50th Anniversary

50 YEARS OF THE DOUBLE HELIX

Celebrating the Double Helix

In October of 1951, James Watson traveled to Cambridge, England to begin his historic collaboration with Francis Crick. Together in 1953, they published a model of the DNA helix (1). As information accumulated, it became clear that DNA held the key to more than just genetic inheritance. Intra- and interspecies differences in DNA sequence could be used for identification purposes. Now 50 years later, DNA analysis has become one of the most definitive methods for human identification in forensic science.

The discovery of a highly polymorphic region in the human genome (2) was a significant advance toward the goal of creating a unique genetic fingerprint, which could be used to identify an individual based solely on his DNA sequence. Scientists soon applied this discovery to human identification and paternity determination (3). However, the low heterozygosity and low number of alleles for early genetic markers meant that these loci also had low powers of discrimination. These shortfalls were overcome as new polymorphic regions scattered throughout the human genome, including variable number of tandem repeat (VNTR) and trimeric and tetrameric short tandem repeat (STR) loci, were characterized (4,5,6).

Initially, restriction fragment length polymorphism (RFLP) analysis (7) was applied to human identification, but it was time-consuming and required high molecular weight DNA. However, a new technique, the polymerase chain reaction (PCR), soon revolutionized DNA analysis and replaced RFLP. Whereas RFLP analysis required microgram amounts of intact DNA, PCR allowed the amplification and detection of nanogram amounts of lower molecular weight DNA in hours rather than days. PCR primers were soon developed to amplify some of the most informative VNTR and STR genetic markers (6,8,9). Speed, sensitivity and specificity make PCR one of the most common methods for genetic analysis in laboratories worldwide.

Up to this point, much of the focus in DNA typing had been on genomic DNA. However, certain DNA sources, such as shed hair shafts, teeth and ancient remains, have too little DNA or are too degraded for nuclear DNA analysis. Mitochondrial DNA, a circular DNA with two hypervariable regions (HV1 and HV2), is an attractive alternative to genomic DNA due to its high copy number within a cell (10). Analysis of mitochondrial DNA can provide results in cases where nuclear DNA analysis fails (11). In addition to its role in human identification, maternally inherited mitochondrial DNA can provide information on a single maternal line on an evolutionary time scale. Similarly, polymorphic regions on the paternally inherited Y chromosome (12,13) can be used to track a single paternal line throughout history and are particularly useful in forensic science to distinguish the male component within a mixture of samples.

Within the last 5 years, much effort has gone into generating population data for statistical analysis and collecting and organizing DNA profiles of convicted criminals into databases, such as the FBI's CODIS database. These databases can be searched by federal, state and local agencies to match DNA profiles left at a crime scene to those of offenders registered in the database. These networks have already proven invaluable in identifying or excluding suspects and in solving crimes as demonstrated in the Commonwealth of Virginia as they recently celebrated their 1000th cold hit. The following timeline highlights a few of the key scientific events leading up to that historical paper of 1953 and chronicles the many discoveries within the past 5 decades which have lead to our current technology and to the Commonwealth of Virginia's accomplishment, which is discussed in more detail on page 16.

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1950
Chargaff uses chromatography to determine that, when DNA is reduced to single nucleotides, the mass of adenine residues is always equal to that of thymine residues and that the mass of guanine residues is always equal to that of cytosine residues. This rule of equality becomes known as Chargaff's rule.[14]

1953
A three-dimensional structure for DNA that is consistent with previously published and unpublished data, including X-ray crystallography data and Chargaff's rule is published. The Watson and Crick model consists of two intertwined DNA strands following right-handed helices with phosphates residues on the outside and purine and pyrimidine bases on the inside, holding the chains together by hydrogen bonds.[1]

1953
X-ray cystallography data strongly suggest that the structure of DNA is helical with a fiber axis of 34Å and with exactly 10 residues per turn of the helix[15]

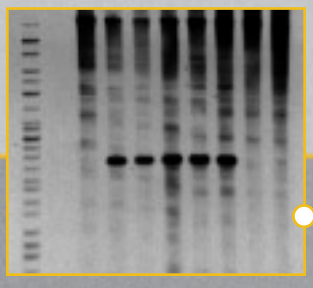
1975
Southern describes restriction fragment length polymorphism (RFLP) analysis. Restriction enzymes, enzymes which cut DNA at a specific sequence, are used to detect small variations in DNA sequence. The restriction fragments are size separated, transferred to a membrane and hybridized to probes for the polymorphic region. The presence or absence of restriction enzyme sites can be determined by the size of the fragments detected by the probe.[7]

1950

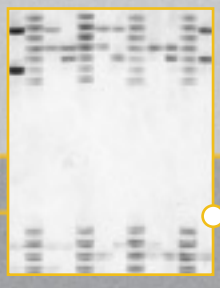
1980
Wyman and White report one of the first highly polymorphic regions of the human genome. This locus, which is not associated with any specific gene, has at least 8 alleles, making it particularly useful for genetic identification.[2]

1985
Jeffreys applies the analysis of hypervariable regions, which he dubs "minisatellite regions", to genetic identification and paternity testing. Analysis of multiple loci will produce a DNA 'fingerprint' which is specific to a single individual.[3]

1986
The polymerase chain reaction (PCR) is first described in scientific literature. PCR using locus-specific primers allows direct amplification of loci from small amounts of DNA, reducing the amount of time and DNA template required. Speed, sensitivity and specificity will soon make PCR the preferred method for genetic analysis in laboratories worldwide.



Restriction fragment length polymorphism using a human Y-specific probe



Silver staining of amplified STR loci

EVOLUTION OF DNA

DNA Typing Timeline

1987
Nakamura *et al.* describe new highly polymorphic genetic loci with variable numbers of tandem repeat (VNTR loci) and develop a series of new VNTR probes for use in RFLP analysis.[4]

In the UK, forensic investigators use DNA testing to help solve the "Black Pad" murders and to identify the killer as Colin Pitchfork, who later confessed to the crimes. This marks the first case in which DNA evidence is used to determine the identity of a murderer. This also marks the first case in which a prime suspect was exonerated due to DNA evidence.

1988
Jeffreys *et al.* show that PCR can be used to faithfully amplify entire VNTR loci, allowing genetic testing to be performed with far less DNA than previous techniques.[8]

Variable regions of mitochondrial DNA are analyzed for human identification purposes.[11]

1989
Promega first introduces probes for isotopic detection of VNTR loci.

1989
Trimeric and tetrameric short tandem repeats (STRs) are found to be highly polymorphic and easily amplified by PCR. Size standards, prepared by combining amplification products from individuals of known genotypes, are used to more accurately determine the number of repeats during data interpretation.[5,6]

1992
Promega first introduces probes for nonisotopic detection of VNTR loci.

STR polymorphisms are discovered on the Y chromosome[12]

1993
Promega releases its first STR detection systems.

1994
The U.S. Congress enacts the DNA Identification Act; the Combined DNA Index System (CODIS) is formally created.

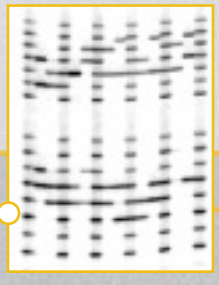
1996
Mitochondrial DNA evidence is first presented in court to help convict Paul Ware of the rape and murder of a four year old girl. Mitochondrial DNA extracted from hairs found at the crime scene and on the child's body was an exact match to that of Ware's.

1998
The FBI Laboratory establishes NDIS (National DNA Index System), which allows federal, state and local forensic agencies to share DNA profiles of convicted offenders and crime scene evidence.

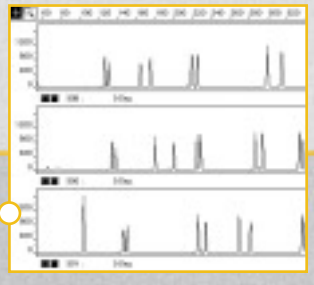
2000
The PowerPlex® 16 System, which allows amplification of all 13 CODIS loci in a single reaction, is made available by Promega.

2002

2002
The Division of Forensic Science Laboratory in the Commonwealth of Virginia becomes the first state laboratory to mark 1,000 "cold hits" from its DNA database.



Fluorescent detection of amplified STR loci



Capillary electrophoresis to separate amplification products or STR loci

TYPING RESULTS

State of Virginia Achieves Milestone of One Thousand "Cold Hits"

On November 13, 2002, the Division of Forensic Science Laboratory in the Commonwealth of Virginia became the first state laboratory to mark 1,000 cold hits from their DNA database. A cold hit occurs when physical evidence from a crime scene is matched to a DNA profile without prior suspicion of the offender's involvement in the crime. Cold hits are often useful in identifying offenders in cases without an eyewitness or suspect and can match DNA from a number of crime scenes, linking multiple crimes to a single perpetrator.

Virginia has long been a leader in fighting crime with DNA evidence. In May of 1989, Virginia became the first state to establish a DNA databank. The Division of Forensic Science Laboratory celebrated its first cold hit in 1993. As of October 31, 2002, matches from Virginia's DNA database have been instrumental in solving 109 homicides, 241 rapes, 12 rape-homicides, 9 malicious woundings, 14 carjackings, 57 robberies, 465 burglaries or larcenies and more than 80 other crimes. Virginia's DNA Data Bank is the most comprehensive in the United States. At present, it contains the genetic profiles of more than 187,000 convicted felons. The end result of Virginia's well-populated database is an ever-increasing number of DNA matches. These matches are leading to arrests and convictions in the Commonwealth of Virginia, making the state a safer and better place to live.

Early on, Virginia selected products from Promega to process its DNA typing samples. Promega's DNA typing systems are widely recognized for their reliability and power of discrimination. The PowerPlex® 16 BIO System[b–f], coupled with automated technologies, has helped the forensic laboratory in Virginia to maintain its leadership position among the states' databases. To learn more about DNA typing products from Promega, contact your local Promega Branch Office or Distributor.

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