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Profiles in DNA
is published by:
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Profiles in DNA is also
available on the Internet at:
www.promega.com/profiles/

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ABOUT THE COVER

An exciting, new approach to real-time quantitative PCR is coming soon to a laboratory near you. Learn more about the technology in this issue, and in the next issue of *Profiles in DNA*, look for more information about a new system that allows you to simultaneously quantitate human and male-specific DNA.

Upcoming Meetings

California Association of Criminalists Spring Seminar
May 9–13, 2005
Oakland, California, USA
www.cacnews.org

2005 Mid-Atlantic Association of Forensic Scientists Annual Meeting
May 18–20, 2005
Pittsburgh, Pennsylvania, USA
www.maafs.org

2005 International Association of Forensic Sciences Meeting
August 21–26, 2005
Hong Kong
www.iafs2005.com

Fourth European-American School in Forensic Genetics and Mayo Clinic Course in Advanced Molecular and Cellular Medicine
September 5–9, 2005
Dubrovnik, Croatia
www.euroamerican-genetics-meetings.org/

21st Congress of the International Society for Forensic Genetics
September 13–17, 2005
Ponta Delgada, Azores, Portugal
www.ipatimup.pt/isfg2005

16th International Symposium on Human Identification
September 26–29, 2005
Grapevine, Texas, USA
www.promega.com/geneticsymp16/

Dear Readers,

In this issue, we look toward the future. The feature article provides us with a view into the future of DNA quantitation. In the first article of a two-part series, Promega scientists Benjamin Krenke, Steve Ekenberg, Susan Frackman, Katharine Hoffmann, Cynthia Sprecher and Douglas Storts describe a novel, real-time, quantitative PCR technology based on the specific interaction between two modified nucleotides. The follow-up article will be published in the fall issue of *Profiles in DNA* and will describe a real-time quantitative PCR system based on this chemistry that allows simultaneous quantitation of human and male-specific DNA.

David Cornacchia and Ian Fitch from the San Diego Police Department Crime Laboratory then recount their courtroom experiences with Y-STR markers. Their success in presenting Y-STR evidence is good news for the increasing number of laboratories using Y-STR markers to obtain DNA evidence in situations where autosomal STR markers cannot provide definitive results.

In our spotlight article, Linda Carne and Ramona Thiss describe how the Virginia Institute of Forensic Science and Medicine is training the forensic analysts of the future. Certified forensic scientists provide hands-on training to all levels of healthcare professionals, legal and law enforcement personnel and emergency medical technicians. The institute also offers public education courses where participants learn the reality of the forensic science depicted in popular television shows.

Kimberly Huston and Curtis Knox then introduce the new PowerPlex® Matrix Standards, 3100—Custom. These matrix standards result in better performance over the entire linear range of fluorescence detection. Finally, in "Tech Tips", Curtis Knox and Eric Vincent answer frequently asked questions about the Differex™ System, a new, improved method for separating sperm and epithelial fractions in sexual assault samples.

What topics would you like to see in future issues? We are continually seeking to improve *Profiles in DNA*, and we would like to hear your comments and ideas. Please contact us by letter at the address given on page 1 or by e-mail at: profilesindna@promega.com.



Terri Sundquist
Editor,
Profiles in DNA

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QUANTITATIVE PCR

Development of a Novel, Fluorescent, Two-Primer Approach to Quantitative PCR

Benjamin E. Krenke, Steve Ekenberg, Susan Frackman, Katharine Hoffmann, Cynthia J. Sprecher and Douglas R. Storts
Promega Corporation

INTRODUCTION

Multiplexed short tandem repeat (STR) analysis has become the dominant technology in DNA-based human identification. Although highly informative, these assays require a defined range of template quantities to produce optimal results. Thus, the ability to accurately assess the extracted DNA quantity and quality has become increasingly important. Quantification of male-specific DNA has also become necessary with the advent of forensic Y-STR testing. All of these issues can be addressed in a quantitative PCR system.

Many STR megaplexes used in forensic applications require 0.5–1 ng of DNA template. Using template quantities below the suggested range increases the likelihood of allelic imbalance and partial amplification. Excessive template can lead to signal saturation during analysis, nonspecific amplification and product imbalance for the different amplified loci. Precise quantification of template DNA conserves resources by reducing the need to reamplify samples that are not interpretable due to insufficient or excessive template.

Common hybridization-based quantification methods produce increased levels of false-negative results (due to lack of sensitivity) or subjective conclusions (due to visual comparison of band intensities). Amplification-based quantification methods provide a high level of sensitivity, and real-time PCR methods can deliver a dynamic range that exceeds that of other methods. The numerical output of real-time quantitative PCR also increases the objectivity of data interpretation.

Here we describe a new technology for real-time quantitative PCR. The background of this chemistry is presented here, and an article in the next issue of *Profiles in DNA* will describe the development of a human-specific and male-specific quantitation system based on this technology.

THE PLEXOR™ TECHNOLOGY

This new real-time PCR^(a) method takes advantage of the specific interaction between two modified nucleotides to achieve quantitative analysis (1–3). As shown in Figure 1, two novel bases, isoguanine (iso-dG) and 5'-methylisocytosine (iso-dC),

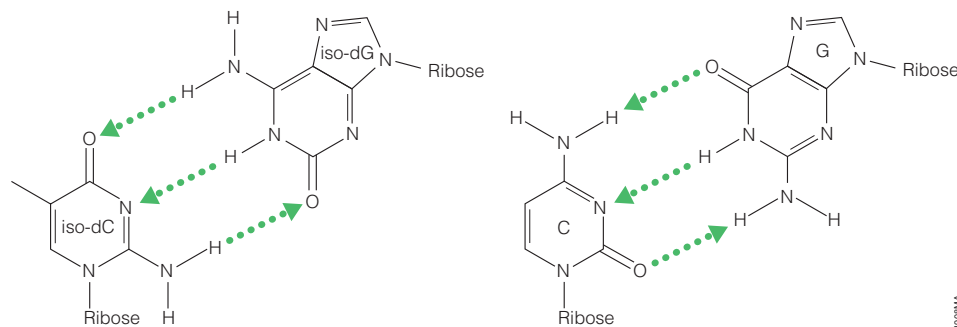


Figure 1. Base pairing between isoguanine (iso-G) and 5'-methylisocytosine (iso-C).

The first predesigned assays will allow quantitation of total human and male-specific DNA in a two-color multiplex assay, with the potential for further multiplexing.

form a unique base pair in double-stranded DNA (2). To perform fluorescent quantitative PCR using this new technology, one primer is synthesized with an iso-dC residue as the 5'-terminal nucleotide and a fluorescent label at the 5'-end; the second primer is unlabeled. During PCR, this labeled primer is annealed and extended, becoming part of the template used during the next round of amplification. During subsequent rounds of amplification, the complementary iso-dGTP, which is available in the nucleotide mix as dabcyL-iso-dGTP, pairs specifically with iso-dC. When the dabcyL-iso-dGTP is incorporated, the close proximity of dabcyL and the fluorescent label on the opposite strand effectively quenches the fluorescent signal. This process is illustrated in Figure 2.

By directly coupling fluorescence detection and thermal cycling, real-time PCR measures the change of fluorescent signal (in relative fluorescent units, RFU) at every cycle. The initial fluorescence level of the labeled primers is high in Plexor™ System^(b) reactions. As amplification product accumulates, the signal decreases. Amplification data present a characteristic three-phase curve (Figure 3, Panel A).

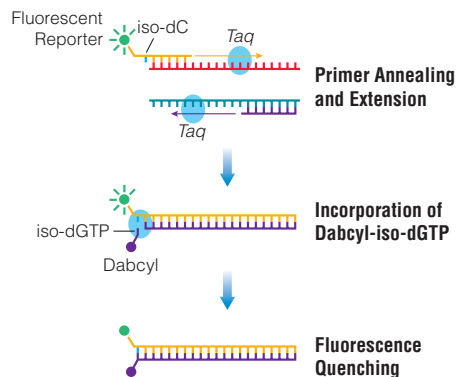


Figure 2. Quenching of the fluorescent signal by dabcyL during product accumulation.

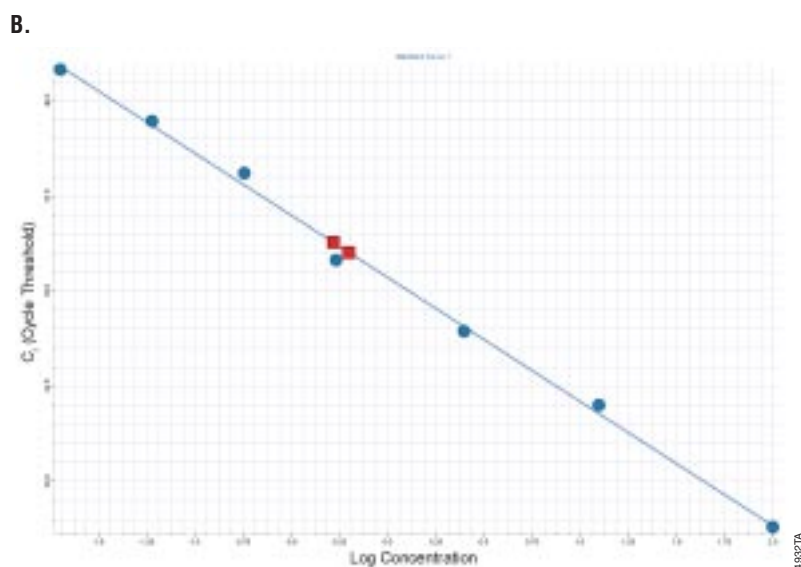
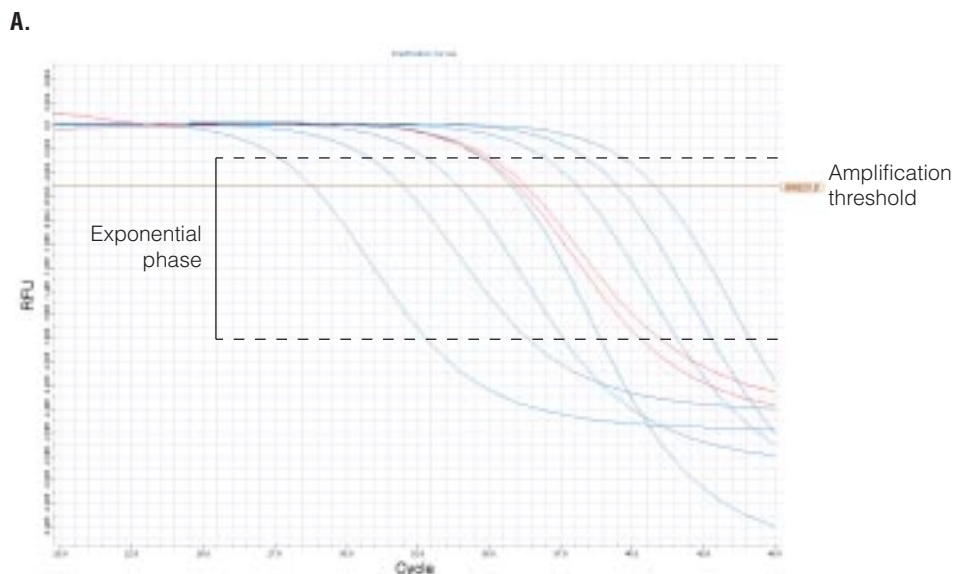


Figure 3. Panel A. A representative amplification curve, which shows the relative fluorescence units (RFU) at each cycle of the reaction. The amplification threshold is indicated by a horizontal line across the graph. This threshold is used to establish the cycle threshold, the cycle at which the amplification curve crosses the amplification threshold, for each sample. The blue circles represent standard samples, and the red squares represent unknown samples. Panel B. A standard curve generated from the amplification curve data shown in Panel A.

The part of the curve with the biggest signal change is the exponential phase. The exponential phase is the most consistent phase and is used to estimate the quantity of starting material. An amplification threshold is set within the exponential phase at a

fluorescence level where all amplification curves exhibit the most significant signal decrease. The point at which an amplification curve crosses that threshold is the cycle threshold (C_t) of the sample. C_t values for a dilution series of a sample of known

QUANTITATIVE PCR

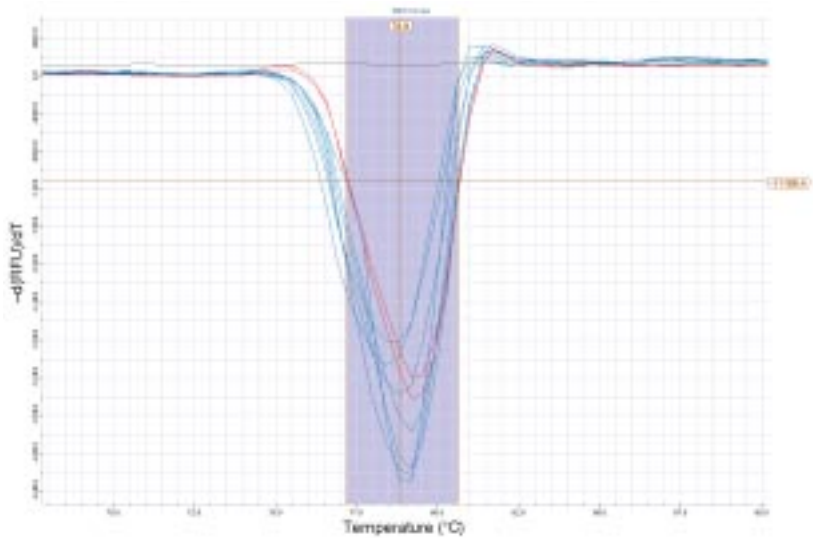


Figure 4. Thermal melt curve. The melting temperature was empirically determined from the data shown in Figure 3 by plotting the change in fluorescence with temperature ($-dRFU/dT$) versus temperature and calculating the temperature at which the biggest change in fluorescence occurs.

DNA quantity are used to generate a standard curve, which is used to quantify samples with unknown amounts of DNA (Figure 3, Panel B).

Quenching of the fluorescent label by dabcyI is a reversible process. Fluorescence is quenched when the product is double-stranded due to the close proximity of dabcyI and the fluorescent label. Denaturing the product separates the label and quencher, resulting in an increased fluorescent signal. Consequently, thermal melt curves can be generated by allowing all product to form double-stranded DNA at a lower temperature (approximately 60°C) and slowly ramping the temperature to denaturing levels (approximately 95°C).

Figure 4 illustrates a “melt curve” with the empirically derived melting temperature (T_m). The product length and sequence impact T_m , so the melt curve is used to characterize amplicon homogeneity in the selected wells. Nonspecific amplification can be identified by broad peaks in the melt curve or peaks with different T_m values. By distinguishing specific and

nonspecific amplification products, the melt curve adds a quality control aspect during routine use of validated or predesigned assays.

A benefit of the Plexor™ technology over detection using simple DNA-binding dyes, such as SYBR® Green, is the capacity for multiplexing. The labeled primer can be tagged with one of many common fluorescent labels used in fluorescent PCR, allowing two- to five-color multiplexing, depending on the instrument used. The simplicity of primer design for the Plexor™ technology is a distinct advantage over probe-based quantitative PCR approaches.

DATA ANALYSIS AND INSTRUMENT COMPATIBILITY

The Plexor™ Analysis Software has been developed to analyze amplification data from a variety of real-time instruments, plot standard curves and calculate DNA concentrations of unknown samples. The software, which will be distributed free of charge, allows freedom of choice for instrument use. Currently, data can be imported from

the ABI PRISM® 7000, 7700 and 7900HT sequence detection systems, Applied Biosystems 7500 Real Time PCR System, Roche LightCycler® 1.0 and 2.0 instruments, Bio-Rad iCycler® thermal cycler, MJ Research DNA Engine Opticon® 2 fluorescence detection system, Cepheid SmartCycler® II system and the Stratagene Mx3000 and Mx3000P™ real-time PCR systems.

APPLICATION OF THE PLEXOR™ TECHNOLOGY

Plexor™ System protocols are being developed for quantitative PCR, quantitative RT-PCR and genotyping. Users of the Plexor™ Systems can design and order fluorescently labeled, iso-dC-containing primers specific for their assays.

Predesigned assays using this technology are in development. The first predesigned assays will allow quantitation of human-specific and male-specific DNA. We have demonstrated simultaneous quantitation of total human and male-specific DNA in a two-color multiplex assay, with the potential for further multiplexing.

Stay tuned for more information about this application of the Plexor™ technology in the next issue of *Profiles in DNA*.

The Plexor™ technology is licensed from EraGen Biosciences.

REFERENCES

- Sherrill, C.B. *et al.* (2004) Nucleic acid analysis using an expanded genetic alphabet to quench fluorescence. *J. Am. Chem. Soc.* **126**, 4550–6.
- Johnson, S.C. *et al.* (2004) A third base pair for the polymerase chain reaction: Inserting isoC and isoG. *Nucl. Acids Res.* **32**, 1937–41.
- Moser, M.J. and Prudent, J.R. (2003) Enzymatic repair of an expanded genetic information system. *Nucl. Acids Res.* **31**, 5048–53.

Introducing Y-STR DNA Testing in the Courts

By David Cornacchia and Ian Fitch
San Diego Police Department Crime Laboratory

In all three cases, the Y-STR testing results were accepted in the courtroom with little specific opposition from the attorneys or the bench.

INTRODUCTION

In July 2004, the San Diego Police Department (SDPD) Forensic Biology unit began using the PowerPlex®Y System[©] for Y-STR DNA testing in casework. To date, we have performed such testing in approximately ten cases and testified in three of those cases. Here we present a summary of these cases, including the corresponding court experiences.

CASE #1: A GANG-RELATED HOMICIDE

by David Cornacchia

On June 15, 2003, 23-year-old Travis Thomas was shot and killed at Mission Bay Park in San Diego. Based in part on video captured by a nearby security camera, homicide detectives arrested eleven documented gang members. Eight of those arrested subsequently pled guilty to charges ranging from manslaughter to assault. Na'il Downey, Darrell Tittle and Jerome Silvels all maintained their innocence and chose to be tried for murder. Although it was unclear from the video, eyewitnesses identified Na'il Downey as the shooter.

In preparation for trial, the District Attorney's Office asked the SDPD crime lab to perform DNA testing on a red leather jacket, a green bandana and a pair of black leather gloves to determine if Na'il Downey could be a habitual wearer of this visually distinctive attire that eyewitnesses attributed to the shooter. The results were anything but distinguishing! Each item yielded a complex DNA mixture from at least three individuals. Based on results at thirteen autosomal-STR markers, Na'il Downey was excluded as a source of the DNA from the bandana but could not be included or excluded as a potential DNA contributor to the gloves and jacket. Based on results at the amelogenin locus, it appeared that at least one female was a major contributor to the DNA mixture obtained from the jacket.

After speaking with the prosecuting attorney assigned to the case, we decided to see if Y-STR testing could clarify whether Na'il Downey's DNA could be a component of the mixture from the jacket. A ten-locus Y-STR profile from at least two males was obtained from the sample. This time Na'il Downey was definitively excluded as being a DNA contributor. I was encouraged by the results. As we had predicted, by eliminating the sizeable female component in the DNA mixture, we got a much clearer look at the male component.

TESTIFYING IN COURT

On September 15, 2004, I got an opportunity to testify to all my DNA results, including for the first time, Y-STRs. As a relative greenhorn, I found myself in the advantageous position of being essentially unchallenged as to the merits of Y-STR testing. The prosecution requested the work be performed and so was in no position to question its use. As you can imagine, the defense fully embraced my results and therefore had no interest in raising a legal challenge.

During my direct examination, I simply was asked if I was able to use DNA from the jacket to exclude the defendant. Clearly, the prosecutor was all too willing to let the topic of Y-STR testing slide. Cross-examination proceeded much the same way. Just when I thought this golden learning opportunity had passed, the attorney

Y-STRs IN COURT

representing Na'il Downey began to ask detailed questions on how and why Y-STR testing was different from standard forms of DNA testing. In preparation for my testimony, I had decided that the easiest way to present Y-STR testing to a jury was to circumvent the concept of chromosomes altogether. In the simplest terms possible, I explained that Y-STRs are unique in that we examine that portion of the DNA molecule that is responsible for defining males. Since females lack this region, only DNA from males produce results. While perhaps oversimplified, I found this approach to be generally well received by both the jurors and the attorneys.

In the end, Na'il Downey was convicted of second degree murder, Darrell Tittle was found guilty of manslaughter, and the jury hung on the case against Jerome Silvels.

CASE #2: ALLEGED SEXUAL ASSAULT OF AN UNCONSCIOUS VICTIM

by Ian Fitch

In May 2004, the victim in this case attended a social event where she apparently drank too much and passed out. She awoke in a hotel room to find herself in bed with three males. Complaining of discomfort to her vagina and anus, she submitted to a sexual assault response team (SART) exam. One of the three males was subsequently developed as a possible rape suspect.

The SART kit and items of the victim's clothing were examined by a criminalist at the SDPD crime lab. Semen was found on several items, and the DNA results implicated the suspect as the source of the sperm DNA from the vaginal swabs, shallow rectal swabs, external anal swabs and a cutting from the victim's dress. DNA from semen

consistent with the victim's boyfriend was also found on cuttings from the victim's underwear, and he appeared to be a minor contributor to the sperm DNA from the vaginal swabs, external anal swabs and dress cutting.

After narrowly avoiding the subject several times in the past, this time I did discuss the product rule and why the closely linked Y-STR markers were not subject to this method of DNA profile frequency determination.

My colleague also examined a deep rectal swab and observed a low number of sperm cells—too few for autosomal-STR testing. However, we thought Y-STR testing might be appropriate for analyzing this sample for two reasons. First, in our hands the PowerPlex®Y System is somewhat more sensitive than the Profiler Plus™ system used for autosomal-STR testing, and second, we reasoned that DNA from the limited number of sperm cells could be analyzed regardless of the amount of nonsperm DNA from the victim.

I was asked to do the Y-STR testing. The resulting Y-STR profile from the sperm fraction indicated a single male (or male lineage) and matched the suspect in this case. The victim's boyfriend was excluded. The occurrence of the profile (or haplotype) in the PowerPlex®Y database of 2,443 profiles was 13. No Y-STR DNA types were detected in the nonsperm fraction from this item.

TESTIFYING IN COURT

I was asked to testify at the preliminary hearing to determine if the suspect should go on trial on rape and sodomy charges. The prosecutor in this case had some knowledge of Y-STR testing but had not tried a case involving its application before. During the hearing, she asked if I'd performed such testing in this case. I stated "yes", and the subsequent line of questioning allowed me to explain the difference between standard STR and Y-STR testing, when and why we performed such testing, and why we did so in this case.

I stressed that the two methods of DNA testing were technologically similar, but the DNA markers examined in Y-STR testing were male-specific and not present in DNA from females. I explained that Y-STR testing was useful in certain cases and, in particular, sexual assaults, where a relatively small amount of DNA from the male perpetrator is often mixed with a much larger amount of DNA from the female victim, which can mask the male component during standard DNA testing.

After introducing the technology, I presented my findings. The prosecutor asked why the profile frequency of about 1 in 200 was in such contrast to the 1 in billions or trillions we have all come to expect for DNA evidence. I explained that Y-STR markers are clustered on the same piece of DNA (the Y chromosome) and are not subject to the same methods for determining DNA profile frequencies as standard STRs. Not wishing to discuss the product rule, I didn't elaborate. The prosecutor had no further questions. My direct examination took no more than about 15 minutes.

The defense attorney on cross-examination seemed unconcerned that a relatively new technology was used in this case or that the Y-STR

profile that matched his client was relatively common in the general male population. However he was curious that sperm DNA matching his client had been found on the deep rectal swab, but no such DNA from skin cells had been found. During the act of anal sex would I not expect male skin cells to be shed? It was not really within my area of expertise. We moved on! The subsequent questioning went smoothly, and attention was shifted to standard STR testing. All in all, my first Y-STR testimony was relatively painless. The suspect was bound over for trial.

CASE #3: ALLEGED SEXUAL ASSAULT OF A MINOR BY A VASECTOMIZED MALE

by Ian Fitch

This case relates to the alleged molestation of a 17-year-old girl by her vasectomized stepfather. A pair of the victim's underwear, collected by her suspicious mother, was submitted to the SDPD crime lab. Stains on the crotch area of the underwear tested positive for acid phosphatase and P30, but microscopically no sperm cells were observed. I received two cuttings from the underwear for DNA testing. Autosomal-STR testing generated a predominant profile consistent with the victim from each cutting. The suspect was included as a possible minor contributor to the DNA from one of the cuttings, but it was inconclusive if he was included or excluded as a possible contributor to the DNA from the second cutting. I performed Y-STR testing on both cuttings and generated a Y-STR profile from a single male (or male lineage) that matched the suspect. The occurrence of the profile (or haplotype) in the PowerPlex®Y database of 2,443 profiles was 0.

TESTIFYING IN COURT

The suspect was in the military, and on February 7, 2005, I testified at an article 32 hearing (the military equivalent of a preliminary hearing). The prosecutor had not tried a DNA case before and seemed enthusiastic. Upon discussing the Y-STR testing, he suggested that a Daubert hearing might be required should the case go to trial.

This acceptance is encouraging in light of the fact that, since going online with PowerPlex® Y, we have realized that its application in casework is notably broader than originally expected.

The prosecutor asked a few introductory questions, and then I was allowed to present my findings without interruption. I explained that tests had suggested the presence of semen from a sterile or vasectomized male on the underwear and that two methods of DNA testing had been used to analyze the DNA on the two cuttings. I discussed the difference between standard and Y-STR testing and why the latter had been used in this case. My direct examination went smoothly.

The same cannot be said for my cross-examination. The defense attorney went through my education, background, training and all aspects of forensic DNA testing with a fine-tooth comb. He was not hostile but rather curious and thorough. He asked why we'd been using Y-STR testing only since July. I explained that it was a relatively new technology but assured him it was generally accepted by the

forensics community. He asked me if I knew of other labs that were using this technology. I knew a few but also explained that, while the forensics community had been discussing Y-STR testing for some time, implementing new technology for use in casework was a slow process.

I spent considerable time talking about statistical approaches. After narrowly avoiding the subject several times in the past, I did discuss the product rule and why the closely linked Y-STR markers, inherited as a unit from father to son, were not subject to this method of DNA profile frequency determination. The defense attorney asked if I knew the source of samples in the PowerPlex®Y database. This was a very good question—one I was pleased I'd asked previously myself. I explained that they were samples available to the different laboratories in North America that participated in the development of the PowerPlex®Y System, which we use for Y-STR testing.

Thus, my second testimony involving Y-STR testing was more grueling than the first but not because the defense mounted any specific attack on the new technology. He wanted details about everything. At the time of writing, the hearing is still ongoing.

CONCLUSION

In all three cases, the Y-STR testing results were accepted in the courtroom with very little specific opposition from the attorneys or the bench. This acceptance is encouraging in light of the fact that, since going online with PowerPlex®Y, we have realized that its application in casework is notably broader than originally expected, and we anticipate using this new system with some regularity.

Editor's Note: As of March 2005, the PowerPlex®Y database has been expanded to 4,004 profiles.

SPOTLIGHT

State of Forensic Science and Medicine Education in Virginia

By Linda P. Carne and Ramona H. Thiss

Virginia Institute of Forensic Science and Medicine, Richmond, Virginia

INTRODUCTION

Interest in forensic science and medicine has increased dramatically in the past five years. With the explosion of television programs such as *CSI: Crime Scene Investigation*, *Cold Case Files*, *Crossing Jordon* and *Forensic Files*, coupled with extensive media coverage of high-profile criminal cases, the public has become fascinated with anything forensic. This is especially true of high school and college students who contact the Virginia Institute of Forensic Science and Medicine (VIFSM) with questions about how to enter the field of forensics. According to an article in the *Wall Street Journal* on February 19, 2002, "The National Science Teachers Association says forensics is growing in popularity and is a good way to motivate students in math and science weaned on crime shows such as CSI." Mary Fran Ernst, past president of the American Academy of Forensic Science wrote, "Middle and high school teachers are now clamoring for information and pleading for resources and assistance to help them incorporate forensic science into their math and science curriculums." This unprecedented interest comes at a time when the demand for highly trained, certified forensic science and medicine personnel is at a peak. The use of sophisticated technology to investigate crime and crime scenes expands to meet an exponentially increasing criminal justice caseload. According to Dr. Paul Ferrara, Director of the Virginia Division of Forensic Science, "Nationally there is an estimated need for 5,000 to 10,000 trained forensic scientists, the competition is keen and there is a limited pool of qualified examiners to draw upon."

With the explosion of television programs such as CSI: Crime Scene Investigation, Cold Case Files, Crossing Jordon and Forensic Files, coupled with extensive media coverage of high-profile criminal cases, the public has become fascinated with anything forensic.

OVERVIEW

VIFSM is one of the first organizations to address the national need for highly trained forensic personnel and has become the prototype for training programs throughout the country. In 2000, VIFSM was the first to offer forensic science and medicine curriculum integration for high school science and math teachers. The institute is a result of a collaborative effort of the Commonwealth of Virginia's Department of Health, Office of the Chief Medical Examiner, Department of Criminal Justice Services and the Division of Forensic Science. VIFSM was founded in 1999 with the mission to further forensic science and medicine education and training.

Forensic evidence has played a crucial role in investigating and resolving thousands of violent crimes over the past several decades, and its value as an investigative tool is likely to increase in the coming years. Forensic science and medicine must apply the latest available technology through established scientific techniques to identify, collect and examine criminal evidence. Scientists then interpret information gained in the laboratories and autopsy rooms and present reports at judicial proceedings. There is a constant need to provide the highest level of training on technology advances in the forensic science environment for those who need forensic science and medicine information on a daily basis. The mission of VIFSM is to further forensic science and medicine education with the goal of bridging the gap between the advances in forensic science and medicine and their applications in the real world of crime scene and death investigations. VIFSM fulfills this mission through federally and privately funded post-graduate fellowships, forensic training of professionals, forensic curriculum design for state and national partners, research and public education.

FELLOWSHIP PROGRAMS

A key component of the Virginia Institute of Forensic Science and Medicine's work for justice is its post-graduate fellowship program. To date, the institute has trained over three dozen individuals through its one-year and two-year fellowship programs, thereby helping to fill the national need for qualified forensic personnel. VIFSM's current fellowship program, a model for the country, consists of 17 post-graduate forensic fellows in the disciplines of DNA, firearms and toolmarks, controlled substances and latent prints.

Laboratory instruction of fellows is under the direct supervision of certified forensic scientists, with literature reviews, research projects and moot court testimony designed to train fellows to proficiency. The fellowship program relieves state and local forensic laboratories from the burdensome task of providing on-the-job training. The program allows qualified examiners to focus on case analysis without having to shift priorities to train newly hired, inexperienced examiners. After completing the year-long fellowship program, many of the qualified graduates will find employment in Virginia's Division of Forensic Science.

SEMINARS IN FORENSIC SCIENCE AND MEDICINE

To meet the growing demand for fully trained experts, VIFSM provides intensive, multi-disciplinary training in a real-world investigatory environment. Courses provide a systematic approach to violent crime and death investigation using applied theory as well as a hands-on learning methodology. VIFSM provides both basic and advanced courses for all levels of healthcare professionals, legal and law enforcement personnel, emergency

and first responders as well as tangential forensic professionals such as firemen, game wardens and state police. Many jurisdictions send teams of professionals to achieve a coordinated, efficient and complete death inquiry.

Since VIFSM's founding in 1999, over 800 professionals, including science teachers, public defenders, prosecuting attorneys, judges, police detectives and investigators, medical examiners, physicians, emergency medical technicians and forensic nurses, have completed the institute's forensic science and medicine seminars. Over 200 world-renowned faculty, many of whom are staff members of the Division of Forensic Science and Office of the Chief Medical Examiner, lend their expertise in all disciplines of forensic science and medicine. Through its training seminars, VIFSM enhances the knowledge and performance of those engaged in forensic investigation.

NATIONAL CURRICULUM TEMPLATE

Through an initiative funded by the Department of Justice under the 2003 President's mandate "Advancing Justice Through Forensic DNA Technology", VIFSM was called upon to produce a national training curriculum in partnership with the Community Oriented Policing Services office in Washington, D.C., through their Virginia affiliate, Virginia Community Policing Institute. This curriculum will be the template for law enforcement officer DNA training throughout the country.

Additionally, VIFSM has provided guidance for universities and the private sector in establishing forensic training programs for the U.S. Army and in California, Illinois, South Carolina, New Jersey, West Virginia, Australia, the United Kingdom and Switzerland.

PUBLIC EDUCATION

In response to the public's heightened interest in forensics, VIFSM has developed a program entitled *Forensic U*, which presents an accurate and fascinating glimpse into the world of forensic science and medicine for the average citizen. Participants delve beneath the entertainment surface of popular television shows and explore the pros and cons of emerging forensic science and medicine applications. The program further helps to educate the public about the Commonwealth's criminal justice system and expertise in forensic science and medicine.

RESEARCH

Research and development in areas of forensic science and medicine are critical to the future of medicolegal investigation efforts and to advance the criminal justice system. VIFSM is currently conducting the first such research project in partnership with the federal government and will continue to work with federal, state and academic research entities to explore and validate new programs and technologies.

FUTURE

Well-qualified forensic scientists and pathologists contribute to restoring faith in judicial processes by using science and technology in the search for truth in civil, criminal and regulatory matters. Society and the criminal justice system will not be able to derive maximal benefit from advances in science and technology if those who use or need the technology are not trained. The Virginia Institute of Forensic Science and Medicine exists to fill the need for sophisticated educational opportunities and ensure an adequate pool of trained forensic personnel.

MATRIX

The New PowerPlex® Matrix Standards, 3100—Custom

By Kimberly Huston and Curtis Knox
Promega Corporation

INTRODUCTION

A spectral calibration on the ABI PRISM® 3100 Genetic Analyzer, also known as a matrix, is a mathematical description of the spectral overlap of a given set of fluorescent dye labels. It is used to correct emission overlap of dyes. A poor matrix can result in raised baseline and bleedthrough of fluorescent signal from one channel into another. Thus proper generation of a spectral calibration file is critical to evaluate multicolor systems, such as the PowerPlex® Systems.

THE NEW POWERPLEX® MATRIX STANDARDS, 3100—CUSTOM

Promega has developed the PowerPlex® Matrix Standards, 3100—Custom (Cat.# X3121), which includes four individual fragments labeled with four different fluorescent dyes, for the ABI PRISM® 3100 Genetic Analyzer. Each matrix fragment is provided in a separate tube: one tube contains a 375bp fragment labeled with fluorescein (FL), two tubes each contain a 350bp fragment labeled with 6-carboxy-4',5'-dichloro-2',7'-dimethoxyfluorescein (JOE A and JOE B), one tube contains a 300bp fragment labeled with carboxy-tetramethylrhodamine (TMR), and one tube contains a 275bp fragment labeled with carboxy-X-rhodamine (CXR).

The new PowerPlex® Matrix Standards, 3100—Custom, will be particularly useful for those applications that require higher peak heights, such as the analysis of DNA mixtures, and in forensic casework. In addition, these improved matrix standards accommodate more sensitive instruments with better resolution. An electropherogram image showing these new matrix standards is provided in Figure 1.

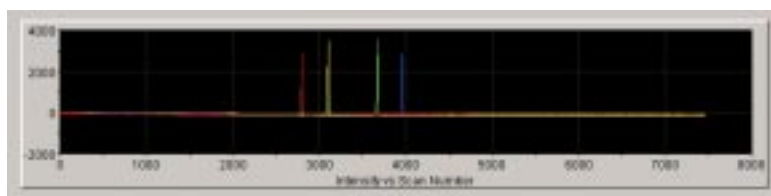


Figure 1. The new PowerPlex® Matrix Standards, 3100—Custom. Five microliters of each matrix standard was mixed with 480µl of HiDi™ formamide, and the mixture was injected onto an ABI PRISM® 3100-Avant Genetic Analyzer using a 5-second, 3kV injection.

WHAT'S NEW?

The new PowerPlex® Matrix Standards, 3100—Custom, includes two JOE matrix standards: JOE A Matrix, Custom, and JOE B Matrix, Custom. To increase the spectral calibration quality, use JOE A Matrix, Custom, to generate a matrix for the PowerPlex®Y or PowerPlex®ES System, and use JOE B Matrix, Custom, for the PowerPlex®16 System.

The high-quality matrix generated with the PowerPlex® Matrix Standards, 3100—Custom, means significantly less bleedthrough and raised baseline and better spectral performance for casework on the ABI PRISM® 3100 Genetic Analyzer.

We have changed the concentration of the DNA fragments in the PowerPlex® Matrix Standards, 3100—Custom, eliminating the need to perform an initial dilution.

The recommended dye set and parameters used when generating the spectral calibration have changed from previous PowerPlex® Matrix Standards, 3100, protocols, depending upon your version of the ABI PRISM® 3100 data collection software. These recommended changes have been made to take advantage of software updates that are present in data collection software versions 1.1 and newer. These software updates are not present in the data collection software version 1.0.1; therefore, users of this software may not see significant improvement in spectral performance.

SUMMARY OF PROTOCOL CHANGES

Preparing the Matrix Standards: To generate a spectral calibration with the new custom matrix standards, add 5µl of each dye fragment to 480µl of Hi-Di™ formamide. This new dilution scheme eliminates the initial 1:10 dilution of each matrix standard required for the PowerPlex® Matrix Standards, 3100 (Cat.# DG3650). The solvent used to prepare the matrix standards has also changed. We find that using HiDi™ formamide rather than water with these new matrix standards results in a higher quality matrix.

Data Collection Software Parameters: For data collection software version 1.1, we still recommend using dye set Z, but the recommended dye set parameters have changed from dye set D parameters to dye set F parameters. For data collection software version 2.0, the recommended dye set has changed from “any 4 dyes” to dye set F. For data collection software version 1.0.1,

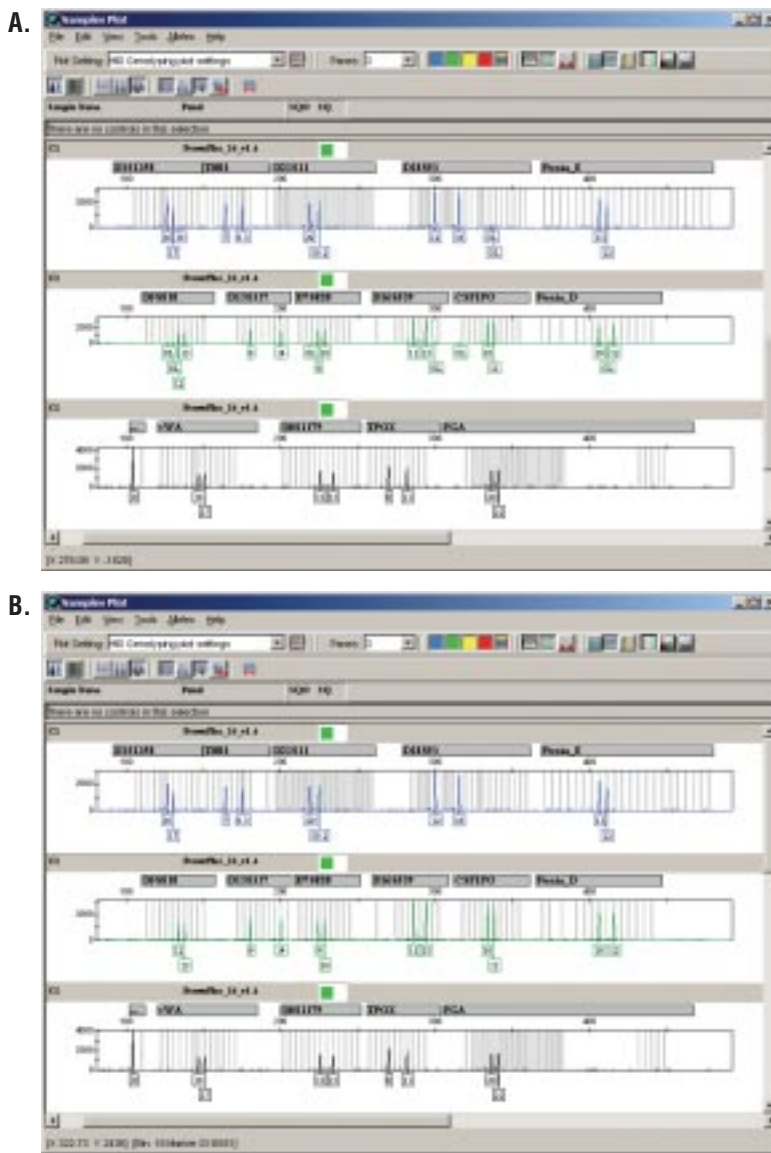


Figure 2. Comparison of spectral performance between the current PowerPlex® Matrix Standards, 3100 (Cat.# DG3650), and the new PowerPlex® Matrix Standards, 3100—Custom (Cat.# X3121). **Panel A.** Sample “C1” was analyzed on an ABI PRISM® 3100 Genetic Analyzer using a spectral created with the current PowerPlex® Matrix Standards, 3100 (Cat.# DG3650). **Panel B.** From the same well on a 96-well plate, sample “C1” was reanalyzed on the same instrument using a spectral created with the new PowerPlex® Matrix Standards, 3100—Custom (Cat.# X3121). This sample was analyzed on GeneMapper™ ID version 3.1 with a cutoff of 50RFU. Note the reduction of off-ladder peaks, which can be directly attributed to improved spectral resolution.

there is no advantage to changing these parameters for the PowerPlex® Matrix Standards, 3100—Custom.

Spectral Parameters: For data collection software versions 1.0.1

and 1.2, change the condition bounds range in the parameter file at: D:\appliedbio\Support Files\Data Collection Support Files\Calibration Data\Spectral Calibration\ParamFiles to [4.0, 11.0]. The condition number

MATRIX

generated can vary between individual instruments. Therefore, you may need to optimize this range for your particular instrument.

Run Module: Change the run module to alter the data delay time (400 seconds) and lengthen the recommended run time (see the PowerPlex® Matrix Standards, 3100—Custom, manual for recommendations).

HOW THESE CHANGES BENEFIT YOU

The high-quality matrix generated with the PowerPlex® Matrix Standards, 3100—Custom, means less bleedthrough between dye channels (3% or less), allowing higher sample peak heights in the range of 4,000–6,000RFU (Figure 2). In addition, as shown in Figure 3, raised baseline is significantly reduced. This translates to better performance over the entire linear range of fluorescence detection. Analysts spend less time manually editing data, which saves time and increases laboratory throughput. Finally, and perhaps most importantly, these improvements result in better spectral performance for the analysis of casework samples with the ABI PRISM® 3100 Genetic Analyzer.

For more information about these improved matrix standards or for a protocol, contact Promega Technical Services at: genetic@promega.com. For those who do not want to switch from the existing set of matrix standards, the PowerPlex® Matrix Standards, 3100 (Cat.# DG3650), is still available.



Figure 3. Close-up view of the sample “C1” from Figure 2 analyzed with a cutoff of 50RFU. **Panel A.** Current PowerPlex® Matrix Standards, 3100 (Cat.# DG3650). **Panel B.** The new PowerPlex® Matrix Standards, 3100—Custom (Cat.# X3121). Note the reduction of bleedthrough from the fluorescein channel into the JOE channel and the reduction of the raised baseline in the fluorescein channel caused by the JOE channel.

The Differex™ System

By Eric B. Vincent and Curtis Knox
Promega Corporation

The Differex™ System^(d) (Cat.# DC6800 and DC6801) is a new, improved method for separating sperm and epithelial fractions in sexual assault samples. Following a standard proteinase K digestion, the sperm pellet is separated from the epithelial fraction using the unique Separation Solution (Figure 1), which allows rapid fractionation with only one centrifugation. In combination with the DNA IQ™ System, the Differex™ System provides a simple, rapid purification system that takes the art out of differential extraction.



Figure 1. Separation of the sperm pellet and epithelial DNA. Following a proteinase K digestion to lyse epithelial cells, the sample and Digestion Buffer are placed in a DNA IQ™ Spin Basket seated in a tube containing the Separation Solution. During a single centrifugation, the sperm collect at the bottom of the tube, separated from the epithelial DNA, which remains in the Digestion Buffer.

What is required to use the Differex™ System?

In addition to the Differex™ System, the following are required:

- Proteinase K (10–20mg/ml) (Cat.# V3021)
- barrier pipette tips
- Microcentrifuge Tubes (Cat.# V1231)
- DNA IQ™ Spin Baskets (Cat.# V1221)
- microcentrifuge
- 56°C or 37°C heat block or oven

To isolate DNA from the epithelial and sperm fractions obtained using the Differex™ System, a DNA purification system, such as the DNA IQ™ System, is also required.

How long does it take to perform the differential extraction? How long to isolate DNA?

Differential extraction of 10 samples can be completed in 90 to 100 minutes using the Differex™ System. The hands-on time is only 30 to 40 minutes with a 1-hour proteinase K digestion at 56°C. Differential extraction followed by manual DNA isolation from both the epithelial and sperm fractions using the DNA IQ™ System requires a total of approximately 3 hours for 10 samples.

At what temperatures can I perform the proteinase K digestion?

We have successfully performed the proteinase K digestion at 37°C for 2 hours and 56°C for 1 hour. In most cases, this is sufficient to lyse all epithelial cells.

Which DNA purification protocols have been used with the Differex™ System?

We have tested and validated the Differex™ System with the DNA IQ™ System. DNA purification by organic extraction has also been successful with epithelial and sperm fractions obtained using the Differex™ System. Other protocols are being tested. Contact Promega Technical Services at: genetic@promega.com for information regarding other DNA purification methods.

Can the Differex™ System be automated?

We are developing protocols and an apparatus to allow differential extraction using the Differex™ System on robotic liquid handlers. Contact your local Promega representative for more information.

TECH TIPS

Can the sperm fraction be analyzed microscopically prior to DNA isolation?

Yes. However, only the sperm heads are detected at this stage since the sperm tails will be digested during the initial proteinase K digestion (Figure 2).

To microscopically examine your sperm pellet, use a pipette to remove as much of the Separation Solution as possible without disturbing the pellet. Add 50–100 μ l of TE⁻⁴ buffer and mix by vortexing to resuspend the pellet. Spot 5–10 μ l of the resuspension on a microscope slide. Follow your laboratory's procedure for the staining and microscopic detection of sperm cells.

Users of the Differex™ System should not attempt to remove a portion of the sperm pellet without first removing the Separation Solution. Vortex mixing in the Separation Solution will not adequately resuspend the pellet, resulting in an inaccurate estimate of the number of cells present in the sperm fraction.

I see residual Digestion Solution at the top of my tube. How can I remove it?

Digestion Solution on the sides of the tube will be removed by the water wash step. If there is Digestion Solution in the cap, remove it with a Kimwipes® tissue or centrifuge the tube briefly to force the liquid to the bottom of the tube.



Figure 2. Microscopic analysis of sperm. Following differential extraction using the Differex™ System, the Separation Solution was removed from the sperm pellet, and 100 μ l of TE⁻⁴ buffer was added. Cells were resuspended by vortex mixing, stained then analyzed microscopically. Sperm heads are indicated by the arrows.

After performing the differential extraction, I observe a large clump of what appears to be cellular material in the Separation Solution. What is this material?

There are two main sources for this material. A large tight cell pellet can be caused by incomplete digestion of epithelial cells. A large diffuse pellet can result from a large amount of mucus in the sample. A longer proteinase K digestion can help (up to 2 hours at 56°C). Diffuse pellets often contain debris with little or no DNA and can usually be removed by carefully pipetting away the solution, leaving the tight sperm pellet behind.

Now that I've performed the separation, can I store my samples prior to purifying the DNA?

We have not investigated the effects of storing the sperm pellet in Separation Solution overnight, so we recommend proceeding with the DNA isolation. If DNA will be purified from the fractions using the DNA IQ™ System, this additional step requires only 30–60 minutes to perform manually, depending on the number of samples.

Career Opportunities



MARKETING MANAGER—FORENSICS AND MOLECULAR DIAGNOSTICS

Join the Promega team. We have an opening for a proven marketing expert to plan and execute the conception, pricing, promotion and distribution of Promega products, services and information related to new and existing opportunities in the forensic and molecular diagnostic markets.

Essential duties include:

- Develop, plan, monitor, communicate and implement worldwide marketing strategies and plans, including market research, situation analysis and product/technology positioning.
- Identify market opportunities for Promega Genetic Identity and Molecular Diagnostics products and technology.
- Collaborate with sales management to plan and coordinate product and technology commercialization efforts.
- Initiate, direct and analyze market research activities, including development and implementation of surveys, focus groups, competitive profile summaries and reports.
- Measure performance against plans, report on results, and adjust strategies and tactics to achieve goals.
- Develop and manage budgets, revenue forecasts and investment plans to support market strategies.

Qualifications include:

- B.S. in life science field, MBA or equivalent, and three or more years of experience in life-science marketing management with a focus on instrumentation and reagent systems.
- In-depth understanding of marketing functions, including strategic and tactical marketing, marketing communications, market research, product management, distribution channels, competitive intelligence and customer relationship management.
- Sound judgment, strategic thinking, creativity and innovation. Good public-speaking skills and exceptional problem-solving, decision-making and negotiation skills.
- Ability to manage cross-functional teams and develop trust, respect and cooperation across all areas of the organization.
- Ability to travel up to 30% of time on a global basis.

For more information, visit: www.promega.com/hr/

Promega is an affirmative action/equal opportunity employer.