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Rapid DNA – a technology for rapid automated DNA profile analysis based on STR *loci* polymorphism

Summary

Since the mid-1990s of the last century, DNA research has become synonymous with scientific progress in forensics. DNA profiling based on the analysis of STR *loci* polymorphism is considered the gold standard and constitutes an invaluable source of information, enabling, inter alia, the identification of suspects and wanted persons and the identification of corpses, including that based on kinship analysis. However, such analysis is laborious and time-consuming. To reduce this inconvenience, a technology described as *rapid DNA* has been developed.

Key words: *rapid DNA*, DNA profile, STR, CODIS, DNA database

Introduction

DNA tests using the analysis of STR (short tandem repeats) *loci* polymorphism are considered the gold standard in forensics, which is an invaluable source of information, enabling, inter alia, the identification of suspects and wanted persons and the identification of corpses, including that based on kinship analysis.

The standard analysis of DNA profiles carried out by qualified staff under laboratory conditions is a multistage process which consists of: isolation and quantitation of DNA, amplification of specific STR regions in polymerase chain reaction (PCR), separation of PCR products via capillary electrophoresis and their detection and analysis of DNA profiles (Butler et al., 2004). Such a scope of analysis takes approx. 7–8 hours of work (Thong et al., 2015), i.e., is both time- and labor-consuming. The testing time is additionally extended by the necessity to deliver samples of biological material to the laboratory. It has therefore become necessary to reduce the time needed to generate DNA profiles, in particular those of persons arrested on suspicion of committing crimes with high criminal sanctions, and to gain the ability to instantly search the obtained DNA profiles in DNA databases in order to link them to unresolved criminal cases, especially those of particular concern to society, such as sexual assault, rape, homicide, kidnapping and terrorism.

In 2010, the Federal Bureau of Investigation (FBI) launched a program in the United States to develop and implement *rapid* DNA technology. The Department of Defense, the National Institute of Standardization and Technology, the National Institute of Justice and other federal agencies and local law enforcement agencies participated in this project.

On August 18, 2017, President of the United States of America Donald Trump signed the Rapid DNA Act of 2017, which amended the 1994 regulations. The new regulations made it possible to use on-site *rapid DNA* analysis equipment and transfer the obtained DNA profiles to a federal DNA CODIS (*Combined DNA Index System*) database. It should be noted, however, that automatic searching of DNA databases is allowed only for the so-called reference (comparative) DNA profiles that have been obtained from buccal swabs of persons with established identity (e.g. persons arrested, suspects, defendants, convicted). This is because only the reference samples guarantee sufficient quantity of DNA to generate full DNA profiles. Also, they carry a low probability of obtaining mixed DNA profiles, which would require an additional analysis carried out by a qualified expert in interpretation. Unlike reference samples, biological traces revealed at the scene of a criminal event are presumed to require analysis and approval by an expert in forensic genetics. In addition, under U.S. law, in order to qualify for transferring to and searching in the CODIS system, all samples secured at the scene of an incident, must be processed by an accredited forensic laboratory that complies with the

FBI Quality Assurance Standards for Forensic DNA Testing Laboratories (*The Public Health and Welfare*, 2009).

1. Definition of *rapid DNA* technology

Rapid DNA is a term borrowed from English, used to describe a fully automated (almost maintenance-free), fast (obtaining a DNA profile in less than two hours) process of generating a DNA profile using STR *loci* polymorphism analysis. The process includes automatic isolation, amplification, electrophoresis, detection and analysis of the DNA profile without human intervention.

This technology enables quick identification of a person on the basis of comparative DNA analysis (STR *loci* polymorphism) between reference samples (buccal swabs) and biological traces (e.g. blood, saliva, cigarette butts, chewing gum, sperm and other forensic samples) left by the offender at the crime scene. The technology has been developed for use not only in laboratory conditions, but primarily at the scene of an incident or mass disaster, to identify victims or perpetrators of terrorist attacks. Due to the fact that the system is automated, it can also be operated by people who are not specialists in forensic genetics. One of the manufacturers, Thermo Fisher Scientific, informs on its website that the equipment can be used for example by law enforcement and judicial personnel, airport security, Border Guard or police officers.

2. Characteristics of devices operating in *rapid DNA* technology

Among the pioneering devices operating in *rapid DNA* technology, three systems should be mentioned, i.e., RapidHIT™ID, RapidHIT™200 (Thermo Fisher Scientific) and *ANDE* (ANDE Corporation). In all three systems, the emphasis has been placed on the protection of sensitive data such as DNA profiles. Only users who are logged on to the system can perform DNA analysis. Depending on the type of account created, the user has different privileges. There are three types of user accounts: administrator, supervisor and operator. The administrator has the highest level of privileges. Among other things, he can create, delete and supervise other users' accounts, change device settings, create backup copies and preview the obtained DNA profile. The supervisor has certain administrative privileges, but cannot create new accounts or backup copies. His main function is to oversee the device and supervise operator's work. In turn, the role of the operator is limited only to applying samples of biological material to the device. Thanks to this permission hierarchy, access to sensitive data is strictly limited and controlled. All three devices support the above mentioned user categories.

RapidHIT™ID (Thermo Fisher Scientific) (Fig. 1) is the most compact and easy-to-use device among the three. It is an automated system for rapid individual identification based on DNA analysis (STR *loci* polymorphism) of biological material.

The system consists of *RapidHIT™ID* device, disposable cartridges for samples of biological material and appropriate software. In the front lower part of the device there is a main cartridge, which contains, among other things, gel and capillaries necessary for the electrophoresis process and is the only element of the device that requires additional attention during regular operation. The capacity of the cartridge allows for about 150 cycles of operation. During its replacement, certain operating parts are also replaced, which, in traditional laboratory systems, are usually prone to failures. This eliminates the need for preventive maintenance. After

Fig. 1. a) Disposable cartridge; b) *RapidHIT™ID* System; c) Computer with the installed *RapidLINK™* Software and *GeneMarker®HID STR Human Identity Software* (Thermo Fisher Scientific).

the installation of the main cartridge, it is also necessary to install a control cartridge containing an allelic ladder (which is a set of common alleles in a given STR *locus*), a negative control (used to check the sterility of the device) and a positive control (of a known genotype). Two types of disposable cartridges are used for DNA extraction and amplification: *ACE Sample Cartridge* for testing of comparative material (buccal swabs from a single source) and *EXT Sample Cartridge* for the analysis of biological traces secured at the scene. Both cartridges use the *GlobalFiler™ Express* reagent kit consisting of 21 autosomal STR *loci* and 3 sex markers (Fig. 2).

The *RapidHIT™ID* system is an automated mobile platform with a single sample processing time of about 1 minute (the time necessary for placing the sample in the cartridge) and the DNA profile analysis time of less than 90 minutes. The dimensions of the machine are: 28 cm width × 53 cm depth × 47 cm height and the weight is about 28.4 kg (including main cartridge). The device operates in the temperature range 15–30°C, at 20–80% air humidity, up to 2600 m above sea level, at 100–240 V (50/60 Hz frequency), with power supply 600 W (*RapidHIT™ID System v 1.0 User Guide*). The sample is subjected to a complete analytical process, from DNA isolation, through PCR and capillary

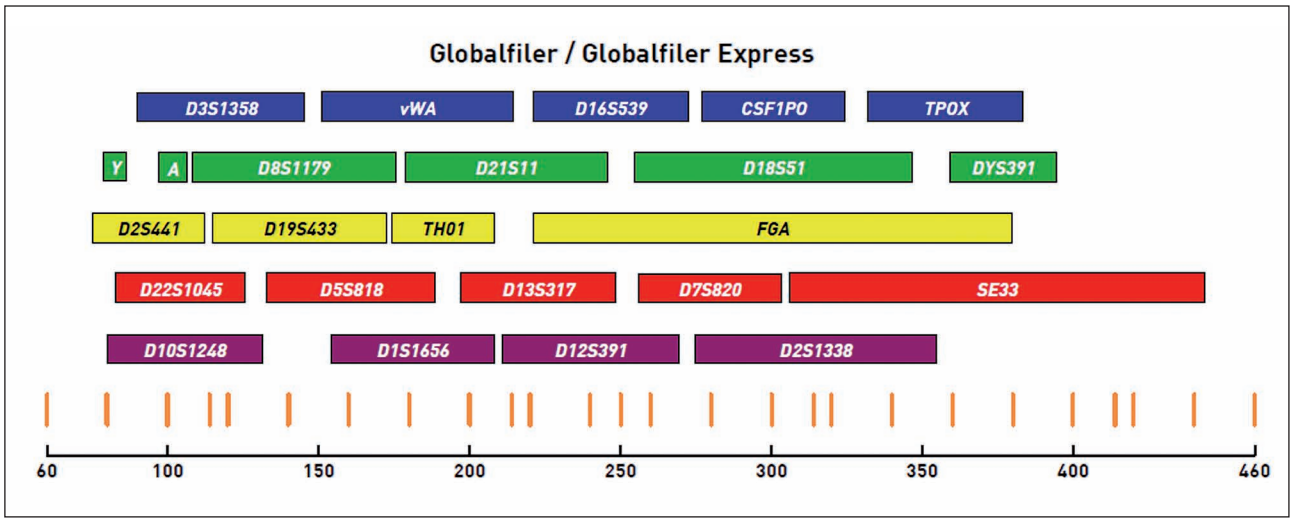

Fig. 2. The size of particular markers in base pairs (bp) for Thermo Fisher Scientific *GlobalFiler® Express* System.

Fig. 3. Example of genealogical tree under *RapidLINK Kinship* tab.

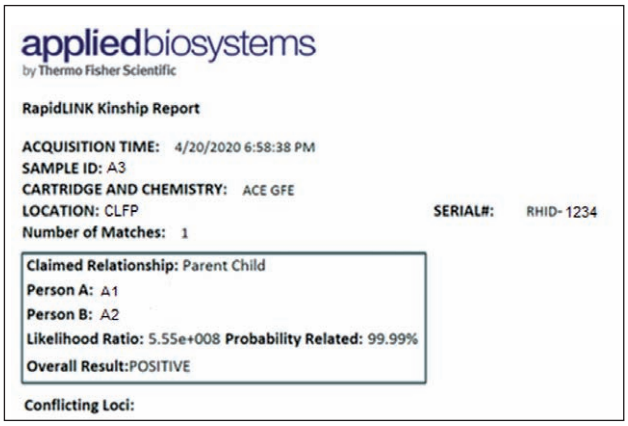

Fig. 4. Exemplary report from kinship analysis carried out using *RapidLINK* software.

separation, to obtaining and analyzing a genotype data. The device is connected to a computer with *RapidLINK™* and *GeneMarker®HID STR Human Identity Software* installed. The analysis is carried out automatically and the result is marked with one of three tags: green when the profile is generated in accordance with acceptance criteria, yellow when the profile does not meet all of the acceptance criteria, red when the system has not generated the profile. DNA analysis results can be sent directly to the national DNA database to be searched. It is also possible to create a local DNA database in *RapidLINK™* and compare the obtained profiles with those collected in this database in one of the software modules (*RapidLINK™ Software v 1.0 User Guide*). *RapidLINK™* also has modules for kinship analysis and for detecting DNA contamination.

Kinship analysis is carried out using *RapidLINK Kinship* module. The tab contains a graphical representation of genealogical tree (Fig. 3), where female sex is marked with the circle and male sex with the square. The profiles selected from the list are dragged onto the genealogical tree diagram and then a report is generated (Fig. 4) containing an estimate of the probability based on the calculation of the *Likelihood Ratio* (LR) and with the presented value of the probability coefficient.

RapidLINK Staff Elimination module is designed to process and collect DNA profiles of individuals who might potentially contaminate the samples analyzed, e.g. operators of the device, individuals performing tests and taking samples for tests. It enables automatic detection of the above-mentioned contamination. The system generates a report containing a list of DNA profiles from the elimination database that match up with the sample subject to verification. In this report, in a tabular form, individual markers within matching DNA profiles are compared in terms of allele compliance, including the calculated likehood ratio (LR).

Another device is *RapidHIT™ 200* (Thermo Fisher Scientific) (Fig. 5).

Like *RapidHIT™ID*, it is an automated mobile platform for fast individual identification based on the analysis DNA isolated from biological material. The sample processing time is about 3 minutes (the time necessary for placing the sample in the cartridge) and the DNA profile analysis time varies between 90–120 minutes, depending on the protocol. The device is also designed to work in both laboratory and field conditions. However, its dimensions and weight are much larger than in the case of *RapidHIT™ID*, i.e., 73 cm width × 71 cm depth × 48 cm height and about 81.5 kg of weight. In addition, the machine needs to be calibrated after relocation. It operates in the temperature range 18–30°C, at 15–75% air humidity, up to 2000 m above sea level, at 100–240 V (50/60 Hz frequency) (Thermo Fisher Scientific – website). From 1 to 8 samples can be analyzed in a single test cycle. The samples are placed in two cartridges that also contain the *GlobalFiler® Expres* reagent kit. One of the cartridges contains an allelic ladder. Two additional cartridges, replaced before each test cycle, are required: “cartridge A” containing anode buffer and polymer and “cartridge B” with cathode buffer, water and waste reservoir. No other chemical reagents are required for operation. The samples are subjected to a full analytical process,

Fig. 5. *RapidHIT™ 200* System with cartridge set (Thermo Fisher Scientific).

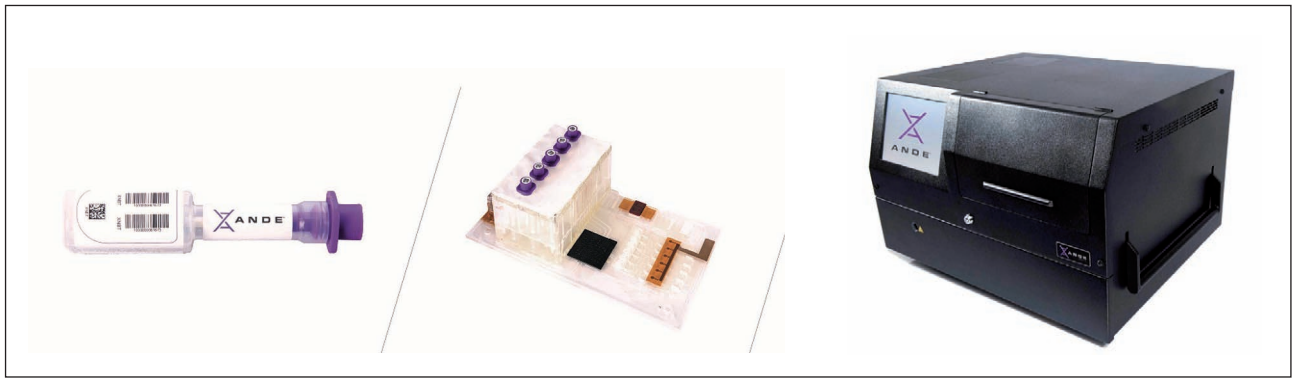

Fig. 6. *ANDE* System, swab stick and A-chip (ANDE Corporation).

similar to that described for *RapidHIT™ID*. The analysis is performed by running the *Run Buccal* protocol for buccal swabs or *Run Other* protocol biological traces secured at the scene. *RapidHIT™200* is also compatible with *RapidLINK™* software.

Another *rapid DNA* device is ANDE (*ANDE 6C System*) (ANDE Corporation), which is also an automated mobile platform for the analysis of DNA profiles from biological material (Fig. 6). The DNA profile analysis time is about 85 minutes.

The dimensions and weight of the device are: 45 cm width × 75 cm depth × 59 cm height, 54 kg weight. It operates in the temperature range 20–30°C, at 20–80% air humidity, at 100–240 V (50/60 Hz frequency) (*ANDE™ 6C Rapid DNA Analysis™ System Product User Manual*). The platform consists of three components: special swabs, an A-chip with five chambers for the swabs and an apparatus (Fig. 6). The A-chip is a disposable component containing all the necessary reagents for DNA analysis, from DNA isolation to electrophoretic separation. The chip is designed to avoid direct contact between the sample of biological material, the device and the reagents. The system containing the swab is closed with a plug to prevent cross-contamination. All fluids inside the chip are pneumatically driven under pressure. A *FlexPlex* reagent kit (Fig. 7) based on the *PowerPlexFusion 6C* kit (Promega Corporation) is used for analysis. This kit enables testing 27 STR *loci*. The device has a computer with a touch-screen panel and a graphical user panel, *FAIRS™* (ANDE Corporation) software to control and manage data collection, and an integrated expert system (*Expert System Software*) for automatic DNA analysis and profile qualification in terms of its suitability for DNA databases. All data are encoded and can be transmitted to DNA databases via the *FAIRS™* system. This software is used, inter alia, to manage the DNA profiles, perform comparative analysis and kinship analysis (Carney et al., 2019).

The device is additionally equipped with a transport box for safe delivery to the place of destination. This solution makes it possible to skip the calibration process after relocation.

3. Practical aspects of *rapid DNA* technology

Prompt identification of human remains after mass disasters is essential to provide family members and friends of victims with the opportunity to bid farewell, arrange a funeral and complete legal formalities related to the death their loved ones (Turingan et al., 2020).

Identification may be difficult due to the nature, location and accessibility of the disaster site, the disassembly of the remains and the time needed to secure them, as well as the time needed to transport the samples to the laboratory. In addition, exposure of biological material to harsh environmental conditions may accelerate its degradation. The above circumstances and factors affect the quality of the samples secured and may be the reason for extending the time of DNA analysis performed by a forensic laboratory. Therefore, the possibility to use the *rapid DNA* technology on site and operate devices by non-experts may contribute to a more efficient processing of cases involving human identification.

The following events may serve as examples of using *rapid DNA* technology in mass disasters.

The first one took place in November 2018. More than 60,000 hectares of land in Butte County, California was completely destroyed by the most destructive fire on record in the state to date. The fire was so violent that, at its peak, it consumed about 30 hectares per minute. The speed and intensity of the approaching flames killed dozens of people. As with most mass disasters, conventional identification methods (e.g. fingerprints, teeth – odontology, personal belongings) were used to identify the victims. However, the intensity and duration of the fire as well as high temperature had caused serious malformations of the victims' bodies, thus making their identification much more difficult. Therefore, conventional methods turned out to be useful only in 22 out of 84 cases. For the remaining victims, it was initially planned to perform a conventional DNA analysis, which could take months or even years. Eventually, however, *ANDE rapid DNA* technology was used for identification. *Rapid DNA* identification was performed on 69 remains, of which 62 (89.9%) yielded STR profiles. The samples used

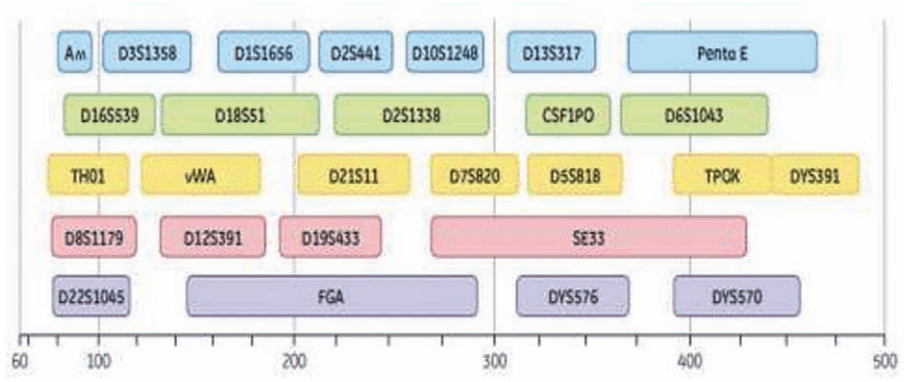

Fig. 7. The size of particular markers in base pairs (bp) for *FlexPlex* System.

for identification were taken from blood, bones, liver, muscles, soft tissue of unknown origin, and brain. Appropriate test protocols were developed for each type of biological material, taking into account its state of degradation. Macerates were obtained from blood and soft tissues, which were then applied to the ANDE swab. The bones were first mechanically ground and then, according to the test protocols, mixed with ANDE buffer. The samples prepared in this way were incubated at room temperature or at 56°C. The solutions obtained were pipetted onto ANDE swabs and applied to the device. All profiles were determined within a few hours of receiving the samples for testing. DNA profiles from 255 members of the victims' families were analyzed in parallel with fire site samples. Using the module for searching relatives, the DNA profiles obtained from the remains were compared against reference profiles, as a result of which, 58 victims were identified. This case was the first successful use of r*apid DNA* technology in a mass disaster identification (Gin et al., 2020).

Another accident happened shortly after Christmas in 2019. In the Hawaiian Islands region, a helicopter crashed in the cliffs on the north-western side of the Kaua`i island. None of the seven people on board survived this tragic accident. Since the Kaua`i police did not have forensic facilities to perform DNA analysis, the tests were commissioned in the continental United States. The *RapidHIT™ID* system was used to shorten the identification process and thus prevent degradation of samples that were additionally charred. The biological material analyzed were tissue, brain and bone samples. By using r*apid DNA* technology, it was possible to quickly identify all seven victims. If similar tests had been carried out according to standard procedures, the identification process would have taken more than a month, due to the need to organize the transport of samples and logistical issues (Thermo Fisher Scientific, The Kaua'i Police Department..., 2020).

Another example of using *Rapid DNA* technology, this time involving a criminal case, was a sexual assault that took place on early April morning, on a homeless woman sleeping in an abandoned house in Louisville, Kentucky. The woman was woken up by a stranger who stretched her bedding over her head and raped her. It should be noted that rape cases are particularly complicated not only in procedural terms, but also because they involve the victim's intimate sphere and provide a particularly traumatic experience for a woman. Therefore, any additional delay, including in the analysis, further aggravates the psychological condition of the victim, which, in turn, negatively affects the subsequent testimony, hinders the investigation, and consequently, the resolution of the case and the judgment of the perpetrator. The rape in question coincided with the process of validating the newly purchased *rapid DNA* system (ANDE Corporation) in the Kentucky State Police laboratory. If this technology were found to work satisfactorily, it could revolutionize the way rape cases are investigated in the U.S., where hundreds of thousands of sex crimes remain unresolved and only a third of the cases end with arrest. Thus, the first ever performed test of r*apid DNA* technology in the context of sexual assault involved taking samples from the rape victim and examining them using the above-mentioned technology. Within three hours, the device generated a DNA profile of the potential perpetrator, who was subsequently arrested within a few weeks. This rape case reflects the strength and potential of the *rapid DNA* technology, which is slowly beginning to be a method acceptable to the justice system for handling criminal cases (Schuppe, 2019). The use of *rapid DNA* in the aforementioned criminal case seems promising, but the FBI recommends extreme caution when examining biological traces using this technology and recommends that judges be cautious when ruling on cases involving the results obtained with the use of *rapid DNA*. It is noteworthy, however, that the police, using this new technology, have been able to push the boundaries of the standard practice of analyzing evidence secured at the scene and taking DNA samples from persons suspected of a crime.

The cases described herein prove that *rapid DNA* technology can be successfully used to complement conventional methods of identification of victims of mass disasters and, in the future, also in criminal cases. The unquestionable advantage of this technology is the ease of use of the analytical equipment, also by people who are not specialists in forensic genetics, its mobility and short waiting time for results (about 90 minutes in the case of reference material obtained from buccal swabs). Thus, the use of r*apid DNA* technology in forensic practice may contribute to increasing the resources of national DNA databases, and create new opportunities for authorities responsible for state security: law enforcement agencies, judiciary, border guards, etc. It should be noted, however, that its use still entails considerable financial outlays.

The development of *rapid DNA* technology also raises justified concerns about the fact that the results obtained from devices of this type may contribute to incorrect identification. This is because the analytical equipment can be operated by officers who have no knowledge of genetics. Moreover, limited pilot studies have raised questions whether the reagents used in the devices may be prone to contamination and whether contamination can be caused by unqualified operators. It is therefore advisable that experts in genetics exercise supervision over individual devices. Another concern is the ease of obtaining sensitive data, such as DNA profiles. Currently, police DNA databases process data on the results of deoxyribonucleic acid analysis limited to information on the non-coding DNA regions. However, should *rapid DNA* technology be further developed in the future, it may become much easier to extract more and more sensitive data from DNA, e.g. on the susceptibility to various diseases. These concerns can be addressed by introducing transparent legal regulations. The legislator may, for example, indicate which entities would be permitted to acquire this type of analytical equipment and specify the scope of its use. It is worth noting that also in Poland it will become necessary to adjust the regulations that will govern the practical use of the above described technology.

Rapid DNA technology seems very promising and may contribute to more effective detection of offenders and, in consequence, improve the efficiency of investigative and judicial authorities.

Source of figures

Figure 1: https://www.thermofisher.com/pl/en/home/industrial/forensics/human-identification/forensic-dna-analysis/dna-analysis/rapidhit-id-system-human-identification/rapidhit-id-system-law-enforcement.html (accessed on 07/08/2020)

Figure 2: https://www.interlabservice.ru/solutions/criminalistics/nabory-reagentov-dlya-amplifikatsii-str-lokusov.php (accessed on 31/08/2020)

Figures 3, 4: authors

Figure 5: https://www.thermofisher.com/pl/en/home/industrial/forensics/human-identification/forensic-dna-analysis/dna-analysis/rapidhit-id-system-human-identification/rapidhit-id-system-crime-labs/integenx-by-thermo-fisher-scientific-rapidhit.html (accessed on 31/08/2020)

Figure 6: https://www.ande.com/what-is-rapid-dna/ oraz https://www.fbinaa.org//FBINAA/Associate/Associate_Magazine.JanFeb.2018/JANFEB2018_Feature_2_JF.aspx (accessed on 31/08/2020)

Figure 7: Carney et al., 2019

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