Hitachi FMBIO® or FMBIO® II Fluorescent Scanner, the ABI

Model 373 or ABI PRISM™ DNA Sequencer or the ABI Model 310 Capillary Electrophoresis Unit.

Figure 1 shows results obtained using the PowerPlex™ System and the Hitachi FMBIO® Fluorescent Scanner. In the PowerPlex™ System, four loci (D16S539, D7S820, D13S317 and D5S818) are labeled with fluorescein and displayed in green, and four loci (CSF1PO, TPOX, TH01 and vWA) are labeled with carboxy-tetramethylrhodamine (TMR) and displayed in red (Panel A). These two sets of loci are scanned separately by the instrument and may be displayed as two distinct black and white images (Panels B and C, respectively). The images shown in Figure 1 illustrate several features of each of the GenePrint™ Fluorescent STR Multiplexes. Alleles for loci detected using the same color are always spatially separated from neighboring systems. Each locus is provided with an allelic ladder which can be used as a visual size marker or analyzed using the sizing software of the instrument. Very few off-ladder alleles exist.

The Fluorescent Ladder (CXR), 60-400 Bases, is an additional size marker of unrelated sequence composed of 16 evenly spaced DNA fragments labeled with carboxy-X-rhodamine. When this marker is included in each gel lane, the instrument is capable of monitoring and correcting lane-to-lane migration differences of samples. Only one STR locus included in the PowerPlex™ System has shown microvariant alleles (TH01 allele 9.3). In systems that contain more microvariant alleles (e.g., FGA, D18S51 or D21S11), the presence of an additional size marker is more important to identify the small lane-to-lane migration differences which may occur.

Table 1. STR Loci Contained in Multiplex Systems Detected Using Silver Stain or Fluorescent Labels.

STR Multiplex System	CSF1PO	TH01	TPOX	vWA	F13A01	FESFPS	F13B	LPL	D5S818	D7S820	D13S317	D16S539
Silver STRs												
CTT												
FFv												
SilverSTR™ III Multiplex												
Fluorescent STRs												
CTTv												
FFFL												
GammaSTR™ Multiplex												
PowerPlex™ System												

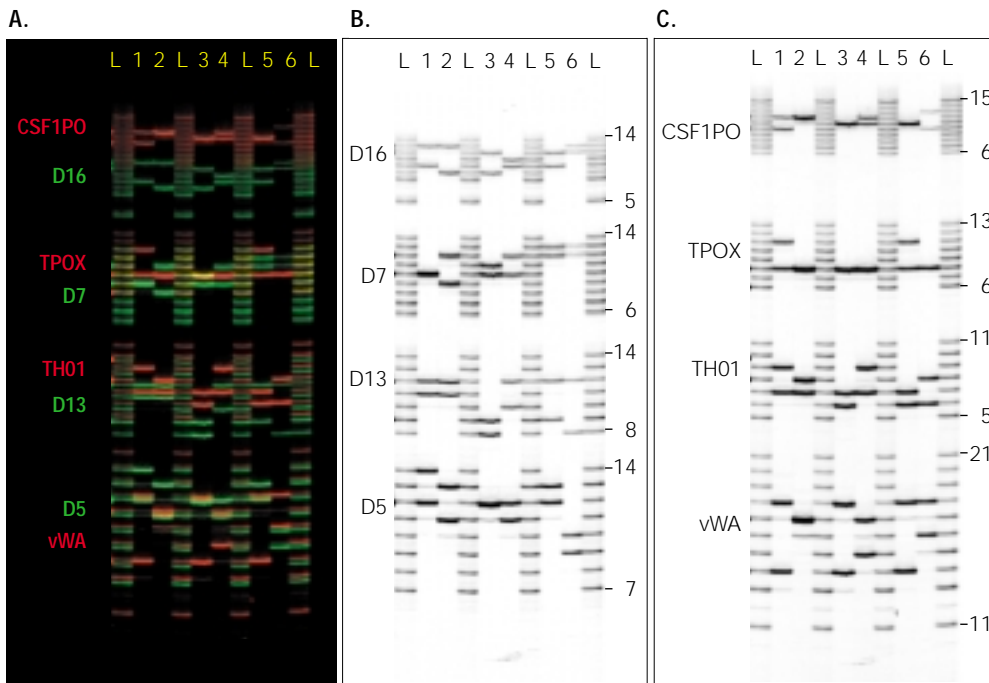


Figure 1. The GenePrint™ PowerPlex™ Fluorescent STR System (Fluorescein/TMR) (Hitachi). Six DNA samples were amplified (Lanes 1-6) and are shown with allelic ladders for the corresponding system (Lanes L). **Panel A:** Two-color image of all eight STR systems which were amplified simultaneously and detected using the Hitachi FMBIO® Fluorescent Scanner. The amplified products of the fluorescein-labeled loci (D16S539, D7S820, D13S317 and D5S818) are shown in green, while the TMR-labeled loci (CSF1PO, TPOX, TH01 and vWA) are shown in red. **Panel B:** A scan using a 505nm filter, which reveals the corresponding black and white image of the fluorescein-labeled loci (D16S539, D7S820, D13S317 and D5S818). **Panel C:** A scan using a 625nm filter, which reveals a black and white image of the TMR-labeled loci (CSF1PO, TPOX, TH01 and vWA). In Panels B and C, each allelic ladder is labeled to its right with the number of copies of the repeated sequence contained within its corresponding largest and smallest alleles. All materials were separated using a 4% denaturing polyacrylamide gel.

Differences in the properties of the lasers contained in the Hitachi and ABI instruments require that a separate configuration of the PowerPlex™ System be used with each instrument. Figure 2 shows results obtained using the PowerPlex™ System and the ABI PRISM™ 377 DNA Sequencer. In this case, the four fluorescein-labeled loci (D16S539, D7S820, D13S317 and D5S818) are displayed in purple, the TMR-labeled loci (CSF1PO, TPOX, TH01 and vWA) are shown in black

and the 16-fragment Fluorescent Ladder (CXR), 60-400 Bases, is displayed in red. Users have reported that this configuration of the PowerPlex™ System also allows three-color detection analysis using the ABI Model 373 DNA Sequencer and the ABI Model 310 Capillary Electrophoresis Unit, or two-color analysis (without the Fluorescent Ladder (CXR), 60-400 Bases) using the GenomexSC™ DNA Sequencing System or the Molecular Dynamics FluorImager™ 595 Fluorescent Scanner.

The fluorescein-labeled FFFL Multiplex (Table 1) contains the STR loci F13A01, FESFPS, F13B and LPL. This system is compatible with all of the instruments mentioned above and also with the Molecular Dynamics FluorImager™ 575 and SI Fluorescent Scanners. The two additional fluorescein-labeled quadriplex systems described in Table 1 (i.e., the CTTv and GammaSTR™ Fluorescent STR Multiplexes) are also compatible with all of these instruments (Figure 3).

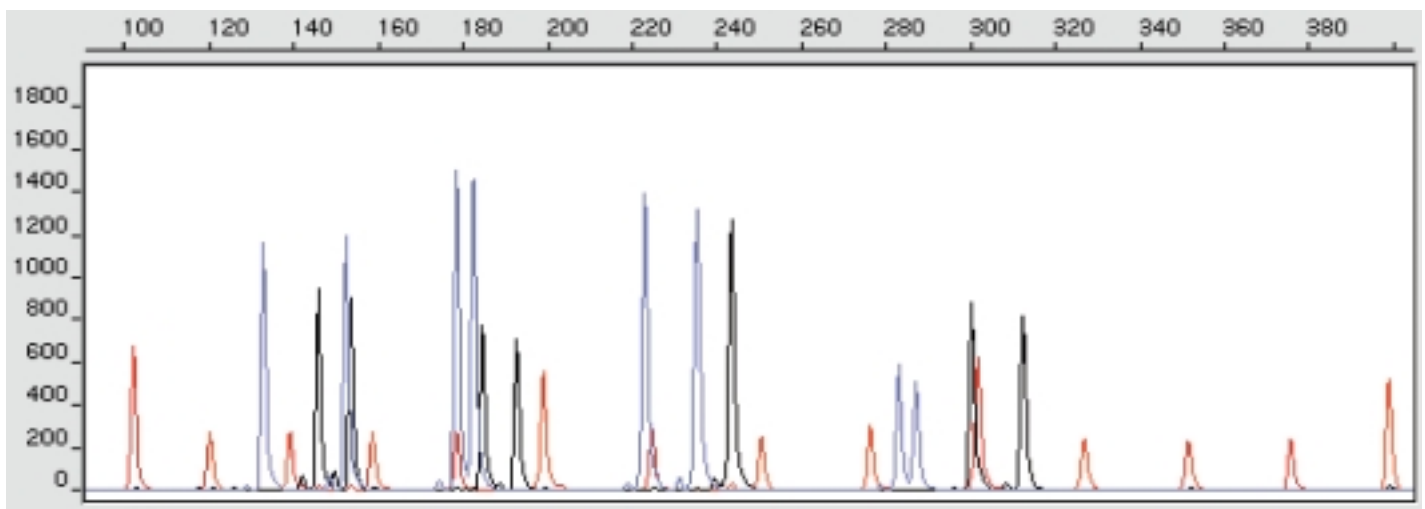


Figure 2. The GenePrint™ PowerPlex™ Fluorescent STR System (Fluorescein/TMR) (ABI). The electropherogram of a DNA sample co-amplified at eight loci is shown. The amplified products of the fluorescein-labeled loci (D16S539, D7S820, D13S317 and D5S818) are shown as purple peaks, while the TMR-labeled loci (CSF1PO, TPOX, TH01 and vWA) are shown as black peaks. The Fluorescent Ladder (CXR), 60-400 Bases, is displayed as red peaks. All materials were separated using a 4% denaturing polyacrylamide gel and detected with the ABI PRISM™ 377 DNA Sequencer.

SILVER DETECTION

Six of the twelve loci included in the PowerPlex™ and FFFL Systems are currently available in multiplexes that may be analyzed using silver stain analysis (Table 1). These are contained in the CTT Multiplex (CSF1PO, TPOX, TH01) and the FFv Multiplex (F13A01, FESFPS, vWA). A third multiplex system, the SilverSTR™ III Multiplex (D16S539, D7S820, D13S317), is being developed. Use of these nine loci in silver detection analyses offers significant discriminating power to laboratories that are not able to afford the capital investment required to purchase an instrument for fluorescent detection formats. With these silver detection systems, nine of the same loci that are available for high throughput screening will also be available in multiplex format for low cost, lower throughput needs. While the silver stain approach is slightly more cumbersome and time consuming than fluorescence detection systems, only a gel rig, a power supply and a few plastic tubs are necessary to set up the detection method. Figure 4 illustrates typical results obtained using the CTT Multiplex.

POWER OF DISCRIMINATION

Use of these STR multiplex systems provides extremely powerful discrimination. Preliminary development of population statistics for the twelve-locus combination of the PowerPlex™ and FFFL Systems (Table 2), the eight-locus PowerPlex™ System alone (Table 3), or the nine-locus combination of the three silver stain-compatible STR

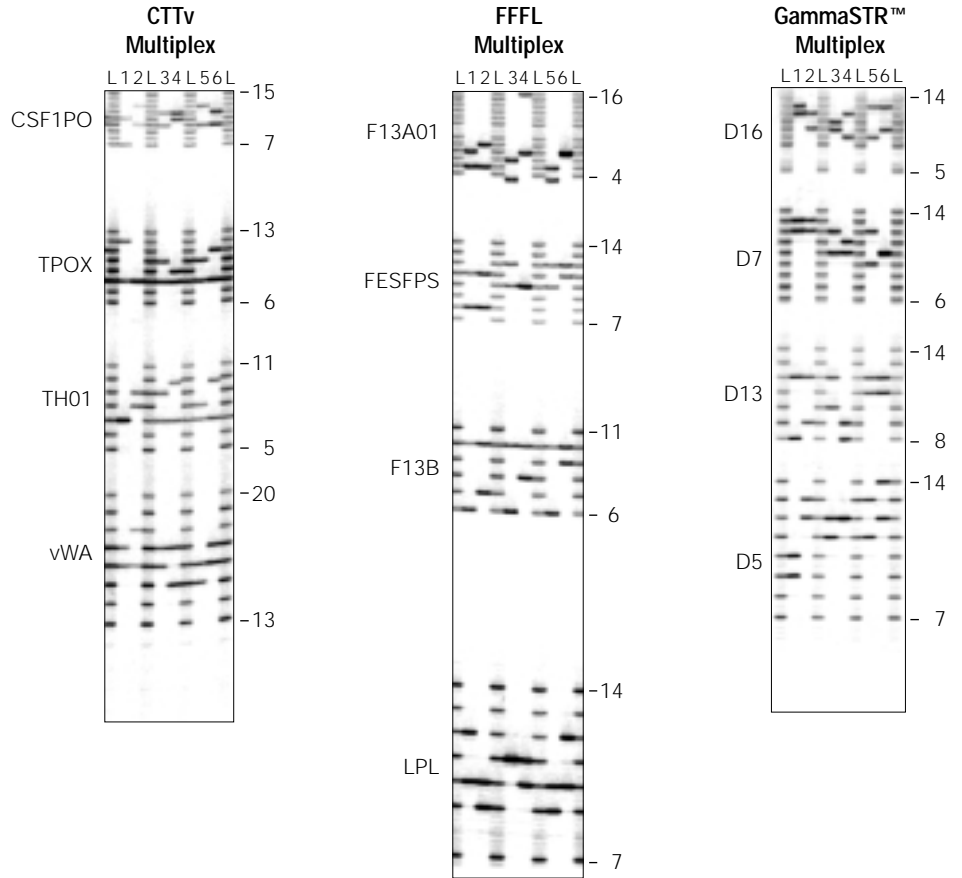


Figure 3. GenePrint™ fluorescein-labeled STR Multiplex Systems. In each panel, six DNA samples have been amplified (Lanes 1-6) and are shown along with allelic ladders for the corresponding system (Lanes L). DNA samples amplified using the CTTv, FFFL and GammaSTR™ Multiplex systems are shown. Each allelic ladder is labeled to its right with the number of copies of the repeated sequence contained within its corresponding largest and smallest alleles. All materials were separated using a 4% denaturing polyacrylamide gel and detected using the Molecular Dynamics FluorImager™ SI Fluorescent Scanner.

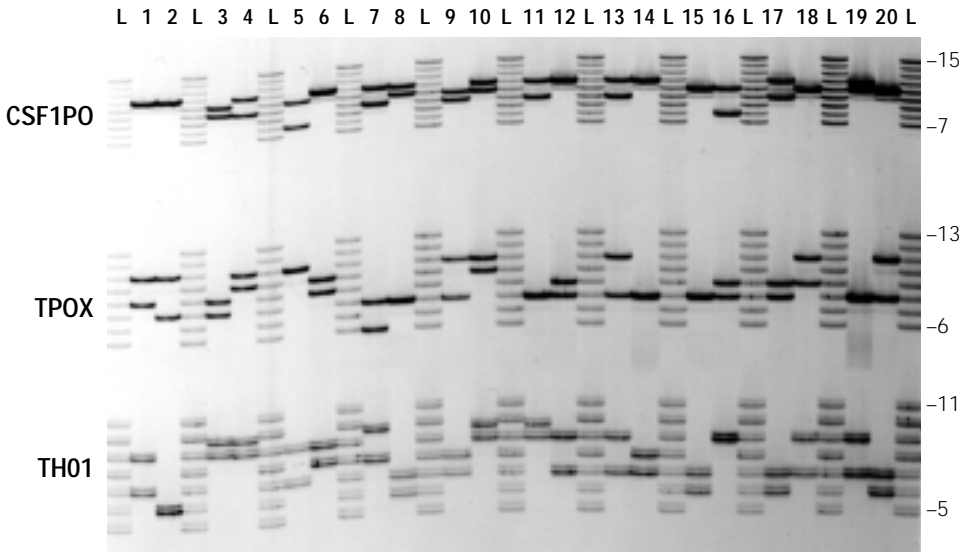


Figure 4. Silver stain detection of the GenePrint™ CTT Multiplex System. Twenty DNA samples were amplified and are shown (Lanes 1-20) along with allelic ladders for the corresponding systems (Lanes L). Regions of the gel containing amplified alleles of the CSF1PO, TPOX and TH01 loci are labeled to the left, with the largest and smallest alleles of the corresponding allelic ladders labeled to the right. All materials were separated using a 4% denaturing polyacrylamide gel and detected using the DNA Silver Staining System. The doublets observed at the TH01 locus result from silver staining of opposing strands of the amplified product which are separated in this denaturing gel system due to their sequence differences. With fluorescent systems, only one strand of the amplified product is fluorescently labeled, generating a single visible band (Figure 1).

Multiplexes (Table 4) are illustrated. These data are based on analysis of more than two hundred individuals from each of three major racial and ethnic groups present in the United States: African-Americans, Caucasian-Americans and Hispanic-Americans.

As an example of the enormous discriminating ability of these systems, consider the combined twelve-locus system (i.e., the PowerPlex™ and FFFL Systems) evaluated in the African-American population. The matching probability has been calculated at nearly 1 in 3 trillion. For paternity applications, these two systems used in combination provide typical paternity indices of greater than 2,500 in each racial group, and power of exclusion of greater than 0.9997, making these systems a competitive alternative to VNTRs or any other available system in terms of discriminating power.

SUMMARY

The *GenePrint™* STR Multiplexes described in this article have been selected and developed because they display a minimum of genetic or amplification artifacts and elicit low mutation rates. This has allowed development of reliable, easily applied systems. Loci containing microvariant alleles are nearly absent from the *GenePrint™* STR Multiplexes. The two loci which do contain microvariants (i.e., TH01 and F13A01) are well characterized and easily included in the analysis. The inclusion of allelic ladders with each system provides a rapid and accurate method of allele determination. The development of both silver stain and fluorescent detection methods for the same STR systems provides universal application across laboratories with different levels of funding and sophistication. Whatever approach for detection is selected, there are enough multiplex systems available in the *GenePrint™* product line to provide adequate discrimination for all routine forensic and paternity applications.

Table 2. Population Statistics for the Twelve-Locus Combined PowerPlex™ and FFFL Systems.

	African-American	Caucasian-American	Hispanic-American
Matching Probability	1 in 2.91 x 10 ¹²	1 in 1.78 x 10 ¹¹	1 in 2.37 x 10 ¹¹
Typical Paternity Index	6,691	5,605	2,617
Typical Power of Exclusion	.99989	.99983	.99973

Table 3. Population Statistics for the Eight-Locus Combined PowerPlex™ System.

	African-American	Caucasian-American	Hispanic-American
Matching Probability	1 in 2.61 x 10 ⁸	1 in 1.18 x 10 ⁸	1 in 1.45 x 10 ⁸
Typical Paternity Index	403	354	319
Typical Power of Exclusion	.9979	.9976	.9973

Table 4. Population Statistics for the Nine-Locus Combined Silver Stain Systems.

	African-American	Caucasian-American	Hispanic-American
Matching Probability	1 in 4.93 x 10 ⁹	1 in 1.05 x 10 ⁹	1 in 1.83 x 10 ⁹
Typical Paternity Index	987	723	556
Typical Power of Exclusion	.9992	.9989	.9986

*STR loci are the subject of German Patent No. DE 38 34 636 C2 issued to the Max-Planck-Gesellschaft zur Förderung der Wissenschaften, eV, Germany. Exclusive rights have been assigned to Promega Corporation for uses in human clinical research and diagnostics applications and all forms of human genetic identity. Exclusive rights to human linkage analysis in the research market are assigned to Research Genetics, Inc., Huntsville, Alabama. All other rights are shared by Research Genetics and Promega.

The development and use of STR loci is covered by U.S. Patent No. 5,364,759 assigned to Baylor College of Medicine, Houston, Texas. Rights have been licensed to Promega Corporation for all applications. Most applications have been licensed on an exclusive basis. U.S. Patent No. 5,599,666 has been issued to Promega Corporation for allelic ladders for the loci CSF1PO, F13A01, FESFPS, LPL and vWA. PCR primers for the STR loci were developed in several laboratories including that of Dr. C. Thomas Caskey while at Baylor College of Medicine (Houston, Texas), Dr. Peter Gill at the Forensic Science Service (Aldermaston, Reading, Berkshire) and Dr. Jeffrey Murray at The University of Iowa (Iowa City, Iowa).

Use of the *GenePrint™* STR System requires performance of the polymerase chain reaction (PCR), which is the subject of European Patent Nos. 201,184 and 200,362, and U.S. Pat. Nos. 4,683,195, 4,965,188 and 4,683,202 owned by Hoffmann-La Roche. Purchase of the *GenePrint™* STR System does not include or provide a license

with respect to these patents or any other PCR-related patent owned by Hoffmann-La Roche or others. Users of the *GenePrint™* STR System may, therefore, be required to obtain a patent license, depending on the country in which the system is used. For more specific information on obtaining a PCR license, please contact Hoffmann-La Roche.

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Ask Us

By Kimberly A. Huston

e-mail: genetics@promega.com

Short tandem repeat (STR) polymorphisms are highly useful tools for human identification, paternity testing and genetic mapping (1). STRs contain tandemly repeated sequences that range from 3 to 7 base pairs in length (2-4). Alleles of STR loci are defined by the number of repeated sequences they contain and are easily amplified using the Polymerase Chain Reaction* (5-8). This allows analysis and interpretation of STR results in 1-2 days.

Q: How do STR systems work?

A: Genomic DNA is purified and the STR regions of interest are amplified. The amplification products, generally less than 400bp in size, are run on a 4% or 6% denaturing polyacrylamide gel. Allelic ladders containing fragments of the same size as several or all known alleles for each locus are run on the gel alongside the amplified samples. Comparison of sample and allelic ladders allows easy interpretation of amplified alleles. After electrophoresis, alleles are detected by silver staining, fluorescent detection, or the use of radioactivity. *GenePrint™* STR Systems that utilize silver and fluorescent detection formats are available from Promega.

Q: Will these systems work with degraded DNA?

A: Degraded DNA is more amenable to STR analysis than VNTR analysis as the amplification products are short, generally under 400bp in size.

Q: How much DNA is needed for use with the *GenePrint™* STR Systems?

A: The amount of template DNA is somewhat system-dependent; in general, the *GenePrint™* STR Systems are designed for use with 1 to 25ng of template DNA.

Q: What are the common amplification artifacts seen with STR analysis?

A: Repeat slippage (9,10), also called "stutter bands", "n-4 bands" or "shadow bands", is a common phenomenon within STR analysis. This is due to the loss of one repeat during amplification and is seen as a band that is a full repeat smaller than the true allele. The frequency of the artifact varies with different loci as well as, to a lesser degree, primer design. We have selected loci and primers carefully to minimize the occurrence of this phenomenon. A second amplification artifact seen with STR analysis is due to terminal nucleotide addition (11,12). During amplification, *Taq* DNA Polymerase** adds a nucleotide, generally adenine, to the ends of the amplified DNA fragments. Terminal nucleotide addition artifacts appear because this process does not occur with 100% efficiency. The occurrence of the n-1 artifact (i.e., bands which have not received the terminal nucleotide) can be minimized by careful primer design as well as by the addition of a 60°C incubation for 30 minutes at the end of the amplification profile (13). The *GenePrint™* STR Systems have been specifically engineered to minimize the occurrence of these artifacts.

Q: Will any *Taq* DNA Polymerase function within the *GenePrint™* STR Systems?

A: Promega's STR systems have been developed for amplification using standard *Taq* DNA Polymerase. Specialized enzymes such as AmpliTaq Gold™ (Roche Molecular Systems) are not required for peak performance. If using AmpliTaq Gold™, use the GeneAmp® (Roche Molecular Systems) PCR buffer that is provided with the enzyme (instead of the 10X STR Buffer included in the *GenePrint™* STR Systems) and add each dNTP to the reaction mix at a final concen-

tration of 200µM. Use of AmpliTaq Gold™ also necessitates an additional incubation at 95°C for 11 minutes prior to the initiation of the thermal cycling profile. The STR Buffer (pH 9.0) currently provided with the *GenePrint™* STR Systems does not function optimally with AmpliTaq Gold™ due to incompatibility with the pH of the modified enzyme.

Q: Are there any special considerations for the thermal cycler used with the *GenePrint™* STR Systems?

A: All of the *GenePrint™* STR Systems are optimized for the Perkin Elmer Model 480 Thermal Cycler with the exception of the *GenePrint™* PowerPlex™ Fluorescent STR Systems which are optimized on the GeneAmp® PCR System 9600 Thermal Cycler. All of the *GenePrint™* STR Systems are provided with protocols for use with both of these thermal cyclers. Customers have reported that the BIOTHERM (MJ Research) and the Perkin Elmer Model 2400 thermal cyclers also perform well.

Q: Are microvariant alleles seen within the *GenePrint™* STR Systems?

A: Microvariants are alleles that differ from one another by lengths other than the repeat length (i.e., 4 bases). The occurrence of microvariants appears to be correlated with highly polymorphic STR regions and increased mutation rates (14,15). Because of this, we have chosen loci which are moderately high with respect to polymorphisms as well as those which have a minimal occurrence of microvariants. Microvariants are more common in many other STR systems which have been developed, adding complexity to allele assignment. There are two loci within the *GenePrint™* STR Systems in which microvariants have been observed.

The TH01 locus contains the common 9.3 microvariant allele as well as the very rare 8.3 allele. The 3.2 allele has been observed within the F13A01 locus. Although microvariants can complicate interpretation, they are easily separated on a 4% denaturing polyacrylamide gel.

Q: What is the matching probability of the GenePrint™ STR Systems?

A: The matching probability increases with the number of STR loci that are amplified. The GenePrint™ PowerPlex™ STR System, in which eight loci are amplified in a single reaction, has a matching probability ranging from 1 in 118,000,000 for Caucasian-Americans to 1 in 261,000,000 for African-Americans. When using this system in combination with the GenePrint™ Fluorescent STR Multiplex-F13A01, F13B, FESFPS, LPL (FFFL Multiplex), the matching probability is 1 in 178,000,000,000 for Caucasian-Americans and 1 in 2,910,000,000,000 for African-Americans in just 2 reactions of 12 loci.

Q: What are the typical paternity indices for the GenePrint™ STR Systems?

A: The typical paternity index (PI) for the GenePrint™ PowerPlex™ STR System is 354 for Caucasian-Americans and 403 for African-Americans. When used in combination with the GenePrint™ Fluorescent STR Multiplex-F13A01, F13B, FESFPS, LPL (FFFL Multiplex) the PI value is 5,605 for Caucasian-Americans and 6,691 for African-Americans.

Q: What is included with the GenePrint™ STR Systems?

A: Each GenePrint™ System contains all of the materials required to amplify STR regions, with the exception of Taq DNA Polymerase. Each system includes sufficient STR Allelic Ladder to load every third lane. This means that each amplified sample can be run next to an Allelic Ladder to allow for

easy identification of alleles. The GenePrint™ PowerPlex™ System also includes a fluorescent ladder which is labeled with carboxy-X-rhodamine. The Fluorescent Ladder (CXR), 60-400 Bases, contains 16 evenly spaced DNA fragments of 60, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, and 400 bases. This ladder can be used as an internal size marker to increase precision in analysis. The Fluorescent Ladder (CXR) and the GenePrint™ Multiplex Allelic Ladder can also be purchased separately.

Q: Which fluorescent dyes are used in the GenePrint™ Fluorescent STR Systems?

A: All of the GenePrint™ Fluorescent STR Systems which amplify 4 STR loci contain locus specific primers which are labeled with fluorescein. Within the GenePrint™ PowerPlex™ Fluorescent STR System, one primer for each of the loci D16S539, D7S820, D13S317 and D5S818 is labeled with fluorescein and one primer for each of the loci CSF1PO, TPOX, TH01 and vWA is labeled with carboxy-tetramethylrhodamine (TMR). The use of different fluorescent dyes allows simultaneous detection of overlapping loci within one reaction, in a single gel lane.

Q: Who do I contact with technical questions on the GenePrint™ STR Systems or any other Promega product questions?

A: Promega's Technical Services Department is committed to helping you with any questions you may have. Contact us either at 1 (800) 356-9526 or by e-mail at: genetics@promega.com. Outside the U.S., please contact your local Promega Branch Office or distributor.

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*PCR is a patented process. Promega does not encourage or support the unauthorized or unlicensed use of the PCR process.

**Some applications in which this product may be used are covered by patents issued and applicable in certain countries. Because purchase of this product does not include a license to perform any patented application, users of the product may be required to obtain a patent license depending upon the particular application and country in which the product is used. For more specific information, please contact Promega.

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AmpliTaq Gold is a trademark and GeneAmp is a registered trademark of Roche Molecular Systems, Inc.

GenePrint™ SHORT TANDEM REPEAT (STR) WORKSHOPS

As part of Promega's worldwide commitment to customer education and exceptional technical support, we are currently offering a series of workshops entitled "DNA Typing With STRs."



Mary Hoenecke demonstrates the proper method of preparing reagents for polyacrylamide gels.

About the Workshops

COMPREHENSIVE

The 2-day workshops provide practical hands-on experience in all aspects of DNA typing using Promega's GenePrint™ STR Systems, including:

- Amplification
- Agarose yield gels
- Polyacrylamide gel preparation
- Polyacrylamide gel electrophoresis
- Silver staining or fluorescent detection
- Data analysis



Workshop participants load their amplified samples onto a polyacrylamide gel for electrophoresis.

WORLDWIDE

GenePrint™ STR workshops are offered throughout the USA and the world.

PROVEN EFFECTIVE

The workshops are well attended and have been extremely popular with our DNA typing customers. Below are comments from former participants.

"The course was extremely well organized. The instructors are excellent and the methodology is elegant...the STR typing method is so well optimized." (Los Angeles Police Department)

"I feel that I can go back to my lab and get reproducible results using these kits...everything worked..." (Biological Laboratory)

"Very well organized!" (Workshop Participant)

"The instructors were very knowledgeable...they were great..." (Texas Department of Public Safety)

HOW TO FIND OUT MORE ABOUT PROMEGA'S WORKSHOPS

- View our Internet site at www.promega.com/genetic/
- Contact Carol Zabit in the USA at
Tel: (608) 277-2670
Fax: (608) 277-2601
e-mail: czabit@promega.com

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LATIN AMERICAN SYMPOSIUM ON HUMAN IDENTIFICATION

August 11-13, 1997

Brazilia, Brazil

STATISTICS WORKSHOP

September 15-16, 1997

Scottsdale, Arizona

8TH INTERNATIONAL SYMPOSIUM ON HUMAN IDENTIFICATION

September 17-20, 1997

Scottsdale, Arizona

2ND EUROPEAN SYMPOSIUM ON HUMAN IDENTIFICATION

June 9-12, 1998

Innsbruck, Austria

Contact: Carol Zabit at (608) 277 2670
(e-mail: czabit@promega.com) for information on any of the symposia listed above.

Promega Workshops

"DNA TYPING WITH STRS"

FLUORESCENT DETECTION:

May 13-14

June 24-25

July 29-30

August 12-13

November 18-19

December 9-10

SILVER STAIN DETECTION:

August 5-6

November 11-12