THE FBI FEDERAL BUREAU OF INVESTIGATION

Laboratory Services

Frequently Asked Questions (FAQs) on the CODIS Program and the National DNA Index System

Please note that these questions and responses refer specifically to the National DNA Index System; state DNA databases operate in accordance with the applicable state law and questions concerning the operation of a particular state DNA database should be directed to that state.

CODIS

Q: What is CODIS?
A: CODIS is the acronym for the "Combined DNA Index System" and is the generic term used to describe the FBI's program of support for criminal justice DNA databases as well as the software used to run these databases. The National DNA Index System or NDIS is considered one part of CODIS, the national level, containing the DNA profiles contributed by federal, state, and local participating forensic laboratories.

CODIS DNA Databases

Q: How do these DNA databases using CODIS work?
A: For example, in the case of a sexual assault where an evidence kit is collected from the victim, a DNA profile of the suspected perpetrator is developed from the swabs in the kit. The forensic unknown profile attributed to the suspected perpetrator is searched against their state database of convicted offender and arrestee profiles (contained within the Convicted Offender and Arrestee Indices, if that state is authorized to collect and database DNA samples from arrestees). If there is a candidate match in the Convicted Offender or Arrestee Index, the laboratory will go through procedures to confirm the match and, if confirmed, will obtain the identity of the suspected perpetrator. The DNA profile from the evidence is also searched against the state's database of crime scene DNA profiles called the Forensic Index. If there is a candidate match in the Forensic Index, the laboratory goes through the confirmation procedures and, if confirmed, the match will have linked two or more crimes together. The law enforcement agencies involved in these cases are then able to share the information obtained on each of the cases and possibly develop additional leads.

Q: What happens after there is a hit in the DNA database?
A: CODIS was designed to compare a target DNA record against the DNA records contained in the database. Once a match is identified by the CODIS software, the laboratories involved in the match exchange information to verify the match and establish coordination between their two agencies. The match of the forensic DNA record against the DNA record in the database may be used to establish probable cause to obtain an evidentiary DNA sample from the suspect. The law enforcement agency can use this documentation to obtain a court order authorizing the collection of a known biological reference sample from the offender. The casework laboratory can then perform a DNA analysis on the known biological sample so that this analysis can be presented as evidence in court.

Q: What DNA information is stored in these databases?
A: The DNA profile also known as a DNA type is stored in the database. For Forensic STR DNA analysis, the DNA profile consists of one or two alleles at the 13 CODIS core loci.

Q: Is any personal information relating to the convicted offenders, arrestees or detainees stored in these DNA databases?
A: No names or other personal identifiers of the offenders, arrestees, or detainees are stored using the CODIS software. Only the following information is stored and can be searched at the national level:

(1) The DNA profile—the set of identification characteristics or numerical representation at each of the various loci analyzed;

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(2) The Agency Identifier of the agency submitting the DNA profile;

(3) The Specimen Identification Number—generally a number assigned sequentially at the time of sample collection. This number does not correspond to the individual's social security number, criminal history identifier, or correctional facility identifier; and

(4) The DNA laboratory personnel associated with a DNA profile analysis.

Q: What precautions are taken for safeguarding the information in these DNA databases?
A: The computer terminals/servers containing the CODIS software are located in physically secure space at a criminal justice agency. Access to these computers is limited to only those individuals authorized to use CODIS and approved by the FBI. Communications between participating federal, state, and local laboratories occur over a wide area network accessible to only criminal justice agencies approved by the FBI.

Pursuant to federal law (the DNA Identification Act of 1994), DNA data is confidential. Access is restricted to criminal justice agencies for law enforcement identification purposes. Defendants are also permitted access to the samples and analyses performed in connection with their case. If all personally identifiable information is removed, DNA profile information may be accessed by criminal justice agencies for a population statistics database, for identification research and protocol development purposes, or for quality control purposes. The unauthorized disclosure of DNA data in the National DNA database is subject to a criminal penalty not to exceed $250,000.

The National DNA Index System

Q: What is the National DNA Index System or NDIS?
A: NDIS is the acronym for the "National DNA Index System" and is one part of CODIS—the national level—containing the DNA profiles contributed by federal, state, and local participating forensic laboratories. NDIS was implemented in October 1998. All 50 states, the District of Columbia, the federal government, the U.S. Army Criminal Investigation Laboratory, and Puerto Rico participate in NDIS.

The DNA Identification Act of 1994 (42 U.S.C. §14132) authorized the establishment of this National DNA Index. The DNA Act specifies the categories of data that may be maintained in NDIS (convicted offenders, arrestees, legal, detainees, forensic (casework), unidentified human remains, missing persons and relatives of missing persons as well as requirements for participating laboratories relating to quality assurance, privacy and expungement.

Q: What are the specific requirements for a state's participation in the National DNA Index?

A: The DNA Identification Act (42 U.S.C. §14132(b)) specifies the requirements for participation in the National DNA Index System (NDIS) and the DNA data that may be maintained at NDIS (convicted offender, arrestees, legal, detainees, forensic (casework), unidentified human remains, missing persons and relatives of missing persons). The DNA Identification Act requires the following:

That the laboratories participating in the National DNA Index comply with the Quality Assurance Standards issued by the FBI Director;

That the laboratories submitting the DNA records are accredited by a nonprofit professional association of persons actively engaged in forensic science that is nationally recognized within the forensic science community;

That the laboratory submitting the DNA record undergoes an external audit every two years to demonstrate compliance with the FBI Director's Quality Assurance Standards;

That the laboratory is federal, state, or local criminal justice agency ("or the Secretary of Defense in accordance with section 1565 of title 10, United States Code"); and

That access to the DNA samples and records is limited in accordance with Federal law.

States seeking to participate in NDIS sign a Memorandum of Understanding with the FBI Laboratory documenting their agreement to abide by the DNA Identification Act requirements as well as record-keeping and other operational procedures governing the uploading of DNA data, expungements, CODIS users, audits, etc.

Q: Are there approved accrediting agencies?
A: Federal law requires that laboratories submitting DNA data to NDIS are accredited by a nonprofit professional association of persons actively engaged in forensic science that is nationally recognized within the forensic science community. The following entities have been determined to satisfy this definition: the American Society of Crime Laboratory Directors/Laboratory Accreditation Board (ASCLD/LAB) and Forensic Quality Services, Inc. (FQS).

DNA Data Requirements

Q: What DNA data is accepted at NDIS?
A: Currently, DNA data generated through PCR Short Tandem Repeat (STR) technology, Y chromosome STR (Y STR) technology, and Mitochondrial DNA (mtDNA) technology are accepted at NDIS.

SCIENTIFIC ANALYSIS

Forensic Analysis
- Cryptanalysis & Racketeering
- Latent Print
- Questioned Documents

Scientific Analysis
- CODIS
- Chemistry
- DNA-Nuclear
- DNA-Mitochondrial
- Trace Evidence
- Firearms/Toolmarks

Operational Response
- CBRN Sciences
- Explosives
- Evidence Response Team
- Hazardous Material Response
- Photographic & Imaging

Forensic Science
- Counterterrorism & Forensic
- Science Research
- Forensic Science Support
- Crime Scene Survey
- Forensic Facial Imaging

More About Us
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TEDAC

Evidence Control
- Evidence Control

Training
- Classes

Y STR and mtDNA data is only searched with the missing person related indexes.

The National DNA Index no longer searches DNA data developed using restriction fragment length polymorphism (RFLP) technology.

Q: Are there specific data requirements for the DNA records submitted to NDIS?
A: Yes. There are several requirements for the DNA data submitted to NDIS:

1. The DNA data must be generated in accordance with the FBI Director's Quality Assurance Standards;
2. The DNA data must be generated by a laboratory that is accredited by an approved accrediting agency;
3. The DNA data must be generated by a laboratory that undergoes an external audit every two years to demonstrate compliance with the FBI Director's Quality Assurance Standards;
4. The DNA data must be one of the categories of data acceptable at NDIS, such as convicted offender, arrestee, detainee, legal, forensic (casework), unidentified human remains, missing person or a relative of missing person;
5. The DNA data must meet minimum loci requirements for the specimen category;
6. The DNA PCR data must be generated using PCR accepted kits; and
7. Participating laboratories must have and follow expungement procedures in accordance with federal law.

Q: What are the 13 core CODIS loci?
A: The 13 core CODIS loci are:

- CSF1PO
- FGA
- THO1
- TPOX
- VWA
- D3S1358
- D5S818
- D7S820
- D8S1179
- D13S317
- D16S539
- D18S51
- D21S11

Q: What are the minimum loci requirements for the STR DNA data submitted to NDIS?
A: The minimum loci required for submission of DNA data to NDIS vary by specimen category. Generally, the 13 core CODIS loci are required for submission of convicted offender, arrestee, detainee, and legal profiles. The 13 core CODIS loci and Amelogenin are required for relatives of missing person profiles.

All 13 core loci must be attempted for other specimen categories with the following limited exceptions:

- For forensic DNA profiles, all 13 core loci must be attempted but at least 10 loci must have generated results for submission to and searching at NDIS.
- For Missing Person and Unidentified Human Remains, all 13 core loci must be attempted but at least eight loci and Amelogenin must have generated results for submission to and searching at NDIS.

Q: What are the requirements for submission of mtDNA data to NDIS?
A: Hypervariable region I ("HV1"; positions 16024-16365) and hypervariable region II ("HV2"; positions 73-340) are required for the submission of mtDNA data to NDIS.

Q: Are there additional requirements for forensic (casework) DNA records?
A: Forensic (casework) DNA samples are considered crime scene evidence. To be classified as a forensic unknown record, the DNA sample must be attributed to the putative perpetrator. Items taken directly from the suspect are considered deduced suspect samples, not forensic unknowns, and are not eligible for upload to NDIS.

Q: Are there any additional requirements for missing persons-related DNA records?
A; For missing person, relatives of missing person and unidentified human (remains) samples, additional DNA technologies (such as mtDNA, Y STR) should always be considered, as appropriate. For purposes of this discussion, "as appropriate" means if relevant. For example, if the missing person is a female, then Y STR technology would not be relevant. The lack of an additional technology will not render a sample ineligible for entry into CODIS but use of an additional appropriate technology will ensure the most robust search possible.

Additionally, creation of a Pedigree Tree for the missing persons-related DNA records is strongly encouraged. A Pedigree Tree is a graphical representation of the relationship of the missing person with two or more relatives. The more robust Pedigree Trees have at least one relative that is a biological mother, biological father, or biological child of the missing person.

Q: What are the expungement requirements?
A: Laboratories participating in the National DNA Index are required to expunge qualifying profiles from the National Index under the following circumstances:

1. For convicted offenders, if the participating laboratory receives a certified copy of a final court order documenting the conviction has been overturned; and

2. For arrestees, if the participating laboratory receives a certified copy of a final court order documenting the charge has been dismissed, resulted in an acquittal or no charges have been brought within the applicable time period.

Partial Matches and Familial Searches

Q: What is a partial match at NDIS?
A: Occasionally a partial match between a forensic profile and an offender profile is observed during a routine NDIS database search. The FBI defines a partial match as a moderate stringency candidate match between two single source profiles having at each locus all of the alleles of one sample represented in the other sample. (See below illustration). A "partial match" is not an exact match of the two profiles. A forensic scientist, when evaluating whether a candidate match is a viable match and should be processed through to confirmation, discovers that the candidate offender profile is, in fact, excluded as the possible source of the profile obtained from crime scene evidence but that, because of a similarity in alleles between the forensic unknown and the candidate offender profile, believes that a close biological relative of the offender may be the source of the forensic unknown.

The following illustrates a hypothetical partial match as seen in the SWGDAM Recommendations to the FBI Director on the "Interim Plan for the Release of Information in the Event of a 'Partial Match' at NDIS" at http://www.fbi.gov/about-us/lab/forensic-science-communications/fsc/archive/oct2009/standard_guidlines/swgdam.html (with correction at http://www.fbi.gov/about-us/lab/forensic-science-communications/fsc/communications/swgdamv3/swgdam.html).

Locus	Forensic Unknown	Candidate Offender	Match Stringency
D8S1179	13	13, 14	Moderate
D21S11	28, 31.2	28, 31.2	High
D7S820	12	10, 12	Moderate
CSF1PO	10, 12	10	Moderate
D3S1358	15, 17	15, 17	High
TH01	8	7, 8	Moderate
D13S317	9, 12	9	Moderate
D16S539	11, 12	12	Moderate
VWA	17	15, 17	Moderate
TPOX	8, 11	8	Moderate
D18S51	24	16, 24	Moderate
D5S818	9, 12	12	Moderate
FGA	24, 25	24, 25	High

Q: Can partial match information at NDIS be disclosed?
A: Since a partial match is not an exact profile match to an offender profile and therefore cannot be subject to NDIS defined confirmation procedures, the FBI has authorized, on an interim basis, procedures for the release of partial match information. NDIS Laboratories that identify a partial match resulting from an NDIS search and wish to identify the offender profile should contact the FBI's CODIS Unit for further information.

Q: Is there any guidance on how to address these partial matches?
A: At the FBI's request, the Scientific Working Group on DNA Analysis Methods (SWGDAM) reviewed the scientific issues relating to partial matches and developed recommendations to assist in the evaluation of this information. Those recommendations are available in Forensic Science Communications at http://www.fbi.gov/about-us/lab/forensic-science-communications/fsc/archive/oct2009/standard_guidlines/swgdam.html (with correction at http://www.fbi.gov/about-us/lab/forensic-science-communications/fsc/communications/swgdamv3/swgdam.html).

Q: How successful are partial matches at locating potential suspects?
A: As explained in SWGDAM's recommendations "Moderate stringency CODIS matches, in general, have very low efficiency in locating true relatives in offender databases. There is little useful probative value in the majority of partial matches using the current CODIS searching rules and algorithms. There are two main reasons for this: (1) true siblings will very rarely share alleles at all 13 CODIS loci; (2) as offender DNA databases get large, the number of unrelated people that do share at least one allele at all loci increases very rapidly. The original intent for allowing moderate stringency CODIS searches was the realization and acknowledgment that crime scene profiles often may be partially degraded and/or contain

profiles. Allowing the detection of partial matches can help accommodate these two scenarios and allow the ultimate detection of full, high-stringency matches that might otherwise not have been found." The Committee's complete list of recommendations is available at http://www.fbi.gov/about-us/lab/forensic-science-communications/fsc/archive/oct2009/standard_guidlines/swgdam.html (with correction at http://www.fbi.gov/about-us/lab/forensic-science-communications/fsc/communications/swgdamv3/swgdam.html).

Q: Are partial matches the same as familial searches?
A: No. A partial match, as indicated above, is the spontaneous product of a routine database search where a candidate offender profile is not identical to the forensic profile but because of a similarity in the number of alleles shared between the forensic profile and the candidate profile, the offender may be a close biological relative of the source of the forensic profile. Familial Searching is an intentional or deliberate search of the database conducted after a routine search for the purpose of potentially identifying close biological relatives of the unknown forensic sample associated with the crime scene profile.

Q: Are familial searches performed at NDIS?
A: No, familial searching is not currently performed at NDIS. See also Federal Register Vol. 73, No. 238 (December 10, 2008 at page 74937).

Q: Are familial searches performed at the state level?
A: Each jurisdiction must determine whether or not they are authorized to perform familial searching, and if so, the criteria and procedures governing their use of this searching process. The FBI does not regulate this type of search at the state level. California and Colorado are two states where familial searching is currently performed and their policies/procedures are available at http://ag.ca.gov/cms_attachments/press/pdfs/n1548_08-bfs-01.pdf and Colorado Bureau of Investigation - DNA Familial Search Policy, respectively. Please note that these jurisdictions use specially designed software (not CODIS software) to perform familial searching of their databases. Two jurisdictions, Maryland (§ 2-506) and the District of Columbia (§ 22-4151), have passed laws specifically prohibiting familial searching. These laws are available at http://law.justia.com/codes/maryland/2010/public-safety/title-2/subtitle-5/2-506/ and http://government.westlaw.com/linkedslice/default.asp?rs=gvt1.0&vr=2.0&sp=dcc-1000, respectively.

Expert Systems

Q: What are Expert Systems and are they approved for use in generating DNA data for NDIS?
A: An Expert System is a software program or set of software programs that interprets the data generated from a DNA analysis instrument (or platform) in accordance with laboratory defined quality assurance rules and accurately identifies the data that does and does not satisfy such rules. Portions of the technical review required by the FBI Director's Quality Assurance Standards may be accomplished by an NDIS approved and internally validated Expert System.

The following Expert Systems are approved for use on offender samples and known reference samples at NDIS. There are no Expert Systems approved for use on casework (forensic unknown) samples.

NDIS Approved Expert Systems (as of February 2011)

Expert System and Version (s)	Manufacturer	Instrument Platform(s)	Kit(s)
GeneMapperIDv.3.2.1	Applied Biosystems (AB)	AB 3130xl (data collection v3.0)	Identifiler®
GeneMapperIDv.3.2.1	AB	AB 3130xl (data collection v3.0)	PowerPlex 16®
GeneMapperID-Xv1.2	AB	AB 3130xl (data collection v3.0)	Identifiler®
GeneMapperID-Xv1.1.1	AB	AB 3130xl (data collection v3.0)	Identifiler®
GeneMapperID-Xv1.0.1	AB	AB 3730 (data collection v3.0)	Identifiler®
GeneMapperID-Xv1.0.1	AB	AB 3100 (data collection v2.0)	Profiler Plus®and COFiler®
GeneMapperID-Xv1.0.1	AB	AB 3130xl (data collection v3.0)	Identifiler®
GeneMapperID-Xv1.0.1	AB	AB 3130xl (data collection v3.0)	Powerplex 16®

GeneMapperID-Xv1.0	AB	AB 3100 (data collection v2.0)	Profiler Plus® and COFiler®
i-Cubed™ v.4.2.2 using	Forensic Science Service (FSS)/Promega and AB	AB 3730 (data collection v3.0)	Identifiler®
GeneMapperIDv.3.2.1			
i-Cubed™ v.4.2.2 using	FSS/Promega and AB	AB 3130xl (data collection v3.0)	Identifiler®
GeneMapperIDv.3.2.1			
i-Cubed™ v.4.2.1 using	FSS/Promega and AB	AB 3130xl (data collection v3.0)	Powerplex 16®
GeneMapperIDv.3.2			
i-Cubed™ v.4.1.3 using	FSS/Promega and AB	AB 3130xl (data collection v3.0)	Identifiler®
GeneMapperIDv.3.2			
i-Cubed™ v.4.0.2 using	Forensic Science Service (FSS)/Promega and AB	AB 3700 (data collection v3.1.1)	Identifiler®
GeneMapperIDv.3.2			
TrueAllele™v.2.9	Cybergenetics	AB 3100 (data collection v1.1)	Profiler Plus® and COFiler®
TrueAllele™v.2.9	Cybergenetics	AB 3130xl (data collection v3.0)	Identifiler®
TrueAllele™v.2.9	Cybergenetics	AB 3130xl (data collection v3.0)	Profiler Plus® and COFiler®
TrueAllele™v.2.7.348	Cybergenetics	AB 3100 (data collection v1.1)	Profiler Plus® and COFiler®

The listing above is not all inclusive; if you have a question concerning a specific Expert System; please contact the FBI's CODIS Unit.

PCR STR Kits

Q: What are the PCR kits accepted for use at NDIS?
A: Following are the most frequently used PCR kits accepted at NDIS (listed by manufacturer):

- Applied Biosystems
- AmpFISTR®Profiler Plus®
- AmpFISTR®COfiler®
- AmpFISTR®Profiler Plus® and AmpFISTR®COfiler®
- AmpFISTR®Profiler Plus®ID
- AmpFISTR®Profiler Plus®ID and AmpFISTR®COfiler®
- AmpFISTR®Identifiler®
- AmpFISTR®Identifiler® Direct
- AmpFISTR®Identifiler® Plus
- AmpFISTR ® MiniFiler®
- Promega
- PowerPlex®1.1
- PowerPlex®1.2
- PowerPlex®2.1
- PowerPlex®16
- PowerPlex®16 BIO
- PowerPlex®16 HS

The listing above is not all inclusive; if you have a question concerning a specific PCR kit; please contact the FBI's CODIS Unit.

Q: What is the process for PCR kits, loci and Expert Systems to be approved for use at NDIS?
A: Laboratories that participate in the National DNA Index and who have validated the kits, loci, or Expert

validation data and other supporting documentation must accompany the request.

International Searches

Q: How are International DNA databases searched?
A: Requests for a search of an international DNA database should be directed to your state CODIS administrator. The state CODIS administrator will forward the request to their state liaison Interpol contact. Those requesting an international search must use the Interpol DNA Profile Search Request Form available at http://www.interpol.int/public/forensic/dna/default.asp.

Q: Can the National DNA Index System be searched by international agencies?
A: An international law enforcement agency may submit a request for a search of the National DNA Index either through the FBI's legal attaché responsible for that jurisdiction or through Interpol. Requests for such a search will be reviewed by the NDIS Custodian to ensure compliance with the Federal DNA Identification Act (criminal justice agency status, authorized specimen category and participation in quality assurance program) as well as the inclusion of a sufficient number of loci for effective searching.

Outsourcing Offender/Arrestee or Casework Samples

Q: Are there specific requirements for outsourcing offender/arrestee or casework samples?
A: New requirements for the outsourcing of DNA samples are contained in Standard 17 of the *Quality Assurance Standards for Forensic DNA Testing and Databasing Laboratories* and took effect July 1, 2009.

For law enforcement agencies seeking to outsource offender and/or casework samples, the technical specifications of the outsourcing agreement must have the prior approval of the technical leader of the NDIS participating laboratory that will be entering that DNA data into CODIS. At a minimum, the outsourced laboratory must follow the FBI's *Quality Assurance Standards* and be accredited.

Standard 17 of the *Quality Assurance Standards* also requires the completion of an on-site visit of the vendor laboratory prior to the beginning of the outsourced analyses and a technical review of the outsourced DNA records by the NDIS participating laboratory.

Quality Assurance Standards

Q: What are the Quality Assurance Standards?
A: Compliance with the Quality Assurance Standards or QAS issued by the FBI Director is required by federal law in order for a laboratory to participate in and contribute DNA records to the National DNA Index System.

The QAS describe the minimum standards for laboratory's quality program if performing forensic DNA analysis and/or databasing. The minimum standards cover the following areas: organization, personnel, facilities, evidence or sample control, validation, analytical procedures, equipment calibration and maintenance, reports, review, proficiency testing, corrective action, audits, safety, and outsourcing. For example, the standards require that DNA examiners undergo external proficiency testing on a semiannual basis.

Q: When did the revised Quality Assurance Standards take effect?
A: The *Quality Assurance Standards for Forensic DNA Testing Laboratories* and *Quality Assurance Standards for Databasing Laboratories* took effect **July 1, 2009**.

Q: Do the approved accrediting agencies use the Quality Assurance Standards?
A: The approved accrediting agencies use the FBI's Quality Assurance Standards when performing audits of forensic DNA and databasing laboratories.

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CASE REPORT

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First Polish DNA "manhunt" – an application of Y-chromosome STRs

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Abstract This study presents the application of Y-chromosomal STR polymorphisms to male identification in the case of a serial rapist and woman murderer in Poland. Since August 1996 a rapist from Swinoujscie (northwest Poland) committed at least 14 rapes. In the year 2000 he brutally raped 8 young girls and murdered a 22-year-old girl. DNA profiles obtained from semen stains left at the scenes of crime gave information that one and the same man had committed all the rapes. The Y-chromosome haplotype (9 loci) obtained was used for the elimination process of 421 suspects. One man was found who had an identical DNA profile in all Y-chromosome STR loci analysed and possessed common alleles in 9 out of 10 autosomal loci, strongly suggesting that the real rapist and the typed man were closely related males. Analysis of reference DNA obtained from the man's brother revealed an identical DNA STR profile to that identified at the crime scenes. To the best of our knowledge this is the first case in Poland and probably in Eastern Europe where DNA typing of a large population was used to identify the offender.

Keywords Multiple rape case · Y-chromosome STRs · Multiplex PCR

Case report

In a period of 6 years 14 young girls and women in the age range of 9–26 years were brutally raped and one was murdered in the Swinoujscie area (a town located in northwest Poland very close to the German border). Information about the offender was very scanty. His face had never been seen because he wore a mask. The victims described him as a tall athletic man armed with a pistol and using very primitive language. Three months after the homicide of the 22-year-old girl, a special police group consisting of 8 highly experienced policemen was established who decided that all young men in the age range between 22 and 38 years living in Swinoujscie area had to be investigated. The number of theoretical checks was estimated to ca. 12,000. During 15 months of intensive work 714 suspects including known rapists, pedophiles etc. were interrogated and 421 men were asked to submit mouth swabs or blood samples for elimination by DNA typing. In the first sample of 420 donors was a 28-year-old man who lived 3 km away from the place of the first reported rape. A detailed analysis of his DNA profile showed that this man could be closely related to the real rapist. A few days after this information was sent to the public prosecutor, the brother of the analysed man was arrested and accused of committing 14 rapes and 1 homicide.

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Materials and methods

DNA extraction

Blood samples or buccal swabs were obtained from 421 males living in northern Poland and DNA was isolated using the Sherlock AX kit (A@A Biotechnology, Gdansk, Poland). DNA from vaginal swabs and semen stains was isolated using mild preferential lysis as described by Wiegand at al. [1]. DNA was quantified fluorimetrically or using the QuantiBlot kit (PE, Foster City, Calif.) with chemiluminescence detection.

Amplification conditions

Amplification of the 10 loci included in the commercial Profiler-Plus kit (Perkin Elmer) was carried out in accordance with the manufacturer's instructions on a 2400 Thermal Cycler (Perkin Elmer). A set of nine Y-STR loci was amplified in two separate multiplex reactions (DYSI: 19, 390, 393 and amelogenin and DYSII: 391, 392, 389I/II 385I/II) using methods worked out in our laboratory (manuscript in preparation). For all analysed loci except DYS385I/II, primer sequences were as described by Kayser at al. [2]. In the case of DYS385I/II and the amelogenin loci, primers described by Schneider et al. [3] and Sullivan et al. [4] were used, respectively. DYS 391, 392 and 393 were labelled with HEX, DYS 19, amelogenin and 389I/II with 6-FAM, and DYS390 and 385I/II with TET.

PCR reactions were performed in a 5 µl volume containing 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 200 µM dNTP, 0.1 U of AmpliTaq Gold polymerase (Perkin Elmer) and 0.5–1 ng of template DNA. The following primer concentrations were used for the DYSI multiplex: amelogenin 0.06 µM, DYS19 0.24 µM, DYS390 0.08 µM, DYS393 0.15 µM and for the DYSII

multiplex: DYS393 0.3 µM, DYS392 0.3 µM, DYS385I/II 0.25 µM and DYS389I/II 0.23 µM. Both multiplexes were run under the same cycling conditions on a 2400 Perkin Elmer Cycler (Perkin Elmer) at 95°C for 11 min, then 30 cycles at 95°C for 1 min, 55°C for 1.5 min and at 72°C for 2 min and finally 60°C for 30 min.

Detection of PCR products

Detection of ProfilerPlus PCR products was done using capillary electrophoresis as described earlier [5]. In the case of the DYS multiplexes, 1 µl of each PCR product was mixed with 12 µl of deionised formamide and 0.5 µl of GS500 size standard, denatured and analysed using an ABI 310 machine. The designation of alleles followed the nomenclature based on the number of repeat units, according to the recommendations of the International Society of Forensic Genetics [6]. Appropriate allelic ladders were kindly donated by B. Brinkmann (Münster), P. deKnijff (Leiden) and P. Schneider (Mainz). The analysis of the electrophoretic data was carried out using the computer program GeneScan v. 2.1.

Results and discussion

The analysis of Y-chromosome polymorphisms is becoming increasingly more important for forensic genetics and particularly for the interpretation of results from vaginal swabs containing male DNA. STR sequences on the Y-chromosome have the potential for wide variation among male individuals and provide a simple and sensitive method to selectively obtain male DNA profiles. In this work we applied Y-chromosome specific markers to identification of a serial rapist from a large population sample.

The results of the first three investigated cases sent to our laboratory, suggested that all rapes were committed by one and the same male. The analysis of the mixed stains revealed the same Y-chromosome haplotype in all nine loci (Table 1). The Y-STR analysis of the next cases gave the same profile except one where a mixture of two males was detected (case 4). In this case the raped girl stated that 4 days before the rape she had had sexual intercourse with her boyfriend. Detailed analysis of the peak height differences showed that the dominant alleles were the same as those identified in previous cases.

The results of autosomal loci analysis of these samples gave DNA profiles with a strong domination of female DNA and so in the beginning it was not possible to deduce the rapist's profile. Complex analysis of the non-victim ProfilerPlus alleles allowed almost the complete DNA profile of the rapist to be deduced also suggesting that one

Table 1 Y-chromosome DNA profiles in samples from the five cases

Samples analysed	DYS locus							
	DYS 19	DYS 390	DYS 393	DYS 392	DYS391	DYS389I	DYS 389II	DYS 385I/II
Case 1	16	25	13	11	10	13	29	11, 15
Case 2	16	25	13	11	10	13	29	11, 15
Case 3	16	25	13	11	10	13	29	11, 15
Case 4	**15, 16**	**24, 25**	12, **13**	11	10	12, **13**	**29**, 30	**11**, 14, **15**,17
Case 5	16	25	13	11	10	13	29	11, 15

Alleles typed in bold represent dominating alleles in the mixture.

Table 2 Deduced autosomal profile of the rapist (ProfilerPlus) in three of the cases

Analysed samples	ProfilerPlus loci									
	D3S1358	VWA	FGA	D5S818	D13S317	D7S820	AMG	D8S1179	D21S11	D18S51
Case 1	15, 16, **17**	14, 15, **17**	22, **22.2**, 23	**11**, 12, 13	**10, 11**, 12	**8**, 10, 12	X>Y	12, 13	29, 30, **31**	15, 18, **20**
Case 2	15, **16**, 17	14, 17	21, **22**, **22.2**, 25	10, 11, **12**	**10**, 11, 14	**8**, 9, 10, 12	X>Y	12, **13**, 14	28, **29**, 31, 31.2	17, **20**
Case 3	15, **16**, 17	**14**, 17, 18	20, **22**, **22.2**, 23	11, 12	**9, 10**, **11**, 12	7, **8**, **12**, 13	X>Y	12, 13	28, 29, **31**	14, 17, **20**
Deduced Rapist's profile	16, 17	14, 17	22, 22.2	11, 12	10, 11	8, 12	XY	12?, 13	29, 31	20

Alleles shown in bold type indicate alleles which were not present in the victim's reference material.

Table 3 Comparison of rapist's DNA profile (ProfilerPlus) with the sample originating from suspect and the one of his brother with an identical Y-haplotype

Sample analysed	ProfilerPlus loci									
	D3S1358	VWA	FGA	D5S818	D13S317	D7S820	AMG	D8S1179	D21S11	D18S51
Deduced offender profile	16, 17	14, 17	22, 22.2	11, 12	10, 11	8, 12	XY	12?, 13	29, 31	20
Suspect (KW)	16, 17	17	22	10, 11	10, 12	10, 11	XY	12, 13	30.2, 31	15, 20
Suspect's brother (TW)	16, 17	14, 17	22, 22.2	11, 12	10, 11	8, 12	XY	12, 13	29, 31	20

and the same man had committed all rapes (Table 2). It was decided (with a scenario of possibly 12,000 samples) that an analysis of 9 Y-chromosome STRs (2 multiplexes) would be used as a screening tool for elimination of suspects. In the case of identical haplotypes, ProfilerPlus loci would be used for further elimination. A total of 714 suspects (including known rapists, pedophiles etc.) were interrogated. A sudden breakthrough was brought by sample number 420, taken from a 28-year-old male (KW), whose Y-chromosome haplotype was identical with the haplotype (in all 9 loci analysed) of the offender. Subsequent analysis of ProfilerPlus loci showed that KW possessed a different profile than identified in the stains. A detailed analysis showed that 9 out of the 10 autosomal loci displayed the same alleles as deduced for the rapist (Table 3). This observation suggested that the real rapist could be a man closely related to KW. Shortly afterwards it was established that KW had a 26-year-old brother (TW) whose mouth swab was obtained and sent for urgent typing and showed that his profile exactly matched all 19 autosomal and Y-chromosome STRs identified in the rape cases. The frequency of the DNA profile identified was estimated to be 1.6×10^{-16}. To the best of our knowledge this is first such case in Poland and in Eastern Europe when DNA typing of large population was used to identify the offender.

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