Patent Landscape in CRISPR/Cas Technology for Biomedical Applications

A REPORT PREPARED BY LAKSHMIKUMARAN AND SRIDHARAN ATTORNEYS (LKS) FOR BLOCKCHAIN FOR IMPACT (BFI) ON CRISPR/CAS TECHNOLOGY

LKS

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Acknowledgement

We (LKS) would like to extend our heartfelt gratitude to Blockchain for Impact (BFI) for entrusting us with the opportunity to work on this CRISPR/Cas project. The collaboration with BFI has been a pivotal step in solidifying our foundations and deepening our understanding in the CRISPR/Cas gene editing technology and its use in biomedical applications.

We are profoundly grateful for the support and trust we have received from Mr. Sandeep Nailwal, Founder, BFI, and Dr. Gaurav Singh, CEO, BFI. We look forward to the continued collaboration and the positive impact this project will have in the scientific community.

Our sincere appreciation goes to Dr. Satya Prakash Dash, Senior Advisor, BFI, whose invaluable guidance, and expertise have played an essential role in steering the project toward success. This project spanned over a period of four months and Dr. Satya's dedication and insights have been pivotal throughout the process, and we are fortunate to have had the opportunity to work closely with him. The successful completion of this project would not have been possible without his unwavering support and mentoring efforts.

Thank you again for this wonderful opportunity.

Dr. Malathi Lakshmikumaran
Executive Director
Lakshmikumaran and Sridharan Attorneys

Messages

Sandeep Nailwal
Founder, BFI

At BFI, our mission has always been to empower innovation through knowledge. This comprehensive report, a collaborative effort with LKS, represents a significant step towards that goal. By analyzing over 4,000 global CRISPR-Cas patents, we've mapped the key areas of development and identified the leading nations in this groundbreaking field. We believe this deep dive into the patent landscape will be an invaluable resource, particularly for researchers in India, providing crucial insights to guide their own pioneering work and accelerate breakthroughs in CRISPR-Cas technology. This report is a testament to the power of strategic analysis in driving scientific advancement. At BFI we are focused on building a series of knowledge reports that would inform researchers, innovators and policy makers and aid in their innovation journeys.

The CRISPR-Cas technology holds immense potential to revolutionize medicine, agriculture, and biotechnology. Understanding the global patent landscape is crucial for strategic decision-making and fostering innovation. This report, a result of the dedicated efforts of LKS and BFI , provides a detailed analysis of CRISPR-Cas patent filings across the globe. It offers critical intelligence on emerging trends, key players, and geographical hotspots of innovation. We are confident that this report will serve as an essential tool for researchers, industry leaders, and policymakers in India, enabling them to navigate the complexities of this rapidly evolving field and capitalize on its transformative opportunities. India can leverage this emerging technology and hopefully this will play an important role in our ambition to be Viksit Bharat. We are hopeful that this report will be useful for advancement of CRISPR-Cas research and its applications in India.

Dr. Gaurav Singh
CEO, BFI

Dr. Satya Dash
Senior Advisor, BFI

Emerging technologies such as CRISPR-Cas have the power to transform several areas of human endeavour. This report is more than just a Patent Landscape analysis, it is a compass for navigating the future of CRISPR-Cas innovation, especially for researchers and innovators in India and across the world. Our analysis, in collaboration with LKS, illuminates not only where the field has been, but where it is heading and how India could strategise to be a global player and create impact. By identifying key patent trends and active regions, we aim to empower Indian biotech & biomed scientists and innovators to strategically focus their research & innovation efforts, foster collaboration, and accelerate the development of CRISPR-Cas applications that address India's unique needs and contribute to the global scientific community. I thank Dr Malathi Lakshmikumaran and her team for diligently working and making this expansive report which will be of benefit to all stakeholders in the biomedical field and beyond.

MAIN INDEX

Executive Summary

The present report delves into both existing literature and the latest advancements in CRISPR/Cas technology. It examines trends in patent filings related to biomedical research applications, particularly in therapeutics, diagnostics, vaccines, novel Cas enzymes, and biological models. By providing a comprehensive analysis, the report aims to assist companies and organizations in understanding current trends in CRISPR/Cas technology filings and grants. Additionally, it offers valuable insights for researchers to strategically plan their research and innovation efforts.

Globally, CRISPR/Cas-related innovations have seen remarkable growth, as revealed by an extensive analysis of 5,527 extended INPADOC patent families.

- **China** has emerged as the top player with the maximum number of filings. It was observed that China mainly leads in priority filings of applications, with many applications exclusively filed in China and not pursued internationally. The Chinese Academy of Sciences and Huazhong Agricultural University were identified as major applicants in China.

- **The United States of America (US/USA)** is the second major contributor in this pool of applications for CRISPR/Cas-based technology. Although applicants from China have filed the highest number of patent applications, the Broad Institute and University of California, University of Vienna, and Emmanuelle Charpentier (CVC) hold some of the seminal patents in this field.

- **India** also holds a prominent position in contributing to CRISPR/Cas technology applications. While a substantial number of applications were filed in biomedicine, the majority in India were related to agriculture. Institutions such as CSIR-Institute of Genomics and Integrative Biology (CSIR-IGIB), the Tata Institute for Genetics and Society (TIGS), the National Institute of Plant Genetic Research (NIPGR) house prominent researchers actively developing new products using CRISPR/Cas technology. Additionally, companies like CrisprBits utilize CRISPR to develop affordable and high-quality solutions in the life sciences sector.

Other significant contributors include Argentina, Europe, South Korea, and Russia. Jurisdictions like the United Kingdom, Japan, Mexico, Singapore, Australia, Brazil, and Spain also have a decent number of applications filed in the field of CRISPR/Cas-based technology.

In terms of the distribution of applications across different sectors of biomedical applications, the maximum number of applications were filed in the fields of CRISPR/Cas-based therapeutics and diagnostics.

- **Therapeutics:** Nearly half of the patent applications focus on cancer treatment, with significant innovations also targeting genetic, neurological, cardiovascular, and infectious diseases. A major chunk of applications has also been filed in the field of CAR-T cell therapy. Cas9 was identified as the star enzyme for therapeutic applications.

- **Diagnostics:** Cas12 and its variants emerged as the most widely used enzymes, highlighting their efficacy and adaptability in diagnostic applications. Multiple CRISPR/Cas-based diagnostic applications were filed for diseases like Human Papilloma Virus, Zika Virus, Influenza, and cancer. The maximum number of collaborations between applicants was observed in the area of diagnostic applications.

Apart from therapeutics and diagnostics, researchers worldwide are employing CRISPR/Cas technology to develop cell and animal models. While the development of *in vitro* cell-based models is being done by all major jurisdictions, the preparation of animal models is mainly done by Chinese researchers.

CRISPR/Cas technology is also being widely used in vaccine development, with the maximum number of applications filed in key jurisdictions such as China, the US, and Europe. Diseases like toxoplasmosis, influenza, cancer, Corona virus and Herpes virus are being targeted for vaccine development.

This report also provides an overview of applications filed for new Cas enzymes. Most of the seminal patents have been filed by US applicants. Whereas China stands out as the dominant player with the maximum number of patent applications. Another important player contributing actively to this field is Korea.

1 Introduction to CRISPR/Cas: Revolutionizing Biotechnology and The Rise of Gene Editing Patents

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1.1. Overview of the CRISPR/Cas Technology

The year 2020 underscored the significance of biotechnology in the life sciences when Emmanuelle Charpentier (Max Planck Unit for the Science of Pathogens, Berlin, Germany) and Jennifer Doudna (University of California, Berkeley, USA) were awarded the Nobel Prize in Chemistry. Their groundbreaking discovery of CRISPR/Cas9 gene editing, a precise tool for manipulating the DNA of plants, animals, and microorganisms, revolutionized recombinant gene technology. Since its discovery, CRISPR/Cas has evolved as a crucial and imperative gene editing tool with diverse applications in all fields of science.

The more we know, the more we realize there is to know

~Jennifer Doudna

You have to be ready for the unexpected

~Emmanuelle Charpentier

1.1.1. History of CRISPR/Cas

Historically, the journey to the discovery of CRISPR/Cas began in the late 1980s (Figure 1.1) when scientists first observed unusual repeating sequences in the DNA of bacteria. In 1987, Japanese scientist Ishino and his team accidentally discovered these unusual repetitive palindromic DNA sequences interrupted by spacers in *Escherichia coli* while analyzing a gene for alkaline phosphatase.

The biological function of these genes was not ascertained immediately. These sequences, later named Clustered Regularly Interspaced Short Palindromic Repeats[1.1], were found to be part of a bacterial immune system that defends against viruses[1.2]. Over the next few decades, researchers gradually

uncovered the mechanisms behind this system. In 2012, Charpentier and Doudna successfully adapted CRISPR/Cas9 for use in gene editing, enabling precise and targeted modifications to DNA. This breakthrough has since transformed genetic research and opened new possibilities in medicine, agriculture, and biotechnology.

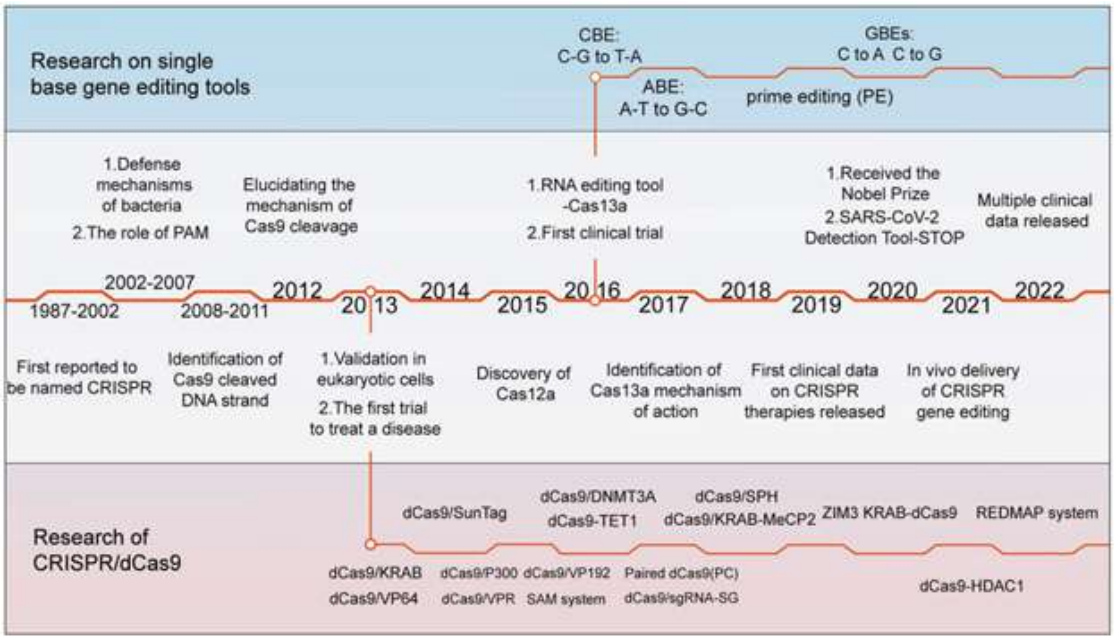


Figure 1.1: History of CRISPR/Cas

(Source: Li, T., Yang, Y., Qi, H. et al. CRISPR/Cas9 therapeutics: progress and prospects. Sig Transduct Target Ther 8, 36 (2023). https://doi.org/10.1038/s41392-023-01309-7[2.1])

In the past decade, there has been a notable rise in patent filings exploring various applications of CRISPR/Cas technology. Equally impressive is the extensive and in-depth research that has enabled scientists to understand the complexities of this technology and utilize it for different purposes.

The present report provides a detailed overview of the patents filed across different sectors of biomedical research, with a particular focus on the use of CRISPR/Cas technology in therapeutics, diagnostics, and vaccines. It also explores the application of CRISPR/Cas technology in developing new cell lines and animal models, which are essential for screening new chemical entities. Additionally, the report examines new or variant Cas enzymes that hold great promise for future biomedical applications.

1.1.2. Role of CRISPR/CAS in Nature:

CRISPR/Cas systems, which stand for Clustered Regularly Interspaced Short Palindromic Repeats and CRISPR Associated proteins, are adaptive immune mechanisms present in most bacterial species and

nearly all sequenced archaeal species[1,3]. These systems have evolved over billions of years to protect microbes from foreign nucleic acids, such as bacteriophage genomes and conjugating plasmids, by targeting and neutralizing their DNA or RNA.

The CRISPR/Cas systems mediate adaptation immunity (immunization and defense) through three general phases (Figure 1.2):

- Adaptation Phase or Acquisition phase
- CRISPR RNA (crRNA) Processing
- Interference Phase

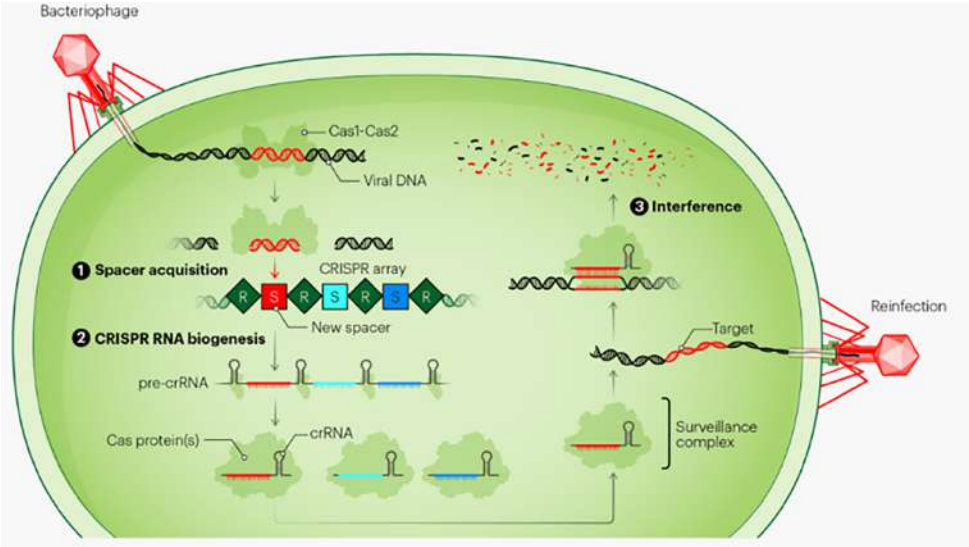


Figure 1.2: CRISPR-Cas systems mediate adaptation immunity in bacteria.

Source: Innovative Genomics Institute. (n.d.). CRISPR in nature. Innovative Genomics Institute. Retrieved March 28, 2025, from https://innovativegenomics.org/crisprpedia/crispr-in-nature/

1.1.3. Classification of CRISPR/Cas System:

Based on their structure and function, Cas proteins are divided into two main classes: Class I and Class II. These classes are further broken down into six types (Types I–VI). Class I encompasses Types I, III, and IV, while Class II includes Types II, V, and VI. The classification within each class is determined by the specific Cas endonuclease involved in the cleavage process and its mechanism of action.

Different Types and Subtypes within the two classes of CRISPR/Cas

Till date, a total of 6 types and 36 subtypes have been identified within the two classes of CRISPR/Cas, as noted hereinabove and depicted herein below:

Different CRISPR/Cas systems have their own characteristics, such as different protospacer adjacent motif (PAM) regions, different Cas protein sizes, and different cleavage sites, which are summarized in Figure 1.3 below.

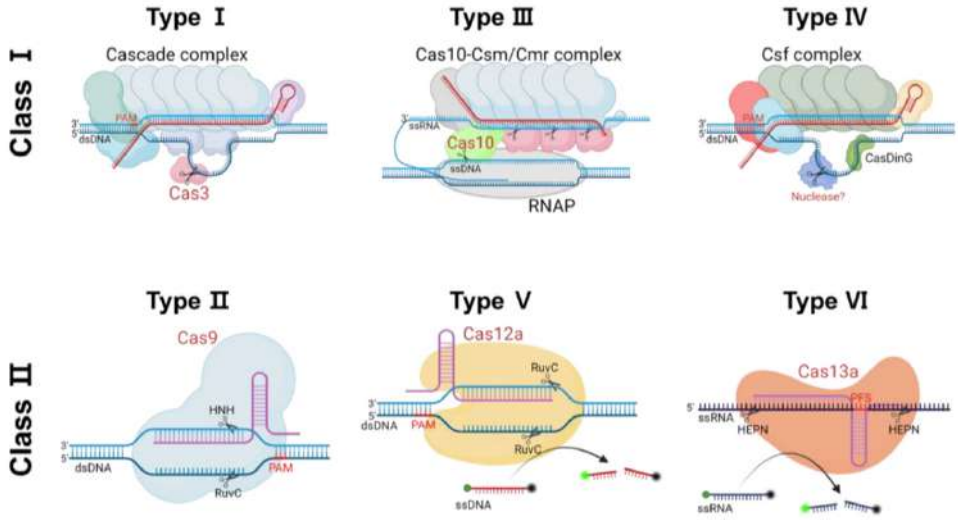


Figure 1.3: Schematics of the mechanisms of the CRISPR/Cas system types.

(Source: Choi, W., Cha, S., & Kim, K. (2024). Navigating the CRISPR/Cas Landscape for Enhanced Diagnosis and Treatment of Wilson's Disease. Cells, 13(14), 1214.)

1.1.4. Mode of Action of CRISPR/Cas in Eukaryotic Cells:

The CRISPR system stands out compared to Zinc Finger Nucleases (ZFNs) and Transcription Activator-Like Effector Nucleases (TALENs), especially when it comes to creating large sets of vectors for targeting multiple sites, including entire genome-wide libraries. Another key advantage of CRISPR is its ability to multiplex, which means it can use multiple guide RNAs at the same time to target different sites within a single cell. This enables the simultaneous manipulation of several genes or the precise engineering of deletions in specific genomic regions[1.4].

For the CRISPR/Cas protein to accurately cut the target DNA, the guide RNA must have a complementary sequence, and the DNA must include a specific protospacer adjacent motif (PAM) sequence (Figure 1.4. This requirement helps ensure that CRISPR does not mistakenly cut its own genetic material. As CRISPR research progressed, it became clear that bacteria have various CRISPR systems, each with distinct biochemical properties[1.5].

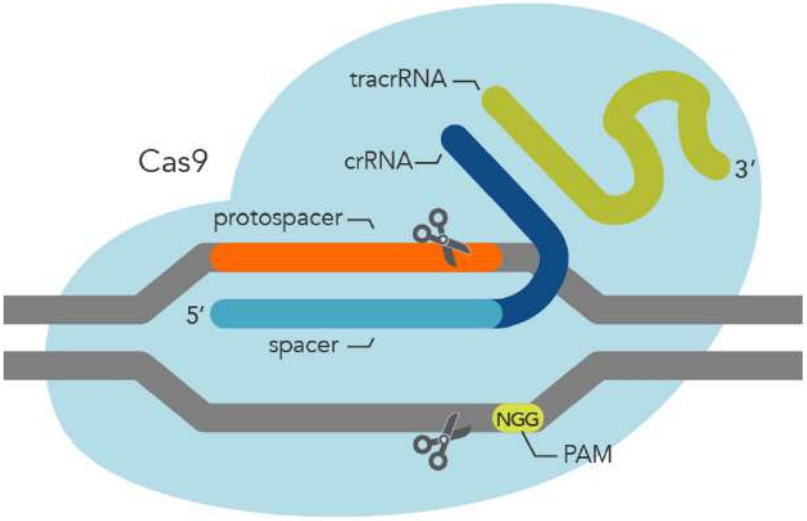


Figure 1.4: Components of a CRISPR/Cas9 system for directing Cas9 endonuclease to genomic targets

(Source: Integrated DNA Technologies. (n.d.). CRISPR/Cas nucleases: Understanding PAM requirements and Cas diversification strategies.)

In CRISPR studies, protospacer adjacent motifs (PAM) are short DNA sequences situated right next to the target modification site. These sequences are crucial for any CRISPR experiment because they are the DNA segments recognized by the Cas nuclease. PAM sequences serve as signals for Cas nucleases, indicating that they have located the correct modification site[1.6].

1.1.5. Application of CRISPR/Cas Technology

The CRISPR/Cas system has become a transformative tool in genome engineering, impacting various fields such as agriculture, research, and medicine (Figure 1.5). In medical science, CRISPR/Cas9 has proven to be both versatile and cost-effective, significantly advancing the potential for gene therapy.

Figure 1.5: Applications of Genome engineering

(Source: Development and Applications of CRISPR/Cas9 for Genome Engineering (Hsu, Patrick D. et al. Cell, Volume 157, Issue 6, 1262 – 1278)

The initial phase of the CRISPR revolution focused on developing Cas9 for genome editing. However, with the recent discovery and advancement of other Cas effectors, especially the RNA-targeting Cas13 family, CRISPR has expanded into new areas. CRISPR-based technologies are now being used in various ways to enhance human health and have the potential to revolutionize disease treatment[1.7].

1.2. Global Search and Data

A comprehensive patent search was carried out using the Innography patent database. This search aimed to analyze the current global landscape of CRISPR/Cas systems and their associated research. The findings provide valuable insights into the advancements and trends in this revolutionary field[1,8]. This comprehensive search process initially identified 16,904 patent applications.

1.2.1. World Map Based on Source Jurisdiction

The analysis of the patent families was conducted to create a detailed map. This map illustrates the number of applications filed across various source jurisdictions. By examining this data, researchers can gain insights into the geographical distribution of CRISPR/Cas related patent filings. This comprehensive mapping helps to identify regions with significant activity and innovation in CRISPR/Cas technology, providing a clearer understanding of global trends and developments in the field.

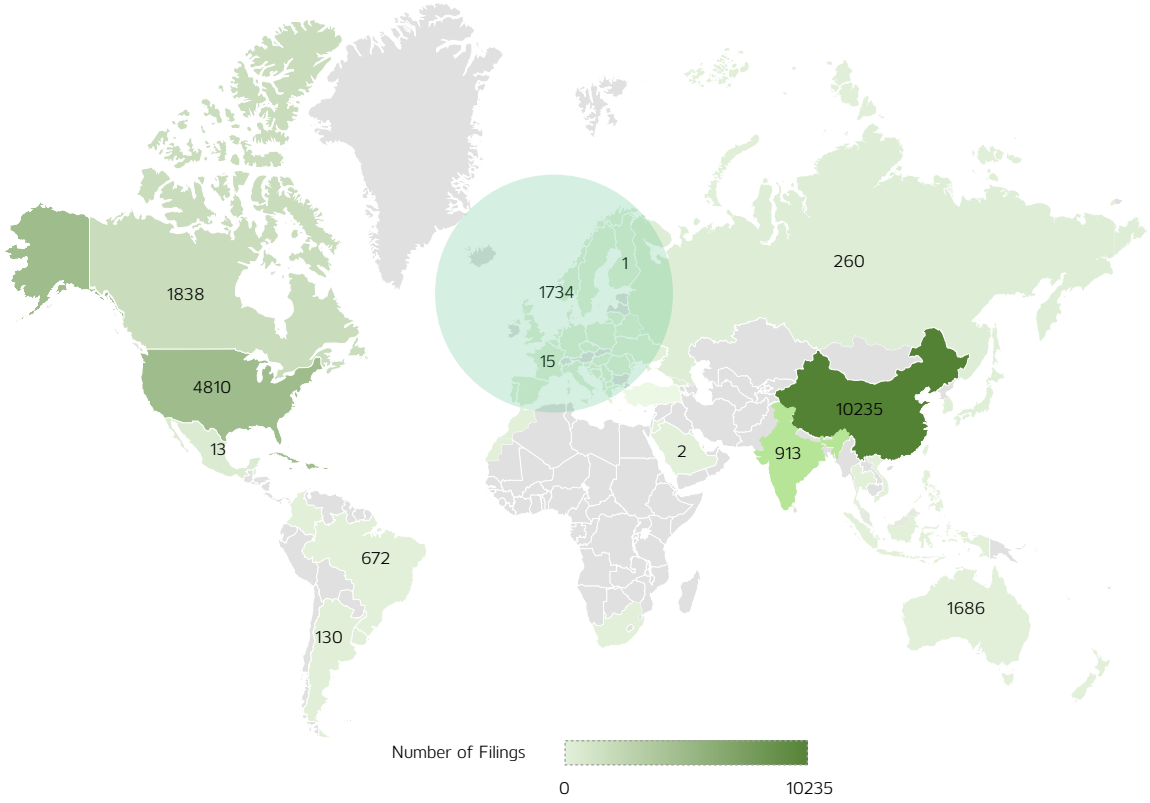


Figure 1.6: World map representing the number of applications filed in different source jurisdictions related to CRISPR/Cas

It is apparent from Figure 1.6, that China leads with 10235 filings, indicating a strong focus on CRISPR research and development. The United States (US) follows with 4810 filings, showcasing its substantial contribution to CRISPR innovation. India, with 913 filings, shows a growing interest in CRISPR technology, while South Korea has 1537 filings, highlighting its active participation in CRISPR research. The European Patent Office (EPO) has 1734 filings representing patents seeking protection across multiple European countries. Other jurisdictions, including Japan (921), Russia (260), and Argentina (130), also contribute to the global CRISPR patent landscape, though to a lesser extent. This data provides a comprehensive view of the global distribution of CRISPR/Cas related priority filings, highlighting regions with significant research and development activity in this field.

1.2.2. Top Applicants

The identified patent families were further utilized to identify the top applicants working in the field of CRISPR/Cas related technologies. These patent families were reviewed to get an insight into the top contributors in advancement of CRISPR/Cas related technology. The results are depicted in Figure 1.7 below.

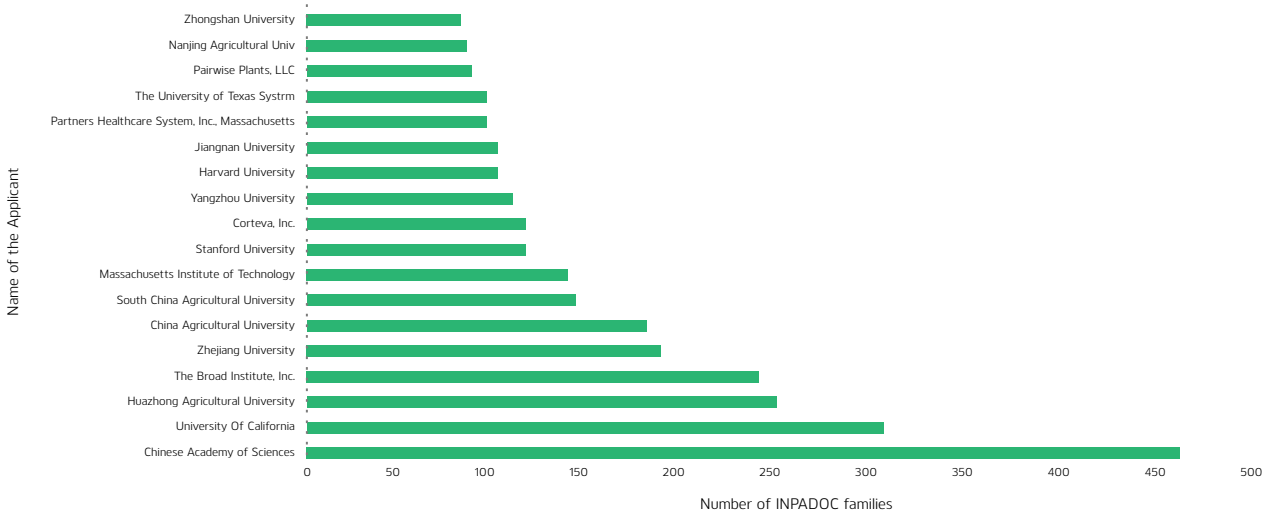


Figure 1.7: World map representing the number of priority applications filed in different jurisdictions related to CRISPR/Cas

Although applicants from China have filed the highest number of patent applications related to CRISPR/Cas technology, the Broad Institute and CVC hold some of the most seminal patents in this field, as discussed in the following chapters. The Broad Institute has partnered with the Massachusetts Institute of Technology (MIT), Harvard College, and Rockefeller University, while CVC represents a collaboration between the University of California, the University of Vienna, and Emmanuelle Charpentier.

1.3. Legal Battle for the CRISPR-Cas9 Patent Rights

The year 2012 marked the rise of CRISPR/Cas and there was a race between different researchers for filing and owning the intellectual property (IP) for this multimillion-dollar technology. Since CRISPR/Cas involved such high stakes, it was not surprising when the researchers got into a battle for claiming the ownership of CRISPR/Cas technology, particularly for its application in eukaryotic cells and organisms. The five entities or the "big five" filed patent applications with partly overlapping scope, protecting the basic CRISPR/Cas9 technologies[1.9].

The respective key applications from the parties are shown in Figure 1.8 below:

Table I
Overview of the "seminal" CRISPR-Cas9 patent portfolios assigned to the big five.

Assignee	Alias	PCT Publicaton	Earliest Priority Date
Vilnius University	CVC	WO2013142578	20.03.2012
		WO2013141680	20.03.2012
University of California		WO2013176772	25.03.2012
Universitat Wien			
Emanuelle Charpentier			
Toolgen		WO2014065596	23.10.2012
Sigma Aldrich (now Merck Milipore)		WO2014089290	06.12.2012
Broad Institute	Broad/ Rockefeller	WO2014093595	12.12.2012
Massachusetts Institute of Technology			
Harvard College			
Rockfeller University			
Broad Institute	Broad	WO2014093694	12.12.2012
Massachusetts Institute of Technology		WO2014093655	
		WO2014093635	
Harvard College		WO2014093712	
		WO2014093661	

Figure 1.8: Overview of the seminal CRISPR/Cas9 patent portfolios assigned to the "big five"

(Source: The CRISPR Cas patent files, part 1: Cas9 – Where do we stand at the 10 year halftime?, Ulrich Storz, Journal of Biotechnology, Journal of Biotechnology 379 (2024) 46–52, e 19 November 2023).

Since the applications were filed for similar inventions, what followed was a patent dispute which involved all the major filers. The paragraphs to follow provide an overview of the legal battle between two of the pioneering groups of researchers, CVC (comprising University of California, University of Vienna, and Emmanuelle Charpentier), and Broad Institute (affiliated with Harvard and MIT).

1.3.1. Broad Vs CVC

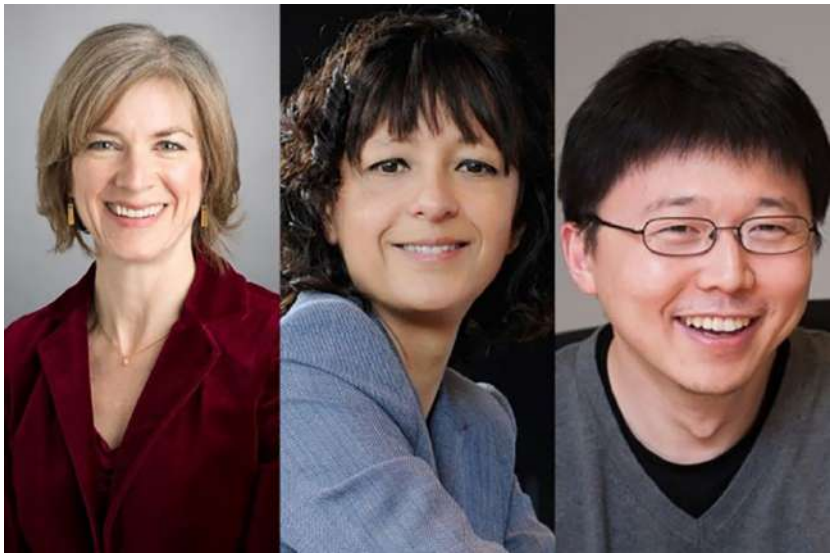


Figure 1.9: From Left to Right, Jennifer Doudna, Emmanuelle Charpentier and Feng Zhang

(Source: Who Owns CRISPR in 2021? It's Even More Complicated Than You Think - SynBioBeta)

In June 2012, Nobel laureates Emmanuelle Charpentier (University of Vienna) and Jennifer Doudna (University of California, Berkeley, USA), published a scientific paper (*Jinek M et al*[1.10]) which outlined how CRISPR, with the help of an enzyme called Cas9, can be transformed into a tool to edit genes. The subject matter was mostly restricted to experiments in test tube. Subsequently, the duo also filed their first CRISPR-related patent application at the United States Patent and Trademark Office (USPTO) in May 2012 (US13/842,859). Subsequently after prosecution, a patent (US 10,266,850 B2), was granted on April 23, 2019.

In January 2013, a group of scientists led by Feng Zhang at the Broad Institute of MIT and Harvard College ("Broad Institute"), reported the utility of CRISPR/Cas9 in editing the cells of mammals *(Le Cong et al*[1.11]*)*. This further sparked interest amongst the scientific community in exploring the blockbuster tool for generating new medical regiments. Before this, in December 2012, Broad Institute researchers filed their first CRISPR-related patent application (US 61/736,527) along with 11 additional applications for

safeguarding the landmark invention. Using a fast-track review process, Broad Institute received a grant (US 8,889,356 B2) from the USPTO in 2014 for its patent on CRISPR technology.

Upon the knowledge of this grant, a legal war was triggered between these two groups of researchers, CVC and Broad Institute. This legal war would later be considered as one of the greatest battles in the history of biotechnology, since the one who would emerge as winner would be entitled to all the profits that will be generated via this technology.

In April 2015, Charpentier and Doudna requested a patent interference proceeding against the patents granted to the Broad Institute in US. Hearings began in January 2016 to determine which of the two parties invented the CRISPR/Cas technology in eukaryotes first. It was for the USPTO to decide if the invention of Broad Institute was actually novel and inventive or was it an obvious invention considering the publication and application filed by CVC.

The Patent Trial and Appeal Board (PTAB), on February 12, 2017, announced its decision stating that the patents of Broad Institute for the use of CRISPR/Cas9 in editing mammalian cells (eukaryotic genomes), did not overlap or interfere with patent claims filed by the UC Berkeley team for the use of the system in any environment. The PTAB thus ruled that the Broad Institute's patent claims (directed to methods of using CRISPR/Cas9 in mammalian cells eukaryotes) were not obvious considering the information provided in UC Berkeley's US patent application (directed to methods of using CRISPR/Cas9 in any cells)[1.12].

In parallel, the aforementioned two groups also filed their applications for patents in other jurisdictions, including Europe (EP). A similar battle was also ignited in EP between CVC and Broad Institute. However, the EPO acknowledged that the CVC patent is sufficiently enabled for the claims related to use in eukaryotes[1.9].

1.3.2. Priority issue with Broad Applications in EP:

In separate proceedings, the patent applications of Broad Institute were almost entirely revoked as an aftermath of series that unfolded due to priority problems. Briefly, Broad Institute filed US provisional applications P1 and P2 in 2012 and 2013 respectively, involving four inventors, including Dr. Marraffini from Rockefeller University. Broad Institute's priority claim was invalidated by the EPO due to a missing transfer of rights from Dr. Marraffini. The Board of Appeal (BoA) confirmed that valid priority claims require identity of applicantship or proper transfer of rights. The missing assignment from Dr. Marraffini could not be rectified by a subsequent submission because his assignment to Rockefeller University was already recorded. Additionally, the BoA affirmed that the validity of a priority claim made before the EPO is determined by the European Patent Convention (EPC) and not by the laws of the country where the priority application was initially filed. In one of the later priority applications having a filing date of 30 January 2013, in which only employees of Broad Institute, MIT and Harvard

College were named as applicants, was deemed valid by the BoA. With this, the "effective" priority date of the entire patent family moved to January 30, 2013. Since this was later than the publication dates of novelty destroying prior arts, considerable portions of Broad Institute's patent application were considered to be non-novel. Consequently, Broad Institute's patent EP2771468B1 was revoked, affecting their European patent portfolio. Rockefeller University filed a parallel PCT application without the priority issue and without the knowledge of Broad Institute, leading to a dispute resolved by a settlement between the two parties. Broad Institute's misunderstanding of the issue was one of inventorship rather than applicantship, which contributed to their procedural failure. As of January 2024, two of the Broad Institute's EP patents (EP2764103B1 and EP2840140B2) are in force but with limited scope[1.9].

1.3.3. Sufficiency of Disclosure Issue with CVC Applications at the EPO:

As an aftermath of the opinion issued by the BoA, that the earliest patent filings of CVC did not sufficiently explain CRISPR/Cas for other scientists to apply it, Emmanuelle Charpentier and Jennifer Doudna have requested the withdrawal of two key European patents (EP2800811 and EP3401400). The omission of disclosure relates to a feature of DNA molecules called "protospacer adjacent motifs," or PAMs (which has been described earlier in this report). The withdrawal aims to avoid an unfavorable legal decision being issued by the EPO[1.12].

Though there have been landmark decisions delivered by patent offices across major jurisdictions, there is still a lurking unclarity in the biotechnology business community, more particularly, the small and medium enterprises, on whom to approach for the licensing of the technology. Thus, the licensing situation of CRISPR/Cas technology remains uncertain. A solution to this problem that is being explored by various researchers is redirecting their focus to other Cas enzymes such as Cas 12, Cas 13 and Cas 14. Other researchers are also working on either finding new Cas enzymes or mutating the current enzymes. This has been discussed in more detail under Chapter 5 of this report.

1.4. CRISPR/Cas in Biomedical Applications

The present report explores the use of CRISPR/Cas technology in four major areas of Biomedical Applications, viz, Therapeutics, Diagnostics, Vaccines, Biological models and further provides an overview of patent filing associated with new Cas enzymes.

Therapeutics:

A detailed overview of CRISPR/Cas based therapeutics is provided in Chapter 2 of this report. The CRISPR technologies also have been actively utilized for the development of *in vivo* and *ex vivo* gene and cell therapies. Research has shown the *ex vivo* application of CRISPR technology in creating cell therapies using patient-derived cells. Researchers worldwide have applied CRISPR/Cas based gene editing for correcting pathogenic mutations, for example in Wolfram syndrome[1.13], in myeloproliferative neoplasm[1.14]; and in Huntington's disease (HD)[1.15]. Casgevy and Lyfgenia are a few examples of FDA approved CRISPR/Cas cell-based therapy in treating sickle cell disease. However, like any genome editing technology, CRISPR/Cas has also been under the radar for ethical concerns, which have been discussed in detail in Chapter 2 of this report.

Base editing technologies have been used both *ex vivo* and *in vivo* to develop gene therapies for neurological diseases, blood disorders treatment of human breast cancer cells and severe combined immunodeficiency (SCID)[1.16].

Prime editing technology enabled genome editing of multiple consecutive DNA bases with relatively less sequence constraints compared to base editors[1.16,1.17].

Diagnostics

Chapter 3 of this report provides a detailed overview of the application of CRISPR/Cas technology in diagnostics. There is a significant social demand for rapid and sensitive diagnostics, particularly highlighted by the COVID-19 pandemic, which has posed a substantial threat to human society.

CRISPR/Cas is considered an ideal next-generation technology for rapid and sensitive nucleic acid detection on-site. Researchers have created numerous diagnostic platforms that offer high sensitivity, high specificity, and low cost by utilizing Cas proteins (such as Cas9, Cas12, Cas13, Cas14) in combination with signal amplification and transformation techniques (like fluorescence methods and lateral flow technology). This approach provides a novel method for the quick detection of pathogen nucleic acids[1.19].

Vaccines:

Chapter 4 of the present report discusses the application of CRISPR/Cas in the field of vaccine generation. Use of CRISPR/Cas9 in development of vaccines have aided in overcoming the limitations associated with conventional recombination methods of preparing vaccines. The advantages of using CRISPR include precision, high efficacy, specificity, and low-cost properties. In virology, CRISPR/Cas9 is employed to modify virus genomes, aiding in the understanding of viral pathogenesis, gene therapy, and virus-host interactions.

Biological Models:

Chapter 5 (5.2) of this report further discusses the development of biological models using CRISPR/Cas technology. CRISPR/Cas9 has been widely used to develop cell models by introducing specific genetic alterations in cultured cells. This allows scientists to investigate the effects of these changes on cellular processes and disease pathways. For example, CRISPR/Cas9 has been used to create cell lines with mutations that mimic human diseases, providing valuable insights into disease mechanisms and potential therapeutic targets[1.18].

New Cas enzymes:

CRISPR/Cas9 systems have been widely used in functional genomics and crop genetic improvement, but the protospacer adjacent motif (PAM) sequence NGG of *Streptococcus pyogenes* Cas9 (SpCas9) limits its targeting scope. Additionally, due to the heavy litigation surrounding the CRISPR/Cas9 system, the researchers are actively exploring for variants of Cas enzymes. While few groups are involved in identifying new Cas enzymes from nature, there are several groups who are working in creating Cas enzymes by performing mutations and fusions. This has been further discussed in detail in Chapter 5 (5.3) of this report.

2 CRISPR/CAS & THERAPEUTICS: Cut, Repair, Cure

INDEX - CHAPTER 2

2.1. Background

CRISPR/Cas systems have been extensively manipulated to target specific regions of the human/animal genome, resulting in multiple therapeutic applications. The road to such modifications, however, is not straightforward. In 2012, Charpentier and Doudna purified Cas9 from *S. thermophilus* and *Streptococcus pyogenes*, enabling *in vitro* cleavage of prokaryotic DNA [2.1.] They discovered that Cas9's cleavage site is controlled by a seed sequence in the crRNA and requires PAM. By altering the seed sequence, the system can function as a gene silencer, allowing gene targeting and editing [2.1 and 2.2.] CVC submitted a patent application for their CRISPR-Cas9 technology on May 25, 2012. The USPTO granted them a patent (US10,000,772) in 2018.

However, the application of CRISPR technology commences in the field of medicine after filing of a provisional patent application and the publishing of a paper by Zhang Feng *et al.* in 2013 (Broad Institute) [2.3.] wherein human-derived 293 T cells were transformed with transactivating crRNA (tracrRNA), pre-crRNA, host factor ribonuclease (RNase) III, and Cas9 from S. pyogenes along with respective promoters and two nuclear localization signals (NLSs) [2.1, and 2.4-2.6]. This opened the gates for several researchers to successfully achieve editing in eukaryotic cells, especially in animals such as mice, fruit flies, and rats [2.7-2.12]. In April 2014, the USPTO granted US Patent No. 8,697,359 to Broad Institute, MIT, and Dr. Feng Zhang. This patent draws priority from a provisional patent application filed on December 12, 2012. For details of patent interference proceedings between CVC and the Broad Institute, refer to Chapter 1 of this report.

It was only in December 2013 that Wu *et al.* (Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences) conducted a study to treat cataracts in a mouse model by base deletions using CRISPR/Cas9 [2.13, 2.14]. Subsequently, Schwank *et al.* (2013) from Hubrecht Institute/ Royal Netherlands Academy of Arts and Sciences (KNAW) isolated intestinal stem cells from two patients with cystic fibrosis transmembrane conductor receptor (CFTR) mutations and corrected the same using CRISPR/Cas9 gene editing [2.15-2.18]. In fact, they established a protocol for *in vitro* editing of genetically mutated stem cells and their subsequent introduction into the body for treatment, laying the foundation for the present-day cell therapies [2.19]. The next challenge was to rectify the mutated base instead of excising it [2.20]. This was achieved by Komor *et al* in 2016 (The Broad Institute of MIT and Harvard) by utilizing cytidine deaminase which catalyzes the deamidation of cytosine into uracil. The single-base gene-editing technology provided a predictable method of achieving single as well as multiple base substitutions. Hence, genetic diseases caused by multiple base substitutions could also be targeted [2.21, 2.22]. The continued research in the therapeutics eventually led to the first clinical trial of CRISPR/Cas9 technology which was conducted by Lu and colleagues at West China Hospital in Sichuan, China [2.23]. During this trial, CRISPR/Cas9 gene-edited T cells were injected back into patients, resulting in the world's first human injection of gene-edited cells (2016). The T-cells obtained from patients were transfected with plasmids encoding Cas9 and sgRNA targeting the Programmed Cell Death 1 (PD-1) gene responsible for regulating immune responses and is implicated in both cancer and autoimmune diseases [2.24, 2.25].

2.2. Search Results

Note: This section is a patent overview in CRISPR/Cas technology with a specific focus on therapeutics.

Methodology

- A total of 1925 patent families were obtained as a result of the patent searches. The identified 1925 families covered the use of CRISPR-based gene technology in a wide range of applications in biomedicine including therapy, vaccine preparation, diagnosis, biological models, drug screening, etc. Hence, a further analysis was carried out to categorize the search results into 5 main categories: *Therapy, Vaccines, Diagnostics, New Cas enzymes and Miscellaneous applications.* The analysis for identifying inventions directed to "*use of CRISPR-based gene editing in therapy*" was divided into three screening processes:

  First screening: The 1925 patent families were obtained at the end of patent search for CRISPR related technology in the field of "biomedicine". These 1925 applications were screened, and 1082 families were further shortlisted to be related to the use/potential use of CRISPR/Cas technologies in "therapy". These 1082 families were analyzed to study the trends in jurisdiction-specific patent filings and priority filings for CRISPR-based human therapies.

  Second screening: The 1082 families were further narrowed down to 242 patent families specifically related to treatment of diseases in humans. In other words, these families had direct application in human therapy based on the claims and as-filed specification. These 242 families were analyzed to identify the trends in top Applicants, collaborations, disease specific inventions, and cell therapy related inventions. In addition, a detailed taxonomy-based classification was prepared for the selected 242 patent families.

  Third screening: Out of the selected 242 patent families, a detailed analysis was performed for 15 patent families to study the recent advances in CRISPR-based gene editing technology for use in human therapy.

2.2.1. World Map for Patent Filings

During the first screening process, 1925 patent families were screened carefully to narrow down the dataset to 1082 patent families. This screening was carried out to restrict the results to Patent Applications specifically related to CRISPR-based gene editing for use in therapy. The results included

both; *Applications claiming for subject matter having direct* ***Application in therapeutics*** (method of treatment, product for use in treatment, and use of a reagent/biological reagent for therapy, etc.); and *Applications claiming subject matter having* ***indirect application in therapy.*** The objective of including both categories was to overcome clever claim drafting practices where claims were specifically restricted to subject matter not related to therapy (commonly done for overcoming objections across different Patent regimes). However, for such Applications, the claims when read with the specification provided hint of further application/use of the invention in therapeutics.

For the shortlisted 1082 patent families, a jurisdiction specific analysis was carried out. The results are depicted in the form of a world map (Figure 2.1). It was observed that China had maximum number of patent Applications filed; *641 out of 1082 families*, followed by US *(584 out of 1082 families)*, Europe (*378 out of 1082 families*), Japan (*250 out of 1082 families*), Canada (*213 out of 1082 families)* and Australia (*205 out of 1082 families*).

A total of 85 out of 1082 Patent Applications were filed in India for CRISPR-based gene editing for use in therapy, all of which were patents filed through Patent Cooperation Treaty (PCT) route.

Notably, 660 families out of the shortlisted 1082 families (61.05% of total Applications) were filed via PCT route.

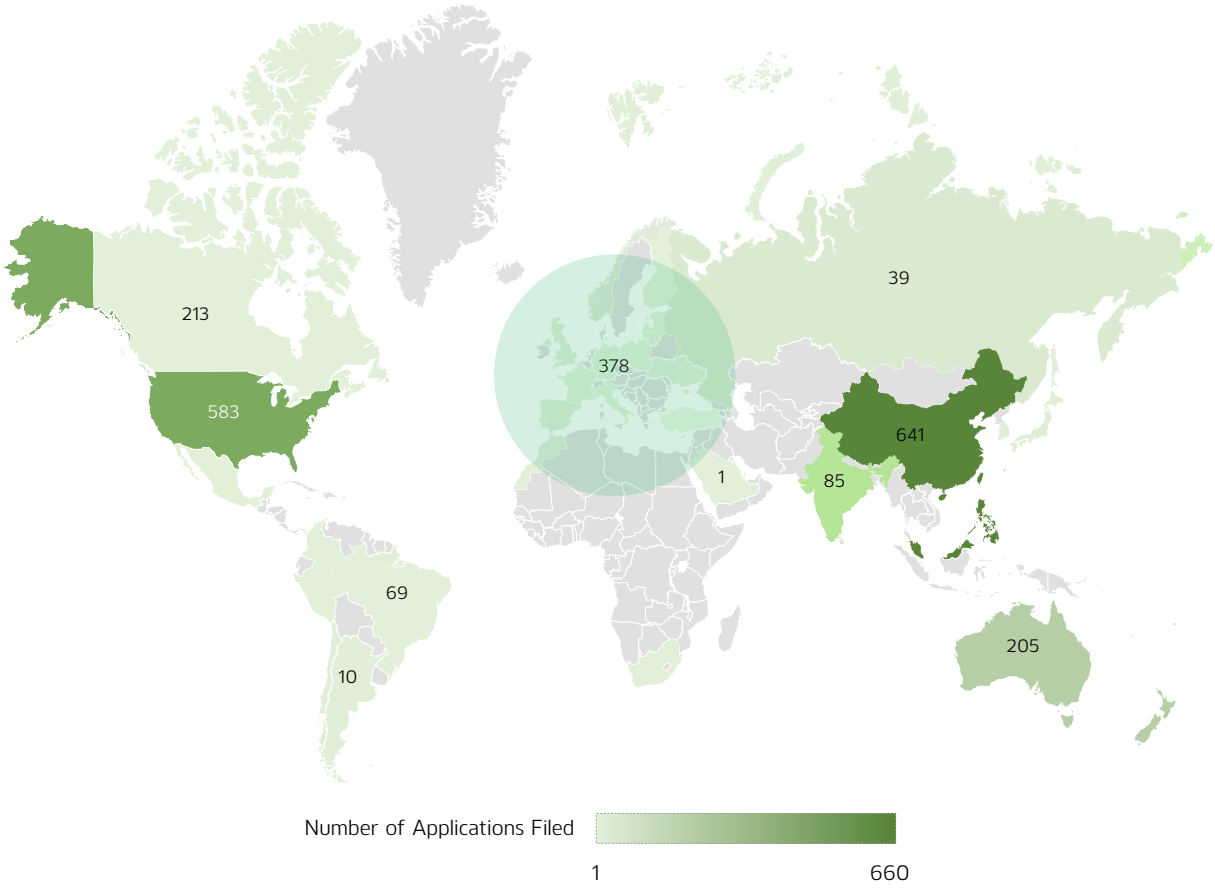


Figure 2.1: World map indicating filing for patent applications related to CRISPR/Cas-based therapeutics

In terms of priority filings, US had maximum number of priority filings (526 filings amounting to 49%), followed by China (423 filings amounting to 39%) and Europe (40 filings amounting to 4%). India, on the other hand, constituted for <1% of priority filings.

2.2.2. Top Applicants

During the second screening process, 1082 patent families were further narrowed down to 242 patent families based on therapeutics. Taxonomy based classification was prepared for these 242 Patent families where the taxonomic categories included:

- Type of enzyme/technology used,
- Target disorder/disease/pathogen,
- Claimed subject matter (product/method/use),
- Target gene/molecule,
- Whether *ex vivo* or *in vivo* treatment is provided,
- Target organism/organ/cell, and
- Mode of CRISPR system delivery.

The 242 Patent families were analyzed to identify the top Applicants across major jurisdictions like China, US, Europe, etc. The top 10 Applicants are represented in Figure 2.2. The shortlisted Applicants are specific to patent families pertaining to use CRISPR technology in therapeutics.
Further, many patent Applications have been filed as a collaborative effort on behalf of different educational institutes (discussed in next section).

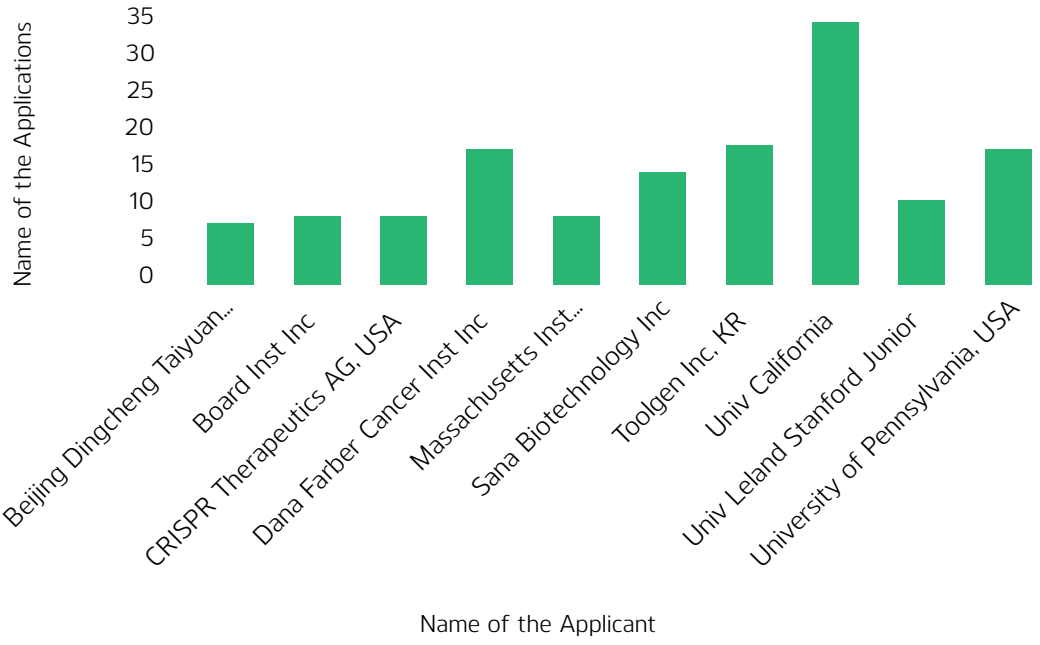


Figure 2.2: Top 10 Applicants for patent Applications related to CRISPR/Cas in therapeutics

In addition to the above, it is worth noting that CRISPR/Cas9 and CRISPR/Cas12a systems have been extensively used in preclinical studies and clinical trials for treating severe genetic diseases, angiogenesis-related eye diseases, cardiac diseases, neural diseases, cancers, infectious diseases and orphan diseases.

2.2.3. Collaborations

CRISPR/Cas technology, like many scientific breakthroughs, is a result of several collaborative efforts. There is currently a dispute between four major groups over the use of CRISPR/Cas9 in eukaryotes: CVC, Broad Institute, Sigma-Aldrich and Toolgen (as noted in further detail in Chapter 1 of this report; 1.3: Legal Battle for the CRISPR/Cas9 patent rights).

In the present landscape, the 242 Patent families were analyzed to identify the top collaborators in filing Patent Applications related to CRISPR-mediated therapy. The collaborations of the above-mentioned groups are also evident in the search results obtained in the present landscape. In addition to the above 4 groups, several other research collaborations were identified as enlisted below.

The University of Pennsylvania has filed numerous patent applications—9 out of 17 identified—in collaboration with partners including Children's Hospital Philadelphia, Dana Farber Cancer Institute, University of California, Stanford University, and Novartis AG.

- Together with Children's Hospital Philadelphia, the University of Pennsylvania has submitted patent applications focused on CRISPR-mediated gene editing techniques for disease treatment. These include methods for *in utero* genome editing (US2024075164A1); genome editing targeting genes in fetal or postnatal lungs to treat monogenic lung diseases (US2022275402A1); editing the 4-hydroxyphenylpyruvate dioxygenase (HPD) gene for hereditary tyrosinemia type I (HT1) (WO2024152058A1); and producing megakaryocytes via gene editing to address thrombocytopenia (US2022177843A1).

- In partnership with Dana Farber Cancer Institute and University of California, the University of Pennsylvania filed a patent application describing engineered mammalian T cells with deletions in gene expression regulatory regions via gene editing, intended for cell therapy in immune disorders (CA3018332A1).

- Collaborating with Novartis AG, the University of Pennsylvania has filed patents related to CRISPR-based treatments for cancers and proliferative diseases.

All the identified 8 Applications of Broad Institute were jointly filed with MIT, Harvard College, Dana-Farber Cancer Institute, and Massachusetts General Hospital. The patent Applications relate to novel nucleic acid targeting systems comprising components of CRISPR systems (EP4085141A1), vectors and vector systems some of which encode one or more components of a CRISPR complex (US2016153004A1), Class 2, type VI CRISPR system for disease treatment (US2020165594A1), etc.

The University of California has submitted 7 out of 31 patent applications as a joint applicant alongside institutions such as the Children's Hospital & Research Center at Oakland, National Yang Ming University, Dana-Farber Cancer Institute, University of Pennsylvania, F. Hoffmann-La Roche Ltd, Johns Hopkins University, and the Regents of the University of California. These applications include patents related to the use of CRISPR technology for treating diseases such as chronic infections or cancer (CA3018332A1), β-thalassemia (WO2023056139A), Huntington's disease (WO2022204543A1), and neurodegenerative diseases (CA3216825A1), among others.

Harvard College has co-filed 2 patent applications with the Children's Medical Center Corporation. Similarly, ToolGen Inc. from South Korea has filed 3 patent applications in collaboration with other entities. These patents cover a broad range of CRISPR-based inventions targeting treatments for general viral infections (US2020165594A1), specific viral infections like HIV (US2022333119A1), and severe combined immunodeficiency (EP3019595A2).

2.2.4. Disease Specific Results

CRISPR gene-editing tools have been used for the treatment of different types of diseases (Figure 2.3) including genetic disorders (Sickle cell diseases [2.26, 2.27] Beta-thalassemia [2.28], Duchenne Muscular Dystrophy [2.29], Cystic Fibrosis [2.30]), brain and neurological genetic diseases [2.31, 2.32], human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS)2.33, cardiovascular diseases (CVDs) [2.34] cardiopulmonary diseases, cancers [2.36-2.37], and ocular diseases [2.38-2.40, 2.41].

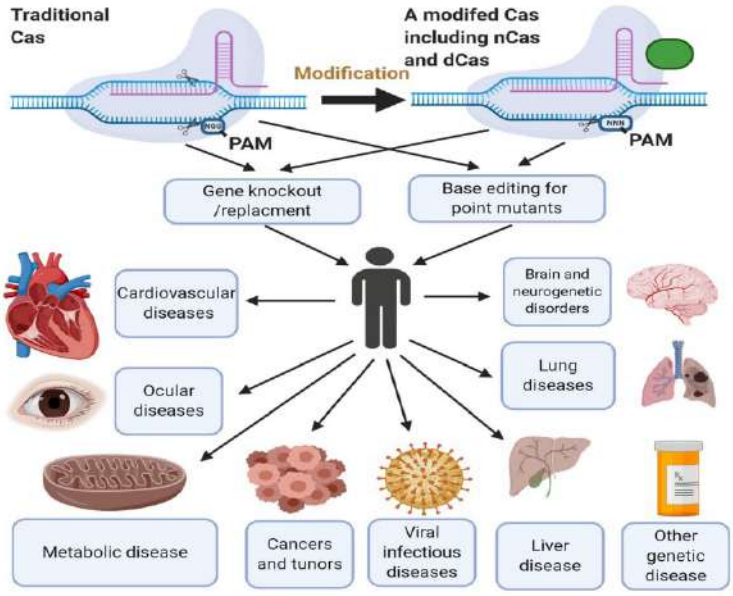


Figure 2.3: Huge potential of CRISPR/Cas genome editing in treating human genetic

Source: Zhang B. CRISPR/Cas gene therapy. J Cell Physiol. 2021 Apr;236(4):2459-2481. doi: 10.1002/jcp.30064. Epub 2020 Sep 22. PMID: 32959897.

Out of 242 Patent families, about 46% related to use of CRISPR-based gene editing for the treatment of cancers (refer, Figure 2.4, Table 2.1), and 15% related to use of CRISPR-based gene editing for the treatment of genetic disorders like Sickle cell disease, beta-thalassemia, Duchenne Muscular Dystrophy and Cystic Fibrosis. Similarly, 15% of Applications related to use of CRISPR-based gene editing for the treatment of brain and neurological genetic diseases like Alzheimer's disease and Huntington's disease. 13% of the Applications related to use of CRISPR-based gene editing for the treatment of Cardiovascular/Cardiopulmonary diseases. 8% and 3% of Applications related to use of CRISPR-based gene editing for the treatment of ocular diseases and HIV/AIDS, respectively.

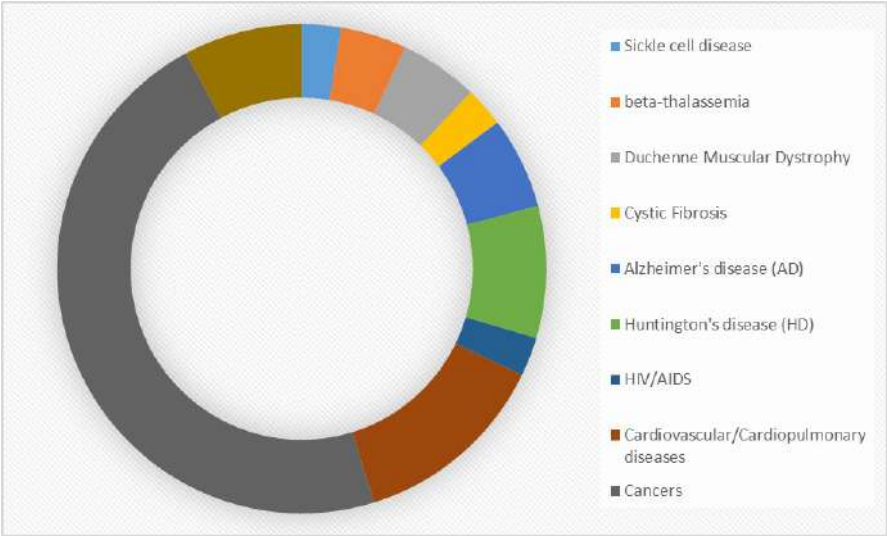


Figure 2.4: Disease specific classification for CRISPR-based therapies.

Type of Disease	Major Applicants and corresponding Patent Applications
Sickle cell disease	HARVARD COLLEGE and CHILDRENS MEDICAL CENTER (EP3019595A2) SANA BIOTECH INC (CN118401653A and CN118382693A)
beta-thalassemia	UNIV CALIFORNIA and CHILDRENS HOSPITAL & RES CENTER AT OAKLAND (WO2023056139A1) HARVARD COLLEGE and CHILDRENS MEDICAL CENTER (EP3019595A2) SANA BIOTECH INC (CN118401653A) (CN118382693A) UNIV WASHINGTON (CA2947466A1)
Duchenne Muscular Dystrophy	UNIV CALIFORNIA (GB2574769A) TOOLGEN INC, and COLLEGE OF MEDICINE POCHON CHA UNIV INDUSTRY ACADEMIC COOPERATION FOUNDATION (EP3640334A1) AVELLINO LAB USA INC (US2021032612A1 and WO2023044510A2) UNIV WASHINGTON (US2017362635A1)
Cystic Fibrosis	UNIV CALIFORNIA (US2019167816A1 and US2021238257A1) SANA BIOTECHNOLOGY INC (WO2024163952A2)
Alzheimer's disease (AD)	UNIV CALIFORNIA (AU2020307538A1) REGENERON PHARMA (US2024415980A1) HARVARD COLLEGE (GB2572918A) SANA BIOTECHNOLOGY INC (WO2024163952A2) TOOLGEN INC (US2023063739A1) SHENZHEN BIOCAN TECH CO LTD (WO2019237379A1)
Huntington's disease (HD)	UNIV PENNSYLVANIA (US2022323553A1) UNIV CALIFORNIA and F HOFFMANN LA ROCHE LTD (WO2022204543A1) SANA BIOTECH INC (CN118401653A and CN118382693A) TOOLGEN INC (US2023063739A1) TOOLGEN INC and COLLEGE OF MEDICINE POCHON CHA UNIV INDUSTRY ACADEMIC COOPERATION FOUNDATION (EP3640334A1) UNIV COLUMBIA (WO2024011224A2) AVELLINO LAB USA INC (WO2023044510A2)
HIV/AIDS	SANA BIOTECHNOLOGY INC (WO2024163952A2, CN118401653A, CN118382693A)

Cardiovascular/ Cardiopulmonary diseases	REGENERON PHARMA (US2024415980A1) SANA BIOTECHNOLOGY INC (US2023062612A1, WO2024163952A2, CN118382693A) TOOLGEN INC (US2024166999A1, EP3690047A2, WO2024253421A1) UNIV COLUMBIA (US2021275646A1) AVELLINO LAB USA INC (US2021032612A1, WO2023044510A2) UNIV FUDAN (CN108265029A, CN105039399A)
Cancers	CRISPR THERAPEUTICS AG (TW202328444A, US2023355761A1) NANJING DRUM TOWER HOSPITAL (CN115927303A) DANA FARBER CANCER INST INC, UNIV PENNSYLVANIA, UNIV CALIFORNIA (CA3018332A1) UNIV PENNSYLVANIA (US2021155667A1, US2022323553A1, WO2024238938A1, EP3619302A1) UNIV PENNSYLVANIA, UNIV LELAND STANFORD JUNIOR (US2023364238A1) NOVARTIS AG, UNIV PENNSYLVANIA (US2019375815A1, CN109072195A, US2020055948A1) UNIV SICHUAN AGRICULTURAL (CN118286428A) INST MILITARY MEDICINE ACADEMY MILITARY SCIENCES PLA INST BIOPHYSICS CAS (CN115725729A) DANA FARBER CANCER INST INC (US2020405720A1, WO2018148378A1, US12050219B2, US11820822B2, US2023201166A1, US2023203485A1, WO2022261183A2, CA3047334A1) BROAD INST INC, MASSACHUSETTS INST TECHNOLOGY, DANA FARBER CANCER INST INC, MASSACHUSETTS GEN HOSPITAL (US2018100201A1) MASSACHUSETTS GEN HOSPITAL, DANA FARBER CANCER INST INC, MASSACHUSETTS INST TECHNOLOGY (WO2023230632A2) BROAD INST INC, MASSACHUSETTS INST TECHNOLOGY, MASSACHUSETTS GEN HOSPITAL, DANA FARBER CANCER INST INC. (US12018080B2) UNIV SOUTHERN CALIFORNIA (CA3107675A1) WHITEHEAD INST BIOMEDICAL RES, HARVARD COLLEGE (WO2024076750A2) HUMANIGEN INC, MAYO FOUND MEDICAL EDUCATION & RES (US2022040229A1) NOVARTIS AG, INTELLIA THERAPEUTICS INC (CN108699557A) SANA BIOTECHNOLOGY INC (AU2022325955A1, CN118434844A, CA3227108A1, US2024425820A1,

	CN118451178A, WO2024163952A2, WO2023069790A1, WO2023183313A1, CN118401653A, CN118382693A) SUNGKWANG MEDICAL FOUND, TOOLGEN INC (KR102213837B1) TOOLGEN INC (US2023063739A1) UNIV LELAND STANFORD JUNIOR (WO2024102797A1) INSIDEOUTBIO INC (US2020262879A1, WO2021118927A1, US2021403519A1) UNIV FUDAN (CN112143806A) UNIV NORTH CAROLINA CHAPEL HILL, RESTIFO NICHOLAS P, XU NUO, US HEALTH (WO2021092593A1) UNIV WASHINGTON (US2024011055A1) UNIV ZHEJIANG (CN118320112A, WO2020093574A1, WO2020093573A1, CN117085134A)
Ocular diseases	COUNCIL OF SCINTIFIC AND INDUSTRIAL RES AN INDIAN REGISTERED BODY INCORPOR (US2024117359A1) UNIV CALIFORNIA (WO2023154749A2) REGENERON PHARMA (US2024415980A1) SANA BIOTECHNOLOGY INC (AU2022325955A1, CA3227108A1, US2024425820A1, WO2024163952A2, CN118401653A, CN118382693A)

Table 2.1: Disease specific trends for CRISPR-based therapies.

2.2.5. CRISPR-Based Gene Editing for CAR-T Cell Therapy

Chimeric antigen receptor T cell (CAR-T cell) therapy has gained a lot of attention for treating malignancies. CAR constructs, which consist of an extracellular antigen-binding domain (single-chain fragment variable, scFv), transmembrane hinges, and intracellular signal domains, enable CAR-T cells to specifically identify, activate, and eradicate tumor cells in an antigen-specific and MHC-independent manner [2,42]. For all six products to be expressed, the introduction of CAR genes into human primary T cells is carried out through infection with lentivirus (LV) or retroviral vector (RV). In CAR/TCR-T cell therapy, CRISPR-mediated gene editing is either based on a Knockout (KO) or a Knockin (KI) strategy. To achieve the formation of CAR/TCR-T cells, sgRNA and Cas9 protein are co-transposed into T cells, while CAR/TCR can enter T cells through two primary methods, eventually resulting in CAR/TCR-T cells. (1) Random insertion via LV/RV: The CAR or TCR is randomly inserted into T cells using LV or RV.

(2) Precise insertion: This method facilitated by a donor template. Various forms of templates such as dsDNA, ssDNA, pDNA, or AAV are employed for site-specific integration of CAR or TCR into the T cells (refer, Figure 2.5). [2.43].

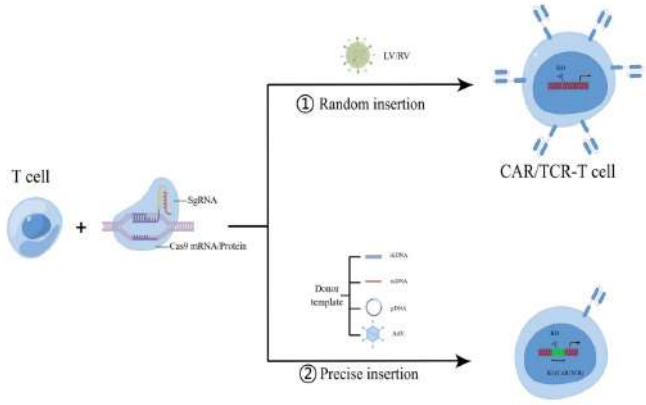


Figure 2.5: CRISPR/Cas-based CAR-T cells.

Source: Song, P., Zhang, Q., Xu, Z. et al. CRISPR/Cas-based CAR-T cells: production and application. Biomark Res 12, 54 (2024). https://doi.org/10.1186/s40364-024-00602-z

At present, there are three strategies for generating CAR-T cells utilizing the CRISPR system, with the most widely used being the CRISPR/Cas9 system, alongside CRISPR/Cas12a and CRISPR/Cas13d. A comparison of the advantages and drawbacks of CRISPR/Cas9 [2.45], CRISPR/Cas12a, and CRISPR/Cas13d [2.44] in CAR-T therapy[2.43]., is presented in Table below.

Feature	CRISPR/Cas9	CRISPR/Cas12a	CRISPR/Cas13d
Target gene editing efficiency	High	Moderate to high	Low
PAM sequence requirements	NGG	TTTN	N/A (targets RNA)
Gene editing precision	High	Moderate	High
Applicability to large-scale genome editing	Yes	Yes	No
Suitability for point mutations and insertions/deletions	Yes	Yes	Yes
Targeting capability for RNA and DNA	DNA and RNA	DNA	RNA
Type of target modified	Genomic DNA	Genomic DNA	RNA
Structural complexity	Larger	Smaller	Moderate
Therapeutic potential	High	Moderate	Moderate to low
Design flexibility	High	Moderate	High
Economic practicality	High	High	Low

Table 2.2 A comparison of the advantages and drawbacks of CRISPR/Cas9, CRISPR/Cas12a, and CRISPR/Cas13d in CAR-T therapy

Source: Song P, Zhang Q, Xu Z, Shi Y, Jing R, Luo D. CRISPR/Cas-based CAR-T cells: production and application. Biomark Res. 2024 May 31;12(1):54. doi: 10.1186/s40364-024-00602-z. Erratum in: Biomark Res. 2024 Jul 23;12(1):69. doi: 10.1186/s40364-024-00616-7. PMID: 38816881; PMCID: PMC11140991.

The emerging application of CRISPR-gene editing for CAR-T cell therapy is evident from the search results of the present patent landscape. Among the 242 Patent Applications, a total of 21 Applications related to use of CRISPR technology for preparing CAR-T cells, accounting for 9% of 242 Applications.

Crispr Therapeutics AG is a leading entity in filing patent applications focused on the use of CRISPR technology in CAR-T cell therapies. Their primary focus is on engineering T cells by targeting genes such as Regnase-1 (Reg1), transforming growth factor beta receptor II (TGFBRII), T cell receptor alpha chain constant region (TRAC), and β-2 microglobulin (β2M) through CRISPR/Cas9 gene editing for applications in cancer treatment (TW202328444A), solid tumor therapy (US2023355761A1), renal cell carcinoma (US2022387572A1, US2022378829A1), and multiple myeloma (US2023220059A1, US2022202859A1).

The University of Pennsylvania, either independently or in collaboration with other institutions, has filed patent applications involving CRISPR technology in CAR-T therapies targeting cancer (US2021155667A1, US2023364238A1, EP3619302A1, US2020055948A1) and diseases linked to tumor antigen expression (CN109072195A).

Similarly, the Broad Institute, alone or with partners, has submitted patent applications for CRISPR-based CAR-T therapies addressing cancer, glioma, melanoma (US2018100201A1, US12018080B2), viral pathogenesis (US2020165594A1), diabetes (US2020384115A1), inflammatory bowel disease (US12105089B2), and genetic disorders (US2016153004A1).

In summary, CRISPR Therapeutics AG, the University of Pennsylvania, and the Broad Institute are key leaders in advancing CRISPR-based CAR-T therapies through extensive patent filings. Their innovations focus on genetically engineering T cells to target a range of cancers and other serious diseases, highlighting the significant potential of CRISPR technology to revolutionize personalized and targeted treatments across oncology and beyond.

2.3.
Detailed Analysis of Selected Patent Applications

Detailed analysis was carried out for 15 Patent families (Table 2.4) selected from the results of the second screening process. Out of 242 Patent families, specific applications were shortlisted in order to study the recent advances in use of CRISPR-based gene editing in human therapy.

Family No	Application No.	Applicant	Corresponding Jurisdictions
1	PCT/US2022/017854	VERTEX PHARMACEUTICALS INCORPORATED (US)	US (18/456,288), EP (22710843.8)
2	PCT/IB2020/060720	CRISPR THERAPEUTICS AG [CH]	US (17/776,871), EP (20817495.3)
3	201810565134.6	CHINESE PLA GENERAL HOSPITAL; HAINAN HOSPITAL OF PLA GENERAL HOSPITAL (CN)	None
4	PCT/US2019/030083	THE TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA [US]; THE CHILDREN'S HOSPITAL OF PHILADELPHIA [US].	US (17/052,170)
5	PCT/US2023/069704	THE REGENTS OF THE UNIVERSITY OF CALIFORNIA [US]	EP (23836272.7)
6	PCT/US2017/023404	DANA-FARBER CANCER INSTITUTE, INC. [US]; THE TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA [US]; THE REGENTS OF THE UNIVERSITY OF CALIFORNIA [US]	US (16/086,719), EP (17770984.7)
7	PCT/US2023/067607	THE GENERAL HOSPITAL CORPORATION [US]; DANA-FARBER CANCER INSTITUTE, INC. [US]; MASSACHUSETTS INSTITUTE OF TECHNOLOGY [US].	None
8	PCT/US2015/054747	PRESIDENT AND FELLOWS OF HARVARD COLLEGE [US]; THE CHILDREN'S MEDICAL CENTER CORPORATION [US].	US (14/485,288; 14/509,787; 15/818,710; 17/728,927), EP

			(23172496.4; 14779492.9)
9	PCT/IB2016/057318	NOVARTIS AG [CH]; INTELLIA THERAPEUTICS, INC. [US].	US (15/780,751; 18/534,209), EP (16820015.2), IN (201817023766)
10	PCT/KR2022/011139	TOOLGEN INC [KR]	US (18/424,920), EP (22849909.1)
11	PCT/US2023/016141	THE TRUSTEES OF COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK [US]	None
12	PCT/US2022/072014	THE BOARD OF TRUSTEES OF THE LELAND STANFORD JUNIOR UNIVERSITY [US]	None
13	PCT/US2017/034070	WASHINGTON UNIVERSITY [US]	US (16/303,790)
14	PCT/US2016/040015	BROAD INST INC [US] MASSACHUSETTS INST TECHNOLOGY [US] DANA FARBER CANCER INST INC [US] MASSACHUSETTS GEN HOSPITAL [US]	EP (16741186.7), US (15/844,601)
15	202410382743	UNIV SICHUAN AGRICULTURAL [CN]	None

Table 2.3. List of 15 Patent shortlisted families for detailed analysis

A brief summary of the inventions covered by the 15 identified families have been provided in the table below.

Family No	Application No.	The Invention Relates to
1	PCT/US2022/017854	Compositions and methods using the Cas9 from *Staphylococcus lugdunensis* (SluCas9) to treat Myotonic Dystrophy Type 1 (DM1). It focuses on guide RNA combinations suitable for SluCas9 to excise a CTG repeat in the Myotonic Dystrophy Protein Kinase (DMPK) gene, with or without a DNA-PK inhibitor.
2	PCT/IB2020/060720	Methods to treat CD70+ solid tumors by first performing lymphodepletion, followed by administering genetically engineered T cells expressing a CAR that targets CD70.

		These T cells are modified using CRISPR/Cas9 to disrupt specific genes, enhancing their therapeutic efficacy.
3	201810565134.6	A FOXC1 gene mutant used in drugs for treating Axelfeld-Riegel syndrome, screening susceptibility, and constructing recombinant cells. It includes a medicament with gene editing agents like CRISPR/Cas9, zinc finger nucleases, and other technologies for modifying the nucleic acid or protein.
4	PCT/US2019/030083	A method of *in utero* genome editing using CRISPR-mediated base editors BE3 or BE4 to introduce mutations in genes causing genetic diseases. It aims to treat conditions like enzyme deficiencies, inherited metabolic diseases, and lung disorders.
5	PCT/US2023/069704	Modifying viral nucleic acids in eukaryotic cells using CRISPR-Cas effector polypeptides, preferably Cas9. It includes various types of CRISPR-Cas effector polypeptides (II, III, IV, V, VI) for this purpose.
6	PCT/US2017/023404	CAR-T cell therapy using engineered mammalian T cells with modified genomic regions to regulate gene expression in exhausted CD8+ T cells. These modifications, achieved through various gene editing techniques like CRISPR-Cas9, aim to treat immune disorders.
7	PCT/US2023/067607	Methods of treating cancer by administering to a subject, one or more therapeutic agents that target one or more cancer-specific genes or gene expression products and such agent is a gene editing system, more specifically, a CRISPR-associated transposase (CAST) system.
8	PCT/US2015/054747	Multiple guide strategy to efficiently delete target polynucleotide sequences (e.g., beta-2-microglobulin (B2M), Hypoxanthine-guanine phosphoribosyltransferase (HPRT), C-C chemokine receptor type 5 (CCR5) and/or C-X-C chemokine receptor type 4 (CXCR4)) in primary somatic cells. These modified cells are then administered to treat disorders like genetic diseases, HIV, AIDS, or cancer.
9	PCT/IB2016/057318	Immunooncology compositions and methods using CRISPR-modified cells targeting specific genome sequences (B2M, CD247, CD3D, CD3E, CD3G, TRAC, TRBC1, TRBC2, HLA-A, HLA-B, HLA-C, DCK, CD52, FKBP1A, CIITA, NLRC5, RFXANK, RFX5, RFXAP, or NR3C1). These modified cells, including engineered T cells with chimeric antigen receptors, are used to treat cancers.

10	PCT/KR2022/011139	Mesenchymal stem cells with engineered Peripheral blood mononuclear cell tissue factor (F3) genes containing indels, reducing the expression of Coagulation Factor III (CD142) compared to wild-type cells. It uses the CRISPR/Cas9 complex for gene modification.
11	PCT/US2023/016141	Inducing multidirectional degranulation by cytotoxic effector cells in tumors by inhibiting vesicle transport proteins using CRISPR. These modified cells are then delivered to the tumor microenvironment for treatment.
12	PCT/US2022/072014	Generating genetically modified stem cells with mutations in the hemoglobin subunit beta (HBB) gene using CRISPR-Cas9 and AAV vectors. These modified cells are used for *ex vivo* treatment of hematological diseases like sickle cell disease.
13	PCT/US2017/034070	Use of targeted adenovirus with CRISPR/Cas9 for *in vivo* editing at specific organ sites, particularly pulmonary cells, to reduce liver toxicity. It includes vectors for delivering therapeutic genes like Factor IX for hemophilia treatment.
14	PCT/US2016/040015	Treating conditions like cancer, glioma, and melanoma by using agents to modify the expression of specific complement system genes. The CRISPR-Cas9 system is employed to either activate or decrease the expression of these genes.
15	202410382743	Using a CRISPR/Cas9 knockout vector to silence the KLRB1 gene for promoting bone formation. This approach provides a theoretical basis for treating bone-related diseases like osteoporosis, osteoarthritis, and bone cancer.

Table 2.4. Summary of inventions for the 15 shortlisted families.

2.4. Current Status of Technology

CRISPR GENOME EDITING VIA DOUBLE STRAND DNA BREAK AND REPAIR

Figure 2.6 depicts the working mechanism of CRISPR/Cas9 technology. The CRISPR gene editing process begins with creating double-strand breaks (DSBs) at specific locations in the eukaryotic genome. The Cas protein, guided by RNA, induces these DSBs at the target site in the cellular DNA. Following this, the cell's DNA repair mechanisms are activated, leading to genome editing at the break points. This editing can result in insertions or deletions of sequences at the target site or the insertion of externally provided DNA sequences. In eukaryotic cells, DSB repair occurs through three main pathways: non-homologous end joining (NHEJ), homologous directed repair (HDR), and microhomology-mediated end joining (MMEJ)[2.46, 2.47, 2.48]. The foregoing repair processes in DNA function in a mutually exclusive or complementary manner, depending on the *in vivo* and cellular context.

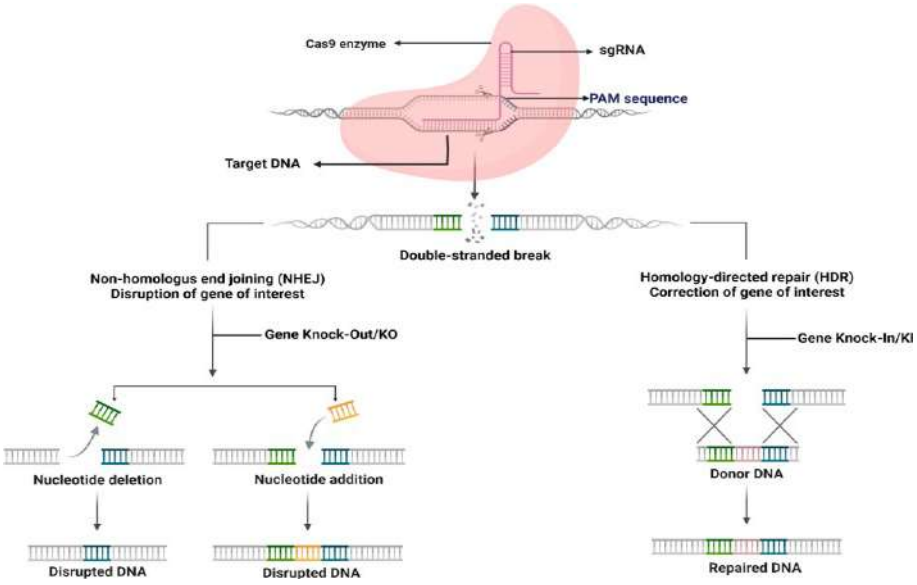


Figure 2.6. Working mechanism of CRISPR/Cas9 technology

(Source: Muhammad Naeem, Hanoof Fahd Alkhodairy, Iqra Ashraf, Amjad Bajes Khalil, CRISPR/Cas System Toward the Development of Next-Generation Recombinant Vaccines: Current Scenario and Future Prospects, Arabian Journal for Science and Engineering (2023) 48:1–11).

Non-homologous end joining (NHEJ): NHEJ functions throughout all phases of the cell cycle and consists of four main steps: 1) detecting DNA breaks, 2) aligning and forming a synapse with the cut DNA, 3) processing the DNA ends, and 4) ligating the DNA ends together. This repair process involves several proteins, such as Ku70/80, MRE11, the Artemis:DNA-PKcs complex, and the XLF:XRCC4:DNA ligase IV complex. NHEJ is naturally error-prone because it lacks a homologous DNA template during the cutting and joining of DNA ends[2.48]. As a result, the DNA base sequence can undergo various changes during NHEJ-mediated repair. If insertions or deletions occur within the coding region, there is a potential risk of gene knockout due to a frameshift or the introduction of a premature stop codon.

Homologous Directed Repair (HDR): Homologous recombination is mainly active during the S and G2 phases of the cell cycle. This process begins with the coordinated action of several proteins, such as Rad51, Rad52, Rad54, BRCA2, and RPA. HR involves several key steps: 1) recognizing and resecting DNA ends, 2) homologous pairing and DNA strand exchange, 3) extending the DNA heteroduplex and branch migration, and 4) resolving Holliday junctions[2.49]. During the repair process, the homologous region on the sister chromatid acts as a template to repair the broken DNA, enabling error-free DNA repair without introducing mutations.

Microhomology-mediated end joining (MMEJ): In addition to canonical NHEJ (c-NHEJ), microhomology-mediated end joining (MMEJ) is an alternative NHEJ pathway that operates during the G1 and early S phases of the cell cycle. Unlike c-NHEJ, MMEJ repairs DNA breaks by using short homologous sequences near the DSB site[2.50, 2.51]. This process begins with proteins such as PARP1, MRE11, CtIP, and XRCC1: DNA ligase 1 or 3 binding to the DNA break site. The cleaved DNA ends are joined using a 10-base microhomologous sequence. This error-prone process often results in the deletion of DNA sequences.

CRISPR technology leverages these repair mechanisms to insert foreign DNA sequences into the genome or remove target genes by creating DSBs and utilizing the subsequent repair processes.

CRISPR GENOME EDITING WITHOUT DOUBLE-STRANDED DNA BREAK

Despite improvements in precision with DBS-mediated CRISPR gene editing, the technology can still introduce variable mutations, insertions, or deletions into target DNA sequences. This makes it challenging to use CRISPR for tasks requiring precise base alterations, such as correcting pathogenic SNVs.

Base Editing: For addressing the concerns associated with DSBs, approaches such as base editor technology, has been explored and it demonstrated the ability to replace a single base at a target site by utilizing a base modification enzyme in conjunction with engineered CRISPR system that induces DNA nick instead of DSB[2.52]. This technique can usually replace single bases within specific sequence regions of 10 or fewer bases in the target DNA. It allows for directed changes in DNA bases, such as switching cytosine to thymine or adenine to guanine. Additionally, depending on the gene editing goals, the sequence region for base substitution can be extended or reduced beyond the 10-base limit. The development of base editing technology offers an alternative approach that avoids the use of DSBs.

Prime Editing: Although base editing techniques have enabled precise single-base edits, challenges like bystander editing, inability to perform transverse mutations, and accurate editing of consecutive bases remain. To address these issues, prime editing was developed[2.53]. Similar to base editing, prime editing performs gene editing without inducing DSBs, but its molecular mechanism differs as compared to the former. A major difference in the prime editing method is the use of reverse transcriptase (RT) instead of a base conversion enzyme. Additionally, the prime editing guide RNA (pegRNA) serves two roles: it acts as an RNA template for DNA reverse transcription and includes a segment that recognizes the target sequence as a guide RNA.

Enzymes

Specifically, Class II Cas9 and Cas12a (also known as Cpf1) proteins have been widely used in the field

of biomedicine due to their higher potential for targeted genetic engineering. Both Cas9 and Cas12a recognize and cleave the target double-stranded DNA with the help of a guide RNA, leading to PAM-dependent Cas nuclease activation resulting in breaks in the target DNA, followed by DNA repair [2.54, 2.55].

In fact, researchers have developed several variants of both the systems to increase the efficiency of CRISPR-mediated gene editing. These variants display higher specificity in comparison to the wild type enzymes [2.56].

Variant name	Resources	Selection Strategy	Mutation Domain	Ref.
SpCas9-HF1	SpCas9	Reduce the interaction between Cas9 and nontarget DNA sites	HNH and REC3 domains	2.57
eSpCas9	SpCas9	Neutralize the positive charges of Cas9 and DNA links and sites.	HNH and PAM-interacting domains	2.58
Sniper-Cas9	SpCas9	Sniper screen, an E. coli-based selection method		2.59
HypaCas9	SpCas9	REC3 and DNA complementation control HNH domain activation	REC3 domain	2.60
evoCas9	SpCas9	Screening method using a yeast reporter strain	REC3 domain	2.61
Cas9TX	SpCas9	Prevent the perfect repair of DNA	Carry optimized TREX2	2.62
HscCas9-v1.2	SpCas9	Substitution of amino acid residues	Multiple domains	2.63
superFi-Cas9	SpCas9	When mismatched, sgRNA, and DNA chains form RuvC loop	RuvC loop	2.64
efSaCas9	SaCas9	Construction of an SaCas9 variant library and directional screening system	REC3 domain	2.65
SaCas9-HF	SaCas9	Modify that residues where the distal region of PAM is linked to the target DNA	Recognition lobe domain and RuvC domain	2.66

Table 2.5: Cas9 variants that have been modified for increased specificity.

Source: Li, T., Yang, Y., Qi, H. et al. CRISPR/Cas9 therapeutics: progress and prospects. Sig Transduct Target Ther 8, 36 (2023). https://doi.org/10.1038/s41392-023-01309-7.2.1

Due to these advancements, Cas enzymes act as an ideal tool for treatment of diseases by either correcting deleterious base mutations or by disrupting disease causing genes.

Carriers for delivering CRISPR technology

CRISPR-mediated therapy is based on two major approaches; *ex vivo* genome editing and *in vivo* genome editing, where the CRISPR components can be delivered into target cells in the form of DNA, RNA, or RNP. *Ex vivo* therapies involve cell extraction, gene editing, and transplantation of modified cells. On the other hand, *in vivo* therapies deliver cells/biomolecules directly inside the body of the subject and for the ethical reasons associated with *in vivo* therapies, very few clinical trials have been performed for such therapies [2.67].

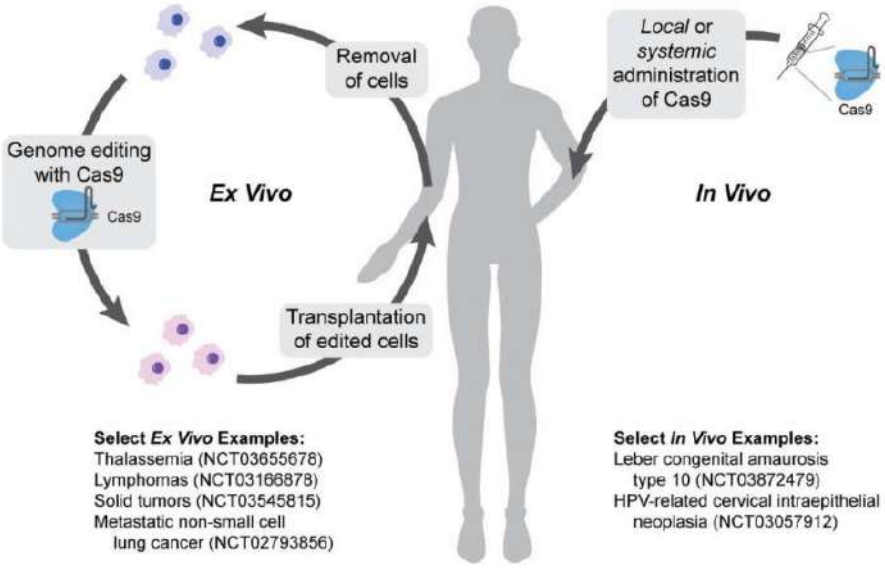


Figure 2.7: CRISPR-based cell-editing strategies in patients. (Left) *Ex vivo* applications of CRISPR/Cas9 gene editing begin with the isolation of cells. Cells are typically expanded and then edited and filtered before being transplanted. (Right) For *in vivo* editing, CRISPR/Cas9 (or dCas9, not shown) is administered locally or systemically with the help of viral packaging or nanoparticles.

Source: Modell AE, Lim D, Nguyen TM, Sreekanth V, Choudhary A. CRISPR-based therapeutics: current challenges and future applications. Trends Pharmacol Sci. 2022 Feb;43(2):151-161. doi: 10.1016/j.tips.2021.10.012. Epub 2021 Dec 21. PMID: 34952739; PMCID: PMC9726229.2.2

Due to the complicated nature of both types of therapies, the selection of mode of delivery of CRISPR/Cas systems is critical to ensure targeted and effective delivery of the CRISPR components. In clinical trials, electroporation is widely used, however, several approaches are available for *in vivo* CRISPR-system deliveries as summarized in the figure below.

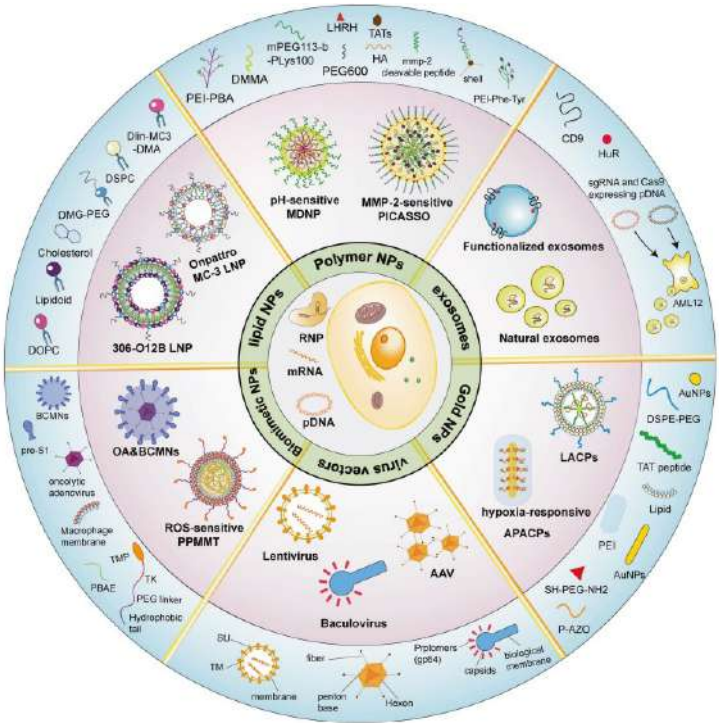


Figure 2.8: Schematic diagram showing multiple types of vectors for the *in vivo* delivery of CRISPR systems. The central region shows three forms of CRISPR action: pDNA, mRNA, and RNP. The middle circle section shows examples of delivery carriers, and the outermost area shows how the carriers are produced or the components.

Source: Li, T., Yang, Y., Qi, H. et al. CRISPR/Cas9 therapeutics: progress and prospects. Sig Transduct Target Ther 8, 36 (2023). https://doi.org/10.1038/s41392-023-01309-7.2.1

2.5. Application of CRISPR/Cas Systems in Therapy

In recent times, the CRISPR/Cas9 system has been broadly utilized for: Gene therapy for genetic disorders (*sickle cell diseases, beta-thalassemia, Duchenne Muscular Dystrophy, Cystic Fibrosis*); Cancer Therapy (*CAR-T, Oncogene editing);* Infectious diseases (*HIV, HPV, Hepatitis B*); and Neurological disorders (*Huntington's disease, Alzheimer's disease, Parkinson's disease*). [2.67]

Ex vivo therapy

Modification of hematopoietic stem cells and progenitor cells (HSPCs) using CRISPR/Cas system has led to development of therapies for hematological diseases like sickle cell disease and transfusion-dependent β-thalassemia[2.68] as well as therapies for X-linked chronic granulomatous disease (X-CGD) [2.69]. In addition, *ex vivo* genome editing of induced pluripotent stem cells (iPSCs) can lead to development of treatment strategies for Duchenne muscular dystrophy (DMD)[2.70]. Application of *ex vivo* gene editing also extends to treatment of rare diseases like recessive dystrophic epidermolysis bullosa (RDEB)2.71, and hereditary tyrosinemia type 1[2.72].

In vivo therapy

In vivo therapies based on CRISPR/Cas system have been a success in treatment of muscle degeneration diseases, eye-related genetic diseases, liver-related genetic diseases and wide range of cancers [2.73-2.76].

Precision medicine

The advancements in the CRISPR/Cas system make it an excellent tool for precision medicine. Beyond gene editing, CRISPR can be used to modulate gene expression and explore non-coding elements through CRISPR interference (CRISPRi) and CRISPR activation (CRISPRa)-based epigenome and transcriptome editing. This adds flexibility in how the genome is reprogrammed[2.77-2.84]. Emerging technologies like epigenome, prime, and base editing are opening new avenues for precision oncology, accelerating basic cancer research, preclinical drug discovery, diagnosis, and treatment (Figure 2.9).

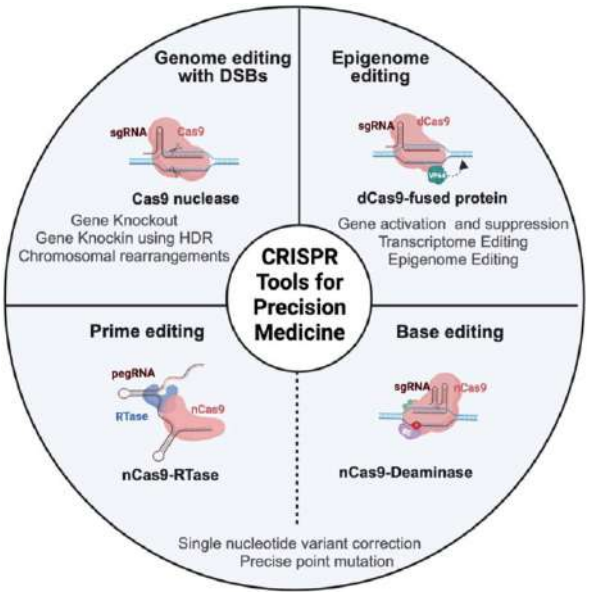


Figure 2.9: Different CRISPR-based genome and epigenome engineering tools for cancer research

Source: Ravichandran M, Maddalo D. Applications of CRISPR/Cas9 for advancing precision medicine in oncology: from target discovery to disease modeling. Front Genet. 2023 Oct 16;14:1273994. doi: 10.3389/fgene.2023.1273994. PMID: 37908590; PMCID: PMC10613999.

CRISPR/Cas mediated epigenome, prime, and base editing have provided a plethora of options for precision oncology and also contribute to cancer research, drug screening, and treatment, as enlisted in the Table 2.6 below.

Cas9 variants	Components	Effector domain(s)	Gene edits	Applications
CRISPR/ Cas9 nuclease	Cas9 endonuclease and sgRNA	None	Gene knockout with indels Gene knock-in using the exogenous HDR template Introduce DSB	High-throughput functional genomics screen in *in vitro* (cellular and organoid models), *ex vivo*, and *in vivo* systems *In vitro* and *in vivo* disease modeling (knock-in and knockout models) Cancer diagnostics
Epigenome editor	dCas9 fused to epigenome editors and sgRNA	Epigenome editors such as TET enzymes, DNMT3A, 3L, and MECP2	Chromatin re-organization Histone modifications DNA methylation and demethylation Non-coding element Does not introduce DSB	Complex genetic modeling *in vitro* as well as *in vivo* albeit less efficiency

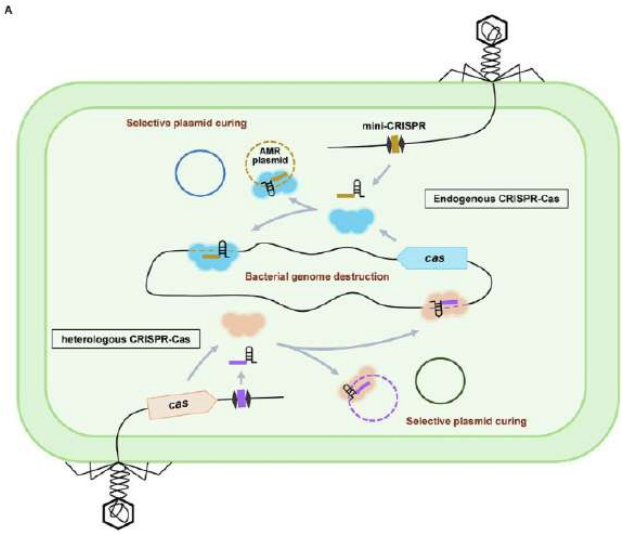
Transcriptome editor	Dead Cas9 fused to gene activators (CRISPRa) or gene suppressors (CRISPRi) and sgRNA	VP64, VP16, and KRAB domains	Transcriptional activation and repression Do not introduce DSB	High-throughput functional genomics screen in *in vitro, ex vivo*, and *in vivo* system
Base editor	Cas9 nickase fused to adenine or cytosine base editor and sgRNA	Adenine deaminase (example: Tad-8e); cytidine deaminase (example: AID/ APOBEC)	Single-nucleotide editing ABE: A to G CBE: C to T Introduces DNA nick	Modeling SNVs *in vitro* and *in vivo* High-throughput SNV screening
Prime editor	Dead Cas9 fused to reverse transcriptase to pegRNA	Reverse transcriptase	All 12 base substitutions Short insertion and deletion without donor plasmid and DSB Requires modified sgRNA	Modeling SNVs Modeling gene knock-in or mutant with single/multiple mutations

Table 2.6: Applications of CRISPR/Cas mediated epigenome, prime, and base editing.

Source: Ravichandran M, Maddalo D. Applications of CRISPR/Cas9 for advancing precision medicine in oncology: from target discovery to disease modeling. Front Genet. 2023 Oct 16;14:1273994. doi: 10.3389/fgene.2023.1273994. PMID: 37908590; PMCID: PMC10613999.

Combating antimicrobial resistance

Antimicrobial-resistant (AMR) bacteria have become a major problem to be tackled in the field of biomedicine. CRISPR/Cas system offers the solution by selectively targeting and disintegrating bacterial DNA other than invasive genetic elements. Therefore, it can be used in development of the next-generation antimicrobials against AMR pathogens. [2.85].



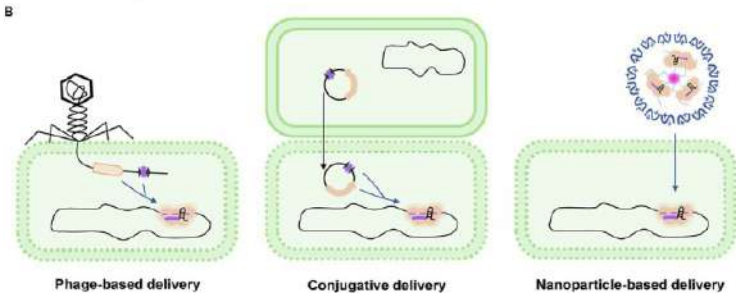


Figure 2.10: Delivery of CRISPR/Cas antimicrobials based on the endogenous and heterogeneous CRISPR/Cas systems.

Source: Duan C, Cao H, Zhang LH, Xu Z. Harnessing the CRISPR-Cas Systems to Combat Antimicrobial Resistance. Front Microbiol. 2021 Aug 20;12:716064. doi: 10.3389/fmicb.2021.716064. PMID: 34489905; PMCID: PMC8418092.

Due to the presence of an extremely strict permeability barrier, Gram-negative bacteria (GNB) display resistance to several antibiotics. For these reasons, multiple drug-resistant (MDR) and widely drug-resistant (XDR)[2.84] GNB have become a reason for concern. Further, due to widespread presence of drug-resistance genes like bla KPC, blaNDM, blaVIM, and MCR-1, a sudden need for therapies against MDR Gram-negative bacteria has been realized[2.84]. The CRISPR/Cas system, due to its ability to target multiple genes at once, offers great potential to curb anti-microbial resistance. In a recent study, it was established that CRISPR/Cas9 system can target two super drug-resistant genes namely, MCR-1 and blaNDM-1 concurrently, leading to elimination of both the genes at once. This helps to maintain the efficacy of carbapenem and colistin antibiotics.[2.86]

On the other hand, drug-resistant Gram-positive bacteria (GPB), which cause various infections, possess thick cell walls, has raised concerns, especially methicillin-sensitive *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococci* (VRE), and highly resistant strains to *Streptococcus penicillin pneumoniae* infections which pose significant threat to humans[2.67]. Recent studies show that CRISPR/Cas systems are effective in combatting drug resistance in Gram-positive bacteria, for instance, CRISPR/Cas acts as an effective barrier to horizontal antibiotic resistance in *E. faecalis*.[2.87]

Antibody Engineering

CRISPR/Cas system allows engineering antibodies in hybridoma cells without sequencing the variable regions of antibody and having to clone the same in cell lines (Figure 2.9). This enables production of site-specific conjugated antibodies in a time and cost-effective way.

In addition, CRISPR/Cas9, in recent studies, was used for modification of mouse and human immunoglobulin genes to induce class-switch recombination and generate different IgH subclasses[2.88]. Further, it has been used to develop a platform to swap the variable chains of the immunoglobulin genes to change their specificity[2.89].

Khoshnejad, M., *et al*,[2.90] made use of CRISPR/Cas9 genomic editing, to incorporate Sortase (LPETGG) and Flag (DYKDDDDK) tags at the C-terminal end of the CH3 heavy chain region in a mouse monoclonal antibody providing conjugation of cargo without loss of antibody affinity, while ensuring optimal orientation of the antibody and minimizing steric hindrance or altered conformation of the complementarity-determining regions (CDRs). This led to the development of genetically encoded modifications to antibodies in a cost-effective manner. The conjugatable antibodies produced can further be employed in therapeutics and diagnostics. [2.90].

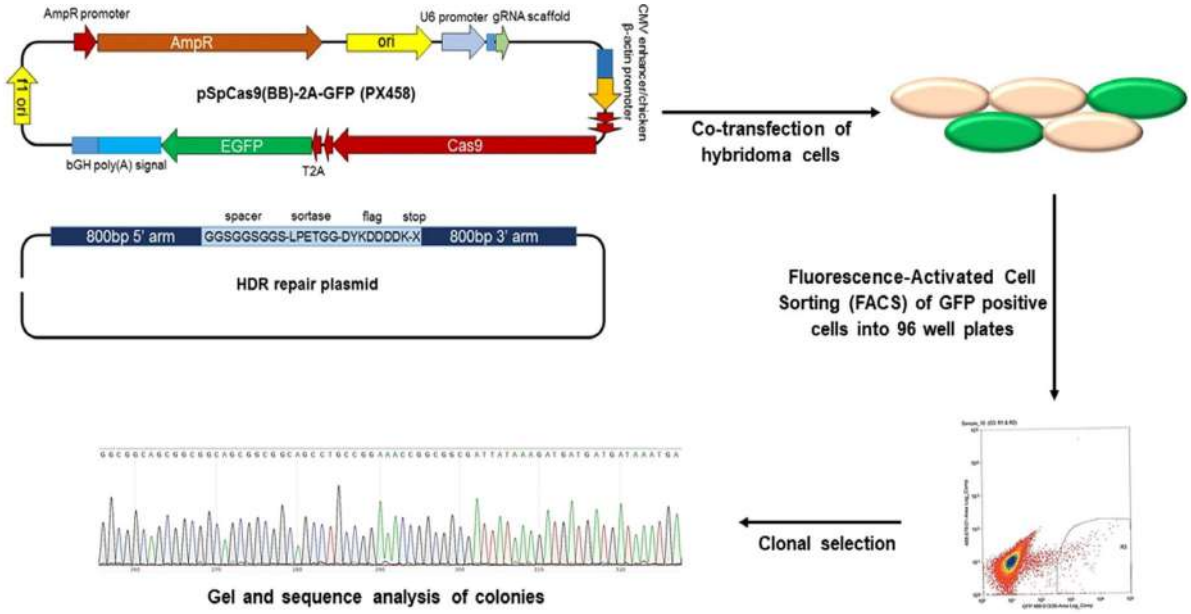


Figure 2.11: Illustration of CRISPR/Cas9 genome editing approach of hybridoma cells for site-specific modification of antibodies. Hybridoma cells were modified by co-transfection with plasmid expressing sgRNA and Cas9, and linearized HDR repair plasmid.

Source: Khoshnejad, M., Brenner, J.S., Motley, W. et al. Molecular engineering of antibodies for site-specific covalent conjugation using CRISPR/Cas9. Sci Rep 8, 1760 (2018). https://doi.org/10.1038/s41598-018-19784-2

2.6. Current Challenges and Future Prospects

CRISPR/Cas systems have proved to be of great utility for both *in vivo* and *ex vivo* therapies. It enables wider coverage of genome editing and helps target multiple genes simultaneously. Technological advancements in CRISPR based technologies have directly shown a positive effect on the advances in present day cell therapies. However, several challenges remain to be countered for achieving an enhanced development of CRISPR-based therapies. These challenges can be broadly divided into two categories:

1. Ethical challenges, and
2. Technical challenges.

Since the application of CRISPR/Cas systems in mammalian cells, there have been several debates to draw the boundaries of gene editing in humans in view of public morality and safety. Three main viewpoints have been reflected in such debates; the first group that supports advancements in CRISPR/Cas technology for human therapies in a liberal manner, the second group that completely negates the application of same in germline editing. In addition, there is a third group of researchers that are focused on promoting CRISPR/Cas related research in biomedicine while complying with the international guidelines on the ethical concerns associated with CRISPR technologies.

One such prominent incident that fueled the debates on ethical concerns, was the experiment conducted by He Jiankui (China) in 2018 [2.91]. He Jiankui's research was never officially published in a peer-reviewed journal. However, excerpts from his unpublished manuscript titled "Birth of Twins After Genome Editing for HIV Resistance" were made public by MIT Technology Review [2.92, 2.93]. The researcher used CRISPR technology to target CCR5 gene in human embryos wherein 32 base pairs were deleted from said gene. This mutation, called CCR5 32, can help prevent infection with HIV in children where one of the parents is HIV infected [2.92, 2.93]. His research received severe backlash from the scientific community questioning the legal basis for the experiment carried out by He Jiankui and his team [2.94, 2.95]. Furthermore, there were speculations that contrary to the reason provided by Jiankui, the real reason for his experiments could be associated with enhancing human intelligence via gene editing as it was well known that CCR5 genes also regulate major brain functions.[2.96].

What received even more criticism was the inefficiency of the experiments conducted[2.94]. The genetically edited human embryos resulted in the birth of two healthy girls, Nana and Lulu. However, the gene editing was not performed uniformly for the twins. In one of the twins, two copies of CCR5 were modified, whereas in the other only one copy was modified (heterozygous expression) [2.94]. This meant that one twin could still become infected, although the evolution of the disease would probably be slower. The objective was to analyze the differences in both the babies as their growth progressed.

This raised the issue of parents' informed consent regarding human experimentation, which follows a much stricter regimen than consent for therapeutic procedures. Further, the data from this research suggested that the embryos were mosaics, and displayed off-target edits which are also one of the major technical concerns associated with emerging CRISPR technologies. In the future, when CRISPR/Cas9 and gene editing in general reaches its maximum potential to tackle human health related issues, an extensive legal guideline would be a prerequisite to monitor consent and moral responsibilities of the people concerned. Further, clear boundaries between biomedical research and research based on mere scientific curiosity would require to be drawn up to ensure that CRISPR/Cas technologies are limited to purely health related issues.

Referring to technical concerns, immune responses in patients, off-targets, efficient delivery, DNA damage, are the major problems associated with CRISPR-based therapies. [2.97-2.99] To overcome these challenges, the development of safe, and stable delivery strategies which prevent modification of non-target cells is required [2.97, 2.100].

For instance, Adeno-Associated Viruses (AAV), the most commonly used vectors for delivering gene drugs, may lead to disease and non-acceptability, in the human genome [2.101]. Therefore, alternatives like safer nonviral vectors like lipid nanoparticles (LNPs), have recently gained attention in clinical trials [2.102]. However, LNPs and AuNPs also frequently trigger immunogenicity in vivo and may be absorbed by digestive organs [2.103]. To overcome this, modification or peptide addition in LNPs and AuNPs have been carried out by scientists.

Further, the efficiency and safety of CRISPR/Cas9 are the other two important factors to consider in clinical applications. In order to avoid off-target CRISPR/Cas9 effects, scientists have developed several variants of Cas enzymes especially for Cas9 that are highly target sensitive without compromising with the efficiency. In addition to modification of Cas9, modification of sgRNA has also displayed reduced off-target effects [2.104].

Another study by Ryotaro Hashizume, *et al* 2.105 used the CRISPR/Cas9 system to cleave the third chromosome in trisomy 21 cell lines derived from both pluripotent cells and skin fibroblasts [2.105, 2.106] (Figure 2.12). The technique was successful in identifying the duplicated chromosome, ensuring that the cell does not end up with two identical copies after removal, but instead has one from each parent. The researchers were able to remove duplicate chromosomes from cells and chromosomal rescue reversibly restored both gene expression and cellular phenotypes[2.105, 2.106]. However, the approach is not yet ready for *in vivo* application, however, in part because the current technique can also change the retained chromosomes. This technique with further refinement would prove quite useful for the treatment of people with Down syndrome. [2.107]

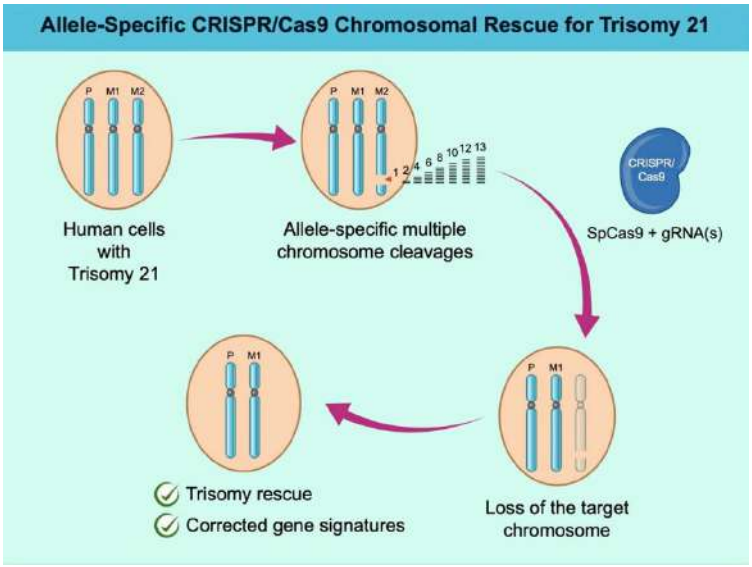


Figure 2.10. CRISPR/Cas9 system to cleave the third chromosome in trisomy 21 cell lines.

Source: https://medicalxpress.com/news/2025-02-crispr-extra-chromosomes-syndrome.html#google_vignette

To summarize, detailed studies of disease etiology, the development of better and effective delivery vectors, and generation of improved Cas9 variants are important for development of any CRISPR/Cas based therapy.

2.7. Key Findings

- During the initial screening, 1925 patent families were carefully reviewed and narrowed down to 1082 families specifically related to CRISPR-based gene editing for therapeutic use. This included applications with both direct and indirect claims to therapy, addressing tactics used to avoid therapeutic subject matter claims. A jurisdictional analysis revealed that China led with 641 filings, followed by the USA (584), Europe (378), Japan (250), Canada (213), and Australia (205). India had 85 filings, all originating from PCT applications. Notably, 61.05% (660 families) of the shortlisted patents were filed via the PCT route, highlighting the global strategic approach in protecting CRISPR therapeutic innovations.

- Out of 1,082 CRISPR-based therapeutic patent families, the US accounted for the highest share of priority filings at 49%, followed by China at 39%, and Europe at 4%, while India represented less than 1%. This disparity highlights limited patent activity in India, likely due to stricter patent laws concerning therapeutic inventions. The data confirms that the US and China are the dominant players in pioneering CRISPR technology for human therapeutics, with China also leading in priority filings for general CRISPR applications globally. This trend underscores the strategic focus of these countries in securing intellectual property rights in this transformative field.

- An analysis of 242 patent families related to CRISPR technology in therapeutics reveals the University of California (UC) as the leading applicant with 31 patent families, focusing on diverse gene targets for treating diseases such as severe combined immunodeficiency, Meniere's disease, renal cell carcinoma, Duchenne muscular dystrophy, glaucoma, and neurodegenerative disorders.

- Dana-Farber Cancer Institute, ToolGen Inc., and the University of Pennsylvania follow closely, each with 17 patent families. Dana-Farber's patents primarily target cancer-related genes, while ToolGen emphasizes engineered stem cells for a wide range of diseases including cardiovascular, autoimmune, neurological, and hematological disorders.

- The University of Pennsylvania's filings focus on cancer and autoimmune therapies targeting immune regulatory genes. In China, the Chinese Academy of Sciences and the Chinese Academy of Agricultural Sciences dominate patent filings in human therapeutics.

- The analysis reveals extensive collaborations among leading institutions in CRISPR-mediated therapy patent filings. The University of Pennsylvania stands out, filing 9 of its 17 applications jointly with partners such as Children's Hospital Philadelphia, Dana-Farber Cancer Institute, University of California, Stanford University, and Novartis AG. These collaborations focus on innovative gene-editing methods for treating diseases ranging from monogenic lung disorders to cancers and immune conditions. Similarly, the Broad Institute collaborates with MIT, Harvard, and Dana-Farber, while the University of California partners with multiple research and medical institutions globally.

- Among 242 patent families related to CRISPR-based gene editing for therapy, 46% focus on cancer treatment, making it the dominant application area. Genetic disorders such as sickle cell disease, beta-thalassemia, Duchenne muscular dystrophy, and cystic fibrosis account for 15% of applications, while another 15% target neurological diseases like Alzheimer's and Huntington's. Cardiovascular and cardiopulmonary diseases represent 13%, with ocular diseases and HIV/AIDS comprising 8% and 3%, respectively.

- The patent landscape reveals a growing focus on CRISPR-based CAR-T cell therapies, with 21 out of 242 applications dedicated to this area, and over 80% utilizing the CRISPR/Cas9 system. Crispr Therapeutics AG leads in this field, targeting genes like Regnase-1, TGFBRII, TRAC, and β2M for treating various cancers, including solid tumors and multiple myeloma. The University of Pennsylvania and the Broad Institute also contribute significantly, filing patents for CAR-T therapies addressing cancer, viral diseases, and autoimmune conditions.

3 CRISPR UNLEASHED: Transforming Medical Diagnostics, One Cut at a Time

INDEX- CHAPTER 3

3.1.
CRISPR/Cas & Diagnostics: Background

Different nucleic acids tests are crucial for detecting specific nucleotide sequences, often used to identify particular species, including pathogens from blood, urine, secretions, and tissues. Advancements in nucleic acid-based diagnostics, such as polymerase chain reaction (PCR) and sequence alignment, have transformed clinical laboratories' ability to diagnose human pathogens. These innovative methods enable accurate and rapid diagnosis of various infectious diseases and help monitor treatment responses for infections like HIV and cytomegalovirus. However, in regions with limited medical resources, the lack of professionals and necessary equipment significantly restricts the use of these advanced tests [3.1-3.6]. CRISPR/Cas technology has emerged as a transformative tool in the field of molecular diagnostics, particularly for its application in the detection of various diseases, including cancer and infectious diseases. This technology utilizes the unique properties of CRISPR/Cas proteins to identify specific nucleic acid sequences with high sensitivity and specificity.

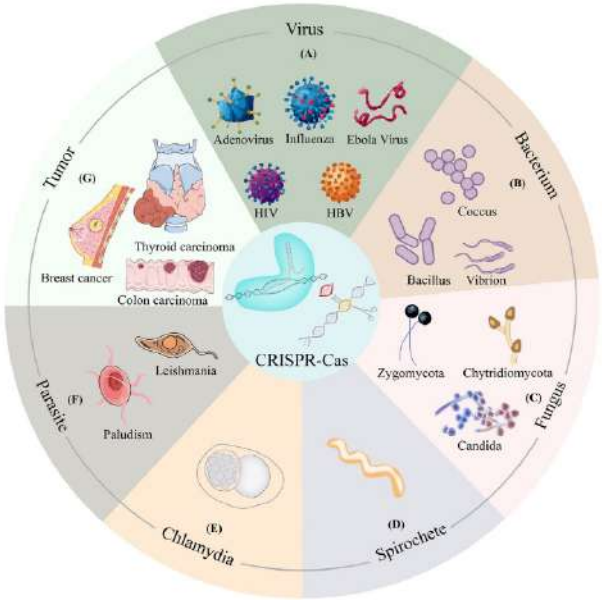


Figure 3.1. Applications of CRISPR/Cas- based in vitro diagnostic platforms in different pathogenic factors detection: (A) Virus, (B) Bacteria, (C) Fungus, (D) Spirochete, (E) Chlamydia, (F) Parasite, (G) Tumor.

Source: Wang, Z., & Cui, W. (2020). CRISPR-Cas system for biomedical diagnostic platforms. View, 1(3), 20200008.

3.2. CRISPR/Cas & Diagnostics: Search Results

Note: This section is a patent overview in CRISPR/Cas technology with a specific focus on Diagnostics.

METHODOLOGY:

- A patent search was conducted using patent databases to identify patent applications related to CRISPR/Cas-based diagnostics in the field of biomedical sciences.

- A total of 1547 Patent families were obtained as a result of the search. The identified 1547 patent families covered the use of CRISPR-based gene technology in Diagnostics. Further, these search results were divided into three screening processes:

  First Screening: The 1547 patent families were obtained at the end of patent search for CRISPR/Cas technology and its Diagnostic applications. These 1547 applications were screened, and 557 families were further shortlisted to be related to the applications of CRISPR/Cas technology in the diagnosis of diseases which may be infectious to humans. These 557 patent families were analyzed to study the trends in jurisdiction-specific patent filings and priority filings for CRISPR-based Diagnostics.

  Second Screening: The 557 patent families were further narrowed down to 105 patent families specifically related to the detection of diseases in humans. In other words, these families had direct application in diagnosis of diseases infectious to humans, based on the claims and as-filed specification. These 105 families were analyzed, and a detailed taxonomy-based classification was prepared.

  Third screening: Out of the 105 patent families, a detailed analysis was performed for 15 patent families that were identified to study the recent advances in CRISPR-based gene editing technology for use in diagnostic applications.

3.2.1. World Map for Patent Filings

During the first screening process, 1547 patent families were screened carefully to narrow down the dataset to 557 patent families. This screening was carried out to restrict our results to Patent Applications specifically related to CRISPR/Cas-based diagnostics for diseases, infections, and pathogens in humans.

For the shortlisted 557 patent families, a jurisdiction specific analysis was conducted, and the results are illustrated on a world map (Figure 3.2). The analysis revealed that the maximum number of patent applications have been filed in China i.e., 445 patent applications, which amounts to 80% of the total filings, followed by US (17%), South Korea (9%), and the EPO (9%). In India, a total of 10 patent applications have been filed for the applications related to the CRISPR/Cas-based diagnostics for diseases and infection in humans. Most of these were the patents filed by the PCT route. Additionally, a total of 117 PCT patent applications were filed directly at the International Bureau of WIPO, accounting for 21% of the 557 patent families.

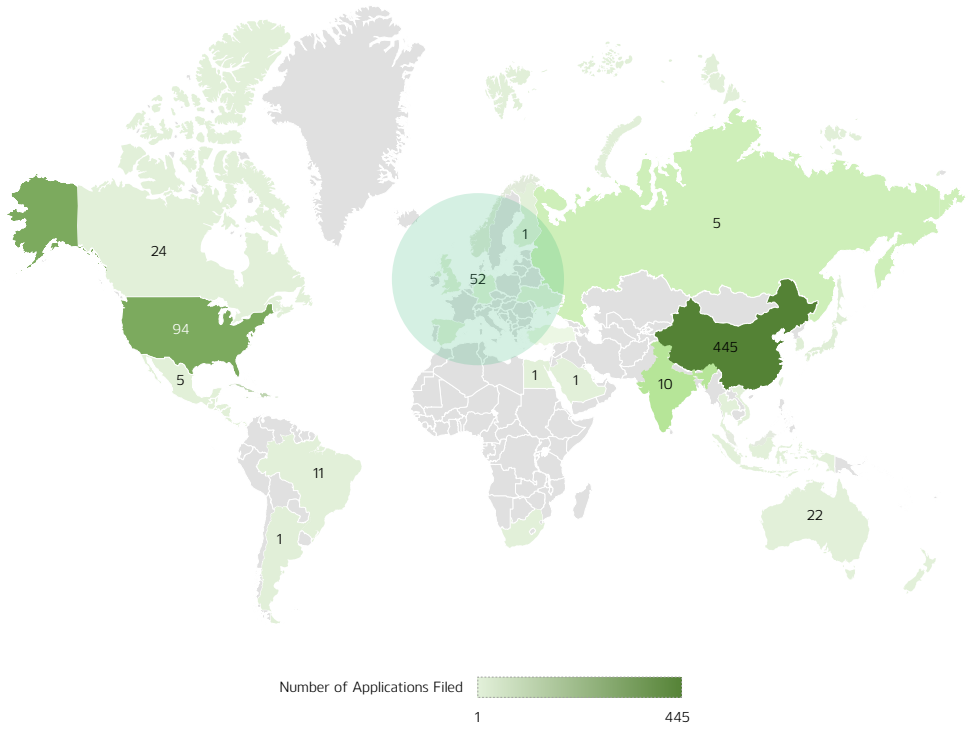


Figure 3.2: World map for patent applications related to CRISPR/Cas-based diagnostics of infectious disease and pathogens in humans

Out of 557 patent families, China has the maximum number of priority filings (75%), followed by US (13%), South Korea (7%), and the EPO (2%). India, on the other hand, does have priority filings but in very low number. A possible reason for this could be the conservative approach followed in India with respect to inventions related to diagnostics.

3.2.2. Top Applicants

During the second screening process, 557 patent families were further narrowed down to 105 patent families. This narrowing down was carried out to reduce the data set to patent applications related to inventions for use in diagnosis of diseases and infections affecting humans. Taxonomy based classification was prepared for the 105 patent families, where the taxonomic categories included: type of Cas enzyme/effector protein used, target disease/pathogen/disorder, target molecule, method of amplification, method of detection, multiplexing, type of claims in the patent application.

For identifying the top applicants across the major jurisdictions like China, US, Europe, etc., total of 557 patent families were analyzed. The top 10 applicants are represented in Figure 3.3. The Broad Institute (US) is the top applicant in filing patent applications related to the CRISPR/Cas-based diagnostics of diseases and infections affecting humans, accounting for 22 patent families. Following them is the INST Military Medicine Academy Military Sciences (CN) with 15 patent families, and both Shandong Shunfeng Biotechnology Co Ltd (CN) and Hainan University (CN) hold 10 patent families each.

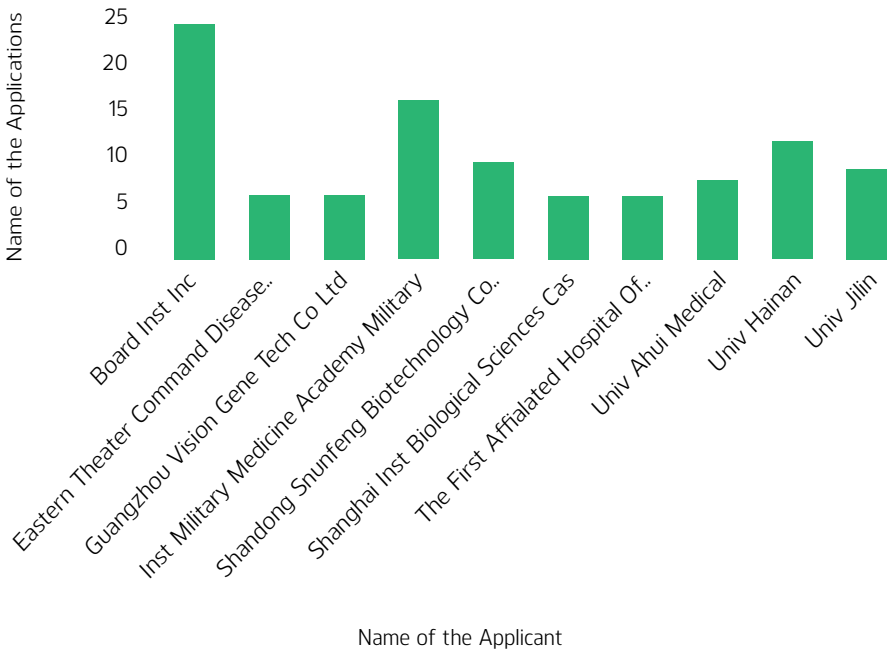


Figure 3.3: Top 10 Applicants for patent applications related to CRISPR/Cas-based diagnostics of infectious disease and pathogens in humans

3.2.3. Collaborations

The 557 patent families were analyzed to identify the top collaborators in filing the patent applications related to CRISPR/Cas-based diagnostics of diseases, infections, and pathogens in humans. The Broad Institute (US) has filed their patent applications in collaboration with either Harvard College, Massachusetts General Hospital, MIT, or Dana-Farber Cancer Institute. The Chinese applicant, INST Military Medicine Academy Military Sciences PLA has filed only 1 application out of their 15 applications in collaboration with Chinese PLA Center for Disease Control & Prevention. Another Chinese applicant, Hainan University have filed 4 out of their 10 applications in collaboration with three different entities.

3.2.4. Different Types of Cas Enzymes Used

The 557 patent families were analyzed to identify the number of patent applications employing different Cas enzymes. In 557 patent families, several applicants employed a different type of Cas enzyme to perform the diagnostic methods. Around 270 patent applications were filed for inventions using Cas12 and/or its variants. Another 96 applications were filed for inventions using Cas13 and/or its variants for diagnostic methods. Another 79 patent applications were filed for inventions using Cas9 and its variants. Interestingly, it was identified that a total of 84 patent families disclosed the use of more than one Cas enzyme for use in diagnosis or detection of diseases (Figure 3.4).

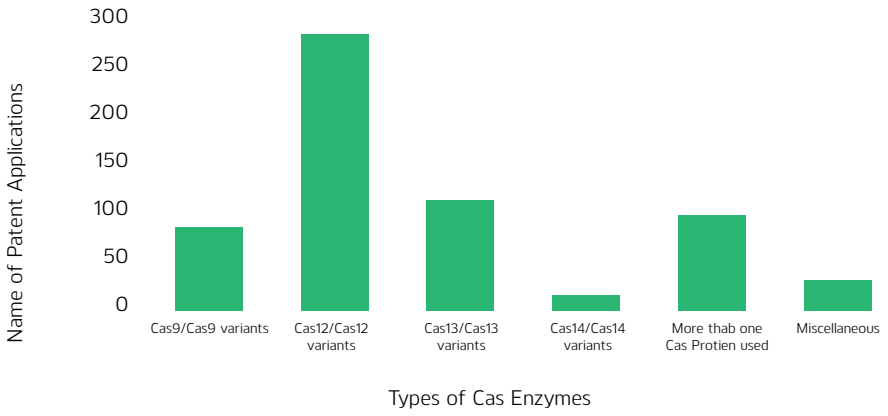


Figure III.IV: Different Cas enzyme used for patent applications related to CRISPR/Cas-based diagnostics of infectious disease and pathogens in humans

3.3. Detailed Analysis of Relevant Applications

Detailed analysis was carried out for 15 patent families selected from the results of the second screening process (Table 3.1).

Family Number	Application Number	Applicant	Corresponding Jurisdictions
1	PCT/US2020/022795	BROAD INSTITUTE INC [US]; MASSACHUSETTS INSTITUTE OF TECHNOLOGY [US]; HARVARD COLLEGE [US]	US17/439,063
2	PCT/US2018/050091	BROAD INSTITUTE INC [US]; MASSACHUSETTS INSTITUTE OF TECHNOLOGY [US]; HARVARD COLLEGE [US]	US16/645,571, EP18853355.8
3	PCT/US2018/066940	BROAD INSTITUTE INC [US]; MASSACHUSETTS INSTITUTE OF TECHNOLOGY [US]; HARVARD COLLEGE [US]	US16/955,380, EP18890190.4
4	PCT/US2020/049257	BROAD INSTITUTE INC [US]; MASSACHUSETTS INSTITUTE OF TECHNOLOGY [US]; DANA-FABER CANCER INSTITUTE [US]	US17/640,016
5	PCT/US2019/054561	BROAD INSTITUTE INC [US]; HARVARD COLLEGE [US]	US17/282,424
6	PCT/IN2020/050993	COUNCIL OF SCIENTIFIC & INDUSTRIAL RESEARCH [IN]	US17/781,726, EP20895727.4
7	US15/952,132	BROAD INSTITUTE INC [US]; MASSACHUSETTS INSTITUTE OF TECHNOLOGY [US]; HARVARD COLLEGE [US]	None
8	CN202410393528	INST MILITARY MEDICINE ACADEMY MILITARY SCIENCES PLA	-

9	CN202010160702	INST MILITARY MEDICINE ACADEMY MILITARY SCIENCES PLA	-
10	CN201910488038	INST MILITARY MEDICINE ACADEMY MILITARY SCIENCES PLA [CN]; CHINESE PLA CENTER FOR DISEASE CONTROL & PREVENTION [CN]	-
11	CN202410745149	SHANDONG SHUNFENG BIOTECHNOLOGY CO LTD	-
12	PCT/CN2022/091332	ANHUI MEDICAL UNIVERSITY [CN]	-
13	CN202211646024	UNIV JILIN	-
14	CN202310163354	EASTERN THEATER COMMAND DISEASE CONTROL AND PREVENTION CENTER [CN]; SUZHOU AOLIKANG BIOTECHNOLOGY CO LTD [CN]	-
15	PCT/CN2015/087557	SHANGHAI INST BIOL SCIENCES [CN]	US15/504,748

Table 3.1: List of 15 Patent shortlisted families for detailed analysis

A brief summary of the inventions covered by the identified families provided below (Table 3.2):

Family Number	Application Number	The Invention Relates to
1	PCT/US2020/022795	Rapid diagnostic systems and methods using CRISPR effector systems with optimized guide sequences, featuring a multiplex lateral flow device with a substrate that includes sample loading and capturing regions, detectable ligands, and CRISPR effector proteins or polynucleotides designed to target specific molecules.
2	PCT/US2018/050091	A highly sensitive CRISPR-based nucleic acid detection system that uses effector proteins, guide RNAs, signal amplification proteins, and RNA-based masking constructs to detect both DNA and RNA with attomolar sensitivity, distinguishing targets from non-targets based on single base pair differences, and is valuable for applications such as viral detection, bacterial strain identification, sensitive genotyping, and detecting disease-related cell-free DNA.

3	PCT/US2018/066940	A nucleic acid detection system with multiple CRISPR systems, each comprising an effector protein and guide molecule for specific target binding, along with a masking construct and optional nucleic acid amplification reagents, where the masking construct includes a cutting motif preferentially cleaved by one of the activated CRISPR systems.
4	PCT/US2020/049257	Rapid diagnostic systems and methods using CRISPR effector systems with optimized guide sequences, including multiplex lateral flow devices for detecting cancer markers, featuring CRISPR systems with Cas polypeptides and guide molecules targeting cancer fusion genes, along with detection constructs.
5	PCT/US2019/054561	A system for detecting hemorrhagic fever viruses in a sample, utilizing CRISPR systems with effector proteins and guide RNAs (selected from specific sequences) to bind target molecules associated with these viruses, along with a masking construct, enabling differentiation between viruses with similar symptoms and between strains.
6	PCT/IN2020/050993	A cost-effective, easy-to-assemble kit for detecting target polynucleotides using a CRISPR effector system with Cas9 from *Francisella novicida* (FnCas9) and synthetic sgRNA, capable of distinguishing between pathogenic and non-pathogenic polynucleotides, identifying single mismatches without sequencing, and applicable for COVID-19 detection, providing a robust, rapid readout suitable for point-of-care use.
7	US15/952,132	A nucleic acid detection system featuring a CRISPR system with a Type VI Cas effector protein exhibiting collateral activity, guide RNAs designed to bind specific target sequences from a mosquito-borne parasite, and an RNA-based masking construct.
8	CN202410393528	A method for detecting the monkeypox virus by mixing the sample with a virus lysis reagent to create a lysed sample and then combining it with an RPA-CRISPR/Cas13a detection composition to form a detection system.
9	CN202010160702	A CRISPR-based nucleic acid detection kit for identifying the novel coronavirus (2019-nCoV), featuring a CRISPR-Cas13a system with separately packaged crRNA and LwCas13a proteins, a 20 U reporter RNA, an RT-RAA amplification primer with a T7 RNA polymerase recognition region, and lateral flow test paper, enabling highly sensitive and specific detection of the virus with a sensitivity of 10 copies per test.

10	CN201910488038	A PCR-CRISPR detection method for identifying HBV drug-resistant mutant genes by performing PCR amplification with specific primers, followed by detection using a system with crRNA, T7 RNA polymerase, Cas13a protein, and an RNase reporter molecule, targeting YIDD or YVDD mutations in the YMDD region of the HBV genome, and includes a kit with the designed crRNA for rapid, sensitive, and specific detection.
11	CN202410745149	A method, system, and kit for detecting Helicobacter pylori by using MIRA amplification and CRISPR-based technology, involving isothermal amplification of nucleic acids followed by detection with a V-type Cas protein, gRNA, and a single-stranded nucleic acid detector, offering flexible detection options such as color-based, fluorescence, or electrochemical signals, and suitable for diagnosing infections in animals.
12	PCT/CN2022/091332	A kit and method for detecting the N439K mutation of the novel coronavirus, involving RNA extraction, RT-RAA amplification, and CRISPR reactions with specific crRNAs and fluorescent probes, using fluorescence or color changes to determine the mutation's presence, and includes all necessary reagents and instructions for the process.
13	CN202211646024	A visual rapid detection kit and method for detecting Clonorchis sinensis using the RPA-CRISPR/Cas12a system, which includes RPA primers, Cas12a protein, crRNA, fluorescent and lateral flow reporter molecules, and a test strip, enabling rapid, on-site detection through fluorescence signals or color changes at 37°C-39°C without specialized equipment.
14	CN202310163354	A CRISPR-Cas12a-based system and kit for detecting Francisella tularensis, which includes Cas12a protein, crRNA targeting the pathogen's gene sequence, and a fluorescent or quenching probe, combined with RAA amplification primers for target sequence amplification and detection via fluorescence or lateral flow methods, suitable for diagnostic purposes and product development for detecting or diagnosing infections.
15	PCT/CN2015/087557	Using Protein C receptor (PROCR) for diagnosing and treating triple-negative breast cancer (TNBC) by detecting elevated PROCR levels, employing methods to reduce PROCR activity (such as antibodies, interfering RNA, or CRISPR/Cas9), and developing diagnostic kits and pharmaceutical compositions with PROCR inhibitors to suppress TNBC cell growth, metastasis, and EMT.

Table 3.2: Summary of inventions for the 15 shortlisted families.

3.4. CRISPR/Cas-based Diagnostics: Current Status

3.4.1 CRISPR/Cas-based Diagnostics Test for Different Types of Organism/Cells

- **CRISPR/Cas-based diagnostic test for Virus**

1. While early nucleic acid amplification and detection methods are sensitive and adaptable, the primary challenge in viral diagnosis is the extensive sample manipulation and significant financial investment in equipment required. CRISPR/Cas based detection offers a desirable alternative to these methods. CRISPR/Cas based detection methods have been successfully explored for: Human Papillomavirus (HPV)

2. ZIKA virus

3. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV2)

- **CRISPR/Cas-based diagnostic test for Bacteria**

Pathogenic bacteria can cause the spread and infection of diseases such as *Staphylococcal pneumonia,* tuberculosis, typhoid fever, syphilis, cholera, and foodborne illness [3.7].
Gootenberg *et al.* [Applicants: Massachusetts Institute of Technology, Broad Institute Inc, and Harvard University; US 10266887B2] utilized a CRISPR/Cas13a-based molecular detection platform to successfully identify *E. coli and P. aeruginosa*, both of which are Gram-negative, rod-shaped, and pathogenic bacteria that cause diseases in humans.

CRISPR/Cas-based diagnostic test for tumor

Cas proteins show great potential in DNA and RNA quantification, particularly for detecting multiple mutations simultaneously. Beyond genomic mutations, there is also significant interest in detecting tumor secretions and certain non-coding RNAs, which are closely linked to cancer progression and metastasis.

3.4.2. CRISPR/Cas-based Detection Technologies for Detecting Pathogens

The discovery of CRISPR-mediated adaptive immunity and various Cas proteins has revolutionized genome editing and advanced next-generation nucleic acid-based diagnostics for diseases and pathogens. Integrating lateral-flow chemistry into CRISPR diagnostics has created opportunities for developing rapid, reliable, specific, and affordable diagnostic kits. The CRISPR system has been utilized to develop molecular diagnostic kits for detecting Dengue virus (DENV), Human papillomavirus (HPV), and Zika virus (ZIKV) in human samples [3.8].

The table hereinbelow summarizes different nucleic acid detection methods based on CRISPR/Cas system:

Technology item	Effector proteins	Target molecule	Mode of amplification	Limit of detection	Pathogens	Detection technologies
DETECTR	Cas12a	DNA, RNA	RPA	amol/L	HPV16/18 SARS- CoV-2	Fluorescence signal
OR-DETECTR	Cas12a	RNA	RT-RPA	1-2.5 copies /ul	SARS-CO V-2 H1 N1	Fluorescence signal
HOLMES	Cas12a	DNA, RNA	PCR	amol/L	JEV	Fluorescence signal
HOLMES v2	Cas12b	DNA, RNA	LAMP	amol/L	JEV	Fluorescence signal
E-CRISPR	Cas12a	DNA	-	pmol/L	HPV1, B19	Electro-chemistry
CRISPR-ENHAN	LbCas12a	RNA	RT-LAMP	-	SARS-Co V-2, HIV, HCV	Lateral flow immunoassay
AIOD-CRISPR	LbCas12a	RNA	RPA	1-2.5 copies /ul	SARS-Co V-2	Fluorescence signal
SCAN	Cas12a	DNA, RNA	RT-PCR/ RT-RPA	13.5 copies /ul	HIV	Nanopore sensor
TB-QUICK	Cas12b	DNA	LAMP	1.3 copies /ul	*Mycobacterium tuberculosis*	Fluorescence signal
DETECTR-Cas14	Cas14a	DNA, RNA	RPA	amol/L	Viruses, Bacteria	Fluorescence signal

SHERLOCK	Cas13a	DNA, RNA	RPA	amol/L	Viruses, Bacteria	Fluorescence signal
HUDSON	Cas13a	RNA	RT-RPA	1 copies/ul	Zika virus, Dengue virus	Fluorescence signal
OR-SHERLOCK	Cas13a	RNA	RT-RPA	1-2 copies/ ul	SARS-Co V-2	Fluorescence signal
SHERLOCK v2	Cas12a	DNA, RNA	RPA	-	SARS-Co V-2	Lateral flow immunoassay

Table 3.3: Different types of CRISPR/Cas based detection systems.

Source: Li, X., Zhong, J., Li, H., Qiao, Y., Mao, X., Fan, H., ... & Li, J. (2023). Advances in the application of CRISPR/Cas technology in rapid detection of pathogen nucleic acid. Frontiers in Molecular Biosciences, 10, 1260883.

1. FnCas9 Editor Linked Uniform Detection Assay (FELUDA)

The Cas9 enzyme is widely used for genetic editing. It binds specifically to a DNA sequence complementary to the guide RNA and performs endonuclease activity at the target site. In the context of CRISPRDx, Azhar *et al.* [Applicant: CSIR, India; IN 201911049432; IN 202111029109] reported FELUDA technique. This assay uses FnCas9, a Cas9 ortholog from *Francisella novicida,* known for its high mismatch sensitivity. FnCas9 does not bind to sequences with mismatches [3.9]. RNA from viral samples is amplified using biotinylated primers through the Recombinase Polymerase Amplification (RPA) method. RPA offers the advantage of conducting the technique under isothermal conditions, which is crucial for point-of-care (POC) applications where sophisticated setups are not available [3.10].

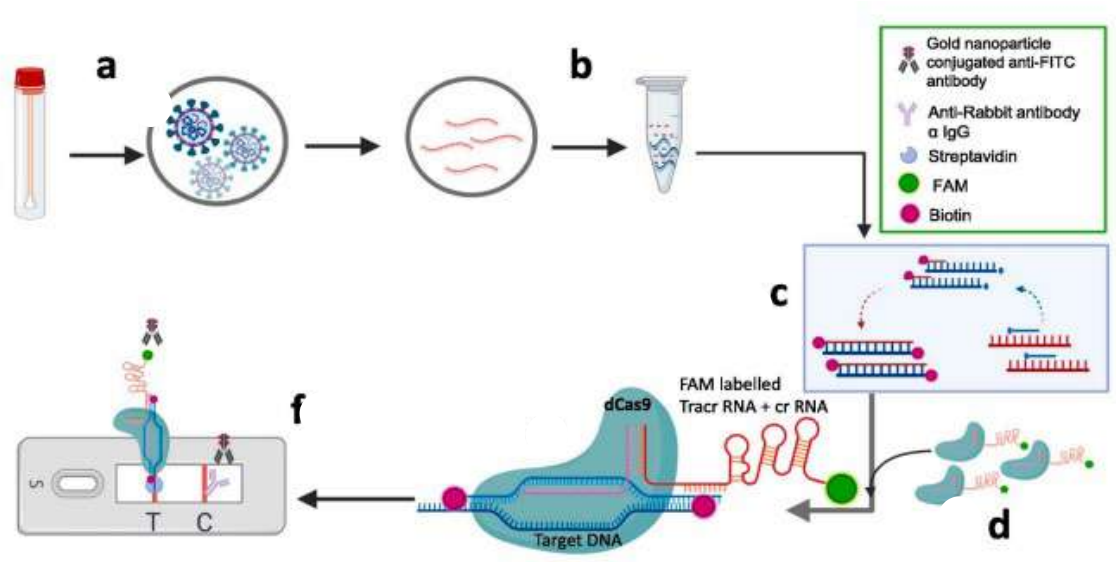


Figure 3.5: Schematic representation of FELUDA.

Source: Javalkote, V. S., Kancharla, N., Bhadra, B., Shukla, M., Soni, B., Sapre, A., ... & Dasgupta, S. (2022). CRISPR-based assays for rapid detection of SARS-CoV-2. Methods, 203, 594-603.

The CRISPR ribonucleoprotein (RNP) FnCas9 complex, labelled with FAM, and the biotin-labelled amplicons are used for detection with commercially available paper strips. The test line of the strip is coated with streptavidin. The biotin-labelled amplicons specific to SARS-CoV-2 bind at the test line along with the FAM-labelled RNP, which then binds to anti-FAM antibody-linked gold nanoparticles as they flow through the applied buffer. In the absence of biotin-labelled amplicons in the sample, the FAM-labelled RNP does not bind at the streptavidin test band line. The control line is coated with anti-rabbit antibody, where unbound gold nanoparticle-conjugated anti-FAM antibody binds and shows a color band [3.11].

2. Specific High-sensitivity Enzymatic Reporter un-Locking (SHERLOCK):

Sherlock Biosciences is a Biotechnology company based in Cambridge, Massachusetts, which is developing diagnostics tests using CRISPR/Cas13. Founded in 2019 by Feng Zhang, Jim Collins, Omar Abudayyeh, and Jonathan Gotenberg from the Broad Institute, the company has made significant strides in the field.

Gootenberg et al. [U.S. 10,266,886; U.S. 10,266,887] chose Cas13a over Cas9 due to its ability to bind and cleave RNA instead of DNA. This makes it suitable for direct viral RNA detection. Unlike Cas9, Cas13a remains enzymatically active and cleaves surrounding RNAs regardless of their sequence. The scientists developed a technology called SHERLOCK, which allows for rapid detection with high sensitivity and single-base specificity on a portable platform. SHERLOCK uses recombinase polymerase amplification (RPA), an isothermal nucleic acid amplification method that eliminates the need for a sophisticated PCR machine [3.12].

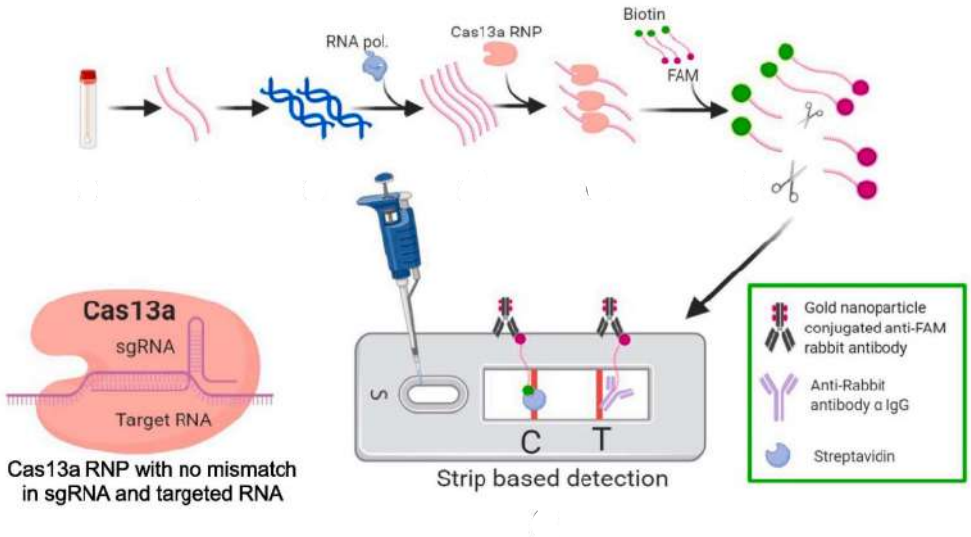


Figure 3.6: Schematic representation of SHERLOCK.

Source: Javalkote, V. S., Kancharla, N., Bhadra, B., Shukla, M., Soni, B., Sapre, A., ... & Dasgupta, S. (2022). CRISPR-based assays for rapid detection of SARS-CoV-2. Methods, 203, 594-603.

SHERLOCK is designed for direct RNA virus detection and can also be adapted for DNA detection by incorporating T7 RNA polymerase to convert DNA into RNA. The amplified RNA molecules are then targeted by Cas13a nuclease with a guide RNA specific to the sequence of interest. Upon binding to the complementary sequence, Cas13a becomes activated and exhibits nonspecific collateral cleavage activity. This activity generates a signal by cleaving a short nucleotide sequence linked to a fluorescent reporter and a quencher. Cas13a has demonstrated the ability to detect RNA at concentrations as low as 2% of total serum. It can differentiate between Zika virus (ZIKV) and dengue virus (DENV) at a minimum concentration of 2000 copies of viral genome per ml (3.2aM) [3.12].

In the latest version of SHERLOCK (SHERLOCKv2), Gootenberg *et al.* achieved four-channel multiplexing with a 3.5-fold increase in signal sensitivity, allowing detection of concentrations as low as 2 aM (2 x 10^{-8} M viral RNA).

Sherlock Biosciences holds a leading IP position in CRISPR-based diagnostics, primarily due to its exclusive US rights to a key patent covering the diagnostic use of the Cas12 enzyme. This patent, granted by the USPTO in February 2023, covers a method for detecting nucleic acids by leveraging the collateral cleavage activity of Cas12, an RNA-guided endonuclease well-suited for DNA and RNA detection rather than gene editing. **Sherlock acquired these exclusive US rights from Shanghai-based Tolo Biotech**, with whom it also holds co-exclusive rights to Cas12 and Cas13 CRISPR diagnostic methods in markets outside the US and Greater China. Additionally, Sherlock has secured further IPs related to Cas12 and Cas13 from the Broad Institute solidifying its dominant and comprehensive patent portfolio in the diagnostic CRISPR space [3.13].

Shanghai-based Tolo Biotech Co., Ltd. has been a prominent player in the biotechnology sector, particularly in the field of molecular diagnostics. Tolo Biotech has filed multiple patent applications related to diagnosis of various diseases. For example, patent publication number CN117887901A, which relates to a one-tube method for detecting foodborne hepatitis A virus combining with CRISPR/Cas12a and RT-LAMP by configuring an RT-LAMP reaction system in a tube, adding the sample, sealing the tube, and placing a CRISPR/Cas12a system with a ssDNA fluorescent probe on the cover, performing constant temperature amplification to obtain an amplified gene fragment, mixing the amplification product with the CRISPR/Cas12a system, and detecting fluorescence intensity, where an increase in fluorescence indicates the presence of hepatitis A virus and no change indicates its absence. Another patent publication number CN118813765A, which relates to a multiple nucleic acid detection method involves amplifying and detecting both the first and second nucleic acid targets in the same container, where the first target is detected using a CRISPR or Ago protein nucleic acid detection method, and the second target is detected using a probe method, with both amplification and detection of the targets preferably occurring simultaneously, using the same amplification method, and with the probe method being a molecular beacon or RNase HII probe, preferably modified or thio-modified.

In India, Tolo Biotech has filed one application, 202017002033 which is a National Phase application of the corresponding PCT Application PCT/CN2018/082769. The application relates to method for detecting a target nucleic acid molecule and a kit, the method for detecting a target nucleic acid molecule comprising: adding a guide RNA, Cas12a and a nucleic acid probe to a reaction system comprising a target nucleic acid molecule to be detected, and carrying out detection after reaction is complete.

3. DNA Endonuclease Targeted CRISPR Trans Reporter (DETECTR)

The Doudna group at the University of California, Berkeley, developed a CRISPR-based detection assay called DETECTR using a Cas12a. Chen et al. [U.S. 10,253,365; U.S. 10, 337, 051] discovered a remarkable phenomenon with Cas12a: when the target sequence complements at least 15 nucleotides of crRNA, Cas12a cleaves double-stranded DNA and activates collateral cleavage activity. This leads to the cleavage of single-stranded DNA without any specificity, a process known as indiscriminate single-stranded DNase activity [3.14].

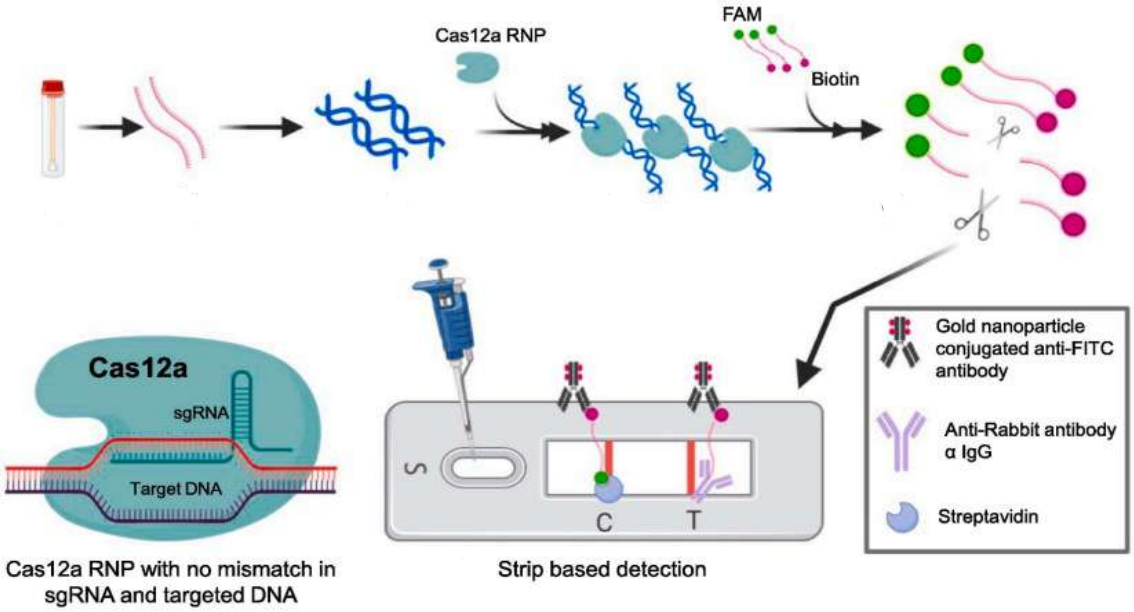


Figure 3.7: Schematic representation of DETECTR.

Source: Javalkote, V. S., Kancharla, N., Bhadra, B., Shukla, M., Soni, B., Sapre, A., ... & Dasgupta, S. (2022). CRISPR-based assays for rapid detection of SARS-CoV-2. Methods, 203, 594-603.

Mammoth Biosciences Inc. has adapted the DETECTR platform SARS-CoV-2 detection, providing results within 30 minutes using a lateral flow strip format.

Mammoth Biosciences has actively licensed and collaborated to commercialize its DETECTR CRISPR-based diagnostic platform. In 2019, Mammoth exclusively licensed two key patents from University of California covering CRISPR collateral cleavage diagnostic systems essential for DETECTR. These patents (U.S. Patent Nos. 10,253,365 and 10,337,051) cover Type V CRISPR-Cas effectors for detecting DNA and RNA targets, enabling Mammoth to develop DNA and RNA detection diagnostics using CRISPR technology [3.15].

Mammoth further expanded its IP portfolio by licensing new CRISPR enzymes such as Cas14 from UC Berkeley, enhancing its diagnostic capabilities beyond Cas12 and Cas13 enzymes initially used in DETECTR.

4. All-in-One Dual CRISPR/Cas12a (AIOD-CRISPR) assay

Ding *et al.* [Applicant: University of Connecticut; U.S. 20240279718A1] used two individual crRNAs to create a pair of Cas12a crRNAs that bind to two different sites on the target sequence near the RPA primer binding site. At 37 °C, RPA amplification begins, and strand displacement exposes the Cas12a-crRNA binding sites on the target sequences. When Cas12a-crRNA binds to the target site, it activates its endonuclease activity, cleaving the ssDNA-FQ reporter (single-stranded DNA sequences labelled with 6-carboxyfluorescein (FAM) and a quencher) to produce fluorescence. This process is continuously repeated on the amplified RPA products, amplifying the detection signal [3.16].

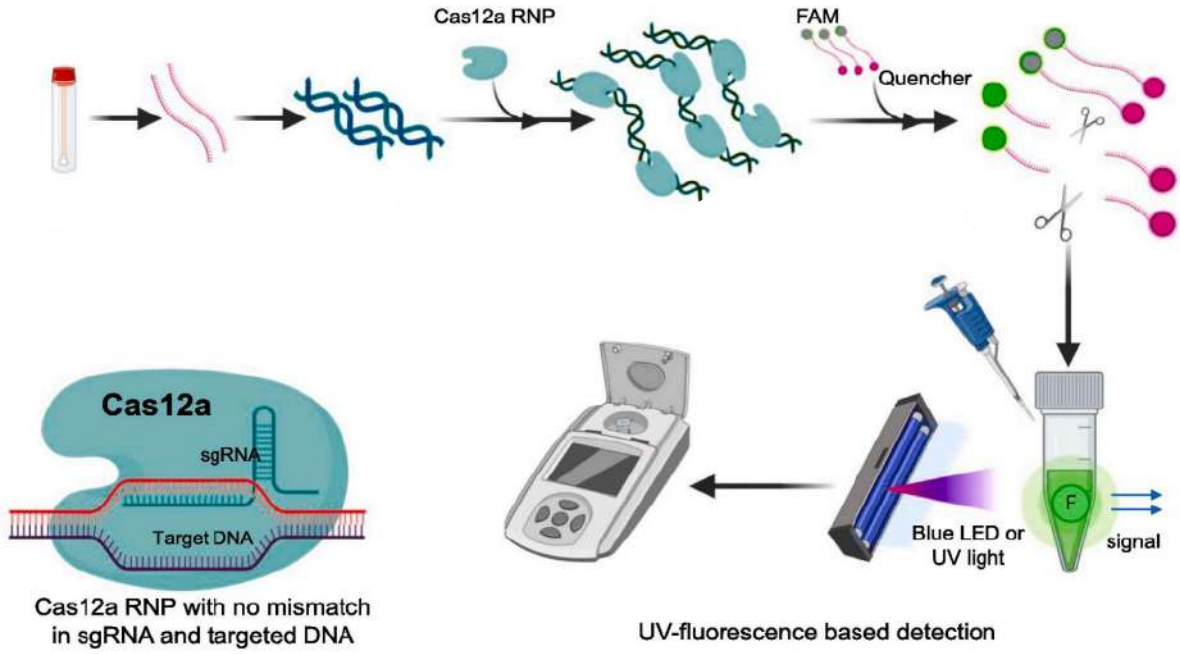


Figure 3.8: Schematic representation of AIOD-CRISPR assay.

Source: Javalkote, V. S., Kancharla, N., Bhadra, B., Shukla, M., Soni, B., Sapre, A., ... & Dasgupta, S. (2022). CRISPR-based assays for rapid detection of SARS-CoV-2. Methods, 203, 594-603.

5. Enhanced Analysis of Nucleic Acids with crRNA Extensions (CRISPR-ENHANCE)

In CRISPR-ENHANCE, the optimization of a CRISPR-based detection assay was achieved. Nguyen *et al.* [3.17] from University of Florida, United States, developed the CRISPR-ENHANCE technique using engineered crRNA + 3'DNA7. Observations indicated that the standard system efficiently detects RNA only when the target strand for crRNA is DNA rather than RNA in a heteroduplex. The engineered crRNA + 3'DNA7 demonstrated higher sensitivity than wild type crRNA for detecting SARS-CoV-2 within 30 minutes. By integrating a paper-based lateral flow assay with an FITC-ssDNA-Biotin reporter, detection time was reduced to 20 minutes, and SARS-CoV-2 cDNA could be detected at concentrations as low as 1nM without any target amplification [3.17].

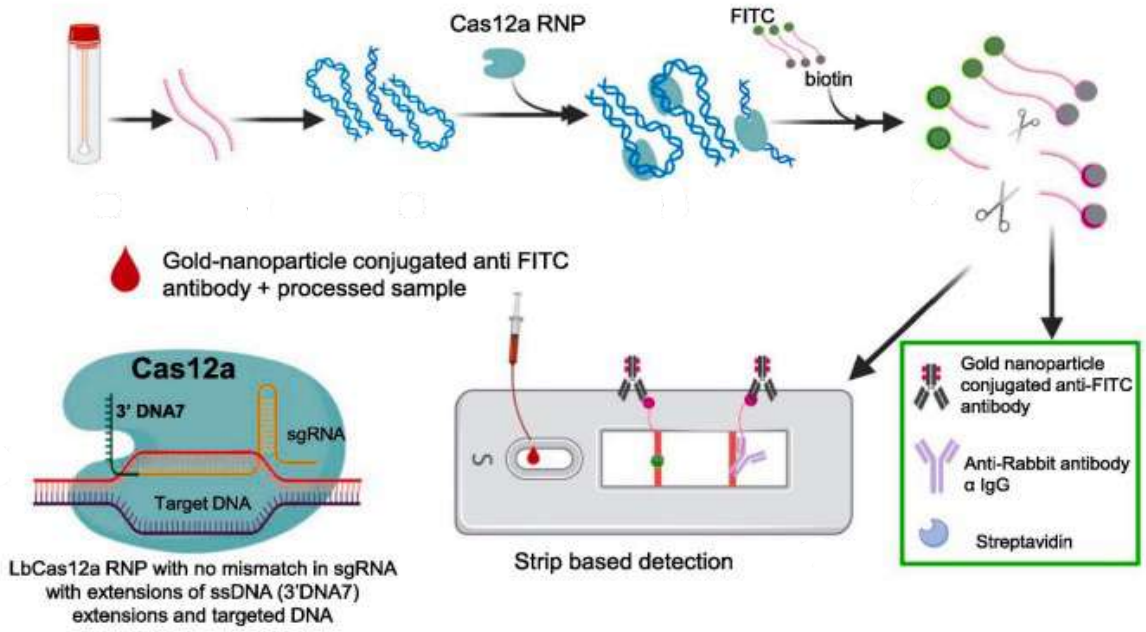


Figure 3.9: Schematic representation of CRISPR-ENHANCE.

Source: Javalkote, V. S., Kancharla, N., Bhadra, B., Shukla, M., Soni, B., Sapre, A., ... & Dasgupta, S. (2022). CRISPR-based assays for rapid detection of SARS-CoV-2. Methods, 203, 594-603.

When RT-LAMP was used, the detection limit was further reduced to 3-300 copies of RNA for both wild-type crRNA and engineered crRNA +3'DNA7. However, the wild type crRNA produced a darker control band compared to the engineered crRNA + 3'DNA7 on the paper strip. Comparing band intensities, CRISPR-ENHANCE technique showed an average 23-fold intensity ratio of the positive band to the control band, whereas the wild type crRNA had an average 7-fold ratio at SARS-CoV-2 RNA concentration between 1nM and 1pM [3.17].

3.5. Key Findings

- Results of the first screening (557 identified patent families) were analyzed, and it was observed that China had maximum number of patent applications filed; 445 patent applications out of 557, which accounts to 80% of the total filings, followed by the US (17%), South Korea (9%), and EPO (9%).

- Out of the 557 patent families, a total of 117 patent applications were filed through the Patent Cooperation Treaty (PCT) route.

- Out of 557 patent families, China had maximum number of priority filings (75%), followed by US (13%), and South Korea (7%). India, on the other hand, does not have any priority filings for the patent applications related to CRISPR/Cas-based diagnostics of infectious disease and pathogens in humans.

- The results were also examined to identify the top applicants in patent filings. The Broad Institute (US) leads the CRISPR landscape for CRISPR/Cas-based diagnostics targeting infectious diseases in humans, with a total of 22 patent families. Following them is the INST Military Medicine Academy Military Sciences (CN) with 15 patent families, and both Shandong Shunfeng Biotechnology Co Ltd (CN) and Hainan University (CN) hold 10 patent families each.

- Several top applicants had filed patent applications in collaboration with other entities indicating the collaborative nature of research related to CRISPR/Cas-based diagnostics of infectious disease and pathogens in humans.

- With respect to the different types of Cas enzymes used in these patent families, most patents were filed using Cas12 and its variants, accounting for 270 patent applications. This is followed by Cas13 and its variants, accounting for 96 applications and Cas9 and its variants, accounting for 79 applications. The total number of 84 patent families have disclosed that their inventions can be performed using more than one type of Cas enzyme.

- While China led in the number of priority filings related to CRISPR/Cas and its applications in diagnostics, the most significant patent applications were submitted by organizations in the US. Notably, the Broad Institute filed the patent for "SHERLOCK", and the University of California filed the "DETECTR" patent. These applications have played a crucial role in advancing the development of CRISPR-based diagnostic technologies. Also, Council of Scientific & Research Institute-IGIB, India, have filed a patent application [IN 201911049432] reporting FELUDA technique, which uses FnCas9, a Cas9 ortholog.

- Sherlock Biosciences acquired exclusive US rights from Shanghai-based Tolo Biotech for patent covering the diagnostic use of the Cas12 enzyme, with co-exclusive rights to Cas12 and Cas13 CRISPR diagnostic methods outside the US and Greater China, while Mammoth Biosciences licensed key patents from the University of California in 2019 to commercialize its DETECTR CRISPR-based diagnostic platform.

- Multiple CRISPR/Cas-based diagnostic patent applications have been filed targeting a wide range of diseases, including Human Papilloma Virus, COVID-19, Zika Virus, Cancer etc.

4 CRISPR/Cas & Vaccines: COWPOX to CRISPR-Based Vaccines

INDEX- CHAPTER 4

4.1. Background

4.1.1. Introduction to Vaccines

The first recorded scientific application of vaccination dates back to Edward Jenner's smallpox experiment (1796) where material from cowpox lesions successfully immunized a young boy against smallpox [4.1], laying foundation for modern immunization, and leading **Dr. Jenner** to coin the term "Vaccine," derived from the Latin word Vacca (cow). This breakthrough laid the foundation for modern immunization practices [4.2]. What followed next was Louis Pasteur's groundbreaking attenuation techniques in the late 19th century for preparing live attenuated vaccines 4.3 and development of recombinant vaccines in the 20th century [4.6, 4.7, 4.8].

From Jenner to Jennifer: The Vaccine Journey

Year	Vaccine	Illustration
1796	Smallpox vaccine: Edward Jenner inoculates with cowpox [4.2]	
1881	Anthrax Vaccine: Pasteur's attenuated bacterial vaccine [4.3, 4.5]	

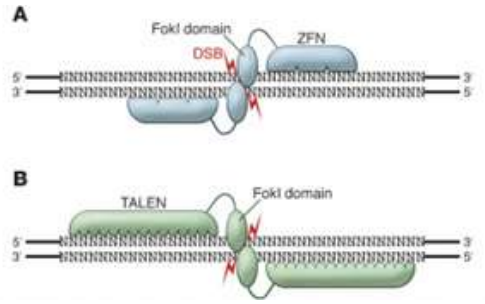
1885	Rabies Vaccine: Pasteur's viral vaccine innovation [4.4]	
1955	Polio vaccine: Salk and Sabin develop inactivated and live forms [4.9, 4.10]	
1986	First Recombinant Hepatitis B: First approved recombinant vaccine using yeast expressions [4.6]	
1990s	Reverse genetics & ZFNs/TALENs: reverse genetics in influenza vaccine[4.11]; ZFNs [4.12]/TALENs [4.13] for targeted gene edits	
2012	CRISPR Cas-9: The rise of efficient, programmable gene editing. [4.14]	

Table 4.1. Illustrating timelines for different innovations related to vaccine

4.2. Search Results

Note: This section is a patent overview in CRISPR technology with a specific focus on Vaccines.

METHODOLOGY

- A total of 819 Patent families were obtained as a result of the patent searches. The identified 819 families covered the use of CRISPR-based gene technology in a wide range of applications in biomedicine including therapy, vaccine preparation, diagnosis, bio models, drug screening, etc. Hence, a further analysis was carried out to categorize the search results into 5 main categories: *Therapy, Vaccines, Diagnosis, New Cas enzymes and Miscellaneous applications.* The analysis for identifying inventions directed to *"use of CRISPR-based gene technology in vaccines"* was divided into three screening processes:

    - **First Screening:** The 819 patent families were obtained at the end of patent search for CRISPR related technology in the field of "vaccine". These 819 Applications were screened, and 147 Patent Applications were shortlisted that related to use/potential use of CRISPR/Cas technology in "vaccine" for both humans and animals.

    - **Second Screening:** Out of the 147 families, 81 patent applications were categorized according to the following taxonomy:

        - Types of vaccines
        - Diseases targeted
        - Types of CRISPR/Cas proteins or enzymes utilized.

    - **Third Screening:** Further classification was carried out to focus on well-recognized groups working on CRISPR-Based vaccine technology such as Dana-Farber Cancer Institute, Catholic University of America and others. These applications were analyzed on the basis of:

        - Types of vaccines,
        - Modes of delivery,
        - Target diseases,
        - Vaccine composition, and
        - Application of CRISPR/Cas technology (direct or indirect).

    Building on this structured approach, a focused and in-depth analysis was performed on 12 selected patent families from the 81 patent applications as shortlisted from the second screening, providing a clearer understanding of key innovations and strategies shaping the domain of vaccine development using CRISPR/Cas technology.

4.2.1. World Map for Patent Filings

An analysis of the 147 shortlisted patent families related to CRISPR-based vaccines provides valuable insights into global innovation trends. The screening included patent applications related to delivery systems (e.g., genetically modified viral vectors), methods of vaccine production and compositions that could potentially be used as vaccines.

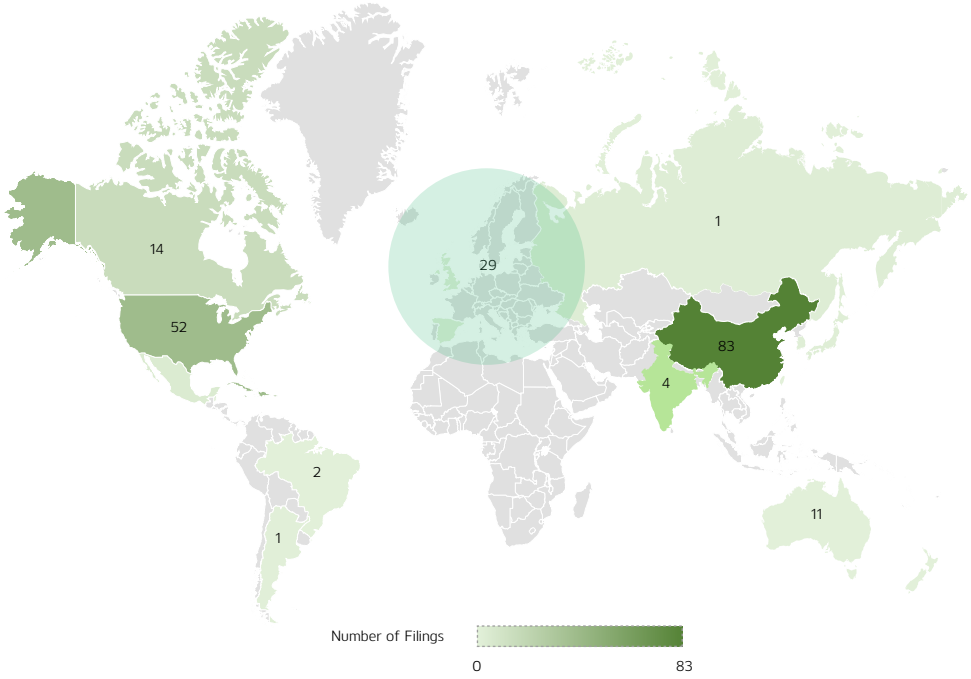


Figure 4.1. World map for patent applications related to CRISPR/Cas-based vaccines

The world map (Figure 4.1) provides a visual distribution of patent applications related to CRISPR-based vaccine innovations, filed across major jurisdictions. This map illustrates the global distribution of applications based on CRISPR-enabled vaccine technologies.

As depicted in **Figure 4.1**, the geographical distribution reveals China's leading position with 83 patent applications (30%), emphasizing its dominance both as a key market and a prolific source of innovation in Vaccine development using CRISPR/Cas. The US follows with 53 applications (19%), solidifying its role as a major player in CRISPR-related vaccine technology. Europe ranks third with 29 applications (10%), reflecting steady activity in this domain. Other notable contributors include Canada and Japan with 14 applications each, South Korea with 9 applications, and Australia with 11 applications. Brazil and Spain contribute with approximately 2 applications each. Notably, India's comparatively low filing activity —with only 4 applications may be due to the stringent non-patentability criteria under Section 3 of the Indian Patents Act. In terms of priority filing, the US leads significantly (61%), followed by China (19%) and United Kingdom (7%).

4.2.2. Top Applicants

The CRISPR/Cas based vaccine patent landscape shows distinct patterns in both the types of vaccines being developed and the diseases they are designed to target. As illustrated in Figure IV.2A, majority of applicants have focused the subject matter of their applications on live attenuated vaccines (50%), followed by cell-based vaccines (16%) the second most common category, and a smaller share on genetically modified live virus vaccines and bacteriophage vaccines.

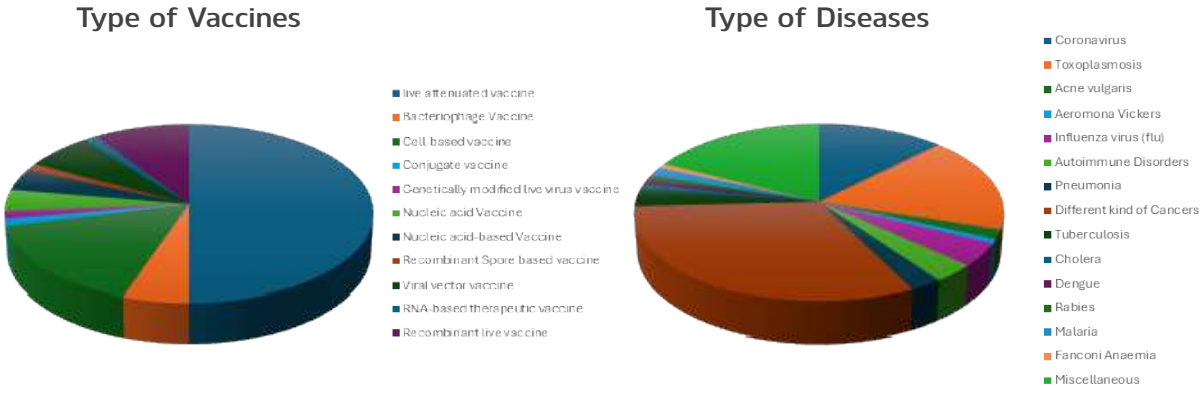


Figure 4.2A **Figure 4.2B**

Figures 4.2A. and 4.2B. illustrate distribution of CRISPR Based Vaccine and targeted diseases.

Corresponding, Figure 4.2B provides a diagrammatic overview of the most frequently targeted diseases in patent filings shortlisted during the first screening.

A total of 38 patents were filed in the area of vaccines related to cancer. These include patent applications by Dana-Farber Cancer Institute (US2022265798A1), applications by Cancervax Inc. (US2024424094A1), applications by Beijing Weiyuan Likang Biotechnology Co Ltd. (CN117965634B).

It is worth noting that **Toxoplasmosis** has been the subject of maximum patents filings that were filed based on CRISPR/Cas-based vaccines. Toxoplasmosis is an infection caused by the parasite *Toxoplasma gondii.* It is mainly transmitted through contact with cat feces or by consuming undercooked, contaminated meat. Toxoplasmosis is emerging as a global health concern, affecting 30-50% of the world's population. Clinically, the parasite's lifelong presence in the tissues of most infected individuals is typically asymptomatic[2.47].

Applicants such as Anhui Medical University (CN114569711B), South China Agricultural University (CN118546788B), and Lanzhou Veterinary Research Institute (CN115992163A) were identified as major players who have filed applications related to Toxoplasmosis. Further, these applications are mostly directed to live attenuated vaccine employing different strains of Toxoplasmosis.

- Patent family CN114569711B uses ME49Δcdpk3 strain wherein CDPK3 in ME49 strain has been knocked out by CRISPR/Cas9-mediated genome editing technology. It was found that immunization with ME49Δcdpk3 elicited a strong immune response.

- Patent family CN118546788B uses *toxoplasma gondii* succinate dehydrogenase α subunit gene-deficient vaccine strain. In this, the Toxoplasma succinate dehydrogenase α subunit TgSDHα gene has been knocked out through CRISRP/Cas9 technology.

- Patent family CN115992163A uses Toxoplasma attenuated strain with TgPP6C gene deletion by knocking out the TgPP6C gene. It was found that *in vitro* proliferation rate and pathogenicity of the deleted attenuated *Toxoplasma gondii* strain were significantly reduced, and the infected mice showed no disease and no death, which was relatively safe for the host.

Trailing Toxoplasmosis are applications relating to Coronavirus. Since the pandemic of the Coronavirus, researchers across the world have been actively working in the development of vaccines for COVID-19. CRISPR-based vaccine development offers a novel approach by utilizing gene-editing techniques to create precise and adaptable vaccine candidates[4,28]. A list of applicants in this area includes Catholic University of America (US2023355738A1), Spanish National Research Council (US2023203536A1), and Weng Binghuan (CN112646823A and CN112646823A).

- Patent family US2023355738A1 relates to a bacteriophage display vaccine against SARS-CoV-2. The vaccine comprising a bacteriophage, a spike protein decorated on the surface of capsid protein of bacteriophage and nucleoprotein is hard-wired by human engineering and packed inner capsid protein of the bacteriophage.

- Patent family US2023203536A1 relates to an RNA replicon derived from a coronavirus has been modified using CRISPR/Cas9. This RNA replicon is self-replicating but propagating defective RNA in nature and thus used in a vaccine composition for coronaviruses, preferably for MERS-CoV.

- Patent family CN112646823A relates to gene-edited new crown vaccine vector that knocks out the new crown virus susceptibility gene hACE2 and transfects the immortalization gene hTERT and/or SV40LT. This gene-edited new crown vaccine vector has the function of natural stem cell therapy and unlimited passage function and deletion characteristics of the new coronavirus infection receptor hACE2.

Other critical diseases that have drawn considerable attention are:

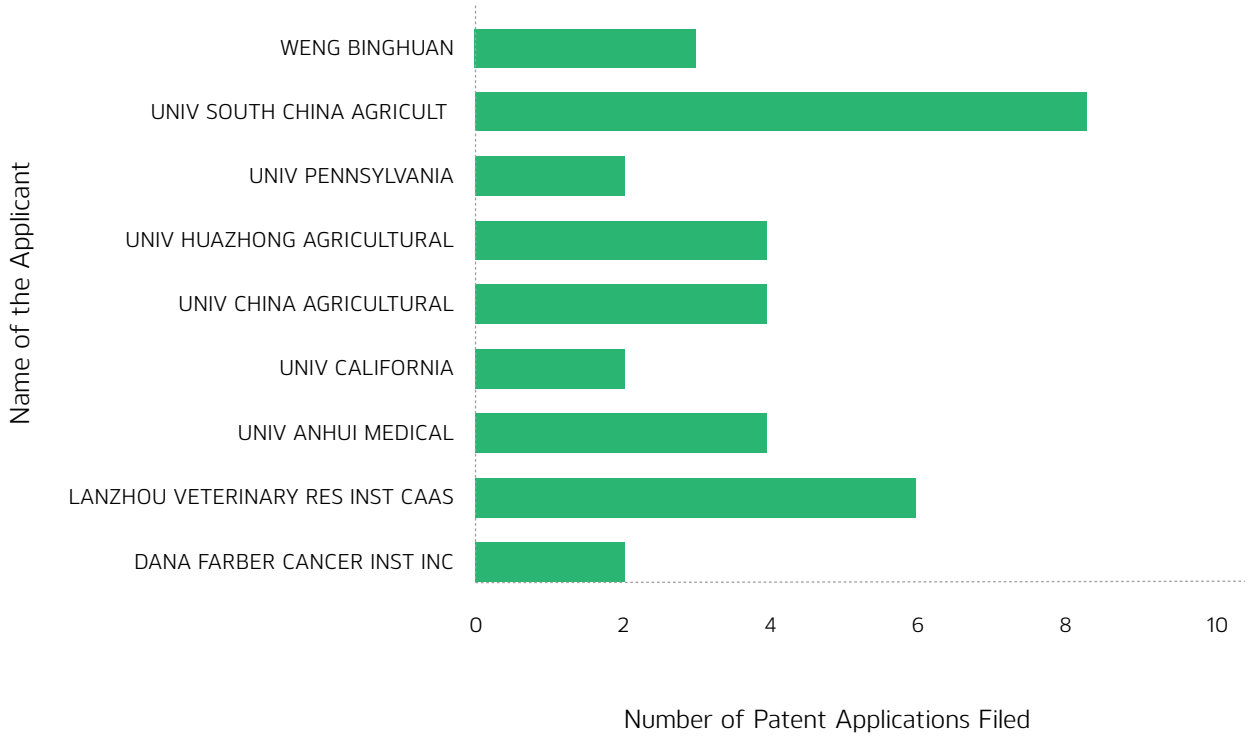
- Influenza: Huazhong Agricultural University (CN114836391A) and Japan's Science and Technology Agency lead (JP6730188B2) with maximum number of filings in the field of CRISPR/Cas based vaccines.

- Acne vulgaris: In the fight against Acne vulgaris, Eligo Bioscience's (US11820989B2) stands out.

- Autoimmune disorders: These include a list of multiple disorders. Applicants like Garvan Institute of Medical Research (US2022056536A1) and CureVac SE (US2024229075A1) have filed applications relating to development of vaccines for autoimmune disorders.

These diverse patent filings underscore the global drive to combat some of the most challenging diseases through cutting-edge CRISPR/Cas advancements.

Out of 147 patent families screened, several leading universities, research institutions, and independent inventors have emerged as key players in CRISPR/Cas-based vaccine development.



Figures 4.3. Top Applicants for patent Applications related to CRISPR/Cas in Vaccines.

As illustrated in Figure 4.3, notably South China Agricultural University leads with respect to the filing of patent applications ((for e.g., CN118308431A and CN118146955A), primarily targeting toxoplasmosis. Similarly, Lanzhou Veterinary Research Institute has also filed eight patents (for e.g., CN115992163A and CN117187070A) focused on toxoplasmosis vaccines. Most of these applications were filed between the period of 2023 and 2024.

Other Chinese institutions, such as Huazhong Agricultural University, have directed their patent applications (for e.g., CN104894075A, CN114836391A and CN114958783A) towards development of vaccines for pseudorabies, influenza, and herpesvirus using CRISPR/Cas technology.

Beyond academic institutions, independent inventors are also actively contributing to this field. For example, Weng Binghuan has filed two patent families (CN112646823A and CN112626624A), signaling the growing accessibility of CRISPR platforms beyond traditional academic and industrial centers. While China dominates the CRISPR/Cas based patent landscape in vaccine development, US-based institutions also feature prominently. For example, the University of California (US2024248080A1, US10876125B2) and Dana-Farber Cancer Institute (US2020268864A1) are developing CRISPR-enabled vaccines targeting human diseases such as cancer. Their filings reflect a broader biomedical focus aligned with therapeutic vaccine development.

Inventors from University of Pennsylvania are developing LNP delivery systems incorporating CRISPR/Cas9 as a therapeutic agent, for instance PCT/US2022/077346. This highlights a novel integration of gene editing with RNA-based delivery systems, positioning CRISPR at the forefront of next-generation vaccine therapy.

4.3. Detailed Analysis of Selected Applications

Detailed analysis was carried out for 12 Patent families selected from the results of the third screening process. Out of 81 Patent families, specific applications were shortlisted in order to study the recent advances in use of CRISPR-based vaccines. The landscape analysis has been performed for the following families using databases like Espacenet, Patentscope, Innography, and Google Patents. In addition, non-patent literature has been referred to for providing a better understanding of technology.

Family Number	Application/ Publication Number	Applicant	Corresponding Jurisdictions
1	PCT/EP2020/065625	INTERVET INTERNATIONAL B.V. (NL)	US17615961, EP20729786
2	PCT/EP2018/066668	TRANSGENE [FR]	US18299858, EP18731146
3	US16596829	RESEARCH FOUNDATION OF THE STATE UNIVERSITY OF NEW YORK (US)	None
4	PCT/KR2017/014027	THE CATHOLIC UNIVERSITY OF KOREA INDUSTRY-ACADEMIC COOPERATION FOUNDATION [KR]	US16465933
5	PCT/JP2015/076681	JAPAN SCIENCE AND TECHNOLOGY AGENCY [JP]	US15511988, US16453464, EP 20167102.1, EP15844041.2
6	PCT/US2019/019311	CELULARITY, INC. [US]	US20210095258A1
7	PCT/EP2022/061863	CUREVAC SE [DE]	US18558531, EP22719599.7

8	PCT/CN2022/095396	SHANGHAI PULMONARY HOSPITAL [CN]	EP22928058A
9	CN202210298777A	ANHUI MEDICAL UNIVERSITY	None
10	PCT/US2020/041886	DANA-FARBER CANCER INSTITUTE, INC. [US]	EP3999112A4, US17626263
11	PCT/IB2023/054229	THE CATHOLIC UNIVERSITY OF AMERICA [US]	EP23795745A, US18138183, IN202427081629
12	PCT/US2022/077346	THE TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA [US]	EP22877609A

Table 4.2: List of 12 Patent shortlisted families for detailed analysis

A brief summary of the inventions covered by the identified families –below–

Family Number	Application/Publication Number	The Invention Relates to
1	PCT/EP2020/065625	MDBK cells with IRF3 and/or IRF7 genes knocked out via CRISPR-Cas9, blocking type I interferon activation. The antiviral response is suppressed, enhancing viral yield in culture. These modified cells produce vaccines targeting Bovine Respiratory Syncytial Virus (BRSV) for improved vaccine development and efficacy.
2	PCT/EP2018/066668	A personalized cancer vaccine uses recombinant vaccinia virus engineered with neopeptides. CRISPR-Cas9 precisely removes a fluorescent reporter gene from the viral genome, allowing insertion of nucleic acids encoding tumor-specific neopeptides for targeted immune activation.
3	US16596829	A cancer vaccine comprising senescent or mitosis-arrested cell which expresses a target antigen. These senescent or mitosis-arrested cells are genetically edited with the help of CRISPR-CAS9 system to suppress the presentation of at least one immunosuppressive (PD-L1) or checkpoint molecule (CD47).

4	PCT/KR2017/014027	Vaccine using artificial antigen-presenting cells derived from HLA-deficient 293T cells. Using multiplex CRISPR-Cas9, exons 2 and 3 of HLA class I genes are deleted, preventing immune mismatches. These cells are sensitized with tumor, pathogens, or auto-antigens to prevent tumors, infections, or autoimmune diseases.
5	PCT/JP2015/076681	A method for producing influenza virus in genetically modified host cells to enhance virus production, primarily for vaccine development. This approach involves suppressing the expression of specific genes in the host cell's chromosome, such as those that inhibit influenza virus proliferation, using the CRISPR-Cas9 system. Propagated viruses are used for vaccine manufacturing.
6	PCT/US2019/019311	Induced pluripotent stem (iPS) cell, or population of iPS cells in which the expression level of one or more factors selected from the Group I-such as an Oct family member, a Sox family member, a Klf family member, a Myc family member, Nanog, Lin28, or their combinations are increased using the gene editing technologies such as CRISPR, C2c2-RNA/CRISPR. The resulting iPS cells are utilized for the production of vaccine that eliciting robust immune-response against cancer-associated antigens.
7	PCT/EP2022/061863	A nucleic acid sequence that includes at least one coding region encoding a therapeutic peptide or protein, along with at least one miRNA binding site sequence located upstream (in the 5' direction) of the coding region, wherein CRISPR-associated endonucleases can be used as therapeutic agent. This nucleic acid sequence is designed to be administered as part of a vaccine.
8	PCT/CN2022/095396	A recombinant Bacillus Calmette-Guérin (BCG) strain, specifically engineered for improved immune protection against tuberculosis (TB). The invention involves knocking out the BCG-1820 gene in the wild-type BCG strain using CRISPR/Cas9 technology.
9	CN202210298777A	A live attenuated ME49Δcdpk3 vaccine for *toxoplasmosis*, developed using the modified ME49Δcdpk3 strain of *Toxoplasma gondii.* ME49Δcdpk3 strain is modified using CRISPR/Cas9-mediated gene editing technology

10	PCT/US2020/041886	A cancer vaccine using cancer cells deficient in PTEN and p53, with activated TGFβ-Smad/p63 signaling via modulation of biomarkers. Activation involves increasing or decreasing specific biomarkers (Tables 1 and 2), with reductions achieved using CRISPR gRNA, RNAi, small molecules, antibodies, or other inhibitory agents.
11	PCT/IB2023/054229	A bacteriophage-based vaccine utilizes a genetically engineered phage. It aims to stimulate a robust immune response against SARS-CoV-2, leveraging cutting-edge CRISPR technology for precise modifications.
12	PCT/US2022/077346	A lipid nanoparticle (LNPs) capable of suppressing unwanted innate immune responses while efficiently delivering mRNA cargo with low toxicity to immune cells, enabling in vivo therapeutic applications. The LNPs include ionizable lipids, cholesterol and/or substitutes, PEG lipids, nucleic acid, and a therapeutic agent comprising CRISPR components such as gRNA and Cas protein. The invention provides a method for gene editing of immune cells by administering LNPs formulated for targeted T cell delivery with nucleoside-modified RNA molecules.

Table 4.3: Summary of inventions for the 12 shortlisted families

4.4. Technology Overview

4.4.1. Bridging Technologies: Reverse Genetics, ZFNs and TALENs

Before CRISPR's emergence, the scientific community explored other precision tools to tackle the limitations of traditional vaccine platforms:

- Reverse Genetics: Enabled controlled attenuation by introducing deliberate genetic changes into viral genomes. This method became critical in developing influenza vaccines, where specific genes were engineered to reduce virulence while preserving immunogenicity[4.11].

- ZFNs[4.12] and TALENs[4.13]: These programmable nucleases offered targeted genome editing capabilities, allowing researchers to knock out or modify virulence genes in microbial pathogens. However, both systems suffered from complex design processes, lower modularity and higher costs.[4.15]

All these platforms pushed the boundaries of vaccine design, they still lacked the efficiency, precision, and scalability demanded by modern vaccinology.

4.4.2. The CRISPR/CAS Breakthrough in Vaccine

The discovery of CRISPR and its associated Cas proteins, originally characterized as a bacterial defense mechanism, has revolutionized molecular biology and is now making significant contribution to vaccine research and manufacturing[4.17, 4.18]. With the introduction of CRISPR/Cas9 (2012) (US10266850B2)[4.14] and related systems (e.g. Cas12a[4.21], Cas13[4.22]), enabled precise genomic modification via Non-Homologous End Joining (NHEJ)[4.16] and Homology-Directed Repair (HDR) pathways[4.16], vaccine development underwent a paradigm shift, CRISPR offered:

- Highly precise genome editing [4.19].
- Multiplexed targeting capabilities (simultaneously editing multiple genes) [4.19].
- Cost-effect, rapid turnaround compared to ZFNs/TALENs[4.19].
- Greater product stability and reduced risk of adverse immunological reactions[4.19].

4.4.3. Application of CRISPR/CAS System in Development of Vaccine

Vaccine types can be categorized as: **Whole-organism Vaccines** [4.20], which include Inactivated (Killed) Vaccines, Live-attenuated Vaccines, and Chimeric Vaccines. **Subunit Vaccines** [4.23 4.24], which include Conjugated Vaccines, Toxoid Vaccines, Recombinant Protein Vaccines, and Nanoparticle Vaccines. **Nucleic Acid Vaccines** [4.25], which include DNA Plasmid Vaccines, mRNA Vaccines, and Recombinant Vector Vaccines. An illustrative figure for each kind of vaccine is provided below (Figure 4.4).

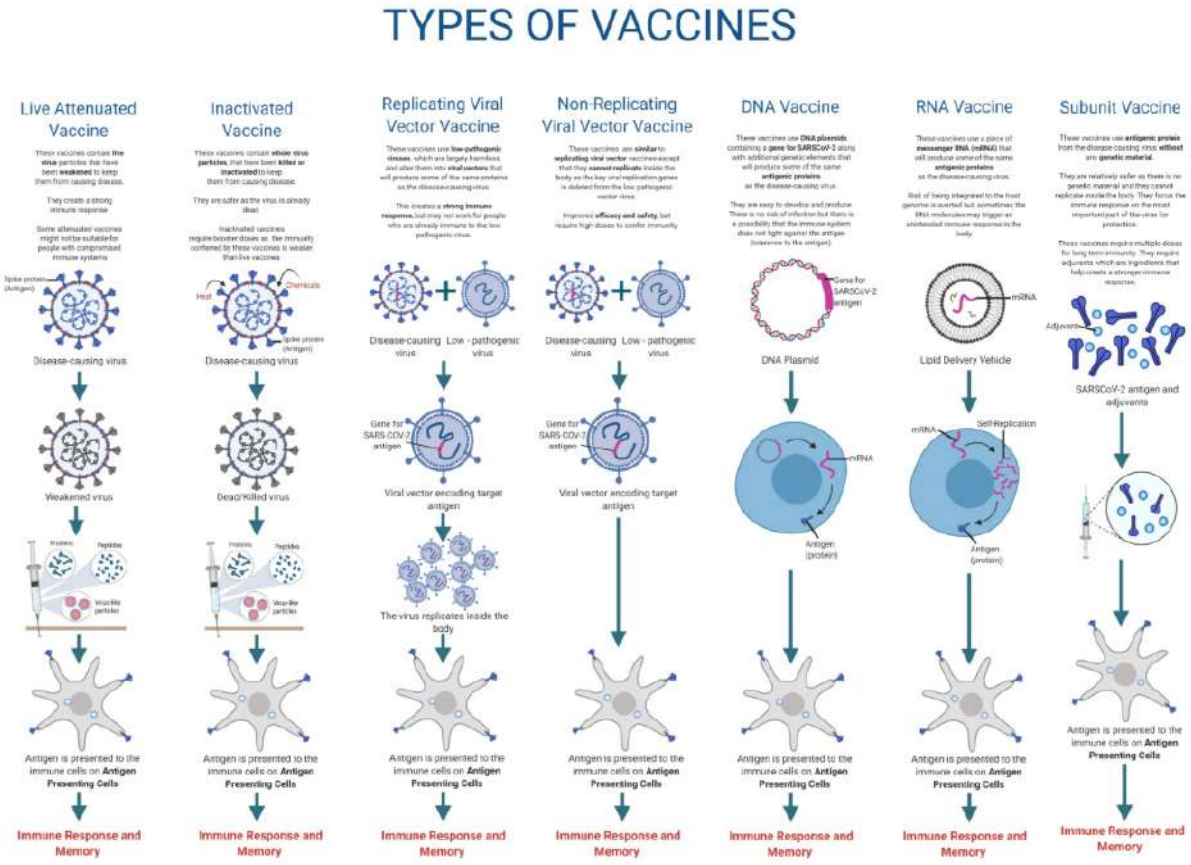


Figure 4.4: Illustrating different kinds of vaccines.

Source: https://sites.bu.edu/covid-corps/projects/science-communication/types-of-vaccines-infographics/

Based on these types of vaccines, CRISPR/Cas was utilized to:

- **Modify Viral Genomes:** CRISPR can edit the genomes of viruses to create attenuated strains that are safe for use in vaccines while still capable of inducing immunity [4.16].

- **Engineer Viral Vectors:** Viral vectors, which serve as delivery vehicles for vaccine antigens, can be optimized using CRISPR to enhance their safety and efficacy. For instance, Herpesvirus of turkey

(HVT) is commonly used as a live vaccine vector against Marek's disease and other avian viral diseases due to its high growth capacity in cell lines, although traditional HVT-based vaccines have shown limitations, such as low immunogenic responses. Recent advancements using CRISPR/Cas9 have significantly improved HVT vector vaccines [4.16].

- **Develop Novel Vaccine Platforms:** CRISPR facilitates the creation of new vaccine platforms, such as DNA or mRNA vaccines, by enabling the precise insertion of immunogenic sequences into delivery systems[4.16].

- **Modify Host Cells or Cell Lines:** CRISPR can modify host cells that are typically used for producing vectors [4.26].

Despite the remarkable advances in CRISPR, several limitations and concerns still exist, which need to be addressed and solved for development of optimized CRISPR/Cas systems. Efficient and targeted delivery of CRISPR components remains a major limitation, alongside concerns over off-target effects [4.26] that may compromise genome integrity. **The immunogenicity of Cas protein**[4.26], due to their bacterial origin poses potential safety risks, **while regulatory uncertainties and limited long-term clinical data**[4.26] further constrain clinical translation. Additionally, scaling up manufacturing for widespread use remain technically demanding. These hurdles must be addressed to fully harness CRISPR's potential in developing vaccines for human health.

4.5. Key Findings

- Results of the first screening (147 identified patent families) were analyzed, and it was observed that China has maximum number of patent Applications filed, followed by US with 53 applications (19%), Europe (10%) and Canada and Japan (5%).

- It was observed that out of 147 identified patent families, USA had maximum number of priority filings (61%), followed by China (19%) and United Kingdom (7%)

- Thus, it could be derived that, the US and China dominate the field of CRISPR-based vaccine development, underscoring their strategic investments in biotechnology and robust research ecosystems.

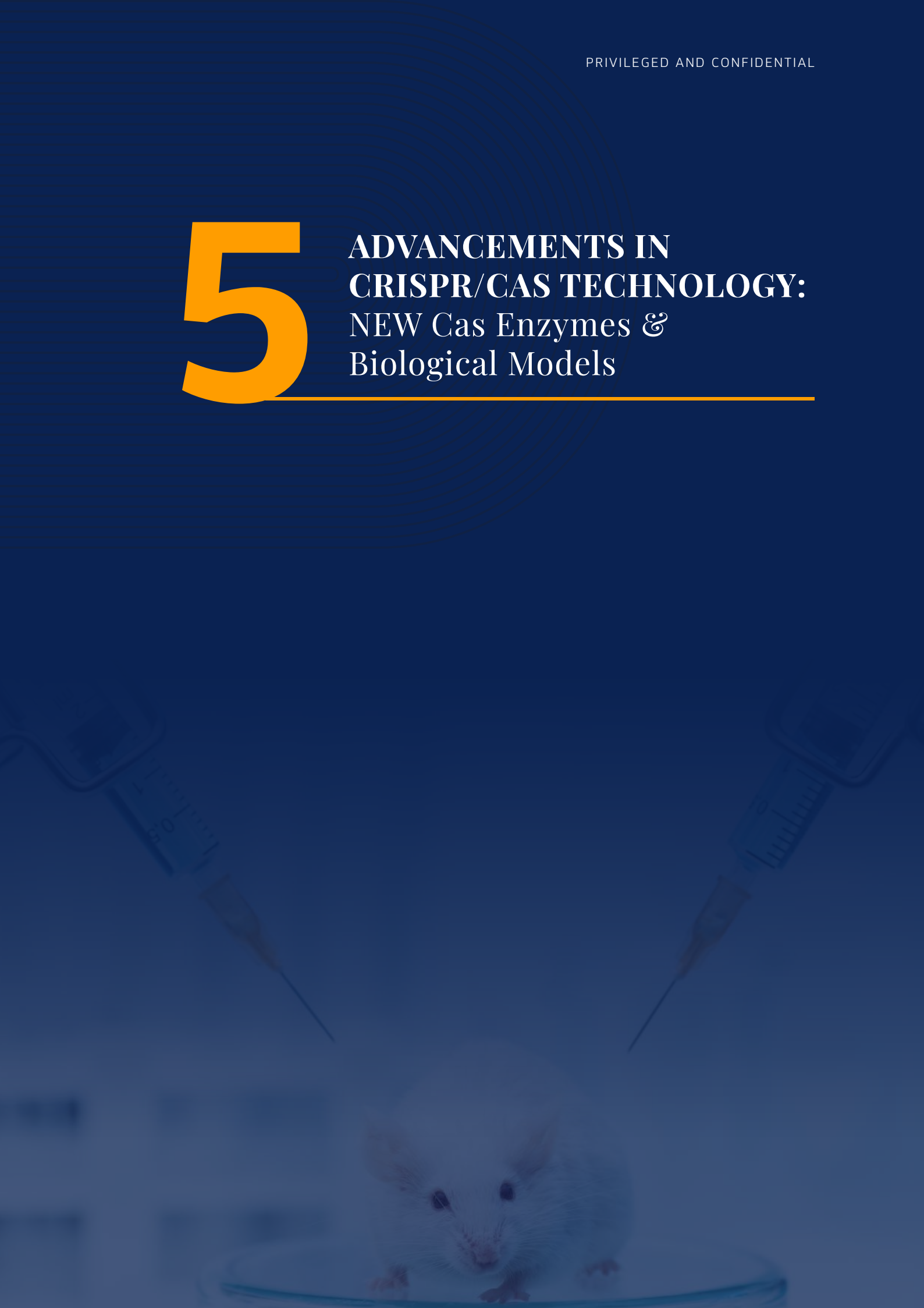
- The results of the second screening (shortlisted 81 patent families) were examined, to identify the top Applicants in patent filings. The University of California (US) was identified as the top player in CRISPR landscape specific to human therapeutics (31 patent families). Dana-Farber Cancer Institute (USA), Toolgen Inc (KR) and University of Pennsylvania (USA) hold the second position with 17 applications each.

- The second screening of 81 patent families revealed that Live attenuated vaccines are the most common, accounting for 50% of filings. Cell-based vaccines follow at 16%, with smaller portions for genetically modified live virus vaccines and bacteriophage vaccines. The most targeted diseases for vaccine applications are cancer (US2022265798A1, US2024424094A1, and CN117965634B), toxoplasmosis (CN114569711B, CN118546788B and CN115992163A), and coronavirus (US2023355738A1, US2023203536A1 and CN112646823A).

- The results of the second screening (shortlisted 81 patent families) were examined, to identify the top Applicants in patent filings. Agricultural University and Lanzhou Veterinary Research Institute are at the forefront of developing vaccines for animal diseases, particularly toxoplasmosis. University of California (US2024248080A1, US10876125B2) and Dana-Farber Cancer Institute (US2020268864A1) are focusing on CRISPR-enabled vaccines targeting human diseases, including cancer and herpesvirus infections. The University of Pennsylvania is exploring the integration of CRISPR/Cas9 with LNP delivery systems (PCT/US2022/077346).

- While China leads in the number of patent applications filed, much of its CRISPR-based vaccine research is directed toward animal welfare, such as improving livestock health and disease resistance. In contrast, the US stands out as primary hub for human health-focused CRISPR vaccine development and European Patent region ranks second (10%). United Kingdom, Austria, Japan and South Korea also show growing activity for human applications. On the other hand, India has a notably low number of patent filings, signaling an opportunity for deeper investment and capacity building for innovations in CRISPR-based human vaccines.

5 ADVANCEMENTS IN CRISPR/CAS TECHNOLOGY: NEW Cas Enzymes & Biological Models



INDEX- CHAPTER 5

5.1.
New Cas Enzymes: Background

CRISPR-associated enzymes are a diverse family of proteins that play a crucial role in the bacterial immune system, and they have revolutionized genetic research and biotechnology through their use in genome editing. Among the most widely studied Cas enzymes is Cas9, which has been employed for precise DNA modifications in a variety of organisms. However, beyond Cas9, there are several other Cas proteins, such as Cas12 and Cas13 complexes, each with distinct mechanisms and applications. These enzymes differ in their structure, function, and target specificity, offering researchers a wide range of tools for genetic manipulation. In recent years, the discovery of new Cas enzymes, including variants like Cas14 and other lesser-known proteins, has further expanded the toolkit available for genetic manipulation, offering even greater precision, versatility, and potential for innovative applications. The classification and types of the Cas enzymes have already been comprehensively covered in Chapter I of this report.

5.1.1.
New Cas Enzymes: Search Results

5.1.1.1
World Map for Patent Filings

The results are depicted in the form of a world map (Figure 5.1). It was observed that China has maximum number of patent Applications filed, followed by US, the EPO, Japan, and South Korea. Further, 32 patent applications were filed through PCT route. India had about 4 patent applications filed for new Cas enzymes, most of which were extended from PCT.

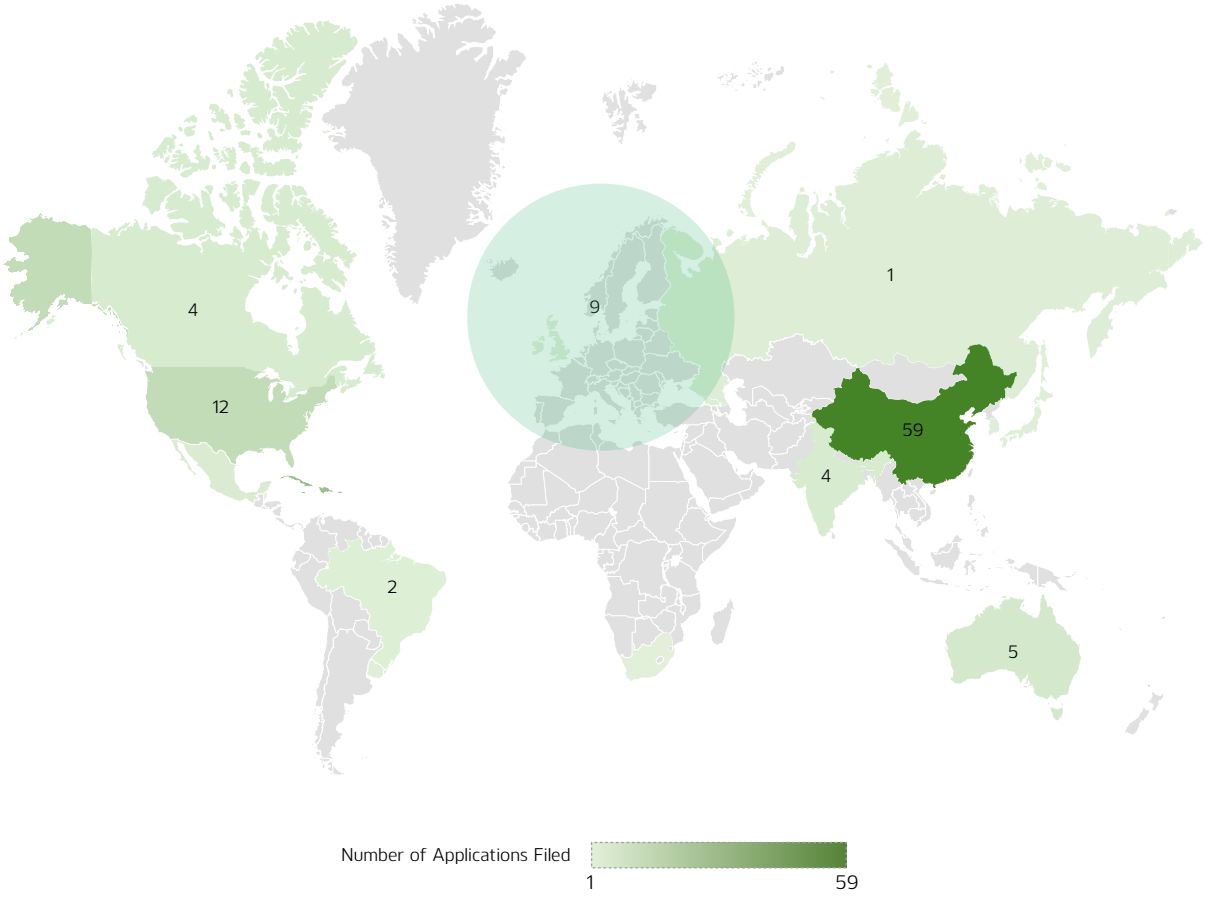


Figure 5.1. World map for patent applications related to New Cas enzymes

With regards to first filing, China had maximum number of priority filings (81%), followed by US (13%) and South Korea (3%).

5.1.1.2 Top Applicants

The top 5 Applicants in the area of New Cas enzymes are represented in Figure 5.2. The Shandong Shunfeng Biotechnology Co LTD (CN) is the top applicant in filing patent applications related to New Cas enzymes, accounting for 67% of the patent families. Following them is the China Agricultural University (CN), Toolgen INC (KR), and Guangzhou Ruifeng Biotechnology Co LTD.

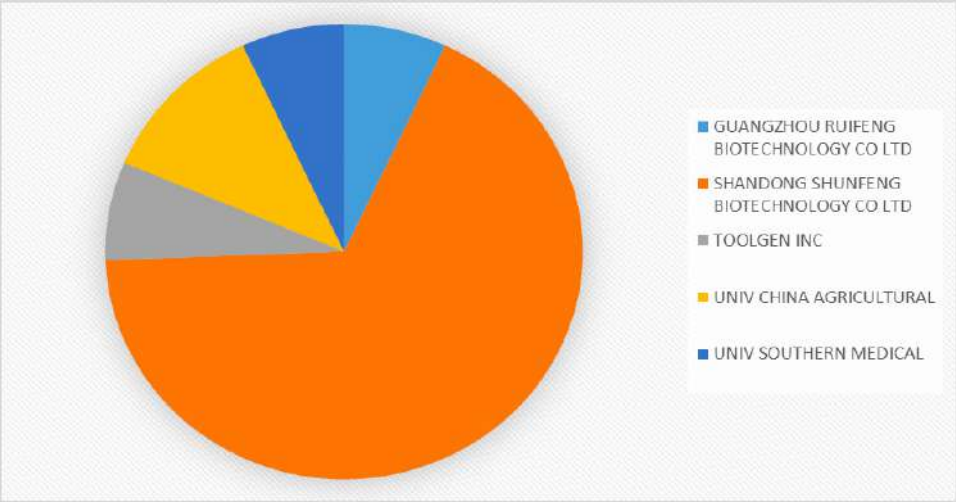


Figure 5.2. Top 5 Applicants for patent applications related to New Cas enzymes

5.1.2. New Cas Enzymes: A Short Review

5.1.2.1 Major Types of Cas Enzymes

- **CRISPR-associated protein 9 (Cas9):**
  Gene-editing technology has progressed through three main generations. The first generation featured ZFNs, followed by the second generation with TALENs. The third and most widely used generation is CRISPR/Cas9. Unlike ZFNs and TALENs, which use proteins to target DNA strands, CRISPR technology guides Cas proteins to specific genome location by altering the base sequence of small RNA molecules, thereby broadening the scope of gene-editing applications [5.1-5.2].

  Cas9 is a versatile and powerful enzyme that has transformed the field of genome engineering. It is one of the most widely utilized Cas nuclease which is used along with CRISPR to be used as CRISPR/Cas9 system, a groundbreaking and a highly effective gene-editing tool. Cas9 is an RNA-guided DNA endonuclease that plays a critical role in bacterial defence mechanisms against foreign genetic material such as viruses and plasmids. After binding to a guide RNA (gRNA) which identifies and binds to specific DNA sequences, Cas9 cleaves the DNA at the target site, effectively neutralizing the invading genetic material.

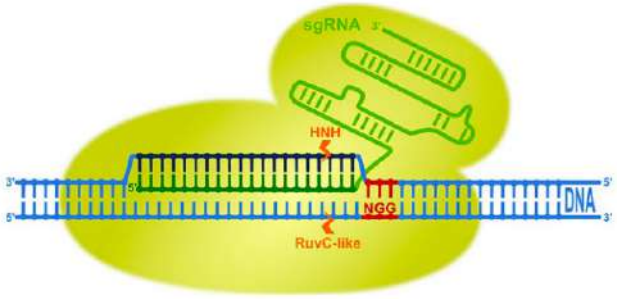


Figure 5.3. Genome editing through programmable RNA-guided DNA endonuclease cleavage with type II CRISPR/Cas9 system from *Streptococcus pyogenes, the most used Cas nuclease.*

Source: Murovec, J., Pirc, Ž., & Yang, B. (2017). New variants of CRISPR RNA-guided genome editing enzymes. Plant biotechnology journal, 15(8), 917-926

Wild-type Cas9 exclusively cuts double-stranded DNA, creating DSBs. These breaks are subsequently repaired by DNA repair mechanisms, specifically HDR and NHEJ [5.3-5.5]. The original gene's base sequence is damaged, which leads to its inactivation. However, inactivating a single harmful gene is insufficient to address the complex processes involved in all disease events. Consequently, researchers explored ways to modify Cas9 by studying its physicochemical structure, the mechanism by which Cas9 cleaves double-stranded DNA, and other properties [5.6].

- **CRISPR-associated protein 12 (Cas12):**
  The Cas12 family is a type of class 2 CRISPR/Cas system, type V effector proteins. The first Cas12 enzyme, classified as type V-A and known as Cas12a (previously Cpf1), was discovered in the genomes of *Prevotella* and *Francisella*, containing a large protein of unknown function. Cas12a is distinct from Cas9. Several *Francisella* species, including F. *novicida*, have Cas12a associated with putative CRISPR arrays. When the F. novicida CRISPR/Cas12a locus was expressed in *E. coli*, it interfered with plasmid DNA transformation, confirming Cas12a as a functional CRISPR/Cas system. Cas12a requires a T-rich PAM sequence before the DNA target site. Unlike Cas9, the Cas12a system does not include a tracrRNA and produces a 5' overhang during DNA cleavage rather than a blunt double-stranded break. Additionally, Cas12a has its own RNase activity, allowing it to process its pre-crRNA array into individual crRNAs, whereas Cas9 relies on host RNase III for CRISPR array processing [5.7-5.8].

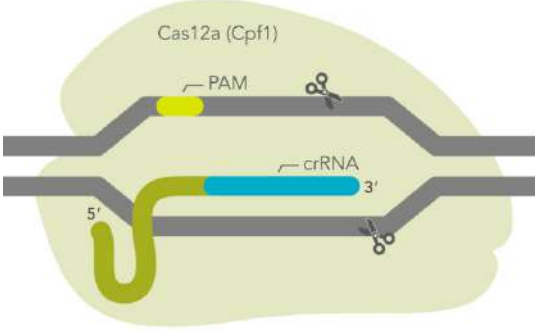


Figure 5.4. Cas12a effector protein. Source: Integrated DNA Technologies. (n.d.). *Alt-R™ CRISPR/Cas12a (Cpf1) genome editing system*. Integrated DNA Technologies.

Source: https://sg.idtdna.com/pages/technology/crispr/crispr-genome-editing/Alt-R-systems/CRISPR/Cas12a

Cas12a-mediated editing offers several advantages over Cas9. It is considerably more specific, making it particularly advantageous for therapeutic applications. Additionally, it simplifies guide design as it does not require tracrRNA. Unlike Cas9, which generates blunt ends, Cas12a produces overhanging ends, which can be beneficial for introducing new sequences. Cas12a also has a smaller molecular size, making it more suitable for viral packaging. Furthermore, it is well-suited for multiplex genome editing, as multiple guide RNAs can be expressed as a single transcript and then processed into individual guide RNAs by Cas12a itself [5.9-5.10].

- **CRISPR-associated protein 13 (Cas13):**
  The type VI CRISPR/Cas systems, characterized by the RNA-guided RNA-targeting Cas13 effector, were initially identified by using the highly conserved adaptation protein Cas1 as a search seed to locate genomic fragments containing potential CRISPR/Cas systems. By focusing on conserved proteins of unknown function within each CRISPR locus, a family of large, well-conserved proteins was discovered. These proteins contained the higher eukaryotic-prokaryotic nuclease (HEPN) domain, indicating they are likely RNases [5.11].

  Cas13a, b, c, and d have been successfully adapted for use in mammalian cells to achieve targeted RNA knockdown. Interestingly, while each Cas13 ortholog in bacteria shows different levels of nucleotide preference in sequences flanking the protospacer (known as the protospacer flanking site or PFS), the presence of PFS is not strictly necessary for RNA targeting in mammalian cells [5.12].

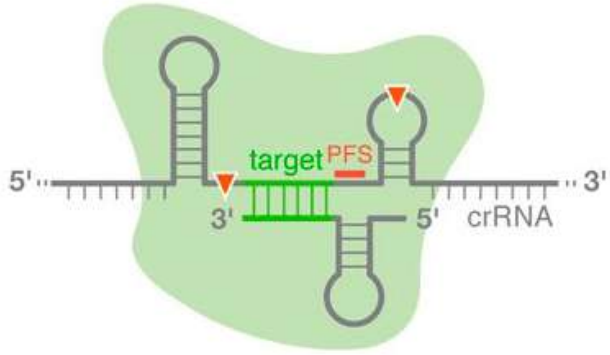


Figure 5.5. Cas13a effector protein.

Source: Zhang, F. (2019). Development of CRISPR/Cas systems for genome editing and beyond. Quarterly Reviews of Biophysics, 52, e6.

- **CRISPR-associated protein 14 (Cas14):**
  Jennifer Doudna and her team explored various Cas systems found in nature and discovered Cas14. This system encodes a smaller Cas protein with a molecular weight ranging from 40,000 to 70,000 Daltons. The Cas14 protein, consisting of 40 to 700 amino acids, is significantly smaller than other known Cas proteins. Due to its compact size, Doudna's lab reported that Cas14 can target single-stranded DNA (ssDNA) without the need for a PAM [5.13].

  Cas14 cleaves ssDNA and provides immunity against viruses with ssDNA genomes or mobile genetic elements (MGEs). The Cas14 protein identifies ssDNA, facilitates seed sequence interaction with the target ssDNA, and cleaves it, but does not cleave double-stranded DNA (dsDNA) or

ssRNA. Similar to Cas9, the Cas14 protein requires both tracrRNA and crRNA to target ssDNA. The cleavage efficiency of Cas14 is more specific than that of Cas9, Cas12, and Cas13 proteins, even without the presence of PAM [5.14].

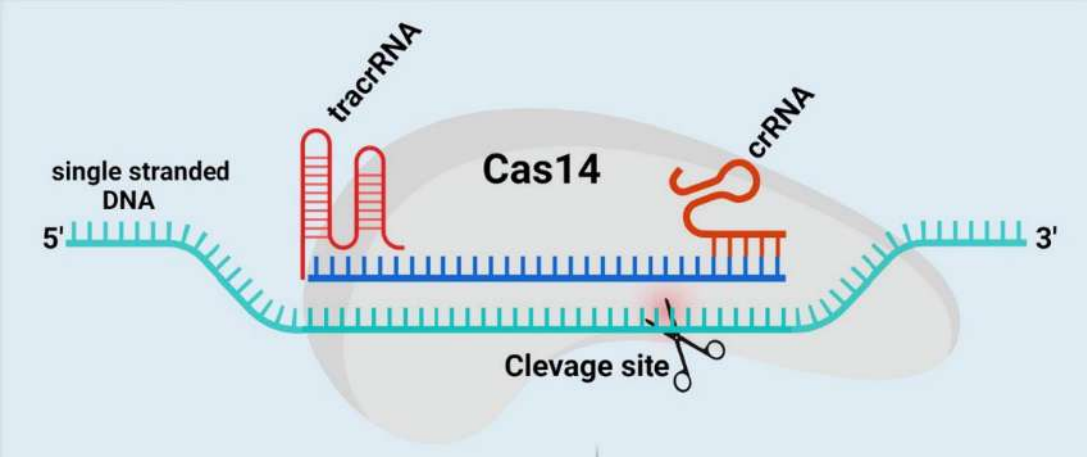


Figure 5.6. Cas14 effector protein.

Source: Hillary, V. E., & Ceasar, S. A. (2023). A review on the mechanism and applications of CRISPR/ Cas9/ Cas12/ Cas13/ Cas14 proteins utilized for genome engineering. Molecular biotechnology, 65(3), 311-325.

The CRISPR/Cas14 system is now considered superior to the Cas13 system. Researchers have combined the CRISPR/Cas14 system with DETECTR for high-fidelity detection of ssDNA. Additionally, Cas14 has been proposed for use in diagnostic applications such as phylogenetic association (identifying new viruses), epidemiological association with other pathogens, and taxonomic analysis. Cas14 has also been employed for viral diagnostics in combination with simplified nucleic acid extraction methods, which do not require complicated sample preparation, such as heating unextracted diagnostic samples to destroy nucleases (HUDSON) [5.15-5.16].

	Cas3	Cas9	Cas7-11	Cas12	Cas13	Cas14
PAM or PFS	None	NGG and Others	None	TTN or TTTN	A, U or C	None
RNA or DNA	DNA	DNA	RNA	DNA	RNA	DNA
Special Features	Eliminates large stretches of DNA	Many variants evolved with unique features	ssRNA specific	Staggered cutting	Non-specifically cleaves non-target RNA	ssDNA specific
Applications	Viral DNA targeting and large DNA detection	Knock ins, knock outs, and many more	mRNA knockdown and RNA editing	Homology directed repair and clinical applications	mRNA lockdown and RNA editing	ssDNA cutting and single nucleotide variant detection

Table 5.1. Cas family proteins and their genome editing abilities.

Source: Addgene. (n.d.). CRISPR 101: Cas9 vs. The other Cas(s). Addgene. Retrieved March 28, 2025, from https://blog.addgene.org/crispr-101-cas9-vs.-the-other-cass

5.1.2.2 Different Variants of Cas9

Several variants of the Cas9 protein have been developed to enhance its functionality in genome editing. These variants primarily focus on improving specificity, expanding the range of targetable DNA sequences, and reducing off-target effects. Some variants are designed to cleave only one strand of DNA, which minimizes the risk of unintended mutations by requiring simultaneous targeting of both strands for a double-stranded break. Others have been altered to recognize non-canonical PAMs, allowing for a broader selection of target sites within the genome. Additionally, catalytically inactive versions of Cas9 can bind to DNA without cutting, enabling applications in gene regulation and epigenetic modifications. Overall, these advancements reflect a significant evolution in the CRISPR toolkit, facilitating more accurate and efficient genetic modifications across various organisms and applications. The table below presents a comprehensive list of various Cas9 variants, each offering unique properties.

Name of the variants	First publication	Patent (if any)	Novelty aspect
Class 2 type VI-A CRISPR effector C2c2 (LshC2c2)	Abudayyeh *et al.* (2016) East-Seletsky *et al.* (2016)	WO2017219027A1	RNase function PFS-C, A or U
Francisella novicida Cas9 (FnCas9)	Sampson *et al.* (2013) Price *et al.* (2015)	WO2014113493A1	RNase function PAM independent
Staphylococcus aureus Cas9 (SaCas9)	Ran *et al.* (2015) Kleinstiver *et al.* (2015a, b)		Smaller size PAM- NNGRRT, NNNRRT
Streptococcus thermophilus Cas9 (St1Cas9)	Kleinstiver *et al.* (2015b)		Smaller size PAM- NNAGAAW
Neisseria meningitidis Cas9 (NmCas9)	Hou *et al.* (2013)		Smaller size PAM- NNNNGMTT
Francisella novicida Cpf1 (FnCpf1)	Zetsche *et al.* (2015)	US9790490B2	Smaller size PAM – TTN, CTA
Acidaminococcus sp Cpf1 (AsCpf1)	Zetsche *et al.* (2015)	US9790490B2	Smaller size PAM- TTTN
Lachnospiraceae bacterium Cpf1 (LbCpf1)	Zetsche *et al.* (2015)		Smaller size PAM- TTN
SpCas9-nickase	Jinek *et al.* (2012), Ran *et al.* (2013)		Enhanced specificity
eSpCas9	Slaymaker *et al.* (2016)	WO2016205613A1	Enhanced specificity
Split-SpCas9	Wright *et al.* (2015)		Smaller size Two-component enzyme

dSpCas9-FokI	Tsai *et al.* (2014) Guilinger *et al.* (2014)		Enhanced specificity
SpCas9-cytidine deaminase	Komor *et al.* (2016) Nishida *et al.* (2016)		Enhanced specificity Gene editing without DSB
dSpCas9-gene expression functional domains	Qi *et al.* (2013)		Modulating gene expression
dSpCas9-Tet1 and -Dnmt3a	Liu et al. (2016), Vojta et al. (2016)		Editing CpG methylation

Table 5.2. New Cas9 variants.

Source: Murovec, J., Pirc, Ž., & Yang, B. (2017). New variants of CRISPR RNA-guided genome editing enzymes. Plant biotechnology journal, 15(8), 917-926.

A few of the variants have been discussed in detail hereinbelow:

Dead Cas9 (dCas9)

Besides their role as nucleases, Cas9 proteins can be inactivated to function as RNA-guided DNA or RNA-binding domains. These inactivated variants can be utilized for numerous powerful applications by acting as programmable nucleic acid binding scaffolds to recruit various effector functions. To deactivate the nuclease activity of Cas9, alanine substitutions are introduced into the catalytic residues of the HNH and RuvC nuclease domains [5.17]. In early 2013, researchers demonstrated that a mutant version of Cas9, known as dead Cas9 (dCas9), could be used to achieve programmable gene repression in both bacteria and mammalian cells. This was accomplished by dCas9 binding to the genome and blocking transcription [5.18]

With Cas9, researchers can utilize truncated guide sequences that do not activate the nuclease function of Cas9. This method allows them to simultaneously use Cas9 as a nuclease to cleave one set of genomic targets by employing guide RNAs with either full-length (20-nt) or truncated (12-nt) sequences. This approach is especially useful when working with transgenic mouse lines that express the nuclease-active form of Cas9. By using this truncated guide RNA strategy, DNA binding experiments can be performed without the need to create an additional line expressing dCas9 [5.19-5.21].

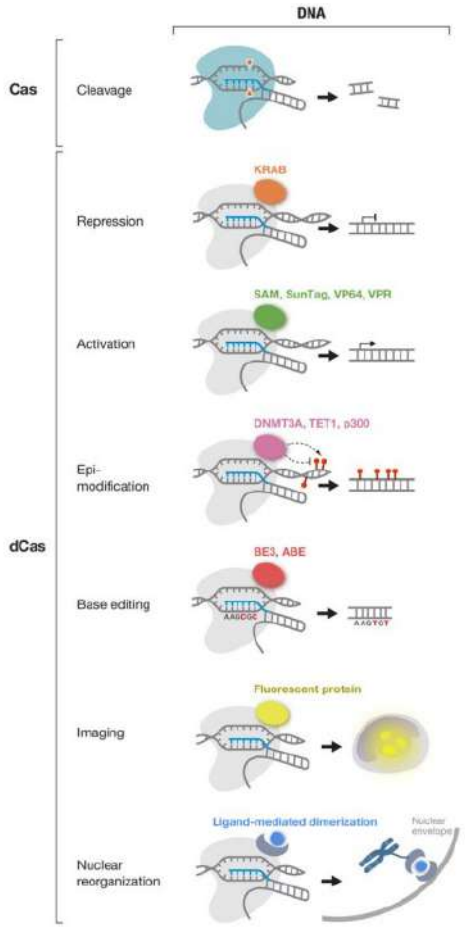


Figure 5.7. Applications of DNA-targeting Cas effectors (Cas9).

Source: Zhang, F. (2019). Development of CRISPR/Cas systems for genome editing and beyond. Quarterly Reviews of Biophysics, 52, e6.

The applications of dCas9 are extensive. Initial studies demonstrated that simply recruiting dCas9 to target loci could repress gene expression in both bacterial and human cells. By fusing dCas9 with transcriptional repressors like the Krüppel-associated box (KRAB), researchers have been able to programmably repress gene expression in human cell lines. These dCas9-KRAB fusions have been combined with inducible Cas9 systems for precise regulatory control of gene networks. Additionally, dCas9 can facilitate the transcriptional activation of target genes. It has also been fused with epigenetic modifiers to achieve targeted histone acetylation, histone demethylation, and DNA methylation and demethylation. Various groups have utilized dCas9 for genomic locus and chromosome imaging, as well as for spatial manipulation of genomic organization. Multiplex locus imaging can be achieved using orthogonal Cas enzymes or aptamers and multiple fluorophores. Furthermore, by fusing dCas9 with the engineered peroxidase APEX2, it can be used to identify proteins associated with specific genomic loci. The CRISPR-X system employs dCas9 and modified guide RNAs to recruit cytidine deaminase variants, creating localized windows of variation, which may be useful for directed evolution [5.22].

Francisella novicida Cas9 (FnCas9)

Francisella novicida is a gram-negative bacterium closely related to *Francisella tularensis*, the causative agent of tularemia. While *F. tularensis* is highly virulent and poses significant public health risks, *F. novicida* is considered less pathogenic, primarily serving as a model organism for studying *Francisella* species due to its lower virulence.

FnCas9, derived from *Francisella novicida*, was discovered in 2013 is an enzyme that targets bacterial mRNA, altering gene expression. By 2015, it was engineered to target and destroy the hepatitis C virus (HCV) in huh-7.5 cells, showcasing its flexible and PAM-independent RNA inhibition method. FnCas9 can target both positive-sense and negative-sense RNA strands, inhibiting RNA viruses by blocking their translation and replication machinery. Notably, mutation in the RuvC and HNH cleavage domains (D11A and H969A, respectively) did not reduce HCV inhibition. However, a mutation in the RNA-binding arginine-rich motif (ARM; R59A), essential for FnCas9's interaction with nucleic acids, led to diminished HCV inhibition [5.23].

FnCas9's targeting of HCV demonstrated that mismatches of up to six bases within the 3' region of the rgRNA were tolerated without losing HCV inhibition. However, longer mismatched regions at either the 3' or 5' ends led to a loss of activity. Because FnCas9 targets cytosolic RNA, the risk of off-target effects on host DNA appears to be limited, even though FnCas9 can also target DNA [5.23].

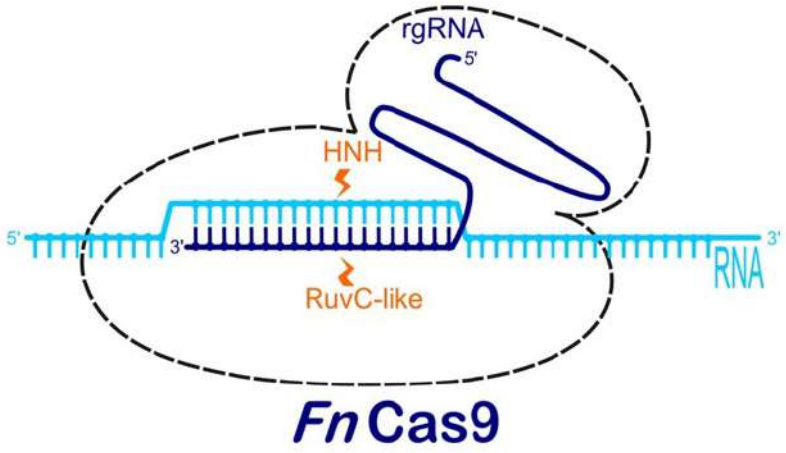


Figure 5.8: FnCas9 cleavage occurring within the complementary region via a combined activity of HNH and like nuclease domains (orange).

Source: Murovec, J., Pirc, Ž., & Yang, B. (2017). New variants of CRISPR RNA-guided genome editing enzymes. Plant biotechnology journal, 15(8), 917-926.

Debojyoti Chakraborty and his team at the Council of Scientific & Industrial Research (CSIR) observed that, despite FnCas9's high precision and negligible affinity for mismatched substrates, its low cellular targeting efficiency limits its therapeutic applications. To address this issue, they engineered enhanced FnCas9 (enFnCas9) variants. These variants exhibit approximately 3.5-fold broader accessibility across human genomic sites. The enFnCas9 proteins, with their single mismatch specificity, have expanded the target range of Fncas9-based CRISPR diagnostics to detect pathogenic DNA signatures [5.24].

As mentioned in patent application PCT/IN2020/050993, the below mentioned application is the one filed by the same applicant (CSIR) who have filed the patent for FELUDA application.

Indian Patent Application: 202111029109

Applicant: Council of Scientific and Industrial Research (an Indian registered body incorporated under the Regn. of Soc. Act (Act XXI of 1860).

Inventors: CHAKRABORTY, Debojyoti, *et al.*

Summary:

In the above-mentioned application relates to an engineered FnCas9 protein from *Francisella novicida* has been developed with enhanced kinetic activity and broader PAM recognition. This variant exhibits increased activity in binding and cleaving polynucleotides. Additionally, it demonstrates high specificity to mismatches in the target sequence, making it ideal for applications such as therapeutic genome editing, disease diagnosis, and genome regulation. The improved kinetic activity of this engineered protein allows it to edit nucleotide loci that was previously inaccessible to the enzyme.

The protein engineering approach introduced specific mutations that stabilize the interaction between the Cas9 enzyme and target DNA. This enhanced kinetic activity boosts NHEJ-mediated editing due to more efficient DSB generation compared to wild-type FnCas9. Additionally, the broadened PAM specificity expands the target range of FnCas9 variants. Consequently, this invention increases the scope and accessibility of CRISPR/Cas9 system targets, producing robust and highly specific engineered FnCas9 variants.

5.2 Biological Models: Background

Investigating the mechanisms of human diseases often begins with the establishment of animal models, which are also widely utilized in agriculture, pharmaceuticals, and clinical research. However, small animals like rodents, commonly used for disease models, frequently fail to accurately replicate the key pathological changes and significant symptoms of human diseases. Consequently, there is a growing need to develop suitable large animal models that can better mimic the important phenotypes of human diseases, aiding in the study of pathogenesis and the development of effective treatments [5.25]. Traditional genetic modification techniques used for creating small animal models are challenging to apply when generating large animal models of human diseases. However, this challenge has been significantly mitigated by the advent of gene editing technologies, particularly CRISPR/Cas9 [5.26].

5.2.1 Biological Models: Search Results

5.2.1.1 World Map for Patent Filings

The results are depicted in the form of a world map (Figure 5.9). It was observed that China had maximum number of patent applications filed, followed by US, South Korea, and the EPO. Further, 35 patent applications were filed through PCT route. India exhibits no filing of patent applications related to CRISPR/Cas and its use in preparing biological models.

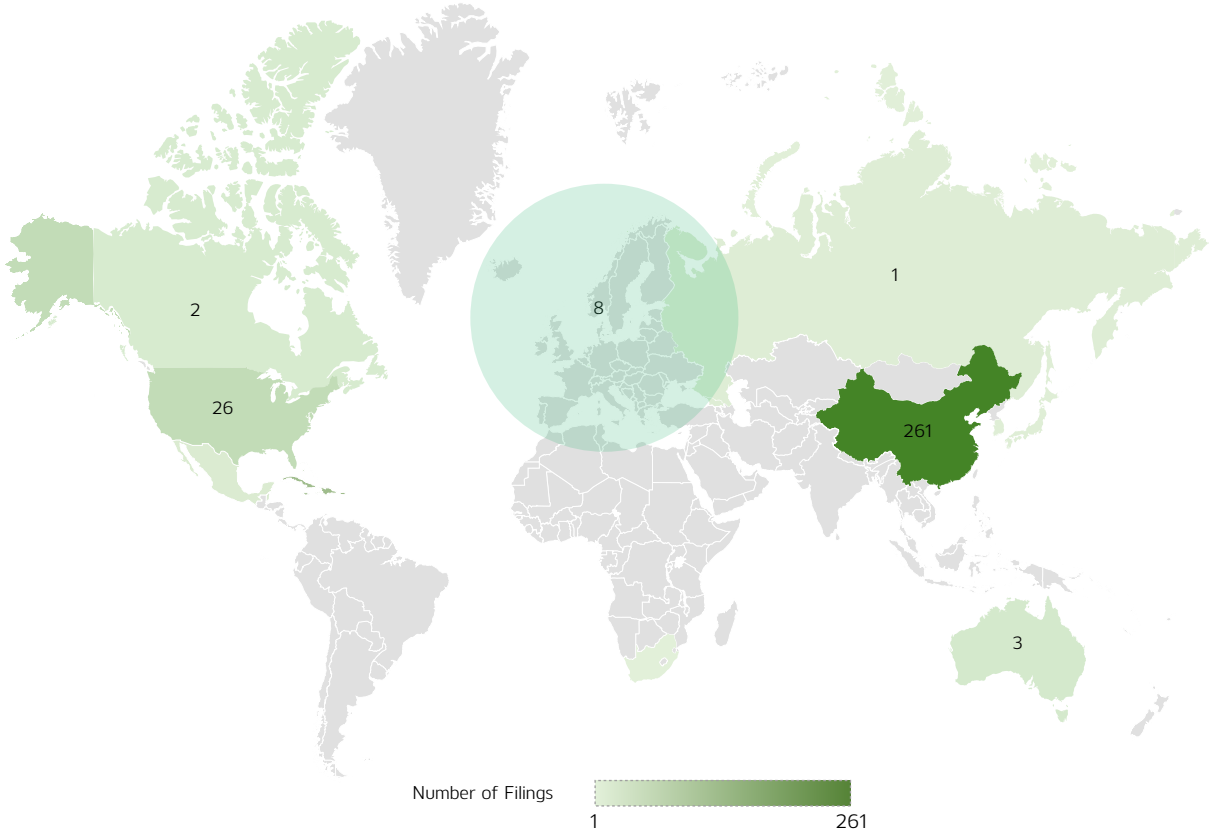


Figure 5.9. World map for patent applications related to CRISPR/Cas and its use in preparing biological models

In terms of priority filing, China has maximum number of priority filings (90%) followed by US (5%) and South Korea (4%). India, on the other hand, appears to not have any priority filings for the patent applications related to biological models using CRISPR/Cas.

5.2.1.2 Top applicants

The top 5 Applicants are enlisted in Table 5.3 and the data is represented in Figure 5.10. The Beijing Biocytogen Co LTD (CN) is the top applicant in filing patent applications related to CRISPR/Cas and its use in preparing biological models, accounting for 36% of the patent families. Following them is Zheijiang University (CN), Sichuan Provincial Peoples Hospital (CN), Hunan Normal University (CN), and Jilin University (CN).

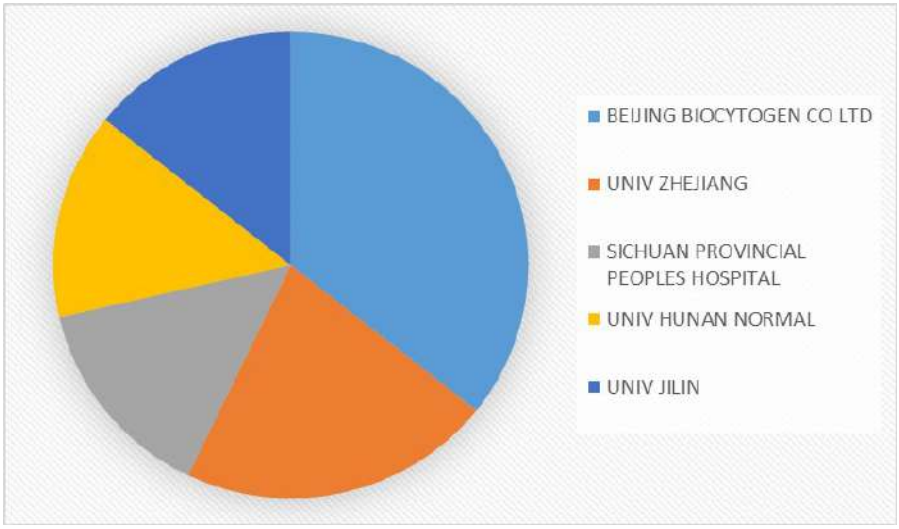


Figure 5.10. Top 5 Applicants for patent applications related to CRISPR/Cas and its use in preparing biological models.

5.2.2 Biological Models: A Short Review

5.2.2.1 CRISPR/Cas9 for Creation of Animal Models

Characterizing disease phenotypes is a primary objective for mouse geneticists. Studying genetic mutations in whole animals can help validate findings from cell culture experiments. Since the creation of the first transgenic mice in 1981, the process has become more versatile and user-friendly. The introduction of Bacterial Artificial Chromosomes (BAC) in the 1990s [5.27] enabled the use of larger genetic constructs. Traditional methods for creating genetically altered mouse models typically require 8 to 13 months, demanding significant time and resources. In contrast, CRISPR/Cas9 technology has been used to produce transgenic mice with an observable albino phenotype after just a single microinjection into C57BL/6J mouse embryos [5.28].

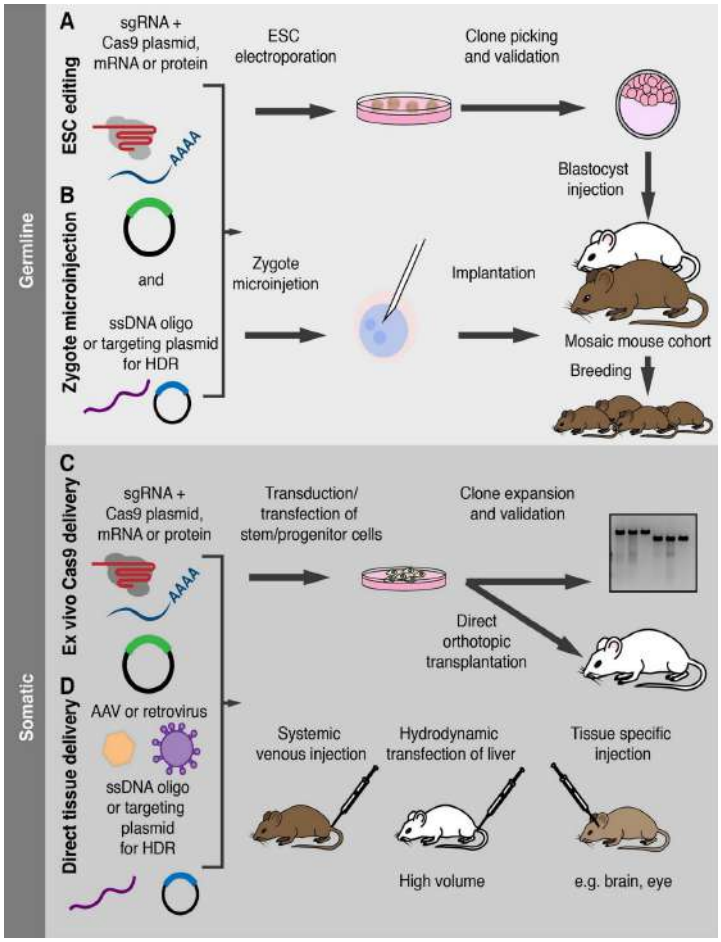


Figure 5.11. Summary of in vivo CRISPR strategies for mouse model production.

Source: Tschaharganeh, D. F., Lowe, S. W., Garippa, R. J., & Livshits, G. (2016). Using CRISPR/Cas to study gene function and model disease in vivo. The FEBS journal, 283(17), 3194-3203.

Pioneering research by the Huang group demonstrated that genetic engineering in eukaryotes could be achieved through the microinjection of CRISPR/Cas9 mRNAs into zebrafish embryos. They advanced this technique further by successfully using it to delete a segment of Enhanced Green Fluorescent Protein (EGFP) in an established transgenic mouse [5.29]. Following this initial proof-of-concept experiment, many research groups have further advanced the field of transgenic mouse models using CRISPR/Cas9. Wang *et al.* demonstrated that multiplexed targeting of multiple genes simultaneously could generate a complex mouse model with just a single injection [5.30]. Mutated Cas9 nickases have proven effective in facilitating the creation of both knock-in and knockout mouse models, offering the added advantage of reduced off-target effects [5.31].

Another challenge researchers often face is producing recessive homozygous loss-of-function mutations. To address this, Gantz and Bier developed an autocatalytic mutation system known as the mutagenic chain reaction (MCR). MCR involves a vector where Cas9 and sgRNA are flanked by two homology arms targeting the region to be cut. This system can effectively propagate itself in both

somatic and germline cells in Drosophila, creating homologous mutant flies that would not typically be possible through Mendelian inheritance. While MCR could be extremely useful, the authors caution that its automatic action poses a biological risk if these animals were accidentally released into the environment [5.32].

5.2.2.2 Expanding Animal Models with CRISPR/Cas9

Beyond the numerous mouse models being developed with CRISPR/Cas9 technology, other organisms have also shown responsiveness to this genetic manipulation method. These include traditional animal models such as *Drosophila melanogaster* [5.33], *Caenorhabditis elegans* [5.34], *Saccharomyces cerevisiae* [5.35], *and Dani rerio* [5.36]. Additionally, CRISPR/Cas9 genetic engineering has been successfully applied to rats, overcoming the limitations of the genetic toolbox. In rats, multi-gene targeting and conditional allele modifications have been effectively performed, leading to the creation of new transgenic models [5.37].

CRISPR/Cas9 technology has also proven effective in non-traditional animal models such as goats and pigs, which are significant for both agricultural and biomedical research [5.38]. This technology has enabled simplified genetic modifications and the creation of disease models in non-human primates, highlighting its potential to enhance our ability to model diseases in animals more closely related to humans [5.39].

An intriguing example of how CRISPR/Cas9 is opening a new research avenue is the development of an animal model, the short-lived African turquoise killifish *(Northbranchius furzeri)*. The killifish serves as an attractive alternative to other vertebrate models used for studying age-related diseases, such as rodents or zebrafish. Its shorter lifespan (4-6 months) reduces the time required for the manifestation of aging phenotypes, including common aging biomarkers [5.40]. Additionally, the killifish offers practical benefits, including lower maintenance costs and rapid offspring production. When these advantages are combined with the ability to manipulate the genome using CRISPR/Cas9, the potential for discoveries in age-related diseases is significantly enhanced [5.41].

5.2.2.3 Utilizing CRISPR/Cas9 in Nonhuman Primate Models

Nonhuman primates are undoubtedly the most representative animals for mimicking human diseases due to their similarities in genetics, physiology, developmental biology, social behaviors, and cognition. However, creating transgenic models in nonhuman primates is significantly more challenging compared to small animals like mice, primarily due to factors such as their long breeding cycles and ethical considerations [5.41].

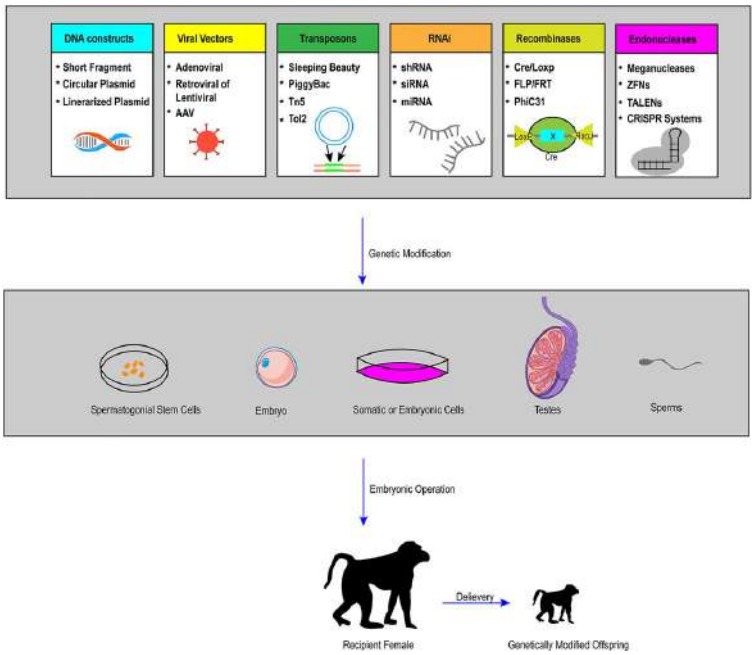


Figure 5.12. Schematic representation of practical and possible pathways of genetic modification in large animals.

Source: : Lin, Y., Li, J., Li, C., Tu, Z., Li, S., Li, X. J., & Yan, S. (2022). Application of CRISPR/Cas9 system in establishing large animal models. Frontiers in cell and developmental biology, 10, 919155.

The first report of generating a gene-modified monkey using CRISPR/Cas9 emerged in 2014[5.42]. Researchers successfully achieved precise gene targeting in cynomolgus monkeys by coinjecting Cas9 mRNA and sgRNAs into one-cell-stage embryos. This method enabled the simultaneous disruption of two target genes, peroxisome proliferator-activated receptor gamma (PPARG) and recombination activating gene 1 (RAG1), in a single step without detectable off-target effects. Additionally, they demonstrated the possibility of germline transmission in Cas9 manipulated monkeys by examining gene targeting in gonads and germ cells [5.43]. However, the resulting transgenic monkey displayed mosaic mutations, with wild type alleles present in different tissue, raising concerns about whether these mosaic mutations could affect functional studies. Subsequently, Chen *et al.* used Cas9 to disrupt the dystrophin gene (DMD) in rhesus monkeys, which showed significantly reduced dystrophin levels and muscle degeneration characteristic of early Duchenne muscular dystrophy (DMD). This demonstrated that CRISPR/Cas9 can effectively generate monkey models of human diseases, regardless of inheritance patterns [5.44].

6 CRISPR-TECHNOLOGY IN BIOMEDICINE: Current Scenario in India

INDEX- CHAPTER 6

6.1. Background

Based on the jurisdiction specific analysis carried out for CRISPR-based inventions for use in biomedicine; for therapy (Chapter II), diagnosis (Chapter III) and vaccines (Chapter IV), it was observed that very few of the PCT Applications entered National phase in India (Table 6.1).

Type of Inventions in Biomedicine	Percentage of Corresponding Applications Fled in India with Respect to Globally Identified PCT Applications.
CRISPR-based gene editing for therapy	13%
CRISPR-based gene editing for Vaccine production	8.3%
CRISPR-based gene editing for disease diagnosis	8.5%

Table 6.1. Number of corresponding applications filed in India for each type of invention in biomedicine

In order to study the CRISPR patenting scenario in India, an additional search was conducted to shortlist patent Applications related to CRISPR-technology.

The shortlisted Applications were analyzed to study the following trends.

6.2. Fields

Out of the shortlisted Applications, the fields where CRISPR-based gene editing is employed were divided into 6 categories as enlisted in the chart below (Figure 6.1).

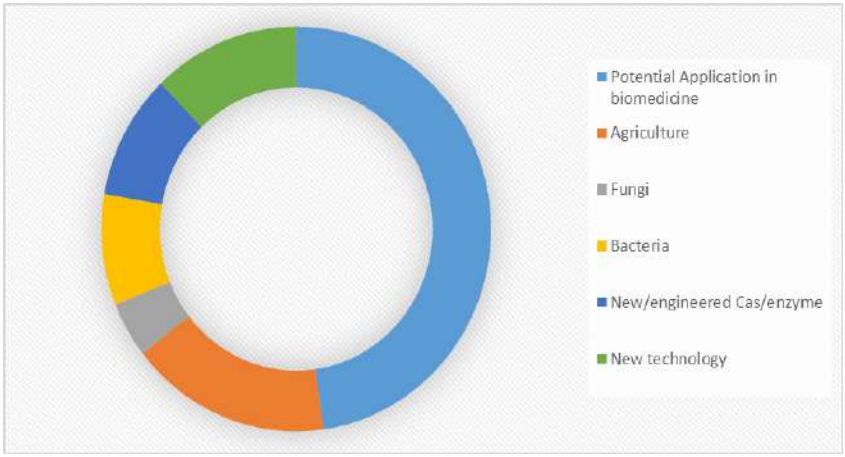


Figure 6.1. Patent applications filed for different applications of CRISPR technology

- The data indicate that out of all the patent Applications filed in India pertaining to CRISPR technology, maximum number of filings in India accounted for the use of CRISPR-based gene editing in the *field of biomedicine* (48% of shortlisted Applications). This includes Applications employing CRISPR-based gene editing for therapies, vaccine production, diagnosis, animal model production, drug screening, etc.

- Of the total applications, 17% of the Applications relate to the use of CRISPR-based gene editing in the field of agriculture especially for improving crop traits like insect resistance, or herbicide tolerance.

- Improved methods of gene editing using CRISPR technology, improved CRISPR systems for gene editing or any improvement in delivery of CRISPR-based systems were covered by 12% of the Applications.

- Several Applications directed to new or improved Cas enzymes were shortlisted which generally aimed at improving enzyme specificity.

- A few of the Applications relating to methods or systems of CRISPR-gene editing in fungi and bacteria were also identified. These inventions generally have applications in areas related to synthetic biology.

- A number of Applications have also been filed in the field of CAR-T technology using CRISPR/Cas from Applicants such as Anurag University (IN202441096335 A).

6.3 Top Applicants in Biomedicine

The identified patent Applications covering inventions related to use of CRISPR based gene editing in the field of biomedicine were analyzed to study the trend in top Applicants. The top players in India in terms of patent filings include Regeneron Pharmaceuticals Inc, Johns Hopkins University, and the Broad group (Broad Institute Inc., MIT, President and Fellows of Harvard College). A list of Applicants has been provided in the Table below:

APPLICANT(S)
DANISCO US INC
E. I. DU PONT DE NEMOURS AND COMPANY; PIONEER HI BRED INTERNATIONAL INC
EDITAS MEDICINE, INC.
GENZYME CORPORATION
JUNO THERAPEUTICS, INC. EDITAS MEDICINE, INC.
REGENERON PHARMACEUTICALS INC.
SHANGHAI TOLO BIOTECHNOLOGY COMPANY LIMITED
SIGMA-ALDRICH CO. LLC
REGENTS OF THE UNIVERSITY OF MINNESOTA
THE JOHNS HOPKINS UNIVERSITY
GREEN BIOLOGICS LIMITED
PIONEER HI BRED INTERNATIONAL INC.
PRESIDENT AND FELLOWS OF HARVARD COLLEGE
PURAC BIOCHEM B.V.
RODOS BIOTARGET GMBH

THE BROAD INSTITUTE INC. MASSACHUSETTS INSTITUTE OF TECHNOLOGY PRESIDENT AND FELLOWS OF HARVARD COLLEGE
THE REGENTS OF THE UNIVERSITY OF CALIFORNIA
TOOLGEN INCORPORATED
VILNIUS UNIVERSITY
WAGENINGEN UNIVERSITEIT

Table 6.2. Applicants filing CRISPR/Cas related applications in India

Most of the Applicants are non-Indian organizations that have entered India through the PCT route. Applications filed by Indian Applicants would be a handful and not identified in the search conducted via InPASS. These Applications were identified while carrying out jurisdiction specific analysis in the previous chapters; IN201911049432, and IN202111029109.

Both of these Applications were filed by CSIR which is the top Government based CRISPR research institute in India with respect to biomedicine. IN201911049432 (filed on December 02, 2019) is currently awaiting examination with claims directed to a kit for detection of a target polynucleotide. The primary application of the claimed invention would be detecting/diagnosing Genetic disorders.

IN202111029109 (filed on June 29, 2021) is also currently awaiting examination with claims directed to a ribonucleoprotein complex for gene editing, having applications in health sector, pharmaceuticals, agriculture, biotechnology, and food sector. Applications in the health sector primarily include therapeutic genome editing, and disease diagnosis.

6.4 Indian Entities Working on CRISPR/Cas Technology

- **CrisprBits:**

  CrisprBits is an Indian biotechnology startup founded in 2020 by Dr. Vijay Chandru and four other BITS Pilani alumni: Sunil Arora, Dr. Rajeev Kohli, Bharat Jobanputra, and Aditya Sarda. CrisprBits utilizes CRISPR to develop affordable and high-quality solutions in the life sciences sector. Their current projects focus on creating diagnostics and surveillance tests, as well as advancing CRISPR applications in critical healthcare areas such as CAR-T cell development and disease tissue models from edited induced pluripotent stem cells (iPSCs). CrisprBits have filed three patent applications in India which are:

  - Indian application number 202411084372, titled "Guide RNA, Kits Containing It, and Associated Methods". The invention provides a guide RNA, a kit, a composition, and a method fordetecting genetic variations such as Sickle Cell Anemia (SCA) and identifying carriers through an optimized point-of-care CRISPR-based assay. The gRNA identifies SCA-specific nucleotide changes using distinct mixes for Hemoglobin A (HbA) and Hemoglobin S (HbS). The guide RNA, featuring multiple SNP recognition sites, is constructed via a hybridization chain reaction, incorporating multiple Cas12b proteins. This guide RNA can determine the homozygous, heterozygous, or carrier state of sickle cell disease.

  - Indian application number 202417027642, titled " ASSAY DEVICE, METHOD FOR DETERMINING GENETIC PREDISPOSITION TO SEVERE FORMS OF SARS-COV2 INFECTION". The present disclosure introduces an assay device and associated methods to determine a subject's risk of developing severe forms of SARS-CoV2 infection. This risk assessment or genetic predisposition determination relies on specifically designed CRISPR/Cas systems. These CRISPR/Cas systems are characterized by unique guide sequences that detect the presence or absence of specific Single Nucleotide Polymorphisms (SNPs) in a sample, with these SNPs being genetically correlated with severe forms of SARS-CoV2 infection.

  - Indian application number 202211074360, titled "SEQUENCES AND METHODS FOR CRISPR-BASED DETECTION OF OMICRON VARIANT OF SARS-COV-2". The present disclosure introduces methods to detect SARS-CoV2 infection using CRISPR sgRNA sequences. These sequences enable the detection of SARS-CoV2 and facilitate the identification and differentiation between variants, such as Omicron and non-Omicron variants. Additionally, the associated methods utilize these specifically designed sgRNA sequences and CRISPR/Cas systems to detect the presence or absence of SARS-CoV2 in a sample and distinguish between different variants.

- **Tata Institute for Genetics and Society (TIGS):**

  TIGS is a research organization focused on leveraging cutting-edge scientific tools to explore genetics and its applications for society benefits. One of the most important technologies

associated with genetic research today is CRISPR/Cas. TIGS has filed two patent applications in India. One of these applications, with the Indian patent application number 202341053245, pertains to BfCas12a Associated Detection Assays and Kits. This invention describes a system and method for detecting target nucleic acids using BfCas12a trans-cleavage activity. The disclosed compositions and methods offer a rapid, sensitive, low cost, and accurate approach for detecting target nucleic acids. These can be utilized to swiftly and precisely identify pathogenic microorganisms or disorders caused by genomic mutations. The system/composition includes BfCas12a, guide RNA, dual-labeled probes, and an assay buffer for detecting the target nucleic acid.

- **National Institute of Plant Genetic Research (NIPGR):**

  NIPGR is a renowned research institute in India, primarily focused on plant genome research and biotechnology. NIPGR utilizes CRISPR/Cas technology in its research to modify plane genomes for the development of crops with improved traits such as resistance to diseases, higher yield, enhanced nutritional contents, or tolerance to environmental stresses like drought or salinity. One of their applications with the Indian patent application number 202211034971, relates to Recombinant Expression Cassettes for Modification of Glucosinolate Content in Plants. This invention introduces a recombinant expression cassette containing guide RNAs and Cas9 protein, which is effective in modifying the glucosinolate content in a plant. Additionally, it provides a method for altering tissue-specific glucosinolate accumulation in a plant and process for producing a transgene-free edited plant using the recombinant expression cassette.

Research Scenario on CRISPR/Cas technology in India

India has witnessed significant progress in CRISPR/Cas technology research over the past decade, with various advancements taking place in both basic research and its application in agriculture, medicine, and biotechnology.

India has a vast agricultural base, and CRISPR/Cas technology holds significant promise for improving crop yield, resistance to pests, and resilience to environmental stress. Indian scientists have been exploring the potential of CRISPR to genetically modify crops like rice, wheat, and mustard.

In the medical field, CRISPR is being studied for its potential to treat genetic diseases like sickle cell anemia, thalassemia, and certain types of cancer. Some institutions in India have focused on developing CRISPR-based gene therapies, which could be groundbreaking for patients from suffering from genetic disorders.

Indian Council of Agricultural Research (ICAR) is the leading CRISPR research institute in India with a significant focus on agriculture research. The CSIR follows closely behind. The most active Indian authors/researchers in the CRISPR field are Anshu Alok and Debojyoti Chakraborty. Dr. Alok is an agriculture researcher from Punjab University, while Dr. Chakraborty specializes in RNA biology at the CSIR-Institute of Genomics and Integrated Biology.[6.1]

Conclusion

Globally, CRISPR/Cas-related innovations have seen remarkable growth, as revealed by an extensive analysis of 5,527 extended INPADOC patent families. From just a handful of priority filings in the early years (2005-2011), CRISPR research has surged significantly, peaking in 2022 with 852 filings. This trend clearly reflects the growing global recognition of CRISPR/Cas technology as a transformative tool in biotechnology medicine. Despite a slight dip in 2023 and 2024- likely due to the delay in publications- the momentum remains strong. Geographically, China has emerged as the global leader with 3,704 priority filings, followed by the US with 938 priority filings, while other countries like India, South Korea, and members of the EPO also demonstrate vibrant activity in this space.

The data also highlights the major institutions and companies driving CRISPR innovation. The Chinese Academy of Sciences stands out as the top contributor, followed by Huazhong Agricultural University and The Broad Institute.

The comprehensive analysis of CRISPR/Cas-related patent filings specific to therapeutic applications reveal a dynamic and rapidly evolving landscape, underscoring the immense potential of gene editing in modern medicine. With the US and China leading in both priority and total filings, and institutions like the University of California and Dana-Farber Cancer Institute, and University of Pennsylvania emerging as key contributors, it is clear that global efforts are intensifying toward clinical translation. Notably, nearly half of the patents focus on cancer treatment, with significant innovations also targeting genetic, neurological, cardiovascular, and infectious diseases. Furthermore, the growing application of CRISPR in CAR-T cell therapy highlights a promising frontier in personalized cancer treatment. The high level of international collaboration further enriches the innovation ecosystem, demonstrating a collective commitment to advancing human health through cutting-edge genetic technologies. This trend is encouraging and illustrates the positive trajectory of CRISPR-based therapeutics toward becoming mainstream in future healthcare solutions.

The patent landscape for CRISPR/Cas-based diagnostics of infectious diseases in human reveal a strong and focused global research effort, with China and the US leading the way in both total and priority filings. The Broad Institute stands out as a major innovator, actively collaborating with leading institutions like Harvard and MIT, reflecting the significance of multidisciplinary partnerships in driving diagnostics advancements. Cas12 and its variants emerged as the most widely used enzymes, highlighting their efficacy and adaptability in diagnostic applications. The presence of several collaborative efforts across jurisdictions and institutions underscores a collective global commitment to developing rapid accurate, and scalable diagnostic tools using CRISPR technology. This growing innovation ecosystem is highly encouraging, signaling a promising future for CRISPR-based diagnostics in combating infectious diseases and safeguarding global public health.

The global patent landscape for CRISPR/Cas-based vaccines reflects dynamic and promising innovation, with notable contributions from key jurisdictions such as China, the US, and Europe. China's leadership in patent filings underscores its significant investment in biotechnological advancements, particularly in the area of vaccines for infectious and zoonotic diseases like toxoplasmosis. Meanwhile, US institutions demonstrate a strong focus on therapeutic vaccines, including cancer and herpesvirus, leveraging CRISPR technology in novel ways. The emergence of universities and independent inventors from various regions highlights the democratization of CRISPR tools, fostering a diverse

range of vaccine innovations. This growing global collaboration and competition signal a bright future for CRISPR-based vaccines, positioning the technology to play a crucial role in addressing both current and emerging health challenges. The continued evolution of CRISPR as a vaccine platform holds immense potential for advancing public health globally.

The global landscape of patent filings for new Cas enzymes and their application in biological models highlights the increasing significance of CRISPR/Cas technologies in biotechnology and medical research. China stands out as the dominant player, particularly in the areas of new Cas enzyme development and biological models, signaling its leadership in advancing gene-editing innovations. The US and South Korea also demonstrate notable contributions, reflecting a strong international interest in CRISPR-based technologies. The emergence of universities and companies in China, such as Shandong Shunfeng Biotechnology Co. and Beijing Biocytogen Co., emphasizes the growing role of academic and commercial collaborations in driving innovation. While India shows limited activity in these areas, the global trend indicates a rapidly expanding and promising field with significant potential for breakthroughs in genetic engineering and biomedical applications. The future of CRISPR technology appears bright, with collaborative global efforts paving the way for novel solutions in medicine and biotechnology.

The CRISPR/Cas patent landscape in India, though currently modest scale, reflects a steadily growing interest and promising potential across diverse fields such as biomedicine, agriculture, and biotechnology. Despite stringent patentability criteria and limited national phase entries through the PCT route, India has demonstrated focused innovation, particularly through leading public institutions like CSIR, NIPGR, and TIGS, and emerging biotechnology startups like CrisprBits. The majority of CRISPR-related filings in India pertain to biomedical applications, indicating a keen interest in leveraging gene-editing technologies for diagnostics, therapeutics, and vaccine development. Encouragingly, Indian scientists and institutions are making significant contributions, with active research and patent filings that align with global advancements. This evolving landscape highlights India's growing capabilities in gene-editing technologies and suggests a bright future for CRISPR innovation, especially with continued support for indigenous research and development and translation into impactful solutions for health, agriculture, and industry.

The legal battle between the Broad Institute and the CVC group over CRISPR/Cas9 technology stands as one of the most pivotal and complex patent disputes in biotechnology history, with far-reaching implications for innovation, licensing, and commercialization. While the USPTO and EPO have delivered key rulings- favoring Broad in the US and CVC in Europe-uncertainty still prevails, particularly for smaller entities seeking clear licensing pathways. Procedural missteps, such as priority transfer issues and sufficiency of disclosure, have further complicated the patent landscape. In light of this ambiguity, the scientific community is increasingly turning toward alternative Cas enzymes like Cas12, Cas13, and Cas14, as well as engineering novel variants to bypass existing patents. This shift underscores the dynamic nature of CRISPR research and reflects the field's resilience and adaptability in navigating legal and commercial hurdles.

Notably, both academic and commercial entities are actively shaping the future of CRISPR, showcasing the technology's broad applicability across agriculture, healthcare, and beyond. These trends are a testament to the thriving research ecosystem and the collaborative global effort to harness CRISPR's full potential and societal advancement.

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Chapter 5

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Chapter 6

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