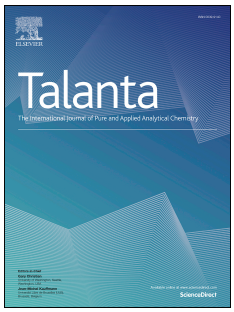
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CRISPR/Cas12a-based biosensing platform for precise and efficient screening of CRISPR/Cas9-induced biallelic mutants

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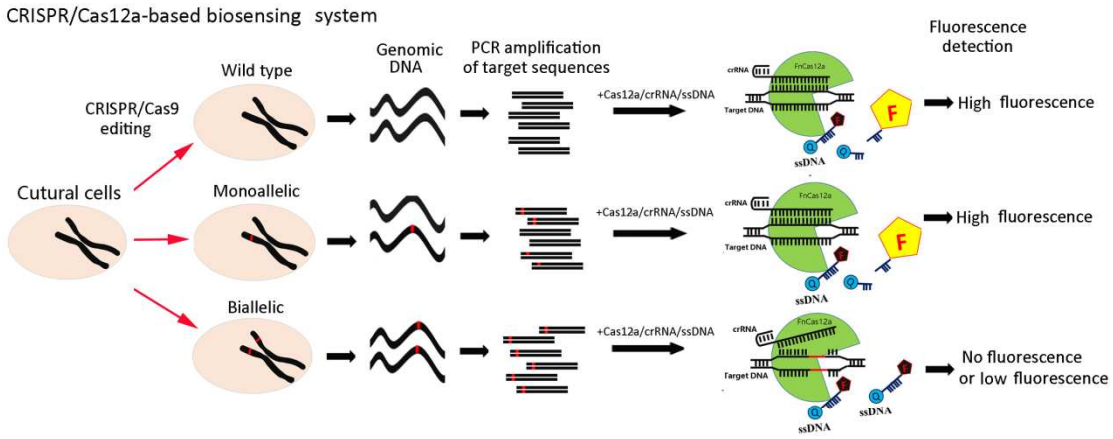
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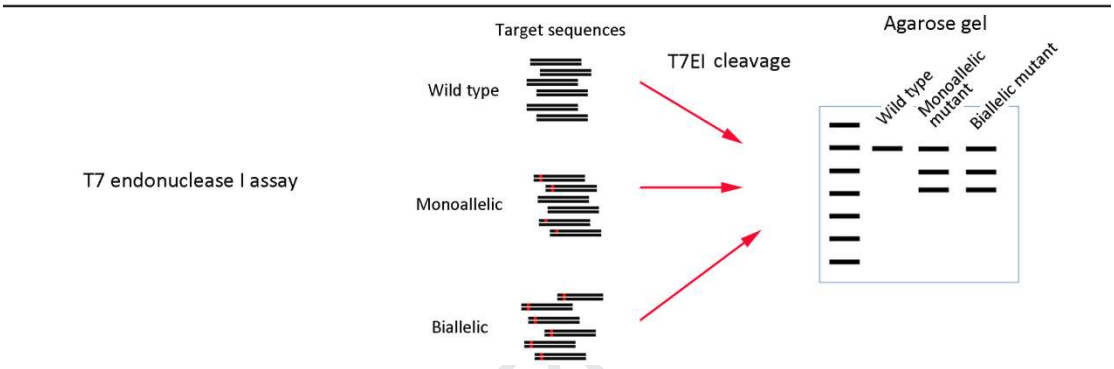
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CRISPR/Cas12a-based biosensing platform for precise and efficient screening of CRISPR/Cas9-induced biallelic mutants

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Abstract

CRISPR/Cas9 is a robust tool to manipulate genes in a wide range of species. Although several methods are introduced to identify the CRISPR/Cas9-induced mutations, they are labor-intensive, costly, and not easy to use or were sequence-limited. Moreover, few of them could identify the biallelic mutants that are the desired outcomes of targeted mutagenesis. Recently, a CRISPR/Cas12a-mediated biosensing platform was developed to detect nucleic acids based on the collateral DNA cleavage activity of Cas12a; it was highly sensitive, specific, rapid, and cost-efficient for genotyping, mutation detection, and single nucleotide polymorphism (SNP) identification, thereby deeming it as an innovative method for screening the CRISPR/Cas9-induced biallelic mutants. Thus, the CRISPR/Cas12a-based biosensing platform has been successfully utilized for screening 23 CRISPR/Cas9-induced biallelic mutants in Thp-1 cells, which were also confirmed by direct sequencing and ELISA. The precision and efficiency of CRISPR/Cas12a-based biosensing platform make it a promising tool for screening of CRISPR/Cas9-induced biallelic mutants in the future.

Keywords: CRISPR/Cas9; CRISPR/Cas12a; biallelic mutants; mutants screening

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Introduction

Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated 9 (Cas9) has been widely applied for genome editing in life science research. In this system, single-strand RNA (sgRNA) guides Cas9 to cleaves the target DNA and forms a double-strand break (DSB) [1]. The subsequent DNA repair by the error-prone non-homologous end-joining (NHEJ) pathway might result in mutations at the target sites [1]. A majority of the mutations generated by the CRISPR/Cas9 system are either insertions or deletions usually located close to the DSB site where is 3 bp upstream of the protospacer adjacent motif (PAM) [2].

Mutations caused by CRISPR/Cas9 in diploid cells present three possible zygosities, including monoallelic mutation, biallelic homozygous mutation, and biallelic heterozygous mutation [3]. Recently, several mutation detection methods have been developed for the screening of CRISPR/Cas9-induced mutants, such as heteroduplex mobility assay on polyacrylamide gel electrophoresis (HMA-PAGE) [4], high-resolution melt analysis (HRM) [5], indel detection by amplicon analysis (IDAA) [6], annealing at critical temperature PCR (ACT-PCR) [7], mutation sites-based specific primers PCR (MSBSP-PCR) [8], and T7 endonuclease I and surveyor nuclease assays [1]. However, each method has specific limitations, such as being complicated, tedious, time-consuming, sequence-limited, low sensitivity, and poor efficiency. Strikingly, few of them can determine the zygosity of individual diploid cell lines without DNA sequencing.

Recently, Cas13a (previously known as C2c2), an RNA-guided RNase belonging to the type VI CRISPR/Cas system, possessed the target RNA-triggered collateral RNA cleavage activity in 2016 [9, 10]. Gootenberg et al. developed the first comprehensive and applicable CRISPR/Cas13a-based nucleic acid detection system and named it as SHERLOCK (Specific High Sensitivity Enzymatic Reporter Unlocking) [11]. Similar to Cas13a, Cas12a, an RNA-guided DNase, possessed the target DNA-triggered collateral DNA cleavage activity as demonstrated by two independent studies. In addition, it was exploited to be a CRISPR/Cas12a-based nucleic acid detection system

termed as HOLMES (one-Hour Low-cost Multipurpose highly Efficient System) or DETECTR (DNA Endonuclease Targeted CRISPR Trans Reporter) [12-14]. In combination with nucleic acid amplification method, these platforms achieved attomolar sensitivity for detecting DNA or RNA targets with a single-base resolution [11]. These findings prompted that CRISPR/Cas12a-based nucleic acid detection system could be used for mutation detection.

In this study, to demonstrate the potential of CRISPR/Cas12a-based biosensing platform in detection of mutations, *CXCL9* gene was modified by CRISPR/Cas9 and individual cell clones were obtained. By designing the specific guide CRISPR RNA (crRNA) targeting the CRISPR/Cas9 editing sites, we demonstrated that the CRISPR/Cas12a-based biosensing platform identifies the biallelic mutants in Thp-1 rapidly and precisely, showing remarkable advantages as compared to that by recent approaches.

Materials and methods

Cells

THP-1 human monocytic cell line was maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 0.1 mg/mL streptomycin at 37 □ in a humidified atmosphere with 5% CO_2.

Construction of *CXCL9* knockout lentiviral vector

To increase the viral titer, we used a dual vector lentiviral GeCKO system, in which Cas9 (lentiCas9-Blast) and sgRNA (lentiGuide-Puro) are delivered using separate viral vectors with distinct antibiotic selection markers. The dual vector system enables the generation of Cas9-expressing cell lines, which can be subsequently used for screening using lentiGuide-Puro. The two viral vectors were a kind gift by Professor Feng at the University of Sydney, and the two *CXCL9* sgRNAs-targeting exons were designed using the Broad Institute sgRNA design tool (https://portals.broadinstitute.org/gpp/public/analysis-tools/sgrna-design) (sgRNA1: 5’-CAGCGACCCTTTCTCACTAC-3’; sgRNA2: 5’- ATTGTAGGTGGATAGTCCC T-3’). Oligonucleotides were synthesized and cloned into lentiGuide-Puro

individually according to the oligo cloning protocol for lentiGuide-Puro [15, 16]. Two targeting clones were validated by sequencing.

Lentiviral production

CXCL9-knockout THP-1 cell lines were constructed according to the method described previously [15, 16]. Briefly, we packaged the mature lentivirus which contained the lentiCas9-Blast plasmid and CXCL9 sgRNA lentiGuide-Puro plasmid, respectively. The transfer plasmids were co-transfected with packaging plasmids pMD2.G and psPAX2, respectively, to HEK293T cells using Lipofectamine 3000. After 6 h, the media was replaced with complete medium (RPIM1640 with 10% FBS). After 60 h, the viral supernatants were harvested and filtered through a 0.45-μm low protein filter (Millipore), and stored at -80 □.

Construction and screening of *CXCL9* knockout single THP-1 cell lines

Firstly, 0.5–1×10^6 THP1 cells in 12-well cell culture plates were infected with 0.5–1 mL packaged virus supernatant, containing the lentiCas9-Blast plasmid. After infection for 24, the cells were washed with 1X PBS, and the media was replaced (RIPM 1640 with 10% FBS and 100U/ml penicillin and streptomycin). After infection for 48 h, blasticidin (10 ng/mL) was added to the supernatant and maintained at 14 days. Subsequently, the blasticidin-resistant cells were harvested and identified with respect to the Cas9 gene and protein as assessed by PCR and Western blot methods. The cells expressing the *Cas9* gene were deemed as Cas9-positive THP1 cells. Secondly, the Cas9-positive THP1 cells were infected with virus supernatant containing CXCL9sgRNA lentiGuide-Puro plasmid through the same method of infection as described above. The resistant screening drug was puromycin (puro) but not blasticidin. After 14 days of screening, the mixed cells were diluted to only 100 cells/mL and further diluted to 10 mL. After blending, the cells with 10 mL volume were sub-divided into 96-well cell culture plate/100 μL. Cultures were expanded using single cells. Finally, the supernatants were used to determine the expression of CXCL9 protein by ELISA. Total DNA of cells was extracted and stored at -80 □ for the downstream experiments.

Design and preparation of crRNAs

crRNAs for guiding Cas12a were designed to target the Cas9/sgRNA editing sites. For the preparation of crRNAs, constructs were ordered as DNA with an appended T7 promoter sequence. crRNA was prepared by annealing the short T7 primer, followed by incubation with T7 polymerase at 37 □ for 2 h using the T7 High Yield RNA Transcription Kit (Vazyme, Nanjing, China). crRNA was purified using VAHTS RNA Clean Beads (Vazyme, Nanjing, China) according to the manufacturer's instructions and quantified using NanoDrop 2000 (ThermoFisher Scientific, Shanghai, China). All oligonucleotides are listed in Table S1.

Preparation of target DNA

For testing the efficiency of transcriptional crRNAs, target sequences containing different insertions (1–5 bp) were synthesized commercially (Table S2). For identification of mutation lines, target sequences were amplified by PCR using genomic DNA from individual clones. Target sequences containing Cas9/sgRNA1 targeting site were amplified using primers *HCXCL9-1*-F (5'-GATGCAGGAACAGCGTTACC-3') and *HCXCL9-1*-R (5'-CCTCCTTCAAAG-GTTTGTGG-3') which could introduce Cas12a-recognized PAM (TTN) sequence. Target sequences containing Cas9/sgRNA2 targeting sites were amplified using primers *HCXCL9-2*-F (5'-GCCCAAATTTATGGAGGGGG-3') and *HCXCL9-1*-R (5'-CCTCCTTCAAAGGTTTGTGG-3'). PCRs were performed using PrimeSTAR Max DNA Polymerase (TaKaRa, Dalian, China). The following amplification program was used: 94 □ for 3 min, 32 cycles of 94 □ for 30 s, 60 □ for 10 s, and 72 □ for 10 s, followed by one cycle at 72 □ for 5 min. To determine the sensitivity of CRISPR/Cas12a-based biosensing platform, DNA extracted from wild-type cells were serially diluted ranging from 100 ng to 0.1pg. PCR were performed using primers *HCXCL9-1*-F and *HCXCL9-1*-R, *HCXCL9-2*-F and *HCXCL9-1*-R, respectively. The PCR products were examined by 1% agarose gel electrophoresis.

FnCas12a-mediated mutation detection

Cas12a protein from *Francisella novicida* was purchased from Tolo Biotech

(Shanghai, China). FnCas12a-mediated cleavage assays were performed as described by Li' et al. [14]. Briefly, the 20-μL reaction volume consisted of 1 pmol FnCas12a, 100 nM synthesized and purified crRNA, target sequences (1 μg synthesized target sequence or 1 μL PCR reaction mixture), 500 nM collateral ssDNA (quenched fluorescent DNA reporter HEX-N12-BHQ1), and 10 U RNase inhibitor (TaKaRa, Dalian, China) at 37 □ for 30 min. Fluorescence emission was excited at 535 nm and detected at 560 nm using a Varioskan Flash (Thermo Fisher Scientific, Shanghai, China), and reactions with no target DNA served as the background.

Sequencing analysis

Targeted regions surrounding Cas9/sgRNA target sites were amplified by PCR using primers *HCXCL9-11-F*: 5-TCTGTGGCTAACCAATTAAGGA-3 and *HCXCL9-12-R*: 5-GCCCAAATTTATGGAGGGGG-3. PCR was performed using PrimeSTAR Max DNA Polymerase (TaKaRa, Dalian, China) in a 50 μL reaction volume as follows: 94 □ for 3 min, then 94 □ for 30 s, 60 □ 30 s, and 72 □ 30 s for 32 cycles, followed by one cycle at 72 □ for 10 min. The PCR products were examined by 1% agarose gel electrophoresis, followed by directly sequencing. Some PCR products were cloned in PMD-19 TA vectors (TaKaRa, Dalian, China) and followed by DNA sequencing.

T7EI cleavage assays

T7EI cleavage assay was performed using the T7 Endonuclease I Kit (Vazyme, Nanjing, China) according to the manufacturer's instructions. Briefly, target sequences containing the nuclease target site were amplified by PCR using primers *HCXCL9-T7-F*: 5-TGTGCACACGAAGCAAGTAAGG-3 and *HCXCL9-T7-R*: 5-TCTGTGGCTAACCAATTAAGGA-3. Then, the PCR products were quantified using the Qubit dsDNA HS Assay Kit (ThermoFisher Scientific, Shanghai, China). Subsequently, 250 ng PCR products were treated at 95 □, 5 min; 95–85 □, decrease 2 □/s; 85–25 □, decrease 0.1 □/s; 4 □ hold to form a heteroduplex DNA. 1 μL T7EI was added to heteroduplex DNA and incubated at 37°C for 30 min. The reaction was stopped by 2 μL of 0.25 M EDTA and analyzed using 1% agarose gel electrophoresis.

Results

Schematic overview of CRISPR/Cas12a-based biosensing platform for the identification of CRISPR/Cas9-induced biallelic mutants

Since the CRISPR/Cas12a-based biosensing platform possesses the ability to specifically and accurately detect the target DNA, it might be able to screen the CRISPR/Cas9-induced mutants. The schematic overview of CRISPR/Cas12a-based biosensing platform to identify CRISPR/Cas9-induced biallelic mutants is shown in Fig. 1. The individual clones edited by CRISPR/Cas9 can take on three genotypes, including biallelic mutants, monoallelic mutants, and wild-type. Using this platform, target sequences containing Cas9/sgRNA target site are amplified by PCR, incubated with FnCas12a protein, and linked with a specific crRNA and a quenched fluorescence reporter ssDNA. When crRNA completely matches with the target sequences, the formation of FnCas12a/crRNA/target DNA ternary complex trans-cleaves the reporter ssDNA, resulting in illuminating fluorescence. In the case of mutation in Cas9/sgRNA target sites, leading to a mismatch between crRNA and target sequences, FnCas12a will not trans-cleave reporter ssDNA. Briefly, PCR amplification from wild-type or monoallelic mutants will result in detectable fluorescence, while those from biallelic mutants will not illuminate fluorescence or illuminate low-fluorescence intensity. Thus, biallelic mutants could be distinguished from wild-type and monoallelic mutants based on the fluorescence intensity. Conversely, T7EI could distinguish mutants from wild-type but could not distinguish biallelic mutants from monoallelic mutants since they share the same banding pattern in gel T7EI enzyme cleavage (Fig. 1).

Application of FnCas12a-based biosensing platform for the identification of CRISPR/Cas9-induced biallelic mutants in Thp-1 cells

To determine the feasibility of FnCas12a-based biosensing platform for screening the CRISPR/Cas9-induced biallelic mutants, two sgRNAs (sgRNA1 and sgRNA2) targeting the exons of *CXCL9* gene were designed and used to construct the *CXCL9* mutants in Thp-1 cells by CRISPR/Cas9. Two crRNAs (crRNA-1 and crRNA-2) targeting the Cas9/sgRNA target sites were designed, as shown in Fig. 2A. First, we

determined the efficiency of designed crRNAs *in vitro* using a series of chemically synthesized target sequences containing different insertions of bases (1–5 bp) and locating in Cas9/sgRNA cleavage sites (Fig. 3a). Fluorescence was detected as described above (Fig. 3b and c). Surprisingly, 1 bp insertion in the cleavage was sufficient to cause a remarkable decline in the fluorescence signal. Moreover, we determined the sensitivity of FnCas12a-based sensing system and established the detection limit of the system as 10 pg DNA (Fig. 3d and e).

To verify the usefulness of the FnCas12a-based biosensing platform in experimental conditions, a number of Thp-1 individual clones edited by CRISPR/Cas9 at the *CXCL9* locus were screened out, and DNA was extracted for PCR. Since a 5'-NGG PAM prior to the target site is required for FnCas12a and FnCas9/sgRNA1 targeting site does not contain a suitable PAM sequence, "TT" bases were inserted into the primer and introduced into target sequences by PCR amplification [14] (Fig. 2B). This approach enabled FnCas12a-based biosensing platform to identify any mutation generated by CRISPR/Cas9 in a sequence-independent manner. PCR amplification products from individual clones were incubated separately with FnCas12a/crRNA in the presence of fluorescence reporter ssDNA. 11 samples from Cas9/sgRNA1 edited individual clones and 13 samples from Cas9/sgRNA2 edited individual clones exhibited dramatically weak fluorescence signal as compared to the wild-type (Fig. 4A and B), suggesting that these individual clones were potentially biallelic mutants. Meanwhile, a number of screened samples still showed fluorescence as strong as the wild-type (Fig.4A and B).

Confirmation of the identified biallelic mutants by sequencing and ELISA

The above results were substantiated by DNA sequencing in order to determine the mutation of the target sequences. Those surrounding the Cas9/sgRNA target sites were amplified by PCR and confirmed by direct sequencing (Fig. 5). These samples showing no fluorescence above exhibited multiple overlapping peaks around Cas9/sgRNA target sites, suggesting that *CXCL9* genes in these individual clones were modified successfully (Fig. 5A and B). However, we also found three samples

(#3, #9 and #6) showing high fluorescence in above Cas9/sgRNA cleavage assays exhibited overlapping peaks, implying they are potentially monoallelic mutants (Fig.5A and B). To confirm these three sample are monoallelic mutants, PCR products were cloned into TA vectors. Single clones were slected for Sanger sequence. Sequence results showed that #3, #9 and #6 have 19 bp, 3bp and 12bp deletion in one of allele, respectively, which suggested that #3, #9 and #6 are monoallelic mutants (Fig.5C).Meanwhile, ELISA was employed to determine the expression of CXCL9 in the supernatants of these modified cell clones. As shown in Fig. 7, all these individual clones showing no fluorescence in above FnCas12a-based assays did not express CXCL9, indicating that both alleles of *CXCL9* in individual clones were modified successfully in these individual clones. Taken together, DNA sequences and ELISA results confirmed that FnCas12a-based biosensing platform is an accurate and reliable approach for screening CRISPR/Cas9-induced biallelic mutants. In comparison, T7EI assays failed to distinguish between the biallelic and monoallelic mutants since both share the same banding pattern (Fig. S1).

Discussion

With the increased use of CRISPR/Cas9 gene editing, new methodologies are required to rapidly and cost-effectively screen a large number of mutations. Several methods have been developed to identify CRISPR/Cas9-induced mutants. Although these approaches can distinguish the mutants from wild-type, few of them can discriminate the biallelic mutants, which are the desired outcomes of targeted mutagenesis, from monoallelic mutants. Recently, the classical method to distinguish biallelic mutants from monoallelic mutants is DNA sequencing. Fragments containing the nuclease target sites were amplified by PCR and cloned into T-vector. Single clones were selected for Sanger sequencing. Thus, this method is time-, cost-, and labor-consuming to identify the biallelic mutants.

In the recent studies, CRISPR/Cas-based biosensing platform including Cas13a, Csm6, and Cas12a has shown great advantages in the detection of nucleic acids and SNP and genotyping as compared to conventional methods [17]. These platforms are

considered to be the next-generation diagnostic biosensing platforms[17]. In the present study, we employed FnCas12a-based biosensing platform to distinguish between biallelic and monoallelic mutants and wild-type in CRISPR/Cas9 modified-Thp-1 cells. This tool remedies the drawbacks of the other mutation detection methods. In addition, we found that FnCas12a-based biosensing platform discriminates the single base insertion in this study. The most common mutation type in CRISPR/Cas9-induced plants is 1-bp indel mutations, and hence, FnCas12a-based biosensing platform would be advantageous in screening the CRISPR/Cas9-induced mutations in plants in the future. Nevertheless, this platform has some limitations. It cannot distinguish between biallelic homozygous and heterozygous mutations. Therefore, sequencing the target site using TA cloning method is essential after the biallelic mutants have been identified. The crRNA required in this method is easily degraded by RNase; hence, the whole process should be prevented from RNase contamination. In addition, expression and purification of recombinant Cas12a is difficult due to its large molecular weight and recently very few companies provide purified Cas12a, which limits its application.

Conclusions

CRISPR/Cas9 is now a widely used tool for gene editing, while mutation screening is time- and money- consuming. In this study, we demonstrated that Cas12a-based biosensing platform enables us to identify the CRISPR/Cas9-induced biallelic mutants easily, accurately, and cost-effectively. Furthermore, this biosensing platform shows high sensitivity for DNA detection with single base resolution. In summary, this method would aid in conserving the time and costs, rendering it as a potential mutant-screening method.

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Figure legends

Figure 1 Schematic illustration of CRISPR/Cas12a-based biosensing platform. The cells edited by CRISPR/Cas9 may have three results, including biallelic mutants, monoallelic mutants, and wild-type. Mutation sites in genomic regions are indicated by red boxes. Target DNA containing mutation sites are firstly amplified from genomic DNA by PCR. Then, FnCas12a protein is used to bind the amplified target, associated with a specific guide RNA and a quenched fluorescence reporter ssDNA. If guide crRNA completely matches with target DNA, FnCas12a will be activated to trans-cleave reporter ssDNA, generating fluorescence. If guide RNA is incompletely matched with target DNA due to the mutation, FnCas12a will not trans-cleave or inefficiently trans-cleave the reporter ssDNA.

Figure 2 Overview of the crRNA (a) and primer (b) design for mutants screening using CRISPR/Cas12a-based biosensing platform. (a) Two crRNAs (crRNA-1 and crRNA-2) were designed to match the Cas9/sgRNA cleavage sites, directly downstream of a requisite 5'-TTN adjacent motif (PAM, in the box). (b) Since SgRNA1 target region does not contain a suitable "5'-TTN" PAM sequence, PAM was inserted into the primer and introduced into target DNA by PCR.

Figure 3 Determination of the signal-to-noise of bases insertion in target sequences and the sensitivity of FnCas12a-based biosensing platform

(a) The representation of the synthesized target sequences. The PAM "TTN" is underlined. Insertion of bases is shown in red. 1 to 5 represents the number of bases inserting into potential CRISPR/Cas9 cleavage sites, respectively. (b) and (c) Detection of fluorescence signal among crRNA-1- and crRNA-2-targeting sequences with different insertion of bases. (d) and (e) Sensitivity detection of FnCas12a-based biosensing platform. Serially diluted DNA amounts ranging from 10 ng to 0.01 pg were used to determine the detection limit of FnCas12a-based biosensing platform. Error bars represent mean ± s.d., where $n = 3$ technical replicates.

Figure 4 Identification of *CXCL9* biallelic mutants using FnCas12a-based biosensing platform

Screened individual clones were used as templates for the PCR amplification of target sequences from the genomic DNA. FnCas12a-based cleavage assay was performed as materials section's description. Fluorescence emission was excited at 535 nm and detected at 560 nM, and reactions with no target DNA were taken as the background. (A) Individual clones derived from Cas9/sgRNA1 edited cell lines. (B) Individual clones derived from Cas9/sgRNA1 edited cell lines. Arabic numerals below abscissa axis represent each individual clone. sgRNA1sgRNA2Error bars represent mean ± s.d., where n = 3 technical replicates.

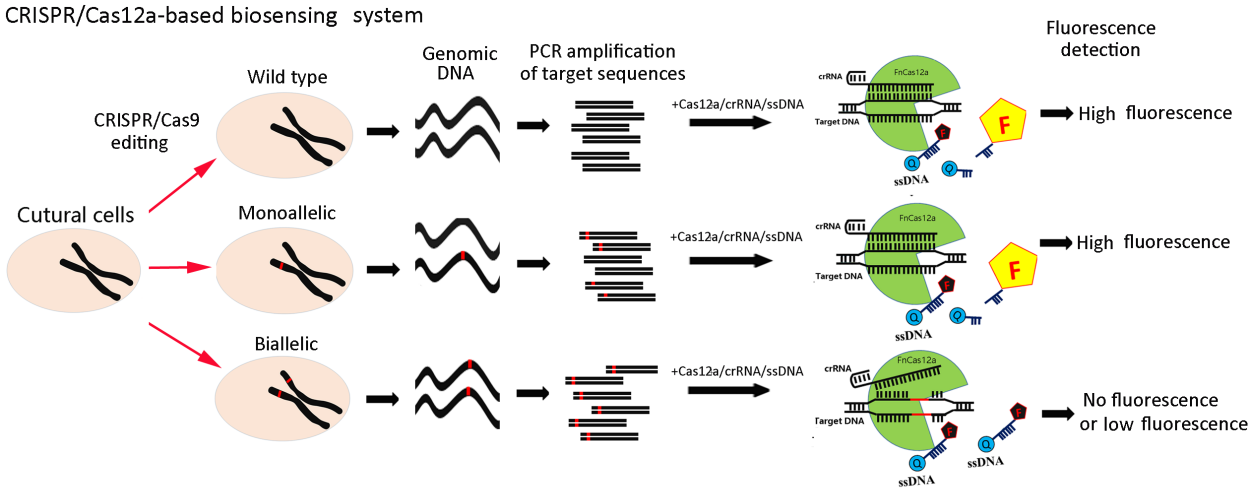
Figure 5 Determination of the mutation of target DNA by Singer sequence.

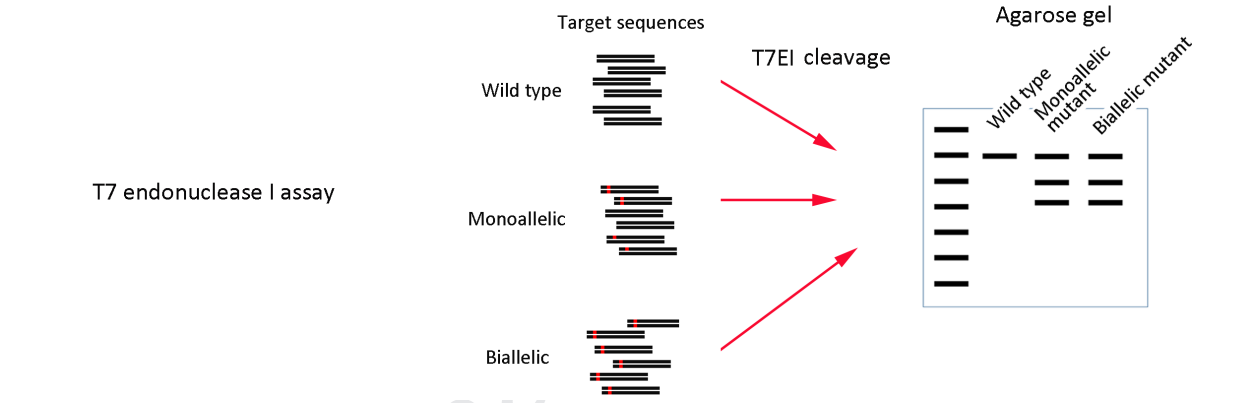
PCR products were amplified from individual clones edited by Cas9/sgRNA1 (A) or Cas9/sgRNA2 (B) and followed by directly sequencing. Overlapping peaks indicate that at least one allele was modified. (C) The genotypes of #3, #9 and #6 were further confirmed by TA clone sequencing. For all these three individuals, base deletion occur in one of the alleles, which confirms they are monoallelic mutants.

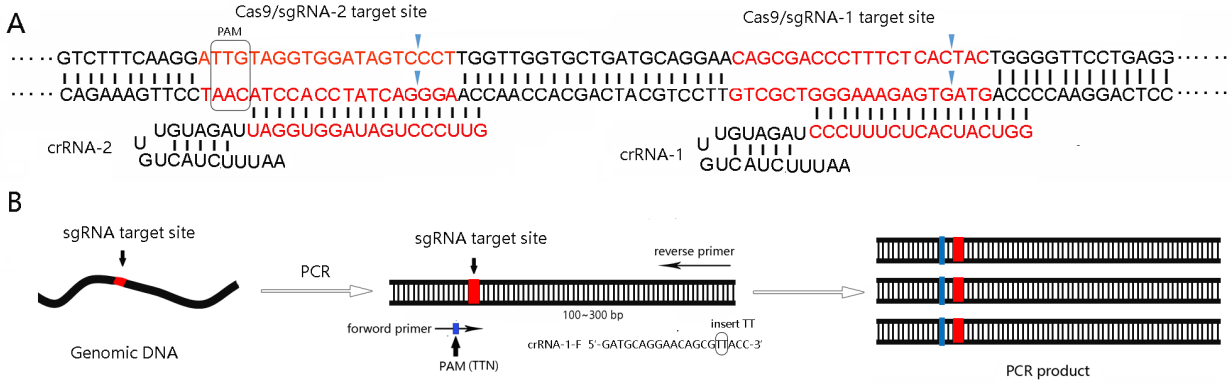
Figure 6 Determination of the expression of CXCL9 by ELISA.

Supernatants collected from the individual clones which showed no fluorescence in FnCas12a/sgRNA cleavage assays were used for the performance of ELISA.Wild-type cells were used as controls. Error bars represent mean ± s.d., where n = 3 technical replicates.

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CRISPR/Cas12a-based biosensing system
Fluorescence detection
Genomic DNA
PCR amplification of target sequences
Wild type
CRISPR/Cas9 editing
Cutural cells
Monoallelic
Biallelic
+Cas12a/crRNA/ssDNA
crRNA
FnCas12a
Target DNA
ssDNA
F
Q
High fluorescence
High fluorescence
No fluorescence or low fluorescence


T7 endonuclease I assay
Target sequences
T7EI cleavage
Agarose gel
Wild type
Monoallelic
Biallelic
Wild type
Monoallelic mutant
Biallelic mutant



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(a)

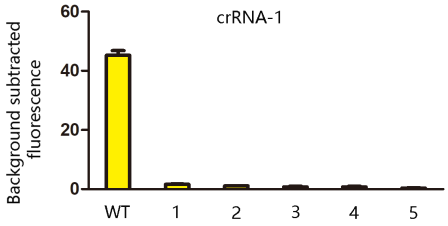
Synthesized crRNA-1 target sequences

WT: 5′···GAACAGCGTTACCCTTTCTCACTACTGGGGTTCCTGA···3′
1: 5′···GAACAGCGTTACCCTTTCTCACCTACTGGGGTTCCTGA···3′
2: 5′···GAACAGCGTTACCCTTTCTCACCCTACTGGGGTTCCTGA···3′
3: 5′···GAACAGCGTTACCCTTTCTCACCCCTACTGGGGTTCCTGA···3
4: 5′···GAACAGCGTTACCCTTTCTCACCCCCTACTGGGGTTCCTGA···3
5: 5′···GAACAGCGTTACCCTTTCTCACCCCCCTACTGGGGTTCCTGA···3′

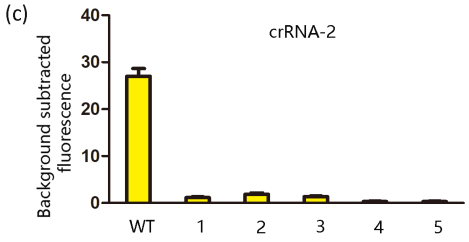
Synthesized crRNA-2 target sequences

5′···GGTCTTTCAAGGATTGTAGGTGGATAGTCCCTTGGTTGGTGCTGATGCAGGAACA···3′
5′···GGTCTTTCAAGGATTGTAGGTGGATAGTCCCCTTGGTTGGTGCTGATGCAGGAACA···3′
5′···GGTCTTTCAAGGATTGTAGGTGGATAGTCCCCCTTGGTTGGTGCTGATGCAGGAACA···3′
5′···GGTCTTTCAAGGATTGTAGGTGGATAGTCCCCCCTTGGTTGGTGCTGATGCAGGAACA···3′
5′···GGTCTTTCAAGGATTGTAGGTGGATAGTCCCCCCCTTGGTTGGTGCTGATGCAGGAACA···3′
5′···GGTCTTTCAAGGATTGTAGGTGGATAGTCCCCCCCCTTGGTTGGTGCTGATGCAGGAACA···3′

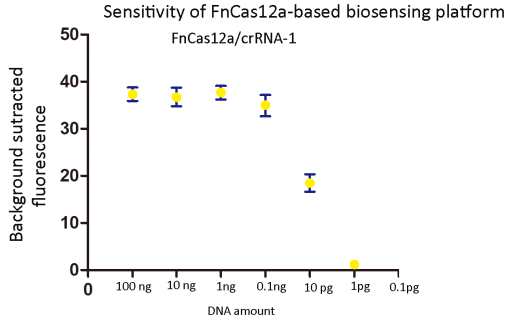
(b)



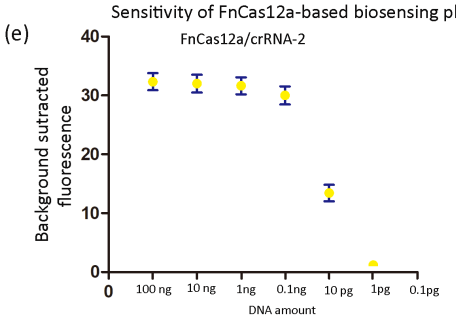
(c)

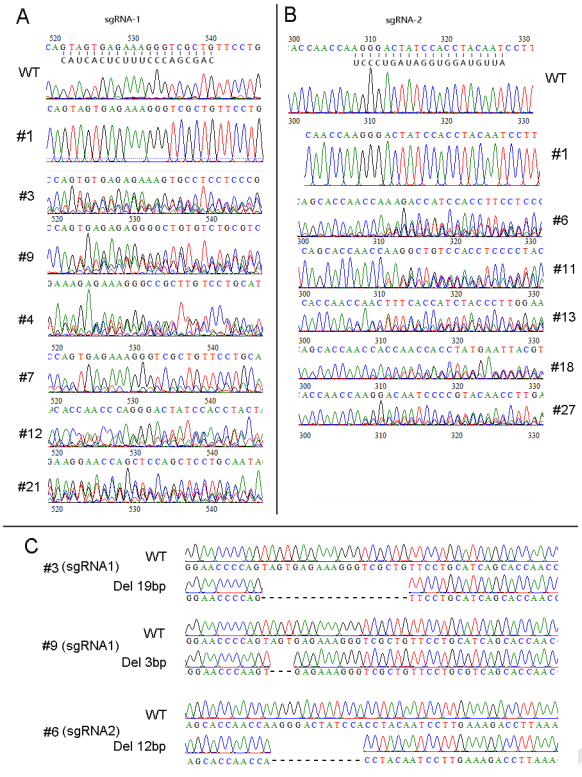


(d)

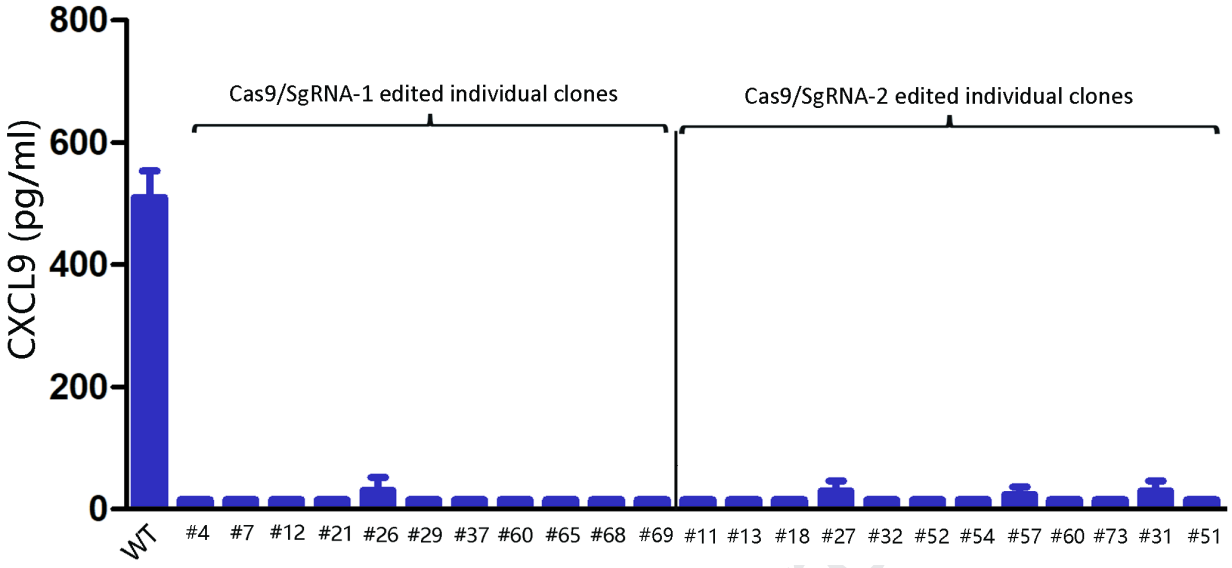


(e)

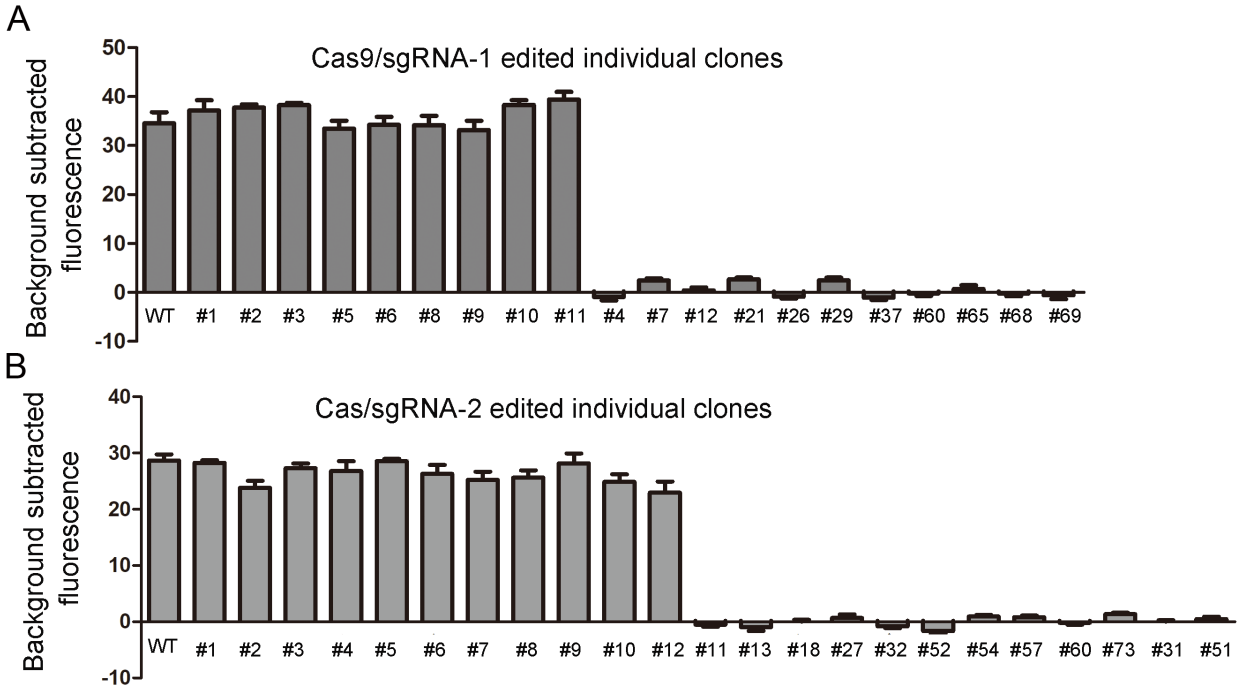




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A
Cas9/sgRNA-1 edited individual clones
Background subtracted fluorescence
50
40
30
20
10
0
-10
WT #1 #2 #3 #5 #6 #8 #9 #10 #11 #4 #7 #12 #21 #26 #29 #37 #60 #65 #68 #69
B
Cas/sgRNA-2 edited individual clones
Background subtracted fluorescence
40
30
20
10
0
-10
WT #1 #2 #3 #4 #5 #6 #7 #8 #9 #10 #12 #11 #13 #18 #27 #32 #52 #54 #57 #60 #73 #31 #51

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CRISPR/Cas12a-based biosensing platform could be applied to screen CRISPR/Cas9-induced biallelic mutants, which are the desired outcomes of targeted mutagenesis.

Compared to conventional methods, it shows multiply advantages, such as more accurate, more efficient, easy to use and more reliable.

Furthermore, it is even able to identify 1 bp indel mutation which is most common mutation type in CRISPR/Cas9-induced plants.

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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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