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High-fidelity telomerase activity assay based on light-triggered nucleic acid separation system for the diagnosis of bladder cancer

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ABSTRACT

In conventional methods for telomerase activity assay, the obtained telomerase sample contains a large amount of impurity, which seriously affect the accuracy of the assay. Herein, we propose a light-triggered nucleic acid separation strategy to realize high fidelity Telomerase activity assay (**Lit-Telo**). In the light-triggered nucleic acid separation system, a 5′ terminal biotinylated and photo-cleavable (PC) linker-functionalized telomerase substrate probe (Bio-PCTS) is designed. Telomerase extends telomeric repeat DNA (TTAGGG) to the 3′ terminal of Bio-PCTS probe to produce telomerase extension product, which can be captured by streptavidin coated 96-well plate. Thus, the impurity can be removed from the reaction to realize the purification of telomerase extension product. A few seconds of UV light irradiation can disrupt the PC-linker in Bio-PCTS probe, allowing the easy and quick release of the telomerase extension product DNA fragment from the bottom of 96-well plate into the reaction solution for subsequent detection. Asymmetric-PCR-based TRAP and LbaCas12a/crRNA system were elucidated and optimized to realize the enhanced detection of telomerase activity. The proposed Lit-Telo platform achieved a limit-of-detection of telomerase activity equivalent to 8 HeLa cells. 26 bladder specimens were collected for telomerase activity assay using both fluorescence detection based Lit-Telo (Fluo Lit-Telo) visual detection based Lit-Telo (Visual Lit-Telo). ROC (receiver operating characteristic curve) analysis of the data indicated the good detection accuracy of Fluo Lit-Telo and Visual Lit-Telo methods with the AUC value of 93.94% and 92.12%, respectively. These results demonstrated the potential of the Lit-Telo platform in the *in vitro* diagnosis of bladder cancer.

1. Introduction

Telomerase is a ribonucleoprotein complex, also known as telomere terminal transferase, which is responsible for the progressive synthesis of telomeric DNA repeats (TTAGGG) on the terminal of chromosome (Ghanim et al., 2021). Telomerase can reverse the loss of DNA in each round of replication, thereby compensating for the gradual shortening of telomeres caused by the end replication problem of telomere(Shay and Wright, 2019; Zhang et al. 2021, 2022). A prevalent feature of cancer is indefinite cell division associated with the activation of telomere maintenance mechanisms, and the researchers have found that telomerase is broadly expressed in cancer cells, with telomerase reactivation observed in most (approximately 90%) cancer cell lines and low or no telomerase activity in normal somatic cells(Guterres and Villanueva, 2020; Shen et al., 2022). Nowadays, a large number of studies clearly show that telomerase is a biomarker that can reflect the early occurrence of tumors, but also as a universal molecular marker and potential therapeutic target for early diagnosis, prognosis, and treatment of

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tumors(Harley, 2008; Shen et al., 2023). The samples for telomerase activity assay can be prepared using cells, tissues, and body fluids (including plasma, pleural effusion, ascites, bronchial lavage, pancreatic duct irrigation fluid, oral rinses, urine, bladder washings, etc.)(Wang et al. 2018, 2021). Therefore, telomerase has been regarded as a crucial molecular marker for non-invasive *in vitro* diagnosis of cancer.

In 1987, Kim and Shay invented the TRAP (telomeric repeat amplification protocol) method for the detection of telomerase activity(Kim et al., 1994). Then, a series of new methods have been developed based on TRAP, such as real-time quantitative TRAP (RQ-TRAP), droplet digital TRAP (ddTRAP) and FCS-TRAP (Fluorescence correlation spectroscopy-TRAP), etc.(Ludlow et al., 2014; Su et al., 2018; Wege et al., 2003). Other optical detection-based methods for telomerase activity detection have also been extensively developed, including TE-RPA (Telomerase extension recombinase polymerase amplification), Cas12a-LSMBs (Cas12a-lighting up single microbeads), colorimetric code strategy, photoelectrochemical (PEC) sensing platform, etc. (Cheng et al., 2021; Wang et al. 2023a, 2023c, 2025). Furthermore, many different sensing platforms based on amplification free strategy have been constructed for telomerase activity assay, but most of these methods require sophisticated preparation process of nanoprobes or rely on specialized apparatus, thus limiting the further application of these platforms(Li et al., 2019; Liu et al., 2022; Wang et al. 2023a, 2023c; Wu et al., 2023; Zou et al., 2020). In the commonly used method for preparing of telomerase samples, the collected cells are lysed to release telomerase into lysis buffer(Herbert et al., 2006). Therefore, the prepared telomerase sample is a crude cell extract containing a large amount of impurity, that is, almost all the soluble components from the cytoplasm, such as enzymes, ions, peptides, etc., which would exert an adverse effect on telomerase activity assay. For instance, the degradation of telomerase extension product by nuclease and the inhibition of enzymatic reactions by peptides, effecting the detection sensitivity leading to the false-negative results when detecting complex sample has tiny telomerase activity(Cheng et al., 2021; Yaku et al., 2017). Therefore, although the existing methodologies for telomerase activity detection have achieved good sensitivity in the analysis of telomerase samples from cultured cell lines, most of these methods cannot eliminate the risk of the false negative result or underestimation of telomerase activity.

The CRISPR system has revolutionized the research of molecular diagnostic technologies. The CRISPR/Cas proteins (e.g., Cas9, Cas12a/b, Cas13a/b/c, Cas14a, etc.) display programmable and targeted gene binding, *cis*-cleavage and *trans*-cleavage activity, which can convert the event of target gene recognition into the changes in optical or electrical signals to realize precise molecular diagnosis(Wang et al., 2023b; Yang et al., 2023; Yin et al., 2024). CRISPR/Cas systems have been employed in the detection of genes, small molecules, and proteins, etc.(Chen et al., 2024; Klebes et al., 2024; Wang et al., 2023b; Yang et al., 2023; Zhu et al., 2023). The CRISPR/Cas technology also demonstrate excellent detection ability that can achieve the detection of target gene at fM to aM level, and CRISPR/Cas-based digital droplet platforms enable amplification free detection of DNA or RNA at the single-molecule level (Moon and Liu, 2023; Tian et al., 2021; Yue et al., 2021). Therefore, CRISPR/Cas has significant potential for the construction of highly sensitive and specific detection platform for telomerase activity assay.

The author has proposed a colorimetric code platform for the monitoring of false-negative detection of telomerase activity assay in a previous work, but the method cannot eliminate the inhibitory effect arising from the high concentrated impurity of complex telomerase sample (Cheng et al., 2021). In this work, we report a **Li**ght-**t**riggered nucleic acid separation system and enhanced CRISPR-Cas12a detection strategy to enable high fidelity **Telo**merase activity assay, which we named the **Lit-Telo**. In the proposed Lit-Telo platform, a biotin and PC (Photo-Cleavable) linker-functionalized telomerase substrate primer is designed to obtain the Bio-PCTS probe. Telomerase recognizes and extends the telomeric DNA repeat at the 3′ terminus of Bio-PCTS probe, which can be captured and purified using a streptavidin coated 96-well plate, enabling the separation of telomerase extension product from telomerase extension mixture. Subsequently, the PC linker inside the enriched Bio-PCTS probe can be broken under 365 nm UV irradiation, allowing the facile and rapid separation and collection of telomerase extension products for further analysis. To enhance the sensitivity and specificity of the detection, CRISPR/Cas12a system for targeting analysis of telomerase extension product has been designed and validated. The proposed Lit-Telo platform achieved a limit of detection of 8 HeLa cells. Finally, the Lit-Telo system was applied for the detection of 26 bladder tissue specimens. In addition to the developed fluorescent detection based Lit-Telo assay (Fluo Lit-Telo), we further demonstrated the feasibility of visual detection based Lit-Telo concept (Visual Lit-Telo) using a conventional CRISPR-LFA method for the testing of clinical specimens with naked eye. The ROC (receiver operating characteristic curve) analysis of the data for detection of clinical specimens revealed that Fluo Lit-Telo and Visual Lit-Telo achieved the AUC (Area Under Curve) value of 93.94% and 92.12%, respectively. These results indicate the potential of the developed Lit-Telo platform in the utilization of cancer diagnosis.

2. Experimental section

2.1. Light-triggered nucleic acid separation

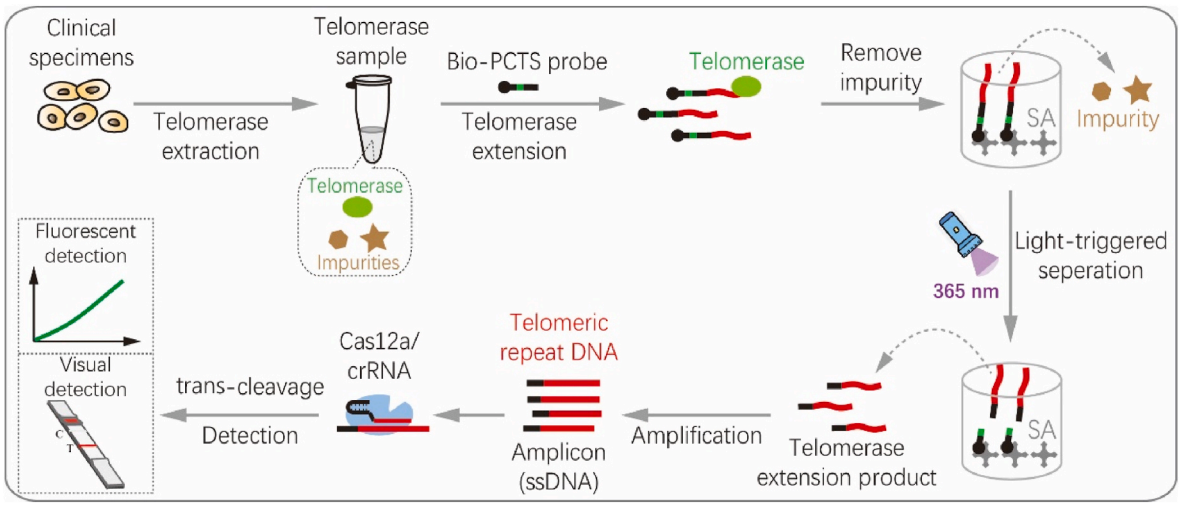
The protocol for light-triggered nucleic acid isolation experiment is shown below. 12.6 μL nuclease free water, 2 μL 10 × TRAP buffer, 0.4 μL dNTP (2.5 mM each), 1 μL λDNA (0.2 mg/mL), 2 μL telomerase sample or NP40 buffer, and 2 μL Bio-PCTS probe (1 μM). The mixture was incubated at 37 °C for 90 min and then transferred into 96-well plate containing 20 μL buffer 1 (10 mM Tris-HCl, 2 M KCl, pH 7.5). The plate was incubated at 37 °C and shaking at 800 rpm for 10 min. Then, the solution was removed and 40 μL buffer 2 (10 mM Tris-HCl, 1 M NaCl, pH 7.5) was added to wash the bottom of the plate. 20 μL 1 × TRAP buffer was introduced into the plate and the plate was irradiated with 365 nm UV (Ultraviolet) for 30 s. Subsequently, the solution was collected and mixed with 2 μL 10 × TRAP buffer, 1 μL dNTP (2.5 mM each), 1 μL TS primer (100 μM), 0.5 μL ACX primer (1 μM), 1 μL BSA (20 mg/mL), 0.5 μL Taq DNA polymerase (5U/μL), 24 μL nuclease free water in a 200 μL PCR tube. The AS-TRAP reaction was performed according to the following procedure: 95 °C for 5 min; 95 °C for 30 s, 60 °C for 30 s, 72 °C for 60 s, 30 cycles; 72 °C for 5 min. The obtained amplicon of AS-TRAP was used for CRISPR/Cas12a assay.

For more details of the experiments, please refer to the Supplementary Material.

3. Results and discussion

3.1. Principle of the Lit-Telo platform

The principle of the proposed Lit-Telo platform is demonstrated in Scheme 1. Firstly, the sample (such as cells, tissues, plasma, etc.) is collected and treated with lysis buffer to obtain the telomerase sample, which is a crude whole cell extract containing telomerase and almost all the soluble components in the cytoplasm, such as enzymes, ions, peptides, etc., which would exert an adverse effect on telomerase activity assay. For instance, DNase can degrade the elongated telomeric repeat DNA by telomerase, or peptides and small molecular may have inhibitory effect on the enzymatic reactions of the detection, thus leading to false-negative assay when evaluating complex sample with tiny telomerase activity. To address this problem, we develop the **Li**ght-**t**riggered nucleic acid separation system and enhanced CRISPR-Cas12a detection strategy to enable high fidelity **Telo**merase activity assay, which we named the **Lit-Telo**. In the Lit-Telo platform, as seen in Scheme 1, the conventional telomerase substrate primer is modified to construct the photo-cleavable Bio-PCTS probe, the 3′ terminus of which is linked to



Scheme 1. Principle illustration of the Lit-Telo platform.

biotin via a PC (Photo-Cleavable) linker group, which can be disrupted under 365 nm UV irradiation(Hu et al., 2022). Telomerase can recognize the TS primer partial of Bio-PCTS probe, on which telomeric repeat DNA (TTAGGG) is continuously added to generate the telomerase extension product. Then, the telomerase extension reaction mixture is added into 96-well plate, the bottom of which is coated with streptavidin. The biotin labeled Bio-PCTS probe carrying telomerase extension product would be captured and enriched to the bottom of 96-well plate through the affinity between biotin and streptavidin. Then, purified telomerase extension product is left in the 96-well plate after the removement of the telomerase extension reaction mixture including the impurities and inhibitors from telomerase sample. Next, a few seconds of 365 nm UV irradiation leads to the break of the PC-linker in the Bio-PCTS probe enriched at the bottom of 96-well plate, allowing the rapid release of the telomerase extension product portion of the Bio-PCTS probe from the bottom of 96-well plate into solution, thus completing the light-triggered nucleic acid separation process. Finally, the separated telomerase extension product is amplified by asymmetric TRAP to produce a large amount of ssDNA amplicon, which would be robustly detected by an optimized CRISPR/Cas12a system without the limitation of PAM (protospacer adjacent motif) recognition and dsDNA unwinding process required in the detection of dsDNA target. Telomerase activity assay using Lit-Telo platform can be realized in both fluorescent detection format and lateral flow assay based visual detection format.

Comparing with conventional one-pot style telomerase activity assay, the proposed light-triggered nucleic acid separation system designed in this study provide two improvements to enhance the accuracy of telomerase activity assay: (1) Remove the telomerase extension product from the telomerase extension mixture, and reduce the degradation of the nuclease on the telomerase extension product and subsequent reaction components; (2) Remove inhibitory components from telomerase samples and eliminate their inhibitory effects on detection reactions, such as the inhibitory effects of DNase, RNase, peptides and other components on nucleic acid amplification and CRISPR reaction.

3.2. Development of detection system targeting telomerase extension product

The Lit-Telo platform is consisted of two parts, that is, the light-triggered nucleic acid separation system and the subsequent DNA detection system, which is constructed based on DNA amplification and CRISPR/Cas12a-based DNA detection modules. Firstly, to realize highly specific and sensitive detection of separated telomerase extension product, we would like to construct the DNA amplification module based on conventional TRAP method. However, considering the distinct

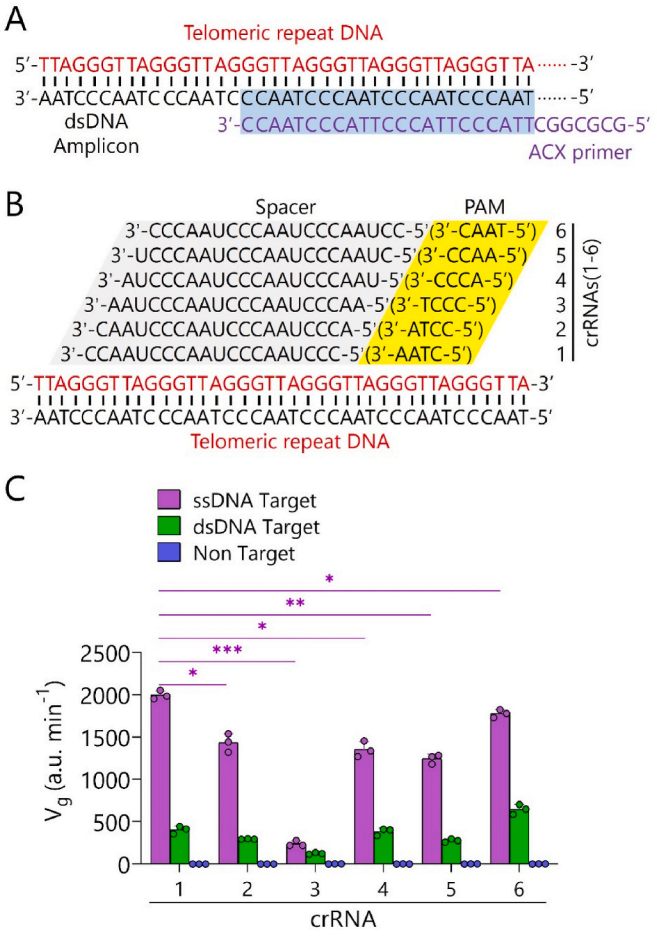


Fig. 1. Design and evaluation of crRNAs targeting telomerase extension product. (A). Schematic illustration of the complementarity of ACX primer with telomeric DNA repeats in conventional TRAP reaction; (B). Schematic demonstration of the design of crRNAs targeting telomeric DNA repeats; (C). The initial reaction rate of the designed LbaCas12a/crRNAs for detection of synthetic DNA targets. (n = 3 technical replicates; two-tailed Student's t-test: *P <0.1; **P <0.01; ***P <0.001; bars represent the mean ± s.d.; *Vg* represents the initial rate of the reaction calculated based on a formula: $Vg = \Delta F/10$, the average fluorescence intensity change (ΔF) between 1 min and 11 min; a.u., arbitrary units.).

DNA sequence arrangement of TRAP amplicon, the design and screening of LbaCas12a/crRNA was firstly performed. As shown in Fig. 1A, the complementary strand of the telomeric repeat DNA (TTAGGG) sequence in the dsDNA amplicon is very similar to the ACX primer used in TRAP, indicating that ACX primer would also be recognized by crRNAs targeting the complementary strand of the telomeric repeat DNA sequence. Thus, only the telomeric repeat DNA strand of the TRAP amplicon can be used as the target of LbaCas12a/crRNA. As demonstrated in Fig. 1B, there were 6 different crRNAs targeting the telomeric repeat DNA were designed. It is noteworthy that neither of the 6 crRNAs had a classical PAM site (TTTN or TTN) flanking the target sequence, so the detection efficiency of these crRNAs for dsDNA amplicons of telomeric DNA repeats was unclear. Thus, the evaluation and screening of the detection efficiency of the designed crRNAs was reasonable and necessary for the construction of the detection system. Fluorescent assay was conducted to monitor the activation of *trans*-cleavage activity of different LbaCas12a/crRNAs toward ssDNA (single-stranded DNA) signal reporter. As seen in Fig. 1C, the designed crRNAs showed significant differences in detection efficiency for synthetic dsDNA target, among which crRNA3 displayed relatively low efficiency when detecting high concentration of synthetic target. We noticed that the PAM sequence corresponding to crRNA3 was one of the most unfavorable for LbaCas12a, and such phenomenon was also consistent with previous study(Yamano et al., 2017). On the other hand, the efficiency of the designed crRNAs was evaluated using a ssDNA target (telomeric DNA repeats) at the same concentration as that of the synthetic dsDNA target. As depicted in Fig. 1C, the detection efficiency of LbaCas12a/crRNA for ssDNA target was significantly superior to that for dsDNA target, as it is not limited by the PAM recognition and dsDNA unwinding process. The results in also show that crRNA1 display the optimal efficiency in the detection of ssDNA target. Thus, crRNA1 was selected for further use in subsequent experiments.

3.3. Establishment of asymmetric TRAP (AS-TRAP)

Based on the results in Fig. 1C, the ratio of TS and ACX primer in conventional TRAP was adjusted from 1.5 to 25 to realize asymmetric PCR-based TRAP (AS-TRAP, TS:ACX = 1 μM:0.04 μM), enabling the production of a large amount of ssDNA amplicon, which displayed stronger activation efficiency upon LbaCas12a/crRNA system. Then the amplicon of AS-TRAP was analyzed using PAGE, and we found a significant reduction in the band of amplicon that had incubated with ExoI enzyme (ssDNA-specific nuclease), while ExoI did not degrade the dsDNA amplicon from conventional TRAP, indicating that the amplicon of AS-TRAP was mainly composed of ssDNA (Fig. 2A). Next, fluorescent CRISPR assay was performed using LbaCas12a/crRNA1 for the analysis of amplicon produced by TRAP and AS-TRAP, respectively. And we also explored the difference in the *trans*-cleavage activation of LbaCas12a detecting equal quality of amplicon from TRAP and AS-TRAP, respectively. As illustrated in Fig. 2B and Fig. S1, amplicon from AS-TRAP induced stronger activation of *trans*-cleavage activity of LbaCas12a than that from conventional TRAP. Such results indicated that the use of AS-TRAP method would help to improve the performance for detection of telomerase activity. Subsequently, we optimized and screened the AS-TRAP system. We tested a combination of different concentrations of TS primers (1–2 μM) and ACX primers (0.01–0.2 μM), and the results (Fig. 2C) showed that different TS/ACX primer concentration and ratio did not induce significant difference in the result of the assay. But we noted that the lower background signal was generated when using a lower concentration of ACX primer, where TS:ACX = 2 μM:0.01 μM showed the best signal-to-noise ratio and this parameter was used for subsequent experiments.

3.4. Construction of light-triggered nucleic acid separation system

Based on established the AS-TRAP and LbaCas12a/crRNA1 systems,

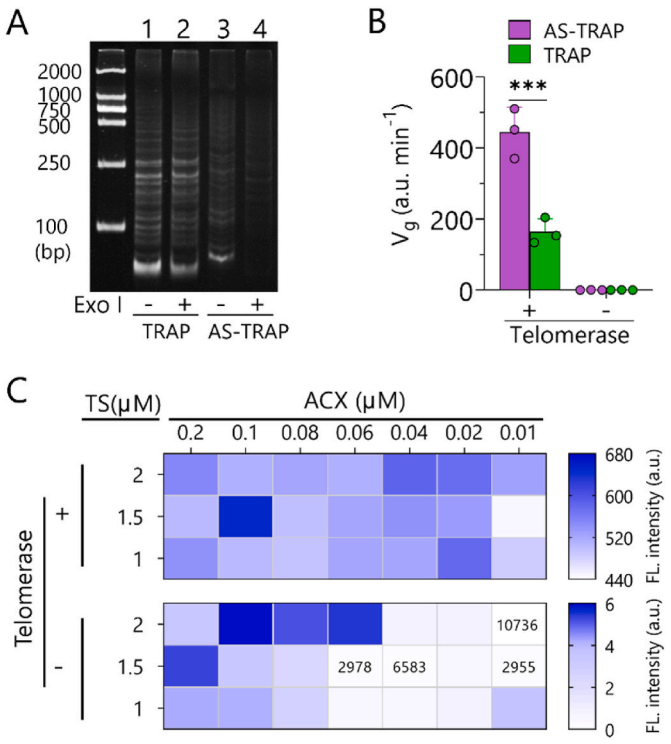


Fig. 2. Construction of the AS-TRAP method. (A). PAGE analysis of amplicons from conventional TRAP and AS-TRAP that have incubated with Exo I. Lane 1–2: TRAP amplicons incubated without and with Exo I; Lane 3–4: AS-TRAP amplicons incubated with and without Exo I; (B). The difference in the *trans*-cleavage activation of LbaCas12a/crRNA1 for detection of amplicons produced by conventional TRAP and AS-TRAP; (C). Optimization of the concentration of TS and ACX primer used in AS-TRAP. Telomerase equivalent to 250 HeLa cells was used in Fig. 2. (n = 3 technical replicates; two-tailed Student's t-test: ***P <0.001; bars represent the mean ± s.d.; *Vg* represents the initial rate of the reaction calculated based on a formula: $Vg = \Delta F/10$, the average fluorescence intensity change (ΔF) between 1 min and 11 min; a.u., arbitrary units.).

we carried out the design and evaluation of the separation system of light-triggered telomerase extension product. First, we constructed the photocleavable Bio-PCTS probe. As shown in Fig. 3A, a biotin and poly C spacer sequence were added to the 5' terminus of TS primer used in TRAP method, enabling the capture and enrichment of telomerase extension product. A photocleavable linker (PC linker) was inserted between the ploy C spacer and TS primer partial to construct the Bio-PCTS probe. The principle of light-triggered nucleic acid separation strategy based on Bio-PCTS probe is demonstrated in Fig. 3B. The prepared telomerase sample was introduced into a mixture containing Bio-PCTS probe. Telomerase recognized the TS primer partial of Bio-PCTS probe, and added telomeric DNA repeats (TTAGGG) to 3' end of the probe, that is, the telomerase extension product. Then, the mixture was transferred into a 96-well plate, the bottom of which was coated with streptavidin. The telomerase extension product was captured and enriched at the bottom of the 96-well plate through the affinity interaction between streptavidin and biotin labeled on the Bio-PCTS probe. Next, telomerase extension mixture containing impurities from the telomerase sample was removed and leaved the captured Bio-PCTS probe at the bottom of the 96-well plate. Finally, the telomerase extension product was released from the bottom of the 96-well plate after a few seconds of 365 nm UV illumination, which broke the PC linker inside the Bio-PCTS probe, allowing the easy and rapid separation and collection of telomerase extension product for further analysis.

We tested the function of the Bio-PCTS probe by treating the captured telomerase extension product in 96-well plate with or without 365 nm UV irradiation and collecting the solution for subsequent AS-

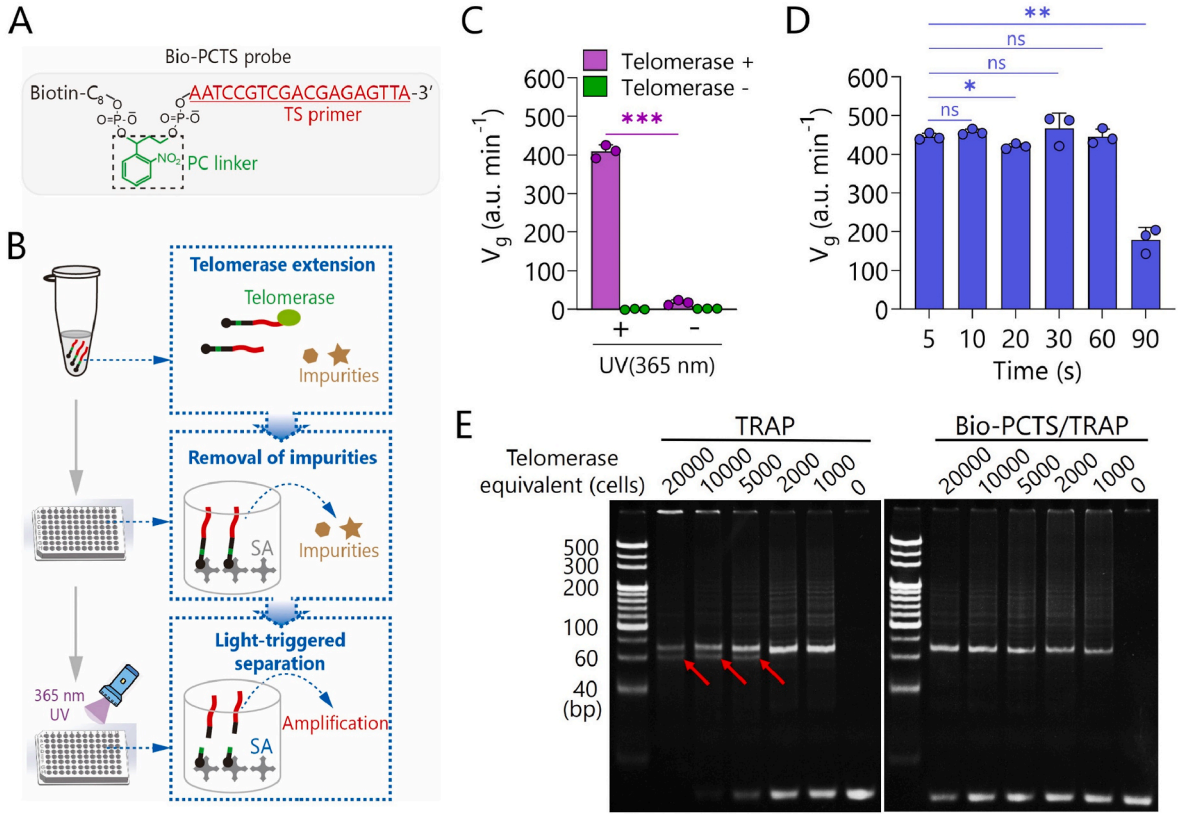


Fig. 3. Design and validation of light-triggered nucleic acid separation system. (A). Schematic demonstration of the design of Bio-PCTS probe; (B). Schematic illustration of the work-flow of the proposed light-triggered telomerase extension product purification and isolation system; (C). Feasibility verification of light-triggered nucleic acid separation system; (D). Optimization of the time required for 365 nm UV irradiation; (E). PAGE analysis of amplicons from TRAP and Bio-PCTS probe system assisted TRAP. Arrows indicate the presence of TRAP amplicon degraded by nuclease from telomerase sample. Telomerase equivalent to 250 HeLa cells was used in figure C–D; 100 nM Bio-PCTS probe was used in figure C–E. (n = 3 technical replicates; two-tailed Student's t-test: *P <0.1; **P <0.01; ***P <0.001; bars represent the mean ± s.d.; Vg represents the initial rate of the reaction calculated based on a formula: Vg = ΔF/10, the average fluorescence intensity change (ΔF) between 1 min and 11 min; a.u., arbitrary units.).

TRAP and CRISPR assay. The results in Fig. 3C show that the telomerase positive detection illuminated with UV generated extremely pronounced signal, while no UV light treatment only induced very weak signal generation. Such results revealed that 365 nm UV can break the PC linker inside Bio-PCTS probe and enable the separation and collection of telomerase extension product. In addition, we also confirmed that a 5 s-UV light illumination was sufficient for the induction of Bio-PCTS probe breaking (Fig. 3D). It is noteworthy that previous studies have proved the stability of PC-linker functionalized probe under ambient light condition, indicating that the stability of the Bio-PCTS probe used in this work would not be affected by ambient light (Chen et al., 2020).

We further investigated whether this light-induced separation strategy could effectively eliminate the adverse effect of impurity (such as enzyme, protein) from telomerase samples. As shown in Fig. 3E, short bands (arrow indicated) can be seen in the amplicon from conventional TRAP, which was consistent with the previous report of Yaku et al., due to the partial degradation of the telomerase extension product by DNase from telomerase sample, resulting in the production of amplicon with a length of approximately 44 bp seen in PAGE (polyacrylamide gel electrophoresis) result (Yaku et al., 2017). However, there were no such short amplificon were observed in the amplification product of Bio-PCTS probe assisted TRAP, revealing that the proposed photo-induced nucleic acid separation strategy can effectively eliminate the inhibitory effect of the detection by removing impurities introduced from telomerase sample. These results indicated the successful construction of Lit-Telo platform.

Furthermore, parameter optimization for the detection platform was conducted. The concentration of Bio-PCTS probe was evaluated and the results in Fig. 4A demonstrated that the use of both 100 and 200 nM Bio-PCTS probe in the assay induced the generation of the highest *trans*-cleavage reaction rate value of LbaCas12a/crRNA1 when detecting telomerase equivalent to 250 HeLa cells. However, for the detection of telomerase equivalent to 50 HeLa cells, the 200 nM Bio-PCTS probe generated a relatively higher signal compared to the 100 nM probe. Therefore, 200 nM was selected as the optimal concentration of Bio-PCTS probe for further use. The time used for telomerase extension was also studied, and the results depicted in Fig. 4B shown that incubation for 90 min was sufficient for efficient telomerase activity assay. Intriguingly, we noted that longer telomerase extension time led to the reduction of detection signal, which maybe arising from the degradation of telomerase extension product by DNase introduced from telomerase sample.

3.5. *Performance evaluation of the Lit-Telo system*

First, we evaluated the detection sensitivity of the developed Lit-Telo system. The activity of telomerase equivalent to different amounts of HeLa cells (1, 2, 4, 8, 20, 40, 100, 200, 400, 1000, 2000, 4000, 8000) was analyzed. As shown in Fig. 5A, the initial *trans*-cleavage reaction rate of LbaCas12a/crRNA1 enhanced gradually with the increasing of telomerase concentration, and reached a plateau with at the telomerase concentration equivalent to 4000 cells. The Lit-Telo platform achieved a limit of detection (LOD) of 8 HeLa cells. In addition, as depicted in Fig. 5B, the reaction rate of CRISPR assay was linear related to the logarithm of cell number in the range of 200–4000 cells ($R^2 = 0.9955$). We further validated the specificity of the Lit-Telo assay by testing for

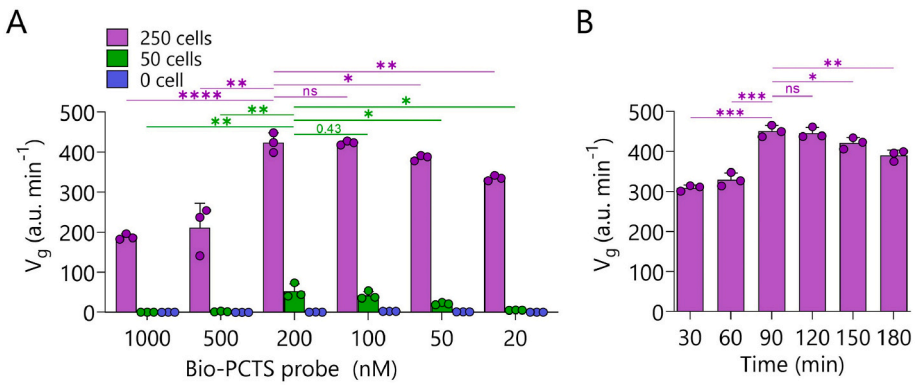


Fig. 4. Parameter optimization of the Lit-Telo platform. (A). Optimization of the concentration of Bio-PCTS probe used in Lit-Telo platform; (B). Optimization of the time for telomerase extension reaction used in Lit-Telo platform. Telomerase equivalent to 250 and 50 HeLa cells was used in Fig. 4. (n = 3 technical replicates; two-tailed Student's t-test: *P <0.1; **P <0.01; ***P <0.001; ****P <0.0001; bars represent the mean ± s.d.; Vg represents the initial rate of the reaction calculated based on a formula: Vg = ΔF/10, the average fluorescence intensity change (ΔF) between 1 min and 11 min; a.u., arbitrary units.).

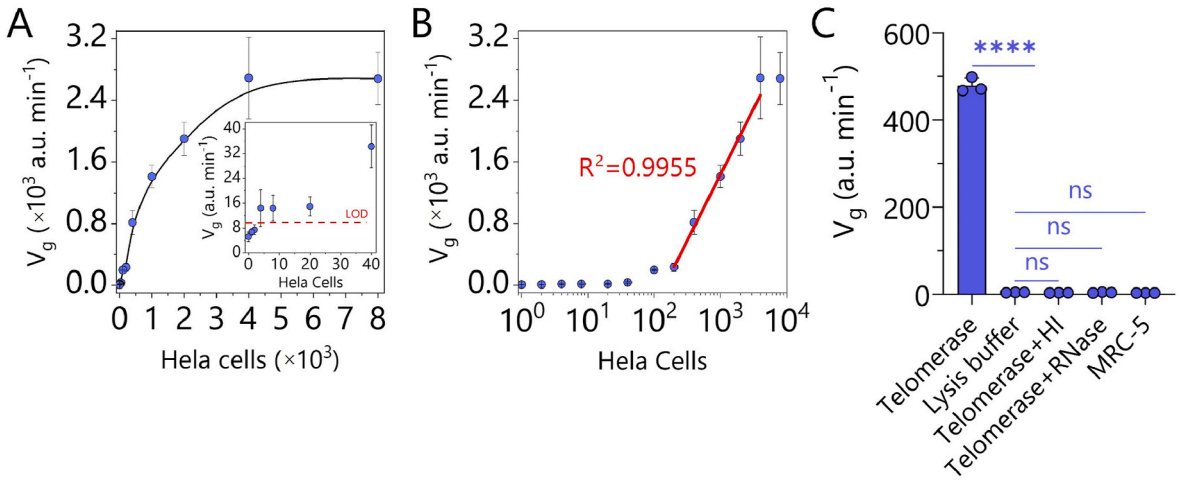


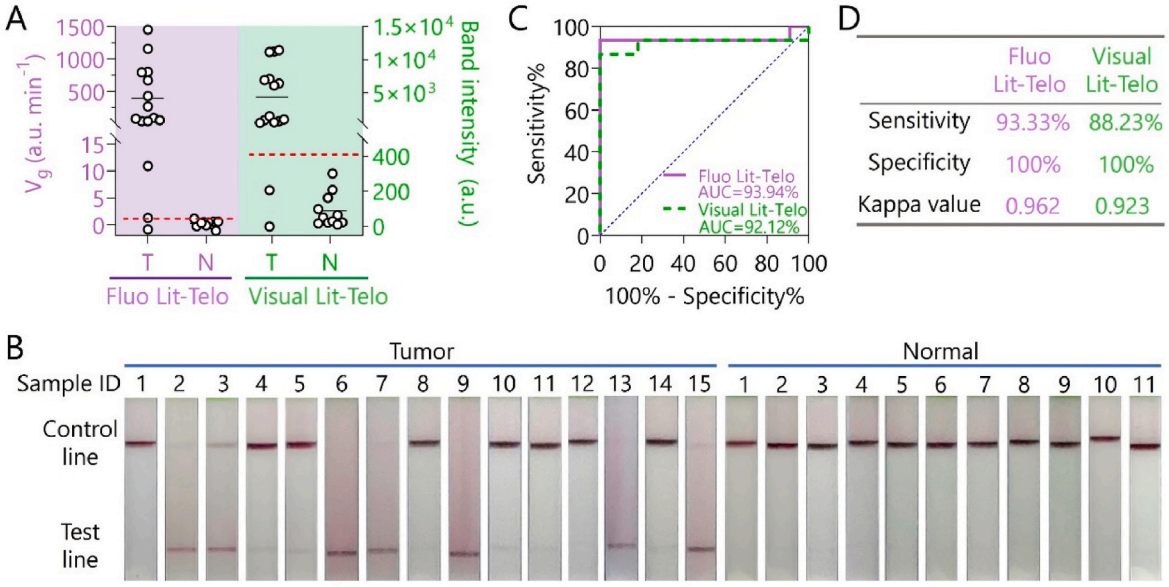
Fig. 5. Analytical performance evaluation of the Lit-Telo system. (A). Correlation curve between the *Vg* value of Lit-Telo assay and the amount of HeLa cells. Inset, limit of detection of the Lit-Telo platform for telomerase activity assay. The red dash line represents LOD (limit of detection), calculated based on the formula: LOD = $Mean_{negative}$+3SD. (B). The linear relationship between the *Vg* value of Lit-Telo assay and the amount of HeLa cells. (C). Examination of the specificity of Lit-Telo platform for telomerase activity assay. Telomerase + HI, heat-inactivated telomerase; Telomerase + RNase, RNase-treated telomerase; extract from MRC-5. (n = 3 technical replicates; two-tailed Student's t-test: ****P <0.0001; bars represent the mean ± s.d.; *Vg* represents the initial rate of the reaction calculated based on a formula: *Vg* = ΔF/10, the average fluorescence intensity change (ΔF) between 1 min and 11 min; a.u., arbitrary units.). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

telomerase, heat-inactivated telomerase, RNase-treated telomerase and extract from normal cell MRC-5 (human embryonic lung fibroblast cell), and the results indicated that heat-inactivated, RNase-treated telomerase and extract from MRC-5 did not induce any activation of the CRISPR system, thus confirming the good detection specificity of Lit-Telo platform (Fig. 5C).

3.6. Analysis of clinical specimens

Bladder cancer is one of the most common malignant tumors of the urinary system, and the bladder epithelial tissue is usually obtained through cystourethroscopy for pathological assessment to determine the occurrence of bladder cancer(Compérat et al., 2022; van Hoogstraten et al., 2023). Patients with bladder cancer need to have regular check cystoscopy after surgical treatment to monitor for recurrence of bladder cancer(van Hoogstraten et al., 2023). However, the pathological detection process of bladder tissue is complex and time-consuming, so bladder cancer detection technology, which is simpler and faster than pathological detection, has significant application potential in the diagnosis of bladder cancer.

Analysis of telomerase activity from clinical specimens using the proposed Lit-Telo platform was conducted to assess the performance of the method. We had collected 26 bladder tissues, including 15 cancer tissue specimens and 11 non-cancerous tissues, for telomerase activity assay using both fluorescence detection based Lit-Telo (Fluo Lit-Telo) visual detection based Lit-Telo (Visual Lit-Telo) (Fig. 6A and B and **S2-3**). It is noteworthy that the analysis of 2 specimens of inverted urothelial papilloma, an uncommon benign tumor, produced negative detection results, indicating the good specificity of the Lit-Telo platform. As shown in Fig. 6C, both Fluo Lit-Telo and Visual Lit-Telo demonstrated good detection accuracy for clinical specimens with the AUC value of 93.94% and 92.12% based on ROC analysis of the detection results. The data of the sensitivity, specificity and Kappa value of the Fluo Lit-Telo and Visual Lit-Telo platforms confirmed the good performance for the assay of telomerase activity from clinical specimens (Fig. 6D), revealing the significant application potential of the Lit-Telo method in clinical diagnosis. It is noteworthy that the detection of some clinical specimens produced very faint test lines (Fig. 6B). This phenomenon can be attributed to two primary factors. Primarily, these samples have intrinsically very weak telomerase activity. Furthermore, the colloidal



	Fluo Lit-Telo	Visual Lit-Telo
Sensitivity	93.33%	88.23%
Specificity	100%	100%
Kappa value	0.962	0.923

Fig. 6. Telomerase activity assay of bladder cancer clinical specimens using Lit-Telo platform. (A). Data analysis of the detection results of the Fluo Lit-Telo and Visual Lit-Telo assay for telomerase activity from bladder tissue specimens. Red dashed lines indicate the cut-off values. (B). Images of the lateral flow assay using Visual Lit-Telo method for telomerase activity from clinical specimens. (C). ROC analysis of the data shown in figure A. (D). Sensitivity, specificity and Kappa value of the developed Lit-Telo platform for detection of telomerase activity from bladder cancer clinical specimens. (Vg represents the initial rate of the reaction calculated based on a formula: Vg = ΔF/10, the average fluorescence intensity change (ΔF) between 1 min and 11 min; a.u., arbitrary units.). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

gold-based LFA demonstrates inherent sensitivity limitations, as it produced results visually indistinguishable from negative controls. Therefore, the use of quantum dot or fluorescent bead-based LFA technology may be required to increase the sensitivity for the detection of samples with weak telomerase activity.

4. Conclusions

In this study, we proposed a method for the isolation and purification of telomerase extension products based on light-triggered nucleic acid separation strategy, which can achieve simple and convenient separation of telomerase extension products from complex telomerase extension reaction. The developed Lit-Telo platform can be used for detection of telomerase activity from complex samples, while conventional one-pot style telomerase activity assay cannot eliminate the inhibitory effect from complex telomerase sample. A comparison between the proposed concept and methods in previous works is shown in Table S1. Yaku et al. developed a method named STAMC, which uses streptavidin-coated magnetic beads to capture biotinylated telomerase extension products, and the magnetic beads loaded with telomerase extension products are mixed with PCR solution(Yaku et al., 2017). The streptavidin is heat-inactivated during PCR process and the captured telomerase extension products are released to allow the amplification by PCR. However, such approach introduces a large amount of other component into the reaction, which may have unknown effects on the detection. In this study, streptavidin-coated 96-well plate is selected as the carrier for the enrichment and isolation of nucleic acids, and the use of this highly light-transmitting matrix not only effectively realizes the light-triggered nucleic acid release process, but also achieves the specific release of specific light-responsive nucleic acids through only 5-s UV light irradiation. We expect that the light-triggered nucleic acid separation strategy can also be widely used in other field of biomedical research and applications. However, it should be noted that matrix influence to telomerase extension reaction and strand degradation during substrate extension cannot be eliminated using this light-triggered separation system. Strategy for simple and rapid telomerase purification is required in further study.

CRediT authorship contribution statement

Meng Cheng: Writing – original draft, Validation, Project administration, Conceptualization. **Ligang Zhang:** Writing – original draft, Methodology, Formal analysis, Data curation. **Xiujing Han:** Resources, Investigation, Formal analysis, Data curation. **Hanjiang Xu:** Resources, Data curation. **Haoqiang Shi:** Resources, Data curation. **Yongping Lin:** Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bios.2025.117355.

Data availability

Data will be made available on request.

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