

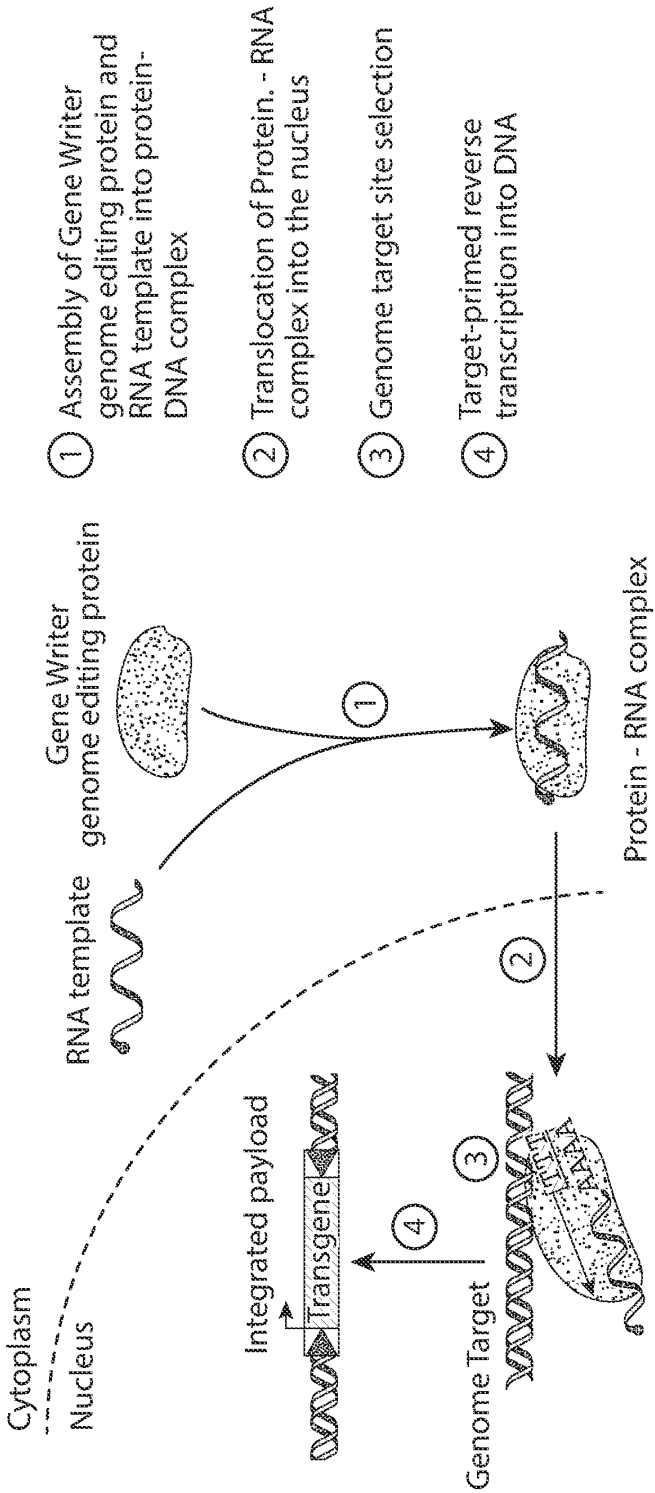


FIG. 1

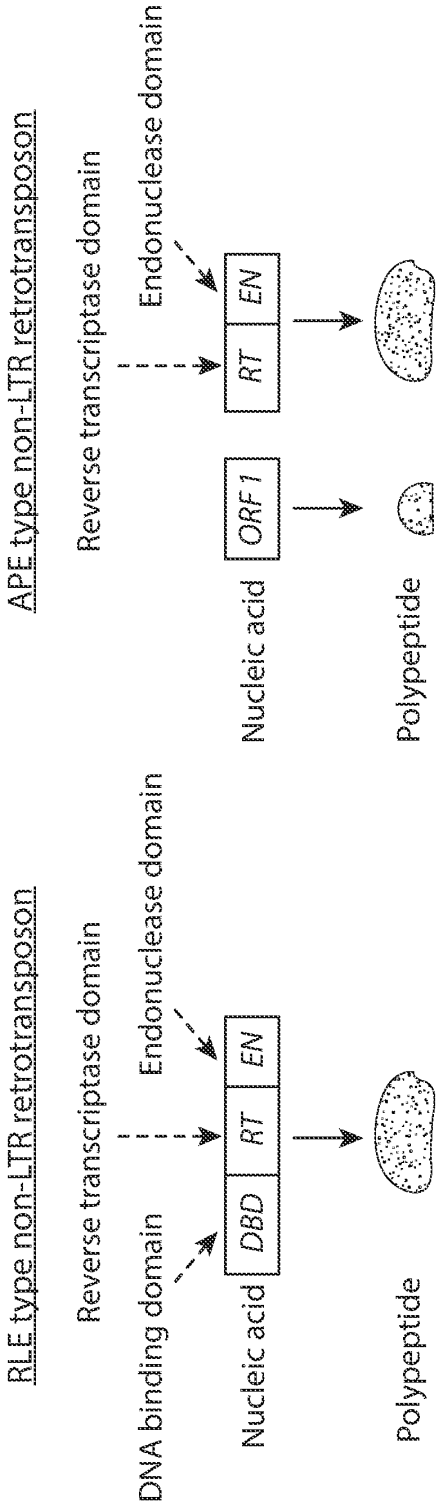


FIG. 2

Examples of Gene Writer Template RNA Structure

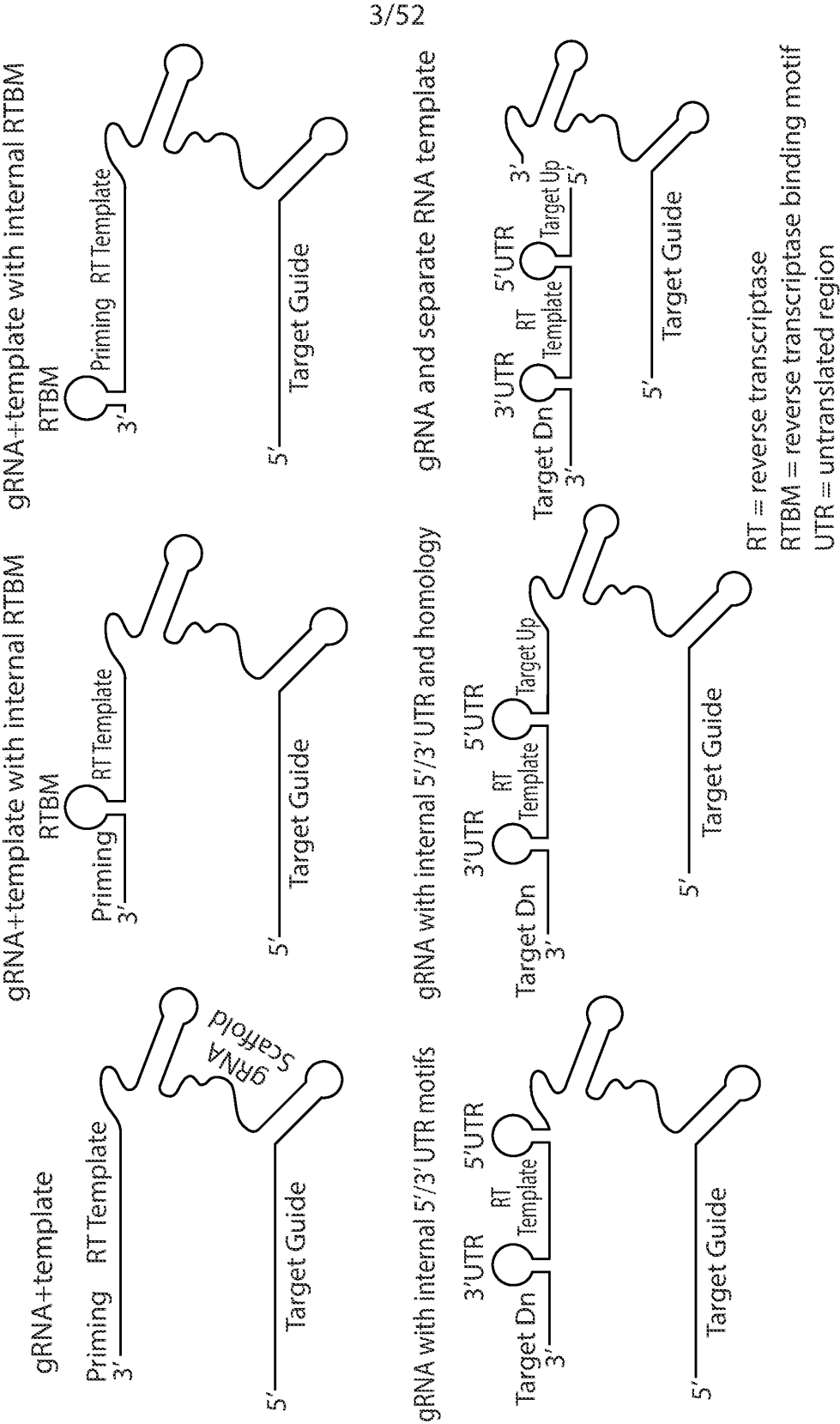


FIG. 3

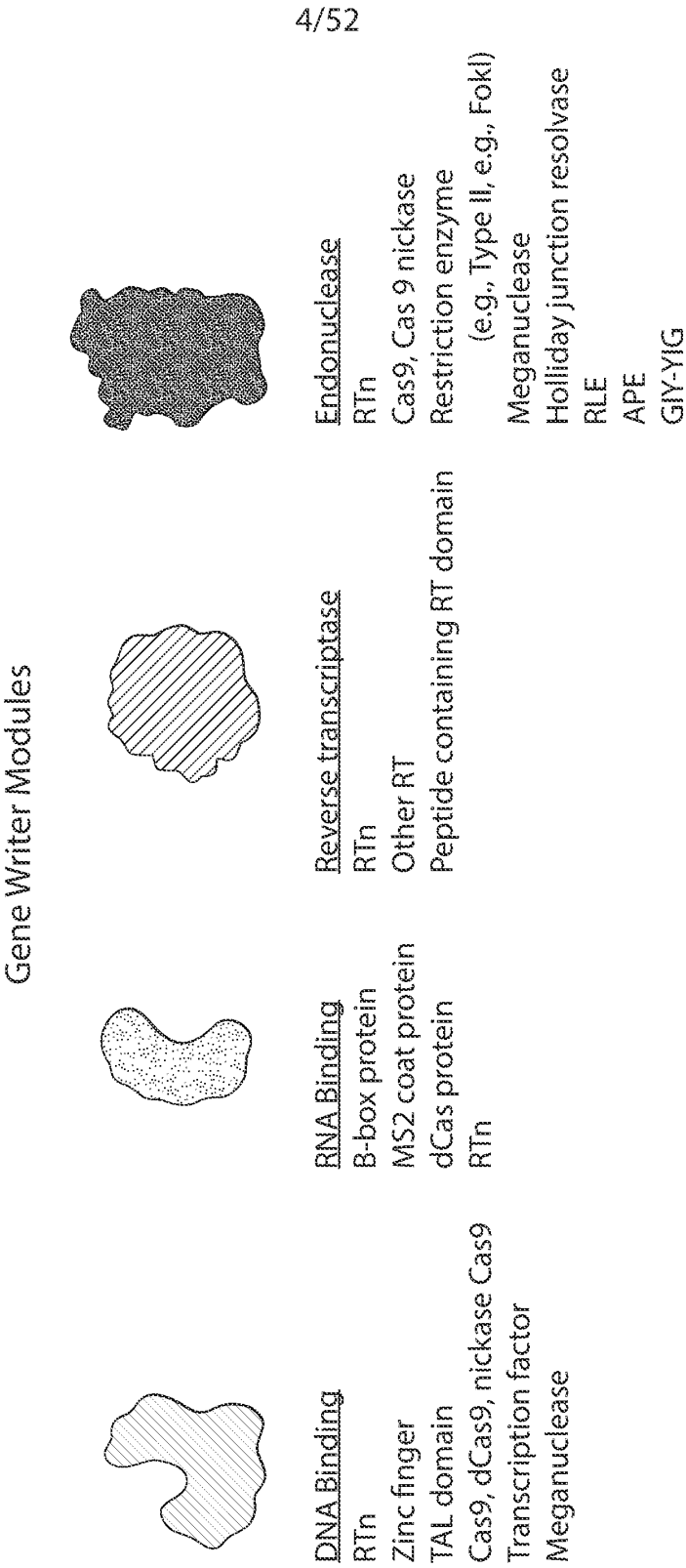


FIG. 4A

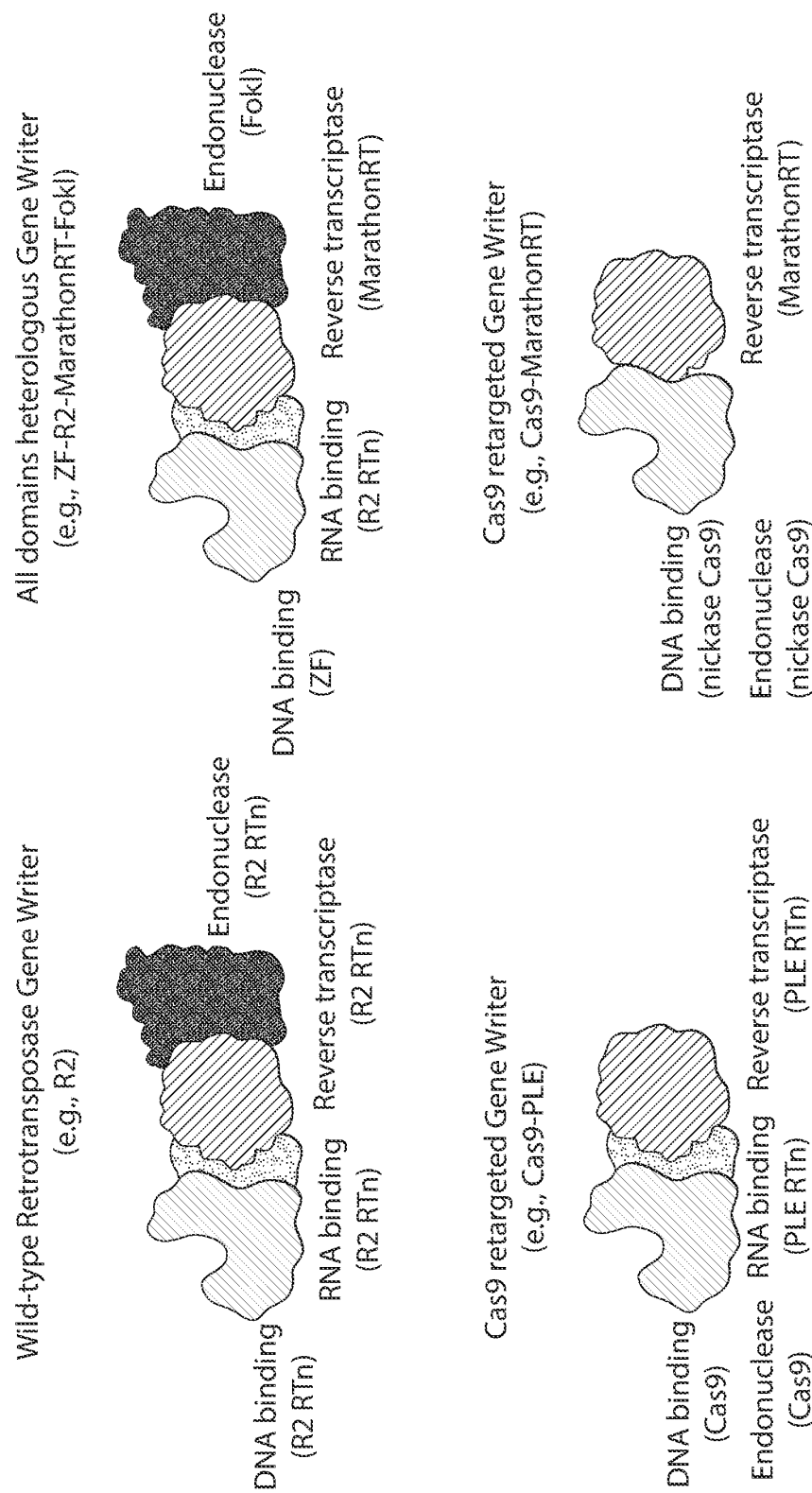


FIG. 4B

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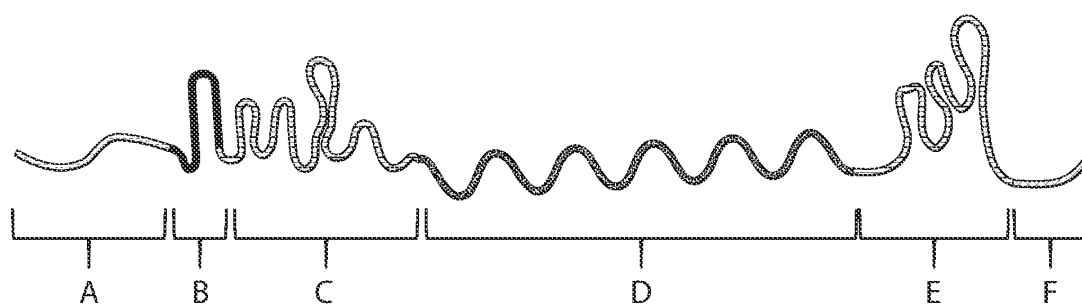


FIG. 5

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| <b>Module</b>                          | <b>Function</b>                                                                                                                                                                                                          |
|----------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>A: 5' homology arm</b>              | The 5' homology arm module is complementary to the DNA sequence 5' to where the GeneWriter system nicks target DNA                                                                                                       |
| <b>B: Ribozyme</b>                     | The ribozyme module is a sub-part of the 5' UTR sequence of the retrotransposase                                                                                                                                         |
| <b>C: 5' UTR</b>                       | The 5' UTR module is the RNA sequence or interacting moiety that the driver of retrotransposase uses in the process of retrotransposition                                                                                |
| <b>D: Heterologous object sequence</b> | The heterologous object sequence is an RNA sequence that serves as the template for the GeneWriter polypeptide to insert a desired payload into the targeted genomic location                                            |
| <b>E: 3' UTR</b>                       | The 3' UTR module is an RNA sequence or interacting moiety that the GeneWriter polypeptide interacts with to bind to the template RNA molecule that is used in whole and/or in part as a template for retrotransposition |
| <b>F: 3' homology arm</b>              | The 3' homology arm module is complementary to the DNA sequence 3' to where the GeneWriter system nicks target DNA                                                                                                       |

FIG. 6

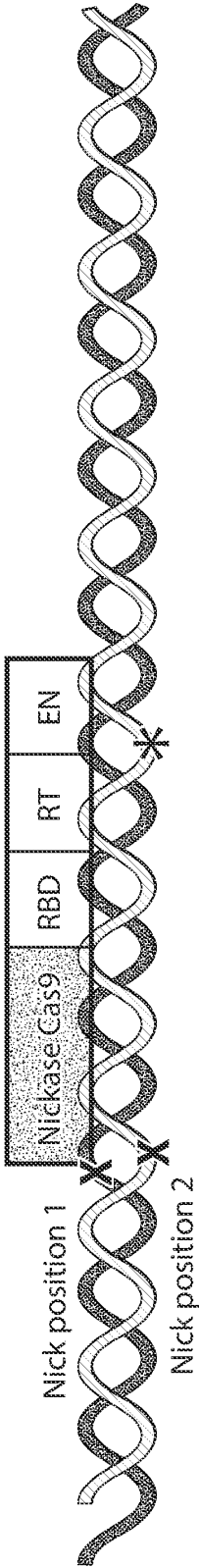


FIG. 7A

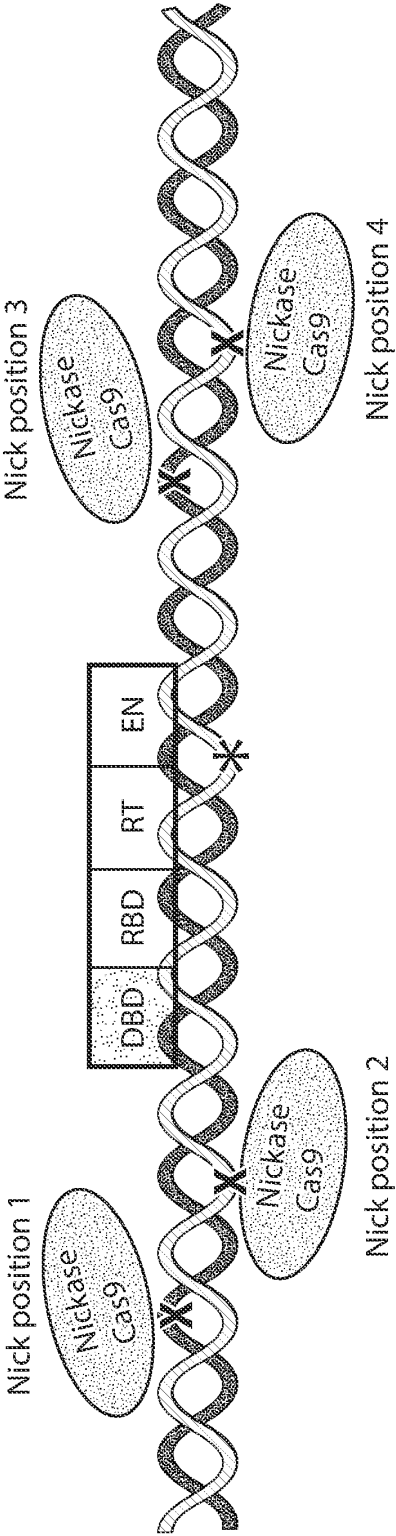


FIG. 7B

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## Natural linker deletion

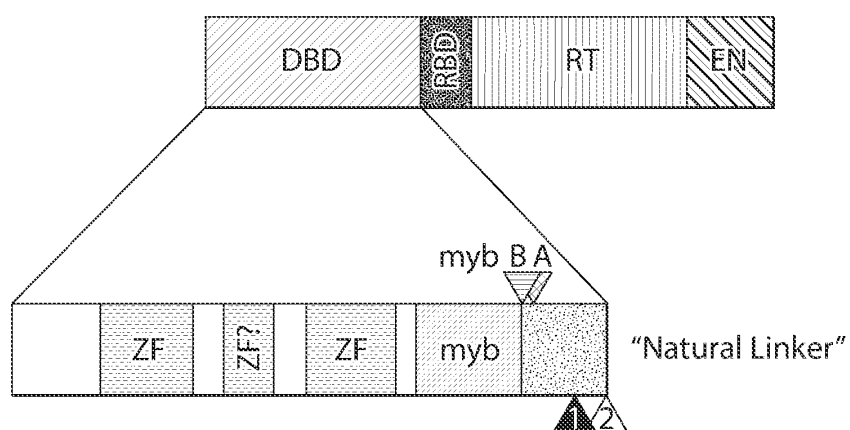


FIG. 8A

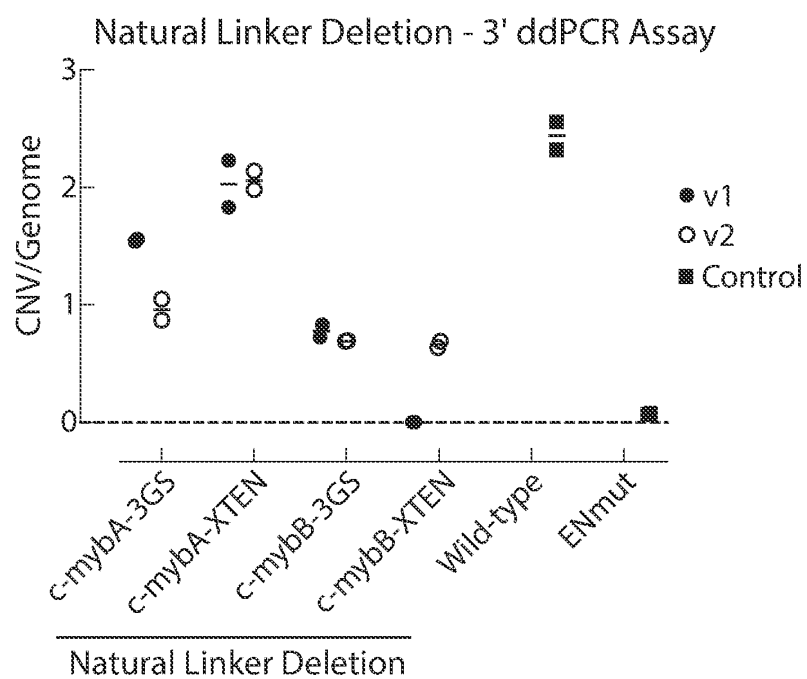


FIG. 8B

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## Landing Pad Figures

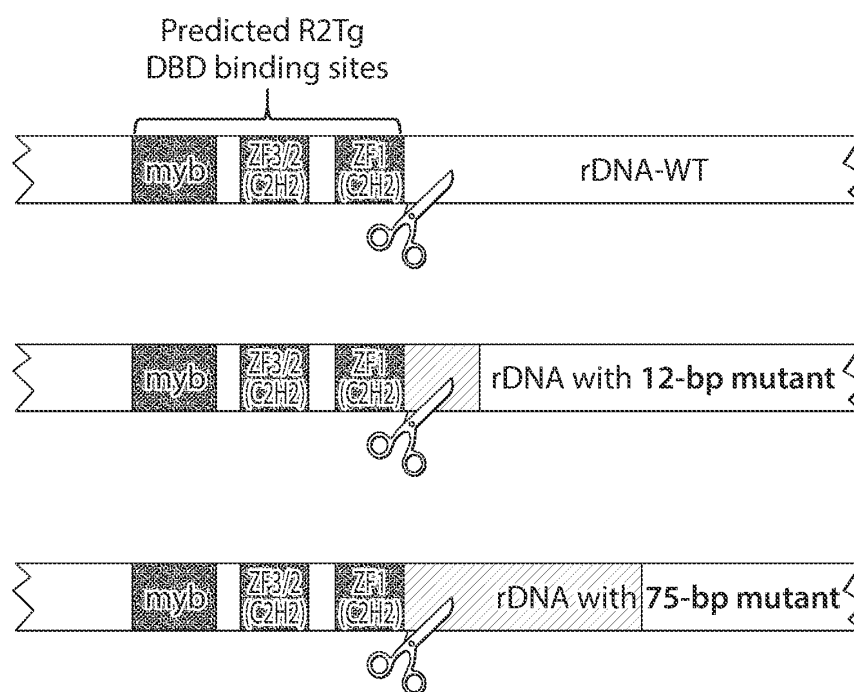


FIG. 9

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## Integration at Mutated R2 site Landing Pads

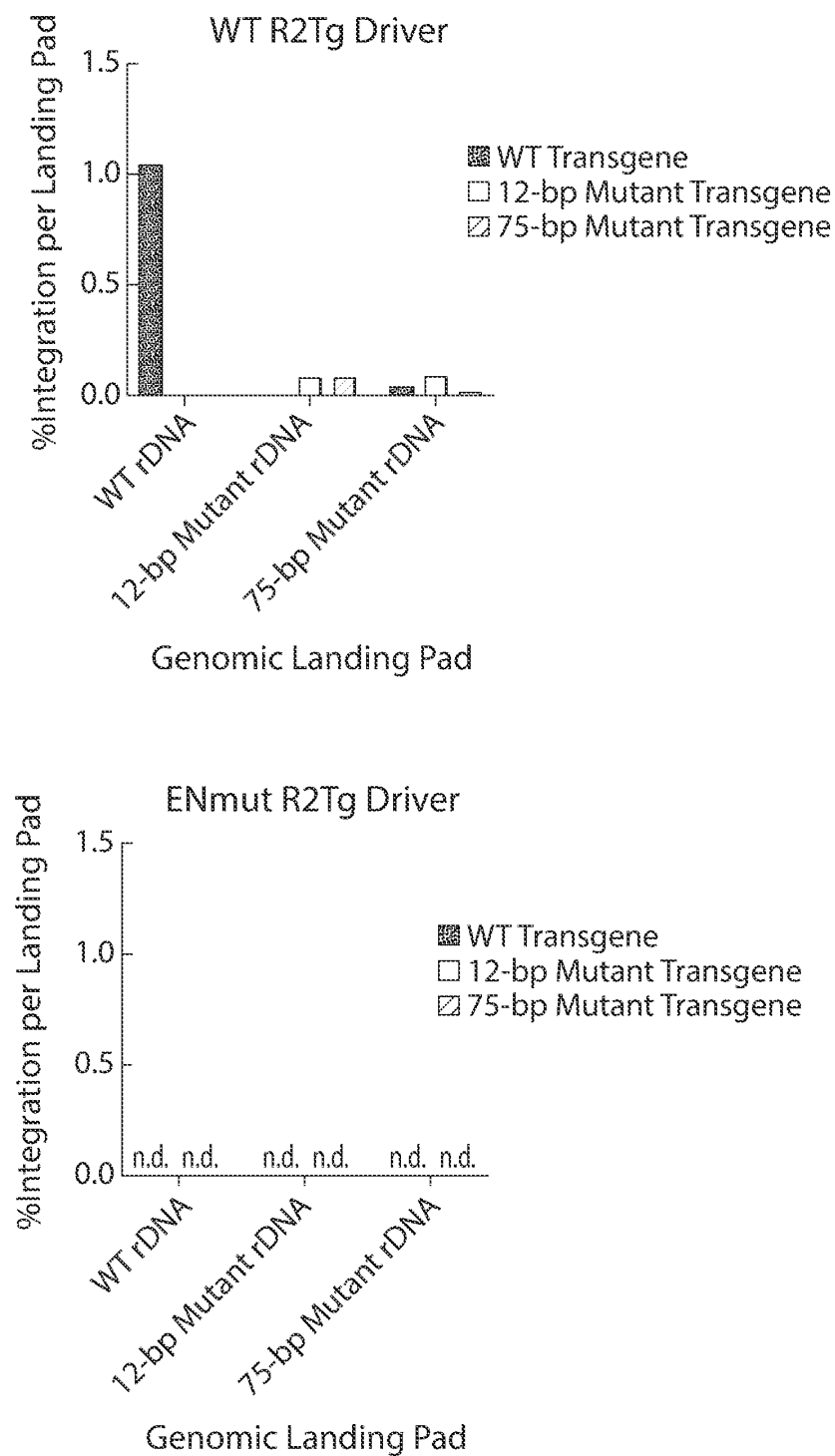


FIG. 10A

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## Indels (Nicking) at Mutated R2 site Landing Pads

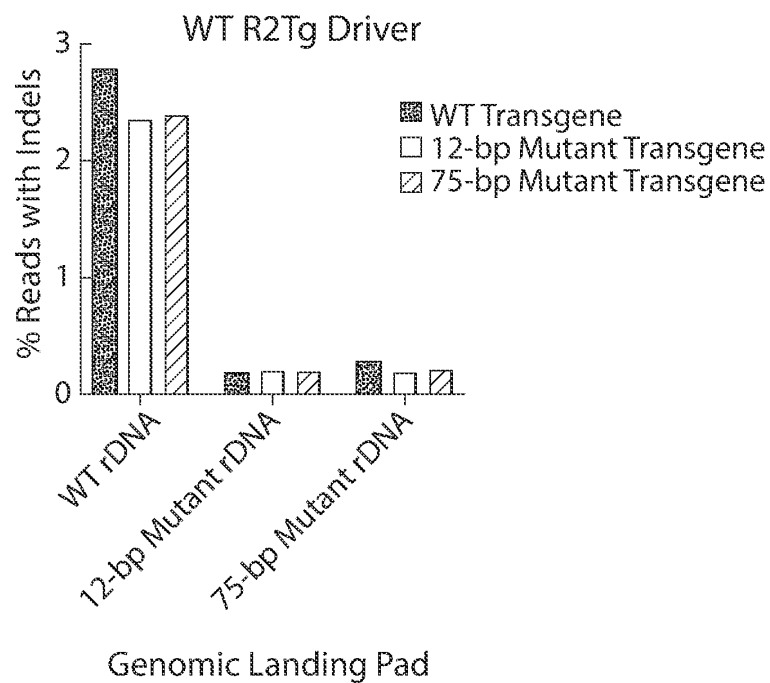
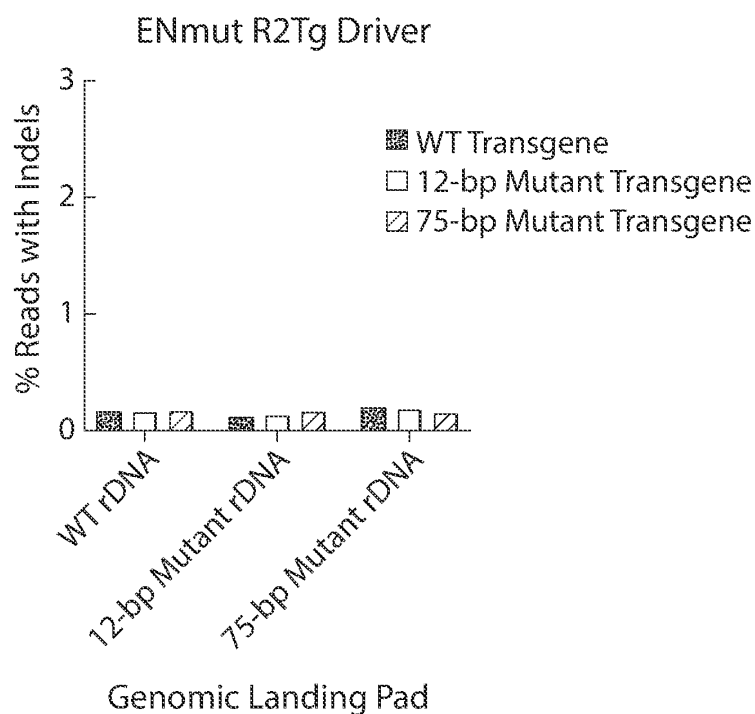


FIG. 10B

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### Examples of Re-targeting Gene Writer polypeptide by replacing DNA binding domain

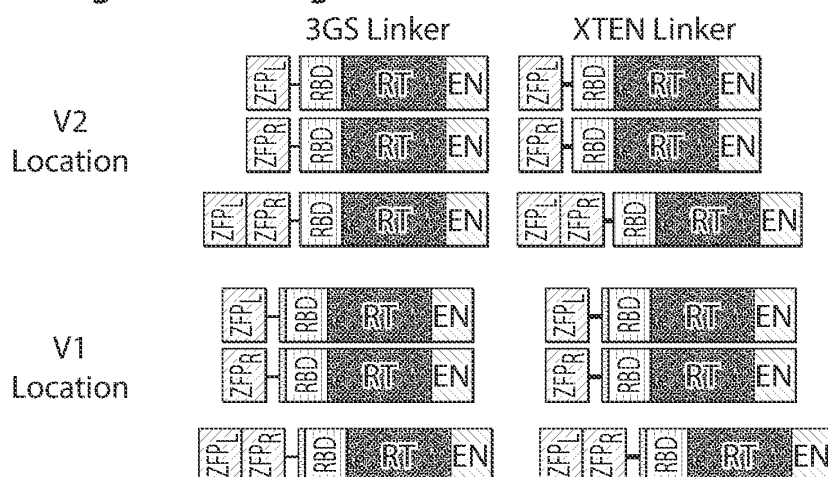


FIG. 11

### Examples of Re-targeting Gene Writer polypeptide by replacing DNA binding domain

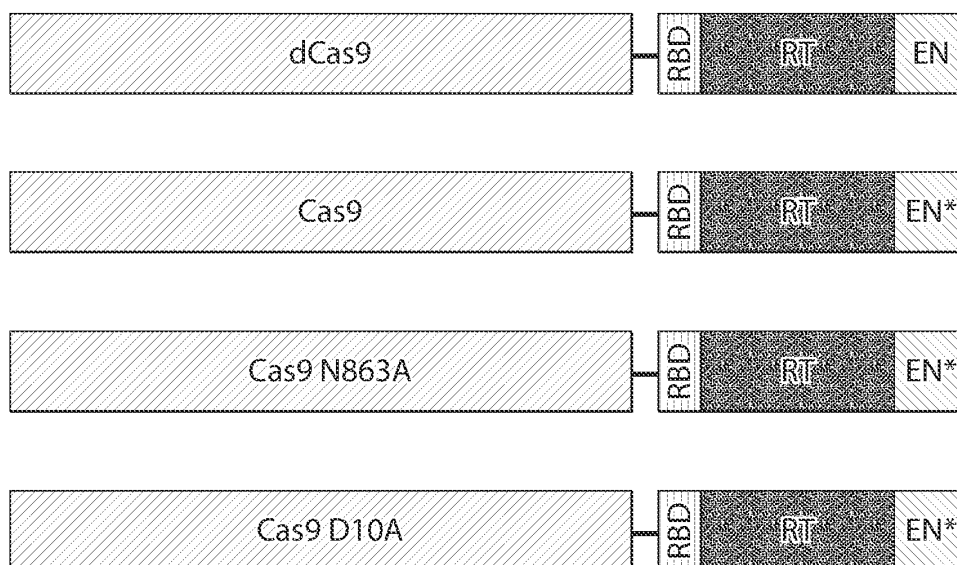


FIG. 12

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Examples of Re-targeting Gene Writer polypeptide by  
Fusion to terminus

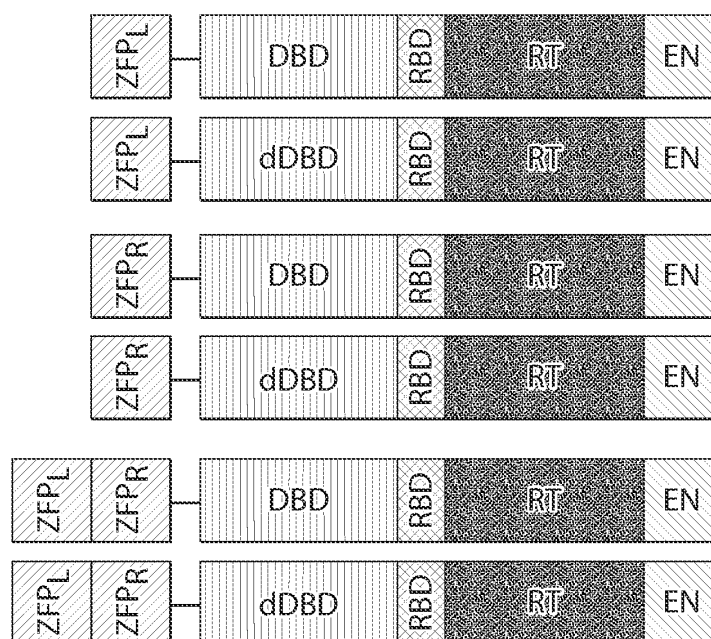


FIG. 13

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## Second Strand Nicking: Cas9-Gene Writer Fusion with 3' extended gRNA donor

### R2Tg Cas9 fusion constructs

Cas9-N863A-R2tg(RBD\*, RT, EN) Gene Writer



Cas9-H840A-R2tg(RBD\*, RT, EN) Gene Writer



Cas9-D10A-R2tg(RBD\*, RT, EN) Gene Writer



dCas9-R2tg(RBD\*, RT, EN) Gene Writer



FIG. 14A

Donor: gRNA extended at 5' end

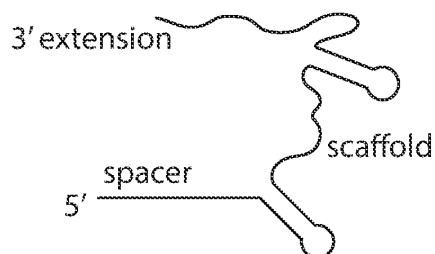
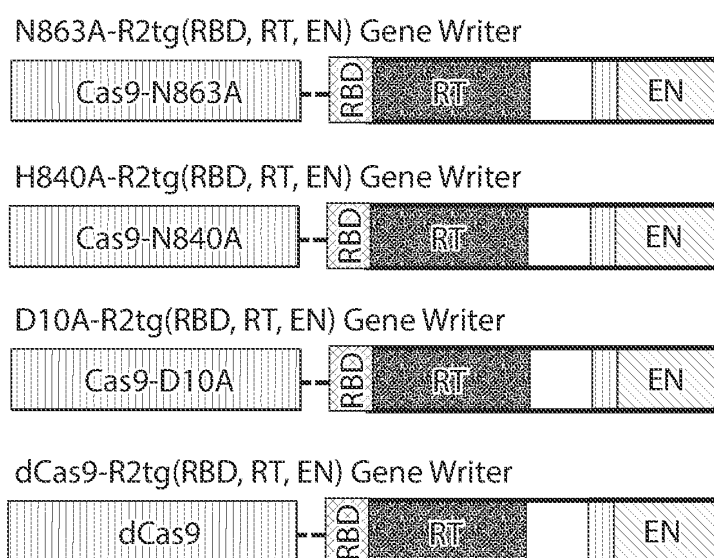
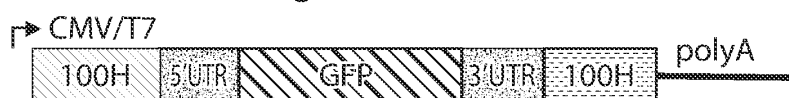


FIG. 14B

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**Second Strand Nicking: Cas9-Gene Writer Fusions with UTR flanked transgene****FIG. 15A****Donor: UTR-transgene****FIG. 15B**

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## Second Strand Nicking: Cas9 Nickase



FIG. 16A

## Donor: UTR-transgene



FIG. 16B

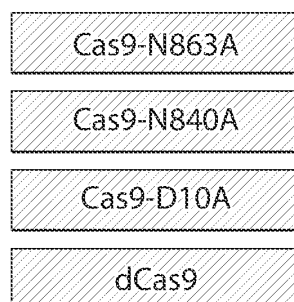


FIG. 16C

Improved Gene Writer polypeptide expression



FIG. 17A

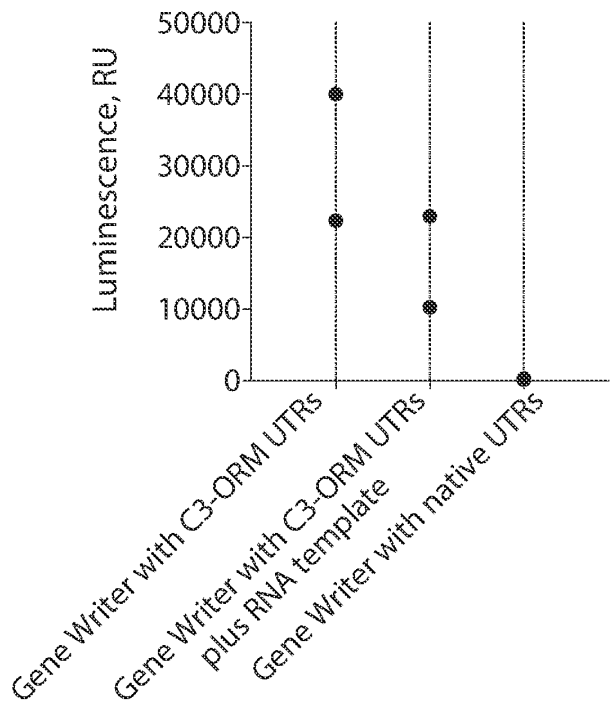
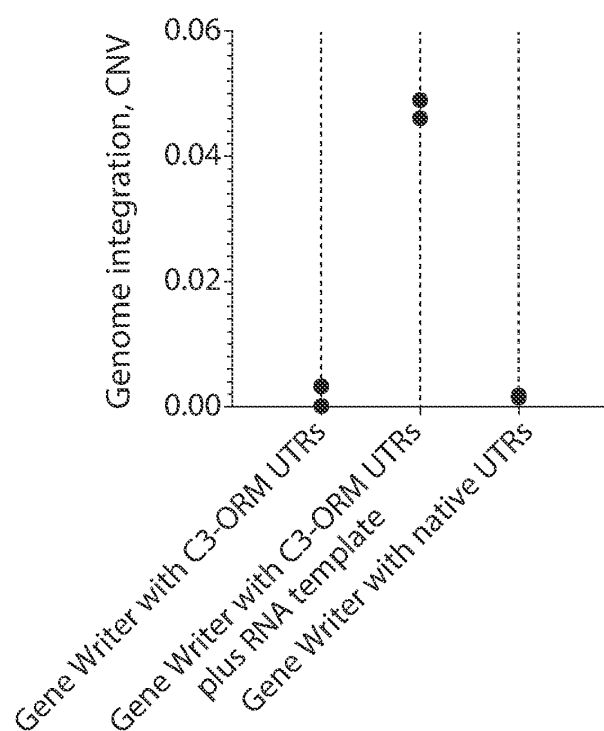
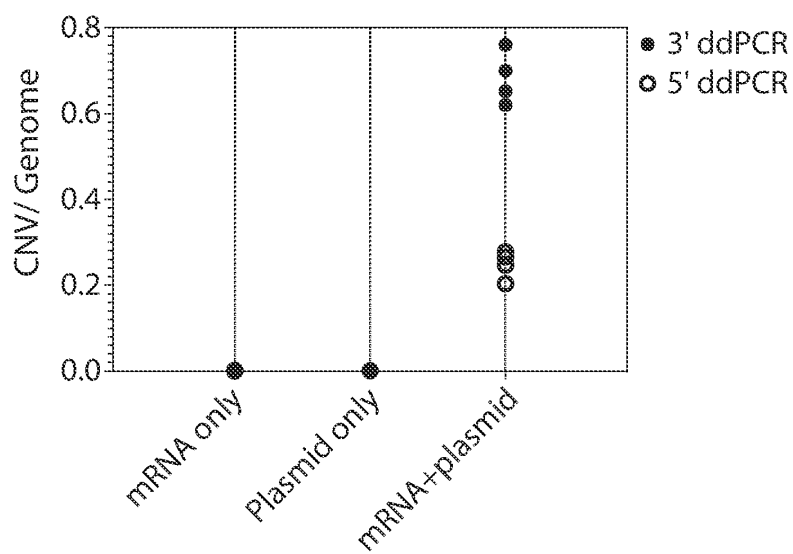
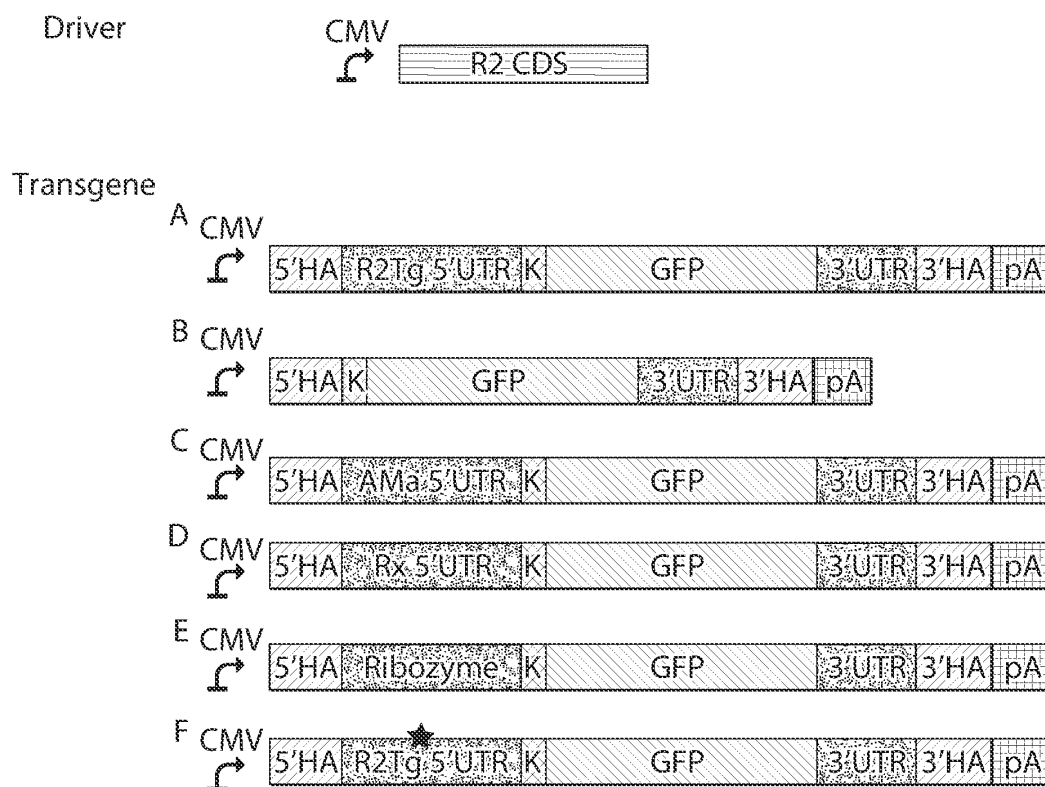


FIG. 17B

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**Improved Gene Writer polypeptide expression****FIG. 18****Mixed RNA/DNA delivery example****FIG. 19**

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**Engineering 5'UTR****FIG. 20**

Introns in template RNA

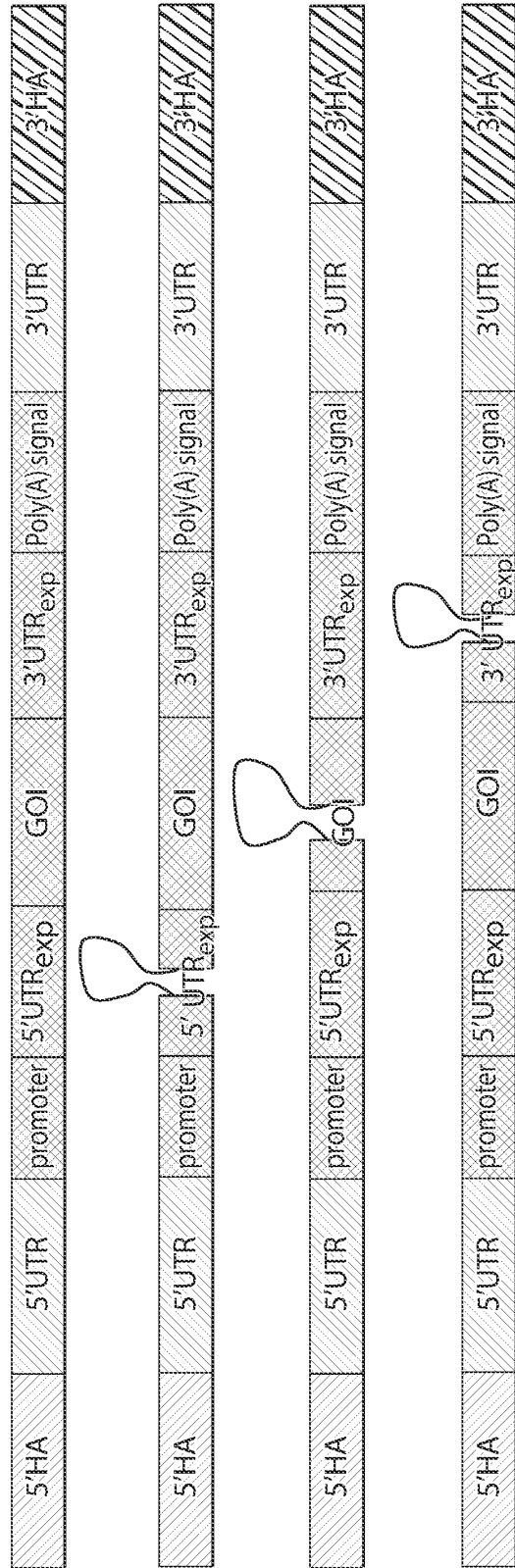


FIG. 21

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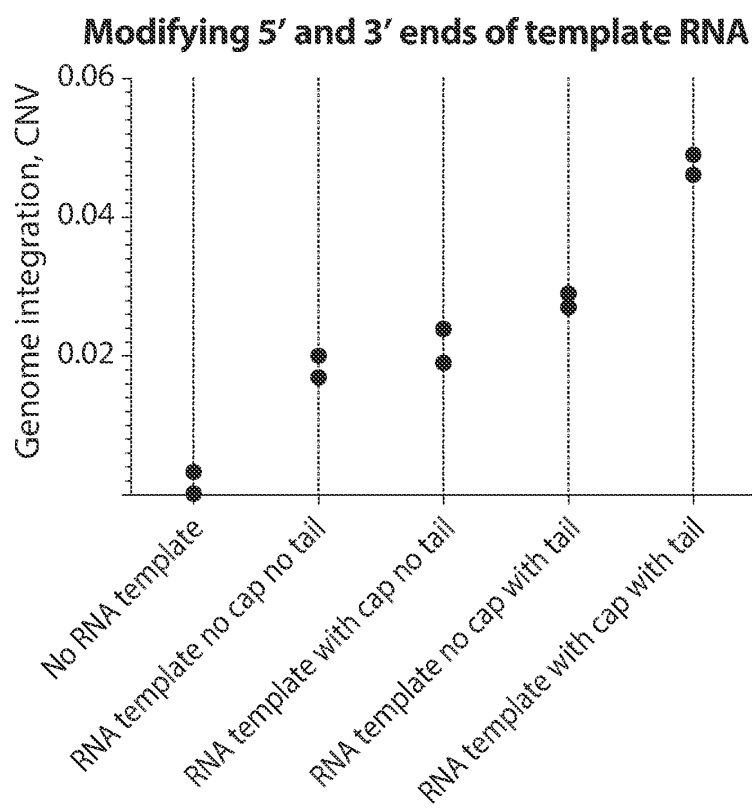


FIG. 22

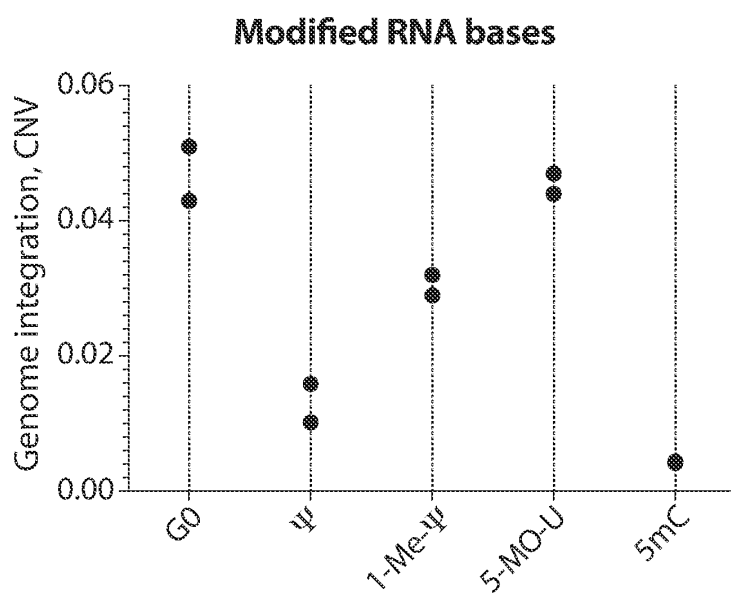


FIG. 23

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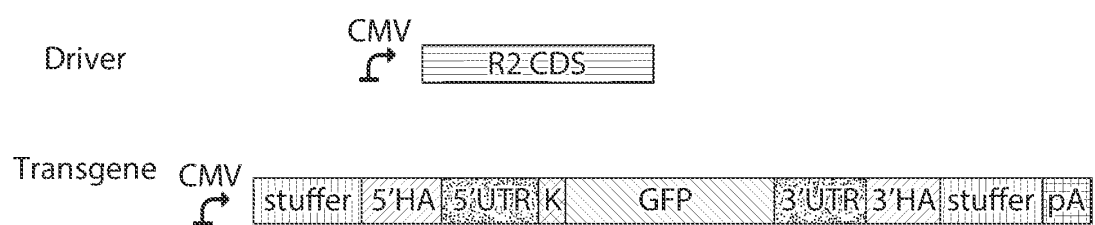
**Driver and transgene for HA experiments**

FIG. 24

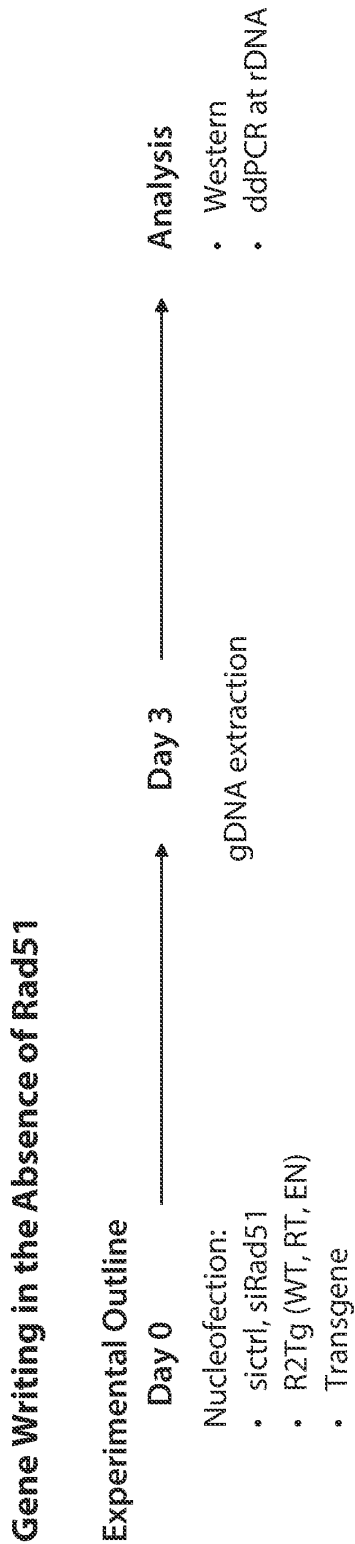


FIG. 25A

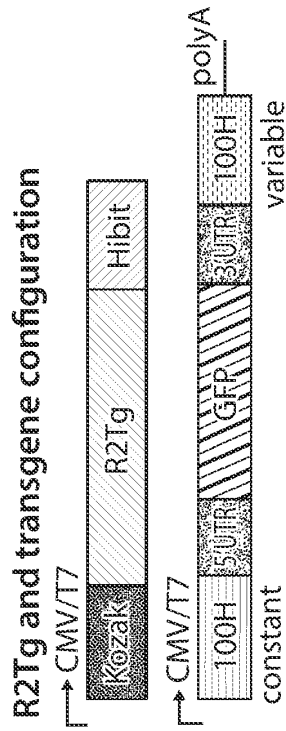


FIG. 25B

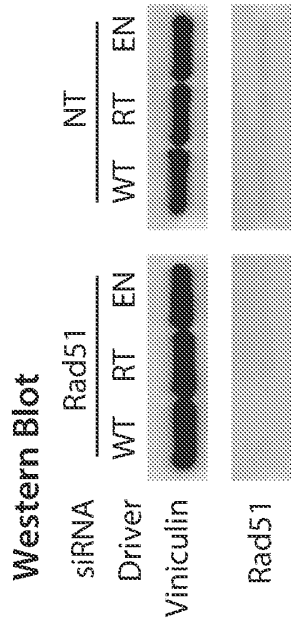


FIG. 25C

Gene Writing in the Absence of Rad51

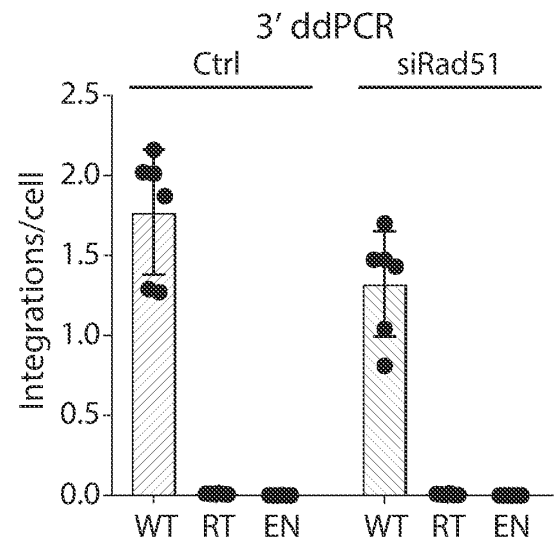


FIG. 26A

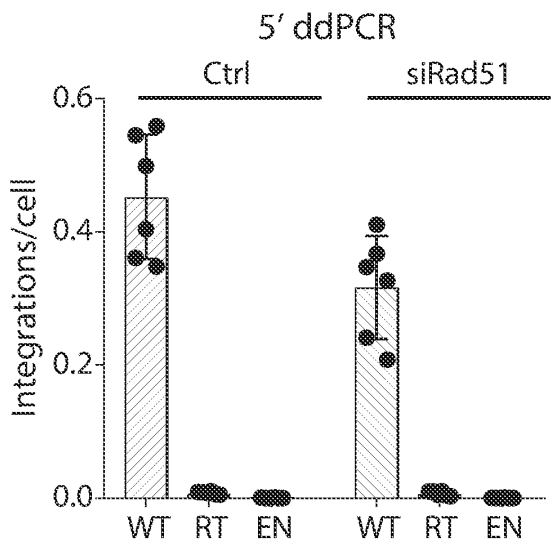


FIG. 26B

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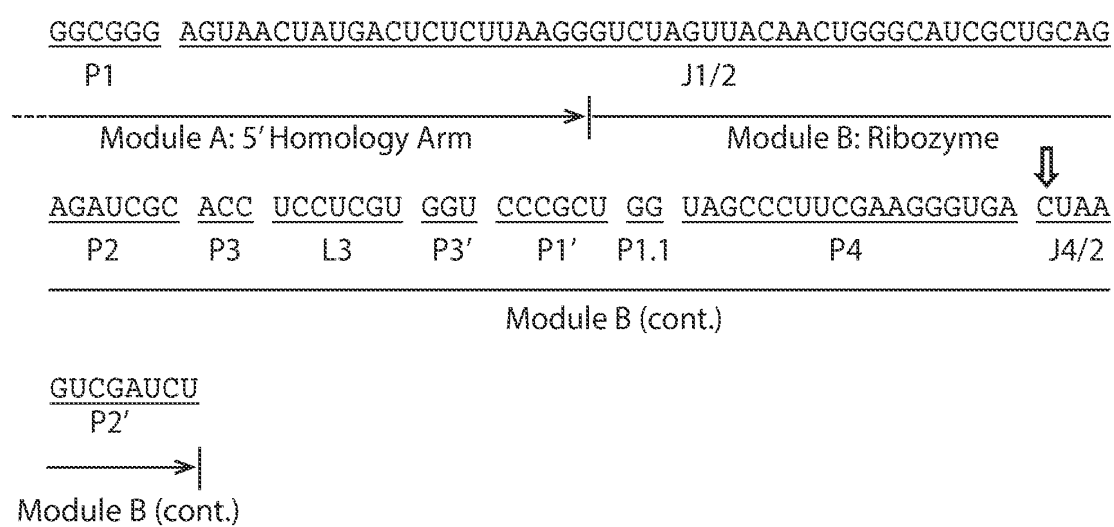
**R2Tg Ribozyme**

FIG. 27A

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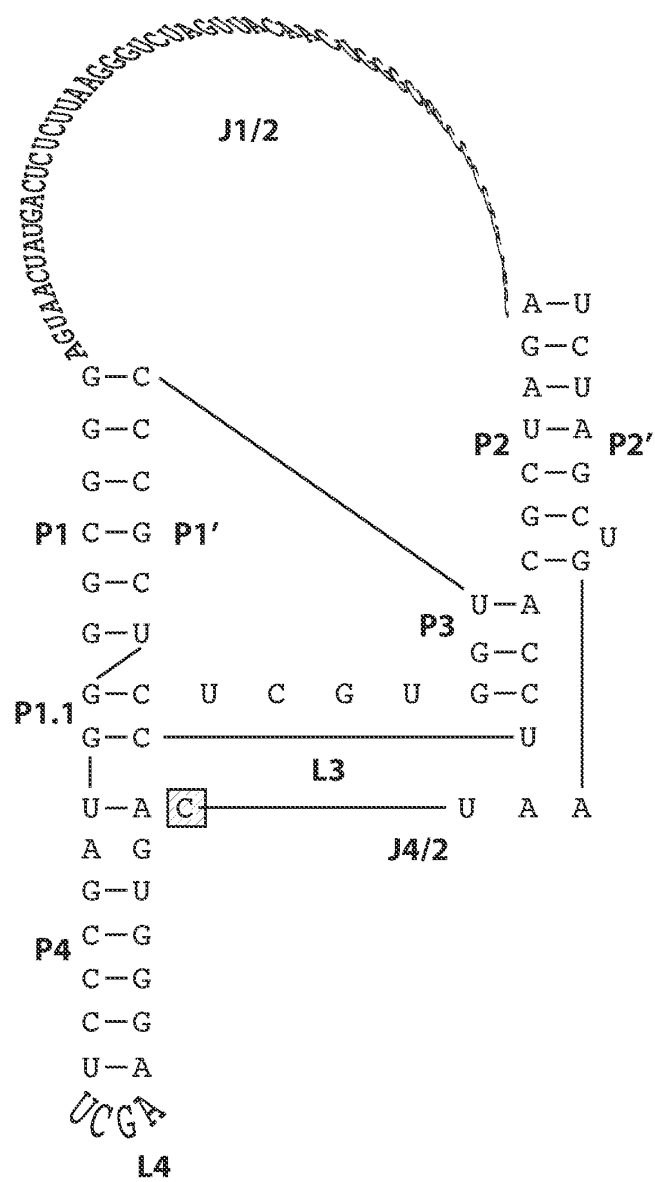


FIG. 27B

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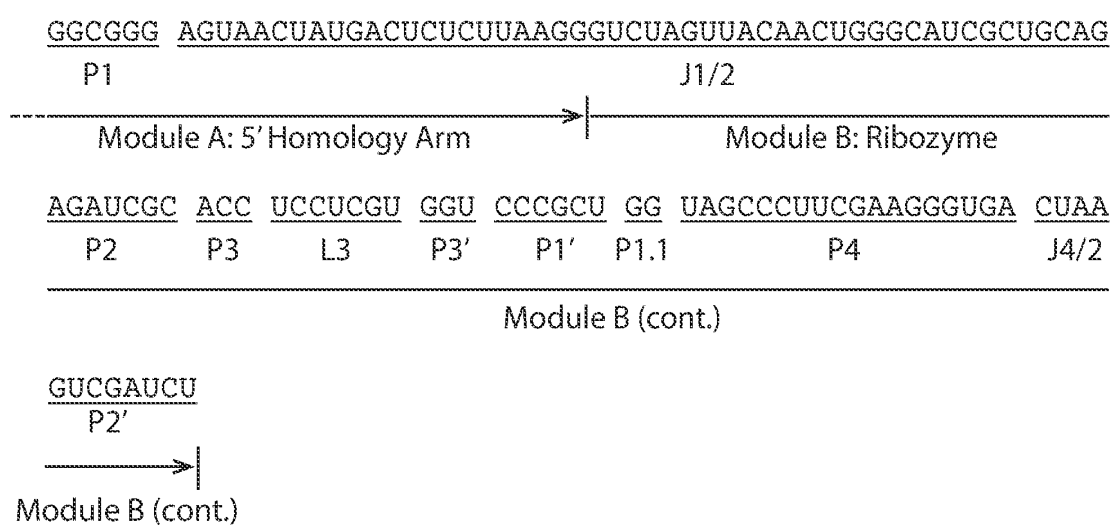
**R2Tg Ribozyme Sequence**

FIG. 28

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## Predicted Secondary Structure of R2Tg Ribozyme

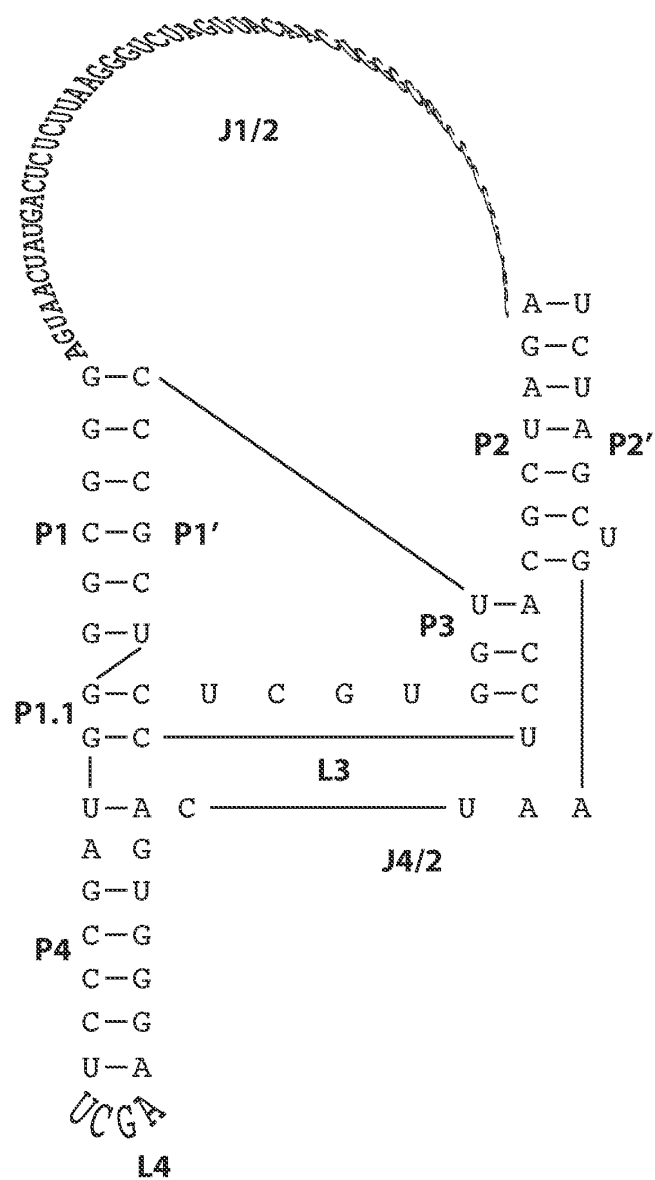


FIG. 29

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5' -XXXXXXXXCATCATCATCATCATCATCATCATCATCATCA TXXXXXXXXX-3'  
3' -YYYYYYYGTAGTAGTAGTAGTAGTAGTAGTAGTAGTAGTAYYYYYYYY-5'

5' -XXXXXXXXCATCATCATCATCATCATCATCATCATCATXXXXXX-3'  
3' -YYYYYYYGTAGTAGTAGTAGTAGTAGTAGTAGTAGTAGTA-3' 5'

RNA template

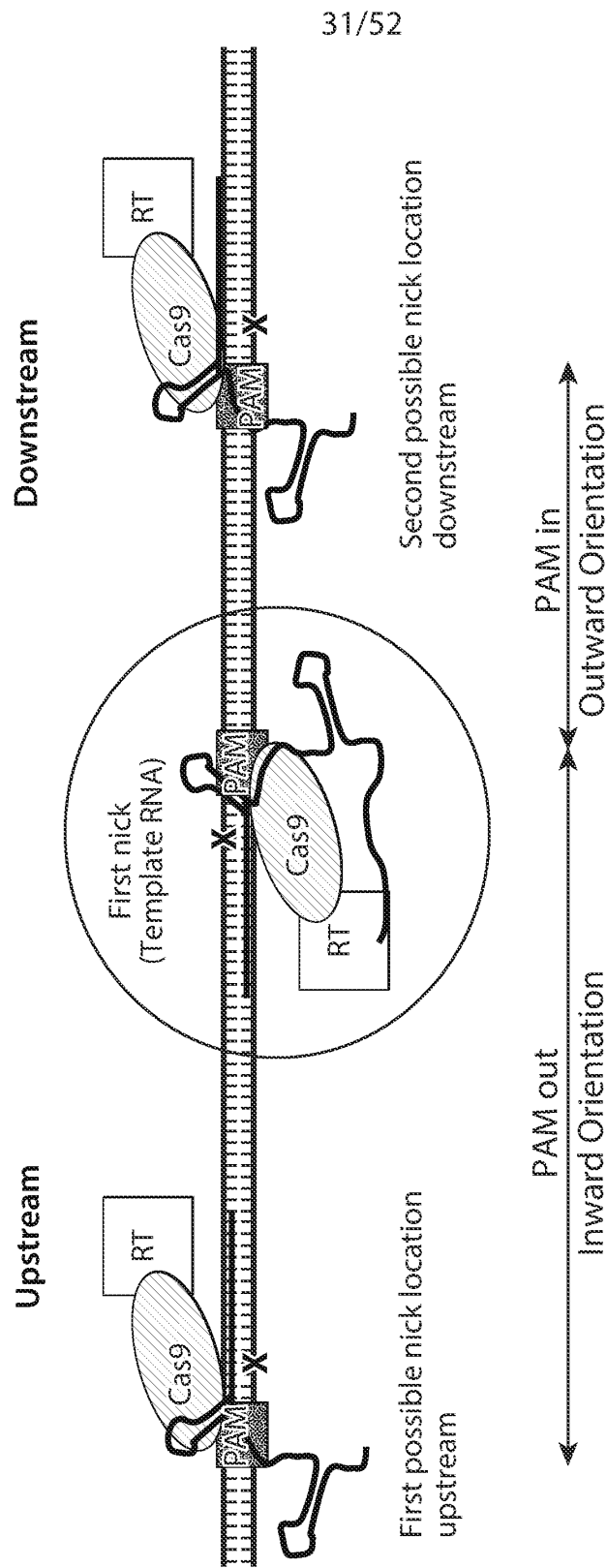
AGTAGTAGTAGTAGTAGTAGTA  
NA template  
5' -XXXXXXXXCATCATCATCATXXX-3'  
GTAYYYYYYY-5'  
←TPRT

5' -XXXXXXXXCATCATCATCATCATCATCATCATCATCATXXXXXX-3'  
3' -YYYYYYY | 3' -YYYYYYYGTAGTAGTAYYYYYYY-5'

5'-XXXXXXXXCATCATCATCATXXXXXX-3'  
3'-YYYYYYYGTAGTAGTAGTAYYYYYYY-5'

FIG. 30

**“PAM-in” and “PAM-out” orientations for second nicking**



A template RNA is directed to the HEK locus to introduce a CTT insertion mediated by the Gene Writer polypeptide. The first nick is indicated with a red X. To enhance the installation of the desired edit, a second nick can be placed on the opposite strand of the first nick. The second nick can be placed upstream or downstream of the first nick. In this example, the PAM orientation is “PAM-out” if the second nick is targeted upstream or “PAM-in” if the second nick is targeted downstream, with respect to the first nick.

**FIG. 31**

**Optimizing PAM orientation and distance between nicks to reduce undesired editing outcomes**

"PAM-in" orientation leads to reduction in undesired editing outcomes resulting from the occurrence of dual nicks

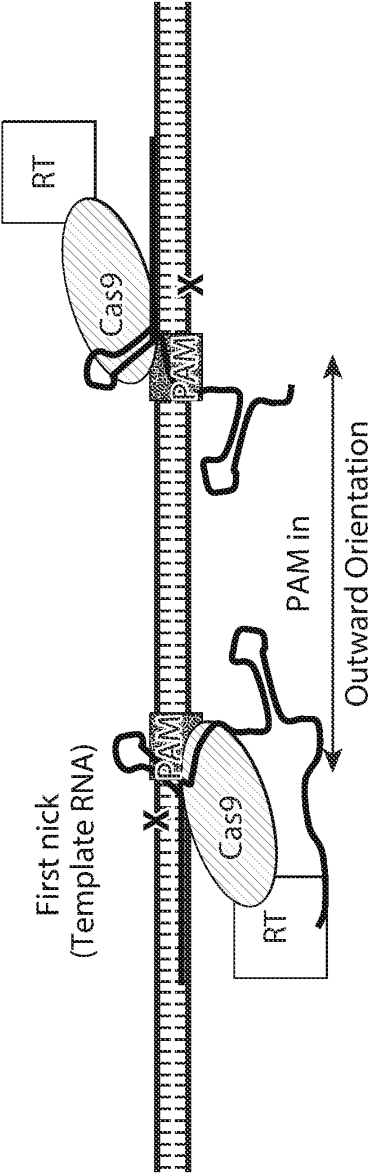


FIG. 32A

"PAM-out" orientation leads to reduction in undesired editing outcomes when the two nicks are separated by an increased distance

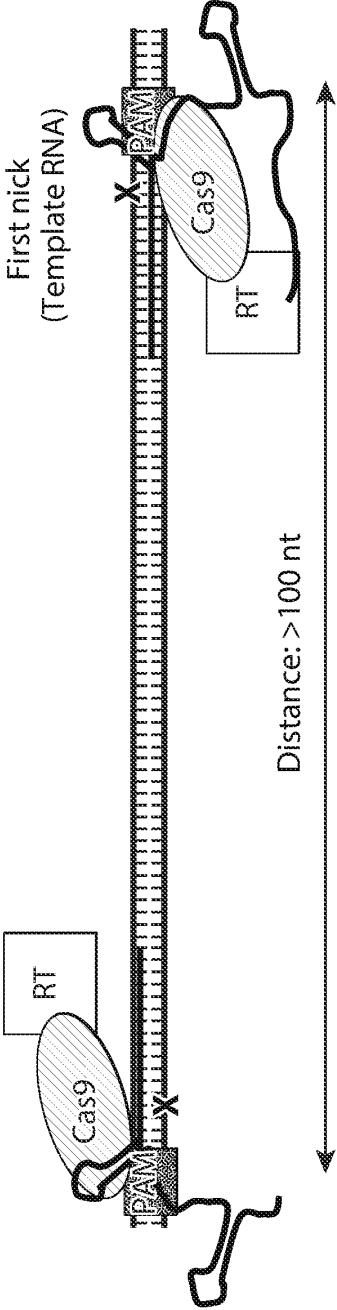


FIG. 32B

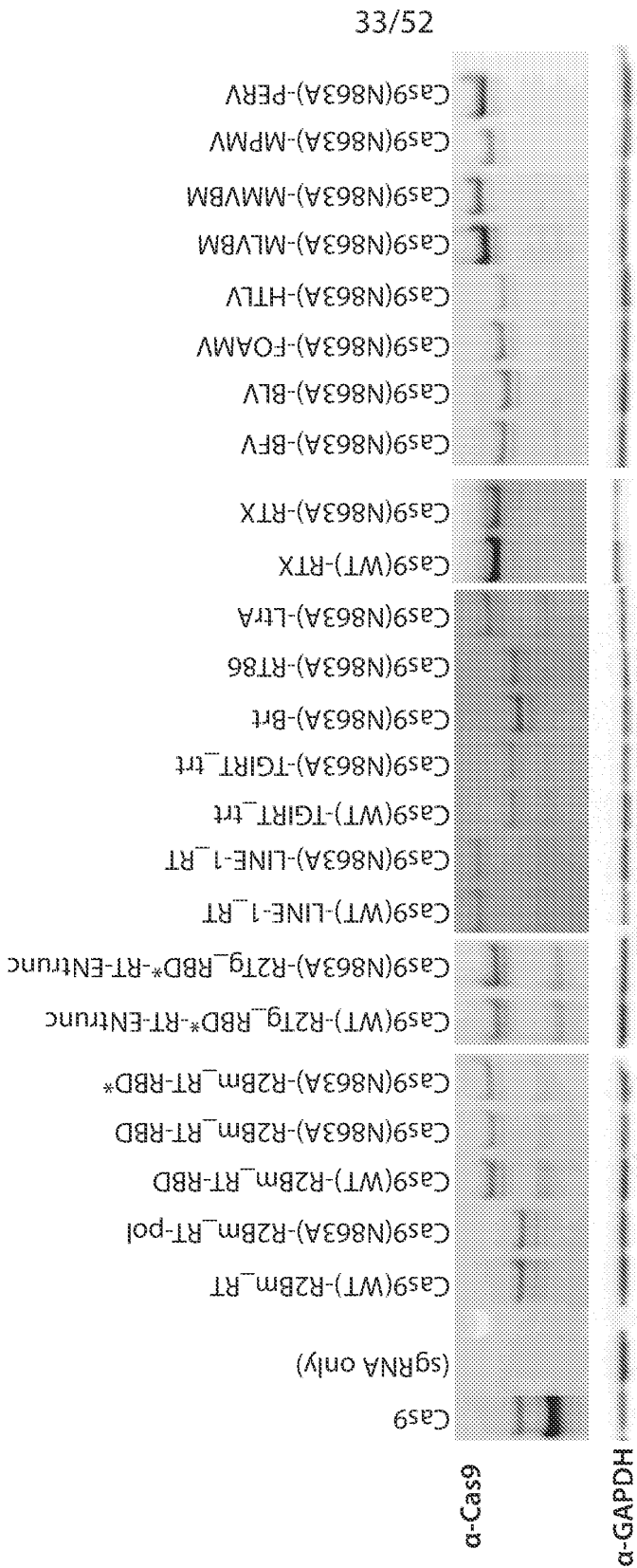
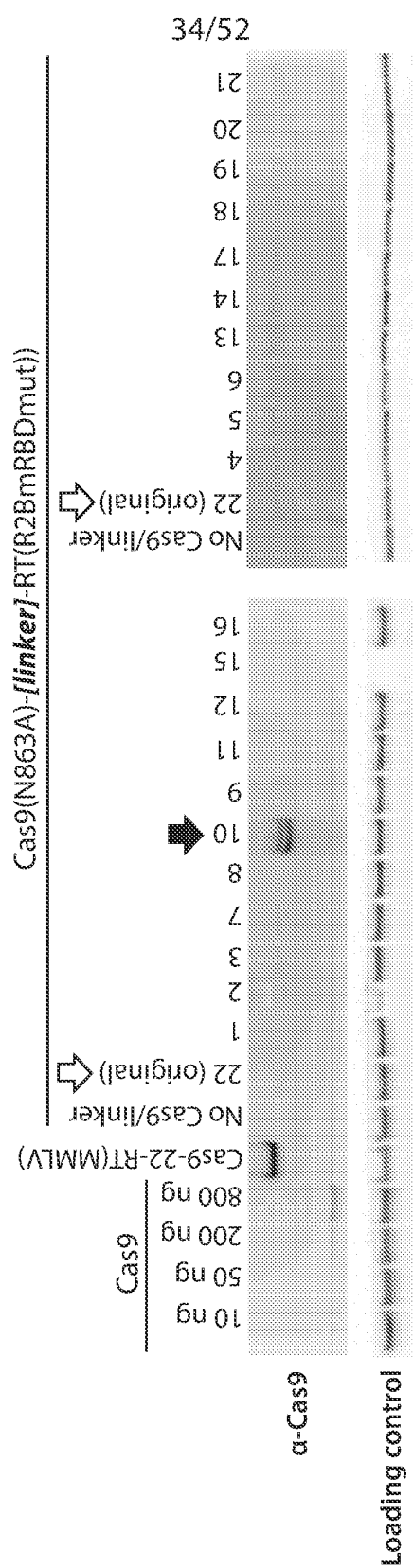


FIG. 33



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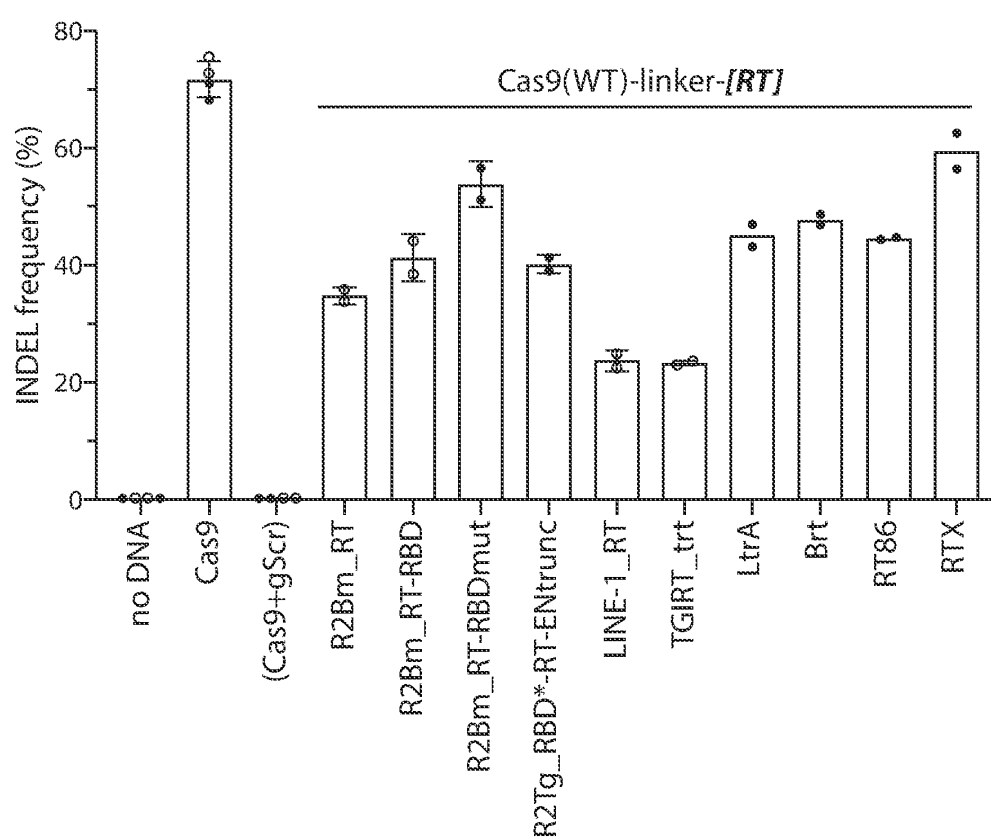


FIG. 35

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| D200N     |                                  | T330P                             |                                  | L603W |  |
|-----------|----------------------------------|-----------------------------------|----------------------------------|-------|--|
| Consensus | WTVLPQGFKNSTPLFDEALARDLADRFRIQHP | WIPGFAELLXPLVLLKGTDP--XISLXPE     | FATAHXLG-----G-LTSAGXIKKXEL      |       |  |
| Gamma     | R.....H.....                     | MAA.....T..G.....--LFWNG.D        | ..I.EIYRRR.L...E.E....D...       |       |  |
|           | R.....H.....                     | MAA.....T..G.....--LFWNG.D        | ..VH.EIYRRR.L...E.E....N...      |       |  |
|           | R.....N.....OG.LD..              | AO.....AATR.GN....--LVNGEK        | ..I.VH.MIYRRR.L...AG.A...AP...   |       |  |
|           | R.....I.....N.....               | FAA.....T..EKEG....--F.WA.        | ..VH.AIKYOR.L...RE....E...       |       |  |
| Epsilon   | R.....H.....                     | MAA.....T..G.....--LFWNG.D        | ..I.EIYRRR.L...E.E....S...       |       |  |
|           | ..I.H.....SO.YOS.HKIKFKIS        | ..E.SHSKFT.BKO..RD.A--EPFQ.DDO    | YGVV.DF.HLMWHR.FV....TP...HK..E  |       |  |
|           | R.....I.....A.....               | CT.TYRRP.OL..SS..LD..RAI.H.S.     | YSLRT.A-----VLG.WLOPDPVPSYA      |       |  |
|           | R.....EMO..HI.OPI.OAF.           | CT.TLROP.HS..CA.OMH..RDO.Y.N.S    | YHYLRT.A-----LGTFOGRSSOAPFO      |       |  |
| Delta     | R.....EMO..HI.OPI.OAF.           | CT.TLROP.HS..CA.OMH..RDO.Y.N.S    | YHYLRT.A-----LGTFOGRSSOAPFO      |       |  |
|           | R.....EMO..AV.NPM.KMF.           | CT.ILRKH.OS..SA.H.VR..RAC.T.T.O   | IKYL.S.A-----IG.FLGTSAGOTLO      |       |  |
|           | R.....MA.....COKYV.TAIHKV.HAWK   | YKLTTGD.K..FDT..DS..NSHR..SK.     | AKSIFL.E-----TVAQI.H.SETAKLF     |       |  |
|           | K.....M.....COKYVDKAILTV.DKYQ    | FLKLTGTE.K.FEI.N.DSN.ISTRK.T..    | TCLFPEIE-----ATLSPTKIYTE.        |       |  |
| Beta      | R.....MA.....COKYVDKAILTV.DKYQ   | YKLTPISA.V..NNI..DEN.LSVRA.T..    | AQSVPL.E-----TVPFL.SST.APPLF     |       |  |
|           | R.....L....A..FA-----VV.LLKEI.   | F..NYS..VK...TIVANANG--KF..WTED   | AES.NKELPYWKS.N.F.NNKK.PLRHVSXWK |       |  |
|           | R.....L....A..FA-----VV.LLKEI.   | F..N.....VO...N.IASARG--KY..EWSE. | AES.NKELPYWKS.N.FVNNKK.PL.HISKWK |       |  |
|           | R.....L....A..FA-----VV.LLKEI.   |                                   |                                  |       |  |
| Spuma     |                                  |                                   |                                  |       |  |

FIG. 36A

| T306K & W313F |                                | L139P                               |                                  | E607K |  |
|---------------|--------------------------------|-------------------------------------|----------------------------------|-------|--|
| Consensus     | PKTLRQLEFLGTIGWCRLLWIPGFAELLXP | DIHATVPGPPBLIS-LPPSLXWYTVLDLKA      | HEXLG-----G-LTSAGXIKKXELIALLK    |       |  |
| Gamma         | P..R...A.F.....MAA.            | ..P..N.YN...G...HO.....             | ..I.H.EIYRRR.L...E.E....D...     |       |  |
|               | P..R...A.F.....MAA.            | ..P..N.YN...G...HO.....             | ..VH.EIYRRR.L...E.E....N...      |       |  |
|               | K..VR...Y.....AO.              | T..P..N.YT...L...DYL..S.....        | ..VH.MIYRRR.L...AG.A...AP...     |       |  |
|               | T..AK.VR...A.F.....T.AA.       | ..P..N.YN...CA...ORS.....           | ..VH.AIKYOR.L...RE....E....S..E  |       |  |
| Epsilon       | P..R...A.F.....MAA.            | ..P..N.YN...G...HO.....             | ..I.H.EIYRRR.L...E.E....S...     |       |  |
|               | T..I..IRA...LV.Y..H..E.SHSKFT  | PLT.V.AS.TTV.N.A...H.F..I..SN.      | ..DF.HLMWHR.FV....TP...HK..EY... |       |  |
|               | ..S.H...TV..DLO.VSRGT.TYRRP.OL | P..P..LS....D..TA..L..YH.FAIIYC.... | RT.A-----VLG.WLOPDPVPSYAL.Y.     |       |  |
|               | RWA.PE..AL..E.O.VSKGT.TLROP.HS | ..LSSSS....D.S...TT.AHLOTI....      | RT.A-----LGTFOGRSSOAPFO...P      |       |  |
| Delta         | RWA.PE..AL..E.O.VSKGT.TLROP.HS | ..LSSSS....D.S...TT.AHLOTI....      | RT.A-----LGTFOGRSSOAPFO...P      |       |  |
|               | OW..TE..VI..E.O.VSKGT.ILRKH.OS | TLTSPS....D.T...TA.PHILOTI..T..     | S.A.E-----IG.FLGTSAGOTLO.A.P     |       |  |
|               | LO..NDF.KL..D.N.L.PYLKLTGTD.K. | LMG.LQ..L.SPVA..I..OGYL-KIIL...C    | PL.E.E-----TVAQI.H.SETAKLFLOCO   |       |  |
|               | ER..NDF.KL..N.I.HFLKLTGTE.K.   | MG.LQ..L.SPVA..V..KGE-III..O.C      | PEIE-----ATLSPTKIYTE.KH.O        |       |  |
| Beta          | L..NDF.KL..D.O.L.HYLKLTGTE.K.  | PMG.LQ..L.SPVA..I..LNY-HKI..I..C    | PE.E.E-----TVPHI.SST.ATPLFSK.O   |       |  |
|               | ..D.K...SI..LLNFA.NF..NYS..VK. | L.A.QNQSAGI..SIRG-KYK.T...TNG       | NKELPYWKS.N.F.NNKK.PLRHVSXWKSI   |       |  |
|               | ..D.K...SI..LLNFA.NF..NYS..VK. | LTA.QNQSAGI..ATIVRO-KYK.T...ANG     | NKELPYWKS.N.FVNNKK.PL.HISKWSIAE  |       |  |
|               | ..D.K...SI..LLNFA.NF..NYS..VK. |                                     |                                  |       |  |
| Spuma         |                                |                                     |                                  |       |  |

FIG. 36B

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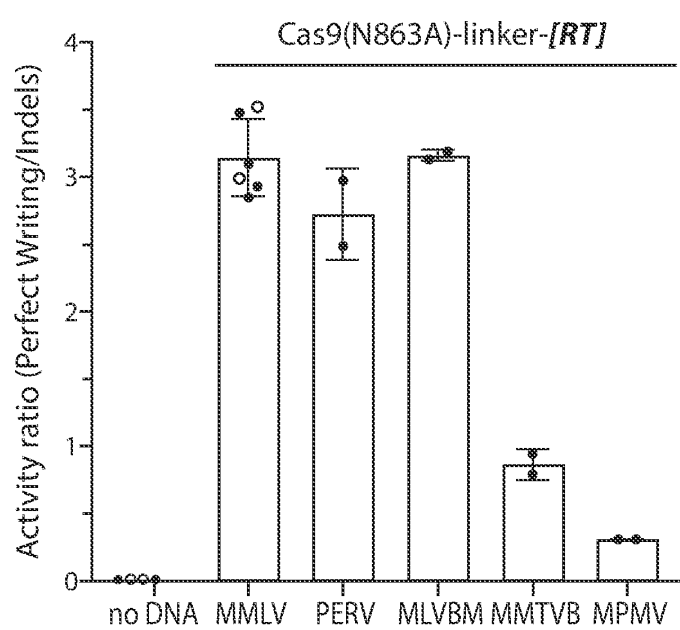


FIG. 37

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FIG. 38

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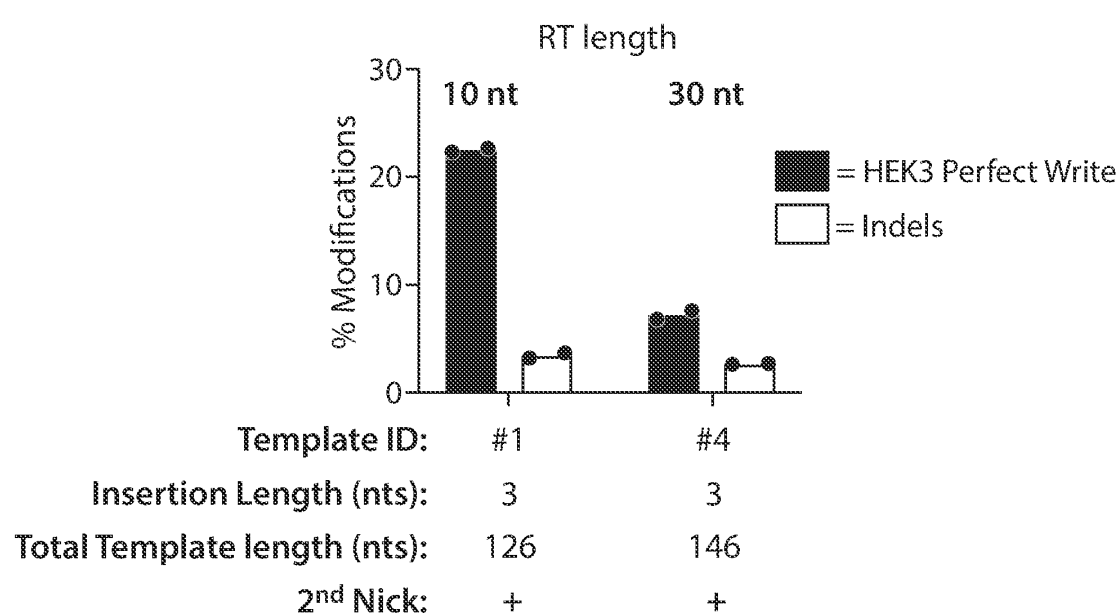


FIG. 39

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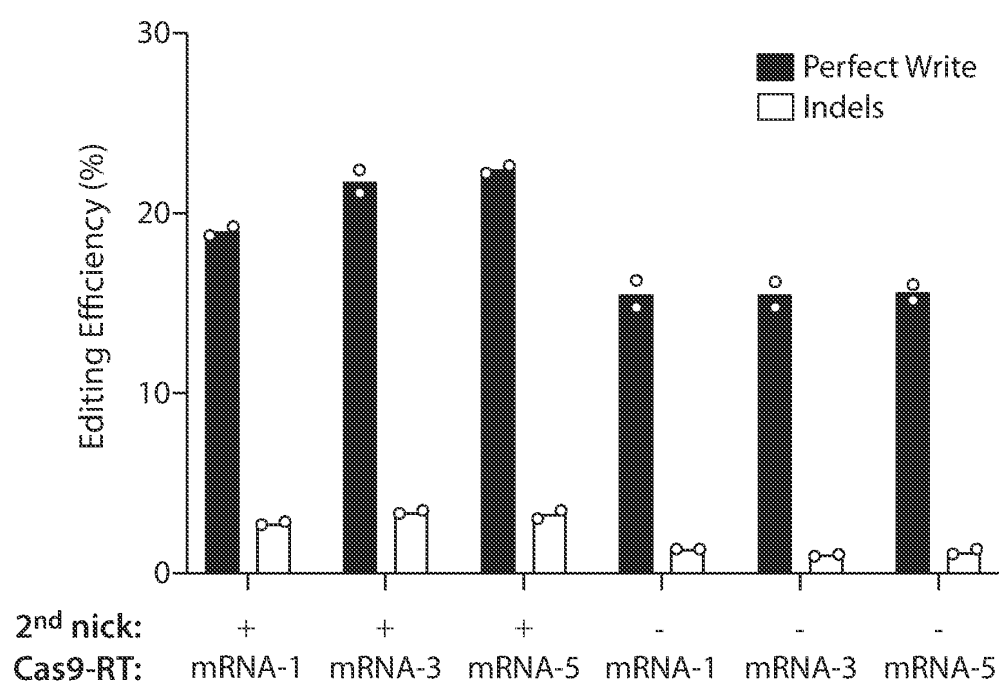


FIG. 40

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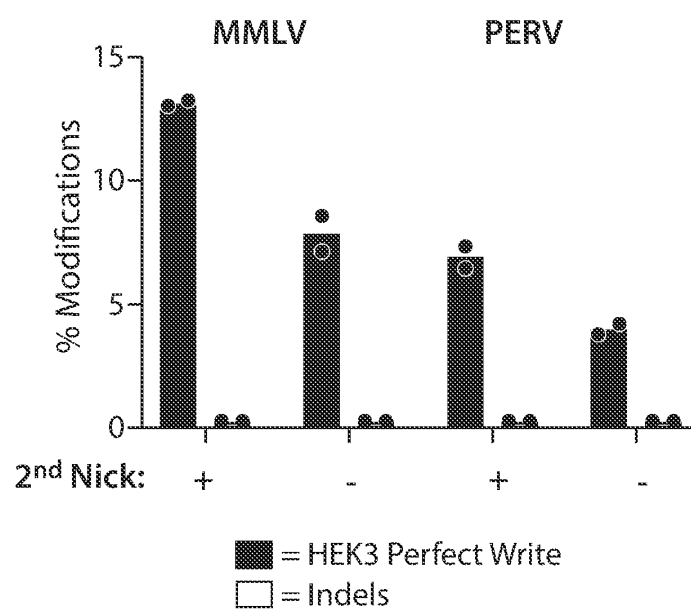


FIG. 41

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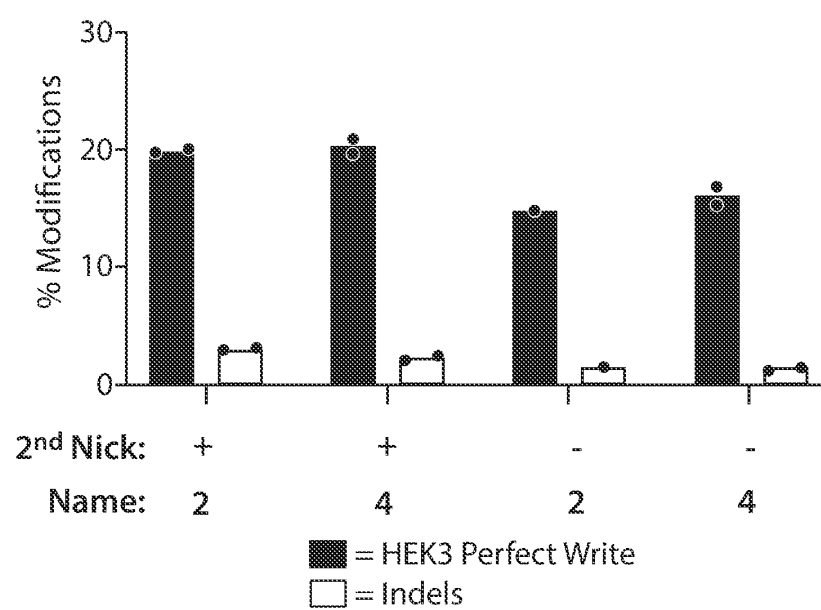


FIG. 42

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## Lipofectamine 3000

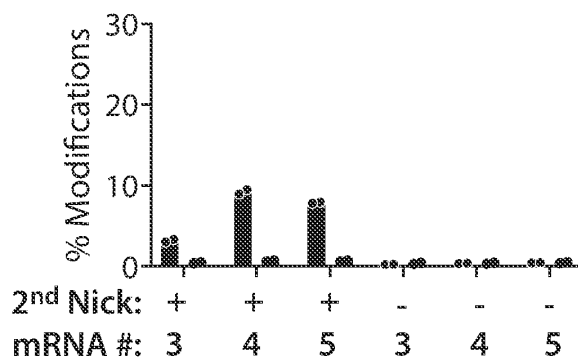


FIG. 43A

## MessengerMax

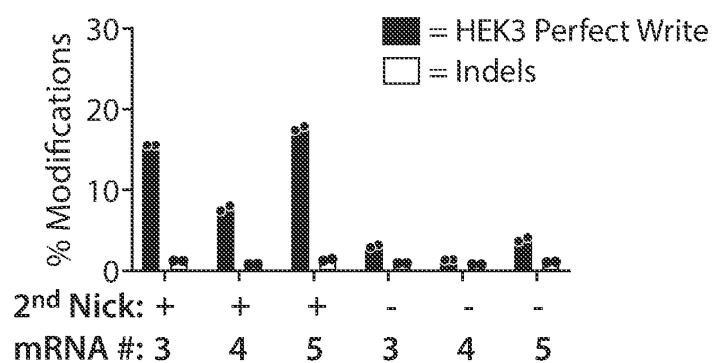


FIG. 43B

## RT length

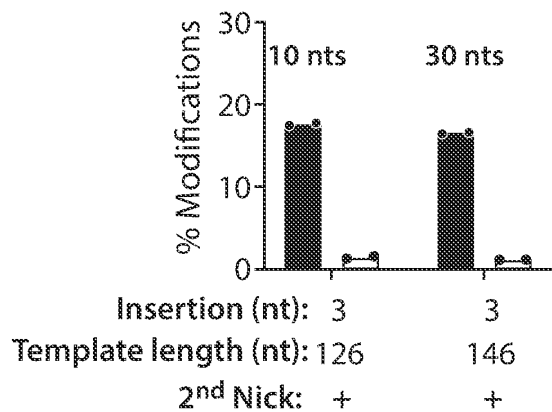


FIG. 43C

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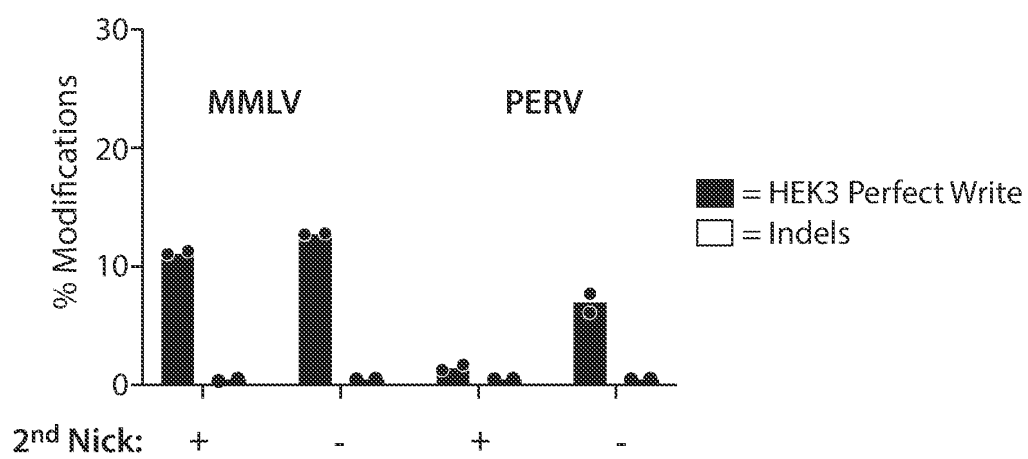
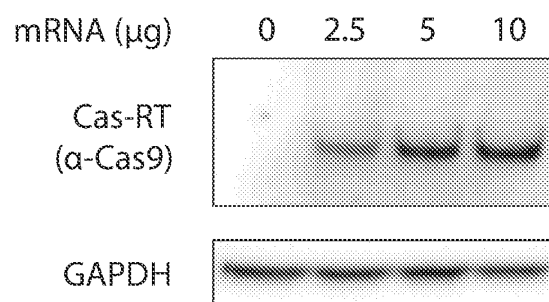
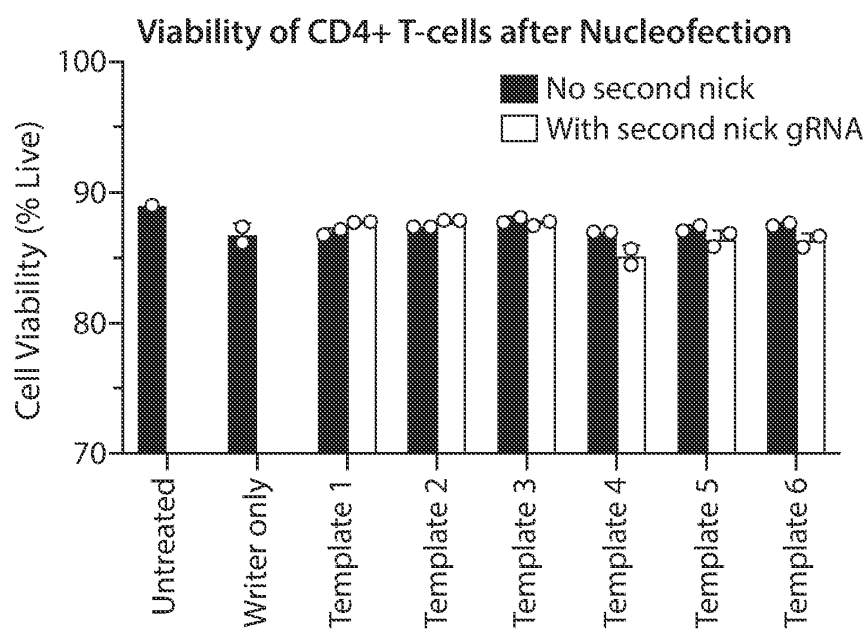


FIG. 44

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**Expression of Gene Writer Polypeptide****FIG. 45A****FIG. 45B**

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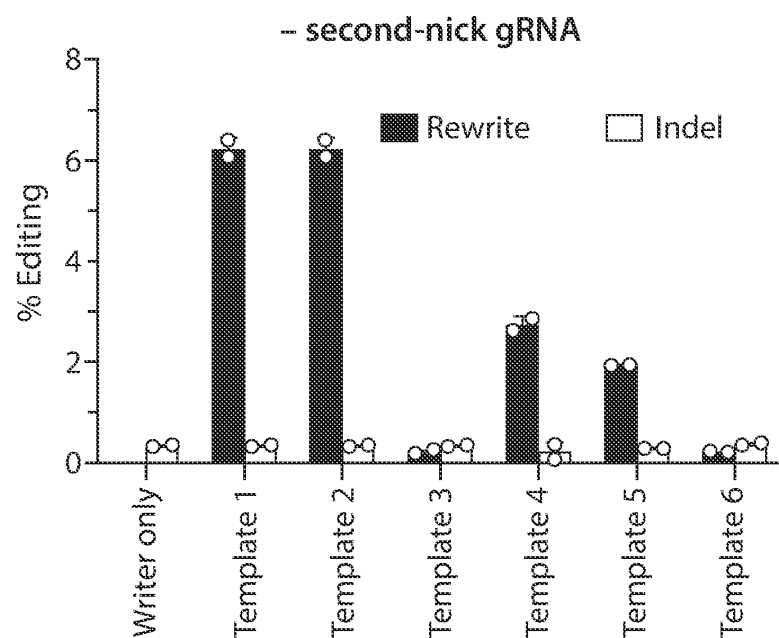


FIG. 46A

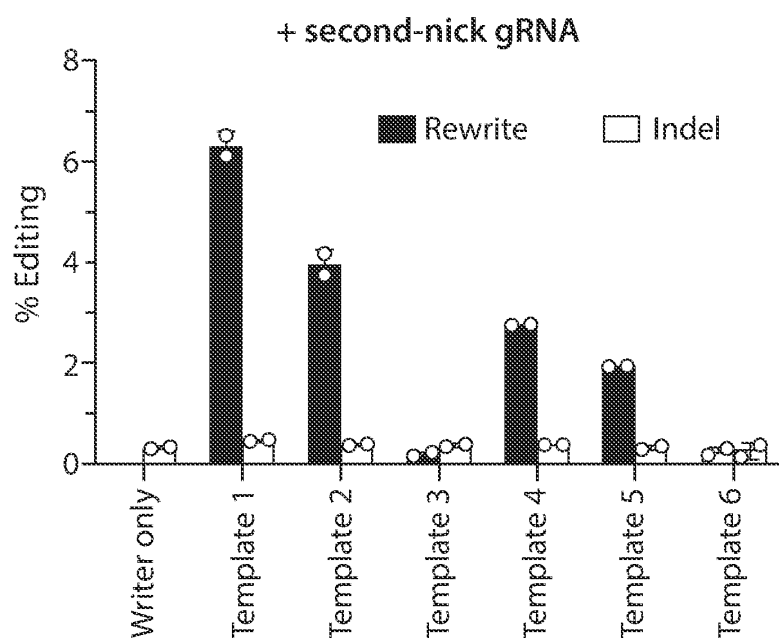


FIG. 46B

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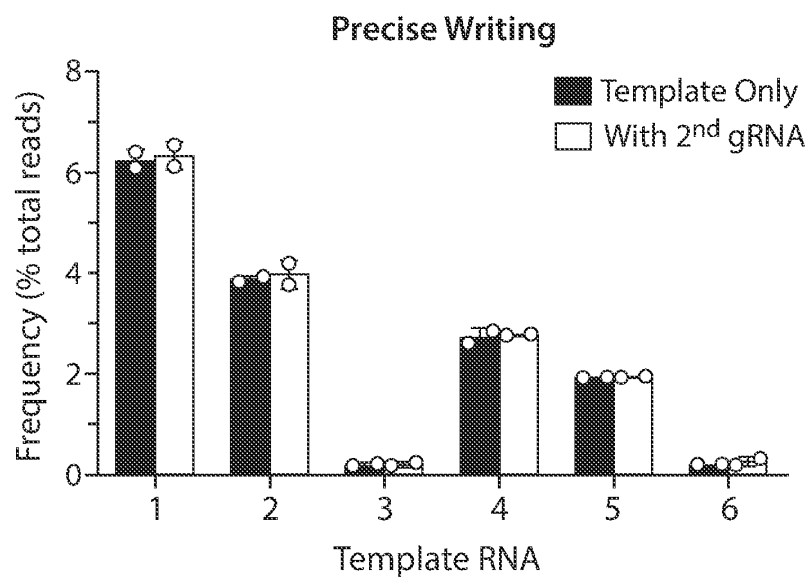


FIG. 47A

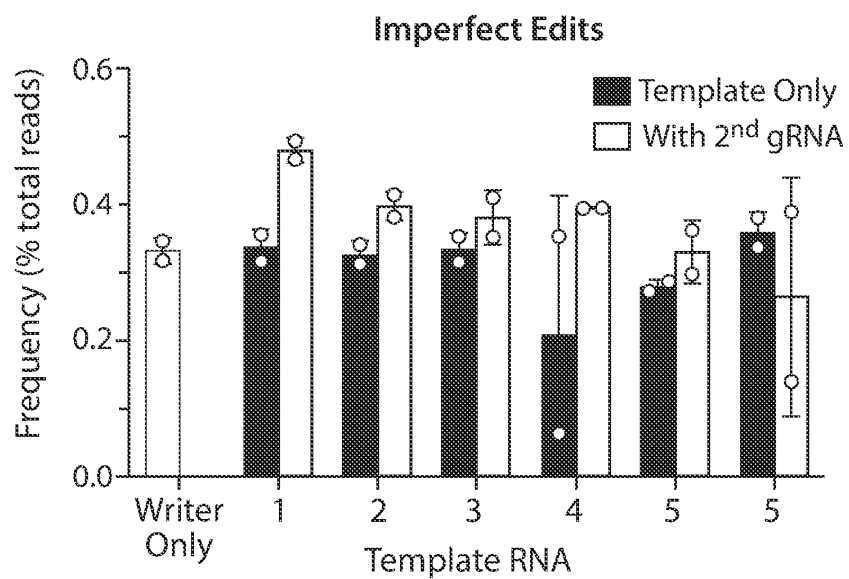


FIG. 47B

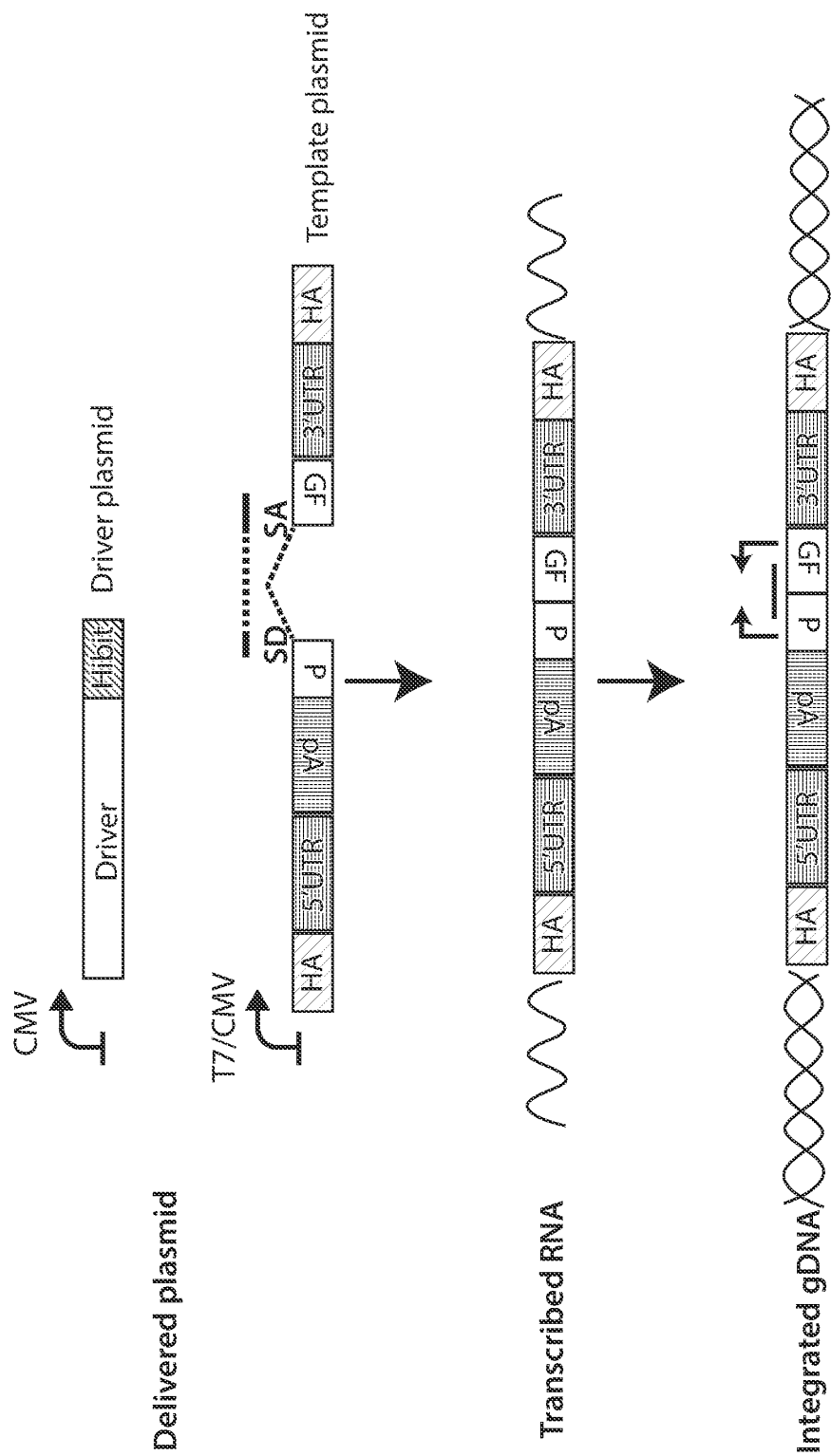


Figure 48

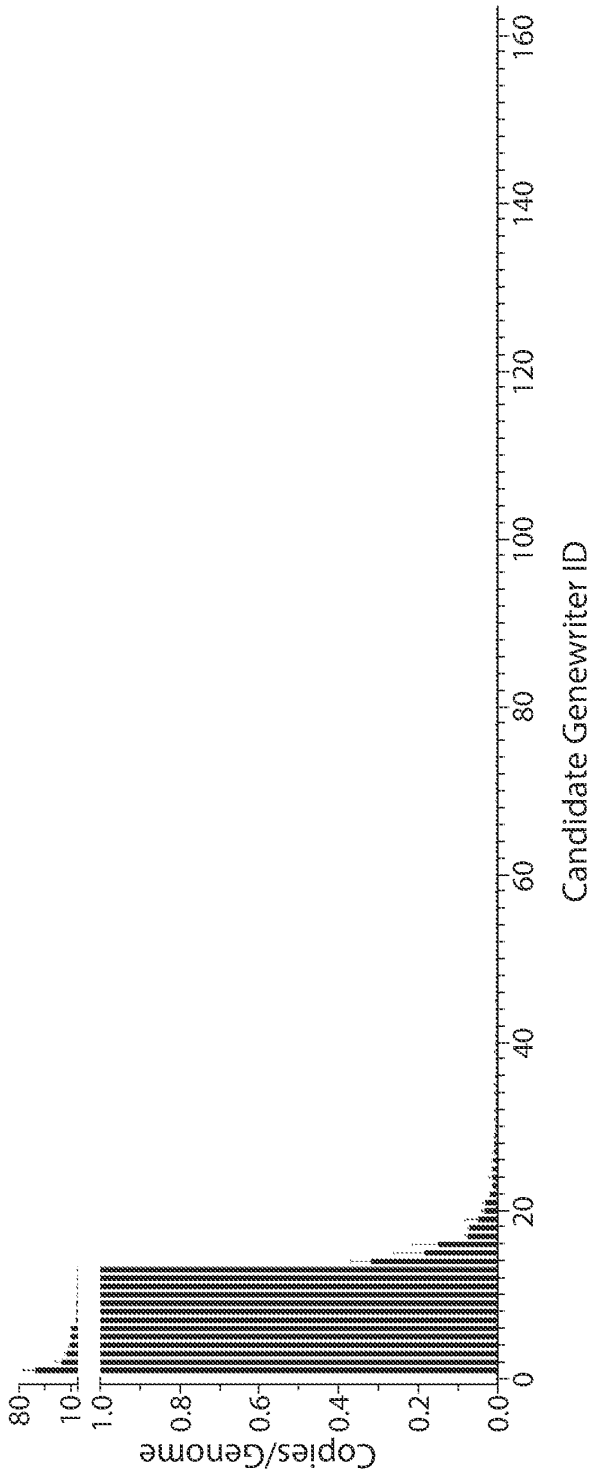


Figure 49

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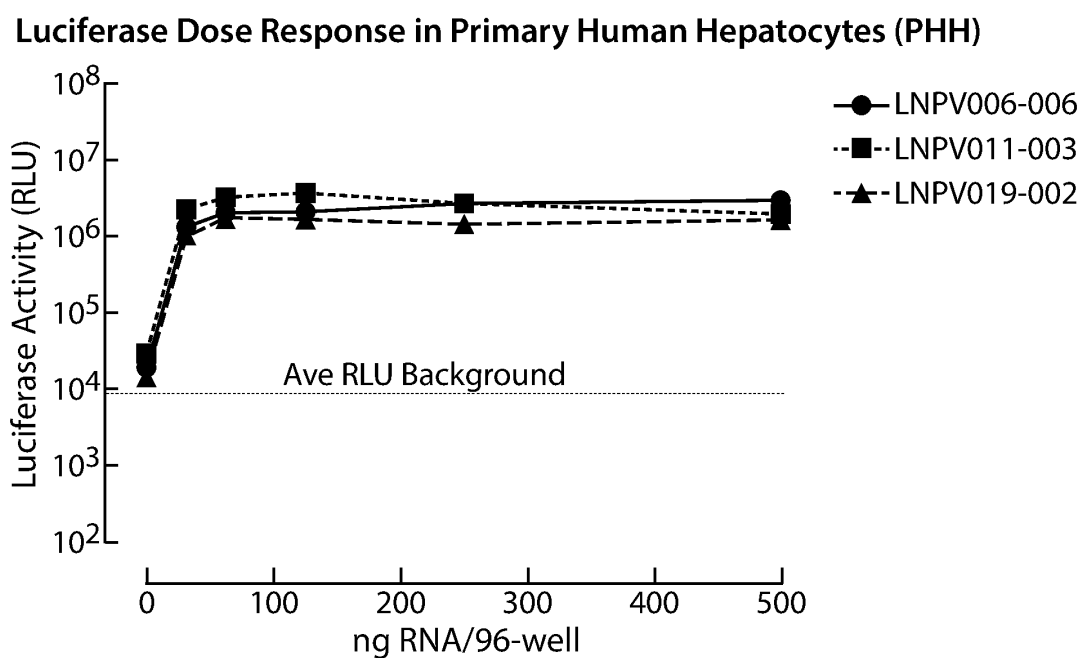
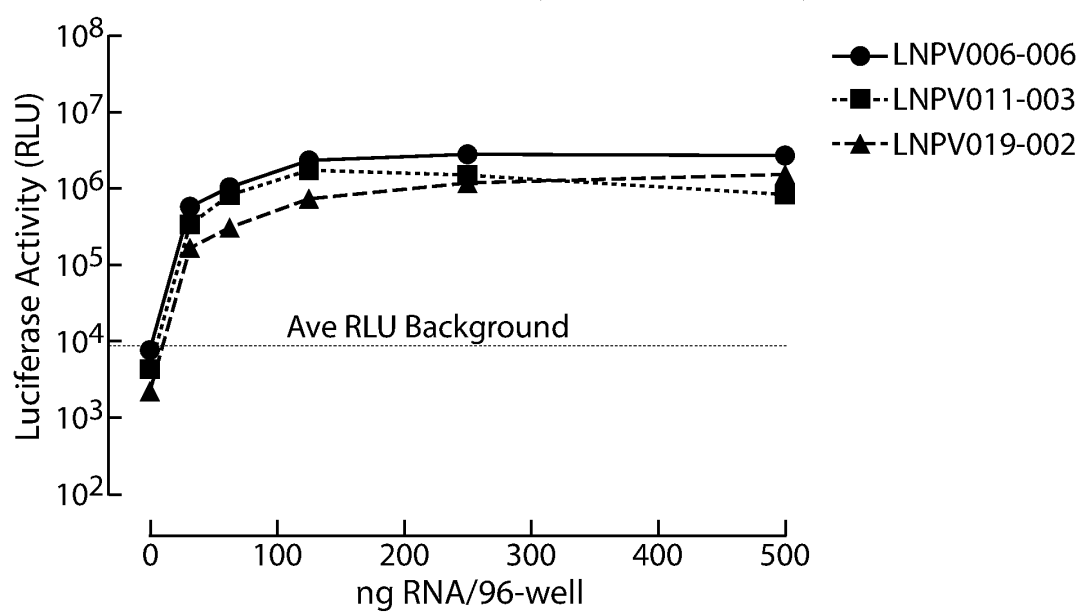


FIG. 50A

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**Luciferase Dose Response in Primary Mouse Hepatocytes (PMH)****FIG. 50B**

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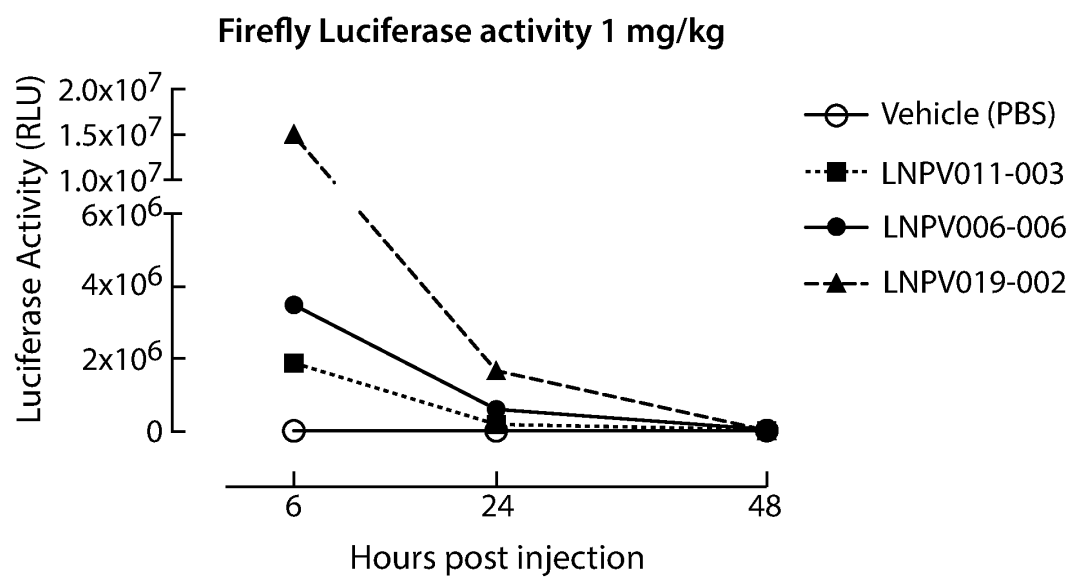


FIG. 51