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# DUAL USE APPLICATIONS OF MOLECULAR BIOLOGY METHODS: SECURITY PERSPECTIVES

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*SUMMARY: Molecular biology methods are frequently discussed in the context of their beneficial and harmful applications. To enhance the regulation of research in medical and biological sciences, the concept of dual-use technology has been introduced. This concept delineates a range of research activities that have the potential to yield products, scientific services, and technologies that, if misused, could pose risks to human health or the environment. Given the exponential advancement in the capabilities of molecular biology methods, this concept remains continuously active and subject to modification. Furthermore, with the integration of digitalization in managing vast datasets from molecular biology research and the incorporation of artificial intelligence, a new protective measure has been developed within the fourth (cyber) dimension of security, termed "cyberbiosecurity." This measure complements traditional biosecurity and biosafety frameworks. The objective of this study is to provide a comprehensive review of the latest capabilities, products, and scientific services of selected molecular biology methods through specific examples of their dual-use applications. By adopting this approach, the aim is to foster greater awareness within the scientific community and to contribute to a more precise definition of the dual-use concept.*

**Key words:** artificial intelligence, biosafety and biosecurity, cyberbiosecurity, dual-use applications, molecular biology methods

## INTRODUCTION

Molecular biology methods represent perhaps the best example of the rapid advancement of a scientific field within less than half a century. Through the lens of the BTWC (Biological and Toxin Weapons Convention), it has become evident that these methods can be applied for both beneficial and harmful purposes (Millett, 2010, Warmbrod et al., 2020).

To achieve better control and a clearer definition of the intentions, products, and services associated with knowledge in the broader life sciences, which encompass these methods, it has been

necessary to introduce concepts and mechanisms at an operational level to address this duality. This has led to the adoption of the term "dual-use technology" (Ashcheulova, Ambrosova, 2021, Beard et al., 2023).

The concept of "dual-use technology" refers to technologies that have legitimate civilian or scientific applications but can also be misused for harmful purposes. In the life sciences, this includes the development of biological weapons or other products derived from molecular biology methods that have intentional or unintentional harmful consequences for humans or ecosystems (Ashcheulova, Ambrosova, 2021, Novossiolova, Perkins, 2021, DiEuliis, Giordano, 2018).

Currently, three main directions have been identified in the development of molecular bio-

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logy methods: DNA reading, writing, and editing. Thanks to NGS (Next-Generation Sequencing) methodologies and the PCR (Polymerase chain reaction) tool, it has become possible to read extensive DNA sequences. CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) technology, combined with synthetic biology, has made significant advancements in DNA editing, while synthetic biology focuses on creating new constructs and innovations, supported by artificial intelligence.

DNA reading, writing, and editing have a direct impact on five distinct areas of life sciences: immunology, neuroscience, reproductive technologies, animal and plant agriculture, and infectious diseases. Research and development in these fields are predominantly conducted for peaceful purposes. However, these same research areas raise numerous ethical, legal, and security concerns, including fears that advancements might lead to new forms of biological weapons with different and potentially more harmful effects than those of the past (*Warmbrod et al., 2020, Mao et al., 2021, Beard et al., 2023, Redman et al., 2016, Li et al., 2023*).

Contemporary global trends emphasize the integration and combination of technologies from various fields to benefit society and the economy. AI (artificial intelligence) has entered a new dimension of shaping the life sciences. On the other hand, scientific efforts to harness the full potential and tools of informatics and AI in life sciences can have adverse consequences for humans and ecosystems. These consequences may arise from insufficient process control, unclear AI algorithms, a lack of trained personnel, or the development of personnel with unethical or questionable intentions (*Anyanwu et al., 2024, Russell, Norvig, 2022, Tugui, Zelinschi, 2024, Mukherji, Oudenaarden, 2009*).

Despite evident advantages and good intentions, measures for biosafety and biosecurity are continuously reassessed and revised. The advent of communication-informatics tools has introduced the fourth dimension of space—cyberspace. Existing biosafety and biosecurity measures are insufficient to protect this fourth dimension, leading to the development of a new field termed "cyberbiosecurity" (*Elgabry, 2023, Liu et al., 2024, Shankar et al., 2024*).

The aim of this work is to provide an up-to-date review of the capabilities, products, and scientific services of certain molecular biology methods through specific examples of their dual-use applications. Additionally, it synthesizes the latest data on the capabilities of molecular biology methods, the integration of AI into life sciences, the associated security challenges, and their ethical implications. Through this approach, the intention is to contribute to greater awareness within the scientific community and to facilitate a more precise definition of the framework for dual-use applications.

## LIFE SCIENCE AND DUAL USE RESEARCH CONCERN

Life sciences, particularly molecular biology methods, are advancing at such a rapid pace that legislative regulations are alarmingly lagging behind. Significant leaps in these scientific fields have occurred with the implementation of digital technologies in research and the introduction of artificial intelligence (AI). Since the mid-20th century, it has been recognized that molecular biology methods can be used for both beneficial and harmful purposes. To improve oversight and define the intent, products, and knowledge-based services, one of the most critical prerequisites was the introduction of the concept of Dual Use Research of Concern (DURC) in medical and biological sciences, which occurred in 2015.

DURC delineates a range of research activities that could potentially yield products, scientific services, or technologies whose misuse might harm humans or the environment. Given the exponential advancement of molecular biology methods, this concept remains dynamic and subject to ongoing refinement. Considering the current capabilities of molecular biology methods, this represents an essential step for the international community to minimize the potential for destructive misuse of biomedical research while simultaneously raising awareness and fostering ethical and moral responsibility (*Moritz, 2022, Ashcheulova, Ambrosova, 2021, Beard et al., 2023, Guraiib et al., 2024*).

The majority of issues surrounding biological safety relate to dual-use technology. However,

from a legal perspective, dual-use refers to both civilian and military applications. In the context of military applications, this primarily pertains to biological weapons, which are prohibited under the BTWC of 1975. Since the Convention lacks verification mechanisms, measures to build trust, including biosafety and biosecurity, have been developed within the framework of biological safety (*Novossiolova, Perkins, 2021, DiEuliis, Giordano, 2018, Trump et al., 2023, Millett, 2010*).

Current debates surrounding the development of biological weapons often center on methods and tools for the gain-of-function of a given (micro)organism. While gain-of-function research can have beneficial applications, these methods and tools can also "weaponize" benign natural microorganisms—whether of chimeric, peptide-based, nucleotide-based, or combined origin—transforming them into biological weapons (*Berche, 2023, Beard et al., 2023*).

The capability of gain-of-function research has already been demonstrated through experiments on potentially epidemic pathogens, such as the avian influenza Myxovirus influenzae and coronaviruses responsible for SARS and MERS. The origin of SARS-CoV-2 remains a mystery, with natural mutation and the possibility of an artificially engineered "biological agent" both under scrutiny. Supporting the latter hypothesis are findings that primary isolates are highly genetically homogeneous, differing by only two nucleotides and showing no evidence of adaptive mutations. Furthermore, the Spike protein, a major virulence factor, contains a furin cleavage site not observed in any other known sarbecovirus. Another critical point is the absence of an identified intermediate host, unlike the outbreaks of SARS and MERS. These factors strongly suggest the possibility of intentional misuse (*Beard et al., 2023, Novossiolova, Perkins, 2021, Vennis et al., 2021, Berche, 2023*).

Beyond this approach, primarily employed for monitoring armament and military scientific potential, dual-use concerns in life sciences are defined much more broadly. Dual-use research is described as "research conducted for legitimate scientific purposes, the results of which could be misused to pose a threat to public or national security" (*Beard et al., 2023, Sandbrink, Koblentz, 2022*).

This broader framework extends beyond the intentional or unintentional creation of biological weapons or their components. It encompasses all molecular and genetic research on human cells, embryos, fetuses, adult humans, plants, and animals. These considerations are exceptionally complex and dynamic for two key reasons.

The first reason is the lack of consensus within the scientific community regarding the scope and direction of such research. The second is that, even with benevolent intentions, deficiencies in methods and strategies can lead to highly concerning outcomes, including the creation of harmful results, products, or organisms that may pose significant risks to human populations and ecosystems (*Beard et al., 2023, Uhlenhaut et al., 2013, Guraiib et al., 2024, Kropf, 2024, Trump et al., 2023*).

## CAPABILITIES AND POTENTIAL OF MOLECULAR BIOLOGY METHODS IN THE CONTEXT OF DUAL-USE APPLICATIONS

### CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)

CRISPR was first discovered serendipitously in 1987 by researcher Yoshizumi Ishino, who observed a peculiar 30-base pair pattern in the *E. coli* genome. This pattern, referred to as palindromes due to its symmetry (readable both forward and backward), became pivotal in understanding DNA repair mechanisms and the subsequent development of the CRISPR technique (*Farokhi et al., 2024*).

The CRISPR-Cas9 system, an ingeniously designed laboratory method introduced in 2012, mimics a natural genome-editing mechanism found in bacteria. This natural system arose from an evolutionary arms race between bacteria and bacteriophages, leading to the development of a bacterial immune defense mechanism critical for survival. Bacteria employ the Cas9 endonuclease to cleave both strands of invading DNA, initiating a repair process that can proceed via two pathways: 1. Non-homologous end joining (NHEJ) – Typically resulting in random DNA insertions or deletions,

## 2. Homology-directed repair (HDR) – Utilizing a homologous DNA template for precise edits.

The HDR pathway enables highly accurate genomic modifications, making it ideal for laboratory manipulations (*Beljouv, Brouns, 2024, Redman et al., 2016*).

Beyond its application in DNA and RNA processing, CRISPR technology has expanded to include protein editing. This was facilitated by the discovery of CRISPR-associated proteases (Craspases), which, upon activation by targeted RNA, cleave host proteins to induce various cellular effects. CRISPR-controlled proteases are considered promising tools for molecular applications, including biotechnology, molecular diagnostics, targeted protein cleavage, and bacterial detection (*Beljouv, Brouns, 2024, Jiang, Doudna 2017, Selvam et al., 2022*).

Due to the CRISPR-Cas9 method's ability to correct and repair genetic structures, its biomedical applications have boldly ventured into editing human genomic sequences at mutated loci. These loci are often associated with hereditary diseases such as muscular dystrophy, thalassemia, and various cancers. In cancer treatment, research focuses on manipulating oncogenes that drive cancer progression, tumor suppressor genes (TSGs) that regulate cell division, and enhancing the functionality of anti-tumor T cells in adaptive immunotherapy. CRISPR is envisioned as a tool that could potentially halt or slow cancer progression (*Farokhi et al., 2024, Ito et al., 2024, Tabibian et al., 2024, Zhan et al., 2019*).

Tumor cell genes, such as those in glioblastomas, have been successfully targeted using the CRISPR-Cas9 method (*Peixoto et al., 2023*). Additionally, research has expanded into the manipulation of stem cells by acting on key transcription factors critical for differentiation phases (*Kim et al., 2022*). However, a significant challenge remains the lack of effective strategies for delivering the CRISPR system to target diseased cells with precision (*Li et al., 2023*).

In the realm of genetic engineering for cognitive impairments, autism, schizophrenia, or bipolar disorder, the suppression of undesirable effects from genes like KCTD13 has been achieved thro-

ugh "knockout" techniques using induced pluripotent stem cells (iPSCs). This process exemplifies the dual-use potential of CRISPR applications, as essential regions of the human genome could potentially be disabled to induce disorders or diseases intentionally (*Kropf, 2024*).

Two specific issues have been identified within the scientific community that raise concerns regarding such research, modifications, and therapies. The first issue pertains to precision and potential off-target effects resulting from gene shifts within populations. This is not only detrimental to individual organisms but also introduces the possibility of transmitting harmful traits of such genes or sequences to other species within the ecosystem (*Farokhi et al., 2024, Jiang, Doudna, 2017*).

The second issue involves the design or editing of the human germline to achieve targeted traits in individuals, such as enhanced cognitive functions, athletic performance, artistic talents, and, most concerning, human capabilities relevant to the execution of military operations (*Farokhi et al., 2024, Mir Gani et al., 2022, DiEuliis, Giordano, 2018*).

Despite these concerns, research has already ventured into editing human embryos. For instance, genome editing was performed on twin girls known as the "CRISPR babies." Although there is no definitive evidence regarding which parts of their genome were altered or how these changes affected their phenotype, the lack of control over the targeted genomic actions is a critical issue. The direction of research into using CRISPR-Cas9 for embryo genome editing has sparked significant concern and represents a substantial leap in this methodology (*Mir Gani et al., 2022, Cyranosky, 2019*).

## NGS (Next-generation Sequencing) and NAT (Nucleic Acid Amplification Technologies)

One significant direction in the advancement of molecular biology methods is the development of technologies capable of rapid and efficient DNA sequence reading. NGS is a technology designed for this purpose and involves a structured process comprising four main steps to enable successful sequencing. NGS encompasses DNA fragmenta-

tion, library preparation, massive parallel sequencing, bioinformatic analysis, and annotation and interpretation of variants/mutations.

During DNA fragmentation, short DNA segments (100–300 bp) are generated using mechanical methods (e.g., ultrasonication), enzymes, or chemical treatments. Target DNA sequences of interest are isolated via hybridization capture assays using specific complementary probes. Another technique involves PCR amplification, wherein primers amplify the desired DNA segments through PCR.

Following amplification, the fragments undergo library selection, quantitative and qualitative library analyses, and sample dilution preparation for sequencing. Quantitative analysis is commonly performed using qPCR, which monitors the amplification process in real-time through fluorescent signals to measure DNA quantity.

The final step, massive parallel sequencing, can be conducted using sequencers like Illumina NGS, which employs flow cells, or Ion Torrent NGS, which utilizes semiconductor chips (*Qin, 2019, Kumar et al., 2019*). Despite variations in sequencer platforms, the ultimate goal of NGS remains consistent: enabling the simultaneous sequencing of all DNA segments in parallel.

Post-sequencing, the resulting sequence data are analyzed using bioinformatics software to generate actionable insights. NGS has proven instrumental in personalized precision medicine and the diagnosis of infectious diseases. Its applications extend beyond diagnostics to include mutation identification for targeted therapies and the identification of high-risk populations for specific hereditary cancers (*Qin, 2019, Kumar et al., 2019, Stenzinger et al., 2024, Nafea et al., 2024, Rodino, Simner, 2024*).

A key outcome of NGS is the ability to read and compare various genetic variants or mutations. This capability provides critical bioinformatic data, which, if misused, could serve harmful purposes, such as the development of race- or ethnicity-specific bioweapons. Consequently, NGS is categorized as a dual-use technology (*Beard et al., 2023*).

Conversely, NGS is also a powerful tool for identifying bacteria, fungi, and viruses, with applications in clinical practice and biomonitoring. Its speed and precision give it a significant advantage over traditional methods, making it a priority in the diagnosis, surveillance, and control of infectious diseases. However, this application also has a dual-use dimension. Data on microorganisms obtained via NGS can facilitate genetic manipulation using methods like CRISPR, enabling gain-of-function modifications that could result in the creation of bioweapons (*Beard et al., 2023, Berche, 2023*).

Beyond infectious disease diagnostics and environmental surveillance, NATTs also play a crucial role. For example, digital PCR (dPCR) utilizes innovative microfluidic crystal technology to achieve high throughput and sensitivity, providing precise quantification of nucleic acids. This precision allows for accurate mutation detection and pathogen identification (*Mirabile, et al., 2024*).

NATTs, fundamentally based on PCR combined with isothermal amplification, include several variants tailored to specific DNA or RNA targets. These variants are:

- LAMP (Loop-mediated isothermal amplification)
- RPA (Recombinase polymerase amplification)
- RCA (Rolling circle amplification)
- SDA (Strand displacement amplification)
- NASBA (Nucleic acid sequence-based amplification)

Like NGS, NATTs are integral to nucleic acid analysis, with applications spanning infectious disease diagnostics, tumor etiology and diagnosis, genetic disease identification, and personalized therapeutics. However, NATTs have limitations, including false-positive and false-negative results, which could compromise their reliability.

To enhance their functionality, NATTs can be combined with advanced technologies such as CRISPR and synthetic biology, further broadening their scope but also raising additional dual-use concerns (*Wang, et al., 2023, Wang et al., 2024*).

## Synthetic Biology and AI

Synthetic biology represents the human endeavor to create new genetic systems, cells, and organisms or to modify existing ones by leveraging biological principles and mechanisms. The methods and tools employed in synthetic biology mimic or modify natural mechanisms, enabling a broad spectrum of applications—from designing synthetic gene expression promoters to controlling cellular, tissue, and organ communication (DiEuliis, Giordano, 2018, Mukherji, Oudenaarden, 2009, Vučemić, 2024).

A review of literature and findings published between 2013 and 2023 highlights synthetic biology's significant innovations in medical therapy through the integration of engineering principles with biological system insights. This progress has driven advancements in drug development, metabolic pathway exploration, targeted therapies, and personalized treatment approaches (Mao et al., 2021, Tan et al., 2021, Udegbe et al., 2024).

One promising avenue in synthetic biology is the development of antitumor microbial vaccines, which exhibit unique properties. Microorganisms engineered through synthetic biology are better adapted to the tumor microenvironment compared to wild-type strains. These microorganisms accumulate and proliferate within tumors, potentially weakening or reversing the functions of immune-suppressive cells and enhancing the presentation of tumor antigens. Such interventions could stimulate innate and adaptive antitumor immune responses, making synthetic biology a pivotal tool in cancer vaccine research (Tan et al., 2021, Tan et al., 2024).

Despite these promising developments, time will be needed to evaluate their safety, efficacy, and therapeutic utility.

Synthetic biology's rapid development is bolstered by tools from artificial intelligence (AI), particularly in optimizing processes and analyzing large datasets. A notable contribution comes from ML („machine learning“), a branch of AI focused on developing algorithms and models that allow computers to learn from collected data and make predictions, decisions, or forecasts. ML operates through steps including data collection,

processing, model training, evaluation, and deployment. However, biases in data can lead to incorrect, harmful, or unjust outcomes, raising significant ethical concerns (Anyanwu et al., 2024, Beard et al., 2023, Goshisht, 2024, Kriegman et al., 2021).

AI tools have proven highly effective in analyzing and comparing vast DNA sequence datasets, significantly reducing research time (Russell, Norvig, 2022). Their greatest contributions are in the creation of artificial biological constructs. For instance, AI tools can predict the functionality of newly designed DNA sequences or the functionality of their peptide products. AI-powered protein engineering tools, such as AlphaFold, enable accurate predictions of protein 3D structures. Practical applications in DNA analysis or message decoding often involve thousands or even millions of steps (Anyanwu et al., 2024, Russell, Norvig, 2022, Tugui, Zelinschi, 2024).

Recent advancements also highlight dialogues between researchers and ChatGPT-4, leveraging its capabilities to discuss synthetic humans and humanoid robots. These discussions analyzed potential applications, challenges, and ethical implications of synthetic biology's influence on human modification, augmentation, and its unintended consequences. ChatGPT-4 synthesized the following areas of synthetic biology's impact:

1. Gene therapy and genetic engineering
2. Regenerative medicine
3. Neuroscience and brain-computer interfaces
4. Personalized medicine
5. Development of novel biomaterials for construction and textiles
6. Environmental bioremediation and terraforming
7. Creation of synthetic ecosystems, including extraterrestrial ecosystems and space colonization (Tugui, Zelinschi, 2024, Jones et al., 2024).

On the darker side, synthetic biology and AI tools can also pose significant risks in the realm of biological weaponry. Constructs such as chimeras, plasmids, and transposons, designed using

synthetic biology and AI, can weaponize harmless microorganisms, evade detection systems, and cause damage akin to weapons of mass destruction. The creation of new biological forms and organisms using synthesized sequences represents a slippery slope, with potential for both beneficial and harmful outcomes, whether intentional or accidental (*Berche, 2023, Kropf, 2024, Sandbrink, Koblentz, 2022, Sanz et al., 2022, Smith et al., 2023*).

## CYBERBIOSECURITY

The introduction of the Internet into the life sciences has facilitated faster sharing of knowledge and skills, which has led to an acceleration of research. However, the same internet technologies and tools that have strongly supported research have also created various forms of crime, as security measures have not adequately kept pace with their development. The implementation of legal frameworks, which mainly focus on assessing security risks and providing guidelines, cannot offer satisfactory results. As a result, criminology, in studying crime perspectives, has assisted the life sciences in designing security measures and combating criminal activities (*Elgabry, 2023*).

The development of molecular biology methods follows three main directions: reading, writing, and editing DNA. The capacities and capabilities for these tasks have grown so large that computer support is now essential for sequencing, comparing, processing, transferring, or storing large amounts of data (*Warmbrod et al., 2020*).

From a biosafety perspective, these systems are vulnerable, primarily because the DNA molecule itself is an informational medium. The steps involved in creating, manipulating, processing, and analyzing DNA are prone to attacks similar to those in conventional information technology and computing. DNA can encode or store malicious programs, such as computer “viruses” or “worms,” when a “contaminated” DNA sequence is inserted. These malicious programs can remotely compromise the connected systems and networks. This can be achieved by attacking the sequencing process using synthetic DNA that

contains a malicious program, creating a software vulnerability. Once the synthetic DNA containing a malicious program is sequenced using NGS and processed, the malicious program is triggered as an application and compromises the computational analysis system (*Liu et al., 2024*).

Cyberbiosecurity encompasses a set of complex protective measures and is based on a multidisciplinary approach, integrating security engineering capabilities, physical security, privacy, infrastructure resilience, and security psychology (*Mueller, 2021*).

Cyberbiosecurity is founded on the assumption that cyberbiological attacks are very similar to conventional cyberattacks, although they are limited by the use of DNA as an informational medium. Accordingly, the key measures implemented in cyberbiosecurity include: 1) security audits and software updates, 2) control of software package authenticity, 3) security of DNA samples for sequencing, which must be ensured from collection to sequencing, and 4) verification of whether synthetic sequences can be decoded into malicious codes during and before synthesis. Furthermore, control of DNA synthesis programs must be maintained to prevent the synthesis of DNA that could yield various pathogens, chimeras, toxins, or other components that could then be used as biological weapons (*Liu et al., 2024, Shankar et al., 2024*).

## DISCUSSION AND CONCLUSIONS

This review article presents a synthesis of the capabilities, products, and scientific services of the most significant molecular biology methods, based on the latest literature, through concrete examples of their dual-use applications. By synthesizing scientific data from the literature, a broad spectrum of applications for these methods is covered, ranging from beneficial human uses in cancer treatment to harmful and monstrous applications in the creation of new synthetic organisms and fifth-generation biological weapons. In addition, to complement the scientific data, well-founded predictions from literature references have been used to more concretely define the future

perspectives of these methods. In this context, AI plays a crucial role, not only as a source of information but also due to its ability to synthesize large amounts of data, which can provide a broader picture and clearer future perspectives for humanity. In this regard, AI undoubtedly has its clearly beneficial role.

It is evident that these methods and the accompanying technologies will develop in three main directions: reading, writing, and editing DNA (Warmbrod et al., 2020, Li et al., 2023, Ashcheulova, Ambrosova, 2021, Guraiib et al., 2024).

Artificial intelligence, with its capabilities and tools and the built algorithms, has been implemented in all three directions. As seen from the data synthesis in Tables 1 and 2, all three directions have found their dual-use applications. In addition to these three directions, there are also three main targets for harmful purposes: the creation of new biological weapons, the synthetic human, and unforeseen mutations in biomedical research, as well as ecosystem modifications (including plants and animals) (Anyanwu et al., 2024, Beard et al., 2023, DiEuliis, Giordano, 2018, Gillum, 2024, Russell, Norvig 2022, Trump et al., 2023).

**Table 1. Overview of capabilities, products, and scientific services of molecular biology methods in human research and biomedicine**

**Tablica 1. Pregled mogućnosti, proizvoda i znanstvenih usluga metoda molekularne biologije u istraživanjima ljudi i biomedicini**

CAPABILITIES	METHODS	PRODUCTS	PURPOSE	REFERENCES
DNA reading	NGS, PCR	Mass sequencing, high-throughput DNA sequencing. DNA Amplification: Large-scale amplification of DNA.	Personalized Medicine: Diagnostics and identification of mutations for targeted therapy. Microbiology and environmental revitalization. Pathogen detection and identification: Applications in both peacetime and military operations.	<i>Qin, 2019, Kumar et al., 2019, Nafea et al., 2024, Rodino, Simner 2024, Mirabile, et al., 2024, Vučemilović, Volf, 2019.</i>
DNA writing	SYNT BIO + AI	Creation and synthesis of entirely new genetic systems and synthetic biomolecules.	Development of novel drugs, exploration of metabolic pathways, and targeted therapies. Personalized approaches to treatment.	<i>Mao et al., 2021, Tan et al., 2021, Udegbe et al., 2024, Mukherji, Oudenaarden, 2009.</i>
DNA editing	CRISPR, Craspase	Targeted protein cleavage, DNA sequence editing, and mutation detection. Integration of "knockout genes."	Applications in molecular diagnostics, cancer research, and inherited diseases designing stem cell lines and applications. Bacterial detection.	<i>Beljouv, Brouns, 2024, Jiang, Doudna 2017, Selvam et al., 2022, Redman et al., 2016, Farokhi et al., 2024, Ito et al., 2024, Tabibian et al., 2024, Zhan et al., 2019, Peixoto et al., 2023, Kim et al., 2022, Li et al., 2023., Kropf, 2024.</i>

NGS - Next-Generation Sequencing, PCR - Polymerase Chain Reaction, SYNT BIO - synthetic biology, AI - artificial intelligence, CRISPR - Clustered Regularly Interspaced Short Palindromic Repeats

**Table 2. Overview of capabilities, products, and scientific services of molecular biology methods in dual use and military use applications****Tablica 2. Pregled mogućnosti, proizvoda i znanstvenih usluga metoda molekularne biologije u dvojnoj namjeni i vojnoj primjeni**

CAPABILITIES	METHODS	DUAL USE & MILITARY USE APPLICATIONS	PURPOSE	REFERENCES
DNA reading	NGS, PCR	Mass sequencing, high-throughput DNA sequencing. DNA Amplification: Large-scale amplification of DNA. Identification of targeted genes within populations and ethnic race.	Development of biological and ethnic weapons.	<i>Beard et al., 2023, Berche, 2023, Nafea et al., 2024, Rodino, Simner 2024, Vučemilović, Volf, 2019,</i>
DNA writing	SYNT BIO + AI	Synthesis of entirely new genetic systems, synthetic biomolecules, and organisms. <i>De novo</i> synthesis of viruses, peptides, plasmids, transposons, and chimeras.	Development of biological and toxic weapons. Modification of abilities and functions in humans, animals, and plants. Design of synthetic ecosystems.	<i>Beard et al., 2023, Berche, 2023, Novossiolova, Perkins, 2021, Vennis et al., 2021, Kropf, 2024, Sandbrink, Koblentz, 2022, Sanz et al., 2022, Smith et al., 2023, Koblentz, 2022, Vučemilović, Volf, 2019.</i>
DNA editing	CRISPR, Craspase	Targeted protein cleavage, DNA sequence editing, and mutatio detection. Integration of "knockout genes." Creating "gain-of-function" modifications.	Development of pathogens, biological, toxic, and ethnic weapons. „Weaponizing" non-pathogenic microorganisms. Design of human embryos— CRISPR babies.	<i>Kropf, 2024, Beard et al., 2023, Jiang, Doudna 2017, Farokhi et al., 2024, Mir Gani et al., 2022, DiEuliis, Giordano, 2018, Cyranosky, 2019.</i>

NGS - Next-Generation Sequencing, PCR - Polymerase Chain Reaction, SYNT BIO - synthetic biology, AI - artificial intelligence, CRISPR - Clustered Regularly Interspaced Short Palindromic Repeats

One of the key concerns is the ability to create new DNA constructs that can generate (functional) synthetic cells, microorganisms, and multicellular organisms, which can have irreversible consequences for humanity and disrupt entire ecosystems. For example, under highly controlled laboratory conditions, scientists have recreated previously extinct pathogens (e.g., horse pox and the 1918 pandemic flu) from commercial DNA fragments, demonstrating the potential to recreate any virus solely from genomic information (*Sanz et al., 2022*).

Scientists are capable of synthesizing and likely using as weapons various endogenous modifiers of biological responses, including cytokines, hormones, neurotransmitters, and plasma proteases. New natural infections that can cause major human diseases and death continuously emerge worldwide, and it is possible that these pathogens could also be used as weapons (*Beard et al., 2023, Berg, Kapler, 2024*).

For the production of "synthetic products," synthetic biology can employ various tools and techniques. One of these is the use of light signals of different frequencies to induce gene expression or the transport of various molecules based on the principles and knowledge gained from optogenetics. This is currently being successfully researched on microorganisms, with concrete results being observed (*Mazraeh, Ventura, 2022, Choi et al., 2022, Smith et al., 2023*).

In addition to new DNA constructs, the threat of gain of function poses a significant concern, as it offers a wide range of possibilities for "arming" non-pathogenic microorganisms, converting them into a new form of biological weapon that can bypass modern detection, diagnostic, and protection systems, similar to "stealth technology" (*Berche, 2023, Li et al., 2023, Vučemilović, Volf, 2019*).

In this perspective, it is important to understand that it is not beneficial to propose a ban on all experiments defined as "gain of function." Such an action would exclude a large part of microbiological research, which is essential for understanding pathogenesis and disease and for the development of new therapeutic agents. Instead, the focus should be on creating and implementing robust risk assessment methodologies and ensuring the enforce-

ment of appropriate biosafety measures. Technology management measures can further reduce barriers that facilitate directed evolution (*Beard et al., 2023, Trump et al., 2023*).

On the other hand, control and security measures may struggle to keep pace with the speed of discoveries in research and methodologies for obtaining both harmful and beneficial products and scientific services. Due to the digitalization of all processes and data in biomedical research, traditional biosafety and biosecurity measures are no longer sufficient to ensure security. Consequently, the concept of "cyberbiosecurity" has been introduced and further developed. In general, cyber warfare is considered the fourth dimension of warfare, alongside land, sea, and air as the three traditional domains. Cyber warfare has significantly transformed the structure of intelligence data, the definition of the theater of military operations, and, consequently, defense doctrines. Analogously, cyber attacks in the field of biomedical sciences have necessitated a defensive response in the form of measures defined as "cyberbiosecurity", albeit only after the first incidents of this kind had occurred. Undoubtedly, cyber attacks targeting biomedicine will induce substantial changes in the near future, similar to the impact that cyber warfare has had on contemporary military conflicts (*Liu et al., 2024, Shankar et al., 2024, Mueller, 2021*).

In this context, it is crucial to develop counterterrorism and intelligence measures for monitoring both state and non-state actors. The advancements in digitalization and the availability of data have facilitated the adoption of NGS, CRISPR, and other methods and technologies in numerous laboratories worldwide, creating vast amounts of genomic data immediately available for systematic research. Using publicly available data, a recent study utilized cloud computing resources to identify over 100,000 new RNA viruses, highlighting the availability and scale at which new pathogens can be detected (*Sanz et al., 2022, Vučemilović, Volf, 2019*).

Therefore, through such a comprehensive approach, the intention is to contribute to greater awareness within the scientific community and a more concrete definition of the concept of dual-use applications.

## Abbreviations

AI - artificial intelligence  
 BTWC - Biological and Toxin Weapons Convention  
 ChatGPT – Chat Generative Pre-trained Transformer  
 CRISPR - Clustered Regularly Interspaced Short Palindromic Repeats  
 DNA - deoxyribonucleic acid  
 dPCR - digital PCR  
 DURC - Dual Use Research of Concern  
 HDR - Homology-directed repair  
 iPSCs - induced pluripotent stem cells  
 LAMP - Loop-mediated isothermal amplification  
 MERS - Middle East Respiratory Syndrome  
 ML - machine learning  
 NASBA - Nucleic acid sequence-based amplification  
 NATT - Nucleic Acid Amplification Technologies  
 NGS - Next-Generation Sequencing  
 NHEJ - Non-homologous end joining  
 PCR - Polymerase chain reaction  
 qPCR – quantitative PCR  
 RCA - Rolling circle amplification  
 RNA - ribonucleic acid  
 RPA - Recombinase polymerase amplification  
 SARS - Severe Acute Respiratory Syndrome  
 SDA - Strand displacement amplification  
 SYNT BIO - synthetic biology  
 TSGs - tumor suppressor genes

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## **DVOJAKOST APLIKACIJA IZ METODA MOLEKULARNE BIOLOGIJE: ASPEKTI SIGURNOSTI**

*SAŽETAK: Metode molekularne biologije često se spominju u kontekstu njihovih korisnih i štetnih aplikacija. Kako bi se poboljšala regulacija istraživanja u medicinskim i biološkim znanostima, uveden je koncept tehnologije s dvojnomo uporabom. Ovaj koncept navodi razne istraživačke radnje koje potencijalno mogu pružiti rezultate, znanstvene usluge i tehnologije koje, ako se pogrešno koriste, mogu postati rizični za zdravlje ljudi i okoliša. S obzirom na eksponencijalni napredak mogućnosti metoda molekularne biologije, ovaj koncept ostaje trajno aktivan i predmet je modifikacije. Nadalje, uz integriranje digitalizacije u upravljanju ogromnim količinama podataka pri istraživanjima u molekularnoj biologiji i uporabom umjetne inteligencije, razvijena je nova zaštitna mjera unutar četvrte (cyber) dimenzije sigurnosti, nazvana "cyberbiosecurity". Mjera je dopuna tradicionalnoj biosigurnosti i biozaštiti. Cilj je istraživanja ponuditi sveobuhvatan pregled najnovijih mogućnosti, proizvoda i znanstvenih usluga odabranih metoda molekularne biologije kroz specifične primjere njihovih dvojakih aplikacija. Ovim pristupom cilj je podići svijest kod znanstvene zajednice i pridonijeti preciznijem definiranju koncepta dvojake uporabe.*

**Ključne riječi:** *umjetna inteligencija, biosigurnost, biozaštita, aplikacije s dvojakom uporabom, metode molekularne biologije*

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