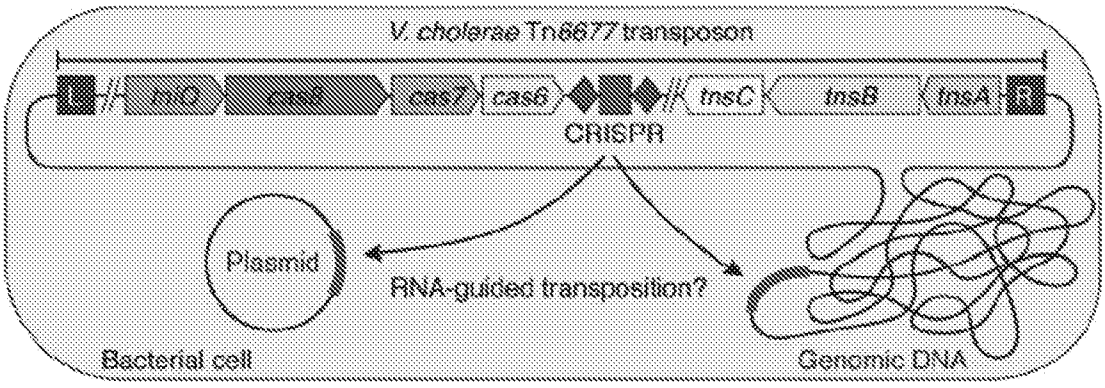

V. cholerae Tn6677 transposon
L
R
cas7
cas6
tnsC
tnsB
tnsA
CRISPR
Plasmid
RNA-guided transposition?
Bacterial cell
Genomic DNA

FIG. 1A

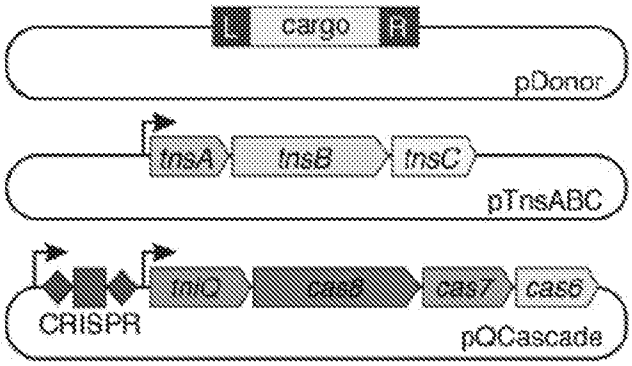

L
cargo
R
pDonor
tnsA
tnsB
tnsC
pTnsABC
CRISPR
cas7
cas6
pQCascade

FIG. 1B

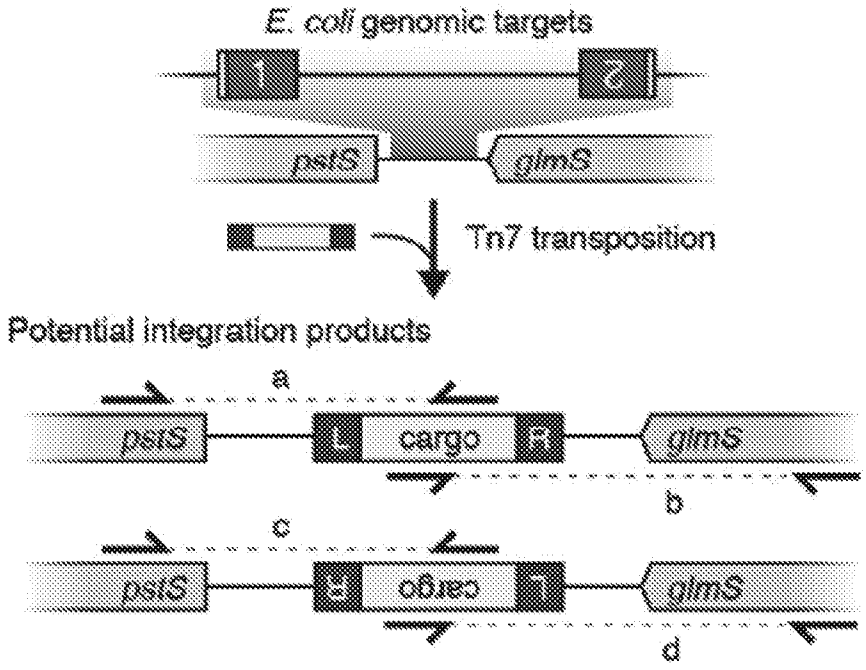

E. coli genomic targets
1
2
pstS
glmS
Tn7 transposition
Potential integration products
a
pstS
L
cargo
R
glmS
b
c
pstS
L
cargo
R
glmS
d

FIG. 1C

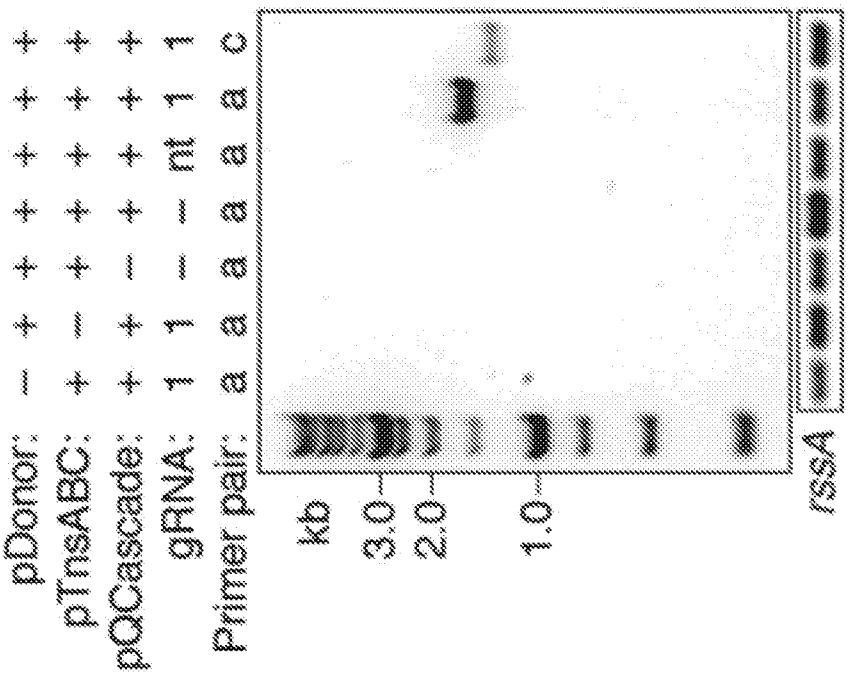

pDonor: – + + + + + + + +
pTnsABC: + – + + + + + + +
pQCascade: + + – + + + + + +
gRNA: 1 1 – – nt 1 1 1
Primer pair: a a a a a a a c
kb
3.0
2.0
1.0
rssA

FIG. 1D

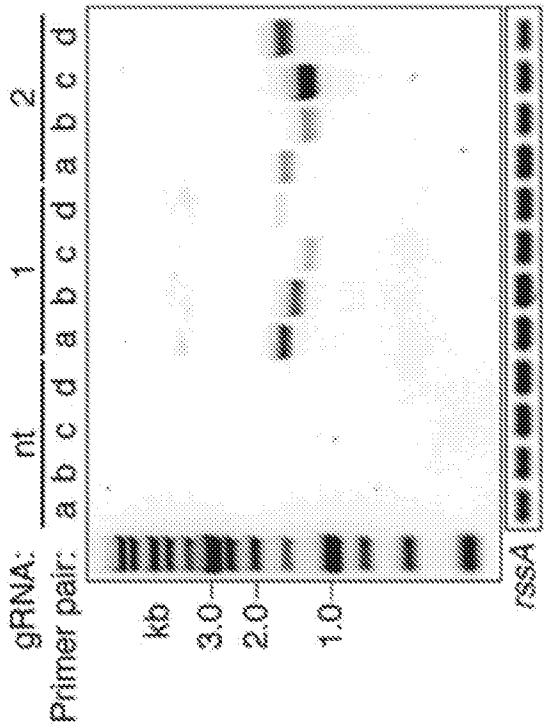

gRNA: nt 1 2
Primer pair: a b c d a b c d a b c d
kb
3.0
2.0
1.0
rssA

FIG. 1E

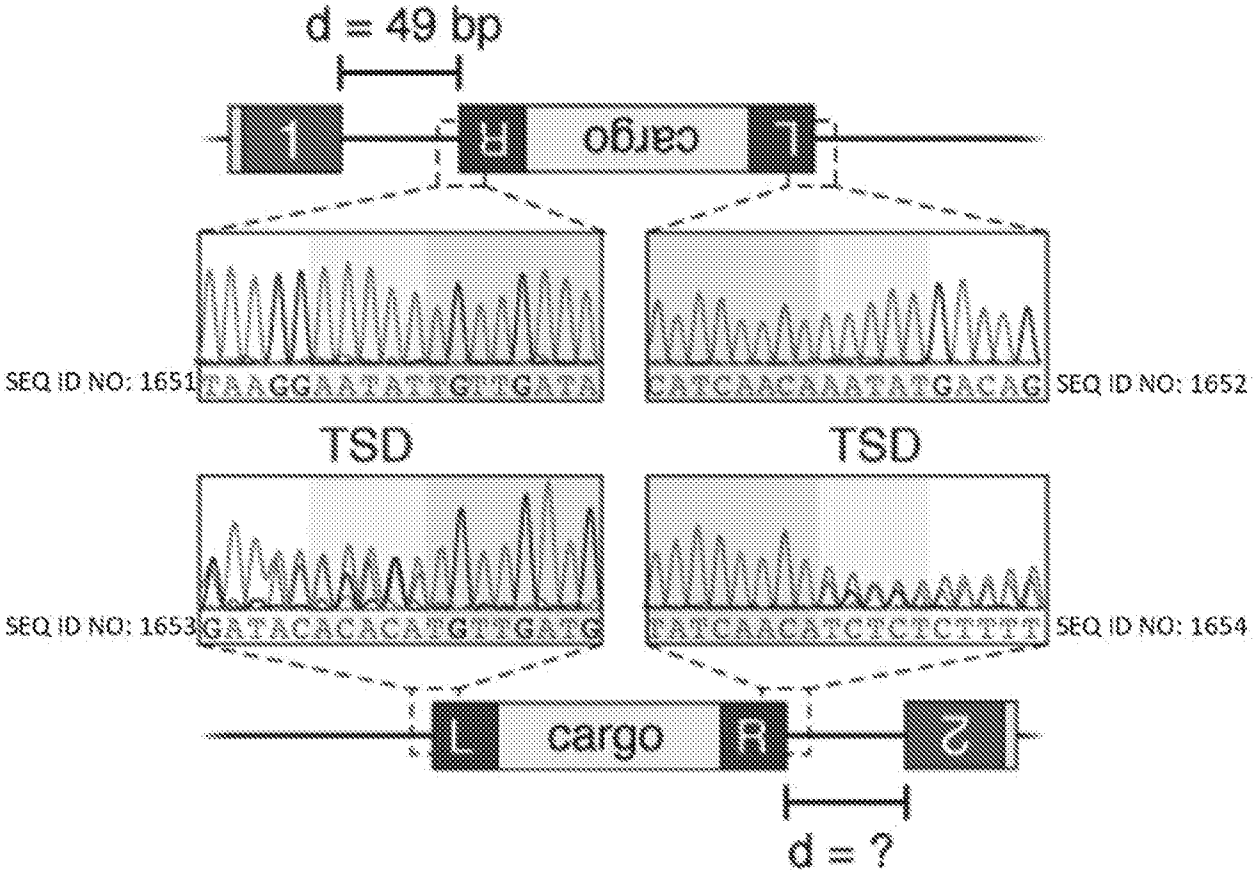

d = 49 bp
1
R
cargo
T
SEQ ID NO: 1651
TAAGGAATATTGTTGATA
CATCAACAAATATGACAG
SEQ ID NO: 1652
TSD
TSD
SEQ ID NO: 1653
GATACACACATGTTGATG
CATCAACATCTCTCTTTT
SEQ ID NO: 1654
L
cargo
R
2
d = ?

FIG. 1F

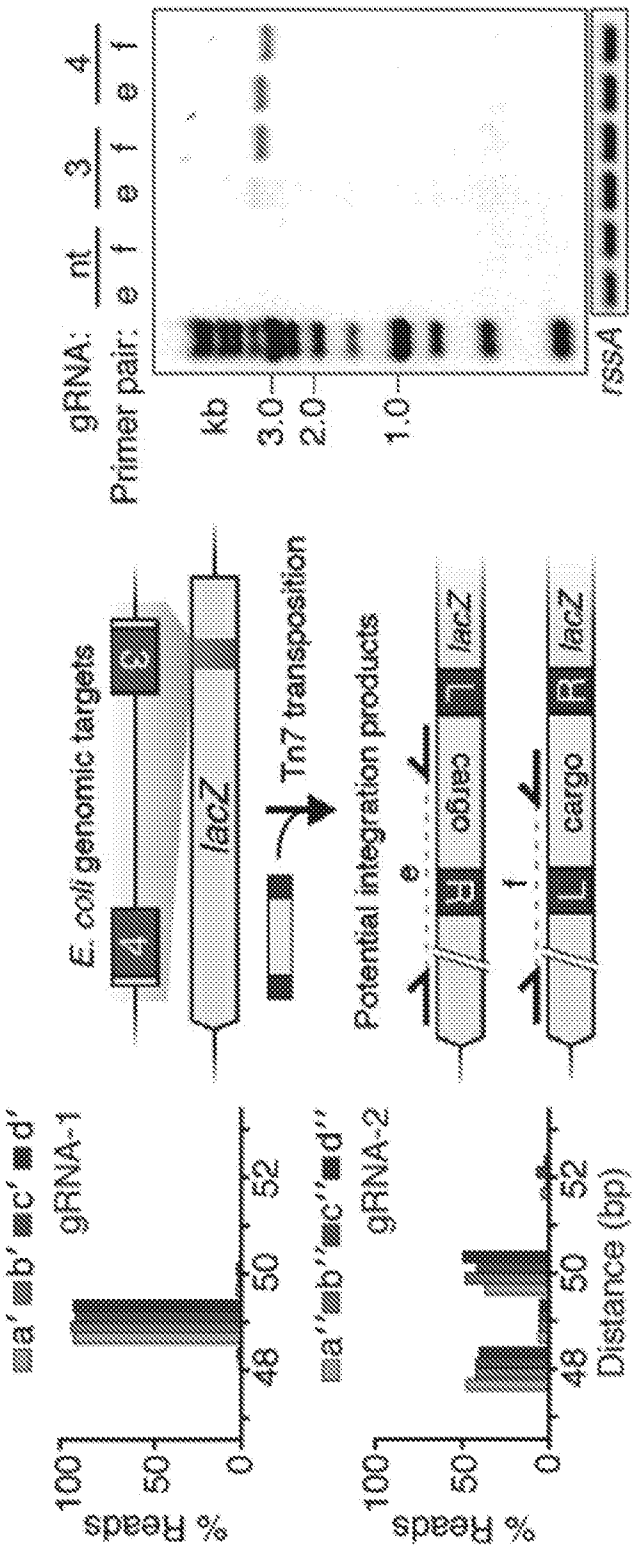


FIG. 1G

FIG. 1H

FIG. 1I

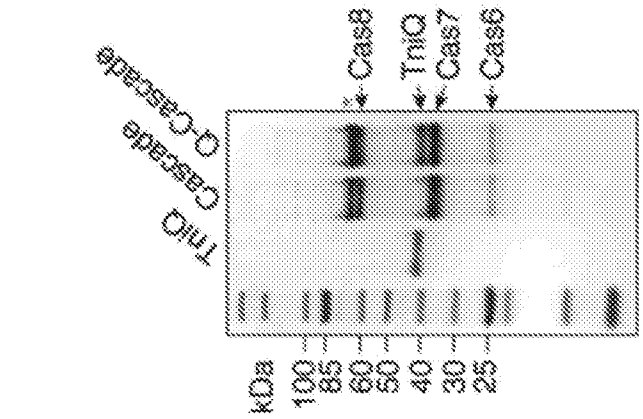

Cas8
TniQ
Cas7
Cas6
TniQ
Cascade
Q-Cascade
kDa
100
85
60
50
40
30
25

FIG. 2B

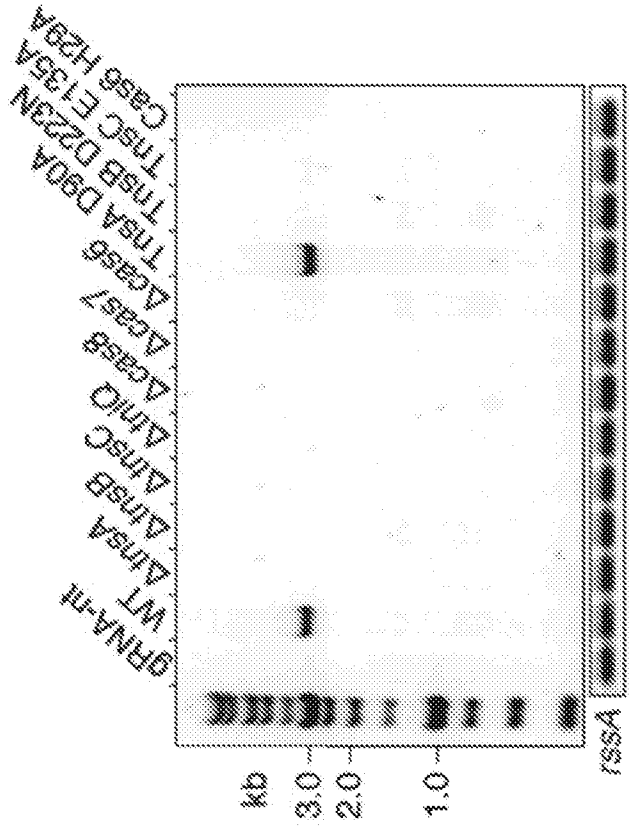

gRNA-mt
WT
ΔInsA
ΔInsB
ΔInsC
ΔInsD
ΔCas8
ΔCas7
ΔCas6
TnsA D90A
TnsB D223N
TnsC E135A
Cas6 H29A
kb
3.0
2.0
1.0
rssA

FIG. 2A

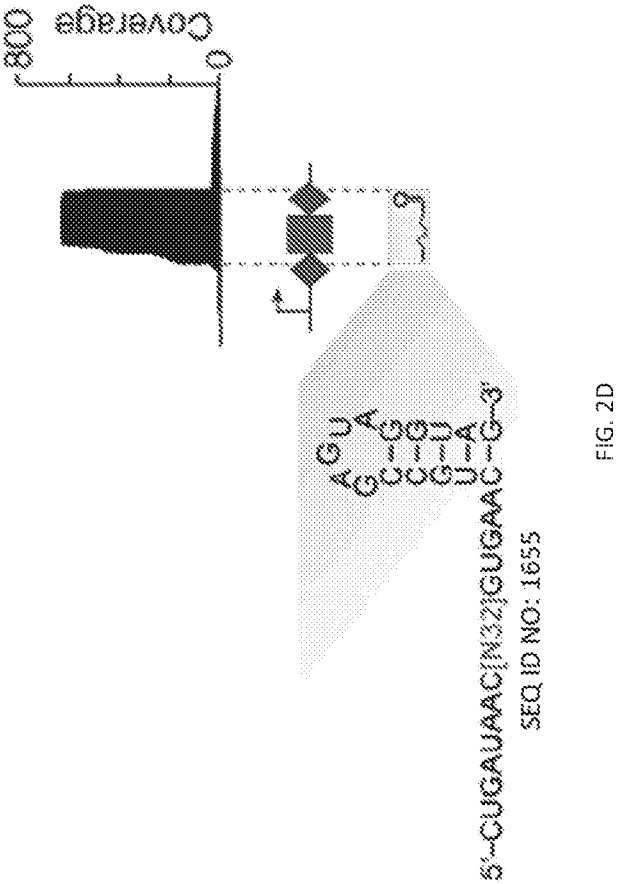

Coverage
0
608
5'-CUGAUAAC(N32)GUGAAC-3'
SEQ ID NO: 1655

FIG. 2D

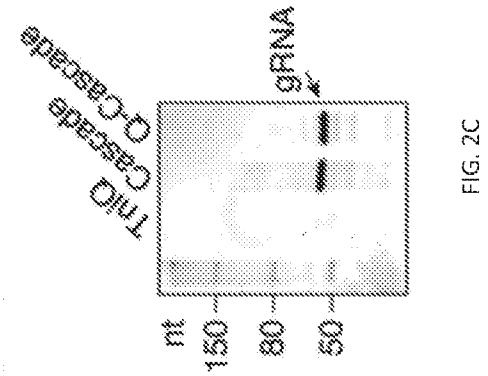

gRNA
TniQ
Cascade
Cas3
nt
150
80
50

FIG. 2C

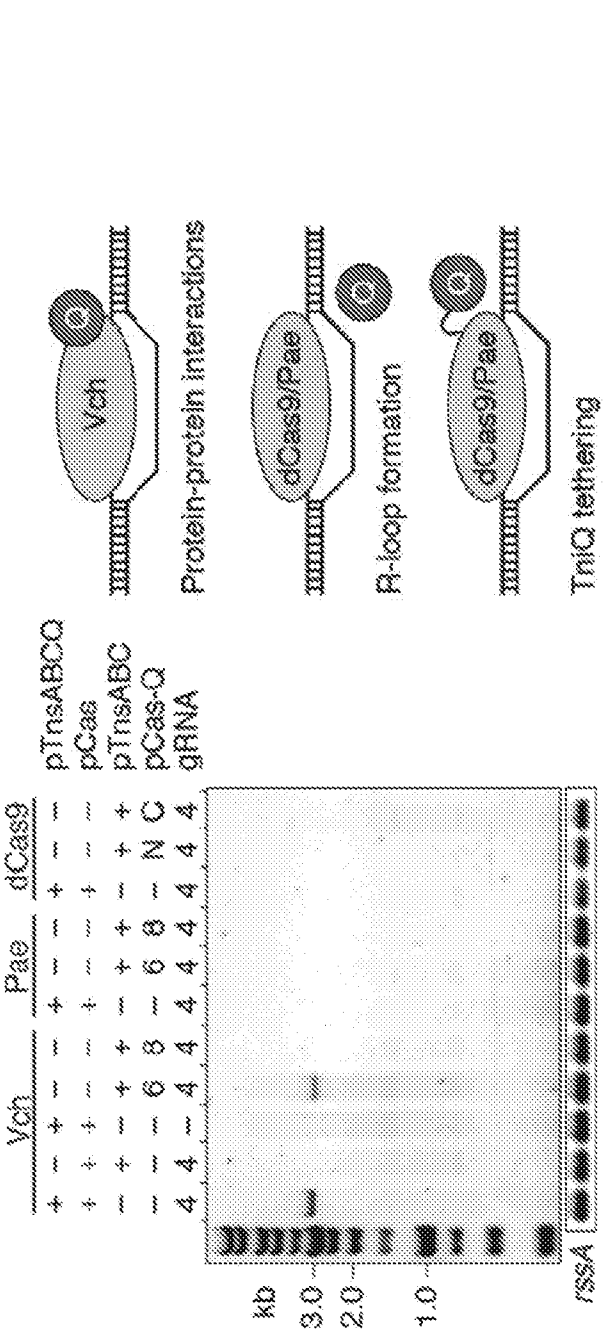

Vch
Pae
dCas9
pTnsABCQ
pCas
pTnsABC
pCas-Q
gRNA
kb
3.0
2.0
1.0
rssA
Vch
Protein-protein interactions
dCas9/Pae
R-loop formation
dCas9/Pae
TniQ tethering

FIG. 2E

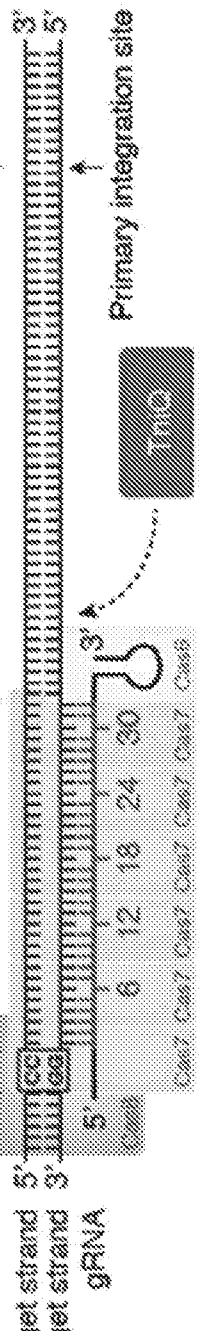

PAM
32 bp target
variable distance
Non-target strand
Target strand
gRNA
5'
3'
Primary integration site
TniQ

FIG. 2F

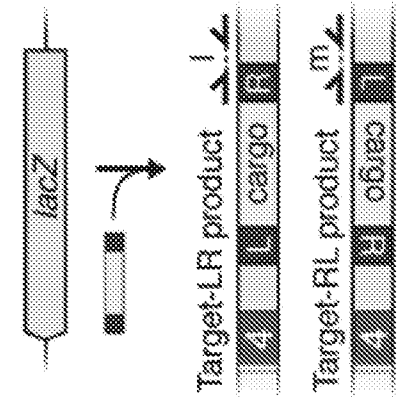


FIG. 3A

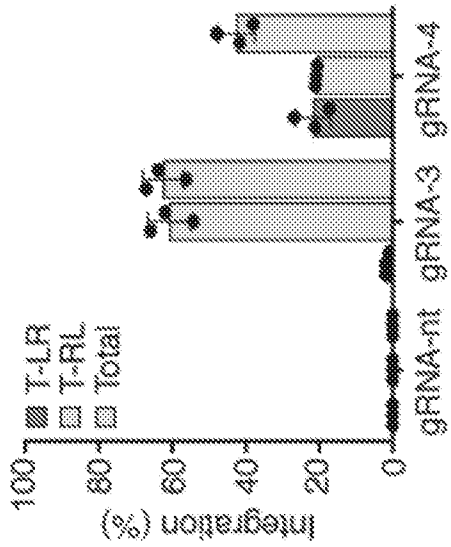


FIG. 3B

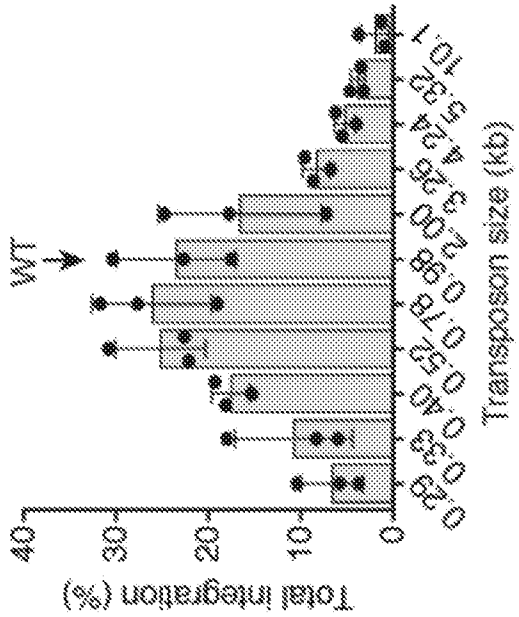


FIG. 3C

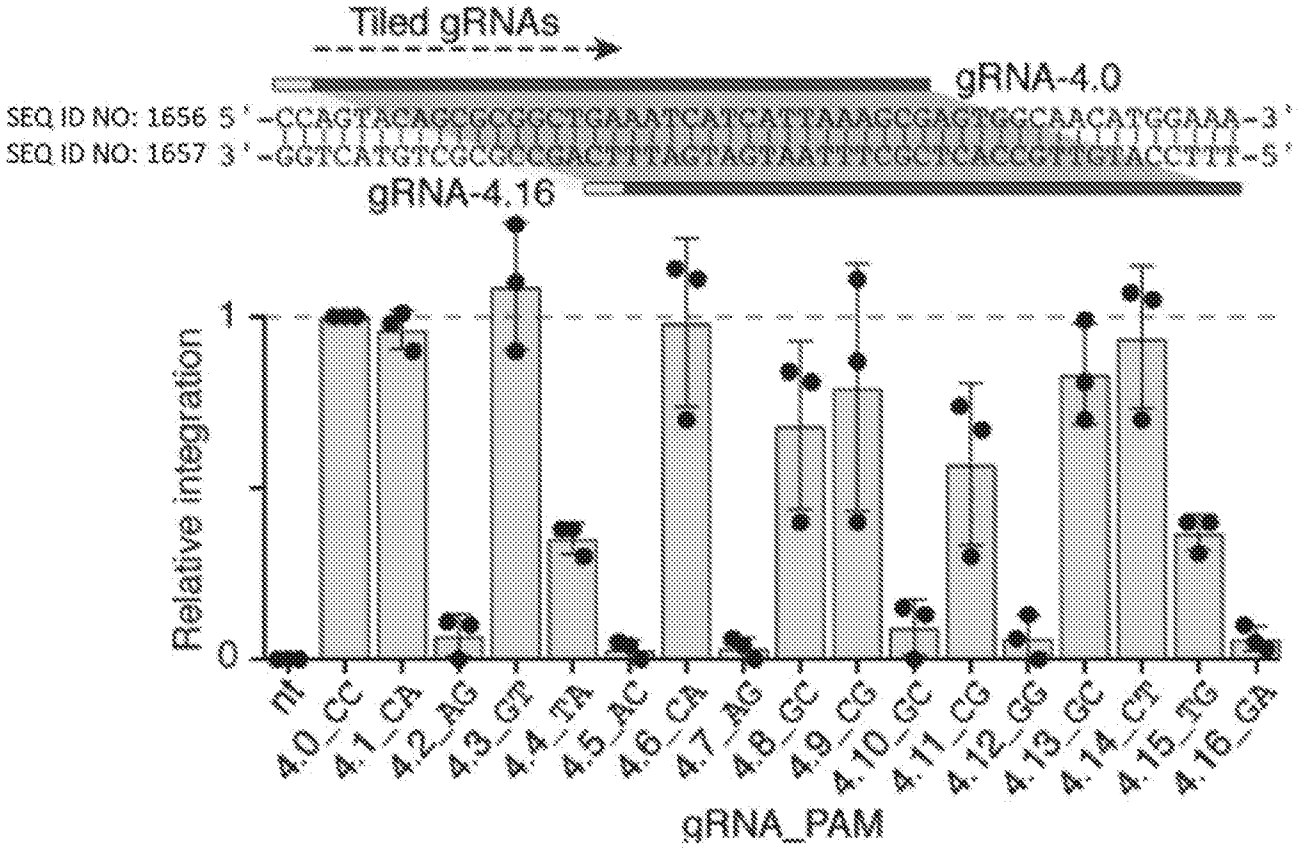

Tiled gRNAs
gRNA-4.0
SEQ ID NO: 1656 5'-CCAGTACAGCCCCGCTGAAATCATCATAAACCCATTGGCAACATCGAAA-3'
SEQ ID NO: 1657 3'-GGTCATGTCGCCCCGACTTTAGTACTAATTTCGGTAACCGTTGTACCTTT-5'
gRNA-4.16
Relative integration
1
0
nt
4.0_CC
4.1_CA
4.2_AG
4.3_GT
4.4_TA
4.5_AC
4.6_CA
4.7_AG
4.8_GC
4.9_CG
4.10_GC
4.11_CG
4.12_GG
4.13_GC
4.14_CT
4.15_TG
4.16_GA
gRNA_PAM

FIG. 3D

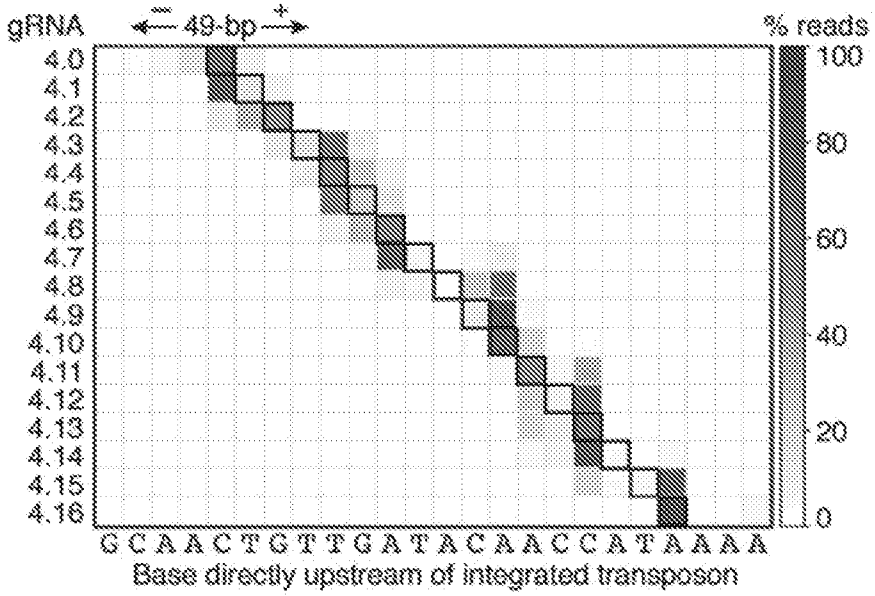

gRNA
49-bp
% reads
4.0
4.1
4.2
4.3
4.4
4.5
4.6
4.7
4.8
4.9
4.10
4.11
4.12
4.13
4.14
4.15
4.16
100
80
60
40
20
0
G C A A C T G T T G A T A C A A C C A T A A A A
Base directly upstream of integrated transposon

FIG. 3E

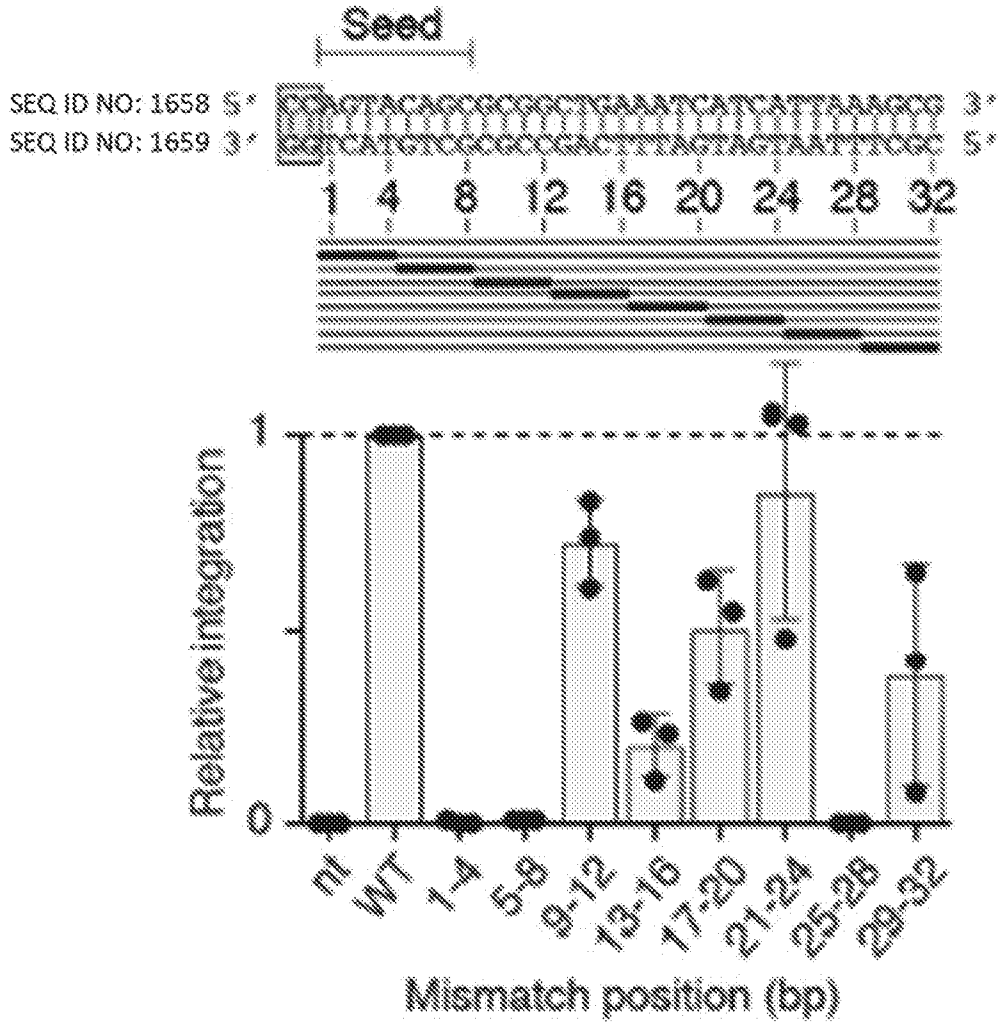

Seed
SEQ ID NO: 1658 5'
SEQ ID NO: 1659 3'
1 4 8 12 16 20 24 28 32
Relative integration
1
0
nt
WT
1-4
5-8
9-12
13-16
17-20
21-24
25-28
29-32
Mismatch position (bp)

FIG. 3F

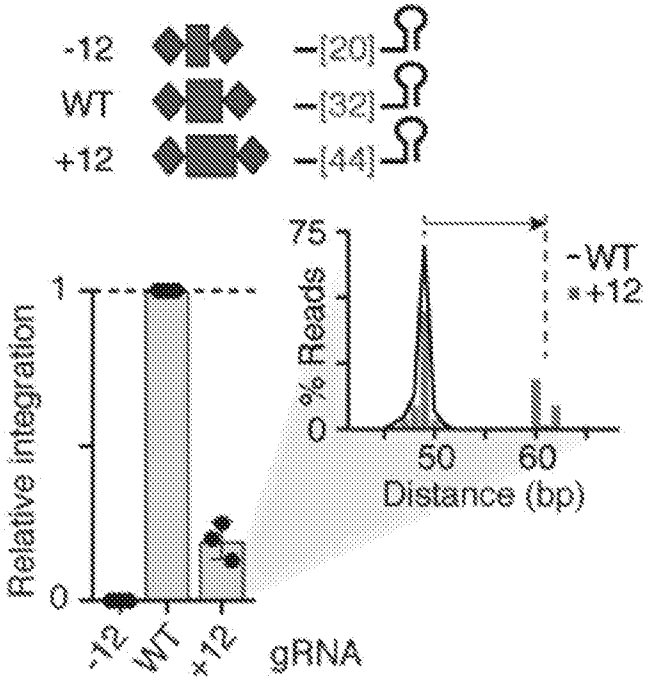

-12
WT
+12
-[20]
-[32]
-[44]
Relative integration
1
0
-12
WT
+12
gRNA
% Reads
75
0
50
60
Distance (bp)
-WT
+12

FIG.3G

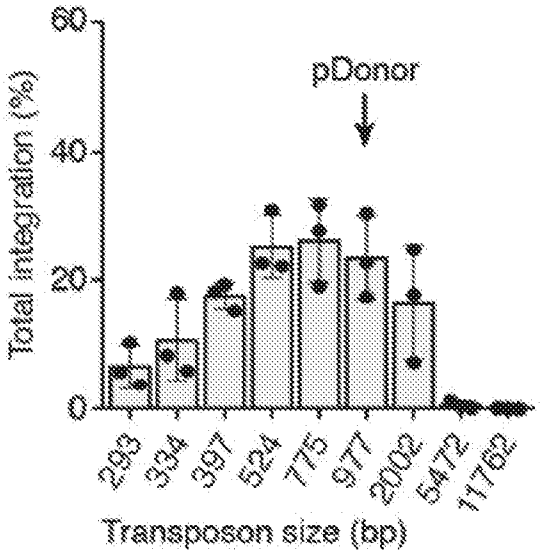

Total integration (%)
60
40
20
0
pDonor
293
334
397
524
775
977
2002
5472
11762
Transposon size (bp)

FIG. 3H

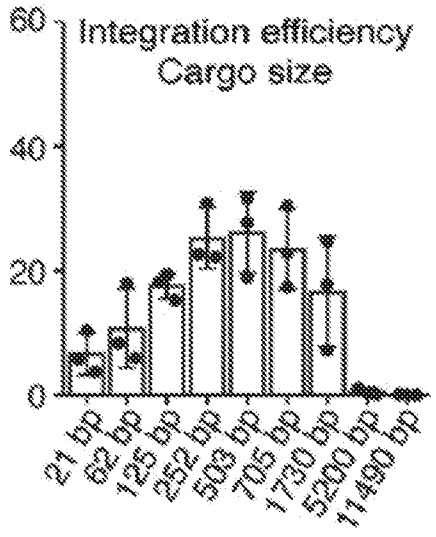

Integration efficiency
Cargo size
60
40
20
0
21 bp
62 bp
125 bp
252 bp
509 bp
705 bp
1730 bp
5200 bp
11490 bp

FIG. 3I

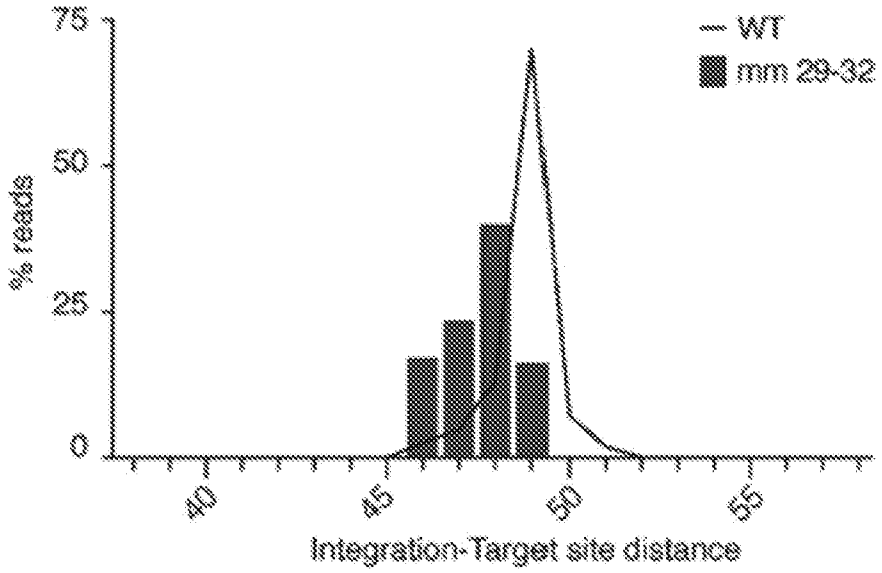

WT
mm 29-32
% reads
75
50
25
0
40
45
50
55
Integration-Target site distance

FIG. 3J

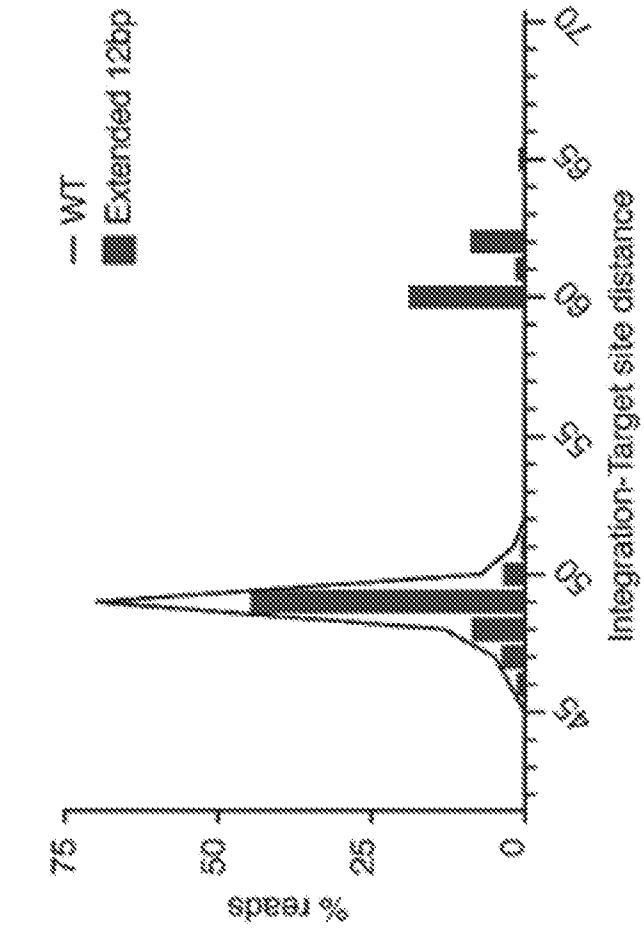
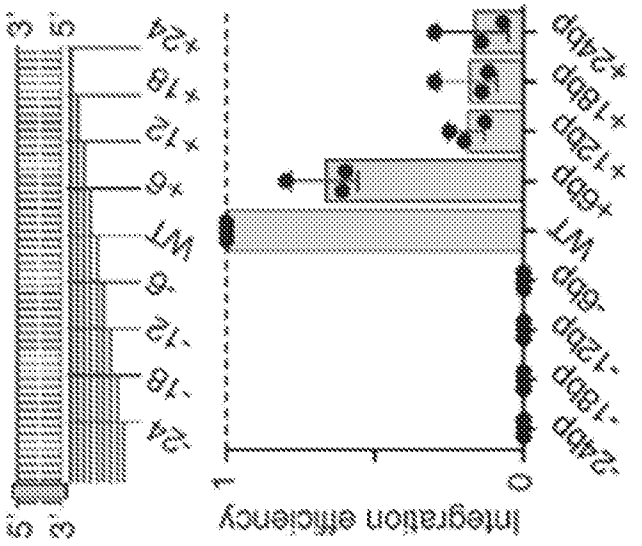

WT
Extended 12bp
% reads
75
50
25
0
45
50
55
60
65
70
Integration-Target site distance

FIG. 3K


Integration efficiency
1
0
-24bp
-18bp
-12bp
-6bp
WT
+6bp
+12bp
+18bp
+24bp
5'
3'
-24
-18
-12
-6
WT
+6
+12
+18
+24

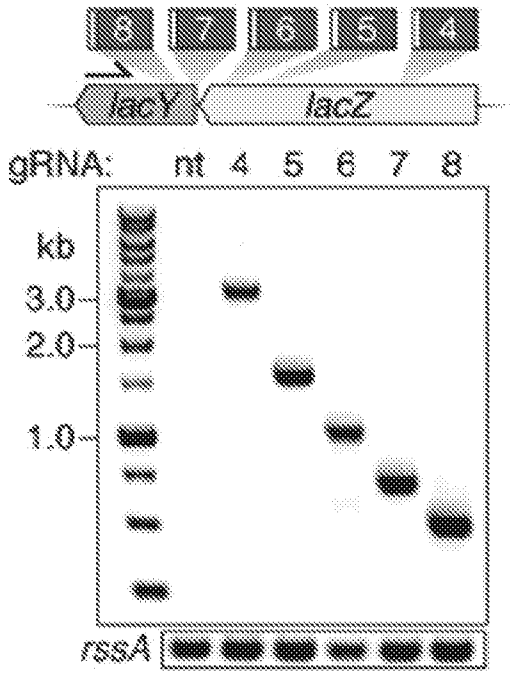

8
7
6
5
4
lacY
lacZ
gRNA:
nt 4 5 6 7 8
kb
3.0
2.0
1.0
rssA

FIG. 4A

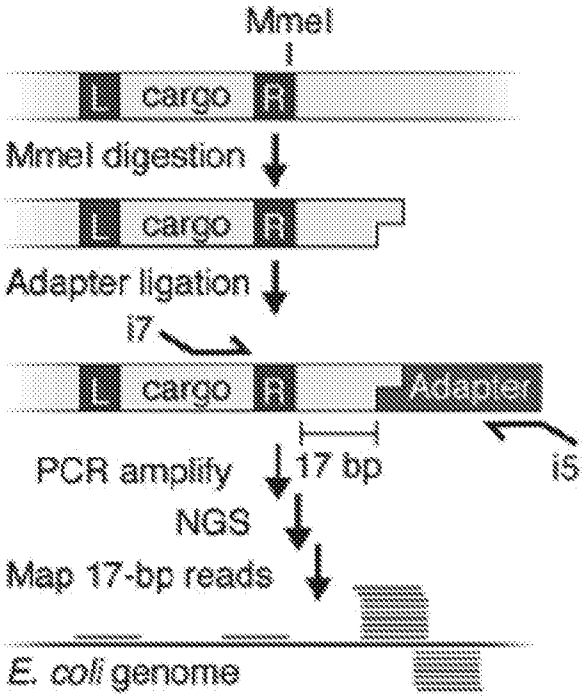

MmeI
L cargo R
MmeI digestion
L cargo R
Adapter ligation
i7
L cargo R Adapter
PCR amplify
17 bp
i5
NGS
Map 17-bp reads
E. coli genome

FIG. 4B

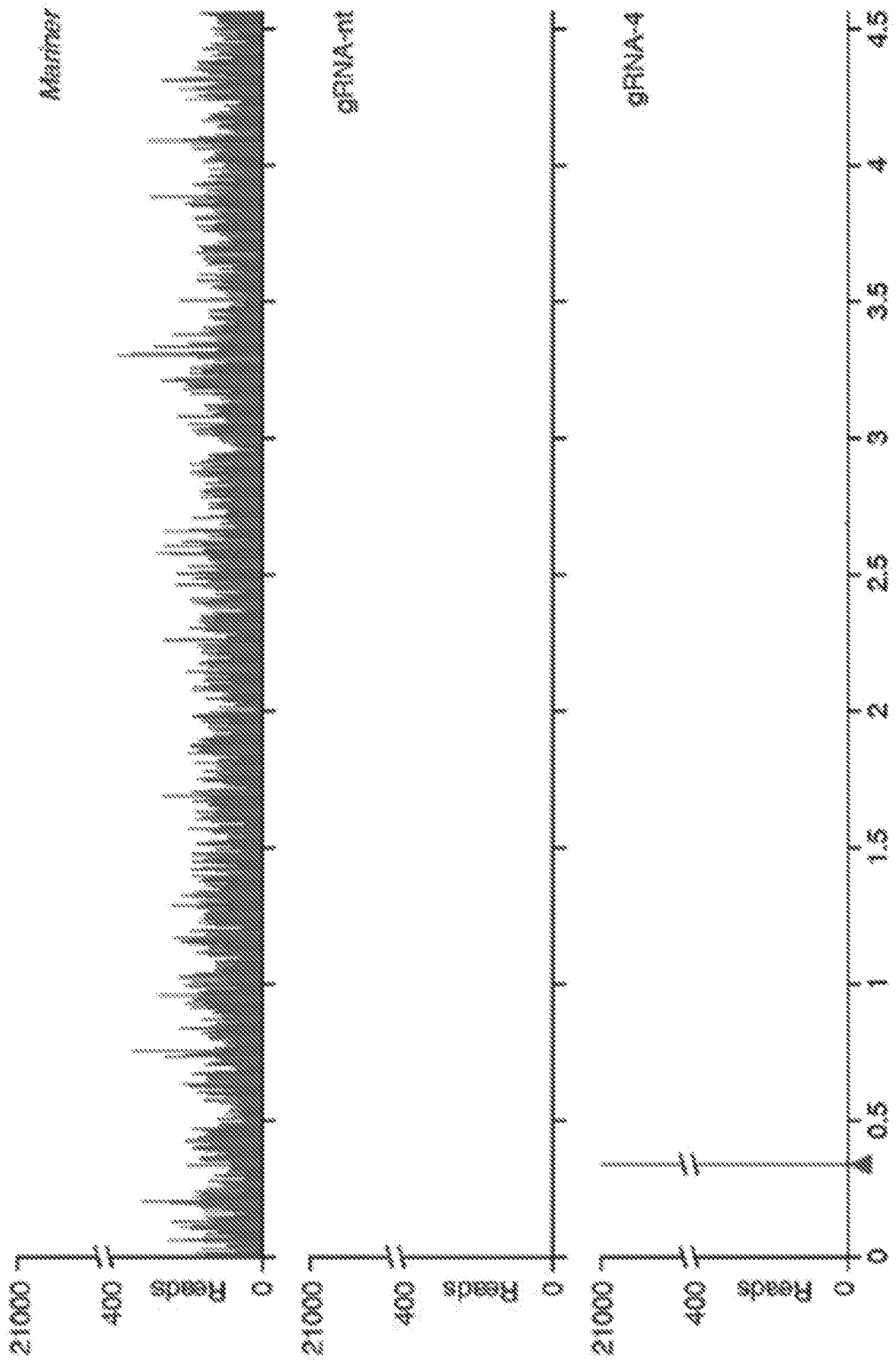

Mariner
gRNA-nt
gRNA-4
Reads
21000
400
0
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
E. coli genome (Mbp)

FIG. 4C

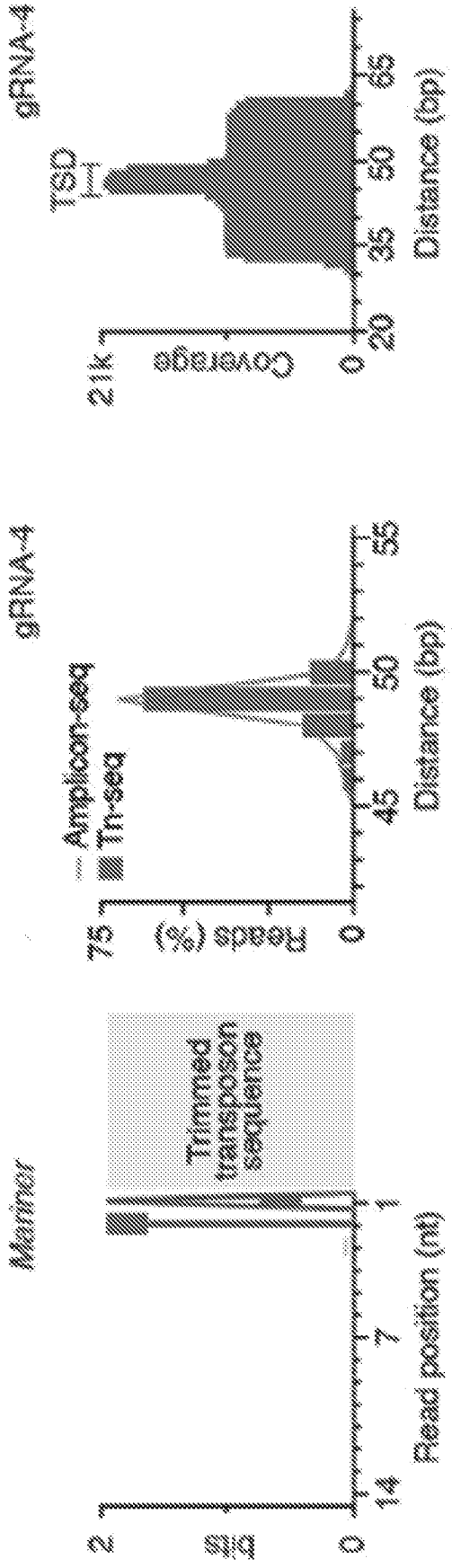


FIG. 4D

FIG. 4E

FIG. 4F

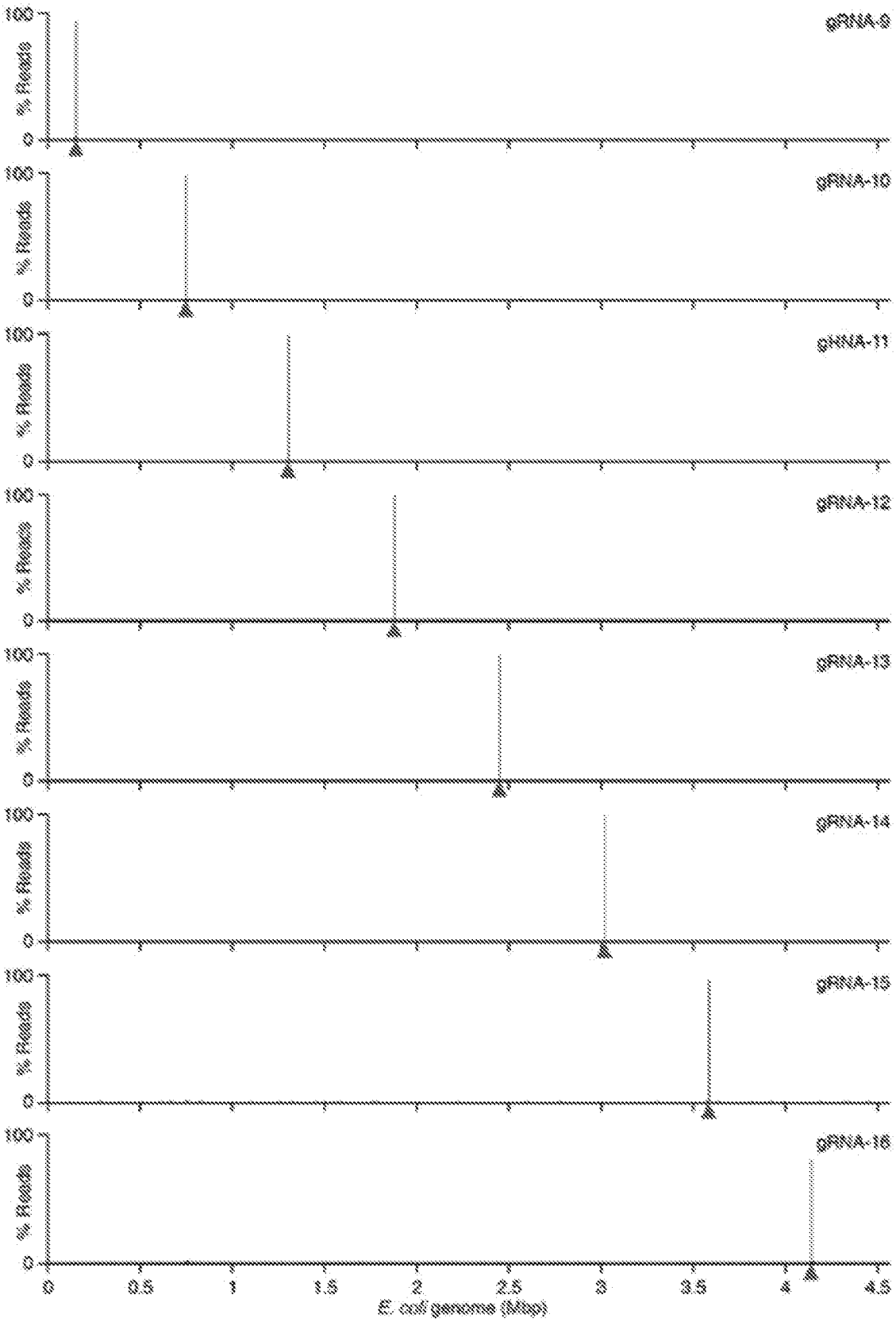

gRNA-9
gRNA-10
gRNA-11
gRNA-12
gRNA-13
gRNA-14
gRNA-15
gRNA-16
% Reads
100
0
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
E. coli genome (Mbp)

FIG. 4G

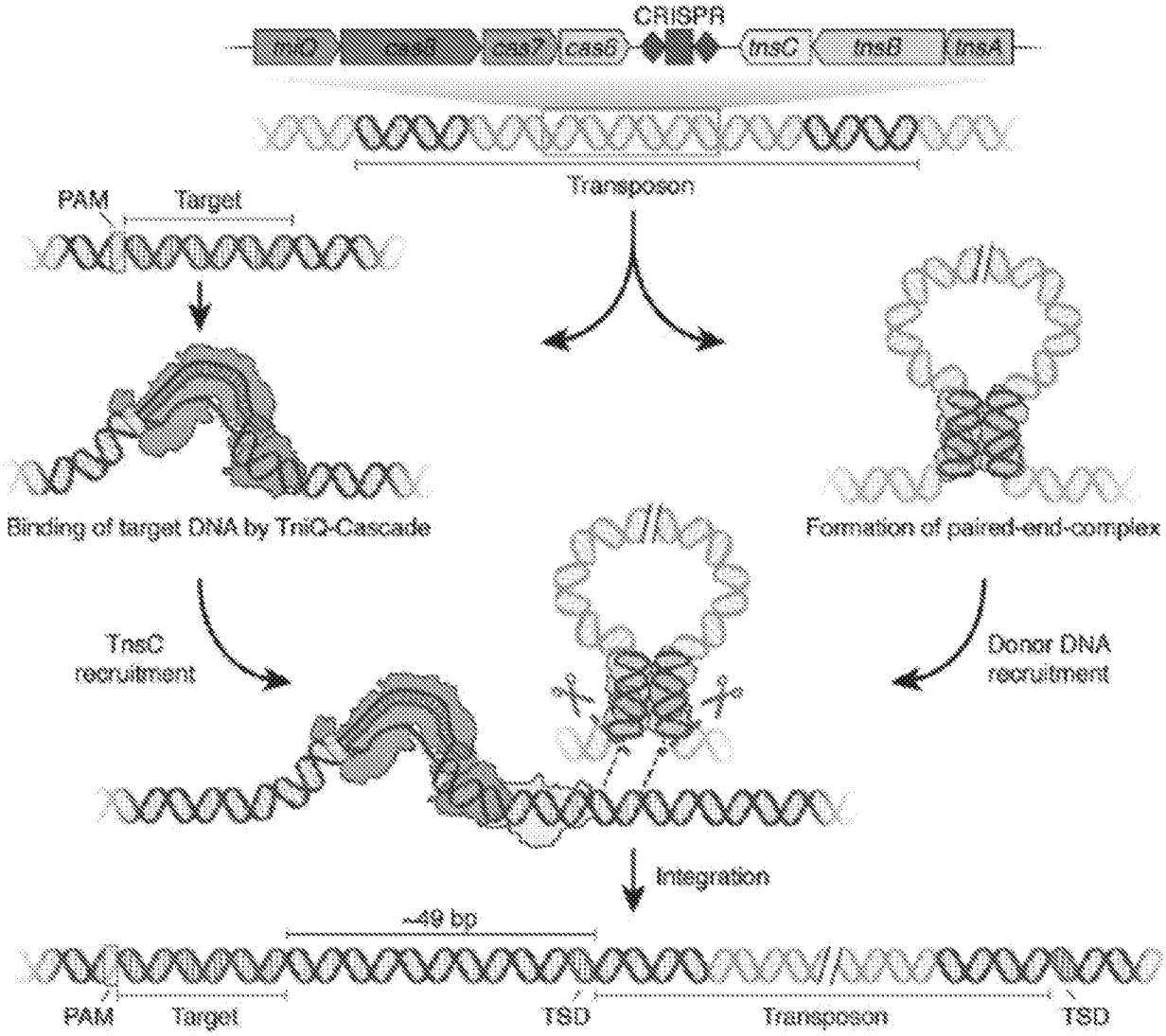

CRISPR
cas7
casD
tnsC
tnsB
tnsA
Transposon
PAM
Target
Binding of target DNA by TniQ-Cascade
Formation of paired-end-complex
TnsC
recruitment
Donor DNA
recruitment
Integration
~49 bp
PAM
Target
TSD
Transposon
TSD

FIG. 5A

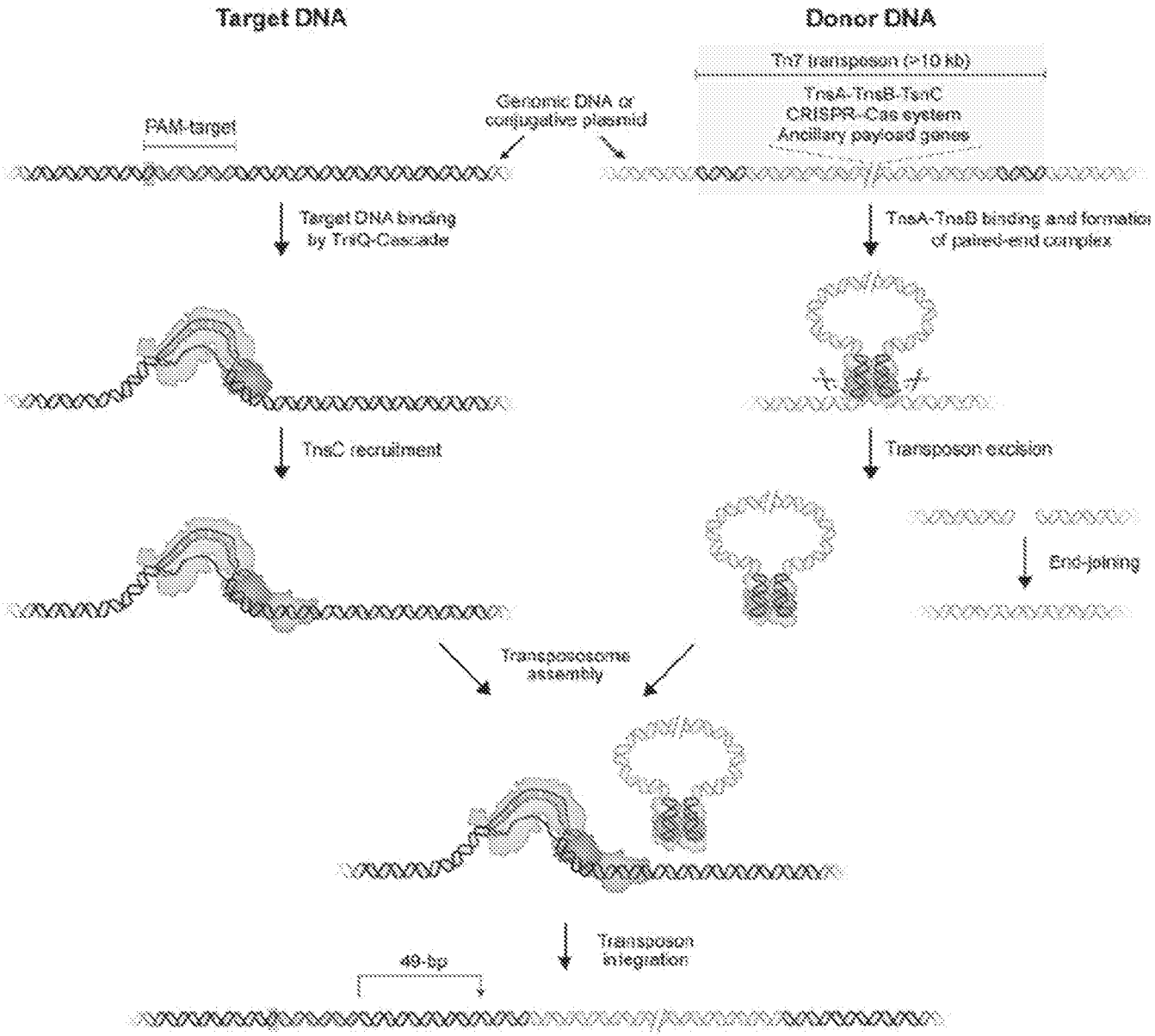

Target DNA
Donor DNA
Tn7 transposon (>10 kb)
TnsA-TnsB-TsnC
CRISPR-Cas system
Ancillary payload genes
PAM-target
Genomic DNA or
conjugative plasmid
Target DNA binding
by TniQ-Cascade
TnsA-TnsB binding and formation
of paired-end complex
TnsC recruitment
Transposon excision
End-joining
Transpososome
assembly
Transposon
integration
49-bp

FIG. 5B

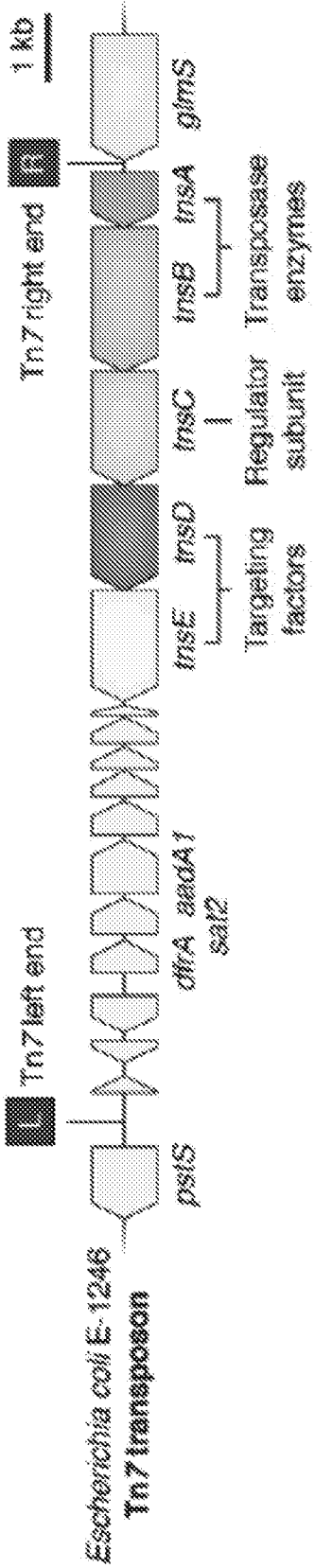

Escherichia coli E-1246
Tn7 transposon
Tn7 left end
pstS
dfrA aadA1
sat2
tnsE tnsD tnsC tnsB tnsA glmS
Targeting factors
Regulator subunit
Transposase enzymes
Tn7 right end
1 kb

FIG. 6A

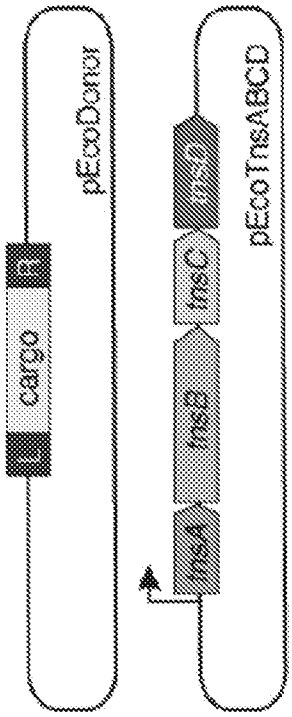

cargo
pEcoDonor
tnsA
tnsB
tnsC
tnsD
pEcoTnsABCD

FIG. 6B

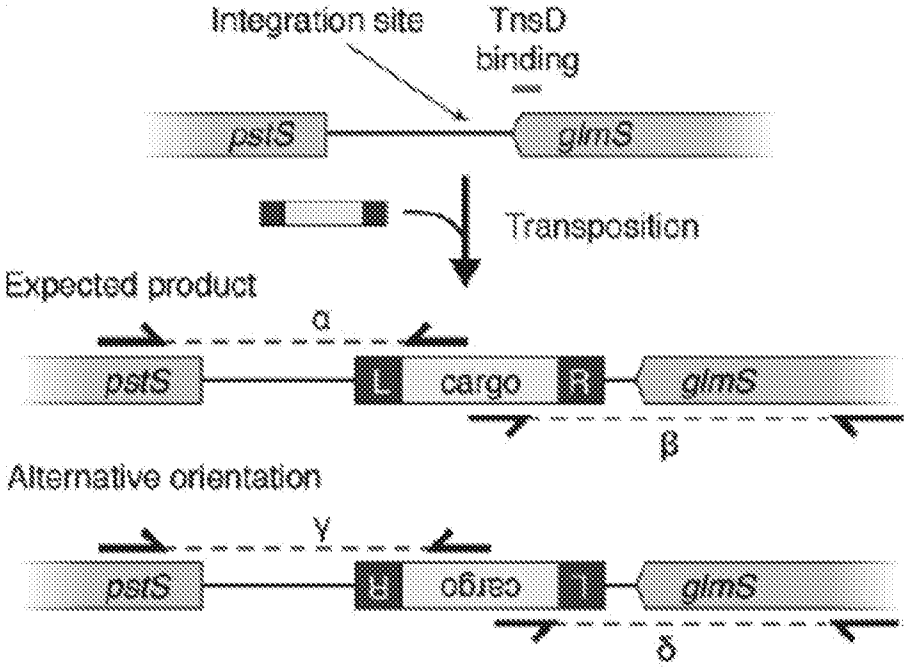

Integration site
TnsD binding
pstS
glmS
Transposition
Expected product
α
pstS
L
cargo
R
glmS
β
Alternative orientation
γ
pstS
R
cargo
L
glmS
δ

FIG. 6C

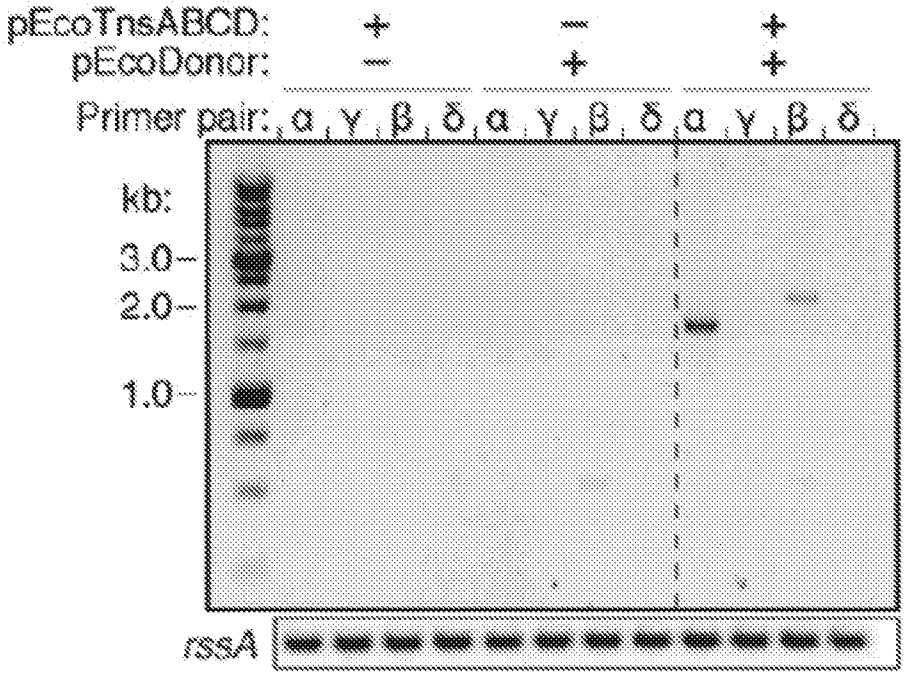

pEcoTnsABCD: + − +
pEcoDonor: − + +
Primer pair: α γ β δ α γ β δ α γ β δ
kb:
3.0
2.0
1.0
rssA

FIG. 6D

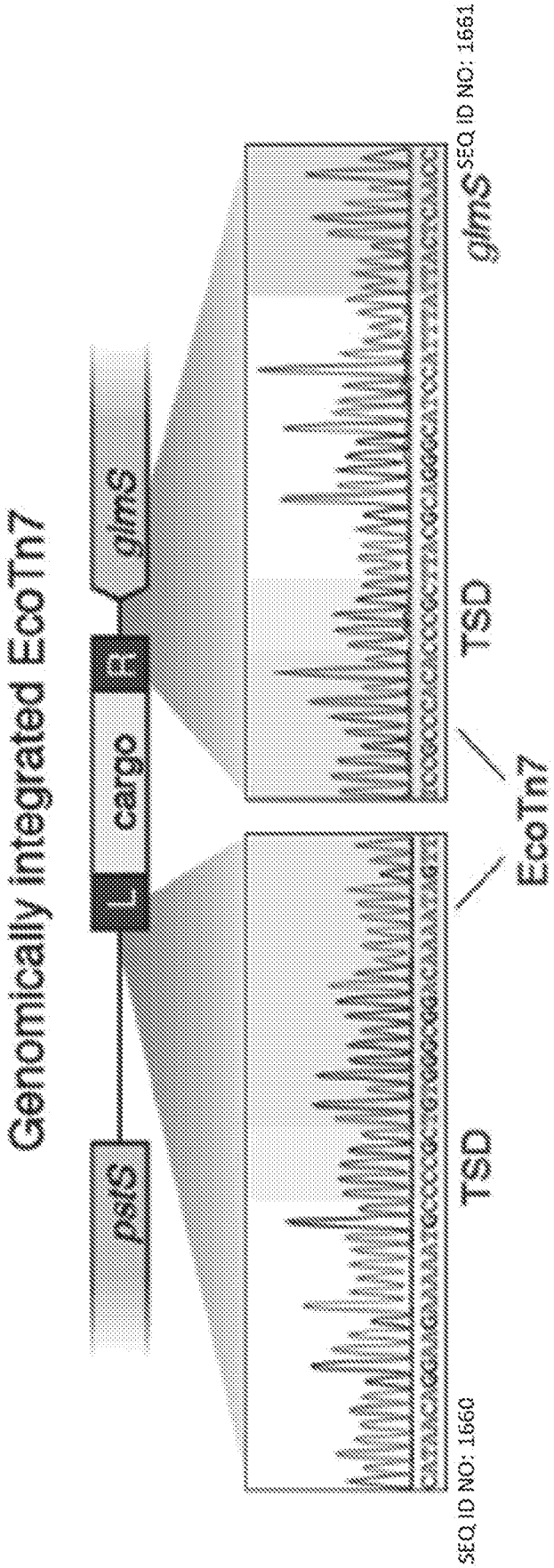

Genomically integrated EcoTn7
pstS
L
cargo
R
glmS
TSO
TSO
EcoTn7
SEQ ID NO: 1660
SEQ ID NO: 1661

FIG. 6E

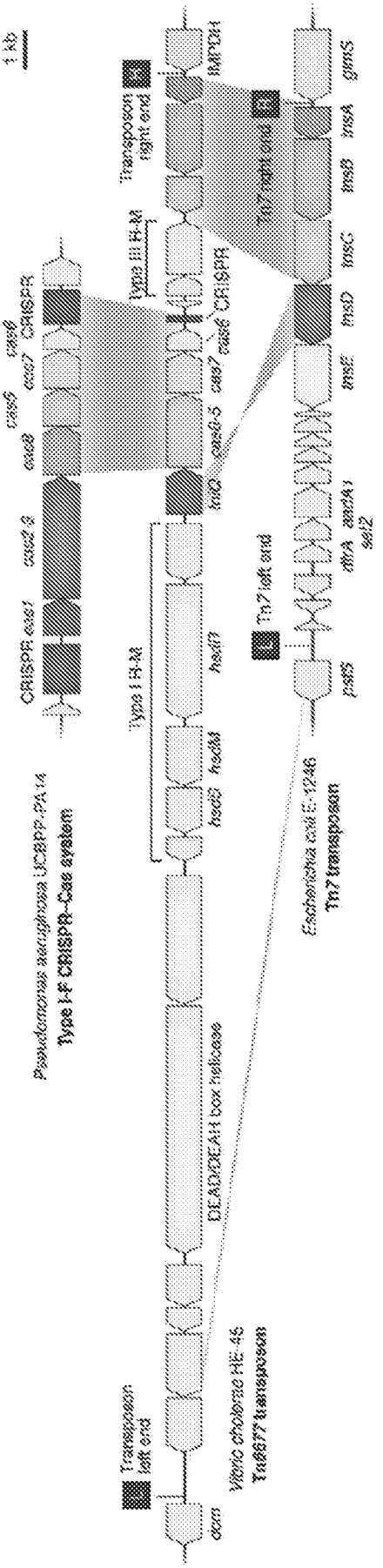

1 kb
Pseudomonas aeruginosa UCBPP-PA14
Type I-F CRISPR–Cas system
CRISPR
cas1
cas2-3
cas8
cas5
cas7
cas6
CRISPR
Type I-M
Transposon right end
IMP/GH
insB
insA
glmS
Type III-M
CRISPR
cas10-5
cas7
cas6
tniQ
tniD
tniC
tniE
Tn7 right end
Tn7 left end
hsdR
hsdM
hsdS
DEAD/DEAH box helicase
Transposon left end
orn
Vibrio cholerae HE-45
Tn6677 transposon
psiS
ultA
ssuA1
set2
Escherichia coli E-1246
Tn7 transposon

FIG. 6F

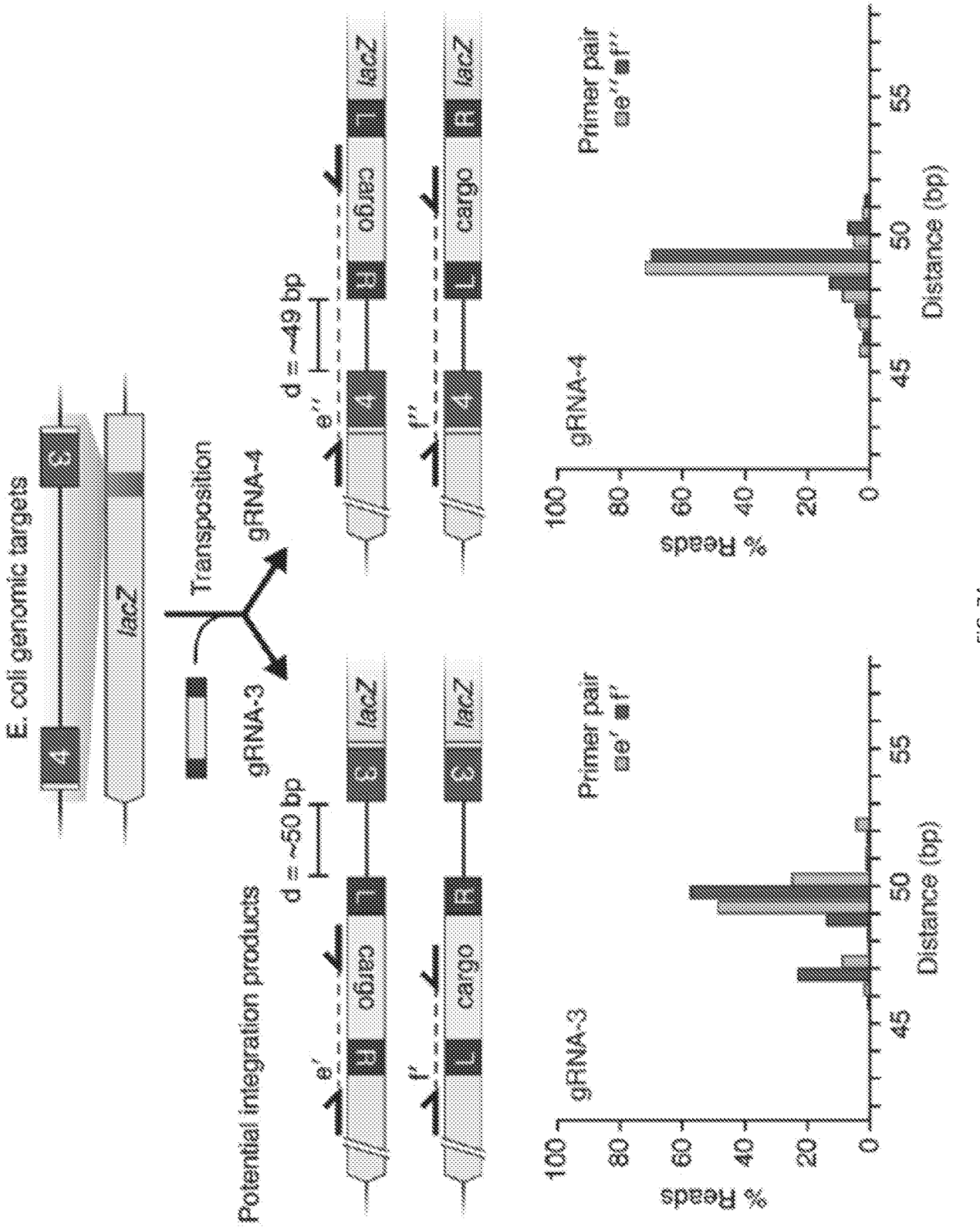

E. coli genomic targets
4
3
lacZ
Transposition
gRNA-3
gRNA-4
Potential integration products
d = ~50 bp
d = ~49 bp
e'
f'
e''
f''
cargo
lacZ
gRNA-3
gRNA-4
Primer pair
e' f'
e'' f''
% Reads
Distance (bp)
0
20
40
60
80
100
45
50
55

FIG. 7A

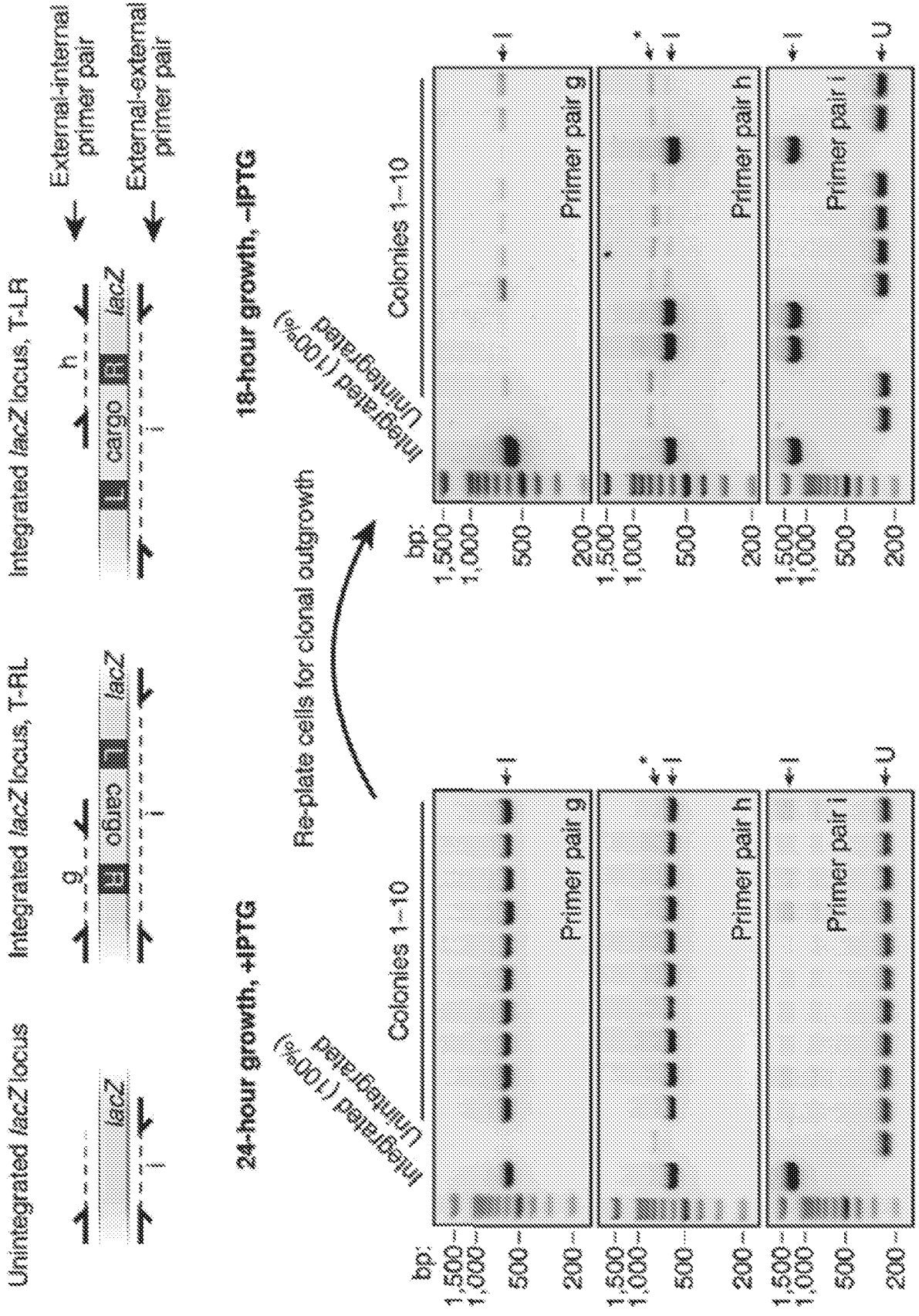

Unintegrated lacZ locus
lacZ
i
Integrated lacZ locus, T-RL
g
cargo
lacZ
i
Integrated lacZ locus, T-LR
h
cargo
lacZ
i
External-internal primer pair
External-external primer pair
24-hour growth, +IPTG
Re-plate cells for clonal outgrowth
18-hour growth, -IPTG
Integrated (100%)
Unintegrated (100%)
Colonies 1–10
bp:
1,500
1,000
500
200
Primer pair g
Primer pair h
Primer pair i
i
*
U

FIG. 7B

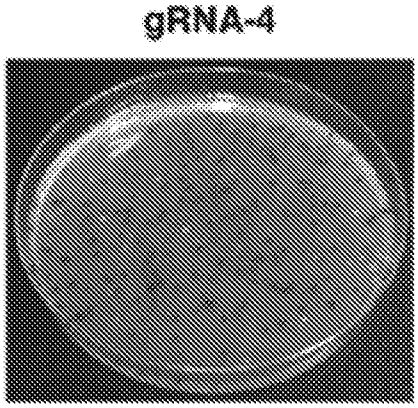

gRNA-4

FIG. 7C

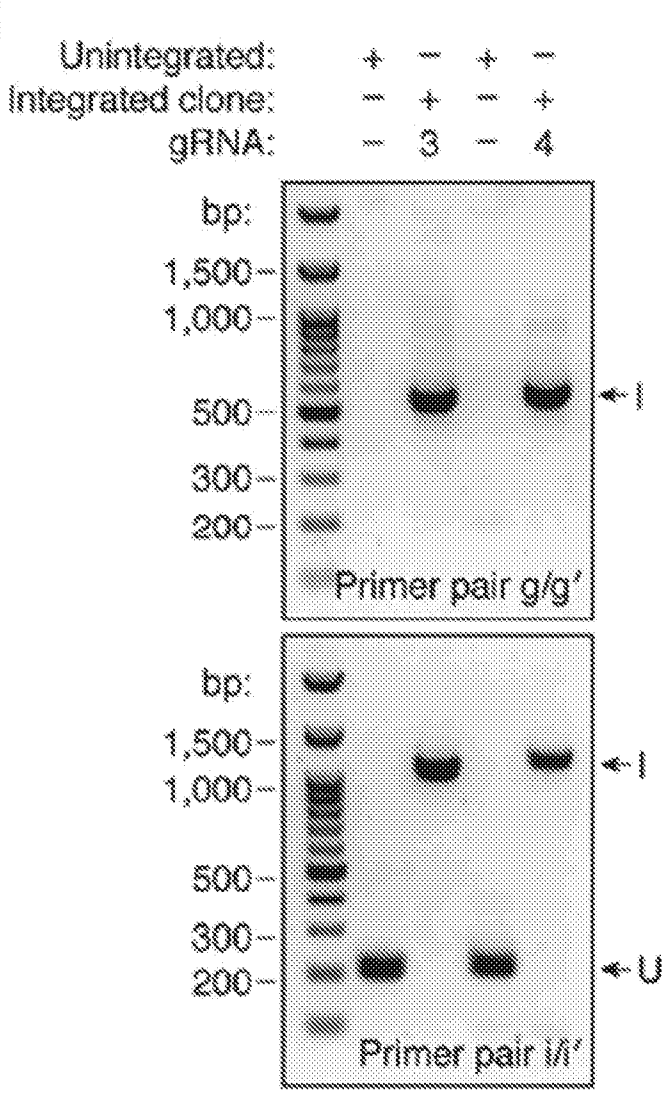

Unintegrated: + − + −
Integrated clone: − + − +
gRNA: − 3 − 4
bp:
1,500
1,000
500
300
200
I
Primer pair g/g'
bp:
1,500
1,000
500
300
200
I
U
Primer pair i/i'

FIG. 7D

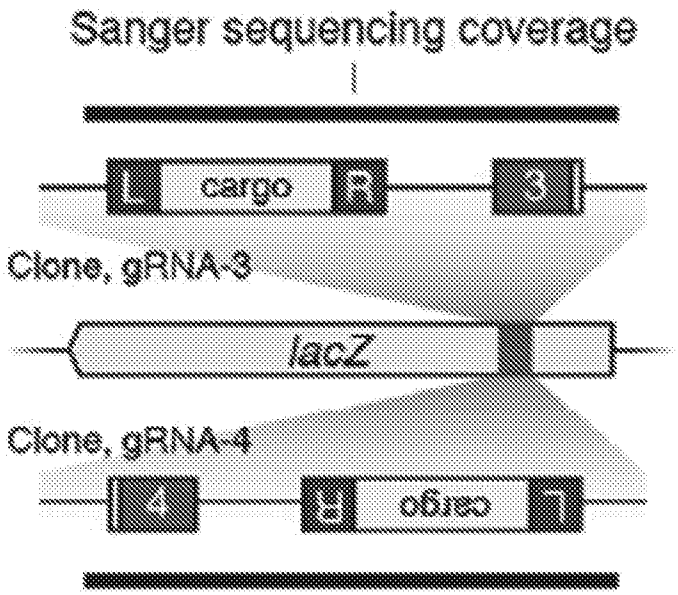

Sanger sequencing coverage
L
cargo
R
3
Clone, gRNA-3
lacZ
Clone, gRNA-4
4
5
cargo
5

FIG. 7E

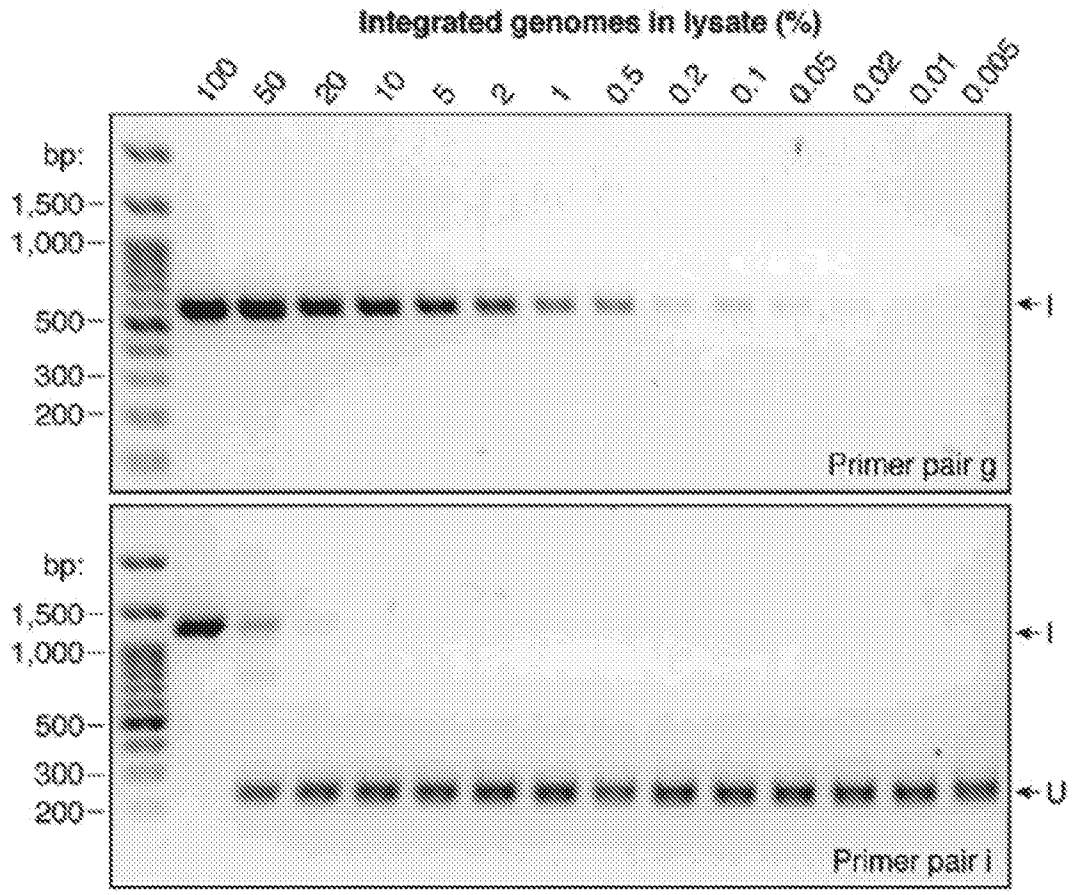

Integrated genomes in lysate (%)
100
50
20
10
5
2
1
0.5
0.2
0.1
0.05
0.02
0.01
0.005
bp:
1,500
1,000
500
300
200
I
Primer pair g
bp:
1,500
1,000
500
300
200
I
U
Primer pair I

FIG. 7F

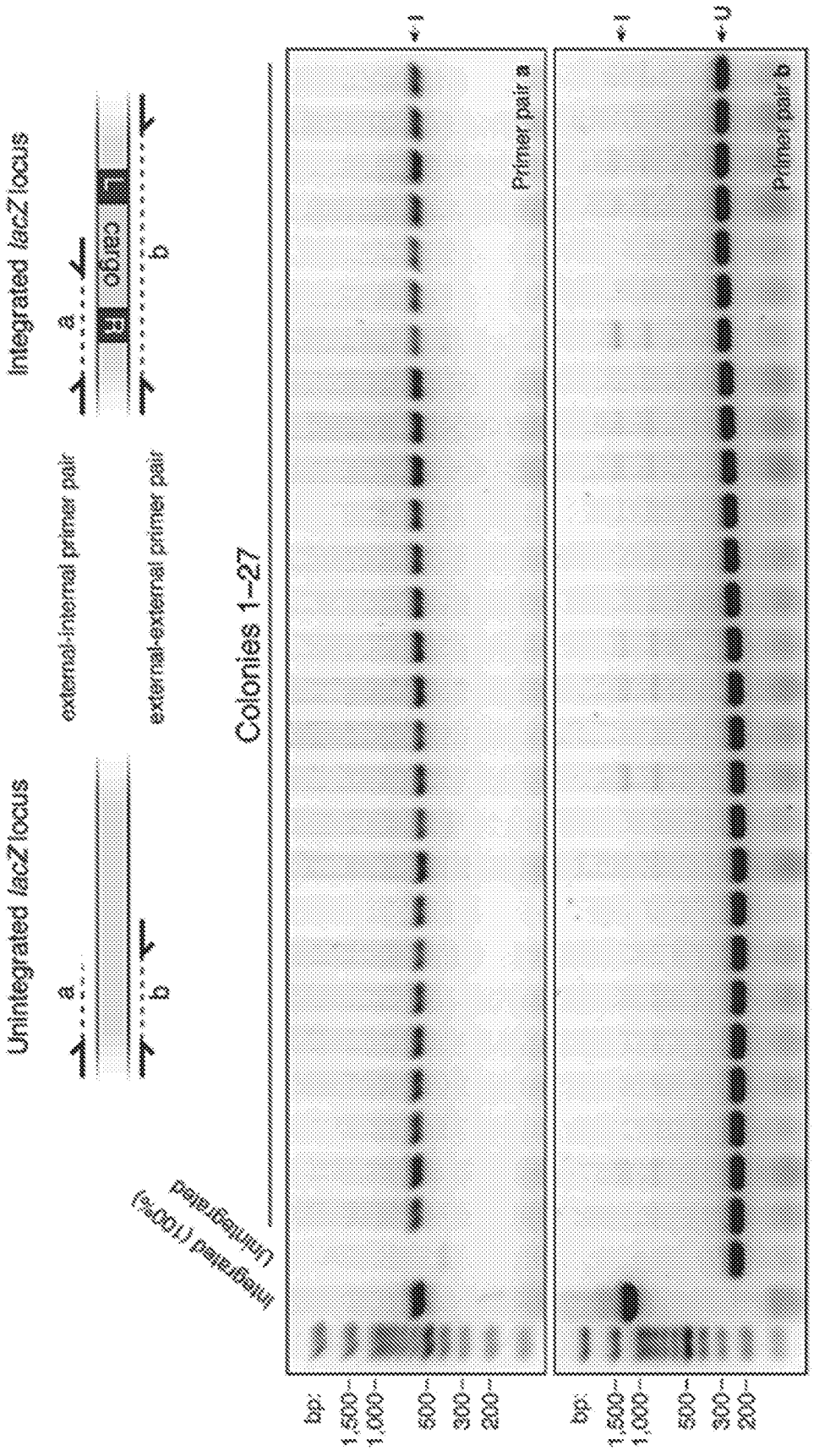

Unintegrated lacZ locus
Integrated lacZ locus
a
b
external-internal primer pair
external-external primer pair
cargo
Colonies 1–27
Integrated (100%)
Unintegrated (100%)
bp
1,500
1,000
500
300
200
I
U
Primer pair a
Primer pair b

FIG. 7G

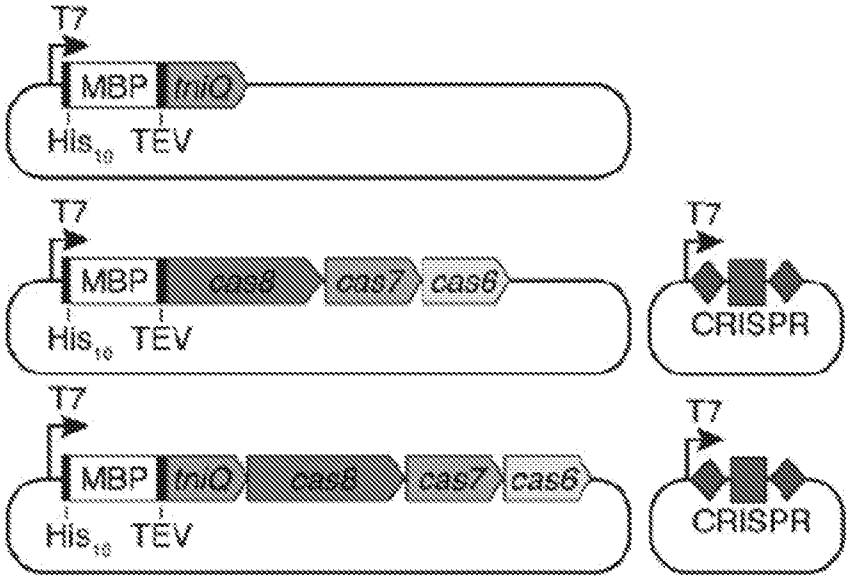

T7
MBP
lnrQ
His10
TEV
T7
MBP
cas8
cas7
cas6
His10
TEV
T7
CRISPR
T7
MBP
lnrQ
cas8
cas7
cas6
His10
TEV
T7
CRISPR

FIG. 8A

Description	MW (kDa)	1	2	3	4	5	6	7	8	9	10
V. cholerae TrriQ	46 kDa	513	0	0	0	0	7	15	210	47	41
V. cholerae Cas3	72 kDa	1	194	67	29	20	108	239	30	18	18
V. cholerae Cas7	40 kDa	65	10	8	352	83	13	11	111	460	115
V. cholerae Cas8	23 kDa	2	1	4	5	114	2	0	1	0	158
His10-MBP-TEVsite	45 kDa	16	11	13	52	30	20	23	22	23	29
GroL	57 kDa	8	396	986	326	91	38	46	17	9	15
HtpG	71 kDa	1	7	2	2	0	391	175	97	46	47
PhoH	41 kDa	3	0	0	28	10	5	6	303	97	43
AmA	74 kDa	0	0	0	0	0	224	83	31	10	49
PPIase	21 kDa	0	5	10	9	82	0	4	4	3	48
GlmS	67 kDa	0	17	2	0	0	11	42	1	0	0
DnaK	69 kDa	8	19	7	5	4	41	37	13	7	8
Monooxygenase	37 kDa	0	0	0	36	2	0	0	0	1	0
DnaJ	41 kDa	1	0	0	7	1	0	1	35	15	2
RecN	61 kDa	0	22	24	2	1	0	14	0	0	2

FIG. 8B

FIG. 8C

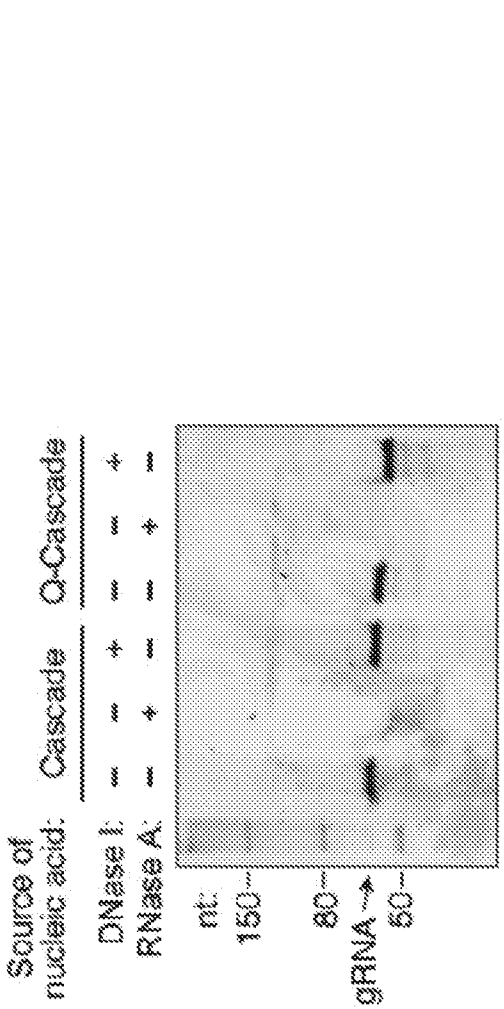

Source of nucleic acid:
Cascade
Q-Cascade
DNase I:
RNase A:
nt:
150
80
gRNA
60

FIG. 8D

TniQ:
Cascade:
Fraction:
Q C
Q C
Q C
kDa
100
85
60
50
40
30
25
Cas8
TniQ
Cas7
Cas6
TniQ
Cascade
Cascade + TniQ (1:5)
Cascade peak (C)
TniQ peak (Q)
Absorbance at 280 nm (mAU)
Retention volume (mL)

FIG. 8E

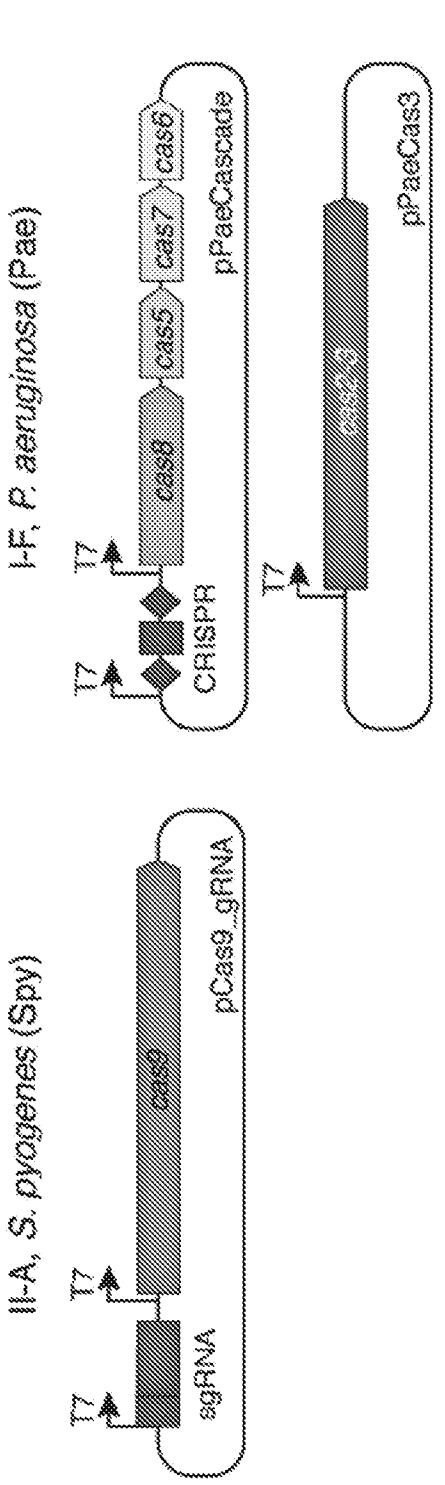


FIG. 9A

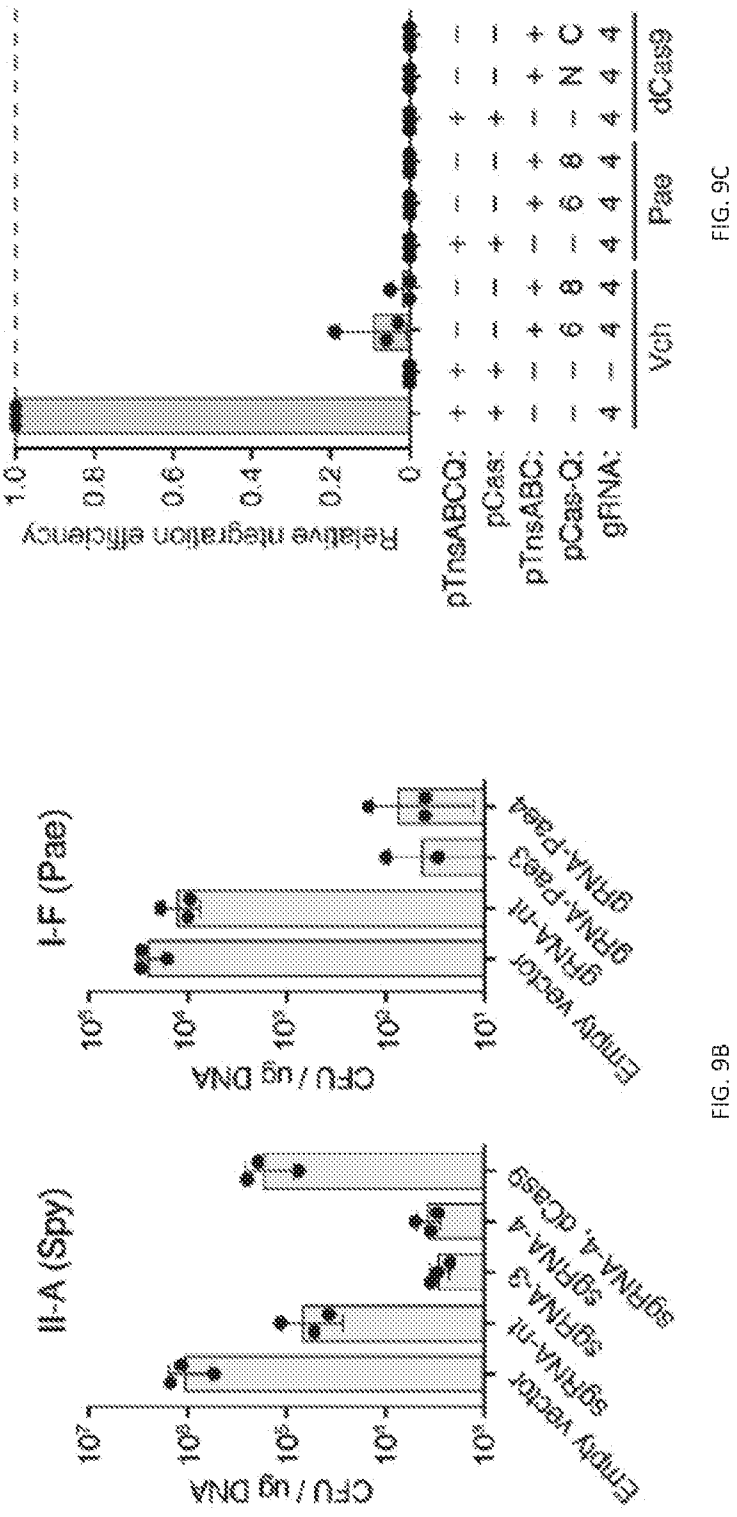


FIG. 9B

FIG. 9C

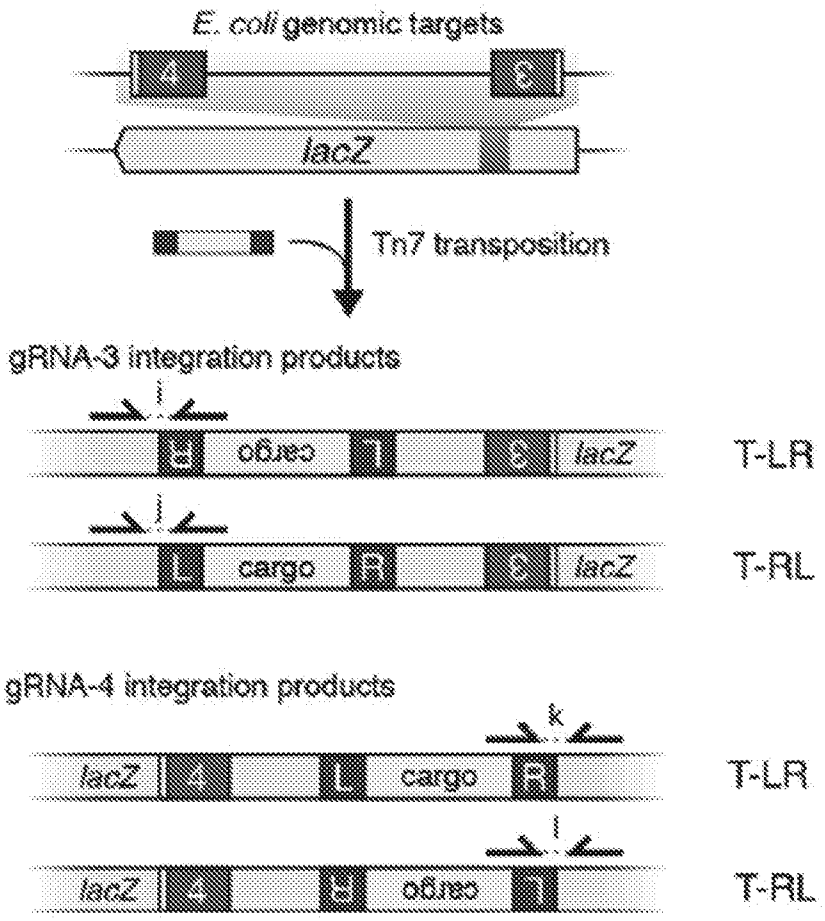

E. coli genomic targets
4
5
lacZ
Tn7 transposition
gRNA-3 integration products
j
R
cargo
T
5
lacZ
T-LR
j
L
cargo
R
5
lacZ
T-RL
gRNA-4 integration products
k
lacZ
4
L
cargo
R
T-LR
l
lacZ
4
L
cargo
T
T-RL

FIG. 10A

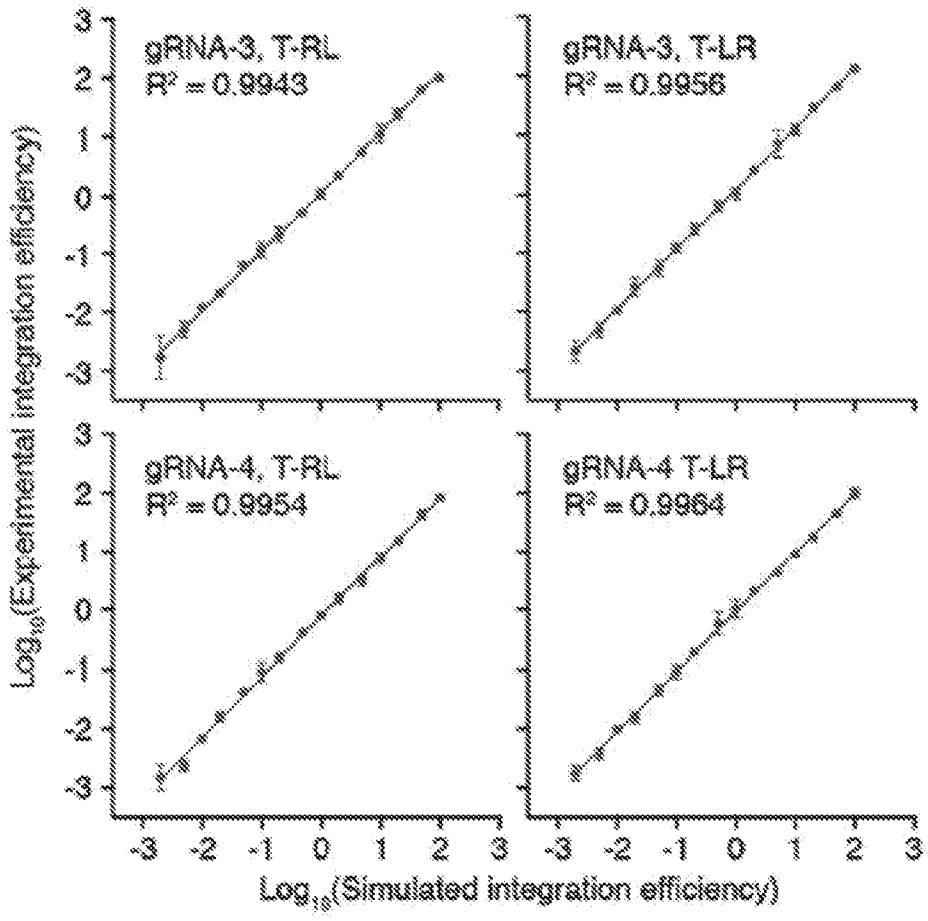


FIG. 10B

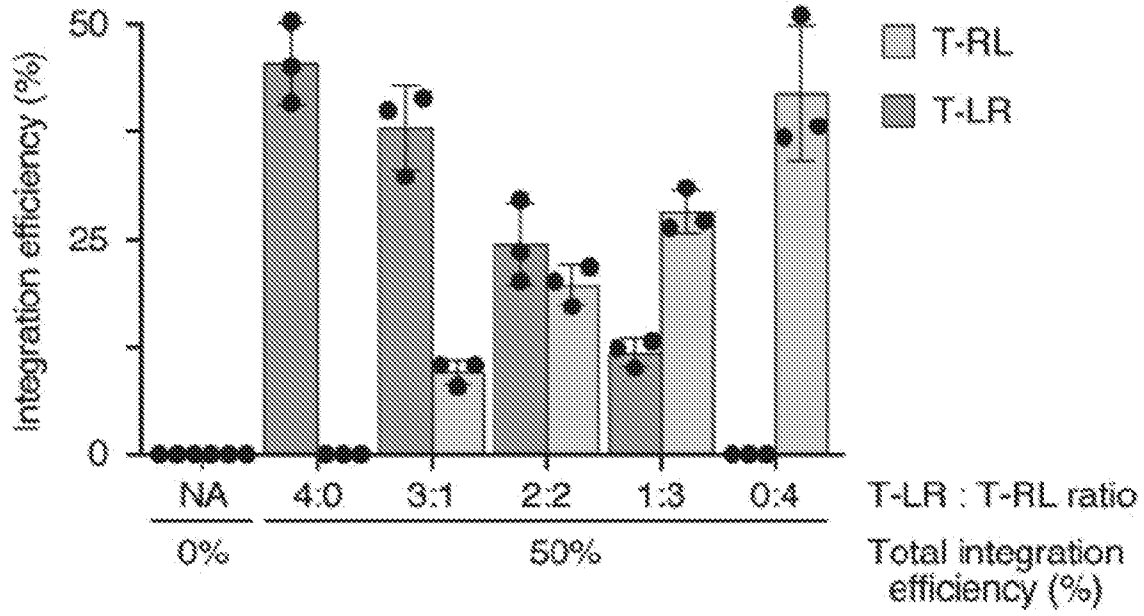


FIG. 10C

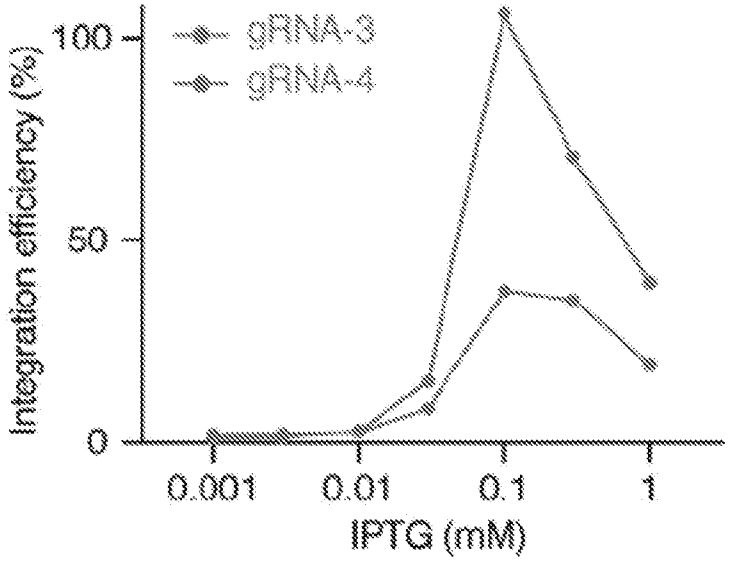


FIG. 10D

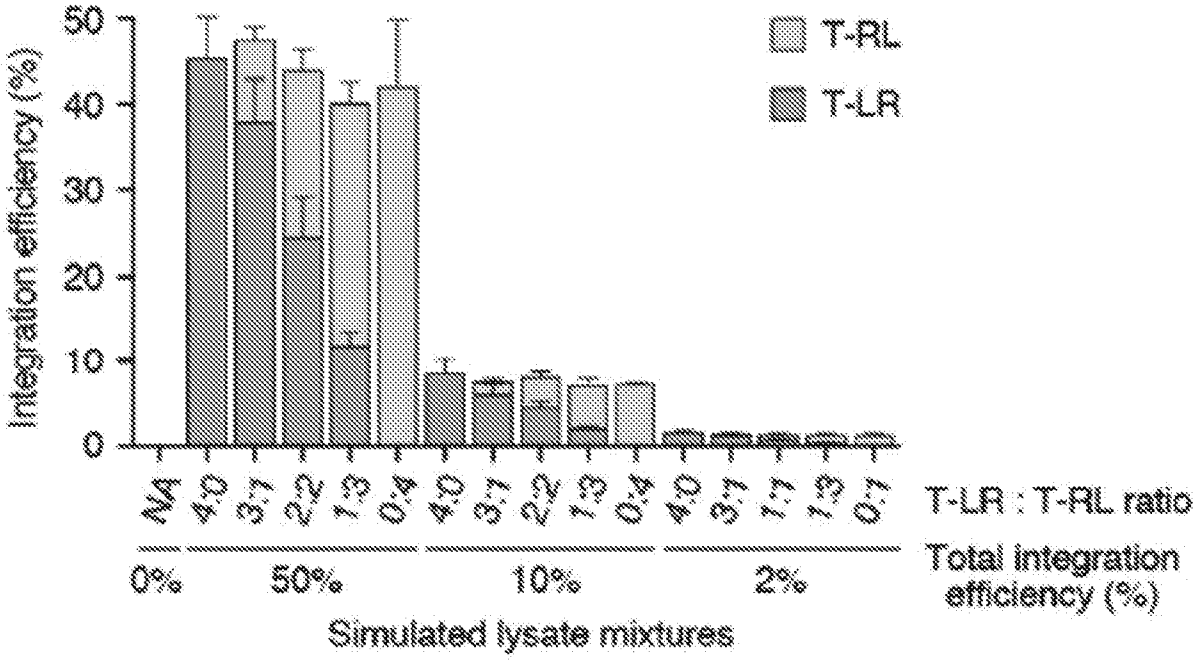


FIG. 10E

FIG. 11A

FIG. 11B

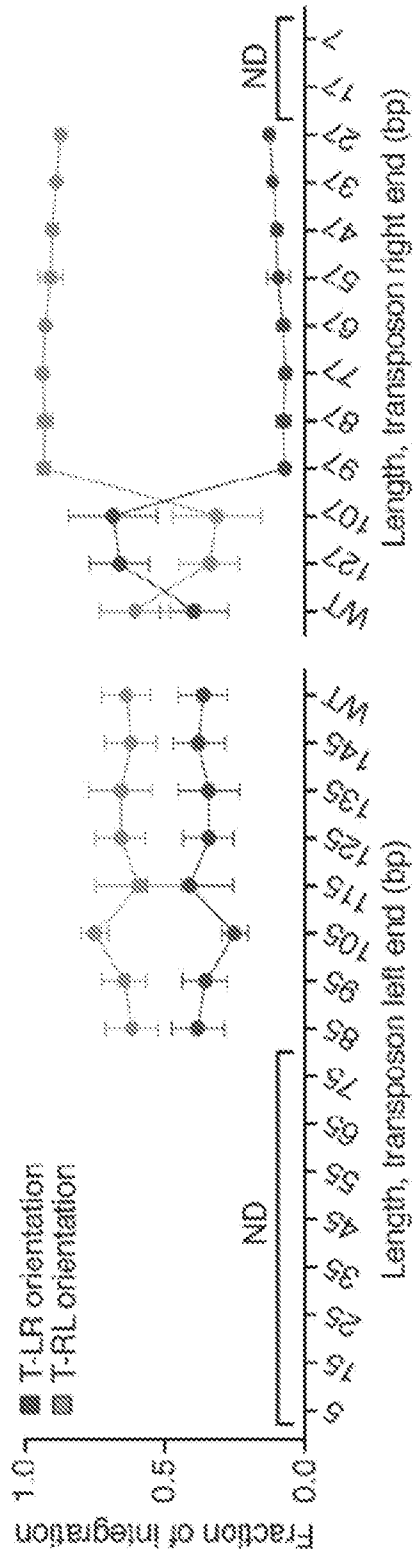


FIG. 11C

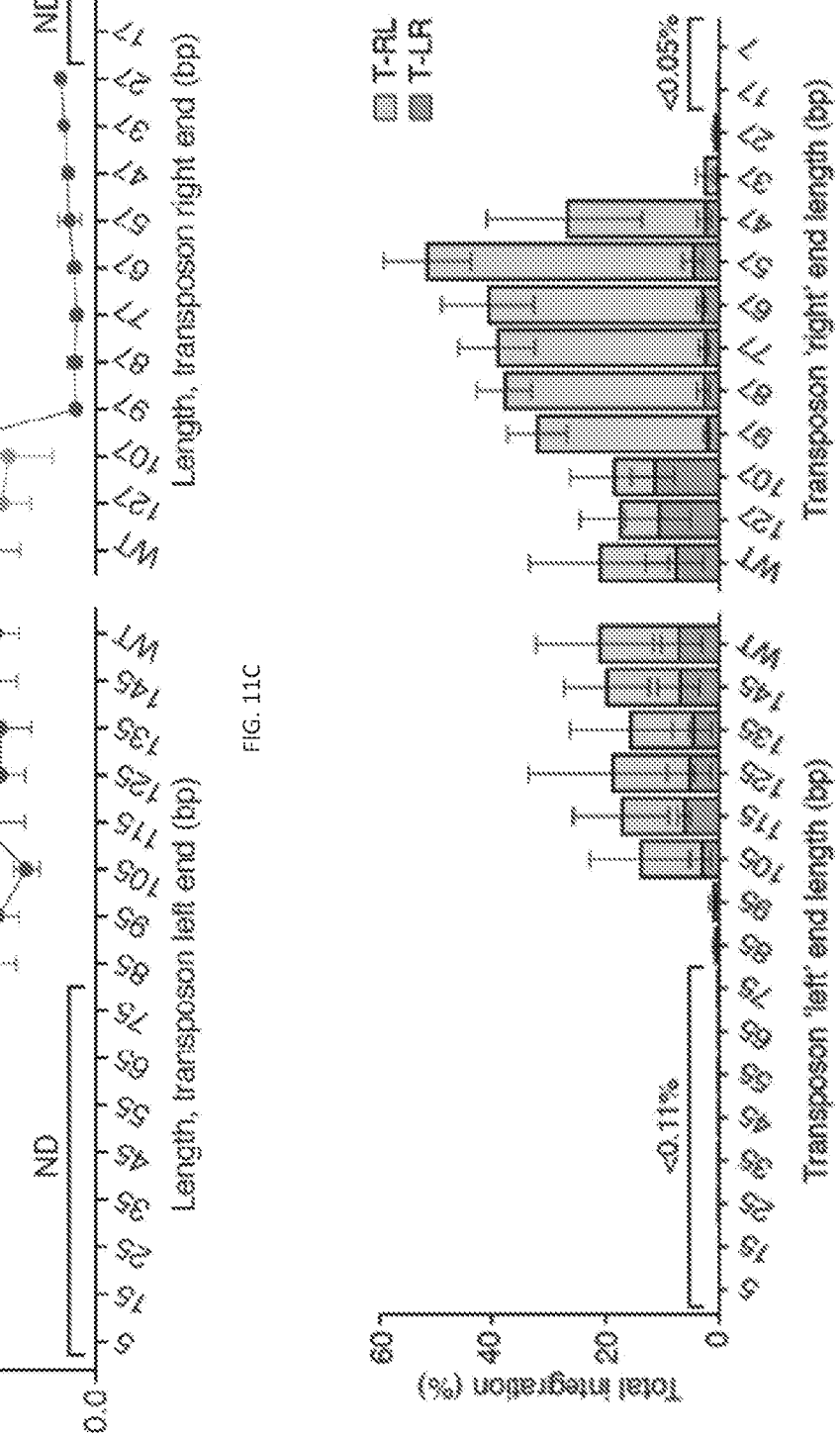


FIG. 11D

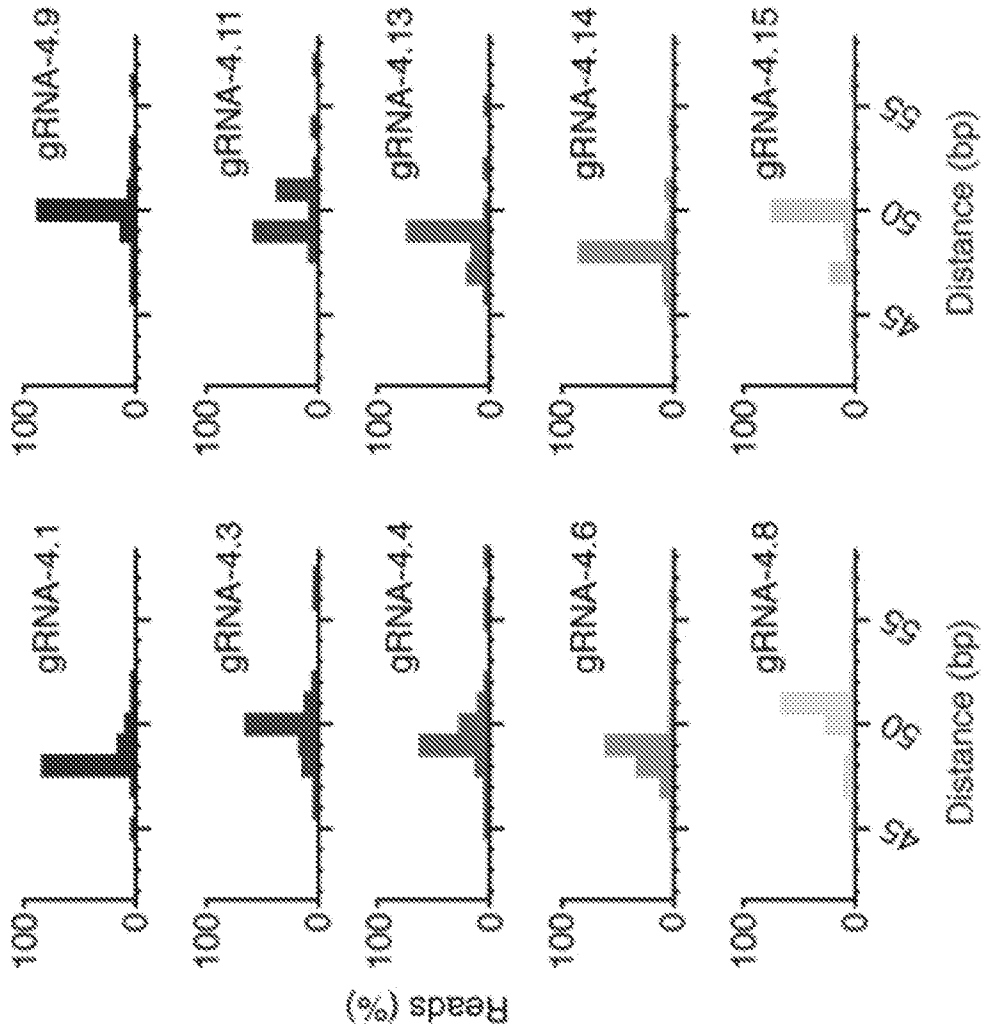

gRNA-4.1
gRNA-4.3
gRNA-4.4
gRNA-4.6
gRNA-4.8
gRNA-4.9
gRNA-4.11
gRNA-4.13
gRNA-4.14
gRNA-4.15
100
0
Reads (%)
45
60
65
Distance (bp)

FIG. 12A

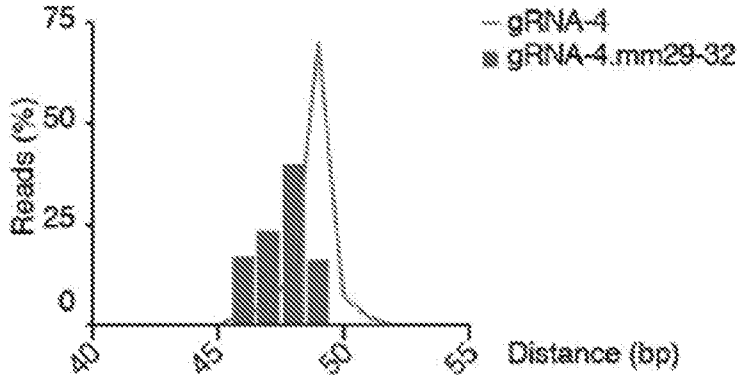


FIG. 12B

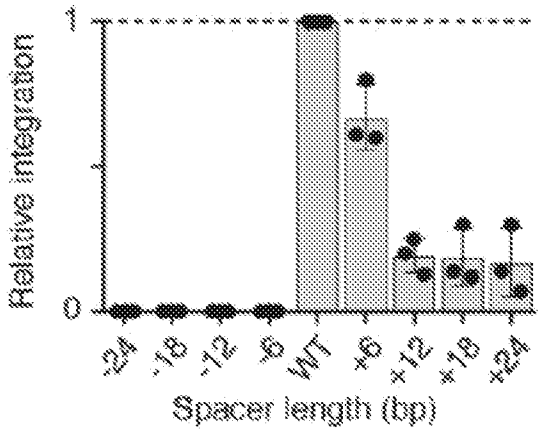


FIG. 12C

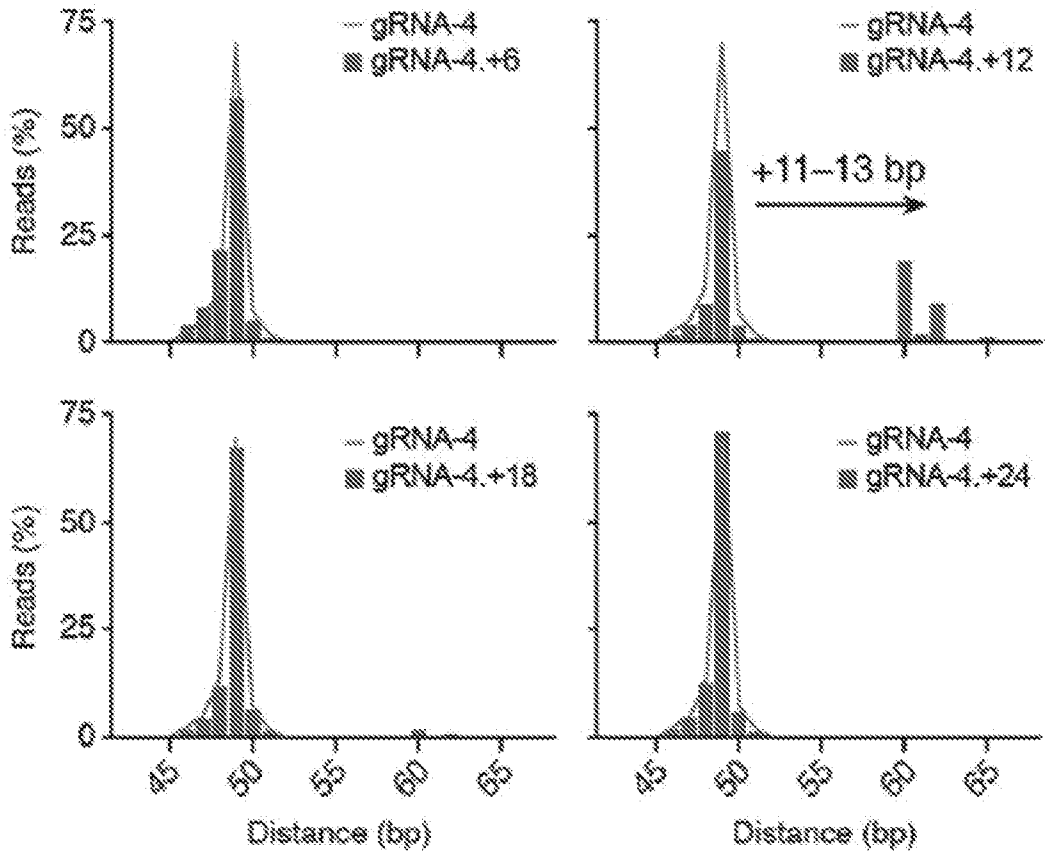


FIG. 12D

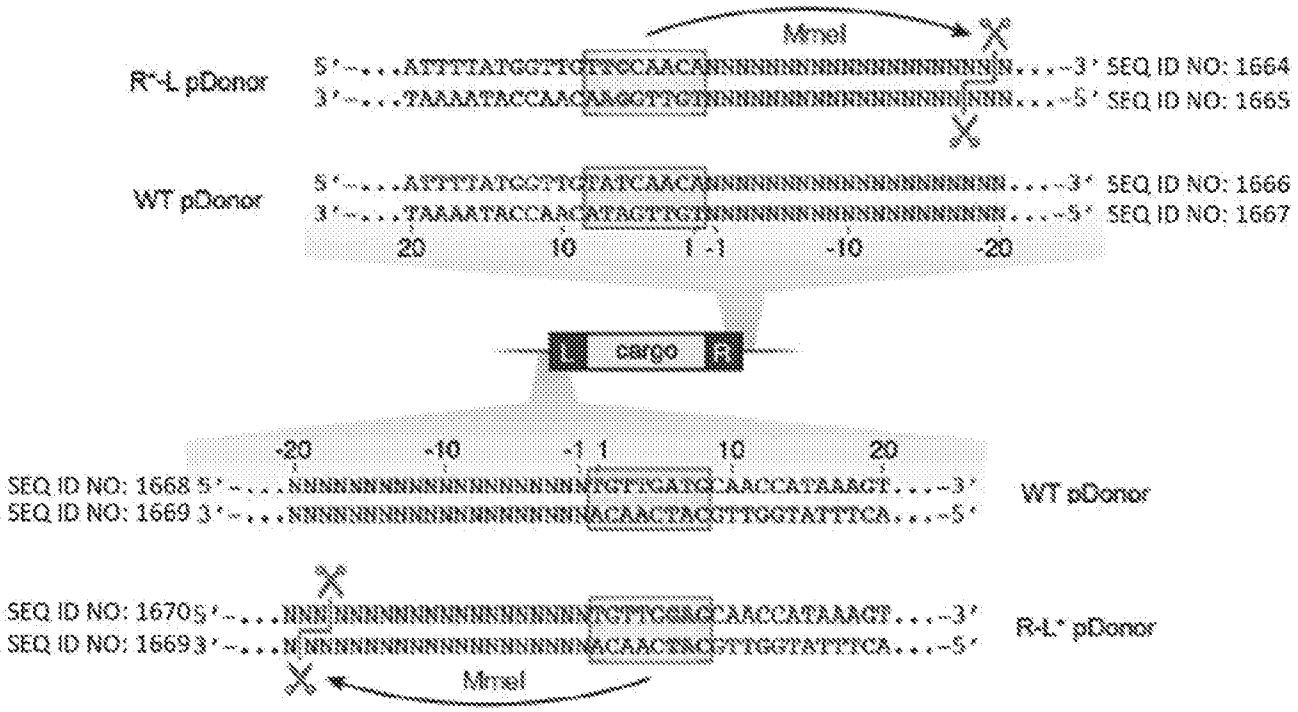


FIG. 13A

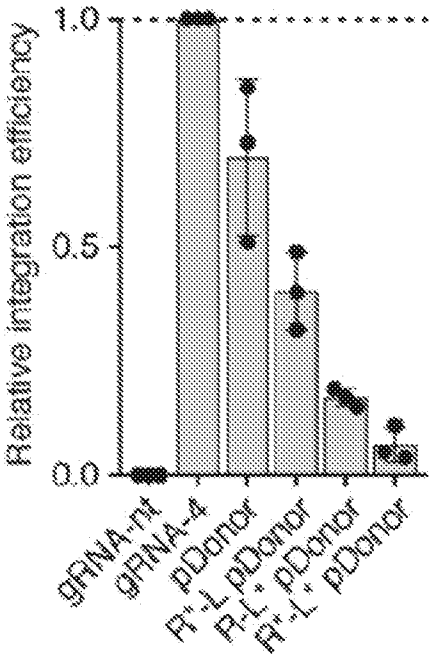


FIG. 13B

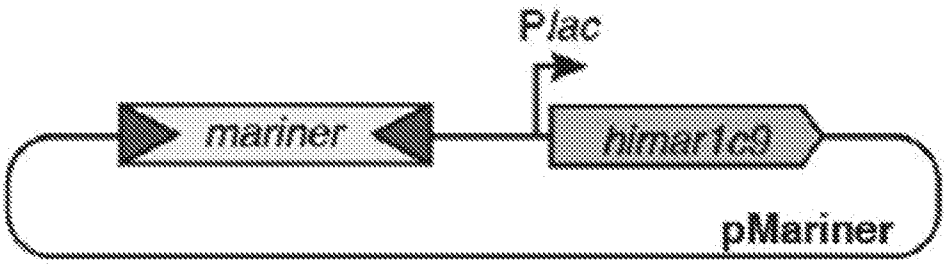


FIG. 13C

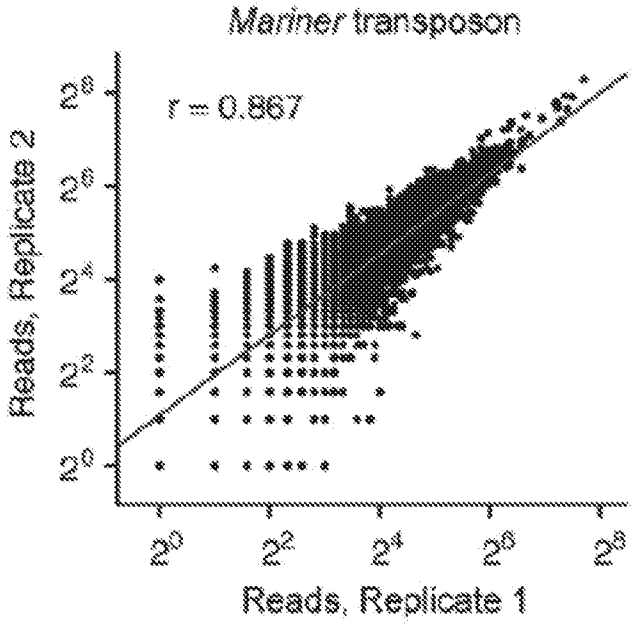


FIG. 13D

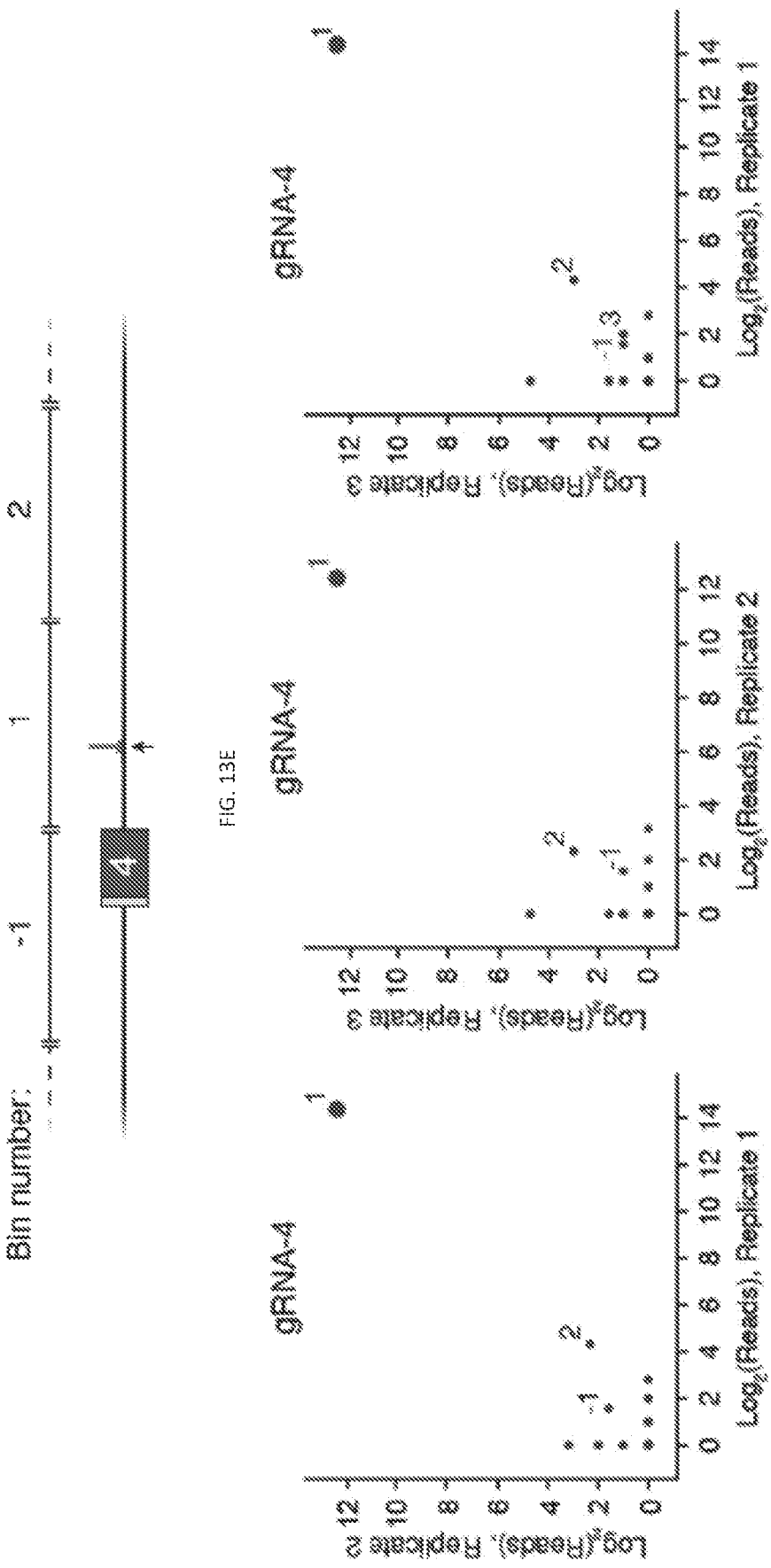


FIG. 13E

FIG. 13F

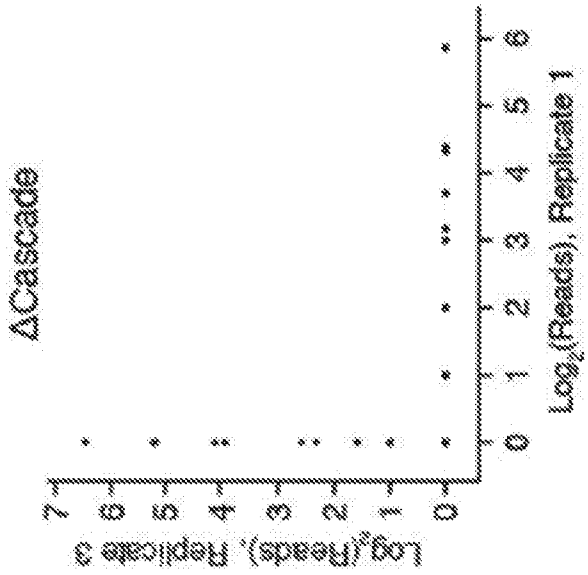


FIG. 13H

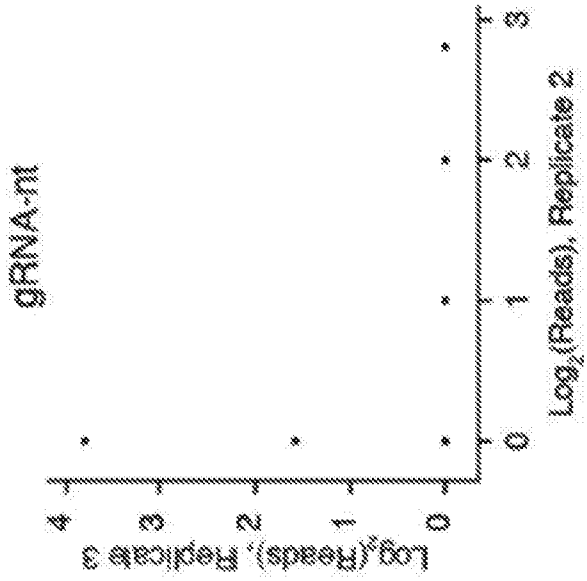


FIG. 13G

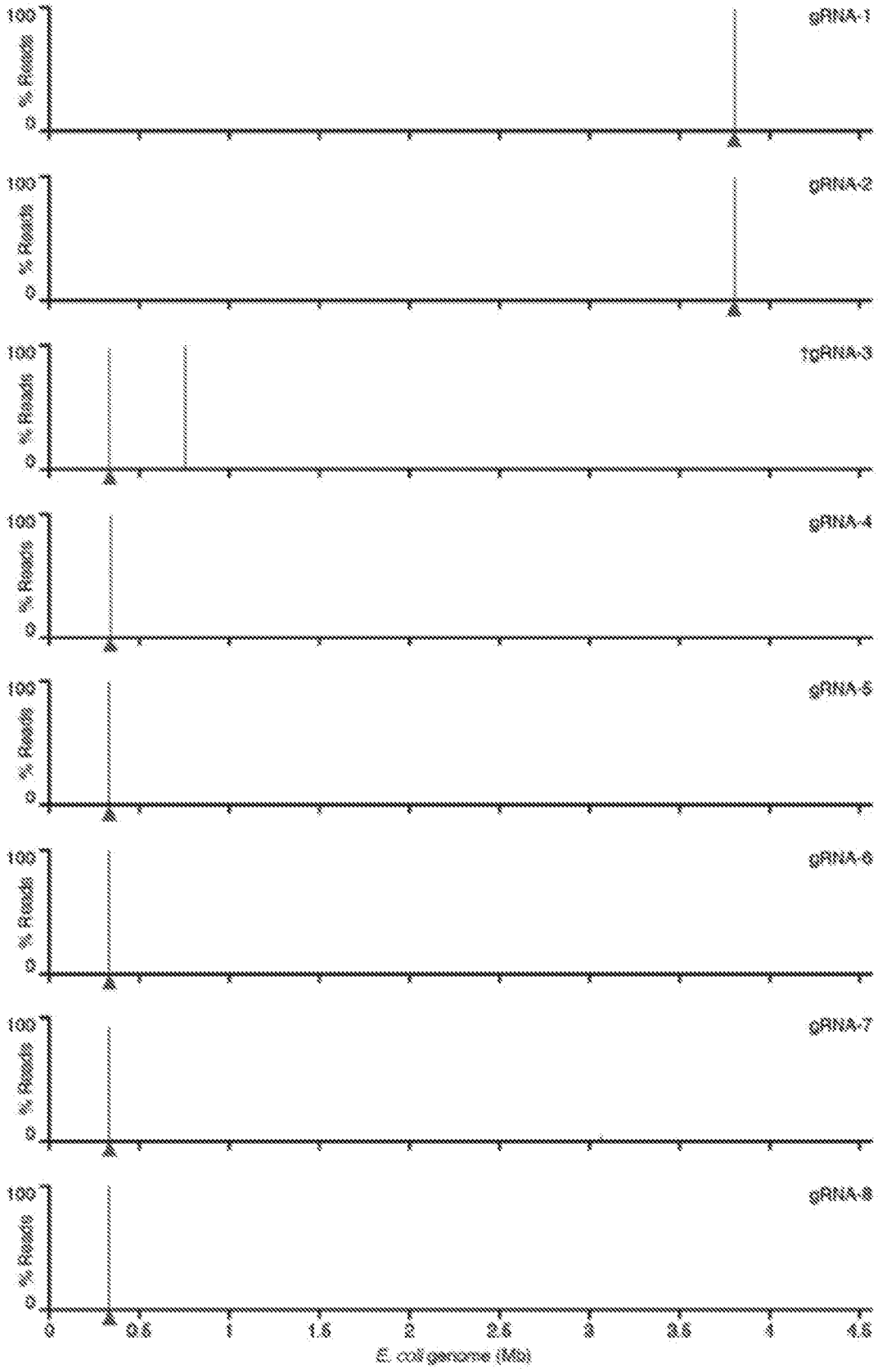

gRNA-1
gRNA-2
gRNA-3
gRNA-4
gRNA-5
gRNA-6
gRNA-7
gRNA-8
100
% Reads
0
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
E. coli genome (Mb)

FIG. 14A

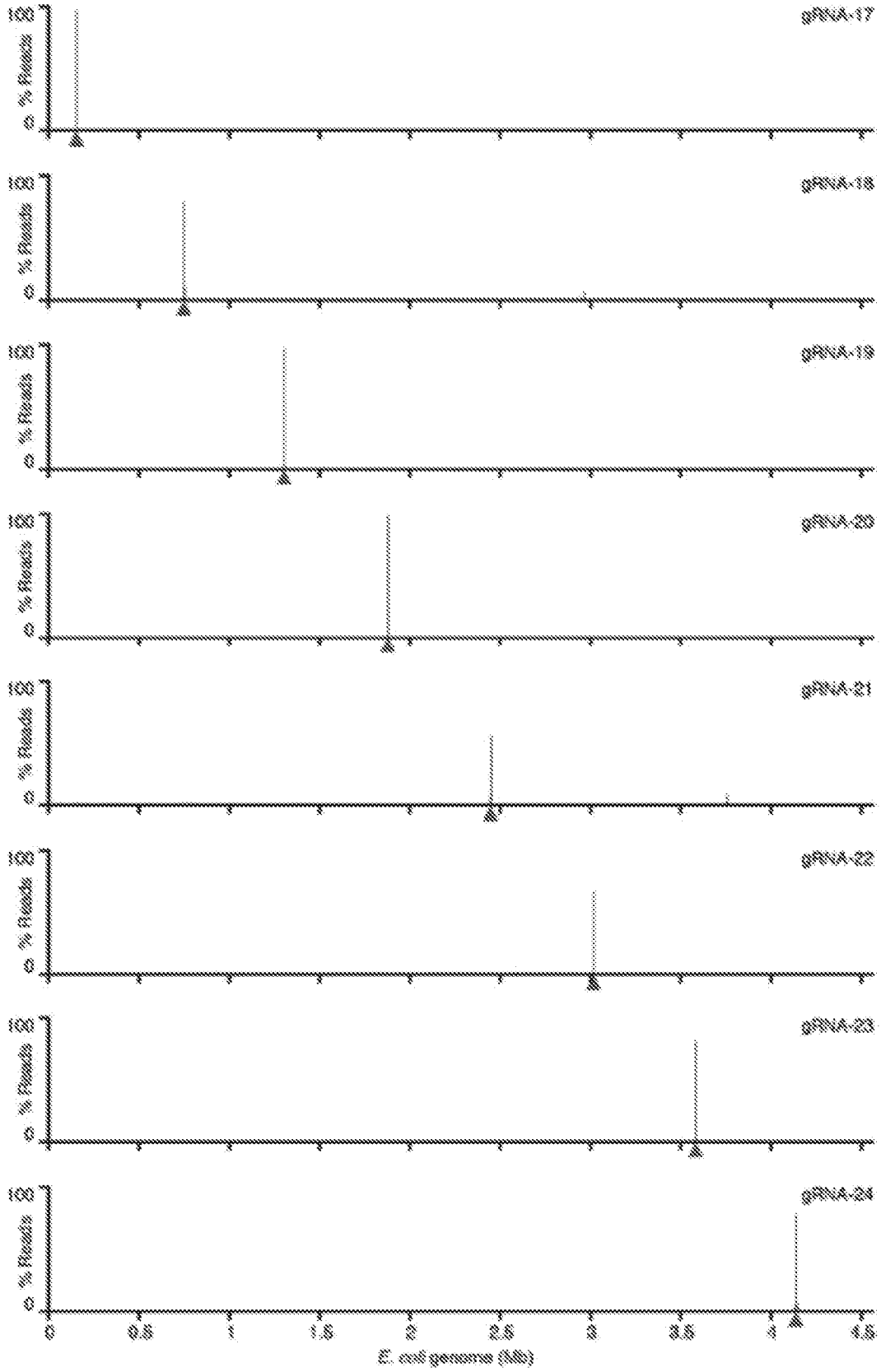

gRNA-17
gRNA-18
gRNA-19
gRNA-20
gRNA-21
gRNA-22
gRNA-23
gRNA-24
100
% Reads
0
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
E. coli genome (Mb)

FIG. 14B

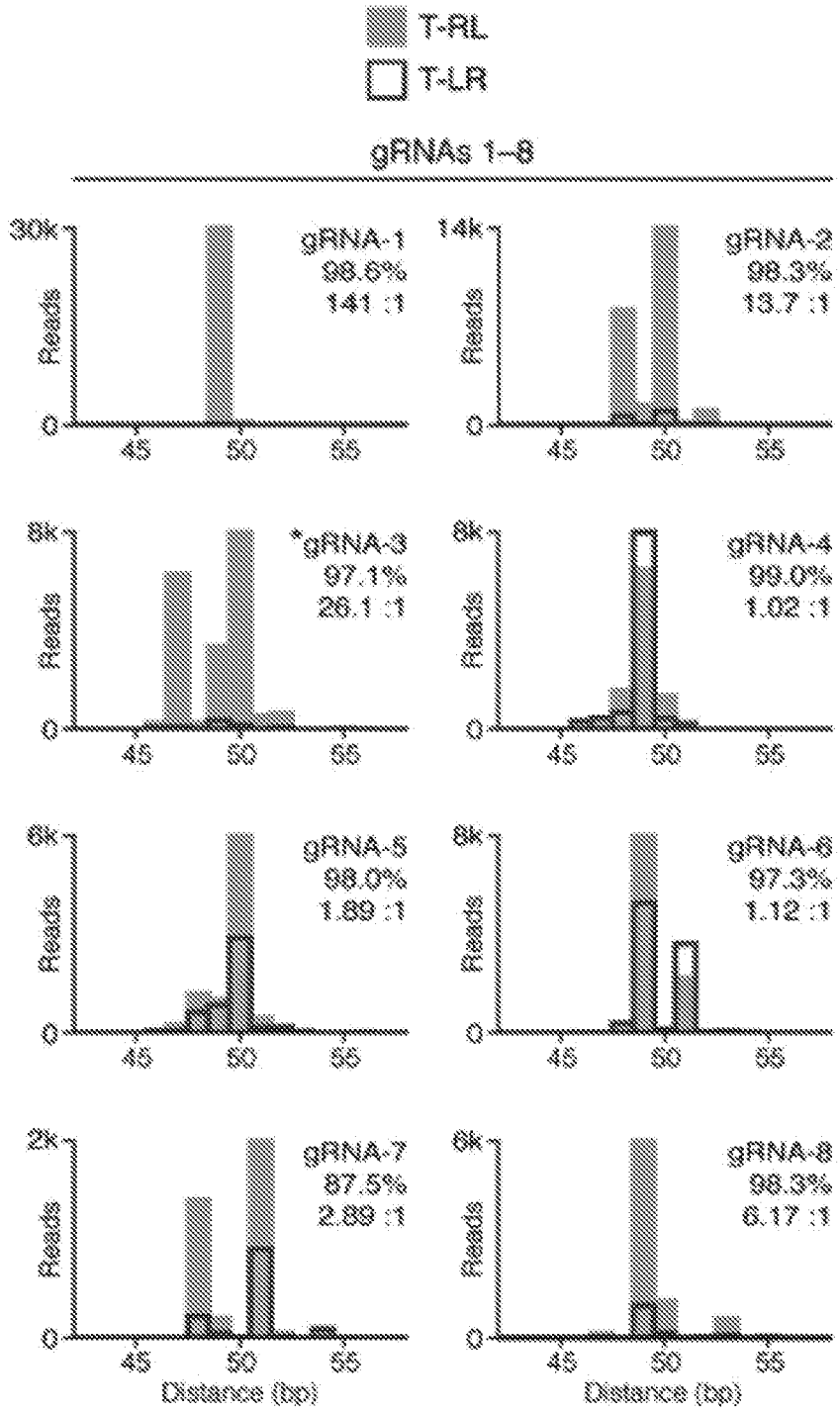

T-RL
T-LR
gRNAs 1–8
gRNA-1
98.6%
141 :1
gRNA-2
98.3%
13.7 :1
*gRNA-3
97.1%
26.1 :1
gRNA-4
99.0%
1.02 :1
gRNA-5
98.0%
1.89 :1
gRNA-6
97.3%
1.12 :1
gRNA-7
87.5%
2.89 :1
gRNA-8
98.3%
6.17 :1
Reads
Distance (bp)
45
50
55

FIG. 14C

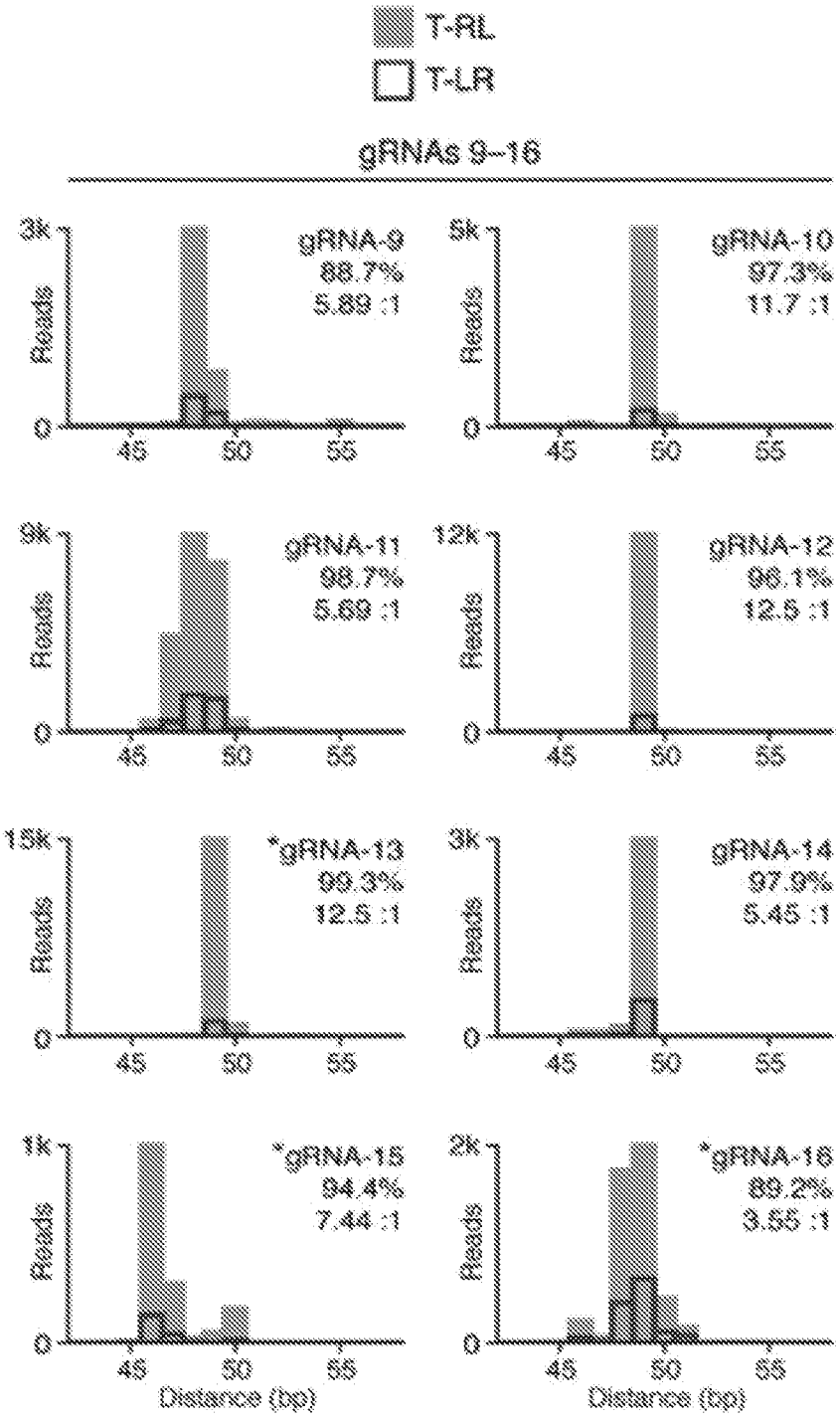

T-RL
T-LR
gRNAs 9–16
gRNA-9
88.7%
5.89 :1
gRNA-10
97.3%
11.7 :1
gRNA-11
98.7%
5.69 :1
gRNA-12
96.1%
12.5 :1
*gRNA-13
99.3%
12.5 :1
gRNA-14
97.9%
5.45 :1
*gRNA-15
94.4%
7.44 :1
*gRNA-16
89.2%
3.55 :1
Reads
Distance (bp)
45
50
55

FIG. 14D

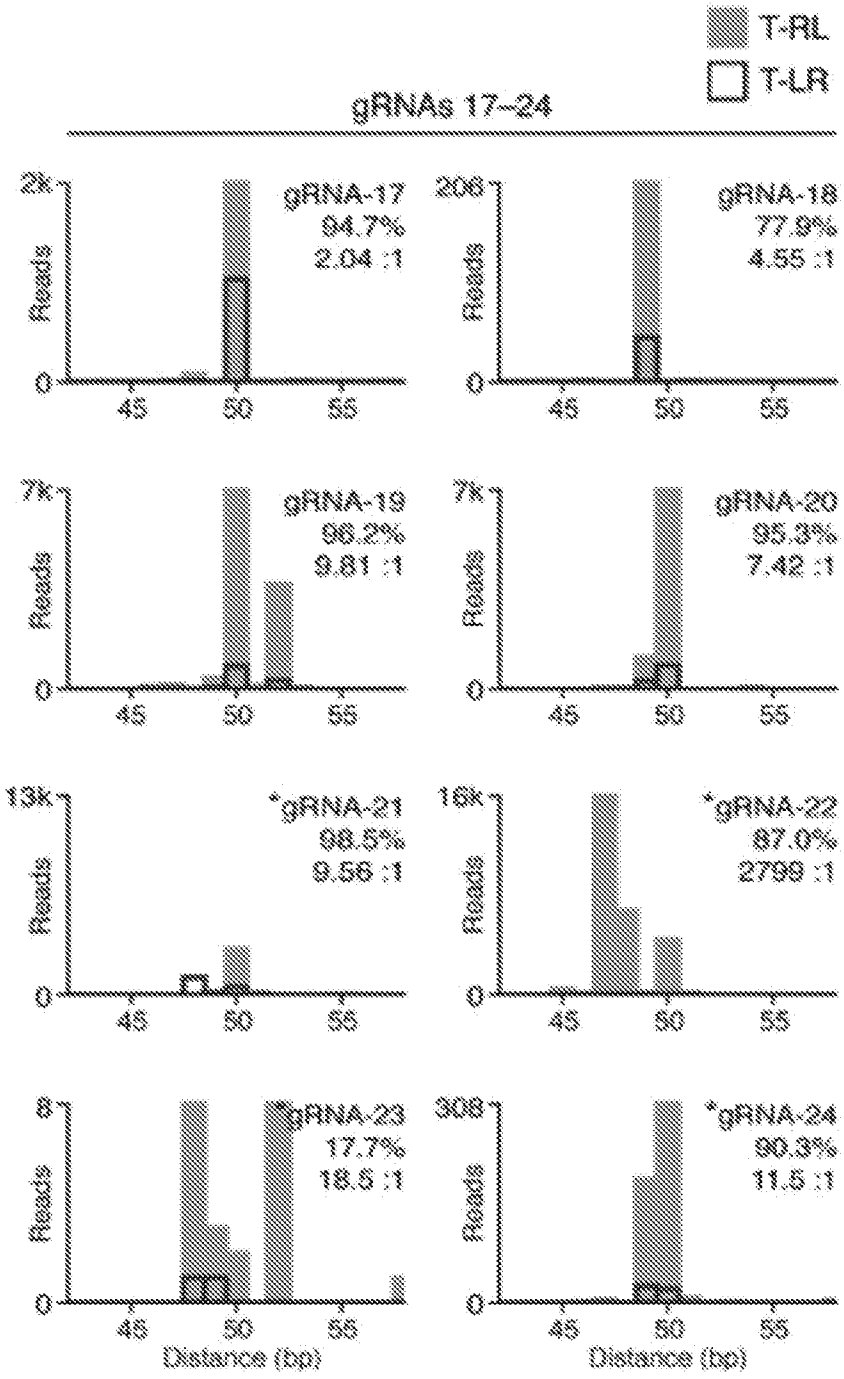

T-RL
T-LR
gRNAs 17–24
gRNA-17
94.7%
2.04 :1
gRNA-18
77.9%
4.55 :1
gRNA-19
96.2%
9.81 :1
gRNA-20
95.3%
7.42 :1
*gRNA-21
98.5%
9.56 :1
*gRNA-22
87.0%
2799 :1
*gRNA-23
17.7%
18.5 :1
*gRNA-24
90.3%
11.5 :1
Reads
Distance (bp)
45
50
55

FIG. 14E

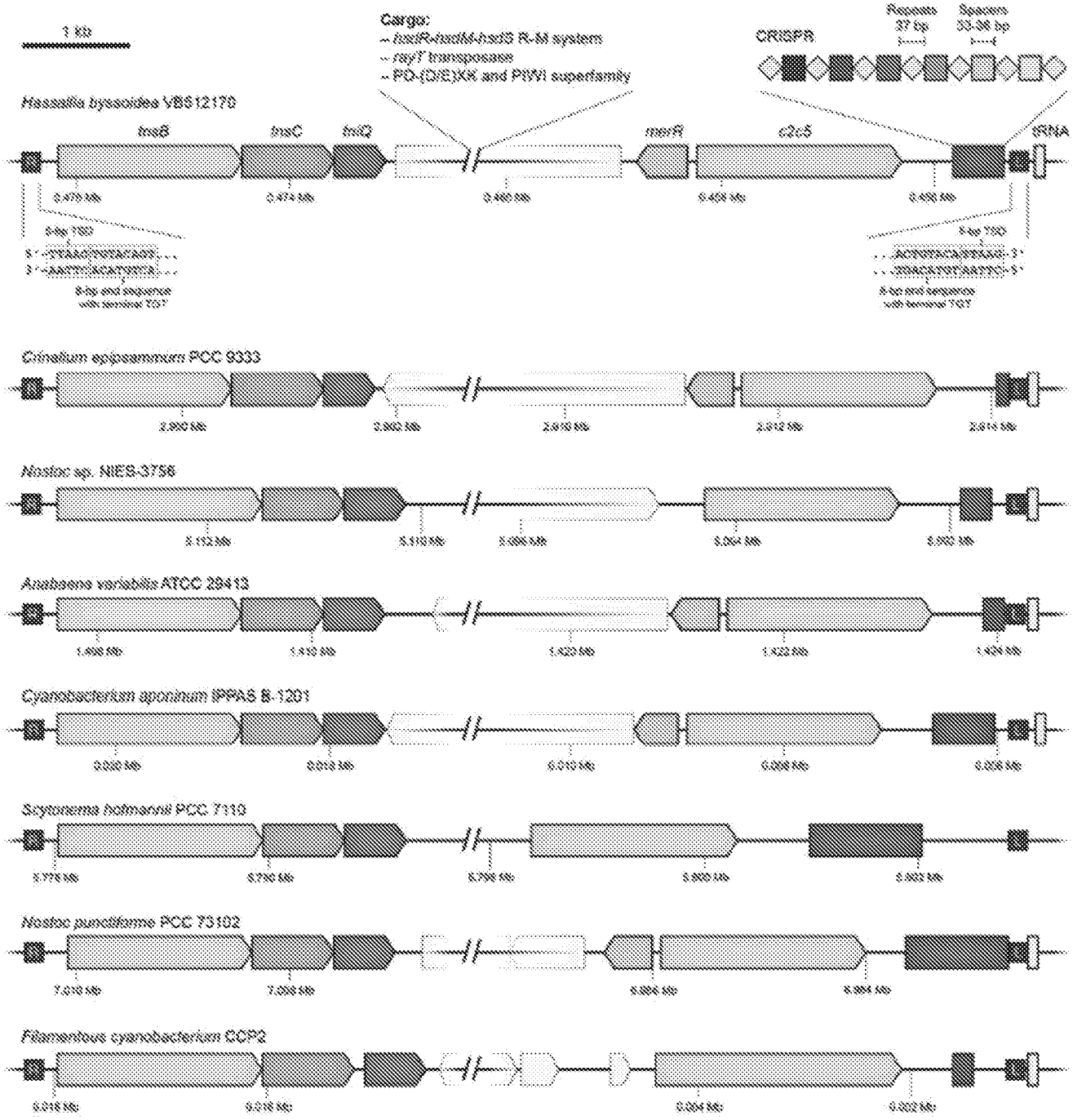

1 kb
Cargo:
– hsdR-hsdM-hsdS R-M system
– rayT transposase
– PD-(D/E)XK and PIWI superfamily
CRISPR
Repeats
37 bp
Spacers
33-38 bp
Haasella hyssoidea VBS12170
insB
insC
insQ
marR
c2c5
tRNA
5-bp TSD
8-bp end sequence
with terminal TGT
Crinalium epipsammum PCC 9333
Nostoc sp. NIES-3756
Anabaena variabilis ATCC 29413
Cyanobacterium aponinum IPPAS B-1201
Scytonema hofmannii PCC 7110
Nostoc punctiforme PCC 73102
Filamentous cyanobacterium CCP2

FIG. 15

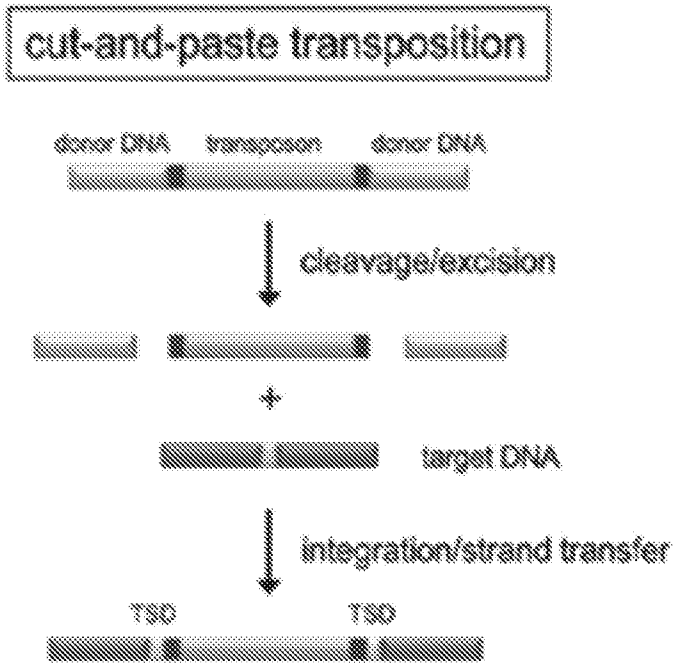


FIG. 16A

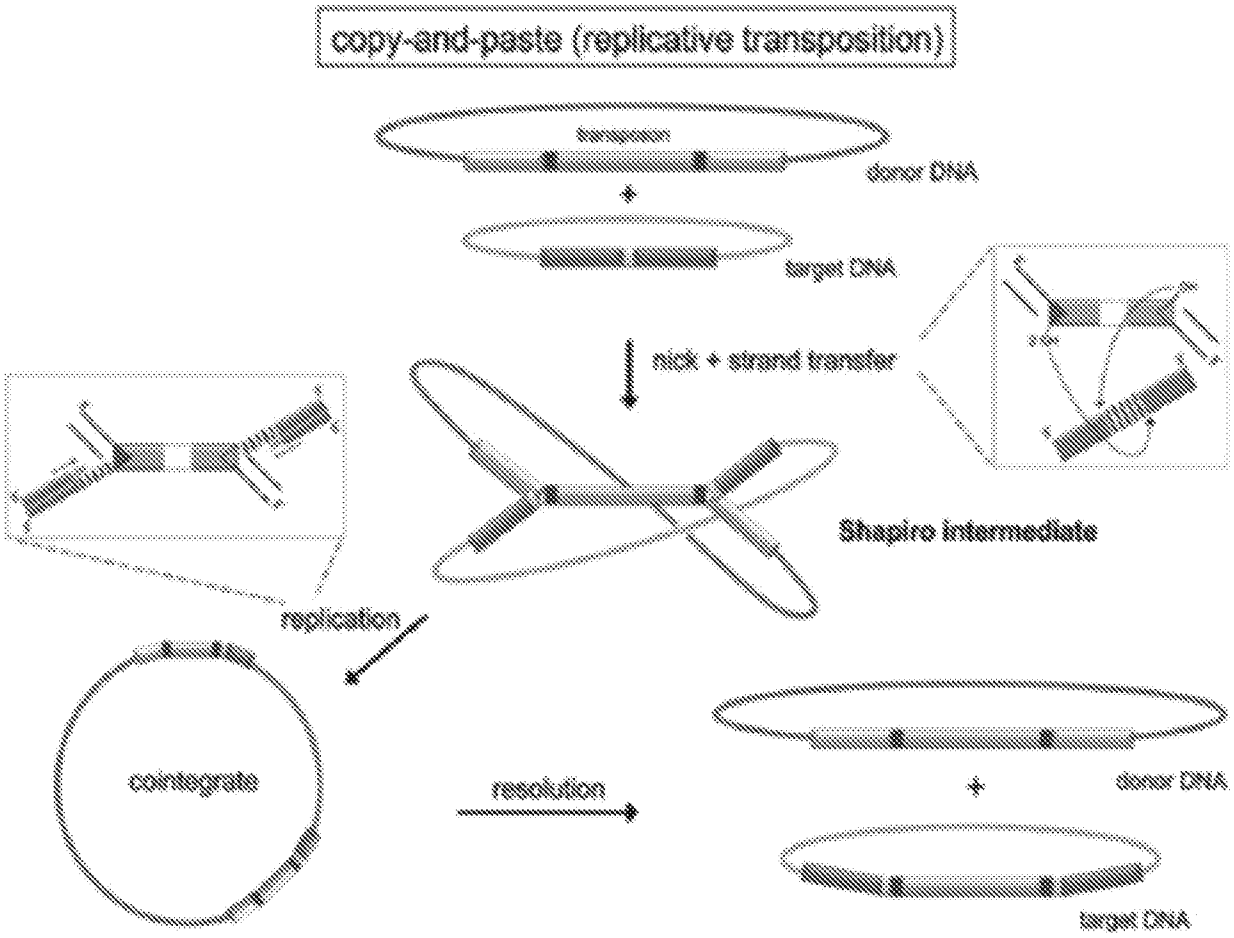


FIG. 16B

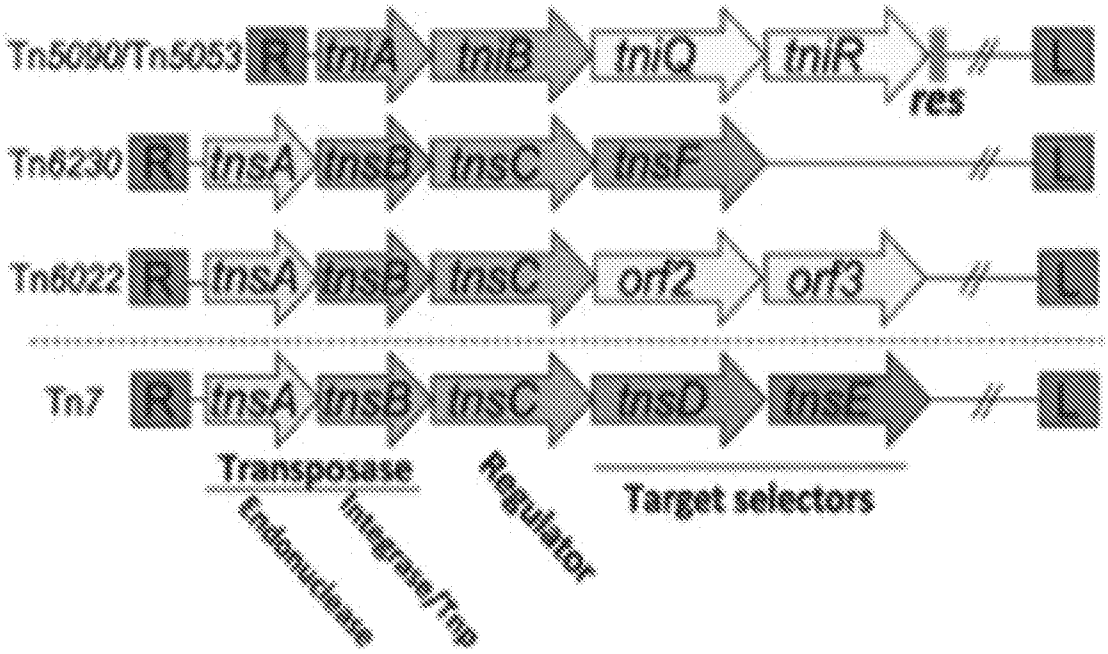


FIG. 17A

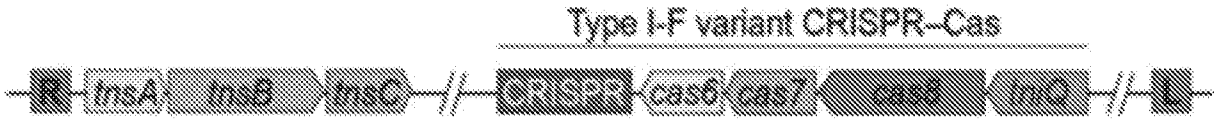


FIG. 17B

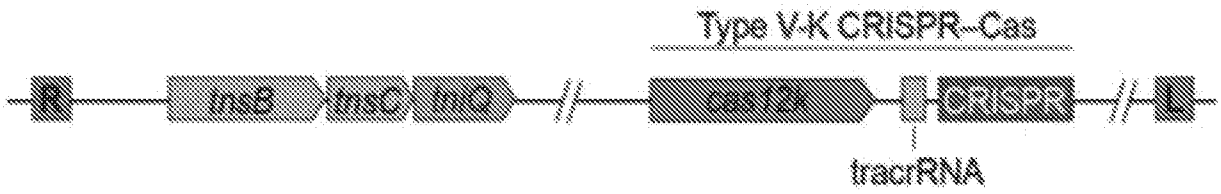


FIG. 17C

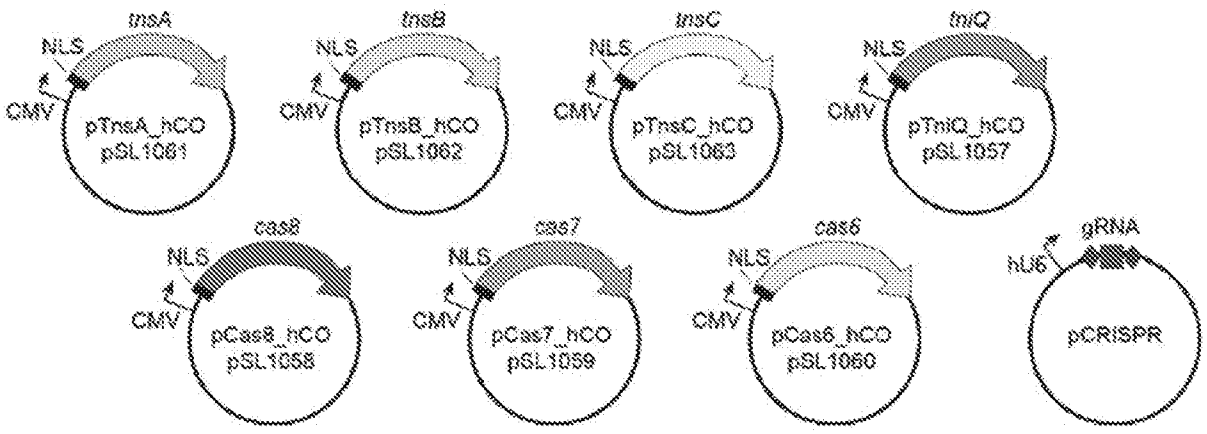


FIG. 18

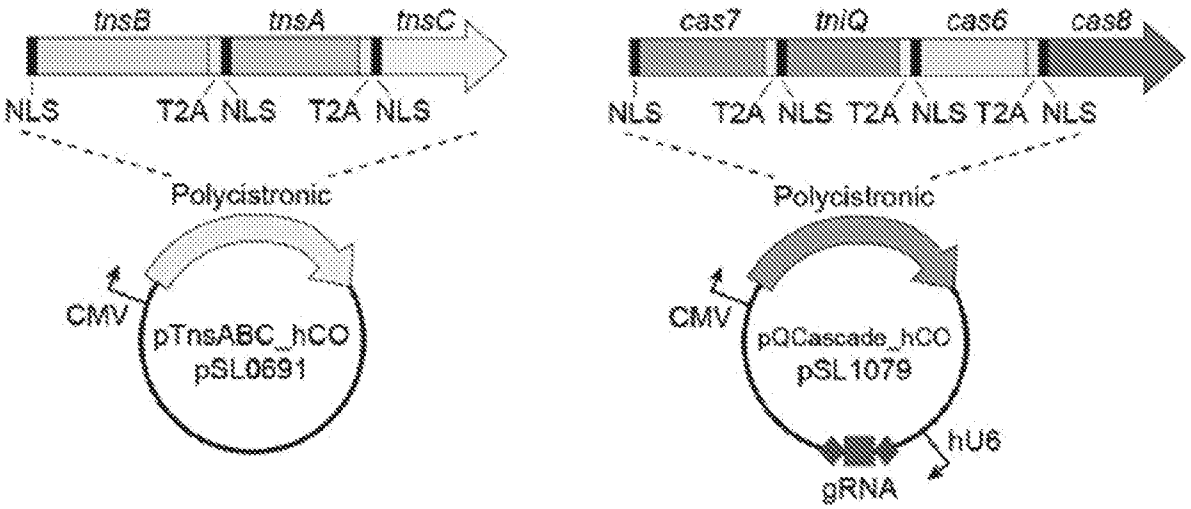


FIG. 19

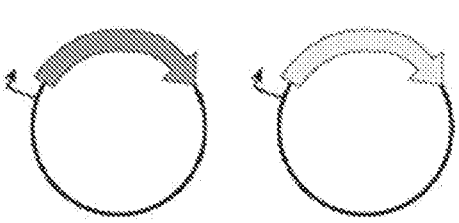


FIG. 20A

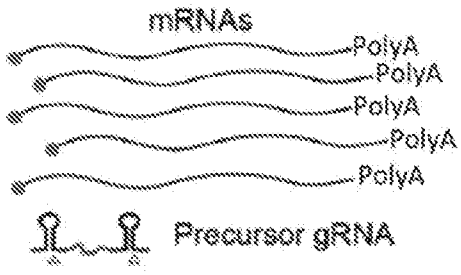


FIG. 20B

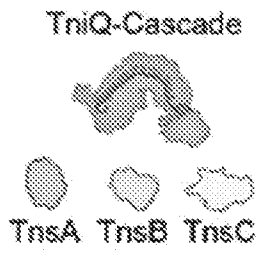


FIG. 20C

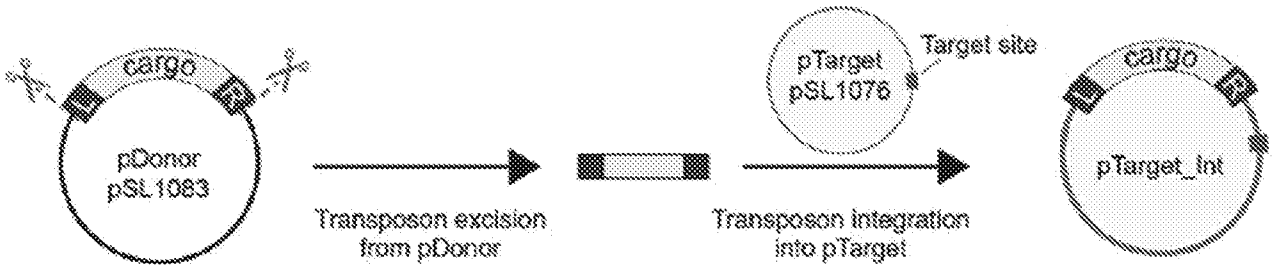


FIG. 21A

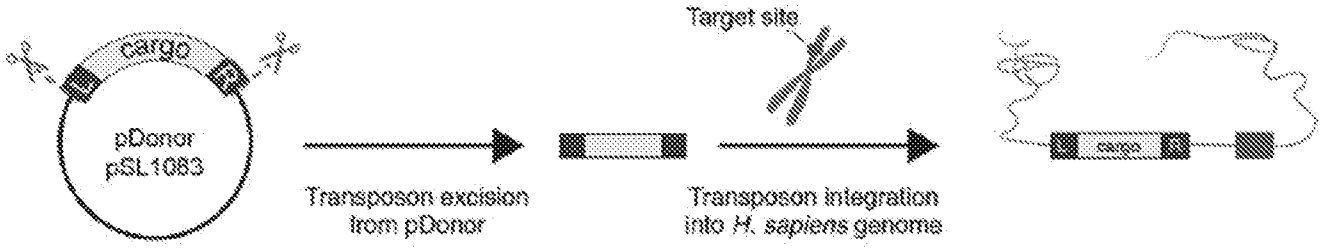


FIG. 21B

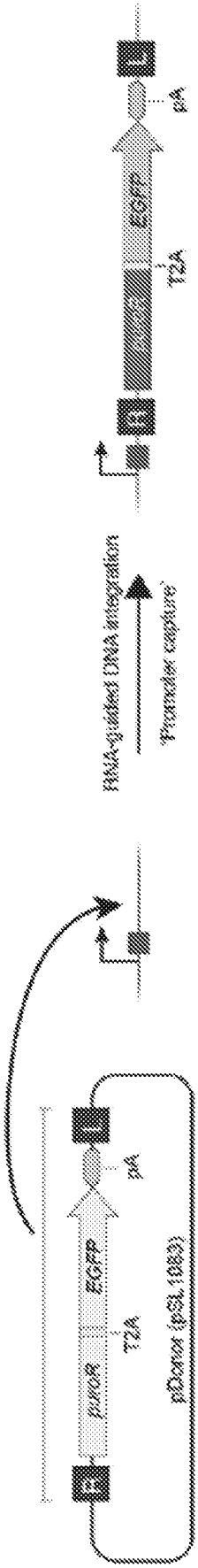


FIG. 22A

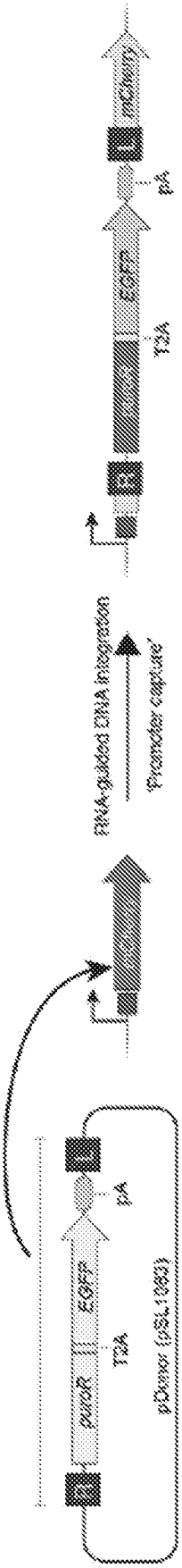


FIG. 22B

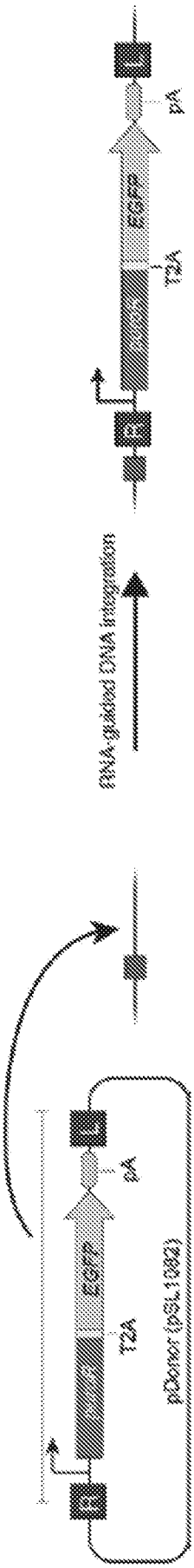


FIG. 22C

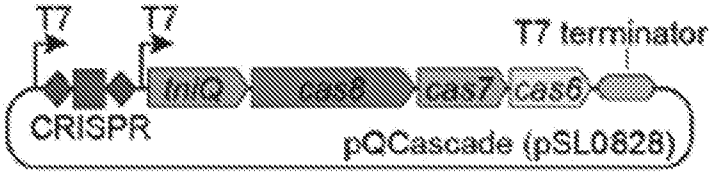


FIG. 23A

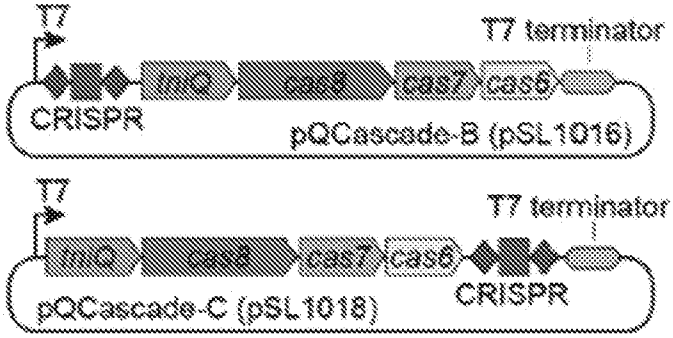


FIG. 23B

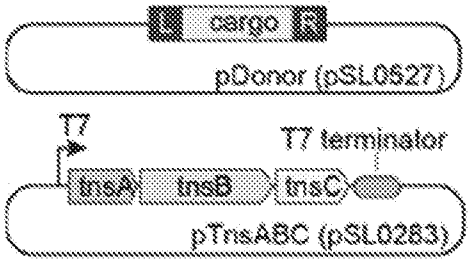


FIG. 23C

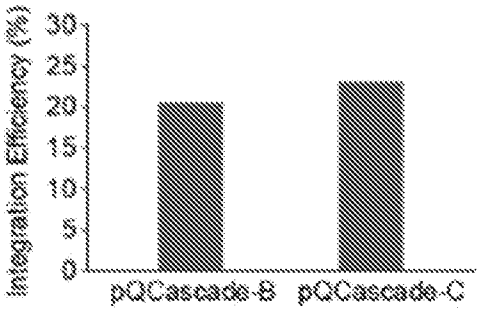


FIG. 23D

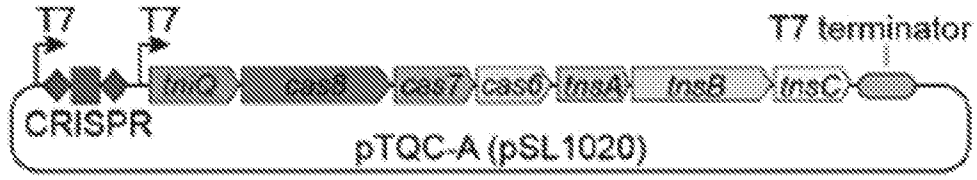


FIG. 24A

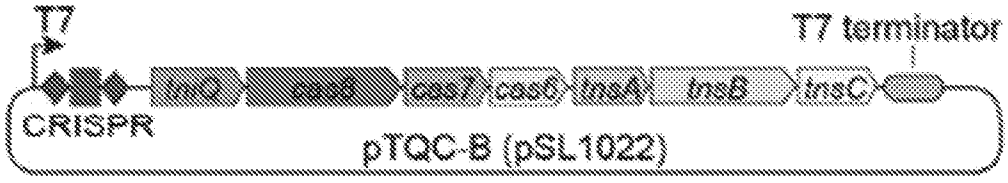


FIG. 24B

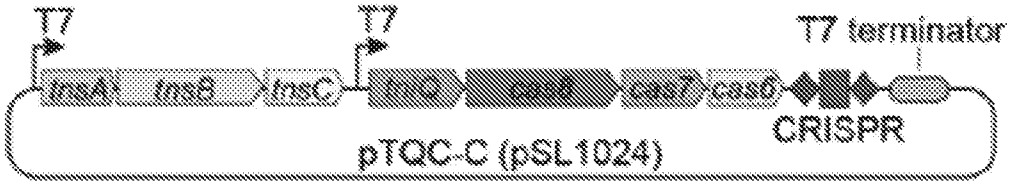


FIG. 24C

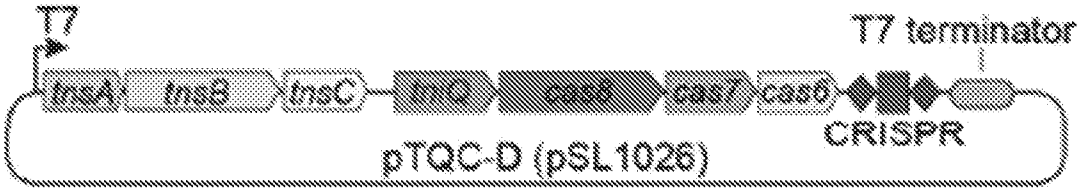


FIG. 24D

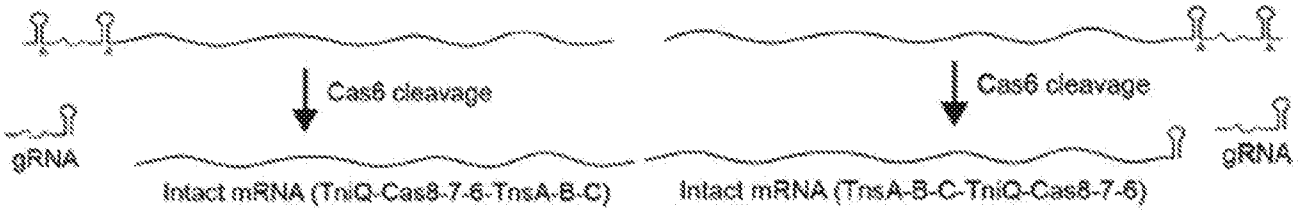


FIG. 24E

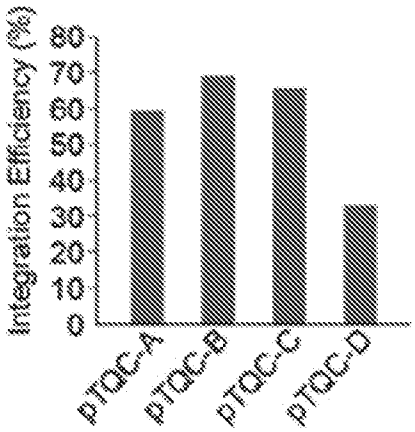


FIG. 24F

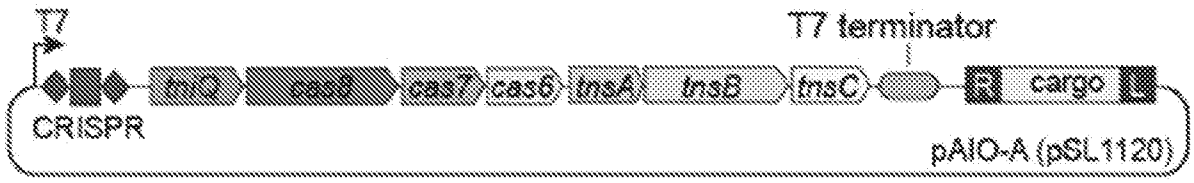


FIG. 25A

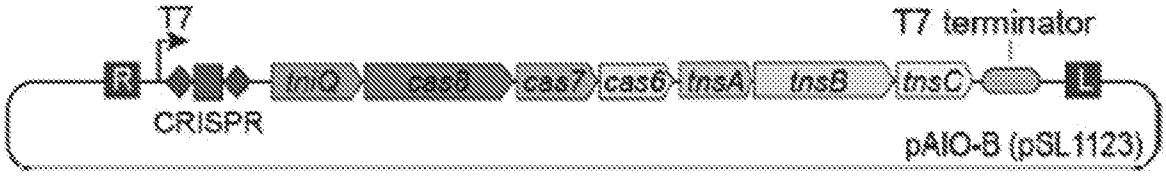


FIG. 25B

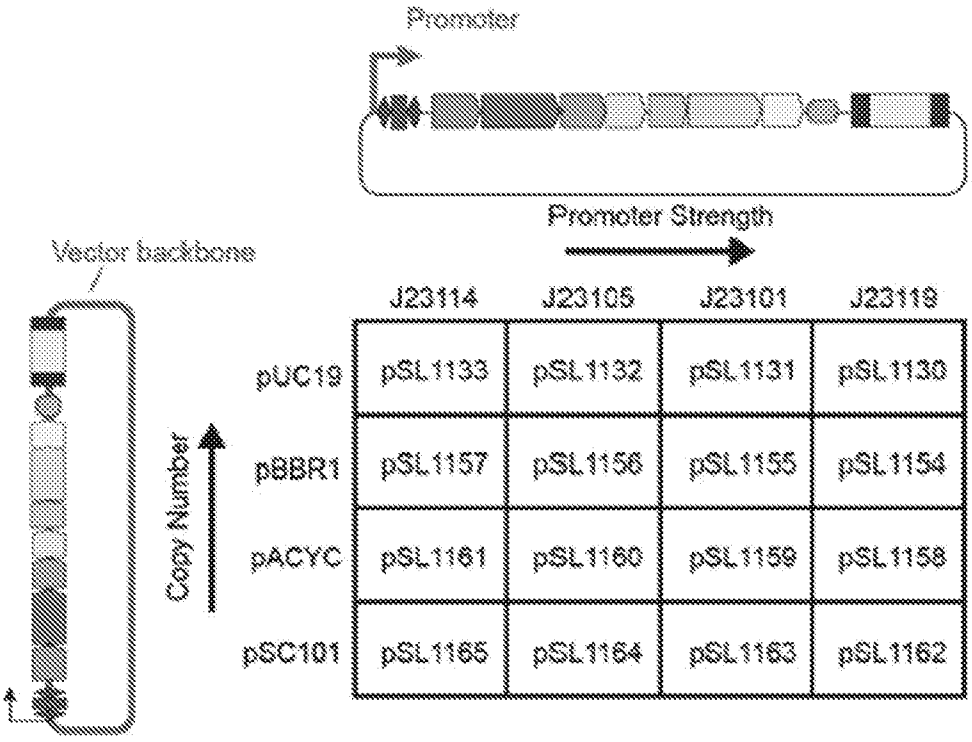


FIG. 26A

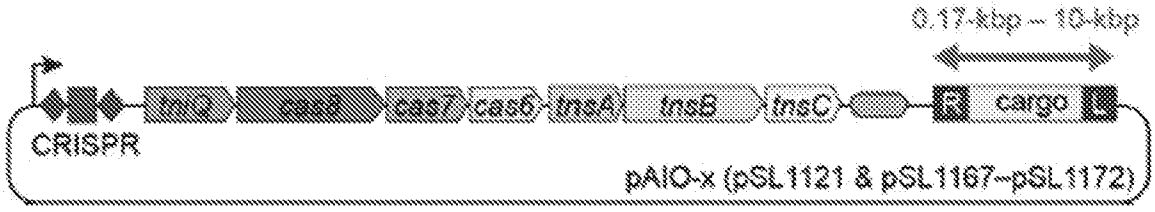


FIG. 26B

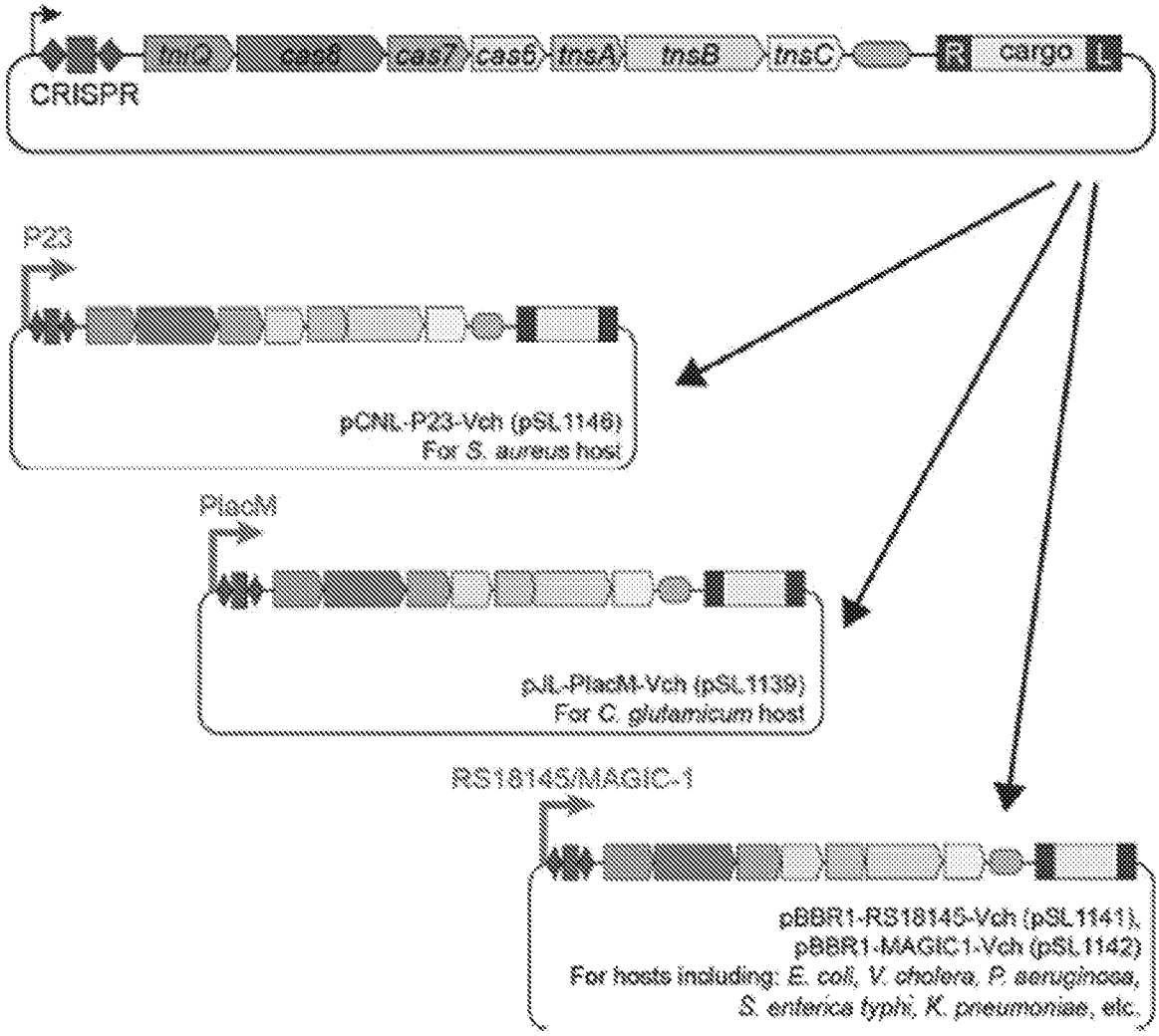

CRISPR
cas7
cas6
tnsA
tnsB
tnsC
R
cargo
L
P23
pCNL-P23-Vch (pSL1146)
For S. aureus host
PlacM
pJL-PlacM-Vch (pSL1139)
For C. glutamicum host
RS18145/MAGIC-1
pBBR1-RS18145-Vch (pSL1141),
pBBR1-MAGIC1-Vch (pSL1142)
For hosts including: E. coli, V. cholera, P. aeruginosa,
S. enterica typhi, K. pneumoniae, etc.

FIG. 27

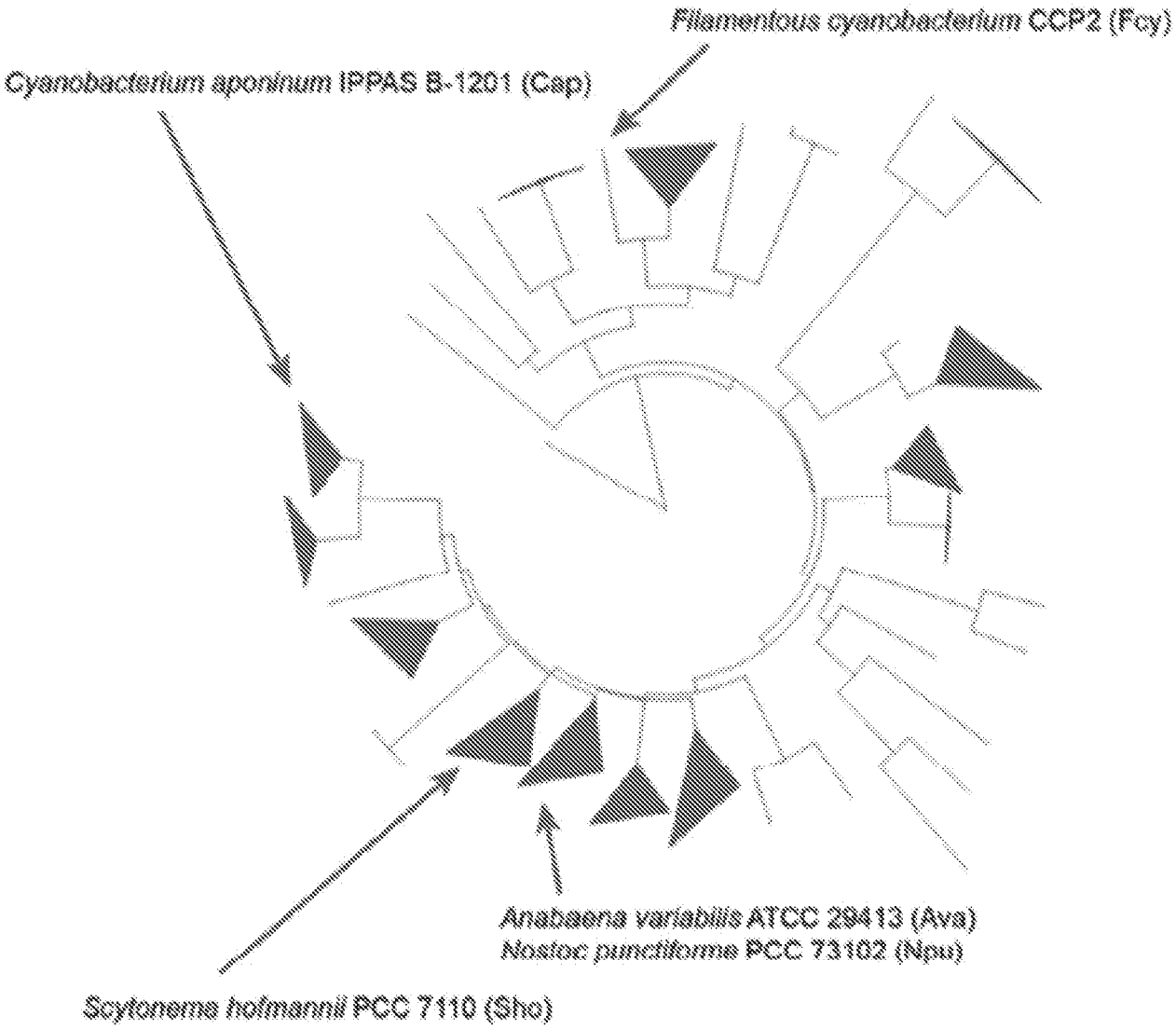

Filamentous cyanobacterium CCP2 (Fcy)
Cyanobacterium aponinum IPPAS B-1201 (Cap)
Anabaena variabilis ATCC 29413 (Ava)
Nostoc punctiforme PCC 73102 (Npu)
Scytonema hofmannii PCC 7110 (Sho)

FIG. 28

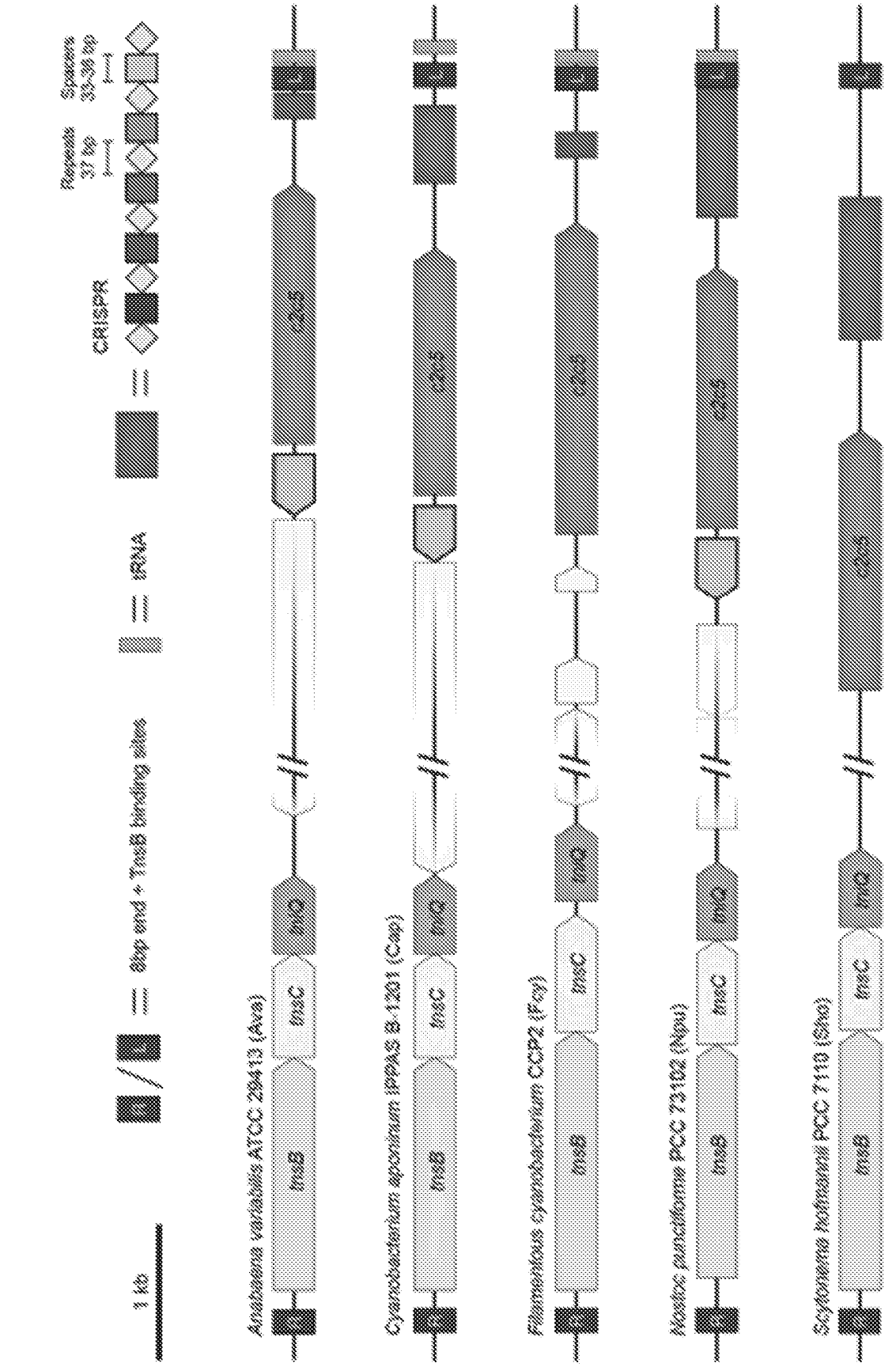

1 kb
8bp end + TnsB binding sites
tRNA
CRISPR
Repeats 37 bp
Spacers 33-36 bp
Anabaena variabilis ATCC 29413 (Ava)
Cyanobacterium aponinum IPPAS B-1201 (Cap)
Filamentous cyanobacterium CCP2 (Fcy)
Nostoc punctiforme PCC 73102 (Npu)
Scytonema hofmanni PCC 7110 (Sho)
tnsB
tnsC
tniQ
cas

FIG. 29

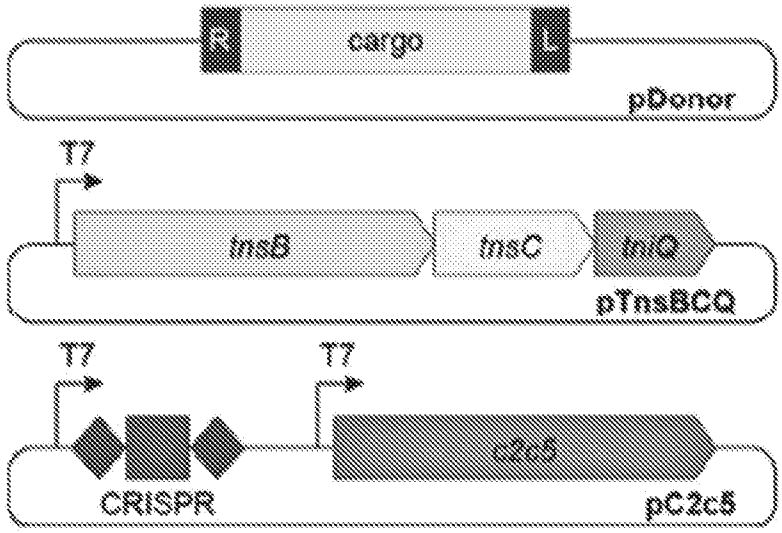


FIG. 30A

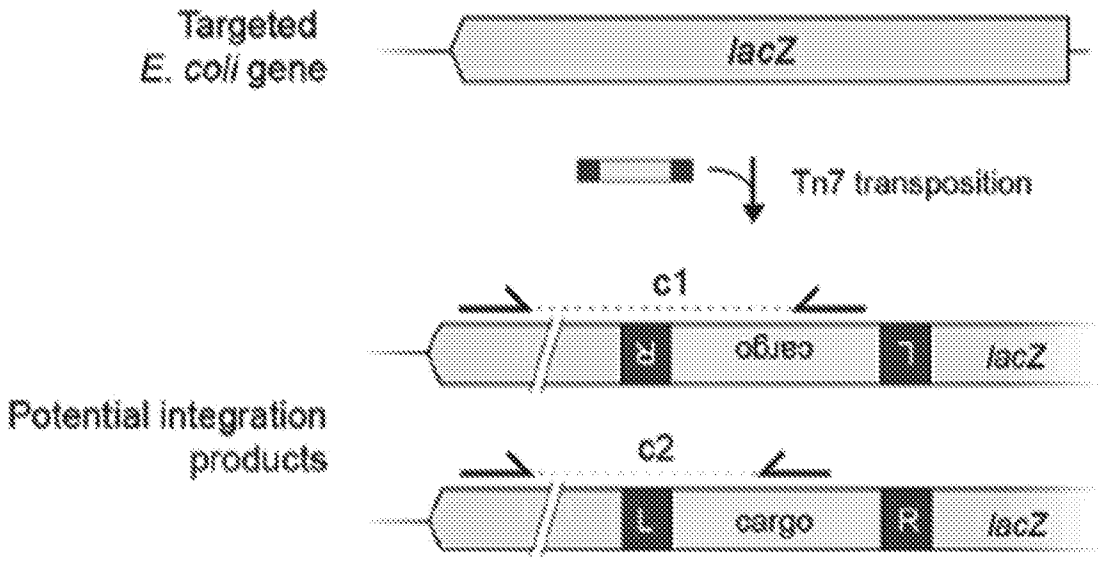


FIG. 30B

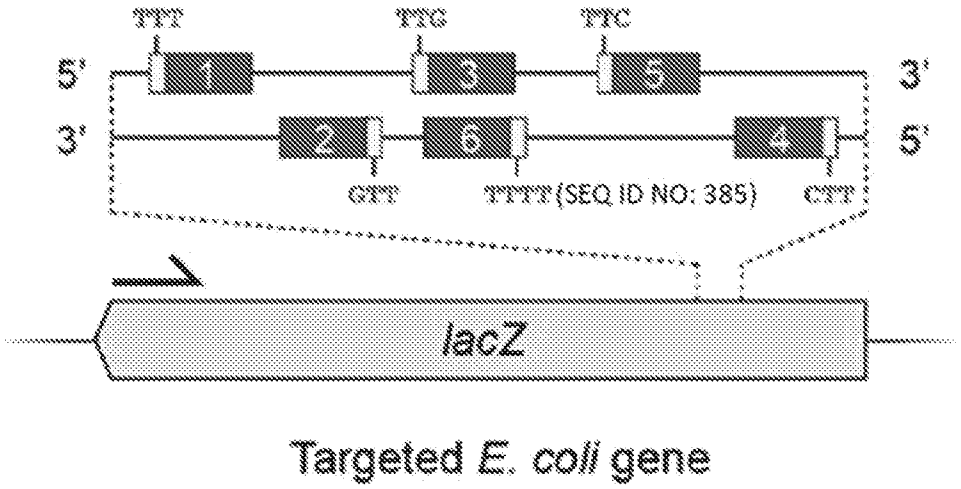


FIG. 31A

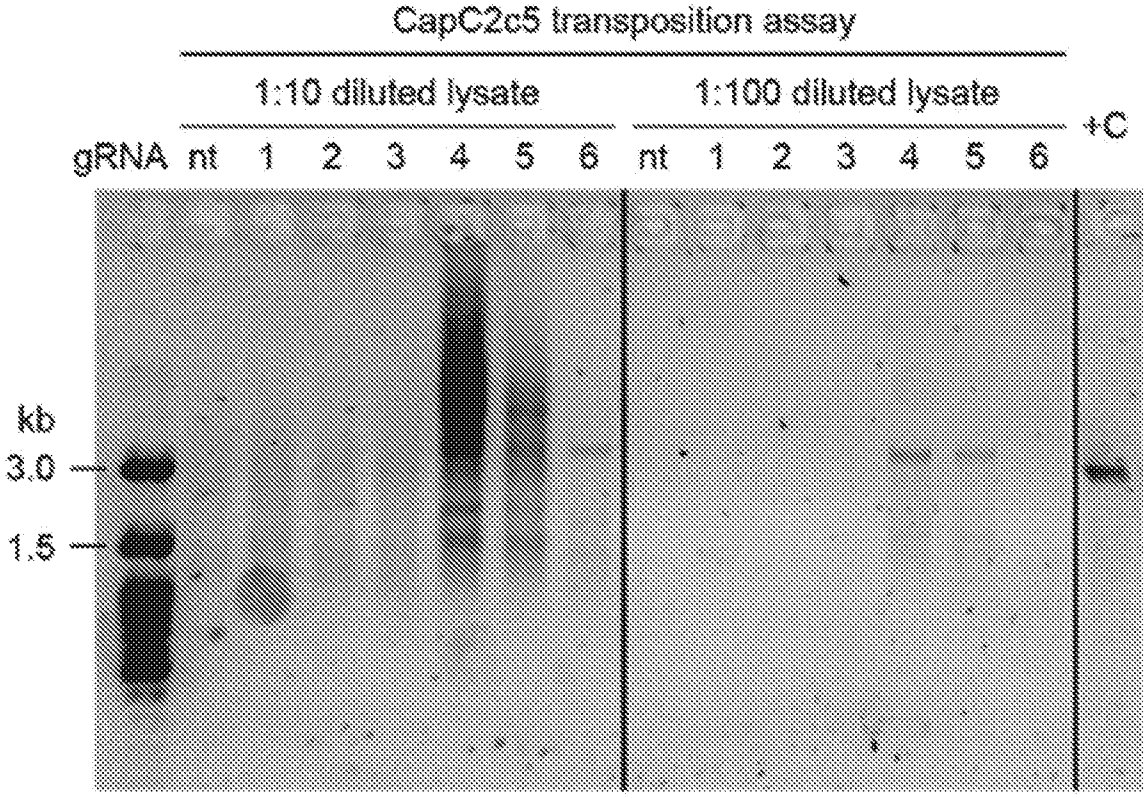


FIG. 31B

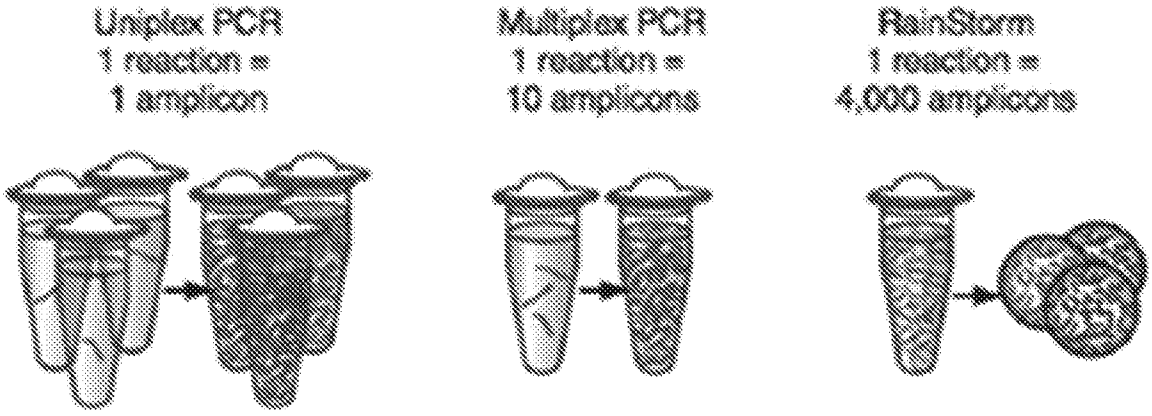


FIG. 32A

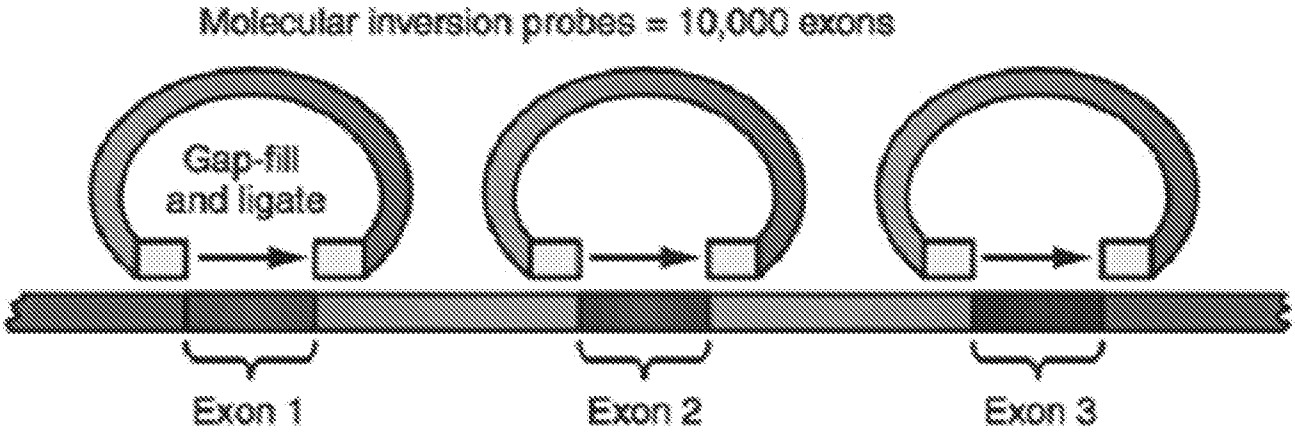


FIG. 32B

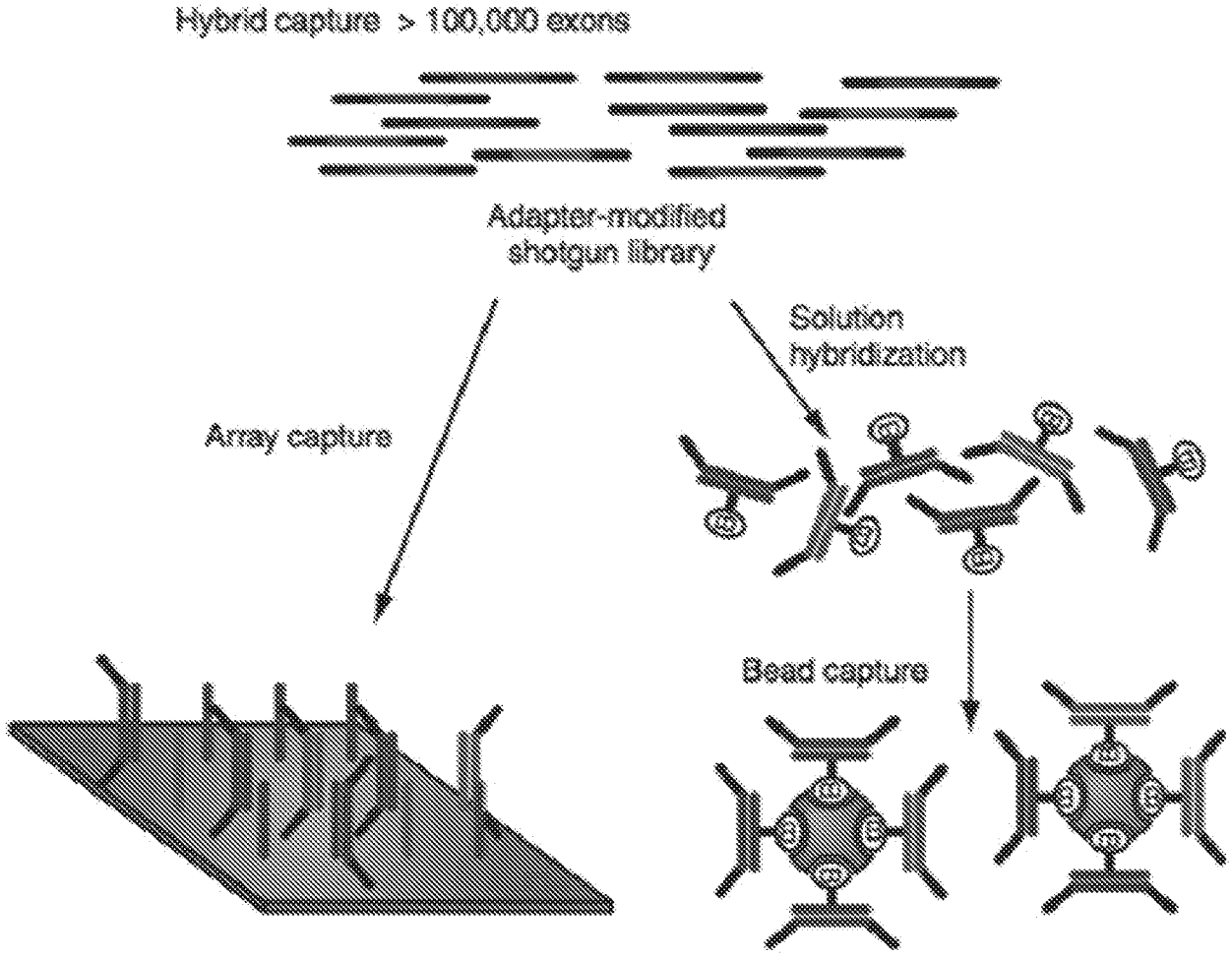

Hybrid capture > 100,000 exons
Adapter-modified shotgun library
Solution hybridization
Array capture
Bead capture

FIG. 32C

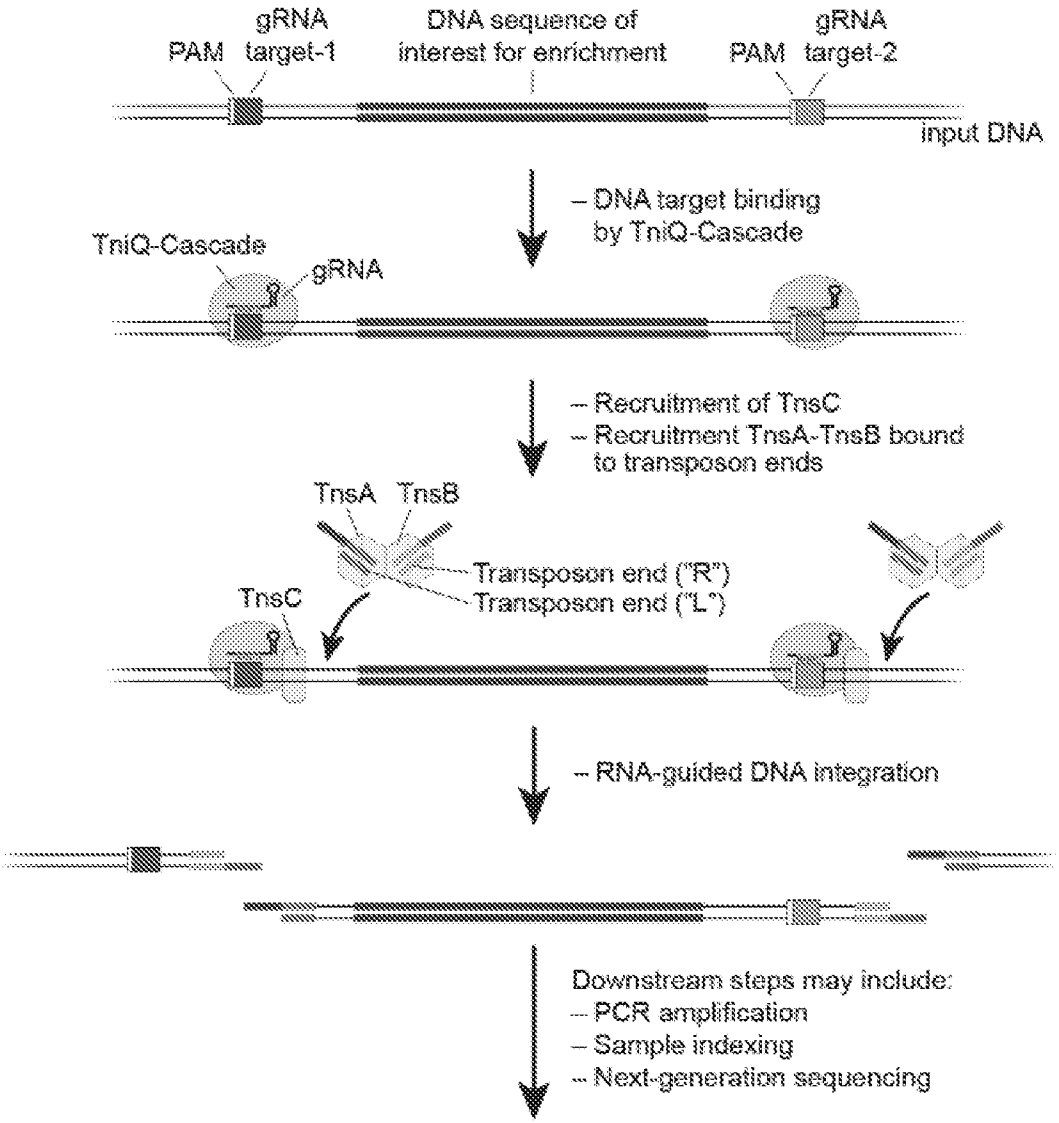


FIG. 33A

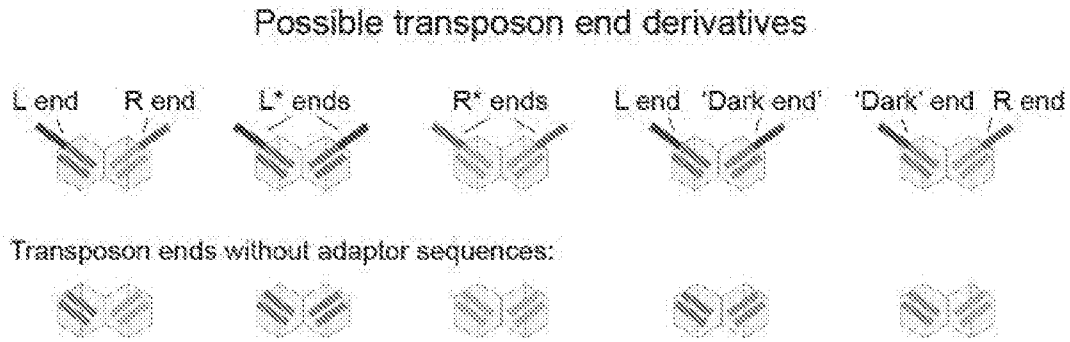


FIG. 33B

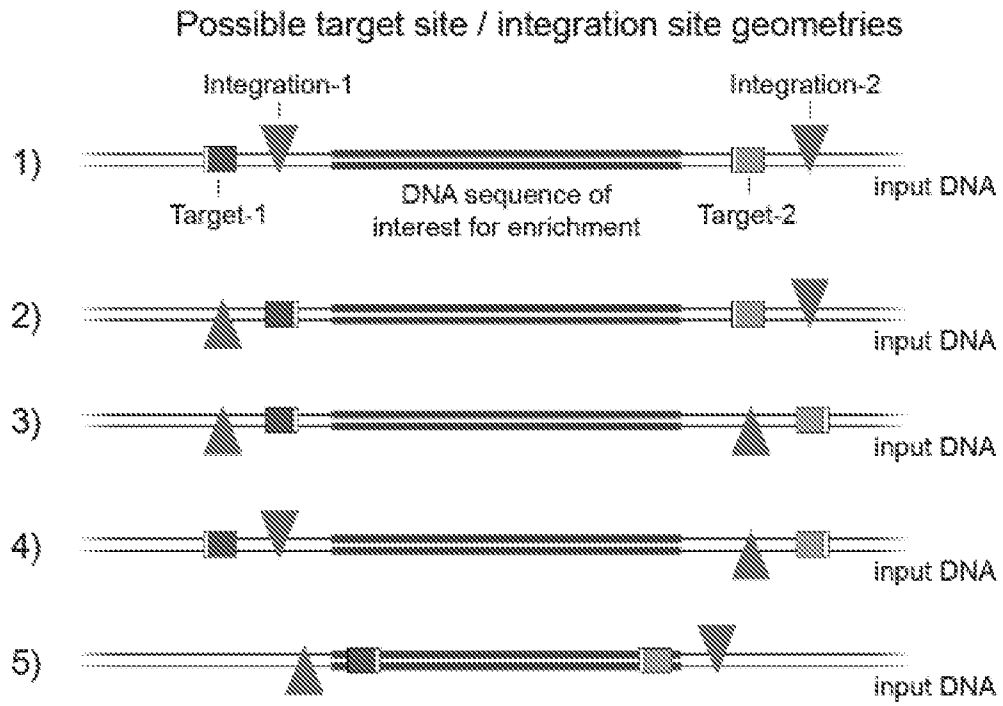


FIG. 33C

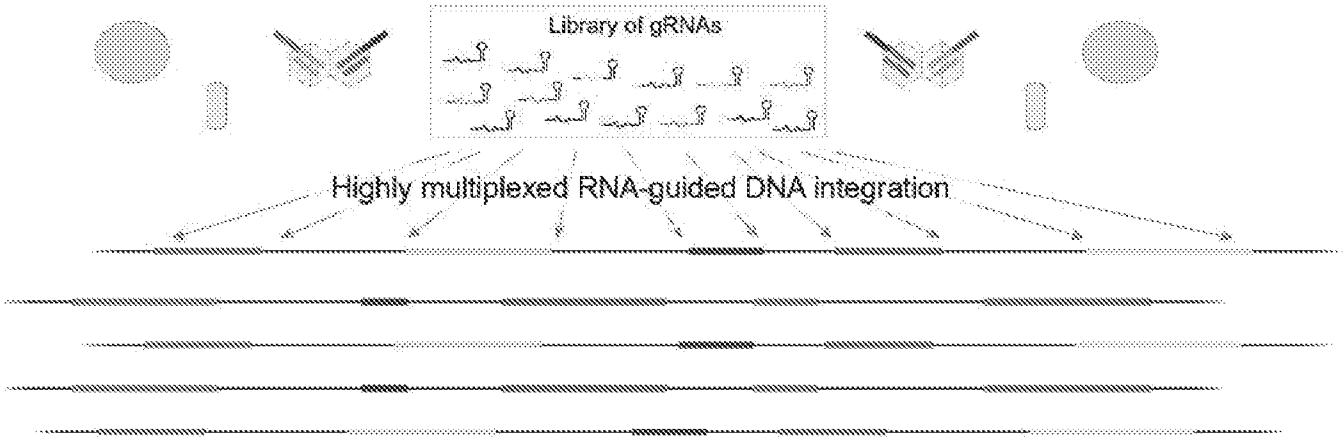


FIG. 33D

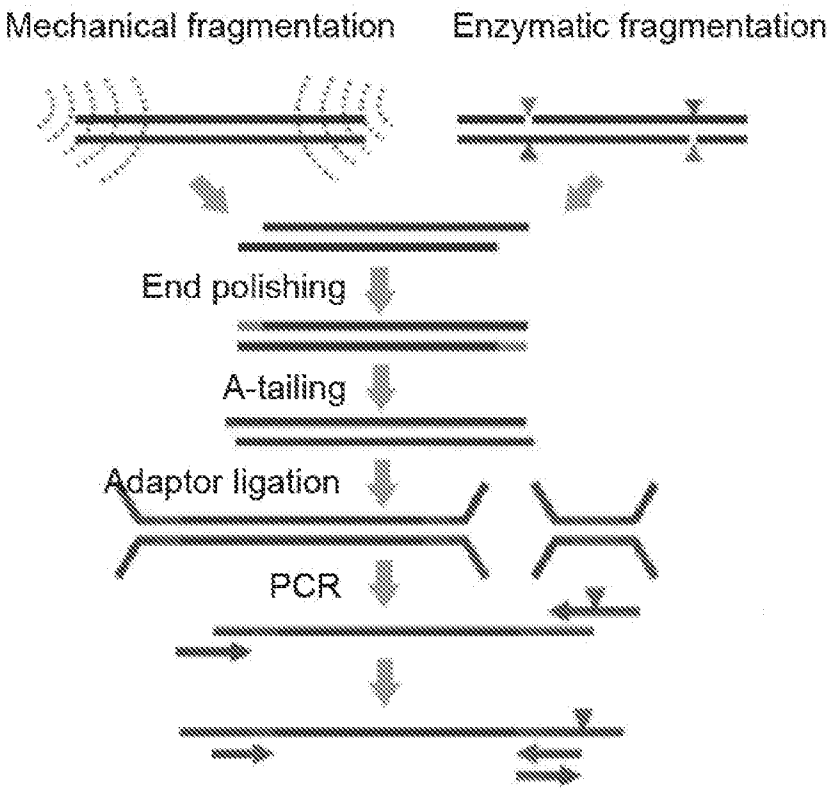


FIG. 34A

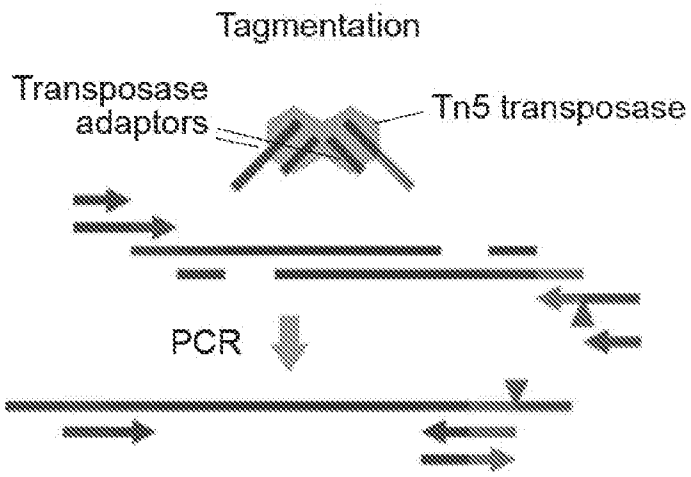


FIG. 34B

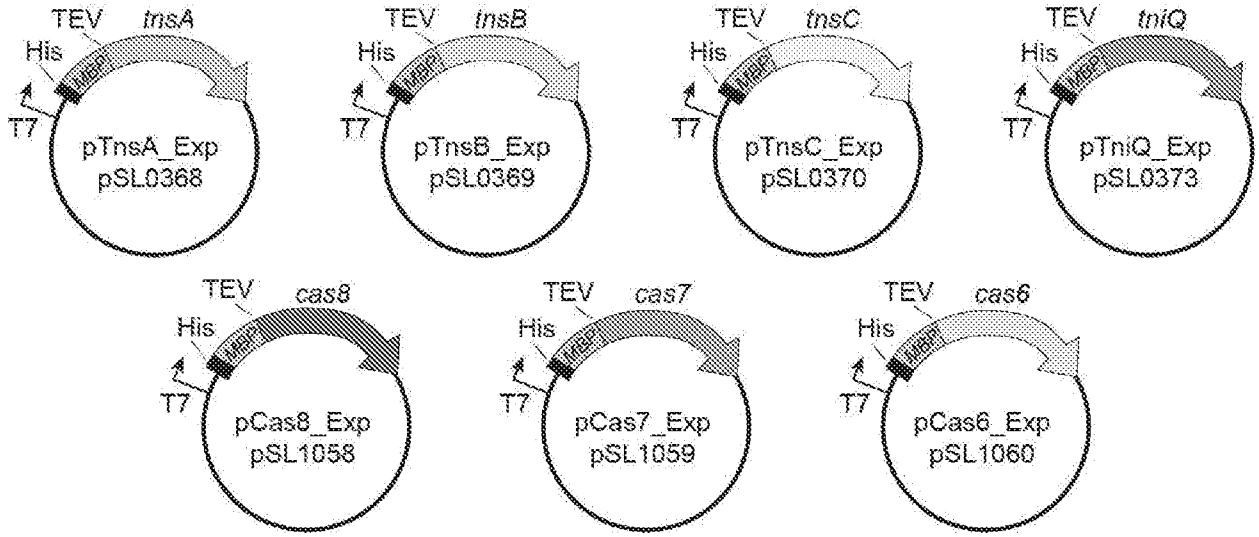


FIG. 35A

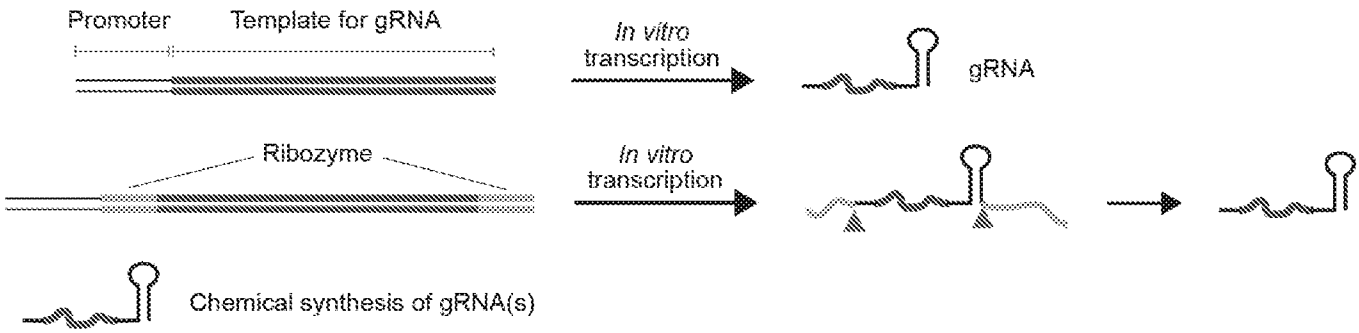


FIG. 35B

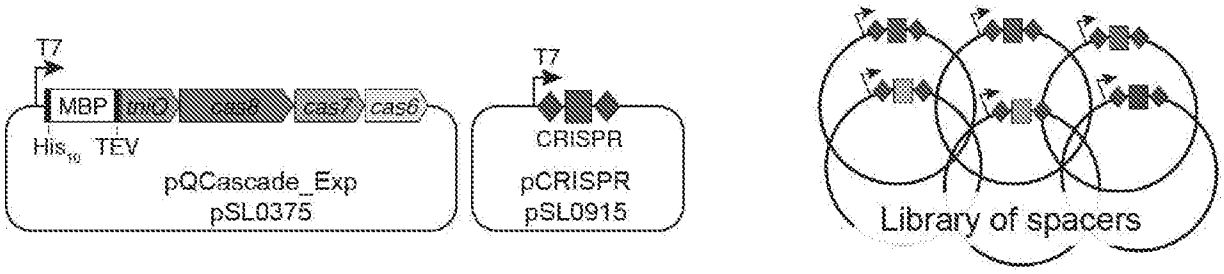


FIG. 35C

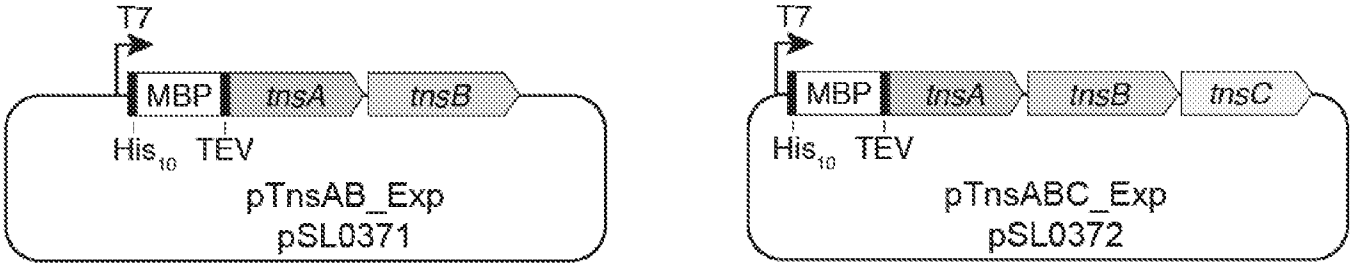


FIG. 35D

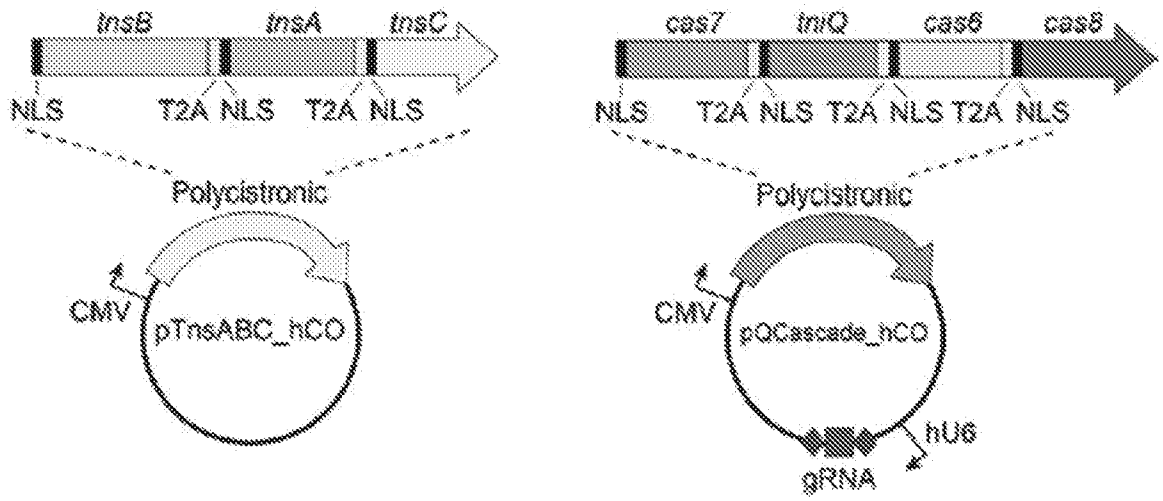


FIG. 35E

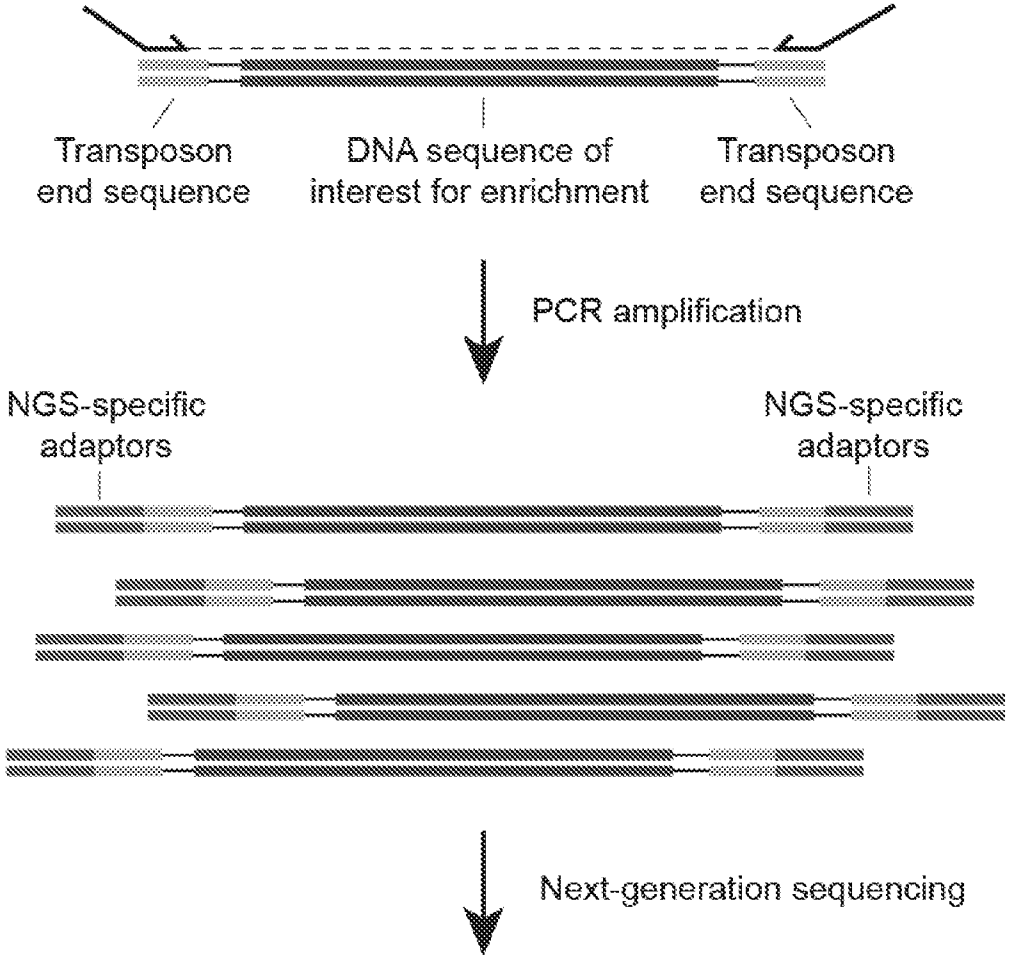

Transposon
end sequence
DNA sequence of
interest for enrichment
Transposon
end sequence
PCR amplification
NGS-specific
adaptors
NGS-specific
adaptors
Next-generation sequencing

FIG. 36

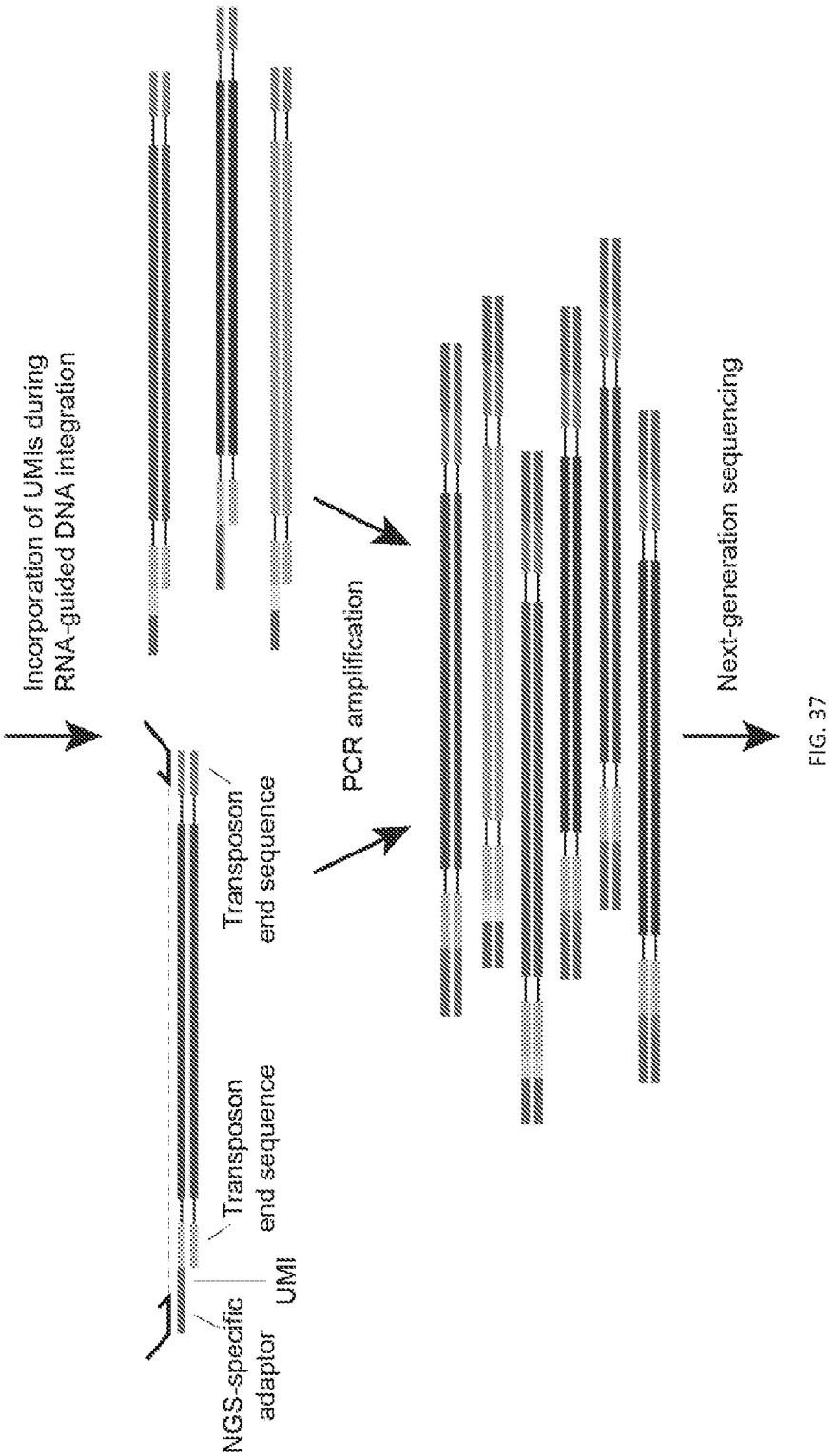

Incorporation of UMIs during
RNA-guided DNA integration
NGS-specific
adaptor
UMI
Transposon
end sequence
Transposon
end sequence
PCR amplification
Next-generation sequencing

FIG. 37

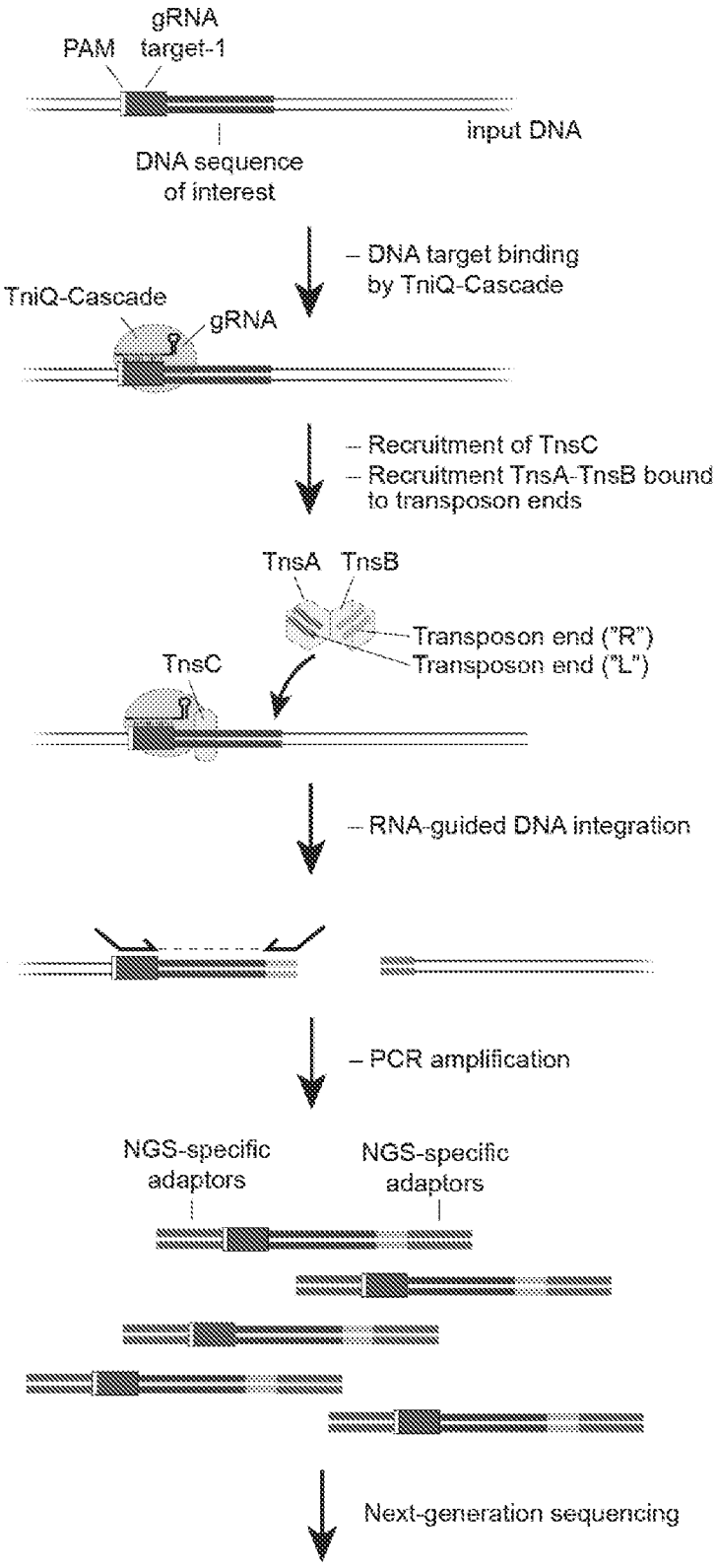

gRNA
PAM
target-1
input DNA
DNA sequence
of interest
– DNA target binding
by TniQ-Cascade
TniQ-Cascade
gRNA
– Recruitment of TnsC
– Recruitment TnsA-TnsB bound
to transposon ends
TnsA
TnsB
Transposon end ("R")
Transposon end ("L")
TnsC
– RNA-guided DNA integration
– PCR amplification
NGS-specific
adaptors
NGS-specific
adaptors
Next-generation sequencing

FIG. 38

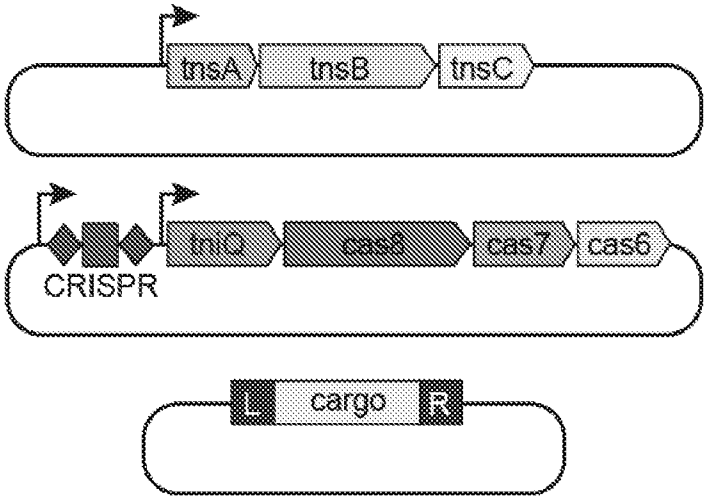


FIG. 39A

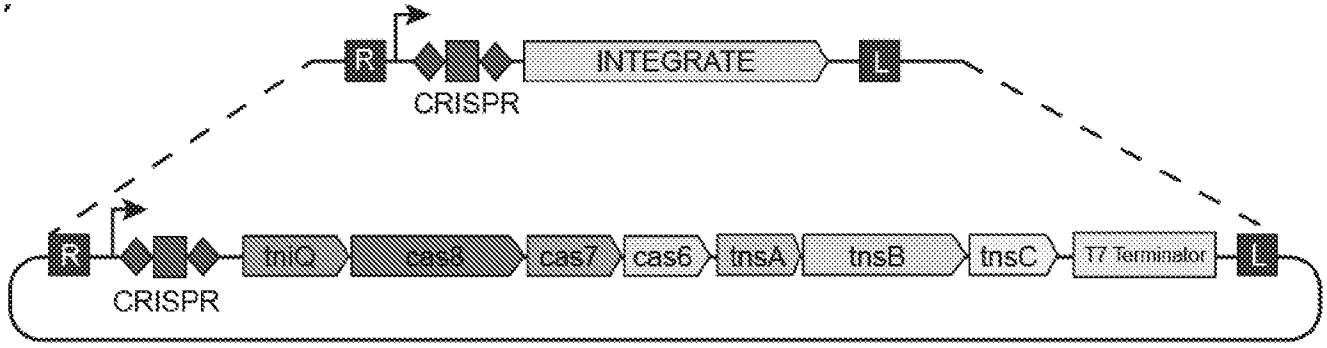


FIG. 39B

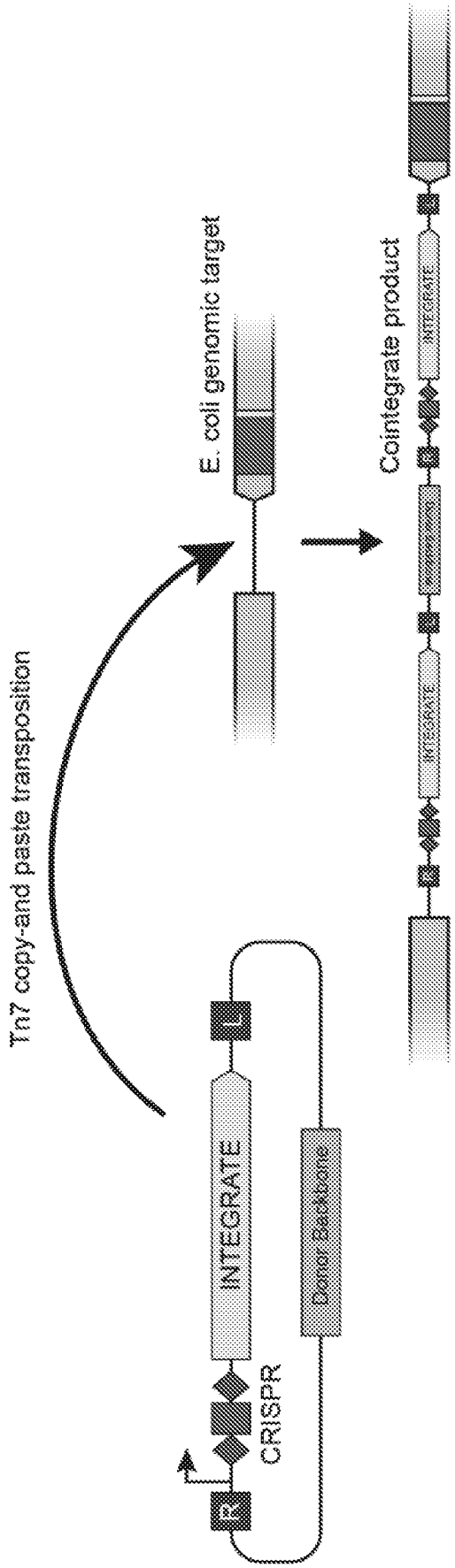

Tn7 copy-and paste transposition
E. coli genomic target
Cointegrate product
Recombination
INTEGRATE
CRISPR
Donor Backbone
R
L

FIG. 40

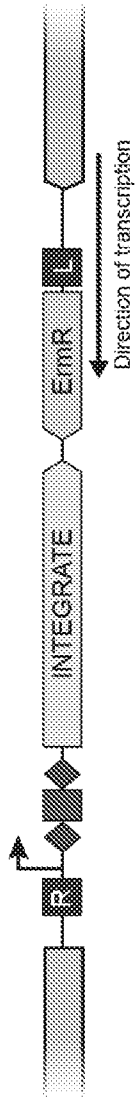

INTEGRATE
EmR
R
L
Direction of transcription

FIG. 41

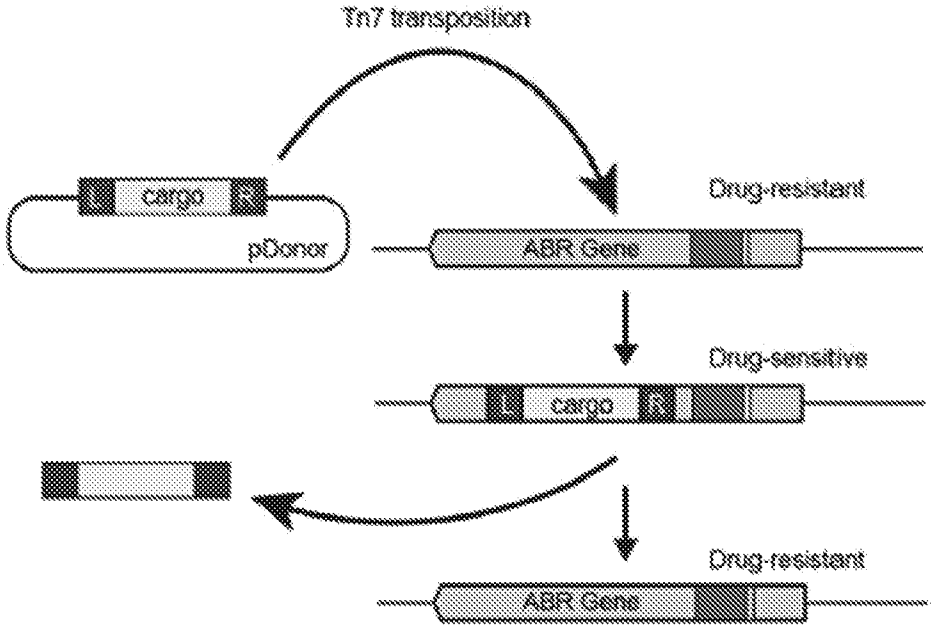

Tn7 transposition
L
cargo
R
pDonor
Drug-resistant
ABR Gene
Drug-sensitive
L
cargo
R
Drug-resistant
ABR Gene

FIG. 42

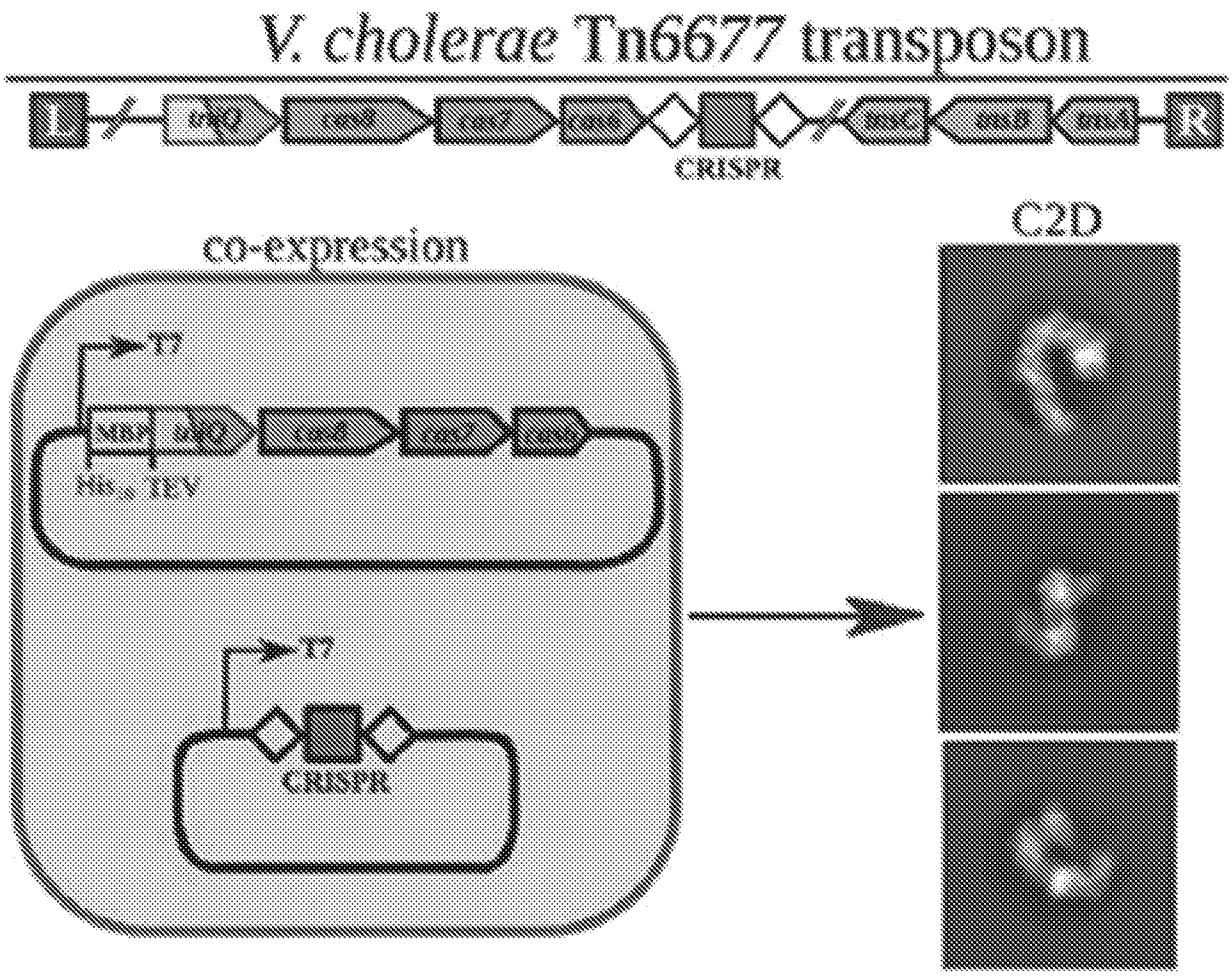

V. cholerae Tn6677 transposon
L
R
CRISPR
co-expression
T7
T7
CRISPR
C2D

FIG. 43A

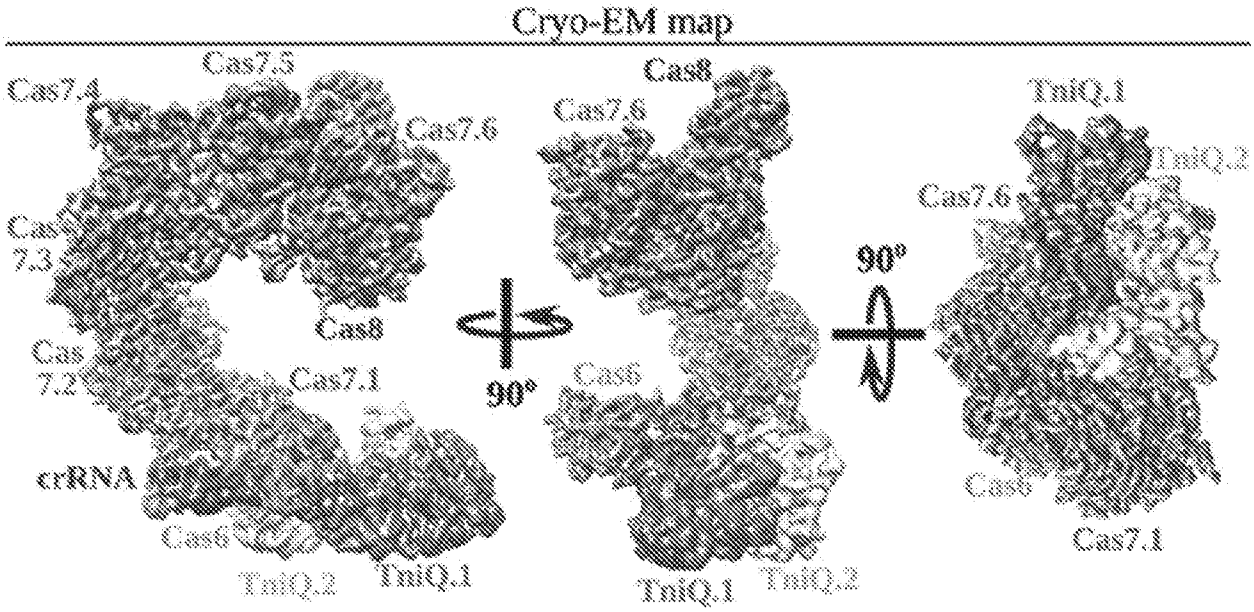


FIG. 43B

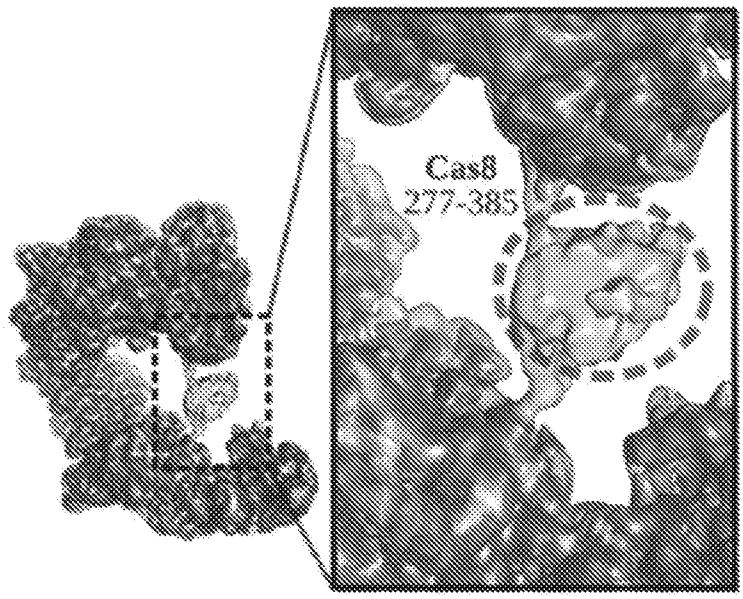


FIG. 43C

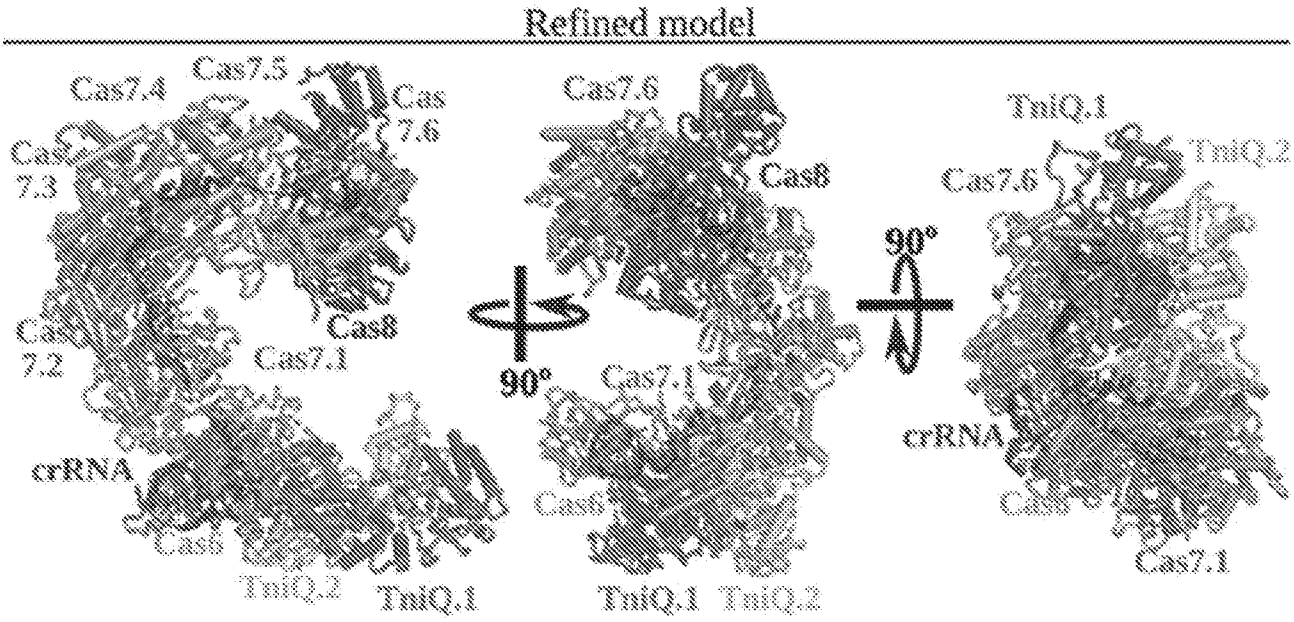


FIG. 43D

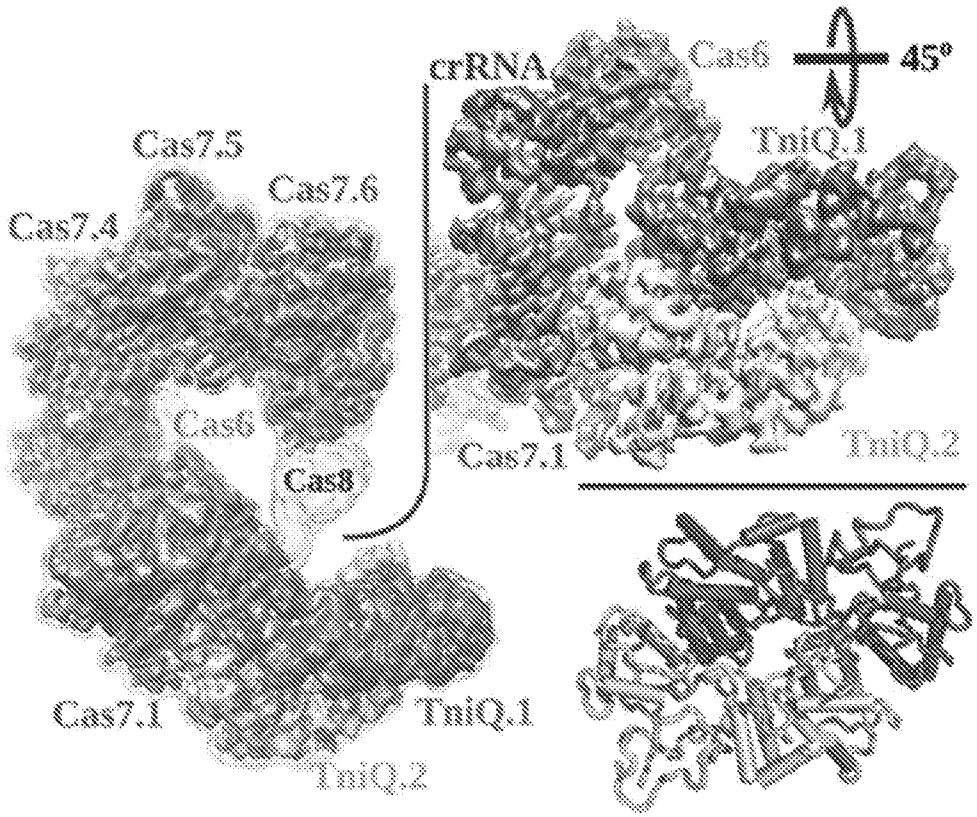


FIG. 44A

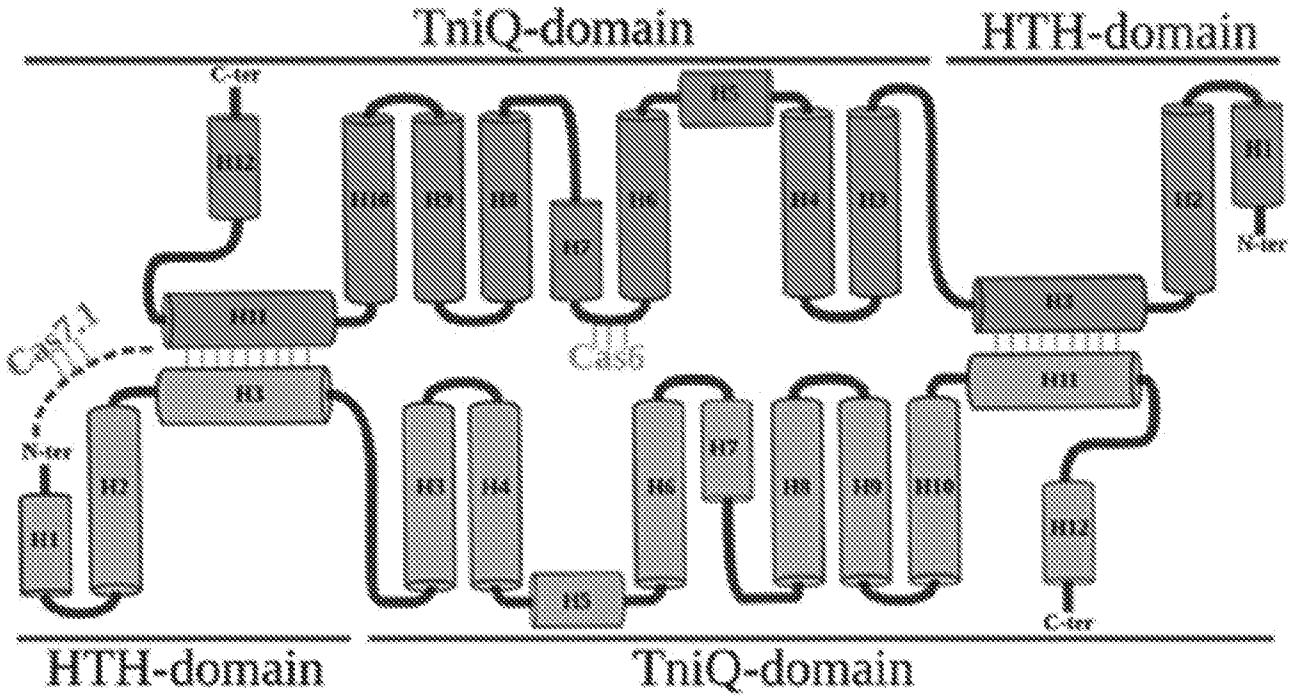


FIG. 44B

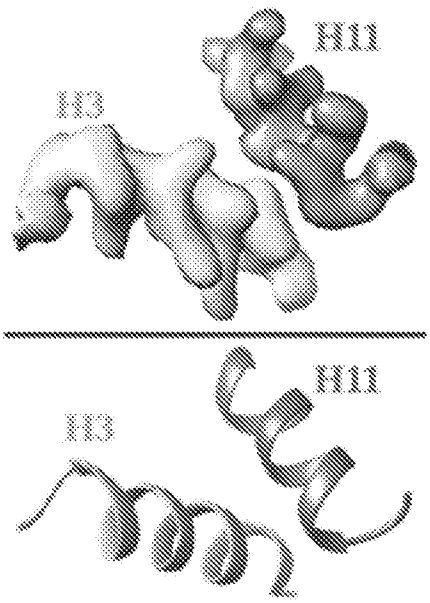


FIG. 44C

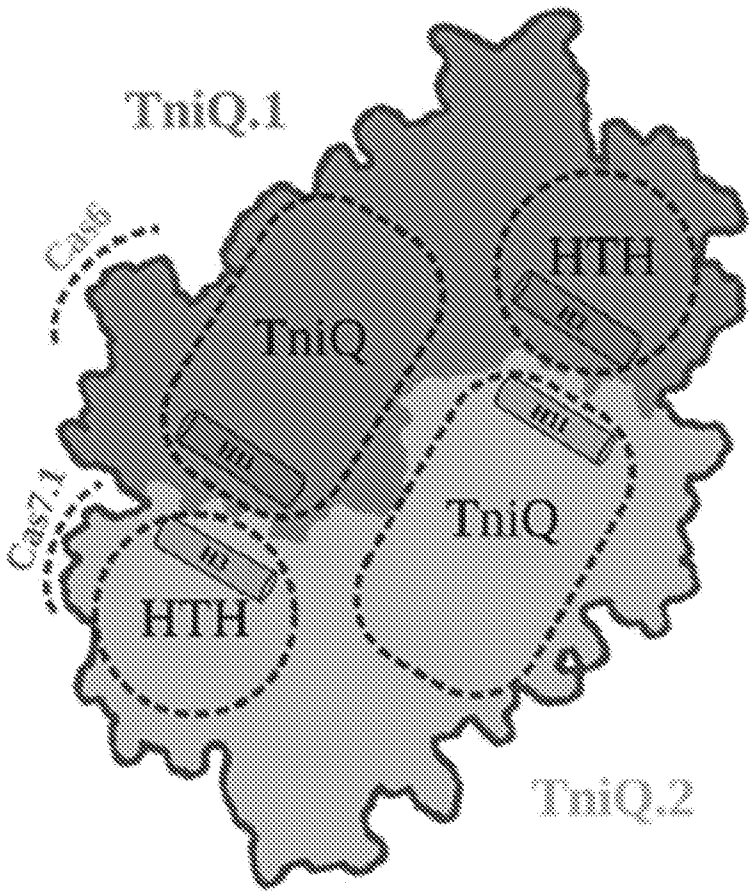


FIG. 44D

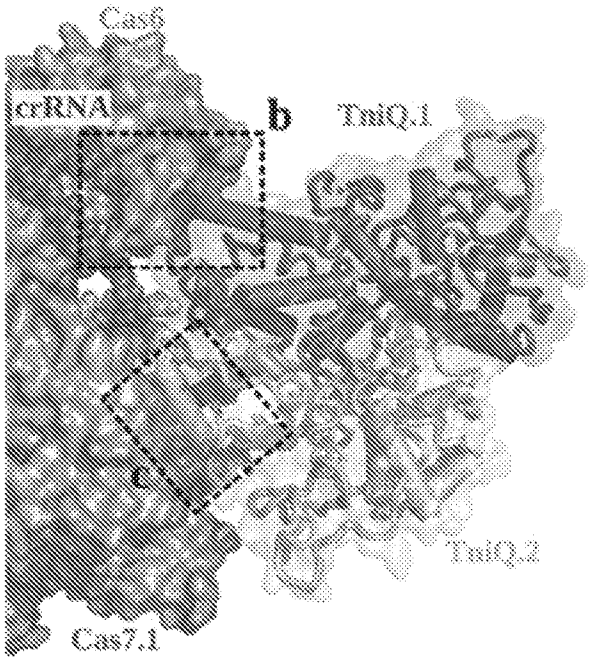


FIG. 45A

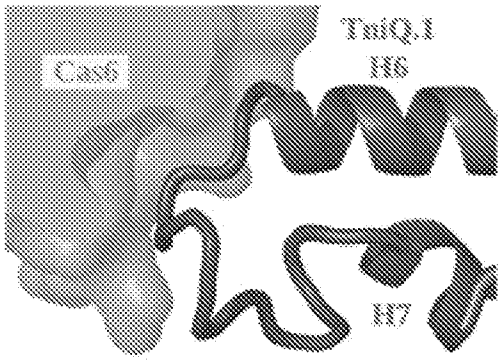


FIG. 45B

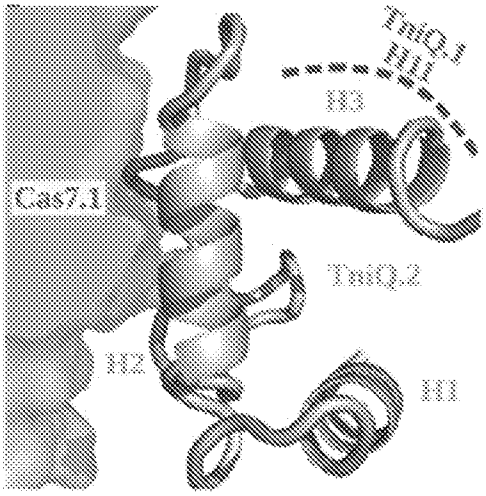


FIG. 45C

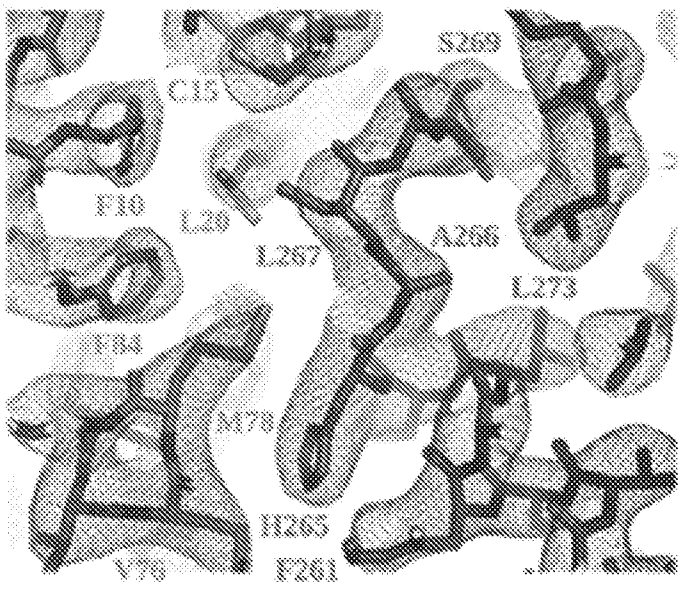


FIG. 45D

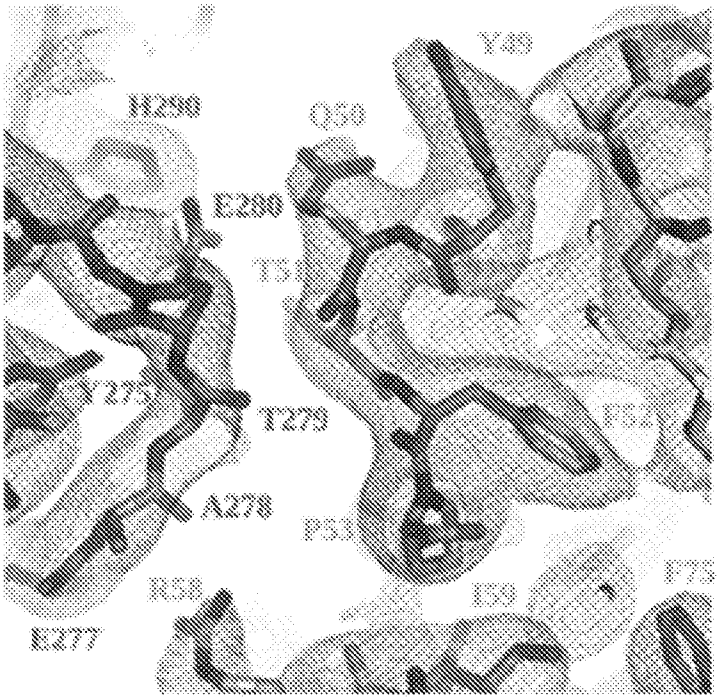


FIG. 45E

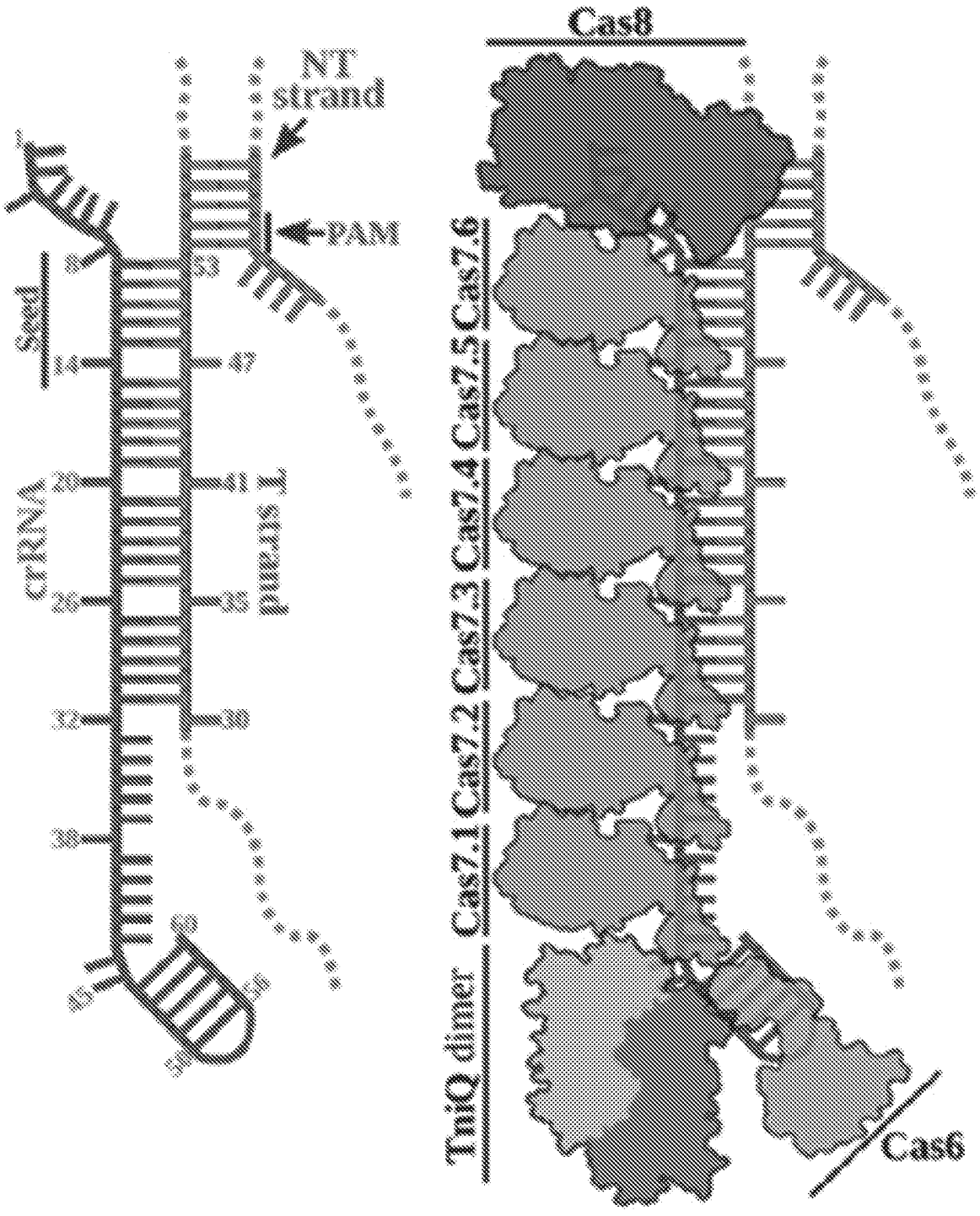

Cas8
NT strand
PAM
Seed
PAM
crRNA
T strand
TniQ dimer
Cas7.1
Cas7.2
Cas7.3
Cas7.4
Cas7.5
Cas7.6
Cas6

FIG. 46A

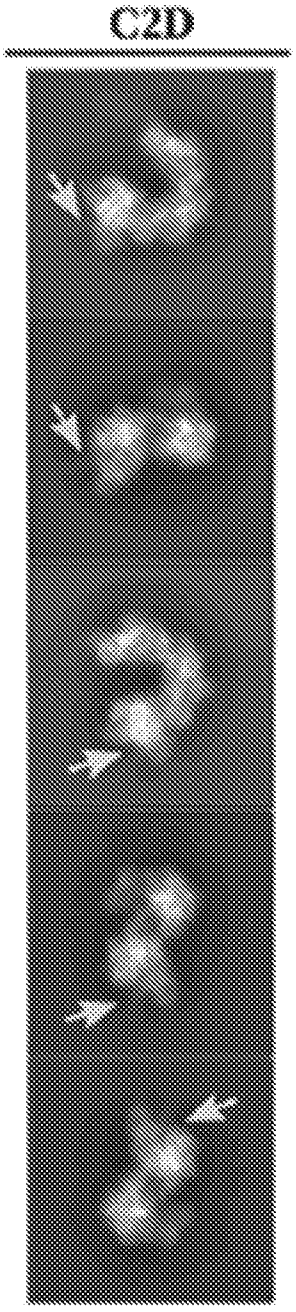


FIG. 46B

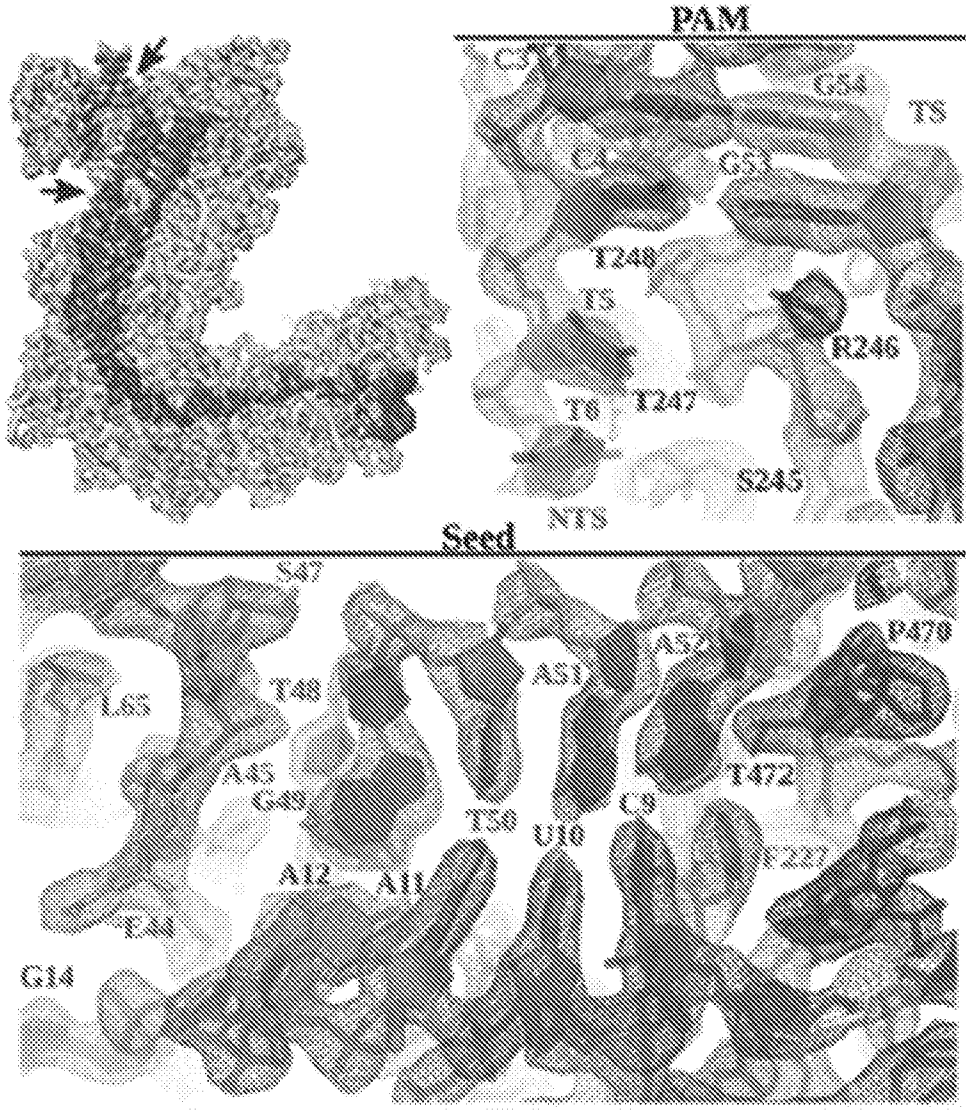


FIG. 46C

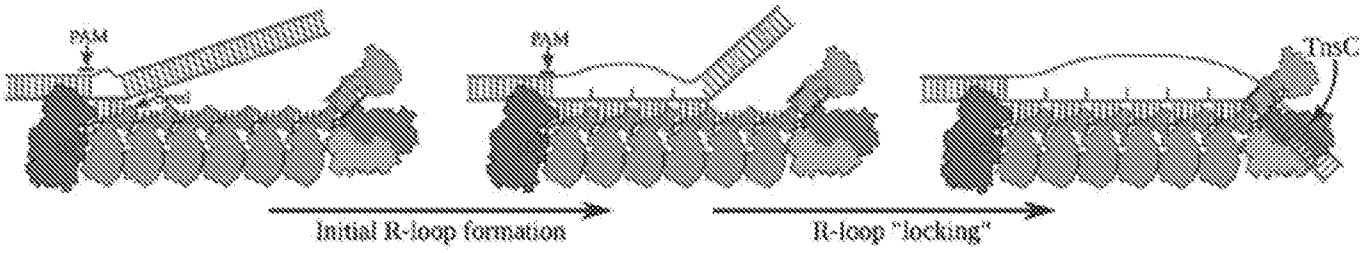


FIG. 46D

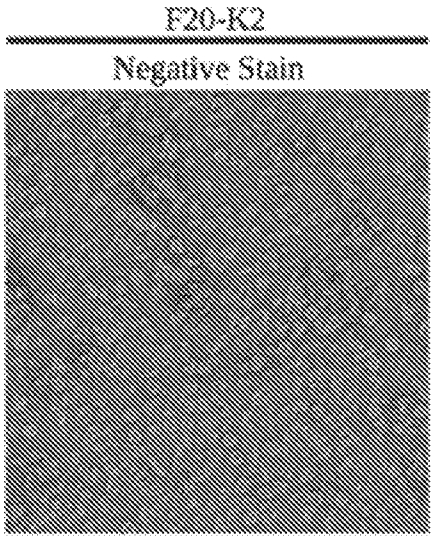


FIG. 47A

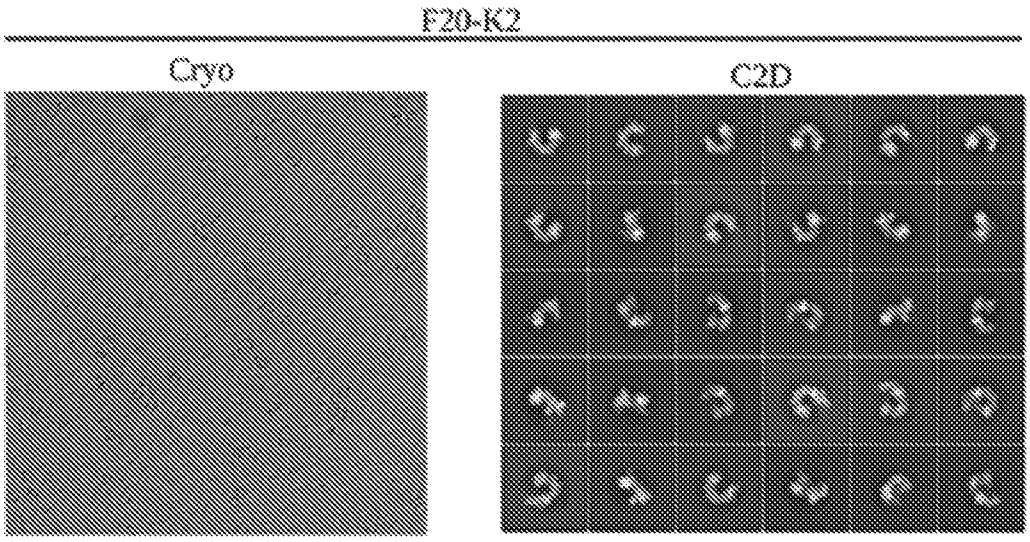


FIG. 47B

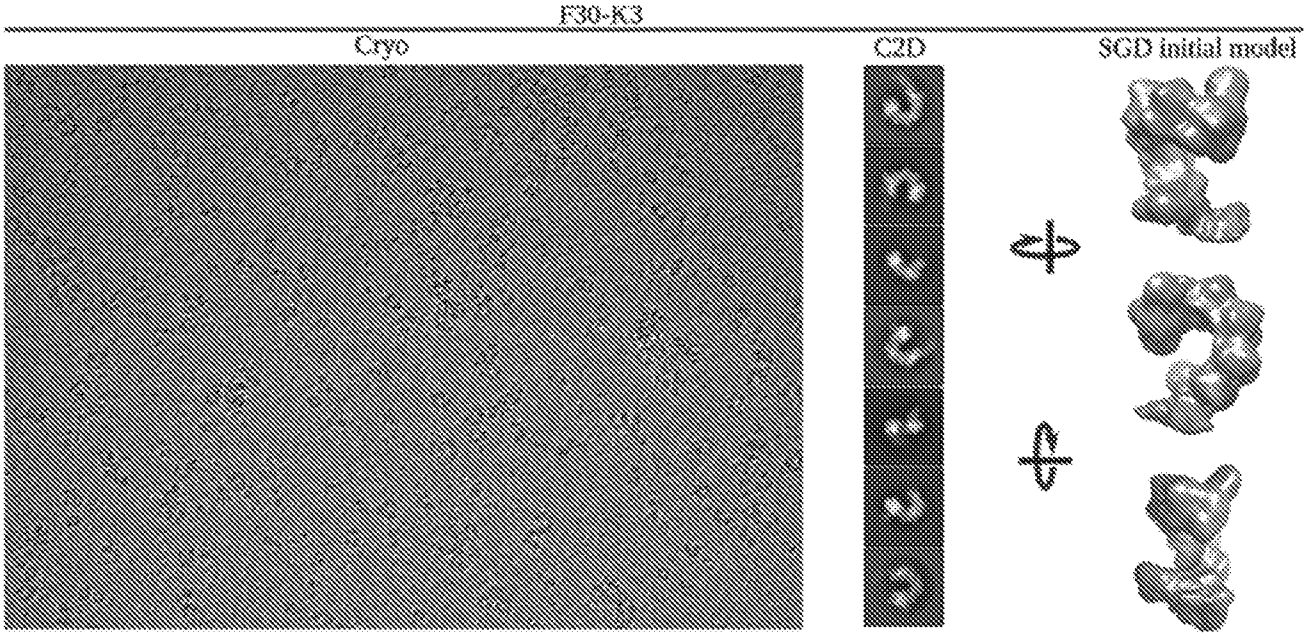


FIG. 47C

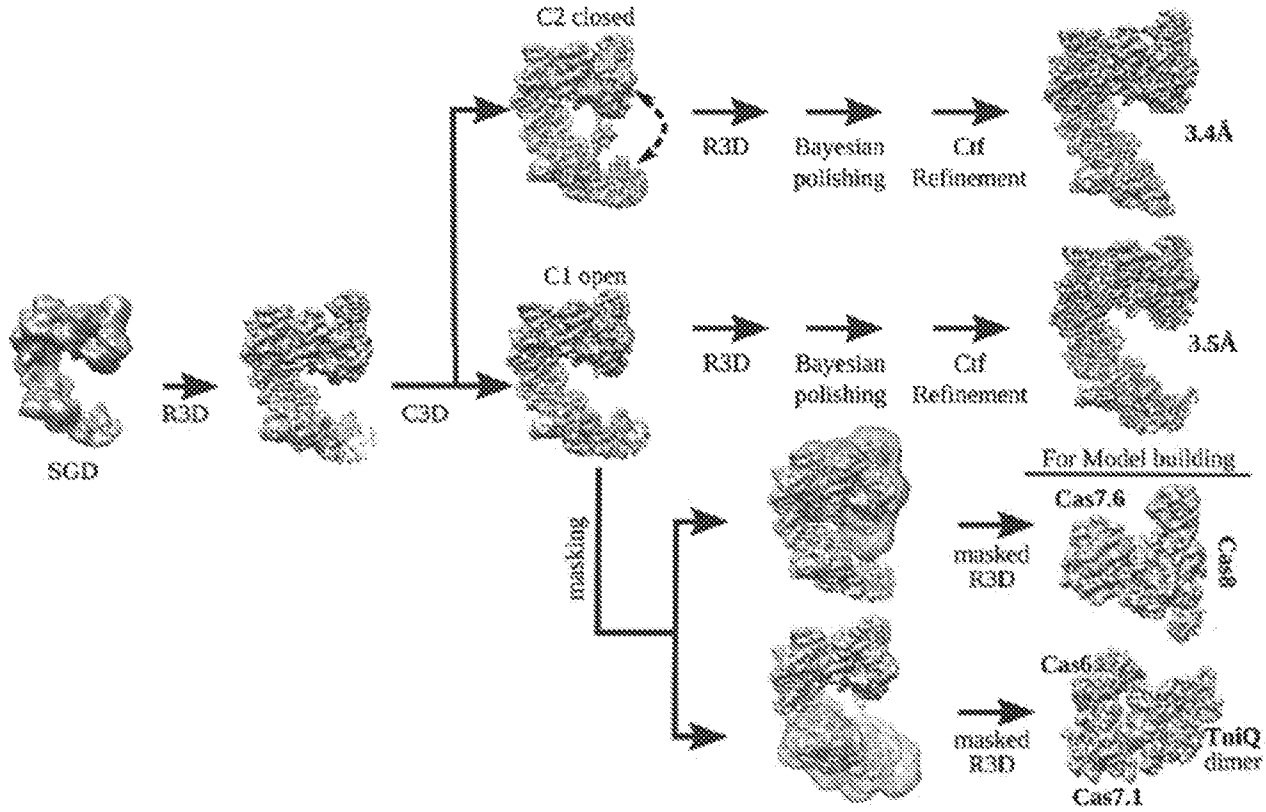


FIG. 47D

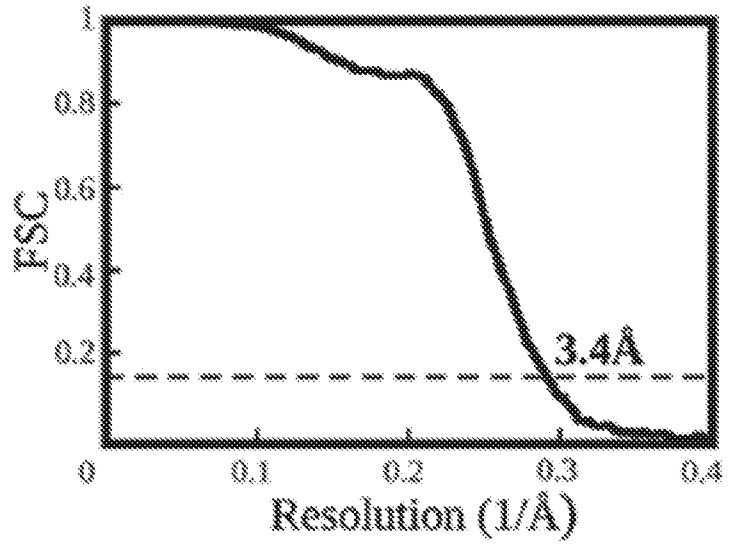


FIG. 48A

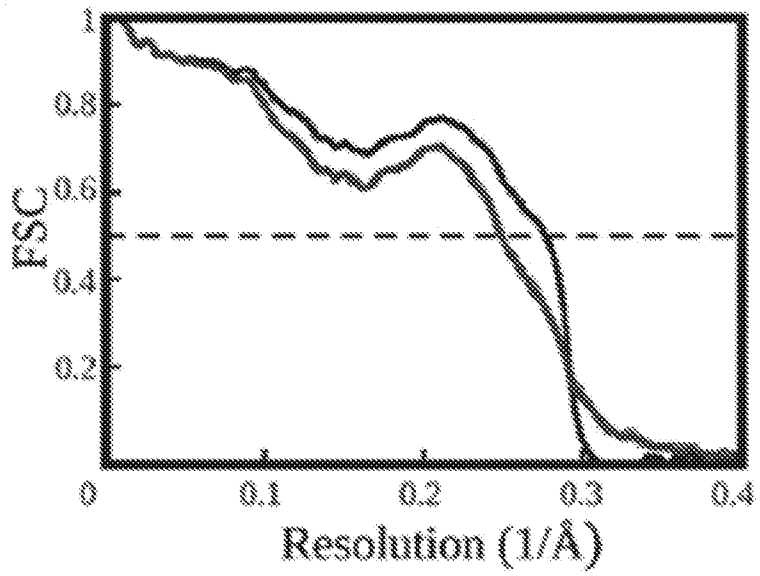


FIG. 48B

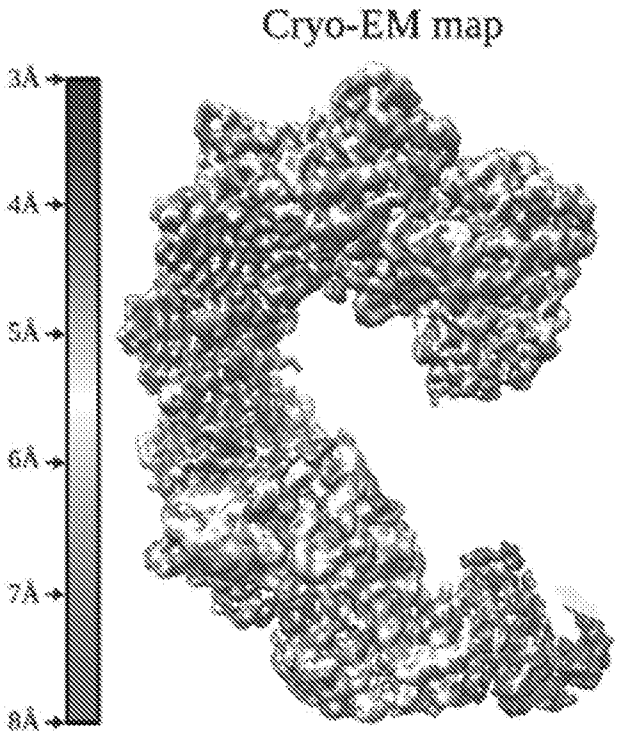


FIG. 48C

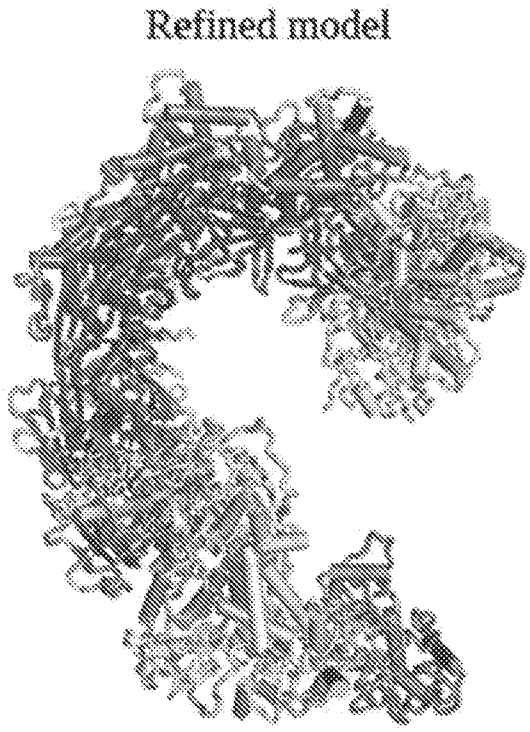


FIG. 48D

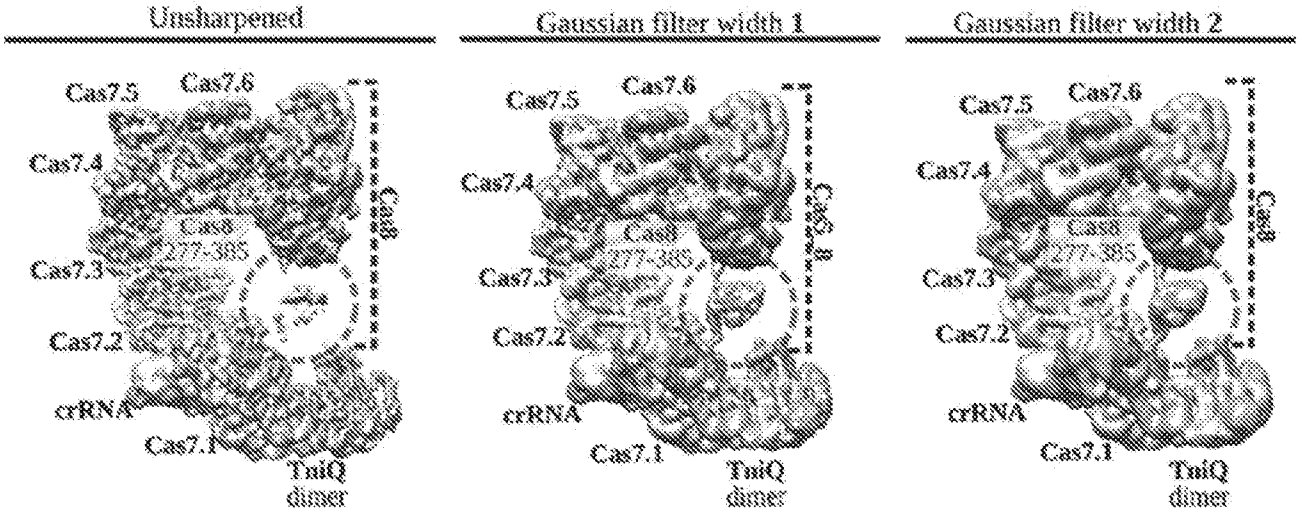


FIG. 48E

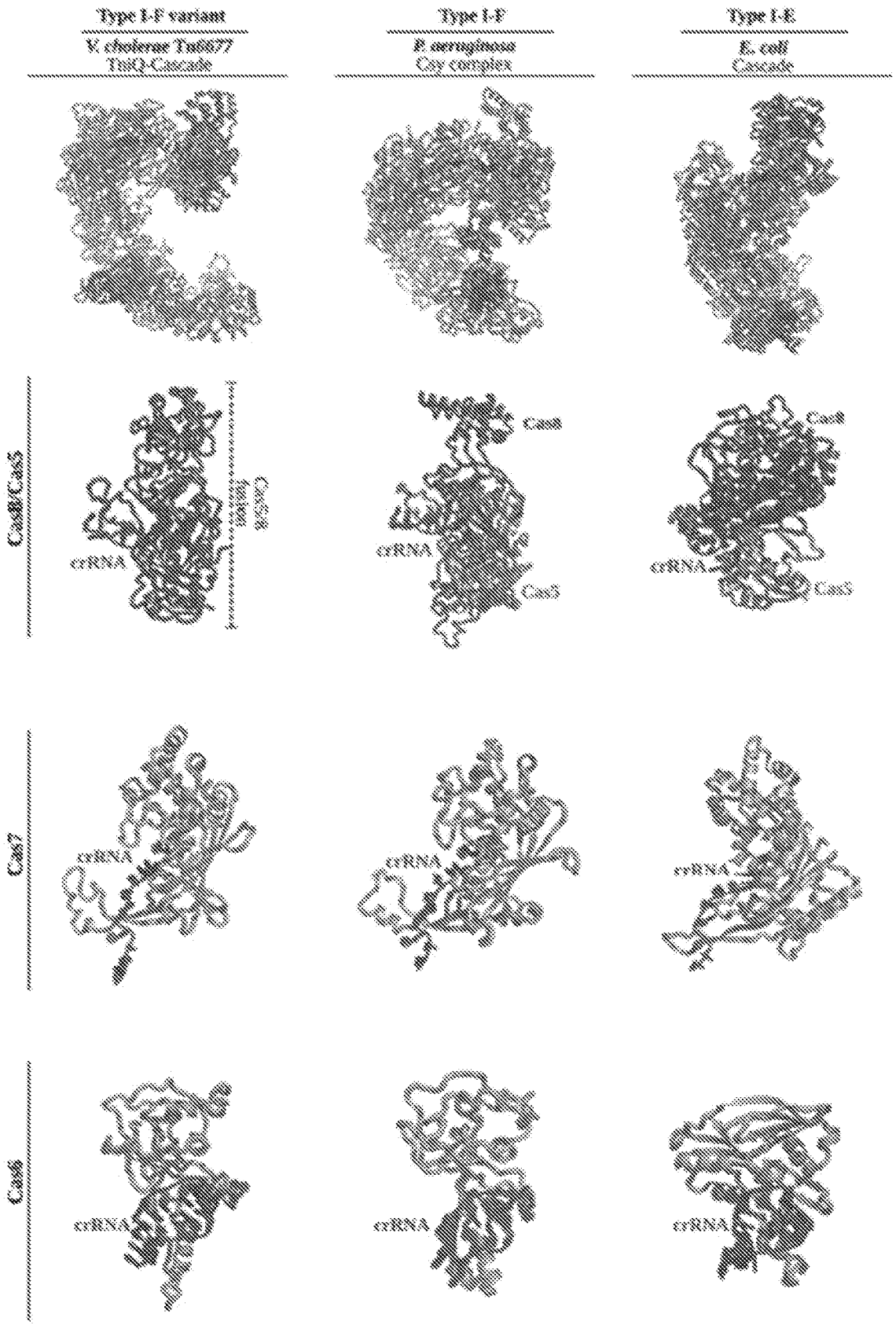

Type I-F variant
V. cholerae Tn6677
TniQ-Cascade
Type I-F
P. aeruginosa
Csy complex
Type I-E
E. coli
Cascade
Cas8/Cas5
Cas8
Cas5
Cas7
Cas6
crRNA

FIG. 49

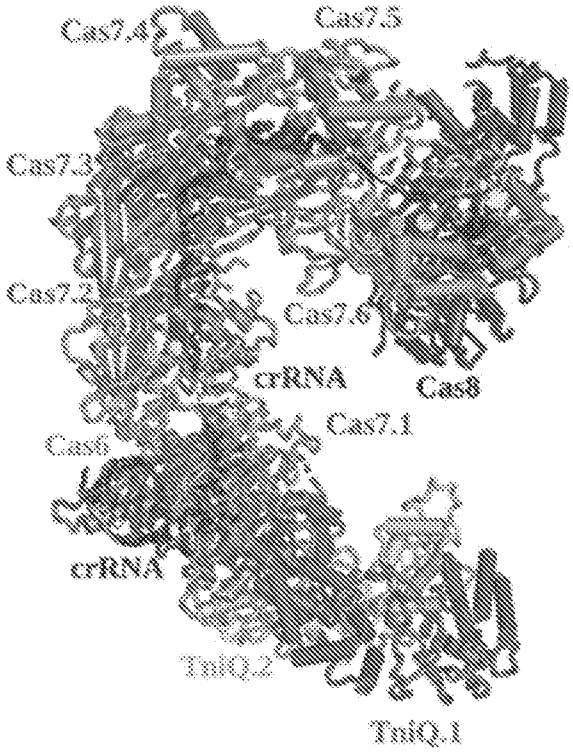


FIG. 50A

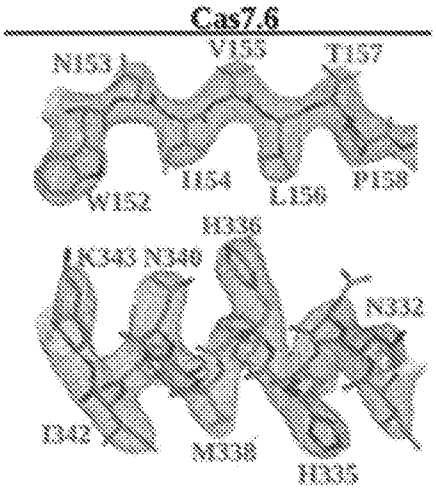


FIG. 50B

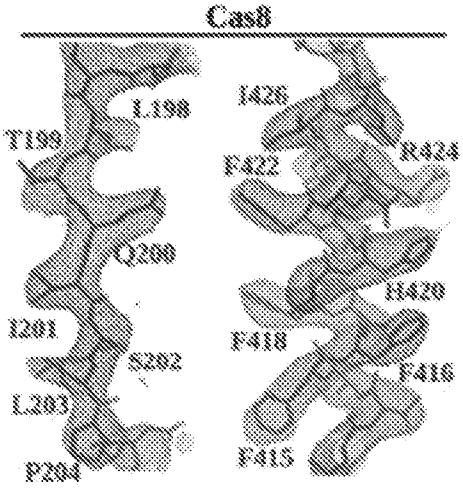


FIG. 50C

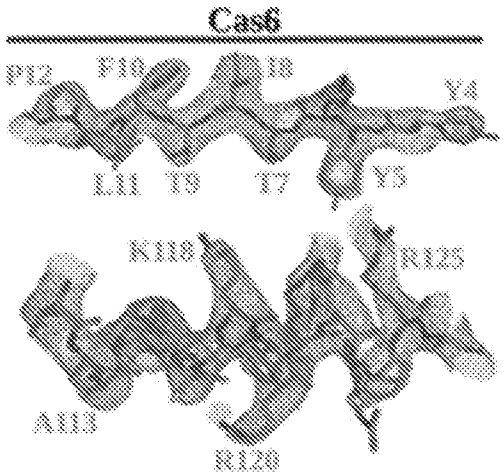


FIG. 50D

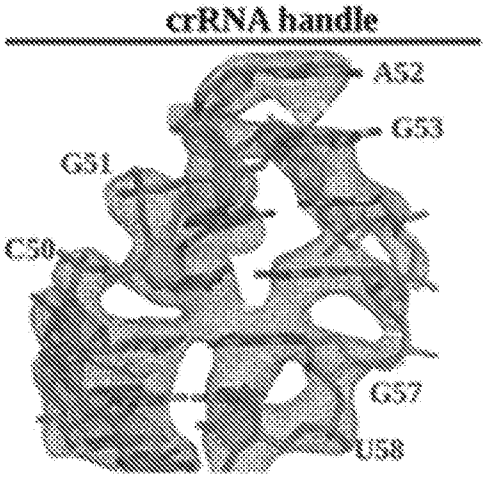


FIG. 50E

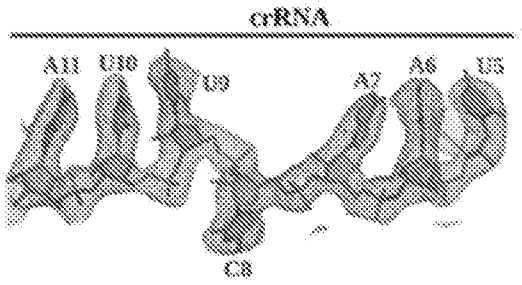


FIG. 50F

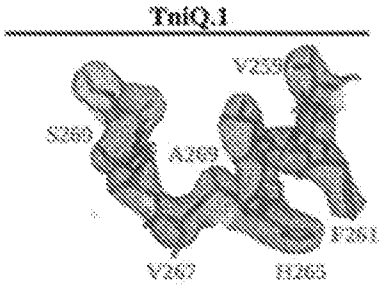


FIG. 50G

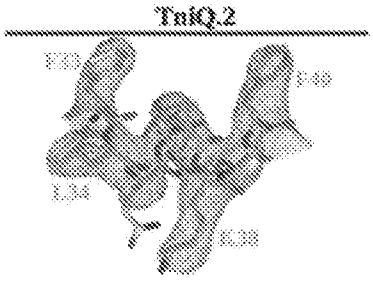


FIG. 50H

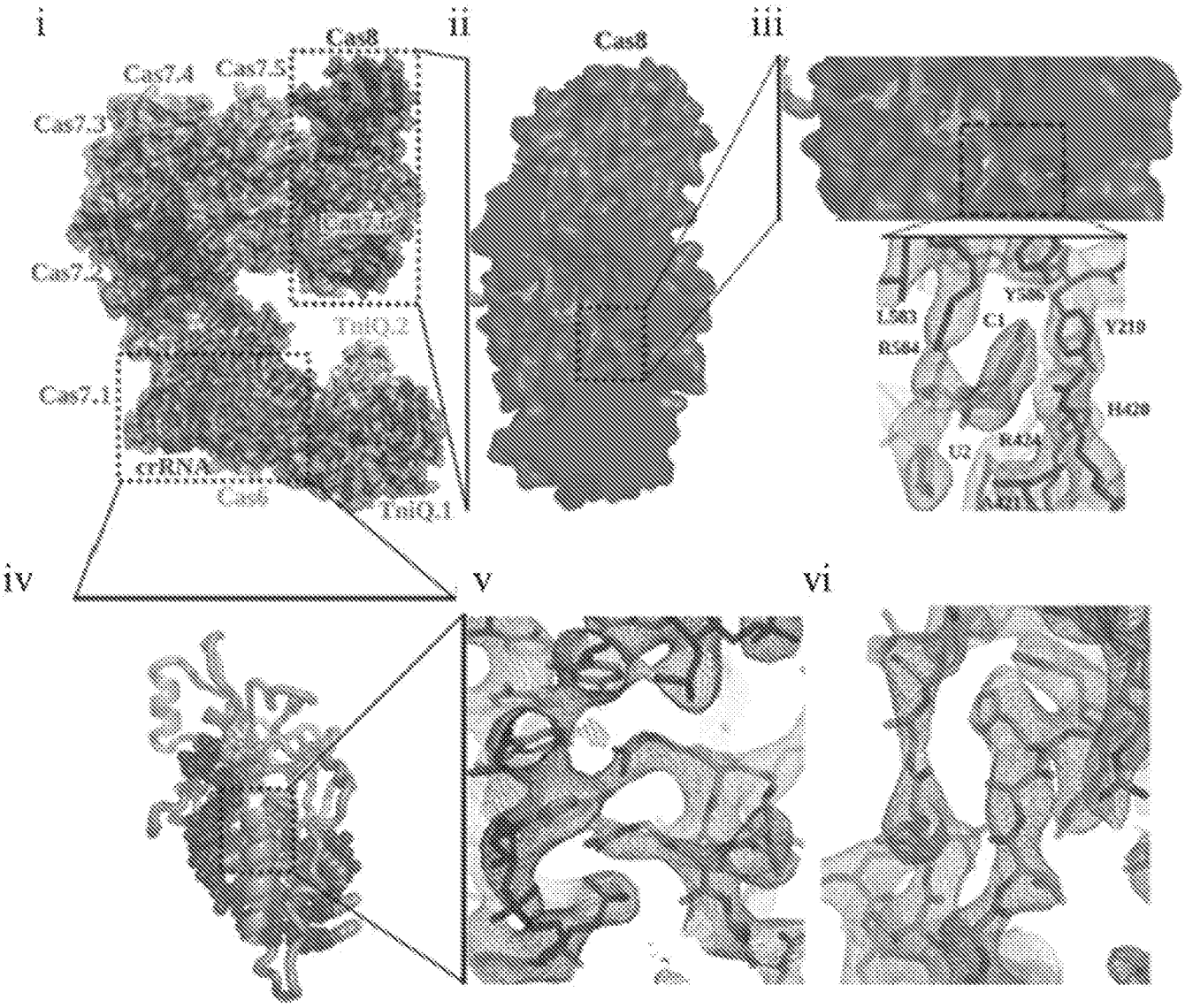

i
ii
iii
iv
v
vi
Cas8
Cas7.4
Cas7.5
Cas7.3
Cas7.2
Cas7.1
TniQ.2
TniQ.1
crRNA
Cas6
Cas8
Y386
L383
C1
Y219
R364
R436
C2
R433
A431

FIG. 51

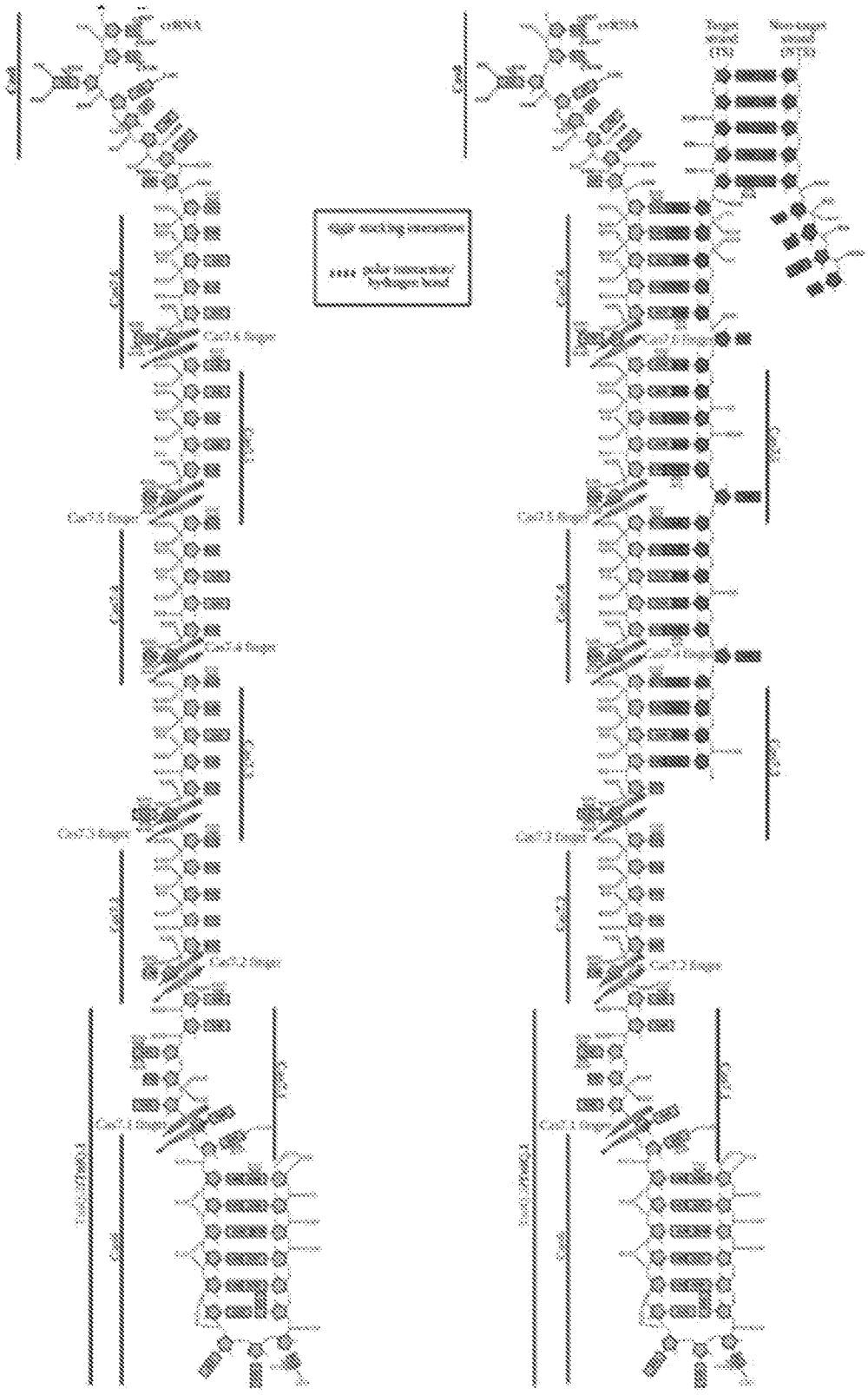


FIG. 52A

FIG. 52B

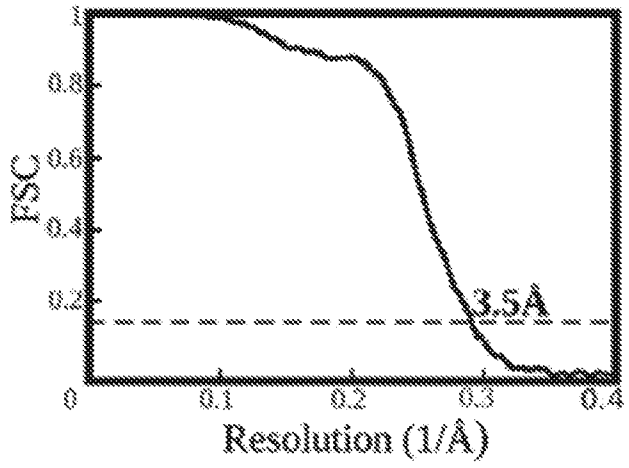


FIG. 53A

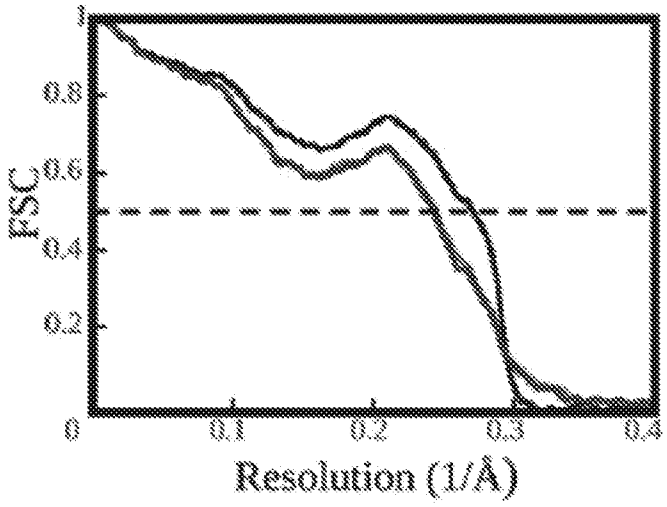


FIG. 53B

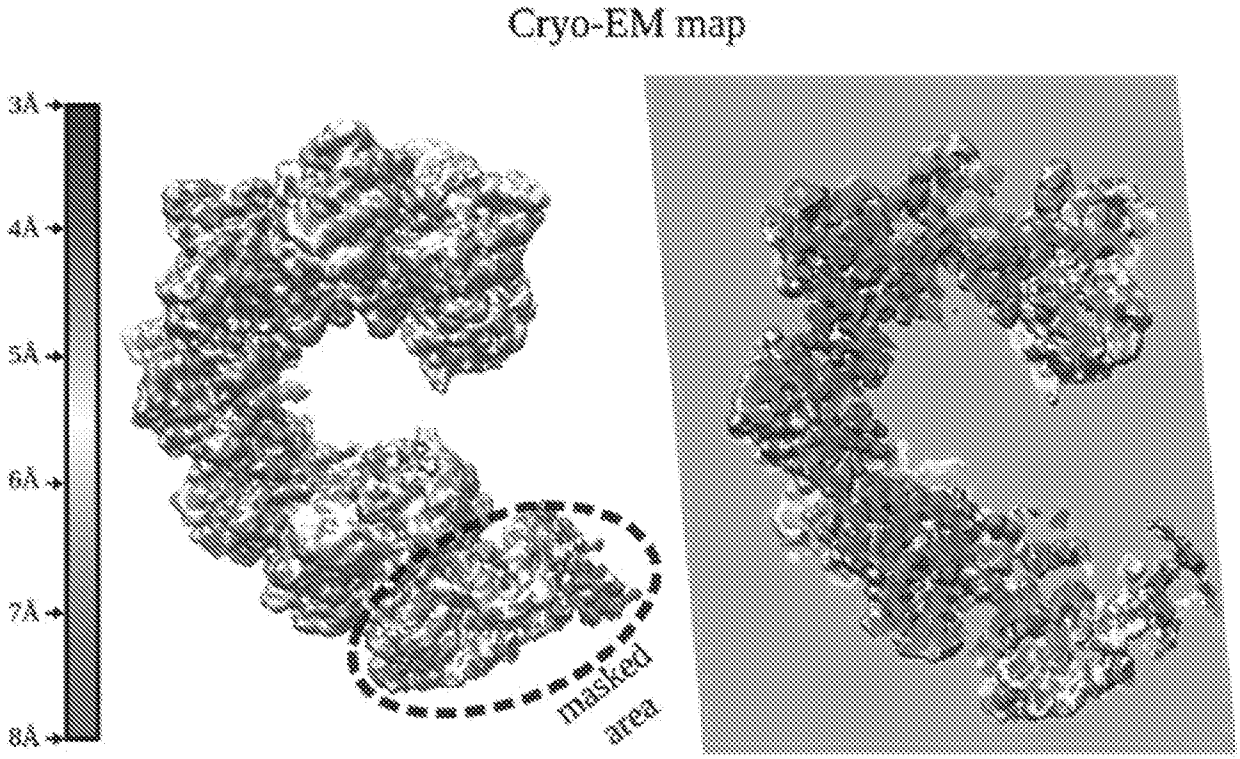


FIG. 53C

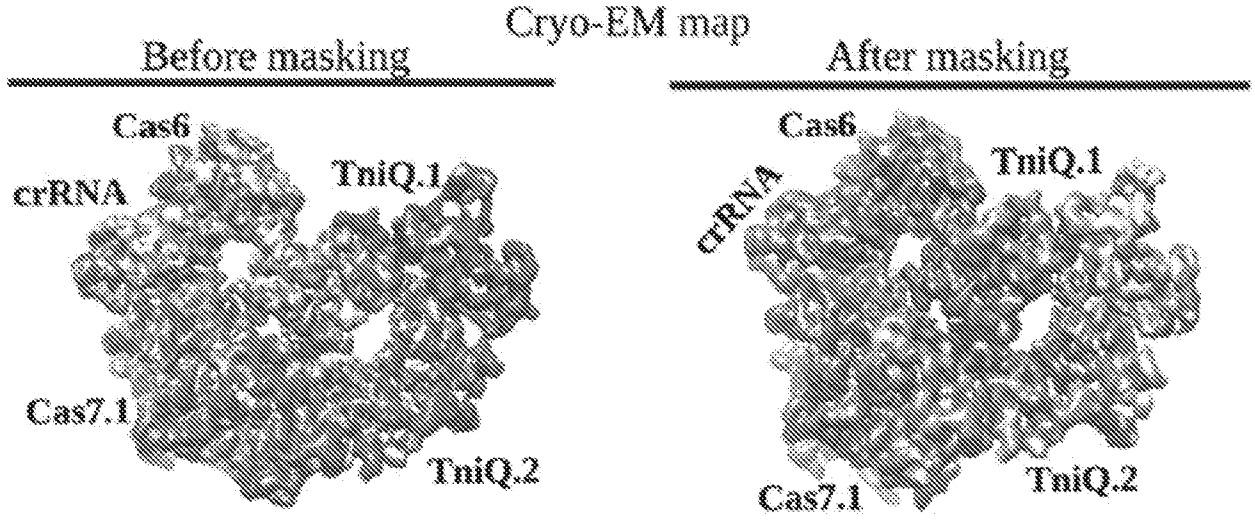


FIG. 53D

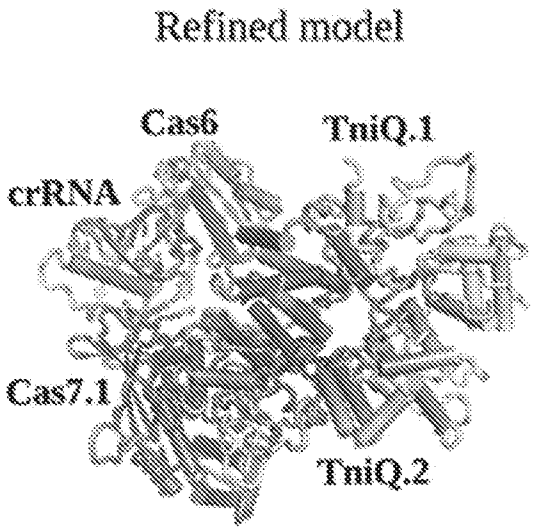


FIG. 53E



FIG. 54A

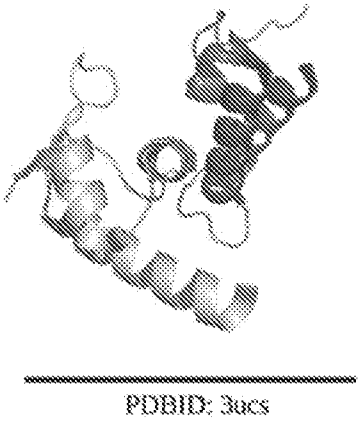


FIG. 54B

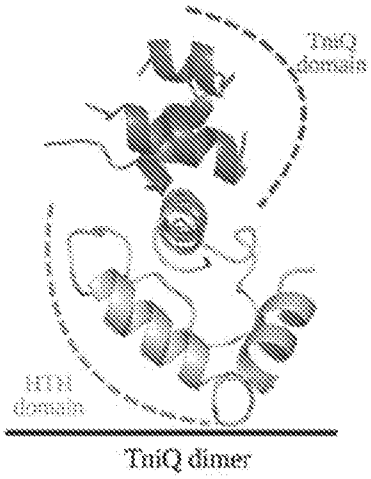


FIG. 54C

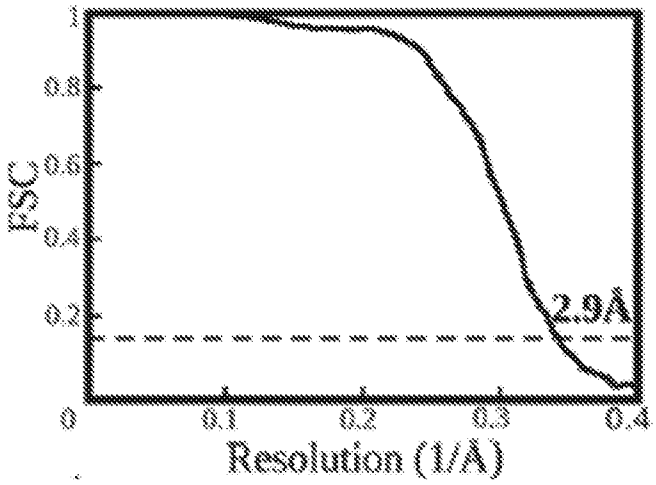


FIG. 55A

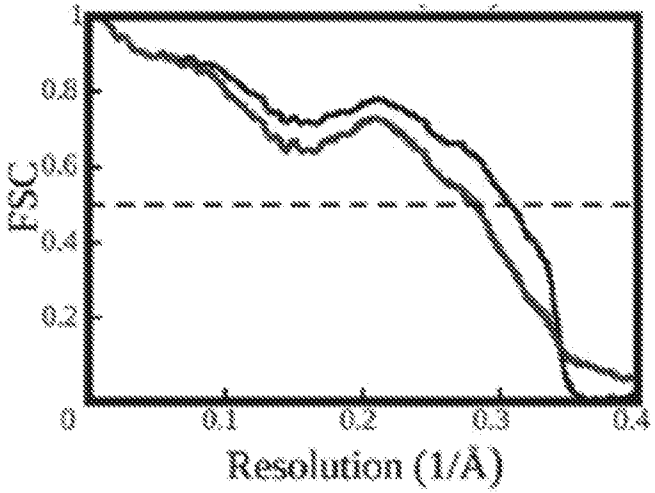


FIG. 55B

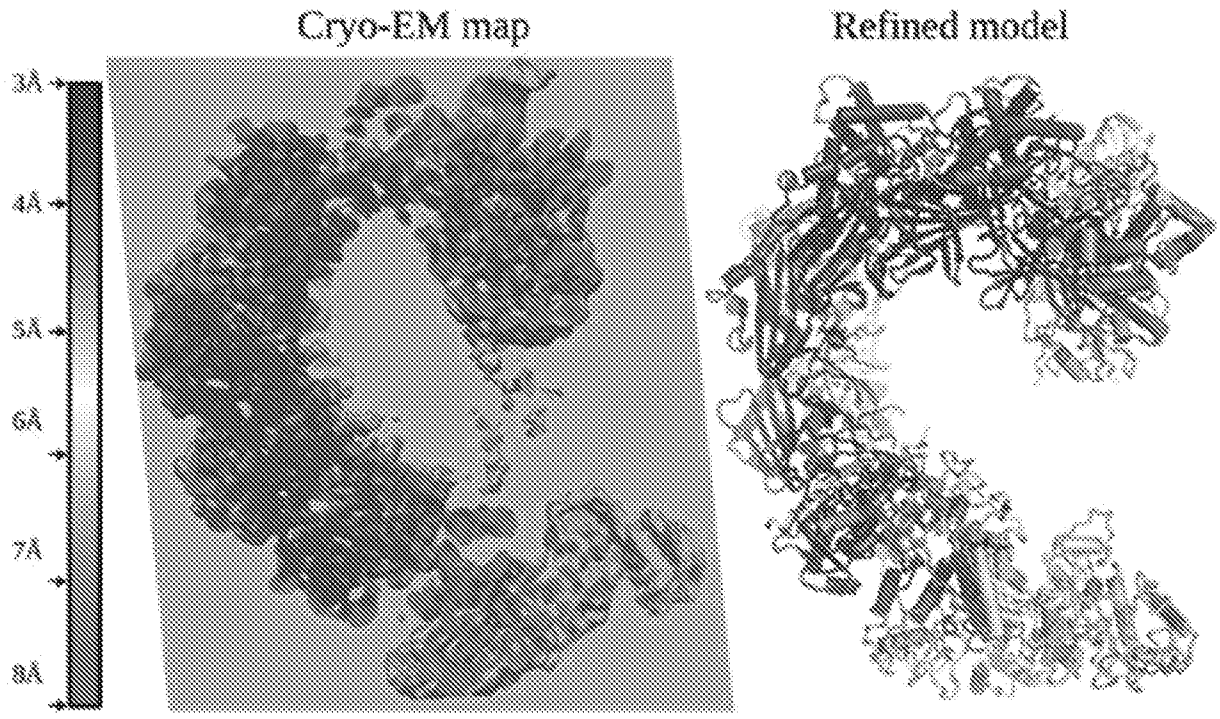


FIG. 55C

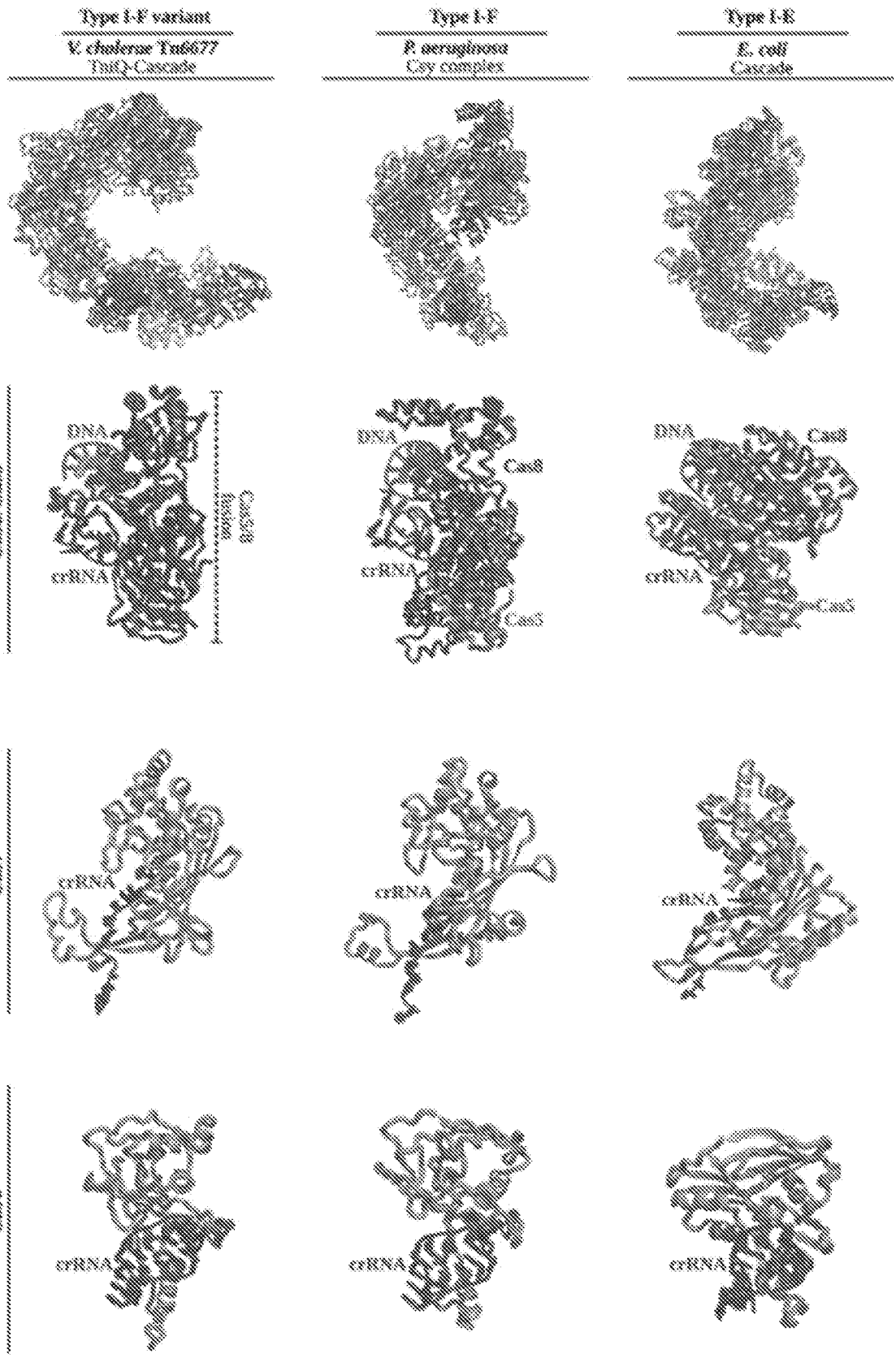

Type I-F variant
V. cholerae Tn6677
Tn6677-Cascade
Type I-F
P. aeruginosa
Csy complex
Type I-E
E. coli
Cascade
Cas8/Cas5
Cas7
Cas6
DNA
crRNA
Cas8
Cas5
Cas7
Cas6
Spacer
5'-handle
3'-handle

FIG. 56

Organism	NCBI Accession ID	WP_012596246.1	WP_012596241.1	WP_106173277.1	WP_066116114.1	WP_067772238.1	WP_096553061.1	WP_017739979.1	WP_012411901.1	WP_099067416.1
Cyan PCC 8801	WP_012596246.1		37	28	42	49	34	47	38	39
Cyan PCC 8801	WP_012596241.1	37		28	42	49	36	47	45	47
Kamptonema CCP2	WP_106173277.1	28	28		27	32	29	31	32	31
Genaio NIES-3709	WP_066116114.1	35	42	27		45	33	41	46	46
Nostoc NIES-3756	WP_067772238.1	39	49	32	45		43	60	57	60
Nostoc NIES-4103	WP_096553061.1	34	36	29	33	43		42	40	40
Scyto PCC 7110	WP_017739979.1	40	47	31	41	60	42		54	57
Nostoc punctiforme	WP_012411901.1	38	45	32	46	57	40	54		65
Nostoc linkia	WP_099067416.1	39	47	31	46	60	40	57	65	

FIG. 57A

Organism	NCBI Accession ID	WP 01 259624 6.1	WP 01 259624 1.1	WP 10 617327 7.1	WP 06 611611 4.1	WP 06 777223 8.1	WP 09 655306 1.1	WP 01 773097 9.1	WP 01 241180 1.1	WP 09 906741 6.1	KIF355 41.1
Hassallia byssoidea	KIF35541.1	39	46	31	47	38	40	55	84	66	
Crinalium epipsammum 2.91 Mbp	WP 015203565.1	40	45	30	53	56	36	51	60	60	63
Another Scytonema... not annotated	WP 096566883.1	34	35	29	34	43	70	44	41	41	41
Leptolyngbya boryana NIES-2135	WP 017289534.1	37	43	30	42	50	36	46	53	55	52
Cyanobacterium aponinum IPPAS B-1201	WP 099434943.1	37	42	27	46	46	34	45	31	50	52
Leptolyngbya ohadii IS1 (no Tns genes)	WP 088893813.1	25	25	35	26	31	27	29	29	30	29
Lyngbya confervoides BDU141931	WP 039728122.1	30	29	38	30	30	34	32	32	30	32
Cyanobacterium aponinum IPPAS B-1201	WP 099436544.1	36	42	29	68	46	33	41	47	46	46
Anabaena variabilis ATCC 29413	ABA20785.1	39	45	30	46	61	40	55	65	87	66

FIG. 57B

Organism	NCBI Accession ID	WP 01 259624 6.1	WP 01 259624 1.1	WP 10 617327 7.1	WP 06 611611 4.1	WP 06 777223 8.1	WP 09 655306 1.1	WP 01 773997 8.1	WP 01 241190 1.1	WP 09 906741 6.1	KIF355 41.1
Anabaena variabilis ATCC 29413	ABA20947.1	39	49	32	44	91	44	60	57	61	59
Anabaena variabilis ATCC 29413	ABA21816.1	39	45	31	41	57	39	54	54	51	55
Cyanothece sp. ATCC 51142	ACB53128.1	43	49	29	52	58	44	54	63	60	64

FIG. 57C

Organism	NCBI Accession ID	WP_0 152035 65.1	WP_0 965668 83.1	WP_0 172895 34.1	WP_0 994349 43.1	WP_0 888938 13.1	WP_0 397281 22.1	WP_0 994365 44.1	ABA2 0785.1	ABA2 0947.1	ABA2 0947.1	ACB5 3128.1
Cyan PCC 8801	WP_012596246.1	40	34	37	37	25	30	36	39	39	39	43
Cyan PCC 8801	WP_012596241.1	45	35	43	42	25	29	42	45	49	45	49
Filamentous CCP2	WP_106173277.1	30	29	30	27	35	38	29	30	32	31	29
Gemino NIES-3709	WP_066116114.1	51	34	42	46	26	30	68	46	44	41	52
Nostoc NIES-3756	WP_067772238.1	56	43	50	46	31	30	46	61	91	57	58
Nostoc NIES-4103	WP_096553061.1	36	70	36	34	27	34	33	40	44	39	44
Scyto PCC 7110	WP_017739979.1	51	44	46	45	29	32	41	55	60	54	54
Nostoc punctiforme	WP_012411901.1	60	41	53	51	29	32	47	65	57	54	63
Nostoc linckia	WP_099087416.1	60	41	55	50	30	30	46	87	61	51	60

FIG. 57D

Organism	NCBI Accession ID	WP_0152035 65.1	WP_0965668 83.1	WP_0172895 34.1	WP_0994349 43.1	WP_0888938 13.1	WP_0397281 22.1	WP_0994365 44.1	ABA20785.1	ABA20947.1	ABA20947.1	ACB53128.1
Hassallia byssoidea	KIF35541.1	63	41	52	52	29	32	46	66	59	55	64
Cylindrospermum stagnale PCC 7417 2.91 Mbp	WP_015203563.1		38	52	52	28	31	50	62	57	51	66
Another *Scytonema*... not annotated	WP_096566863.1	38		37	34	26	31	34	41	43	42	44
Leptolyngbya boryana NIES-2135	WP_017289534.1	52	37		45	27	28	41	54	49	47	54
Cyanobacterium aponinum IPPAS B-1201	WP_099434943.1	52	34	45		25	28	46	48	46	45	55
Leptolyngbya sp. O-77 (no Tnz genes)	WP_088893813.1	28	26	27	25		34	26	30	32	29	28
Lyngbya confervoides BDU141951	WP_039728122.1	31	31	28	28	34		30	29	32	31	30
Cyanobacterium aponinum IPPAS B-1201	WP_099436344.1	50	34	41	46	26	30		46	44	40	52
Anabaena variabilis ATCC 29413	ABA20785.1	62	41	54	48	30	29	46		61	52	63

FIG. 57E

Organism	NCBI Accession ID	WP_015203565.1	WP_096566883.1	WP_017289534.1	WP_099434943.1	WP_088893813.1	WP_039728122.1	WP_099436544.1	ABA20785.1	ABA20947.1	ABA20947.1	ACB53128.1
Anabaena variabilis ATCC 29413	ABA20947.1	57	43	49	46	32	32	44	61		57	57
Anabaena variabilis ATCC 29413	ABA21816.1	51	42	47	45	29	31	40	52	57		55
Cyanothece sp. ATCC 51142	ACB53128.1	66	44	54	55	28	30	52	63	57	55	

FIG. 57F

Organism	NCBI Accession ID	CRISPR length	C2c5 length	tRNA	MerR	Cargo	Tns length	Additional Information
Cyan PCC 8801	WP_012596246.1	4 repeats	1983	tRNA Pro	no	Single gene, AAA+	3438	Immediately flanking TnsBCD operon
Cyan PCC 8801	WP_012596241.1	5 repeats	2049	no	no; has CopG-like ribbon-helix-helix domain	RM system, transposase, HNH endonuclease	3082	Flanked on other side by Tns operon from a separate transposon-C2c5 complex
Filamentous CCP2	WP_106173277.1	4 repeats	2268	tRNA Ala	no; has CopG-like ribbon-helix-helix domain	PaaB/HNH, hypothetical, NACHT domain,	3460	
Gemino NIES-3709	WP_066116114.1	5 repeats	1851	tRNA Arg	yes	alkyl sulfatase	2987	Cargo looks like not defense-associated
Nostoc NIES-3756	WP_067772238.1	5 repeats	1908	tRNA Ala	no	R-M system, peptidase/cell wall associated	3223	
Nostoc NIES-4103	WP_096553061.1	3 repeats	1998	no	no; has CopG-like ribbon-helix-helix domain	No cargo in between C2c5 and Tns	3161	Tns operon orientation, relative to C2c5, is atypical
Scyto PCC 7110	WP_017739979.1	15 repeats	1938	no		NACHT, WD40 protein, ATPase	3232	

FIG. 58A

Organism	NCBI Accession ID	CRISPR length	C2c5 length	tRNA	MerR	Cargo	Tns length	Additional Information
Nostoc punctiforme	WP_012411901.1	13 repeats	1920	tRNA Val	yes	BREX system	3089	
Nostoc linckia	WP_099067416.1	15-20 repeats	1938	no	yes	R-M system, NACHT domain	3038	
Hassallia byssoidea	KIF35541.1	7 repeats	1920	tRNA Leu	yes	R-M system, Piwi	3092	
Cronalium epipsammum 2.91 Mbp	WP 015203565.1	2 repeats	1845	tRNA Lys	yes	R-M system, PIN domain, transposase, histidine-like kinase	3001	
Another Scytonema... not annotated	WP_096366883.1	–	N/A	N/A	N/A	N/A	N/A	N/A
Leptolyngbya boryana NIES-2135	WP_017289534.1	–	N/A	N/A	N/A	N/A	N/A	N/A
Cyanobacterium aponinum IPPAS B-1201	WP 099434943.1	4 repeats	1839	tRNA Pro	yes	R-M system, phage-associated protein	3216	
Leptolyngbya ohadii IS1 (no Tns genes)	WP_088893813.1	–	N/A	N/A	N/A	N/A	N/A	No Tns genes

FIG. 58B

Organism	NCBI Accession ID	CRISPR length	C2c5 length	tRNA	MerR	Cargo	Tns length	Additional Information
Lyngbya confervoides BDU141951	WP_039728122.1	3 repeats	1590	no	no	DNA phosphorothioation system; transposase	3291	
Cyanobacterium aponinum IPPAS B-1201	WP_099436544.1	9 repeats	1872	tRNA Ala	yes	R-M module	3086	
Anabaena variabilis ATCC 29413	ABA20785.1	3 repeats	1929	tRNA Pro	yes	R-M module, ser-thr protein kinase	3026	
Anabaena variabilis ATCC 29413	ABA20947.1	3 repeats	1908	no	no	R-M system, ATPase	4703	Orientation of Tns operon is backwards compared to C2c5 and other systems
Anabaena variabilis ATCC 29413	ABA21816.1	4 repeats	1932	tRNA Thr	transcriptional regulator, XRE family	R-M like, ATPase	3783	
Cyanothece sp. ATCC 51142	ACB53128.1	3 repeats	1095; possibly truncated					

FIG. 58C

FIG. 59

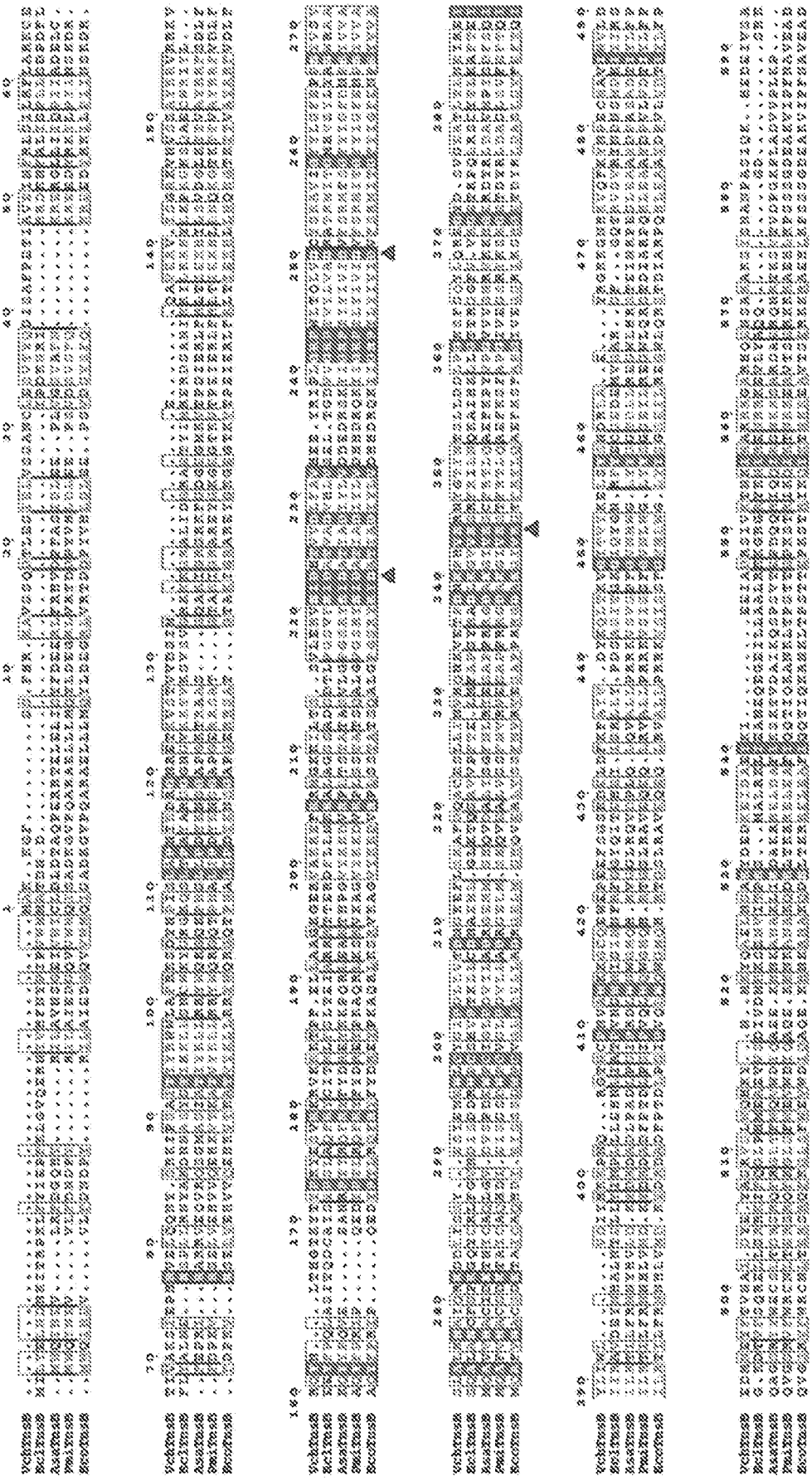


FIG. 60

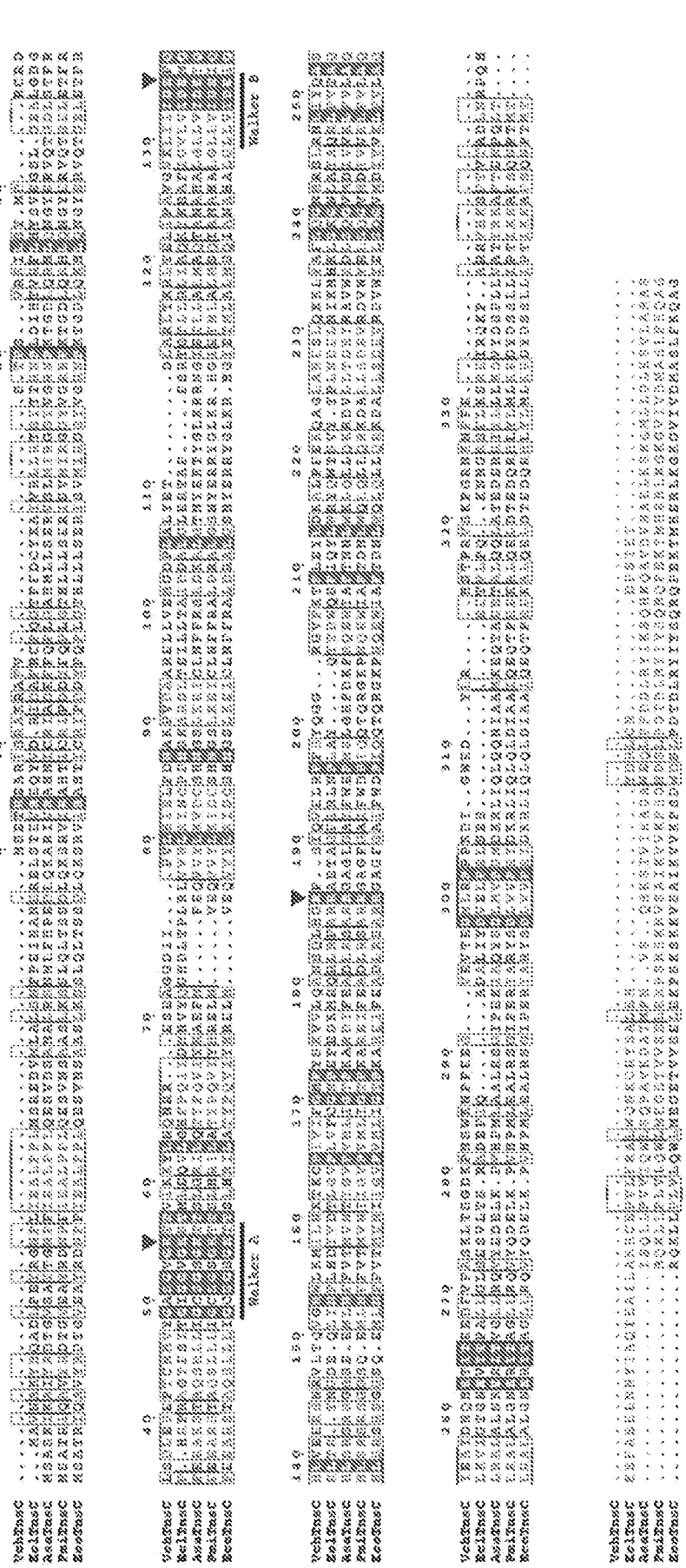


FIG. 61

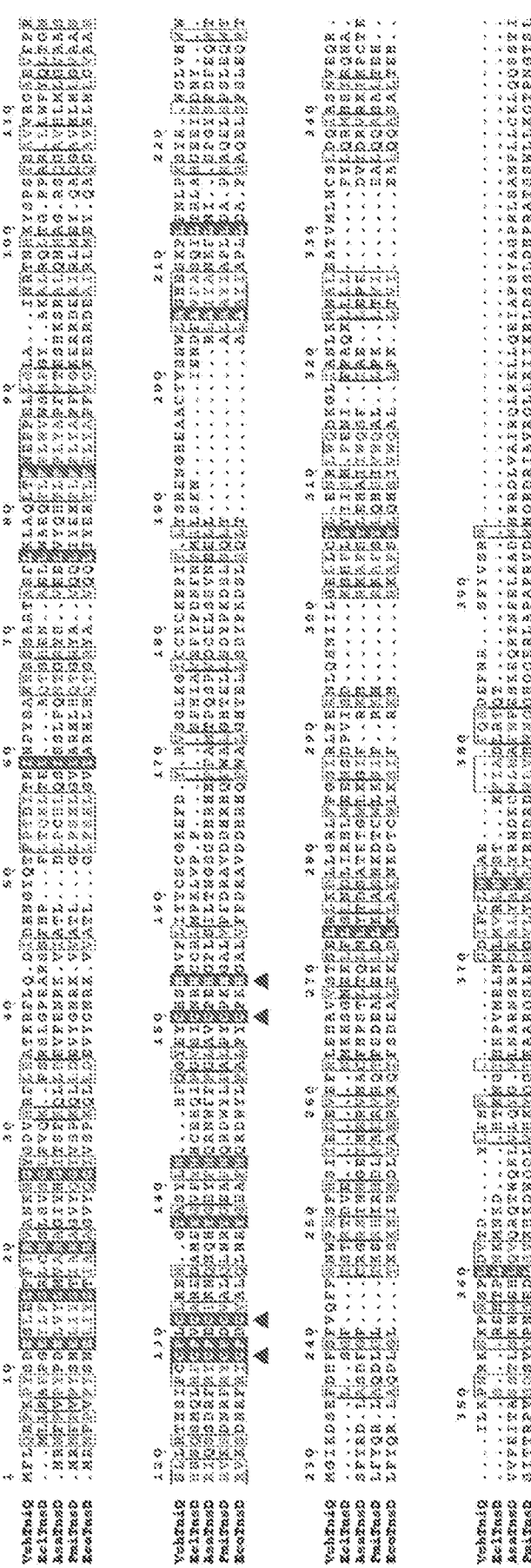


FIG. 62

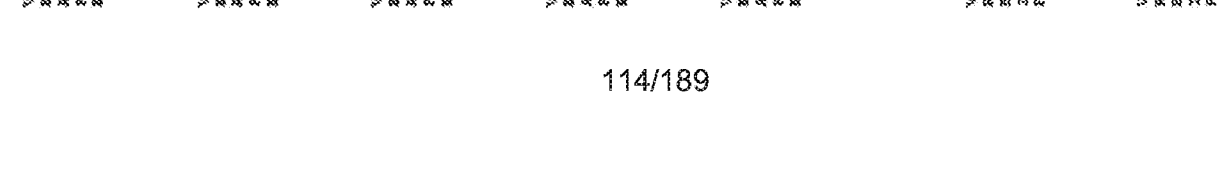


FIG. 63

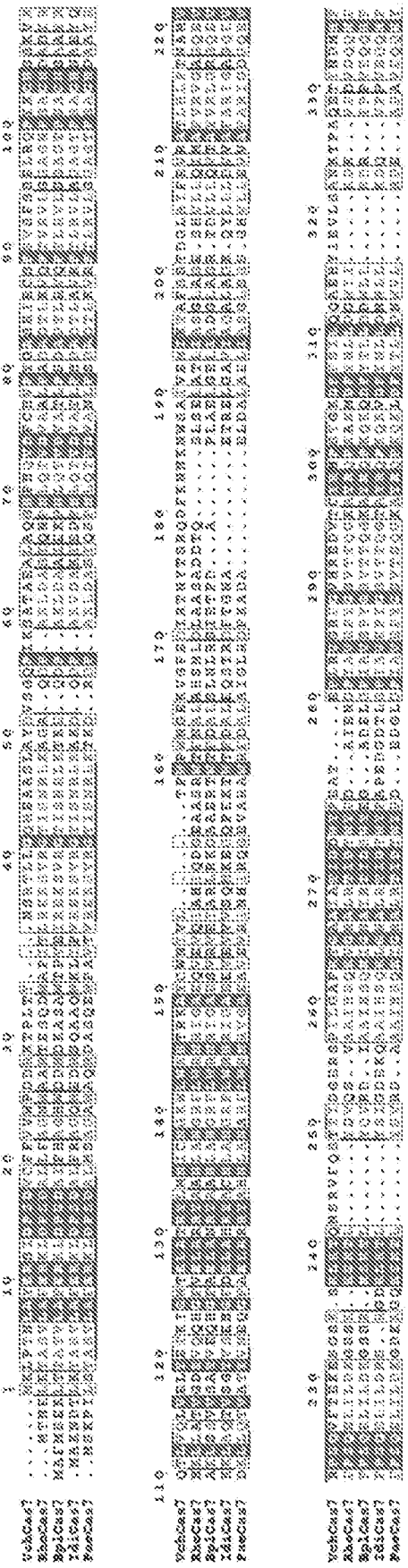


FIG. 64

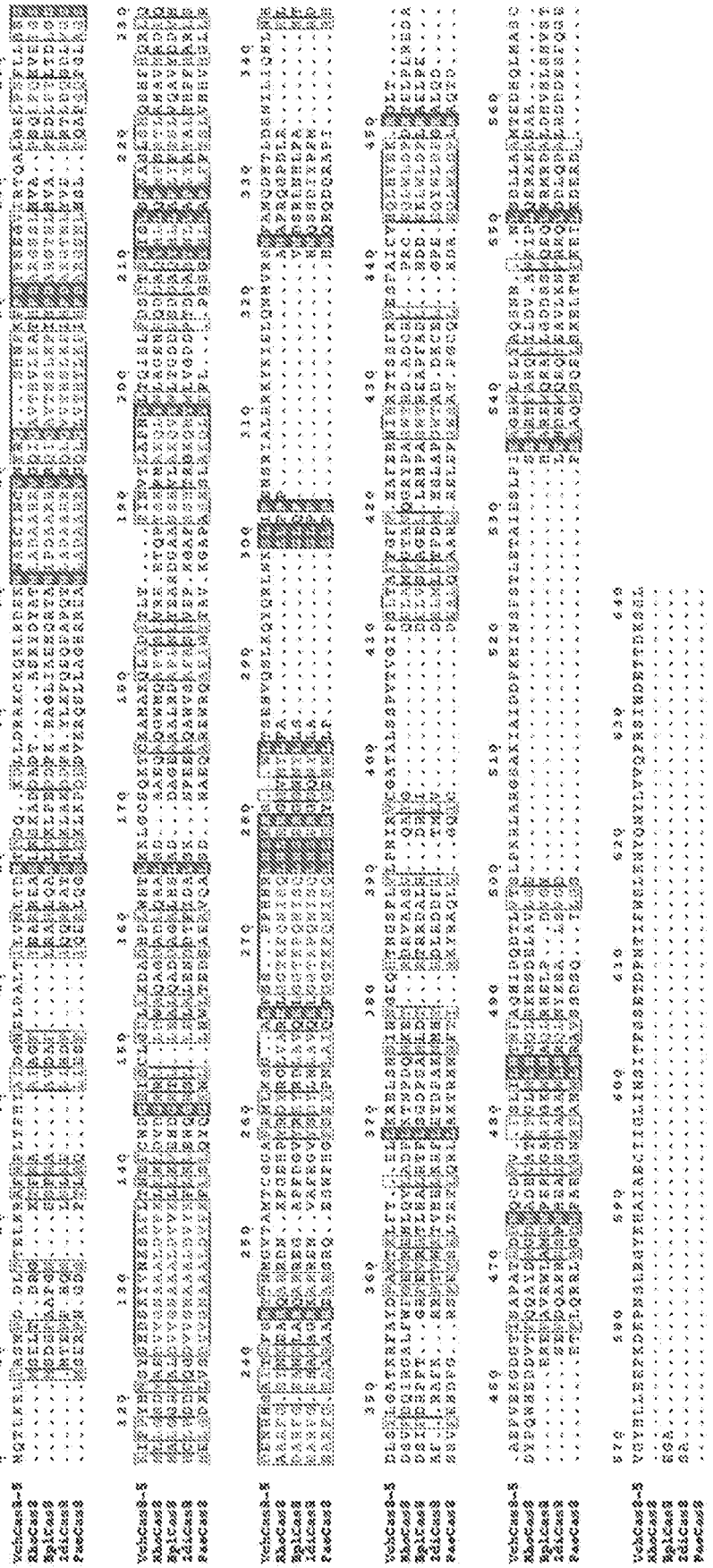


FIG. 65A

FIG. 65B

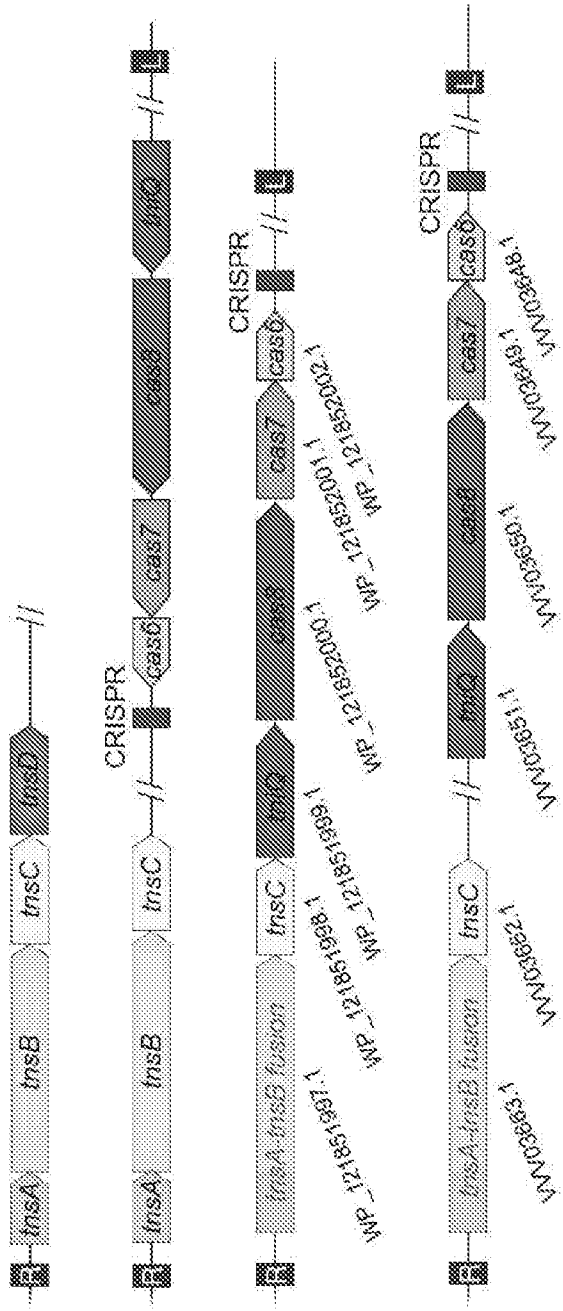


FIG. 66

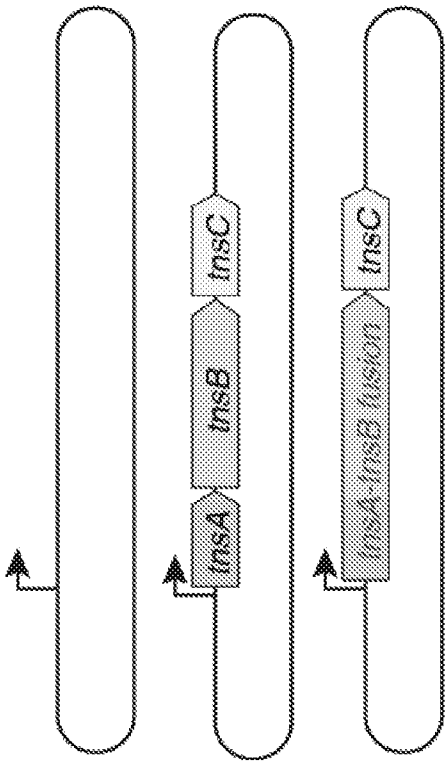


FIG. 67A

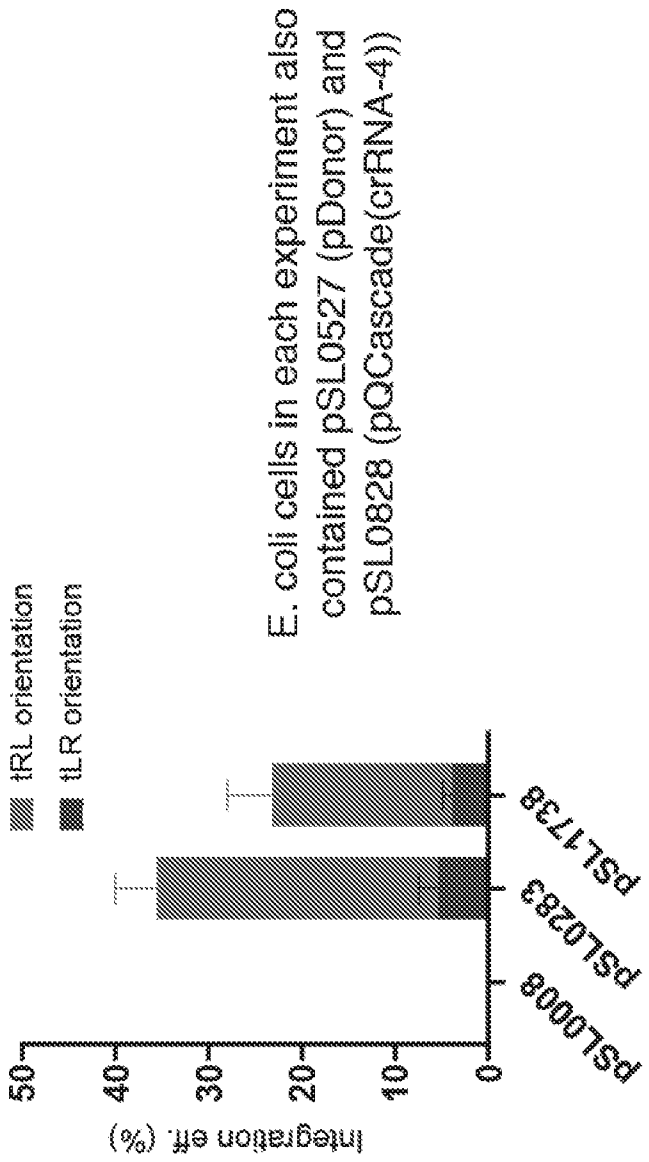

E. coli cells in each experiment also contained pSL0527 (pDonor) and pSL0828 (pQCascade(crRNA-4))
tRL orientation
tLR orientation
Integration eff. (%)
50
40
30
20
10
0
pSL0008
pSL0283
pSL1738

FIG. 67B

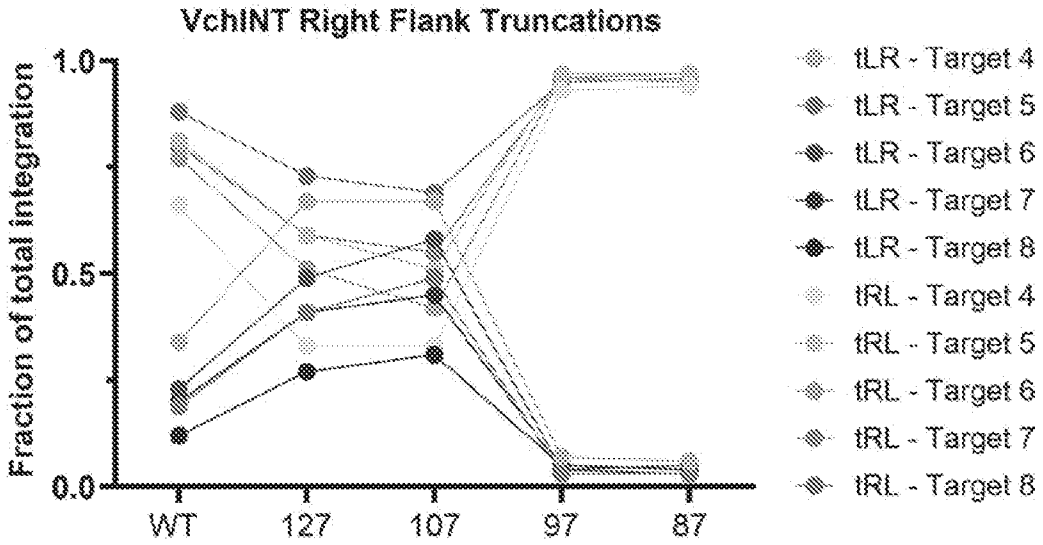
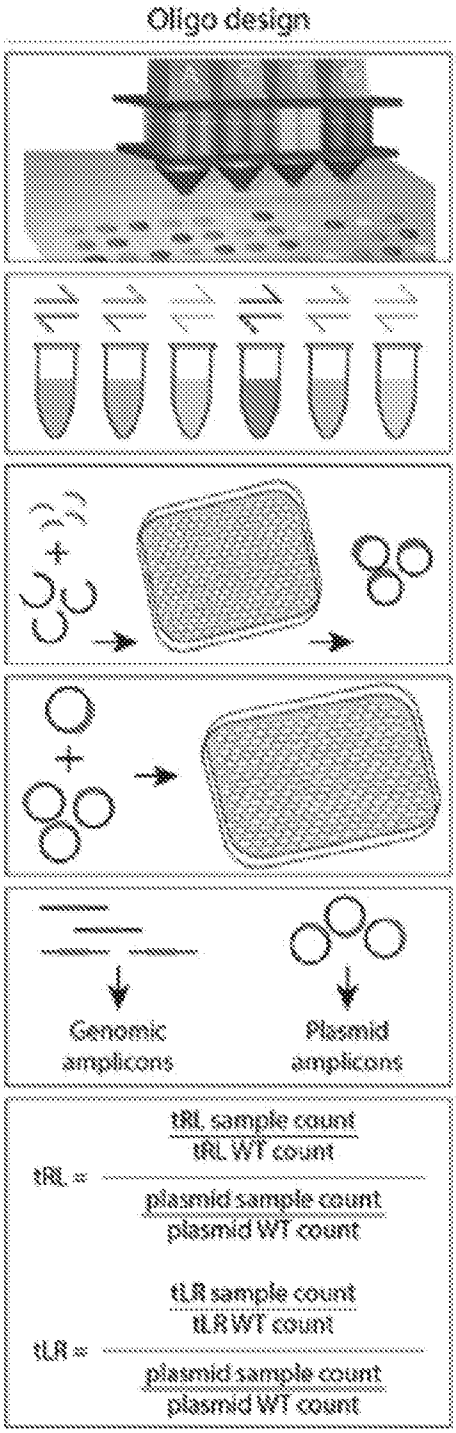

VchINT Right Flank Truncations
Fraction of total integration
1.0
0.5
0.0
WT
127
107
97
87
tLR - Target 4
tLR - Target 5
tLR - Target 6
tLR - Target 7
tLR - Target 8
tRL - Target 4
tRL - Target 5
tRL - Target 6
tRL - Target 7
tRL - Target 8

FIG. 68



Agilent Oligo Chip

Amplify individual libraries using PCR

Digest purified amplicons and entry vectors
Ligate and transform Electrocompetent NEBTurbo cells
Plate on large bioassay dishes with antibiotic
Scrape the colonies and extract the plasmid DNA

Co-transform the library and INTEGRATE machinery into
Electrocompetent BL21(DE3) cells
Plate on large bioassay dishes with antibiotics and inducer

Scrape the colonies and extract genomic DNA
Amplify target locations with a transposon- and a genome- or pDonor- specific primer that have illumina adapter overhangs

Sequence the abundance of the library members in the plasmid entry library as well as the genomically integrated products
Calculate the relative integration efficiency for both orientations

FIG. 69

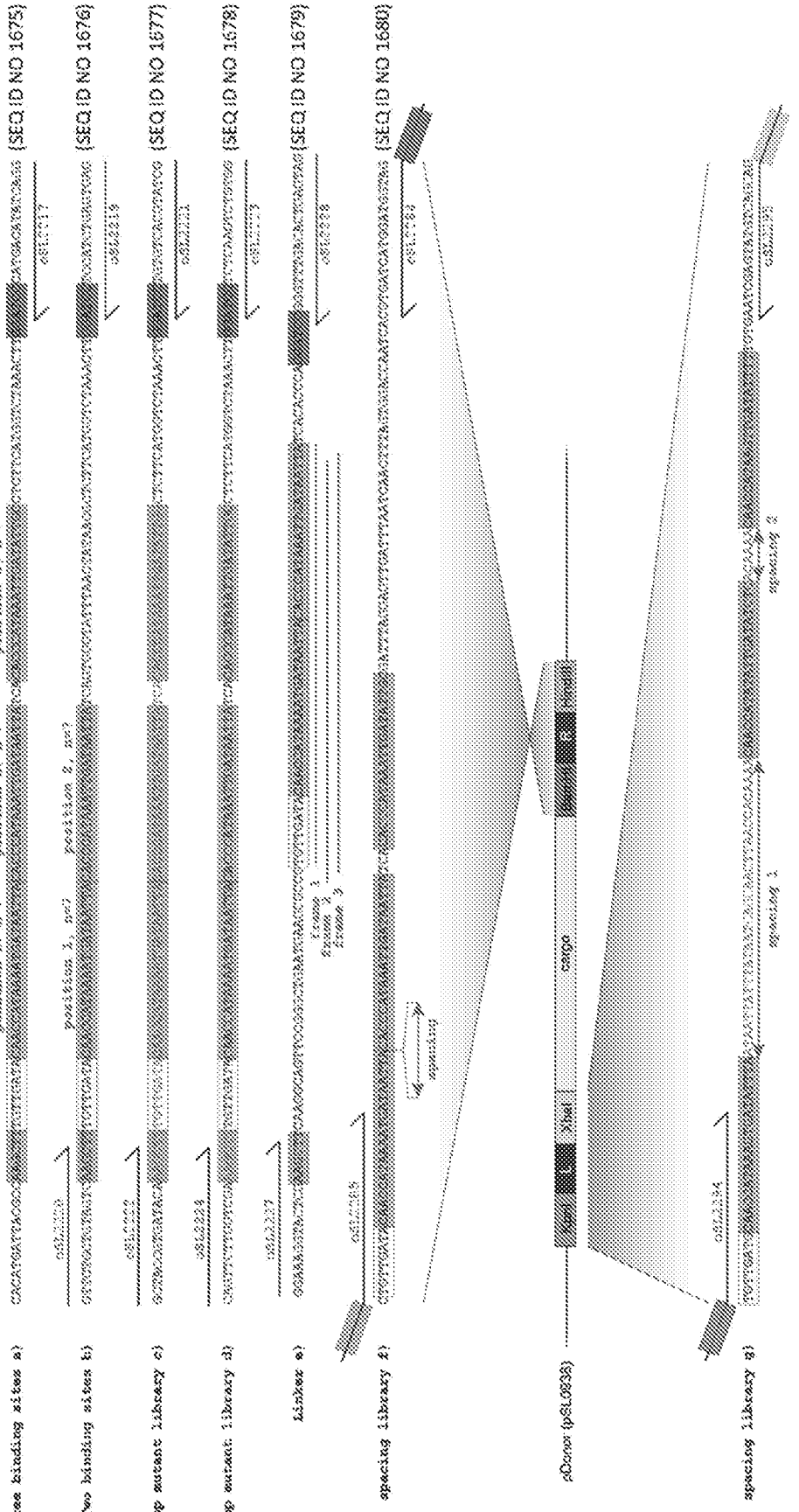

HindIII
Sbp end
Cas
BsmBI
Three binding sites a)
Two binding sites b)
2bp mutant library c)
1bp mutant library d)
Linker e)
spacing library f)
pDonor (pSL0938)
spacing library g)
position 1, nt7
position 2, nt7
position 3, nt7
(SEQ ID NO 1675)
(SEQ ID NO 1676)
(SEQ ID NO 1677)
(SEQ ID NO 1678)
(SEQ ID NO 1679)
(SEQ ID NO 1680)
Exon 1
Exon 2
Exon 3
spacing
sacB
XbaI
cargo
HindIII
spacing 1
spacing 2

FIG. 70

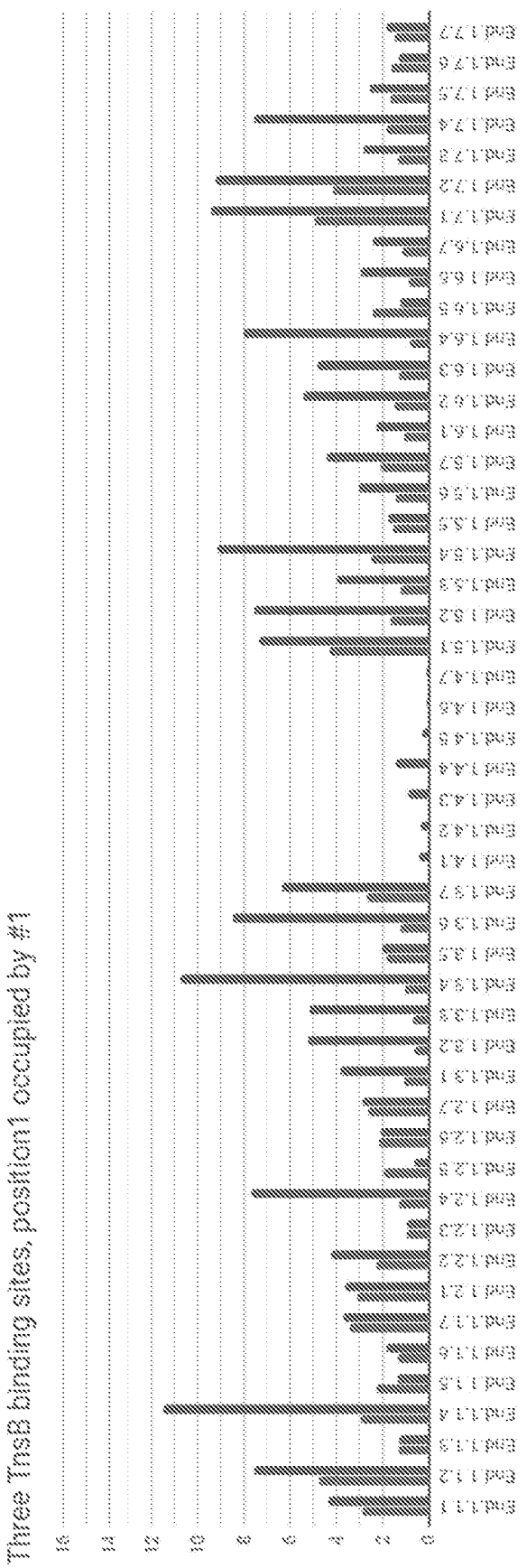


FIG. 71A

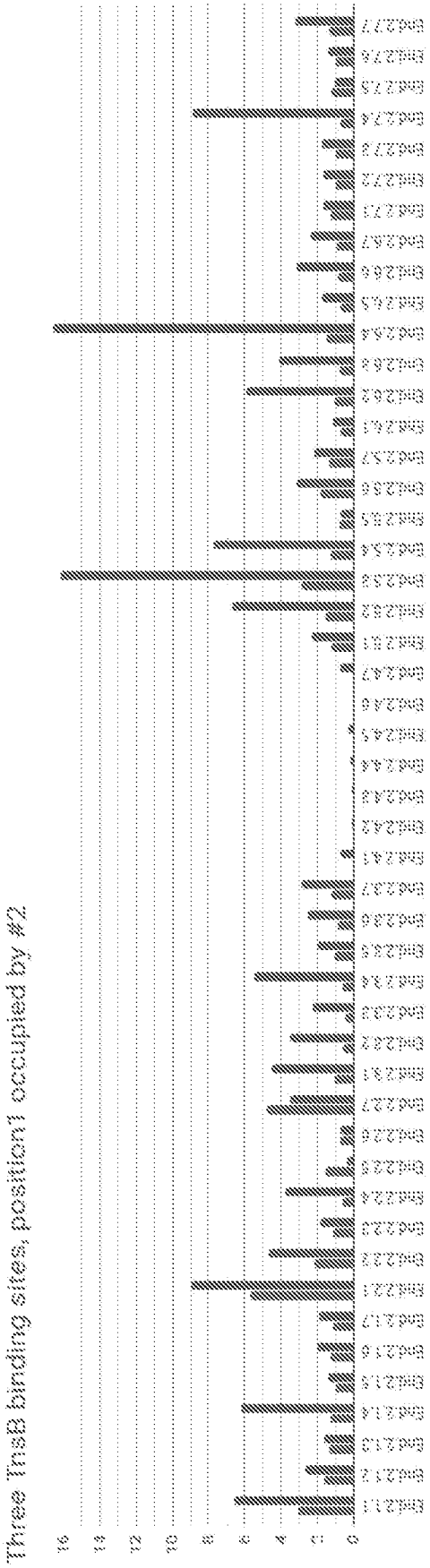


FIG. 71B

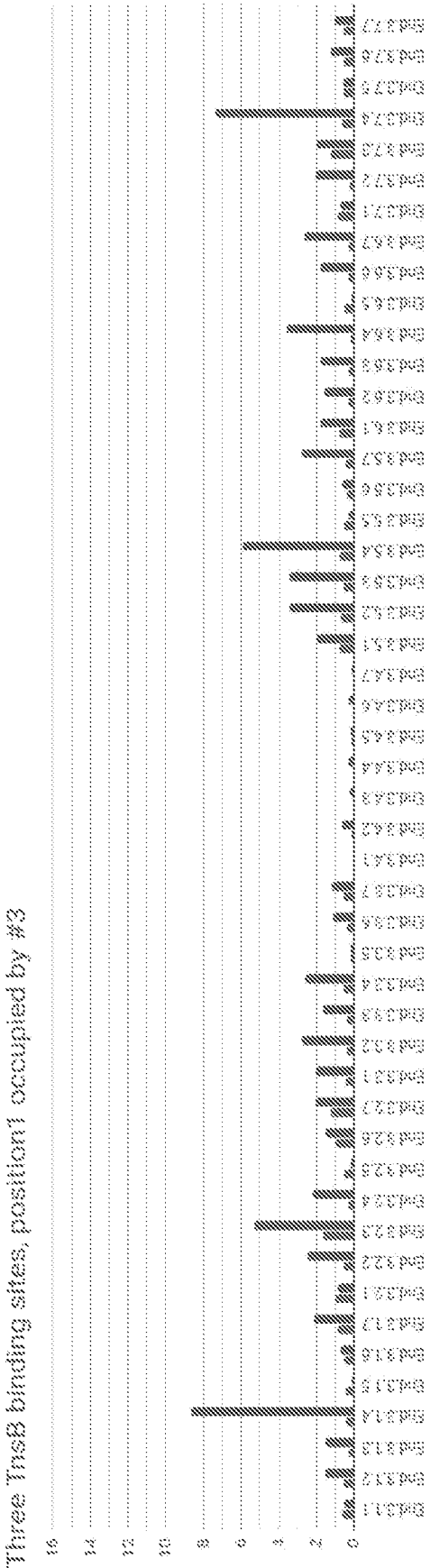


FIG. 71C

FIG. 71D

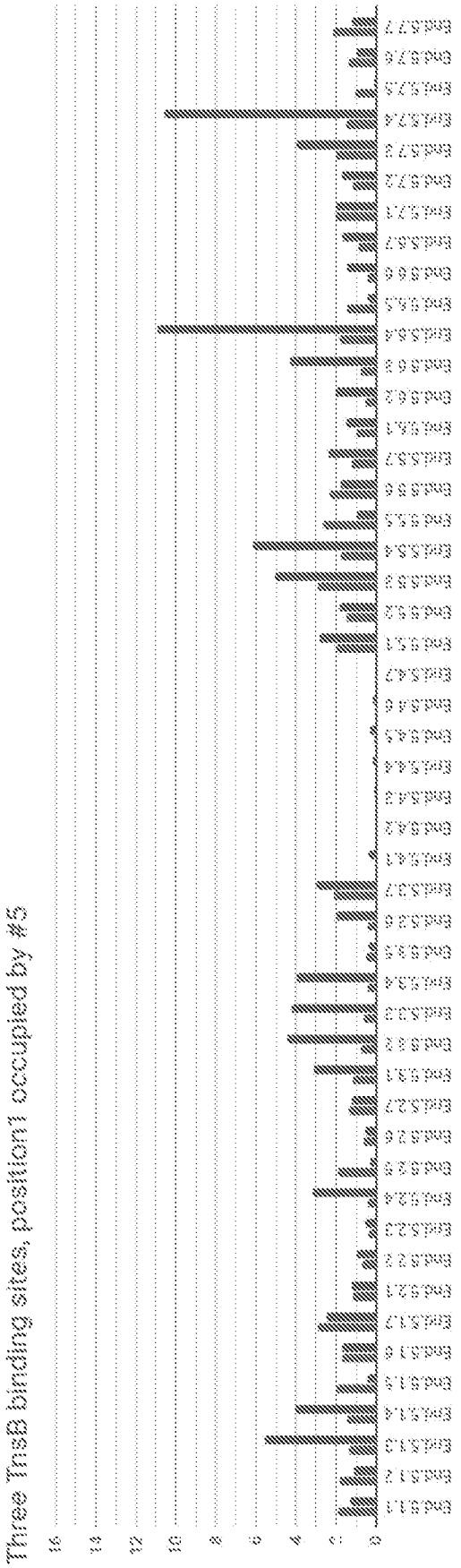


FIG. 71E

FIG. 71F

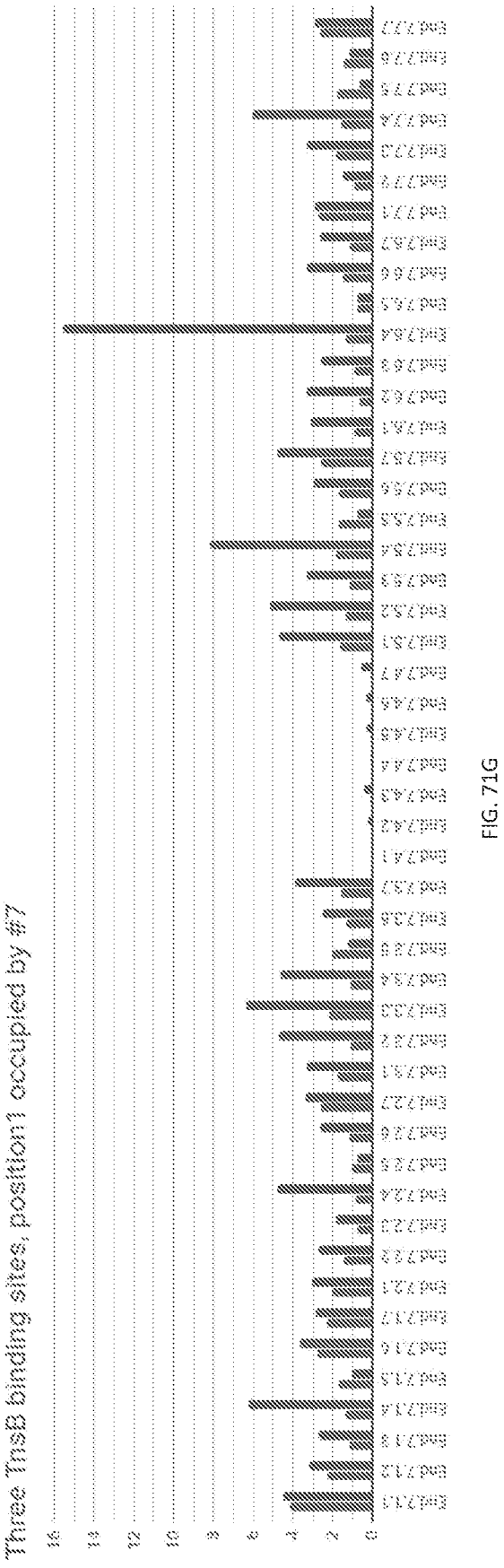

Three TnsB binding sites, position1 occupied by #7
16
14
12
10
8
6
4
2
0
end711
end712
end718
end714
end715
end716
end717
end721
end722
end723
end724
end725
end726
end727
end731
end732
end733
end734
end735
end736
end737
end741
end742
end743
end744
end745
end746
end747
end751
end752
end753
end754
end755
end756
end757
end761
end762
end763
end764
end765
end766
end767
end771
end772
end773
end774
end775
end776
end777

FIG. 71G

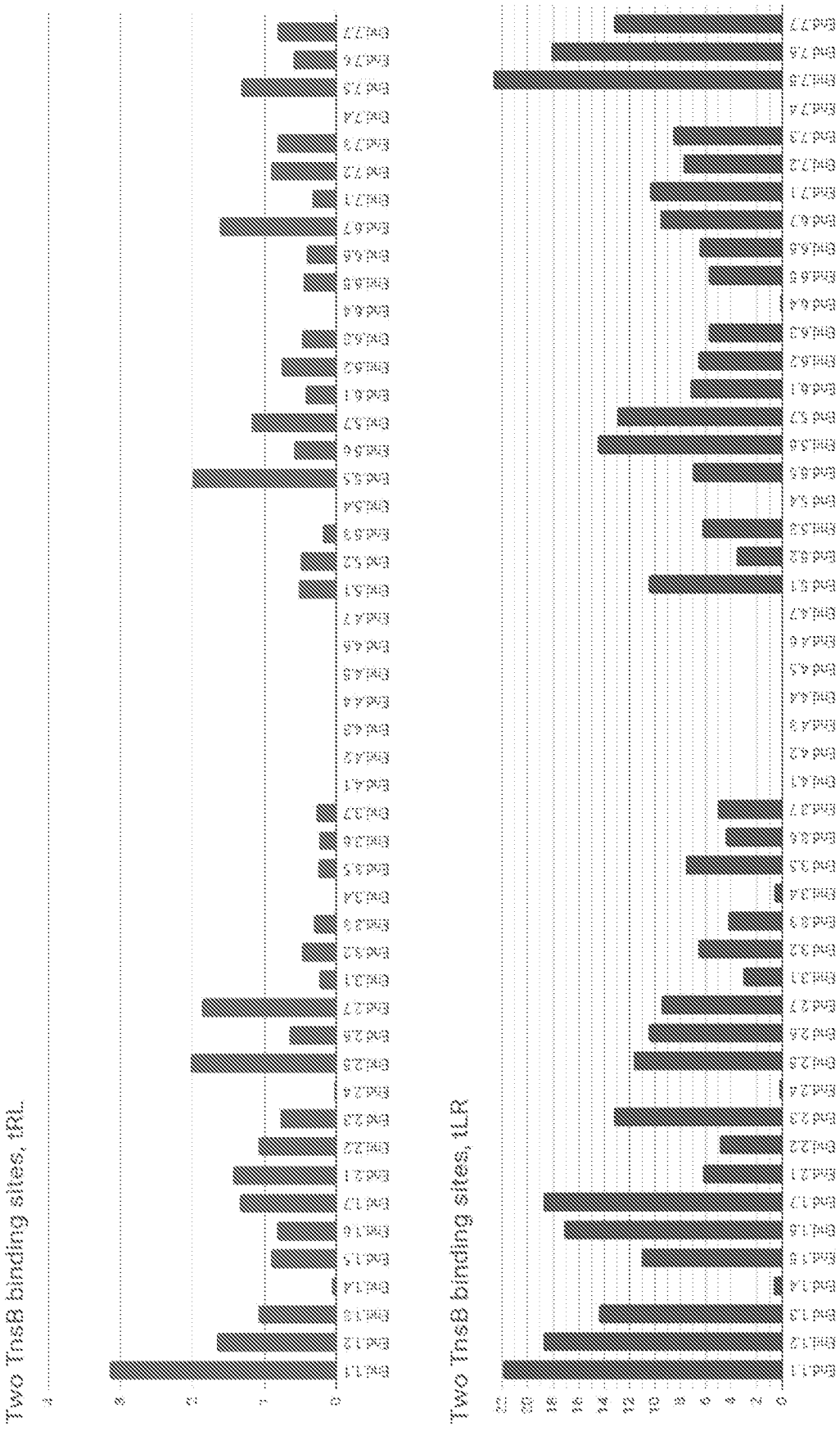


FIG. 72

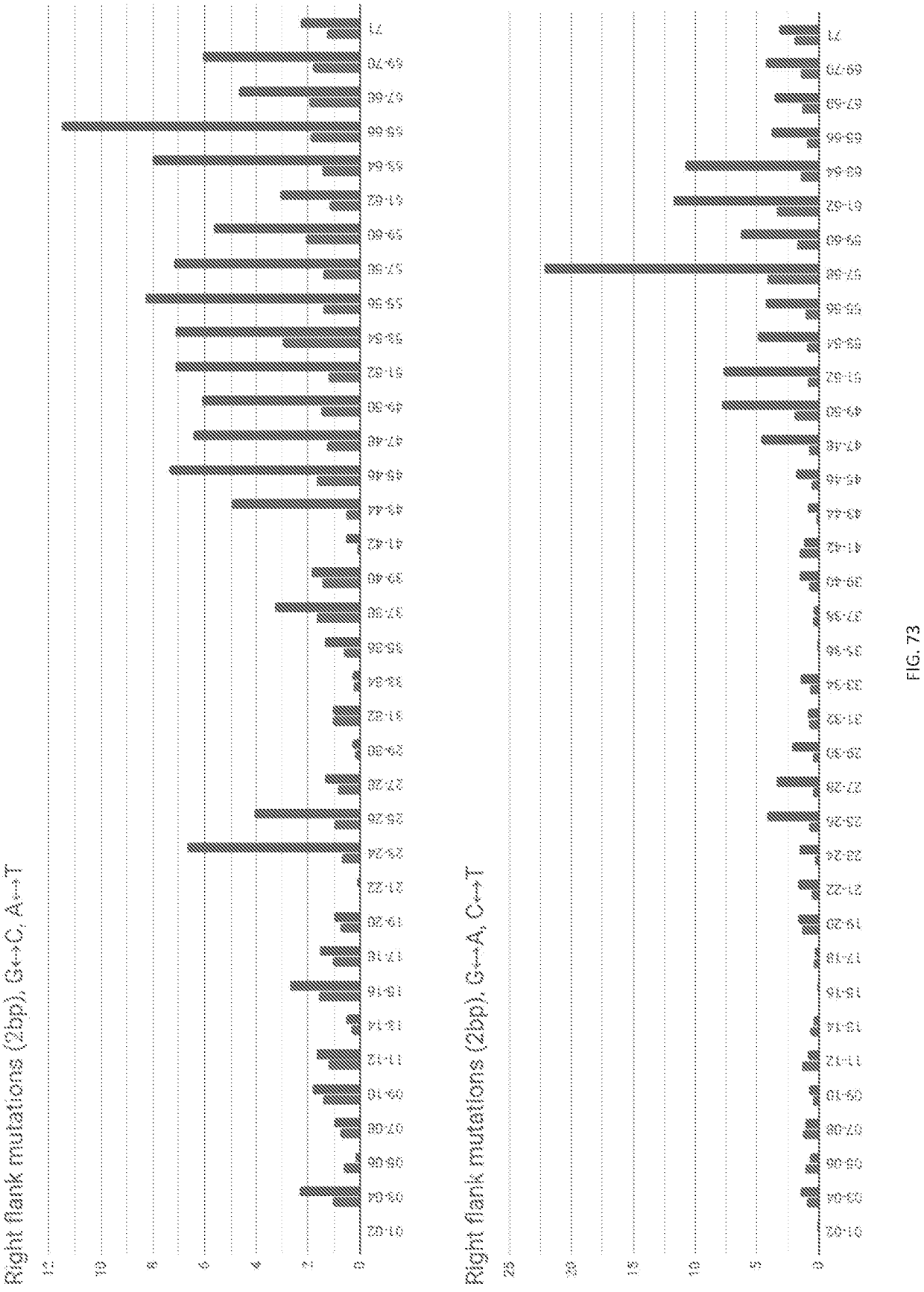

Right flank mutations (2bp), G↔C, A↔T
Right flank mutations (2bp), G↔A, C↔T

FIG. 73

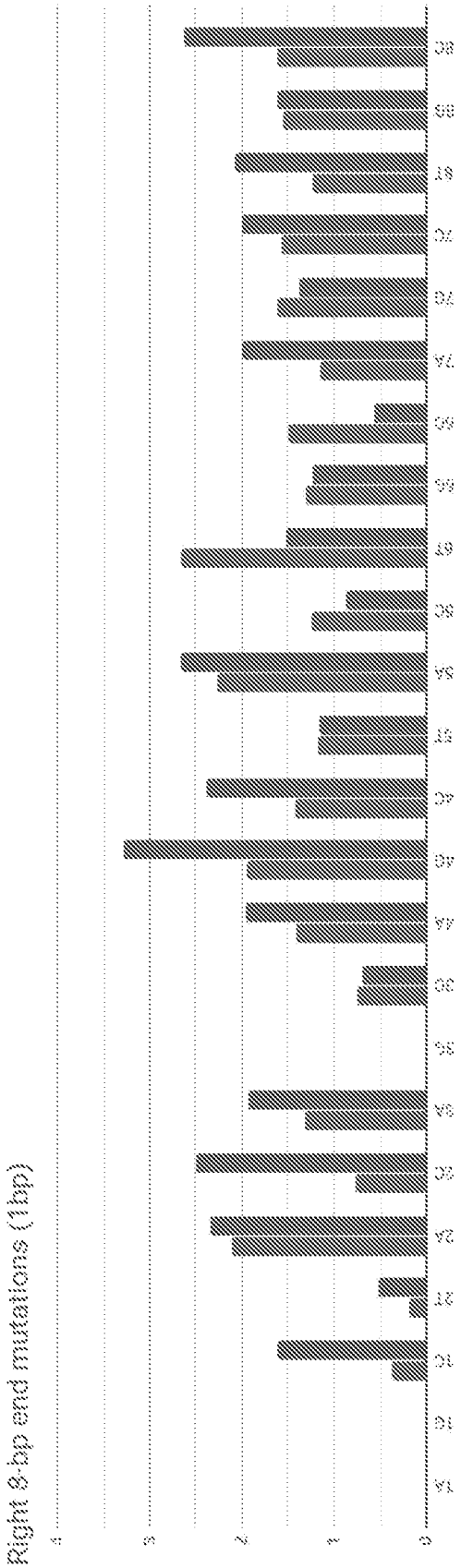

Right 8-bp end mutations (1bp)
0
1
2
3
4
1A
1C
1G
1T
2A
2C
2G
2T
3A
3C
3G
3T
4A
4C
4G
4T
5A
5C
5G
5T
6A
6C
6G
6T
7A
7C
7G
7T
8A
8C
8G
8T

FIG. 74

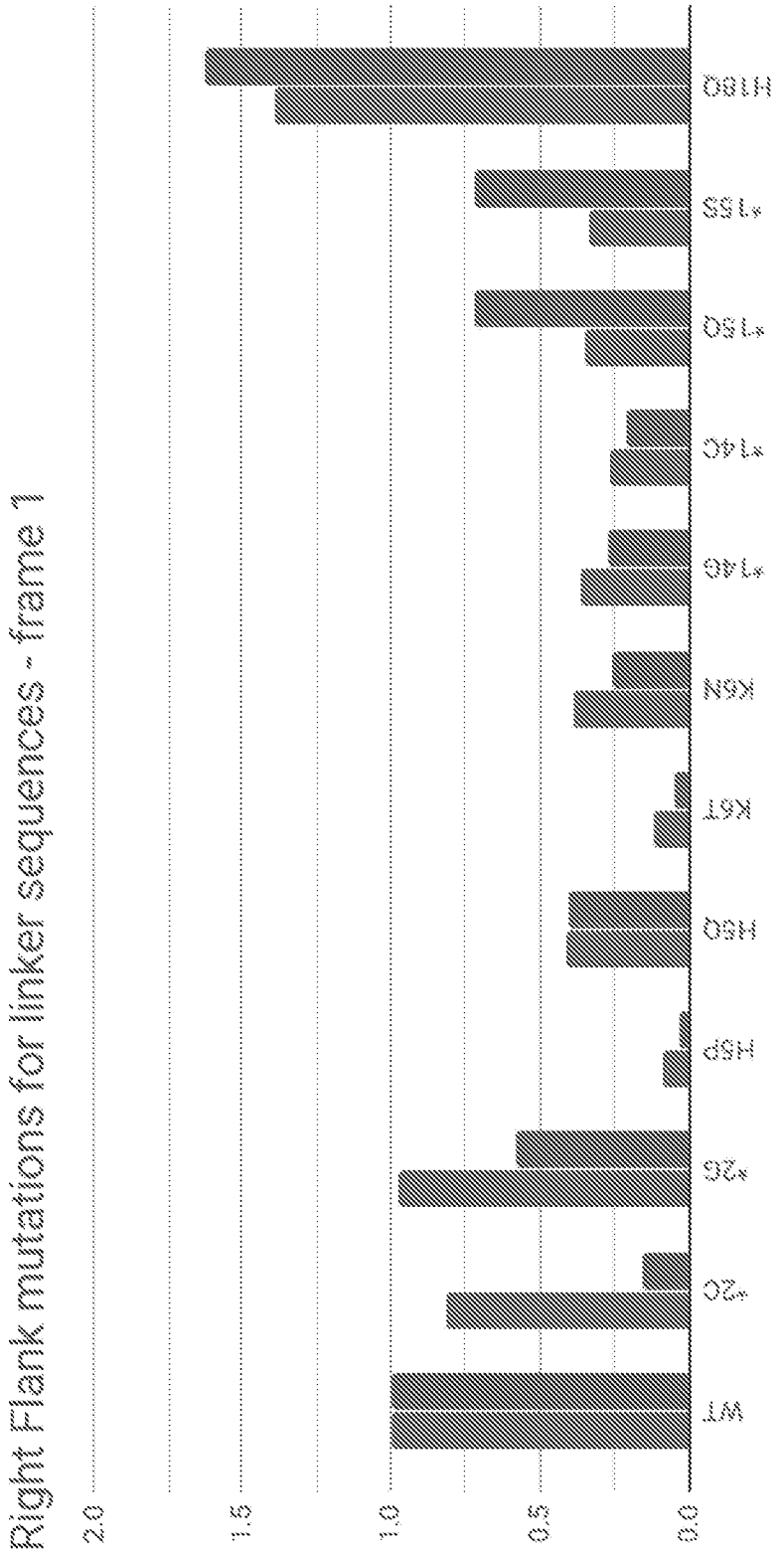

Right Flank mutations for linker sequences - frame 1
2.0
1.5
1.0
0.5
0.0
WT
*2C
*2G
H5P
H5Q
K6T
K6N
*14G
*14C
*15Q
*15S
H18Q

FIG. 75A

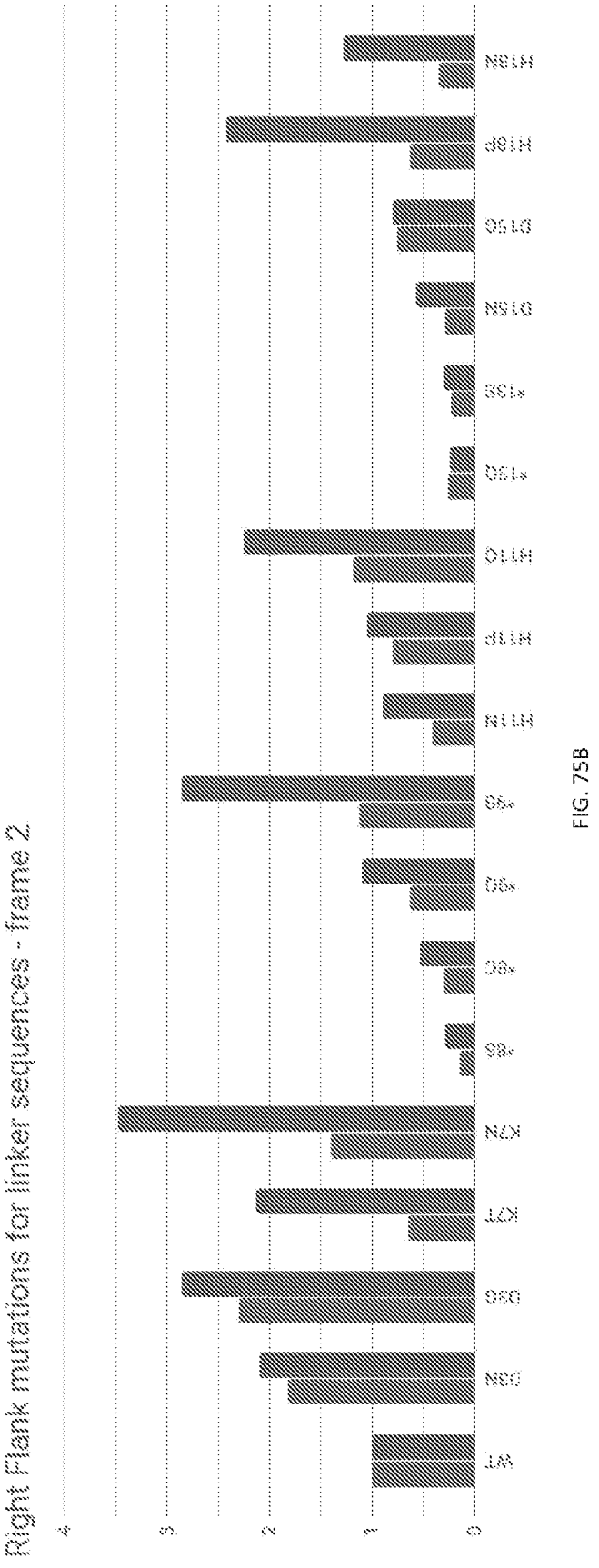

Right Flank mutations for linker sequences - frame 2
4
3
2
1
0
WT
D3N
D3G
K7T
K7N
*8S
*8C
*9Q
*9S
H11N
H11P
H11O
*13O
*13E
D15N
D15G
H18P
H18N

FIG. 75B

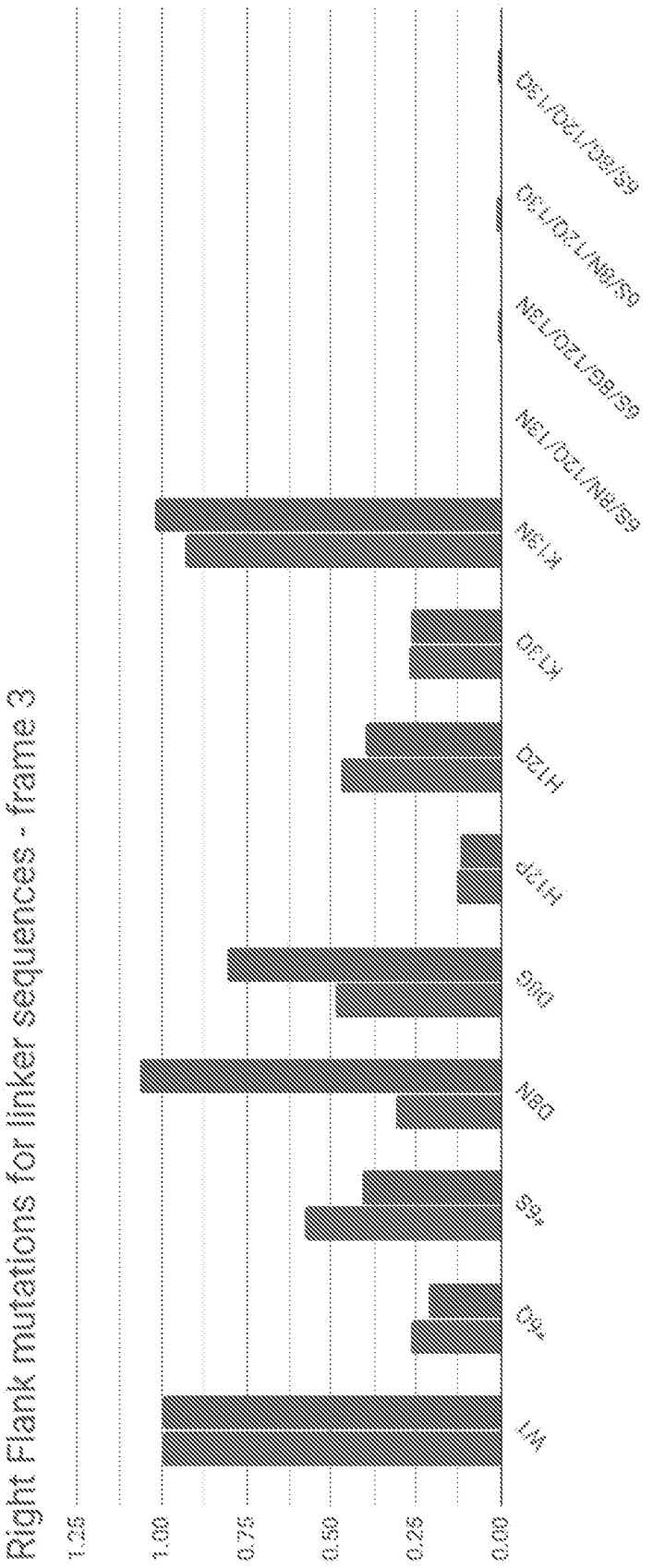

Right Flank mutations for linker sequences - frame 3
1.25
1.00
0.75
0.50
0.25
0.00
LM
0%
S%
N8Q
99Q
JQ1H
D21H
K13Q
N81%
N81/D21L/N8/S9
N81/D21L/S8/S9
N81/D21L/N8Q/S9
D81/D21/S9/S9

FIG. 75C

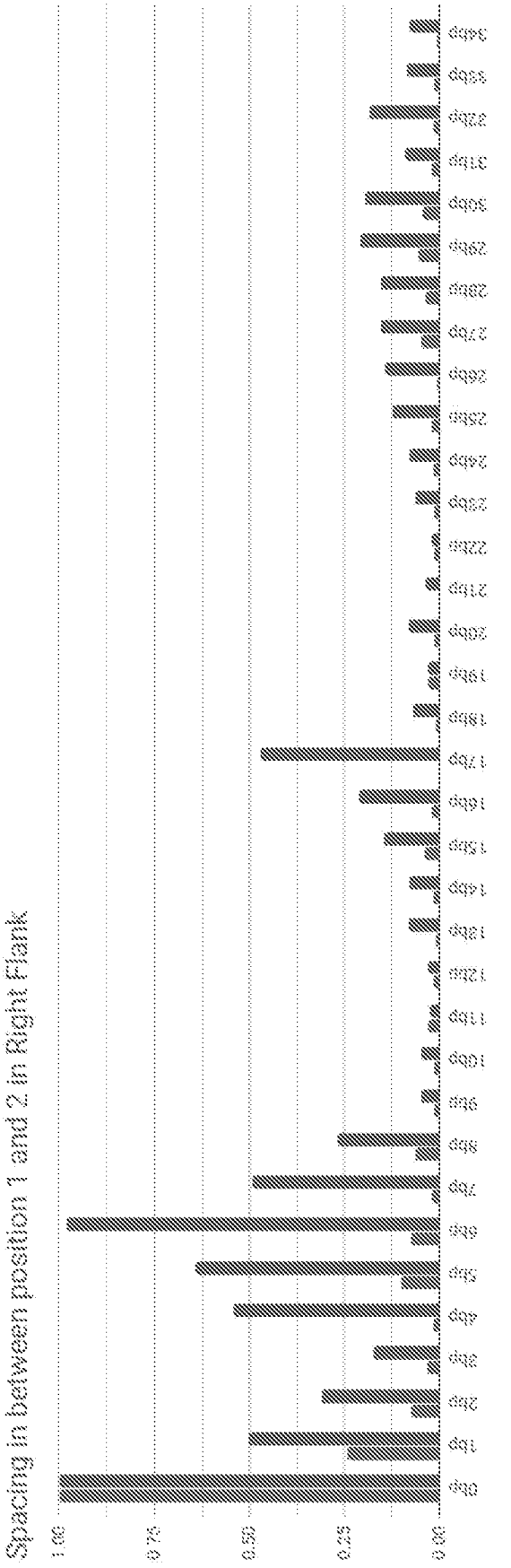


FIG. 76

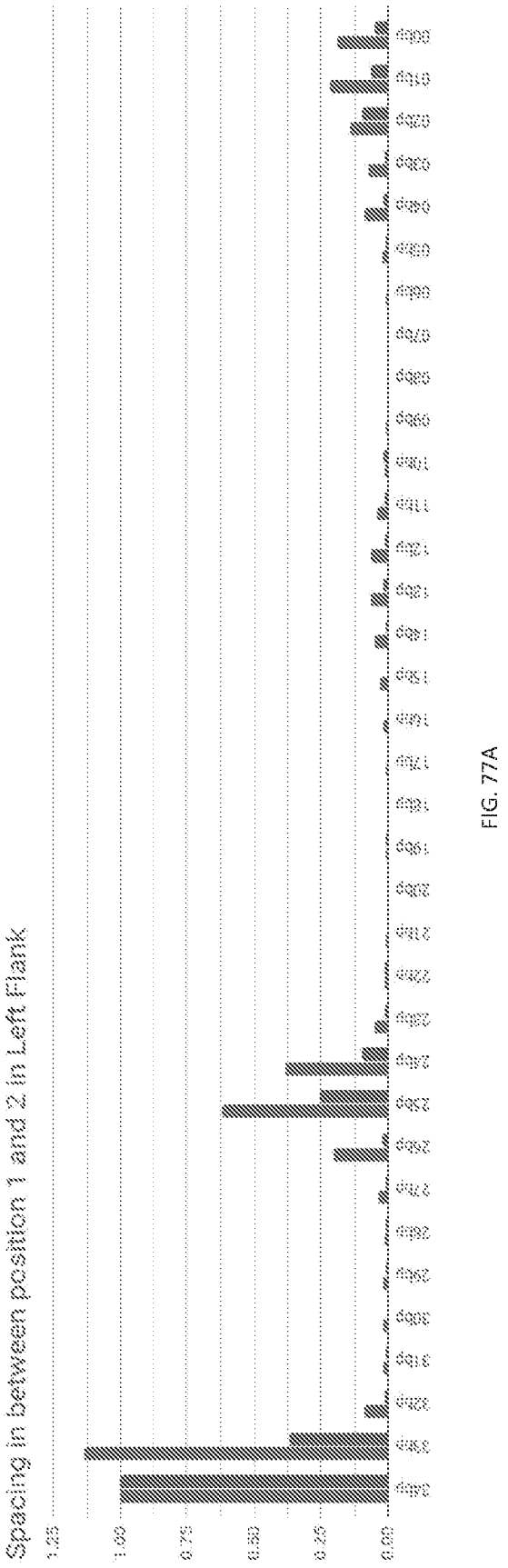


FIG. 77A

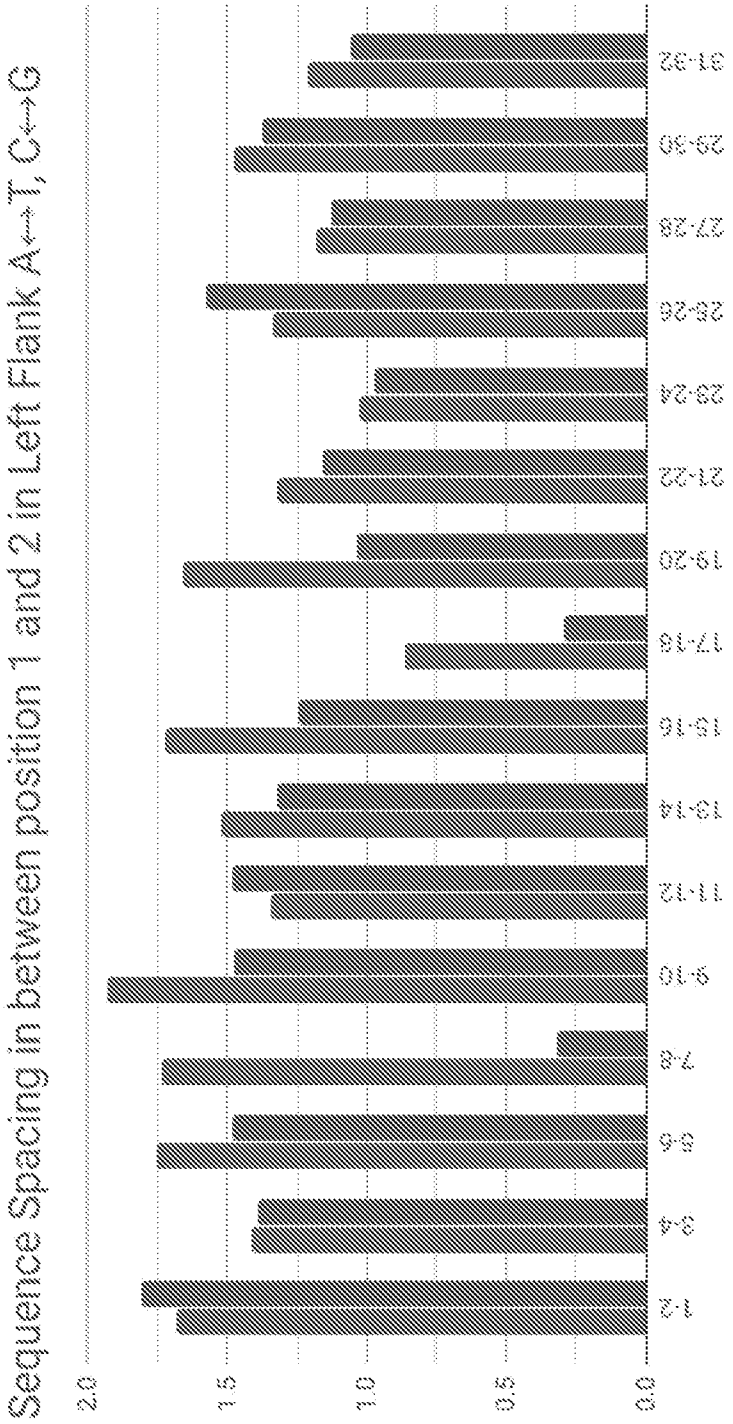

Sequence Spacing in between position 1 and 2 in Left Flank A↔T, C↔G
2.0
1.5
1.0
0.5
0.0
1-2
3-4
5-6
7-8
9-10
11-12
13-14
15-16
17-18
19-20
21-22
23-24
25-26
27-28
29-30
31-32

FIG. 77B

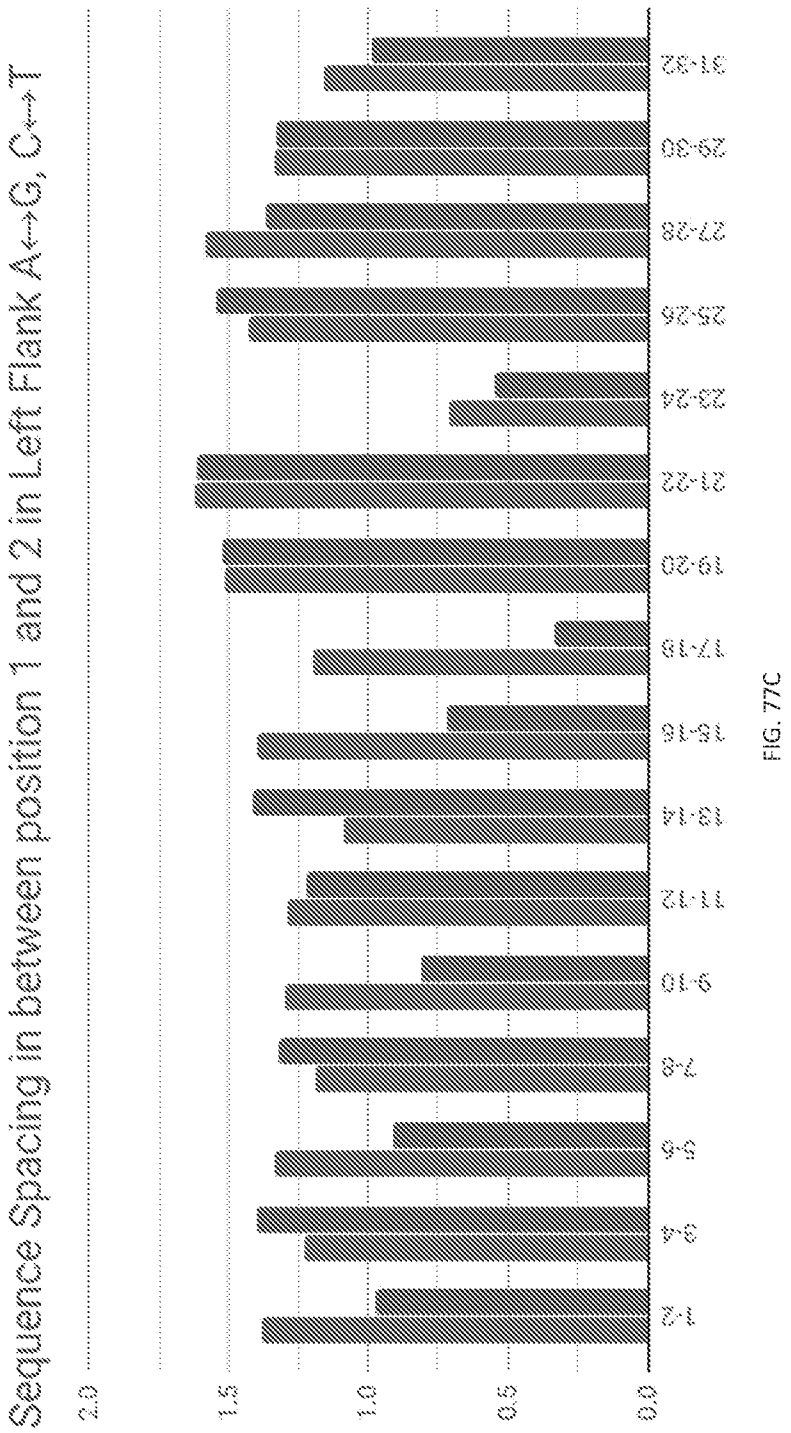

Sequence Spacing in between position 1 and 2 in Left Flank A↔G, C↔T
2.0
1.5
1.0
0.5
0.0
1-2
3-4
5-6
7-8
9-10
11-12
13-14
15-16
17-19
19-20
21-22
23-24
25-26
27-28
29-30
31-32

FIG. 77C

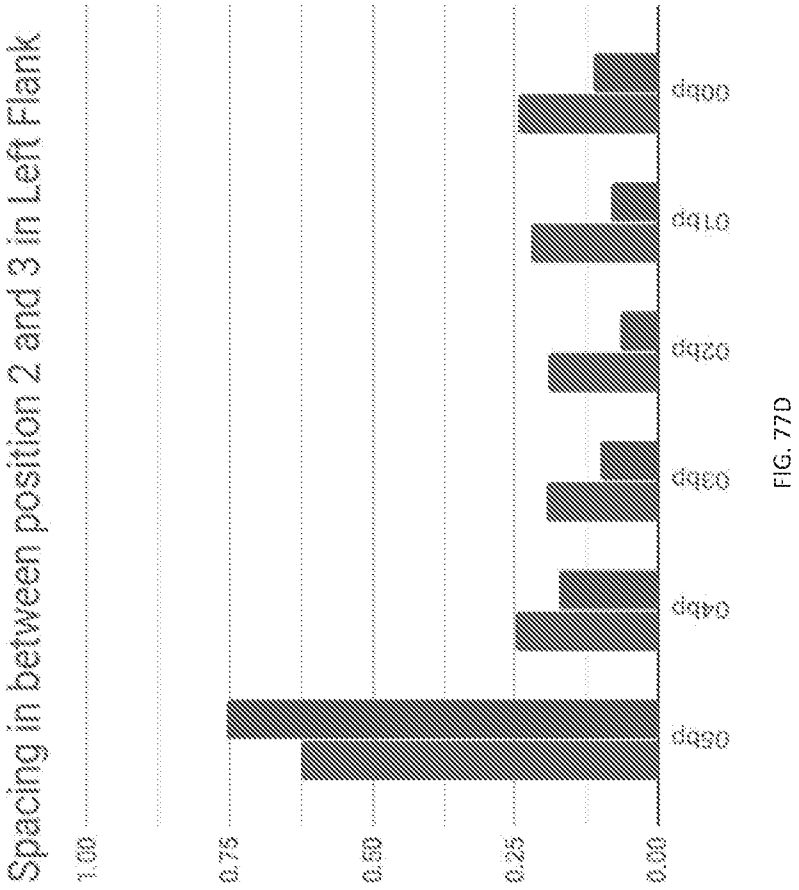

Spacing in between position 2 and 3 in Left Flank
1.00
0.75
0.50
0.25
0.00
dq00
dq10
dq20
dq30
dq40
dq50

FIG. 77D

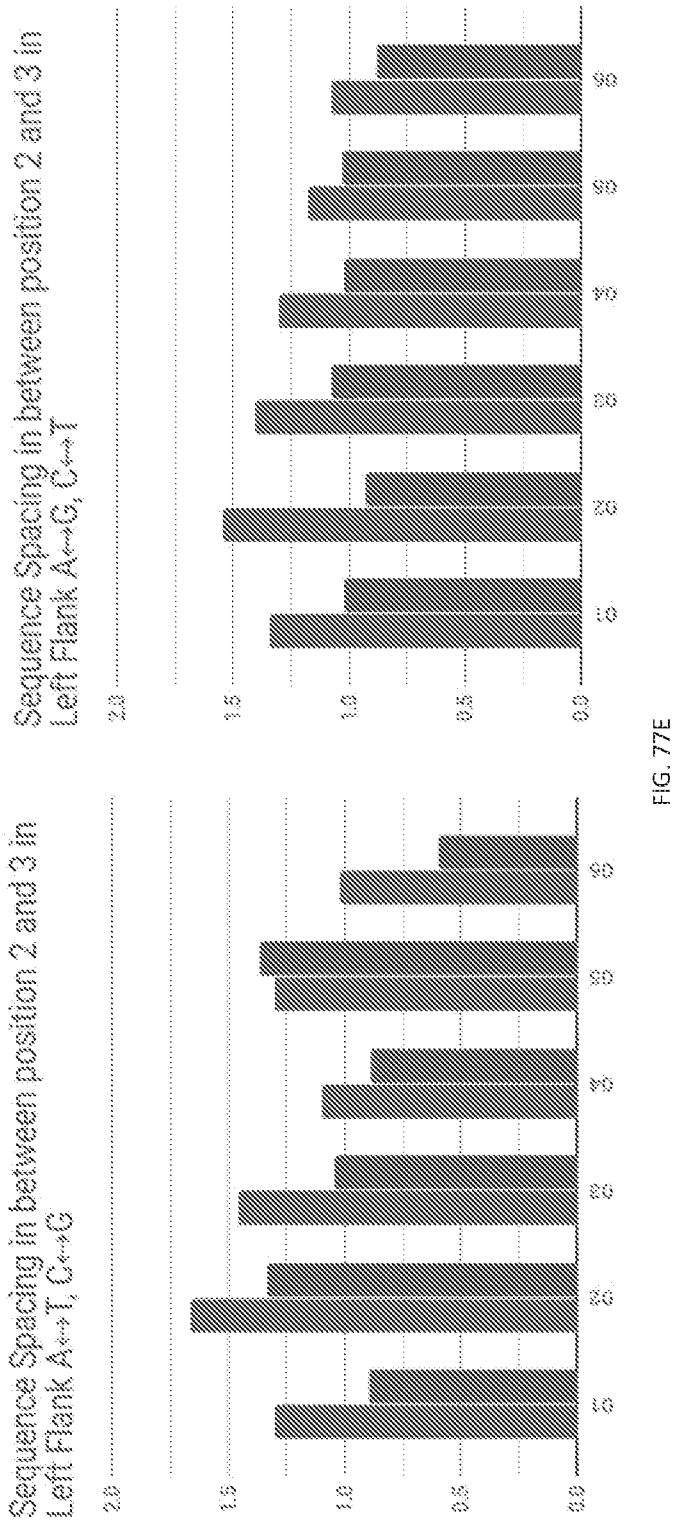

Sequence Spacing in between position 2 and 3 in Left Flank A↔T, C↔G
2.0
1.5
1.0
0.5
0.0
5
10
15
20
25
30
35
40
Sequence Spacing in between position 2 and 3 in Left Flank A↔G, C↔T
2.0
1.5
1.0
0.5
0.0
10
20
30
40
50
60
70
80
90

FIG. 77E

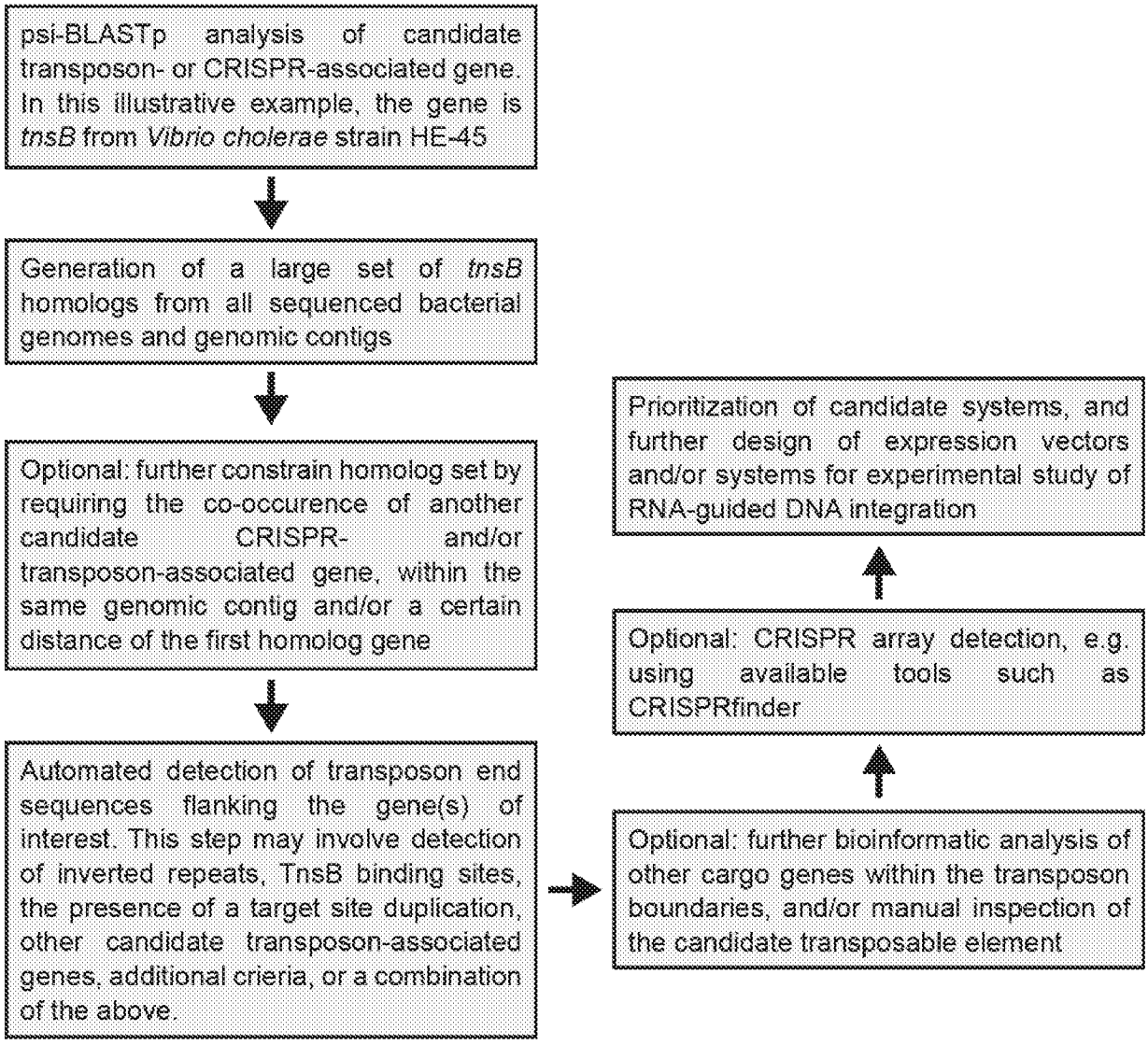

psi-BLASTp analysis of candidate transposon- or CRISPR-associated gene. In this illustrative example, the gene is *tnsB* from *Vibrio cholerae* strain HE-45
Generation of a large set of *tnsB* homologs from all sequenced bacterial genomes and genomic contigs
Optional: further constrain homolog set by requiring the co-occurence of another candidate CRISPR- and/or transposon-associated gene, within the same genomic contig and/or a certain distance of the first homolog gene
Automated detection of transposon end sequences flanking the gene(s) of interest. This step may involve detection of inverted repeats, TnsB binding sites, the presence of a target site duplication, other candidate transposon-associated genes, additional crieria, or a combination of the above.
Optional: further bioinformatic analysis of other cargo genes within the transposon boundaries, and/or manual inspection of the candidate transposable element
Optional: CRISPR array detection, e.g. using available tools such as CRISPRfinder
Prioritization of candidate systems, and further design of expression vectors and/or systems for experimental study of RNA-guided DNA integration

FIG. 78

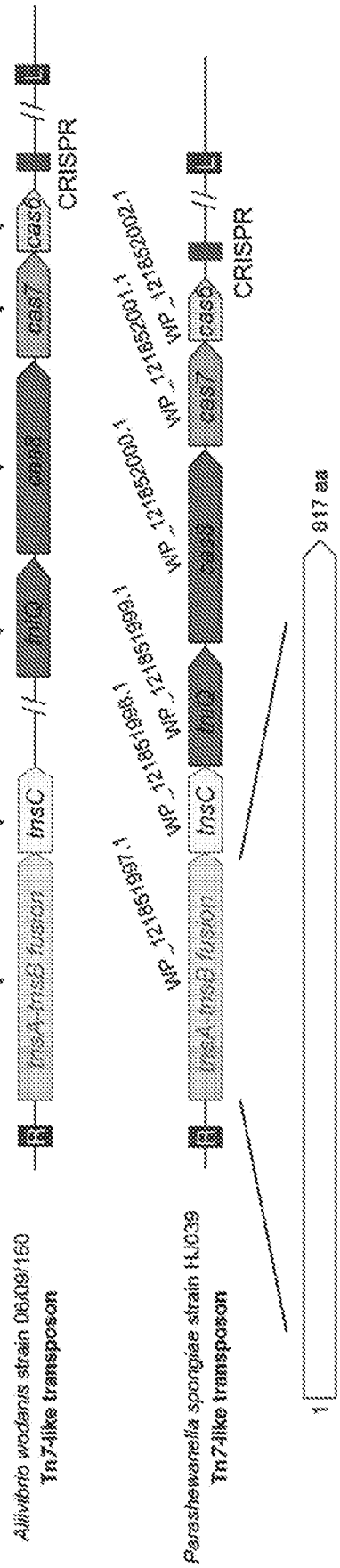

Allivibrio wodanis strain 06/09/160
Tn7-like transposon
tnsA-tnsB fusion
tnsC
CRISPR
cas7
cas6
Parashewanella spongiae strain HU039
Tn7-like transposon
817 aa
PF05367.11
Phage_endo_I
PF09039.11
HTH_Tnp_Mu_2
PF02914.15
DDE_2

FIG. 79

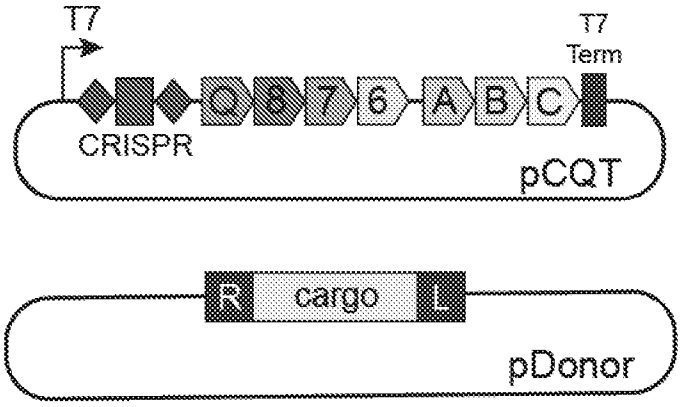


FIG. 80A

Organism ID	pCQT (crRNA-4)	pDonor
Vibrio cholerae strain HE-45	pSL1022 (SEQ ID NO: 855)	pSL1235 (SEQ ID NO: 1614)
Vibrio cholerae strain 4874	pSL1781 (SEQ ID NO: 1623)	pSL1711 (SEQ ID NO: 1615)
Photobacterium iliopiscarium strain NCIMB	pSL1782 (SEQ ID NO: 1624)	pSL1712 (SEQ ID NO: 1616)
Pseudoalteromonas sp. P1-25	pSL1783 (SEQ ID NO: 1625)	pSL1713 (SEQ ID NO: 1617)
Pseudoalteromonas ruthenica strain S3245	pSL1784 (SEQ ID NO: 1626)	pSL1714 (SEQ ID NO: 1618)
Photobacterium ganghwense strain JCM	pSL1785 (SEQ ID NO: 1627)	pSL1715 (SEQ ID NO: 1619)
Shewanella sp. UCD-KL21	pSL1786 (SEQ ID NO: 1628)	pSL1716 (SEQ ID NO: 1620)
Vibrio diazotrophicus strain 60.6F	pSL1787 (SEQ ID NO: 1903)	pSL1717 (SEQ ID NO: 1897)
Vibrio cholerae strain OYP7G04	pSL1788 (SEQ ID NO: 1629)	pSL1718 (SEQ ID NO: 1621)
Vibrio sp. 16	pSL1789 (SEQ ID NO:1904)	pSL1719 (SEQ ID NO: 1898)
Vibrio sp. F12	pSL1790 (SEQ ID NO:1905)	pSL1720 (SEQ ID NO: 1899)
Vibrio cholerae strain M1517	pSL1792 (SEQ ID NO: 1630)	pSL1722 (SEQ ID NO: 1622)
Vibrio splendidus strain UCD-SED10	pSL1793 (SEQ ID NO: 1906)	pSL1723 (SEQ ID NO: 1900)
Aliivibrio wodanis 06/06/160	pSL1794 (SEQ ID NO: 1907)	pSL1724 (SEQ ID NO: 1901)
Parashewanella spongiae strain HJ039	pSL1795 (SEQ ID NO: 1908)	pSL1725 (SEQ ID NO: 1902)

FIG. 80B

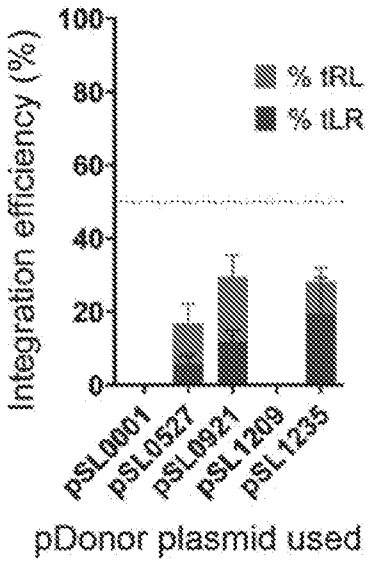


FIG. 81

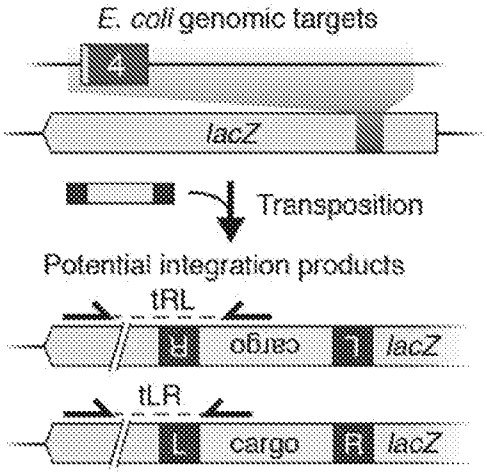
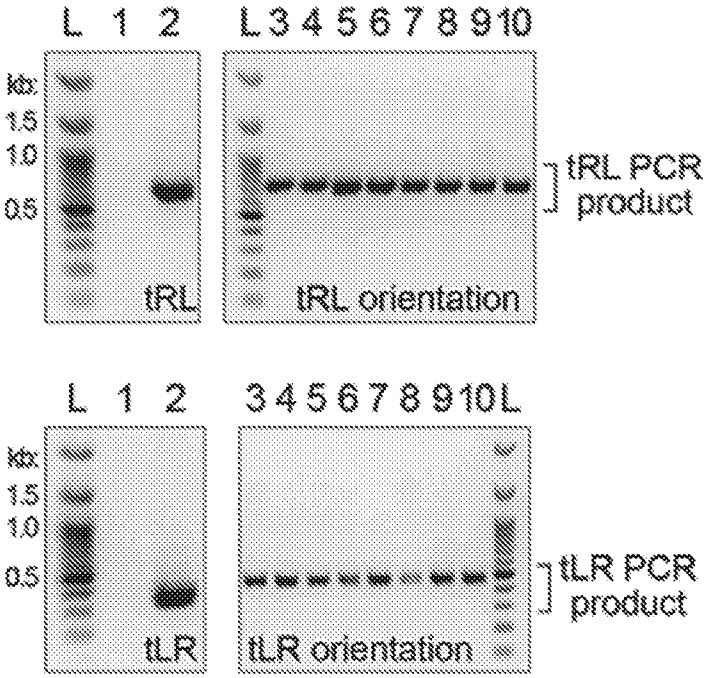


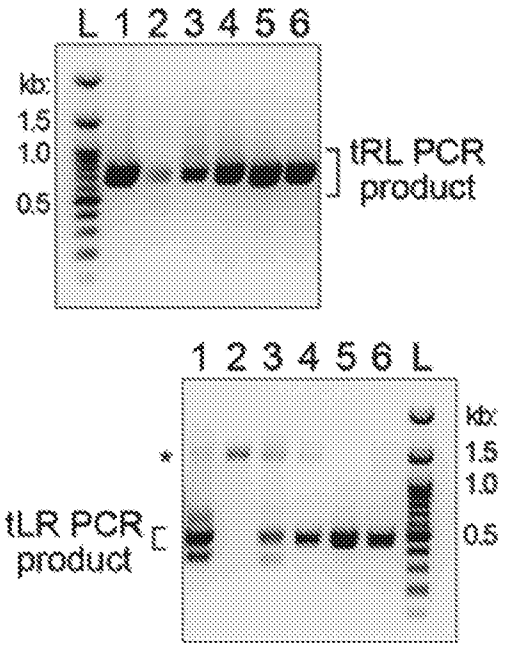
FIG. 82A



Legend:

L: ladder
1: pSL1022 + pSL0001
2: pSL1022 + pSL1235
3: pSL1781 + pSL1711
4: pSL1782 + pSL1712
5: pSL1783 + pSL1713
6: pSL1784 + pSL1714
7: pSL1785 + pSL1715
8: pSL1786 + pSL1716
9: pSL1788 + pSL1718
10: pSL1792 + pSL1722

FIG. 82B



Legend:

L: ladder
1: pSL1787 + pSL1717
2: pSL1789 + pSL1719
3: pSL1790 + pSL1720
4: pSL1793 + pSL1723
5: pSL1794 + pSL1724
6: pSL1795 + pSL1725

FIG. 82C

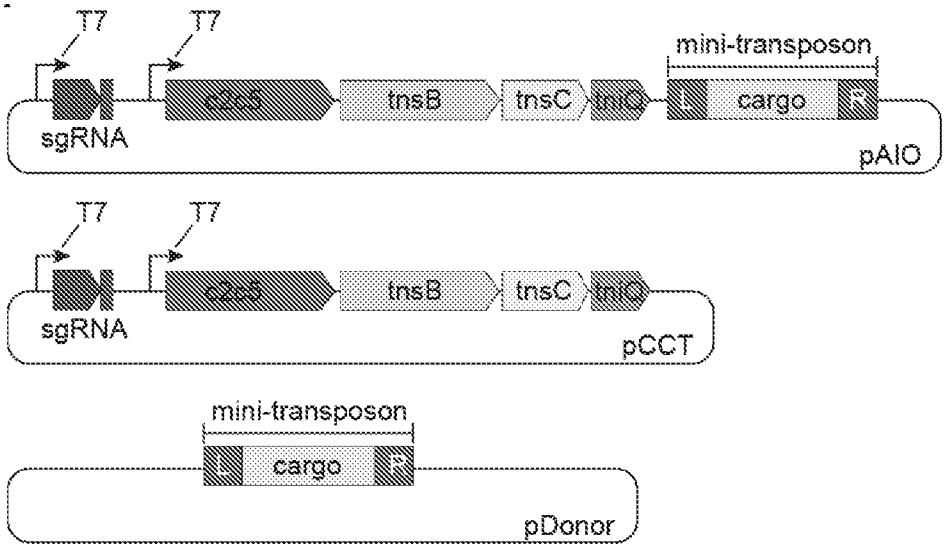


FIG. 83A

Scytonema hofmannii strain PCC 7110

	plasmid ID
pAIO (NT/cloning)	pSL1117
pCQT (NT/cloning)	pSL1114
pDonor (2kb transposon)	pSL0948

FIG. 83B

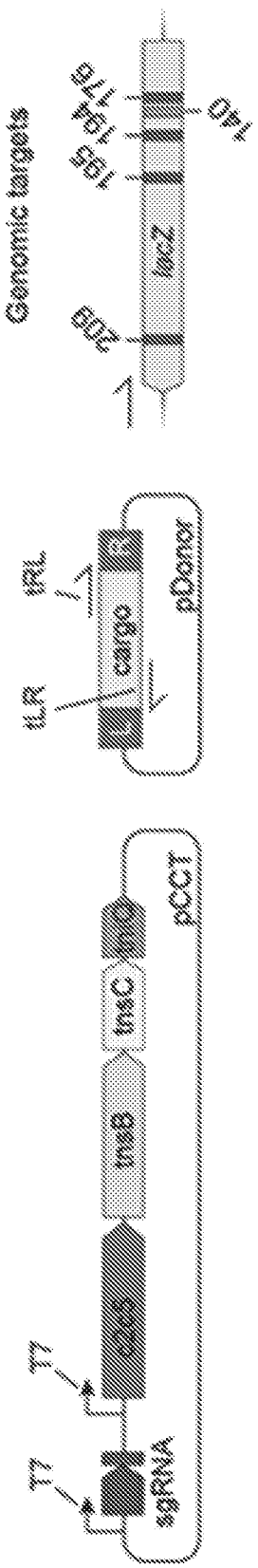


FIG. 84A

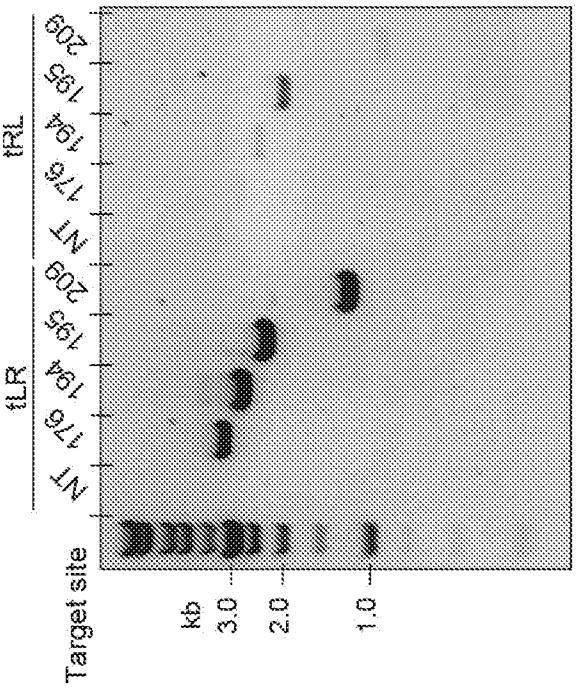


FIG. 84B

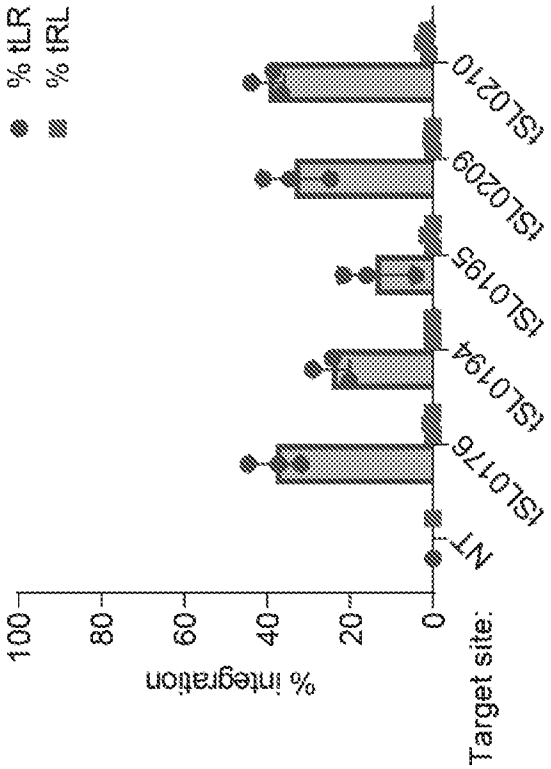
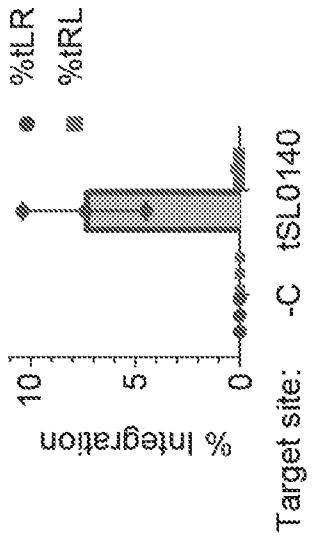


FIG. 84C



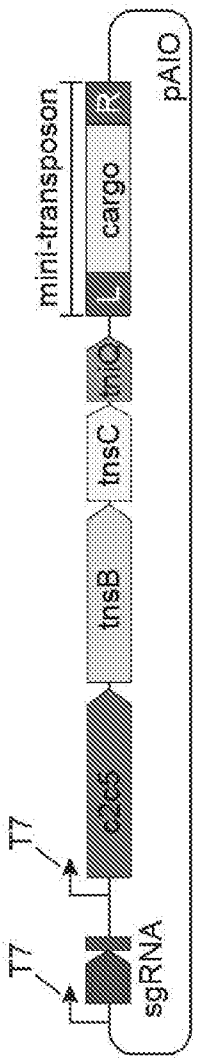


FIG. 84D

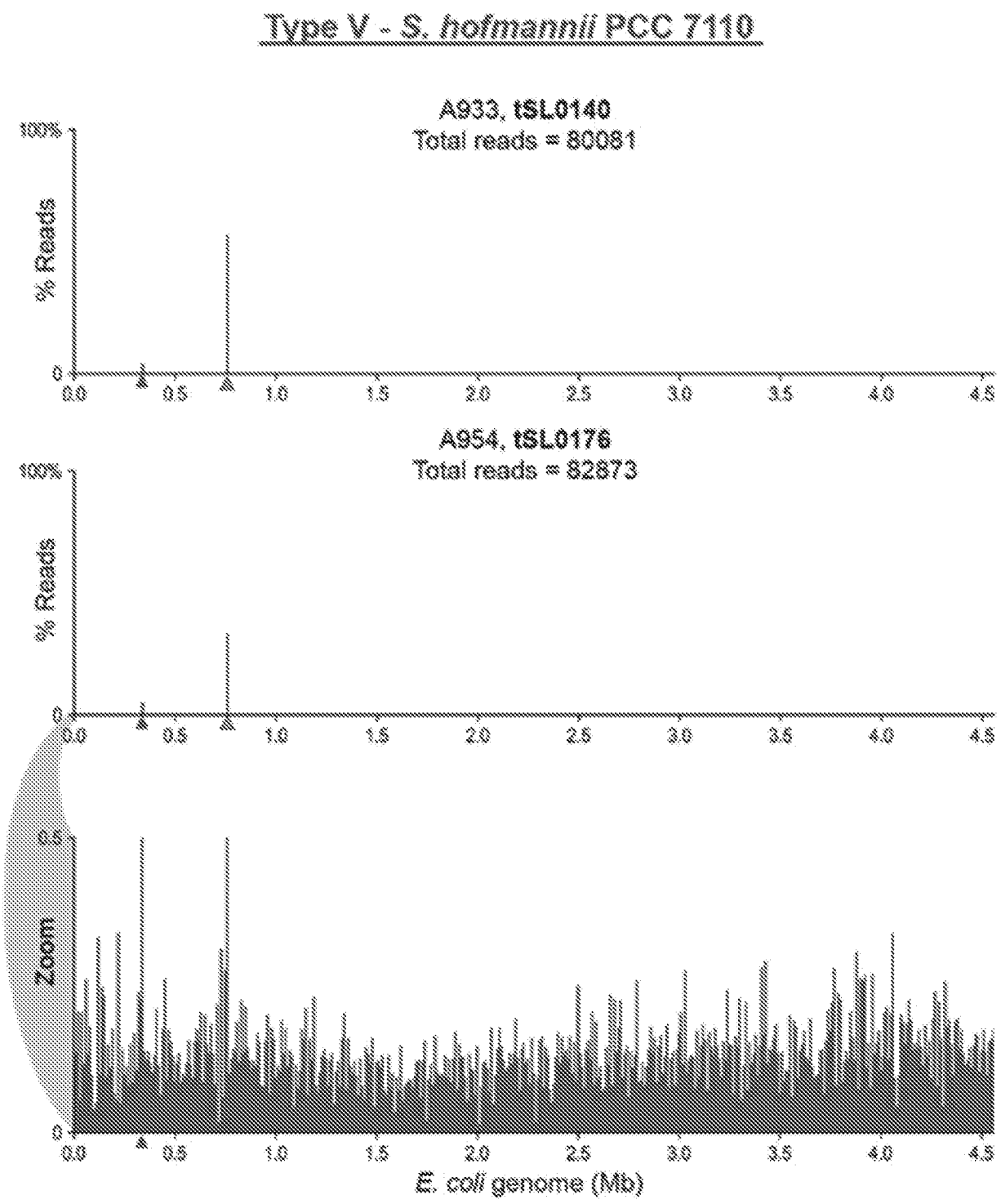

Type V - S. hofmannii PCC 7110
A933, tSL0140
Total reads = 80081
100%
% Reads
0
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
A954, tSL0176
Total reads = 82873
100%
% Reads
0
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
Zoom
0.5
0
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
E. coli genome (Mb)

FIG. 85A

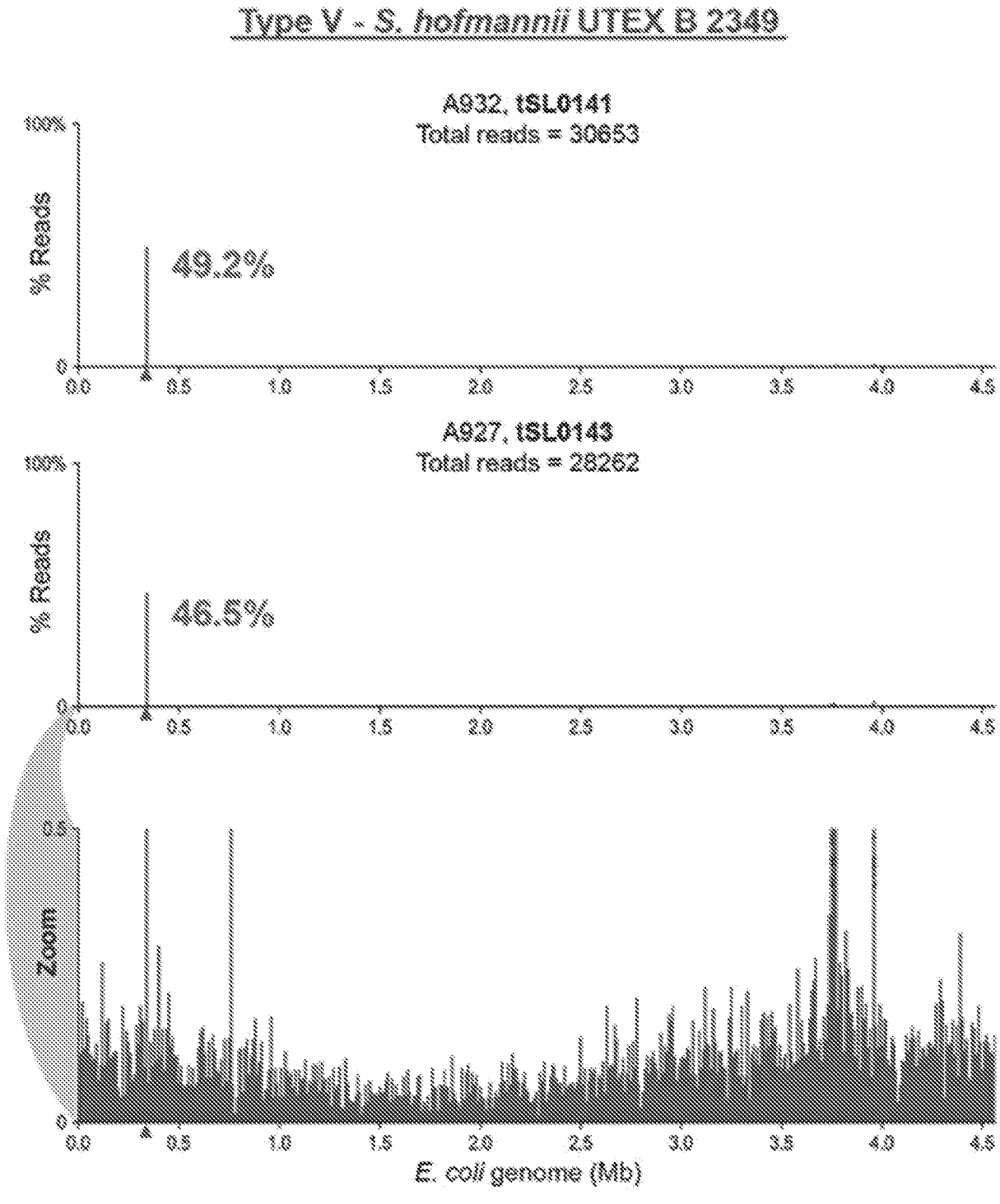

Type V - S. hofmannii UTEX B 2349
A932, tSL0141
Total reads = 30653
% Reads
100%
0
49.2%
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
A927, tSL0143
Total reads = 28262
% Reads
100%
0
46.5%
Zoom
0.5
0
E. coli genome (Mb)

FIG. 85B

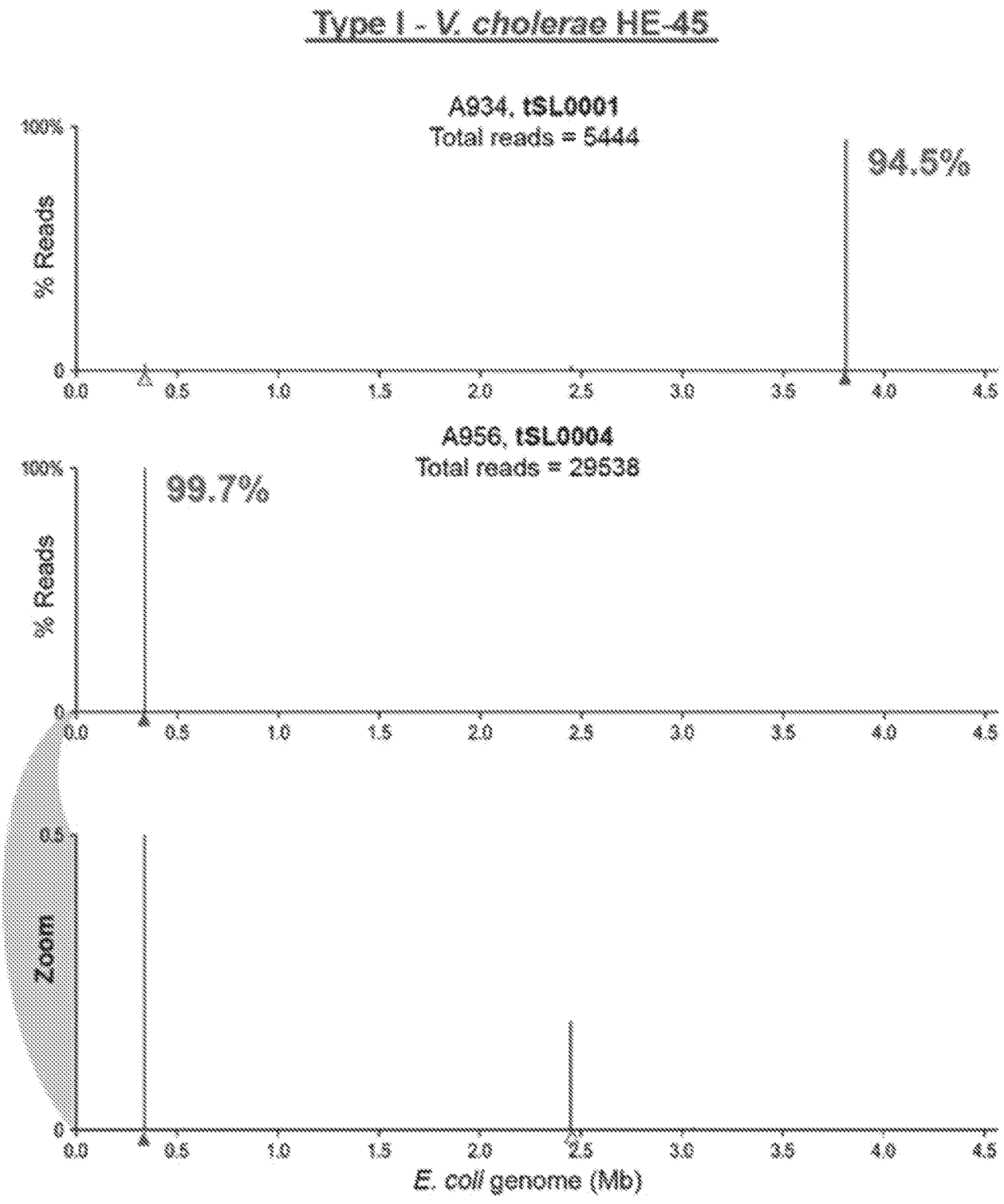

Type I - V. cholerae HE-45
A934, tSL0001
Total reads = 5444
94.5%
% Reads
100%
0
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
A956, tSL0004
Total reads = 29538
99.7%
% Reads
100%
0
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
Zoom
0.5
0
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
E. coli genome (Mb)

FIG. 85C

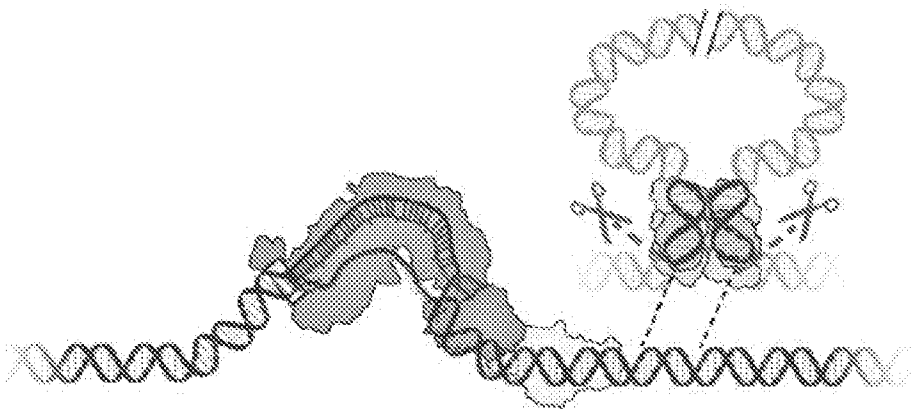


FIG. 86A

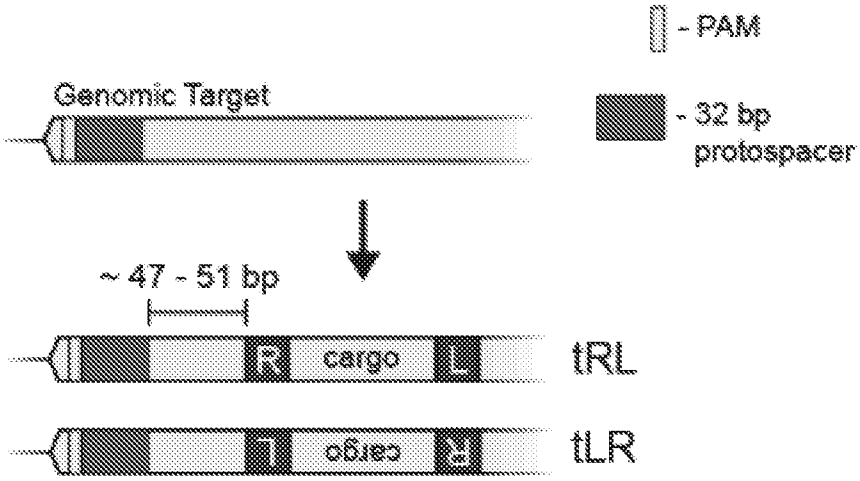


FIG. 86B

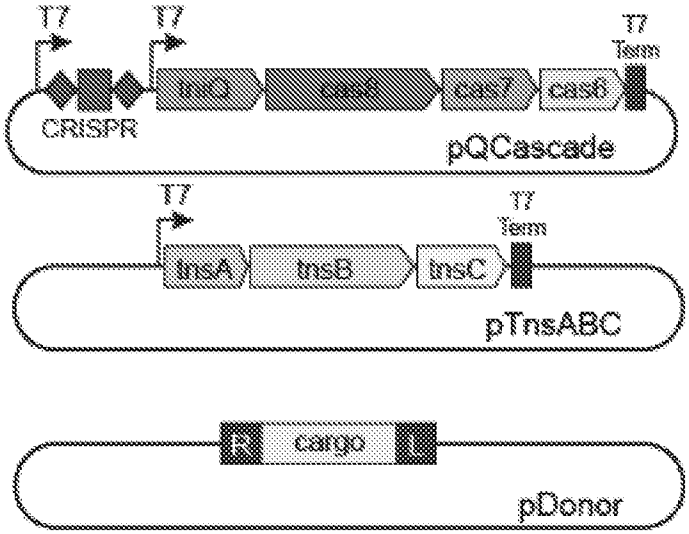


FIG. 86C

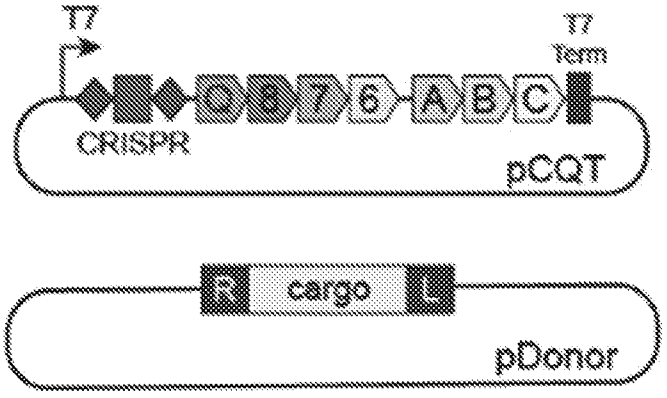


FIG. 86D

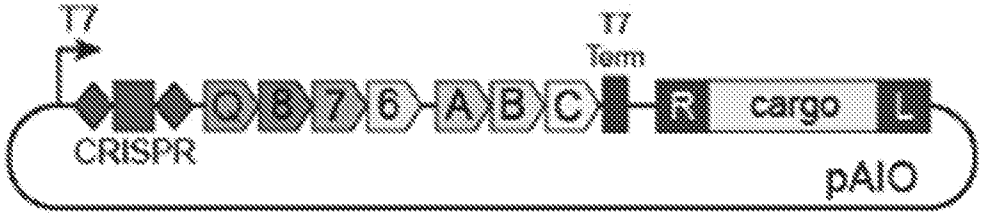


FIG. 86E

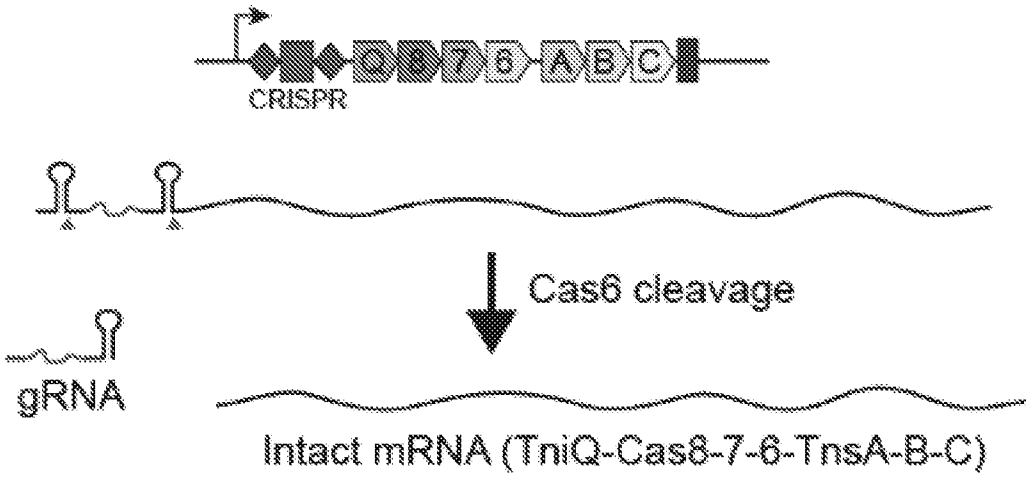


FIG. 86F

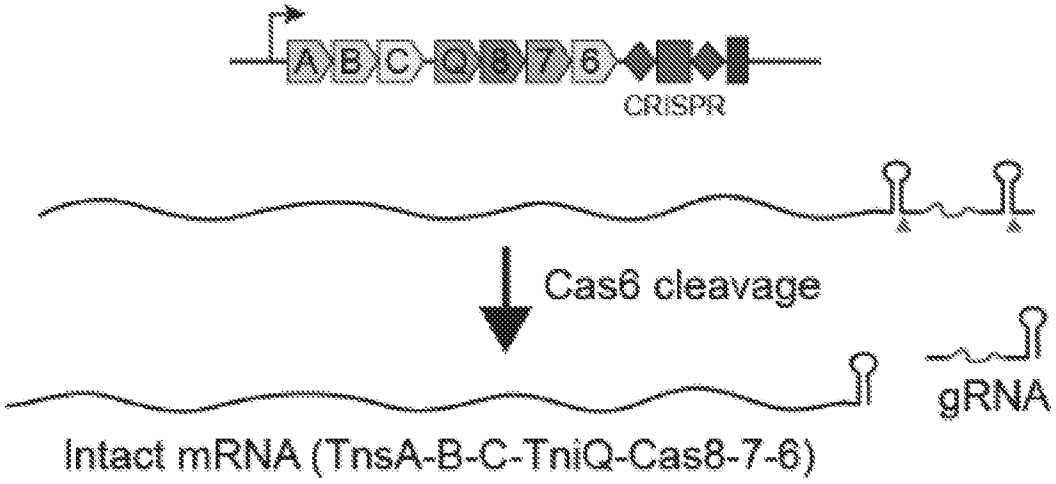
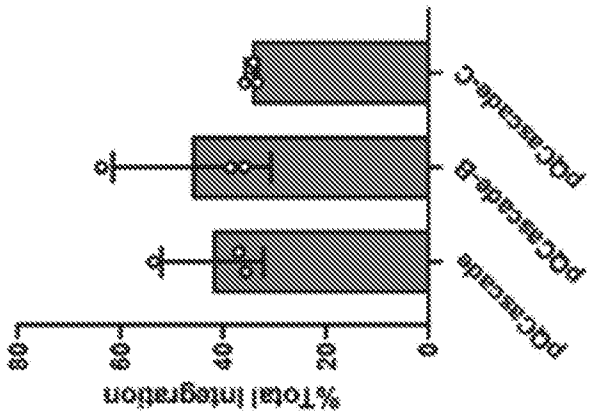

A
B
C
7
6
CRISPR
Cas6 cleavage
gRNA
Intact mRNA (TnsA-B-C-TniQ-Cas8-7-6)

FIG. 86G


pQCascade
pQCascade-B
pQCascade-C
% Total Integration
0
20
40
60
80

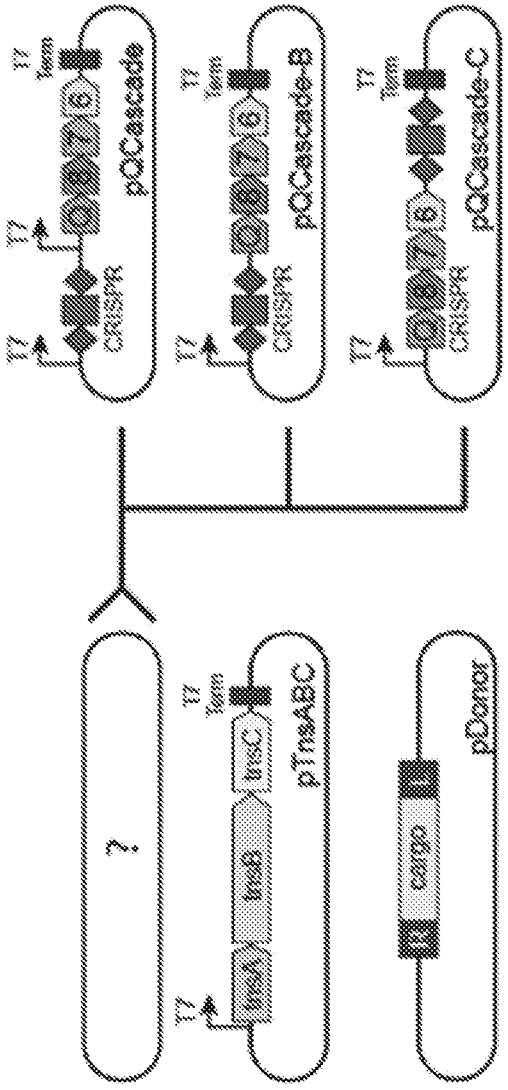

T7
Term
pQCascade
CRISPR
pQCascade-B
pQCascade-C
?
pTnsABC
tnsA
tnsB
tnsC
pDonor
cargo

FIG. 87A

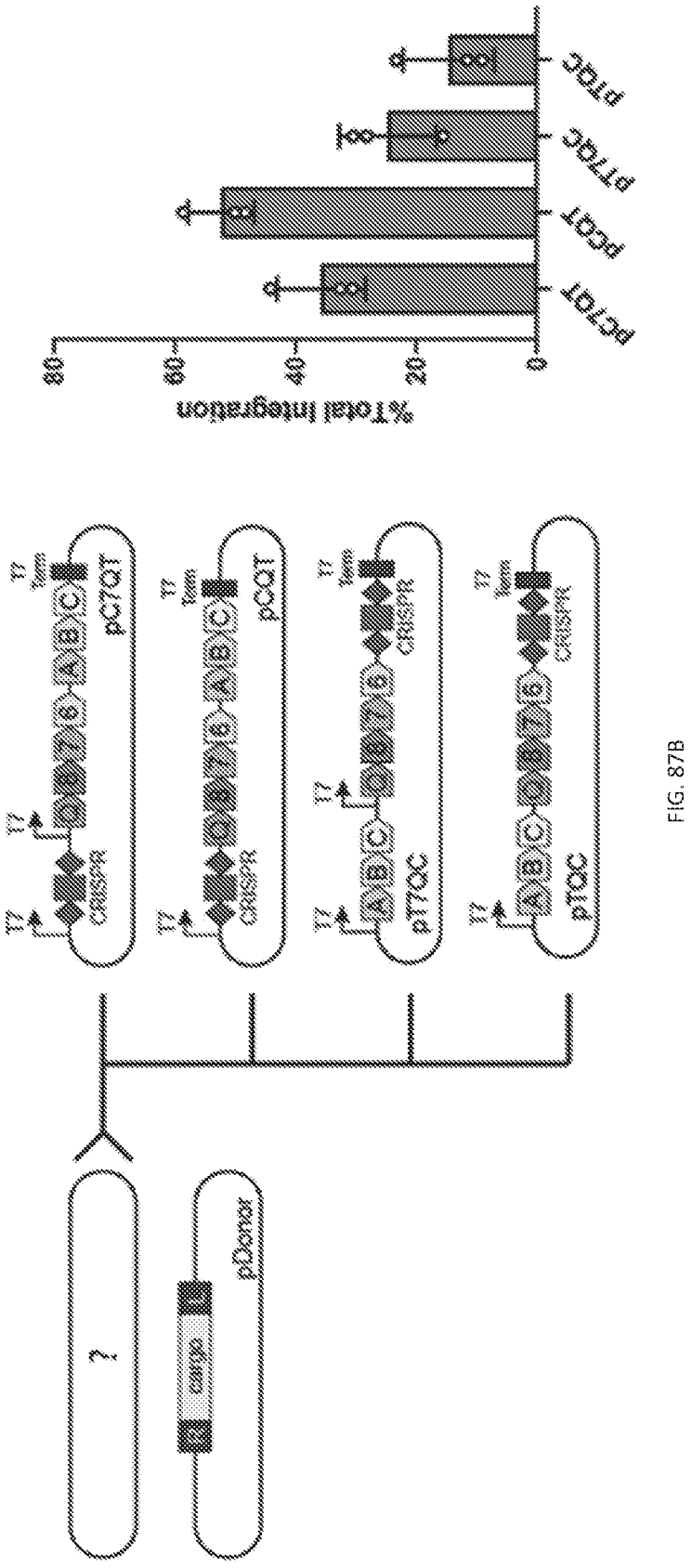

%Total integration
80
60
40
20
0
pC7QT
pCQT
pTTQC
pTQC
T7
Term
CRISPR
A
B
C
D
E
F
G
?
cargo
pDonor

FIG. 87B

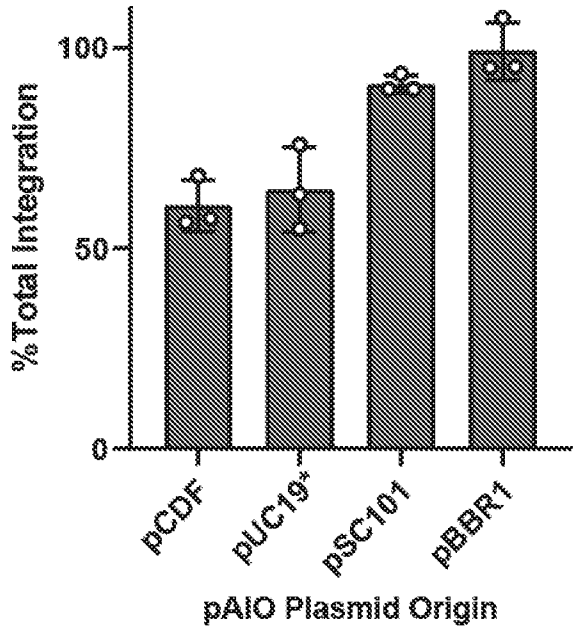


FIG. 88A

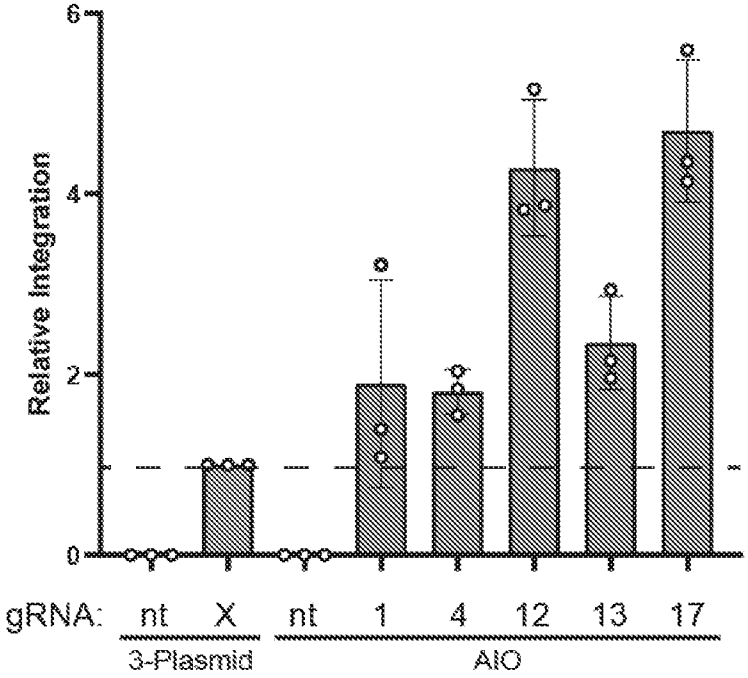


FIG. 88B

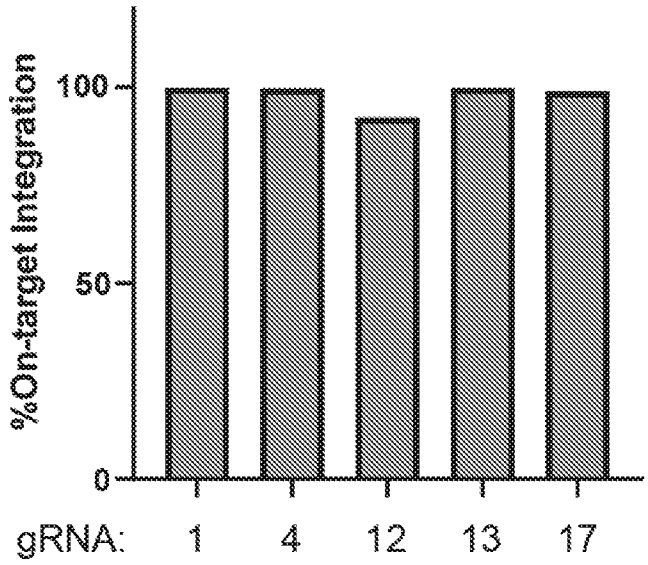


FIG. 88C

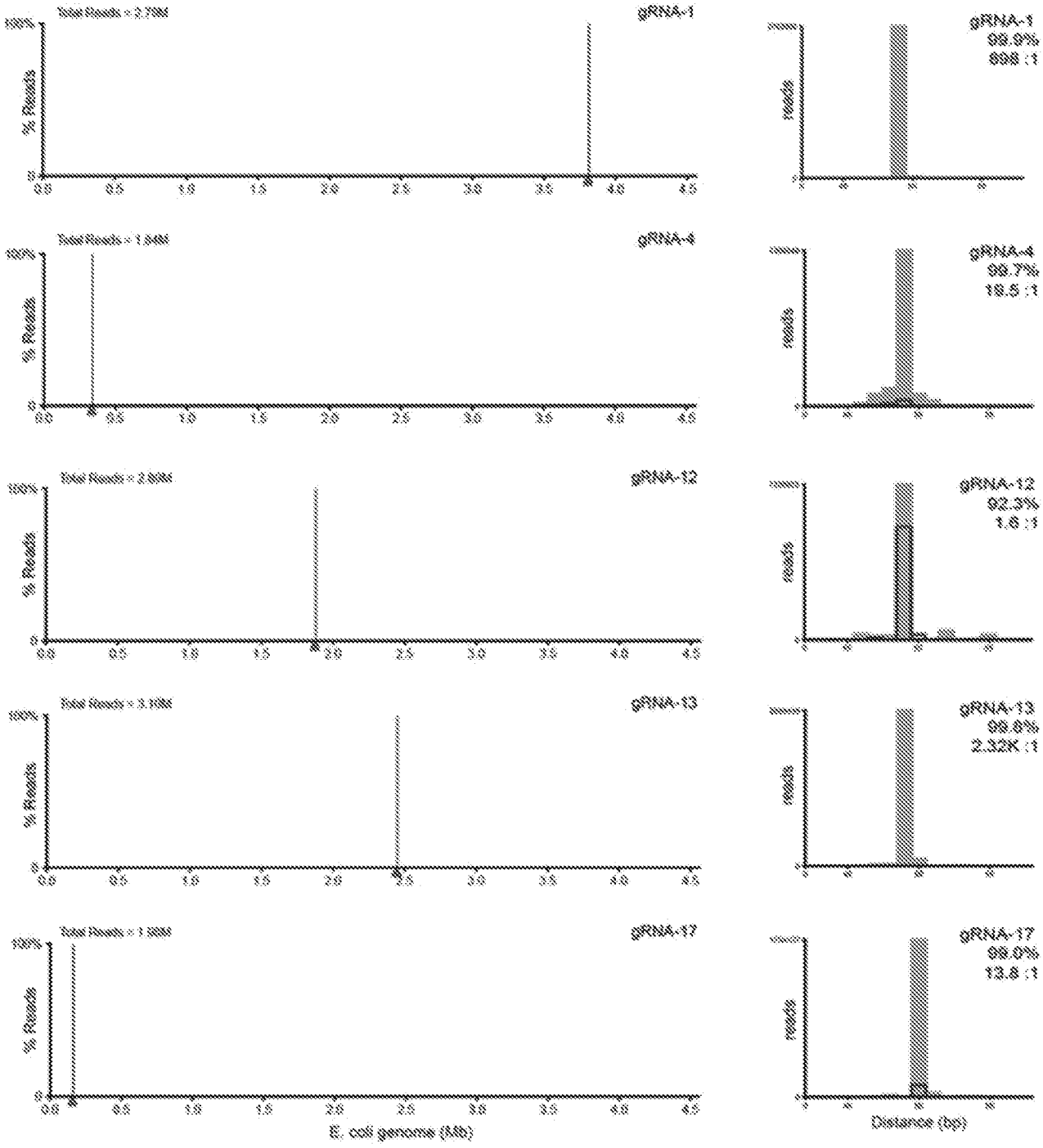

gRNA-1
gRNA-1
99.9%
698 :1
gRNA-4
gRNA-4
99.7%
18.5 :1
gRNA-12
gRNA-12
92.3%
1.6 :1
gRNA-13
gRNA-13
99.8%
2.32K :1
gRNA-17
gRNA-17
99.0%
13.8 :1
% Reads
Reads
E. coli genome (Mb)
Distance (bp)

FIG. 89

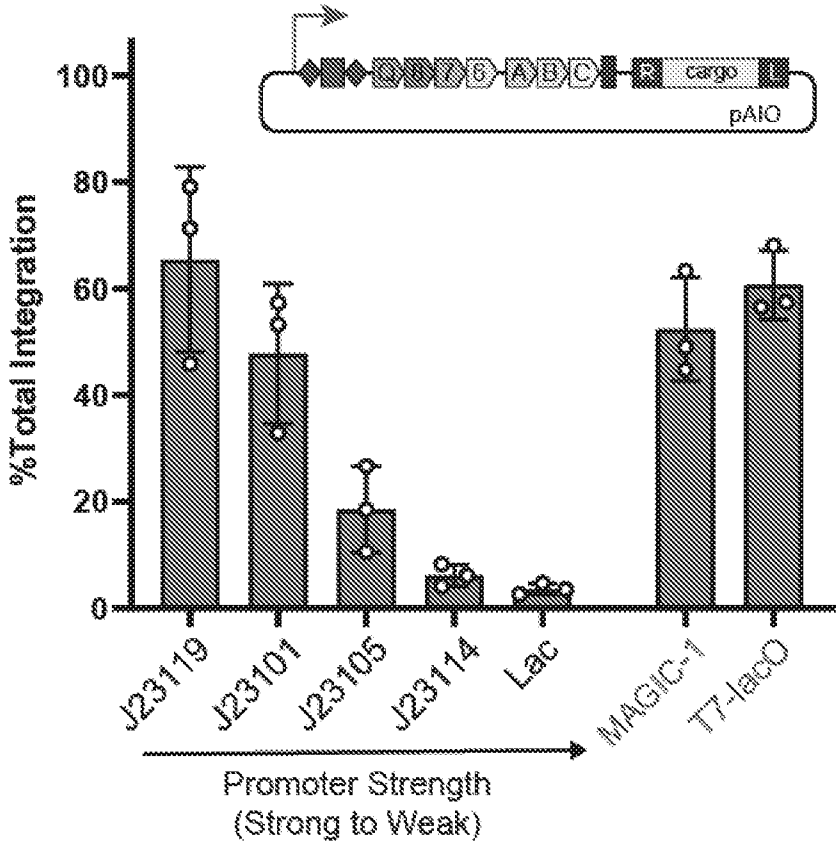


FIG. 90A

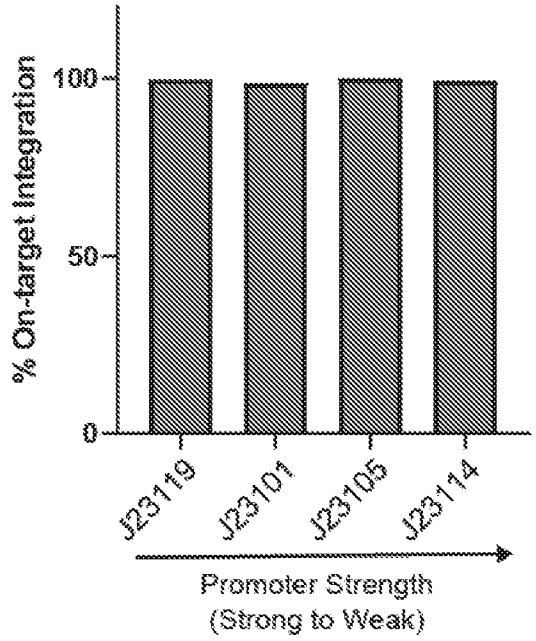


FIG. 90B

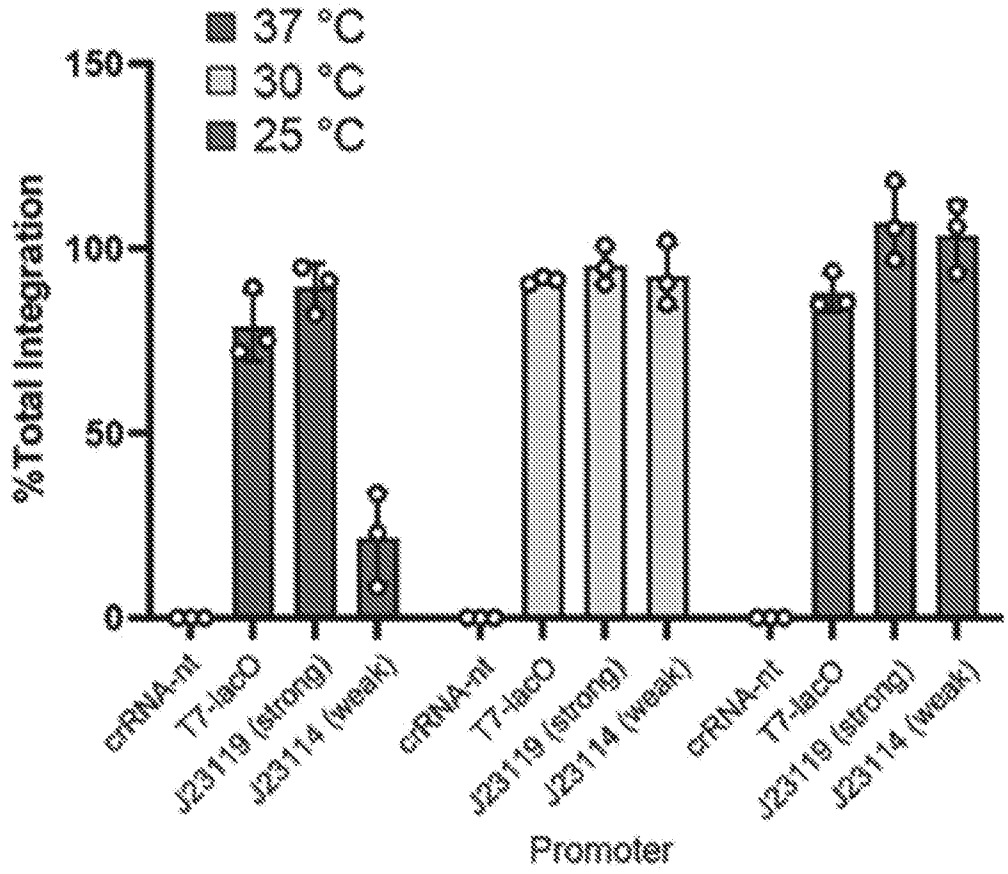

37 °C
30 °C
25 °C
%Total Integration
150
100
50
0
crRNA-nt
T7-lacO
J23119 (strong)
J23114 (weak)
crRNA-nt
T7-lacO
J23119 (strong)
J23114 (weak)
crRNA-nt
T7-lacO
J23119 (strong)
J23114 (weak)
Promoter

FIG. 90C

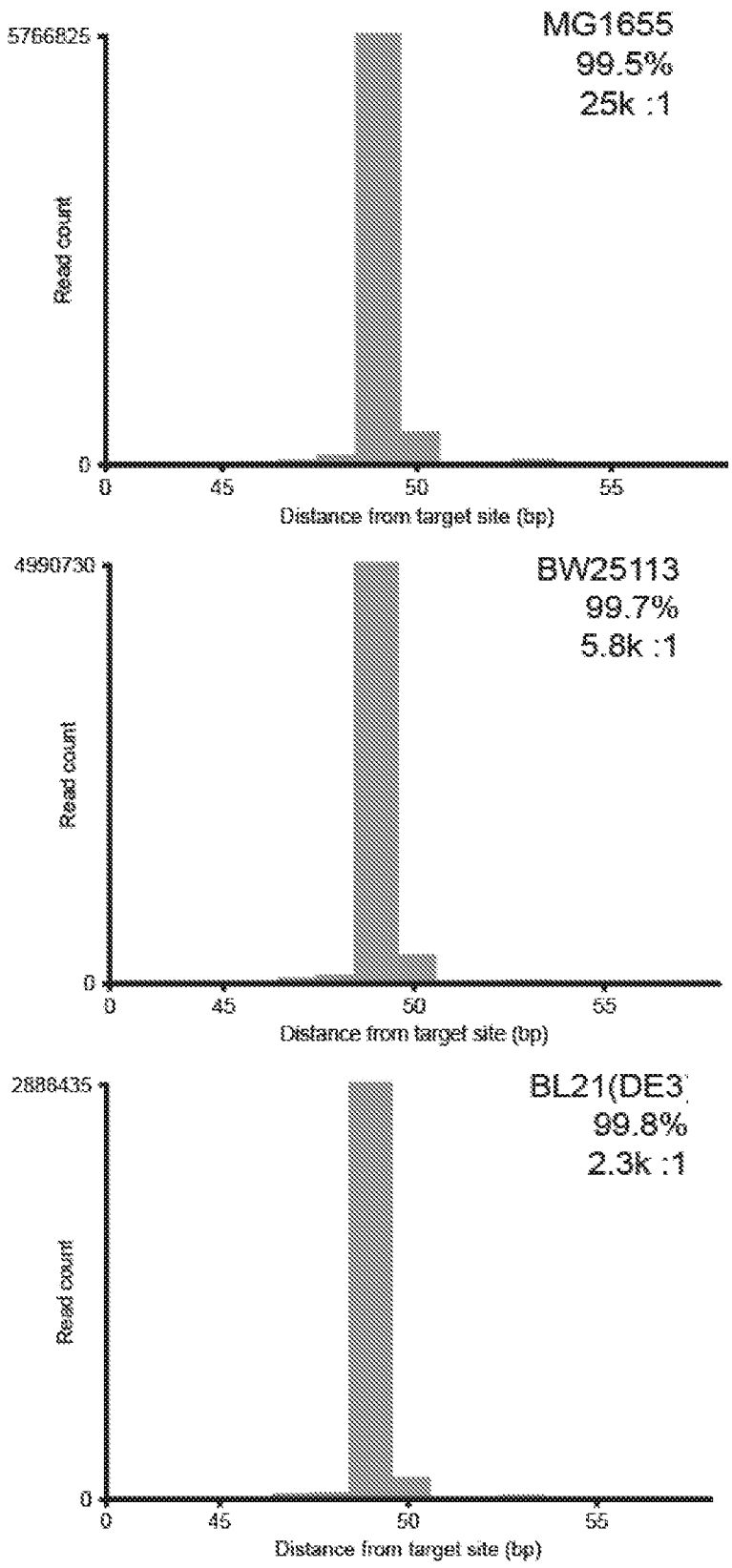

MG1655
99.5%
25k :1
5766825
Read count
0
0
45
50
55
Distance from target site (bp)
BW25113
99.7%
5.8k :1
4990730
Read count
0
0
45
50
55
Distance from target site (bp)
BL21(DE3
99.8%
2.3k :1
2888435
Read count
0
0
45
50
55
Distance from target site (bp)

FIG. 91A

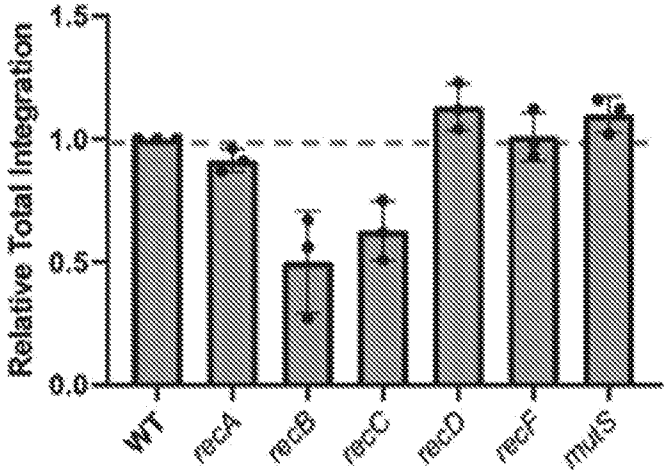


FIG. 91B

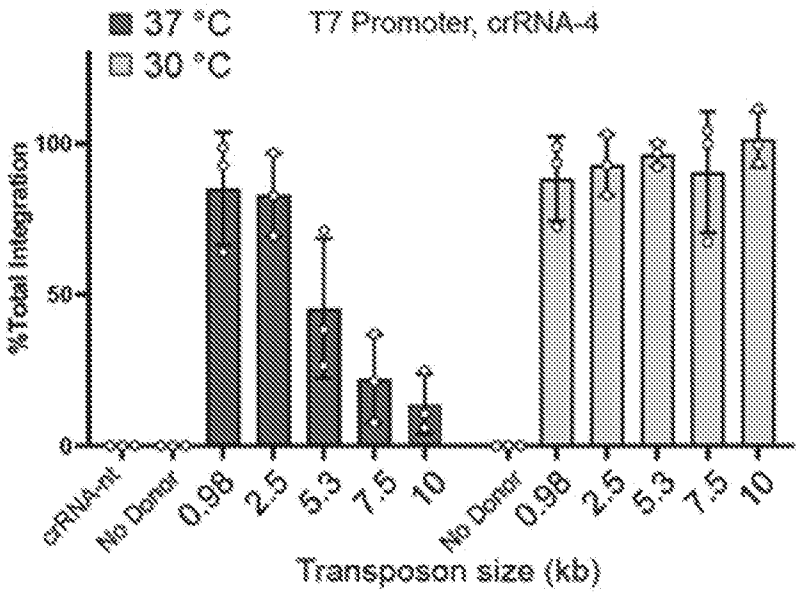


FIG. 92A

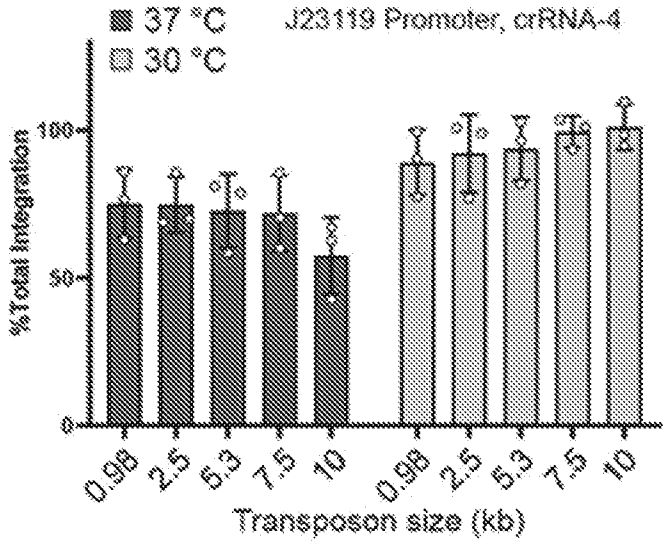


FIG. 92B

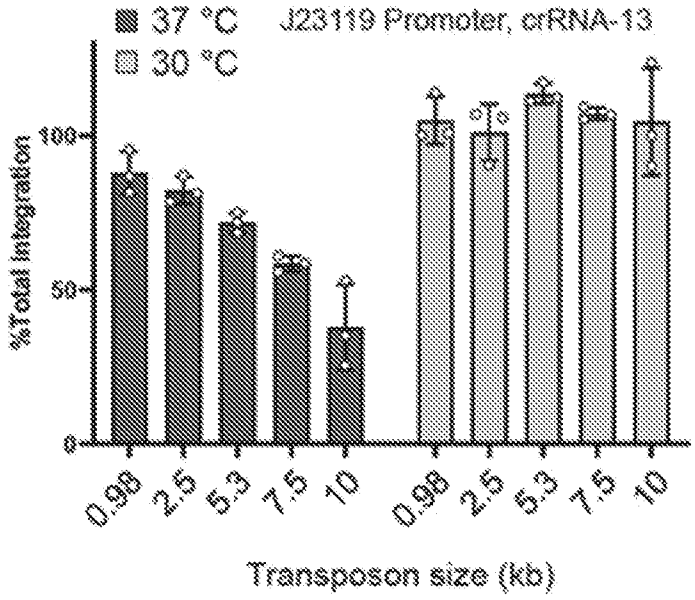


FIG. 92C

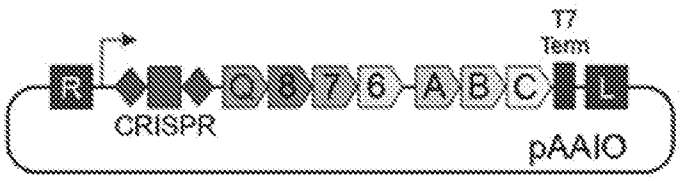


FIG. 93A

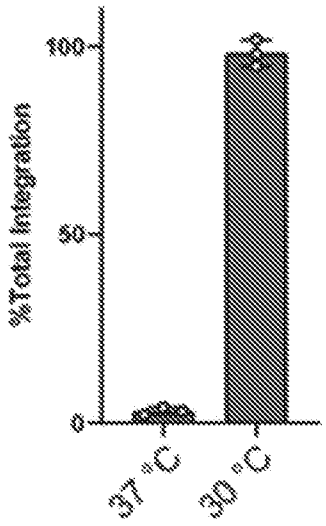


FIG. 93B

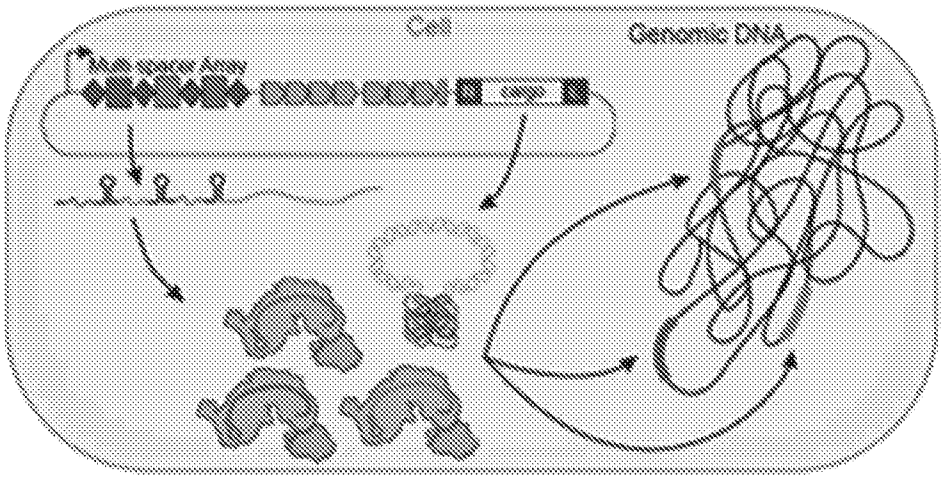


FIG. 94A

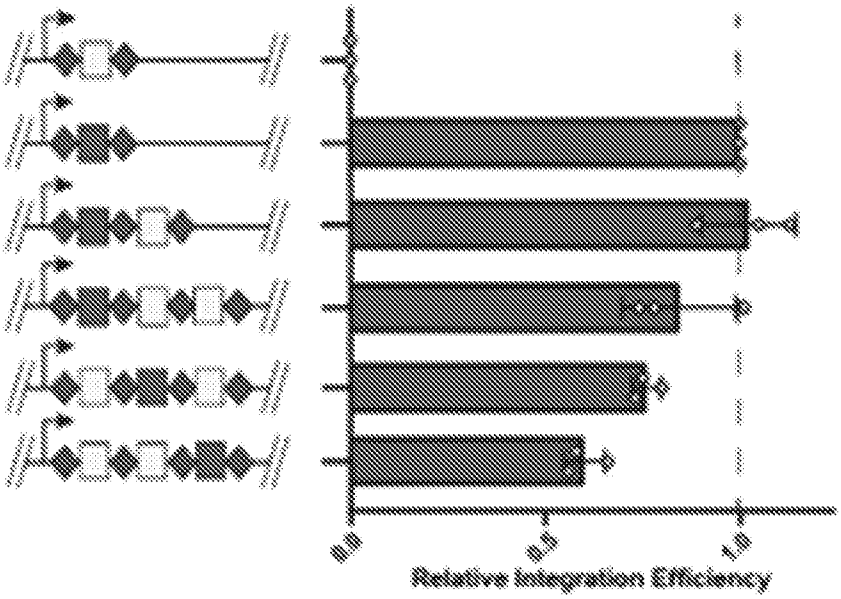


FIG. 94B

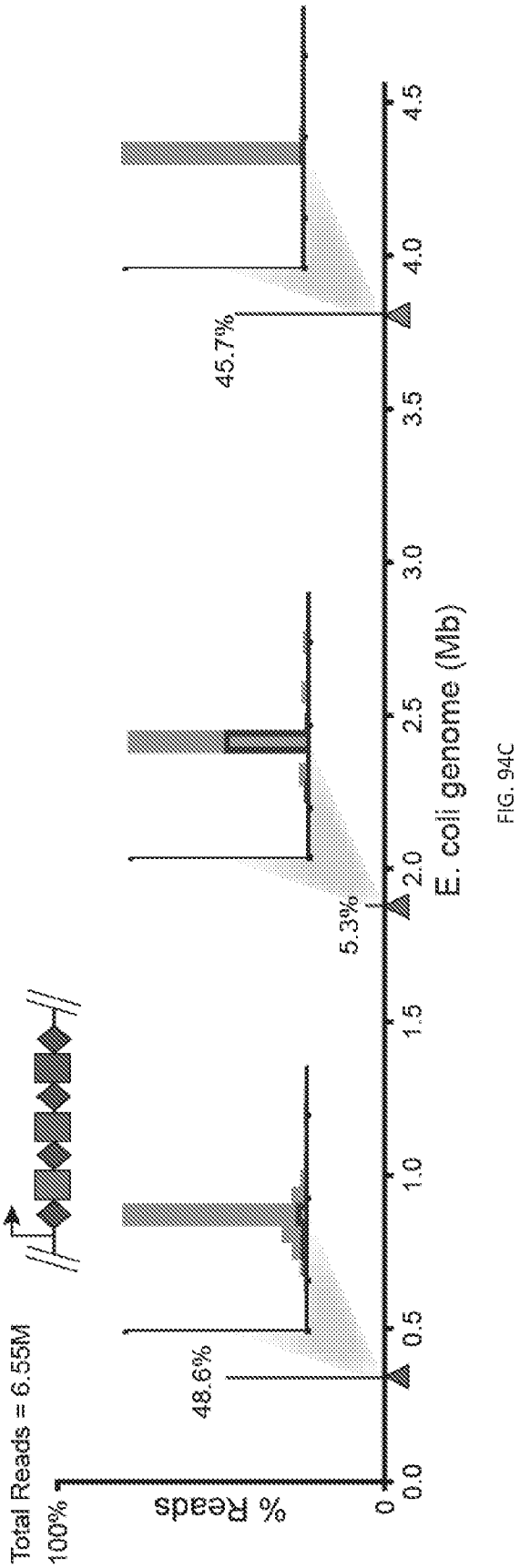

Total Reads = 6.55M
100%
% Reads
0
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
E. coli genome (Mb)
48.6%
5.3%
45.7%

FIG. 94C

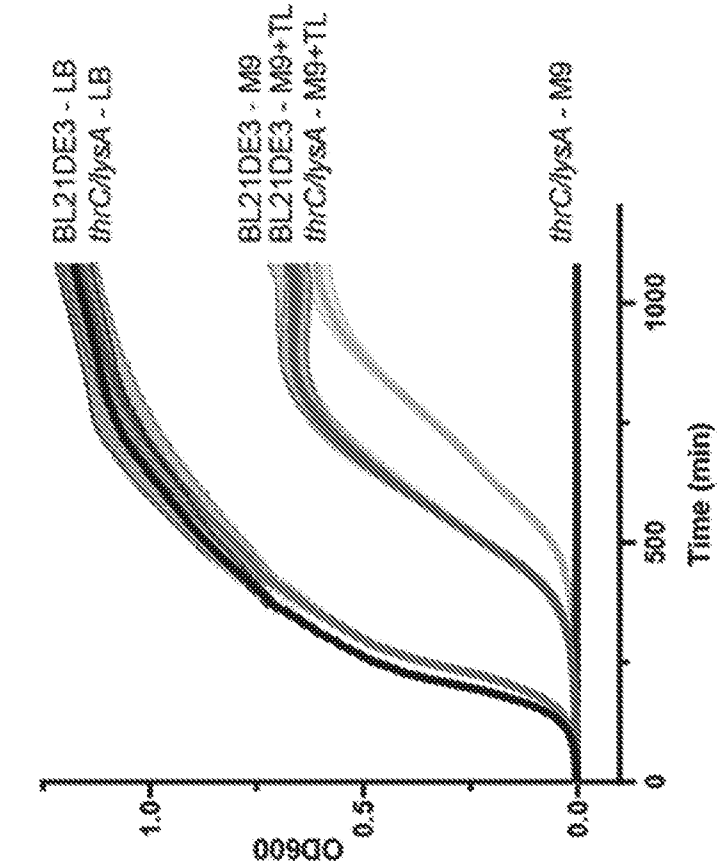


FIG. 95B

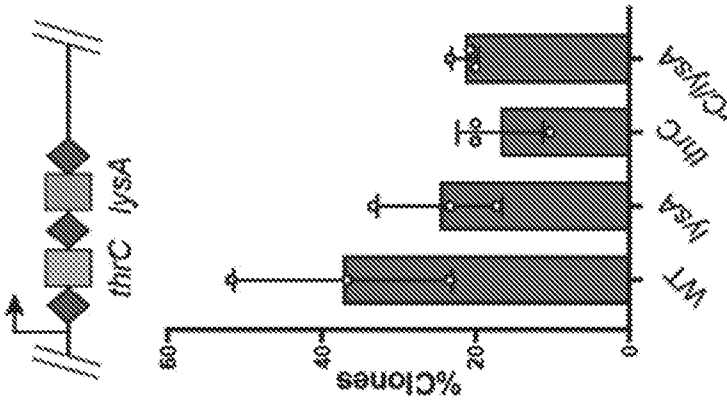


FIG. 95A

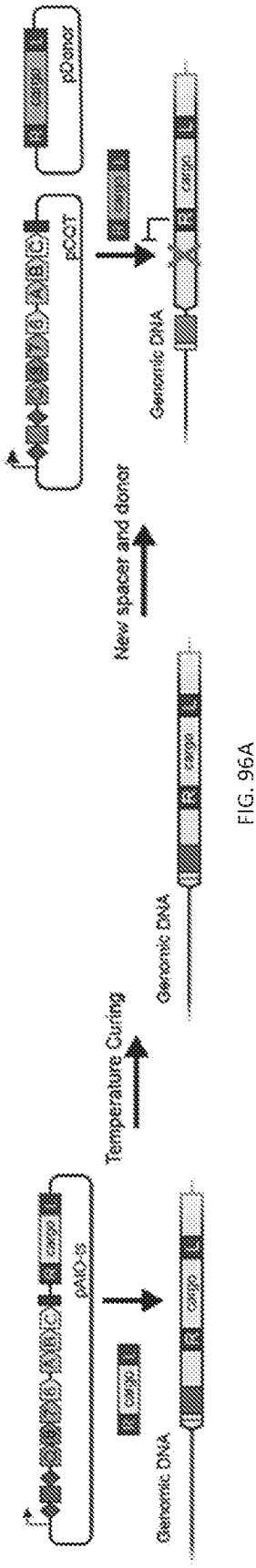


FIG. 96A

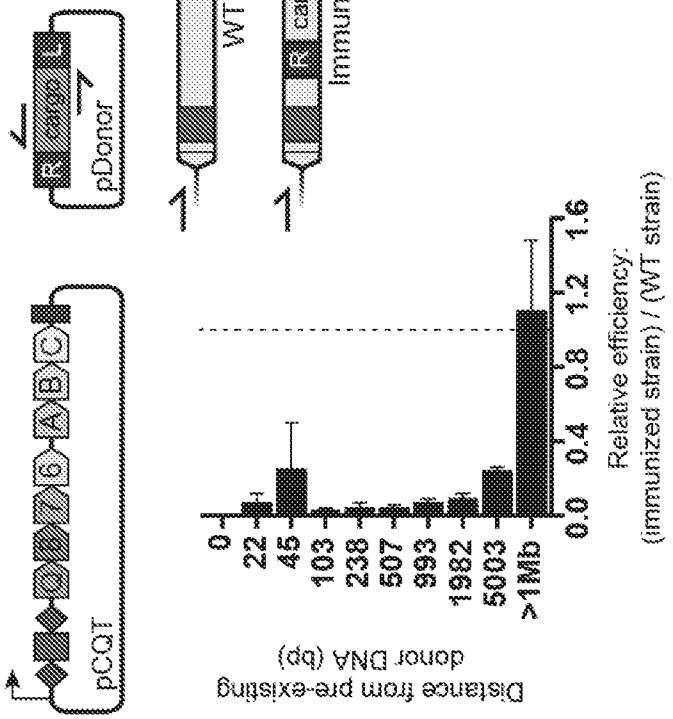


FIG. 96B

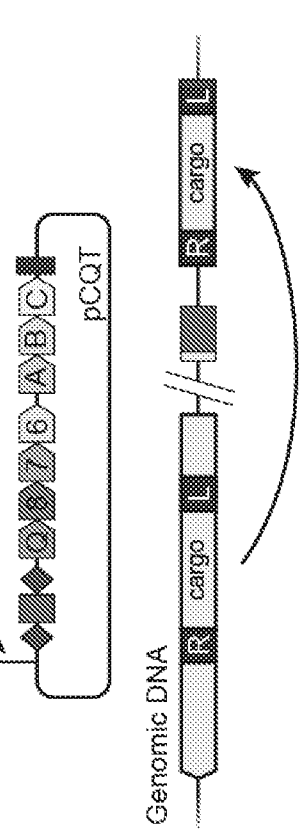


FIG. 96C

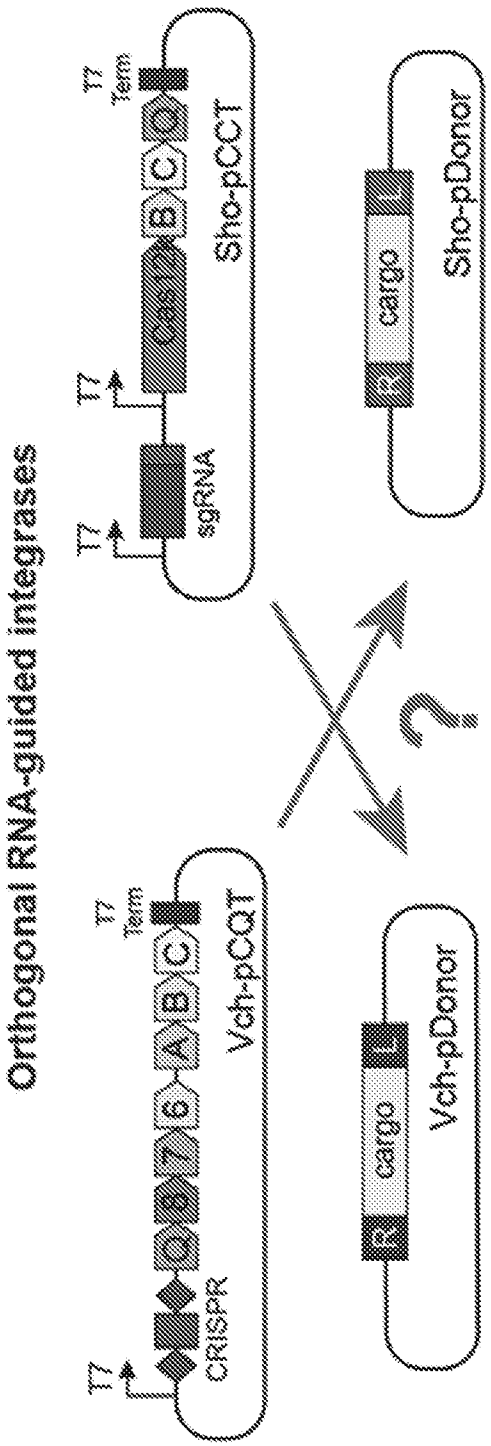


FIG. 97A

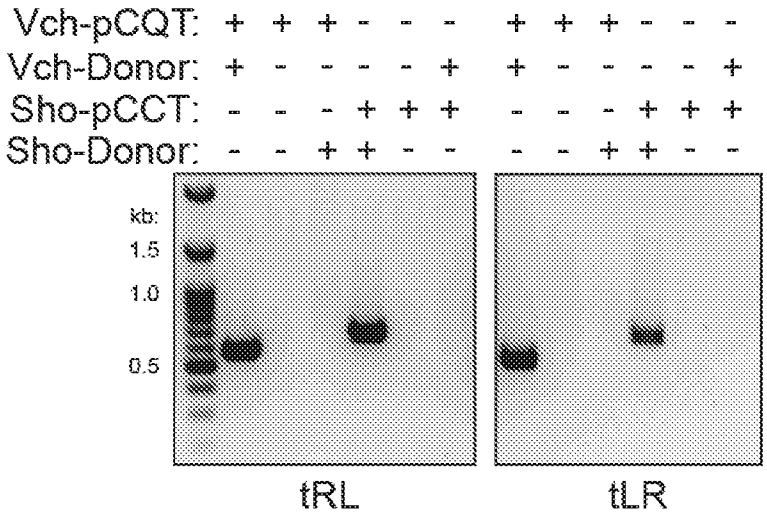


FIG. 97B

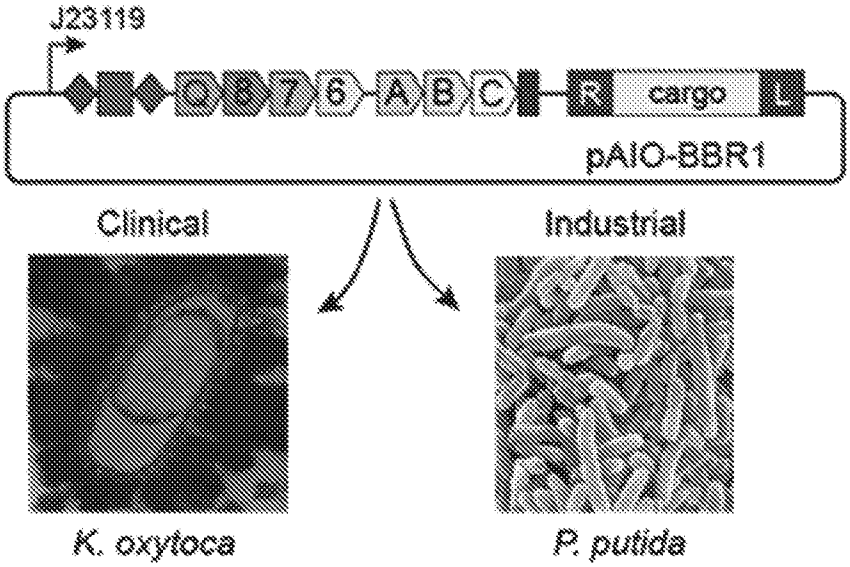


FIG. 98A

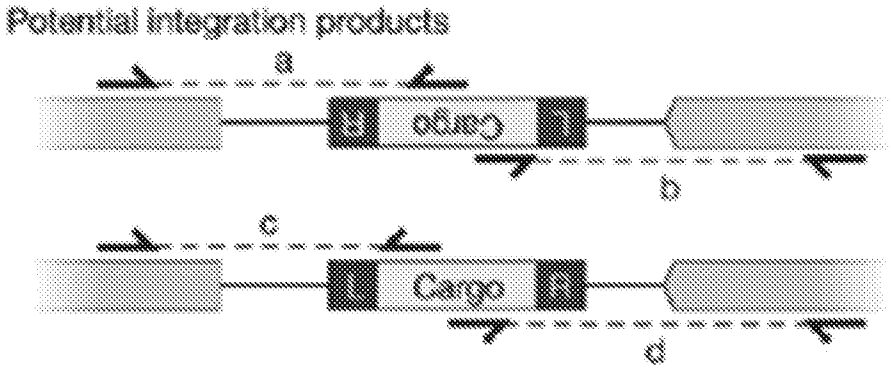


FIG. 98B

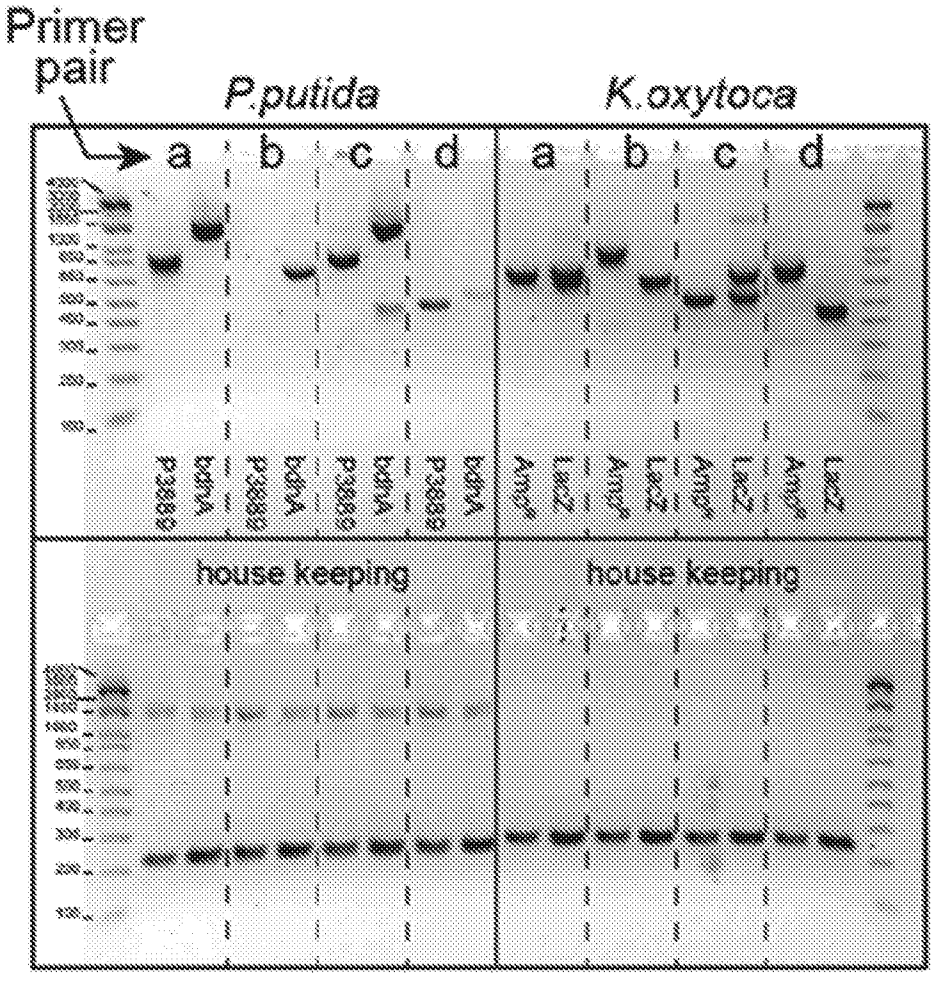


FIG. 98C

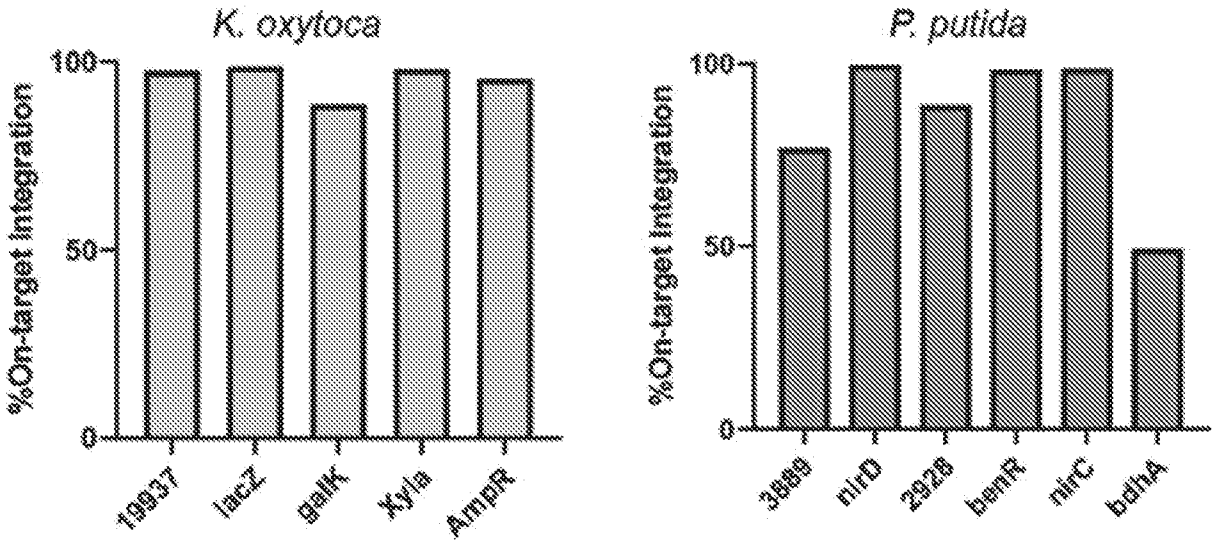


FIG. 98D

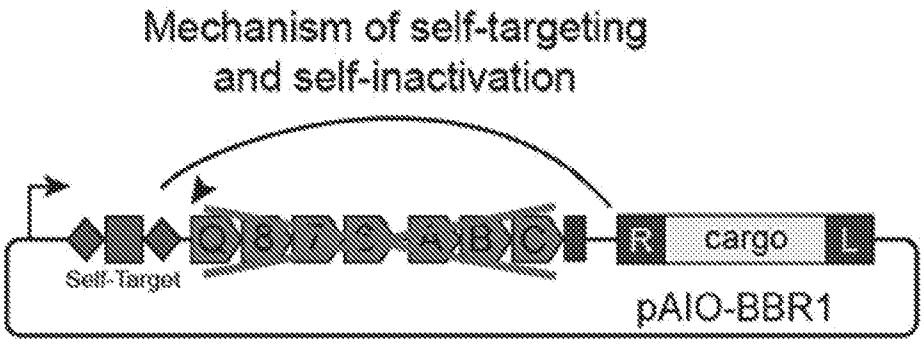


FIG. 99A

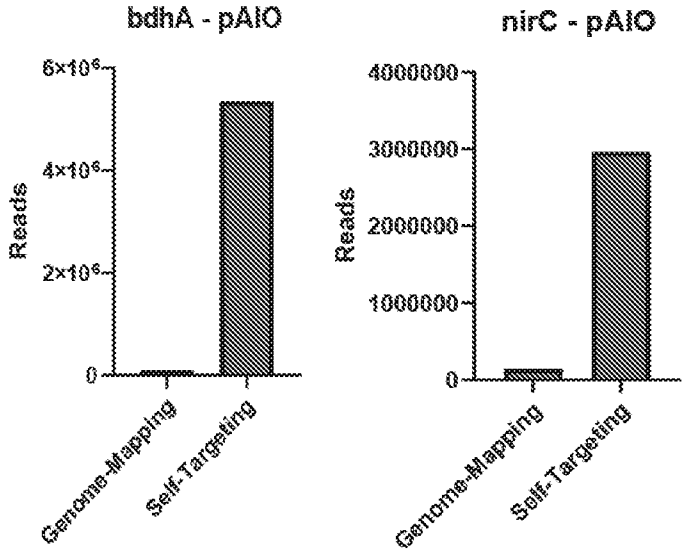


FIG. 99B



FIG. 99C

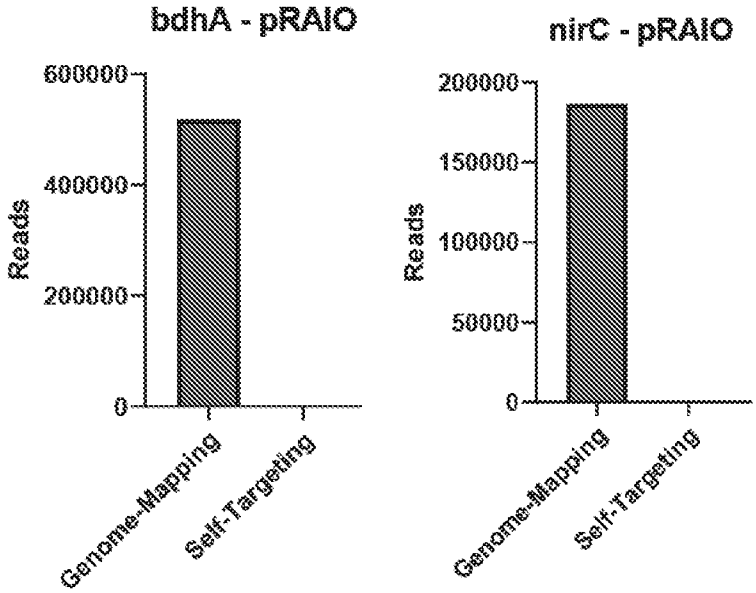


FIG. 99D

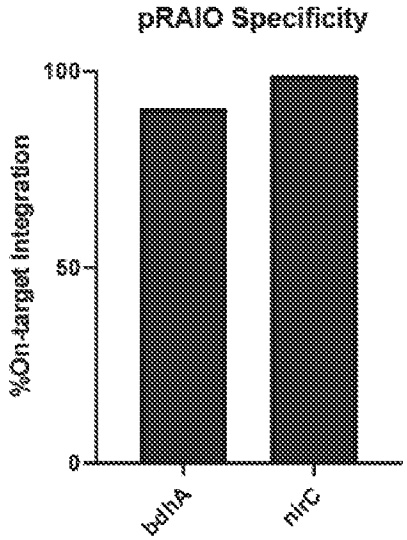


FIG. 99E

gRNA ID	Encoded by	Description	Targeting complex	CRISPR repeat sequence (5'→3')	Spacer sequence (5'→3')	Full length gRNA sequence (5'→3')	Genomic coordinates of target site*	PAM†
gRNA-nt	pSL0762	Non-targeting	V. cholerae Cascade	(SEQ ID No. 154) GTGAACTGCCG AGTAGGTAGCT GATAAC	(SEQ ID No. 155) GTTGTCTGACAC TTGTCACAAACC GCTAGGAG	(SEQ ID No. 156) CUGAUAACGUUGUCUGAC ACUUGUCACAAACCGCUA GGAGGUGAACUGCCGAGU AGGUAG	-	-
gRNA-1	pSL0826	Target-1 (glmS locus)	V. cholerae Cascade	(SEQ ID No. 157) GTGAACTGCCG AGTAGGTAGCT GATAAC	(SEQ ID No. 158) TGGGAGGATTCA TAAAGCATTGTT TGTTGGCT	(SEQ ID No. 159) CUGAUAACUGGGAGGAUU CAUAAAGCAUUGUUUGUU GGCUGUGAACUGCCGAGU AGGUAG	3800336-3800367	CC
gRNA-2	pSL0827	Target-2 (glmS locus)	V. cholerae Cascade	(SEQ ID No. 160) GTGAACTGCCG AGTAGGTAGCT GATAAC	(SEQ ID No. 161) GTCATCTTCGGC TACTTTTCTCT GTCACAGA	(SEQ ID No. 162) CUGAUAACGUCAUCUUCG GCUACUUUUCUCUGUCA CAGAGUGAACUGCCGAGU AGGUAG	complement (3800324-3800355)	CC
gRNA-3	pSL0778 /pSL091 5	Target-3 (lacZ locus)	V. cholerae Cascade	(SEQ ID No. 163) GTGAACTGCCG AGTAGGTAGCT GATAAC	(SEQ ID No. 164) TATCCCATTACG GTCAATCCGCCG TTTGTTCC	(SEQ ID No. 165) CUGAUAACUAUCCCAUUA CGGUCAAUCCGCCGUUUG UUCCGUGAACUGCCGAGU AGGUAG	complement (335494-335525)	CC
gRNA-4	pSL0828	Target-4 (lacZ locus)	V. cholerae Cascade	(SEQ ID No. 166) GTGAACTGCCG AGTAGGTAGCT GATAAC	(SEQ ID No. 167) AGTACAGCGCG GCTGAAATCATC ATTAAAGCG	(SEQ ID No. 168) CUGAUAACAGUACAGCGC GGCUGAAAUCAUCAUUAA AGCGGUGAACUGCCGAGU AGGUAG	335119-335150	CC
gRNA-5	pSL0829	Target-5 (lacZ locus)	V. cholerae Cascade	(SEQ ID No. 169) GTGAACTGCCG AGTAGGTAGCT GATAAC	(SEQ ID No. 170) AGCGGTGCACG GGTGAACTGATC GCGCAGCCGG	(SEQ ID No. 171) CUGAUAACAGCGGUGCAC GCGUGAACUGAUCGCGCA GCGGCUGAACUGCCGAGU AGGUAG	333472-333503	CC
gRNA-6	pSL0830	Target-6 (lacZ locus)	V. cholerae Cascade	(SEQ ID No. 172) GTGAACTGCCG AGTAGGTAGCT GATAAC	(SEQ ID No. 173) GCGCCACTGGTG TGGCCATAATT CAATTCGC	(SEQ ID No. 174) CUGAUAACGCGCCACUGG UGUGGCCAUAAUUCAAU UCGCGUGAACUGCCGAGU AGGUAG	332982-333013	CC

FIG. 100A

gRNA ID	Encoded by:	Description	Targeting complex	CRISPR repeat sequence (5'→3')	Spacer sequence (5'→3')	Full length gRNA sequence (5'→3')	Genomic coordinates of target site*	PAM†
gRNA-7	pSL0831	Target-7 (lacY locus)	V. cholerae Cascade	**(SEQ ID No. 175)** GTGAACTGCCG AGTAGGTAGCT GATAAC	**(SEQ ID No. 176)** AAAAGTTTGTGT TTTTTAAATAGT ACATAATG	**(SEQ ID No. 177)** CUGAUAACAAAAGUUUGU GUUUUUUAAAUAGUACAU AAUGGUGAACUGCCGAGU AGGUAG	332687-332718	CC
gRNA-8	pSL0832	Target-8 (lacY locus)	V. cholerae Cascade	**(SEQ ID No. 178)** GTGAACTGCCG AGTAGGTAGCT GATAAC	**(SEQ ID No. 179)** CGAGTTTGTCAG AAAGCAGACCA AACAGCGGT	**(SEQ ID No. 180)** CUGAUAACCGAGUUUGUC AGAAAGCAGACCAAACAG CGGUGUGAACUGCCGAGU AGGUAG	332504-332535	CC
gRNA-9	pSL0910	Target-9 (yadD-panC locus)	V. cholerae Cascade	**(SEQ ID No. 181)** GTGAACTGCCG AGTAGGTAGCT GATAAC	**(SEQ ID No. 182)** GCCTGGCTTGGC GATGCTCGCCTG ATCGACAA	**(SEQ ID No. 183)** CUGAUAACGCCUGGCUUG GCGAUGCUCGCCUGAUCG ACAAGUGAACUGCCGAGU AGGUAG	complement (1507701-1508011)	CC
gRNA-10	pSL0903	Target-10 (amiG-gmhA locus)	V. cholerae Cascade	**(SEQ ID No. 184)** GTGAACTGCCG AGTAGGTAGCT GATAAC	**(SEQ ID No. 185)** GTCAGAGTGGC GTATCCGATGAA TCACCACAG	**(SEQ ID No. 186)** CUGAUAACGUCAGAGUGG CGUAUCCGAUGAAUCACC ACAGGUGAACUGCCGAGU AGGUAG	745115-745146	CC
gRNA-11	pSL0904	Target-11 (ompW-yciE locus)	V. cholerae Cascade	**(SEQ ID No. 187)** GTGAACTGCCG AGTAGGTAGCT GATAAC	**(SEQ ID No. 188)** TCGATCCGTGGC TGTTTATGTTCT CAGCAGGA	**(SEQ ID No. 189)** CUGAUAACUCGAUCCGUG GGUGUUUAUGUUCUCAGC AGGAGUGAACUGCCGAGU AGGUAG	1300207-1300238	CC
gRNA-12	pSL0905	Target-12 (purT-ada locus)	V. cholerae Cascade	**(SEQ ID No. 190)** GTGAACTGCCG AGTAGGTAGCT GATAAC	**(SEQ ID No. 191)** GCATTACTAAGC TGGCGCGTGAA GCTGTAGAA	**(SEQ ID No. 192)** CUGAUAACGCCAUUACUAA GCUGGCGCGGUGAAGCUGU AGAAGUGAACUGCCGAGU AGGUAG	complement (1877834-1877885)	CC
gRNA-13	pSL0898	Target-13 (ttrB-yqfG locus)	V. cholerae Cascade	**(SEQ ID No. 193)** GTGAACTGCCG AGTAGGTAGCT GATAAC	**(SEQ ID No. 194)** GAGAATATTGTG GCAAAAGCGCA TAAGGTGCT	**(SEQ ID No. 195)** CUGAUAACGAGAAUAUUG UGGCAAAAGCGCAUAAGG UGCUGUGAACUGCCGAGU AGGUAG	2446078-2446109	CC

FIG. 10B

gRNA ID	Encoded by	Description	Targeting complex	CRISPR repeat sequence (5'→3')	Spacer sequence (5'→3')	Full length gRNA sequence (5'→3')	Genomic coordinates of target site*	PAM†
gRNA-14	pSL0899	Target-14 (yghA-entD locus)	V. cholerae Cascade	**(SEQ ID No. 196)** GTGAACTGCCG AGTAGGTAGCT GATAAC	**(SEQ ID No. 197)** GCAGAAGTGCA CGGCGTGTGCCG CGGCGAGCA	**(SEQ ID No. 198)** CUGAUAACGCAGAAGUGC ACGGCGUGUGCCGGCGCG AGCAGUGAACUGCCGAGU AGGUAG	3017942-3017973	CC
gRNA-15	pSL0908	Target-15 (cspA-hokA locus)	V. cholerae Cascade	**(SEQ ID No. 199)** GTGAACTGCCG AGTAGGTAGCT GATAAC	**(SEQ ID No. 200)** TTACTTACGGCG TTGCGCATTCTC ATTGCACC	**(SEQ ID No. 201)** CUGAUAACUUACUUACGG CGUUGCGCAUUCUCAUUG CACCGUGAACUGCCGAGU AGGUAG	complement (3582542-3583573)	CC
gRNA-16	pSL0901	Target-16 (dusF-ypbD locus)	V. cholerae Cascade	**(SEQ ID No. 202)** GTGAACTGCCG AGTAGGTAGCT GATAAC	**(SEQ ID No. 203)** GGGGCGTAAAA AGAAGGGGCGC TGATTAACGC	**(SEQ ID No. 204)** CUGAUAACGGGGCGCGUAAA AAGAAGGCGGCGCUGAUUA ACGCCUGAACUGCCGAGU AGGUAG	4138373-4138604	CC
gRNA-17	pSL0902	Target-17 (yadD-pauC locus)	V. cholerae Cascade	**(SEQ ID No. 205)** GTGAACTGCCG AGTAGGTAGCT GATAAC	**(SEQ ID No. 206)** TCTTGCTGAGAT TGATAAGGTAAT TAACCTTA	**(SEQ ID No. 207)** CUGAUAACUCUUGCUGAG AUUGAUAAGGUAAUUAAC CUUAGUGAACUGCCGAGU AGGUAG	150638-150669	CC
gRNA-18	pSL0911	Target-18 (arcG-gpmA locus)	V. cholerae Cascade	**(SEQ ID No. 208)** GTGAACTGCCG AGTAGGTAGCT GATAAC	**(SEQ ID No. 209)** GACTGCAAGTG AGTCGGCTTTTT TGTTGCTAA	**(SEQ ID No. 210)** CUGAUAACGACUGCAAGU GAGUCGGCUUUUUUGUUG CUAAGUGAACUGCCGAGU AGGUAG	complement (745192-745223)	CC
gRNA-19	pSL0912	Target-19 (ompW-ycfE locus)	V. cholerae Cascade	**(SEQ ID No. 211)** GTGAACTGCCG AGTAGGTAGCT GATAAC	**(SEQ ID No. 212)** GCAACAACTG AGAAATTTTTAA TTCGCTCTG	**(SEQ ID No. 213)** CUGAUAACGCAACAACUG GAGAAAUUUUUAAUUCGC UCUGGUGAACUGCCGAGU AGGUAG	complement (1300339-1300370)	CC
gRNA-20	pSL0897	Target-20 (gnrT-eda locus)	V. cholerae Cascade	**(SEQ ID No. 214)** GTGAACTGCCG AGTAGGTAGCT GATAAC	**(SEQ ID No. 215)** ATTGAACGCGCG AAGCACGCCGC CGGACAGGT	**(SEQ ID No. 216)** CUGAUAACAUUGAACGCG CGAAGCACGCCGCCGGAC AGGUGUGAACUGCCGAGU AGGUAG	1877736-1877767	CC

FIG. 100C

gRNA ID	Encoded by	Description	Targeting complex	CRISPR repeat sequence (5'→3')	Spacer sequence (5'→3')	Full length gRNA sequence (5'→3')	Genomic coordinates of target site*	PAM†
gRNA-21	pSL0906	Target-21 (tatB-ypfG locus)	V. cholerae Cascade	(SEQ ID No. 217) GTGAACTGCCG AGTAGGTAGCT GATAAC	(SEQ ID No. 218) TGGCCCACGTTG TGGATCACCCGT TAAACAAA	(SEQ ID No. 219) CUGAUAACUGGCCCACGU UGUUGGAUCACCCGUUAAA CAAAGUGAACUGCCGAGU AGGUAG	complement (2446218-2446249)	CC
gRNA-22	pSL0907	Target-22 (yghA-exbD locus)	V. cholerae Cascade	(SEQ ID No. 220) GTGAACTGCCG AGTAGGTAGCT GATAAC	(SEQ ID No. 221) TGAAGATAGGTC TGGTCGGCGAA GAAACCGCC	(SEQ ID No. 222) CUGAUAACUGAAGAUAGG UCUUGGUUGGCGAAGAAAC CGCCGUGAACUGCCGAGU AGGUAG	complement (3018267-3018298)	CC
gRNA-23	pSL0910	Target-23 (cspA-lolA locus)	V. cholerae Cascade	(SEQ ID No. 223) GTGAACTGCCG AGTAGGTAGCT GATAAC	(SEQ ID No. 224) AGCCTGTAATCT CTGCTTAAAAGC ACAGAATC	(SEQ ID No. 225) CUGAUAACAGCCUGUAAU CUCUGCUUAAAAGCACAG AAUCGUGAACUGCCGAGU AGGUAG	3382407-3382438	CC
gRNA-24	pSL0909	Target-24 (rluF-yjbD locus)	V. cholerae Cascade	(SEQ ID No. 226) GTGAACTGCCG AGTAGGTAGCT GATAAC	(SEQ ID No. 227) TGAGATTTTCAC TCGTCAGTGTGA ACACAATT	(SEQ ID No. 228) CUGAUAACUGAGAUUUUC ACUCGUCAGUGUGAACAC AAUUGUGAACUGCCGAGU AGGUAG	complement (4138683-4138714)	CC
gRNA-4.1	pSL0835	PAM tiling, Target-4, shifted +1-nt	V. cholerae Cascade	(SEQ ID No. 229) GTGAACTGCCG AGTAGGTAGCT GATAAC	(SEQ ID No. 230) GTACAGCGCGG CTGAAATCATCA TTAAAGCGA	(SEQ ID No. 231) CUGAUAACGUACAGCGCG GCUGAAAUCAUCAUUAAA GCGAGUGAACUGCCGAGU AGGUAG	335120-335151	CA
gRNA-4.2	pSL0834	PAM tiling, Target-4, shifted +2-nt	V. cholerae Cascade	(SEQ ID No. 232) GTGAACTGCCG AGTAGGTAGCT GATAAC	(SEQ ID No. 233) TACAGCGCGGCT GAAATCATCATT AAAGCGAG	(SEQ ID No. 234) CUGAUAACUACAGCGCGG CUGAAAUCAUCAUUAAAG CGAGUGAACUGCCGAGU AGGUAG	335121-335152	AG
gRNA-4.3	pSL0833	PAM tiling, Target-4, shifted +3-nt	V. cholerae Cascade	(SEQ ID No. 235) GTGAACTGCCG AGTAGGTAGCT GATAAC	(SEQ ID No. 236) ACAGCGCGGCT GAAATCATCATT AAAGCGAGT	(SEQ ID No. 237) CUGAUAACACAGCGCGGC UGAAAUCAUCAUUAAAGC GAGUGUGAACUGCCGAGU AGGUAG	335122-335153	GT

FIG. 100D

gRNA ID	Encoded by	Description	Targeting complex	CRISPR repeat sequence (5'→3')	Spacer sequence (5'→3')	Full length gRNA sequence (5'→3')	Genomic coordinates of target site*	PAM†
gRNA-4.4	pSL0852	PAM tiling, Target-4, shifted +4-nt	V. cholerae Cascade	(SEQ ID No. 238) GTGAACTGCCG AGTAGGTAGCT GATAAC	(SEQ ID No. 239) CAGCCGGCTG AAATCATCATTA AAGCGAGTG	(SEQ ID No. 240) CUGAUAACCAGCCGGCU GAAAUCAUCAUUAAAGCG AGUGGUGAACUGCCGAGU AGGUAG	333123-333154	TA
gRNA-4.5	pSL0851	PAM tiling, Target-4, shifted +5-nt	V. cholerae Cascade	(SEQ ID No. 241) GTGAACTGCCG AGTAGGTAGCT GATAAC	(SEQ ID No. 242) AGCGCCGCTGA AATCATCATTAA AGCGAGTGG	(SEQ ID No. 243) CUGAUAACAGCGCCGCUG AAAUCAUCAUUAAAGCGA GUGGUGAACUGCCGAGU AGGUAG	333124-333155	AC
gRNA-4.6	pSL0763	PAM tiling, Target-4, shifted +6-nt	V. cholerae Cascade	(SEQ ID No. 244) GTGAACTGCCG AGTAGGTAGCT GATAAC	(SEQ ID No. 245) GCGCGGCTGAA ATCATCATTAAA GCGAGTGGC	(SEQ ID No. 246) CUGAUAACGCGCGGCUGA AAUCAUCAUUAAAGCGAG UGGCGUGAACUGCCGAGU AGGUAG	333125-333156	CA
gRNA-4.7	pSL0764	PAM tiling, Target-4, shifted +7-nt	V. cholerae Cascade	(SEQ ID No. 247) GTGAACTGCCG AGTAGGTAGCT GATAAC	(SEQ ID No. 248) CGCGGCTGAAAT CATCATTAAAGC GAGTGGCA	(SEQ ID No. 249) CUGAUAACCGCGGCUGAA AUCAUCAUUAAAGCGAGU GGCAGUGAACUGCCGAGU AGGUAG	333126-333157	AG
gRNA-4.8	pSL0765	PAM tiling, Target-4, shifted +8-nt	V. cholerae Cascade	(SEQ ID No. 250) GTGAACTGCCG AGTAGGTAGCT GATAAC	(SEQ ID No. 251) GCGGCTGAAATC ATCATTAAAGCG AGTGGCAA	(SEQ ID No. 252) CUGAUAACGCGGCUGAAA UCAUCAUUAAAGCGAGUG GCAAGUGAACUGCCGAGU AGGUAG	333127-333158	GC
gRNA-4.9	pSL0766	PAM tiling, Target-4, shifted +9-nt	V. cholerae Cascade	(SEQ ID No. 253) GTGAACTGCCG AGTAGGTAGCT GATAAC	(SEQ ID No. 254) CGGCTGAAATCA TCATTAAAGCGA GTGGCAAC	(SEQ ID No. 255) CUGAUAACCGGCUGAAAU CAUCAUUAAAGCGAGUGG CAACGUGAACUGCCGAGU AGGUAG	333128-333159	CG
gRNA-4.10	pSL0767	PAM tiling, Target-4, shifted +10-nt	V. cholerae Cascade	(SEQ ID No. 256) GTGAACTGCCG AGTAGGTAGCT GATAAC	(SEQ ID No. 257) GGCTGAAATCAT CATTAAAGCGA GTGGCAACA	(SEQ ID No. 258) CUGAUAACGGCUGAAAUC AUCAUUAAAGCGAGUGGC AACAGUGAACUGCCGAGU AGGUAG	333129-333160	GC

FIG. 10DE

gRNA ID	Encoded by	Description	Targeting complex	CRISPR repeat sequence (5'—3')	Spacer sequence (5'—3')	Full length gRNA sequence (5'—3')	Genomic coordinates of target site*	PAM†
gRNA-4.11	pSL0755	PAM tiling, Target-4, shifted +11-nt	V. cholerae Cascade	(SEQ ID No. 259) GTGAACTGCCG AGTAGGTAGCT GATAAC	(SEQ ID No. 260) GCTGAAATCATC ATTAAAGCGAGT GGCAACAT	(SEQ ID No. 261) CUGAUAACGCCUGAAAUCA UCAUUAAAGCGAGUGGCA ACAUGUGAACUGCCGAGU AGGUAG	335130-335161	CG
gRNA-4.12	pSL0768	PAM tiling, Target-4, shifted +12-nt	V. cholerae Cascade	(SEQ ID No. 262) GTGAACTGCCG AGTAGGTAGCT GATAAC	(SEQ ID No. 263) CTGAAATCATCA TTAAAGCGAGTG GCAACATG	(SEQ ID No. 264) CUGAUAACCUGAAAUCAU CAUUAAAGCGAGUGGCAA CAUGGUGAACUGCCGAGU AGGUAG	335131-335162	GG
gRNA-4.13	pSL0769	PAM tiling, Target-4, shifted +13-nt	V. cholerae Cascade	(SEQ ID No. 265) GTGAACTGCCG AGTAGGTAGCT GATAAC	(SEQ ID No. 266) TGAAATCATCAT TAAAGCGAGTG GCAACATGG	(SEQ ID No. 267) CUGAUAACUGAAAUCAUC AUUAAAGCGAGUGGCAAC AUGGGUGAACUGCCGAGU AGGUAG	335132-335163	GC
gRNA-4.14	pSL0737	PAM tiling, Target-4, shifted +14-nt	V. cholerae Cascade	(SEQ ID No. 268) GTGAACTGCCG AGTAGGTAGCT GATAAC	(SEQ ID No. 269) GAAATCATCATT AAAGCGAGTGG CAACATGGA	(SEQ ID No. 270) CUGAUAACGAAAUCAUCA UUAAAGCGAGUGGCAACA UGGAGUGAACUGCCGAGU AGGUAG	335133-335164	CT
gRNA-4.15	pSL0770	PAM tiling, Target-4, shifted +15-nt	V. cholerae Cascade	(SEQ ID No. 271) GTGAACTGCCG AGTAGGTAGCT GATAAC	(SEQ ID No. 272) AAATCATCATTA AAGCGAGTGGC AACATGGAA	(SEQ ID No. 273) CUGAUAACAAAUCAUCAU UAAAGCGAGUGGCAACAU GGAAGUGAACUGCCGAGU AGGUAG	333134-335165	TG
gRNA-4.16	pSL0771	PAM tiling, Target-4, shifted +16-nt	V. cholerae Cascade	(SEQ ID No. 274) GTGAACTGCCG AGTAGGTAGCT GATAAC	(SEQ ID No. 275) AATCATCATTAA AGCGAGTGGCA ACATGGAAA	(SEQ ID No. 276) CUGAUAACAAUCAUCAUU AAAGCGAGUGGCAACAUG GAAAGUGAACUGCCGAGU AGGUAG	335135-335166	GA
gRNA-4.mm1-4	pSL0864	Target-4, mismatches 1-4	V. cholerae Cascade	(SEQ ID No. 277) GTGAACTGCCG AGTAGGTAGCT GATAAC	(SEQ ID No. 278) TCATCAGCGCGG CTGAAATCATCA TTAAAGCG	(SEQ ID No. 279) CUGAUAACUCAUCAGCGC GGCUGAAAUCAUCAUUAA AGCGGUGAACUGCCGAGU AGGUAG	335119-335150	CC

FIG. 10DF

gRNA ID	Encoded by	Description	Targeting complex	CRISPR repeat sequence (5'→3')	Spacer sequence (5'→3')	Full length gRNA sequence (5'→3')	Genomic coordinates of target site*	PAM†
gRNA-4.mm5-8	pSL0865	Target-4, mismatches bp 5-8	V. cholerae Cascade	(SEQ ID No. 280) GTGAACTGCCG AGTAGGTAGCT GATAAC	(SEQ ID No. 281) AGTAGTCGGCG GCTGAAATCATC ATTAAAGCG	(SEQ ID No. 282) CUGAUAACAGUAGUCGGCC GGCUGAAAUCAUCAUUAA AGCGGUGAACUGCCGAGU AGGUAG	335119-335150	CC
gRNA-4.mm9-12	pSL0866	Target-4, mismatches bp 9-12	V. cholerae Cascade	(SEQ ID No. 283) GTGAACTGCCG AGTAGGTAGCT GATAAC	(SEQ ID No. 284) AGTACAGCCGCC CTGAAATCATCA TTAAAGCG	(SEQ ID No. 285) CUGAUAACAGUACAGCCG CCCUGAAAUCAUCAUUAA AGCGGUGAACUGCCGAGU AGGUAG	335119-335150	CC
gRNA-4.mm13-16	pSL0867	Target-4, mismatches bp 13-16	V. cholerae Cascade	(SEQ ID No. 286) GTGAACTGCCG AGTAGGTAGCT GATAAC	(SEQ ID No. 287) AGTACAGCCGCC GGACTAATCATC ATTAAAGCG	(SEQ ID No. 288) CUGAUAACAGUACAGCCGC GGGACUAAAUCAUCAUUAA AGCGGUGAACUGCCGAGU AGGUAG	335119-335150	CC
gRNA-4.mm17-20	pSL0868	Target-4, mismatches bp 17-20	V. cholerae Cascade	(SEQ ID No. 289) GTGAACTGCCG AGTAGGTAGCT GATAAC	(SEQ ID No. 290) AGTACAGCGCCG GCTGATTAGATC ATTAAAGCG	(SEQ ID No. 291) CUGAUAACAGUACAGCGCC GGCUGAUUAGAUCAUUAA AGCGGUGAACUGCCGAGU AGGUAG	335119-335150	CC
gRNA-4.mm21-24	pSL0869	Target-4, mismatches bp 21-24	V. cholerae Cascade	(SEQ ID No. 292) GTGAACTGCCG AGTAGGTAGCT GATAAC	(SEQ ID No. 293) AGTACAGCGCG GCTGAAATCTAG TTTAAAGCG	(SEQ ID No. 294) CUGAUAACAGUACAGCGCC GGCUGAAAUCUAGUUUAA AGCGGUGAACUGCCGAGU AGGUAG	335119-335150	CC
gRNA-4.mm25-28	pSL0870	Target-4, mismatches bp 25-28	V. cholerae Cascade	(SEQ ID No. 295) GTGAACTGCCG AGTAGGTAGCT GATAAC	(SEQ ID No. 296) AGTACAGCCGCG GCTGAAATCATC AAATTAGCG	(SEQ ID No. 297) CUGAUAACAGUACAGCGCC GGCUGAAAUCAUCAAAUU AGCGGUGAACUGCCGAGU AGGUAG	335119-335150	CC
gRNA-4.mm29-32	pSL0871	Target-4, mismatches bp 29-32	V. cholerae Cascade	(SEQ ID No. 298) GTGAACTGCCG AGTAGGTAGCT GATAAC	(SEQ ID No. 299) AGTACAGCGCG GCTGAAATCATC ATTAATCGC	(SEQ ID No. 300) CUGAUAACAGUACAGCGCC GGCUGAAAUCAUCAUUAA UCGCGUGAACUGCCGAGU AGGUAG	335119-335150	CC

FIG. 100G

gRNA ID	Encoded by	Description	Targeting complex	CRISPR repeat sequence (5'→3')	Spacer sequence (5'→3')	Full length gRNA sequence (5'→3')	Genomic coordinates of target site[a]	PAM[b]
gRNA-4.-24nt	pSL0959	Target-4, -24 shortened spacer length (8-nt)	V. cholerae Cascade	(SEQ ID No. 301) GTGAACTGCCG AGTAGGTAGCT GATAAC	(SEQ ID No. 302) AGTACAGC	(SEQ ID No. 303) CUGAUAACAGUACAGCCGU GAACUGCCGAGUAGGUAG	333119-333126	CC
gRNA-4.-18nt	pSL0960	Target-4, -18 shortened spacer length (14-nt)	V. cholerae Cascade	(SEQ ID No. 304) GTGAACTGCCG AGTAGGTAGCT GATAAC	(SEQ ID No. 305) AGTACAGCGCG GCT	(SEQ ID No. 306) CUGAUAACAGUACAGCGC GGCUGUGAACUGCCGAGU AGGUAG	333119-333132	CC
gRNA-4.-12nt	pSL0961	Target-4, -12 shortened spacer length (20-nt)	V. cholerae Cascade	(SEQ ID No. 307) GTGAACTGCCG AGTAGGTAGCT GATAAC	(SEQ ID No. 308) AGTACAGCGCG GCTGAAATC	(SEQ ID No. 309) CUGAUAACAGUACAGCGC GGCUGAAAUUCGUGAACUG CCGAGUAGGUAG	333119-333138	CC
gRNA-4.-6nt	pSL0962	Target-4, -6 shortened spacer length (26-nt)	V. cholerae Cascade	(SEQ ID No. 310) GTGAACTGCCG AGTAGGTAGCT GATAAC	(SEQ ID No. 311) AGTACAGCGCG GCTGAAATCATC ATT	(SEQ ID No. 312) CUGAUAACAGUACAGCGC GGCUGAAAUCAUCAUUGU GAACUGCCGAGUAGGUAG	333119-333144	CC
gRNA-4.+6nt	pSL0963	Target-4, +6 extended spacer length (38-nt)	V. cholerae Cascade	(SEQ ID No. 313) GTGAACTGCCG AGTAGGTAGCT GATAAC	(SEQ ID No. 314) AGTACAGCGCG GCTGAAATCATC ATTAAAGCGAGT GGC	(SEQ ID No. 315) CUGAUAACAGUACAGCGC GGCUGAAAUCAUCAUUAA AGCGAGUGGCGUGAACUG CCGAGUAGGUAG	333119-333156	CC
gRNA-4.+12nt	pSL0964	Target-4, +12 extended spacer length (44-nt)	V. cholerae Cascade	(SEQ ID No. 316) GTGAACTGCCG AGTAGGTAGCT GATAAC	(SEQ ID No. 317) AGTACAGCGCG GCTGAAATCATC ATTAAAGCGAGT GGCAACATG	(SEQ ID No. 318) CUGAUAACAGUACAGCGC GGCUGAAAUCAUCAUUAA AGCGAGUGGCAACAUGGU GAACUGCCGAGUAGGUAG	333119-333162	CC
gRNA-4.+18nt	pSL0965	Target-4, +18 extended spacer length (50-nt)	V. cholerae Cascade	(SEQ ID No. 319) GTGAACTGCCG AGTAGGTAGCT GATAAC	(SEQ ID No. 320) AGTACAGCGCG GCTGAAATCATC ATTAAAGCGAGT GGCAACATGGA AATC	(SEQ ID No. 321) CUGAUAACAGUACAGCGC GGCUGAAAUCAUCAUUAA AGCGAGUGGCAACAUGGA AAUCGUGAACUGCCGAGU AGGUAG	333119-333168	CC

FIG. 100H

gRNA ID	Encoded by	Description	Targeting complex	CRISPR repeat sequence (5'→3')	Spacer sequence (5'→3')	Full length gRNA sequence (5'→3')	Genomic coordinates of target site*	PAM†
lgRNA-4.+24nt	pSL0966	Target-4, +24 extended spacer length (56-nt)	V. cholerae Cascade	(SEQ ID No. 322) GTGAACTGCCG AGTAGGTAGCT GATAAC	(SEQ ID No. 323) AGTACAGCGCG GCTGAAATCATC ATTAAAGCGAGT CGCAACATGGA AATCGCTGAT	(SEQ ID No. 324) CUGAUAACAGUACAGCGC GGCUGAAAUCAUCAUUAA AGCGAGUGGCAACAUGGA AAUCGCUGAUGUGAACUG CGGAGUAGGUAG	335119-335174	CC
gRNA-sp5	pSL0426	Spacer-3 (native spacer)	V. cholerae Cascade	(SEQ ID No. 325) GTGAACTGCCG AGTAGGTAGCT GATAAC	(SEQ ID No. 326) TTACAGGACGCT TTGGCTTCATTG CTTTTCAG	(SEQ ID No. 327) CUGAUAACUUACAGGACG CUUUGGCUUCAUUGCUUU UCAGGUGAACUGCGGAGU AGGUAG	-	-
sgRNA-nt (BsaI)	pSL0728	Cas9 non-targeting (BsaI stuffer)	S. pyogenes Cas9	N/A	(SEQ ID No. 328) CGAGACCAGTCT AGGTCTCG	(SEQ ID No. 329) GGAGACCAGUCUAGGUC UCGGUUUUAGAGCUAGAA AUAGCAAGUUAAAAUAAG GCUAGUCCGUUAUCAACU UGAAAAAGUGGCACCGAG UCGGUGCUUUUUUU	-	-
sgRNA-3	pSL0685	Cas9 target proximal to Vch Target-3	S. pyogenes Cas9	N/A	(SEQ ID No. 330) CGGATTGACCGT AATGCGAT	(SEQ ID No. 331) GCGGAUUGACCGUAAUGG GAUGUUUUAGAGCUAGAA AUAGCAAGUUAAAAUAAG GCUAGUCCGUUAUCAACU UGAAAAAGUGGCACCGAG UCGGUGCUUUUUUU	335505-335524	AGG
sgRNA-4	pSL0686 /pSL0688	(dXCas9 target proximal to Vch Target-4	S. pyogenes Cas9	N/A	(SEQ ID No. 332) TGATTTCAGCCG CGCTGTAC	(SEQ ID No. 333) GUGAUUUCAGCCGCGCUG UACGUUUUAGAGCUAGAA AUAGCAAGUUAAAAUAAG GCUAGUCCGUUAUCAACU UGAAAAAGUGGCACCGAG UCGGUGCUUUUUUU	complement (335120-335139)	TGG
gRNA-Parent (BsaI)	pSL0295	Pac non-targeting (BsaI stuffer)	P. aeruginosa Cascade	(SEQ ID No. 334) GTTCACTGCCG TATAGGCAGCT AAGAAA	(SEQ ID No. 335) CGAGACCTCAAT TGGTCTCC	(SEQ ID No. 336) CUAAGAAACGAGACCUCA AUUGGUCUCCGUUCACUG CCGUAUAGGCAG	-	-

FIG. 100I

gRNA ID	Encoded by	Description	Targeting complex	CRISPR repeat sequence (5'→3')	Spacer sequence (5'→3')	Full length gRNA sequence (5'→3')	Genomic coordinates of target site*	PAM†
gRNA-Pae3	pSL0872	Pae target, same as Vch Target-3	P. aeruginosa Cascade	(SEQ ID No. 337) GTTCACTGCCG TATAGGCAGCT AAGAAA	(SEQ ID No. 338) TATCCCATTACG GTCAATCCGCCG TTTGTTCC	(SEQ ID No. 339) CUAAGAAAUAUCCCAUUA CGGUCAAUCCGCCGUUUG UUCCGUUCACUGCCGUAU AGGCAG	complement (333494-333523)	CC
gRNA-Pae4	pSL0873	Pae target, same as Vch Target-4	P. aeruginosa Cascade	(SEQ ID No. 340) GTTCACTGCCG TATAGGCAGCT AAGAAA	(SEQ ID No. 341) AGTACAGCGCG GCTGAAATCATC ATTAAAGCG	(SEQ ID No. 342) CUAAGAAAAGUACAGCGCG GCUGAAAUCAUCAUUAA AGCGGUUCACUGCCGUAU AGGCAG	335119-335150	CC

FIG. 100J

Oligo ID	Use	Sequence
oSL0763	PCR	GTGGTATTCACTCCAGAGCG (SEQ ID No. 343)
oSL0388	PCR	CAACAGTACTGCGATGAG (SEQ ID No. 344)
oSL1712	PCR	CGAACCTGTTCAACGAC (SEQ ID No. 345)
oSL1709	PCR	CGAACTGCTGTCGAAG (SEQ ID No. 346)
oSL0842	PCR	GCCCGAGTTTGTCAGAAAGC (SEQ ID No. 347)
oSL0311	PCR	CGTCCTGTGGATCCTCTAC (SEQ ID No. 348)
oSL0400	PCR	GCATCAACGCATATAGCG (SEQ ID No. 349)
oSL1177	PCR	CATGCAGTATTCCAGGACTC (SEQ ID No. 350)
oSL1178	PCR	GGAGAGCAAATCTTGTTGC (SEQ ID No. 351)
oSL1303	cPCR	CACCACAGATGAAACGCCG (SEQ ID No. 352)
oSL1186	cPCR	CATCTACACCAACGTGACCTATCC (SEQ ID No. 379)
oSL1164	(c)PCR	CGCCGCACATCTGAACTTC (SEQ ID No. 353)
oSL1163	(c)PCR	GTCTGAATTTGACCTGAGCGC (SEQ ID No. 354)

FIG. 101A

Oligo ID	Use	Sequence
oSL1177	qPCR	CATGCAGTATTCCAGGACTC (SEQ ID No. 355)
oSL1178	qPCR	GGAGAGCAAATCTTGTTGC (SEQ ID No. 356)
oSL1304	qPCR	GCGCTCAGGTCAAATTCAGAC (SEQ ID No. 357)
oSL1307	qPCR	GGAGTGACGGCAGTTATCTG (SEQ ID No. 358)
oSL1164	qPCR	CGCCGCACATCTGAACTTC (SEQ ID No. 359)
oSL1184	qPCR	CTGAAGTTTAGACCATGAAGAGGC (SEQ ID No. 360)
oSL1185	qPCR	GGTTGTTTTGTGGTTAAGTTGCTG (SEQ ID No. 361)

FIG. 101B

Oligo ID	Use	Sequence
oSL1623	NGS	CTCTTTCCCTACACGACGCTCTTCCGATCTGCATTGTTTGTTGGCTACGAG **(SEQ ID No. 362)**
oSL1624	NGS	CTCTTTCCCTACACGACGCTCTTCCGATCTGTTTGTAATTGACTGAATATCAACGC **(SEQ ID No. 363)**
oSL1625	NGS	CTCTTTCCCTACACGACGCTCTTCCGATCTGGTGACAGTTATATGTAAGGAATATGACAG **(SEQ ID No. 364)**
oSL1626	NGS	CTCTTTCCCTACACGACGCTCTTCCGATCTGCTACTTTTTCTCTGTCACAGAATG **(SEQ ID No. 365)**
oSL1310	NGS	CTCTTTCCCTACACGACGCTCTTCCGATCTCACCACAGATGAAACGCC **(SEQ ID No. 366)**
oSL1309	NGS	CTCTTTCCCTACACGACGCTCTTCCGATCTCATCATTAAAGCGAGTGGCAAC **(SEQ ID No. 367)**
oSL1098	NGS	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTGAAGTTTAGACCATGAAGAGGC **(SEQ ID No. 368)**
oSL1101	NGS	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGTTGTTTTGTGGTTAAGTTGCTG **(SEQ ID No. 369)**
oSL1323	NGS	CTGGAGTTCAGACGTGTGCTCTTCCGATCTGCGTTTCTACCTGCAGGG **(SEQ ID No. 370)**
oSL1368	NGS	CTCTTTCCCTACACGACGCTCTTCCGATCTNN **(SEQ ID No. 371)**
oSL1369	NGS	P-AGATCGGAAGAGCGTCGTGTAGGGAAAGAG **(SEQ ID No. 372)**
oSL1371	NGS	ACACTCTTTCCCTACACGACGC **(SEQ ID No. 373)**
oSL1723	NGS	5rApp/AGATCGGAAGAGCACACGTCTGAACTCCAG/3ddC **(SEQ ID No. 374)**
oSL1724	NGS	CTGGAGTTCAGACGTGTGCTCTTCCGATCT **(SEQ ID No. 375)**
oSL1725	NGS	CUCUUUCCCUACACGACGCUCUUCCGAUCU **(SEQ ID No. 376)**
N/A	NGS	AATGATACGGCGACCACCGAGATCTACACNNNNNNNNACACTCTTTCCCTACACGACGC **(SEQ ID No. 377)**
N/A	NGS	CAAGCAGAAGACGGCATACGAGATNNNNNNNNGTGACTGGAGTTCAGACGTGTGC **(SEQ ID No. 378)**

FIG. 101C

NCBI Genome accession ID	Transposon startCoord	Transposon EndCoord	NCBI accession ID for TnsB	TnsB StartCoord	TnsB EndCoord	TnsB direction
NZ_NOJI01000009.1	15104	59319	WP_119464978.1	15918	17729	+
NZ_PYLX01000025.1	17023	42612	WP_107250937.1	41801	39990	-
NZ_LIXE01000015.1	20888	51613	WP_119464619.1	21702	23513	+
NZ_SYUM01000004.1	28898	81452	WP_136985358.1	29649	31520	+
NZ_PYLU01000010.1	124311	153260	WP_107274795.1	152449	150638	-
NZ_PYOO01000001.1	261740	290689	WP_107274795.1	262551	264362	+
NZ_AIDL02000029.1	10522	56314	WP_016795651.1	55500	53689	-
NZ_APHW01000105.1	46265	128761	WP_038885460.1	47083	48909	+
NZ_FNVG01000001.1	63510	121776	WP_103878368.1	120962	119157	-
NZ_MCVS01000025.1	396241	435706	WP_102538708.1	396092	398863	+
NZ_MCWR01000077.1	397294	436737	WP_102526002.1	398045	399835	+
NZ_MVJE01000005.1	91720	121245	WP_086712134.1	120431	118620	-
NZ_NNHQ01000001.1	174925	209858	WP_053305643.1	209044	207233	-
NZ_PYNV01000008.1	80491	104282	WP_107246830.1	81305	83116	+
NZ_QLYY01000013.1	133649	175929	WP_114786278.1	175177	173303	-
NZ_SPOT01000009.1	26582	67009	WP_136677344.1	27334	29208	+
NZ_SYVM01000070.1	41164	73042	WP_136982639.1	72291	70420	-
NZ_JSEN01000004.1	1885	26805	WP_082828034.1	15035	13683	-
NZ_JSEO01000015.1	2134	27054	WP_082828034.1	15284	13932	-
NZ_SNUB01000030.1	3352	37730	WP_134291474.1	36888	35047	-
NZ_VIOO01000139.1	98959	126013	WP_142542587.1	125266	123359	-
NZ_MPIK01000004.1	223025	256059	WP_076338387.1	255215	253362	-
NZ_QEBK01000011.1	252760	291982	WP_114778930.1	291235	289328	-
NZ_POSI01000001.1	295534	323062	WP_102952857.1	296275	298182	+
NZ_QEAX01000001.1	357345	396567	WP_114778930.1	395820	393913	-
NZ_QECQ01000002.1	424314	463536	WP_114778930.1	462789	460882	-

FIG. 102A

NCBI Genome accession ID	Transposon startCoord	Transposon EndCoord	NCBI accession ID for TnsB	TnsB StartCoord	TnsB EndCoord	TnsB direction
NZ_ANFM02000036.1	44605	60502	WP_002540938.1	59765	57885	-
NZ_BAOG01000014.1	11676	44082	WP_038875868.1	12418	14334	+
NZ_BBLF01000006.1	135215	166075	WP_045419481.1	165333	163417	-
NZ_JPTP01000003.1	16836	56214	WP_050907889.1	17578	19485	+
NZ_LBFX01000002.1	272375	307337	WP_057552674.1	306590	304683	-
NZ_LBGC01000006.1	143553	178515	WP_057552674.1	144300	146207	+
NZ_NMSU01000018.1	66778	101740	WP_057552674.1	67525	69432	+
NZ_NMSV01000007.1	51699	86661	WP_057552674.1	52446	54353	+
NZ_QEBU01000008.1	66763	101725	WP_057552674.1	67510	69417	+
NZ_QNVL01000001.1	296900	330099	WP_113628400.1	329352	327445	-
NZ_PNCB01000022.1	3789	27014	WP_138590899.1	26278	24440	-
NZ_PNCF01000018.1	4182	27407	WP_138590899.1	26671	24833	-
NZ_LKDW01000019.1	20342	38562	WP_055018246.1	37827	36004	-
NZ_AUTR01000037.1	31979	52181	WP_024590737.1	32714	34537	+
NZ_AUTS01000021.1	31979	52181	WP_024590737.1	32714	34537	+
NZ_ML064597.1	19722	51969	WP_121851997.1	42643	45096	+
NZ_ML064597.1	42457	65763	WP_121851997.1	42643	45096	+
NZ_JPMC01000002.1	84103	111073	WP_036953812.1	84838	86661	+
NZ_LRUE01000024.1	419856	446473	WP_062566900.1	420589	422430	+
NZ_AHCF02000042.1	111880	134320	WP_010388169.1	133585	131765	-
NZ_BCAD01000009.1	90757	106936	WP_053910945.1	106200	104362	-
NZ_PYMT01000003.1	36741	54623	WP_053061935.1	38028	39347	+
NZ_AUGM01000008.1	29221	83548	WP_035387132.1	82811	80910	-
NZ_AUGM01000008.1	57742	83548	WP_035387132.1	82811	80910	-
NZ_CH902601.1	215989	238175	WP_005370417.1	216727	218574	+
NZ_LNTE01000007.1	86371	101548	WP_058119882.1	100812	98953	-

FIG. 102B

NCBI Genome accession ID	Transposon startCoord	Transposon EndCoord	NCBI accession ID for TnsB	TnsB StartCoord	TnsB EndCoord	TnsB direction
NZ_QJIG01000019.1	22653	106032	WP_110276361.1	105199	102704	-
NZ_MAJT01000003.1	249701	272248	WP_065591601.1	271510	269666	-
NZ_MAJS01000006.1	250062	272609	WP_065591601.1	271871	270027	-
NZ_AJYI02000040.1	34788	62152	WP_017019107.1	34904	37393	+
NZ_LIZL01000007.1	259520	283009	WP_054541713.1	260262	262127	+
NZ_CCKD01000050.1	10899	47808	WP_048668972.1	11659	13524	+
NZ_MCUC01000076.1	63487	96200	WP_102502386.1	95440	93575	-
NZ_DS999339.1	53329	84030	WP_005472985.1	54070	55935	+
NZ_SLWJ01000005.1	129837	166746	WP_048668972.1	165986	164121	-
NZ_BBLD01000063.1	12734	42453	WP_045403567.1	13495	15360	+
NZ_LK934360.1	133	26203	WP_057623761.1	25462	23597	-
NZ_MCTU01000138.1	10708	37782	WP_102538610.1	11448	13313	+
NZ_SMAX01000007.1	112	26182	WP_057623761.1	25441	23576	-
NZ_SMBB01000007.1	207612	233682	WP_057623761.1	208353	210218	+
NZ_SMBF01000014.1	110	26180	WP_057623761.1	25439	23574	-
NZ_SODC01000006.1	207401	233471	WP_057623761.1	208142	210007	+
NZ_QEDB01000011.1	51671	80635	WP_001134621.1	52412	54277	+
NZ_VTZZ01000001.1	51861	80825	WP_001134621.1	52602	54467	+
NZ_AMBR01000012.1	158575	187539	WP_001134621.1	186798	184933	-

FIG. 102C

FIG. 103A

FIG. 103B

FIG. 103C

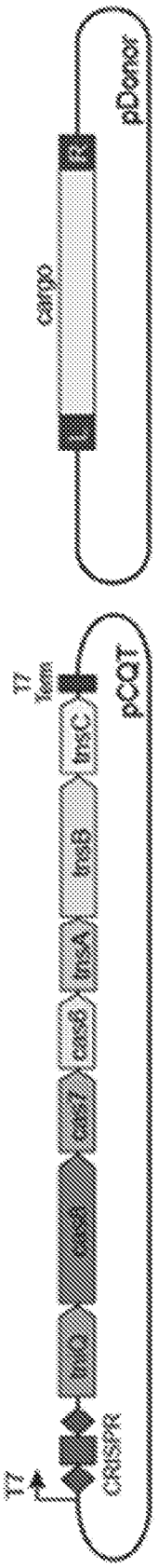


FIG. 104A

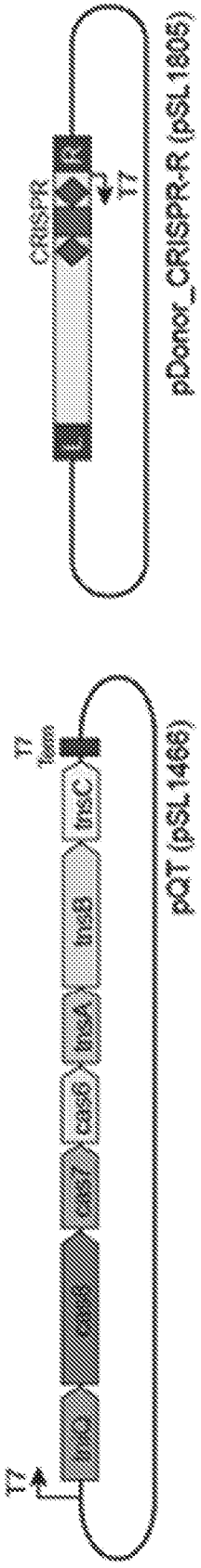


FIG. 104B

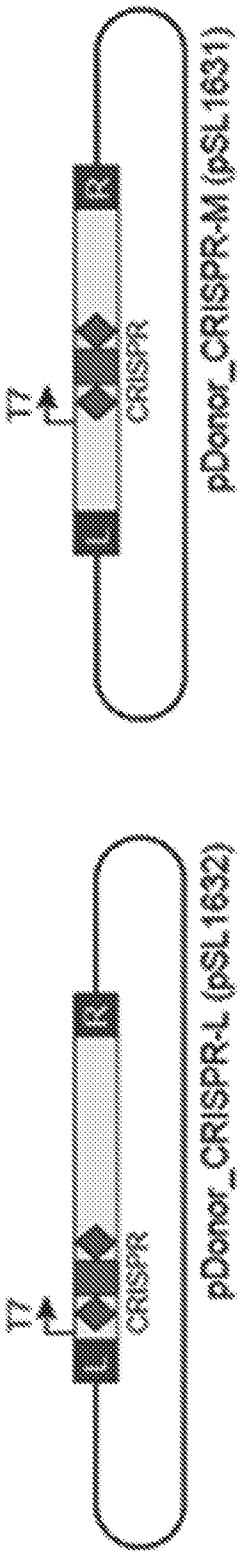


FIG. 104C

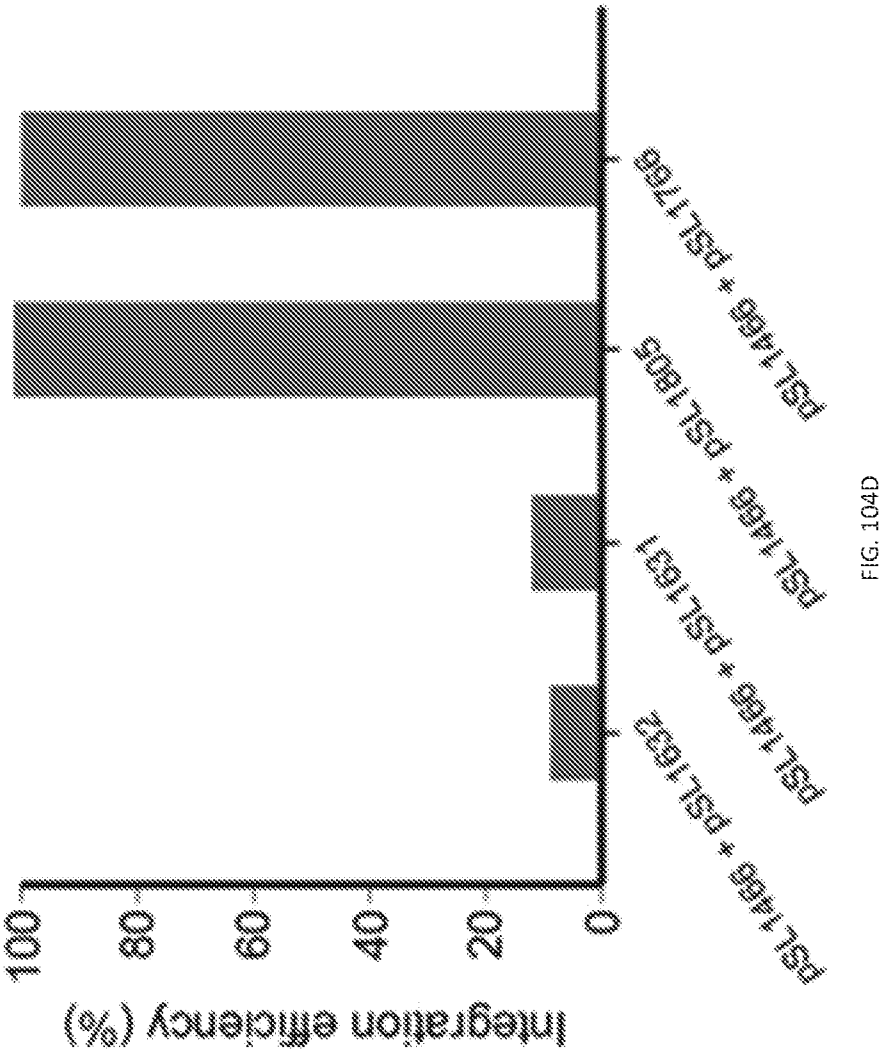

Integration efficiency (%)
100
80
60
40
20
0
PSL1466 + PSL1632
PSL1466 + PSL1631
PSL1466 + PSL1605
PSL1466 + PSL1766

FIG. 104D