PERIODICUM BIOLOGORUM
VOL. 106, No 3, 205–207, 2004

UDC 57:61
CODEN PDBIAD
ISSN 0031-5362

Editorial

Molecular medicine – a new star on horizon

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Received July 7, 2004.

Every individual, with the exception of identical twins, has a different DNA sequence. Using these sequences, every person can be identified solely by the sequence of their base pairs. However, this task is very time-consuming because there are so many millions of base pairs. Instead, scientists are able to use a shorter method, due to the repeating patterns in DNA. These patterns do not, give an individual »fingerprint,« but they are able to determine whether two DNA samples are from the same person, related people, or non-related people. Scientists use a small number of sequences of DNA that are known to vary among individuals a great deal, and analyze them to determine the certain probability of a match.

DNA technology today has an irreplaceable position in the field of the forensic sciences. A restriction fragment length polymorphism (RFLP) technique was first applied in 1985 by Alec Jeffreys to examine the length variation of DNA repeat regions known as variable number of tandem repeats (VNTRs). As VNTRs could differ from individual to individual, RFLP actually offered the ability to perform human identity tests. Ever since this method was first used in forensics, human identity testing using DNA typing methods has been widespread and there was tremendous growth in the use of DNA evidence in crime scene investigations as well as paternity testing.

Forensics is the application of science to law. Forensic science uses highly developed technologies to uncover scientific evidence and it is most commonly used to investigate criminal cases involving a victim, such as assault, robbery, kidnapping, rape, or murder. Modern forensic science is used in civil cases such as forgeries, fraud, or negligence. It can help determine if laws or regulations were violated in the marketing of foods and drinks, the manufacture of medicines, or the use of pesticides on crops. Miller et al discuss the use of non-human DNA in forensics. Some of reported use of trace plant material as physical evidence in criminal casework include the analysis of wood evidence in the Charles Lindbergh baby kidnapping, the use of pollen in establishing the location of a sexual assault, and pollen analysis to determine the time of year for burial in a mass grave. Additional cases discuss the use of plant growth rates to determine the time of a body deposit in a field, the use of diatoms to link individuals to a crime scene, and plant DNA typing to match seedpods to a tree under which a body was discovered. New DNA methods in development for plant species identification and individualization for forensic applications may be useful for linking an individual to a crime scene or physical evidence to a geographic location, or tracking marijuana distribution patterns.

Today many forensic laboratories all over the world conduct hundreds of thousands of DNA tests annually. The number of laboratories around the world conducting DNA testing will continue to grow as the technique gains in popularity within the law enforcement community. Two main types of forensic DNA testing are RFLP analysis and PCR (polymerase chain reaction)-based analysis.

Generally, RFLP analysis requires larger amounts of DNA and the DNA must not be degraded. Crime-scene evidence that is old or that is present in small amounts is often unsuitable for RFLP testing. Warm moist conditions may accelerate DNA degradation rendering it unsuitable for RFLP in a relatively short period of time.

PCR testing often requires less DNA than RFLP testing and the DNA may be partially degraded, more so than is the case with RFLP. However, PCR still has sample size and degradation limitations. PCR tests are also extremely sensitive to contaminating DNA at the crime scene and within the test laboratory. Prevention of false results involves the use of carefully applied controls and techniques. Term PCR applies to a wide variety of different DNA tests that differ in reliability and effectiveness. The reliability of each kind of PCR test needs independent verification. PCR itself does not accomplish DNA typing; it only increases the amount of DNA available for typing. Real-time PCR is a relatively new technology that provides a broad dynamic range for detecting specific gene sequences with excellent sensitivity and precision. The broader the dynamic range means more accurate the quantitation. Real-time PCR monitors the fluorescence emitted during the reaction as an indicator of amplicon production during each PCR cycle (i.e., in real time) as opposed to the endpoint detection. The development of real-time quantitative PCR has eliminated the variability traditionally associated with quantitative PCR, thus allowing the routine and reliable quantification of PCR products.

DNA typing, since it was introduced in the mid-1980s, has revolutionized forensic science and the ability of law enforcement to match perpetrators with crime scenes. In many situations, multiple technologies may be used to help solve an important case.

The best solution including a high power of discrimination and a rapid analysis speed has been achieved with short tandem repeat (STR) DNA. STR DNA can be analyzed three or more at a time. Multiplex STRs are valuable because they can produce highly discriminating results and can successfully measure sample mixtures and biological materials containing degraded DNA molecules. In addition, the detection of multiplex STRs can be automated, which is an important benefit as demand for DNA testing increases. The array of types of markers is expanded STR multiplexes (a few loci co-amplified [3–9]) have become »megaplexes« (15–16 loci). The Federal Bureau of Investigation (FBI) uses a standard set of 13 specific STR regions for CODIS. CODIS is a software program that operates local, state, and national databases of DNA profiles from convicted offenders, unsolved crime scene evidence, and missing persons.

Mitochondrial DNA (mtDNA) provides a valuable locus for forensic DNA typing in certain circumstances such are forensic cases involving severely degraded DNA samples or when associating maternally related individuals. As mtDNA molecules are present in hundreds to thousands of copies per cell, compared to the nuclear complement of two copies per cell, the likelihood of recovering mtDNA in small or degraded biological samples is greater than for nuclear DNA. In addition, mtDNA is inherited from the mother only, so that in situations where an individual is not available for a direct comparison with a biological sample, any maternally related individual may provide a reference sample. While mtDNA is useful for forensic examinations, it also has been used extensively by the medical profession to study and understand the mode of inheritance of a number of serious human diseases caused by deleterious mutations in gene-coding regions of the mtDNA molecule. In addition, molecular anthropologists have been using it examine both the extent of genetic variation in humans and the relatedness of populations all over the world. Despite the information offered by mtDNA can never provide the resolution of individuality that nuclear typing can. For this primary reason, mtDNA forensic testing should be reserved primarily in situations where nuclear DNA typing is not an option, or in the event that nuclear typing has been attempted and is unsuccessful.

The Y chromosome is passed directly from father to son, so the analysis of genetic markers on the Y chromosome is especially useful for tracing relationships among males or for analyzing biological evidence involving multiple male contributors. Genetic variation on the Y chromosome, originally studied as a specialty, is becoming a common and a useful adjunct marker system.

In forensic science, DNA analysis has become »the new form of scientific evidence« and an indisputable proof of identification. Though it has come under public scrutiny and questioned as to its competence, more and more courts admit the DNA based evidence. It is believed that in the near future this technology will be generally accepted in the legal system.

Forensic science is also being applied more frequently as to its competence in Croatian laboratories. After the war, Croatia faced a great number (over 3000), of unidentified bodies found in numerous mass graves throughout the country. During the last ten years, significant efforts were allocated into the identification of discovered individuals. Though the majority of identifications had been achieved by standard forensic methods, there were still many cases that needed to undergo DNA analysis, due to lack of ante mortem data and body decomposition. For the first time our country was confronted by the difficult task of applying DNA technology to such cases. PCR-based testing needed to be introduced. With the help of worldwide forensic scientists, colleagues, and friends, three DNA laboratories were founded and started this

very important challenge of identification process. For many it was learning experience. The formation of a database was one of the first steps. Several Croatian population databases, with total of more than 3000 profiles, have been created for use in forensic analyses to estimate the frequency of a multiple locus DNA profile.

Degradation and contamination of DNA extracted from bone and teeth samples were often obstacles for PCR and that made the entire process more difficult. During many years of this identification process, DNA laboratories experimented with different techniques in attempt to overcome the problems encountered. Techniques included decalcification, repurification, testing new DNA extraction and DNA quantitation procedures, and validation of a different amplification systems. Along with STR analysis of nuclear DNA, we are in the process of applying mtDNA typing which is proving to be valuable. Unfortunately, the identification process is not over yet, but it has become more successful as the DNA technology develops. Croatia started as pioneers in this field of forensic science but today it can stand next to the many respectable forensic laboratories. As a result of foreign collaboration, a meeting was initiated 7 years ago as an intensive training course in PCR-based testing for forensic and clinical scientists. Today it is recognized among scientists all over the world and it is a great honor to country. Still, there is always more to learn, especially when forensic science is moving forward so fast with automated equipment and emergent multiplex technology. Laboratories in Croatia will certainly strive the best to keep up with magnificent work and will reach for even higher goals.