

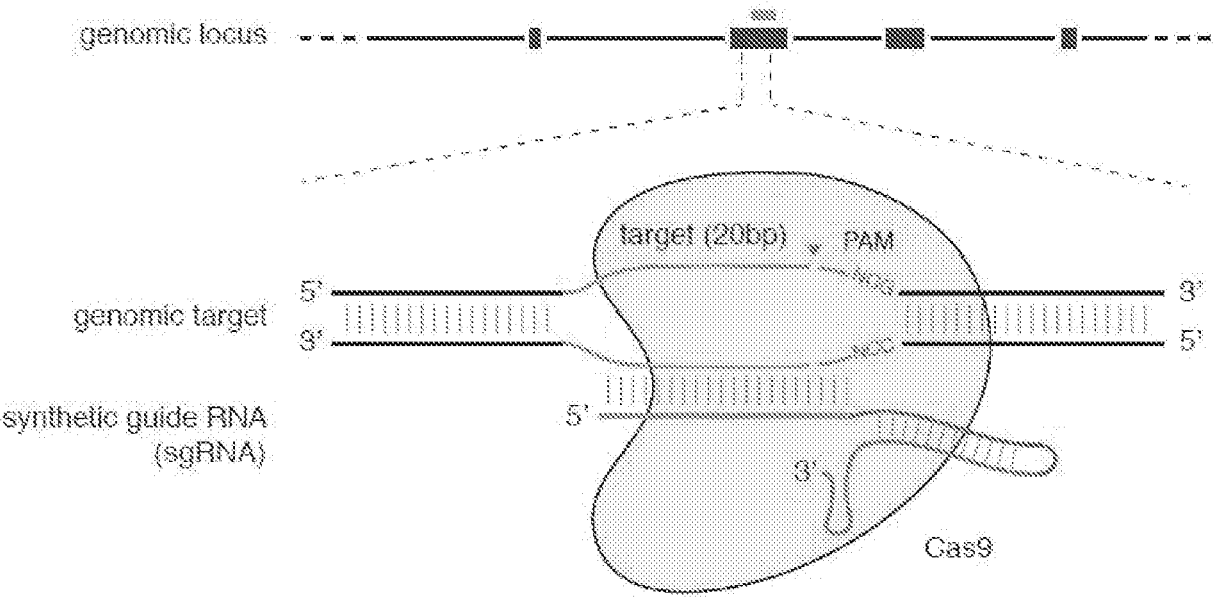


FIG. 1

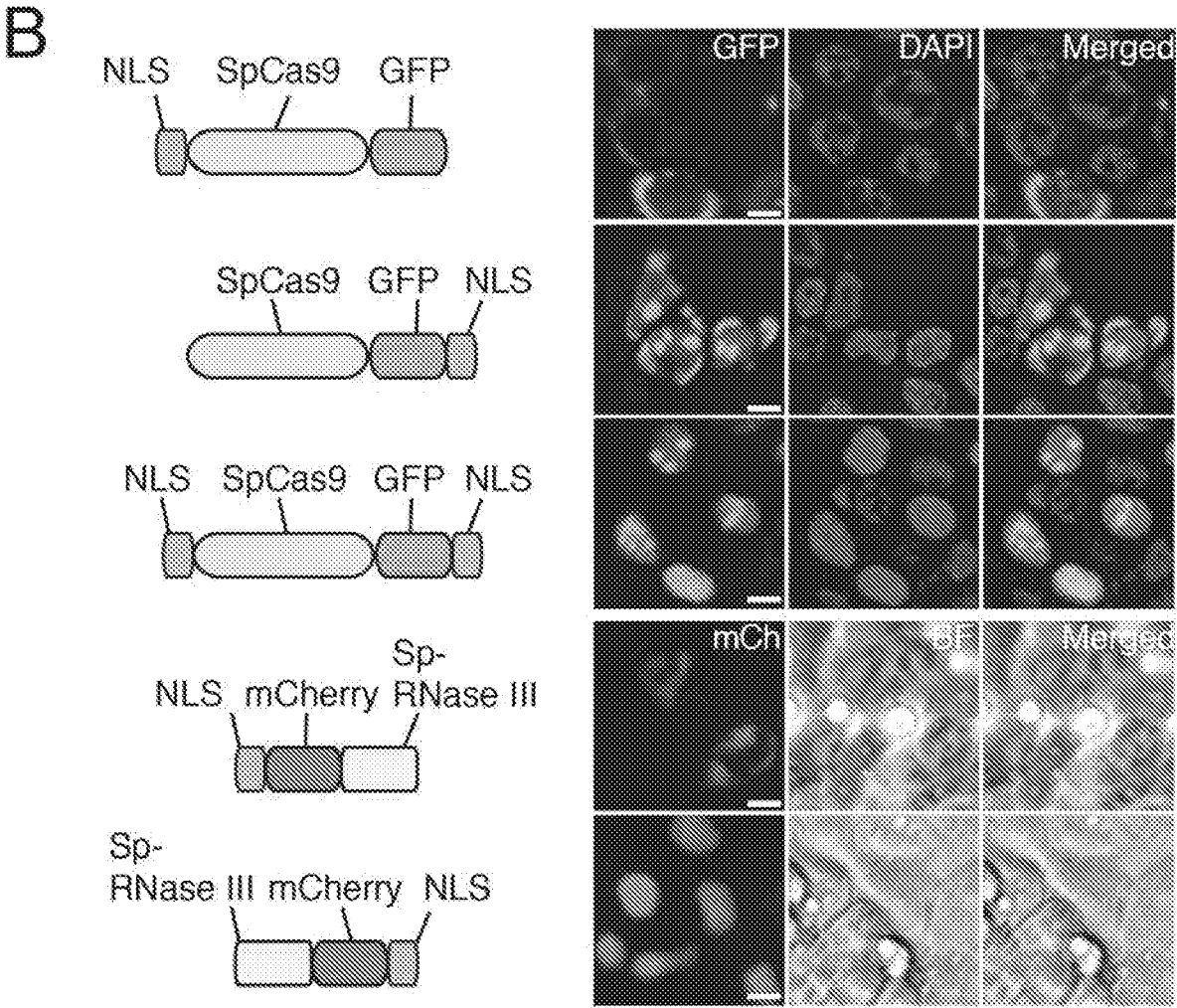


FIG. 2B

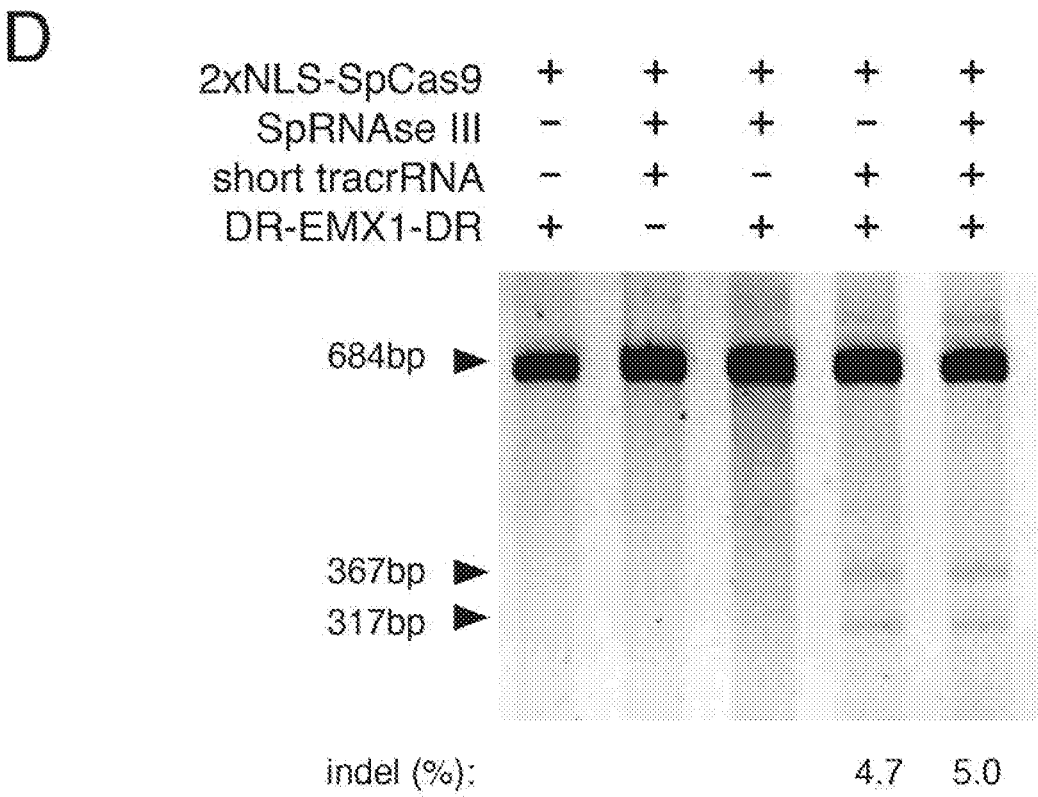
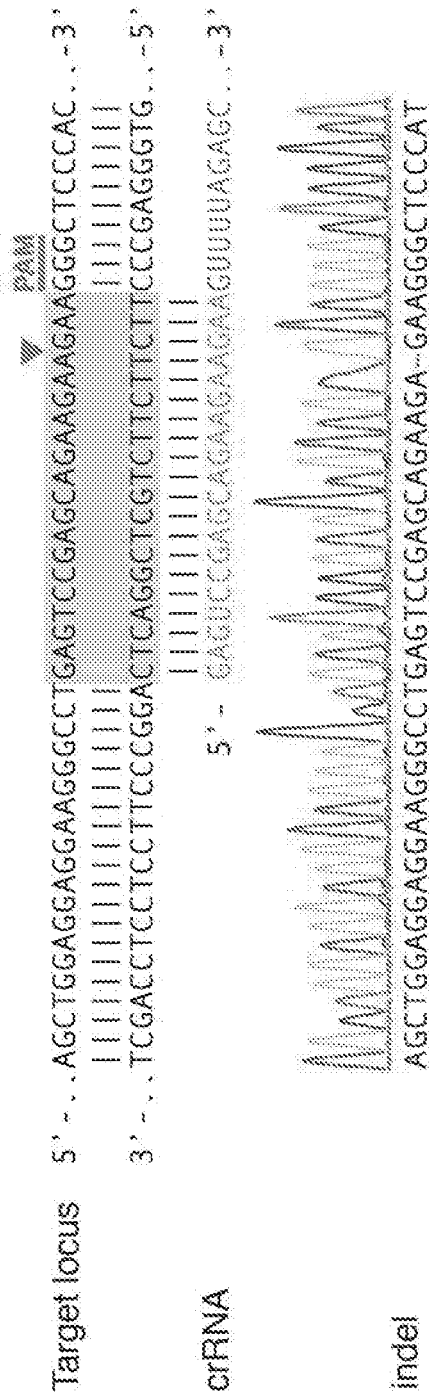


FIG. 2D

E



F

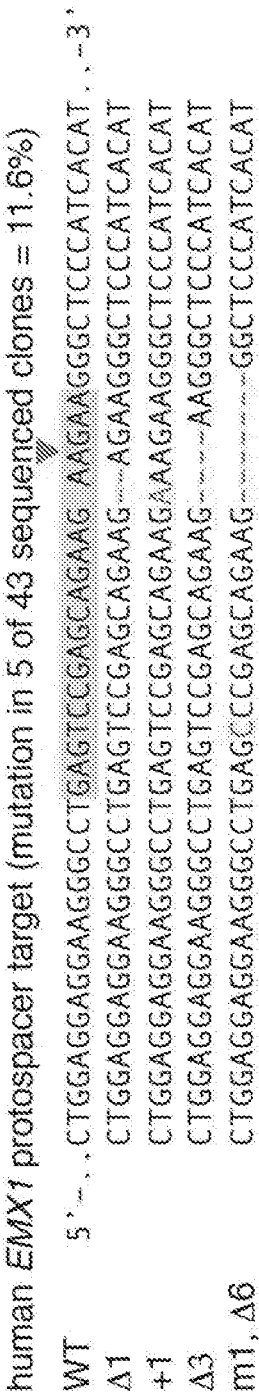
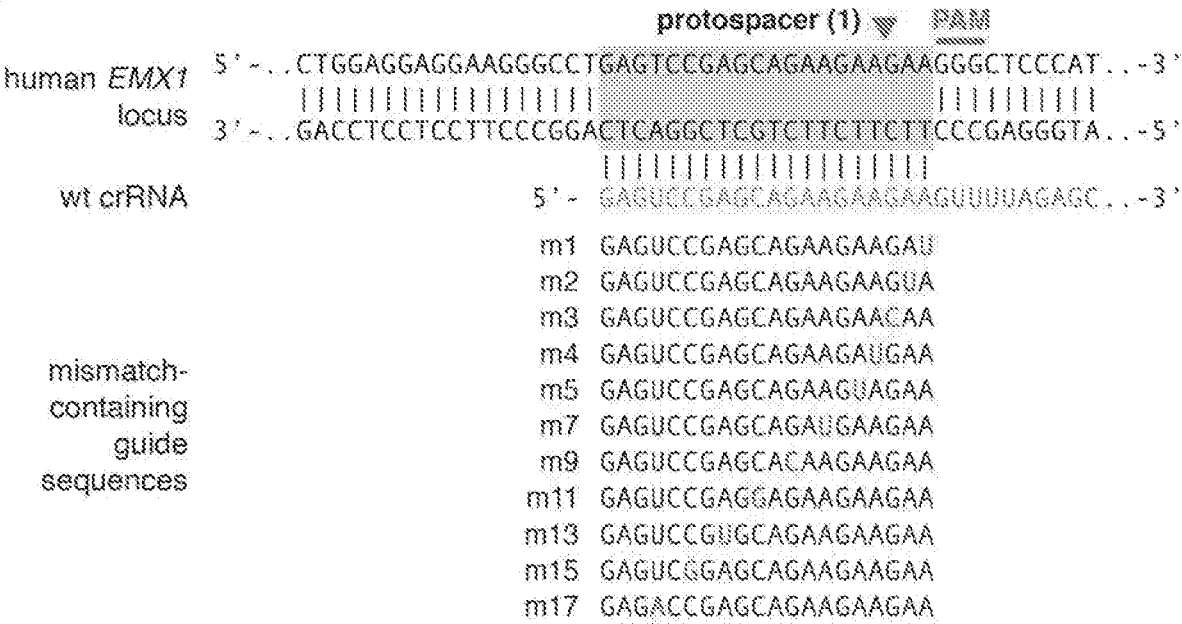
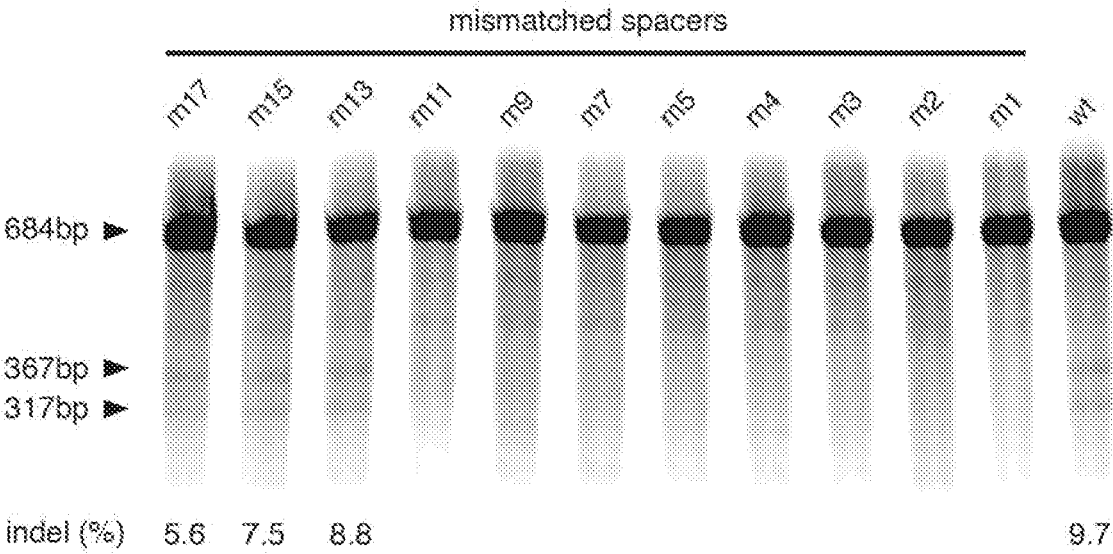


FIG. 2E-F

A



B



C

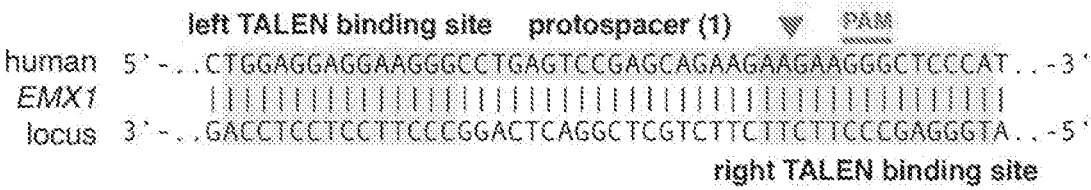


FIG. 3A-C

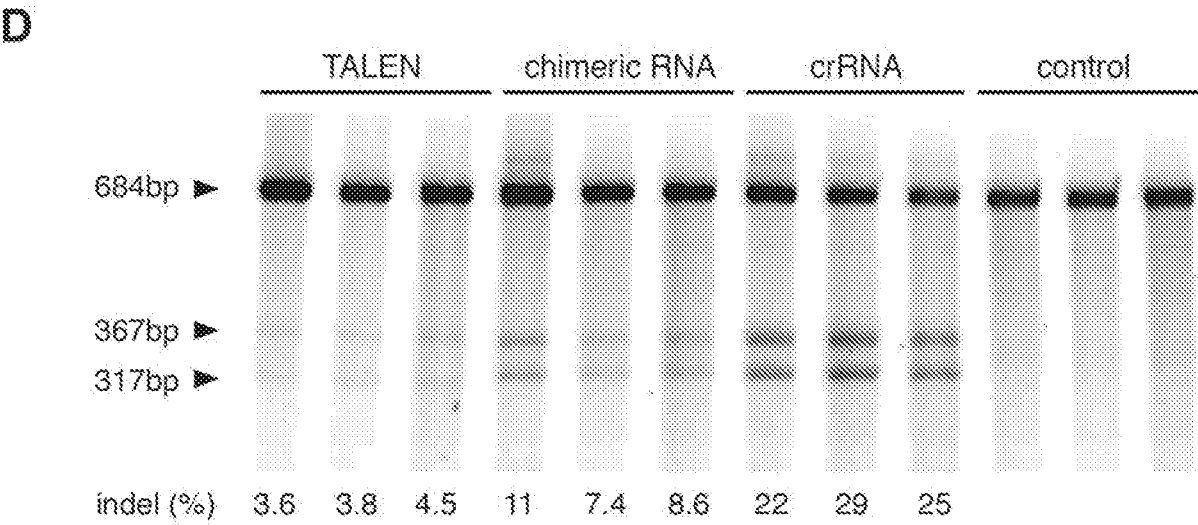
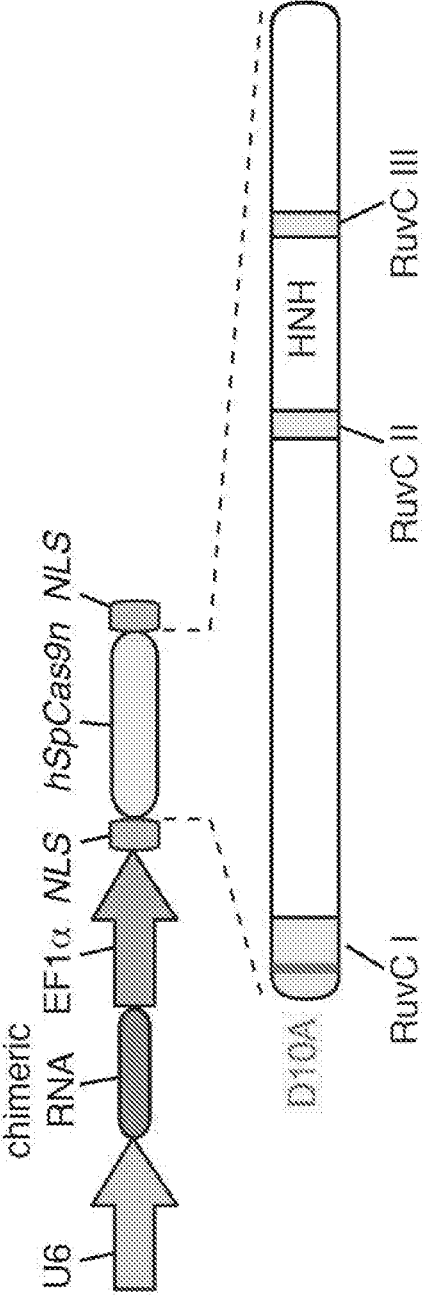


FIG. 3D

A



B

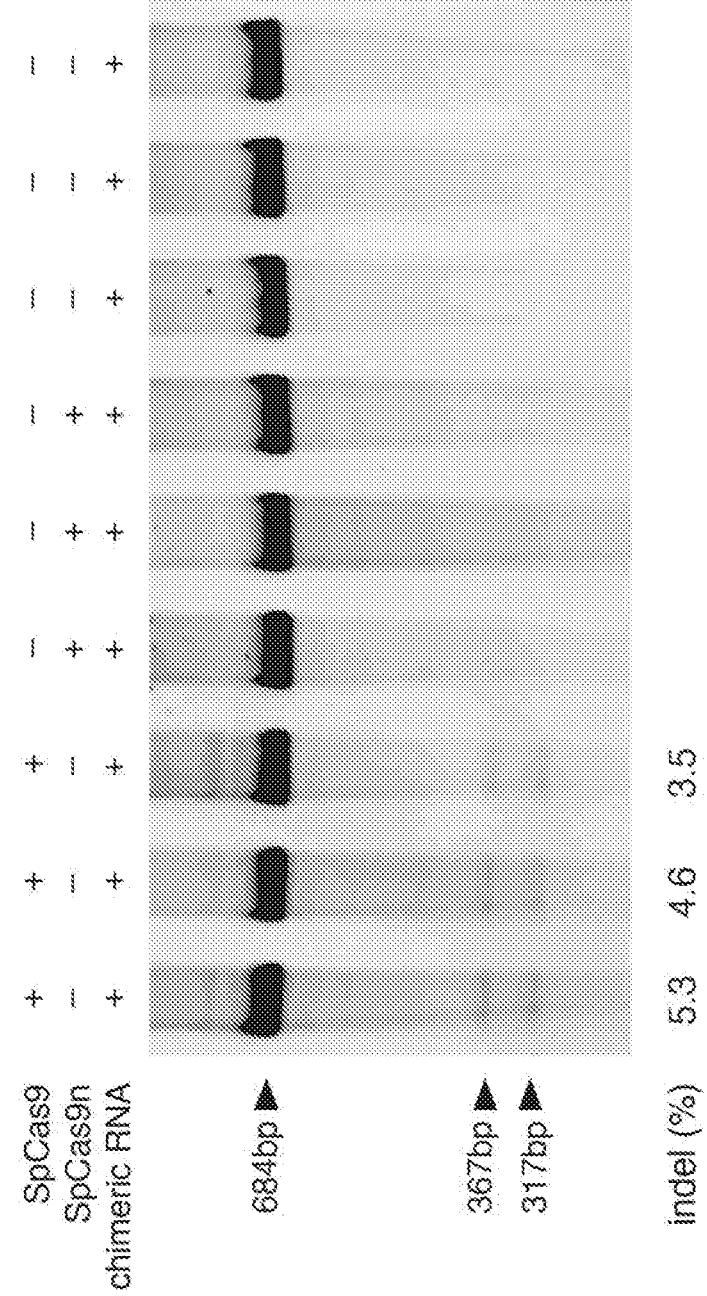


FIG. 4A-B

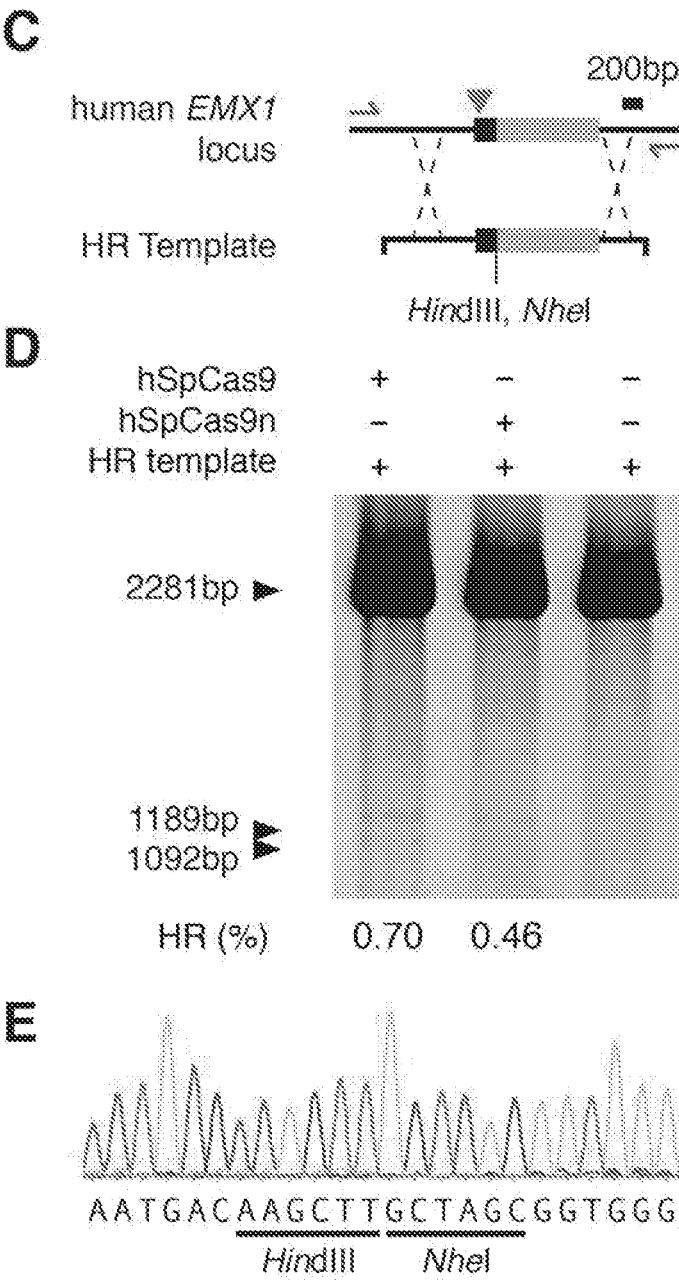


FIG. 4C-E

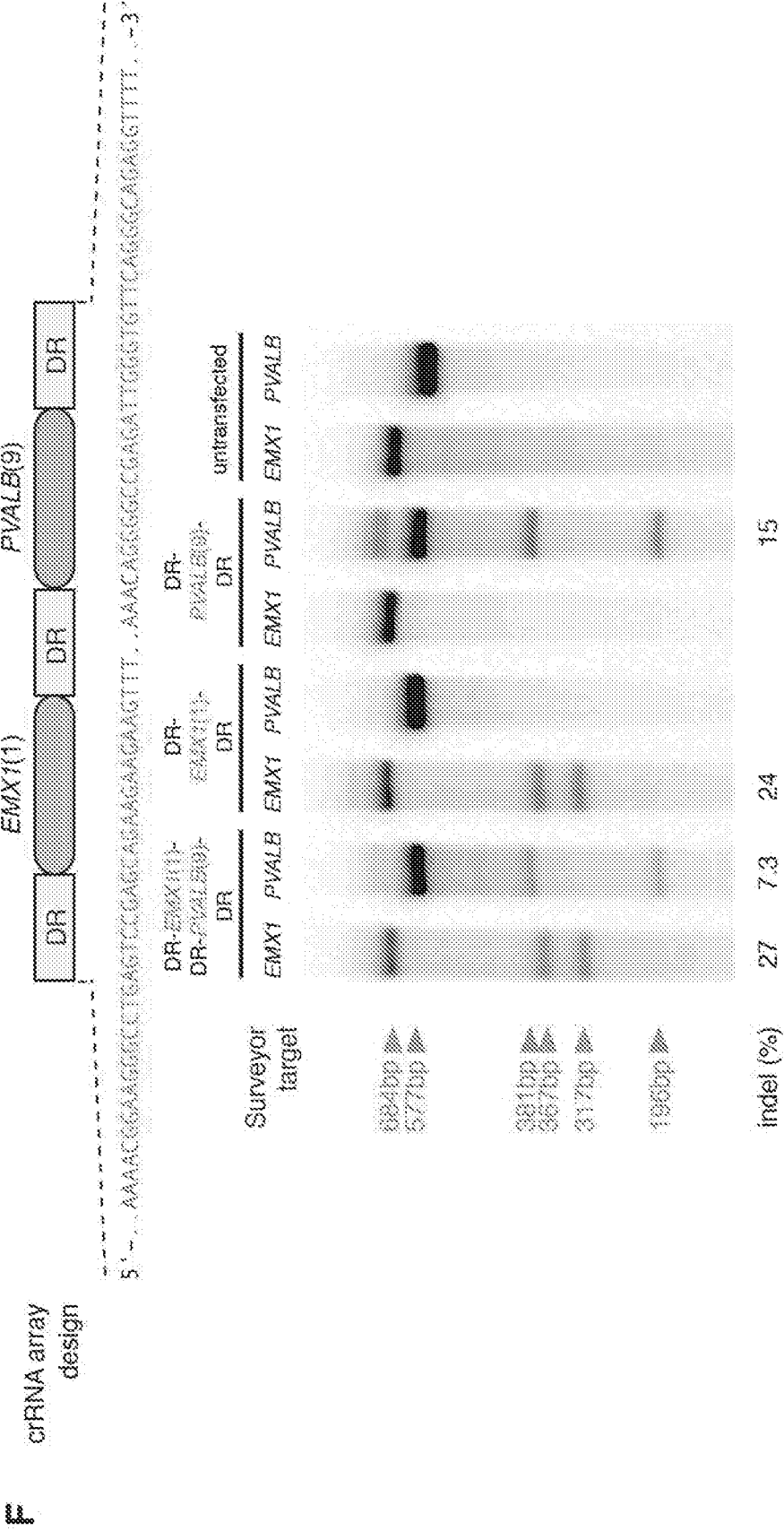


FIG. 4F

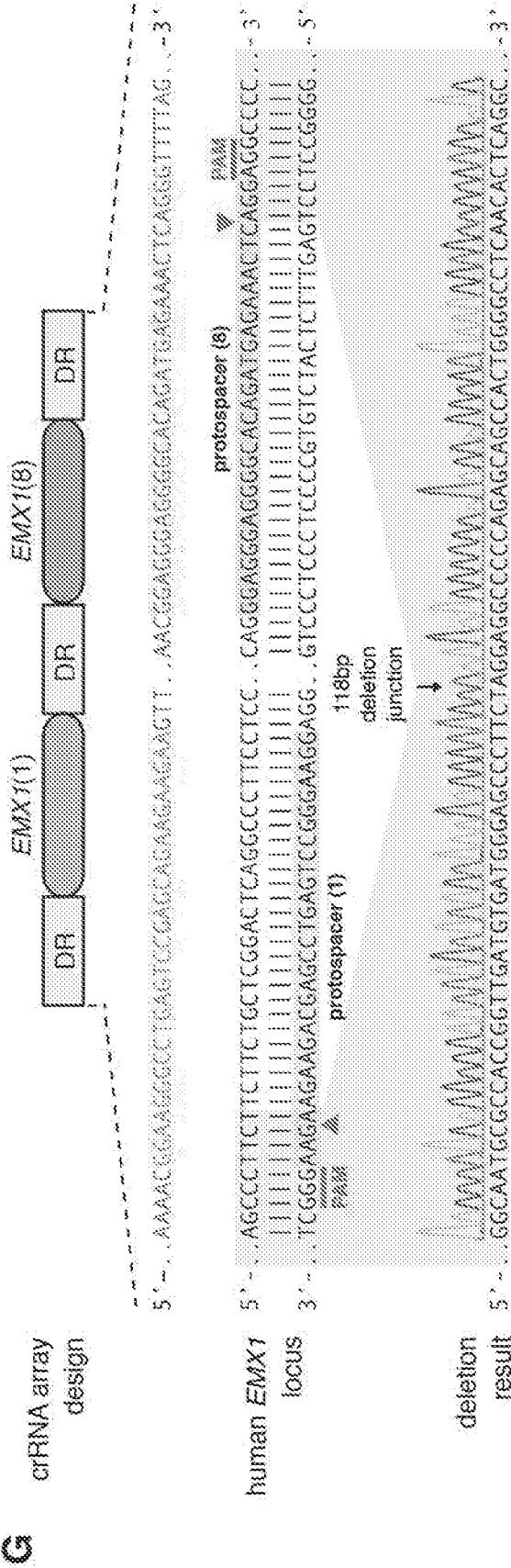


FIG. 4G

Cas9	target species	gene	protospacer ID	protospacer sequence (5' to 3')	PAM	strand	cell line tested	% indel (pre-crRNA + tracrRNA)	% indel (chimeric RNA)
<i>S. pyogenes</i> SF370 type II	Homo sapiens	EMX1	1	GGAGGGCTGAGTCCGAGCAGAGAGAA	GGG	+	293FT	20 ± 1.8	6.7 ± 0.82
			2	CATTGGAGGTGACATGATGCTCTCCCAT	TGG	-	293FT	2.1 ± 0.31	N.D.
			3	GGACATCGATGTCACCTCCCAATCAGTAGG	TGG	+	293FT	14 ± 1.1	N.D.
			4	CATCGATGCTCTCCCATGCTGCTCTCG	TGG	-	293FT	11 ± 1.7	N.D.
			5	TTCGTGGCANTGCGCCACCGGTTGATGTGA	TGG	-	293FT	4.3 ± 0.48	2.1 ± 0.51
			6	TGGTGGCAATGCCCGACCGGTTGATGTGAT	GGG	-	293FT	4.0 ± 0.88	0.41 ± 0.25
			7	TCCAGCTTCTGCGCTTGTGACTTTGCTCTC	CGG	-	293FT	1.5 ± 0.12	N.D.
			8	GGAGGGAGGGGACAGATGAGAACTCAGG	AGG	-	293FT	7.8 ± 0.83	2.3 ± 1.2
CRISPR	Homo	PVALB	9	AGGGCCGAGATTGGGTGTTGAGGGCAGAG	AGG	+	293FT	21 ± 2.6	6.5 ± 0.32
	sapiens	PVALB	10	ATGCAGGAGGTGGCGAGAGGGCCGAGAT	TGG	+	293FT	N.D.	N.D.
		PVALB	11	GGTGGCAGAGGGGCGAGATTGGGTCTTC	AGG	+	293FT	N.D.	N.D.
<i>Mus musculus</i>	Th		12	CAGCACATGAGTGCCATTAGCTAATGTCAT	AGG	-	Neuro2A	27 ± 4.3	4.1 ± 2.2
	Th		13	AATGCATAGGTATCCACCCACAGAGGTGTCAG	GGG	-	Neuro2A	4.8 ± 1.2	N.D.
	Th		14	ACACACATGGGAAGCCCTCTGGGCCAGGA	AGG	+	Neuro2A	11.3 ± 1.3	N.D.
<i>S. thermophilus</i> UMD-9 CRISPR	Homo	EMX1	15	GGAGAGGTAGTATACAGAAACACACAGAA	GTAGCAT	-	293FT	14 ± 0.88	N.T.
	sapiens	EMX1	16	AGANTGTAGAGGAGTCCACAGAACTCAGCA	CTAGGAA	-	293FT	7.8 ± 0.77	N.T.

FIG. 5

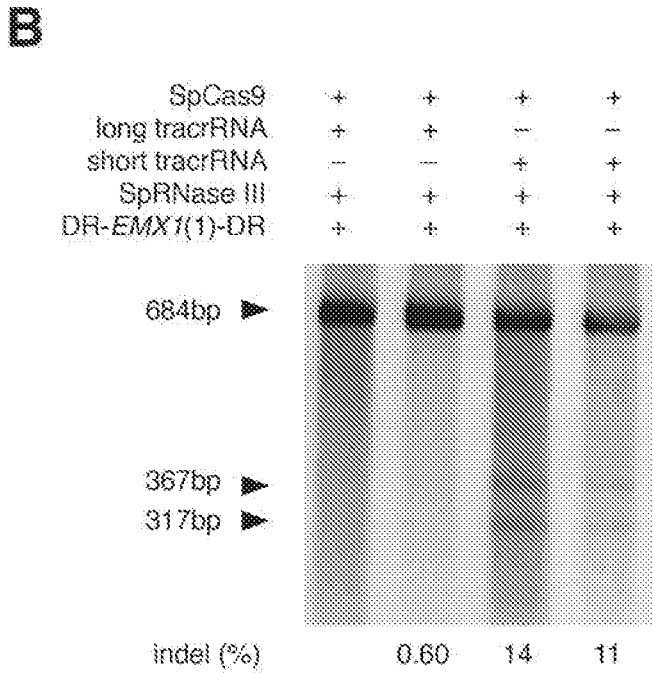
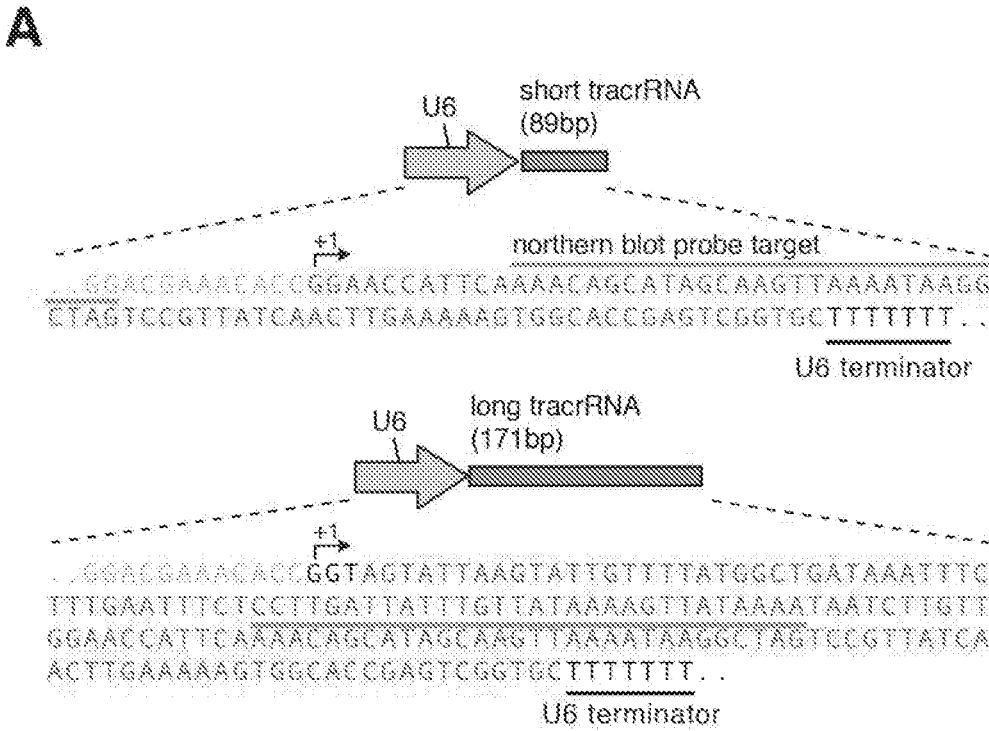


FIG. 6A-B

C

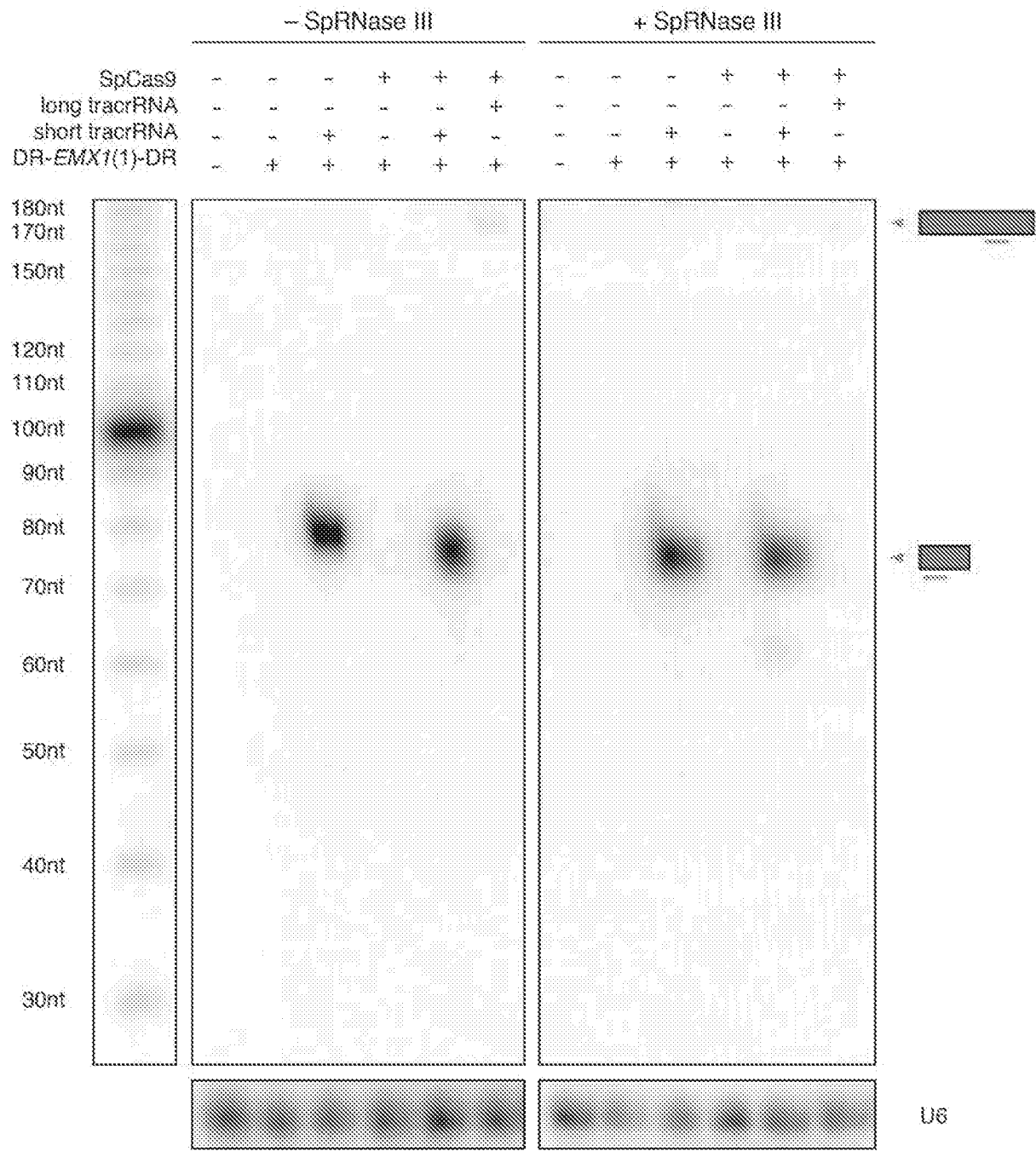
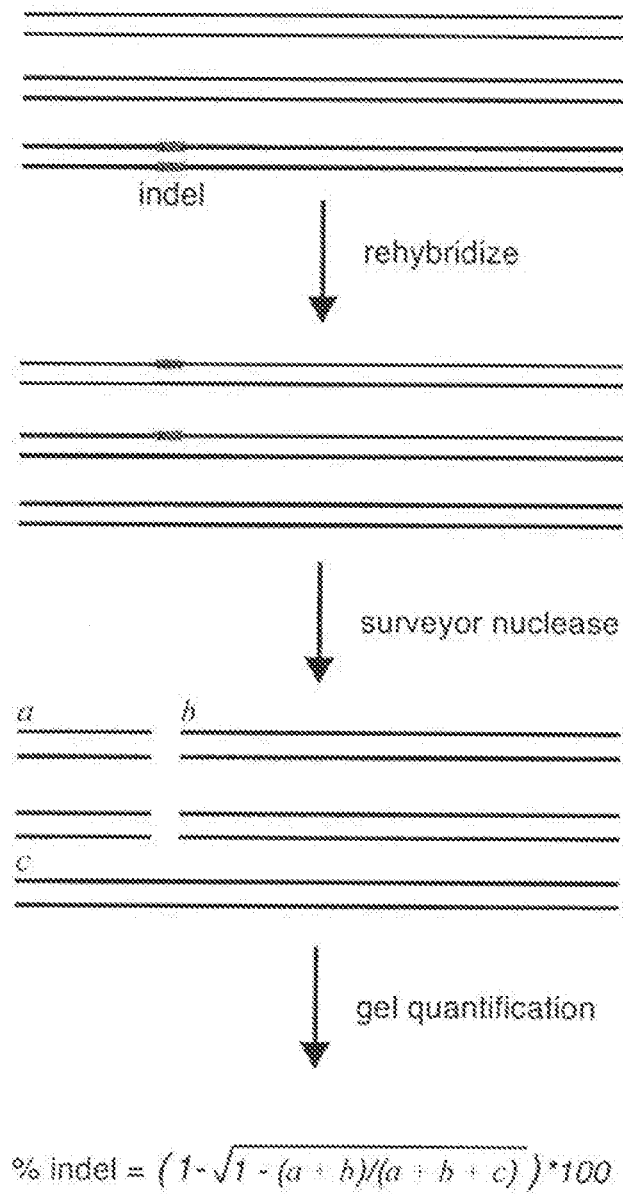
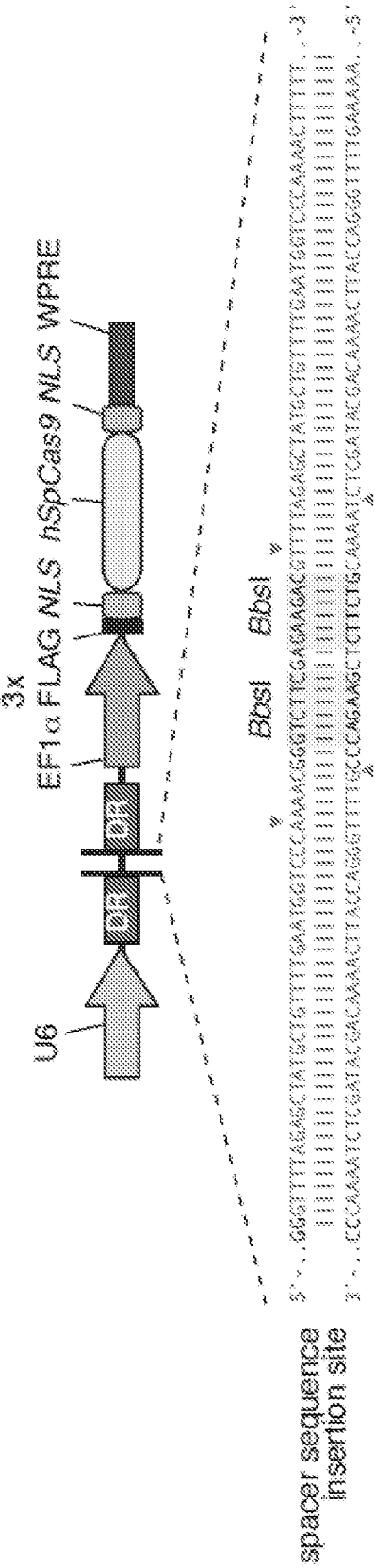


FIG. 6C

**FIG. 7**

A



B

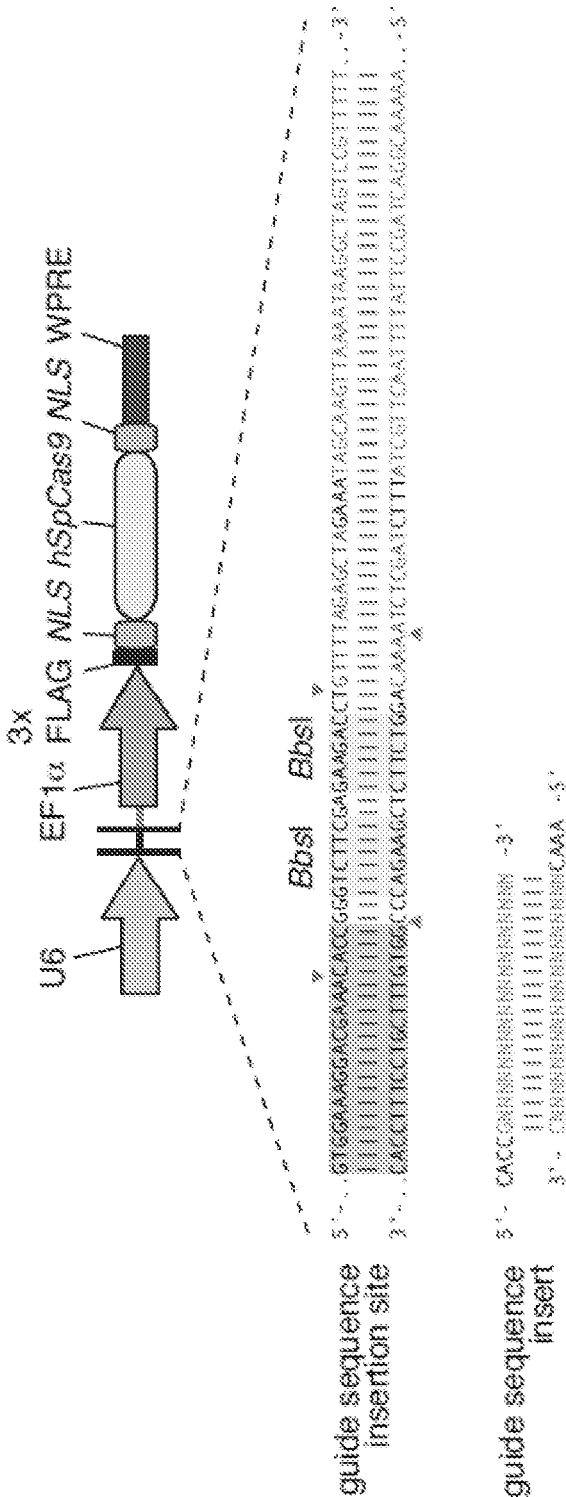
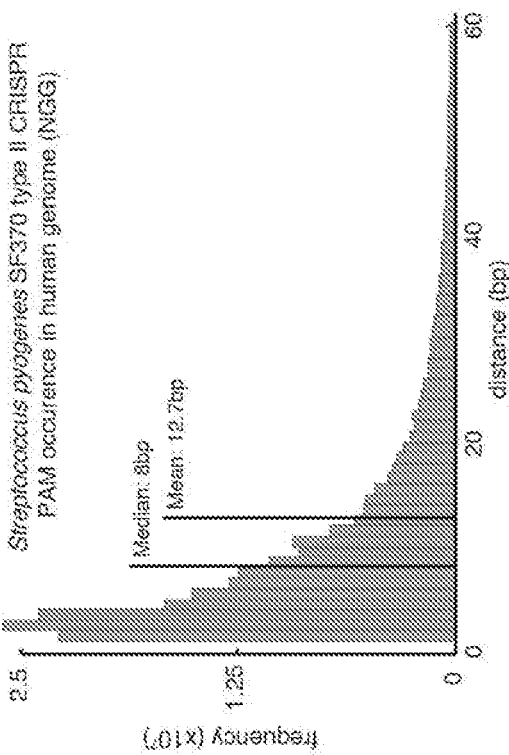
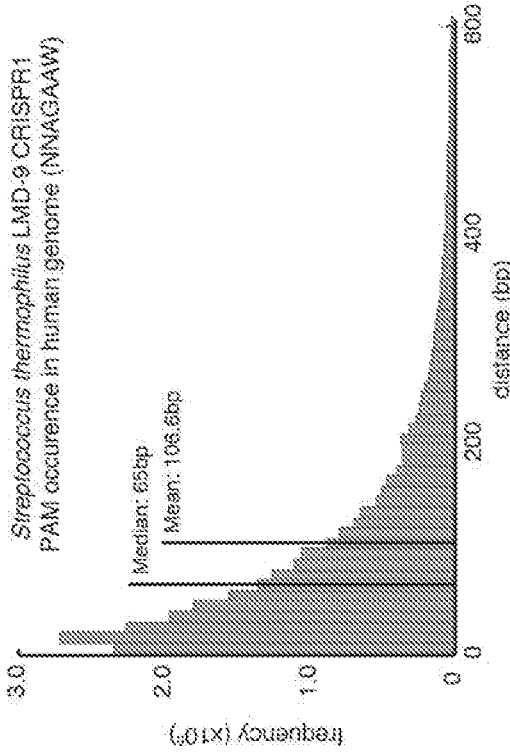


FIG. 8A-B

C



B



Chr	NGG		NNAGAAW	
	median	mean	median	mean
1	7	12.8	67	115.8
2	8	12.7	64	100.8
3	8	13.0	63	98.5
4	9	14.0	61	94.5
5	8	13.1	63	97.9
6	8	13.1	63	98.5
7	8	12.4	64	102.9
8	8	12.8	64	100.9
9	7	13.9	65	120.5
10	7	12.1	66	107.0
11	7	12.0	65	105.8
12	8	12.4	65	103.5
13	8	13.6	62	94.6
14	8	12.0	65	101.5
15	7	11.5	68	107.7
16	7	11.7	74	136.8
17	6	10.3	76	127.9
18	8	13.4	63	101.8
19	6	9.4	82	145.4
20	7	11.1	72	121.8
21	7	13.4	64	111.4
22	6	9.2	85	140.3
X	8	13.2	63	99.0
Y	8	29.2	62	223.7

FIG. 9A-C

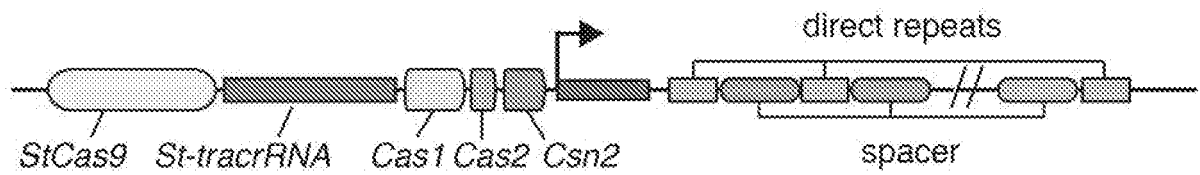
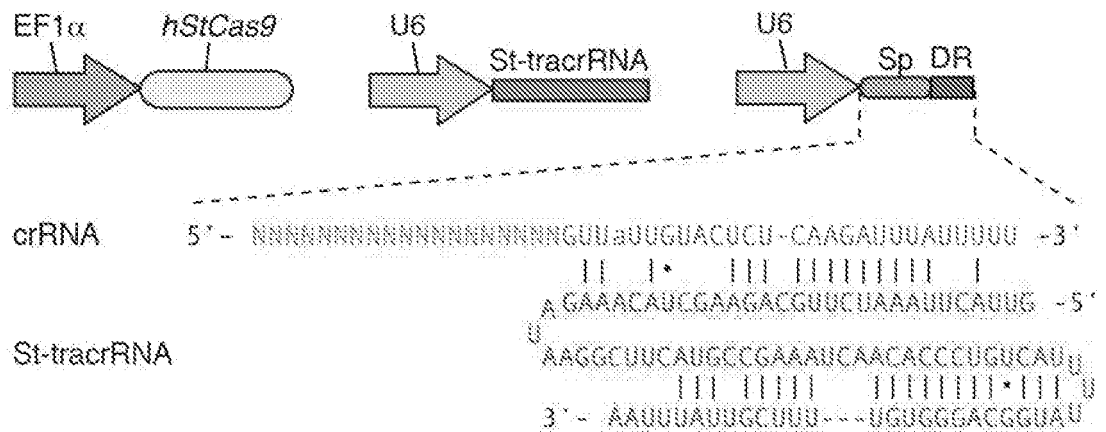
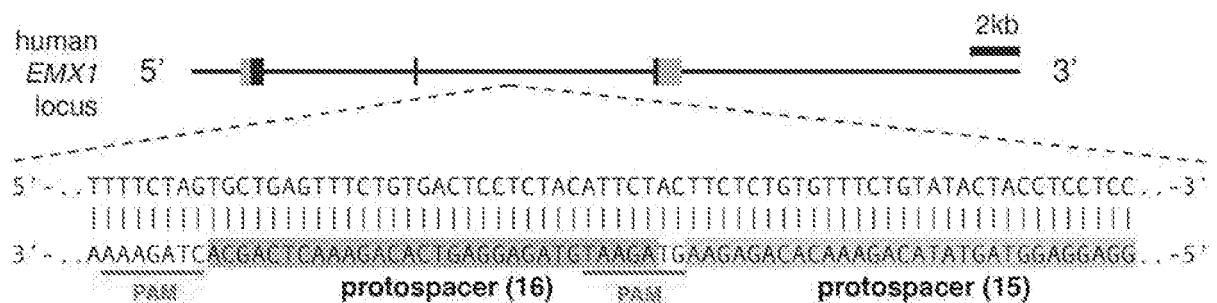
A *Streptococcus thermophilus* LMD-9 CRISPR1**B****C**

FIG. 10A-C

D

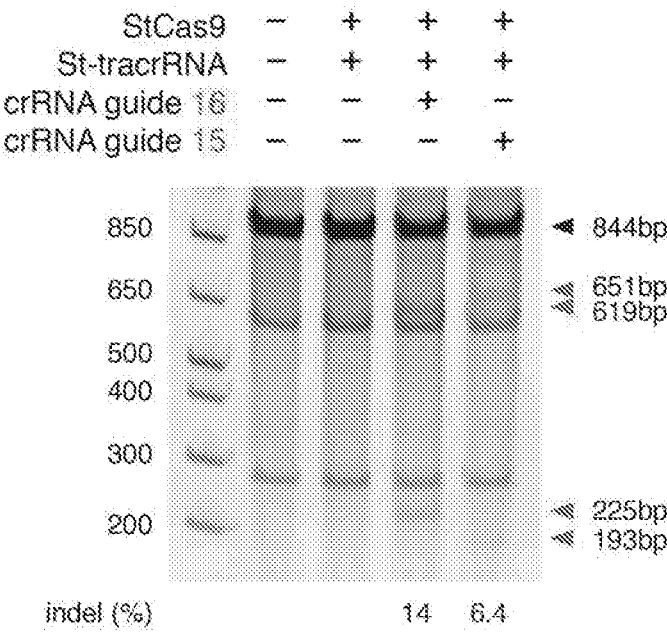
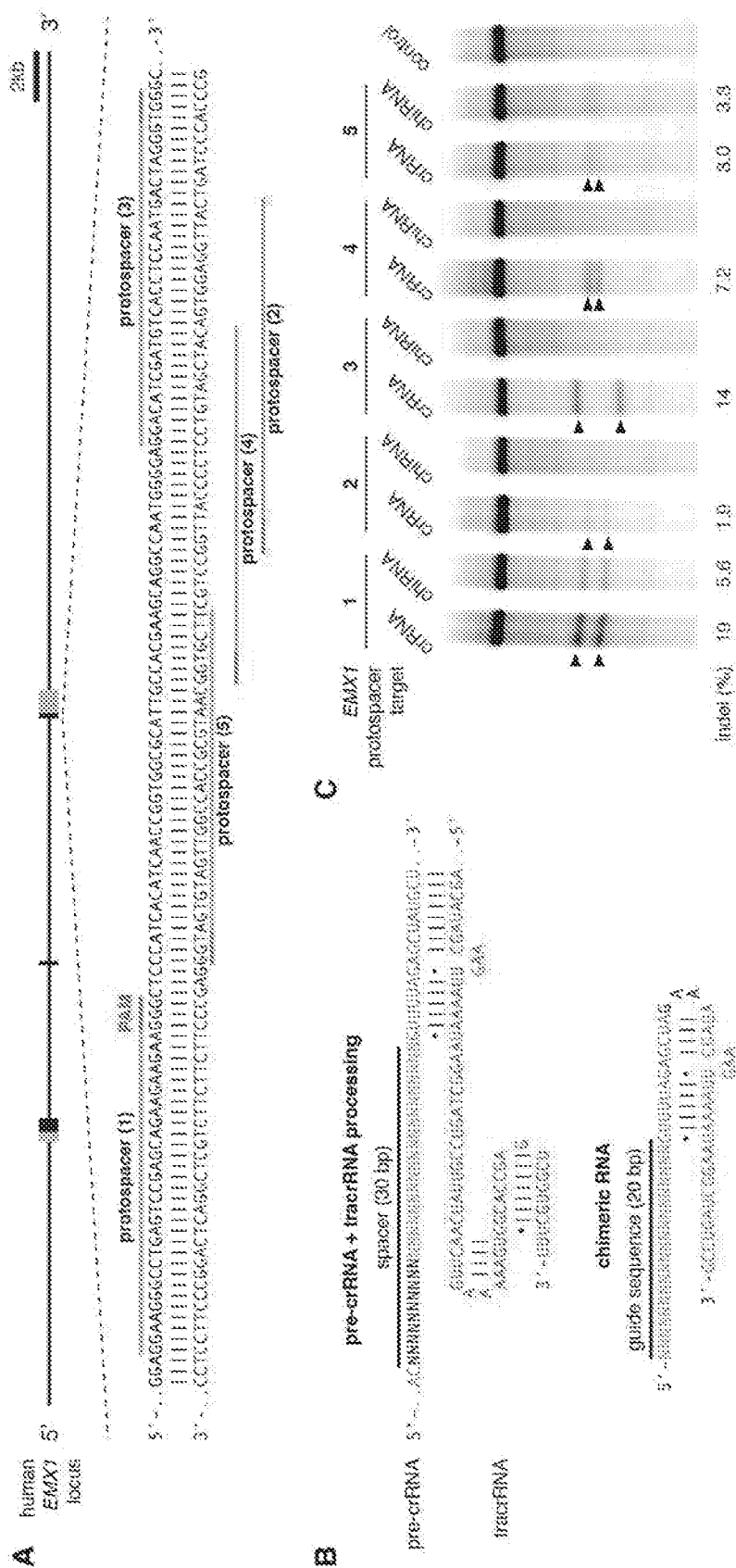
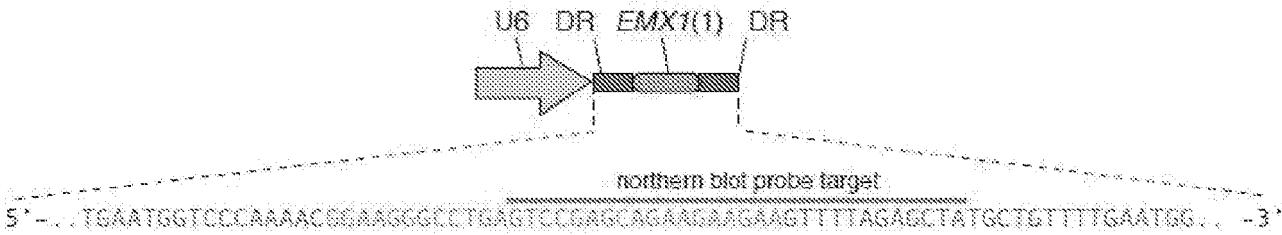


FIG. 10D



A



B

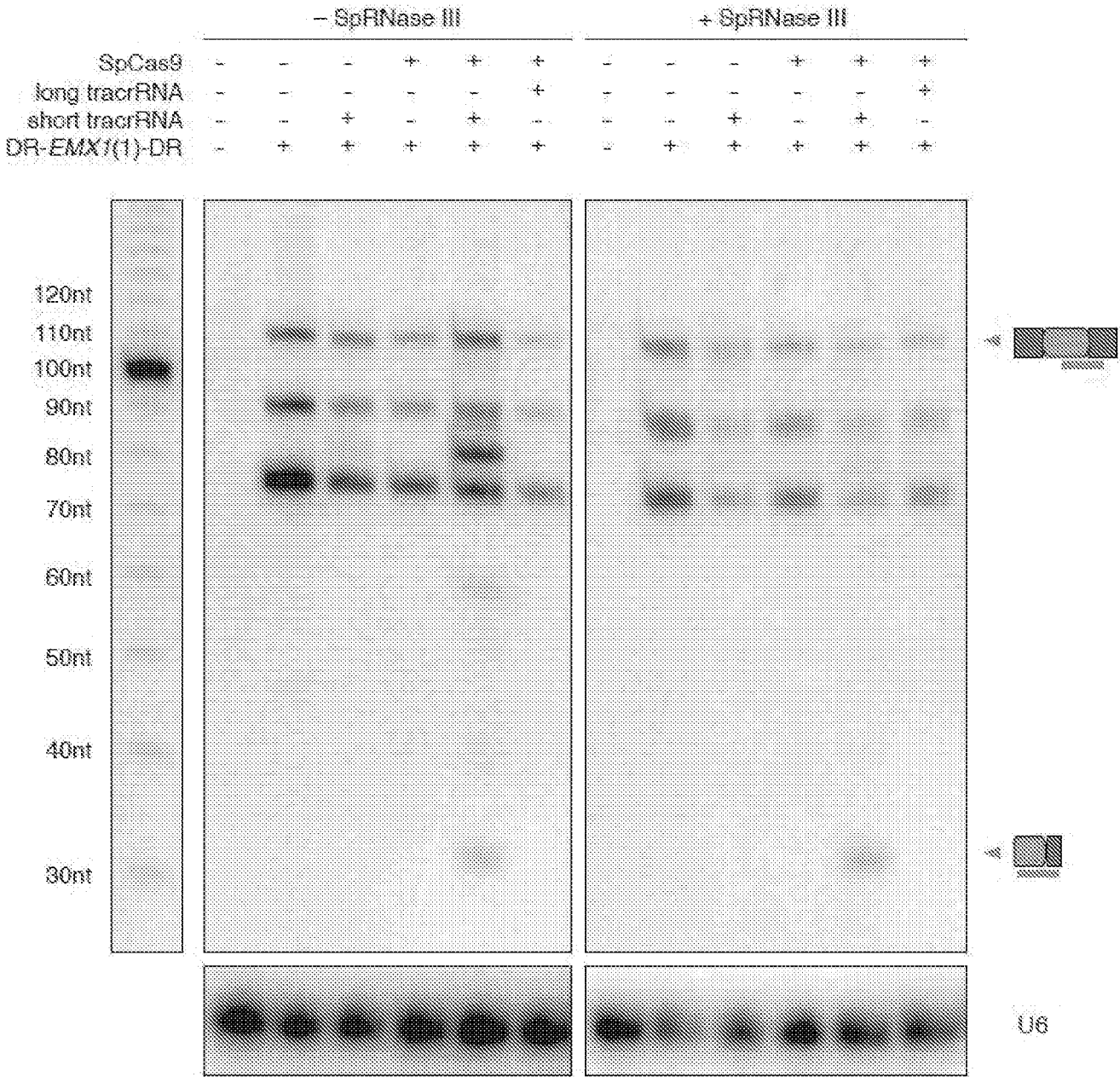


FIG. 12A-B

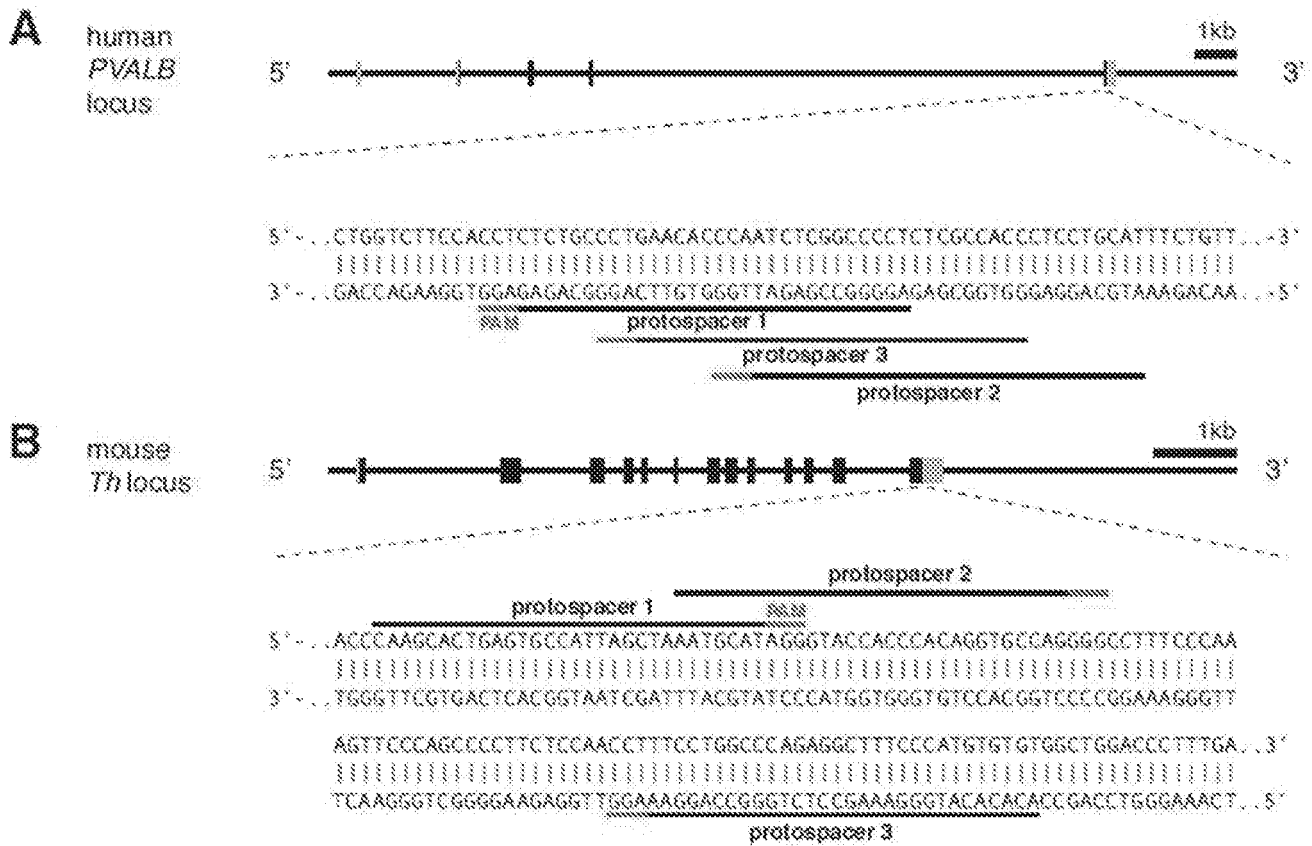


FIG. 13A-B

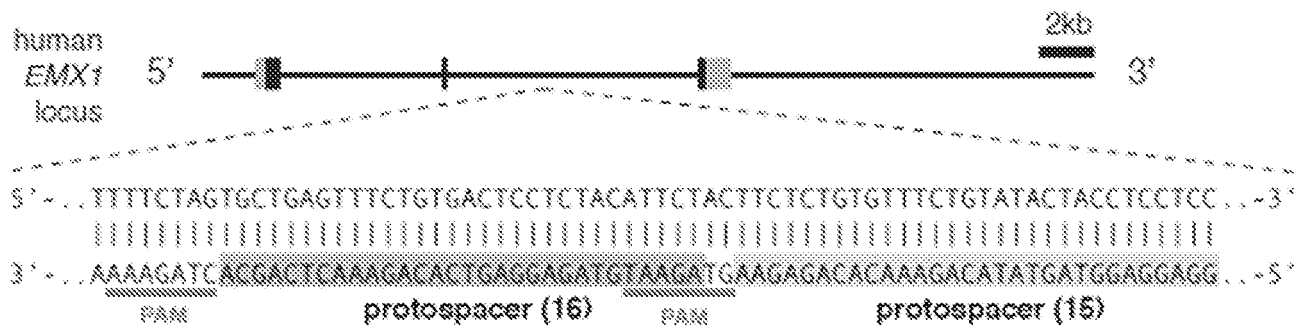
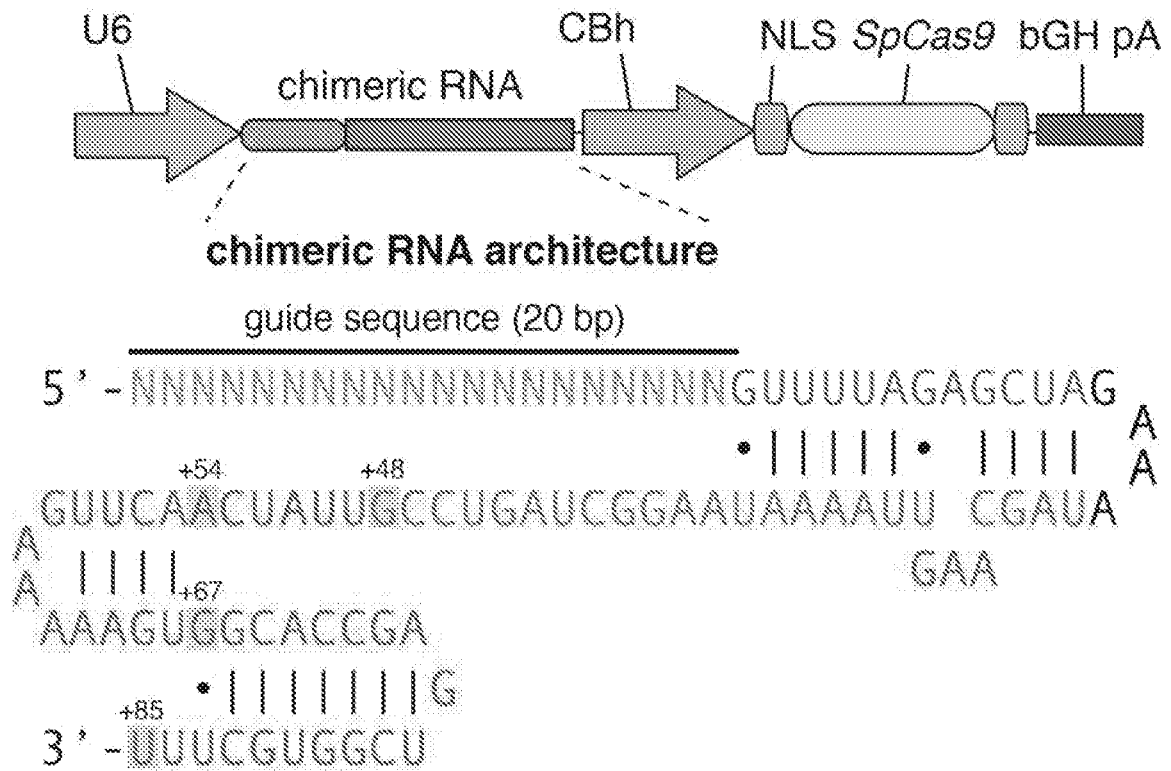


FIG. 14

Primer name	Assay	Genomic Target	Primer sequence
Sp-EMX1-F	SURVEYOR assay, sequencing	<i>EMX1</i>	AAAACCACCCTTCTCTCTGGC
Sp-EMX1-R	SURVEYOR assay, sequencing	<i>EMX1</i>	GGAGATTGGAGACACGGAGAG
Sp-PVALB-F	SURVEYOR assay, sequencing	<i>PVALB</i>	CTGGAAAGCCAATGCCTGAC
Sp-PVALB-R	SURVEYOR assay, sequencing	<i>PVALB</i>	GGCAGCAAACCTCCTTGTCCT
Sp-Th-F	SURVEYOR assay, sequencing	<i>Th</i>	GTGCTTTGCAGAGGCCTACC
Sp-Th-R	SURVEYOR assay, sequencing	<i>Th</i>	CCTGGAGCGCATGCAGTAGT
St-EMX1-F	SURVEYOR assay, sequencing	<i>EMX1</i>	ACCTTCTGTGTTTCCACCATTC
St-EMX1-R	SURVEYOR assay, sequencing	<i>EMX1</i>	TTGGGGAGTGCACAGACTTC
Sp-EMX1- RFLP-F	RFLP, sequencing	<i>EMX1</i>	GGCTCCCTGGGTTCAAAGTA
Sp-EMX1- RFLP-R	RFLP, sequencing	<i>EMX1</i>	AGAGGGGTCTGGATGTCGTAA
Pb_EMX1_sp1	Northern Blot Probe	Not applicable	TAGCTCTAAAACTTCTTCTTCTGCTCGGAC
Pb_tracrRNA	Northern Blot Probe	Not applicable	CTAGCCTTATTTTAACTTGCTATGCTGTTT

FIG. 15

a**FIG. 16A**

b

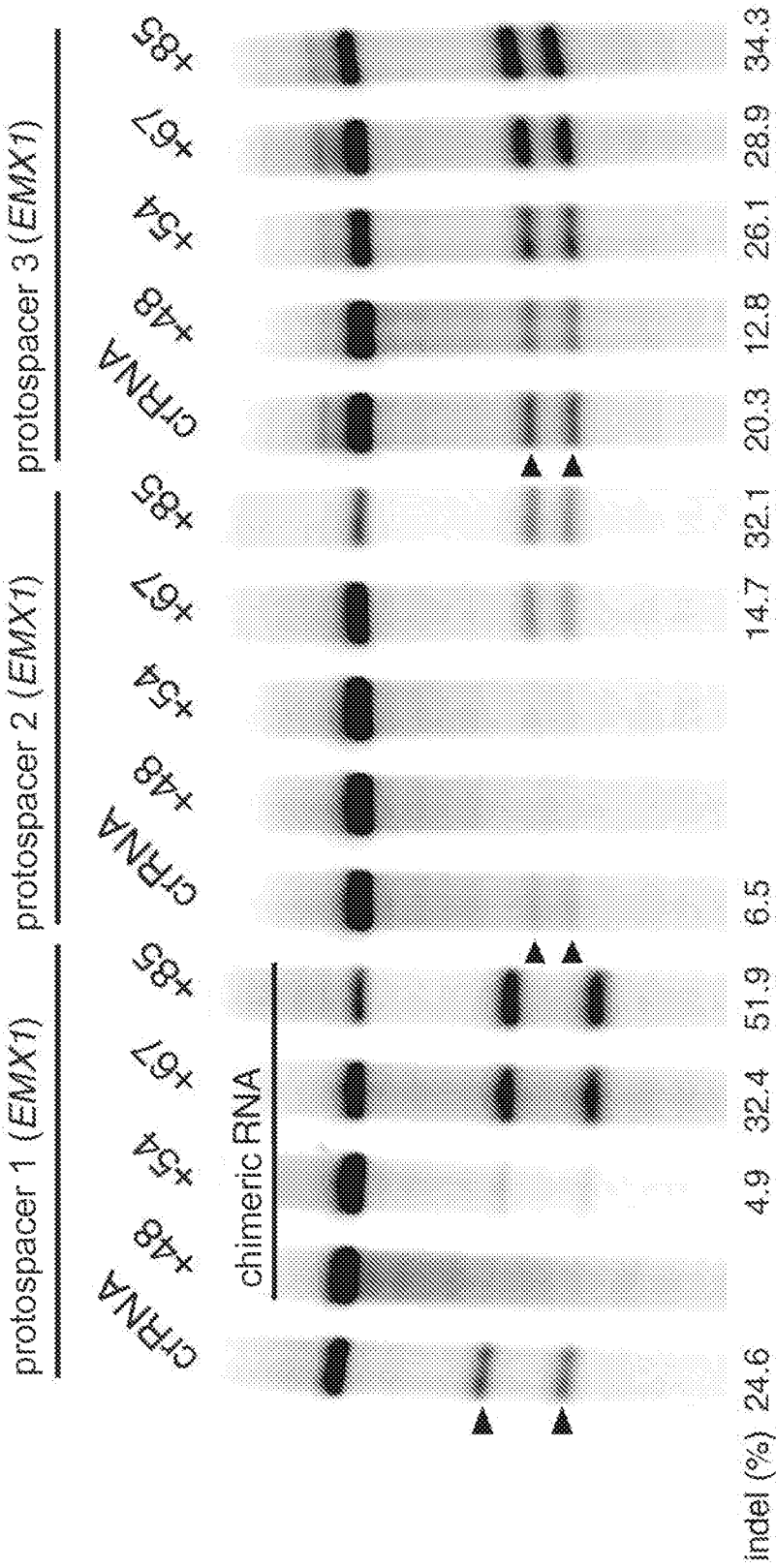


FIG. 16B

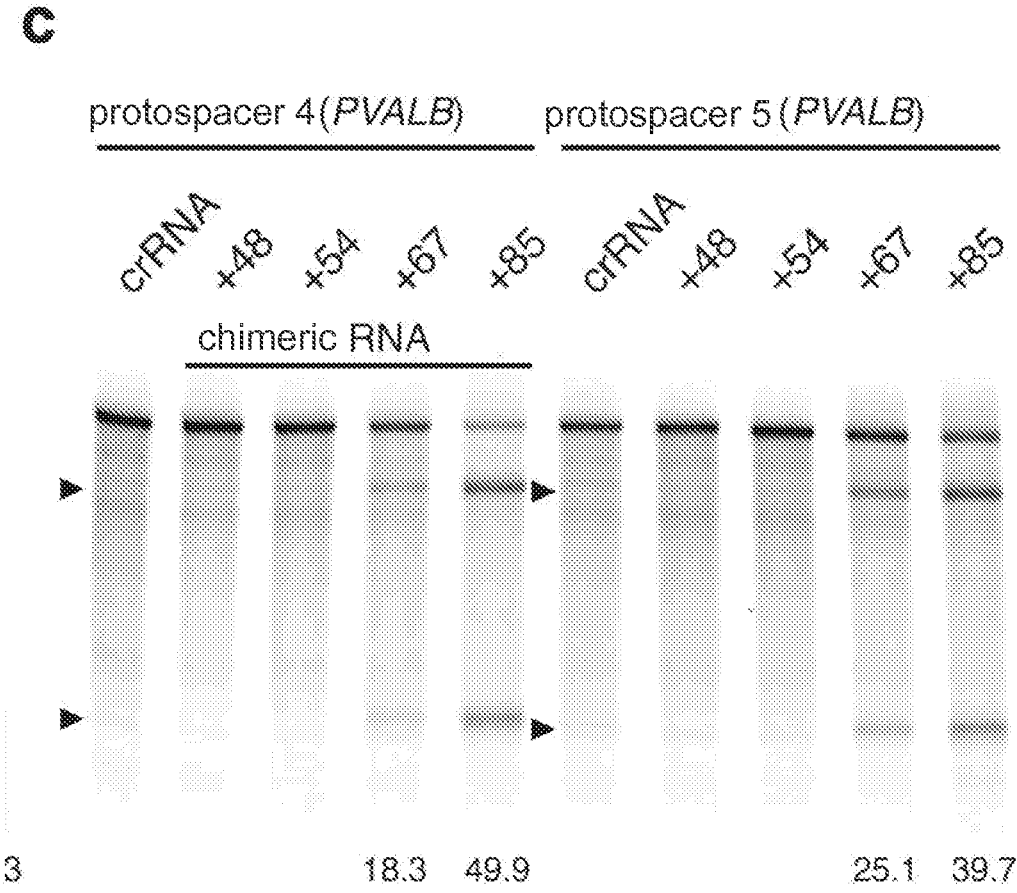


FIG. 16C

29/110

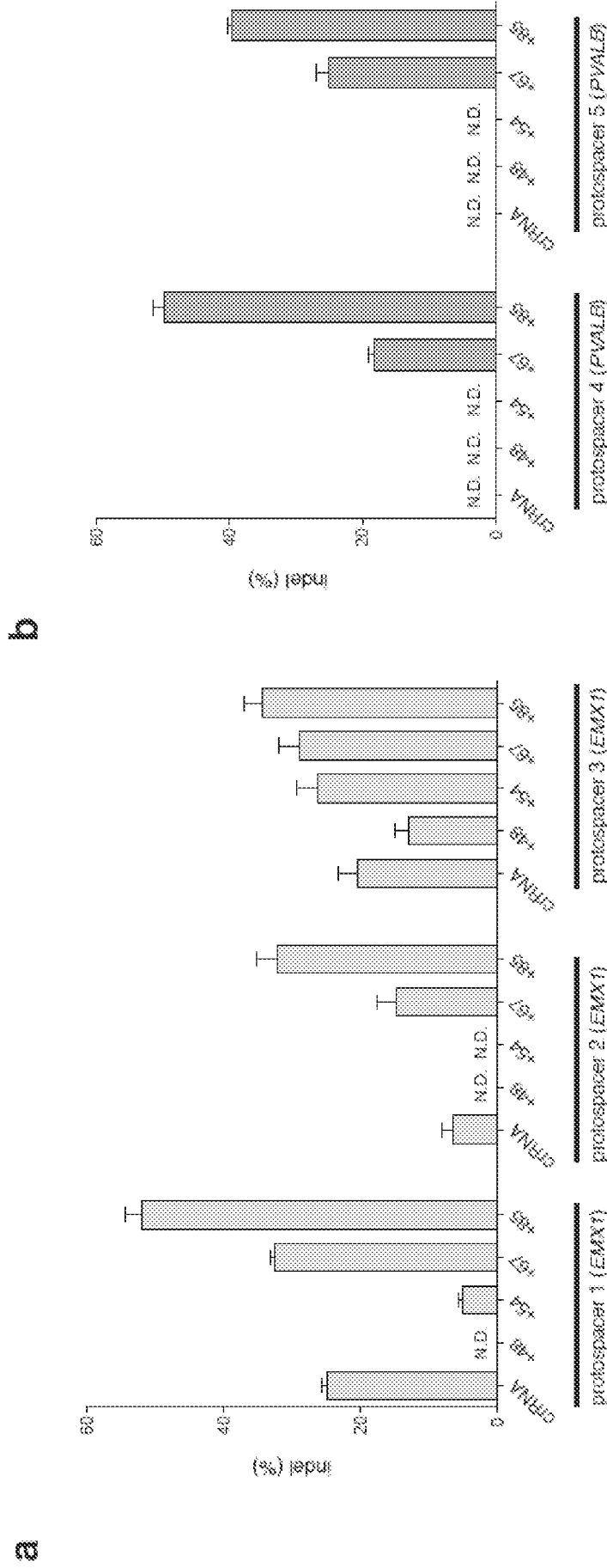


FIG. 17A-B

UCSC Genome Browser on Human Feb. 2009 (GRCh37/hg19) Assembly

move: <<< << < > >> >>> zoom in 1.5x 3x 10x base zoom out 1.5x 3x 10x
chr12:2,614,008-2,675,748 61,741 bp. enter position, gene symbol or search terms go
chr12 (p13.33)

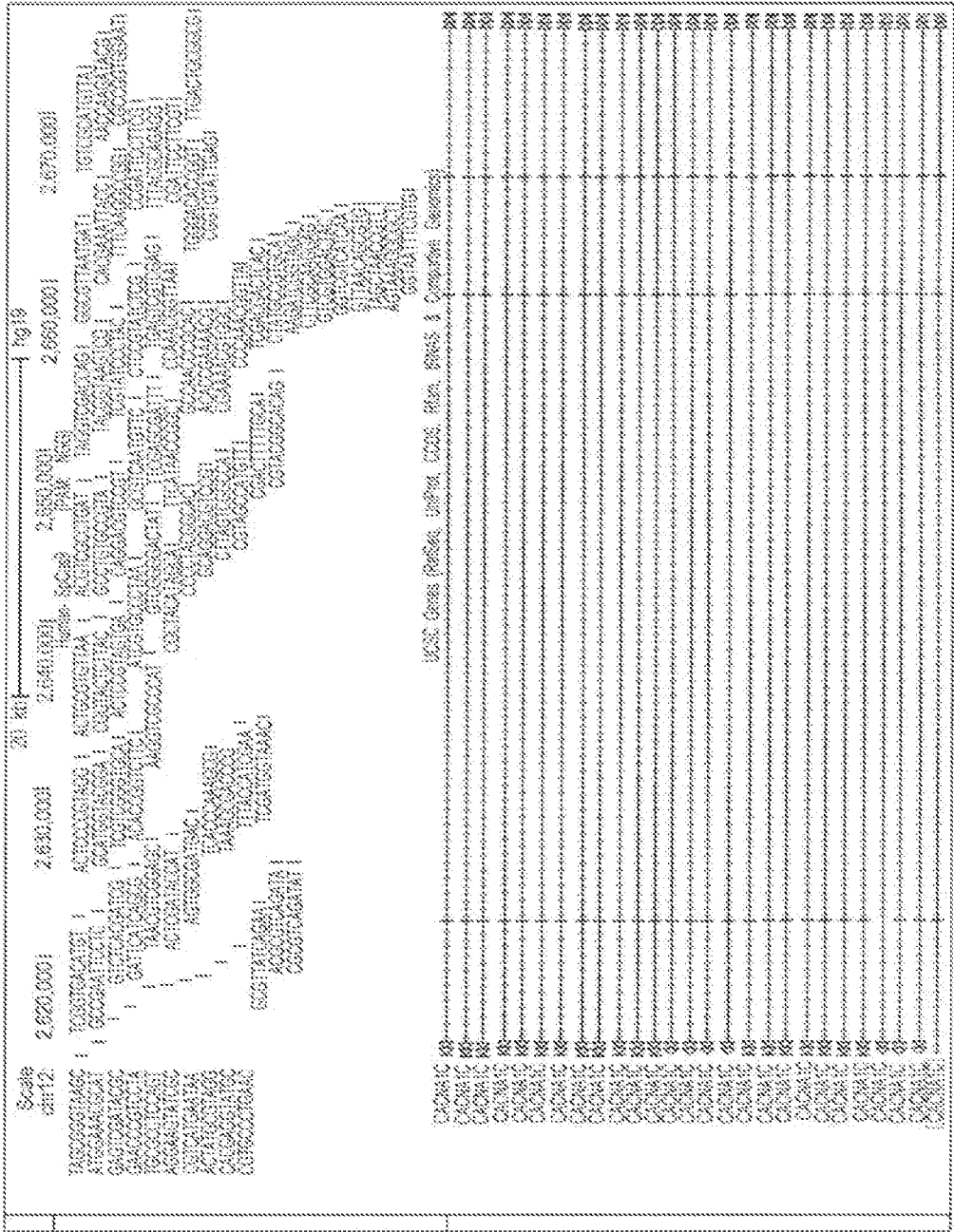
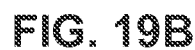
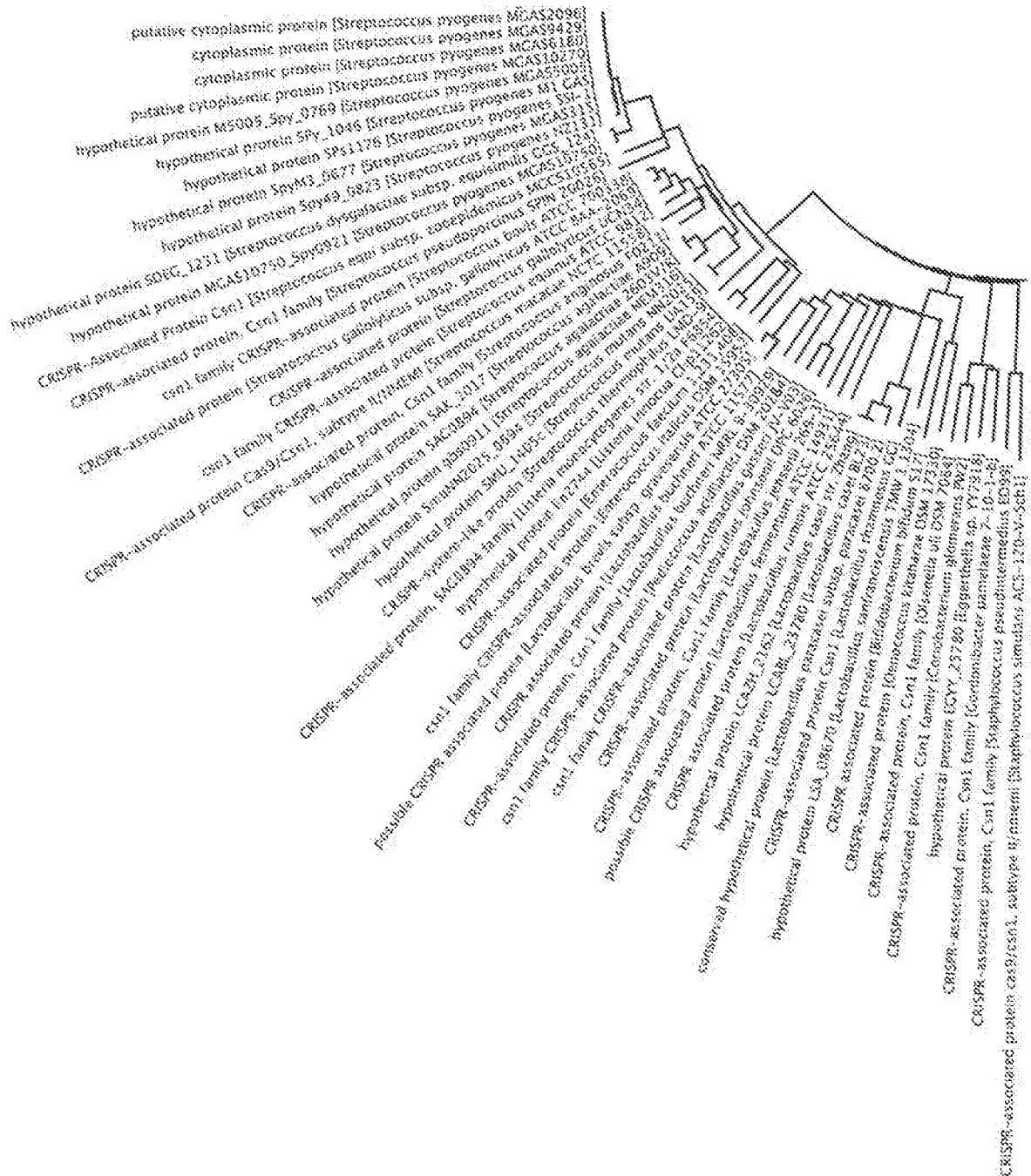


FIG. 18





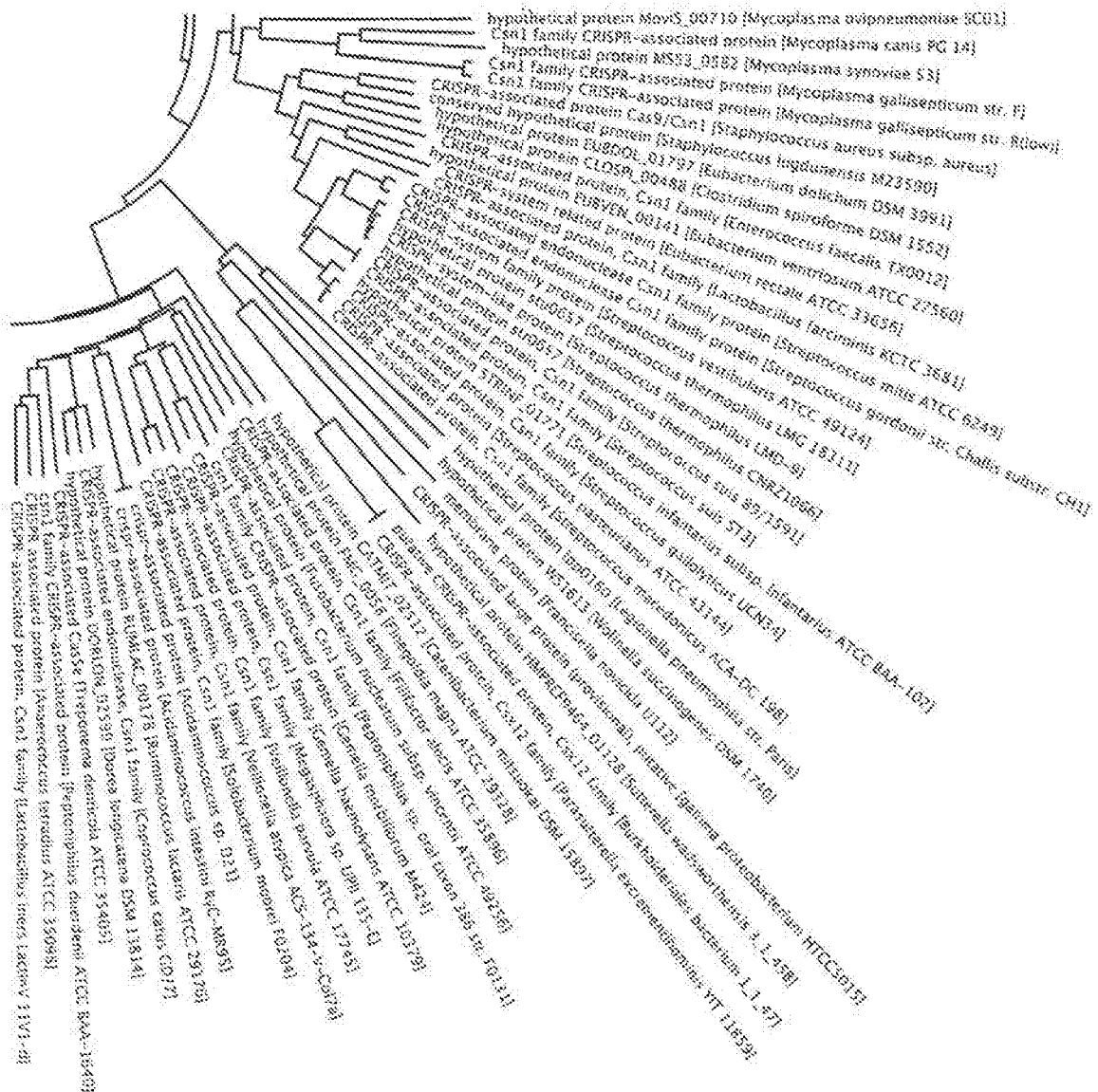


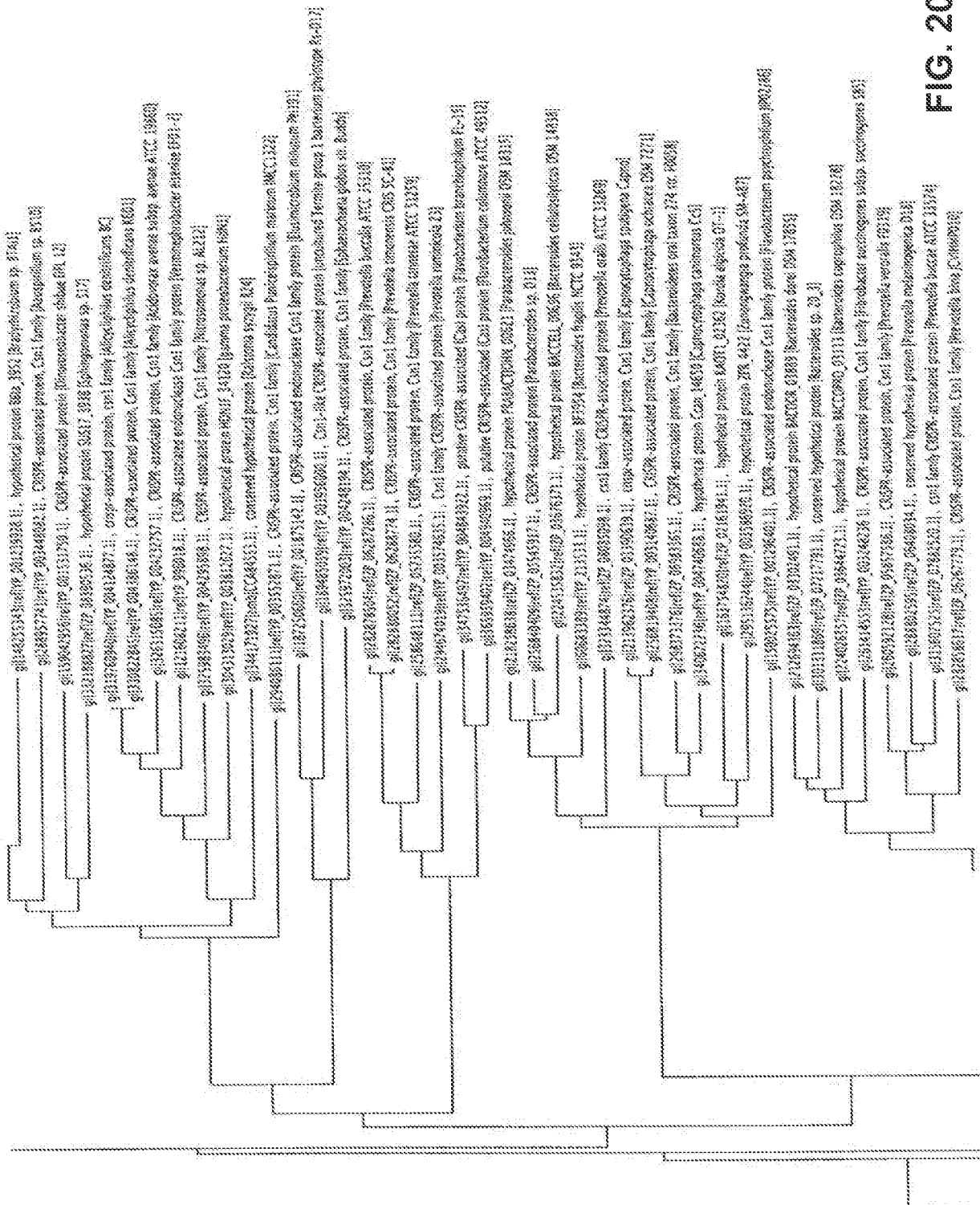
FIG. 19D

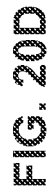


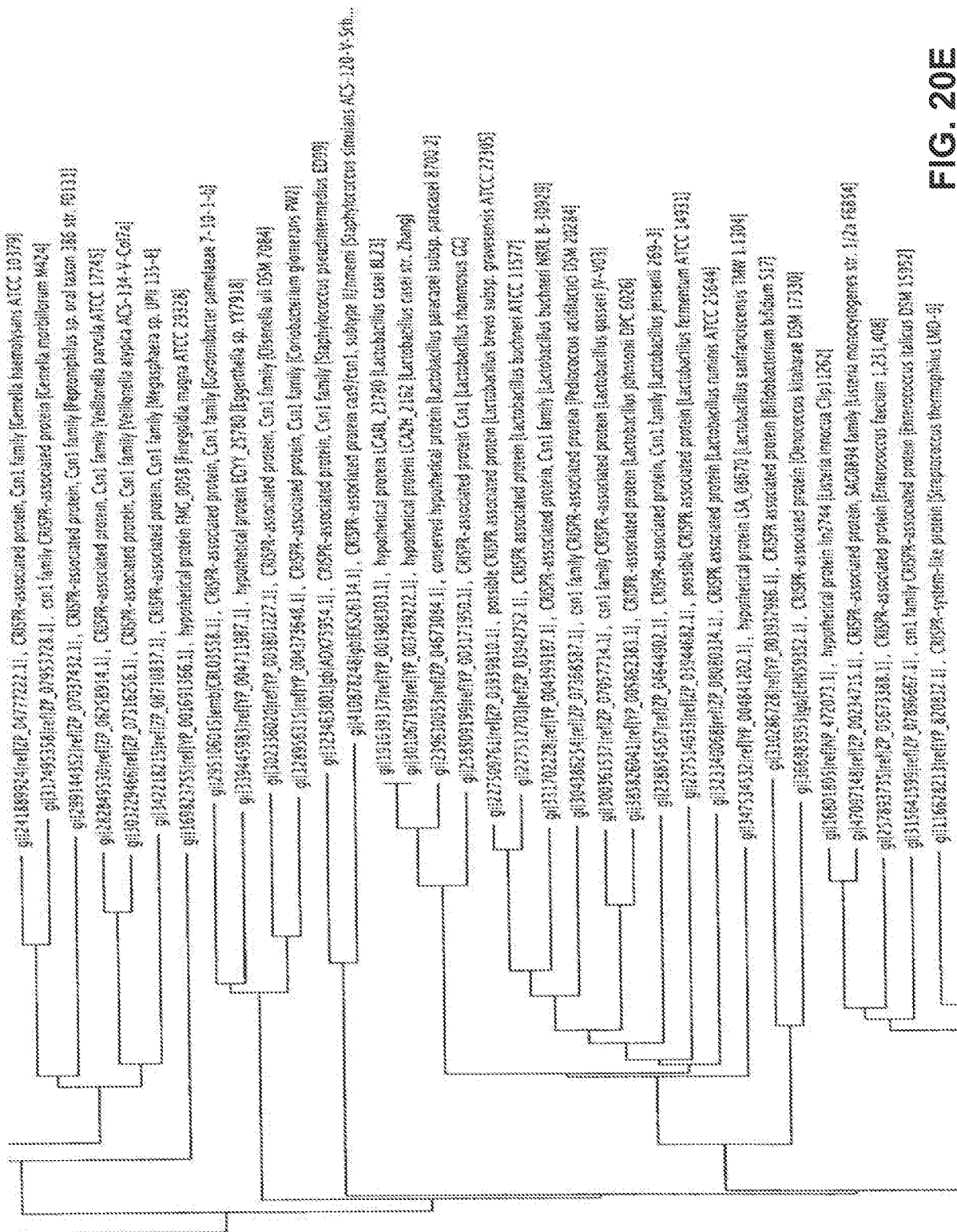
FIG. 20A



FIG. 20B







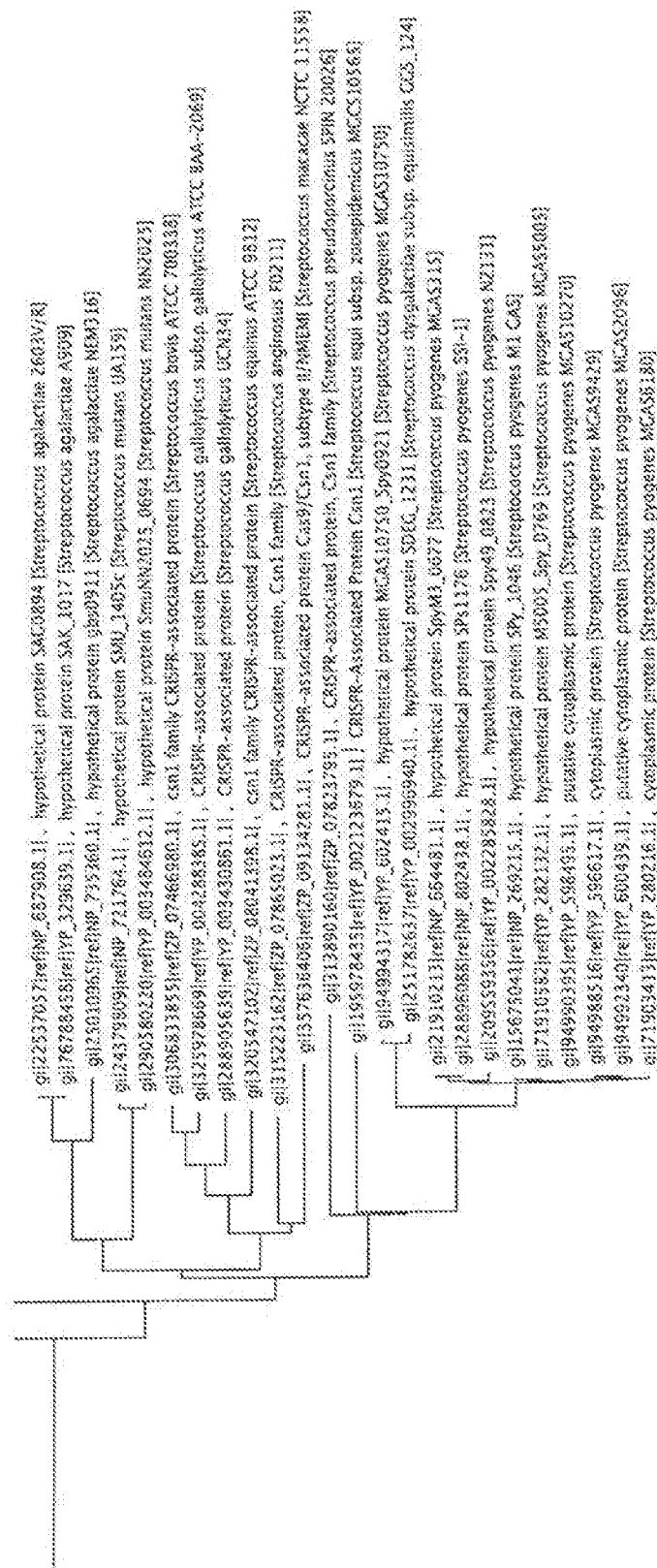


FIG. 20F

a

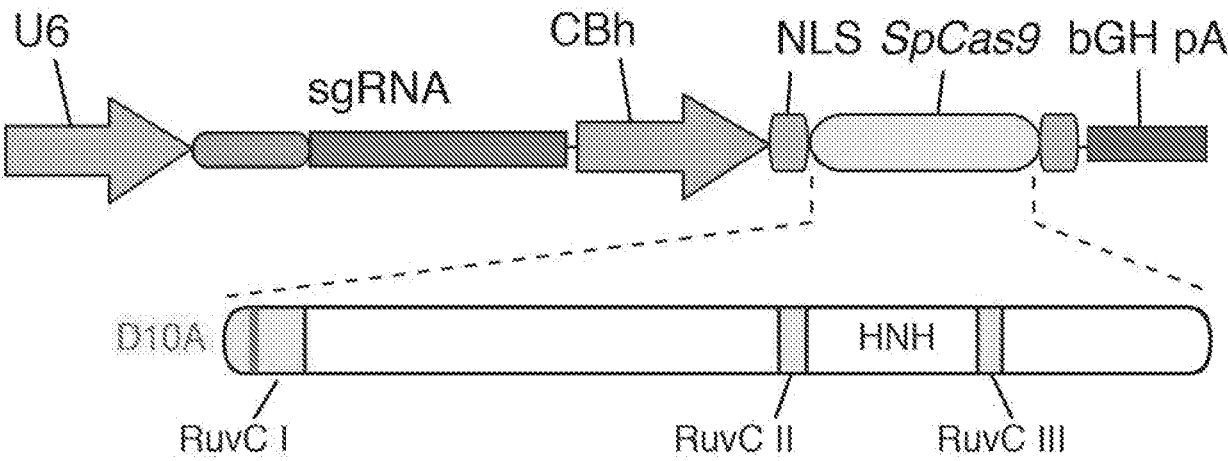


FIG. 21A

b

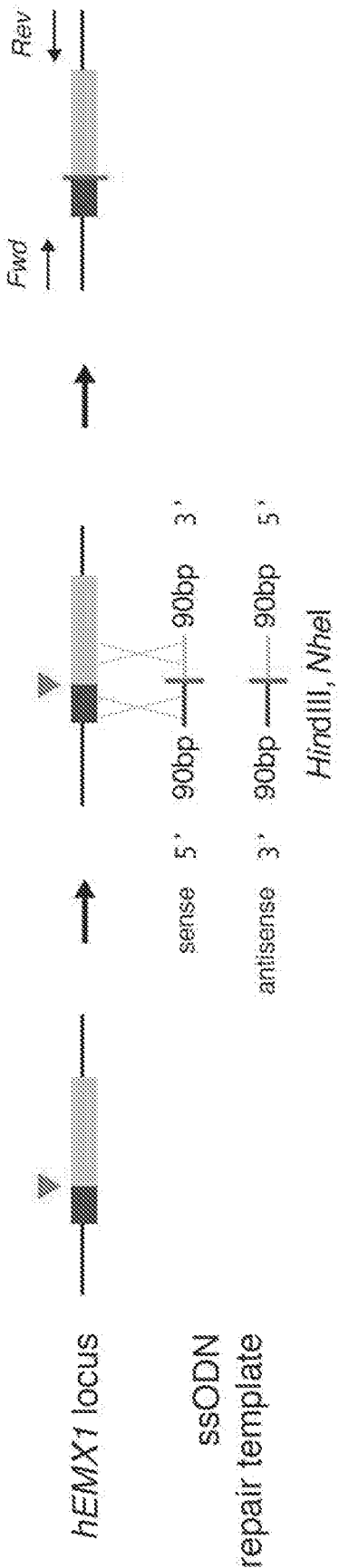


FIG. 21B

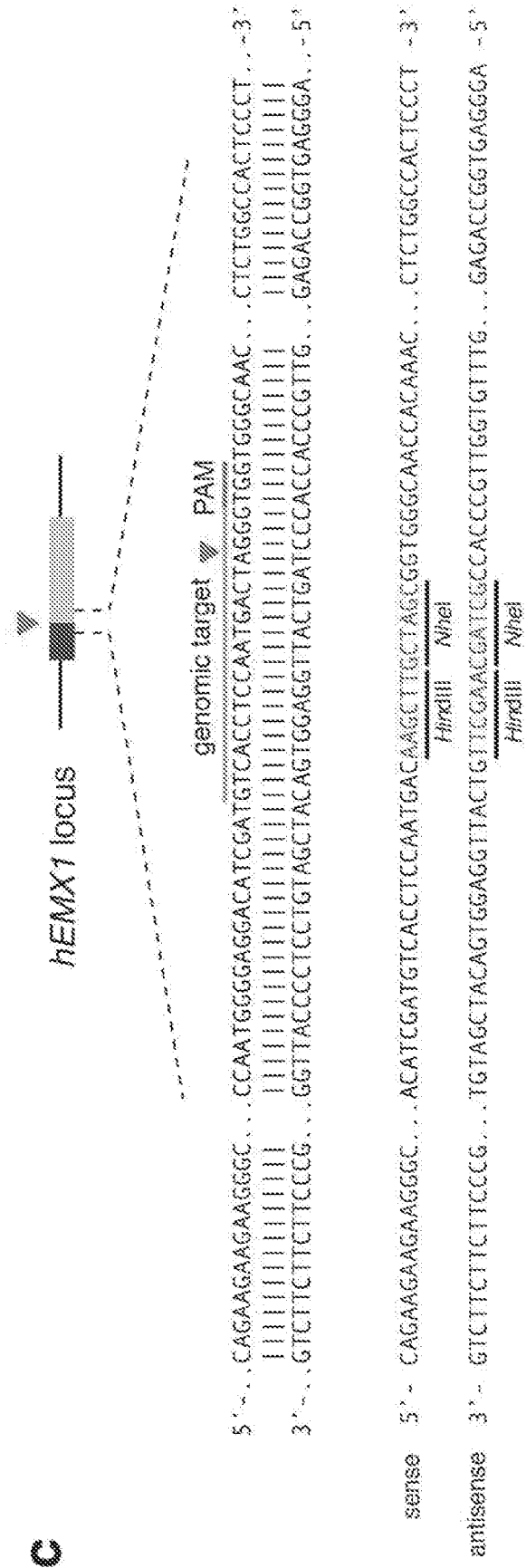


FIG. 21C

d

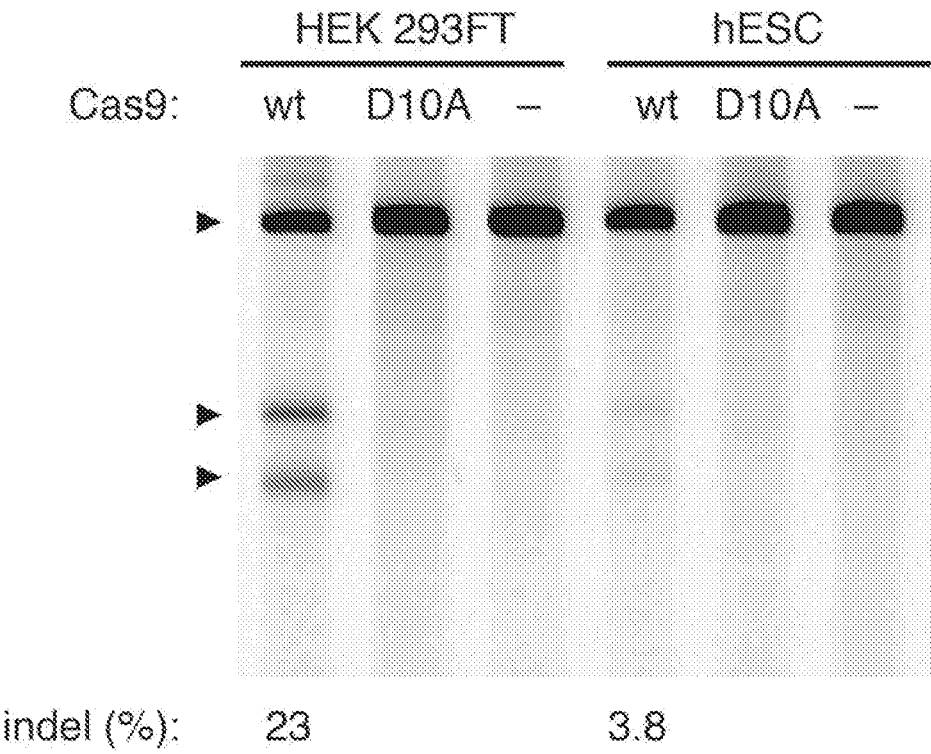


FIG. 21D

b

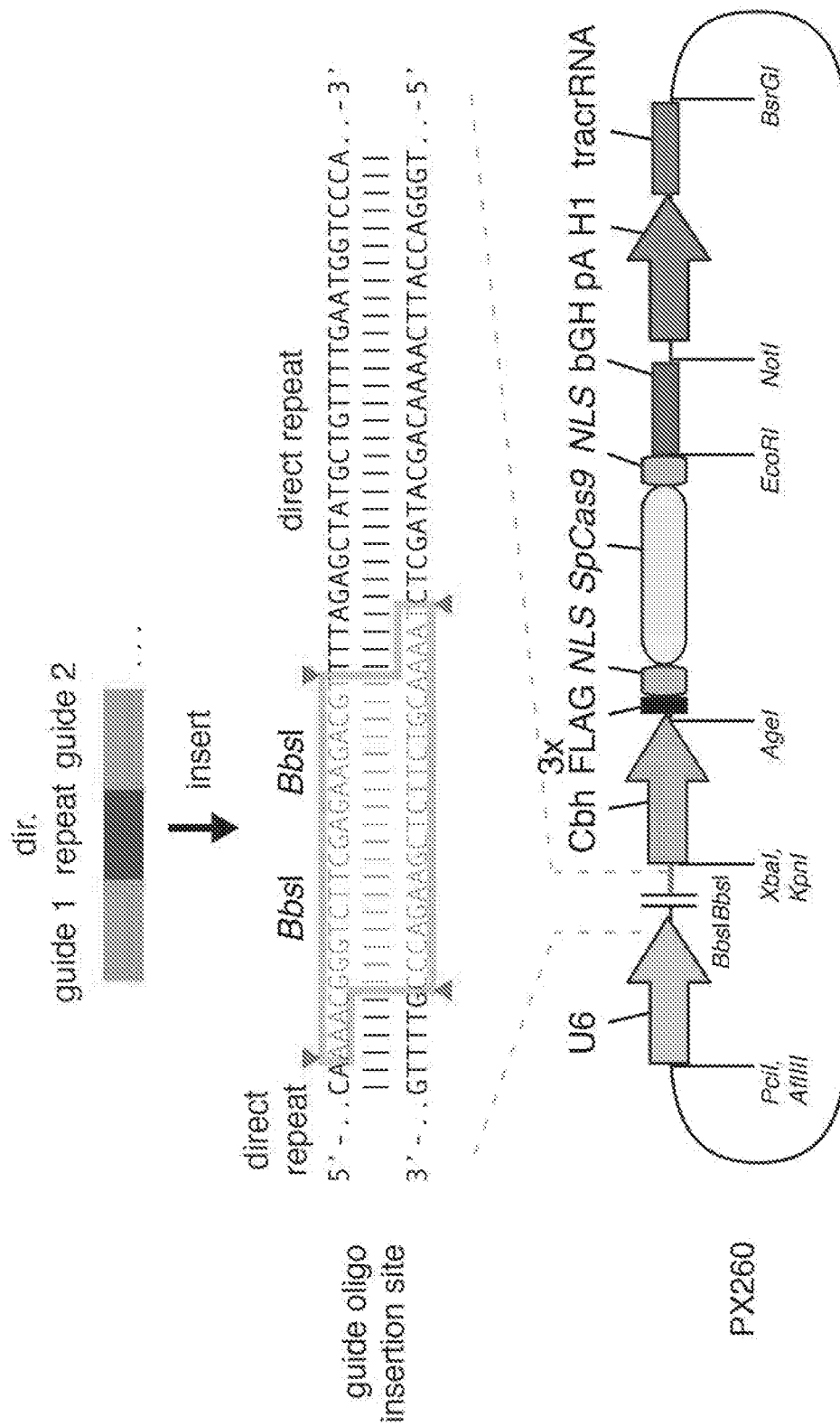


FIG. 22B

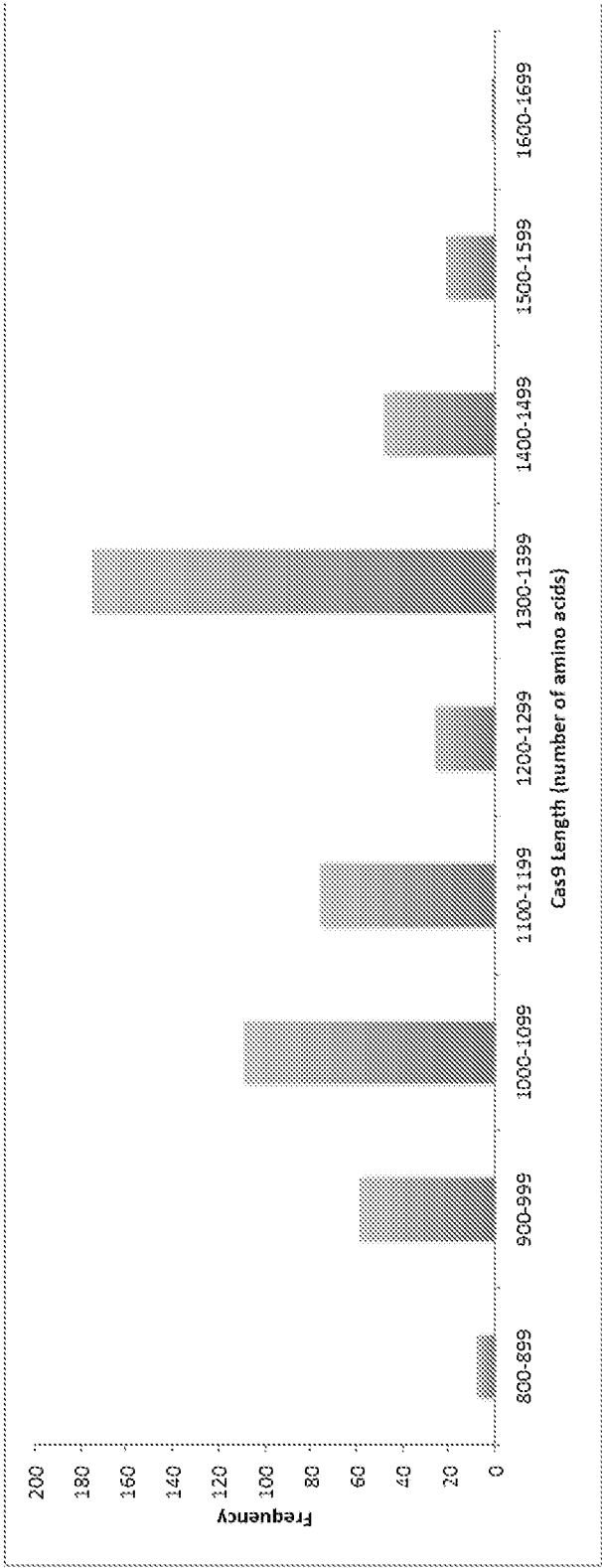


FIG. 23

SpCas9 mutation positions

hSpCas9

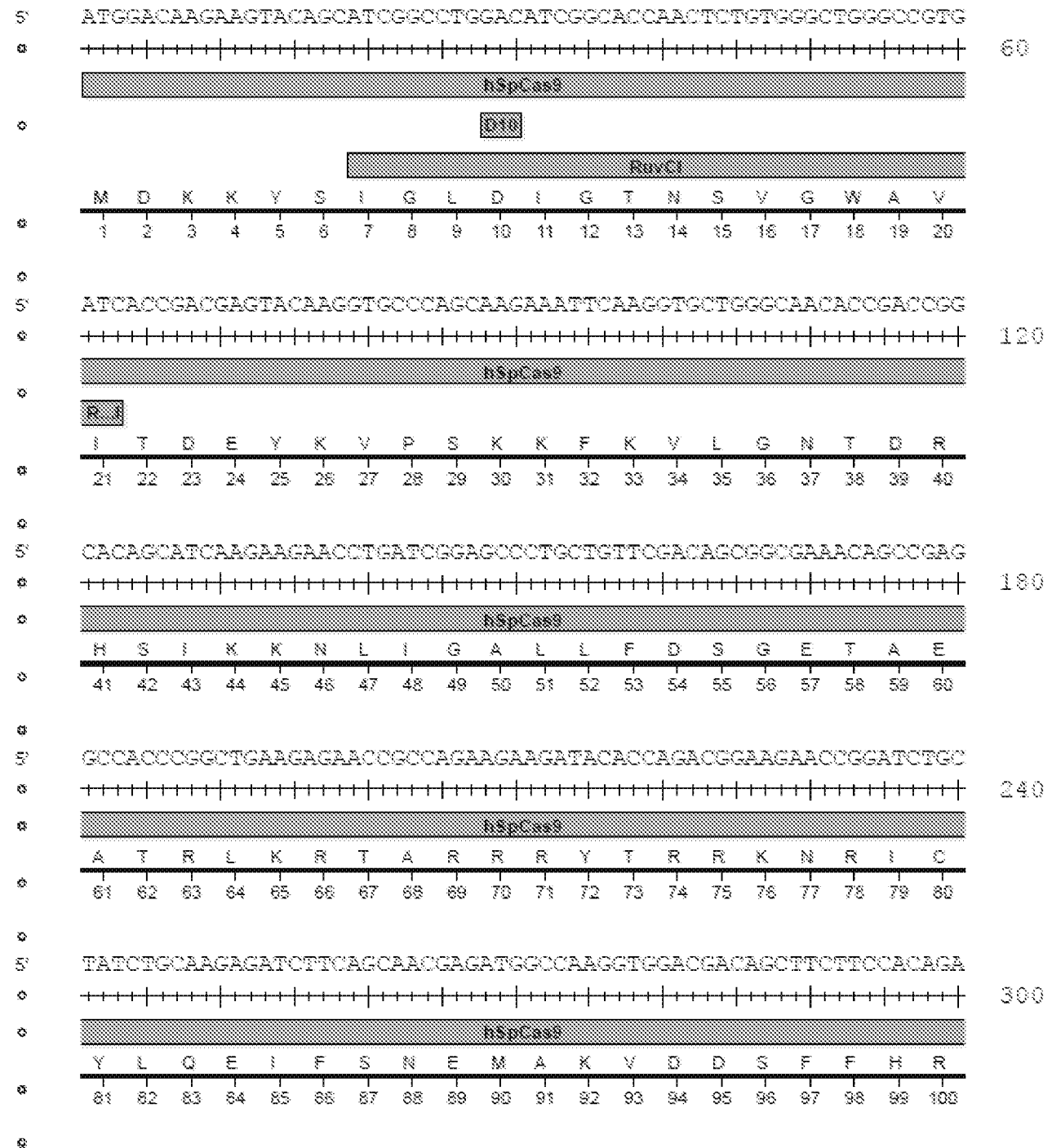


FIG. 24A

hSpCas9

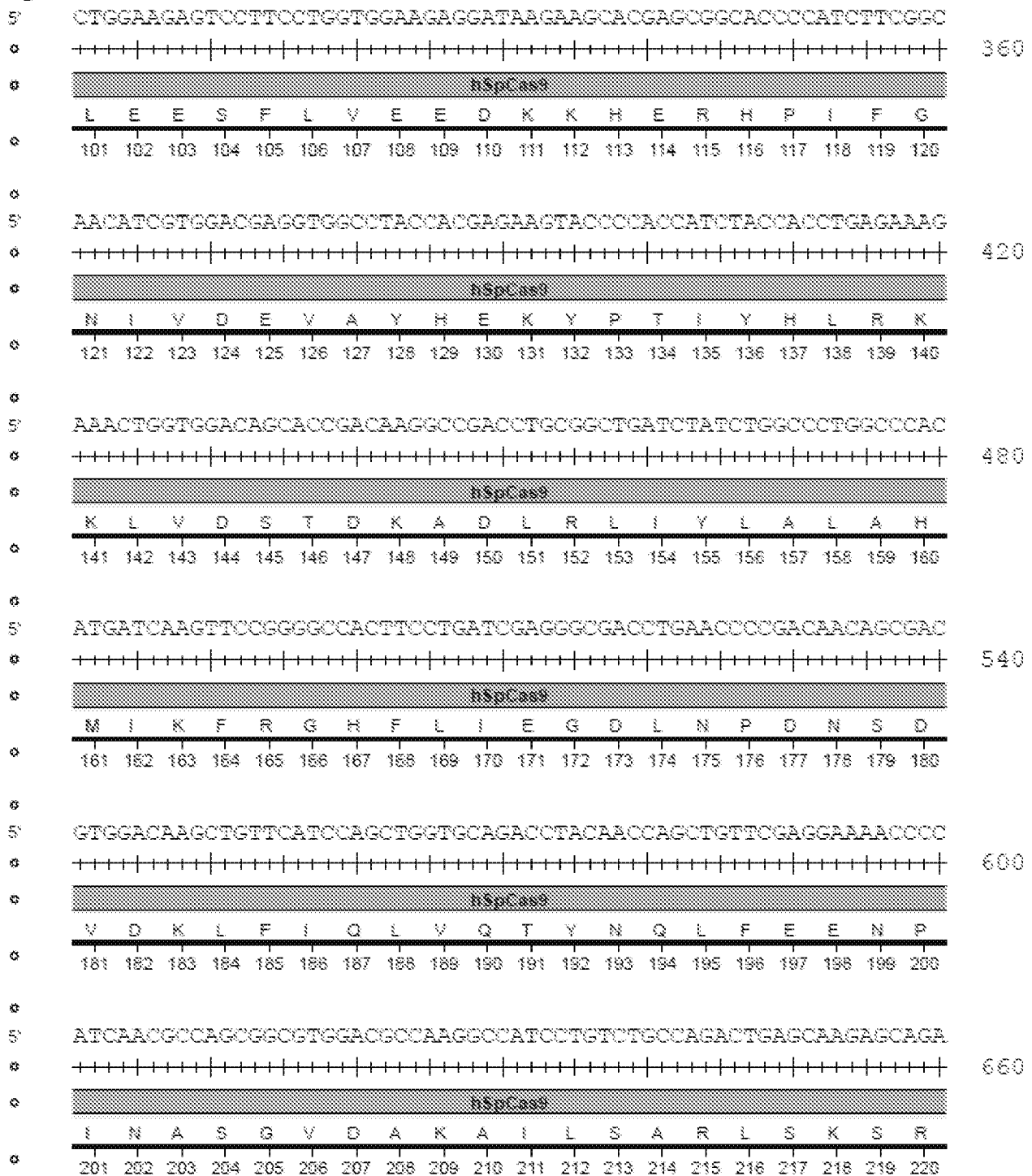


FIG. 24B

hSpCas9

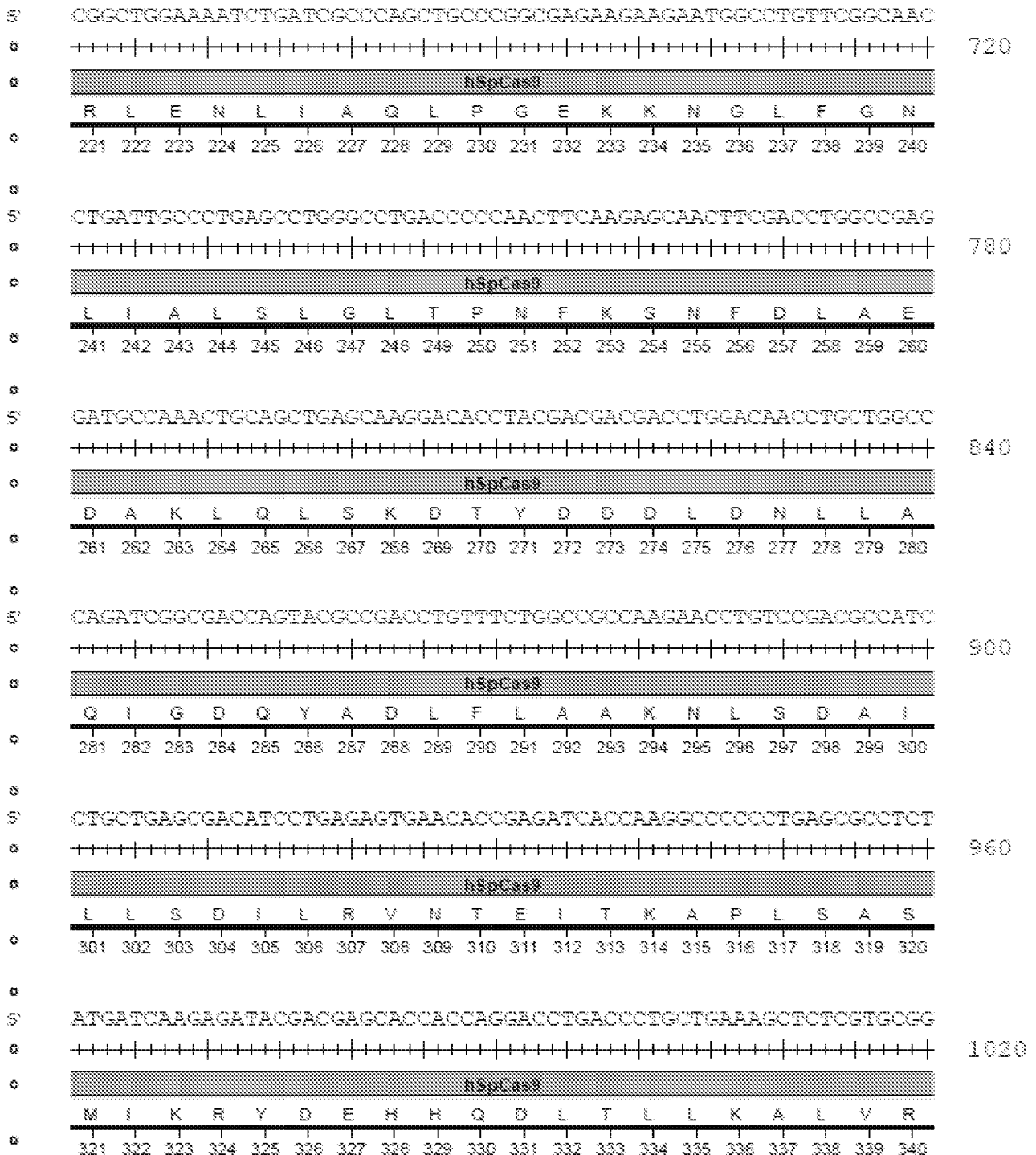


FIG. 24C

hSpCas9

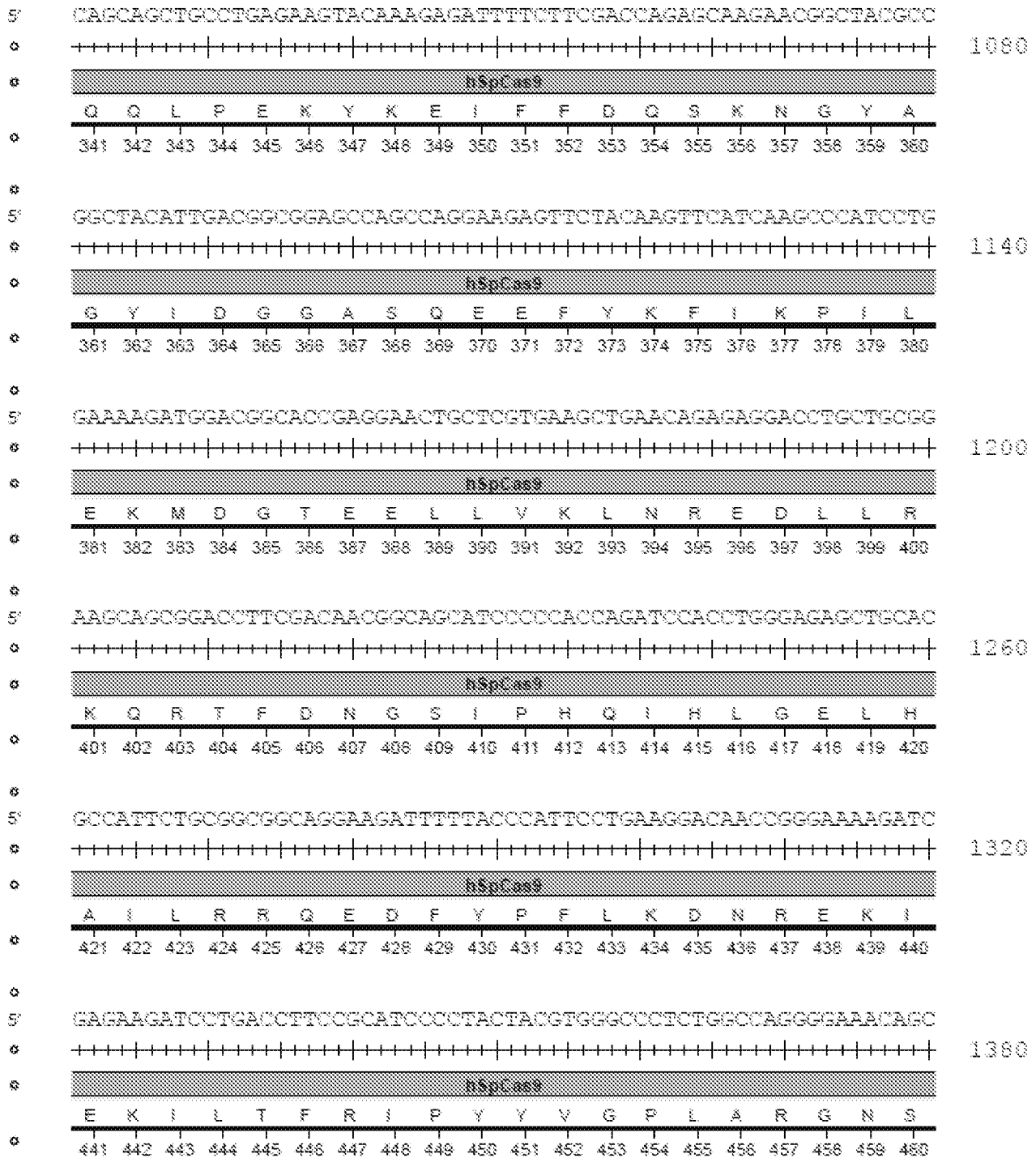


FIG. 24D

hSpCas9

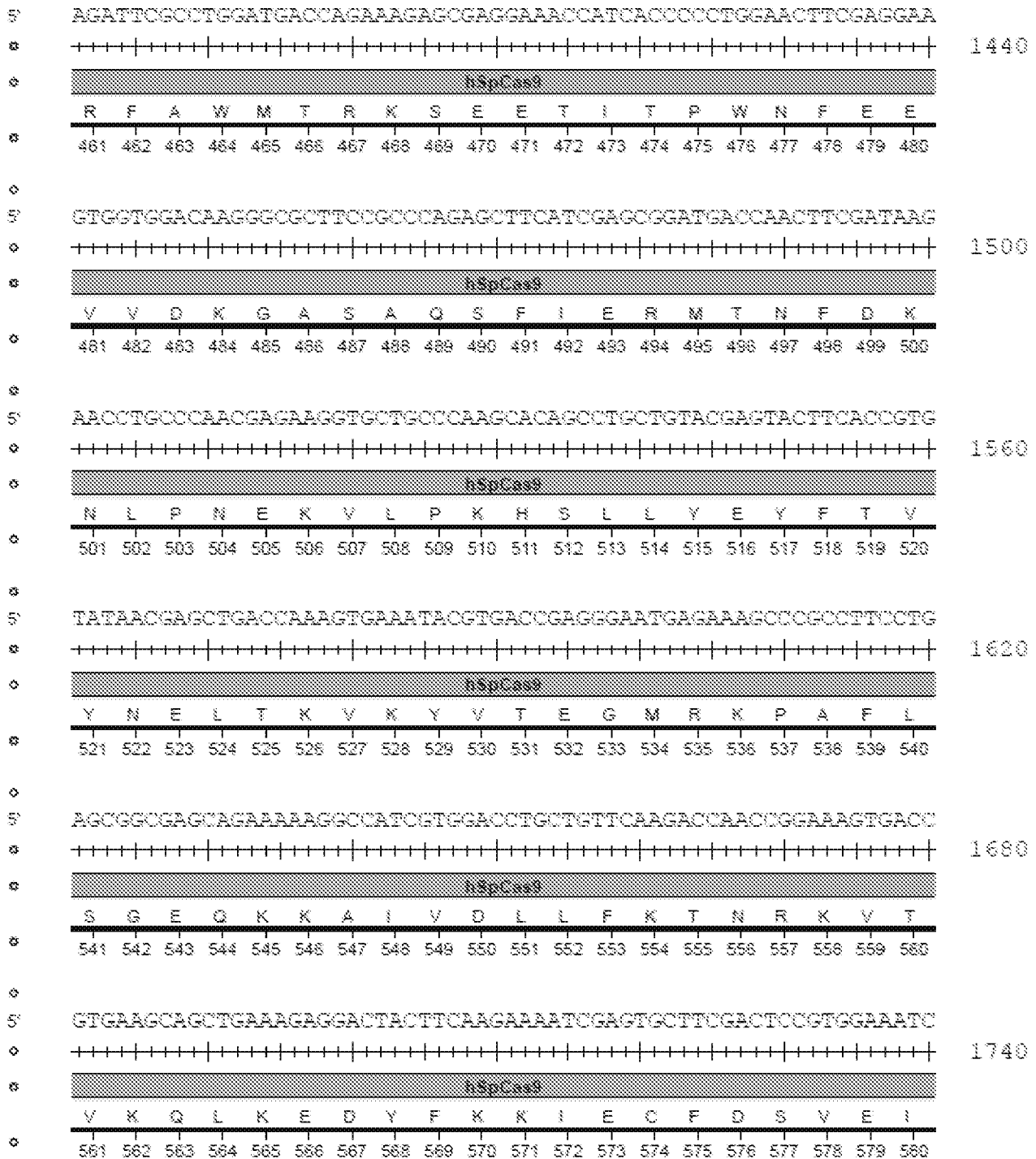


FIG. 24E

hSpCas9

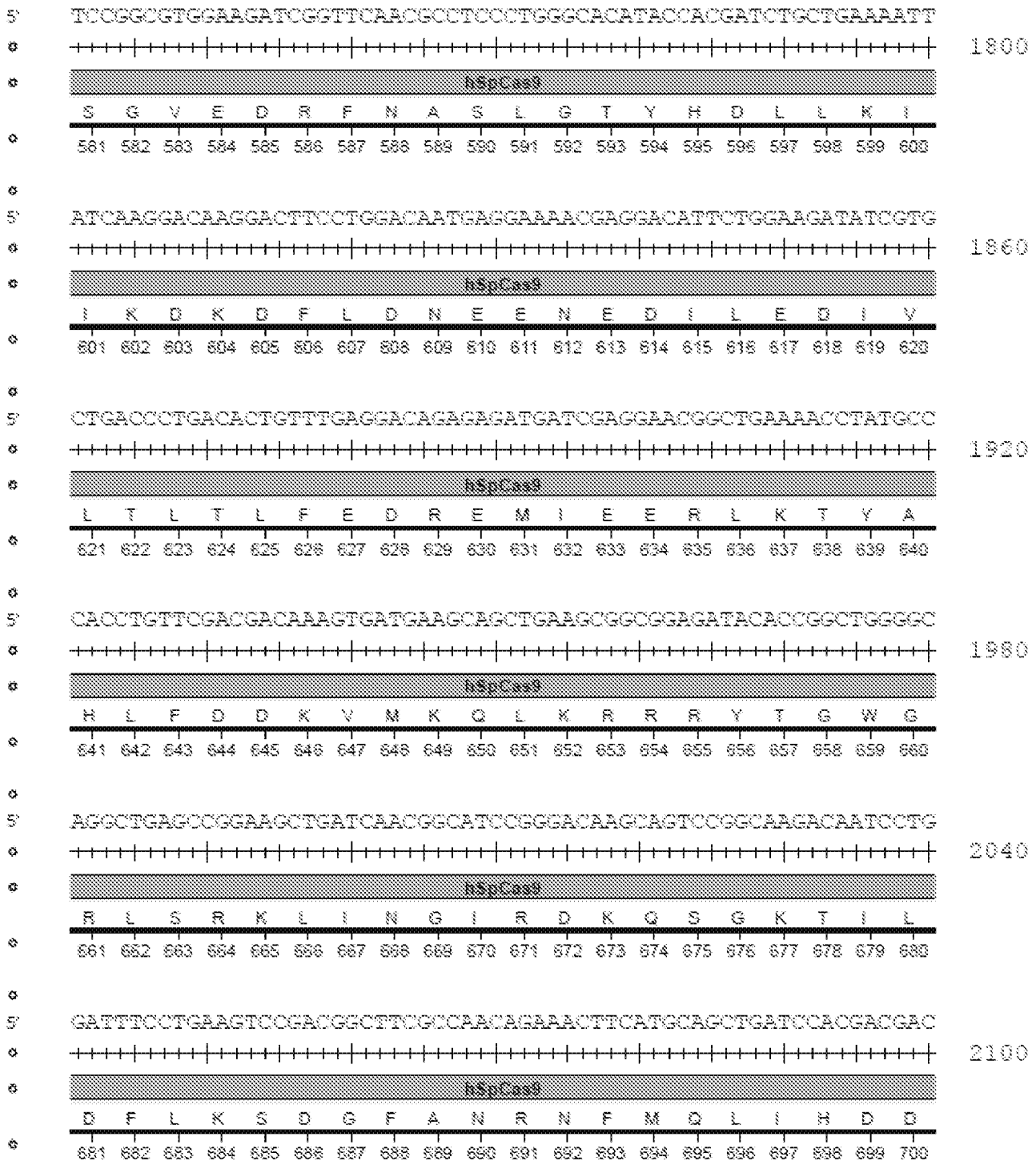


FIG. 24F

hSpCas9

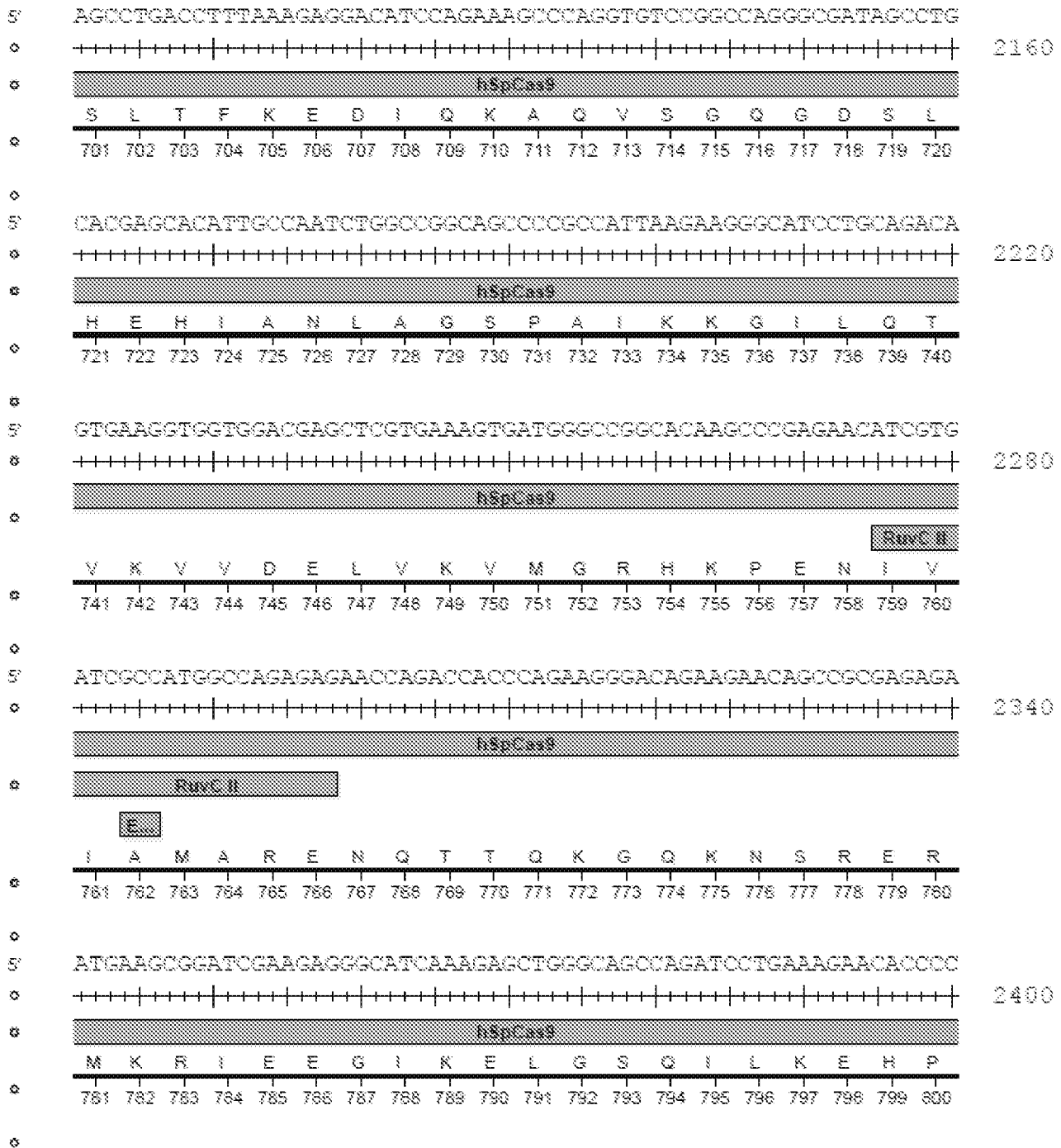


FIG. 24G

hSpCas9

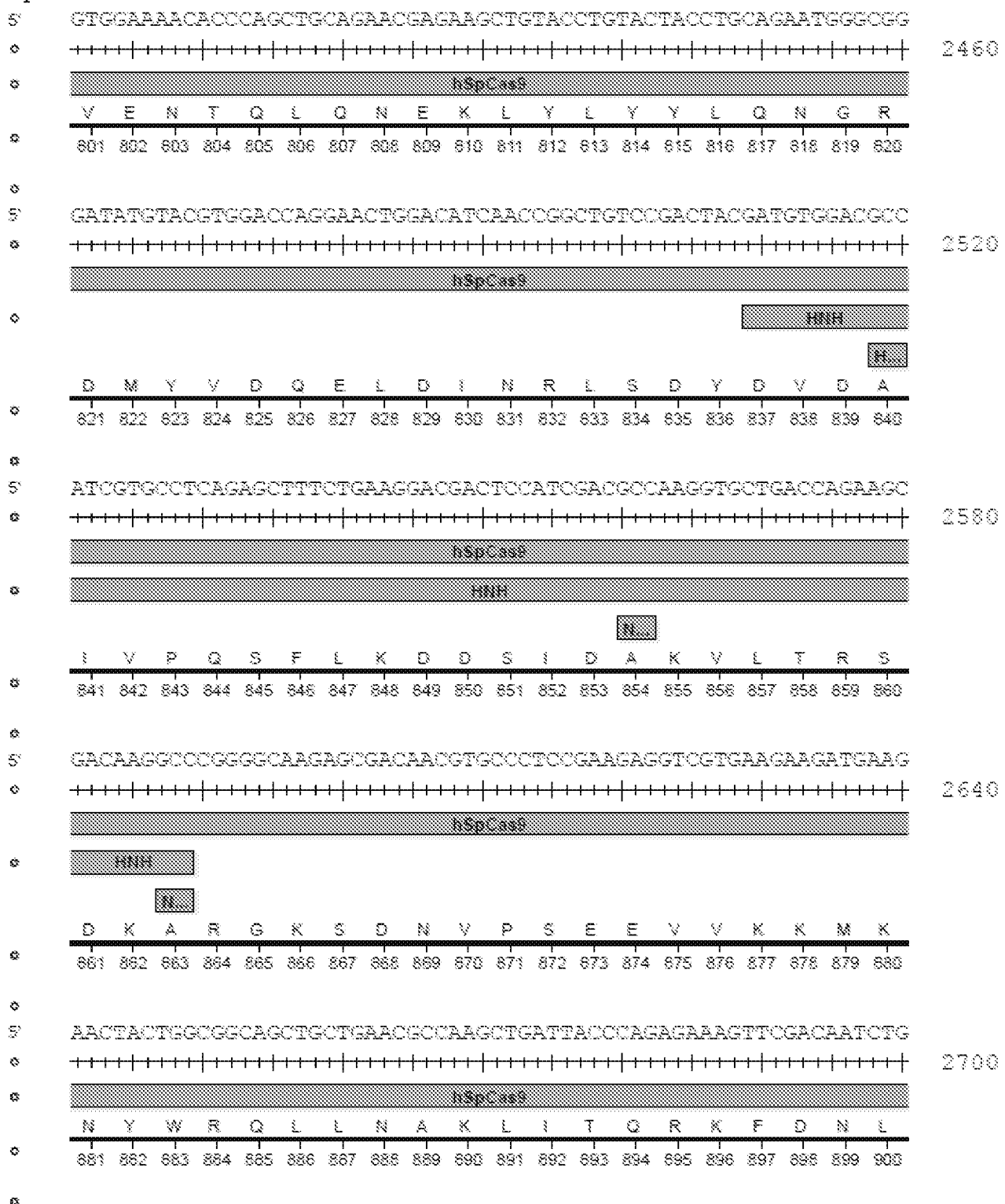


FIG. 24H

hSpCas9

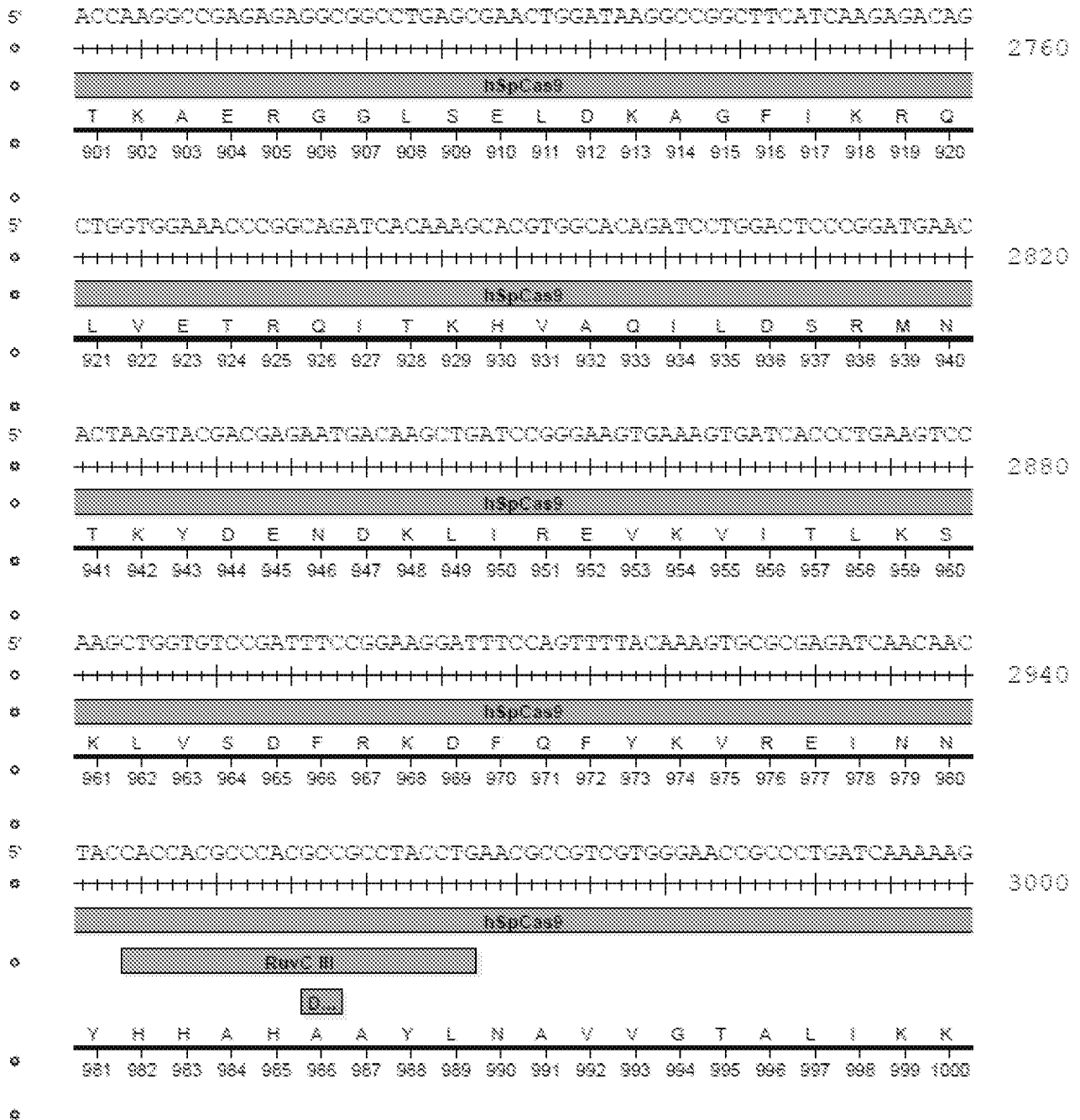


FIG. 24I

hSpCas9

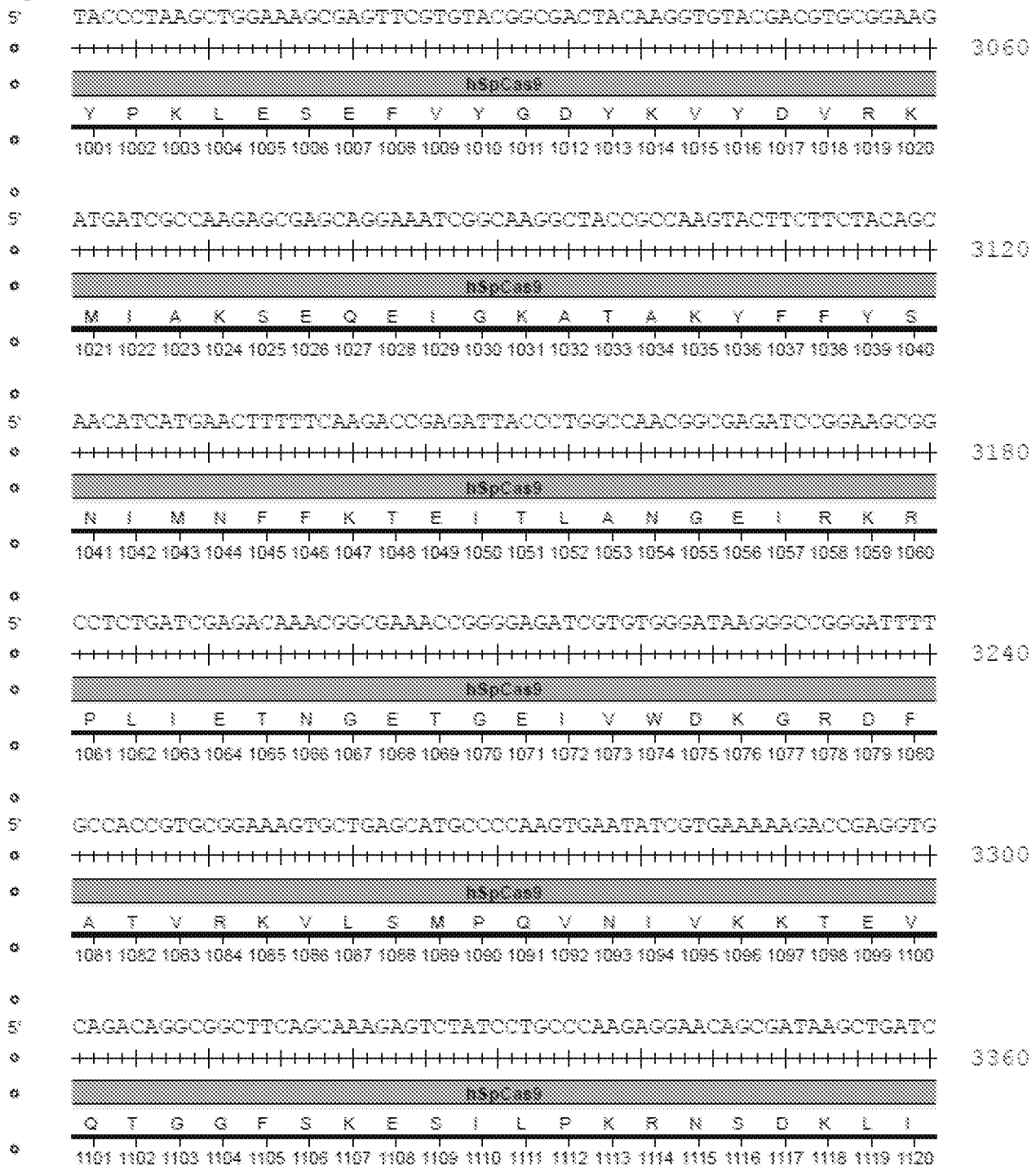


FIG. 24J

hSpCas9

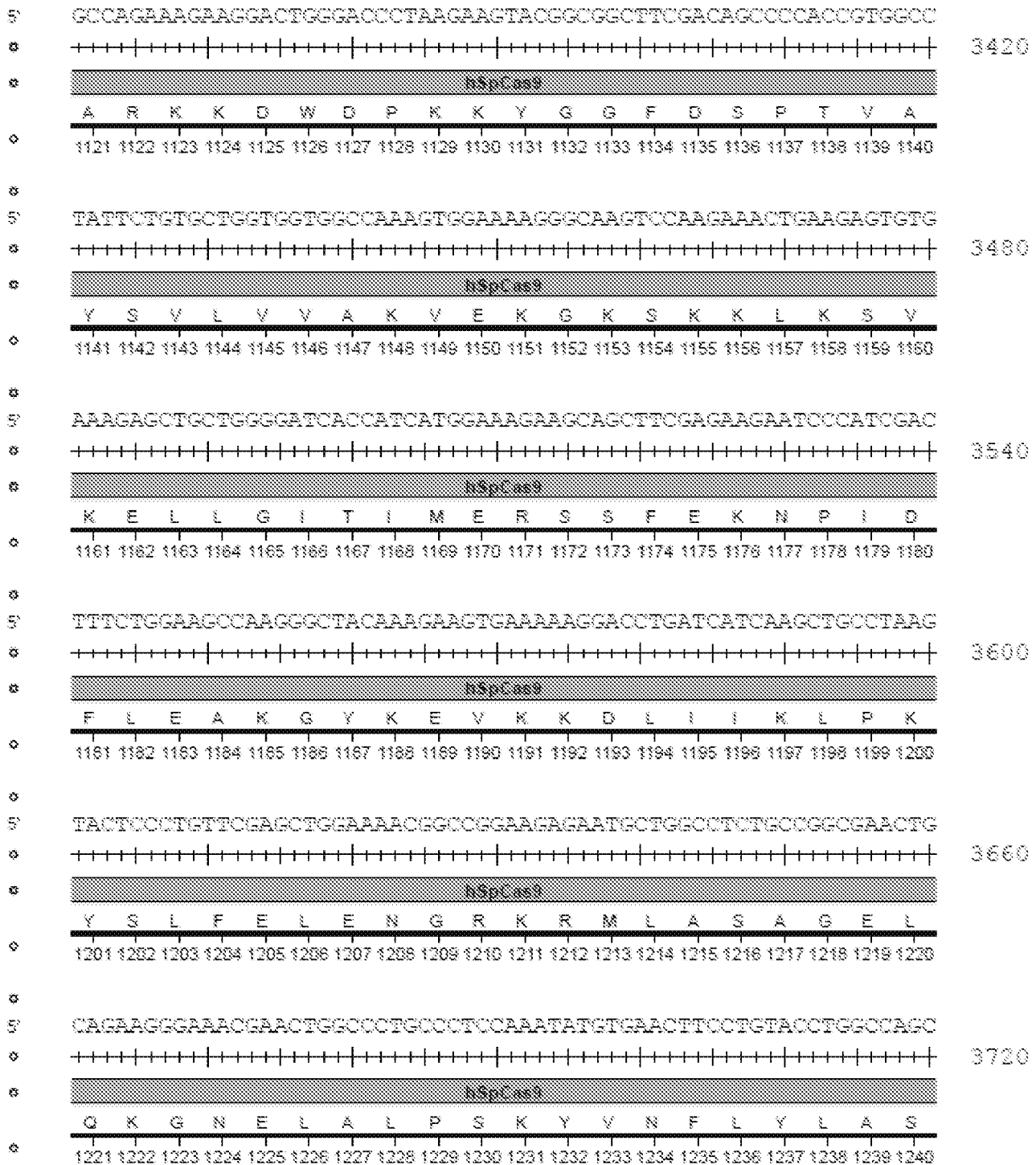


FIG. 24K

hSpCas9

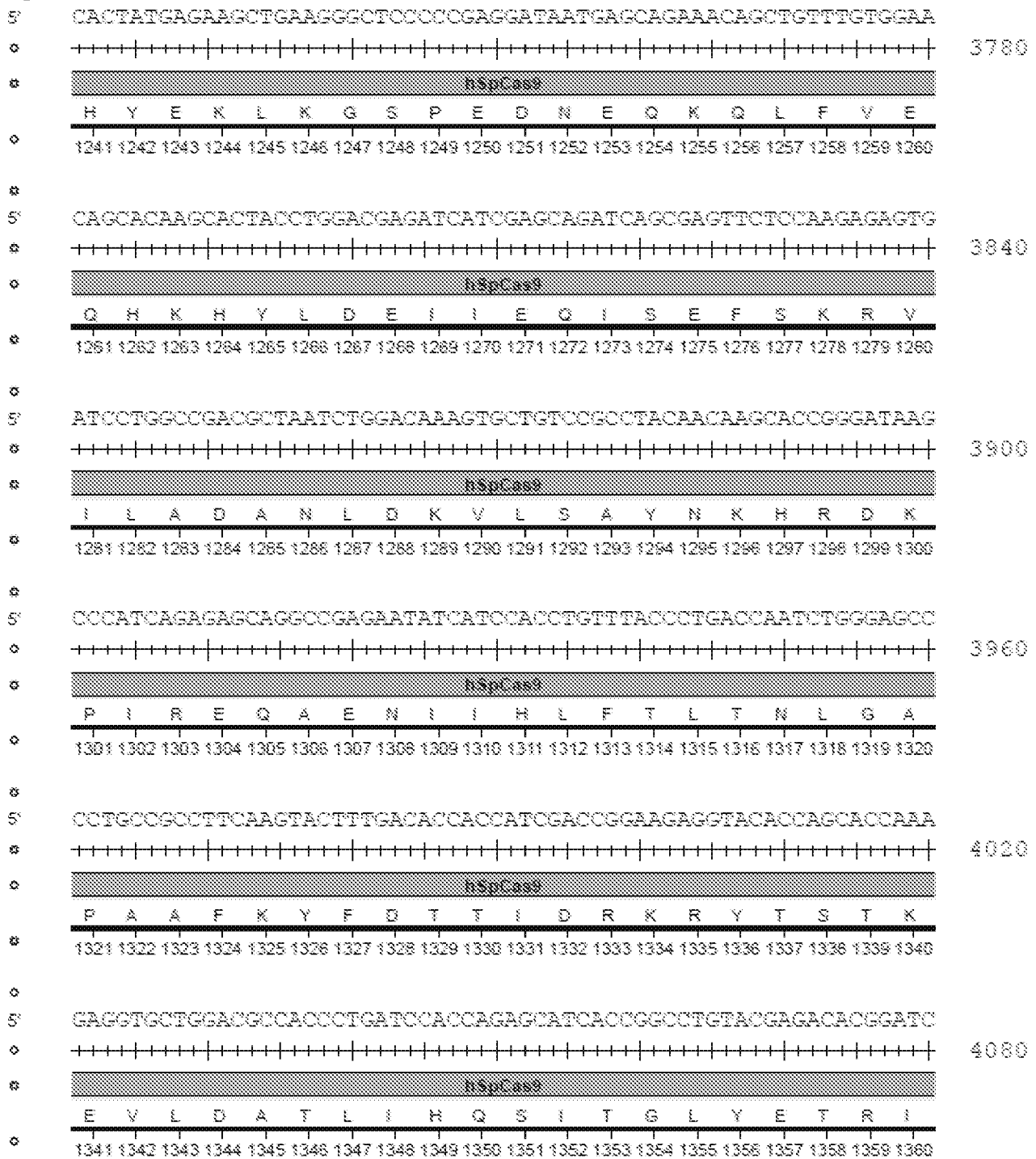


FIG. 24L

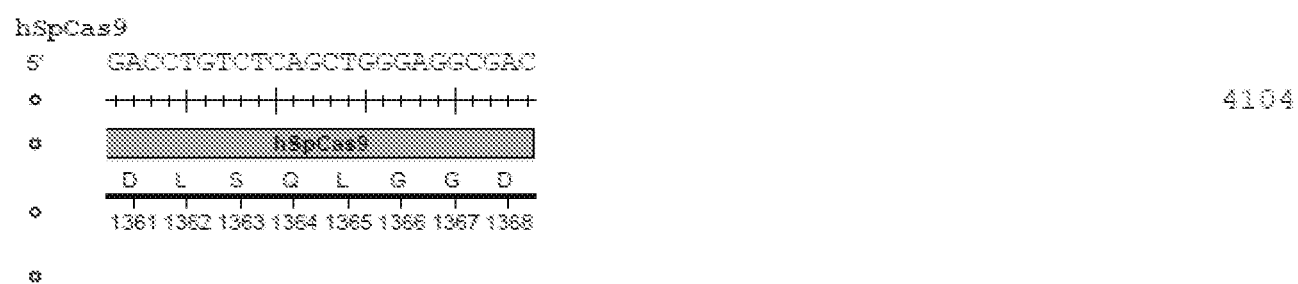


FIG. 24M

Conditional Cas9, Rosa26 targeting vector map

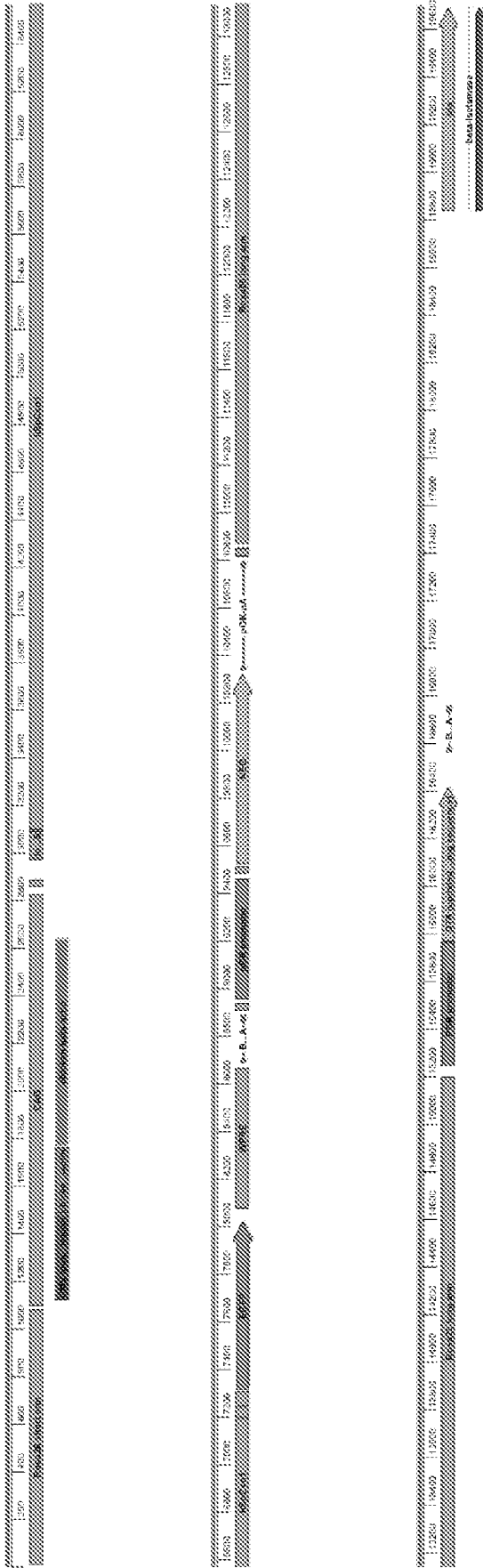


FIG. 25A

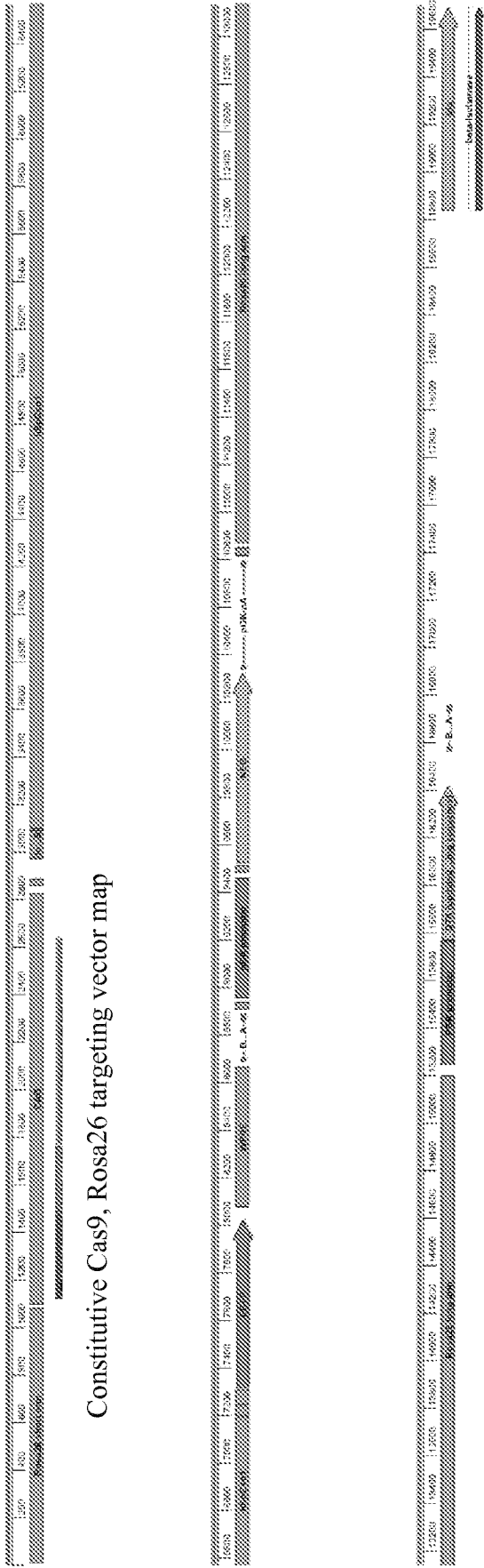


FIG. 25B

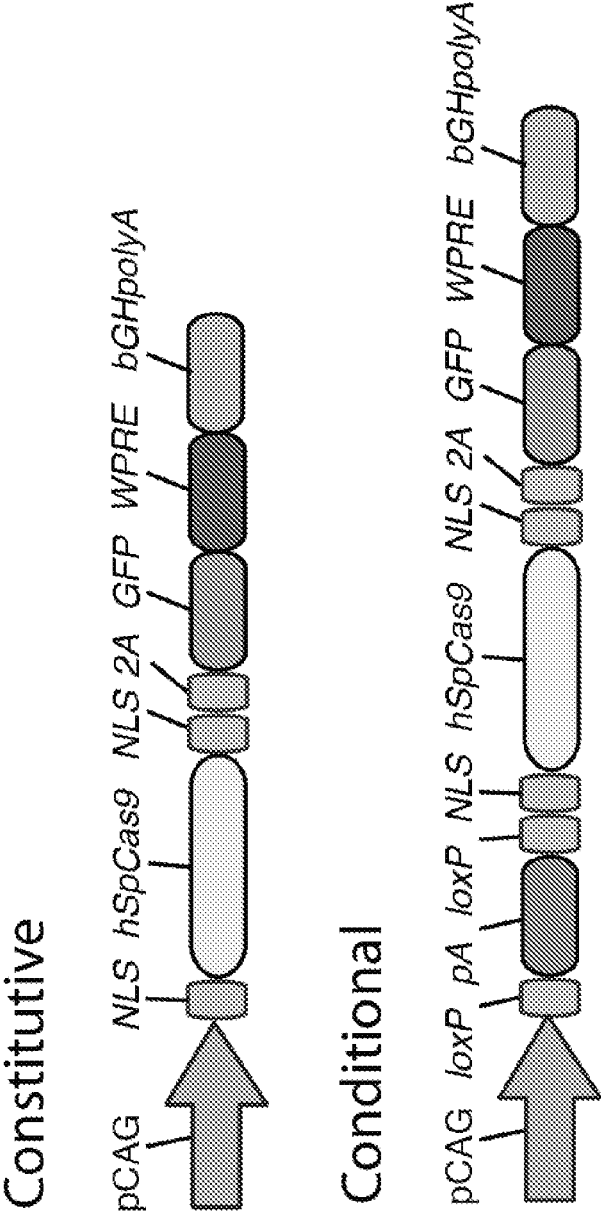
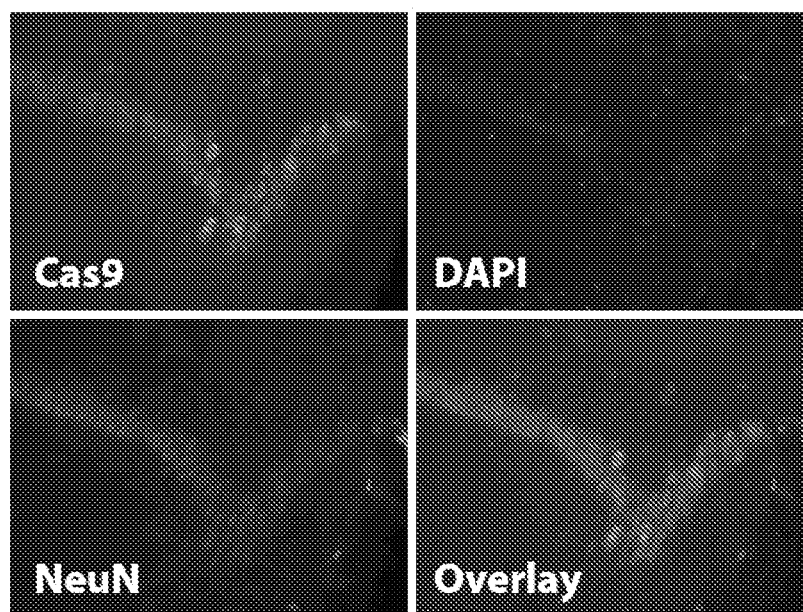


FIG. 26

Cas9 Expression in Mouse Hippocampus (AAV)



Cas9 Expression in Mouse Cortex (AAV)

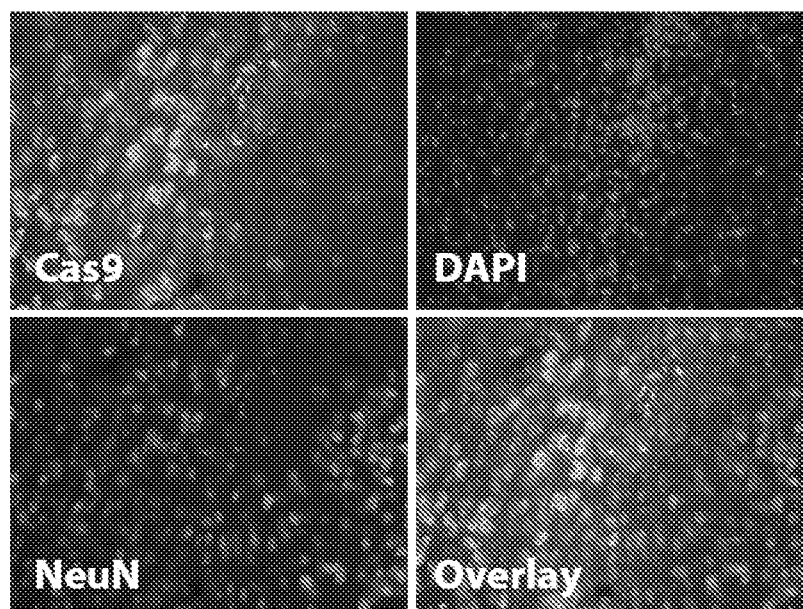


FIG. 27

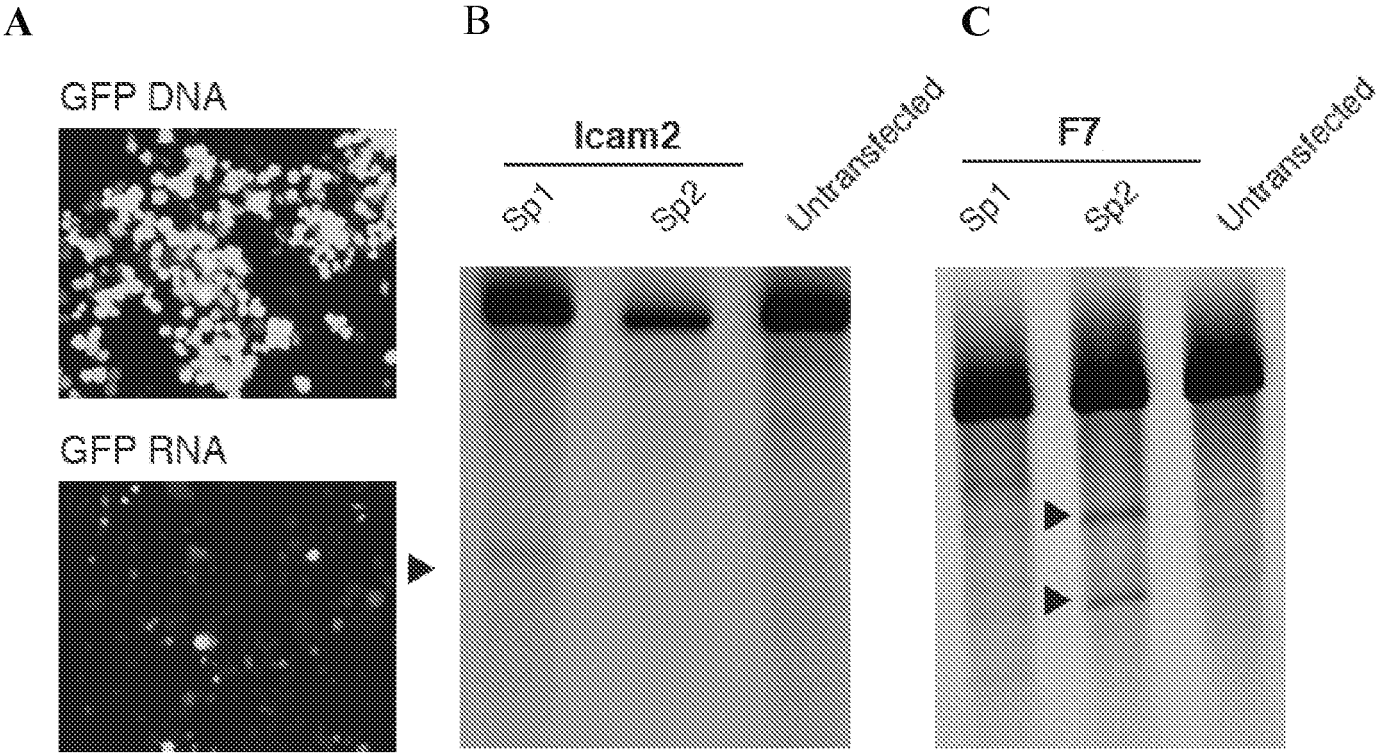


FIG. 28

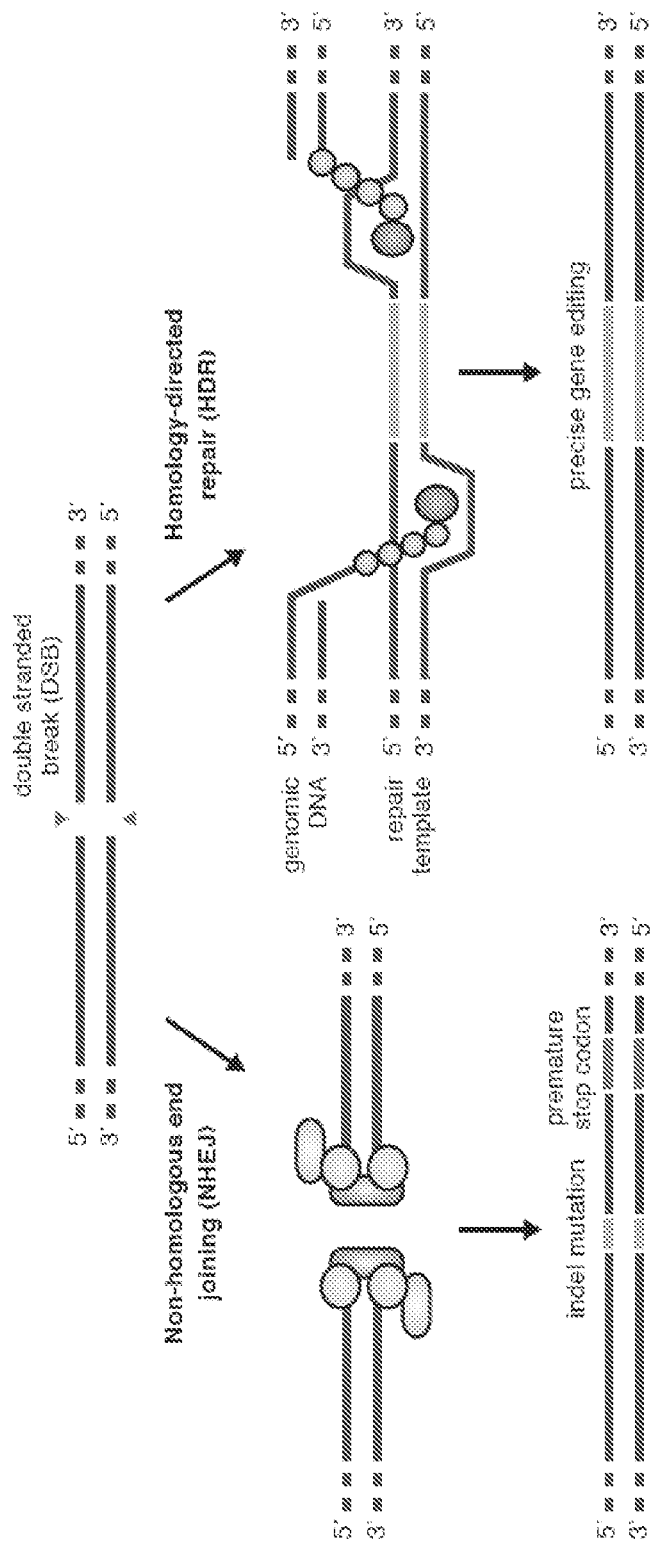


FIG. 29

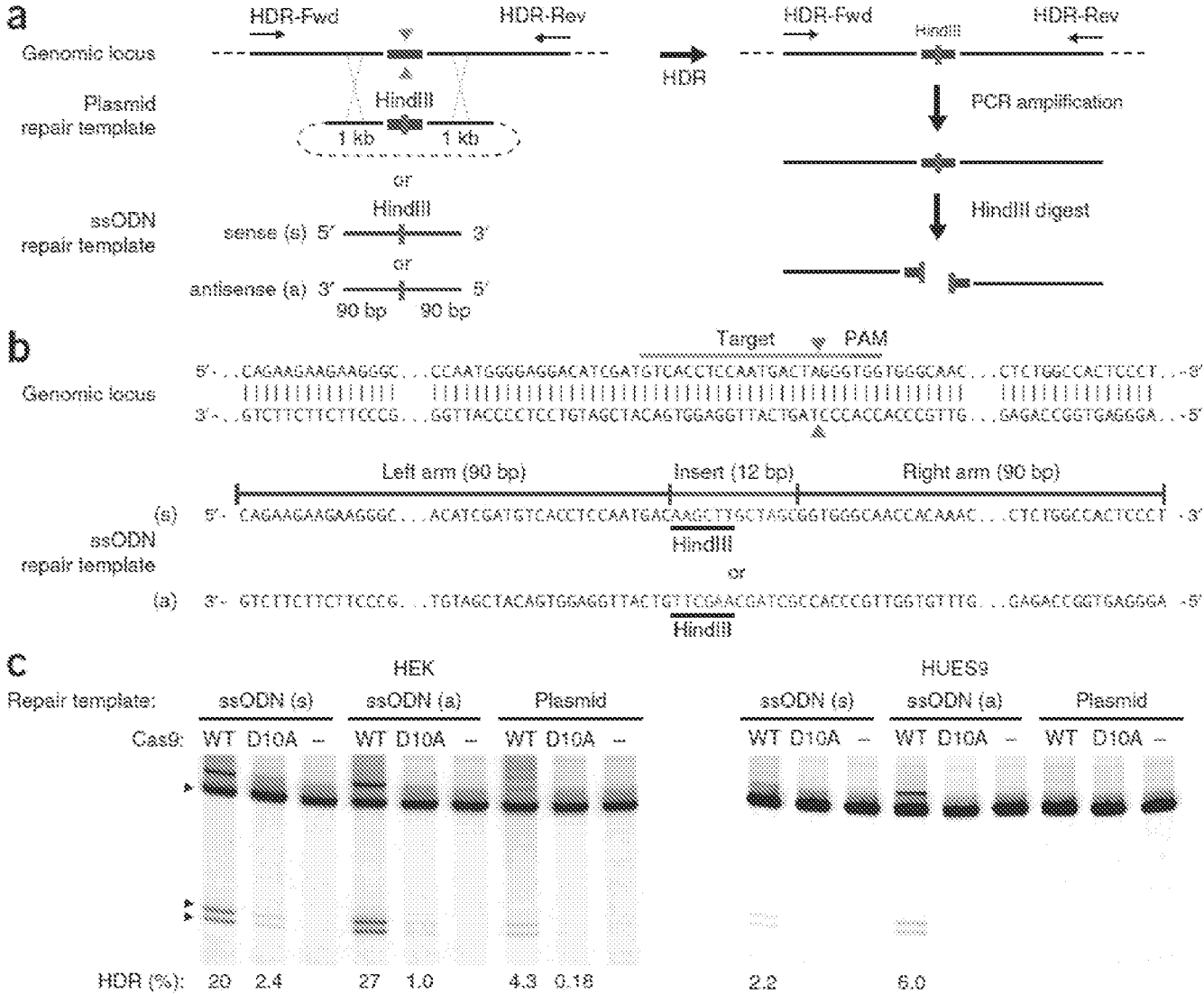


FIG. 30A-C

Repair Strategy for Cystic Fibrosis deltaF508 Mutation

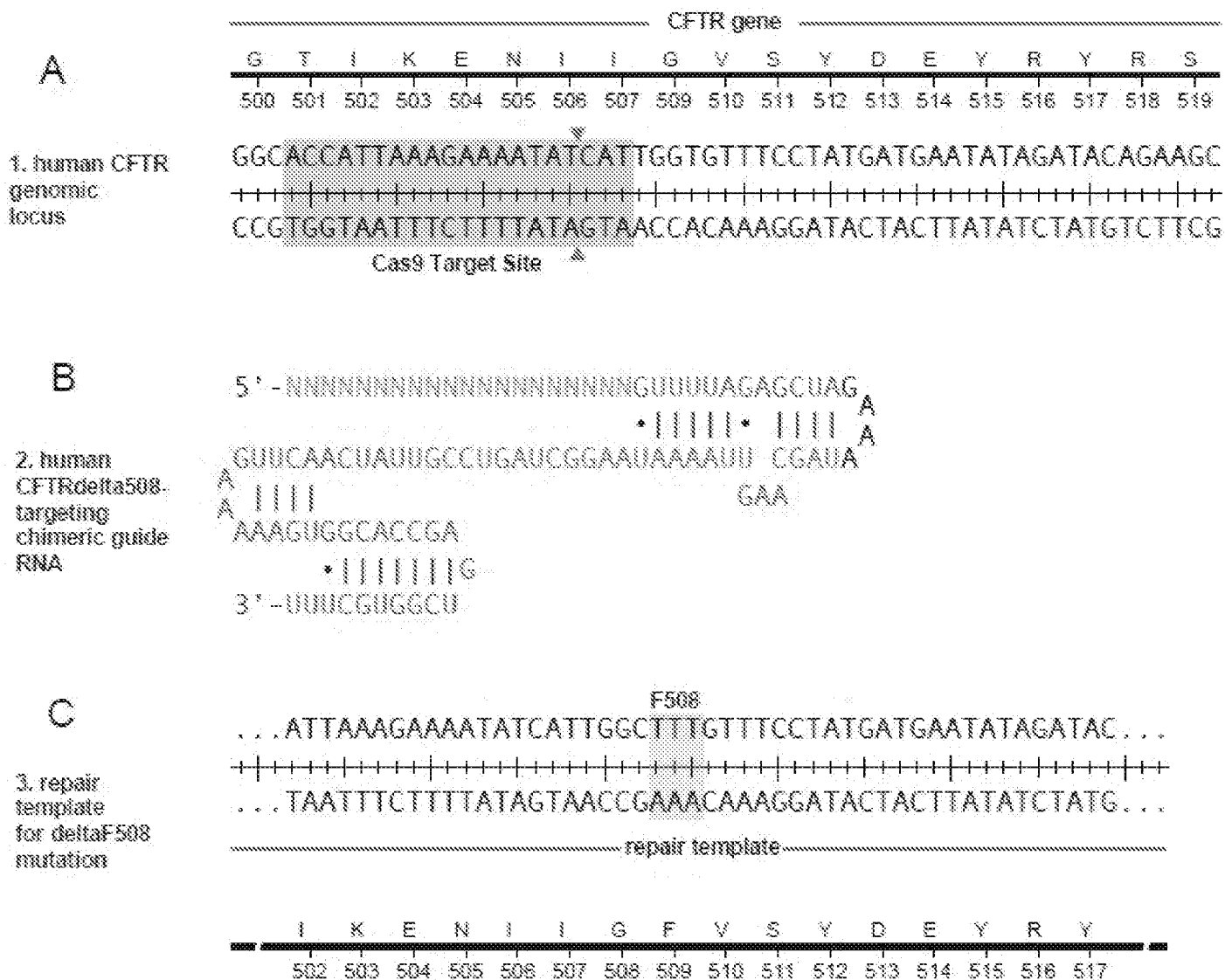


FIG. 31-A-C

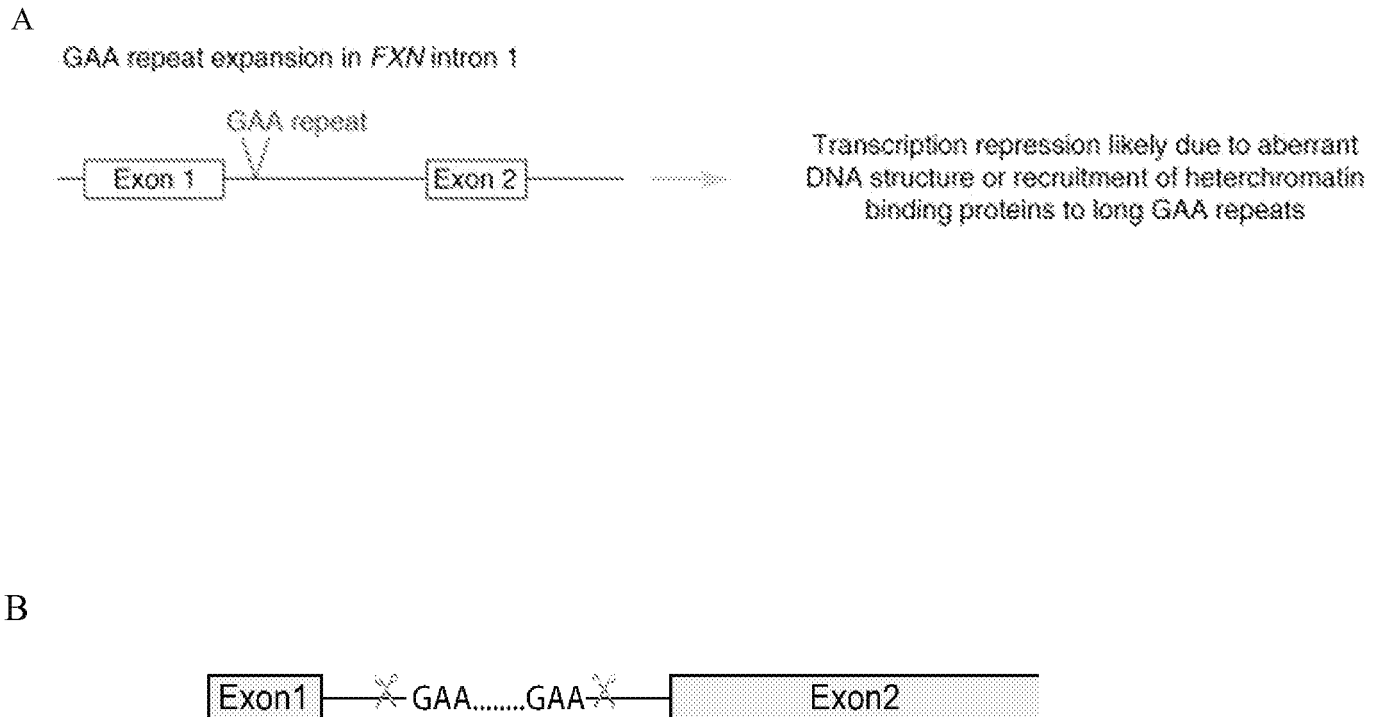


FIG. 32A-B

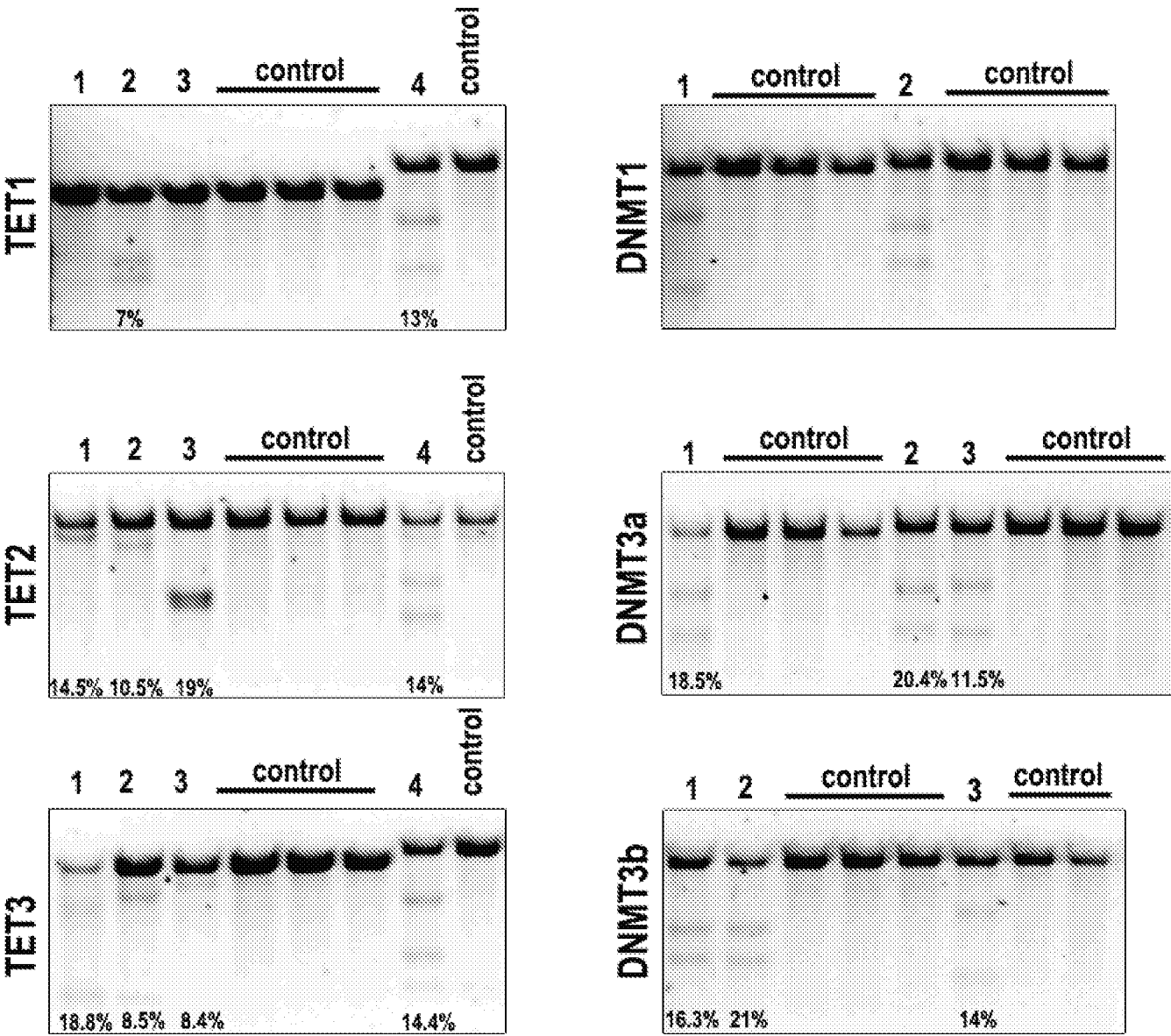


FIG. 33

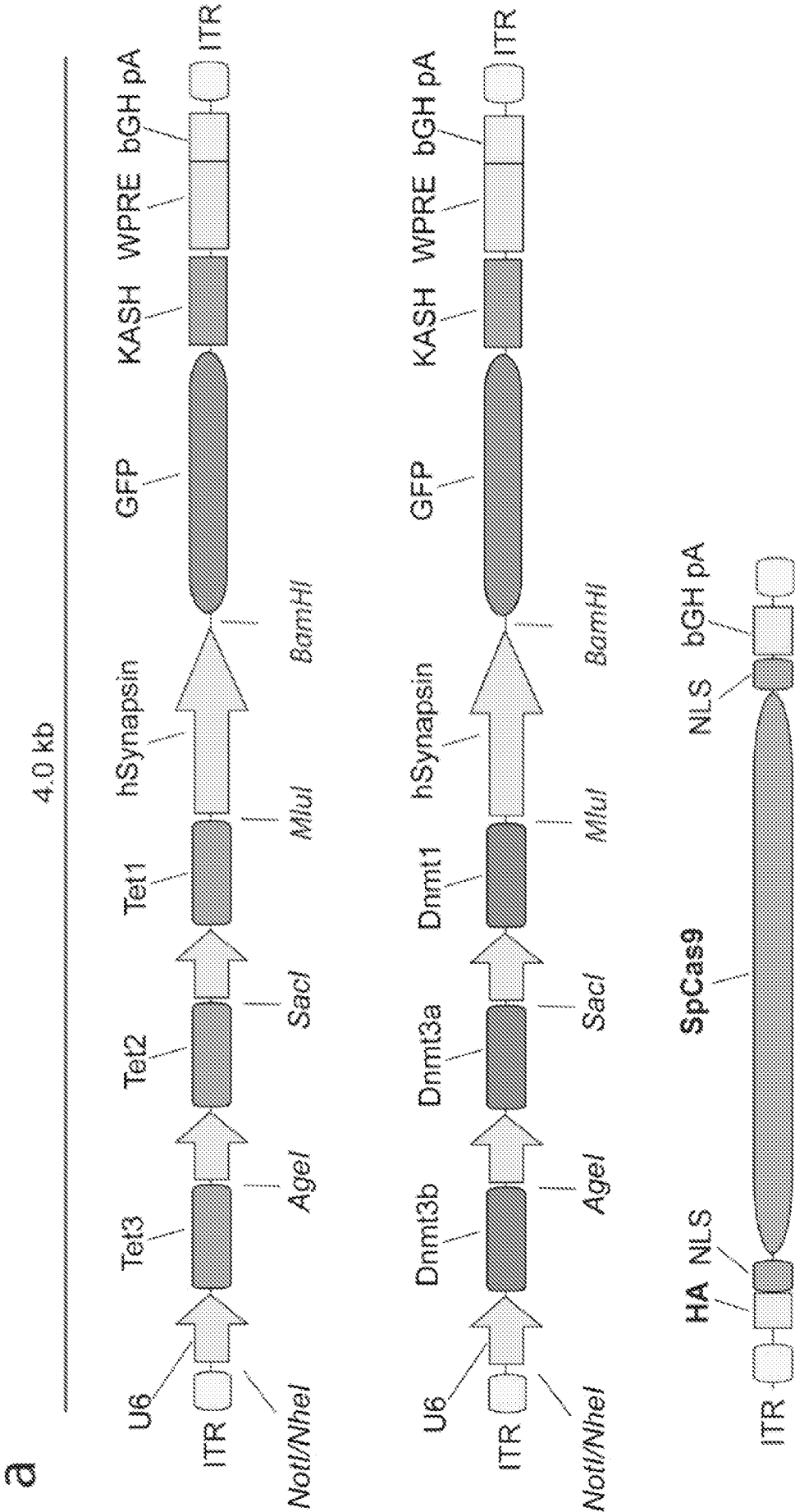


FIG. 34

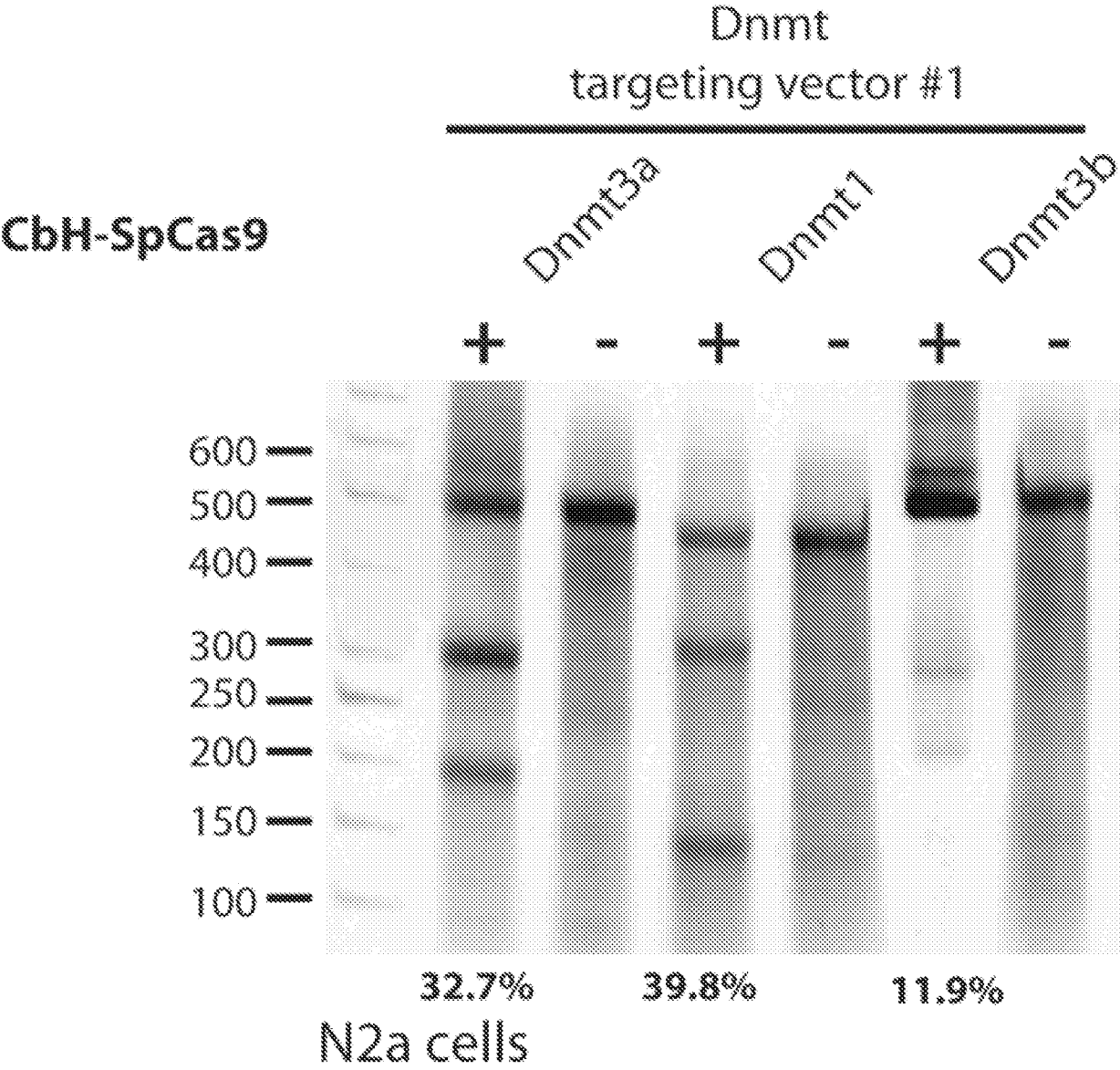


FIG. 35

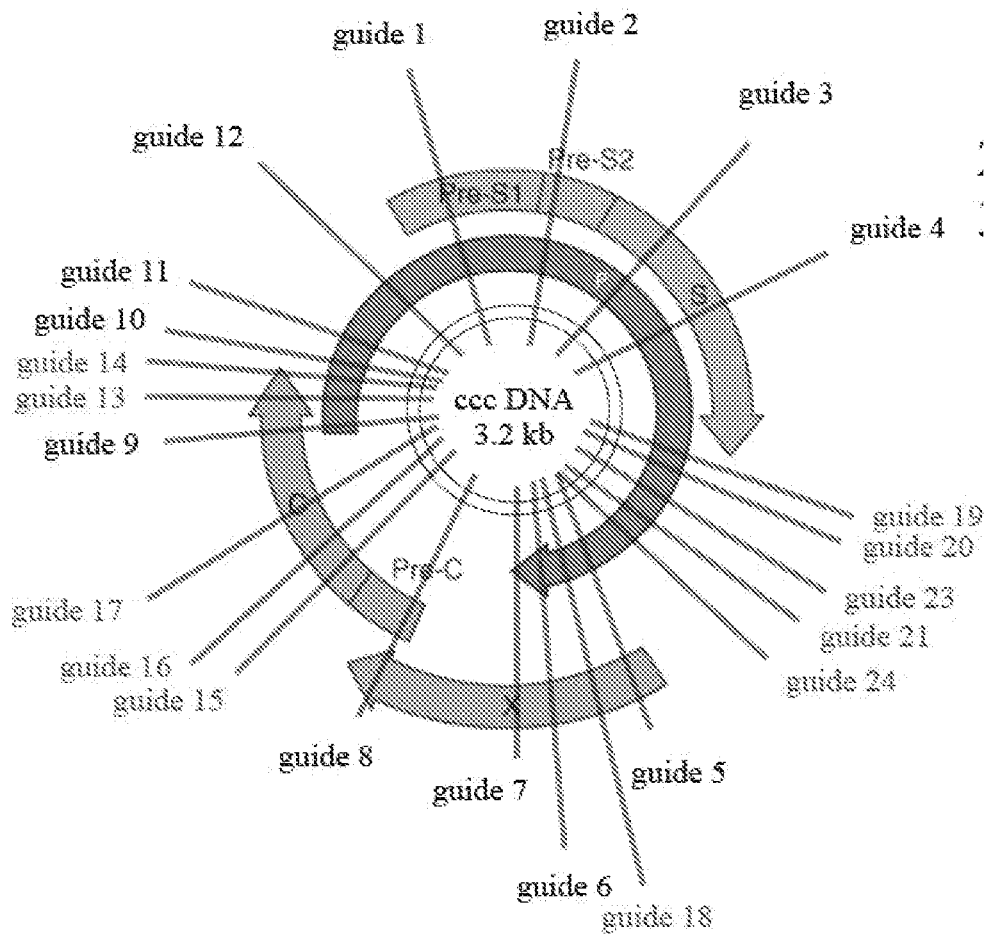


FIG. 36

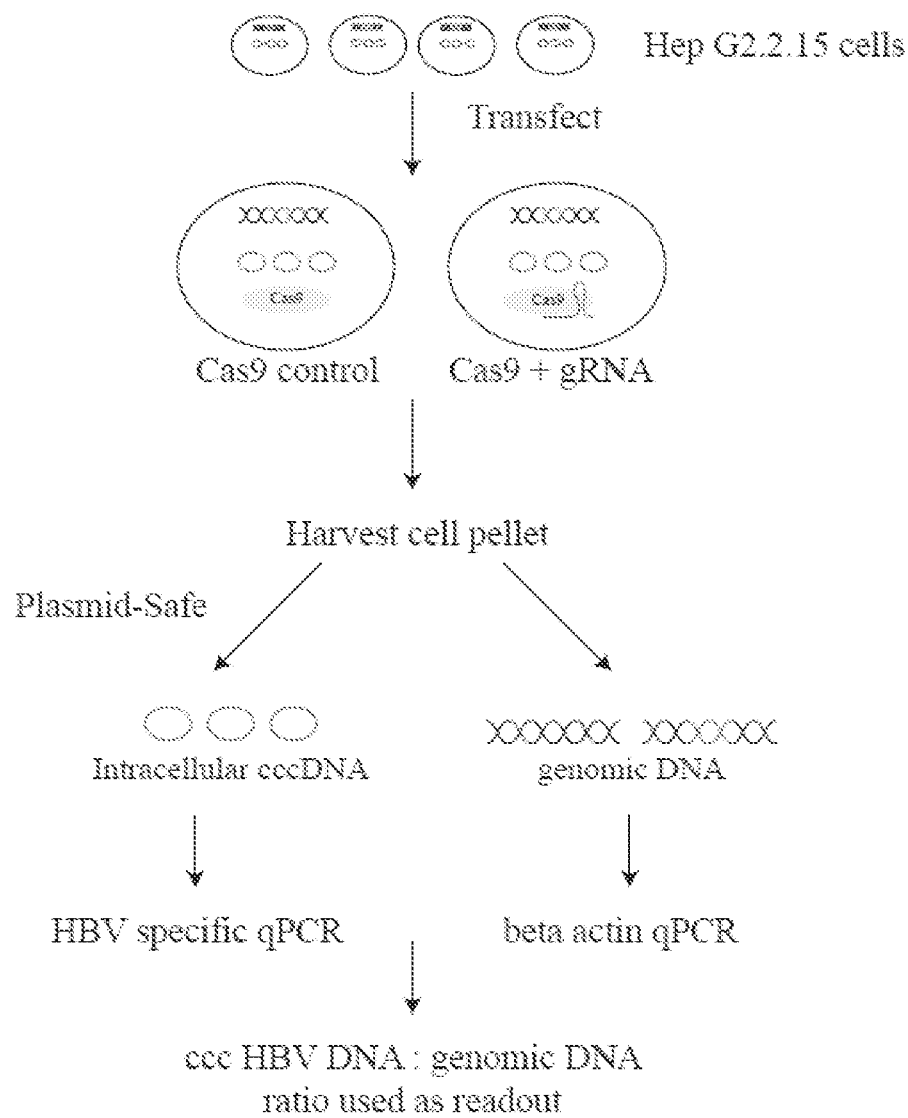


FIG. 37

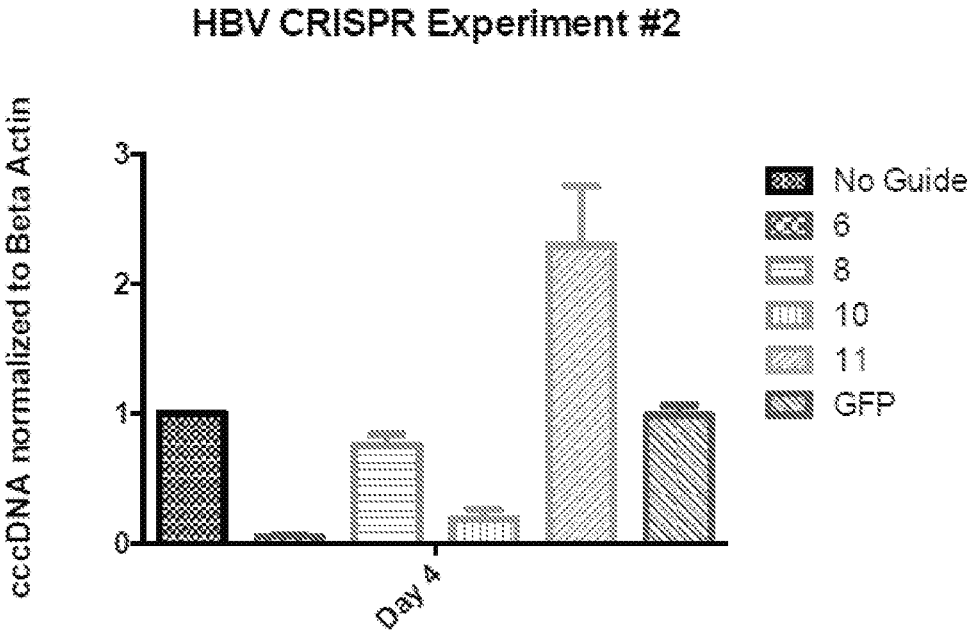


FIG. 38

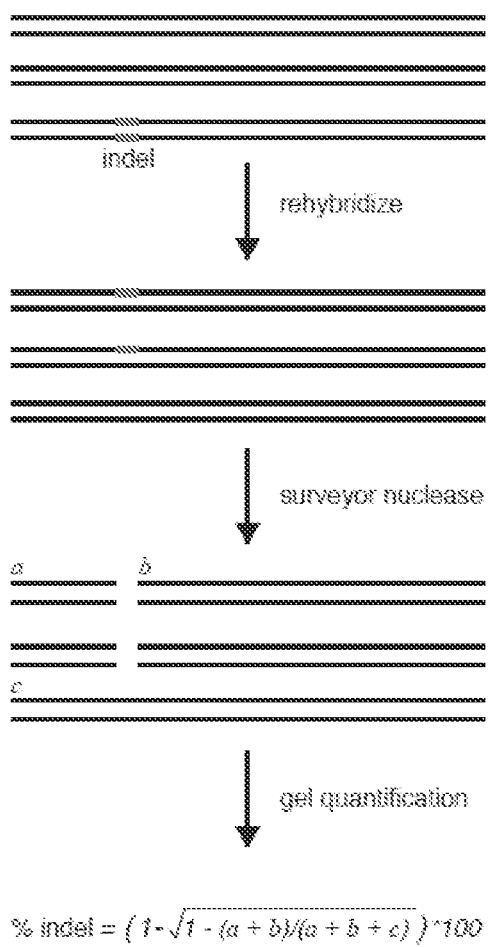
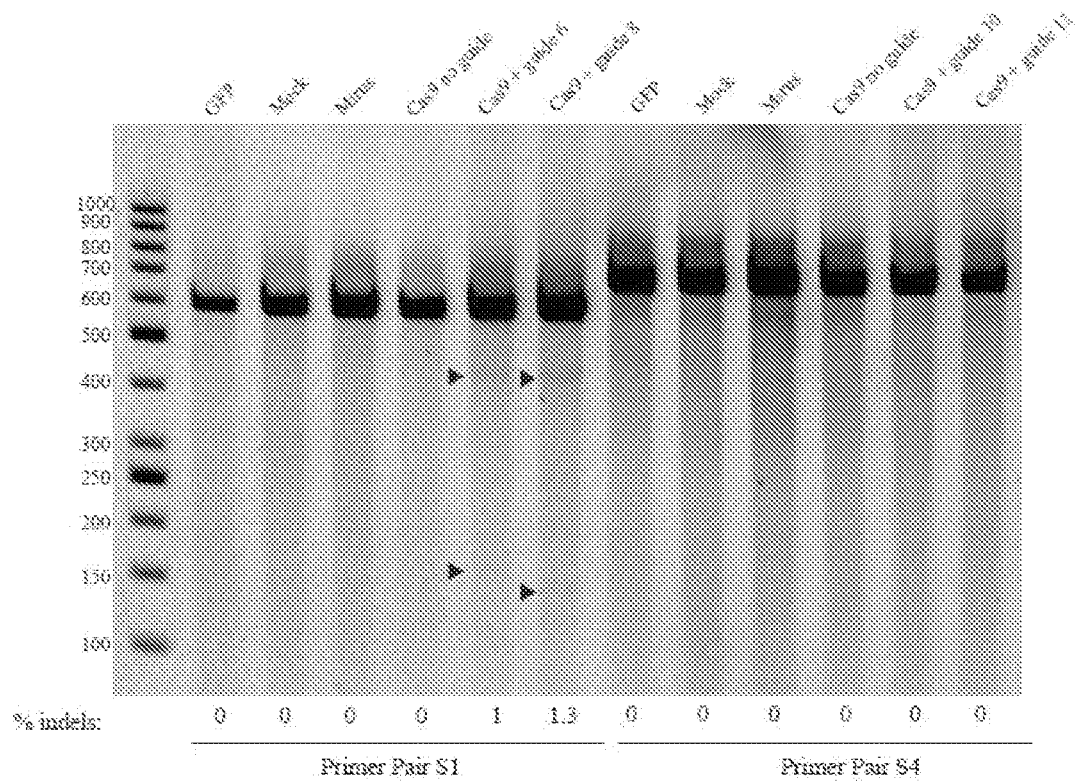


FIG. 39



Predicted band sizes for guide 6 indel formation: 172 + 472 + 599 (undigested PCR product)
 Predicted band sizes for guide 8 indel formation: 153 + 446 + 599 (undigested PCR product)
 Predicted band sizes for guide 10 indel formation: 242 + 431 + 673 (undigested PCR product)
 Predicted band sizes for guide 11 indel formation: 207 + 466 + 673 (undigested PCR product)

FIG. 40

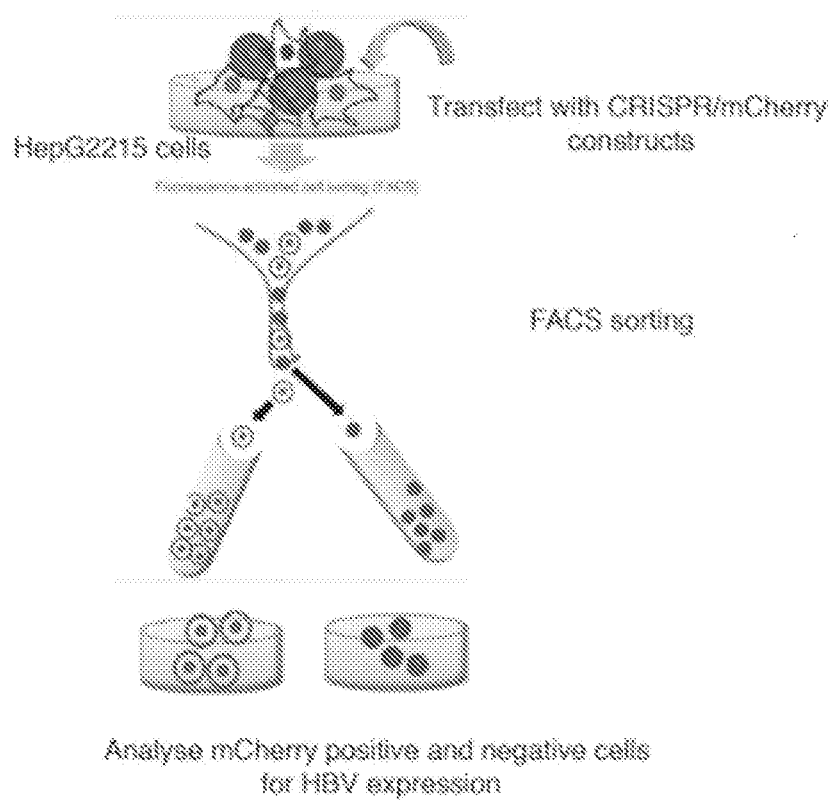


FIG. 41

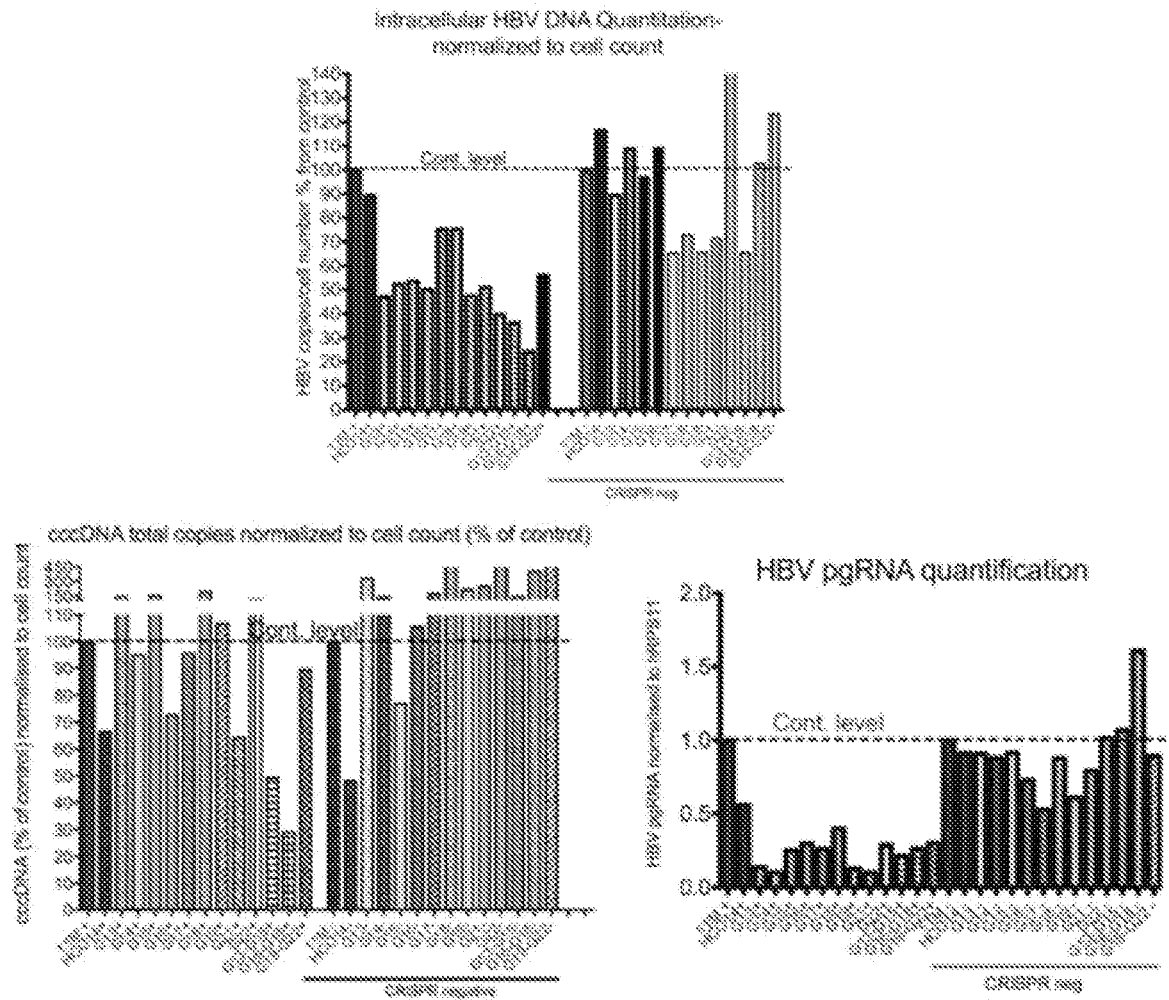


FIG. 42

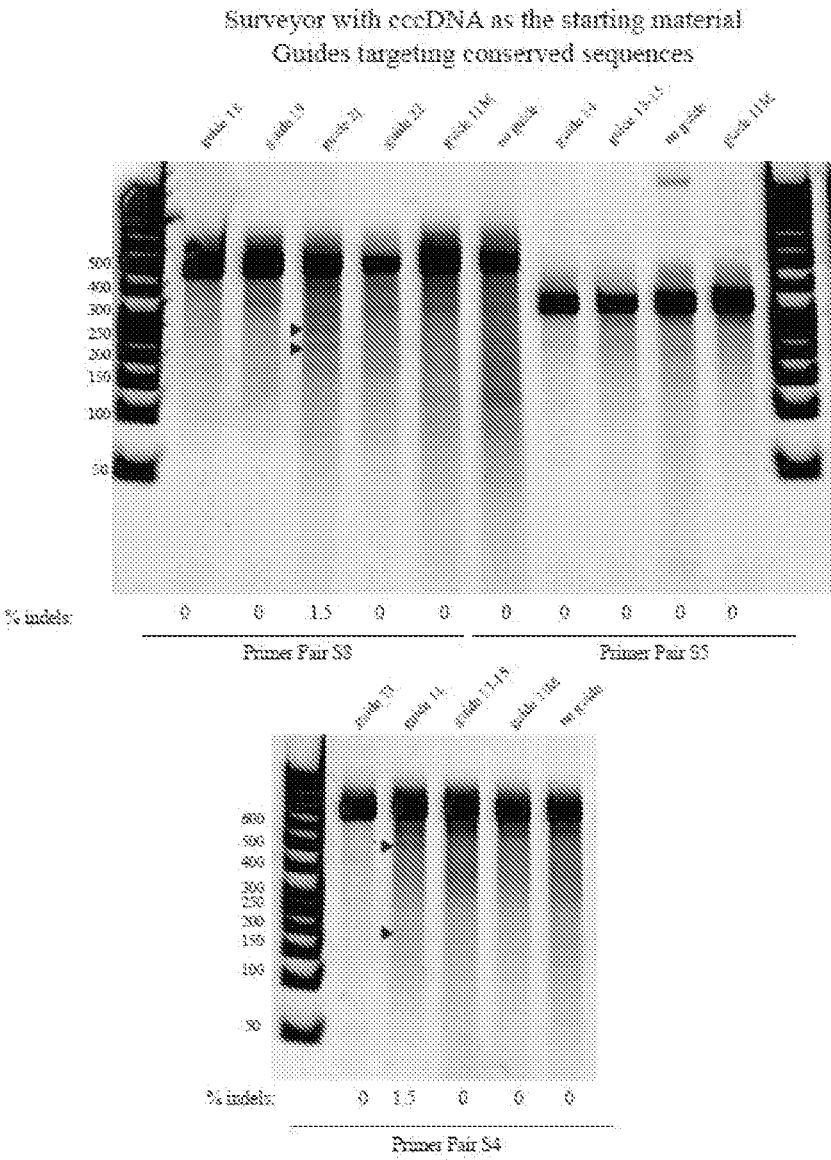


FIG. 43

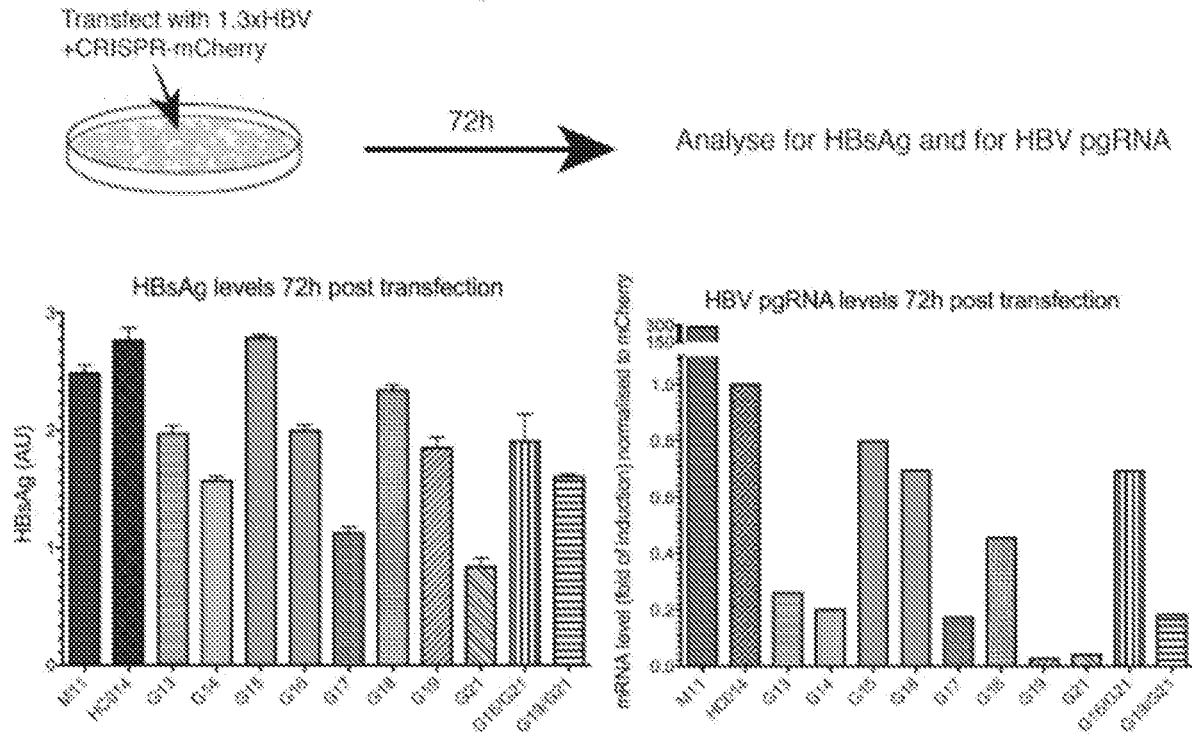


FIG. 44

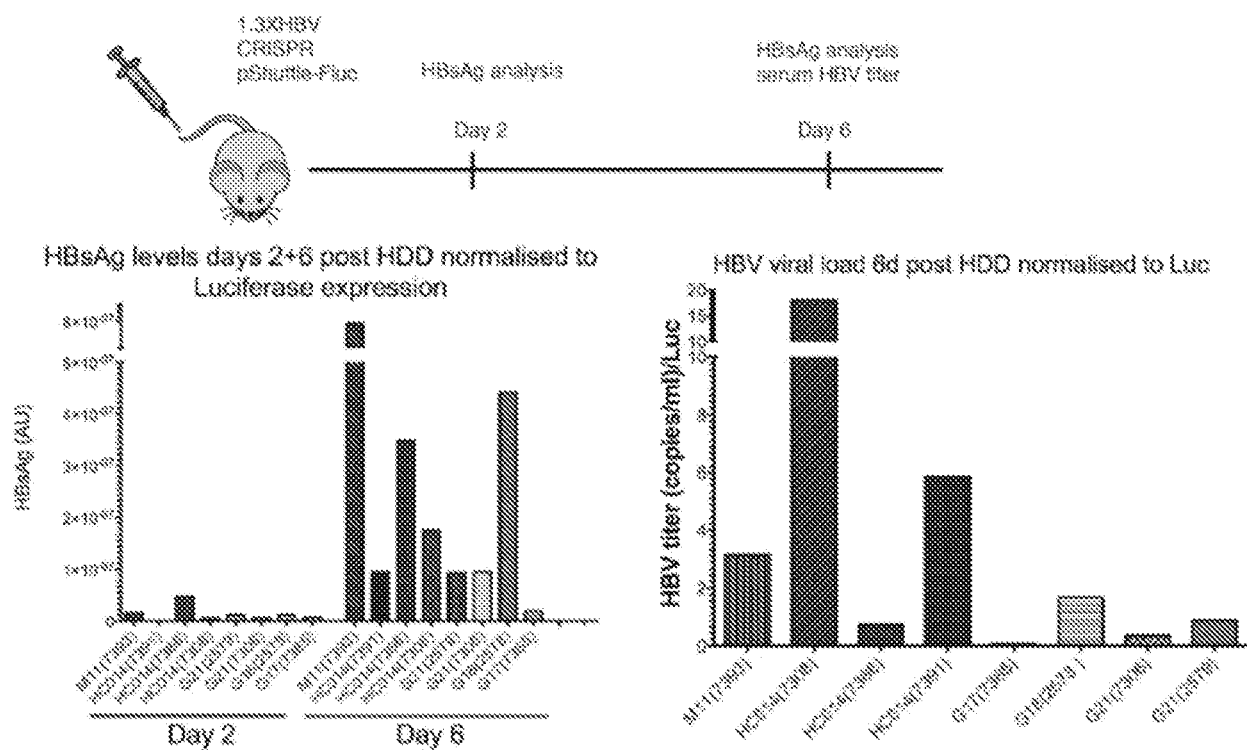


FIG. 45

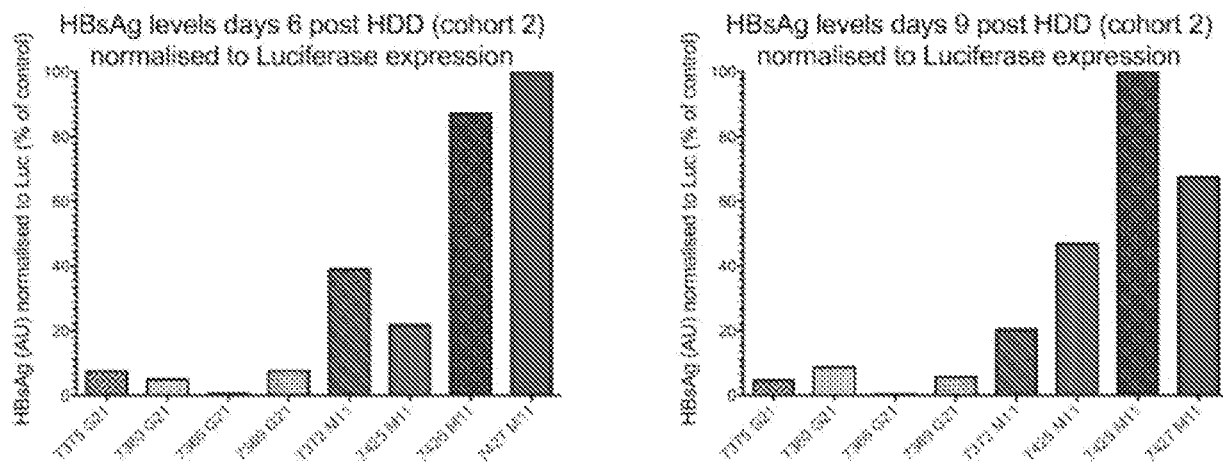


FIG. 46

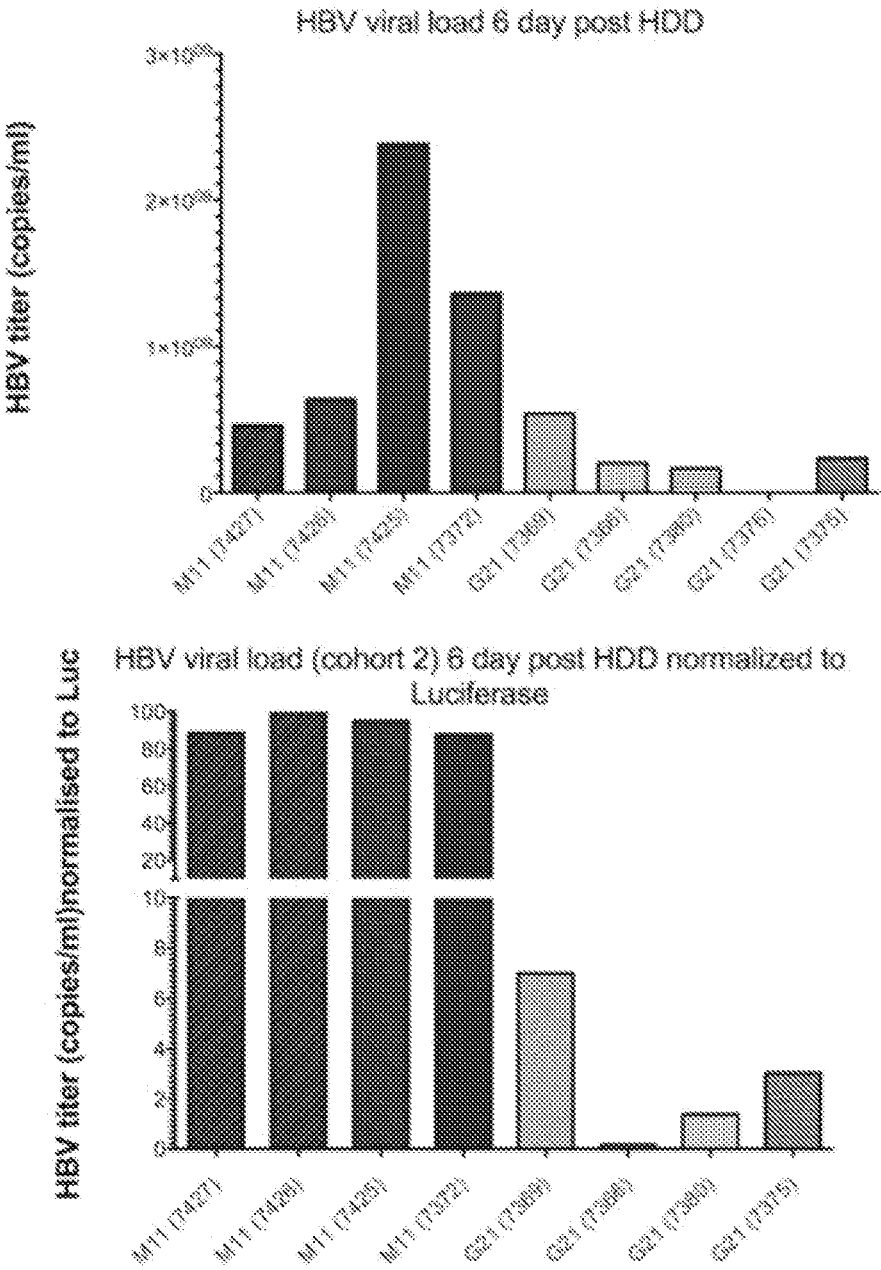


FIG. 47

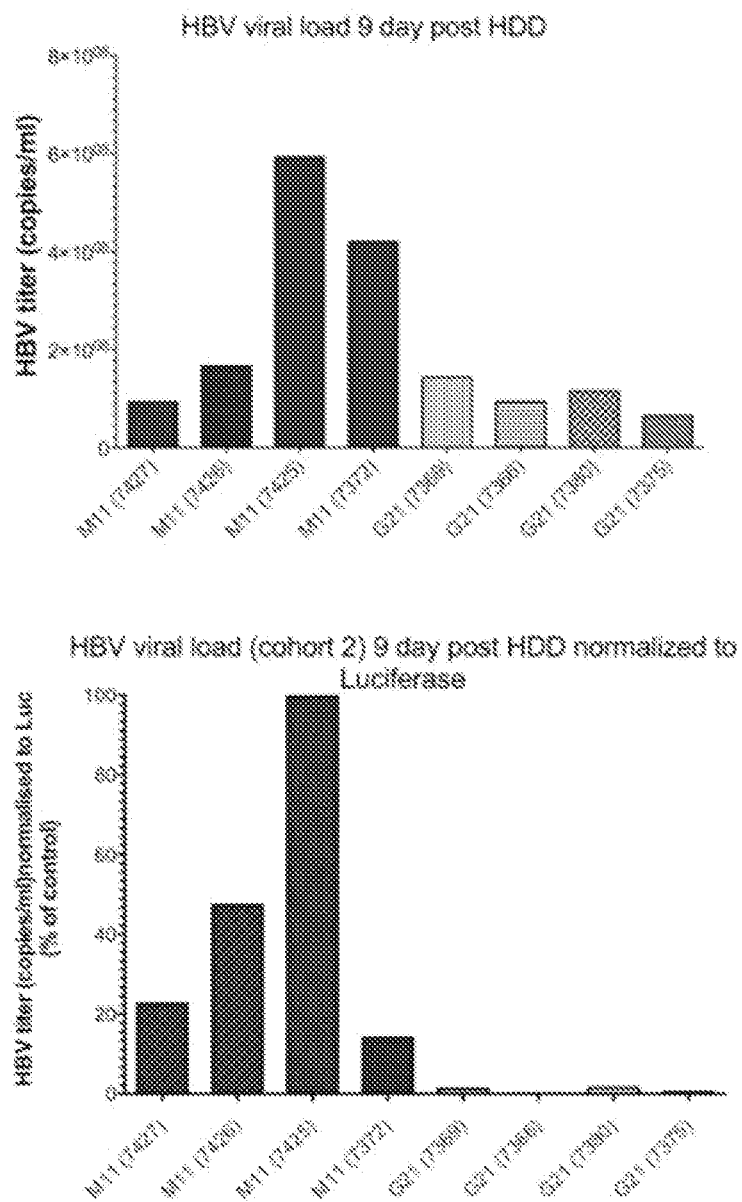


FIG. 48

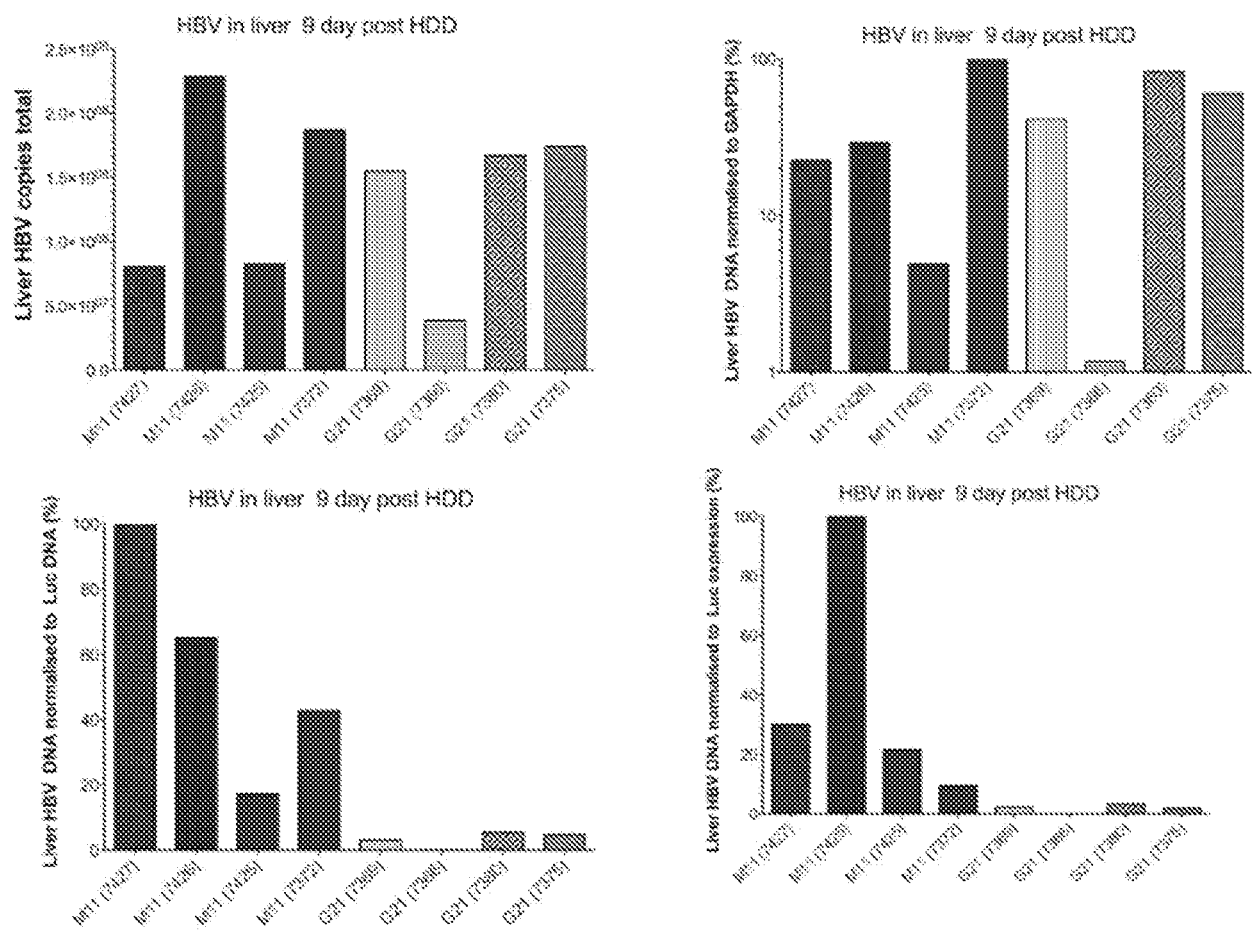


FIG. 49

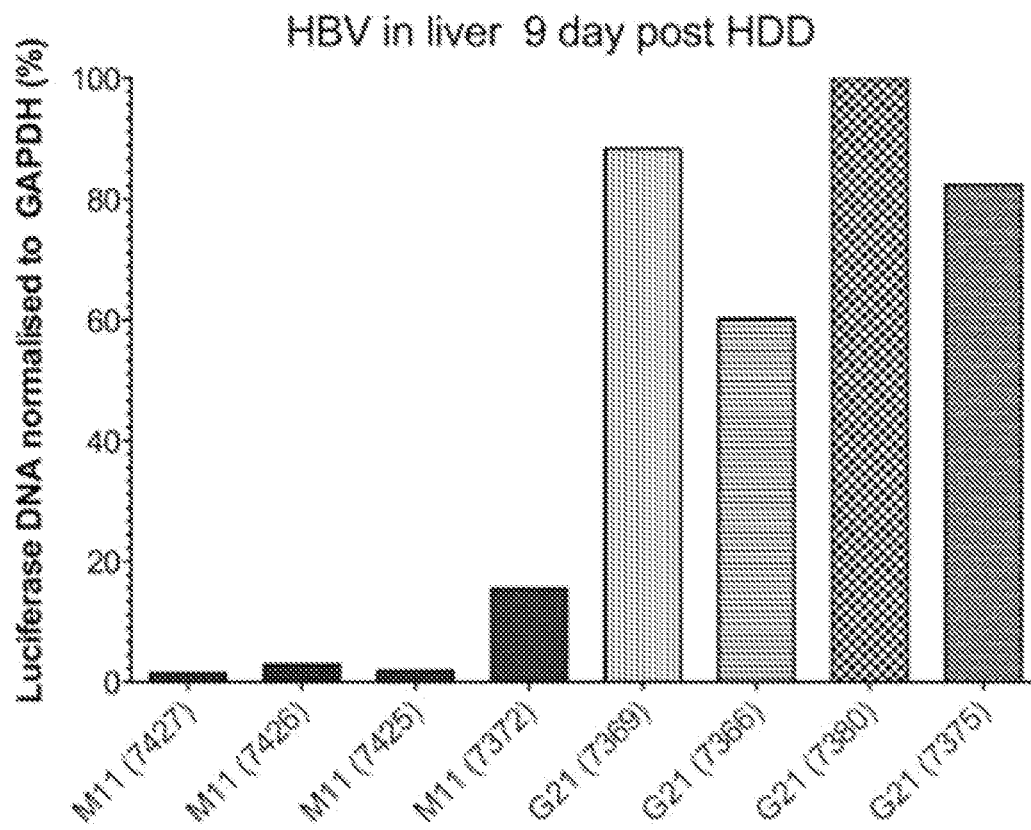


FIG. 50

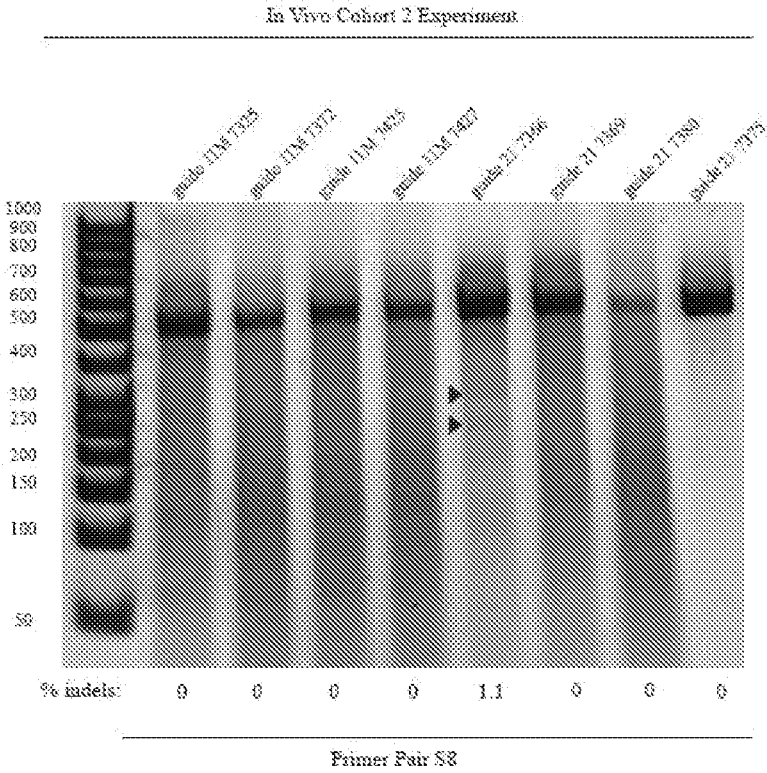


FIG. 51

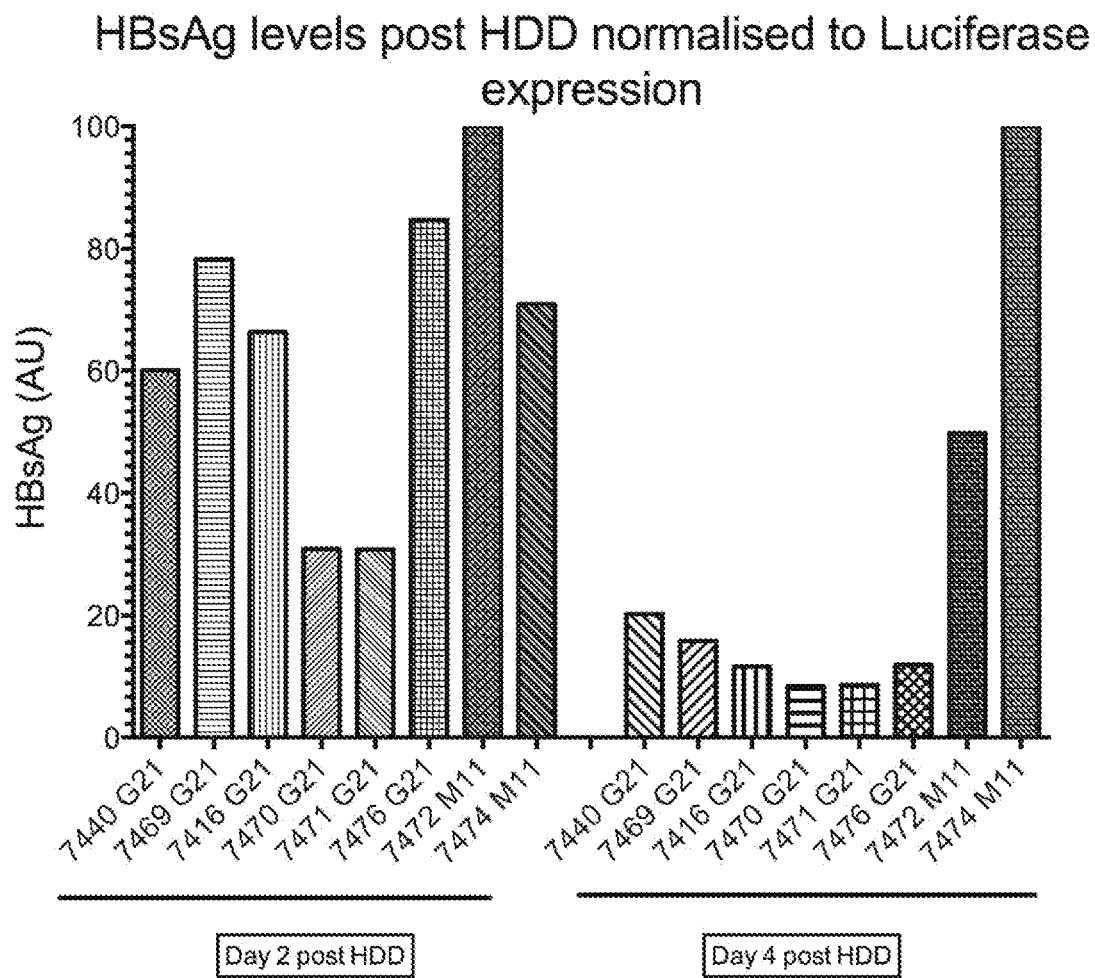


FIG. 52

Cohort 3: Viral Load Normalized to Luciferase

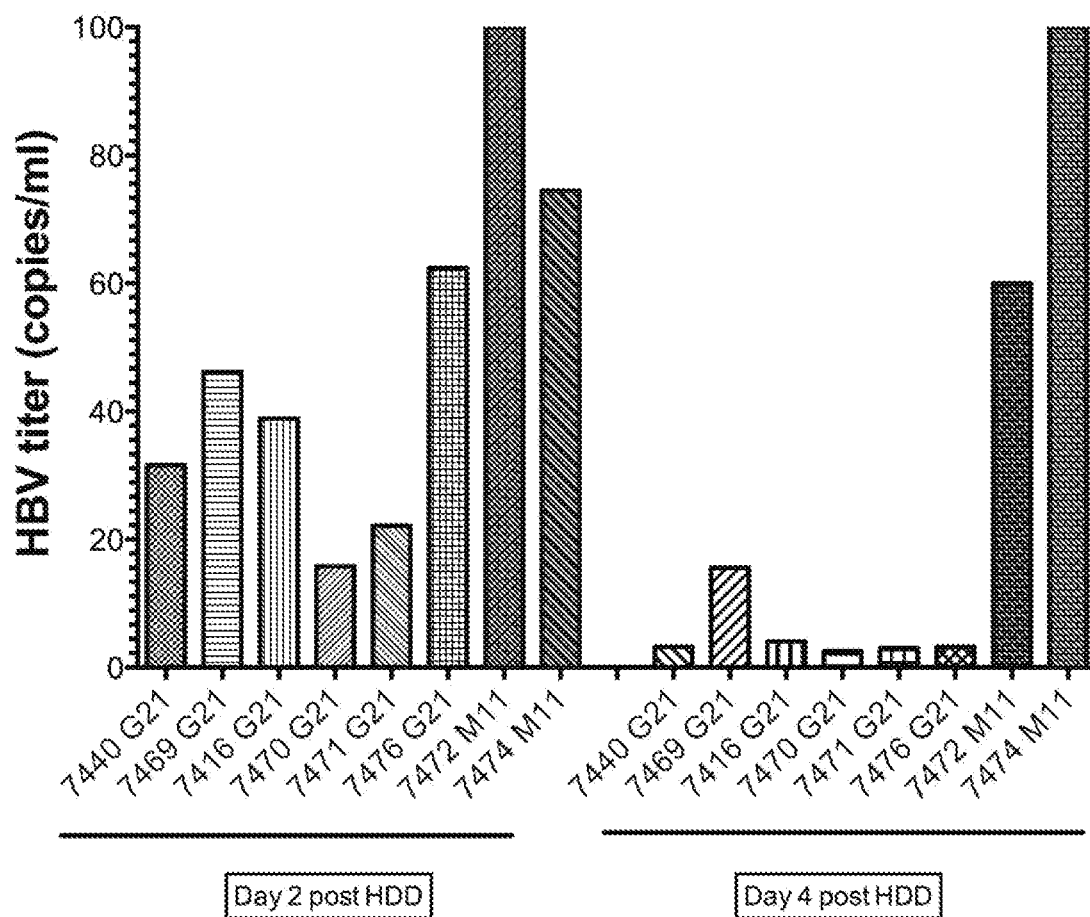


FIG. 53

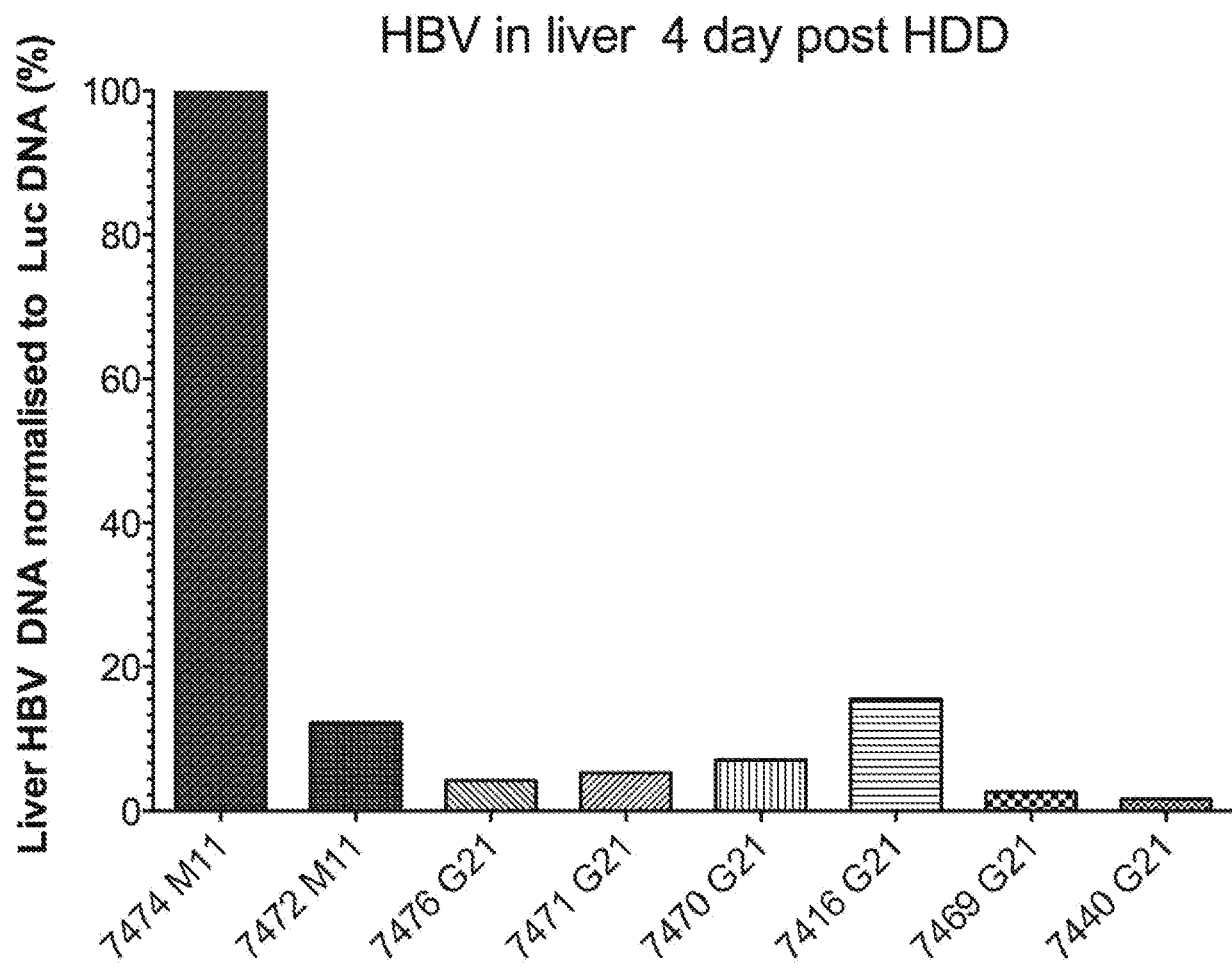


FIG. 54

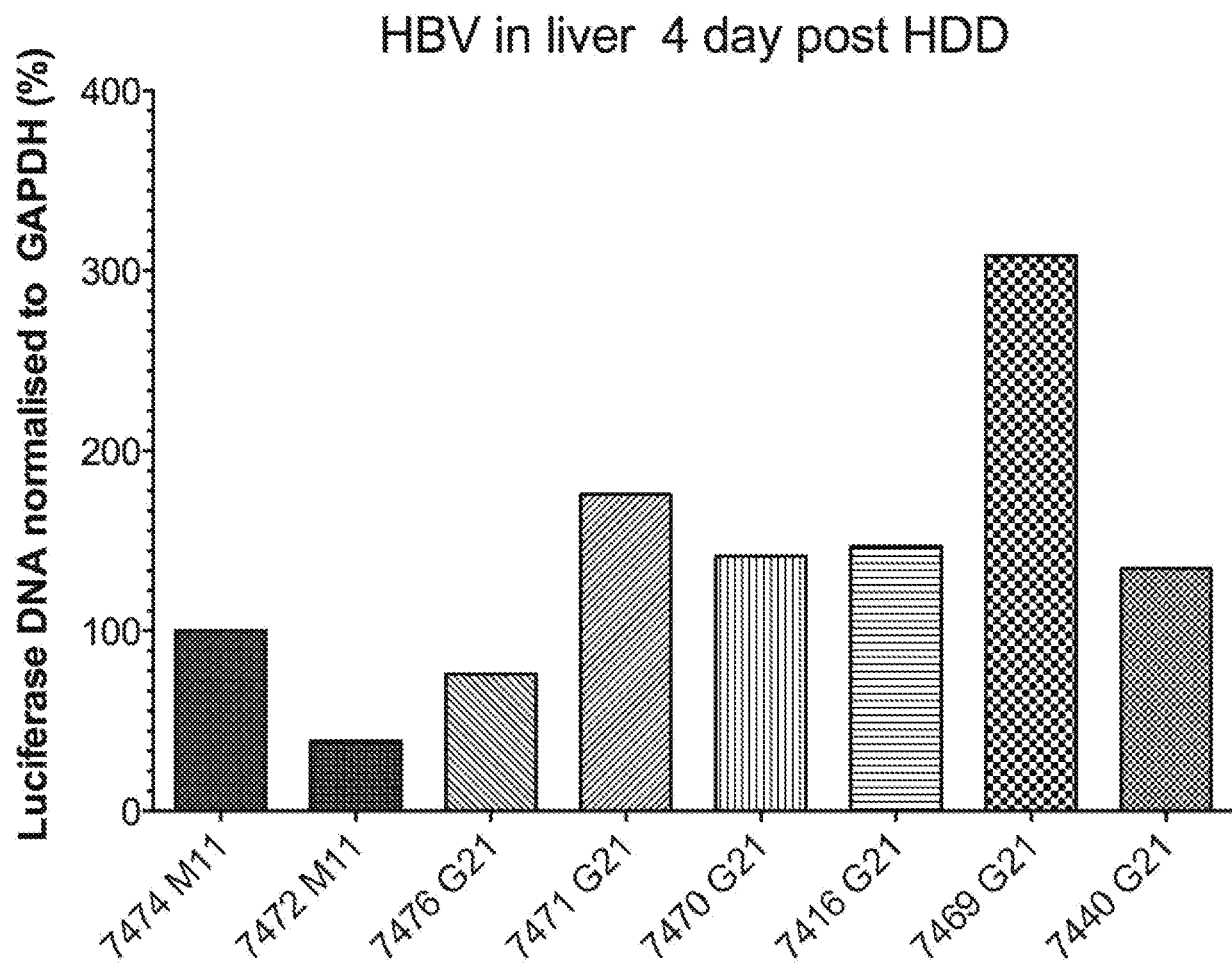


FIG. 55

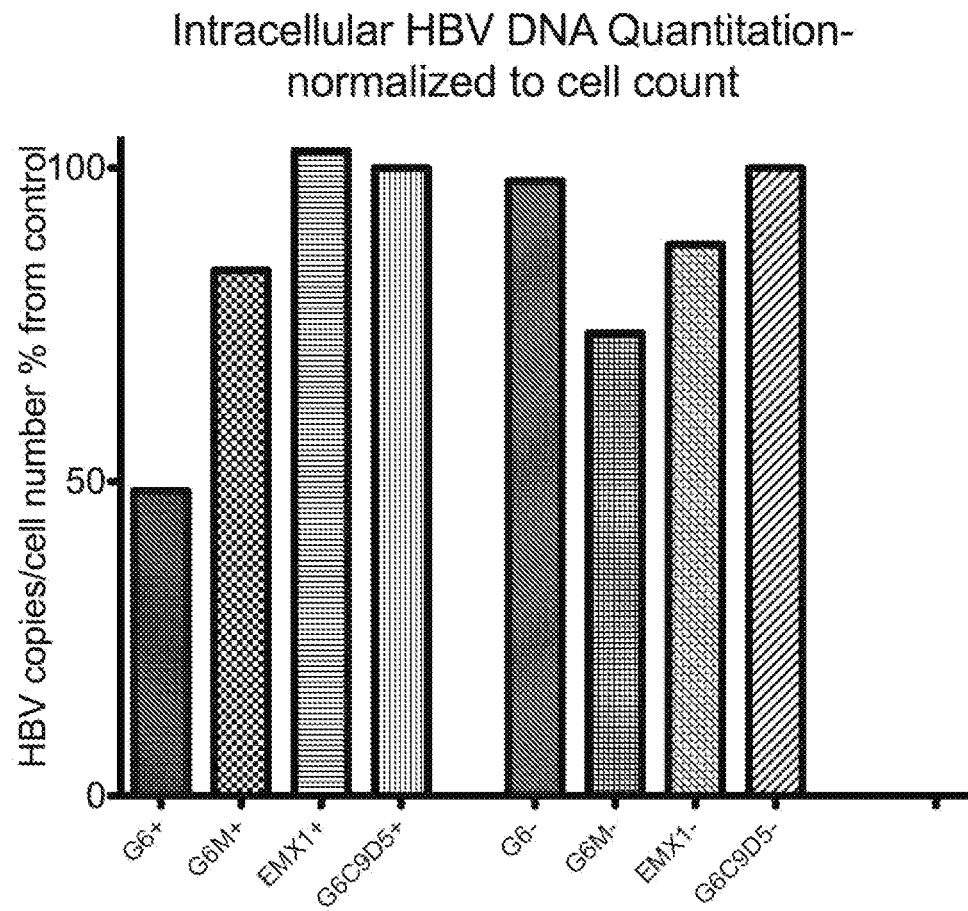


FIG. 56

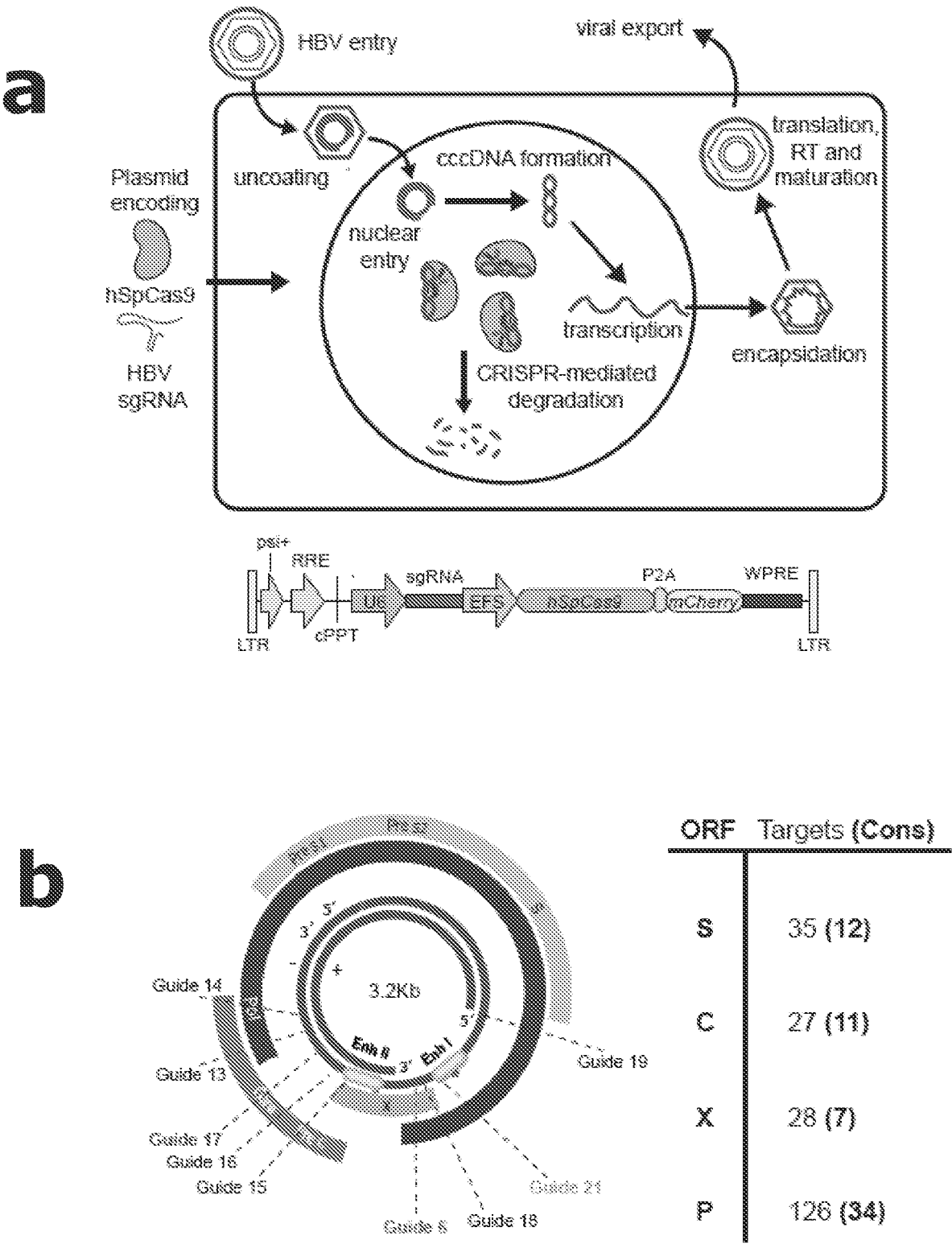
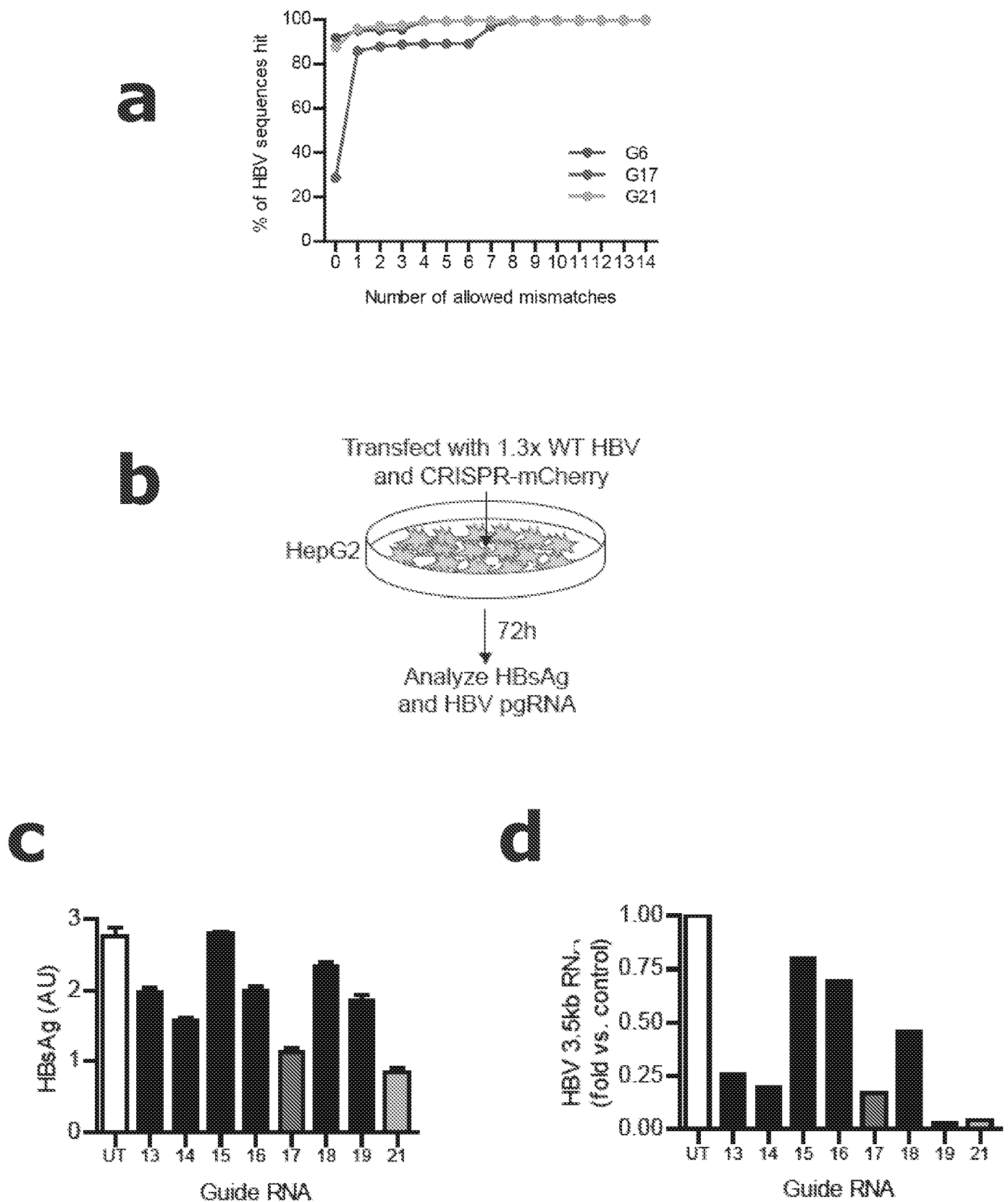
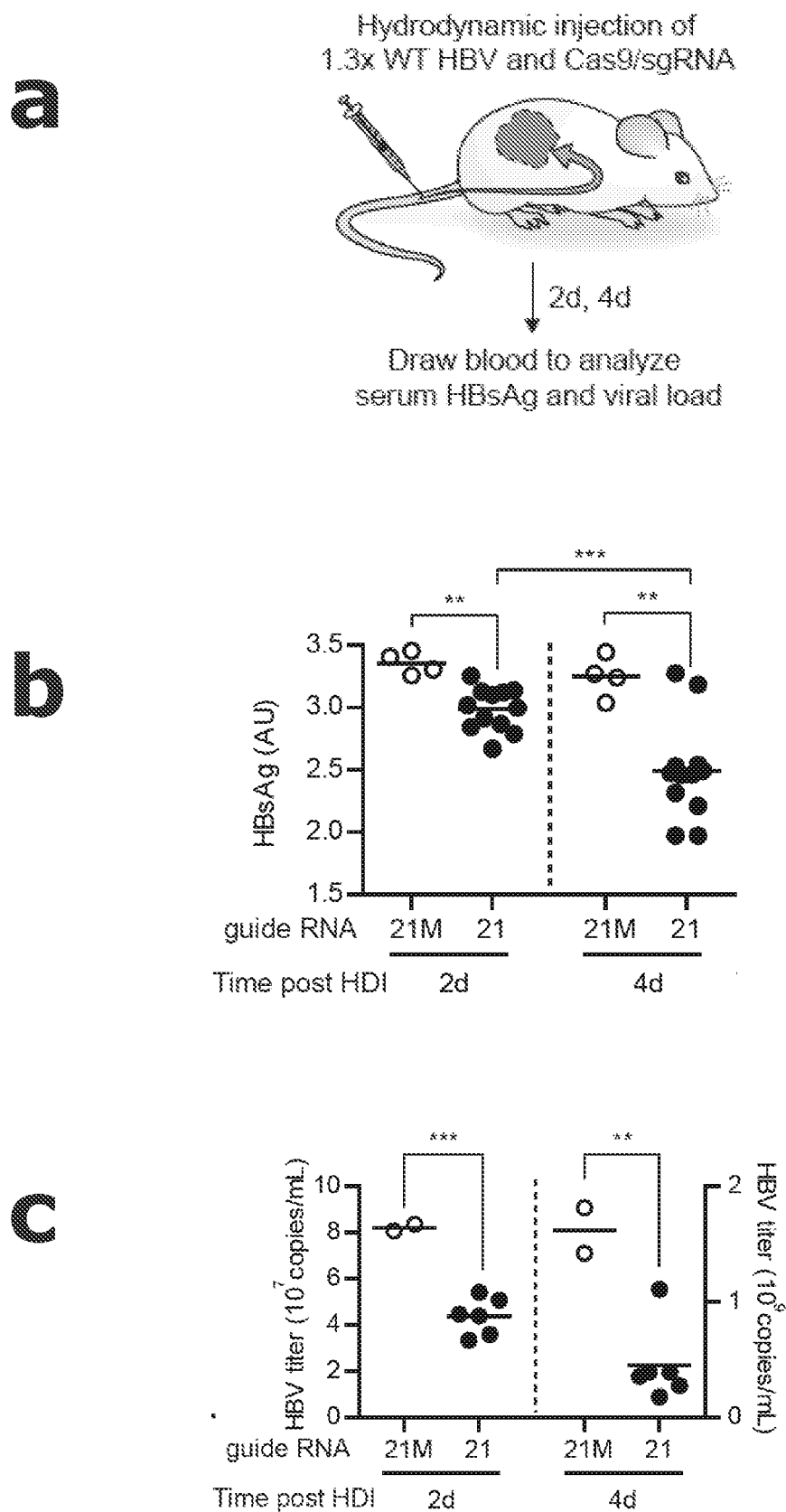
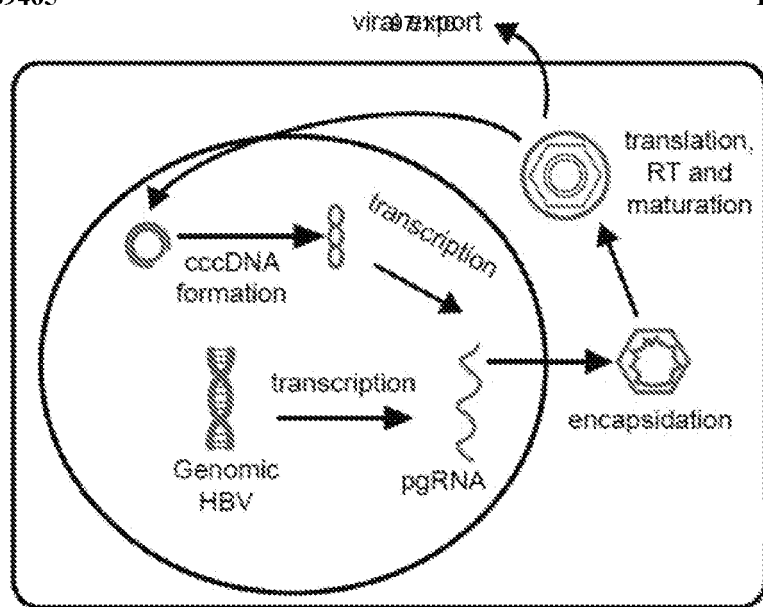
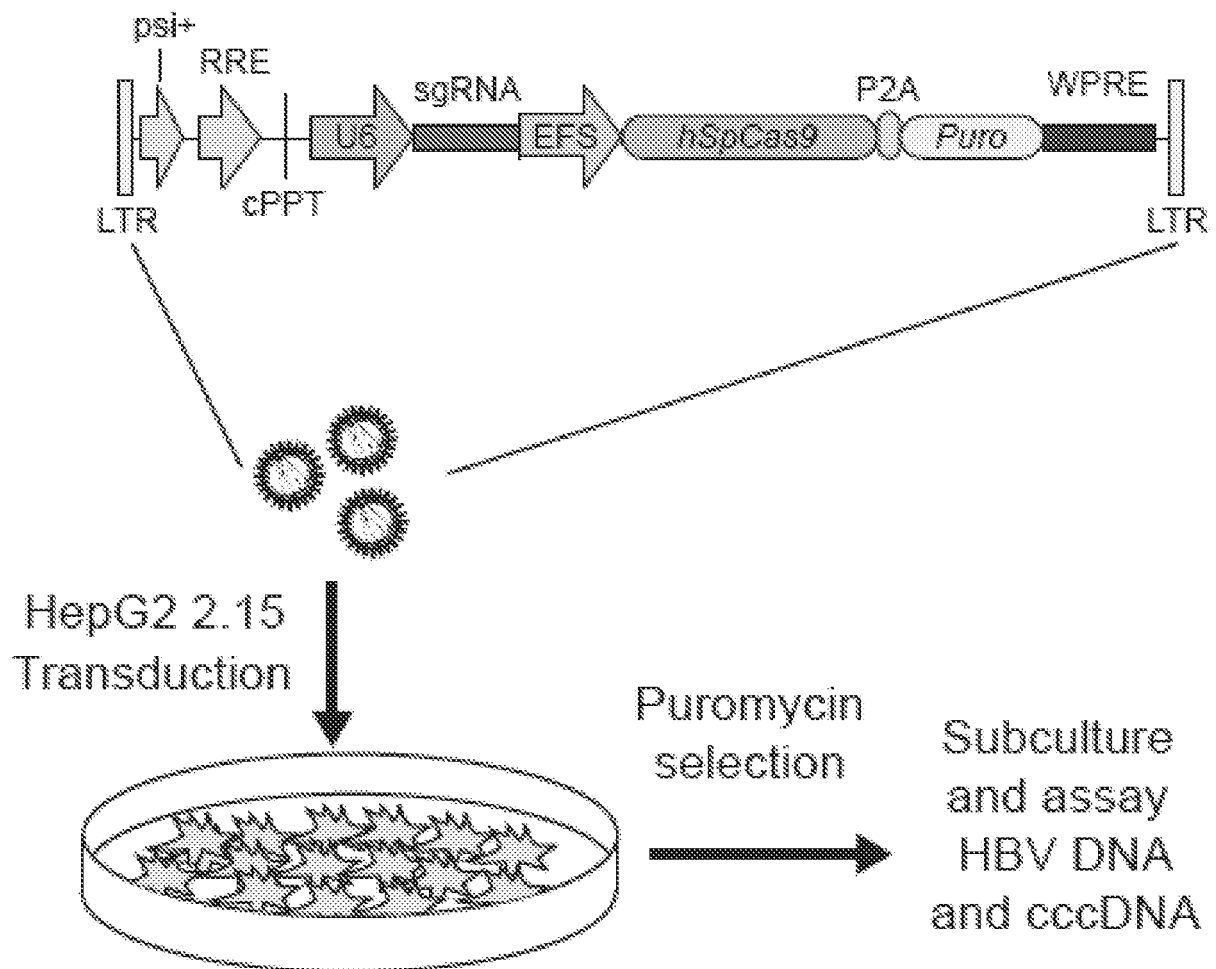


FIG. 57A-B

**FIG. 58A-D**

**FIG. 59A-C**

a**Figure S2****b****FIG. 60A-B**

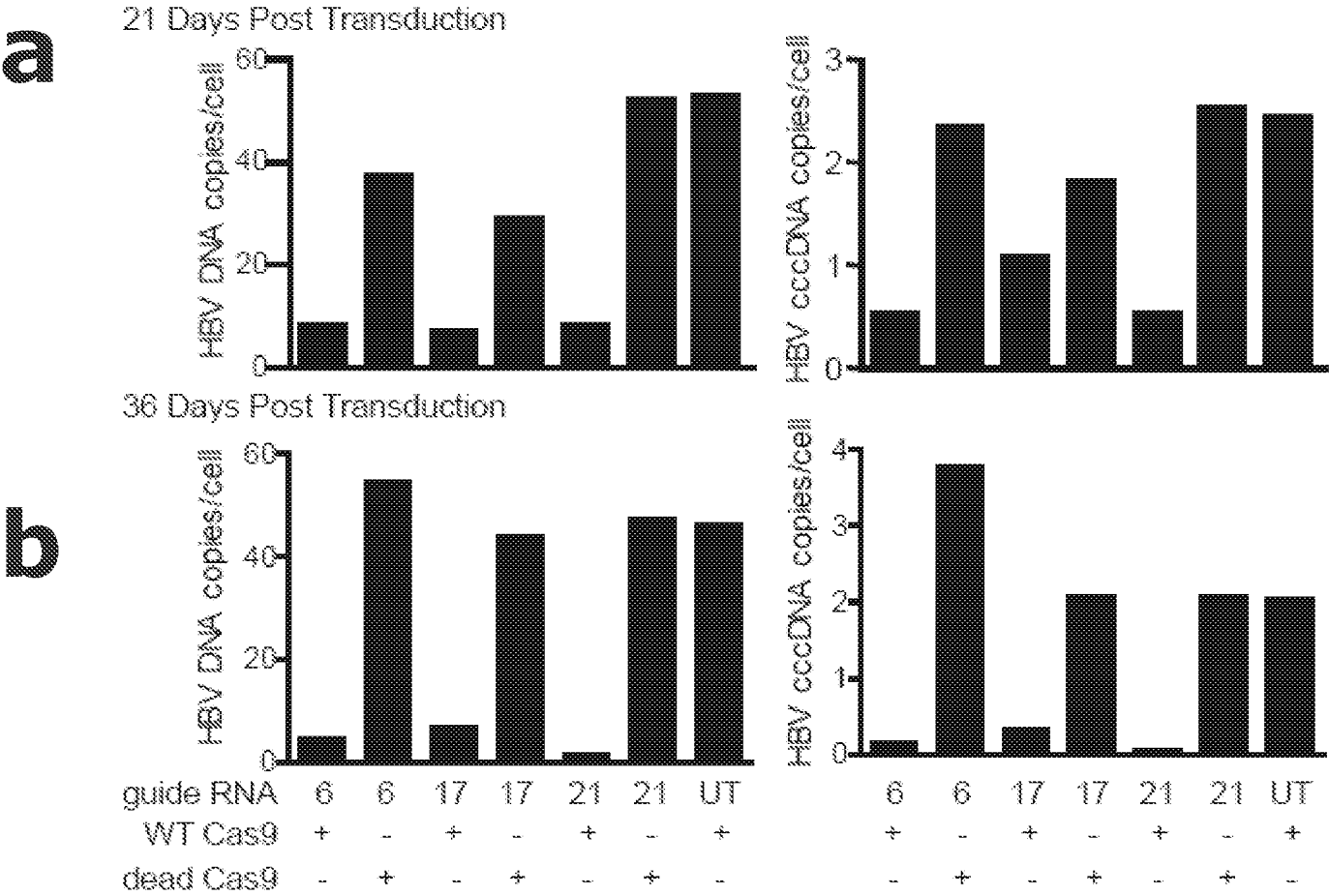
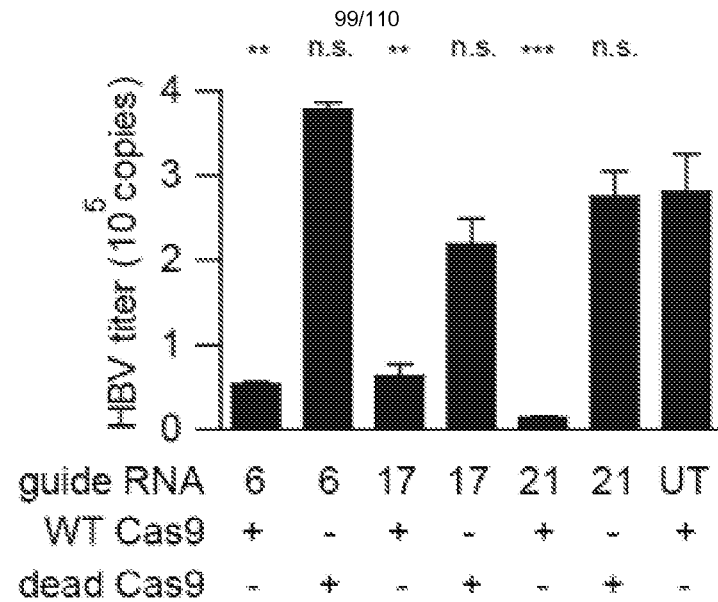
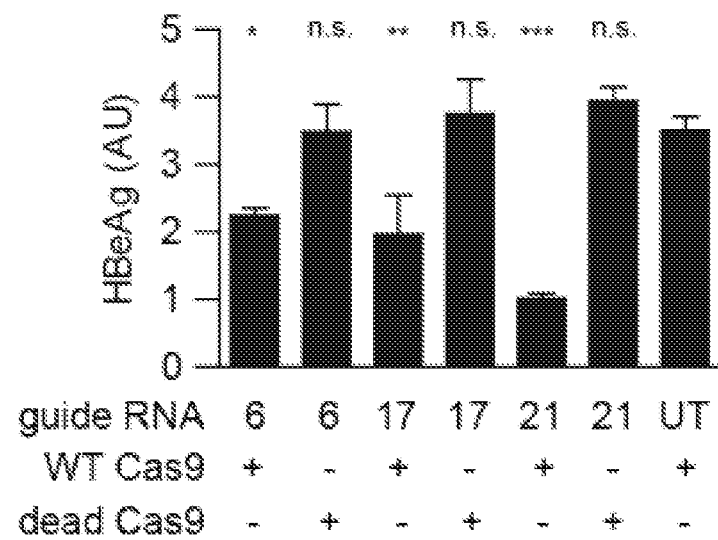
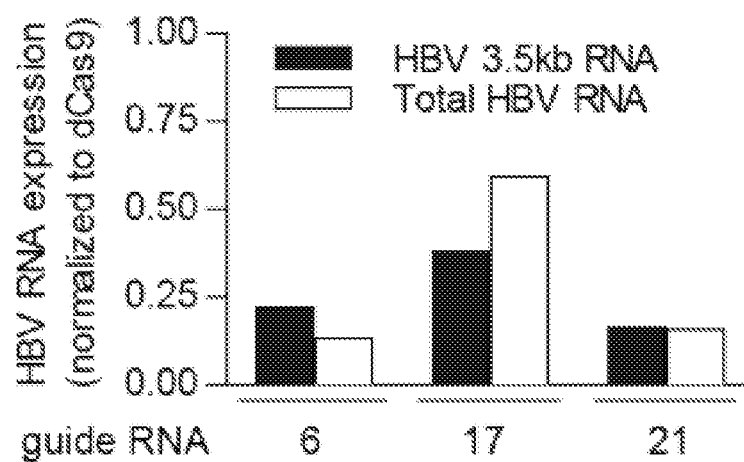
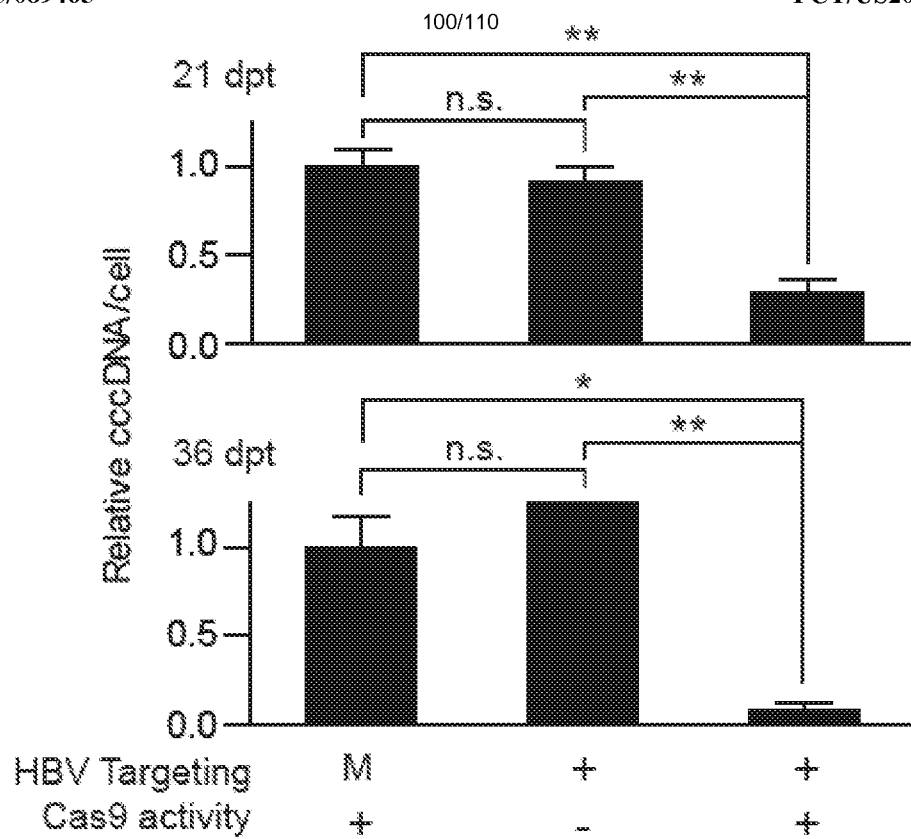
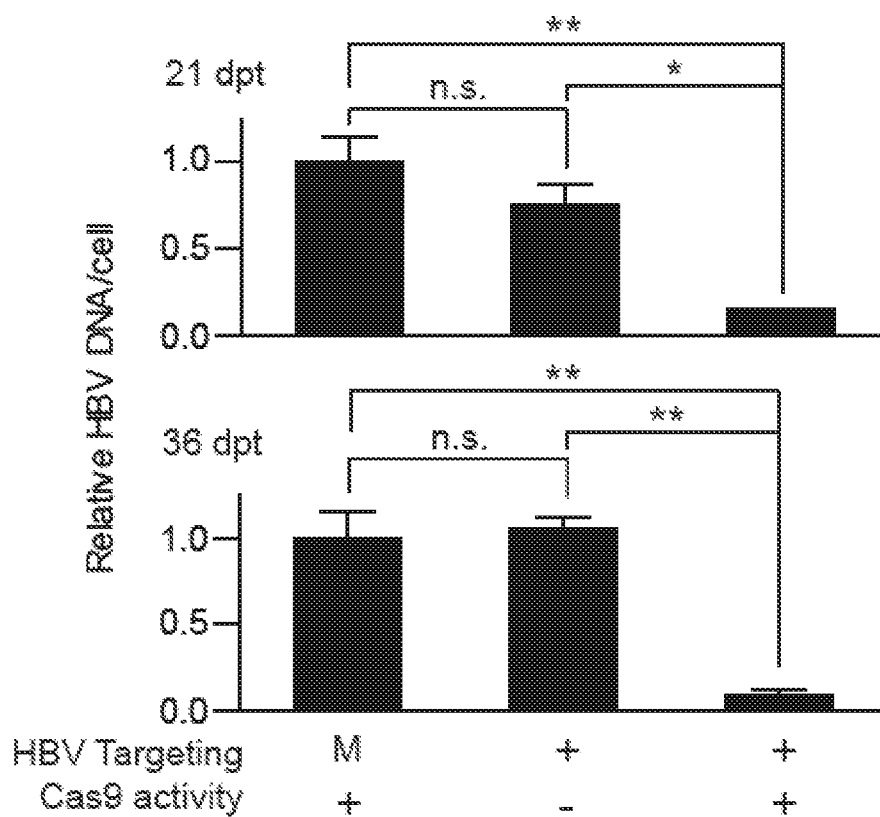
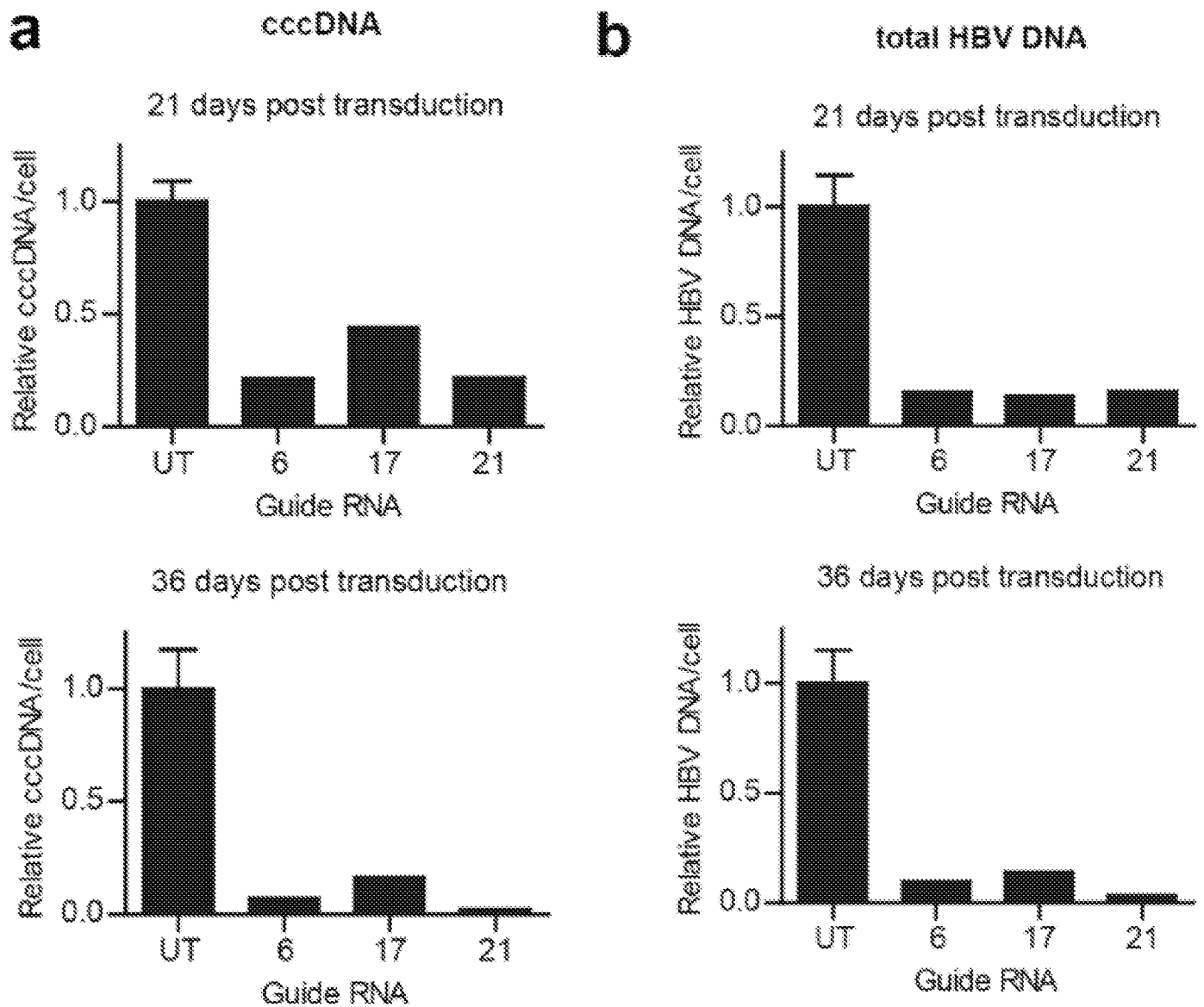


FIG. 61A-B

a**b****c****FIG. 62A-C**

a**b****FIG. 63A-B**

**FIG. 64A-B**

Day 29 post transduction

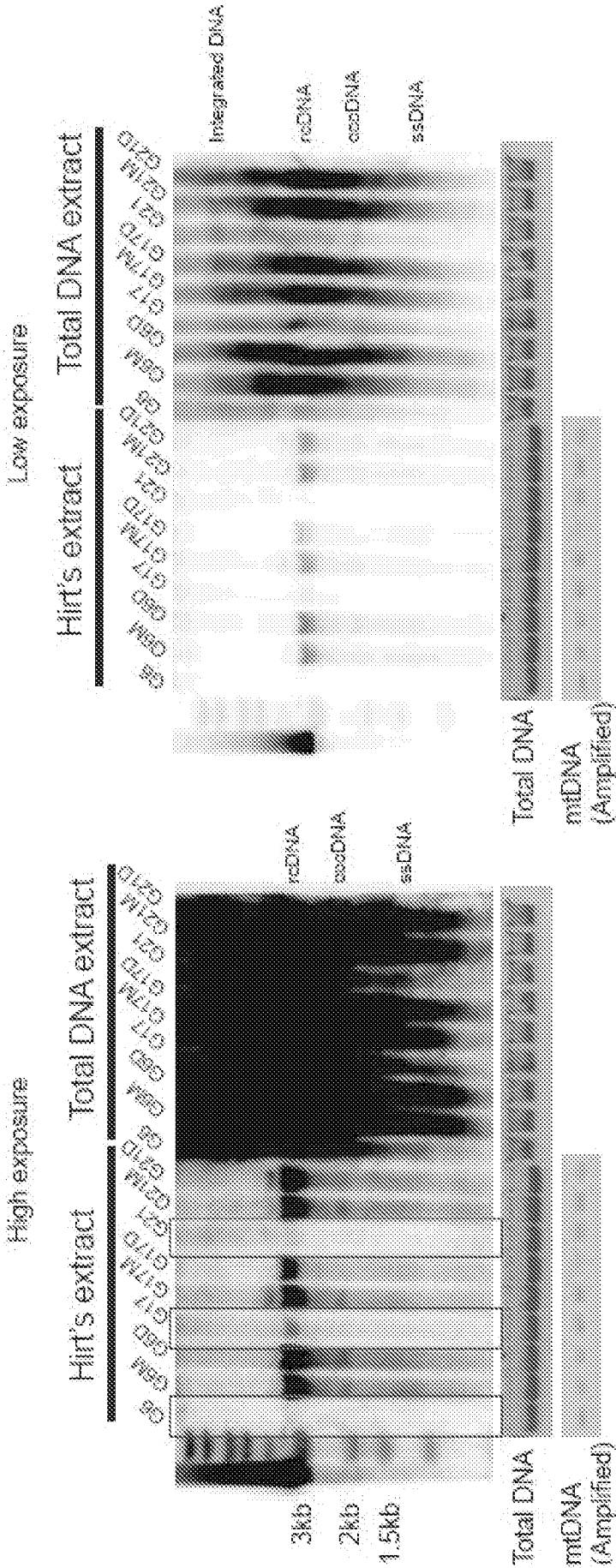


FIG. 65

36 days post transduction

Guide RNA	17	UT	17	21	UT	21
Nuclease activity	+	+	-	+	+	-

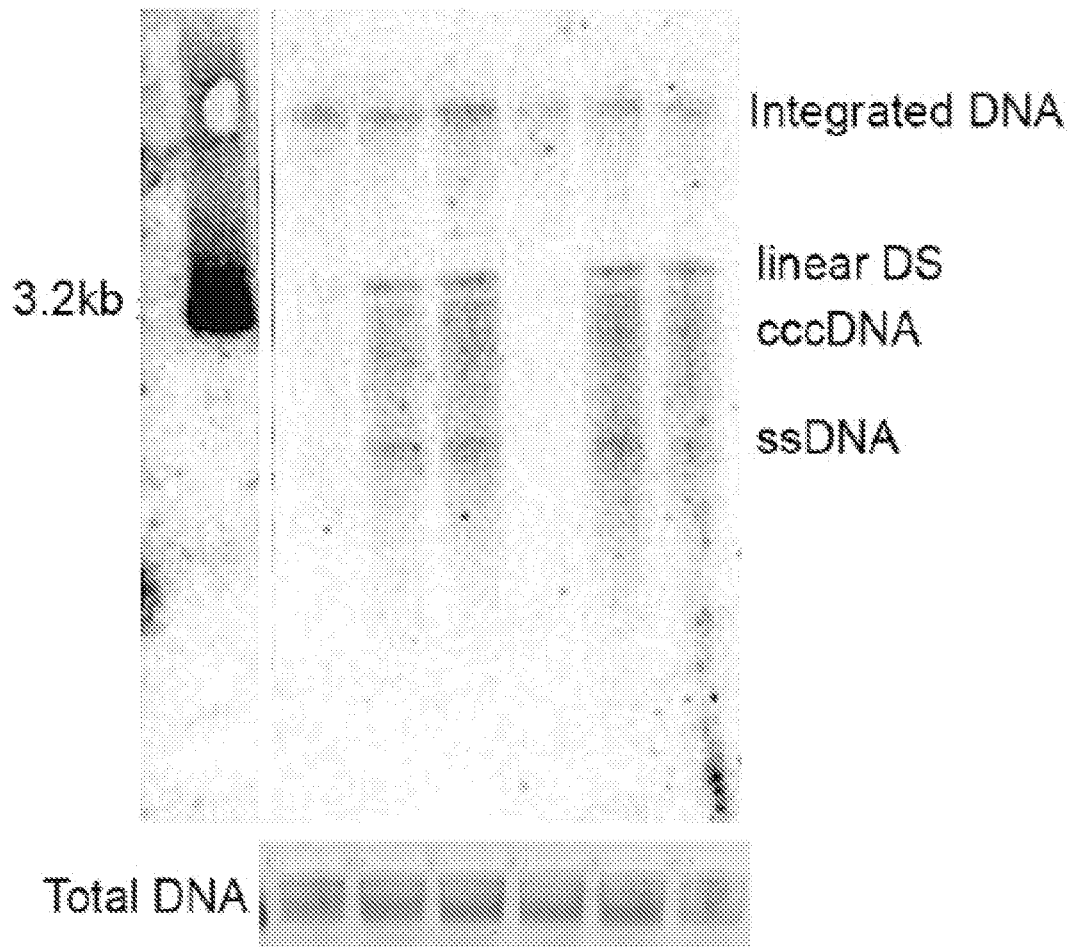


FIG. 66

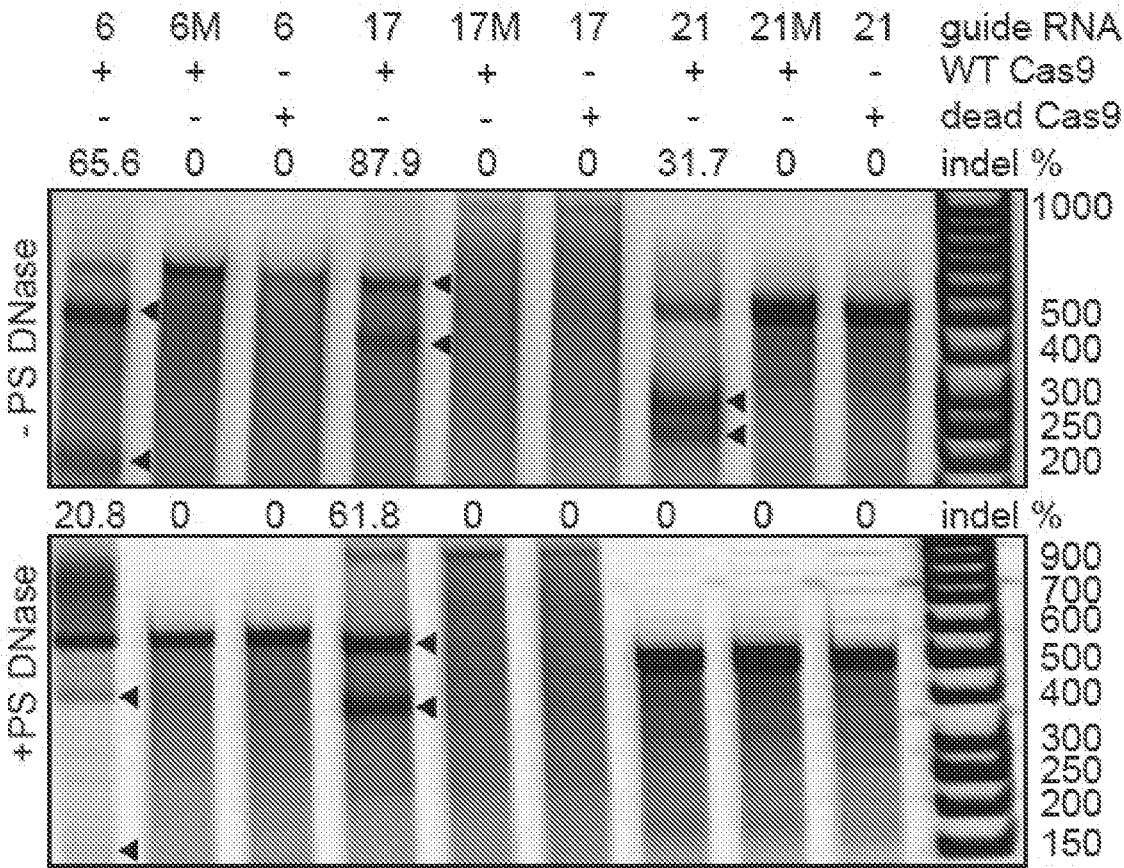
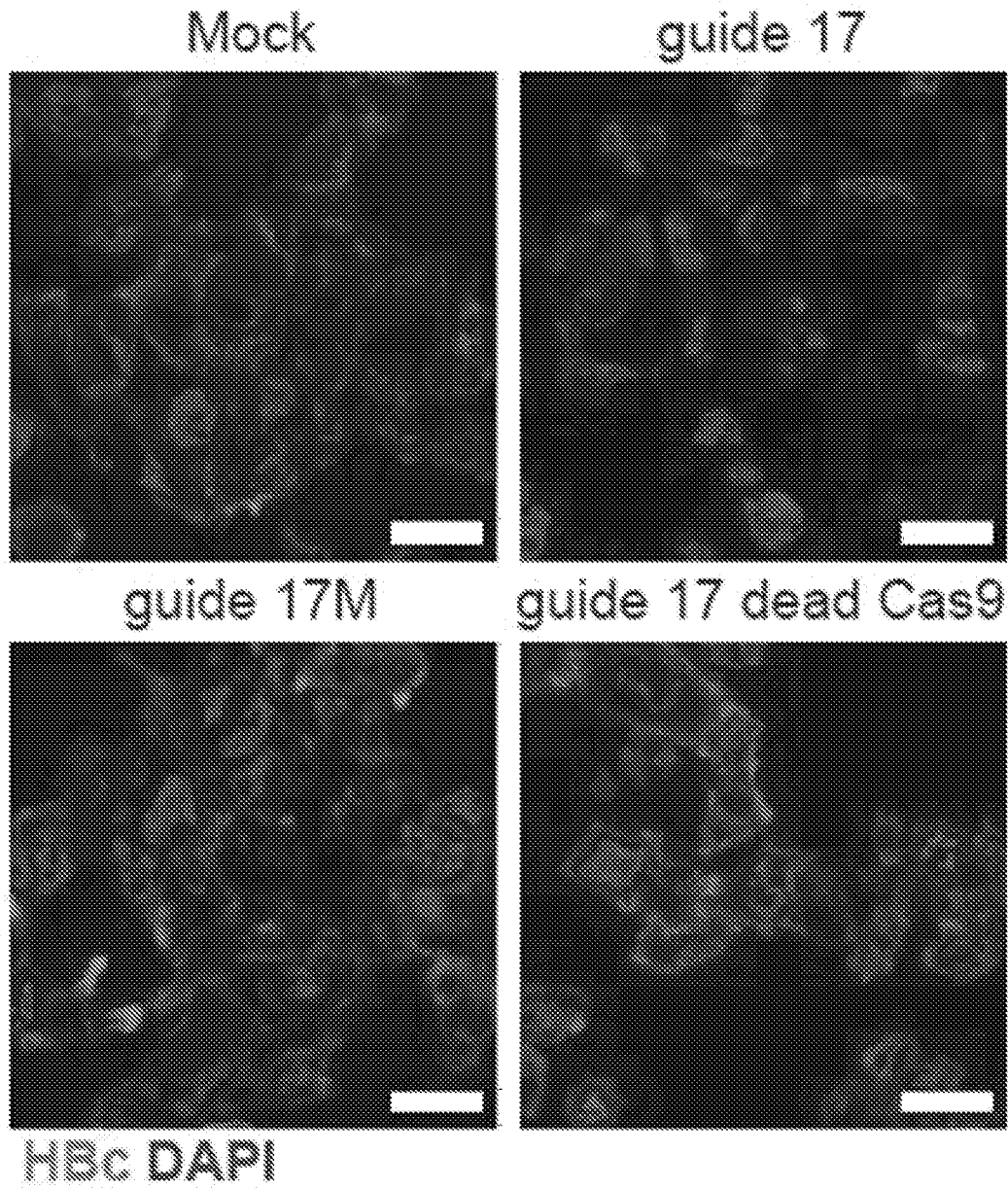
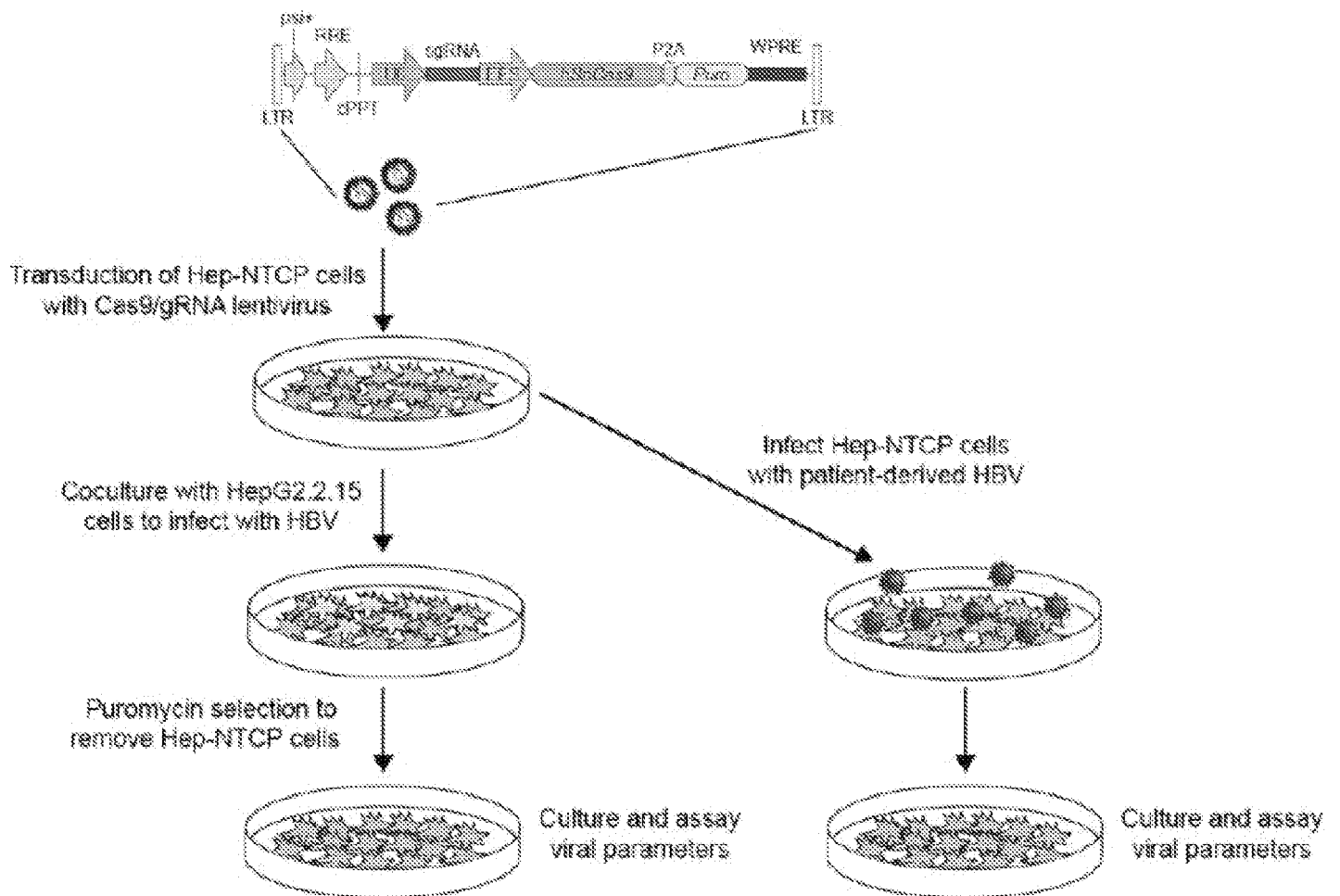


FIG. 67

**FIG. 68**

**FIG. 69**

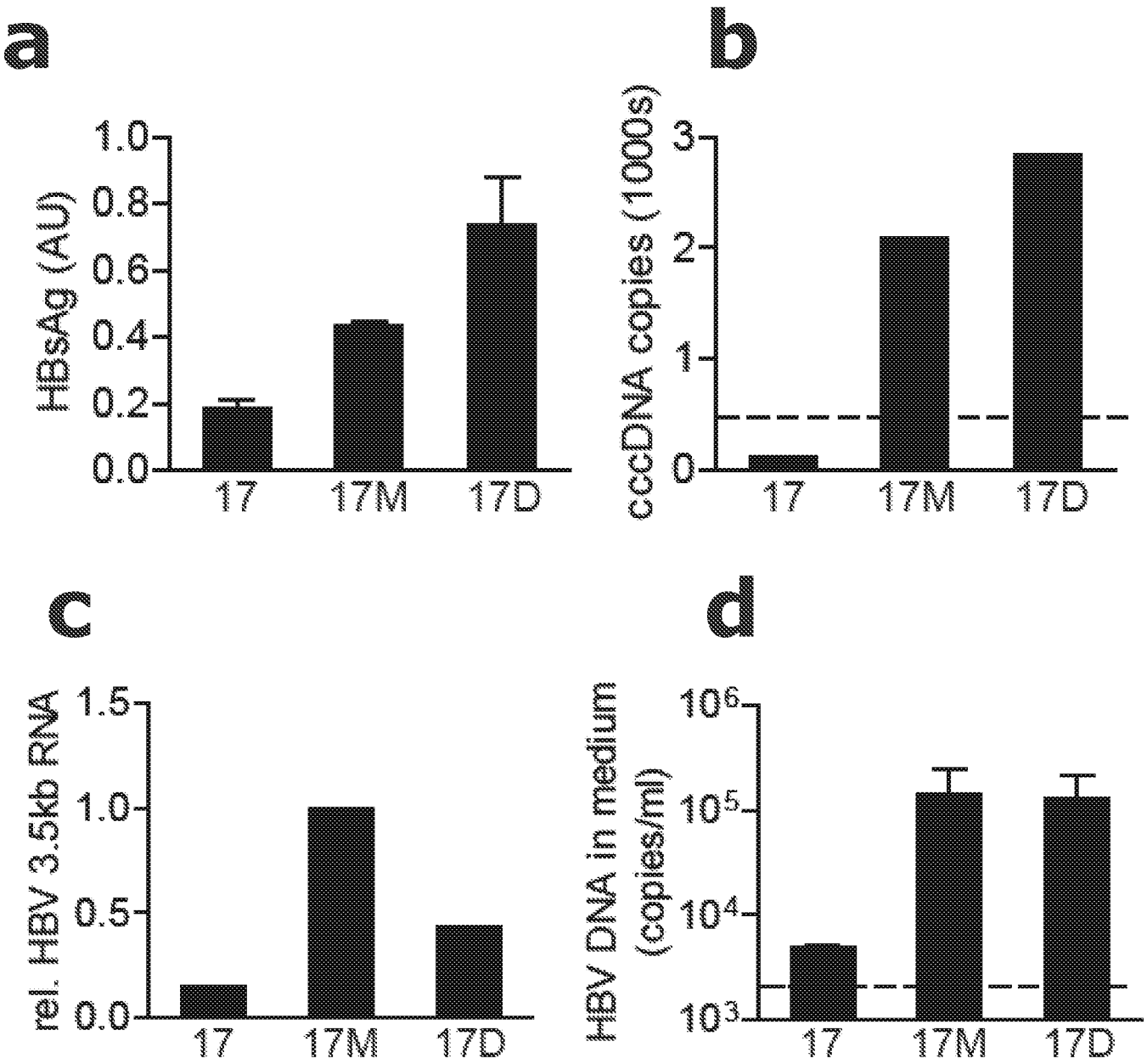


FIG. 70A-D

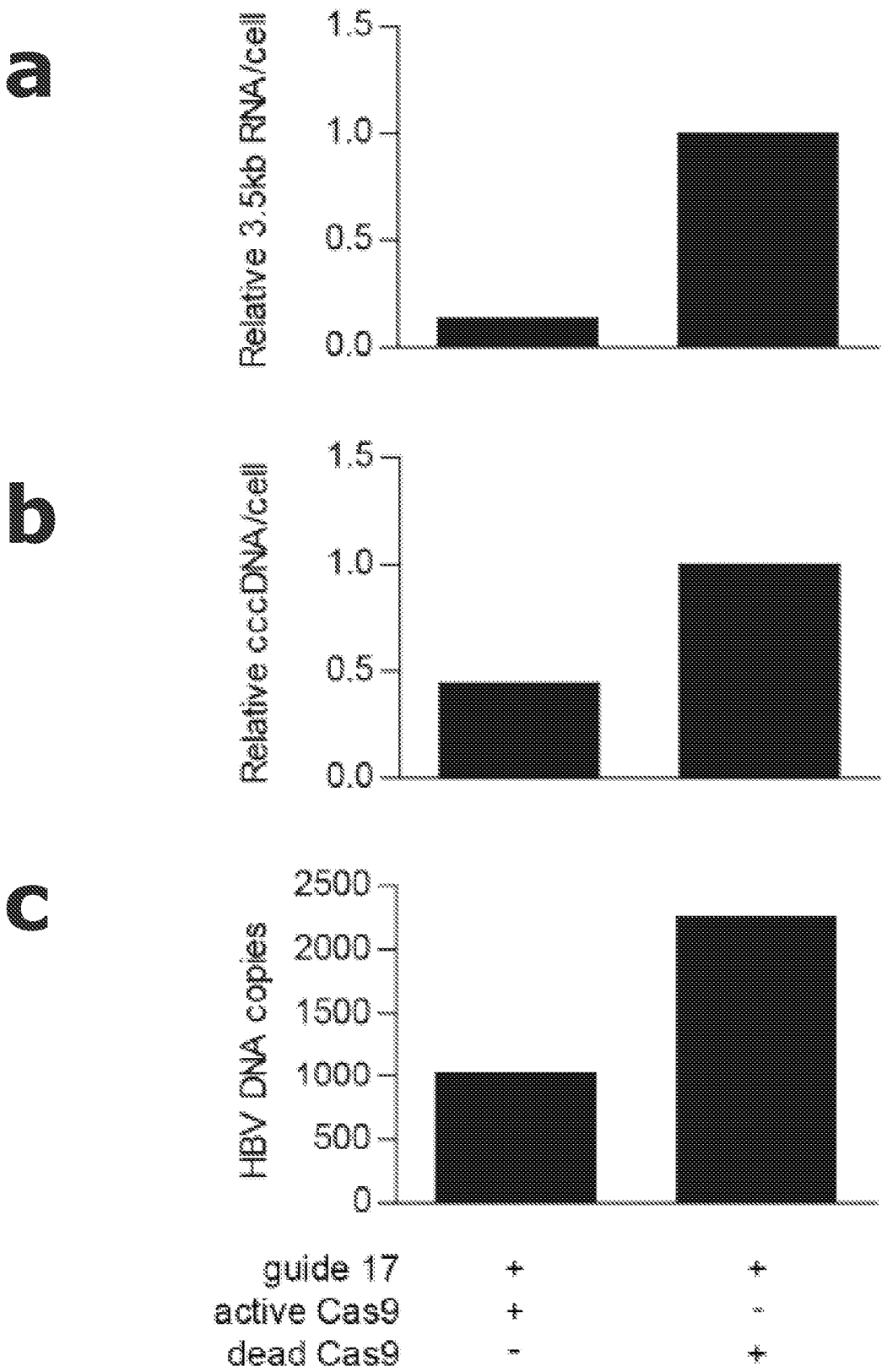


FIG. 71A-C

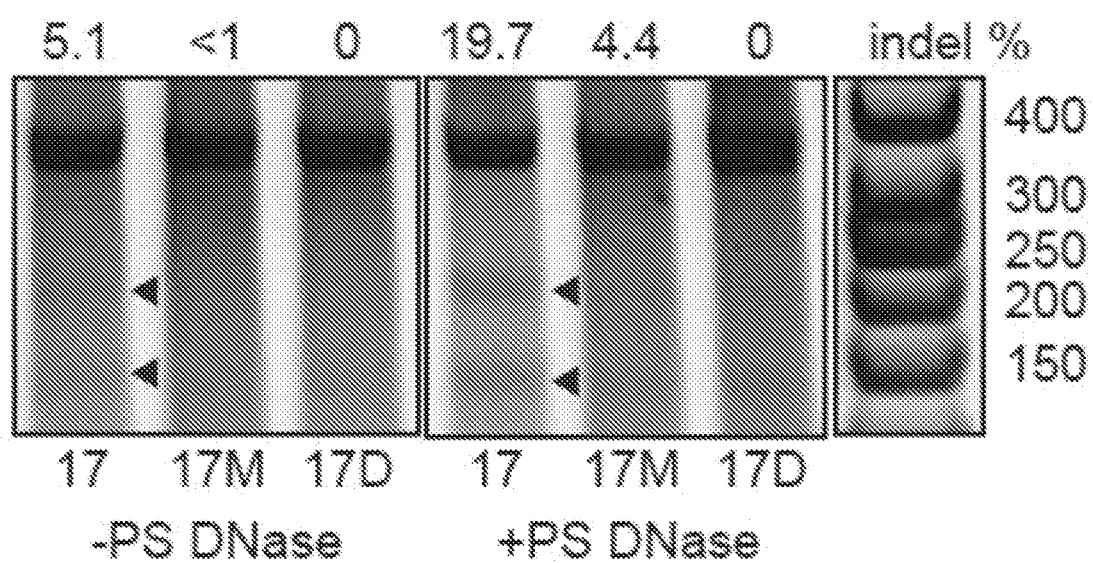
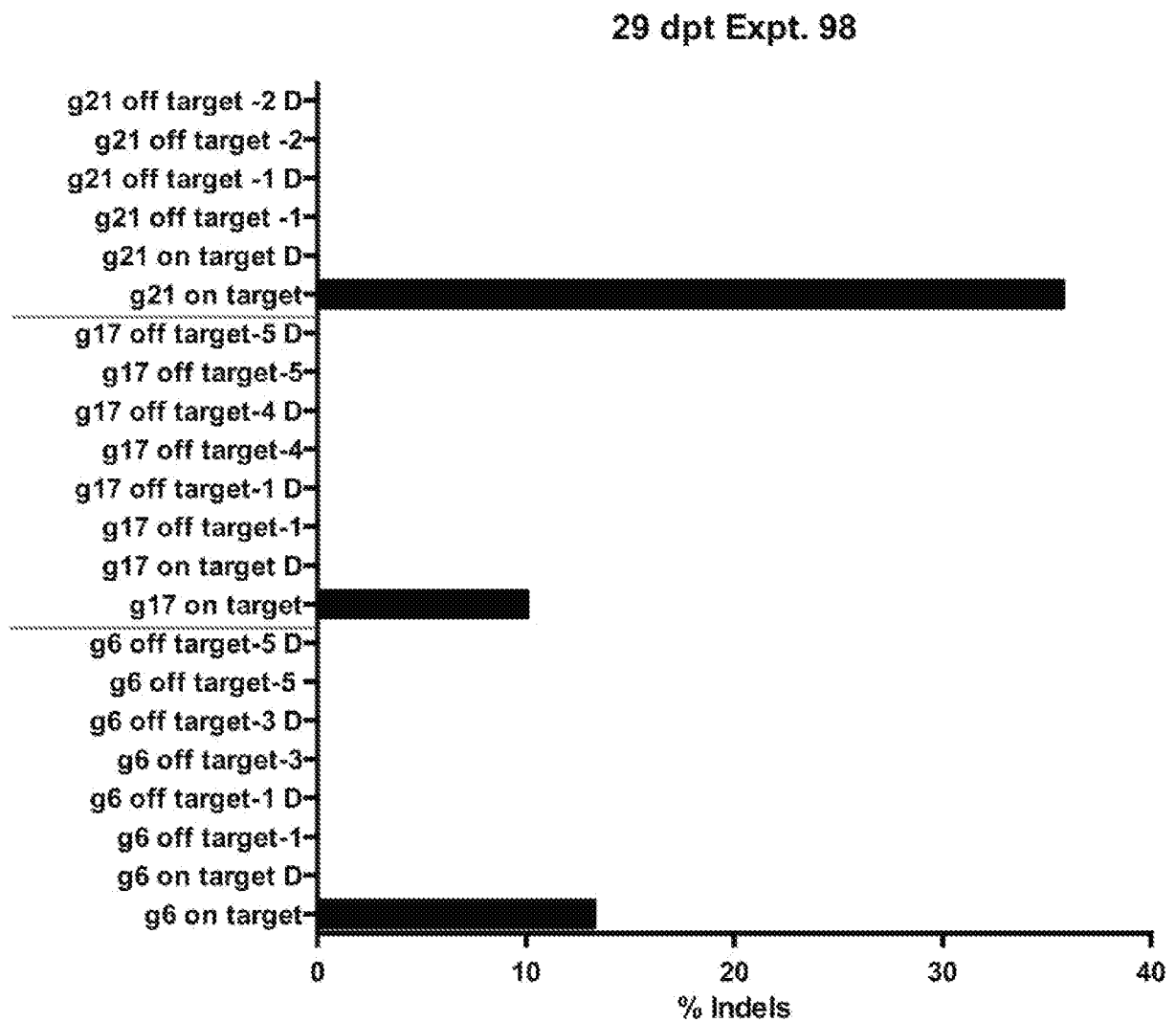


FIG. 72

**FIG. 73**