Establishment of A PEG-mediated Protoplast Transformation System Based on DNA and CRISPR/Cas9 Ribonucleoprotein Complexes For Banana

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Research article

Keywords: PEG-mediated, protoplast transformation, Deep amplicon sequencing, genome editing, DNA-free.

Posted Date: June 22nd, 2020

DOI: https://doi.org/10.21203/rs.3.rs-32229/v1

Version of Record: A version of this preprint was published on September 15th, 2020. See the published version at https://doi.org/10.1186/s12870-020-02609-8.

Abstract

Background: To date, CRISPR/Cas9 RNPs editing tools have not been applied to genetic modification of banana. Here, the establishment of PEG-mediated banana protoplast transformation system makes it possible to build an efficient DNA- free method for the site-directed mutagenesis system.

Results: Protoplasts are a versatile platform for transient expression in plant science. In this study, we established a PEG-mediated banana protoplast transformation system. This system was further optimized for successfully delivering plasmids of CRISPR/Cas9 and CRISPR/Cas12a , CRISPR/Cas9 ribonucleoproteins (RNPs) that targeted *PDS* gene into banana protoplasts. Specific bands were observed in PCR-Restriction Enzyme Digestion (PCR-RE) assays and monoclonal sequencing further confirmed the occurring of indels at target sites. Deep amplicon sequencing results showed that the editing efficiency of CRISPR/Cas9 system was higher than that of the other two systems.

Conclusions: The PEG-mediated banana protoplast transformation system can serve as a rapid and effective tool for transient expression assays and sgRNA validation in banana.The application of CRISPR/Cas9 RNPs system enables the generation of DNA-free genome edited banana plants.

Background

Protoplast is a cell of a plant, fungus, bacterium, or archaeon from which the cell wall has been removed by plasmolysis, leaving the protoplasm and plasma membrane. As early as 1892, Klercker obtained protoplasts by mechanical method [1], which caused a low yield, difficult operation and poor applicability. In 1962, Cocking successfully isolated tomato root tip protoplasts for the first time by enzymatic hydrolysis [2]. This method was widely used because of its high yield, high activity, easy operation and wide adaptability. The transient transformation system of plant protoplasts without unique characteristics of cell wall is extensively used in the study of gene researches covering gene function identification, subcellular localization and gene editing. The common methods used for plant protoplast transformation include PEG-mediated transformation [3, 4], electroporation-mediated transformation [5–9] and microinjection-based transformation [10]. Among them, PEG-mediated method is widely used due to its easy operation, low cost, no requirements for specific equipment and generation of stable results. Up to date, mature and stable genetic transformation systems for protoplast transient expression have been established in Arabidopsis [11–14], wheat [15], rice [16], maize [17] and other species.

Nowadays, there are many reports on PEG-mediated protoplast transformation of DNA, but few reports on PEG-mediated protoplast transformation of ribonucleoprotein (RNP). In 2015, Woo *et al*. directly transferred RNP into the protoplasts of *Arabidopsis thaliana*, tobacco and rice for the first time. The genome-edited mutant regenerated from protoplasts contained no transgenic ingredients [18]. In 2016, Malnoy *et al*. successfully transferred RNP into the protoplasts in grapes and apples, and the mutation efficiency was up to 6.9% [19]. To date, several studies have been reported on the application of CRISPR/Cas9 gene editing technology in bananas [20–23], yet transgenic plants containing the T-DNA

were all generated for the CRISPR/Cas9 gene editing, and a DNA-free genome editing method has not been developed in banana. In this study, a PEG-mediated transformation system was established, which provided an effective method for the detection of gRNA activity. This system was used to successfully deliver RNP into banana protoplasts for the first time, and the RNP system was detected to be working through Deep amplicon sequencing, which laid a good foundation for the further study of banana genome editing.

Results

Establishment of PEG-mediated protoplast transformation system in banana

To establish PEG-mediated banana protoplasts transformation system, we optimized the transformation method based on transformation protocols of rice and wheat. The rice and wheat protocol work very well in rice or wheat protoplast transformation,and the transformation efficiency is up to 58.4% and 64.5%,respectively, confirmed by flow cytometry detection [24]. The transformation efficiency of this protocol in banana was much lower than that in rice and wheat, after 5-day incubation at dark only few spot GFP fluorescence appeared (Figure 1a). Subsequently, the PEG concentration and incubated time were optimized in banana transformation with pUbi-GFP plasmid based on rice and wheat protocol. The highest transformation rate was observed when PEG concentration was increased to 50% and induction time was up to 30 mins (Figure 1d). The transformation efficiency was 5.6%, determined by flow cytometry detection(Figure 1e). To determining the editing efficiency of different editing methods in banana, CRISPR/Cas9-*PDS* plasmids, CRISPR/Cas12a-*PDS* plasmids and CRISPR/Cas9 RNP-*PDS* complex (RNPs) were examined using optimized protoplast transformation protocol with the following flow chart. (Figure 2).

PEG - mediated PCR - RE assay of gene editing in banana protoplast through Plasmid DNA transformation

To explore whether our protoplast transformation system can be applied to genome editing by transferring plasmid DNAs, we designed 9 sgRNAs, in which two sgRNAs target the 2nd (target 3: OsU3p-PDSt3) and 7th exon (target 4: OsU3p-PDSt4) of banana PDS gene, respectively. These two sgRNAs both contain recognition sites of *Eco*47I that can be used for PCR-RE assay(Figure 3). Each of these 9 guided RNAs were fused with an enhanced scaffold RNA (Figure 3a). These plasmid DNAs and Cas9 plasmid were transformed into the banana protoplasts using the above-mentioned method. DNA isolated from these transformed and non-transformed (control) protoplasts were used for PCR-RE assay and sequencing analysis. PCR-RE results showed that PCR product from control sample is completely digested into two band (t3wt-dig, t4wt-dig), but PCR product from plasmid transformated sample is only partially digested under same condition (t3ko-dig, t4ko-dig), suggesting that the restriction enzyme site is mutated by gene editing cassette (Figure 4a).To further characterize the mutation type (i.e. insertion, deletion etc) created by Cas9, the undigested PCR fragments by *Eco*47I were recovered and cloned into T-blunt vectors for sanger sequencing. Ten single clones for OsU3p- PDSt3 and three single clones for

OsU3p- PDSt4 were selected for Sanger sequencing. The results indicated that a 16bp deletion was present in two cloned fragments of OsU3p- PDSt3, and all three cloned fragments of OsU3p- PDSt4 exhibited 1 bp insertion. The insertion and deletion started at the fourth nucleotide from the PAM sequence(Figure 4b). We also designed 11 sgRNAs of banana PDS gene for LbCas12a. and no any recognition site of restriction enzyme was existed in all sgRNAs.So we cann't check the results by PCR-RE assay.

PCR-RE assay of gene editing in banana protoplast transformed with RNP

To detect whether RNP complex can be successfully delivered into protoplasts, and mutated at target sites, we have transferred the complex of purified Cas9 protein with target sgRNAs into the banana protoplasts according to the method reported by Liang [25,26]. We first tested whether RNP could efficiently edit the targets in vitro prior to their transformation into the protoplasts. As results shown in Figure 5a, RNP could cleave the targets included PCR product into two fragments. We next isolated DNA from the transformed and nontransformed protoplasts for PCR amplifications. Amplified fragments containing target sequences were subjected to digestion with *Eco*47I. The results showed that substantial amount of amplified fragment could not be digested in RNP transformed sample, whereas amplicon from control sample could be completely digested, suggesting the disruption of recognition site of *Eco*47 I by RNP in RNP transformed sample, (Figure 5 b).

Deep amplicon sequencing of DNA transformation mediated by PEG in banana protoplast

The pUbi - Cas9 plasmid mixed with 9 sgRNAs plasmids targeting PDS respectively and 11 Cas12a - PDS plasmids (Cas12a - PDSt1 to Cas12a - PDSt11) were transformed into Cavendish banana protoplast. To examine the editing efficiency, genomic DNAs were isolated from protoplasts transformed with each sgRNA and Cas9 or LbCas12a plasmids for Deep amplicon sequencing. The sequencing results showed that 5 target sites (PDSt4, PDSt6, PDSt7, PDSt8 and PDSt9) were edited in the protoplasts transformed with sgRNAs and Cas9 plasmids. Among these edited target sites, the target site PDSt8 exhibited the highest editing efficiency of 1.04%, and 320 inserts and 800 deletions out of 107568 sequencing reads for target site PDSt8 contained deletion and insertion mutations, respectively. The lowest editing efficiency of 0.18% happened to the target site PDSt6. Mutations were observed out of 80384 sequencing reads, and 147 sequencing reads contained various deletions (Supplemental Data Set1).

For LbCas12a system, four target sites (Cas12a- PDSt1, Cas12a- PDSt7, Cas12a- PDSt9 and Cas12a- PDSt10) were successfully edited. The highest editing was observed in the target site Cas12a- PDSt9 with an efficiency of 0.39%, and deletion type editing was found in 114 out of 29008 sequencing reads for Cas12a- PDSt9. In contrast, only 78 (3 insertions and 75 deletions) of 52965 sequencing reads contained mutations created in the target site Cas12a-PDSt7, resulting in the lowest editing efficiency (0.15%) at this target site(Supplemental Data Set2).

Deep amplicon sequencing of RNP transformation mediated by PEG in banana protoplast

Cas9 proteins combined respectively with 9 sgRNAs were transformed into the banana protoplast. To examine the editing efficiency by transforming RNPs, we also performed Deep amplicon sequencing of genomic DNA isolated from the protoplasts transformed with the 9 complexes of Cas9 and sgRNAs targeting 9 different PDS sites. We have detected editing by this RNP system at five target sites including PDS-sgRNAt2, PDS -sgRNAt4, PDS -sgRNAt6, PDS -sgRNAt7 and PDS -sgRNAt9. The target site PDS -sgRNAt9 with the lowest editing efficiency (0.19 %) 166 insertion reads and 26 deletion reads out of 102866 reads. The target site PDS -sgRNAt6 with the highest editing efficiency (0.92 %) ,included 169 insertion reads and 1104 deletion reads out of 138085 reads (Supplemental Data Set3).

Off-target detection

In order to analyze the off-target effect of CRISPR/Cas9-*PDS* plasmids and CRISPR/Cas9 RNP-*PDS* complex (RNPs) gene editing system, A potential off-target site (AGCTTCGTGTACCGCAGTAGTGG), GSMUA_Achr6G21680_001, was predicted via CRISPR-P 2.0 website. There are four base mismatches between the off-target site and sgRNA sequence. Through Deep amplicon sequencing, only one site was detected in CRISPR/Cas9-*PDS* and RNPs gene editing system, and the off-target efficiency was 0.01%. (Supplemental data set4 ,Supplemental data set5, Supplemental data set6).

Discussion

Banana is a kind of tropical and subtropical monocots perennial herbaceous plant. Most of cultivated varieties are triploid, with high fertility. As there is no seed in edible bananas, it is very difficult to achieve fine varieties with good quality and strong disease-resistance through the traditional crossbreeding cultivate. However, there is a certain blindness and longer cycle requirement in breeding new varieties by mutation breeding and mutant screening. For stable CRISPR/Cas9 gene editing by Agrobacterium-mediated transformation in major banana cultivars, the sterility of pollen makes it difficult to remove the exogenous integrated DNA by crossing as other diploid plants do. Due to no exogenous DNA inetergationin genome for gene editing with CRISPR/Cas9 RNP complex, it provides an effective option for banana molecular breeding by gene editing.

Woo *et al.* (2015) reported CRISPR/Cas9 RNP complex can be deliver into the protoplasts of *Arabidopsis thaliana*, tobacco, lettuce and rice for gene editing, and the mutation efficiency of the target in tobacco was up to 44% [18]. Malnoy *et al.*(2016) dilivered RNP into grape and apple protoplasts by PEG, and the mutation efficiency in apples reached 6.9% by Deep amplicon sequencing [19]. In 2016, Svitashev *et al*. directly deliverred RNP complex into immature embryos of corn by biolistic bombardment, and enable targeted gene editing with 0.69% mutation efficiency [27]. Similarly Liang *et al.* (2017) developed an efficient DNA-free genome editing method in bread wheat using Cas9 RNP complexes by particle bombardment with 0.56% mutation efficiency [25]. These results indicate that the mutation efficiency of RNP complex using PEG-mediated protoplast transformation is much higher than that using biolistic bombardment.

Since CRSPR/Cas9 gene editing technology was first reported in 2013, it has been widely used and rapidly developed. Protoplast transient transformation system can effectively detect the activity of target gene sites, providing an effective means for screening gRNA, and is a very good auxiliary tool for the application of CRSPR/Cas9 gene editing technology. In this study, Cas9 and OsU3p- PDS, RNP, and Cas12a-PDS were transformed into banana protoplast system. Through Deep amplicon sequencing, mutation efficiency of Cas9 system was found to be greater than that of RNP at the same target, such as Cas9 and OsU3p- PDSt4 (0.65%) > PDSt4-RNP (0.27%), and Cas9 and OsU3p- PDSt7 (0.55%) > PDSt7-RNP (0.42%), Cas9 and OsU3p- PDSt9 (0.42%) > PDSt9-RNP (0.19%). Among 9 target sites, the highest editing efficiency of the detected Cas9 system was Cas9 and OsU3p - PDSt8, with a mutation efficiency of 1.04%; the highest mutation efficiency of the detected RNP system was PDSt6-RNP, with a mutation efficiency of 0.92%; the highest editing efficiency of the detected Cas12a system was Cas12a- PDSt9, with an mutation efficiency of 0.39%, mostly consistent with the predicted results (Fig. 6).

Nowadays, CRISPR/Cas9 technology has been applied to knock out *PDS* gene in banana to achieve albino mutant [20–22], and disrupt *MaGA20ox2* gene to obtain semi-dwarf banana material [23]. However, there have been no reports about transformation RNP into banana protoplast or to deliver RNP into embryogenic cell suspension(ECS) of banana. Although it has been reported that regenerated plants could be obtained by nursing culture of banana protoplasm [28, 29], this method displays low regeneration rate, poor reproducibility, and difficult regeneration. Therefore, further researches on improving plant regeneration efficiency from Cas9 RNP complex transformed protoplasts is very important for the applications of this technology in banana breeding. The mutation efficiency of transformation RNP into protoplast is much higher than that of delivery RNP into ECS by biolistic bombardment. It is very potential that the DNA-Free regeneration plants can be achieved from the banana protoplast transformed with RNP by PEG mediated method, as long as the increase in banana protoplast regeneration efficiency. This proposal has the vital significance in genetic improvement of banana and generation of new non-GMO germplasms.

Conclusion

In this study, we optimized and established a banana protoplast transformation method base on rice and wheat protocol, which is useful for gRNA activity validation. In addition, CRISPR/Cas9, CRISPR/Cas12a and Cas9 RNP complex can successfully edited endogenous gene via optimized protocol. The efficiency of CRISPR/Cas9 system is ahead of the other two systems. In addition, delivering Cas9 RNP complex into protoplasts using PEG-mediated method shows apparent advantages, compared to that into ECS by biolistic bombardment, and further researches on plant regeration from protoplasts is critial for sucessfully establishing DNA-free gene editing system in banana.

Methods

Plant material

Plant material used in this study is Cavendish Banana (*Musa* spp. Cavendish; AAA Group cv. 'Baxi'), the 'Baxi' banana is one banana cultivar that has been grown in China for many years and is one of the main banana cultivar in China.We obtained male flower buds of 'Baxi' banana from Institute of Fruit Tree Research, Guangdong Academy of Agricultural Sciences, Guangzhou, P. R. of China. ECS was induced by our laboratory in Institute of Fruit Tree Research, Guangdong Academy of Agricultural Sciences.

Banana protoplast preparation

Small and evenly distributed subculture of ECS for about 10 days was selected, the M2 medium was removed, and 10ml of enzymatic hydrolysate (3.0 % cellulose R-10, 1 % segregation enzyme R-10, 0.2 % pectinase Y-23, 15.2g/L KCl, 7.8g/L $CaCl_2$, 100 mg/L MES, 10 % mannitol, pH 5.7) was added, and the cell were incubated in a shaking table at 50 rpm/min for 6-8 hours. The yield of protoplasts was observed by microscope. If the enzymatic hydrolysis was sufficient, the hydrolysate was diluted with 10 ml W5 solution and shaken for 10 s to separate the protoplasts. A 75 micron membrane was used to filter the enzymatic solution into a round bottom centrifugal tube. Centrifugation was performed at 100 g for 3min, and the supernatant was removed by pipette. Protoplasts were suspended in 15 ml W5 Solution, incubated on ice for 30 min and discard the supernatant.

gRNA design and vector construction

A total of 9 targets aiming at *PDS* gene in banana were designed by SnapGene software. The OsU3p vector was digested by Bsa1 and the fragments was recovered from agarose gel. The serial joint primers(Supplemental data set7) were linked to the recycled OsU3p vector by T4 ligase to generate OsU3p- PDSt1 to OsU3p- PDSt9, and then transformed into *E. coli DH5a* competent cells. After overnight culture at 37 ℃, single colonies verified by sequencing were inoculated in the LB liquid medium with ampicillin. After overnight culture at 37 ℃ 220 rpm, plasmids were extracted.

CRISPR/Cas12a vector construction

A total of 11 targets aiming at *PDS* gene in banana were designed by SnapGene software. The Cas12a vector was digested by Bsa1 and the fragments was recovered from agarose gel. The serial joint primers(Supplemental data set8) were linked to the recycled Cas12a vector by T4 ligase to generate Cas12a- PDSt1 to Cas12a- PDSt11, and then transformed into *E. coli DH5a* competent cells. After overnight culture at 37 ℃, single colonies verified by sequencing were inoculated in the LB liquid medium with Kanamycin. After overnight culture at 37 ℃ 220 RPM, plasmids were extracted.

Transcription of sgRNAs *in vitro*

Specific primers of target sites (Supplemental data set9)were designed, using OsU3p- PDSt1 to OsU3p- PDSt9 plasmids as templates, amplified by high-fidelity enzyme FastPfu and purified by EasyPure PCR Kit. Transcription of purified PCR products *in vitro* was performed by NEB HiScribe ™ T7 *in vitro* Transcription Kit. In Vitro Transcription products were purified by TIANGEN RNA Purification Kit.

Protoplast transformation with plasmid or Cas9 RNP complex

The protoplast concentration was adjusted to 2 ×10^6 – 2 ×10^7 with MMG, and protoplasts were incubated on ice. 20 μg plasmids were added to 2 ml centrifuge tube, precipitated to the bottom of the tube by centrifugation. 200μl protoplasts was added into the tube, and mixed lightly. 250 μl 50 % PEG 4000 was added and induced transformation for 30 mins in dark. Addition of 900 μl W5 Solution stopped transfection. The sample was centrifuged at 100 g for 3min, and supernatant was discarded. The protoplast was resuspended with 1ml W5 and cultured in dark at 26-28 ℃.

According to the method described above, 10 μg plasmids pUbi-Cas9 was mixed respectively with 10 μg plasmids from OsU3p- PDSt1 to OsU3p- PDSt9, 20 μg plasmids from Cas12a- PDSt1 to Cas12a- PDSt11, Cas9 protein (20 μg) and sgRNA (20 μg). The prepared samples were respectively transformed into banana protoplast by PEG method and then dark cultured. The pUbi-GFP was transformed as a control.

PCR-RE test and monoclonal sequencing

After DNA and RNP transformation, the target sites with specific endonuclease sites were selected for PCR - RE test. Genomic DNA of protoplasts was extracted. The sequence with the length of about 1000 bp containing target sites was amplified by PFU enzyme. The specific PCR products was digested by *Eco*471 at 37 ℃ for 2 hours and analyzed on 2 % agarose gel by electrophoresis. In order to determine whether there were specific fragments with base mutations, the specific fragments were recovered after electrophoresis, connected to the T-blunt vector, transformed into *E. coli DH5a* competent cells and then selected for monoclonal sequencing.

RNP cleavage *in vitro*

DNA fragments containing target 3 and target 4 were amplified by PCR, purified by EasyPure PCR Purification Kit, and eluted by RNase-free water. The cleavage reaction system *in vitro* was as follows: Cas9 protein (1 μg), sgRNA (1 μg), target fragment (100 ng), 10×Cas9 reaction buffer (20 mM HEPES, pH 7.5, 150 mM KCl, 10 mM $MgCl_2$, 0.5 mM DTT) 2 μl, RNase-free water up to total volume of 20 μl. Samples were incubated at 37 °C for 1 hour and then at 65 °C for 10 mins. Finally samples were tested by electrophoresis on 2 % agarose gel.

Deep amplicon sequencing

The prepared samples of DNA or RNP were transformed into banana protoplast. After 4-5 days dark culture, the banana protoplast were collected by centrifugation at 12000 RPM and then genomic DNA was extracted by TIANGEN DNA extracted Kit. Deep amplicon sequencing primers(Supplemental data set10, Supplemental data set11)were designed and nested PCR was performed to amplify fragments with the length of approximate 200 bp. After gel purification, samples were sent for Deep amplicon sequencing by Shanghai shenggong biology co., Ltd to determine whether there were base mutations in the target sequences and the types of mutations.

List Of Abbreviations

PEG, Polyethylene glycol; CRISPR, Clustered regularly interspaced short palindromic repeats; RNP, Ribonucleoprotein; PCR-RE, PCR-Restriction Enzyme; PDS, Phytoene dehydrogenase; GFP, Green fluorescent protein; ECS, Embryogenic cell suspension; sgRNA, Single-guide RNA.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

All listed coauthors have contributed to the manuscript, reviewed the manuscript and agreed with its publication in *BMC Plant Biology*.

Availability of data and materials

Deep amplicon sequencing data are available under BioProject IDs PRJNA637446 (https://www.ncbi.nlm.nih.gov/sra/PRJNA637446), PRJNA637703 (https://www.ncbi.nlm.nih.gov/sra/PRJNA637703) and PRJNA637699 (https://www.ncbi.nlm.nih.gov/sra/PRJNA637699).

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by the National Key R&D Program of China (2018YFD1000302 and 2019YFD1000901), National Natural Science Foundation of China (31872939), Modern Agricultural Innovation Team Project of Guangdong Province (2018LM2150 and 2019KJ106), R&D Plan of Guangdong Province Key Fields (2018B020202005), Science and Technology Plan Project of Guangdong Province (2015B070701011), and Guangzhou Scientific Research Plan (201904020033) , Special fund for scientific innovation strategy-construction of high level Academy of Agriculture Science (R2017PY-JX001).Thanks to grant No. 2015B070701011No. R2017PY-JX001 and No. 31872939 ,we were able to pay for reagents, Thanks to grant No. 2018YFD1000302 and No. 2018LM2150, we were able to pay for all kinds of kits, Thanks to grant No. 2018B020202005, No. 2019YFD1000901, No. 2019KJ106 and No. 201904020033 , we were able to pay for primers and sequencing. The design of the study and collection, analysis, and interpretation of data and writing the manuscript were performed by all the authors. The funding bodies just provide the financial support.

Authors' contributions

G.Y., K.C. designed research; S.W., H.Z., J.L., Q.Y., H.S., F. B., C.H., performed research; S.W., H. Z. analyzed data; S.W., H.Z. ,H.H., Q.Y. wrote the paper. all authors have read and approved the manuscript

Acknowledgements

The authors would like to thank Caixia Gao for technical support and kindly providing the Cas9, gRNA and Cas12a vectors.

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Figures

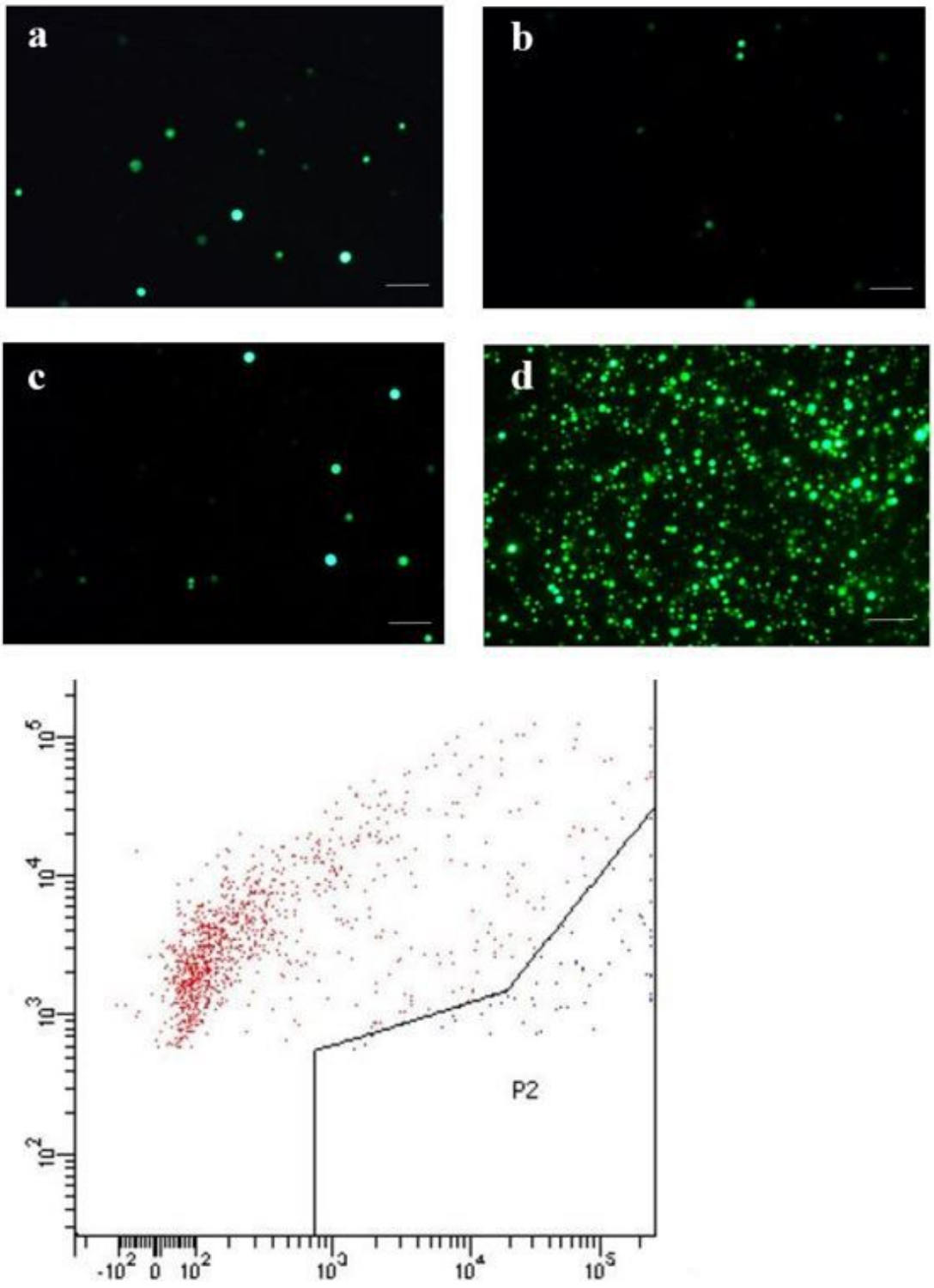


Figure 1

Detection of GFP fluorescence in transformed protoplasts by fluorescence microscope. pUbi-GFP plasmid was used for protoplasts transformation under different transformtaion condition, 40% PEG and 20 mins incubation (a), 40% PEG and 30 mins incubation (b), 50% PEG and 20 mins incubation (c), 50% PEG and 30 mins incubation (d). The protoplasts from 50 % PEG with 30 mins combination were examined by flow cytometry (e). All pictures were taken after 5 days incubation in dark. Scale bars were 75 μm.

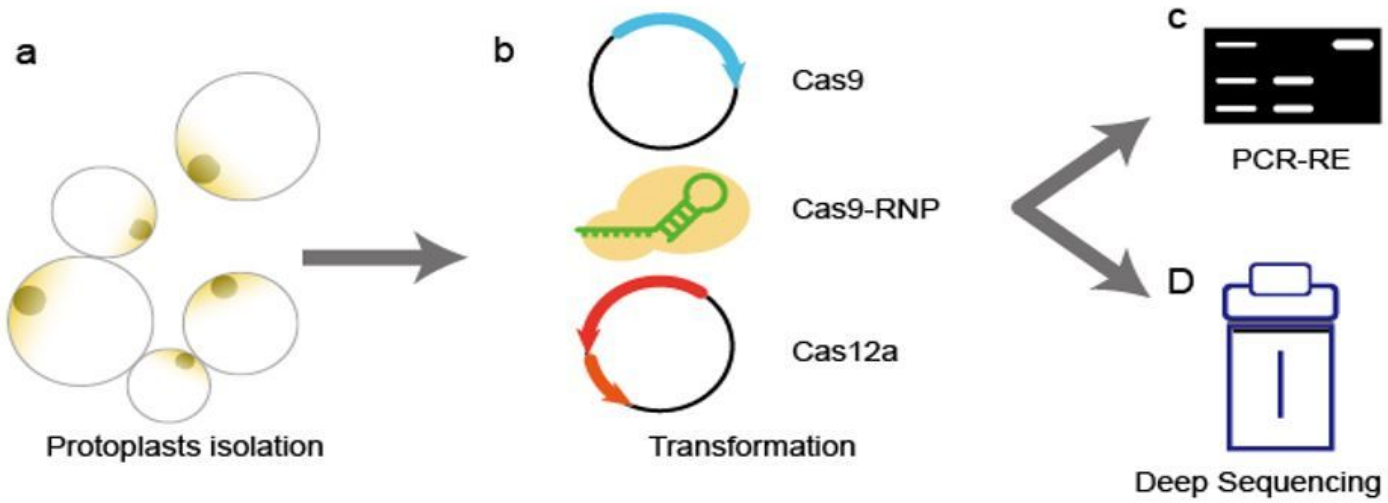


Figure 2

Experimental design and workflow of the transient genome editing system in banana protoplasts. The banana protoplasts were isolated from embryogenic cell suspension of Cavendish banana□AAA□(a), and then plasmids or RNP were transformed into the banana protoplasts through polyethylene glycol (PEG)-mediated (b). The genome DNA of protoplasts was extracted 4 or 5 days after the transformation. The mutation efficiency was measured by PCR-RE (c) or Deep amplicon sequencing (d).

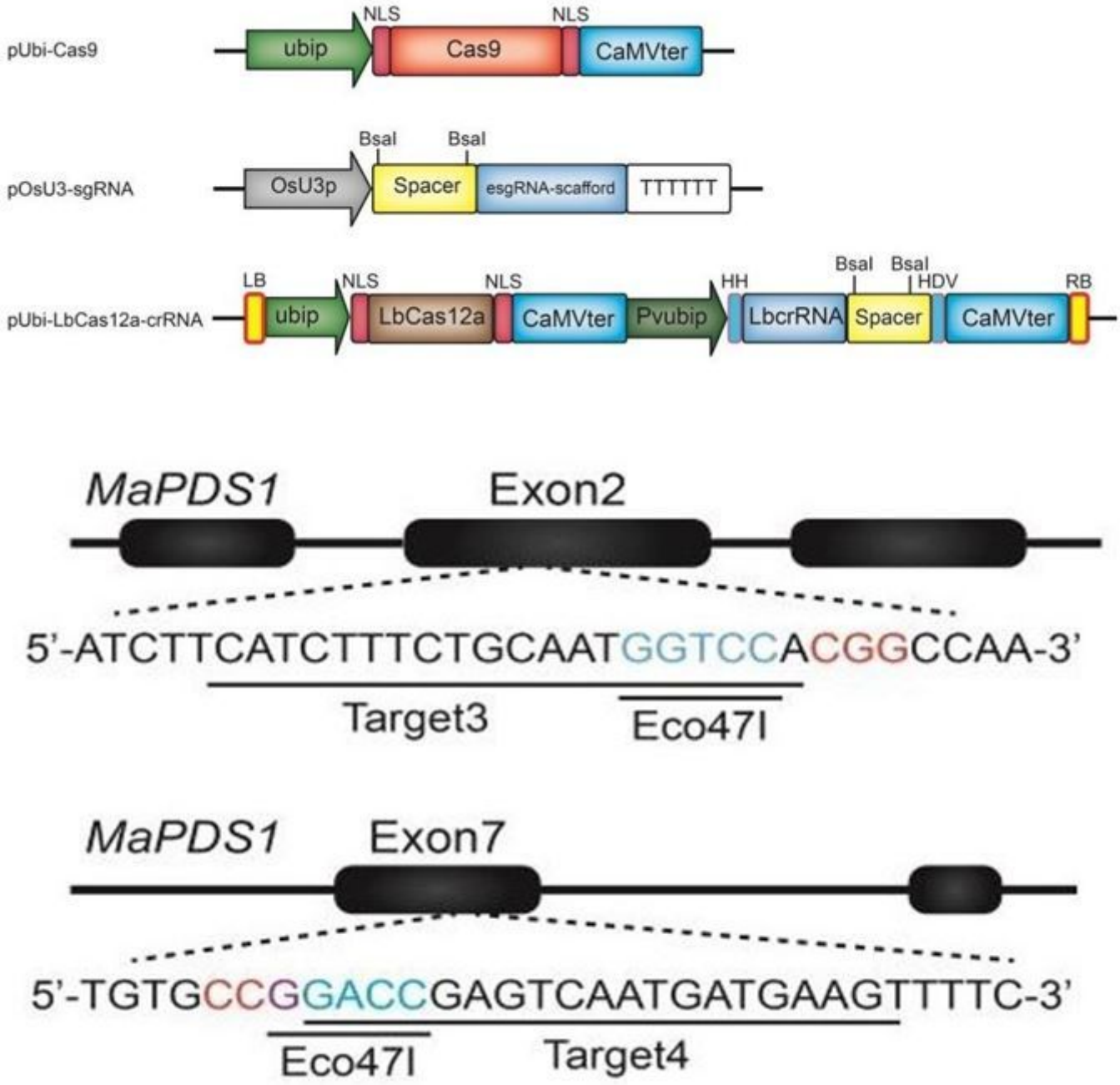


Figure 3

Schematic representation of the vectors and targets implemented in this study. Diagrams of pUbi-Cas9, sgRNA plasmid and Cas12a vector. ubip, ubiquitin promoter from Zea mays. Pvubip, ubiquitin promoter from Phaseolus vulgaris. CaMVter, termination sequence of cauliflower mosaic virus (a). Details of two targets against the PDS gene in banana. The position of target 3 and target 4 on the gene and the endonuclease sites were illustrated (b).

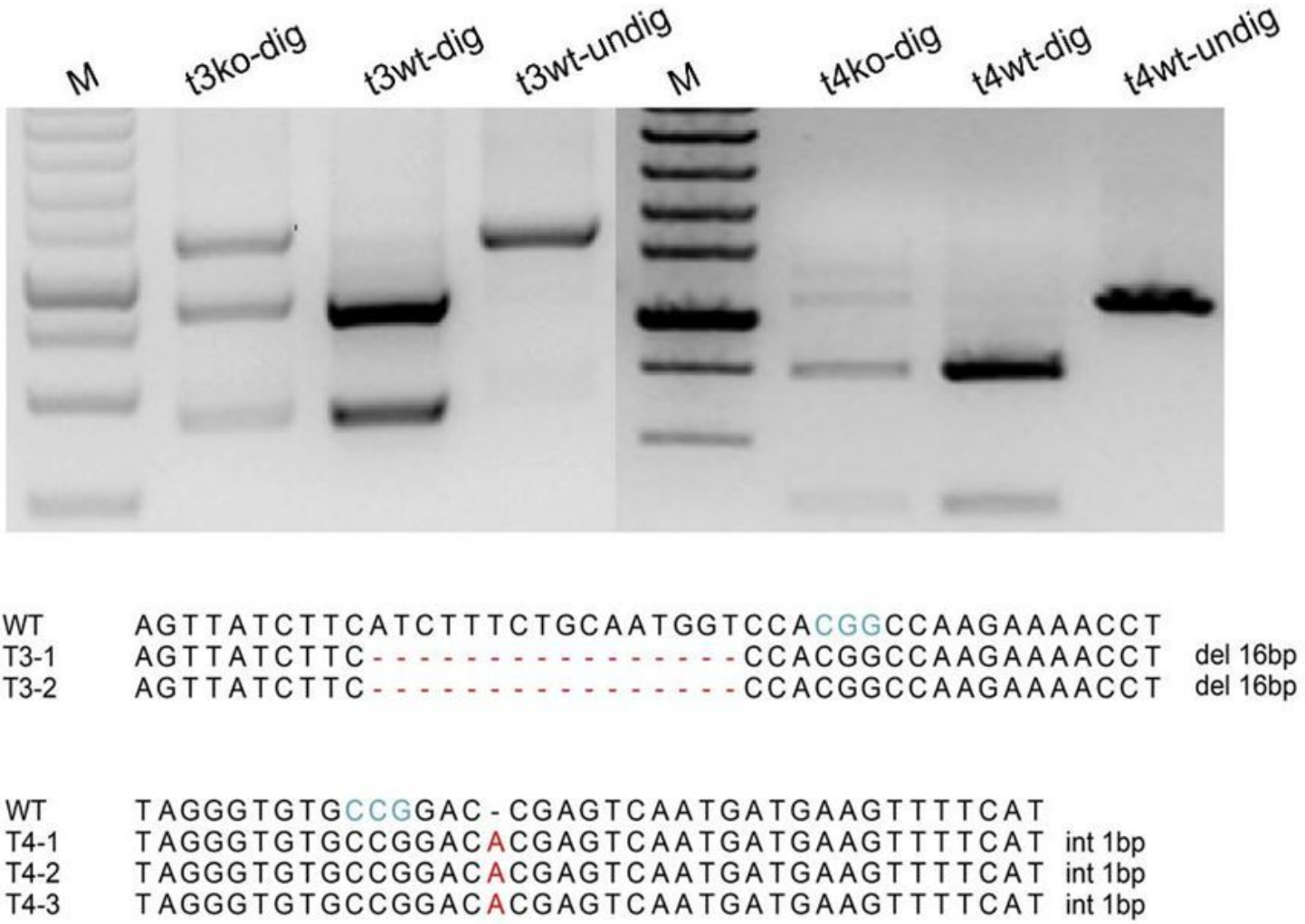
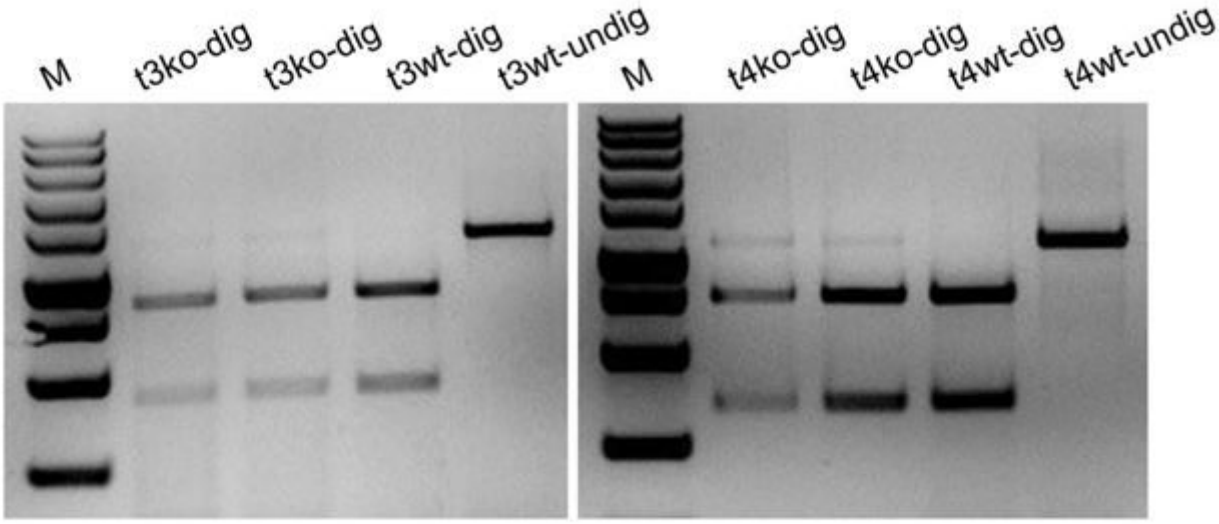


Figure 4

Protoplast transformed Cas9 system, target 3 and target 4 PCR-RE assay and specific band monoclonal sequencing. PCR-digestion essay. The PCR amplified fragment of edited target 3 (t3ko) or its wild type (t3wt), edited target 4 (t4ko) or its wild type (t4wt) were digested by Eco47I (dig) or were not digested (undig). Gene edited fragments were amplified with the DNA from transformed protoplast, while wild type target fragments were amplified with the DNA from untransformed protoplast. M indicates DNA marker (a). Sanger sequencing of single clone of undigested fragments from Figure 4a (b).



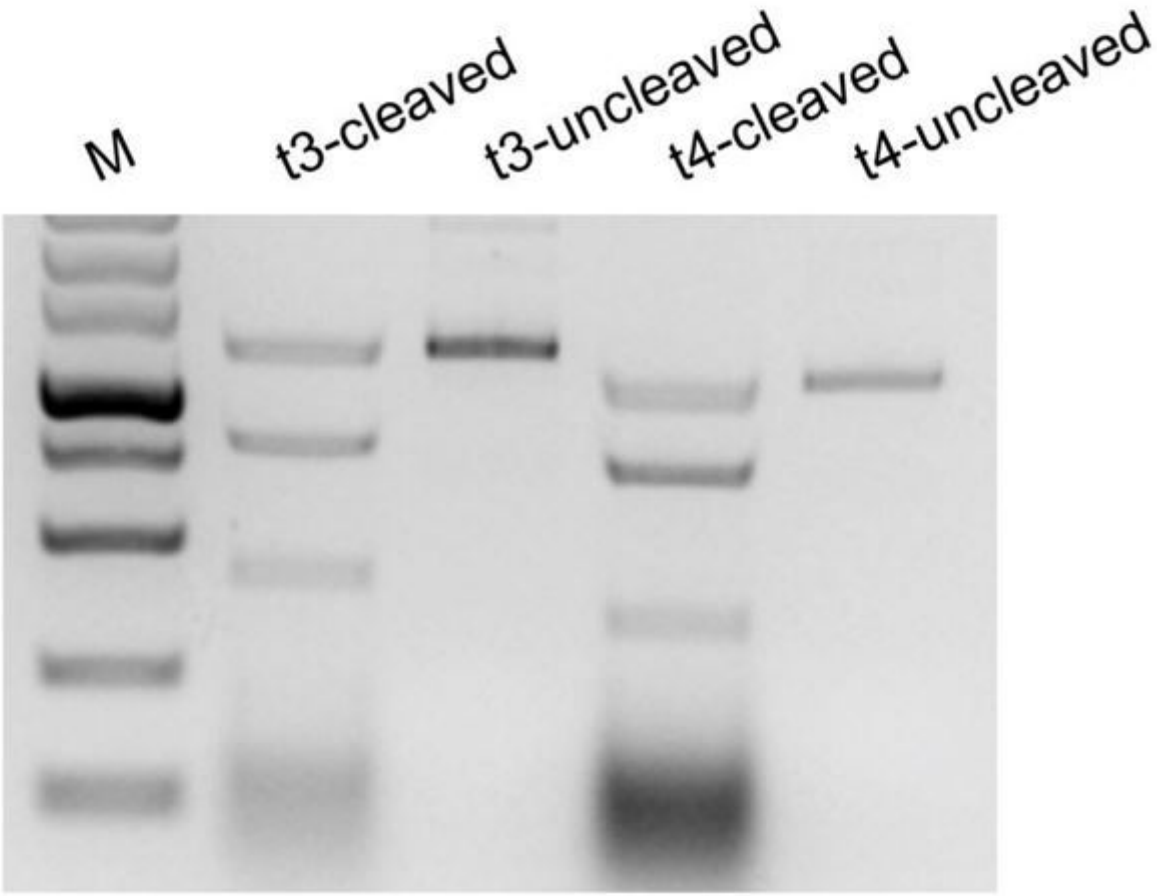


Figure 5

RNP cleavage test in vitro and PCR-RE test after protoplast transformation. RNP cleavage test in vitro, t3-cleaved and t4-cleaved were used to cut the target 3 and target 4 PCR sequence by RNP, while t3-uncleaved, as a control, was the sequence of target 3 PCR, as well as t4-uncleaved (a). ko-dig referred to Eco471 enzyme digestion of PCR amplification target sequences after RNP transformation into protoplast; wt-dig referred to Eco471 enzyme digestion of target sequences of PCR amplification after

blank transformation into protoplast; wt-undig referred to PCR amplification after blank transformation into protoplast, as a control. M indicates DNA marker (b).

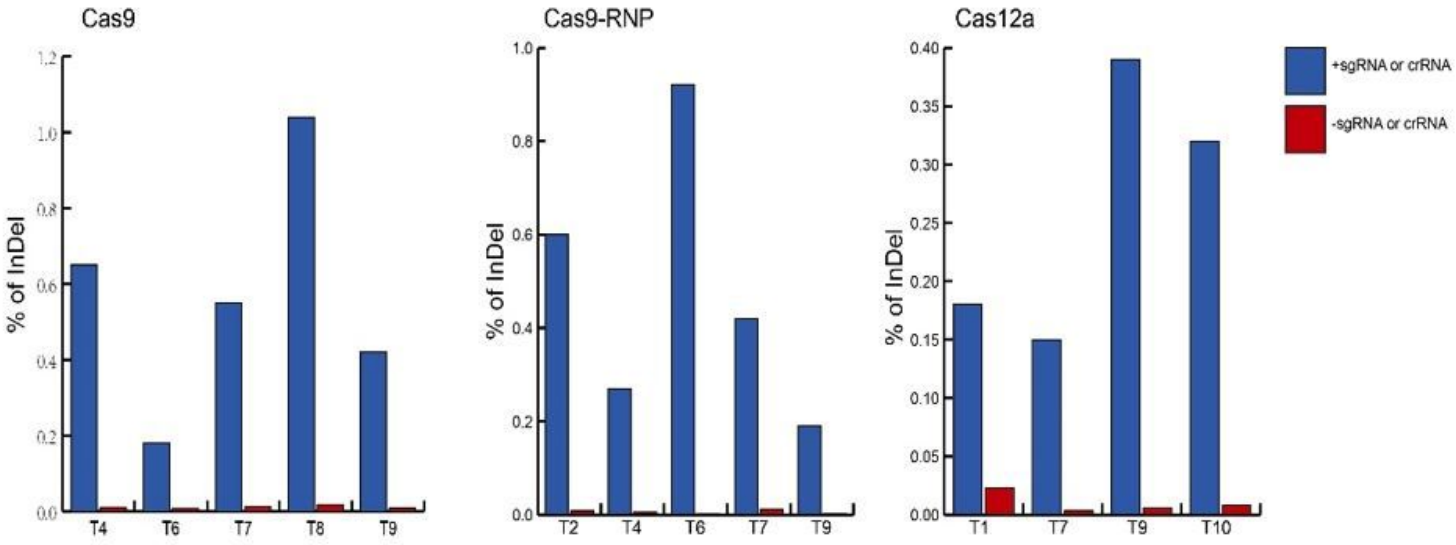


Figure 6

Frequencies of indels introduced by Cas9 , RNP or Cas12a genome editing system.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementalData.pdf
- OriginalpictureofFigure1a.jpg
- OriginalpictureofFigure1b.jpg
- OriginalpictureofFigure1c.jpg
- OriginalpictureofFigure1d.jpg
- OriginalpictureofFigure1e.jpg
- OriginalelectrophoreticgelspictureofFigure4a1.TIF
- OriginalelectrophoreticgelspictureofFigure4a2.TIF
- OriginalelectrophoreticgelspictureofFigure5a.TIF
- OriginalelectrophoreticgelspictureofFigure5b.JPG