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# Recent progress in prompt molecular detection of liquid biopsy using Cas enzymes: innovative approaches for cancer diagnosis and analysis

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**Abstract**
Creating fast, non-invasive, precise, and specific diagnostic tests is crucial for enhancing cancer treatment outcomes. Among diagnostic methods, those relying on nucleic acid detection are highly sensitive and specific. Recent developments in diagnostic technologies, particularly those leveraging Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR), are revolutionizing cancer detection, providing accurate and timely results. In clinical oncology, liquid biopsy has become a noninvasive and early-detectable alternative to traditional biopsies over the last two decades. Analyzing the nucleic acid content of liquid biopsy samples, which include Circulating Tumor Cells (CTCs), Circulating Tumor DNA (ctDNA), Circulating Cell-Free RNA (cfRNA), and tumor extracellular vesicles, provides a noninvasive method for cancer detection and monitoring. In this review, we explore how the characteristics of various Cas (CRISPR-associated) enzymes have been utilized in diagnostic assays for cancer liquid biopsy and highlight their main applications of innovative approaches in monitoring, as well as early and rapid detection of cancers.

**Keywords** CRISPR-Cas, Cancer, Liquid biopsy, Diagnosis

## Background

Cancer has become a major public health issue globally, with cancer screening and diagnosis being key strategies in reducing its impact. Diagnosing cancer in its early stages can significantly improve the cure rate and 5-year survival rate for patients [1]. Genetic changes in cancer are now a priority for cancer treatment. Samples from primary tumors or metastasis are typically used to assess these variations. Of course, there are several challenges in examining this type of data [2]. As obtaining tissue biopsies usually requires surgery, it restricts the ability to sample biopsies. It can make it difficult to access particular tumor tissues depending on where the tumor is located. Additionally, intra-tumor heterogeneity, particularly spatial heterogeneity, could pose a challenge. The

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biomarker detection results can be dubious when a single biopsy is tested. These factors may have collectively led to this issue [3–5]. Acquiring tumor specimens from long-term patients may be difficult, and testing these archived tumor samples may not be desirable as the tumors may have evolved. Furthermore, it is impractical to constantly use painful biopsies to monitor and track tumor growth or changes. Therefore, it is necessary to find alternative solutions for serial monitoring to make it more efficient. The use of accessible and non-invasive materials is in high demand to efficiently monitor cancer-related molecular changes in real-time. This would ensure more systematic progress tracking and facilitate better patient monitoring. The medical field does not rely heavily on conventional biopsies as the primary go-to method for tumor analyses. Instead, modern research has shifted its focus to Liquid Biopsies (LBs), which analyze various bodily fluids to detect potential tumor components. In recent years, liquid biopsy has become one of the most favored procedures in many applications. Advancements seen in this field have been nothing short of impressive [6].

Tissue biopsy is not a suitable option for cancer screening when tumors have not yet been formed. Fortunately, other effective methods can be used for both the prevention and early diagnosis of cancer. Regular tests, such as the Pap test for cervical cancer, mammograms for breast cancer, and colorectal cancer screening, as well as Low-Dose CT scanning to detect lung cancers, can help with early detection and reduce mortality from these illnesses (Fig. 1) [7]. Although existing cancer screening methods are effective for certain types of cancers, they have limited sensitivity and specificity. To facilitate large-scale screening of healthy people at a lower cost, an improved approach is needed. Over the past few years, liquid biopsy has garnered considerable interest from scientists and organizations [8–11].

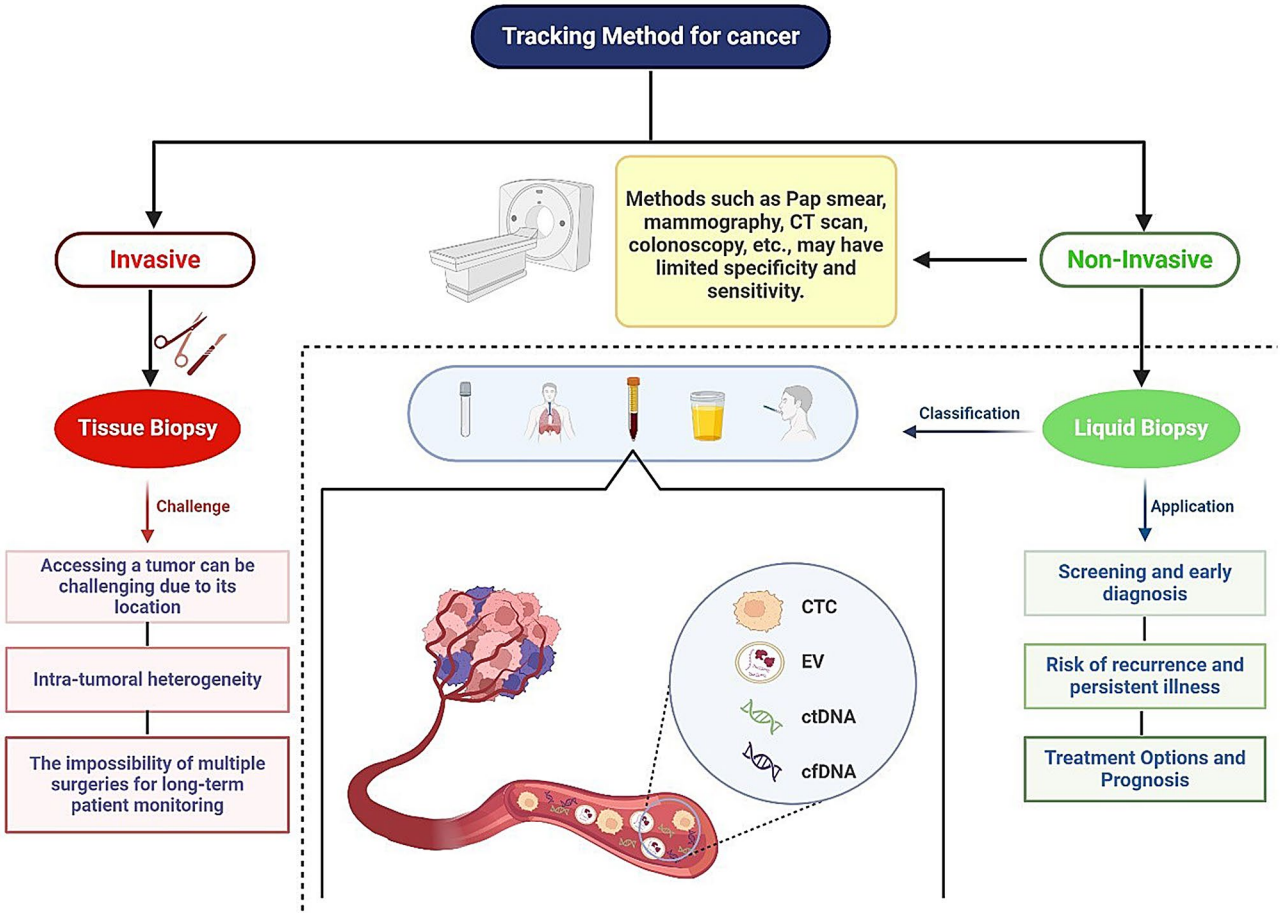


**Fig. 1** An outline of the differences between tissue and LBs, as well as the applications and classification of LB. Cancer diagnosis and staging can be approached through both invasive and non-invasive methodologies. The invasive technique, primarily tissue biopsy, presents several challenges, as illustrated in the accompanying figure. Conversely, non-invasive methods encompass various imaging modalities, which are often limited by issues related to sensitivity and specificity. However, another non-invasive approach, LB detection, offers distinct advantages, including reduced challenges and enhanced benefits. The central portion of the figure delineates the types of samples analyzed in LBs, as well as the various types of LB

LBs involve assessing both proteomic and genomic elements from tumor-derived substances like Circulating Tumor Cells (CTCs) released from primary or metastatic tumors, as well as circulating tumor DNA (ctDNA), circulating cell-free RNA (cfRNA) composed of small RNAs and micro-RNAs (miRNAs), tumor-derived extracellular vehicles (EVs) that contain membrane-bound nucleic acids and proteins and Tumor Educated Platelets (TEPs) are also found in the body. Collectively, these tumor-derived elements can provide invaluable longitudinal data and insights for pathologists to make more precise prognoses for primary and metastatic tumors [12].

LBs typically involve data such as mutations in DNA, transcriptome/proteome profiling, copy number alterations (CNAs) of crucial genes, metabolite profiling, and epigenetic modifications [13–16]. Research has embraced the use of bioinformatics tools to investigate disease patterns using LBs [17]. In less than five years, CRISPR-based diagnostics have evolved from an experimental nucleic acid sensing tool to a clinically relevant diagnostic technology for the fast, affordable, and ultrasensitive sensing of biomarkers. In this review, we outline how the properties of different Cas enzymes have been leveraged for diagnostic assays in cancer LB.

## Types of LBs

Initially, LB was used for CTCs. However, it has now been developed to identify the many components released by tumor cells that are present in body effluents mainly in blood, such as circulating cell-free nucleic acids (DNA, mRNA, miRNA, or long non-coding RNA (lncRNA)), TEPs, and exosomes, which are being studied for their potential use in cancer diagnosis (Fig. 1) [6]. LB technology has evolved tremendously over the years, making it common for cancer diagnosis. This remarkable progress has not only made the diagnostic process faster but also opened up vast research avenues that could help us understand the cause of malignant tumors. Through LBs, people have access to a highly efficient and minimally invasive approach to retrieve information concerning tumors through body fluids. Blood is the most commonly used fluid for this procedure, but many other body fluids can be biopsied as well [18].

### Circulating tumor cells (CTCs)

Cancer cells, known as CTCs, detach from the primary site of tumor growth and/or metastatic lesions. These cells circulate throughout the body in the bloodstream and can last for 1–2.4 h. Even in metastatic cancers, they are present in low abundance (<10 cells/mL of blood). These cells may manifest in the blood, as single cells or clusters, and the counts vary depending on the type of cancer [14, 19].

The main source of metastases is considered to be CTCs [20]. Metastatic cancer can be particularly difficult to detect and monitor due to the lower number of CTC – down to 1 CTC per $1\times10^9$ blood cells [21]. Fortunately, research has indicated that the presence of CTCs in one's blood can be used as an accurate prognostic biomarker, as it is highly associated with reduced progression-free and overall survival (OS) periods [20, 22]. Despite the advantages of CTCs, their use in clinical practice is not straightforward due to the variation in CTC counts among different types of tumors. Additionally, their properties such as size, different cell surface markers, and clustering ability make them difficult to isolate [23]. The only CTC quantification platform to have received approval from the US Food and Drug Administration (FDA) for metastatic breast, colorectal, and prostate cancers as of 2018 was the CELLSEARCH® CTC test. Circulating tumor cells (CTCs), which include the DNA, RNA, and proteins of malignancies, have the potential to provide important information. For instance, the AR-V7 CTC LB test has been clinically confirmed for accurate prediction and is the only one covered by Medicare [24].

CTCs have become an important tool in tumor detection, replacing traditional tissue biopsies because of their simplicity and ability to provide 'real-time' data regarding the tumor state. Research has revealed that CTC levels are a more accurate indicator of tumor conditions than the usual blood biomarkers [25, 26]. Additionally, CTCs have demonstrated encouraging outcomes in the early identification of numerous forms of cancer, including lung cancer, but only in a small subset of individuals with chronic obstructive pulmonary disease [27]. It is interesting to note that LBs utilizing CTCs have recently been demonstrated to distinguish between benign and malignant lung lesions [28]. Research indicates that CTC counts can act as an accurate gauge of treatment efficacy, with lower levels closely correlating with improved OS rates in a large cohort of breast cancer patients [29].

### Circulating cell-free DNA

A cancer biomarker called circulating cell-free DNA (ccfDNA) has been proposed, but only the study of the percentage that comes directly from the tumor (ctDNA) offers the required specificity and sensitivity. The levels of cell-free DNA (cfDNA) have been linked to a variety of pathological and non-pathological processes. Additionally, ctDNA found in body effluent is highly fragmented. With a blood half-life of 114 min [30], cancer can be dynamically monitored in real-time using this biomarker. Depending on the type of cancer, ctDNA has been found in different body fluids such as urine [30], cerebrospinal fluid (CSF) [31], pleural fluid [32], and saliva [33]. The amount of ctDNA detected was mostly related to the location of the primary tumor and metastases [14].

The concentration of cfDNA in patients with advanced lung and colorectal cancer [34] was found to be higher in patients with liver metastases. Studies have suggested that the amount of ctDNA increases in the Case of advanced cancers compared to localized tumors [35]. Moreover, ctDNA concentration and tumor load were correlated [36, 37].

An important barrier to the detection of cancer-specific genetic or epigenetic alterations in ctDNAs is the ability to reach acceptable levels of sensitivity and specificity. The detection of ctDNA has long been difficult because of low blood levels of ctDNA and dilution within ccfDNA. The detection of uncommon mutations and, more recently, methylated sequences has become possible owing to recent advancements in a Droplet-based Digital Polymerase Chain Reaction (ddPCR) and Next-Generation Sequencing (NGS) [2, 38]. It should be highlighted that ctDNA only makes up 0.1–10% of the entire amount of ccfDNA, which typically exists in plasma at levels between 10 and 100 ng/ml [39]. Additionally, known to increase cfDNA levels are physiological conditions such as exercise or inflammation, which are not always indicative of the presence of malignancy [40]. Moreover, tumor stage, tumor load, and therapy response affect plasma ctDNA levels [41]. The clinical use of ctDNA in precision medicine enables the investigation of ctDNA variants in plasma in addition to quantification. Recent research has revealed that ctDNA is shorter than the pool of circulating cfDNA, with data indicating that ctDNA fractions in cancer patients are 20–50 bp shorter than cfDNA [42].

#### Extracellular vesicles

Extracellular Vehicles (EVs) have garnered significant interest as an innovative modality of intercellular communication. Especially within the field of cancer, there is increasing evidence that EVs have a significant involvement in the spread of tumors [43]. This includes their contribution to various processes such as the creation of a premetastatic environment [44], promotion of angiogenesis [45], damage to the peritoneum [46] or blood-brain barrier [47], development of resistance to drugs [48], and the formation of heterogeneity cancer-associated fibroblasts [49]. Therefore, the use of EVs for intercellular communication within the tumor microenvironment has become a promising focus for innovative therapeutic approaches [50]. Moreover, EVs have been documented to transport numerous molecules, including proteins and nucleic acids, which mirror the characteristics of their originating cells. Additionally, EVs are present in various body fluids and can serve as valuable biomarkers for detecting diseases [51]. Many studies have found that EVs obtained from individuals with cancer are linked to the progression of the disease and can be detected even in the initial stages of cancer [52].

EVs can be generally classified according to their size and origin: exosomes (50–200 nm) [53], microvesicles (100–1,000 nm) [54], apoptotic bodies (50–4,000 nm) [55], and prostasomes (40–500 nm) [56]. Exosomes are formed when the endosomal membrane buds inward and are released when multivesicular bodies (MVBs) merge with the plasma membrane [57]. EVs can be consistently detected in different body fluids, including blood [58], saliva [59], urine [60], bronchoalveolar fluid [61], breast milk [62], and semen [63]. Additionally, they indicate the present condition of the disease by transporting certain substances, such as proteins, miRNAs, mRNAs, lncRNAs, and lipids, from the original cells. Hence, by focusing on the contents of EVs, we can evaluate essential molecular details regarding the condition of the disease. In addition, EVs can be collected sequentially due to the less intrusive sampling process and their convenient accessibility. For multiple reasons, EVs have significant potential as biomarkers that can be clinically valuable. They can offer non-invasive snapshots of disease status. EV-associated proteins and RNAs have been specifically documented as tumor biomarkers in the diagnosis of cancer or the monitoring of cancer progression.

miRNA is small non-coding, endogenous RNAs that range in size from 21 to 25 nucleotides. They circulate through the blood in the form of exosomes or TEPs, apoptotic bodies, protein-miRNA complexes, or apoptotic bodies and are the majority of RNA molecules that circulate in the blood. Under specific circumstances, they could control various cellular pathways and act as either tumor suppressors or oncogenes. miRNAs are highly stable (perhaps because they are shielded by protein and/or exosome complexes). They have been shown to play an essential function in therapy resistance and tumor development [64]. The profiles of blood miRNAs and solid tumors were correlated. Exosomal miRNAs have different amounts and compositions in cancer patients compared to those in healthy individuals, and these differences might serve as potential biomarkers for cancer [65]. miRNAs have also been suggested as possible predictive, prognostic, and diagnostic biomarkers for several malignancies [66, 67].

## Clinical applications of LB

### Screening and early diagnosis

The technique known as LB has emerged as a powerful tool for detecting and treating cancers at various stages. In particular, it has shown promise in identifying cancer patients among asymptomatic populations, facilitating earlier diagnosis, and more effective intervention. Indeed, growing evidence supports the potential of ctDNA as a useful tool for cancer screening. According

to certain cohort studies, ctDNA can be utilized to detect lung cancer in its early stages because it offers a great combination of sensitivity and specificity [68]. The first blood-based screening test for Colorectal Cancer (CRC) authorized by the US FDA uses SEPT9 gene methylation detection. Compared to protein markers, it has better specificity and sensitivity [69, 70].

#### Risk of recurrence and persistent illness

Despite successful treatment, many cancer patients are at risk of relapse, and timely detection of residual disease can be challenging with imaging or tissue biopsy methods. Currently, very few reliable markers are available. Recent research demonstrates that ctDNA-based diagnostics identify illness persist for several weeks sooner than radiological imaging, and patients who have positive ctDNA findings suffer negative effects (such as shorter disease-free survival and OS time) than those whose ctDNA is negative. These patients also have a higher risk of relapse [71, 72]. Patients with cancer may be categorized into various adjuvant therapies using the obtained profiles to avoid overtreatment [73].

#### Treatment options and prognosis

After being diagnosed with cancer, ctDNA sequencing has the potential to produce a molecular profile that is specific to the tumor of each patient. This profile can be used to help guide targeted therapy, which is an important component of precision medicine. This approach can help optimize cancer treatment and improve patient outcomes. The cfDNA has a half-life, ranging from 16 min to 2.5 h in blood circulation [41]. Because of this, ctDNA may be viewed as a "real-time" picture of the overall progression of lesions. By enabling long-term and continuous monitoring of treatment efficacy, ctDNA sequencing facilitates the ability to modify treatments as needed, leading to improved prognosis for patients. Additionally, ctDNA makes it possible to dynamically monitor clonal evolution and detect the appearance of resistant subclones [73]. Currently, both the European Medicines Agency and the FDA have approved the use of ctDNA for epidermal growth factor receptor (EGFR) mutation testing in Non-Small Cell Lung Cancer (NSCLC) patients to guide therapy decisions [74, 75]. Additionally, compared to other systemic treatments, several recently authorized immunotherapies are known to cause distinct tumor response patterns. It might not be prudent to continue with the standard approach of evaluating therapy effectiveness. It has been demonstrated that ctDNA is an early indicator of therapy success and can more accurately forecast survival outcomes for patients treated with immune checkpoint inhibitors for NSCLC (Non-Small Cell Lung Cancer) [76]. Accordingly, evaluation of the genetic and protein content of LB using new methods such as the CRISPR system opens a new window in the treatment process.

### CRISPR-Cas system

CRISPR, known as Clustered Regularly Interspaced Short Palindromic Repeats, is present in about half of bacterial genomes and 87% of archaeal genomes. It consists of an array of short DNA sequences that are interspaced with unique spacer sequences [77]. In 1987, scientists identified repeats of 29-nt fragments in the Escherichia coli genome that were separated by 32-nt fragments. In 2005, the CRISPR-Cas system was found to be part of an adaptive immune system in archaea and bacteria to protect them against the invasion of nucleic acids (Fig. 2) [78–82]. In 1987, Ishino and his colleagues were credited with being the first to uncover the CRISPR array [82]. This finding was made up of 14 repeats with a length of 29 bp with alternating non-repeating spacer sequences that ranged from 32 to 33 bp [83].

The CRISPR system consists of two parts that vary significantly between different microbial species [84, 85]. In an array, any duplicate elements are typically the same size and appear in the same order [85]. The scientists found multiple CRISPR typically match with DNA from phages and other extrachromosomal elements [81, 86–88]. When looking at the underrepresented areas of content research, 2,156 were examined in a separate study [87]. Also, there is a sequence right adjacent to the first repeat which contains up to 550 bp and is located 5' to most CRISPR loci [85, 88]. This common sequence has been denoted the 'leader' and is usually AT-rich [85]. The new repeat spacer unit is added to the CRISPR array between the leader and the previous unit. The leader could function as a recognition sequence for adding new spacers [89, 90]. The leader has many responsibilities, including acting as the promoter of the transcribed CRISPR array, which is found right upstream of the first repeat [91, 92].

There is a voluminous set of gene families associated with CRISPR arrays that have been shown in two recent studies [86, 93]. The CRISPR system includes several genes that are loosely associated with each other, including RAMPs (Repeat Associated Mysterious Protein) [86, 94]. Specific functional domains found in Cas proteins include exonuclease and endonuclease domains, RNA- and DNA-binding domains, helicases, and domains involved in transcription regulation [85, 86, 93, 95].

The CRISPR-Cas technology offers a high level of precision for target recognition in nucleic acids, as it can distinguish between single base mismatches [96–98]. CRISPR-Cas has been explored for various purposes, such as fundamental research, animal models, genome imaging in cells, clinical medicine, functional gene screening, generation, epigenetic control, and even as a

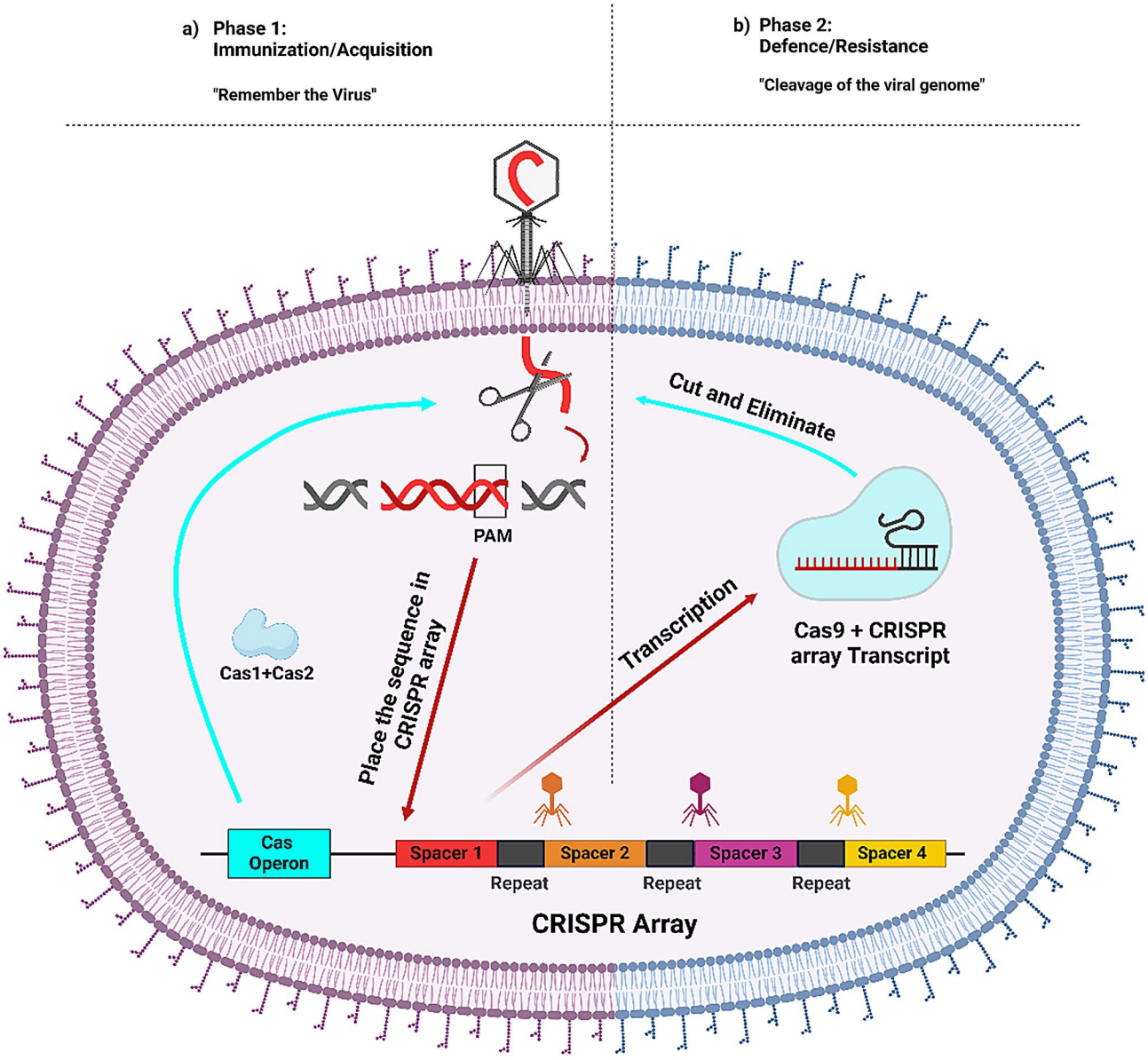


**Fig. 2** The mechanism of the CRISPR-Cas system in the bacterial/archaeal immune system in nature. **a**) In Phase 1, the identification of the virus (bacteriophage) by the bacteria/archaea occurs; where: first, the viral DNA enters the bacterial cell and is recognized and cleaved by the Cas1 and Cas2 complex. This process involves the transcription and translation of the Cas genes in the CRISPR-Array. Finally, a segment of the DNA, with a specific sequence at the end called PAM (Protospacer Adjacent Motif), is added to the end of the CRISPR-Array, so that in the event of a subsequent encounter (Phase 2) with the same virus, the immune system can more rapidly destroy the bacteriophage. **b**) Phase 2, which is the repeated encounter of that bacteria with the virus, involves the rapid transcription from the CRISPR-Array (contains a portion of the viral sequence), and the resulting RNA sequence, along with the Cas9 protein, identifies, cleaves, and destroys the virus. Figure created with BioRender

treatment for genetic diseases and in-vitro diagnostics [99–109].

### CRISPR-Cas classification

CRISPR-based methods for disease diagnosis have recently been gaining attention. The number of different CRISPR–Cas systems has expanded rapidly due to the strong success of CRISPR technology [110–112]. CRISPR-Cas systems can be divided into two different classes, six types, and several subtypes according to their evolutionary relationships [111]. The CRISPR-Cas system is given a broad distinction into two classes: Class 1 and Class 2. Class 2 is more complex than Class 1. Class 1 possesses multiple protein effector complexes, while Class 2 contains single protein effectors, which results in smaller effector complexes [113]. Of several CRISPR systems, class 2 systems hold the most ground in this practical field, especially in diagnosis, as these systems are

easier to reconstitute. Class 2 systems include enzymes with collateral activity, which stand as the backbone of some CRISPR-based diagnostic assays [114, 115].

Additionally, the type II CRISPR-Cas system contains three distinct Cas subtypes—Cas9, Cas12, and Cas13—for type II, type V, and type VI. Three subtypes are frequently utilized [114, 115]. Class 1 systems (such as the type III effector nuclease Cas10 or Csm6) have also been developed for diagnostic purposes, either alone or in conjunction with natural type III complexes or class 2 system components [114, 115]. In the following, the most widely used and important Cas proteins are described.

#### Cas9

Cas9, composed of the HNH catalytic domain and the RuvC catalytic domain, can recognize and cleave double-stranded DNA that contains the 5'-NGG-3' PAM (Protospacer Adjacent Motifs) sequence in the presence of single-guide RNA (sgRNA) by disrupting a phosphodiester bond. Following this, CRISPR RNA (crRNA) and trans-activating crRNA (tracrRNA) are used [116, 117]. CRISPR-Cas9 is a gene-editing system used in bacteria and archaea. Its components and working mechanisms differ from species to species [112, 118]. Here are some of the most commonly used Cas9 variants: SaCas9 [119] and SpCas9 [120]. The Streptococcus pyogenes Cas9 (SpCas9) was the first to be used outside prokaryotic cells [120] and reprogrammed for genome editing in mammalian cells [121]. SpCas9 is a type of nucleotide editor that relies on an included 20-nucleotide 'spacer' at the start and end of the sequence and a 'PAM' 5'NGG (N represents any nucleotide) [122]. Nishimasu et al. have discovered that SaCas9 (Staphylococcus aureus Cas9) is capable of recognizing the PAM sequences of NNGRRT (where R refers to A and G, and N refers to T, A, G, and C) in mammalian cells and exhibits exceptional cutting activity and targeting accuracy [123].

It is possible to generate DNA "nickases" using Cas9 nuclease by independently mutating its nuclease domains, which can introduce a single-strand cut with the same precision as a conventional CRISPR-Cas9 nuclease [124]. One notable distinction between the utilization of Cas9 nickases and the conventional CRISPR-Cas9 system is that when two Cas9 nickases are employed, they generate long overhangs at both ends of the cleaved DNA instead of blunt ends. This provides an even greater level of precision and control over the integration and insertion of genes [125, 126]. The CRISPR-Cas system can be utilized for various purposes by modifying it into a Dead Cas (dCas). dCas9 is created by introducing two mutations (D10A and H840A) in the cleavage domains of Cas9. Although dCas9 can still bind to DNA through sgRNA-mediated recognition, it is no longer capable of cleaving DNA. Various effector domains, including base editing domains, repressors, and transcriptional activators, can be fused to dCas9. When these derivatives are introduced into the cells, they can be guided by sgRNA to activate or repress gene expression or perform base editing at the targeted locus. Furthermore, dCas9 can be utilized for fluorescent imaging. Further details regarding the use the usage of these Cas9 derivatives will be discussed further down [127].

#### Cas12

Cas12a only contains the RuvC catalytic domain and can make the necessary bond with a specific double-strand (dsDNA) containing a 5' TT TN-3' PAM sequence to degrade it [128]. When the dsDNA is cleaved, Cas12a will initiate a collateral cleavage activity. It triggered Cas12a to cleave nonspecifically and single-strand DNA (ssDNA) can amplify the signal [129]. Additionally, CRISPR-Cas12a's reporter ssDNA cleavage activity can also be activated by ssDNA targets, expanding the biosensing application [97, 130]. The two important types of Cas12 are Cas12a and Cas12b. Cpf1, or Cas12a, is similar to CRISPR-Cas9 in that it can generate targeted DSB. It seems like Cpf1 could be useful in the future of genome editing [130]. Although, Cas12a only needs guide RNA to accurately target a site on DNA with its enzyme that requires a T-rich PAM for targeting to detect and cleave at the PAM-distal site on DNA [131]. Cas12a enzymes have been shown to have utility for combinatorial library screens [132], gene activation [133, 134], as well as for multiplex gene editing [135].

The cleavage activity of CRISPR-Cas12a can greatly enhance detection sensitivity, making it a valuable tool. When a Cas12a guide RNA (gRNA) complex binds to a target DNA sequence, it triggers the cleavage of a quenched fluorescent probe. This enzymatic signal amplification leads to even more accurate and sensitive detection capabilities that can greatly improve the identification of target sequences with low concentrations [136, 137]. Exploring beyond the widely utilized SpCas9, researchers are delving into the study of alternative CRISPR nucleases, such as Cas12a orthologs [131, 138, 139]. Cas12a nucleases, such as the Lachnospiraceae bacterium ND2006 and Cas12a (LbCas12a) AsCas12a, have a remarkable ability to identify target sites that contain T-rich PAMs [131, 140]. Although Cas12a orthologs from Moraxella bovoculi 237 (MbCas12a) and Francisella novicida (FnCas12a) were previously reported to recognize an increased number of PAMs in vitro [131].

Chen et al. developed an innovative technique, known as the DNA Endonuclease Targeted CRISPR Trans Reporter (DETECTR), which involves the integration of isothermal amplification of recombinase polymerase with LbCas12a. This results in nucleic acid detection with attomolar sensitivity. They demonstrate the rapid

and specific detection of human papillomavirus (HPV) in human patient samples using DETECTR, thereby establishing a more efficient platform for nucleic acid-based disease detection [130]. Research has revealed that the Dead Cas12a protein from Eubacterium eligens (EedCas12a) is more effective at repressing genes on the target DNA template strand than the Dead Cas9 protein from Streptococcus pyogenes (SpdCas9) [141].

#### Cas13

The class II type-VI CRISPR-Cas13 system is beneficial for RNA editing in eukaryotic cells [142, 143]. Guided by a single CRISPR RNA, Cas13 (C2c2) can be programmed to specifically cleave single-stranded RNA targets containing complementary protospacers [144]. Through improvements made to the Cas13 effector, the RNA Editing for Programmable Adenosine to Inosine Replacement (REPAIR) system has been developed, enabling the editing of full-length transcripts that contain pathogenic mutations [145]. In terms of functionality, the CRISPR effector Cas13 exhibits potent antiviral activity against single-stranded RNA (ssRNA) viruses [146]. In addition, Cas13 is employed as a versatile tool for targeted virus knockdown and investigation of viral replication, localization, and evolution. Notably, the catalytically dead variant of Cas13 (dCas13) can be utilized to study viral RNA localization, while dCas13 fusion proteins possessing RNA editing capabilities can be employed to functionally characterize specific viral polymorphisms [138, 147].

In contrast to CRISPR-Cas9, which operates at the DNA level, the discovery of a new CRISPR-Cas system effector protein in 2016, known as c2c2 (now named Cas13a), revealed its ability to specifically bind and cleave RNA with the aid of crRNA guidance. Cas13a can also regulate gene activity at the RNA level [143]. It has been demonstrated that CRISPR-Cas13 systems primarily possess RNA-cleaving capabilities and lack DNase (Deoxyribonuclease) activity. This feature provides unique targeting advantages for RNA, making it an attractive tool for RNA-specific applications [148]. In addition, they are unique among CRISPR-Cas systems in that they lack the third component, tracrRNA [141]. The absence of tracrRNA in CRISPR-Cas13 systems created a new avenue for targeted RNA editing. Since its inception, Cas13 has found widespread use in various fields. For instance, in 2019, Zhang Feng's research group developed a new CRISPR-Cas13 system called CARVER (Cas13-assisted Restriction of Viral Expression and Readout), which combines the antiviral activity and diagnostic potential of Cas13. This innovative system holds promise for the diagnosis and treatment of viral infections [146]. Cas13a is distinct from Cas9 and Cas12a in that it contains two higher eukaryotes and prokaryotes nucleotide-binding (HEPN) domains. The CRISPR-Cas13a system utilizes cis-cleavage to target RNA containing the protospacer flanking site (PFS) sequence, followed by trans-cleavage of the reporter ssRNA [149, 150].

In addition to target-specific binding and cleavage, some Cas proteins, such as Cas12a and Cas13a, have been reported to possess trans-cleavage activity. This activity involves the cleavage of surrounding single-stranded DNA or RNA upon the binding of the Cas-gRNA complex to the target [151]. The class II CRISPR-Cas system, specifically CRISPR-Cas12a and CRISPR-Cas13a, offers unique advantages for in vitro diagnostics, including high detection sensitivity and specificity, as well as the potential for developing point-of-care diagnostic technology. When incorporated with isothermal nucleic acid amplification methods, CRISPR-Cas-based nucleic acid amplification strategies exhibit a high detection sensitivity comparable to that of PCR (Polymerase Chain Reaction) [118, 152]. The Cas13a protein variant, dCas13a, can bind to specific RNA molecules and has been employed as a tool for visualizing RNA in living cells [153].

#### Cas14

In recent years, scientists have identified a new kind of Cas protein called Cas14. These proteins are relatively small, typically composed of 400–700 amino acids, and are part of the CRISPR-Cas type V family of systems [154]. Researchers have found that Cas14 proteins can break down single-stranded DNA without needing PAM, which is different from other types of Cas proteins. Furthermore, some Cas12 and Cas14 proteins have collateral activity, which involves non-specific destruction of any adjacent DNA [110]. Cas14 is relatively smaller in size compared to previously identified class 2 CRISPR RNA-guided enzymes. They consist of about 400–700 amino acids, which is approximately half the length of the larger class 2 enzymes that range between 950 and 1400 amino acids. Despite the significant sequence diversity among the identified Cas14 proteins, they all possess a predicted RuvC nuclease domain, which is a typical characteristic of Type V CRISPR-Cas enzymes that target DNA [138, 139, 155]. When examining the evolutionary history of Cas14 proteins, scientists have noted that they are remarkably diverse and display similarities with two bacterial protein families, C2c10 and C2c9. These protein families contain RuvC domains and are sometimes located near a CRISPR array, although not always in conjunction with other Cas genes [139]. By analyzing the sequences of various Cas14 genes, researchers have identified 24 different variants that can be divided into three subgroups (Cas14a-c) based on shared sequence characteristics [154]. In Fig. 3, you can observe the diverse varieties of Cas proteins that exhibit the greatest diagnostic utility for cancer detection, as described previously.

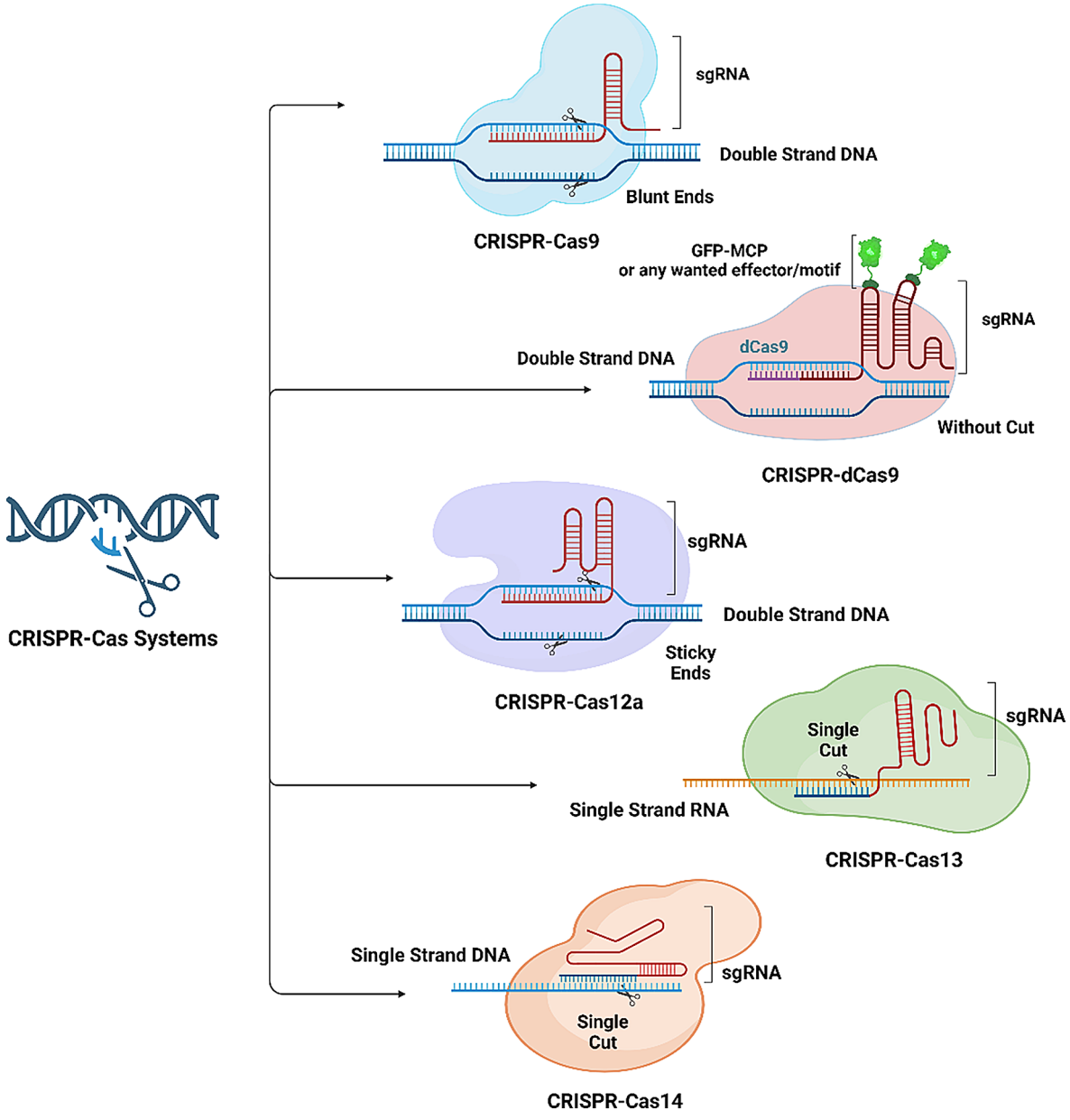


**Fig. 3** The various types of CRISPR-Cas systems that have the most widespread applications in detection (and are mentioned in this study): Generally, each system has a Cas protein and a sgRNA. **a**) CRISPR-Cas9: (1) Recognition of double-stranded DNA. (2) Blunt-end cleavage of both strands. **b**) CRISPR-dCas9: (1) Recognition of double-stranded DNA. (2) No cleavage of both strands (inactivation of nuclease domains) and has the ability to add other factors such as Transcription activators, Transcription Inhibitors, fluorescent tags, and more. **c**) CRISPR-Cas12a: (1) Recognition of double-stranded DNA. (2) Sticky-end cleavage of both strands. **d**) CRISPR-Cas13: (1) Recognition of single-stranded RNA. (2) Cleavage of single-strands. **e**) CRISPR-Cas14: (1) Recognition of single-stranded DNA. (2) Cleavage of single-strands. Figure created with BioRender

## Application of CRISPR-Cas in the diagnosis of LB in cancers

Accurate and prompt diagnosis of a disease is imperative for effective treatment and to mitigate any potential long-term consequences [156]. Nucleic-acid-based biomarkers associated with disease are essential for diagnostics because DNA and RNA can be amplified from trace amounts, which enables their highly specific detection via the pairing of complementary nucleotides. Nucleic-acid-based diagnostics have become the go-to choice for the diagnosis of several acute and chronic medical conditions, mainly those caused by infections [157].

The CRISPR-Cas system is becoming an important part of diagnostics due to its ability to identify specific genes accurately and quickly. Cas enzymes, when combined with guide RNA, can effectively target and cleave DNA or RNA molecules, making it an extremely powerful tool for

**Table 1** Summary of characteristics of the different CRISPR-Cas systems

| | Cas9 | Cas12 | Cas13 | Cas14 |
|---|---|---|---|---|
| **Type** | RNA-guided endonuclease | RNA-guided endonuclease | RNA-guided RNase | RNA-guided endonuclease |
| **Target** | DNA | DNA | RNA | RNA |
| **PAM Sequence** | NGG | TTTV | Varies by subtype | Varies by subtype |
| **Cleavage Mechanism** | Double-strand break | Double-strand break | Single-strand cleavage | Double-strand break |

genetic engineering. This has resulted in its widespread application in the field. CRISPR-Cas can carry out different activities due to its target-oriented binding, making it ideal for creating diagnostic techniques to identify disease-related genes, miRNAs, and genetic variations such as Single Nucleotide Polymorphisms (SNP) and DNA methylation [158]. Accurately detecting nucleic acid biomarkers is of utmost importance for monitoring agricultural and food safety, environmental protection, and identifying biological warfare agents. In the subsequent text, we will elucidate the ongoing advancements in employing CRISPR-Cas systems for more precise and efficient cancer diagnostics through LBs. Table 1 presents a summary of the differences among various types of CRISPR-Cas.

The CRISPR-Cas9 technology has a wide range of applications in research, development, and diagnostics. Its primary function is as a genetic tool for modifying target genes (DNA/RNA) in a host, but it also has multiple roles in genome library screening, transcriptional regulation (repression /activation), epigenetic modification, live cell imaging, and therapeutic applications [159]. Table 2 presents a comprehensive overview of research conducted on the application of CRISPR-Cas9 and CRISPR-dCas9 technology in the identification of cancer biomarkers through LB, with a focus on enhancing cancer diagnostic sensitivity. At first, investigations were carried out regarding the detection of cfDNA. A study focused on Ovarian Clear Cell Carcinoma (OCCC) aimed to identify mutations in the PIK3CA and KRAS genes present in cfDNA patients using ddPCR. In this particular study, the CRISPR-Cas9 system was employed to target and cleave the wild-type PIK3CA, resulting in an enhanced sensitivity of ddPCR in detecting cfDNA harboring the PIK3CA-H1047R mutation [160] (Fig. 4-A).

A similar approach was used to the previous study in a separate study focusing on NSCLC, specifically examining gene EGFR mutations, by utilizing the CRISPR-Cas9 system to selectively eliminate wild-type fragments, the researchers successfully identified mutant fragments even at low concentrations using Sanger sequencing and NGS. It is worth noting that biomarker concentrations

**Table 2** Studies that have employed the CRISPR-Cas9 and CRISPR-dCas9 systems for the detection of various cancers

| Type of LB | Type of cancer | Type of CRISPR-Cas System | Effect | Year | Reference |
|---|---|---|---|---|---|
| cfDNA | OCCC | Cas9 | Improve the sensitivity of the ddPCR method | 2018 | Morikava [160] |
| | NSCLC | Cas9 | The results showed that after CRISPR-Cas9 enrichment, a low concentration of mutant DNA fragments (0.01%) could be detected by Sanger sequencing, which represented a 1000-fold increase compared with the untreated samples. | 2020 | Li Wang [161] |
| | NSCLC | Cas9 | CRISPR-CPPC aided detection of T790M with 93.9% sensitivity and 100% specificity. T790M mutant copies were sensitively detected achieving an approximately 13-fold increase in the detected allele frequency. | 2022 | Boyeon Kim [164] |
| | Colorectal cancer, Pancreatic cancer, and Lung cancer | Cas9 | Reduce the cost and time of cancer screening and genotyping, and enable targeted therapies in resource-limited settings. | 2022 | Junman Chen [165] |
| | NSCLC | dCas9 | Enriching minor alleles by use of dCas9 protein and immunomagnetic separation | 2018 | Amin Aalipour [167] |
| miRNA | cultured cancer cells and NSCLC | Cas9 | RACE is a powerful tool for multiplexed, specific detection of nucleic acids in point-of-care diagnostics and field-deployable analysis. | 2020 | Ruixuan Wang [162] |
| | HeLa cell and CHO-K1cell | Cas9 | The sensitivity of this method was determined to be 23 fM | 2020 | Yang Liu [163] |
| ctDNA | PDAC | Cas9 | PASEA technology provides a reliable, cost-effective, and minimally invasive method for detecting ctDNA of PDAC. | 2023 | Yue Shen [166] |
| | Breast Cancer | dCas9 | "Use of CRISPR/Cas technology as a label-free tool that can be used in an impedimetric system for the detection of ctDNA's" | 2020 | Zihni Onur Uygun [168] |

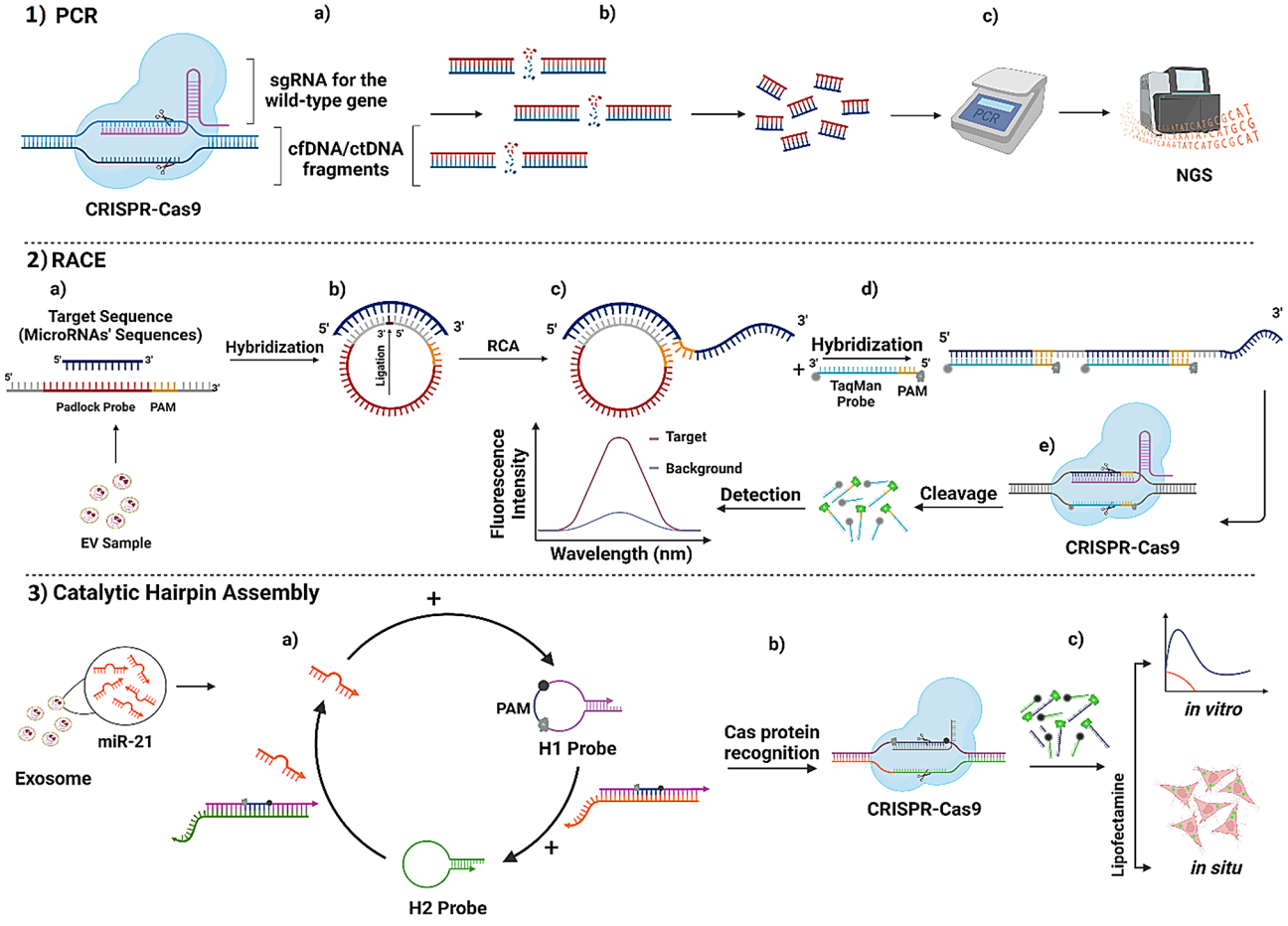


**Fig. 4** The methods that have used the CRISPR-Cas9 system for diagnostic applications in various studies: 1) In these two studies, the CRISPR-Cas9 system was used to identify mutated genes (the first study: PIK3CA and KRAS genes in patient cfDNA samples, and the second study: EGFR gene in patient ctDNA samples): **a**) sgRNAs were designed to target the wild-type sequences of these genes. **b**) The Cas9 protein recognized and cleaved these gene sequences. **c**) PCR was performed to amplify the mutated gene fragments (wild-type samples could not be amplified due to fragmentation). Finally, they were analyzed by NGS (in the second study). 2) In this study, miRNA samples in EVs were amplified using the Rolling Circle method with a designed probe, and then labeled with a fluorescent probe for detection. **a**) The EV sample containing miRNA from the patient was selected, and a Padlock probe complementary to the miRNAs containing the PAM sequence was designed. **b**) Ligation occurred at the two ends of the probe, making it circular. **c**) The complementary strand of the probe (which is the miRNA sequence) was amplified using the Rolling Circle method. **d**) Another probe (Taqman probe, containing the PAM sequence) labeled with quenched fluorescence hybridized with the amplified sample. **e**) The formed complex was recognized and cleaved by the Cas9 protein, which activated the fluorescence signal for evaluation. 3) In this study, miR-21 in the patient's exosome sample was detected using two probes and the Cas9 protein. **a**) Two probes were designed: H1, which is complementary to miR-21, contains the PAM sequence, and has quenched fluorescence; and H2, which is complementary to H1. Both probes are in a hairpin structure and become single-stranded upon encountering miR-21. **b**) The Cas9 protein recognized the H1-H2 complex and cleaved it, activating the fluorescence signal. **c**) The emitted fluorescence was analyzed. The system was also introduced into HeLa and CHO-K1 cells using Lipofectamine, and the fluorescence from the cells was examined. Figure created with BioRender

are typically quite low in the early stages of cancer, underscoring the significance of developing methods to enhance the diagnostic sensitivity of these biomarkers at such low concentrations [161] (Fig. 4-A).

Two distinct methods leveraging CRISPR-Cas9 have been developed for the detection of miRNA. Rolling Circular Amplification (RCA) is a nifty isothermal technique used to amplify circular DNA molecules. Instead of needing to cycle through different temperatures like in PCR, RCA operates at a constant temperature. This method can generate long single-stranded DNA or RNA molecules, making it super useful for various applications, including diagnostics, pathogen detection, and even as a tool in CRISPR techniques. The first method, known as RACE (Rolling Circular Amplification-assisted CRISPR-Cas9 cleavage), involves the utilization of RCA to assist in the cleavage process by CRISPR-Cas9.

[162] (Fig. 4-B).

Catalytic Hairpin Assembly (CHA) is a fascinating nucleic acid amplification technique that leverages the hybridization of two DNA hairpins. This process is catalyzed by a specific DNA input, leading to signal amplification without the need for enzymes. CHA is widely used for detecting nucleic acids and small molecules due

to its simplicity, isothermal conditions, and powerful amplification capabilities. The second method combines CHA with CRISPR-Cas9, aiming to enhance the diagnostic sensitivity of miRNA detection. This integration of CHA with CRISPR-Cas9 offers improved capabilities for detecting miRNA molecules with higher sensitivity and precision [163] (Fig. 4-C).

Boyeon Kim et al. introduced a novel approach named CRISPR-CPPC (CRISPR system combined with post PCR) cfDNA in their research. This method was developed to detect the T790M mutation in NSCLC patients, leveraging the CRISPR-Cas9 system. By integrating it with post-PCR analysis of cfDNA, the diagnostic sensitivity and specificity of the ddPCR method were significantly enhanced [164]. PASEA (Programmable Enzyme Assisted Selective Exponential Amplification) is an innovative technique that combines CRISPR-Cas9 with isothermal polymerase amplification to selectively enrich and detect rare mutant alleles while simultaneously eliminating wild-type alleles. Furthermore, another research group successfully employed the method above to identify KRAS ctDNA mutations in the plasma of pancreatic ductal adenocarcinoma (PDAC) patients. As a result, it enables the detection of mutant alleles even at low concentrations within a short timeframe of 20 min. This method not only facilitates rapid genotyping but also proves to be cost-effective in the process [165, 166] (Fig. 5).

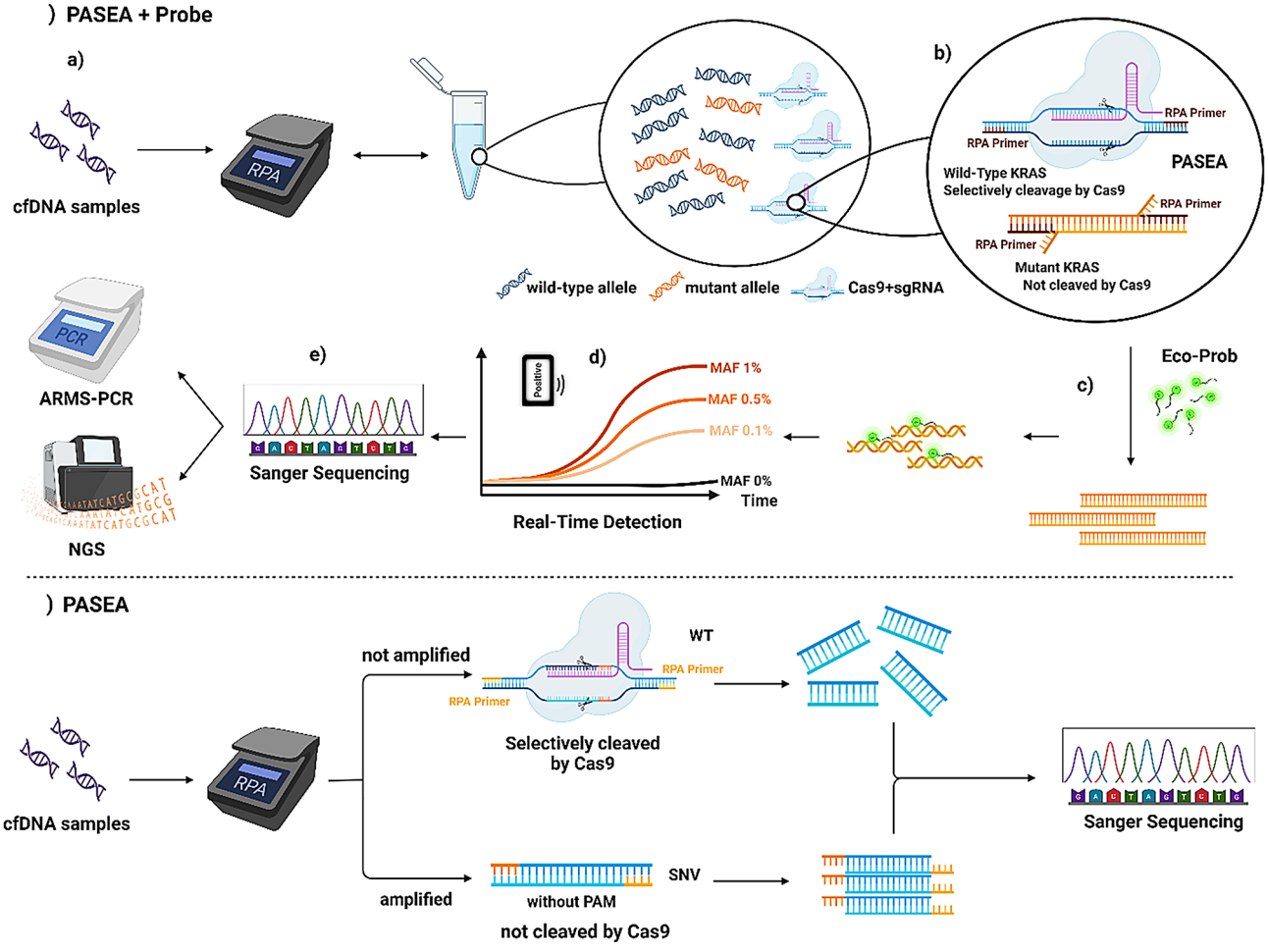


**Fig. 5** The studies that have been reported in two publications utilized the PASEA method to enhance the abundance of the mutant gene and eliminate the wild-type gene: 1) In this study, the CRISPR-Cas9 system was used to achieve more robust detection of KRAS gene mutations from patient cfDNA samples using the PASEA method: **a**) First, the patient cfDNA sample in plasma, along with the Cas9 protein and sgRNA designed for the wild-type KRAS gene, was amplified using RPA (Recombinase Polymerase Amplification). **b**) The wild-type KRAS gene was cleaved and fragmented by Cas9, while the mutant sequences remained intact. **c**) Fluorescently-labelled probes complementary to the uncleaved, mutant strands were used to label the mutant sequences. **d**) The emitted fluorescent signals were then quantified by real-time PCR. **e**) Sanger sequencing was performed to confirm the mutations, followed by ARMS-PCR and NGS. 2) In this study, the PASEA method was used to enrich the mutant KRAS gene fraction in the patient cfDNA sample for better tracking and analysis. The RPA-prepared sample contained the patient cfDNA and Cas9 with sgRNA designed for the wild-type KRAS gene. During RPA, the wild-type KRAS sequences were recognized and cleaved by Cas9. As a result, only the mutant samples were amplified, which were then analyzed by Sanger sequencing to identify the mutations. Figure created with BioRender

CRISPR-dCas9 technology can be utilized to identify biomarkers for cancer diagnosis, particularly when their abundance is low. In the context of NSCLC, a study demonstrated that the diagnostic potential of DNA was enhanced through the application of dCas9 [167] (Fig. 6-A). Furthermore, Zihni Onur Uygun et al., in their study conducted in 2020, developed an impedimetric biosensor powered by CRISPR-dCas9. This innovative biosensor enabled label-free detection of circulating tumor DNAs, offering a promising approach for sensitive and specific detection of cancer biomarkers [168] (Fig. 6-B). Thus, these studies demonstrate that by fine-tuning the conditions for employing CRISPR-Cas9 to detect and identify genetic mutations through LBs from various cancer samples, significant advancements can be achieved with high sensitivity and in a short timeframe.

## CRISPR-Cas12a

The CRISPR-Cas12a system has shown significant development in cancer diagnosis through LBs. Various techniques have been developed based on the application and capabilities of Cas12a (Table 3).

### *Some basic CRISPR-Cas12a diagnostic mechanisms*

Ning Shao et al. introduced a novel method that combines a Magnet-Assisted V-chip (MAV-chip), Platinum Nanoparticles (PtNP) reporter, and CRISPR-Cas12a to accurately detect the quantity of SNVs (Single Nucleotide Variant) and SNPs. This method was tested to investigate single nucleotide variation (SNVs) related to liver cancer, successfully identifying the target SNVs (Fig. 7-a) [169]. In another study, a CRISPR-Cas12a-based nucleic acid amplification-free fluorescent biosensor was developed to detect ccfDNA in breast cancer. This biosensor utilized Metal-Enhanced Fluorescence (MEF) using

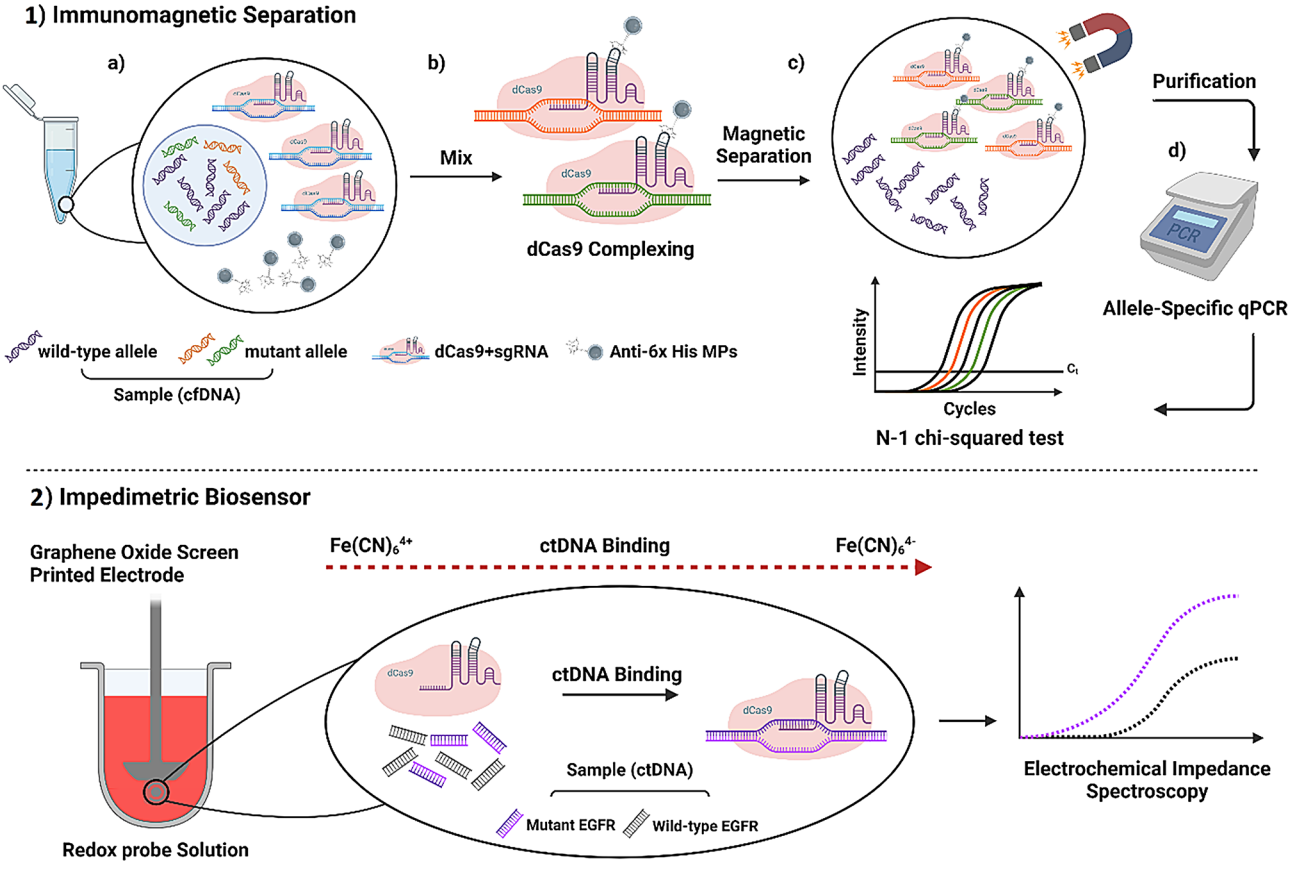


**Fig. 6** The methods that have used the CRISPR-dCas9 system for diagnostic applications in various studies: 1) In this study, magnetic particles (Anti-6x-His) were used to separate the wild-type (bound to dCas9) and mutant samples. **a**) In a microtubeeto, dCas9 (sgRNA: EGFR WT) complementary to magnetic particles and the patient's cfDNA sample containing a mixture of wild-type and mutant EGFR genes were combined. **b**) After mixing, the magnetic particles bound to the dCas9 and paired with the wild-type EGFR. **c**) Using a magnet, the complex formed in "b" was used to separate the wild-type samples from the mutant ones. **d**) Allele-specific qPCR was then performed, and the results were analyzed using the N-1 chi-squared test. 2) In this study, a biosensor was designed using dCas9 and an electrode made with nanoparticles to quantify the frequency of cancer-related mutant samples. When the mutant ctDNA sample (in this case, the PIK3CA gene) bound to the dCas9 (with sgRNA designed to target the mutant sequence), the $Fe(CN)_6^{3-}$ groups were converted to $Fe(CN)_6^{4-}$, and the extent of this conversion (quantifying the mutant genes) was measured using Electrochemical Impedance Spectroscopy. Figure created with BioRender

**Table 3** Research studies that have utilized the CRISPR-Cas12a technologies for the detection of different types of cancers

| Type of cancer | Type of LB | Results | Year | References |
|---|---|---|---|---|
| Lung cancer | cfDNA | This preliminary research shows that the CRISPR-Cas12a system has the potential to quickly and accurately identify *EGFR* mutations in the bloodstream | 2020 | Jen-Hui Tsou [171] |
| | cfDNA | CASMART is a single-step CRISPR Cas12a-based isothermal amplification technique that enables rapid and high-precision digital detection of rare mutant alleles | 2023 | Chanqiong Zhang [175] |
| | Exosome | This method offers a highly sensitive and specific way to detect exosomes, which could lead to future exosome-based diagnostic applications for diseases | 2020 | Xianxian Zhao [176] |
| | Exosome | The described method exhibits high sensitivity and specificity for the electrochemical detection of EV PD-L1, with the ability to quantify exosome concentrations across a wide dynamic range down to a low detection limit | 2022 | Lingjun Sha [180] |
| | EV | The method for EV PD-L1 measurement could be a useful tool that provides valuable information to support the precise diagnosis and personalized treatment of lung cancer patients | 2023 | Bing Bo [181] |
| | Exosomal miRNA-21 | The optimized fluorescence biosensor showed excellent sensitivity and specificity for the quantification of exosomal miR-21, with a wide linear range from 10 to 100,000 fM and a very low detection limit of around 0.89 fM | 2022 | Qing Liu [182] |
| Liver cancer | cfDNA | It allows for the detection and quantification of multiple cancer-related genetic mutations in both pure DNA samples and cell-free DNA samples from blood serum, even when the mutant alleles are present at very low frequencies down to 0.01% | 2019 | Ning Shao [169] |
| Breast cancer | cfDNA | This system allows for the detection of *BRCA-1* with extremely high sensitivity within a 30-minute timeframe | 2020 | Jin-Ha Choi [170] |
| | Exosomal miRNA-155 | This biosensor could effectively discriminate breast cancer patients by analyzing exosomal miR-155, and its results were in agreement with the standard qRT-PCR technique | 2023 | Bo Shen [183] |
| NPC | TEV | The described platform was simple and easy to use, and this approach appears useful for the sensitive and versatile quantification of TEV proteins in clinical samples | 2020 | Shan Xing [173] |
| | | The described aptamer-CRISPR-Cas12a assay can sensitively and conveniently detect very low concentrations of specific TEVs in serum, which could be useful for NPC diagnosis and prognosis | 2021 | Huilan Li [174] |
| UM | ctDNA | The method, when coupled with allele-specific PCR, provides a sensitive platform for LB detection that can sense the *GNAQ Q209P* mutation in plasma samples even when the ctDNA concentration and fractional abundance are low. | 2023 | Carmen Escalona-Noguero [172] |
| Pancreatic cancer | EV | This method showed high sensitivity and selectivity in identifying low amounts of miR-1290 in serum extracellular vesicles, enabling it to effectively distinguish early-stage pancreatic cancer patients from healthy controls, indicating its strong potential for clinical applications | 2024 | Tenghua Zhang [177] |
| Colorectal cancer | ncRNAs | The BRCACas platform, which is a one-step, isothermal, and highly specific approach, exhibited excellent performance, highlighting its promise as a rapid, adaptable, and practical tool for cancer screening, diagnosis, and prognosis | 2023 | Hui Chen [178] |
| Papillary thyroid carcinoma | ctDNA | This approach offers not just a platform for the fast detection of circulating tumor DNA, but also shows strong potential for early clinical diagnosis and biomedical research purposes | 2022 | Wenxiu Zhang [179] |

DNA-functionalized gold nanoparticles (AuNP). Upon activation of the CRISPR-Cas12a complex by the target cfDNA, MEF occurred, resulting in color changes that enabled the detection of breast cancer gene-1 (BRCA-1) with high sensitivity within 30 min (Fig. 7-b) [170]. A study compared the sensitivity and diagnostic power of CRISPR-Cas12a with ddPCR for detecting EGFR mutations in lung cancer. The Cas12a system was used to amplify the target DNA through PCR and then exposed to Cas12a, which was attached to gRNA and a ssDNA Fluorophore-Quencher (FQ) reporter. Cas12a activation led to the cleavage of the reporter DNA, activating the fluorophore and generating a signal. This method demonstrated higher sensitivity and faster results compared to ddPCR. In addition, allele-specific PCR and CRISPR-Cas12a are utilized in two studies; however, they focused on detecting the GNAQ Q209P mutation, which is associated with uveal melanoma (UM) (Fig. 7-c) [171, 172].

#### *The methods for detecting NPC-associated TEV based on CRISPR-Cas12a*

Shan Xing and his colleagues have devised two distinct approaches utilizing CRISPR-Cas12a for the detection of TEVs in nasopharyngeal carcinoma cancer (NPC). In their initial study conducted in 2020, they developed the apta-HCR-CRISPR assay, which involved a dual amplification strategy incorporating Hybridization Chain Reaction (HCR) and CRISPR-Cas12a. By employing

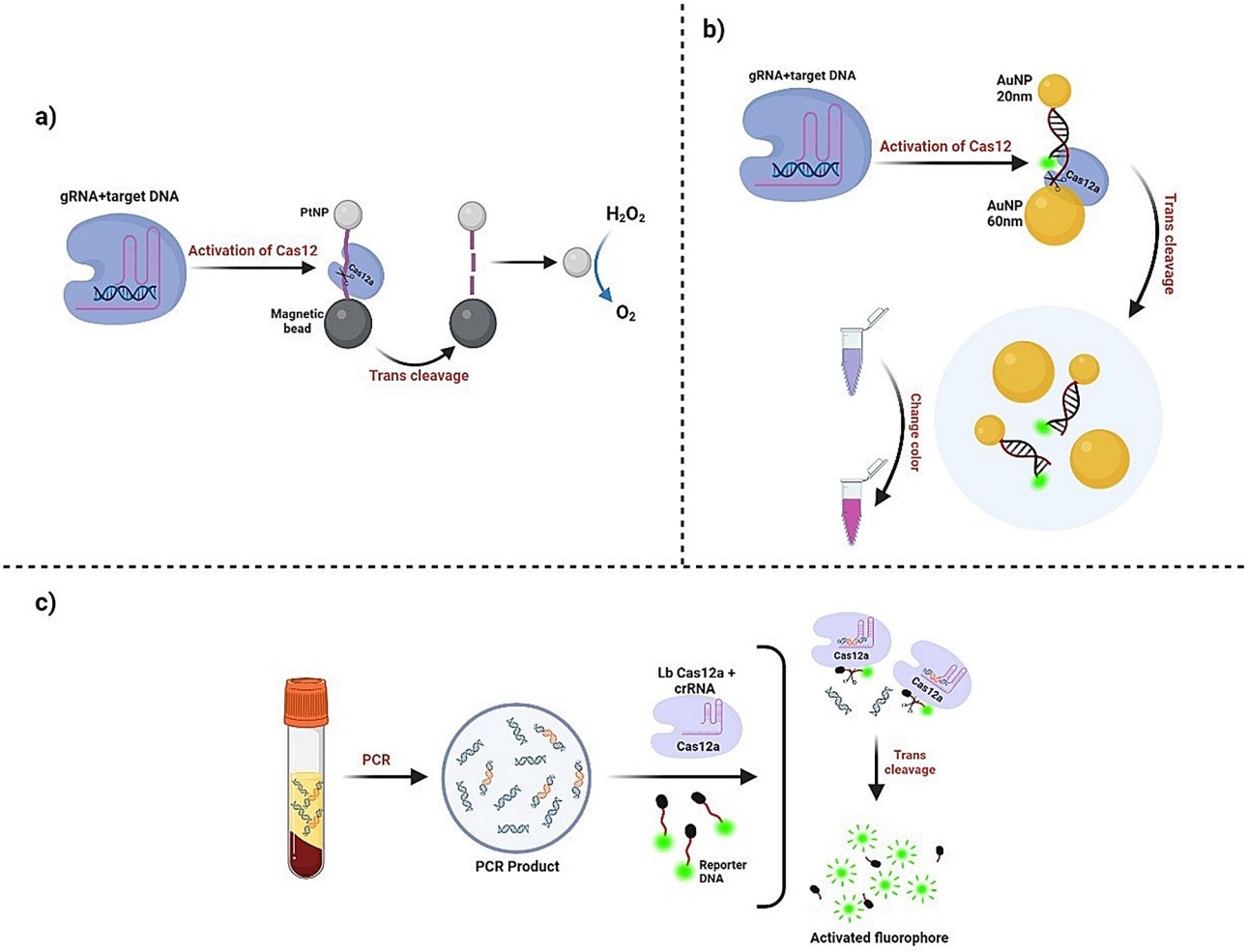


**Fig. 7** Some basic CRISPR-Cas12a diagnostic mechanisms. (**a**) PtNPs are linked to a streptavidin-coated magnetic bead via ssDNA that has been altered to have thiol and biotin groups on opposite ends. Specific guide RNA attaches to its target DNA and initiates the trans-cleavage of Cas12a when the CRISPR-Cas12a system is introduced to this complex. The ssDNA linker that connects the magnetic bead and PtNP is broken by activated Cas12a. The released PtNPs are then transported to catalyze the production of oxygen by H2O2, after being magnetically separated from the magnetic beads throughout the reaction. A reaction's target DNA content can be determined by measuring the quantity of oxygen that is generated. (**b**) To induce MEF with target DNA and fluorescence quenching without target DNA, two different-sized AuNP pairs (20 and 60 nm) were inserted, coupled by a 7 nm long dsDNA and a 2 nm long ssDNA. Because of its close vicinity to the 60-AuNPs, fluorescein isothiocyanate (FITC) remained in a quenching condition when it was linked to one of the ends of the dsDNA that was not coupled to the 60-AuNPs. The active CRISPR-Cas12a complex cleaved the ssDNA between the 20- and 60-AuNPs if target cfDNA was present, resulting in MEF and color changes from purple to red-purple. (**c**) For simple PCR amplification, cultured cell supernatant or plasma was prepared. A combination of LbCas12a, crRNA, and ssDNA FQ reporter made up the PCR product. The reaction was carried out on a plate reader for fluorescence readout for up to two hours at room temperature. Figure created with BioRender

HCR, they amplified TEV protein-targeted aptamers, generating a lengthy repetitive sequence containing multiple crRNA-targetable barcodes. Subsequently, CRISPR-Cas12a was employed to further amplify the signals through collateral cleavage activities, ultimately resulting in the generation of a detectable fluorescence signal. In a subsequent study carried out in 2021, the researchers introduced a novel technique known as the aptamer-CRISPR-Cas12a assay. This method combined aptamers specifically binding to protein targets on TEVs, PCR-based exponential amplification, and real-time DNA detection through CRISPR-Cas12a. By integrating these components, the assay demonstrated the capability to rapidly and conveniently detect extremely low concentrations of CD109+and EGFR+TEVs directly in serum, thus holding potential utility in the diagnosis and prognosis of NPC. These investigations by Shan Xing his team present innovative CRISPR-Cas12a-based methodologies that offer sensitivity and specificity in detecting TEVs associated with nasopharyngeal carcinoma cancer. The findings hold promise for applications in cancer diagnosis and monitoring, particularly in NPC (Fig. 8) [173, 174].

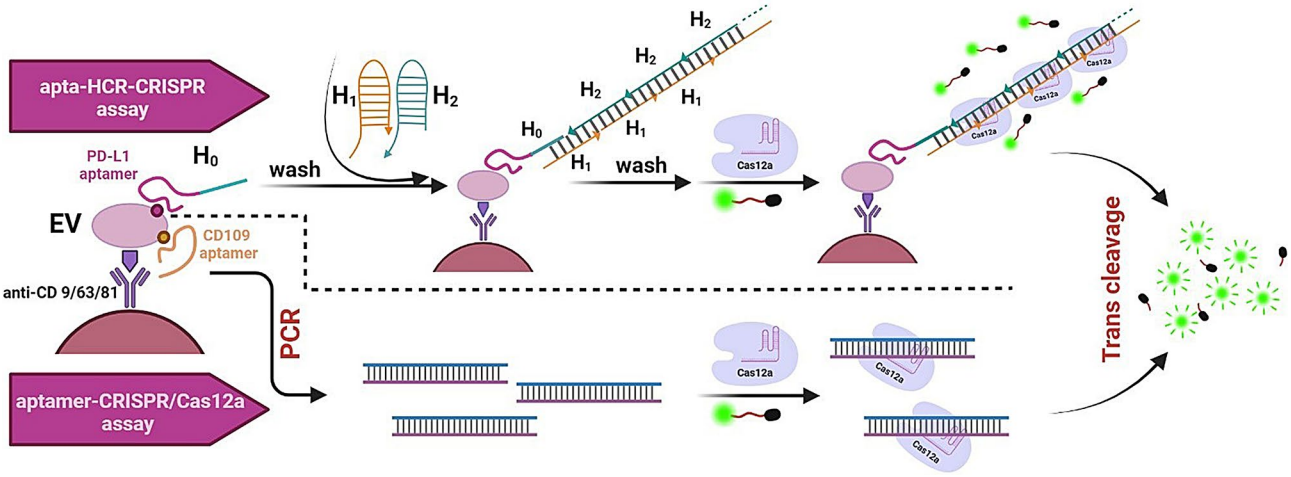


**Fig. 8** Two different methods for detecting NPC-associated TEV. In apta-HCR-CRISPR assay, anti-CD63, anti-CD81, and anti-CD9 antibody-coated beads were used to collect the EVs, and H0 was used to identify the targets. Should the EV membranes express target proteins, H0 will attach to the target with the aid of matching aptamers, forming antibody-protein-H0 complexes that will open up H1s. This will set off a Cascade reaction that will open up H2s, resulting in a long-nicked dsDNA with tens to hundreds of repeating units that contain target sequences and PAM structures that will be specifically recognized by the predesigned crRNA. Once unattached nucleic acids have been removed, the inserted crRNA/Cas12a duplex attaches to the repeating units in H1/H2 inside the HCR assembly. This causes Cas12a to initiate its trans-cleavage activity, which causes nontarget ssDNA cleavage to occur nearby. A fluorescent signal is created after the ssDNA reporter is transcleaved by target-activated Cas12a. In aptamer-CRISPR-Cas12a assay, Similar to prior studies, EVs are captured on microplates coated with anti-CD9, CD63, and CD81. After removing any non-specifically bound material, CD109-apt is introduced and attaches itself to CD109 + TEVs. The bound CD109-apt, which is proportionate to the starting concentration of CD109 + TEVs, is amplified exponentially by PCR in situ after the unbound CD109-apt is washed off. When a crRNA-guided PCR yields dsDNA products, Cas12a is concurrently activated and proceeds to cleave a neighboring ssDNA reporter collaterally. Since the intensity of the fluorescence is directly correlated with the quantity of CD109-apt, the amounts of CD109 + TEVs are indirectly reflected. Figure created with BioRender

***CRISPR-Cas12a-based technology in rapid LB detection***

CASMART (CRISPR-associated Mutation Allele Rapid Test) is an additional CRISPR-Cas12a-based platform that offers several advantages for diagnostic purposes. CASMART operates by targeting the specific mutation site within a DNA sample, where the Cas12a-crRNA complex binds to the complementary sequence. Upon target recognition, the collateral cleavage activity of Cas12a is activated, leading to the cleavage of nearby reporter molecules, thereby producing a detectable signal. This signal is then quantified, allowing for the accurate measurement of the mutant allele frequency.

Notably, it stands out for its high speed and single-step operation. Validation of the CASMART platform involved the identification of EGFR L858R mutations in complex sample backgrounds, including simulated multiplex samples cfDNA reference standards) and genomic samples from lung cancer patients. Overall, CASMART serves as a powerful proof-of-concept for a digital CRISPR-based diagnostic platform, delivering rapid and sensitive quantification of rare variant alleles with digital resolution (Fig. 9-a) [175].

Another study, conducted by Xianxian Zhao et al., developed a rapid diagnostic method utilizing LB for cancer. The approach involved the detection of exosomes using a combination of CD63 aptamer and CRISPR-Cas12a. The CD63 aptamer facilitated the recognition of exosomal membrane proteins, while CRISPR-Cas12a provided signal amplification. The outcomes of this study led to the development of a sensitive, specific, and rapid method for exosome detection in cancer (Fig. 9-b) [176].

In a similar vein, a rapid assay was developed for EV aggregation-induced in-situ miRNA detection. This technique employed cationic lipid-polymer hybrid nanoparticles encapsulating a Cascade system of CHA and CRISPR-Cas12a (CLHN-CCC). It allowed for the enrichment of EVs in a three-dimensional space and facilitated in-situ detection of internal miRNAs in just one step, within a 30-minute timeframe. This technology exhibited the capability to sensitively and selectively identify low amounts of miR-1290 in serum EVs, enabling the distinction between early-stage pancreatic cancer patients and healthy subjects. Consequently, it demonstrated high potential for clinical applications (Fig. 9-c) [177].

The BRCACas platform (Branched Rolling Circle Amplification and CRISPR-Cas12a) represents another rapid assay for the one-pot detection of non-coding RNAs (ncRNAs) associated with CRC. This method involved the design of primers that incorporated ncRNA sequences of circulating CRC-associated RNAs (piRNA or miRNA), specifically hybridizing with circular probes to initiate the BRCA process. Subsequently, the generation of dendritic DNA products triggered Cas12a trans-cleavage activity, resulting in a fluorescent signal. The

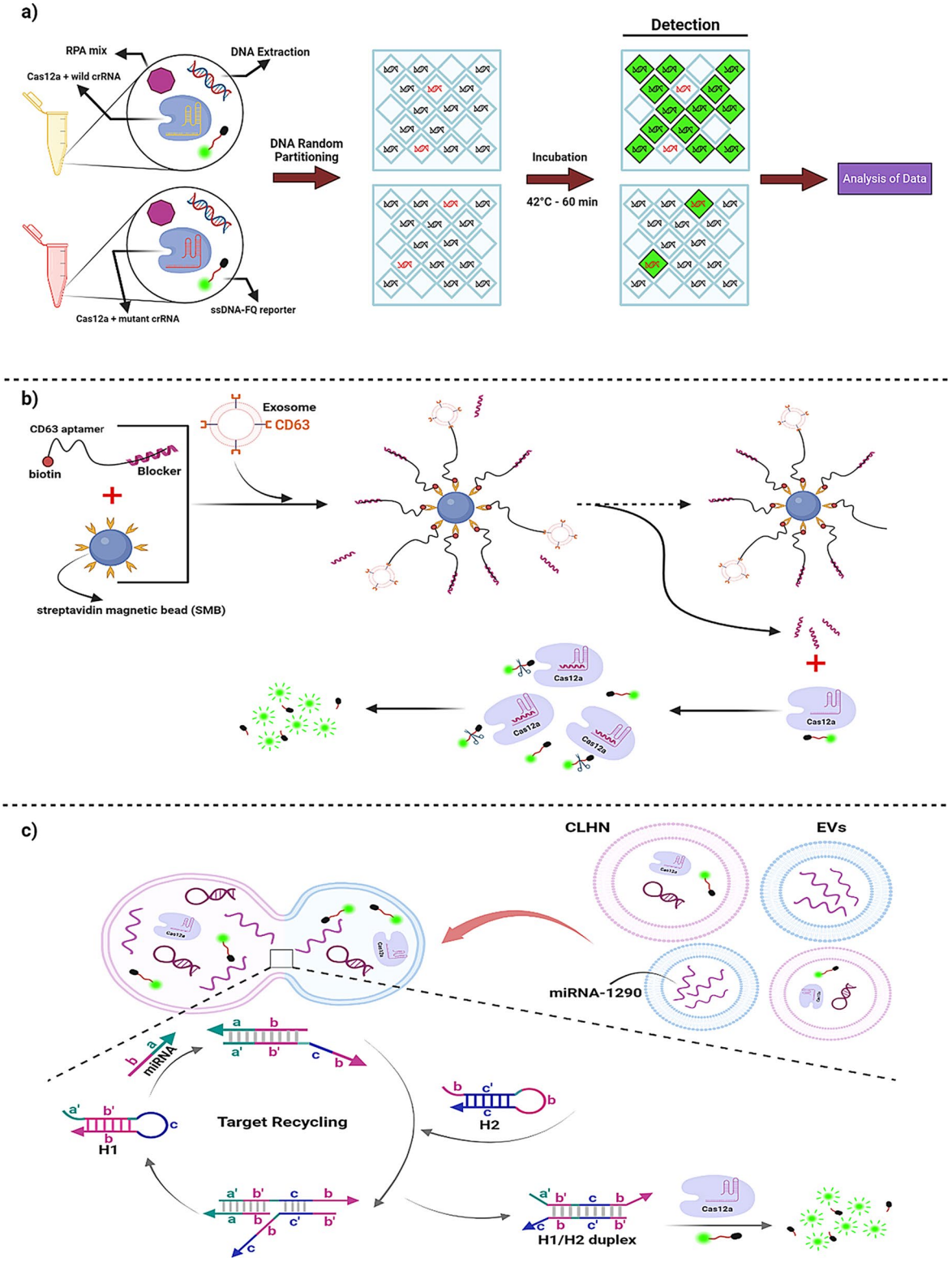


**Fig. 9** (See legend on next page.)

(See figure on previous page.)

**Fig. 9** Pointing to some rapid CRISPR-Cas12a diagnostic assays. (**a**) CASMART procedure: The LbCas12a/crRNA complex, the RPA reaction system, and the target DNA were combined to create two tubes of reaction mixtures. The crRNA in the two tubes was different, and we evenly distributed them across two digital chips using a blade. The Mutant-crRNA2/Cas12a complex identified the mutant alleles in a particular VAF sample throughout the incubation period at 42 ∘C. This recognition led to the trans-cleavage of the ssDNA-FQ reporter, producing a fluorescence signal. The VAF was then ascertained, the quantities of mutant and wild-type alleles were approximated using a Poisson distribution, and the endpoint fluorescence pictures were assessed using ImageJ software. (**b**) Exosomes containing CD63 are captured using the CD63 aptamer, which is partly inhibited by complementary DNA strands (blocker). Exosomes and CD63 aptamer work together to cause CD63 aptamer to undergo a conformational shift that releases the blocker. CRISPR-Cas12a recognizes the released blocker, which initiates the trans cleavage. The exosomes that the magnetic beads were able to collect are positively connected with the fluorescence signal that was produced. (**c**) Two functions are carried out by the CLHN-CCC: CCC for in-situ miRNA detection and CLHNs for EV enrichment. With biodegradable polymers representing the middle shell, cationic lipids serving as the outer corona, and an inner hollow core serving as a central hub for all CCC components, CLHN has a core-shell-corona structure. As many negatively charged EVs as possible are captured in solution by the exterior cationic lipids of CLHN-CCC, which then fuse with the EVs' membrane to create CLHN-CCC: EV aggregates. As this is going on, the encapsulated cargo in CLHN-CCC: EV aggregates can move and mix through CLHN's porous shell, enabling the target miRNA to activate CCC, the dual amplifier, and produce a fluorescent signal. H1 and H2 DNA hairpin molecules are used in the first-stage signal amplification (CHA) step of the CCC process. Following the introduction of the target miRNA, H1 and H2 unfold one after the other to produce the H1/H2 duplex, which contains the PAM sequence and moves the target miRNA into the subsequent catalytic cycle. The resulting H1/H2 duplex then takes part in the CRISPR-Cas12a second-stage signal amplification. The trans-cleavage activity of Cas12a is triggered to cleave multiple DNA reporters, resulting in an enhanced fluorescence signal and lighting up inside the CLHN-CCC: EV aggregates, when the binding site of the H1/H2 duplex hybridizes with the Cas12a: crRNA binary complex. Figure created with BioRender

results of this study indicate that the BRCACas platform has the potential to serve as a rapid, adaptable, and practical diagnostic/prognostic cancer screening method [178].

In addition to the aforementioned rapid diagnostic methods for exosomes and ccfDNA, Wenxiu Zhang et al. developed a technique capable of rapidly detecting ctDNA. Specifically, the BRAF V600E gene was selected as an in vitro biomarker, and a fluorescence detection strategy combining a 3D DNA walker with CRISPR-Cas12a was established. In the presence of target ctDNA, the 3D DNA walker could identify and bind to it, thereby releasing a large number of output DNAs through cyclic cleavage with the assistance of a specific endonuclease (Nb.BbvCI). The output DNAs then bound specifically to crRNA and activated the non-specific trans-cleavage activity of Cas12a, leading to a significant enhancement of the fluorescence signal. This method not only provides a platform for the rapid detection of ctDNA (the entire process took only a maximum of 70 min) but also exhibits great potential for early clinical diagnosis and biomedical research [179].

#### *EV PD-L1 determination with the CIRSPR-Cas 12a system*

Two separate studies conducted by researchers in Shanghai have yielded distinct approaches for detecting EV PD-L1 in lung cancer. EV PD-L1, a biomarker closely linked to the development and progression of lung cancer, holds immense potential for diagnostic and immunotherapy applications. In the first study, the researchers developed a programmable DNA-based electrochemical analysis strategy to identify EV PD-L1. This involved enriching PD-L1-expressing exosomes onto magnetic beads coated with PD-L1 antibodies, facilitating interaction with cholesterol-modified hairpin templates. Subsequently, programmable DNA synthesis was initiated through a Primer Exchange Reaction (PER) triggered by the hairpin template, generating numerous extension products that activate the trans-cleavage activity of CRISPR-Cas12a. This, in turn, promoted the degradation of methylene blue-labeled signaling strands via CRISPR-Cas12a-mediated random cleavage. The electro-active methylene blue molecules were then concentrated on a cucurbit [7] uril-modified electrode for quantitative assessment (Fig. 10-a) [180]. In the second study, the researchers proposed a fluorescent biosensing method for the precise detection of circulating EV PD-L1. They employed a phosphatidylserine-targeting peptide-assisted magnetic enrichment technique, followed by the design of a programmable DNA circuit. This circuit facilitated the translation of PD-L1 presence into the appearance of numerous duplex DNA probes on the surface of circulating EVs. Upon fructose treatment, these newly formed duplex DNA probes were released from the EV surface, activating the trans-cleavage activity of the CRISPR-Cas12a system and resulting in a significant fluorescence signal (Fig. 10-b) [181]. Consequently, both of these studies offer promising methodologies for determining EV PD-L1, which could provide valuable insights for the accurate diagnosis and treatment of lung cancer.

#### *Combination of nanoparticle and biosensors with CRISPR-Cas12a allows for the detection of miRNAs*

Qing Liu et al. employed a combination of three techniques, namely magnetic nanoparticles (MNPs), Cascade Strand Displacement Reaction (CSDR), and CRISPR-Cas12a, to develop a successful method for identifying microRNA-21 (miRNA-21, miR-21) as a promising biomarker for early detection of lung cancer. The MNPs demonstrated strong separation capabilities, effectively eliminating potential interference from the sample matrix and reducing background signals. This characteristic greatly enhanced the specificity and sensitivity of

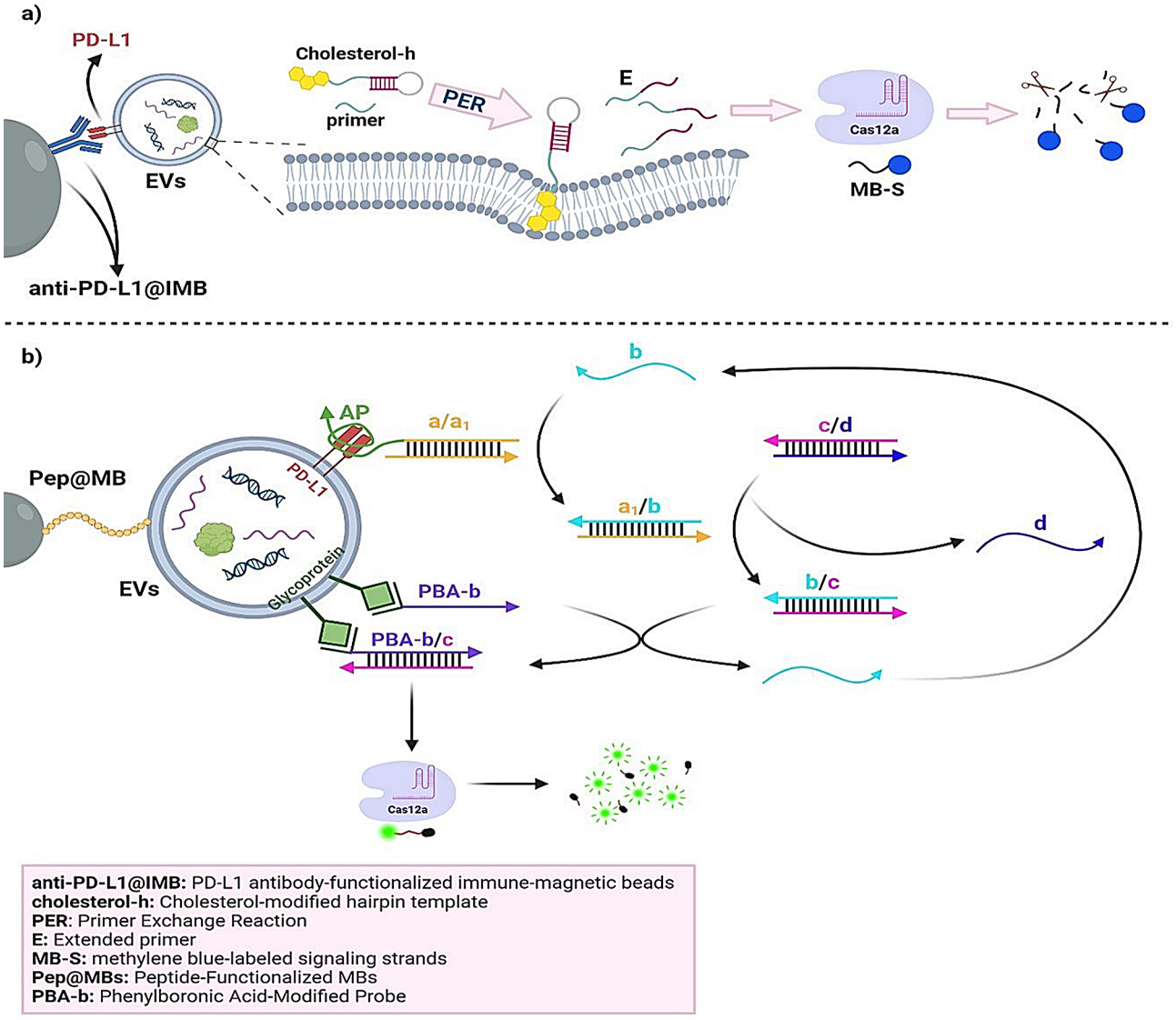


**Fig. 10** EV PD-L1 determination with the CIRSPR/Cas 12a system. **a**) First anti-PD-L1@IMBs are used to enrich PD-L1-expressing exosomes. Then, through the hydrophobic interaction of cholesterol and membrane-forming lipids, the cholesterol-H inserts into the exosome membrane. This process is quick and does not involve the alteration of lipids or the production of biomarkers. PER can be carried out using the catalytic hairpin as an extension template. In order to produce a large number of MB-labeled short segments, a programmable DNA-fueled Cascade signal amplification reaction is carried out. This reaction consists of PER-accelerated synthesis of extended primer (E) and then activated CRISPRCas12a-catalyzed degradation of MB-S. Ultimately, through host-guest interaction, MB molecules are concentrated onto a cucurbit [7] uril (CB [7])-modified electrode, eliciting increased electrochemical signals for the quantitative identification of PDL1-expressing exosomes. **b**) The phosphatidylserine-peptide interaction at the Pep@MB interface is the initial step in the collection and enrichment of circulating EVs. Subsequently, the enhanced EVs are labeled using two DNA probes. The first is the aptamer probe AP, which recognizes PD-L1 using an aptamer motif and activates the downstream DNA circuit with an activation motif (**a**); the second is PBA-b, which binds glycoprotein expressed on the EV membrane reversibly and functions as an assistant probe to reflect the expression of EV PD-L1. Two DNA probes, a1/b1 and c/d, are used in a programmable DNA circuit at the biomembrane interface following the labeling of enriched EVs. To be more precise, the release of single-stranded b1 through toehold-mediated strand displacement occurs when the activation motif of AP first interacts with the probe a1/b1. After that, the liberated b1 switches from c/d to b1/c by taking part in the DNA circuit. In order to start the next cycle of the DNA circuit, the freshly generated b1/c exposes a toehold that the surface-attached PBA-b can identify. This immediately starts the creation of duplex PBA-b/c and the regeneration of single-stranded b1. Large volumes of duplex PBA-b/c are generated at the EV surface following many cycles. These PBA-b/c may be freed from the EV surface by employing fructose to competitively bind boronic acid. This releases the CRISPR-Cas12a-crRNA system, which effectively cleaves dual-labeled reporters FAM-DNA – BHQ to provide a notable fluorescent signal. Figure created with BioRender

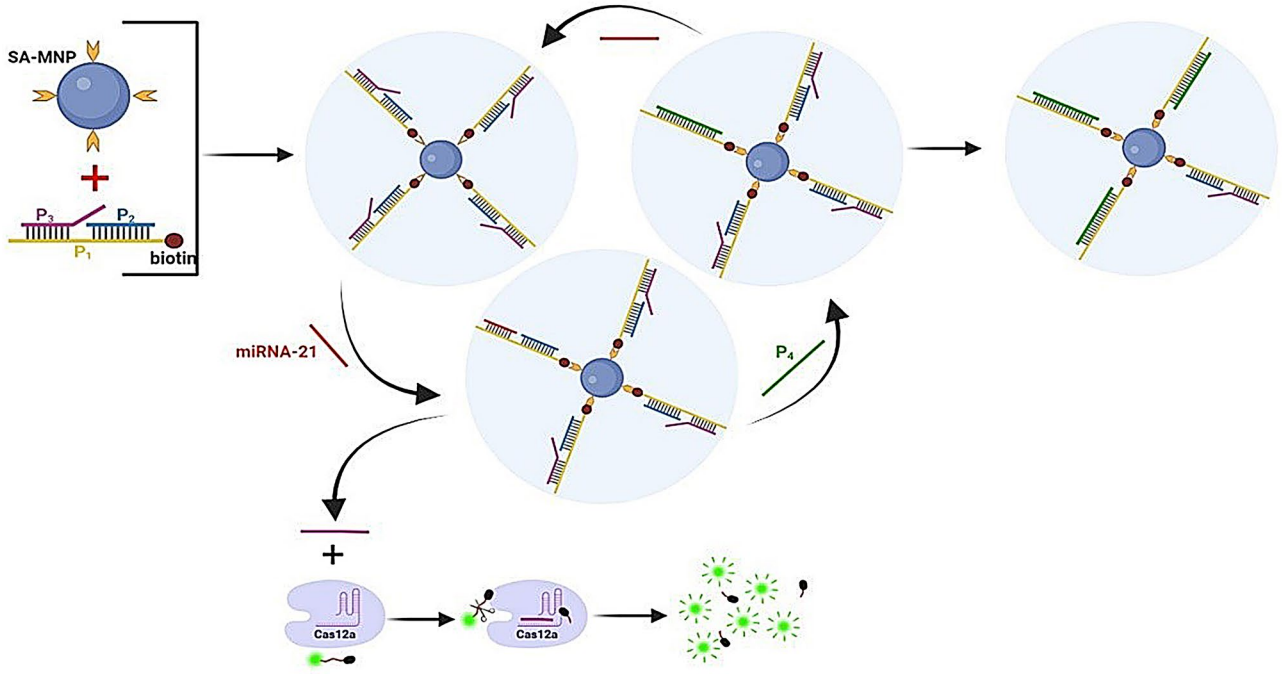


**Fig. 11** The fluorescence biosensor's schematic depiction for determining exosomal miR-21 using CSDR in conjunction with CRISPR-Cas12a. First, complementary base pairing causes P2 and P3 to join with P1 to create the biotin-P1/P2/P3 complex. Because of the unique way that biotin and streptavidin combine, the complex of biotin-P1/P2/P3 combines with SA-MNPs to create the biotin-P1/P2/P3/SA-MNPs. When miR-21 is present, it will start the first cycle of SDR and take P3's position to create biotin-P1/P2/miR-21/SA-MNPs. miR-21 replaces P3 and creates a number of additional unpaired bases that support entropy-driven binding between P4 and P1. Subsequently, P4 initiates the second round SDR, forming the biotin-P1/P4/SA-MNP complex, which releases P2 and miR-21. The following SDR is started with the release of miR-21. Therefore, using this SDR, one miR-21 may produce a significant amount of P3, which is released from the biotin-P1/P2/P3/SA-MNPs. Additionally, free P3 has the ability to bind to the Cas12a/crRNA complex, activating Cas12a's trans cleavage activity to cleave the ssDNA-FQ probe and producing the fluorescence signal. Figure created with BioRender

the technique. The CSDR technique enabled the specific conversion of miR-21 into multiple DNA strands, which acted as triggers to activate the trans-cleavage nuclease activity of Cas12a. Consequently, this resulted in the cleavage of single-stranded DNA labeled with both a fluorescent marker and a quencher (Fig. 11) [182]. Another technique employed in the study focused on miRNA detection using an ultrasensitive electrochemiluminescent (ECL) biosensor. This biosensor was constructed by utilizing three-dimensional (3D) walking nanomotor-mediated CRISPR-Cas12a and tetrahedral DNA nanostructures (TDNs)-modified nanoemitters (TCPP-Fe@HMUiO@Au-ABEI) to detect exomiR-155. The 3D walking nanomotor-mediated CRISPR-Cas12a strategy effectively converted the target exomiR-155 into amplified biological signals, thereby enhancing sensitivity and specificity. Additionally, TCPP-Fe@HMUiO@Au nanozymes with excellent catalytic performance were employed to amplify the ECL signals. The TDNs served as scaffolds for constructing "bottom-up" anchor bioprobes, thereby improving the trans-cleavage efficiency of Cas12a. The biosensor demonstrated the ability to effectively discriminate breast cancer patients by analyzing exomiR-155, and these results were consistent with those obtained using qRT-PCR [183]. Based on these studies, the combination of CRISPR-cas12a with aptamer and CASMART, BRCACas, 3D DNA walker, ESL-Biosensor techniques enables rapid and convenient diagnosis and prediction of low concentrations in LB samples.

#### CRISPR-Cas13a and CRISPR-Cas14a

CRISPR-Cas13 is a genome editing system that targets RNA molecules. Upon recognition of single-stranded RNA, Cas13a (C2c2) becomes activated [140]. The Cas13a subtype of CRISPR enzyme cleaves the nitrogenous base uracil at any position following binding to the target RNA, a phenomenon referred to as collateral cleavage. This process results in the cleavage of nearby untargeted RNAs. Cas13a has been observed to spontaneously cleave specific target mRNAs in both bacterial and eukaryotic cells [140, 153].

In addition, Cas13 has numerous applications in research and development. For example, Cas13a plays a distinct role in RNA imaging. The dCas13a can be fused with KRAB (Kruppel-associated box domain), ZFN (Zinc Finger Nucleases), and GFP (Green Fluorescent Protein) [135]. In response to cellular pathogen infection, non-specific RNA degradation mediated by Cas13a activates

apoptosis, a programmed cell death mechanism, within the cell [184]. The application of inactivated Cas13 has emerged as a vital tool in the field of RNA editing technology, particularly in the context of REPAIRv1 [153].

Cas13a, among the Cas nucleases, is utilized for detecting specific EV miRNA molecules using crRNA. By directly hybridizing the target RNA with crRNA, the ribonuclease (RNase) activity of Cas13a is activated. The activated Cas13a cleaves the target RNA bound to crRNA (cis-cleavage) as well as unbound single-stranded RNA molecules (trans-cleavage). This trans-cleavage process, along with FQ probes connected via single-stranded RNA linkers, can be employed for biosensing applications. This approach eliminates the need for reverse transcription from RNA to cDNA, simplifying EV miRNA detection [185, 186].

In one study, an amplification-free and extraction-free method for detecting EV miRNA using a CRISPR-Cas13a sensing system was introduced. The CRISPR-Cas13a components were encapsulated in liposomes and delivered into EVs through liposome-EV fusion. The results exhibited a strong correlation between bulk analyses using the gold-standard method (RT-qPCR). This developed EV miRNA sensing system enables specific miRNA detection within intact EVs without the need for RNA extraction, offering the potential for multiplexed analysis of single EVs for protein and RNA markers [186].

In another study by Junli Zhang et al., a highly sensitive and user-friendly method called liposome-mediated membrane fusion strategy (MFS) was utilized to transfect CRISPR-Cas13a into exosomes. Termed MFS-CRISPR, this approach directly measured exosomal miR-21 in plasma. The MFS approach confined fluorescent signals within fused vesicles, enabling analysis of exosome heterogeneity. The expression of miR-21 achieved through this method showed significant differences between breast cancer patients and healthy donors [187].

In a recent study published in February 2024, a novel and sensitive in situ detection strategy for exosomal miR-1246 was developed by integrating the CRISPR-Cas13a system with the formation of hybrids between exosomes and cationic liposomes. The liposomes were loaded with CRISPR-Cas13a, crRNA, and RNA reporter probes. In the presence of exosomes, liposome-exosome hybrids were formed through electrostatic interactions, activating CRISPR-Cas13a to cleave the reporter probes via exosomal miR-1246. This method provides an advantage in detecting serum exosomal miR-1246 in breast cancer patients and, importantly, distinguishing between early-stage and advanced disease [188].

Jing et al. conducted a study that utilized a combination of the PER and CRISPR-Cas14a to construct an electrochemical biosensor for detecting ctDNA EGFR L858R. This study represents the first instance of using CRISPR-Cas14a in this context. The target, EGFR L858R, triggered isothermal amplification through the PER reaction, leading to the activation of the CRISPR-Cas14a system. Consequently, the substrate ssDNA-MB was cleaved, causing the transfer of electrons on the surface of the gold electrode and resulting in fluctuations in the electrochemical redox signal on the electrode surface. When EGFR L858R was absent, the electrochemical signal remained stable. Therefore, the concentration of EGFR L858R could be quantified through analysis of the electrochemical signal. This platform exhibited excellent specificity, reproducibility, stability, and a good recovery rate. The study presents an efficient and innovative strategy for detecting ctDNA EGFR L858R, offering significant potential for applications in the diagnosis and treatment of NSCLC [189].

## Conclusion and future directions

The potential of LB in cancer is remarkable. Over the past decade, this innovative approach has transformed the field of clinical oncology by offering several key advancements. By non-invasive sampling, entities including CTCs, ctDNA, and tumor extracellular vesicles have emerged as valuable diagnostic tools in cancer research and clinical practice. LBs enable continuous monitoring by repeated sampling, allowing clinicians to track disease progression, treatment response, and genetic changes over time. With personalized therapeutic regimens, clinicians can customize precision medicine approaches by analyzing genomic and proteomic data from LBs. By screening for therapeutic resistance, LBs contribute to identifying therapeutic resistance. LB assays approved by the US FDA are already in use demonstrating their practical application and impact in clinical settings. These assays enable clinicians to make informed decisions, enhance patient care, and contribute to the future advancement of personalized medicine.

The discovery of CRISPR-Cas systems has created a new revolution in the landscape of precise cancer diagnosis and targeted therapies. CRISPR-Cas systems offer a versatile and potent alternative to traditional diagnostic methods, with the potential for applications in a wide range of settings. This development was a significant step forward in the field of molecular diagnostics. The CRISPR-Cas systems, including Cas9, Cas12, and Cas13, possess unique properties that render them well-suited for various applications in LB. Each of these CRISPR-Cas systems provides distinct benefits for LB applications. Their integration into diagnostic platforms is propelling the field of non-invasive cancer diagnostics, equipping clinicians with tools for early detection, monitoring treatment response, and identifying therapeutic targets with remarkable precision. In a recent study, the use of aptamer-CRISPR-cas12a assay, CASMART, and

nanodevice technology along with biosensors in LB samples yielded valuable results in molecular diagnosis and early detection of cancers.

Both the LB and CRISPR-Cas systems hold significant promise for diagnosing various diseases, though they do have some limitations. Sensitivity and specificity can be issues for LBs, as they sometimes struggle with detecting low levels of biomarkers, potentially leading to false negatives or positives [190, 191]. Standardization is another challenge, with a lack of standardized protocols and guidelines affecting the reproducibility and reliability of results. Additionally, the complexity of data interpretation from LBs can be daunting, given the presence of various genetic materials and the need for advanced bioinformatics tools. One major challenge for the CRISPR system is the potential for off-target effects, where unintended parts of the genome may be edited. Ethical and regulatory concerns also arise with the use of CRISPR technology, especially in human applications, necessitating careful consideration. Despite these challenges, ongoing research and technological advancements continue to enhance the accuracy and applicability of both liquid biopsy and CRISPR-Cas systems in clinical diagnostics [192–194].

In summary, this paper provides a comprehensive review of the application of CRISPR-based diagnostics in LBs for cancer. We have delved into the cutting-edge CRISPR-based diagnostic tools designed for nucleic acid-based detection. These innovative approaches play a pivotal role in monitoring, as well as early and rapid detection of cancer. By harnessing the precision of CRISPR technology, researchers and clinicians are poised to revolutionize cancer diagnostics, ultimately enhancing patient outcomes.

In the not-so-distant future, a new horizon will emerge, revolutionizing the landscape of cancer diagnosis and personalized medicine. CRISPR-based diagnostics, LBs, and advanced technologies will empower clinicians to tailor treatments with unprecedented precision, finally improving patient outcomes. Promising directions for future studies in this field can be exploring the integration of liquid biopsy data with advanced machine learning algorithms, exploring single-cell liquid biopsy techniques using CRISPR-Cas systems, investigating the impact of liquid biopsy-based diagnostics on patient outcomes, and developing methods for long-term monitoring using LBs.

**Abbreviations**

| | |
|---|---|
| anti-PD-L1@IMB | PD-L1 antibody-functionalized immune-magnetic beads |
| AuNP | DNA-functionalized gold nanoparticles |
| BRCACas | Branched Rolling Circle Amplification and CRISPR-Cas12a |
| BRCA-1 | Breast cancer gene-1 |
| Cas | CRISPR-associated |
| CASMART | CRISPR-associated Mutation Allele Rapid Test |
| CB[7] | Cucurbit[7]uril |
| ccfDNA | Cell-free DNA |
| cfDNA | Cell-free DNA |
| cfRNA | Cell-Free RNA |
| cfRNA | Circulating cell-free RNA |
| CHA | Catalytic Hairpin Assembly |
| cholesterol-h | Cholesterol-modified hairpin domain |
| CLHN-CCC | Cascade system of CHA and CRISPR-Cas12a |
| CNAs | Copy number alterations |
| CRC | Colorectal Cancer |
| CRISPR | Clustered Regularly Interspaced Short Palindromic Repeats |
| crRNA | CRISPR RNA |
| CSDR | Cascade Strand Displacement Reaction |
| CTCs | Circulating Tumor Cells |
| ctDNA | Circulating Tumor DNA |
| dCas | Dead Cas |
| dCas13 | Dead variant of Cas13 |
| ddPCR | Droplet-based Digital Polymerase Chain Reaction |
| DETECTR | DNA Endonuclease Targeted CRISPR Trans Reporter |
| DNase | Deoxyribonuclease |
| dsDNA | Double-strand DNA |
| ECL | Electrochemiluminescent |
| EedCas12a | Eubacterium eligens Cas12a |
| EGFR | Epidermal growth factor receptor |
| EV | Extracellular vehicles |
| FDA | US Food and Drug Administration |
| FITC | Fluorescein isothiocyanate |
| FnCas12a | Francisella novicida Cas12a |
| FQ | Fluorophore-Quencher |
| GFP | Green Fluorescent Protein |
| gRNA | Guide RNA |
| HCR | Hybridization Chain Reaction |
| HEPN | Higher eukaryotes and prokaryotes nucleotide-binding |
| HPV | Human papillomavirus |
| KRAB | Kruppel-associated box domain |
| LB | Liquid Biopsy |
| LbCas12a | Lachnospiraceae bacterium Cas12a |
| lncRNA | Long non-coding RNA |
| MAV-chip | Magnet-Assisted V-chip |
| MbCas12a | Moraxella bovoculi Cas12a |
| MB-S | Methylene blue-labeled signaling strands |
| MEF | Metal-Enhanced Fluorescence |
| MFS | Membrane fusion strategy |
| miRNAs | Micro-RNAs |
| MNPs | Magnetic nanoparticles |
| ncRNAs | Non-coding RNAs |
| NGS | Next-Generation Sequencing |
| NPC | Nasopharyngeal carcinoma cancer |
| NSCLC | Non-Small Cell Lung Cancer |
| OCCC | Ovarian Clear Cell Carcinoma |
| OS | Overall survival |
| PAM | Protospacer Adjacent Motif |
| PASEA | Programmable Enzyme Assisted Selective Exponential Amplification |
| PBA | Phenylboronic Acid-Modified probe |
| PCR | Polymerase Chain Reaction |
| PDAC | Pancreatic ductal adenocarcinoma |
| Pep@MBs | peptide-functionalized methylene blue |
| PER | Primer Exchange Reaction |
| PFS | Protospacer flanking site |
| PtNP | Platinum Nanoparticles |
| RACE | Rolling Circular Amplification-assisted CRISPR-Cas9 cleavage |
| RAMPs | Repeat Associated Mysterious Protein |
| RCA | Rolling Circular Amplification |
| REPAIR | RNA Editing for Programmable Adenosine to Inosine Replacement |

| | |
|---|---|
| RNase | Ribonuclease |
| RPA | Recombinase Polymerase Amplification |
| SaCas9 | Staphylococcus aureus Cas9 |
| sgRNA | Single-guide RNA |
| SNP | Single Nucleotide Polymorphisms |
| SNV | Single nucleotide variation |
| SpCas9 | The Streptococcus pyogenes Cas9 |
| ssDNA | Single-strand DNA |
| ssRNA | Single-stranded RNA |
| TCPP-Fe@HMUiO@Au-ABEI | Tetrahedral DNA nanostructures (TDNs)-modified nanoemitters |
| TEPs | Tumor Educated Platelets |
| UM | Uveal melanoma |
| ZFN | Zinc Finger Nucleases |
| 3D | Three-dimensional |

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During the preparation of this work the authors used Chat-GPT3.5 in order to get a better translation. After using this tool/service, the authors reviewed and edited the content as needed and takes full responsibility for the content of the publication.

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