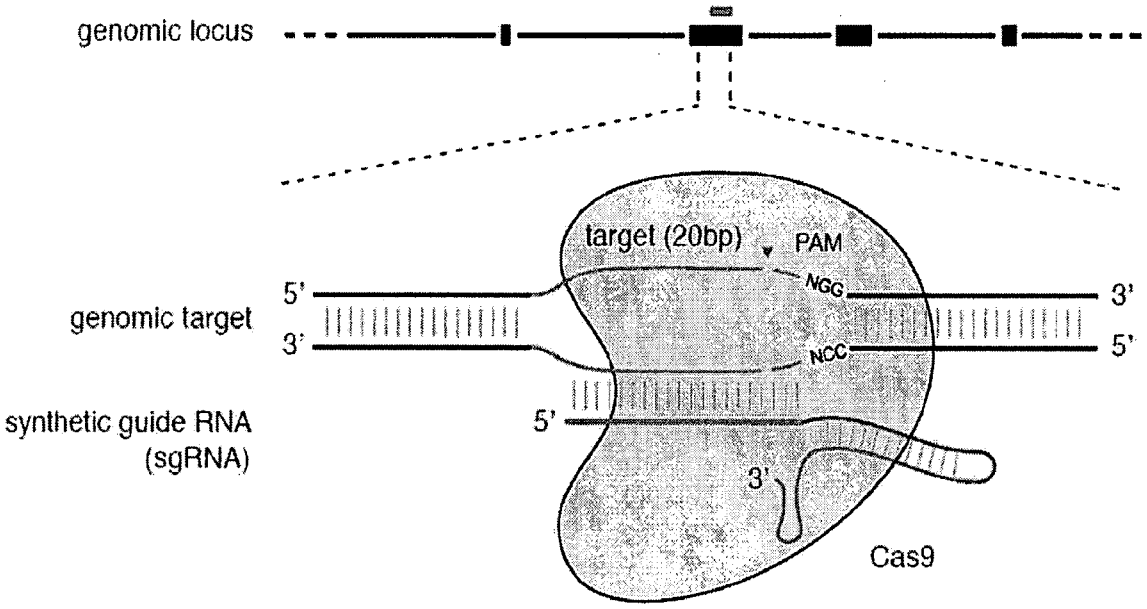

genomic locus
target (20bp)
PAM
NGG
NCC
5'
3'
genomic target
3'
5'
synthetic guide RNA
(sgRNA)
5'
3'
Cas9

FIG. 1

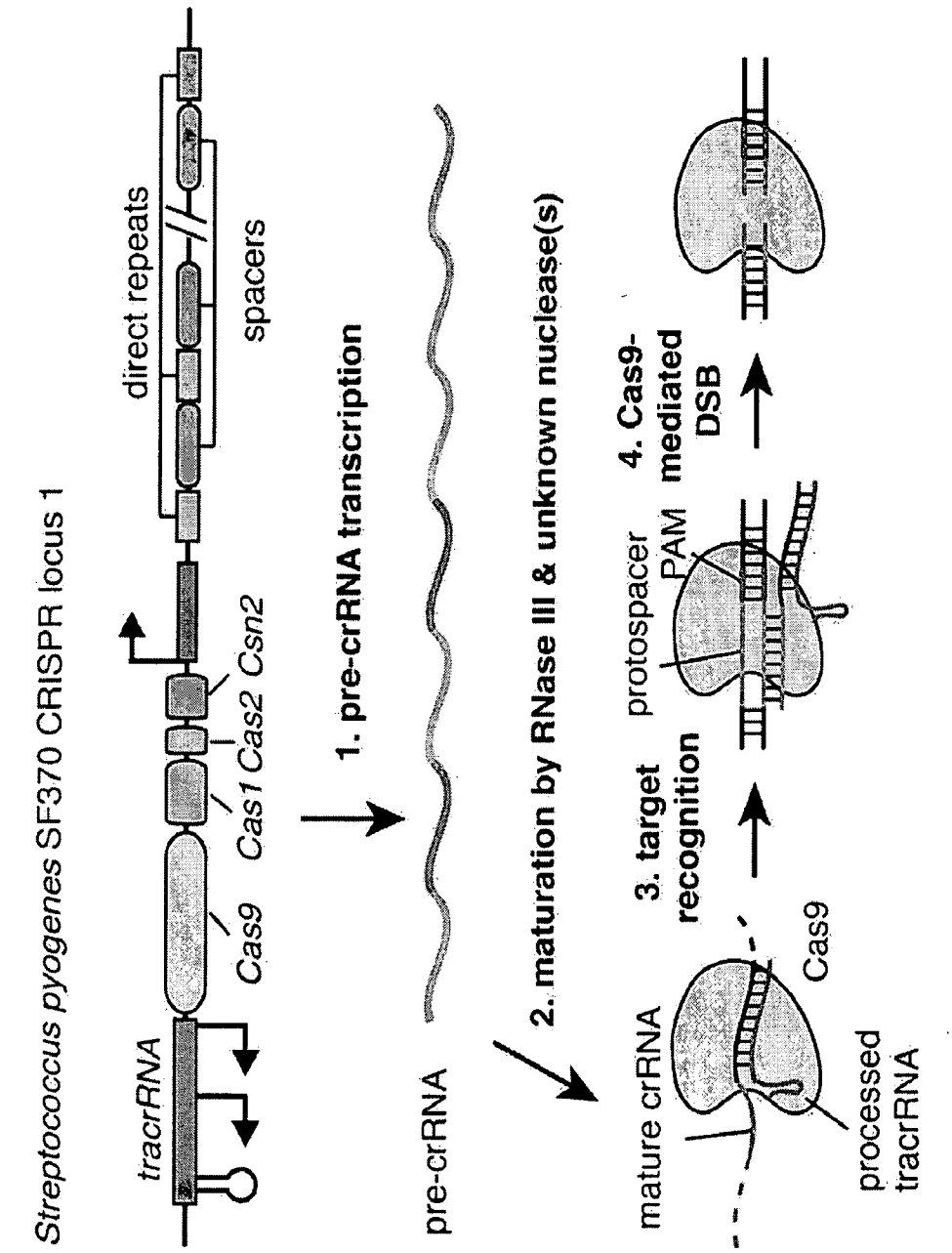

A
Streptococcus pyogenes SF370 CRISPR locus 1
tracrRNA
Cas9
Cas1
Cas2
Csn2
direct repeats
spacers
1. pre-crRNA transcription
pre-crRNA
2. maturation by RNase III & unknown nuclease(s)
mature crRNA
Cas9
processed tracrRNA
3. target recognition
protospacer
PAM
4. Cas9-mediated DSB

FIG. 2A

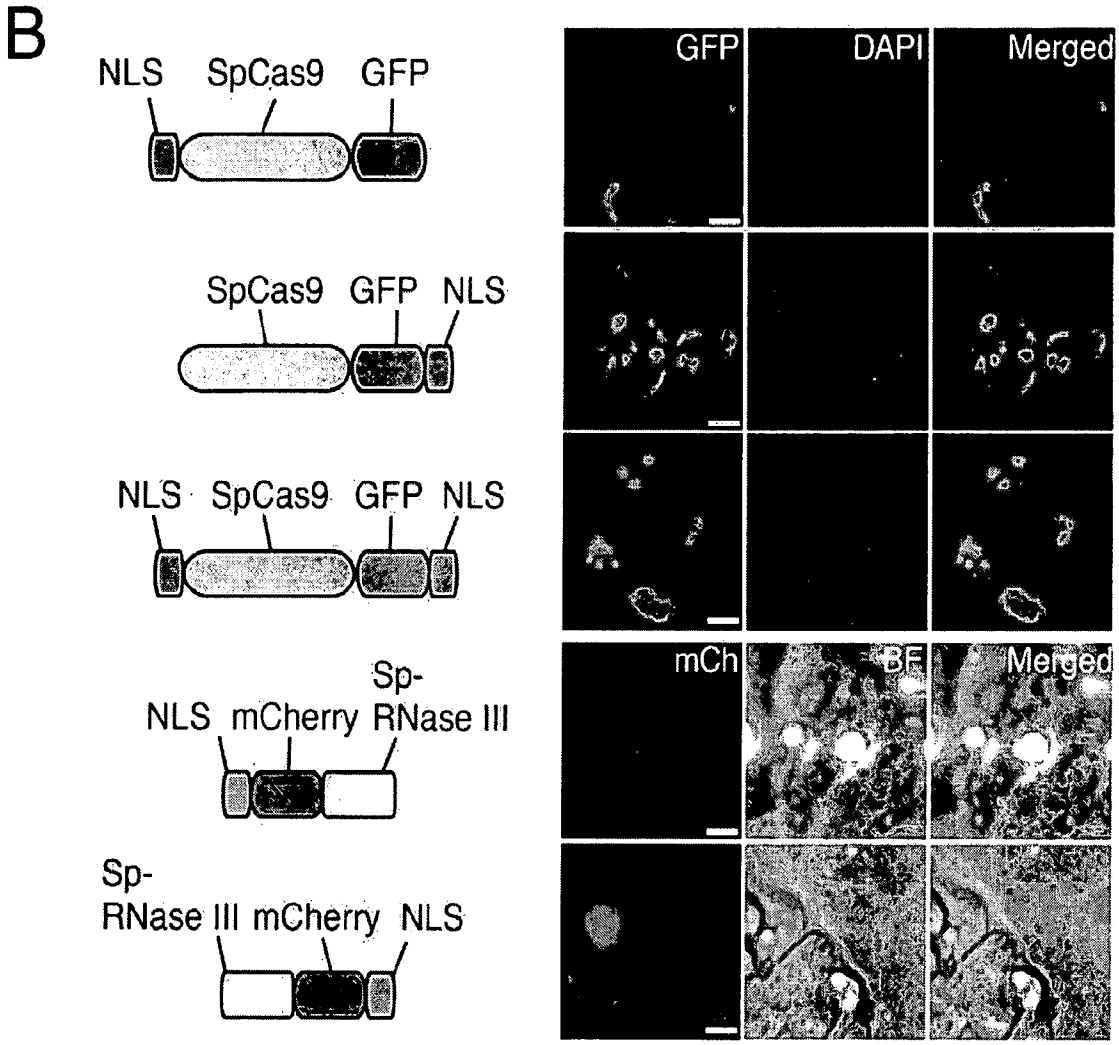

B
NLS SpCas9 GFP
SpCas9 GFP NLS
NLS SpCas9 GFP NLS
NLS mCherry Sp-RNase III
Sp-RNase III mCherry NLS
GFP
DAPI
Merged
mCh
BF
Merged

FIG. 2B

C

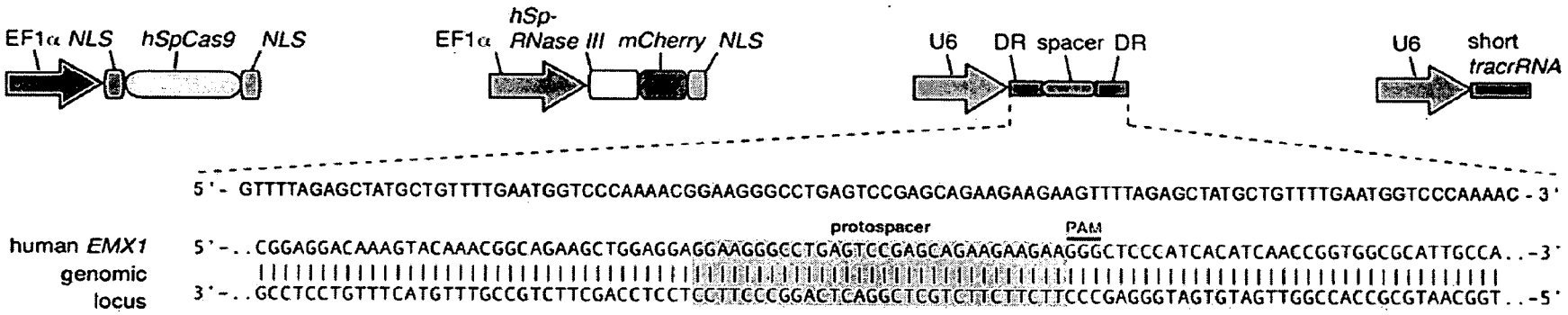

EF1α NLS
hSpCas9
NLS
EF1α
hSp-
RNase III
mCherry
NLS
U6
DR
spacer
DR
U6
short
tracrRNA
5'- GTTTTAGAGCTATGCTGTTTTGAATGGTCCCAAAACGGAAGGGCCTGAGTCCGAGCAGAAGAAGAAGTTTTAGAGCTATGCTGTTTTGAATGGTCCCAAAAC - 3'
protospacer
PAM
human EMX1 genomic locus
5'-..CGGAGGACAAAGTACAAACGGCAGAAGCTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAAGAAGGGCTCCCATCACATCAACCGGTGGCGCATTGCCA..-3'
3'-..GCCTCCTGTTTCATGTTTGCCGTCTTCGACCTCCTCCTTCCCGGACTCAGGCTCGTCTTCTTCTTCCCGAGGGTAGTGTAGTTGGCCACCGCGTAACGGT..-5'

**FIG. 2C**

D

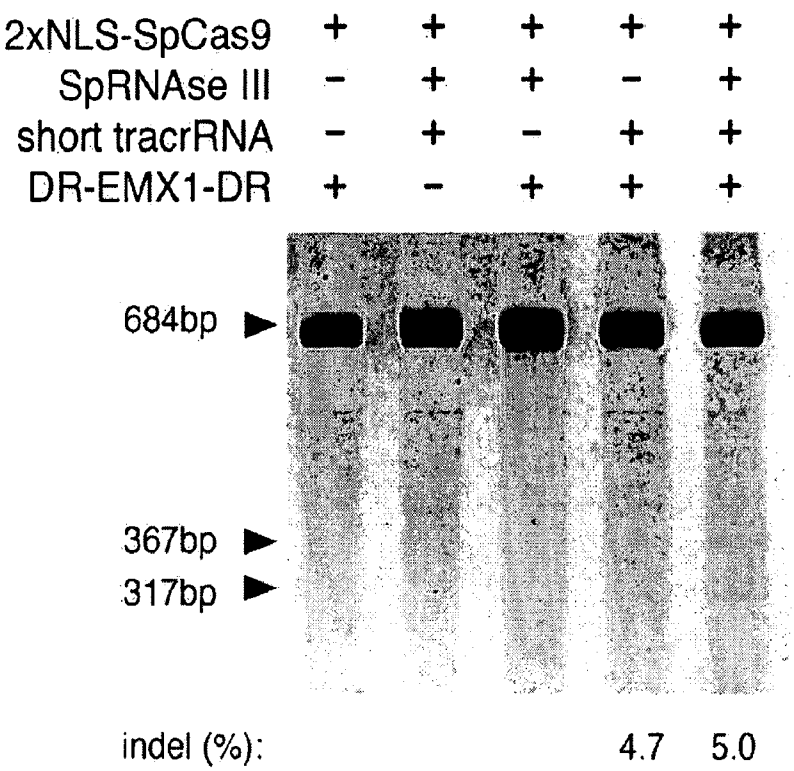
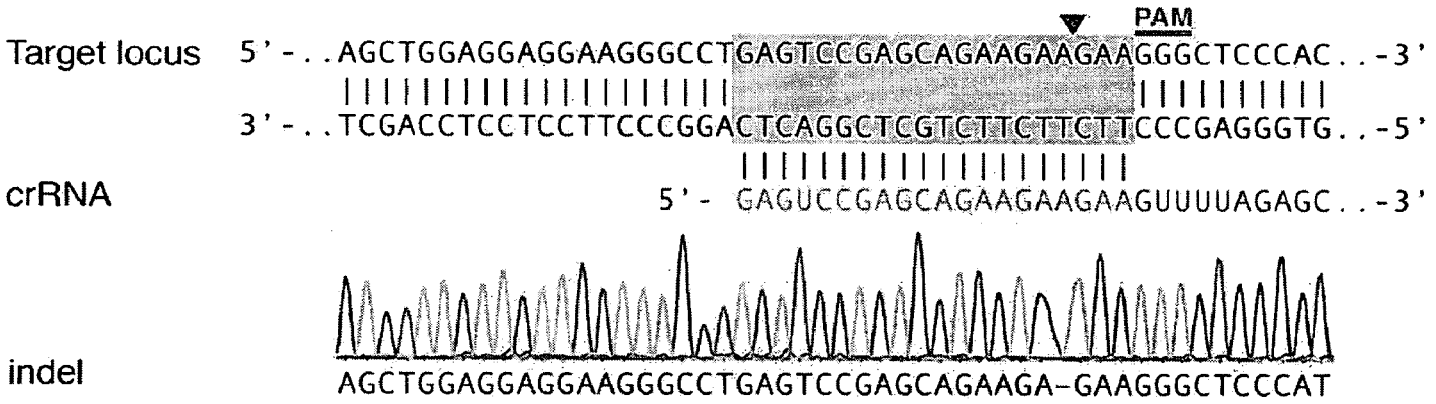

2xNLS-SpCas9 + + + + +
SpRNAse III - + + - +
short tracrRNA - + - + +
DR-EMX1-DR + - + + +
684bp
367bp
317bp
indel (%): 4.7 5.0

FIG. 2D

E




F

human *EMX1* protospacer target (mutation in 5 of 43 sequenced clones = 11.6%)

| | |
|---|---|
| WT | 5'-..CTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAG AAGAAGGGCTCCCATCACAT..-3' |
| Δ1 | CTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAG--AGAAGGGCTCCCATCACAT |
| +1 | CTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAAAGAAGGGCTCCCATCACAT |
| Δ3 | CTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAG----AAGGGCTCCCATCACAT |
| m1, Δ6 | CTGGAGGAGGAAGGGCCTGAGCCCGAGCAGAAG-------GGCTCCCATCACAT |

FIG. 2E-F

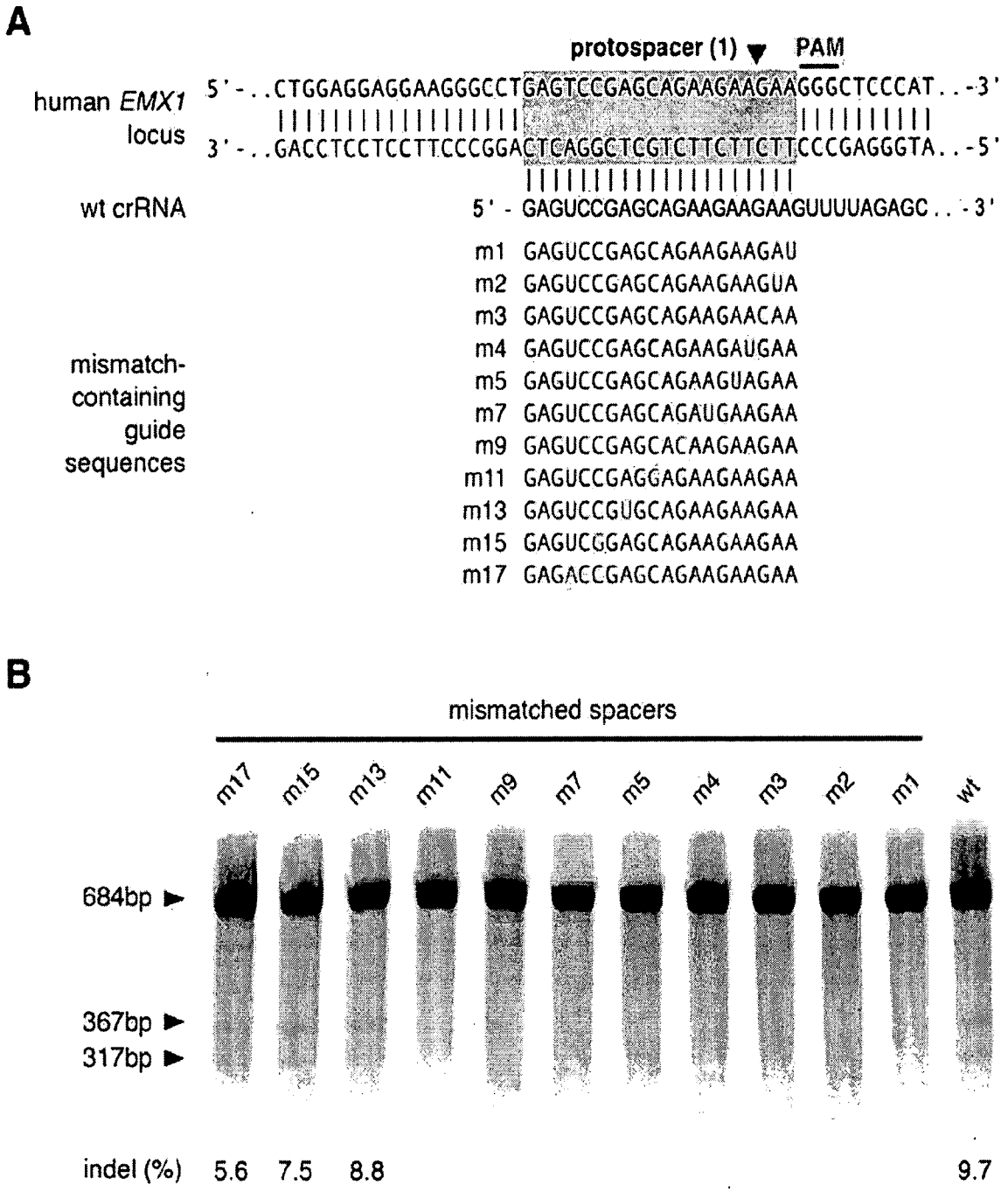

A
protospacer (1)
PAM
human EMX1 locus
5'-..CTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAAGAAGGGCTCCCAT..-3'
3'-..GACCTCCTCCTTCCCGGACTCAGGCTCGTCTTCTTCTTCCCGAGGGTA..-5'
wt crRNA
5' - GAGUCCGAGCAGAAGAAGAAGUUUUAGAGC..-3'
mismatch-containing guide sequences
m1 GAGUCCGAGCAGAAGAAGAU
m2 GAGUCCGAGCAGAAGAAGUA
m3 GAGUCCGAGCAGAAGAACAA
m4 GAGUCCGAGCAGAAGAUGAA
m5 GAGUCCGAGCAGAAGUAGAA
m7 GAGUCCGAGCAGAUGAAGAA
m9 GAGUCCGAGCACAAGAAGAA
m11 GAGUCCGAGGAGAAGAAGAA
m13 GAGUCCGUGCAGAAGAAGAA
m15 GAGUCGGAGCAGAAGAAGAA
m17 GAGACCGAGCAGAAGAAGAA
B
mismatched spacers
m17
m15
m13
m11
m9
m7
m5
m4
m3
m2
m1
wt
684bp
367bp
317bp
indel (%)
5.6
7.5
8.8
9.7
C
left TALEN binding site
protospacer (1)
PAM
human EMX1 locus
5'-..CTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAAGAAGGGCTCCCAT..-3'
3'-..GACCTCCTCCTTCCCGGACTCAGGCTCGTCTTCTTCTTCCCGAGGGTA..-5'
right TALEN binding site

FIG. 3A-C

D

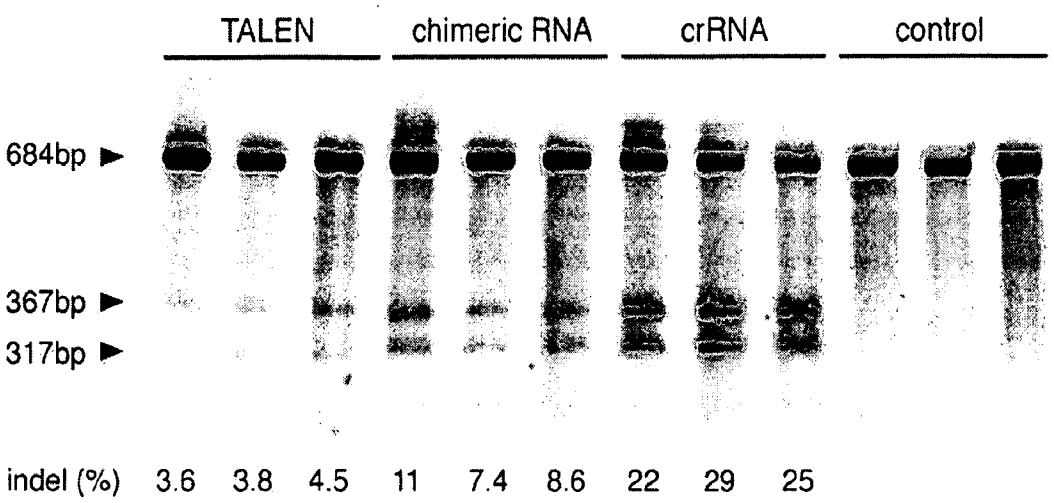

TALEN
chimeric RNA
crRNA
control
684bp
367bp
317bp
indel (%) 3.6 3.8 4.5 11 7.4 8.6 22 29 25

FIG. 3D

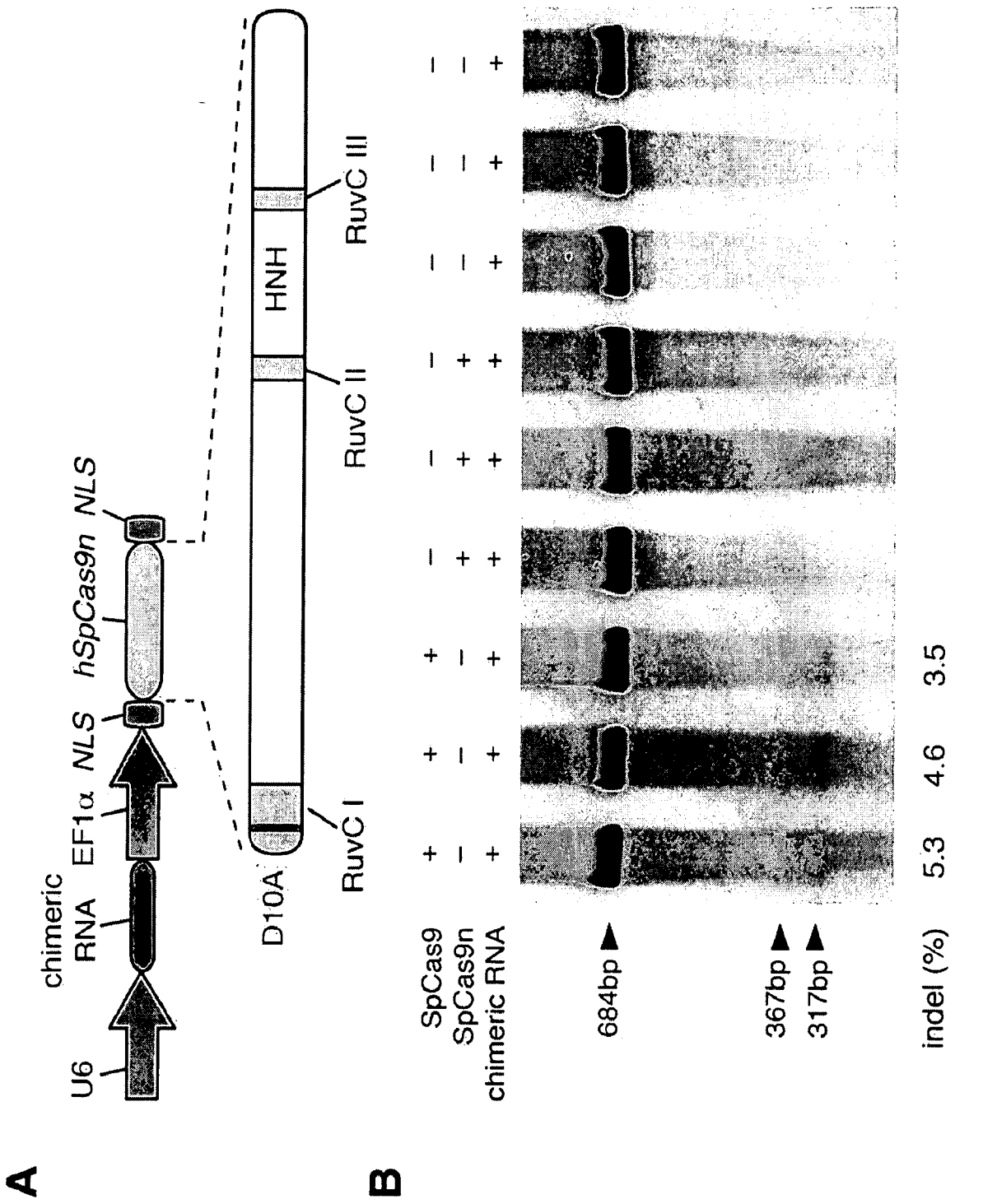

A
U6
chimeric RNA
EF1α
NLS
hSpCas9n
NLS
D10A
RuvC I
RuvC II
HNH
RuvC III
B
SpCas9
SpCas9n
chimeric RNA
684bp
367bp
317bp
indel (%)
5.3
4.6
3.5

FIG. 4A-B

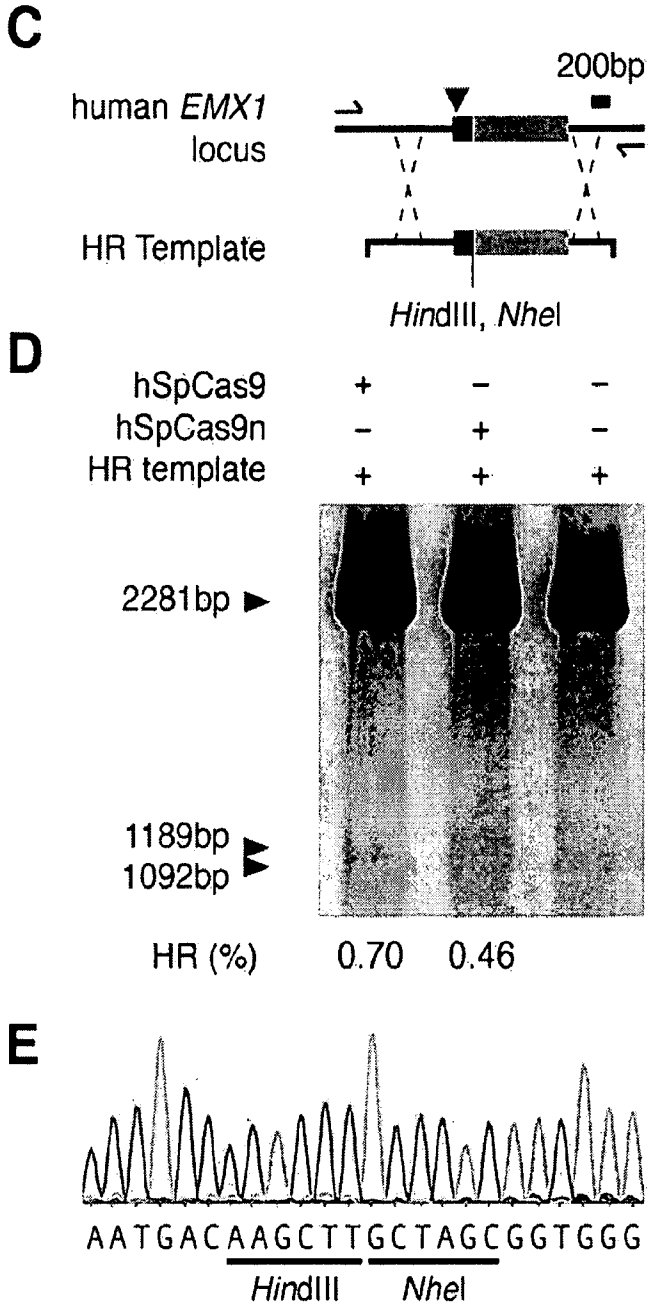

C
200bp
human EMX1
locus
HR Template
HindIII, NheI
D
hSpCas9
hSpCas9n
HR template
+ − −
− + −
+ + +
2281bp
1189bp
1092bp
HR (%)
0.70 0.46
E
AATGACAAGCTTGCTAGCGGTGGG
HindIII
NheI

FIG. 4C-E

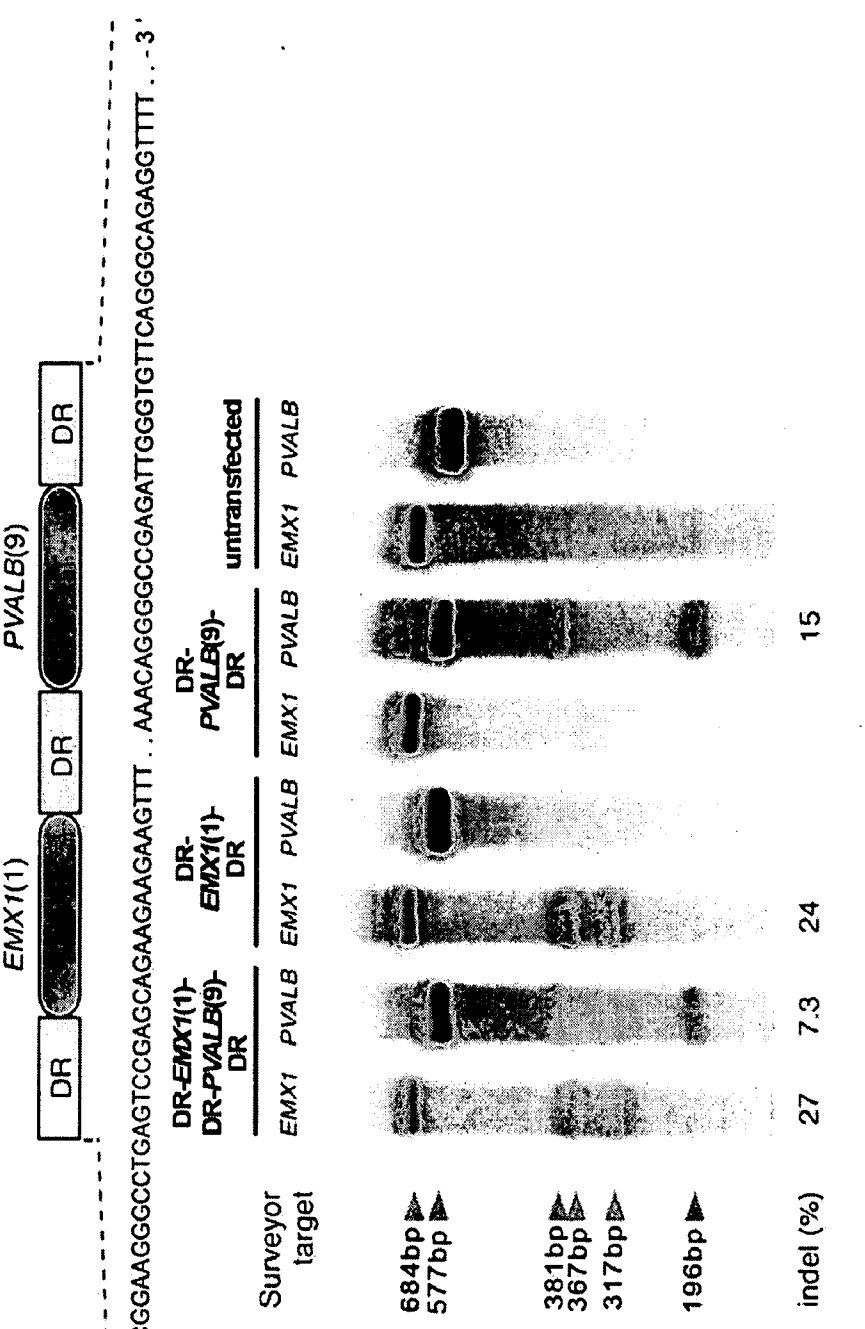

F
crRNA array design
EMX1(1)
PVALB(9)
DR
DR
DR
DR
5' - . . AAAACGGAAGGGCCTGAGTCCGAGCAGAAGAAGAAGTTT . . AAACAGGGGCCGAGATTGGGTGTTCAGGGCAGAGGTTTT . . - 3'
DR-EMX1(1)-DR-PVALB(9)-DR
DR-EMX1(1)-DR
DR-PVALB(9)-DR
untransfected
EMX1 PVALB
EMX1 PVALB
EMX1 PVALB
EMX1 PVALB
Surveyor target
684bp
577bp
381bp
367bp
317bp
196bp
indel (%)
27
7.3
24
15

FIG. 4F

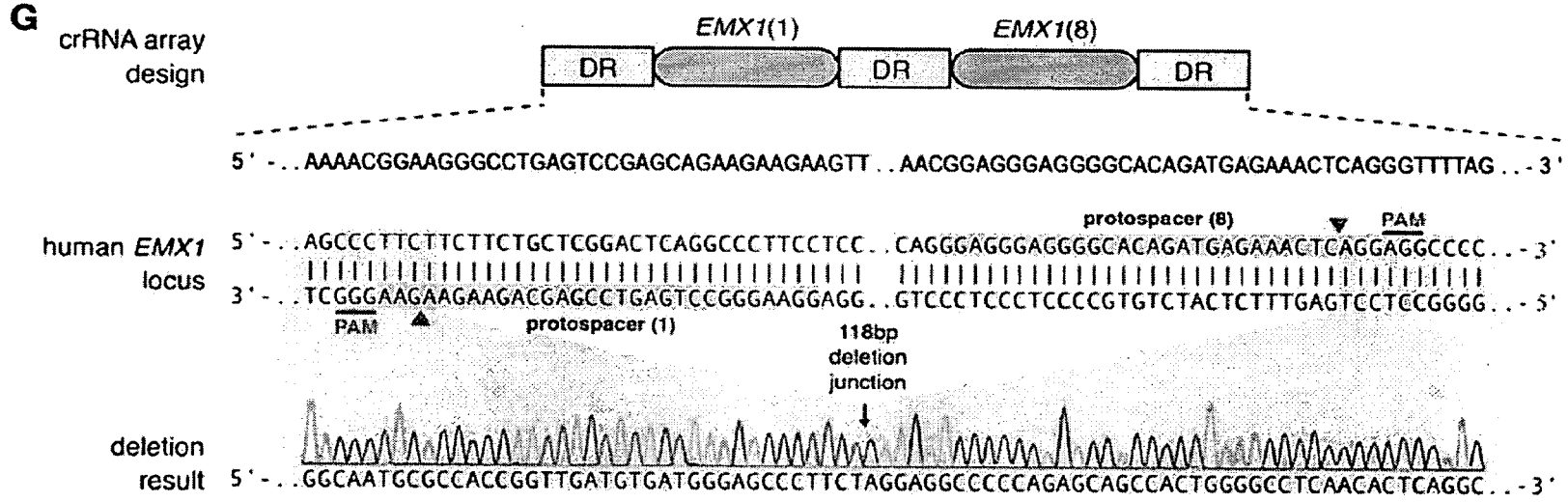

G
crRNA array design
EMX1(1)
EMX1(8)
DR
DR
DR
5' - . . AAAACGGAAGGGCCTGAGTCCGAGCAGAAGAAGAAGTT . . AACGGAGGGAGGGGCACAGATGAGAAACTCAGGGTTTTAG . . . 3'
human EMX1 locus
protospacer (8)
PAM
5' - . . AGCCCTTCTTCTTCTGCTCGGACTCAGGCCCTTCCTCC . . CAGGGAGGGAGGGGCACAGATGAGAAACTCAGGAGGCCCC . . . 3'
3' - . . TCGGGAAGAAGAAGACGAGCCTGAGTCCGGGAAGGAGG . . GTCCCTCCCTCCCCGTGTCTACTCTTTGAGTCCTCCGGGG . . . 5'
PAM
protospacer (1)
118bp deletion junction
deletion result
5' - . . GGCAATGCGCCACCGGTTGATGTGATGGGAGCCCTTCTAGGAGGCCCCCAGAGCAGCCACTGGGGCCTCAACACTCAGGC . . . 3'

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) |
|---|---|---|---|---|---|---|---|---|---|
| *S. pyogenes SF370 type II CRISPR* | *Homo sapiens* | *EMX1* | 1 | GGAAGGGCCTGAGTCCGAGCAGAAGAAGAA | GGG | + | 293FT | 20 ± 1.8 | 6.7 ± 0.62 |
| | | *EMX1* | 2 | CATTGGAGGTGACATCGATGTCCTCCCCAT | TGG | – | 293FT | 2.1 ± 0.31 | N.D. |
| | | *EMX1* | 3 | GGACATCGATGTCACCTCCAATGACTAGGG | TGG | + | 293FT | 14 ± 1.1 | N.D. |
| | | *EMX1* | 4 | CATCGATGTCCTCCCCATTGGCCTGCTTCG | TGG | – | 293FT | 11 ± 1.7 | N.D. |
| | | *EMX1* | 5 | TTCGTGGCAATGCGCCACCGGTTGATGTGA | TGG | – | 293FT | 4.3 ± 0.46 | 2.1 ± 0.51 |
| | | *EMX1* | 6 | TCGTGGCAATGCGCCACCGGTTGATGTGAT | GGG | – | 293FT | 4.0 ± 0.66 | 0.41 ± 0.25 |
| | | *EMX1* | 7 | TCCAGCTTCTGCCGTTTGTACTTTGTCCTC | CGG | – | 293FT | 1.5 ± 0.12 | N.D. |
| | | *EMX1* | 8 | GGAGGGAGGGGCACAGATGAGAAACTCAGG | AGG | – | 293FT | 7.8 ± 0.83 | 2.3 ± 1.2 |
| | *Homo sapiens* | *PVALB* | 9 | AGGGGCCGAGATTGGGTGTTCAGGGCAGAG | AGG | + | 293FT | 21 ± 2.6 | 6.5 ± 0.32 |
| | | *PVALB* | 10 | ATGCAGGAGGGTGGCGAGAGGGGCCGAGAT | TGG | + | 293FT | N.D. | N.D. |
| | | *PVALB* | 11 | GGTGGCGAGAGGGGCCGAGATTGGGTGTTC | AGG | + | 293FT | N.D. | N.D. |
| | *Mus musculus* | *Th* | 12 | CAAGCACTGAGTGCCATTAGCTAAATGCAT | AGG | – | Neuro2A | 27 ± 4.3 | 4.1 ± 2.2 |
| | | *Th* | 13 | AATGCATAGGGTACCACCCACAGGTGCCAG | GGG | – | Neuro2A | 4.8 ± 1.2 | N.D. |
| | | *Th* | 14 | ACACACATGGGAAAGCCTCTGGGCCAGGAA | AGG | + | Neuro2A | 11.3 ± 1.3 | N.D. |
| *S. thermophilus LMD-9 CRISPR1* | *Homo sapiens* | *EMX1* | 15 | GGAGGAGGTAGTATACAGAAACACAGAGAA | GTAGAAT | – | 293FT | 14 ± 0.88 | N.T. |
| | | *EMX1* | 16 | AGAATGTAGAGGAGTCACAGAAACTCAGCA | CTAGAAA | – | 293FT | 7.8 ± 0.77 | N.T. |

**FIG. 5**

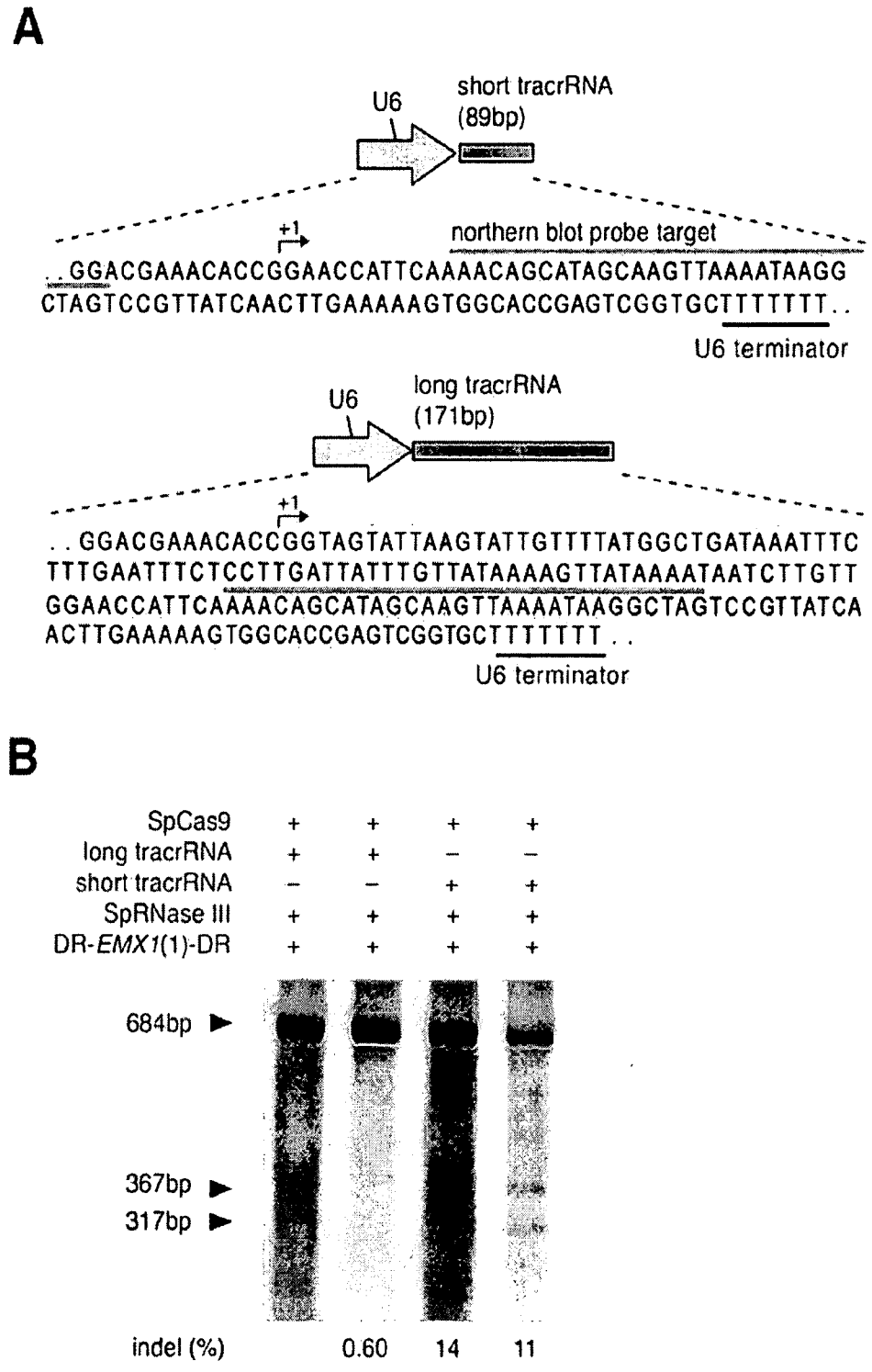

A
U6
short tracrRNA
(89bp)
+1
northern blot probe target
..GGACGAAACACCGGAACCATTCAAAACAGCATAGCAAGTTAAAATAAGG
CTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTT..
U6 terminator
U6
long tracrRNA
(171bp)
+1
..GGACGAAACACCGGTAGTATTAAGTATTGTTTTATGGCTGATAAATTTC
TTTGAATTTCTCCTTGATTATTTGTTATAAAAGTTATAAAATAATCTTGTT
GGAACCATTCAAAACAGCATAGCAAGTTAAAATAAGGCTAGTCCGTTATCA
ACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTT..
U6 terminator
B
SpCas9 + + + +
long tracrRNA + + – –
short tracrRNA – – + +
SpRNase III + + + +
DR-EMX1(1)-DR + + + +
684bp
367bp
317bp
indel (%) 0.60 14 11

**FIG. 6A-B**

C

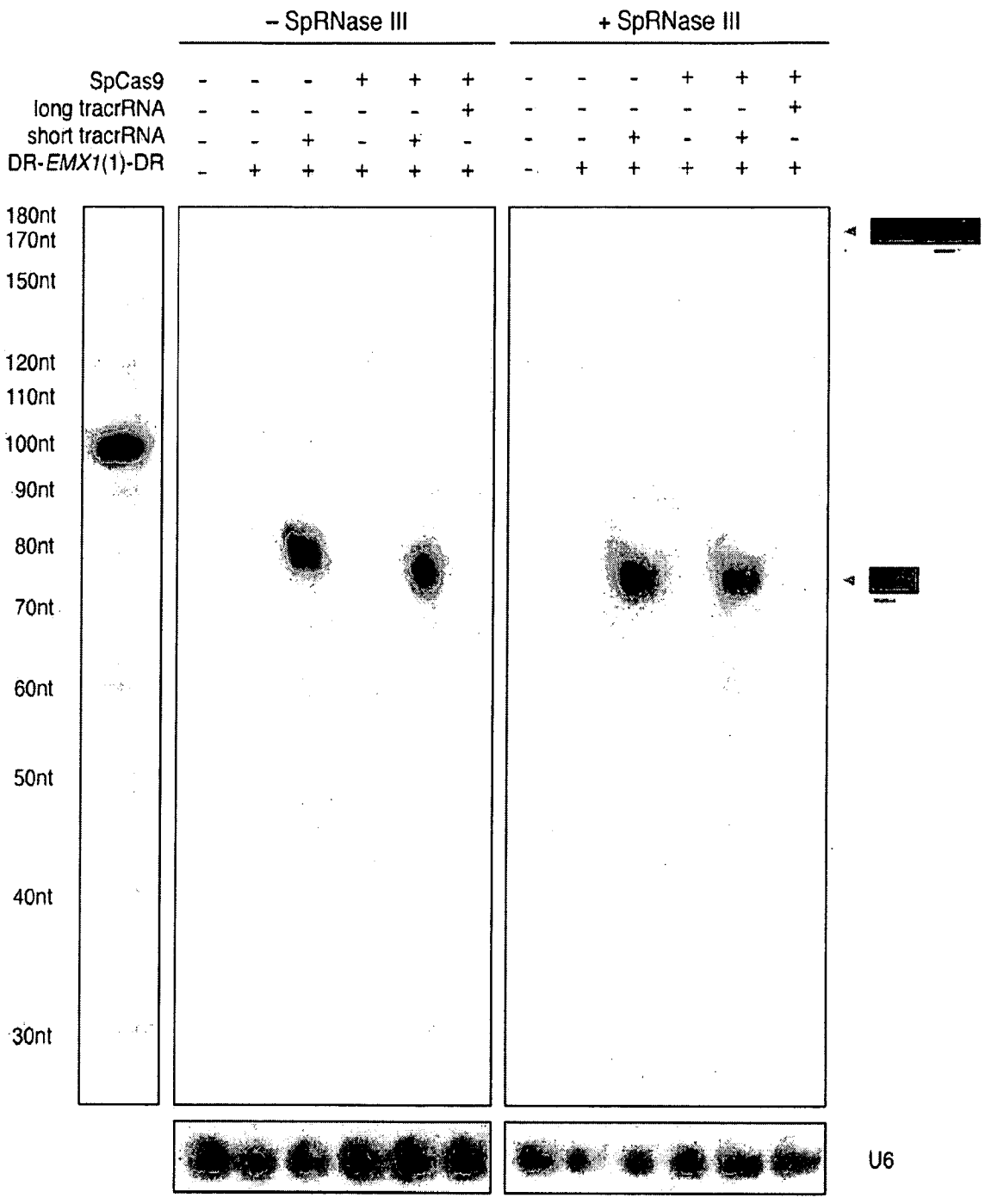
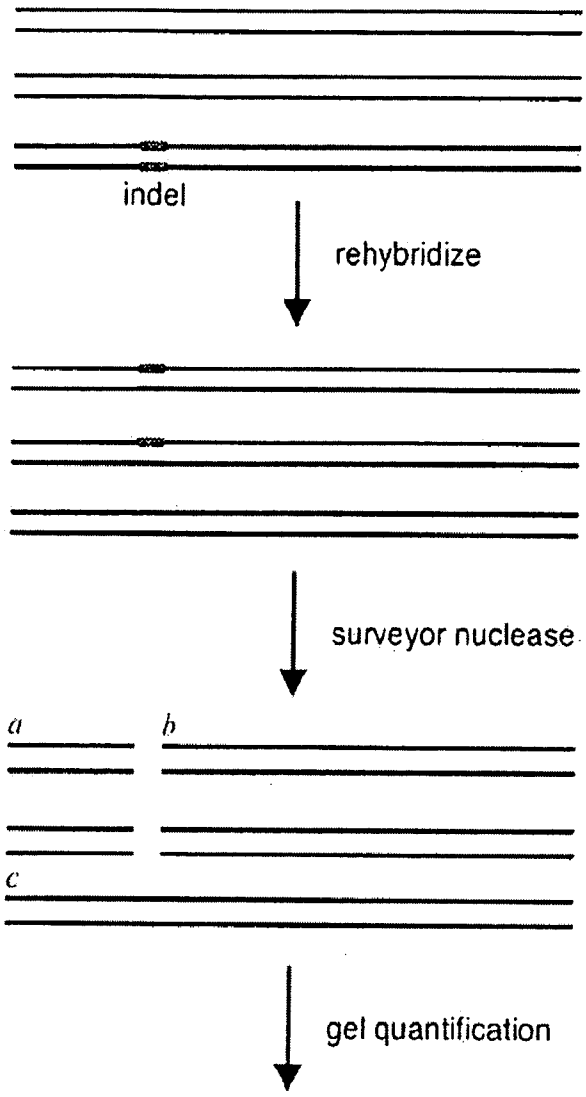

– SpRNase III
+ SpRNase III
SpCas9
long tracrRNA
short tracrRNA
DR-EMX1(1)-DR
180nt
170nt
150nt
120nt
110nt
100nt
90nt
80nt
70nt
60nt
50nt
40nt
30nt
U6

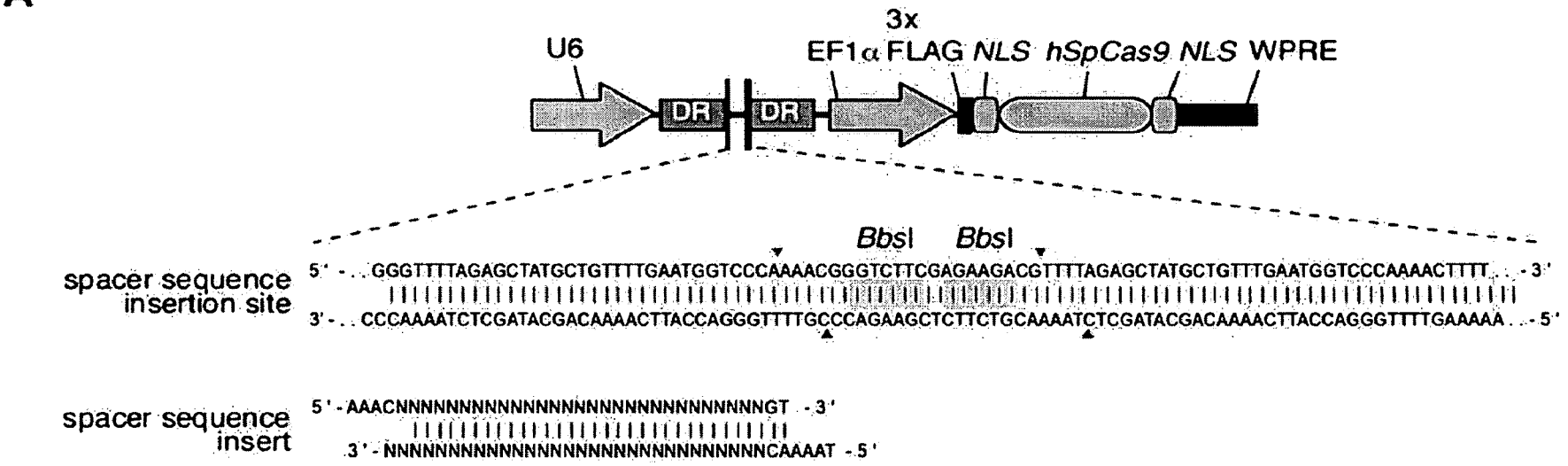
FIG. 6C




$$\% \text{ indel} = \left(1 - \sqrt{1 - (a + b)/(a + b + c)}\right) * 100$$

FIG. 7

A


3x
U6
EF1α FLAG NLS hSpCas9 NLS WPRE
DR
DR
BbsI
BbsI
spacer sequence insertion site
5' - . . GGGTTTTAGAGCTATGCTGTTTTGAATGGTCCCAAAACGGGTCTTCGAGAAGACGTTTTAGAGCTATGCTGTTTGAATGGTCCCAAAACTTTT . . - 3'
3' - . . CCCAAAATCTCGATACGACAAAACTTACCAGGGTTTTGCCCAGAAGCTCTTCTGCAAAATCTCGATACGACAAAACTTACCAGGGTTTTGAAAAA . . - 5'
spacer sequence insert
5' - AAACNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNGT - 3'
3' - NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNCAAAAT - 5'

B

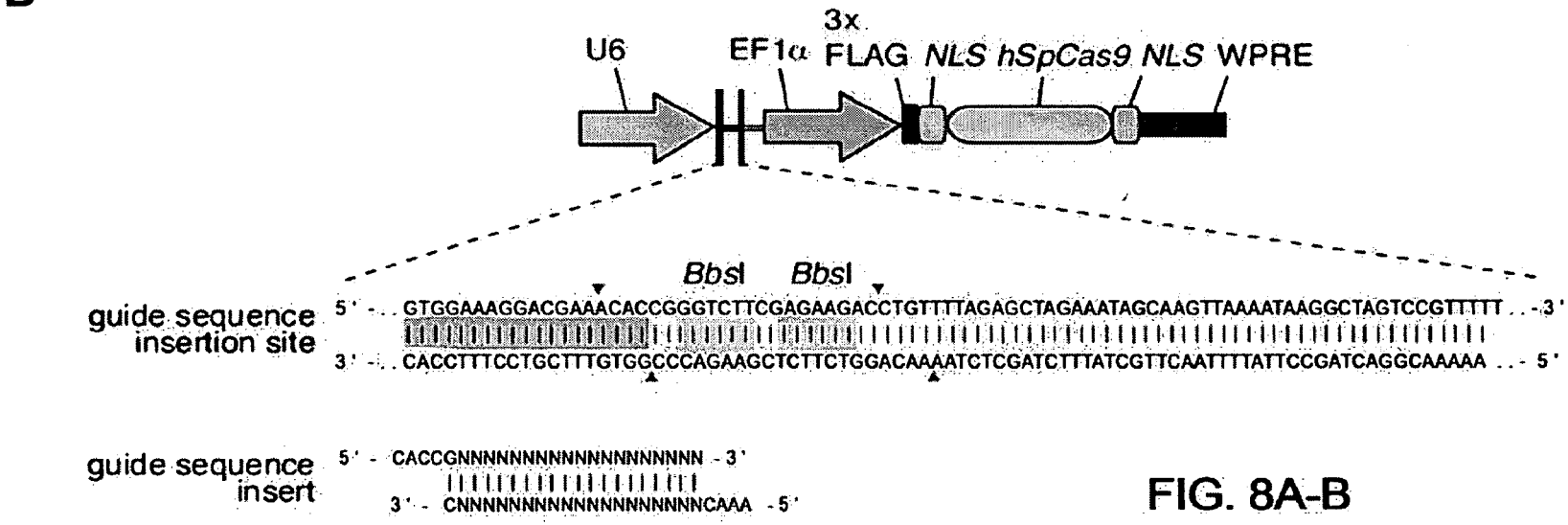
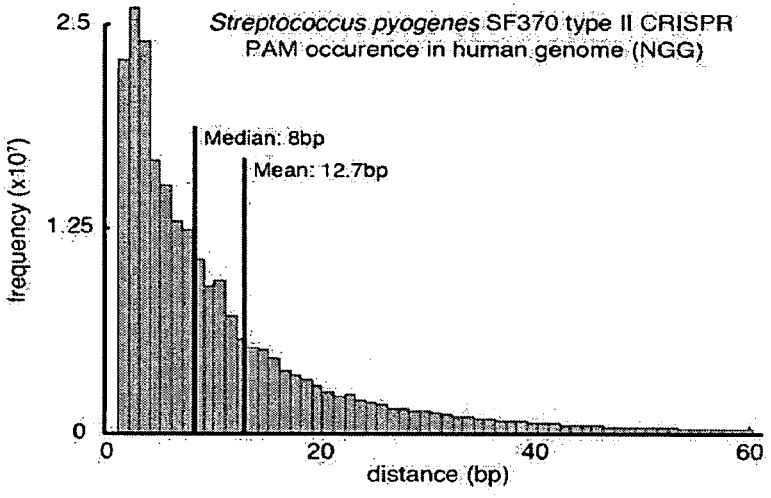
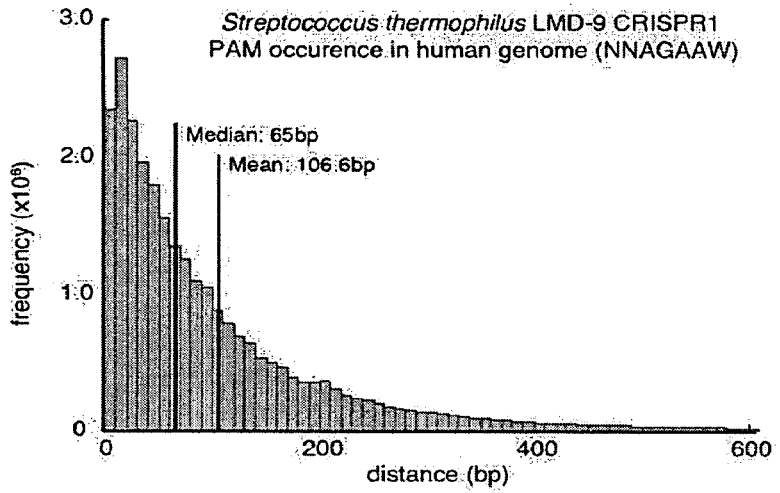

3x
U6
EF1α FLAG NLS hSpCas9 NLS WPRE
BbsI
BbsI
guide sequence insertion site
5' - . . GTGGAAAGGACGAAACACCGGGTCTTCGAGAAGACCTGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTTTT . . - 3'
3' - . . CACCTTTCCTGCTTTGTGGCCCAGAAGCTCTTCTGGACAAAATCTCGATCTTTATCGTTCAATTTTATTCCGATCAGGCAAAAA . . - 5'
guide sequence insert
5' - CACCGNNNNNNNNNNNNNNNNNN - 3'
3' - CNNNNNNNNNNNNNNNNNNNCAAA - 5'

FIG. 8A-B

A




B




C

| | NGG | | NNAGAAW | |
|---|---|---|---|---|
| Chr | 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

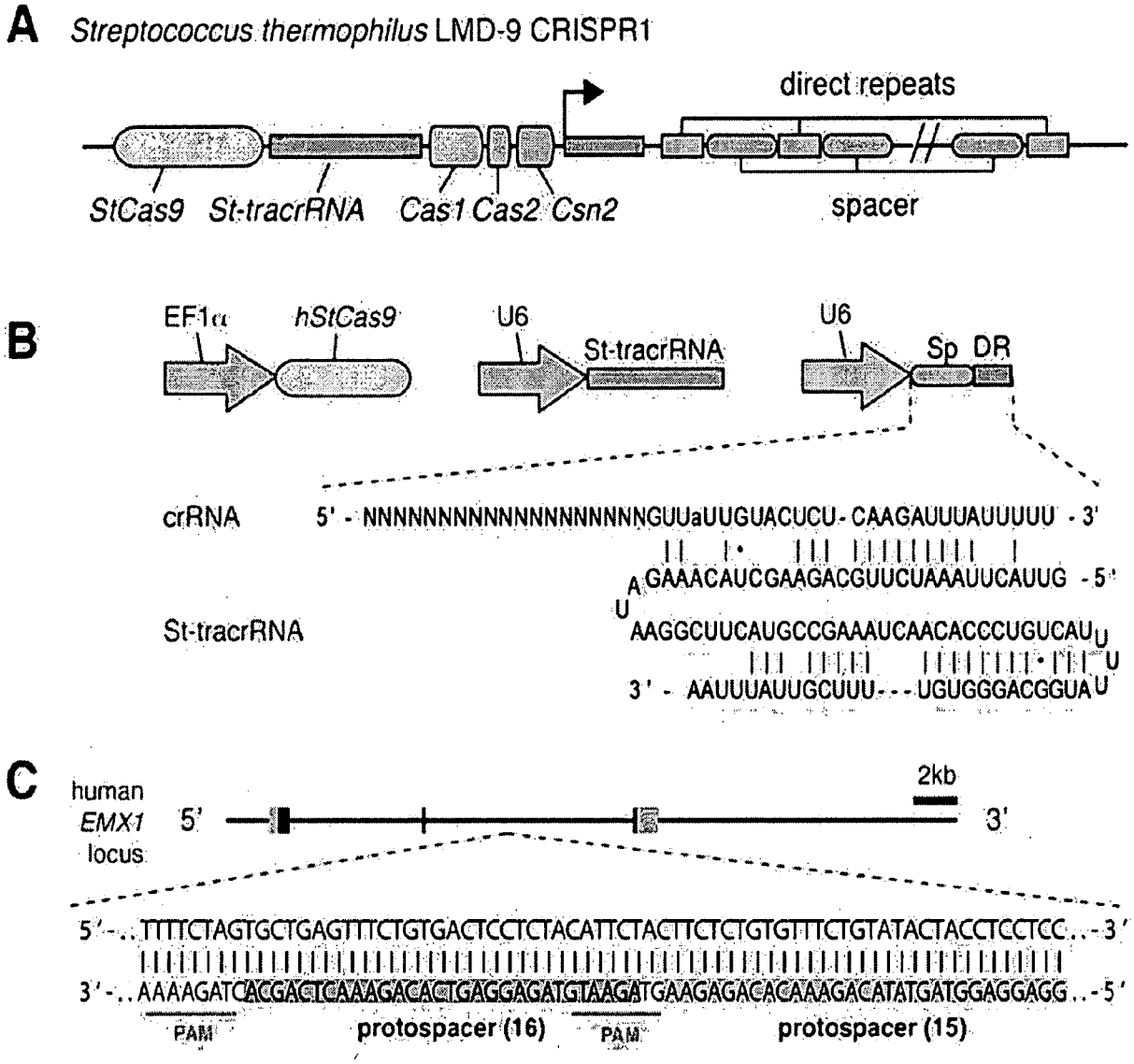

A
Streptococcus thermophilus LMD-9 CRISPR1
direct repeats
StCas9
St-tracrRNA
Cas1
Cas2
Csn2
spacer
B
EF1α
hStCas9
U6
St-tracrRNA
U6
Sp
DR
crRNA
5' - NNNNNNNNNNNNNNNNNNGUUaUUGUACUCU- CAAGAUUUAUUUUU - 3'
GAAACAUCGAAGACGUUCUAAAUUCAUUG - 5'
St-tracrRNA
AAGGCUUCAUGCCGAAAUCAACACCCUGUCAU
3' - AAUUUAUUGCUUU- - - UGUGGGACGGUA
C
human
EMX1
locus
5'
3'
2kb
5'-..TTTTCTAGTGCTGAGTTTCTGTGACTCCTCTACATTCTACTTCTCTGTGTTTCTGTATACTACCTCCTCC..-3'
3'-..AAAAGATCACGACTCAAAGACACTGAGGAGATGTAAGATGAAGAGACACAAAGACATATGATGGAGGAGG..-5'
PAM
protospacer (16)
PAM
protospacer (15)

FIG. 10A-C

D

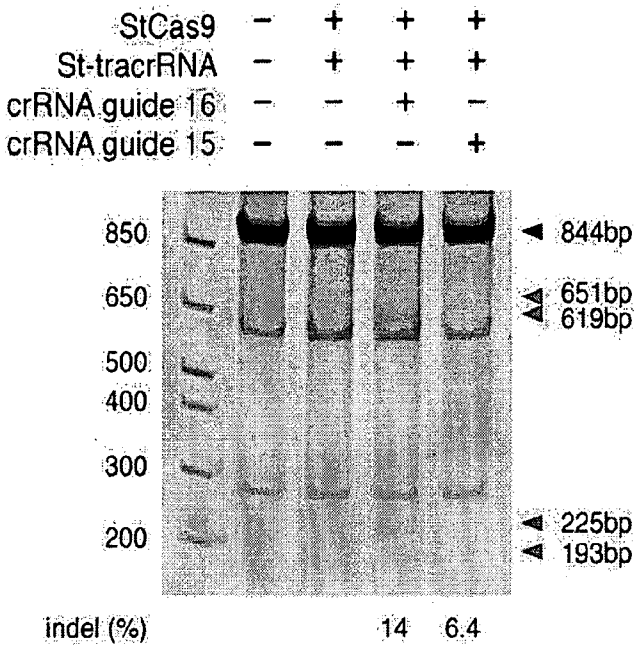

StCas9 − + + +
St-tracrRNA − + + +
crRNA guide 16 − − + −
crRNA guide 15 − − − +
850
650
500
400
300
200
844bp
651bp
619bp
225bp
193bp
indel (%)
14
6.4

FIG. 10D

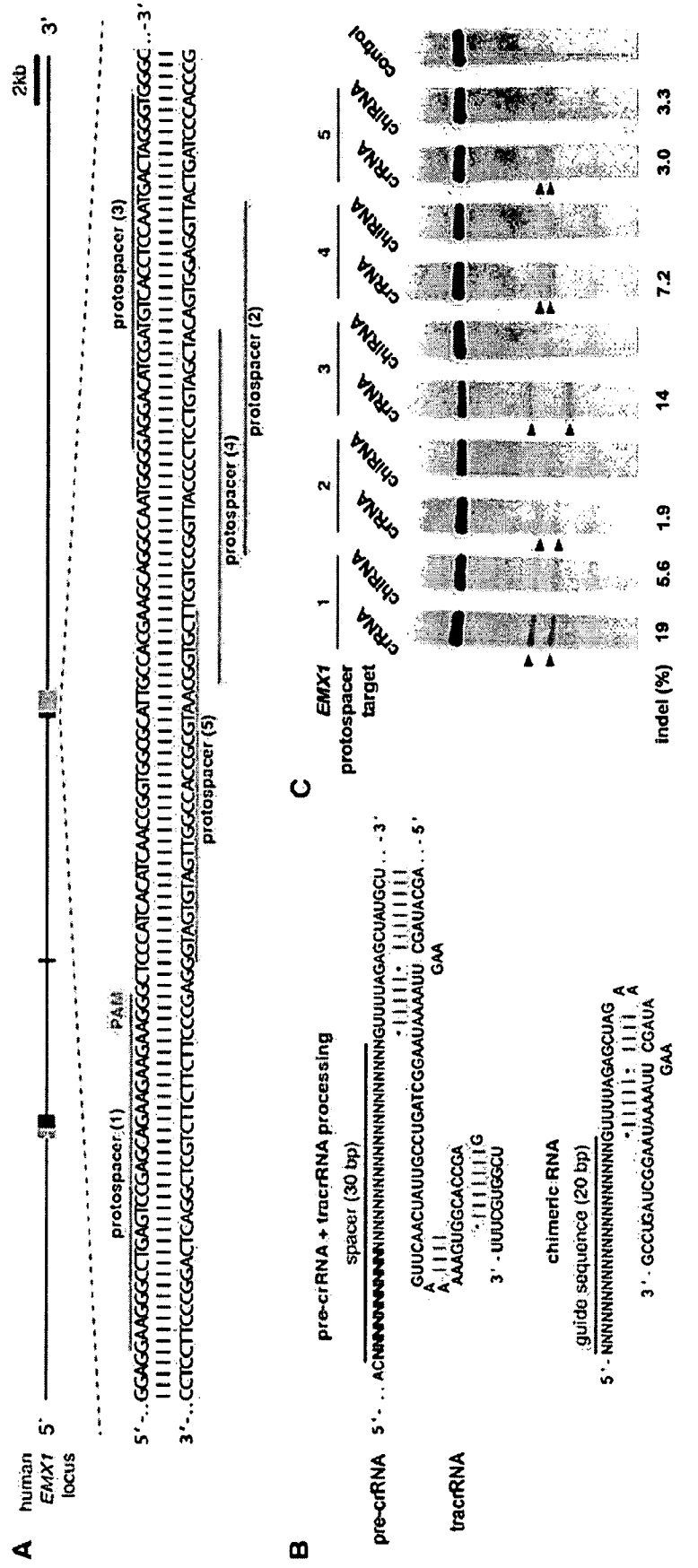

A
human EMX1 locus
2kb
5'
3'
protospacer (1)
PAM
protospacer (3)
protospacer (4)
protospacer (5)
protospacer (2)
B
pre-crRNA
tracrRNA
pre-crRNA + tracrRNA processing
spacer (30 bp)
chimeric RNA
guide sequence (20 bp)
C
EMX1 protospacer target
crRNA
chiRNA
Control
indel (%)
19
5.6
1.9
14
7.2
3.0
3.3

FIG. 11A-C

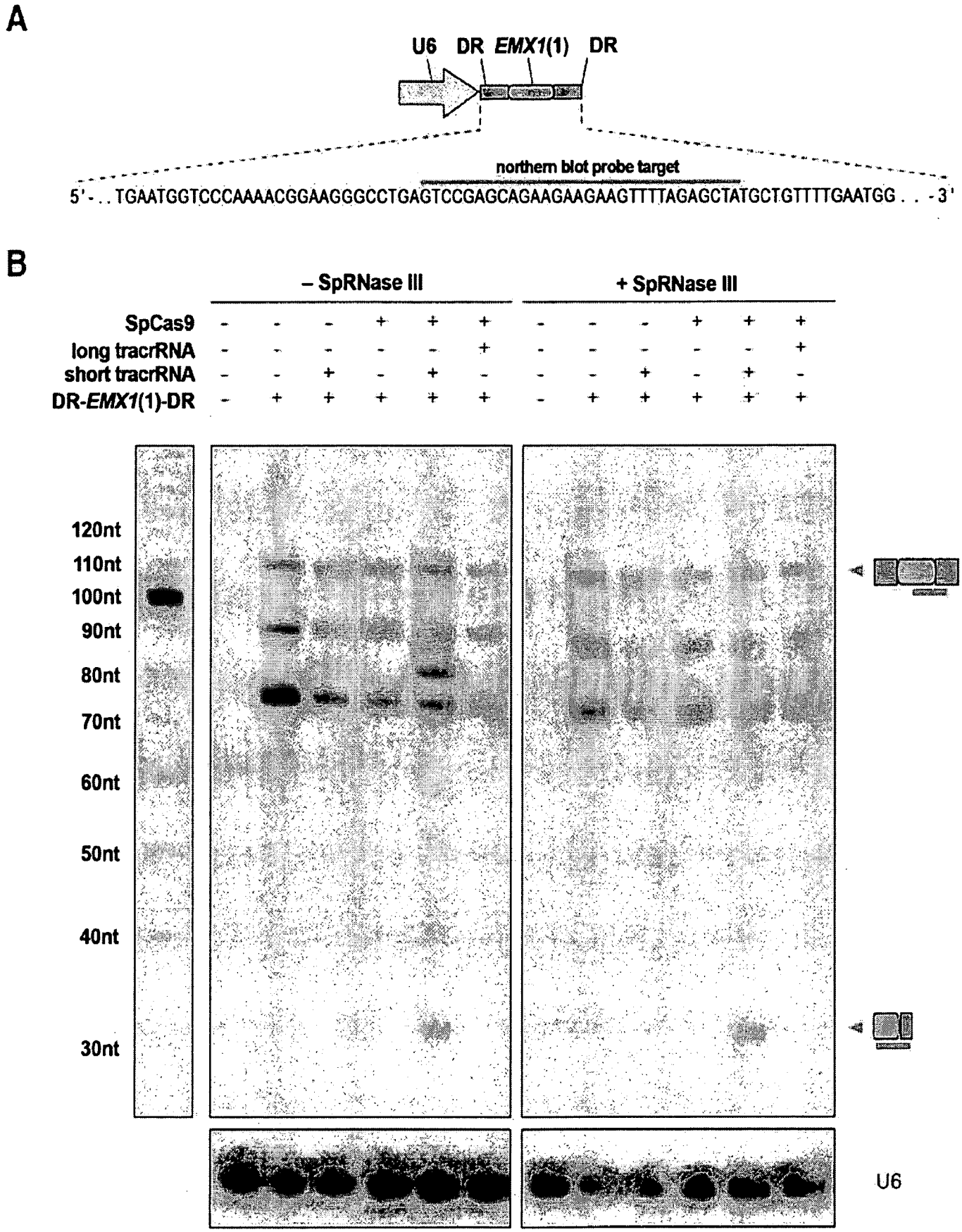

A
U6
DR
EMX1(1)
DR
northern blot probe target
5'- . . TGAATGGTCCCAAAACGGAAGGGCCTGAGTCCGAGCAGAAGAAGAAGTTTTAGAGCTATGCTGTTTTGAATGG . . - 3'
B
– SpRNase III
+ SpRNase III
SpCas9
long tracrRNA
short tracrRNA
DR-EMX1(1)-DR
120nt
110nt
100nt
90nt
80nt
70nt
60nt
50nt
40nt
30nt
U6

FIG. 12A-B

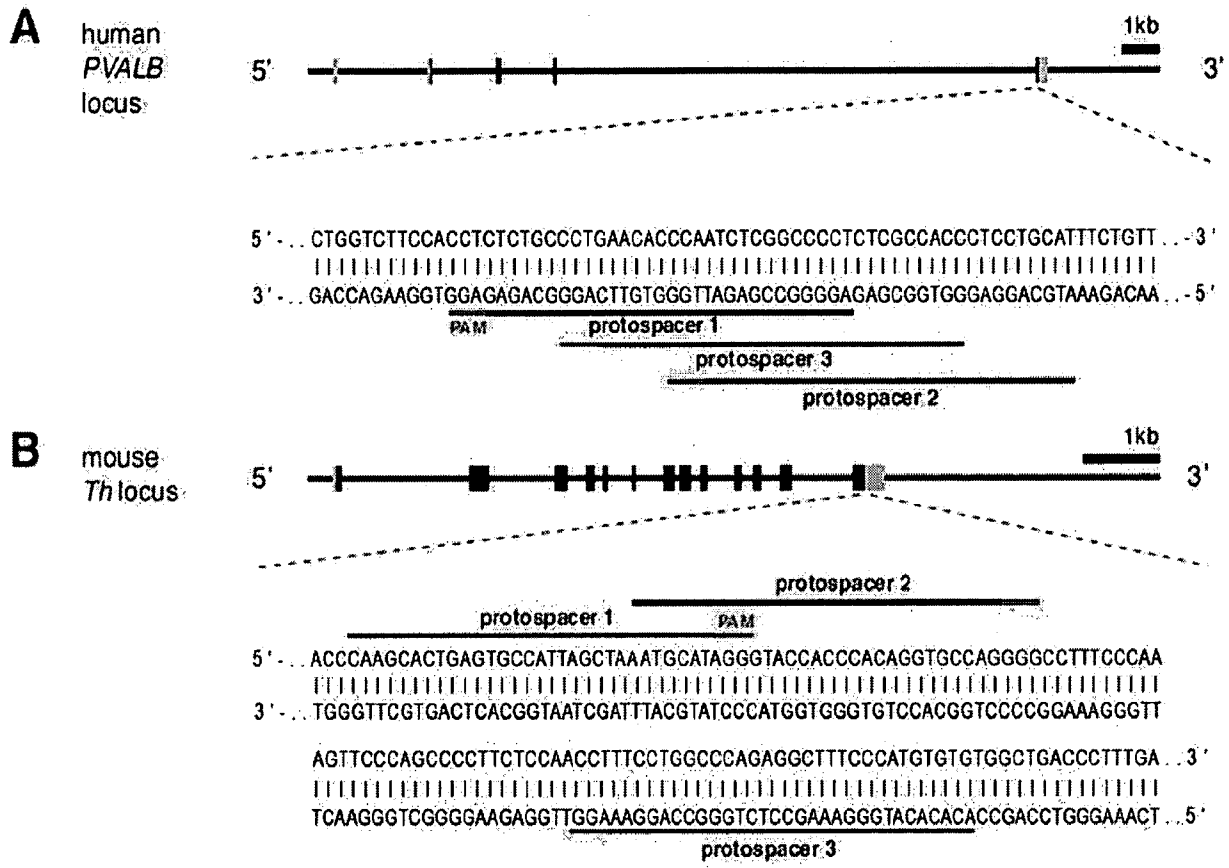

A
human
PVALB
locus
5'
1kb
3'
5'-...CTGGTCTTCCACCTCTCTGCCCTGAACACCCAATCTCGGCCCCTCTCGCCACCCTCCTGCATTTCTGTT...-3'
3'-...GACCAGAAGGTGGAGAGACGGGACTTGTGGGTTAGAGCCGGGGAGAGCGGTGGGAGGACGTAAAGACAA...-5'
PAM
protospacer 1
protospacer 3
protospacer 2
B
mouse
Th locus
5'
1kb
3'
protospacer 2
protospacer 1
PAM
5'-...ACCCAAGCACTGAGTGCCATTAGCTAAATGCATAGGGTACCACCCACAGGTGCCAGGGGCCTTTCCCAA
3'-...TGGGTTCGTGACTCACGGTAATCGATTTACGTATCCCATGGTGGGTGTCCACGGTCCCCGGAAAGGGTT
AGTTCCCAGCCCCTTCTCCAACCTTTCCTGGCCCAGAGGCTTTCCCATGTGTGTGGCTGACCCTTTGA...3'
TCAAGGGTCGGGGAAGAGGTTGGAAAGGACCGGGTCTCCGAAAGGGTACACACACCGACCTGGGAAACT...5'
protospacer 3

FIG. 13A-B

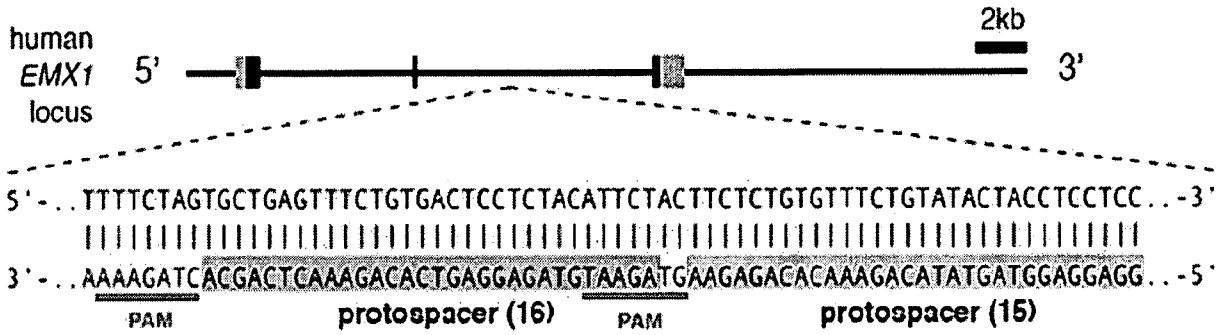

human
EMX1
locus
5'
2kb
3'
5'-..TTTTCTAGTGCTGAGTTTCTGTGACTCCTCTACATTCTACTTCTCTGTGTTTCTGTATACTACCTCCTCC..-3'
3'-..AAAAGATCACGACTCAAAGACACTGAGGAGATGTAAGATGAAGAGACACAAAGACATATGATGGAGGAGG..-5'
PAM
protospacer (16)
PAM
protospacer (15)

FIG. 14

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

FIG. 15

**a**

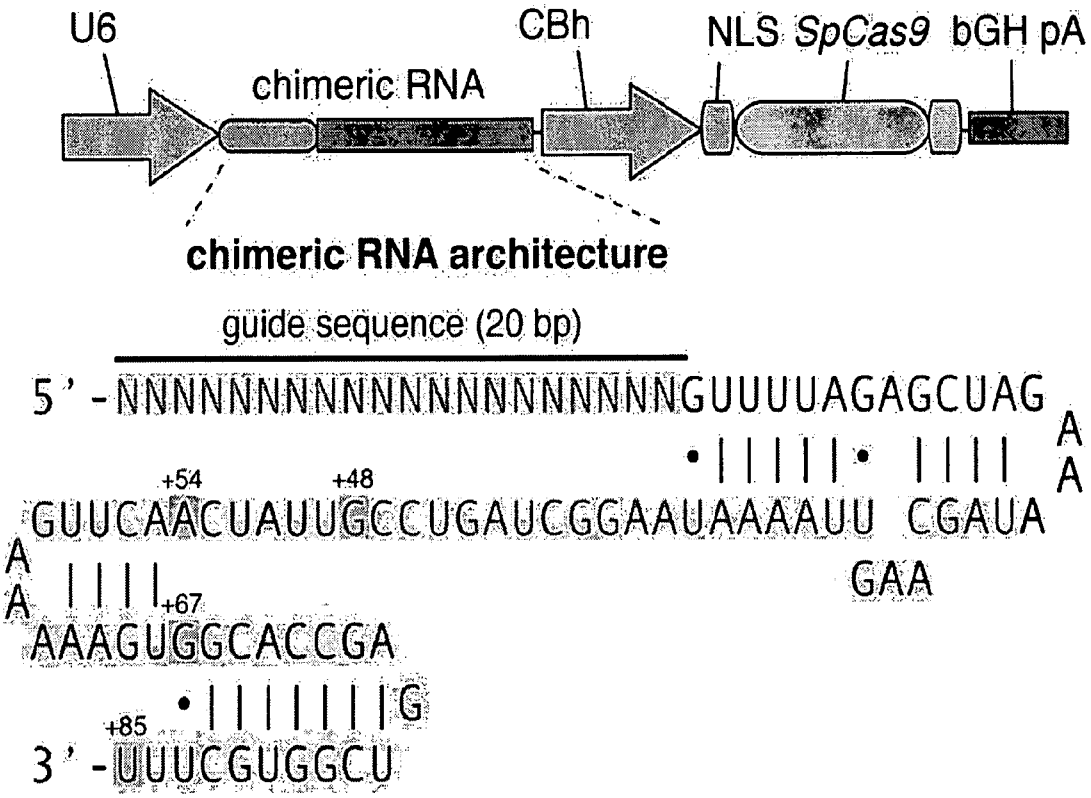

U6
chimeric RNA
CBh
NLS SpCas9 bGH pA
chimeric RNA architecture
guide sequence (20 bp)
5'-NNNNNNNNNNNNNNNNNNNNGUUUUAGAGCUAG
A
A
+54
+48
GUUCAACUAUUGCCUGAUCGGAAUAAAAUU CGAUA
A
A
GAA
+67
AAAGUGGCACCGA
G
+85
3'-UUUCGUGGCU

FIG. 16A

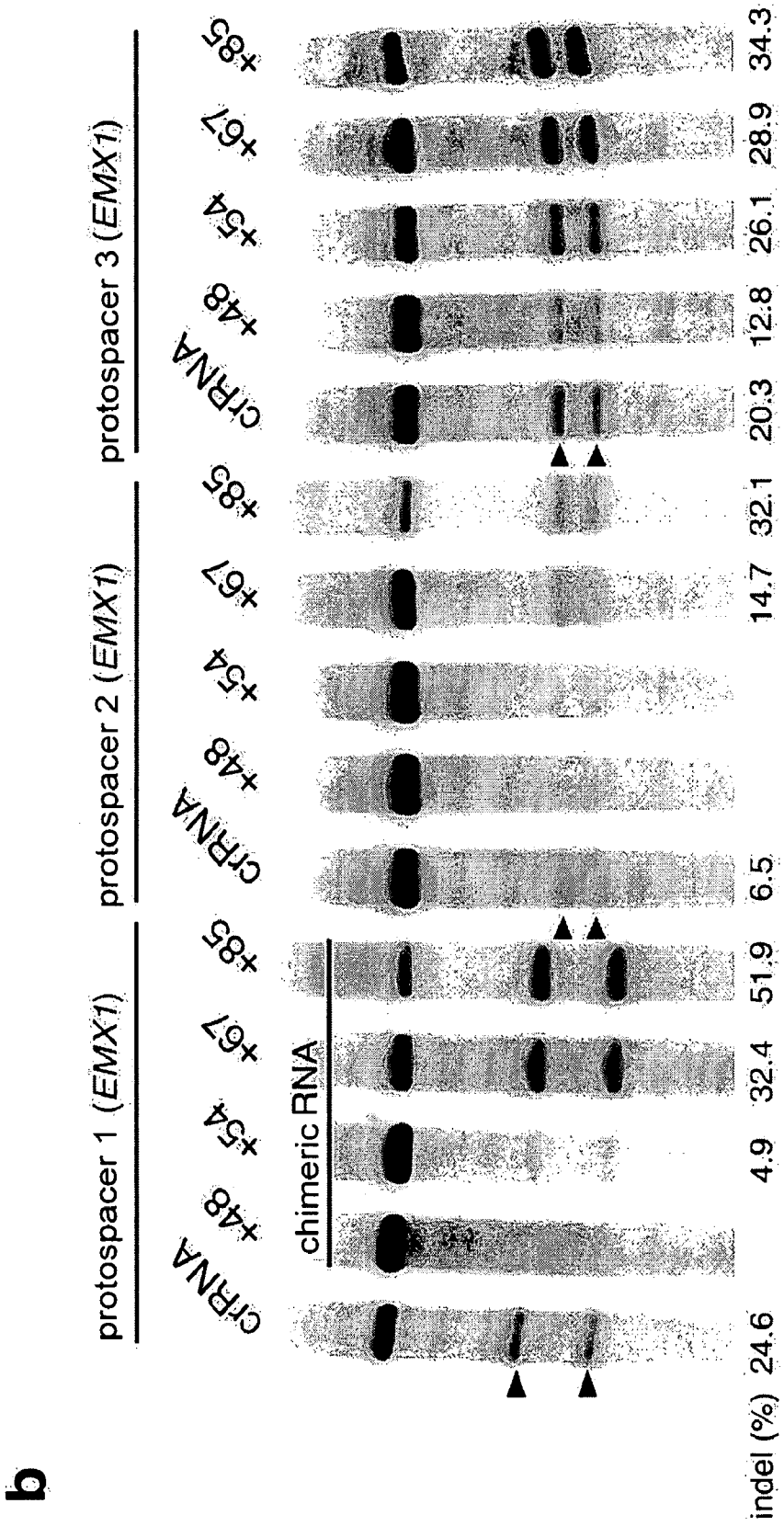

b
protospacer 1 (EMX1)
protospacer 2 (EMX1)
protospacer 3 (EMX1)
crRNA
+48
+54
+67
+85
chimeric RNA
indel (%)
24.6
4.9
32.4
51.9
6.5
14.7
32.1
20.3
12.8
26.1
28.9
34.3

FIG. 16B

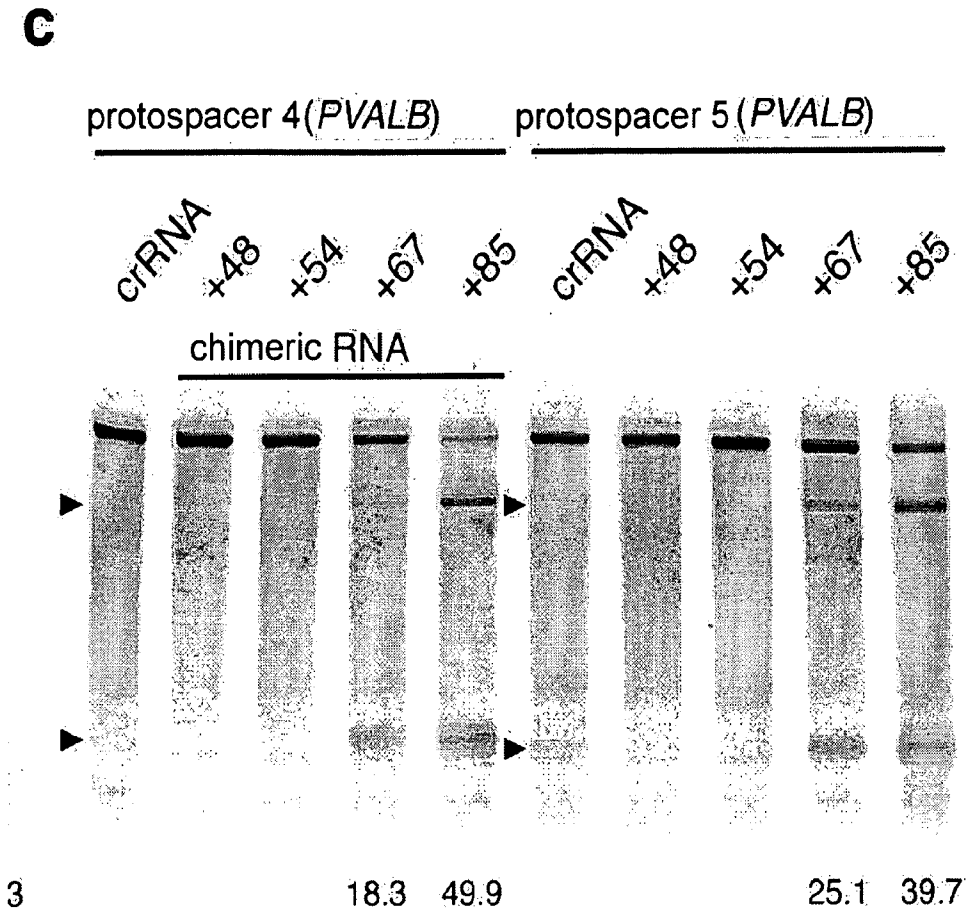
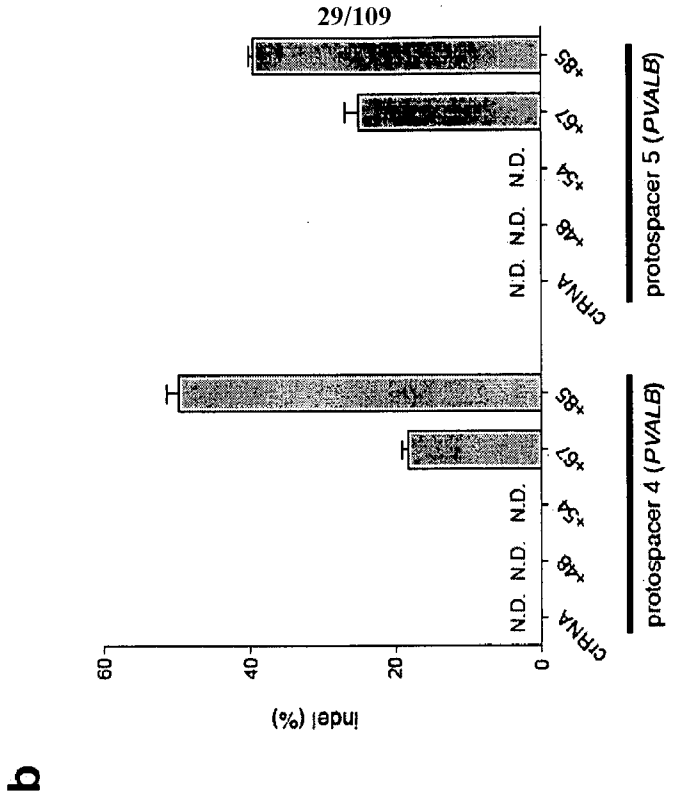

C
protospacer 4 (PVALB)
protospacer 5 (PVALB)
crRNA
+48
+54
+67
+85
crRNA
+48
+54
+67
+85
chimeric RNA
3
18.3
49.9
25.1
39.7

FIG. 16C


b
Indel (%)
0
20
40
60
protospacer 4 (PVALB)
protospacer 5 (PVALB)
crRNA
*A8
*S4
*G7
*8S
N.D.

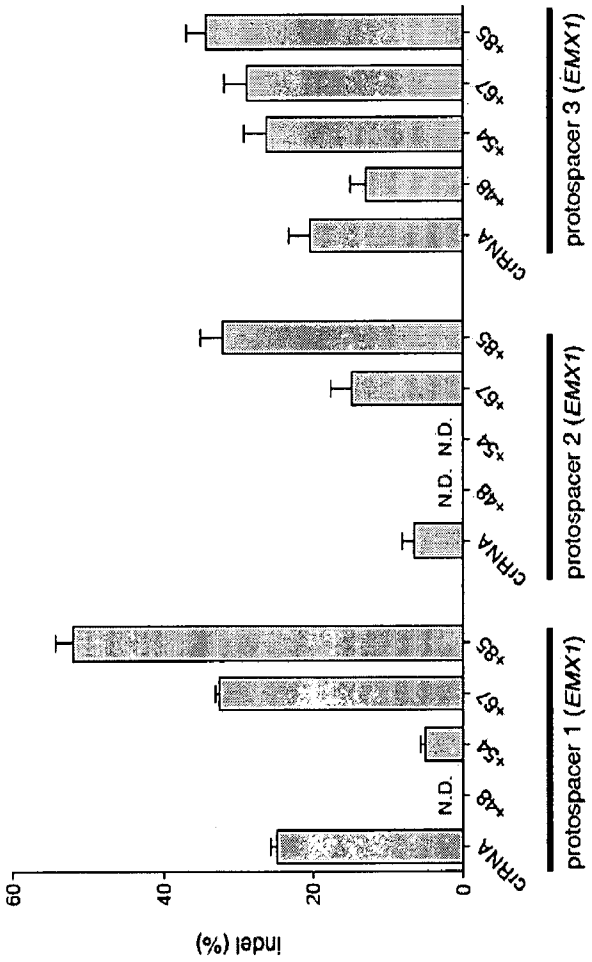

a
Indel (%)
0
20
40
60
protospacer 1 (EMX1)
protospacer 2 (EMX1)
protospacer 3 (EMX1)
crRNA
*A8
*S4
*G7
*8S
N.D.

FIG. 17A-B

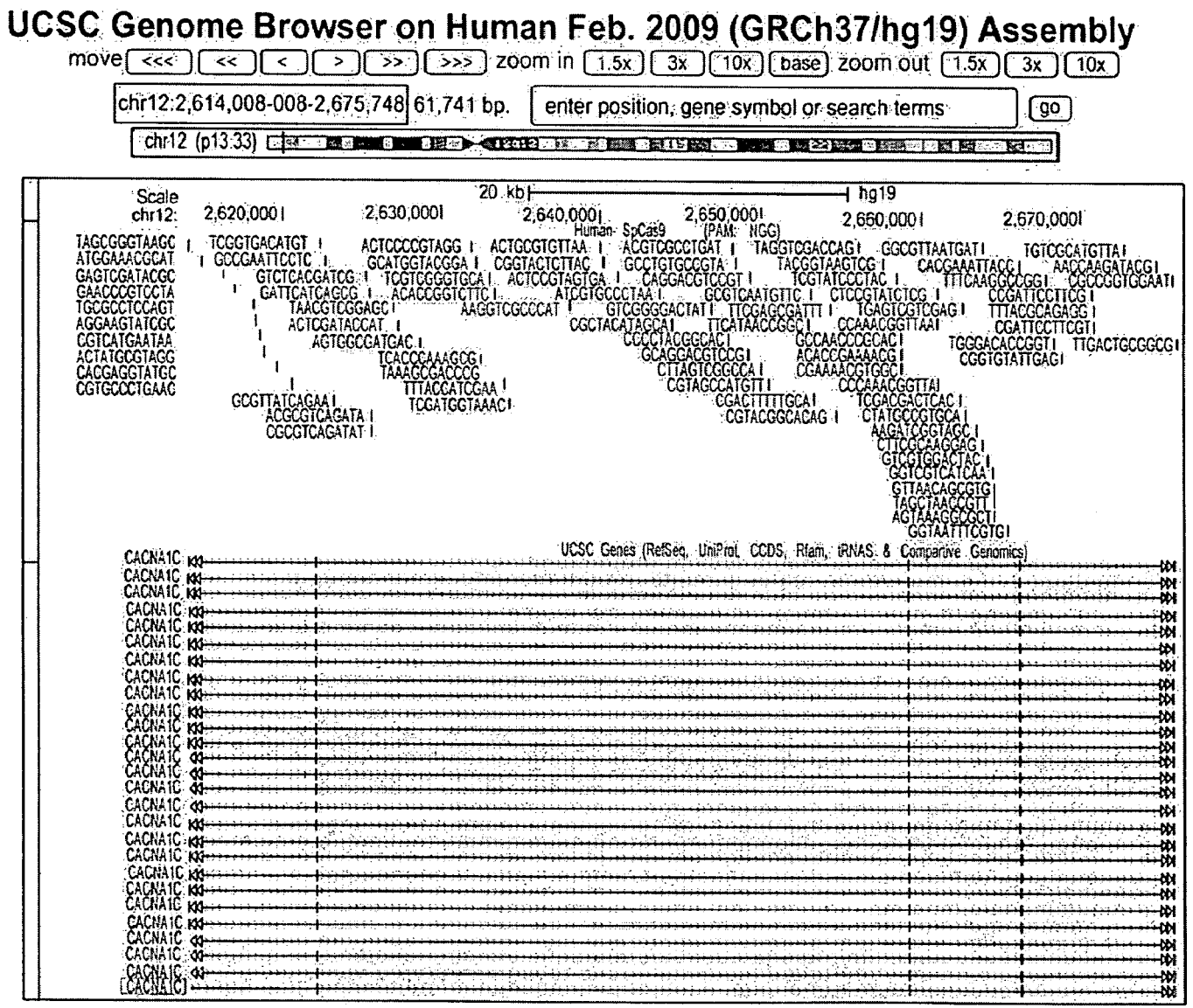

UCSC Genome Browser on Human Feb. 2009 (GRCh37/hg19) Assembly
move <<< << < > >> >>> zoom in 1.5x 3x 10x base zoom out 1.5x 3x 10x
chr12:2,614,008-008-2,675,748 61,741 bp.
enter position, gene symbol or search terms
go
chr12 (p13.33)
Scale
20 kb
hg19
chr12:
2,620,000
2,630,000
2,640,000
2,650,000
2,660,000
2,670,000
Human SpCas9 (PAM: NGG)
UCSC Genes (RefSeq, UniProt, CCDS, Rfam, tRNAs & Comparative Genomics)
CACNA1C

FIG. 18

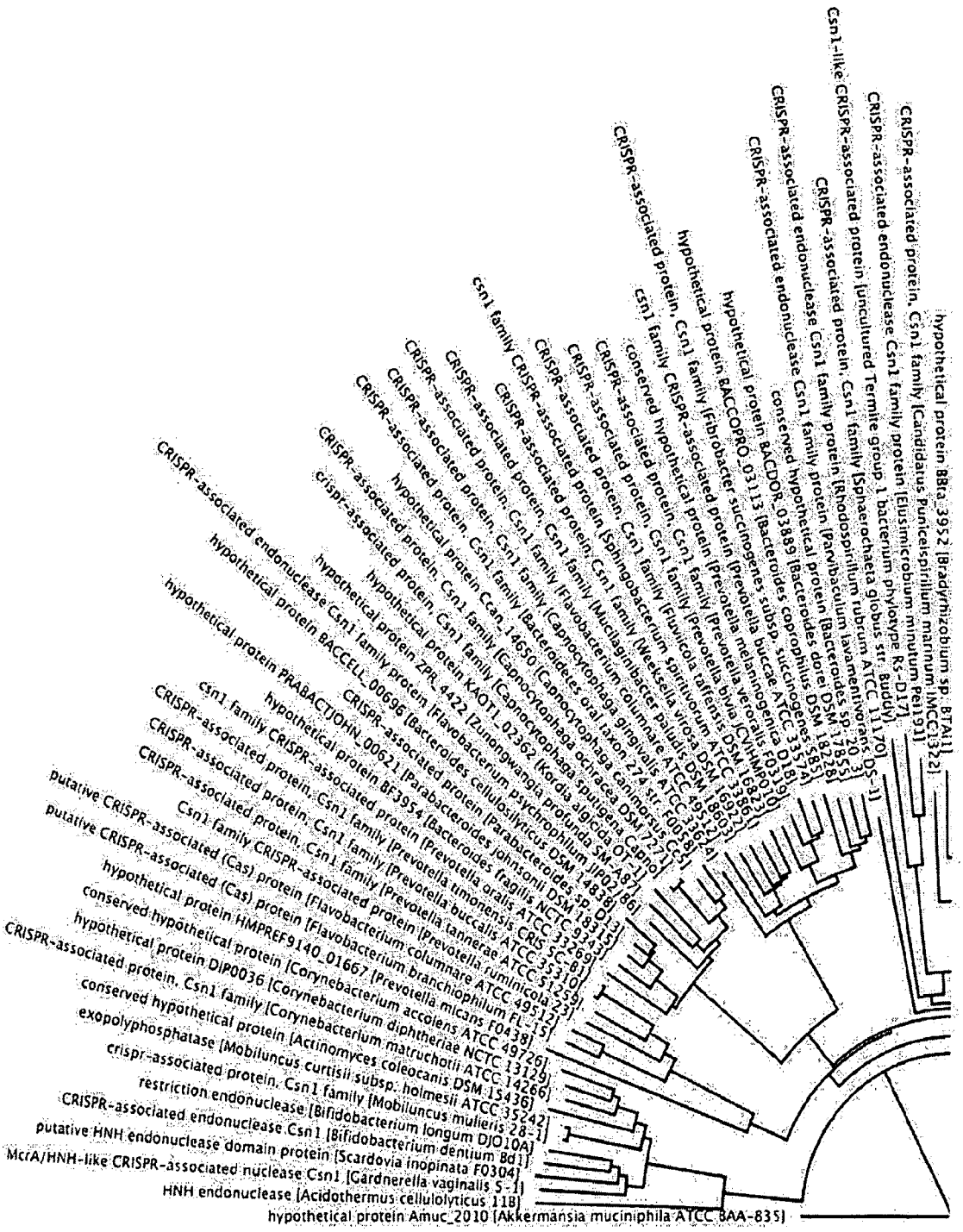

hypothetical protein BBta_3952 [Bradyrhizobium sp. BTAi1]
CRISPR-associated protein, Csn1 family [Candidatus Puniceispirillum marinum IMCC1322]
CRISPR-associated endonuclease Csn1 family protein [Elusimicrobium minutum Pei191]
CRISPR-associated protein [uncultured Termite group 1 bacterium phylotype Rs-D17]
Csn1-like CRISPR-associated protein [Sphaerochaeta globus str. Buddy]
CRISPR-associated protein, Csn1 family [Rhodospirillum rubrum ATCC 11170]
CRISPR-associated endonuclease Csn1 family protein [Parvibaculum lavamentivorans DS-1]
CRISPR-associated endonuclease Csn1 family protein [Rhodobacter sp. 20-53]
conserved hypothetical protein [Bacteroides dorei DSM 17855]
hypothetical protein BACCOPRO_03113 [Bacteroides coprophilus DSM 18228]
CRISPR-associated endonuclease [Bacteroides subsp. succinogenes S85]
hypothetical protein [Prevotella melaninogenica D18]
CRISPR-associated protein, Csn1 family [Fibrobacter succinogenes subsp. succinogenes S85]
conserved hypothetical protein [Prevotella veroralis F0319]
hypothetical protein BACCOPRO_03889 [Bacteroides coprophilus DSM 18228]
CRISPR-associated protein, Csn1 family [Prevotella bivia JCVIHMP022]
hypothetical protein BACCOPRO_03113 [Bacteroides coprophilus DSM 18228]
CRISPR-associated protein, Csn1 family [Prevotella tannerae ATCC 51259]
csn1 family CRISPR-associated protein [Prevotella tannerae ATCC 51259]
CRISPR-associated protein, Csn1 family [Flavobacterium psychrophilum JIP02/86]
CRISPR-associated protein, Csn1 family [Capnocytophaga ochracea DSM 7271]
CRISPR-associated protein, Csn1 family [Capnocytophaga gingivalis ATCC 33624]
CRISPR-associated protein, Csn1 family [Capnocytophaga canimorsus Cc5]
CRISPR-associated protein, Csn1 family [Sphingobacterium spiritivorum ATCC 33861]
CRISPR-associated protein, Csn1 family [Mucilaginibacter paludis DSM 18603]
conserved hypothetical protein, Csn1 family [Weeksella virosa DSM 16922]
hypothetical protein, Csn1 family CRISPR-associated protein [Fluviicola taffensis DSM 16823]
CRISPR-associated protein, Csn1 family [Prevotella bivia JCVIHMP022]
hypothetical protein BACCOPRO_03889 [Bacteroides coprophilus DSM 18228]
hypothetical protein BACCELL_00696 [Bacteroides cellulosilyticus DSM 14838]
CRISPR-associated protein, Csn1 family [Bacteroides johnsonii DSM 18315]
hypothetical protein PRABACTJOHN_00621 [Parabacteroides johnsonii DSM 18315]
CRISPR-associated protein, Csn1 family [Bacteroides fragilis NCTC 9343]
hypothetical protein BF3954 [Bacteroides fragilis NCTC 9343]
CRISPR-associated protein, Csn1 family [Prevotella oralis ATCC 33269]
hypothetical protein HMPREF9140_01667 [Prevotella oralis ATCC 33269]
CRISPR-associated protein, Csn1 family [Prevotella timonensis CRIS 5C-B1]
hypothetical protein DIP0036 [Corynebacterium diphtheriae NCTC 13129]
CRISPR-associated protein, Csn1 family [Corynebacterium accolens ATCC 49726]
conserved hypothetical protein [Corynebacterium matruchotii ATCC 14266]
exopolyphosphatase [Actinomyces coleocanis DSM 15436]
crispr-associated protein, Csn1 family [Mobiluncus curtisii subsp. holmesii ATCC 35242]
restriction endonuclease [Bifidobacterium longum DJO10A]
CRISPR-associated endonuclease Csn1 [Bifidobacterium dentium Bd1]
putative HNH endonuclease domain protein [Scardovia inopinata F0304]
McrA/HNH-like CRISPR-associated nuclease Csn1 [Gardnerella vaginalis 5-1]
HNH endonuclease [Acidothermus cellulolyticus 11B]
hypothetical protein Amuc_2010 [Akkermansia muciniphila ATCC BAA-835]

FIG. 19A

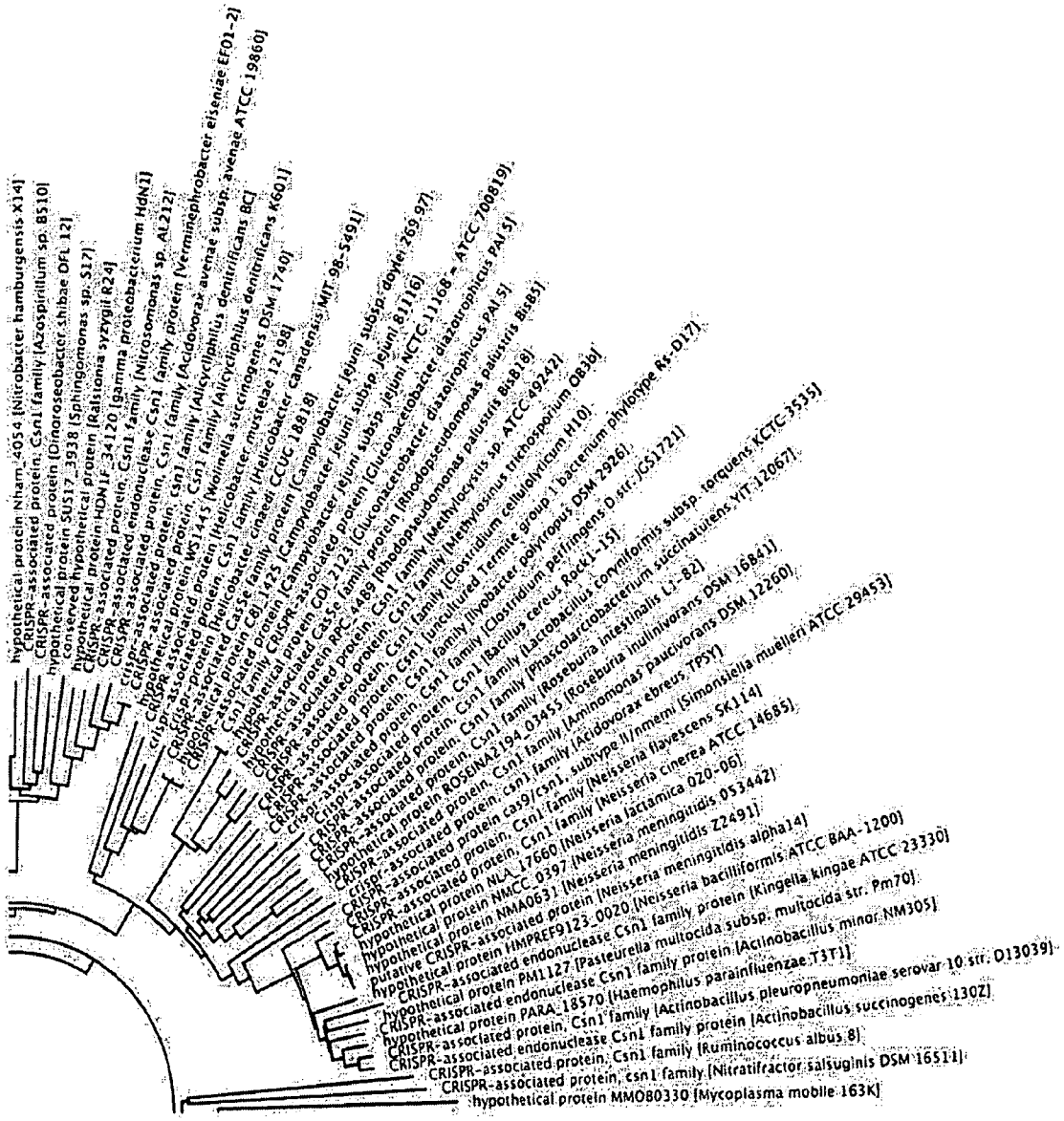

hypothetical protein Nham_4054 [Nitrobacter hamburgensis X14]
CRISPR-associated protein, Csn1 family [Azospirillum sp. B510]
hypothetical protein [Oinorosebacter shibae DFL 12]
conserved hypothetical protein SU517_3938 [Sphingomonas sp. S17]
hypothetical protein HDN1F_34120 [gamma proteobacterium HdN1]
CRISPR-associated protein, Csn1 family [Ralstonia syzygii R24]
CRISPR-associated endonuclease Csn1 family [Nitrosomonas sp. AL212]
hypothetical protein WS1445 [Wolinella succinogenes DSM 1740]
CRISPR-associated protein, Csn1 family [Alicyclobacillus denitrificans K601]
CRISPR-associated protein, Csn1 family [Helicobacter mustelae 12198]
CRISPR-associated protein, Csn1 family [Helicobacter canadensis MIT 98-5491]
CRISPR-associated protein, Csn1 family [Campylobacter jejuni subsp. doylei 269.97]
CRISPR-associated protein, Csn1 family [Gluconacetobacter diazotrophicus PAL 5]
CRISPR-associated protein, Csn1 family [Rhodopseudomonas palustris BisB18]
CRISPR-associated protein, Csn1 family [Methylocystis sp. ATCC 49242]
CRISPR-associated protein, Csn1 family [Methylosinus trichosporium OB3b]
CRISPR-associated protein, Csn1 family [Clostridium cellulolyticum H10]
CRISPR-associated protein, Csn1 family [uncultured Termite group 1 bacterium phylotype Rs-D17]
CRISPR-associated protein, Csn1 family [Ilyobacter polytropus DSM 2926]
CRISPR-associated protein, Csn1 family [Clostridium perfringens D str. JGS1721]
CRISPR-associated protein, Csn1 family [Bacillus cereus Rock1-15]
CRISPR-associated protein, Csn1 family [Lactobacillus coryniformis subsp. torquens KCTC 3535]
CRISPR-associated protein, Csn1 family [Phascolarctobacterium succinatutens YIT 12067]
CRISPR-associated protein, Csn1 family [Roseburia intestinalis L1-82]
CRISPR-associated protein, Csn1 family [Roseburia inulinivorans DSM 16841]
CRISPR-associated protein, Csn1 family [Aminomonas paucivorans DSM 12260]
hypothetical protein ROSEINA2194_03455 [Roseburia inulinivorans DSM 16841]
CRISPR-associated protein, Csn1 family [Acidovorax ebreus, TPSY]
CRISPR-associated protein, csn1 family [Simonsiella muelleri ATCC 29453]
CRISPR-associated protein cas9/csn1, subtype II/nmeni [Simonsiella muelleri ATCC 29453]
CRISPR-associated protein, Csn1 family [Neisseria flavescens SK114]
CRISPR-associated protein, Csn1 family [Neisseria cinerea ATCC 14685]
CRISPR-associated protein, Csn1 family [Neisseria lactamica 020-06]
hypothetical protein NLA_17660 [Neisseria lactamica 020-06]
CRISPR-associated protein, Csn1 family [Neisseria meningitidis 053442]
hypothetical protein NMCC_0397 [Neisseria meningitidis Z2491]
hypothetical protein NMA0631 [Neisseria meningitidis alpha14]
CRISPR-associated protein, Csn1 family [Neisseria bacilliformis ATCC BAA-1200]
hypothetical protein [Neisseria meningitidis ATCC 23330]
hypothetical protein [Kingella kingae ATCC 23330]
putative CRISPR-associated protein [Acidovorax sp. Pm70]
hypothetical protein HMPREF9123_0020 [Neisseria bacilliformis ATCC BAA-1200]
CRISPR-associated endonuclease Csn1 family protein [Actinobacillus minor NM305]
hypothetical protein PM1127 [Pasteurella multocida subsp. multocida str. Pm70]
CRISPR-associated endonuclease Csn1 family protein [Actinobacillus parainfluenzae T3T1]
hypothetical protein PARA_18570 [Haemophilus parainfluenzae T3T1]
CRISPR-associated protein, Csn1 family [Actinobacillus pleuropneumoniae serovar 10 str. D13039]
hypothetical protein [Actinobacillus pleuropneumoniae serovar 10 str. D13039]
CRISPR-associated endonuclease Csn1 family protein [Actinobacillus succinogenes 130Z]
CRISPR-associated protein, Csn1 family [Ruminococcus albus 8]
CRISPR-associated protein, csn1 family [Nitratifractor salsuginis DSM 16511]
hypothetical protein MMOB0330 [Mycoplasma mobile 163K]

FIG. 19B

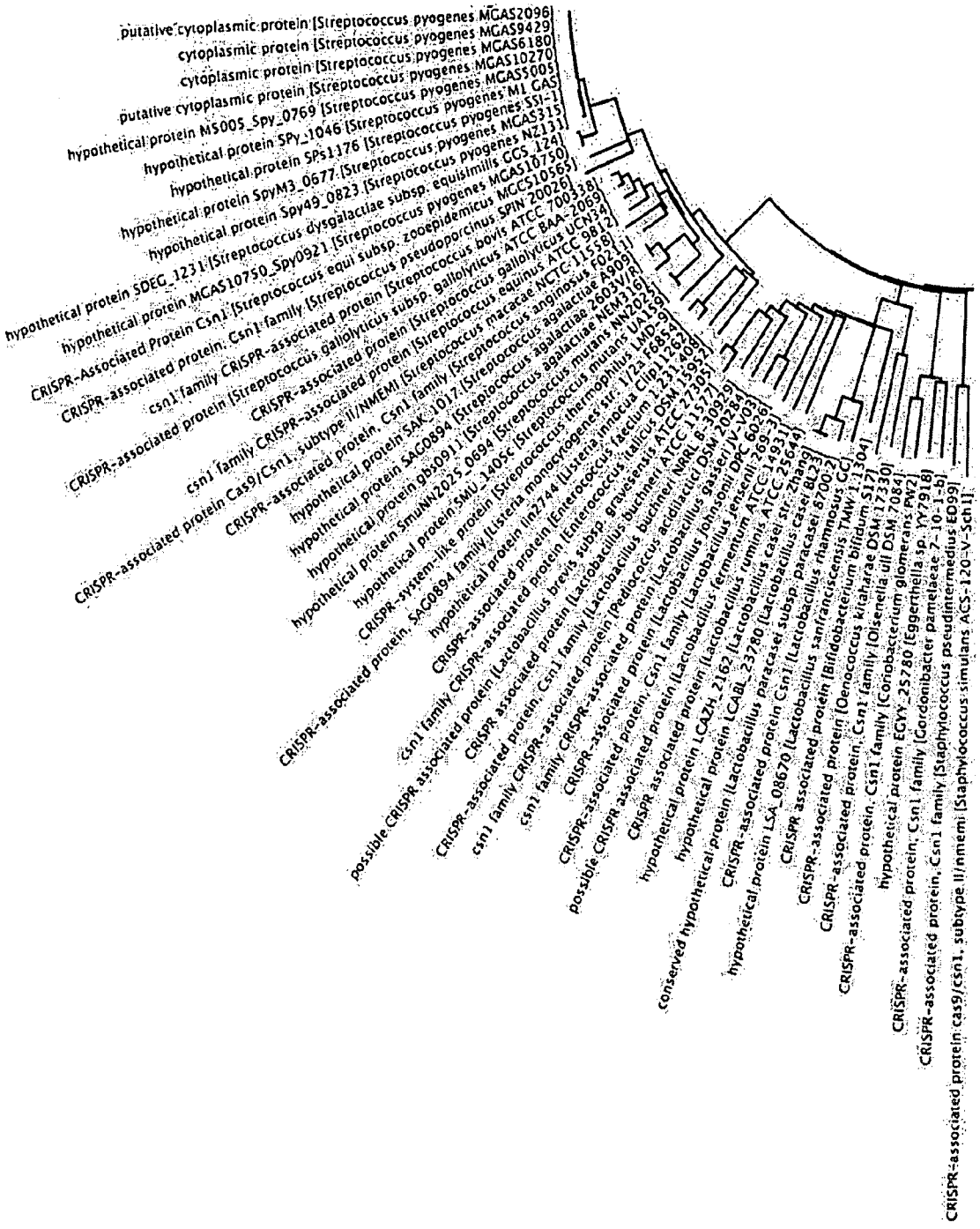

putative cytoplasmic protein [Streptococcus pyogenes MGAS2096]
cytoplasmic protein [Streptococcus pyogenes MGAS9429]
cytoplasmic protein [Streptococcus pyogenes MGAS6180]
putative cytoplasmic protein [Streptococcus pyogenes MGAS10270]
hypothetical protein MS005_SPy_0769 [Streptococcus pyogenes MGAS5005]
hypothetical protein SPy_1046 [Streptococcus pyogenes M1 GAS]
hypothetical protein SPs1176 [Streptococcus pyogenes SSI-1]
hypothetical protein SpyM3_0677 [Streptococcus pyogenes MGAS315]
hypothetical protein Spy49_0823 [Streptococcus pyogenes NZ131]
hypothetical protein SDEG_1231 [Streptococcus dysgalactiae subsp. equisimilis GGS_124]
hypothetical protein MGAS10750_Spy0921 [Streptococcus pyogenes MGAS10750]
CRISPR-Associated Protein Csn1 [Streptococcus equi subsp. zooepidemicus MGCS10565]
CRISPR-associated protein, Csn1 family [Streptococcus pseudoporcinus SPIN 20026]
csn1 family CRISPR-associated protein [Streptococcus gallolyticus subsp. gallolyticus ATCC BAA-2069]
CRISPR-associated protein [Streptococcus gallolyticus subsp. gallolyticus UCN34]
csn1 family CRISPR-associated protein [Streptococcus bovis ATCC 700338]
CRISPR-associated protein Cas9/Csn1, subtype II/NMEMI [Streptococcus macacae NCTC 11558]
CRISPR-associated protein, Csn1 family [Streptococcus equinus ATCC 9812]
hypothetical protein SAK_1017 [Streptococcus agalactiae A909]
hypothetical protein SAG0894 [Streptococcus agalactiae 2603V/R]
hypothetical protein gbs0911 [Streptococcus agalactiae NEM316]
hypothetical protein SmuNN2025_0694 [Streptococcus mutans NN2025]
hypothetical protein SMU_1405c [Streptococcus mutans UA159]
CRISPR-system-like protein [Streptococcus thermophilus LMD-9]
CRISPR-associated protein, SAG0894 family [Listeria monocytogenes str. 1/2a F6854]
hypothetical protein lin2744 [Listeria innocua Clip11262]
hypothetical protein [Enterococcus italicus DSM 15952]
CRISPR-associated protein [Enterococcus gravesensis ATCC 27305]
csn1 family CRISPR-associated protein [Lactobacillus buchneri NRRL B-30929]
CRISPR-associated protein [Lactobacillus brevis subsp. gravesensis ATCC 27305]
possible CRISPR associated protein [Lactobacillus acidilactici DSM 20284]
csn1 family CRISPR-associated protein [Pediococcus acidilactici 7_4]
CRISPR-associated protein, Csn1 family [Lactobacillus buchneri ATCC 11577]
CRISPR-associated protein [Lactobacillus johnsonii DPC 6026]
possible CRISPR associated protein [Lactobacillus gasseri JV-V03]
CRISPR associated protein [Lactobacillus fermentum ATCC 14931]
hypothetical protein LCAZH_2162 [Lactobacillus fermentum IFO 3956]
conserved hypothetical protein [Lactobacillus jensenii 269-3]
CRISPR-associated protein [Lactobacillus ruminis ATCC 25644]
hypothetical protein LSA_08670 [Lactobacillus casei str. Zhang]
CRISPR associated protein Csn1 [Lactobacillus paracasei subsp. paracasei 8700:2]
CRISPR-associated protein [Bifidobacterium animalis subsp. lactis BL23]
CRISPR-associated protein, Csn1 family [Oenococcus kitaharae DSM 17330]
CRISPR-associated protein [Olsenella uli DSM 7084]
hypothetical protein EGYY_25780 [Eggerthella sp. YY7918]
CRISPR-associated protein, Csn1 family [Cordonibacter pamelaeae 7-10-1-b]
CRISPR-associated protein Cas9/Csn1, subtype II/nmemi [Staphylococcus pseudintermedius ED99]
CRISPR-associated protein, Csn1 family [Staphylococcus simulans ACS-120-V-Sch1]

FIG. 19C


hypothetical protein MovIS_007-10 [Mycoplasma ovipneumoniae SC01]
Csn1 family CRISPR-associated protein [Mycoplasma canis PG 14]
hypothetical protein MS53_0582 [Mycoplasma synoviae 53]
Csn1 family CRISPR-associated protein [Mycoplasma gallisepticum str. F]
CRISPR-associated protein Cas9/Csn1 [Staphylococcus aureus subsp. aureus]
conserved hypothetical protein [Staphylococcus lugdunensis M23590]
hypothetical protein EUBDOL_01797 [Eubacterium dolichum DSM 3991]
hypothetical protein CLOSPI_00488 [Clostridium spiroforme DSM 1552]
CRISPR-associated protein, Csn1 family [Enterococcus faecalis TX0012]
hypothetical protein EUBVEN_00141 [Eubacterium ventriosum ATCC 27560]
CRISPR-system related protein [Eubacterium rectale ATCC 33656]
CRISPR-associated endonuclease Csn1 family protein [Lactobacillus farciminis KCTC 3681]
CRISPR-system family protein [Streptococcus vestibularis ATCC 49124]
hypothetical protein stu0657 [Streptococcus thermophilus LMD-9]
CRISPR-system-like protein str0657 [Streptococcus thermophilus CNRZ1066]
hypothetical protein, Csn1 family [Streptococcus infantarius subsp. infantarius ATCC BAA-102]
CRISPR-associated protein, Csn1 family [Streptococcus suis 89/1591]
hypothetical protein STRINF_01771 [Streptococcus suis 873]
CRISPR-associated protein, Csn1 family [Streptococcus gallolyticus UCN34]
hypothetical protein [Streptococcus pasteurianus ATCC 43144]
CRISPR-associated protein, Csn1 family [Streptococcus macedonicus ACA-DC 198]
membrane protein [Francisella novicida U112]
hypothetical protein lpp0160 [Legionella pneumophila str. Paris]
Wolinella succinogenes DSM 1740
putative CRISPR-associated large protein (provisional) putative [gamma proteobacterium HTCC5015]
CRISPR-associated protein, Csx12 family [Catenibacterium mitsuokai DSM 15897]
hypothetical protein HMPREF9464_01128 [Suiterella wadsworthensis 3_1_45B]
CATMIT_02512 [Catenibacterium mitsuokai DSM 15897]
CRISPR-associated protein, Csx12 family [Parasutterella excrementihominis YIT 11859]
hypothetical protein FMG_0058 [Finegoldia magna ATCC 29328]
Burkholderiales bacterium 1_1_47
hypothetical protein [Filifactor alocis ATCC 35896]
CRISPR-associated protein, Csn1 family [Peptoniphilus sp. oral taxon 386 str. F0131]
hypothetical protein [Fusobacterium nucleatum subsp. vincentii ATCC 49256]
CRISPR-associated protein, Csn1 family [Peptoniphilus sp. oral taxon 836 str. F0429]
hypothetical protein [Fusobacterium nucleatum subsp. nucleatum ATCC 25586]
CRISPR-associated protein, Csn1 family [Gemella morbillorum M424]
hypothetical protein [Megasphaera sp. UPII 135-E]
CRISPR-associated protein, Csn1 family [Gemella haemolysans ATCC 10379]
CRISPR-associated protein, Csn1 family [Veillonella atypica ACS-134-V-Col7a]
Csn1 family CRISPR-associated protein [Veillonella parvula ATCC 17745]
CRISPR-associated protein, Csn1 family [Veillonella sp. 6_1_27]
CRISPR-associated protein, Csn1 family [Solobacterium moorei F0204]
CRISPR-associated protein, Csn1 family [Solobacterium sp. D21]
CRISPR-associated protein [Acidaminococcus intestini RyC-MR95]
CRISPR-associated protein [Acidaminococcus fermentans DSM 20731]
crispr-associated protein RUMLAC_00178 [Ruminococcus lactaris ATCC 29176]
hypothetical protein endonuclease, Csn1 family [Coprococcus catus GD/7]
CRISPR-associated protein DORLON_02599 [Dorea longicatena DSM 13814]
hypothetical protein [Treponema denticola ATCC 35405]
CRISPR-associated Cas5e [Treponema denticola ATCC 35405]
hypothetical protein [Peptoniphilus duerdenii ATCC BAA-1640]
CRISPR-associated protein [Anaerococcus tetradius ATCC 35098]
csn1 family CRISPR-associated protein [Lactobacillus iners LactinV 11V1-d]
CRISPR-associated protein, Csn1 family [Lactobacillus iners LactinV 01V1-a]

FIG. 19D

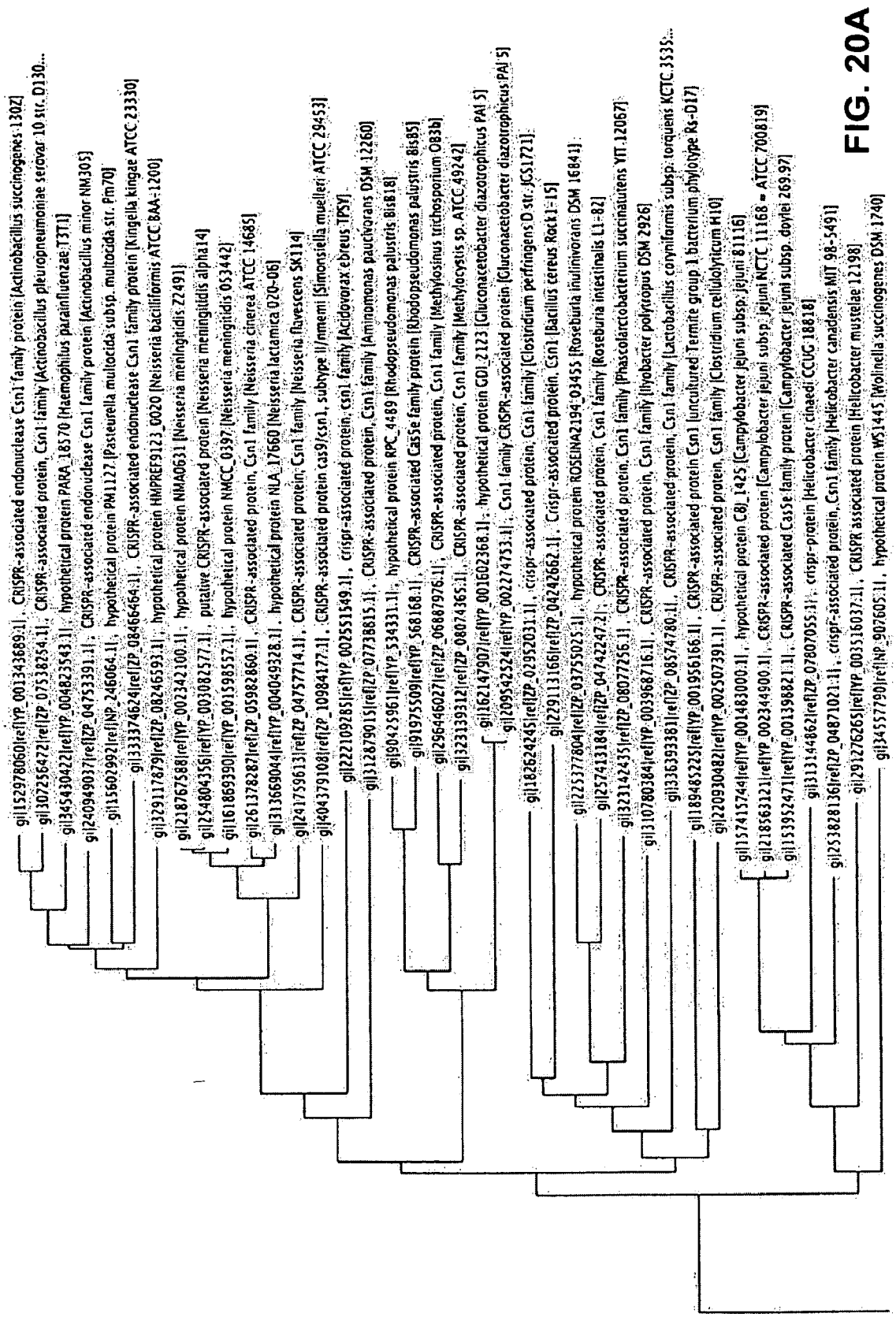

gi|152978060|ref|YP_001343689.1|, CRISPR-associated endonuclease Csn1 family protein [Actinobacillus succinogenes 130Z]
gi|307256472|ref|ZP_07538254.1|, CRISPR-associated protein, Csn1 family [Actinobacillus pleuropneumoniae serovar 10 str. D130...
gi|345430422|ref|YP_004823543.1|, hypothetical protein PARA_18570 [Haemophilus parainfluenzae T3T1]
gi|240949037|ref|ZP_04753391.1|, CRISPR-associated endonuclease Csn1 family protein [Actinobacillus minor NM305]
gi|115602992|ref|NP_246064.1|, hypothetical protein PM1127 [Pasteurella multocida subsp. multocida str. Pm70]
gi|333374624|ref|ZP_08466464.1|, CRISPR-associated endonuclease Csn1 family protein [Kingella kingae ATCC 23330]
gi|329117879|ref|ZP_08246593.1|, hypothetical protein HMPREF9123_0020 [Neisseria bacilliformis ATCC BAA-1200]
gi|218767588|ref|YP_002342100.1|, hypothetical protein NMA0631 [Neisseria meningitidis Z2491]
gi|254804356|ref|YP_003082577.1|, putative CRISPR-associated protein [Neisseria meningitidis alpha14]
gi|161869390|ref|YP_001598557.1|, hypothetical protein NMCC_0397 [Neisseria meningitidis 053442]
gi|261378287|ref|ZP_05982860.1|, CRISPR-associated protein, Csn1 family [Neisseria cinerea ATCC 14685]
gi|313669044|ref|YP_004049328.1|, hypothetical protein NLA_17660 [Neisseria lactamica 020-06]
gi|241759613|ref|ZP_04757714.1|, CRISPR-associated protein, Csn1 family [Neisseria flavescens SK114]
gi|404379108|ref|ZP_10984177.1|, CRISPR-associated protein cas9/csn1, subtype II/nmeni [Simonsiella muelleri ATCC 29453]
gi|222109285|ref|YP_002551549.1|, crispr-associated protein, csn1 family [Acidovorax ebreus TPSY]
gi|312879015|ref|ZP_07738815.1|, CRISPR-associated protein, Csn1 family [Aminomonas paucivorans DSM 12260]
gi|90425961|ref|YP_534331.1|, hypothetical protein RPC_4489 [Rhodopseudomonas palustris BisB18]
gi|91975509|ref|YP_568168.1|, CRISPR-associated Cas5e family protein [Rhodopseudomonas palustris BisB5]
gi|296446027|ref|ZP_06887976.1|, CRISPR-associated protein, Csn1 family [Methylosinus trichosporium OB3b]
gi|323139312|ref|ZP_08074365.1|, CRISPR-associated protein, Csn1 family [Methylocystis sp. ATCC 49242]
gi|16214790|ref|YP_001602368.1|, hypothetical protein GDI_2123 [Gluconacetobacter diazotrophicus PAl 5]
gi|209542524|ref|YP_002274753.1|, Csn1 family CRISPR-associated protein [Gluconacetobacter diazotrophicus PAl 5]
gi|182624245|ref|ZP_02952031.1|, crispr-associated protein, Csn1 family [Clostridium perfringens D str. JGS1721]
gi|229113166|ref|ZP_04242662.1|, Crispr-associated protein, Csn1 [Bacillus cereus Rock1-15]
gi|225377804|ref|ZP_03755025.1|, hypothetical protein ROSEINA2194_03455 [Roseburia inulinivorans DSM 16841]
gi|257413184|ref|ZP_04742247.1|, CRISPR-associated protein, Csn1 family [Roseburia intestinalis L1-82]
gi|322142435|ref|ZP_08077256.1|, CRISPR-associated protein, Csn1 family [Phascolarctobacterium succinatutens YIT 12067]
gi|310780384|ref|YP_003968716.1|, CRISPR-associated protein, Csn1 family [Hyobacter polytropus DSM 2926]
gi|336393381|ref|ZP_08574780.1|, CRISPR-associated protein, Csn1 family [Lactobacillus coryniformis subsp. torquens KCTC 3535...
gi|189485225|ref|YP_001956166.1|, CRISPR-associated protein Csn1 [uncultured Termite group 1 bacterium phylotype Rs-D17]
gi|220930482|ref|YP_002507391.1|, CRISPR-associated protein, Csn1 family [Clostridium cellulolyticum H10]
gi|115741574|ref|YP_001483000.1|, hypothetical protein CJE_1425 [Campylobacter jejuni subsp. jejuni 81116]
gi|218563121|ref|YP_002344900.1|, CRISPR-associated protein [Campylobacter jejuni subsp. jejuni NCTC 11168 = ATCC 700819]
gi|153952471|ref|YP_001398821.1|, CRISPR-associated Cas5e family protein [Campylobacter jejuni subsp. doylei 269.97]
gi|313144862|ref|ZP_07807055.1|, crispr-protein [Helicobacter cinaedi CCUG 18818]
gi|253828136|ref|ZP_04871021.1|, crispr-associated protein, Csn1 family [Helicobacter canadensis MIT 98-5491]
gi|291276265|ref|YP_003516037.1|, CRISPR associated protein [Helicobacter mustelae 12198]
gi|345577901|ref|NP_907605.1|, hypothetical protein WS1445 [Wolinella succinogenes DSM 1740]

FIG. 20A

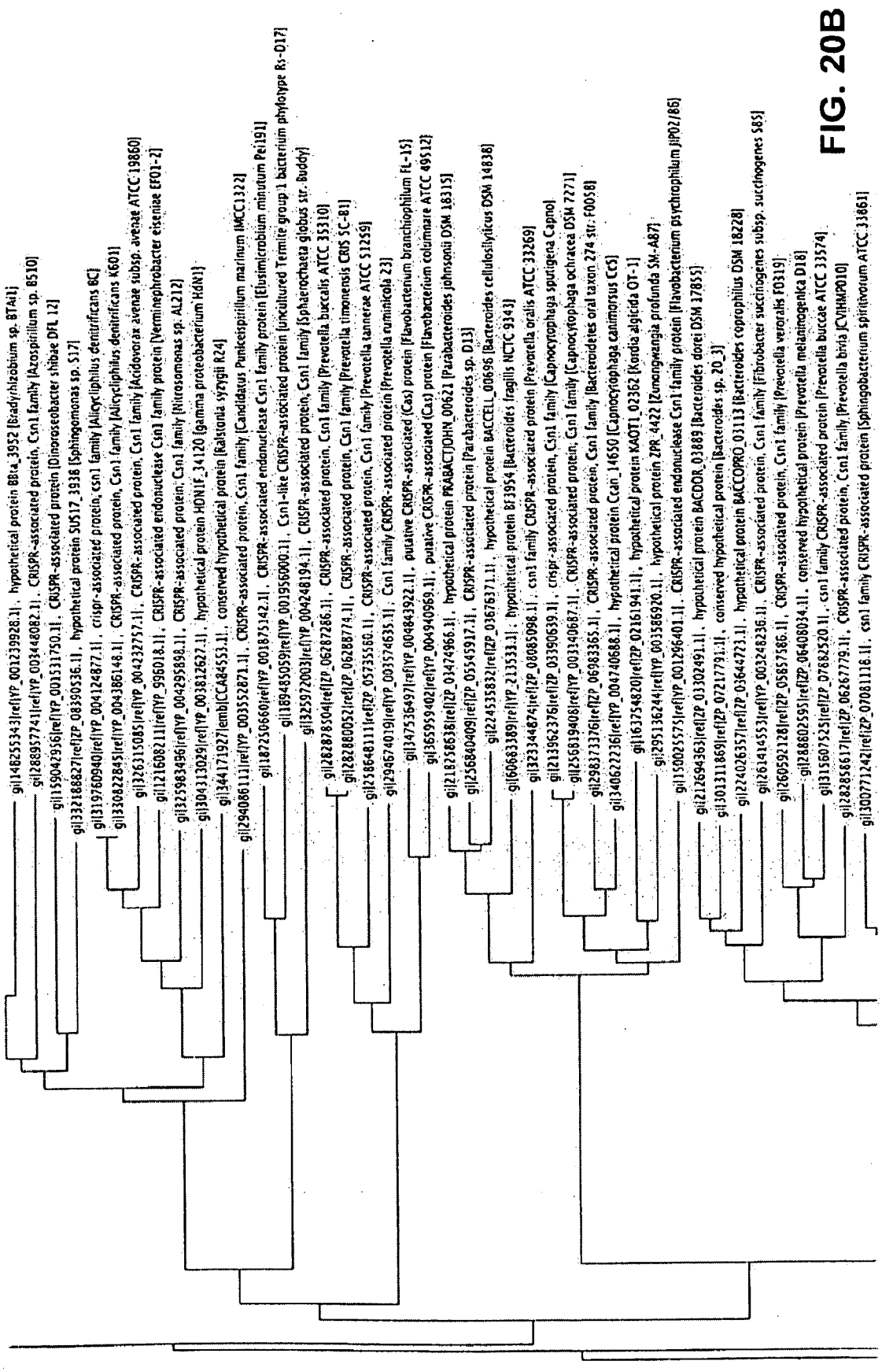

gi|148255343|ref|YP_001239928.1|, hypothetical protein BBta_3952 [Bradyrhizobium sp. BTAi1]
gi|288957741|ref|YP_003448082.1|, CRISPR-associated protein, Csn1 family [Azospirillum sp. B510]
gi|315904956|ref|YP_003931750.1|, CRISPR-associated protein [Dinoroseobacter shibae DFL 12]
gi|332188827|ref|ZP_08390536.1|, hypothetical protein SU57_3938 [Sphingomonas sp. S17]
gi|319760940|ref|YP_004124872.1|, crispr-associated protein, csn1 family [Alicycliphilus denitrificans BC]
gi|330822845|ref|YP_004386148.1|, CRISPR-associated protein, Csn1 family [Alicycliphilus denitrificans K601]
gi|326315085|ref|YP_004232757.1|, CRISPR-associated protein, Csn1 family [Acidovorax avenae subsp. avenae ATCC 19860]
gi|121608211|ref|YP_996018.1|, CRISPR-associated endonuclease Csn1 family protein [Verminephrobacter eiseniae EF01-2]
gi|325983496|ref|YP_004295898.1|, CRISPR-associated protein, Csn1 family [Nitrosomonas sp. AL212]
gi|304313029|ref|YP_003812627.1|, hypothetical protein HDN1F_34120 [gamma proteobacterium HdN1]
gi|344171927|emb|CCA84553.1|, conserved hypothetical protein [Ralstonia syzygii R24]
gi|294086111|ref|YP_003552871.1|, CRISPR-associated protein, Csn1 family [Candidatus Puniceispirillum marinum IMCC1322]
gi|187250660|ref|YP_001875142.1|, CRISPR-associated endonuclease Csn1 family protein [Elusimicrobium minutum Pei191]
gi|118948059|ref|YP_001956000.1|, Csn1-like CRISPR-associated protein [uncultured Termite group 1 bacterium phylotype Rs-D17]
gi|325972003|ref|YP_004248194.1|, CRISPR-associated protein, Csn1 family [Spherochaeta globus str. Buddy]
gi|282878504|ref|ZP_06287286.1|, CRISPR-associated protein, Csn1 family [Prevotella buccalis ATCC 35310]
gi|282880052|ref|ZP_06288774.1|, CRISPR-associated protein, Csn1 family [Prevotella timonensis CRIS 5C-B1]
gi|258648111|ref|ZP_05735560.1|, CRISPR-associated protein, Csn1 family [Prevotella tannerae ATCC 51259]
gi|294674019|ref|YP_003574635.1|, Csn1 family CRISPR-associated protein [Prevotella ruminicola 23]
gi|347535649|ref|YP_004843922.1|, putative CRISPR-associated (Cas) protein [Flavobacterium branchiophilum FL-15]
gi|365959402|ref|YP_004940969.1|, putative CRISPR-associated (Cas) protein [Flavobacterium columnare ATCC 49512]
gi|218258638|ref|ZP_03474966.1|, hypothetical protein PRABACTJOHN_00621 [Parabacteroides johnsonii DSM 18315]
gi|256840409|ref|ZP_05545917.1|, CRISPR-associated protein [Parabacteroides sp. D13]
gi|224535832|ref|ZP_03676371.1|, hypothetical protein BACCELL_00698 [Bacteroides cellulosilyticus DSM 14838]
gi|60683389|ref|YP_213533.1|, hypothetical protein BF3954 [Bacteroides fragilis NCTC 9343]
gi|323344874|ref|ZP_08085098.1|, csn1 family CRISPR-associated protein [Prevotella oralis ATCC 33269]
gi|213962376|ref|ZP_03390639.1|, crispr-associated protein, Csn1 family [Capnocytophaga sputigena Capno]
gi|256819408|ref|YP_003340687.1|, CRISPR-associated protein, Csn1 family [Capnocytophaga ochracea DSM 7271]
gi|298373376|ref|ZP_06983365.1|, CRISPR-associated protein, Csn1 family [Bacteroidetes oral taxon 274 str. F0058]
gi|340622236|ref|YP_004740688.1|, hypothetical protein Ccan_14650 [Capnocytophaga canimorsus Cc5]
gi|163754820|ref|ZP_02161941.1|, hypothetical protein KAOT1_02362 [Kordia algicida OT-1]
gi|295136244|ref|YP_003586920.1|, hypothetical protein ZPR_4422 [Zunongwangia profunda SM-A87]
gi|150025575|ref|YP_001296401.1|, CRISPR-associated endonuclease Csn1 family protein [Flavobacterium psychrophilum JIP02/86]
gi|212694363|ref|ZP_03302491.1|, hypothetical protein BACDOR_03889 [Bacteroides dorei DSM 17855]
gi|301311869|ref|ZP_07217791.1|, conserved hypothetical protein [Bacteroides sp. 20_3]
gi|224026357|ref|ZP_03644723.1|, hypothetical protein BACCOPRO_03133 [Bacteroides coprophilus DSM 18228]
gi|261414553|ref|YP_003248236.1|, CRISPR-associated protein, Csn1 family [Fibrobacter succinogenes subsp. succinogenes S85]
gi|260592128|ref|ZP_05857586.1|, CRISPR-associated protein, Csn1 family [Prevotella veroralis F0319]
gi|288802595|ref|ZP_06408034.1|, conserved hypothetical protein [Prevotella melaninogenica D18]
gi|315507525|ref|ZP_07862520.1|, csn1 family CRISPR-associated protein [Prevotella buccae ATCC 33574]
gi|282856617|ref|ZP_06267779.1|, CRISPR-associated protein, Csn1 family [Prevotella bivia JCVIHMP010]
gi|300771242|ref|ZP_07081118.1|, csn1 family CRISPR-associated protein [Sphingobacterium spiritivorum ATCC 33861]

FIG. 20B

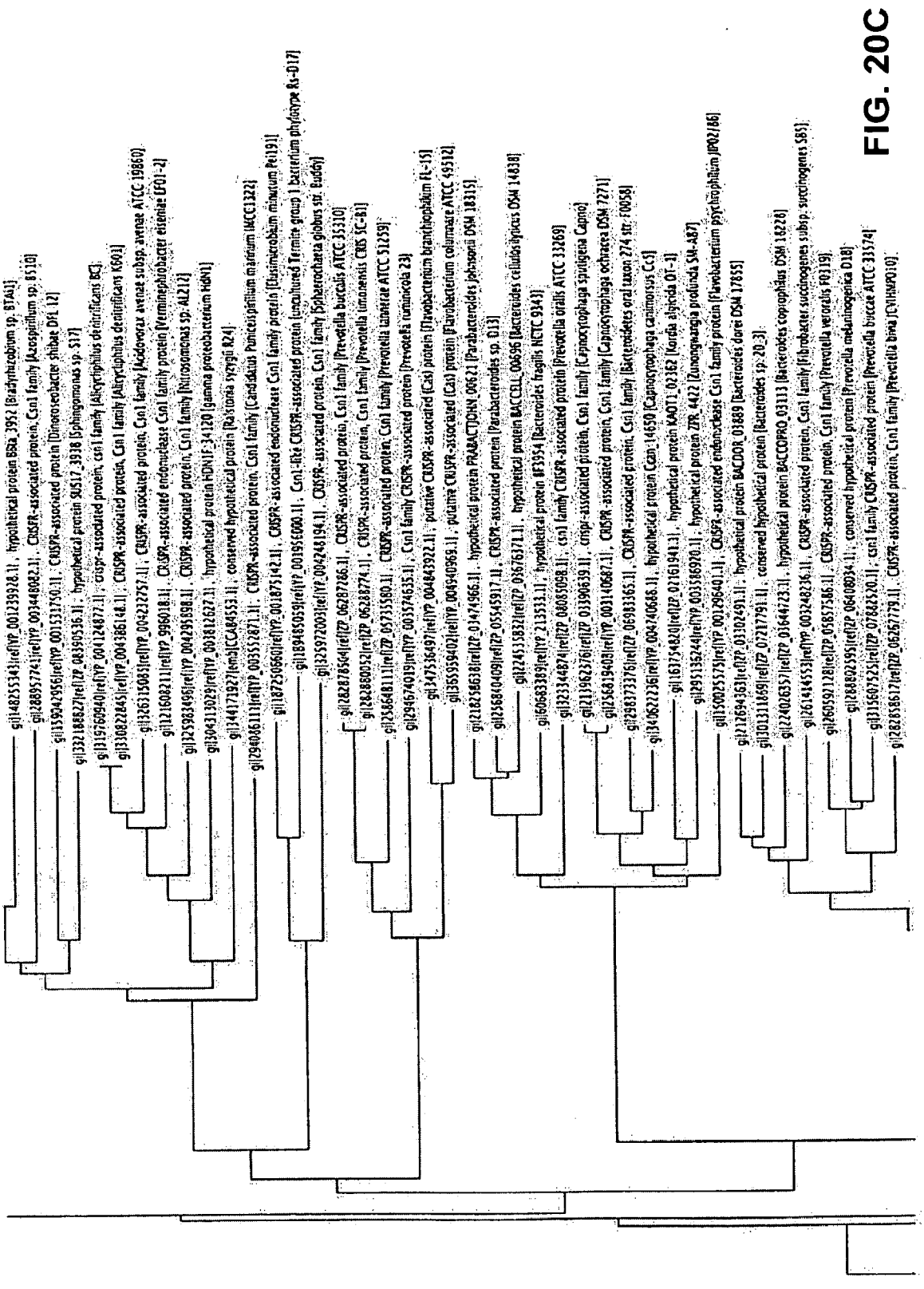


FIG. 20C

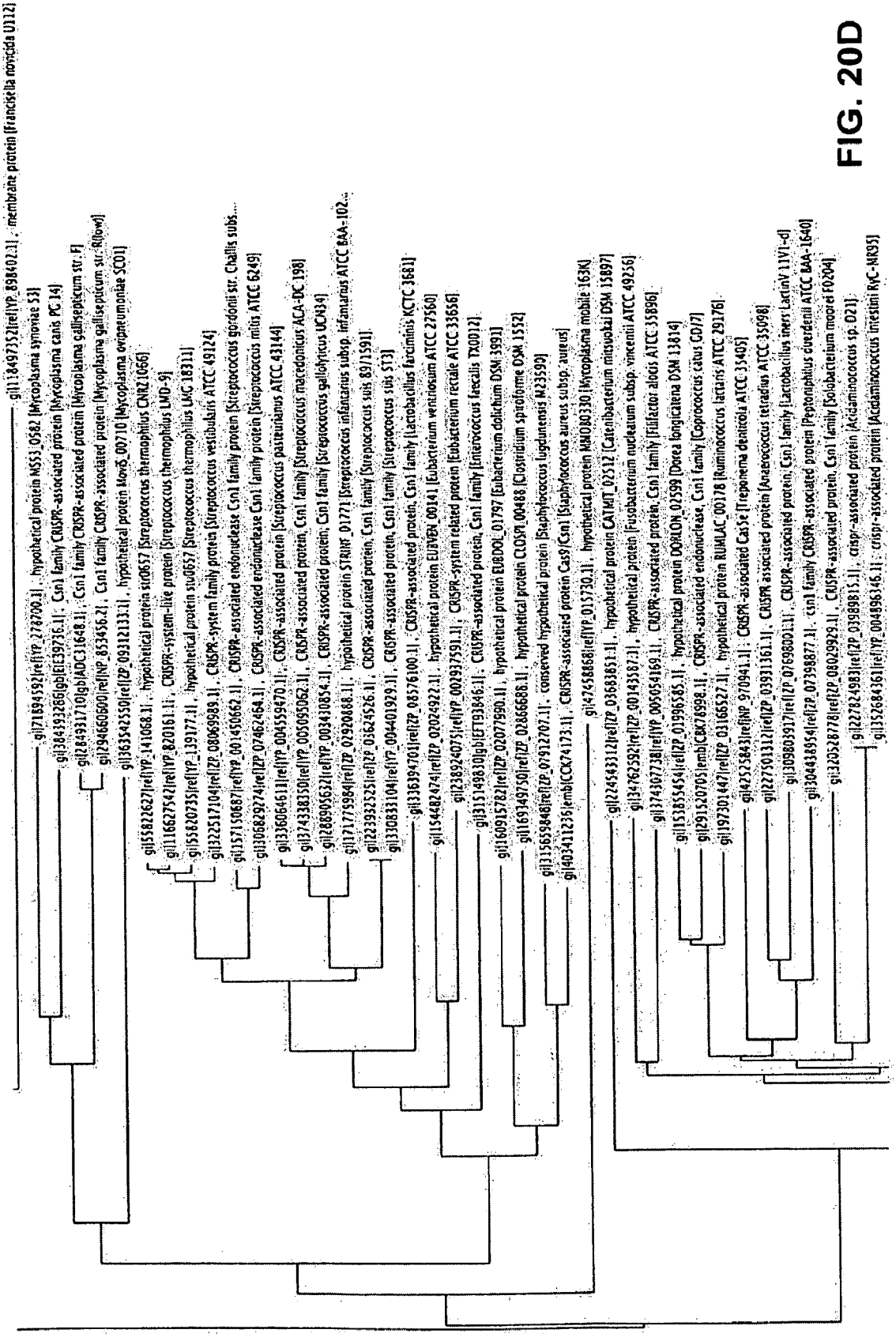

gi|118497352|ref|YP_898402.1|, membrane protein [Francisella novicida U112]
gi|71894592|ref|YP_278700.1|, hypothetical protein MSS3_0582 [Mycoplasma synoviae 53]
gi|384393286|gb|EIE39736.1|, Csn1 family CRISPR-associated protein [Mycoplasma canis PG 14]
gi|284931710|gb|ADC31648.1|, Csn1 family CRISPR-associated protein [Mycoplasma gallisepticum str. F]
gi|294660600|ref|NP_853456.2|, Csn1 family CRISPR-associated protein [Mycoplasma gallisepticum str. R(low)]
gi|363542550|ref|ZP_09312133.1|, hypothetical protein MovS_0710 [Mycoplasma ovipneumoniae SC01]
gi|55822627|ref|YP_141068.1|, hypothetical protein str0657 [Streptococcus thermophilus CNRZ1066]
gi|111662754|ref|YP_820161.1|, CRISPR-system-like protein [Streptococcus thermophilus LMD-9]
gi|55820735|ref|YP_139177.1|, hypothetical protein stu0657 [Streptococcus thermophilus LMG 18311]
gi|322517104|ref|ZP_08069983.1|, CRISPR-system family protein [Streptococcus vestibularis ATCC 49124]
gi|157150687|ref|YP_001450662.1|, CRISPR-associated endonuclease Csn1 family protein [Streptococcus gordonii str. Challis subs...
gi|306829774|ref|ZP_07462464.1|, CRISPR-associated endonuclease Csn1 family protein [Streptococcus mitis ATCC 6249]
gi|336064611|ref|YP_004559470.1|, CRISPR-associated protein [Streptococcus pasteurianus ATCC 43144]
gi|374338350|ref|YP_005095062.1|, CRISPR-associated protein, Csn1 family [Streptococcus macedonicus ACA-DC 198]
gi|288905627|ref|YP_003430854.1|, CRISPR-associated protein, Csn1 family [Streptococcus gallolyticus UCN34]
gi|171779984|ref|ZP_02920688.1|, hypothetical protein STRINF_01771 [Streptococcus infantarius subsp. infantarius ATCC BAA-102...
gi|223932525|ref|ZP_03624526.1|, CRISPR-associated protein, Csn1 family [Streptococcus suis 89/1591]
gi|330833104|ref|YP_004401929.1|, CRISPR-associated protein, Csn1 family [Streptococcus suis 873]
gi|336394701|ref|ZP_08576100.1|, CRISPR-associated protein, Csn1 family [Lactobacillus farciminis KCTC 3681]
gi|154482474|ref|ZP_02024922.1|, hypothetical protein EUBVEN_00141 [Eubacterium ventriosum ATCC 27560]
gi|238924075|ref|YP_002937591.1|, CRISPR-system related protein [Eubacterium rectale ATCC 33656]
gi|315149830|gb|EFT93846.1|, CRISPR-associated protein, Csn1 family [Enterococcus faecalis TX0012]
gi|160915782|ref|ZP_02077990.1|, hypothetical protein EUBDOL_01797 [Eubacterium dolichum DSM 3991]
gi|116934750|ref|ZP_02866688.1|, hypothetical protein CLOSPI_00488 [Clostridium sporosphaeroides DSM 1552]
gi|315659848|ref|ZP_07912707.1|, conserved hypothetical protein [Staphylococcus lugdunensis M23590]
gi|140311236|emb|CCX74173.1|, CRISPR-associated protein Cas9/Csn1 [Staphylococcus aureus subsp. aureus]
gi|474588658|ref|YP_015730.1|, hypothetical protein MMOB0330 [Mycoplasma mobile 163K]
gi|224543312|ref|ZP_03683851.1|, hypothetical protein CATMIT_02512 [Catenibacterium mitsuokai DSM 15897]
gi|347625921|ref|ZP_08143367.1|, hypothetical protein [Fusobacterium nucleatum subsp. vincentii ATCC 49256]
gi|374360773|ref|YP_005054169.1|, CRISPR-associated protein, Csn1 family [Filifactor alocis ATCC 35896]
gi|153855454|ref|ZP_01996585.1|, hypothetical protein DORLON_02599 [Dorea longicatena DSM 13814]
gi|291520705|emb|CBK76998.1|, CRISPR-associated endonuclease, Csn1 family [Coprococcus catus GD/7]
gi|197301447|ref|ZP_03166527.1|, hypothetical protein RUMLAC_00178 [Ruminococcus lactaris ATCC 29176]
gi|42525843|ref|NP_970041.1|, CRISPR-associated Cas9e [Treponema denticola ATCC 35405]
gi|227503112|ref|ZP_03931161.1|, CRISPR associated protein [Anaerococcus tetradius ATCC 35098]
gi|309803917|ref|ZP_07698001.1|, CRISPR-associated protein, Csn1 family [Lactobacillus iners LactinV 11V1-d]
gi|304418954|ref|ZP_07398877.1|, csn1 family CRISPR-associated protein [Peptoniphilus duerdenii ATCC BAA-1640]
gi|320528778|ref|ZP_08029929.1|, CRISPR-associated protein, Csn1 family [Solobacterium moorei F0204]
gi|227824983|ref|ZP_03989815.1|, crispr-associated protein [Actinomyces sp. oral taxon 171 str. F0337]
gi|352684361|ref|YP_004896146.1|, crispr-associated protein [Actinomyces sp. oral taxon 848 str. F0332]

FIG. 20D

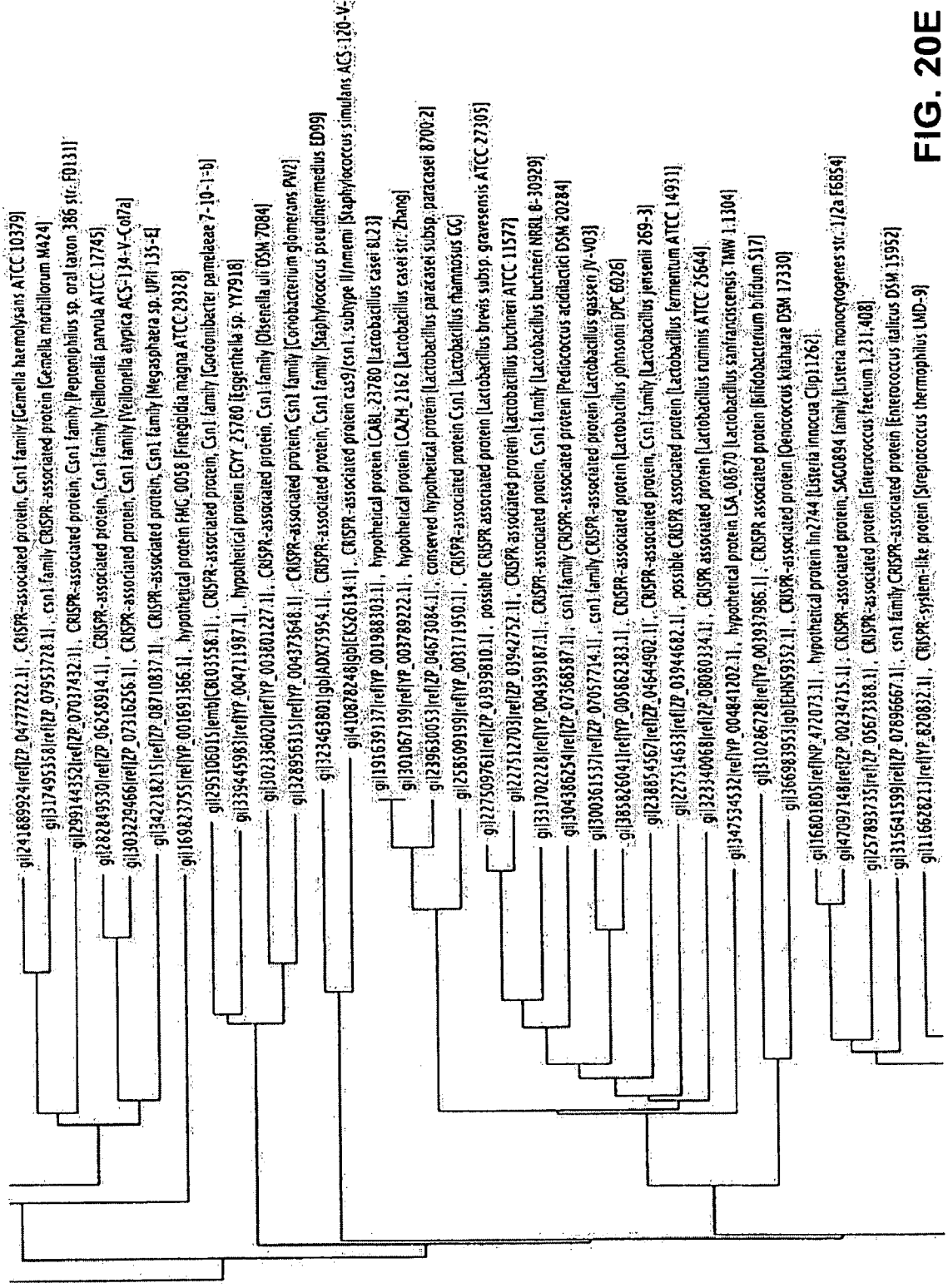

gi|24168992 4|ref|ZP_04777222.1| CRISPR-associated protein, Csn1 family [Gemella haemolysans ATCC 10379]
gi|317495358|ref|ZP_07953728.1| csn1 family CRISPR-associated protein [Gemella morbillorum M424]
gi|299144352|ref|ZP_07037432.1| CRISPR-associated protein, Csn1 family [Peptoniphilus sp. oral taxon 386 str. F0131]
gi|282849530|ref|ZP_06258914.1| CRISPR-associated protein, Csn1 family [Veillonella parvula ATCC 17745]
gi|303229466|ref|ZP_07316256.1| CRISPR-associated protein, Csn1 family [Veillonella atypica ACS-134-V-Col7a]
gi|342218215|ref|ZP_08710837.1| CRISPR-associated protein, Csn1 family [Megasphaera sp. UPII 135-E]
gi|169823755|ref|YP_001691366.1| hypothetical protein FMG_0058 [Finegoldia magna ATCC 29328]
gi|295106601|emb|CBL03558.1| CRISPR-associated protein, Csn1 family [Gordonibacter pamelaeae 7-10-1-b]
gi|339645983|ref|YP_004711987.1| hypothetical protein EGYY_25780 [Eggerthella sp. YY7918]
gi|302336020|ref|YP_003801227.1| CRISPR-associated protein, Csn1 family [Olsenella uli DSM 7084]
gi|328956315|ref|YP_004373648.1| CRISPR-associated protein, Csn1 family [Coriobacterium glomerans PW2]
gi|323463801|gb|ADX75954.1| CRISPR-associated protein, Csn1 family [Staphylococcus pseudintermedius ED99]
gi|410878248|gb|EKS26134.1| CRISPR-associated protein cas9/csn1 subtype II/nmemi [Staphylococcus simulans ACS-120-V-Sch...
gi|191639137|ref|YP_001988303.1| hypothetical protein LCABL_23780 [Lactobacillus casei BL23]
gi|301067199|ref|YP_003789222.1| hypothetical protein LCAZH_2162 [Lactobacillus casei str. Zhang]
gi|239636053|ref|ZP_04673084.1| conserved hypothetical protein [Lactobacillus paracasei subsp. paracasei 8700:2]
gi|258509199|ref|YP_003171950.1| CRISPR-associated protein Csn1 [Lactobacillus rhamnosus GG]
gi|227509761|ref|ZP_03939810.1| possible CRISPR associated protein [Lactobacillus brevis subsp. gravesensis ATCC 27305]
gi|227512703|ref|ZP_03942752.1| CRISPR associated protein [Lactobacillus buchneri ATCC 11577]
gi|331702228|ref|YP_004399187.1| CRISPR-associated protein, Csn1 family [Lactobacillus buchneri NRRL B-30929]
gi|304386254|ref|ZP_07368587.1| csn1 family CRISPR-associated protein [Pediococcus acidilactici DSM 20284]
gi|300361537|ref|ZP_07057714.1| csn1 family CRISPR-associated protein [Lactobacillus gasseri JV-V03]
gi|385826041|ref|YP_005862383.1| CRISPR-associated protein [Lactobacillus johnsonii DPC 6026]
gi|238854367|ref|ZP_04644902.1| CRISPR-associated protein, Csn1 family [Lactobacillus jensenii 269-3]
gi|227514633|ref|ZP_03944682.1| possible CRISPR associated protein [Lactobacillus fermentum ATCC 14931]
gi|323340068|ref|ZP_08080334.1| CRISPR associated protein [Lactobacillus ruminis ATCC 25644]
gi|347534332|ref|YP_004841202.1| hypothetical protein LSA_08670 [Lactobacillus sanfranciscensis TMW 1.1304]
gi|310286728|ref|YP_003937986.1| CRISPR associated protein [Bifidobacterium bifidum S17]
gi|366983953|gb|EHN59352.1| CRISPR-associated protein [Oenococcus kitaharae DSM 17330]
gi|166018051|ref|NP_472073.1| hypothetical protein lin2744 [Listeria innocua Clip11262]
gi|470971481|ref|ZP_00234715.1| CRISPR-associated protein, SAG0894 family [Listeria monocytogenes str. 1/2a F6854]
gi|257893735|ref|ZP_05673388.1| CRISPR-associated protein [Enterococcus faecium 1,231,408]
gi|315641599|ref|ZP_07896667.1| csn1 family CRISPR-associated protein [Enterococcus italicus DSM 15952]
gi|116628213|ref|YP_820832.1| CRISPR system-like protein [Streptococcus thermophilus LMD-9]

FIG. 20E

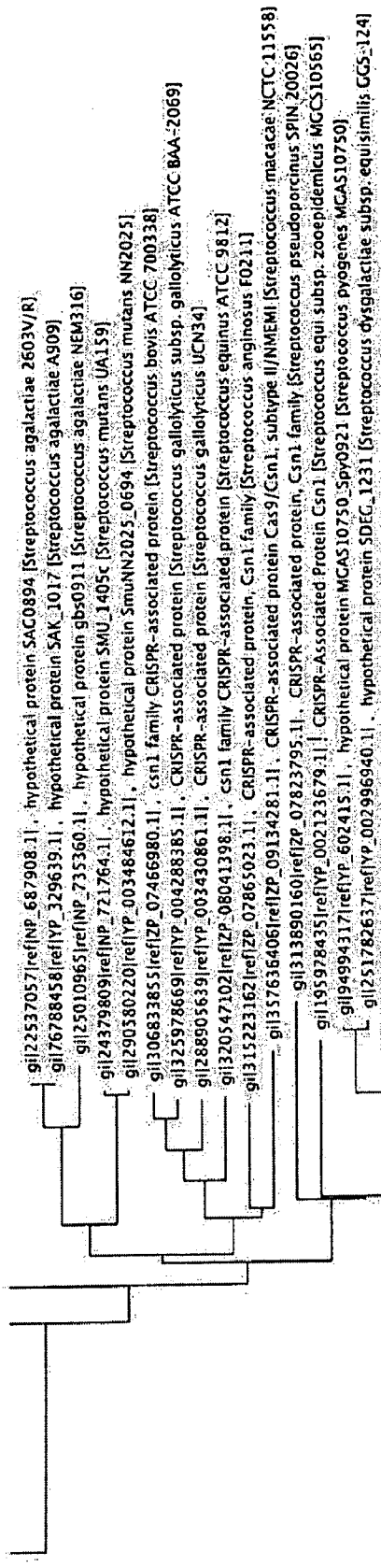

gi|22537057|ref|NP_687908.1| hypothetical protein SAG0894 [Streptococcus agalactiae 2603V/R]
gi|76788458|ref|YP_329639.1| hypothetical protein SAK_1017 [Streptococcus agalactiae A909]
gi|25010965|ref|NP_735360.1| hypothetical protein gbs0911 [Streptococcus agalactiae NEM316]
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gi|29038022O|ref|YP_003484612.1| hypothetical protein SmuNN2025_0694 [Streptococcus mutans NN2025]
gi|30683385S|ref|ZP_07466980.1| csn1 family CRISPR-associated protein [Streptococcus bovis ATCC 700338]
gi|32597866g|ref|YP_004288385.1| CRISPR-associated protein [Streptococcus gallolyticus subsp. gallolyticus ATCC BAA-2069]
gi|28890563g|ref|YP_003430861.1| CRISPR-associated protein [Streptococcus gallolyticus UCN34]
gi|32054710Z|ref|ZP_08041398.1| csn1 family CRISPR-associated protein [Streptococcus equinus ATCC 9812]
gi|31522316Z|ref|ZP_07865023.1| CRISPR-associated protein, Csn1 family [Streptococcus anginosus F0211]
gi|35763640G|ref|ZP_09134281.1| CRISPR-associated protein Cas9/Csn1, subtype II/NMEMI [Streptococcus macacae NCTC 11558]
gi|31389016O|ref|ZP_07823795.1| CRISPR-associated protein, Csn1 family [Streptococcus pseudoporcinus SPIN 20026]
gi|19597843S|ref|YP_002123679.1| CRISPR-Associated Protein Csn1 [Streptococcus equi subsp. zooepidemicus MGCS10565]
gi|94994317|ref|YP_602415.1| hypothetical protein MGAS10750_Spy0921 [Streptococcus pyogenes MGAS10750]
gi|25178263Z|ref|YP_002996940.1| hypothetical protein SDEG_1231 [Streptococcus dysgalactiae subsp. equisimilis GGS_124]
gi|21910213|ref|NP_664481.1| hypothetical protein SpyM3_0677 [Streptococcus pyogenes MGAS315]
gi|28896088|ref|NP_802438.1| hypothetical protein SPs1176 [Streptococcus pyogenes SSI-1]
gi|20955935G|ref|YP_002285828.1| hypothetical protein Spy49_0823 [Streptococcus pyogenes NZ131]
gi|15675041|ref|NP_269215.1| hypothetical protein SPy_1046 [Streptococcus pyogenes M1 GAS]
gi|71910582|ref|YP_282132.1| hypothetical protein M5005_Spy_0769 [Streptococcus pyogenes MGAS5005]
gi|94990395|ref|YP_598495.1| putative cytoplasmic protein [Streptococcus pyogenes MGAS10270]
gi|94988516|ref|YP_596617.1| cytoplasmic protein [Streptococcus pyogenes MGAS9429]
gi|94992340|ref|YP_600439.1| putative cytoplasmic protein [Streptococcus pyogenes MGAS2096]
gi|71903413|ref|YP_280216.1| cytoplasmic protein [Streptococcus pyogenes MGAS6180]

FIG. 20F

a

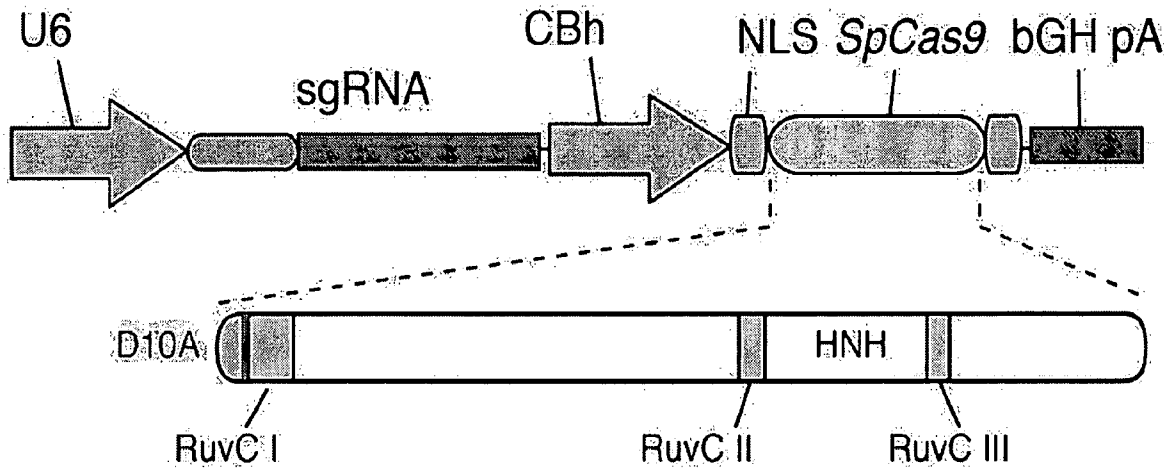



FIG. 21A

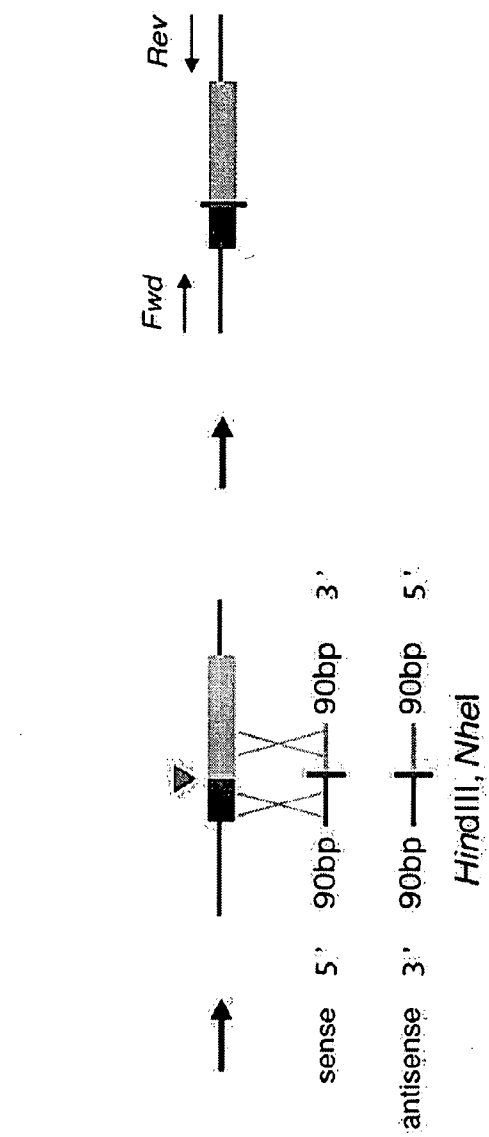

b
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ssODN
repair template
Fwd
Rev
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antisense
5'
3'
90bp
90bp
90bp
90bp
3'
5'
HindIII, NheI

FIG. 21B

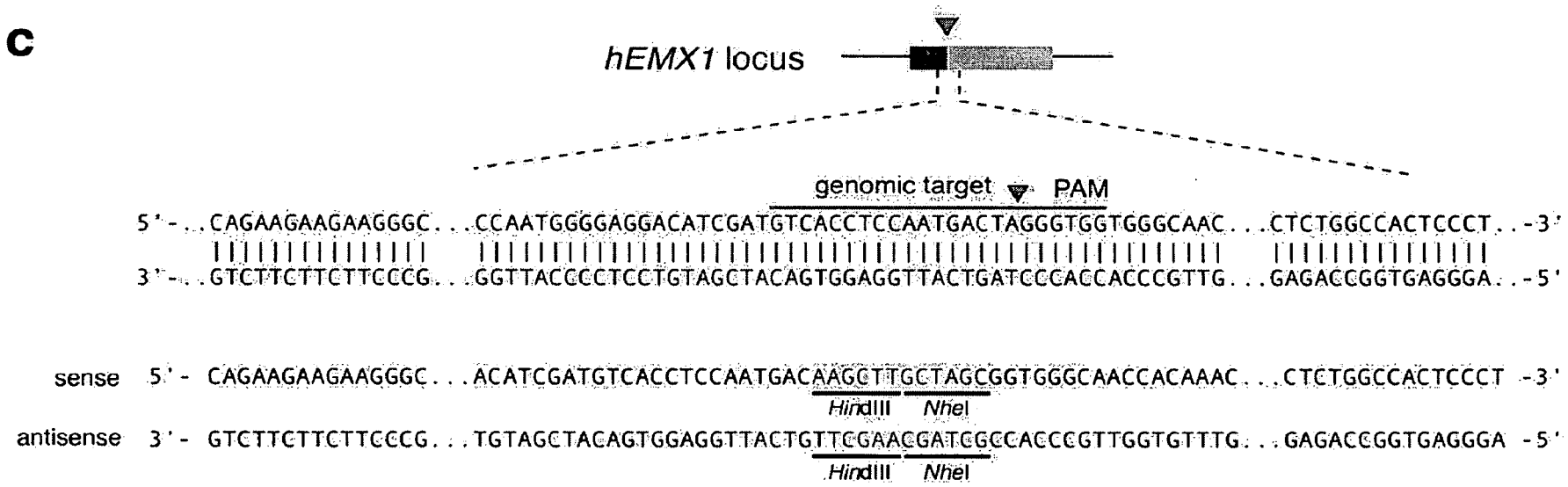

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5'-..CAGAAGAAGAAGGGC...CCAATGGGGAGGACATCGATGTCACCTCCAATGACTAGGGTGGTGGGCAAC...CTCTGGCCACTCCCT..-3'
3'-..GTCTTCTTCTTCCCG...GGTTACCCCTCCTGTAGCTACAGTGGAGGTTACTGATCCCACCACCCGTTG...GAGACCGGTGAGGGA..-5'
sense 5'- CAGAAGAAGAAGGGC...ACATCGATGTCACCTCCAATGACAAGCTTGCTAGCGGTGGGCAACCACAAAC...CTCTGGCCACTCCCT -3'
HindIII NheI
antisense 3'- GTCTTCTTCTTCCCG...TGTAGCTACAGTGGAGGTTACTGTTCGAACGATCGCCACCCGTTGGTGTTTG...GAGACCGGTGAGGGA -5'
HindIII NheI

FIG. 21C

d

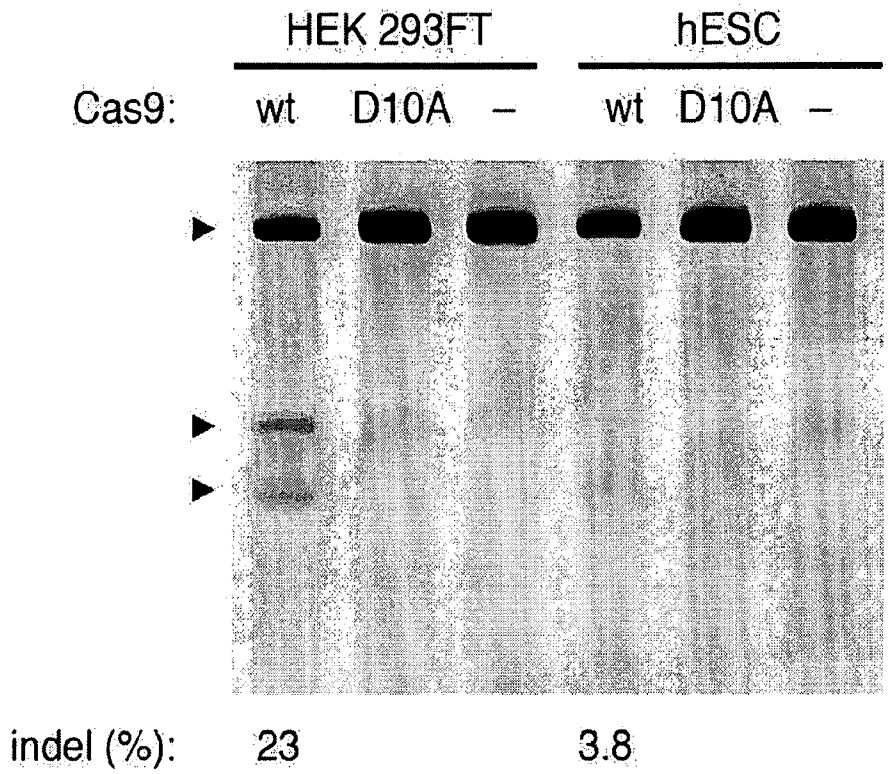



FIG. 21D

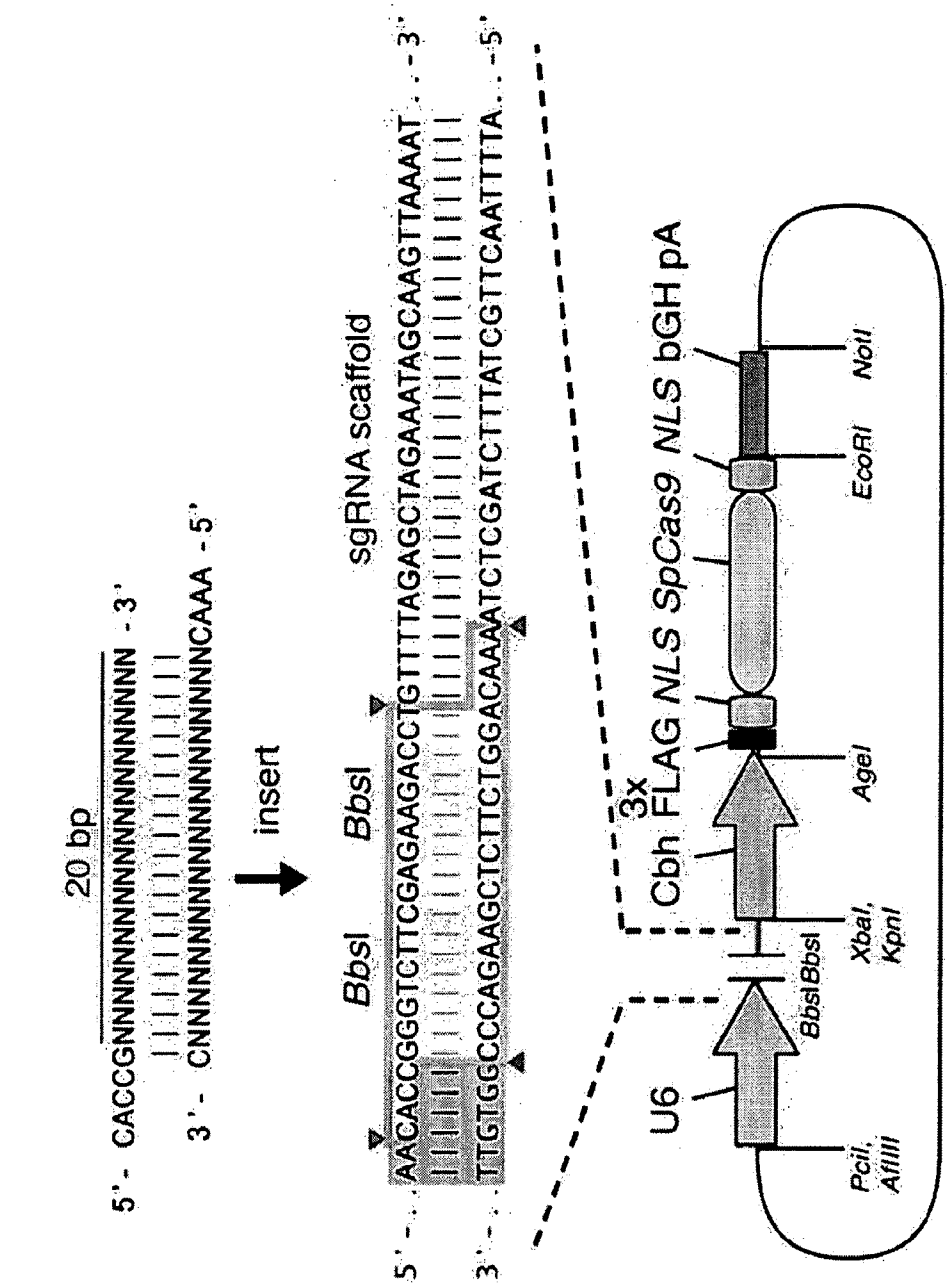

a
guide oligo (top)
(bottom)
20 bp
5' - CACCGNNNNNNNNNNNNNNNNNNN - 3'
3' - CNNNNNNNNNNNNNNNNNNNNCAAA - 5'
insert
guide oligo insertion site
BbsI
BbsI
sgRNA scaffold
5' - AACACCGGGTCTTCGAGAAGACCTGTTTTAGAGCTAGAAATAGCAAGTTAAAAT - 3'
3' - TTGTGGCCCAGAAGCTCTTCTGGACAAAATCTCGATCTTTATCGTTCAATTTTA - 5'
PX330
U6
3x
Cbh FLAG NLS SpCas9 NLS bGH pA
PciI, AflIII
BbsI BbsI
XbaI, KpnI
AgeI
EcoRI
NotI

FIG. 22A

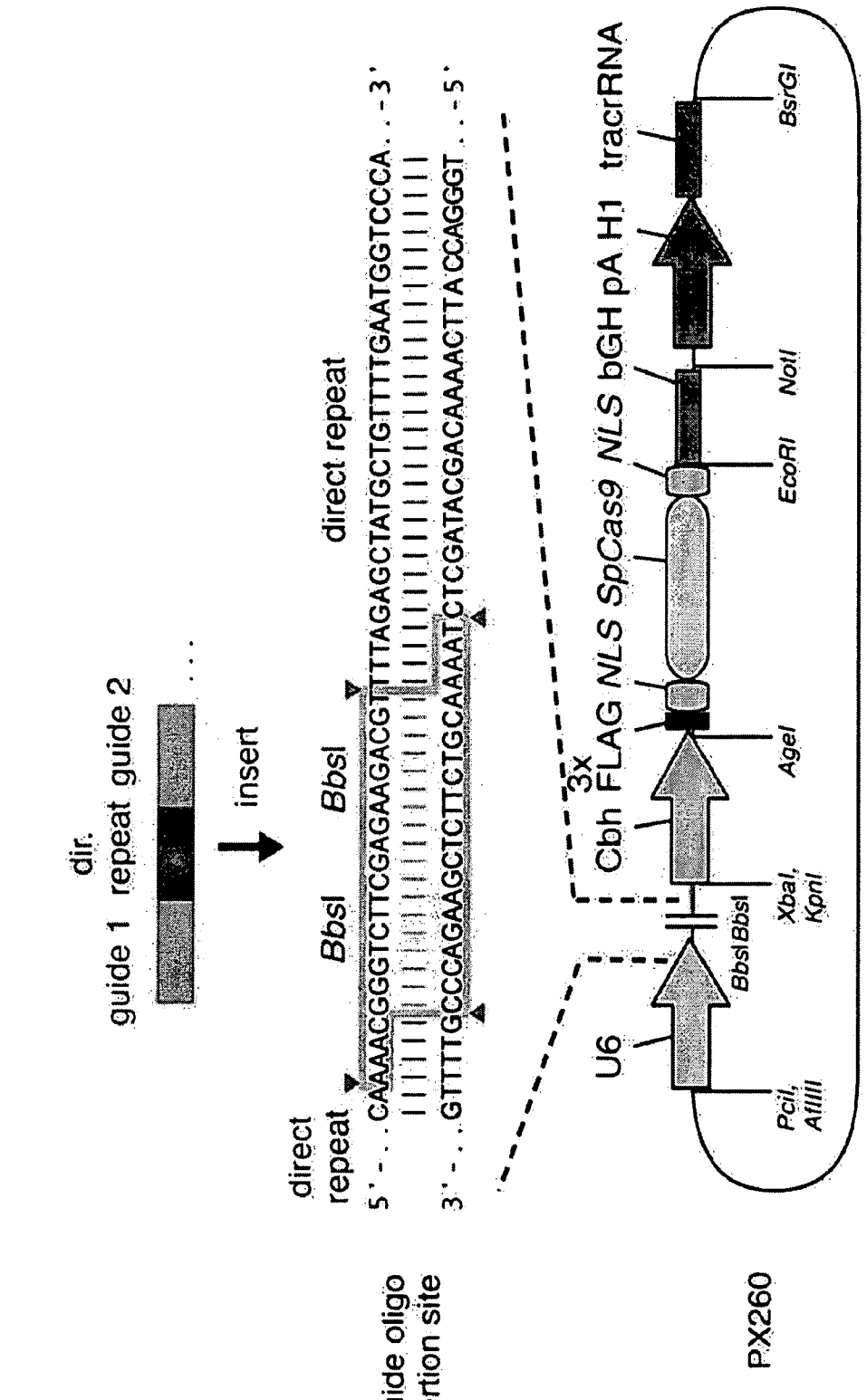

b
dir.
guide 1 repeat guide 2
insert
BbsI
BbsI
direct repeat
direct repeat
5' - . . . CAAAACGGGTCTTCGAGAAGACGTTTTAGAGCTATGCTGTTTTGAATGGTCCCA. . . - 3'
3' - . . . GTTTTGCCCAGAAGCTCTTCTGCAAAATCTCGATACGACAAAACTTACCAGGGT . . . - 5'
guide oligo
insertion site
3x
Cbh FLAG NLS SpCas9 NLS bGH pA H1 tracrRNA
U6
BbsI BbsI
XbaI,
KpnI
AgeI
EcoRI
NotI
BsrGI
PciI,
AflIII
PX260

FIG. 22B

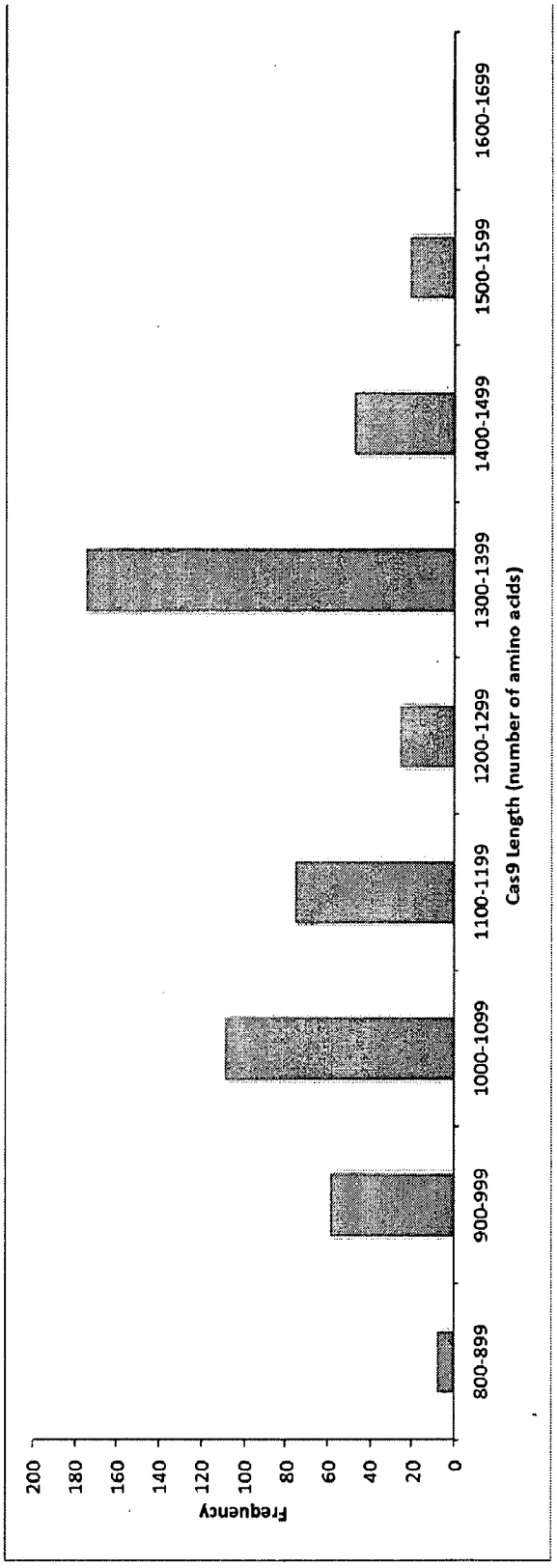

Frequency
200
180
160
140
120
100
80
60
40
20
0
800-899
900-999
1000-1099
1100-1199
1200-1299
1300-1399
1400-1499
1500-1599
1600-1699
Cas9 Length (number of amino acids)

FIG. 23

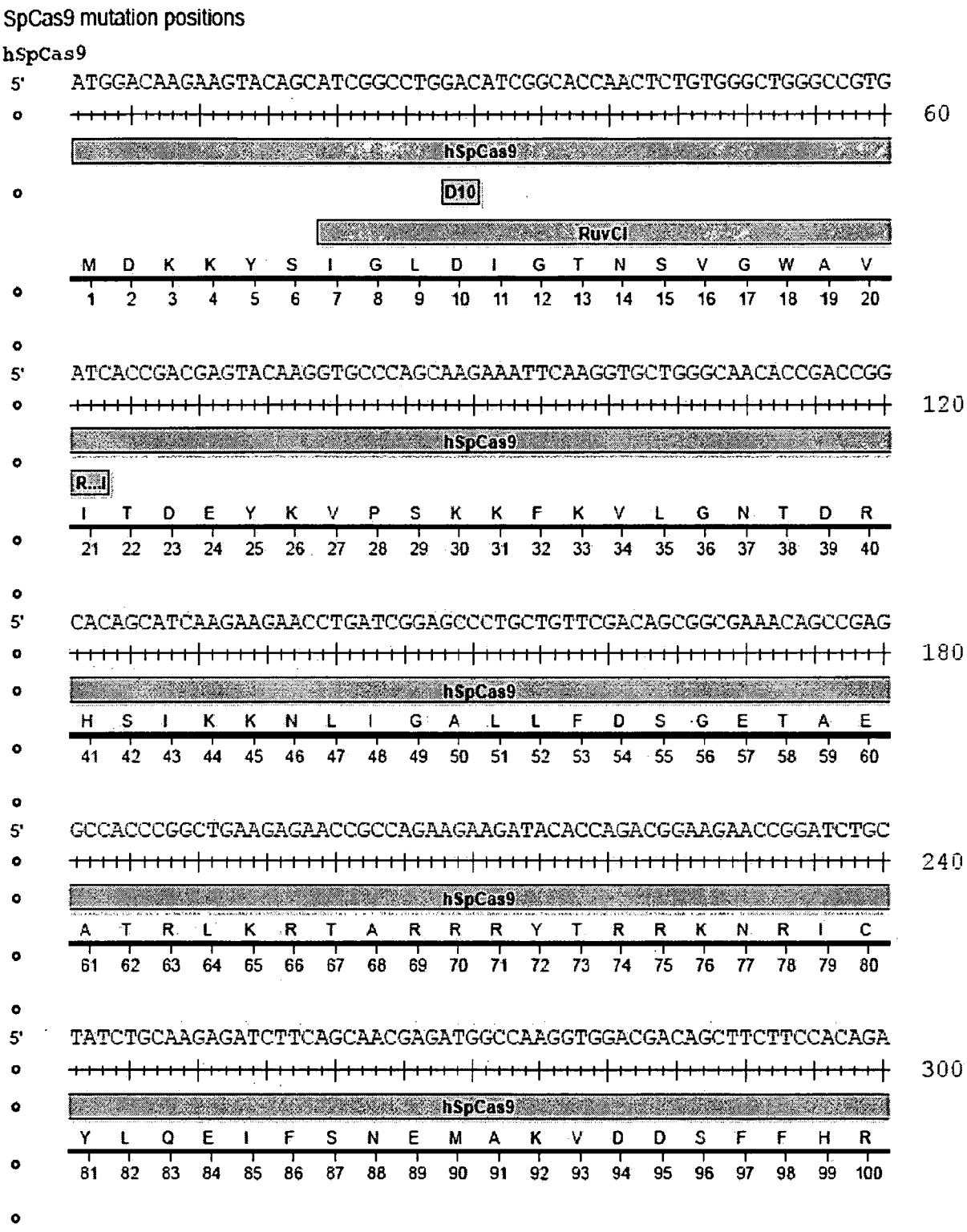

SpCas9 mutation positions
hSpCas9
5' ATGGACAAGAAGTACAGCATCGGCCTGGACATCGGCACCAACTCTGTGGGCTGGGCCGTG
60
hSpCas9
D10
RuvCI
M D K K Y S I G L D I G T N S V G W A V
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
5' ATCACCGACGAGTACAAGGTGCCCAGCAAGAAATTCAAGGTGCTGGGCAACACCGACCGG
120
hSpCas9
R...I
I T D E Y K V P S K K F K V L G N T D R
21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40
5' CACAGCATCAAGAAGAACCTGATCGGAGCCCTGCTGTTCGACAGCGGCGAAACAGCCGAG
180
hSpCas9
H S I K K N L I G A L L F D S G E T A E
41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60
5' GCCACCCGGCTGAAGAGAACCGCCAGAAGAAGATACACCAGACGGAAGAACCGGATCTGC
240
hSpCas9
A T R L K R T A R R R Y T R R K N R I C
61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80
5' TATCTGCAAGAGATCTTCAGCAACGAGATGGCCAAGGTGGACGACAGCTTCTTCCACAGA
300
hSpCas9
Y L Q E I F S N E M A K V D D S F F H R
81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100

FIG. 24A

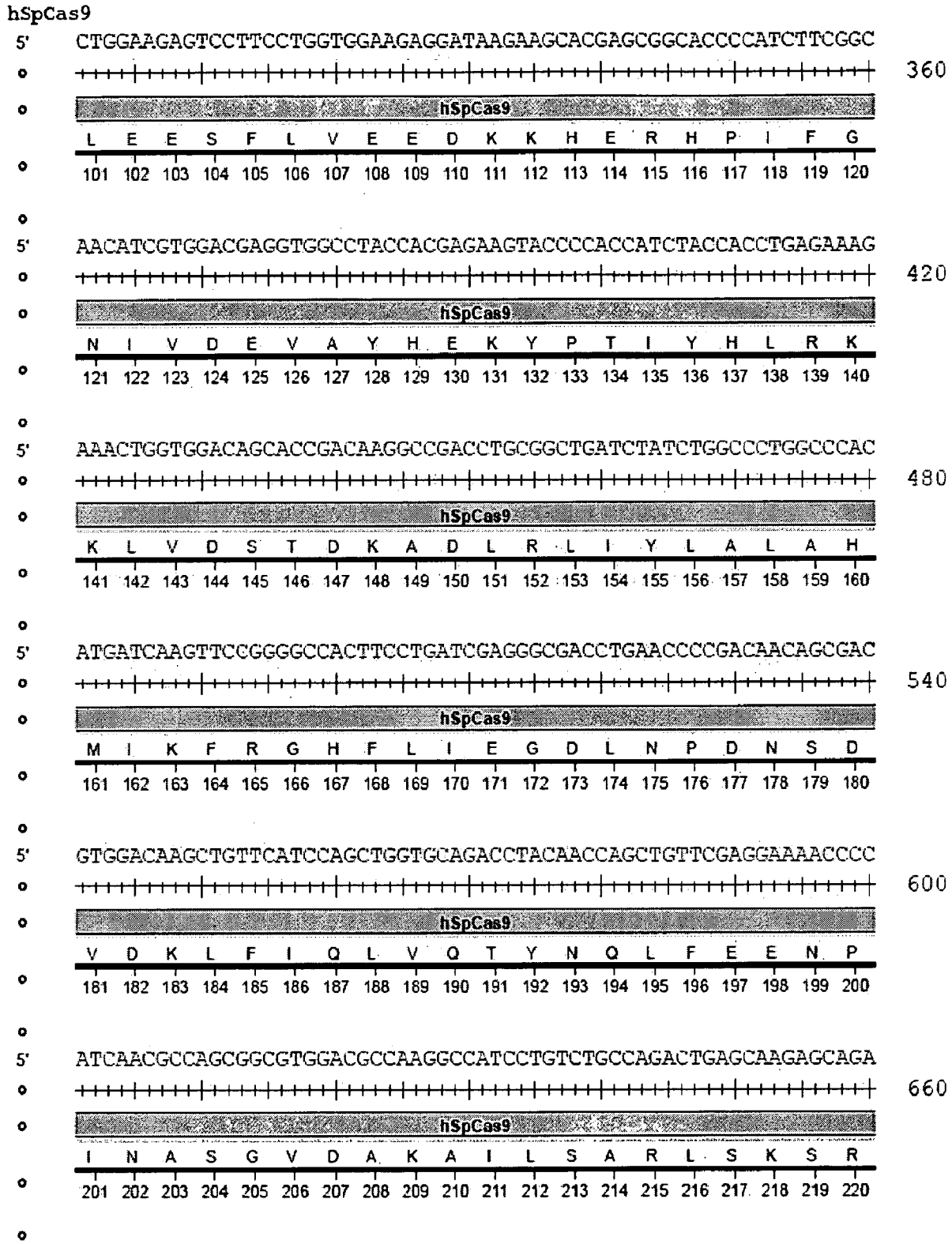

hSpCas9
5' CTGGAAGAGTCCTTCCTGGTGGAAGAGGATAAGAAGCACGAGCGGCACCCCATCTTCGGC
360
hSpCas9
L E E S F L V E E D K K H E R H P I F G
101 102 103 104 105 106 107 108 109 110 111 112 113 114 115 116 117 118 119 120
5' AACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCCCACCATCTACCACCTGAGAAAG
420
hSpCas9
N I V D E V A Y H E K Y P T I Y H L R K
121 122 123 124 125 126 127 128 129 130 131 132 133 134 135 136 137 138 139 140
5' AAACTGGTGGACAGCACCGACAAGGCCGACCTGCGGCTGATCTATCTGGCCCTGGCCCAC
480
hSpCas9
K L V D S T D K A D L R L I Y L A L A H
141 142 143 144 145 146 147 148 149 150 151 152 153 154 155 156 157 158 159 160
5' ATGATCAAGTTCCGGGGCCACTTCCTGATCGAGGGCGACCTGAACCCCGACAACAGCGAC
540
hSpCas9
M I K F R G H F L I E G D L N P D N S D
161 162 163 164 165 166 167 168 169 170 171 172 173 174 175 176 177 178 179 180
5' GTGGACAAGCTGTTCATCCAGCTGGTGCAGACCTACAACCAGCTGTTCGAGGAAAACCCC
600
hSpCas9
V D K L F I Q L V Q T Y N Q L F E E N P
181 182 183 184 185 186 187 188 189 190 191 192 193 194 195 196 197 198 199 200
5' ATCAACGCCAGCGGCGTGGACGCCAAGGCCATCCTGTCTGCCAGACTGAGCAAGAGCAGA
660
hSpCas9
I N A S G V D A K A I L S A R L S K S R
201 202 203 204 205 206 207 208 209 210 211 212 213 214 215 216 217 218 219 220

FIG. 24B

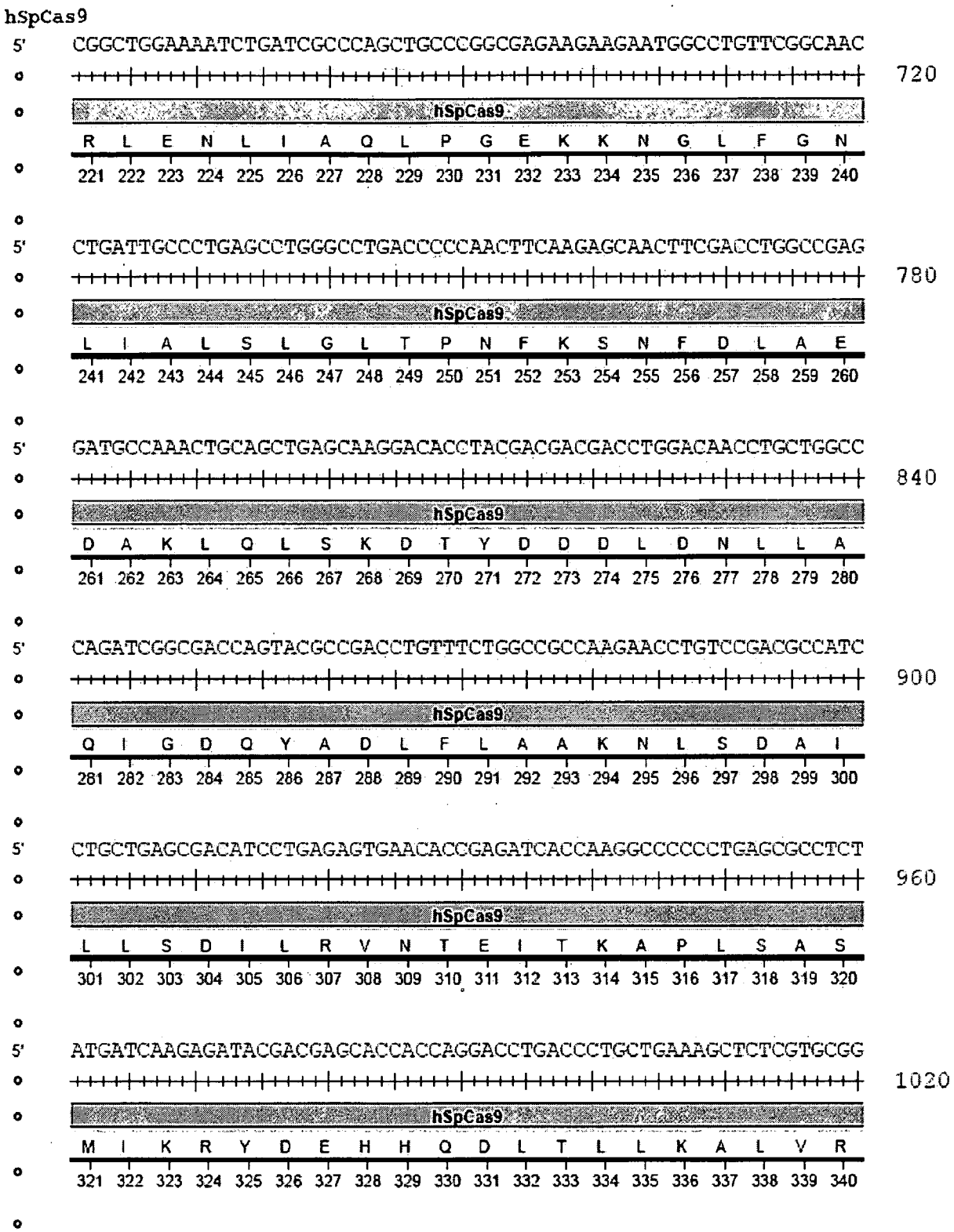

hSpCas9
5' CGGCTGGAAAATCTGATCGCCCAGCTGCCCGGCGAGAAGAAGAATGGCCTGTTCGGCAAC
720
hSpCas9
R L E N L I A Q L P G E K K N G L F G N
221 222 223 224 225 226 227 228 229 230 231 232 233 234 235 236 237 238 239 240
5' CTGATTGCCCTGAGCCTGGGCCTGACCCCCAACTTCAAGAGCAACTTCGACCTGGCCGAG
780
hSpCas9
L I A L S L G L T P N F K S N F D L A E
241 242 243 244 245 246 247 248 249 250 251 252 253 254 255 256 257 258 259 260
5' GATGCCAAACTGCAGCTGAGCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCC
840
hSpCas9
D A K L Q L S K D T Y D D D D L D N L L A
261 262 263 264 265 266 267 268 269 270 271 272 273 274 275 276 277 278 279 280
5' CAGATCGGCGACCAGTACGCCGACCTGTTTCTGGCCGCCAAGAACCTGTCCGACGCCATC
900
hSpCas9
Q I G D Q Y A D L F L A A K N L S D A I
281 282 283 284 285 286 287 288 289 290 291 292 293 294 295 296 297 298 299 300
5' CTGCTGAGCGACATCCTGAGAGTGAACACCGAGATCACCAAGGCCCCCCTGAGCGCCTCT
960
hSpCas9
L L S D I L R V N T E I T K A P L S A S
301 302 303 304 305 306 307 308 309 310 311 312 313 314 315 316 317 318 319 320
5' ATGATCAAGAGATACGACGAGCACCACCAGGACCTGACCCTGCTGAAAGCTCTCGTGCGG
1020
hSpCas9
M I K R Y D E H H Q D L T L L K A L V R
321 322 323 324 325 326 327 328 329 330 331 332 333 334 335 336 337 338 339 340

FIG. 24C

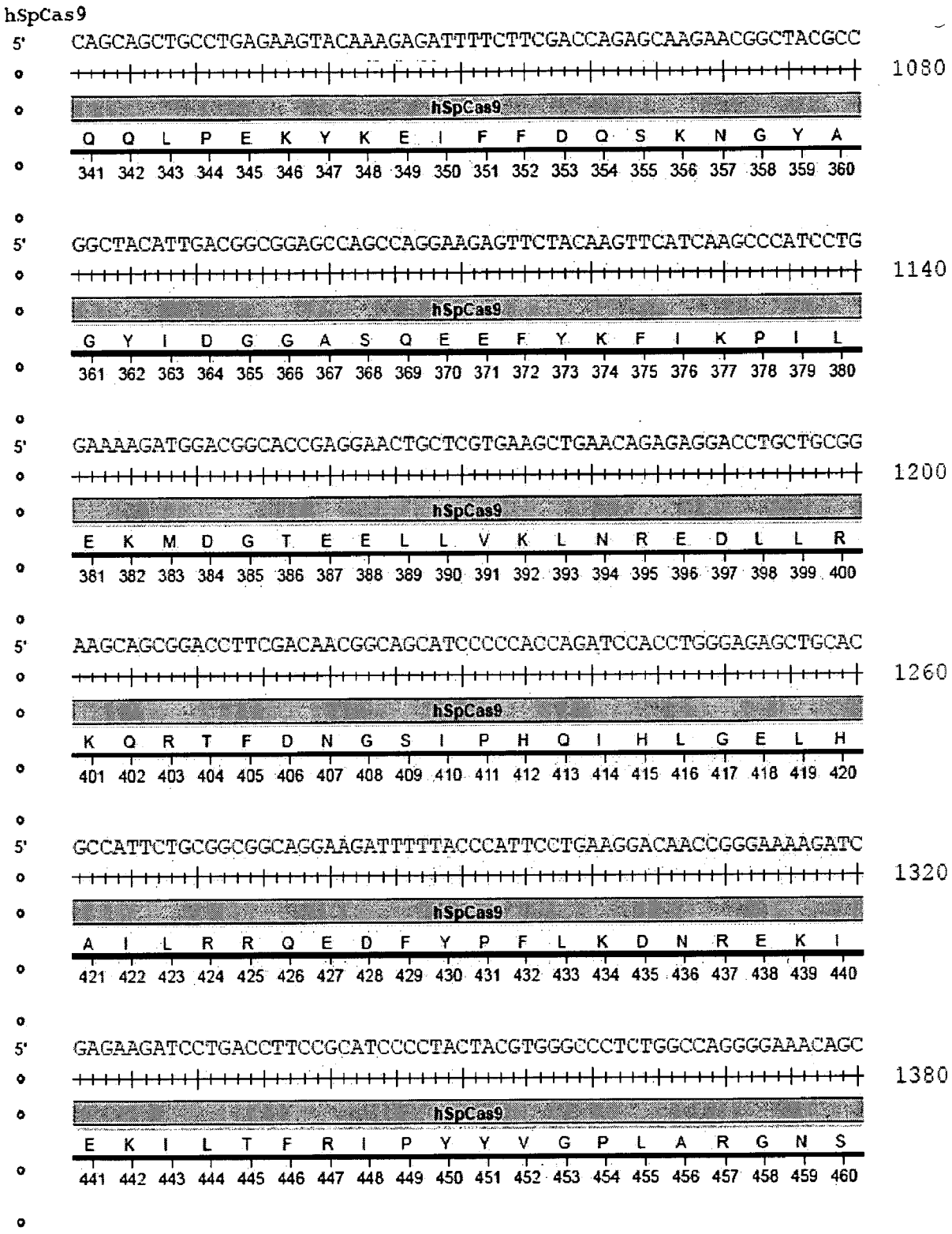

hSpCas9
5' CAGCAGCTGCCTGAGAAGTACAAAGAGATTTTCTTCGACCAGAGCAAGAACGGCTACGCC
1080
hSpCas9
Q Q L P E K Y K E I F F D Q S K N G Y A
341 342 343 344 345 346 347 348 349 350 351 352 353 354 355 356 357 358 359 360
5' GGCTACATTGACGGCGGAGCCAGCCAGGAAGAGTTCTACAAGTTCATCAAGCCCATCCTG
1140
hSpCas9
G Y I D G G A S Q E E F Y K F I K P I L
361 362 363 364 365 366 367 368 369 370 371 372 373 374 375 376 377 378 379 380
5' GAAAAGATGGACGGCACCGAGGAACTGCTCGTGAAGCTGAACAGAGAGGACCTGCTGCGG
1200
hSpCas9
E K M D G T E E L L V K L N R E D L L R
381 382 383 384 385 386 387 388 389 390 391 392 393 394 395 396 397 398 399 400
5' AAGCAGCGGACCTTCGACAACGGCAGCATCCCCCACCAGATCCACCTGGGAGAGCTGCAC
1260
hSpCas9
K Q R T F D N G S I P H Q I H L G E L H
401 402 403 404 405 406 407 408 409 410 411 412 413 414 415 416 417 418 419 420
5' GCCATTCTGCGGCGGCAGGAAGATTTTTACCCATTCCTGAAGGACAACCGGGAAAAGATC
1320
hSpCas9
A I L R R Q E D F Y P F L K D N R E K I
421 422 423 424 425 426 427 428 429 430 431 432 433 434 435 436 437 438 439 440
5' GAGAAGATCCTGACCTTCCGCATCCCCTACTACGTGGGCCCTCTGGCCAGGGGAAACAGC
1380
hSpCas9
E K I L T F R I P Y Y V G P L A R G N S
441 442 443 444 445 446 447 448 449 450 451 452 453 454 455 456 457 458 459 460

FIG. 24D

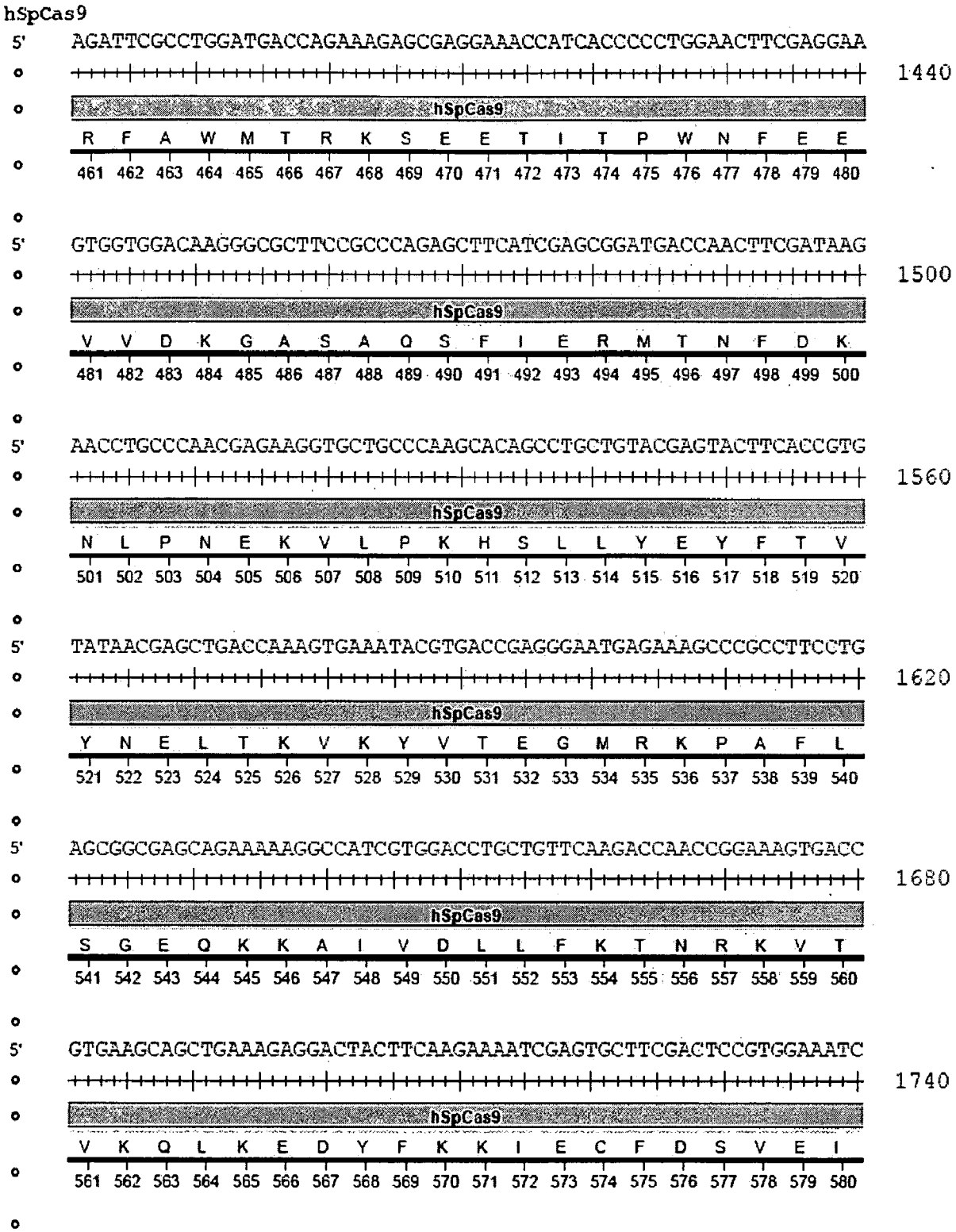

hSpCas9
5' AGATTCGCCTGGATGACCAGAAAGAGCGAGGAAACCATCACCCCCTGGAACTTCGAGGAA
1440
hSpCas9
R F A W M T R K S E E T I T P W N F E E
461 462 463 464 465 466 467 468 469 470 471 472 473 474 475 476 477 478 479 480
5' GTGGTGGACAAGGGCGCTTCCGCCCAGAGCTTCATCGAGCGGATGACCAACTTCGATAAG
1500
hSpCas9
V V D K G A S A Q S F I E R M T N F D K
481 482 483 484 485 486 487 488 489 490 491 492 493 494 495 496 497 498 499 500
5' AACCTGCCCAACGAGAAGGTGCTGCCCAAGCACAGCCTGCTGTACGAGTACTTCACCGTG
1560
hSpCas9
N L P N E K V L P K H S L L Y E Y F T V
501 502 503 504 505 506 507 508 509 510 511 512 513 514 515 516 517 518 519 520
5' TATAACGAGCTGACCAAAGTGAAATACGTGACCGAGGGAATGAGAAAGCCCGCCTTCCTG
1620
hSpCas9
Y N E L T K V K Y V T E G M R K P A F L
521 522 523 524 525 526 527 528 529 530 531 532 533 534 535 536 537 538 539 540
5' AGCGGCGAGCAGAAAAAGGCCATCGTGGACCTGCTGTTCAAGACCAACCGGAAAGTGACC
1680
hSpCas9
S G E Q K K A I V D L L F K T N R K V T
541 542 543 544 545 546 547 548 549 550 551 552 553 554 555 556 557 558 559 560
5' GTGAAGCAGCTGAAAGAGGACTACTTCAAGAAAATCGAGTGCTTCGACTCCGTGGAAATC
1740
hSpCas9
V K Q L K E D Y F K K I E C F D S V E I
561 562 563 564 565 566 567 568 569 570 571 572 573 574 575 576 577 578 579 580

FIG. 24E

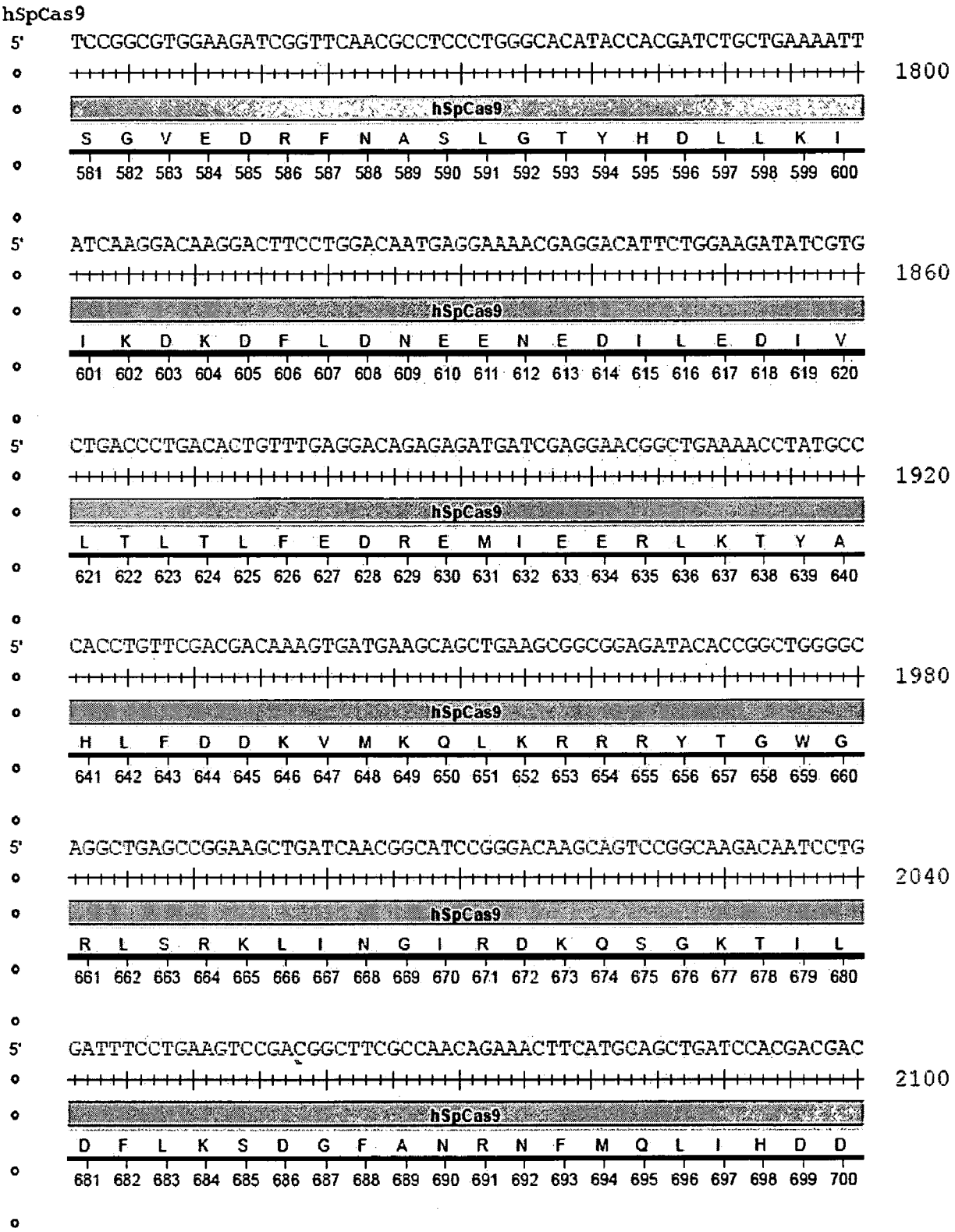

hSpCas9
5' TCCGGCGTGGAAGATCGGTTCAACGCCTCCCTGGGCACATACCACGATCTGCTGAAAATT
1800
hSpCas9
S G V E D R F N A S L G T Y H D L L K I
581 582 583 584 585 586 587 588 589 590 591 592 593 594 595 596 597 598 599 600
5' ATCAAGGACAAGGACTTCCTGGACAATGAGGAAAACGAGGACATTCTGGAAGATATCGTG
1860
hSpCas9
I K D K D F L D N E E N E D I L E D I V
601 602 603 604 605 606 607 608 609 610 611 612 613 614 615 616 617 618 619 620
5' CTGACCCTGACACTGTTTGAGGACAGAGAGATGATCGAGGAACGGCTGAAAACCTATGCC
1920
hSpCas9
L T L T L F E D R E M I E E R L K T Y A
621 622 623 624 625 626 627 628 629 630 631 632 633 634 635 636 637 638 639 640
5' CACCTGTTCGACGACAAAGTGATGAAGCAGCTGAAGCGGCGGAGATACACCGGCTGGGGC
1980
hSpCas9
H L F D D K V M K Q L K R R R Y T G W G
641 642 643 644 645 646 647 648 649 650 651 652 653 654 655 656 657 658 659 660
5' AGGCTGAGCCGGAAGCTGATCAACGGCATCCGGGACAAGCAGTCCGGCAAGACAATCCTG
2040
hSpCas9
R L S R K L I N G I R D K Q S G K T I L
661 662 663 664 665 666 667 668 669 670 671 672 673 674 675 676 677 678 679 680
5' GATTTCCTGAAGTCCGACGGCTTCGCCAACAGAAACTTCATGCAGCTGATCCACGACGAC
2100
hSpCas9
D F L K S D G F A N R N F M Q L I H D D
681 682 683 684 685 686 687 688 689 690 691 692 693 694 695 696 697 698 699 700

FIG. 24F

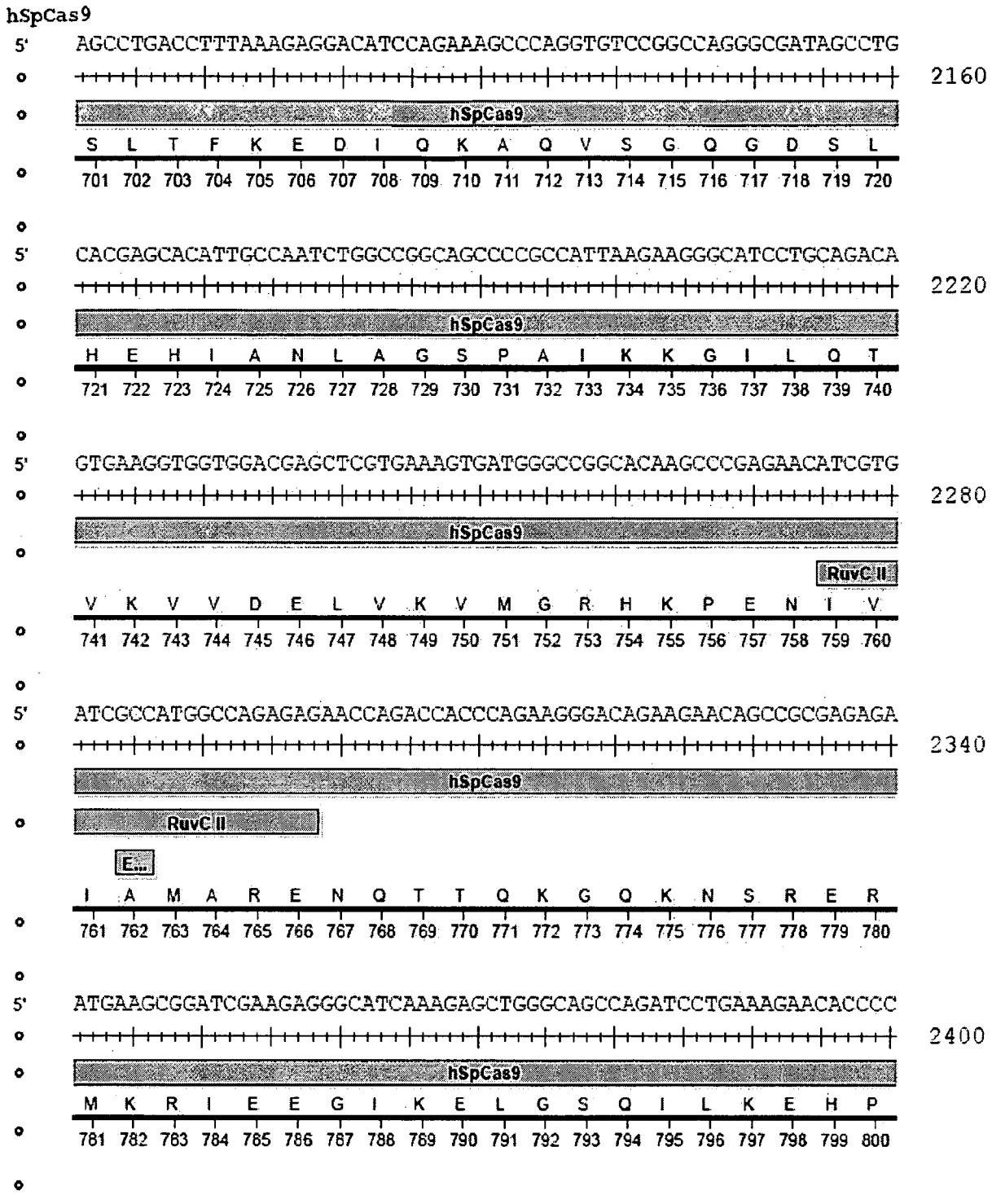

hSpCas9
5' AGCCTGACCTTTAAAGAGGACATCCAGAAAGCCCAGGTGTCCGGCCAGGGCGATAGCCTG
2160
hSpCas9
S L T F K E D I Q K A Q V S G Q G D S L
701 702 703 704 705 706 707 708 709 710 711 712 713 714 715 716 717 718 719 720
5' CACGAGCACATTGCCAATCTGGCCGGCAGCCCCGCCATTAAGAAGGGCATCCTGCAGACA
2220
hSpCas9
H E H I A N L A G S P A I K K G I L Q T
721 722 723 724 725 726 727 728 729 730 731 732 733 734 735 736 737 738 739 740
5' GTGAAGGTGGTGGACGAGCTCGTGAAAGTGATGGGCCGGCACAAGCCCGAGAACATCGTG
2280
hSpCas9
RuvC II
V K V V D E L V K V M G R H K P E N I V
741 742 743 744 745 746 747 748 749 750 751 752 753 754 755 756 757 758 759 760
5' ATCGCCATGGCCAGAGAGAACCAGACCACCCAGAAGGGACAGAAGAACAGCCGCGAGAGA
2340
hSpCas9
RuvC II
E...
I A M A R E N Q T T Q K G Q K N S R E R
761 762 763 764 765 766 767 768 769 770 771 772 773 774 775 776 777 778 779 780
5' ATGAAGCGGATCGAAGAGGGCATCAAAGAGCTGGGCAGCCAGATCCTGAAAGAACACCCC
2400
hSpCas9
M K R I E E G I K E L G S Q I L K E H P
781 782 783 784 785 786 787 788 789 790 791 792 793 794 795 796 797 798 799 800

FIG. 24G

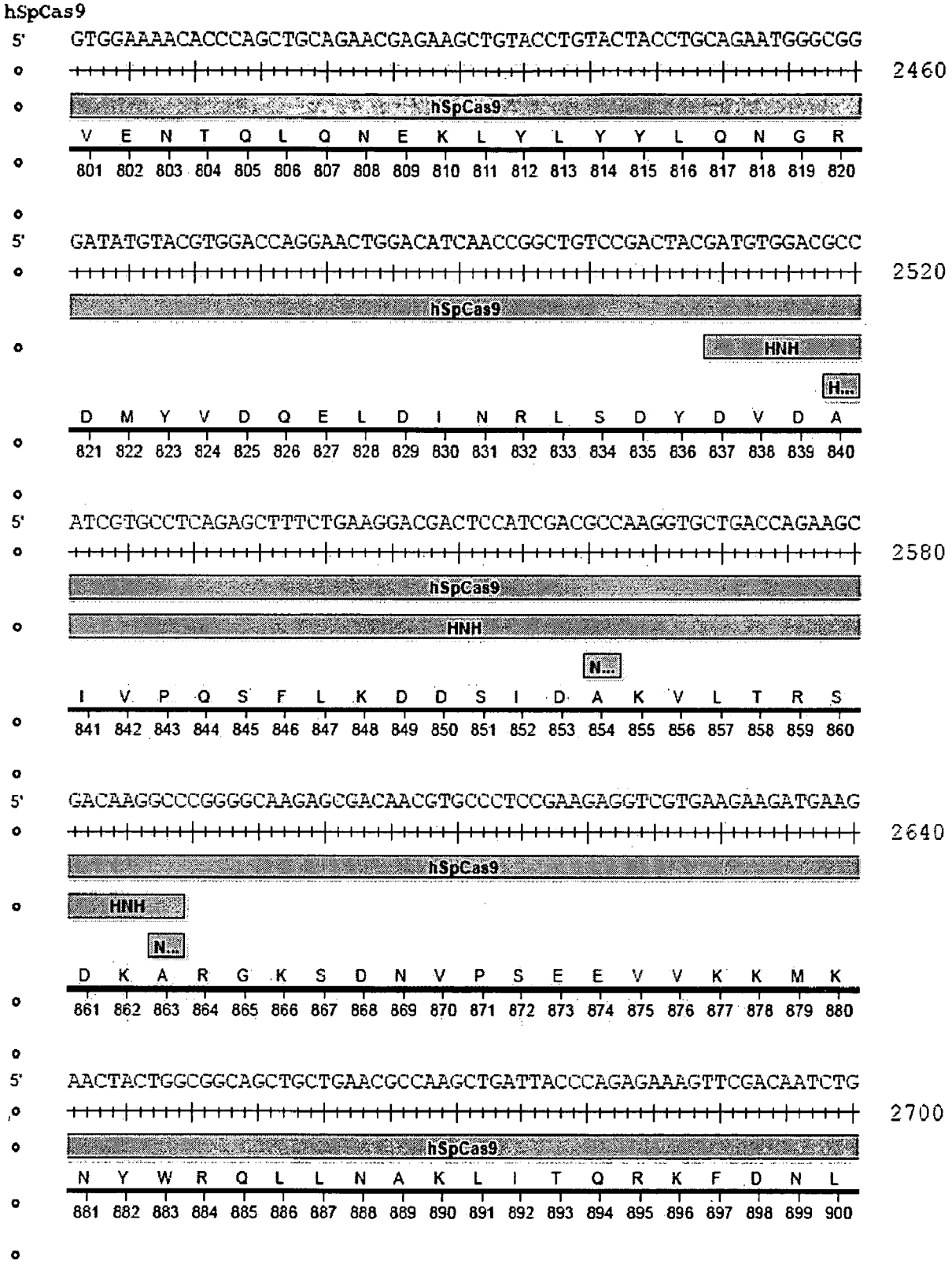

hSpCas9
5' GTGGAAAACACCCAGCTGCAGAACGAGAAGCTGTACCTGTACTACCTGCAGAATGGGCGG
2460
hSpCas9
V E N T Q L Q N E K L Y L Y Y L Q N G R
801 802 803 804 805 806 807 808 809 810 811 812 813 814 815 816 817 818 819 820
5' GATATGTACGTGGACCAGGAACTGGACATCAACCGGCTGTCCGACTACGATGTGGACGCC
2520
hSpCas9
HNH
H...
D M Y V D Q E L D I N R L S D Y D V D A
821 822 823 824 825 826 827 828 829 830 831 832 833 834 835 836 837 838 839 840
5' ATCGTGCCTCAGAGCTTTCTGAAGGACGACTCCATCGACGCCAAGGTGCTGACCAGAAGC
2580
hSpCas9
HNH
N...
I V P Q S F L K D D S I D A K V L T R S
841 842 843 844 845 846 847 848 849 850 851 852 853 854 855 856 857 858 859 860
5' GACAAGGCCCGGGGCAAGAGCGACAACGTGCCCTCCGAAGAGGTCGTGAAGAAGATGAAG
2640
hSpCas9
HNH
N...
D K A R G K S D N V P S E E V V K K M K
861 862 863 864 865 866 867 868 869 870 871 872 873 874 875 876 877 878 879 880
5' AACTACTGGCGGCAGCTGCTGAACGCCAAGCTGATTACCCAGAGAAAGTTCGACAATCTG
2700
hSpCas9
N Y W R Q L L N A K L I T Q R K F D N L
881 882 883 884 885 886 887 888 889 890 891 892 893 894 895 896 897 898 899 900

FIG. 24H

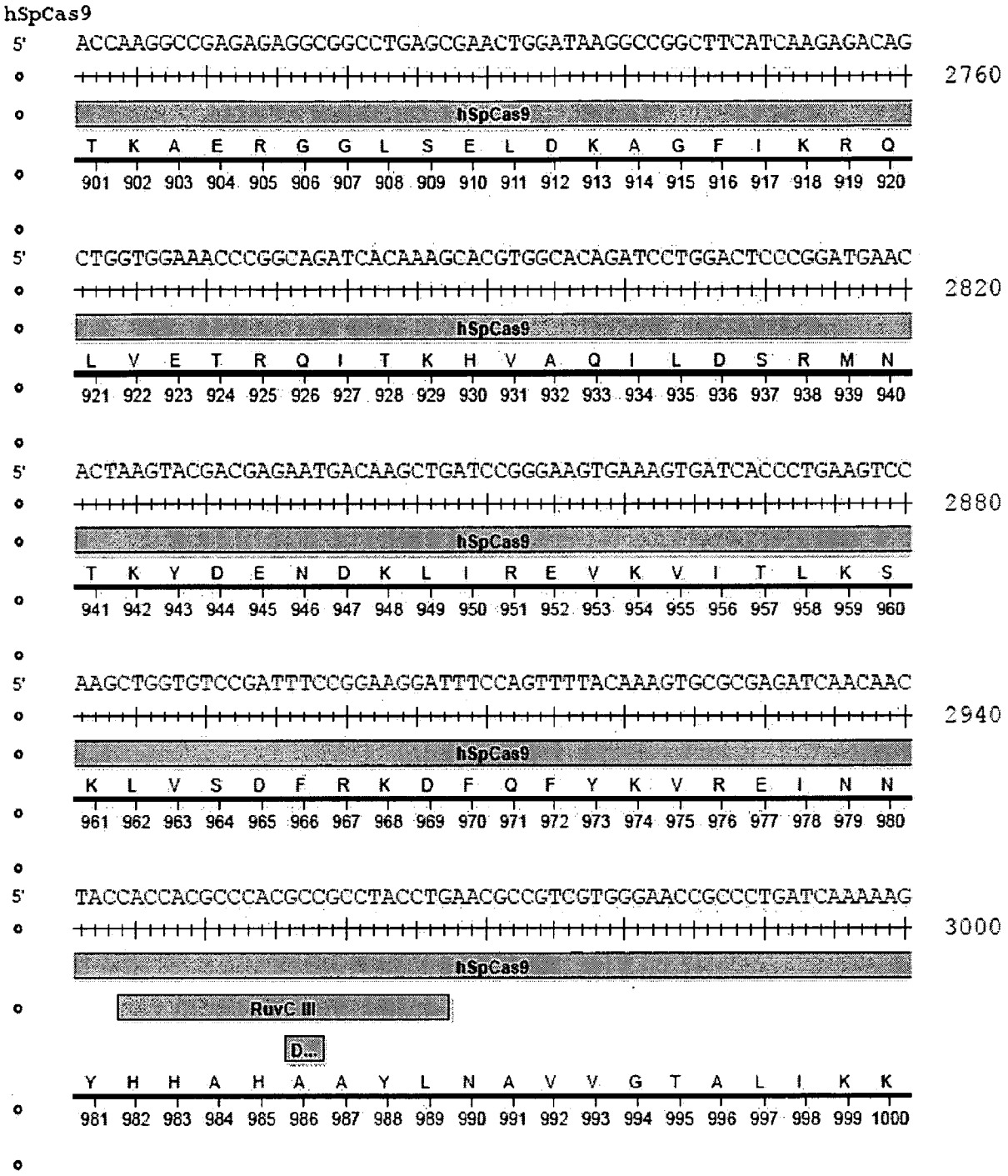

hSpCas9
5' ACCAAGGCCGAGAGAGGCGGCCTGAGCGAACTGGATAAGGCCGGCTTCATCAAGAGACAG
2760
hSpCas9
T K A E R G G L S E L D K A G F I K R Q
901 902 903 904 905 906 907 908 909 910 911 912 913 914 915 916 917 918 919 920
5' CTGGTGGAAACCCGGCAGATCACAAAGCACGTGGCACAGATCCTGGACTCCCGGATGAAC
2820
hSpCas9
L V E T R Q I T K H V A Q I L D S R M N
921 922 923 924 925 926 927 928 929 930 931 932 933 934 935 936 937 938 939 940
5' ACTAAGTACGACGAGAATGACAAGCTGATCCGGGAAGTGAAAGTGATCACCCTGAAGTCC
2880
hSpCas9
T K Y D E N D K L I R E V K V I T L K S
941 942 943 944 945 946 947 948 949 950 951 952 953 954 955 956 957 958 959 960
5' AAGCTGGTGTCCGATTTCCGGAAGGATTTCCAGTTTTACAAAGTGCGCGAGATCAACAAC
2940
hSpCas9
K L V S D F R K D F Q F Y K V R E I N N
961 962 963 964 965 966 967 968 969 970 971 972 973 974 975 976 977 978 979 980
5' TACCACCACGCCCACGCCGCCTACCTGAACGCCGTCGTGGGAACCGCCCTGATCAAAAAG
3000
hSpCas9
RuvC III
D...
Y H H A H A A Y L N A V V G T A L I K K
981 982 983 984 985 986 987 988 989 990 991 992 993 994 995 996 997 998 999 1000

FIG. 24I

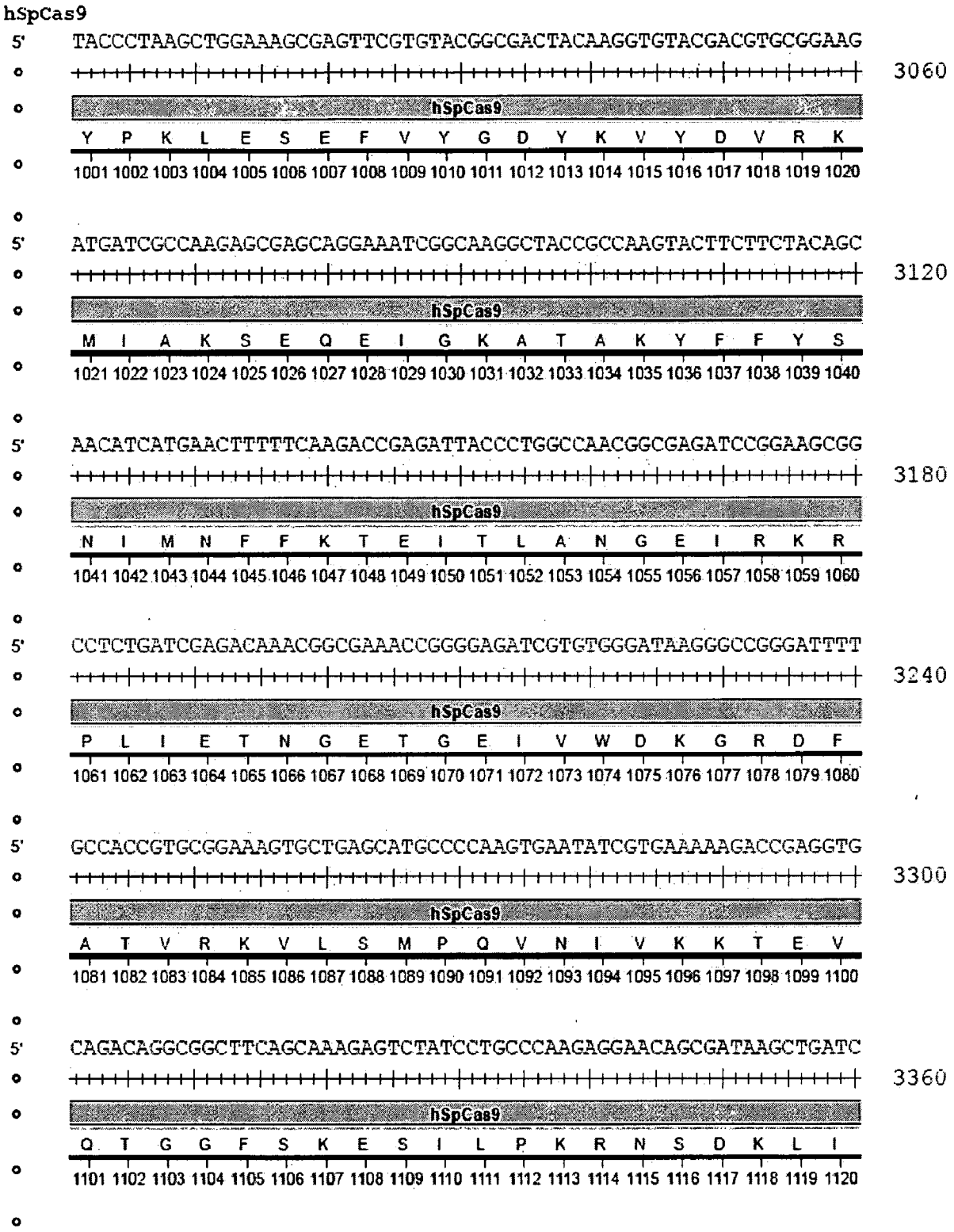

hSpCas9
5' TACCCTAAGCTGGAAAGCGAGTTCGTGTACGGCGACTACAAGGTGTACGACGTGCGGAAG
3060
hSpCas9
Y P K L E S E F V Y G D Y K V Y D V R K
1001 1002 1003 1004 1005 1006 1007 1008 1009 1010 1011 1012 1013 1014 1015 1016 1017 1018 1019 1020
5' ATGATCGCCAAGAGCGAGCAGGAAATCGGCAAGGCTACCGCCAAGTACTTCTTCTACAGC
3120
hSpCas9
M I A K S E Q E I G K A T A K Y F F Y S
1021 1022 1023 1024 1025 1026 1027 1028 1029 1030 1031 1032 1033 1034 1035 1036 1037 1038 1039 1040
5' AACATCATGAACTTTTTCAAGACCGAGATTACCCTGGCCAACGGCGAGATCCGGAAGCGG
3180
hSpCas9
N I M N F F K T E I T L A N G E I R K R
1041 1042 1043 1044 1045 1046 1047 1048 1049 1050 1051 1052 1053 1054 1055 1056 1057 1058 1059 1060
5' CCTCTGATCGAGACAAACGGCGAAACCGGGGAGATCGTGTGGGATAAGGGCCGGGATTTT
3240
hSpCas9
P L I E T N G E T G E I V W D K G R D F
1061 1062 1063 1064 1065 1066 1067 1068 1069 1070 1071 1072 1073 1074 1075 1076 1077 1078 1079 1080
5' GCCACCGTGCGGAAAGTGCTGAGCATGCCCCAAGTGAATATCGTGAAAAAGACCGAGGTG
3300
hSpCas9
A T V R K V L S M P Q V N I V K K T E V
1081 1082 1083 1084 1085 1086 1087 1088 1089 1090 1091 1092 1093 1094 1095 1096 1097 1098 1099 1100
5' CAGACAGGCGGCTTCAGCAAAGAGTCTATCCTGCCCAAGAGGAACAGCGATAAGCTGATC
3360
hSpCas9
Q T G G F S K E S I L P K R N S D K L I
1101 1102 1103 1104 1105 1106 1107 1108 1109 1110 1111 1112 1113 1114 1115 1116 1117 1118 1119 1120

FIG. 24J

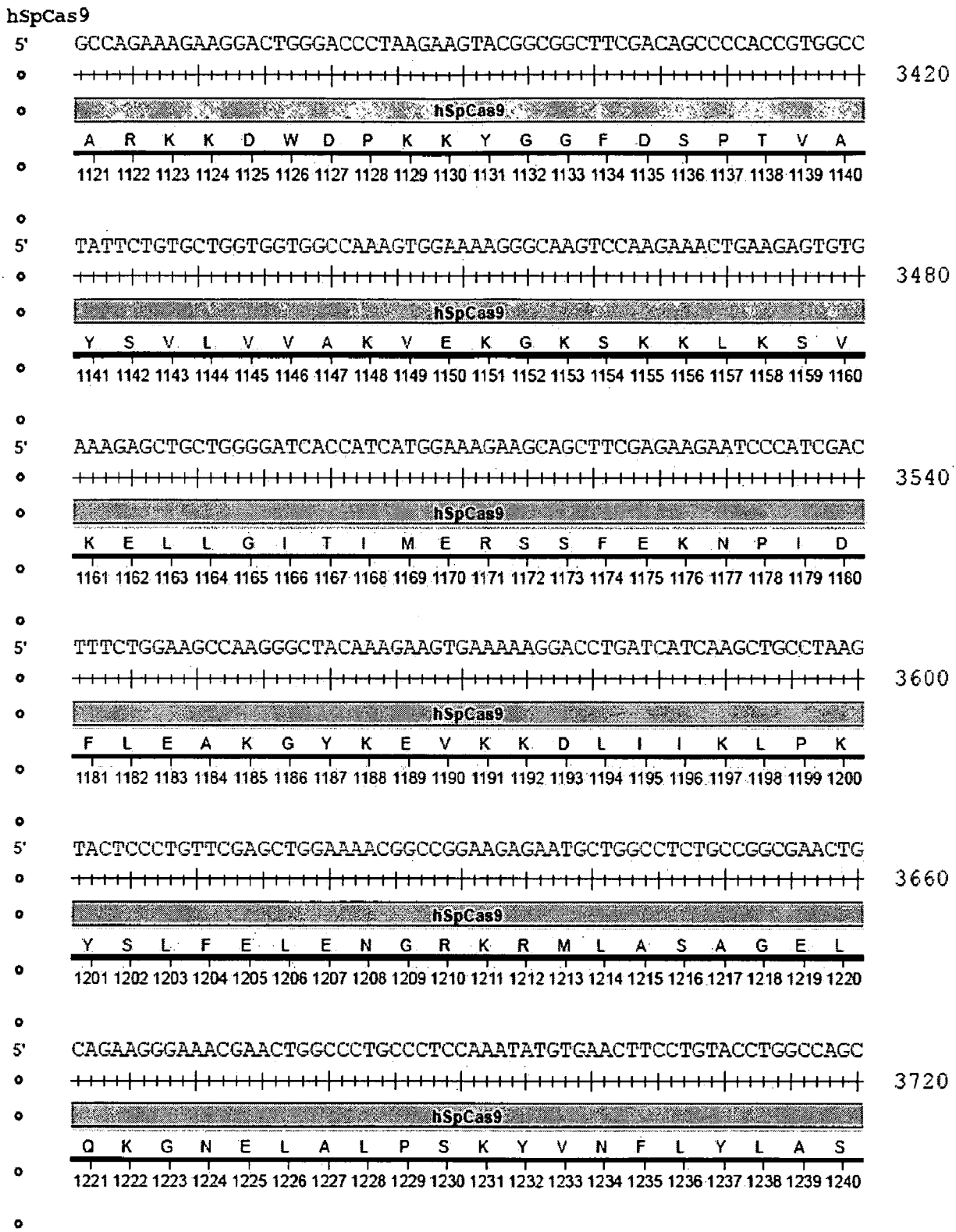

hSpCas9
5' GCCAGAAAGAAGGACTGGGACCCTAAGAAGTACGGCGGCTTCGACAGCCCCACCGTGGCC
3420
hSpCas9
A R K K D W D P K K Y G G F D S P T V A
1121 1122 1123 1124 1125 1126 1127 1128 1129 1130 1131 1132 1133 1134 1135 1136 1137 1138 1139 1140
5' TATTCTGTGCTGGTGGTGGCCAAAGTGGAAAAGGGCAAGTCCAAGAAACTGAAGAGTGTG
3480
hSpCas9
Y S V L V V A K V E K G K S K K L K S V
1141 1142 1143 1144 1145 1146 1147 1148 1149 1150 1151 1152 1153 1154 1155 1156 1157 1158 1159 1160
5' AAAGAGCTGCTGGGGATCACCATCATGGAAAGAAGCAGCTTCGAGAAGAATCCCATCGAC
3540
hSpCas9
K E L L G I T I M E R S S F E K N P I D
1161 1162 1163 1164 1165 1166 1167 1168 1169 1170 1171 1172 1173 1174 1175 1176 1177 1178 1179 1180
5' TTTCTGGAAGCCAAGGGCTACAAAGAAGTGAAAAAGGACCTGATCATCAAGCTGCCTAAG
3600
hSpCas9
F L E A K G Y K E V K K D L I I K L P K
1181 1182 1183 1184 1185 1186 1187 1188 1189 1190 1191 1192 1193 1194 1195 1196 1197 1198 1199 1200
5' TACTCCCTGTTCGAGCTGGAAAACGGCCGGAAGAGAATGCTGGCCTCTGCCGGCGAACTG
3660
hSpCas9
Y S L F E L E N G R K R M L A S A G E L
1201 1202 1203 1204 1205 1206 1207 1208 1209 1210 1211 1212 1213 1214 1215 1216 1217 1218 1219 1220
5' CAGAAGGGAAACGAACTGGCCCTGCCCTCCAAATATGTGAACTTCCTGTACCTGGCCAGC
3720
hSpCas9
Q K G N E L A L P S K Y V N F L Y L A S
1221 1222 1223 1224 1225 1226 1227 1228 1229 1230 1231 1232 1233 1234 1235 1236 1237 1238 1239 1240

FIG. 24K

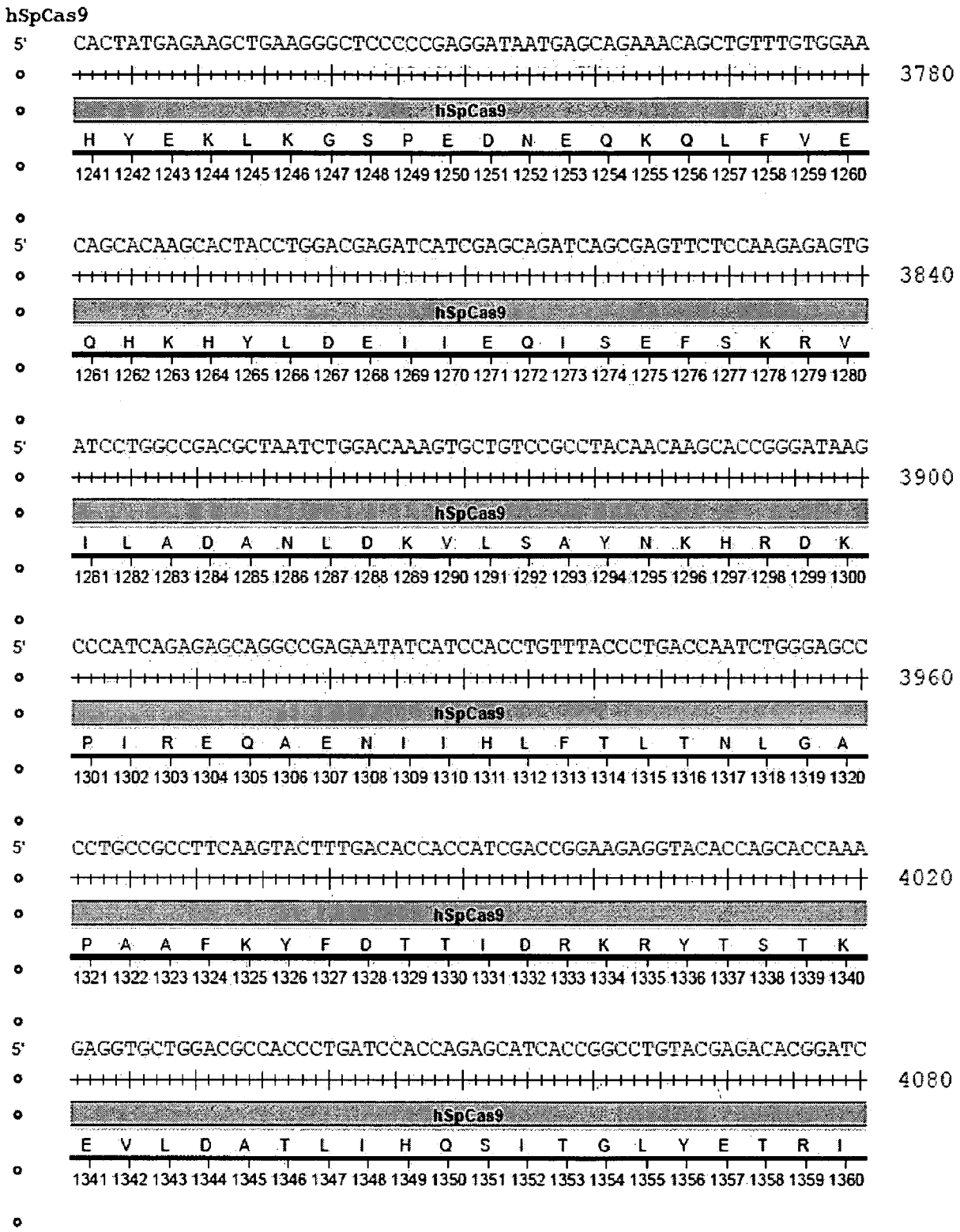

hSpCas9
5' CACTATGAGAAGCTGAAGGGCTCCCCCGAGGATAATGAGCAGAAACAGCTGTTTGTGGAA
3780
hSpCas9
H Y E K L K G S P E D N E Q K Q L F V E
1241 1242 1243 1244 1245 1246 1247 1248 1249 1250 1251 1252 1253 1254 1255 1256 1257 1258 1259 1260
5' CAGCACAAGCACTACCTGGACGAGATCATCGAGCAGATCAGCGAGTTCTCCAAGAGAGTG
3840
hSpCas9
Q H K H Y L D E I I E Q I S E F S K R V
1261 1262 1263 1264 1265 1266 1267 1268 1269 1270 1271 1272 1273 1274 1275 1276 1277 1278 1279 1280
5' ATCCTGGCCGACGCTAATCTGGACAAAGTGCTGTCCGCCTACAACAAGCACCGGGATAAG
3900
hSpCas9
I L A D A N L D K V L S A Y N K H R D K
1281 1282 1283 1284 1285 1286 1287 1288 1289 1290 1291 1292 1293 1294 1295 1296 1297 1298 1299 1300
5' CCCATCAGAGAGCAGGCCGAGAATATCATCCACCTGTTTACCCTGACCAATCTGGGAGCC
3960
hSpCas9
P I R E Q A E N I I H L F T L T N L G A
1301 1302 1303 1304 1305 1306 1307 1308 1309 1310 1311 1312 1313 1314 1315 1316 1317 1318 1319 1320
5' CCTGCCGCCTTCAAGTACTTTGACACCACCATCGACCGGAAGAGGTACACCAGCACCAAA
4020
hSpCas9
P A A F K Y F D T T I D R K R Y T S T K
1321 1322 1323 1324 1325 1326 1327 1328 1329 1330 1331 1332 1333 1334 1335 1336 1337 1338 1339 1340
5' GAGGTGCTGGACGCCACCCTGATCCACCAGAGCATCACCGGCCTGTACGAGACACGGATC
4080
hSpCas9
E V L D A T L I H Q S I T G L Y E T R I
1341 1342 1343 1344 1345 1346 1347 1348 1349 1350 1351 1352 1353 1354 1355 1356 1357 1358 1359 1360

FIG. 24L

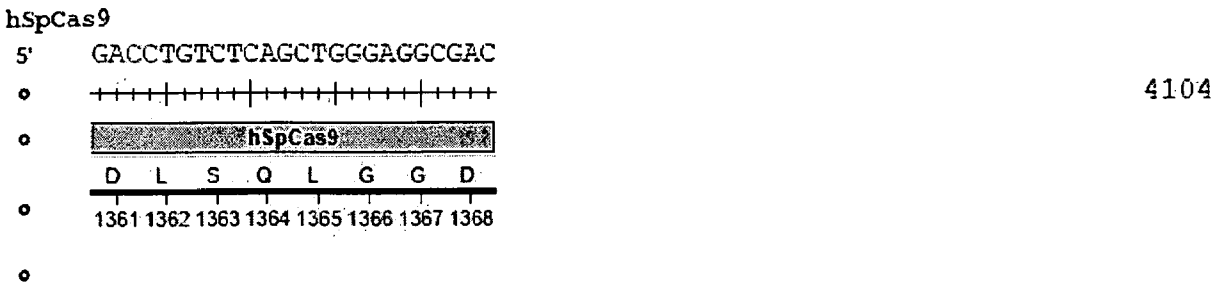

hSpCas9
5'
GACCTGTCTCAGCTGGGAGGCGAC
4104
hSpCas9
D L S Q L G G D
1361 1362 1363 1364 1365 1366 1367 1368

FIG. 24M

Conditional Cas9, Rosa26 targeting vector map

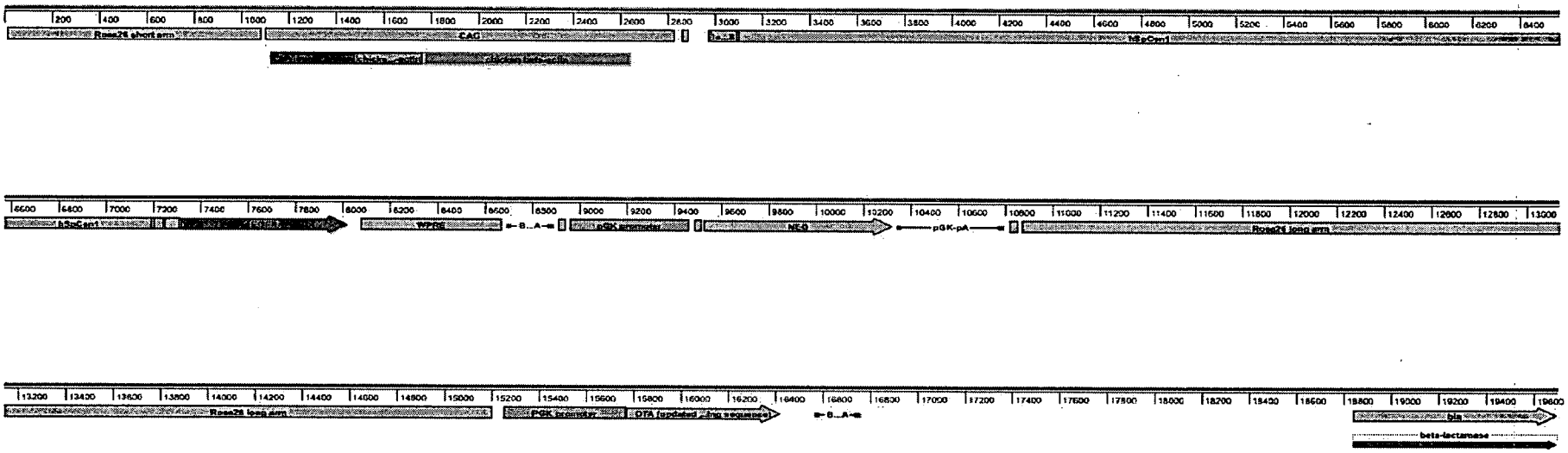



FIG. 25A

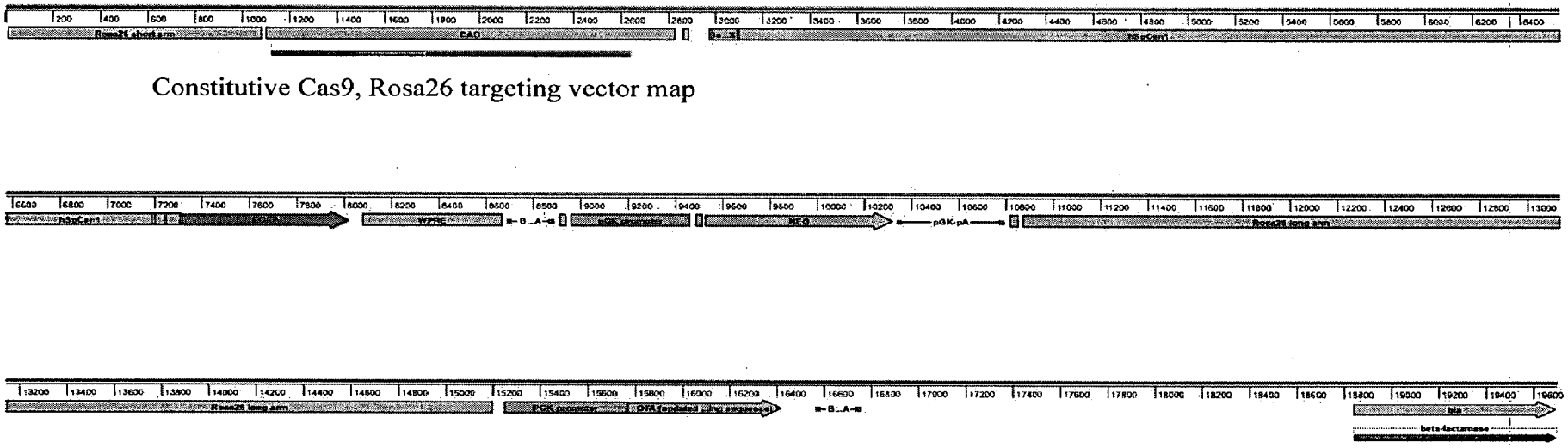





FIG. 25B

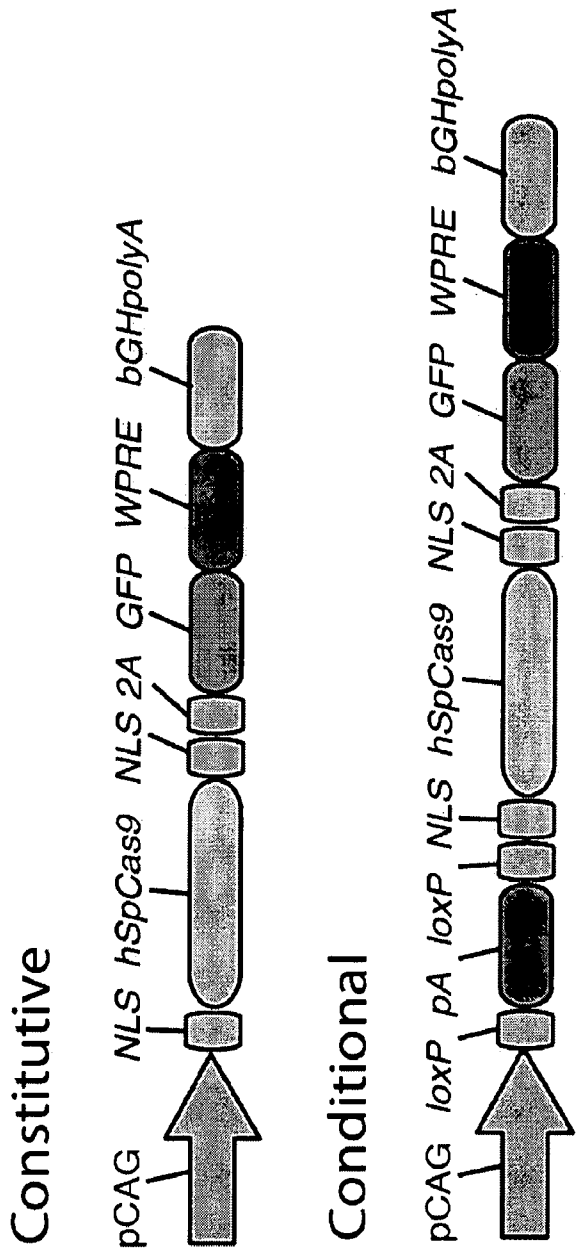
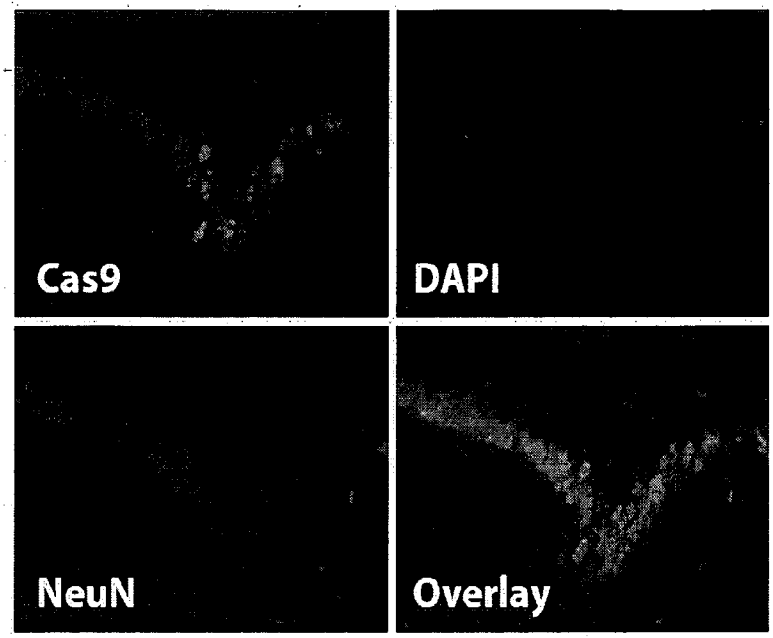

Constitutive
pCAG
NLS
hSpCas9
NLS
2A
GFP
WPRE
bGHpolyA
Conditional
pCAG
loxP
pA
loxP
NLS
hSpCas9
NLS
2A
GFP
WPRE
bGHpolyA

FIG. 26

Cas9 Expression in Mouse Hippocampus (AAV)




Cas9 Expression in Mouse Cortex (AAV)

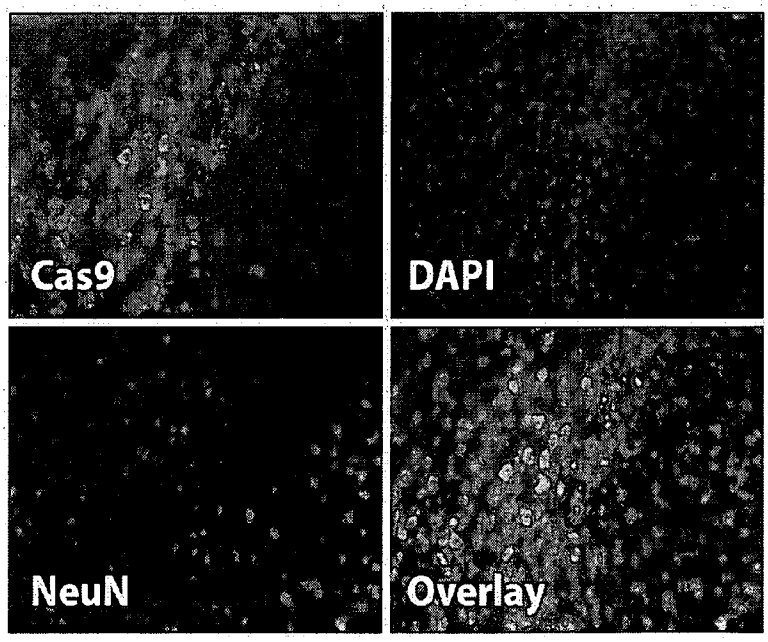



FIG. 27

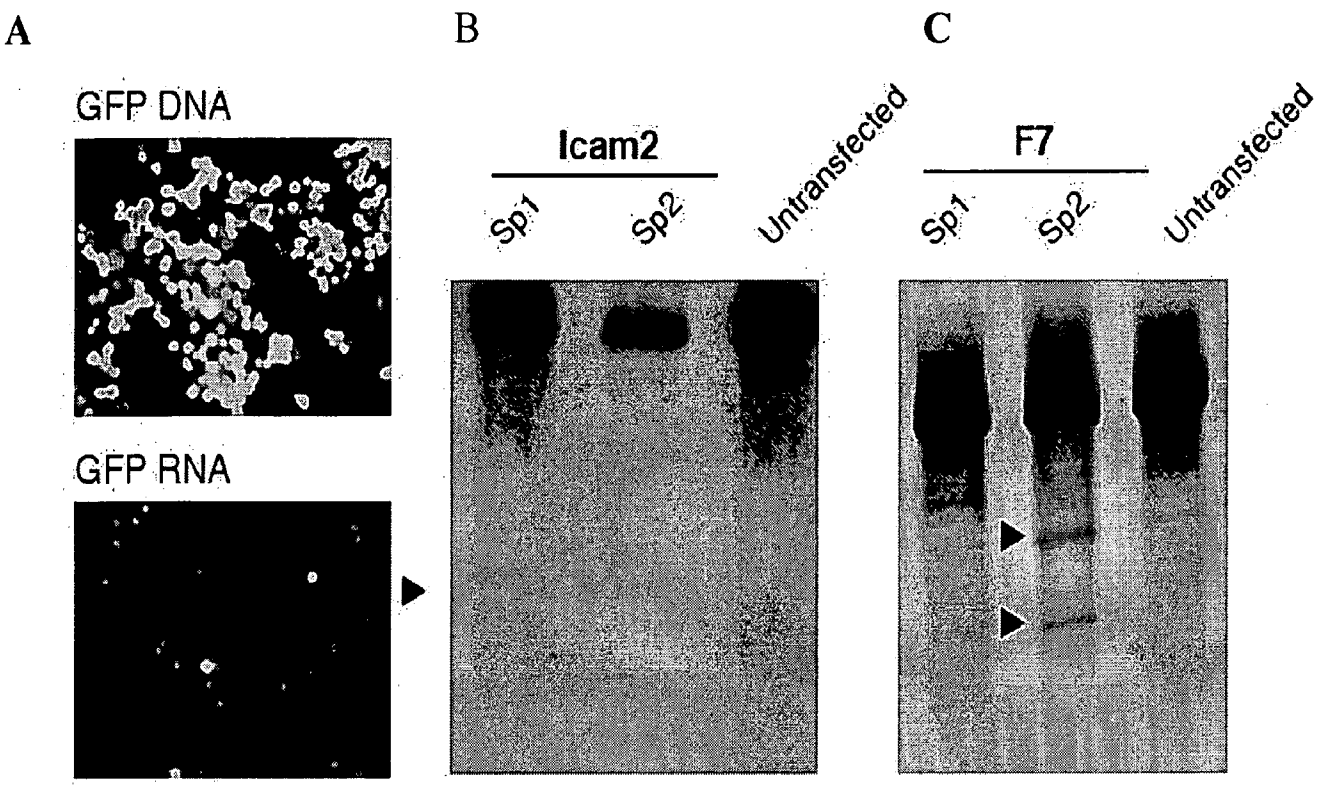

A
B
C
GFP DNA
GFP RNA
Icam2
Sp1
Sp2
Untransfected
F7
Sp1
Sp2
Untransfected

FIG. 28

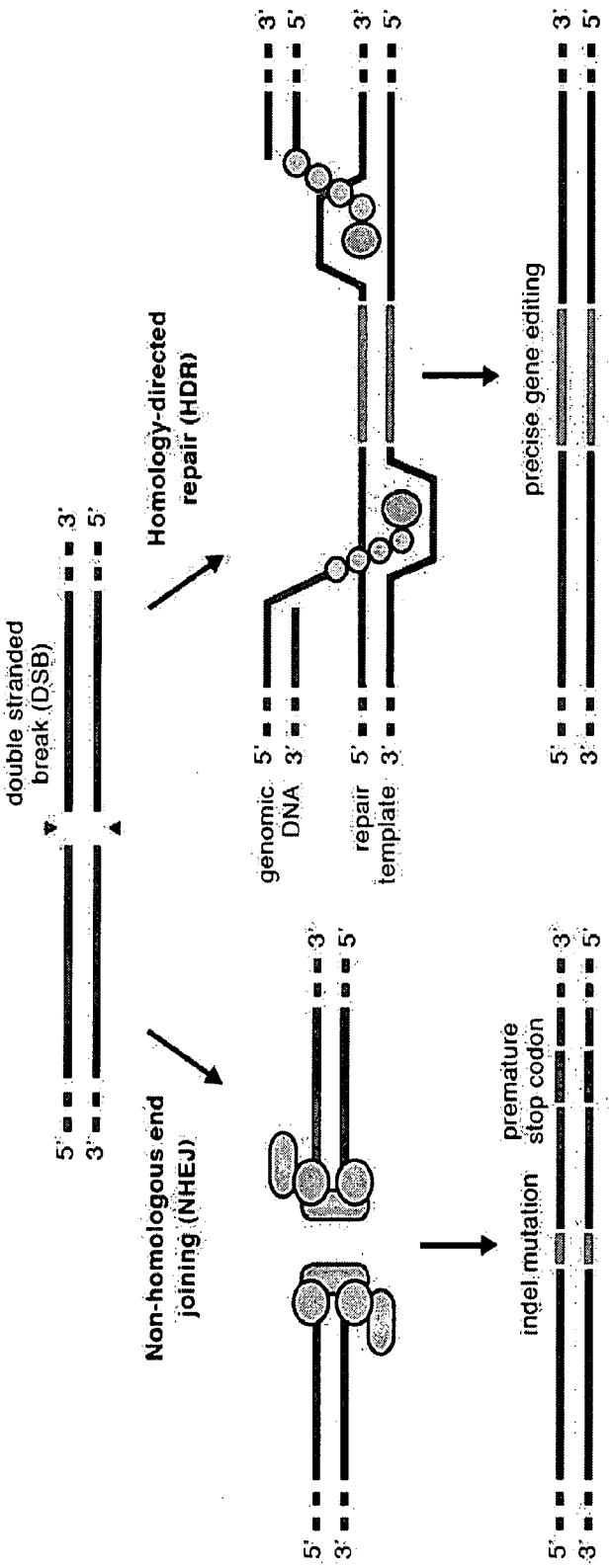

double stranded break (DSB)
5'
3'
3'
5'
Homology-directed repair (HDR)
genomic DNA
repair template
precise gene editing
Non-homologous end joining (NHEJ)
indel mutation
premature stop codon

FIG. 29

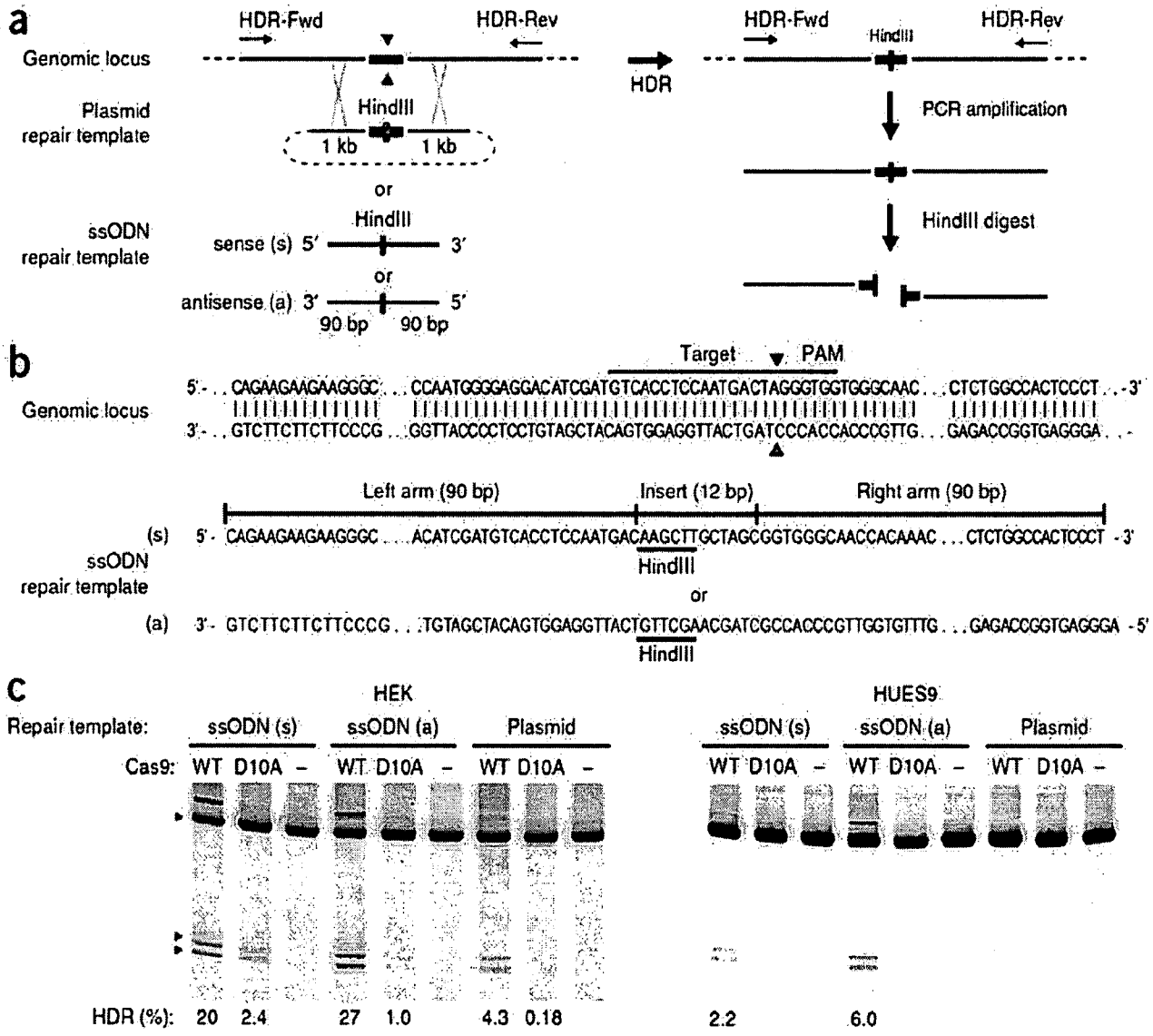

a
HDR-Fwd
HDR-Rev
Genomic locus
Plasmid repair template
HindIII
1 kb
1 kb
or
ssODN repair template
sense (s) 5′
3′
antisense (a) 3′
5′
90 bp
90 bp
HDR
HDR-Fwd
HindIII
HDR-Rev
PCR amplification
HindIII digest
b
Target
PAM
Genomic locus
5′- . . CAGAAGAAGAAGGGC . . . CCAATGGGAGGACATCGATGTCACCTCCAATGACTAGGGTGGTGGGCAAC . . . CTCTGGCCACTCCCT . . -3′
3′- . . GTCTTCTTCTTCCCG . . . GGTTACCCCTCCTGTAGCTACAGTGGAGGTTACTGATCCCACCACCCGTTG . . . GAGACCGGTGAGGGA . . -
Left arm (90 bp)
Insert (12 bp)
Right arm (90 bp)
(s) 5′- CAGAAGAAGAAGGGC . . . ACATCGATGTCACCTCCAATGACAAGCTTGCTAGCGGTGGGCAACCACAAAC . . . CTCTGGCCACTCCCT -3′
HindIII
ssODN repair template
or
(a) 3′- GTCTTCTTCTTCCCG . . . TGTAGCTACAGTGGAGGTTACTGTTCGAACGATCGCCACCCGTTGGTGTTTG . . . GAGACCGGTGAGGGA -5′
HindIII
c
HEK
HUES9
Repair template:
ssODN (s)
ssODN (a)
Plasmid
ssODN (s)
ssODN (a)
Plasmid
Cas9: WT D10A – WT D10A – WT D10A – WT D10A – WT D10A – WT D10A –
HDR (%): 20 2.4 27 1.0 4.3 0.18 2.2 6.0

FIG. 30A-C

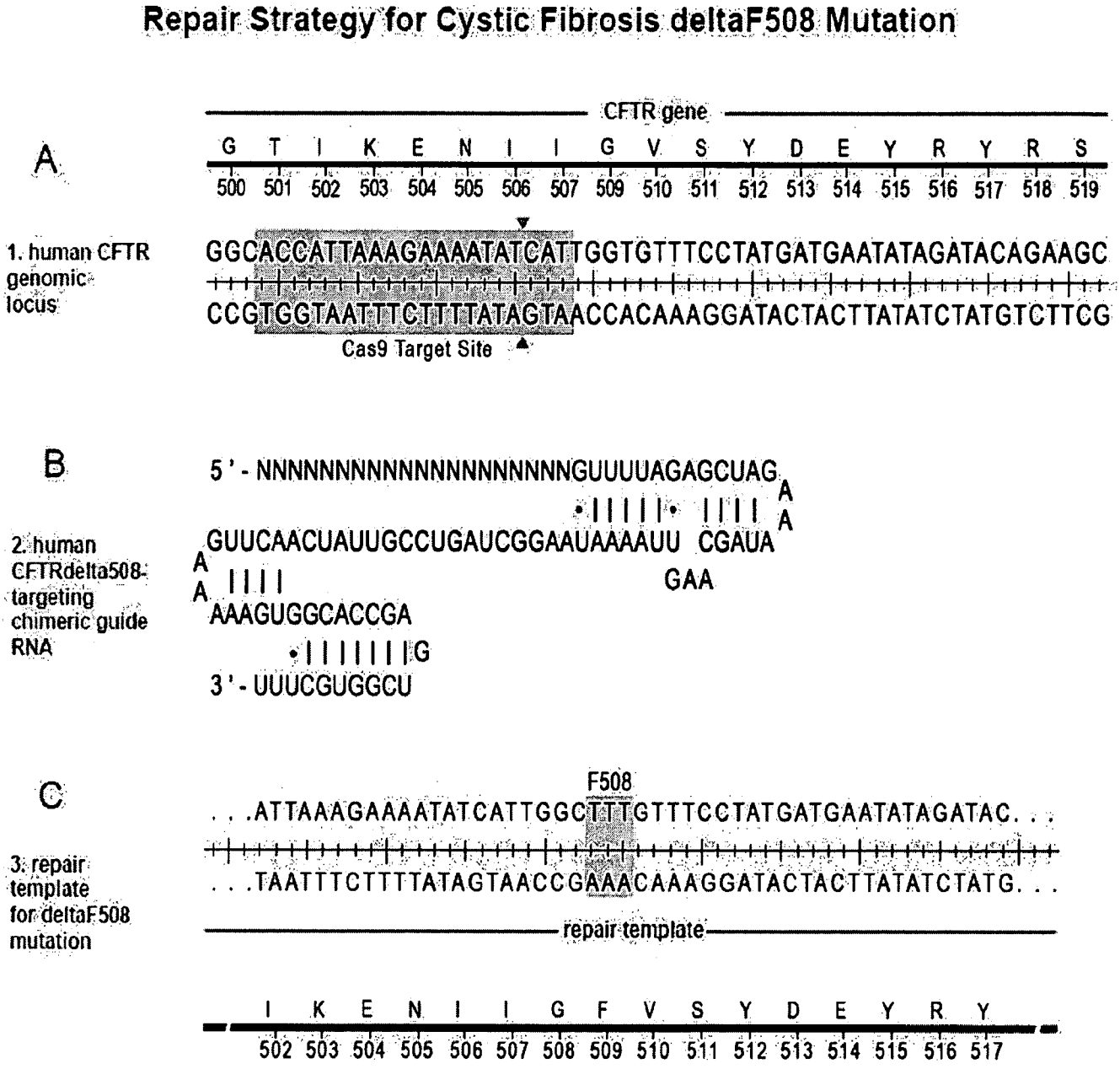

Repair Strategy for Cystic Fibrosis deltaF508 Mutation
A
CFTR gene
G T I K E N I I G V S Y D E Y R Y R S
500 501 502 503 504 505 506 507 509 510 511 512 513 514 515 516 517 518 519
1. human CFTR genomic locus
GGCACCATTAAAGAAAATATCATTGGTGTTTCCTATGATGAATATAGATACAGAAGC
CCGTGGTAATTTCTTTTATAGTAACCACAAAGGATACTACTTATATCTATGTCTTCG
Cas9 Target Site
B
5 ' - NNNNNNNNNNNNNNNNNNNGUUUUAGAGCUAG
GUUCAACUAUUGCCUGAUCGGAAUAAAAUU CGAUA
AAAGUGGCACCGA
3 ' - UUUCGUGGCU
2. human CFTRdelta508-targeting chimeric guide RNA
C
F508
. . .ATTAAAGAAAATATCATTGGCTTTGTTTCCTATGATGAATATAGATAC. . .
. . .TAATTTCTTTTATAGTAACCGAAACAAAGGATACTACTTATATCTATG. . .
repair template
I K E N I I G F V S Y D E Y R Y
502 503 504 505 506 507 508 509 510 511 512 513 514 515 516 517
3. repair template for deltaF508 mutation

FIG. 31

A


GAA repeat expansion in *FXN* intron 1
GAA repeat
Exon 1
Exon 2
Transcription repression likely due to aberrant DNA structure or recruitment of heterchromatin binding proteins to long GAA repeats

B

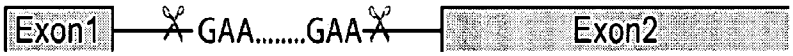

Exon1
GAA.....GAA
Exon2

FIG. 32

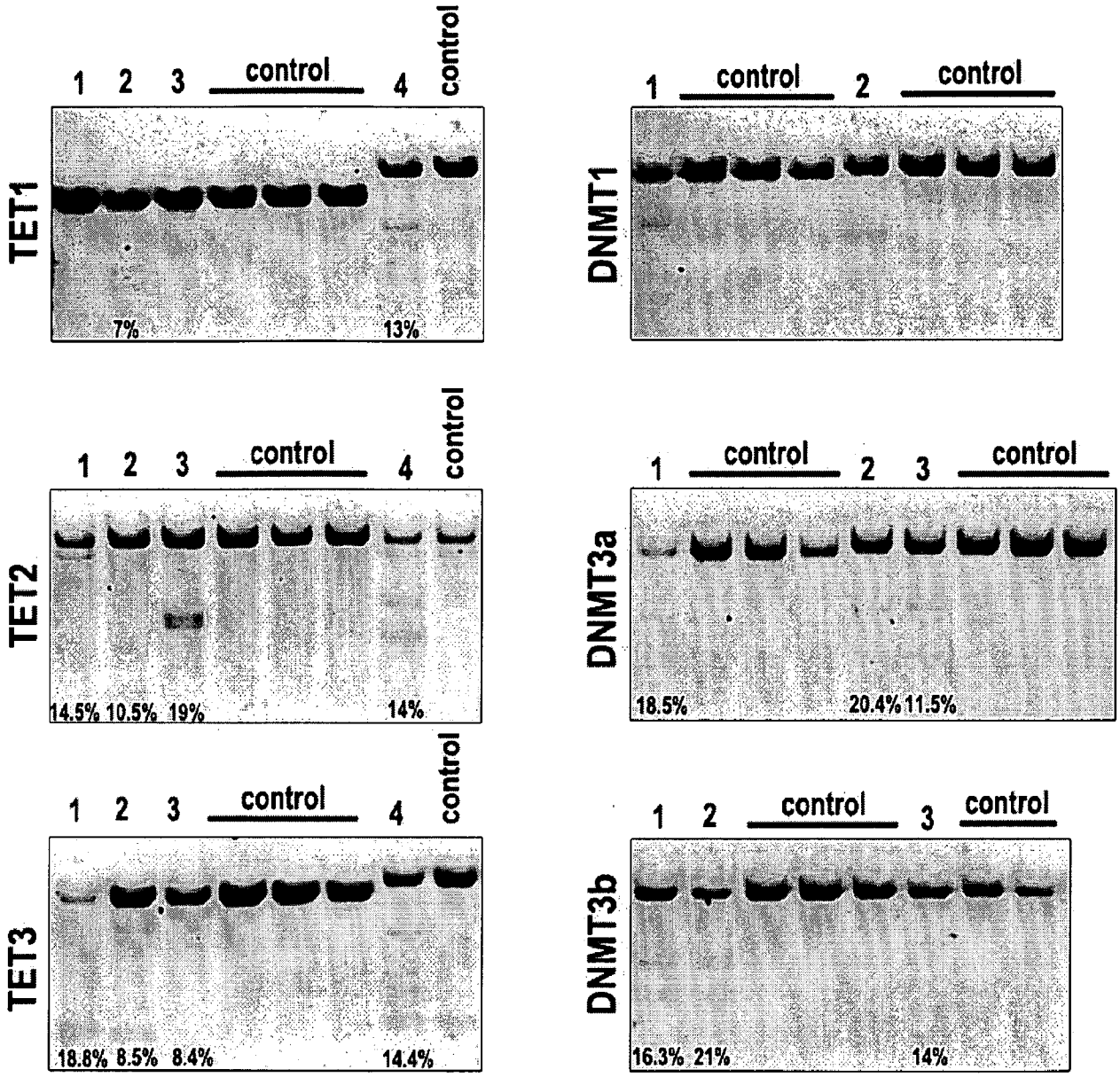

TET1
1 2 3 control 4 control
7%
13%
DNMT1
1 control 2 control
TET2
1 2 3 control 4 control
14.5% 10.5% 19%
14%
DNMT3a
1 control 2 3 control
18.5%
20.4% 11.5%
TET3
1 2 3 control 4 control
18.8% 8.5% 8.4%
14.4%
DNMT3b
1 2 control 3 control
16.3% 21%
14%

FIG. 33

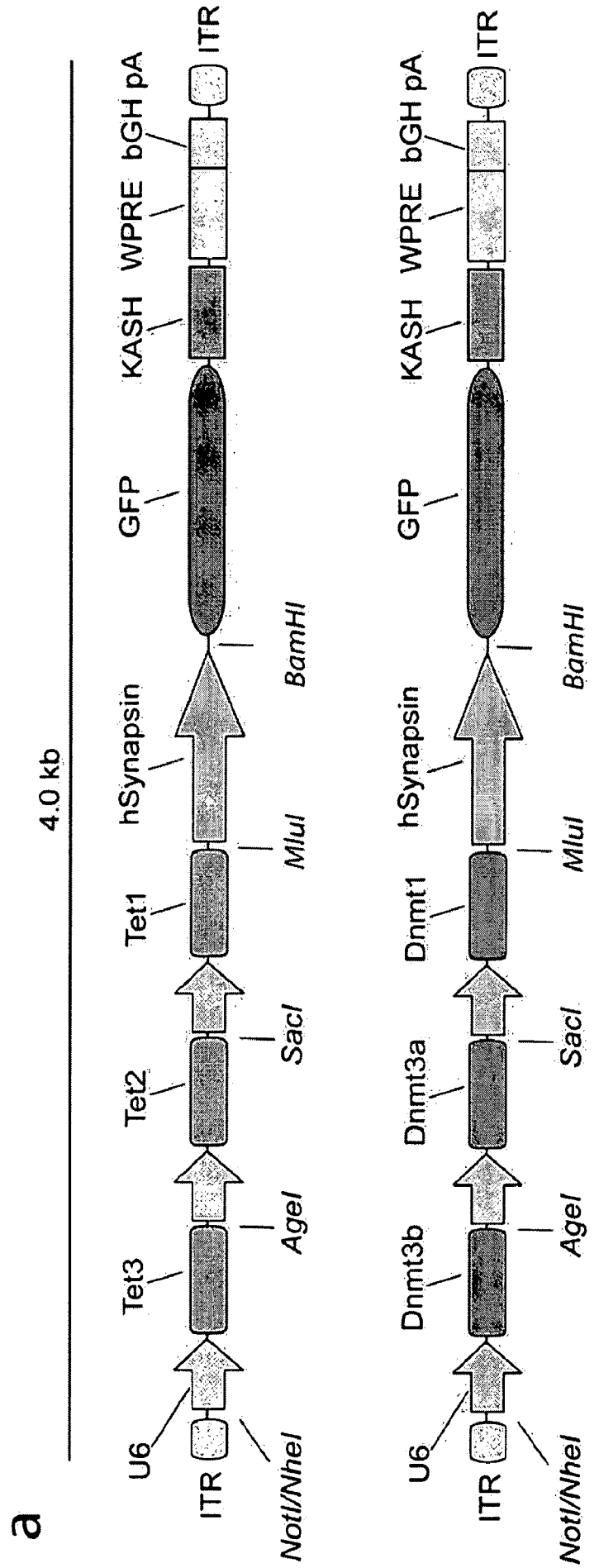

a
4.0 kb
ITR
U6
NotI/NheI
Tet3
Tet2
AgeI
SacI
Tet1
hSynapsin
MluI
GFP
KASH
WPRE
bGH pA
BamHI
ITR
Dnmt3b
Dnmt3a
Dnmt1
HA
NLS
SpCas9

FIG. 34

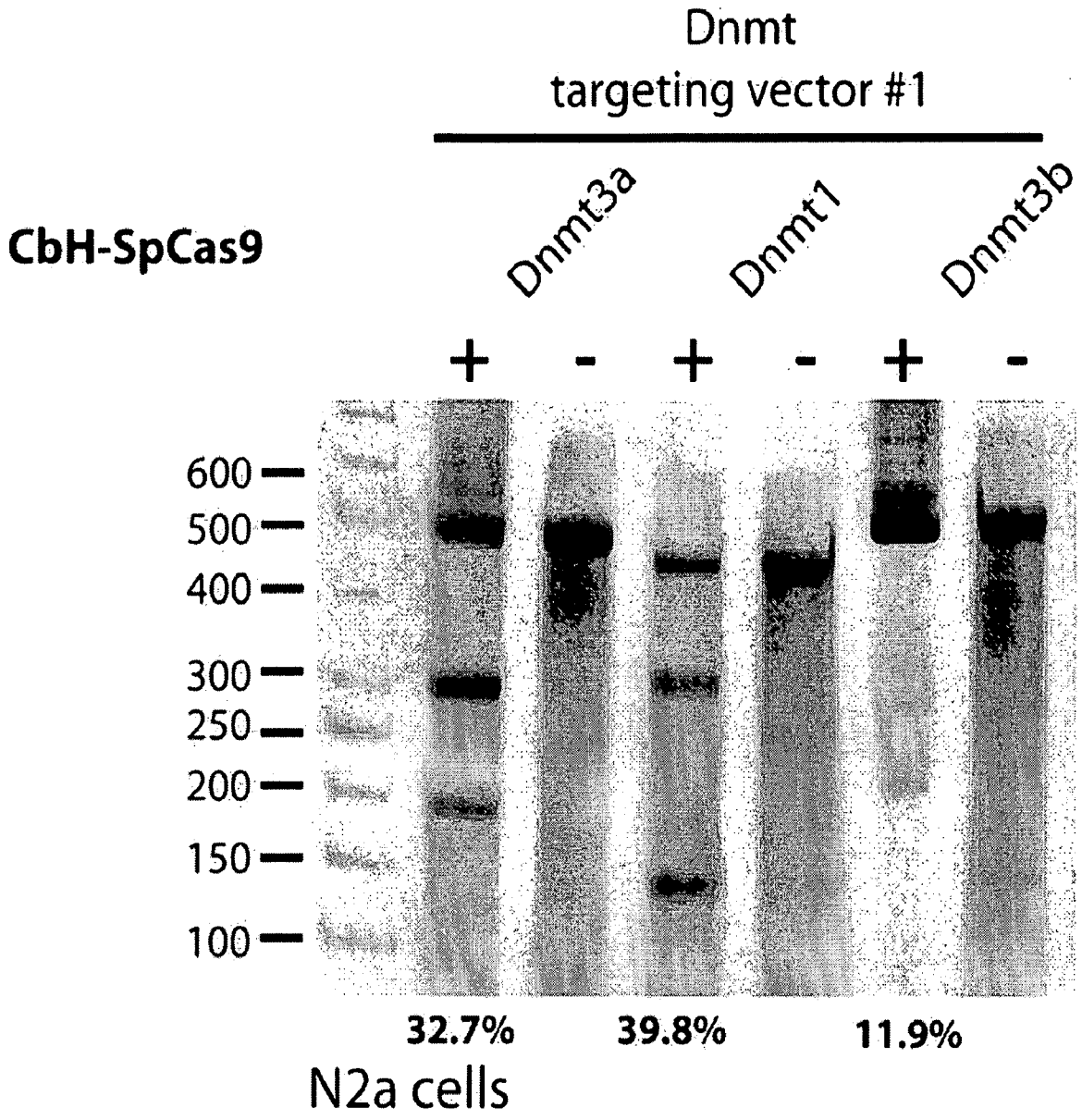

Dnmt
targeting vector #1
CbH-SpCas9
Dnmt3a
Dnmt1
Dnmt3b
+ - + - + -
600
500
400
300
250
200
150
100
32.7%
39.8%
11.9%
N2a cells

FIG. 35

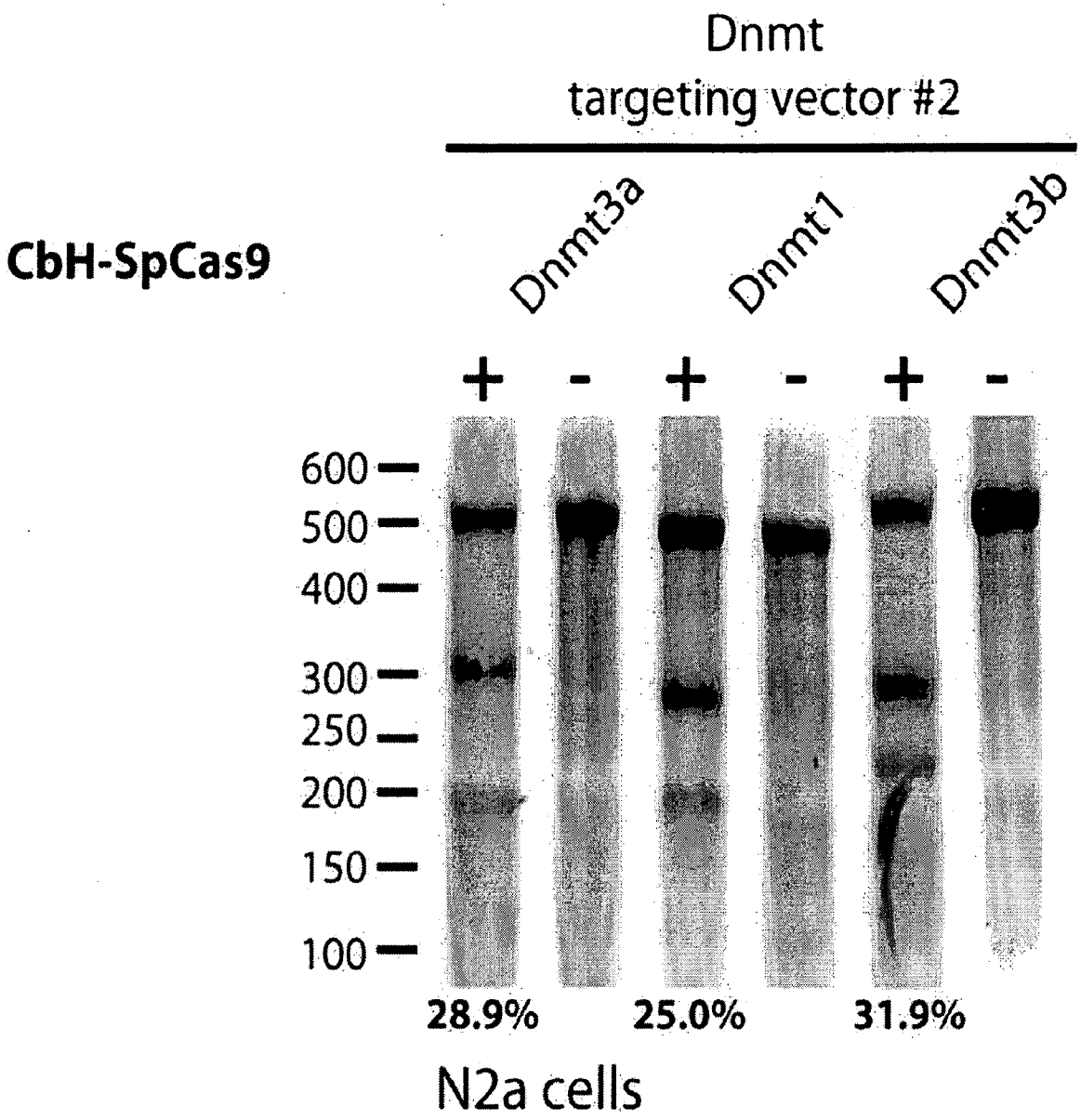

Dnmt
targeting vector #2
CbH-SpCas9
Dnmt3a
Dnmt1
Dnmt3b
+ - + - + -
600
500
400
300
250
200
150
100
28.9%
25.0%
31.9%
N2a cells

FIG. 36

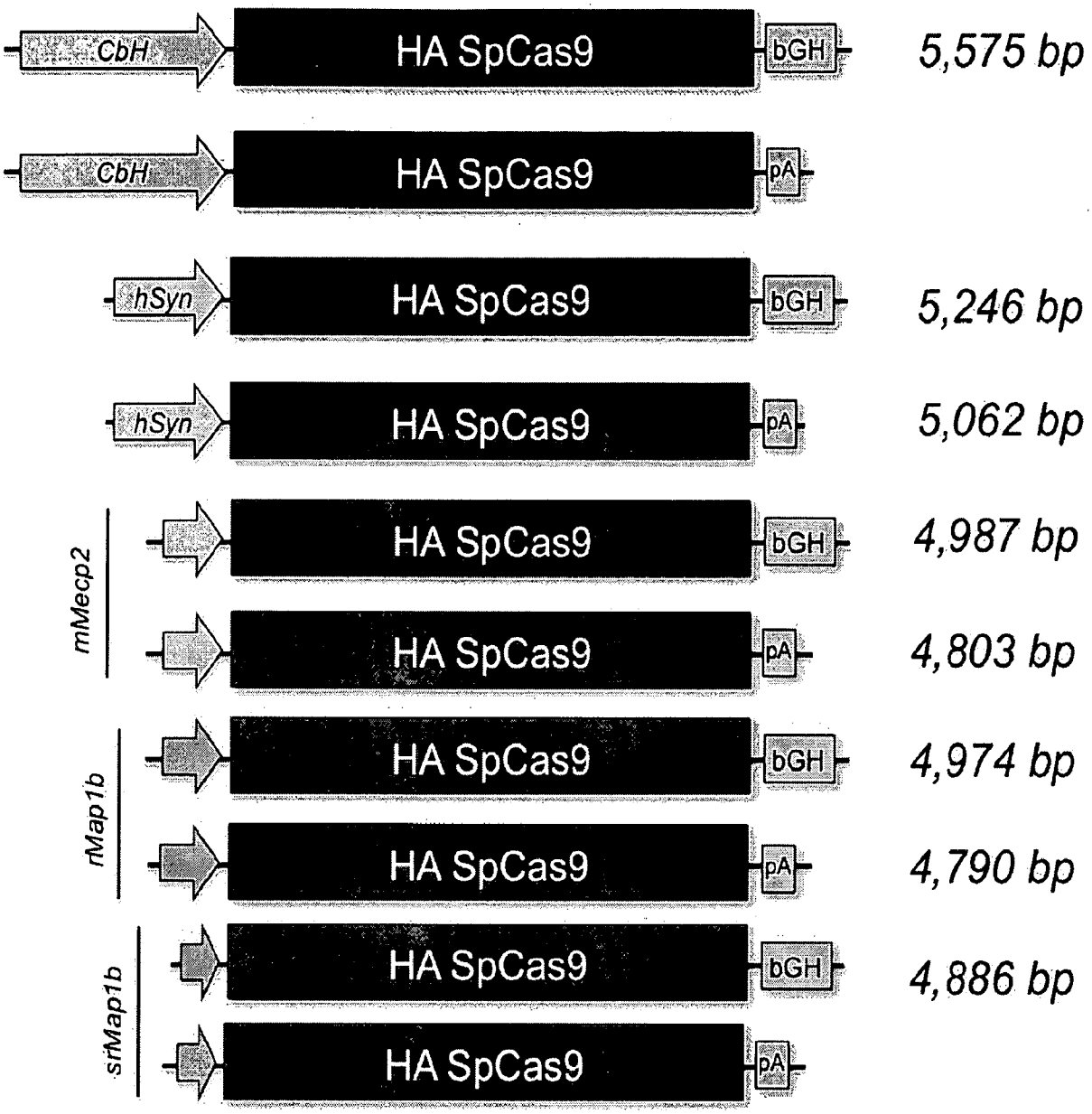

CbH
HA SpCas9
bGH
5,575 bp
CbH
HA SpCas9
pA
hSyn
HA SpCas9
bGH
5,246 bp
hSyn
HA SpCas9
pA
5,062 bp
mMecp2
HA SpCas9
bGH
4,987 bp
HA SpCas9
pA
4,803 bp
rMap1b
HA SpCas9
bGH
4,974 bp
HA SpCas9
pA
4,790 bp
srMap1b
HA SpCas9
bGH
4,886 bp
HA SpCas9
pA

FIG. 37

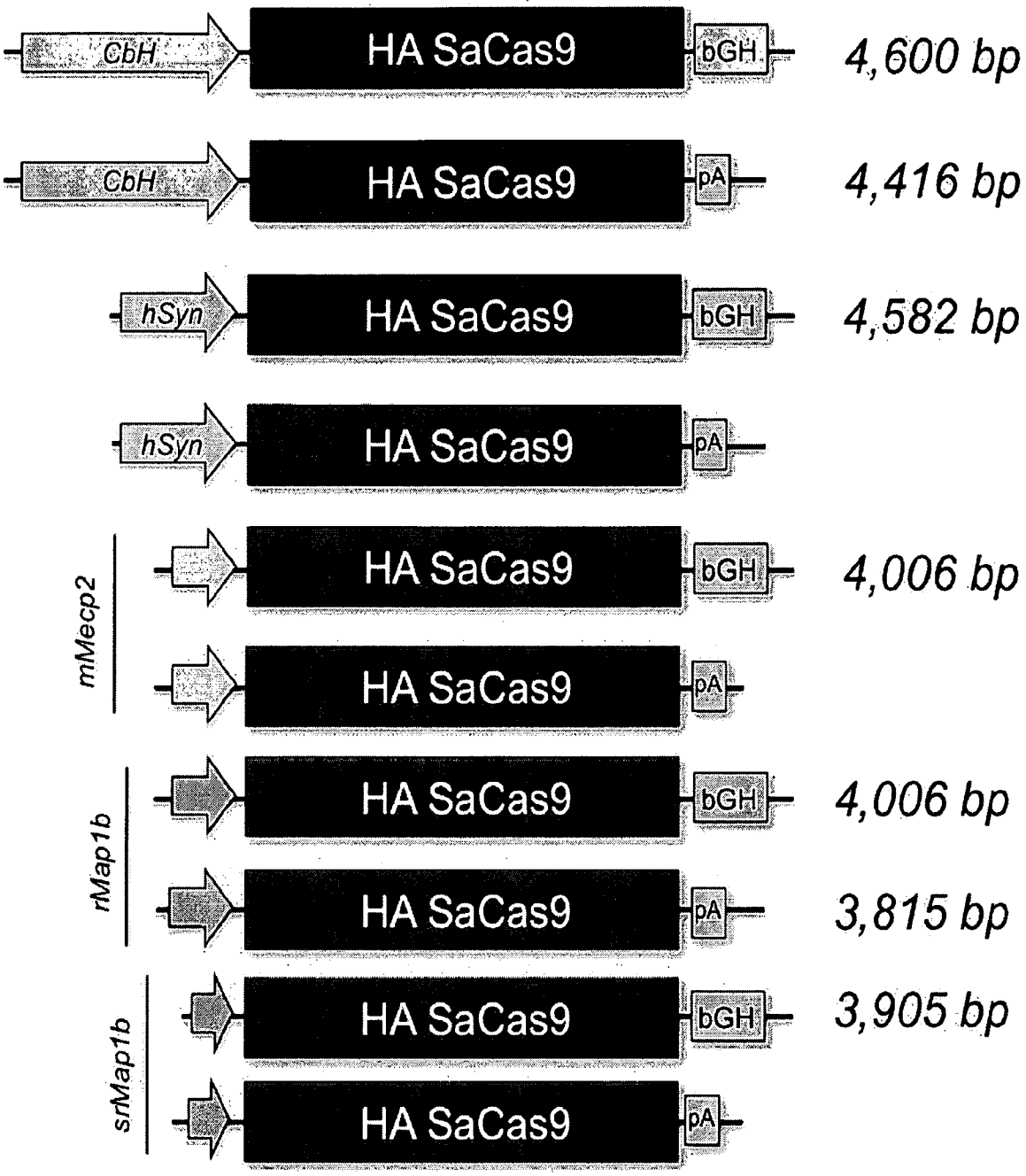

CbH
HA SaCas9
bGH
4,600 bp
CbH
HA SaCas9
pA
4,416 bp
hSyn
HA SaCas9
bGH
4,582 bp
hSyn
HA SaCas9
pA
mMecp2
HA SaCas9
bGH
4,006 bp
HA SaCas9
pA
rMap1b
HA SaCas9
bGH
4,006 bp
HA SaCas9
pA
3,815 bp
srMap1b
HA SaCas9
bGH
3,905 bp
HA SaCas9
pA

FIG. 38

# Expression of SpCas9 & SaCas9 in N2a cells

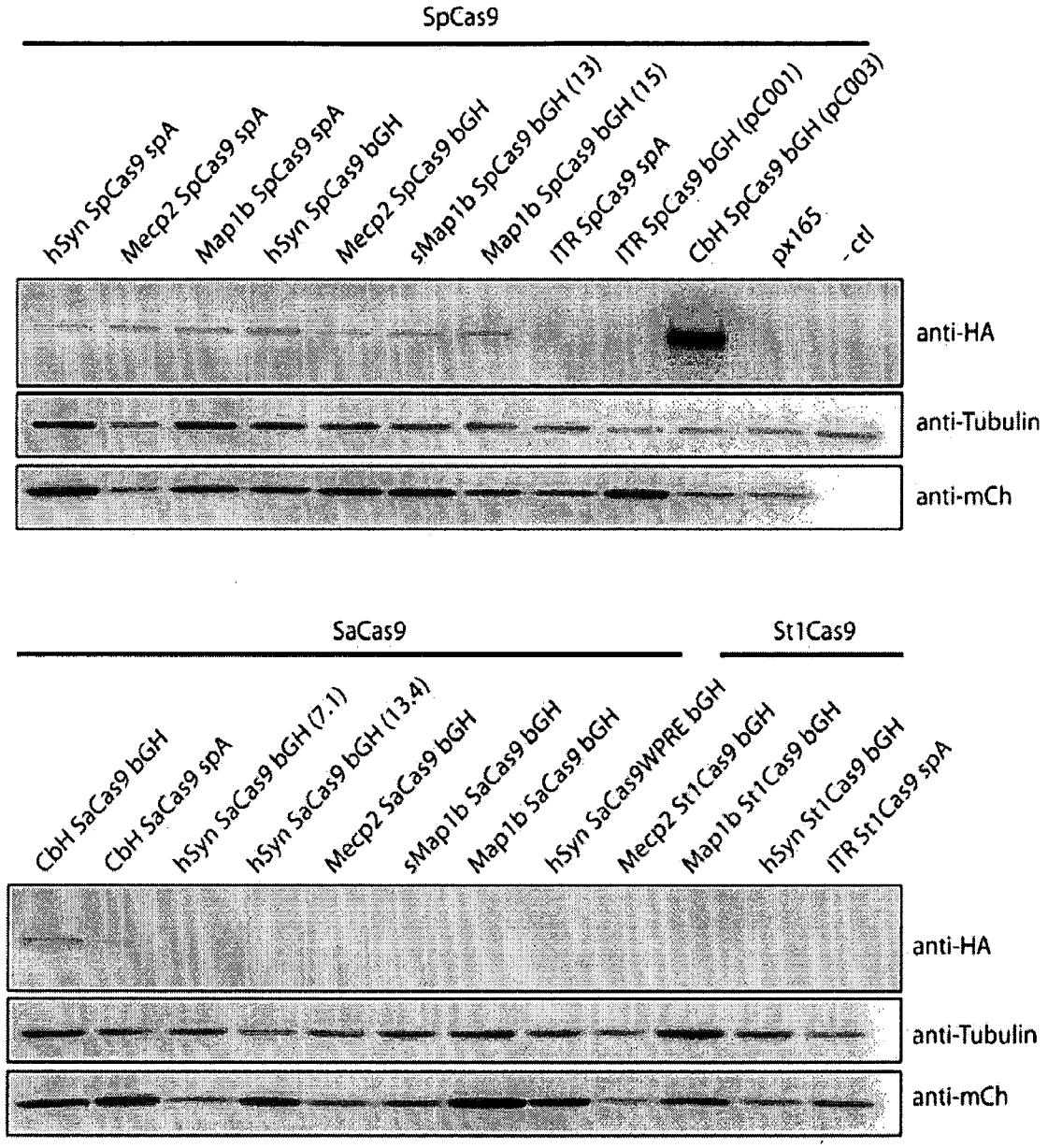



FIG. 39

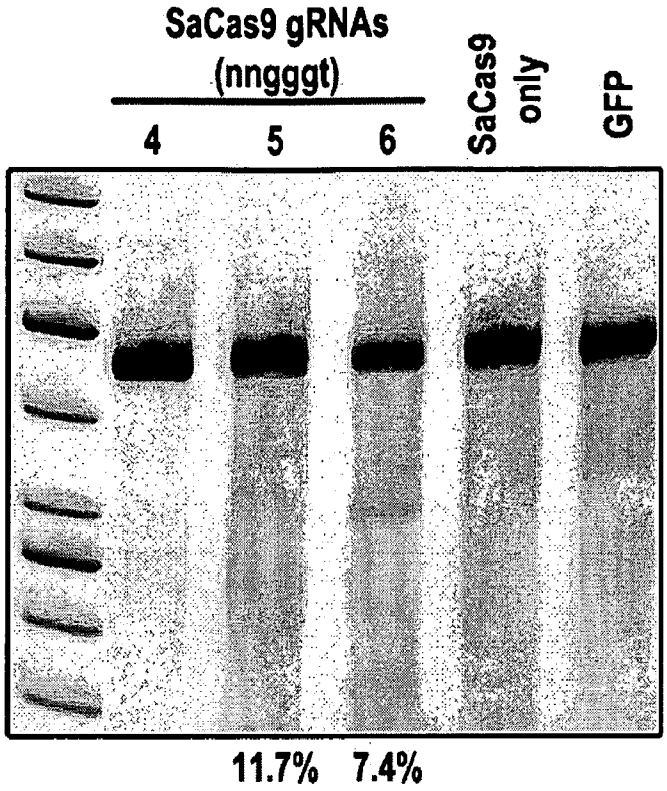



FIG. 40

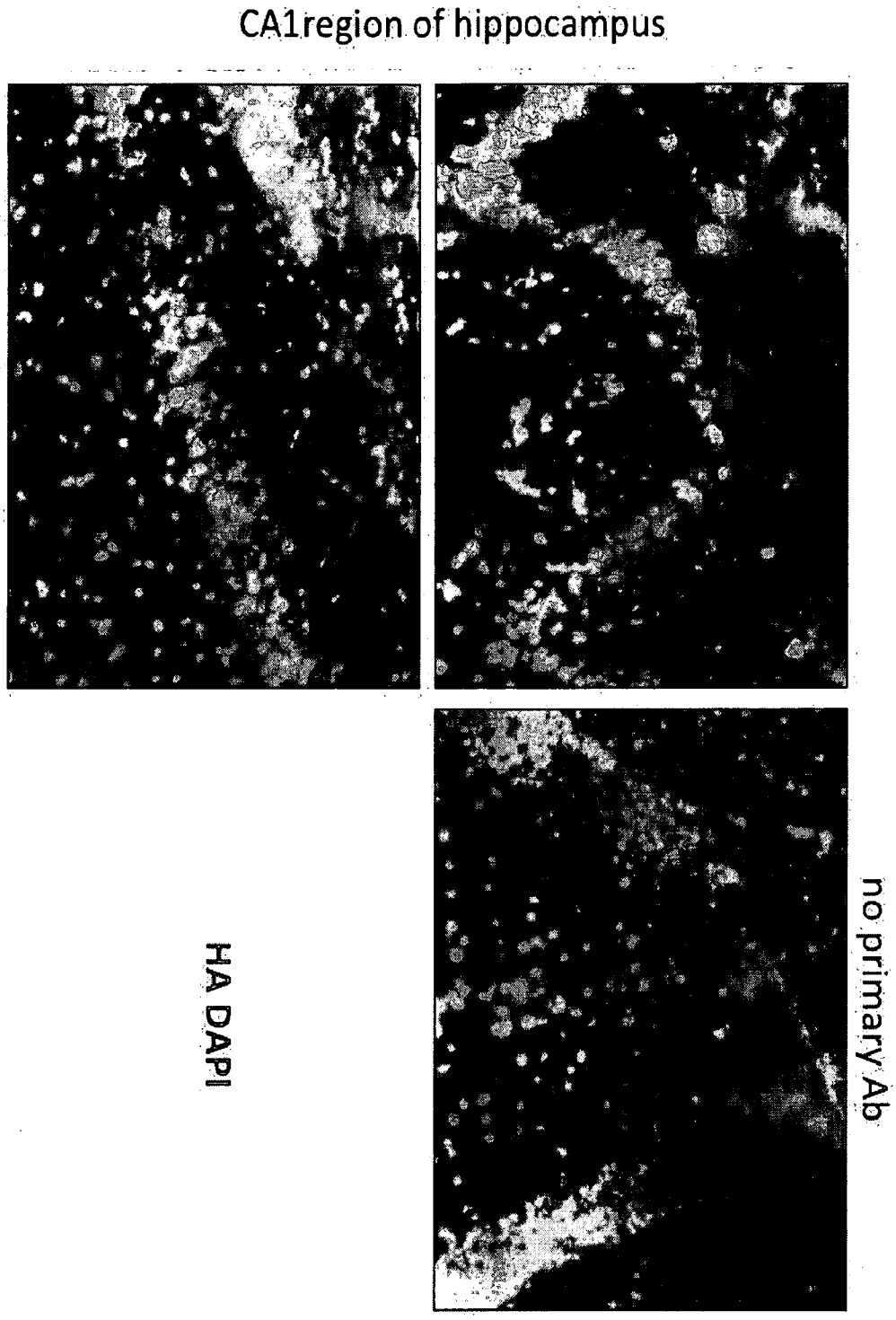



FIG. 41

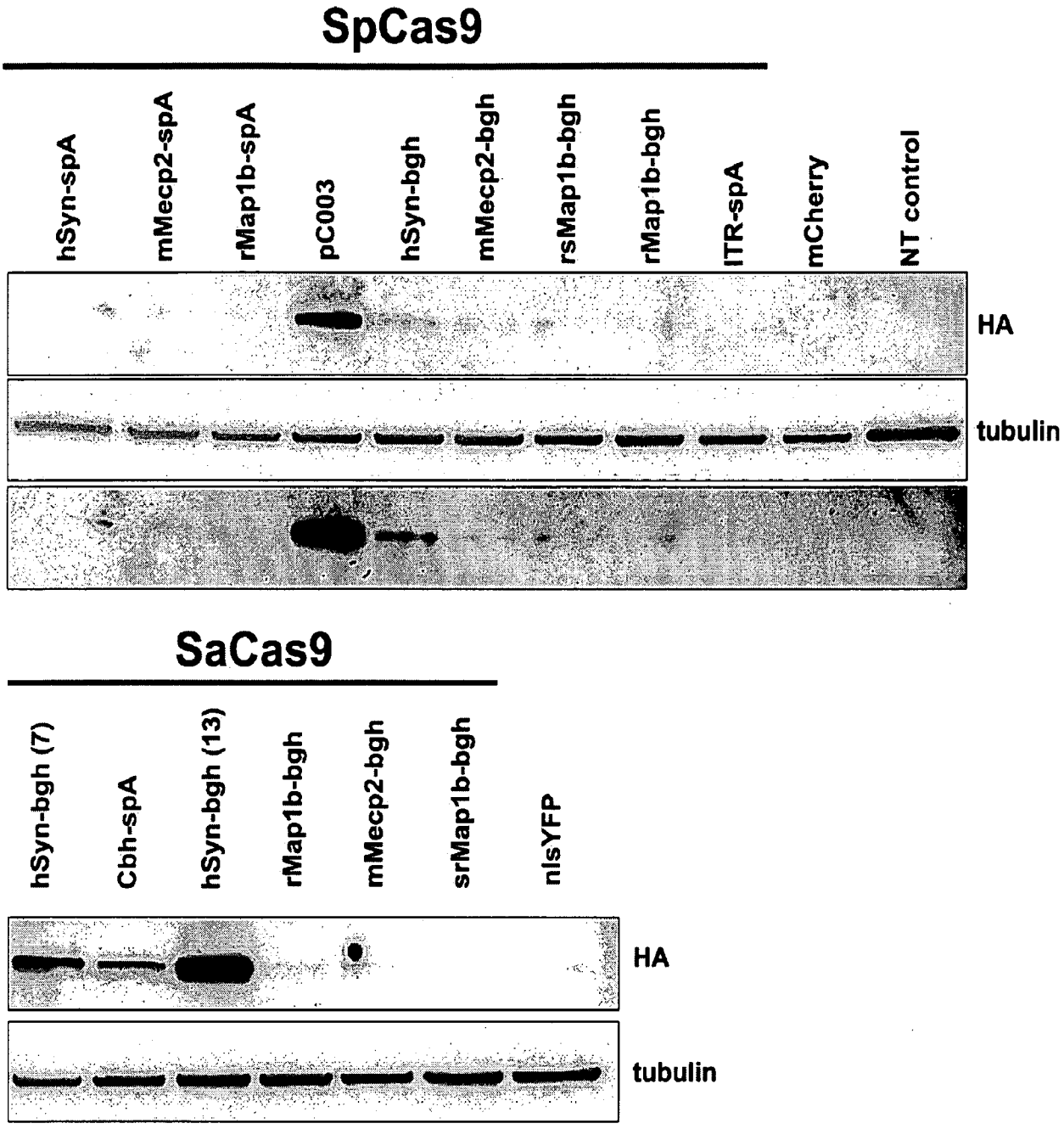

SpCas9
hSyn-spA
mMecp2-spA
rMap1b-spA
pC003
hSyn-bgh
mMecp2-bgh
rsMap1b-bgh
rMap1b-bgh
ITR-spA
mCherry
NT control
HA
tubulin
SaCas9
hSyn-bgh (7)
Cbh-spA
hSyn-bgh (13)
rMap1b-bgh
mMecp2-bgh
srMap1b-bgh
nlsYFP
HA
tubulin

FIG. 42

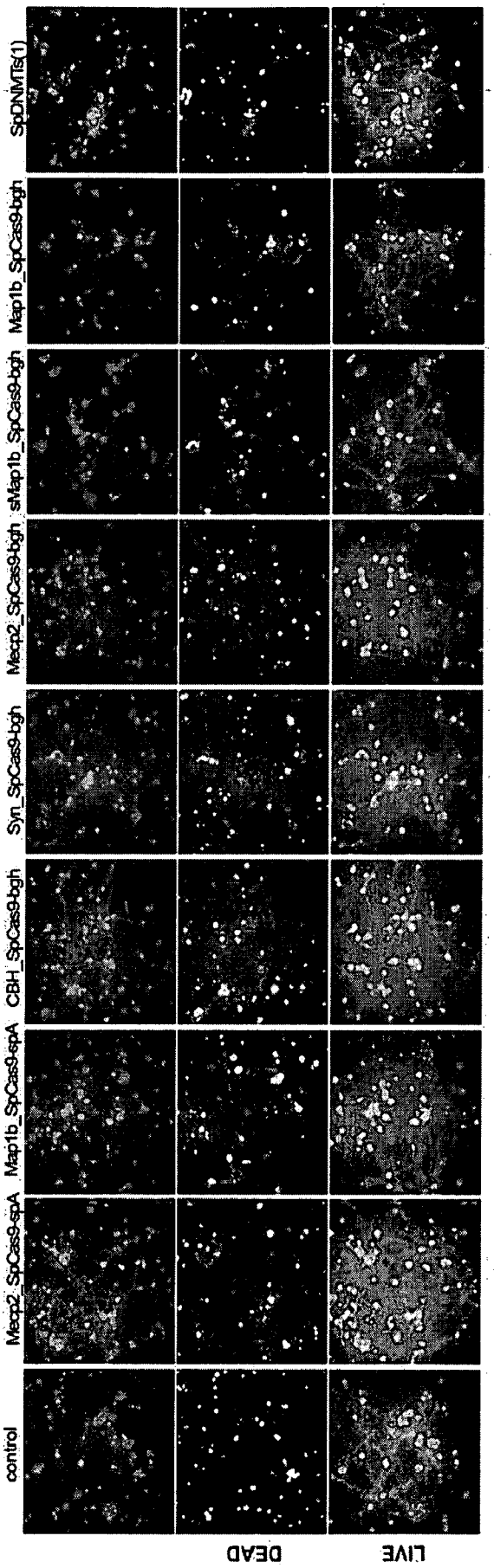

control
Mecp2_SpCas9-spA
Map1b_SpCas9-spA
CBH_SpCas9-bgh
Syn_SpCas9-bgh
Mecp2_SpCas9-bgh
sMap1b_SpCas9-bgh
Map1b_SpCas9-bgh
SpDNMTs(1)
DEAD
LIVE

FIG. 43

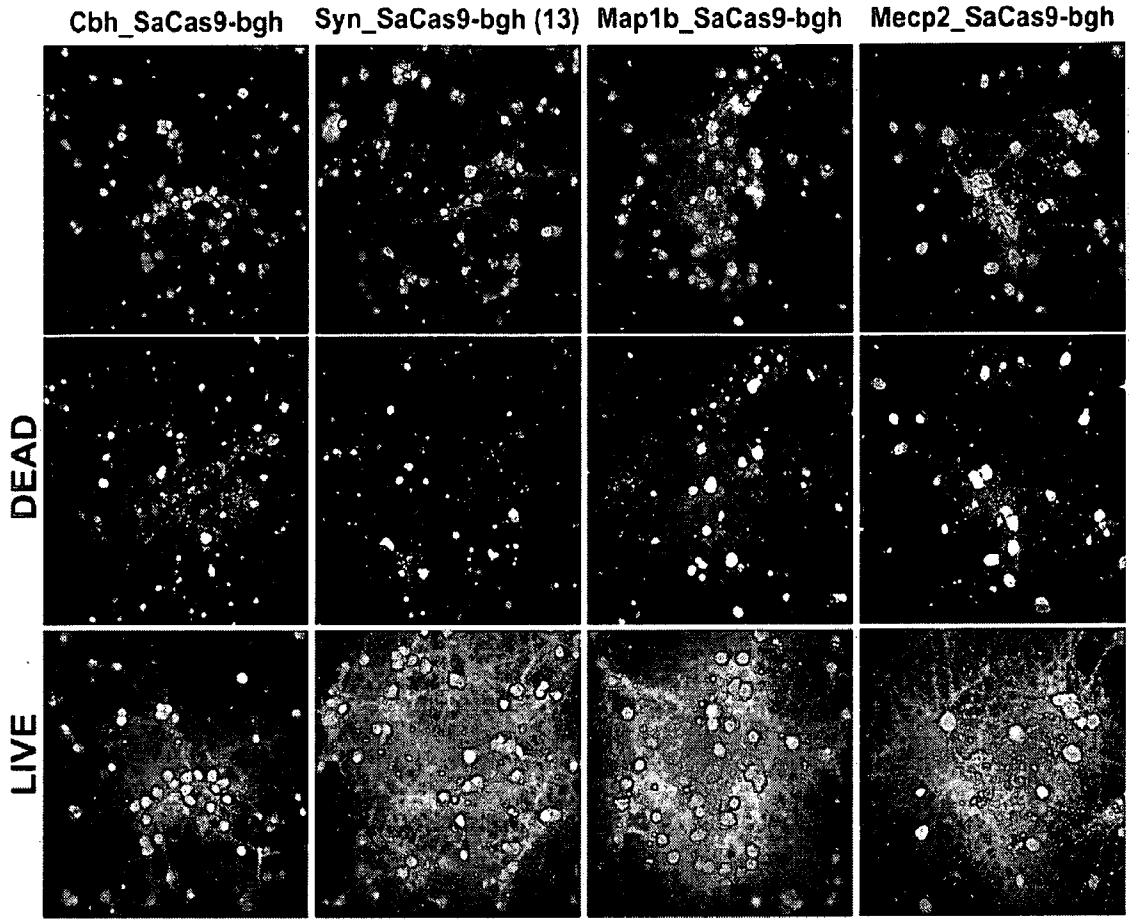

Cbh_SaCas9-bgh
Syn_SaCas9-bgh (13)
Map1b_SaCas9-bgh
Mecp2_SaCas9-bgh
DEAD
LIVE

FIG. 44

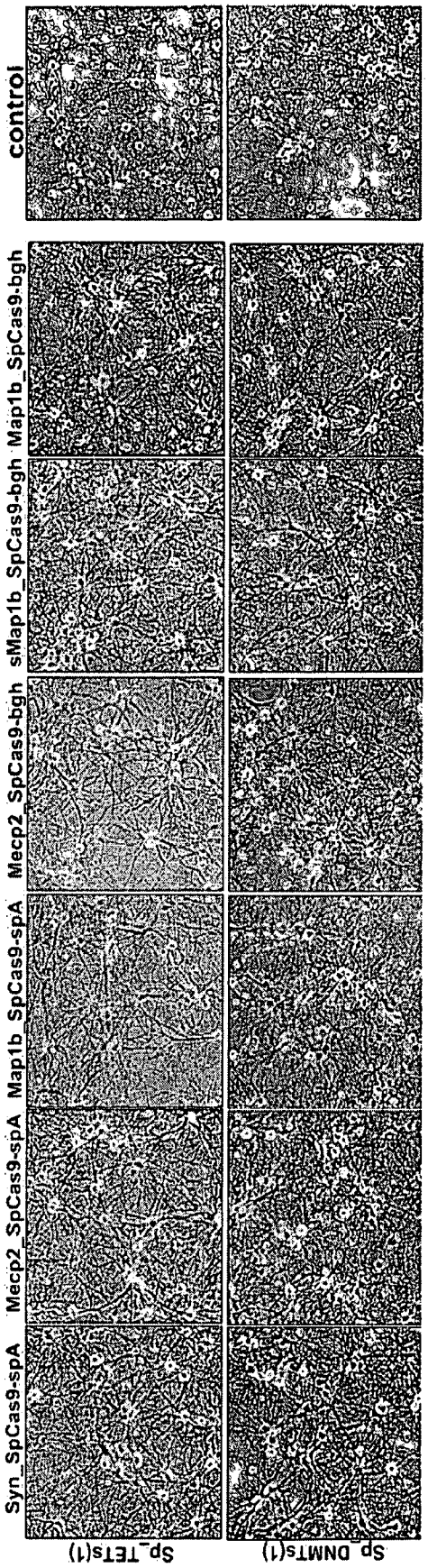

Syn_SpCas9-spA
Mecp2_SpCas9-spA
Map1b_SpCas9-spA
Mecp2_SpCas9-bgh
sMap1b_SpCas9-bgh
Map1b_SpCas9-bgh
control
Sp_TETs(1)
Sp_DNMTs(1)

FIG. 45

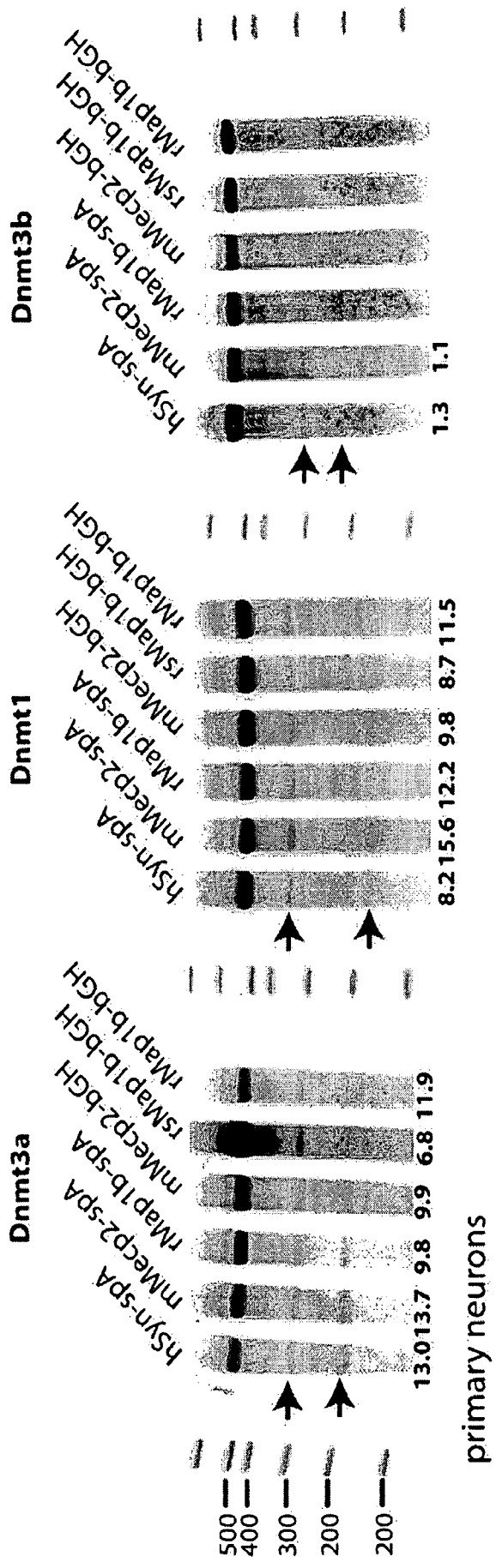

Dnmt3b
Dnmt1
Dnmt3a
hSyn-spA
mMecp2-spA
rMap1b-spA
rsMecp2-bGH
rsMap1b-bGH
rMap1b-bGH
1.3
1.1
8.2
15.6
12.2
9.8
8.7
11.5
13.0
13.7
9.8
9.9
6.8
11.9
500
400
300
200
200
primary neurons

FIG. 46

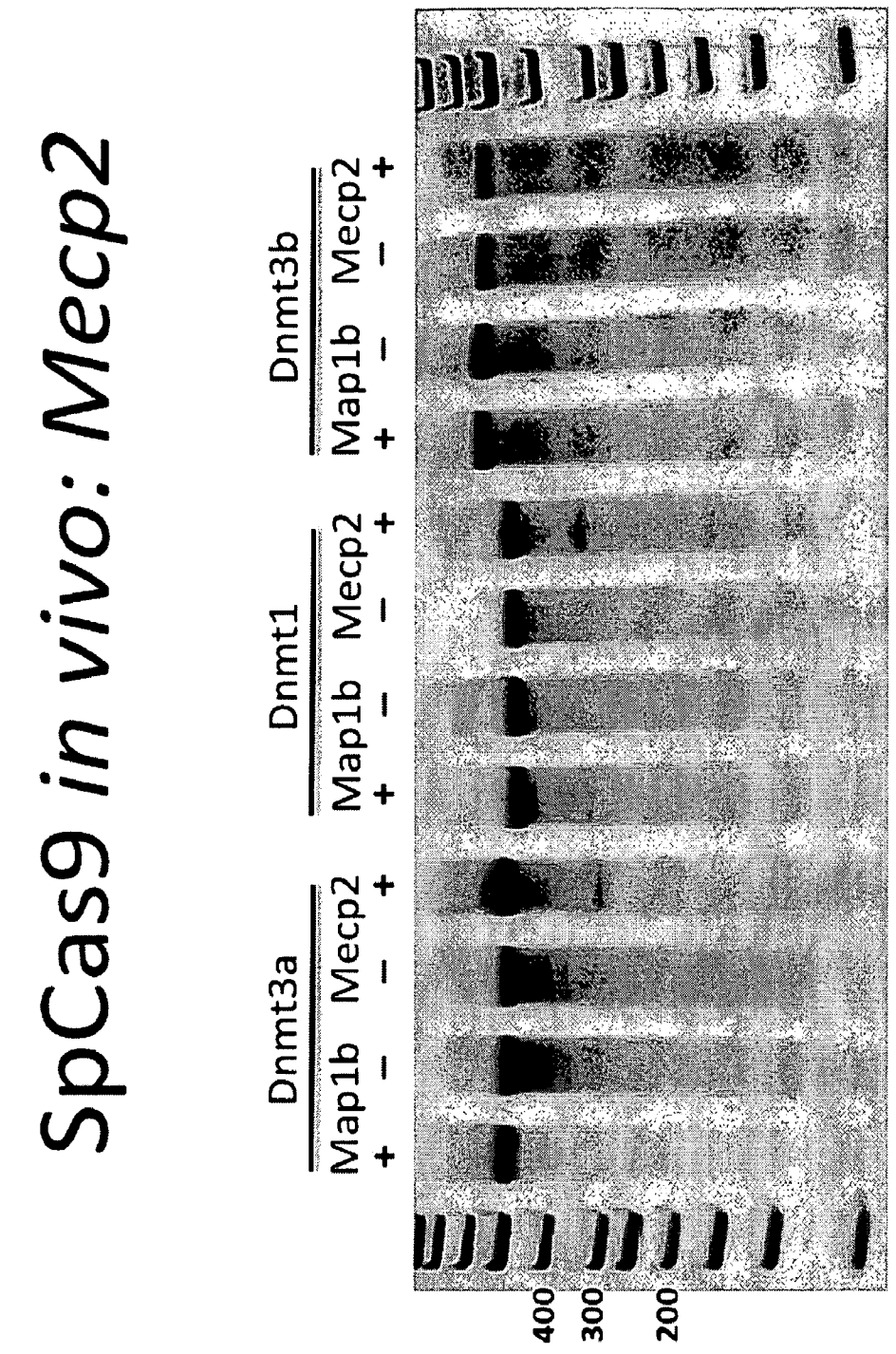

SpCas9 in vivo: Mecp2
Dnmt3a
Map1b + Mecp2 +
Dnmt1
Map1b + Mecp2 +
Dnmt3b
Map1b + Mecp2 +
400
300
200
12.0%
17.0%

FIG. 47

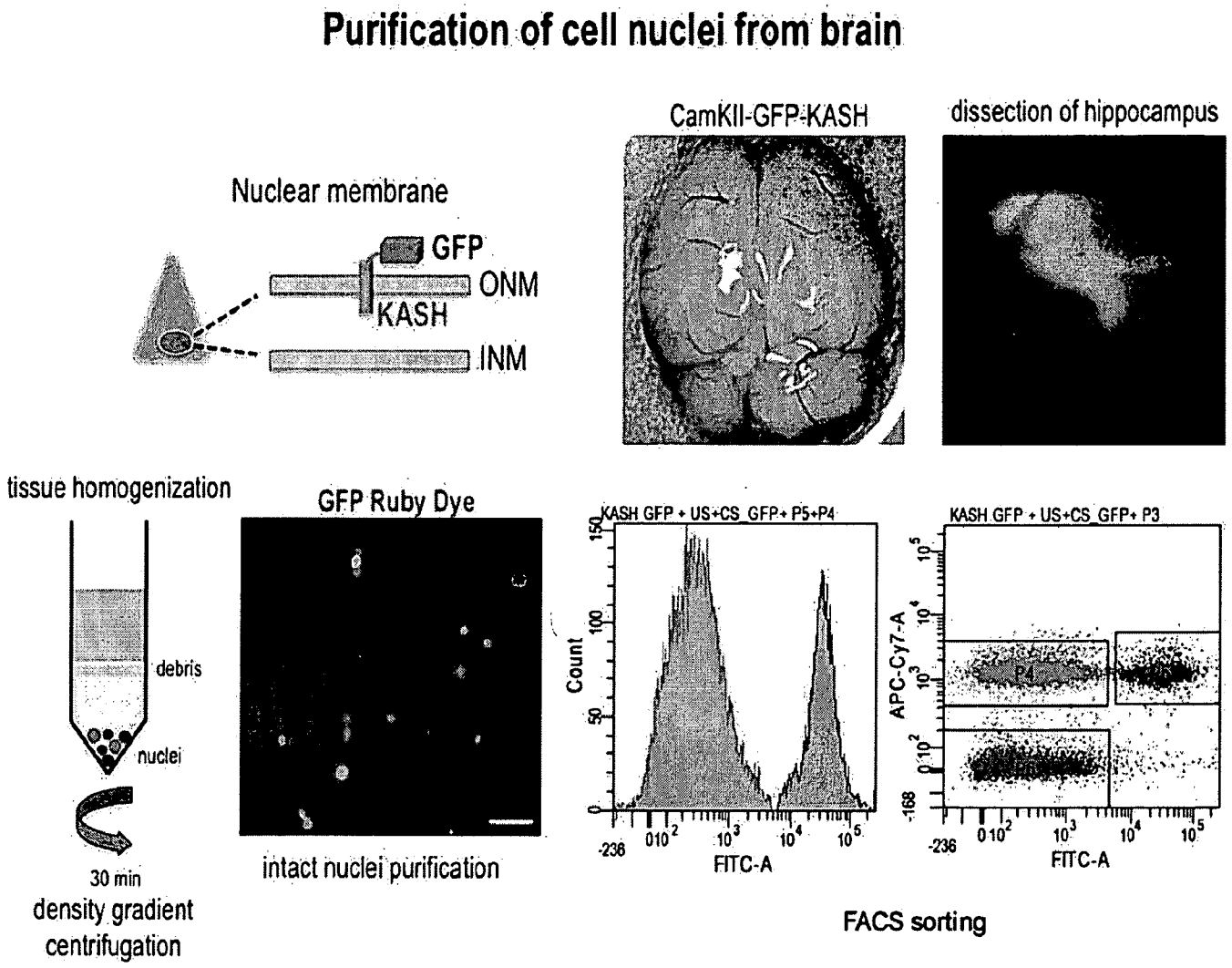

Purification of cell nuclei from brain
Nuclear membrane
GFP
ONM
KASH
INM
CamKII-GFP-KASH
dissection of hippocampus
tissue homogenization
debris
nuclei
30 min
density gradient
centrifugation
GFP Ruby Dye
intact nuclei purification
KASH GFP + US+CS_GFP+ P5+P4
Count
FITC-A
KASH GFP + US+CS_GFP+ P3
APC-Cy7-A
P4
FITC-A
FACS sorting

FIG. 48

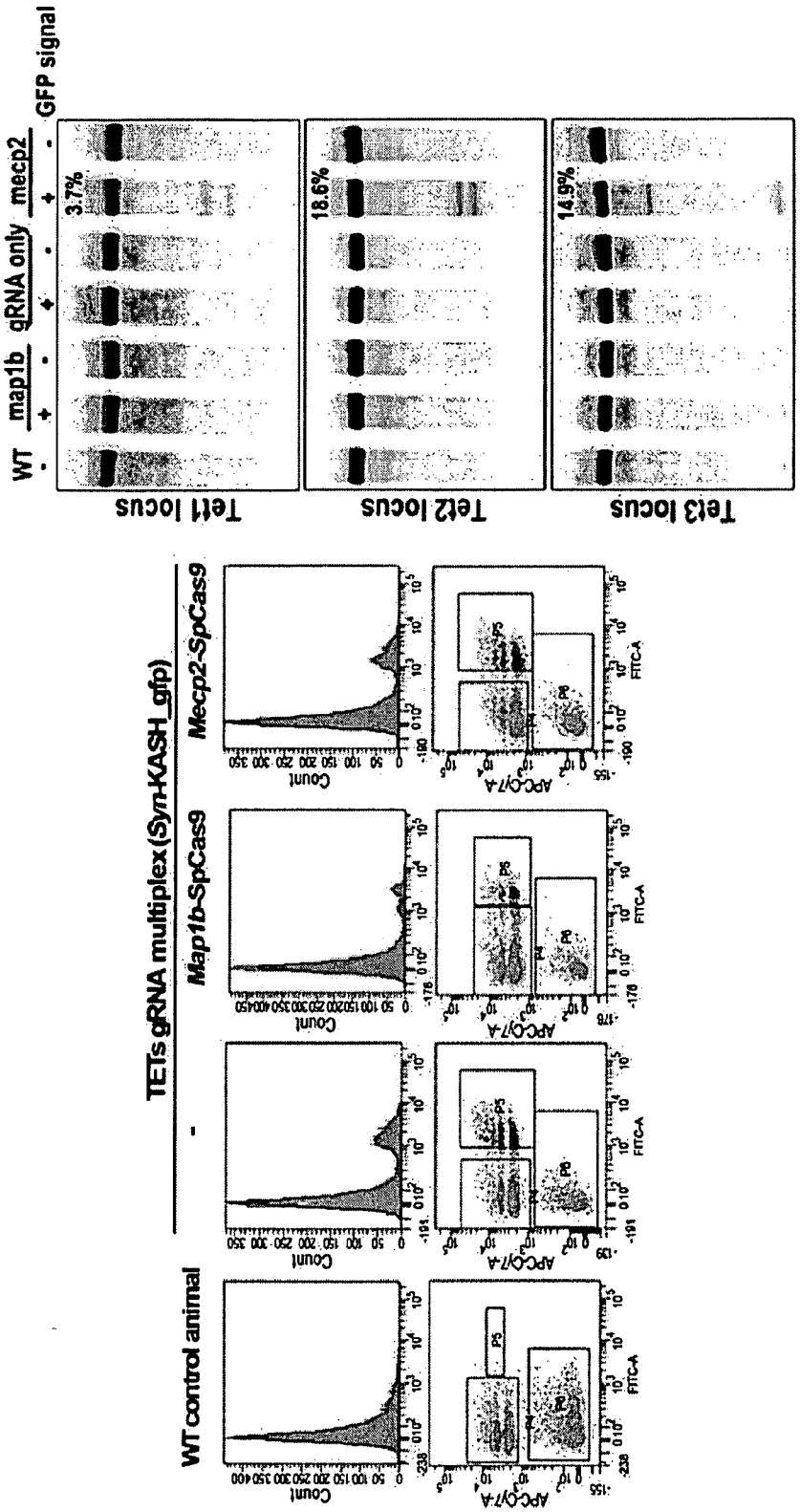

WT
map1b
gRNA only
mecp2
GFP signal
3.7%
18.6%
14.9%
Tet1 locus
Tet2 locus
Tet3 locus
TETs gRNA multiplex (Syn-KASH_gfp)
Map1b-SpCas9
Mecp2-SpCas9
WT control animal
Count
APC-Cy7-A
FITC-A
P5
P6

FIG. 49

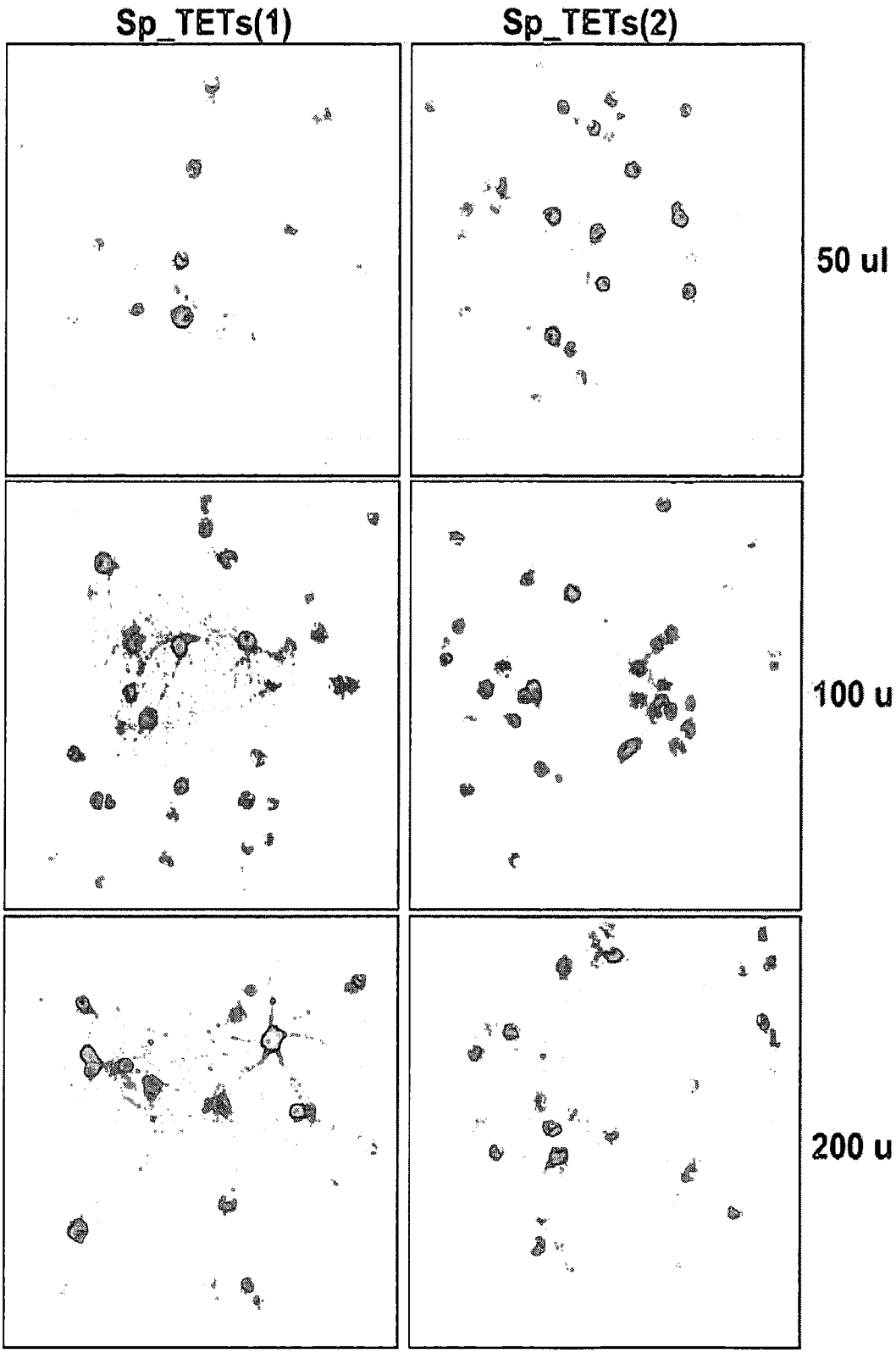
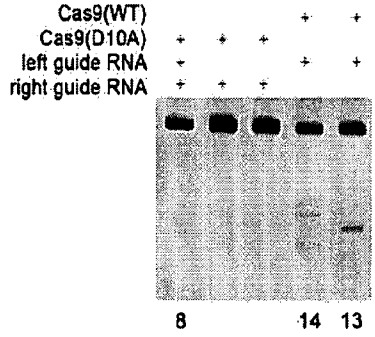
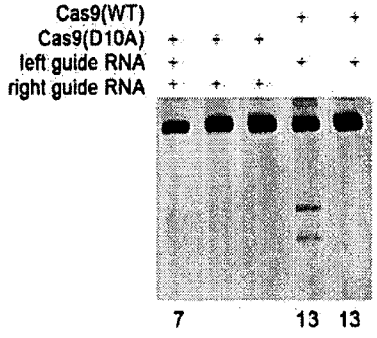



FIG. 50

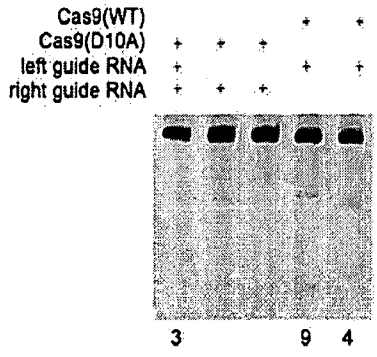
| | pattern | overhang |
|---|---|---|
| 1 | CCnNNNNNNNNNNNNNNNnnnnnnnnnnnnNNNNNNNNNNNNNNNnGG | 38 |
| 2 | CCnNNNNNNNNNNNNNNNNnnnnnnnnnNNNNNNNNNNNNNNNnGG | 34 |
| 3 | CCnNNNNNNNNNNNNNNNNnnnnnNNNNNNNNNNNNNNNNnGG | 30 |
| 4 | CCnNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNnGG | 26 |
| 5 | CCnNNNNNNNNNNNNNNNNNNNNNNNNNNNNnGG | 22 |
| 6 | CCnNNNNNNNNNNNNNNNNNNNNNNNNNnGG | 18 |
| 7 | CCnNNNNNNNNNNNNNNNNNNNNnGG | 14 |
| 8 | CCnNNNNNNNNNNNNNNNNnGG | 10 |
| 9 | CCnNNNNNNNNNNNNnGG | 6 |
| 10 | CCnNNNNNNNNNNnGG | 4 |
| 11 | CCnNNNNNNNNNnGG | 3 |
| 12 | CCnNNNNNNNNnGG | 2 |
| 13 | CCnNNNNNNNnGG | 1 |
| 14 | CCnNNNNNNnGG | 0 |
| 15 | CCnNNNNNnGG | 1 |
| 16 | CCnNNNNnGG | 2 |
| 17 | CCnNNNnGG | 3 |
| 18 | CCnnGG | 4 |
| 19 | CCGG | 8 |
| 20 | GGCC | 10 |
| 21 | GGNNCC | 12 |
| 22 | GGNNNCC | 14 |
| 23 | GGNNNNCC | 16 |
| 24 | GGNNNNNNNCC | 20 |



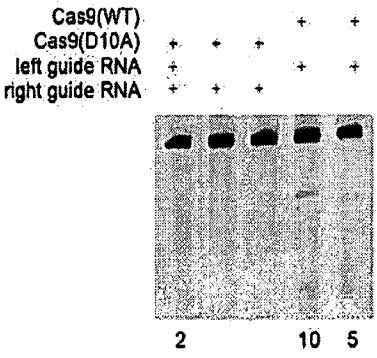

Pattern 1




Pattern 2




Pattern 3




Pattern 4

FIG. 51

**Reverse PCR primers for generating U6-guide RNA expression Cassette (pair with U6 forward primer)**

| | | |
|---|---|---|
| Z0260 | AAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAACTCACATCAACCGGTGGCGCAGGTGTTTCGTCCTTTCCACAAG | Left guide RNA 1 |
| Z0261 | AAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAACTCACATCAACCGGTGGCGCAGGTGTTTCGTCCTTTCCACAAG | Left guide RNA 2 |
| Z0262 | AAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAACGAGGACAAAGTACAAACGGCGGTGTTTCGTCCTTTCCACAAG | Left guide RNA 3 |
| Z0263 | AAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAACGAGGACAAAGTACAAACGGCGGTGTTTCGTCCTTTCCACAAG | Left guide RNA 4 |
| Z0264 | AAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAACGTGGCGCATTGCCACGAAGCGGTGTTTCGTCCTTTCCACAAG | Left guide RNA 5 |
| Z0265 | AAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAACCGAGGGCAGAGTGCTGCTTGGGTGTTTCGTCCTTTCCACAAG | Left guide RNA 6 |
| Z0266 | AAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAACGAGTCCGAGCAGAAGAAGAAGGTGTTTCGTCCTTTCCACAAG | Left guide RNA 7 |
| Z0267 | AAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAACGAGGACAAAGTACAAACGGCGGTGTTTCGTCCTTTCCACAAG | Left guide RNA 8 |
| Z0268 | AAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAACAGCAGAAGAAGAAGGGCTCCGGTGTTTCGTCCTTTCCACAAG | Left guide RNA 9 |
| Z0269 | AAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAACTCACATCAACCGGTGGCGCAGGTGTTTCGTCCTTTCCACAAG | Left guide RNA 10 |
| Z0270 | AAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAACCCCTGGCCCAGGTGAAGGTGGGTGTTTCGTCCTTTCCACAAG | Left guide RNA 11 |
| Z0271 | AAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAACTCCCTCCCTGGCCCAGGTGAGGTGTTTCGTCCTTTCCACAAG | Left guide RNA 12 |
| Z0272 | AAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAACGAACCGGAGGACAAAGTACAGGTGTTTCGTCCTTTCCACAAG | Left guide RNA 13 |
| Z0273 | AAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAACAGGTGAAGGTGTGGTTCCAGGGTGTTTCGTCCTTTCCACAAG | Left guide RNA 14 |
| Z0274 | AAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAACGGTGAAGGTGTGGTTCCAGAGGTGTTTCGTCCTTTCCACAAG | Left guide RNA 15 |
| Z0275 | AAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAACGAACCGGAGGACAAAGTACAGGTGTTTCGTCCTTTCCACAAG | Left guide RNA 16 |
| Z0276 | AAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAACCCCTGGCCCAGGTGAAGGTGGGTGTTTCGTCCTTTCCACAAG | Left guide RNA 17 |
| Z0277 | AAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAACAGGTGAAGGTGTGGTTCCAGGGTGTTTCGTCCTTTCCACAAG | Left guide RNA 18 |
| Z0278 | AAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAACGAGGACAAAGTACAAACGGCGGTGTTTCGTCCTTTCCACAAG | Left guide RNA 19 |
| Z0279 | AAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAACGGGAGGGAGGGGCACAGATGGGTGTTTCGTCCTTTCCACAAG | Left guide RNA 20 |
| Z0280 | AAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAACCACCTTCACCTGGGCCAGGGGGTGTTTCGTCCTTTCCACAAG | Left guide RNA 21 |
| Z0281 | AAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAACACCCTAGTCATTGGAGGTGAGGTGTTTCGTCCTTTCCACAAG | Left guide RNA 22 |
| Z0282 | AAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAACCAGAGCAGCCACTGGGGCCTGGTGTTTCGTCCTTTCCACAAG | Left guide RNA 23 |
| Z0283 | AAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAACCACCTTCACCTGGGCCAGGGGGTGTTTCGTCCTTTCCACAAG | Left guide RNA 24 |
| Z0284 | AAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAACCCCCATTGGCCTGCTTCGTGGGTGTTTCGTCCTTTCCACAAG | Right guide RNA 1 |
| Z0285 | AAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAACATTGGCCTGCTTCGTGGCAAGGTGTTTCGTCCTTTCCACAAG | Right guide RNA 2 |
| Z0286 | AAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAACTCCTCCTCCAGCTTCTGCCGGGTGTTTCGTCCTTTCCACAAG | Right guide RNA 3 |
| Z0287 | AAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAACCCTCCAGCTTCTGCCGTTTGGGTGTTTCGTCCTTTCCACAAG | Right guide RNA 4 |
| Z0288 | AAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAACATTGGCCTGCTTCGTGGCAAGGTGTTTCGTCCTTTCCACAAG | Right guide RNA 5 |
| Z0289 | AAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAACGCAGCAAGCAGCACTCTGCCGGTGTTTCGTCCTTTCCACAAG | Right guide RNA 6 |
| Z0290 | AAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAACTTCTTCTTCTGCTCGGACTCGGTGTTTCGTCCTTTCCACAAG | Right guide RNA 7 |
| Z0291 | AAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAACACCGGAGGACAAAGTACAAAGGTGTTTCGTCCTTTCCACAAG | Right guide RNA 8 |
| Z0292 | AAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAACTCTTCTTCTGCTCGGACTCAGGTGTTTCGTCCTTTCCACAAG | Right guide RNA 9 |
| Z0293 | AAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAACGTTGATGTGATGGGAGCCCTGGTGTTTCGTCCTTTCCACAAG | Right guide RNA 10 |
| Z0294 | AAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAACGGGCCAGGGAGGGAGGGGCAGGTGTTTCGTCCTTTCCACAAG | Right guide RNA 11 |
| Z0295 | AAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAACGGGAGGGAGGGGCACAGATGGGTGTTTCGTCCTTTCCACAAG | Right guide RNA 12 |
| Z0296 | AAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAACCCGGTTCTGGAACCACACCTGGTGTTTCGTCCTTTCCACAAG | Right guide RNA 13 |
| Z0297 | AAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAACTCACCTGGGCCAGGGAGGGAGGTGTTTCGTCCTTTCCACAAG | Right guide RNA 14 |
| Z0298 | AAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAACTCACCTGGGCCAGGGAGGGAGGTGTTTCGTCCTTTCCACAAG | Right guide RNA 15 |
| Z0299 | AAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAACGTTCTGGAACCACACCTTCAGGTGTTTCGTCCTTTCCACAAG | Right guide RNA 16 |
| Z0300 | AAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAACGGGAGGGAGGGGCACAGATGGGTGTTTCGTCCTTTCCACAAG | Right guide RNA 17 |
| Z0301 | AAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAACGGGCCAGGGAGGGAGGGGCAGGTGTTTCGTCCTTTCCACAAG | Right guide RNA 18 |
| Z0302 | AAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAACGTTCTGGAACCACACCTTCAGGTGTTTCGTCCTTTCCACAAG | Right guide RNA 19 |
| Z0303 | AAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAACAGGTGAAGGTGTGGTTCCAGGGTGTTTCGTCCTTTCCACAAG | Right guide RNA 20 |
| Z0304 | AAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAACGAACCGGAGGACAAAGTACAGGTGTTTCGTCCTTTCCACAAG | Right guide RNA 21 |
| Z0305 | AAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAACCAAACCCACGAGGGCAGAGTGGTGTTTCGTCCTTTCCACAAG | Right guide RNA 22 |
| Z0306 | AAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAACGAGTTTCTCATCTGTGCCCCGGTGTTTCGTCCTTTCCACAAG | Right guide RNA 23 |
| Z0307 | AAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAACGAGGACAAAGTACAAACGGCGGTGTTTCGTCCTTTCCACAAG | Right guide RNA 24 |

FIG. 52

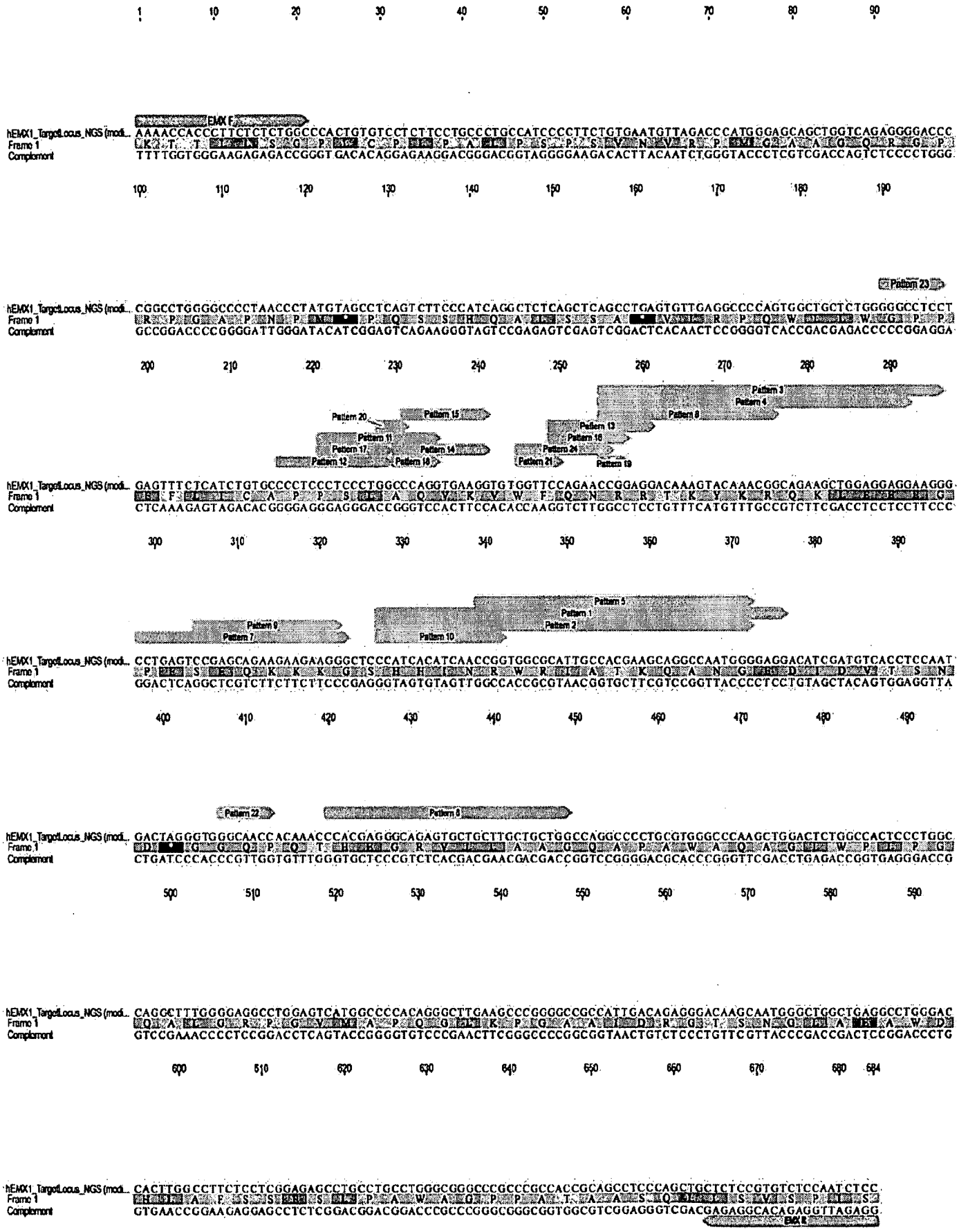

EMX F
Pattern 23
Pattern 3
Pattern 4
Pattern 20
Pattern 15
Pattern 8
Pattern 13
Pattern 11
Pattern 16
Pattern 17
Pattern 14
Pattern 24
Pattern 12
Pattern 18
Pattern 21
Pattern 19
Pattern 5
Pattern 1
Pattern 9
Pattern 7
Pattern 2
Pattern 10
Pattern 22
Pattern 6
EMX R

FIG. 53

| lane # | Cas9 to use | Left gRNA | Right gRNA | Overhang (D10A) - number of bases protruding from 5' |
|---|---|---|---|---|
| 1 | D10A | left 23 | left 12 | -36 |
| 2 | D10A | right 4 | left 9 | -25 |
| 3 | D10A | left 23 | right 23 | -16 |
| 4 | D10A | right 7 | left 10 | -15 |
| 5 | D10A | right 16 | left 3 | -8 |
| 6 | D10A | right 22 | right 6 | 26 |
| 7 | D10A | left 12 | right 16 | 31 |
| 8 | D10A | left 12 | right 13 | 34 |
| 9 | D10A | left 10 | right 1 | 38 |
| 10 | D10A | right 23 | right 16 | 51 |
| 11 | D10A | right 23 | right 13 | 54 |
| 12 | D10A | left 3 | right 7 | 57 |
| 13 | D10A | left 12 | right 4 | 65 |
| 14 | D10A | left 12 | right 3 | 69 |
| 15 | D10A | left 3 | right 10 | 76 |
| 16 | D10A | right 23 | right 4 | 85 |
| 17 | D10A | left 12 | right 9 | 95 |
| 18 | D10A | left 12 | right 10 | 115 |
| 19 | D10A | right 23 | right 10 | 135 |
| 20 | D10A | left 12 | right 2 | 145 |
| 21 | D10A | left 12 | left 22 | 181 |
| 22 | D10A | right 23 | left 22 | 201 |
| 23 | D10A | left 12 | right 6 | 222 |
| 24 | D10A | right 23 | right 6 | 242 |

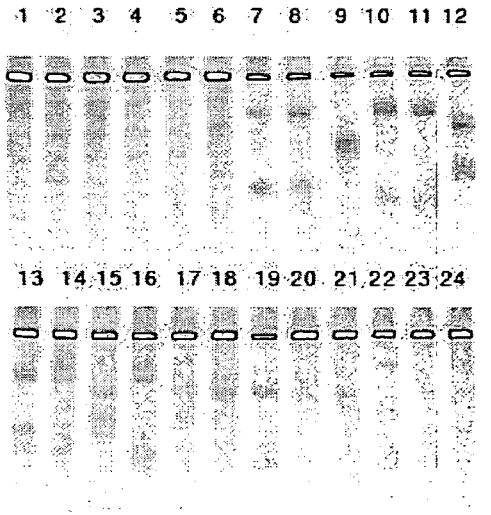



FIG. 54

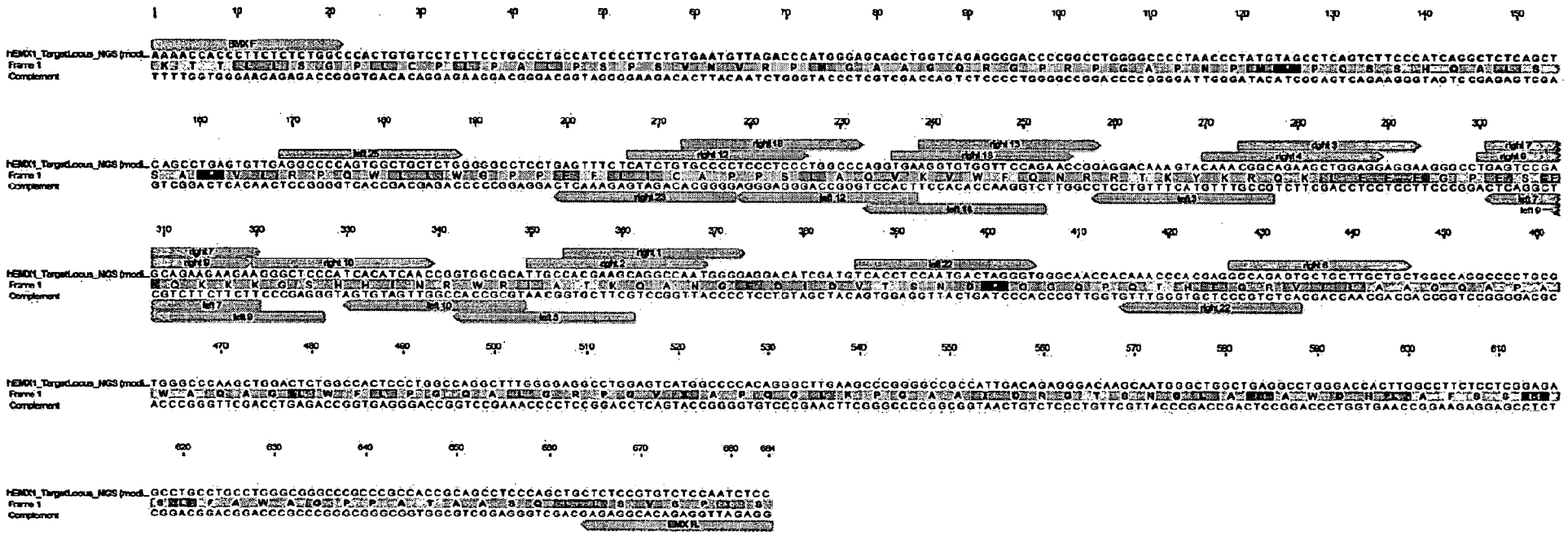


FIG. 55

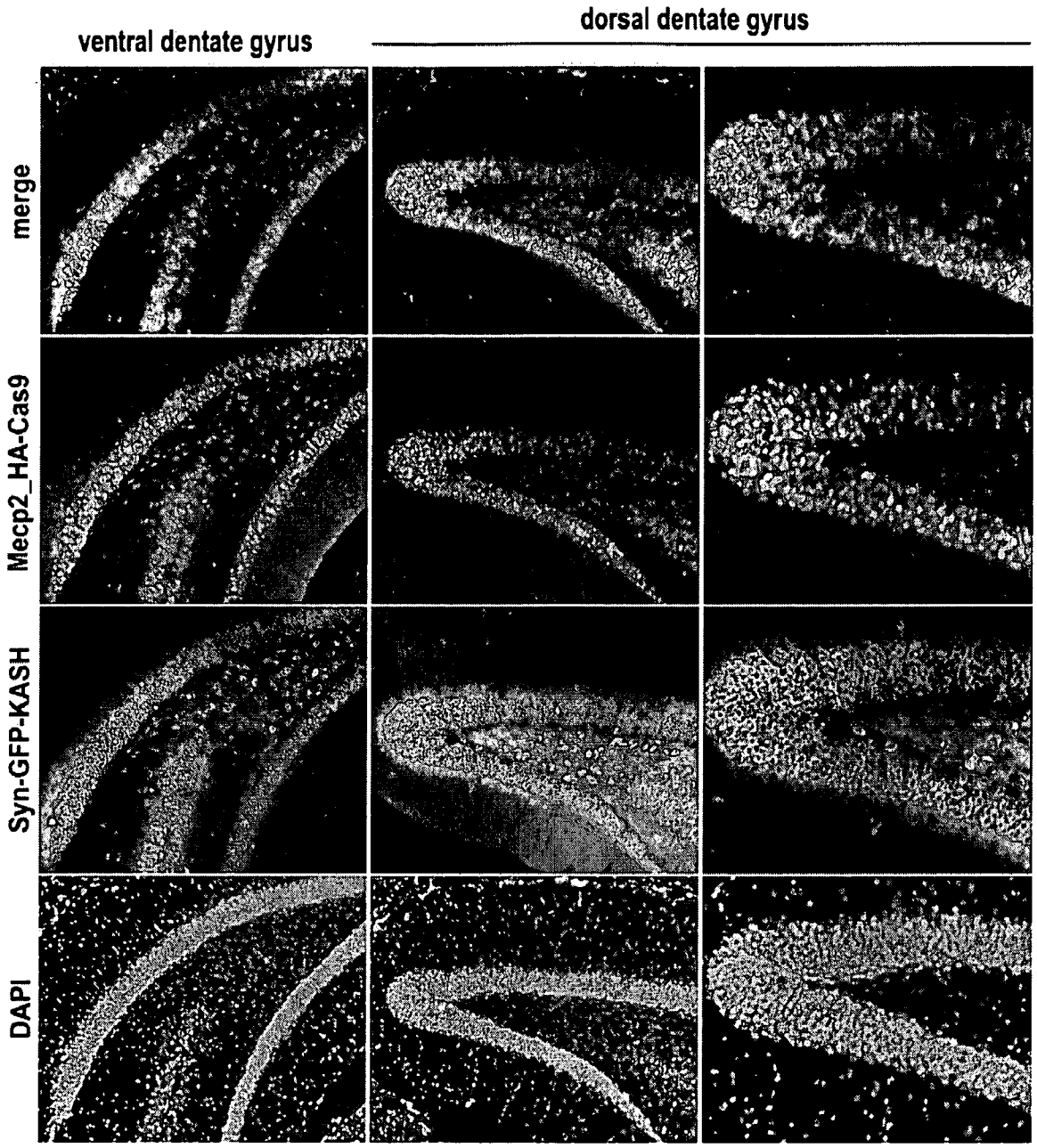
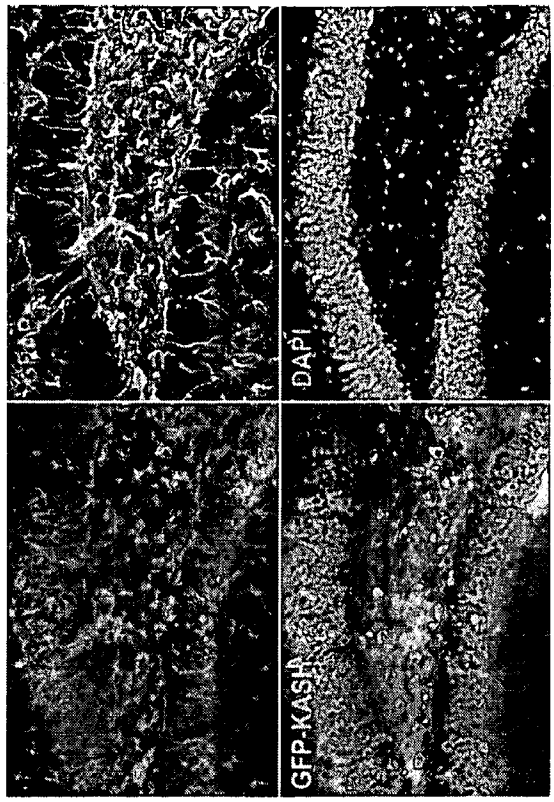

ventral dentate gyrus
dorsal dentate gyrus
merge
Mecp2_HA-Cas9
Syn-GFP-KASH
DAPI

FIG. 56



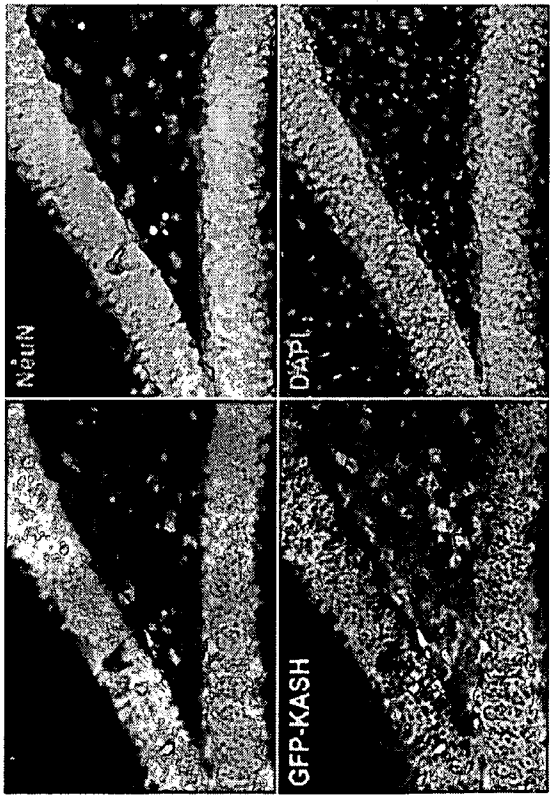




FIG. 57

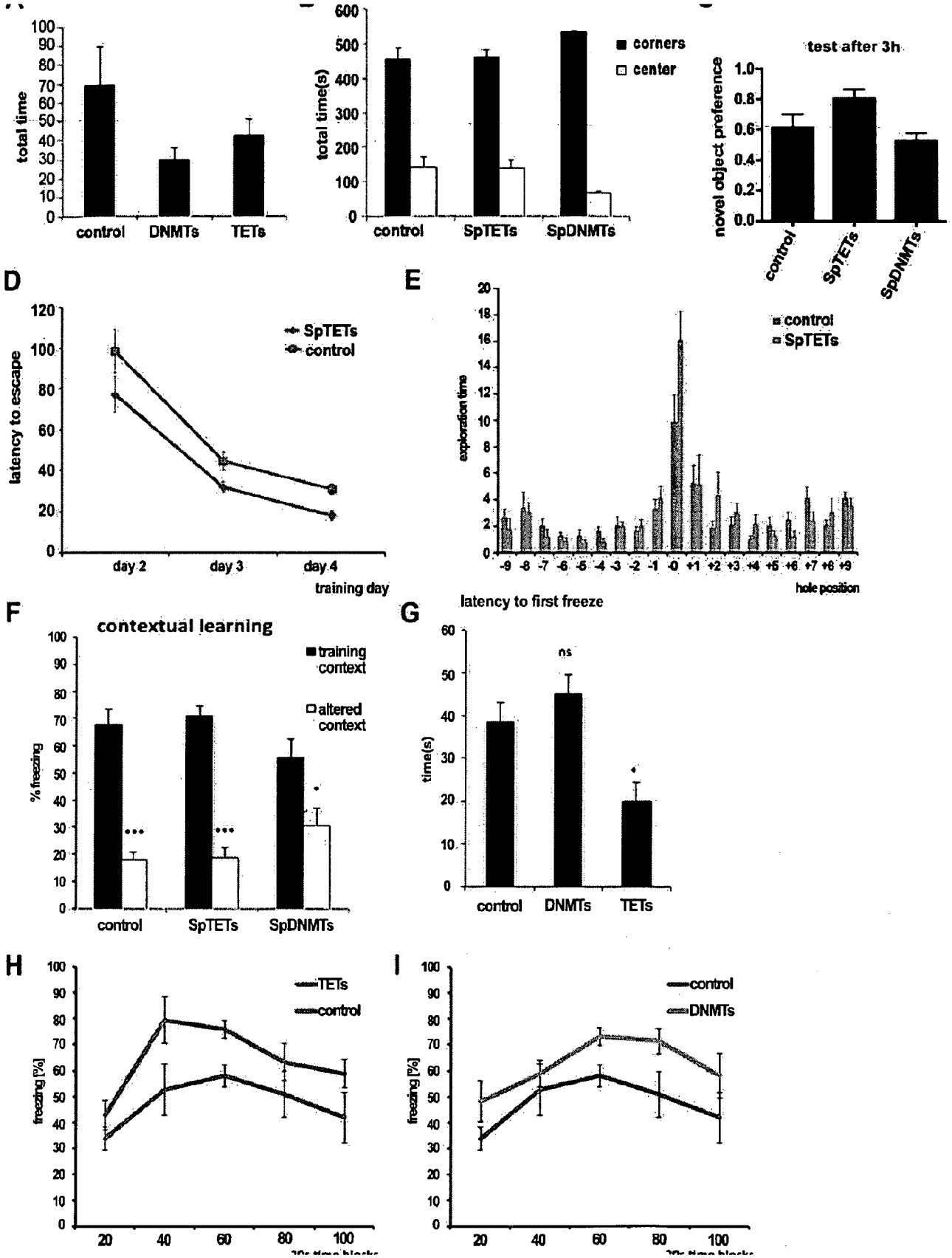

total time
control
DNMTs
TETs
total time(s)
corners
center
SpTETs
SpDNMTs
test after 3h
novel object preference
D
latency to escape
SpTETs
control
day 2
day 3
day 4
training day
E
exploration time
hole position
F
contextual learning
training context
altered context
% freezing
G
latency to first freeze
ns
time(s)
H
freezing [%]
I

FIG. 58

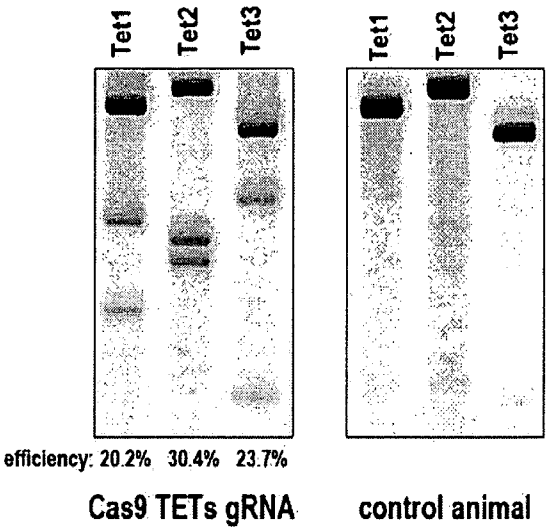

Tet1
Tet2
Tet3
Tet1
Tet2
Tet3
efficiency: 20.2% 30.4% 23.7%
Cas9 TETs gRNA
control animal

FIG. 59

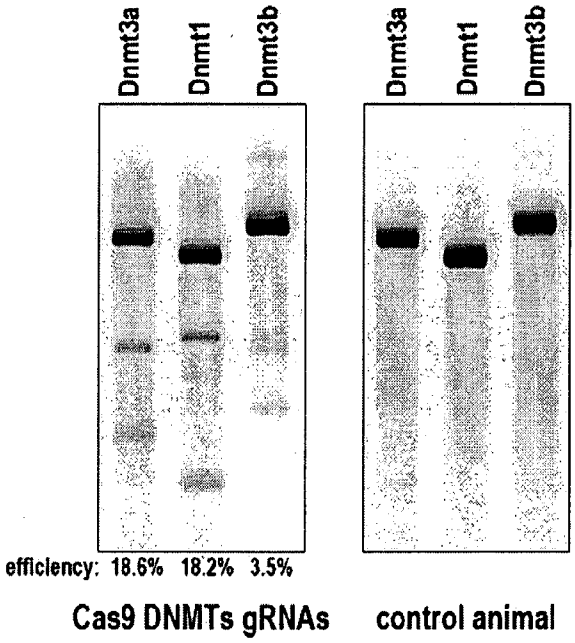

Dnmt3a
Dnmt1
Dnmt3b
Dnmt3a
Dnmt1
Dnmt3b
efficiency: 18.6% 18.2% 3.5%
Cas9 DNMTs gRNAs
control animal

FIG. 60

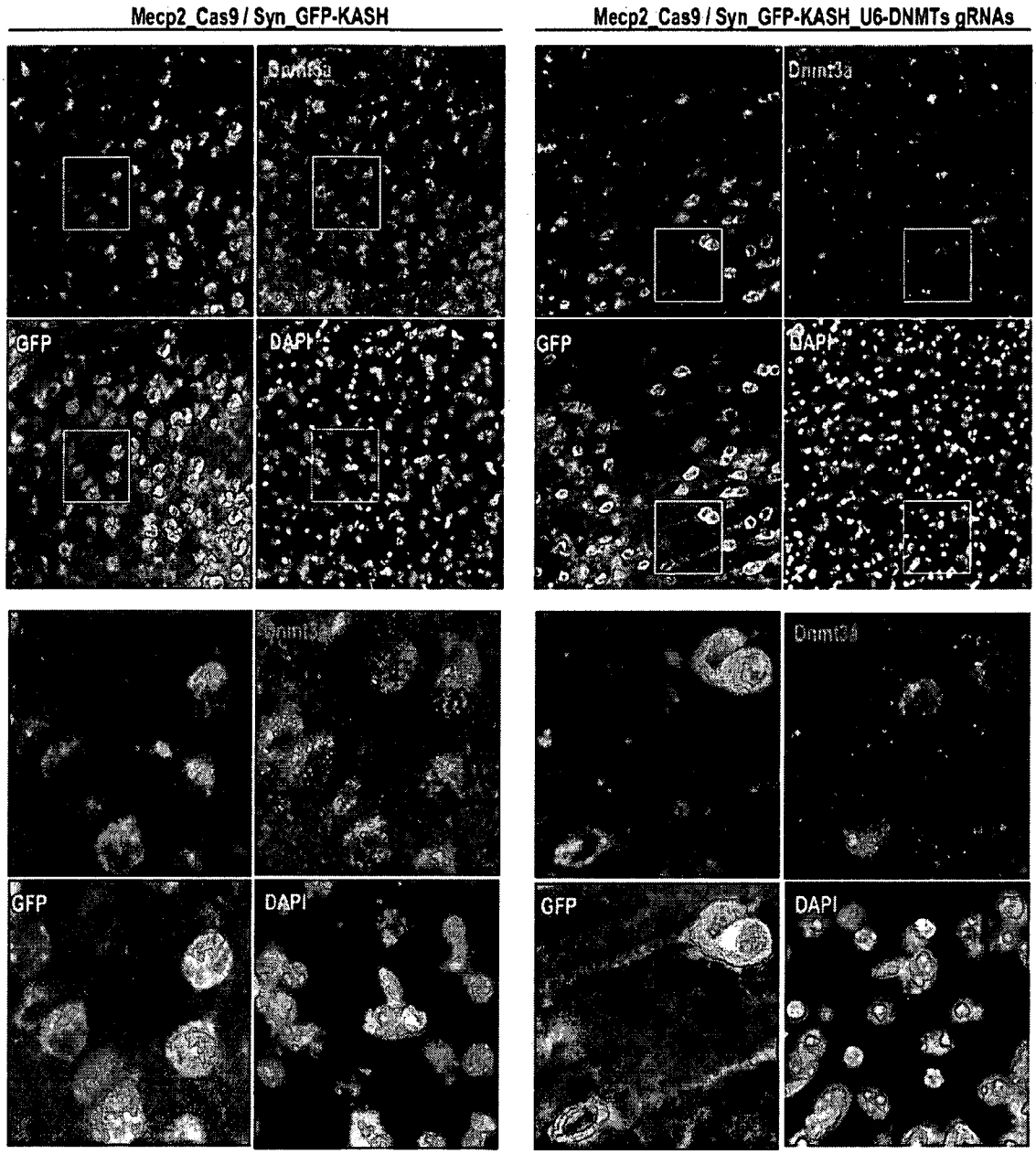

Mecp2_Cas9 / Syn_GFP-KASH
Mecp2_Cas9 / Syn_GFP-KASH_U6-DNMTs gRNAs
Dnmt3a
GFP
DAPI

FIG. 61

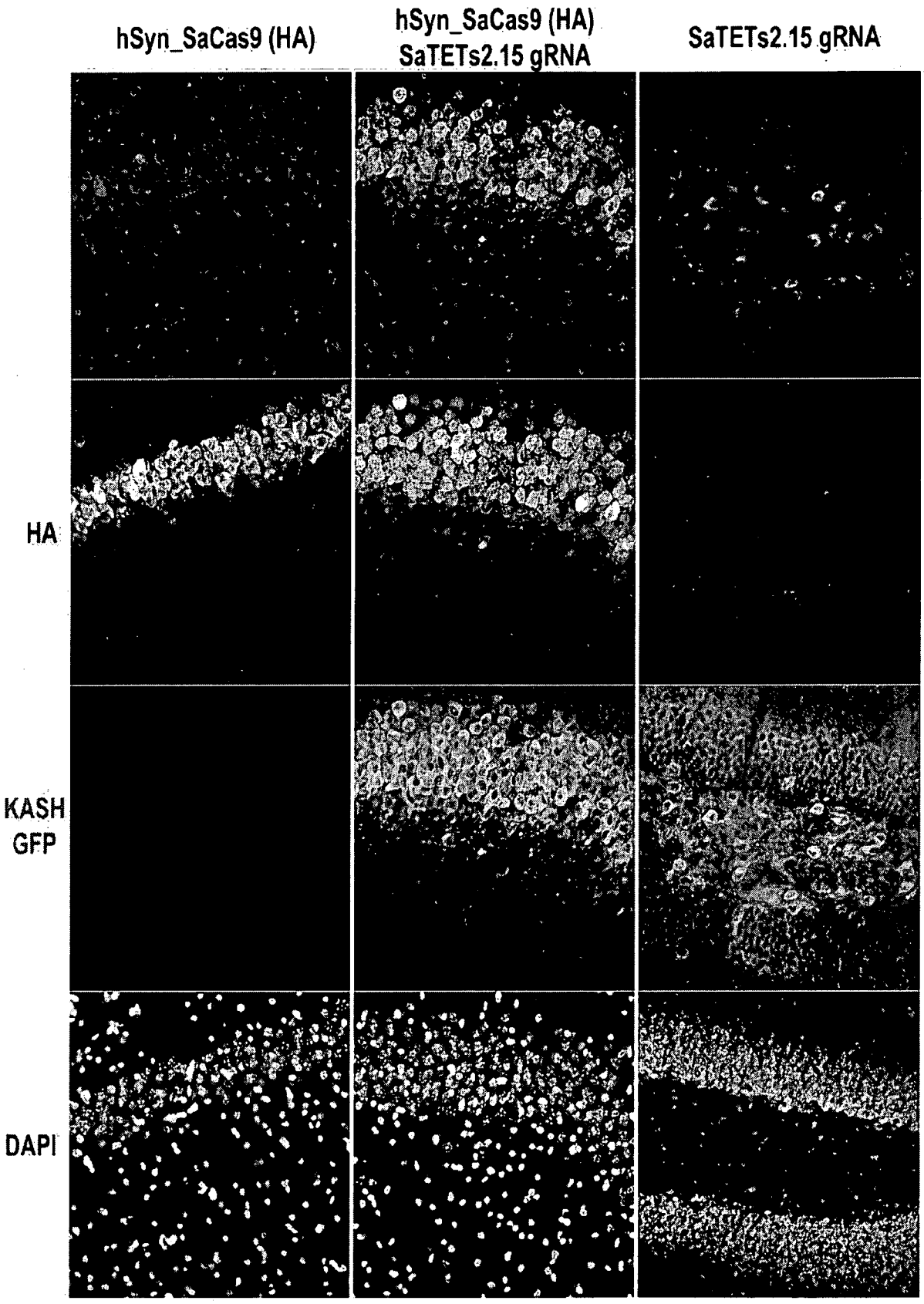

hSyn_SaCas9 (HA)
hSyn_SaCas9 (HA)
SaTETs2.15 gRNA
SaTETs2.15 gRNA
HA
KASH
GFP
DAPI

FIG. 62

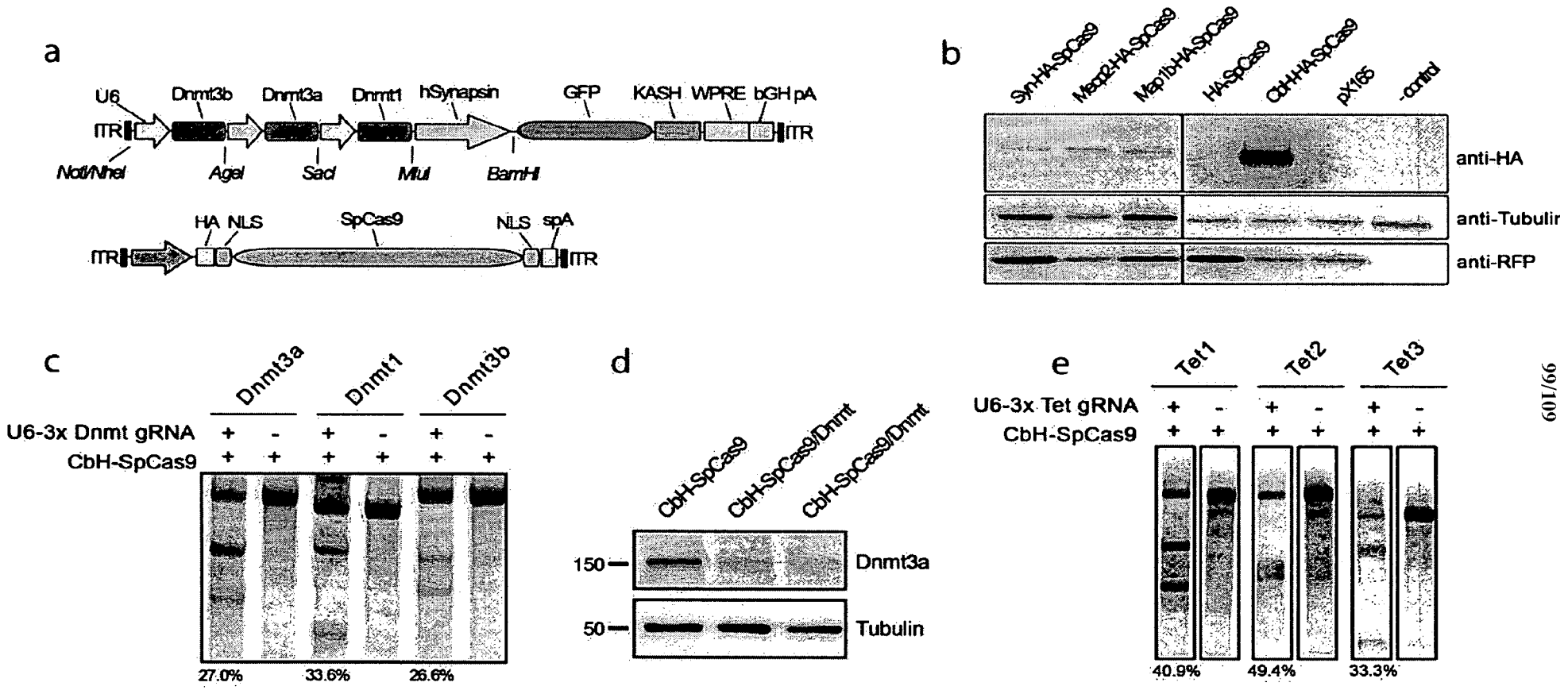

a
U6
Dnmt3b
Dnmt3a
Dnmt1
hSynapsin
GFP
KASH
WPRE
bGH pA
ITR
ITR
NotI/NheI
AgeI
SacI
MluI
BamHI
HA
NLS
SpCas9
NLS
spA
ITR
ITR
b
SynHA-SpCas9
Mecp2HA-SpCas9
Map1bHA-SpCas9
HA-SpCas9
CbHHA-SpCas9
pX165
-control
anti-HA
anti-Tubulin
anti-RFP
c
Dnmt3a
Dnmt1
Dnmt3b
U6-3x Dnmt gRNA
CbH-SpCas9
27.0%
33.6%
26.6%
d
CbH-SpCas9
CbH-SpCas9/Dnmt
CbH-SpCas9/Dnmt
150
50
Dnmt3a
Tubulin
e
Tet1
Tet2
Tet3
U6-3x Tet gRNA
CbH-SpCas9
40.9%
49.4%
33.3%

FIG. 63

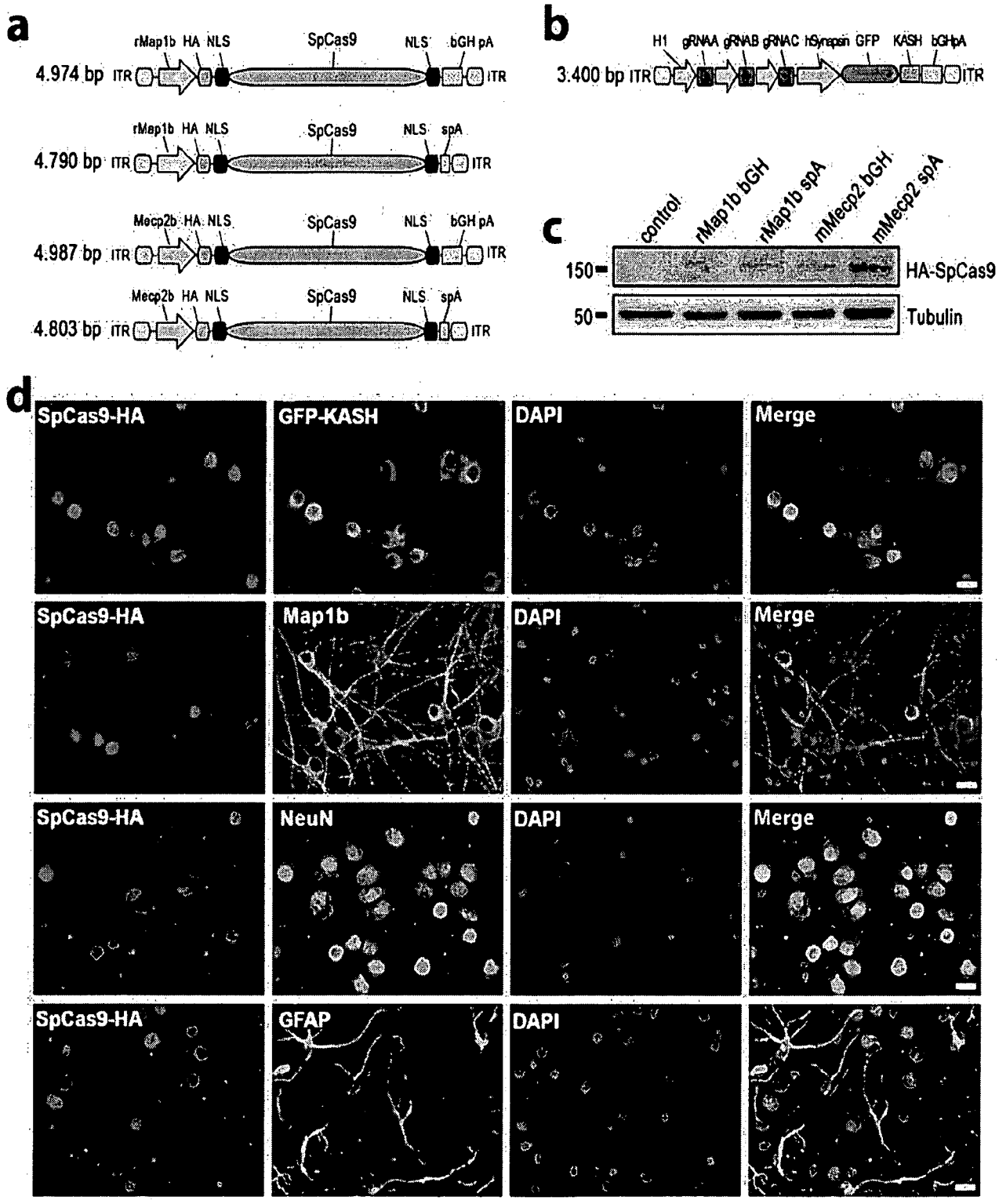

a
rMap1b HA NLS SpCas9 NLS bGH pA
4.974 bp ITR ITR
rMap1b HA NLS SpCas9 NLS spA
4.790 bp ITR ITR
Mecp2b HA NLS SpCas9 NLS bGH pA
4.987 bp ITR ITR
Mecp2b HA NLS SpCas9 NLS spA
4.803 bp ITR ITR
b
H1 gRNAA gRNAB gRNAC hSynapsin GFP KASH bGHpA
3.400 bp ITR ITR
c
control
rMap1b bGH
rMap1b spA
mMecp2 bGH
mMecp2 spA
150
HA-SpCas9
50
Tubulin
d
SpCas9-HA
GFP-KASH
DAPI
Merge
SpCas9-HA
Map1b
DAPI
Merge
SpCas9-HA
NeuN
DAPI
Merge
SpCas9-HA
GFAP
DAPI

FIG. 64

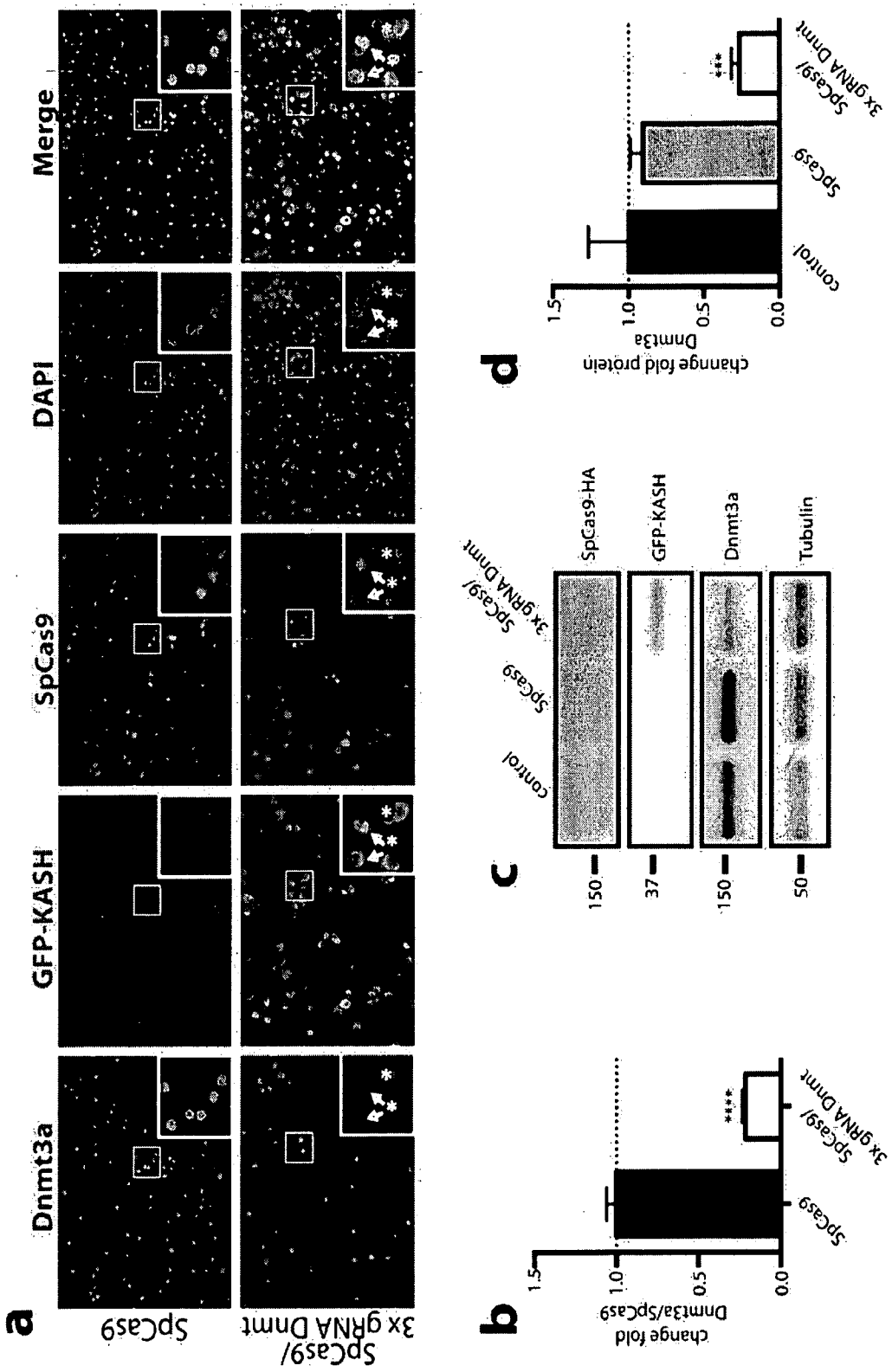

a
Dnmt3a
GFP-KASH
SpCas9
DAPI
Merge
SpCas9
SpCas9/ 3x gRNA Dnmt
b
change fold Dnmt3a/SpCas9
1.5
1.0
0.5
0.0
****
SpCas9
SpCas9/ 3x gRNA Dnmt
c
control
SpCas9
SpCas9/ 3x gRNA Dnmt
SpCas9-HA
GFP-KASH
Dnmt3a
Tubulin
150
37
150
50
d
change fold protein Dnmt3a
1.5
1.0
0.5
0.0
control
SpCas9
SpCas9/ 3x gRNA Dnmt
**

FIG. 65

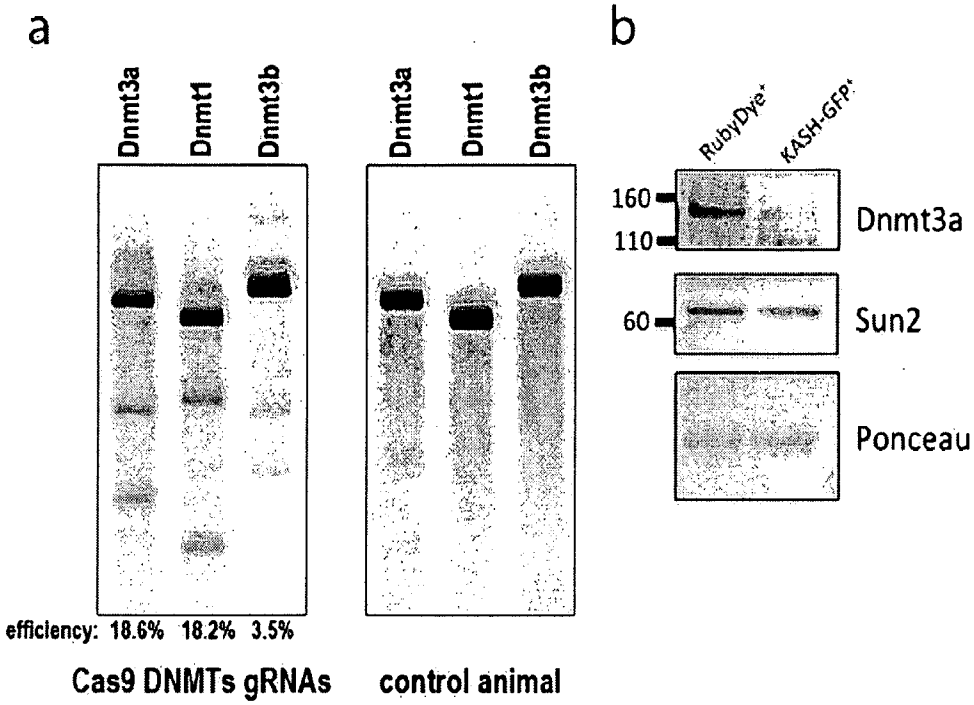

a
Dnmt3a
Dnmt1
Dnmt3b
Dnmt3a
Dnmt1
Dnmt3b
efficiency: 18.6% 18.2% 3.5%
Cas9 DNMTs gRNAs
control animal
b
RubyDye+
KASH-GFP+
160
110
Dnmt3a
60
Sun2
Ponceau

FIG. 66

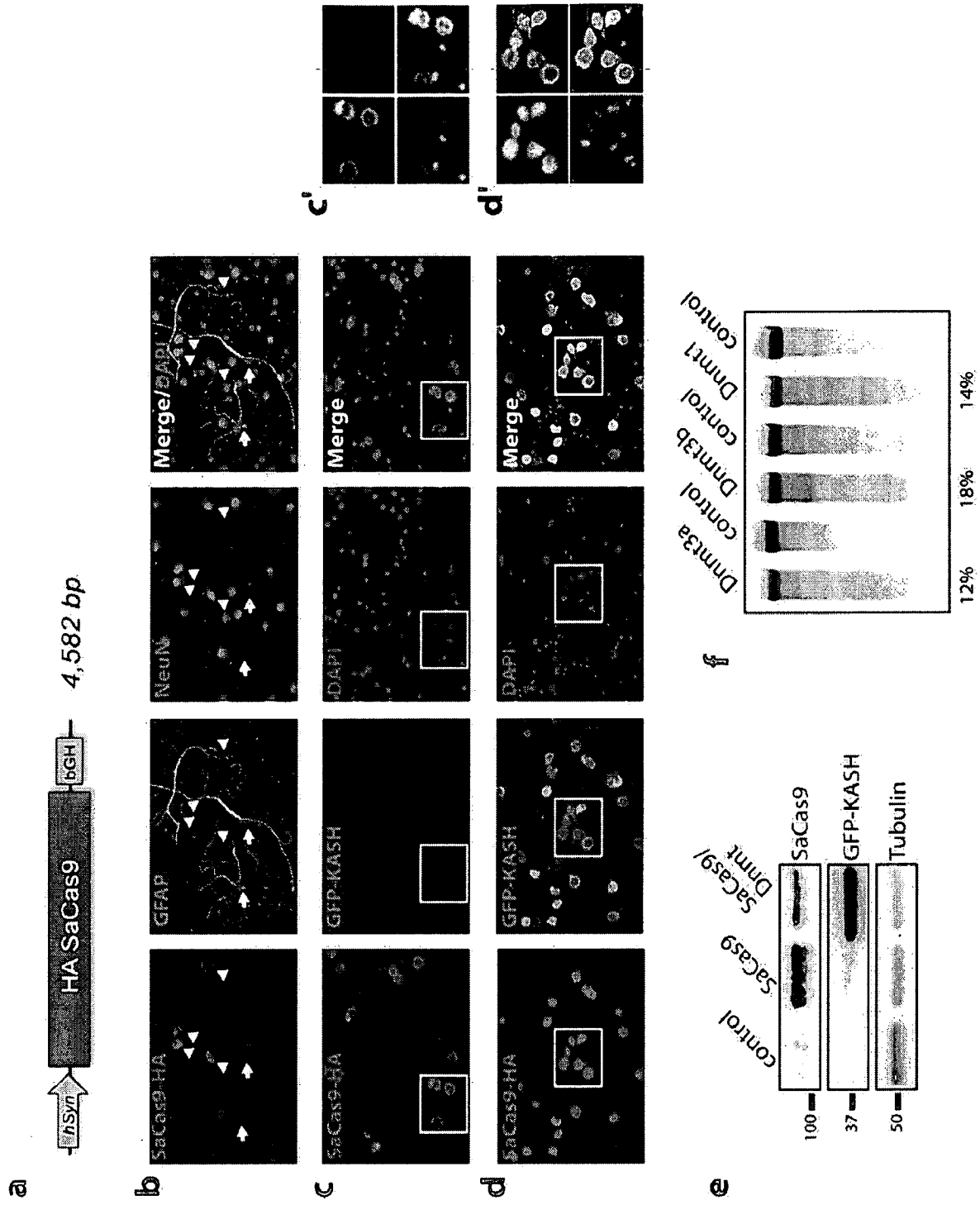

a
hSyn
HA SaCas9
bGH
4,582 bp
b
SaCas9-HA
GFAP
NeuN
Merge/DAPI
c
SaCas9-HA
GFP-KASH
DAPI
Merge
d
SaCas9-HA
GFP-KASH
DAPI
Merge
c'
d'
e
control
saCas9
saCas9/ Dnmt
SaCas9
GFP-KASH
Tubulin
100
37
50
f
Dnmt3a
control
Dnmt3b
control
Dnmt1
control
12%
18%
14%

FIG. 67

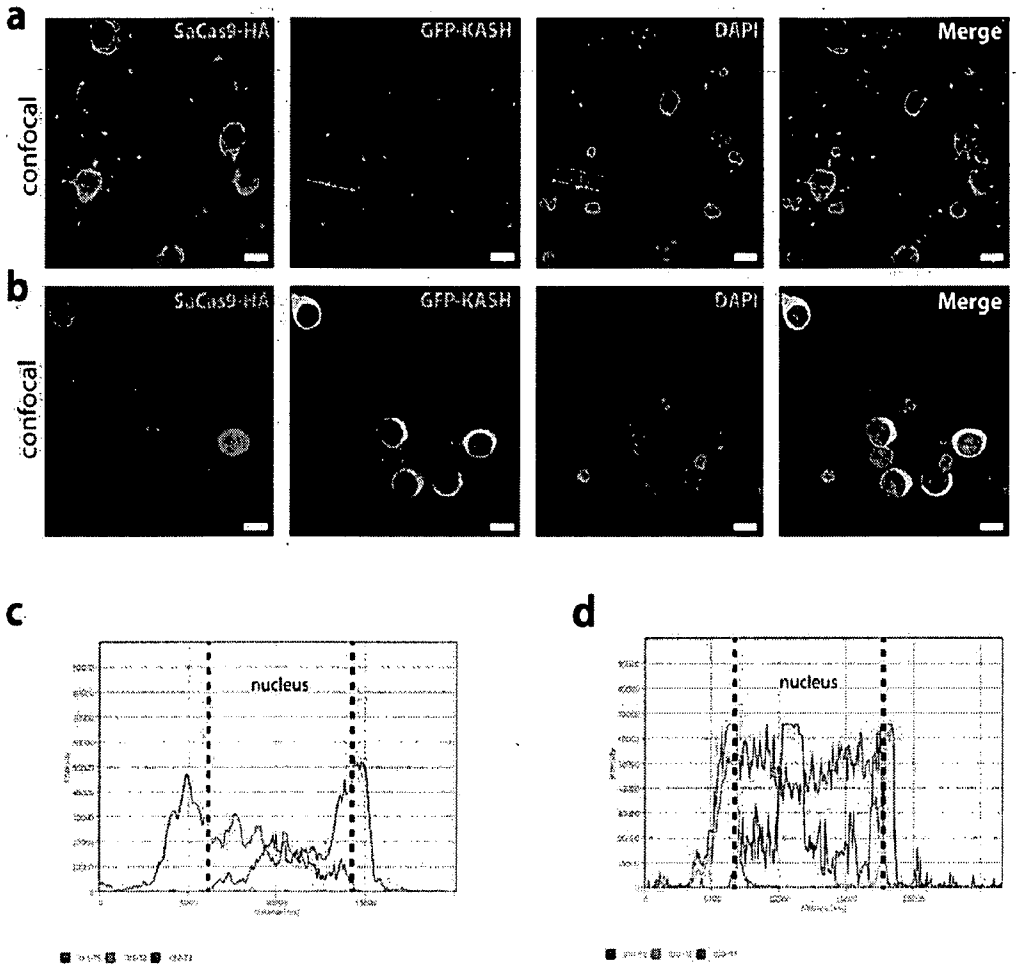
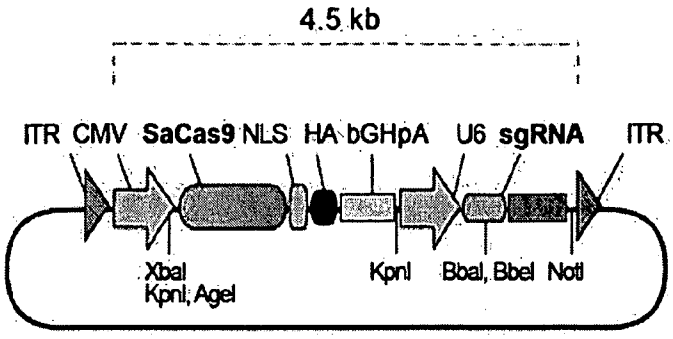
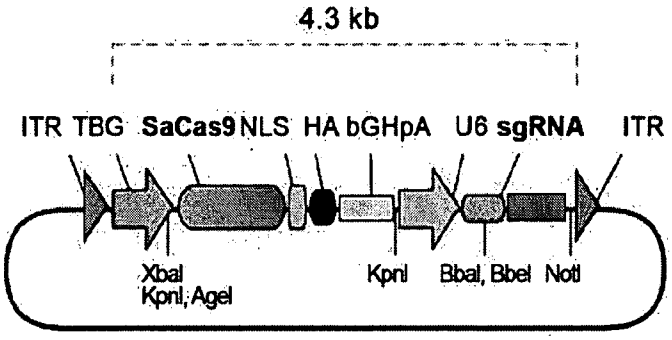

a
SaCas9-HA
GFP-KASH
DAPI
Merge
confocal
b
SaCas9-HA
GFP-KASH
DAPI
Merge
confocal
c
nucleus
d
nucleus
e
SaCas9
SpCas9
cytoplasm
nuclear
cytoplasm
nuclear
- + - + - + - +
250
150
100
75
37
anti-HA
anti-Sun2
anti-Tubulin

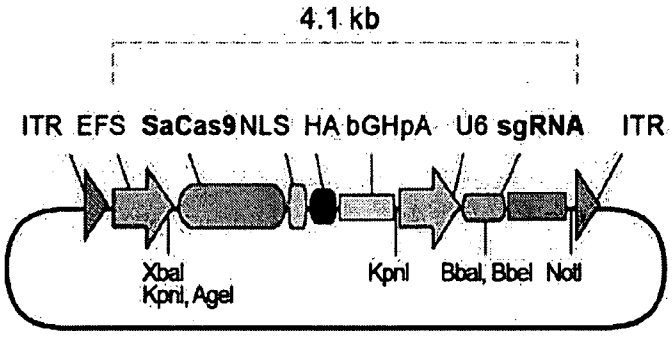
FIG. 68




**AAV-Sa-Cas9 vector:**
CMV-SaCas9-NLS-HA-U6-sgRNA




**Liver-specific AAV-Sa-Cas9 vector:**
TBG-SaCas9-NLS-HA-U6-sgRNA




**Alternate AAV-Sa-Cas9 vector:**
EFS-SaCas9-NLS-HA-U6-sgRNA

FIG. 69

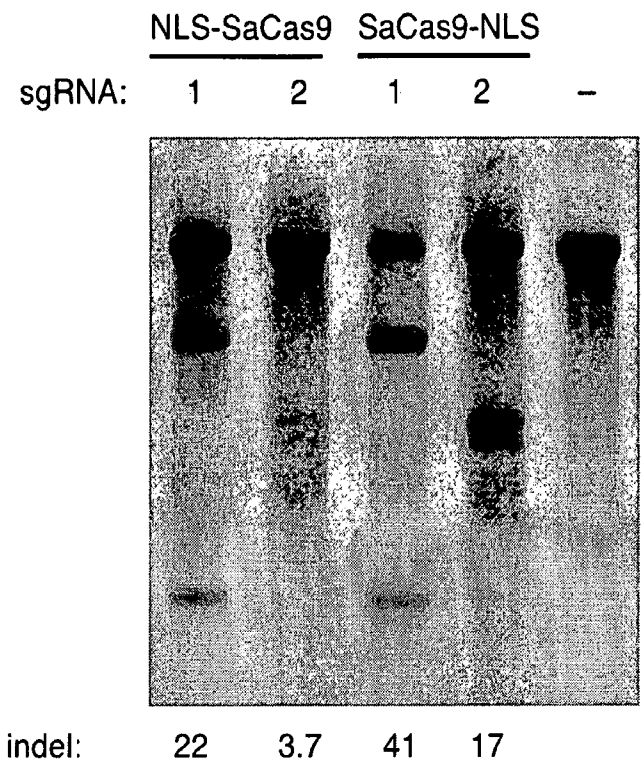



FIG. 70

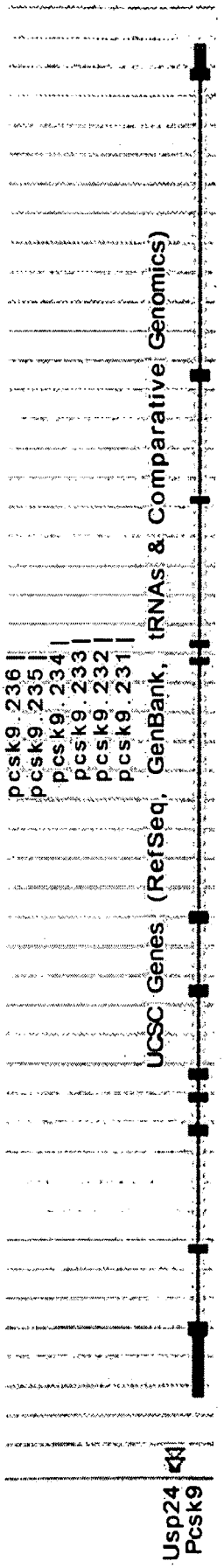

Usp24
Pcsk9
UCSC Genes (RefSeq, GenBank, tRNAs & Comparative Genomics)
pcsk9_236
pcsk9_235
pcsk9_234
pcsk9_233
pcsk9_232
pcsk9_231

FIG. 71

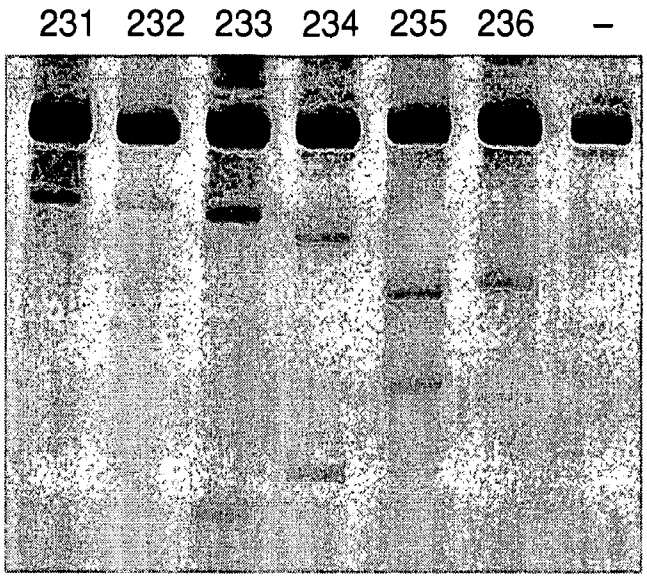



FIG. 72

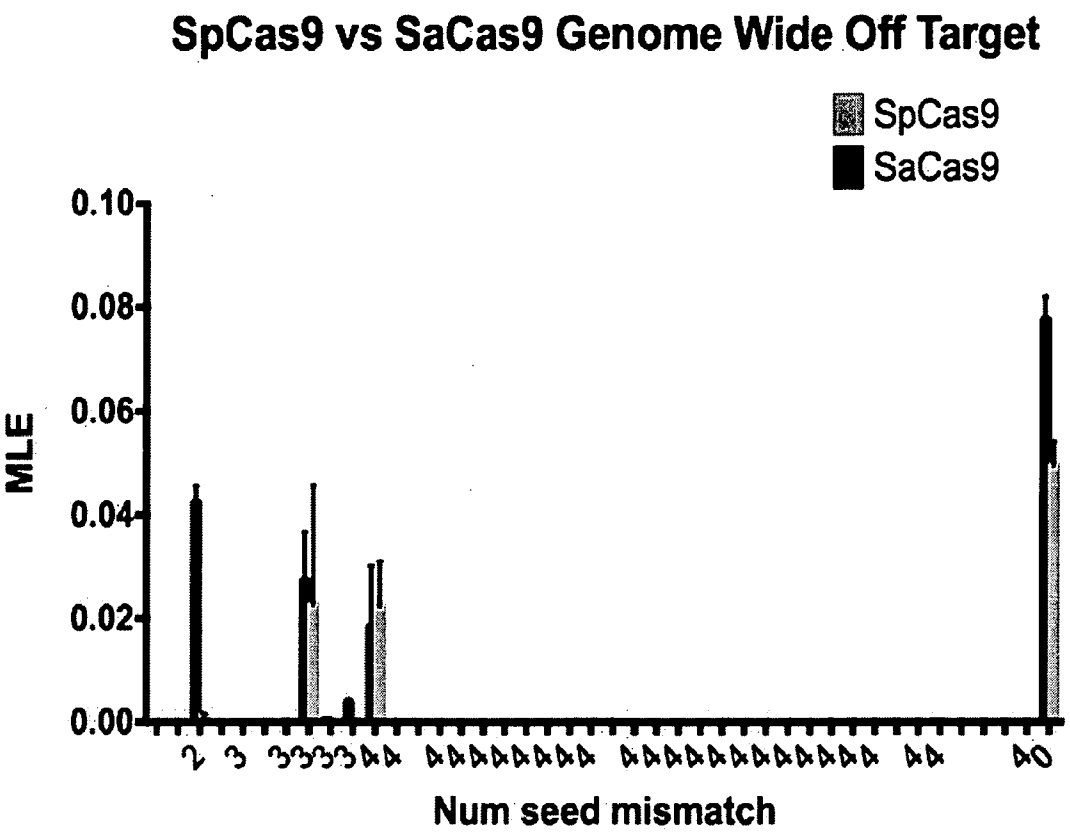

SpCas9 vs SaCas9 Genome Wide Off Target
SpCas9
SaCas9
0.10
0.08
0.06
0.04
0.02
0.00
MLE
Num seed mismatch

FIG. 73