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(54) Title: COMPOSITIONS, CONSTRUCTS, PLANT VIRAL VECTORS, AND METHODS FOR PLANT GENE EDITING

(57) Abstract: Disclosed herein are compositions, constructs, viral vectors, and methods for plant gene editing. The compositions can include a TnpB bacterial enzyme and a polynucleotide having a first portion adapted to bind to at least a portion of the TnpB bacterial enzyme and a second portion for binding to a plant genomic target.

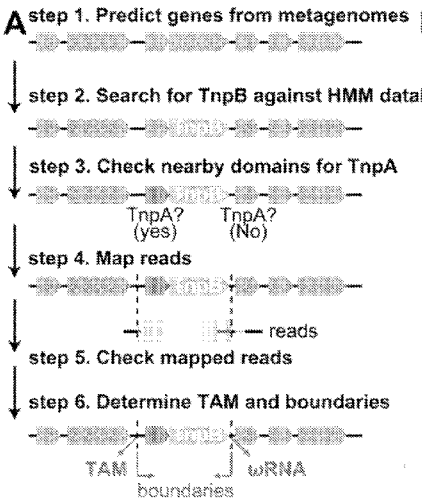

FIG. 1A

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[Continued on next page]

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COMPOSITIONS, CONSTRUCTS, PLANT VIRAL VECTORS, AND METHODS FOR PLANT GENE EDITING

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional App. No. 63/511,070, filed on June 29, 2023, U.S. Provisional App. No. 63/520,258, filed on August 17, 2023, and U.S. Provisional App. No. 63/566,666, filed on March 18, 2024. The entire contents of each of the aforementioned applications are incorporated by reference herein.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] This invention was made with government support under award number 2334027 awarded by the National Science Foundation. The government has certain rights in the invention.

REFERENCE TO AN ELECTRONIC SEQUENCE LISTING

[0003] The contents of the electronic sequence listing (790482.00489.xml; Size: 785,615 bytes; and Date of Creation: June 26, 2024) is herein incorporated by reference in its entirety.

BACKGROUND

[0004] Given current crop productivity projections, agricultural practices will be inadequate to meet the global food demands by the year 2050 (Ray et al., 2013). Our ability to address this problem will largely depend on the efficiency that novel genetic diversity can be created and introduced into current plant breeding pipelines. Historically, novel phenotypes were generated through random mutation or transgenic breeding, followed by successive backcrossing to an elite crop variety, taking upwards of 10-12 years to complete (Chen et al., 2019). With the advent of genome editing, we now have the ability to more efficiently genetically modify crop genomes, resulting in beneficial phenotypes(Haun et al., 2014; Lemmon et al., 2018; Liu et al., 2021; Rodriguez-Leal et al., 2017; Zsogon et al., 2018). Despite this advance, a primary bottleneck remains: fast and efficient delivery of the gene editing reagents into crop plants (Altpeter et al., 2016).

SUMMARY

[0005] In one aspect, a composition is provided. The composition can include a TnpB bacterial enzyme. The composition can also include a polynucleotide including a first portion adapted to bind to at least a portion of the TnpB bacterial enzyme and a second portion for binding to a plant genomic target.

[0006] In another aspect, a construct is provided. The construct can include a plant promotor operably connected to a polynucleotide encoding for a TnpB bacterial enzyme.

[0007] In yet another aspect, a plant viral vector is provided. The plant viral vector can include a construct that can include a plant promotor operably connected to a polynucleotide encoding for a TnpB bacterial enzyme.

[0008] In another aspect, a plant cell is provided. The plant cell can include a construct that can include a plant promotor operably connected to a polynucleotide encoding for a TnpB bacterial enzyme, or a plant viral vector that includes the construct.

[0009] In another aspect, a method for gene editing a plant is provided. The method can include introducing a construct or a plant viral vector into one or more plant cells. The construct can include a plant promotor operably connected to a polynucleotide encoding for a TnpB bacterial enzyme. The plan viral vector can include the construct.

BRIEF DESCRIPTION OF THE FIGURES

[0010] FIGS. 1 A and 1B. (FIG. 1A)The "DOTS" pipeline for detecting bona-fide RNA-guided TnpB systems in metagenomes. (FIG. 1B) A phylogenetic tree of TnpBs from metagenomes (red) as well as a public IS database, ISfinder (black), with the corresponding amino acid length shown in the bar graph below. Distinct TAM sequences and small-sized TnpB enzymes from metagenomes are also highlighted in red circles and black arrows, respectively.

[0011] FIGS. 2A-2C. (FIG. 2A) Representative loci architectures for TnpB-associated systems characterized in this study. (FIG. 2B) The Web logo of the TAMs for ISBag01 and ISXfa01 TnpB systems. (FIG. 2C) Comparable *E. coli* interference activities between Cas12a, ISBag01 and ISXfa01, when the target site is flanked by a PAM/TAM at 26°C.

[0012] FIGS. 3A-3C. (FIG. 3A) Schematics of the constructs used to test gene editing activity by ISBag01. Top panel, ISBag01 ωRNA with a 20bp spacer targeting the *AtPDS3* gene were driven by the AtU6-26 promoter and followed by the HDV ribozyme. 2X indicates that the construct also contains a second similar AtU6-26 driven ωRNA cassette with a second gRNA sequence that is not shown. *Zea mays* codon optimized ISBag01 coding sequence was expressed in a separate transcription cassette driven by the *UBQ10* gene promoter and an rbcS-E9 terminator. Bottom panel, native ISBag01 sequence encoding the ISBag01 protein and the overlapping ωRNA were driven by the *UBQ10* gene promoter, followed by a 20bp spacer targeting the *AtPDS3* gene the HDV ribozyme, and the rbcS-E9 terminator. NLS, nuclear localization signal. (FIG. 3B) Left panel, editing efficiency of ISBag01 at the *AtPDS3* gene at regions targeted by four different guide RNAs in *Arabidopsis* protoplasts, with ISBag01 protein coding sequence and ωRNA coding sequence in split cassettes. Right panel, editing efficiency by ISBag01 and *AtPDS3* gRNA1, with native ISBag01 coding sequence and ωRNA sequence

in the same cassette. (FIG. 3C) Examples of detailed editing profiles by ISBag01 in Arabidopsis protoplasts detected by amplicon sequencing. Left panel, *AtPDS3* gRNA1. Right panel, *AtPDS3* gRNA18. Editing events are shown on the left as: (position where the editing starts):(number of nucleotides of)D(deletion). position 0 is between the 18th and 19th nucleotides of the guide RNA sequence, such that the 18th nucleotide is position -1, the 19th nucleotide is position +1. Read number, number of reads showing a particular edit. AtPDS3 gRNA1 reference sequence is SEQ ID NO: 610, amplicons in descending order are SEQ ID NOs: 611-620. AtPDS3 gRNA18 reference sequence is SEQ ID NO: 621, amplicons in descending order are SEQ ID NOs: 622-625.

[0013] FIG. 4A and 4B. Schematics of a bacteria selection assay. (FIG. 4A) The *E.coli* cell harbors the selection plasmid with the inducible toxic gene induced by Arabinose. The cell can survive in the absence of Arabinose but not in the presence of Arabinose. (FIG. 4B) Transforming another plasmid encoding TnpB and ωRNA targeting the toxic gene will result in survival of the cell.

[0014] FIGS. 5A-5C. (FIG. 5A) The distribution of amino acid lengths of Cas9, Cas12, IscB and TnpB endonucleases. (FIG. 5B) Representative loci architecture of CRISPR-Cas systems versus TnpB-associated transposon systems (FIG. 5C) Molecular mechanism of DNA targeting by CRISPR-Cas enzymes and TnpB-ωRNA.

[0015] FIG. 6. An example of the split expression cassette to express the ωRNA and the TnpB as described in Example 8. In this example, the UBQ10 promoter is used to drive expression of the TnpB, followed by the rbcS-E9 terminator. Two SV40 NLS signals and two FLAG tags are located at the 3' end, however these sequences may also be tested at the 5' end of the TnpB. In this example the ωRNA is being driven by the U6 promoter, with an HDV ribozyme sequence downstream of the spacer; however, a variety of promoters and RNA processing sequences may be tested.

[0016] FIG. 7A and 7B. (FIG. 7A) An example schematic of the TnpB and ωRNA architecture commonly found in nature, where the ωRNA overlaps with the TnpB at the 3' end of the TnpB sequence. (FIG. 7B) An example of a single expression cassette to express the TnpB and ωRNA. In this example the UBQ10 promoter is used to drive expression of the TnpB-ωRNA sequence, and terminated by the rbcS-E9 terminator. In this example the HDV ribozyme sequence downstream of the spacer is depicted, however a variety of RNA processing systems such as tRNA-Gly may be used.

[0017] FIG. 8. An example of the single expression cassette to express the TnpB and ωRNA, followed by a tandem repeat of the ωRNA and spacer sequences. In this example the

UBQ10 promoter is used to drive expression of the TnpB-ωRNA sequence, and terminated by the rbcS-E9 terminator. In this example the HDV ribozyme sequence upstream of the terminator is depicted, however a variety of RNA processing systems such as tRNA-Gly may be used.

[0018] FIGS. 9A-9D. TRV2 schematic depicting the RNA virus sequence. The red box indicates the part of the virus where TnpB and ωRNA will most likely be cloned. The light gray boxes indicate the promoter and terminator used to drive initial expression of the RNA virus. The light blue boxes indicate the RNA virus. (FIG. 9A) TnpB-ωRNA single expression cassette cloned into the "cargo" site. (FIG. 9B) TnpB and ωRNA split expression design. (FIG. 9C) TRV2 schematic showing an example of a RNA processing sequence, HDV ribozyme. (FIG. 9D) TnpB and tandem ωRNA cloned into the "cargo" design.

[0019] FIG. 10A-10C. ISYmu1, ISDra2 and ISAam1 editing frequency. The target sites are plotted along the X-axis and the editing efficiency (percent indel reads (%)) are plotted on the Y-axis. For ISYmu1 and ISDra2, additional transfections were performed to replicate initial results, as indicated by "experiment_2" and "experiment_3" along the X-axis. Each dot indicates a single transfection. The standard error of the mean (SEM) was calculated for each target site.

[0020] FIGS. 11. Editing frequency for TnpBs capable of editing an average greater than 0.1% at least one target site. The target sites are plotted along the X-axis and the editing efficiency (percent indel reads (%)) are plotted on the Y-axis. Each dot indicates a single transfection. The standard error of the mean (SEM) was calculated for each target site.

[0021] FIG. 12A-12D. Small RNA-Seq and structure analysis of ISBag01 and ISYmu1 ωRNA sequences. (FIG. 12A and FIG. 12B) Small RNA-Seq results for ISYmu1 and ISBag01 with the read count plotted along the Y-axis, and the TnpB and ωRNA positions in the bacterial expression vectors plotted along the X-axis. (FIG. 12C and FIG. 12D). The secondary structures of ωRNA for ISYmu1 and ISBag01 predicted by Vienna RNA Fold (Gruber et al. 2008), consisting of ωRNA scaffold (ISYmu1=127-nt; ISBag01=186-nt) and guide regions. The stem-loops and pseudoknot (PK) interactions are also indicated on the structures. ISYmu1 (SEQ ID NO: 626) and ISBag01 (SEQ ID NO: 627).

[0022] FIG. 13A and 13B. ISBag01 ωRNA variants for improved editing efficiency. (FIG. 13A) ωRNA (WT, SEQ ID NO: 628) variant structure for 1.1 (SEQ ID NO: 629) and 1.2 (SEQ ID NO: 630). (FIG. 13B) ISBag01 g1 was used in this experiment. The target sites are plotted along the X-axis and the editing efficiency (percent indel reads (%)) are plotted on the Y-axis. The X-axis label g1_native indicates the TnpB with ωRNA overlap Native DNA sequence;

g1_variant_1.1 and g1_variant_1.2 are novel ωRNA variants. Each dot indicates a single transfection. The standard error of the mean (SEM) was calculated for each target site. P value was calculated using a two-tailed t-Test assuming equal variances.

[0023] FIG. 14. Editing frequency for ISYmu1 using TRV vectors for genome editing in Arabidopsis protoplast cells. The ISYmu1 TRV2 cargo configurations are plotted along the X-axis and the editing efficiency (percent indel reads (%)) are plotted on the Y-axis. The vertical dashed line separates experiment one and two. Each dot indicates a single transfection. The standard error mean (SEM) was calculated for each target site.

[0024] FIG. 15A-15G. Gene editing outcomes in transgenic T1 plants expressing ISYmu1 or ISDra2 TnpB. (FIG. 15A-15D) The genotypes are plotted along the X-axis and the editing efficiency (percent indel reads (%)) are plotted on the Y-axis. Each dot indicates an independent T1 plant. The standard error of the mean (SEM) was calculated for each target site. The number above each bar indicates the average editing frequency. WT is an abbreviation for wild type, and *rdr6* is an abbreviation for the mutant in the *RNA-DEPENDENT RNA POLYMERASE 6* gene. (FIG. 15E-15G) DNA repair indel profile for ISDra2 g9 and ISYmu1 g12 wildtype samples. The six, two, and twenty most common indels are shown for ISDra2 g9, ISDra2 g12, and ISYmu1 g12, respectively. The indel type is listed on the left. For example -3 means a deletion of three base pairs. The read counts for each indel are listed on the right. The TAM is identified by the red box, and the target site is located by the black box in the Reference sequence. ISDra2 g9 WT reference sequence is SEQ ID NO: 631, amplicons in descending order are SEQ ID NOs: 632-637. ISDra2 g12 WT reference sequence is SEQ ID NO: 638, amplicons in descending order are SEQ ID NO 639-640. ISYmu1 g12 WT reference sequence is SEQ ID NO: 641, amplicons in descending order are SEQ ID NOs: 642-661.

[0025] FIG. 16A-16E. TRV delivery of ISYmu1 for editing in whole plants. (FIG. 16A and 16B) Violin plots depicting the genotypes along the X-axis and the editing efficiency (percent indel reads (%)) on the Y-axis. Each dot indicates an independent plant. The standard error of the mean (SEM) was calculated for each target site as indicated by the blue dot and vertical line. WT is an abbreviation for wild type, and *rdr6* is an abbreviation for mutant in RNA-DEPENDENT RNA POLYMERASE 6 gene. (FIG. 16C and 16D) DNA repair indel profile for ISYmu1 g2. The indel type is listed on the left. The read counts for each indel are listed on the right. The TAM is identified by the red box, and the target site is located by the black box in the Reference sequence. ISYmu1-g2-tRNA reference sequence is SEQ ID NO: 662, amplicons in descending order are SEQ ID NOs: 663-672. ISYmu1-g2-HDV-tRNA reference sequence is SEQ ID NO: 673, amplicons in descending order are SEQ ID NOs: 674-

686. (FIG. 16E) Examples of plants infected with TRV vectors showing white sectors, an ISYmu1-g2-tRNA-Isoleucine TRV infected plant (top) and an ISYmu1-g2-HDV-tRNA-Isoleucine TRV infected plant (bottom). Yellow arrow points to a leaf with clear white sectors.

[0026] FIGS. 17A-17E. Testing activities of novel TnpBs in bacteria. (FIG. 17A) Cassette of dual plasmids for plasmid interference assay. (FIG. 17B) Normalized CFU (Norm. CFU) of five identified novel TnpBs. All the interference assays were performed at 26°C. Error bars represent the standard error of the mean (SEM) of three replicas. (FIG. 17C) TAM sequences of TnpB_30 from the TAM depletion assay. (FIG. 17D) Small RNA-seq confirmed expression of the ωRNA of TnpB_30. (FIG. 17E) Secondary structures of TnpB_30 ωRNA (SEQ ID NO 687).

[0027] FIG. 18. TnpB_30 editing efficiency in *Arabidopsis* protoplast cells. The target sites are plotted along the X-axis and the editing efficiency (percent indel reads (%)) is plotted on the Y-axis. Each dot indicates a single transfection. The standard error of the mean (SEM) was calculated for each target site.

[0028] FIGS. 19A-19D. Spacer length optimization for ISDra2, ISYmu1, ISBag01, and TnpB_30. The spacer lengths (nucleotides) are plotted along the X-axis and the editing efficiency (percent indel reads (%)) is plotted on the Y-axis. Each dot indicates a single transfection. The standard error of the mean (SEM) was calculated for each target site. (FIG. 19A) ISDra2. (FIG. 19B) ISYmu1. (FIG. 19C) ISBag01. (FIG. `9D) TnpB_30.

[0029] FIG. 20A-20C. Comparison of ISYmu1 expression using the single or split expression cassette designs. (FIG. 20A) Plasmid design for the single and split plasmids. Green arrow boxes symbolize promoters, red boxes symbolize terminators, and the black arrows indicate the orientation of the expression cassette. (FIG. 20B) Small RNA-seq results for ISYmu1 with the read count plotted along the Y-axis, and the TnpB and ωRNA positions in the bacterial expression vectors plotted along the X-axis. (FIG. 20C) TnpB and ωRNA single and split expression configurations are plotted along the X-axis and the editing efficiency (percent indel reads (%)) is plotted on the Y-axis. Each dot indicates a single transfection. The standard error of the mean (SEM) was calculated for each target site.

[0030] FIG. 21. ISYmu1 ωRNA variants for improved editing efficiency. (A) ISYmu1 g2 was used in this experiment. The ωRNA variants are plotted along the X-axis and the editing efficiencies (percent indel reads (%)) are plotted on the Y-axis. The X-axis label WT v0.0 indicates the TnpB with ωRNA overlap native DNA sequence; all other variants along the X-axis are novel ISYmu1 ωRNA variants. Each dot indicates a single transfection. The standard error of the mean (SEM) was calculated for each ωRNA design tested.

[0031] FIG. 22. Representative ISYmu1 ωRNA variants at stem-loop 2. The stem-loop 2 are highlighted in the secondary structures of ISYmu1 ωRNA variants. Representative ωRNA variants of stem-loop 2 are compared to the wild-type sequences. The topologies of the RNA two-way junctions are labeled within the region of interest. Stem-loop 2 (top) is SEQ ID NO: 688, WT is SEQ ID NO: 689, v3.2 is SEQ ID NO: 690, v3.16 is SEQ ID NO: 691, v2.1 is SEQ ID NO: 692.

[0032] FIG. 23A and 23B. ISYmu1 somatic editing in T1 transgenic plants. ISYmu1 g2 and ISYmu1 g12 were used in this experiment. Panels A and B display box and whisker plots. Each dot indicates a single T1 transgenic plant. The room and HS treatments stand for room temperature and heat shock plant growth conditions, respectively. The genotypes are plotted along the X-axis and the editing efficiencies (percent indel reads (%)) are plotted on the Y-axis. WT is an abbreviation for wild type, and *rdr6* is an abbreviation for mutant in *RNA-DEPENDENT RNA POLYMERASE 6* gene. (FIG. 23A) ISYmu1 g2 T1. (FIG. 23B) ISYmu1 g12 T1.

[0033] FIG. 24A and 24B. ISDra2 somatic editing in T1 transgenic plants. ISDra2 g9 and ISDra2 g12 were used in this experiment. Panels A and B display box and whisker plots. Each dot indicates a single T1 transgenic plant. The room and HS treatments stand for room temperature and heat shock plant growth conditions, respectively. The genotypes are plotted along the X-axis and the editing efficiencies (percent indel reads (%)) are plotted on the Y-axis. WT is an abbreviation for wild type, and *rdr6* is an abbreviation for mutant in RNA-DEPENDENT RNA POLYMERASE 6 gene. (FIG. 24A) ISDra2 g9 T1. (FIG. 24B) ISDra2 g12 T1.

[0034] FIG. 25A-25D. TRV delivery of ISYmu1 for editing in whole plants. (FIG. 25A) Schematic of the TRV1 and TRV2 plasmids. (FIG. 25B and 25C) The TRV cargo configurations are plotted along the X-axis and the editing efficiency (percent indel reads (%)) is plotted on the Y-axis. Each dot indicates a single plant. The standard error of the mean (SEM) was calculated for each target site. WT samples are black, *rdr6* samples are blue, and *ku70* samples are red. WT is an abbreviation for wild type, and *rdr6* is an abbreviation for mutant in RNA-DEPENDENT RNA POLYMERASE 6 gene. (FIG. 25B) ISYmu1 g2. (FIG. 25C) ISYmu1 g12. (FIG. 25D) DNA repair indel profile for ISYmu1 g12 using TRV to deliver ISYmu1-g12-HDV-tRNA-Isoleucine. The top six most common indel types are listed on the left. The read counts for each indel are listed on the right. The TAM is identified by the red box, and the target site is located by the black box in the reference sequence. The reference sequence is SEQ ID NO: 693, amplicons in descending order are SEQ ID NOs: 694-699.

[0035] FIG. 26A-26C. Heritability of edits generated with ISYmu1 g2 encoded on the TRV vector. (FIG. 26A) Picture of a plant that underwent TRV delivery using ISYmu1-g2-HDV-tRNA-Isoleucine. The white sectors, indicated by the yellow arrows, indicate biallelic mutations in the *PDS3* gene. The 54.54% in the upper left corner is the editing frequency determined by random tissue sampling of three leaves distal from the TRV delivery location. (FIG. 26B) Image of progeny seedlings from the plant in Figure 26A, depicting two albino seedlings. (FIG. 26C) Sanger sequencing trace file screenshot for one of the albino plants in FIG. 26B (top sequence, SEQ ID NO: 700, bottom sequence SEQ ID NO: 701).

[0036] FIG. 27A-27F. Testing the impact of ωRNA processing on the activities of TnpB in bacteria. (FIG. 27A) Different cassettes of TnpB and ωRNA expression in bacteria. (FIG. 27B) Normalized CFU of ISDra2, ISBag01, ISYmu1 and ISAam1 TnpB expressed in cassette (a). (FIG. 27C) Normalized CFU of ISDra2, ISBag01, ISYmu1 and ISAam1 TnpB expressed in cassette (b). (FIG. 27D) Normalized CFU of ISBag01 expressed in cassette (c) and (d). (FIG. 27E) Normalized CFU of ISYmu1 expressed in cassette (c) and (e). (FIG. 27F) Normalized CFU of ISYmu1 expressed in cassette (f) and (g). All the interference assays were performed in 26°C. Error bars represent the standard error of the mean (SEM) of three replicas.

DETAILED DESCRIPTION

[0037] Overview

[0038] The most common method of delivery of gene editing reagents into plants is to encode RNA-guided genome editors (e.g. CRISPR-Cas enzymes) within transgenes and use standard tissue culture transformation approaches (Altpeter et al., 2016; Chen et al., 2019; Nasti & Voytas, 2021). However, this approach has substantial disadvantages. Tissue culture methods require considerable time, resources, technical expertise, and can cause unintended changes to the genome and epigenome (Altpeter et al., 2016). Further, tissue culture is restricted in its application, as only a limited number species and genotypes are amenable to this technique (Altpeter et al., 2016). As such, approaches have recently been developed to circumvent tissue culture transformation, such as *de novo* induction of meristems (Maher et al., 2020), the "graft-mobile" gene editing system(Yang et al., 2023), and viral delivery of editing reagents (Ellison et al., 2020; Ghoshal et al., 2020; Liu et al., 2022; Nagalakshmi et al., 2022). These techniques hint at the potential to overcome this bottleneck; however, they are limited by the need for pre-established transgenic lines, they require highly experienced personnel, are likely difficult to scale, and appear to be species and genotype dependent.

[0039] A second, slightly more efficient way that works in some crops, is to introduce CRISPR protein and guide RNAs directly into plant cells during the tissue culture process,

followed by plant regeneration and backcrossing to eliminate epigenetic variation induced by tissue culture. However, this method also requires difficult tissue culture procedures that are costly, time consuming, and that only work in some crop species.

[0040] Plant viruses are ideal vectors for delivering editing reagents into plants given their natural ability to amplify and spread throughout the plant. In addition, many plant viruses, for example the broad host range RNA virus *Tobacco rattle virus* (TRV), are able to infect almost all parts of the plant, and yet the virus is rarely transmitted into future sexual generations because viral genome does not integrate into the plant genome (Bradamante et al., 2021). Recently, it has been shown that delivery of single guide RNA (sgRNA) sequences fused to mobile tRNA sequences encoded in TRV vectors can induce highly efficient biallelic gene editing or epigenome editing in plants (Ellison et al., 2020; Ghoshal et al., 2020; Nagalakshmi et al., 2022). However, delivery of both the Cas nuclease and the sgRNA has not yet been possible with TRV or other viruses as their low cargo capacity (~1.5-2 kb RNA) limits the size of the Cas nuclease to be fewer than 400 amino acids (aa). For example, *Spy*Cas9 (1369 aa) and *Lb*Cas12a (1228 aa), two of the most extensively utilized CRISPR-Cas genome editors in plants, are substantially larger than this cargo limit (FIG. 5A). Even the most compact Cas9d (750 aa) (Aliaga Goltsman et al., 2022) and Cas12 (420 aa) (Bigelyte et al., 2021; Wu et al., 2021) genome editors still don't meet such a stringent limit. Several ancestral Cas9 and Cas12 enzymes (~400 aa or less) have recently been demonstrated to be RNA-guided endonucleases with genome editing activities in mammalian cells (Altae-Tran et al., 2021; Karvelis et al., 2021). These ancestral enzymes, including IscB (Cas9 ancestors) and TnpB (Cas12 ancestors), are derived from prokaryotic transposons (e.g., IS200/IS605) rather than CRISPR systems (FIG. 5B). Yet, they share common structural architectures and similar mechanisms of RNA-guided DNA cleavage with CRISPR-Cas9 and Cas12 (FIG. 5C). For example, the target DNA binding and cleavage of both systems involve the recognition of a protospacer adjacent motif (PAM) or transposon adjacent motif (TAM) and the formation of an *R*-loop structure between the target DNA and guide RNA (FIG. 5C) (Nakagawa et al., 2023; Sasnauskas et al., 2023; Schuler et al., 2022). In contrast to CRISPR-Cas enzymes, these ancestral proteins typically form a complex with a relatively large (~200 nt) guide RNA called OMEGA (for obligate mobile element-guided activity) RNA (ωRNA) (Altae-Tran et al., 2021) which is encoded at either the left or right boundary of the transposons (FIG. 5B). Interestingly, while the PAM sequences of CRISPR-Cas systems are often difficult to predict, due to the natural function of these transposon systems, the TAM sequences of these ancestral RNA-guided systems are directly encoded on one end of the transposon boundary, providing a catalog of "TAM"

sequences from nature (FIG. 5B). Compared to IscB, TnpB enzymes bear significantly larger diversities (Altae-Tran et al., 2021) and higher genome editing efficiencies (Altae-Tran et al., 2021; Karvelis et al., 2021) and the more compact size of TnpB offers advantages for the viral delivery underlying our strategy (FIG. 5A).

[0041] The present disclosure herein relates, in part, to one or more small TnpB type bacterial enzymes that are less than about 500 amino acids in length and are capable of editing plant cells. An advantage of this small enzyme is that it is small enough to be cloned in a plant viral vector, such as for example TRV. This allows for an easier method to edit plant genomes that does not require tissue culture or plant transformation.

[0042] Definitions and Terminology

[0043] The disclosed polypeptides, compositions, constructs, viral vectors, and methods for plant gene editing may be further described using definitions and terminology as follows. The definitions and terminology used herein are for the purpose of describing particular aspects only and are not intended to be limiting.

[0044] As used in this specification and the claims, the singular forms "a," "an," and "the" include plural forms unless the context clearly dictates otherwise.

[0045] As used herein, "about", "approximately," "substantially," and "significantly" will be understood by persons of ordinary skill in the art and will vary to some extent on the context in which they are used. If there are uses of the term which are not clear to persons of ordinary skill in the art given the context in which it is used, "about" and "approximately" will mean up to plus or minus 10% of the particular term and "substantially" and "significantly" will mean more than plus or minus 10% of the particular term.

[0046] As used herein, the terms "include" and "including" have the same meaning as the terms "comprise" and "comprising." The terms "comprise" and "comprising" should be interpreted as being "open" transitional terms that permit the inclusion of additional components further to those components recited in the claims. The terms "consist" and "consisting of" should be interpreted as being "closed" transitional terms that do not permit the inclusion of additional components other than the components recited in the claims. The term "consisting essentially of" should be interpreted to be partially closed and allowing the inclusion only of additional components that do not fundamentally alter the nature of the claimed subject matter.

[0047] The phrase "such as" should be interpreted as "for example, including." Moreover, the use of any and all exemplary language, including but not limited to "such as", is intended

merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed.

[0048] Furthermore, in those instances where a convention analogous to "at least one of A, B and C, etc." is used, in general such a construction is intended in the sense of one having ordinary skill in the art would understand the convention (*e.g.*, "a system having at least one of A, B and C" would include but not be limited to systems that have A alone, B alone, C alone, A and B together, A and C together, B and C together, and/or A, B, and C together.). It will be further understood by those within the art that virtually any disjunctive word and/or phrase presenting two or more alternative terms, whether in the description or figures, should be understood to contemplate the possibilities of including one of the terms, either of the terms, or both terms. For example, the phrase "A or B" will be understood to include the possibilities of "A" or "B" or "A and B."

[0049] All language such as "up to," "at least," "greater than," "less than," and the like, include the number recited and refer to ranges which can subsequently be broken down into ranges and subranges. A range includes each individual member. Thus, for example, a group having 1-3 members refers to groups having 1, 2, or 3 members. Similarly, a group having 6 members refers to groups having 1, 2, 3, 4, or 6 members, and so forth.

[0050] The modal verb "may" refers to the preferred use or selection of one or more options or choices among the several described embodiments or features contained within the same. Where no options or choices are disclosed regarding a particular embodiment or feature contained in the same, the modal verb "may" refers to an affirmative act regarding how to make or use an aspect of a described embodiment or feature contained in the same, or a definitive decision to use a specific skill regarding a described embodiment or feature contained in the same. In this latter context, the modal verb "may" has the same meaning and connotation as the auxiliary verb "can."

[0051] The terms "identical" or percent "identity," in the context of two or more nucleic acids or polypeptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same (i.e., 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identity over a specified region, e.g., of an entire nucleic acid or polypeptide sequence or individual portions or domains of a nucleic acid or polypeptide), when compared and aligned for maximum correspondence over a comparison window, or designated region as measured using one of the following sequence comparison algorithms or by manual alignment and visual inspection. Such sequences are then said to be "substantially identical."

This definition also refers to the complement of a test sequence, in the context of nucleic acids. By way of example, in embodiments, the identify exists over a region that is about or at least about 5, 10, 15, 20, 50, 100, or 1000, amino acids in length, to about, less than about, or at least about 220, 100 or 1000 amino acids or nucleotides in length. Optionally, the identity exists over a region that is at least about 5, 10, 15, or 16 amino acids in length to about 100, about 20 to about 75, about 30 to about 50 amino acids or nucleotides in length.

[0052] For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are entered into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. Preferably, default program parameters can be used, or alternative parameters can be designated. The sequence comparison algorithm then calculates the percent sequence identities for the test sequences relative to the reference sequence, based on the program parameters.

[0053] An example of algorithms suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul et al., *Nuc. Acids Res.* 25:3389-3402 (1977) and Altschul et al., J. Mol. Biol. 215:403-410 (1990), respectively. As will be appreciated by one of skill in the art, the software for performing BLAST analyses is publicly available through the website of the National Center for Biotechnology Information (NCBI). In embodiments, BLAST and BLAST 2.0 are used, with the parameters described herein, to determine percent sequence identity for the nucleic acids and proteins. In embodiments, a BLAST algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. In embodiments, T is referred to as the neighborhood word score threshold (Altschul et al., supra). In embodiments, these initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. In embodiments, the word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. In embodiments, cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always >0) and N (penalty score for mismatching residues; always <0). In embodiments, for amino acid sequences, a scoring matrix is used to calculate the cumulative score. In embodiments, extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the

end of either sequence is reached. In embodiments, the BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. In embodiments, the NCBI BLASTN or BLASTP program is used to align sequences. In embodiments, the BLASTN or BLASTP program uses the defaults used by the NCBI. In embodiments, the BLASTN program (for nucleotide sequences) uses as defaults: a word size (W) of 28; an expectation threshold (E) of 10; max matches in a query range set to 0; match/mismatch scores of 1, −2; linear gap costs; the filter for low complexity regions used; and mask for lookup table only used. In embodiments, the BLASTP program (for amino acid sequences) uses as defaults: a word size (W) of 3; an expectation threshold (E) of 10; max matches in a query range set to 0; the BLOSUM62 matrix (see Henikoff & Henikoff, Proc. Natl. Acad. Sci. USA 89:10915 (1992)); gap costs of existence: 11 and extension: 1; and conditional compositional score matrix adjustment.

[0054] The terms "polypeptide," "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally occurring amino acid polymer.

[0055] The term "amino acid" refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, e.g., hydroxyproline, γ-carboxyglutamate, and O-phosphoserine. Amino acid analogs refers to compounds that have the same basic chemical structure as a naturally occurring amino acid, i.e., an a carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, e.g., homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs have modified R groups (e.g., norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. Amino acid mimetics refers to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but that functions in a manner similar to a naturally occurring amino acid.

[0056] Amino acids may be referred to herein by either their commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Nucleotides, likewise, may be referred to by their commonly accepted single-letter codes.

[0057] "Conservatively modified variants" applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, conservatively modified variants refers to those nucleic acids which encode identical or essentially identical amino acid sequences, or where the nucleic acid does not encode an amino acid sequence, to essentially identical sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given protein. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are "silent variations," which are one species of conservatively modified variations. Every nucleic acid sequence herein which encodes a polypeptide also describes every possible silent variation of the nucleic acid. One of skill will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine, and TGG, which is ordinarily the only codon for tryptophan) can be modified to yield a functionally identical molecule. Accordingly, each silent variation of a nucleic acid which encodes a polypeptide is implicit in each described sequence with respect to the expression product, but not with respect to actual probe sequences.

[0058] As to amino acid sequences, one of skill will recognize that individual substitutions to a peptide, polypeptide, or protein sequence which alters a single amino acid is a "conservatively modified variant" where the alteration results in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art. Such conservatively modified variants are in addition to and do not exclude polymorphic variants, interspecies homologs, and alleles.

[0059] The following eight groups each contain amino acids that are conservative substitutions for one another: 1) Alanine (A), Glycine (G); 2) Aspartic acid (D), Glutamic acid (E); 3) Asparagine (N), Glutamine (Q); 4) Arginine (R), Lysine (K); 5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W); 7) Serine (S), Threonine (T); and 8) Cysteine (C), Methionine (M) (see, e.g., Creighton, Proteins (1984)).

[0060] The terms "polynucleotide," "polynucleotide sequence," "nucleic acid" and "nucleic acid sequence" refer to a nucleotide, oligonucleotide, polynucleotide (which terms may be used interchangeably), or any fragment thereof. These phrases also refer to DNA or RNA of genomic, natural, or synthetic origin (which may be single-stranded or double-stranded and may represent the sense or the antisense strand).

[0061] The terms "nucleic acid" and "oligonucleotide," as used herein, may refer to polydeoxyribonucleotides (containing 2-deoxy-D-ribose), polyribonucleotides (containing D-ribose), and to any other type of polynucleotide that is an N glycoside of a purine or pyrimidine base. There is no intended distinction in length between the terms "nucleic acid", "oligonucleotide" and "polynucleotide", and these terms will be used interchangeably. These terms refer only to the primary structure of the molecule. Thus, these terms include double- and single-stranded DNA, as well as double- and single-stranded RNA. For use in the present methods, an oligonucleotide also can comprise nucleotide analogs in which the base, sugar, or phosphate backbone is modified as well as non-purine or non-pyrimidine nucleotide analogs.

[0062] Oligonucleotides can be prepared by any suitable method, including direct chemical synthesis by a method such as the phosphotriester method of Narang *et al.*, 1979, *Meth. Enzymol.* 68:90-99; the phosphodiester method of Brown *et al.*, 1979, *Meth. Enzymol.* 68:109-151; the diethylphosphoramidite method of Beaucage *et al.*, 1981, *Tetrahedron Letters* 22:1859-1862; and the solid support method of U.S. Pat. No. 4,458,066, each incorporated herein by reference. A review of synthesis methods of conjugates of oligonucleotides and modified nucleotides is provided in Goodchild, 1990, *Bioconjugate Chemistry* 1(3): 165-187, incorporated herein by reference.

[0063] Regarding polynucleotide sequences, the terms "percent identity" and "% identity" refer to the percentage of residue matches between at least two polynucleotide sequences aligned using a standardized algorithm. Such an algorithm may insert, in a standardized and reproducible way, gaps in the sequences being compared in order to optimize alignment between two sequences, and therefore achieve a more meaningful comparison of the two sequences. Percent identity for a nucleic acid sequence may be determined as understood in the art. (*See, e.g.*, U.S. Patent No. 7,396,664, which is incorporated herein by reference in its entirety). A suite of commonly used and freely available sequence comparison algorithms is provided by the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST), which is available from several sources, including the NCBI, Bethesda, Md., at its website. The BLAST software suite includes various sequence analysis programs including "blastn," that is used to align a known polynucleotide sequence with other polynucleotide sequences from a variety of databases. Also available is a tool called "BLAST 2 Sequences" that is used for direct pairwise comparison of two nucleotide sequences. "BLAST 2 Sequences" can be accessed and used interactively at the NCBI website. The "BLAST 2 Sequences" tool can be used for both blastn and blastp (discussed above).

[0064] Regarding polynucleotide sequences, percent identity may be measured over the length of an entire defined polynucleotide sequence, for example, as defined by a particular SEQ ID number, or may be measured over a shorter length, for example, over the length of a fragment taken from a larger, defined sequence, for instance, a fragment of at least 20, at least 30, at least 40, at least 50, at least 70, at least 100, or at least 200 contiguous nucleotides. Such lengths are exemplary only, and it is understood that any fragment length supported by the sequences shown herein, in the tables, figures, or Sequence Listing, may be used to describe a length over which percentage identity may be measured.

[0065] Regarding polynucleotide sequences, "variant," "mutant," or "derivative" may be defined as a nucleic acid sequence having at least 50% sequence identity to the particular nucleic acid sequence over a certain length of one of the nucleic acid sequences using blastn with the "BLAST 2 Sequences" tool available at the National Center for Biotechnology Information's website. (*See* Tatiana A. Tatusova, Thomas L. Madden (1999), "Blast 2 sequences - a new tool for comparing protein and nucleotide sequences", FEMS Microbiol Lett. 174:247-250). Such a pair of nucleic acids may show, for example, at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% or greater sequence identity over a certain defined length.

[0066] Nucleic acid sequences that do not show a high degree of identity may nevertheless encode similar amino acid sequences due to the degeneracy of the genetic code where multiple codons may encode for a single amino acid. It is understood that changes in a nucleic acid sequence can be made using this degeneracy to produce multiple nucleic acid sequences that all encode substantially the same protein. For example, polynucleotide sequences as contemplated herein may encode a protein and may be codon-optimized for expression in a particular host. In the art, codon usage frequency tables have been prepared for a number of host organisms including humans, mouse, rat, pig, *E. coli*, plants, and other host cells.

[0067] A "recombinant nucleic acid" is a sequence that is not naturally occurring or has a sequence that is made by an artificial combination of two or more otherwise separated segments of sequence. This artificial combination is often accomplished by chemical synthesis or, more commonly, by the artificial manipulation of isolated segments of nucleic acids, *e.g.*, by genetic engineering techniques known in the art. The term recombinant includes nucleic acids that have been altered solely by addition, substitution, or deletion of a portion of the nucleic acid. Frequently, a recombinant nucleic acid may include a nucleic acid sequence operably linked to

a promoter sequence. Such a recombinant nucleic acid may be part of a vector that is used, for example, to transform a cell.

[0068] The nucleic acids disclosed herein may be "substantially isolated or purified." The term "substantially isolated or purified" refers to a nucleic acid that is removed from its natural environment, and is at least 60% free, preferably at least 75% free, and more preferably at least 90% free, even more preferably at least 95% free from other components with which it is naturally associated.

[0069] The polynucleotide sequences contemplated herein may be present in expression vectors. For example, the vectors may comprise a polynucleotide encoding an ORF of a protein operably linked to a promoter. "Operably linked" refers to the situation in which a first nucleic acid sequence is placed in a functional relationship with a second nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. Operably linked DNA sequences may be in close proximity or contiguous and, where necessary to join two protein coding regions, in the same reading frame. Vectors contemplated herein may comprise a heterologous promoter operably linked to a polynucleotide that encodes a protein. A "heterologous promoter" refers to a promoter that is not the native or endogenous promoter for the protein or RNA that is being expressed.

[0070] As used herein, "expression" refers to the process by which a polynucleotide is transcribed from a DNA template (such as into mRNA or another RNA transcript) and/or the process by which a transcribed mRNA is subsequently translated into peptides, polypeptides, or proteins. Transcripts and encoded polypeptides may be collectively referred to as "gene product."

[0071] The term "vector" refers to some means by which nucleic acid (*e.g.*, DNA) can be introduced into a host organism or host tissue. There are various types of vectors including plasmid vector, bacteriophage vectors, cosmid vectors, bacterial vectors, and viral vectors. As used herein, a "vector" may refer to a recombinant nucleic acid that has been engineered to express a heterologous polypeptide (*e.g.*, the fusion proteins disclosed herein). The recombinant nucleic acid typically includes *cis*-acting elements for expression of the heterologous polypeptide.

[0072] Compositions, Constructs, Viral Vectors, and Plant Cells

[0073] In certain aspects, disclosed herein are compositions for plant gene editing. In various aspects, the composition can include a TnpB bacterial enzyme. In various aspects, the TnpB bacterial enzyme can be from any bacterial TnpB system. In certain aspects, the TnpB

bacterial enzyme can have a length of less than about 500 amino acids, less than about 450 amino acids, less than about 420 amino acids, or less than about 400 amino acids.

[0074] In various aspects, the TnpB can be from, or derived from, *Brevibacillus agri*. A TnpB derived from *Brevibacillus agri* refers to a variant of the TnpB of *Brevibacillus agri*. In the same or alternative aspects, the TnpB can have an amino acid sequence that is 70 % or more, 75 % or more, 80 % or more, 85 % or more, 90 % or more, 95 % or more, or 99 % or more identical to the amino acid sequence of the TnpB of *Brevibacillus agri*. In various aspects, the TnpB can have an amino acid sequence that is 70 % or more, 75 % or more, 80 % or more, 85 % or more, 90 % or more, 95 % or more, or 99 % or more identical to the amino acid sequence of SEQ ID NO: 5. In various aspects, the TnpB can have the amino acid sequence of SEQ ID NO: 5. It should be understood that a variant of the TnpB of *Brevibacillus agri* can be a synthetically designed variant or can be a TnpB that is of similar sequence but from another species or genus of bacteria.

[0075] In various aspects, the TnpB can be from, or derived from, *Xylella fastidiosa*. A TnpB derived from *Xylella fastidiosa* refers to a variant of the TnpB of *Xylella fastidiosa*. In the same or alternative aspects, the TnpB can have an amino acid sequence that is 70 % or more, 75 % or more, 80 % or more, 85 % or more, 90 % or more, 95 % or more, or 99 % or more identical to the amino acid sequence of the TnpB of *Xylella fastidiosa*. In various aspects, the TnpB can have an amino acid sequence that is 70 % or more, 75 % or more, 80 % or more, 85 % or more, 90 % or more, 95 % or more, or 99 % or more identical to the amino acid sequence of SEQ ID NO: 10. In various aspects, the TnpB can have the amino acid sequence of SEQ ID NO: 10. It should be understood that a variant of the TnpB of *Xylella fastidiosa* can be a synthetically designed variant or can be a TnpB that is of similar sequence but from another species or genus of bacteria.

[0076] In various aspects, the TnpB can have an amino acid sequence that is 70 % or more, 75 % or more, 80 % or more, 85 % or more, 90 % or more, 95 % or more, or 99 % or more, or 100 % identical to one or more of the amino acid sequences of SEQ ID NOs: 46, 47, 48, 49, and 702.

[0077] In various aspects, the TnpB can comprise a peptide tag. In such aspects, the peptide tag can include any suitable peptide tag known in the art. As one example aspect, the peptide tag can be a FLAG tag. In some aspects, the peptide tag(s) is located at the N-terminus of the TnpB protein, while in others, the peptide tag(s) is located at the C-terminus of the TnpB protein, while in still further aspects, the peptide tag(s) is located within the interior portion of the TnpB, or any combination thereof.

[0078] In various aspects, the compositions disclosed herein can also include a polynucleotide. In various aspects, the polynucleotide can include a first portion adapted to bind to at least a portion of the TnpB and a second portion for binding to a plant genomic target. In various aspects, the polynucleotide can be RNA. In one or more aspects, the polynucleotide can comprise an ωRNA. In one or more aspects, the ωRNA is about 100-400 nucleotides in length, or about 200-300 nucleotides in length. In various aspects, a DNA sequence that is 70 % or more, 75 % or more, 80 % or more, 85 % or more, 90 % or more, 95 % or more, or 99 % or more, or 100% identical to the polynucleotide sequence of one or more of SEQ ID NOs: 11-13, 50-53, 359-430, and 580-606 can encode for the ωRNA. In certain aspects, the polynucleotide can include a spacer element. In various aspects, the space element can be any length suitable for use in plant gene editing using the systems and methods disclosed herein. It should be understood that in alternative aspect, the first portion of the polynucleotide and the second portion of the polynucleotide can also be present as separate polynucleotides. In such an aspect, the separate polynucleotides may include sequences to allow for joining or hybridization in a plant cell.

[0079] In various aspects, constructs are disclosed herein. The constructs can include a plant promoter operably connected to a polynucleotide encoding for a TnpB. The TnpB encoded for in the polynucleotide can exhibit any or all of the properties discussed above with respect to TnpB.

[0080] In various aspects, the polynucleotide encoding for a TnpB can include a polynucleotide sequence that is 70 % or more, 75 % or more, 80 % or more, 85 % or more, 90 % or more, 95 % or more, or 99 % or more identical to the polynucleotide sequence of one or more of SEQ ID NOs: 1-4, 6-9, or 34-45. In various aspects, the polynucleotide encoding for a TnpB can have the polynucleotide sequence of one or more of SEQ ID NOs: 1-4, 6-9, or 34-45.

[0081] In various aspects, the polynucleotide encoding for a TnpB can be codon optimized for expression in plant cells. In one or more aspects, the polynucleotide encoding for a TnpB can be *Zea mays* codon optimized.

[0082] As discussed above, in various aspects, the construct can include a plant promoter operably connected to the polynucleotide encoding for a TnpB. The plant promoter can be any suitable promoter for expressing the TnpB in the plant cell of interest. In one example aspect, the plant promoter can include a UBQ10 gene promoter.

[0083] In certain aspects, the polynucleotide can further encode for an ωRNA having a first portion adapted to bind to at least a portion of the TnpB bacterial enzyme and a second portion

for binding to a plant genomic target. The ωRNA can include any or all of the features and parameters discussed above with respect to ωRNA. In one or more aspects, the polynucleotide encoding for TnpB and the ωRNA can utilize the same promoter and/or be part of the same cassette. In various aspects, a sequence of the ωRNA and a sequence of the TnpB bacterial enzyme can at least partly overlap in a cassette or construct. For instance, in certain aspects, a polynucleotide that encodes for an ωRNA and a TnpB that at least partly overlap can have a sequence that is 70 % or more, 75 % or more, 80 % or more, 85 % or more, 90 % or more, 95 % or more, or 99 % or more identical to the polynucleotide sequence of one or more of SEQ ID NOs: 140-147, 150-170, 431-433, or 475-481. In alternative aspects, a sequence of the ωRNA and a sequence of the TnpB bacterial enzyme can be present as a split cassette and under the control of separate promoters.

[0084] In various aspects, the polynucleotide encoding for an ωRNA can be a separate polynucleotide and/or construct than the one encoding for the TnpB.

[0085] In various aspects, plant viral vectors are disclosed. In one or more aspects, the plant viral vector can include one or more of the constructs mentioned above. In various aspects, the plant viral vector can be any suitable vector for use in plant gene editing. In one example aspect, the plant viral vector can be, or can be derived from, the *Tobacco rattle virus* (TRV).

[0086] In various aspects, plant cells are disclosed. In certain aspects, the plant cells can include one or more of the constructs mentioned above and/or the plant viral vectors mentioned above. In one or more aspects, the constructs and plant viral vectors can include any or all of the respective parameters and properties described above. For example, the cell can be a cell of a major agricultural plant, e.g., Barley, Beans (Dry Edible), Canola, Corn, Cotton (Pima), Cotton (Upland), Flaxseed, Hay (Alfalfa), Hay (Non-Alfalfa), Oats, Peanuts, Rice, Sorghum, Soybeans, Sugarbeets, Sugarcane, Sunflowers (Oil), Sunflowers (Non-Oil), Sweet Potatoes , Tobacco (Burley), Tobacco (Flue-cured), Tomatoes, Wheat (Durum), Wheat (Spring), Wheat (Winter), and the like. As another example, the cell is a cell of a vegetable crops which include but are not limited to, *e.g.*, alfalfa sprouts, aloe leaves, arrow root, arrowhead, artichokes, asparagus, bamboo shoots, banana flowers, bean sprouts, beans, beet tops, beets, bittermelon, bok choy, broccoli, broccoli rabe (rappini), brussels sprouts, cabbage, cabbage sprouts, cactus leaf (nopales), calabaza, cardoon, carrots, cauliflower, celery, chayote, chinese artichoke (crosnes), chinese cabbage, chinese celery, chinese chives, choy sum, chrysanthemum leaves (tung ho), collard greens, corn stalks, corn-sweet, cucumbers, daikon, dandelion greens, dasheen, dau mue (pea tips), donqua (winter melon), eggplant, endive, escarole, fiddle head

ferns, field cress, frisee, gai choy (chinese mustard), gailon, galanga (siam, thai ginger), garlic, ginger root, gobo, greens, hanover salad greens, huauzontle, jerusalem artichokes, jicama, kale greens, kohlrabi, lamb's quarters (quilete), lettuce (bibb), lettuce (boston), lettuce (boston red), lettuce (green leaf), lettuce (iceberg), lettuce (lolla rossa), lettuce (oak leaf - green), lettuce (oak leaf - red), lettuce (processed), lettuce (red leaf), lettuce (romaine), lettuce (ruby romaine), lettuce (russian red mustard), linkok, lo bok, long beans, lotus root, mache, maguey (agave) leaves, malanga, mesculin mix, mizuna, moap (smooth luffa), moo, moqua (fuzzy squash), mushrooms, mustard, nagaimo, okra, ong choy, onions green, opo (long squash), ornamental corn, ornamental gourds, parsley, parsnips, peas, peppers (bell type), peppers, pumpkins, radicchio, radish sprouts, radishes, rape greens, rape greens, rhubarb, romaine (baby red), rutabagas, salicornia (sea bean), sinqua (angled/ridged luffa), spinach, squash, straw bales, sugarcane, sweet potatoes, swiss chard, tamarindo, taro, taro leaf, taro shoots, tatsoi, tepeguaje (guaje), tindora, tomatillos, tomatoes, tomatoes (cherry), tomatoes (grape type), tomatoes (plum type), tumeric, turnip tops greens, turnips, water chestnuts, yampi, yams, yu choy, yuca (cassava), and the like. In one aspect, in addition to agricultural plants, ornamental plants, conifer plants, forage and turf grass are also contemplated for use with the systems disclosed herein.In various aspects, any plant cell and/or type of plant may be used in the present disclosure so long as it remains viable after being transformed with a sequence of nucleic acids. In certain aspects, the plant cell is not adversely affected by the transduction of the necessary nucleic acid sequences, the subsequent expression of the proteins or the resulting intermediates.

[0087] Methods

[0088] In various aspects, methods for gene editing in a plant are disclosed. In one or more aspects, the methods can include introducing one or more of the constructs mentioned above and/or one or more of the plant viral vectors mentioned above into one or more plant cells. The contracts and/or plant viral vectors can be introduced into the plant cells using any suitable techniques known by those of skill in the art.

[0089] The methods for gene editing in a plant can be utilized on any desired plant genus or species. In certain aspects, the methods for gene editing can be performed on any agricultural crop or other plant.

EXAMPLES

[0090] The following Examples are illustrative and are not intended to limit the scope of the claimed subject matter.

[0091] Example 1 – Detection of bona-fide RNA-guided TnpB systems in metagenomes

[0092] In order to determine the ωRNA architecture in functional RNA-guided TnpB systems and the TAM sequences, the boundaries of the TnpB-associated transposons need to be precisely determined. TnpB associated transposons are moving from one location to another and the genomic regions flanking the transposon ends therefore are different. The exact region of the system can be determined by common strategies such as multiple genome alignments (Altae-Tran et al., 2021). However, multiple genome alignments require the genomes to be from the species or strains that are relatively highly abundant in the corresponding samples or that can be cultivated; otherwise, no genome will be obtained from such analyses. Alternatively, in metagenomic analyses based on short paired-end reads, the same region of the transposon systems can be determined by read mapping (called the read mapping approach hereafter), if only a fraction of the corresponding genomes encode the system while the others do not. Based on this knowledge, a high-throughput computational pipeline was developed based on the read mapping approach to identify the transposon boundaries (LE and RE) and the TAM of the TnpB-associated transposon systems from metagenomic datasets. This pipeline was named "DOTS" (for Detectives of Transposon Systems) (FIG. 1A). The read mapping approach has several advantages over multiple genome alignments: (1) it requires only one metagenomic sample; (2) the species or strains representing the genome do not need to be dominant in the sample; and (3) the analysis can essentially be applied to any assembled sequences, including mobile genetic elements which typically require detection in multiple habitats or samples. In a preliminary analysis, we applied the "DOTS" pipeline to identify a total of 96 putative RNA-guided IS605 TnpB systems from only 500 ggKbase metagenomes (FIG. 1B). These RNA-guided IS605 TnpB systems bear diverse TAM sequences, including CTAT, TCAC and ACAG, which are not present in public IS databases such as ISfinder, have a range of molecular sizes (336-478 aa) (FIG. 1B), and have not been reported previously.

[0093] Example 2 -TnpB systems with robust RNA-guided activities in *E.coli*

[0094] To establish a robust pipeline for experimentally onboarding RNA-guided TnpB systems, ten compact TnpB (< 420 aa) systems were selected that are from publicly accessible genomes on the National Center for Biotechnology Information (NCBI) for reconstituting their RNA-guided DNA cleavage activities in *E.coli*. These TnpB-associated transposons are all from organisms in ambient environments, such as soil and plant-associated microbes, making them ideal candidates for plant editing. Preliminary results show that two TnpB systems have robust RNA-guided activities in *E.coli*: one is a hypercompact IS200/IS605 TnpB (359 aa) from *Brevibacillus agri* (ISBag01), the other one is an IS607 TnpB (398 aa) from *Xylella fastidiosa* (ISXfa01) which is a plant pathogen (FIGS. 2A-2C). Interestingly, the RNA-guided

DNA cleavage activities of TnpB from IS607 transposons have yet to be reported, and our preliminary results suggests that beyond IS200/IS605, it should be fruitful to mine TnpBs from additional IS607s.

[0095] Example 3 -TnpB activity in Arabidopsis protoplasts

[0096] The ISBag01 sequence and 18 different gRNA sequences targeting the *AtPDS3* gene were cloned into plasmids and tested for activity in Arabidopsis protoplasts (FIG. 3A). Ten of the gRNA sequences tested have thus far shown a low level of editing activity, with highest editing efficiencies yielded by gRNA1, gRNA10 and gRNA18 (FIG. 3B), showing deletions of between 4 and 17 base pairs (FIG. 3C). Two configurations were tested, one in which the ISBag01 protein and the ωRNA were expressed as separate expression cassettes, and one resembling the native bacterial architecture in which the native sequence encoding the ISBag01 protein and the ωRNA were partially overlapping in a single expression cassette (FIG. 3A). Editing activity was detected with both configurations (FIG. 3B). Importantly, the single expression cassette architecture should allow the system to be further compacted, decreasing the cargo DNA size that will need to be cloned into plant viral vectors. While the editing efficiency of ISBag01 has thus far been very low (FIG. 3B), we are highly encouraged to see activity of this tiny nuclease in plant cells, as it is the first candidate we have tested. A very similar set of experiments was carried out with ISXfa01, however we did not observe any editing activity. These initial experiments suggest that a wise course of action will be to test many TnpB candidates to find ones with the highest editing efficiency in plant cells. Table 1 includes the construct sequences for the constructs used in Example 3. Table 2 lists the spacer sequences of ISBag01 that were tested.

Table 1: Construct Sequences for Example 3

Plasmid sequence of pCAMBIA1300_native_ISBag01_wRNA_in_one_cassette_AtPDS3_gRNA1 and pCAMBIA1300_maize_codon_ISBag01_U6wRNA_in_split_cassettes_AtPDS3_gRNA10_gRNA18. Note that the guide RNA cassette in pCAMBIA1300_maize_codon_ISBag01_U6wRNA_in_split_cassettes_AtPDS3_gRNA10_gRNA18 is going in reverse direction compared to the ISBag01 protein encoding cassette. Letters in bold indicate ISBag01 DNA sequence (native sequence or *Zea mays* codon optimized). Underlined letters indicate wRNA sequence before the spacer part. Letters in italic indicate guide RNA sequence (spacer part).	
plasmid	**sequence**
pCAMBIA1300_native_ISBag01_wRNA_in_one_casse	GTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATT CCACACAACATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAA TGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAG TCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGG GAGAGGCGGTTTGCGTATTGGCTAGAGCAGCTTGCCAACATGGTGGAGCA

tte_AtPDS3_ gRNA1 (SEQ ID NO: 14)	CGACACTCTCGTCTACTCCAAGAATATCAAAGATACAGTCTCAGAAGACCAA AGGGCTATTGAGACTTTTCAACAAAGGGTAATATCGGGAAACCTCCTCGGA TTCCATTGCCCAGCTATCTGTCACTTCATCAAAAGGACAGTAGAAAAGGAAG GTGGCACCTACAAATGCCATCATTGCGATAAAGGAAAGGCTATCGTTCAAG ATGCCTCTGCCGACAGTGGTCCCAAAGATGGACCCCCACCCACGAGGAGC ATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAGTGGATTGA TGTGATAACATGGTGGAGCACGACACTCTCGTCTACTCCAAGAATATCAAA GATACAGTCTCAGAAGACCAAAGGGCTATTGAGACTTTTCAACAAAGGGTA ATATCGGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTCATCA AAAGGACAGTAGAAAAGGAAGGTGGCACCTACAAATGCCATCATTGCGATA AAGGAAAGGCTATCGTTCAAGATGCCTCTGCCGACAGTGGTCCCAAAGATG GACCCCCACCCACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACG TCTTCAAAGCAAGTGGATTGATGTGATATCTCCACTGACGTAAGGGATGAC GCACAATCCCACTATCCTTCGCAAGACCTTCCTCTATATAAGGAAGTTCATT TCATTTGGAGAGGACACGCTGAAATCACCAGTCTCTCTCTACAAATCTATCT CTCTCGAGCTTTCGCAGATCCCGGGGGGCAATGAGATATGAAAAAGCCTGA ACTCACCGCGACGTCTGTCGAGAAGTTTCTGATCGAAAAGTTCGACAGCGT CTCCGACCTGATGCAGCTCTCGGAGGGCGAAGAATCTCGTGCTTTCAGCTT CGATGTAGGAGGGCGTGGATATGTCCTGCGGGTAAATAGCTGCGCCGATG GTTTCTACAAAGATCGTTATGTTTATCGGCACTTTGCATCGGCCGCGCTCCC GATTCCGGAAGTGCTTGACATTGGGGAGTTTAGCGAGAGCCTGACCTATTG CATCTCCCGCCGTGCACAGGGTGTCACGTTGCAAGACCTGCCTGAAACCG AACTGCCCGCTGTTCTACAACCGGTCGCGGAGGCTATGGATGCGATCGCT GCGGCCGATCTTAGCCAGACGAGCGGGTTCGGCCCATTCGGACCGCAAG GAATCGGTCAATACACTACATGGCGTGATTTCATATGCGCGATTGCTGATC CCCATGTGTATCACTGGCAAACTGTGATGGACGACACCGTCAGTGCGTCCG TCGCGCAGGCTCTCGATGAGCTGATGCTTTGGGCCGAGGACTGCCCCGAA GTCCGGCACCTCGTGCACGCGGATTTCGGCTCCAACAATGTCCTGACGGA CAATGGCCGCATAACAGCGGTCATTGACTGGAGCGAGGCGATGTTCGGGG ATTCCCAATACGAGGTCGCCAACATCTTCTTCTGGAGGCCGTGGTTGGCTT GTATGGAGCAGCAGACGCGCTACTTCGAGCGGAGGCATCCGGAGCTTGCA GGATCGCCACGACTCCGGGCGTATATGCTCCGCATTGGTCTTGACCAACTC TATCAGAGCTTGGTTGACGGCAATTTCGATGATGCAGCTTGGGCGCAGGGT CGATGCGACGCAATCGTCCGATCCGGAGCCGGGACTGTCGGGCGTACACA AATCGCCCGCAGAAGCGCGGCCGTCTGGACCGATGGCTGTGTAGAAGTAC TCGCCGATAGTGGAAACCGACGCCCCAGCACTCGTCCGAGGGCAAAGAAA TAGAGTAGATGCCGACCGGATCTGTCGATCGACAAGCTCGAGTTTCTCCAT AATAATGTGTGAGTAGTTCCCAGATAAGGGAATTAGGGTTCCTATAGGGTTT CGCTCATGTGTTGAGCATATAAGAAACCCTTAGTATGTATTTGTATTTGTAAA ATACTTCTATCAATAAAATTTCTAATTCCTAAAACCAAAATCCAGTACTAAAAT CCAGATCCCCCGAATTAATTCGGCGTTAATTCAGTACATTAAAAACGTCCGC AATGTGTTATTAAGTTGTCTAAGCGTCAATTTGTTTACACCACAATATATCCT GCCACCAGCCAGCCAACAGCTCCCCGACCGGCAGCTCGGCACAAAATCAC CACTCGATACAGGCAGCCCATCAGTCCGGGACGGCGTCAGCGGGAGAGC CGTTGTAAGGCGGCAGACTTTGCTCATGTTACCGATGCTATTCGGAAGAAC GGCAACTAAGCTGCCGGGTTTGAAACACGGATGATCTCGCGGAGGGTAGC ATGTTGATTGTAACGATGACAGAGCGTTGCTGCCTGTGATCACCGCGGTTT CAAAATCGGCTCCGTCGATACTATGTTATACGCCAACTTTGAAAACAACTTT GAAAAAGCTGTTTTCTGGTATTTAAGGTTTTAGAATGCAAGGAACAGTGAAT TGGAGTTCGTCTTGTTATAATTAGCTTCTTGGGGTATCTTTAAATACTGTAGA AAAGAGGAAGGAAATAATAAATGGCTAAAATGAGAATATCACCGGAATTGAA AAAACTGATCGAAAAATACCGCTGCGTAAAAGATACGGAAGGAATGTCTCC TGCTAAGGTATATAAGCTGGTGGGAGAAAATGAAAACCTATATTTAAAAATG ACGGACAGCCGGTATAAAGGGACCACCTATGATGTGGAACGGGAAAAGGA CATGATGCTATGGCTGGAAGGAAAGCTGCCTGTTCCAAAGGTCCTGCACTT TGAACGGCATGATGGCTGGAGCAATCTGCTCATGAGTGAGGCCGATGGCG TCCTTTGCTCGGAAGAGTATGAAGATGAACAAAGCCCTGAAAAGATTATCG AGCTGTATGCGGAGTGCATCAGGCTCTTTCACTCCATCGACATATCGGATT GTCCCTATACGAATAGCTTAGACAGCCGCTTAGCCGAATTGGATTACTTACT GAATAACGATCTGGCCGATGTGGATTGCGAAAACTGGGAAGAAGACACTCC

	ATTTAAAGATCCGCGCGAGCTGTATGATTTTTTAAAGACGGAAAAGCCCGAA GAGGAACTTGTCTTTTCCCACGGCGACCTGGGAGACAGCAACATCTTTGTG AAAGATGGCAAAGTAAGTGGCTTTATTGATCTTGGGAGAAGCGGCAGGGC GGACAAGTGGTATGACATTGCCTTCTGCGTCCGGTCGATCAGGGAGGATAT CGGGGAAGAACAGTATGTCGAGCTATTTTTTGACTTACTGGGGATCAAGCC TGATTGGGAGAAAATAAAATATTATATTTTACTGGATGAATTGTTTTAGTACC TAGAATGCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTC AGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGC GTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGT TTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGC AGAGCGCAGATACCAAATACTGTCCTTCTAGTGTAGCCGTAGTTAGGCCAC CACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGT TACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACT CAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGG TTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATA CCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGG CGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAG GGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCG CCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGA GCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTT GCTGGCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGA TAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAGCCGAAC GACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCTGATG CGGTATTTTCTCCTTACGCATCTGTGCGGTATTTCACACCGCATATGGTGCA CTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAAGCCAGTATACACTCCG CTATCGCTACGTGACTGGGTCATGGCTGCGCCCCGACACCCGCCAACACC CGCTGACGCGCCCTGACGGGCTTGTCTGCTCCCGGCATCCGCTTACAGAC AAGCTGTGACCGTCTCCGGGAGCTGCATGTGTCAGAGGTTTTCACCGTCAT CACCGAAACGCGCGAGGCAGGGTGCCTTGATGTGGGCGCCGGCGGTCGA GTGGCGACGGCGCGGCTTGTCCGCGCCCTGGTAGATTGCCTGGCCGTAG GCCAGCCATTTTTGAGCGGCCAGCGGCCGCGATAGGCCGACGCGAAGCG GCGGGGCGTAGGGAGCGCAGCGACCGAAGGGTAGGCGCTTTTTGCAGCT CTTCGGCTGTGCGCTGGCCAGACAGTTATGCACAGGCCAGGCGGGTTTTA AGAGTTTTAATAAGTTTTAAAGAGTTTTAGGCGGAAAAATCGCCTTTTTTCTC TTTTATATCAGTCACTTACATGTGTGACCGGTTCCCAATGTACGGCTTTGGG TTCCCAATGTACGGGTTCCGGTTCCCAATGTACGGCTTTGGGTTCCCAATG TACGTGCTATCCACAGGAAAGAGACCTTTTCGACCTTTTTCCCCTGCTAGG GCAATTTGCCCTAGCATCTGCTCCGTACATTAGGAACCGGCGGATGCTTCG CCCTCGATCAGGTTGCGGTAGCGCATGACTAGGATCGGGCCAGCCTGCCC CGCCTCCTCCTTCAAATCGTACTCCGGCAGGTCATTTGACCCGATCAGCTT GCGCACGGTGAAACAGAACTTCTTGAACTCTCCGGCGCTGCCACTGCGTTC GTAGATCGTCTTGAACAACCATCTGGCTTCTGCCTTGCCTGCGGCGCGGC GTGCCAGGCGGTAGAGAAAACGGCCGATGCCGGGATCGATCAAAAAGTAA TCGGGGTGAACCGTCAGCACGTCCGGGTTCTTGCCTTCTGTGATCTCGCG GTACATCCAATCAGCTAGCTCGATCTCGATGTACTCCGGCCGCCCGGTTTC GCTCTTTACGATCTTGTAGCGGCTAATCAAGGCTTCACCCTCGGATACCGT CACCAGGCGGCCGTTCTTGGCCTTCTTCGTACGCTGCATGGCAACGTGCG TGGTGTTTAACCGAATGCAGGTTTCTACCAGGTCGTCTTTCTGCTTTCCGCC ATCGGCTCGCCGGCAGAACTTGAGTACGTCCGCAACGTGTGGACGGAACA CGCGGCCGGGCTTGTCTCCCTTCCCTTCCCGGTATCGGTTCATGGATTCG GTTAGATGGGAAACCGCCATCAGTACCAGGTCGTAATCCCACACACTGGCC ATGCCGGCCGGCCCTGCGGAAACCTCTACGTGCCCGTCTGGAAGCTCGTA GCGGATCACCTCGCCAGCTCGTCGGTCACGCTTCGACAGACGGAAAACGG CCACGTCCATGATGCTGCGACTATCGCGGGTGCCCACGTCATAGAGCATC GGAACGAAAAAATCTGGTTGCTCGTCGCCCTTGGGCGGCTTCCTAATCGAC GGCGCACCGGCTGCCGGCGGTTGCCGGGATTCTTTGCGGATTCGATCAGC GGCCGCTTGCCACGATTCACCGGGGCGTGCTTCTGCCTCGATGCGTTGCC GCTGGGCGGCCTGCGCGGCCTTCAACTTCTCCACCAGGTCATCACCCAGC GCCGCGCCGATTTGTACCGGGCCGGATGGTTTGCGACCGCTCACGCCGAT TCCTCGGGCTTGGGGGTTCCAGTGCCATTGCAGGGCCGGCAGACAACCCA

	GCCGCTTACGCCTGGCCAACCGCCCGTTCCTCCACACATGGGGCATTCCA CGGCGTCGGTGCCTGGTTGTTCTTGATTTTCCATGCCGCCTCCTTTAGCCG CTAAAATTCATCTACTCATTTATTCATTTGCTCATTTACTCTGGTAGCTGCGC GATGTATTCAGATAGCAGCTCGGTAATGGTCTTGCCTTGGCGTACCGCGTA CATCTTCAGCTTGGTGTGATCCTCCGCCGGCAACTGAAAGTTGACCCGCTT CATGGCTGGCGTGTCTGCCAGGCTGGCCAACGTTGCAGCCTTGCTGCTGC GTGCGCTCGGACGGCCGGCACTTAGCGTGTTTGTGCTTTTGCTCATTTTCT CTTTACCTCATTAACTCAAATGAGTTTTGATTTAATTTCAGCGGCCAGCGCC TGGACCTCGCGGGCAGCGTCGCCCTCGGGTTCTGATTCAAGAACGGTTGT GCCGGCGGCGGCAGTGCCTGGGTAGCTCACGCGCTGCGTGATACGGGAC TCAAGAATGGGCAGCTCGTACCCGGCCAGCGCCTCGGCAACCTCACCGCC GATGCGCGTGCCTTTGATCGCCCGCGACACGACAAAGGCCGCTTGTAGCC TTCCATCCGTGACCTCAATGCGCTGCTTAACCAGCTCCACCAGGTCGGCGG TGGCCCATATGTCGTAAGGGCTTGGCTGCACCGGAATCAGCACGAAGTCG GCTGCCTTGATCGCGGACACAGCCAAGTCCGCCGCCTGGGGCGCTCCGTC GATCACTACGAAGTCGCGCCGGCCGATGGCCTTCACGTCGCGGTCAATCG TCGGGCGGTCGATGCCGACAACGGTTAGCGGTTGATCTTCCCGCACGGCC GCCCAATCGCGGGCACTGCCCTGGGGATCGGAATCGACTAACAGAACATC GGCCCCGGCGAGTTGCAGGGCGCGGGCTAGATGGGTTGCGATGGTCGTC TTGCCTGACCCGCCTTTCTGGTTAAGTACAGCGATAACCTTCATGCGTTCC CCTTGCGTATTTGTTTATTTACTCATCGCATCATATACGCAGCGACCGCATG ACGCAAGCTGTTTTACTCAAATACACATCACCTTTTTAGACGGCGGCGCTC GGTTTCTTCAGCGGCCAAGCTGGCCGGCCAGGCCGCCAGCTTGGCATCAG ACAAACCGGCCAGGATTTCATGCAGCCGCACGGTTGAGACGTGCGCGGGC GGCTCGAACACGTACCCGGCCGCGATCATCTCCGCCTCGATCTCTTCGGT AATGAAAAACGGTTCGTCCTGGCCGTCCTGGTGCGGTTTCATGCTTGTTCC TCTTGGCGTTCATTCTCGGCGGCCGCCAGGGCGTCGGCCTCGGTCAATGC GTCCTCACGGAAGGCACCGCGCCGCCTGGCCTCGGTGGGCGTCACTTCCT CGCTGCGCTCAAGTGCGCGGTACAGGGTCGAGCGATGCACGCCAAGCAGT GCAGCCGCCTCTTTCACGGTGCGGCCTTCCTGGTCGATCAGCTCGCGGGC GTGCGCGATCTGTGCCGGGGGTGAGGGTAGGGCGGGGGCCAAACTTCACG CCTCGGGCCTTGGCGGCCTCGCGCCCGCTCCGGGTGCGGTCGATGATTA GGGAACGCTCGAACTCGGCAATGCCGGCGAACACGGTCAACACCATGCGG CCGGCCGGCGTGGTGGTGTCGGCCCACGGCTCTGCCAGGCTACGCAGGC CCGCGCCGGCCTCCTGGATGCGCTCGGCAATGTCCAGTAGGTCGCGGGT GCTGCGGGCCAGGCGGTCTAGCCTGGTCACTGTCACAACGTCGCCAGGG CGTAGGTGGTCAAGCATCCTGGCCAGCTCCGGGCGGTCGCGCCTGGTGC CGGTGATCTTCTCGGAAAACAGCTTGGTGCAGCCGGCCGCGTGCAGTTCG GCCCGTTGGTTGGTCAAGTCCTGGTCGTCGGTGCTGACGCGGGCATAGCC CAGCAGGCCAGCGGCGGCGCTCTTGTTCATGGCGTAATGTCTCCGGTTCT AGTCGCAAGTATTCTACTTTATGCGACTAAAACACGCGACAAGAAAACGCC AGGAAAAGGGCAGGGCGGCAGCCTGTCGCGTAACTTAGGACTTGTGCGAC ATGTCGTTTTCAGAAGACGGCTGCACTGAACGTCAGAAGCCGACTGCACTA TAGCAGCGGAGGGGTTGGATCAAAGTACTTTGATCCCGAGGGGAACCCTG TGGTTGGCATGCACATACAAATGGACGAACGGATAAACCTTTTCACGCCCT TTTAAATATCCGTTATTCTAATAAACGCTCTTTTCTCTTAGGTTTACCCGCCA ATATATCCTGTCAAACACTGATAGTTTAAACTGAAGGCGGGAAACGACAATC TGATCCAAGCTCAAGCTGCTCTAGCATTCGCCATTCAGGCTGCGCAACTGT TGGGAAGGGCGATCGGTGCGGGCCTCTTCGCTATTACGCCAGCTGGCGAA AGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTAACGCCAGGGTTTTCCC AGTCACGACGTTGTAAAACGACGGCCAGTGCCAAGCTTGCATGCCTGCAG GTCGACTCTAGATCACTAGTATCCTAGGAAGGTACCAGTCTAGCTCAACAG AGCTTTTAACCCAAATTGGTACAATAGAATACAACTTTAGATCATAATTCTCA AAAGAAAGAGATTCCTTAGCTATTCTATCTGCCACTCCATTTCCTTCTCGGC TTGTATGCACAAGCATAAAATCCTCAAACTTGCTAAGTAGATACTTTATGTCT TGGATAATTGGATTGAGACTTGACAAGCATAACTTTCATGTAACCAAAGACA CAAGTTGCTGAGAATCCACCTCAAAAATGATCTTCCTATAATTGAATCGGGA TAATGACAGCACAGCCCATCTAAGAGCCTCCACTTCTACTTCCAGCACGCT TCTTACTTTTACCACAGCTCTTGCACCTAACCATAACACCTTCCCTGTATGAT CGCGAAGCACCCACCCTAAGCCACATTTTAATCCTTCTGTTGGCCATGCCC

	CATCAAAGTTGCACTTAACCCAAGATTGTGGTGGAGCTTCCCATGTTTCTCG TCTGTCCCGACGGTGTTGTGGTTGGTGCTTTCCTTACATTCTGAGCCTCTTT CCTTCTAATCCACTCATCTGCATCTTCTTGTGTCCTTACTAATACCTCATTGG TTCCAAATTCCCTCCCTTTAAGCACCAGCTCGTTTCTGTTCTTCCACAGCCT CCCAAGTATCCAAGGGACTAAAGCCTCCACATTCTTCAGATCAGGATATTCT TGTTTAAGATGTTGAACTCTATGGAGGTTTGTATGAACTGATGATCTAGGAC CGGATAAGTTCCCTTCTTCATAGCGAACTTATTCAAAGAATGTTTTGTGTATC ATTCTTGTTACATTGTTATTAATGAAAAAATATTATTGGTCATTGGACTGAAC ACGAGTGTTAAATATGGACCAGGCCCCAAATAAGATCCATTGATATATGAAT TAAATAACAAGAATAAATCGAGTCACCAAACCACTTGCCTTTTTTAACGAGA CTTGTTCACCAACTTGATACAAAAGTCATTATCCTATGCAAATCAATAATCAT ACAAAAATATCCAATAACACTAAAAAATTAAAAGAAATGGATAATTTCACAAT ATGTTATACGATAAAGAAGTTACTTTTCCAAGAAATTCACTGATTTTATAAGC CCACTTGCATTAGATAAATGGCAAAAAAAAACAAAAAGGAAAAGAAATAAAG CACGAAGAATTCTAGAAAATACGAAATACGCTTCAATGCAGTGGGACCCAC GGTTCAATTATTGCCAATTTTCAGCTCCACCGTATATTTAAAAAATAAAACGA TAATGCTAAAAAAATATAAATCGTAACGATCGTTAAATCTCAACGGCTGGAT CTTATGACGACCGTTAGAAATTGTGGTTGTCGACGAGTCAGTAATAAACGG CGTCAAAGTGGTTGCAGCCGGCACACACGAGTCGTGTTTATCAACTCAAAG CACAAATACTTTTCCTCAACCTAAAAATAAGGCAATTAGCCAAAAACAACTTT GCGTGTAAACAACGCTCAATACACGTGTCATTTTATTATTAGCTATTGCTTCA CCGCCTTAGCTTTCTCGTGACCTAGTCGTCCTCGTCTTTTCTTCTTCTTCTT CTATAAAACAATACCCAAAGAGCTCTTCTTCTTCACAATTCAGATTTCAATTT CTCAAAATCTTAAAAACTTTCTCTCAATTCTCTCTACCGTGATCAAGGTAAAT TTCTGTGTTCCTTATTCTCTCAAAATCTTCGATTTTGTTTTCGTTCGATCCCA ATTTCGTATATGTTCTTTGGTTTAGATTCTGTTAATCTTAGATCGAAGACGAT TTTCTGGGTTTGATCGTTAGATATCATCTTAATTCTCGATTAGGGTTTCATAG ATATCATCCGATTTGTTCAAATAATTTGAGTTTTGTCGAATAATTACTCTTCG ATTTGTGATTTCTATCTAGATCTGGTGTTAGTTTCTAGTTTGTGCGATCGAAT TTGTCGATTAATCTGAGTTTTTCTGATTAACAGATGGATTACAAGGATGATG ATGATAAGGATTACAAGGATGATGATGATAAGATGGCTCCAAAGAAGAAGA GAAAGGTTGGAATCCACGGAGTTCCAGCTGCT**ATGTCGCAGACCATCACA** **GTCAAAGTGAAATTGCTTCCAACAAAAGAACAGGCTTCTATTTTGCGTGA** **GATGAGTCAAACGTACCTCTCCACTATCAATACTCTCGTTTCCGAAATGGT** **TGCTGAAAAGAAAAGCACAAAGAAGTCGAGTAAGGATATTTCTGCTTCTT** **TGCCAAGTGCCGTTAAAAATCAAGCTATCAAGGACGCGAAAAGTGTGTTT** **AAGAAAGCGAAGAAAAGCAAATTCACTACTGTTCCTGTTTTGAAGAAACC** **TGTCTGCATTTGGAACAATCAAAACTATTCGTTTGATTTCACGCACATTTC** **CATGCCGATTATGATTGACGGCAAGGTAACTAAAACACCTATCCGTGCTT** **TGTTAGTTGATAAAGACAACCGTAACTTTGCTTTGCTAAAACACAAATTAG** **GTACGCTTCGAATCACACAGAAAGCAAATAAATGGATTGCCCAAATTTCT** **GTCACCATACCTACTATGGAAGGAACAGGAGTAAAAGTCATGGGGGTTG** **ATTTGGGTTTAAAAGTTCCTGCCGTTGCTGTAACAGATGATGAAAAGGTAC** **GTTTCTTTGGGAATGGCAGACAAAATAAGTACATGAAGCGTAAGTTCCGT** **TCTGAACGCAAAGCATTAGGCAAAAAGAAAAAGGTAAATGCGATTCGTAG** **TTCAAAAGATAAAGAACAACGTTGGATGAAAGACCAAGACCATAAGGTTA** **GTCGTGCTATCGTAAATTTTGCAAAAGACAATAAAATTTCTGTCATTCGCT** **TGGAACAACTGGCGAATATTAGACAGACGGCAAGAACAAGCCGTAAAAA** **CGAAAAGAATCTGCATACTTGGTCATTTTATCGCCTGTCTCAGTTCATTGA** **ATATAAAGCGAATTTAGTTGGTATTAGAGTTGAGCATGTGAATCCTGCATA** **CACAAGTCAGACATGCCCTAAATGCTCTGAGAAGAATAAGGCACAAGAC** **CGTAAGTAT**<u>**AAATGCAAATGTGGATTCGAAAAACATCGTGATTTAGTCGG** **TGCTATGAACATTCGATATGCACCTGTGATTGATGGTAACAGTCAATCAGC** **CTAA**GATGCTATATGCACTGTCTTAGGAGGGGCAATGAGATGCCCTCATCT TGAAGGCTGTTCAAAACAGAAATGGACTGCGAACGCTTAGTCATTCAAGAA TCCCACCCGTACACCGCAAGGTGGAAGGCTTGCGGCTTTAGCCGTGGGAG TCTCAA*GAATTGATGACCATATGATT*GGCCGGCATGGTCCCAGCCTCCTCG CTGGCGCCGGCTGGGCAACATGCTTCGGCATGGCGAATGGGACAGAGCTT TCGTTCGTATCATCGGTTTCGACAACGTTCGTCAAGTTCAATGCATCAGTTT CATTGCGCACACACCAGAATCCTACTGAGTTTGAGTATTATGGCATTGGGA

	AAACTGTTTTTCTTGTACCATTTGTTGTGCTTGTAATTTACTGTGTTTTTTATT CGGTTTTCGCTATCGAACTGTGAAATGGAAATGGATGGAGAAGAGTTAATG AATGATATGGTCCTTTTGTTCATTCTCAAATTAATATTATTTGTTTTTTCTCTT ATTTGTTGTGTGTTGAATTTGAAATTATAAGAGATATGCAAACATTTTGTTTT GAGTAAAAATGTGTCAAATCGTGGCCTCTAATGACCGAAGTTAATATGAGGA GTAAAACACTTGTAGTTGTACCATTATGCTTATTCACTAGGCAACAAATATAT TTTCAGACCTAGAAAAGCTGCAAATGTTACTGAATACAAGTATGTCCTCTTG TGTTTTAGACATTTATGAACTTTCCTTTATGTAATTTTCCAGAATCCTTGTCA GATTCTAATCATTGCTTTATAATTATAGTTATACTCATGGATTTGTAGTTGAG TATGAAAATATTTTTTAATGCATTTTATGACTTGCCAATTGATTGACAACGAA TTC
pCAMBIA1300_maize_codon_ISBag01_U6wRNA_in_split_cassettes_AtPDS3_gRNA10_gRNA18 (SEQ ID NO: 15)	GTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATT CCACACAACATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAA TGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAG TCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGG GAGAGGCGGTTTGCGTATTGGCTAGAGCAGCTTGCCAACATGGTGGAGCA CGACACTCTCGTCTACTCCAAGAATATCAAAGATACAGTCTCAGAAGACCAA AGGGCTATTGAGACTTTTCAACAAAGGGTAATATCGGGAAACCTCCTCGGA TTCCATTGCCCAGCTATCTGTCACTTCATCAAAAGGACAGTAGAAAAGGAAG GTGGCACCTACAAATGCCATCATTGCGATAAAGGAAAGGCTATCGTTCAAG ATGCCTCTGCCGACAGTGGTCCCAAAGATGGACCCCCACCCACGAGGAGC ATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAGTGGATTGA TGTGATAACATGGTGGAGCACGACACTCTCGTCTACTCCAAGAATATCAAA GATACAGTCTCAGAAGACCAAAGGGCTATTGAGACTTTTCAACAAAGGGTA ATATCGGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTCATCA AAAGGACAGTAGAAAAGGAAGGTGGCACCTACAAATGCCATCATTGCGATA AAGGAAAGGCTATCGTTCAAGATGCCTCTGCCGACAGTGGTCCCAAAGATG GACCCCCACCCACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACG TCTTCAAAGCAAGTGGATTGATGTGATATCTCCACTGACGTAAGGGATGAC GCACAATCCCACTATCCTTCGCAAGACCTTCCTCTATATAAGGAAGTTCATT TCATTTGGAGAGGACACGCTGAAATCACCAGTCTCTCTCTACAAATCTATCT CTCTCGAGCTTTCGCAGATCCCGGGGGGCAATGAGATATGAAAAAGCCTGA ACTCACCGCGACGTCTGTCGAGAAGTTTCTGATCGAAAAGTTCGACAGCGT CTCCGACCTGATGCAGCTCTCGGAGGGCGAAGAATCTCGTGCTTTCAGCTT CGATGTAGGAGGGCGTGGATATGTCCTGCGGGTAAATAGCTGCGCCGATG GTTTCTACAAAGATCGTTATGTTTATCGGCACTTTGCATCGGCCGCGCTCCC GATTCCGGAAGTGCTTGACATTGGGGAGTTTAGCGAGAGCCTGACCTATTG CATCTCCCGCCGTGCACAGGGTGTCACGTTGCAAGACCTGCCTGAAACCG AACTGCCCGCTGTTCTACAACCGGTCGCGGAGGCTATGGATGCGATCGCT GCGGCCGATCTTAGCCAGACGAGCGGGTTCGGCCCATTCGGACCGCAAG GAATCGGTCAATACACTACATGGCGTGATTTCATATGCGCGATTGCTGATC CCCATGTGTATCACTGGCAAACTGTGATGGACGACACCGTCAGTGCGTCCG TCGCGCAGGCTCTCGATGAGCTGATGCTTTGGGCCGAGGACTGCCCCGAA GTCCGGCACCTCGTGCACGCGGATTTCGGCTCCAACAATGTCCTGACGGA CAATGGCCGCATAACAGCGGTCATTGACTGGAGCGAGGCGATGTTCGGGG ATTCCCAATACGAGGTCGCCAACATCTTCTTCTGGAGGCCGTGGTTGGCTT GTATGGAGCAGCAGACGCGCTACTTCGAGCGGAGGCATCCGGAGCTTGCA GGATCGCCACGACTCCGGGCGTATATGCTCCGCATTGGTCTTGACCAACTC TATCAGAGCTTGGTTGACGGCAATTTCGATGATGCAGCTTGGGCGCAGGGT CGATGCGACGCAATCGTCCGATCCGGAGCCGGGACTGTCGGGCGTACACA AATCGCCCGCAGAAGCGCGGCCGTCTGGACCGATGGCTGTGTAGAAGTAC TCGCCGATAGTGGAAACCGACGCCCCAGCACTCGTCCGAGGGCAAAGAAA TAGAGTAGATGCCGACCGGATCTGTCGATCGACAAGCTCGAGTTTCTCCAT AATAATGTGTGAGTAGTTCCCAGATAAGGGAATTAGGGTTCCTATAGGGTTT CGCTCATGTGTTGAGCATATAAGAAACCCTTAGTATGTATTTGTATTTGTAAA ATACTTCTATCAATAAAATTTCTAATTCCTAAAACCAAAATCCAGTACTAAAAT CCAGATCCCCCGAATTAATTCGGCGTTAATTCAGTACATTAAAAACGTCCGC AATGTGTTATTAAGTTGTCTAAGCGTCAATTTGTTTACACCACAATATATCCT GCCACCAGCCAGCCAACAGCTCCCCGACCGGCAGCTCGGCACAAAATCAC CACTCGATACAGGCAGCCCATCAGTCCGGGACGGCGTCAGCGGGAGAGC

	CGTTGTAAGGCGGCAGACTTTGCTCATGTTACCGATGCTATTCGGAAGAAC GGCAACTAAGCTGCCGGGTTTGAAACACGGATGATCTCGCGGAGGGTAGC ATGTTGATTGTAACGATGACAGAGCGTTGCTGCCTGTGATCACCGCGGTTT CAAAATCGGCTCCGTCGATACTATGTTATACGCCAACTTTGAAAACAACTTT GAAAAAGCTGTTTTCTGGTATTTAAGGTTTTAGAATGCAAGGAACAGTGAAT TGGAGTTCGTCTTGTTATAATTAGCTTCTTGGGGTATCTTTAAATACTGTAGA AAAGAGGAAGGAAATAATAAATGGCTAAAATGAGAATATCACCGGAATTGAA AAAACTGATCGAAAAATACCGCTGCGTAAAAGATACGGAAGGAATGTCTCC TGCTAAGGTATATAAGCTGGTGGGAGAAAATGAAAACCTATATTTAAAAATG ACGGACAGCCGGTATAAAGGGACCACCTATGATGTGGAACGGGAAAAGGA CATGATGCTATGGCTGGAAGGAAAGCTGCCTGTTCCAAAGGTCCTGCACTT TGAACGGCATGATGGCTGGAGCAATCTGCTCATGAGTGAGGCCGATGGCG TCCTTTGCTCGGAAGAGTATGAAGATGAACAAAGCCCTGAAAAGATTATCG AGCTGTATGCGGAGTGCATCAGGCTCTTTCACTCCATCGACATATCGGATT GTCCCTATACGAATAGCTTAGACAGCCGCTTAGCCGAATTGGATTACTTACT GAATAACGATCTGGCCGATGTGGATTGCGAAAACTGGGAAGAAGACACTCC ATTTAAAGATCCGCGCGAGCTGTATGATTTTTTAAAGACGGAAAAGCCCGAA GAGGAACTTGTCTTTTCCCACGGCGACCTGGGAGACAGCAACATCTTTGTG AAAGATGGCAAAGTAAGTGGCTTTATTGATCTTGGGAGAAGCGGCAGGGC GGACAAGTGGTATGACATTGCCTTCTGCGTCCGGTCGATCAGGGAGGATAT CGGGGAAGAACAGTATGTCGAGCTATTTTTTGACTTACTGGGGATCAAGCC TGATTGGGAGAAAATAAAATATTATATTTTACTGGATGAATTGTTTTAGTACC TAGAATGCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTC AGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGC GTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGT TTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGC AGAGCGCAGATACCAAATACTGTCCTTCTAGTGTAGCCGTAGTTAGGCCAC CACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGT TACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACT CAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGG TTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATA CCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGG CGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAG GGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCG CCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGA GCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTT GCTGGCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGA TAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAGCCGAAC GACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCTGATG CGGTATTTTCTCCTTACGCATCTGTGCGGTATTTCACACCGCATATGGTGCA CTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAAGCCAGTATACACTCCG CTATCGCTACGTGACTGGGTCATGGCTGCGCCCCGACACCCGCCAACACC CGCTGACGCGCCCTGACGGGCTTGTCTGCTCCCGGCATCCGCTTACAGAC AAGCTGTGACCGTCTCCGGGAGCTGCATGTGTCAGAGGTTTTCACCGTCAT CACCGAAACGCGCGAGGCAGGGTGCCTTGATGTGGGCGCCGGCGGTCGA GTGGCGACGGCGCGGCTTGTCCGCGCCCTGGTAGATTGCCTGGCCGTAG GCCAGCCATTTTTGAGCGGCCAGCGGCCGCGATAGGCCGACGCGAAGCG GCGGGGCGTAGGGAGCGCAGCGACCGAAGGGTAGGCGCTTTTTGCAGCT CTTCGGCTGTGCGCTGGCCAGACAGTTATGCACAGGCCAGGCGGGTTTTA AGAGTTTTAATAAGTTTTAAAGAGTTTTAGGCGGAAAAATCGCCTTTTTTCTC TTTTATATCAGTCACTTACATGTGTGACCGGTTCCCAATGTACGGCTTTGGG TTCCCAATGTACGGGTTCCGGTTCCCAATGTACGGCTTTGGGTTCCCAATG TACGTGCTATCCACAGGAAAGAGACCTTTTCGACCTTTTTCCCCTGCTAGG GCAATTTGCCCTAGCATCTGCTCCGTACATTAGGAACCGGCGGATGCTTCG CCCTCGATCAGGTTGCGGTAGCGCATGACTAGGATCGGGCCAGCCTGCCC CGCCTCCTCCTTCAAATCGTACTCCGGCAGGTCATTTGACCCGATCAGCTT GCGCACGGTGAAACAGAACTTCTTGAACTCTCCGGCGCTGCCACTGCGTTC GTAGATCGTCTTGAACAACCATCTGGCTTCTGCCTTGCCTGCGGCGCGGC GTGCCAGGCGGTAGAGAAAACGGCCGATGCCGGGATCGATCAAAAAGTAA TCGGGGTGAACCGTCAGCACGTCCGGGTTCTTGCCTTCTGTGATCTCGCG

	GTACATCCAATCAGCTAGCTCGATCTCGATGTACTCCGGCCGCCCGGTTTC GCTCTTTACGATCTTGTAGCGGCTAATCAAGGCTTCACCCTCGGATACCGT CACCAGGCGGCCGTTCTTGGCCTTCTTCGTACGCTGCATGGCAACGTGCG TGGTGTTTAACCGAATGCAGGTTTCTACCAGGTCGTCTTTCTGCTTTCCGCC ATCGGCTCGCCGGCAGAACTTGAGTACGTCCGCAACGTGTGGACGGAACA CGCGGCCGGGCTTGTCTCCCTTCCCTTCCCGGTATCGGTTCATGGATTCG GTTAGATGGGAAACCGCCATCAGTACCAGGTCGTAATCCCACACACTGGCC ATGCCGGCCGGCCCTGCGGAAACCTCTACGTGCCCGTCTGGAAGCTCGTA GCGGATCACCTCGCCAGCTCGTCGGTCACGCTTCGACAGACGGAAAACGG CCACGTCCATGATGCTGCGACTATCGCGGGTGCCCACGTCATAGAGCATC GGAACGAAAAAATCTGGTTGCTCGTCGCCCTTGGGCGGCTTCCTAATCGAC GGCGCACCGGCTGCCGGCGGTTGCCGGGATTCTTTGCGGATTCGATCAGC GGCCGCTTGCCACGATTCACCGGGGCGTGCTTCTGCCTCGATGCGTTGCC GCTGGGCGGCCTGCGCGGCCTTCAACTTCTCCACCAGGTCATCACCCAGC GCCGCGCCGATTTGTACCGGGCCGGATGGTTTGCGACCGCTCACGCCGAT TCCTCGGGCTTGGGGGTTCCAGTGCCATTGCAGGGCCGGCAGACAACCCA GCCGCTTACGCCTGGCCAACCGCCCGTTCCTCCACACATGGGGCATTCCA CGGCGTCGGTGCCTGGTTGTTCTTGATTTTCCATGCCGCCTCCTTTAGCCG CTAAAATTCATCTACTCATTTATTCATTTGCTCATTTACTCTGGTAGCTGCGC GATGTATTCAGATAGCAGCTCGGTAATGGTCTTGCCTTGGCGTACCGCGTA CATCTTCAGCTTGGTGTGATCCTCCGCCGGCAACTGAAAGTTGACCCGCTT CATGGCTGGCGTGTCTGCCAGGCTGGCCAACGTTGCAGCCTTGCTGCTGC GTGCGCTCGGACGGCCGGCACTTAGCGTGTTTGTGCTTTTGCTCATTTTCT CTTTACCTCATTAACTCAAATGAGTTTTGATTTAATTTCAGCGGCCAGCGCC TGGACCTCGCGGGCAGCGTCGCCCTCGGGTTCTGATTCAAGAACGGTTGT GCCGGCGGCGGCAGTGCCTGGGTAGCTCACGCGCTGCGTGATACGGGAC TCAAGAATGGGCAGCTCGTACCCGGCCAGCGCCTCGGCAACCTCACCGCC GATGCGCGTGCCTTTGATCGCCCGCGACACGACAAAGGCCGCTTGTAGCC TTCCATCCGTGACCTCAATGCGCTGCTTAACCAGCTCCACCAGGTCGGCGG TGGCCCATATGTCGTAAGGGCTTGGCTGCACCGGAATCAGCACGAAGTCG GCTGCCTTGATCGCGGACACAGCCAAGTCCGCCGCCTGGGGCGCTCCGTC GATCACTACGAAGTCGCGCCGGCCGATGGCCTTCACGTCGCGGTCAATCG TCGGGCGGTCGATGCCGACAACGGTTAGCGGTTGATCTTCCCGCACGGCC GCCCAATCGCGGGCACTGCCCTGGGGATCGGAATCGACTAACAGAACATC GGCCCCGGCGAGTTGCAGGGCGCGGGCTAGATGGGTTGCGATGGTCGTC TTGCCTGACCCGCCTTTCTGGTTAAGTACAGCGATAACCTTCATGCGTTCC CCTTGCGTATTTGTTTATTTACTCATCGCATCATATACGCAGCGACCGCATG ACGCAAGCTGTTTTACTCAAATACACATCACCTTTTTAGACGGCGGCGCTC GGTTTCTTCAGCGGCCAAGCTGGCCGGCCAGGCCGCCAGCTTGGCATCAG ACAAACCGGCCAGGATTTCATGCAGCCGCACGGTTGAGACGTGCGCGGGC GGCTCGAACACGTACCCGGCCGCGATCATCTCCGCCTCGATCTCTTCGGT AATGAAAAACGGTTCGTCCTGGCCGTCCTGGTGCGGTTTCATGCTTGTTCC TCTTGGCGTTCATTCTCGGCGGCCGCCAGGGCGTCGGCCTCGGTCAATGC GTCCTCACGGAAGGCACCGCGCCGCCTGGCCTCGGTGGGCGTCACTTCCT CGCTGCGCTCAAGTGCGCGGTACAGGGTCGAGCGATGCACGCCAAGCAGT GCAGCCGCCTCTTTCACGGTGCGGCCTTCCTGGTCGATCAGCTCGCGGGC GTGCGCGATCTGTGCCGGGGTGAGGGTAGGGCGGGGGCCAAACTTCACG CCTCGGGCCTTGGCGGCCTCGCGCCCGCTCCGGGTGCGGTCGATGATTA GGGAACGCTCGAACTCGGCAATGCCGGCGAACACGGTCAACACCATGCGG CCGGCCGGCGTGGTGGTGTCGGCCCACGGCTCTGCCAGGCTACGCAGGC CCGCGCCGGCCTCCTGGATGCGCTCGGCAATGTCCAGTAGGTCGCGGGT GCTGCGGGCCAGGCGGTCTAGCCTGGTCACTGTCACAACGTCGCCAGGG CGTAGGTGGTCAAGCATCCTGGCCAGCTCCGGGCGGTCGCGCCTGGTGC CGGTGATCTTCTCGGAAAACAGCTTGGTGCAGCCGGCCGCGTGCAGTTCG GCCCGTTGGTTGGTCAAGTCCTGGTCGTCGGTGCTGACGCGGGCATAGCC CAGCAGGCCAGCGGCGGCGCTCTTGTTCATGGCGTAATGTCTCCGGTTCT AGTCGCAAGTATTCTACTTTATGCGACTAAAACACGCGACAAGAAAACGCC AGGAAAAGGGCAGGGCGGCAGCCTGTCGCGTAACTTAGGACTTGTGCGAC ATGTCGTTTTCAGAAGACGGCTGCACTGAACGTCAGAAGCCGACTGCACTA TAGCAGCGGAGGGGTTGGATCAAAGTACTTTGATCCCGAGGGGAACCCTG

	TGGTTGGCATGCACATACAAATGGACGAACGGATAAACCTTTTCACGCCCT TTTAAATATCCGTTATTCTAATAAACGCTCTTTTCTCTTAGGTTTACCCGCCA ATATATCCTGTCAAACACTGATAGTTTAAACTGAAGGCGGGAAACGACAATC TGATCCAAGCTCAAGCTGCTCTAGCATTCGCCATTCAGGCTGCGCAACTGT TGGGAAGGGCGATCGGTGCGGGCCTCTTCGCTATTACGCCAGCTGGCGAA AGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTAACGCCAGGGTTTTCCC AGTCACGACGTTGTAAAACGACGGCCAGTGCCAAGCTTGCATGCCTGCAG GTCGACTCTAGATCACTAGTATGATCAGATGCAGAGAGACTAACCTCAGAT AGGCGACTTCAAAACCCCCCGAAAATTTGACAATGGAGCTTGAAAAGAATG GCAAAAAAAAGTCCCATTCGCCATGCCGAAGCATGTTGCCCAGCCGGCGC CAGCGAGGAGGCTGGGACCATGCCGGCC*CACTTAACCTTACTCTCTTCA*TTG AGACTCCCACGGCTAAAGCCGCAAGCCTTCCACCTTGCGGTGTACGGGTG GGATTCTTGAATGACTAAGCGTTCGCAGTCCATTTCTGTTTTGAACAGCCTT CAAGATGAGGGCATCTCATTGCCCCTCCTAAGACAGTGCATATAGCATCTT AGGCTGATTGACTGTTACCATCAATCACAGGTGCATATCGAATGTTCATAGC ACCGACTAAATCACGATGTTTTTCGAATCCACATTTGCATTTCAATCACTACT TCGACTCTAGCTGTATATAAACTCAGCTTCGTTTTCTTATCTAAGCGATGTG GGACTTTTGAAGATTGTTTTCAACTTAAATGGGCCTATATAAGAAATACTATT GTTCTTTCCCATATAAATGGGCCTGCTTCTCTTCTTTCAGATTCCCAGGGGC CTTTTGAAGATTATCTTCATATCTTAAGAATGAAGATGTTTTATTCAATCAAAT TCTTGAAGGTTCGATGCCTAATCATTCTAATCCTGGGACAAACTATGAAACA AGATACAAAAACTCCGAATGGAAAGTTAAAAAGAAGAAAACGAAAGCTACG GTTCAAGAAAATGTAAGCTGATAAACAAAAAAAAACTGTATGAACGAAGAAG AAGAAAAAAAGCTAAGAAGAAATGATGTATTGTGCGGAAGGCAAGTCGAGT TTCCGTTGTTCAACGAAGCTTTGATCAGATGCAGAGAGACTAACCTCAGATA GGCGACTTCAAAACCCCCCGAAAATTTGACAATGGAGCTTGAAAAGAATGG CAAAAAAAAGTCCCATTCGCCATGCCGAAGCATGTTGCCCAGCCGGCGCC AGCGAGGAGGCTGGGACCATGCCGGCC*ATAGCCCAAATACCTACAAC*TTG AGACTCCCACGGCTAAAGCCGCAAGCCTTCCACCTTGCGGTGTACGGGTG GGATTCTTGAATGACTAAGCGTTCGCAGTCCATTTCTGTTTTGAACAGCCTT CAAGATGAGGGCATCTCATTGCCCCTCCTAAGACAGTGCATATAGCATCTT AGGCTGATTGACTGTTACCATCAATCACAGGTGCATATCGAATGTTCATAGC ACCGACTAAATCACGATGTTTTTCGAATCCACATTTGCATTTCAATCACTACT TCGACTCTAGCTGTATATAAACTCAGCTTCGTTTTCTTATCTAAGCGATGTG GGACTTTTGAAGATTGTTTTCAACTTAAATGGGCCTATATAAGAAATACTATT GTTCTTTCCCATATAAATGGGCCTGCTTCTCTTCTTTCAGATTCCCAGGGGC CTTTTGAAGATTATCTTCATATCTTAAGAATGAAGATGTTTTATTCAATCAAAT TCTTGAAGGTTCGATGCCTAATCATTCTAATCCTGGGACAAACTATGAAACA AGATACAAAAACTCCGAATGGAAAGTTAAAAAGAAGAAAACGAAAGCTACG GTTCAAGAAAATGTAAGCTGATAAACAAAAAAAAACTGTATGAACGAAGAAG AAGAAAAAAAGCTAAGAAGAAATGATGTATTGTGCGGAAGGCAAGTCGAGT TTCCGTTGTTCAACGAAGCTTTATCCTAGGAAGGTACCAGTCTAGCTCAACA GAGCTTTTAACCCAAATTGGTACAATAGAATACAACTTTAGATCATAATTCTC AAAAGAAAGAGATTCCTTAGCTATTCTATCTGCCACTCCATTTCCTTCTCGG CTTGTATGCACAAGCATAAAATCCTCAAACTTGCTAAGTAGATACTTTATGTC TTGGATAATTGGATTGAGACTTGACAAGCATAACTTTCATGTAACCAAAGAC ACAAGTTGCTGAGAATCCACCTCAAAAATGATCTTCCTATAATTGAATCGGG ATAATGACAGCACAGCCCATCTAAGAGCCTCCACTTCTACTTCCAGCACGC TTCTTACTTTTACCACAGCTCTTGCACCTAACCATAACACCTTCCCTGTATGA TCGCGAAGCACCCACCCTAAGCCACATTTTAATCCTTCTGTTGGCCATGCC CCATCAAAGTTGCACTTAACCCAAGATTGTGGTGGAGCTTCCCATGTTTCTC GTCTGTCCCGACGGTGTTGTGGTTGGTGCTTTCCTTACATTCTGAGCCTCTT TCCTTCTAATCCACTCATCTGCATCTTCTTGTGTCCTTACTAATACCTCATTG GTTCCAAATTCCCTCCCTTTAAGCACCAGCTCGTTTCTGTTCTTCCACAGCC TCCCAAGTATCCAAGGGACTAAAGCCTCCACATTCTTCAGATCAGGATATTC TTGTTTAAGATGTTGAACTCTATGGAGGTTTGTATGAACTGATGATCTAGGA CCGGATAAGTTCCCTTCTTCATAGCGAACTTATTCAAAGAATGTTTTGTGTAT CATTCTTGTTACATTGTTATTAATGAAAAAATATTATTGGTCATTGGACTGAA CACGAGTGTTAAATATGGACCAGGCCCCAAATAAGATCCATTGATATATGAA TTAAATAACAAGAATAAATCGAGTCACCAAACCACTTGCCTTTTTTAACGAG

	ACTTGTTCACCAACTTGATACAAAAGTCATTATCCTATGCAAATCAATAATCA TACAAAAATATCCAATAACACTAAAAAATTAAAAGAAATGGATAATTTCACAA TATGTTATACGATAAAGAAGTTACTTTTCCAAGAAATTCACTGATTTTATAAG CCCACTTGCATTAGATAAATGGCAAAAAAAAACAAAAAGGAAAAGAAATAAA GCACGAAGAATTCTAGAAAATACGAAATACGCTTCAATGCAGTGGGACCCA CGGTTCAATTATTGCCAATTTTCAGCTCCACCGTATATTTAAAAAATAAAACG ATAATGCTAAAAAAATATAAATCGTAACGATCGTTAAATCTCAACGGCTGGA TCTTATGACGACCGTTAGAAATTGTGGTTGTCGACGAGTCAGTAATAAACG GCGTCAAAGTGGTTGCAGCCGGCACACACGAGTCGTGTTTATCAACTCAAA GCACAAATACTTTTCCTCAACCTAAAAATAAGGCAATTAGCCAAAAACAACT TTGCGTGTAAACAACGCTCAATACACGTGTCATTTTATTATTAGCTATTGCTT CACCGCCTTAGCTTTCTCGTGACCTAGTCGTCCTCGTCTTTTCTTCTTCTTC TTCTATAAAACAATACCCAAAGAGCTCTTCTTCTTCACAATTCAGATTTCAAT TTCTCAAAATCTTAAAAACTTTCTCTCAATTCTCTCTACCGTGATCAAGGTAA ATTTCTGTGTTCCTTATTCTCTCAAAATCTTCGATTTTGTTTTCGTTCGATCC CAATTTCGTATATGTTCTTTGGTTTAGATTCTGTTAATCTTAGATCGAAGACG ATTTTCTGGGTTTGATCGTTAGATATCATCTTAATTCTCGATTAGGGTTTCAT AGATATCATCCGATTTGTTCAAATAATTTGAGTTTTGTCGAATAATTACTCTT CGATTTGTGATTTCTATCTAGATCTGGTGTTAGTTTCTAGTTTGTGCGATCG AATTTGTCGATTAATCTGAGTTTTTCTGATTAACAG**ATGTCTCAGACTATCA** **CAGTTAAGGTGAAGCTGTTGCCGACGAAGGAGCAGGCATCAATCCTGCG** **CGAGATGTCGCAGACATATTTGAGCACAA**TCAACACTCTGGTTTCTGAAA **TGGTTGCAGAAAAGAAGAGCACTAAGAAGAGCTCGAAGGATATCTCTGCT** **TCCCTGCCCTCAGCCGTTAAGAACCAAGCGATCAAGGATGCCAAGTCAGT** **TTTCAAGAAGGCGAAGAAGTCAAAGTTCACGACGGTTCCTGTTCTCAAGA** **AGCCTGTTTGCATCTGGAATAATCAAAACTATAGCTTCGATTTCACACACA** **TCTCCATGCCTATCATGATCGATGGGAAGGTCACTAAGACCCCAATCCGC** **GCTCTCCTTGTTGATAAGGATAATCGCAATTTCGCGTTGCTCAAGCATAAG** **CTCGGAACTCTTCGCATCACCCAGAAGGCCAATAAGTGGATCGCCCAAAT** **CTCGGTGACCATCCCCACGATGGAGGGTACAGGTGTCAAGGTCATGGGA** **GTGGATCTTGGATTGAAGGTGCCCGCTGTCGCTGTCACTGACGATGAAAA** **GGTCCGCTTCTTCGGGAATGGGCGCCAGAATAAGTACATGAAGCGCAAG** **TTCCGCTCTGAACGCAAGGCATTGGGGAAGAAGAAGAAGGTCAACGCGA** **TCCGCTCGTCCAAGGACAAGGAGCAGCGCTGGATGAAGGACCAAGACCA** **CAAGGTGTCAAGGGCCATCGTGAATTTCGCCAAGGACAACAAGATCTCTG** **TTATCAGGCTGGAGCAGCTTGCGAACATCCGCCAGACTGCCCGCACCTC** **GCGCAAGAACGAAAAGAA**TTTGCATACGTGGAGCTTCTACCGCCTCTCAC **AATTCATCGAATACAAGGCGAATC**TCGTGGGAATCAGGGTGGAACATGTC **AACCCTGCATACACATCGCAAACCTGCCCTAAGTGCTCCGAAAAGAATAA** **GGCACAGGATCGCAAGTATAAGTGTAAGTGTGGATTCGAAAAGCACCGC** **GATTTGGTTGGTGCCATGAACATCCGCTACGCCCCGGTGATCGACGGGAA** **TTCGCAGTCTGCC**GGATCCGGACCGAAGAAGAAGCGAAAGGTAGAGGATC CTAAGAAGAAGCGTAAAGTCTCCTTGGGTTCTGGCTCCGACTATAAGGATG ACGATGACAAAGACTATAAGGATGACGACGACAAGTGAAGAGCTTTCGTTC GTATCATCGGTTTCGACAACGTTCGTCAAGTTCAATGCATCAGTTTCATTGC GCACACACCAGAATCCTACTGAGTTTGAGTATTATGGCATTGGGAAAACTGT TTTTCTTGTACCATTTGTTGTGCTTGTAATTTACTGTGTTTTTTTATTCGGTTTT CGCTATCGAACTGTGAAATGGAAATGGATGGAGAAGAGTTAATGAATGATA TGGTCCTTTTGTTCATTCTCAAATTAATATTATTTGTTTTTTCTCTTATTTGTT GTGTGTTGAATTTGAAATTATAAGAGATATGCAAACATTTTGTTTTGAGTAAA AATGTGTCAAATCGTGGCCTCTAATGACCGAAGTTAATATGAGGAGTAAAAC ACTTGTAGTTGTACCATTATGCTTATTCACTAGGCAACAAATATATTTTCAGA CCTAGAAAAGCTGCAAATGTTACTGAATACAAGTATGTCCTCTTGTGTTTTA GACATTTATGAACTTTCCTTTATGTAATTTTCCAGAATCCTTGTCAGATTCTA ATCATTGCTTTATAATTATAGTTATACTCATGGATTTGTAGTTGAGTATGAAA ATATTTTTTAATGCATTTTATGACTTGCCAATTGATTGACAACGAATTC

Table 2: spacer sequences of ISBag01 that were tested in Example 3

wRNA	sequence
ISBag01 AtPDS3 gR1 (SEQ ID NO: 16)	gaattgatgaccatatgatt
ISBag01 AtPDS3 gR2 (SEQ ID NO: 17)	ctagcgagtaatcgtgaagt
ISBag01 AtPDS3 gR3 (SEQ ID NO: 18)	tggaagatatcttttggata
ISBag01 AtPDS3 gR4 (SEQ ID NO: 19)	ctatcttattggagagcgat
ISBag01 AtPDS3 gR5 (SEQ ID NO: 20)	atattttctgtaagtccaaa
ISBag01 AtPDS3 gR6 (SEQ ID NO: 21)	ttggcatagcaaaaatcatg
ISBag01 AtPDS3 gR7 (SEQ ID NO: 22)	attcaacaagtgtgtgcaca
ISBag01 AtPDS3 gR8 (SEQ ID NO: 23)	tcaattacatcatactttgt
ISBag01 AtPDS3 gR9 (SEQ ID NO: 24)	agtttgattacccatcctaa
ISBag01 AtPDS3 gR10 (SEQ ID NO: 25)	gttgtaggtatttgggctat
ISBag01 AtPDS3 gR11 (SEQ ID NO: 26)	ccaaacaatacttgagtttg
ISBag01 AtPDS3 gR12 (SEQ ID NO: 27)	taccatgctgtcttgtgggt
ISBag01 AtPDS3 gR13 (SEQ ID NO: 28)	cagatccctggaaagaaata
ISBag01 AtPDS3 gR14 (SEQ ID NO: 29)	attgataaattcaacatctt
ISBag01 AtPDS3 gR15 (SEQ ID NO: 30)	ctgaacaatagactgagagc
ISBag01 AtPDS3 gR16 (SEQ ID NO: 31)	ctgattttgtctcactatta
ISBag01 AtPDS3 gR17 (SEQ ID NO: 32)	tggttatacaggattacgag
ISBag01 AtPDS3 gR18 (SEQ ID NO: 33)	tgaagagagtaaggttaagt

[0097] Example 4 - Identification and testing of tiny TnpB systems in plant cells

[0098] Utilizing ggKbase, new RNA-guided TnpB systems will be identified from IS605 (with IS605 TnpA), IS607 (with IS607 TnpA), and IS1341 (without TnpA) transposons using the "DOTS" approach described above. Thousands of new RNA-guided TnpB systems in the range of 300-400 amino acids in size will be selected, manually curated and subjected to subsequent sanity checks: (1) the catalytic domain is not broken (2) structure prediction algorithms (e.g., Alphafold2) can produce a reasonable 3D folding of the TnpB protein and (3) the RE of the transposons comprise putative ωRNA-like secondary structures by RNA folding programs (e.g., RNAfold). A large number of these TnpB (~100) will be tested, first in bacteria to identify their TAM sequences and confirm that they are active RNA-guided nucleases. Tests in bacteria will be run at 26 ^{0}C to screen for nucleases that are active at temperatures that are optimal for most crop plants. All active nucleases will then be tested in *Arabidopsis* and maize protoplasts to quickly test if these enzymes work in plant cells, and to screen for ones that work in both monocots and dicots. Enzymes whose catalytic activity is optimal around 23-28 ^{0}C will be prioritized as these are the temperatures that most crop plants are grown. This will be tested

by running parallel protoplast editing assays at different temperatures. Protoplasts will be transfected with the small Cas systems encoded on plasmids, and editing outcomes will be assayed by deep amplicon sequencing to determine cutting efficiency, similar to the methods used above to test ISBag01. Prioritized Cas systems will initially be tested to determine if (1) they are compatible with a single expression cassette format containing both the TnpB protein and ωRNA (FIG. 3A), (2) an N terminal or C terminal nuclear localization sequence shows activity, (3) if codon optimization is necessary. We will also vary the length of the ωRNA and spacer sequences and we will test a number of different gRNA sequences. It is believed that several TnpB systems with good editing activity will be identified, ideally ones with different TAM sequences which will allow for editing of a larger variety of plant sequences.

[0099] Example 5 – Testing tiny TnpB systems *in planta*

[00100] Small RNA-guided TnpB systems that show good activity in protoplasts will be tested in whole plant settings. We will test in a *Nicotiana benthamiana* transient expression system. We will test in transgenic *Arabidopsis* plants to test for germline editing and heritable transmission. The constructs which are used to test TnpB activities in protoplasts are binary vectors, thus, they can be used directly for Agrobacterium-mediated transformation of the *Arabidopsis* plants. In addition, the Arabidopsis protoplast assays will target the *AtPDS3* gene, which provides a convenient readout for editing in whole plants. Constructs with TnpB systems and the best performing guide RNA sequences will be transformed into *Arabidopsis* plants and the T0 plants used for transformation, as well as the T1 transgenic plants will be cultured at the optimum temperature determined for the specific TnpB system being tested. The somatic editing efficiency of target regions in transgenic T1 plants will be evaluated by amplicon sequencing. In addition, systems with high efficiency will be screened for white sectors caused by the loss of function of the *AtPDS3* gene, as seen previously for CasΦ (Li et al., 2023). T2 populations of T1 plants with relatively high somatic editing efficiency will be screened for albino seedlings and tested for transgene segregation. Fully albino seedlings in the T2 population in which the transgene has been segregated away (null segregants) indicate that the mutation in the *AtPDS3* gene has been meiotically inherited.

[00101] Example 6 – Testing tiny TnpB systems in plant viral systems

[00102] The most successful tiny RNA-guided TnpB systems will be initially encoded into tobacco rattle virus (TRV) derived viral vectors together with the isoleucine tRNA sequence which was shown to be the most effective RNA mobility sequence in our previous study (Nagalakshmi et al., 2022). *Arabidopsis* and *Nicotiana benthamiana* plants will be infected in order to test for both somatic and germline editing, using protocols established in our previous

work (Ghoshal et al., 2020; Nagalakshmi et al., 2022). For *Arabidopsis*, the best performing AtPDS3 guide RNA sequences will be used so that we can observe white sectors on the initially infected plants, and also screen for albino seedling in the next generation which will be indicative of germline transmission of edits (Li et al., 2023).

[00103] Those systems showing the most promise will also be encoded into viral vectors being developed that are capable of infecting monocots such as maize, and tested for both somatic and germline editing.

[00104] Example 7 – Optimizing the efficiency of tiny TnpB systems

[00105] The efficiency of the best systems will be further optimized by protein and guide RNA engineering using both directed evolution and rational design in *E.coli* and/or *in vitro* assays, following retesting in protoplasts and whole plants using the approaches in Examples 4-6. We will first employ a bacterial selection system to perform high-throughput directed evolution on the TnpB proteins of candidate systems for higher efficiency (Ma et al., 2022). In the selection system, TnpB-mediated cleavage of a toxic gene in a selection plasmid enables bacterial survival (FIG. 4A and 4B). Candidate TnpBs will be selectively mutagenized in all regions of the protein using standard protocols (Hand et al., 2019). Mutants with fast DNA targeting kinetics will be selected under conditions such as shortened bacterial recovery time after plasmid transformation, where faster targeting kinetics can compensate for the shortened time to destroy the toxic gene.

[00106] As a second approach we will determine the 3D structures of the candidate TnpB systems and use structural guided mutagenesis of both the TnpB protein and ωRNA sequences, and then test variants for improved activity in *in vitro* assays. We will first express and reconstitute the candidate TnpB and ωRNA complexes in *E.coli* and conduct *in-vitro* biochemical analysis on these candidate systems. Using polyacrylamide gel electrophoresis (PAGE), the DNA cleavage activity of these systems on short oligonucleotides as well as plasmid DNA will be assessed. We will then use cryo-electron microscopy to perform structural studies on systems that are biochemically well-behaved with detectable DNA binding and/or cleavage activities. We will identify potential engineering sites based on the 3D structures and prior CRISPR-Cas enzyme engineering efforts (Kim et al., 2022; Tsuchida et al., 2022; Xu et al., 2021) and design variants that may improve genome editing activities.

[00107] Variants from these approaches will then be tested in protoplast editing assays, after which they will be incorporated into the best performing viral editing systems.

[00108] Example 8 – Testing vecotrs for TnpB-mediated editing in plants

[00109] This Example describes exemplary experimental guidelines for constructing and testing vectors expressing the TnpB protein and the associated ωRNA. These vectors will be used to modify plant genomic DNA in protoplast cells, somatic whole plant cells, and germ cells.

[00110] Identification of TnpB target sites

[00111] Four TnpBs capable of targeted mutagenesis in bacteria and human cells were selected for testing in plants: ISDra2, ISYmu1, ISAam1, and ISDge10 (Xiang et al. 2023; Karvelis et al. 2021). We will test these TnpBs using their native DNA sequence, maize codon optimized sequence, and Arabidopsis codon optimized DNA sequence (see sequences provided below in informal sequence listing). For each TnpB, a library of spacer sequences targeting the Arabidopsis PDS3 gene (AT4G14210), will be designed according to their Transposon associated motif (TAM) sequence (Table 3). In Table 3, the gRNA sequences are listed as DNA sequences that are the equivalent RNA sequences (except the RNA will have the T replaced with a U). A similar approach will be used to test for editing capabilities in other species, such as maize.

Table 3: TAM and gRNA sequences

TnpB	TAM Sequence	gRNA ID	gRNA sequence	gRNA sequence SEQ ID NO:
ISYmu1	TTGAT	1	ttacgaattgatgacc	54
ISYmu1	TTGAT	2	aaggcaaattcgccgc	55
ISYmu1	TTGAT	3	tcacattaagcctaga	56
ISYmu1	TTGAT	4	cccaagttctccaaat	57
ISYmu1	TTGAT	5	tacccatcctaaagta	58
ISYmu1	TTGAT	6	gttgattaacttgtac	59
ISYmu1	TTGAT	7	aaattcaacatctttc	60
ISYmu1	TTGAT	8	tggtctcactttccga	61
ISYmu1	TTGAT	9	agctttgaaccggttt	62
ISYmu1	TTGAT	10	taacttgtactacctc	63
ISYmu1	TTGAT	11	cgaaaactgaagaaca	64
ISYmu1	TTGAT	12	gcgttggagcatataa	65
ISYmu1	TTGAT	13	taaagagaggaaattg	66
ISYmu1	TTGAT	14	ggtagagctgataaga	67
ISYmu1	TTGAT	15	aaaattggattaatgt	68
ISYmu1	TTGAT	16	tactattaaatgtcaa	69
ISYmu1	TTGAT	17	caatacaaataaatac	70
ISYmu1	TTGAT	18	caattcaagctaatta	71

TnpB	TAM Sequence	gRNA ID	gRNA sequence	gRNA sequence SEQ ID NO:
ISYmu1	TTGAT	19	gagcttaacttggtag	72
ISYmu1	TTGAT	20	ttgtcagctttcttat	73
ISAam1	TTTAA	1	gtccactagttttaga	74
ISAam1	TTTAA	2	cggagccatccgaatc	75
ISAam1	TTTAA	3	tccatctacatgtttc	76
ISAam1	TTTAA	4	cttggagttctcgaaa	77
ISAam1	TTTAA	5	ccagtgttttgcaact	78
ISAam1	TTTAA	6	gggtgctggtaggaca	79
ISAam1	TTTAA	7	ttacttgaagcagtaa	80
ISAam1	TTTAA	8	actatctgatgaaaac	81
ISAam1	TTTAA	9	tagtaatcaaagaact	82
ISAam1	TTTAA	10	ttatttgccagaaaca	83
ISAam1	TTTAA	11	caattcatctggtatc	84
ISAam1	TTTAA	12	ccaagaacaagcctta	85
ISAam1	TTTAA	13	gttttgtcctcttctc	86
ISAam1	TTTAA	14	caatttacctatctta	87
ISAam1	TTTAA	15	cacataattgaaaaga	88
ISAam1	TTTAA	16	aatttgttacacaact	89
ISAam1	TTTAA	17	tttgtgtggtatttaa	90
ISAam1	TTTAA	18	aaagtaattactgtcg	91
ISAam1	TTTAA	19	aaagagagacataatg	92
ISAam1	TTTAA	20	aacctttctggtaca	93
ISDge10	TTAT	1	cggctacttccactag	94
ISDge10	TTAT	2	ctgcatccttccgtag	95
ISDge10	TTAT	3	ccgaatgtgcagaatt	96
ISDge10	TTAT	4	ttggagaacttgggat	97
ISDge10	TTAT	5	gttgaggcccaagatg	98
ISDge10	TTAT	6	cagtcaaagaatggat	99
ISDge10	TTAT	7	tgccatgtcaaaggcg	100
ISDge10	TTAT	8	aaaccctgatgaactg	101
ISDge10	TTAT	9	acaggattacgagcta	102
ISDge10	TTAT	10	tgtgttctctggttct	103
ISDge10	TTAT	11	cagctctaccatcaat	104
ISDge10	TTAT	12	tctctcaccatttaag	105
ISDge10	TTAT	13	caaaccaaatacatgg	106
ISDge10	TTAT	14	atcgtcttgccttgac	107
ISDge10	TTAT	15	tttctctggccatgtc	108
ISDge10	TTAT	16	ggatagctctgacttt	109
ISDge10	TTAT	17	ctcactggcaatcata	110

TnpB	TAM Sequence	gRNA ID	gRNA sequence	gRNA sequence SEQ ID NO:
ISDge10	TTAT	18	tgggatgaggtagtac	111
ISDge10	TTAT	19	gtgtttgccgctccag	112
ISDge10	TTAT	20	ttagcttcaaatgagg	113
ISDra2	TTGAT	1	ttacgaattgatgaccatat	114
ISDra2	TTGAT	2	aaggcaaattcgccgcagaa	115
ISDra2	TTGAT	3	tcacattaagcctagaaact	116
ISDra2	TTGAT	4	cccaagttctccaaataaat	117
ISDra2	TTGAT	5	tacccatcctaaagtatggg	118
ISDra2	TTGAT	7	aaattcaacatctttctcta	119
ISDra2	TTGAT	8	tggtctcactttccgaatta	120
ISDra2	TTGAT	9	agctttgaaccggtttcttc	121
ISDra2	TTGAT	11	cgaaaactgaagaacacata	122
ISDra2	TTGAT	12	gcgttggagcatataacaga	123
ISDra2	TTGAT	13	taaagagaggaaattgcagg	124
ISDra2	TTGAT	14	ggtagagctgataagatata	125
ISDra2	TTGAT	15	aaaattggattaatgtgcac	126
ISDra2	TTGAT	16	tactattaaatgtcaaaatc	127
ISDra2	TTGAT	17	caatacaaataaatacatgc	128
ISDra2	TTGAT	18	caattcaagctaattataga	129
ISDra2	TTGAT	19	gagcttaacttggtagagta	130
ISDra2	TTGAT	20	ttgtcagctttcttatggat	131

[00112] Creating vectors for TnpB-mediated genome editing

[00113] To test whether the TnpBs as described herein may be used to target DNA modifications in the plant genome, a variety of constructs will be created. Based on reports published in human cells, we will test a split system for expression of the TnpB and ωRNA sequences (Xiang et al. 2023; Karvelis et al. 2021). Here the TnpB and ωRNA will be expressed by a separate promoter and terminator. The TnpB sequence will be cloned into a binary vector with NLS sequence(s) at the 5' and/or 3' end of the TnpB sequence. The TnpB will be expressed using a Pol II promoter and terminator such as UBQ10 and rbcS E9, respectively (Table 20 below). As we expect the presence of an intron will improve TnpB protein expression, the IV2 intron (Table 20), of various copy numbers, will be cloned into the TnpB coding sequence to test for enhanced editing. The ωRNA sequence will be cloned into a separate intermediate vector driven by either a Pol III promoter (such as AtU6) or a Pol II promoter (such as UBQ10) (Table 20). The spacer sequences will then be cloned into the

intermediate ωRNA vector using a typeII restriction enzymes such as PaqCI. RNA processing sequences, such as Hammerhead ribozyme, HDV ribozyme, or tRNA-Gly will be tested for improved editing (Table 20). Once the binary vector containing the TnpB sequence and intermediate vector for each of the ωRNA-spacer is created, the ωRNA expression cassette from the intermediate vector will get assembled into the TnpB-containing binary vector (FIG. 6). Of the TnpBs capable of editing, further optimization will be done, such as testing various ωRNA sequence lengths and spacer sequence lengths.

[00114] Testing TnpBs for editing in protoplast cells

[00115] The constructs described above will be tested in Arabidopsis mesophyll protoplast cells and maize protoplast cells using previously reported methods (Yoo, Cho, and Sheen 2007; Niu et al. 2019). To test for sensitivity to temperature, protoplast cells will be subjected to varying temperature regimens, such as 26 °C, 32 °C, 37 °C, and various combinations of temperatures including heat shock treatments. The heat shock treatments will be performed at varying times-after-transfection, and varying lengths and temperatures, such as a 2-hour incubation at 37 °C twelve hours after transfection.

[00116] Testing TnpBs for somatic and germline editing in whole plants

[00117] The ability of TnpBs to edit whole plants and faithfully transmit those edits to the next generation is crucial for the creation of novel stable genotypes. To evaluate the TnpBs for somatic editing in stable transgenic plants, and transmission of germline edits to transgene-free progenies, the TnpBs along with promoter, terminator, and RNA processing combinations capable of generating targeted DNA modifications in protoplasts will be transformed into Arabidopsis using the floral dip technique (Zhang et al. 2006).

[00118] Following floral dip, the T1 seeds will be harvested, subjected to selection to identify transgenic plants, and quantified for editing with somatic tissue such as leaves of T1 plants using deep amplicon next generation sequencing (NGS). In addition to the ubiquitous promoter in Example 8, we suspect highly active germ-cell promoters may improve germline editing and heritability of mutations. We will test additional pol II promoters such as RPS5A, YAO1 and EC1.2 promoters. Heritability of edits which disrupts the PDS3 gene function can be easily identified due to the bleached phenotype of biallelic mutations in the PDS3 (AT4G14210) gene being targeted for editing in the T2 generation. Albino plants in T2 generation in which the TnpB transgene has already segregated away indicates the heritability of the edits in the PDS3 gene. These plants will also be subjected to Sanger sequencing to confirm the edits in the PDS3 gene.

[00119] Example 9 - Single expression cassettes for improved editing efficiency

[00120] This Example describes exemplary experimental guidelines for constructing and testing vectors expressing the TnpB protein and the associated ωRNA in a single expression cassette. These vectors may be used to modify plant genomic DNA in protoplasts, whole plants and to generate heritable edits in genomic sequences using similar methods as described in Example 8. We expect these results to translate to eukaryotic species other than those being tested here.

[00121] In all previous reports of TnpB-mediated genome editing the TnpB has been separated from the ωRNA, resulting in separate expression cassettes, similar to what is outlined in Example 8. However, in nature, the ωRNA overlaps with the TnpB sequence (FIG. 7A).

[00122] We expect the single expression cassette will work as efficiently or better than the split systems previously described. To test this, we will clone the TnpB and overlapping ωRNA into a single expression cassette (see informal sequence listing below – SEQ ID NOs: 140-147) on a binary vector. As an example, the single expression cassette will contain a promoter (such as UBQ10), TnpB coding sequence, ωRNA sequence, spacer sequence, RNA processing sequence, and terminator, as depicted in (FIG. 7B). Further optimization of TnpB and ωRNA expression will be performed using various promoters, terminators, and RNA processing sequences such as tRNA-Gly, Hammerhead ribozyme and HDV ribozyme (Table 20).

[00123] Example 10 - TnpBs for multiplexed editing

[00124] This Example describes exemplary experimental guidelines for constructing and testing vectors expressing the TnpB protein and the associated ωRNA with a tandem repeat of the ωRNA in a single expression cassette. These vectors may be used to modify genomic DNA in protoplasts, whole plants and to generate heritable edits in genomic sequences using similar methods as described in Example 8.

[00125] The ability to make multiple edits in a single generation can greatly enhance genome editing possibilities.

[00126] Because TnpBs have been shown to self-process their own ωRNA (Nety et al. 2023), we will test tandem repeats of the ωRNA for improved ωRNA processing, editing, and multiplexing capabilities; an example with one tandem ωRNA repeat is depicted in FIG. 8, however the copy number may vary.

[00127] Example 11 - Constructing and testing viral vectors for TnpB-mediated somatic and germ cell editing

[00128] This Example describes exemplary experimental guidelines for constructing and testing vectors expressing the TnpB protein and the associated ωRNA in viral vectors such as Tobacco Rattle Virus (TRV).

[00129] Delivery of viral vectors for genome editing has the potential to expedite the creation of novel genetic diversity, and at the same time avoid the process of generating transgenic plants. However, due to the sequence size limitation, viruses harboring the RNA-guided endonuclease and the gRNA (such as Cas9 and Cas12a) cannot fit in single viral vectors such as TRV.

[00130] We will test and optimize the TnpBs identified in the prior examples as viral vector reagents. We expect these vectors to create somatic, and germline mutations that are transmitted to the following generation. This work will primarily be conducted in Arabidopsis, however due to TRV's wide host range this technology will be applicable to many other plant species. In addition, we expect the results with TRV to be applicable to a wide range of viral vectors, further broadening the range of plant species to which this technology can apply.

[00131] TRV is a bipartite positive-sense single stranded RNA virus consisting of two components: TRV1 and TRV2 (E. E. Ellison, Chamness, and Voytas 2021). TRV1 contains the RNA-dependent RNA polymerase (RdRP) which replicates both TRV1 and TRV2 and expresses sub genomic RNAs of other coding regions. TRV1 also encodes the movement protein (MP), responsible for enabling cell-to-cell movement, and the viral suppressor of RNA silencing (VSR), responsible for suppression of the host immune response. TRV2 contains the coat protein (CP) and can be modified for expression of heterologous sequences. The native TRV2 contains coding sequences to enable transmission between plants, which are removed in the vector. Heterologous sequences are expressed from a sub genomic promoter.

[00132] Cloning of the viral vectors

[00133] We will take advantage of what we learned in the previous Examples to reengineer TRV2 to express the TnpB and the ωRNA. In one instance we will drive the expression of the single expression cassette, containing the TnpB and ωRNA-spacer, using the sub genomic pPEBV promoter (FIG. 9A). To test the split system, where the TnpB and ωRNA are driven by separate promoters, one example will be to express the TnpB protein coding sequence in-frame with the CP sequence, separated by a (P or T)2A sequence, with the ωRNA being driven by the sub genomic pPEBV promoter (FIG. 9B) (Tian et al. 2014). We will also test the strategy of delivering multiple TRV2 vectors for editing capabilities. For example, placing pPEBV:TnpB on one TRV2 and the pPEBV:ωRNA on a second TRV2 vector, and simultaneously delivering them using the methods described above. These construct designs will be tested using the most efficient TnpBs identified in the previous Examples.

[00134] Movement sequences such as tRNA-Ile (SEQ ID NO: 148) will be used to improve the movement of the genome editing reagents (Liu et al. 2022; Nagalakshmi et al. 2022; Evan E. Ellison et al. 2020).

[00135] We expect 3' processing of the ωRNA-spacer sequence to greatly influence the editing capabilities of these TnpB systems in viral vectors. As such, we will test a variety of RNA processing sequences downstream of the spacer, such as HDV ribozyme, tRNA-Ile, and the tandem repeat ωRNA (FIG. 9C and 9D).

[00136] Evaluating viral vectors for somatic and germ cell editing

[00137] TRV1 and TRV2 vectors will be delivered to Arabidopsis plants using previously described approaches such as syringe infiltration of leaves, agro-pricking, and agro-flooding methods (Nagalakshmi et al. 2022).

[00138] Somatic editing will be quantified using methods similar to those described in Example 8. Briefly, two approaches will be used: deep amplicon NGS, and visually via the photobleaching phenotype caused by a bi-allelic frameshift mutation in the AtPDS3 gene (AT4G14210).

[00139] Heritable germline editing will also be assayed in the offspring generations using methods similar to those described in Example 8.

[00140] Example 12 – Utilizing TnpBs for genome editing in plant cells

[00141] This Example describes experimental guidelines for constructing and testing vectors expressing the TnpB protein and the associated ωRNA. These vectors were used to modify plant genomic DNA in *Arabidopsis* mesophyll protoplast cells. This example is related to the prophetic Example 8, and the experiments were performed as described in that example.

[00142] Creating vectors for TnpB-mediated genome editing

[00143] Three TnpBs capable of targeted mutagenesis in bacteria and human cells were selected for testing in plants: ISDra2, ISYmu1, and ISAam1 (Xiang et al. 2023; Karvelis et al. 2021). In all previous reports of TnpB-mediated genome editing, the TnpB protein coding sequences have been separated from the DNA sequence encoding the ωRNA, resulting in separate expression cassettes. However, in the natural host genomes of the TnpBs, the ωRNA overlaps with or is directly downstream of the TnpB sequence, as revealed by metagenomic data in Example 1 and publicly available genomic data (FIG. 7A). Here it is demonstrated that targeted genome editing in plant cells is achieved by TnpB systems encoded in the single expression cassette format. The native DNA sequence encoding the TnpB and the ωRNA was cloned into a single expression cassette, driven by the UBQ10 promoter, followed by the desired spacer sequence, HDV ribozyme RNA processing sequence, and rbcS-E9 terminator

on a binary vector, as depicted in FIG. 7B (Tables 4 and 5 below). For each TnpB, gRNA sequences targeting the Arabidopsis PDS3 gene (AT4G14210) were designed according to their Transposon associated motif (TAM) sequence (Table 6).

[00144] Testing TnpBs for editing in protoplast cells

[00145] The constructs described above were tested in *Arabidopsis* mesophyll protoplast cells using the previously reported method (Yoo, Cho, and Sheen 2007). Protoplast cells were subjected to a 37°C heat shock treatment for 2 hours at 16 hours post transfection. At 48 hours post transfection, protoplasts were harvested for genomic DNA extraction and analysis for targeted mutagenesis using Next Generation Sequencing (NGS). Editing frequencies of up to 13.34% (experiment_3_g2) for ISYmu1, 11.76% (experiment_2_g9) for ISDra2, and 0.31% (g17) for ISAam1. (FIG. 10, Table 7) were observed.

[00146] Example 13 - Identifying unpublished TnpBs for genome editing in plant cells

[00147] This Example describes experimental guidelines for testing vectors expressing the TnpB protein and the associated ωRNA. These vectors were used to modify plant genomic DNA in protoplast cells.

[00148] Identification of novel TnpBs capable of targeted editing in plant cells

[00149] 21 novel TnpBs were identified using the pipeline described in Example 1. Using the same approach described above, the 21 TnpBs targeting the PDS3 gene (AT4G14210) were cloned into individual binary vectors (Table 8 (SEQ ID NOs: 150-170) and Table 9 (SEQ ID NOs: 171-358). Each vector was transfected into Arabidopsis mesophyll protoplast cells. Protoplast cells were subjected to a 37°C heat shock treatment for 2 hours at 16 hours post transfection. At 48 hours post transfection, protoplasts were harvested for genomic DNA extraction and analysis for targeted mutagenesis using NGS. Of the 21 TnpBs tested, 14 TnpBs displayed little to no editing activity, with an average single target site editing frequency less than 0.1% (Table 7); Seven TnpBs exhibited higher activity, capable of editing an average greater than 0.1% for at least one target site (FIG. 11, Table 7). Notably, TnpB_20, TnpB_25, TnpB_15 and TnpB_21 reached up to an editing frequency of 0.53%, 0.57%, 0.83, and 1.36% at an individual target site, respectively. (FIG. 11, Table 7).

[00150] Example 14 - ωRNA engineering for improved TnpB editing efficiency

[00151] This Example describes exemplary experimental guidelines for testing vectors expressing the TnpB protein and the associated ωRNA.

[00152] ωRNA engineering for increased editing efficiency

[00153] It was recently demonstrated that RNA engineering of the ISDra2 ωRNA is a strategy for improving the TnpBs ability to edit genomic DNA in mammalian cells (Li et al.

2024). In the single cassette architecture, TnpB and ωRNA are co-expressed as a single transcript where sometimes there is an overlapping region between the C-terminus of TnpB coding sequences (CDS) and the 5'-end of the ωRNA. For example, the ISDra2 ωRNA scaffold sequence overlaps with the TnpB CDS except for 7 nucleotides. Such overlap between the CDS and ωRNA introduces additional complexity and difficulty for RNA engineering, i.e. changing ωRNA sequences can also interfere with the CDS. To test for ωRNA engineering potential in plant cells, ISBag01 and ISYmu1 TnpB systems were selected, with 32-nt overlap and no overlap between TnpB CDS and ωRNA, respectively.

[00154] Small RNA-seq and RNA folding analysis on ISYmu1 and ISBag01 ωRNA suggests a three stem-loop (127-nt) and a five stem-loop (186-nt) ωRNA architecture, respectively (FIG. 12). The three stem-loop architectures of ISYmu1 ωRNA resembles the ISDra2 ωRNA (Sasnauskas et al. 2023; Nakagawa et al. 2023), in which the apical loop of stem-loop 1 forms pseudoknot interactions with the 3'-end of the ωRNA scaffold. Compared to ISDra2 and ISYmu1, the ωRNA of ISBag01 bears two additional stem-loops at the 5'-end forming an RNA structure comprised of five stem-loops, where the pseudoknot interactions are still preserved at similar positions. Given the fact that the ωRNA architectures of ISYmu1 and ISBag01 are similar to ISDra2 and their evolutionary descendant enzyme CRISPR-Cas12f which has been structurally characterized and engineered (Sasnauskas et al. 2023; Nakagawa et al. 2023; Takeda et al. 2021; Hino et al. 2023; Kim et al. 2021), we plan to explore ωRNA engineering of ISYmu1 and ISBag01 for improved editing capabilities.

[00155] There are two primary strategies for RNA engineering for TnpB and CRISPR-Cas12f: stem truncation and base-pair substitution. For stem truncation, it has been shown that trimming or deleting RNA stems that are in structurally disordered regions and/or not interacting with proteins can improve the editing activities for ISDra2 TnpB and CRISPR-Cas12f (Nakagawa et al. 2023; Li et al. 2024; Kim et al. 2021). For base-pair substitution, it has been shown that replacing certain A-U or G-U base pairs with G-C base pairs at certain stems can stabilize the RNA folding and also improve the editing activities for CRISPR-Cas12f (Kong et al. 2023). Moreover, base-pair substitution has been applied to silent internal premature expression terminator signals (i.e. an internal penta-uridinylate region under U6 promoter) (Kim et al. 2021; Xu et al. 2021). In addition to the above two strategies, additional strategies are sought for ωRNA engineering including stabilizing pseudoknot interactions and RNA internal loop engineering (nucleotide insertions/deletions at the RNA internal loop). For ISYmu1, five groups of ωRNA variants were designed: (1) stem-loop 3 truncation (2) stem-loop 2 truncation (3) stem-loop 2 internal-loop insertion/deletion (4) stem-loop 1 base-pair

substitution and (5) PK base-pair substitution. For ISBag01, five groups of ωRNA variants were designed: (1) stem-loop 5 truncation (2) stem-loop 4 truncation (3) stem-loop 4 internal-loop insertion/deletion (4) stem-loop 3 base-pair substitution and (5) stem-loop 2 truncation. All sequences for the engineered ωRNA variants are listed in Table 10 (SEQ ID NOs: 359-430). To further increase editing capabilities using the ωRNA variants, stacking of ωRNA mutations that improve editing will also be explored.

[00156] Of the ωRNA variants described above, two of the ISBag01 variants have thus far been tested in protoplasts. For ISBag01, variants 1.1 and 1.2 were tested by trimming stem-loop 5 (FIG. 13A and Table 11 (SEQ ID NOs: 431-436)). These TnpB and ωRNA sequences were expressed as described in the above examples, with a single promoter and terminator used to express the overlapping TnpB-ωRNA sequence and a 16bp spacer sequence (g1) targeting the PDS3 gene (AT4G14210). A significant increase in editing efficiency was observed for the ISBag01 TnpB with ωRNA variant 1.1 (3.31% editing) compared to the ISBag01 TnpB with the native ωRNA sequence (2.17% editing), as depicted in FIG. 13B.

[00157] Example 15 - TnpB-mediated genome editing using TRV viral vectors in Arabidopsis protoplast cells

[00158] As outlined in Example 11, viral vectors for TnpB-mediated genome editing of somatic and germ cells in plant species, such as *Arabidopsis* and major crop species are to be developed. It is expected the 5' and 3' processing of the ωRNA-spacer sequence greatly influence the editing capabilities of TnpBs in viral vectors. To test the impact of HDV ribozyme, tRNA-Ile, or the tandem repeat ωRNAs on ωRNA processing and ultimately TnpB mediated genome editing, these sequences were encoded along with the ISYmu1 TnpB, ωRNA, and gRNA 2 as the TRV2 cargo as depicted in FIGS. 9A, 9C, and 9D, and Table 12 (SEQ ID NOs: 437-474).

[00159] Each TRV2 plasmid was co-transfected into *Arabidopsis* mesophyll protoplast cells together with the TRV1 plasmid. Protoplast cells were subjected to a 37°C heat shock treatment for 2 hours at 16 hours post transfection. At 48 hours post transfection, protoplasts were harvested for genomic DNA extraction and analysis for targeted mutagenesis using NGS. Detectable editing was observed in all three configurations tested, with a similar trend in both experiments one and two. An average 0.15% and 1.09% editing using the ISYmu1-g2-tRNA–Isoleucine configuration, and 0.84% and 1.61% editing using the ISYmu1-g2-tandem-200bp-wRNA–tRNA–Isoleucine configuration (FIG. 14) was observed. The highest editing, 4.93% and 11.61% using the ISYmu1-g2-HDV–tRNA–Isoleucine sequence, likely due to the HDV ribozyme's highly efficient RNA processing, resulting in a precise 3' end of the spacer

sequence (FIG. 14) was observed. Without being bound by any particular theory, it is possible that the use of the HDV ribozyme in TRV delivery to whole plants such as *Arabidopsis* seedlings may not result in as efficient editing as in the transfected protoplast cells due to the highly efficient RNA processing of the HDV ribozyme sequence, which may hinder the TRV's ability to replicate and spread throughout the plant.

[00160] Therefore, to further optimize this system for the successful viral delivery in plants, strategies will be explored to precisely process the 3' spacer and second ωRNA junction, without preventing the virus's ability to replicate and spread from cell-to-cell. The initial experiment using ISYmu1-g2-tandem-200bp-wRNA–tRNA–Isoleucine contained a 200 nucleotide second ωRNA. The optimal length of the second ωRNA in the ISYmu1-g2-tandem ωRNA–tRNA–Isoleucine configuration will be identified to create a precisely processed end at the 3' end of the first spacer sequence. A screen will be performed using the same ISYmu1-g2-tandem ωRNA–tRNA–Isoleucine design, but with varying lengths for the second ωRNA, ranging from 100-250 nucleotides in increments of ten nucleotides (Table 12). Based on ISYmu1 small RNA-seq data in E.coli, it is suspected that 127 nucleotides may be an optimal length for the second ωRNA, which will also be tested (FIG. 12A). Once the range with highest editing efficiencies is found, all ωRNA sequence lengths in that range to identify the optimal length of the second ωRNA sequence will be tested.

[00161] Example 16 – TnpB-mediated editing in transgenic plants

[00162] This Example describes exemplary experimental guidelines for constructing vectors to express the TnpB protein and the associated ωRNA in transgenic plants by *Agrobacterium* transformation, as well as testing the corresponding editing outcomes in these transgenic plants. This example is related to Examples 5, 8, and 9.

[00163] TnpBs for gene editing in transgenic plants

[00164] Because *Agrobacterium* mediated transformation is still widely used to insert gene editing systems into plant genomes to generate desired edits, the ability of TnpBs to edit in transgenic plants is an important approach for the creation of novel genetic materials. To evaluate TnpB-mediated editing in transgenic plants, the two TnpBs, ISDra2, and ISYmu1, demonstrating the highest editing efficiency in *Arabidopsis* mesophyll protoplast cells were selected (FIG. 10). The native DNA sequence encoding the TnpB and the ωRNA were cloned into a single expression cassette (SEQ ID NOs: 140 and 143), driven by the UBQ10 promoter (SEQ ID NO: 132), followed by the desired spacer sequence (SEQ ID NOs: 55, 65, 121, 123), HDV ribozyme RNA processing sequence (SEQ ID NO: 137), and rbcS-E9 terminator (SEQ

ID NO: 135) on a binary vector, as depicted in Figure 7B (Tables 4, 5, and 6) and used floral dipped according to Zhang et al. (Zhang et al. 2006).

[00165] Following floral dip, the T1 seeds were harvested and subjected to hygromycin selection on ½ MS plates to select for transgenic plants. Seedlings that passed selection were then transplanted to soil and grown at room temperature for three weeks. Next, leaf tissue samples were randomly collected from each transgenic plant and editing efficiencies were quantified by amplicon sequencing using Illumina next generation sequencing (NGS). Editing in leaf tissue for ISDRa2 (g9 and g12) and ISYmu1 (g2 and g12) was observed. ISDra2 g9 demonstrated an average editing frequency in wild type (WT) of 0.32% and in *rdr6* of 0.04% (FIG. 15A). ISDra2 g12 demonstrated an average editing frequency of 0.28% and 0.37% in wild type and *rdr6*, respectively (FIG. 15B). For ISYmu1 g2 we observed an editing frequency in wild type of 0.14%, and in *rdr6* of 4.59% (FIG. 15C). ISYmu1 g12 demonstrated a very high editing frequency in wildtype (48.96%) and in *rdr6* (79.73%) (FIG. 15D). Furthermore, analysis of the repair profiles for ISDra2 g9 and ISYmu1 g12 revealed deletion-dominant repair outcomes (FIGS. 15E-15G).

[00166] Example 17 - TnpB-mediated editing via delivery of a TRV vector to whole plants

[00167] This Example describes exemplary experimental guidelines for constructing vectors to express the TnpB protein and the associated ωRNA via the Tobacco Rattle Virus (TRV) delivery. These vectors were used to edit plant DNA in whole plants. This example is related to the previously filed Examples 6, 11 and 15.

[00168] Here, TRV vectors for TnpB-mediated genome editing of *Arabidopsis* were designed. Two different viral vector configurations were cloned into the TRV "cargo" site for testing: 1. ISYmu1-g2-tRNA–Isoleucine (SEQ ID NO: 456) and 2. ISYmu1-g2-HDV–tRNA–Isoleucine (SEQ ID NO: 457), as shown in FIGS. 9A and 9C, respectively (Table 12).

[00169] Each TRV2 plasmid was co-delivered to wild type or *rdr6 Arabidopsis* seedlings together with the TRV1 plasmid at 9 days post germination using the agroflood method (Nagalakshmi et al. 2022). After four days of agroflood co-culture, seedlings were transplanted to soil and grown for 18 days. Next, leaf tissue samples were randomly sampled from each plant for genomic DNA extraction and analysis for targeted mutagenesis using Illumina sequencing. Editing was detected in both vector configurations tested. An average 0.03% and 0.12% editing efficiency using the ISYmu1-g2-tRNA–Isoleucine configuration in the wild type and *rdr6* samples, respectively (FIG. 16A) was observed. Notably, editing efficiency up to 1.26% in the *rdr6* genotype (FIG. 16A) was observed. Additionally, an average of 0.29%

and 0.28% using the ISYmu1-g2-HDV–tRNA–Isoleucine configuration in wild type and *rdr6*, respectively (FIG. 16B) was observed. Notably, moderately high editing efficiency up to 5.54% and 8.93% in the wild type and *rdr6* samples, respectively (FIG. 16B) was observed. Additionally, a deletion-dominant indel profile, similar to the transgenic T1 data (FIGS. 16C-16D) was observed. The editing target of these experiments is the *PDS3* gene. When both copies of the *PDS3* gene are mutated (biallelic edits), the mutated plant cells are white rather than green. On several of the plants infected with either of these TRV vectors white sectors were observed, indicating biallelic editing of *PDS3* in these areas of the plant (FIG. 16E). The efficiency of editing observed in these experiments by amplicon sequencing, together with the appearance of white sectors on the plants, suggests that editing of the *PDS3* gene will likely occur in some of the germ cells resulting in germ line transmission of edits. This will be confirmed in the next generation by growing plants and looking for white seedlings that contain biallelic edits in the *PDS3* gene.

[00170] Example 18 - Investigating the activities of novel TnpBs in bacteria

[00171] This Example describes exemplary experimental guidelines for constructing vectors to express the TnpB protein and the associated ωRNA in bacteria. These vectors were used to test the activities of novel TnpB proteins in bacteria.

[00172] Thirty novel TnpBs were identified using either the DART approach outlined in Examples 1 and 13 or a protein homology-based search, including seven new examples of TnpBs (Table 13 below). A subset of these TnpBs was confirmed for their plasmid interference activities in bacteria. The native DNA sequence encoding the TnpB and the ωRNA was cloned into a single expression cassette (bearing a CmR gene) driven by the TetR promoter (FIG. 17A), followed by the desired spacer sequence targeting another vector bearing an AmpR gene (FIG. 17A). Both vectors were co-transformed into E.coli, which were then subjected to serial dilution and grown on plates with either the single antibiotic (Cam) or double antibiotic (Cam and Amp). The experiments were performed at 26°C. The activities of TnpB were calculated by the ratio of colony-forming units (normalized CFU) between the single antibiotic plate and the double antibiotic plate. An active TnpB will target the AmpR vector resulting in plasmid interference and reduced normalized CFU. The normalized CFU for each TnpB were also compared under conditions with and without the predicted TAM sequences on the AmpR vector. We tested the plasmid interference activities of five selected TnpBs (TnpB_5, TnpB_15, TnpB_19, TnpB_21 and TnpB_30) and observed the highest activities in TnpB_30 in bacteria (FIG. 17B).

[00173] The TAM of TnpB_30 was further confirmed by a TAM depletion assay as the "AGGAG" motif (FIG. 17C). Small RNA-seq of TnpB_30 (FIG. 17D) revealed a ωRNA scaffold size of 138 nucleotides, consisting of a three stem-loop architecture (Fig. 17E).

[00174] Example 19 - Identifying novel TnpBs for genome editing in plant cells

[00175] This Example describes exemplary experimental guidelines for testing vectors expressing the TnpB protein and the associated ωRNA. These vectors were used to modify plant genomic DNA in protoplast cells. It is expected that these results translate to eukaryotic species other than those being tested in this Example.

[00176] Seven novel TnpBs for genome editing capabilities in plant cells (Table 13) were identified and tested. Using the same approach outlined in previous Examples, the native DNA sequence encoding the TnpB and the ωRNA was cloned into a single expression cassette, driven by the UBQl0 promoter (SEQ ID NO: 132), followed by the desired spacer sequence, HDV ribozyme RNA processing sequence (SEQ ID NO: 137), and rbcS-E9 terminator (SEQ ID NO: 135) on a binary vector, as depicted in (FIG. 7B, Tables 13 and 14). Each vector was transfected into *Arabidopsis* mesophyll protoplast cells. Protoplast cells were subjected to a 37°C heat shock treatment for 2 hours at 16 hours post transfection. At 48 hours post transfection, protoplasts were harvested for genomic DNA extraction and targeted mutagenesis analysis using amplicon sequencing. Of the seven TnpBs tested, six TnpBs displayed little to no editing activity, with all target sites averaging less than 0.1% editing efficiency (Table 15). Notably, TnpB_30, the most active novel TnpB in bacteria in Example 18, generated edits at an average 0.45% across nine target sites, reaching up to and average of 1.2% at target site g2 (FIG. 18, Table 15).

[00177] Example 20 - The spacer length for ISYmu1, ISDra2, ISBag01, and TnpB_30 can impact editing efficacy

[00178] This Example describes exemplary experimental guidelines for testing vectors expressing the TnpB protein and the associated ωRNA. These vectors were used to modify plant genomic DNA in protoplast cells. It is expected that these results translate to eukaryotic species other than those being tested in this Example.

[00179] It has been shown in Cas9 and Cas12s that the spacer sequence length used for targeted genome editing can have a major impact on nuclease efficacy. Therefore, we tested different spacer lengths for the TnpBs ISYmu1 (SEQ ID NO: 140), ISDra2 (SEQ ID NO: 143), ISBag01 (SEQ ID NO: 14), and TnpB_30 (SEQ ID NO: 481). Using the same single expression cassette described in previous Examples, we cloned plasmids using a single expression cassette, driven by the UBQl0 promoter (SEQ ID NO: 132), followed by the desired spacer

sequence length (ranging from 14-20 nucleotides for ISYmu1, ISDra2, and ISBag01; for TnpB_30 we tested spacer lengths ranging from 13-21 nucleotides (Table 16)), HDV ribozyme RNA processing sequence (SEQ ID NO: 137), and rbcS-E9 terminator (SEQ ID NO: 135) as depicted in FIG. 7B.

[00180] Each vector was transfected into *Arabidopsis* mesophyll protoplast cells. Protoplast cells were subjected to a 37°C heat shock treatment for 2 hours at 16 hours post transfection. At 48 hours post transfection, protoplasts were harvested for genomic DNA extraction and targeted mutagenesis analysis using amplicon sequencing. Analysis of the editing frequencies revealed very little difference between the spacer lengths for ISDra2 (FIG. 19A). ISYmu1 displayed a preference for spacer lengths of 16 and 19 nucleotides, with 16 being the optimal length (FIG. 19B). ISBag01 appeared to have a strong preference for a 16 nucleotide spacer length (FIG. 19C). TnpB_30 showed increased editing for spacer lengths greater than 15 nucleotides, with 19 nucleotides demonstrating the highest editing efficiency (FIG. 19D). In summary, these data indicate the spacer length can influence TnpB-mediated genome editing efficacy.

[00181] Example 21 - Expression of TnpB-ωRNA using a single promoter for optimal TnpB-mediated genome editing

[00182] This Example describes exemplary experimental guidelines for constructing and testing vectors expressing the TnpB protein and the associated ωRNA in a single expression cassette, or a split expression cassette design. These vectors may be used to modify plant genomic DNA in protoplasts, whole plants and to generate heritable edits in genomic sequences using similar methods as described in other Examples in this patent. It is expected that these results translate to eukaryotic species, and TnpBs, other than those being tested in this Example. This Example is related to Example 9.

[00183] In all previous reports of TnpB-mediated genome editing the TnpB has been separated from the ωRNA, resulting in separate expression cassettes. However, in nature, the ωRNA is directly downstream of, or overlaps with, the TnpB sequence (FIG. 7 A). We hypothesized the single expression cassette will work as efficiently or more efficiently than the split systems described in the literature. To compare the single expression cassette with the split expression cassette we cloned the TnpB and ωRNA into a single expression cassette (SEQ ID NO: 140) on a binary vector (FIG. 20A). The single expression cassette contained a pol II promoter (such as UBQ 10 (SEQ ID NO: 132)), TnpB coding sequence, ωRNA sequence, spacer sequence, HDV ribozyme RNA processing sequence (SEQ ID NO: 137), and terminator (such as rbcS-E9 (SEQ ID NO: 135)) (FIG. 20A). As a comparison, we created split designs

where the TnpB coding sequence is driven by a Pol II promoter (such as UBQ10 (SEQ ID NO: 132)) and the ωRNA sequence, spacer sequence, and HDV ribozyme (SEQ ID NO: 137) sequence are under the expression of a pol III U6 promoter (SEQ ID NO: 134) (FIG. 20A). In total 11 split design plasmids were designed, with the ωRNA length varying from 100-250 nucleotides (FIG. 20A, Table 17). Based on small RNA-seq performed in E.coli (Example 14 and FIG. 20B), we hypothesized the ωRNA is processed at 127 nucleotides. Therefore, we included a split design with 127 nucleotides ωRNA (Table 17).

[00184] The single versus split expression configurations using ISYmu1 g2 (SEQ ID NOs: 140 and 55, Table 17) was tested, however, it is expected these results translate to other TnpBs and target sites. Each vector was transfected into *Arabidopsis* mesophyll protoplast cells. Protoplast cells were subjected to a 37°C heat shock treatment for 2 hours at 16 hours post transfection. At 48 hours post transfection, protoplasts were harvested for genomic DNA extraction and targeted mutagenesis analysis using amplicon sequencing.

[00185] Analysis of the amplicon sequencing data revealed the single expression cassette to be the top performer at 0.70% editing efficiency (FIG. 20C). By comparison, all of the split designs demonstrated much lower editing efficiency, ranging from 0.01-0.23% for the 150 and 127 nucleotides ωRNA, respectively (FIG. 20C). As expected, the 127 nucleotides ωRNA in the split design generated the highest editing efficiency out of all of the split designs tested, indicating the proper length and processing of the ωRNA is critical for TnpB editing efficacy. In summary, we demonstrated that the single expression cassette is an optimal configuration to express the ISYmu1 and wRNA sequences for TnpB-mediated genome editing. We expect these results to translate to other TnpBs and eukaryotic species.

[00186] Example 22 - ISYmu1 ωRNA engineering for improved plant genome editing

[00187] This Example describes exemplary experimental guidelines for testing vectors expressing the TnpB protein and the associated ωRNA. This Example is related to Example 14. It is expected that these results translate to TnpBs and eukaryotic species other than those being tested in this Example.

[00188] It was recently demonstrated that RNA engineering of the ISDra2 ωRNA is a strategy for improving the TnpB's ability to edit genomic DNA in mammalian cells (Li et al. 2024). In the single cassette architecture, the TnpB and ωRNA are co-expressed as a single transcript where sometimes there is an overlapping region between the C-terminus of TnpB coding sequences (CDS) and the 5'-end of the ωRNA. For example, the ISDra2 ωRNA scaffold sequence overlaps with the TnpB CDS except for 7 nucleotides. Such overlap between

the CDS and ωRNA introduces additional complexity and difficulty for RNA engineering, i.e. changing ωRNA sequences can also interfere with the CDS.

[00189] To test for ωRNA engineering potential in plant cells, 32 unique ISYmu1 ωRNA variants were cloned, as outlined in Example 14, into individual binary vectors using the single expression cassette design (Table 10). Each vector was transfected into *Arabidopsis* mesophyll protoplast cells. Protoplast cells were subjected to a 37°C heat shock treatment for 2 hours at 16 hours post-transfection. At 48 hours post-transfection, protoplasts were harvested for genomic DNA extraction and targeted mutagenesis analysis using amplicon sequencing. We observed three trends in the ωRNA variants relative to the wild type (WT) ωRNA: (1) increased editing frequency (ωRNA variants v2.1, v3.2, v3.3, v3.4, v3.7, v3.10, v3.11, v3.16, v1.1, and v5.2), (2) decreased editing frequency (ωRNA variants v2.2, v3.1, v3.6, v3.9, v3.12, v3.13, v3.14, v3.15, v1.2, v1.3, v1.4, v1.5, v1.6, v2.4, v4.1, v4.2, v5.1, v5.3, and v5.4), or (3) similar editing frequency (v2.3, v3.5, v3.7, and v3.8) (FIG. 21). Notably we observed 2.1, 1.63, 1.38-fold increased editing for ωRNA variants v3.2, v3.16, and v2.1, respectively (FIG. 21).

[00190] RNA secondary structure analysis revealed that v3.2 features an S1S1 rather than an S2S1 two-way junction topology at lower stem-loop 2; v3.16 features an S2S0 rather than an S1S0 two-way junction topology at the middle of stem-loop 2; and v2.1 features stem truncation at upper stem-loop 2 (FIG. 22). Since modulation of RNA two-way junctions in stem-loop 2, as seen with v3.2, enhances genome editing activities the most, we hypothesize that maintaining similar topologies in stem-loop 2 are critical for ISYmu1 TnpB activity. Consequently, 9 additional variants based on the ISYmu1 ωRNA variant v3.2 with a new mutation (Table 18) were designed. Furthermore, as indicated in Example 14, we plan to combine ωRNA variant mutations to test for improved editing. Stacked ωRNA variants using combinations of v3.2, v3.16, v3.4, v5.2, and/or v2.1 to evaluate enhanced ISYmu1 editing capabilities (Table 18) have been designed. It is anticipated that further exploration of ωRNA engineering variants and stacking will significantly improve ISYmu1 genome editing efficacy.

[00191] Example 23 - Highly efficient ISYmu1-mediated somatic editing in transgenic Arabidopsis

[00192] This Example describes exemplary experimental guidelines for constructing vectors to express the TnpB protein and the associated ωRNA in transgenic plants by Agrobacterium transformation, as well as testing the corresponding editing outcomes in these transgenic plants. This Example is related to Example 16.

[00193] Because Agrobacterium mediated transformation is still widely used to insert gene editing systems into plant genomes to generate targeted edits, the ability of TnpBs to edit in

transgenic plants is an important approach for the creation of novel genetic materials. To evaluate TnpB-mediated editing in transgenic plants, ISYmu1 g2 and ISYmu1 g12 were selected. The native DNA sequence encoding the TnpB and the ωRNA were cloned into a single expression cassette (SEQ ID NO: 140), driven by the UBQ 10 promoter (SEQ ID NO: 132), followed by the desired spacer sequence (SEQ ID NOs: 55 and 65), HDV ribozyme RNA processing sequence (SEQ ID NO: 137), and rbcS-E9 terminator (SEQ ID NO: 135) on a binary vector, as depicted in FIG. 7B, and used for floral dipped according to Zhang et al. 2006. Floral dip was performed using wild type (WT) and RNA-DEPENDENT RNA POLYMERASE 6 (*rdr6*) genotypes, as *rdr6* has been shown to have reduced transgene silencing.

[00194] Following floral dip, the Tl seeds were harvested and subjected to hygromycin selection on ½ MS plates to select for transgenic plants. Seedlings that passed selection were then transplanted to soil and grown at room temperature for one week. After one week, plants continued to grow at room temperature for two more weeks; however, plants that underwent heat shock treatment were exposed to 8 hours of heat exposure at 37°C every day for 5 days, followed by 2 days of recovery at room temperature. This heat shock regime lasted for two weeks. Next, leaf tissue samples were randomly collected from each transgenic plant and editing efficiencies were quantified by amplicon sequencing using Illumina next generation sequencing (NGS). Editing in leaf tissue for ISYmu1 g2 and ISYmu1 g12 was detected. For ISYmul g2 we observed an average editing frequency in WT of 1.56%, and 2.49% in *rdr6* for the room temperature samples (FIG. 23A). ISYmul g12 demonstrated a high editing frequency in WT (44.86%) and in *rdr6* (75.55%) for the room temperature grown plants (FIG. 23B). Analysis of editing efficiency in the heat shock treatment samples revealed increased editing at ISYmu1 g2 target site in both WT and *rdr6*, averaging 9.81% and 32.47%, respectively (FIG. 23A). The ISYmu1 g12 plants that underwent the heat shock treatment showed increased editing in WT, averaging 63.73%; whereas the *rdr6* samples saw no difference in editing efficiency, averaging 75.43% (FIG. 23B).

[00195] Example 24 - Efficient ISDra2-mediated somatic editing in transgenic *Arabidopsis* requires heat shock treatment

[00196] This Example describes exemplary experimental guidelines for constructing vectors to express the TnpB protein and the associated ωRNA in transgenic plants by Agrobacterium transformation, as well as testing the corresponding editing outcomes in these transgenic plants. This Example is related to Examples 16.

[00197] Because Agrobacterium mediated transformation is still widely used to insert gene editing systems into plant genomes to generate targeted edits, the ability of TnpBs to edit in

transgenic plants is an important approach for the creation of novel genetic materials. To evaluate TnpB-mediated editing in transgenic plants, ISDra2 g9 and ISDra2 g12 were selected. The native DNA sequence encoding the TnpB and the ωRNA were cloned into a single expression cassette (SEQ ID NO: 143), driven by the UBQ IO promoter (SEQ ID NO: 132), followed by the desired spacer sequence (SEQ ID NOs: 121 and 123), HDV ribozyme RNA processing sequence (SEQ ID NO: 137), and rbcS-E9 terminator (SEQ ID NO: 135) on a binary vector, as depicted in FIG. 7B 7B, and used for floral dipped according to Zhang et al. 2006. Floral dip was performed using wild type (WT) and RNA-DEPENDENT RNA POLYMERASE 6 (*rdr6*) genotypes, as *rdr6* has been shown to have reduced transgene silencing.

[00198] Following floral dip, the Tl seeds were harvested and subjected to hygromycin selection on ½ MS plates to select for transgenic plants. Seedlings that passed selection were then transplanted to soil and grown at room temperature for one week. After one week, room temperature plants continued to grow at room temperature for two more weeks; however, plants that underwent heat shock treatment were exposed to 8 hours of heat exposure at 37°C every day for 5 days, followed by 2 days of recovery at room temperature. This heat shock regime lasted for two weeks. Next, leaf tissue samples were randomly collected from each transgenic plant and editing efficiencies were quantified by amplicon sequencing using Illumina next generation sequencing (NGS). Editing in leaf tissue for ISDra2 g9 and ISDra2 gl2 was detected. For ISDra2 g9 we observed an average editing frequency in WT of 0.16%, and in *rdr6* of 0.10% for the room temperature samples (FIG. 24A). For ISDra2 g12 we observed an average editing frequency in WT of 7.62%, and in *rdr6* of 1.14% for the room temperature samples (FIG. 24B). Comparison of editing efficiency between room temperature and heat shock treatment revealed a substantial difference for both ISDra2 g9 and ISDra2 g12 (FIG. 24A-24B). For ISDra2 g9 we observed an average editing frequency in WT of 12.40%, and 41.20% in *rdr6* for the heat shock treatment samples (FIG. 24A). For ISDra2 g12 we observed an average editing frequency in WT of 18.92%, and 58.40% in *rdr6* for the heat shock treatment plants (FIG. 24B). These data indicate an elevated temperature treatment is critical for efficient plant gene editing using ISDra2.

[00199] Example 25 - Somatic editing of whole plants using TRV vectors expressing the ISYmu1 TnpB and ωRNA

[00200] This Example describes exemplary experimental guidelines for constructing vectors to express the TnpB protein and the associated ωRNA via Tobacco Rattle Virus (TRV)

delivery. These vectors were used to edit plant DNA in whole plants. This Example is related to the previously filed Example 17.

[00201] First, TRV vectors for TnpB-mediated genome editing in *Arabidopsis* were designed targeting ISYmu1 g2 target site. Three different viral vector configurations were cloned into the TRV "cargo" site for testing: (1) ISYmu1-g2-tRNA-Isoleucine (SEQ ID NO: 437), (2) ISYmu1-g2-HDV-tRNA-Isoleucine (SEQ ID NO: 438), and (3) ISYmu1-g2-tandem-200bp-wRNA-tRNA-Isoleucine (SEQ ID NO: 439) (FIG. 25A).

[00202] Each TRV2 plasmid was co-delivered with the TRV1 plasmid to wild type (WT), *ku70*, or RNA-DEPENDENT RNA POLYMERASE 6 (*rdr6*) *Arabidopsis* seedlings at 9 days post germination using the agroflood method (Nagalakshmi et al. 2022). Ku70 is a nonhomologous end joining DNA repair mutant. We hypothesized the *ku70* genetic background may show increased editing efficiency.

[00203] After four days of agroflood co-culture, seedlings were transplanted to soil and grown for 18-22 days. For the heat shock treatment, one week after seedlings were transplanted from agroflood plates to soil, they were exposed to 8 hours of heat exposure at 37°C every day for 5 days, followed by 2 days of recovery at room temperature. This heat shock regime lasted for two weeks (or until the plants started flowering). Next, three leaf tissue samples distal from the TRV delivery location were randomly collected and pooled from each individual plant for genomic DNA extraction and analysis for targeted mutagenesis using Illumina amplicon sequencing. Editing was detected in all three vector configurations tested.

[00204] An average 0.06%, 0.09%, and 0.02% editing efficiency using the ISYmu1-g2-tRNA-Isoleucine configuration in the WT, *rdr6*, and *ku70* samples, respectively, was observed for the room temperature treatment samples (FIG. 25B). An average 0.36%, 0.25%, and 0.68% editing efficiency using the ISYmu1-g2-tRNA-Isoleucine configuration in the WT, *rdr6*, and *ku70* samples, respectively, was observed for the heat shock treatment samples (FIG. 25B). Additionally, an average of 0.56%, 0.81%, and 1.96% editing efficiency using the ISYmu1-g2-HDV-tRNA-Isoleucine configuration in WT, *rdr6*, and *ku70*, respectively, was observed for the room temperature samples (FIG. 25B). An average of 3.29%, 0.16%, and 8.93% editing efficiency using the ISYmu1-g2-HDV-tRNA-Isoleucine configuration in WT, *rdr6*, and *ku70*, respectively, was observed for the heat shock treatment samples (FIG. 25B). Additionally, we detected editing using the ISYmu1-g2-tandem-200bp-wRNA-tRNA-Isoleucine TRV vector design. An average of 0.01% using the ISYmu1-g2-tandem-200bp-wRNA-tRNA-Isoleucine configuration in all three genotypes was observed for the room temperature samples (FIG. 25B). An average of 0.14%, 5.26%, and 0.17% using the ISYmu1-g2-tandem-200bp-wRNA-

tRNA-Isoleucine configuration in wild type, *rdr6*, and *ku70*, respectively, was observed for the heat shock treatment samples (Figure 26B).

[00205] Next we designed TRV vectors to target the ISYmu1 g12 target site. Here, the ISYmu1-g12-HDV-tRNA-Isoleucine TRV vector was used (Table 19). The TRV2 plasmid was co-delivered with TRV1 to WT or *rdr6 Arabidopsis* seedlings using the same agroflood methods described above (Nagalakshmi et al. 2022). Additionally, the plant growth and heat shock treatment was the same as described above. Next, three leaf tissue samples distal from the TRV delivery location were randomly collected and pooled from each individual plant for genomic DNA extraction and analysis for targeted mutagenesis using Illumina amplicon sequencing. Editing was detected in both genotypes tested. An average 8.51% and 13.54% editing efficiency using the ISYmu1-g12-HDV-tRNA-Isoleucine configuration in the WT and *rdr6* samples, respectively, was observed for the room temperature grown plants (FIG. 25C). Additionally, an average of 4.27% and 1.34% using the ISYmu1-g12-HDV-tRNA-Isoleucine configuration in WT and *rdr6*, respectively, was observed for the plants that underwent heat shock treatment (FIG. 25C). Further, 6 out of 57 WT plants displayed editing greater than 40%, with four of those being greater than 75%, for the ISYmu1-g12-HDV-tRNA-Isoleucine vector with room temperature treatment (FIG. 25C). A deletion dominant editing pattern for ISYmu1 g12 was observed, similar to the mutation profile generated using TRV with ISYmu1 g2 (FIG. 25D).

[00206] These results indicate that both ISYmu1 g2 and g12 are capable of somatic editing when delivered to whole plants using the TRV vector. Further, these data demonstrate that all three TRV vector designs are capable of targeted genome editing in plants. Additionally, we demonstrate the use of genetic mutants for improved TRV-mediated genome editing efficiency using ISYmu1. It is expected that these results translate to TnpBs, viruses, and eukaryotic species other than those tested in this Example.

[00207] Example 26 - Heritable editing using TRV vectors expressing the ISYmu1 TnpB and ωRNA in *Arabidopsis*

[00208] This Example describes exemplary experimental guidelines for constructing vectors to express the TnpB protein and the associated ωRNA via Tobacco Rattle Virus (TRV) delivery. These vectors were used to edit plant DNA in whole plants. This Example is related to the previously filed Example 17.

[00209] White sectors were observed on several of the plants infected with the TRV vectors in Example 17 and Example 25, indicating biallelic editing of the *PHYTOENE DESATURASE3* gene (*PDS3*) in these areas of the plant (FIG. 16E, FIG. 26A). To test for heritability of edited

alleles to the next generation, seeds were collected from a WT plant showing 54.54% somatic editing using the ISYmu1-g2-HDV-tRNA-Isoleucine TRV design with the heat shock treatment (FIG. 26A). In total, 2318 seeds were sown on ½ MS plates containing 3% sucrose. After 8-10 days 68 albino seedlings were observed, indicating biallelic mutations in the *PDS3* gene (FIG. 26B). To confirm that the *PDS3* gene is mutated we performed Sanger sequencing on the two white seedlings shown in FIG. 26B. Sanger sequencing revealed that both plants are homozygous for a 4bp deletion (FIG. 26C). To further characterize transmission of edited alleles, NGS was performed on 58 seedlings, of which 31 were albino. Analysis of NGS revealed biallelic mutations for all of the albino seedlings, with the majority of mutations being the 4bp deletion observed in FIG. 26C. Of the 27 green seedlings, two of them were heterozygous (WT/4bp del). These data indicate that TRV-mediated edits using ISYmu1 are heritable. We anticipate higher heritability rates for the highly edited plants targeting ISYmu1 g12 from Example 25. Further, we expect the approaches described in this Example will extend to other TnpBs, viral vectors, and plant species.

[00210] Example 27 - Efficient plasmid interference activities of TnpBs with matured ωRNA

[00211] This Example describes exemplary experimental guidelines for constructing vectors to express the TnpB protein and the associated ωRNA in bacteria. These vectors were used to test the activities of TnpB proteins with various mechanisms of ωRNA expression and processing.

[00212] Different expression cassettes for TnpB and ωRNA in bacteria were created as depicted in (FIG. 27A): (a) A single expression cassette for TnpB and ωRNA with the 3'-end of the guide uncapped; (b) A single expression cassette for TnpB and ωRNA with a 16-nt guide region with the 3'-end capped by HDV ribozyme; (c) A split expression cassette for TnpB and ωRNA, with the ωRNA scaffold length inferred from small RNA-seq experiments, a 16-nucleotide guide region with the 3'-end capped by HDV ribozyme; (d) A split expression cassette for TnpB and ωRNA, with the ωRNA scaffold truncated by 52 nucleotides at the 5'-end, a 16-nucleotide guide region with the 3'-end capped by HDV ribozyme; (e) A split expression cassette for TnpB and ωRNA, with the ωRNA scaffold extended by 73 nucleotides at the 5'-end, a 16-nucleotide guide region with the 3'-end capped by HDV ribozyme; (f) A single expression cassette for TnpB and ωRNA, with the 3'-end of the guide capped by another ωRNA scaffold of a size inferred from small RNA-seq experiments; and (g) A single expression cassette for TnpB and ωRNA, with the 3'-end of the guide capped by another ωRNA scaffold extended by 73 nucleotides at the 5'-end.

[00213] First, the importance of 3'-end processing of ωRNA for TnpB activity was assessed. TnpB was expressed from ISDra2, ISBag01, ISYmu1, and ISAam1 using cassette (a). Strong plasmid interference activities were observed for ISDra2 and ISBag01 but not for ISYmu1 and ISAam1 (FIG. 27B). When the HDV ribozyme was introduced at the 3'-end of the guide in cassette (b), the activities of ISYmu1 and ISAam1 were restored (FIG. 27C), indicating that the lack of 3'-end processing was responsible for their weak activities in cassette (a).

[00214] Next, the importance of 5'-end processing of ωRNA for TnpB's activities was examined. ISBag01 and ISYmu1 were expressed using the split cassette (c), where the 3'-end of the ωRNA was capped by the HDV ribozyme, resulting in efficient plasmid interference activities for both TnpBs (FIG. 27D and 27E). When the 5'-end of the ωRNA for ISBag01 was truncated by 52 nucleotides, plasmid interference activity diminished (FIG. 27D). Extending the 5'-end of the ωRNA for ISYmu1 by 73 nucleotides did not affect its activity (FIG. 27E). These findings suggest that the proper size of the ωRNA scaffold is crucial for TnpB activity and that 5'-end extension does not impact TnpB function, likely because the 5'-end can be processed into mature ωRNA by TnpB or by other host nucleases.

[00215] Utilizing these insights into ωRNA processing, a single cassette (f) was designed where the 16-nucleotide guide region was capped by an intact 127-nucleotide ωRNA scaffold from ISYmu1. This design demonstrated efficient plasmid interference activity (FIG. 27F). However, capping the 16-nucleotide guide region with an extended ωRNA scaffold resulted in diminished activity (FIG. 27F), suggesting inefficient 3'-end processing. The design of cassette (f) can potentially be leveraged for multiplex editing by alternating the intact 127-nucleotide ωRNA scaffold and guide region.

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Table 4: Vectors for TnpB-mediated genome editing

TnpB name	**TnpB with wRNA overlap Native DNA sequence (single)**	**SEQ ID NO:**
ISYmu1	ATGCTGCAGCATAAAGCCTATGAATACCGTATCTATCCAGATAAGA AGCAGGAAACACTGATTGCCAAGACAATAGGCAGTTCAAGGTATG TATACAACCATTTCCTGGAACTTTGGAACAAGGAATATGAGGAGAC CGGAAAAGGTCTGACTTATTACGCATGCTCCAAACTTCTGACAAAG CTTAAGAGGGACCCGGAAACAGTATGGCTTTGTGAAGTGGATAAGT TCTCGCTCCAGAATTCGCTTCGTAACCTGTCGGACGCCTTCTCCCGC TTTTTCAAAGGACAGAACGAGCATCCCCAGTTCAAGAGCAAGAAG AGCCCGAGGCAAAGCTACACGACACAATATACGAACAACAACATC GCAGTTTCCGGAAATTGTCTGAAGCTGCCTAAGCTGGGCCTTGTCA AGTTTGCAGACAGTAGGGAGATGAAAGGCCGTATACTGAATGCCA CAGTACGAAGGAAATCGAGCGGGAAATTCTTCGTATCGATCCTATG CGAAGAGGAGATCTGTGAATTGCCAAAGACTGACTCATCTGTCGGA ATTGACCTCGGAATCATTGACTTTGCGGTTATGTCAGACGGAAGCA GGCATGACAACAATCATTTTACCAGGCAAATGGAAGAAAGGCTTA GACGGGAGCAGCGAAAGCTTGCAAGGCGTGCACTTGCTGCGGAGA AAAGGGGCATTTCCCTTTCTGAAGCCAGGAACTATCAGAAGCAAAG GCGGAAGGTAGCGAGACTTTATGAAAAGGTTGCAAACCAGCGAAA AGAATATCTTAACAAACTCAGTACAGAGATAGTCAAAAACCACGAT ATCATCTGTATCGAGGACCTTAACGTTAAGGGCATGATGCGCAATC ATAAACTGGCAAAAAGCATCTCTGATGTATCATGGACGAGCCTTGT ATCGAAACTGCAGTACAAGGCTTCCTGGTATGGAAAAGAAGTCATC AGGATAAGCAGATGGTTTCCGTCAAGTCAGATATGCTCAGAGTGCG GCCACAAGGACAGGAAGAAGCCTCTCCATGTAAGGGAGTGGACCT GTCCTGTTTGTCATGCCCACCATGACCGTGACGTCAATGCAGCAAG AAATATACTGGCTGAGGGACTCAGGATAAGAGCTCTGACTCCGGGG TCTTAGCAATACAGAAGCAAACAGGAACCGCAGGAATTGCGGGGG	140

TnpB name	TnpB with wRNA overlap Native DNA sequence (single)	SEQ ID NO:
	TAGCTTGGTAAACAAGAGAAACCTCTGCCGGCAAAGAAATAAGCC GGTAAGTATGCTCTGTTCCCAAGAATCTCGTGACTTTAGTCATGAG AGTTTCAA	
ISAam1	ATGGTAAACAAATCATATAAATTCCGTCTGTACCCAACAAAAGAAC AAGAACAGCTGCTTGCCAAAACCTTCGGTTGTGTCCGTTTCGTTTAT AACAAAATGCTTGAAGAACGCATACAAATTTATGAAAAGTTCAAA GACGACAAAGAAGCTTTGAAAAAACAAACATTTCCGACCCCTGCCA AGTACAAAAAGGAGTTTCCTTGGCTTAAAGAAGTGGATAGCCTTGC GTTAGCAAACGCCCAATTGAATTTGCAGAAAGCGTTTCAAAATTTC TTTTCGGGTCGTGCTGGATTTCCAAAGTTCAAAAACCGCAAGGCGA AACAGTCGTACACCACAAATGTGGTCAATGGCAATATTCAACTTTC AGATGGCTATATCAAGTTACCCAAACTGAAATGGGTCAAGTTCAAG CAACATCGGGAGATTCCTGCCCACCATATCATCAAGGCTTGTACGA TCAAGAAAACAAAAACAGGAAAATACTATGTTTCTATTCTCACCGA ATACGAACATCAACCTGTCCCCAAAGAAATACAAACGGTTGTTGGG CTTGATTTTTCCATGAATGGTTTATTTGTCGATAGCGAGGGTAAGAG AGCCAATTATCCTCGTTTCTATCGGCAAGCATTGGAAAAATTAGCG AAAGAACAACGGATATTATCACGCCGAAAGAAGGGTTCTAATCGTT GGCACAAACAGCGTTTGAAAGTAGCGAAGCTACATGAGAAAATAG CGAACCAACGAAAAGACTTTCTACACAAAAAATCATACGAATTAGC GAAACAGTATGATTGCGTGGTCATCGAAAACCTCAACATGAAAGG GATGTCGCAAGTCCTGAATTTCGGCAAGAGCGTTCATGACAACGGC TGGGGGATGTTCACGACATTTCTCCAATACAAGTTAGAGGAGCAAG GGAAAAAGCTTATCAAAATAGATAAGTGGTTTCCGTCATCTAAAAC TTGTTCATGTTGCGATCAAGTAAAGGAATCTTTATCGCTTTCTGAGC GTACATTCCGCTGTGAATGTGGATTCGAGAGCGACAGGGACGTCAA TGCGGCAATCAATATCAAACATGAGGGCATGAAACGATTAGCAAT AGCCTAACTTGTCCTCGAACCGTGGGACACACGGGGATCGCTCAGT CAACTTCCCGTCATGAGATGGGATTACCTGAGAAGCCCCCACCTCT AAGCGAAGCGTAGGTGGTGGGAGCATGTCAC	141
ISDra2	ATGATAAGGAATAAGGCTTTCGTGGTCAGGCTGTACCCAAATGCGG CTCAGACTGAACTGATTAACCGCACGCTGGGTAGCGCAAGGTTCGT CTACAACCACTTCCTTGCCCGTCGCATTGCGGCCTACAAGGAAAGC GGGAAGGGACTGACCTACGGGCAAACGAGTAGCGAACTGACCCTT CTGAAGCAGGCTGAAGAAACCTCCTGGCTCTCGGAAGTAGATAAGT TTGCTTTGCAGAACTCGCTGAAAAACCTTGAaACCGCGTACAAGAA CTTCTTTCGGACTGTGAAGCAGTCCGGTAAAAAGGTAGGATTCCCA CGTTTCAGAAAGAAGCGCACGGGAGAGTCCTACCGGACTCAATTCA CCAACAACAACATCCAAATTGGGGAAGGTAGGCTCAAACTTCCTAA GCTGGGATGGGTGAAAACCAAGGGCCAGCAGGATATTCAAGGGAA GATTCTGAATGTCACTGTGCGCCGTATTCACGAAGGCCATTACGAA GCGTCCGTTCTCTGTGAAGTCGAGATTCCCTACCTGCCTGCGGCTCC CAAGTTTGCAGCGGGTGTGGATGTCGGCATCAAGGATTTTGCCATC GTGACCGATGGCGTGAGGTTTAAGCATGAACAGAATCCGAAATATT ACCGCTCCACCCTGAAAAGACTTCGTAAAGCTCAGCAAACCCTGTC	143

TnpB name	TnpB with wRNA overlap Native DNA sequence (single)	SEQ ID NO:
	CAGACGGAAGAAGGGCAGCGCACGTTACGGGAAAGCGAAAACCAA GCTGGCTCGGATTCACAAGCGCATTGTCAATAAGCGTCAGGATTTC CTTCACAAGCTCACCACCTCCCTGGTGCGTGAGTACGAAATCATCG GAACCGAACACCTTAAACCCGACAACATGCGGAAAAATCGCCGCC TTGCACTGAGCATCAGTGATGCGGGCTGGGGTGAGTTCATCCGGCA GTTGGAATACAAGGCAGCGTGGTACGGGCGACTGGTATCTAAAGTC AGCCCCTACTTTCCATCTAGCCAGTTGTGTCATGACTGCGgattcaagaat cccgaagtgaagaatcttgccgtccgtacatggacttgcccgaactgtggggaaacccatgaccgagaCgag aacgctgcgctgaacattcggcgtgaagcgttggtggctgcgggaatctcagacaccttaaacgctcatggagg ctatgtcagacctgcttcggcgggcaatggtctgcgaagtgagaatcacgcgactttagtcgtgtgaGGTTC AA	

Table 5: Selected sequences for Example 12

Name	DNA Sequence	SEQ ID NO:
AtUBQ10 pol II promoter	agtctagctcaacagagcttttaacccaaattggtacaatagaatacaactttagatcataattctcaaaagaaagagattcctt agctattctatctgccactccatttccttctcggcttgtatgcacaagcataaaatcctcaaacttgctaagtagatactttatgtct tggataattggattgagacttgacaagcataactttcatgtaaccaaagacacaagttgctgagaatccacctcaaaaatgatc ttcctataattgaatcgggataatgacagcacagcccatctaagagcctccacttctacttccagcacgcttcttacttttaccac agctcttgcacctaaccataacaccttccctgtatgatcgcgaagcacccaccctaagccacattttaatccttctgttggccat gccccatcaaagttgcacttaacccaagattgtggtggagcttcccatgtttctcgtctgtcccgacggtgttgtggttggtgct ttccttacattctgagcctctttccttctaatccactcatctgcatcttcttgtgtccttactaatacctcattggttccaaattccctcc ctttaagcaccagctcgtttctgttcttccacagcctcccaagtatccaagggactaaagcctccacattcttcagatcaggata ttcttgtttaagatgttgaactctatggaggtttgtatgaactgatgatctaggaccggataagttcccttcttcatagcgaacttat tcaaagaatgttttgtgtatcattcttgttacattgttattaatgaaaaaatattattggtcattggactgaacacgagtgttaaatat ggaccaggccccaaataagatccattgatatatgaattaaataacaagaataaatcgagtcaccaaaccacttgccttttttaa cgagacttgttcaccaacttgatacaaaagtcattatcctatgcaaatcaataatcatacaaaaatatccaataacactaaaaaa ttaaaagaaatggataatttcacaatatgttatacgataaagaagttacttttccaagaaattcactgattttataagcccacttgc attagataaatggcaaaaaaaaacaaaaaggaaaagaaataaagcacgaagaattctagaaaatacgaaatacgcttcaat gcagtgggacccacggttcaattattgccaattttcagctccaccgtatatttaaaaaataaaacgataatgctaaaaaaatata aatcgtaacgatcgttaaatctcaacggctggatcttatgacgaccgttagaaattgtggttgtcgacgagtcagtaataaacg gcgtcaaagtggttgcagccggcacacacgagtcgtgtttatcaactcaaagcacaaatacttttcctcaacctaaaaataag gcaattagccaaaaacaacttttgcgtgtaaacaacgctcaatacacgtgtcattttattattagctattgcttcaccgccttagctt tctcgtgacctagtcgtcctcgtcttttcttcttcttcttctataaaacaatacccaaagagctcttcttcttcacaattcagatttcaa tttctcaaaatcttaaaaacttttctctcaattctctctaccgtgatcaaggtaaatttctgtgttccttattctctcaaaatcttcgatttt gttttcgttcgatcccaatttcgtatatgttctttggtttagattctgttaatcttagatcgaagacgattttctgggtttgatcgttaga tatcatcttaattctcgattagggtttcatagatatcatccgatttgttcaaataatttgagttttgtcgaataattactcttcgatttgt gatttctatctagatctggtgttagtttctagtttgtgcgatcgaatttgtcgattaatctgagtttttctgattaacag	132
rbcS E9 terminator	agagctttcgttcgtatcatcggtttcgacaacgttcgtcaagttcaatgcatcagtttcattgcgcacacaccagaatcctact gagtttgagtattatggcattgggaaaactgtttttcttgtaccatttgttgtgcttgtaatttactgtgtttttttattcggttttcgctat cgaactgtgaaatggaaatggatggagaagagttaatgaatgatatggtccttttgttcattctcaaattaatattatttgttttttct cttatttgttgtgtgttgaatttgaaattataagagatatgcaaacattttgttttgagtaaaaatgtgtcaaatcgtggcctctaatg accgaagttaatatgaggagtaaaacacttgtagttgtaccattatgcttattcactaggcaacaaatatattttcagacctagaa aagctgcaaatgttactgaatacaagtatgtcctcttgtgttttagacatttatgaactttcctttatgtaattttccagaatccttgtc	135

Name	DNA Sequence	SEQ ID NO:
	agattctaatcattgctttataattatagttatactcatggatttgtagttgagtatgaaaatatttttaatgcattttatgacttgccaattgattgacaac	
HDV ribozyme	ggccggcatggtcccagcctcctcgctggcgccggctgggcaacatgcttcggcatggcgaatgggac	137

Table 6: Additional Sequences for Example 12 (In Table 6, the gRNA sequences are listed as DNA sequences that are the equivalent RNA sequences (except the RNA will have the T replaced with a U)).

TnpB	TAM Sequence	gRNA ID	gRNA sequence	gRNA - SEQ ID NO:
ISYmu1	TTGAT	1	ttacgaattgatgacc	54
ISYmu1	TTGAT	2	aaggcaaattcgccgc	55
ISYmu1	TTGAT	3	tcacattaagcctaga	56
ISYmu1	TTGAT	4	cccaagttctccaaat	57
ISYmu1	TTGAT	5	tacccatcctaaagta	58
ISYmu1	TTGAT	7	aaattcaacatctttc	60
ISYmu1	TTGAT	8	tggtctcactttccga	61
ISYmu1	TTGAT	9	agctttgaaccggttt	62
ISYmu1	TTGAT	10	taacttgtactacctc	63
ISYmu1	TTGAT	12	gcgttggagcatataa	64
ISAam1	TTTAA	11	caattcatctggtatc	84
ISAam1	TTTAA	13	gttttgtcctcttctc	86
ISAam1	TTTAA	14	caatttacctatctta	87
ISAam1	TTTAA	16	aatttgttacacaact	89
ISAam1	TTTAA	17	tttgtgtggtatttaa	90
ISAam1	TTTAA	12	ccaagaacaagcctta	85
ISAam1	TTTAA	15	cacataattgaaaaga	88
ISDra2	TTGAT	1	ttacgaattgatgaccatat	114
ISDra2	TTGAT	2	aaggcaaattcgccgcagaa	193
ISDra2	TTGAT	4	cccaagttctccaaataaat	117
ISDra2	TTGAT	7	aaattcaacatctttctcta	119
ISDra2	TTGAT	8	tggtctcactttccgaatta	120
ISDra2	TTGAT	9	agctttgaaccggtttcttc	121
ISDra2	TTGAT	11	cgaaaactgaagaacacata	122
ISDra2	TTGAT	12	gcgttggagcatataacaga	123

TnpB	TAM Sequence	gRNA ID	gRNA sequence	gRNA - SEQ ID NO:
ISDra2	TTGAT	13	taaagagaggaaattgcagg	124
ISDra2	TTGAT	14	ggtagagctgataagatata	125
ISDra2	TTGAT	15	aaaattggattaatgtgcac	126
ISDra2	TTGAT	17	caatacaaataaatacatgc	128
ISDra2	TTGAT	18	caattcaagctaattataga	129
ISDra2	TTGAT	19	gagcttaacttggtagagta	130
ISDra2	TTGAT	21	gttgattaacttgtactacc	608
ISDra2	TTGAT	22	taacttgtactacctcatcc	609
ISDra2	TTGAT	3	tcacattaagcctagaaact	116
ISDra2	TTGAT	5	tacccatcctaaagtatggg	118
ISDra2	TTGAT	16	tactattaaatgtcaaaatc	127
ISDra2	TTGAT	20	ttgtcagctttcttatggat	131

Table 7: TnpB editing frequency associated with Example 13

Nuclease	Target Site	Percent Indel Reads (%)
ISYmu1	experiment_2_g2	4.99939666
ISYmu1	experiment_2_g2	5.18979911
ISYmu1	experiment_2_g2	6.59212917
TnpB_20	g1	0
TnpB_20	g1	0
TnpB_20	g1	0
TnpB_20	g11	0
TnpB_20	g11	0.02944011
TnpB_20	g11	0.14436613
TnpB_20	g12	0
TnpB_20	g12	0
TnpB_20	g12	0
TnpB_20	g3	0.48007453
TnpB_20	g3	0.58319602
TnpB_20	g6	0
TnpB_20	g6	0.04450675
TnpB_20	g6	0.04663422
TnpB_20	g7	0
TnpB_20	g7	0
TnpB_20	g7	0.03337859
TnpB_20	g8	0.16805624

Nuclease	Target Site	Percent Indel Reads (%)
TnpB_20	g8	0.20184454
TnpB_20	g8	0.26755937
TnpB_20	g9	0
TnpB_20	g9	0
TnpB_20	g9	0
TnpB_25	g13	0
TnpB_25	g13	0
TnpB_25	g13	0
TnpB_25	g14	0
TnpB_25	g14	0
TnpB_25	g14	0
TnpB_25	g3	0.21571548
TnpB_25	g3	0.92843864
TnpB_25	g4	0
TnpB_25	g4	0
TnpB_25	g4	0.02969945
TnpB_25	g5	0
TnpB_25	g5	0.03236991
TnpB_25	g5	0.06313702
TnpB_25	g6	0
TnpB_25	g6	0
TnpB_25	g6	0
TnpB_25	g7	0
TnpB_25	g7	0
TnpB_25	g7	0
TnpB_25	g8	0
TnpB_25	g8	0
TnpB_25	g8	0.01938655
TnpB_26	g10	0
TnpB_26	g10	0
TnpB_26	g10	0
TnpB_26	g11	0
TnpB_26	g11	0
TnpB_26	g11	0
TnpB_26	g12	0
TnpB_26	g12	0
TnpB_26	g12	0
TnpB_26	g13	0
TnpB_26	g13	0
TnpB_26	g13	0

Nuclease	Target Site	Percent Indel Reads (%)
TnpB_26	g18	0
TnpB_26	g18	0
TnpB_26	g18	0
TnpB_26	g19	0
TnpB_26	g19	0
TnpB_26	g19	0
TnpB_26	g20	0
TnpB_26	g20	0
TnpB_26	g20	0
TnpB_26	g7	0
TnpB_26	g7	0
TnpB_26	g7	0
TnpB_26	g8	0
TnpB_26	g8	0
TnpB_26	g8	0
TnpB_26	g9	0
TnpB_26	g9	0
TnpB_26	g9	0
TnpB_14	g1	0
TnpB_14	g1	0
TnpB_14	g1	0
TnpB_14	g2	0
TnpB_14	g2	0
TnpB_14	g2	0
TnpB_14	g3	0
TnpB_14	g3	0
TnpB_14	g3	0
TnpB_14	g4	0
TnpB_14	g4	0
TnpB_14	g4	0
TnpB_14	g5	0
TnpB_14	g5	0
TnpB_14	g5	0
TnpB_14	g6	0
TnpB_14	g6	0
TnpB_14	g6	0
TnpB_14	g8	0
TnpB_14	g8	0
TnpB_14	g8	0
TnpB_17	g1	0.0474025

Nuclease	Target Site	Percent Indel Reads (%)
TnpB_17	g1	0.07664723
TnpB_17	g1	0.48188682
TnpB_17	g2	0
TnpB_17	g2	0
TnpB_17	g2	0
TnpB_17	g4	0.06649092
TnpB_17	g4	0.06732075
TnpB_17	g4	0.10331211
TnpB_17	g5	0.00885216
TnpB_17	g5	0.01125574
TnpB_17	g5	0.0187792
TnpB_17	g6	0
TnpB_17	g6	0.00584416
TnpB_17	g8	0
TnpB_17	g8	0
TnpB_17	g8	0
TnpB_17	g9	0
TnpB_17	g9	0
TnpB_17	g9	0
ISYmu1	g1	0.06807423
ISYmu1	g1	0.03501401
ISYmu1	g2	3.87065402
ISYmu1	g2	4.85665356
ISYmu1	g2	3.73225424
ISYmu1	g3	0.03904572
ISYmu1	g3	0.05631763
ISYmu1	g3	0.05424299
ISYmu1	g4	3.03061996
ISYmu1	g4	1.02974571
ISYmu1	g4	2.30006822
ISYmu1	g5	0.91378045
ISYmu1	g5	1.00381244
ISYmu1	g5	1.24186136
ISYmu1	g7	4.40465197
ISYmu1	g7	3.20201486
ISYmu1	g7	3.7924399
ISYmu1	g8	2.1193445
ISYmu1	g8	2.40385213
ISYmu1	g8	1.45945795
ISYmu1	g9	2.65415622

Nuclease	Target Site	Percent Indel Reads (%)
ISYmu1	g9	2.54991149
ISYmu1	g10	0.96151829
ISYmu1	g10	1.17677782
ISYmu1	g12	4.1012075
ISYmu1	g12	4.86845113
ISYmu1	g12	3.50081659
TnpB_17	g10	0.01511163
TnpB_17	g10	0.00784551
TnpB_17	g10	0.01535367
TnpB_17	g12	0
TnpB_17	g12	0
TnpB_17	g12	0
TnpB_17	g13	0
TnpB_17	g13	0.0051364
TnpB_17	g13	0.00297286
ISAam1	g11	0.07409578
ISAam1	g11	0.09470404
ISAam1	g11	0.06725845
ISAam1	g13	0.05148402
ISAam1	g13	0.01602903
ISAam1	g13	0.03973599
ISAam1	g14	0.01040386
ISAam1	g14	0.0213991
ISAam1	g14	0.0283879
ISAam1	g16	0.13147888
ISAam1	g16	0.04481922
ISAam1	g16	0.03761465
ISAam1	g17	0.26847586
ISAam1	g17	0.40177172
ISAam1	g12	0
ISAam1	g12	0.01209118
ISAam1	g12	0
ISAam1	g15	0
ISAam1	g15	0
ISAam1	g15	0
ISAam1	g17	0.25393659
ISDra2	g1	0.55853462
ISDra2	g1	0.46957429
ISDra2	g1	0.9810104
ISDra2	g2	1.40258604

Nuclease	Target Site	Percent Indel Reads (%)
ISDra2	g2	2.23323844
ISDra2	g2	1.73645833
ISDra2	g4	0.14778109
ISDra2	g4	0.04415036
ISDra2	g4	0.08451919
ISDra2	g7	1.35635556
ISDra2	g7	2.7381535
ISDra2	g7	3.31914326
ISDra2	g8	0.48614276
ISDra2	g8	0.29793718
ISDra2	g8	0.4177285
ISDra2	g9	2.21785539
ISDra2	g9	2.28263261
ISDra2	g9	2.53381496
ISDra2	g11	0.17854765
ISDra2	g11	0.09384926
ISDra2	g11	0.08775334
ISDra2	g12	5.18974375
ISDra2	g12	4.4706969
ISDra2	g12	4.75029671
ISDra2	g13	0.71739028
ISDra2	g13	0.6314373
ISDra2	g13	0.69918794
ISDra2	g14	2.21683555
ISDra2	g14	2.40058149
ISDra2	g14	2.0527062
ISDra2	g15	0.05108264
ISDra2	g15	0.03754849
ISDra2	g15	0.00969994
ISDra2	g17	0.11477872
ISDra2	g17	0.26120757
ISDra2	g17	0.1044236
ISDra2	g18	0.11586156
ISDra2	g18	0.15800264
ISDra2	g18	0.09118095
ISDra2	g19	2.80269827
ISDra2	g19	6.10379583
ISDra2	g19	2.96128925
ISDra2	g21	0.05366757
ISDra2	g21	0.07866299

Nuclease	Target Site	Percent Indel Reads (%)
ISDra2	g21	0.10937967
ISDra2	g22	0.04286668
ISDra2	g22	0.23421461
ISDra2	g22	0.10892352
ISDra2	g3	0.00636573
ISDra2	g3	0.01198017
ISDra2	g3	0
ISDra2	g5	0
ISDra2	g5	0
ISDra2	g5	0.00787662
ISDra2	g16	0.01941211
ISDra2	g16	0
ISDra2	g16	0.03912356
ISDra2	g20	0.00653203
ISDra2	g20	0.01491622
ISDra2	g20	0
TnpB_5	g1	0.1422721
TnpB_5	g1	0.11666551
TnpB_5	g1	0.11666551
TnpB_5	g3	0.04641557
TnpB_5	g3	0.03033334
TnpB_5	g3	0.00995026
TnpB_5	g7	0.01852631
TnpB_5	g7	0.01685874
TnpB_5	g7	0.00972563
TnpB_5	g9	0.02616883
TnpB_5	g9	0.01539763
TnpB_5	g9	0.02862544
TnpB_5	g2	0.0038306
TnpB_5	g2	0
TnpB_5	g2	0
TnpB_5	g5	0.01123323
TnpB_5	g5	0
TnpB_5	g5	0.00277553
TnpB_5	g6	0
TnpB_5	g6	0
TnpB_5	g6	0
TnpB_5	g8	0
TnpB_5	g8	0
TnpB_5	g8	0.00374798

Nuclease	Target Site	Percent Indel Reads (%)
TnpB_8	g1	0.07297516
TnpB_8	g1	0.02865991
TnpB_8	g1	0.04638062
TnpB_8	g3	0
TnpB_8	g3	0
TnpB_8	g3	0
TnpB_8	g4	0
TnpB_8	g4	0
TnpB_8	g4	0
TnpB_8	g8	0
TnpB_8	g8	0
TnpB_8	g8	0
TnpB_8	g15	0
TnpB_8	g15	0
TnpB_8	g15	0
TnpB_8	g16	0
TnpB_8	g16	0
TnpB_8	g16	0
TnpB_8	g17	0
TnpB_8	g17	0
TnpB_8	g17	0
TnpB_8	g20	0
TnpB_8	g20	0
TnpB_8	g20	0
TnpB_15	g1	0.0482157
TnpB_15	g1	0.01107948
TnpB_15	g1	0.10464789
TnpB_15	g2	0.05880987
TnpB_15	g2	0.05806362
TnpB_15	g2	0.05268206
TnpB_15	g4	0.82558903
TnpB_15	g4	0.49090878
TnpB_15	g4	0.40665773
TnpB_15	g5	0.52063957
TnpB_15	g5	0.89599651
TnpB_15	g5	0.63621293
TnpB_15	g3	0
TnpB_15	g3	0
TnpB_15	g3	0
TnpB_15	g6	0

Nuclease	Target Site	Percent Indel Reads (%)
TnpB_15	g6	0.0139435
TnpB_15	g6	0
TnpB_2	g1	0
TnpB_2	g1	0
TnpB_2	g1	0.01613277
TnpB_2	g3	0
TnpB_2	g3	0
TnpB_2	g3	0
TnpB_2	g4	0
TnpB_2	g4	0
TnpB_2	g4	0
TnpB_2	g5	0
TnpB_2	g5	0
TnpB_2	g5	0
TnpB_2	g6	0
TnpB_2	g6	0
TnpB_2	g6	0
TnpB_2	g7	0
TnpB_2	g7	0
TnpB_2	g7	0
TnpB_2	g8	0
TnpB_2	g8	0
TnpB_2	g8	0
TnpB_2	g9	0
TnpB_2	g9	0
TnpB_2	g9	0
TnpB_2	g10	0
TnpB_2	g10	0
TnpB_2	g10	0
TnpB_2	g11	0
TnpB_2	g11	0
TnpB_2	g11	0
TnpB_2	g12	0
TnpB_2	g12	0
TnpB_2	g12	0
TnpB_4	g1	0
TnpB_4	g1	0
TnpB_4	g1	0
TnpB_4	g2	0
TnpB_4	g2	0

Nuclease	Target Site	Percent Indel Reads (%)
TnpB_4	g2	0
TnpB_4	g3	0
TnpB_4	g3	0
TnpB_4	g3	0
TnpB_4	g4	0
TnpB_4	g4	0
TnpB_4	g4	0
TnpB_4	g5	0
TnpB_4	g5	0
TnpB_4	g5	0
TnpB_4	g6	0.13786897
TnpB_4	g6	0.0079869
TnpB_4	g6	0
TnpB_4	g8	0
TnpB_4	g8	0
TnpB_4	g8	0
TnpB_4	g9	0
TnpB_4	g9	0
TnpB_4	g9	0
TnpB_4	g10	0
TnpB_4	g10	0
TnpB_4	g10	0
TnpB_4	g11	0
TnpB_4	g11	0
TnpB_4	g11	0
TnpB_6	g1	0
TnpB_6	g1	0
TnpB_6	g1	0
TnpB_6	g2	0
TnpB_6	g2	0
TnpB_6	g2	0
TnpB_6	g3	0
TnpB_6	g3	0
TnpB_6	g3	0
TnpB_6	g5	0
TnpB_6	g5	0
TnpB_6	g5	0
TnpB_6	g6	0
TnpB_6	g6	0
TnpB_6	g6	0

Nuclease	Target Site	Percent Indel Reads (%)
TnpB_6	g8	0
TnpB_6	g8	0
TnpB_6	g8	0
TnpB_6	g9	0
TnpB_6	g9	0
TnpB_6	g9	0
TnpB_6	g10	0
TnpB_6	g10	0
TnpB_6	g10	0
TnpB_6	g11	0
TnpB_6	g11	0
TnpB_6	g11	0
TnpB_6	g12	0
TnpB_6	g12	0
TnpB_6	g12	0
TnpB_3	g1	0
TnpB_3	g1	0
TnpB_3	g1	0
TnpB_3	g3	0
TnpB_3	g3	0
TnpB_3	g3	0
TnpB_3	g4	0
TnpB_3	g4	0
TnpB_3	g4	0
TnpB_3	g5	0
TnpB_3	g5	0
TnpB_3	g5	0
TnpB_3	g6	0
TnpB_3	g6	0
TnpB_3	g6	0
TnpB_3	g8	0
TnpB_3	g8	0
TnpB_3	g8	0
TnpB_3	g9	0
TnpB_3	g9	0
TnpB_3	g9	0
TnpB_3	g10	0
TnpB_3	g10	0
TnpB_3	g10	0
TnpB_3	g12	0

Nuclease	Target Site	Percent Indel Reads (%)
TnpB_3	g12	0
TnpB_3	g12	0
TnpB_3	g13	0
TnpB_3	g13	0
TnpB_3	g13	0
TnpB_7	g2	0
TnpB_7	g2	0
TnpB_7	g2	0
TnpB_7	g13	0
TnpB_7	g13	0
TnpB_7	g13	0
TnpB_7	g14	0
TnpB_7	g14	0
TnpB_7	g14	0
TnpB_7	g15	0
TnpB_7	g15	0
TnpB_7	g15	0
TnpB_7	g16	0
TnpB_7	g16	0
TnpB_7	g16	0
TnpB_7	g17	0.00658738
TnpB_7	g17	0
TnpB_7	g17	0
TnpB_7	g18	0
TnpB_7	g18	0
TnpB_7	g18	0
TnpB_7	g19	0
TnpB_7	g19	0
TnpB_7	g19	0
TnpB_7	g20	0
TnpB_7	g20	0
TnpB_7	g20	0
TnpB_10	g2	0
TnpB_10	g2	0
TnpB_10	g2	0
TnpB_10	g3	0.00481498
TnpB_10	g3	0
TnpB_10	g3	0
TnpB_10	g4	0
TnpB_10	g4	0.00211751

Nuclease	Target Site	Percent Indel Reads (%)
TnpB_10	g4	0
TnpB_10	g5	0
TnpB_10	g5	0
TnpB_10	g5	0
TnpB_10	g6	0
TnpB_10	g6	0
TnpB_10	g6	0
TnpB_10	g8	0
TnpB_10	g8	0
TnpB_10	g8	0
TnpB_10	g9	0
TnpB_10	g9	0
TnpB_10	g9	0
TnpB_10	g10	0
TnpB_10	g10	0
TnpB_10	g10	0
TnpB_12	g1	0
TnpB_12	g1	0
TnpB_12	g1	0
TnpB_12	g2	0
TnpB_12	g2	0
TnpB_12	g2	0
TnpB_12	g4	0
TnpB_12	g4	0
TnpB_12	g4	0
TnpB_12	g5	0
TnpB_12	g5	0
TnpB_12	g5	0
TnpB_12	g6	0
TnpB_12	g6	0
TnpB_12	g6	0
TnpB_12	g7	0
TnpB_12	g7	0
TnpB_12	g7	0
TnpB_12	g8	0
TnpB_12	g8	0
TnpB_12	g8	0
TnpB_18	g12	0
TnpB_18	g12	0
TnpB_18	g12	0

Nuclease	Target Site	Percent Indel Reads (%)
TnpB_18	g13	0
TnpB_18	g13	0
TnpB_18	g13	0
TnpB_27	g1	0
TnpB_27	g1	0
TnpB_27	g1	0.00581465
TnpB_27	g2	0
TnpB_27	g2	0
TnpB_27	g2	0
TnpB_27	g3	0
TnpB_27	g3	0
TnpB_27	g3	0
TnpB_27	g4	0
TnpB_27	g4	0
TnpB_27	g4	0
TnpB_27	g5	0
TnpB_27	g5	0
TnpB_27	g5	0
TnpB_27	g6	0
TnpB_27	g6	0
TnpB_27	g6	0
TnpB_27	g9	0
TnpB_27	g9	0
TnpB_27	g9	0.0022781
TnpB_27	g10	0
TnpB_27	g10	0
TnpB_27	g10	0
TnpB_27	g12	0
TnpB_27	g12	0
TnpB_27	g12	0
TnpB_27	g13	0
TnpB_27	g13	0
TnpB_27	g13	0
TnpB_27	g14	0
TnpB_27	g14	0
TnpB_27	g14	0
TnpB_12	g3	0
TnpB_12	g3	0
TnpB_12	g3	0
TnpB_15	g7	1.01043429

Nuclease	Target Site	Percent Indel Reads (%)
TnpB_15	g7	0.60818778
TnpB_15	g7	0.85717843
TnpB_15	g8	0.02444636
TnpB_15	g8	0.01170427
TnpB_15	g8	0.00649966
TnpB_17	g6	0
TnpB_18	g1	0
TnpB_18	g1	0
TnpB_18	g1	0
TnpB_18	g2	0
TnpB_18	g2	0
TnpB_18	g2	0
TnpB_18	g3	0
TnpB_18	g3	0
TnpB_18	g3	0
TnpB_18	g5	0
TnpB_18	g5	0
TnpB_18	g5	0
TnpB_18	g6	0.00375251
TnpB_18	g6	0
TnpB_18	g6	0.00615671
TnpB_18	g7	0
TnpB_18	g7	0
TnpB_18	g7	0
TnpB_18	g9	0
TnpB_18	g9	0
TnpB_18	g9	0
TnpB_19	g1	0
TnpB_19	g1	0
TnpB_19	g1	0
TnpB_19	g2	0
TnpB_19	g2	0
TnpB_19	g2	0
TnpB_20	g10	0.03092221
TnpB_20	g10	0.01444471
TnpB_20	g10	0.05469983
TnpB_20	g13	0.13739101
TnpB_20	g13	0.15661732
TnpB_20	g13	0.09219925
TnpB_20	g2	0.00732489

Nuclease	Target Site	Percent Indel Reads (%)
TnpB_20	g2	0.05910549
TnpB_20	g2	0
TnpB_21	g11	0
TnpB_21	g11	0
TnpB_21	g11	0
TnpB_21	g1	0.04492266
TnpB_21	g1	0
TnpB_21	g1	0.1063435
TnpB_21	g2	0
TnpB_21	g2	0.01750824
TnpB_21	g2	0
TnpB_21	g3	0
TnpB_21	g3	0
TnpB_21	g3	0
TnpB_21	g5	0.63130476
TnpB_21	g5	0.37620356
TnpB_21	g5	0.85033465
TnpB_21	g6	0
TnpB_21	g6	0
TnpB_21	g6	0.02059266
TnpB_21	g7	1.36535623
TnpB_21	g7	1.95204648
TnpB_21	g7	0.77122314
TnpB_21	g8	0.00526684
TnpB_21	g8	0
TnpB_21	g8	0
TnpB_21	g9	0
TnpB_21	g9	0.04454654
TnpB_21	g9	0
TnpB_22	g10	0
TnpB_22	g10	0
TnpB_22	g10	0
TnpB_22	g12	0
TnpB_22	g12	0
TnpB_22	g12	0
TnpB_22	g1	0.00198156
TnpB_22	g1	0
TnpB_22	g1	0
TnpB_22	g2	0.00277837
TnpB_22	g2	0

Nuclease	Target Site	Percent Indel Reads (%)
TnpB_22	g2	0
TnpB_22	g3	0
TnpB_22	g3	0
TnpB_22	g3	0
TnpB_22	g4	0
TnpB_22	g4	0
TnpB_22	g4	0
TnpB_22	g5	0.02111244
TnpB_22	g5	0
TnpB_22	g5	0.05552971
TnpB_22	g6	0
TnpB_22	g6	0
TnpB_22	g6	0
TnpB_22	g7	0.02565817
TnpB_22	g7	0
TnpB_22	g7	0
TnpB_22	g8	0
TnpB_22	g8	0
TnpB_22	g8	0.0351499
TnpB_22	g9	0
TnpB_22	g9	0
TnpB_22	g9	0
TnpB_23	g1	0
TnpB_23	g1	0
TnpB_23	g1	0
TnpB_23	g2	0
TnpB_23	g2	0
TnpB_23	g2	0
TnpB_23	g3	0.01806173
TnpB_23	g3	0
TnpB_23	g3	0
TnpB_26	g1	0.17616486
TnpB_26	g1	0.07570503
TnpB_26	g1	0.16238373
TnpB_26	g2	0
TnpB_26	g2	0
TnpB_26	g2	0
TnpB_26	g3	0
TnpB_26	g3	0
TnpB_26	g3	0

Nuclease	Target Site	Percent Indel Reads (%)
TnpB_26	g4	0
TnpB_26	g4	0
TnpB_26	g4	0
TnpB_26	g5	0
TnpB_26	g5	0
TnpB_26	g5	0
TnpB_27	g7	0
TnpB_27	g7	0
TnpB_27	g7	0
ISYmu1	experiment_3_g2	15.0011521
ISYmu1	experiment_3_g2	15.1141811
ISYmu1	experiment_3_g2	9.8916315
ISDra2	experiment_2_g9	10.9095437
ISDra2	experiment_2_g9	12.6114455

Table 8: Various TnpB sequences associated with Example 13

TnpB Name	TnpB with wRNA overlap Native DNA sequence (single)	SEQ ID NO:
TnpB_5	ATGATCAAAACCTTCAAATACAGGCTTTATCCTACAAAAAAACAAGTCCAGAAACTTAACT GGACTTTGGATAAGTGCCGTGTTCTTTATAATTCTTGTTTGCTTGATAAAAAGAACCGTTAC GAACAAACAGGCAAGGGGCTCTCTCGTATAGACCAGCAGGTTATCCTTAAAAACGATAAA GATAGATTTGATTTTCTTAATGATATCCATTCTCAAGTCCTTCAAGATGTGCTTTTCAGAGTA GATAAAGCCTTTCAAAGTTTCTTTCGCAGGGTTAAGGCTAAAAAGAGGGCTGGTTATCCTC GTTTTAAAAGTGAAGGCAGATATGACTCCATTACTTATCCCCAAAAACCTGCATTTCAGATA ACAGAACAAGGACTTAAACTTTCTAAGATAGGCTCTATTAAGATTAAACTTCATAGGTCTAT TGTTGGAAAGCCTAAAACCTGCGTTATCAAAAAGCAAAACGGTAAGTGGTATGCCTGTTTC TCTGTTGAATATATTAATCAACCCCTTCCTCATTCTGATAAGGTTGTGGGCATAGATGTCGG TTTGGAGAATTTTGCTATTCTTTCTAATCAAGAGAAAATAAAAAATCCCCGCTTTTTTAAGA TTGACCAAAATGCCCTTGCGAAGGCTCAAAGAAAATTGTCTAAACAAACTAAAAGCTCTCC TAAAAGAAAGAAGGCTAAAAAGGTTGTTATTCGTATTCACGAAAGAATTGTTAATAGACGT CATAATTTTATCCATCAAGAAGCAAGAAAGATTGTTAATAACTTTGGCGTAATCTGTATTGA AAAACTTAATGTCAAAGATATGATGAGTAATCAAATCAAAGTGTTTGGTCATAAACTCAAC AGGAGCATTGCAGATGCCGCCTGGAGTCAATTTGCTCAACAACTTTTCTTTAAAGCGGAAG ATGCTGGAAGAAAAATCATAGCAATTAACCCAAAAGGAACAAGCCAACGATGCAGTCAAT GCGGGACTATTGTTAAAAAAGATTTGTCTGTTCGTTGGCATGATTGTCCTGTTTGCAATGTT CATCTTCATAGAGACCTCAATGCTTCTTATAATATTTTGAGACTGGGAACACAGTCTCTTGG CTTCTCCATAGAAGCCCCTTCCTATCTTTGATAGGTGGGGGAATGTTCAC	150

TnpB Name	TnpB with wRNA overlap Native DNA sequence (single)	SEQ ID NO:
TnpB_8	ATGTTGCGATTGCAAGCCTACAAATTCCATATCCTGCCAAACGGCGAGCAGCAACGCGCCA TGCGTCGCTTCGCTGGCAATGCGCGCAAAGTGTGGAATCTAGCCTTGGCTCGCCAACAAG AAAAACATGCGTTAGGTGAGAAATTCACCAACGCCTTCGGGATGAGCGCGTGGCTCTTGG AATGGAAAAAAGAATACCCATTTCTCGTTGAATCCCCCTCTCAAACACTACAACAAGTAAT GGGCAACTTGCAGTCCGGGTTTAAAAACTTCTTCGAGAAACGCGCAGCGCATCCTGAGTTT AAAAAGAAAGGGAAGTCGTCCGACAGCGTCCGCTTTCCTCAAGGCTTCGAGATTGATGCG AAAAACAACCGTATCAAACTTCCCAAGCTAGGTTGGATACGCTATCGCAACAGCCGCGACA TTCTCGGCACCCCAAAAAACATCACCGTCTCCTGCGTCGCGGGTAAATGGTACGCCGCTAT CCAGACAGAGCGTGAAGTTGAAGATGTGAGGCATCCCGCATCGACTAGCGTCGGTATCGA TGTCGGGATCGCGCGCTTCGCGACGCTCTCAGACGGAAGCCACATCGAACCACTCAACAG CTTCAAGAAACACCAAGCACGGCTGGCACGGTACCAGCGTGCCATGGCGCGCAAGGTTAA ATTCAGCAGCAATTGGAAGAAAGCAAAAGCAAAAGTAACGAAGTTGCATCAAAAGATCG GACATGTAAGGCAGGACTTTCTACACAAGACTTCCACGATCCTCAGCAAAAACCACGCGCT GATCGTGATCGAAGATCTGAAAGTCCGGAACATGAGCCGAAGTGCGAGCGGCACCAAGG CCGTGCCAGGACGTTCCGTCCGGGCCAAGTCCGGCTTGAACCGCTCGATCCTCGACCAGG GGTGGGGCGAGTTCCGGCGTCAGCTGGAATACAAGCAAGCTCGGTCAGGCGGAGACGTC CTGGCCGTCAACCCTCGAAACACCAGCCGGACCTGTCCGGCTTGCGGGCATGTGTCTGCTG AGAATCGCAGAACCCAAGCCCGGTTCGCGTGCGTGGAATGCGGCTATGAGAATAACGCCG ACGTAGTCGGGGCAATCAATATTTTAGAGCGAGGGCATCGCTTGTTAGCCTGTGGAGAAG TCGCCGAAGTAAGGCGCTCTGTGAAGCAGGAACCCACCGAAGCGACTACGGGAGAGCTT GCTCTCGTGTAGCGCCGTAGGAATCCCCTTCCTTTAGGTGGGGGAGGATGTCAA	151
TnpB_17	ATGAGACTATTGCTAAAGGCGTTCAAAACGGAGATAGCCCCCACTGAAGGACAGAAAACC AAAATCATTCGTTCTATCGGCGTGGCTAGATTTCTGTATAACCAATATATCGCCTGCAATCG CCGTCTGTATAAGATGTATCAGCGTGGCATGCTTGACGAAAAACAGAAACGTTTTGTTACT GCTAACGATTTTGACAAGTATGTTAATCATAAGCTGAAAAAGGAACTGCCATGGATTGACC AGTGCGGCTCTAAGGCACGTAAGAAAGCCTTAGTAAACGCTGAACAGGCATTTCGGCGGT TCTTCTCTGGTACGTCTGGGTTTCCAAGTTTCAAAAAGAAAGCGAATCAGGACGTAAAGCT GTATTTTCCCAAGAATAATAACGGCGACTGGACGATATGGCGACATAAACTAATGATACCT ACGCTAAAACAAGTCAGACTTAAAGAGTTTGGCTATTTGCCCGTGGGTGCTAAAGTCACCA ATGGTACAGTATCTTATGTGGCTGGACGGTTTTATGTCTCCGTTGTGGTGGATATTGACGA AAAATCCAAGTATAACAAGGATTTGGAAGCATCGTATCATACGGTGACGGATGGTGTGGG CATAGATTTAGGCGTGAAAGACCTTGCTATTGTCAGTGATGGCAAGGTTTTCAAGAATATC AATAAGAGTTCAAAGGTCAAACGACTGGAGAGACGGCTTCGGAGAGAGCAGAGGTGCCT TAGCCGTAAATATGAGTTTATAAAAAAGAAAGGTGGTAAGACTGCTACTGCCTCTGCAAAC ATAGAAAAACAAAAGTTAAAGGTACAGATGCTTCATCAGCGTATAACAAAAATCCGAGAG GATTATGAAAACAAAACGGTTCACGAAATAGTGAAGCAAAAACCACGCTTCATTACGGTG GAAGATTTGAATGTTAAAGGTATGATGAAGAATCGGCATTTAGCAAAAGCTATAGCGGCA CAGAGATTCAATCATCTGTTAGCCAAGCTAAAACGCAAAGCAGAAATAATAGGGATTGAA CTTCGGGAAGTTGATAGATTCTATCCCAGCTCCAAGTCCTGCCATTCTTGCGGACATATATA TAAAGGTTTGAAACTCAAAGACAGAGTGTATGTTTGTCCAAAATGTGGTTATACAGCGGAT AGAGACTACAATGCTTCGCTTAATTTGCGGGATGCTAAAGAATATCGGGTAGCTTAATAAC AAAAGTTCCGATATATGTACCGATGGCTAGTCGGGAATTTACGCCTTTGGAGTGCTGCACA AACGCTAGTAGCTTGCCCCTTTGGGACGGCGAGAGCGAGCACGATGAACAAGGAATTTTT CTAAGACATATACACTTTTGTCTATGTTTTTAGTAGCag	152

TnpB Name	TnpB with wRNA overlap Native DNA sequence (single)	SEQ ID NO:
TnpB_20	ATGGTAAAAGCCATAAAAGTAATGTTGATACCAAATAACATACAGAAAACGAAAATGTTCC AGTACGCTGGTGCTTCAAGATTTGCTTATAACTGGGCTTTAGCCAGGGAAAAAGAGAGCT ATGAAAAAGAGGGCAGATTTCTCTCAGACTCAGAACTTAGAAAAGAATTTACAAGACTTA GAAATTCGGATGAATATGCATGGCTGCGGAATATTTCGAACAATGTAACCAAACAGACAA TTAAGGATGCTTGTACGGCTTATAAAAACTTTTTCAAAGGATTACAAAACTATCCAAGGTTT AAGTCAAAAAAGAAATCAACACCGAAGTTCTATCAGGACAACATGAAGATACGGTTTTGT GATACCCATGTTAAGTTTGAGGGTTTTGCTGTTTCTAAAAAGAAGAATAAGCAAAAACGAA ACTGGGTAAGGCTTGCAGAACGTGGTCGTATTCCTACAGATGCCAAATACAGGAATCCAA GAATCTCTTTTGATGGATTGAATTGGTGGATTAGTGTATGTGTGGAAGTTCCGGACTGTAA AAAAAGATTAAAGGATGATGGAATTGGGATTGATTTAGGAATTAAAGACCTGGCGATATG TTCAGATGCTACGAAGTATAAGAATATCAATAAAAGCCAAACCGTAAAGAAACTGGAGAA ACGCAGGCGCAGATTACAACGCAACGTATCTCGCAAATATGAACAAAATAAAGAGAAAGG AAAATACTGTAAAACAAATAATATAGTTAAGAAAGAAAAACTTTTGTTAAAAGTAAATCAC AGATTAACAAATATTCGTAAGAATTATTTACATCAAACCACATCGGAAATCGTAAATCGAA AACCAAGATTTATCTGTATTGAGGATTTGAATGTGAGTGGAATGATGAAAAACAGGCATTT ATCCAAAGCGATACAGAATCAGGGCTTTTTTGAGTTTCGAAAGCAGCTGGAGTACAAATGT AAGAATAATGGGATTCCACTTATTGTAGCAGATCGATTTTATCCGTCATCAAAACATTGCA GTTGCTGTGGAAAGATTAAAAAAGACTTGAAACTGTCAGACAGAATTTATAGATGCGAAT GTGGAAATGTTATTGACAGGGATTTTCAAGCATCTATAAATCTTAGAGTTTATGGAGAACA ATTTGTAAGTTAACACTTAAAAGTTACTACAAATATGTACGGGTACGTTAATTCGGAATTTA CGCCTATGGAGAGTACAGGAACTTGTTAGTAGACAGATATTTCTATCGTCAAAAGCATACT CGTTGAAGTAGGAATGAAACCTAAAAGTTTATAACTTTTTATAAGTTTTCAGTAACgg	153
TnpB_25	ATGATCAAAGCCCACAAAATACGCCTTCACCCCACAGAAGAGCAACAAGTGTATTTCGCCA AAGCCGCAGGAACAGCAAGATTTGTGTGGAATTGGGCGCTTGCAGAGTGGAATCGGCAA TATGAAGCAGGCGAGAAACCCACCGCTCTCTCGTTAAAGAAGCAATTTAATGCGATCAGG CGCGAGCAGTTCCCCTGGACATGGGAAGTGACCAAAAACGCCTCTGACCAGCCGTTTCTTG ACTTGGGAAAGGCATTTAAGGCGTTCTTTAAAGGATTGAAGAACGGGAAACGCAAGGGC CGACCAAAGTTTAAAAGTAAGAAGAGAAGCAAACCGAGCTTTTATCTGGCAAACGATCAA TTCGAGCTTGGCGATCATCGCATTTGGATCCCCAAATTGGGGTGGGTCAATCTGGCAGAG AACCTGCGCTTTCAAGGGAAGGTGACAGGTGCACGGATCACCAAAACGGCTGATTGGTGG TTTGTGAGTATTACCGTCGAACTTTCAGATGAACGCCTGGACAAGAGAAGAGCAGCAGTT GGGATAGACGTTGGTCTGAATCGACTCGCTACCTTGAGCACCGCAGAGGGATACGAGAAC CAAGCCTTCCTCAAAACGTCGCTCTCGAAGCTGCGTCAGGCCAACAAGCGGCTCCATCGAA GGAAACCTGGATCAAAGAATCGCGAGAAAGCTCGTCGTCAGGTGGCACGGTTGCACTACC GGATTAGCTGCATGCGCGAGGATGTCCTGCACAAACTGACCACCAGCGTAGCTTCCTGCTA TGGGATTGTTGGCATCGAAGACCTCAATCTCAAGGGTCTGTTCAAGAACCGGCGCTTGTCC CGTTCGTTCTCTGATGCGGCGTTAGGCAAACTGCTCACGCTGCTCACGAGCAAAGTAGAGC AGCGCGGTGGGCAGGTcATCAAGGTTGGGCGTTTCTTTCCCTCCAGCAAAACCTGTCATGG GTGTGGCTGGAAATGGGAGGACATGGAGCTGTCCGATCGCATCTTTCTTTGCCAAAACGG GGAGTGTGTGTATTACCAGCGTGCGCAAGATCGTGATCACAACGCATCACAGAATATCCT GTGTGAAGCCCTTCGCTTGATCGGGCTGGTTGATCAAGCCGTCAACGGTATTGGCTCAGAC GTGACGTAAACTTGGCTGTGGACCTGACGTAAGACCAAAAATGCTCCGGCATTGAGGCTG TTGGGGTTGAAACAGCAACAACAAAAGCTGTGGTGAAGCTATGCTTGCATACGTTTTACTA CATTAATTGGAGCag	154

TnpB Name	TnpB with wRNA overlap Native DNA sequence (single)	SEQ ID NO:
TnpB_15	ATGCGAAAGAGCTTCAAGACGGAAATAAACCCGTCAGAGGAACAGAAGATCAAGATCCG TAAGACAATAGGAACTTGCAGGTTTATCTACAACTTCTATCTTGCTTATAATAAAGAACTTT ATAATAATGGCGAAAAATTTATGAGCAGCAATAAGTTCAGAGTCTGGCTTAATAATGAATA TCTTCCACAACATCCGGAGTATTCATGGATCAAGGAAGCTTATTCAAAAGCAGTAACGCAG GCAGTGAACAACGGACAGACCGCATTTACAAGATTTTTCAATCATGAAAGTGCCTTTCCTA ATTTCAAAAAGAAAGGCAGATCTGATGTGAAGATGTATTTTGTAAAGAATAATCCAAAAG ATTGTCGATGCGAAAGACATAGGATCAACATCCCGTCACTTGGCTGGGTTCGTATCAAAGA AAAAGGATATATCCCAACCACAAAAGATGGATATGTTATTAAAAGCGGTTCCGTTTCAATA AAAGCTGACAGATTTTATGTTTCCGTTCTTGTAGAGATCCCTGATAACAAAATAACTGATGA TCCTAATGAGGGAATAGGCATTGATCTTGGATTAAAGGAATTCGCCATTGTTTCTAATGGT AAAACTTATAAGAACATTAACAGATCAGCAAGACTTAAAAAGCTTGAAAAACAACTTATTC GGAAACAGAGAAGTCTCTCGCGAAAATATGAAAATTTAAAGAAAGGAGAGTCCACTCAAA GAGCAAATTTACAAAAACAAAAGCGCAAGGTACAGAAACTTCATCATAAGATAGATAATA TCCGCACTGATTACATTAACAAAACAATCGCTGAGATAGTAAAAACCAAACCATCTTATATA ACGATTGAAGACTTAAATGTAAAAGGCATGATGAAAAACAGACATCTTTCAAAAGCAGTT GCATCACAGAAGTTTTATGAATTTAGAACAAAACTTCAAGCCAAATGCAGGGAAAAAGGA ATTGAACTTCGAGTAGTAGATAGATGGTATCCATCGTCTAAAACCTGTCACTGTTGCGGTG CTGTCAAGAATGATTTAAAACTTTCAGATAGGATCTTTAAATGCAGTTGTGGTTATGTCGA AGATAGGGATTTCAATGCTGCTTTAAATCTGAGAGATGTCACGACTTACGAAATTGCATAA CGAAACGCAAACGTGAGTATGTACCGAAGGCTATTTCGGGAATTTACGACTATGGAGTGT ACAAGAACCTGTGAGTAGATATGATCTCGGTCAATCCAAAGCATACACGATGAAGTAGTA AGTAGAACTCGTGAGAGTCTACATTATCTCGATGTGCGTATATTTGCACACGTTTTGAGTG GCag	155
TnpB_26	ATGATCAAGGCGCACAAAATCCGGCTCCATCCGACGCCTGAACAAGCAAGCTATTTTGCCA AGGCTGCTGGCACAGCGAGATTTACCTTCAATTGGGCGCTGGCCGAATGGAAGCGACAGT ATGAAGCGGATGGAAAACCATCGGCTTTTTCCCTCAAGAAGCAATTCCACGCCATCCGCCG CGAGCAGTTCCCCTGGTCGTATGAGGTCTCCAAGTCGGTGATAGAAGGTGCGTTCATAGA TGTTGCGGCGGCCTTCAAACAGTTCTTTGAGGGACTCAAAACCGGCAAAAAGCGCGGGTA TCCCAAGTTTAAGAGCAAAAAACGCTCAAAACAGGCCTTCTATTTAGCAAATGATCGTTTT GAAGTGGGCGACCATTGGGTGGAGATTTCTAAGCTCGGGCGCGTCAACAGTGCGGAGAA ATTGCGCTTTTCAGGCAAGATTCTTTCTGCACGGATCTCAAAAACTGCCTCCTGGTGGTTTA TTTCCATCACGGTTGAACTTCCCGATGAAACACCAGTCCCACACACCAAACCACCTGTCGGC ATCGATGTCGGCTTAAATCGCCTTGCGACCTTGAGCGGTGGCAAACAGTTTGAGAACCAA AAACCGTTGCGCACGCTCCTCAAGCAGCTTCGTACCGCTAACAAAAAGTTGTCTCGTTGTG TCAAAGGCTCTGCGAACTGGCAGAAGGCCAAAAAGAAGCTAAGCAGGCTGCACTATCGCA TTGCGTGCATCCGCGATGACCTCCTGCACAAGCTCACCACAGAAGTTGCCCGCACTTTTGG GTTGGTGGGGATCGAAGCCTTGCATGTGAAGGGACTCATCCAGAATCGCAAGCTTGCTTT GTCTTTCTCCGATGCTGCACTCGGCAAGCTCTTGCGTCTCCTTGAGAGCAAGGTGGCACAT GGGGGTGGCACGGTAGTCAAGGTGGACCGTTTCTTTCCTTCGAGCCAATTGTGTCATCAAT GCGGCTGGCGTTGGCACGCCATCACGCTTGCTGATCGCGTCTTTGTCTGCCAGAATCCCAC CTGTGGCTGGTGTGGGGATCGGGACTACAACGCTGCTGTGAACATTTTGAACGAAGCCCT TCGCCTCATCGGGGTTGCATTATCTGATGAGGTGGTGCCGGTAGTGGCTACGACGGCACC TAAAATCGCTGCTGGACTTGGGGTAAGACCAAAAAGGCGCTAGCCTTCAGGCACCTGGGG ATGAAGGAAGTACATCAAAGGATGTGCTAGGATTCACTTTCGAGTACATCCGATGTAGCag	156

TnpB Name	TnpB with wRNA overlap Native DNA sequence (single)	SEQ ID NO:
TnpB_14	ATGCACCAAGCGCACAAGATACGCCTGAATCCCACACCTGACCAGGCGGCCTACTTTCGCC GCGCGACTGGGACCCAGCGCTTTGTCTTCAATTGGGGACTGGCGCGGATCAAAGAGGCCC TGGATGCTGGCGAGACACCACCCCATGTGTTAGAACTGAAGAAAGCGTTTAACGCGATCA AGGGGGAACAATTCCCGTGGGTCTACGAGGTGACCAAGTGTGCGGTCGAAGGGGGATTT CAGAACCTCCAGGCTGCCCTGAGTAACTTCTTCCAGAGCAAGAAAGGTCAACGCAAAGGC AAGCGTATGGGCTTTCCCCACCGCAAATCCCGCAAGTGGGGGTATGGATCGCTCACGCTG GCCAACGATAAGGTGTCGCACGAGGGGCACACCGTCACTATCCCCCGGTTGGGCAAGGTG AACATGACGGAAGCGGTGCGGTTTGAAGGCAAACTACTCAAAGCCACCGTGTCCTACCTC GCGGGCTGGTGGTGGATCAGCTTTACCCTCGATGTCCCCCATGACACGCCTGAGCATCAAG GACATGCCATTGGTATTGATGTCGGCGTGAAAGACCTGGCGGTCGACTCTGATGGACAGC GGTACGAAAACCAAGCACCGCTTCGTCGCAGTCTCCGTCAGCTCCGTCGTGCCTGTCGCAA GGTGAGCCGTCGGATCAAAGGCAGCAAGAATCACCGGAAGGCCGTACTCAAGGTCGCCA GGCTCCACTACCGGATACGGTGTCAGCGGGTGGATGCCATCCACAAAGCCACCGCGGGCC TTGTGCAACGCGCGTCACTCATCGGTATTGAAGATCTCCACATCGCGGGCATGCTGCGCAA TCGCCGCTTAGCCCGCAGTCTCAGTGATGCGTCCATGAGCGAATTCCACCGTCAGCTCACG TATAAAGCCGAGTGGCATGGTGCGGTTGTGCAGCGGATTGGCCGCTTCTTCCCTTCGAGCC AGATCCACCATGGCTGTGGGGGACGCAAGACGGACCTCACGTTGAACGAGCGACATTGG GTGTGCCCGGCATGCGGGGCACTGGTCGAGCGTGACCTGAATGCGGCGCTCAATATTCGG GATGAAGCGCTGCGGTTGGTGCAGAGGGCAGCGTAACGGTGTTGGACGTCCCGGTCGTG GCTTCGTCGGGACGCAAATCGCCTGTGGATGCGGTGTCAGACTGGCCTTGCCAGCAGTCG CAGTCGAAGCAGGAACTATCAATGCGCACAAGTGCGCACGTAATAGGAAGCag	157
TnpB_2	ATGATCCTGACCTACAAGATCCGCCACAATACCGATTTCAGTGATGAACTGAAACAGGCGT TCAAGATAGCGGAGTTTGTAGTCAGGAATCCAAAATGCAGAACGTCAAAAGACGTTAAAG AGTTCGGACTGAAATCAGCAATATCAAATCAGATCCTCAAAAAGTATGGCAACCAGAAAA CCATCAAACAAGTCCACAACGTAAATTTGGTAATACCGAATCAGGCGATCAAAGTTGACAA GGACGCCAGAACGATCCGGATTGTACCGTTGAAACTGGTTCTGAACTATCAGTTCCCTGAT TTCCAAAAGGTCAATCAGATTGAACTTGACAAAGAGTTTGCCTATATTTCATGCACGGTGG CAGAACAACCGGAGATGATACCTCAACAATTCATTGGGGTTGACCGGAATACTACCGGAC ACGTTGCAGTCACAGCCAACCCGGATACCGGGAAGATTGAGAAACTTGGTAAGTCTGCAT TGCACATCCACCGTAAGTACTCTGCGATCAGGAAACAACTTCAACGAGAGGGAAAGTTCC GGAAGTTGGCAGTTGTTAAAGATCGTGAGAGTAGAATCGTGAAAGACTTGAACCATAAAA TCAGTCGTAAGATCGTTGATATGGCACAGGTACAACACGCAGCACTTGTCTTTGAAGATTT GGGCGGTATCCGTCAGACAAGGAAACAACACCGTTCGTTCAAATATGCCCTGCACAGTTG GTCTTTCTATCAACTACAGACGTTTGTAGAATACAAAGCCAAGCTGCTCGGTGTCCCTGTCC TCTACGTAGACCCGGCATATACCAGTCAGGATTGTTCCCGATGCGGTACACGCGGTCAACG AAATGGCAAGGATTTCAAGTGTCCGACTTGTGGACACGTTGATCATGCAGACGTTAATGC GGCATTCAATATTGCATTAAAAGTCAAAGACATGATCGATCGTACGCAGAAAGAGATGTG TACGATGGGAGCACTGATACCCCACGACGCAATGCTGGAATGCCCGGCAACGTTAGAACC CCACGCGCTTTAGCCGTGGGAGTATGTCAG	158

TnpB Name	TnpB with wRNA overlap Native DNA sequence (single)	SEQ ID NO:
TnpB_4	ATGCGCACCGCCTGCATTCGAGTGAAGGTGACCCCGGAGCAGGCCGCCAGATTGAGTGCG TTGCAGAGGGCGTATGCGGAGGCCTGCAATCGGCTCGTGCCGCTGGTGCAAGCCGCGCG CTGTTGGAATCGGGTGGCCTTGCATCAGCTCGGCTACCGAGCCTTGCGGCAGCAGACCTC GCTGGGCGCGCAGATGGTGTGCAACGCCATCTTCTCGGTGTGCAAGGCGTATCGCTCGCA AGGAGCTCTGGGACGCATCCCCCAAGACACGCCGGTCCCACCCCTGTCTTTTCACCGCACC AGCGTCCATTTCGACCATCGCACCTATACGCTCAAGGACGAGACGGTCTCGCTCAACACCC TGCAGGGACGCATGCGGGTGCTGATGATCCTCGGGGATCACCAGCGAAAGATCCTGACCA GCGGTCTGCCGAGGGAGGCGGAACTGGTGTTTCGTCGAGGCCAATGGTTCTTCAATCTCG TCGTAGAGTCGGCCGACGGCGAACGGGTGGCATCCGGCCCCGTGATGGGTGTGGATGTC GGGGAAAATACTCTCGCCGCCACCAGCACGGGCCGGGTCTGGGGCGGAGAGACACTGCG CCATCGGCGTGATCAACATCTGGCCCTGCGTCGTCGCCTCCAGTCCAACGGCAGCCAGAGT GCGACTCAACGACTCCGGCAGGTCTCCGGCAAGGAACGGCGGCGTGTCAGACACGTCAAT CACGAAACGAGCAAGGCGATTCTCGAGGAAGCCCGACGCATTGGCGCGGCCACGATCGT GATGGAGGACCTCACCCACATCCGCGATCGCCTTCGGGCCGGCCGGCGCATGCGCGCAAG GCTCCACCGCTGGGCGTTCCGGCAATTGCAGGGCTTCCTCGAGTACAAGGCCCGTGCCATT GGCATCTCCGTCGTGTATGTCAATCCGGCCTACAGCAGCCAGACCTGTTCGGCCTGCGGGC AGTTGGGTACGCGGCGCAAGCATCGTTTCGAGTGTTCCTGTGGTCTCCGGGCGCACGCTG ACTTGAACGCAAGTCGGAATCTTGCCCGGATTGGCGAGACTGCCGTTTCGCCAAGGGCGG TTGTAAATACGCCTGATGTGGGTTGCGTGGCTTGCCATGCTTCACCATAAAGCCTGCGACT TCAGTCGCGGGTTATTTAC	159
TnpB_6	ATGAAACGGCTGCAAGCCTTCAAATTTGTACTTCTGCCCAATGGCGCACAACAGCGCGACA TGCGCCGCTTTGCCGGGTCTTGCCGCTTCGTCTTTAATAGGGCTCTGGCGCTGCAACAGGC CAACCATCAGTCCGGCGGGAGATTCATCGGCTACCTCGCCATGGCAAAGCACCTGACCGA GTGGCGCCATGGCGCTGAGACGCCCTGGCTCAAGGATGCGCCAGTTCACCCCCTGCAGCA CGCGCTCAAGGATTTGGATCGGGCCTACAAGAACTTCTTTGCCAGGCGAGCCGGCTTCCCG CGATTCAAGCGCAAGGGCAGTGGCGAGAGCTTTCGCTACCCCGATGCCAAGCAGTTCGAA ATCGACCAGTCCAATAGTCGCATCAAATTGCCCAAACTGGGCTGGATCCGCTATCGCAACA GCCGCGAGATTCTGGGAGCGGCCAAGAACATCACCTTGGCCACCTTGAGCGATGGCAGTT TCATTGCCCCGCTCAACAGCTTCAGGAAGCACCAGCAACGGCTGGCACGTTATCAACGGC GCATGAGCCGCAAAGCCAAGTTCAGCAACAACTGGAAGAAGGCAAAAATCAAAGTACAG AAGGTTTACACGCAAATCGCCAACGCGCGTAGGGACTTCCTGCACAAGACCACTACAACG ATCAGCAAAAACCACGCGCTCGTCTGTATTGAGGATTTGCAGGTCAGGAACATGTCCAGG TGTTCGAAGGGGACAAGCGAGCAGCCGGGTAGCCGGGTCAGGCAAAAATCCGGGCTGAA CCGCGCCATTCTCGATCAGGGATGGGCAGAGTTCAGGCGGCAACTGGCATACAAGACCCA ATGGGCAGGCGGCATGCTGGTGGCAGTGCCTGCACACCACACCAGTCAGACCTGCCCCTG CTGCGGGCACGTGGCGCCCGCAAACCGACGCACCCAAGCCCAATTCCTATGCGTCGAATG TGCGCACACCGCCAATGCAGATGTGGTTGGCGCGATCAATGTTTTAGAGCGCGGACAGCG CTTGGTAGCCTGTGGAGAGGACGGCTCTGGCCGTGGTCGCAAGACGACGACGCAACCAG CCTCTGTGAAGCAGGAACCCACCGAAGCGACTATGCAAGAGTTAGTTCATGCATAGCGCC GTAGGAATCTCCGCCGTTCACGGCGGGGAGGACGTCAA	160

TnpB Name	TnpB with wRNA overlap Native DNA sequence (single)	SEQ ID NO:
TnpB_3	ATGATTCTGACATACAACATCAAACATGAAAGAGATTTTTCAAGCGAGTTAATCCAGGCAA AAAAGATAGCTGATTTTGCCATAAGGACAAAAAGTAACTCAAGCAAGGATGTACGTCACTT GGGATTAAAATCAGCAATAGCAAATCAGATATTAAGAAAATACAGAAAGTCTGGAATCAA AAAAACATCCAGTGTCAATCTGACCGTACCTGGACAATCAGTCAAATTTGACAATACTGAA AAACTGGCCAAAATTTCATGTCTAAATCTTGAGCTTGATTGTATGTATCTACCAGAATTTAA AAGATAAGGCAGATAGAGATTGGAAAAACACTGGCTCATGTATCAGTTGAGATTTCAGA ACCTGAGACAAGAGCATCTGAAAAATTCATTGGCATTGACTGTAACACGACAGGTCATGCA GCAGTAATTGCCATACCTCACACAGGCAAAATACACAAACTTGGCAAATCAGCAATTCACA CACATACAAAATACAAACAAATTAGAAAAAAACTACAGAAAAAAGGCAAGTTCTCACTGCT CAAAAAGATAAAAGACAAGGAATCTCACATTCTAAAAAACATCAACCATCACATATCAAAA AAAATCATACAGATTGCCGTCTCTGAAAAGTCAGGAATTCGATTTGAGAATTTGAAAGGTA TAAGAAAGAACAAAAAGCATTCCCGAAAATTTCGCTATTCTCTAAACTCTTGGTCGTACTAC CAGCTACAACAGTTCACACAGTACAAGGCCAAAAAGCAGGGAATAGATGTTGCCTATGTC GCACCCGCATACACAAGTCAGACTTGTTCAAGATGCGGGTCTTTGGGGGACCGAGACGGA AAAAAATTTCAATGTGTCTGCGGACACGCCGACCATGCAGATGTCAATGCGTCATTTAATA TTGGAAAACCAGTATCGCATTGTCTCATAGATCCTACAAGGATTAAAAGCAAGAGTCAGTT GCACAAAGAAAGTGATTTGTGCAAGGGGAGCACTGATACCCCACAAACGGCAATGTCAGA AATGATGATAACCGTAGAACCCATGAAGCTTTAGCGAGTGGAGTATGTCAG	161
TnpB_7	ATGAAAGTAATTCACAAATCATATAAGTTTAAAATTGCTCCTGATAATGAGCAAAAAGAAC TTCTTGCAAAACACTTTGGTGCTTGTAGGTTTGTGTTTAATCGTTACTTAAACAGTAGAAAA GAAACATATCTTGAAGAAAAGAAATCGCTTAACTACTACGATAATGCAAATGACCTTACTG TTTTAAAAAAGGATGAGCAGTTTGTTTGGCTTAAAGAAATCAACTCACAAAGTTTGCAATC ATCACTTAGGAATTTAGATACTGCATATAATAAGTTTTTTAGAAAGCAAACTAAATTCCCTC GATTTAAAAGTAAGTTTGACAAACAAAGTTTTACCATTCCACAATCGGTATATATTGAAGAT GGTAAACTGCAAATACCAAAATTTAAAAAAGGTATTGAGATAAATATTCATAGAGAAATTG AAGGTAAACTTCTTTTTGCAACAATATCAAAATCTACAACAGGTAAATACTATGTAAGCATT ACTTGCGAAGTTGAATATATTCCATTTGAAAAAACAAACTCAAAGGTTGGTATTGATACAG GAATAAAGGATTTAGCAATACTTTCAGATGGTAAGGTGTACGAAAACATTAAGACACTTAA AACCAATTTAAAGAAGCTAAAATATAACCAAAGGCAATTATCTAAAAAAGTAAAAGGAAG CAATTCCAGATTAAGACAAAAATCAAAACTTGCAACAATACACGAAAAAGTAACCAATATC AGAAAAGATTATTTGCACAAAGTCAGTACAGAAATCATCAAAAACCACGATGTAATTTGTA TTGAAGATTTGGCTGTGAAAAATATGATGAAGAATCATAAATTGGCACAAGCATTTTCTGA TGTTTCGTTGGGTACTTTTTACACTATGCTTGAATATAAAGCTAATTGGAATGACAAGGTAA TCGTAAAGATTGACAGATTCTTTCCAAGCAGCAAGACTTGTTCAAACTGTAATTATATCAAT CAAGACCTAACTCTAAAAGATAGAGAGTGGACTTGCAAAAGTTGTAATACAGTACACGAC AGAGATTTTAACGCAAGCATAAACATTAAAAAACAAGGTTTAAAAATACTGTCTGGTTCAG GAATTGAGTCGGACATTAAACAAAAACGGAGTAAGGCGTTGCCATTAGGCGAGTCTATGA CCTCCGAAGCCCATCCCATCAGCTTTGCTGTGGGTGGGTAGTTCAC	162

TnpB Name	TnpB with wRNA overlap Native DNA sequence (single)	SEQ ID NO:
TnpB_10	ATGAGAACATATAAATTCAGGATTTACCCCTCAGATTCACAGATCACCATACTGAACAATAC TCTTGAACTGTGCAGTGAATTATACAATTCCATGTTACAGCAGCGAATATACGCTTACAGAT CGGGGAAGAAGGTCAATTACAAATCTCAGCAGAACGAAATACCTGAAATAAAGAATACAT TTCCGGAATACCATCATATCCATTCGCTTGTCATACAGGATGTTGCAAGGAGATTAGATAA GGCATATGGCAACTTCTTCAGAAGGGTGGGAGAGAAGAAGAACGGAAAGAACATAAAGG CGGGATTTCCAAGATTCAAATCTAAAGACAGGTACAGTTCCATCACATACACCCAGTCTGG ATTCAGCATACTGGATAACGGCCATGTGTGGTTATCAAAGATCGGTGAAGTGAGGATGTT CATGCACCGATCCGTTAATGGAGATATCAGGACAATATCAGTAAAACGTGATTCTGTTGGA GACTGGTTCATAACATTTACTGTTGAAACACATAGCAACAGGGATAAATCGGAAGGAAAC GAAATCATGCAGGATTCACAATTCCAACATGTCAAACCGGTTGGCATAGATATGGGCCTGA AATCCATCATCACCACCAGTGACGGTATACAGATCGATCCTCCCCGTTCTCTGGAAAAATCA GACAAGAAACTTTCAAAGGCGCAGAGGAATCTATCCATGAAGAAGAAAGGATCGGGAAA AAGAAGGAAAGCAAAAACAAGGGTGGCAAAGATCCACAGGAAGATAGAGAGGCAGAGA AATGATTTCGCACATAAACTTTCCAAATACCTTGTGGAAAATCATGATCTCATTGTTTTCGA GTCCCTGAACATAAACGGGATGGTTAAGAATCACCATCTTGCAAAAAGCATAATCGATGCA GGATGGAATCATATCATCCAGTATACAACATACAAGGCTGAAAGTGCCGGTAAGGTTGTA ATACTGGTCAATCCCAGGAATACGTCAAGAAAATGCTCAAAATGCGGCAACATTAAACAC GATCTGAAACTTTCAGACCGCACATACCACTGCAACACATGTGGTCTTACCATAGACAGGG ACCTGAATGCAGCAATAAATATCCGCAATATGGGACTGATAAAGATAGGGGGGGGCACTC CCGAATTCACGCCCGTGGAGATCCGAGCTCTGCCAGCAATGGCAACTCCCGTCGTTGAAAC GGGAAGCCCCCTGCGTTAGCCAGGGGAGGATGTCAC	163
TnpB_12	ATGAAAAGAAGTCTTAAATTTAGTCTTAATTTTAGCAATCTAAAAAAGAGAGTTCTTCTGG ATAATTTATATATAGAATACCAACAGATAGTCAATCTATTTCTCCAAAAACTTCTCAACAAA GAGGGATTAGCAGAATCTTATCTTAAATCTATCTTCTCTCCGTTGTCTTATCGTTACAGGCA ATGTGCCAAGAGACAAGCGCTCCAGATATTCAAAGTTTGGTGTAGGAGTAAAAAGAAAGG GGAAAAGCCAATGTTTAATGGTTCTCTGGTTTTAGATGGTAGGTTTATCGAAATTCAGAAA GGCCGGAACTCATTTGATTACTGGATCAAAATTTCAACCTTGAATAAAGGTAATCCCCTCCT AATTCCTATCAAATCCTATAATTATGCCAATAAATATTTCACAGAATGGAACTTAGTTAAAG GTGGTAGATTGCAAAAGACGGATAAGGGCTGGTTTATAATTCTTACATTTGAAAAGGAAA CCCCTCCAAAAAAAGAAATAGGTAAAACTATTGGAATAGATATTGGAATTAAAAAACTTAT GGTTGATAGCGATGGTAATACCTATGGTCAGCAGATAGAACCTTTGATGAACAAAATTCA GAGGAAACAACAAGGTTCTCTGGCATTCAAAAGAGCCTTAAAAGAGAGGGATTGTTACAT CAATAAAACAGTTAAAGAAGTTCCTCTTGATGAAGTAAAGACTATTGTTCTGGAGAACATT AAAGACATTAAAATAAATACTAAGAAAGAGAGGAGATTAAATAAAGAGTTCAGAAGTAA GTTTCAGAGATGGACTTACTCTAAACTTATAGACAGGATAAAGCAGTTAGCTGAAGTAAGT GGCGTCCACTGTCAGCTGATTGCTCCTACCTATACATCGCAAACTTGCAGTAAATGTGGGT TTGTCCACAAGCTAAACAGGACTAATGAGATGTTTAGGTGTAGGAATTGTGGTTACACTTT GGATGCGGATTATAATGCGTCTCTGAACATCTTGAATCTTGACTTAGCCCAGCAGTCTATG GTCGCTGGAAATATGAAAGGCAATATTTGTCTATAAAATTGTCAACTATGTTGAGACATAT TTTTATTTCAATCCAGTGGCTGGTACCCAGAAAGGATTTCATAAGTACAACGACCTTCTGG GAAGATATGACCTTATGGCATAAGAGAGTAAATTAATAAAGTAATAAATGATTTCAAAAAA TGGTTAGATGAAATATCACTCTTCATCTGAAATGCTTTTGGA	164

TnpB Name	TnpB with wRNA overlap Native DNA sequence (single)	SEQ ID NO:
TnpB_18	ATGCAGAGGGCGCATGTGATCAGACTGAATACCACGCCAGCACAAGAGACGTACTTCCGT AAAGCCTGTGGGGTCTCAAGGCACGCCTATAACTGGGCACTGGCTCGCTGGAAAGAAGCT CGGGCAAAAGGTGAGCGGGTCAAGATGAAAGACCTCAAGCGCGAGTACAACCAGATCAA GGGTGAGCAATTTCCCTGGGTCTACGAAGTGACGAAATGCGCTCCAGAGCAGGCATTTGC TGATCTTGGGCAAGCCTTCGCCAACTACTGGCGCATGAAAGAGGAAGCAACCCAGCCCAA GCTCAAACATCCACGGAAGGATGGCGAAGAGGCAGGCTTCCCGCACTTCAAAAGCAAAAA GCATGATCGATTGAGCTTCTACCTTGCCAACGATAAGTTCTCGGTCGATGGGCATACGCTC CACGTTCCAAAGTTGGGCGCTGTCAACATGACTGAAGCGCTCCGGTTTCAAGGCAAGATC ATGAGTGCCACTATTTCCTATCGGGCGGGCTGGTGGTTCGTTGCTATCGCGGTTGAGGTG GAATGTGAGATGCCGCCTCATCACGGCGGAACTGTTGGGATAGACCTGGGCATTAAGACG CTGGCAACCTTAAGCGACGGTGAGAAGTTTGAGAACCAAAAACACTATCGTCGGAACTTA GGACGCATCAAGGGCTTATCCCGCAAAGTCGAGGGCAGTCAGAACTGGTGGAAGAACGC GAAGAAACTGGCCCGTGCCCACTATCGCTTCGCCTGCCAACGAGAAGACACGCTGCACAA GATGACCACGCAAGTGGCACAAACCTATGCGCTTATTGGCCTGGAAAACCTCAACGTGAA AGGCATGCTGGCTAACCATTGCTTAGCGCAAGCAGTAAGCGATGCCAGTTTCTTTGAGGTC AAGCGTCAGCTTTTCTACAAGTCTGAGCAGTACGGCGGCTCTGTGCAACTGGTCGACCGAT GGTTTCCCTCATCGAAGACCTGTCACGCCTGTGGCTGGGTCAAGGAAGATTTAACACTTGC CGACCGTGTCTGGGTGTGCGCACAGTGCGGTACCGTTCATGACCGTGATTTGAACGCCGC CATCATGGTCGAAATTGAAGCTCTCCGTCTGGTGACGGAGGTTCCGGCAGTGGCTTCGTCG GAACGTAAAATCGCCTGTGGAGCGGAACGCTCTGGCCCAGCAATGGGTGAAACGGTCTGC GGTGAAGCAGGTACGACGATGCTGTAAACACGTTTTACTACATTTGAGAAAGCag	165
TnpB_27	ATGAGCAAGACGGTACAAAAATCTTGGTTCTCAACCAAGACGGTTTATCCCCAGAGCGAG AATTGGTGGAAGACATACTTGCCATTGTTCACGTCTTCAGTTGTAGATTATACGGACTCAG AAAGTACAAATCTGCAATCAAAGAAGATCCGGATCTACCCAAGTCCTGAACTAAAAAAGG TATGGAAGAAATGGTTGGCGGCTTGTCGTTATTGCTATAACCAAGCTATTGCTATACAGCG TCAGTCACACAAAAAACTCAGTAAGTTAAATCTGAGAAATTTGGTCATGCAATCTGATTTA CCAGCGTGGGTAAAAGAAACACCATGTCATATTAGACAAAACGCCACGTTTGACGCTCATC AAGCTTATACAGCTAGTCGTGATGCTAAGTTTAAAAGTGTGCGGGATGCTAAACAAACGA TAAAGTTTAACAGTAGTAATTTTACCCAAGGAAAGTGGTATAGTCAGCTTACCAAAAAACT CGGATTCGAGTCTAGTGAACCAATACCAGAATCTTGTGATTATGGTACTCAGTTAGTCTATC GCCGGGGTAAATGGTTTGCCATTTTTCCTGAACCATTAGTTAAATTATCTCACCAAGCTCAA AAAATCATCGCTATAGATCCGGGAGTGAGAAGTTTTTTAACTGGATACGATGGCGACAAA GTGATTGAAATTGGTGGTGGTGATATAGGGAAAATTACTCGTTTGTGTCATCACTTAGATA ATTTGATGAGTCGTTCTACACAGGTAAATGCTCAACGTCGCCGTGGGATGAGGAAAGCGG CAAATCGGATGCGAGAACGAATCCAAAACTTGATTGATGATGTGCATAAAAAATCAGCTA ATTATCTTACCAGTAATTACAGATTAATCTTTTTACCTAGATTTGAGACATCCCAAATGGTA GCCAAAACTAGGAGAAAAATTAAATCTAAAACTGTGAGAAGTATGTTGACTTGGGCGCAT TATAGATTCAAGTTAATTGTTAAACATCAAGGATTAAAGCGTGGTTGCGTGGTACTTGATG TTAGTGAAGCCCATACATCTAAAACTTGTGGTGTATGTGGACATCAGCACTCTAAATTAGG TGGCTCTAAGGTGCATAAATGCCCGAATTGTGGAGCGCAAACACCGAGAGATGCTAATGG CGCACGTAATATTATGTTACGTGCTTTGAGGGATTCCTCCTTTACTGTCAGTAATGATGGTA TTGCTATCGTTACAGTTCGAGCTTTGTCAATCAATGCTCAGAAATGTTCAGCGTAAATGAAT Cag	166

TnpB Name	TnpB with wRNA overlap Native DNA sequence (single)	SEQ ID NO:
TnpB_19	ATGCTAAAGGCATTCAAGACAGAAATAGCACCGACTAGAGAGCAGAAACAAAAGATAATA CGCTCAATAGGTATAGCAAGATTTTTATATAACCAATACATTGCTTATAATAAAAAGTTATA TAAAATGTATCAGCGAGGTTTATTAGATATAAATCAGAAACATTTTATATCTGCTAATGACT TTGATAAATATGTAAACCATAATCTGAAGTTGAAATTGCCGTGGATTGACGAATGTGGTTC TAAAGCACGAAAAAAAGCGCAAGTCAATGCCGAAGCAGCGTTCAAGAAGTTTTTCAAAGG CGAAGCCGGCTTCCCGAGGTTCAAGAAGAAATCTAAGCAAGATGTAAAGCTATATTTTCCT AAAAATAATAAAGGTGATTGGACGATATGGCGGCATAAGCTAATGATACCTACGTTAAAA CAGGTTAAGCTAAAAGAATTTGGCTACTTACCGGTAGGTGCTAAAGTCACTAACGGCACA GTATCTTATGAGGCTGGCAGGTTCTATATTTCTGTCGTGATAGATATTGATGAAAACTCCAA GTACAACAAGGATTTGGAAACTTCATATCGTACAACAACCAAAGGCATTGGTATAGATTTA GGCATCAAAGAATTGGCTATTGTAAGCAATGGCAAAATATTTAAAAATATCAACAAAAGTT CAAAAATCAAGAAACTTGAGAAACGGCTTCGGAGAGAGCAGAGACGCCTTAGCCGTAAAT ATGAGTCTAAGAAAAAGAAAGGTGGTAAGACTGCTACTGTCTCTGCAAACATAGAAAAGC AAAAGTTAAAGGTACAAAAACTTCATCATCGCATTGATAAAATCCGCGAGGATTATGAAAA CAAAGTCATCCATGAAATTGTAAAGCAAAAACCACGCTTTATTACGGTGGAAGATTTGAAT GTCAAAGGCATGATGAAGAACAGGCATCTGGCAAAAGCTGTGGCAGCACAAAGATTTAAT TATCTGCTGACTAAGCTGAAACGCAAATCAGAAATCATTGGCATAGAAATGCGTATAGTTG ACAGATTCTATCCAAGTTCCAAAACTTGTCATTTTTGTGGGCATATCCATAAAGGTCTGAAA CTCAAAGACCGTGTATATATTTGTCCTGAATGTGGCTATATACAAAATAGGGATTTCAATG CTTCACTAAATTTGCGGGATGCCAAAGAATATCGGGTAGCTTAATAACAAAAGGCCCGATA TATGTACCGATGGCTAGTCGGGAATTTACGCCTTCGGAGTGCTATACAAACGTTAGTAGCT TGTCTTTCAAGGCAGCGAGAGCGAGTACGATGAACAAGGAATATTTCTAAGGCATAAACA CTTTTGTCTATGTTTTTAGTAGCag	167
TnpB_21	ATGAGGAAATTGCTAAAGAGCTTCAAGACGGAAATAAATCCGACGATTGAGCAAAAAATC AAGATTAACAAGACTATTGGCACTTGTAGGTACGTCTATAACTTCTATCTCGGTCACAACAA AACTTTATATGATAACGGTGAAAAGTTTATGCCTGGCAAGGGTTTCAGCGTATGGCTTAAT AATGAGTATATTCCTAATAATCCTGATAAAATATGGATTAAAGAAGCATATTCAAAAGCTG TAAAAAAGTCTATTGAAGATGGATGTACTGCATTTACAAGATTTTTTAAACATCAAAGCGC TTTTCCTAATTTCAAAAAGAAAGGTAAATCTGATGTAAAAATGTATTTCGTAAAGAACAATA CTAAAGACTGTAGATGTGAGAGACATAGGCTGAACATACCCACTTTAGGTTGGGTACGCA TTAAAGAAAAAGGTTATATACCAACAACTAAAGACGGATGGAAAATCAAAAGCGGTACAG TATCCGTCAAAGCAGACAGATACTATGTGTCAGTTCTTGTAGAAATTCCCGACGTTAAGAT TGCTAATAATAGCAATGGTGGTATAGGAATTGACTTGGGTTTAAAAGACTTGGCGATTGTT TCCAATGGTAAAACTTATAAAAATATCAATAAGTCAACAAGAATTAAAAAATTGGAAAAGA AACTGCGTAGAGAACAAAGATGTCTCTCACGAAAATATGAGAACTTAAAGAAAGGAGAGT CCACTCAAAAGAATATACAAAAGCAAAAGCTCAAAGTACAAAGACTTCATCATAAAATAGA TAATATCCGTACTGATTATATCAATAAATCAATAGCTGAGATAGTGAAAACCAAGCCATCTT ATATAACTATTGAAAATTTGAATGTATCAGGAATGATGAAGAACAGCCATCTTTCAAAAGC TGTTGCGTCACAGAAGTTCTATGAATTTAGAACCAAGCTTAAAGCAAAATGTGATGAAAAT GGTATTGAATTAAGAGTCGTAGACAGATGGTATCCATCATCCAAAATATGTCATTGCTGTG GTGCTATCAAGAAAGATTTGAAGCTTTCAGATAGAATATATCGTTGTGATTGTGGCTATGT TGAGGATAGGGATTTCAATGCTGCTCTTAATCTAAGAGATGCTTTAACTTACGAAGTTGCA TAATAAACGCAAACGTAAGTATGTACTGCGGGCTATCGCAGGAATTTACGACTGTGGAGT GTACACGAACTTGTGAGTAGCGTATTGTTTACAATCGCCAAAGCATACACATCGAAGCAGT AAGAAGTATCCGCAAGGACTTTAATTTCTCGATGTGTTTGAGTATATTTGAACACATTTTGA GTGGCag	168

TnpB Name	TnpB with wRNA overlap Native DNA sequence (single)	SEQ ID NO:
TnpB_22	ATGACGATCAAGACACAAGTAGTTAAGCTAAAAGTTAATAAGACCATGCAAAAGCATCTT GATGCTCTATGCGACTATCGTCGATACTGCTGGAATAAAGGCTTAGAAACTTGGCAATTAA TGTATGAAGCTCATACATTAAACAAAAAAGATAATCCCAGTCCTAACGAACGCAGAGTCCG TGATGAACTAGTCGCAAATAAAGCCGACTGGCAATATGATTTGTCTGCCAGATGTTTACAA TTAGCGATTAAAGACTTAGCTAATGCTTGGAAGAACTTCTTTGATAAGTCACAACCTGATT GGGGAATACCTAGTTTTAAATCTAAGAAAGCTCCCAGACAAGGCTTTAAAACTGATAGAGC TAAGATCGTTAATGGTAAGCTTCGCCTTGATCGCCCAAGAAGCATTTCAAAAGAGTCTTGG TTTGATTTAAAAAGCTATGAAGCTCTAAAGATGGATGAAGTCAAAGTAGTAAGTGTCTTCA AAGAAAAAGATAATTATTATGCGGCTCTTCCTTATGAAGAAGAAATTGAACTAAAGGCTAA AACCCAGCAAAAGACAGCAGTTGATGTCAATGTTGGTCACTTTAACTATACCGAGGGGCA AATCAATATTTTGCCTGCTAAATTGCAAAAGCTTTATAAGCGTATTAAGCATTATCAAAGAA TGCTGGCACGTAAAAGAGAAGTTAACGGTAAGTTAGCTACAAAATCAAATAATTACTTTGC AGTGAGAACCAAACTGCAAAGAGATTATCGCAAAGCAGCTAATATTCAAAACGATCTATTA CAAAAATTTACTACTAAGCTTGTTAATAATTACGATCAAATTGTAATTGAAGATTTGGCAGT CAAAGAAATGATGATGACGCATGTAGCTTCAAAAGGAATGCAGAGATCACTTTTTAGCAA GTTTAGACGAATACTAACTTATAAGTGTGATTGGTATGGCAAAGAATTAATCTTGGCTAAT AAAACATACCCATCAACTCAAAGATGTGCTGCGTGCGGTTATGGCAAAAAAGGCGAGGAA AAGATCACTTTGCAGGGCAATAAAAAGCATGGTACCAAACATAATGAGTATGTCTGTTATG AGTGTGGCTACAAGAATGATCGCGATGAAAATGCAGTTTTAAATCTTTTAGCTTTGGCAAA ATAAAGAAAATAAACAGGGCTGGCTAGGTCCTTAAGCTGTAAGAGCTAGTCAATGTGATT ACTCCTATTTGGAATATCAGAATACTAGTGAAGACGACAGTAAATGAAACAAAGAAAGGA AAAATATATCTTTCTGATATGTAGGAAATTCGTATTCTTCTACATATTTCCATGTTTTATATA GCag	169
TnpB_23	ATGATCCGAACGCAAAAGGTCAGGCTGTATCCGAACAAAACGATGCAAAAGGCGATTGAT CAGCTTTGCGATTATCGGCGCTACTGCTGGAATCAAGGCTTGGCGCTTTGGAATGATATGT ACGATGCTTCGTTGATTTTAGAAGACAAAAAGTTGCGTCCAAATGAGCGCAAGGTTCGTG ATGAACTGGTCGCCAATAAAGCTGACTGGCAGTATCAGCTATCCGCTCGGTGCCTACAGCT TGCAATTGCCGATCTTAGCAAGGCTTGGAAGAACTTCTTTAACAAGGCTCAACCAGATTGG GGCAAGCCCAAGTTCAAATCAAAGAAAGCTCCAAGACAAGGATTTAAAACTGACCGGGCT AAGATTGTTGATGGCAGGTTGCGCTTAGATAAGCCGCATGGTGTTGAGAAATGGTACGAC ATTCGGTTTAATGGTGCCAAATCTTTGAACGGTGAATTAAAAGTCGTTTCAATTTACCGGG AAAACGGTAAATACTGGGCTAGCTTACCCTTTGAAAGAAAGACAGAAGCAAAAGCTGATA CCGCTAAAAAGACGGCAGTTGATGTCAATGTAGGGCATTTTAATTATACTAACGGGCGGA TTAACACTTTACCGCATAATCTAAATAATTTATACAAGCGCATCAAGCATTACCAACGTCTA TTGGCTAGGAAACGAGCAGTAAATGGACAGAAAGCAACGCAGACAAACAATTACGTTAA GACGAGAGCCAAGTTGCAGCGTGATTATCGGCGGGTTGCCAATATCCAGCATGACATTGT CCAAAAATTCACCACTAAGCTGGTTAATGATTATGATCAGATCGTTATTGAAGATTTAGAC GTAAAGCAAATGCAGATGAGCCATGTTGCCTCTAAAGGATTACAGCGGTCGTTATTTGGCT ATTTCAGACAGGTGCTAACTTATAAATGCGAATGGTATAACAAGCATTTATTATTAGCCGA TAAGTTTTATCCAAGCACTCAACGGTGTTCTCAATGTGGGTTTGTTAAGACAGGTAAAGAT AAAATCGGTCTTAATGGCAACATCAAACATCACACTAAGCATAACGAATATATTTGTTATGC ATGCGGTGCTGTTATGGATAGAGATAGAAATGCAGTGATGAACTTGCTTGCATTATTATAA AGAATTAATGGGTGGGCTACACCCGCAAGCTATAAGAGTTAGTCAATGTCATTACCCTCTA GTTAGGATATGGGAATACTAATGTTGACGATAGTAAATAAAATTAAGAAAGGAAAACTAT ATTCTTTCTAATAACATAGAAAACTGTATTACGGTTTCTACGTTATTCTACATTTTATATAGC ag	170

Table 9: TAM Sequences and gRNA Sequences for Selected TnpBs (In Table 9, the gRNA sequences are listed as DNA sequences that are the equivalent RNA sequences (except the RNA will have the T replaced with a U)

TnpB	TAM Sequence	gRNA ID	gRNA sequence	gRNA SEQ ID NO:
TnpB _5	TTTAA	g1	gtccactagttttaga	171
TnpB _5	TTTAA	g3	tccatctacatgtttc	172
TnpB _5	TTTAA	g7	ttacttgaagcagtaa	173
TnpB _5	TTTAA	g9	tagtaatcaaagaact	174
TnpB _5	TTTAA	g2	cggagccatccgaatc	175
TnpB _5	TTTAA	g5	ccagtgttttgcaact	176
TnpB _5	TTTAA	g6	gggtgctggtaggaca	177
TnpB _5	TTTAA	g8	actatctgatgaaaac	178
TnpB _8	TTTAT	g1	tcggtgagagagtggt	179
TnpB _8	TTTAT	g3	tttgtcgtagttactt	180
TnpB _8	TTTAT	g4	agcttcaatttttaga	181
TnpB _8	TTTAT	g8	ttggagaacttgggat	182
TnpB _8	TTTAT	g15	aaaccctgatgaactg	183
TnpB _8	TTTAT	g16	aaagtttagcgccttt	184
TnpB _8	TTTAT	g17	ctcactggcaatcata	185
TnpB _8	TTTAT	g20	ctgtaggaatattacg	186
TnpB _17	AGGAG	g1	gaggagtatttggttt	187
TnpB _17	AGGAG	g2	gagtatttggttttta	188
TnpB _17	AGGAG	g4	gctagtggaagtagcc	189
TnpB _17	AGGAG	g5	gaggctagtggaagta	190
TnpB _17	AGGAG	g6	acgcagcgaaggagga	191
TnpB _17	AGGAG	g8	gagtactgctggtcct	192
TnpB _17	AGGAG	g9	tactgctggtcctttg	193
TnpB _17	AGGAG	g10	cactacggaaggatgc	194
TnpB _17	AGGAG	g12	acgcttatgtgtttgc	195
TnpB _17	AGGAG	g13	cttcaggatatcgact	196
TnpB _20	GGAGG	g1	aggagtatttggtttt	197
TnpB _20	GGAGG	g11	attaccatccaagaat	198
TnpB _20	GGAGG	g12	aacacgtttagaaaaa	199
TnpB _20	GGAGG	g3	aaatgcaatgggaaca	200
TnpB _20	GGAGG	g6	cactttcatctggagg	201
TnpB _20	GGAGG	g7	ttgtgaactaatggga	202
TnpB _20	GGAGG	g8	aggagtactgctggtc	203
TnpB _20	GGAGG	g9	agtactgctggtcctt	204
TnpB _25	AAGAG	g13	gacaaaacttaaagat	205
TnpB _25	AAGAG	g14	agtaaggttaagtttg	206

TnpB	TAM Sequence	gRNA ID	gRNA sequence	gRNA SEQ ID NO:
TnpB _25	AAGAG	g3	aaaatgcaaggaggag	207
TnpB _25	AAGAG	g4	atttggaggaaatgca	208
TnpB _25	AAGAG	g5	cttggtctgaaaacca	209
TnpB _25	AAGAG	g6	aggaaattgcaggtag	210
TnpB _25	AAGAG	g7	atgttcttggtggaaa	211
TnpB _25	AAGAG	g8	agtatcagtaacgaaa	212
TnpB _15	AGGAG	g1	gaggagtatttggttt	213
TnpB _15	AGGAG	g2	gagtatttggttttta	214
TnpB _15	AGGAG	g4	gctagtggaagtagcc	215
TnpB _15	AGGAG	g5	gaggctagtggaagta	216
TnpB _15	AGGAG	g3	tatttggtttttatat	217
TnpB _15	AGGAG	g6	acgcagcgaaggagga	218
TnpB_2	TTTAC	g1	gaattgatgaccatat	219
TnpB_2	TTTAC	g3	tggaagatatcttttg	220
TnpB_2	TTTAC	g4	ctatcttattggagag	221
TnpB_2	TTTAC	g5	atattttctgtaagtc	222
TnpB_2	TTTAC	g6	ttggcatagcaaaaat	223
TnpB_2	TTTAC	g7	attcaacaagtgtgtg	224
TnpB_2	TTTAC	g8	tcaattacatcatact	225
TnpB_2	TTTAC	g9	agtttgattacccatc	226
TnpB_2	TTTAC	g10	gttgtaggtatttggg	227
TnpB_2	TTTAC	g11	ccaaacaatacttgag	228
TnpB_2	TTTAC	g12	taccatgctgtcttgt	229
TnpB_4	TTCAT	g1	atctatttgacaccaa	230
TnpB_4	TTCAT	g2	tgaagcagttgtgagt	231
TnpB_4	TTCAT	g3	ctggaggttgtgaact	232
TnpB_4	TTCAT	g4	ctagtgtataagtgta	233
TnpB_4	TTCAT	g5	ttcttactgctttgtt	234
TnpB_4	TTCAT	g6	gtgatgaagttctttt	235
TnpB_4	TTCAT	g8	ccttccatgcagctat	236
TnpB_4	TTCAT	g9	gttcttgttggaagtc	237
TnpB_4	TTCAT	g10	cagatagtttaaaagt	238
TnpB_4	TTCAT	g11	cagttctttgattact	239
TnpB_6	TTGAT	g1	ttacgaattgatgacc	240
TnpB_6	TTGAT	g2	aaggcaaattcgccgc	241
TnpB_6	TTGAT	g3	tcacattaagcctaga	242
TnpB_6	TTGAT	g5	tacccatcctaaagta	243
TnpB_6	TTGAT	g6	gttgattaacttgtac	244
TnpB_6	TTGAT	g8	tggtctcactttccga	245
TnpB_6	TTGAT	g9	agctttgaaccggttt	246
TnpB_6	TTGAT	g10	taacttgtactacctc	247

TnpB	TAM Sequence	gRNA ID	gRNA sequence	gRNA SEQ ID NO:
TnpB_6	TTGAT	g11	cgaaaactgaagaaca	248
TnpB_6	TTGAT	g12	gcgttggagcatataa	249
TnpB_3	ATTAC	g1	tcgctaggtaaaagat	250
TnpB_3	ATTAC	g3	aactttcaaaggctta	251
TnpB_3	ATTAC	g4	ttattgttgtttgcat	252
TnpB_3	ATTAC	g5	ttttcgttactgatac	253
TnpB_3	ATTAC	g6	tgcttcaagtaattaa	254
TnpB_3	ATTAC	g8	atcatactttgtttta	255
TnpB_3	ATTAC	g9	tattaaatgtcaaaat	256
TnpB_3	ATTAC	g10	ccatcctaaagtatgg	257
TnpB_3	ATTAC	g12	atgcaggtcttaactg	258
TnpB_3	ATTAC	g13	acaaaacagaagtact	259
TnpB_7	TTTAT	g2	ttttattttgtcgtag	260
TnpB_7	TTTAT	g13	cagtcaaagaatggat	261
TnpB_7	TTTAT	g14	tgccatgtcaaaggcg	262
TnpB_7	TTTAT	g15	aaaccctgatgaactg	263
TnpB_7	TTTAT	g16	aaagtttagcgccttt	264
TnpB_7	TTTAT	g17	ctcactggcaatcata	265
TnpB_7	TTTAT	g18	cctagaattaagttgc	266
TnpB_7	TTTAT	g19	ccaatttcttgaagta	267
TnpB_7	TTTAT	g20	ctgtaggaatattacg	268
TnpB_10	TTTAA	g2	cggagccatccgaatc	269
TnpB_10	TTTAA	g3	tccatctacatgtttc	270
TnpB_10	TTTAA	g4	cttggagttctcgaaa	271
TnpB_10	TTTAA	g5	ccagtgttttgcaact	272
TnpB_10	TTTAA	g6	gggtgctggtaggaca	273
TnpB_10	TTTAA	g8	actatctgatgaaaac	274
TnpB_10	TTTAA	g9	tagtaatcaaagaact	275
TnpB_10	TTTAA	g10	ttatttgccagaaaca	276
TnpB_12	GTGAT	g1	atctcaagttctgtta	277
TnpB_12	GTGAT	g2	tcccaagtatacccta	278
TnpB_12	GTGAT	g4	atttgtcttcctccag	279
TnpB_12	GTGAT	g5	cgaatatgatccacta	280
TnpB_12	GTGAT	g6	catatgtgttcttcag	281
TnpB_12	GTGAT	g7	atccattcctctgctg	282
TnpB_12	GTGAT	g8	ctttgtagaggacgac	283
TnpB_18	AGGAG	g12	acgcttatgtgtttgc	284
TnpB_18	AGGAG	g13	cttcaggatatcgact	285
TnpB_27	GAGAG	g1	tggtgatatctcaagt	286
TnpB_27	GAGAG	g2	agtggtgatatctcaa	287
TnpB_27	GAGAG	g3	gtcaatttctttttcc	288

TnpB	TAM Sequence	gRNA ID	gRNA sequence	gRNA SEQ ID NO:
TnpB_27	GAGAG	g4	accatgcatctcaacg	289
TnpB_27	GAGAG	g5	agaccatgcatctcaa	290
TnpB_27	GAGAG	g6	gaaattgcaggtagtt	291
TnpB_27	GAGAG	g9	ggtatgagttttggac	292
TnpB_27	GAGAG	g10	tatcagtaacgaaaag	293
TnpB_27	GAGAG	g12	gacagcgccttccatg	294
TnpB_27	GAGAG	g13	cagaatttgccagaga	295
TnpB_27	GAGAG	g14	taaggttaagtttgga	296
TnpB_26	TGGAG	g10	gaagacaaatatcaca	297
TnpB_26	TGGAG	g11	aacttgggatcaatga	298
TnpB_26	TGGAG	g12	tgttccttccactgca	299
TnpB_26	TGGAG	g13	aatttagtagatttga	300
TnpB_26	TGGAG	g18	ctagtatttgcaccag	301
TnpB_26	TGGAG	g19	tcttaacgacatggta	302
TnpB_26	TGGAG	g20	attacacaaaacagaa	303
TnpB_26	TGGAG	g7	ttgtgaactaatggga	304
TnpB_26	TGGAG	g8	ttctcgaaatttcatc	305
TnpB_26	TGGAG	g9	agcgatatttatgatg	306
TnpB_14	AGGAG	g1	gaggagtatttggttt	307
TnpB_14	AGGAG	g2	gagtatttggttttta	308
TnpB_14	AGGAG	g3	tatttggtttttatat	309
TnpB_14	AGGAG	g4	gctagtggaagtagcc	310
TnpB_14	AGGAG	g5	gaggctagtggaagta	311
TnpB_14	AGGAG	g6	acgcagcgaaggagga	312
TnpB_14	AGGAG	g8	gagtactgctggtcct	313
TnpB_12	GTGAT	g3	gaagttcttttggctc	314
TnpB_15	AGGAG	g7	gaggagtactgctggt	315
TnpB_15	AGGAG	g8	gagtactgctggtcct	316
TnpB_17	AGGAG	g6	acgcagcgaaggagga	317
TnpB_18	AGGAG	g1	gaggagtatttggttt	318
TnpB_18	AGGAG	g2	gagtatttggttttta	319
TnpB_18	AGGAG	g3	tatttggtttttatat	320
TnpB_18	AGGAG	g5	gaggctagtggaagta	321
TnpB_18	AGGAG	g6	acgcagcgaaggagga	322
TnpB_18	AGGAG	g7	gaggagtactgctggt	323
TnpB_18	AGGAG	g9	tactgctggtcctttg	324
TnpB_19	GGGAG	g1	caacagagagggtatg	325
TnpB_19	GGGAG	g2	tacctgagcgcgtgac	326
TnpB_20	GGAGG	g10	aagacaaatatcacat	327
TnpB_20	GGAGG	g13	caacagtatcatcatc	328
TnpB_20	GGAGG	g2	agtatttggtttttat	329

TnpB	TAM Sequence	gRNA ID	gRNA sequence	gRNA SEQ ID NO:
TnpB_21	AGGAG	g11	ctgtaacactctaaac	330
TnpB_21	AGGAG	g1	gaggagtatttggttt	331
TnpB_21	AGGAG	g2	gagtatttggttttta	332
TnpB_21	AGGAG	g3	tatttggtttttatat	333
TnpB_21	AGGAG	g5	gaggctagtggaagta	334
TnpB_21	AGGAG	g6	acgcagcgaaggagga	335
TnpB_21	AGGAG	g7	gaggagtactgctggt	336
TnpB_21	AGGAG	g8	gagtactgctggtcct	337
TnpB_21	AGGAG	g9	tactgctggtcctttg	338
TnpB_22	AGGAG	g10	cactacggaaggatgc	339
TnpB_22	AGGAG	g12	acgcttatgtgtttgc	340
TnpB_22	AGGAG	g1	gaggagtatttggttt	341
TnpB_22	AGGAG	g2	gagtatttggttttta	342
TnpB_22	AGGAG	g3	tatttggtttttatat	343
TnpB_22	AGGAG	g4	gctagtggaagtagcc	344
TnpB_22	AGGAG	g5	gaggctagtggaagta	345
TnpB_22	AGGAG	g6	acgcagcgaaggagga	346
TnpB_22	AGGAG	g7	gaggagtactgctggt	347
TnpB_22	AGGAG	g8	gagtactgctggtcct	348
TnpB_22	AGGAG	g9	tactgctggtcctttg	349
TnpB_23	GGGAG	g1	caacagagagggtatg	350
TnpB_23	GGGAG	g2	tacctgagcgcgtgac	351
TnpB_23	GGGAG	g3	ataacggtttcactaa	352
TnpB_26	TGGAG	g1	gaaatgcaatgggaac	353
TnpB_26	TGGAG	g2	catataacagaacttg	354
TnpB_26	TGGAG	g3	ttggtgtcaaatagat	355
TnpB_26	TGGAG	g4	tattcgagtgcccata	356
TnpB_26	TGGAG	g5	aaaatggttgtgtttg	357
TnpB_27	GAGAG	g7	ccaaaagaacttcatc	358

Table 10: Sequences Associated with Example 14 (In Table 10, the ωRNA sequences are listed as DNA sequences that are the equivalent RNA sequences (except the RNA will have the T replaced with a U))

TnpB	Description	Version	Size	ωRNA Sequence	SEQ ID NO:
ISYmu1	wild-type	v0.0	127	CAAACAGGAACCGCAGGAATTGCGGGGGTAGCTTGGTAAACAAGAGAAACCTCTGCCGGCAAAGAAATAAGCCGGTAAGTATGCTCTGTTCCCAAGAATCTCGTGACTTTAGTCATGAGAGTTTCAA	359
ISYmu1	stem-loop 2 truncation	v2.1	94	CAAACAGGAACCGCAGGAATTGCGGGGGTAGCTTGGTAAACAAGAGgaaCTCTGTTCCCAAGAATCTCGTGACTTTAGTCATGAGAGTTTCAA	360
ISYmu1	stem-loop 2 truncation	v2.2	98	CAAACAGGAACCGCAGGAATTGCGGGGGTAGCTTGGTAAACAAGAGAAgaaaTGCTCTGTTCCCAAGAATCTCGTGACTTTAGTCATGAGAGTTTCAA	361
ISYmu1	stem-loop 2 internal-loop indel	v3.1	126	CAAACAGGAACCGCAGGAATTGCGGGGGTAGCTTGGAAACAAGAGAAACCTCTGCCGGCAAAGAAATAAGCCGGTAAGTATGCTCTGTTCCCAAGAATCTCGTGACTTTAGTCATGAGAGTTTCAA	362
ISYmu1	stem-loop 2 internal-loop indel	v3.2	126	CAAACAGGAACCGCAGGAATTGCGGGGGTAGCTTGGTAACAAGAGAAACCTCTGCCGGCAAAGAAATAAGCCGGTAAGTATGCTCTGTTCCCAAGAATCTCGTGACTTTAGTCATGAGAGTTTCAA	363
ISYmu1	stem-loop 2 internal-	v3.3	126	CAAACAGGAACCGCAGGAATTGCGGGGGTAGCTTGGTAAAAAGAGAAACCTCTGCCGGCAAAGAAATAAGCCGGTAAGTATGCTCTGTTCCCAAGAATCTCGTGACTTTAGTCATGAGAGTTTCAA	364

TnpB	Description	Version	Size	ωRNA Sequence	SEQ ID NO:
	loop indel				
ISYmu1	stem-loop 2 internal-loop indel	v3.4	126	CAAACAGGAACCGCAGGAATTGCGGGGGTAGCTTGGTAAACAGAGAA ACCTCTGCCGGCAAAGAAATAAGCCGGTAAGTATGCTCTGTTCCCAAGA ATCTCGTGACTTTAGTCATGAGAGTTTCAA	365
ISYmu1	stem-loop 2 internal-loop indel	v3.5	126	CAAACAGGAACCGCAGGAATTGCGGGGGTAGCTTGGTAAACAAGAGA AACCTCTGCCGGCAAAGAAATAAGCCGGTAAGTATGCTCGTTCCCAAGA ATCTCGTGACTTTAGTCATGAGAGTTTCAA	366
ISYmu1	stem-loop 2 internal-loop indel	v3.6	126	CAAACAGGAACCGCAGGAATTGCGGGGGTAGCTTGGTAAACAAGAGA AACCTCTGCCGGCAAAGAAATAAGCCGGTAAGTATGCTCTTTCCCAAGA ATCTCGTGACTTTAGTCATGAGAGTTTCAA	367
ISYmu1	stem-loop 2 internal-loop indel	v3.7	126	CAAACAGGAACCGCAGGAATTGCGGGGGTAGCTTGGTAAACAAGAGA AACCTCTGCCGGCAAAGAAATAAGCCGGTAAGTATGCTCTGTCCCAAGA ATCTCGTGACTTTAGTCATGAGAGTTTCAA	368
ISYmu1	stem-loop 2 inter	v3.8	126	CAAACAGGAACCGCAGGAATTGCGGGGGTAGCTTGGTAAACAAGAGA AACCTCTGCCGGCAAAGAAATAAGCCGGTAAGTATGCTCTGTTCCAAGA ATCTCGTGACTTTAGTCATGAGAGTTTCAA	369

TnpB	Description	Version	Size	ωRNA Sequence	SEQ ID NO:
	nal-loop indel				
ISYmu1	stem-loop 2 internal-loop indel	v3.9	125	CAAACAGGAACCGCAGGAATTGCGGGGGTAGCTTGGAACAAGAGAAA CCTCTGCCGGCAAAGAAATAAGCCGGTAAGTATGCTCTGTTCCCAAGAA TCTCGTGACTTTAGTCATGAGAGTTTCAA	370
ISYmu1	stem-loop 2 internal-loop indel	v3.10	125	CAAACAGGAACCGCAGGAATTGCGGGGGTAGCTTGGTACAAGAGAAA CCTCTGCCGGCAAAGAAATAAGCCGGTAAGTATGCTCTGTTCCCAAGAA TCTCGTGACTTTAGTCATGAGAGTTTCAA	371
ISYmu1	stem-loop 2 internal-loop indel	v3.11	125	CAAACAGGAACCGCAGGAATTGCGGGGGTAGCTTGGTAAAAGAGAAA CCTCTGCCGGCAAAGAAATAAGCCGGTAAGTATGCTCTGTTCCCAAGAA TCTCGTGACTTTAGTCATGAGAGTTTCAA	372
ISYmu1	stem-loop 2 internal-loop indel	v3.12	125	CAAACAGGAACCGCAGGAATTGCGGGGGTAGCTTGGTAAACGAGAAA CCTCTGCCGGCAAAGAAATAAGCCGGTAAGTATGCTCTGTTCCCAAGAA TCTCGTGACTTTAGTCATGAGAGTTTCAA	373
ISYmu1	stem-loop 2	v3.13	125	CAAACAGGAACCGCAGGAATTGCGGGGGTAGCTTGGTAAACAAGAGA AACCTCTGCCGGCAAAGAAATAAGCCGGTAAGTATGCTCTTCCCAAGAA TCTCGTGACTTTAGTCATGAGAGTTTCAA	374

TnpB	Description	Version	Size	ωRNA Sequence	SEQ ID NO:
	internal-loop indel				
ISYmu1	stem-loop 2 internal-loop indel	v3.14	125	CAAACAGGAACCGCAGGAATTGCGGGGGTAGCTTGGTAAACAAGAGA AACCTCTGCCGGCAAAGAAATAAGCCGGTAAGTATGCTCTGCCCAAGA ATCTCGTGACTTTAGTCATGAGAGTTTCAA	375
ISYmu1	stem-loop 2 internal-loop indel	v3.15	125	CAAACAGGAACCGCAGGAATTGCGGGGGTAGCTTGGTAAACAAGAGA AACCTCTGCCGGCAAAGAAATAAGCCGGTAAGTATGCTCTGTCCAAGAA TCTCGTGACTTTAGTCATGAGAGTTTCAA	376
ISYmu1	stem-loop 2 internal-loop indel	v3.16	126	CAAACAGGAACCGCAGGAATTGCGGGGGTAGCTTGGTAAACAAGAGA AACCTCTGCCGGCAAAGAAATAAGCCGGTAAGTATGCTTGTTCCCAAGA ATCTCGTGACTTTAGTCATGAGAGTTTCAA	377
ISYmu1	stem-loop 3 truncation	v1.1	125	CAAACAGGAACCGCAGGAATTGCGGGGGTAGCTTGGTAAACAAGAGA AACCTCTGCCGGCAAAGAAATAAGCCGGTAAGTATGCTCTGTTCCCAAG AATCTCGTGATTTATCATGAGAGTTTCAA	378
ISYmu1	stem-loop 3	v1.2	123	CAAACAGGAACCGCAGGAATTGCGGGGGTAGCTTGGTAAACAAGAGA AACCTCTGCCGGCAAAGAAATAAGCCGGTAAGTATGCTCTGTTCCCAAG AATCTCGTGTTTACATGAGAGTTTCAA	379

TnpB	Description	Version	Size	ωRNA Sequence	SEQ ID NO:
	truncation				
ISYmu1	stem-loop 3 truncation	v1.3	121	CAAACAGGAACCGCAGGAATTGCGGGGGTAGCTTGGTAAACAAGAGAAACCTCTGCCGGCAAAGAAATAAGCCGGTAAGTATGCTCTGTTCCCAAGAATCTCGTTTTAATGAGAGTTTCAA	380
ISYmu1	stem-loop 3 truncation	v1.4	119	CAAACAGGAACCGCAGGAATTGCGGGGGTAGCTTGGTAAACAAGAGAAACCTCTGCCGGCAAAGAAATAAGCCGGTAAGTATGCTCTGTTCCCAAGAATCTCGTTTATGAGAGTTTCAA	381
ISYmu1	stem-loop 3 truncation	v1.5	117	CAAACAGGAACCGCAGGAATTGCGGGGGTAGCTTGGTAAACAAGAGAAACCTCTGCCGGCAAAGAAATAAGCCGGTAAGTATGCTCTGTTCCCAAGAATCTCTTTAGAGAGTTTCAA	382
ISYmu1	stem-loop 3 truncation	v1.6	115	CAAACAGGAACCGCAGGAATTGCGGGGGTAGCTTGGTAAACAAGAGAAACCTCTGCCGGCAAAGAAATAAGCCGGTAAGTATGCTCTGTTCCCAAGAATCTTTTAAGAGTTTCAA	383
ISYmu1	stem-loop 2 truncation	v2.3	90	CAAACAGGAACCGCAGGAATTGCGGGGGTAGCTTGGTAAACAAGgaaaCTGTTCCCAAGAATCTCGTGACTTTAGTCATGAGAGTTTCAA	384

TnpB	Description	Version	Size	ωRNA Sequence	SEQ ID NO:
ISYmu1	stem-loop 2 truncation	v2.4	87	CAAACAGGAACCGCAGGAATTGCGGGGGTAGCTTGGTAAACAgaaaTGTTCCCAAGAATCTCGTGACTTTAGTCATGAGAGTTTCAA	385
ISYmu1	stem-loop 1 base-pair substitution	v4.1	127	CAAACAGGAACCGCgGGAATcGCGGGGGTAGCTTGGTAAACAAGAGAAACCTCTGCCGGCAAAGAAATAAGCCGGTAAGTATGCTCTGTTCCCAAGAATCTCGTGACTTTAGTCATGAGAGTTTCAA	386
ISYmu1	stem-loop 1 base-pair substitution	v4.2	127	CAAACAGGAACCGCcGGAATgGCGGGGGTAGCTTGGTAAACAAGAGAAACCTCTGCCGGCAAAGAAATAAGCCGGTAAGTATGCTCTGTTCCCAAGAATCTCGTGACTTTAGTCATGAGAGTTTCAA	387
ISYmu1	PK base-pair substitution	v5.1	127	CAAACAGGAACCGCAGGAAgTGCGGGGGTAGCTTGGTAAACAAGAGAAACCTCTGCCGGCAAAGAAATAAGCCGGTAAGTATGCTCTGTTCCCAAGAATCTCGTGACTTTAGTCATGAGAGTTTCAA	388
ISYmu1	PK base-pair substitution	v5.2	127	CAAACAGGAACCGCAGGAAaTGCGGGGGTAGCTTGGTAAACAAGAGAAACCTCTGCCGGCAAAGAAATAAGCCGGTAAGTATGCTCTGTTCCCAAGAATCTCGTGACTTTAGTCATGAGAGTTTCAA	389

TnpB	Description	Version	Size	ωRNA Sequence	SEQ ID NO:
ISYmu1	PK base-pair substitution	v5.3	127	CAAACAGGAACCGCAtGAATTGCGGGGGTAGCTTGGTAAACAAGAGAAACCTCTGCCGGCAAAGAAATAAGCCGGTAAGTATGCTCTGTTCCCAAGAATCTCGTGACTTTAGTCATGAGAGTTTCAA	390
ISYmu1	PK base-pair substitution	v5.4	127	CAAACAGGAACCGCAGGAATTGCGGGGGTAGCTTGGTAAACAAGAGAAACCTCTGCCGGCAAAGAAATAAGCCGGTAAGTATGCTCTGTTCCCAAGAATCTCGTGACTTTAGTCATGAGAGgTTCAA	391
ISBag01	wild-type	v0.0	186	CTGTGATTGATGGTAACAGTCAATCAGCCTAAGATGCTATATGCACTGTCTTAGGAGGGGCAATGAGATGCCCTCATCTTGAAGGCTGTTCAAAACAGAAATGGACTGCGAACGCTTAGTCATTCAAGAATCCCACCCGTACACCGCAAGGTGGAAGGCTTGCGGCTTTAGCCGTGGGAGTCTCAA	392
ISBag01	stem-loop 5 truncation	v1.1	153	CTGTGATTGATGGTAACAGTCAATCAGCCTAAGATGCTATATGCACTGTCTTAGGAGGGGCAATGAGATGCCCTCATCTTGAAGGCTGTTCAAAACAGAAATGGACTGCGAACGCTTAGTCATTCAAGAATCCCACgaaaGTGGGAGTCTCAA	393
ISBag01	stem-loop 4 truncation	v2.1	172	CTGTGATTGATGGTAACAGTCAATCAGCCTAAGATGCTATATGCACTGTCTTAGGAGGGGCAATGAGATGCCCTCATCTTGAAGGCTGTTCgaaaGAACGCTTAGTCATTCAAGAATCCCACCCGTACACCGCAAGGTGGAAGGCTTGCGGCTTTAGCCGTGGGAGTCTCAA	394
ISBag01	stem-loop 4 truncation	v2.2	160	CTGTGATTGATGGTAACAGTCAATCAGCCTAAGATGCTATATGCACTGTCTTAGGAGGGGCAATGAGATGCCCTCATCTTGAAGGCTgaaaAGTCATTCAAGAATCCCACCCGTACACCGCAAGGTGGAAGGCTTGCGGCTTTAGCCGTGGGAGTCTCAA	395

TnpB	Description	Version	Size	ωRNA Sequence	SEQ ID NO:
ISBag01	stem-loop 5 truncation	v1.2	164	CTGTGATTGATGGTAACAGTCAATCAGCCTAAGATGCTATATGCACTGTCTTAGGAGGGGCAATGAGATGCCCTCATCTTGAAGGCTGTTCAAAACAGAAATGGACTGCGAACGCTTAGTCATTCAAGAATCCCACCCGTACACTTTAGCCGTGGGAGTCTCAA	396
ISBag01	Undetermined	v999.999	165	CTtagGCCTAAGATGCTATATGCACTGTCTTAGGAGGGGCAATGAGATGCCCTCATCTTGAAGGCTGTTCAAAACAGAAATGGACTGCGAACGCTTAGTCATTCAAGAATCCCACCCGTACACCGCAAGGTGGAAGGCTTGCGGCTTTAGCCGTGGGAGTCTCAA	397
ISBag01	Undetermined	v999.999	158	CTGTGATTGATGGTAACAGTCAATCAtAGGGGCAATGAGATGCCCTCATCTTGAAGGCTGTTCAAAACAGAAATGGACTGCGAACGCTTAGTCATTCAAGAATCCCACCCGTACACCGCAAGGTGGAAGGCTTGCGGCTTTAGCCGTGGGAGTCTCAA	398
ISBag01	Undetermined	v999.999	176	CTGTGATTGATGGTAACAGTCAATCAGCCTAAGAgaaaTCTTAGGAGGGGCAATGAGATGCCCTCATCTTGAAGGCTGTTCAAAACAGAAATGGACTGCGAACGCTTAGTCATTCAAGAATCCCACCCGTACACCGCAAGGTGGAAGGCTTGCGGCTTTAGCCGTGGGAGTCTCAA	399
ISBag01	Undetermined	v999.999	132	CTtagGCCTAAGATGCTATATGCACTGTCTTAGGAGGGGCAATGAGATGCCCTCATCTTGAAGGCTGTTCAAAACAGAAATGGACTGCGAACGCTTAGTCATTCAAGAATCCCACgaaaGTGGGAGTCTCAA	400
ISBag01	Undetermined	v999.999	125	CTGTGATTGATGGTAACAGTCAATCAtAGGGGCAATGAGATGCCCTCATCTTGAAGGCTGTTCAAAACAGAAATGGACTGCGAACGCTTAGTCATTCAAGAATCCCACgaaaGTGGGAGTCTCAA	401
ISBag01	Undetermined	v999.999	104	CTTAGGAGGGGCAATGAGATGCCCTCATCTTGAAGGCTGTTCAAAACAGAAATGGACTGCGAACGCTTAGTCATTCAAGAATCCCACgaaaGTGGGAGTCTCAA	402
ISBag01	stem-loop 5 trun	v1.3	151	CTGTGATTGATGGTAACAGTCAATCAGCCTAAGATGCTATATGCACTGTCTTAGGAGGGGCAATGAGATGCCCTCATCTTGAAGGCTGTTCAAAACAGAAATGGACTGCGAACGCTTAGTCATTCAAGAATCCCAgaaaTGGGAGTCTCAA	403

TnpB	Description	Version	Size	ωRNA Sequence	SEQ ID NO:
	cation				
ISBag01	stem-loop 5 truncation	v1.4	149	CTGTGATTGATGGTAACAGTCAATCAGCCTAAGATGCTATATGCACTGTCTTAGGAGGGGCAATGAGATGCCCTCATCTTGAAGGCTGTTCAAAACAGAAATGGACTGCGAACGCTTAGTCATTCAAGAATCCCgaaaGGGAGTCTCAA	404
ISBag01	stem-loop 5 truncation	v1.5	147	CTGTGATTGATGGTAACAGTCAATCAGCCTAAGATGCTATATGCACTGTCTTAGGAGGGGCAATGAGATGCCCTCATCTTGAAGGCTGTTCAAAACAGAAATGGACTGCGAACGCTTAGTCATTCAAGAATCCgaaaGGAGTCTCAA	405
ISBag01	stem-loop 5 truncation	v1.6	145	CTGTGATTGATGGTAACAGTCAATCAGCCTAAGATGCTATATGCACTGTCTTAGGAGGGGCAATGAGATGCCCTCATCTTGAAGGCTGTTCAAAACAGAAATGGACTGCGAACGCTTAGTCATTCAAGAATCgaaaGAGTCTCAA	406
ISBag01	stem-loop 4 internal-loop indel	v3.1	185	CTGTGATTGATGGTAACAGTCAATCAGCCTAAGATGCTATATGCACTGTCTTAGGAGGGGCAATGAGATGCCCTCATCTTGAAGCTGTTCAAAACAGAAATGGACTGCGAACGCTTAGTCATTCAAGAATCCCACCCGTACACCGCAAGGTGGAAGGCTTGCGGCTTTAGCCGTGGGAGTCTCAA	407
ISBag01	stem-loop 4 internal-	v3.2	185	CTGTGATTGATGGTAACAGTCAATCAGCCTAAGATGCTATATGCACTGTCTTAGGAGGGGCAATGAGATGCCCTCATCTTGAAGGTGTTCAAAACAGAAATGGACTGCGAACGCTTAGTCATTCAAGAATCCCACCCGTACACCGCAAGGTGGAAGGCTTGCGGCTTTAGCCGTGGGAGTCTCAA	408

TnpB	Description	Version	Size	ωRNA Sequence	SEQ ID NO:
	loop indel				
ISBag01	stem-loop 4 internal-loop indel	v3.3	185	CTGTGATTGATGGTAACAGTCAATCAGCCTAAGATGCTATATGCACTGTCTTAGGAGGGGCAATGAGATGCCCTCATCTTGAAGGCGTTCAAAACAGAAATGGACTGCGAACGCTTAGTCATTCAAGAATCCCACCCGTACACCGCAAGGTGGAAGGCTTGCGGCTTTAGCCGTGGGAGTCTCAA	409
ISBag01	stem-loop 4 internal-loop indel	v3.4	185	CTGTGATTGATGGTAACAGTCAATCAGCCTAAGATGCTATATGCACTGTCTTAGGAGGGGCAATGAGATGCCCTCATCTTGAAGGCTTTCAAAACAGAAATGGACTGCGAACGCTTAGTCATTCAAGAATCCCACCCGTACACCGCAAGGTGGAAGGCTTGCGGCTTTAGCCGTGGGAGTCTCAA	410
ISBag01	stem-loop 4 internal-loop indel	v3.5	185	CTGTGATTGATGGTAACAGTCAATCAGCCTAAGATGCTATATGCACTGTCTTAGGAGGGGCAATGAGATGCCCTCATCTTGAAGGCTGTCAAAACAGAAATGGACTGCGAACGCTTAGTCATTCAAGAATCCCACCCGTACACCGCAAGGTGGAAGGCTTGCGGCTTTAGCCGTGGGAGTCTCAA	411
ISBag01	stem-loop 4 internal-loop indel	v3.6	185	CTGTGATTGATGGTAACAGTCAATCAGCCTAAGATGCTATATGCACTGTCTTAGGAGGGGCAATGAGATGCCCTCATCTTGAAGGCTGTTAAAACAGAAATGGACTGCGAACGCTTAGTCATTCAAGAATCCCACCCGTACACCGCAAGGTGGAAGGCTTGCGGCTTTAGCCGTGGGAGTCTCAA	412

TnpB	Description	Version	Size	ωRNA Sequence	SEQ ID NO:
ISBag01	stem-loop 4 internal-loop indel	v3.7	185	CTGTGATTGATGGTAACAGTCAATCAGCCTAAGATGCTATATGCACTGT CTTAGGAGGGGCAATGAGATGCCCTCATCTTGAAGGCTGTTCAAACAG AAATGGACTGCGAACGCTTAGTCATTCAAGAATCCCACCCGTACACCGC AAGGTGGAAGGCTTGCGGCTTTAGCCGTGGGAGTCTCAA	413
ISBag01	stem-loop 4 internal-loop indel	v3.8	185	CTGTGATTGATGGTAACAGTCAATCAGCCTAAGATGCTATATGCACTGT CTTAGGAGGGGCAATGAGATGCCCTCATCTTGAAGGCTGTTCAAAACA GAAATGGACTGGAACGCTTAGTCATTCAAGAATCCCACCCGTACACCGC AAGGTGGAAGGCTTGCGGCTTTAGCCGTGGGAGTCTCAA	414
ISBag01	stem-loop 4 internal-loop indel	v3.9	185	CTGTGATTGATGGTAACAGTCAATCAGCCTAAGATGCTATATGCACTGT CTTAGGAGGGGCAATGAGATGCCCTCATCTTGAAGGCTGTTCAAAACA GAAATGGACTGCAACGCTTAGTCATTCAAGAATCCCACCCGTACACCGC AAGGTGGAAGGCTTGCGGCTTTAGCCGTGGGAGTCTCAA	415
ISBag01	stem-loop 4 internal-loop indel	v3.10	185	CTGTGATTGATGGTAACAGTCAATCAGCCTAAGATGCTATATGCACTGT CTTAGGAGGGGCAATGAGATGCCCTCATCTTGAAGGCTGTTCAAAACA GAAATGGACTGCGACGCTTAGTCATTCAAGAATCCCACCCGTACACCGC AAGGTGGAAGGCTTGCGGCTTTAGCCGTGGGAGTCTCAA	416
ISBag01	stem-loop 4 internal-	v3.11	185	CTGTGATTGATGGTAACAGTCAATCAGCCTAAGATGCTATATGCACTGT CTTAGGAGGGGCAATGAGATGCCCTCATCTTGAAGGCTGTTCAAAACA GAAATGGACTGCGAAGCTTAGTCATTCAAGAATCCCACCCGTACACCGC AAGGTGGAAGGCTTGCGGCTTTAGCCGTGGGAGTCTCAA	417

TnpB	Description	Version	Size	ωRNA Sequence	SEQ ID NO:
	loop indel				
ISBag01	stem-loop 4 internal-loop indel	v3.12	185	CTGTGATTGATGGTAACAGTCAATCAGCCTAAGATGCTATATGCACTGT CTTAGGAGGGGCAATGAGATGCCCTCATCTTGAAGGCTGTTCAAAACA GAAATGGACTGCGAACCTTAGTCATTCAAGAATCCCACCCGTACACCGC AAGGTGGAAGGCTTGCGGCTTTAGCCGTGGGAGTCTCAA	418
ISBag01	stem-loop 4 internal-loop indel	v3.13	185	CTGTGATTGATGGTAACAGTCAATCAGCCTAAGATGCTATATGCACTGT CTTAGGAGGGGCAATGAGATGCCCTCATCTTGAAGGCTGTTCAAAACA GAAATGGACTGCGAACGTTAGTCATTCAAGAATCCCACCCGTACACCGC AAGGTGGAAGGCTTGCGGCTTTAGCCGTGGGAGTCTCAA	419
ISBag01	stem-loop 4 internal-loop indel	v3.14	185	CTGTGATTGATGGTAACAGTCAATCAGCCTAAGATGCTATATGCACTGT CTTAGGAGGGGCAATGAGATGCCCTCATCTTGAAGGCTGTTCAAAACA GAAATGGACTGCGAACGCTAGTCATTCAAGAATCCCACCCGTACACCGC AAGGTGGAAGGCTTGCGGCTTTAGCCGTGGGAGTCTCAA	420
ISBag01	stem-loop 4 internal-loop indel	v3.15	185	CTGTGATTGATGGTAACAGTCAATCAGCCTAAGATGCTATATGCACTGT CTTAGGAGGGGCAATGAGATGCCCTCATCTTGAAGGCTGTTCAAAACA GAAATGGACTGCGAACGCTTGTCATTCAAGAATCCCACCCGTACACCGC AAGGTGGAAGGCTTGCGGCTTTAGCCGTGGGAGTCTCAA	421

TnpB	Description	Version	Size	ωRNA Sequence	SEQ ID NO:
ISBag01	stem-loop 4 internal-loop indel	v3.16	185	CTGTGATTGATGGTAACAGTCAATCAGCCTAAGATGCTATATGCACTGT CTTAGGAGGGGCAATGAGATGCCCTCATCTTGAAGGCTGTTCAAAACA GAAATGGACTGCGAACGCTTATCATTCAAGAATCCCACCCGTACACCGC AAGGTGGAAGGCTTGCGGCTTTAGCCGTGGGAGTCTCAA	422
ISBag01	stem-loop 4 internal-loop indel	v3.17	185	CTGTGATTGATGGTAACAGTCAATCAGCCTAAGATGCTATATGCACTGT CTTAGGAGGGGCAATGAGATGCCCTCATCTTGAAGGCTGTTCAAAACA GAAATGGACTGCGAACGCTTAGCATTCAAGAATCCCACCCGTACACCGC AAGGTGGAAGGCTTGCGGCTTTAGCCGTGGGAGTCTCAA	423
ISBag01	stem-loop 4 internal-loop indel	v3.18	185	CTGTGATTGATGGTAACAGTCAATCAGCCTAAGATGCTATATGCACTGT CTTAGGAGGGGCAATGAGATGCCCTCATCTTGAAGGCTGTTCAAAACA GAAATGGACTGCGAACGCTTAGTATTCAAGAATCCCACCCGTACACCGC AAGGTGGAAGGCTTGCGGCTTTAGCCGTGGGAGTCTCAA	424
ISBag01	stem-loop 4 internal-loop indel	v3.19	185	CTGTGATTGATGGTAACAGTCAATCAGCCTAAGATGCTATATGCACTGT CTTAGGAGGGGCAATGAGATGCCCTCATCTTGAAGGCTGTTCAAAACA GAAATGGACTGCGAACGCTTAGTCTTCAAGAATCCCACCCGTACACCGC AAGGTGGAAGGCTTGCGGCTTTAGCCGTGGGAGTCTCAA	425
ISBag01	stem-loop 3 base-pair subs	v4.1	186	CTGTGATTGATGGTAACAGTCAATCAGCCTAAGATGCTATATGCACTGT CTTAGGAGGGGCgATGAGAcGCCCTCATCTTGAAGGCTGTTCAAAACAG AAATGGACTGCGAACGCTTAGTCATTCAAGAATCCCACCCGTACACCGC AAGGTGGAAGGCTTGCGGCTTTAGCCGTGGGAGTCTCAA	426

TnpB	Description	Version	Size	ωRNA Sequence	SEQ ID NO:
	tituti on				
ISBag 01	stem - loop 3 base -pair subs tituti on	v4.2	186	CTGTGATTGATGGTAACAGTCAATCAGCCTAAGATGCTATATGCACTGT CTTAGGAGGGGCcATGAGAgGCCCTCATCTTGAAGGCTGTTCAAAACAG AAATGGACTGCGAACGCTTAGTCATTCAAGAATCCCACCCGTACACCGC AAGGTGGAAGGCTTGCGGCTTTAGCCGTGGGAGTCTCAA	427
ISBag 01	stem - loop 2 trun catio n	v5.1	176	CTGTGATTGATGGTAACAGTCAATCAGCCTAAGAgaaaTCTTAGGAGGG GCAATGAGATGCCCTCATCTTGAAGGCTGTTCAAAACAGAAATGGACTG CGAACGCTTAGTCATTCAAGAATCCCACCCGTACACCGCAAGGTGGAAG GCTTGCGGCTTTAGCCGTGGGAGTCTCAA	428
ISBag 01	stem - loop 2 trun catio n	v5.2	172	CTGTGATTGATGGTAACAGTCAATCAGCCTAAgaaaTTAGGAGGGGCAA TGAGATGCCCTCATCTTGAAGGCTGTTCAAAACAGAAATGGACTGCGAA CGCTTAGTCATTCAAGAATCCCACCCGTACACCGCAAGGTGGAAGGCTT GCGGCTTTAGCCGTGGGAGTCTCAA	429
ISBag 01	stem - loop 2 trun catio n	v5.3	170	CTGTGATTGATGGTAACAGTCAATCAGCCTAAttTTAGGAGGGGCAATG AGATGCCCTCATCTTGAAGGCTGTTCAAAACAGAAATGGACTGCGAACG CTTAGTCATTCAAGAATCCCACCCGTACACCGCAAGGTGGAAGGCTTGC GGCTTTAGCCGTGGGAGTCTCAA	430

Table 11: Additional Sequences Associated with Example 14

TnpB Name	TnpB with wRNA SEQ ID NO:	TnpB with wRNA overlap Native DNA sequence (single)	gRNA SEQ ID NO:	gRNA sequence
ISBag01	431	atgtcgcagaccatcacagtcaaagtgaaattgcttccaacaaaagaacaggcttctattttgcgtgagatgagtcaaacgtacctctccactatcaatactctcgtttccgaaatggttgctgaaaagaaaagcacaaagaagtcgagtaaggatatttctgcttctttgccaagtgccgttaaaaatcaagctatcaaggacgcgaaaagtgtgtttaagaaagcgaagaaaagcaaattcactactgttcctgttttgaagaaacctgtctgcatttggaacaatcaaaactattcgtttgatttcacgcacatttccatgccgattatgattgacggcaaggtaactaaaacacctatccgtgctttgttagttgataaagacaaccgtaactttgctttgctaaaacacaaattaggtacgcttcgaatcacacagaaagcaaataaatggattgcccaaatttctgtcaccatacctactatggaaggaacaggagtaaaagtcatgggggttgatttgggtttaaaagttcctgccgttgctgtaacagatgatgaaaaggtacgtttctttgggaatggcagacaaaataagtacatgaagcgtaagttccgttctgaacgcaaagcattaggcaaaaagaaaaaggtaaatgcgattcgtagttcaaaagataaagaacaacgttggatgaaagaccaagaccataaggttagtcgtgctatcgtaaattttgcaaaagacaataaaatttctgtcattcgcttggaacaactggcgaatattagacagacggcaagaacaagccgtaaaaacgaaaagaatctgcatacttggtcattttatcgcctgtctcagttcattgaatataaagcgaatttagttggtattagagttgagcatgtgaatcctgcatacacaagtcagacatgccctaaatgctctgagaagaataaggcacaagaccgtaagtataaatgcaaatgtggattcgaaaaacatcgtgatttagtcggtgctatgaacattcgatatgcacctgtgattgatggtaacagtcaatcagcctaagatgctatatgcactgtcttaggaggggcaatgagatgccctcatcttgaaggctgttcaaaacagaaatggactgcgaacgcttagtcattcaagaatcccacccgtacaccgcaaggtggaaggcttgcggctttagccgtgggagtctcaa	434	gaattgatgaccatat
ISBag01 variant 1.1	432	atgtcgcagaccatcacagtcaaagtgaaattgcttccaacaaaagaacaggcttctattttgcgtgagatgagtcaaacgtacctctccactatcaatactctcgtttccgaaatggttgctgaaaagaaaagcacaaagaagtcgagtaaggatatttctgcttctttgccaagtgccgttaaaaatcaagctatcaaggacgcgaaaagtgtgtttaagaaagcgaagaaaagcaaattcactactgttcctgttttgaagaaacctgtctgcatttggaacaatcaaaactattcgtttgatttcacgcacatttccatgccgattatgattgacggcaaggtaactaaaacacctatccgtgctttgttagttgataaagacaaccgtaactttgctttgctaaaacacaaattaggtacgcttcgaatcacacagaaagcaaataaatggattgcccaaatttctgtcaccatacctactatggaaggaacaggagtaaaagtcatgggggttgatttgggtttaaaagttcctgccgttgctgtaacagatgatgaaaaggtacgtttctttgggaatggcagacaaaataagtacatgaagcgtaagttccgttctgaacgcaaagcattaggcaaaaagaaaaaggtaaatgcgattcgtagttcaaaagataaagaacaacgttggatgaaagaccaagaccataaggttagtcgtgctatcgtaaattttgcaaaagacaataaaatttctgtcattcgcttggaacaactggcgaatattagacagacggcaagaacaagccgtaaaaacgaaaagaatctgcatacttggtcattttatcgcctgtctcagttcattgaatataaagcgaatttagttggtattagagttgagcatgtgaatcctgcatacacaagtcagacatgccctaaatgctctgagaagaataaggcacaagaccgtaagtataaatgcaaatgtggattcgaaaaacatcgtgatttagtcggtgctatgaacattcgatatgcacctgtgattgatggtaacagtcaatcagcctaagatgctatatgcactgtcttaggaggggcaatgagatgccctcatcttgaaggctgttcaaaacagaaatggactgcgaacgcttagtcattcaagaatcccacgaaagtgggagtctcaa	435	gaattgatgaccatat

TnpB Name	TnpB with wRNA SEQ ID NO:	TnpB with wRNA overlap Native DNA sequence (single)	gRNA SEQ ID NO:	gRNA sequence
ISBag01 variant 1.2	433	atgtcgcagaccatcacagtcaaagtgaaattgcttccaacaaaagaacaggcttctattttgcgtga gatgagtcaaacgtacctctccactatcaatactctcgtttccgaaatggttgctgaaaagaaaagca caaagaagtcgagtaaggatatttctgcttctttgccaagtgccgttaaaaatcaagctatcaaggac gcgaaaagtgtgtttaagaaagcgaagaaaagcaaattcactactgttcctgttttgaagaaacctgt ctgcatttggaacaatcaaaactattcgtttgatttcacgcacatttccatgccgattatgattgacggc aaggtaactaaaacacctatccgtgctttgttagttgataaagacaaccgtaactttgctttgctaaaa cacaaattaggtacgcttcgaatcacacagaaagcaaataaatggattgcccaaatttctgtcaccat acctactatggaaggaacaggagtaaaagtcatgggggttgatttgggtttaaaagttcctgccgttg ctgtaacagatgatgaaaaggtacgtttctttgggaatggcagacaaaataagtacatgaagcgtaa gttccgttctgaacgcaaagcattaggcaaaaagaaaaaggtaaatgcgattcgtagttcaaaagat aaagaacaacgttggatgaaagaccaagaccataaggttagtcgtgctatcgtaaattttgcaaaag acaataaaatttctgtcattcgcttggaacaactggcgaatattagacagacggcaagaacaagccg taaaaacgaaaagaatctgcatacttggtcattttatcgcctgtctcagttcattgaatataaagcgaa tttagttggtattagagttgagcatgtgaatcctgcatacacaagtcagacatgccctaaatgctctga gaagaataaggcacaagaccgtaagtataaatgcaaatgtggattcgaaaaacatcgtgatttagtc ggtgctatgaacattcgatatgcacctgtgattgatggtaacagtcaatcagcctaagatgctatatgc actgtcttaggaggggcaatgagatgccctcatcttgaaggctgttcaaaacagaaatggactgcga acgcttagtcattcaagaatcccacccgtacactttagccgtgggagtctcaa	436	gaattg atgacc atat

Table 12: Sequences associated with Example 15

Description	TnpB Name	DNA sequence SEQ ID NO:	DNA sequence cloned into TRV cargo site	gRNA sequence	gRNA SEQ ID NO:
ISYmu1-g2-tRNA–Isoleucine	ISYmu1	437	atggattacaaggatgatgatgataaggattacaaggatgatgatgataagatggctccaaagaagaagagaaaggttggaatccacggagttccagctgctATGCTGCAGCATAAAGCCTATGAATACCGTATCTATCCAGATAAGAAGCAGGAAACACTGATTGCCAAGACAATAGGCAGTTCAAGGTATGTATACAACCATTTCCTGGAACTTTGGAACAAGGAATATGAGGAGACCGGAAAAGGTCTGACTTATTACGCATGCTCCAAACTTCTGACAAAGCTTAAGAGGGACCCGGAAACAGTATGGCTTTGTGAAGTGGATAAGTTCTCGCTCCAGAATTCGCTTCGTAACCTGTCGGACGCCTTCTCCCGCTTTTTCAAAGGACAGAACGAGCATCCCCAGTTCAAGAGCAAGAAGAGCCCGAGGCAAAGCTACACGACACAATATACGAACAACAACATCGCAGTTTCCGGAAATTGTCTGAAGCTGCCTAAGCTGGGCCTTGTCAAGTTTGCAGACAGTAGGGAGATGAAAGGCCGTATACTGAATGCCACAGTACGAAGGAAATCGAGCGGGAAATTCTTCGTATCGATCCTATGCGAAGAGGAGATCTGTGAATTGCCAAAGACTGACTCATCTGTCGGAATTGACCTCGGAATCATTGACTTTGCGGTTATGTCAGACGGAAGCAGGCATGACAACAATCATTTTACCAGGCAAATGGAAGAAAGGCTTAGACGGGAGCAGCGAAAGCTTGCAAGGCGTGCACTTGCTGCGGAGAAAAGGGGCATTTCCCTTTCTGAAGCCAGGAACTATCAGAAGCAAAGGCGGAAGGTAGCGAGACTTTATGAAAAGGTTGCAAACCAGCGAAAAGAATATCTTAACAAACTCAGTACAGAGATAGTCAAAAACCACGATATCATCTGTATCGAGGACCTTAACGTTAAGGGCATGATGCGCAATCATAAACTGGCAAAAAGCATCTCTGATGTATCATGGACGAGCCTTGTATCGAAACTGCAGTACAAGGCTTCCTGGTATGGAAAAGAAGTCATCAGGATAAGCAGATGGTTTCCGTCAAGTCAGATATGCTCAGAGTGCGGCCACAAGGACAGGAAGAAGCCTCTCCATGTAAGGGAGTGGACCTGTCCTGTTTGTCATGCCCACCATGACCGTGACGTCAATGCAGCAAGAAATATACTGGCTGAGGGACTCAGGATAAGAGCTCTGACTCCGGGGTCTTAGCAATACAGAAGCAAACAGGAACCGCAGGAATTGCGGGGGTAGCTTGGTAAACAAGAGAAACCTCTGCCGGCAAAGAAATAAGCCGGTAAGTATGCTCTGTTCCCAAGAATCTCGTGACTTTAGTCATGAGAGTTTCAAaaggcaaattcgccgcCCGTAGCTCAGTTGGTTAGAGCGTTGGTCTTATGAGCCGAAGGTCGCGGGTTCGAGCCCCGCCGGAAGCA	aaggcaaattcgccgc	456

Description	TnpB Name	DNA sequence SEQ ID NO:	DNA sequence cloned into TRV cargo site	gRNA sequence	gRNA SEQ ID NO:
ISYmu1-g2-HDV-tRNA-Isoleucine	ISYmu1	438	atggattacaaggatgatgatgataaggattacaaggatgatgatgataagatggctccaaagaaga agagaaaggttggaatccacggagttccagctgctATGCTGCAGCATAAAGCCTATGAAT ACCGTATCTATCCAGATAAGAAGCAGGAAACACTGATTGCCAAGACAATAGG CAGTTCAAGGTATGTATACAACCATTTCCTGGAACTTTGGAACAAGGAATATG AGGAGACCGGAAAAGGTCTGACTTATTACGCATGCTCCAAACTTCTGACAAA GCTTAAGAGGGACCCGGAAACAGTATGGCTTTGTGAAGTGGATAAGTTCTCG CTCCAGAATTCGCTTCGTAACCTGTCGGACGCCTTCTCCCGCTTTTTCAAAGGA CAGAACGAGCATCCCCAGTTCAAGAGCAAGAAGAGCCCGAGGCAAAGCTAC ACGACACAATATACGAACAACAACATCGCAGTTTCCGGAAATTGTCTGAAGCT GCCTAAGCTGGGCCTTGTCAAGTTTGCAGACAGTAGGGAGATGAAAGGCCGT ATACTGAATGCCACAGTACGAAGGAAATCGAGCGGGAAATTCTTCGTATCGA TCCTATGCGAAGAGGAGATCTGTGAATTGCCAAAGACTGACTCATCTGTCGG AATTGACCTCGGAATCATTGACTTTGCGGTTATGTCAGACGGAAGCAGGCAT GACAACAATCATTTTACCAGGCAAATGGAAGAAAGGCTTAGACGGGAGCAGC GAAAGCTTGCAAGGCGTGCACTTGCTGCGGAGAAAAGGGGCATTTCCCTTTC TGAAGCCAGGAACTATCAGAAGCAAAGGCGGAAGGTAGCGAGACTTTATGA AAAGGTTGCAAACCAGCGAAAAGAATATCTTAACAAACTCAGTACAGAGATA GTCAAAAACCACGATATCATCTGTATCGAGGACCTTAACGTTAAGGGCATGAT GCGCAATCATAAACTGGCAAAAAGCATCTCTGATGTATCATGGACGAGCCTTG TATCGAAACTGCAGTACAAGGCTTCCTGGTATGGAAAAGAAGTCATCAGGAT AAGCAGATGGTTTCCGTCAAGTCAGATATGCTCAGAGTGCGGCCACAAGGAC AGGAAGAAGCCTCTCCATGTAAGGGAGTGGACCTGTCCTGTTTGTCATGCCCA CCATGACCGTGACGTCAATGCAGCAAGAAATATACTGGCTGAGGGACTCAGG ATAAGAGCTCTGACTCCGGGGTCTTAGCAATACAGAAGCAAACAGGAACCGC AGGAATTGCGGGGGTAGCTTGGTAAACAAGAGAAACCTCTGCCGGCAAAGA AATAAGCCGGTAAGTATGCTCTGTTCCCAAGAATCTCGTGACTTTAGTCATGA GAGTTTCAAaaggcaaattcgccgcggccggcatggtcccagcctcctcgctggcgccggctggg caacatgcttcggcatggcgaatgggacCCGTAGCTCAGTTGGTTAGAGCGTTGGTCTT ATGAGCCGAAGGTCGCGGGTTCGAGCCCCGCCGGAAGCA	aaggcaaattcgccgc	457

Description	TnpB Name	DNA sequence SEQ ID NO:	DNA sequence cloned into TRV cargo site	gRNA sequence	gRNA SEQ ID NO:
ISYmu1-g2-tandem-200bp-wRNA–tRNA–Isoleucine	ISYmu1	439	atggattacaaggatgatgatgataaggattacaaggatgatgatgataagatggctccaaagaaga agagaaaggttggaatccacggagttccagctgctATGCTGCAGCATAAAGCCTATGAAT ACCGTATCTATCCAGATAAGAAGCAGGAAACACTGATTGCCAAGACAATAGG CAGTTCAAGGTATGTATACAACCATTTCCTGGAACTTTGGAACAAGGAATATG AGGAGACCGGAAAAGGTCTGACTTATTACGCATGCTCCAAACTTCTGACAAA GCTTAAGAGGGACCCGGAAACAGTATGGCTTTGTGAAGTGGATAAGTTCTCG CTCCAGAATTCGCTTCGTAACCTGTCGGACGCCTTCTCCCGCTTTTTCAAAGGA CAGAACGAGCATCCCCAGTTCAAGAGCAAGAAGAGCCCGAGGCAAAGCTAC ACGACACAATATACGAACAACAACATCGCAGTTTCCGGAAATTGTCTGAAGCT GCCTAAGCTGGGCCTTGTCAAGTTTGCAGACAGTAGGGAGATGAAAGGCCGT ATACTGAATGCCACAGTACGAAGGAAATCGAGCGGGAAATTCTTCGTATCGA TCCTATGCGAAGAGGAGATCTGTGAATTGCCAAAGACTGACTCATCTGTCGG AATTGACCTCGGAATCATTGACTTTGCGGTTATGTCAGACGGAAGCAGGCAT GACAACAATCATTTTACCAGGCAAATGGAAGAAAGGCTTAGACGGGAGCAGC GAAAGCTTGCAAGGCGTGCACTTGCTGCGGAGAAAAGGGGCATTTCCCTTTC TGAAGCCAGGAACTATCAGAAGCAAAGGCGGAAGGTAGCGAGACTTTATGA AAAGGTTGCAAACCAGCGAAAAGAATATCTTAACAAACTCAGTACAGAGATA GTCAAAAACCACGATATCATCTGTATCGAGGACCTTAACGTTAAGGGCATGAT GCGCAATCATAAACTGGCAAAAAGCATCTCTGATGTATCATGGACGAGCCTTG TATCGAAACTGCAGTACAAGGCTTCCTGGTATGGAAAAGAAGTCATCAGGAT AAGCAGATGGTTTCCGTCAAGTCAGATATGCTCAGAGTGCGGCCACAAGGAC AGGAAGAAGCCTCTCCATGTAAGGGAGTGGACCTGTCCTGTTTGTCATGCCCA CCATGACCGTGACGTCAATGCAGCAAGAAATATACTGGCTGAGGGACTCAGG ATAAGAGCTCTGACTCCGGGGTCTTAGCAATACAGAAGCAAACAGGAACCGC AGGAATTGCGGGGGTAGCTTGGTAAACAAGAGAAACCTCTGCCGGCAAAGA AATAAGCCGGTAAGTATGCTCTGTTCCCAAGAATCTCGTGACTTTAGTCATGA GAGTTTCAAaaggcaaattcgccgcATGCAGCAAGAAATATACTGGCTGAGGGACT CAGGATAAGAGCTCTGACTCCGGGGTCTTAGCAATACAGAAGCAAACAGGAA CCGCAGGAATTGCGGGGGTAGCTTGGTAAACAAGAGAAACCTCTGCCGGCA AAGAAATAAGCCGGTAAGTATGCTCTGTTCCCAAGAATCTCGTGACTTTAGTC ATGAGAGTTTCAAaaggcaaattcgccgcCCGTAGCTCAGTTGGTTAGAGCGTTGG TCTTATGAGCCGAAGGTCGCGGGTTCGAGCCCCGCCGGAAGCA	aaggcaaattcgccgc	458

Description	TnpB Name	DNA sequence SEQ ID NO:	DNA sequence cloned into TRV cargo site	gRNA sequence	gRNA SEQ ID NO:
ISYmu1-g2-tandem 100bp wRNA–tRNA–Isoleucine	ISYmu1	440	atggattacaaggatgatgatgataaggattacaaggatgatgatgataagatggctccaaagaaga agagaaaggttggaatccacggagttccagctgctatgctgcagcataaagcctatgaataccgtatc tatccagataagaagcaggaaacactgattgccaagacaataggcagttcaaggtatgtatacaacc atttcctggaactttggaacaaggaatatgaggagaccggaaaaggtctgacttattacgcatgctcc aaacttctgacaaagcttaagagggacccggaaacagtatggctttgtgaagtggataagttctcgct ccagaattcgcttcgtaacctgtcggacgccttctcccgctttttcaaaggacagaacgagcatcccca gttcaagagcaagaagagcccgaggcaaagctacacgacacaatatacgaacaacaacatcgcagt ttccggaaattgtctgaagctgcctaagctgggccttgtcaagtttgcagacagtagggagatgaaag gccgtatactgaatgccacagtacgaaggaaatcgagcgggaaattcttcgtatcgatcctatgcgaa gaggagatctgtgaattgccaaagactgactcatctgtcggaattgacctcggaatcattgactttgcg gttatgtcagacggaagcaggcatgacaacaatcattttaccaggcaaatggaagaaaggcttagac gggagcagcgaaagcttgcaaggcgtgcacttgctgcggagaaaaggggcatttccctttctgaagcc aggaactatcagaagcaaaggcggaaggtagcgagactttatgaaaaggttgcaaaccagcgaaaa gaatatcttaacaaactcagtacagagatagtcaaaaaccacgatatcatctgtatcgaggaccttaa cgttaagggcatgatgcgcaatcataaactggcaaaaagcatctctgatgtatcatggacgagccttgt atcgaaactgcagtacaaggcttcctggtatggaaaagaagtcatcaggataagcagatggtttccgt caagtcagatatgctcagagtgcggccacaaggacaggaagaagcctctccatgtaagggagtggac ctgtcctgtttgtcatgcccaccatgaccgtgacgtcaatgcagcaagaaatatactggctgagggact caggataagagctctgactccggggtcttagcaatacagaagcaaacaggaaccgcaggaattgcgg gggtagcttggtaaacaagagaaacctctgccggcaaagaaataagccggtaagtatgctctgttccc aagaatctcgtgactttagtcatgagagtttcaaaaggcaaattcgccgcgtagcttggtaaacaaga gaaacctctgccggcaaagaaataagccggtaagtatgctctgttcccaagaatctcgtgactttagtc atgagagtttcaaaaggcaaattcgccgcccgtagctcagttggttagagcgttggtcttatgagccga aggtcgcgggttcgagccccgccggaagca	aaggcaaatt cgccgc	459

Description	TnpB Name	DNA sequence SEQ ID NO:	DNA sequence cloned into TRV cargo site	gRNA sequence	gRNA SEQ ID NO:
ISYmu1-g2-tandem 110bp wRNA–tRNA–Isoleucine	ISYmu1	441	atggattacaaggatgatgatgataaggattacaaggatgatgatgataagatggctccaaagaaga agagaaaggttggaatccacggagttccagctgctatgctgcagcataaagcctatgaataccgtatc tatccagataagaagcaggaaacactgattgccaagacaataggcagttcaaggtatgtatacaacc atttcctggaactttggaacaaggaatatgaggagaccggaaaaggtctgacttattacgcatgctcc aaacttctgacaaagcttaagagggacccggaaacagtatggctttgtgaagtggataagttctcgct ccagaattcgcttcgtaacctgtcggacgccttctcccgctttttcaaaggacagaacgagcatcccca gttcaagagcaagaagagcccgaggcaaagctacacgacacaatatacgaacaacaacatcgcagt ttccggaaattgtctgaagctgcctaagctgggccttgtcaagtttgcagacagtagggagatgaaag gccgtatactgaatgccacagtacgaaggaaatcgagcgggaaattcttcgtatcgatcctatgcgaa gaggagatctgtgaattgccaaagactgactcatctgtcggaattgacctcggaatcattgactttgcg gttatgtcagacggaagcaggcatgacaacaatcattttaccaggcaaatggaagaaaggcttagac gggagcagcgaaagcttgcaaggcgtgcacttgctgcggagaaaaggggcatttccctttctgaagcc aggaactatcagaagcaaaggcggaaggtagcgagactttatgaaaaggttgcaaaccagcgaaaa gaatatcttaacaaactcagtacagagatagtcaaaaaccacgatatcatctgtatcgaggaccttaa cgttaagggcatgatgcgcaatcataaactggcaaaaagcatctctgatgtatcatggacgagccttgt atcgaaactgcagtacaaggcttcctggtatggaaaagaagtcatcaggataagcagatggtttccgt caagtcagatatgctcagagtgcggccacaaggacaggaagaagcctctccatgtaagggagtggac ctgtcctgtttgtcatgcccaccatgaccgtgacgtcaatgcagcaagaaatatactggctgagggact caggataagagctctgactccggggtcttagcaatacagaagcaaacaggaaccgcaggaattgcgg gggtagcttggtaaacaagagaaacctctgccggcaaagaaataagccggtaagtatgctctgttccc aagaatctcggtgactttagtcatgagagtttcaaaaggcaaattcgccgcaattgcgggggtagcttgg taaacaagagaaacctctgccggcaaagaaataagccggtaagtatgctctgttcccaagaatctcgt gactttagtcatgagagtttcaaaaggcaaattcgccgcccgtagctcagttggttagagcgttggtctt atgagccgaaggtcgcgggttcgagccccgccggaagca	aaggcaaatt cgccgc	460

Description	TnpB Name	DNA sequence SEQ ID NO:	DNA sequence cloned into TRV cargo site	gRNA sequence	gRNA SEQ ID NO:
ISYmu1-g2-tandem 120bp wRNA–tRNA–Isoleucine	ISYmu1	442	atggattacaaggatgatgatgataaggattacaaggatgatgatgataagatggctccaaagaaga agagaaaggttggaatccacggagttccagctgctatgctgcagcataaagcctatgaataccgtatc tatccagataagaagcaggaaacactgattgccaagacaataggcagttcaaggtatgtatacaacc atttcctggaactttggaacaaggaatatgaggagaccggaaaaggtctgacttattacgcatgctcc aaacttctgacaaagcttaagagggacccggaaacagtatggctttgtgaagtggataagttctcgct ccagaattcgcttcgtaacctgtcggacgccttctcccgctttttcaaaggacagaacgagcatcccca gttcaagagcaagaagagcccgaggcaaagctacacgacacaatatacgaacaacaacatcgcagt ttccggaaattgtctgaagctgcctaagctgggccttgtcaagtttgcagacagtagggagatgaaag gccgtatactgaatgccacagtacgaaggaaatcgagcgggaaattcttcgtatcgatcctatgcgaa gaggagatctgtgaattgccaaagactgactcatctgtcggaattgacctcggaatcattgactttgcg gttatgtcagacggaagcaggcatgacaacaatcattttaccaggcaaatggaagaaaggcttagac gggagcagcgaaagcttgcaaggcgtgcacttgctgcggagaaaaggggcatttccctttctgaagcc aggaactatcagaagcaaaggcggaaggtagcgagactttatgaaaaggttgcaaaccagcgaaaa gaatatcttaacaaactcagtacagagatagtcaaaaaccacgatatcatctgtatcgaggaccttaa cgttaagggcatgatgcgcaatcataaactggcaaaaagcatctctgatgtatcatggacgagccttgt atcgaaactgcagtacaaggcttcctggtatggaaaagaagtcatcaggataagcagatggtttccgt caagtcagatatgctcagagtgcggccacaaggacaggaagaagcctctccatgtaagggagtggac ctgtcctgtttgtcatgcccaccatgaccgtgacgtcaatgcagcaagaaatatactggctgagggact caggataagagctctgactccggggtcttagcaatacagaagcaaacaggaaccgcaggaattgcgg gggtagcttggtaaacaagagaaacctctgccggcaaagaaataagccggtaagtatgctctgttccc aagaatctcgtgactttagtcatgagagtttcaaaaggcaaattcgccgcgaaccgcaggaattgcgg gggtagcttggtaaacaagagaaacctctgccggcaaagaaataagccggtaagtatgctctgttccc aagaatctcgtgactttagtcatgagagtttcaaaaggcaaattcgccgcccgtagctcagttggttag agcgttggtcttatgagccgaaggtcgcgggttcgagccccgccggaagca	aaggcaaatt cgccgc	461

Description	TnpB Name	DNA sequence SEQ ID NO:	DNA sequence cloned into TRV cargo site	gRNA sequence	gRNA SEQ ID NO:
ISYmu1-g2-tandem 127bp wRNA–tRNA–Isoleucine	ISYmu1	443	atggattacaaggatgatgatgataaggattacaaggatgatgatgataagatggctccaaagaaga agagaaaggttggaatccacggagttccagctgctatgctgcagcataaagcctatgaataccgtatc tatccagataagaagcaggaaacactgattgccaagacaataggcagttcaaggtatgtatacaacc atttcctggaactttggaacaaggaatatgaggagaccggaaaaggtctgacttattacgcatgctcc aaacttctgacaaagcttaagagggacccggaaacagtatggctttgtgaagtggataagttctcgct ccagaattcgcttcgtaacctgtcggacgccttctcccgctttttcaaaggacagaacgagcatcccca gttcaagagcaagaagagcccgaggcaaagctacacgacacaatatacgaacaacaacatcgcagt ttccggaaattgtctgaagctgcctaagctgggccttgtcaagtttgcagacagtagggagatgaaag gccgtatactgaatgccacagtacgaaggaaatcgagcgggaaattcttcgtatcgatcctatgcgaa gaggagatctgtgaattgccaaagactgactcatctgtcggaattgacctcggaatcattgactttgcg gttatgtcagacggaagcaggcatgacaacaatcattttaccaggcaaatggaagaaaggcttagac gggagcagcgaaagcttgcaaggcgtgcacttgctgcggagaaaaggggcatttccctttctgaagcc aggaactatcagaagcaaaggcggaaggtagcgagactttatgaaaaggttgcaaaccagcgaaaa gaatatcttaacaaactcagtacagagatagtcaaaaaccacgatatcatctgtatcgaggaccttaa cgttaagggcatgatgcgcaatcataaactggcaaaaagcatctctgatgtatcatggacgagccttgt atcgaaactgcagtacaaggcttcctggtatggaaaagaagtcatcaggataagcagatggtttccgt caagtcagatatgctcagagtgcggccacaaggacaggaagaagcctctccatgtaagggagtggac ctgtcctgtttgtcatgcccaccatgaccgtgacgtcaatgcagcaagaaatatactggctgagggact caggataagagctctgactccggggtcttagcaatacagaagcaaacaggaaccgcaggaattgcgg gggtagcttggtaaacaagagaaacctctgccggcaaagaaataagccggtaagtatgctctgttccc aagaatctcgtgactttagtcatgagagtttcaaaaggcaaattcgccgccaaacaggaaccgcagg aattgcgggggtagcttggtaaacaagagaaacctctgccggcaaagaaataagccggtaagtatgc tctgttcccaagaatctcgtgactttagtcatgagagtttcaaaaggcaaattcgccgcccgtagctcag ttggttagagcgttggtcttatgagccgaaggtcgcgggttcgagccccgccggaagca	aaggcaaat tcgccgc	462

Description	TnpB Name	DNA sequence SEQ ID NO:	DNA sequence cloned into TRV cargo site	gRNA sequence	gRNA SEQ ID NO:
ISYmu1-g2-tandem 130bp wRNA–tRNA–Isoleucine	ISYmu1	444	atggattacaaggatgatgatgataaggattacaaggatgatgatgataagatggctccaaagaaga agagaaaggttggaatccacggagttccagctgctatgctgcagcataaagcctatgaataccgtatc tatccagataagaagcaggaaacactgattgccaagacaataggcagttcaaggtatgtatacaacc atttcctggaactttggaacaaggaatatgaggagaccggaaaaggtctgacttattacgcatgctcc aaacttctgacaaagcttaagagggacccggaaacagtatggctttgtgaagtggataagttctcgct ccagaattcgcttcgtaacctgtcggacgccttctcccgctttttcaaaggacagaacgagcatcccca gttcaagagcaagaagagcccgaggcaaagctacacgacacaatatacgaacaacaacatcgcagt ttccggaaattgtctgaagctgcctaagctgggccttgtcaagtttgcagacagtagggagatgaaag gccgtatactgaatgccacagtacgaaggaaatcgagcgggaaattcttcgtatcgatcctatgcgaa gaggagatctgtgaattgccaaagactgactcatctgtcggaattgacctcggaatcattgactttgcg gttatgtcagacggaagcaggcatgacaacaatcattttaccaggcaaatggaagaaaggcttagac gggagcagcgaaagcttgcaaggcgtgcacttgctgcggagaaaaggggcatttccctttctgaagcc aggaactatcagaagcaaaggcggaaggtagcgagactttatgaaaaggttgcaaaccagcgaaaa gaatatcttaacaaactcagtacagagatagtcaaaaaccacgatatcatctgtatcgaggaccttaa cgttaagggcatgatgcgcaatcataaactggcaaaaagcatctctgatgtatcatggacgagccttgt atcgaaactgcagtacaaggcttcctggtatggaaaagaagtcatcaggataagcagatggtttccgt caagtcagatatgctcagagtgcggccacaaggacaggaagaagcctctccatgtaagggagtggac ctgtcctgtttgtcatgcccaccatgaccgtgacgtcaatgcagcaagaaatatactggctgagggact caggataagagctctgactccggggtcttagcaatacagaagcaaacaggaaccgcaggaattgcgg gggtagcttggtaaacaagagaaacctctgccggcaaagaaataagccggtaagtatgctctgttccc aagaatctcgtgactttagtcatgagagtttcaaaaggcaaattcgccgcaagcaaacaggaaccgc aggaattgcgggggtagcttggtaaacaagagaaacctctgccggcaaagaaataagccggtaagt atgctctgttcccaagaatctcgtgactttagtcatgagagtttcaaaaggcaaattcgccgcccgtagc tcagttggttagagcgttggtcttatgagccgaaggtcgcgggttcgagccccgccggaagca	aaggcaaattcgccgc	463

Description	TnpB Name	DNA sequence SEQ ID NO:	DNA sequence cloned into TRV cargo site	gRNA sequence	gRNA SEQ ID NO:
ISYmu1-g2-tandem 140bp wRNA–tRNA–Isoleucine	ISYmu1	445	atggattacaaggatgatgatgataaggattacaaggatgatgatgataagatggctccaaagaaga agagaaaggttggaatccacggagttccagctgctatgctgcagcataaagcctatgaataccgtatc tatccagataagaagcaggaaacactgattgccaagacaataggcagttcaaggtatgtatacaacc atttcctggaactttggaacaaggaatatgaggagaccggaaaaggtctgacttattacgcatgctcc aaacttctgacaaagcttaagagggacccggaaacagtatggctttgtgaagtggataagttctcgct ccagaattcgcttcgtaacctgtcggacgccttctcccgctttttcaaaggacagaacgagcatcccca gttcaagagcaagaagagcccgaggcaaagctacacgacacaatatacgaacaacaacatcgcagt ttccggaaattgtctgaagctgcctaagctgggccttgtcaagtttgcagacagtagggagatgaaag gccgtatactgaatgccacagtacgaaggaaatcgagcgggaaattcttcgtatcgatcctatgcgaa gaggagatctgtgaattgccaaagactgactcatctgtcggaattgacctcggaatcattgactttgcg gttatgtcagacggaagcaggcatgacaacaatcattttaccaggcaaatggaagaaaggcttagac gggagcagcgaaagcttgcaaggcgtgcacttgctgcggagaaaaggggcatttccctttctgaagcc aggaactatcagaagcaaaggcggaaggtagcgagactttatgaaaaggttgcaaaccagcgaaaa gaatatcttaacaaactcagtacagagatagtcaaaaaccacgatatcatctgtatcgaggaccttaa cgttaagggcatgatgcgcaatcataaactggcaaaaagcatctctgatgtatcatggacgagccttgt atcgaaactgcagtacaaggcttcctggtatggaaaagaagtcatcaggataagcagatggtttccgt caagtcagatatgctcagagtgcggccacaaggacaggaagaagcctctccatgtaagggagtggac ctgtcctgtttgtcatgcccaccatgaccgtgacgtcaatgcagcaagaaatatactggctgagggact caggataagagctctgactccggggtcttagcaatacagaagcaaacaggaaccgcaggaattgcgg gggtagcttggtaaacaagagaaacctctgccggcaaagaaataagccggtaagtatgctctgttccc aagaatctcgtgactttagtcatgagagtttcaaaaggcaaattcgccgcagcaatacagaagcaaac aggaaccgcaggaattgcgggggtagcttggtaaacaagagaaacctctgccggcaaagaaataag ccggtaagtatgctctgttcccaagaatctcgtgactttagtcatgagagtttcaaaaggcaaattcgcc gcccgtagctcagttggttagagcgttggtcttatgagccgaaggtcgcgggttcgagccccgccggaa gca	aaggcaaatt cgccgc	464

Description	TnpB Name	DNA sequence SEQ ID NO:	DNA sequence cloned into TRV cargo site	gRNA sequence	gRNA SEQ ID NO:
ISYmu1-g2-tandem 150bp wRNA–tRNA–Isoleucine	ISYmu1	446	atggattacaaggatgatgatgataaggattacaaggatgatgatgataagatggctccaaagaaga agagaaaggttggaatccacggagttccagctgctatgctgcagcataaagcctatgaataccgtatc tatccagataagaagcaggaaacactgattgccaagacaataggcagttcaaggtatgtatacaacc atttcctggaactttggaacaaggaatatgaggagaccggaaaaggtctgacttattacgcatgctcc aaacttctgacaaagcttaagagggacccggaaacagtatggctttgtgaagtggataagttctcgct ccagaattcgcttcgtaacctgtcggacgccttctcccgctttttcaaaggacagaacgagcatcccca gttcaagagcaagaagagcccgaggcaaagctacacgacacaatatacgaacaacaacatcgcagt ttccggaaattgtctgaagctgcctaagctgggccttgtcaagtttgcagacagtagggagatgaaag gccgtatactgaatgccacagtacgaaggaaatcgagcgggaaattcttcgtatcgatcctatgcgaa gaggagatctgtgaattgccaaagactgactcatctgtcggaattgacctcggaatcattgactttgcg gttatgtcagacggaagcaggcatgacaacaatcattttaccaggcaaatggaagaaaggcttagac gggagcagcgaaagcttgcaaggcgtgcacttgctgcggagaaaaggggcatttccctttctgaagcc aggaactatcagaagcaaaggcggaaggtagcgagactttatgaaaaggttgcaaaccagcgaaaa gaatatcttaacaaactcagtacagagatagtcaaaaaccacgatatcatctgtatcgaggaccttaa cgttaagggcatgatgcgcaatcataaactggcaaaaagcatctctgatgtatcatggacgagccttgt atcgaaactgcagtacaaggcttcctggtatggaaaagaagtcatcaggataagcagatggtttccgt caagtcagatatgctcagagtgcggccacaaggacaggaagaagcctctccatgtaagggagtggac ctgtcctgtttgtcatgcccaccatgaccgtgacgtcaatgcagcaagaaatatactggctgagggact caggataagagctctgactccggggtcttagcaatacagaagcaaacaggaaccgcaggaattgcgg gggtagcttggtaaacaagagaaacctctgccggcaaagaaataagccggtaagtatgctctgttccc aagaatctcgtgactttagtcatgagagtttcaaaaggcaaattcgccgcccggggtcttagcaatacа gaagcaaacaggaaccgcaggaattgcgggggtagcttggtaaacaagagaaacctctgccggcaa agaaataagccggtaagtatgctctgttcccaagaatctcgtgactttagtcatgagagtttcaaaagg caaattcgccgcccgtagctcagttggttagagcgttggtcttatgagccgaaggtcgcgggttcgagc cccgccggaagca	aaggcaaatt cgccgc	465

Description	TnpB Name	DNA sequence SEQ ID NO:	DNA sequence cloned into TRV cargo site	gRNA sequence	gRNA SEQ ID NO:
ISYmu1-g2-tandem 160bp wRNA–tRNA–Isoleucine	ISYmu1	447	atggattacaaggatgatgatgataaggattacaaggatgatgatgataagatggctccaaagaaga agagaaaggttggaatccacggagttccagctgctatgctgcagcataaagcctatgaataccgtatc tatccagataagaagcaggaaacactgattgccaagacaataggcagttcaaggtatgtatacaacc atttcctggaactttggaacaaggaatatgaggagaccggaaaaggtctgacttattacgcatgctcc aaacttctgacaaagcttaagagggacccggaaacagtatggctttgtgaagtggataagttctcgct ccagaattcgcttcgtaacctgtcggacgccttctcccgctttttcaaaggacagaacgagcatcccca gttcaagagcaagaagagcccgaggcaaagctacacgacacaatatacgaacaacaacatcgcagt ttccggaaattgtctgaagctgcctaagctgggccttgtcaagtttgcagacagtagggagatgaaag gccgtatactgaatgccacagtacgaaggaaatcgagcgggaaattcttcgtatcgatcctatgcgaa gaggagatctgtgaattgccaaagactgactcatctgtcggaattgacctcggaatcattgactttgcg gttatgtcagacggaagcaggcatgacaacaatcattttaccaggcaaatggaagaaaggcttagac gggagcagcgaaagcttgcaaggcgtgcacttgctgcggagaaaaggggcatttccctttctgaagcc aggaactatcagaagcaaaggcggaaggtagcgagactttatgaaaaggttgcaaaccagcgaaaa gaatatcttaacaaactcagtacagagatagtcaaaaaccacgatatcatctgtatcgaggaccttaa cgttaagggcatgatgcgcaatcataaactggcaaaaagcatctctgatgtatcatggacgagccttgt atcgaaactgcagtacaaggcttcctggtatggaaaagaagtcatcaggataagcagatggtttccgt caagtcagatatgctcagagtgcggccacaaggacaggaagaagcctctccatgtaagggagtggac ctgtcctgtttgtcatgcccaccatgaccgtgacgtcaatgcagcaagaaatatactggctgagggact caggataagagctctgactccggggtcttagcaatacagaagcaaacaggaaccgcaggaattgcgg gggtagcttggtaaacaagagaaacctctgccggcaaagaaataagccggtaagtatgctctgttccc aagaatctcgtgactttagtcatgagagtttcaaaaggcaaattcgccgcagctctgactccggggtct tagcaatacagaagcaaacaggaaccgcaggaattgcgggggtagcttggtaaacaagagaaacct ctgccggcaaagaaataagccggtaagtatgctctgttcccaagaatctcgtgactttagtcatgagag tttcaaaaggcaaattcgccgcccgtagctcagttggttagagcgttggtcttatgagccgaaggtcgc gggttcgagccccgccggaagca	aaggcaaat tcgccgc	466

Description	TnpB Name	DNA sequence SEQ ID NO:	DNA sequence cloned into TRV cargo site	gRNA sequence	gRNA SEQ ID NO:
ISYmu1-g2-tandem 170bp wRNA–tRNA–Isoleucine	ISYmu1	448	atggattacaaggatgatgatgataaggattacaaggatgatgatgataagatggctccaaagaaga agagaaaggttggaatccacggagttccagctgctatgctgcagcataaagcctatgaataccgtatc tatccagataagaagcaggaaacactgattgccaagacaataggcagttcaaggtatgtatacaacc atttcctggaactttggaacaaggaatatgaggagaccggaaaaggtctgacttattacgcatgctcc aaacttctgacaaagcttaagagggacccggaaacagtatggctttgtgaagtggataagttctcgct ccagaattcgcttcgtaacctgtcggacgccttctcccgctttttcaaaggacagaacgagcatcccca gttcaagagcaagaagagcccgaggcaaagctacacgacacaatatacgaacaacaacatcgcagt ttccggaaattgtctgaagctgcctaagctgggccttgtcaagtttgcagacagtagggagatgaaag gccgtatactgaatgccacagtacgaaggaaatcgagcgggaaattcttcgtatcgatcctatgcgaa gaggagatctgtgaattgccaaagactgactcatctgtcggaattgacctcggaatcattgactttgcg gttatgtcagacggaagcaggcatgacaacaatcattttaccaggcaaatggaagaaaggcttagac gggagcagcgaaagcttgcaaggcgtgcacttgctgcggagaaaaggggcatttccctttctgaagcc aggaactatcagaagcaaaggcggaaggtagcgagactttatgaaaaggttgcaaaccagcgaaaa gaatatcttaacaaactcagtacagagatagtcaaaaaccacgatatcatctgtatcgaggaccttaa cgttaagggcatgatgcgcaatcataaactggcaaaaagcatctctgatgtatcatggacgagccttgt atcgaaactgcagtacaaggcttcctggtatggaaaagaagtcatcaggataagcagatggtttccgt caagtcagatatgctcagagtgcggccacaaggacaggaagaagcctctccatgtaagggagtggac ctgtcctgtttgtcatgcccaccatgaccgtgacgtcaatgcagcaagaaatatactggctgagggact caggataagagctctgactccggggtcttagcaatacagaagcaaacaggaaccgcaggaattgcgg gggtagcttggtaaacaagagaaacctctgccggcaaagaaataagccggtaagtatgctctgttccc aagaatctcgtgactttagtcatgagagtttcaaaaggcaaattcgccgctcaggataagagctctgac tccggggtcttagcaatacagaagcaaacaggaaccgcaggaattgcgggggtagcttggtaaacaa gagaaacctctgccggcaaagaaataagccggtaagtatgctctgttcccaagaatctcgtgacttta gtcatgagagtttcaaaaggcaaattcgccgcccgtagctcagttggttagagcgttggtcttatgagc cgaaggtcgcgggttcgagccccgccggaagca	aaggcaaatt cgccgc	467

Description	TnpB Name	DNA sequence SEQ ID NO:	DNA sequence cloned into TRV cargo site	gRNA sequence	gRNA SEQ ID NO:
ISYmu1-g2-tandem 180bp wRNA–tRNA–Isoleucine	ISYmu1	449	atggattacaaggatgatgatgataaggattacaaggatgatgatgataagatggctccaaagaaga agagaaaggttggaatccacggagttccagctgctatgctgcagcataaagcctatgaataccgtatc tatccagataagaagcaggaaacactgattgccaagacaataggcagttcaaggtatgtatacaacc atttcctggaactttggaacaaggaatatgaggagaccggaaaaggtctgacttattacgcatgctcc aaacttctgacaaagcttaagagggacccggaaacagtatggctttgtgaagtggataagttctcgct ccagaattcgcttcgtaacctgtcggacgccttctcccgctttttcaaaggacagaacgagcatcccca gttcaagagcaagaagagcccgaggcaaagctacacgacacaatatacgaacaacaacatcgcagt ttccggaaattgtctgaagctgcctaagctgggccttgtcaagtttgcagacagtagggagatgaaag gccgtatactgaatgccacagtacgaaggaaatcgagcgggaaattcttcgtatcgatcctatgcgaa gaggagatctgtgaattgccaaagactgactcatctgtcggaattgacctcggaatcattgactttgcg gttatgtcagacggaagcaggcatgacaacaatcattttaccaggcaaatggaagaaaggcttagac gggagcagcgaaagcttgcaaggcgtgcacttgctgcggagaaaaggggcatttccctttctgaagcc aggaactatcagaagcaaaggcggaaggtagcgagactttatgaaaaggttgcaaaccagcgaaaa gaatatcttaacaaactcagtacagagatagtcaaaaaccacgatatcatctgtatcgaggaccttaa cgttaagggcatgatgcgcaatcataaactggcaaaaagcatctctgatgtatcatggacgagccttgt atcgaaactgcagtacaaggcttcctggtatggaaaagaagtcatcaggataagcagatggtttccgt caagtcagatatgctcagagtgcggccacaaggacaggaagaagcctctccatgtaagggagtggac ctgtcctgtttgtcatgcccaccatgaccgtgacgtcaatgcagcaagaaatatactggctgagggact caggataagagctctgactccggggtcttagcaatacagaagcaaacaggaaccgcaggaattgcgg gggtagcttggtaaacaagagaaacctctgccggcaaagaaataagccggtaagtatgctctgttccc aagaatctcgtgactttagtcatgagagtttcaaaaggcaaattcgccgcgctgagggactcaggata agagctctgactccggggtcttagcaatacagaagcaaacaggaaccgcaggaattgcgggggtagc ttggtaaacaagagaaacctctgccggcaaagaaataagccggtaagtatgctctgttcccaagaatc tcgtgactttagtcatgagagtttcaaaaggcaaattcgccgcccgtagctcagttggttagagcgttgg tcttatgagccgaaggtcgcgggttcgagccccgccggaagca	aaggcaaat tcgccgc	468

Description	TnpB Name	DNA sequence SEQ ID NO:	DNA sequence cloned into TRV cargo site	gRNA sequence	gRNA SEQ ID NO:
ISYmu1-g2-tandem 190bp wRNA–tRNA–Isoleucine	ISYmu1	450	atggattacaaggatgatgatgataaggattacaaggatgatgatgataagatggctccaaagaaga agagaaaggttggaatccacggagttccagctgctatgctgcagcataaagcctatgaataccgtatc tatccagataagaagcaggaaacactgattgccaagacaataggcagttcaaggtatgtatacaacc atttcctggaactttggaacaaggaatatgaggagaccggaaaaggtctgacttattacgcatgctcc aaacttctgacaaagcttaagagggacccggaaacagtatggctttgtgaagtggataagttctcgct ccagaattcgcttcgtaacctgtcggacgccttctcccgctttttcaaaggacagaacgagcatcccca gttcaagagcaagaagagcccgaggcaaagctacacgacacaatatacgaacaacaacatcgcagt ttccggaaattgtctgaagctgcctaagctgggccttgtcaagtttgcagacagtagggagatgaaag gccgtatactgaatgccacagtacgaaggaaatcgagcgggaaattcttcgtatcgatcctatgcgaa gaggagatctgtgaattgccaaagactgactcatctgtcggaattgacctcggaatcattgactttgcg gttatgtcagacggaagcaggcatgacaacaatcattttaccaggcaaatggaagaaaggcttagac gggagcagcgaaagcttgcaaggcgtgcacttgctgcggagaaaaggggcatttccctttctgaagcc aggaactatcagaagcaaaggcggaaggtagcgagactttatgaaaaggttgcaaaccagcgaaaa gaatatcttaacaaactcagtacagagatagtcaaaaaccacgatatcatctgtatcgaggaccttaa cgttaagggcatgatgcgcaatcataaactggcaaaaagcatctctgatgtatcatggacgagccttgt atcgaaactgcagtacaaggcttcctggtatggaaaagaagtcatcaggataagcagatggtttccgt caagtcagatatgctcagagtgcggccacaaggacaggaagaagcctctccatgtaagggagtggac ctgtcctgtttgtcatgcccaccatgaccgtgacgtcaatgcagcaagaaatatactggctgagggact caggataagagctctgactccggggtcttagcaatacagaagcaaacaggaaccgcaggaattgcgg gggtagcttggtaaacaagagaaacctctgccggcaaagaaataagccggtaagtatgctctgttccc aagaatctcgtgactttagtcatgagagtttcaaaaggcaaattcgccgcaaatatactggctgaggg actcaggataagagctctgactccggggtcttagcaatacagaagcaaacaggaaccgcaggaattg cggggtagcttggtaaacaagagaaacctctgccggcaaagaaataagccggtaagtatgctctgtt cccaagaatctcgtgactttagtcatgagagtttcaaaaggcaaattcgccgcccgtagctcagttggt tagagcgttggtcttatgagccgaaggtcgcgggttcgagccccgccggaagca	aaggcaaat tcgccgc	469

Description	TnpB Name	DNA sequence SEQ ID NO:	DNA sequence cloned into TRV cargo site	gRNA sequence	gRNA SEQ ID NO:
ISYmu1-g2-tandem 210bp wRNA–tRNA–Isoleucine	ISYmu1	451	atggattacaaggatgatgatgataaggattacaaggatgatgatgataagatggctccaaagaaga agagaaaggttggaatccacggagttccagctgctatgctgcagcataaagcctatgaataccgtatc tatccagataagaagcaggaaacactgattgccaagacaataggcagttcaaggtatgtatacaacc atttcctggaactttggaacaaggaatatgaggagaccggaaaaggtctgacttattacgcatgctcc aaacttctgacaaagcttaagagggacccggaaacagtatggctttgtgaagtggataagttctcgct ccagaattcgcttcgtaacctgtcggacgccttctcccgctttttcaaaggacagaacgagcatcccca gttcaagagcaagaagagcccgaggcaaagctacacgacacaatatacgaacaacaacatcgcagt ttccggaaattgtctgaagctgcctaagctgggccttgtcaagtttgcagacagtagggagatgaaag gccgtatactgaatgccacagtacgaaggaaatcgagcgggaaattcttcgtatcgatcctatgcgaa gaggagatctgtgaattgccaaagactgactcatctgtcggaattgacctcggaatcattgactttgcg gttatgtcagacggaagcaggcatgacaacaatcattttaccaggcaaatggaagaaaggcttagac gggagcagcgaaagcttgcaaggcgtgcacttgctgcggagaaaaggggcatttccctttctgaagcc aggaactatcagaagcaaaggcggaaggtagcgagactttatgaaaaggttgcaaaccagcgaaaa gaatatcttaacaaactcagtacagagatagtcaaaaaccacgatatcatctgtatcgaggaccttaa cgttaagggcatgatgcgcaatcataaactggcaaaaagcatctctgatgtatcatggacgagccttgt atcgaaactgcagtacaaggcttcctggtatggaaaagaagtcatcaggataagcagatggtttccgt caagtcagatatgctcagagtgcggccacaaggacaggaagaagcctctccatgtaagggagtggac ctgtcctgtttgtcatgcccaccatgaccgtgacgtcaatgcagcaagaaatatactggctgagggact caggataagagctctgactccggggtcttagcaatacagaagcaaacaggaaccgcaggaattgcgg gggtagcttggtaaacaagagaaacctctgccggcaaagaaataagccggtaagtatgctctgttccc aagaatctcgtgactttagtcatgagagtttcaaaaggcaaattcgccgccgtgacgtcaatgcagca agaaatatactggctgagggactcaggataagagctctgactccggggtcttagcaatacagaagca aacaggaaccgcaggaattgcgggggtagcttggtaaacaagagaaacctctgccggcaaagaaat aagccggtaagtatgctctgttcccaagaatctcgtgactttagtcatgagagtttcaaaaggcaaatt cgccgc	aaggcaaat tcgccgc	470

Description	TnpB Name	DNA sequence SEQ ID NO:	DNA sequence cloned into TRV cargo site	gRNA sequence	gRNA SEQ ID NO:
ISYmu1-g2-tandem 220bp wRNA–tRNA–Isoleucine	ISYmu1	452	atggattacaaggatgatgatgataaggattacaaggatgatgatgataagatggctccaaagaaga agagaaaggttggaatccacggagttccagctgctatgctgcagcataaagcctatgaataccgtatc tatccagataagaagcaggaaacactgattgccaagacaataggcagttcaaggtatgtatacaacc atttcctggaactttggaacaaggaatatgaggagaccggaaaaggtctgacttattacgcatgctcc aaacttctgacaaagcttaagagggacccggaaacagtatggctttgtgaagtggataagttctcgct ccagaattcgcttcgtaacctgtcggacgccttctcccgctttttcaaaggacagaacgagcatcccca gttcaagagcaagaagagcccgaggcaaagctacacgacacaatatacgaacaacaacatcgcagt ttccggaaattgtctgaagctgcctaagctgggccttgtcaagtttgcagacagtagggagatgaaag gccgtatactgaatgccacagtacgaaggaaatcgagcgggaaattcttcgtatcgatcctatgcgaa gaggagatctgtgaattgccaaagactgactcatctgtcggaattgacctcggaatcattgactttgcg gttatgtcagacggaagcaggcatgacaacaatcattttaccaggcaaatggaagaaaggcttagac gggagcagcgaaagcttgcaaggcgtgcacttgctgcggagaaaaggggcatttccctttctgaagcc aggaactatcagaagcaaaggcggaaggtagcgagactttatgaaaaggttgcaaaccagcgaaaa gaatatcttaacaaactcagtacagagatagtcaaaaaccacgatatcatctgtatcgaggaccttaa cgttaagggcatgatgcgcaatcataaactggcaaaaagcatctctgatgtatcatggacgagccttgt atcgaaactgcagtacaaggcttcctggtatggaaaagaagtcatcaggataagcagatggtttccgt caagtcagatatgctcagagtgcggccacaaggacaggaagaagcctctccatgtaagggagtggac ctgtcctgtttgtcatgcccaccatgaccgtgacgtcaatgcagcaagaaatatactggctgagggact caggataagagctctgactccggggtcttagcaatacagaagcaaacaggaaccgcaggaattgcgg gggtagcttggtaaacaagagaaacctctgccggcaaagaaataagccggtaagtatgctctgttccc aagaatctcgtgactttagtcatgagagtttcaaaaggcaaattcgccgcccaccatgaccgtgacgtc aatgcagcaagaaatatactggctgagggactcaggataagagctctgactccggggtcttagcaata cagaagcaaacaggaaccgcaggaattgcgggggtagcttggtaaacaagagaaacctctgccggc aaagaaataagccggtaagtatgctctgttcccaagaatctcgtgactttagtcatgagagtttcaaaa ggcaaattcgccgcccgtagctcagttggttagagcgttggtcttatgagccgaaggtcgcgggttcga gccccgccggaagca	aaggcaaattcgccgc	471

Description	TnpB Name	DNA sequence SEQ ID NO:	DNA sequence cloned into TRV cargo site	gRNA sequence	gRNA SEQ ID NO:
ISYmu1-g2-tandem 230bp wRNA–tRNA–Isoleucine	ISYmu1	453	atggattacaaggatgatgatgataaggattacaaggatgatgatgataagatggctccaaagaaga agagaaaggttggaatccacggagttccagctgctatgctgcagcataaagcctatgaataccgtatc tatccagataagaagcaggaaacactgattgccaagacaataggcagttcaaggtatgtatacaacc atttcctggaactttggaacaaggaatatgaggagaccggaaaaggtctgacttattacgcatgctcc aaacttctgacaaagcttaagagggacccggaaacagtatggctttgtgaagtggataagttctcgct ccagaattcgcttcgtaacctgtcggacgccttctcccgctttttcaaaggacagaacgagcatcccca gttcaagagcaagaagagcccgaggcaaagctacacgacacaatatacgaacaacaacatcgcagt ttccggaaattgtctgaagctgcctaagctgggccttgtcaagtttgcagacagtagggagatgaaag gccgtatactgaatgccacagtacgaaggaaatcgagcgggaaattcttcgtatcgatcctatgcgaa gaggagatctgtgaattgccaaagactgactcatctgtcggaattgacctcggaatcattgactttgcg gttatgtcagacggaagcaggcatgacaacaatcattttaccaggcaaatggaagaaaggcttagac gggagcagcgaaagcttgcaaggcgtgcacttgctgcggagaaaaggggcatttccctttctgaagcc aggaactatcagaagcaaaggcggaaggtagcgagactttatgaaaaggttgcaaaccagcgaaaa gaatatcttaacaaactcagtacagagatagtcaaaaaccacgatatcatctgtatcgaggaccttaa cgttaagggcatgatgcgcaatcataaactggcaaaaagcatctctgatgtatcatggacgagccttgt atcgaaactgcagtacaaggcttcctggtatggaaaagaagtcatcaggataagcagatggtttccgt caagtcagatatgctcagagtgcggccacaaggacaggaagaagcctctccatgtaagggagtggac ctgtcctgtttgtcatgcccaccatgaccgtgacgtcaatgcagcaagaaatatactggctgagggact caggataagagctctgactccggggtcttagcaatacagaagcaaacaggaaccgcaggaattgcgg gggtagcttggtaaacaagagaaacctctgccggcaaagaaataagccggtaagtatgctctgttccc aagaatctcgtgactttagtcatgagagtttcaaaaggcaaattcgccgctttgtcatgcccaccatga ccgtgacgtcaatgcagcaagaaatatactggctgagggactcaggataagagctctgactccggggt cttagcaatacagaagcaaacaggaaccgcaggaattgcgggggtagcttggtaaacaagagaaac ctctgccggcaaagaaataagccggtaagtatgctctgttcccaagaatctcgtgactttagtcatgag agtttcaaaaggcaaattcgccgcccgtagctcagttggttagagcgttggtcttatgagccgaaggtc gcgggttcgagccccgccggaagca	aaggcaaatatcgccgc	472

Description	TnpB Name	DNA sequence SEQ ID NO:	DNA sequence cloned into TRV cargo site	gRNA sequence	gRNA SEQ ID NO:
ISYmu1-g2-tandem 240bp wRNA–tRNA–Isoleucine	ISYmu1	454	atggattacaaggatgatgatgataaggattacaaggatgatgatgataagatggctccaaagaagaagagaaaggttggaatccacggagttccagctgctatgctgcagcataaagcctatgaataccgtatctatccagataagaagcaggaaacactgattgccaagacaataggcagttcaaggtatgtatacaaccatttcctggaactttggaacaaggaatatgaggagaccggaaaaggtctgacttattacgcatgctccaaacttctgacaaagcttaagagggacccggaaacagtatggctttgtgaagtggataagttctcgctccagaattcgcttcgtaacctgtcggacgccttctcccgctttttcaaaggacagaacgagcatcccccagttcaagagcaagaagagcccgaggcaaagctacacgacacaatatacgaacaacaacatcgcagtttccggaaattgtctgaagctgcctaagctgggccttgtcaagtttgcagacagtagggagatgaaagccgtatactgaatgccacagtacgaaggaaatcgagcgggaaattcttcgtatcgatcctatgcgaagaggagatctgtgaattgccaaagactgactcatctgtcggaattgacctcggaatcattgactttgcggttatgtcagacggaagcaggcatgacaacaatcattttaccaggcaaatggaagaaaggcttagacgggagcagcgaaagcttgcaaggcgtgcacttgctgcggagaaaaggggcatttccctttctgaagccaggaactatcagaagcaaaggcggaaggtagcgagactttatgaaaaggttgcaaaccagcgaaaagaatatcttaacaaactcagtacagagatagtcaaaaaccacgatatcatctgtatcgaggaccttaacgttaagggcatgatgcgcaatcataaactggcaaaaagcatctctgatgtatcatggacgagccttgtatcgaaactgcagtacaaggcttcctggtatggaaaagaagtcatcaggataagcagatggtttccgtcaagtcagatatgctcagagtgcggccacaaggacaggaagaagcctctccatgtaagggagtggacctgtcctgtttgtcatgcccaccatgaccgtgacgtcaatgcagcaagaaatatactggctgagggactcaggataagagctctgactccggggtcttagcaatacagaagcaaacaggaaccgcaggaattgcgggggtagcttggtaaacaagagaaacctctgccggcaaagaaataagccggtaagtatgctctgttcccaagaatctcgtgacttagtcatgagagtttcaaaaggcaaattcgccgcccgtagctcagttggttagagcgttggtcttatgagccgaaggtcgcgggttcgagccccgccggaagca	aaggcaaatatcgccgc	473

Description	TnpB Name	DNA sequence SEQ ID NO:	DNA sequence cloned into TRV cargo site	gRNA sequence	gRNA SEQ ID NO:
ISYmu1-g2-tandem 250bp wRNA–tRNA–Isoleucine	ISYmu1	455	atggattacaaggatgatgatgataaggattacaaggatgatgatgataagatggctccaaagaagaagagaaaggttggaatccacggagttccagctgctatgctgcagcataaagcctatgaataccgtatctatccagataagaagcaggaaacactgattgccaagacaataggcagttcaaggtatgtatacaaccatttcctggaactttggaacaaggaatatgaggagaccggaaaaggtctgacttattacgcatgctccaaacttctgacaaagcttaagagggacccggaaacagtatggctttgtgaagtggataagttctcgctccagaattcgcttcgtaacctgtcggacgccttctcccgctttttcaaaggacagaacgagcatccccagttcaagagcaagaagagcccgaggcaaagctacacgacacaatatacgaacaacaacatcgcagtttccggaaattgtctgaagctgcctaagctgggccttgtcaagtttgcagacagtagggagatgaaagccgtatactgaatgccacagtacgaaggaaatcgagcgggaaattcttcgtatcgatcctatgcgaagaggagatctgtgaattgccaaagactgactcatctgtcggaattgacctcggaatcattgactttgcggttatgtcagacggaagcaggcatgacaacaatcattttaccaggcaaatggaagaaaggcttagacgggagcagcgaaagcttgcaaggcgtgcacttgctgcggagaaaaggggcatttccctttctgaagccaggaactatcagaagcaaaggcggaaggtagcgagactttatgaaaaggttgcaaaccagcgaaaagaatatcttaacaaactcagtacagagatagtcaaaaaccacgatatcatctgtatcgaggaccttaacgttaagggcatgatgcgcaatcataaactggcaaaaagcatctctgatgtatcatggacgagccttgtatcgaaactgcagtacaaggcttcctggtatggaaaagaagtcatcaggataagcagatggtttccgtcaagtcagatatgctcagagtgcggccacaaggacaggaagaagcctctccatgtaagggagtggacctgtcctgtttgtcatgcccaccatgaccgtgacgtcaatgcagcaagaaatatactggctgagggactcaggataagagctctgactccggggtcttagcaatacagaagcaaacaggaaccgcaggaattgcgggggtagcttggtaaacaagagaaacctctgccggcaaagaaataagccggtaagtatgctctgttcccaagaatctcgtgactttagtcatgagagtttcaaaaggcaaattcgccgcccgtagctcagttggttagagcgttggtcttatgagccgaaggtcgcgggttcgagccccgccggaagca	aaggcaaatatcgccgc	474

Table 13: Example TnpBs associated with Example 18

TnpB Name	**TnpB with wRNA overlap Native DNA sequence (single)**	source	SEQ ID NO:
TnpB_11	ATGCAGCGGCTTCAAGCCTTCAAATTTTTACTCATGCCCGATGGT GAACAGCAGCGTGATATGCGCCGCTTCGCCGGGTCGTGCCGCTT CGTGTTCAACAAGGCGCTGGCGCTGCAAAAGGCCCACTACGAGG CAGGCGGCAAGTTTATCGGCTATGTAGCGATGGCAAAGCACCTG ACCGAGTGGCGCAATGGCGCTGAAACACCGTGGCTCAAAGAGG CCCCGGTACACCCACTGCAGCATGCGCTCAAAGATTTGGAGCGA GCGTACAGAAACTTCTTTGCCAAACGAGCCATGTTCCCCCGCTTC AAGCGCAAAGGCCGGGGCGAGAGTTTTCGTTACCCGGACGCCCA GCAATTCGAGATAGACGAGGCCAACAGCCGTATCAGGCTGCCCA AGCTGGGTTGGATTCGATATCGCAACAGCCGCAACATTCTGGGG GCGGCCAAGAACATCACCGTCAGCCAGTCCGCGGGCAAGTGGTA TGCCAGCATTCAGACGATGCGCGAGGTTGAACAACCCGTACCCA GCGCCACCAGCGCGGTGGGAATCGACGTGGGGATTGTGCGCTTC GCTACGCTGAGCGATGGCAGCTTTGTAGCTCCGCTCAACAGCTTC AAGAAGCATGAGGCCCGATTGCGCCGCTATCAGCGGGCGCTGAG CCGCAAAGCCAAATTCAGCAAGAACCGGCAAAAAGCCCAATGG AGAGTGCAGAACATCCACACCCGAATCGGCAATGCTCGGCGCGA CTTCCTGCACAAGACCACGACCACGATCAGCAAAAACCACGCGA TGGTGTGTATCGAGGACTTGCAaGTGAAGAATATGTCCAGGTCG AGCAAAGGCAACGCTCAGAAAGCAGGTAAGAATGTTCGAGCCA AGTCCGGTCTGAACAAATCCATTCTCGACCAGGGCTGGTTCGAG TTCCGCCGCCAGTTGGAATACAAGCTGACCTGGAATGGCGGAAT GCTGGTAGCCGTGCCGCCACACAACACGAGCCGGACCTGTCCGG CCTGCGGGCGGGTGACAGCAGAGAATCGGCAAACGCAAGCACA TTTCGAGTGTGTGAATTGTGGTTACGAGAACAACGCCGATGTGG TCGGCGCAATGAATATCTTGGCGCGGGGGCACCGCGTTGCAGCC TGTGGAGAGGACGGCTCTGGCCTGGGTCGTAAGACTCGGACGAA ACCAGCCTCTGTGAAAGCGACTACGTGCGAGGCAGCTCATGCGT AGCACCGTAGGAATTCCCTGTCTTCAGGCAGGGGAGGATGTCAA	Example 1 pipeline	475

TnpB Name	**TnpB with wRNA overlap Native DNA sequence (single)**	source	SEQ ID NO:
TnpB_16	ATGAAGAGATTGCTAAAGAGCTTCAAGACGGAAATCAACCCAAC TGAAGAACAGAAAGCCAGGATCCGCAGGACGATAGGCACTTGC CGATATGTCTATAACTTCTACCTTGGTCACAACAAAGCCCTGCAT GATAATGGTGAAAAGTTTATGACTGGTAAGGACTTTAGTCTATG GCTTAACAACGAATACATTCCGGATAATCCTGACAAAACATGGA TCAGGGAAGTGTATTCAAAAGCCGTAAAAAAGTCTATCGAAGAT GGATGTACTGCTTTTACAAGATTTTTTAAACATCAGAGTGATTTT CCTAAATTTAAAAAGAAGGGTAAATCTGATGTGAAGATGTATTT CGTCAGAAATAACCCAAAGGATTGTCAATGCGAAAGGCACAGA CTTAAGATCCCAACTCTGGGCTGGATCCGTATCAAAGAAAAAGG ATATATCCCAACAACAAAGGATGGATATATGATCAGAAGTGGTA CAGTGTCTGTTAAGGCTGGCAGATTTTATGTTTCTGTTCTTGTAG AGATTCCTGATATCAATATCGACAATAACTCAAACGAGGGAATT GGTATAGACCTTGGACTAAAAGATTTTGCCATTGTTTCAAATGGA AAGACATACAGGAACATCAACAAATCGGCAGGATTAAAGAAAC TTGAAAAACAACTGATCCGGGAACAGAGAAGTCTTTCCCGAAAG TATGAAAATTTAAAGAAAGGAGAGTCCACTCAAAGAGCAAATAT ACAGAAACAAAAGCTTAAGGTACAAAAACTTCATCATAAAATGG ATAATATCCGTACGGATCACATTAACAAGACAATCGCTGAGATA GTGAAAACCAAACCGTCTTACATAACGATTGAAGATTTAAATGT AAAAGGAATGATGAAAAACAGGTGTCTTTCAAAAGCTGTTGCAT CTCAGAAGTTTTATGAATTTAGAACAAGGCTTAAAGCCAAGTGT GATGAAAACGGCATTGAATTAAGGGTAGCAGATAGATTTTATCC GTCTTCAAAGACCTGTCACCATTGTGGTTCTGTCAGGAAAAATTT AAAGCTTTCAGATAGAATTTACAGATGTGAATGCGGCTATGTTG CAGACAGGGATCTCAATGCGGCACTTAATTTAAAAGATGCTAAA ACTTACAGGATTGCATGATTAAAAGCAAATGTAAGTATGTACCG AAGGCTATTTCGGGAATTAACGACTGTGGAGTGTACAAGAACCT GTGAGTAGCGTATTGCCTGCAATCGTCAAAACATACACAATGAA GCAGTAAGAAGTATTCGTAAGGACTTCAATTTCTCGATGTGTTTG GGTATATTTAAACATATTTGGAGTGGCag	Example 1 pipeline	476

TnpB Name	**TnpB with wRNA overlap Native DNA sequence (single)**	source	SEQ ID NO:
TnpB_19	ATGCTAAAGGCATTCAAGACAGAAATAGCACCGACTAGAGAGC AGAAACAAAAGATAATACGCTCAATAGGTATAGCAAGATTTTTA TATAACCAATACATTGCTTATAATAAAAAGTTATATAAAATGTAT CAGCGAGGTTTATTAGATATAAATCAGAAACATTTTATATCTGCT AATGACTTTGATAAATATGTAAACCATAATCTGAAGTTGAAATT GCCGTGGATTGACGAATGTGGTTCTAAAGCACGAAAAAAAGCGC AAGTCAATGCCGAAGCAGCGTTCAAGAAGTTTTTCAAAGGCGAA GCCGGCTTCCCGAGGTTCAAGAAGAAATCTAAGCAAGATGTAAA GCTATATTTTCCTAAAAATAATAAAGGTGATTGGACGATATGGC GGCATAAGCTAATGATACCTACGTTAAAACAGGTTAAGCTAAAA GAATTTGGCTACTTACCGGTAGGTGCTAAAGTCACTAACGGCAC AGTATCTTATGAGGCTGGCAGGTTCTATATTTCTGTCGTGATAGA TATTGATGAAAACTCCAAGTACAACAAGGATTTGGAAACTTCAT ATCGTACAACAACCAAAGGCATTGGTATAGATTTAGGCATCAAA GAATTGGCTATTGTAAGCAATGGCAAAATATTTAAAAATATCAA CAAAAGTTCAAAAATCAAGAAACTTGAGAAACGGCTTCGGAGA GAGCAGAGACGCCTTAGCCGTAAATATGAGTCTAAGAAAAAGA AAGGTGGTAAGACTGCTACTGTCTCTGCAAACATAGAAAAGCAA AAGTTAAAGGTACAAAAACTTCATCATCGCATTGATAAAATCCG CGAGGATTATGAAAACAAAGTCATCCATGAAATTGTAAAGCAAA AACCACGCTTTATTACGGTGGAAGATTTGAATGTCAAAGGCATG ATGAAGAACAGGCATCTGGCAAAAGCTGTGGCAGCACAAAGAT TTAATTATCTGCTGACTAAGCTGAAACGCAAATCAGAAATCATT GGCATAGAAATGCGTATAGTTGACAGATTCTATCCAAGTTCCAA AACTTGTCATTTTTGTGGGCATATCCATAAAGGTCTGAAACTCAA AGACCGTGTATATATTTGTCCTGAATGTGGCTATATACAAAATAG GGATTTCAATGCTTCACTAAATTTGCGGGATGCCAAAGAATATC GGGTAGCTTAATAACAAAAGGCCCGATATATGTACCGATGGCTA GTCGGGAATTTACGCCTTCGGAGTGCTATACAAACGTTAGTAGC TTGTCTTTCAAGGCAGCGAGAGCGAGTACGATGAACAAGGAATA TTTCTAAGGCATAAACACTTTTGTCTATGTTTTTAGTAGCag	Example 1 pipeline	477

TnpB Name	**TnpB with wRNA overlap Native DNA sequence (single)**	source	SEQ ID NO:
TnpB_1	ATGATCCTGACCTACAAGATCCGCCACAATACTGATTTCAGCAA TGAACTGAAACGAGCGTTCAAGATAGCGGAGTTTGCAGTCAGAA ATCCAAAATGCAGAACGTCAAAAGACGTTAAAGAGTTCGGACTG AAATCAGCAATATCAAATCAGATCCTCAAAAAGTATGGCAACCA GAAAACCATCAAACAAGTCCACAACGTAAATTTGGTAATACCGA ATCAGGCGATCAAAGTTGACAAGGACGCCAGAACGATCCGGATT GTACCGTTGAAACTGGTTCTGAACTATCAGTTCCCTGATTTCCAA AAGGTCAATCAGATTGAACTTGACAAAGAGTTTGCCTATATTTC ATGCACCGTGGCAGAACAACCGGAGATGATACCTCAACAATTCA TTGGGGTTGATCGGAATACTACTGGACACGTTGCAGTCACAGCC AACCCGGATACCGGGAAGATTGAGAAACTTGGCAAATCTGCACT GCACATCCACCGTAAGTACTCTGCGATCAGGAAACAACTTCAAC GAGACGGAAAGTTCCGGAAGTTGGCAGTTGTTAAAGATCGTGAG AGCAGAATCGTGAAAGACCTGAACCATAAGATCAGTCGTAAGAT TGTTAATATGGCGCAGGTACAACATGCAGCACTCGTCTTTGAAG ATTTGGGCGGTATCCGTCAGACAAGGAAACACCACCGTTCGTTC AAGTATGCCCTGCACAGTTGGTCTTTCTTTCAGTTGCAAACTTTC GTGGAATATAAAGCCAAGCTGCTTGGTGTCCCTGTCCTCTACGTT GATCCAGCATATACCAGTCAGGATTGTTCCCGGTGTGGTACACG TGGTCAACGAAATGGCAAGGATTTCAAGTGTCCGACTTGTGGAC ACGTTGATCATGCAGACGTTAATGCGGCATTCAATATTGCATTAA AAGTCAAAGACATGATCGATCGTACGCAGAAAGAGATGTGTACG ATGGGAGCACTGATACCCCACGACGCAATGCTGGAATGCCCGGC AACGTCAGAACCCCACGCGCTTTAGCTGTGGGAGTATGTCAG	Example 1 pipeline	478

TnpB Name	**TnpB with wRNA overlap Native DNA sequence (single)**	source	SEQ ID NO:
TnpB_28	ATGCTGATTGCTCACCGCATCGCCCTCGATCCGAACAACGCGCA AGCCACGTATCTGGCGCGTGCCGCGGGCACGGCGCGCTTCGCCT ATAACTGGGCGCTGGCCGAGTGGAAGCGCCAGTACGAGGCGTG GAAaACGGACAACGCGCTGCCCAAGCCGTCGCAGGCAGCGTTGC GACGCCAACTGAACGCGATCAAGCGCGAGCAGTTTCCGTGGATG ATGGAAGTCACCAAGAATGCGCCGCAGATGGCGATCATCCAGTT GGGCGACGCGTTCAAGAATTTCTTCGCAGGCCGCGCCCGCTATC CGCAGTTCCGCAAGAAGGGCGTGCACGACCGCTTTACGCTCACC AACGATCAGTTCAGTATCGACGCCTCACGCATTCGCATCCCCAAT CTCGGCTGGGTGCGCGTGCGCGAGACGTTGCGCTTTGCTGGCAA GATCATGTCGGCCACGGTCTCCCGTGTGGCCGACCGCTGGTTCGT CAGCATCACCGTGGACACACCCGACAGTTCGCATCTGCCCGAAG CCGAAAACCAAGGCGCAGTGGGTGTCGATCTGGGTGTGTCGGCG CTGGCCACGCTCTCGACGGGAGAGGCGATTCCTGGTCCCAAGCC GCACAAGGCTTTGCTAGCCCGCCTGCGCAAGCTATCGCGCAGCC TGTCGCGCAAGGTCAAGGGATCGGCCAATCGTCGCAAGGCGAAG GCAAAGCTGGCGAAGCTGCATGCCCGCATCGCAACCATCCGCTC GGATGCCCTGCACAAGCTCACGACTGATCTCACTCGCCGCTTCCA CACCATCGGCATTGAAGAtCTGAACGTGCGCGGCATGGTGAAGA ACCGGCACCTGGCGCGTTCGATCGCCGACATGAGCTTCTTCGAA CTGAGGCGGCAGCTGGAGTACAAGGCGGACATGCGCGGAGCCA AGGTGGTGGTGGCCGACCGCTTCTATGCCAGCAGCAAGACGTGC TCGGCGTGCGGACACAAGCTGGAGAGCTTGCCGCTGTCCATGCG CGAATGGATGTGTCCCCCGTGCGGGACGATTCACGACCGCGATG TGAACGCGGCCGTGAACCTGTGCAACCACGCCGTGAGTTCCACG GTGTCAGCCCGTGGAGAGGAAGGCTCTGGCTCGGATCGCAAGAC CCGAGTGAAACCGTCCTCCGCGAAGCGGGAAGTCAGCTTTGTTC CTGTTTGAGCAGGAATGAGCAAGTCTGATGGAACGG	homology search against ISXfa01	479

TnpB Name	**TnpB with wRNA overlap Native DNA sequence (single)**	source	SEQ ID NO:
TnpB_29	ATGCTGATCGCGCATCGCATCGCGCTTGATCCGAACAACGTACA GGCGACCTACTTTGCCCGCGCAGCAGGCACGGCCCGCTTTGCTT ACAATTGGGCGCTGGCTGAGTGGAAATGCCAATATGAGGCATGG AAGTTGGACAACCGCCAAACCAAGCCCACACAGGCGGCGCTAC GCCGCCAGTTGAATGCCATCAAGCGCGAGCAATTTCCCTGGATG CTTGAAGTCACCAAGAACGCGCCACAAATGGCGATCATTCAGTT GGGGCAAGCATTTCAGAACTTCTTCGCTGGCCGCGCCAAATATC CGCAGCCCCGCAAGAAGGGCGTGCACGACCGTTTCACGCTCACC AATGACCAGTTCAGCATCGACGGCTGCCGCATACGCATCCCCAA TCTGGGATGGGTGCGCATGCGCGAGTCGTTGCGCTTCGCGGGCA AGATCCTGTCGGCCACGGTCTCCCGTGTGGCCGACCGCTGGTTTG TCAGCATTGCCGTGGATACCCAAGACGATTCACATCTACCCCAA GCTAAAAACCAAGGCGTGGTGGGCGTGGATTTGGGTGTTGCGGC GCTGGCGACGCTCTCAACGGGAGAGCCGCCGATCCCCGGTCCGA AGCCTCACAAGGCGCTGCAGGCCCGGTTGCAACGGCTCTCGCGC AGTCTGAGCCGCAAACAAAAAGGATCAGCCAATCGCAAGAAGG CCAAGGCGAAGCTGGCGAGGCTGCATGCCCGCATTGCCGCGATC CGTTCGGACGCCCTGCACAAGCTCACGACGGACCTCACGCGCCG ATTCCACACCATTGGCATTGAGGACCTGAACGTGCGCGGCATGG TGAGGAACCGCCATCTGGCCCGCTCCATCGCCGATATGGGCTTCT TCGAGTTCCGTCGGCAACTGGAATACAAGACGGTGATGCGCGGC GGGCAGGTCGTTGTGGCAGATCGGTTCTACCCGAGCAGCAAGAC GTGTTCGGCTTGCGGGCACAGGCTCGAAGCTCTGCCGCTGGTCG TGCGCGAGTGGACGTGCCCCGCCTGTGGCGTGGTCCATGACCGC GACCTGAACGCGGCAACCAATCTGAAGAACATGGCGGTGAGTTC CACCGTGTCAGCCTGTGGAGAGGAAGGCGCTGGCCGCACTCGCA AGAGCGCGGTGAAACCGGCCTCGGTGAAGCAGGAAGCAAGCAG CAGATTTAGTCAGAAATGACTGGTTTTGCGTAGGTATTGCAGAA CGG	homology search against ISXfa01	480

TnpB Name	TnpB with wRNA overlap Native DNA sequence (single)	source	SEQ ID NO:
TnpB_30	ATGAAAAGAGCATATTTAATAGAAATCAAACCGACAACTGAACA AGTTAGTAAAATCCGTCAAACTATTGGGGTGTGTCGATATGTAT ATAACCTGTATATCTCTAAAAATAAAGAGGCATATGAGTCGGAT GGGAAATTTCTTAGTGGCTATGACTTTTCTAAATGGTTAAACAAC ATTCATACAAAAGAGTGTGACCAGTGGATTAAAGAAGTGTCGAG CAAAGCGGTAAAACAAGCGATTATGAATGGAGATAAAGCTTTTA AGCGATTTTTTAAAGGTTTATCTAAGTTTCCACGATATAAAAAAA AGAAGAAGCAAGATGTGAAGTGCTACTTTCCGAAAAACAACAA AACAGATTGGACAGTAGAAAGACATAGAGTAAAAGTCCCTACA ATTGGTTGGATAAGGTTAAAAGAGTATGGATATATTTCAAAAAA TACAACTGTAAAAAGTGGTACAGTATATCAAAGAGCAGGAAGAT ACTATGTGTCTATACTCTGTGAAGTGAAAGAGGTAAAACCAAAT TCAATTCTCAATGCAGTAGGTATAGGAATTGATTTGGGAATTAA AGACTTCGCTGTACGTAGCGATGGAAAAACTCACAAAAATATAA ATAAAACAGTACAAGTAAAAAAGATAGAAAAACGGTTAAAACG TGAACAACGCTCTTTATCACGCAAATTTGAAAATATAAGAAAGA GAGGTGAACAACCTGTTACTAATAAAAGAGCGAATATAAACAA AAATATTCTTAGGGTGCAACAGCTACATGCTAGGCTAGCAAATA TTCGATTAGCGTATGTAAAGTCTATTGTAAATGATGTGGTGAAA ACCAAGCCAATGTTTATTACAGTAGAAGATTTGAATGTGAAAGG TATGATGAAGAACAAACATCTATCTAAAGCTGTAGCGCAACAAT GTTTTTATGCTTTTAAAACATGGTTATCAACGAAATGTAGGGAAT ATGGAATTGAACTACGGCAAGTGGACAGATTCTATCCATCTTCT AAAATATGTTCATGTTGCGGTCAAAAAAAGTCGGATTTAAAGCT GTCTGACCGAGTATATACATGTGATTGTGGAAATGTAATGGATA GAGATTTAAATGCATCTATCAACCTTTTACAAGCAAAAAAATAT ACAATACTAACGTAAATAGATTGTATATATGTACGGTGGGCTAC ATCGGAATTTACGCCTGTGGAGTGTCACAGCAAACTGTAGTAGC AAGTGATTAGCGAGGCAGGGCACGTAGAAGCAGGAATACTCTA AGAATATACATTTGTCTATATTTTTAGTAGCAG	Isfinder	481

Table 14: Selected TAM and gRNA sequences (In Table 14, the gRNA sequences are listed as DNA sequences that are the equivalent RNA sequences (except the RNA will have the T replaced with a U)

TnpB	TAM Sequence	gRNA ID	gRNA sequence	SEQ ID NO:
TnpB_11	TTGAT	g10	taacttgtactacctc	482
TnpB_11	TTGAT	g3	tcacattaagcctaga	483
TnpB_11	TTGAT	g4	cccaagttctccaaat	484
TnpB_11	TTGAT	g5	tacccatcctaaagta	485
TnpB_11	TTGAT	g6	gttgattaacttgtac	486
TnpB_11	TTGAT	g7	aaattcaacatctttc	487
TnpB_11	TTGAT	g8	tggtctcactttccga	488

TnpB	TAM Sequence	gRNA ID	gRNA sequence	SEQ ID NO:
TnpB_11	TTGAT	g9	agctttgaaccggttt	489
TnpB_16	AGGAG	g11	ctgtaacactctaaac	490
TnpB_16	AGGAG	g12	acgcttatgtgtttgc	491
TnpB_16	AGGAG	g1	gaggagtatttggttt	492
TnpB_16	AGGAG	g2	gagtatttggttttta	493
TnpB_16	AGGAG	g4	gctagtggaagtagcc	494
TnpB_16	AGGAG	g6	acgcagcgaaggagga	495
TnpB_16	AGGAG	g7	gaggagtactgctggt	496
TnpB_16	AGGAG	g8	gagtactgctggtcct	497
TnpB_16	AGGAG	g9	tactgctggtcctttg	498
TnpB_19	GGGAG	g10	ttgatcgccggaagga	499
TnpB_19	GGGAG	g4	atagaggaactggtaa	500
TnpB_19	GGGAG	g5	aagaattgtctgttat	501
TnpB_19	GGGAG	g6	ctaaacctctatatcg	502
TnpB_19	GGGAG	g7	gaggaaggttttgagg	503
TnpB_19	GGGAG	g8	atagaggaactggaaa	504
TnpB_19	GGGAG	g9	tttatcaacatgactg	505
TnpB_1	ATTAC	g11	catccaagaatgccat	506
TnpB_1	ATTAC	g1	tcgctaggtaaaagat	507
TnpB_1	ATTAC	g2	acttatacactagatg	508
TnpB_1	ATTAC	g3	aactttcaaaggctta	509
TnpB_1	ATTAC	g4	ttattgttgtttgcat	510
TnpB_1	ATTAC	g5	ttttcgttactgatac	511
TnpB_1	ATTAC	g6	tgcttcaagtaattaa	512
TnpB_1	ATTAC	g7	ttgaagcagtaatatt	513
TnpB_1	ATTAC	g8	atcatactttgtttta	514
TnpB_1	ATTAC	g9	tattaaatgtcaaaat	515
TnpB_28	GGG	g1	aatgtttctgcggcga	516
TnpB_28	GGG	g2	tttttggaggcacttt	517
TnpB_28	GGG	g3	actggtatgagactgg	518
TnpB_28	GGG	g4	atcaatgatcggttgc	519
TnpB_28	GGG	g5	ctattttgcggaacaa	520
TnpB_28	GGG	g6	cctcaacataagcctg	521
TnpB_28	GGG	g7	agtacctgagcgcgtg	522
TnpB_28	GGG	g8	tttataaagtttagcg	523
TnpB_28	GGG	g9	acctagtgatcgaata	524
TnpB_29	GGG	g10	atctggtaaaaggagc	525
TnpB_29	GGG	g2	tttttggaggcacttt	526
TnpB_29	GGG	g3	actggtatgagactgg	527
TnpB_29	GGG	g4	atcaatgatcggttgc	528
TnpB_29	GGG	g5	ctattttgcggaacaa	529

TnpB	TAM Sequence	gRNA ID	gRNA sequence	SEQ ID NO:
TnpB_29	GGG	g6	cctcaacataagcctg	530
TnpB_29	GGG	g7	agtacctgagcgcgtg	531
TnpB_29	GGG	g8	tttataaagtttagcg	532
TnpB_30	AGGAG	g12	acgcttatgtgtttgc	533
TnpB_30	AGGAG	g13	cttcaggatatcgact	534
TnpB_30	AGGAG	g1	gaggagtatttggttt	535
TnpB_30	AGGAG	g2	gagtatttggttttta	536
TnpB_30	AGGAG	g3	tatttggtttttatat	537
TnpB_30	AGGAG	g4	gctagtggaagtagcc	538
TnpB_30	AGGAG	g6	acgcagcgaaggagga	539
TnpB_30	AGGAG	g7	gaggagtactgctggt	540
TnpB_30	AGGAG	g8	gagtactgctggtcct	541

Table 15: Editing Efficeincy of Selected TnpBs – Example 19

Nuclease	Target Site	Percent Indel Reads (%)
TnpB_11	g10	0
TnpB_11	g10	0
TnpB_11	g10	0
TnpB_11	g10	0
TnpB_11	g10	0
TnpB_11	g10	0
TnpB_11	g3	0
TnpB_11	g3	0
TnpB_11	g3	0
TnpB_11	g4	0
TnpB_11	g4	0
TnpB_11	g4	0
TnpB_11	g5	0
TnpB_11	g5	0
TnpB_11	g5	0
TnpB_11	g6	0
TnpB_11	g6	0
TnpB_11	g6	0
TnpB_11	g7	0
TnpB_11	g7	0
TnpB_11	g7	0
TnpB_11	g8	0.00103653
TnpB_11	g8	0

Nuclease	Target Site	Percent Indel Reads (%)
TnpB_11	g8	0
TnpB_11	g9	0.01832356
TnpB_11	g9	0
TnpB_11	g9	0.03262498
TnpB_16	g11	0.04138435
TnpB_16	g11	0
TnpB_16	g11	0.08382637
TnpB_16	g12	0
TnpB_16	g12	0
TnpB_16	g12	0
TnpB_16	g1	0.06505488
TnpB_16	g1	0.09178863
TnpB_16	g1	0.08619932
TnpB_16	g2	0
TnpB_16	g2	0
TnpB_16	g2	0.00464633
TnpB_16	g4	0
TnpB_16	g4	0.00964559
TnpB_16	g4	0.00650832
TnpB_16	g6	0
TnpB_16	g6	0
TnpB_16	g6	0
TnpB_16	g7	0.05295308
TnpB_16	g7	0.08824942
TnpB_16	g7	0
TnpB_16	g8	0
TnpB_16	g8	0
TnpB_16	g8	0
TnpB_16	g9	0.00735415
TnpB_16	g9	0
TnpB_16	g9	0
TnpB_19	g10	0
TnpB_19	g10	0
TnpB_19	g10	0
TnpB_19	g4	0
TnpB_19	g4	0
TnpB_19	g4	0
TnpB_19	g5	0
TnpB_19	g5	0
TnpB_19	g5	0
TnpB_19	g6	0

Nuclease	Target Site	Percent Indel Reads (%)
TnpB_19	g6	0
TnpB_19	g6	0.00594487
TnpB_19	g7	0.00508214
TnpB_19	g7	0
TnpB_19	g7	0
TnpB_19	g8	0
TnpB_19	g8	0
TnpB_19	g8	0
TnpB_19	g9	0.02133676
TnpB_19	g9	0.00868165
TnpB_19	g9	0
TnpB_1	g11	0
TnpB_1	g11	0
TnpB_1	g11	0
TnpB_1	g1	0
TnpB_1	g1	0
TnpB_1	g1	0
TnpB_1	g2	0.01143549
TnpB_1	g2	0
TnpB_1	g2	0
TnpB_1	g3	0
TnpB_1	g3	0
TnpB_1	g3	0
TnpB_1	g4	0
TnpB_1	g4	0
TnpB_1	g4	0
TnpB_1	g5	0
TnpB_1	g5	0
TnpB_1	g5	0.00858699
TnpB_1	g6	0
TnpB_1	g6	0
TnpB_1	g6	0
TnpB_1	g7	0
TnpB_1	g7	0
TnpB_1	g7	0
TnpB_1	g8	0.003873
TnpB_1	g8	0
TnpB_1	g8	0
TnpB_1	g9	0
TnpB_1	g9	0
TnpB_1	g9	0

Nuclease	Target Site	Percent Indel Reads (%)
TnpB_28	g1	0.00493657
TnpB_28	g1	0.00852208
TnpB_28	g1	0.03809841
TnpB_28	g2	0
TnpB_28	g2	0
TnpB_28	g2	0
TnpB_28	g3	0
TnpB_28	g3	0
TnpB_28	g3	0
TnpB_28	g4	0
TnpB_28	g4	0
TnpB_28	g4	0
TnpB_28	g5	0
TnpB_28	g5	0
TnpB_28	g5	0
TnpB_28	g6	0
TnpB_28	g6	0
TnpB_28	g6	0
TnpB_28	g7	0
TnpB_28	g7	0
TnpB_28	g7	0
TnpB_28	g8	0
TnpB_28	g8	0
TnpB_28	g8	0
TnpB_28	g9	0
TnpB_28	g9	0
TnpB_28	g9	0
TnpB_29	g10	0
TnpB_29	g10	0
TnpB_29	g10	0
TnpB_29	g2	0
TnpB_29	g2	0
TnpB_29	g2	0.02945918
TnpB_29	g3	0
TnpB_29	g3	0
TnpB_29	g3	0
TnpB_29	g4	0
TnpB_29	g4	0
TnpB_29	g4	0
TnpB_29	g5	0
TnpB_29	g5	0

Nuclease	Target Site	Percent Indel Reads (%)
TnpB_29	g5	0
TnpB_29	g6	0
TnpB_29	g6	0
TnpB_29	g6	0
TnpB_29	g7	0
TnpB_29	g7	0.00503534
TnpB_29	g7	0
TnpB_29	g8	0
TnpB_29	g8	0
TnpB_29	g8	0
TnpB_30	g12	0
TnpB_30	g12	0.01551192
TnpB_30	g12	0.01496752
TnpB_30	g13	0.14227364
TnpB_30	g13	0.00831163
TnpB_30	g13	0.12509186
TnpB_30	g1	1.36321414
TnpB_30	g1	0.98309346
TnpB_30	g1	1.08502968
TnpB_30	g2	1.43133107
TnpB_30	g2	0.84878823
TnpB_30	g2	1.32200479
TnpB_30	g3	1.4893998
TnpB_30	g3	1.00685743
TnpB_30	g3	0.5329295
TnpB_30	g4	0.19160955
TnpB_30	g4	0.08809576
TnpB_30	g4	0.18942693
TnpB_30	g6	0.02445936
TnpB_30	g6	0
TnpB_30	g6	0.06426399
TnpB_30	g7	0.59288801
TnpB_30	g7	0.27331858
TnpB_30	g7	0.24378092
TnpB_30	g8	0.01102657
TnpB_30	g8	0
TnpB_30	g8	0

Table 16: Various spacer sequences for selected TnpBs associated with Example 20

TnpB	target site	Spacer length (nucleotides)	Spacer sequence	gRNA SEQ ID NO:
ISDra2	g9	14	agctttgaaccggt	542
ISDra2	g9	15	agctttgaaccggtt	543
ISDra2	g9	16	agctttgaaccggttt	544
ISDra2	g9	17	agctttgaaccggtttc	545
ISDra2	g9	18	agctttgaaccggtttct	546
ISDra2	g9	19	agctttgaaccggtttctt	547
ISDra2	g9	20	agctttgaaccggtttcttc	121
ISYmu1	g2	14	aaggcaaattcgcc	548
ISYmu1	g2	15	aaggcaaattcgccg	549
ISYmu1	g2	16	aaggcaaattcgccgc	55
ISYmu1	g2	17	aaggcaaattcgccgca	550
ISYmu1	g2	18	aaggcaaattcgccgcag	551
ISYmu1	g2	19	aaggcaaattcgccgcaga	552
ISYmu1	g2	20	aaggcaaattcgccgcagaa	553
ISBag01	g1	14	gaattgatgaccat	554
ISBag01	g1	15	gaattgatgaccata	555
ISBag01	g1	16	gaattgatgaccatat	556
ISBag01	g1	17	gaattgatgaccatatg	557
ISBag01	g1	18	gaattgatgaccatatga	558
ISBag01	g1	19	gaattgatgaccatatgat	559
ISBag01	g1	20	gaattgatgaccatatgatt	16
TnpB_30	g2	13	gagtatttggttt	560
TnpB_30	g2	14	gagtatttggtttt	561
TnpB_30	g2	15	gagtatttggttttt	562
TnpB_30	g2	16	gagtatttggtttta	563
TnpB_30	g2	17	gagtatttggttttat	564
TnpB_30	g2	18	gagtatttggttttata	565
TnpB_30	g2	19	gagtatttggttttatat	566
TnpB_30	g2	20	gagtatttggttttatatt	567
TnpB_30	g2	21	gagtatttggttttatattt	568

Table 17: Sequences associated with Example 21

SEQ ID NO:	ISYmu1 split TnpB DNA sequence	ISYmu1 split wRNA length (nucleotides)	ISYmu1 split wRNA sequence	wRNA SEQ ID NO:
569	atgctgcagcataaagcctatgaataccgtatctatccaga taagaagcaggaaacactgattgccaagacaataggca gttcaaggtatgtatacaaccatttcctggaactttggaaca aggaatatgaggagaccggaaaaggtctgacttattacg catgctccaaacttctgacaaagcttaagagggacccgg aaacagtatggctttgtgaagtggataagttctcgctccag aattcgcttcgtaacctgtcggacgccttctcccgcttttca aaggacagaacgagcatccccagttcaagagcaagaag agcccgaggcaaagctacacgacacaatatacgaacaa caacatcgcagtttccggaaattgtctgaagctgcctaag ctgggccttgtcaagtttgcagacagtagggagatgaaa ggccgtatactgaatgccacagtacgaaggaaatcgagc gggaaattcttcgtatcgatcctatgcgaagaggagatct gtgaattgccaaagactgactcatctgtcggaattgacctc ggaatcattgactttgcggttatgtcagacggaagcaggc atgacaacaatcattttaccaggcaaatggaagaaaggct tagacgggagcagcgaaagcttgcaaggcgtgcacttg ctgcggagaaaaggggcatttccctttctgaagccagga actatcagaagcaaaggcggaaggtagcgagactttatg aaaaggttgcaaaccagcgaaaagaatatcttaacaaact cagtacagagatagtcaaaaaccacgatatcatctgtatc gaggaccttaacgttaagggcatgatgcgcaatcataaac tggcaaaaagcatctctgatgtatcatggacgagccttgta tcgaaactgcagtacaaggcttcctggtatggaaaagaa gtcatcaggataagcagatggtttccgtcaagtcagatatg ctcagagtgcggccacaaggacaggaagaagcctctcc atgtaagggagtggacctgtcctgtttgtcatgcccaccat	100	gtagcttggtaaacaagagaaacctctgccggcaa agaaataagccggtaagtatgctctgttcccaagaa tctcgtgactttagtcatgagagtttcaa	580

SEQ ID NO:	ISYmu1 split TnpB DNA sequence	ISYmu1 split wRNA length (nucleotides)	ISYmu1 split wRNA sequence	wRNA SEQ ID NO:
	gaccgtgacgtcaatgcagcaagaaatatactggctgag ggactcaggataagagctctgactccggggtcttag			

SEQ ID NO:	ISYmu1 split TnpB DNA sequence	ISYmu1 split wRNA length (nucleotides)	ISYmu1 split wRNA sequence	wRNA SEQ ID NO:
570	atgctgcagcataaagcctatgaataccgtatctatccaga taagaagcaggaaacactgattgccaagacaataggca gttcaaggtatgtatacaaccatttcctggaactttggaaca aggaatatgaggagaccggaaaaggtctgacttattacg catgctccaaacttctgacaaagcttaagagggacccgg aaacagtatggctttgtgaagtggataagttctcgctccag aattcgcttcgtaacctgtcggacgccttctcccgcttttca aaggacagaacgagcatccccagttcaagagcaagaag agcccgaggcaaagctacacgacacaatatacgaacaa caacatcgcagtttccggaaattgtctgaagctgcctaag ctgggccttgtcaagtttgcagacagtagggagatgaaa ggccgtatactgaatgccacagtacgaaggaaatcgagc gggaaattcttcgtatcgatcctatgcgaagaggagatct gtgaattgccaaagactgactcatctgtcggaattgacctc ggaatcattgactttgcggttatgtcagacggaagcaggc atgacaacaatcattttaccaggcaaatggaagaaaggct tagacgggagcagcgaaagcttgcaaggcgtgcacttg ctgcggagaaaaggggcatttccctttctgaagccagga actatcagaagcaaaggcggaaggtagcgagactttatg aaaaggttgcaaaccagcgaaaagaatatcttaacaaact cagtacagagatagtcaaaaaccacgatatcatctgtatc gaggaccttaacgttaagggcatgatgcgcaatcataaac tggcaaaaagcatctctgatgtatcatggacgagccttgta tcgaaactgcagtacaaggcttcctggtatggaaaagaa gtcatcaggataagcagatggtttccgtcaagtcagatatg ctcagagtgcggccacaaggacaggaagaagcctctcc atgtaagggagtggacctgtcctgtttgtcatgcccaccat	110	aattgcgggggtagcttggtaaacaagagaaacct ctgccggcaaagaaataagccggtaagtatgctct gttcccaagaatctcgtgactttagtcatgagagtttc aa	581

SEQ ID NO:	ISYmu1 split TnpB DNA sequence	ISYmu1 split wRNA length (nucleotides)	ISYmu1 split wRNA sequence	wRNA SEQ ID NO:
	gaccgtgacgtcaatgcagcaagaaatatactggctgag ggactcaggataagagctctgactccggggtcttag			

SEQ ID NO:	ISYmu1 split TnpB DNA sequence	ISYmu1 split wRNA length (nucleotides)	ISYmu1 split wRNA sequence	wRNA SEQ ID NO:
571	atgctgcagcataaagcctatgaataccgtatctatccaga taagaagcaggaaacactgattgccaagacaataggca gttcaaggtatgtatacaaccatttcctggaactttggaaca aggaatatgaggagaccggaaaaggtctgacttattacg catgctccaaacttctgacaaagcttaagagggacccgg aaacagtatggctttgtgaagtggataagttctcgctccag aattcgcttcgtaacctgtcggacgccttctcccgcttttca aaggacagaacgagcatccccagttcaagagcaagaag agcccgaggcaaagctacacgacacaatatacgaacaa caacatcgcagtttccggaaattgtctgaagctgcctaag ctgggccttgtcaagtttgcagacagtagggagatgaaa ggccgtatactgaatgccacagtacgaaggaaatcgagc gggaaattcttcgtatcgatcctatgcgaagaggagatct gtgaattgccaaagactgactcatctgtcggaattgacctc ggaatcattgactttgcggttatgtcagacggaagcaggc atgacaacaatcattttaccaggcaaatggaagaaaggct tagacgggagcagcgaaagcttgcaaggcgtgcacttg ctgcggagaaaaggggcatttccctttctgaagccagga actatcagaagcaaaggcggaaggtagcgagactttatg aaaaggttgcaaaccagcgaaaagaatatcttaacaaact cagtacagagatagtcaaaaaccacgatatcatctgtatc gaggaccttaacgttaagggcatgatgcgcaatcataaac tggcaaaaagcatctctgatgtatcatggacgagccttgta tcgaaactgcagtacaaggcttcctggtatggaaaagaa gtcatcaggataagcagatggtttccgtcaagtcagatatg ctcagagtgcggccacaaggacaggaagaagcctctcc atgtaagggagtggacctgtcctgtttgtcatgcccaccat	120	gaaccgcaggaattgcgggggtagcttggtaaac aagagaaacctctgccggcaaagaaataagccgg taagtatgctctgttcccaagaatctcgtgactttagt catgagagtttcaa	582

SEQ ID NO:	ISYmu1 split TnpB DNA sequence	ISYmu1 split wRNA length (nucleotides)	ISYmu1 split wRNA sequence	wRNA SEQ ID NO:
	gaccgtgacgtcaatgcagcaagaaatatactggctgag ggactcaggataagagctctgactccggggtcttag			

SEQ ID NO:	ISYmu1 split TnpB DNA sequence	ISYmu1 split wRNA length (nucleotides)	ISYmu1 split wRNA sequence	wRNA SEQ ID NO:
572	atgctgcagcataaagcctatgaataccgtatctatccaga taagaagcaggaaacactgattgccaagacaataggca gttcaaggtatgtatacaaccatttcctggaactttggaaca aggaatatgaggagaccggaaaaggtctgacttattacg catgctccaaacttctgacaaagcttaagagggacccgg aaacagtatggctttgtgaagtggataagttctcgctccag aattcgcttcgtaacctgtcggacgccttctcccgcttttca aaggacagaacgagcatccccagttcaagagcaagaag agcccgaggcaaagctacacgacacaatatacgaacaa caacatcgcagtttccggaaattgtctgaagctgcctaag ctgggccttgtcaagtttgcagacagtagggagatgaaa ggccgtatactgaatgccacagtacgaaggaaatcgagc gggaaattcttcgtatcgatcctatgcgaagaggagatct gtgaattgccaaagactgactcatctgtcggaattgacctc ggaatcattgactttgcggttatgtcagacggaagcaggc atgacaacaatcattttaccaggcaaatggaagaaaggct tagacgggagcagcgaaagcttgcaaggcgtgcacttg ctgcggagaaaaggggcatttccctttctgaagccagga actatcagaagcaaaggcggaaggtagcgagactttatg aaaaggttgcaaaccagcgaaaagaatatcttaacaaact cagtacagagatagtcaaaaaccacgatatcatctgtatc gaggaccttaacgttaagggcatgatgcgcaatcataaac tggcaaaaagcatctctgatgtatcatggacgagccttgta tcgaaactgcagtacaaggcttcctggtatggaaaagaa gtcatcaggataagcagatggtttccgtcaagtcagatatg ctcagagtgcggccacaaggacaggaagaagcctctcc atgtaagggagtggacctgtcctgtttgtcatgcccaccat	127	caaacaggaaccgcaggaattgcgggggtagctt ggtaaacaagagaaacctctgccggcaaagaaat aagccggtaagtatgctctgttcccaagaatctcgt gactttagtcatgagagtttcaa	583

SEQ ID NO:	ISYmu1 split TnpB DNA sequence	ISYmu1 split wRNA length (nucleotides)	ISYmu1 split wRNA sequence	wRNA SEQ ID NO:
	gaccgtgacgtcaatgcagcaagaaatatactggctgag ggactcaggataagagctctgactccggggtcttag			

SEQ ID NO:	ISYmu1 split TnpB DNA sequence	ISYmu1 split wRNA length (nucleotides)	ISYmu1 split wRNA sequence	wRNA SEQ ID NO:
573	atgctgcagcataaagcctatgaataccgtatctatccaga taagaagcaggaaacactgattgccaagacaataggca gttcaaggtatgtatacaaccatttcctggaactttggaaca aggaatatgaggagaccggaaaaggtctgacttattacg catgctccaaacttctgacaaagcttaagagggacccgg aaacagtatggctttgtgaagtggataagttctcgctccag aattcgcttcgtaacctgtcggacgccttctcccgcttttca aaggacagaacgagcatccccagttcaagagcaagaag agcccgaggcaaagctacacgacacaatatacgaacaa caacatcgcagtttccggaaattgtctgaagctgcctaag ctgggccttgtcaagtttgcagacagtagggagatgaaa ggccgtatactgaatgccacagtacgaaggaaatcgagc gggaaattcttcgtatcgatcctatgcgaagaggagatct gtgaattgccaaagactgactcatctgtcggaattgacctc ggaatcattgactttgcggttatgtcagacggaagcaggc atgacaacaatcattttaccaggcaaatggaagaaaggct tagacgggagcagcgaaagcttgcaaggcgtgcacttg ctgcggagaaaaggggcatttccctttctgaagccagga actatcagaagcaaaggcggaaggtagcgagactttatg aaaaggttgcaaaccagcgaaaagaatatcttaacaaact cagtacagagatagtcaaaaaccacgatatcatctgtatc gaggaccttaacgttaagggcatgatgcgcaatcataaac tggcaaaaagcatctctgatgtatcatggacgagccttgta tcgaaactgcagtacaaggcttcctggtatggaaaagaa gtcatcaggataagcagatggtttccgtcaagtcagatatg ctcagagtgcggccacaaggacaggaagaagcctctcc atgtaagggagtggacctgtcctgtttgtcatgcccaccat	130	aagcaaacaggaaccgcaggaattgcgggggta gcttggtaaacaagagaaacctctgccggcaaaga aataagccggtaagtatgctctgttcccaagaatctc gtgactttagtcatgagagtttcaa	584

SEQ ID NO:	ISYmu1 split TnpB DNA sequence	ISYmu1 split wRNA length (nucleotides)	ISYmu1 split wRNA sequence	wRNA SEQ ID NO:
	gaccgtgacgtcaatgcagcaagaaatatactggctgag ggactcaggataagagctctgactccggggtcttag			

SEQ ID NO:	ISYmu1 split TnpB DNA sequence	ISYmu1 split wRNA length (nucleotides)	ISYmu1 split wRNA sequence	wRNA SEQ ID NO:
574	atgctgcagcataaagcctatgaataccgtatctatccaga taagaagcaggaaacactgattgccaagacaataggca gttcaaggtatgtatacaaccatttcctggaactttggaaca aggaatatgaggagaccggaaaaggtctgacttattacg catgctccaaacttctgacaaagcttaagagggacccgg aaacagtatggctttgtgaagtggataagttctcgctccag aattcgcttcgtaacctgtcggacgccttctcccgcttttca aaggacagaacgagcatccccagttcaagagcaagaag agcccgaggcaaagctacacgacacaatatacgaacaa caacatcgcagtttccggaaattgtctgaagctgcctaag ctgggccttgtcaagtttgcagacagtagggagatgaaa ggccgtatactgaatgccacagtacgaaggaaatcgagc gggaaattcttcgtatcgatcctatgcgaagaggagatct gtgaattgccaaagactgactcatctgtcggaattgacctc ggaatcattgactttgcggttatgtcagacggaagcaggc atgacaacaatcattttaccaggcaaatggaagaaaggct tagacgggagcagcgaaagcttgcaaggcgtgcacttg ctgcggagaaaaggggcatttccctttctgaagccagga actatcagaagcaaaggcggaaggtagcgagactttatg aaaaggttgcaaaccagcgaaaagaatatcttaacaaact cagtacagagatagtcaaaaaccacgatatcatctgtatc gaggaccttaacgttaagggcatgatgcgcaatcataaac tggcaaaaagcatctctgatgtatcatggacgagccttgta tcgaaactgcagtacaaggcttcctggtatggaaaagaa gtcatcaggataagcagatggtttccgtcaagtcagatatg ctcagagtgcggccacaaggacaggaagaagcctctcc atgtaagggagtggacctgtcctgtttgtcatgcccaccat	150	ccggggtcttagcaatacagaagcaaacaggaac cgcaggaattgcgggggtagcttggtaaacaaga gaaacctctgccggcaaagaaataagccggtaagt atgctctgttcccaagaatctcgtgactttagtcatga gagtttcaa	585

SEQ ID NO:	ISYmu1 split TnpB DNA sequence	ISYmu1 split wRNA length (nucleotides)	ISYmu1 split wRNA sequence	wRNA SEQ ID NO:
	gaccgtgacgtcaatgcagcaagaaatatactggctgag ggactcaggataagagctctgactccggggtcttag			
575	atgctgcagcataaagcctatgaataccgtatctatccaga taagaagcaggaaacactgattgccaagacaataggca gttcaaggtatgtatacaaccatttcctggaactttggaaca aggaatatgaggagaccggaaaaggtctgacttattacg catgctccaaacttctgacaaagcttaagagggacccgg aaacagtatggctttgtgaagtggataagttctcgctccag aattcgcttcgtaacctgtcggacgccttctcccgcttttca aaggacagaacgagcatccccagttcaagagcaagaag agcccgaggcaaagctacacgacacaatatacgaacaa caacatcgcagtttccggaaattgtctgaagctgcctaag ctgggccttgtcaagtttgcagacagtagggagatgaaa ggccgtatactgaatgccacagtacgaaggaaatcgagc gggaaattcttcgtatcgatcctatgcgaagaggagatct	170	tcaggataagagctctgactccggggtcttagcaat acagaagcaaacaggaaccgcaggaattgcggg ggtagcttggtaaacaagagaaacctctgccggca aagaaataagccggtaagtatgctctgttcccaaga atctcgtgactttagtcatgagagtttcaa	586

SEQ ID NO:	ISYmu1 split TnpB DNA sequence	ISYmu1 split wRNA length (nucleotides)	ISYmu1 split wRNA sequence	wRNA SEQ ID NO:
	gtgaattgccaaagactgactcatctgtcggaattgacctc ggaatcattgactttgcggttatgtcagacggaagcaggc atgacaacaatcattttaccaggcaaatggaagaaaggct tagacgggagcagcgaaagcttgcaaggcgtgcacttg ctgcggagaaaaggggcatttccctttctgaagccagga actatcagaagcaaaggcggaaggtagcgagactttatg aaaaggttgcaaaccagcgaaaagaatatcttaacaaact cagtacagagatagtcaaaaaccacgatatcatctgtatc gaggaccttaacgttaagggcatgatgcgcaatcataaac tggcaaaaagcatctctgatgtatcatggacgagccttgta tcgaaactgcagtacaaggcttcctggtatggaaaagaa gtcatcaggataagcagatggtttccgtcaagtcagatatg ctcagagtgcggccacaaggacaggaagaagcctctcc atgtaagggagtggacctgtcctgtttgtcatgcccaccat gaccgtgacgtcaatgcagcaagaaatatactggctgag ggactcaggataagagctctgactccggggtcttag			

SEQ ID NO:	ISYmu1 split TnpB DNA sequence	ISYmu1 split wRNA length (nucleotides)	ISYmu1 split wRNA sequence	wRNA SEQ ID NO:
576	atgctgcagcataaagcctatgaataccgtatctatccaga taagaagcaggaaacactgattgccaagacaataggca gttcaaggtatgtatacaaccatttcctggaactttggaaca aggaatatgaggagaccggaaaaggtctgacttattacg catgctccaaacttctgacaaagcttaagagggacccgg aaacagtatggctttgtgaagtggataagttctcgctccag aattcgcttcgtaacctgtcggacgccttctcccgcttttca aaggacagaacgagcatccccagttcaagagcaagaag agcccgaggcaaagctacacgacacaatatacgaacaa caacatcgcagtttccggaaattgtctgaagctgcctaag ctgggccttgtcaagtttgcagacagtagggagatgaaa ggccgtatactgaatgccacagtacgaaggaaatcgagc gggaaattcttcgtatcgatcctatgcgaagaggagatct gtgaattgccaaagactgactcatctgtcggaattgacctc ggaatcattgactttgcggttatgtcagacggaagcaggc atgacaacaatcattttaccaggcaaatggaagaaaggct tagacgggagcagcgaaagcttgcaaggcgtgcacttg ctgcggagaaaaggggcatttccctttctgaagccagga actatcagaagcaaaggcggaaggtagcgagactttatg aaaaggttgcaaaccagcgaaaagaatatcttaacaaact cagtacagagatagtcaaaaaccacgatatcatctgtatc gaggaccttaacgttaagggcatgatgcgcaatcataaac tggcaaaaagcatctctgatgtatcatggacgagccttgta tcgaaactgcagtacaaggcttcctggtatggaaaagaa gtcatcaggataagcagatggtttccgtcaagtcagatatg ctcagagtgcggccacaaggacaggaagaagcctctcc atgtaagggagtggacctgtcctgtttgtcatgcccaccat	190	aaatatactggctgagggactcaggataagagctct gactccggggtcttagcaatacagaagcaaacag gaaccgcaggaattgcgggggtagcttggtaaac aagagaaacctctgccggcaaagaaataagccgg taagtatgctctgttcccaagaatctcgtgactttagt catgagagtttcaa	587

SEQ ID NO:	ISYmu1 split TnpB DNA sequence	ISYmu1 split wRNA length (nucleotides)	ISYmu1 split wRNA sequence	wRNA SEQ ID NO:
	gaccgtgacgtcaatgcagcaagaaatatactggctgag ggactcaggataagagctctgactccggggtcttag			
577	atgctgcagcataaagcctatgaataccgtatctatccaga taagaagcaggaaacactgattgccaagacaataggca gttcaaggtatgtatacaaccatttcctggaactttggaaca aggaatatgaggagaccggaaaaggtctgacttattacg catgctccaaacttctgacaaagcttaagagggacccgg aaacagtatggctttgtgaagtggataagttctcgctccag aattcgcttcgtaacctgtcggacgccttctcccgcttttca aaggacagaacgagcatccccagttcaagagcaagaag agcccgaggcaaagctacacgacacaatatacgaacaa caacatcgcagtttccggaaattgtctgaagctgcctaag ctgggccttgtcaagtttgcagacagtagggagatgaaa ggccgtatactgaatgccacagtacgaaggaaatcgagc gggaaattcttcgtatcgatcctatgcgaagaggagatct	210	cgtgacgtcaatgcagcaagaaatatactggctga gggactcaggataagagctctgactccggggtctt agcaatacagaagcaaacaggaaccgcaggaatt gcgggggtagcttggtaaacaagagaaacctctgc cggcaaagaaataagccggtaagtatgctctgttcc caagaatctcgtgactttagtcatgagagtttcaa	588

SEQ ID NO:	ISYmu1 split TnpB DNA sequence	ISYmu1 split wRNA length (nucleotides)	ISYmu1 split wRNA sequence	wRNA SEQ ID NO:
	gtgaattgccaaagactgactcatctgtcggaattgacctc ggaatcattgactttgcggttatgtcagacggaagcaggc atgacaacaatcattttaccaggcaaatggaagaaaggct tagacgggagcagcgaaagcttgcaaggcgtgcacttg ctgcggagaaaaggggcatttccctttctgaagccagga actatcagaagcaaaggcggaaggtagcgagactttatg aaaaggttgcaaaccagcgaaaagaatatcttaacaaact cagtacagagatagtcaaaaaccacgatatcatctgtatc gaggaccttaacgttaagggcatgatgcgcaatcataaac tggcaaaaagcatctctgatgtatcatggacgagccttgta tcgaaactgcagtacaaggcttcctggtatggaaaagaa gtcatcaggataagcagatggtttccgtcaagtcagatatg ctcagagtgcggccacaaggacaggaagaagcctctcc atgtaagggagtggacctgtcctgtttgtcatgcccaccat gaccgtgacgtcaatgcagcaagaaatatactggctgag ggactcaggataagagctctgactccggggtcttag			
578	atgctgcagcataaagcctatgaataccgtatctatccaga taagaagcaggaaacactgattgccaagacaataggca gttcaaggtatgtatacaaccatttcctggaactttggaaca aggaatatgaggagaccggaaaaggtctgacttattacg catgctccaaacttctgacaaagcttaagagggacccgg aaacagtatggctttgtgaagtggataagttctcgctccag aattcgcttcgtaacctgtcggacgccttctcccgcttttca aaggacagaacgagcatccccagttcaagagcaagaag agcccgaggcaaagctacacgacacaatatacgaacaa caacatcgcagtttccggaaattgtctgaagctgcctaag ctgggccttgtcaagtttgcagacagtagggagatgaaa ggccgtatactgaatgccacagtacgaaggaaatcgagc	230	tttgtcatgcccaccatgaccgtgacgtcaatgcag caagaaatatactggctgagggactcaggataaga gctctgactccggggtcttagcaatacagaagcaa acaggaaccgcaggaattgcgggggtagcttggt aaacaagagaaacctctgccggcaaagaaataag ccggtaagtatgctctgttcccaagaatctcgtgact ttagtcatgagagtttcaa	589

SEQ ID NO:	ISYmu1 split TnpB DNA sequence	ISYmu1 split wRNA length (nucleotides)	ISYmu1 split wRNA sequence	wRNA SEQ ID NO:
	gggaaattcttcgtatcgatcctatgcgaagaggagatct gtgaattgccaaagactgactcatctgtcggaattgacctc ggaatcattgactttgcggttatgtcagacggaagcaggc atgacaacaatcatttttaccaggcaaatggaagaaaggct tagacgggagcagcgaaagcttgcaaggcgtgcacttg ctgcggagaaaaggggcatttccctttctgaagccagga actatcagaagcaaaggcggaaggtagcgagactttatg aaaaggttgcaaaccagcgaaaagaatatcttaacaaact cagtacagagatagtcaaaaaccacgatatcatctgtatc gaggaccttaacgttaagggcatgatgcgcaatcataaac tggcaaaaagcatctctgatgtatcatggacgagccttgta tcgaaactgcagtacaaggcttcctggtatggaaaagaa gtcatcaggataagcagatggtttccgtcaagtcagatatg ctcagagtgcggccacaaggacaggaagaagcctctcc atgtaagggagtggacctgtcctgtttgtcatgcccaccat gaccgtgacgtcaatgcagcaagaaatatactggctgag ggactcaggataagagctctgactccggggtcttag				

SEQ ID NO:	ISYmu1 split TnpB DNA sequence	ISYmu1 split wRNA length (nucleotides)	ISYmu1 split wRNA sequence	wRNA SEQ ID NO:
579	atgctgcagcataaagcctatgaataccgtatctatccaga taagaagcaggaaacactgattgccaagacaataggca gttcaaggtatgtatacaaccatttcctggaactttggaaca aggaatatgaggagaccggaaaaggtctgacttattacg catgctccaaacttctgacaaagcttaagagggacccgg aaacagtatggctttgtgaagtggataagttctcgctccag aattcgcttcgtaacctgtcggacgccttctcccgcttttca aaggacagaacgagcatccccagttcaagagcaagaag agcccgaggcaaagctacacgacacaatatacgaacaa caacatcgcagtttccggaaattgtctgaagctgcctaag ctgggccttgtcaagtttgcagacagtagggagatgaaa ggccgtatactgaatgccacagtacgaaggaaatcgagc gggaaattcttcgtatcgatcctatgcgaagaggagatct gtgaattgccaaagactgactcatctgtcggaattgacctc ggaatcattgactttgcggttatgtcagacggaagcaggc atgacaacaatcattttaccaggcaaatggaagaaaggct tagacgggagcagcgaaagcttgcaaggcgtgcacttg ctgcggagaaaaggggcatttccctttctgaagccagga actatcagaagcaaaggcggaaggtagcgagactttatg aaaaggttgcaaaccagcgaaaagaatatcttaacaaact cagtacagagatagtcaaaaaccacgatatcatctgtatc gaggaccttaacgttaagggcatgatgcgcaatcataaac tggcaaaaagcatctctgatgtatcatggacgagccttgta tcgaaactgcagtacaaggcttcctggtatggaaaagaa gtcatcaggataagcagatggtttccgtcaagtcagatatg ctcagagtgcggccacaaggacaggaagaagcctctcc atgtaagggagtggacctgtcctgtttgtcatgcccaccat	250	aagggagtggacctgtcctgtttgtcatgcccacca tgaccgtgacgtcaatgcagcaagaaatatactgg ctgagggactcaggataagagctctgactccggg gtcttagcaatacagaagcaaacaggaaccgcag gaattgcgggggtagcttggtaaacaagagaaacc tctgccggcaaagaaataagccggtaagtatgctct gttcccaagaatctcgtgactttagtcatgagagtttc aa	590

SEQ ID NO:	ISYmu1 split TnpB DNA sequence	ISYmu1 split wRNA length (nucleotides)	ISYmu1 split wRNA sequence	wRNA SEQ ID NO:
	gaccgtgacgtcaatgcagcaagaaatatactggctgag ggactcaggataagagctctgactccggggtcttag			

Table 18: Selected ωRNA sequences associated with Example 22 (In Table 18, the ωRNA sequences are listed as DNA sequences that are the equivalent RNA sequences (except the RNA will have the T replaced with a U))

TnpB	**Description**	**Version**	**Size**	**SEQ ID NO:**	**wRNA Sequence**
ISYmu1	stem-loop 2 internal-loop indel	v3.17	126	591	CAAACAGGAACCGCAGGAATTGCGGGGGTAGCTTGGTA ACAAGAGAAACCTCTGCCGGCAAAGAAATAAGCCGGTA AGTATGCTCTGTTaCCAAGAATCTCGTGACTTTAGTCATG AGAGTTTCAA
ISYmu1	stem-loop 2 internal-loop indel	v3.18	126	592	CAAACAGGAACCGCAGGAATTGCGGGGGTAGCTTGGTA ACAAGAGAAACCTCTGCCGGCAAAGAAATAAGCCGGTA AGTATGCTCTGTTgCCAAGAATCTCGTGACTTTAGTCATG AGAGTTTCAA

TnpB	Description	Version	Size	SEQ ID NO:	wRNA Sequence
ISYmu1	stem-loop 2 internal-loop indel	v3.19	126	593	CAAACAGGAACCGCAGGAATTGCGGGGGTAGCTTGGTA ACAAGAGAAACCTCTGCCGGCAAAGAAATAAGCCGGTA AGTATGCTCTGTTtCCAAGAATCTCGTGACTTTAGTCATG AGAGTTTCAA
ISYmu1	stem-loop 2 internal-loop indel	v3.20	126	594	CAAACAGGAACCGCAGGAATTGCGGGGGTAGCTTGGgA ACAAGAGAAACCTCTGCCGGCAAAGAAATAAGCCGGTA AGTATGCTCTGTTCCCAAGAATCTCGTGACTTTAGTCATG AGAGTTTCAA
ISYmu1	stem-loop 2 internal-loop indel	v3.21	126	595	CAAACAGGAACCGCAGGAATTGCGGGGGTAGCTTGGcA ACAAGAGAAACCTCTGCCGGCAAAGAAATAAGCCGGTA AGTATGCTCTGTTCCCAAGAATCTCGTGACTTTAGTCATG AGAGTTTCAA
ISYmu1	stem-loop 2 internal-loop indel	v3.22	127	596	CAAACAGGAACCGCAGGAATTGCGGGGGTAGCTTGGTA ACAAGAGAAACCTCTGCCGGCAAAGAAATAAGCCGGTA AGTATGCTCTGTTCcCCAAGAATCTCGTGACTTTAGTCAT GAGAGTTTCAA
ISYmu1	stem-loop 2 internal-loop indel	v3.23	128	597	CAAACAGGAACCGCAGGAATTGCGGGGGTAGCTTGGTA ACAAGAGAAACCTCTGCCGGCAAAGAAATAAGCCGGTA AGTATGCTCTGTTCccCCAAGAATCTCGTGACTTTAGTCA TGAGAGTTTCAA
ISYmu1	stem-loop 2 internal-loop indel	v3.25	125	598	CAAACAGGAACCGCAGGAATTGCGGGGGTAGCTTGGTA ACAGAGAAACCTCTGCCGGCAAAGAAATAAGCCGGTAA GTATGCTCTGTTCCCAAGAATCTCGTGACTTTAGTCATGA GAGTTTCAA
ISYmu1	stem-loop 2 internal-loop indel	v3.26	127	599	CAAACAGGAACCGCAGGAATTGCGGGGGTAGCTTGGTA ACAAaGAGAAACCTCTGCCGGCAAAGAAATAAGCCGGT AAGTATGCTCTGTTCCCAAGAATCTCGTGACTTTAGTCAT GAGAGTTTCAA
ISYmu1	stem-loop 2 internal-loop indel	v3.27	125	600	CAAACAGGAACCGCAGGAATTGCGGGGGTAGCTTGGTA ACAGAGAAACCTCTGCCGGCAAAGAAATAAGCCGGTAA GTATGCTCTGTTaCCAAGAATCTCGTGACTTTAGTCATGA GAGTTTCAA
ISYmu1	stem-loop 2 truncation +	v6.1	93	601	CAAACAGGAACCGCAGGAATTGCGGGGGTAGCTTGGTA ACAAGAGgaaaCTCTGTTCCCAAGAATCTCGTGACTTTAGT CATGAGAGTTTCAA

TnpB	Description	Version	Size	SEQ ID NO:	wRNA Sequence
	internal-loop indel				
ISYmu1	stem-loop 2 truncation + internal-loop indel	v6.2	93	602	CAAACAGGAACCGCAGGAATTGCGGGGGTAGCTTGGTAAACAAGAGgaaaCTTGTTCCCAAGAATCTCGTGACTTTAGTCATGAGAGTTTCAA
ISYmu1	stem-loop 2 internal-loop indel + PK base pair substitution	v7.1	126	603	CAAACAGGAACCGCAGGAAaTGCGGGGGTAGCTTGGTAACAAGAGAAACCTCTGCCGGCAAAGAAATAAGCCGGTAAGTATGCTCTGTTCCCAAGAATCTCGTGACTTTAGTCATGAGAGTTTCAA
ISYmu1	stem-loop 2 truncation + internal-loop indel + PK base pair substitution	v8.1	93	604	CAAACAGGAACCGCAGGAAaTGCGGGGGTAGCTTGGTAACAAGAGgaaaCTCTGTTCCCAAGAATCTCGTGACTTTAGTCATGAGAGTTTCAA
ISYmu1	stem-loop 2 truncation + internal-loop indel + PK base pair substitution	v8.2	93	605	CAAACAGGAACCGCAGGAAaTGCGGGGGTAGCTTGGTAAACAAGAGgaaaCTTGTTCCCAAGAATCTCGTGACTTTAGTCATGAGAGTTTCAA

TnpB	Description	Vers-ion	Size	SEQ ID NO:	wRNA Sequence
ISYmu1	stem-loop 2 truncation + internal-loop indel + PK base pair substitution	v8.3	92	606	CAAACAGGAACCGCAGGAAaTGCGGGGGTAGCTTGGTA ACAAGAGgaaaCTTGTTCCCAAGAATCTCGTGACTTTAGTC ATGAGAGTTTCAA

Table 19: Vector and gRNA sequence associated with Example 25

Description	**TnpB Name**	**DNA sequence cloned into TRV cargo site (SEQ ID NO: 607)**
ISYmu1-g12-HDV–tRNA–Isoleucine	ISYmu1	atggattacaaggatgatgatgataaggattacaaggatgatgatgataagatggctccaaagaagaagagaaaggttgg aatccacggagttccagctgctATGCTGCAGCATAAAGCCTATGAATACCGTATCTAT CCAGATAAGAAGCAGGAAACACTGATTGCCAAGACAATAGGCAGTTCAA GGTATGTATACAACCATTTCCTGGAACTTTGGAACAAGGAATATGAGGA GACCGGAAAAGGTCTGACTTATTACGCATGCTCCAAACTTCTGACAAAGC TTAAGAGGGACCCGGAAACAGTATGGCTTTGTGAAGTGGATAAGTTCTC GCTCCAGAATTCGCTTCGTAACCTGTCGGACGCCTTCTCCCGCTTTTTCAA AGGACAGAACGAGCATCCCCAGTTCAAGAGCAAGAAGAGCCCGAGGCA AAGCTACACGACACAATATACGAACAACAACATCGCAGTTTCCGGAAAT TGTCTGAAGCTGCCTAAGCTGGGCCTTGTCAAGTTTGCAGACAGTAGGGA GATGAAAGGCCGTATACTGAATGCCACAGTACGAAGGAAATCGAGCGGG AAATTCTTCGTATCGATCCTATGCGAAGAGGAGATCTGTGAATTGCCAAA GACTGACTCATCTGTCGGAATTGACCTCGGAATCATTGACTTTGCGGTTA TGTCAGACGGAAGCAGGCATGACAACAATCATTTTACCAGGCAAATGGA AGAAAGGCTTAGACGGGAGCAGCGAAAGCTTGCAAGGCGTGCACTTGCT GCGGAGAAAAGGGGCATTTCCCTTTCTGAAGCCAGGAACTATCAGAAGC AAAGGCGGAAGGTAGCGAGACTTTATGAAAAGGTTGCAAACCAGCGAA AAGAATATCTTAACAAACTCAGTACAGAGATAGTCAAAAACCACGATAT CATCTGTATCGAGGACCTTAACGTTAAGGGCATGATGCGCAATCATAAAC TGGCAAAAAGCATCTCTGATGTATCATGGACGAGCCTTGTATCGAAACTG CAGTACAAGGCTTCCTGGTATGGAAAAGAAGTCATCAGGATAAGCAGAT GGTTTCCGTCAAGTCAGATATGCTCAGAGTGCGGCCACAAGGACAGGAA GAAGCCTCTCCATGTAAGGGAGTGGACCTGTCCTGTTTGTCATGCCCACC ATGACCGTGACGTCAATGCAGCAAGAAATATACTGGCTGAGGGACTCAG GATAAGAGCTCTGACTCCGGGGTCTTAGCAATACAGAAGCAAACAGGAA CCGCAGGAATTGCGGGGGTAGCTTGGTAAACAAGAGAAACCTCTGCCGG CAAAGAAATAAGCCGGTAAGTATGCTCTGTTCCCAAGAATCTCGTGACTT TAGTCATGAGAGTTTCAAgcgttggagcatataaggccggcatggtcccagcctcctcgctggcgccg gctgggcaacatgcttcggcatggcgaatgggacCCGTAGCTCAGTTGGTTAGAGCGTTGGT CTTATGAGCCGAAGGTCGCGGGTTCGAGCCCCGCCGGAAGCA
gRNA sequence	SEQ ID NO: 65	gcgttggagcatataa

Table 20: Selected sequences associated with Example 8

Name	SEQ ID NO:	DNA sequence
AtUBQ10 pol II promoter	132	agtctagctcaacagagcttttaacccaaattggtacaatagaatacaactttagatcataattctcaaaagaaagagattcctta gctattctatctgccactccatttccttctcggcttgtatgcacaagcataaaatcctcaaacttgctaagtagatactttatgtcttg gataattggattgagacttgacaagcataacttttcatgtaaccaaagacacaagttgctgagaatccacctcaaaaatgatcttc ctataattgaatcgggataatgacagcacagcccatctaagagcctccacttctacttccagcacgcttcttacttttaccacag ctcttgcacctaaccataacaccttccctgtatgatcgcgaagcacccaccctaagccacattttaatccttctgttggccatgcc ccatcaaagttgcacttaacccaagattgtggtggagcttcccatgtttctcgtctgtcccgacggtgttgtggttggtgctttcct tacattctgagcctctttccttctaatccactcatctgcatcttcttgtgtccttactaatacctcattggttccaaattcccctccttta agcaccagctcgtttctgttcttccacagcctcccaagtatccaagggactaaagcctccacattcttcagatcaggatattcttg tttaagatgttgaactctatggaggtttgtatgaactgatgatctaggaccggataagttcccttcttcatagcgaacttattcaaa gaatgttttgtgtatcattcttgttacattgttattaatgaaaaaatattattggtcattggactgaacacgagtgttaaatatggacc aggccccaaataagatccattgatatatgaattaaataacaagaataaatcgagtcaccaaaccacttgccttttttaacgagac ttgttcaccaacttgatacaaaagtcattatcctatgcaaatcaataatcatacaaaaatatccaataacactaaaaaattaaaag aaatggataatttcacaatatgttatacgataaagaagttacttttccaagaaattcactgattttataagcccacttgcattagata aatggcaaaaaaaaacaaaaaggaaaagaaataaagcacgaagaattctagaaaatacgaaatacgcttcaatgcagtgg gacccacggttcaattattgccaattttcagctccaccgtatatttaaaaaataaaacgataatgctaaaaaaatataaatcgtaa cgatcgttaaatctcaacggctggatcttatgacgaccgttagaaattgtggttgtcgacgagtcagtaataaacggcgtcaaa gtggttgcagccggcacacacgagtcgtgtttatcaactcaaagcacaaatacttttcctcaacctaaaaataaggcaattagc caaaaacaacttttgcgtgtaaacaacgctcaatacacgtgtcattttattattagctattgcttcaccgccttagctttctcgtgacc tagtcgtcctcgtctttttcttcttcttctataaaacaatacccaaagagctcttcttcttcacaattcagatttcaatttctcaaaat cttaaaaactttctctcaattctctctaccgtgatcaaggtaaatttctgtgttccttattctctcaaaatcttcgattttgttttcgttcg atcccaatttcgtatatgttctttggtttagattctgttaatcttagatcgaagacgattttctgggtttgatcgttagatatcatcttaat tctcgattagggtttcatagatatcatccgatttgttcaaataatttgagttttgtcgaataattactcttcgatttgtgatttctatctag atctggtgttagtttctagtttgtgcgatcgaatttgtcgattaatctgagtttttctgattaacag

Name	SEQ ID NO:	DNA sequence
Maize UBQ1 pol II promoter	133	GTCGTGCCCCTCTCTAGAGATAATGAGCATTGCATGTCTAAGTTATAAAAA ATTACCACATATTTTTTTTGTCACACTTGTTTGAAGTGCAGTTTATCTATCTT TATACATATATTTAAACTTTACTCTACGAATAATATAATCTATAGTACTACA ATAATATCAGTGTTTTAGAGAATCATATAAATGAACAGTTAGACATGGTCT AAAGGACAATTGAGTATTTTGACAACAGGACTCTACAGTTTTATCTTTTTA GTGTGCATGTGTTCTCCTTTTTTTTTTGCAAATAGCTTCACCTATATAATACTT CATCCATTTTATTAGTACATCCATTTAGGGTTTAGGGTTAATGGTTTTTATA GACTAATTTTTTTAGTACATCTATTTTATTCTATTTTAGCCTCTAAATTAAGA AAACTAAAACTCTATTTTAGTTTTTTTATTTAATAATTTAGATATAAAATAG AATAAAATAAAGTGACTAAAAATTAAACAAATACCCTTTAAGAAATTAAA AAAACTAAGGAAACATTTTTCTTGTTTCGAGTAGATAATGCCAGCCTGTTA AACGCCGTCGACGAGTCTAACGGACACCAACCAGCGAACCAGCAGCGTCG CGTCGGGCCAAGCGAAGCAGACGGCACGGCATCTCTGTCGCTGCCTCTGG ACCCCTCTCGAGAGTTCCGCTCCACCGTTGGACTTGCTCCGCTGTCGGCATC CAGAAATTGCGTGGCGGAGCGGCAGACGTGAGCCGGCACGGCAGGCGGCC TCCTCCTCCTCTCACGGCACCGGCAGCTACGGGGGATTCCTTTCCCACCGCT CCTTCGCTTTCCCTTCCTCGCCCGCCGTAATAAATAGACACCCCCTCCACAC CCTCTTTCCCCAACCTCGTGTTGTTCGGAGCGCACACACACACAACCAGAT CTCCCCCAAATCCACCCGTCGGCACCTCCGCTTCAAGGTACGCCGCTCGTC CTCCCCCCCCCCCCCCCTCTCTACCTTCTCTAGATCGGCGTTCCGGTCCATGG TTAGGGCCCGGTAGTTCTACTTCTGTTCATGTTTGTGTTAGATCCGTGTTTG TGTTAGATCCGTGCTGCTAGCGTTCGTACACGGATGCGACCTGTACGTCAG ACACGTTCTGATTGCTAACTTGCCAGTGTTTCTCTTTGGGGAATCCTGGGAT GGCTCTAGCCGTTCCGCAGACGGGATCGATTTCATGATTTTTTTTGTTTCGT TGCATAGGGTTTGGTTTGCCCTTTTCCTTTATTTCAATATATGCCGTGCACT TGTTTGTCGGGTCATCTTTTCATGCTTTTTTTTGTCTTGGTTGTGATGATGTG GTCTGGTTGGGCGGTCGTTCTAGATCGGAGTAGAATTAATTCTGTTTCAAA CTACCTGGTGGATTTATTAATTTTGGATCTGTATGTGTGTGCCATACATATT CATAGTTACGAATTGAAGATGATGGATGGAAATATCGATCTAGGATAGGT ATACATGTTGATGCGGGTTTTACTGATGCATATACAGAGATGCTTTTTGTTC GCTTGGTTGTGATGATGTGGTGTGGTTGGGCGGTCGTTCATTCGTTCTAGAT CGGAGTAGAATACTGTTTCAAACTACCTGGTGTATTTATTAATTTTGGAACT GTATGTGTGTGTCATACATCTTCATAGTTACGAGTTTAAGATGGATGGAAA TATCGATCTAGGATAGGTATACATGTTGATGTGGGTTTTACTGATGCATAT ACATGATGGCATATGCAGCATCTATTCATATGCTCTAACCTTGAGTACCTA TCTATTATAATAAACAAGTATGTTTTATAATTATTTTGATCTTGATATACTT GGATGATGGCATATGCAGCAGCTATATGTGGATTTTTTTAGCCCTGCCTTC ATACGCTATTTATTTGCTTGGTACTGTTTCTTTTGTCGATGCTCACCCTGTTG TTTGGTGTTACTTCTGCAG
AtU6 pol III promoter	134	cttcgttgaacaacggaaactcgacttgccttccgcacaatacatcatttcttcttagcttttttttcttcttcttcgttcatacagttttttttgtttatcagcttacatttcttgaaccgtagctttcgttttcttcttttttaactttccattcggagttttttgtatcttgtttcatagtttgtccccaggattagaatgattaggcatcgaaccttcaagaatttgattgaataaaacatcttcattcttaagatatgaagataatcttcaaaaggcccctgggaatctgaaagaagagaagcaggcccatttatatgggaaagaacaatagtatttcttatataggcccatttaagttgaaaacaatcttcaaaagtcccacatcgcttagataagaaaacgaagctgagtttatatacagctagagtcgaagtagtgatt

Name	SEQ ID NO:	DNA sequence
rbcS E9 terminator	135	agagctttcgttcgtatcatcggtttcgacaacgttcgtcaagttcaatgcatcagtttcattgcgcacacaccagaatcctactg agtttgagtattatggcattgggaaaactgtttttcttgtaccatttgttgtgcttgtaatttactgtgtttttattcggttttcgctatcg aactgtgaaatggaaatggatggagaagagttaatgaatgatatggtccttttgttcattctcaaattaatattatttgtttttctctt atttgttgtgtgttgaatttgaaattataagagatatgcaaacattttgttttgagtaaaaatgtgtcaaatcgtggcctctaatgacc gaagttaatatgaggagtaaaacacttgtagttgtaccattatgcttattcactaggcaacaaatatattttcagacctagaaaag ctgcaaatgttactgaatacaagtatgtcctcttgtgttttagacatttatgaactttccttttatgtaattttccagaatccttgtcagat tctaatcattgctttataattatagttatactcatggatttgtagttgagtatgaaaatatttttttaatgcattttatgacttgccaattgat tgacaac
tRNA-Gly	136	aacaaagcaccagtggtctagtggtagaatagtaccctgccacggtacagacccgggttcgattcccggctggtgca
HDV ribozyme	137	ggccggcatggtcccagcctcctcgctggcgccggctgggcaacatgcttcggcatggcgaatgggac
Hammerhead ribozyme	138	ctgatgagtccgtgaggacgaaacgagtaagctcgtc
IV2 intron	139	gtaagtttctgcttctacctttgatatatatataataattatcattaattagtagtaatataatatttcaaatatttttttcaaaataaaaga atgtagtatatagcaattgcttttctgtagtttataagtgtgtatattttaatttataacttttctaatatatgaccaaaatttgttgatgtg cag

[00297] Informal Sequence Listing:

[00298] ISBag01 – TnpB DNA sequence without codon optimization (SEQ ID NO: 1):

[00299] atgtcgcagaccatcacagtcaaagtgaaattgcttccaacaaaagaacaggcttctattttgcgtgagatgagtcaaa cgtacctctccactatcaatactctcgtttccgaaatggttgctgaaaagaaaagcacaaagaagtcgagtaaggatatttctgcttctttg ccaagtgccgttaaaaatcaagctatcaaggacgcgaaaagtgtgtttaagaaagcgaagaaaagcaaattcactactgttcctgttttg aagaaacctgtctgcatttggaacaatcaaaactattcgtttgatttcacgcacatttccatgccgattatgattgacggcaaggtaactaa aacacctatccgtgctttgttagttgataaagacaaccgtaactttgctttgctaaaacacaaattaggtacgcttcgaatcacacagaaag

caaataaatggattgcccaaatttctgtcaccatacctactatggaaggaacaggagtaaaagtcatgggggttgatttgggtttaaaagt
tcctgccgttgctgtaacagatgatgaaaaggtacgtttctttgggaatggcagacaaaataagtacatgaagcgtaagttccgttctgaa
cgcaaagcattaggcaaaaagaaaaaggtaaatgcgattcgtagttcaaaagataaagaacaacgttggatgaaagaccaagaccat
aaggttagtcgtgctatcgtaaattttgcaaaagacaataaaatttctgtcattcgcttggaacaactggcgaatattagacagacggcaa
gaacaagccgtaaaaacgaaaagaatctgcatacttggtcattttatcgcctgtctcagttcattgaatataaagcgaatttagttggtatta
gagttgagcatgtgaatcctgcatacacaagtcagacatgccctaaatgctctgagaagaataaggcacaagaccgtaagtataaatg
caaatgtggattcgaaaaacatcgtgatttagtcggtgctatgaacattcgatatgcacctgtgattgatggtaacagtcaatcagcc

[00300] ISBag01 – TnpB DNA sequence with *Arabidopsis thaliana* codon optimization (SEQ ID NO: 2):

[00301] atgtcccaaactattaccgttaaggtgaaacttctacctacgaaggagcaagcaagtattctacgtgagatgtctcagac
atatttatctactattaacaccctagtatctgagatggtagctgagaagaagtccacaaagaagtcttcaaaagacatctctgcttccctgc
cctctgctgttaagaaccaagcgattaaggacgcgaagtcagttttcaagaaggctaagaagtccaagttcacaacagtgccggttttg
aagaagcctgtatgtatatggaacaatcagaactattccttcgacttcacccatatctccatgcctattatgattgatggtaaggtaacaaa
gacgcccatccgagcactattagttgataaagataacagaaacttcgcgttgctgaagcacaagcttgggactctaagaattacccaaa
aggctaacaagtggattgcacaaatctcagtgactatccctactatggagggaacaggcgttaaggtcatgggggttgacttgggggttg
aaggtccctgctgtagcagtgacggacgacgagaaggtccgtttcttcggtaacggtagacagaataagtacatgaagaggaagttca
ggtcagaaaggaaggctttaggcaagaagaagaaggttaatgcgataaggtcctctaaggataaggagcagcgttggatgaaggat
caagaccacaaggtatctagggccatcgtgaacttgccaaggataacaagatctccgtaataaggctcgaacagcttgccaatatccg
acagacagcacgtacctcccgaaagaatgaaaagaatctgcatacctggtcattctacagactttcacaatttatagagtataaggcaaa
cttagttggtatccgtgttgagcatgtaaaccccgcgtatacatcacaaacctgcccgaagtgctcagagaagaacaaggcgcaagac
cgaaagtataagtgcaagtgtggctttgagaagcatagagatctagttggagctatgaatattaggtatgctccggtaattgatggaaact
ctcaaagcgca

[00302] ISBag01 – TnpB DNA sequence with *Zea mays* codon optimization (SEQ ID NO: 3):

[00303] atgtctcagactatcacagttaaggtgaagctgttgccgacgaaggagcaggcatcaatcctgcgcgagatgtcgca
gacatatttgagcacaatcaacactctggtttctgaaatggttgcagaaaagaagagcactaagaagagctcgaaggatatctctgcttc
cctgccctcagccgttaagaaccaagcgatcaaggatgccaagtcagttttcaagaaggcgaagaagtcaaagttcacgacggttcct
gttctcaagaagcctgtttgcatctggaataatcaaaactatagcttcgatttcacacacatctccatgcctatcatgatcgatgggaaggt
cactaagaccccaatccgcgctctccttgttgataaggataatcgcaatttcgcgttgctcaagcataagctcggaactcttcgcatcacc
cagaaggccaataagtggatcgcccaaatctcggtgaccatccccacgatggagggtacaggtgtcaaggtcatgggagtggatctt
ggattgaaggtgcccgctgtcgctgtcactgacgatgaaaaggtccgcttcttcgggaatgggcgccagaataagtacatgaagcgc
aagttccgctctgaacgcaaggcattggggaagaagaagaaggtcaacgcgatccgctcgtccaaggacaaggagcagcgctgga

tgaaggaccaagaccacaaggtgtcaagggccatcgtgaatttcgccaaggacaacaagatctctgttatcaggctggagcagcttgc
gaacatccgccagactgcccgcacctcgcgcaagaacgaaaagaatttgcatacgtggagcttctaccgcctctcacaattcatcgaa
tacaaggcgaatctcgtgggaatcagggtggaacatgtcaaccctgcatacacatcgcaaacctgccctaagtgctccgaaaagaata
aggcacaggatcgcaagtataagtgtaagtgtggattcgaaaagcaccgcgatttggttggtgccatgaacatccgctacgccccggt
gatcgacgggaattcgcagtctgcc

[00304] ISBag01 – TnpB DNA sequence *Arabidopsis thaliana* codon optimization of the N-terminal up to the overlap with ωRNA sequence (SEQ ID NO: 4):

[00305] Atgtcccaaactattaccgttaaggtgaaacttctacctacgaaggagcaagcaagtattctacgtgagatgtctcaga
catatttatctactattaacaccctagtatctgagatggtagctgagaagaagtccacaaagaagtcttcaaaagacatctctgcttccctg
ccctctgctgttaagaaccaagcgattaaggacgcgaagtcagttttcaagaaggctaagaagtccaagttcacaacagtgccggtttt
gaagaagcctgtatgtatatggaacaatcagaactattccttcgacttcacccatatctccatgcctattatgattgatggtaaggtaacaa
agacgcccatccgagcactattagttgataaagataacagaaacttcgcgttgctgaagcacaagcttgggactctaagaattacccaa
aaggctaacaagtggattgcacaaatctcagtgactatccctactatggagggaacaggcgttaaggtcatgggggttgacttggggtt
gaaggtccctgctgtagcagtgacggacgacgagaaggtccgtttcttcggtaacggtagacagaataagtacatgaagaggaagtt
caggtcagaaaggaaggctttaggcaagaagaagaaggttaatgcgataaggtcctctaaggataaggagcagcgttggatgaagg
atcaagaccacaaggtatctagggccatcgtgaactttgccaaggataacaagatctccgtaataaggctcgaacagcttgccaatatc
cgacagacagcacgtacctcccgaaagaatgaaaagaatctgcatacctggtcattctacagactttcacaatttatagagtataaggca
aacttagttggtatccgtgttgagcatgtaaaccccgcgtatacatcacaaacctgcccgaagtgctcagagaagaacaaggcgcaag
accgaaagtataagtgcaagtgtggctttgagaagcatagagatctagttggagctatgaatattaggtatgctcctgtgattgatggtaa
cagtcaatcagcctaa

[00306] ISBag01 – TnpB amino acid sequence (SEQ ID NO: 5):

[00307] MSQTITVKVKLLPTKEQASILREMSQTYLSTINTLVSEMVAEKKSTKKSSK
DISASLPSAVKNQAIKDAKSVFKKAKKSKFTTVPVLKKPVCIWNNQNYSFDFTHISM
PIMIDGKVTKTPIRALLVDKDNRNFALLKHKLGTLRITQKANKWIAQISVTIPTMEGT
GVKVMGVDLGLKVPAVAVTDDEKVRFFGNGRQNKYMKRKFRSERKALGKKKKVN
AIRSSKDKEQRWMKDQDHKVSRAIVNFAKDNKISVIRLEQLANIRQTARTSRKNEKN
LHTWSFYRLSQFIEYKANLVGIRVEHVNPAYTSQTCPKCSEKNKAQDRKYKCKCGF
EKHRDLVGAMNIRYAPVIDGNSQSA

[00308] ISXfa01 – TnpB DNA sequence without codon optimization (SEQ ID NO: 6):

[00309] atgctgatggcccaccgtattgcactggaccctaacaatgtgcaggcgacccacctgtcgcgtgtggccggtgtggc
ccgttttgcctataactgggcgttagccgaatggaggcatcaatatgaagcatgtacgttggacagtgcccttcccaagccatcgcaac
attccttgcggcggcaactgaacgcgatcaagcgcgagcagtttccatggatgggcgaagtcaccaagaacgcgcctcaaatggcg

atcatccagttggggcaggcgtttcagaacttcttcacaggtcgtgccaagtatccgaagttccgtaaaaagggcgcgcatgaccgatt
cacgctgaccaatgaccagtttgaccttaacgcttcacgcattcgcattcctcggctgggctgggtgcgcatgcgtgaaacgttgcgctt
tgccggcagaatcatgtcggccaccgtgtcgcgtgtggctgcacgttggtttgttagcatcaccgtggacgtgcccgatccatcacatct
acctcaagctgaaaaccaaggcgcggtgggtgttgatctgggtgtgttggcgctggcaacgctctcaaccggagaaacaatctgtgg
ccccaggccccacagggcactgctgggtcgcgtgcgcaggctttcacgcagtgtgtcgcgtaaagtcaaagattctgccaatcgtca
caaagccaaagccacattggcgcatctacatgcacggattgcagcaattcgcttagatgcactgcacaaactcaccagcgatcttaccc
ggcgctttcacaccattggcattgagaacctgaatgtcaaaggcatggttaagaatcgccatttggcacgttccatcgccgatatgggct
tttttgaattcaggcggcagcttgaatataaggcggcgatgcgtggtgggcaggttgtggtggctgatcgtttctttgccagtagcaagat
gtgctcgacgtgtgggcacacgctcaaggaattgccgctctcggtgcgccagtgggcgtgtttggggtgcggtacacgtcatgatcgc
gacgtgaacgctgcggttaatttgaagaacatggccgtgagttccacggtatcagcctgtggagaggaaggcactggccctcgtcgc
aagacggcggtgaaaccggcctcggtgaagcaggaagtcagctttgaatctgtttaa

[00310] ISXfa01 – TnpB DNA sequence with *Arabidopsis thaliana* codon optimization (SEQ ID NO: 7):

[00311] atgcttatggctcacagaatagccctagatccaaacaacgttcaggcaacacatttatccagggtggcgggtgtagct
agattcgcgtacaattgggcgctagctgagtggcgtcaccaatatgaggcttgcactctagactccgcccttcctaaaccctctcaacac
tcactgaggaggcagcttaacgcgatcaagcgagagcaattcccgtggatgggggaagtcacaaagaatgctccgcaaatggcgat
aattcaactggggcaagccttccagaatttcttcactggtagagccaaatacccgaaattccgtaagaaggggcccatgatcgttttac
gctcacgaacgatcaatttgacttgaacgcaagtagaatccgtattccaagactggggtgggtacgaatgagagaaactctaagattcg
ctgggaggattatgagtgcaactgtttctagggttgcagcgaggtggttcgtatccatcactgttgatgttcctgatccgtcccacctgcct
caagccgaaaaccagggagcggttggcgtagatctgggcgttcttgcccttgctactttatcaactggggagacgatctgtggaccaa
gaccgcacagggctcttctcggaagagtgcgacgtctatcccgttctgtgtcaagaaaggtgaaggacagtgcaaacaggcataaag
ccaaggcgacgctggcgcatctacacgcgagaatagccgctatcaggctggacgcacttcacaagttgacttctgacttaactcgacg
attccacacaatcggtattgagaacctgaacgtgaaggggatggttaagaacaggcaccttgcaagatctatagcggatatgggcttct
tcgaatttcgtcgtcagctcgaatacaaagccgccatgcgaggggggcaagtagtagttgcggacagattcttcgcatcttccaagatg
tgttctacttgtggccatactctaaaggagttacccctgagcgtgcgtcaatgggcatgtctcggatgtgggaccagacatgaccgagat
gtgaacgcggcagttaacctcaagaacatggcagtcagcagcaccgttagcgcatgcggtgaagagggaactggaccacgtagga
agacggcagtgaaaccggccagcgtaaaacaggaggtttccttcgaaagcgta

[00312] ISXfa01 – TnpB DNA sequence with *Zea mays* codon optimization (SEQ ID NO: 8):

[00313] atgttgatggctcaccgcatcgcattggatccaaataatgtgcaggccacgcatctgagccgcgttgccggcgttgcc
cggtttgcgtataattgggcgcttgccgagtggcggcaccaatatgaggcttgcacgcttgactcagcccttcccaaaccgtcccagca
ctcactcagacgccaactgaatgccatcaaacgcgagcagttcccgtggatgggggaagtgaccaagaatgcccctcagatggcaat
catacagctcggccaggctttccagaatttcttcactggtagggctaagtatcccaagttcagaaagaagggcgcccatgaccggttta

ctcttacgaatgatcagtttgatctgaacgcgtctaggattcgcatcccccgccttggctgggttcggatgcgcgagacgcttaggtttgc
cggtagaataatgtcagccacagtttcaagggtcgctgcgagatggtttgttagcataactgtcgatgtcccggatccgtcgcaccttcc
gcaagcagagaatcaaggtgcggtgggtgtggatcttggagtgctggcgctggcaactctttctaccggcgagactatctgcggtccc
cggccgcatagggctctgcttgggcgggttcgccgcctttcacggtcggtttcacgcaaggtgaaagactcagccaatagacataaag
cgaaagccactcttgcccatctgcatgccaggattgccgcgatacgcctggacgctctccataaactcacctctgaccttaccagaagg
ttccacactattggcatcgagaatttgaatgttaaagggatggtcaagaaccggcaccttgcacggagcattgcggatatgggcttcttc
gagtttaggcggcaacttgaatataaggctgccatgaggggaggtcaagtggttgttgcagacaggttcttcgcgtcatccaagatgtg
ttcgacctgcggacacacacttaaagaacttcccctagcgtccgccagtgggcgtgtcttgggtgtgggaccagacacgatagagac
gtgaatgctgctgtcaatctgaagaacatggctgtgtcttccaccgtctctgcatgtggcgaggagggaactgggccgcgccggaaga
ccgctgtcaaacccgcgtccgtgaagcaagaagtgtccttcgagtcggtc

[00314] ISXfa01 – TnpB DNA sequence *Arabidopsis thaliana* codon optimization of the N-terminal up to the overlap with ωRNA sequence (SEQ ID NO: 9):

[00315] atgctgatggcgcatcgtattgcgctggaccctaataacgtgcaggccacacatctatctcgtgttgcgggtgtagccc
gattcgcctacaactgggcgttagcagagtggcgacaccaatatgaggcatgtaccttggactctgcgcttcctaaaccgtctcagcatt
ctctccgacgacagctcaatgctatcaaaagggaacaattcccgtggatgggtgaagtaacgaagaatgctccacagatggccatcat
ccagttaggacaagccttccagaacttcttcacaggacgtgcgaagtaccctaagtttcgtaagaaaggagctcatgacagatttacttt
gaccaacgaccaatttgacctgaacgcttctcgtataagaatcccacgtctgggttgggtacgtatgagggagacactgcgttttgcag
gaaggatcatgtctgcgaccgtgagtcgagttgcagctcgatggttcgtttccataacggtcgacgtacctgatccaagccacctaccg
caagctgaaaatcagggtgcggttggagtcgacttgggtgtcctggcactcgcgacccctttcaactggcgaaactatctgtgggccaa
gacctcatcgagcgttactgggtagagtacgtcgactgtccagatctgtgtctaggaaggtgaaggacagcgccaatagacataaggc
gaaggcaacactggctcatctacatgcccgaatagcagctataagacttgatgcattacataagctcacttctgacttgactaggagattc
cacaccatcggtatcgagaaccttaatgtaaagggcatggtgaagaatagacacctagctcgatccatagccgatatgggtttcttcga
gtttaggaggcaattggaatacaaagctgcgatgagaggtggccaggtagtagtcgccgacagattctttgcatccagcaagatgtgtt
caacttgtggacacacactgaaagaattgcctctttcagtgcgacaatgggcttgcttaggttgtgggaccagacatgatcgagacgtg
aatgccgctgtcaatctaaagaacatggccgtgagttccacggtatcagcctgtggagaggaaggcactggccctcgtcgcaagacg
gcggtgaaaccggcctcggtgaagcaggaagtcagctttgaatctgtt

[00316] ISXfa01 – TnpB amino acid sequence (SEQ ID NO: 10):

[00317] MLMAHRIALDPNNVQATHLSRVAGVARFAYNWALAEWRHQYEACTLD
SALPKPSQHSLRRQLNAIKREQFPWMGEVTKNAPQMAIIQLGQAFQNFFTGRAKYPK
FRKKGAHDRFTLTNDQFDLNASRIRIPRLGWVRMRETLRFAGRIMSATVSRVAARW
FVSITVDVPDPSHLPQAENQGAVGVDLGVLALATLSTGETICGPRPHRALLGRVRRLS
RSVSRKVKDSANRHKAKATLAHLHARIAAIRLDALHKLTSDLTRRFHTIGIENLNVK

GMVKNRHLARSIADMGFFEFRRQLEYKAAMRGGQVVVADRFFASSKMCSTCGHTL
KELPLSVRQWACLGCGTRHDRDVNAAVNLKNMAVSSTVSACGEEGTGPRRKTAVK
PASVKQEVSFESV*

[00318] DNA sequence (250 nt) encoding for the ωRNA sequence for ISBag01 TnpB (SEQ ID NO: 11):

[00319] aaatgcaaatgtggattcgaaaaacatcgtgatttagtcggtgctatgaacattcgatatgcacctgtgattgatggtaac
agtcaatcagcctaagatgctatatgcactgtcttaggaggggcaatgagatgccctcatcttgaaggctgttcaaaacagaaatggact
gcgaacgcttagtcattcaagaatcccacccgtacaccgcaaggtggaaggcttgcggctttagccgtgggagtctcaa

[00320] DNA sequence (186 nt) encoding for the ωRNA sequence for ISBag01 TnpB (SEQ ID NO: 12):

[00321] ctgtgattgatggtaacagtcaatcagcctaagatgctatatgcactgtcttaggaggggcaatgagatgccctcatctt
gaaggctgttcaaaacagaaatggactgcgaacgcttagtcattcaagaatcccacccgtacaccgcaaggtggaaggcttgcggctt
tagccgtgggagtctcaa

[00322] DNA sequence (144 nt) encoding for the ωRNA sequence for ISXfa01 TnpB (SEQ ID NO: 13):

[00323] acatggccgtgagttccacggtatcagcctgtggagaggaaggcactggccctcgtcgcaagacggcggtgaaac
cggcctcggtgaagcaggaagtcagctttgaatctgtttaaacagatttgagtaggtatgacagaacgg

[00324] ISYmu1 TnpB Native DNA Sequence (SEQ ID NO: 34)

[00325] ATGCTGCAGCATAAAGCCTATGAATACCGTATCTATCCAGATAAGAAG
CAGGAAACACTGATTGCCAAGACAATAGGCAGTTCAAGGTATGTATACAACCAT
TTCCTGGAACTTTGGAACAAGGAATATGAGGAGACCGGAAAAGGTCTGACTTAT
TACGCATGCTCCAAACTTCTGACAAAGCTTAAGAGGGACCCGGAAACAGTATGG
CTTTGTGAAGTGGATAAGTTCTCGCTCCAGAATTCGCTTCGTAACCTGTCGGACG
CCTTCTCCCGCTTTTTCAAAGGACAGAACGAGCATCCCCAGTTCAAGAGCAAGAA
GAGCCCGAGGCAAAGCTACACGACACAATATACGAACAACAACATCGCAGTTTC
CGGAAATTGTCTGAAGCTGCCTAAGCTGGGCCTTGTCAAGTTTGCAGACAGTAGG
GAGATGAAAGGCCGTATACTGAATGCCACAGTACGAAGGAAATCGAGCGGGAA
ATTCTTCGTATCGATCCTATGCGAAGAGGAGATCTGTGAATTGCCAAAGACTGAC
TCATCTGTCGGAATTGACCTCGGAATCATTGACTTTGCGGTTATGTCAGACGGAA
GCAGGCATGACAACAATCATTTTACCAGGCAAATGGAAGAAAGGCTTAGACGGG
AGCAGCGAAAGCTTGCAAGGCGTGCACTTGCTGCGGAGAAAAGGGGCATTTCCC

TTTCTGAAGCCAGGAACTATCAGAAGCAAAGGCGGAAGGTAGCGAGACTTTATG
AAAAGGTTGCAAACCAGCGAAAAGAATATCTTAACAAACTCAGTACAGAGATAG
TCAAAAACCACGATATCATCTGTATCGAGGACCTTAACGTTAAGGGCATGATGC
GCAATCATAAACTGGCAAAAAGCATCTCTGATGTATCATGGACGAGCCTTGTATC
GAAACTGCAGTACAAGGCTTCCTGGTATGGAAAAGAAGTCATCAGGATAAGCAG
ATGGTTTCCGTCAAGTCAGATATGCTCAGAGTGCGGCCACAAGGACAGGAAGAA
GCCTCTCCATGTAAGGGAGTGGACCTGTCCTGTTTGTCATGCCCACCATGACCGT
GACGTCAATGCAGCAAGAAATATACTGGCTGAGGGACTCAGGATAAGAGCTCTG
ACTCCGGGGTCTTAG

[00326] ISAam1 TnpB Native DNA Sequence (SEQ ID NO: 35)

[00327] ATGGTAAACAAATCATATAAATTCCGTCTGTACCCAACAAAAGAACAA
GAACAGCTGCTTGCCAAAACCTTCGGTTGTGTCCGTTTCGTTTATAACAAAATGC
TTGAAGAACGCATACAAATTTATGAAAAGTTCAAAGACGACAAAGAAGCTTTGA
AAAAACAAACATTTCCGACCCCTGCCAAGTACAAAAAGGAGTTTCCTTGGCTTA
AAGAAGTGGATAGCCTTGCGTTAGCAAACGCCCAATTGAATTTGCAGAAAGCGT
TTCAAAATTTCTTTTCGGGTCGTGCTGGATTTCCAAAGTTCAAAAACCGCAAGGC
GAAACAGTCGTACACCACAAATGTGGTCAATGGCAATATTCAACTTTCAGATGG
CTATATCAAGTTACCCAAACTGAAATGGGTCAAGTTCAAGCAACATCGGGAGAT
TCCTGCCCACCATATCATCAAGGCTTGTACGATCAAGAAAACAAAAACAGGAAA
ATACTATGTTTCTATTCTCACCGAATACGAACATCAACCTGTCCCCAAAGAAATA
CAAACGGTTGTTGGGCTTGATTTTTCCATGAATGGTTTATTTGTCGATAGCGAGG
GTAAGAGAGCCAATTATCCTCGTTTCTATCGGCAAGCATTGGAAAAATTAGCGA
AAGAACAACGGATATTATCACGCCGAAAGAAGGGTTCTAATCGTTGGCACAAAC
AGCGTTTGAAAGTAGCGAAGCTACATGAGAAAATAGCGAACCAACGAAAAGAC
TTTCTACACAAAAAATCATACGAATTAGCGAAACAGTATGATTGCGTGGTCATCG
AAAACCTCAACATGAAAGGGATGTCGCAAGTCCTGAATTTCGGCAAGAGCGTTC
ATGACAACGGCTGGGGGATGTTCACGACATTTCTCCAATACAAGTTAGAGGAGC
AAGGGAAAAAGCTTATCAAAATAGATAAGTGGTTTCCGTCATCTAAAACTTGTTC
ATGTTGCGATCAAGTAAAGGAATCTTTATCGCTTTCTGAGCGTACATTCCGCTGT
GAATGTGGATTCGAGAGCGACAGGGACGTCAATGCGGCAATCAATATCAAACAT
GAGGGCATGAAACGATTAGCAATAGCCTAA

[00328] ISDge10 TnpB Native DNA Sequence (SEQ ID NO: 36)

[00329] ATGACGACGCACCGCAAGGTCTACAGGTATCGGATTGAGCCGACCCCG
GTTCAAGAGTCGAAGCTGTACATGCTGGCGGGAAGTCGGCGCTTCGTCTTCAACT
GGGCTCTTGCGCGTCGAAGGGAACACTACGCCGAAACGGGCAAGACCCTGGGGT
ACAACGCTCAGGCGGGAGAGTTGACGGCCCTGAAGAACCAGGAGGAAACCTCCT
GGCTGAAGGAATCGGACAGCCAGCTTCTCCAGCAGGCCCTCAAGGACGTGGAGC
GGGCCTTCGTCAACTTCTTTGAGAAGCGGGCGAGGTTCCCCCGGTTCAAGAGCAA
AAAGACGGATACTCCGCGCTTCCGTATTCCCCAGCGGGTGCGGATAGAGGGGAG
CCGTGTGTATGTCCCGAAGGTGGGATGGGTTAAGCTCCGCAAGTCTCAGGAGAT
AGAGGGCAAGACCAAGAGCGCGACGTTCAAGCGGGAGGCAGACGGTCACTGGT
ACGTCTTGCTCGTCTCCGAGTTTGAGATGCCCGATGTACCGCTGCCCCCCGTCCCT
GAGTCCGAGGTGGTCGGGATTGACCTCGGCCTGAAGGATTTCTACGTGTTGTCCG
ACGGCGGGCGGAAAGAGGCCCCGAGGTTTGCCCGCAAGGGGCAGCGGAAACTC
CGCCGCGCTGCCCGTCGTCACTCCAAATGCACCAGGGGGAGCAACCGCAAGGCG
AAGGCCAAGCGGAAGCTCGCCCGTGTTCACCGCCAGATTGCGAATCAGAGAAAG
GACTTCGTTCACAAGGCCACCTCCGGTCTTGTTCAGCAGTACCAGGGTTTCTGCA
TCGAGAACTTGAGCATCAAGGGGATGGCGAAAACCAAGCTGTCCAAGAGCGTTC
TCGACGCCGCCCTGGGAGAGTTTCGCCGCCAGCTCGCCTACAAGGCCCAGTGGC
ACCGGAAGTGGCTGGCGGTCATAGACCGCTGGTTTCCGTCCAGCAAGCTGTGTG
GGGAATGCGGCAGCATCAACGCAGACCTGACCCTGAGTGACCGGGAATGGACGT
GCGAATGTGGAGCGGTTCACGACCGCGACCTCAACGCCGCCCGGAACATCAAGC
GGGAAGGGCTTTCGCAAATCGTCGTCGCGGGGCACGCGGAGACGTTAAACGCTC
GGGGAGAGGGTGTCAGACCTGCGATAGCGGGCAGCCCTCGATGA

[00330] ISDra2 TnpB Native DNA Sequence (SEQ ID NO: 37)

[00331] ATGATAAGGAATAAGGCTTTCGTGGTCAGGCTGTACCCAAATGCGGCT
CAGACTGAACTGATTAACCGCACGCTGGGTAGCGCAAGGTTCGTCTACAACCAC
TTCCTTGCCCGTCGCATTGCGGCCTACAAGGAAAGCGGGAAGGGACTGACCTAC
GGGCAAACGAGTAGCGAACTGACCCTTCTGAAGCAGGCTGAAGAAACCTCCTGG
CTCTCGGAAGTAGATAAGTTTGCTTTGCAGAACTCGCTGAAAAACCTTGAaACCG
CGTACAAGAACTTCTTTCGGACTGTGAAGCAGTCCGGTAAAAAGGTAGGATTCC
CACGTTTCAGAAAGAAGCGCACGGGAGAGTCCTACCGGACTCAATTCACCAACA
ACAACATCCAAATTGGGGAAGGTAGGCTCAAACTTCCTAAGCTGGGATGGGTGA
AAACCAAGGGCCAGCAGGATATTCAAGGGAAGATTCTGAATGTCACTGTGCGCC
GTATTCACGAAGGCCATTACGAAGCGTCCGTTCTCTGTGAAGTCGAGATTCCCTA

CCTGCCTGCGGCTCCCAAGTTTGCAGCGGGTGTGGATGTCGGCATCAAGGATTTT
GCCATCGTGACCGATGGCGTGAGGTTTAAGCATGAACAGAATCCGAAATATTAC
CGCTCCACCCTGAAAAGACTTCGTAAAGCTCAGCAAACCCTGTCCAGACGGAAG
AAGGGCAGCGCACGTTACGGGAAAGCGAAAACCAAGCTGGCTCGGATTCACAA
GCGCATTGTCAATAAGCGTCAGGATTTCCTTCACAAGCTCACCACCTCCCTGGTG
CGTGAGTACGAAATCATCGGAACCGAACACCTTAAACCCGACAACATGCGGAAA
AATCGCCGCCTTGCACTGAGCATCAGTGATGCGGGCTGGGGTGAGTTCATCCGGC
AGTTGGAATACAAGGCAGCGTGGTACGGGCGACTGGTATCTAAAGTCAGCCCCT
ACTTTCCATCTAGCCAGTTGTGTCATGACTGCGgattcaagaatcccgaagtgaagaatcttgccgtcc
gtacatggacttgcccgaactgtggggaaacccatgaccgagaCgagaacgctgcgctgaacattcggcgtgaagcgttggtggct
gcgggaatctcagacaccttaaacgctcatggaggctatgtcagacctgcttcggcgggcaatggtctgcgaagtgagaatcacgcg
actttagtcgtgtga

[00332] ISYmu1 TnpB Arabidopsis codon optimized DNA Sequence (SEQ ID NO: 38)

[00333] TTGCTACAgCATAAGGCTTAcGAgTATAGAATcTACCCTGACAAgAAGC
AgGAgACCTTAATcGCTAAGACCATcGGCAGCAGTCGTTAcGTGTAcAAcCACTTCC
TGGAgCTGTGGAACAAgGAgTACGAGGAgACCGGCAAgGGATTGACTTAcTAcGCC
TGTAGTAAGCTCTTGACAAAGCTAAAgCGAGACCCAGAGACCGTATGGCTGTGTG
AGGTAGACAAgTTcAGTTTACAGAAcTCTTTGAGGAACCTGTCTGACGCTTTcAGT
CGTTTCTTcAAgGGACAGAACGAgCACCCTCAATTCAAGTCAAAGAAGAGTCCCC
GTCAgAGCTACACCACTCAgTACACAAAcAACAAcATcGCAGTCAGTGGAAACTGC
TTGAAGCTCCCAAAGCTAGGGCTAGTTAAgTTCGCGGACTCCCGTGAgATGAAgG
GCAGAATcTTAAACGCTACCGTTCGTAGAAAgTCAAGCGGAAAGTTCTTcGTGTCA
ATcTTATGTGAGGAgGAGATCTGTGAgCTCCCAAAGACGGATAGCTCAGTGGGGA
TcGACTTGGGCATcATcGACTTCGCGGTTATGTCAGACGGGTCTAGGCATGATAAC
AAcCACTTCACTCGACAGATGGAgGAGCGACTGAGACGAGAgCAgAGGAAgCTTG
CGAGAAGAGCACTCGCGGCCGAgAAgCGTGGCATTTCCCTGAGCGAGGCTAGAA
ACTACCAgAAGCAGCGACGAAAGGTTGCTAGATTGTACGAgAAGGTTGCCAACC
AGCGTAAGGAGTATTTGAACAAGTTGTCAACTGAGATcGTTAAgAAcCACGACAT
CATcTGCATcGAGGATCTAAACGTGAAgGGAATGATGAGGAAcCATAAGCTCGCG
AAgTCAATTAGTGACGTCAGTTGGACTAGCCTGGTGAGTAAgTTACAGTACAAGG
CATCTTGGTAcGGCAAgGAgGTAATCCGTATcAGTAGATGGTTcCCTAGTAGTCAG
ATTTGTTCCGAgTGCGGGCACAAgGATCGTAAGAAGCCCTTACACGTCAGGGAgT

GGACCTGTCCAGTCTGTCACGCTCACCACGACCGAGATGTAAAcGCGGCCAGAA
ACATTCTTGCAGAgGGTCTGAGGATTAGGGCGCTTACTCCAGGAAGCTAG

[00334] ISAam1 TnpB Arabidopsis codon optimized DNA Sequence (SEQ ID NO: 39)

[00335] ATGGTAAACAAGAGTTACAAGTTCCGTTTGTACCCAACAAAGGAgCAG
GAgCAgCTCCTAGCTAAGACATTCGGCTGTGTCAGGTTCGTTTACAACAAGATGC
TAGAGGAgCGAATcCAGATCTAcGAgAAGTTCAAGGACGATAAGGAgGCTCTCAA
GAAGCAGACTTTCCCGACTCCAGCTAAGTACAAGAAGGAgTTCCCGTGGCTGAA
GGAgGTCGACTCACTGGCCCTAGCTAAcGCCCAgCTCAAcCTGCAgAAGGCTTTCC
AGAAcTTCTTCTCAGGGAGAGCAGGGTTCCCTAAGTTCAAGAAcAGAAAGGCGAA
GCAGTCCTACACGACAAAcGTAGTAAAcGGGAACATcCAgCTCTCCGATGGCTAC
ATCAAGCTGCCTAAGCTGAAGTGGGTTAAGTTCAAGCAgCACCGTGAgATCCCTG
CGCATCATATCATcAAGGCTTGCACTATcAAGAAGACTAAGACAGGTAAGTAcTA
CGTGTCTATcTTGACAGAGTAcGAGCACCAgCCCGTCCCGAAGGAGATcCAgACAG
TAGTTGGACTTGACTTCTCCATGAATGGTCTGTTCGTAGATTCTGAGGGCAAGAG
AGCTAACTACCCCAGATTCTACAGACAgGCTCTAGAGAAGCTGGCTAAGGAgCAg
AGGATcCTAAGTAGGCGAAAGAAGGGCAGTAAcCGTTGGCATAAGCAgAGGCTC
AAGGTTGCGAAGCTCCACGAgAAGATCGCCAACCAgCGTAAAGACTTCTTACACA
AGAAGAGTTACGAGTTAGCAAAGCAGTACGATTGCGTTGTGATCGAGAATTTGA
ATATGAAGGGAATGAGTCAgGTGCTTAACTTCGGGAAGTCAGTGCACGACAACG
GCTGGGGAATGTTCACAACGTTCCTCCAGTACAAGTTAGAGGAgCAgGGAAAGA
AGTTGATCAAGATCGACAAGTGGTTCCCTTCCTCAAAGACATGCTCCTGCTGTGA
TCAgGTTAAGGAgTCCCTTAGTCTATCCGAgCGAACATTCAGATGT

[00336] ISDge10 TnpB Arabidopsis codon optimized DNA Sequence (SEQ ID NO: 40)

[00337] ATGACGACGCATAGAAAGGTCTACAGGTACAGGATAGAGCCGACTCC
GGTGCAAGAATCAAAgCTGTATATGTTGGCTGGAAGTAGACGATTCGTATTTAAT
TGGGCATTGGCCCGTAGAAGGGAGCATTACGCGGAGACGGGGAAGACCCTTGGA
TATAACGCCCAAGCAGGAGAGCTTACGGCTCTAAAgAACCAgGAGGAGACGAGT
TGGCTGAAgGAgAGCGACTCCCAgCTGTTACAgCAgGCACTCAAgGACGTCGAACG
TGCATTcGTTAACTTcTTcGAgAAGCGTGCTCGTTTCCCACGTTTcAAGTCTAAGAA
GACCGATACACCCAGATTcCGTATCCCGCAACGTGTTCGAATTGAGGGTAGTAGA
GTGTATGTTCCGAAgGTTGGTTGGGTCAAgCTACGAAAGTCCCAAGAGATAGAGG

GAAAGACTAAGAGCGCCACCTTCAAGCGAGAGGCGGATGGACACTGGTACGTGC
TGCTTGTGAGTGAGTTCGAGATGCCCGACGTGCCCCTTCCTCCAGTGCCAGAGTC
TGAAGTGGTCGGAATAGATTTAGGGCTAAAgGACTTCTACGTTCTATCCGACGGA
GGGCGTAAGGAAGCTCCGCGATTcGCTCGAAAgGGTCAACGTAAGCTTAGGAGA
GCAGCGCGTAGGCACTCAAAGTGTACACGAGGTTCCAATCGTAAgGCAAAgGCTA
AgAGAAAGCTGGCTAGGGTACACCGACAAATCGCGAACCAGCGAAAgGATTTCG
TCCATAAgGCTACTTCTGGTCTTGTACAGCAGTATCAGGGCTTCTGTATTGAgAAC
CTCAGCATTAAgGGAATGGCCAAgACAAAGTTAAGCAAGTCAGTACTAGACGCA
GCGCTAGGAGAATTcCGTAGGCAACTGGCCTACAAGGCACAGTGGCACCGAAAg
TGGTTAGCCGTCATAGATAGATGGTTcCCGTCTTCCAAgCTTTGTGGCGAATGTGG
CTCTATAAACGCAGACCTCACGCTTTCAGACCGAGAATGGACATGCGAATGTGG
CGCTGTTCACGATAGAGATTTAAATGCTGCGCGTAACATTAAGAGAGAAGGGTT
GAGCCAAATAGTTGTAGCTGGCCACGCAGAgACCTTGAATGCCAGAGGAGAAGG
TGTACGACCGGCAATAGCCGGTTCTCCAAGGTGA

[00338] ISDra2 TnpB Arabidopsis codon optimized DNA Sequence (SEQ ID NO: 41)

[00339] ATGATTAGAAATAAGGCATTcGTTGTCAGATTATATCCCAATGCAGCAC
AAACTGAGTTGATAAACCGAACACTGGGCAGCGCGCGTTTCGTCTATAACCATTT
cTTGGCTAGAAGGATTGCTGCTTACAAGGAGAGCGGTAAgGGGTTAACTTATGGA
CAGACGAGTTCTGAGCTTACCCTCCTTAAGCAGGCAGAGGAAACATCCTGGTTGT
CTGAGGTCGATAAgTTcGCGTTACAAAATTCTCTCAAgAATTTGGAGACGGCATAC
AAGAATTTcTTCCGTACGGTCAAgCAGTCTGGGAAgAAgGTGGGTTTcCCGCGTTTC
AGAAAGAAGAGAACTGGAGAATCCTACCGTACTCAGTTcACTAACAACAACATT
CAAATAGGAGAAGgtaagtttctgcttctacctttgatatatatataataattatcattaattagtagtaatataatatttcaaatatt
tttttcaaaataaaagaatgtagtatatagcaattgcttttctgtagtttataagtgtgtatattttaatttataacttttctaatatatgaccaaaatt
tgttgatgtgcagGTAGATTAAAGCTCCCTAAgCTAGGTTGGGTGAAGACAAAgGGCCAA
CAAGACATTCAAGGAAAGATCTTAAATGTAACTGTGAGGCGAATCCACGAGGGC
CACTATGAGGCCTCCGTGCTATGCGAAGTCGAAATACCGTATCTTCCGGCTGCAC
CCAAgTTcGCTGCCGGCGTAGACGTTGGTATTAAGGACTTCGCCATCGTCACCGA
CGGTGTACGATTcAAGCATGAGCAAAATCCTAAgTATTACCGATCTACGCTAAAg
CGTTTACGTAAgGCTCAACAAACCCTATCCAGGAGGAAGAAgGGTTCCGCCCGTT
ACGGTAAgGCTAAgACCAAGTTGGCAAGAATCCACAAgAGAATAGTCAACAAGA
GGCAGGACTTCCTTCACAAGCTTACTACTAGCCTTGTAAGAGAATACGAGATTAT

TGGGACGGAGCATCTAAAgCCAGATAATATGAGAAAGAACCGTAGGCTCGCGCT
GTCAATCAGCGACGCCGGGTGGGGTGAATTcATCAGGCAATTGGAGTACAAGGC
TGCATGGTACGGGCGTCTAGTTTCTAAAGTATCACCATATTTCCCATCCAGTCAG
CTTTGCCATGATTGCGGTTTCAAGAACCCGGAGGTTAAgAATCTCGCAGTTAGGA
CGTGGACCTGTCCGAACTGCGGGGAGACTCACGACAGAGACGAGAACGCAGCCC
TGAACATAAGGAGAGAGGCTTTAGTGGCGGCTGGAATATCTGATACTCTAAACG
CTCACGGAGGGTACGTGCGTCCGGCAAGCGCAGGAAATGGCCTACGAAGTGAAA
ATCATGCCACTCTTGTTGTT

[00340] ISYmu1 TnpB Maize codon optimized DNA Sequence (SEQ ID NO: 42)

[00341] TTGCTcCAgCAcAAGGCCTACGAGTAcAGAATCTATCCAGATAAGAAgC
AGGAGACTCTcATCGCTAAgACCATcGGCTCTTCCAGGTAcGTTTACAACCACTTT
CTCGAgTTGTGGAATAAGGAgTACGAGGAgACTGGGAAgGGACTGACTTACTAcG
CATGCTCGAAgCTCCTcACCAAgTTGAAgAGAGACCCGGAgACCGTGTGGCTGTGT
GAGGTTGACAAgTTCTCTCTCCAgAAcTCACTGAGGAACCTCTCTGACGCCTTcTC
ACGCTTCTTcAAGGGGCAgAACGAGCATCCGCAGTTcAAgTCCAAGAAGTCACCCC
GGCAgTCCTACACAACGCAgTATACTAAcAAcAATATcGCCGTGAGCGGTAACTGC
CTTAAgCTcCCGAAGCTcGGcTTGGTGAAGTTCGCCGACTCACGGGAgATGAAgGG
CCGGATcCTCAAcGCCACTGTCAGACGGAAgAGCAGCGGGAAgTTCTTCGTgTCGA
TcTTGTGTGAGGAgGAgATCTGTGAGCTCCCGAAgACTGACTCATCTGTCGGTATC
GATCTcGGGATcATcGACTTcGCAGTGATGTCCGACGGTTCACGGCATGATAAcAA
CCACTTcACTCGGCAGATGGAgGAgCGCCTGCGGCGGGAGCAGAGgAAgCTCGCC
CGCAGAGCCCTcGCAGCCGAgAAGCGCGGGATCTCGCTCTCAGAGGCGCGGAAcT
ACCAgAAGCAGCGGCGGAAGGTCGCGAGATTGTAcGAgAAgGTGGCTAAcCAgCG
CAAGGAgTAcCTcAAcAAgCTcAGCACGGAgATcGTTAAgAAcCACGATATcATcTGT
ATCGAGGACCTCAATGTTAAGGGGATGATGCGGAAcCACAAGTTGGCCAAgTCAA
TCAGCGACGTGTCCTGGACTTCTCTCGTgTCCAAGCTGCAATAcAAgGCGTCGTGG
TACGGcAAgGAgGTCATcAGAATcTCGCGGTGGTTCCCCTCTTCGCAGATcTGCTCC
GAGTGCGGTCACAAgGACCGCAAGAAGCCTCTCCATGTTAGGGAGTGGACTTGC
CCCGTGTGCCACGCGCATCATGACAGAGATGTTAACGCAGCAAGGAACATcTTGG
CTGAgGGCCTGAGAATcCGGGCACTcACACCAGGAAGCTAG

[00342] ISAam1 TnpB Maize codon optimized DNA Sequence (SEQ ID NO: 43)

[00343] ATGGTgAACAAgTCATAcAAgTTCCGTCTGTACCCAACcAAgGAgCAgGA
gCAGCTGCTgGCCAAgACCTTCGGTTGTGTCCGTTTCGTgTAcAACAAgATGCTcGA
gGAgCGCATcCAgATcTAcGAgAAGTTCAAgGACGACAAgGAgGCcTTGAAgAAgCAg
ACcTTcCCGACCCCTGCCAAGTACAAgAAGGAGTTcCCTTGGCTcAAgGAgGTGGAT
AGCCTTGCGctgGCcAACGCCCAgTTGAAcTTGCAGAAgGCGTTcCAgAAcTTCTTcTC
GGGTCGTGCcGGATTcCCAAAGTTCAAgAACCGCAAGGCGAAgCAGTCGTACACC
ACcAAcGTGGTCAAcGGCAAcATcCAgCTcTCAGATGGCTAcATCAAGctcCCCAAgCT
GAAgTGGGTCAAGTTCAAGCAgCAcCGGGAGATcCCTGCCCACCAcATCATCAAGG
CTTGTACGATCAAGAAgACcAAgACcGGcAAgTACTAcGTgTCTATcCTCACCGAgTA
CGAgCAcCAgCCTGTCCCCAAgGAgATcCAgACGGTcGTcGGGCTcGAcTTcTCCATG
AAcGGcctgTTcGTCGATAGCGAGGGTAAGAGAGCCAATTAcCCTCGTTTCTAcCGG
CAgGCATTGGAgAAgctcGCGAAgGAgCAgCGGATcctcTCACGCCGcAAGAAGGGTTC
TAAcCGTTGGCACAAgCAGCGccTGAAgGTgGCGAAGCTcCAcGAGAAgATcGCGAA
CCAgCGcAAgGACTTcCTcCACAAgAAgTCATACGAgctcGCGAAgCAGTAcGAcTGCG
TGGTCATCGAgAACCTCAACATGAAgGGGATGTCGCAgGTCCTGAAcTTCGGCAA
GAGCGTgCAcGACAACGGCTGGGGGATGTTCACGACgTTcCTCCAgTACAAGctcGA
GGAGCAgGGGAAgAAGCTcATCAAgATcGAcAAGTGGTTcCCGTCATCTAAgACTTG
cTCATGcTGCGAcCAgGTgAAGGAgTCcctgTCGCTcTCTGAGCGTACcTTCCGCTGcG
AgTGcGGATTCGAGAGCGACAGGGACGTCAAcGCGGCAATCAAcATCAAgCAcGA
GGGCATGAAgCGcctgGCAATcGCCTAA

[00344] ISDge10 TnpB Maize codon optimized DNA Sequence (SEQ ID NO: 44)

[00345] ATGACCACTCATCGCAAGGTCTACAGGTATCGGATTGAGCCGACCCCG
GTTCAAGAGTCGAAGCTGTACATGCTGGCGGGAAGTCGGAGGTTCGTCTTCAATT
GGGCTCTTGCGAGGCGAAGGGAACACTACGCAGAAACTGGCAAGACCCTGGGCT
ATAATGCTCAGGCTGGCGAGTTGACGGCCCTGAAGAATCAGGAGGAAACATCCT
GGCTGAAGGAATCGGATAGCCAGCTTCTACAGCAGGCCCTCAAGGATGTGGAGA
GAGCATTCGTCAACTTCTTTGAGAAGAGAGCGAGGTTCCCCCGGTTCAAGTCAAA
gAAGACGGATACTCCACGCTTCCGTATTCCCCAAAGAGTGAGAATAGAGGGCAG
TCGTGTGTATGTACCAAAGGTGGGATGGGTTAAGCTCCGCAAGTCTCAGGAGAT
AGAGGGCAAgACCAAGAGCGCGACTTTCAAACGAGAGGCAGACGGTCACTGGTA
CGTGTTGCTAGTCTCTGAATTTGAGATGCCCGATGTACCACTGCCACCCGTCCCT
GAGTCCGAGGTGGTGGGCATTGACCTCGGCctcAAGGATTTCTACGTGTTGTCCGA

TGGCGGACGGAAgGAGGCCCCGAGGTTTGCCCGCAAGGGGCAGCGGAAACTCCG
CCGCGCTGCCCGTAGGCATTCCAAgTGCACCAGGGGGAGCAACCGAAAGGCGAA
GGCCAAgCGGAAgCTCGCCCGTGTGCACCGCCAGATTGCTAATCAAAGAAAGGA
CTTCGTTCATAAGGCAACATCCGGTCTTGTTCAACAATACCAGGGTTTCTGCATC
GAGAACTTGTCAATCAAGGGGATGGCGAAgACCAAGTTATCTAAgAGCGTTCTCG
ACGCCGCCCTGGGAGAGTTTCGCCGCCAGCTCGCATACAAGGCCCAGTGGCACA
GGAAGTGGCTTGCGGTCATCGACCGCTGGTTTCCGTCAAGCAAGCTGTGTGGGG
AATGCGGCTCAATCAACGCAGACCTGACACTCAGTGACAGGGAATGGACGTGCG
AATGTGGAGCAGTTCATGACCGCGACCTCAACGCCGCCAGAAACATCAAGAGGG
AAGGGCTTTCGCAAATCGTCGTCGCTGGTCACGCTGAGACGctcAACGCTCGGGGC
GAGGGTGTGAGACCTGCGATCGCGGGCTCTCCTCGATGA

[00346] ISDra2 TnpB Maize codon optimized DNA Sequence (SEQ ID NO: 45)

[00347] ATGATTAGGAACAAgGCATTCGTCGTCCGCCTCTATCCAAACGCGGCC
CAAACGGAGTTGATAAACCGCACACTCGGTTCTGCCAGgtaagtttctgcttctacctttgatatatat
ataataattatcattaattagtagtaatataatatttcaaatatttttttcaaaataaaagaatgtagtatatagcaattgcttttctgtagtttataa
gtgtgtatattttaatttataacttttctaatatatgaccaaaatttgttgatgtgcagGTTTGTCTATAATCATTTcCTCG
CTAGGAGGATTGCGGCTTATAAGGAATCGGGGAAgGGGTTGACGTATGGGCAAA
CGTCTAGCGAGCTGACACTTCTCAAACAAGCAGAAGAGACATCTTGGCTCTCAG
AGGTTGATAAGTTCGCACTGCAAAACTCGTTGAAGAACTTGGAGACTGCCTACA
AgAACTTCTTCAGAACAGTGAAGCAATCGGGGAAgAAGGTGGGGTTcCCCCGGTT
CAGGAAGAAgAGGACTGGTGAATCTTACAGGACACAGTTcACTAACAACAATATT
CAAATAGGTGAGGGAAGGCTCAAGCTTCCTAAgTTGGGATGGGTCAAGACAAAg
GGGCAGCAGGATATCCAGGGAAAGATACTTAATGTTACTGTGCGCAGGATACAT
GAAGGCCACTATGAAGCTTCCGTTTTGTGCGAGGTCGAAATTCCCTACTTGCCCG
CCGCACCCAAGTTCGCTGCGGGAGTGGATGTGGGGATTAAgGACTTTGCGATAGT
TACAGATGGAGTTCGCTTcAAGCACGAGCAAAACCCAAAGTACTATAGATCCACC
TTGAAgCGGCTCAGAAAGGCGCAACAAACCCTTAGCAGGCGCAAgAAgGGCTCG
GCCAGGTACGGTAAgGCGAAGACCAAGCTCGCTAGGATTCATAAGCGGATTGTG
AATAAgAGACAAGACTTcCTGCACAAGTTGACTACATCTTTGGTGAGGGAATATG
AAATCATAGGGACCGAACACTTGAAGCCAGATAATATGCGCAAgAATCGGCGCC
TGGCTCTTTCAATTTCAGATGCGGGTTGGGGAGAATTTATTAGGCAGTTGGAGTA
CAAgGCGGCCTGGTACGGTCGGCTTGTCTCAAAGGTGTCCCCGTATTTcCCGAGCT

CGCAACTGTGCCATGACTGTGGCTTCAAGAACCCTGAAGTCAAgAACCTGGCCGT
TAGAACCTGGACATGCCCAAATTGCGGCGAAACACACGACAGAGACGAAAATGC
GGCCCTTAATATACGCCGCGAGGCGTTGGTTGCAGCTGGAATCTCAGACACCCTG
AATGCTCACGGCGGCTATGTTCGCCCAGCGTCAGCAGGTAACGGACTTAGATCTG
AAAACCATGCAACTCTTGTCGTT

[00348] ISYmu1 TnpB amino acid sequence (SEQ ID NO: 46)

[00349] MLQHKAYEYRIYPDKKQETLIAKTIGSSRYVYNHFLELWNKEYEETGKGL
TYYACSKLLTKLKRDPETVWLCEVDKFSLQNSLRNLSDAFSRFFKGQNEHPQFKSKK
SPRQSYTTQYTNNNIAVSGNCLKLPKLGLVKFADSREMKGRILNATVRRKSSGKFFV
SILCEEEICELPKTDSSVGIDLGIIDFAVMSDGSRHDNNHFTRQMEERLRREQRKLARR
ALAAEKRGISLSEARNYQKQRRKVARLYEKVANQRKEYLNKLSTEIVKNHDIICIED
LNVKGMMRNHKLAKSISDVSWTSLVSKLQYKASWYGKEVIRISRWFPSSQICSECGH
KDRKKPLHVREWTCPVCHAHHDRDVNAARNILAEGLRIRALTPGS*

[00350] ISAam1 TnpB amino acid sequence (SEQ ID NO: 47)

[00351] MVNKSYKFRLYPTKEQEQLLAKTFGCVRFVYNKMLEERIQIYEKFKDDK
EALKKQTFPTPAKYKKEFPWLKEVDSLALANAQLNLQKAFQNFFSGRAGFPKFKNR
KAKQSYTTNVVNGNIQLSDGYIKLPKLKWVKFKQHREIPAHHIIKACTIKKTKTGKY
YVSILTEYEHQPVPKEIQTVVGLDFSMNGLFVDSEGKRANYPRFYRQALEKLAKEQR
ILSRRKKGSNRWHKQRLKVAKLHEKIANQRKDFLHKKSYELAKQYDCVVIENLNM
KGMSQVLNFGKSVHDNGWGMFTTFLQYKLEEQGKKLIKIDKWFPSSKTCSCCDQV
KESLSLSERTFRCECGFESDRDVNAAINIKHEGMKRLAIA*

[00352] ISDge10 TnpB amino acid sequence (SEQ ID NO: 48)

[00353] MTTHRKVYRYRIEPTPVQESKLYMLAGSRRFVFNWALARRREHYAETGK
TLGYNAQAGELTALKNQEETSWLKESDSQLLQQALKDVERAFVNFFEKRARFPRFK
SKKTDTPRFRIPQRVRIEGSRVYVPKVGWVKLRKSQEIEGKTKSATFKREADGHWYV
LLVSEFEMPDVPLPPVPESEVVGIDLGLKDFYVLSDGGRKEAPRFARKGQRKLRRAA
RRHSKCTRGSNRKAKAKRKLARVHRQIANQRKDFVHKATSGLVQQYQGFCIENLSI
KGMAKTKLSKSVLDAALGEFRRQLAYKAQWHRKWLAVIDRWFPSSKLCGECGSIN
ADLTLSDREWTCECGAVHDRDLNAARNIKREGLSQIVVAGHAETLNARGEGVRPAI
AGSPR*

[00354] ISDra2 TnpB amino acid sequence (SEQ ID NO: 49)

[00355] MIRNKAFVVRLYPNAAQTELINRTLGSARFVYNHFLARRIAAYKESGKGL TYGQTSSELTLLKQAEETSWLSEVDKFALQNSLKNLETAYKNFFRTVKQSGKKVGFP RFRKKRTGESYRTQFTNNNIQIGEGRLKLPKLGWVKTKGQQDIQGKILNVTVRRIHE GHYEASVLCEVEIPYLPAAPKFAAGVDVGIKDFAIVTDGVRFKHEQNPKYYRSTLKR LRKAQQTLSRRKKGSARYGKAKTKLARIHKRIVNKRQDFLHKLTTSLVREYEIIGTE HLKPDNMRKNRRLALSISDAGWGEFIRQLEYKAAWYGRLVSKVSPYFPSSQLCHDC GFKNPEVKNLAVRTWTCPNCGETHDRDENAALNIRREALVAAGISDTLNAHGGYVR PASAGNGLRSENHATLVV*

[00356] ISYmu1 ωRNA DNA sequence (SEQ ID NO: 50)

[00357] ATGCAGCAAGAAATATACTGGCTGAGGGACTCAGGATAAGAGCTCTG ACTCCGGGGTCTTAGCAATACAGAAGCAAACAGGAACCGCAGGAATTGCGGGGG TAGCTTGGTAAACAAGAGAAACCTCTGCCGGCAAAGAAATAAGCCGGTAAGTAT GCTCTGTTCCCAAGAATCTCGTGACTTTAGTCATGAGAGTTTCAA

[00358] ISAam1 ωRNA DNA sequence (SEQ ID NO: 51)

[00359] GAATGTGGATTCGAGAGCGACAGGGACGTCAATGCGGCAATCAATAT CAAACATGAGGGCATGAAACGATTAGCAATAGCCTAACTTGTCCTCGAACCGTG GGACACACGGGGATCGCTCAGTCAACTTCCCGTCATGAGATGGGATTACCTGAG AAGCCCCCACCTCTAAGCGAAGCGTAGGTGGTGGGAGCATGTCAC

[00360] ISDge10 ωRNA DNA sequence (SEQ ID NO: 52)

[00361] GGAATGGACGTGCGAATGTGGAGCGGTTCACGACCGCGACCTCAACG CCGCCCGGAACATCAAGCGGGAAGGGCTTTCGCAAATCGTCGTCGCGGGGCACG CGGAGACGTTAAACGCTCGGGGAGAGGGTGTCAGACCTGCGATAGCGGGCAGCC CTCGATGAAGCGAGAATCCAACGGCTTTAGCCGTTGGAGTGTCAA

[00362] ISDra2 ωRNA DNA sequence (SEQ ID NO: 53)

[00363] gattcaagaatcccgaagtgaagaatcttgccgtccgtacatggacttgcccgaactgtggggaaacccatgaccga gaCgagaacgctgcgctgaacattcggcgtgaagcgttggtggctgcgggaatctcagacaccttaaacgctcatggaggctatgtc agacctgcttcggcgggcaatggtctgcgaagtgagaatcacgcgactttagtcgtgtgaGGTTCAA

[00364] ISYmu1 TnpB with ωRNA overlap native DNA sequence (single) (SEQ ID NO: 140)

[00365] ATGCTGCAGCATAAAGCCTATGAATACCGTATCTATCCAGATAAGAAG
CAGGAAACACTGATTGCCAAGACAATAGGCAGTTCAAGGTATGTATACAACCAT
TTCCTGGAACTTTGGAACAAGGAATATGAGGAGACCGGAAAAGGTCTGACTTAT
TACGCATGCTCCAAACTTCTGACAAAGCTTAAGAGGGACCCGGAAACAGTATGG
CTTTGTGAAGTGGATAAGTTCTCGCTCCAGAATTCGCTTCGTAACCTGTCGGACG
CCTTCTCCCGCTTTTTCAAAGGACAGAACGAGCATCCCCAGTTCAAGAGCAAGAA
GAGCCCGAGGCAAAGCTACACGACACAATATACGAACAACAACATCGCAGTTTC
CGGAAATTGTCTGAAGCTGCCTAAGCTGGGCCTTGTCAAGTTTGCAGACAGTAGG
GAGATGAAAGGCCGTATACTGAATGCCACAGTACGAAGGAAATCGAGCGGGAA
ATTCTTCGTATCGATCCTATGCGAAGAGGAGATCTGTGAATTGCCAAAGACTGAC
TCATCTGTCGGAATTGACCTCGGAATCATTGACTTTGCGGTTATGTCAGACGGAA
GCAGGCATGACAACAATCATTTTACCAGGCAAATGGAAGAAAGGCTTAGACGGG
AGCAGCGAAAGCTTGCAAGGCGTGCACTTGCTGCGGAGAAAAGGGGCATTTCCC
TTTCTGAAGCCAGGAACTATCAGAAGCAAAGGCGGAAGGTAGCGAGACTTTATG
AAAAGGTTGCAAACCAGCGAAAAGAATATCTTAACAAACTCAGTACAGAGATAG
TCAAAAACCACGATATCATCTGTATCGAGGACCTTAACGTTAAGGGCATGATGC
GCAATCATAAACTGGCAAAAAGCATCTCTGATGTATCATGGACGAGCCTTGTATC
GAAACTGCAGTACAAGGCTTCCTGGTATGGAAAAGAAGTCATCAGGATAAGCAG
ATGGTTTCCGTCAAGTCAGATATGCTCAGAGTGCGGCCACAAGGACAGGAAGAA
GCCTCTCCATGTAAGGGAGTGGACCTGTCCTGTTTGTCATGCCCACCATGACCGT
GACGTCAATGCAGCAAGAAATATACTGGCTGAGGGACTCAGGATAAGAGCTCTG
ACTCCGGGGTCTTAGCAATACAGAAGCAAACAGGAACCGCAGGAATTGCGGGGG
TAGCTTGGTAAACAAGAGAAACCTCTGCCGGCAAAGAAATAAGCCGGTAAGTAT
GCTCTGTTCCCAAGAATCTCGTGACTTTAGTCATGAGAGTTTCAA

[00366] ISAam1 TnpB with ωRNA overlap native DNA sequence (single) (SEQ ID NO: 141)

[00367] ATGGTAAACAAATCATATAAATTCCGTCTGTACCCAACAAAAGAACAA
GAACAGCTGCTTGCCAAAACCTTCGGTTGTGTCCGTTTCGTTTATAACAAAATGC
TTGAAGAACGCATACAAATTTATGAAAAGTTCAAAGACGACAAAGAAGCTTTGA
AAAAACAAACATTTCCGACCCCTGCCAAGTACAAAAAGGAGTTTCCTTGGCTTA

AAGAAGTGGATAGCCTTGCGTTAGCAAACGCCCAATTGAATTTGCAGAAAGCGT
TTCAAAATTTCTTTTCGGGTCGTGCTGGATTTCCAAAGTTCAAAAACCGCAAGGC
GAAACAGTCGTACACCACAAATGTGGTCAATGGCAATATTCAACTTTCAGATGG
CTATATCAAGTTACCCAAACTGAAATGGGTCAAGTTCAAGCAACATCGGGAGAT
TCCTGCCCACCATATCATCAAGGCTTGTACGATCAAGAAAACAAAAACAGGAAA
ATACTATGTTTCTATTCTCACCGAATACGAACATCAACCTGTCCCCAAAGAAATA
CAAACGGTTGTTGGGCTTGATTTTTCCATGAATGGTTTATTTGTCGATAGCGAGG
GTAAGAGAGCCAATTATCCTCGTTTCTATCGGCAAGCATTGGAAAAATTAGCGA
AAGAACAACGGATATTATCACGCCGAAAGAAGGGTTCTAATCGTTGGCACAAAC
AGCGTTTGAAAGTAGCGAAGCTACATGAGAAAATAGCGAACCAACGAAAAGAC
TTTCTACACAAAAAATCATACGAATTAGCGAAACAGTATGATTGCGTGGTCATCG
AAAACCTCAACATGAAAGGGATGTCGCAAGTCCTGAATTTCGGCAAGAGCGTTC
ATGACAACGGCTGGGGGATGTTCACGACATTTCTCCAATACAAGTTAGAGGAGC
AAGGGAAAAAGCTTATCAAAATAGATAAGTGGTTTCCGTCATCTAAAACTTGTTC
ATGTTGCGATCAAGTAAAGGAATCTTTATCGCTTTCTGAGCGTACATTCCGCTGT
GAATGTGGATTCGAGAGCGACAGGGACGTCAATGCGGCAATCAATATCAAACAT
GAGGGCATGAAACGATTAGCAATAGCCTAACTTGTCCTCGAACCGTGGGACACA
CGGGGATCGCTCAGTCAACTTCCCGTCATGAGATGGGATTACCTGAGAAGCCCCC
ACCTCTAAGCGAAGCGTAGGTGGTGGGAGCATGTCAC

[00368] ISDge10 TnpB with ωRNA overlap native DNA sequence (single) (SEQ ID NO: 142)

[00369] ATGACGACGCACCGCAAGGTCTACAGGTATCGGATTGAGCCGACCCCG
GTTCAAGAGTCGAAGCTGTACATGCTGGCGGGAAGTCGGCGCTTCGTCTTCAACT
GGGCTCTTGCGCGTCGAAGGGAACACTACGCCGAAACGGGCAAGACCCTGGGGT
ACAACGCTCAGGCGGGAGAGTTGACGGCCCTGAAGAACCAGGAGGAAACCTCCT
GGCTGAAGGAATCGGACAGCCAGCTTCTCCAGCAGGCCCTCAAGGACGTGGAGC
GGGCCTTCGTCAACTTCTTTGAGAAGCGGGCGAGGTTCCCCCGGTTCAAGAGCAA
AAAGACGGATACTCCGCGCTTCCGTATTCCCCAGCGGGTGCGGATAGAGGGGAG
CCGTGTGTATGTCCCGAAGGTGGGATGGGTTAAGCTCCGCAAGTCTCAGGAGAT
AGAGGGCAAGACCAAGAGCGCGACGTTCAAGCGGGAGGCAGACGGTCACTGGT
ACGTCTTGCTCGTCTCCGAGTTTGAGATGCCCGATGTACCGCTGCCCCCCGTCCCT
GAGTCCGAGGTGGTCGGGATTGACCTCGGCCTGAAGGATTTCTACGTGTTGTCCG

ACGGCGGGCGGAAAGAGGCCCCGAGGTTTGCCCGCAAGGGGCAGCGGAAACTC
CGCCGCGCTGCCCGTCGTCACTCCAAATGCACCAGGGGGAGCAACCGCAAGGCG
AAGGCCAAGCGGAAGCTCGCCCGTGTTCACCGCCAGATTGCGAATCAGAGAAAG
GACTTCGTTCACAAGGCCACCTCCGGTCTTGTTCAGCAGTACCAGGGTTTCTGCA
TCGAGAACTTGAGCATCAAGGGGATGGCGAAAACCAAGCTGTCCAAGAGCGTTC
TCGACGCCGCCCTGGGAGAGTTTCGCCGCCAGCTCGCCTACAAGGCCCAGTGGC
ACCGGAAGTGGCTGGCGGTCATAGACCGCTGGTTTCCGTCCAGCAAGCTGTGTG
GGGAATGCGGCAGCATCAACGCAGACCTGACCCTGAGTGACCGGGAATGGACGT
GCGAATGTGGAGCGGTTCACGACCGCGACCTCAACGCCGCCCGGAACATCAAGC
GGGAAGGGCTTTCGCAAATCGTCGTCGCGGGGCACGCGGAGACGTTAAACGCTC
GGGGAGAGGGTGTCAGACCTGCGATAGCGGGCAGCCCTCGATGAAGCGAGAATC
CAACGGCTTTAGCCGTTGGAGTGTCAA

[00370] ISDra2 TnpB with ωRNA overlap native DNA sequence (single) (SEQ ID NO: 143)

[00371] ATGATAAGGAATAAGGCTTTCGTGGTCAGGCTGTACCCAAATGCGGCT
CAGACTGAACTGATTAACCGCACGCTGGGTAGCGCAAGGTTCGTCTACAACCAC
TTCCTTGCCCGTCGCATTGCGGCCTACAAGGAAAGCGGGAAGGGACTGACCTAC
GGGCAAACGAGTAGCGAACTGACCCTTCTGAAGCAGGCTGAAGAAACCTCCTGG
CTCTCGGAAGTAGATAAGTTTGCTTTGCAGAACTCGCTGAAAAACCTTGAaACCG
CGTACAAGAACTTCTTTCGGACTGTGAAGCAGTCCGGTAAAAAGGTAGGATTCC
CACGTTTCAGAAAGAAGCGCACGGGAGAGTCCTACCGGACTCAATTCACCAACA
ACAACATCCAAATTGGGGAAGGTAGGCTCAAACTTCCTAAGCTGGGATGGGTGA
AAACCAAGGGCCAGCAGGATATTCAAGGGAAGATTCTGAATGTCACTGTGCGCC
GTATTCACGAAGGCCATTACGAAGCGTCCGTTCTCTGTGAAGTCGAGATTCCCTA
CCTGCCTGCGGCTCCCAAGTTTGCAGCGGGTGTGGATGTCGGCATCAAGGATTTT
GCCATCGTGACCGATGGCGTGAGGTTTAAGCATGAACAGAATCCGAAATATTAC
CGCTCCACCCTGAAAAGACTTCGTAAAGCTCAGCAAACCCTGTCCAGACGGAAG
AAGGGCAGCGCACGTTACGGGAAAGCGAAAACCAAGCTGGCTCGGATTCACAA
GCGCATTGTCAATAAGCGTCAGGATTTCCTTCACAAGCTCACCACCTCCCTGGTG
CGTGAGTACGAAATCATCGGAACCGAACACCTTAAACCCGACAACATGCGGAAA
AATCGCCGCCTTGCACTGAGCATCAGTGATGCGGGCTGGGGTGAGTTCATCCGGC
AGTTGGAATACAAGGCAGCGTGGTACGGGCGACTGGTATCTAAAGTCAGCCCCT

ACTTTCCATCTAGCCAGTTGTGTCATGACTGCGgattcaagaatcccgaagtgaagaatcttgccgtcc
gtacatggacttgcccgaactgtggggaaacccatgaccgagaCgagaacgctgcgctgaacattcggcgtgaagcgttggtggct
gcgggaatctcagacaccttaaacgctcatggaggctatgtcagacctgcttcggcgggcaatggtctgcgaagtgagaatcacgcg
actttagtcgtgtgaGGTTCAA

[00372] ISYmu1 TnpB with wRNA overlap Arabidopsis codon optimized DNA sequence (single) (SEQ ID NO: 144)

[00373] TTGTTACAGCACAAgGCATAcGAgTAcAGGATATAcCCGGATAAGAAgC
AGGAgACCTTGATAGCTAAgACCATCGGATCAAGTAGGTAcGTCTAcAACCATTTc
CTGGAgCTTTGGAATAAgGAGTACGAGGAGACCGGAAAgGGGTTAACGTACTACG
CTTGTAGCAAgCTGCTCACCAAGCTCAAGCGAGACCCCGAGACCGTATGGTTGTG
CGAgGTGGATAAgTTcTCTCTGCAgAATAGTCTGCGTAATCTATCAGATGCTTTcTC
TAGGTTCTTCAAGGGTCAGAACGAGCACCCCCAGTTCAAGTCAAAGAAgAGTCCC
AGACAGTCCTACACAACACAGTACACAAACAACAATATcGCAGTGTCTGGCAAC
TGTCTCAAgTTGCCTAAGCTTGGATTAGTCAAGTTcGCTGATAGCCGTGAgATGAA
gGGGAGGATTCTCAACGCAACAGTTCGTCGTAAGAGTAGTGGGAAGTTcTTcGTA
AGCATACTCTGCGAgGAGGAGATcTGTGAgCTACCGAAgACAGACAGTTCAGTCG
GAATcGATCTAGGGATcATCGATTTcGCTGTAATGTCCGACGGGAGCAGACACGA
TAACAAcCATTTcACCAGGCAGATGGAGGAgCGTCTACGAAGGGAGCAgAGAAAg
CTTGCGAGGAGGGCGTTGGCGGCTGAgAAgAGAGGCATcTCACTAAGTGAGGCAC
GTAACTAcCAGAAgCAGCGTAGGAAgGTGGCTCGTCTATAcGAGAAGGTAGCGAA
CCAgAGGAAGGAGTAcCTTAATAAGCTTAGTACAGAGATCGTAAAGAACCACGA
TATCATcTGCATAGAgGACTTGAAcGTCAAGGGAATGATGAGGAACCACAAGTTG
GCAAAgTCTATCAGTGACGTGAGCTGGACCTCCCTAGTTTCTAAGCTTCAgTACAA
GGCAAGCTGGTAcGGTAAGGAgGTTATACGAATATCTCGATGGTTcCCGAGTTCTC
AgATcTGTAGTGAGTGTGGGCATAAgGACCGAAAGAAgCCTTTGCATGTAAGGGA
GTGGACCTGCCCAGTGTGCCACGCACACCACGATCGAGACGTCAATGCAGCAAG
AAATATACTGGCTGAGGGACTCAGGATAAGAGCTCTGACTCCGGGGTCTTAG

[00374] ISAam1 TnpB with wRNA overlap Arabidopsis codon optimized DNA sequence (single) (SEQ ID NO: 145)

[00375] ATGGTAAAcAAGAGTTAcAAGTTCCGACTGTAcCCTACGAAgGAgCAAG
AgCAGTTGCTAGCGAAgACTTTCGGTTGTGTTCGTTTCGTTTAcAAcAAGATGCTGG

AGGAGAGGATcCAgATCTACGAgAAgTTCAAGGATGATAAGGAgGCCCTCAAgAA
GCAgACATTCCCGACTCCTGCGAAGTAcAAgAAgGAgTTTCCATGGTTAAAgGAgG
TGGACTCATTGGCTCTCGCTAACGCACAGTTAAAcCTCCAGAAgGCGTTcCAGAAC
TTcTTcTCAGGTAGAGCTGGCTTcCCCAAGTTCAAgAACCGTAAgGCTAAGCAgAGC
TAcACAACCAAcGTCGTGAACGGCAAcATcCAgTTGTCAGATGGGTAcATCAAgCTC
CCTAAGCTCAAGTGGGTCAAgTTcAAgCAGCACCGTGAgATcCCTGCACATCACAT
CATCAAGGCTTGTACCATcAAGAAGACAAAGACGGGTAAGTAcTACGTAAGCATc
CTTACAGAGTACGAgCATCAgCCGGTGCCTAAgGAgATcCAgACCGTTGTGGGTCT
TGACTTCTCTATGAAcGGTTTATTcGTGGACTCAGAGGGAAAGCGTGCAAAcTACC
CGCGATTcTACCGACAGGCATTAGAgAAgTTAGCGAAgGAGCAgCGTATcCTGAGC
CGAAGAAAgAAgGGCAGCAAcCGATGGCACAAgCAgCGATTGAAGGTCGCAAAgC
TGCACGAgAAGATcGCTAAcCAgCGTAAgGACTTCTTGCATAAgAAgAGTTACGAgT
TGGCTAAGCAGTACGATTGTGTGGTGATcGAGAACCTTAACATGAAGGGCATGTC
CCAgGTGTTAAACTTcGGGAAGAGTGTTCACGACAAcGGCTGGGGGATGTTCACG
ACTTTcCTGCAGTACAAgTTAGAgGAgCAgGGCAAgAAGCTAATcAAgATcGACAAg
TGGTTCCCAAGTTCCAAgACCTGCAGTTGCTGCGATCAgGTAAAGGAGTCTCTAA
GTTTATCCGAAAGGACATTCCGTTGTGAATGTGGATTCGAGAGCGACAGGGACG
TCAATGCGGCAATCAATATCAAACATGAGGGCATGAAACGATTAGCAATAGCCT
AA

[00376] ISDge10 TnpB with wRNA overlap Arabidopsis codon optimized DNA sequence (single) (SEQ ID NO: 146)

[00377] ATGACGACTCATCGAAAGGTTTACCGTTATAGGATCGAGCCCACGCCT
GTTCAAGAGAGTAAgCTTTACATGCTAGCCGGGAGTAGGAGGTTTGTCTTCAACT
GGGCTCTAGCCAGGAGGAGGGAACATTATGCAGAAACAGGCAAGACATTGGGTT
ACAATGCACAAGCTGGAGAGCTCACCGCACTCAAGAATCAGGAGGAGACATCCT
GGCTCAAgGAATCTGACTCCCAGCTATTACAACAGGCACTTAAGGATGTAGAAA
GGGCTTTCGTCAATTTcTTCGAGAAgAGGGCTAGGTTCCCACGTTTcAAgTCCAAG
AAGACTGACACCCCGAGGTTcCGAATACCTCAACGAGTACGAATTGAGGGAAGC
CGTGTGTACGTCCCAAAGGTGGGTTGGGTAAAGTTGAGAAAGAGTCAGGAGATT
GAGGGGAAGACAAAGTCTGCGACGTTcAAgAGAGAAGCCGACGGGCATTGGTAT
GTCTTACTGGTGTCAGAATTcGAgATGCCGGACGTTCCTTTGCCTCCCGTCCCGGA
GAGTGAAGTAGTAGGGATAGACTTGGGCTTGAAGGACTTCTACGTTCTTTCTGAT

GGTGGTCGAAAGGAGGCTCCTCGATTcGCACGAAAGGGGCAAAGAAAgCTAAGA
CGAGCGGCACGTAGGCACTCAAAgTGCACTAGGGGATCTAATAGAAAGGCAAAG
GCCAAGAGGAAgCTAGCCCGTGTCCATCGTCAGATTGCCAACCAACGAAAGGAT
TTcGTCCACAAGGCGACGAGTGGATTAGTACAACAGTATCAAGGCTTCTGTATCG
AGAATCTTTCCATTAAgGGGATGGCAAAGACAAAGCTGTCTAAGAGTGTGTTAGA
TGCCGCTCTCGGAGAGTTcCGTAGGCAGTTGGCATATAAgGCGCAGTGGCACAGG
AAGTGGCTTGCAGTGATAGATCGTTGGTTCCCTTCCTCCAAgTTGTGTGGTGAATG
TGGAAGTATCAACGCAGATTTGACGCTGTCCGACCGGGAATGGACGTGCGAATG
TGGAGCGGTTCACGACCGCGACCTCAACGCCGCCCGGAACATCAAGCGGGAAGG
GCTTTCGCAAATCGTCGTCGCGGGGCACGCGGAGACGTTAAACGCTCGGGGAGA
GGGTGTCAGACCTGCGATAGCGGGCAGCCCTCGATGAAGCGAGAATCCAACGGC
TTTAGCCGTTGGAGTGTCAA

[00378] ISDra2 TnpB with wRNA overlap Arabidopsis codon optimized DNA sequence (single) (SEQ ID NO: 147)

[00379] ATGATAAGGAATAAGGCTTTcGTAGTCAGACTCTACCCAAACGCGGCG
CAGACGGAGTTGATTAATCGAACATTAGGAAGTGCCCGTTTCGTTTACAACCATT
TcCTTGCGAGAAGAATCGCTGCCTACAAGGAgTCAGGGAAGGGTCTcACGTATGG
TCAgACAAGTTCTGAGTTgACACTCTTgAAGCAgGCTGAgGAGACGAGTTGGCTTT
CCGAGGTTGATAAgTTCGCATTGCAgAACAGTCTCAAgAACCTAGAGACGGCTTA
CAAGAACTTCTTCAGAACAGTTAAgCAgTCCGGGAAgAAgGTCGGGTTcCCGAGGT
TCCGTAAgAAgCGTACTGGCGAGTCATACAGGACACAGTTCACCAACAACAAcAT
TCAGATAGGCGAGGGAAGActtAAgCTcCCTAAgTTGGGGTGGGTGAAGACCAAGG
GTCAgCAGGACATTCAgGGTAAGATTCTcAACGTTACTGTAAGGAGAATACATGA
AGGCCACTATGAGGCATCTGTACTCTGTGAGGTTGAGATACCTTATTTGCCCGCC
GCGCCGAAgTTcGCGGCTGGCGTTGACGTAGGCATAAAgGATTTCGCTATAGTCA
CGGATGGAGTAAGGTTCAAGCACGAGCAgAACCCCAAgTACTATCGTTCCACCCT
CAAGCGTCTCAGAAAGGCACAGCAgACCttgTCCCGTCGAAAGAAgGGTAGTGCAC
GATATGGCAAGGCAAAGACGAAgCTcGCAAGGATTCACAAgAGGATCGTGAACA
AGAGGCAGGACTTcTTGCATAAgTTGACAACGAGCCTcGTTCGAGAATACGAAAT
CATTGGTACAGAACACcttAAgCCTGATAATATGAGGAAGAACAGACGACTCGCCC
TCAGCATCAGCGACGCGGGTTGGGGCGAGTTcATCCGTCAGCTGGAATATAAGGC
TGCTTGGTACGGCAGACTTGTGTCTAAGGTCTCACCGTAcTTCCCGAGTAGTCAGT

TGTGCCATGACTGCGgattcaagaatcccgaagtgaagaatcttgccgtccgtacatggacttgcccgaactgtgggga aacccatgaccgagaCgagaacgctgcgctgaacattcggcgtgaagcgttggtggctgcgggaatctcagacaccttaaacgctc atggaggctatgtcagacctgcttcggcgggcaatggtctgcgaagtgagaatcacgcgactttagtcgtgtgaGGTTCAA

[00380] tRNA-Ile (SEQ ID NO: 148)

[00381] CCGTAGCTCAGTTGGTTAGAGCGTTGGTCTTATGAGCCGAAGGTCGCG GGTTCGAGCCCCGCCGGAAGCA

[00382] pPEBV (SEQ ID NO: 149)

[00383] GCACACAAGGTTAAAAACGCTGTAGTAATACATGCGCAAGAACAGGC TGAGCATCTTGTTCTGGGGTTTCACACTATCTTTAGAGAAAGTGTTAAGTTAATTA AGTTATCTTAATTAAGAGCATAATTATACTGATTTGTCTCTCGTTGATAGAGTCTA TCATTCTGTTATTAAAAATTTGACAACTCGGTTTGCTGACCTACTGGTCACTGTAT CACTTACCCGAGTTAACGAG

[00384] TnpB30 amino acid sequence (SEQ ID NO: 702)

[00385] MKRAYLIEIKPTTEQVSKIRQTIGVCRYVYNLYISKNKEAYESDGKFLSGY DFSKWLNNIHTKECDQWIKEVSSKAVKQAIMNGDKAFKRFFKGLSKFPRYKKKKKQ DVKCYFPKNNKTDWTVERHRVKVPTIGWIRLKEYGYISKNTTVKSGTVYQRAGRYY VSILCEVKEVKPNSILNAVGIGIDLGIKDFAVRSDGKTHKNINKTVQVKKIEKRLKRE QRSLSRKFENIRKRGEQPVTNKRANINKNILRVQQLHARLANIRLAYVKSIVNDVVK TKPMFITVEDLNVKGMMKNKHLSKAVAQQCFYAFKTWLSTKCREYGIELRQVDRF YPSSKICSCCGQKKSDLKLSDRVYTCDCGNVMDRDLNASINLLQAKKYTILT

[00386] In the foregoing description, it will be readily apparent to one skilled in the art that varying substitutions and modifications may be made to the invention disclosed herein without departing from the scope and spirit of the invention. The invention illustratively described herein suitably may be practiced in the absence of any element or elements, limitation or limitations which is not specifically disclosed herein. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention. Thus, it should be understood that although the present

invention has been illustrated by specific embodiments and optional features, modification and/or variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention.

[00387] All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention.

[00388] Citations to a number of patent and non-patent references are made herein. The cited references are incorporated by reference herein in their entireties. In the event that there is an inconsistency between a definition of a term in the specification as compared to a definition of the term in a cited reference, the term should be interpreted based on the definition in the specification.

CLAIMS

1. A composition, comprising:

a TnpB bacterial enzyme; and

a polynucleotide comprising a first portion adapted to bind to at least a portion of the TnpB bacterial enzyme and a second portion for binding to a plant genomic target.

2. The composition of claim 1, wherein the polynucleotide is RNA.

3. The composition of claim 1 or 2, wherein the TnpB bacterial enzyme comprises less than about 500 amino acids, less than about 450 amino acids, less than about 420 amino acids, or less than about 400 amino acids.

4. The composition of any one of claims 1-3, wherein the TnpB bacterial enzyme is from, or derived from, tnpB from *Brevibacillus agri*.

5. The composition of any one of claims 1-3, wherein the TnpB bacterial enzyme comprises ISBag01, ISYMu1, or TnpB30.

6. The composition of any one of claims 1-5, wherein the TnpB bacterial enzyme comprises a peptide tag.

7. The composition of claim 6, wherein the peptide tag comprises a FLAG tag.

8. A construct comprising a plant promotor operably connected to a polynucleotide encoding for a TnpB bacterial enzyme.

9. The construct of claim 8, wherein the polynucleotide further encodes for an ωRNA having a first portion adapted to bind to at least a portion of the TnpB bacterial enzyme and a second portion for binding to a plant genomic target.

10. The construct of claim 9, wherein the ωRNA is about 100-400 nucleotides in length.

11. The construct of any one of claims 8-10, wherein the polynucleotide encoding for a TnpB bacterial enzyme is codon optimized for expression in plant cells.

12. The construct of any one of claims 8-11, wherein the plant promotor comprises a UBQ10 gene promoter.

13. The construct of any one of claims 9-12, wherein a coding sequence of the ωRNA and a coding sequence of the TnpB bacterial enzyme at least partly overlap.

14. The construct of any one of claims 8-13, wherein the TnpB bacterial enzyme comprises less than about 500 amino acids, less than about 450 amino acids, less than about 420 amino acids, or less than about 400 amino acids.

15. The construct of any one of claims 8-14, wherein the TnpB bacterial enzyme is from, or derived from, tnpB from *Brevibacillus agri*.

16. The construct of any one of claims 8-14, wherein the TnpB bacterial enzyme comprises ISBag01, ISYMu1, or TnpB30.

17. The construct of any one of claims 8-16, wherein the TnpB bacterial enzyme comprises a peptide tag.

18. The construct of claim 17, wherein the peptide tag comprises a FLAG tag.

19. A plant viral vector comprising the construct of any one of claims 8-18, 26, 27, 29, 31, or 32.

20. The plant viral vector of claim 19, wherein the plant viral vector is derived from the *Tobacco rattle virus* (TRV).

21. A plant cell comprising the construct of any one of claims 8-18, 26, 27, 29, 31, or 32 or the plant viral vector of claims 19 or 20.

22. A method for gene editing a plant, comprising:

introducing the construct of any one of claims 8-18, 26, 27, 29, 31, or 32 and the plant viral vector of claims 19 or 20 into one or more plant cells.

23. The method of claim 22, wherein the one or more plant cells are *Arabidopsis* cells.

24. The composition of any one of claims 1-4, 6, and 7, wherein the TnpB bacterial enzyme comprises an amino acid sequence that is 70 % or more, 75 % or more, 80 % or more, 85 % or more, 90 % or more, 95 % or more, or 99 % or more identical to the amino acid sequence of SEQ ID NO: 5.

25. The composition of any one of claims 1-3 and 5-7, wherein the TnpB bacterial enzyme comprises an amino acid sequence that is 70 % or more, 75 % or more, 80 % or more, 85 % or more, 90 % or more, 95 % or more, or 99 % or more identical to the amino acid sequence of SEQ ID NO: 46.

26. The composition of any one of claims 1-3 and 5-7, wherein the TnpB bacterial enzyme comprises an amino acid sequence that is 70 % or more, 75 % or more, 80 % or more, 85 % or more, 90 % or more, 95 % or more, or 99 % or more identical to the amino acid sequence of SEQ ID NO: 702.

27. The construct of any one of claims 8-15, 17, and 18, wherein the polynucleotide encoding for the TnpB bacterial enzyme comprises a polynucleotide sequence that is 70 % or more, 75 % or more, 80 % or more, 85 % or more, 90 % or more, 95 % or more, or 99 % or more identical to the polynucleotide sequence of one or more of SEQ ID NOs: 1-4.

28. The construct of any one of claims 8-14 and 16-18, wherein the polynucleotide encoding for the TnpB bacterial enzyme comprises a polynucleotide sequence that is 70 % or more, 75 % or more, 80 % or more, 85 % or more, 90 % or more, 95 % or more, or 99 % more identical to the polynucleotide sequence of one or more of SEQ ID NOs: 34, 38, or 42.

29. The composition of any one of claims 1-7, 24, 25, and 26 wherein the polynucleotide is complementary to a sequence that is 70 % or more, 75 % or more, 80 % or more, 85 % or more, 90 % or more, 95 % or more, or 99 % or more identical to the polynucleotide sequence of one or more of SEQ ID NOs: 11-13.

30. The construct of any one of claims 9-18, 26, and 27, wherein the polynucleotide comprises a sequence that is 70 % or more, 75 % or more, 80 % or more, 85 % or more, 90 % or more, 95 % or more, or 99 % or more identical to the polynucleotide sequence of one or more of SEQ ID NOs: 11-13.

31. The construct of any one of claims 8-14, 17, or 18 wherein the polynucleotide encoding for the TnpB bacterial enzyme comprises a polynucleotide sequence that is 70 % or more, 75 % or more, 80 % or more, 85 % or more, 90 % or more, 95 % or more, or 99 % or more identical to the polynucleotide sequence of one or more of SEQ ID NOs: 34-45.

32. The construct of claim 13, wherein the polynucleotide encoding for the TnpB bacterial enzyme and the ωRNA comprises a polynucleotide sequence that is 70 % or more, 75 % or more, 80 % or more, 85 % or more, 90 % or more, 95 % or more, or 99 % or more identical to the polynucleotide sequence of one or more of SEQ ID NOs: 140-147, 150-170, 431-433, or 475-481.

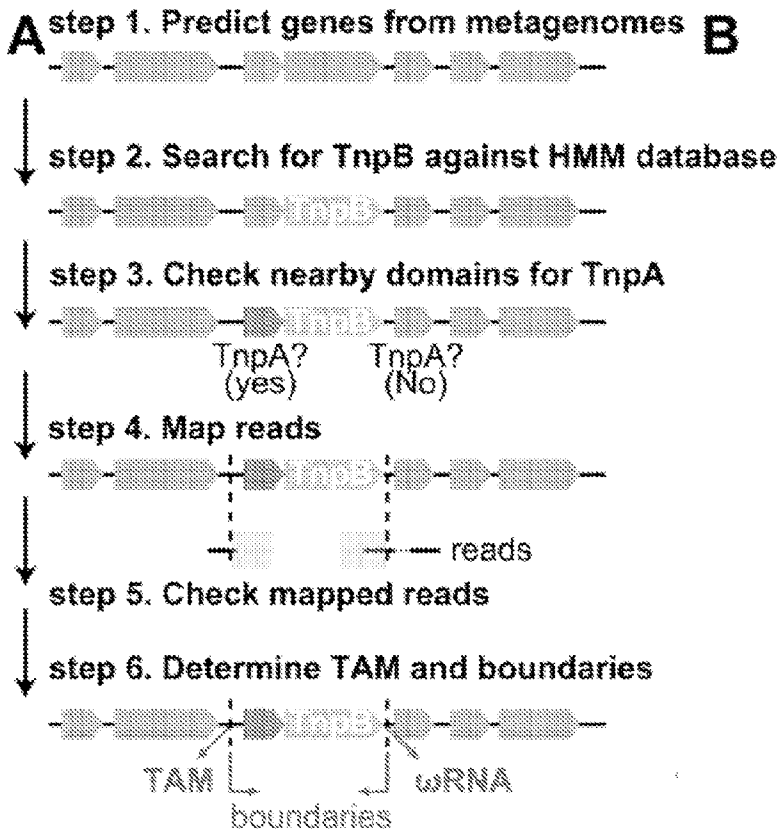

A
step 1. Predict genes from metagenomes
B
step 2. Search for TnpB against HMM database
step 3. Check nearby domains for TnpA
TnpA?
(yes)
TnpA?
(No)
step 4. Map reads
reads
step 5. Check mapped reads
step 6. Determine TAM and boundaries
TAM
boundaries
ωRNA

FIG. 1A

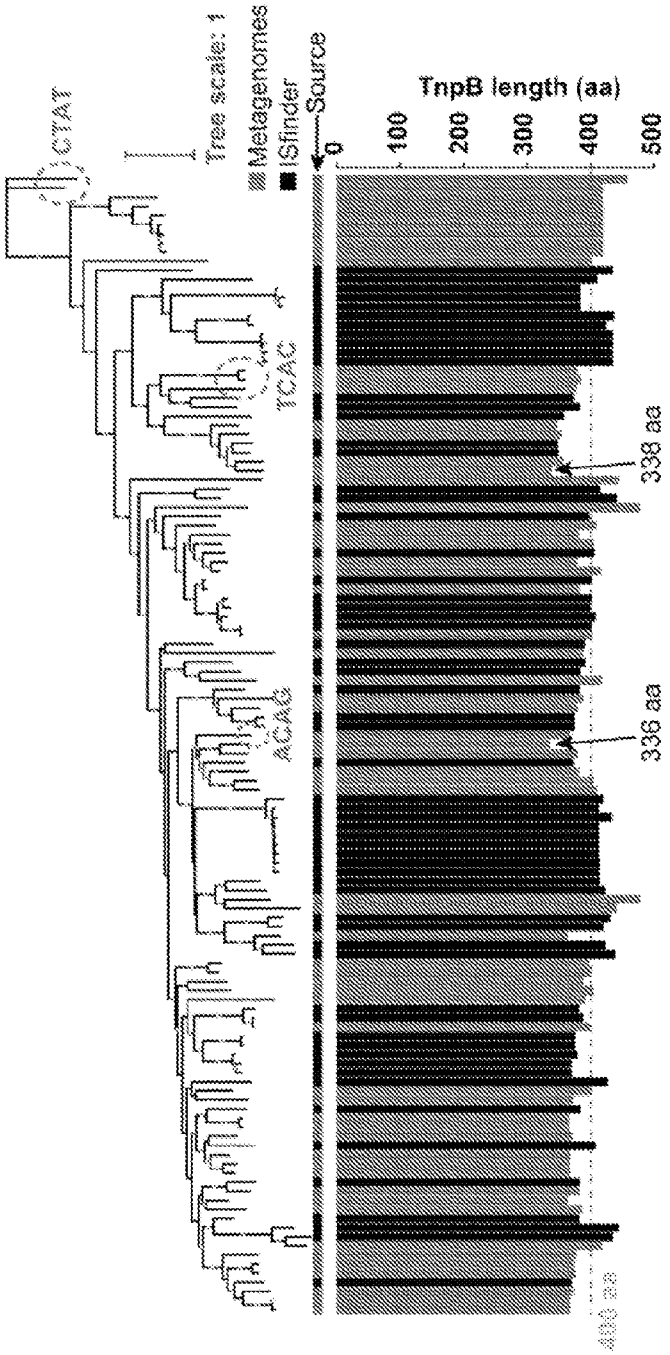

Tree scale: 1
Metagenomes
ISfinder
Source
TnpB length (aa)
0
100
200
300
400
500
CTAT
TCAC
ACAG
338 aa
336 aa
400 aa

FIG. 1B

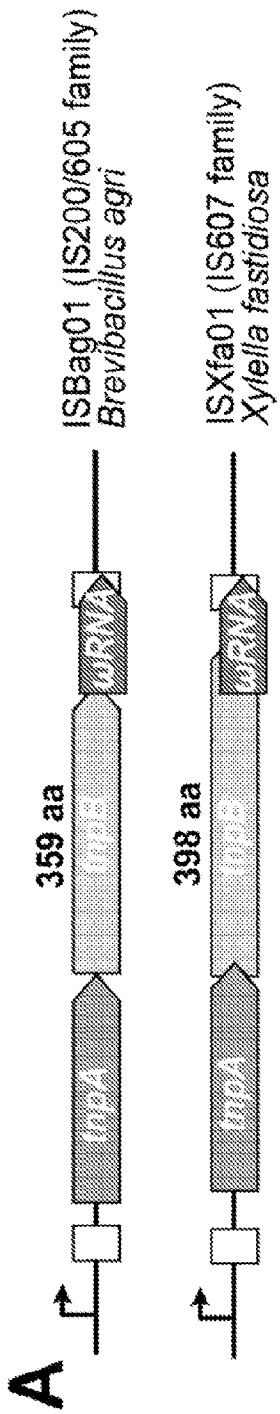

A
ISBag01 (IS200/605 family)
Brevibacillus agri
359 aa
tnpA
tnpB
tRNA
ISXfa01 (IS607 family)
Xylella fastidiosa
398 aa
tnpA
tnpB
tRNA

FIG. 2A

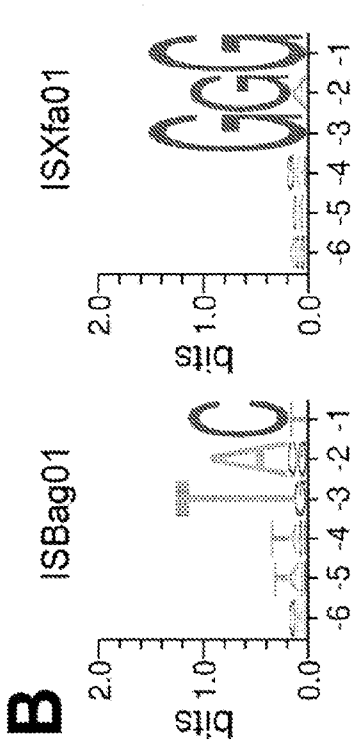

B
ISBag01
ISXfa01
bits
2.0
1.0
0.0
-6 -5 -4 -3 -2 -1

FIG. 2B

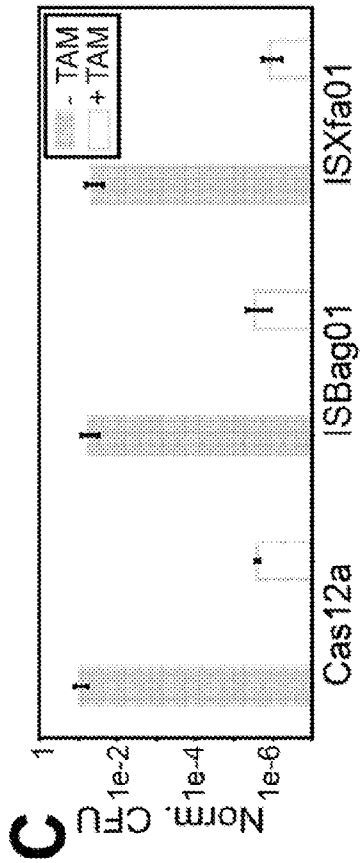

C
Norm. CFU
1
1e-2
1e-4
1e-6
- TAM
+ TAM
Cas12a
ISBag01
ISXfa01

FIG. 2C

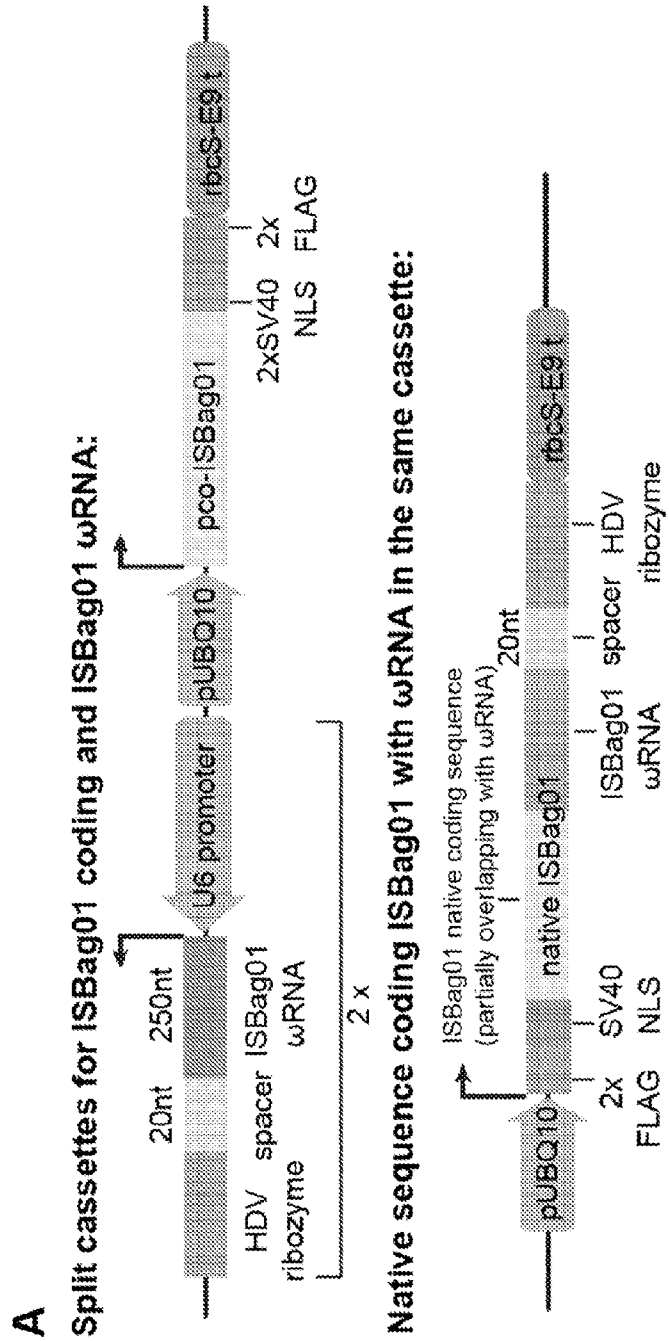

A
Split cassettes for ISBag01 coding and ISBag01 ωRNA:
20nt
250nt
U6 promoter
pUBQ10
pco-ISBag01
rbcS-E9 t
HDV ribozyme
spacer
ISBag01 ωRNA
2xSV40 NLS
2x FLAG
2 x
Native sequence coding ISBag01 with ωRNA in the same cassette:
ISBag01 native coding sequence (partially overlapping with ωRNA)
20nt
pUBQ10
2x FLAG
SV40 NLS
native ISBag01
ISBag01 ωRNA
spacer
HDV ribozyme
rbcS-E9 t

FIG. 3A

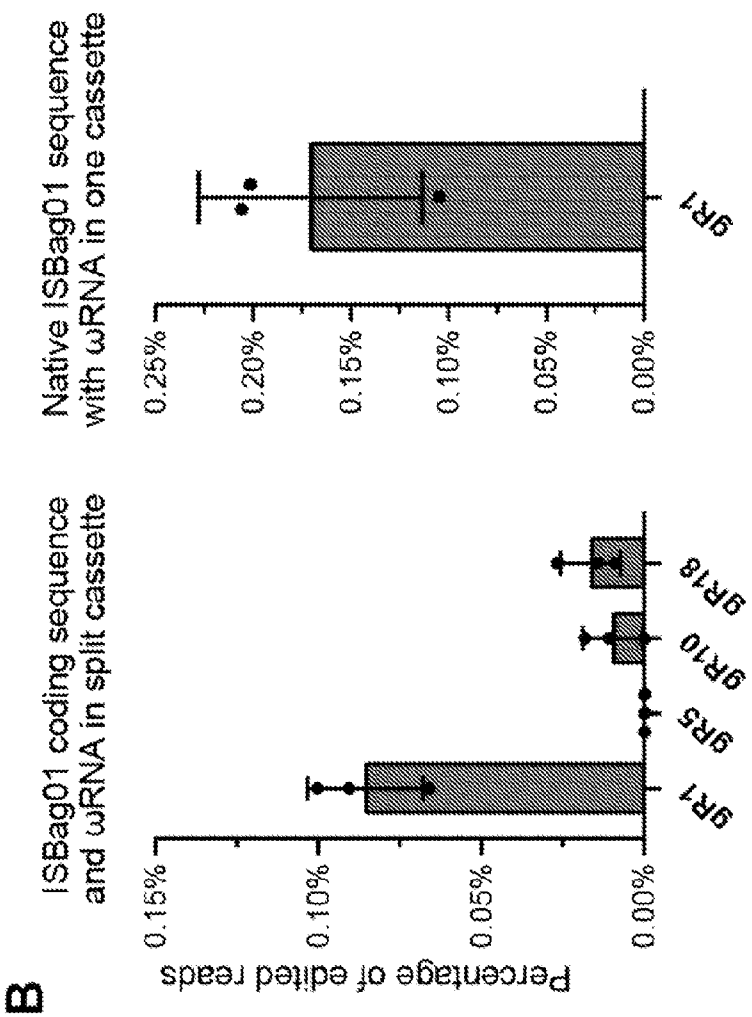

B
ISBag01 coding sequence and ωRNA in split cassette
Native ISBag01 sequence with ωRNA in one cassette
Percentage of edited reads
0.15%
0.10%
0.05%
0.00%
0.25%
0.20%
0.15%
0.10%
0.05%
0.00%
gR1
gR5
gR10
gR18
gR1

FIG. 3B

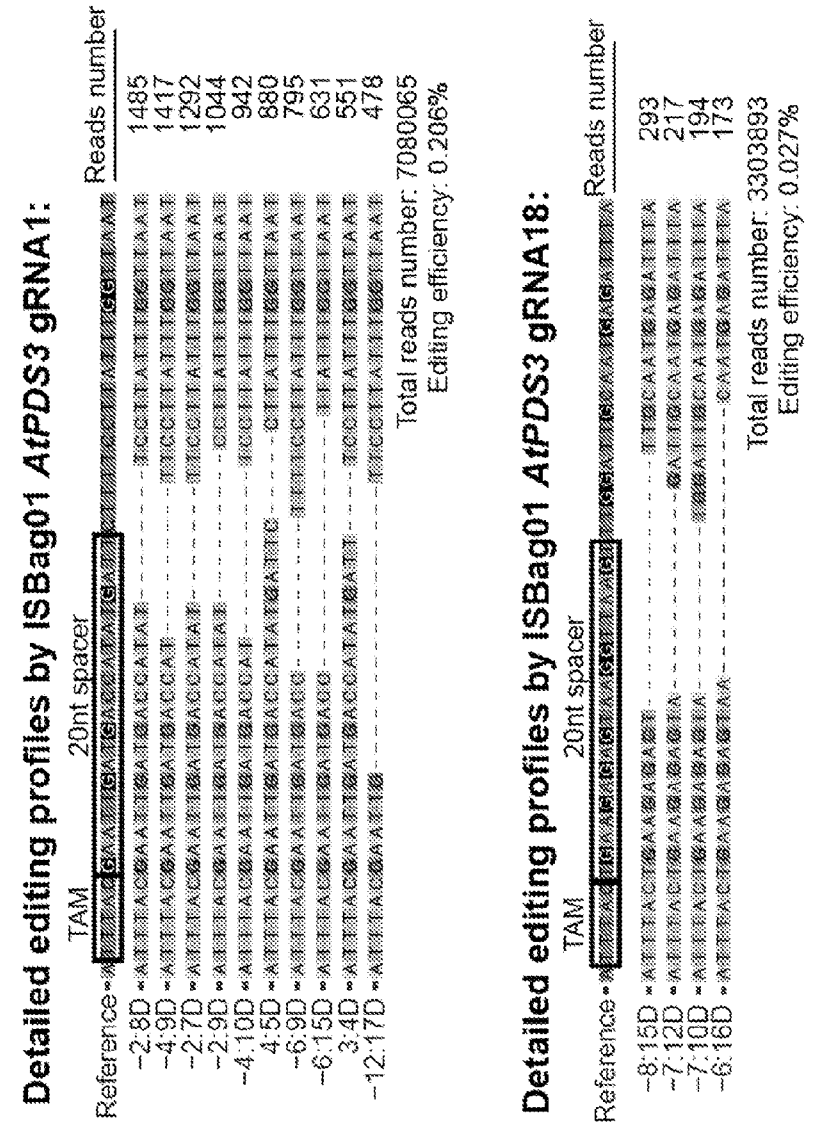

C
Detailed editing profiles by ISBag01 AtPDS3 gRNA1:
TAM
20nt spacer
Reads number
Reference
-2:8D
-4:9D
-2:7D
-2:9D
-4:10D
4:5D
-6:9D
-6:15D
3:4D
-12:17D
1485
1417
1292
1044
942
880
795
631
551
478
Total reads number: 7080065
Editing efficiency: 0.206%
Detailed editing profiles by ISBag01 AtPDS3 gRNA18:
TAM
20nt spacer
Reads number
Reference
-8:15D
-7:12D
-7:10D
-6:16D
293
217
194
173
Total reads number: 3303893
Editing efficiency: 0.027%

FIG. 3C

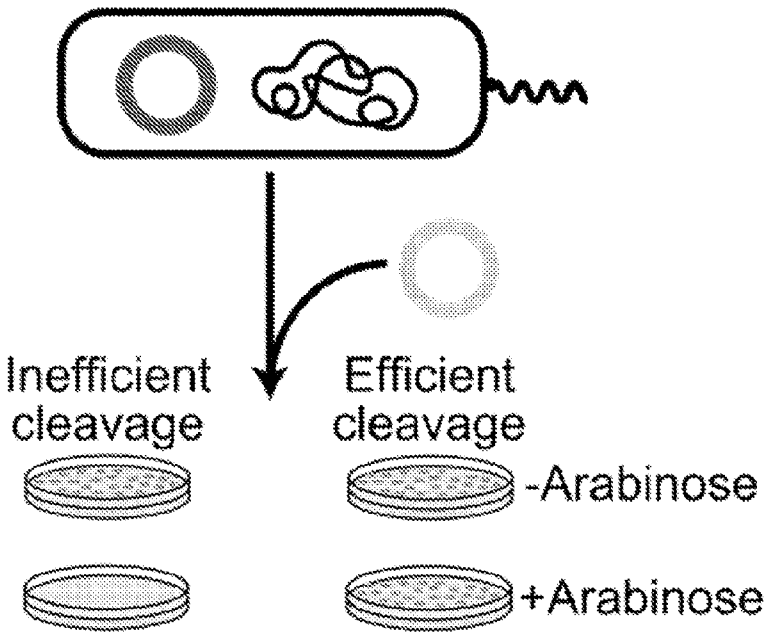

Inefficient cleavage
Efficient cleavage
-Arabinose
+Arabinose

FIG. 4A

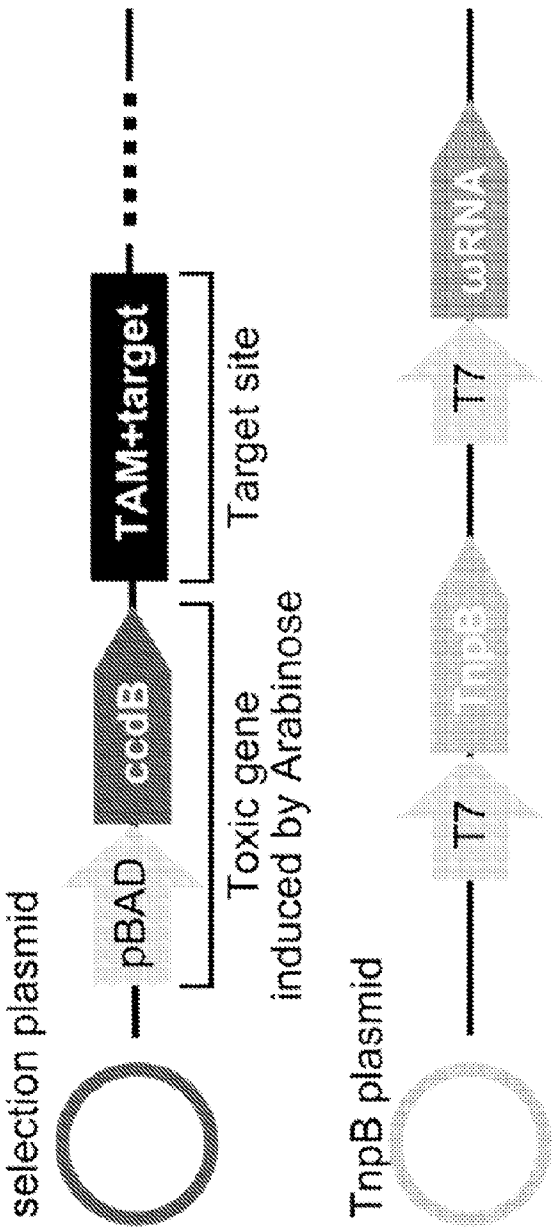

selection plasmid
pBAD
ccdB
TAM+target
Toxic gene
induced by Arabinose
Target site
TnpB plasmid
T7
TnpB
T7
gRNA

FIG. 4B

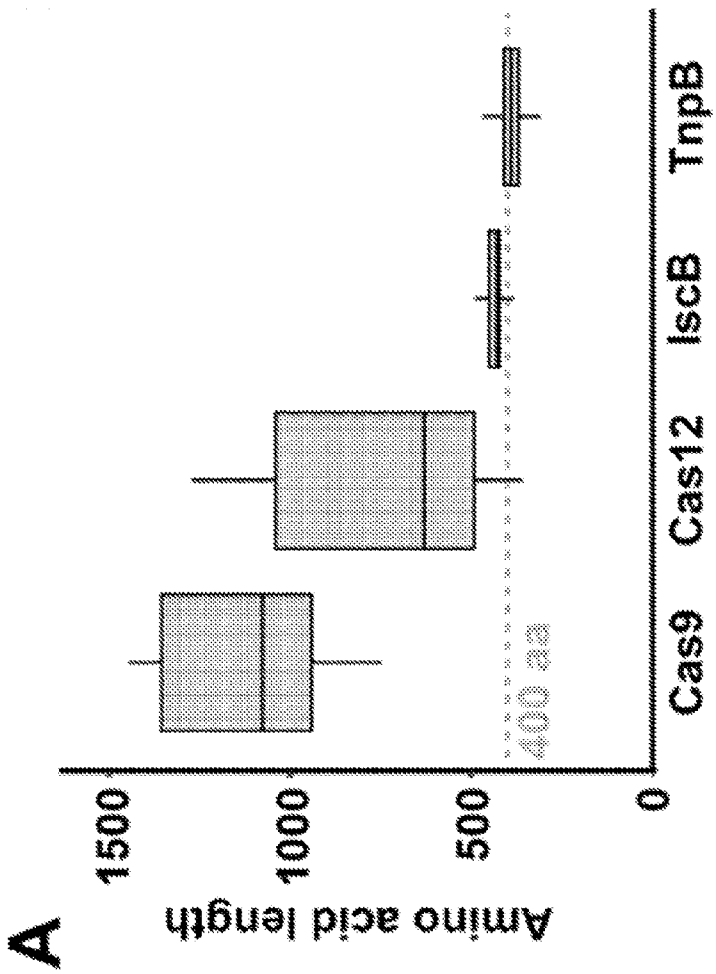

A
Amino acid length
1500
1000
500
0
400 aa
Cas9
Cas12
IscB
TnpB

FIG. 5A

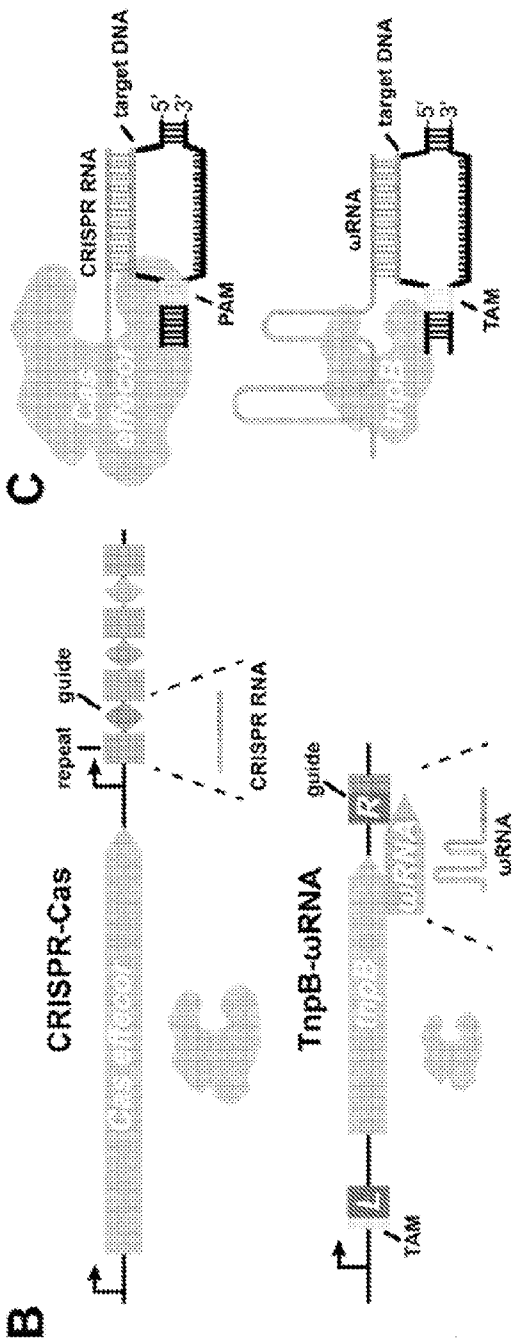

B
CRISPR-Cas
repeat
guide
CRISPR RNA
TnpB-ωRNA
guide
ωRNA
TAM
C
CRISPR RNA
target DNA
5'
3'
PAM
ωRNA
target DNA
5'
3'
TAM

FIG. 5B

FIG. 5C

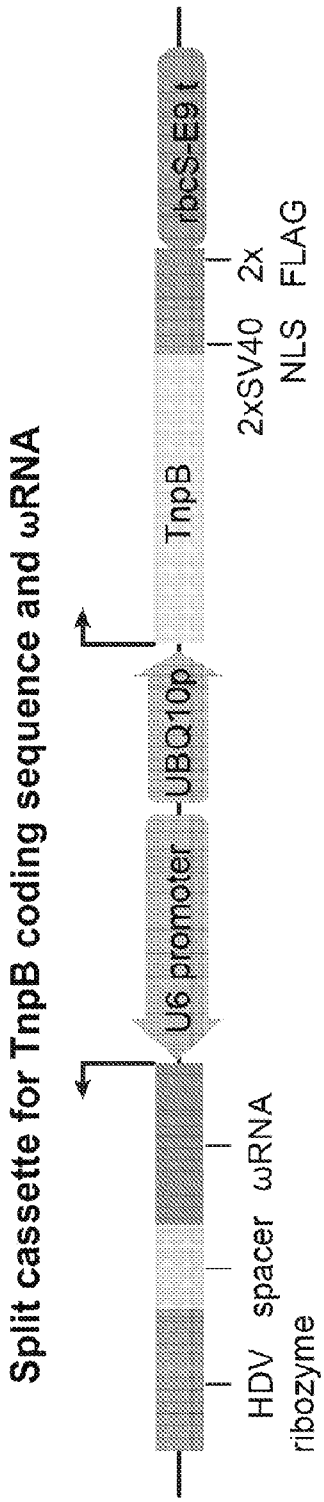

Split cassette for TnpB coding sequence and ωRNA
HDV ribozyme
spacer
ωRNA
U6 promoter
UBQ10p
TnpB
2xSV40 NLS
2x FLAG
rbcS-E9 t

FIG. 6

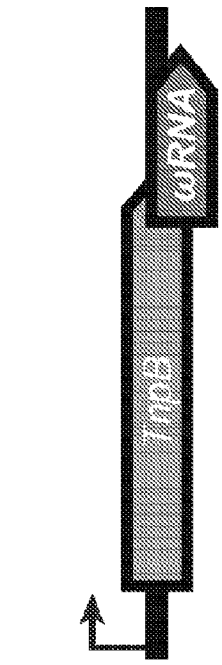

A
TnpB
ωRNA

FIG. 7A

B
Single cassette for TnpB coding sequence with ωRNA
TnpB
(partially overlapping with ωRNA)
UBQ10p
2x FLAG
SV40 NLS
TnpB
ωRNA spacer
HDV ribozyme
rbcS-E9 t

FIG. 7B

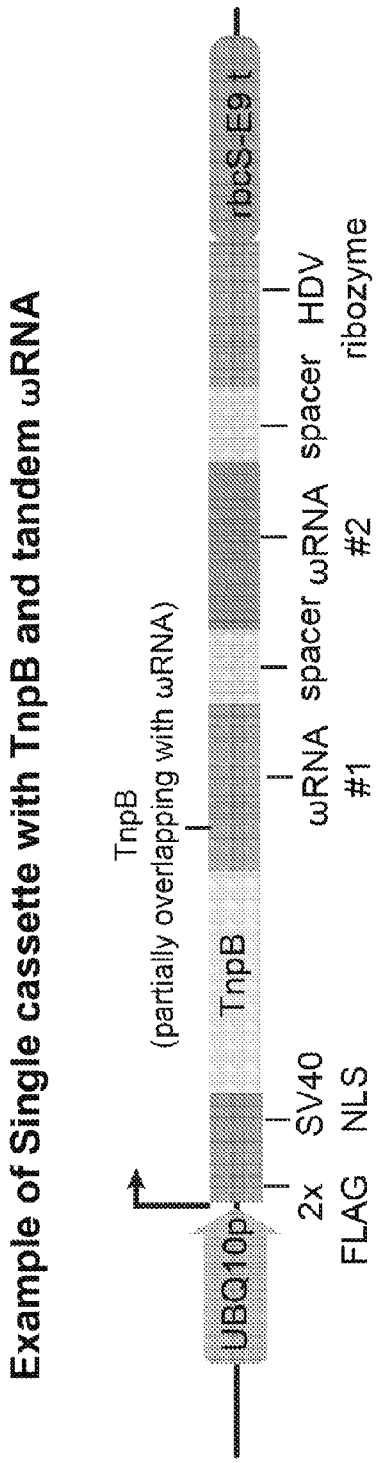

Example of Single cassette with TnpB and tandem ωRNA
UBQ10p
2x FLAG
SV40 NLS
TnpB
TnpB (partially overlapping with ωRNA)
ωRNA #1
spacer
ωRNA #2
spacer
HDV ribozyme
rbcS-E9 t

FIG. 8

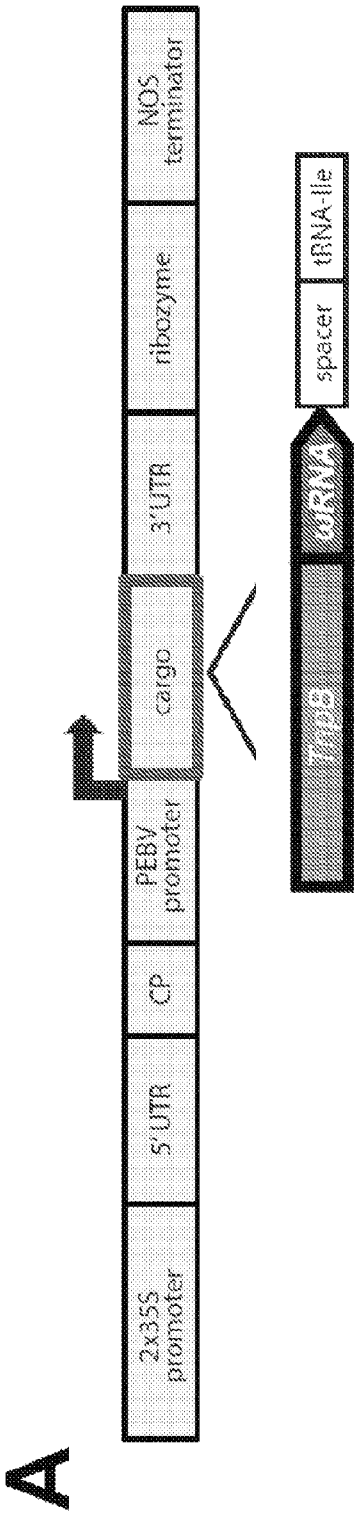

A
2x35S promoter
5'UTR
CP
PEBV promoter
cargo
3'UTR
ribozyme
NOS terminator
gRNA
spacer
tRNA-Ile

FIG. 9A

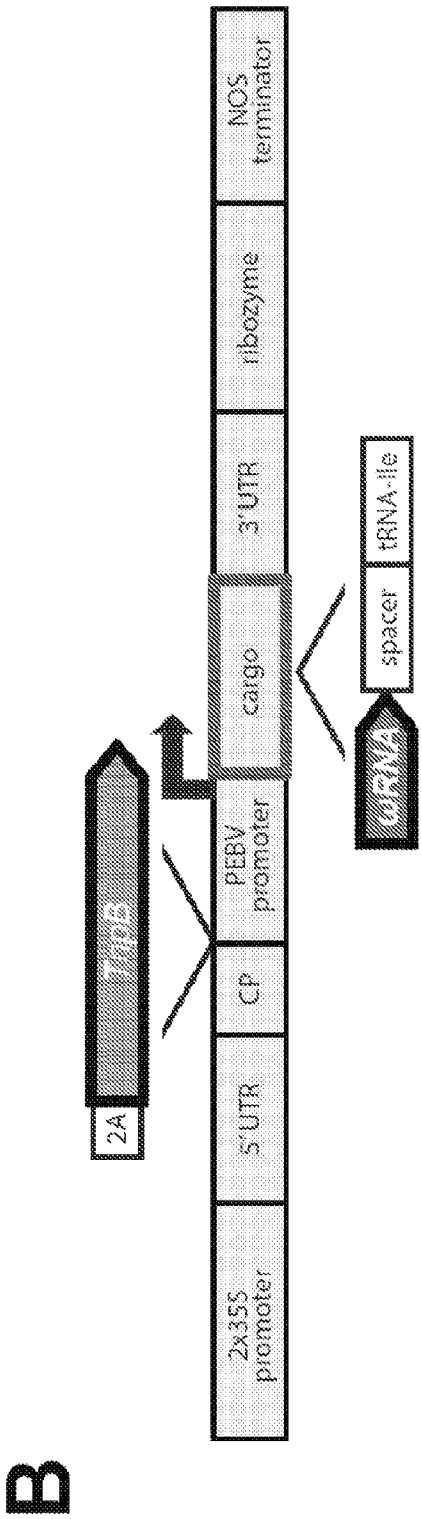

B
2x35S promoter
5'UTR
CP
PEBV promoter
2A
P19/B
cargo
3'UTR
ribozyme
NOS terminator
spacer
tRNA-Ile
gRNA

FIG. 9B

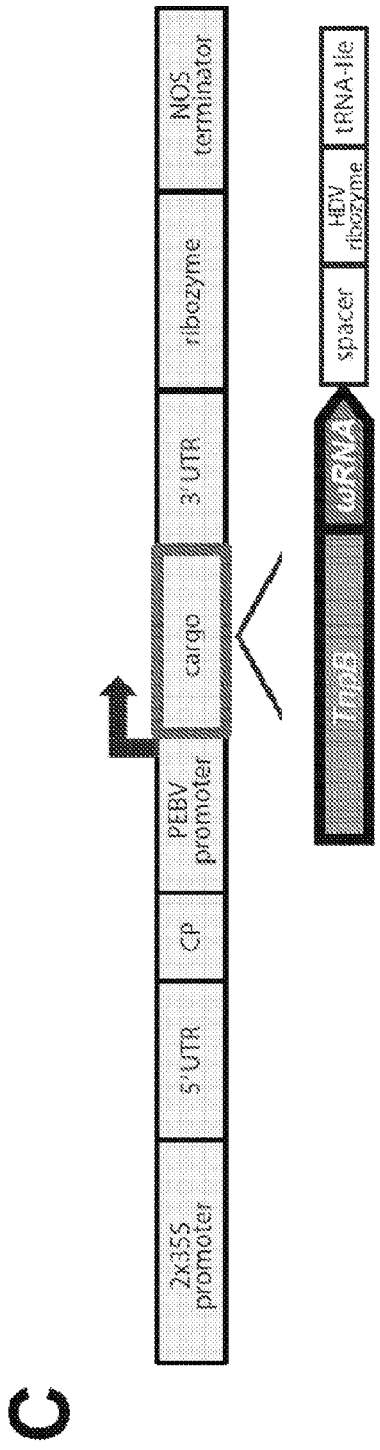

C
2x35S promoter
5'UTR
CP
PEBV promoter
cargo
3'UTR
ribozyme
NOS terminator
Virus
UTRNA
spacer
HDV ribozyme
tRNA-Ile

FIG. 9C

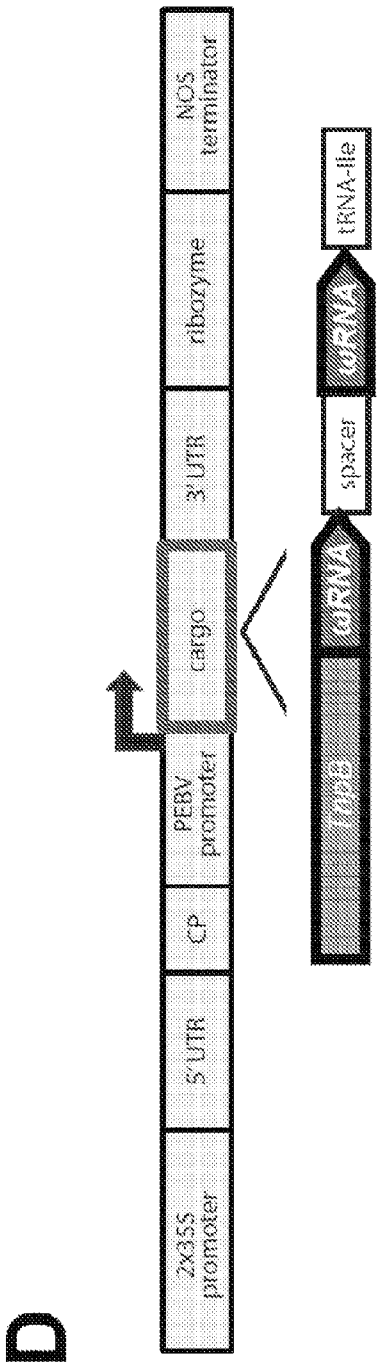

D
2x35S promoter
5'UTR
CP
PEBV promoter
cargo
3'UTR
ribozyme
NOS terminator
tRNA
spacer
gRNA
tRNA-Ile

FIG. 9D

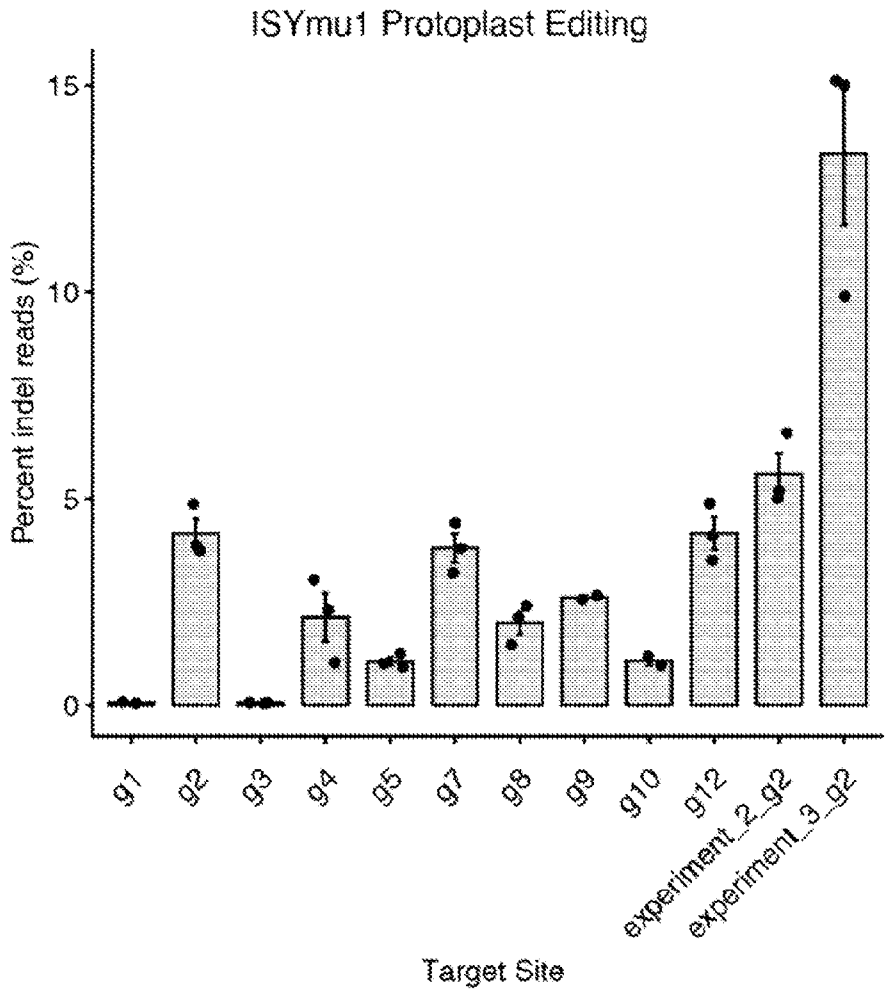

ISYmu1 Protoplast Editing
Percent indel reads (%)
15
10
5
0
g1
g2
g3
g4
g5
g7
g8
g9
g10
g12
experiment_2_g2
experiment_3_g2
Target Site

FIG. 10A

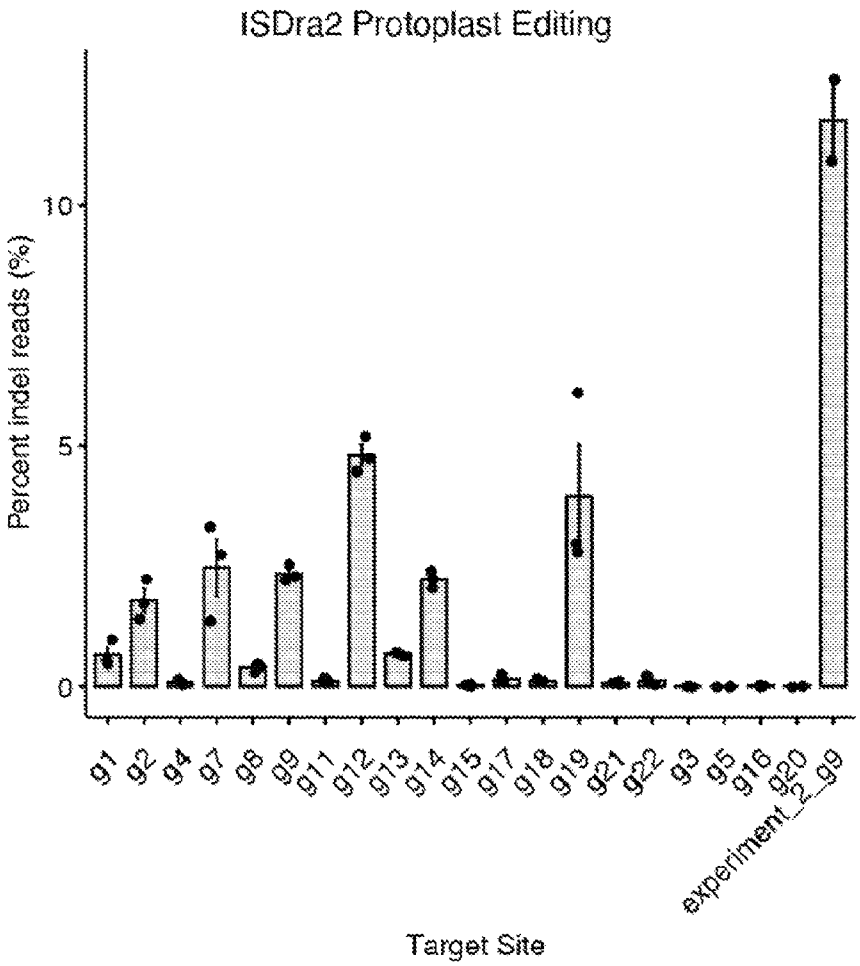

ISDra2 Protoplast Editing
Percent indel reads (%)
0
5
10
g1
g2
g4
g7
g8
g9
g11
g12
g13
g14
g15
g17
g18
g19
g21
g22
g3
g5
g16
g20
experiment_2_g9
Target Site

FIG. 10B

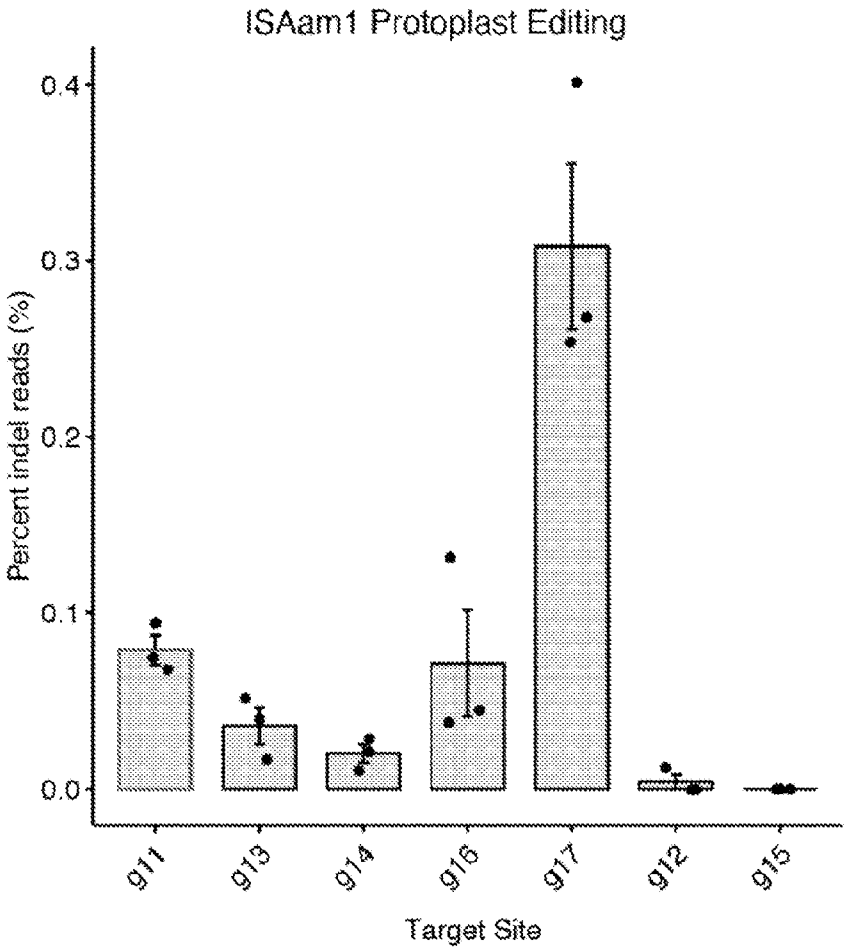

ISAam1 Protoplast Editing
Percent indel reads (%)
0.4
0.3
0.2
0.1
0.0
g11
g13
g14
g16
g17
g12
g15
Target Site

FIG. 10C

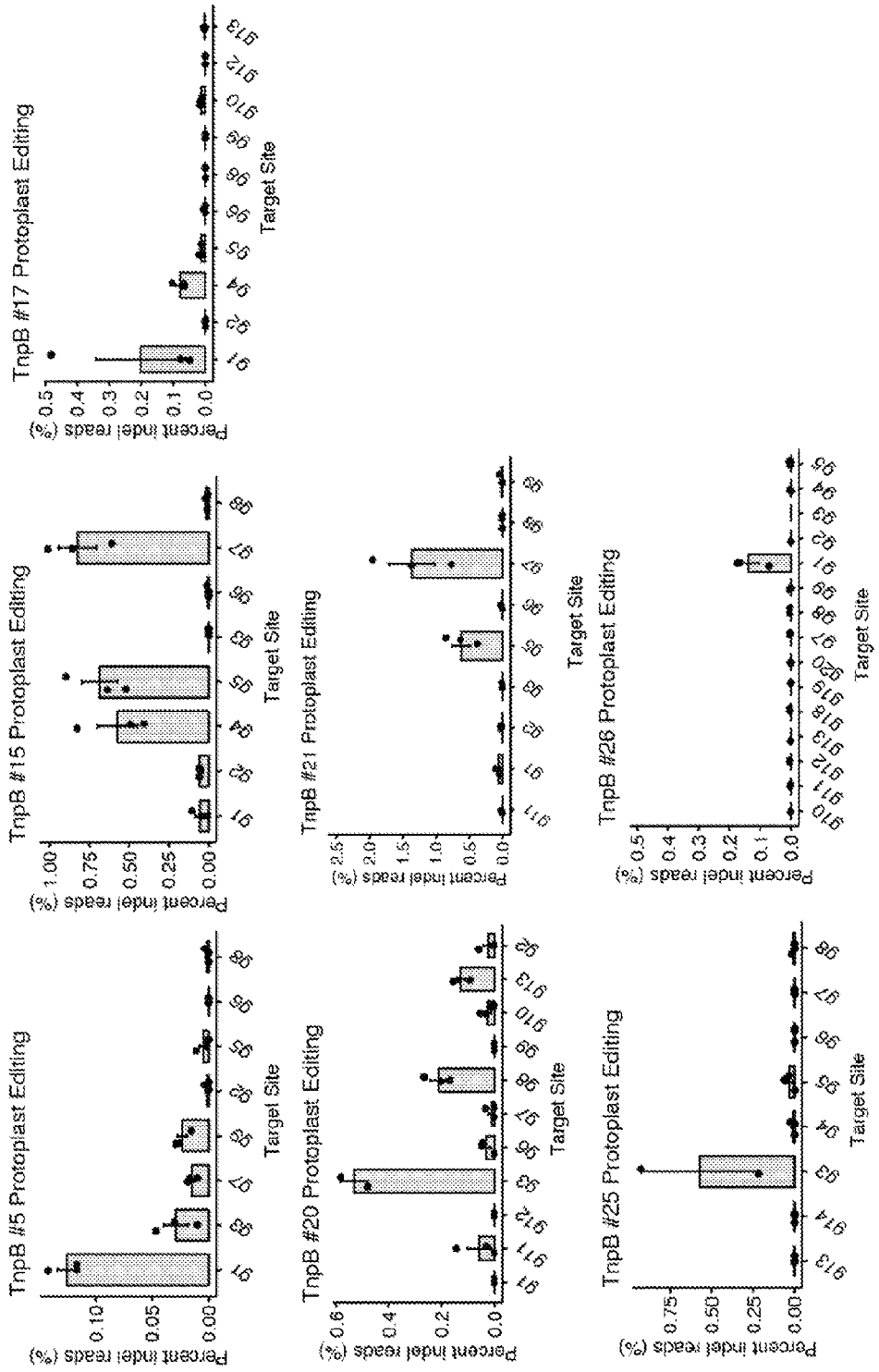

TnpB #5 Protoplast Editing
TnpB #15 Protoplast Editing
TnpB #17 Protoplast Editing
TnpB #20 Protoplast Editing
TnpB #21 Protoplast Editing
TnpB #25 Protoplast Editing
TnpB #26 Protoplast Editing
Percent indel reads (%)
Target Site

FIG. 11

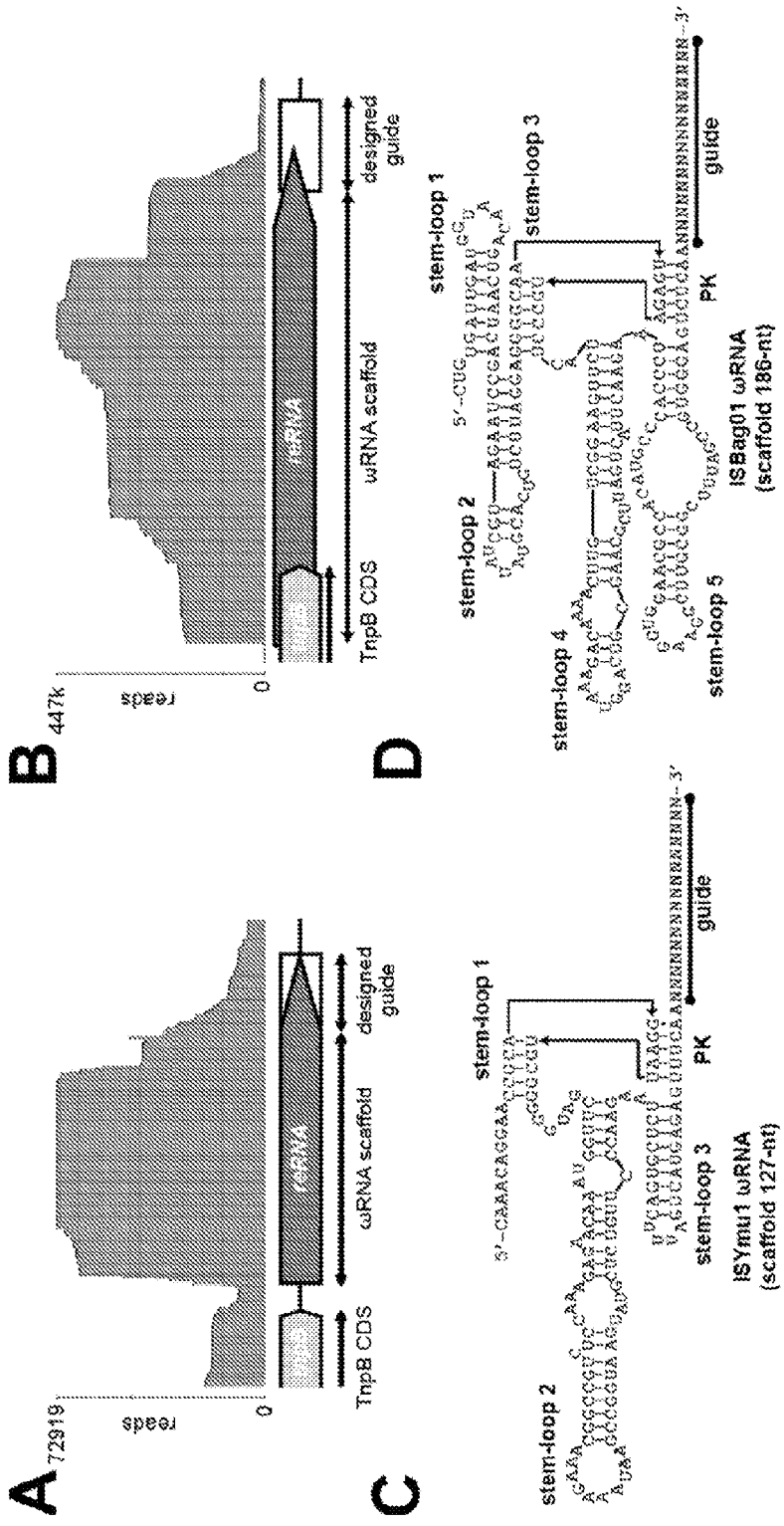

A
72919
reads
0
TnpB CDS
ωRNA scaffold
designed guide
B
447k
reads
0
TnpB CDS
ωRNA scaffold
designed guide
C
stem-loop 1
stem-loop 2
stem-loop 3
PK
guide
ISYmu1 ωRNA
(scaffold 127-nt)
D
stem-loop 1
stem-loop 2
stem-loop 3
stem-loop 4
stem-loop 5
PK
guide
ISBag01 ωRNA
(scaffold 186-nt)

FIG. 12

FIG. 13A

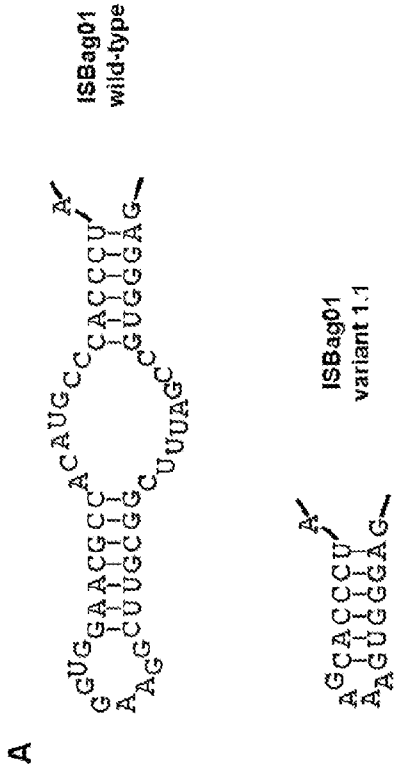
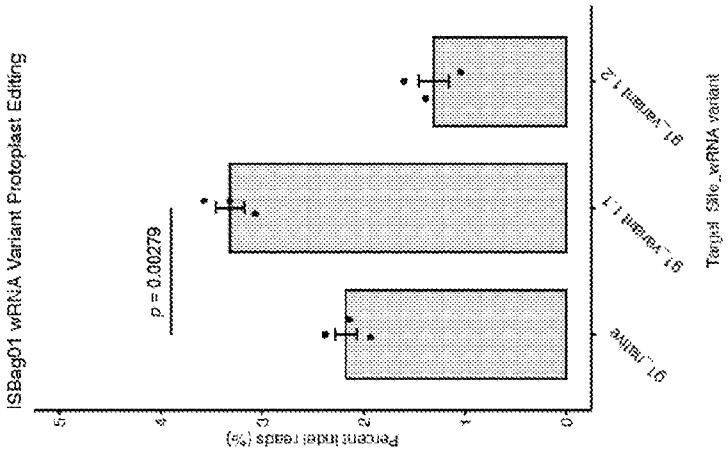


FIG. 13B



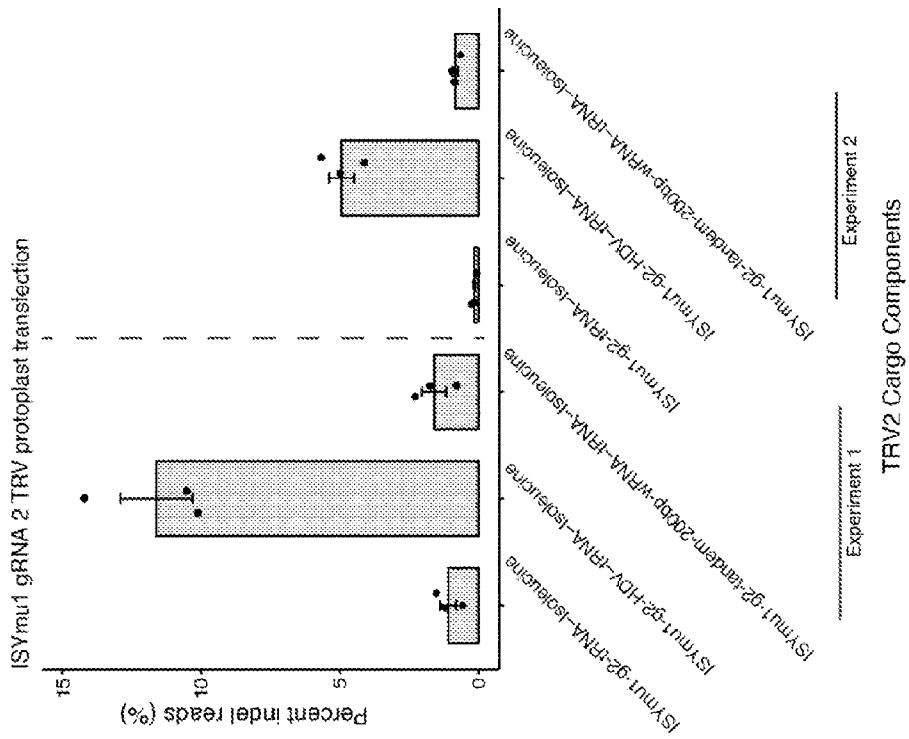

ISYmu1 gRNA 2 TRV protoplast transfection
Percent indel reads (%)
0
5
10
15
ISYmu1-g2-tRNA-Isoleucine
ISYmu1-g2-HDV-tRNA-Isoleucine
ISYmu1-g2-tandem-20bp-wtRNA-tRNA-Isoleucine
ISYmu1-g2-tRNA-Isoleucine
ISYmu1-g2-HDV-tRNA-Isoleucine
ISYmu1-g2-tandem-20bp-wtRNA-tRNA-Isoleucine
Experiment 1
Experiment 2
TRV2 Cargo Components

FIG. 14

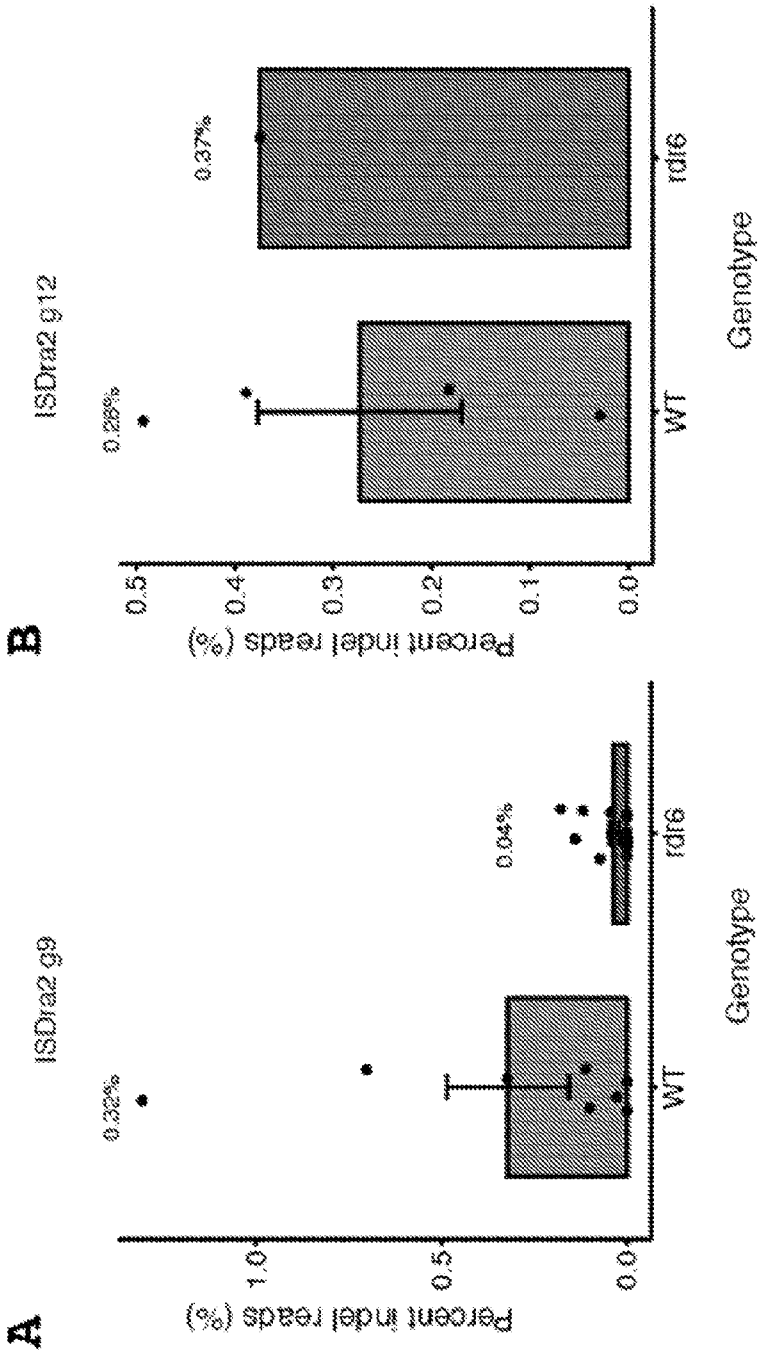

A
ISDra2 g9
Percent indel reads (%)
1.0
0.5
0.0
0.32%
0.04%
WT
rdr6
Genotype
B
ISDra2 g12
Percent indel reads (%)
0.5
0.4
0.3
0.2
0.1
0.0
0.28%
0.37%
WT
rdr6
Genotype

FIG. 15A-15B

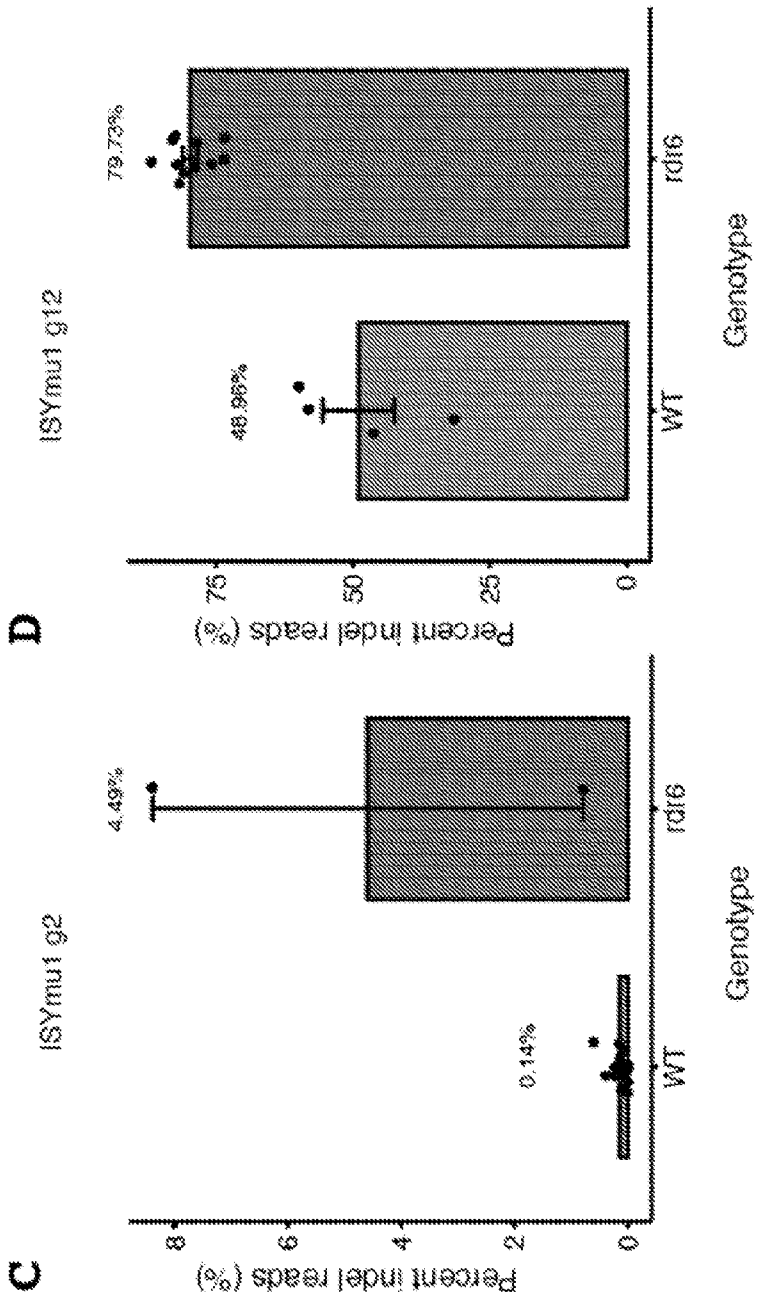

C
ISYmu1 g2
Percent indel reads (%)
0
2
4
6
8
0.14%
4.49%
WT
rdr6
Genotype
D
ISYmu1 g12
Percent indel reads (%)
0
25
50
75
46.96%
79.73%
WT
rdr6
Genotype

FIG. 15C-15D

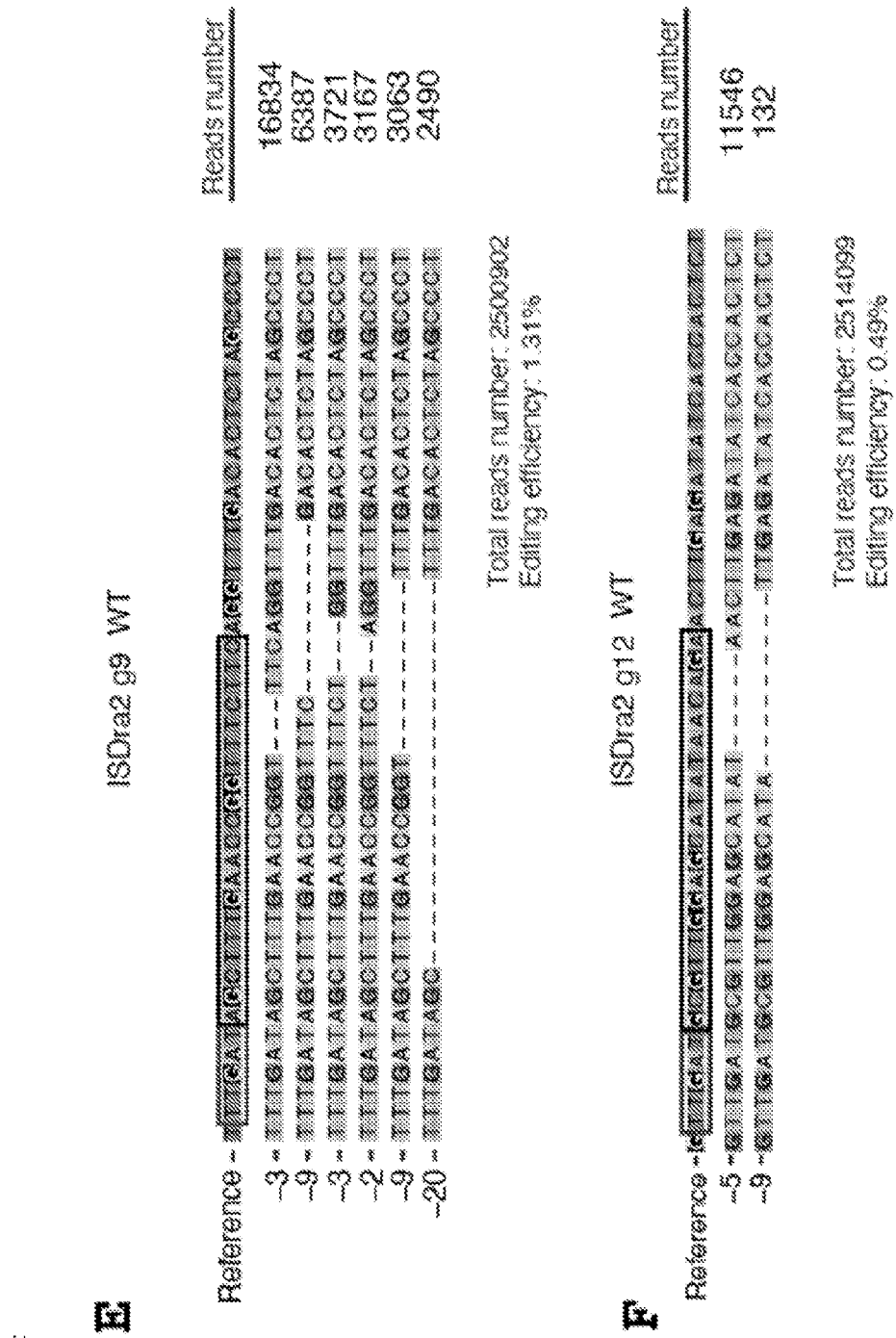

E
ISDra2 g9 WT
Reads number
16834
6387
3721
3167
3063
2490
Total reads number: 2500902
Editing efficiency: 1.31%
F
ISDra2 g12 WT
Reads number
11546
132
Total reads number: 2514099
Editing efficiency: 0.49%

FIG. 15E-15F

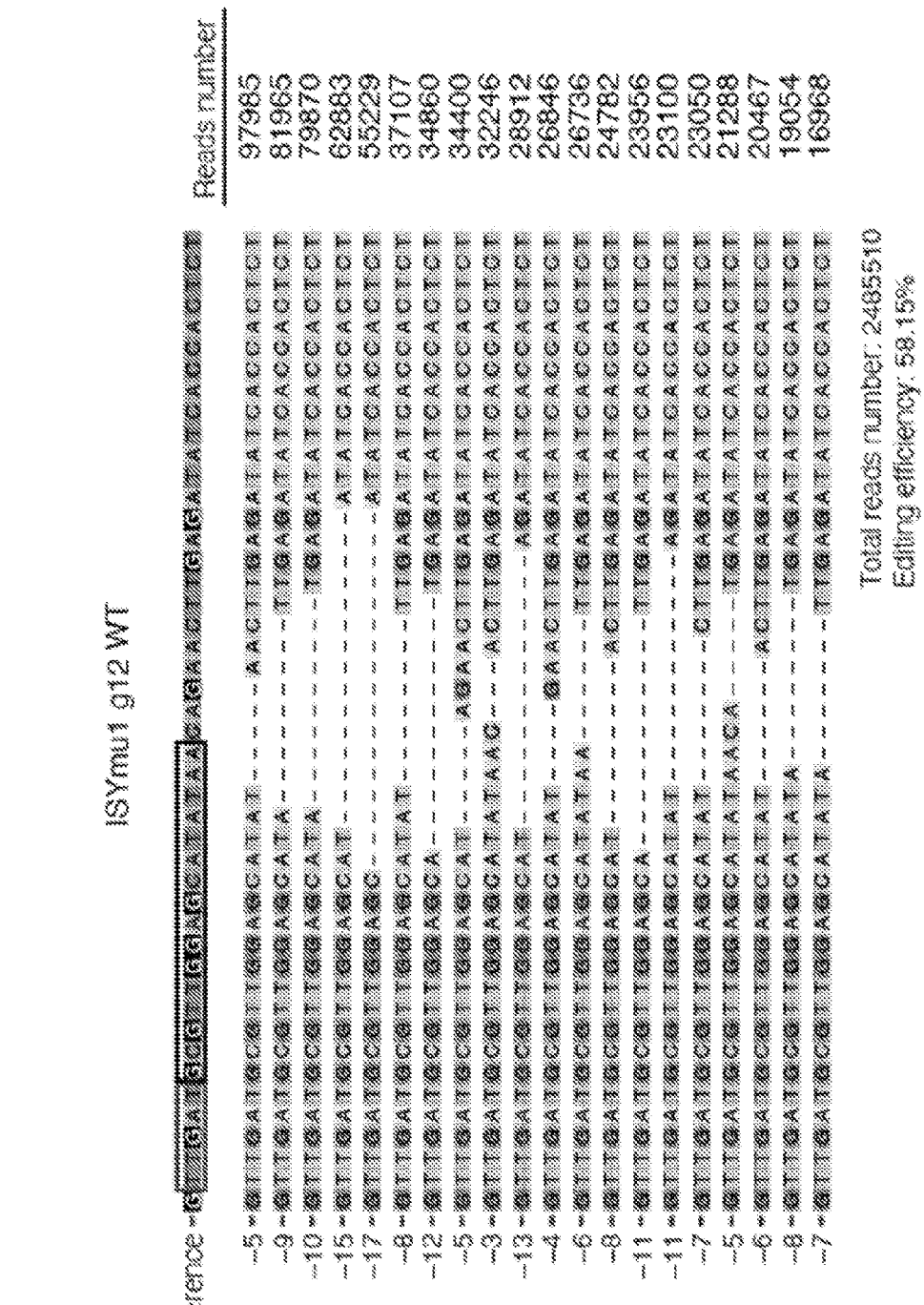

G
ISYmu1 g12 WT
Reference
Reads number
97985
81965
79870
62883
55229
37107
34860
34400
32246
28912
26846
26736
24782
23956
23100
23050
21288
20467
19054
16968
Total reads number: 2485510
Editing efficiency: 58.15%

FIG. 15G

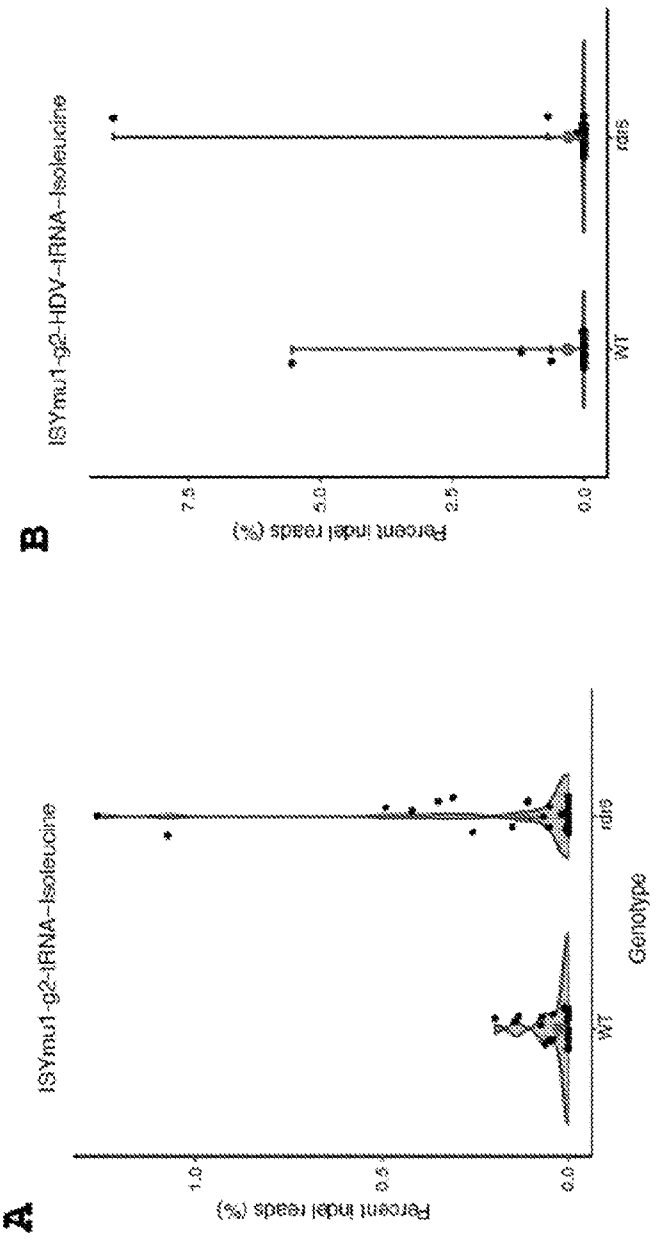

A
ISYmu1-g2-tRNA-Isoleucine
Percent indel reads (%)
1.0
0.5
0.0
WT
rds
Genotype
B
ISYmu1-g2-HDV-tRNA-Isoleucine
Percent indel reads (%)
7.5
5.0
2.5
0.0
WT
rds

FIG. 16A – 16B

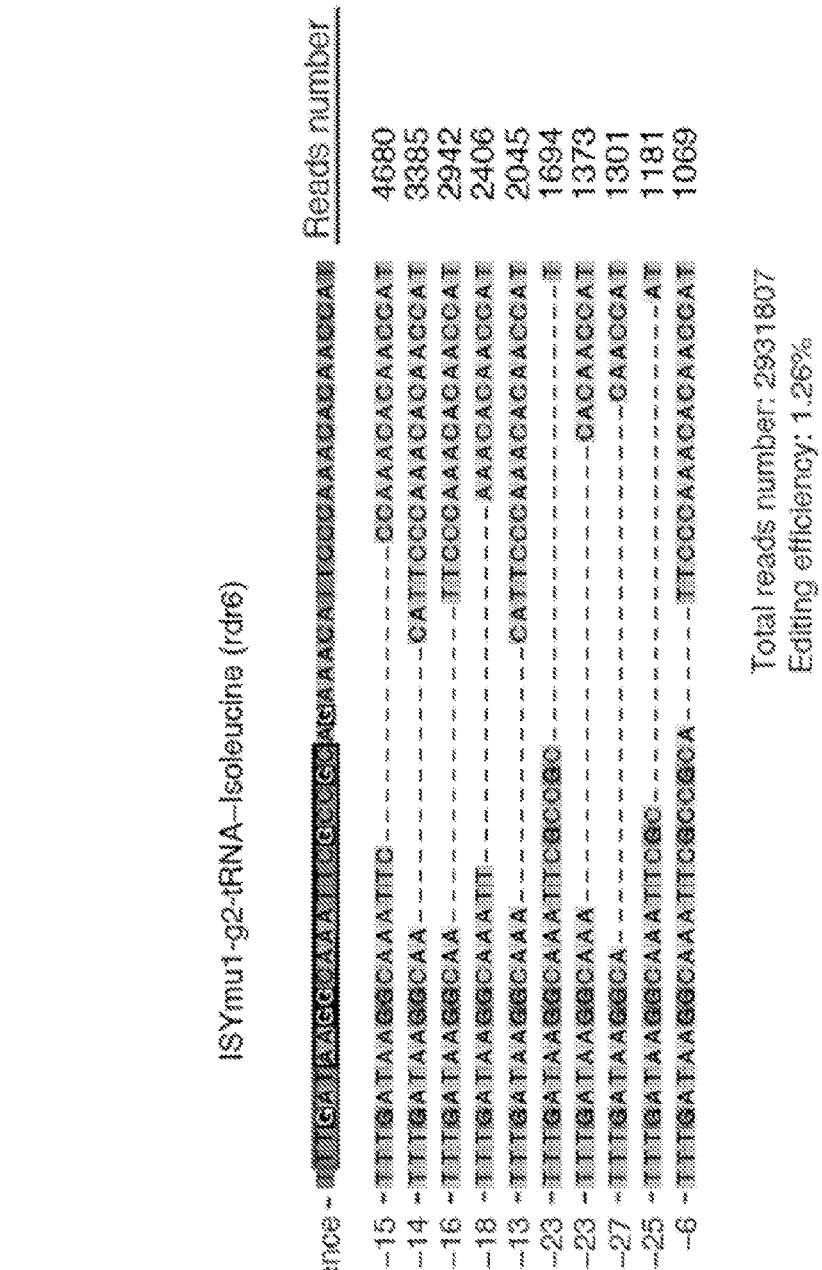

ISYmut1-g2-tRNA–Isoleucine (rdr6)
Reference
Reads number
-15
4680
-14
3385
-16
2942
-18
2406
-13
2045
-23
1694
-23
1373
-27
1301
-25
1181
-6
1069
Total reads number: 2931807
Editing efficiency: 1.26%

FIG. 16C

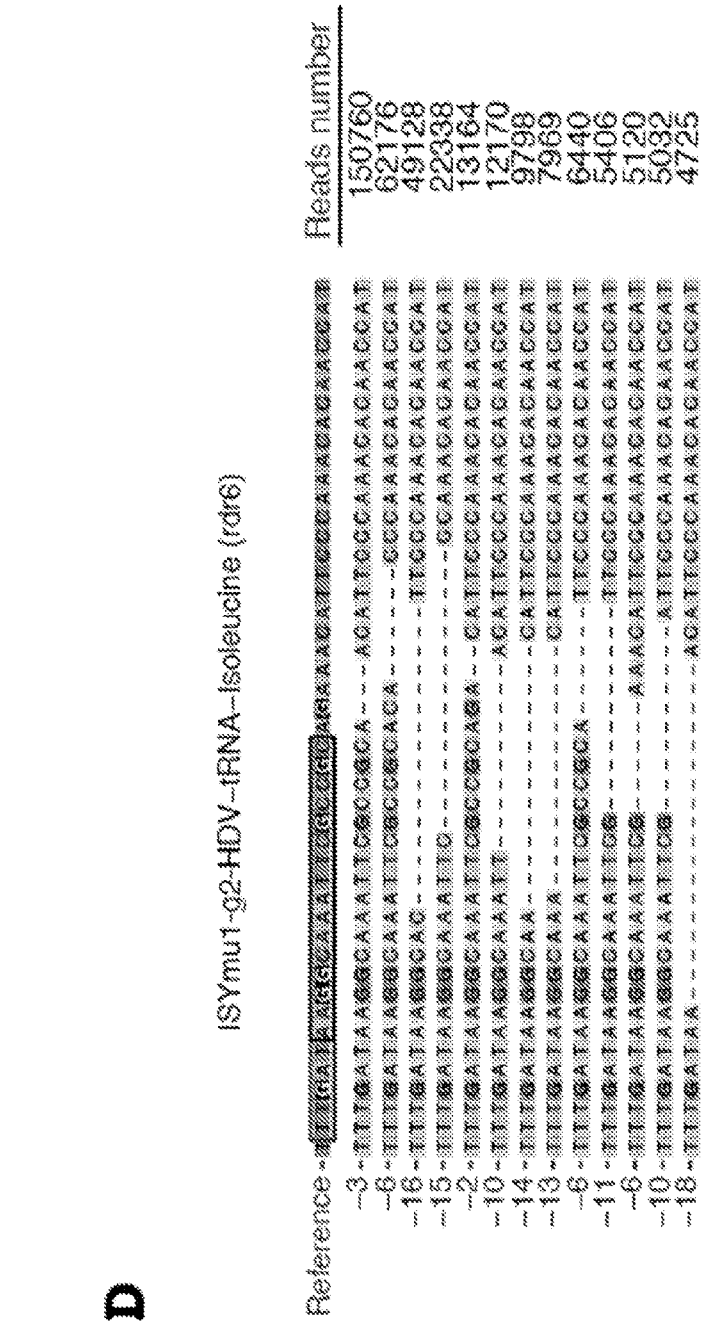
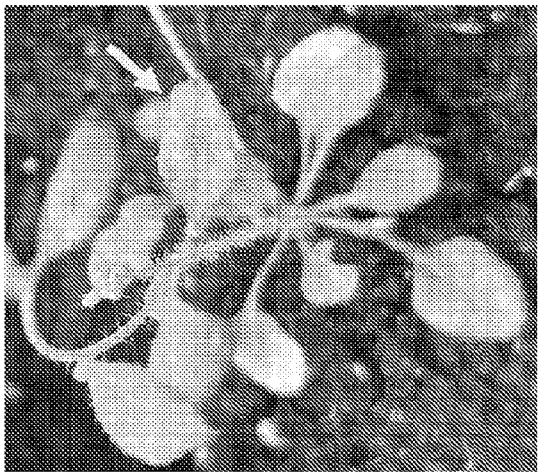

D
ISYmu1-g2-HDV–tRNA–Isoleucine (rdr6)
Reference
Reads number
150760
62176
49128
22338
13164
12170
9798
7969
6440
5406
5120
5032
4725
Total reads number: 41223156
Editing efficiency: 8.93%

FIG. 16D



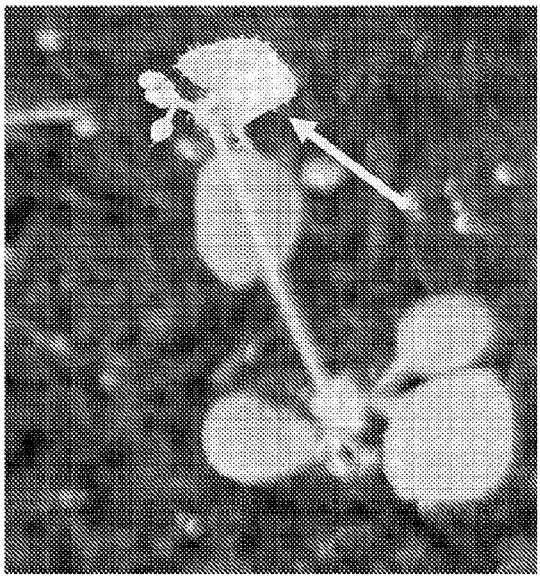


FIG. 16E

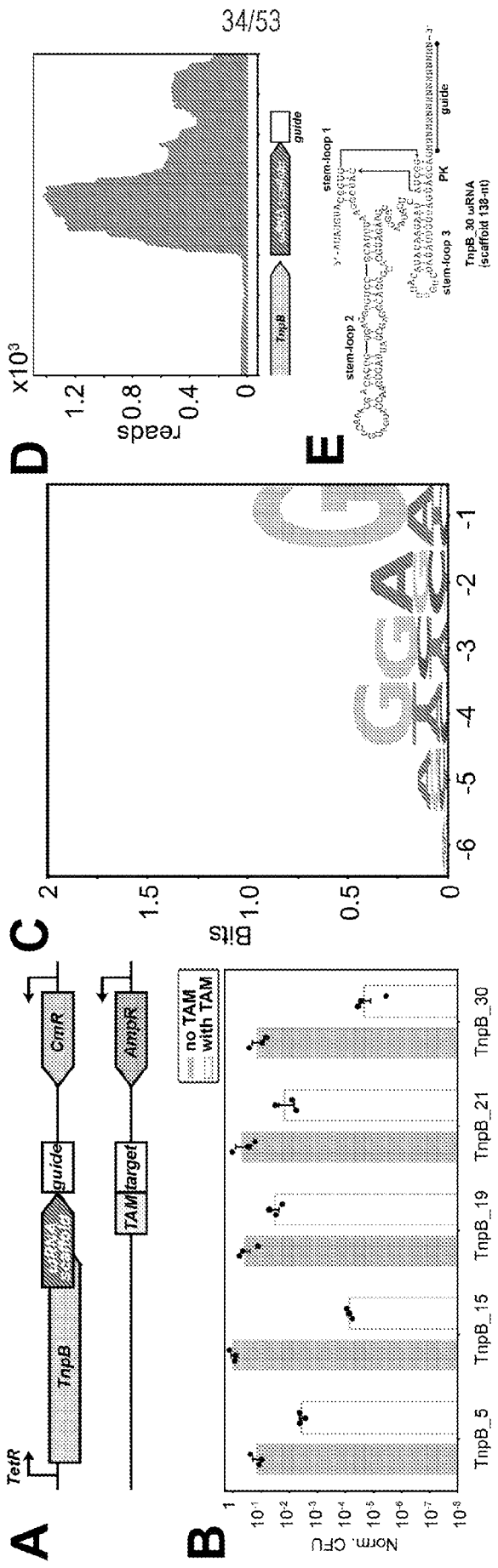

A
TetR
TnpB
guide
CmR
TAM
target
AmpR
B
no TAM
with TAM
Norm. CFU
TnpB_5
TnpB_15
TnpB_19
TnpB_21
TnpB_30
C
Bits
D
x10³
reads
TnpB
guide
E
stem-loop 1
stem-loop 2
stem-loop 3
PK
guide
TnpB_30 ωRNA
(scaffold 138-nt)

FIG. 17A-17E

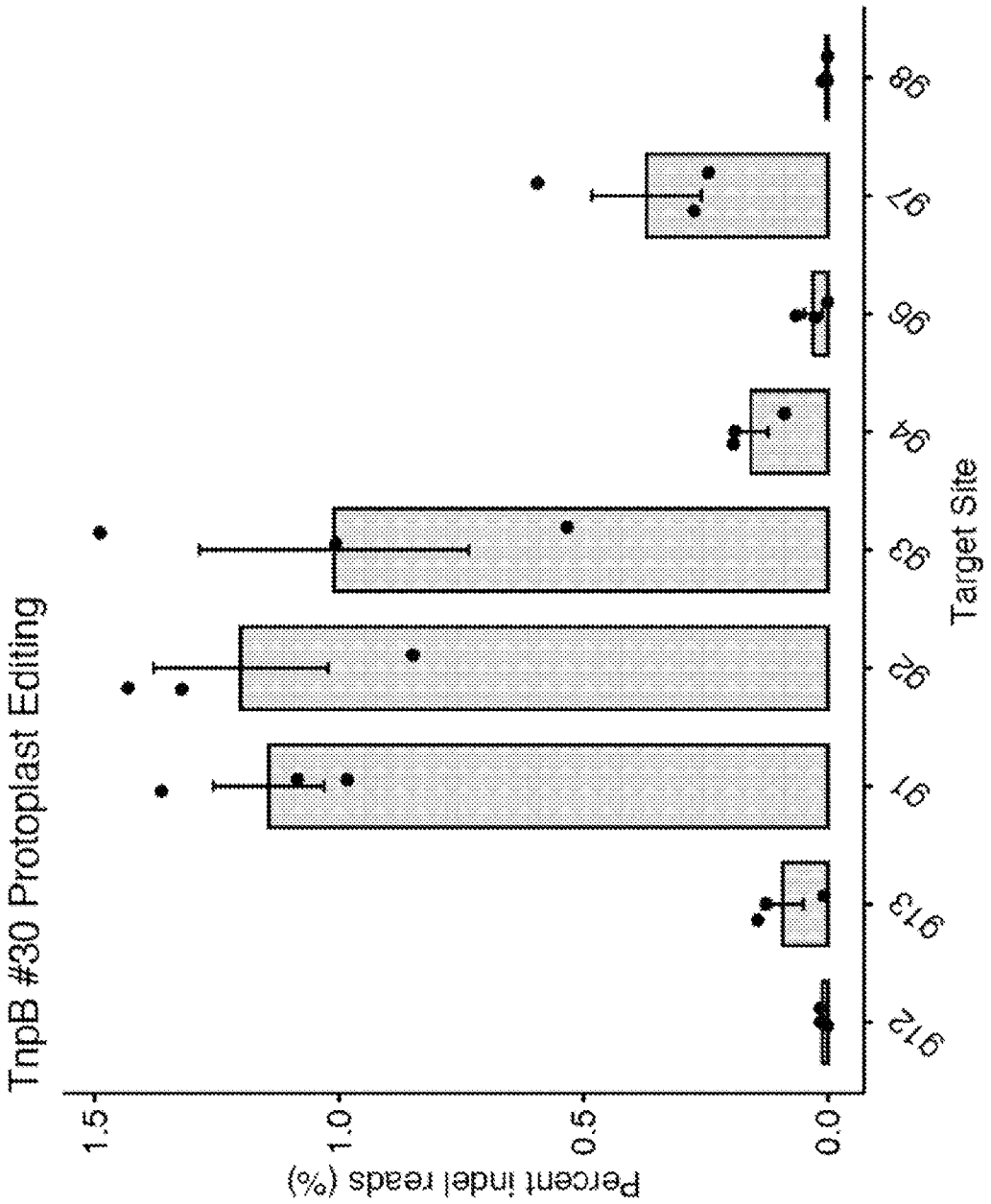
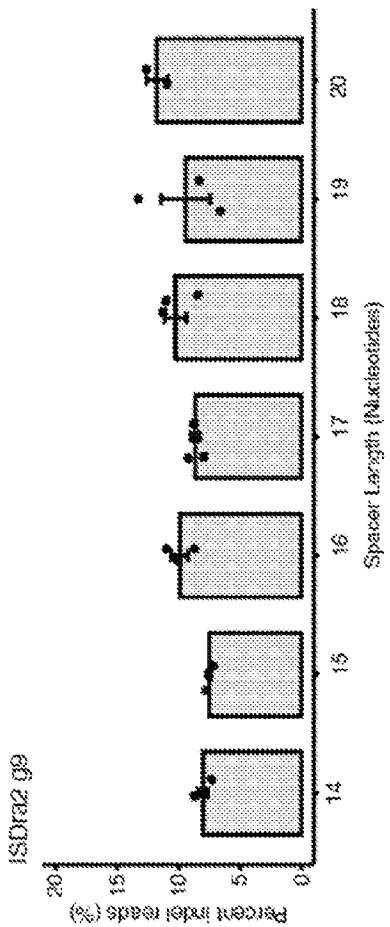

TnpB #30 Protoplast Editing
Percent Indel reads (%)
0.0
0.5
1.0
1.5
Target Site
g12
g13
g1
g2
g3
g4
g6
g7
g8

FIG. 18

FIG. 19A-19B



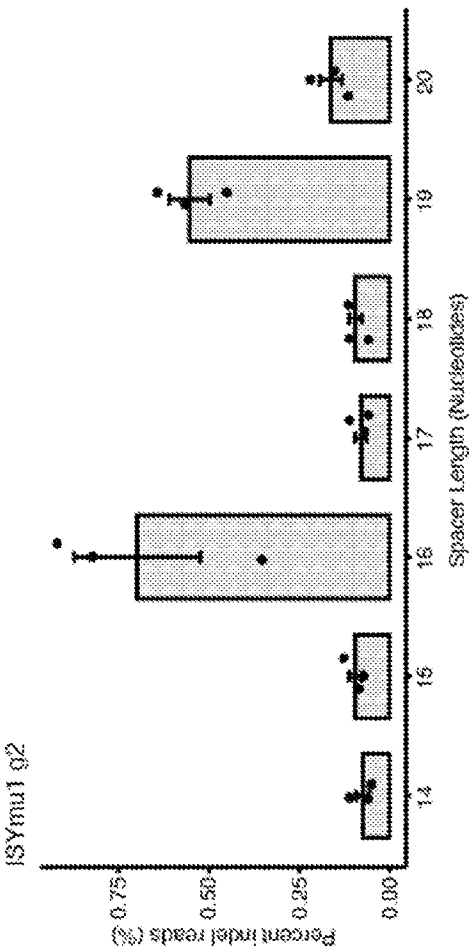
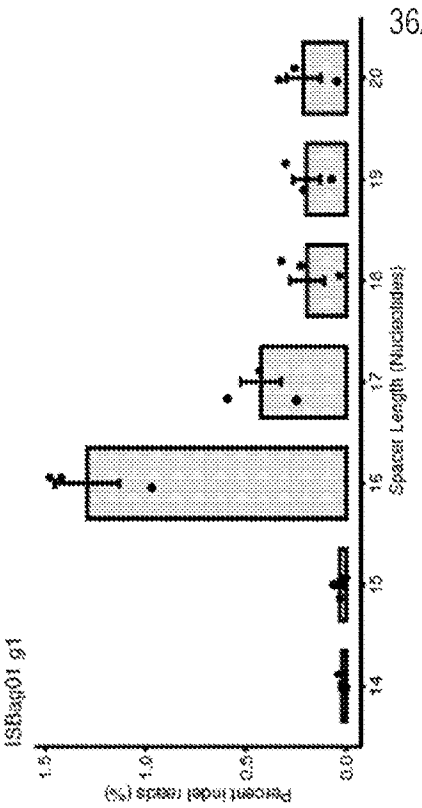
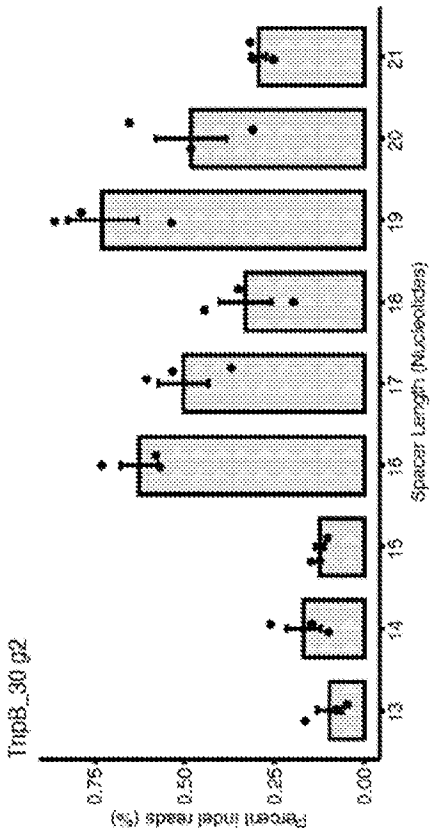


FIG. 19C-19D





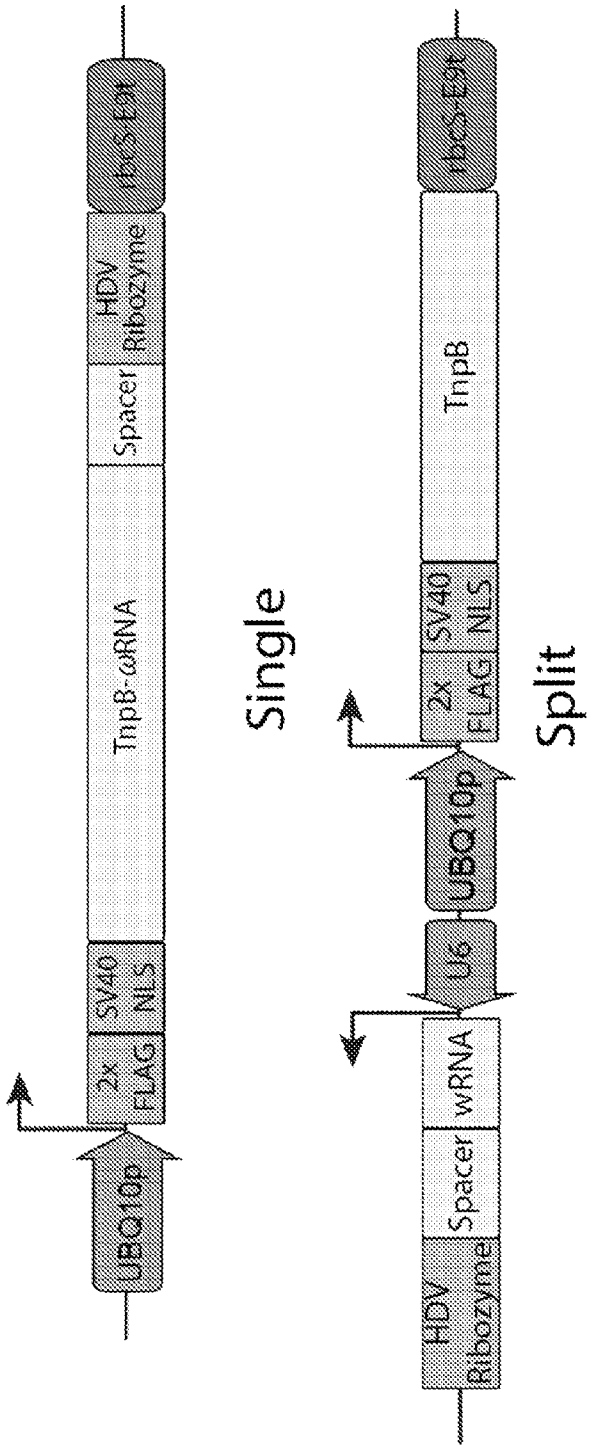

Single
UBQ10p
2x FLAG
SV40 NLS
TnpB-ωRNA
Spacer
HDV Ribozyme
rbcS Ter
Split
HDV Ribozyme
Spacer
wRNA
U6
UBQ10p
2x FLAG
SV40 NLS
TnpB
rbcS Ter

FIG. 20A

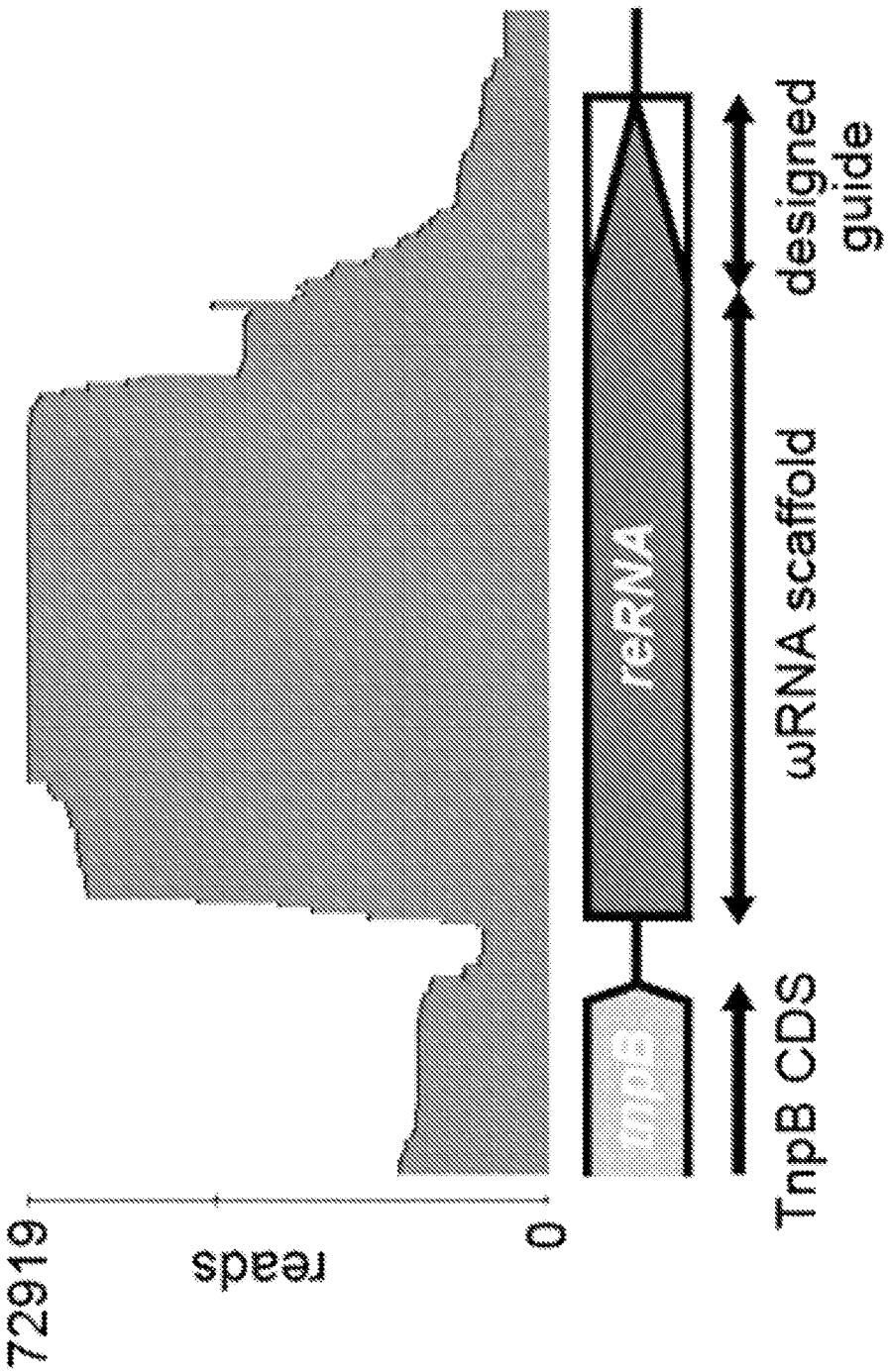


FIG. 20B

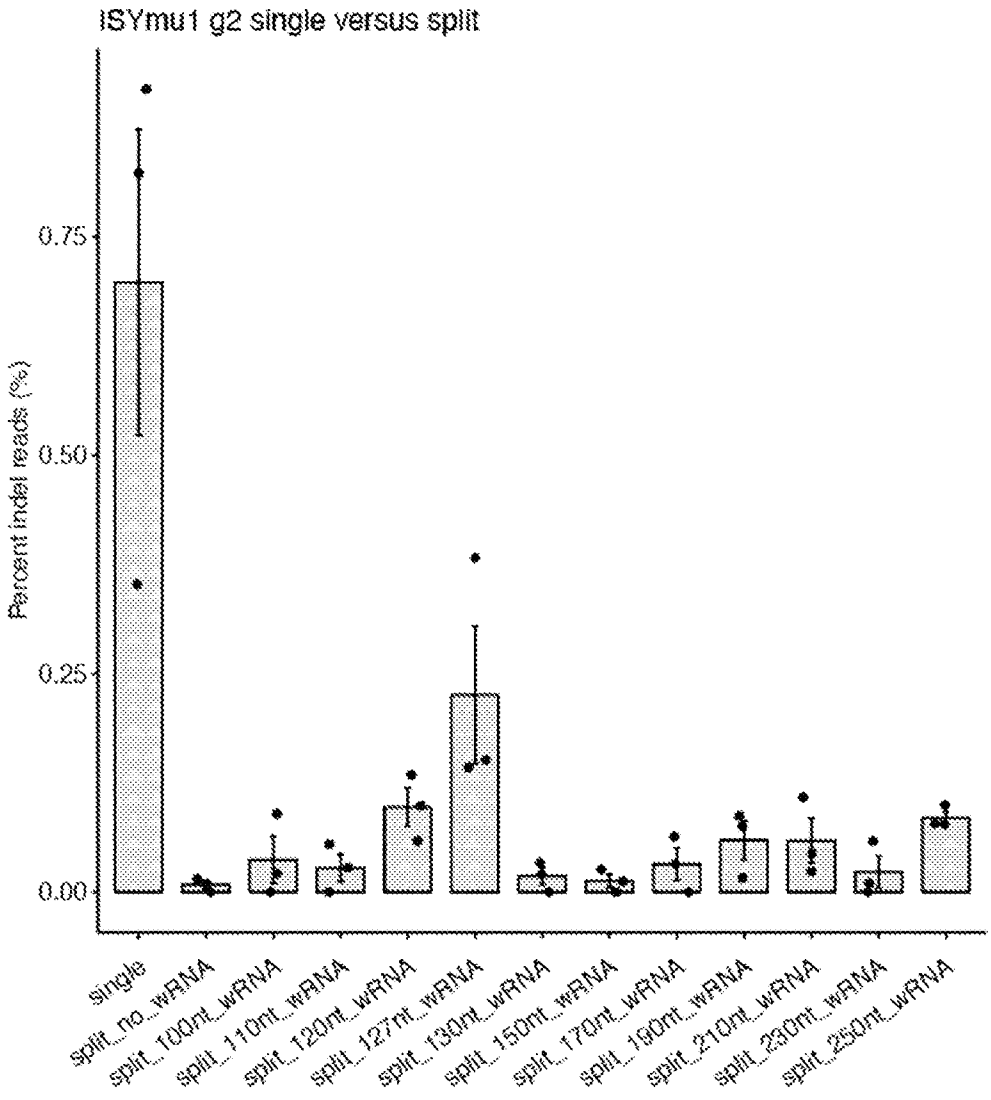

ISYmu1 g2 single versus split
Percent indel reads (%)
0.75
0.50
0.25
0.00
single
split_no_wRNA
split_100nt_wRNA
split_110nt_wRNA
split_120nt_wRNA
split_127nt_wRNA
split_130nt_wRNA
split_150nt_wRNA
split_170nt_wRNA
split_190nt_wRNA
split_210nt_wRNA
split_230nt_wRNA
split_250nt_wRNA

FIG. 20C

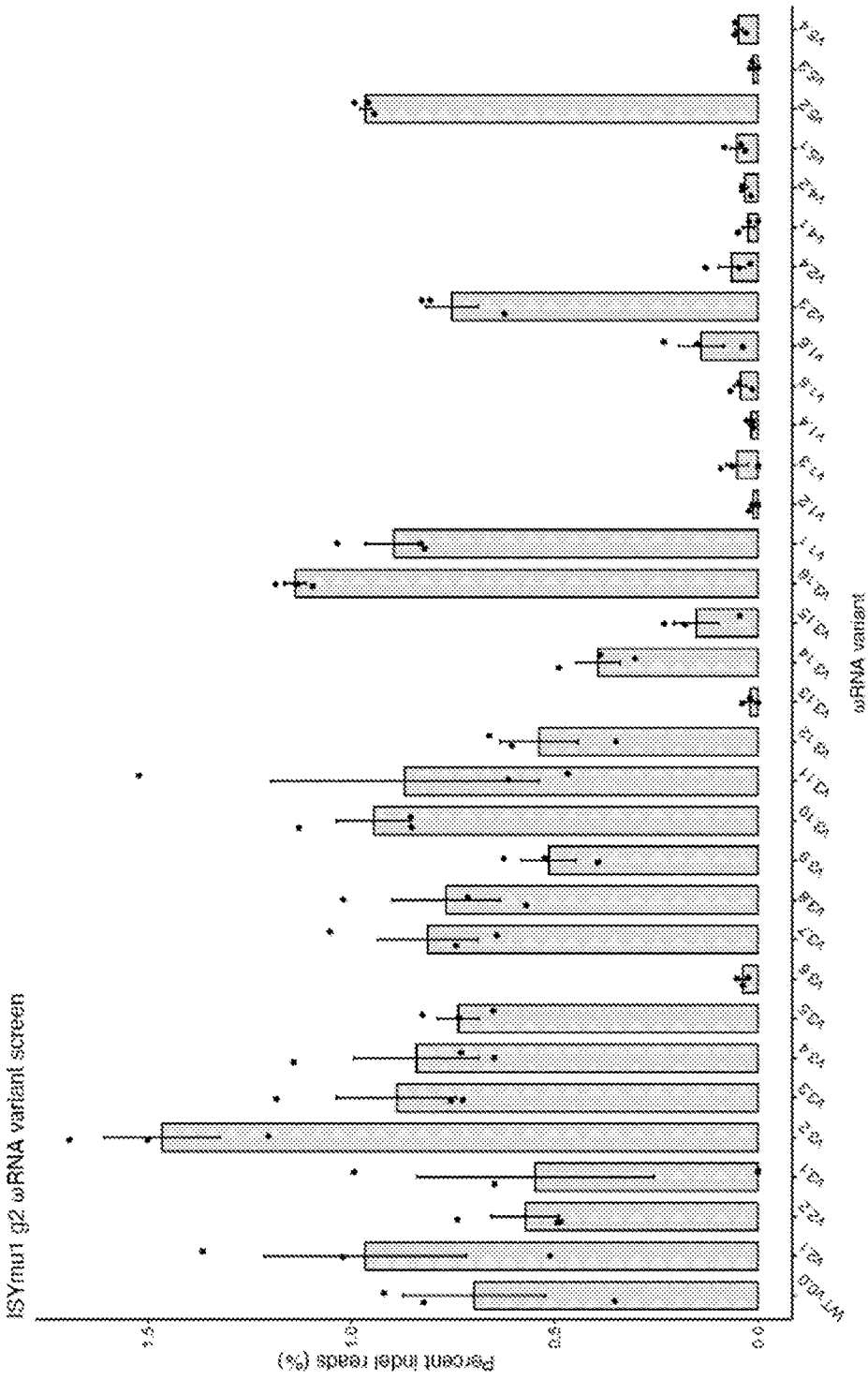

ISYmu1 g2 ωRNA variant screen
Percent indel reads (%)
0.0
0.5
1.0
1.5
ωRNA variant
WT.00

FIG. 21

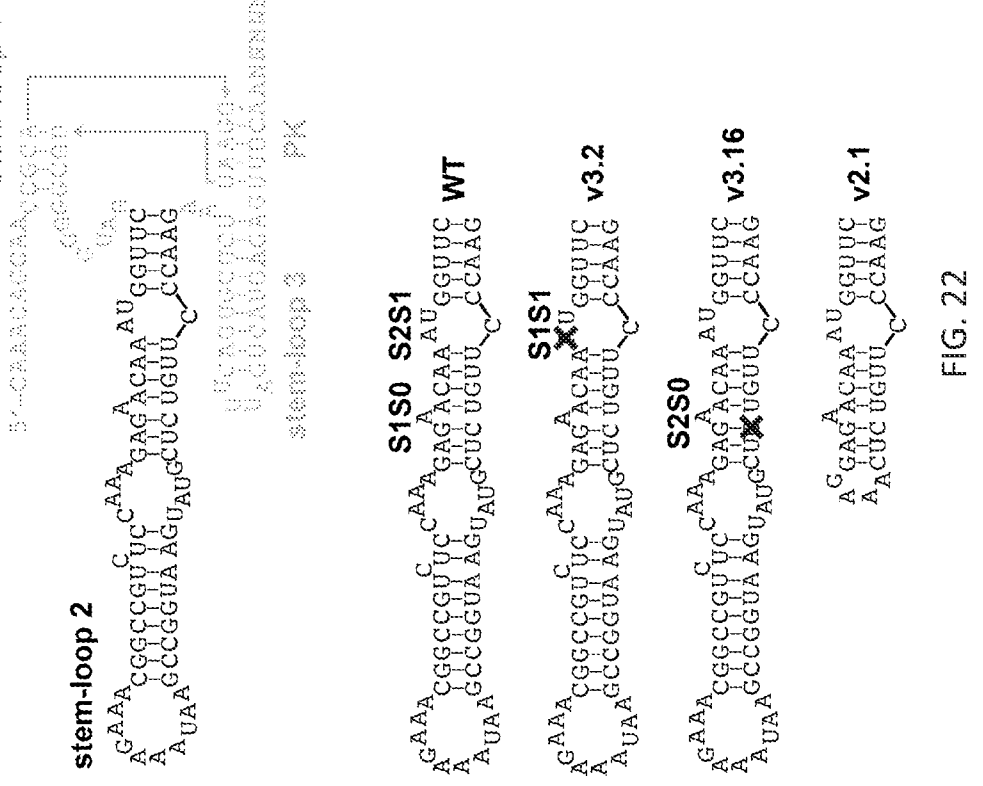

stem-loop 1
stem-loop 2
stem-loop 3
PK
S1S0 S2S1
WT
S1S1
v3.2
S2S0
v3.16
v2.1

FIG. 22

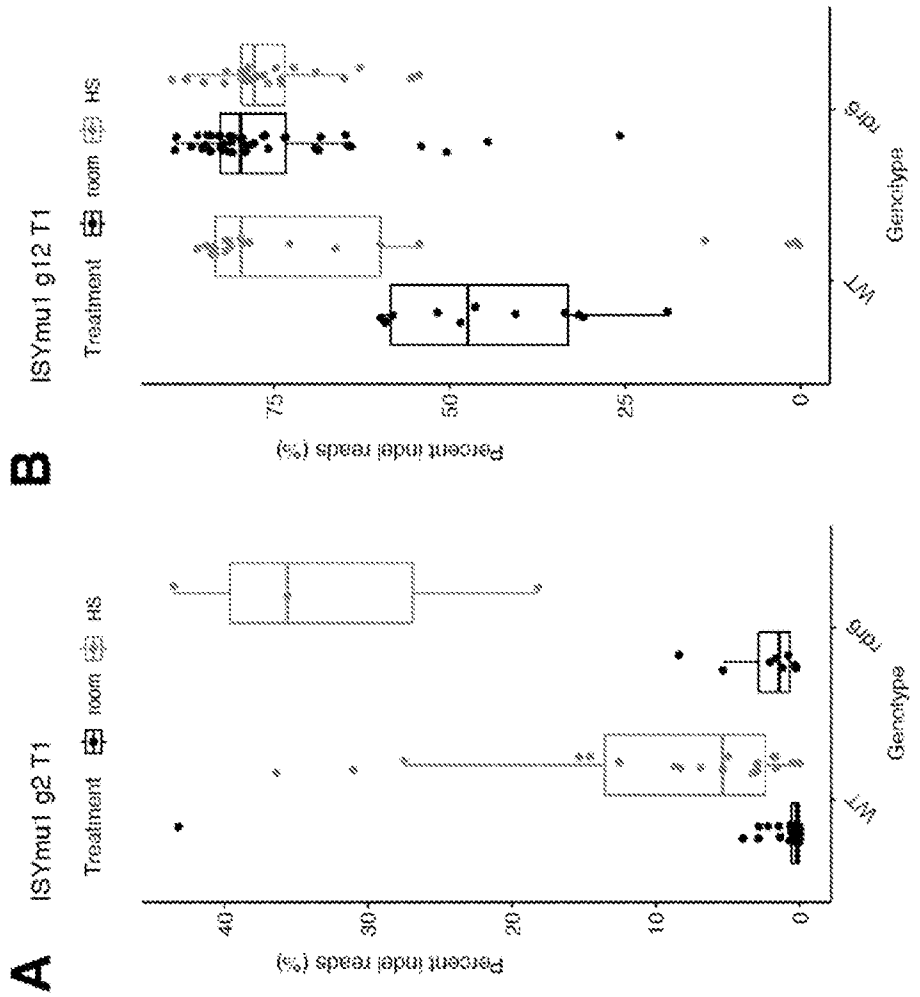

A
ISYmu1 g2 T1
Treatment
room
HS
Percent indel reads (%)
40
30
20
10
0
WT
tdT6
Genotype
B
ISYmu1 g12 T1
Treatment
room
HS
Percent indel reads (%)
75
50
25
0
WT
tdT6
Genotype

FIG. 23A-23B

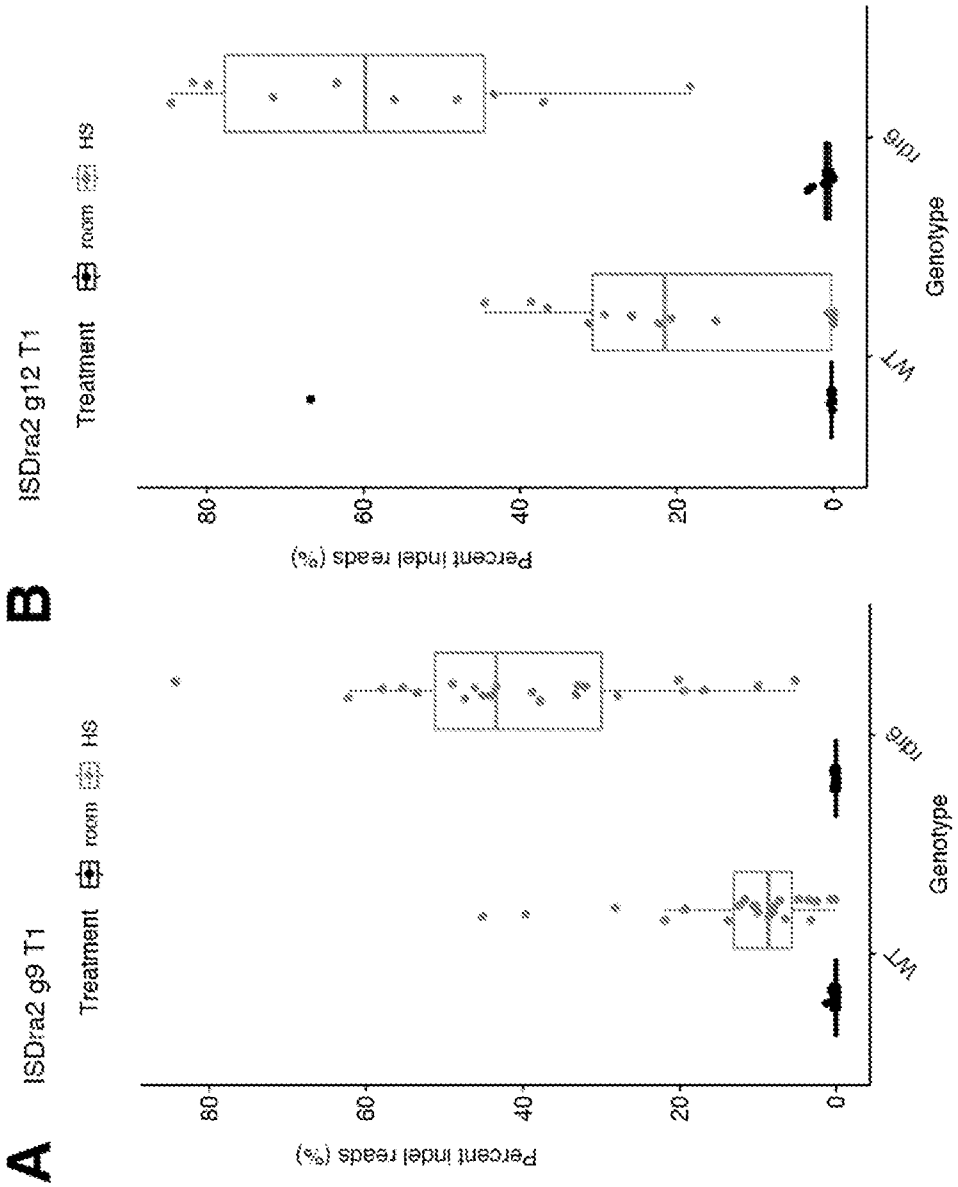

A
ISDra2 g9 T1
Treatment
room
HS
Percent indel reads (%)
0
20
40
60
80
WT
data
Genotype
B
ISDra2 g12 T1
Treatment
room
HS
Percent indel reads (%)
0
20
40
60
80
WT
data
Genotype

FIG. 24A-24B

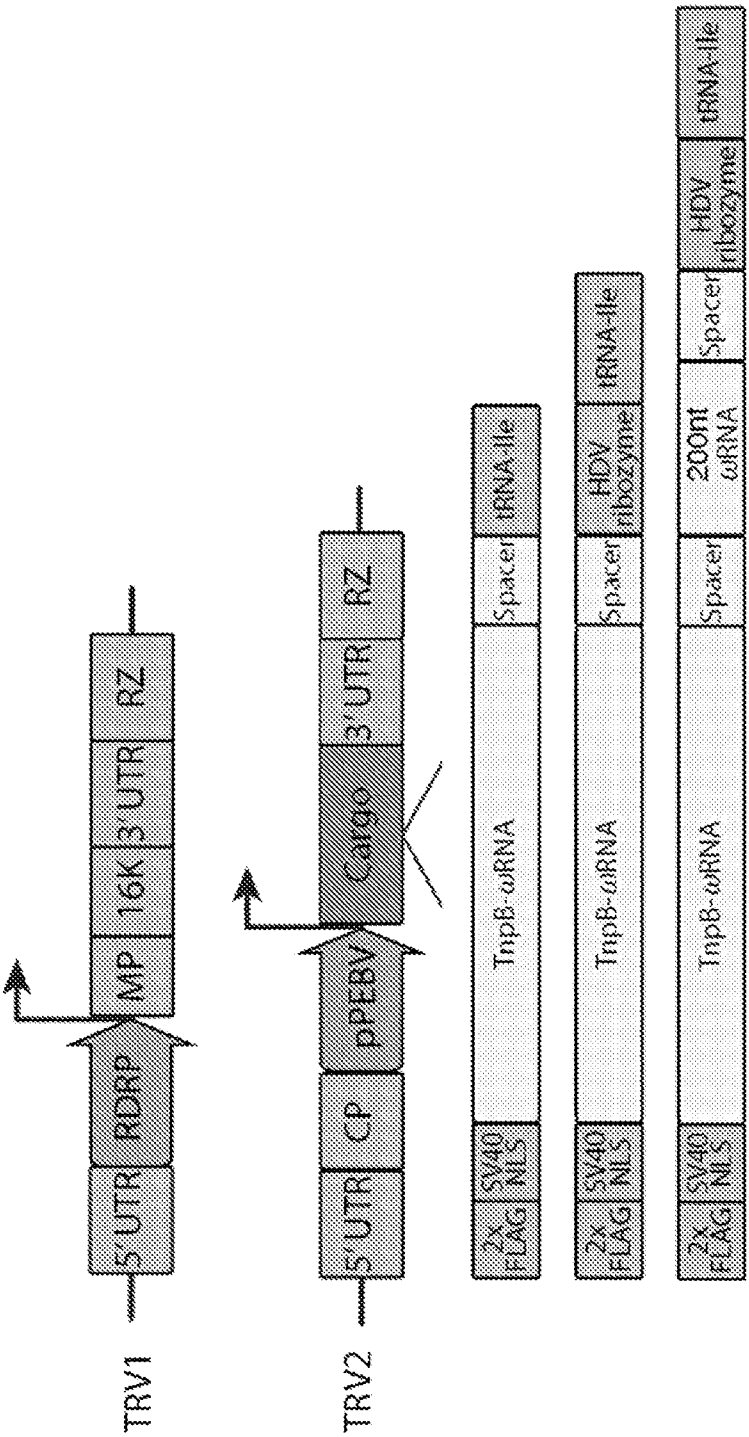

TRV1
5'UTR
RDRP
MP
16K
3'UTR
RZ
TRV2
5'UTR
CP
pPEBV
Cargo
3'UTR
RZ
2x FLAG
SV40 NLS
TnpB-ωRNA
Spacer
tRNA-Ile
2x FLAG
SV40 NLS
TnpB-ωRNA
Spacer
HDV ribozyme
tRNA-Ile
2x FLAG
SV40 NLS
TnpB-ωRNA
Spacer
20nt ωRNA
Spacer
HDV ribozyme
tRNA-Ile

FIG. 25A

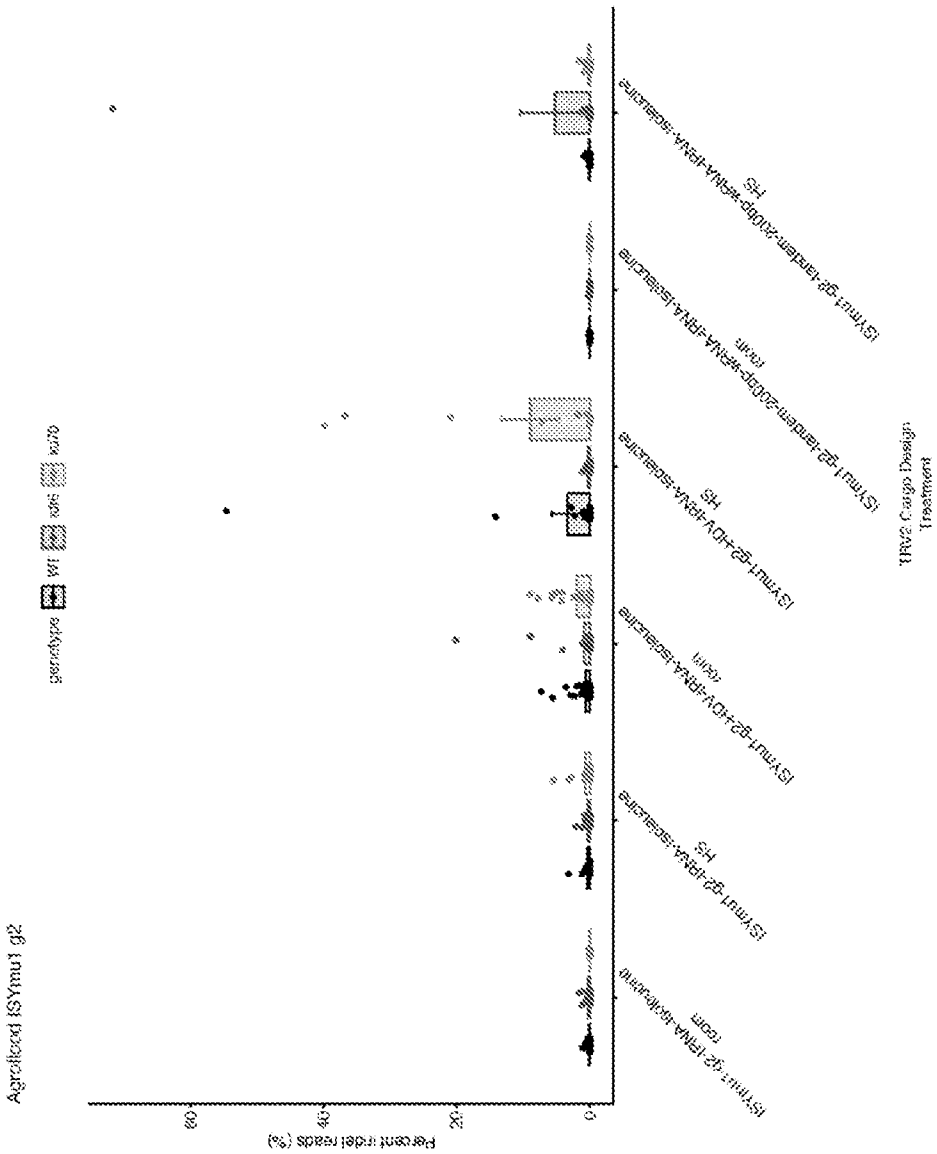

Agrobacterium tSYmut1 g2
genotype
WT
ins
sub
Percent indel reads (%)
0
20
40
60
tSYmut1-g2-tRNA-lysine-scaffold
tSYmut1-g2-tRNA-lysine-scaffold
tSYmut1-g2-HDV-tRNA-isoleucine
tSYmut1-g2-HDV-tRNA-isoleucine
tSYmut1-g2-tandem-20080-peNA-tRNA-isoleucine
tSYmut1-g2-tandem-20080-peNA-tRNA-isoleucine
tSYmut1-g2-tandem-20080-peNA-tRNA-isoleucine
tSYmut1-g2-tandem-20080-peNA-tRNA-isoleucine
TRV2 Cargo Design
Treatment

FIG. 25B

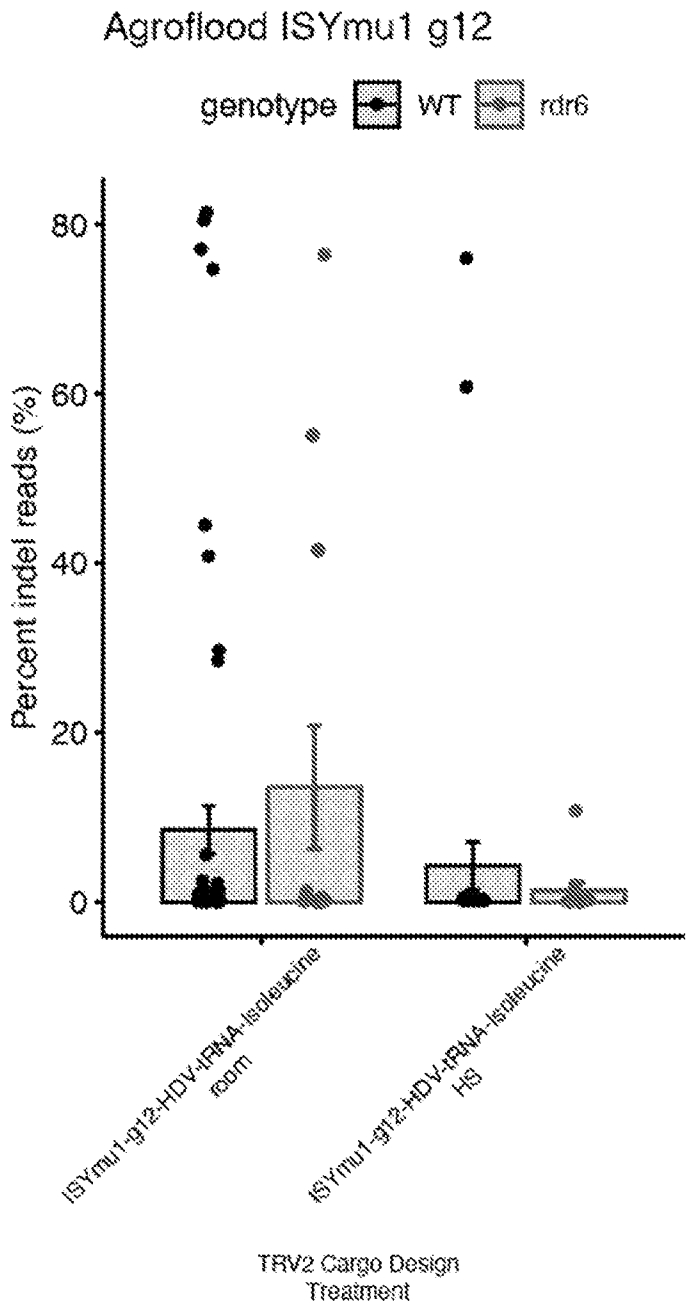
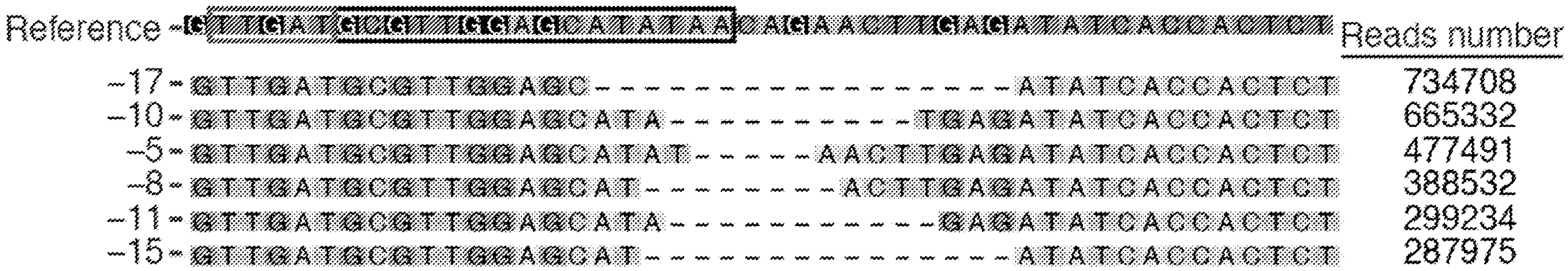

Agroflood ISYmu1 g12
genotype
WT
rdr6
Percent indel reads (%)
80
60
40
20
0
ISYmu1-g12-HDV-tRNA-Isoleucine room
ISYmu1-g12-HDV-tRNA-Isoleucine HS
TRV2 Cargo Design Treatment

FIG. 25C



Total reads number: 12850120
Editing efficiency: 81.42%

FIG. 25D

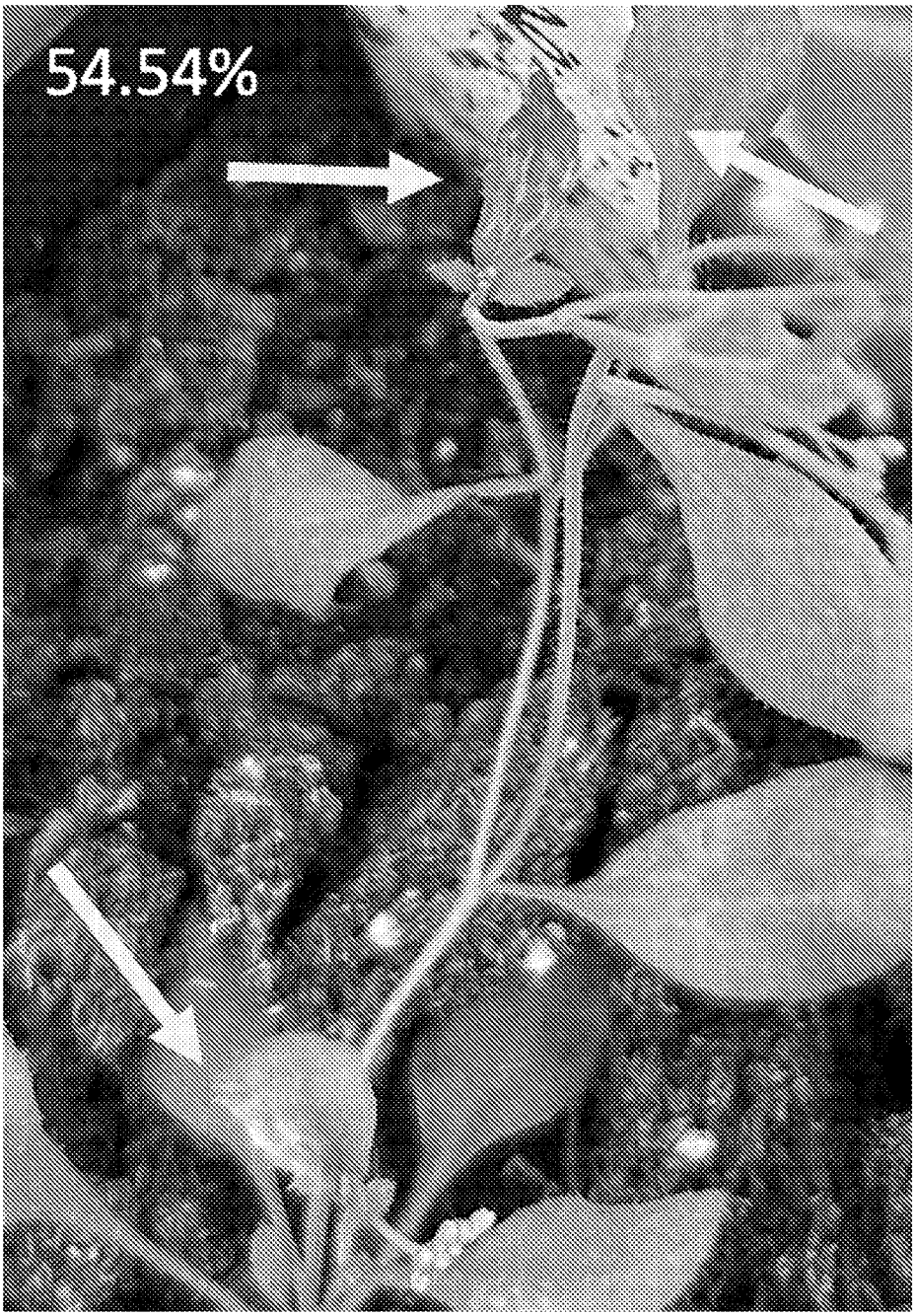


FIG. 26A

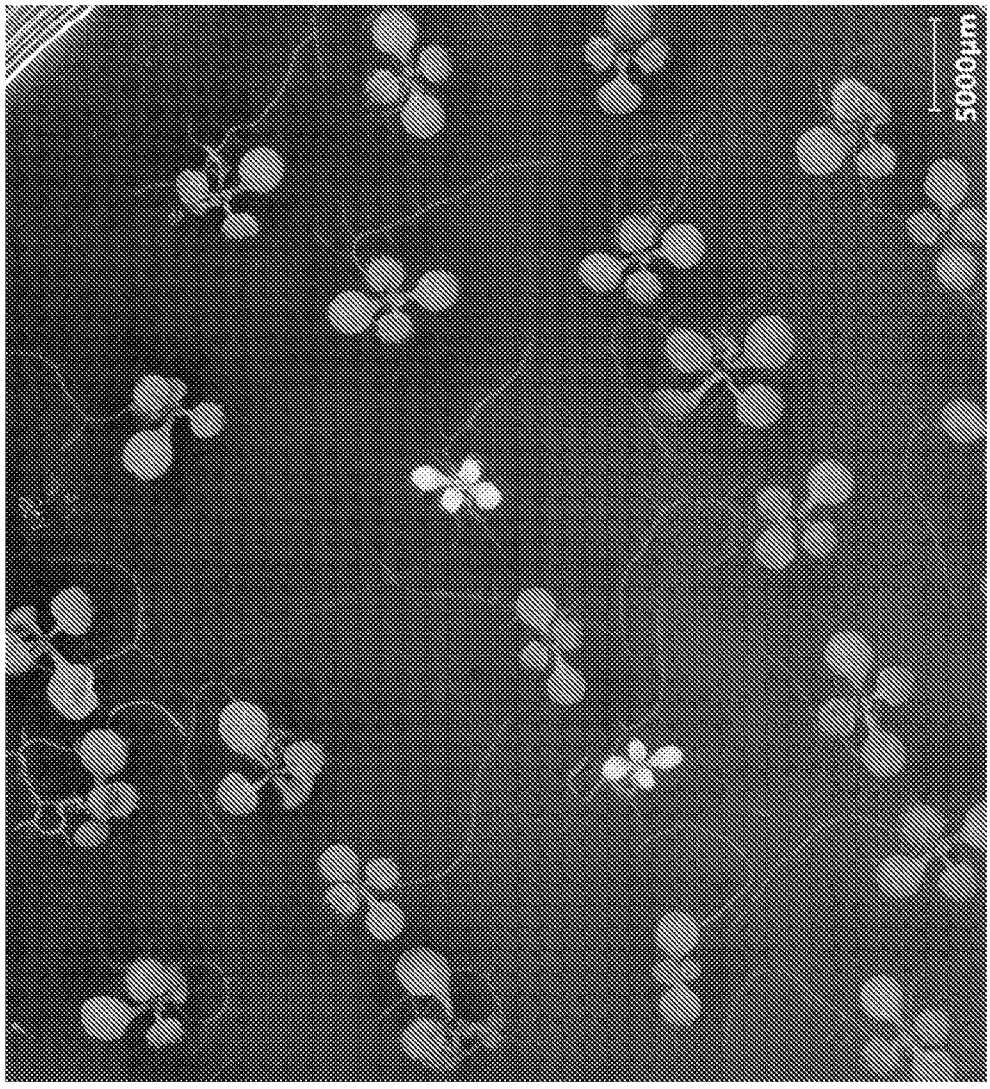


FIG. 26B

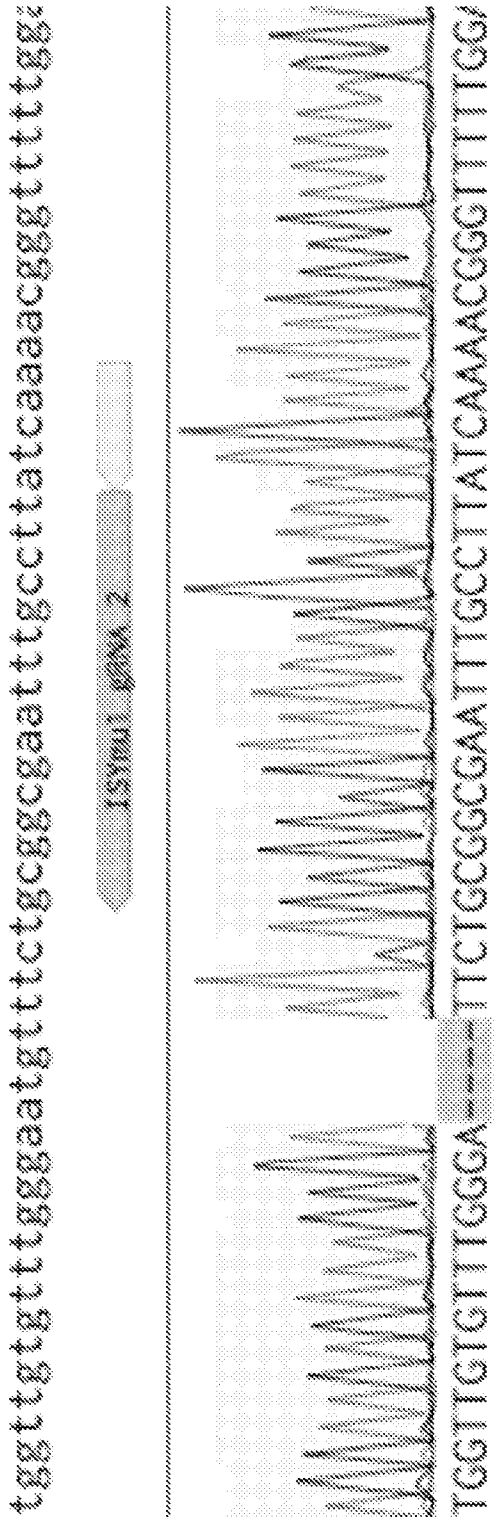

tggttgttgtttggaatgtttctgcggcgaatttgccttatcaaaacgggtttttgg
Ismat gRNA 2
TGGTTGTGTTTGGA----TTCTGCGGCGAATTTGCCTTATCAAAACGGGTTTTTGG

FIG. 26C

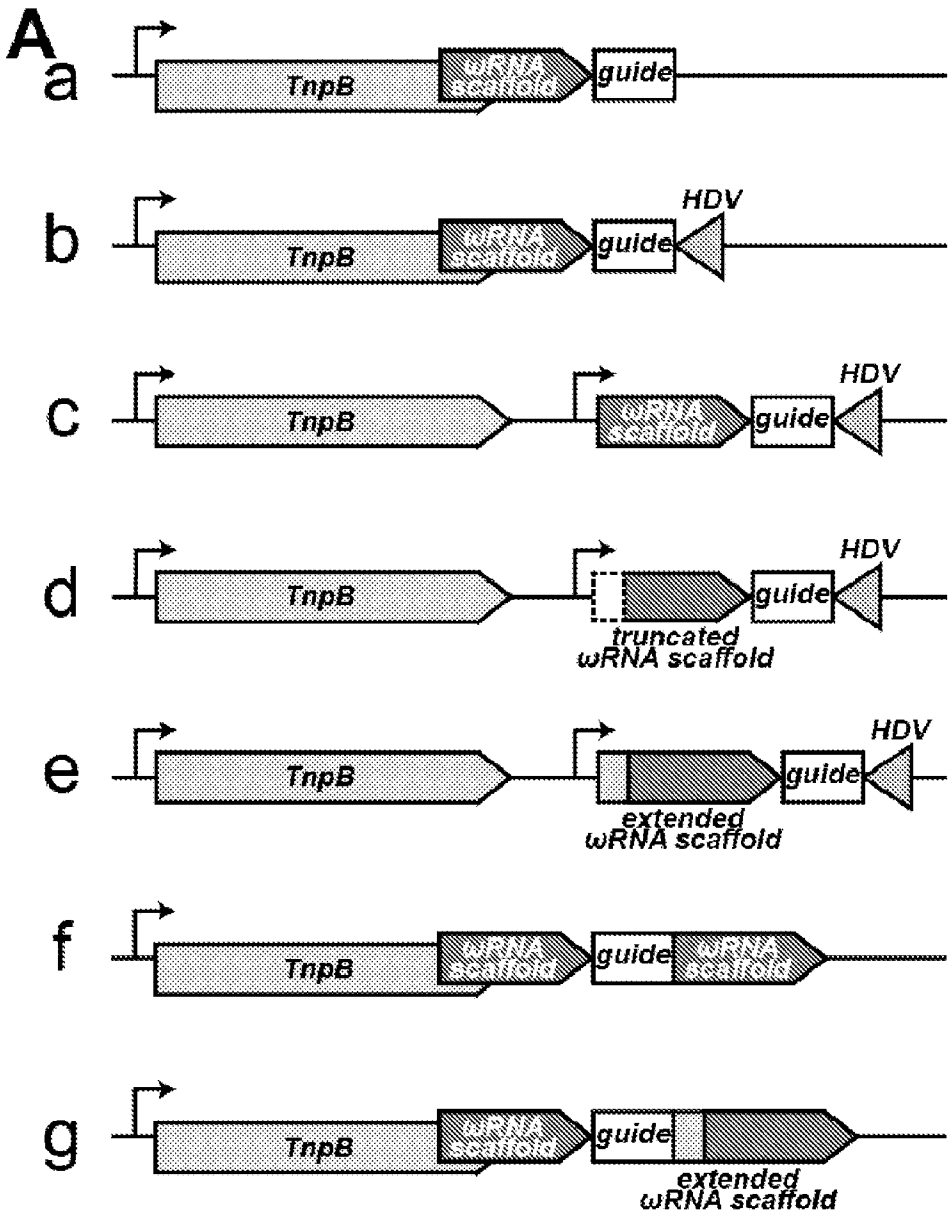

A
a
TnpB
ωRNA scaffold
guide
b
HDV
c
d
truncated ωRNA scaffold
e
extended ωRNA scaffold
f
g

FIG. 27A

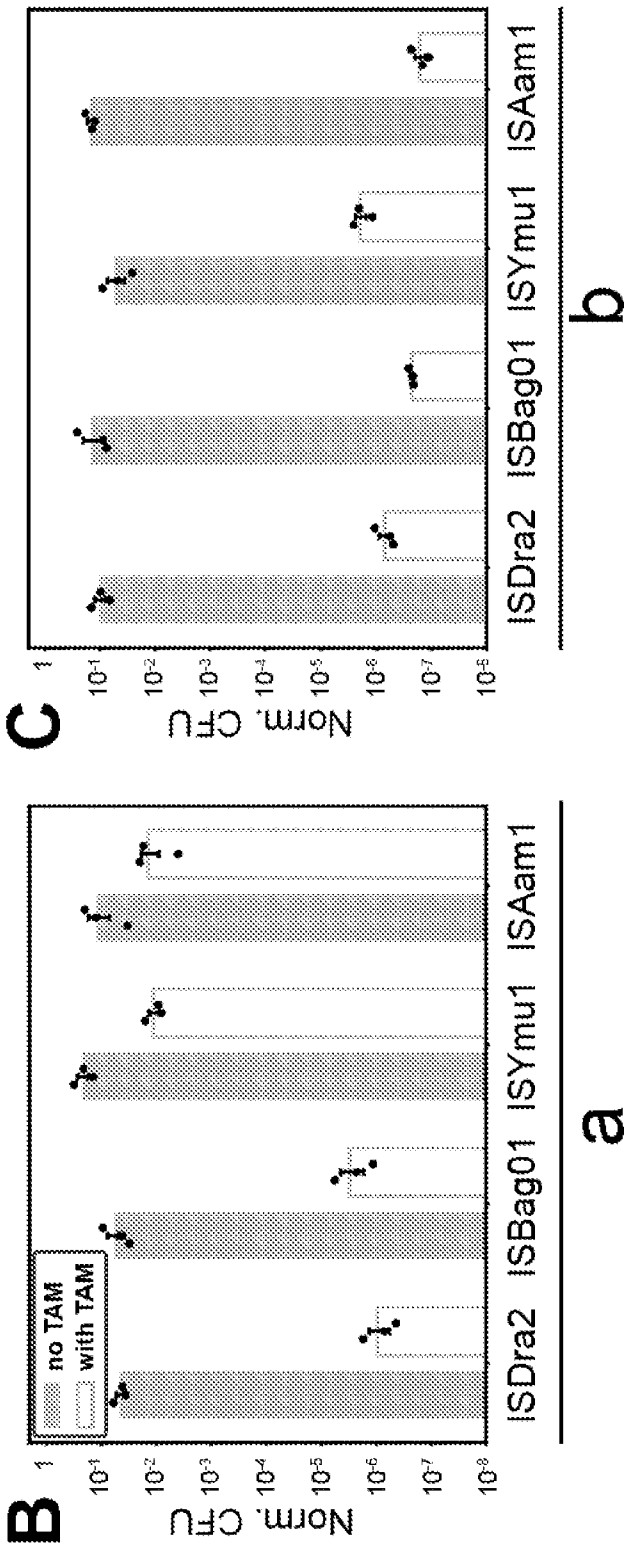


FIG. 27B-27C

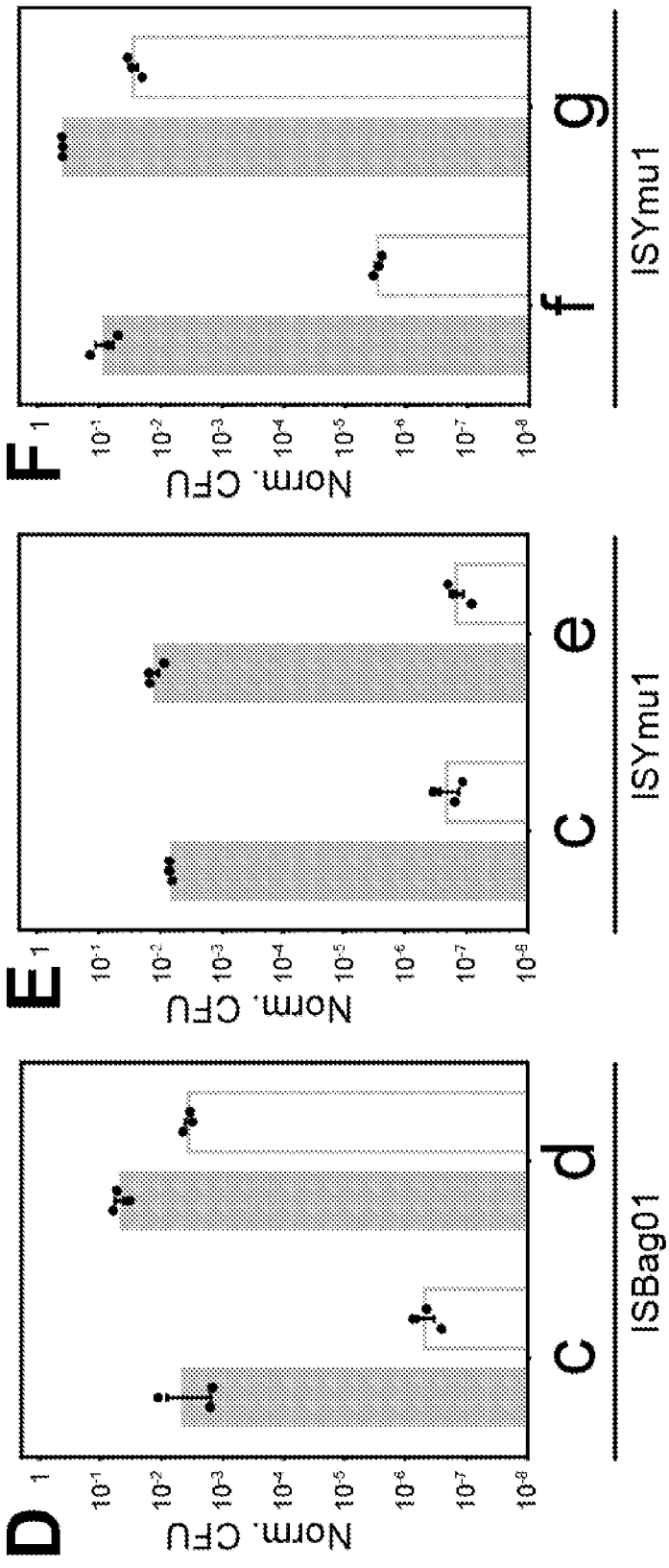


FIG. 27D-F