Forensic DNA analysis - invisible science

By Dean Hildebrand, PhD

"Molecular consciousness". That's the term used by a colleague to describe the essence of his work. As a seasoned forensic DNA scientist working in the Armed Forces DNA Identification Laboratory in Maryland, his job is to apply the latest DNA identification methods (both nuclear and mitochondrial) to the remains of U.S. soldiers killed in the various conflicts of the century. The quotation emphasizes the sometimes frustrating job of a forensic biologist faced with having to isolate traces of unseen biological evidence (DNA) from a bone fragment while protecting it from inadvertent mixing with the sea of cellular debris that surrounds us at all times. The thousands of cells we exude (sloughed or falling from us naturally or even expelled in a cough) act like genetic "bread crumbs" leaving an invisible biological trail everywhere we go. Potential contamination like this is the nemesis of the forensic biologist and its control is a primary concern. Hence the effort and expense devoted to proper training of laboratory personnel and the special design of forensic DNA laboratories. Although the above-mentioned discipline represents the most challenging application of forensic DNA analysis, its use is becoming ubiquitous in even routine investigations that require human identification.

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This article on DNA is written by Dean Hildebrand, PhD, who is Coordinator of Forensic Sciences at British Columbia Institute of Technology (BCIT) which has campuses in the lower mainland of BC. Dean was instrumental in creating the opportunity for the first forensic nursing course in BC which took place July 7-11, 2003 at the Vancouver campus, BCIT.

The forensic nursing course is a three-credit course taught by Virginia Lynch, MSN, RN, FAAFS, FAAN, of Beth El College of Nursing, University of Colorado, Colorado Springs, Colorado, USA, and other guest speakers. Virginia has been described as the "architect of forensic nursing" as she formalized the forensic nursing care model on which current forensic nursing practice is based.

The emergency nurse of 2003 is a frontline health care professional who needs to recognize the significant role in DNA collection and preservation that nurses have in caring for trauma patients, be it the child who has an unusual fracture, the victim of MVA who may or may not be a driver, the male or female patient who has been sexually assaulted or involved in interpersonal violence.

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A successful forensic DNA analysis, however, does not begin at the front door of the laboratory. The education and training about what constitutes good potential DNA evidence and how one should collect and protect that evidence is not only the realm of the forensic biologist, but also that of the many "first responders" who may come into contact with it long before laboratory personnel: the police officer, the forensic pathologist, the forensic dentist, the forensic anthropologist, or the emergency nurse are examples. The responsibility to understand the biology of DNA and the capabilities (and limitations) of the DNA analysis technology should include all of the professionals tasked with collecting DNA evidence.

It is from this premise that this article was conceived in an effort to assist in the education of the forensic nurse in areas of DNA. Although obviously well-versed in many areas of biology, the forensic nurse may be years removed from the education they received on aspects of molecular biology. The aim of this article is to present a brief primer on DNA – "Biology 101" if you will.

Deoxyribonucleic acid (DNA) is an amazing molecule. The genetic "blueprint" of all living organisms, this molecule is a paradox – simplistic, but infinitely diverse. From a single-celled prokaryote to the most complex, multi-celled eukaryote, every species on Earth uses DNA as its genetic code. A phosphate connected to a sugar which in turn is connected to one of only four possible nitrogen-containing bases (given the one-letter symbols G, A, T, and C), this building block is repeated over and over in a double-stranded polymer. The only difference between a plant and a human (or any two species) is the order in which these building blocks are connected. Termed the "double helix", its structure was determined in the early 1950s by James Watson and Francis Crick, and is an example of how function follows form. Studying the unique three-dimensional structure determined by these Nobel laureates eventually led scientists to understand how this polymer is replicated (copied) in cell division and transcribed (used as a template for making RNA) in the complex mechanisms that produce proteins. This in turn led to breakthroughs in recombinant DNA technologies (harnessing the capabilities of DNA outside the cell for use in molecular biology) and led to modern day *in vitro* techniques used in forensic biology.

The human (nuclear) genome consists of approximately three billion basepairs and has now been completely sequenced. Divided between 46 chromosomes (23 pairs of autosomes and one pair of sex chromosomes), the genome contains tens of thousands of genes distributed throughout the genome. Every single nucleated cell contains a complete complement of the genome, an amazing example of biological organization considering that each nucleus must replicate, organize and repair almost a metre of DNA! A striking realization that has come out

of the Human Genome Project is that only a small fraction of the genome is "functional" or acts as a direct code for making protein, with the rest being non-functional. Some have even called it "junk" DNA, but this is probably a misnomer because it undoubtedly has an important, albeit under-appreciated, role. Much of this DNA is unique (occurs only once in the genome), but much of it is repetitive (interspersed many times throughout the genome or arranged in a tandem fashion in one area of the genome). One such class of the latter are the short tandem repeats (STRs), an important class of repetitive DNA that is now analyzed throughout the world for the purposes of forensic identification and paternity testing. STRs have been found to be relatively polymorphic (variable) within the population, a trait that is useful for distinguishing one person from another.

As eluded to earlier, DNA is a long polymeric molecule. Its chemical stability varies and, under conditions that facilitate degradation, the backbone of the polymer is broken down into successively shorter and shorter fragments. When properly preserved, however, DNA can linger for thousands of years (hence the growing subspecialty of archaeology that utilizes ancient DNA analysis in studies of ancient species and populations). Alternatively, certain conditions can degrade DNA in a matter of hours or days. Understanding the conditions that degrade DNA is important for the forensic personnel who collect, preserve and/or analyze DNA. Moist conditions, for example, foster microorganism growth that results in nuclease-induced degradation of DNA. It is for precisely this reason that samples like water-moistened swabs used to collect saliva, blood or sloughed skin cells must be adequately dried and packaged in a material that "breathes" (like paper envelopes or cardboard evidence boxes). These swabs, if properly dried and stored in a cool environment, will remain viable sources of DNA evidence for a prolonged period of time.

Degradation is always a concern when dealing with DNA evidence, but today's technology does overcome some of the challenges inherent with highly degraded samples. A molecular "Xeroxing" technique for DNA called polymerase chain reaction (PCR) is used to copy, and thus amplify, the STR regions of interest. As the name implies, short tandem repeats are short and thus still able to be analyzed when the DNA is highly degraded (fragmented). It is not unheard of to get a usable DNA profile from the equivalent of a couple dozen cells and from DNA that no longer contains large intact tracts of DNA. This was unheard of in days prior to the advent of PCR and has opened the floodgates, in terms of the types of materials that can be used for DNA analysis, and brought a new tool to investigators.

But perhaps PCR's greatest strength is also its greatest weakness, for it plays no favourites – it will amplify DNA in a sample whether it be from the crime scene or from the person who collected it. Contamination control is more crucial than ever when dealing with PCR. Any personnel involved in the collection of DNA evidence must show their own "molecular consciousness" by taking some simple but crucial precautions: wear protective clothing (gloves, lab coat or covering, and face mask). Gloves, in particular, should be changed often. Non-disposable instruments that might be used in sample collection or preparation (like tweezers) must be decontaminated between each use with, for example, bleach or commercially available products like "DNA Away" (VWR Canlab). One sample should never touch another and they should be individually packaged. Forensic laboratory personnel must have DNA profiles on file, but this precaution has not been extended to the frontline personnel involved in the process – a consideration long overdue in the author's opinion.

So, how do emergency nurses fit in with this discussion? Like police officers, emergency medical professionals often have the first contact with victims or perpetrators of crimes and, therefore, represent the first link of the investigative chain. Obviously medical issues take priority in this situation but, at the same time, whenever feasible, evidence should be safeguarded. Potential evidence is at stake. This is the reason our Forensic Science Technology Program at BCIT offered its first forensic nursing course this summer. Emergency rooms need personnel who are versed in areas of basic forensic science covering such things as firearms and weapons (including the recognition of wound patterns they produce), odontology (bitemark recognition), photography (to document any evidence), hair and fibres, DNA and others. Most are familiar with the role of the sexual assault nurse examiner, but there is a much bigger role for nurses to take in forensic investigations, particularly in Canada. This applies to DNA evidence as well, and nurses should recognize what can be used as evidence. There are the obvious items, such as clothing stained with bodily fluids (blood, semen and saliva), or the same substances on a person's skin that can be collected by swabbing. In reality, anything that has been contacted by another person can contain traces of DNA in the form of skin cells. The author did a case once from a piece of discarded clothing that contained a tiny bloodstain (~2mm diameter) that matched the victim of the crime and at the same time a small section of the clothing itself (with no visible staining) yielded a perfect DNA match to the suspect who allegedly wore the clothing. Another case in point deals with the victims of assaults or child abuse who have been bitten (or conversely the perpetrator who has been bitten by the victim). Research and casework has shown that bitemarks can contain saliva from the biter that can be removed by simply swabbing the area. The documentation of the wound and the swabbing should be done as soon as possible and need not necessarily be done by the forensic dentists.

DNA technology provides forensic investigators with a powerful identification tool. As mentioned, standard nuclear DNA tests require only a few dozen cells to produce a result. Even more sensitive tests are available using mitochondrial DNA where even a single cell has enough genetic material to type! So the take-home message when it comes to working with DNA evidence – do as we do in the forensic laboratory and learn "molecular consciousness". Because the words spoken in 1877 by Edmond Locard, one of the pioneers of modern forensic science, have never been more poignant – "Wherever he steps, whatever he touches, whatever he leaves, even unconsciously, will serve as a silent witness against him".

About the author

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