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Advances in ligase-based nucleic acid amplification technology for detecting gene mutations: a review

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Abstract

Gene mutation has been a concern for researchers because it results in genetic variations with base changes in molecular structure. Researchers continue to explore methods to detect gene mutations, which may help in disease diagnosis, medication guidance, and so on. Currently, the detection methods, such as whole-genome sequencing and polymerase chain reaction, have some limitations in terms of cost and sensitivity. Ligase (an enzyme) can recognize base mismatch as a commonly used tool in genetic engineering. Therefore, the ligase-related nucleic acid amplification technology for detecting gene mutations has become a research hotspot. In this study, the main techniques explored for detecting gene mutations included the ligase detection reaction, ligase chain reaction, rolling circle amplification reaction, enzyme-assisted polymerase chain reaction, and loop-mediated isothermal amplification reaction. This review aimed to analyze the aforementioned techniques and mainly present their advantages and disadvantages, sensitivity, specificity, cost, detection time, applications, and so on. The findings may help develop sufficient grounds for further studies on detecting gene mutations.

Keywords Amplification technique · Genetic mutations · Ligase · Nucleic acid

Abbreviations

Fc	Ferrocene
LAMP	Loop-mediated isothermal amplification
LCR	Ligase chain reaction
LDR	Ligase detection reaction
iMLDR	Improved multiplex LDR
PCR	Polymerase chain reaction
PfAgo	Pyrococcus furiosus Argonaute
RCA	Rolling circle amplification
JAK2	Janus kinase 2
EGFR	Epidermal growth factor receptor
CYP2C19	Cytochrome P450 family 2 subfamily C member 19
KRAS	Kirsten rat sarcoma viral oncogene homolog
NEAT1	Nuclear-enriched abundant transcript1
TP53	Tumor protein 53
STK11	Serine/threonine kinase 11
SLC30A8	Solute carrier family 30, member 8
BRAF	B-raf proto-oncogene
PD-L1	Programmed death-1 ligand
ALDH2	Acetaldehyde dehydrogenase 2
MDM4	Murine double minute 4
ATG5	Autophagy related 5
IL-27	Interleukin-27
ABCA1	ATP-binding cassette transporter A1
CTLA-4	Cytotoxic T lymphocyte-associated antigen-4
MIF-AS1	Macrophage migration inhibitory factor 1
AHR	Aryl hydrocarbon receptor
CRISPR	Clustered regularly interspaced short palindromic repeats

Introduction

Gene mutation is a common phenomenon in biological growth and evolution. Base mismatch in DNA molecules easily causes gene mutations, especially point mutations, which are often an important cause of hereditary and genetic predisposition diseases [1]. In recent years, the detection and analysis of gene mutations have become a topic of increasing interest, such as pathogenic gene screening and prenatal diagnosis, which can provide the basis for early diagnosis and precise treatment of diseases [2]. At present, many methods are in place for detecting gene mutations, such as

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whole-genome sequencing, but they still have certain limitations in terms of experimental cost, sensitivity, and accuracy. Ligase is an enzyme that can catalyze the formation of chemical bonds between the head and tail of two molecules or a molecule and can be used as a tool in DNA replication and repair. Ligase recognition ability can effectively identify mismatched base pairs [3–5]. Therefore, the application of ligase for detecting gene mutations is of increasing interest and a research hotspot. This review mainly focused on the latest research progress in ligase and several related nucleic acid amplification technologies and also on their advantages, disadvantages, and applications.

Types and modes of action of ligases

Two types of ligases have been found to catalyze nucleic acid ligations: DNA ligase and RNA ligase. DNA ligase forms a phosphodiester bond by connecting the adjacent 5'-terminal phosphate group with the 3'-terminal hydroxyl group on the catalytic chain so that the single-stranded nick can be repaired, which is known as "the needle and thread of genes" [6]. Ligase can be divided into room-temperature and thermophilic DNA ligases based on the thermal stability of DNA ligase. The room-temperature DNA ligases include SplintR Ligase, T3 DNA ligase, and T4 DNA ligase. They are suitable for the reaction below 37 °C, and the reaction is fast but has low specificity. The thermophilic DNA ligases include Thermus aquaticus ligase (commonly known as HiFi, and so on, with high fidelity), 9°N ligase, Ampligase, Thermotoga maritima ligase, Pfu ligase, and so forth. The reaction temperature of the thermophilic DNA ligases is higher, and they are not inactivated even at 90 °C [7, 8]. RNA ligase can catalyze the intermolecular or intramolecular connection of DNA or RNA and can also be used for the specific modification of transfer RNA. The optimum reaction temperature is 37 °C, and T4 RNA ligase is common. T4 RNA ligase 2 is commonly used and specifically catalyzes linear intermolecular ligation and intramolecular circular ligation with high efficiency, low background, and good stability [9].

Ligase-related nucleic acid amplification techniques

Ligase chain reaction

Since its invention by Backman in 1995, ligase chain reaction (LCR) has been one of the techniques for detecting point mutations in target gene sequences [10]. LCR is an exponential amplification technology based on DNA ligase. The principle of LCR technology is shown by a schematic in Fig. 1. The LCR needs to be carried out in a thermal cycler. Two pairs of complementary oligonucleotide probes are designed and hybridized with the perfectly matched target

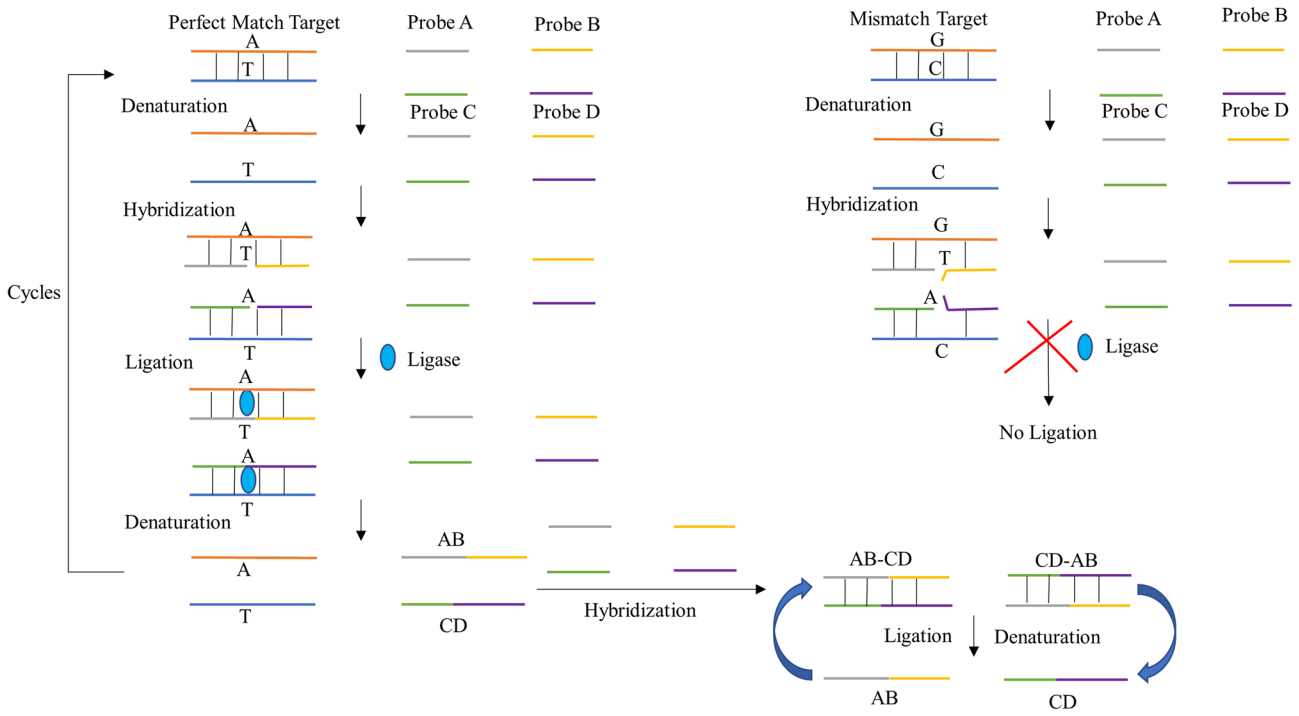

Fig. 1 Schematic diagram depicting the principle of LCR technology

sequence. The 5'-terminal phosphate group of one probe is combined with the 5'-terminal phosphate group of another probe by the action of DNA ligase. The 3'-terminal hydroxyl group of the other probe is connected, and the new fragment obtained is denatured again and used as a template to continue the reaction. The other two probes are hybridized into the second template strand in a similar manner. After cycling, a large number of ligation products are formed to achieve the purpose of exponential amplification. Therefore, this method is more sensitive than ligase detection reaction (LDR) [11]. The adjacent probes cannot be connected if a base mutation occurs at the ligation site, and no further amplifications occur. Therefore, another advantage of LCR is that it has a stronger ability to identify base mutations while ensuring the same amplification efficiency as polymerase chain reaction (PCR). Hence, LCR is currently the best method for detecting point mutations in known sequences. However, it requires the use of a thermal cycler, and the biggest drawback is the possibility of target-independent connections that increase the background signal.

LCR is widely used in detecting gene mutations. Malik used this technology to screen the hepatitis B virus G1896A mutation in patients with chronic liver disease negative for hepatitis B surface antigen in northern India and found that the G1896A mutation caused one-third of the pronuclear stop-codon mutations. Experiments showed that this method had lower cost and higher detection efficiency compared with sequencing. However, the results obtained were reliable and consistent with the sequencing results [12, 13]. The product of LCR can be detected by electrophoresis, autoradiography, and so on, but the process is complicated. With the gradual deepening of nucleic acid research, various combined application methods are being used for detecting gene mutations based on LCR, such as combined fluorescence, electrochemistry, single quantum dots, cationic polymers, and nanoparticles.

Combining LCR with fluorescent biosensors

Feng Rui et al. developed a new method combining LCR and flow cytometry microspheres to detect the tumor-associated Janus kinase 2 (JAK2) gene V617F mutation site. They labeled the LCR products with biotin and fluorophores, fixed them on the microspheres, and quantitatively detected them via flow imaging. The results showed that the fluorescence gradually increased with the increase in the mutant DNA concentration [14]. This method could detect mutant DNA in a linear range of 10^{-15} to 10^{-11} mol/L, and the detection limit was as low as 10^{-15} mol/L. The process was simple and involved one-tube typing. The quantitative imaging results were intuitive and clear, but the sensitivity needed improvement.

Combining LCR with electrochemical biosensor

Hu et al. designed an LCR method combined with a ferrocene (Fc)-labeled electrochemical sensor for the ultrasensitive detection of the mutation site of the gene epidermal growth factor receptor (EGFR) T790M. They hybridized the capture probe on the nanotube-modified electrode using the Fc-labeled LCR product to measure the current, which could be identified by comparing the difference in electrical signals. The method had good specificity and high precision. The linear dynamic range of the detected mutant target sequence concentration was 10^{-18} to 10^{-11} mol/L, and the detection limit was 10^{-19} mol/L. Moreover, the product did not need separation and purification [15]. However, this method also had drawbacks. For example, the modification of carboxyl multi-wall carbon nanotube was cumbersome. Chen et al. transformed the thiol- or biotin-labeled short double-stranded DNA into a recombined complete double-stranded DNA via LCR exponential amplification in the presence of the target sequence, that is, "probe lengthening" and then immobilized it on the surface of the gold electrode to measure the electrical signal. The results showed distinct differences in DNA electrical signals between perfectly paired and single-base mismatches, allowing easy identification of genetic mutations. The linear dynamic range of the mutant DNA concentration measured using this method was 10^{-16} to 10^{-11} mol/L, and the sensitivity was 1000 times higher than that of gel electrophoresis. It also had the characteristics of strong specificity and high resolution and could be used for genomic DNA mutations in clinical serum samples [16]. Liu et al. tested cytochrome P450 family 2 subfamily C member 19 (CYP2C19) gene mutation site in whole blood with high sensitivity. Experiments showed that when the concentration of the mutant target sequence was as low as 10^{-16} mol/L, the method still had good specificity for the presence of more than 104 times the wild-type gene [17].

Combining LCR with DNA melting temperature

In 2020, Hu et al. developed a marker-free and economical method to analyze the mutation sites of the Kirsten rat sarcoma viral oncogene homolog (KRAS) gene by analyzing the DNA melting temperature based on LCR. Compared with the original target DNA, the double-stranded DNA bases produced by LCR and the melting temperature significantly increased. They accurately screened out site-specific mutations from guanine to adenine, thymine, or cytosine in the KRAS gene, and the linear dynamic range of mutant DNA concentration was 0 to 10^{-7} mol/L. Experiments showed that this method was highly sensitive, had good selectivity and reliability, did not need purification products and expensive and sophisticated instruments, and avoided cumbersome chemically

modified probes [18]. At the same time, it could also amplify the signal. This method might be used for the early diagnosis of tumors.

Combining LCR with argonaute pyrococcus

Argonaute *Pyrococcus* (*Pyrococcus furiosus* Argonaute, PfAgo) coupled with LCR was a new method proposed by Wang et al. in 2021 that could rapidly distinguish between the simulated wild-type new coronavirus and the single-base mutation of the spike D614G gene. PfAgo is a thermophilic DNA-guided programmed enzyme that specifically cleaves phosphorylated target DNAs longer than 14 bases [19]. Wang et al. added the LCR product to a solution containing PfAgo protein and a fluorophore. When the target gene was present, PfAgo could cleave the phosphorylated DNA and release the fluorophore for detection. Experiments showed that the detection limit of this method was 10^{-17} mol/L, the linear dynamic range of the mutant concentration was 10^{-17} to 10^{-11} mol/L, the time used was less than that for PCR, and the sensitivity and specificity were better [20]. This method provided new ideas for detecting novel coronaviruses and their more infectious mutated genes. However, this method also had some shortcomings. For example, the LCR and PfAgo cleavage assays could not be carried out in the same tube, which needs to be improved in future.

Ligase detection reaction

LDR is a method that uses DNA ligase to repeat thermal cycling for linear amplification. The principle of the LDR technique is shown in Fig. 2. This reaction requires the design of a pair of specific oligonucleotide probes upstream and downstream of the mutation site. When one of the probes is fully complementary to the target gene, the 3' end of the probe forms a gapped duplex with the 5' end of the adjacent probe, the gap is connected by ligase, and the amplified product is detected using capillary electrophoresis after multiple thermal cycles [21]. LDR overcomes the shortcomings of traditional LCR. It only uses a pair of oligonucleotides to reduce the background signal of false ligation. The biggest advantage is that it can quickly screen mutant genes at multiple sites and is simpler than quantitative PCR, but the signal amplification is lesser than that of LCR.

Many researchers combined PCR with LDR for gene identification. For example, Ruiz et al. applied this method to establish a specific, rapid, and practical liquid biopsy strategy [22]. Zhang Xinya et al. used this method to detect the D614G mutation in the fragmented coronavirus S gene down to a 40-nt fragment length, which was a shorter template length than that required for the probe method of fluorescent quantitative PCR and had an advantage in detecting fragmented templates [23]. Zhang et al. used this method to integrally detect the mutation site of the EGFR T790M gene. The results showed that the linear dynamic range of the concentration of the mutant target sequence was 10^{-16} to

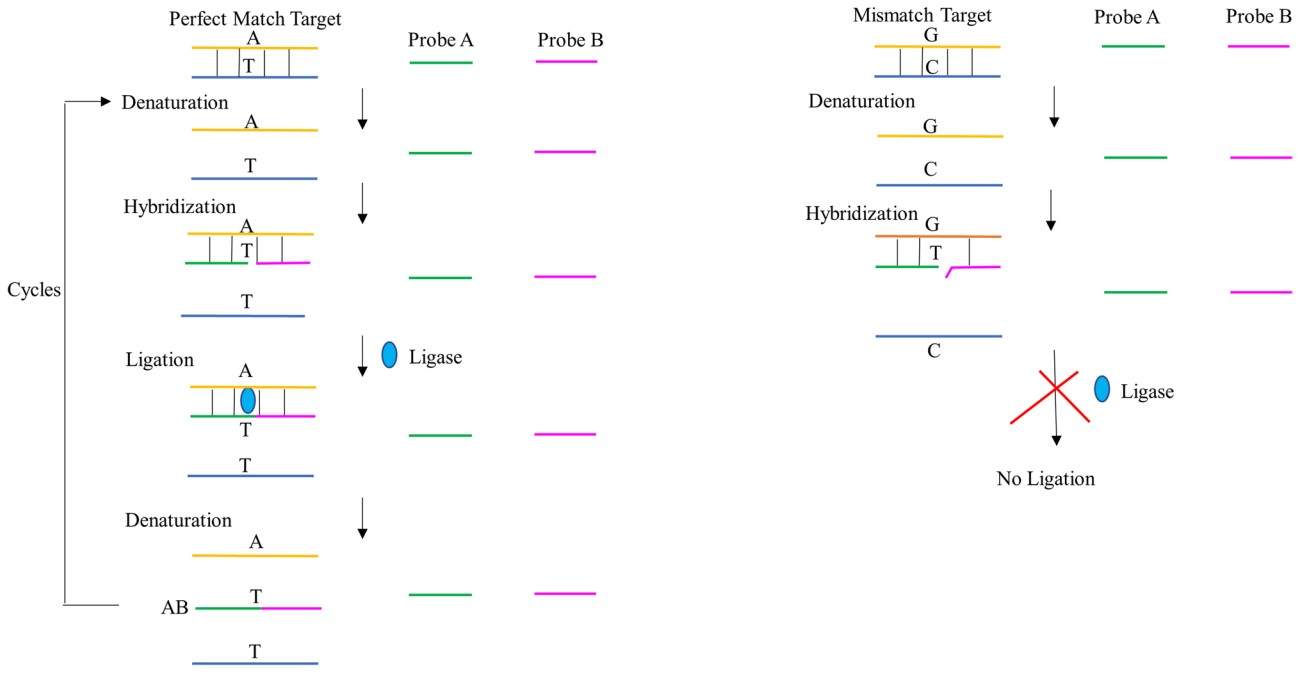

Fig. 2 Schematic diagram depicting LDR technology principles

10^{-12} mol/L, and the detection limit could reach 10^{-17} mol/L [24]. Li et al. used an improved multiplex LDR (iMLDR), that is, multiplex PCR products as templates for ligation, and found that the lncRNA nuclear enriched abundant transcript1 (NEAT1) rs3825071 locus variant was significantly associated with sputum smear positivity in patients with pulmonary tuberculosis [25]. The applications of LDR in recent years are presented in Table 1.

Rolling circle amplification technology

Rolling circle amplification (RCA) is an isothermal amplification reaction in which ligase participates in specific ligation. The key to this reaction is the construction of a single-stranded DNA circle. The principle of probe looping involving DNA ligase is schematically shown in Fig. 3. When the target gene and the probe are completely matched, the 3'-terminal hydroxyl group and the 5'-terminal phosphate group of the probe are connected to form a circular closed loop under the action of ligase and then the subsequent amplification reaction is carried out. The reaction can achieve the effect of amplifying the signal hundreds of times. For a situation where the bases cannot be matched, the probe cannot be closed into a loop and signal amplification cannot be achieved [39, 40]. The RCA reaction that ligase participates in improves the specificity and sensitivity of the detection, requires less experimental temperature conditions, and does not require a thermal cycler.

Qian et al. reported a method of RCA combined with fluorescence detection to identify mutations simply and

Table 1 Current applications of LDR

Format	Target	Disease	Reference
LDR–fluorescence–magnetic nanospheres	SLC30A8 gene	Type 2 diabetes	[26]
LDR–PCR	BRAF gene	Colon adenocarcinoma	[22]
	CYP2C19 gene	Screening before clopidogrel treatment	[27]
	T790M gene	Non-small-cell lung cancer	[24]
	CASP7 gene	Noise-induced hearing loss	[28]
	PD-L1 3'-UTR	Epithelial ovarian cancer	[29]
	ALDH2 gene	Coronary artery disease	[30]
	MDM4 gene	Esophageal squamous cell carcinoma	[31]
	Calgranulin gene	Ischemic stroke	[32]
	ATG5 gene	Thyroid-related eye disease	[33]
	IL-27 gene	Aplastic anemia	[34]
	ABCA1 gene	Hypertension	[35]
	CTLA-4 gene	Epithelial ovarian cancer	[36]
	MIF-AS1 gene	Stomach cancer	[37]
	Novel coronavirus S gene D614G	Novel coronavirus pneumonia	[23]
iMLDR	ncRNA NEAT1gene	Tuberculosis	[25]
	AHR signaling pathway gene	Rheumatoid arthritis	[38]

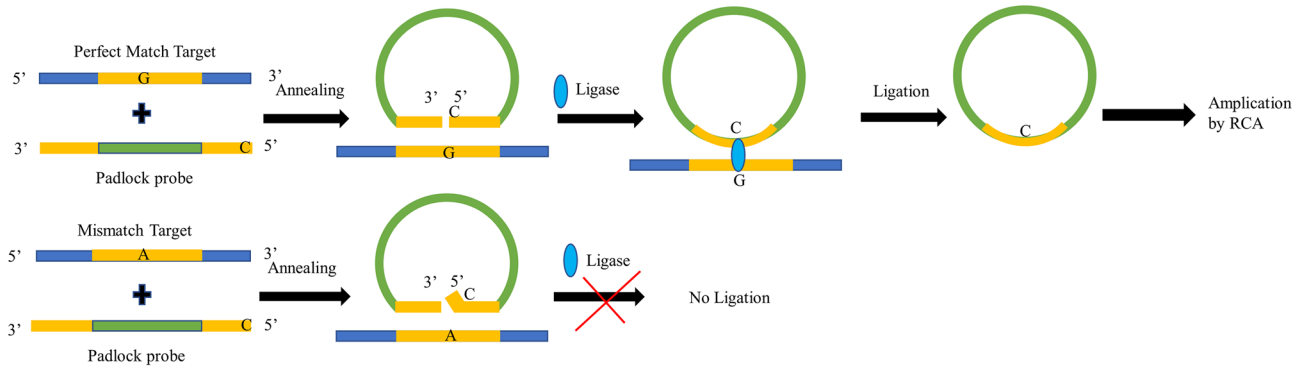

Fig. 3 Schematic diagram of the principle of probe looping involving DNA ligase

efficiently. A large number of pyrophosphate molecules could be generated using this method during the signal amplification process, and the fluorescence intensity could be determined to detect gene mutations. This method could measure the concentration of mutant DNA up to 10^{-13} mol/L and specifically distinguish the difference in the base site of the target gene. This method was less expensive than sequencing and provided a new option for detecting gene mutations [41]. Similarly, Kim et al. also described a method for detecting low-abundance EGFR exon 19-del mutant genomic DNA: generation of long segments containing G-quadruplex structures in RCA by thioflavin T detection based on the fluorescence intensity of single-stranded DNA. This method detected as low as 0.01% of mutant genes in pooled normal plasma [42]. Chung et al. used RCA combined with surface-enhanced Raman scattering to detect multiple point mutations in the KRAS gene. When the linear probe hybridizes with a matched target gene, the probe forms a specific loop structure. Experiments showed that the scattering intensity increased proportionally with the increase in the target mutant gene concentration in the range of 10^{-11} to 10^{-8} mol/L. This method turns on Raman scattering at the same time as the ligation reaction, shortens the detection time, and the clever design of the probe avoids false-positive results caused by self-hybridization. This method can be considered as an alternative method to PCR for detecting gene mutations under restricted conditions [43].

Ligase-assisted PCR

PCR generally uses DNA polymerase to achieve amplification through continuous cycles of denaturation, annealing, and extension. However, this method has disadvantages, such as being prone to false results and a long detection time. Considering these shortcomings, it was proposed to use ligase-assisted PCR with better recognition ability, as shown in Fig. 4. When the target gene is completely matched and complementary to the two oligonucleotide probes, under the action of DNA ligase, probes 1 and 2 connect the gap to form a long DNA chain and then carry out subsequent PCR amplification. However, for a base mutation in the target gene, the two probes cannot be connected, indicating the detection of the base mutation of the target gene. Li Bo developed a simple method, using T4 DNA ligase-assisted PCR, to detect mutations in various pathogenic genes such as tumor protein 53 (TP53) and serine/threonine kinase 11 (STK11) related to lung squamous cell carcinoma. This method could detect 0.1% of mutant genes and was expected to be a powerful tool for detecting gene mutations [44]. However, this method had disadvantages in terms of low ligation efficiency and time consumption. Although these defects were corrected by optimizing the experimental conditions, the activity of T4 DNA ligase was weakened, thereby inhibiting the ligation reaction. Therefore, this method needs further improvement.

Ligase-assisted loop-mediated isothermal amplification technology

Loop-mediated isothermal amplification (LAMP) can amplify a small amount of template multiplicity in a short period under constant-temperature conditions and its sensitivity is higher than that of traditional PCR and the determination is faster [45]. However, the ability of LAMP to identify base mismatches is not adequate. Therefore, it has been proposed to use high-specificity recognition ligase to assist in detecting gene mutations, thus improving the accuracy and lowering the cost of sequencing. The schematic diagram depicting the principle of DNA ligase-assisted LAMP ligation reaction is shown in Fig. 5. In the ligation reaction, a pair of stem-loop probes 1 and 2 are designed: probe 1 is completely complementary to the gene to be tested (perfect match/mismatch) and probe 2 is only complementary to the perfectly matched target gene. In the catalysis of ligase, probes 1 and 2 can be connected to form a dumbbell-shaped

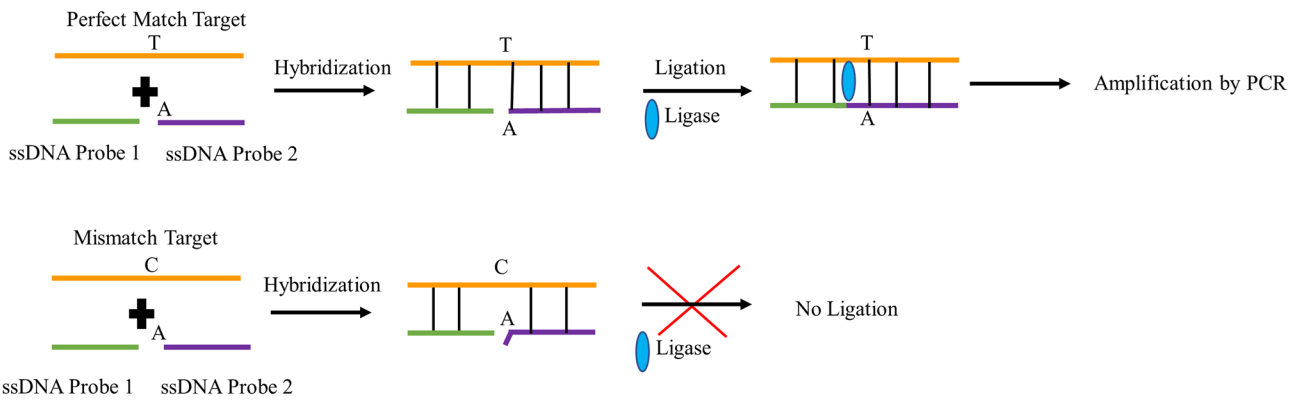

Fig. 4 Schematic diagram depicting the ligation reaction principle of the DNA ligase-assisted PCR

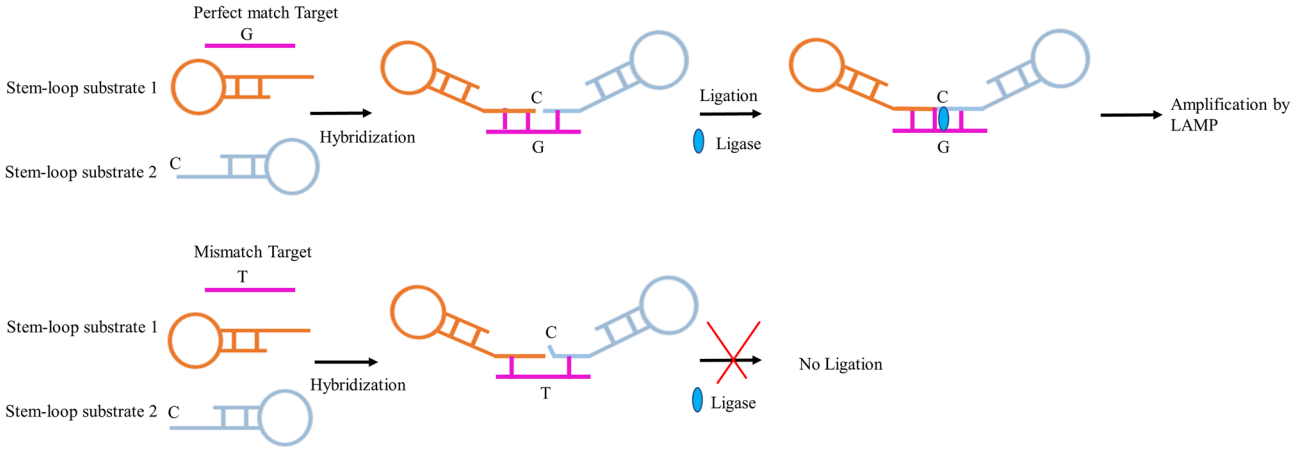

Fig. 5 Schematic illustration of the principle of DNA ligase-assisted LAMP ligation reaction

product, which served as an initial template for the LAMP reaction. When the target sequence is mutated, the bases cannot be matched and the ligation will not occur, thus distinguishing the mutation of the gene sites. For example, Wang et al. designed two stem-loop probes complementary to mutant DNA to carry out ligase reaction to initiate LAMP and quickly identified the mutation site via a fluorescence curve. The results showed that the fluorescence signal increased with an increase in the concentration of mutant DNA, and the mutant DNA with a concentration as low as 10^{-17} mol/L could be accurately detected. The method was simple and universal [46]. Zhang et al. also used this method to screen for breast cancer nucleic acid markers with high sensitivity [47]. Sun et al. reported a novel technique based on artificial mismatch ligation probes combined with ligase-assisted LAMP amplification to detect mutation sites in the exons of the p53 gene. They designed the position of mismatch introduction at the third position of the 3' end of the probe ligation range. They generated three overhanging bases, effectively avoiding the false-positive signal caused by the mismatch target as a template ligation. Their proposed method could clearly measure the target DNA concentration as low as 10^{-17} mol/L with ultra-high specificity, and the detection results were completely consistent with the sequencing results. Therefore, this method might be used in clinical and medical diagnosis [48]. Recently, Choi et al. developed a new double-site ligation-assisted LAMP (dLig-LAMP) method for bedside detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA. They designed three DNA oligonucleotide templates that bound perfectly to the target sequence and used the SplintR ligase double-site ligation to form complementary DNA, which was then further amplified. The ligation is impossible for a mismatch in the sequence to be tested or if one of the templates is not bound. This method is more specific in its selection compared with the reverse transcription LAMP, allowing selection at the ligation binding step without being performed in reverse transcription, thus overcoming problems, such as primer mismatches and false results. Studies showed that this method could achieve a detection limit of 10^{-15} mol/L in 1 h and also had the advantage in terms of high sensitivity and clear clinical selectivity [49]. This method may improve reverse transcription-related selectivity and bedside RNA detection.

Conclusions and future outlook

In the past, most people used sequencing or PCR to detect gene mutations. Certain new developments have been made in ligase-based nucleic acid amplification technology in recent years. The comparative analysis of these methods is presented in Table 2. In the past 5 years, many applications of LDR–PCR and LCR combined with electrochemical sensors have emerged, especially in the screening of genetic diseases and tumors and significant achievements have been made in guiding clinical medication. At present, SARS-CoV-2 mutations still occur frequently. Therefore, sensitive, specific, and field-applicable diversified detection techniques need to be developed. dLig-LAMP and LDR–PCR techniques in this study may provide new alternative ideas for SARS-CoV-2 detection. Unlike LCR, LDR, and PCR requiring a thermal cycler, RCA and LAMP need low experimental temperature. Moreover, they also exhibit improved sensitivity and specificity and hence may be considered for potential use in detecting gene mutations. However, this method can still be improved a lot in terms of cost and detection time.

In conclusion, the techniques described in this study reduced the sample requirements and could not only detect DNA in serum samples but also directly detect genomic DNA mutations in whole blood. Compared with PCR,

Table 2 Comparison of ligase-related nucleic acid amplification techniques for detecting gene mutations

Method	Applications (detection of diseases)	Sensitivity (mol/L)	Linear dynamic range (mol/L)	Specificity (%)	Cost	Time (min)
LDR	Novel coronavirus pneumonia	10^{-12}	10^{-12} to 10^{-11}	0.01	Usually relatively cheap	160
	Non-small-cell lung cancer	10^{-17}	10^{-16} to 10^{-12}	0.1	Relatively inexpensive with real-time PCR systems	50
LCR	Hematological tumor	10^{-15}	10^{-15} to 10^{-11}	0.1	Cost-effective LCR amplification with multiplex detection	90
	Non-small-cell lung cancer	10^{-19}	10^{-18} to 10^{-11}	0.01	Electrochemical probe using Fc-labeled DNA double strand, no purification required, but more expensive than conventional LCR	118
	Abnormal clopidogrel drug metabolism	10^{-16}	10^{-14} to 10^{-10}	0.01	Miniaturized, easy to operate, and relatively inexpensive instrument	110
	Novel coronavirus pneumonia	10^{-17}	10^{-17} to 10^{-11}	Not reported	Relatively inexpensive compared with CRISPR, which does not require the use of costly single-guide RNA	70
	Tumors	10^{-10}	0–10^{-7}	5	More convenient and economical than traditional PCR techniques or nanoparticle-based methods	33
RCA	Tumors	10^{-11}	10^{-11} to 10^{-8}	1	Direct connection of nanoparticles for SERS amplification without further signal amplification; cost-effective with inexpensive instrumentation	60
Ligase-assisted PCR	Squamous lung cancer	Not reported	Not reported	0.1	Relatively cheap with real-time PCR systems	110
Ligase-assisted LAMP	Breast cancer	10^{-16}	10^{-14} to 10^{-11}	Not reported	Lower cost than ribonucleotide-modified DNA probes	60
	Tumors	10^{-17}	10^{-17} to 10^{-11}	0.01	Modified probe using simple manual mismatch, slightly more expensive than traditional LAMP	66
	Novel coronavirus pneumonia	10^{-15}	10^{-18} to 10^{-8}	Not reported	Use of colorimetric and fluorescent detection is not expensive, and costs are relatively low	60

most of these techniques improved in terms of detection specificity. Compared with sequencing, these techniques improved in terms of reduction in the detection cost and were economically competitive. In future, it may be possible to replace sequencing and widely use these techniques in routine and large-scale rapid genetic mutation screening. Therefore, it is believed that the method of detecting gene mutations based on ligase nucleic acid

amplification technology will be continuously innovated and may help in disease diagnosis and designing a precise treatment regime.

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Author contributions All authors contributed to the work conception and design. Data collection and analysis were performed by YL, XJW, MHW, MYL, and HLW. The manuscript was written by YL and reviewed by LML and WX. All authors read and approved the final manuscript.

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Data availability Not applicable.

Declarations

Conflict of interest The authors declare no conflict of interest.

Ethical approval This work does not include any experiments conducted on human or animal participants by any of the authors.

Informed consent Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

References

1. Wang W, Wang X, Yang K, Fan Y (2021) Association of BCL2 polymorphisms and the IL19 single nucleotide polymorphism rs2243188 with systemic lupus erythematosus. J Int Med Res 49(5):3000605211019187. https://doi.org/10.1177/03000605211019187
2. Chen Y, Xie Y, Jiang Y, Luo Q, Shi L, Zeng S, Zhuang J, Lyu G (2021) The genetic etiology diagnosis of fetal growth restriction using single-nucleotide polymorphism-based chromosomal microarray analysis. Front Pediatr 9:743639. https://doi.org/10.3389/fped.2021.743639
3. Li T, Zou H, Zhang J, Ding H, Li C, Chen X, Li Y, Feng W, Kageyama K (2022) High-efficiency and high-fidelity ssDNA circularisation via the pairing of five 3'-terminal bases to assist LR-LAMP for the genotyping of single-nucleotide polymorphisms. Analyst 147(18):3993–3999. https://doi.org/10.1039/d2an01042a
4. Potapov V, Ong JL, Langhorst BW, Bilotti K, Cahoon D, Canton B, Knight TF, Evans TC Jr, Lohman GJS (2018) A single-molecule sequencing assay for the comprehensive profiling of T4 DNA ligase fidelity and bias during DNA end-joining. Nucleic Acids Res 46(13):e79. https://doi.org/10.1093/nar/gky303
5. Yan X, Zhang J, Jiang Q, Jiao D, Cheng Y (2022) Integration of the ligase chain reaction with the CRISPR-Cas12a system for homogeneous, ultrasensitive, and visual detection of microRNA. Anal Chem 94(9):4119–4125. https://doi.org/10.1021/acs.analchem.2c00294
6. Azuara-Liceaga E, Betanzos A, Cardona-Felix CS, Castañeda-Ortiz EJ, Cárdenas H, Cárdenas-Guerra RE, Pastor-Palacios G, García-Rivera G, Hernández-Álvarez D, Trasviña-Arenas CH, Diaz-Quezada C, Orozco E, Brieba LG (2018) The sole DNA ligase in entamoeba histolytica is a high-fidelity DNA ligase involved in DNA damage repair. Front Cell Infect Microbiol 8:214. https://doi.org/10.3389/fcimb.2018.00214
7. Lei Y, Washington J, Hili R (2019) Efficiency and fidelity of T3 DNA ligase in ligase-catalysed oligonucleotide polymerisations. Org Biomol Chem 17(7):1962–1965. https://doi.org/10.1039/c8ob01958d
8. Bilotti K, Potapov V, Pryor JM, Duckworth AT, Keck JL, Lohman GJS (2022) Mismatch discrimination and sequence bias during end-joining by DNA ligases. Nucleic Acids Res 50(8):4647–4658. https://doi.org/10.1093/nar/gkac241
9. Chen H, Cheng K, Liu X, An R, Komiyama M, Liang X (2020) Preferential production of RNA rings by T4 RNA ligase 2 without any splint through rational design of precursor strand. Nucleic Acids Res 48(9):e54. https://doi.org/10.1093/nar/gkaa181
10. Shimer GH Jr, Backman KC (1995) Ligase chain reaction. Methods Mol Biol 46:269–278. https://doi.org/10.1385/0-89603-297-3:269
11. Zhong GX, Ye CL, Wei HX, Yang LY, Wei QX, Liu ZJ, Fu LX, Lin XH, Chen JY (2021) Ultrasensitive detection of RNA with single-base resolution by coupling electrochemical sensing strategy with chimeric DNA probe-aided ligase chain reaction. Anal Chem 93(2):911–919. https://doi.org/10.1021/acs.analchem.0c03563
12. Malik A, Kumar D, Khan AA, Khan AA, Chaudhary AA, Husain SA, Kar P (2018) Hepatitis B virus precore G1896A mutation in chronic liver disease patients with HBeAg negative serology from north India. Saudi J Biol Sci 25(7):1257–1262. https://doi.org/10.1016/j.sjbs.2016.05.004
13. Malik A, Aldakheel F, Rabbani S, Alshehri M, Chaudhary AA, Alkholief M, Alshamsan A (2021) Based quick detection of hotspot G1896A mutation in patients with different spectrum of hepatitis B. J Infect Public Health 14(5):651–654. https://doi.org/10.1016/j.jiph.2021.01.013
14. Feng R (2021) Detection of gene mutations and 5-hydroxymethylcytosine by ligase chain reaction combined with flow technique (Master's thesis, Hebei University). https://doi.org/10.27103/d.cnki.ghebu.2021.001481
15. Hu F, Zhang W, Meng W, Ma Y, Zhang X, Xu Y, Wang P, Gu Y (2020) Ferrocene-labeled and purification-free electrochemical biosensor based on ligase chain reaction for ultrasensitive single nucleotide polymorphism detection. Anal Chim Acta 1109:9–18. https://doi.org/10.1016/j.aca.2020.02.062
16. Chen JY, Liu ZJ, Wang XW, Ye CL, Zheng YJ, Peng HP, Zhong GX, Liu AL, Chen W, Lin XH (2019) Ultrasensitive electrochemical biosensor developed by probe lengthening for detection of genomic DNA in human serum. Anal Chem 91(7):4552–4558. https://doi.org/10.1021/acs.analchem.8b05692
17. Liu ZJ, Yang LY, Wei QX, Ye CL, Xu XW, Zhong GX, Zheng YJ, Chen JY, Lin XH, Liu AL (2020) A novel ligase chain reaction-based electrochemical biosensing strategy for highly sensitive point mutation detection from human whole blood. Talanta 216:120966. https://doi.org/10.1016/j.talanta.2020.120966
18. Xue C, Yu X, Hu S, Luo M, Shen Z, Yuan P, Wu ZS (2020) Biocomputing label-free security system based on homogenous ligation chain reaction-induced dramatic change in melting temperature for screening single nucleotide polymorphisms. Talanta 218:121141. https://doi.org/10.1016/j.talanta.2020.121141

19. He R, Wang L, Wang F, Li W, Liu Y, Li A, Wang Y, Mao W, Zhai C, Ma L (2019) Pyrococcus furiosus argonaute-mediated nucleic acid detection. Chem Commun (Camb) 55(88):13219–13222. https://doi.org/10.1039/c9cc07339f
20. Wang L, He R, Lv B, Yu X, Liu Y, Yang J, Li W, Wang Y, Zhang H, Yan G, Mao W, Liu L, Wang F, Ma L (2021) Pyrococcus furiosus argonaute coupled with modified ligase chain reaction for detection of SARS-CoV-2 and HPV. Talanta 227:122154. https://doi.org/10.1016/j.talanta.2021.122154
21. Deng H, Wang J, Kong X, Zhang H, Wang T, Tian W, Yi T, Wang L (2019) Associations of BAFF rs2893321 polymorphisms with myasthenia gravis susceptibility. BMC Med Genet 20(1):168. https://doi.org/10.1186/s12881-019-0906-8
22. Ruiz C, Huang J, Giardina SF, Feinberg PB, Mirza AH, Bacolod MD, Soper SA, Barany F (2020) Single-molecule detection of cancer mutations using a novel PCR-LDR-qPCR assay. Hum Mutat 41(5):1051–1068. https://doi.org/10.1002/humu.23987
23. Xinya Z, Zhang Jian Lu, Chen XY, Zhidan L (2022) Detection of fragmented new coronavirus S gene D614G mutation by ligase detection reaction. J Biol 39(03):107–110. https://doi.org/10.3969/j.issn
24. Zhang W, Liu K, Zhang P, Cheng W, Zhang Y, Li L, Yu Z, Chen M, Chen L, Li L, Zhang X (2021) All-in-one approaches for rapid and highly specific quantifcation of single nucleotide polymorphisms based on ligase detection reaction using molecular beacons as turn-on probes. Talanta 224:121717. https://doi.org/10.1016/j.talanta.2020.121717
25. Li HM, Wang LJ, Tang F, Pan HF, Zhang TP (2022) Association between genetic polymorphisms of lncRNA NEAT1 and pulmonary tuberculosis risk, clinical manifestations in a Chinese population. Infect Drug Resist 12(15):2481–2489. https://doi.org/10.2147/IDR.S354863
26. Chen Y, Zhao Y, Li YB, Wang YJ, Zhang GZ (2018) Detection of SNPs of T2DM susceptibility genes by a ligase detection reaction-fluorescent nanosphere technique. Anal Biochem 540–541:38–44. https://doi.org/10.1016/j.ab.2017.11.003
27. Zhang J, Zhang H, Li K, Shi M (2018) Development of a polymerase chain reaction/ligase detection reaction assay for detection of CYP2C19 polymorphisms. Genet Test Mol Biomarkers 22(1):62–73. https://doi.org/10.1089/gtmb.2017.0086
28. Ruan Y, Zhang J, Mai S, Zeng W, Huang L, Gu C, Liu K, Ma Y, Wang Z (2021) Role of CASP7 polymorphisms in noise-induced hearing loss risk in Han Chinese population. Sci Rep 11(1):1803. https://doi.org/10.1038/s41598-021-81391-5
29. Sun H, Li Y, Si W, Hua T, Chen J, Kang S (2022) Genetic variation of PD-L1 gene affects its expression and is related to clinical outcome in epithelial ovarian cancer. Front Oncol 26(12):763134. https://doi.org/10.3389/fonc.2022.763134
30. Ye CY, Xin JR, Li Z, Yin XY, Guo SL, Li JM, Zhao TY, Wang L, Yang L (2022) ALDH2, ADCY3 and BCMO1 polymorphisms and lifestyle-induced traits are jointly associated with CAD risk in Chinese Han people. Gene 10(807):145948. https://doi.org/10.1016/j.gene.2021.145948
31. Zhou R, Li Y, Wang N, Niu C, Huang X, Cao S, Huo X (2022) MDM4 polymorphisms associated with the risk but not the prognosis of esophageal cancer in Cixian high-incidence region from northern China. Asia Pac J Clin Oncol. https://doi.org/10.1111/ajco.13746
32. Chen L, Chen X, Wang Y, Li S, Huang S, Wu Z, He J, Chen S, Deng F, Zhu P, Zhong W, Zhao B, Ma G, Li Y (2022) Polymorphisms of calgranulin genes and ischemic stroke in a Chinese population. J Inflamm Res 9(15):3355–3368. https://doi.org/10.2147/JIR.S360775
33. Wang W, Yu ZY, Song RH, He ST, Shi LF, Zhang JA (2022) Polymorphisms of ATG5 gene are associated with autoimmune thyroid diseases, especially thyroid eye disease. J Immunol Res 26(2022):3881417. https://doi.org/10.1155/2022/3881417
34. Yu W, Yang W (2022) Interlukin-27 rs153109 polymorphism confers the susceptibility and prognosis of aplastic anemia in Chinese population. Int J Lab Hematol 44(1):150–156. https://doi.org/10.1111/ijlh.13700
35. Ren Y, Tong E, Di C, Zhang Y, Xu L, Tan X, Yang L (2022) Association between ABCA1 gene polymorphisms and the risk of hypertension in the Chinese Han population. Front Public Health 20(10):878610. https://doi.org/10.3389/fpubh.2022.878610
36. Chen J, Kang S, Wu J, Zhao J, Si W, Sun H, Li Y (2022) CTLA-4 polymorphism contributes to the genetic susceptibility of epithelial ovarian cancer. J Obstet Gynaecol Res 48(5):1240–1247. https://doi.org/10.1111/jog.15186
37. Ni P, Wang G, Wang Y, Liu K, Chen W, Xiao J, Fan H, Ma X, Li Z, Shen K, Xu Z, Yang L (2022) Correlation of MIF-AS1 polymorphisms with the risk and prognosis of gastric cancer. Pathol Res Pract 233:153850. https://doi.org/10.1016/j.prp.2022.153850
38. Zhang TP, Li R, Li HM, Xiang N, Tan Z, Wang GS, Li XM (2022) The contribution of genetic variation and aberrant methylation of aryl hydrocarbon receptor signaling pathway genes to rheumatoid arthritis. Front Immunol 2(13):823863. https://doi.org/10.3389/fimmu.2022.823863
39. Woo CH, Jang S, Shin G, Jung GY, Lee JW (2020) Sensitive fluorescence detection of SARS-CoV-2 RNA in clinical samples via one-pot isothermal ligation and transcription. Nat Biomed Eng 4(12):1168–1179. https://doi.org/10.1038/s41551-020-00617-5
40. Jonchhe S, Selvam S, Karna D, Mandal S, Wales-McGrath B, Mao H (2020) Ensemble sensing using single-molecule DNA copolymers. Anal Chem 92(19):13126–13133. https://doi.org/10.1021/acs.analchem.0c02196
41. Ma Q, Gao Z (2018) A simple and ultrasensitive fluorescence assay for single-nucleotide polymorphism. Anal Bioanal Chem 410(13):3093–3100. https://doi.org/10.1007/s00216-018-0874-4
42. Kim DM, Zhang S, Kim M, Kim DE (2020) Fluorometric detection of low-abundance EGFR exon 19 deletion mutation using tandem gene amplification. J Microbiol Biotechnol 30(5):662–667. https://doi.org/10.4014/jmb.2004.04010
43. Chung CH, Kim JH (2018) One-step isothermal detection of multiple KRAS mutations by forming SNP specific hairpins on a gold nanoshell. Analyst 143(15):3544–3548. https://doi.org/10.1039/c8an00525g
44. Li B (2021) T4 DNA ligase-assisted single base mutation detection by PCR (Master's thesis, Inner Mongolia University). https://doi.org/10.27224/d.cnki.gnmdu.2021.000336
45. Liu Z, Guo C, Zhang Y, Zhao L, Hao Z (2021) Rapid and sensitive detection of the colistin resistance gene mcr-3 by loop-mediated isothermal amplification and visual inspection. Microb Drug Resist 27(10):1328–1335. https://doi.org/10.1089/mdr.2020.0129
46. Wang H, Wang H, Sun Y, Liu X, Liu Y, Wang C, Zhang P, Li Z (2020) A general strategy for highly sensitive analysis of genetic biomarkers at single-base resolution with ligase-based isothermal-lyexponential amplification. Talanta 212:120754. https://doi.org/10.1016/j.talanta.2020.120754
47. Zhang M, Wang H, Wang H, Wang F, Li Z (2021) CRISPR/Cas12a-Assisted ligation-initiated loop-mediated isothermal amplification (CAL-LAMP) for highly specific detection of microRNAs. Anal Chem 93(22):7942–7948. https://doi.org/10.1021/acs.analchem.1c00686
48. Sun Y, Han B, Sun F (2021) Ultra-specific genotyping of single nucleotide variants by ligase-based loop-mediated isothermal amplification coupled with a modified ligation probe. RSC Adv 11(28):17058–17063. https://doi.org/10.1039/d1ra00851j
49. Choi MH, Lee J, Seo YJ (2022) Dual-site ligation-assisted loop-mediated isothermal amplification (dLig-LAMP) for colorimetric

and point-of-care determination of real SARS-CoV-2. Mikrochim Acta 189(5):176. https://doi.org/10.1007/s00604-022-05293-7