# TRANSFORMATIVE INNOVATIONS DRIVEN BY RECOMBINANT DNA RESEARCH

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## Contents

# RECOMBINANT DNA TECHNOLOGY : *Agrobacterium tumefaciens*-MEDIATED GENE TRANSFER METHODS IN PLANTS

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## Introduction

Today, humanity is affected by a wide range of factors, including food scarcity, disruption of natural balance, loss of biodiversity, and ecological crises. As a result of these challenges, people are confronted with numerous health-related problems. Among the most critical societal issues threatening humanity are food shortages and the unsustainable exploitation of natural resources (Bayramoğlu, Tekin & Ağızan, 2018). The continuous growth of the human population, coupled with the declining interest in agriculture, has led to insufficient food resources. Meeting the increasing demand in an affordable and safe manner has become a fundamental objective of nations. Food resources constitute the most essential factor for sustaining life, and beyond ensuring their availability, the processes of production, proper processing, and appropriate storage are also of great importance (Bass

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& ark., 2022; Demirel, Akveç & Can, 2022). In direct proportion to population growth, food prices have also risen, and since equal access to produced food resources cannot be guaranteed across all societies, a globally uneven distribution has emerged, thereby becoming a major issue (DaMatta & ark., 2010; Demirel, Akveç & Can, 2022; Hanjra & Qureshi, 2010; Premanandh, 2011).

Considering all these challenges, the application of modern technological methods is of critical importance in addressing the increasing demand for food resources (Khan & ark., 2016). Consequently, the production of essential agricultural commodities, with an emphasis on public health and under the principle of "adequate and balanced nutrition," forms the foundation of agricultural policies, aiming to yield high-productivity and nutritionally rich crops (Ranabhatt & Kapor, 2017). Alongside traditional agricultural practices, innovative agricultural approaches have introduced biotechnological methods to cultivate greater quantities of crops with higher efficiency and quality for humanity. Plant biotechnology, in addition to classical techniques, employs modern biotechnological advancements such as tissue culture and molecular methods to both improve quality and yield while simultaneously protecting plants against diseases and pests (Kayın & Turan, 2023).

Recombinant DNA technology stands at the forefront of groundbreaking techniques in plant biotechnology. To construct recombinant DNA molecules, the term "clone," derived from the Ancient Greek word meaning "twig or branch," was originally used to describe the vegetative generation of a new plant from a single branch. Today, however, it refers to genetically identical copies at both the molecular and cellular/organismal levels, encompassing cell lines derived from a single cell or genetically similar organisms (Ashwini & ark., 2016). Clones obtained through recombinant DNA technology are subsequently isolated, identified, and purified for utilization across

multiple fields, including agriculture, public health, gene therapy, environmental science and pollution studies, clinical pharmacy, hormone development, and vaccines (Khan & ark., 2016).

The process by which a DNA sequence is isolated, replicated without any modification to the original sequence, transferred into a vector, and subsequently monitored through appropriate procedures to obtain identical copies is known as gene cloning or molecular cloning (Ashwini & ark., 2016). The genome constitutes the complete set of DNA within an organism, and recombinant DNA technology represents a technique that enables the selection and large-scale production of a single gene from the genome of a living organism (Klug, Cummings & Spencer, 2009). With the advent of recombinant DNA technology, microorganisms, animals, and plants have been modified to produce medically beneficial substances, thereby facilitating the development of therapeutic products (Galambos & Sturchio, 1998; Steinberg & Raso, 1998).

**Recombinant DNA Technology**

Recombinant DNA technology is a fundamental genetic engineering method that has extensive applications in the field of molecular biology. The genetic basis of recombinant DNA technology is recombination, which is the exchange of DNA fragments between regions of high homology that would not normally coexist within the natural flow of DNA molecules. The greatest advantage of this technology is the ability to obtain a large number of copies of specific target DNA sequences from complex genomes, such as the human genome. The first essential step in this technique is the isolation of the DNA molecule to be amplified from the targeted tissue, organ, or cells. DNA samples that have been isolated are digested with restriction endonucleases to generate DNA fragments, which are then combined with suitable vectors to produce recombinant DNA molecules. A DNA fragment ligated into another DNA molecule is transferred into a host

cell, where it can be replicated to produce multiple copies. With the replication of the host cell, numerous copies of the recombinant DNA molecule are also generated (Klug, Cummings & Spencer, 2009). The application steps of classical recombinant DNA technology can be summarized under the following headings.

**Isolation of the DNA Molecule to be Cloned**

In eukaryotic organisms, DNA is packaged within chromosomes by being wrapped around positively charged proteins known as histones, owing to the negative charges carried by the phosphate groups of DNA. The isolation of DNA constitutes the first step of recombinant DNA technology. At this stage, the primary goal is to obtain DNA in a clean and high-quality form by separating it from proteins and other cellular components with which it is packaged in chromosomes. This process is referred to as DNA extraction. The extraction phase essentially comprises three steps. The first step involves the release of DNA located in the nucleus using mechanical (e.g., mortar, homogenizer) and enzymatic (e.g., SDS, CTAB, proteinase K) methods. The second step consists of the precipitation of DNA using alcohol (ethanol, isopropanol), whereby high-molecular-weight DNA molecules, once released, are fully separated from other cellular components and rendered soluble. The third step is the purification phase, in which the integrity of the DNA is preserved and it is obtained in pure form, free from contamination, and in high yield (Kalendar, Boronnikova & Seppänen, 2021).

**Cleavage of the DNA Molecule with Restriction Enzymes**

Restriction enzymes are naturally produced by bacteria as part of their defense mechanisms (Lüleyap, 2008). These enzymes have the ability to bind to double-stranded DNA and cleave it specifically at recognition sites within the sugar-phosphate backbone. Restriction enzymes are classified into three groups (Type I, Type II, Type III). Among these, Type II enzymes are highly specific and cleave DNA at

defined recognition sequences. Some of them perform asymmetric cuts, generating sticky ends, while others produce blunt ends. Restriction enzymes are generally named using a three-letter abbreviation: the first letter denotes the genus, and the second and third letters represent the species from which the enzyme was isolated (Boyer, 1971; Carter & ark., 2022; Lüleyap, 2008; Meselson, Yuan & Heywood, 1972; Klug, Cummings & Spencer, 2009) .

**Ligation of DNA Molecules Cleaved by Restriction Enzymes with Suitable Vectors**

DNA molecules cleaved by restriction enzymes cannot directly enter a host cell. Instead, they are introduced into laboratory-designed DNA molecules called vectors, which are capable of carrying and replicating the inserted DNA fragments. In general, desirable characteristics of cloning vectors include small size, ease of isolation, ability to replicate both themselves and the inserted DNA fragments, the presence of a multiple cloning site (MCS), selectable markers (e.g., antibiotic resistance genes), and structural stability. Types of vectors include plasmids, bacteriophages, cosmid vectors, bacterial artificial chromosomes (BACs), yeast artificial chromosomes (YACs), mammalian artificial chromosomes (MACs), and human artificial chromosomes. In plants, the most commonly used vector is the natural Ti plasmid of *Agrobacterium tumefaciens*, a Gram-negative soil bacterium. For the ligation of DNA molecules cleaved by restriction enzymes with suitable vectors, the ligation step is essential. Sticky-end ligation is efficiently carried out by T4 DNA ligase, whereas blunt-end ligation can also be performed by DNA ligase but with lower efficiency. To improve efficiency, linkers/adapters may be added to the ends or blunt ends can be converted into sticky ends. Terminal deoxynucleotidyl transferase (TdT) can be used to randomly add nucleotides to blunt ends, although it does not catalyze the ligation

reaction itself (Carter & ark., 2022; Lüleyap, 2008; Klug, Cummings & Spencer, 2009)).

**Transfer and Cloning of Recombinant DNA into Host Cells**

Once the recombinant DNA molecule is obtained, it must be introduced into a host organism to generate multiple copies. The most commonly used host cell is *E. coli*. If the vector is a plasmid, the process of introducing it into the host cell is called transformation, whereas if the vector is a virus (bacteriophage), the process is referred to as transfection (Yin & Stotzky, 1997). There are several methods for delivering recombinant DNA into host organisms, including electroporation, microinjection, gene gun technology, and polyethylene glycol treatment (Demirel, Akveç & Can, 2022). Among these, electroporation is the most widely employed technique. It involves the application of a high-intensity electrical field to cells for a short period, thereby enabling the genetic material (DNA) to enter the cell. Electroporation is frequently used in molecular biology studies as an efficient gene transfer method (Finer & Dhillon, 2008).

**Isolation and Analysis of Cloned DNA from Progeny Host Cells**

As the host cell replicates itself, the vector also undergoes replication, thereby producing multiple clones. To determine whether the recombinant DNA molecule has been successfully formed, recombinant screening is conducted. Bacterial colonies are cultured on a medium containing ampicillin, X-Gal, and IPTG (isopropyl β-D-1-thiogalactopyranoside). The lacZ gene encodes the β-galactosidase enzyme, and the DNA fragment intended for cloning is inserted into the multiple cloning site (MCS) located within this gene. Blue-colored colonies arise when β-galactosidase cleaves X-Gal, indicating that the lacZ gene remains intact. However, if DNA has been successfully inserted into the lacZ gene, the gene is disrupted, preventing the synthesis of β-galactosidase. As a result, X-Gal cannot be cleaved, and the colonies appear white.

It is important to note that not every white colony necessarily contains the insert. To confirm the presence of the insert, further verification must be performed using agarose gel electrophoresis or PCR (Green & Sambrook, 2019).

**Recombinant DNA Technology in Plants**

Türkiye is among the countries that are particularly rich in terms of natural habitats and plant diversity. With the advancement of molecular-level studies, extensive research has been initiated on nuclear, chloroplast, and mitochondrial genomes for plant systematics (Doğan, 2007) . Classical gene cloning methods are frequently employed in the production of hormones and enzymes. Through gene transfer, plants with desired traits are developed using plant tissue culture techniques and genetic manipulation methods, resulting in the production of transgenic plants (Low & ark., 2018). To improve plant quality and yield, molecular techniques are increasingly integrated into plant breeding, enabling the transfer of genes critical for resistance to climatic conditions, pesticides, and diseases. Consequently, the use of transgenes has become an indispensable strategy in contemporary agricultural production (Demirel, Akveç & Can, 2022; Ranabhatt & Kapor, 2017). Gene transfer in plants is carried out in two principal ways: direct gene transfer and vector-mediated gene transfer (Demirel, Akveç & Can, 2022).

**Vector-Mediated Gene Transfer (*Agrobacterium tumefaciens*)**

Today, *Agrobacterium tumefaciens*-mediated gene transfer is the most widely preferred method of genetic transformation, as it minimizes the transfer of undesirable plasmid DNA fragments and enables the transfer of only the target genes (Anayol, 2014). For gene transfer in plants, a bacterial plasmid carried by the soil bacterium *A. tumefaciens* is used as the vector. *Agrobacterium*-mediated transformation was first employed in 1983 (Nand, 2018). *A. tumefaciens* is a plant pathogen capable of delivering a DNA segment

carried on a large plasmid, known as the tumor-inducing or Ti plasmid, into plant cells (Khan & ark., 2016). *A. tumefaciens* belongs to the class Alphaproteobacteria, and its replicons range in size from ~100 kb to approximately 2 Mb, typically encoding functions essential for cell physiology, pathogenesis, or symbiosis. Most of these elements rely on a conserved gene cassette called repABC for replication and partitioning, and they are maintained in only one or a few copies per cell (Pinto, Pappas & Winans, 2012). Although all pathogenic *Agrobacterium* species carry megaplasmids, non-pathogenic strains either completely lack these plasmids or contain mutant forms. Ti plasmids cause a disease known as crown gall, which is characterized by the formation of plant tumors. Within the T-DNA region, Ti plasmids carry genes responsible for opine synthesis, whereas genes responsible for opine catabolism are located outside the T-DNA in other plasmid regions; thus, they are not transferred to the plant genome. Moreover, *Agrobacterium* strains carrying Ti plasmids not only induce the formation of plant tumors but also stimulate the production of a variety of amino acid and sugar-phosphate derivatives called opines. Transformed plant cells secrete these opines, which are subsequently taken up and metabolized by the infecting bacteria as nutrient sources.

The activation of cytokinin- and auxin-synthesizing genes within the T-DNA region of the plant genome causes hormonal imbalance and uncontrolled cell division, thereby triggering the formation of tumors (crown galls). Meanwhile, opine synthesis genes within the same T-DNA region enable plant cells to produce various amino acid and sugar derivatives (opines), which serve as nitrogen and carbon sources for *A. tumefaciens* (Chilton & ark., 1977; Chilton & ark., 1980; Özcan & ark., 2004; Watson & ark., 1975). Among the critical regions required for gene transfer within the Ti plasmid is the virulence (vir) region, which contains the operons VirA, VirB, VirC, VirD, VirE, and VirG. In response to phenolic compounds produced as a result of plant wounding, these genes direct the synthesis of proteins that mediate

cleavage of the T-DNA region, ensuring its complete integration into the plant chromosome (Amiri, 2018; Gelvin, 2000; Özcan & ark., 2004; Zupan & Zambryski, 1995). Another essential region in the Ti plasmid is the T-DNA region itself, which contains genes encoding cytokinin, auxin, and opine synthesis. This region is flanked by 24 base pairs (bp) at the right border (RB) and left border (LB), and the presence of the RB is particularly crucial for successful gene transfer (Özcan & ark., 2004; Yadav & ark., 1982). Finally, the origin of replication region is responsible for replication, ensuring the propagation of the plasmid (Gordon & Christie, 2014).

## Steps of *Agrobacterium tumefaciens*-Mediated Gene Transfer

### Procurement of Plant Material to Be Used

Plants, which form the basis of life, are the primary food sources that regulate oxygen and moisture balance in the biosphere and prevent erosion. Of approximately 350,000 plant species capable of growing in diverse regions and soils, only about 5% are edible and suitable for active agriculture (FAO, 2023). Transgenic products such as maize, cotton, and soybean—produced through recombinant DNA technologies and plant tissue culture—are widely prevalent. Plant tissue culture refers to the controlled production of tissues, plants, or plant-derived products from explants (plant parts) in an artificial medium. Genetically modified organisms are those created through gene transfer using recombinant DNA technology. This approach is employed to preserve existing plant diversity, enhance diversity, and enable the routine production of plants that are otherwise difficult and time-consuming to cultivate. The purposeful selection of plant material thus constitutes the first step of vector-mediated gene transfer.

**Selection of the Desired Strain of *Agrobacterium tumefaciens***

To perform studies targeting specific genes (e.g., herbicide resistance, disease resistance genes), an appropriate *A. tumefaciens* plasmid is selected (Figure 1-1).

**Sterilization of Plant Material to Be Used**

Surface sterilization of the material to be used in the study (seeds) is carried out. After this procedure is carefully completed, explants obtained from germinated seeds are subjected to in vitro sterilization through different methods such as sterile distilled water, mercuric chloride ($HgCl_2$), sodium hypochlorite (NaClO), sodium dodecyl sulfate (SDS), or hydrogen peroxide ($H_2O_2$) (Anayol, 2014) (Figures 1-2).

**Shoot Regeneration Suitable for Gene Transfer**

Following sterilization, explants from germinated seeds are transferred to culture. During this process, all necessary tools (scissors, forceps, scalpel) must be sterilized in an autoclave and prepared in a sterile manner. The basic medium used includes macronutrients ($NH_4NO_3$, $KNO_3$, $CaCl_2 \cdot 2H_2O$, $MgSO_4 \cdot 7H_2O$, $KH_2PO_4$), micronutrients (KI, $H_3BO_3$, $MnSO_4 \cdot 4H_2O$, $ZnSO_4 \cdot 7H_2O$, $Na_2MoO_4 \cdot 2H_2O$, $CuSO_4 \cdot 5H_2O$, $CoCl_2 \cdot 6H_2O$, $FeSO_4 \cdot 7H_2O$, $Na_2EDTA \cdot 2H_2O$), vitamins (inositol, nicotinic acid, pyridoxine-HCl, thiamine-HCl, glycine), and mineral salts (Amiri, 2018; Murashige & Skoog, 1962) (Figure 1-3).

**Cloning of Genes with Desired Traits**

The insertion of the target gene into the T-DNA region is performed using the T4 DNA ligase enzyme. Vectors prepared through cleavage with the appropriate restriction enzyme are transferred into *E. coli* for large-scale amplification. These vectors are subsequently introduced into *A. tumefaciens* strains using direct gene transfer

methods such as electroporation. As a result of this process, the target gene can be delivered into plant cells through *A. tumefaciens* (Anayol, 2014) (Figure 1-4).

**Gene Transfer into the Target Plant**

Explants obtained from seedlings germinated *in vitro* are inoculated in the inoculation medium, subsequently subjected to co-cultivation, and kept for a defined period before being transferred to the selection medium and placed in the growth chamber. After several weeks, the regenerated shoots are rooted, transferred into pots, and tested by PCR to determine whether they are indeed transgenic plants (Anayol, 2014) (Figure 1-5).

**Transfer of Putative Transgenic Plants into Soil**

Putative plants that have developed roots but whose transgenic status has not yet been fully confirmed are removed from the culture medium, carefully cleaned, and transferred into soil. They are then cultivated under appropriate conditions to ensure their growth.

**Verification of Putative Transgenic Plants**

To confirm the presence of the desired gene in putative transgenic plants, initial examination may focus on morphological features such as rooting and greening. However, as these characteristics can be influenced by environmental conditions and do not provide conclusive evidence of gene presence, they are insufficient for definitive confirmation. Therefore, molecular techniques are employed to fully verify the existence of the transgene, including PCR, qRT-PCR, gel electrophoresis, and ELISA (Figure 1-6).

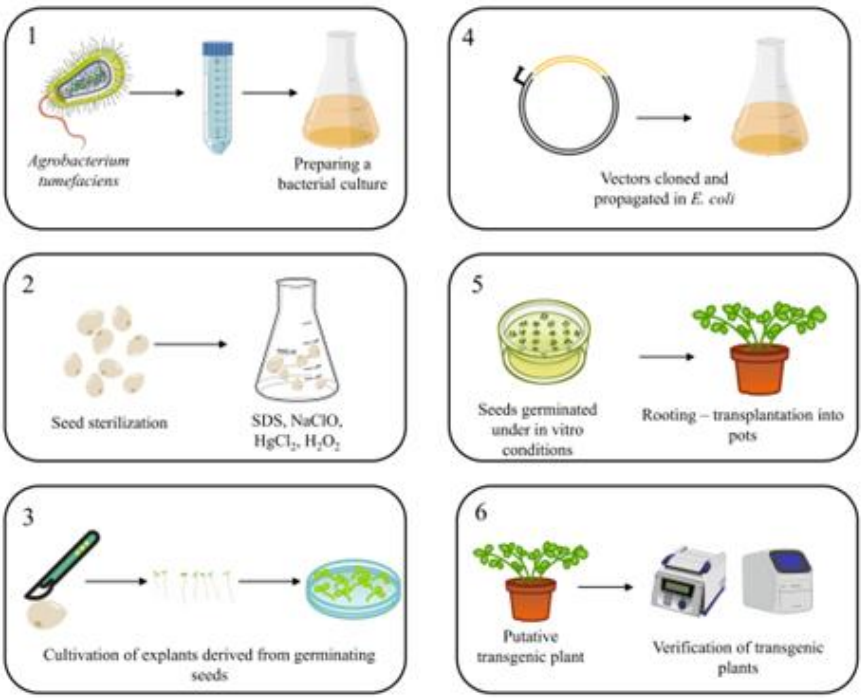


***Figure 1.*** *Workflow of Agrobacterium tumefaciens-Mediated Gene Transfer Adapted from (Paes de Melo & ark., 2020).*



## Direct Transfer of Genes

Direct gene transfer refers to the transformation of foreign DNA directly into plant cells without the need for a bacterial host cell (Rao & ark., 2009; Tzfira & Citovsky, 2006). Methods of direct gene transfer include electroporation, gene gun technology, microinjection, polyethylene glycol (PEG), and liposomes (Finer & Dhillon, 2008).

**Electroporation**

Electroporation is a frequently used method for the transformation of both monocot and dicot plants due to its low cost, speed, and high efficiency (Barampuram & Zhang, 2011; Danilova, 2007; Hjouj & Rubinsky, 2010). In this technique, electrical pulses with high field strength disrupt the polarity of the cell membrane, rendering it permeable and facilitating the entry of genetic material into the cell (Demirel, Akveç & Can, 2022; Fromm, Taylor & Walbot, 1986; Saulis, Venslauskas, & Naktinis, 1991). However, the disadvantages include the fact that it is typically applied to plant protoplasts, the procedure is relatively laborious, and the transformation efficiency is lower compared to other methods (Kalefetoğlu & ark., 2017).

**Gene Gun (Biolistics – Particle Bombardment)**

In the gene gun method, the DNA to be delivered is coated onto microparticles (approximately 2 μm in diameter), typically composed of gold, tungsten, or platinum, and rapidly propelled into plant cells using helium gas pressure (Southgate & ark., 1995). Advantages of this method include its applicability to a wide range of cell types (bacteria, fungi, some animal cells, organelles), the absence of a need for vectors, and the short duration of the procedure (Rakoczy-Trojanowska, 2002). Despite being one of the most preferred methods alongside *A. tumefaciens*-mediated transfer, improper optimization of bombardment velocity can cause damage to the target cells within plants. Additional disadvantages include its high cost and the frequent occurrence of chimeric plants (Demirel & ark., 2022; Kalefetoğlu Macar & ark., 2017).

**Micro- and Macroinjection**

Microinjection involves the direct delivery of DNA into plant targets using a microcapillary injection pipette (mineral oil glass pipette), whereas macroinjection is performed by introducing a DNA

solution into developing shoots. Using a micromanipulator, injections are carried out, after which the plants are transferred to tissue culture for regeneration monitoring (Barampuram & Zhang, 2011; Crossway & ark., 1986; Demirel, Akveç & Can, 2022; Rao & ark., However, the disadvantages include high costs and the difficulty of DNA transfer in plants with thick cellulose layers.

**Polyethylene Glycol (PEG)**

PEG-mediated transformation, one of the most commonly used and effective methods, is generally applied to protoplasts and is employed to achieve transient gene expression. The initial step of this method resembles electroporation, but DNA uptake is facilitated through the application of polyethylene glycol (Barampuram & Zhang, 2011). The disadvantages of this method include its relatively low transformation efficiency and the difficulty of plant regeneration due to its reliance on protoplasts (Kalefetoğlu Macar & ark., 2017).

**Liposomes**

Liposomes, which have been used for many purposes in recent years, were first described in the 1960s and are known as drug delivery systems. They are closed vesicles, ranging from 0.02 to 3.5 μm in diameter, surrounded by lipid bilayers resembling the cell membrane. They are non-toxic, can be designed in desired forms, and serve as versatile carriers (Susar & Karahan, 2019). In direct gene transfer using liposomes, once an appropriate liposome design is created, the liposomes adhere to the surface of protoplasts, undergo fusion into the target, and complete the process through the integration of plasmids. To achieve higher efficiency, optimization of the liposome content, size, surface properties, and DNA quantity is required (Demirel, Akveç & Can, 2022).

## Conclusion

Recombinant DNA technology involves the selection of a single gene from among the genes in a genome and the generation of thousands of its copies. This process requires the use of vectors. Vectors are small, laboratory-engineered molecules that can easily replicate themselves and the DNA fragments they carry, and typically contain resistance genes. Today, due to factors such as population growth, climate change, and the reduction of agricultural land, food resources are steadily diminishing. Plant biotechnology, through the use of recombinant DNA technology, enables the production of larger quantities of plants with higher quality and yield.

Although various gene transfer methods are available in plants, *Agrobacterium tumefaciens*-mediated transformation remains one of the most preferred methods. This soil bacterium, isolated from the environment, carries out transformation through its Ti plasmid. Another method of gene transfer in plants is direct gene transfer, which does not require the use of a vector. However, the efficiency and cost-effectiveness of direct gene transfer methods are generally lower than those of vector-mediated techniques.

This chapter has provided information on recombinant DNA technology, emphasizing the *Agrobacterium tumefaciens*-mediated gene transfer method, which plays a pivotal role in plant biotechnology. In addition, direct gene transfer methods, which do not rely on vectors, have also been discussed.

In order to meet the growing demand for food worldwide, researchers must make effective and safe use of technological advancements to achieve improvements in agriculture. Recombinant DNA technology enables the synthesis of products of great importance to humanity, ranging from agriculture to public health, from gene therapy to enzyme and hormone production. This technology encompasses a variety of techniques, and in plant biotechnology, in

particular, it has become essential for producing economically valuable, high-quality, high-yield, and pest-resistant crops. Among plant gene transfer methods, *Agrobacterium tumefaciens*-mediated gene transfer is the most widely used, as it is target-specific, applicable to a broad range of plant species, easily integrates into the genome, and is an efficient technique. The appropriate, safe, and effective use of technological methods will contribute to achieving sustainable agriculture worldwide and ensuring sufficient food resources for humanity.

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# HIGH – THROUGHPUT BIOSENSOR IN RECOMBINANT DNA TECHNOLOGIES

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## Introduction

The foundations of high-throughput screening (HTS) were established in the 1980s in drug discovery and pharmaceutical research. During this period, the integration of automation systems into laboratories created a growing need for methods capable of rapidly testing thousands of chemical compounds against biological targets (Hertzberg & Pope, 2000:6). The first HTS platforms combined microplate readers and liquid-handling robots to rapidly analyze many samples at once. (Macorron & ark., 2011:7). In the 1990s, with the advancement of directed evolution and recombinant DNA (rDNA) technologies, HTS began to be applied not only to chemical libraries but also to the evaluation of large genetic and protein variant libraries (Arnold,1998:6). During this period, technologies such as fluorescence-based biosensors, reporter gene systems, and flow cytometry facilitated the integration of HTS into biological applications (Zhang, Chung & Oldenburg, 1999: 6). Since the 2000s, microfluidic systems, next-

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generation sequencing (NGS), and artificial intelligence–assisted analytical methods have significantly enhanced both the speed and accuracy of HTS and selection (Dittrich & Manz, 2006: 8). The necessity of HTS and selection is intrinsically linked to the large-scale data complexity and diversity encountered in modern biotechnology and molecular biology (Shin & Noireaux, 2012: 12). rDNA technologies enable the creation of extensive libraries containing thousands to millions of genetic sequences or protein variants. Identifying candidates with desired traits from this diversity quickly and accurately is not feasible with conventional methods. HTS systems make it possible to analyze many samples simultaneously, saving time and labor. (Packer & Liu, 2015: 15).

**Fundamentals of Biosensors**

Biosensors are analytical devices that integrate biological recognition elements with physical or chemical transducers. These devices detect target molecules specifically recognized by biological components such as enzymes, antibodies, nucleic acids, or cells, converting this interaction into a measurable signal. Thus, biosensors are employed for the rapid, sensitive, and selective detection of specific analytes within complex biological environments (Turner, 2013:12).

The fundamental components of biosensors consist of three main parts: the biological recognition element, the transducer, and the signal processor. The biological recognition element selectively binds or transforms the target analyte. The transducer converts this biological recognition event into an electrical, optical, thermal, or mass-based signal. Finally, the signal processor processes this signal and provides a quantitative output. This configuration allows biosensors to be flexible and versatile for applications across diverse fields (D'Orazio, 2003: 28).

Currently, biosensor technology holds critical importance in numerous fields such as medical diagnostics, environmental monitoring, food safety, biotechnology, and industrial process control.

In particular, biosensors are widely preferred in clinical diagnostics due to their rapid response times and cost-effectiveness. Moreover, the integration of nanotechnology and microfluidic systems has enhanced biosensor sensitivity, enabling high performance even with minimal sample volumes (Wang, 2006: 15).

**High-Throughput Screening Approaches**

High-throughput screening (HTS) technologies have been developed to rapidly and systematically identify target molecules or biological activities from biological and chemical libraries. These methods enable the simultaneous analysis of thousands of compounds or biological samples through the integration of robotic automation, miniaturization, and data analysis software (Macorron & et al., 2011:7). HTS is particularly critical in drug discovery and biotechnology, as it provides significantly higher speed and cost- effectiveness compared to traditional screening methods (Swinney & Anthony, 2011: 12). Currently, HTS platforms employ a variety of detection techniques, including fluorescence, luminescence, and mass spectrometry, facilitating the functional and structural analysis of biomolecules (Anthony &. Ark., 2019: 7).

The efficiency of HTS approaches has been further enhanced by the integration of advanced biosensor technologies and artificial intelligence-based data processing methods. Biosensor-based HTS systems offer real-time and highly sensitive measurements by directly interacting with living cells or biological macromolecules. Moreover, machine learning algorithms provide significant advantages in the analysis of HTS data and the optimization of hit compounds, enabling the reduction of false positives and improving the accuracy of the screening process (Puhl & et al., 2019: 6). Thus, high-throughput screening, through multidisciplinary approaches, contributes substantially to accelerating biomedical research and the discovery of novel biomolecules.

## Applications of Recombinant DNA Technology

Recombinant DNA technology, which enables the artificial modification and rearrangement of genetic material, has found wide-ranging applications in biotechnology, medicine, agriculture, and industry. Notably, the production of biopharmaceutical products such as insulin, growth hormone, and vaccines has been made possible through recombinant DNA techniques (Walsh, 2018: 9). In the field of gene therapy, recombinant vectors are employed to develop personalized treatment approaches aimed at correcting genetic defects responsible for diseases (Hacein-Bey-Abina & et al., 2008:4). In agriculture, genetically modified organisms (GMOs) have been engineered to enhance resistance against diseases and environmental stresses, thereby improving crop yields (James, 2017:5). Moreover, recombinant DNA technology plays a crucial role in the development of biological pesticides and nutritionally enhanced products.

## Enzyme Engineering and Directed Evolution

Enzyme engineering is an interdisciplinary field focused on optimizing the catalytic properties of enzymes or imparting novel functions through structural and functional modifications. One of the most effective methods in this field is directed evolution, which mimics the natural evolutionary process in the laboratory to generate genetic diversity and select mutant enzymes with desired traits (Arnold, 1998: 6). Directed evolution is supported by techniques such as error-prone PCR, DNA shuffling, and high-throughput screening, leading to significant improvements in enzyme stability, activity, and substrate specificity (Turner, 2009: 6). These advances enable the development of more efficient and sustainable enzymes for biotechnological applications, pharmaceutical production, and environmentally friendly industrial processes.

## Intracellular Sensors in Metabolic Engineering

Intracellular sensors in metabolic engineering are biological tools used to monitor the metabolic state of living cells in real time and with high sensitivity. These sensors detect intracellular metabolite concentrations, ion levels, or critical parameters such as pH, enabling dynamic control of metabolic pathways (Yu & et al., 2023:5). Through intracellular sensors, metabolic engineers can optimize pathways to enhance the production of desired compounds with high yield, thereby improving the efficiency and economic sustainability of bioprocesses.

The development of intracellular sensors commonly involves genetically encoded fluorescent proteins and riboswitch-based regulators. These sensors generate signals in response to the presence or concentration of metabolites, providing direct feedback on metabolic activities (Liu, Liu, & Wang, 2017: 7). Furthermore, these technologies are integrated with high-throughput screening and artificial intelligence–assisted analytical methods, allowing precise control and improvement of complex metabolic networks. Consequently, intracellular sensors play a critical role in advancing innovative biotechnological applications within the field of metabolic engineering.

## Positive and Negative Selection System Strategies

In recombinant DNA technologies, high-throughput biosensor-based screening and selection systems are critically important for the efficient isolation of cells or molecules exhibiting the desired phenotype from genetic libraries. In positive selection systems, cells possessing the target molecule or activity gain a survival advantage or produce a distinct detectable signal. This strategy focuses on the presence of the desired activity, enabling rapid identification of target mutants (Blazeck & Alper, 2013: 9). Positive selection systems can be easily implemented using, for example, antibiotic resistance genes or biosensors that emit fluorescent signals, allowing effective screening across large libraries due to their high sensitivity.

In contrast, negative selection systems aim to eliminate cells exhibiting undesired phenotypes. This strategy triggers cell death or growth inhibition as a result of off-target activities or toxic effects (Ellefson &. et al., 2013: 4). Negative selection is particularly employed to enhance functional specificity and reduce side effects. Often, both selection systems are combined, applying positive and negative selection steps sequentially; thus, increasing the target activity while eliminating unwanted functions. This multilayered approach significantly improves the efficiency and specificity of biosensor-based high-throughput selections.

**PACS, Microfluidic Systems, and Cell-Based Sensors**

Protein Array Chip Systems (PACS) play a significant role in high-throughput screening and selection processes within recombinant DNA technologies. PACS provide platforms capable of simultaneously analyzing numerous protein interactions and functions. This technology enhances the efficiency of biosensor- based screening systems by enabling the highly accurate detection of protein-protein interactions, enzyme activities, and biomolecular bindings (Smith &. et al., 2021:5). Consequently, it allows for the rapid and reliable selection of functional protein variants from extensive genetic libraries.

Microfluidic systems offer miniaturization, automation, and high parallelism in high-throughput biosensor-based screening workflows. These systems facilitate the simultaneous execution of numerous experiments with precise control of fluids at the microscale, using minimal reagent volumes (Wang, Barahona &. Buch, 2013:8). Microfluidic technologies provide high-throughput and cost-effective solutions in recombinant DNA research by enabling the isolation of cells and biomolecules, accelerating reactions, and allowing real-time monitoring.

Cell-based sensors detect the natural metabolic and physiological states of living cells, producing highly sensitive and

specific signals. Designed using genetically encoded fluorescent proteins or riboswitch-based regulators, these sensors enable real- time monitoring of metabolites and biochemical activities within the intracellular environment (Dittrich &. Manz, 2013: 8). The integration of cell-based sensors with microfluidic platforms represents a critical technological advancement in high-throughput screening processes, enhancing both data quality and the evaluation of dynamic biological responses.

**Recombinant DNA Technologies and High-Throughput Biosensor-Based Screening: Recent Examples and Applications**

Recombinant DNA technologies have significantly advanced through the integration of high-throughput biosensor-based screening methods, enabling the rapid identification and optimization of biomolecules with desired functionalities. A prominent example is fluorescence-activated cell sorting (FACS) combined with genetically encoded biosensors to isolate enzyme variants with enhanced catalytic properties. For instance, a recent study by Zhang et al. (2022) demonstrated the use of a biosensor responsive to intracellular NADH levels to screen for improved variants of alcohol dehydrogenase, leading to enzymes with higher activity and stability suitable for industrial biocatalysis (Zhang, Chen & Wang, 2022: 9).

Another compelling application involves microfluidic droplet-based screening platforms integrated with biosensors that allow compartmentalization of single cells or enzymes. Tu et al. (2023) utilized this approach to conduct ultrahigh-throughput screening of recombinant antibody libraries, where cell-secreted antibody binding was detected via fluorescence signal amplification within picoliter droplets. The platform enabled sorting of high- affinity antibodies at rates exceeding 10,000 variants per second, vastly accelerating antibody engineering processes for therapeutic development (Tu &. et al., 2021:6).

Moreover, biosensor-based screening has been applied in metabolic engineering for dynamic regulation of engineered pathways. For example, a case study by Kumar and Singh (2021) showcased a riboswitch-based biosensor that monitors intracellular levels of key metabolites, facilitating real-time adjustment of gene expression to optimize production of valuable biochemicals in recombinant microbial strains. This feedback-controlled system improved product yield and reduced metabolic burden, illustrating how biosensors can transform strain development pipelines (Kumar &. Sing, 2021:11).

These examples highlight how the convergence of recombinant DNA technologies with high-throughput biosensor platforms, including FACS, microfluidics, and genetically encoded sensors, enables precise, efficient, and scalable biomolecular screening and selection. Such integrated systems continue to push the boundaries of synthetic biology, enzyme engineering, and biopharmaceutical discovery.

## Industrial, Clinical, and Academic Applications of High-Throughput Biosensor-Based Screening in Recombinant DNA Technologies

In industrial biotechnology, high-throughput biosensor- based screening systems play a crucial role, particularly in enzyme engineering and metabolic pathway optimization. The efficient production of commercially valuable compounds such as biofuels, bioplastics, and amino acids by recombinant microorganisms is largely dependent on the ability to screen genetic libraries for optimal variants. For example, a transcription factor-based biosensor developed for use in Escherichia coli enables rapid identification of strains overproducing L-lysine by responding specifically to high intracellular concentrations of the metabolite (Zhang, Li &. Zhao, 2020: 6). This approach significantly enhances the efficiency and sustainability of large-scale bioproduction processes

In clinical settings, biosensor-based screening is widely applied for the discovery of disease biomarkers, identification of novel drug candidates, and implementation of personalized medicine approaches. Recombinant protein and antibody libraries are screened using cell-based fluorescent biosensors to detect disease-related targets with high sensitivity, particularly for cancer, infectious diseases, and metabolic disorders (Chen, Yang &. Ding, 2021: 5). In academic research, these technologies support the exploration of dynamic genetic regulatory networks, the design of synthetic biology circuits, and the investigation of fundamental biological questions. Innovative strategies, such as integrating intracellular sensors with CRISPR-Cas systems, further enable precise functional genomic analyses and enhanced control over engineered biological systems (Kumaran & et al., 2023:7).

**Biosensor Design and Optimization: Challenges and Future Perspectives**

To enhance the efficiency of biosensor-based high- throughput screening systems, key parameters such as specificity, sensitivity, dynamic range, and response time must be carefully designed and optimized. However, balancing these parameters often poses technical challenges; for instance, achieving high sensitivity while maintaining a low background signal can be difficult (Turner, 2013: 12). Moreover, intracellular biosensors must function compatibly within living systems without causing toxicity, consuming minimal energy, and being easily integrated into genetic circuits. Therefore, biosensor design requires a holistic approach that incorporates both biological and engineering perspectives (Lowe, 2007: 6).

In the future, it is anticipated that artificial intelligence (AI) and machine learning (ML) algorithms will be more widely employed in biosensor design and optimization processes. These technologies can analyze large biological datasets to predict new biosensor designs and reduce experimental workload (Zhang, Petersen &. Radivojevic, 2022:

6). Particularly in metabolic engineering and synthetic biology applications, AI-assisted biosensor design enables precise manipulation of complex metabolic networks and allows for system-level optimization (Carbonell, Radivojevic &. Martin, 2019: 3). Nevertheless, challenges such as the lack of standardized biosensor components, environmental variability affecting sensor performance, and difficulties in integrating with systems biology remain significant. In this context, the development of advanced synthetic biology tools and open- access databases is expected to shape the future of biosensor-based screening systems.

**Conclusion and General Evaluation**

High-throughput biosensor-based screening and selection systems have become fundamental tools in recombinant DNA technology and modern biotechnology. These systems offer significant advantages in terms of time, cost, and labor for the functional evaluation of genetic diversity, enabling innovative solutions in drug discovery, enzyme engineering, synthetic biology, and metabolic engineering. Particularly, the integration of positive/negative selection strategies, microfluidic platforms, cell- based sensors, and artificial intelligence-assisted analyses has significantly improved the precision and efficiency of screening processes by enhancing biosensor sensitivity and specificity.

However, current biosensor technologies still face several limitations. Technical challenges such as achieving full compatibility with biological systems, optimizing signal-to-noise ratios, and the lack of standardized biological components persist. In the future, it is expected that AI-based design approaches, synthetic biology tools, and high-throughput data analytics will substantially overcome these limitations. Ultimately, biosensor-based high- throughput screening systems will become more integrated, precise, and scalable through

multidisciplinary innovations, facilitating more effective and sustainable applications of recombinant DNA technologies.

In this context, the development of biosensor-based high-throughput screening systems not only accelerates existing biotechnological processes but also paves the way for innovative applications in diverse fields such as personalized medicine, environmental monitoring, and sustainable agriculture. Particularly, the use of living cell-based biosensors enables real-time monitoring of intracellular metabolic responses, allowing for more precise and targeted selection processes.

Moreover, as the industrial applicability of these technologies increases, biotechnological production processes are expected to become more efficient, cost-effective, and environmentally friendly. Platforms integrated with automation systems reduce experimental repetitions and minimize human error, thereby enhancing data reliability. This enables faster and more accurate results, significantly shortening the time required to bring new products to market.

In conclusion, the advancement of biosensor-based high-throughput screening systems should be regarded not only as a technical improvement but also as a strategic step toward the sustainability of biotechnological innovations. In the coming years, as these systems become more integrated with artificial intelligence, synthetic biology, and advanced materials science, a profound transformation in both basic research and applied biotechnology is inevitable.

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# CRISPR-BASED MOLECULAR DIAGNOSTICS

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## Introduction

Recent striking developments in molecular biology and genetics have led to transformative innovations in both basic science research and clinical applications. Among these innovations, the Clustered Regularly Interspaced Short Palindromic Repeats-CRISPR-associated proteins (CRISPR-Cas) system, initially identified as a bacterial immune mechanism, stands out as one of the most revolutionary technologies. First identified as short, repetitive sequences in the bacterium Escherichia coli in 1987, CRISPR was later understood to provide adaptive immunity against foreign genetic elements in prokaryotes (Ishino & et al., 1987: 5429-5433; Mojica & et al., 2005: 174-182).

A groundbreaking study by Jennifer Doudna and Emmanuelle Charpentier in 2012 demonstrated that the CRISPR-Cas9 system could

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be used as a precise and programmable genome editing tool. Researchers have managed to create target-specific double-stranded breaks in target DNA sequences by combining guide RNA (sgRNA) and the Cas9 protein (Jinek et al., 2012: 816-821). This discovery revolutionized genetic engineering and earned Doudna and Charpentier the Nobel Prize in Chemistry in 2020.

The development of CRISPR technology has not been limited to the Cas9 protein alone, but has expanded further with the discovery of effector proteins such as Cas12 and Cas13. While Cas12 targets double-stranded DNA, Cas13 has the ability to target and cut single-stranded RNA (Chen et al., 2018a: 436-439; Gootenberg et al., 2017: 438-442). In addition to recognizing target DNA or RNA sequences with high specificity, these effector proteins have the activity of cutting non-target molecules (trans-cleavage) after target recognition (Li et al., 2021: 1481-1489). This diversity has enabled CRISPR systems to be used in a wide range of applications, not only gene editing, but also gene expression regulation, RNA monitoring, and most importantly, molecular diagnosis (Chen et al., 2018: 1-9.; Abudayyeh et al., 2016). Today, CRISPR systems are used in a wide range of applications, from the treatment of human genetic diseases to agricultural product development, from the diagnosis of infections to the genetic reprogramming of living organisms (Li et al., 2021a: 723-732).

**Molecular Functioning of the CRISPR-Cas System**

CRISPR-Cas systems are molecular defense mechanisms that form the adaptive immune system developed by bacteria and archaea against mobile genetic elements (e.g. phages and plasmids). CRISPR sequences and their associated Cas proteins enable bacteria and archaea to develop defenses against future attacks by creating genetic memory against previous viral infections. This system is based on the principle that foreign genetic material is recognized by specific guide RNAs and then cut by Cas nucleases. This natural defense mechanism has been

artificially programmed in modern biotechnology and adapted for human benefit.

The molecular functioning of CRISPR-Cas systems generally occurs in three basic stages:

1. Adaptation: Integration of short sequences (spacers) belonging to foreign genetic material into the CRISPR locus,
2. Expression and Processing: Conversion of pre-crRNAs formed by the transcription of CRISPR arrays into mature crRNAs by a special RNA endonuclease complex (e.g. RNase III and trans-activation RNA (tracrRNA)),
3. Interference: Specific recognition and cutting of target DNA or RNA by the complex consisting of crRNA and Cas proteins (Hille et al., 2018:1239-1259).

**Classification of the CRISPR-Cas System**

The classification of the CRISPR-Cas system is mainly based on structural, evolutionary and phylogenetic criteria (Makarova et al., 2015: 722-736). This classification is based on the operon organization, composition of Cas genes and especially the presence of proteins such as Cas1, which are evolutionarily conserved. With more comprehensive analyses in 2019, CRISPR-Cas systems were redefined as two main classes (Class 1 and Class 2), six types (Types I–VI) and thirty-three subtypes (Makarova et al., 2020: 67-83).

Class 1 systems consist of multi-subunit effector complexes, while Class 2 systems have simpler effector modules, usually consisting of only a single large Cas protein. In Class 1 systems, the target DNA is cut by the CRISPR-associated protein complex (Cascade) for antiviral defense and the Cas3 enzyme. Class 2 systems have simpler effector modules such as Cas9, Cas12, and Cas13 alone. This structural

simplicity has led to Class 2 systems being preferred in gene editing applications (Figure 1) (Hille et al., 2018: 1239-1259; Makarova et al., 2020: 67-83).

**Class 1 CRISPR-Cas System**

It consists of three basic types, Type I, Type III, and Type IV, which include their own molecular organization, target recognition, and nucleic acid degradation mechanisms (Figure 1) .

Initiation of the immune response in Class 1 systems occurs through the biogenesis of crRNA molecules. Precursor crRNA (pre-crRNA) transcribed from the CRISPR locus is usually cut by an RNase such as Cas6 and converted into mature crRNAs, each of which contains a repeat sequence and a spacer sequence derived from the intervening foreign DNA. These crRNAs combine with effector complexes to provide specific target recognition (Makarova et al., 2020: 67-83).

In Type I and Type IV systems, the 3' hairpin structure of crRNAs is preserved and their stability is ensured within the complex (Brouns et al., 2008: 960-964). In Type III systems, the 3' end of the crRNA is cut by host nucleases to variable lengths depending on the size of the complex. This structure reveals that each subtype exhibits specific differences in the guide RNA processing mechanism (Hatoum-Aslan et al., 2014: 310-317).

**Class 1 Type I CRISPR-Cas System**

It represents the most widespread and evolutionarily oldest CRISPR-Cas subgroup. This system consists of cas genes, which are usually found as a single operon in the genome and organized in a certain order. These systems usually encode effector complex components such as Cas5, Cas6, Cas7, Cas8, which recognize and process target DNA, together with cas1, cas2 and cas4 adaptation genes. One of its most distinctive features, the Cas3 gene, exhibits both a

helicase and a nuclease activity and is the signature protein of the system (Figure 1) (Hochstrasser & Doudna, 2015: 58-66). Cascade, the effector complex of type I systems, plays a key role in recognizing target DNA. Cascade binds to the target DNA sequence under the guidance of crRNA that matches it and directs Cas3 to the target region. This process occurs especially depending on the Short Nucleotide Sequence Adjacent to Protospacer (PAM) sequence and enables specific recognition of DNA (Jinek et al., 2012: 816-821).

**Class 1 Type III CRISPR-Cas Systems**

They stand out with their ability to produce effective defense responses against both RNA and DNA targets. The characteristic feature of Type III systems is that they have RNA-specific target recognition capacity and can trigger a series of secondary defense mechanisms after binding the target RNA (Figure 1) (Makarova et al., 2020: 67-83).

In addition, Type III systems have significant potential in terms of biotechnological applications. In particular, the use of Csm6 together with other RNA targeting systems such as Cas13 has enabled the detection of viral RNAs with high sensitivity (Rauch et al., 2019: 122-134). In addition, the fact that these systems work at the RNA level has allowed them to be used for temporary suppression of gene expression instead of gene editing. In fact, Type III systems have been successfully applied for RNA suppression in model organisms such as zebrafish (Kushawah et al., 2020: 805-817).

**Class 1 Type IV CRISPR-Cas Systems**

It is thought to function in defense against extrachromosomal DNA elements such as plasmids (Pinilla-Redondo et al., 2020: 2000-2012). Unlike other CRISPR-Cas systems, Type IV systems mostly lack cas1 and cas2 genes and are often not associated with identifiable CRISPR sequences. In this respect, they are the only system in some

bacteria that does not contain a CRISPR cassette (Figure 1) (Makarova et al., 2020: 67-83).

Type IV systems are associated with the Csf4 protein. Although the function of Csf4 has not yet been fully elucidated, it has been shown to play a key role in the suppression of plasmids. The interactions of Type IV systems with mobile genetic elements suggest that these systems may be involved not only in defense but also in processes such as genetic regulation and mobile gene transfer (Pinilla-Redondo et al., 2020: 2000-2012). These systems are defense modules that are notable for their genetic diversity, plasmid-associated nature, and potential functionality despite incomplete CRISPR cassettes, but their mechanisms are not yet fully understood.

**Class 2 CRISPR-Cas System**

Class 2 systems, especially with their single-component nucleases such as Cas9, Cas12 and Cas13, have pioneered groundbreaking applications in the fields of genetic engineering and molecular biology (Figure 1). Comprehensive analyses of the distribution and evolutionary diversity of Class 2 CRISPR-Cas systems have shown that the existence of these systems in nature is divided into three basic types: Type II, V and VI (Makarova et al., 2015: 722-736).

**Class 2 Type II CRISPR-Cas Systems**

It is one of the earliest identified CRISPR subtypes among Class 2 systems and is currently the most widely used in biotechnological applications. The main feature of these systems is that all basic functions such as recognition, binding and cutting of target DNA are performed by the Cas9 protein (Figure 1) (Jinek et al., 2012: 816-821; Makarova et al., 2015: 722-736). The Cas9 protein is capable of creating double-strand breaks (DSB) on target DNA together with crRNA and tracrRNA (Hille et al., 2018: 1239-1259). The Cas9 protein contains two different nuclease domains, RuvC-like and HNH, to

specifically cut both strands of the target DNA. The HNH domain cuts the strand of the target DNA that matches the crRNA, while the RuvC domain cuts the non-complementary strand. This mechanism enables Cas9 to perform programmable DNA cutting with high specificity (Nishimasu et al., 2014: 935-949). Since Cas9 can cut with a single guide RNA (sgRNA), this system has become an indispensable tool in many application areas (Jinek et al., 2012: 816-821).

**Class 2 Type V CRISPR-Cas Systems**

It can recognize and cut target DNA via the Cas12 effector protein. These proteins differ from Cas9 structurally and functionally. Cas12 contains only a RuvC-like domain and can cut both strands of target DNA via this domain (Zetsche et al., 2015: 759-771). This feature of Cas12 has made it an important tool in DNA-targeted genome editing applications (Figure 1). Cas12 effectors are dependent on the PAM sequence to recognize target DNA and require the guidance of the guide RNA during the recognition process. However, it has been shown that Cas12 proteins also exhibit nonspecific single-stranded DNA (ssDNA) nuclease activity that is triggered after cutting the target DNA. This offers significant advantages in diagnostic-based applications (Chen et al., 2018a: 436-439).

Type V systems, especially due to the small structure of Cas12 proteins, different PAM recognition capacities and side-effect ssDNAase activities, have found a wide place in various biotechnological applications (Chen et al., 2018a: 436-439). Systems such as DNA Endonuclease Targeted CRISPR Trans Reporter (DETECTR), which exploit the side-effect activity of these proteins, are pioneers in the development of rapid and sensitive diagnostic tests (Figure 2) (Gootenberg et al., 2018: 439-444). In addition to diagnosis, different versions of proteins belonging to the Cas12 family can also be used in various areas such as genome engineering and RNA-guided transposition (Strecker, et al. 2022:48-53).

## Class 2 Type VI CRISPR-Cas Systems

They stand out especially with their RNA targeting capabilities. These systems are the only group among CRISPR-Cas systems that can only target and cut RNA (Shmakov et al., 2017: 169-182).

Cas13 proteins serve as the effector component of this system (Figure 1). The Cas13 family structurally contains two Higher Eukaryotes and Prokaryotes Nucleotide binding (HEPN) ribonuclease domains. These domains have "collateral RNase" activity, which is activated when the target RNA is recognized and breaks down not only the target RNA but also other RNA molecules within the cell. This can suppress viral replication through RNA degradation in the infected cell as well as cause cell death (Abudayyeh et al., 2016).

Systems such as the Specific and Highly Sensitive Enzymatic Reporter Lock-Unlocking System (SHERLOCK), which uses the collateral RNase activity of Cas13 proteins (Figure 3), are pioneers in the development of rapid and sensitive diagnostic tests (Gootenberg et al., 2017: 438-442).

## The Applications of CRISPR-Cas System

CRISPR-Cas technology offers significant advantages over traditional methods in terms of both efficiency and accuracy in gene editing processes and is used in a wide range of applications from biomedical research to agricultural biotechnology, from molecular diagnostic systems to environmental applications (Li et al., 2021a: 723-732).

In its applications in the field of medicine, CRISPR has a groundbreaking feature in the treatment of monogenic diseases (diseases originating from a single gene). Especially in hereditary diseases such as sickle cell anemia and Duchenne muscular dystrophy, CRISPR-based gene editing methods are used intensively both in the creation of disease models and in treatment-oriented interventions. In

this way, studies are ongoing on the development of personalized treatment strategies aimed at improving the quality of life of patients (Long et al., 2016: 1349-1355). In addition, the use of CRISPR-Cas9 in the genetic modification of cancer cells in cancer research has made it possible to develop targeted immunotherapy (immunotherapy) approaches (Rupp et al., 2017: 1-10).

In agriculture, CRISPR-Cas systems stand out as a tool that can provide much faster and more precise results compared to traditional breeding methods. By editing specific genes in plant genomes, targets such as developing resistance to diseases, increasing stress tolerance and increasing nutritional values are achieved. For example, CRISPR modifications developed in basic agricultural products such as rice and wheat enable plants to gain resistance to fungal and viral diseases. In this way, it is possible to both increase productivity and reduce the use of chemical drugs. The widespread use of CRISPR applications in agricultural production also contributes to the development of sustainable and environmentally friendly agricultural policies (Chen et al., 2019: 667-697; Wang et al., 2021: 13260-13269).

In the field of biotechnology, CRISPR technology is an important tool in genetically editing microorganisms. Thanks to the modification of microbial metabolic pathways with CRISPR, efficiency and cost-effectiveness can be increased in processes such as biofuel production, drug synthesis and industrial enzyme production. Especially in industrial microbiology, CRISPR-based approaches play a critical role in optimizing biosynthesis processes and reducing unwanted by-products. This allows biotechnology to develop in a sustainable and environmentally friendly way (Jinek et al., 2012: 816-821).

In the field of molecular diagnosis, CRISPR has taken on a pioneering role in the development of sensitive and rapid diagnostic methods. Effector proteins such as Cas12 and Cas13 have been used on

platforms such as DETECTR and SHERLOCK to develop rapid, specific and highly sensitive diagnostic tools for the detection of viral and bacterial infections. These technologies contribute to the strengthening of global health systems by providing effective diagnostic opportunities outside the laboratory, in field environments and in resource-limited regions, especially under pandemic conditions (Gootenberg et al., 2018: 439-444).

**CRISPR-Based Diagnosis**

The discovery of enzymes that exhibit enzymatic activity against off-target nucleic acid substrates, especially Cas12 and Cas13, has enabled the development of CRISPR-based diagnostic platforms that are specific to nucleic acid targets and are rapid (Gootenberg et al., 2018: 439-444; Broughton et al., 2020: 870-874).

CRISPR-based diagnostic systems identify target DNA or RNA sequences through sgRNAs designed specifically for them. When Cas12 and Cas13 enzymes bind to the target sequence, they not only cut it, but also cleave short off-target DNA or RNA sequences, producing a signal. This feature, especially when included in the analysis with fluorescence or lateral flow (strip test) systems, allows the development of highly sensitive and rapid diagnostic tools (Kaminski et al., 2021: 643-656). The first prominent examples of these systems are DETECTR and SHERLOCK. The DETECTR platform provided high specificity in the detection of DNA viruses using the Cas12a enzyme. Similarly, the SHERLOCK platform developed for RNA-targeted diagnosis can distinguish various microorganisms (Zika, Dengue, and COVID-19 viruses) with the Cas13a enzyme (Figure 2, Figure 3) (Gootenberg et al., 2017: 438-442).

The COVID-19 pandemic has triggered the rapid testing and development of CRISPR-based diagnostic technologies in the field. The SHERLOCK system attracted attention as the first FDA-approved CRISPR diagnostic test for the detection of SARS-CoV-2. This test can

provide results in approximately 1 hour when used in conjunction with reverse transcription and isothermal amplification (RT-LAMP) (Fozouni et al., 2021: 323-333).

CRISPR-based diagnostic systems have various target molecules, including nucleic acids, proteins and peptides, and pathogenic microorganisms. Thus, specific and sensitive diagnostic systems can be developed for a wide spectrum from infectious diseases to genetic disorders, and these systems are expected to become standard in healthcare in the near future. These systems provide faster results than classical diagnostic methods and can be used effectively in public health screening beyond clinical applications (Gootenberg et al., 2017: 438-442; Broughton et al., 2020: 870-874).

**Nucleic Acid**

CRISPR-based diagnostic systems developed in recent years are among the pioneering biotechnological methods that offer selective diagnosis at the nucleic acid (DNA or RNA) level (Kaminski et al. 2021: 643-656).

**DNA**

In CRISPR-based DNA diagnostic systems developed in recent years, proteins belonging to the CRISPR-Cas12 family stand out. In the DETECTR platform developed in this direction, short DNA probes labeled with fluorescence are used together with the Cas12a enzyme (Figure 2). The system enables rapid and specific detection of DNA viruses, especially human papillomavirus (HPV), at the type and subtype level (Gootenberg et al., 2018: 439-444).

Cas12a-based DNA diagnostic systems are not limited to viral infections only, but are also used in the detection of bacterial pathogens and hereditary diseases. For example, high sensitivity and specificity were achieved with Cas12a in systems developed to recognize sequences specific to the genetic structure of the Mycobacterium

tuberculosis bacterium (Kaminski et al., 2021: 643-656). In addition, studies on the detection of single nucleotide changes (SNP) show that these systems are also promising in the field of cancer genetics and hereditary diseases (Li et al., 2018). In this way, early diagnosis leads to personalized medical interventions.

Adequate amplification of the target DNA sequence is an important step in the success of CRISPR-based DNA diagnostic systems. For this purpose, methods such as recombinase polymerase amplification (RPA) and isothermal cyclic amplification (LAMP) are preferred. These amplification techniques increase the amount of DNA without the need for temperature changes required by PCR, making it easier for Cas12a to reach the target. Thus, the detection of even low amounts of pathogenic DNA becomes possible (Fozouni et al., 2021: 323-333).

Recently, studies on the use of CRISPR-Cas systems for the recognition and analysis of methylated DNA have attracted attention. dCas9, a non-catalytic form of the Cas9 enzyme, is directed to specific DNA sequences with the help of guide RNA and is effective in targeting methylated regions. Although this system does not provide direct information about the methylation status, it allows selective epigenetic analyses to be performed by combining it with proteins that specifically bind to methylated DNA. Thus, intracellular methylation maps have become available and epigenetic regulation processes at the level of gene expression have been examined in more detail (Vojta et al., 2016: 5615-5628).

In addition, there are studies using other CRISPR proteins such as Cas12a and Cas13 to create indirect indicators for the detection of methylated DNA (Zhou et al., 2020). Such systems are especially promising in the detection of cancer biomarkers from liquid biopsy samples.

**RNA**

Among CRISPR-based diagnostic technologies, the Cas13 protein family has an important place in RNA-targeted diagnostic applications (Zhang et al., 2021: 94-99). The SHERLOCK platform, one of the most well-known examples of CRISPR-based diagnostic systems at the RNA level (Figure 3), works in harmony with isothermal amplification techniques and enables the development of portable diagnostic kits.

In addition, the RNA-specific cutting ability of Cas13 is used not only in the diagnosis of viral infections but also for the detection of RNA biomarkers expressed at the gene level in diseases such as cancer. For example, in some studies, RNA sequences specific to prostate cancer were successfully identified with Cas13a-based systems, and this approach was effective in a shorter time and with a lower sample size compared to classical molecular diagnostic methods (Kellner et al., 2019: 2986-3012).

A significant advantage of the CRISPR-Cas13 system is that it can distinguish small differences in RNA sequences, i.e. single nucleotide changes (SNPs). This feature provides a valuable tool for the early detection of RNA variations that cause genetic diseases. In addition, the Cas13 system reduces the need for pre-processing of samples, leading to the development of low-cost and field-usable diagnostic applications (Fozouni et al., 2021: 323-333).

**Small Molecules**

Although CRISPR-based diagnostic systems initially focused on nucleic acid targets, the coverage area of the system has been expanded over time and indirect recognition of small molecules has become possible. Although small molecules such as hormones, toxins and metabolites cannot be directly recognized by CRISPR-Cas enzymes, they trigger CRISPR activity thanks to biosensor systems that

change their structure depending on the presence of such molecules. Thus, a specific diagnostic signal is obtained in the presence of the targeted small molecule (Lee et al., 2020: 313-319). One of the systems developed for this purpose is the ligand-responsive aptamer-CRISPR platform. In these systems, a specific aptamer is constructed that binds to the target small molecule, and this binding leads to a series of structural changes that initiate the CRISPR-Cas system. Nucleases such as Cas12a are activated and signal production begins (Zhou et al., 2020).

**Proteins and Peptides**

Proteins and peptides are key biomarkers associated with many diseases in biological systems. Therefore, studies have intensified towards the use of CRISPR technology in protein and peptide diagnosis. However, since Cas enzymes naturally work specifically for DNA or RNA sequences, direct recognition of proteins and peptides is not possible. This obstacle has been overcome with unique biosensor designs that perform protein diagnosis indirectly. The most common approach in protein-based diagnosis is to create a nucleic acid-based signal depending on the presence of target proteins. For example, by using antibodies or aptamers specific to target proteins, a specific DNA or RNA sequence is released in the presence of these proteins. These sequences are then recognized by guide RNA specifically designed for the CRISPR system and signal production occurs (Chen et al., 2020: 489-494).

In addition, indirect protein diagnosis can be made by RNA-targeting systems such as CRISPR-Cas13a by recognizing specific RNA biomarkers released by the effect of proteins. For example, some tumor cells secrete certain microRNAs, and the presence of these microRNAs can be detected with high sensitivity by CRISPR-based systems. Protein and peptide-based CRISPR diagnostic systems are candidates to become an important component of clinical diagnosis in

the future, especially with their ability to enable multiple biomarker analysis, rapid response time, and portability (Fozouni et al., 2021: 323-333).

**Pathogenic Microorganisms**

Pathogenic microorganisms are the main agents of infectious diseases that have serious effects on human, animal and plant health. These harmful structures can be in the form of bacteria, viruses, fungi or single-celled elements. Early, specific and rapid detection of these microorganisms is extremely important in terms of preventing the spread of diseases, initiating appropriate treatment in a timely manner and protecting public health.

CRISPR-based diagnostic approaches developed in recent years have pioneered a significant transformation in this field. Such systems have been successfully used in the diagnosis of viruses that are of great importance in terms of public health, such as SARS-CoV-2, Zika, Ebola and Influenza (Fozouni et al., 2021: 323-333).

CRISPR-based systems have also been used effectively in the diagnosis of bacterial pathogens. In particular, by targeting the genetic markers of antibiotic-resistant bacteria, resistant strains can be directly identified from the sample. This is of great value in preventing incorrect antibiotic use and directing patients to appropriate treatment (Kellner et al., 2019: 2986-3012).

**Circulatory Tumor Cell**

Circulatory tumor cells (CTCs) are cells that have separated from primary tumors or metastatic foci and are involved in the bloodstream and have the ability to spread to distant organs. The presence of CTCs is closely related to the progression of cancer and the metastasis process. Therefore, early and sensitive detection of these cells is of critical importance in the diagnosis and monitoring of cancer. Traditional diagnostic methods cannot provide sufficient sensitivity and

specificity due to the low density and cellular diversity of CTCs in the blood. However, in recent years, advances in CRISPR-based diagnostic technologies have offered new and powerful approaches in the detection of these cells. In particular, the specific target recognition and cutting ability of the Cas12a enzyme allows the sensitive detection of CTCs (Lv et al., 2021: 12921-12929; Chen et al., 2018a:436-439).

A CRISPR/Cas12a-based diagnostic method developed using multivalent double-stranded aptamer networks (MDANs) enables rapid and reliable detection of CTCs from blood samples. In this method, special aptamers attached to magnetic beads undergo a structural change by interacting with CTCs. As a result of this change, the released activator DNA molecules activate the Cas12a enzyme. The activated Cas12a directly indicates the presence of CTCs by cutting the marker DNA sequences that create a fluorescent signal. This method reaches a detection threshold of only 26 cells per milliliter in human blood samples and completes the diagnostic process in less than an hour (Lv et al., 2021: 12921-12929).

In addition, another CRISPR-based diagnostic platform called "FINDER" has been developed. This system works by bringing together guide RNA (sgRNA) and Cas proteins that recognize specific gene sequences associated with cancer. When interacting with target genes, the enzymatic activity of Cas proteins is triggered and real-time imaging of living cells is provided through fluorescent signals. This method offers high sensitivity and speed by identifying over 80% of target cells within 20 minutes, which is only 0.1% (Li et al., 2022). CRISPR-based diagnostic approaches surpass existing methods in terms of specificity, sensitivity, speed and portability in the detection of CTCs, allowing the development of early cancer diagnosis and personalized treatment approaches (Lv et al., 2021: 12921-12929).

**Metal ions**

While metal ions play important roles in the metabolic functions of living things, certain types and concentrations of metal ions pose serious health risks in terms of environmental pollution and toxicity. In particular, heavy metals such as lead ($Pb^{2+}$), mercury ($Hg^{2+}$), cadmium ($Cd^{2+}$), and arsenic ($As^{3+}$) are associated with various diseases such as nervous system disorders, cancer, and organ failure when mixed into drinking water, soil, and the food chain. Therefore, the detection of these ions with rapid, reliable, and portable diagnostic systems is extremely important for environmental and public health (Chen et al., 2022).

Traditional metal ion diagnostic methods are based on advanced laboratory techniques such as atomic absorption spectroscopy, mass spectrometry, and electroanalytical measurements. However, these methods generally require expensive equipment, expert personnel, and laboratory environments, which limits on-site and low-cost diagnostic applications. In this context, CRISPR-Cas technologies are known as an innovative tool for indirect detection of metal ions in environmental and biological samples with their specific and sensitive diagnostic capabilities (Chen et al., 2022; Su et al., 2023).

Although CRISPR systems do not directly detect metal ions, they can indirectly detect the presence of these ions with the help of biomolecular structures that undergo structural transformation. In these systems, DNA staples designed specifically for the metal ion are generally used. These structures, which interact with the metal ion, cause a DNA sequence to be released, and this DNA sequence is recognized and triggered by CRISPR/Cas12a or Cas13 systems. The activated Cas enzyme creates a luminous signal by cutting fluorescent marker sequences, and thus the presence of the metal ion is revealed with high specificity (Zhou et al., 2023). In the literature, there are systems developed specifically for $Hg^{2+}$ (Su et al., 2023) ions.

## Advantages and challenges of CRISPR-based diagnostics

CRISPR technology has revolutionized the field of gene editing and has also pioneered groundbreaking developments in molecular diagnostic methods. In particular, the sensitive diagnostic properties of CRISPR-Cas systems have enabled them to be used in a wide range of areas from infectious diseases to cancer diagnosis. However, as with every advanced technology, in addition to the obvious advantages offered by these systems, there are some technical and practical challenges that need to be resolved.

### Advantages

### High Sensitivity and Specificity

CRISPR-based diagnostic systems can work sensitively enough to distinguish target nucleic acid sequences with a single base difference. The secondary cleavage activities of Cas12 and Cas13 proteins provide signal amplification and can produce positive results even in the presence of very low amounts of target. This means much lower detection limits compared to traditional PCR-based methods (Gootenberg et al., 2018: 439-444).

### Fast Diagnosis Time

CRISPR-based diagnostic systems often work at a speed that can produce results within 30–60 minutes. Especially when integrated with isothermal amplification techniques (e.g. RPA, LAMP), it allows rapid diagnosis in non-laboratory environments. This feature provides a great advantage in the early detection of epidemic diseases in the field (Kellner et al., 2019: 2986-3012).

### Portability and Field Suitability

New generation CRISPR-based diagnostic platforms have become systems that can operate without the need for laboratory infrastructure by being integrated with microfluidic chips and portable

optical readers. This enables effective diagnosis in both developed and resource-limited regions (Fozouni et al., 2021: 323-333).

## Challenges

### Sample Processing and Purification Processes

Although CRISPR-based systems offer high specificity, inhibitory substances (blood protein, mucus, etc.) found in clinical samples can negatively affect the performance of the system. Therefore, pretreatment and purification steps are still time-consuming and can prolong the diagnostic process (Kellner et al., 2019: 2986-3012)

### Signal Noise and False Positives

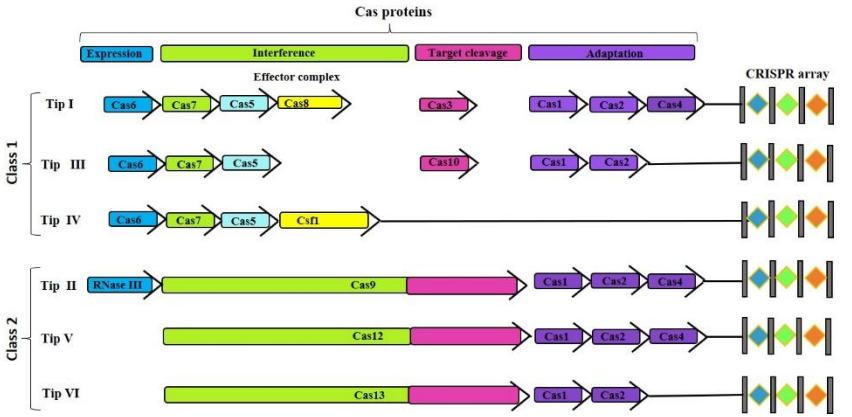
The continuous trans-cutting activity of Cas enzymes after binding to the target can cause the signal to saturate over time. This can lead to false positive results, especially at low target densities (Hendriks et al., 2022).

### Lack of Industrial Production and Standardization

Commercial-scale production of CRISPR-based diagnostic kits is currently carried out by a limited number of companies, and standard protocols need to be developed for widespread use. Validation processes must be completed in order to be widespread in clinical applications (Fozouni et al., 2021: 323-333).

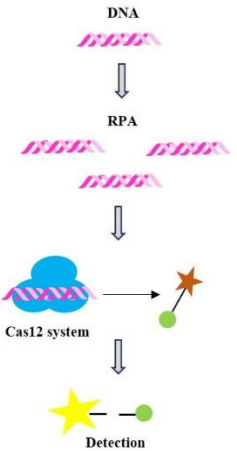
In order for CRISPR technology to fully realize its potential in the field of diagnostics in the future, technical developments as well as improvements in legislation and manufacturing processes are required (Zhang et al.,2020:1651-1669).

*Figure 1: The classification of CRISPR-Cas systems*



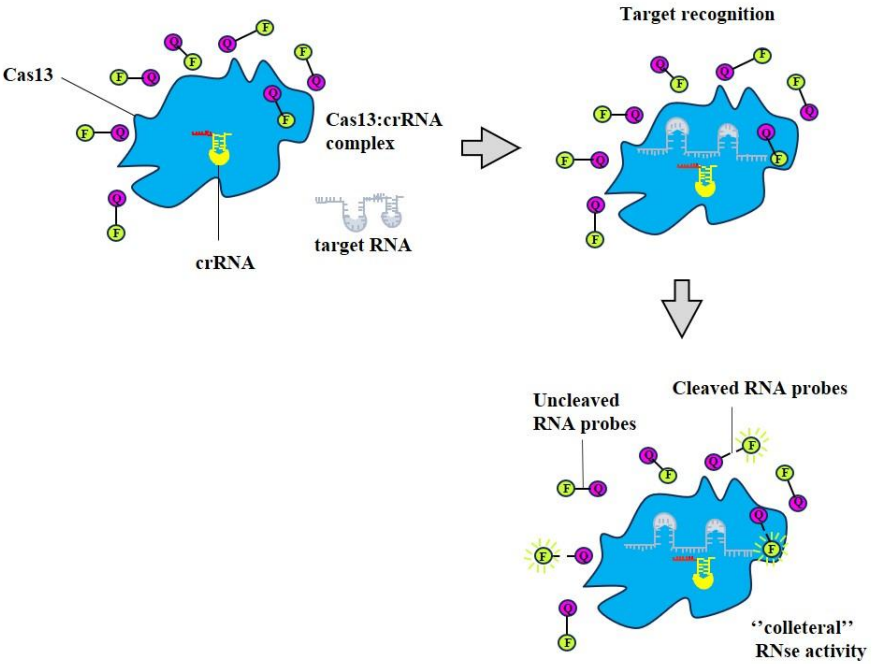

*Makarova, etl al.. (2020) Evolutionary classification of CRISPR–Cas systems: a burst of class 2 and derived variants. Nature Reviews Microbiology, 18(2), 67-83.*

*Figure 2: The shematic respresentation of DETECTR systems*




*Sauvagère, S., & Siatka, C. (2023). CRISPR-Cas: 'the multipurpose molecular tool' for gene therapy and diagnosis. Genes, 14(8), 1542.*

*Figure 3: The shematic respresentation of SHERLOCK systems*

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# INTEGRATING RECOMBINANT DNA TECHNOLOGY IN FOOD SCIENCE AND TECHNOLOGY: INNOVATIONS AND IMPLICATIONS

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## Introduction

Recombinant DNA (rDNA) technology comprises the manipulation and recombination of genetic material from diverse sources to generate unique DNA sequences by using enzymatic activity and various laboratory techniques (Lone & Shah, 2023). rDNA has been discovered by biotechnological and molecular biological researches since the 1970s. The starting point was to understand gene function and expression (Roberts, 2019). rDNA technology has progressed to accomplish different purposes including the creation of genetically modified organisms (GMOs), biopharmaceuticals, and agricultural traits (Feldman et al., 2015; Vipra et al., 2022). Food science and technology is a multidisciplinary field that brings together multidisciplines from engineering, microbiology, chemistry, and physical sciences for the processing, preservation, and distribution of

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food (Bremner, 2010). The integration of rDNA technology into food science and technology has revealed an important progress in food processes. The particular genetic modications has enabled the creation of novel food ingredients, surpassing microbiological safety, production functional foods. The rising levels of global food consumption coupled with growing concerns about food security, sustainability, and health, emphasize the potential of integrating rDNA technology to food science and technology in order to deal with such complicated problems (Lensh et al., 2022). This chapter covers potential applications of rDNA technology in food science, technology, and nutrition, underlining its pros and cons within food systems.

## Recombinant DNA Technology in Agricultural and Livestock Productions

In agricultural biotechnology, recombinant DNA techniques are utilized to integrate genes that provide resistance to insecticides, herbicides, or other environmental challenges. The introduction of novel genes into cotton accelerated the production of insecticidal proteins, thus reducing the necessity of chemical pesticides and improving production stability (Li et al., 2015). Furthermore, genes that enhance drought tolerance or fertilizer performance were employed to create climate-resilient cultivars. Certain genes have been identified in crop-wild relations and successfully transferred to other crops, rendering them adaptable to changing climates (Lusser et al., 2012).

Plant transformation is an elementary method in plant biotechnology performed for implementing desirable traits into an existing genome while maintaining the original genetic material of the germplasm. This is mostly accomplished using Agrobacterium-mediated gene transformation, electroporation, or gene gun techniques. The simplicities of this procedure are the ability to transfer larger DNA fragments and reproducibility. The fundamental characteristics of this method make the Agrobacterium-mediated

approach preferable to alternates (Anand & Jones, 2018; Tian et al., 2021)

rDNA technology has been employed to enhance the expression of genes related to mineral absorption and storage. Genes encoding iron transporters and ferritin, a protein responsible for iron storage, have been incorporated into rice and wheat to enhance their iron content and improve its stability and bioavailability (Beyer, 2010). Similarly zinc transporter genes and genes that regulate the synthesis of metal-chelating compounds, such as nicotianamine or phytosiderophores, which improve zinc absorption, have been introduced into cereals (Hensel, 2020). These technologies allow the development of nutritionally altered crops without requiring chemical fortification or additional supplements (Thakor & Charles, 2025).

Genetically modified crops are designed to increase manufacturing, maximize profit and crop management, lower labor demand, and enable the spread of safer management techniques. The labor-intensive nature, difficulties with trait introgression and regulation, time constraints, and high costs are some of the limitations of this approach (Munaweera et al., 2022).

Transgenic crops are thought to be an essential solution to several climate change-related issues, particularly as it comes to reducing $CO_2$ emissions. Transgenic plants that can be adjusted to harsher climate conditions by using less energy and fertilizer, they were also able to appease agricultural producers (Arora, 2019).

Since rDNA technology allows for precise genetic modifications that improve product quality, productivity, and animal

health, it has opened up new possibilities in the livestock production industry. The most popular method is incorporating advantageous exogenous genes into transgenic animal reproduction. For example, growth hormone genes have been introduced into fish and pigs to increase growth rates and maximize feed conversion efficiency. Cattle have been produced using rDNA techniques to produce animals with improved immunity to diseases such as mastitis and bovine tuberculosis. These changes minimize the need for antibiotics and veterinarian treatments. Also, improvements in gene editing technologies like CRISPR-Cas9, which allows for the simultaneous generation of multiple characteristics, have improved the precision of targeted genomic alterations (Hayes et al., 2013; Pech et al., 2020; Zhang et al., 2020).

One of the main uses of rDNA technology in livestock is the creation of biological agents and recombinant therapeutic proteins in transgenic animals. Goats and calves, for example, have been genetically modified to produce human proteins, such as lactoferrin and antithrombin, providing a varied and affordable source of raw materials for the manufacturing of biopharmaceuticals. An alternative to traditional cell culture systems could be the separation and purification of proteins from milk-presenting cells (Bhat et al., 2010).

Additionally, test kits and vaccines for animal diseases are developed using rDNA methodologies (Gama Sosa et al., 2010). Subunit vaccines made from recombinant microorganisms or plants have proven to offer better protection and specificity in vaccinations against foot-and-mouth disease and classical swine fever (Liu et al., 2010). By eliminating potential risks related to the spread of zoonotic illnesses and antibiotic resistance, these developments protect public health and food safety while also improving the safety and health of livestock. A scientific solution to the increasing demand for sustainable animal-derived sustenance worldwide is the application of

rDNA technology in livestock production (Georges et al., 2019).Despite the fact that transgenic animals have been developed to enhance growth rates, disease resistance, and feed production capacity, their common application is currently restricted by legal and ethical considerations. These possible uses illustrate the potential of rDNA technology to deal with global issues related to food security and sustainable livestock production through targeted genetic modification (Sprink et al., 2016).

## rDNA Technology for Microbial Cultures in Fermentation-Based Food Production

rDNA technology has greatly improved the growth and purification of microbial cultures used in fermentation-based food production. It may be possible to improve metabolic pathways that produce desired molecules for fermentation, including organic acids, alcohols, aroma compounds, and bioactive peptides, by precisely altering microbial genes using rDNA techniques (Terefe, 2022). *Saccharomyces cerevisiae* recombinant strains have been developed to improve ethanol yield, boost resistance to fermentation stressors (such as high temperature and ethanol concentration), and find substitute carbon sources, like xylose from lignocellulosic biomass (Olofsson et al., 2008). Lactic acid bacteria (LAB) play an important role in the production of many fermented products. LAB went through genetic modification to elevate the amounts of enzymes involved in flavor, bacteriocin synthesis, and lactose metabolism. As a result, fermented foods now have longer shelf life, better flavor profiles, and more beneficial characteristics (Raj et al., 2020; Wu et al., 2021).

Moreover, metabolically modified microbial systems that are designed to produce particular fermented products can be developed with the assist of rDNA technology. While recombinant strains of

*Corynebacterium glutamicum* and E. *coli* are commonly used for the commercial production of amino acids, such as glutamate and lysine, have created genetically modified strains of *Lactococcus lactis* to produce vitamins (such as folate and riboflavin) and bioactive peptides that offer health benefits (Yi et al., 2014). In addition, complex starter cultures that can not only carry out conventional fermentation but also produce essential proteins like enzymes or antimicrobial peptides or provide probiotics during food fermentation could be constructed from recombinant microbial cultures (Cho et al., 2020). Significant advancements in fermentation control, efficiency, and functionality are demonstrated by rDNA technology (El Sheikha, 2018).

**Biosynthesis of Functional Food Ingredients by rDNA Techniques**

One of the emerging field in food biotechnology is the biosynthesis of functional food ingredients using rDNA techniques. It encourages the efficient and long-term synthesis of bioactive substances that put health above plain dietary requirements. Prebiotics, probiotics, bioactive peptides, polyphenols, omega-3 fatty acids, and plant sterols are examples of functional food ingredients that could be produced or supplemented using genetically modified microorganisms or plant systems (Joana Gil- Chávez et al., 2013; Miras-Moreno et al., 2016). In order to produce more conjugated linoleic acid (CLA), γ-aminobutyric acid, or short- chain fatty acids which are vital for the control of metabolism, cardiovascular health, and the intestinal microbiota, recombinant strains of *Lactobacillus* species or *E. coli* have been designed (Gorissen et al., 2015).

Another possible nutrient synthesis by recombinant microbial cultures is the production of vitamins, including riboflavin, folate, and vitamin B12 (cobalamin). There might be

some restrictions on the natural synthesis of these vitamins using conventional fermentation techniques. However, crucial regulatory enzymes involved in the pathways leading to vitamin biosynthesis could be overexpressed due to rDNA-based biotechnology. Lactococcus lactis produces more folate due to the overexpression of the folP and folK genes, which makes it easier to use as a functional ingredient in dairy products. Different microorganims have been genetically engineered to increase riboflavin production on industrial scales (Averianova et al., 2020). These biotechnological innovations minimize the dependency on synthetic chemicals, provide more sustainable production techniques, and offer the enrichment of functional foods without changing sensory properties of foods (Acevedo-Rocha et al., 2019; Saxena, 2015).

An important application area of rDNA technology is synthesis bioactive peptides and proteins with intended health- promoting effects, including antioxidant, antihypertensive, immunomodulatory, or antibacterial properties. (Li, 2011). Genes which are encoded by these peptides could be integrated into microbial hosts or plant systems for improving regulated expression and extensive manufacturing (Bonander & Bill, 2012). Antimicrobial peptides, including bacteriocins, have been expressed by recombinant *Lactobacillus* species. These peptides encourage gastrointestinal health and improve food safety by inhibiting pathogenic microorganisms (Wingfield, 2015). Furthermore, using recombinant yeast or plant-based systems therapeutic proteins such as human lysozyme and lactoferrin have been synthesized to improve the nutritional and immunological quality of food products including infant formulas and functional dairy products (Wang et al., 2022).

Organic trace minerals, such as selenium-enriched yeast and bioavailable zinc complexes, are produced in microbial systems

using rDNA technology. These minerals are easier to absorb in the human gastrointestinal tract than their inorganic equivalents. Recombinant yeast strains often accumulate selenium as selenocysteine or selenomethionine, which are more bioactive and less toxic. To encourage the consumption of minerals, these microorganisms could be added to functional foods or taken as dietary supplements (Akram et al., 2020; Khan et al., 2022; Mattanovich et al., 2012).

Furthermore, in addition to improving component digestibility and production rate, the effective application of rDNA techniques in the biosynthesis of functional food components offers many opportunities to solve particular nutritional issues. This encourages the development of novel functional foods targeted at specific health issues like diabetes, inflammation, or neurodegeneration. Personalized nutrition and health promotion strategies in modern food systems are expected to be built on rDNA- based biosynthesis (Carocho et al., 2014).

**Recombinant Enzymes in Food Processings**

Recombinant enzymes are important items for modern food processing since they tend to improve the efficiency, stability, and specificity of various food processes (Hua et al., 2018). The cloning genes are capable of creating particular enzymes in microbial organisms such as *Aspergillus niger*, *Saccharomyces cerevisiae*, and

*E. Coli*. The food industry benefits greatly from the production of large quantities of food-grade enzymes with consistent quality and smaller variations from recombinant enzymes compared to conventional extraction from animal or plant sources (Udhaya Kumar et al., 2025). The development of particular enzyme types with remarkable catalytic efficiency, thermal stability, and pH adaptability is also encouraged by this approach, which improves the control and optimization of food processes (Thapa et al., 2019).

An outstanding recombinant enzyme used in the food industry is chymosin. Previously, it had been conventionally derived from stomachs of calves and utilized in the manufacturing of cheese in order to coagulate milk. The gene from bovine chymosin has been successfully incorporated into microbial hosts permitting the animal- free, recombinant synthesis of this enzyme. Recombinant chymosin eliminates demand on animal rennet while raising purity and activity of enzyme resulting in more consistent cheese quality and product yield (Kumar et al., 2010). Regulating organizations, including the Food Drug Administration (FDA) and The European Food Safety Authority (EFSA) have approved its utilization and recombinant chymosin is widely used in commercial cheesemaking process. Similar achievement has also been accomplished with numerous food enzymes, including lipases, proteases, amylases, and pectinases which are essential for the processing of bakery and dairy products, beverages, and fruit juices (Efimochkina et al., 2023).

Recombinant amylases are utilized in bakery industry to breakdown starch into other fermentable sugars, improving dough handling, crust color and shelf life (Movahedpour et al., 2022a). Recombinantly generated xylanases and glucose oxidases have been used to improve bread texture and retard staling (Khatami et al., 2022). Pectinases from genetically modified fungi are widely applied in the beverage industry to clarify wine, fruit and vegetable juices (Ahmed et al., 2021). β-galactosidase enzyme produced through recombinant expression is employed in the dairy sector to hydrolyze lactose, providing the production of lactose-free products for individuals with lactose intolerance (Movahedpour et al., 2022b).The availability of recombinant enzymes designed for specific process conditions optimizes efficiency and product quality and also increasing sustainability by lowering energy consumption and waste production (Albayati et al., 2024).

Other developments in protein engineering as well as controlled selection techniques offers better optimization for enzyme performance at the molecular levels. Recombinant enzymes can be optimized for exhibiting specific characteristics, such as inhibitor resistance, strengthened substrate selectivity, or compatibility with new substrates through site-directed mutagenesis or high-throughput screening of mutant collections. The enzymes as essential for the advancement of novel food processing technologies, such as enzyme-assisted extraction, encapsulation, and creation of functional food ingredients. The utilization of recombinant enzymes in food processings will continue to rise according to governmental acceptability and consumer knowledge of biotechnology in order to create more efficient, sustainable, and healthy food systems (Albayati et al., 2024; Liu et al., 2024).

**Genetically Modified Organisms (GMOs) in the Food Industry**

Genetically Modified Organisms (GMOs) are comprising plants, animals, or microorganisms whose genetic composition has been deliberately modified by modern biotechnology, notably rDNA technology, to attach, remove, or replace particular genes. The genetic modification of organisms allows exact manipulation at the molecular level by involving gene transfer between unrelated species (transgenesis) or targeted modifications within the genome (Sandhu et al., 2025).

GMOs are valuable to the food industry since they raise agricultural output, improve nutritional value and prolong shelf life of food products. rDNA technology enables the incorporation of genes from many species into the genomes of crops or microorganisms, establishing the expression of advantageous characteristics that can not be achieved by regular breeding procedures. In agricultural crops, adaptation processes often correlate with the integration of genes that provide resistance to

insects, herbicides, or harsher environmental challenges. Ultimately, these genetic modifications point to a more sustainable agricultural production strategy by lowering the need for agricultural chemicals and maintaining production under challenging conditions (Noak et al., 2024; Yadav et al., 2024).

GMOs are widely used in the food industry to create crops that are more nutritious. The most commonly grown genetically modified crops are canola, corn, and soybeans (Vahdani et al., 2024). The most famous example of genetically modified rice is Golden Rice. It is altered to include genes from maize that help the rice endosperm produce β-carotene, which is a precursor to vitamin A (Arvas, 2025). In populations that largely depend on rice as their main food source, vitamin A deficiency is expected to be treated with this methodology. Various concentrations of essential amino acids, minerals such as zinc, and iron have been biofortified into maize and cassava (Majumder, 2024). In order to improve the productivity of processing, flavor, and texture, the brewing, baking, and dairy industries carry out genetically modified yeasts and microorganisms. These applications illustrate the frequently underestimated but widespread importance of GMOs within modern food production systems. GMOs are needed for preventing hunger and expanding global health, particularly among underdeveloped nations (Mirsalami & Mirsalami, 2025)

Global laws and regulations for GMOs aim to guarantee food safety, environmental security, and consumer transparency through strict scientific investigations and policy implementations (Weimer, 2025). The most significant authorities, including FDA, EFSA, and international organizations such as the Codex Alimentarius Commission, have formulated comprehensive risk assessments based on molecular characterization, toxicology, allergenicity, nutritional composition, and environmental impacts. These

assessments frequently implement an individual cases methodology, considering both the origin of the transgene and the particular alterations applied (Paola Ferretti, 2007). For example, EFSA conducts a comprehensive investigation between the GMO and its regular equivalent, incorporating in vitro and in vivo research if required. The FDA necessitates voluntary consultation prior to commercialization and requires labeling solely when nutritional or safety profiles vary significantly. The general opinion regarding GMOs continues to be divided despite the fact that widespread scientific agreement over their safety. The aspects encouraging doubt include misinformation, ethical concerns over 'tampering with nature' and suspicion in commercial goals (Strauss & Sax). Consequently, science-based risk communication, public education, and transparent regulatory strategies are fundamental to build public trust and encourage enlightened customer choices for GMO acceptance (Tahir et al., 2024).

## Environmental and Economic Impacts of rDNA Technology for Food Industry

The implementation of rDNA technology in the food industry has produced considerable environmental advantages by enabling more efficient and sustainable production. Genetically modified crops created with rDNA technology, including herbicide- tolerant and insect-resistant types, have resulted in decreased pesticide and herbicide usage, thereby reducing chemical discharge, soil degradation, and other adverse effects. Moreover, rDNA- enhanced microbial fermentation methods for the production of enzymes, amino acids, and vitamins substitute traditional organic synthesis pathways also while lowering consumption of energy, greenhouse gas emissions, and industrial waste (Bawa & Anilakumar, 2013).

rDNA technology helps to improve productivity and cost-efficiency in agricultural and food production in terms of financial perspectives. Furthermore, recombinant enzymes and microorganisms

employed in food processing offer high-yield production under regulated conditions and also are able to decrease production costs and assure higher end product quality. These advances have created new possibilities for marketing, improved supply chain efficiency, and supported the growth of the bioeconomy (Sharma et al. 2022)

## Future Prospects and Ethical Considerations of rDNA Technology

Developments based on rDNA present significant ethical concerns that must be solved along with scientifical investigations. The ethical considerations contain the possibility of accidental ecological consequences, including gene transfer to non-target species, issues related to equality and availability. The rDNA-based inventions may result in some economical disadvantages and marketing reliability. Furthermore, misinformation regarding GMOs remains frequently related to the absence of transparency, false information, and ethical concerns about 'unnatural' modifications in food production. It is critical to bring together bioethical concepts, inclusive governance, strong rules and regulations that prioritize safety and transparency in order to eliminate ethical considerations. Public discussions, scientific communications, and interdisciplinary collaboration will be necessary for providing trust and driving the ethical implementation of rDNA technologies into the future of food (Hundleby & Harwood, 2018; Qaim, 2020).

## Conclusion

rDNA technology signifies a major revolution in food science and technology by permitting exact genetic modification to improve food quality, safety, sustainability, and nutritional value. The combination of molecular biology, biotechnology, and food engineering has enabled rDNA techniques to produce genetically modified crops, recombinant enzymes, microbial fermentation systems, and biofortified foods which are dealing with key problems consisting of food security, resource efficiency, and nutritional deficiency. The scientific rigor of

rDNA applications underlies, spanning gene identification, expression, safety assessments, and regulatory compliance. rDNA technology is set to assume a more crucial role in developing resilient, health-promoting, and environmentally sustainable food systems. Ongoing interdisciplinary research, ethical supervision, and transparent interaction will be essential to fully realize the potential of rDNA in the global food industry.

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# CELL-FREE SYNTHETIC BIOLOGY FOR COMPLEX BIOCHEMICAL PRODUCTION

**DİDEM MİMİROĞLU**[1]

## Introduction

Synthetic biology is a multidisciplinary area that aims to modulate and re-organize biological functions by designing living systems using engineering principles (A. P. Liu et al., 2022; Tang et al., 2021). Advances in this area have led to significant developments, particularly in the design of genetic circuits, the engineering of metabolic pathways, and the construction of new biological functions. However, traditionally conducted using living cell-based systems are related with numerous challenges and limitations due to the cellular complex metabolic regulations, susceptibility to toxic products, growth and proliferation limitations. Therefore, cell-free synthetic biology, which has significant attention in recent years, offers a new platform that allows the isolation of cellular components (e.g., DNA, ribosomes, enzymes, tRNAs, and energy sources) to use them for many biological reactions and produce many cellular molecules without the need for

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a living intact cell (Koo, Yang, & Park, 2020; Swartz, 2006). Cell-free synthetic biology, also known as the cell-free system, refers to an *in vitro* technology in which separates production of the target molecules and cellular growth, and then, uses the main cellular pathways for producing the molecules are implemented outside the living cell. The cell lysate is used as a basic cell-like environment where the reactions take place (Copeland, Langlois, Kim, & Kwon, 2021). All of these systems allow for the controlled processing of transcription, translation, and metabolic reactions in a single microtube, while also facilitating the production of toxic intermediates, biosynthetic pathway optimization, and rapid prototyping (Rollin, Tam, & Zhang, 2013; Ullah, Khattak, Ul-Islam, Khan, & Park, 2016; Whittaker, 2013).

There is not a living intact cell, but the molecules that carry out specific processes of interest are present in the environment. With this way, the desired products, molecules, proteins, etc. Can be produced, and if a reaction needs to be developed, its continuity can be ensured. Considering the reactions that occur within the intact cell, if there are molecules or products that need to enter the cell, these processes may be difficult due to the cellular membrane. However, when the cell is not an intact, the flow and release of molecules, substrates, or products will occur quite easily. Additionally, undesirable or waste products will not be formed (Swartz, 2006).

Although cell-free synthetic biology has become popular in recent years, its origins comes from to the mid-1800s. Cell-free synthetic biology began to develop in the 1850s with Louis Pasteur's wine fermentation using living yeast cells. Following this discovery, Moritz Traube proposed that biological processes are driven by proteins. Approximately 10 years later, Eduard Buchner achieved cell-free ethanol fermentation using yeast extract and was awarded the Nobel Prize in Chemistry in 1907. In 1961, the use of cell-free

systems for polypeptide synthesis was considered. Additionally, Nirenberg used *Escherichia coli (E.coli)* extracts and this led them to get Nobel Prize in 1968. By the mid-2000s, researchers had started to using metabolic engineering applications into cell-free systems, leading to more efficient metabolite production. In addition, Protein synthesis Using Recombinant Elements (PURE) system which will be described in below, were developed (Koo et al., 2020; Rollin et al., 2013; Ullah et al., 2016; Vilkhovoy, Adhikari, Vadhin, & Varner, 2020).

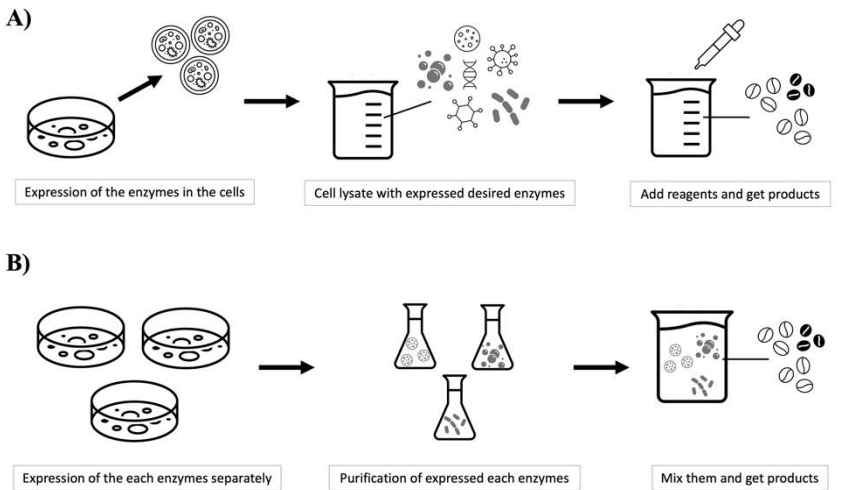
One of the best advantages of cell-free biological systems is that biosynthetic processes can be carried out with less variability, in a more flexible environment and in shorter time periods. These systems, particularly used in the production of natural products, peptides, or enzymes, some toxic products and high-cost macromolecules offer significant contributions to biotechnological applications at both research and industrial scales. Furthermore, cell-free systems have become an alternative and powerful tool for the functional analysis of gene clusters derived from microorganisms that are difficult to genetically manipulate (Claassens, Burgener, Vögeli, Erb, & Bar-Even, 2019; Karig, 2017).

To conclude, cell-free synthetic biology is a revolutionary approach in a multidisciplinary area, with a wide range of applications, including sustainable bioproduction, drug development, biosensor design, and artificial cellular metabolic fabrication. By overcoming the limitations of living intact cells and providing a more productable environment, these systems can play a central role in both fundamental research and applied biological processes in the future.

## Classes of Cell-Free Biological Systems

Cell-free systems essentially refer to the use of cellular molecules such as nucleotides, metabolites, RNAs and proteins etc. outside the cell. However, how this cell-free synthetic biology is carried out is classified differently. When considering the setup of the system, cell-free synthetic biology is divided into two categories: top-down and bottom-up systems (Fig. 1). Basically, top-down systems are studied by obtaining soluble extracts from lysed cells, while bottom-up systems are constructed through purified components [12].

*Figure 1. The two main types of setups for cell-free synthetic biology systems. A) Top-down system (the cell lysate method), B) Bottom-up system.*




In a cell lysate, also known as a top-down system, the macromolecules or pathways of interest are first expressed/produced in natural organisms such as *E. coli, Streptomyces* and etc. After that, the cell membrane is disrupted, effectively converting the cells into lysates. Finally, different types of purification steps can be applied

to remove unwanted components, waste products and molecules (Moore, Lai, Li, & Freemont, 2022; Moritz et al., 2021; Vilkhovoy et al., 2020). In this way, the complex cellular content is preserved, and the natural biochemical environment remains largely sustainable.

Using this cell lysate method rather than using intact cell, is more advantageous since it is simple and low-cost process. Furthermore, the necessary cofactors, enzymes, proteins, RNAs, soluble organic/inorganic elements and etc. are readily available, and therefore, additional specific molecules are not required leads to reducing costs. In addition, higher protein yields are obtained. The cell lysate approach is the most preferred method for the commercial cell-free biological synthesis, especially protein production and the production of natural products (Table 1) (Batista, Soudier, Kushwaha, & Faulon, 2021; Chiba, Knirsch, Azzoni, Moreira, & Stephano, 2021; R. J. R. Kelwick, Webb, & Freemont, 2020; J. Liu et al., 2023). On the other hand, there are also some disadvantages of the cell lysate method include low component/content control, limited experimental flexibility, and the need for additional engineering methods to preserve many metabolic pathways for the desired molecule production and eliminate unwanted pathways (Bowie et al., 2020; Ji, Liu, & Li, 2022; A. P. Liu et al., 2022; Rasor & Erb, 2025).

The second method is the bottom-up system which is related with developing and setting-up new cellular environment. In this method, subcellular components such as ribosomes, tRNA, enzymes, specific proteins for the desired pathways etc., are purified separately and then, added to the process medium at the desired concentrations for using together. Since the purified components can be combined at the desired concentrations, this method can yield better results than the top-down process (Batista et al., 2021; da Silva et al., 2024; A. P. Liu, 2019). The most important advantages of this

system are that the components are controllable and also traceable, there are no complex intracellular systems, and it is ideal for newly designing and modeling biological systems (Table 1) (Batista et al., 2021; Chiba et al., 2021; R. J. R. Kelwick et al., 2020; J. Liu et al., 2023). In addition, due to the absence of metabolic activity, experimental flexibility is quite high. It is particularly preferred for protein engineering studies and fundamental cellular research (Batista et al., 2021; da Silva et al., 2024).

*Table 1. The examples of some products for producing with cell-free synthetic biology systems in the last 10 years*

| Types of cell-free systems | Products | References |
|---|---|---|
| **Top-down system** | Bio-cellulose | (Ullah, Ul-Islam, Khan, Kim, & Park, 2015) |
| | Gold nanoparticles | (Krishnan, Narayan, & Chadha, 2016) |
| | Silver nanoparticles | (Costa Silva et al., 2017) |
| | Monoclonal antibodies | (Martin et al., 2017) |
| | Limonene | (Dudley, Nash, & Jewett, 2019) |
| **Bottom-up system** | Polyhydroxybutyrate | (Opgenorth, Korman, & Bowie, 2016) |
| | Lactic acid | (Kopp, Willows, & Sunna, 2019) |
| | Isoprene | (Cheng et al., 2017) |
| | Isoprenoids | (Ward, Chatzivasileiou, & Stephanopoulos, 2019) |
| | D-1,2,4-butanetriol | (Gao et al., 2019) |
| **Hybrid system** | Epidermal growth factor receptor (EGFR) | (Quast, Sonnabend, Stech, Wustenhagen, & Kubick, 2016) |
| | Pinene | (Niu, Huang, Shen, Ji, & Liu, 2020) |

As it is mentioned before, cell-free synthetic biology offers a platform that enables biological processes to be carried out outside of cellular structures. Thus far, cell-free synthetic biology was divided into two main categories based on the strategies how

constructing the cell-free systems. In addition to these categories, cell-free synthetic biology is further divided into two other categories based on how it is used in relation to the platform on which it is built: transcription-translation based systems (TX-TL systems) and hybrid cell-free enzymatic systems.

TX-TL systems comprise the different steps whic are the transcription of DNA to mRNA, subsequent translation processes *in vitro.* The desired product is obtained by adding the required substances for these processes which are cell lysate, ribosomes, amino acids, tRNAs, and all necessary supporting molecules to the same medium. *E. coli* cell extract is generally preferred for this purpose. Since the all processes occurs *in vitro*, one of their greatest advantages is the ability to produce toxic proteins.

In addition, the production of synthetic amino acids, the ability to facilitate rapid prototyping cycles, the ability to create different production ways outside the cell, and the minimal or no producing of waste or side products are among the most important factors in favoring this system. However, it also has some disadvantages such as rapid depletion of energy resources, the potential for some enzymes in the lysate to cause deterioration of DNA and proteins, and lower yields compared to living systems. For this reasons, PURE system was developed for better and more controlable processes (Bowie et al., 2020). This system includes two processes which are producing recombinantly all the necessary elements for protein synthesis, such as ribosomes, tRNAs, and aminoacyl-tRNA synthetases, and mixing them together in one pot. The RNA polymerase used in the PURE system is generally T7 RNA polymerase, enabling the system to work with enzymes derived from prokaryotic organisms, especially *E. coli* (Batista et al., 2021; Brookwell, Oza, & Caschera, 2021; Copeland et al., 2021; Ji et al., 2022).

Hybrid systems have been developed to solve some challenges come from classical systems in cell-free synthetic biology. Therefore, they combine the advantages of top-down and bottom-up systems based on the system setup perspective. In other words, hybrid systems have the principles of TX-TL systems and cell-free enzymatic systems. This allows many benefits to the systems which are the biochemical advantages of cellular lysates and the controllability of purified components using systems like TX-TL systems. In addition, a key advantage of these hybrid systems is their ability to eliminate the biological complexity that can arise during system setup through biological engineering (Bowie et al., 2020; Rasor & Erb, 2025). For example, it is possible to purify and incorporate the enzymes required for the pathways used for the desired molecule in a system initiated using *E. coli* lysate. Furthermore, the natural cellular microenvironment found in the lysate allows for more efficient folding and modification processes required for a protein (Bowie et al., 2020). Hybrid systems are also being used for microfluidic systems, nanomaterial-supported enzyme stabilization, mimicking the cellular environments, and developing artificial lipid membrane models owing to technological approaches developed (Baranwal & Maerkl, 2024).

In addition to all these classifications, cell-free systems can also be evaluated based on the cell lysate source. This type of classification can significantly have effects on the protein/other molecule synthesis capacity of cell-free systems and the cost of the product. Classification for the cell lysate source is divided into prokaryotic and eukaryotic, depending on the source from which the lysate is obtained. While *E. coli*, *Bacillus subtilis*, and Streptomyces species can be given as examples for prokaryotes, rabbit reticulocytes and various mammalian cells can be given for eukaryotic cells (Batista et al., 2021; R. Kelwick, Webb, MacDonald, & Freemont, 2016; W. Q. Liu, Zhang, Chen, & Li, 2019; Moore et

al., 2022). Furthermore, while prokaryotic lysates are preferred for rapid and low-cost production, eukaryotic systems are preferred for the production of molecules that need more complex post-translational modifications.

## Conclusion

Cell-free synthetic biology has emerged as a powerful technological platform that enhances speed, flexibility, and control to biomolecular production processes without requiring a living intact cell. Classifications of production systems based on top-down and bottom-up methods demonstrate how the setup of the systems differ, while hybrid systems extend this technology to more complex biological production processes, increasing the applicability of synthetic biology in many areas such as pharmaceuticals, biofuels, biosensors, and diagnostic technologies.

Cell-free synthetic biology, which has begun to using in biological sciences since the mid-1800s, has a role for playin a more progressive in both pure biological sciences and industrial applications in the following years.

In particular, standardization of system components and the integration of microfluidic systems and artificial intelligence algorithms will enable the development of more predictable, faster, and more efficient production platforms. This will increase the suitability for long-term, high-volume production through the development of low-cost and more sustainable energy regeneration systems. These developments have the potential to enhance and change in a better not only the biotechnology industry but also the fundamental understanding of life sciences.

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# TRANSFORMATIVE INNOVATIONS DRIVEN BY RECOMBINANT DNA RESEARCH