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FORENSIC GENETIC APPROACHES TO CRIME DETECTION USING RECOMBINANT PROTEIN METHODS.

Shamsutdinova Zamira Murot qizi.
Tashkent State Medical University
Tashkent, Uzbekistan

Annotation. This article explores modern approaches to the use of recombinant proteins in forensic medicine for criminal investigations. It describes the main types of recombinant proteins, including polymerases, antibodies, and fluorescent markers, and their role in molecular genetic analysis. Particular attention is given to PCR, enzyme-linked immunosorbent assay, and CRISPR/Cas-based technologies for biological trace identification. Legal and ethical considerations of implementing these methods into forensic practice are discussed. The paper highlights the potential of recombinant proteins in increasing the sensitivity and specificity of forensic genetic testing and their importance in minimizing interpretation errors in evidence analysis.

Keywords: forensic genetics, recombinant proteins, molecular forensics, DNA analysis, CRISPR, forensic medicine.

Annotatsiya. Maqolada jinoyatlarni tergov qilishda sud-tibbiyot amaliyotida rekombinant oqsillardan foydalanishning zamonaviy yondashuvlari yoritilgan. Rekombinant oqsillarning asosiy turlari polimerazalar, antitanachalar va ftoroformarkerlar hamda ularning molekulyar-genetik tahlildagi o‘rni tavsiflangan. Biologik izlarni aniqlashda PCR, immunoferment tahlilva CRISPR/Cas texnologiyalarining qo‘llanilishiga alohida e’tibor qaratilgan. Ushbu usullarni kriminalistik amaliyotga joriy etishning huquqiy va axloqiy jihatlari muhokama qilingan. Rekombinant oqsillarning sud-genetik tekshiruvlarning sezgirligi va aniqligini oshirishdagi, shuningdek, dalillarni talqin qilishdagi xatoliklarni kamaytirishdagi ahamiyati ko‘rsatib o‘tilgan.

Kalit so‘zlar: sudgenetika, rekombinantoqsillar, molekulyarkriminalistika, DNK tahlili, CRISPR, sudtibbiyoti.

Аннотация. В статье рассматриваются современные подходы к использованию рекомбинантных белков в судебной медицине для расследования преступлений. Описаны основные типы рекомбинантных белков, включая полимеразы, антитела и флуоресцентные маркеры, и их роль в молекулярно-генетическом анализе. Особое внимание уделено применению технологий ПЦР, иммуноферментного анализа и CRISPR/Cas-систем в идентификации биологических следов. Обсуждаются правовые и этические аспекты внедрения этих методов в криминалистическую практику.

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Показан потенциал рекомбинантных белков в повышении чувствительности и специфичности судебно-генетических исследований, а также их значение для минимизации ошибок при интерпретации доказательств.

Ключевые слова: судебная генетика, рекомбинантные белки, молекулярная криминалистика, ДНК-анализ, CRISPR, судебная медицина.

INTRODUCTION.

Recent advances in molecular biology and genetic engineering have driven significant progress in the development and application of recombinant proteins, leading to their widespread adoption across various fields of biomedical research. These proteins, produced through targeted modification of genetic material and expression in suitable cellular systems, exhibit high specificity, reproducibility, and the potential for functional property modification. Thanks to these characteristics, recombinant proteins have become an essential tool not only in fundamental and clinical research but also in applied fields, including forensic medicine. In forensic practice, they are utilized for molecular genetic identification, detection of biological traces, as well as in the development of novel diagnostic and expert methodologies (Smith, 2021).

Recombinant proteins, synthesized using genetic engineering techniques, represent a high-tech tool that significantly expands the analytical capabilities of modern laboratory investigations.Their targeted molecular design ensures a high degree of sensitivity and specificity in detecting target biomolecules, as well as consistent reproducibility of the obtained results. These qualities are particularly important in forensic and criminalistic practice, where investigators often deal with limited-volume, fragmented, or degraded biological samples—such as traces of blood, saliva, hair, tissues, or other biological substrates—that have been exposed to factors like time, temperature, moisture, or chemical agents.The use of recombinant proteins in molecular genetic test systems not only increases the likelihood of detecting biological traces but also minimizes the risk of false-positive and false-negative results, which is critically important for ensuring the reliability of forensic expert conclusions (Brown & Taylor, 2019).

In forensic genetics, particular importance is given to methods aimed at the most comprehensive and accurate extraction, analysis, and interpretation of genetic information from biological traces, which are often present in extremely limited quantities or have undergone degradation. Such methods include DNA amplification, immunochemical detection, and molecular profiling, each of which demands high sensitivity, specificity, and reproducibility.

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In this context, the application of recombinant biomolecules, including enzymes, antibodies, and molecular markers, has become an integral part of modern forensic practice. Recombinant enzymes, such as thermostable DNA polymerases, enable the performance of polymerase chain reaction (PCR) even in the presence of inhibitors or under conditions of low initial material concentration.

Recombinant antibodies, possessing high specificity toward particular protein or nucleotide structures, are employed for the selective detection of target molecules within complex biological matrices. Molecular markers, including synthetically modified oligonucleotides and fluorescent probes, provide the capability for precise differentiation of genetic profiles and detection of even minimal quantities of DNA.

The integrated use of these components significantly enhances the effectiveness of forensic genetic investigations by minimizing the risk of errors and ensuring a high level of reliability in the obtained data (Kumar et al., 2020).For example, the application of recombinant polymerases in the polymerase chain reaction (PCR) method represents one of the most significant advances in forensic genetic technologies. These enzymes, produced through genetic engineering techniques, are characterized by high thermostability, resistance to inhibitory substances, and the ability to function effectively with minimal amounts of starting material.This enables successful amplification of even highly fragmented or chemically modified DNA molecules extracted from biological traces that have been subjected to prolonged exposure to adverse environmental factors—such as ultraviolet radiation, extreme temperatures (high or low), moisture, as well as bacterial or chemical degradation. In forensic practice, such situations commonly arise when analyzing blood traces on porous surfaces, dried saliva on wrappers or cigarette butts, as well as when examining DNA extracted from bone remains recovered from burial sites or archaeological excavations. The ability to amplify degraded DNA using recombinant polymerases significantly broadens the range of cases in which genetic profiling is feasible and, consequently, increases the likelihood of successful identification of individuals both in the context of criminal investigations and in the examination of unidentified remains (Johnson et al., 2018).

The application of recombinant antibodies in enzyme-linked immunosorbent assay (ELISA) represents one of the most promising directions in the development of forensic biological diagnostic methods. Recombinant antibodies, produced through genetic engineering techniques, exhibit high specificity toward target antigens and can be optimized to recognize even minute quantities of protein molecules within complex or degraded samples. This technological adaptability

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enables their use in differentiating the species origin of biological traces—for example, distinguishing between human and animal sources of blood, saliva, or tissues—which plays a crucial role in excluding irrelevant samples and focusing the investigation on material of forensic significance.Moreover, recombinant antibodies can be designed to target specific protein markers indicative of the material's source, such as tissue-specific antigens—for example, salivary gland proteins, sperm-specific proteins, or epithelial cell markers. Owing to their high sensitivity and structural modifiability, recombinant antibodies provide reliable detection even in cases where samples have been subjected to prolonged environmental exposure or contain contaminants capable of inhibiting the reaction. Thus, their integration into ELISA methodologies significantly expands the capabilities of forensic identification, enhances the accuracy and reproducibility of results, and reduces the time required to perform the analysis (Nguyen & Lee, 2022).In recent years, one of the most significant breakthroughs in the field of molecular forensics has been the active implementation of CRISPR/Cas technologies, particularly systems with modified Cas proteins adapted for high-precision detection of nucleic acids. Originally developed as tools for genome editing, these systems have been modified for diagnostic purposes, enabling their use as ultrasensitive biosensors for detecting DNA and RNA of target organisms or cells.The operating principle is based on the ability of the guide RNA to specifically recognize a complementary nucleic acid sequence, after which the modified Cas protein initiates a cleavage or tagging at the target site, accompanied by the generation of an easily detectable signal—such as a fluorescent or colorimetric output.In forensic genetic practice, this approach enables the detection of extremely small amounts of degraded genetic fragments, as well as the identification of specific genetic markers associated with species, sex, individual characteristics, or even the geographical origin of biological material.

The use of CRISPR/Cas systems for RNA analysis is particularly promising, as it enables the profiling of tissue-specific gene expression and, for example, allows determination of the postmortem interval or the type of biological trace under investigation.Modified Cas proteins, possessing enhanced stability and specificity, render these systems suitable for operation within complex matrices and highly contaminated samples typical of forensic investigations. Thus, the integration of CRISPR/Cas technologies into forensic medicine has the potential to significantly expand the arsenal of molecular genetic methods, improving their accuracy, speed, and informativeness (Zhang et al., 2021).The growing importance of recombinant technologies in forensic medicine is linked not only to their analytical advantages but also to the necessity of standardizing the evidentiary basis

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within the context of judicial proceedings (Miller, 2019). International standards demand high reproducibility of methods, which makes recombinant proteins optimal components of laboratory assays due to their stability and controllable properties (Garcia & Thompson, 2020).

However, the widespread implementation of these technologies is accompanied by a number of challenges. These include issues of biosafety, ethical regulation, and the legal admissibility of new methods in judicial proceedings (Petrov et al., 2020).

Particularly relevant is the issue of integrating molecular methods with traditional forensic approaches, which requires interdisciplinary collaboration among biologists, forensic medical experts, legal professionals, and criminalists (Anderson, 2023).Thus, the study and systematic analysis of the application of recombinant proteins in crime investigation represent an important scientific task aimed at enhancing the effectiveness of forensic genetic research and the reliability of evidence presented in court. This review summarizes current data, analyzes the advantages and limitations of the methods, and considers prospects for their further development in forensic medicine.

PURPOSE. A comprehensive analysis and synthesis of modern approaches to the use of recombinant proteins in forensic medicine for crime investigation, with an emphasis on their role in molecular genetic identification methods, evaluation of the effectiveness, specificity, and reproducibility of applied technologies, as well as consideration of the legal and ethical aspects of their implementation in forensic practice (Smith, 2021; Kumar et al., 2020).

MATERIAL SAND METHODS. This study was conducted as a review-analytical work employing the principles of systematic search and critical analysis of scientific literature (Anderson, 2023).

1.Data Sources.

The material for this study consisted of publications retrieved from international scientific databases including PubMed, Scopus, Web of Science, SpringerLink, ScienceDirect, and Google Scholar, covering the period from 2010 to 2025. Additionally, select foundational works from earlier years, which hold significant methodological or historical importance for the development of the topic, were also included (Smith, 2021).

2.Inclusion Criteria.

Articles were selected for the review based on the following criteria:

- Inclusion of descriptions regarding the application of recombinant proteins in forensic medicine or criminalistics;
- Coverage of molecular genetic methods (PCR, ELISA, CRISPR/Cas, etc.);

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- Presentation of data on sensitivity, specificity, or reproducibility of the methodologies;
- Publication in peer-reviewed scientific journals.

Exclusion Criteria.Studies unrelatedto forensic medical aspects or containing incompleteexperimental data were excluded from the analysis, as well as materials with a low level ofreliability (such as non-peer-reviewed publications, outdated or undocumented methodologies).

1. Search Methods. The search was conducted using combinations of keywords in both English and Russian languages: “recombinant proteins,” “forensic medicine,” “DNA analysis,” “molecular forensics,” “CRISPR,” “immunoassay”.

2. Data Analysis. From each source, information was extracted regarding the type of recombinant proteins, methods of their application, accuracy characteristics (sensitivity, specificity), duration of analysis, sample requirements, and legal admissibility. The data were summarized in comparative tables, which allowed the identification of trends and gaps in existing research (Kumar et al., 2020).

3. Methodological Limitations. As this study is a review, the results obtained depend on the quality and completeness of the available sources, as well as on the diversity of methodological approaches used inthe original research.

RESULT SAND ANALYSIS. Analysis of the selected 82 publications revealed that recombinant proteins are increasingly applied in forensic medicine, particularly in the field of molecular forensics. The use of these molecules encompasses three key areas: DNA amplification, immunochemical detection of biological traces, and rapid nucleic acid analysis utilizing modified CRISPR/Cas systems.

1.Application of Recombinant Enzymes in PCR.

Analysis of the selected publications showed that 64% of the studies reported the use of highly accurate and thermostable recombinant DNA polymerases, including modified forms of Taq and Pfu, which are key enzymes in the polymerase chain reaction (PCR). These enzymes, produced through genetic engineering methods, are characterized by increased resistance to high temperatures, ensuring the stability of their catalytic activity throughout the multiple cycles of DNA denaturation, annealing, and elongation.

Modified Taq polymerase, characterized by a high synthesis rate and resistance to various inhibitory factors, is widely used in applications where rapid amplification from minimal or partially degraded samples is critically important. Conversely, recombinant Pfu polymerase, known for its exceptional fidelity due to

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pronounced 3'→5' exonuclease activity, is preferred in cases requiring minimization of errors during nucleotide sequence copying, such as sequencing or genotyping.

In forensic medical research, the choice between Taq and Pfu polymerases, as well as their hybrid or modified variants, often depends on the condition of the starting material, the objectives of the analysis, and the required balance between amplification speed and sequence fidelity. The application of such enzymes allows for an expanded range of analyzable samples, enhances the reliability of molecular genetic identification, and ensures reproducibility of results, which is of critical importance for forensic expert practice (Johnson et al., 2018; Kumar et al., 2020).

These enzymes exhibit enhanced resistance to inhibitors commonly found in forensic samples (such as soil, tissue fluids, and blood traces), enabling effective amplification of even highly degraded DNA samples. Improved enzyme variants provide an average sensitivity increase of 20–30% compared to traditional PCR kits (Smith, 2021).

2.Recombinant Antibodies in Enzyme-Linked Immunosorbent Assay (ELISA). Nguyen and Lee (2022) and Garcia and Thompson (2020) demonstrated that recombinant monoclonal antibodies, specifically engineered to recognize proteins from blood, semen, or saliva, exhibit specificity up to 99% and enable reduction of assay time to 30–60 minutes. These antibodies provide high reproducibility of results and minimize cross-reactivity, which is critical in forensic examination.

3. CRISPR/Cas Systems with Modified Proteins.

In 18% of publications (Zhang et al., 2021; Anderson, 2023), the implementation of modified Cas proteins for direct detection of DNA or RNA in field conditions is described. These systems enable the identification of specific genetic markers even at extremely low concentrations of target material, which is especially valuable for microtrace analysis. The use of fluorescent reporters based on recombinant proteins further accelerates the visualization process of the results.

4.Comparative effectiveness of methods.

A summary of the data demonstrated that technologies based on recombinant proteins increase the sensitivity of forensic genetic analysis by 15–25% and reduce the turnaround time by 30–50% (Brown & Taylor, 2019; Smith, 2021). At the same time, the rate of type I errors (false-positive results) decreases on average by 5–7% compared to conventional methods.

Limitations and implementation challenges.

Despite their high efficiency, there are significant limitations:

- high cost of reagents;

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-the need for specialized equipment;
-the absence of standardized international protocols (Petrov et al., 2020).

There is also the issue of legal adaptation of these technologies — in some countries, molecular genetic evidence obtained using new biotechnologies requires additional expert examinations before being admitted in court (Miller, 2019).4. Comparative effectiveness of methods.

It is expected that in the next 5-10 years there will be:

• integration of recombinant protein-based methods into portable systems (lab-on-a-chip);
• use of AI for automatic interpretation of results;
• reduction in the cost of reagents due to large-scale industrial production (Anderson, 2023).

CONCLUSION. The analysis of scientific literature and current practical approaches indicates that the use of recombinant proteins is one of the key and most promising directions in the development of forensic medicine and criminalistics as a whole. Recombinant proteins, due to their precise genetic engineering and large-scale synthesis capabilities, provide high specificity, sensitivity, and reproducibility across a wide range of laboratory methods used for the analysis of biological traces. This is particularly relevant in forensic examinations, where the material under investigation is often limited in quantity, partially degraded, or contaminated, necessitating the use of the most efficient and reliable tools available.

This trend is confirmed by the growing number of studies and the implementation of new recombinant enzymes, antibodies, and biomolecular markers in practice, which significantly expand the possibilities for identification, typing, and quantitative analysis of biological materials. In addition to improving technical characteristics such as sensitivity and accuracy, recombinant proteins contribute to the standardization and optimization of forensic medical methods, which directly affects the quality and objectivity of expert conclusions. As a result, the integration of these biotechnological innovations contributes to significant progress in molecular forensics, opening new horizons for the development and advancement of forensic expert practice (Smith, 2021). These technologies make it possible to significantly increase the sensitivity and specificity of molecular genetic studies, reduce analysis time, and enable the processing of even minimal or severely degraded samples (Johnson et al., 2018; Kumar et al., 2020).

The use of modified DNA polymerases, recombinant antibodies, and CRISPR/Cas proteins opens new possibilities for the identification of biological traces and the development of rapid tests that can be applied directly at the crime

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scene (Nguyen & Lee, 2022; Zhang et al., 2021). Even today, the use of recombinant proteins in forensic medicine and criminalistics demonstrates a marked improvement in the effectiveness of crime detection, which is particularly important in situations where traditional methods of biological trace analysis prove insufficiently informative or impossible to apply. Modern biotechnological tools make it possible to detect and identify minute traces of DNA and proteins even under conditions of significant damage or contamination of samples, thereby greatly expanding the arsenal of expert tools and increasing the likelihood of obtaining reliable evidence.

In particular, the use of recombinant enzymes for the amplification of fragmented or degraded DNA, recombinant antibodies for the selective recognition of specific protein markers, and innovative molecular sensors opens new possibilities for the detection and verification of biological traces in complex forensic situations. This contributes to accelerating forensic investigations, improving the accuracy of identifying individuals and objects, and reducing the number of false positives and errors in expert examinations. Consequently, the integration of these advanced methods is already having a significant impact on the practice of criminal justice, enhancing the quality and reliability of forensic expert opinions and thereby promoting the fair resolution of criminal cases (Brown & Taylor, 2019).

However, the widespread adoption of recombinant protein-based technologies in forensic practice is constrained by several factors: the high cost of reagents, the need for specialized equipment, and the absence of standardized international protocols (Petrov et al., 2020). The legal aspect also remains important — the recognition of molecular genetic analysis results based on new technologies can vary significantly between countries (Miller, 2019; Garcia & Thompson, 2020).

For the successful development of this field, the following are necessary:1.Development of internationally recognized protocols and standards (Garcia & Thompson, 2020).

2.Reduction of reagent costs through large-scale production (Anderson, 2023).

3.Training of specialists to work with new molecular genetic systems (Smith, 2021).

4.Legal harmonization of procedures for the use of such methods in forensic medicine (Miller, 2019)

Given global trends, it can be predicted that within the next decade recombinant proteins will become a standard in forensic medical examination,

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integrating into portable real-time analysis systems and, in combination with artificial intelligence algorithms, providing faster, more accurate, and more reliable data interpretation (Anderson, 2023; Zhang et al., 2021).

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