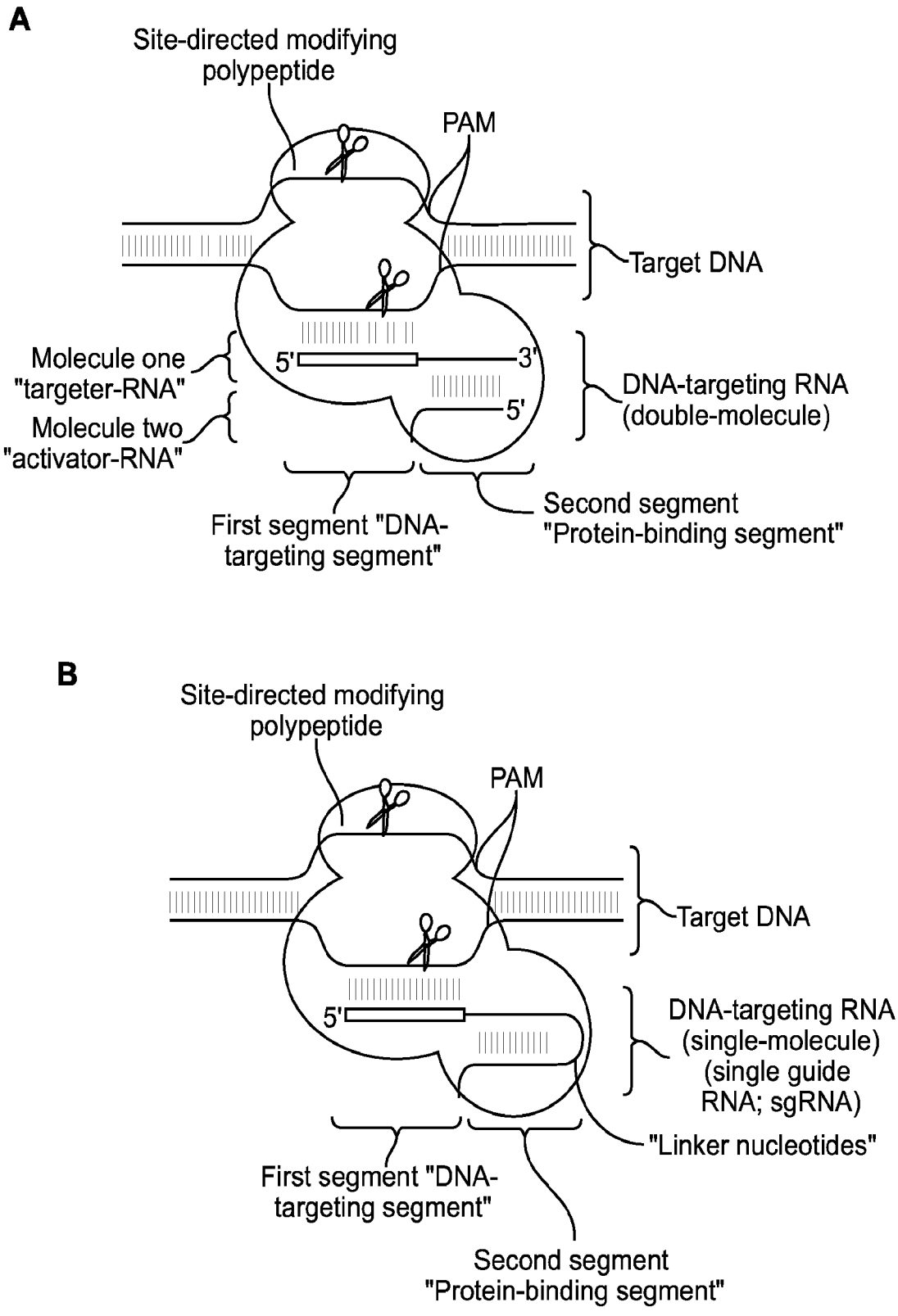

A
Site-directed modifying polypeptide
PAM
Target DNA
Molecule one "targeter-RNA"
Molecule two "activator-RNA"
5'
3'
5'
DNA-targeting RNA (double-molecule)
First segment "DNA-targeting segment"
Second segment "Protein-binding segment"
B
Site-directed modifying polypeptide
PAM
Target DNA
5'
DNA-targeting RNA (single-molecule) (single guide RNA; sgRNA)
"Linker nucleotides"
First segment "DNA-targeting segment"
Second segment "Protein-binding segment"

FIG. 1

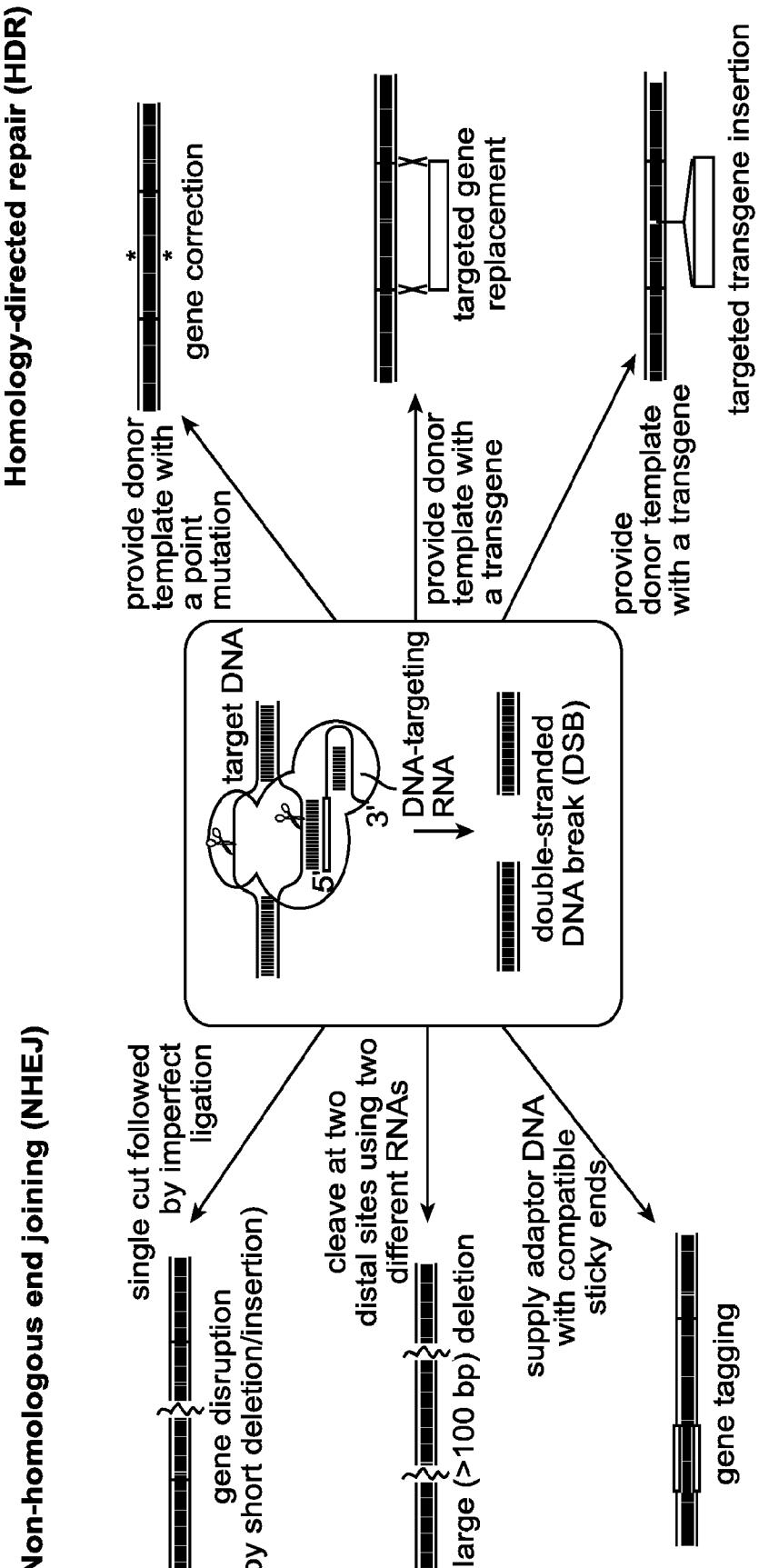

Homology-directed repair (HDR)
provide donor template with a point mutation
gene correction
provide donor template with a transgene
targeted gene replacement
provide donor template with a transgene
targeted transgene insertion
target DNA
5'
3'
DNA-targeting RNA
double-stranded DNA break (DSB)
Non-homologous end joining (NHEJ)
single cut followed by imperfect ligation
gene disruption (by short deletion/insertion)
cleave at two distal sites using two different RNAs
large (>100 bp) deletion
supply adaptor DNA with compatible sticky ends
gene tagging

FIG. 2

Cas9/Csn1 Streptococcus pyogenes

motifs

1 MDKKYS**IGLDIGTNSVGWAVI**TDDYKVPSKKLKGLGNTDRHGIKKNLIGALL
FDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEE
SFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLADSTDKVDLRLIYL
ALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASRVDA
KAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAED
AKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDATLLSDILRVNSEITK
APLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGG
ASQEEFYKFIKPILEKMDGTEELLAKLNREDLLRKQRTFDNGSIPYQIHLGEL
HAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEE
TITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYN
ELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIEC
FDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFED
REMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTI
LDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGS
2 PAIKKGILQTVKVVDELVKVMGRHKPEN**IVIEMARE**NQTTQKGQKNSRERM
KRIEEGIKELGSDILKEYPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRL
3 SDY**DVDHIVPQSFLKDDSIDNKVLTRSDKN**RGKSDNVPSEEVVKKMKNYW
RQLLNAKLITQRKFDNLTKAERGGLSELDKVGFIKRQLVETRQITKHVAQILD
4 SRMNTKYDENDKLIREVRVITLKSKLVSDFRKDFQFYKVREINNY**HHAHDAY**
LNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYS
NIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQV
NIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVL
VVAKVEKGKSKKLKSVKELLGITIMERSSFEKDPIDFLEAKGYKEVRKDLIIKL
PKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSP
EDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPI
REQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLY
ETRIDLSQLGGD

FIG. 3A

Cas9/Csn1 Streptococcus pyogenes

Domains

1 MDKKYSI**GLDIGTNSVGWAVITDDYKVPSKKLKGLGNTDRHGIKKNLIGAL**
LFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLE
ESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLADSTDKVDLRLI
YLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASRV
DAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLA
EDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDATLLSDILRVNSEI
TKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYID
GGASQEEFYKFIKPILEKMDGTEELLAKLNREDLLRKQRTFDNGSIPYQIHL
GELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRK
SEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTV
YNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKI
ECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLF
EDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSG
KTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLA
2 GS**PAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSR**
ERMKRIEEGIKELGSDILKEYPVENTQLQNEKLYLYYLQNGRDMYVDQEL
DINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKK
MKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKVGFIKRQLVETRQIT
KHVAQILDSRMNTKYDENDKLIREVRVITLKSKLVSDFRKDFQFYKVREIN
NYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIG
KATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATV
RKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGF
DSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKDPIDFLEAKGY
KEVRKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLA
SHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVL
SAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLD
ATLIHQSITGLYETRIDLSQLGGD

FIG. 3B

A. **Sequence identities relative to: *S. pyogenes* Cas9/Csn1**

species	reference sequence	Sequence identities - MUSCLE alignment		
		Full-length	*Domain 1*	*Domain 2*
		% identity	% identity	% identity
Streptococcus pyogenes M1 GAS	NP_269215	100.0	100.0	100.0
Streptococcus pyogenes MGAS5005	YP_282132.1	99.9	99.4	100.0
Listeria innocua Clip11262	NP_472073	54.3	60.0	64.9
Pasteurella multocida subsp. multocida str. Pm70	NP_246064.1	19.7	29.0	25.9
Streptococcus thermophilus LMD-9 Csn1-A	YP_820832	59.2	75.6	72.4
Streptococcus thermophilus LMD-9 Csn1-B	YP_820161.1	20.6	27.3	26.8
Neisseria meningitidis Z2491	YP_002342100.1	20.2	33.6	28.1
Streptococcus mutans UA159	NP_721764	64.9	78.1	74.1
Streptococcus gordonii str. Challis substr. CH1	YP_001450662.1	19.8	28.2	27.0
Campylobacter jejuni subsp. jejuni NCTC 11168	YP_002344900.1	19.6	30.3	26.3
Treponema denticola ATCC 35405	NP_970941	32.5	47.3	38.8

B. **Sequence identities relative to: *N. meningitidis* Cas9/Csn1**

species	reference sequence	Sequence identities - MUSCLE alignment		
		Full-length	*Domain 1*	*Domain 2*
		% identity	% identity	% identity
Streptococcus pyogenes M1 GAS	NP_269215	20.2	33.6	28.1
Streptococcus pyogenes MGAS5005	YP_282132.1	20.3	34.5	28.1
Listeria innocua Clip11262	NP_472073	18.8	33.6	25.9
Pasteurella multocida subsp. multocida str. Pm70	NP_246064.1	64.3	72.1	69.0
Streptococcus thermophilus LMD-9 Csn1-A	YP_820832	19.6	35.3	25.9
Streptococcus thermophilus LMD-9 Csn1-B	YP_820161.1	25.8	35.7	35.1
Neisseria meningitidis Z2491	YP_002342100.1	100.0	100.0	100.0
Streptococcus mutans UA159	NP_721764	19.2	36.1	25.5
Streptococcus gordonii str. Challis substr. CH1	YP_001450662.1	25.3	37.5	35.8
Campylobacter jejuni subsp. jejuni NCTC 11168	YP_002344900.1	34.7	45.0	41.0
Treponema denticola ATCC 35405	NP_970941	18.8	31.5	25.5

FIG. 4

SUBSTITUTE SHEET (RULE 26)

	Motif 1	Motif 2	Motif 4
	*		
S. pyogenes	...IGLDIGTNSVGWAVI...	...IVIEMARE...	...HHAHDAYL...
L. pneumophila	...IGIDLGGKFTGVCLS...	...MMQRLAYE...	...SHAIDATL...
G. proteobacterium	...IAIDLGAKFTGVALY...	...IIEHIARK...	...SHVVDAVC...
L. innocua	...IGLDIGTNSVGWAVL...	...IVVEMARE...	...HHAHDAYL...
L. gasseri	...VGLDVGTNSCGWVAM...	...IAIEFTRD...	...HHAIDAYL...
E. rectale	...LALDIGIASVGWAIL...	...IVIEMPRD...	...HHAVDAML...
S. lugdunensis	...LGLDIGITSVGYGLI...	...IIIELARE...	...HHAEDALI...
M. synoviae	...IGFDLGVASVGWSIV...	...VVIEMARE...	...HHAVDASI...
M. mobile	...LGLDLGIASVGWCLT...	...IVVEVTRS...	...HHAEDAYF...
W.succinogenes	...LGVDLGISSLGWAIV...	...VHFELARE...	...HHAVDAII...
F. columnare	...LGLDLGTNSIGWAIR...	...IHIEMARE...	...HHTIDAIT...
F. succinogenes	...LGLDLGTNSIGWAVV...	...IHLELGRD...	...HHAMDAIV...
B. fragilis	...LGLDLGTNSIGWALV...	...IRVELARE...	...HHAMDALT...
A. cellulolyticus	...LGVDVGERSIGLAAV...	...IVVELARG...	...HHAVDAVV...
B. dentium	...IGIDVGLMSVGLAAI...	...VQIEHVRE...	...HHAVDAAV...

	Motif 3
	*
S. pyogenes	...DVDHIVPQSFLKD------DSIDNKVLTRSDKN...
L. pneumophila	...EIDHIYPRSLSKKHFGVIFNSEVNLIYCSSQGN...
G. proteobacterium	...EIDHIIPRSLTGRTKKTVFNSEANLIYCSSKGN...
L. innocua	...DIDHIVPQSFITD------NSIDNLVLTSSAGN...
L. gasseri	...DIDHILPQSFIKD------DSLENRVLVKKAVN...
E. rectale	...EIDHIIPRSISFD------DARSNKVLVYRSEN...
S. lugdunensis	...EVDHIIPRSVSFD------NSYHNKVLVKQSEN...
M. synoviae	...EIDHVIPYSKSAD------DSWFNKLLVKKSTN...
M. mobile	...DIDHIVPRSISFD------DSFSNLVIVNKLDN...
W.succinogenes	...EIDHILPRSRSAD------DSFANKVLCLARAN...
F. columnare	...DIEHTIPRSISQD------NSQMNKTLCSLKFN...
F. succinogenes	...EIEHVIPQSLYFD------DSFSNKVICEAEVN...
B. fragilis	...DIEHIIPQARLFD------DSFSNKTLEARSVN...
A. cellulolyticus	...ELDHIVPRTDGG------SNRHENLAITCGACN...
B. dentium	...EMDHIVPRKGVGS-----TNTRVNLAAACAACN...

FIG. 5

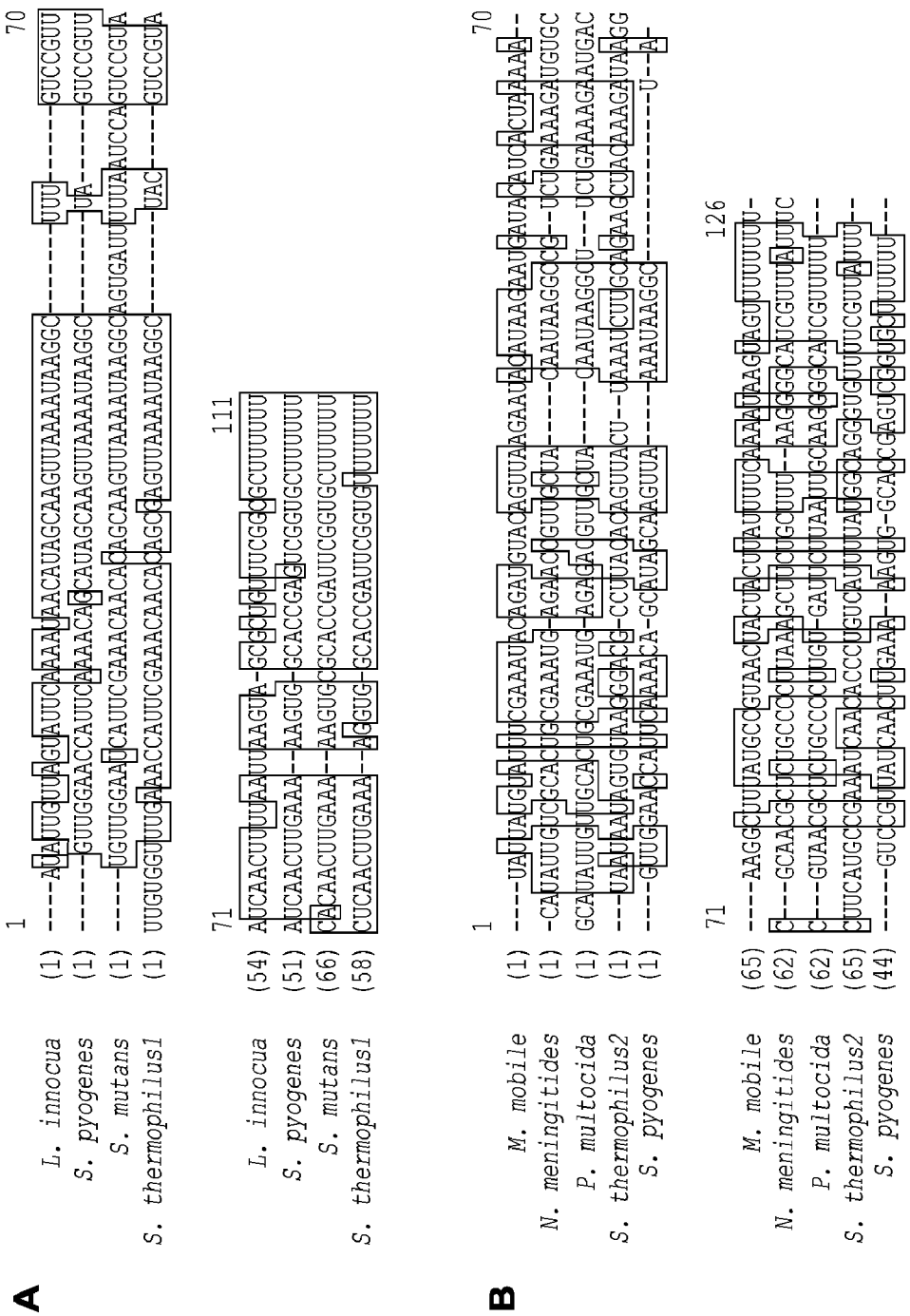


FIG. 6

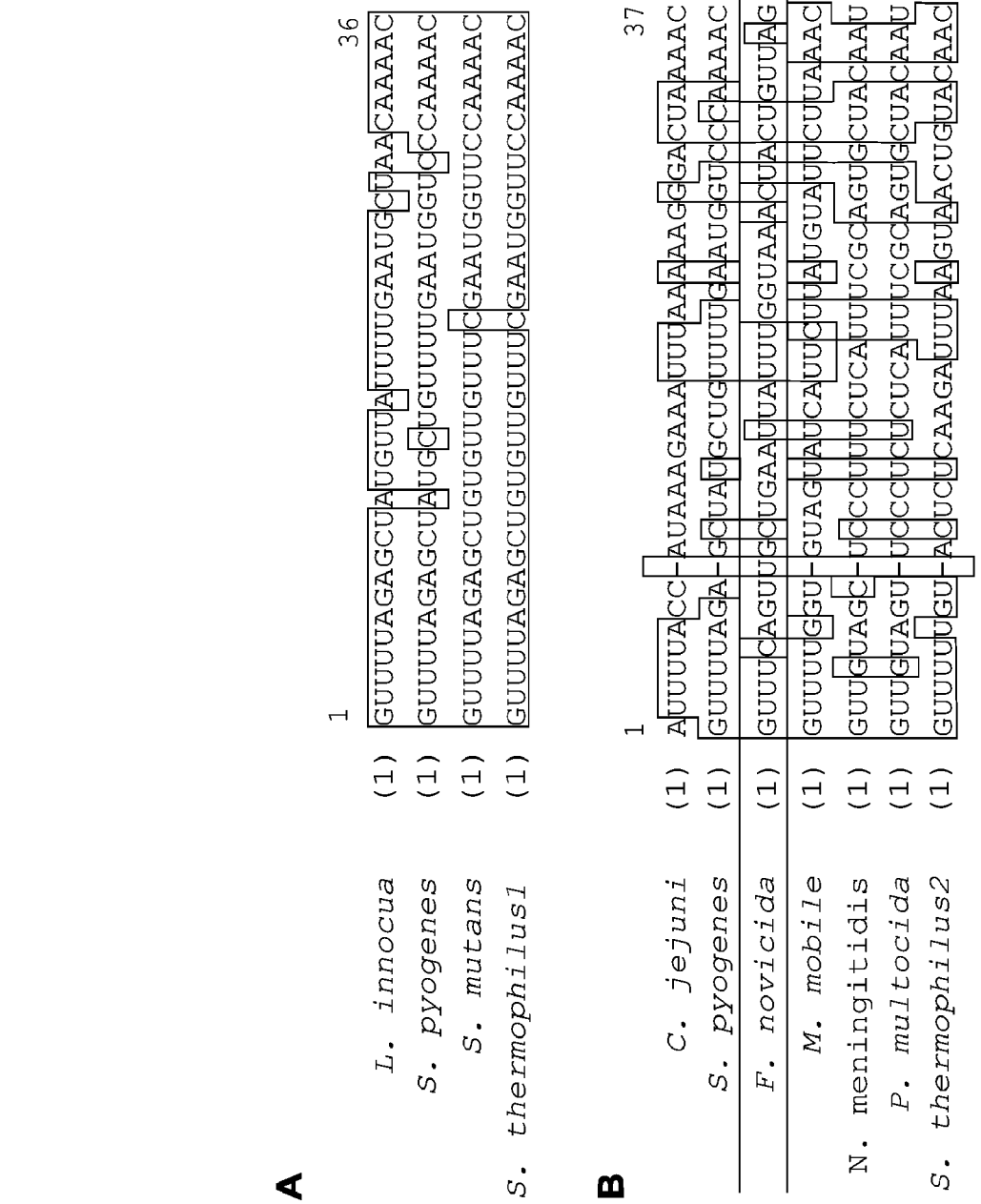
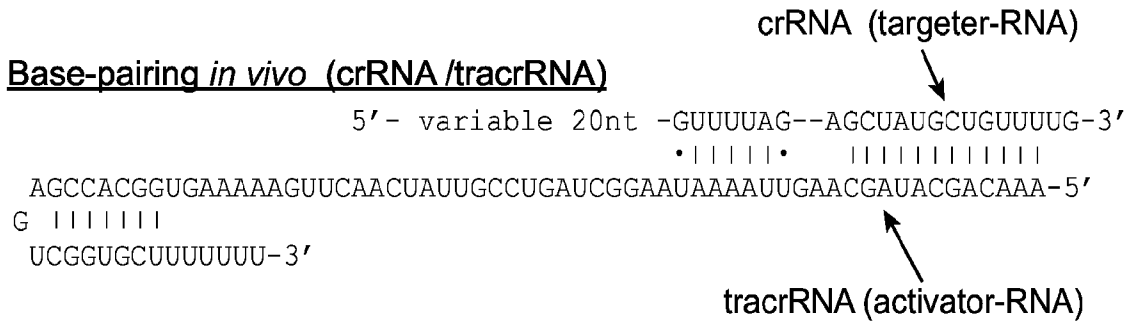

A
1
36
L. innocua (1) GUUUUAGAGCUAUGUUAUUUUUGAAUGCUUAACAAAAC
S. pyogenes (1) GUUUUAGAGCUAUGCUGUUUUUGAAUGGUCCCAAAAC
S. mutans (1) GUUUUAGAGCUGUGUUGUUUCGAAUGGUUCCCAAAAC
S. thermophilus1 (1) GUUUUAGAGCUGUGUUGUUUCGAAUGGUUCCCAAAAC
B
1
37
C. jejuni (1) AUUUUACC-AUAAAGAAAUUUAAAAAGGACUAAAAAC
S. pyogenes (1) GUUUUAGA-GCUAUGCUGUUUUGAAUGGUCCCAAAAAC
F. novicida (1) GUUUCAGUUGCUGAAUUAUUGGUAAACUACUGUUAG
M. mobile (1) GUUUUGGU-GUAGUAUCAUUCUUAUGUAUUCUUAAAAC
N. meningitidis (1) GUUGUAGC-UCCCUUUCUCAUUUCGCAGUGCUACAAU
P. multocida (1) GUUGUAGU-UCCCUCUCUCAUUUCGCAGUGCUACAAU
S. thermophilus2 (1) GUUUUUGU-ACUCUCAAGAUUUAAGUAACUGUACAAC

FIG. 7

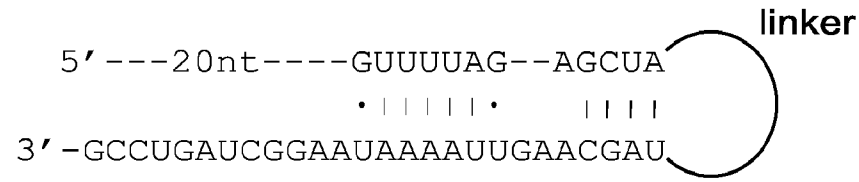
STRAIN	NUMBER OF CRISPRs	CASS4 CRISPR identifier[a]	CRISPR REPEAT:tracrRNA BASEPAIRING[b]
streptococcus pyogenes SF370	2	NC_002737_1	5'GUUUUAG--AGCUAUGCUGUUUUGAAUGGUCCCAAAAC 3' ||||• |||||||||||||||||| |||| | 3' AAAUUGAACGAUACGACAAAACUUACCAAGGUUGUU 5'
Streptococcus mutans UA159	1	NC_004350_1	GUUUUAG--AGCUGUGUUGUUUCGAAUGGUUCCAAAAC ||||• ||||||||||||||•| |||| || AAAUUGAACGACACAACAAAGCUUACUAAGGUUGUG
Streptococcus thermophilus LMD-9	3	NC_008532_5	GUUUUAG--AGCUGUGUUGUUUCGAAUGGUUCCAAAAC ||||• ||||||||||||||||| ||| | AAAUUGAGCGACACAACAAAGCUUACCAAAGUUUGG
		NC_008532_2	GUUUUUGUACUCU-CAAGAUUUAAGUAACUGUACAAC |||||• |||||||||||||||||| || AAACAUCGAAGACGUUCUAAAUUCAUUGACACAUUC
Listeria innocua Clip11262	1	NC_003212_2	GUUUUAG--AGCUAUGUUAUUUUGAAUGCUAACAAAAC ||||• |||||||||||||•|||||| | AAAUUGAACGAUACAAUAAAACUUAUGAUUGUUAUA
Treponema denticola ATCC35405	1	NC_002967_1	GUUUGAG--AGUUGUGUAAUUUAAGAUGGAUCUCAAAC ||||• ||||||||||||||•|||||| || AACUUGAGCAACACAUUAAAUUCUACCUAGAAUUUA
Neisseria meningitidis Z2491	2	NC_003116_10	GUUGUAGCUCCCUUUCUCAUUUCGCAGUGCUACAAU •||||||| | |||||||||||||| ||||| UAACAUCGUUGCCAAGAGUAAAGCGUCACGCUGUUA
streptococcus gordonii str. Challis substr. CH1	1	NC_009785_2	GUUUUUGUACUCU-CAAGAUUUAAGUAACUGUACAAC |||||• |||||||||||||||||| ||| AAACAUCGAAGACGUUCUAAAUUCAUUGACACAUUC
Bifidobacterium bifidum S17	1	NC_014616_1	GUUUCA-AUGCCUGUCAGAUCAAUGACUUUGACCAC • |||| ||||||||| ||||||||•||| || AGUUAAAUACGGACAGUCCAGUUACUGGAACUAGUA
Lactobacillus salivarius UCC118	1	NC_007929_1	GUUUCAGAAGUAUGUUAAAUCAAUAAGGUUAAGACC |• || |||||||||| || ||||•||•||| AAGUUGAGUCUUACAAUUUAGUUACUCCAGUUUUGG
Francisella tularensis subsp.novicida U112	2	NC_008601_1[c]	CUAACAGUAGUUUACCAAAUAAUUCAGCAACUGAAAC |||• •• |||||||||||| |•||||||||| UUGUGUUCAUGUAUGGUUUAUUAGAUUGUUGACUUUG CUAACAGUAGUUUACCAAAUAAUUCA-GCAACUGAAAC | | |•| |||||||||||| | || ||| || UUUAAUAUUUACAUGGUUUAUUAAUUACG-AGACAUUA
Legionella pneumophila str. Paris	1	NC_006368_1	CCAAUAAUCCCUCAUCUAAAAAUCCA-ACCACUGAAAC ||| | ||||||||| || | ||| |||||| AUUUAAUCUUUAGUAGAUUUAAAGCUAUGG-GACUUUA

FIG. 8

Streptococcus pyogenes

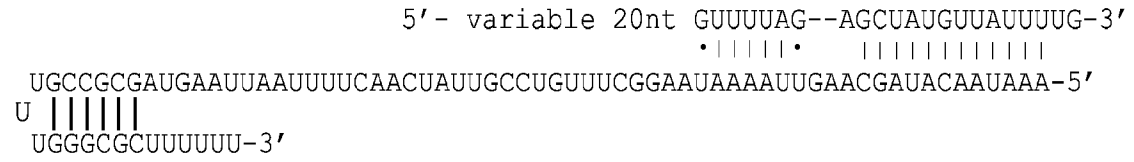


Example of a single-molecule DNA-targeting RNA



Listeria innocua

Base-pairing *in vivo* (crRNA/tracrRNA)



Example of a single-molecule DNA-targeting RNA

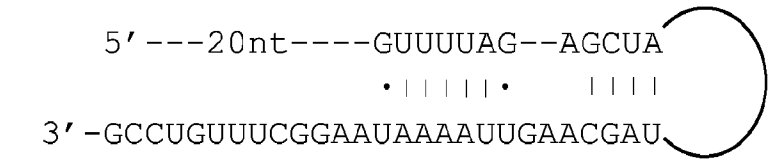


FIG. 9

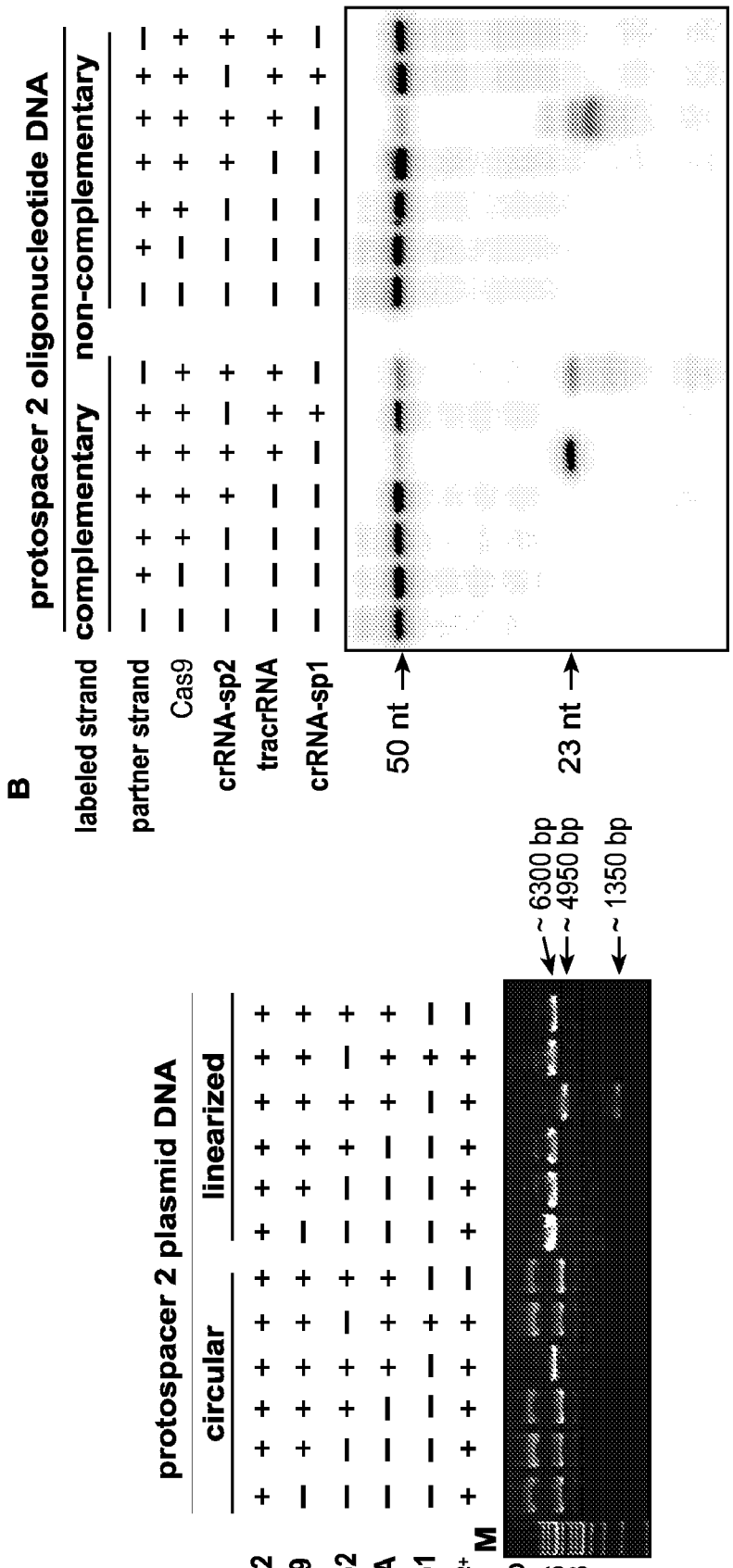
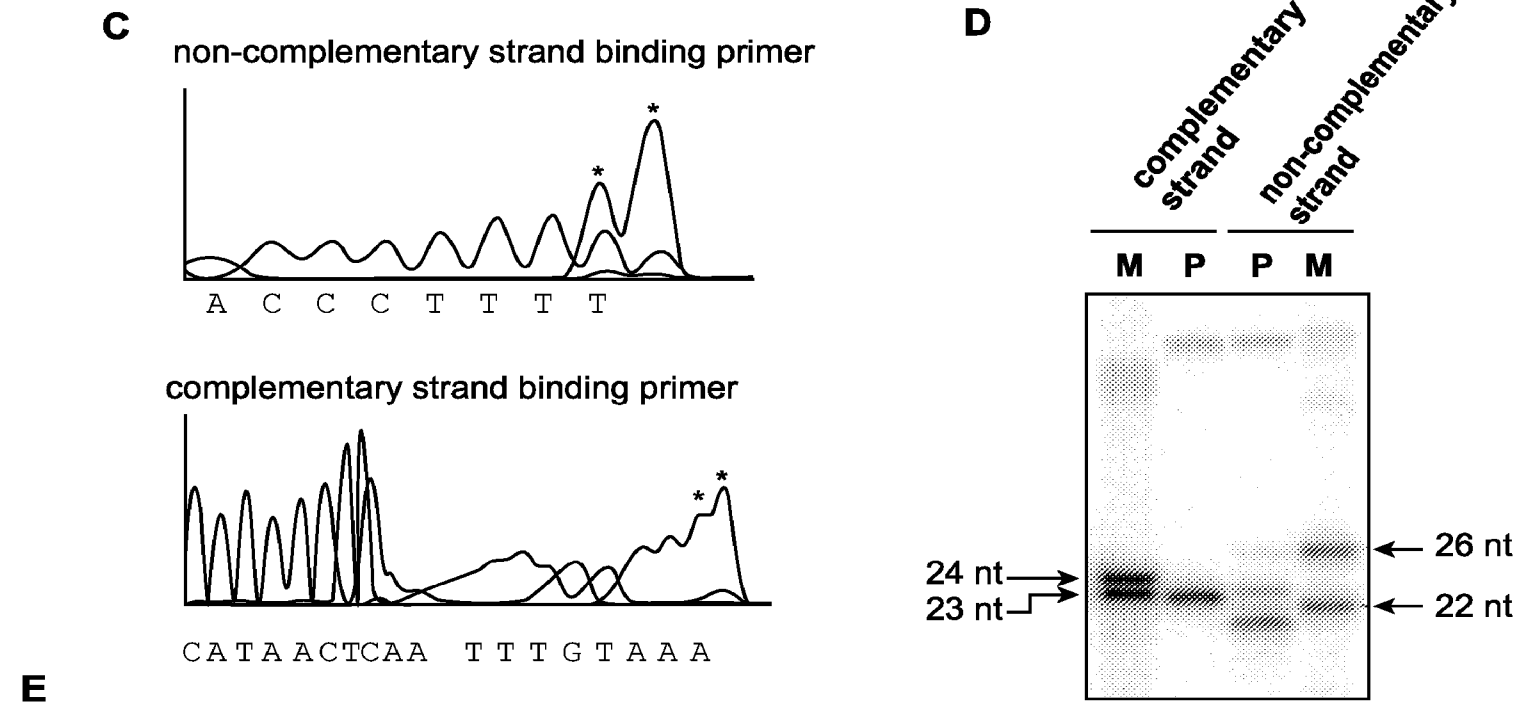

A
protospacer 2 plasmid DNA
circular
linearized
plasmid-sp2
Cas9
crRNA-sp2
tracrRNA
crRNA-sp1
Mg2+
M
kbp
6
3
1.5
~ 6300 bp
~ 4950 bp
~ 1350 bp
B
protospacer 2 oligonucleotide DNA
complementary
non-complementary
labeled strand
partner strand
Cas9
crRNA-sp2
tracrRNA
crRNA-sp1
50 nt
23 nt

FIG. 10



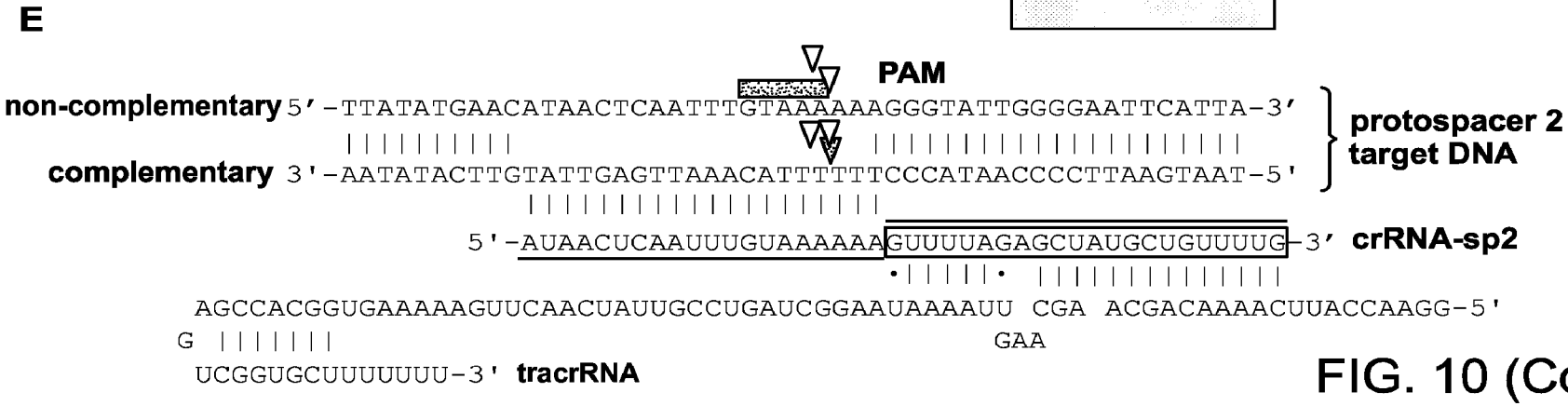


FIG. 10 (Cont.)

SUBSTITUTE SHEET (RULE 26)

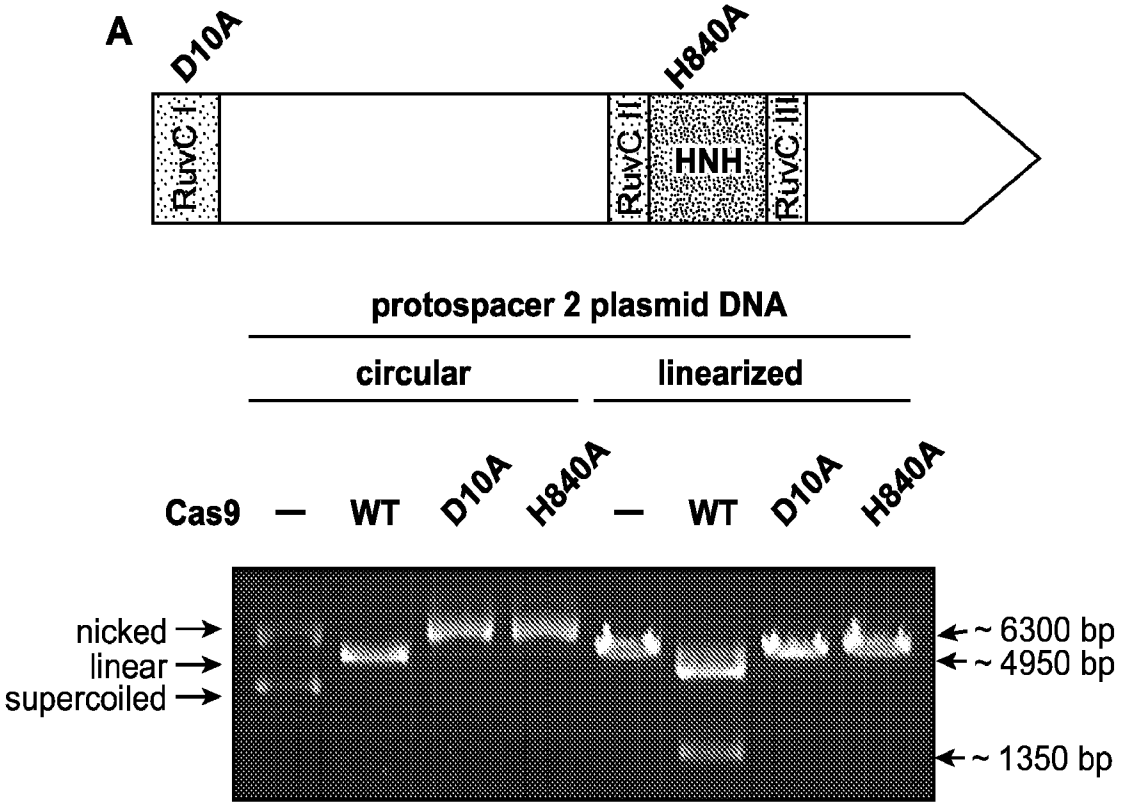

A
D10A
H840A
RuvC I
RuvC II
HNH
RuvC III
protospacer 2 plasmid DNA
circular
linearized
Cas9 — WT D10A H840A — WT D10A H840A
nicked
linear
supercoiled
~ 6300 bp
~ 4950 bp
~ 1350 bp
B
protospacer 2 oligonucleotide DNA
complementary strand
non-complementary strand
Cas9 — WT D10A H840A — WT D10A H840A
50 nt
23 nt

FIG. 11

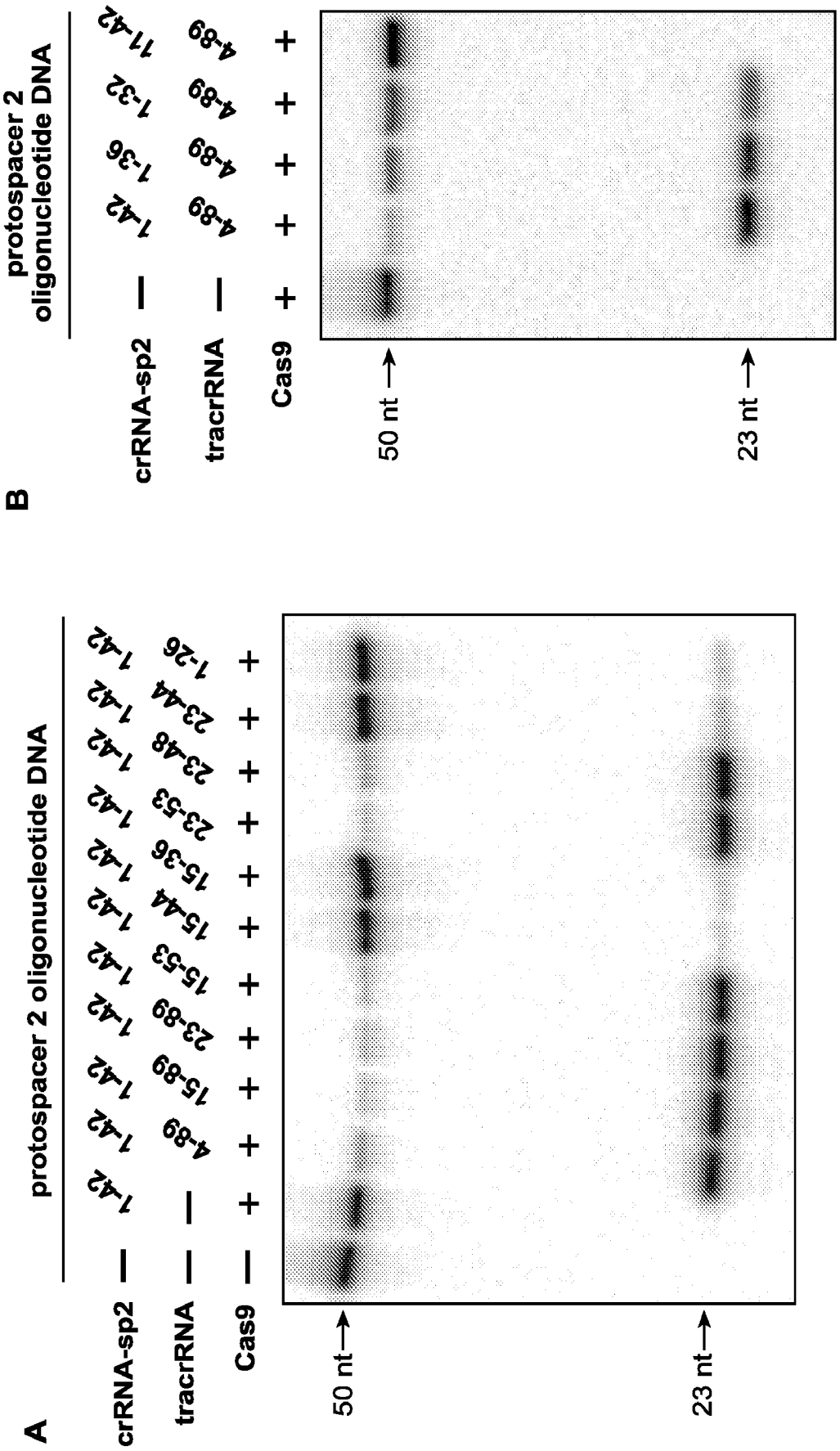

A
protospacer 2 oligonucleotide DNA
crRNA-sp2
tracrRNA
Cas9
1-42 — + 1-26
1-42 4-89 + 1-42
1-42 15-89 + 23-44
1-42 23-89 + 1-42
1-42 15-53 + 23-53
1-42 15-44 + 23-48
1-42 15-36 + 1-42
1-42 23-53 + 1-42
1-42 23-48 + 1-42
1-42 23-44 + 1-42
1-42 1-26 + 1-42
50 nt
23 nt
B
protospacer 2 oligonucleotide DNA
crRNA-sp2
tracrRNA
Cas9
— 4-89 + 11-42
— 4-89 + 1-32
— 4-89 + 1-36
— 4-89 + 1-42
50 nt
23 nt

FIG. 12

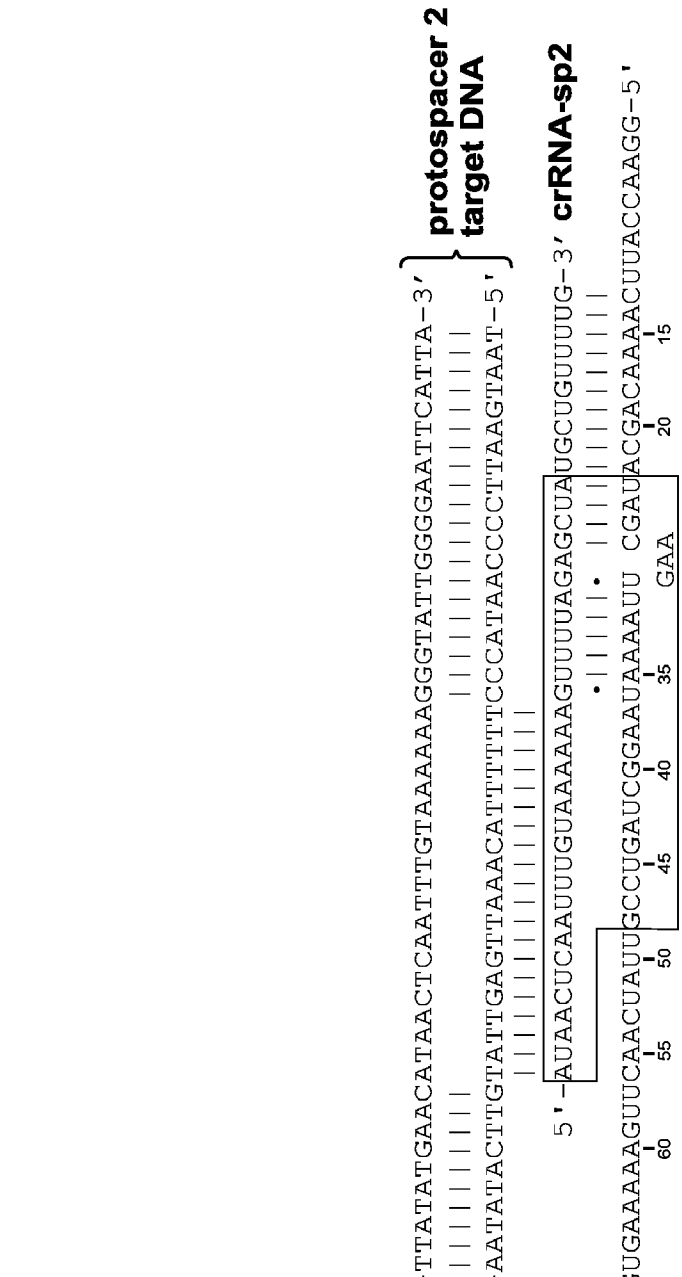

c
non-complementary
complementary
protospacer 2
target DNA
crRNA-sp2
tracrRNA

FIG. 12 (Cont. 1)

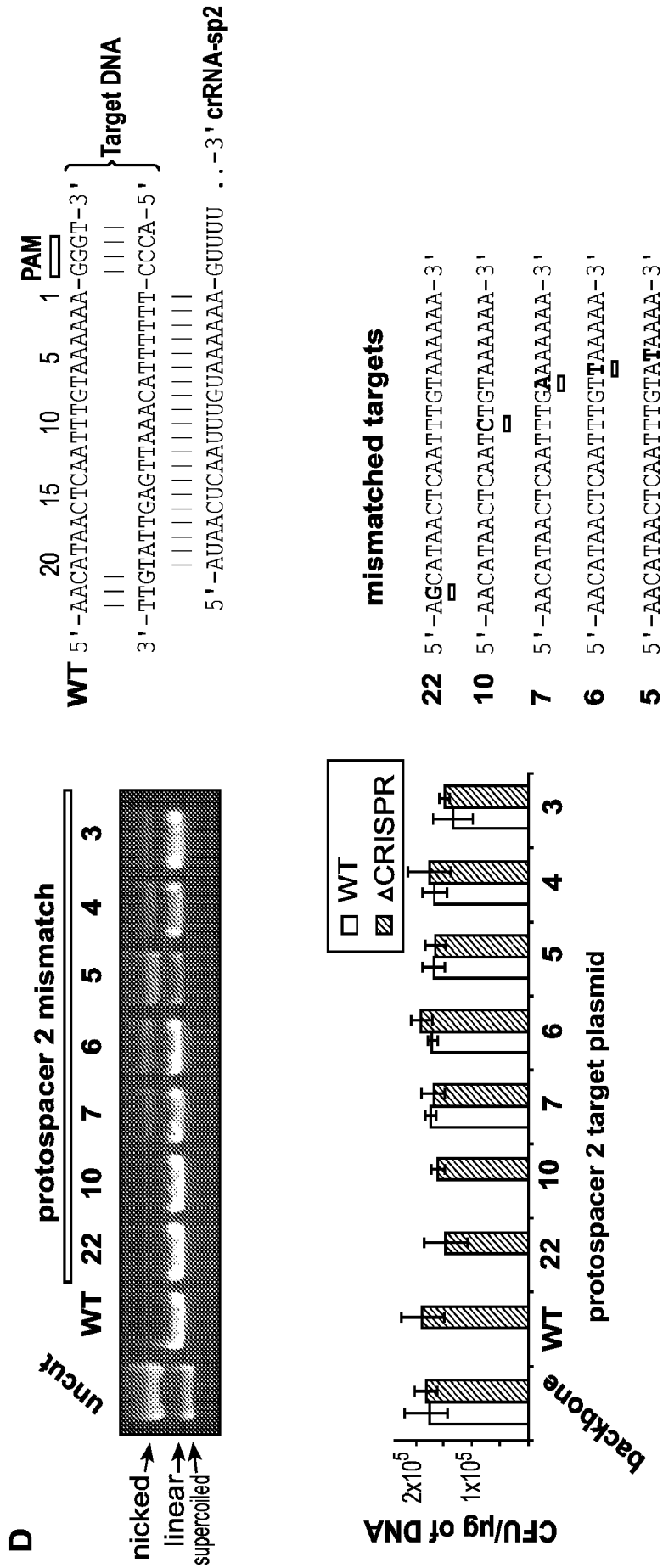

D
uncut
WT 22 10 7 6 5 4 3
protospacer 2 mismatch
nicked
linear
supercoiled
WT 5'-AACATAACTCAATTTGTAAAAAA-GGGT-3'
3'-TTGTATTGAGTTAAACATTTTTT-CCCA-5'
5'-AUAACUCAAUUUGUAAAAAA-GUUUU ..-3' crRNA-sp2
20 15 10 5 1 PAM
Target DNA
mismatched targets
22 5'-AGCATAACTCAATTTGTAAAAAA-3'
10 5'-AACATAACTCAATCTGTAAAAAA-3'
7 5'-AACATAACTCAATTGAAAAAAA-3'
6 5'-AACATAACTCAATTTGTTAAAAA-3'
5 5'-AACATAACTCAATTTGTATAAAA-3'
4 5'-AACATAACTCAATTTGTAATAAA-3'
3 5'-AACATAACTCAATTTGTAAATAA-3'
CFU/μg of DNA
2x10^5
1x10^5
WT
ΔCRISPR
backbone
WT 22 10 7 6 5 4 3
protospacer 2 target plasmid

FIG. 12 (Cont. 2)

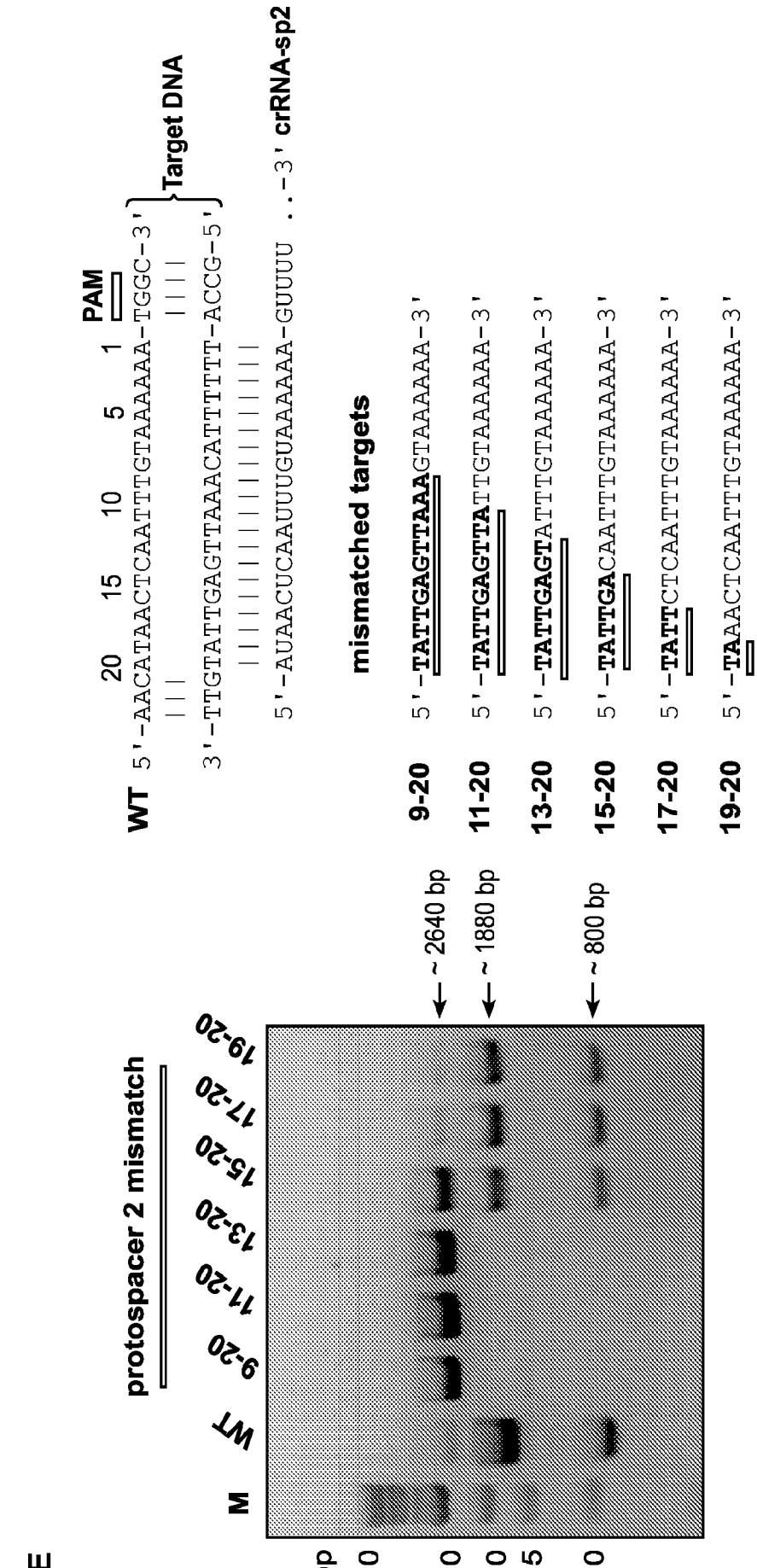

E
protospacer 2 mismatch
M
WT
9-20
11-20
13-20
15-20
17-20
19-20
kbp
10
3.0
2.0
1.5
1.0
~ 2640 bp
~ 1880 bp
~ 800 bp
PAM
20 15 10 5 1
WT 5'-AACATAACTCAATTTGTAAAAAA-TGGC-3'
3'-TTGTATTGAGTTAAACATTTTTT-ACCG-5'
5'-AUAACUCAAUUUGUAAAAAA-GUUUU ..-3' crRNA-sp2
Target DNA
mismatched targets
9-20 5'-TATTGAGTTAAAGTAAAAAA-3'
11-20 5'-TATTGAGTTATTGTAAAAAA-3'
13-20 5'-TATTGAGTATTTGTAAAAAA-3'
15-20 5'-TATTGACAATTTGTAAAAAA-3'
17-20 5'-TATTCTCAATTTGTAAAAAA-3'
19-20 5'-TAAACTCAATTTGTAAAAAA-3'

FIG. 12 (Cont. 3)

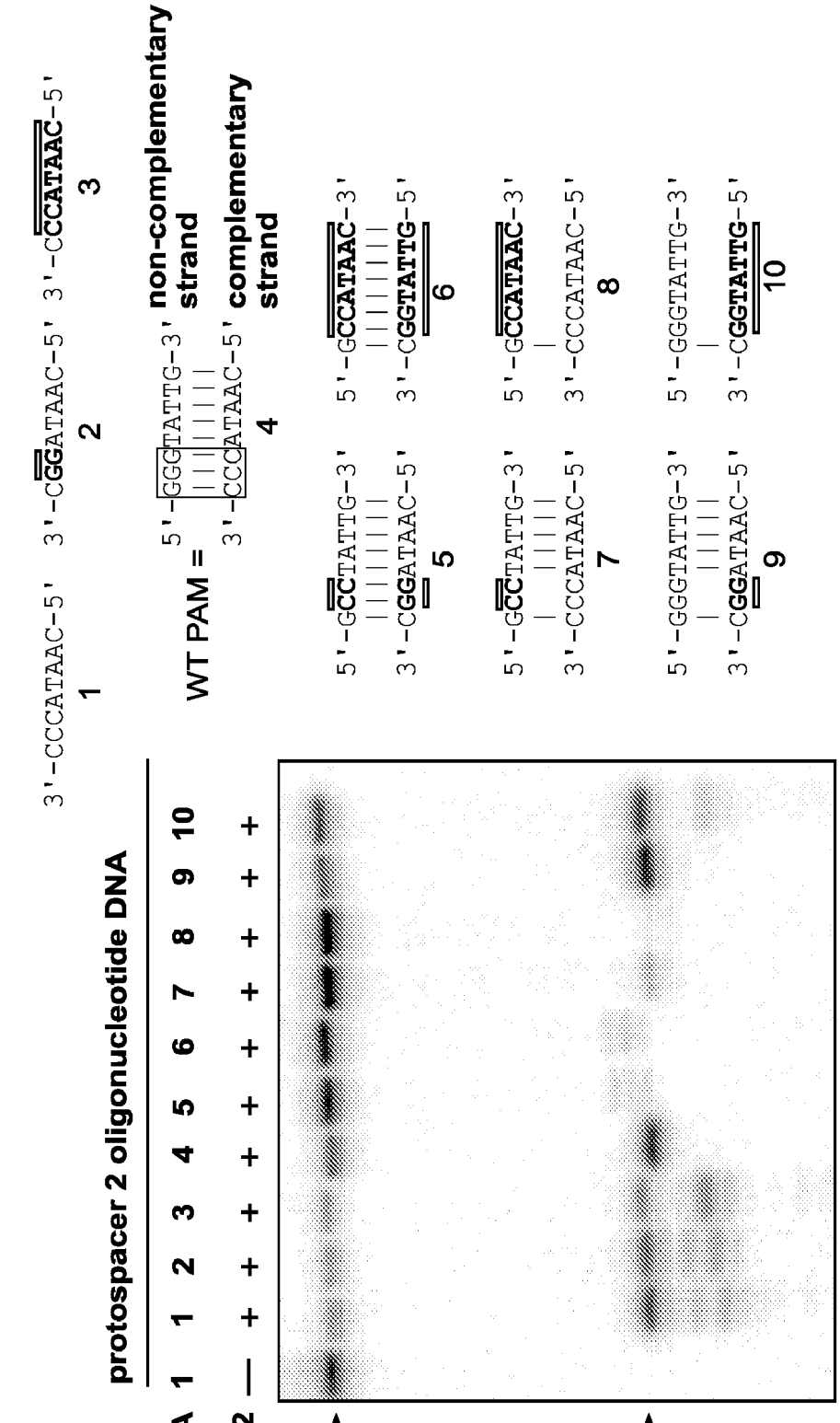

A
target DNA
protospacer 2 oligonucleotide DNA
1 1 2 3 4 5 6 7 8 9 10
tracrRNA:crRNA-sp2 — + + + + + + + + + +
50 nt
23 nt
3'-CCCATAAC-5' 3'-CGGATAAC-5' 3'-CCCATAAC-5'
1 2 3
WT PAM =
5'-GGGTATTG-3' non-complementary strand
3'-CCCATAAC-5' complementary strand
4
5'-GCCTATTG-3'
3'-CGGATAAC-5'
5
5'-GCCATAAC-3'
3'-CGGTATTG-5'
6
5'-GCCTATTG-3'
3'-CCCATAAC-5'
7
5'-GCCATAAC-3'
3'-CCCATAAC-5'
8
5'-GGGTATTG-3'
3'-CGGATAAC-5'
9
5'-GGGTATTG-3'
3'-CGGTATTG-5'
10

FIG. 13

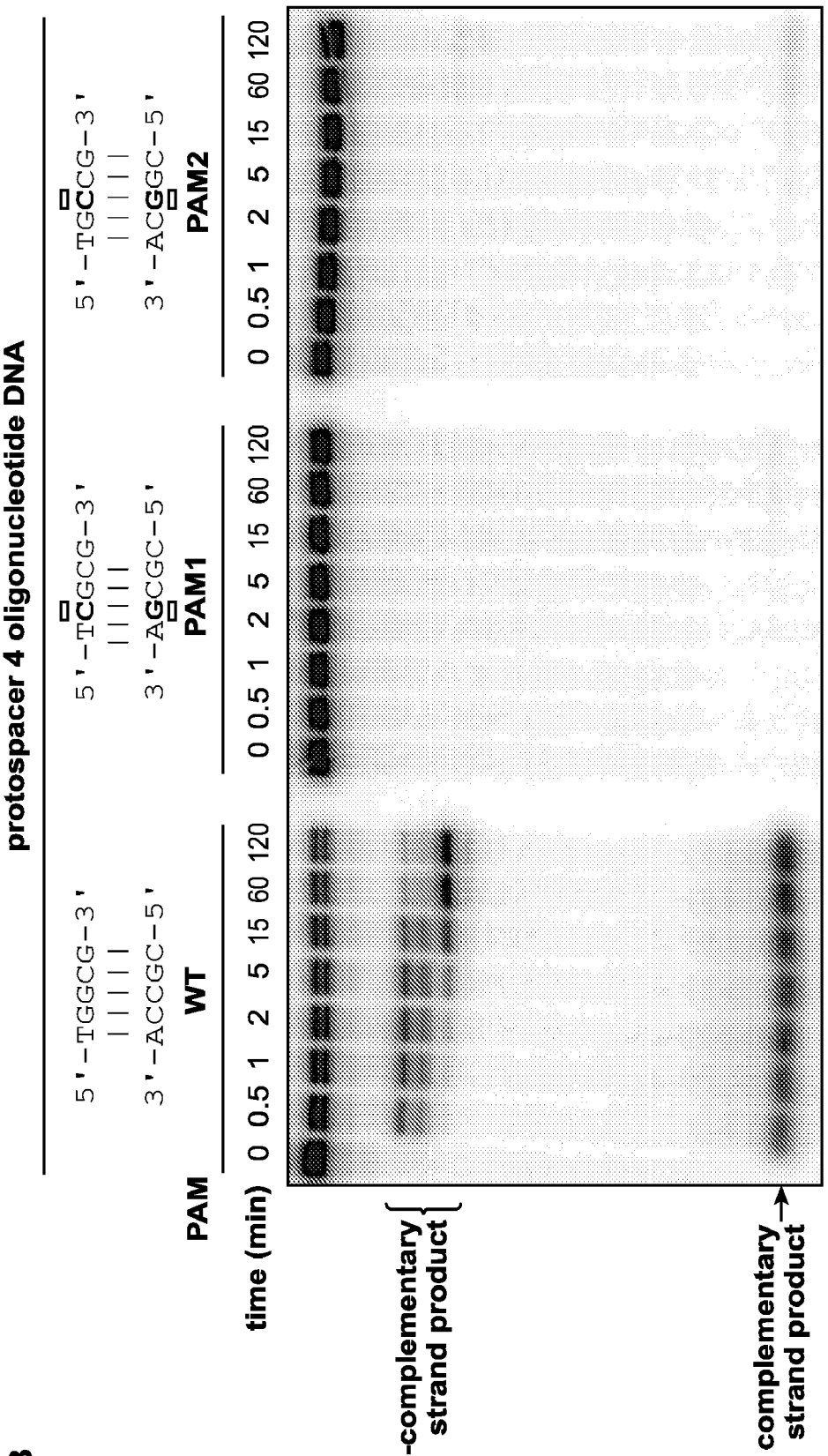

B
protospacer 4 oligonucleotide DNA
5'-TGGCG-3'
3'-ACCGC-5'
PAM
WT
5'-TCGCG-3'
3'-AGCGC-5'
PAM1
5'-TGCCG-3'
3'-ACGGC-5'
PAM2
time (min) 0 0.5 1 2 5 15 60 120
0 0.5 1 2 5 15 60 120
0 0.5 1 2 5 15 60 120
non-complementary strand product
complementary strand product

FIG. 13 (Cont. 1)

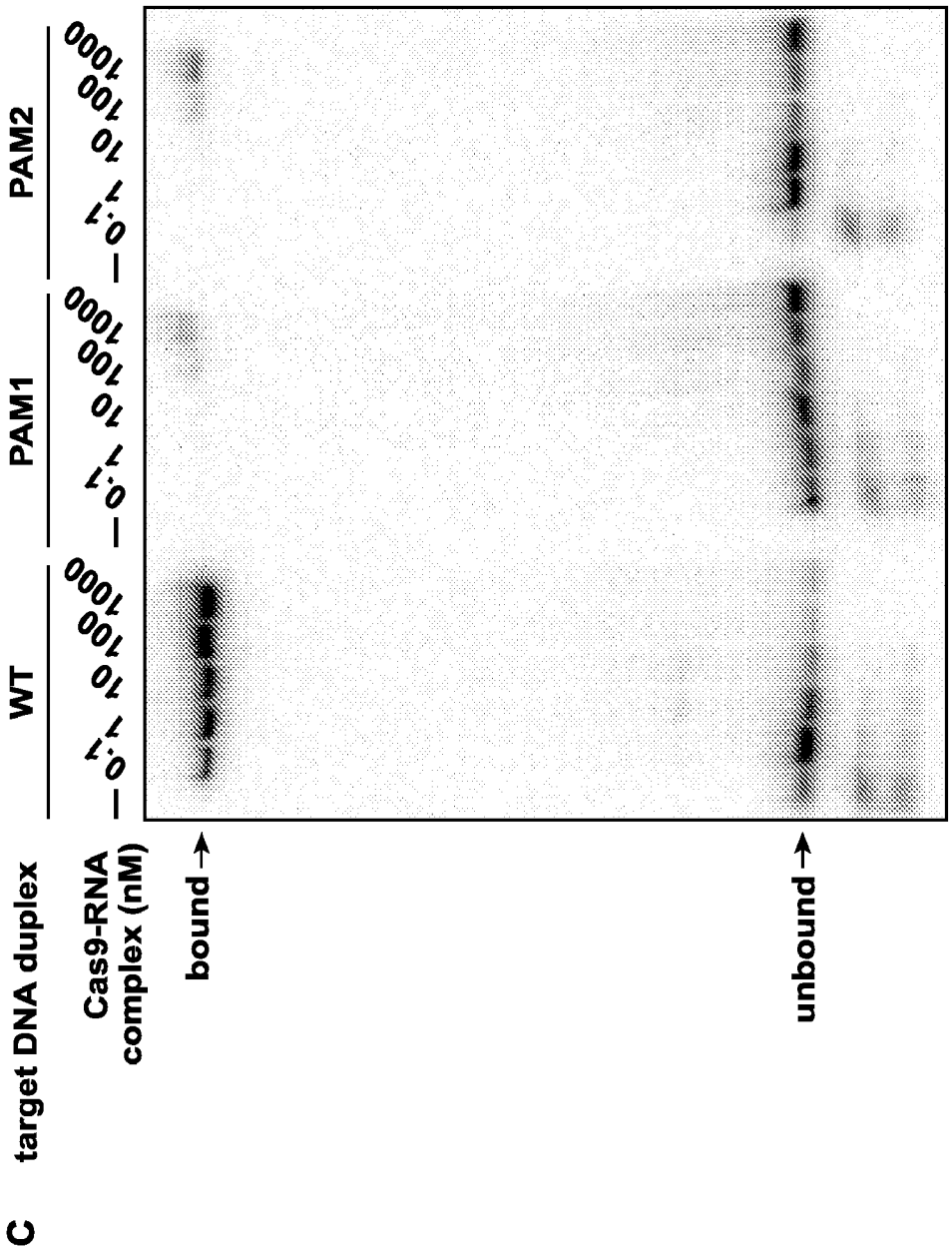
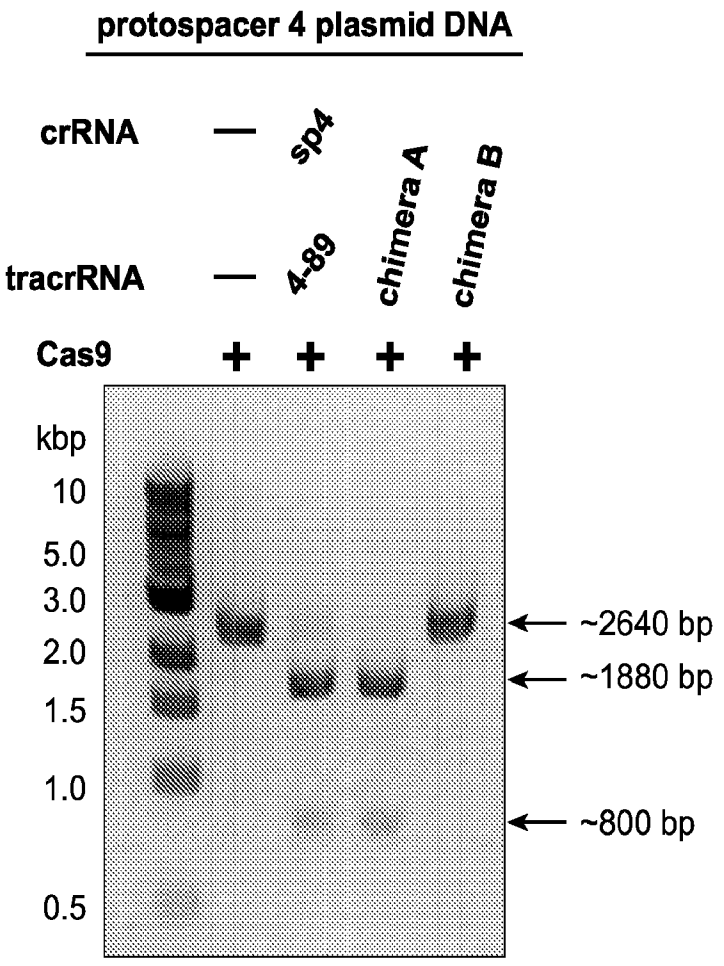
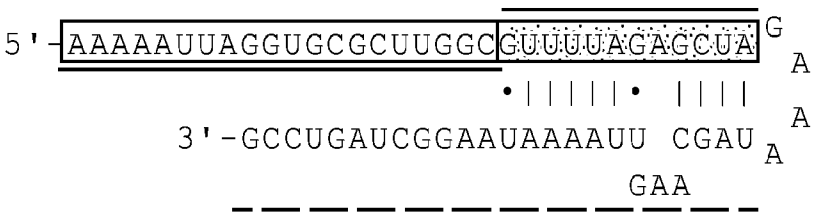

C
target DNA duplex
WT
PAM1
PAM2
Cas9-RNA complex (nM)
0
0.1
1
10
100
1000
bound
unbound

FIG. 13 (Cont. 2)

A



chimera A



chimera B

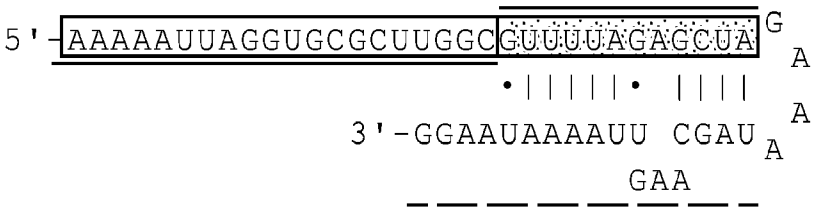


FIG. 14

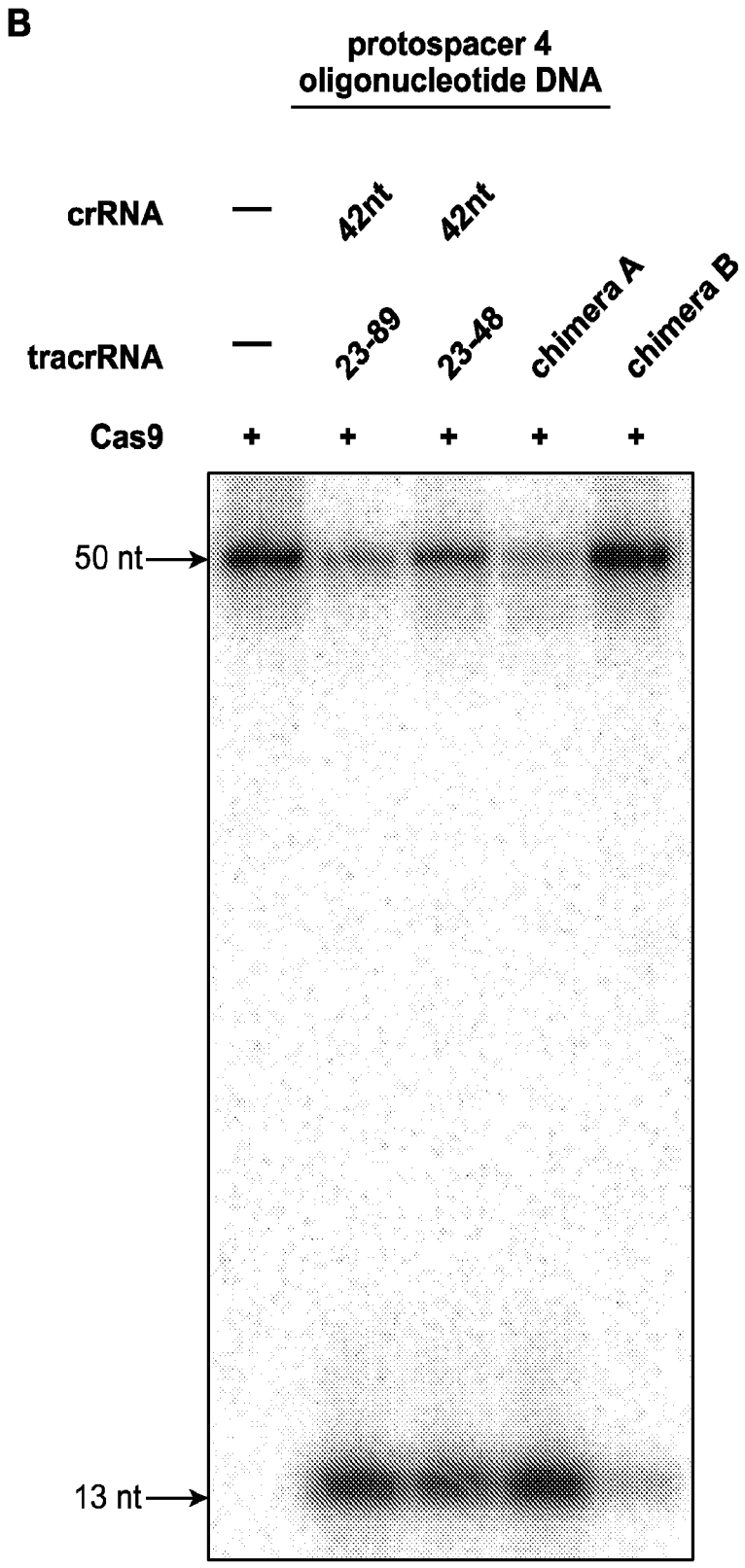

B
protospacer 4
oligonucleotide DNA
crRNA
tracrRNA
Cas9
—
42nt
42nt
—
23-89
23-48
chimera A
chimera B
+
+
+
+
+
50 nt
13 nt

FIG. 14 (Cont. 1)

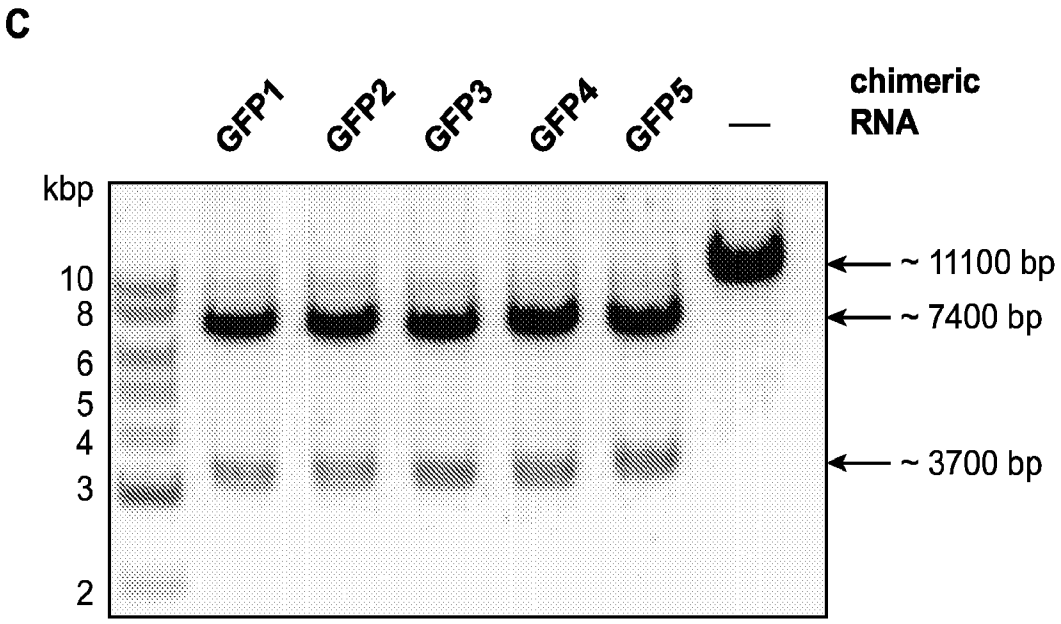

C
GFP1
GFP2
GFP3
GFP4
GFP5
—
chimeric RNA
kbp
10
8
6
5
4
3
2
~ 11100 bp
~ 7400 bp
~ 3700 bp

FIG. 14 (Cont. 2)

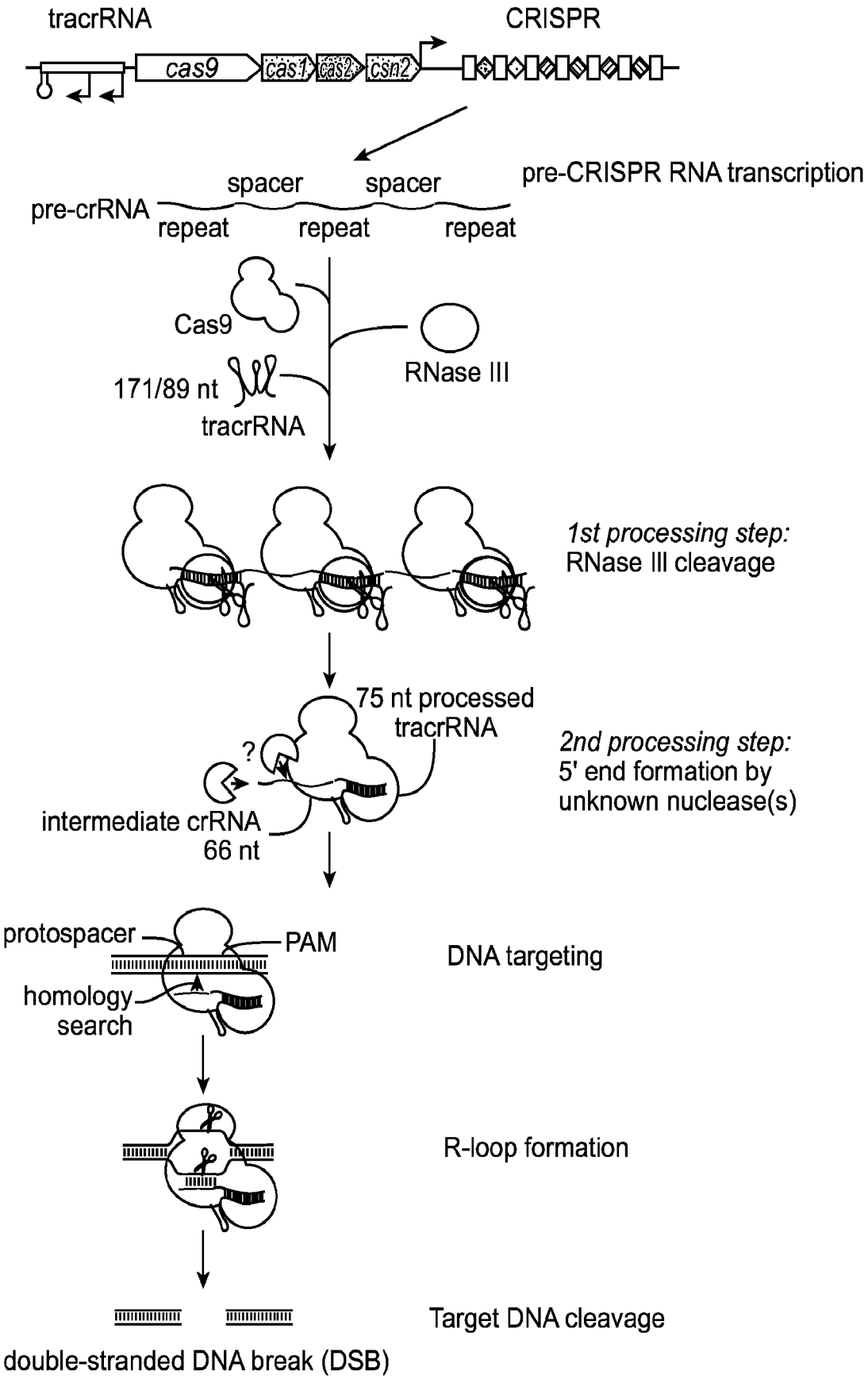

tracrRNA
CRISPR
cas9
cas1
cas2
csn2
pre-CRISPR RNA transcription
spacer
spacer
pre-crRNA
repeat
repeat
repeat
Cas9
RNase III
171/89 nt
tracrRNA
1st processing step:
RNase III cleavage
75 nt processed
tracrRNA
?
intermediate crRNA
66 nt
2nd processing step:
5' end formation by
unknown nuclease(s)
protospacer
PAM
homology
search
DNA targeting
R-loop formation
Target DNA cleavage
double-stranded DNA break (DSB)

FIG. 15

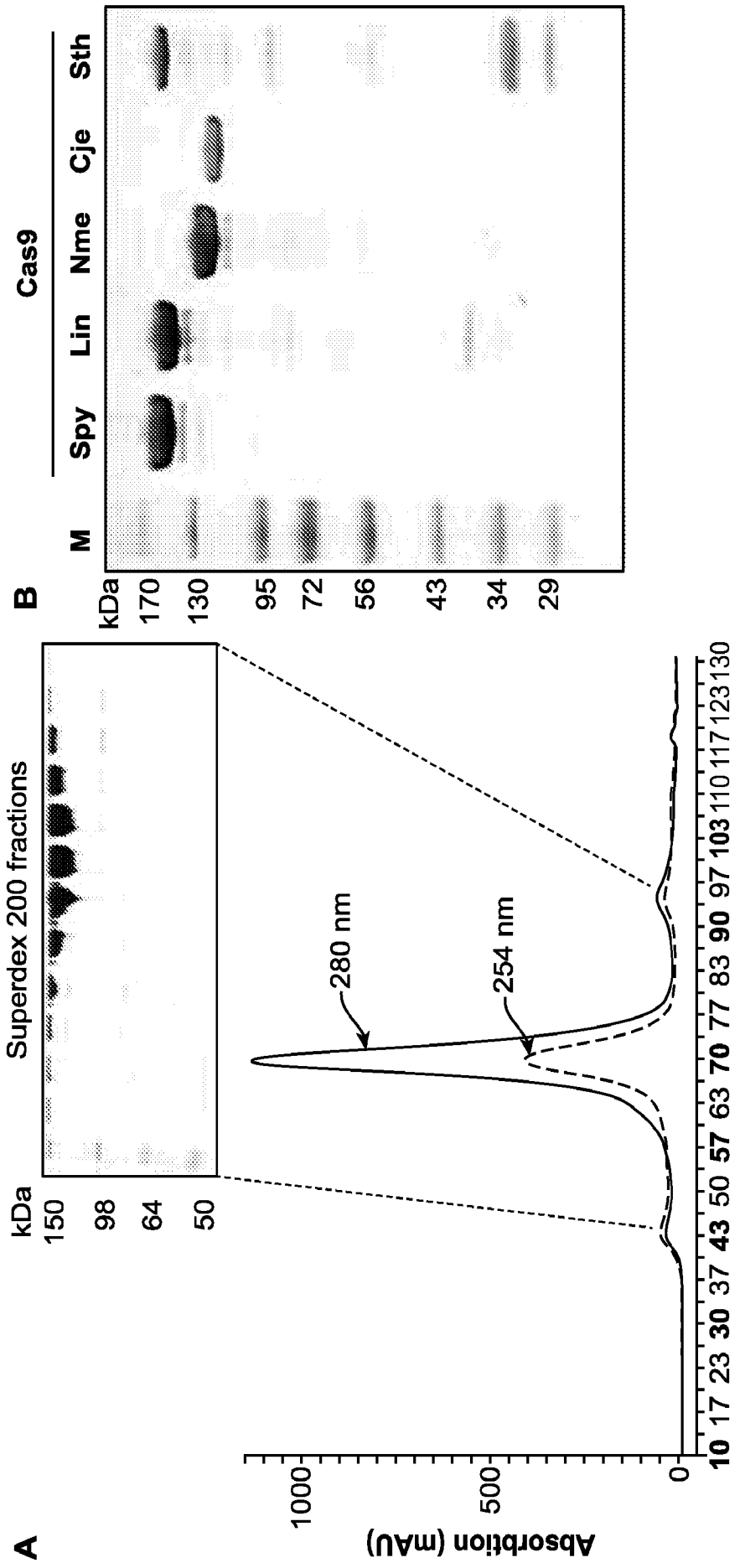

A
Absorption (mAU)
1000
500
0
280 nm
254 nm
Elution volume (ml)
10 17 23 30 37 43 50 57 63 70 77 83 90 97 103 110 117 123 130
Superdex 200 fractions
kDa
150
98
64
50
B
Cas9
M
Spy
Lin
Nme
Cje
Sth
kDa
170
130
95
72
56
43
34
29

FIG. 16

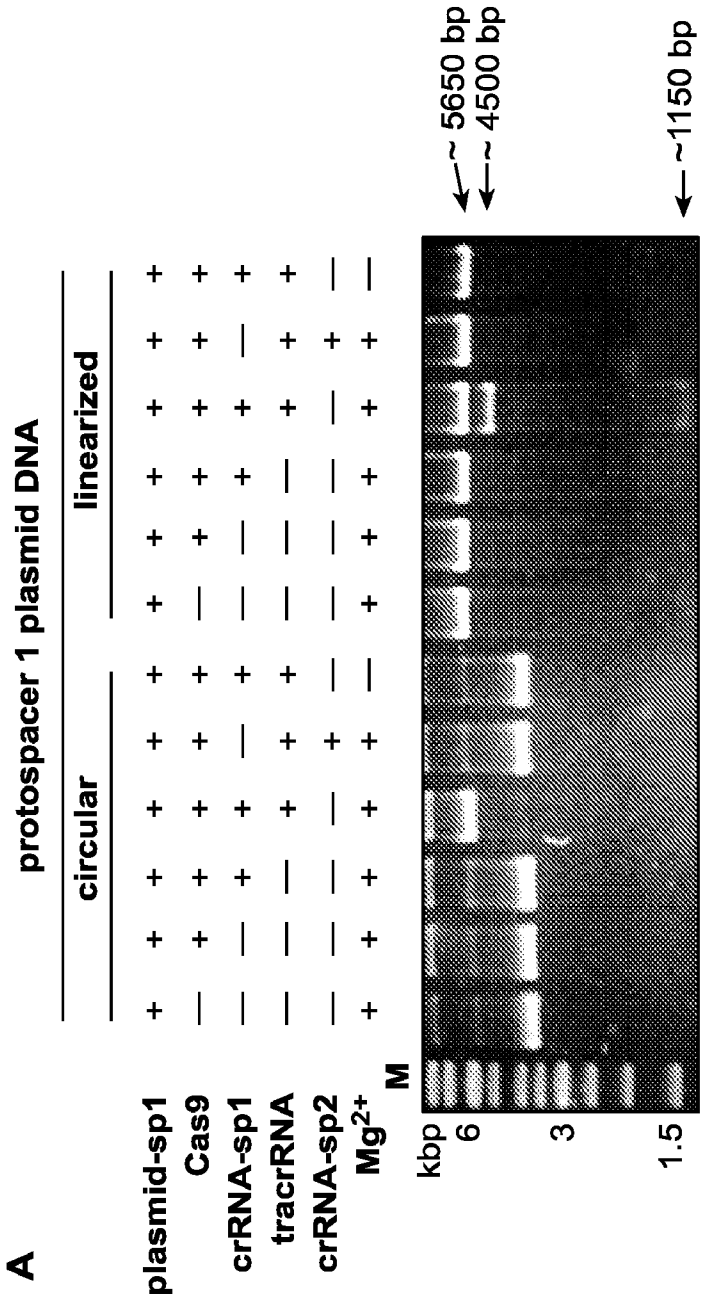

A
protospacer 1 plasmid DNA
circular
linearized
plasmid-sp1
Cas9
crRNA-sp1
tracrRNA
crRNA-sp2
Mg2+
M
kbp
6
3
1.5
~ 5650 bp
~ 4500 bp
~1150 bp

FIG. 17

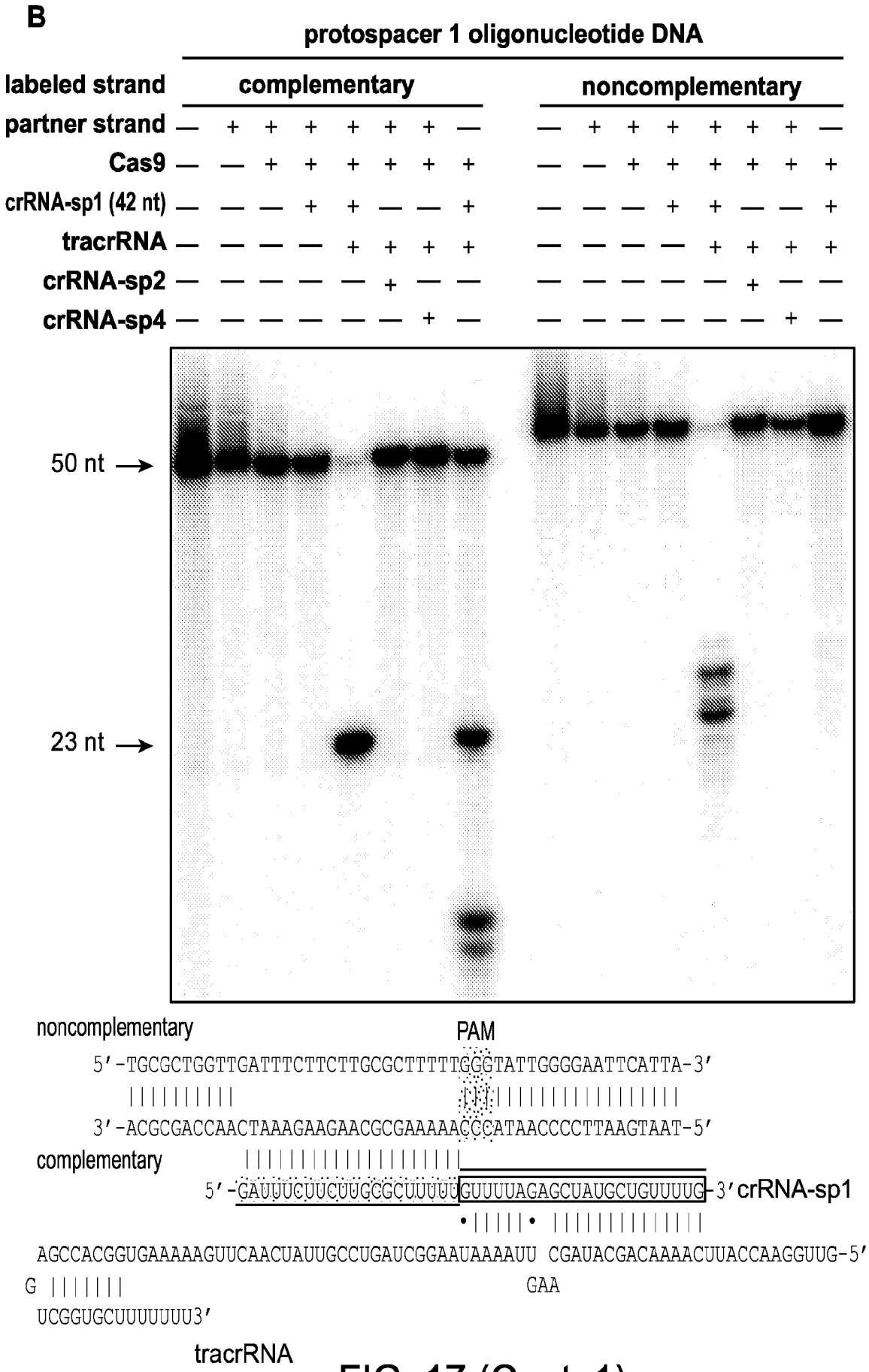
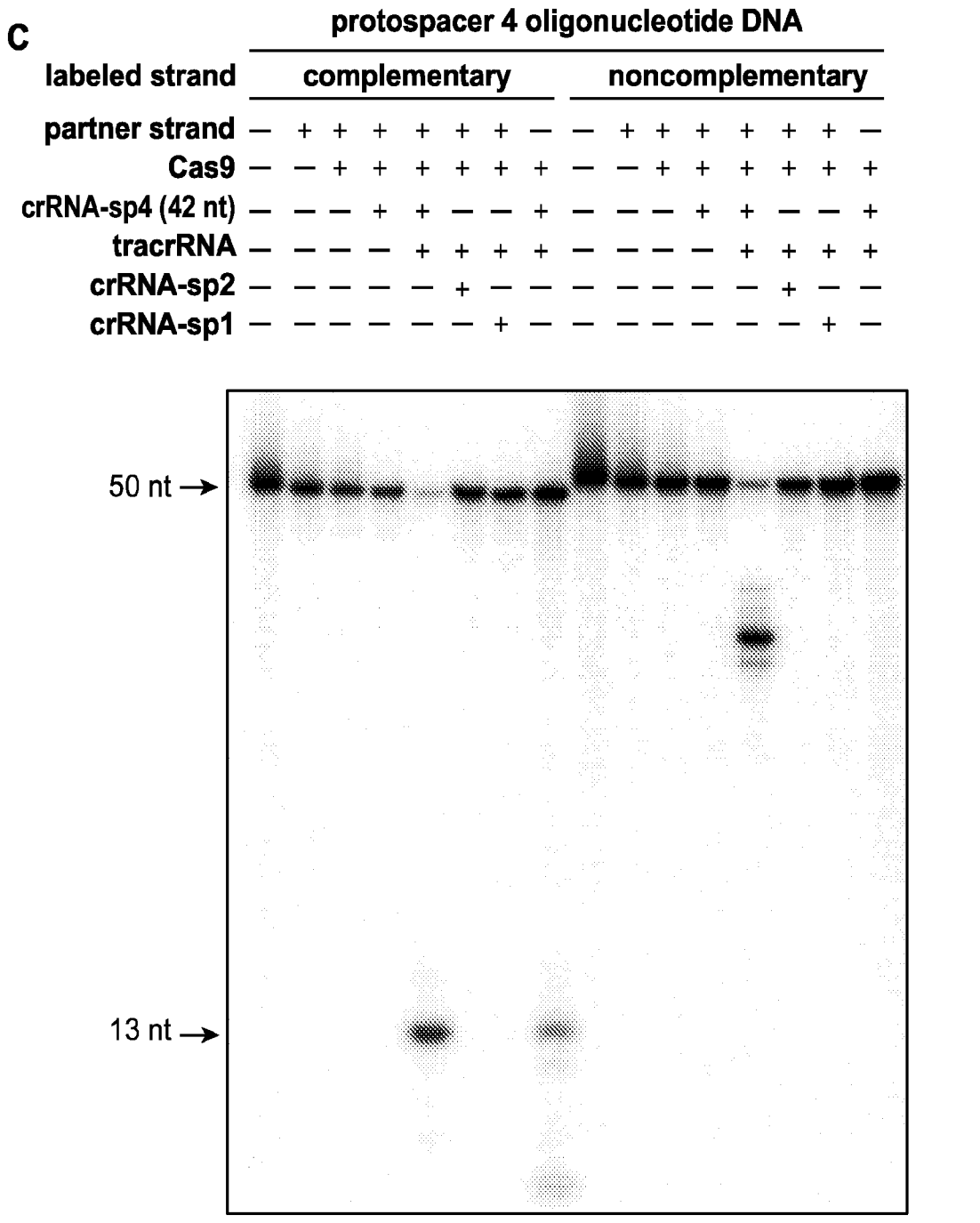

B
protospacer 1 oligonucleotide DNA
labeled strand
complementary
noncomplementary
partner strand — + + + + + + — — + + + + + + + —
Cas9 — — + + + + + + — — + + + + + + + +
crRNA-sp1 (42 nt) — — — + + — — + — — — + + — — +
tracrRNA — — — — + + + + — — — — + + + +
crRNA-sp2 — — — — — + — — — — — — — + — —
crRNA-sp4 — — — — — — + — — — — — — — + —
50 nt
23 nt
noncomplementary
PAM
5′-TGCGCTGGTTGATTTCTTCTTGCGCTTTTTGGGTATTGGGGAATTCATTA-3′
3′-ACGCGACCAACTAAAGAAGAACGCGAAAAACCCATAACCCCTTAAGTAAT-5′
complementary
5′-GAUUUCUUCUUGCGCUUUUUGUUUUAGAGCUAUGCUGUUUUG-3′crRNA-sp1
AGCCACGGUGAAAAAGUUCAACUAUUGCCUGAUCGGAAUAAAAUU CGAUACGACAAAACUUACCAAGGUUG-5′
G
GAA
UCGGUGCUUUUUUU3′
tracrRNA

FIG. 17 (Cont. 1)


C
protospacer 4 oligonucleotide DNA
labeled strand
complementary
noncomplementary
partner strand
Cas9
crRNA-sp4 (42 nt)
tracrRNA
crRNA-sp2
crRNA-sp1
50 nt
13 nt

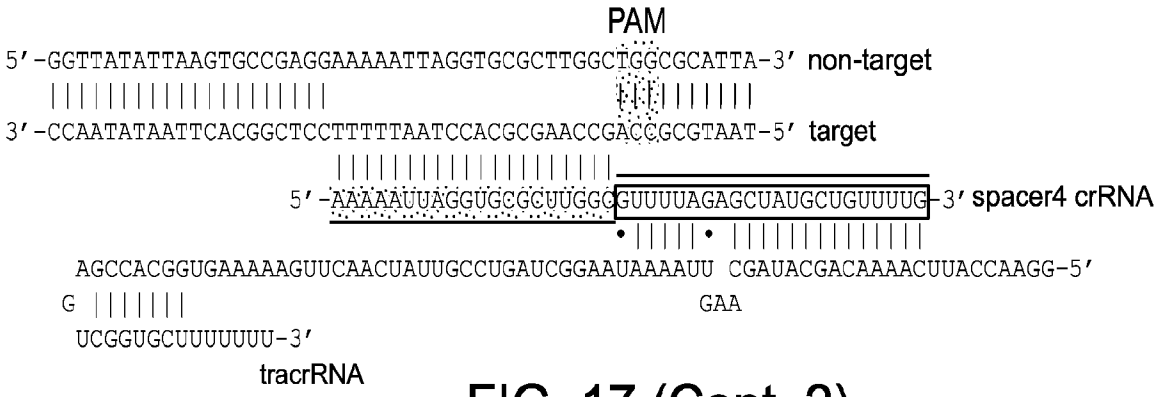

PAM
5′-GGTTATATTAAGTGCCGAGGAAAAATTAGGTGCGCTTGGCTGGCGCATTA-3′ non-target
3′-CCAATATAATTCACGGCTCCTTTTTAATCCACGCGAACCGACCGCGTAAT-5′ target
5′-AAAAAUUAGGUGCGCUUGGGCGUUUUAGAGCUAUGCUGUUUUG-3′ spacer4 crRNA
AGCCACGGUGAAAAAGUUCAACUAUUGCCUGAUCGGAAUAAAAUU CGAUACGACAAAACUUACCAAGG-5′
G
GAA
UCGGUGCUUUUUUU-3′
tracrRNA

FIG. 17 (Cont. 2)

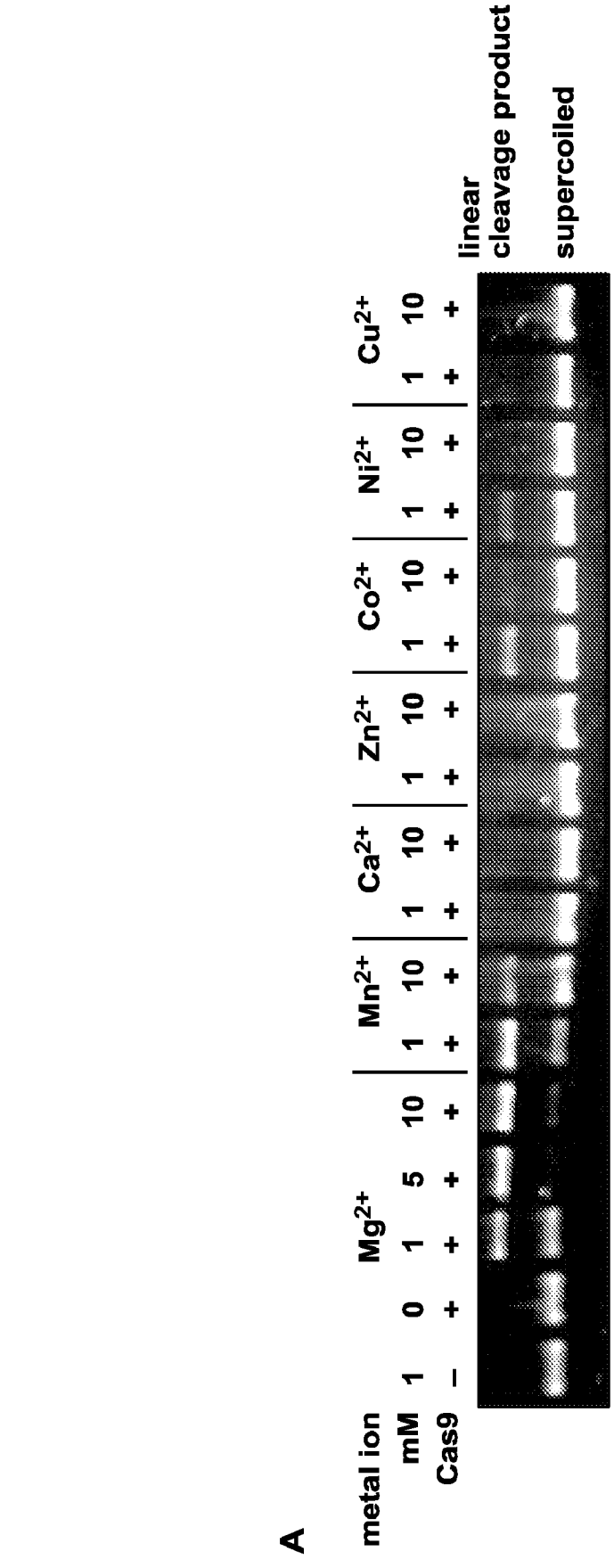

A
metal ion
mM
Cas9
1
–
0
+
Mg2+
1
+
5
+
10
+
Mn2+
1
+
10
+
Ca2+
1
+
10
+
Zn2+
1
+
10
+
Co2+
1
+
10
+
Ni2+
1
+
10
+
Cu2+
1
+
10
+
linear
cleavage product
supercoiled

FIG. 18

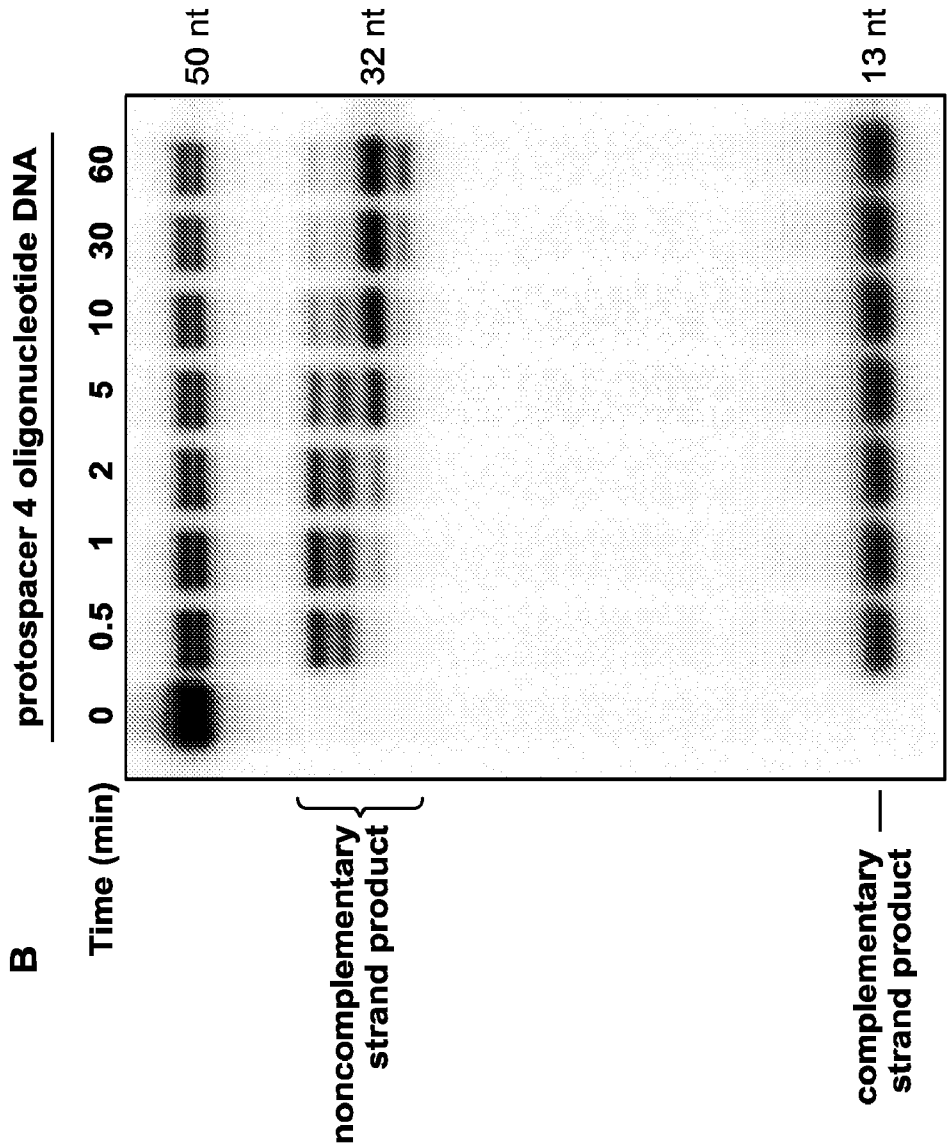

B
protospacer 4 oligonucleotide DNA
Time (min)
0
0.5
1
2
5
10
30
60
50 nt
32 nt
13 nt
noncomplementary strand product
complementary strand product

FIG. 18 (Cont.)

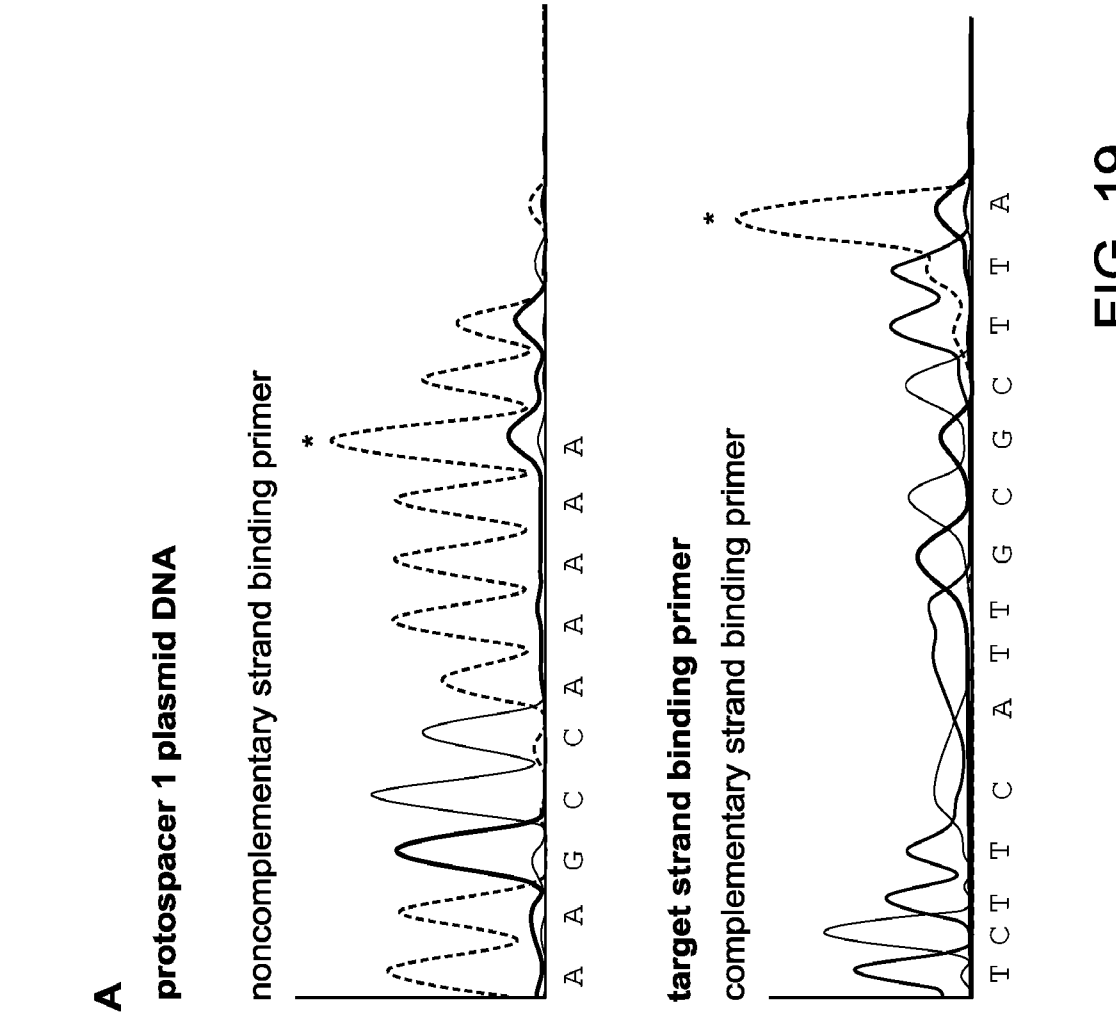

A
protospacer 1 plasmid DNA
noncomplementary strand binding primer
*
A A G C C A A A A A A
target strand binding primer
complementary strand binding primer
*
T C T T C A T T G C G C T T A
B
protospacer 4
oligonucleotide DNA
M P P M
complementary strand
noncomplementary strand
37 nt
36 nt
31 nt
14 nt
13 nt

FIG. 19

C
non-target
target
PAM
Protospacer 1
target DNA
crRNA-sp1
tracrRNA
Protospacer 4
target DNA
crRNA-sp4
tracrRNA

FIG. 19 (Cont.)

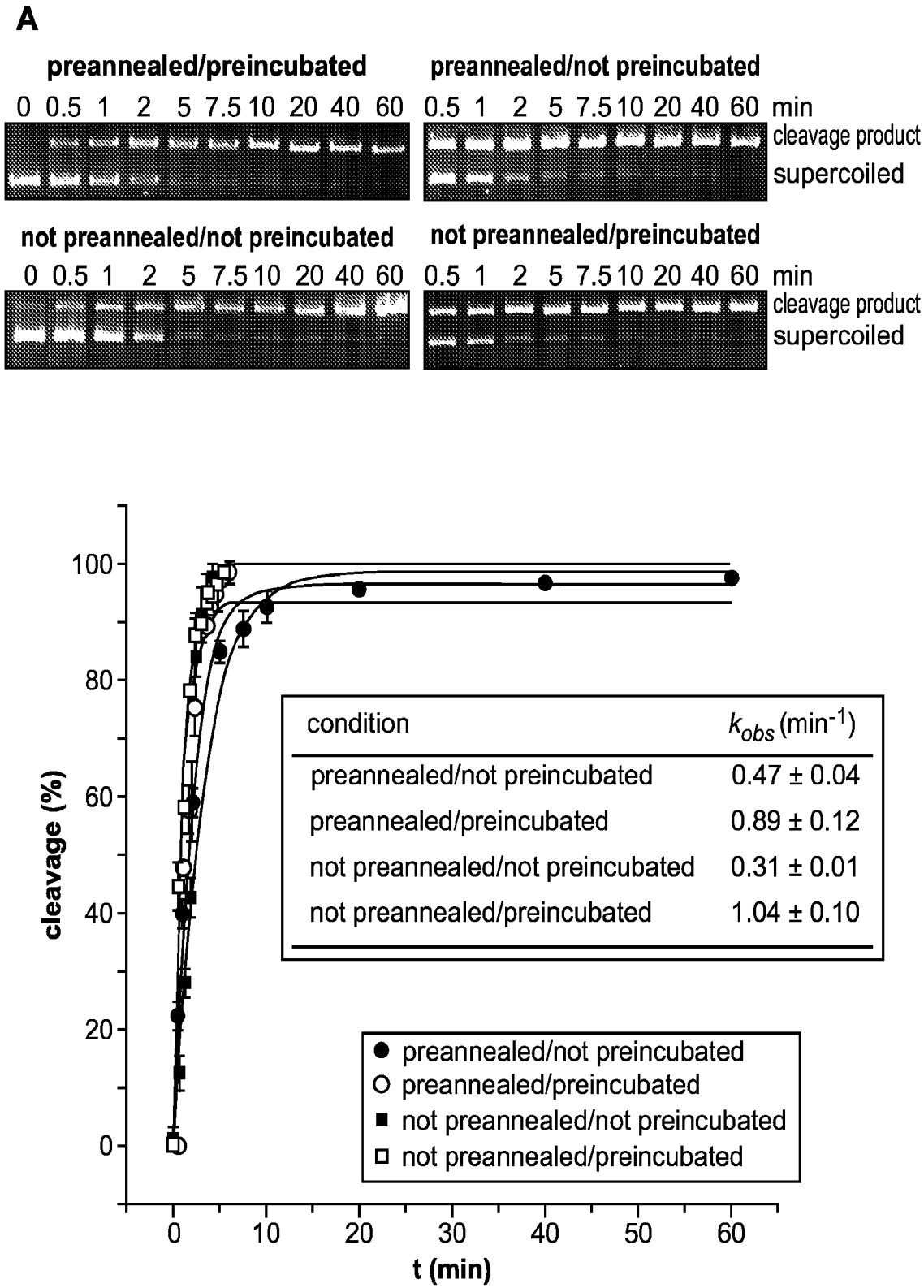

A
preannealed/preincubated
preannealed/not preincubated
0 0.5 1 2 5 7.5 10 20 40 60
0.5 1 2 5 7.5 10 20 40 60 min
cleavage product
supercoiled
not preannealed/not preincubated
not preannealed/preincubated
0 0.5 1 2 5 7.5 10 20 40 60
0.5 1 2 5 7.5 10 20 40 60 min
cleavage product
supercoiled
cleavage (%)
100
80
60
40
20
0
0 10 20 30 40 50 60
t (min)
condition
k_{obs} (min^{-1})
preannealed/not preincubated 0.47 ± 0.04
preannealed/preincubated 0.89 ± 0.12
not preannealed/not preincubated 0.31 ± 0.01
not preannealed/preincubated 1.04 ± 0.10
preannealed/not preincubated
preannealed/preincubated
not preannealed/not preincubated
not preannealed/preincubated

FIG. 20

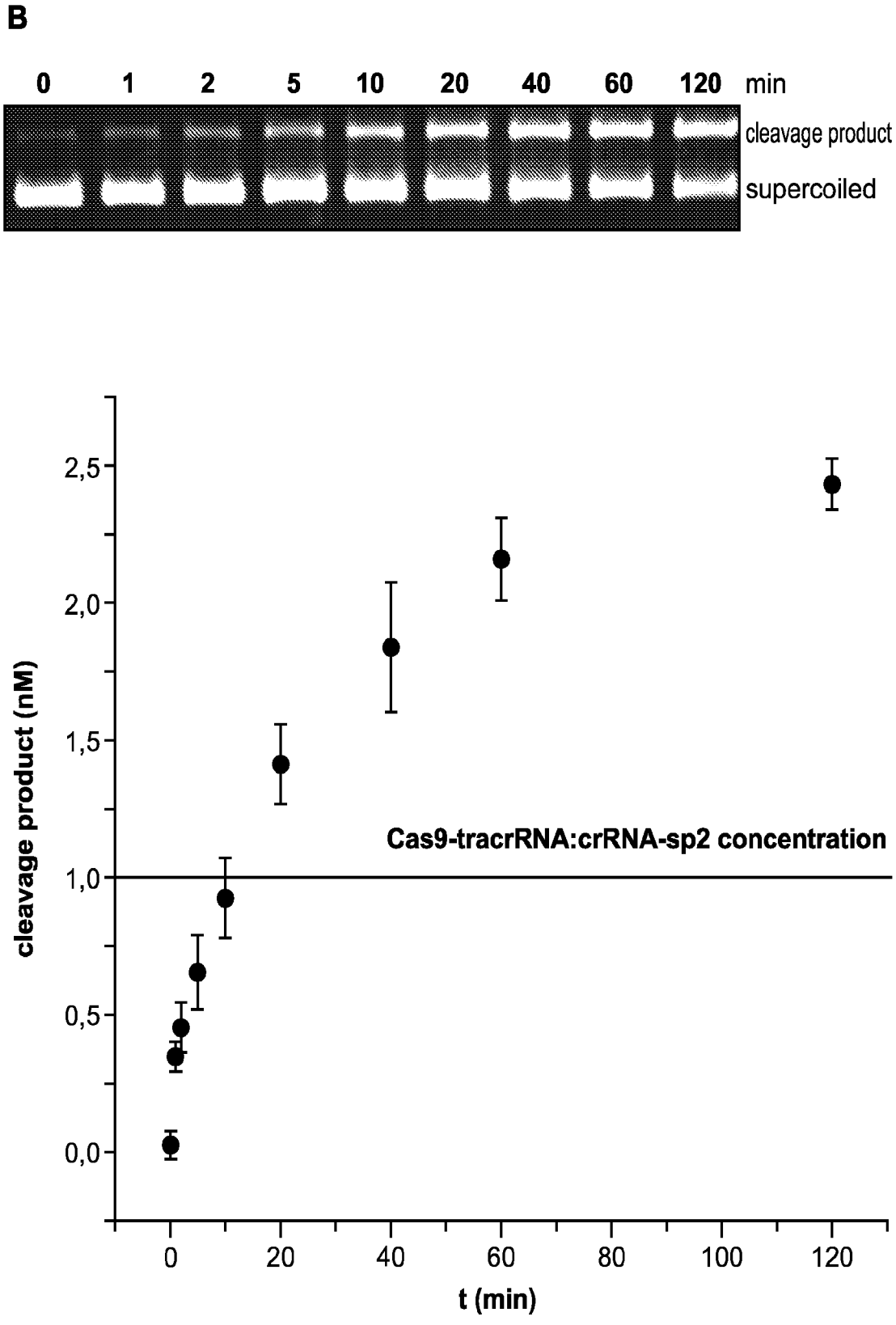

B
0
1
2
5
10
20
40
60
120
min
cleavage product
supercoiled
cleavage product (nM)
2,5
2,0
1,5
1,0
0,5
0,0
Cas9-tracrRNA:crRNA-sp2 concentration
0
20
40
60
80
100
120
t (min)

FIG. 20 (Cont.)

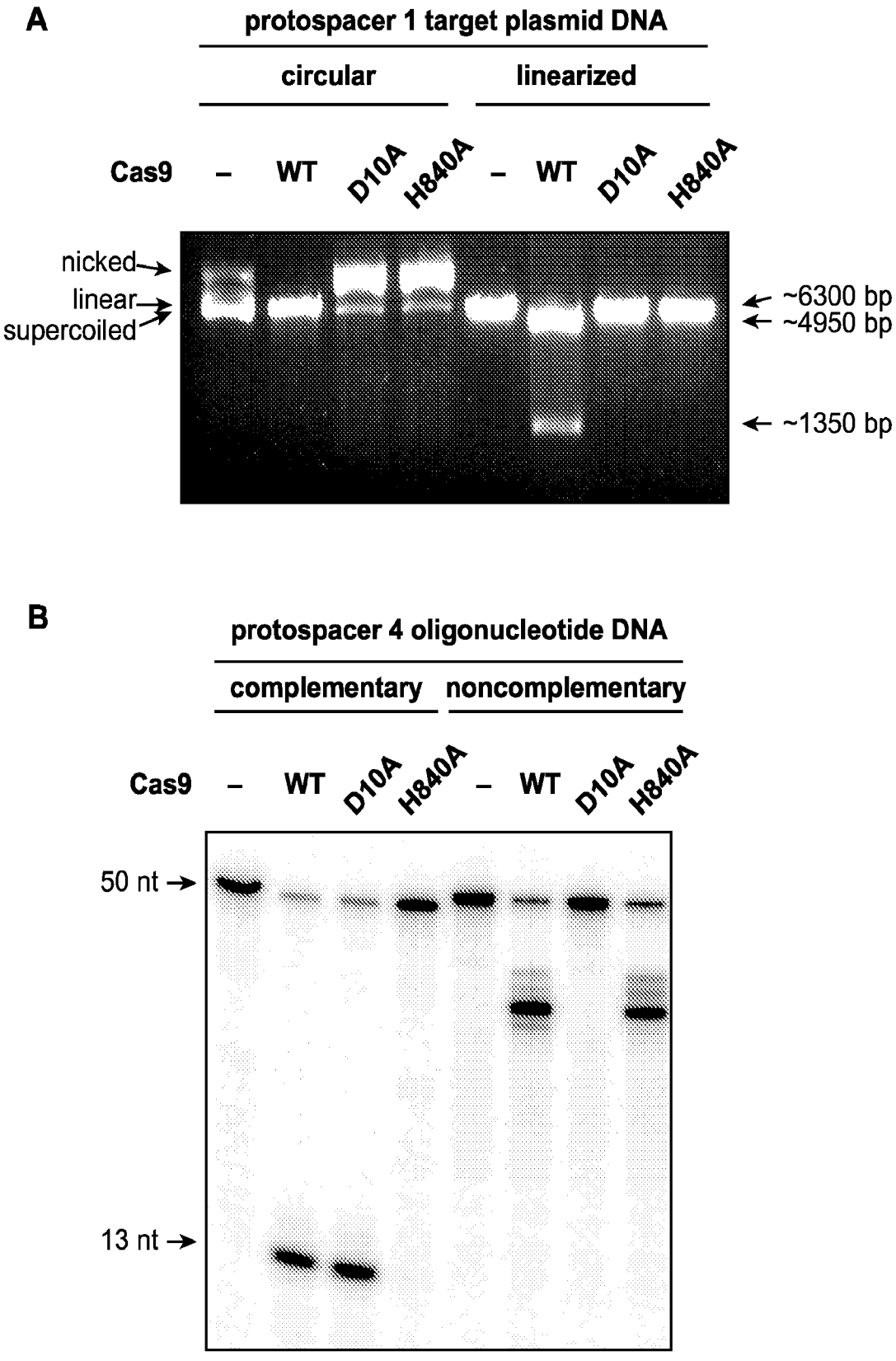

A
protospacer 1 target plasmid DNA
circular
linearized
Cas9
–
WT
D10A
H840A
–
WT
D10A
H840A
nicked
linear
supercoiled
~6300 bp
~4950 bp
~1350 bp
B
protospacer 4 oligonucleotide DNA
complementary
noncomplementary
Cas9
–
WT
D10A
H840A
–
WT
D10A
H840A
50 nt
13 nt

FIG. 21

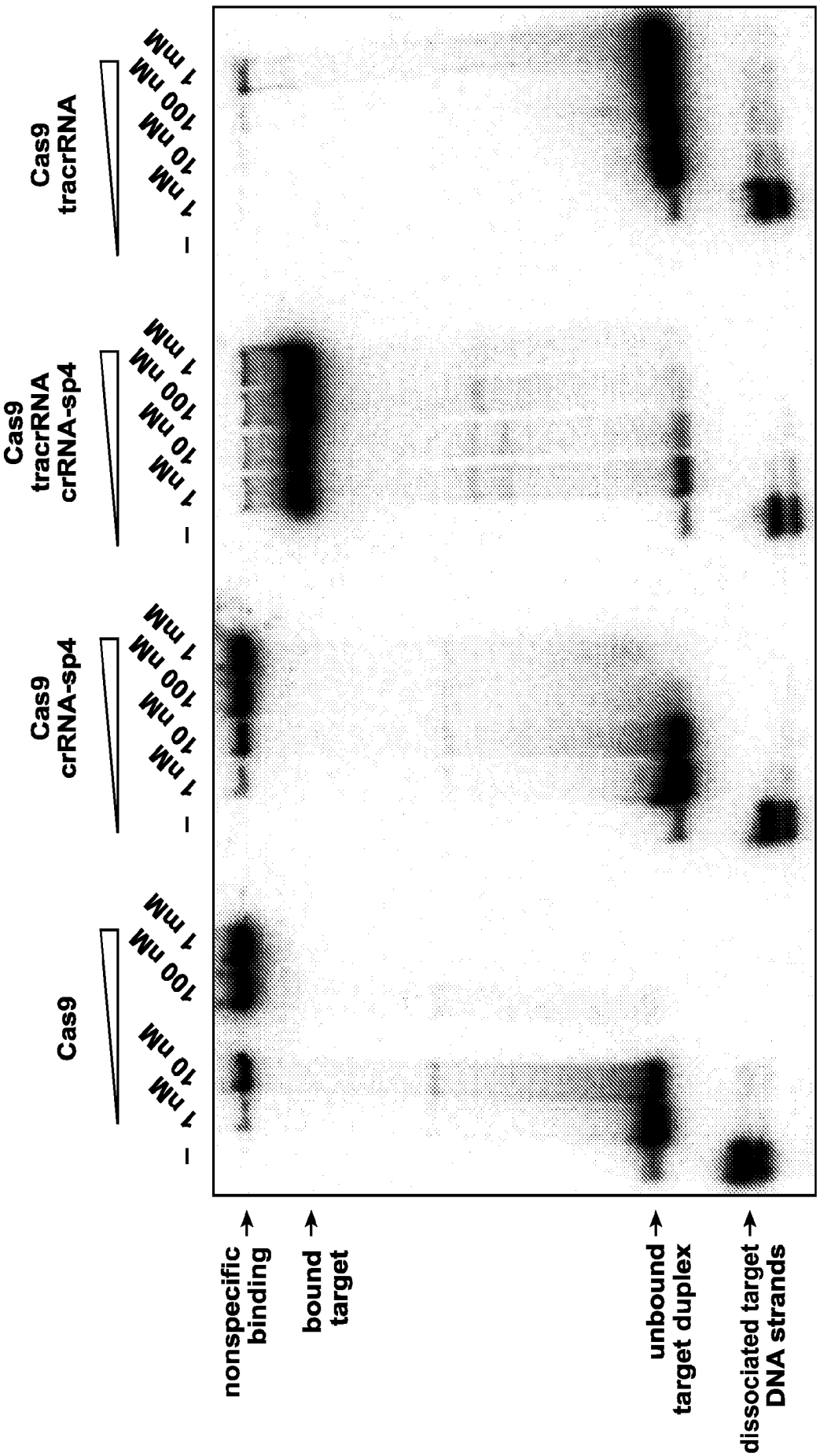

Cas9
–
1 nM
10 nM
100 nM
1 mM
Cas9
crRNA-sp4
–
1 nM
10 nM
100 nM
1 mM
Cas9
tracrRNA
crRNA-sp4
–
1 nM
10 nM
100 nM
1 mM
Cas9
tracrRNA
–
1 nM
10 nM
100 nM
1 mM
nonspecific binding
bound target
unbound target duplex
dissociated target DNA strands

FIG. 22

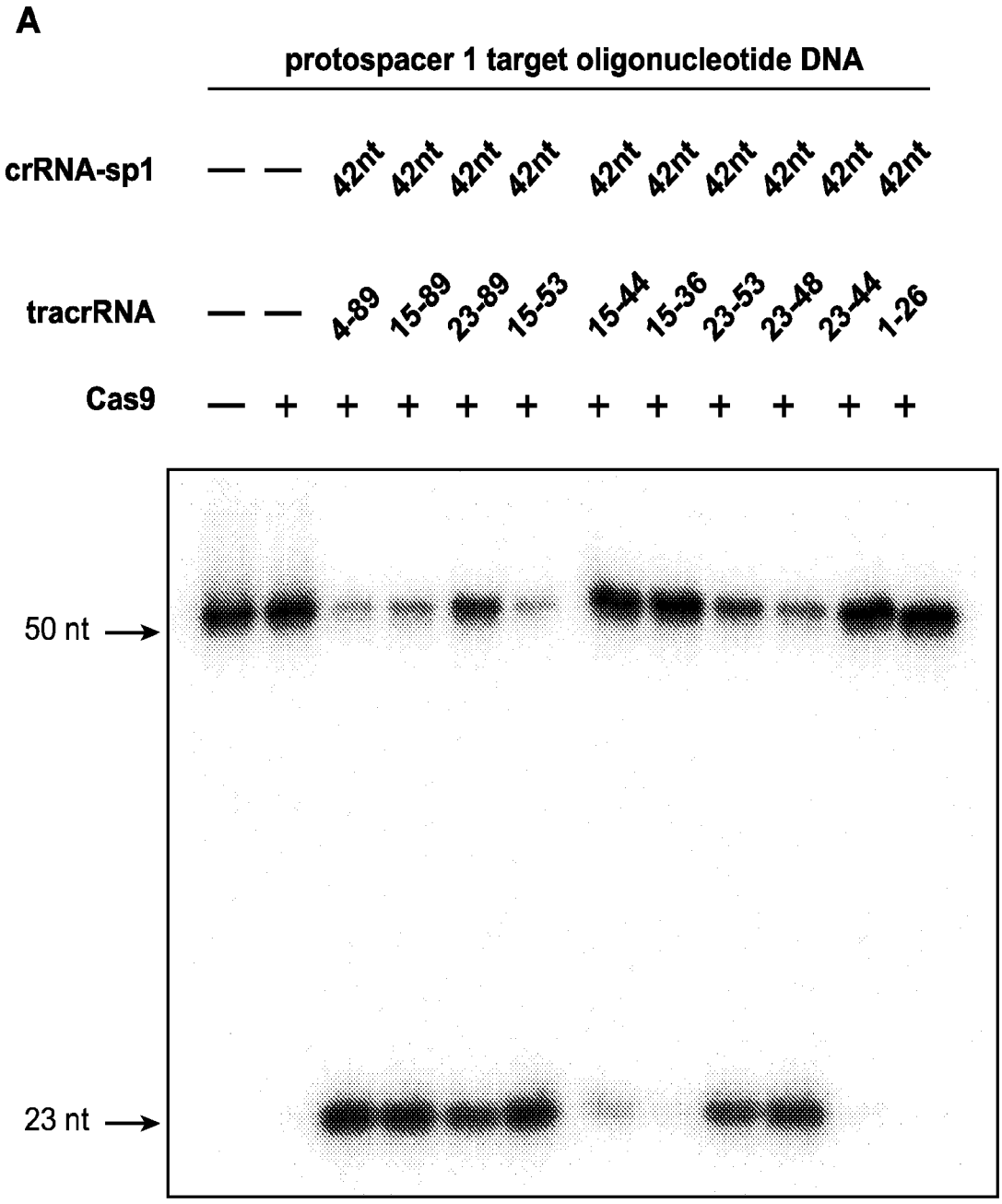

A
protospacer 1 target oligonucleotide DNA
crRNA-sp1
— — 42nt 42nt 42nt 42nt 42nt 42nt 42nt 42nt 42nt 42nt
tracrRNA
— — 4-89 15-89 23-89 15-53 15-44 15-36 23-53 23-48 23-44 1-26
Cas9
— + + + + + + + + + + +
50 nt
23 nt

FIG. 23

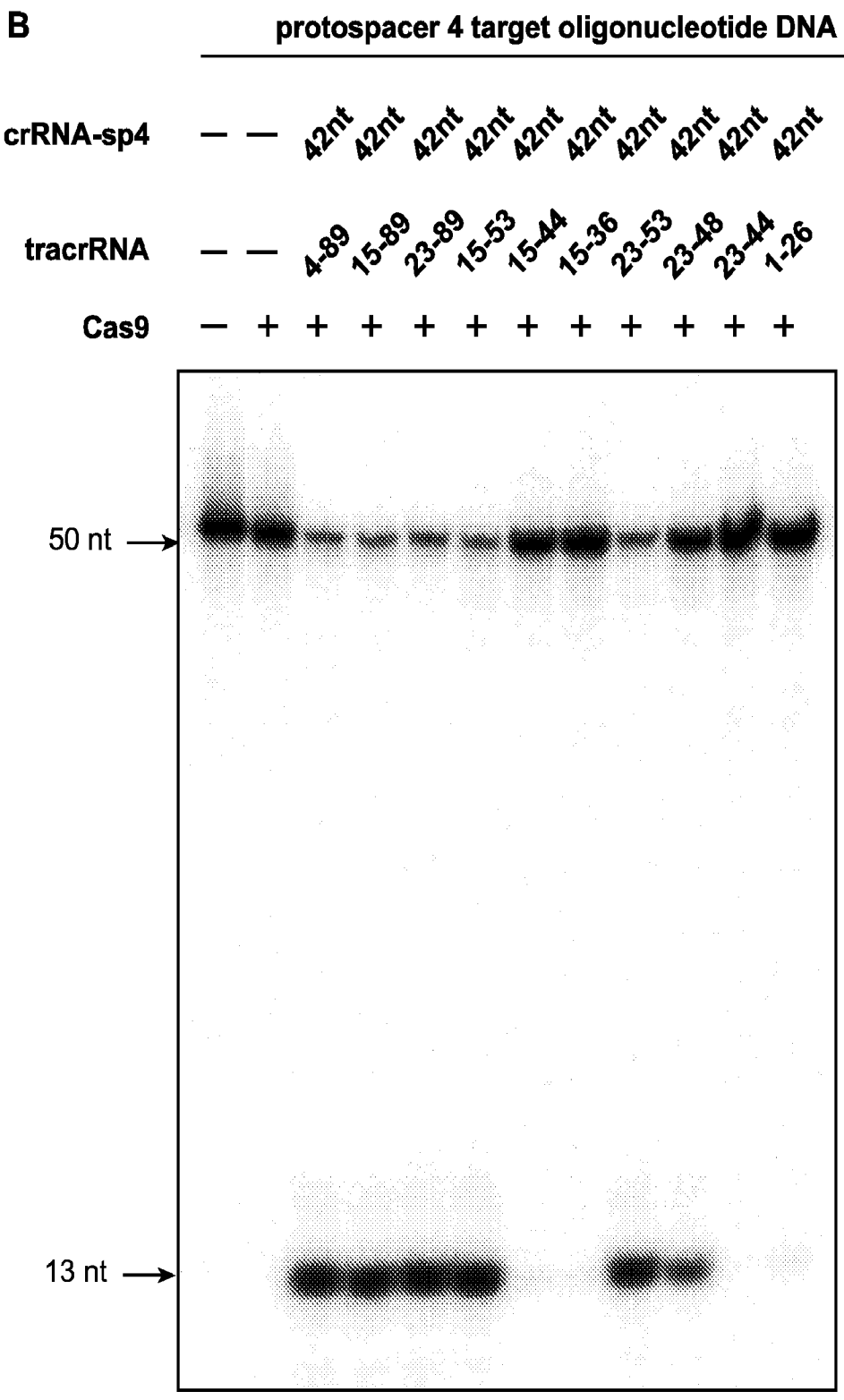

B
protospacer 4 target oligonucleotide DNA
crRNA-sp4
— — 42nt 42nt 42nt 42nt 42nt 42nt 42nt 42nt 42nt 42nt
tracrRNA
— — 4-89 15-89 23-89 15-53 15-44 15-36 23-53 23-48 23-44 1-26
Cas9
— + + + + + + + + + + +
50 nt
13 nt

FIG. 23 (Cont.)

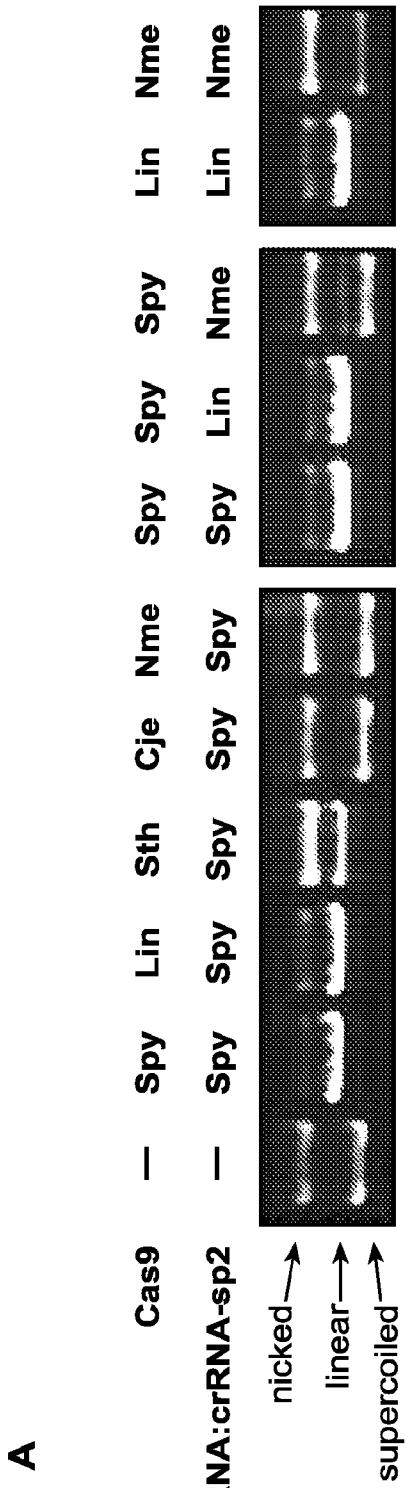

A
Cas9
tracrRNA:crRNA-sp2
—
—
Spy
Spy
Lin
Spy
Sth
Spy
Cje
Spy
Nme
Spy
Spy
Spy
Spy
Lin
Spy
Nme
Lin
Lin
Nme
nicked
linear
supercoiled

FIG. 24

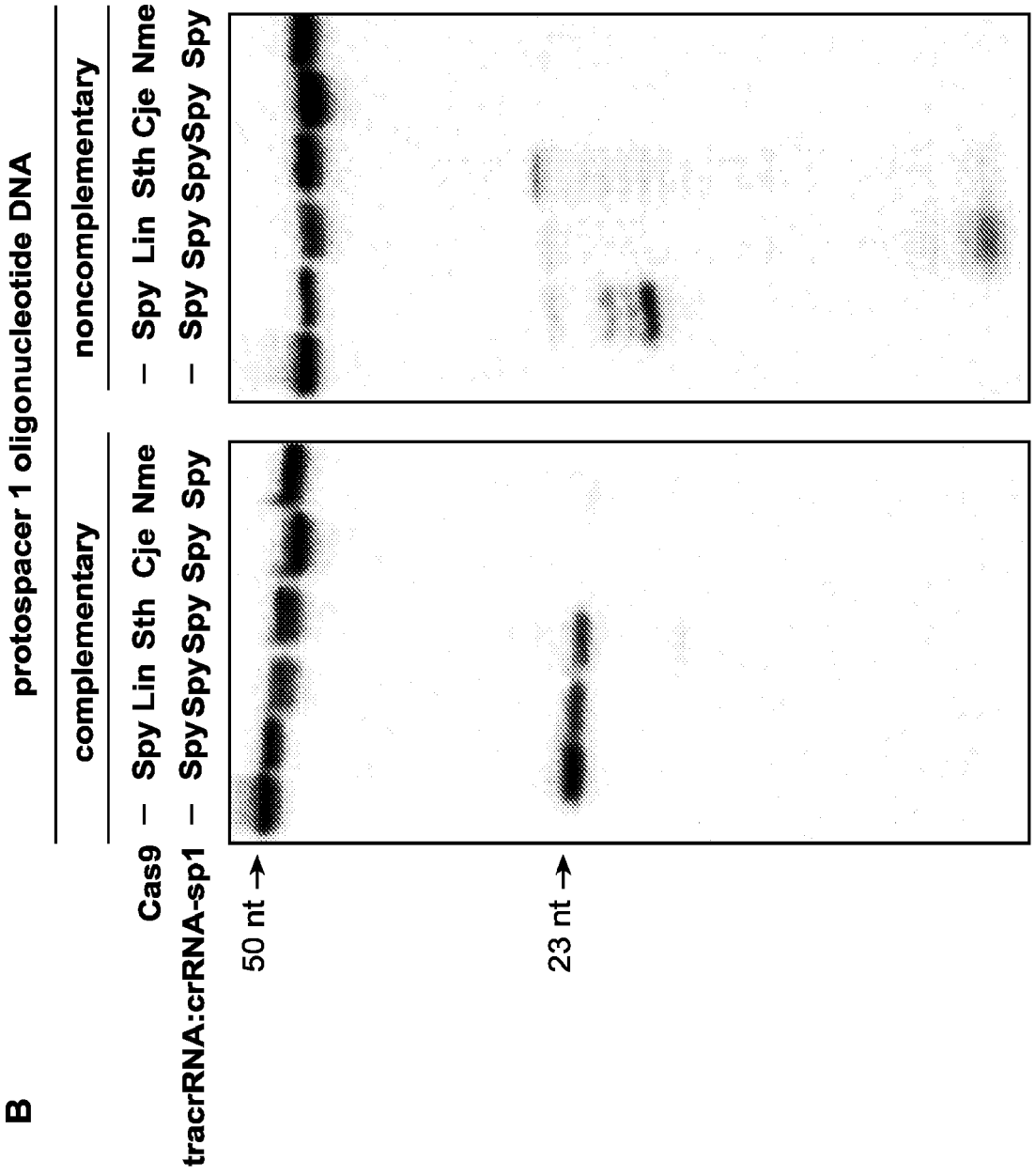

B
protospacer 1 oligonucleotide DNA
complementary
noncomplementary
Cas9 – Spy Lin Sth Cje Nme – Spy Lin Sth Cje Nme
tracrRNA:crRNA-sp1 – SpySpySpy Spy Spy – Spy Spy SpySpy Spy
50 nt
23 nt

FIG. 24 (Cont. 1)

c

	S. pyogenes	L. innocua	S. thermophilus	C. jejuni	N. meningitidis
S. pyogenes	x	54	58	16	16
L. innocua	54	x	52	15	14
S. thermophilus	58	52	x	16	15
C. jejuni	16	15	16	x	32
N. meningitidis	16	14	15	32	x

FIG. 24 (Cont. 2)

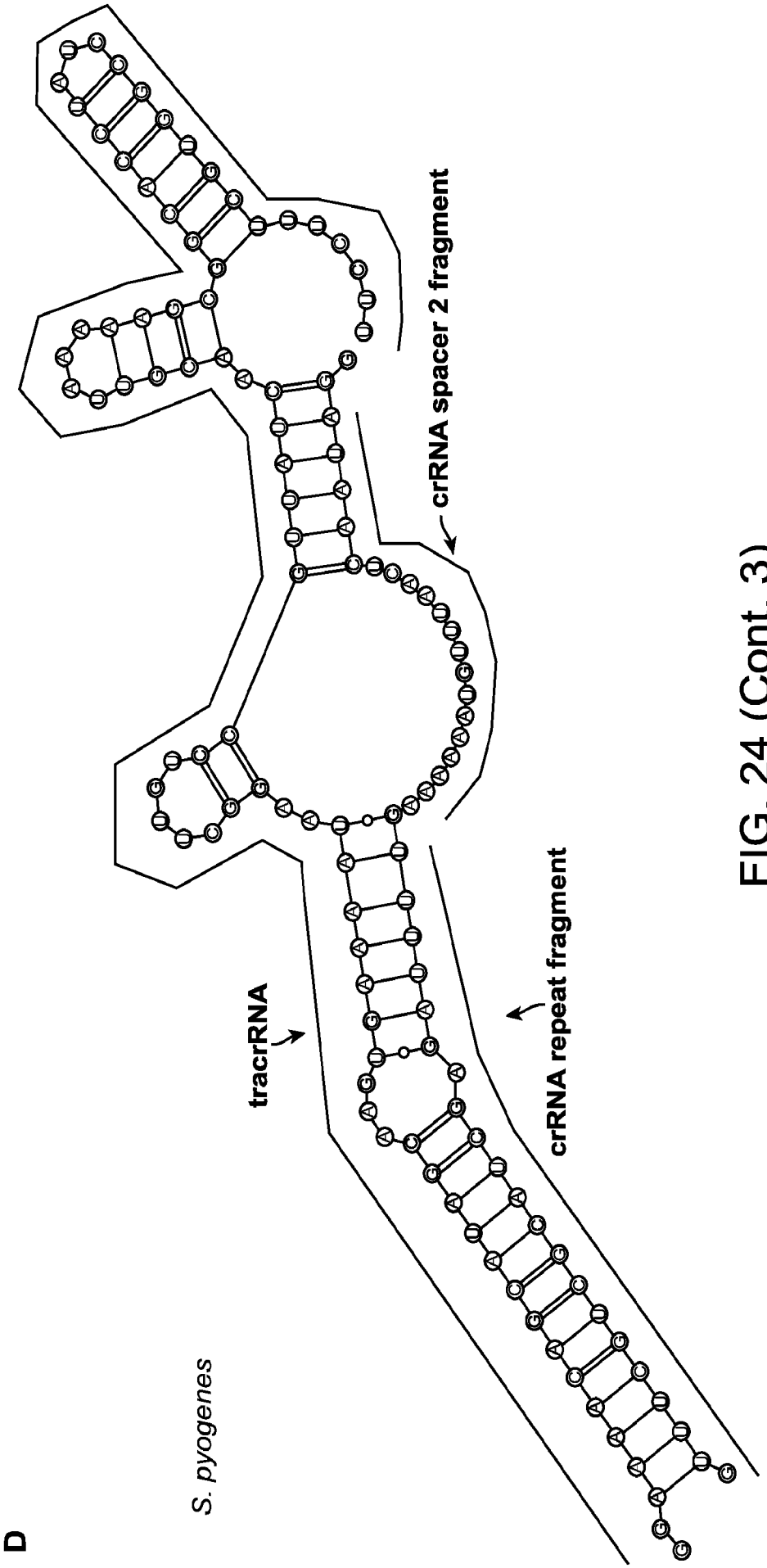

D
S. pyogenes
tracrRNA
crRNA repeat fragment
crRNA spacer 2 fragment

FIG. 24 (Cont. 3)

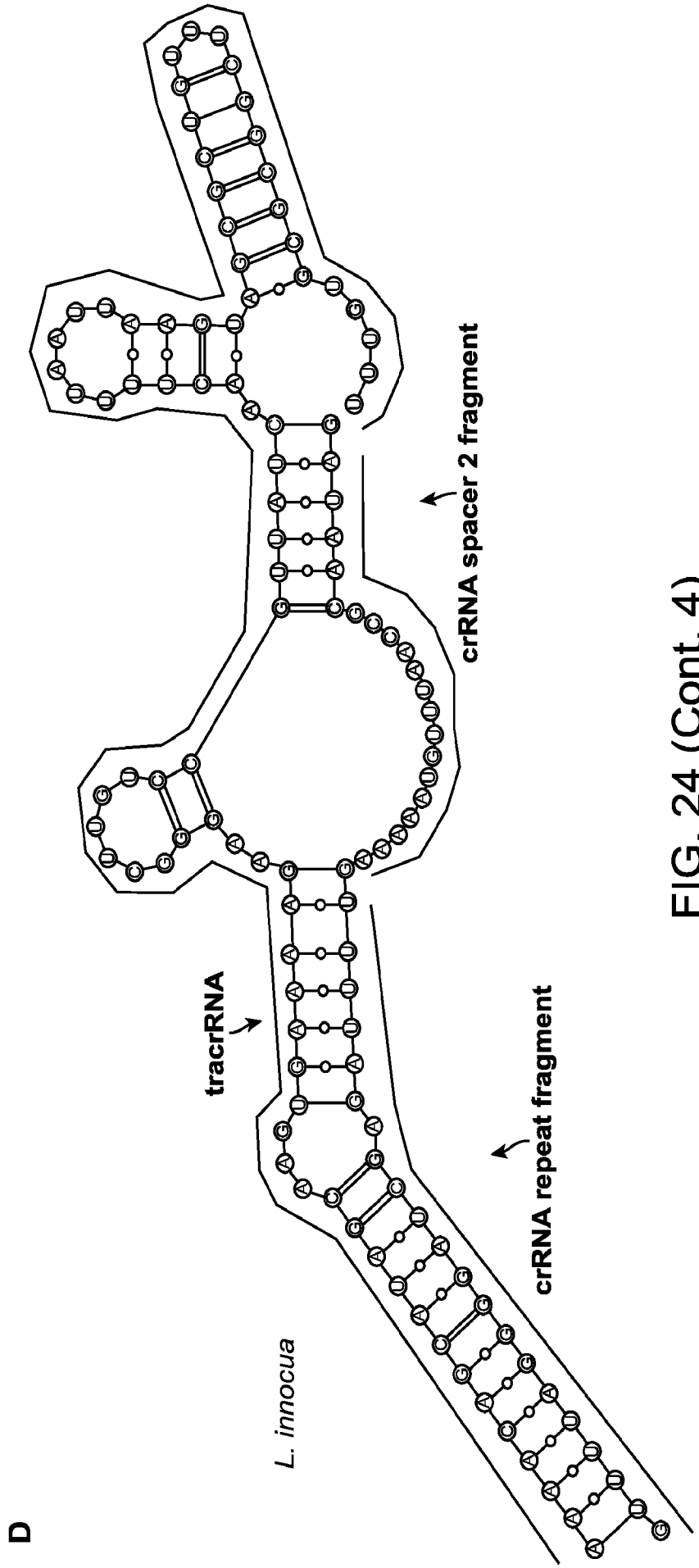

D
L. innocua
tracrRNA
crRNA repeat fragment
crRNA spacer 2 fragment

FIG. 24 (Cont. 4)

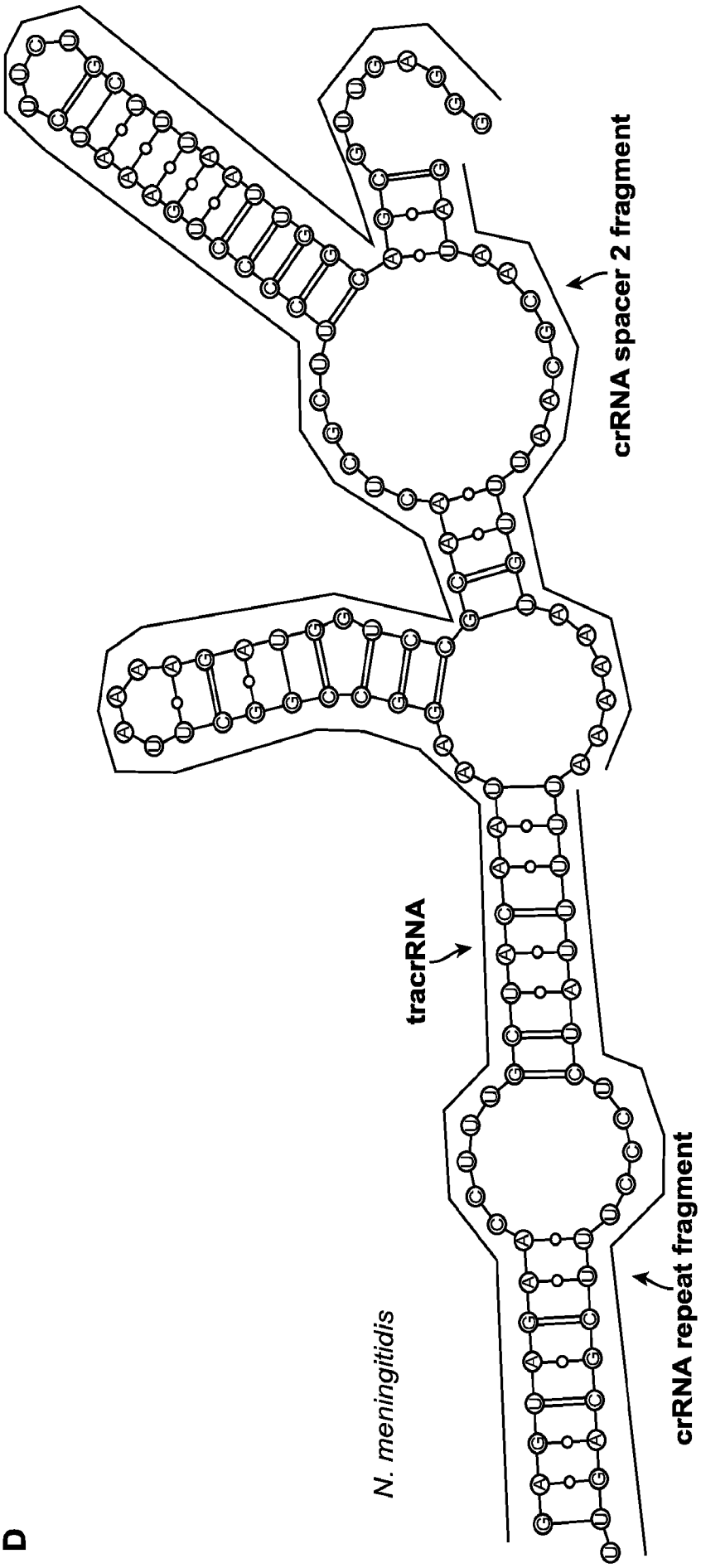

D
N. meningitidis
tracrRNA
crRNA repeat fragment
crRNA spacer 2 fragment

FIG. 24 (Cont. 7)

A
Target DNA probe
5
3'-TTTTAAT-5'
4
3'-TAATCCAC-5'
3
3'-CCACGCGA-5'
2
3'-GCGAACCG-5'
1
3'-ACCGACCGCGTAAT-5'
5'-AAAAAUUAGGUGCGCUUGGC GUUUUAGAGCUAUGCUGUUUUG-3' spacer 4 crRNA (1-42)
AGCCACGGUGAAAAGUUCAACUAUUGCCUGAUCGGAAUAAAAUU CGAUACGACAA-5'
G
UCGGUGCUUUUUUUU-3' tracrRNA (15-89)
GAA

FIG. 25

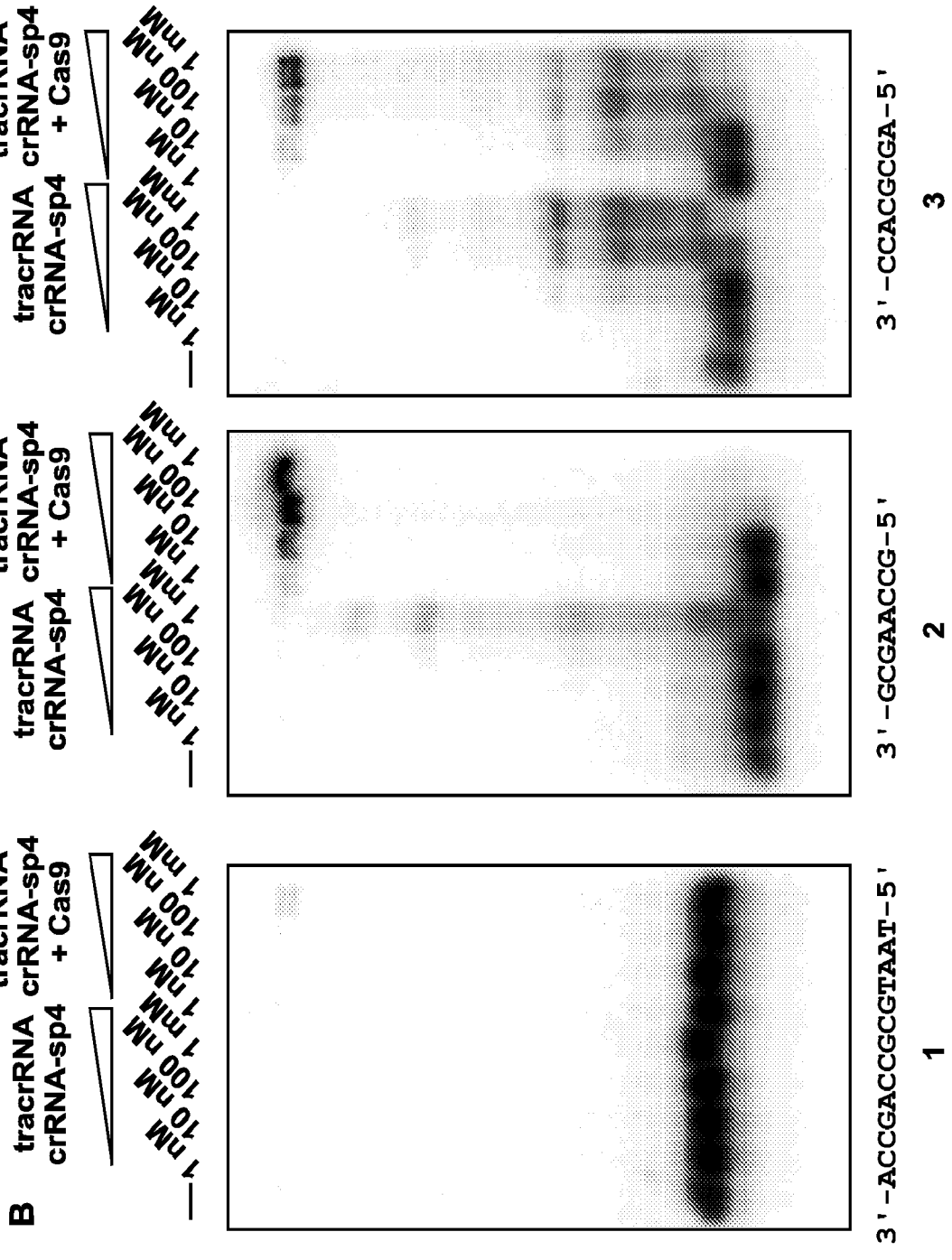

B
tracrRNA crRNA-sp4 + Cas9
tracrRNA crRNA-sp4
—
1 nM
10 nM
100 nM
1 μM
3'-ACCGACCGCGTAAT-5'
1
3'-GCGAACCG-5'
2
3'-CCACGCGA-5'
3

FIG. 25 (Cont. 1)

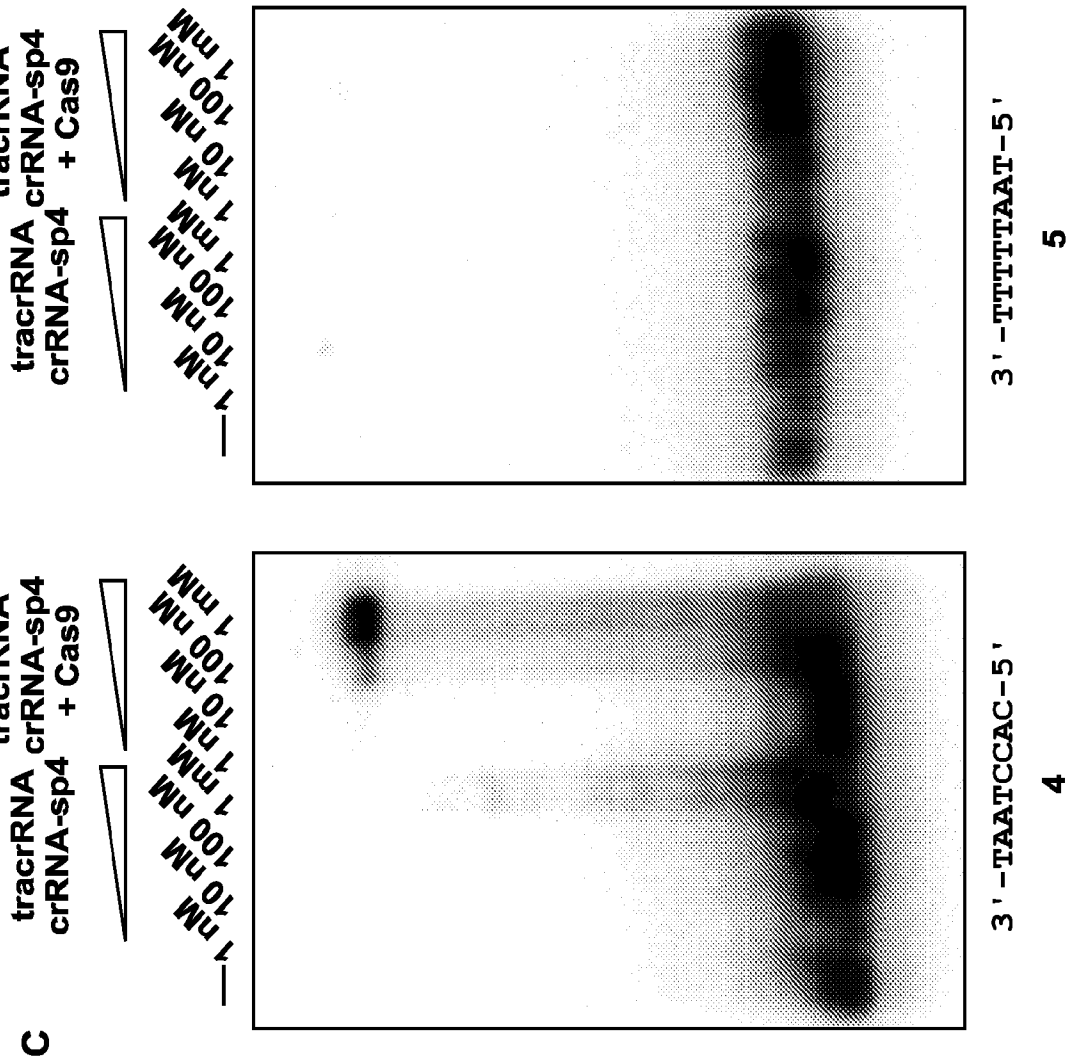

C
tracrRNA crRNA-sp4 + Cas9
tracrRNA crRNA-sp4
1 nM
10 nM
100 nM
1 nM
10 nM
100 nM
1 nM
3'-TAATCCAC-5'
4
3'-TTTTTAAT-5'
5

FIG. 25 (Cont. 2)

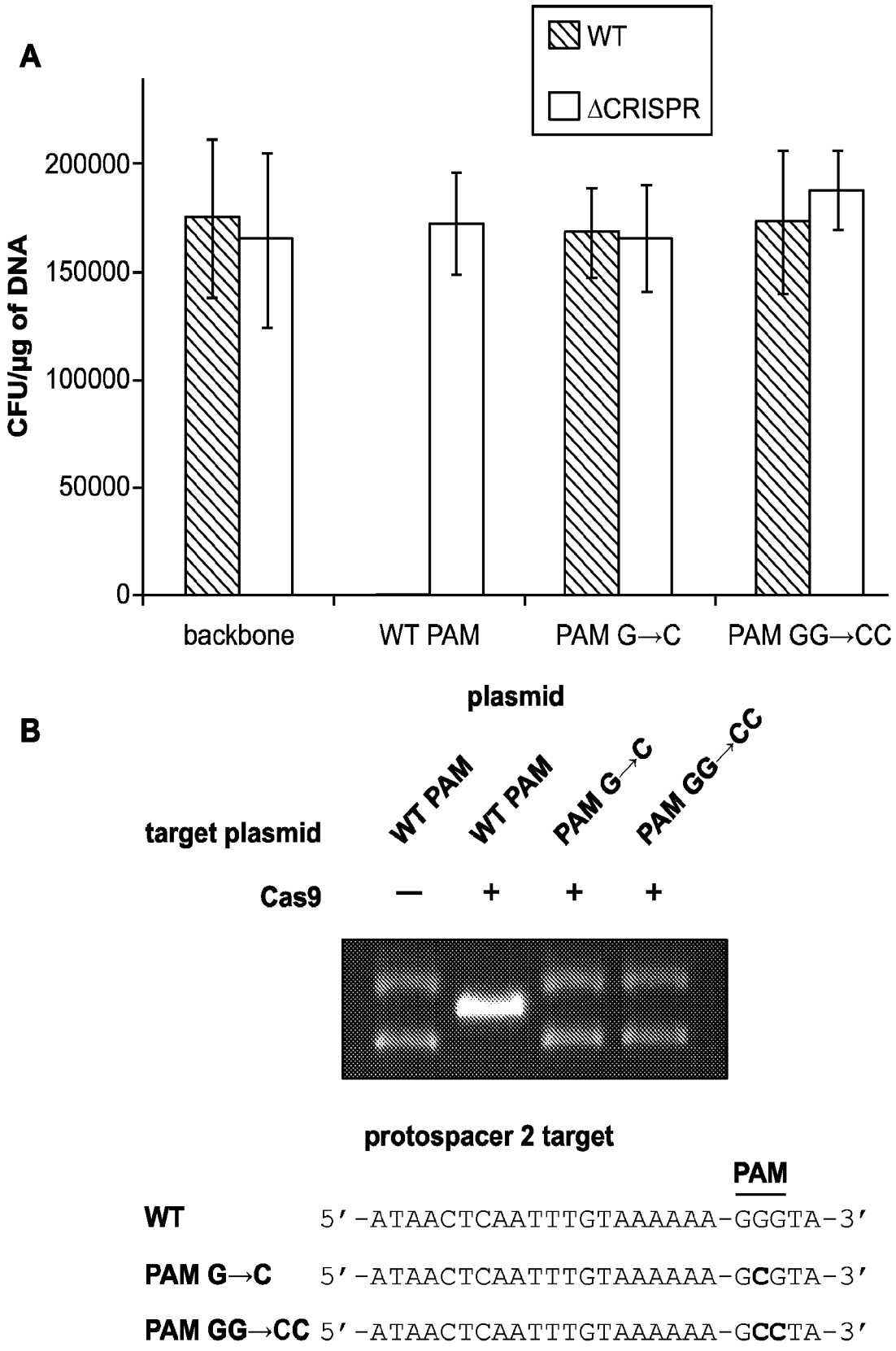
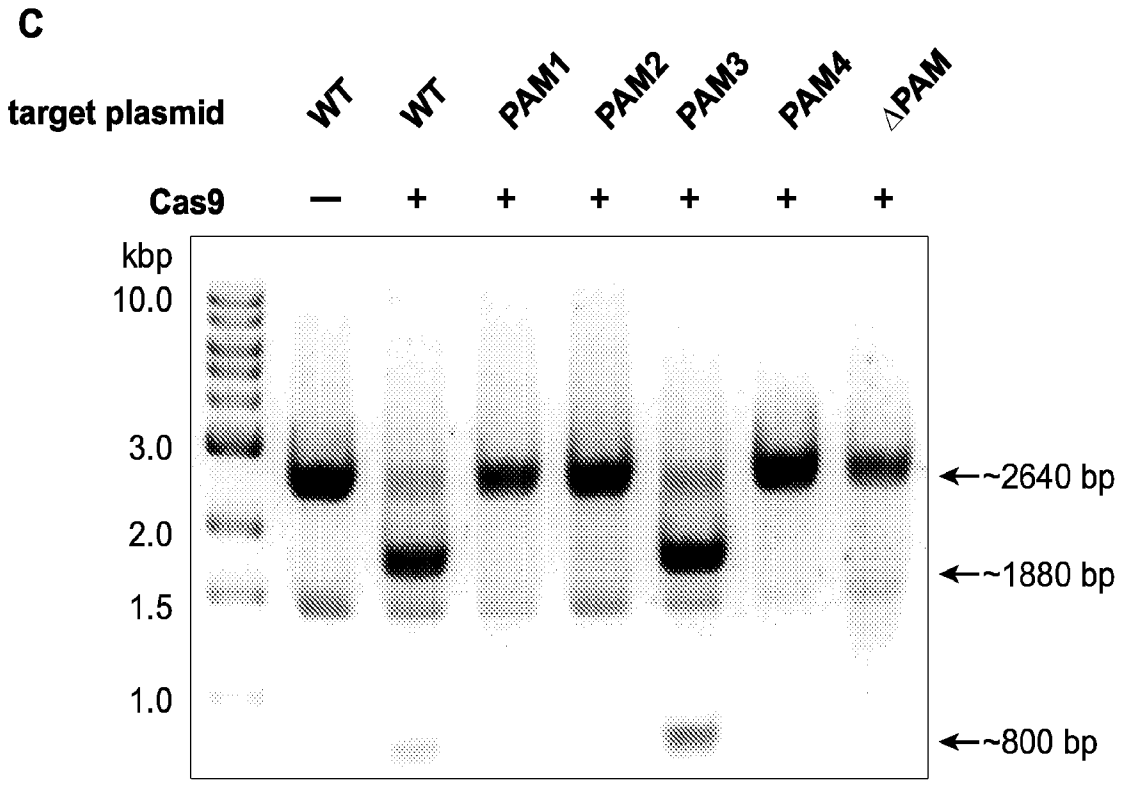

A
WT
ΔCRISPR
CFU/μg of DNA
200000
150000
100000
50000
0
backbone
WT PAM
PAM G→C
PAM GG→CC
plasmid
B
target plasmid
WT PAM
WT PAM
PAM G→C
PAM GG→CC
Cas9
—
+
+
+
protospacer 2 target
PAM
WT 5′-ATAACTCAATTTGTAAAAAA-GGGTA-3′
PAM G→C 5′-ATAACTCAATTTGTAAAAAA-GCGTA-3′
PAM GG→CC 5′-ATAACTCAATTTGTAAAAAA-GCCTA-3′

FIG. 26



protospacer 4 target

PAM

WT	5′-AAAAATTAGGTGCGCTTGGC-TGGCG-3′
PAM1	5′-AAAAATTAGGTGCGCTTGGC-T**C**GCG-3′
PAM2	5′-AAAAATTAGGTGCGCTTGGC-TG**C**CG-3′
PAM3	5′-AAAAATTAGGTGCGCTTGGC-TGGC**C**-3′
PAM4	5′-AAAAATTAGGTGCGCTTGGC-T**CCCC**-3′
DPAM	5′-AAAAATTAGGTGCGCTTGGC-T-3′

FIG. 26 (Cont.)

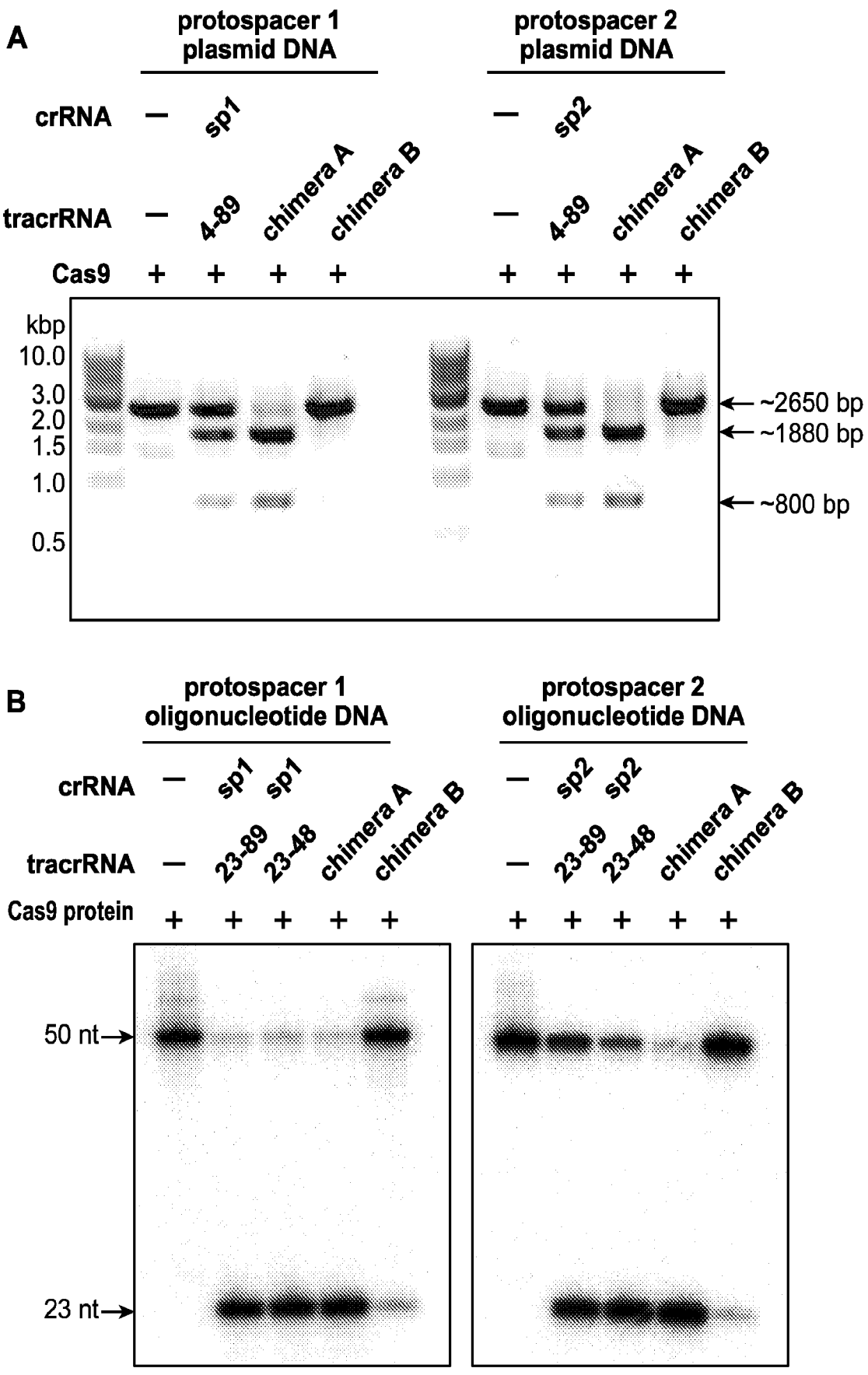
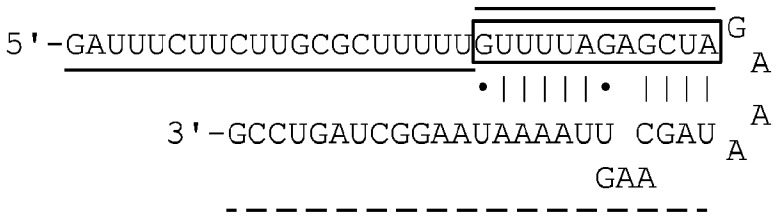

A
protospacer 1 plasmid DNA
protospacer 2 plasmid DNA
crRNA
tracrRNA
Cas9
sp1
sp2
4-89
chimera A
chimera B
kbp
10.0
3.0
2.0
1.5
1.0
0.5
~2650 bp
~1880 bp
~800 bp
B
protospacer 1 oligonucleotide DNA
protospacer 2 oligonucleotide DNA
crRNA
tracrRNA
Cas9 protein
sp1
sp2
23-89
23-48
chimera A
chimera B
50 nt
23 nt

FIG. 27

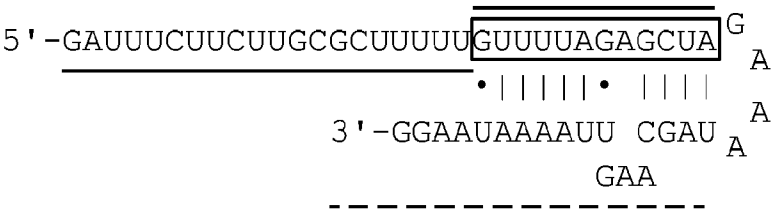
C

protspacer 1 targeting chimeric RNAs

chimera A

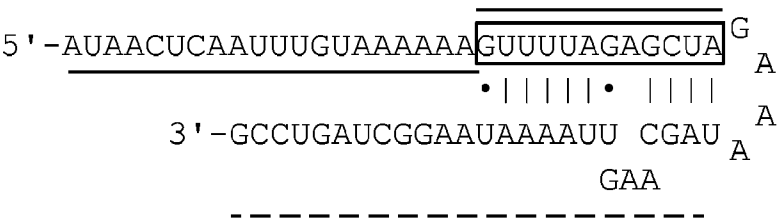


chimera B



protospacer 2 targeting chimeric RNAs

chimera A



chimera B

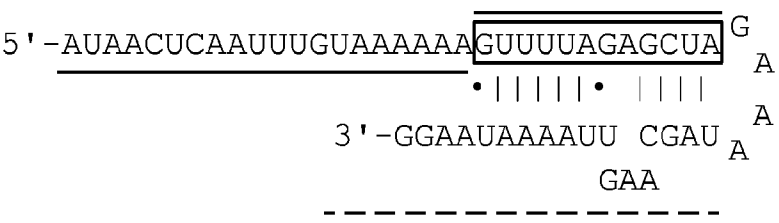


FIG. 27 (Cont.)

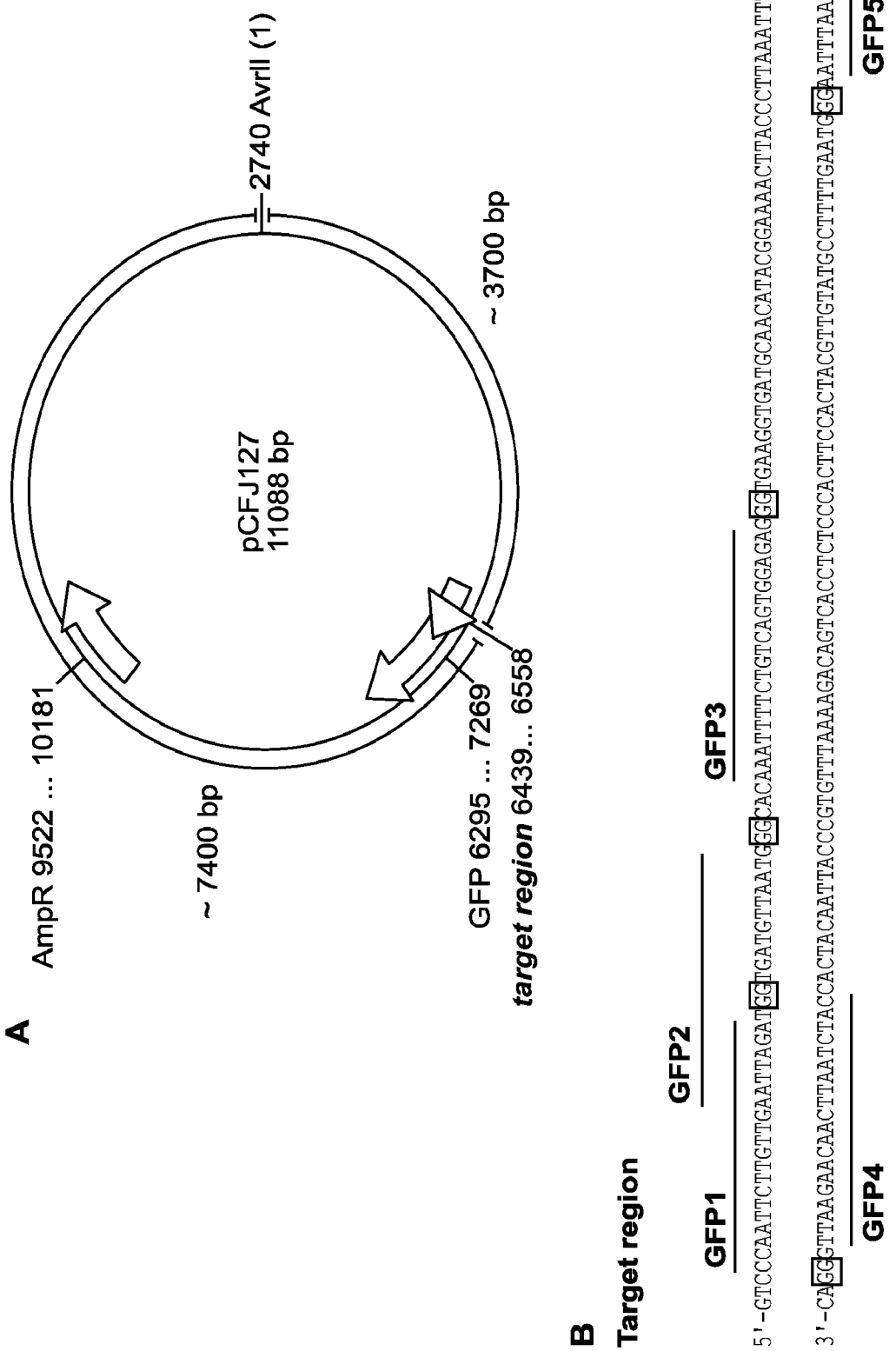

A
AmpR 9522 ... 10181
~ 7400 bp
pCFJ127
11088 bp
2740 AvrII (1)
~ 3700 bp
GFP 6295 ... 7269
target region 6439... 6558
B
Target region
GFP1
GFP2
GFP3
GFP4
GFP5
5'-GTCCCAATTCTTGTTGAATTAGATGGTGATGTTAATGGGCACAAATTTTCTGTCAGTGGAGAGGGTGAAGGTGATGCAACATACGGAAAACTTACCCTTAAATTTATTTGCACTACTGGA-3'
3'-CAGGGTTAAGAACAACTTAATCTACCACTACAATTACCCGTGTTTAAAAGACAGTCACCTCTCCCACTTCCACTACGTTGTATGCCTTTTGAATGGGAATTTAAATAAACGTGATGACCT-5'

FIG. 28

C
Target sequence
PAM
GFP1
CCAATTCTTGTTGAATTAGA-TGGTGA
5′-CCAAUUCUUGUUGAAUUAGAGUUUUAGAGCUAGAA
3′-GCCUGAUCGGAAUAAAUU CGAUUGAA
GFP2
AATTAGATGGTGATGTTAAT-GGGCAC
5′-AAUUAGAUGGUGAUGUUAAUGUUUUAGAGCUAGAA
3′-GCCUGAUCGGAAUAAAUU CGAUUGAA
GFP3
AAATTTTCTGTCAGTGGAGA-GGGTGA
5′-AAAUUUUCUGUCAGUGGAGAGUUUUAGAGCUAGAA
3′-GCCUGAUCGGAAUAAAUU CGAUUGAA
GFP4
CATCTAATTCAACAAGAATT-GGGACA
5′-CAUCUAAUUCAACAAGAAUUGUUUUAGAGCUAGAA
3′-GCCUGAUCGGAAUAAAUU CGAUUGAA
GFP5
CAGTAGTGCAAATAAATTTA-AGGGTA
5′-CAGUAGUGCAAAUAAAUUUAGUUUUAGAGCUAGAA
3′-GCCUGAUCGGAAUAAAUU CGAUUGAA

FIG. 28 (Cont. 1)

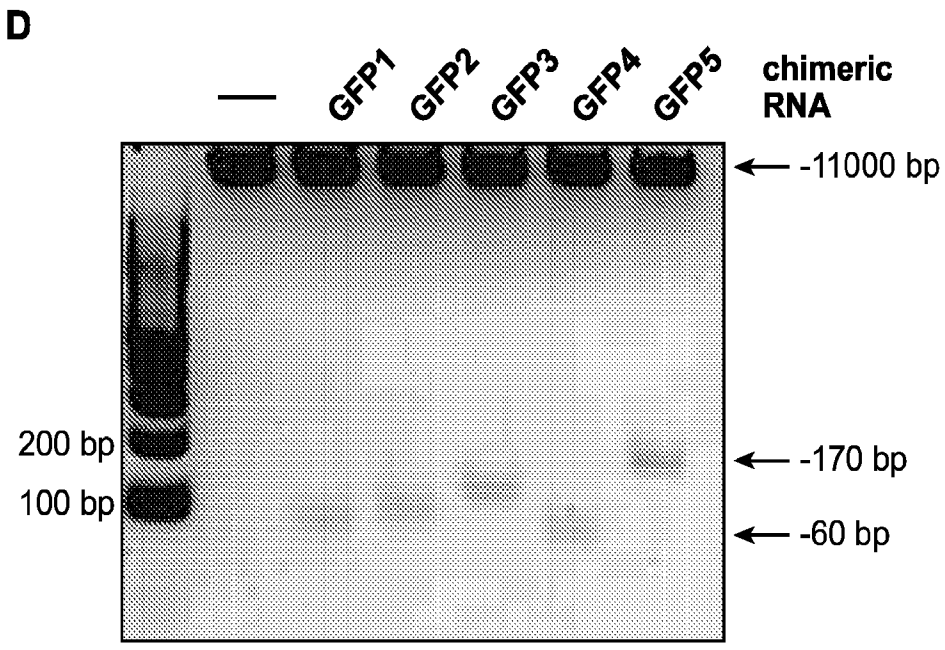

D
—
GFP1
GFP2
GFP3
GFP4
GFP5
chimeric RNA
-11000 bp
200 bp
100 bp
-170 bp
-60 bp

FIG. 28 (Cont. 2)

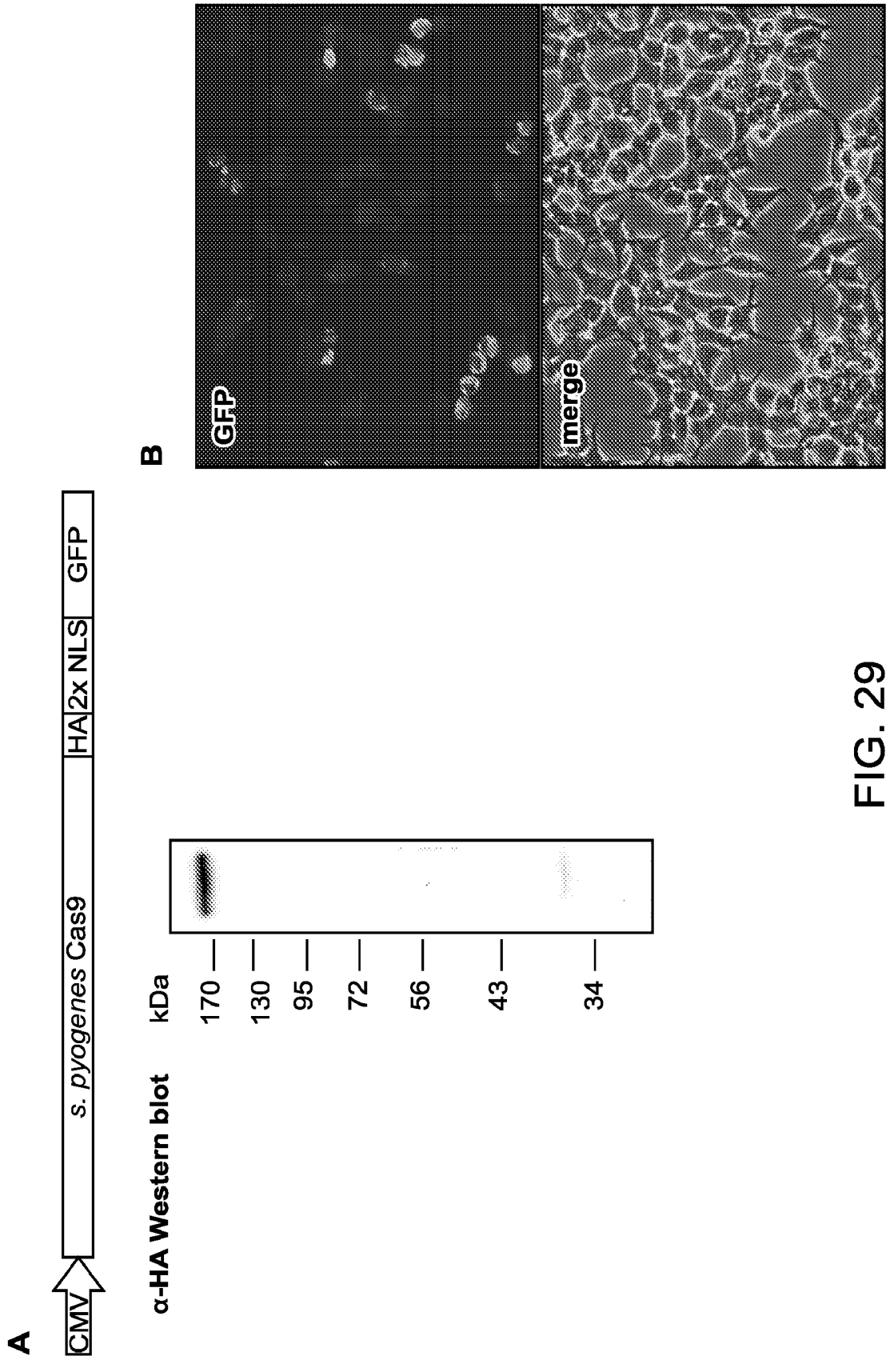

A
CMV
s. pyogenes Cas9
HA
2x NLS
GFP
α-HA Western blot
kDa
170
130
95
72
56
43
34
B
GFP
merge

FIG. 29

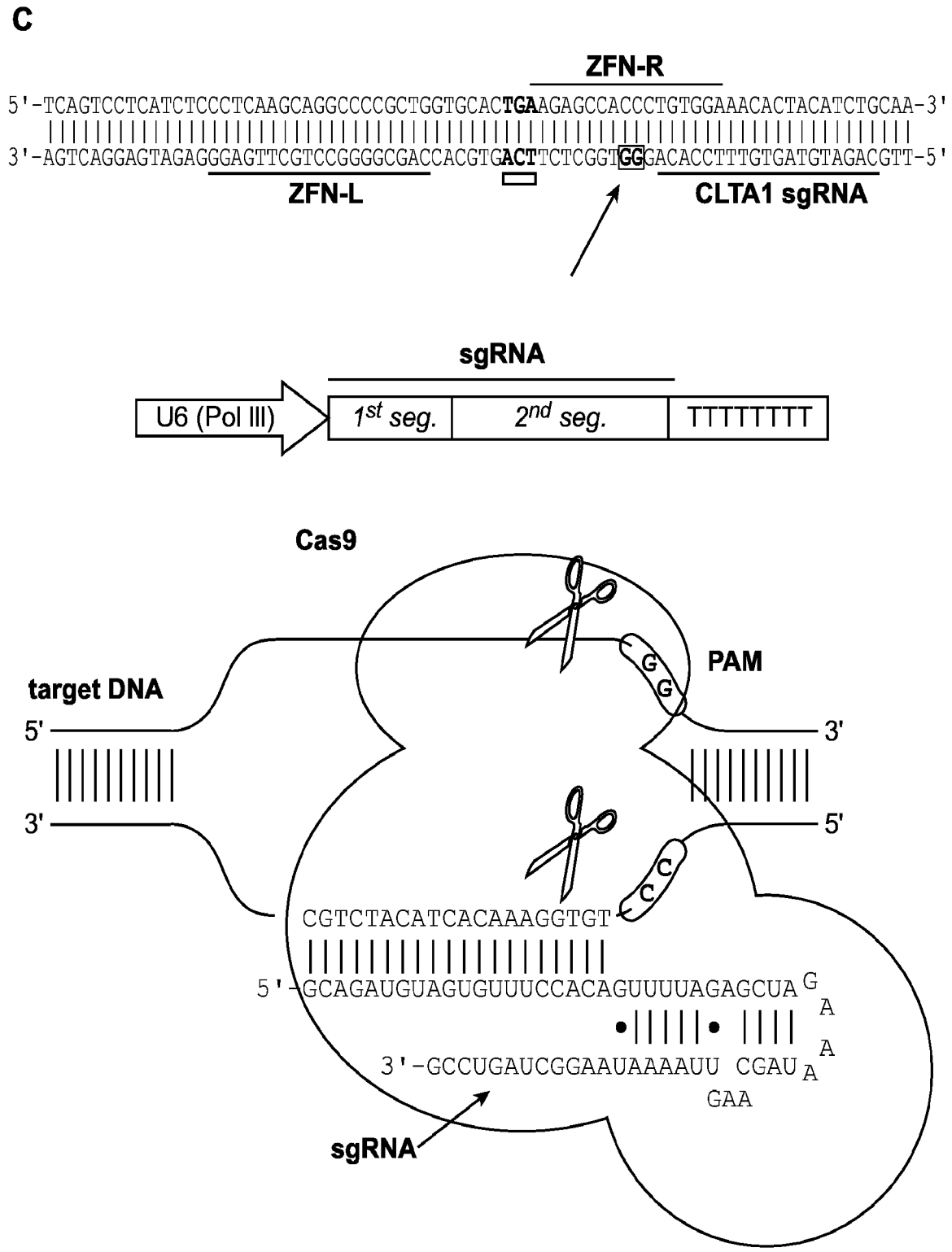
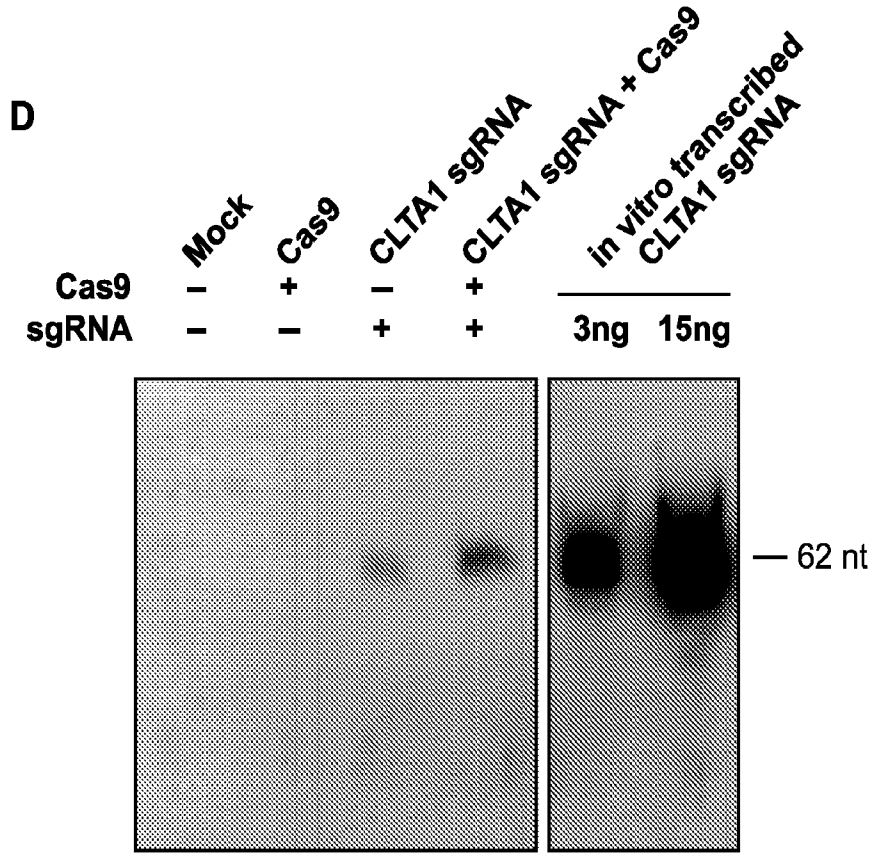

C
ZFN-R
5'-TCAGTCCTCATCTCCCTCAAGCAGGCCCCGCTGGTGCACTGAAGAGCCACCCTGTGGAAACACTACATCTGCAA-3'
3'-AGTCAGGAGTAGAGGGAGTTCGTCCGGGGCGACCACGTGACTTCTCGGTGGGACACCTTTGTGATGTAGACGTT-5'
ZFN-L
CLTA1 sgRNA
sgRNA
U6 (Pol III)
1st seg.
2nd seg.
TTTTTTTT
Cas9
target DNA
5'
3'
PAM
G
G
C
C
CGTCTACATCACAAAGGTGT
5'-GCAGAUGUAGUGUUUCCACAGUUUUAGAGCUA G A
3'-GCCUGAUCGGAAUAAAAUU CGAU A A
GAA
sgRNA

FIG. 29 (Cont. 1)


D
Mock
Cas9
CLTA1 sgRNA
CLTA1 sgRNA + Cas9
in vitro transcribed CLTA1 sgRNA
Cas9 – + – +
sgRNA – – + +
3ng 15ng
62 nt

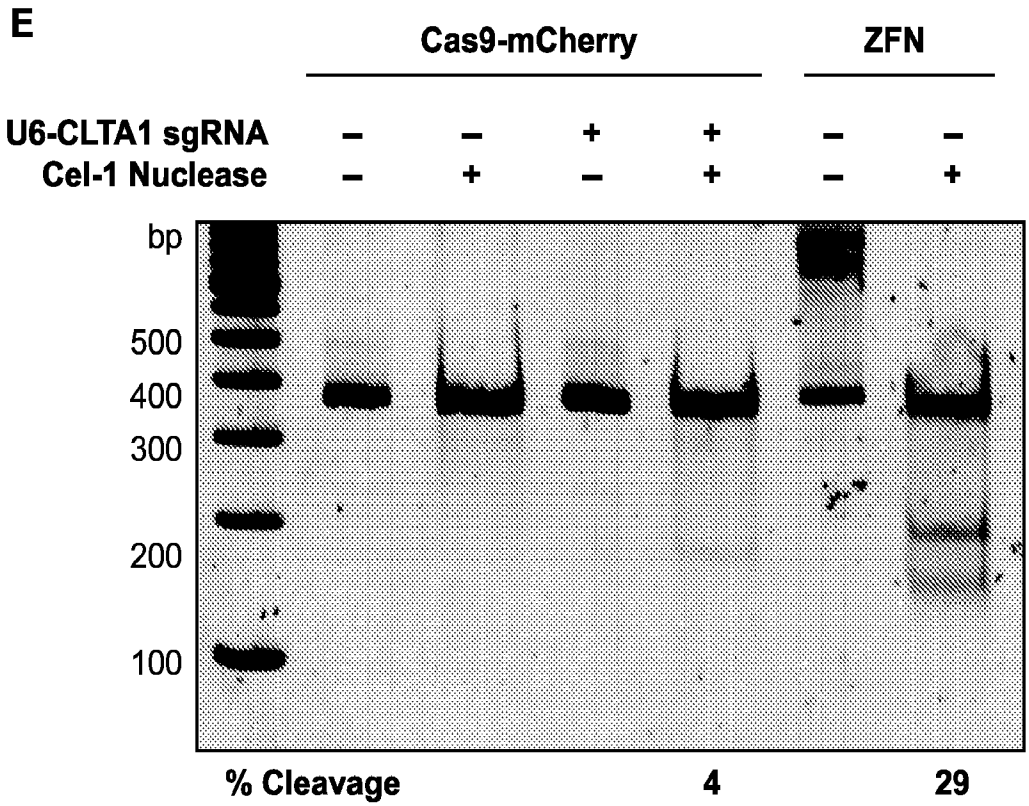

E
Cas9-mCherry
ZFN
U6-CLTA1 sgRNA – – + + – –
Cel-1 Nuclease – + – + – +
bp
500
400
300
200
100
% Cleavage 4 29

FIG. 29 (Cont. 2)

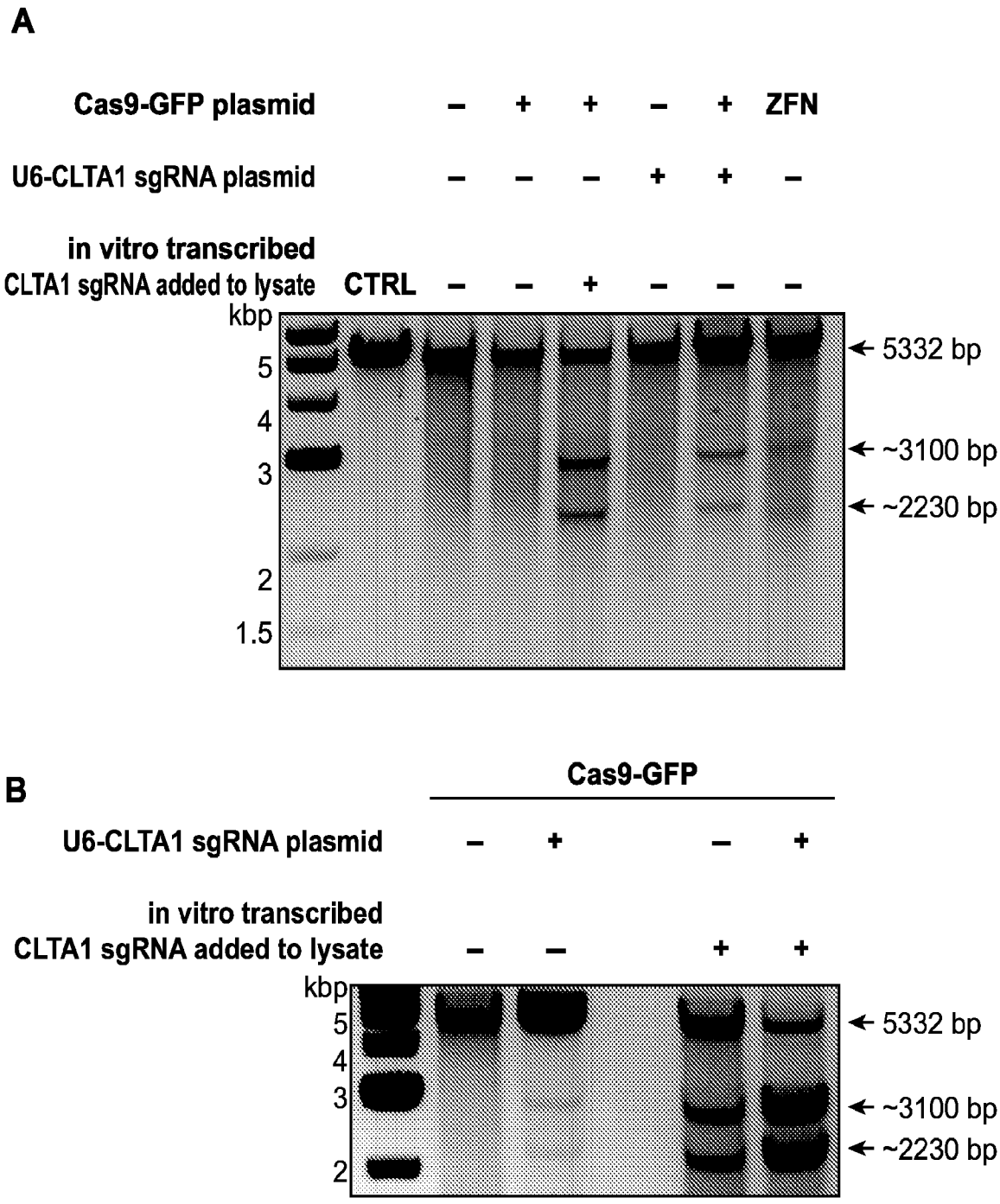

A
Cas9-GFP plasmid
U6-CLTA1 sgRNA plasmid
in vitro transcribed CLTA1 sgRNA added to lysate
CTRL
ZFN
kbp
5
4
3
2
1.5
5332 bp
~3100 bp
~2230 bp
B
Cas9-GFP
U6-CLTA1 sgRNA plasmid
in vitro transcribed CLTA1 sgRNA added to lysate
kbp
5
4
3
2
5332 bp
~3100 bp
~2230 bp

FIG. 30

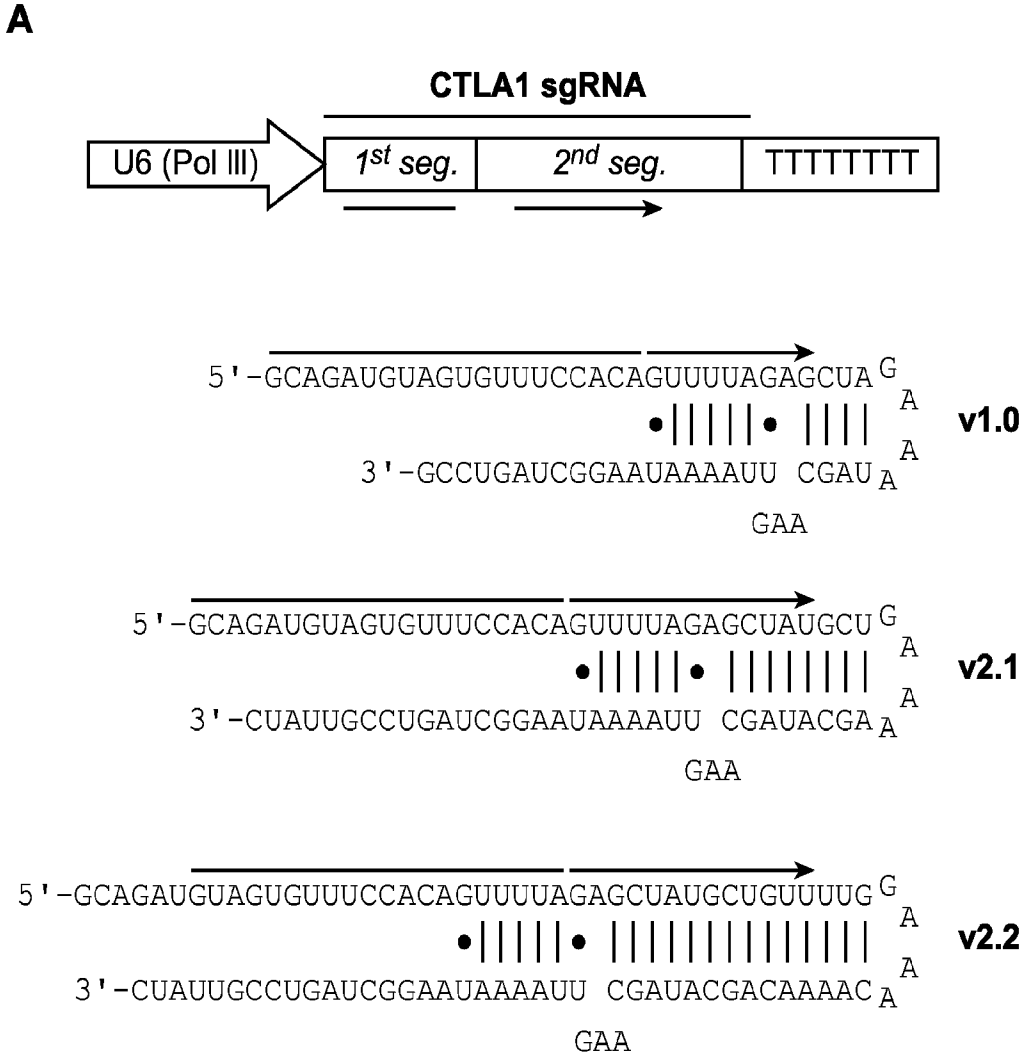

A
CTLA1 sgRNA
U6 (Pol III)
1st seg.
2nd seg.
TTTTTTTT
5'-GCAGAUGUAGUGUUUCCACAGUUUUAGAGCUA G A A
3'-GCCUGAUCGGAAUAAAAUU CGAU A
GAA
v1.0
5'-GCAGAUGUAGUGUUUCCACAGUUUUAGAGCUAUGCU G A A
3'-CUAUUGCCUGAUCGGAAUAAAAUU CGAUACGA A
GAA
v2.1
5'-GCAGAUGUAGUGUUUCCACAGUUUUAGAGCUAUGCUGUUUUG G A A
3'-CUAUUGCCUGAUCGGAAUAAAAUU CGAUACGACAAAAC A
GAA
v2.2

FIG. 31

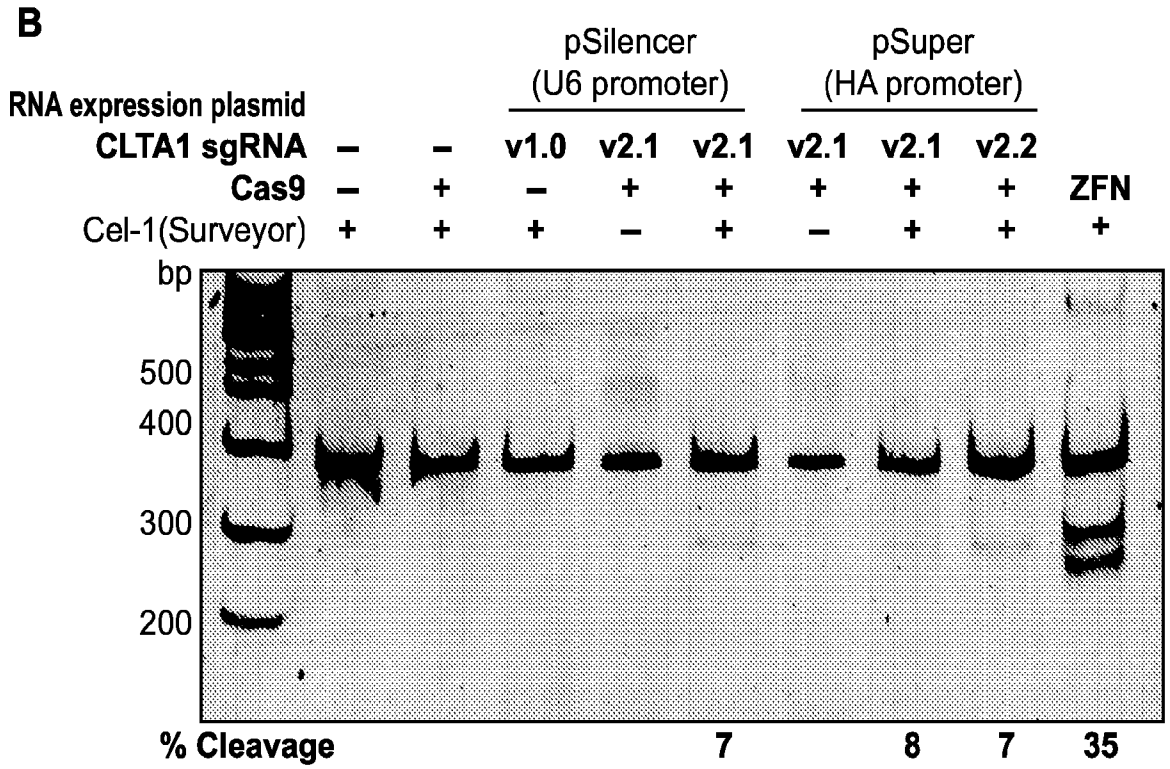

B
pSilencer
(U6 promoter)
pSuper
(HA promoter)
RNA expression plasmid
CLTA1 sgRNA – – v1.0 v2.1 v2.1 v2.1 v2.1 v2.2
Cas9 – + – + + + + + ZFN
Cel-1(Surveyor) + + + – + – + + +
bp
500
400
300
200
% Cleavage 7 8 7 35

FIG. 31 (Cont.)

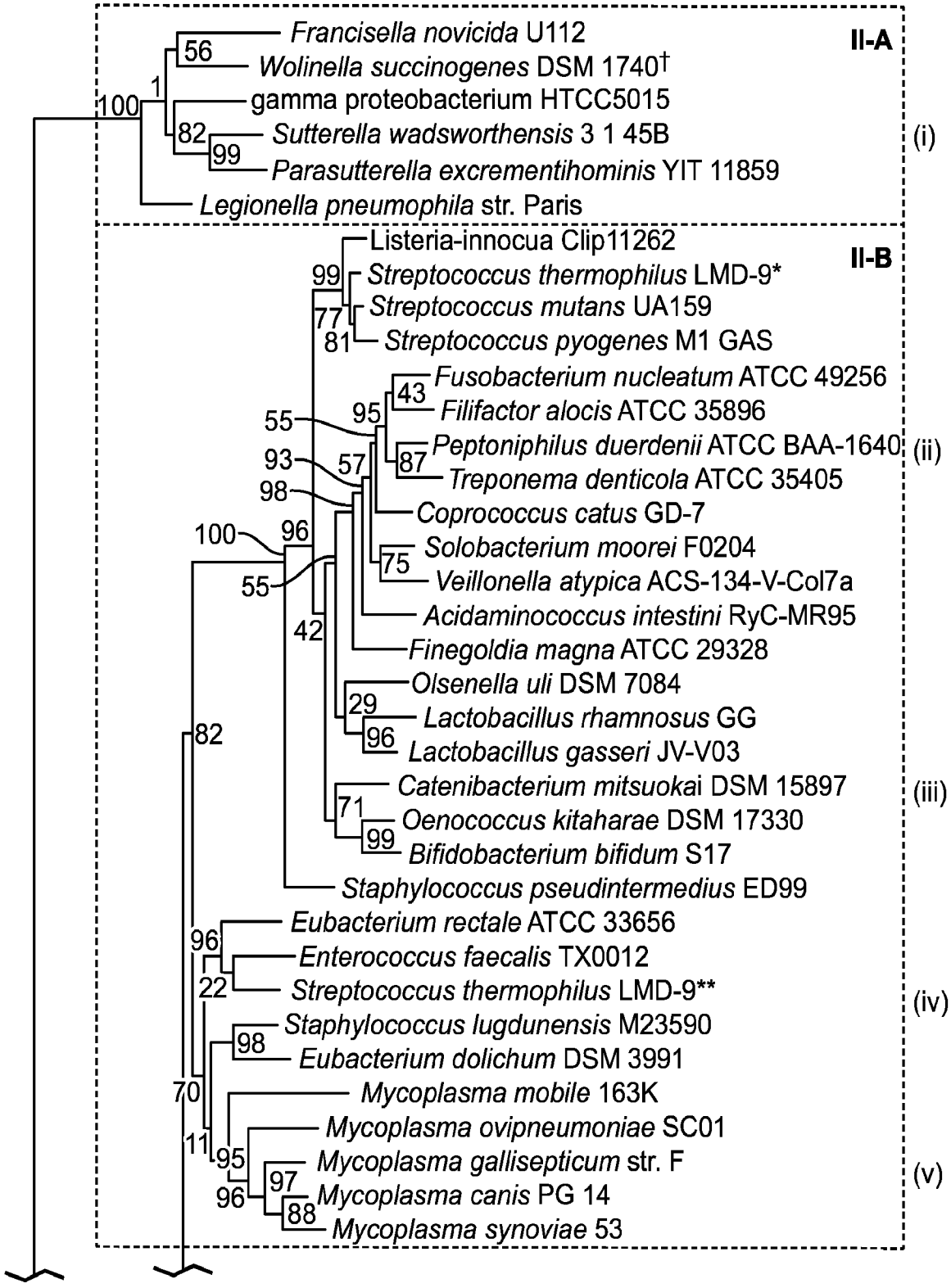

II-A
II-B
(i)
(ii)
(iii)
(iv)
(v)
Francisella novicida U112
Wolinella succinogenes DSM 1740†
gamma proteobacterium HTCC5015
Sutterella wadsworthensis 3 1 45B
Parasutterella excrementihominis YIT 11859
Legionella pneumophila str. Paris
Listeria-innocua Clip11262
Streptococcus thermophilus LMD-9*
Streptococcus mutans UA159
Streptococcus pyogenes M1 GAS
Fusobacterium nucleatum ATCC 49256
Filifactor alocis ATCC 35896
Peptoniphilus duerdenii ATCC BAA-1640
Treponema denticola ATCC 35405
Coprococcus catus GD-7
Solobacterium moorei F0204
Veillonella atypica ACS-134-V-Col7a
Acidaminococcus intestini RyC-MR95
Finegoldia magna ATCC 29328
Olsenella uli DSM 7084
Lactobacillus rhamnosus GG
Lactobacillus gasseri JV-V03
Catenibacterium mitsuokai DSM 15897
Oenococcus kitaharae DSM 17330
Bifidobacterium bifidum S17
Staphylococcus pseudintermedius ED99
Eubacterium rectale ATCC 33656
Enterococcus faecalis TX0012
Streptococcus thermophilus LMD-9**
Staphylococcus lugdunensis M23590
Eubacterium dolichum DSM 3991
Mycoplasma mobile 163K
Mycoplasma ovipneumoniae SC01
Mycoplasma gallisepticum str. F
Mycoplasma canis PG 14
Mycoplasma synoviae 53

FIG. 32A

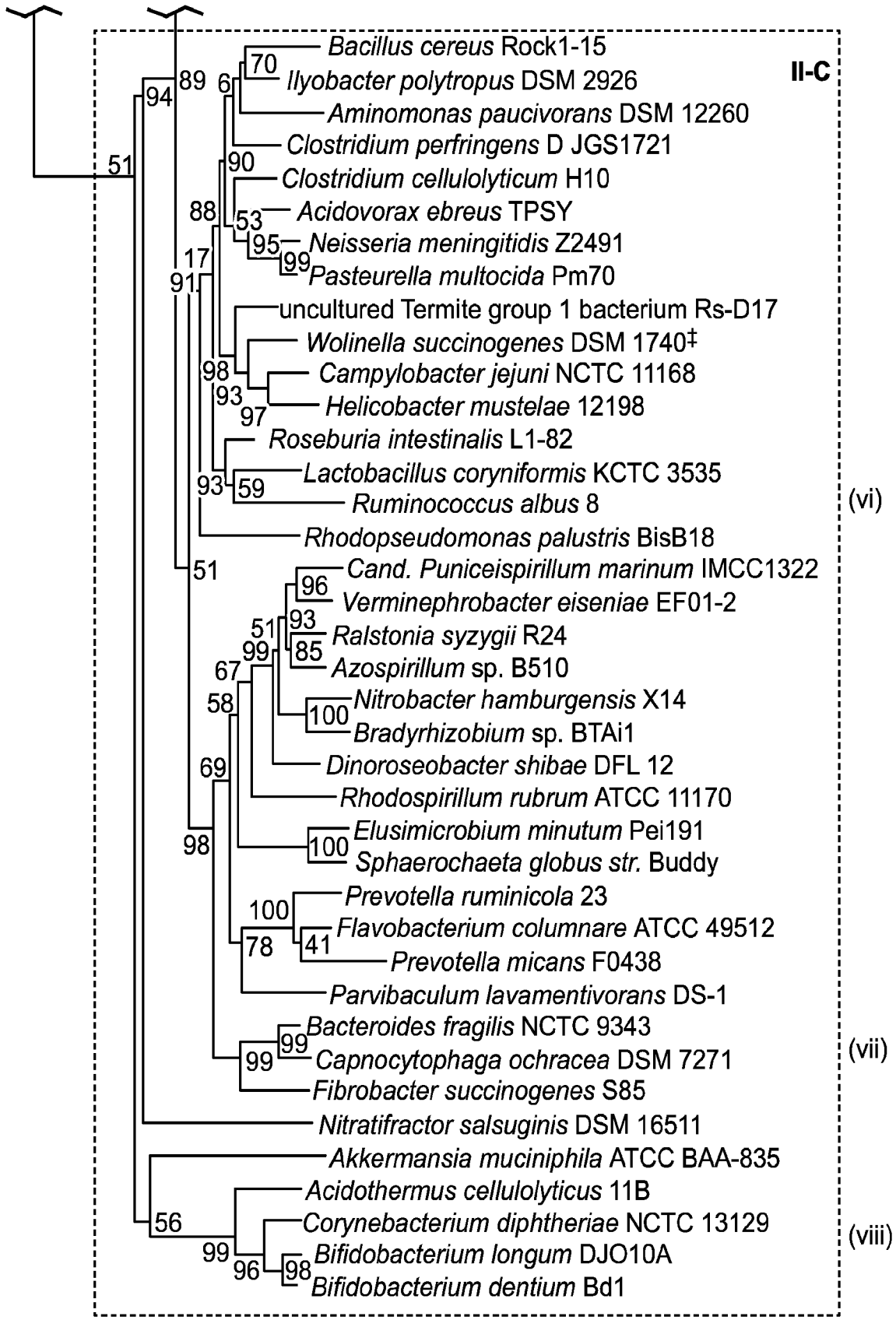

II-C
Bacillus cereus Rock1-15
Ilyobacter polytropus DSM 2926
Aminomonas paucivorans DSM 12260
Clostridium perfringens D JGS1721
Clostridium cellulolyticum H10
Acidovorax ebreus TPSY
Neisseria meningitidis Z2491
Pasteurella multocida Pm70
uncultured Termite group 1 bacterium Rs-D17
Wolinella succinogenes DSM 1740‡
Campylobacter jejuni NCTC 11168
Helicobacter mustelae 12198
Roseburia intestinalis L1-82
Lactobacillus coryniformis KCTC 3535
Ruminococcus albus 8
Rhodopseudomonas palustris BisB18
Cand. Puniceispirillum marinum IMCC1322
Verminephrobacter eiseniae EF01-2
Ralstonia syzygii R24
Azospirillum sp. B510
Nitrobacter hamburgensis X14
Bradyrhizobium sp. BTAi1
Dinoroseobacter shibae DFL 12
Rhodospirillum rubrum ATCC 11170
Elusimicrobium minutum Pei191
Sphaerochaeta globus str. Buddy
Prevotella ruminicola 23
Flavobacterium columnare ATCC 49512
Prevotella micans F0438
Parvibaculum lavamentivorans DS-1
Bacteroides fragilis NCTC 9343
Capnocytophaga ochracea DSM 7271
Fibrobacter succinogenes S85
Nitratifractor salsuginis DSM 16511
Akkermansia muciniphila ATCC BAA-835
Acidothermus cellulolyticus 11B
Corynebacterium diphtheriae NCTC 13129
Bifidobacterium longum DJO10A
Bifidobacterium dentium Bd1
(vi)
(vii)
(viii)

FIG. 32A (Cont.)

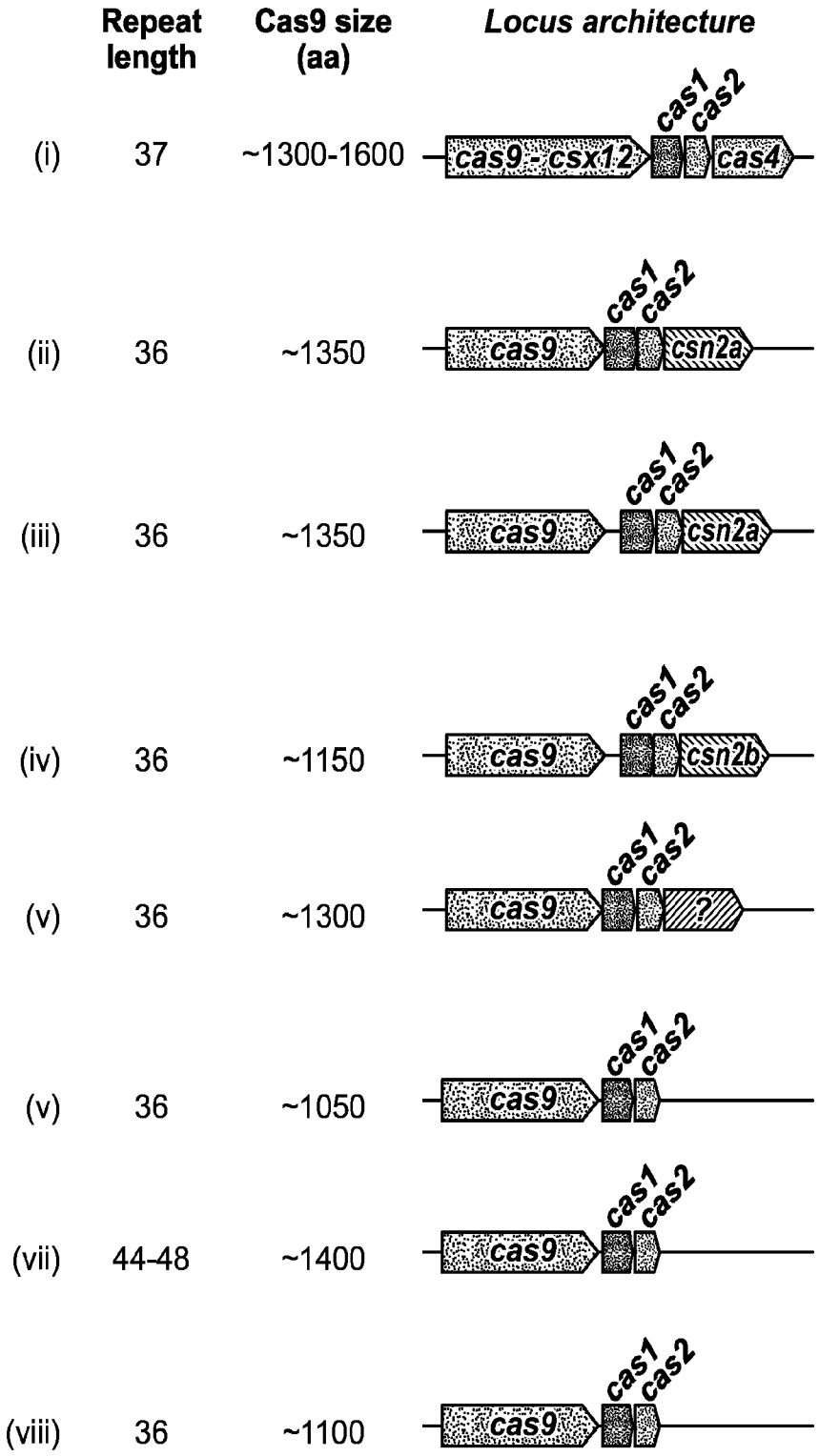

Repeat length
Cas9 size (aa)
Locus architecture
(i)
37
~1300-1600
cas9
csx12
cas1
cas2
cas4
(ii)
36
~1350
cas9
cas1
cas2
csn2a
(iii)
36
~1350
cas9
cas1
cas2
csn2a
(iv)
36
~1150
cas9
cas1
cas2
csn2b
(v)
36
~1300
cas9
cas1
cas2
?
(v)
36
~1050
cas9
cas1
cas2
(vii)
44-48
~1400
cas9
cas1
cas2
(viii)
36
~1100
cas9
cas1
cas2

FIG. 32B

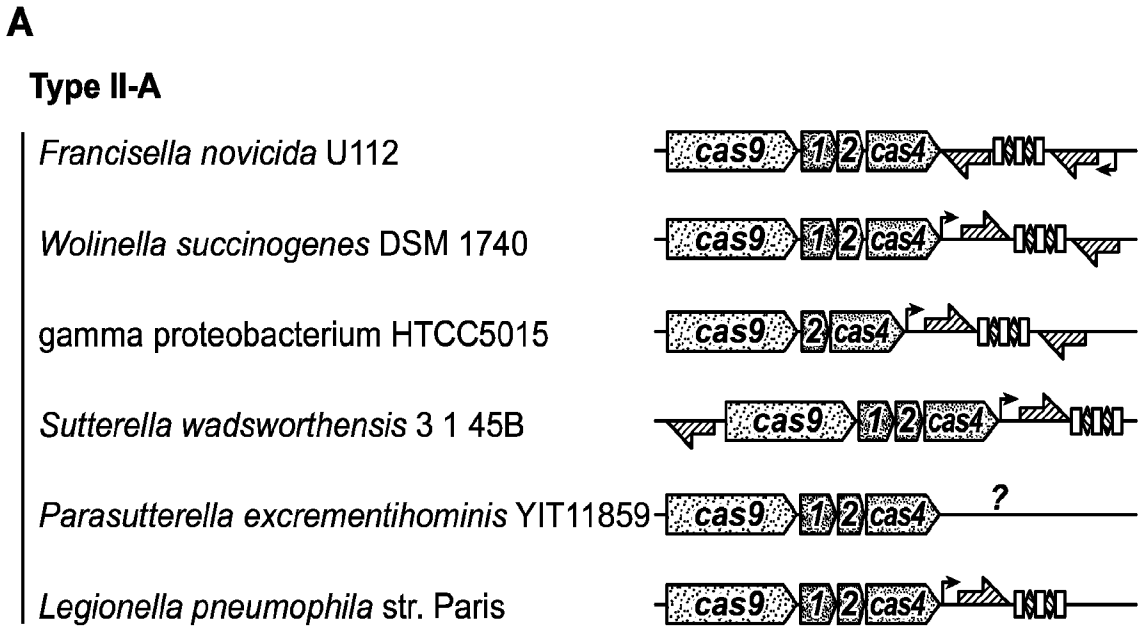

A
Type II-A
Francisella novicida U112
Wolinella succinogenes DSM 1740
gamma proteobacterium HTCC5015
Sutterella wadsworthensis 3 1 45B
Parasutterella excrementihominis YIT11859
Legionella pneumophila str. Paris
cas9
1
2
cas4
?

FIG. 33

B

Type II-B

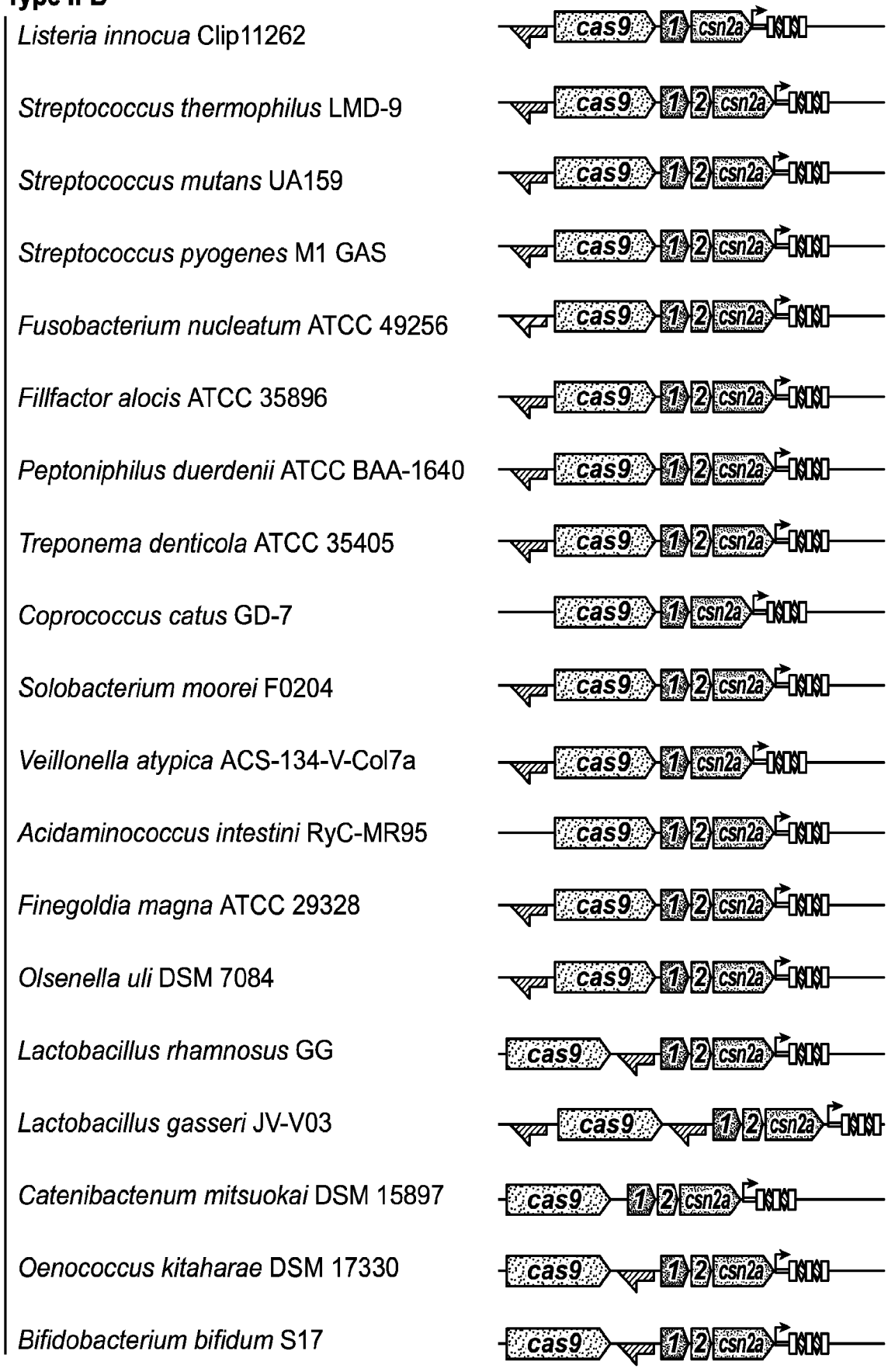


FIG. 33 (Cont. 1)

C

Type II-B (Continued)

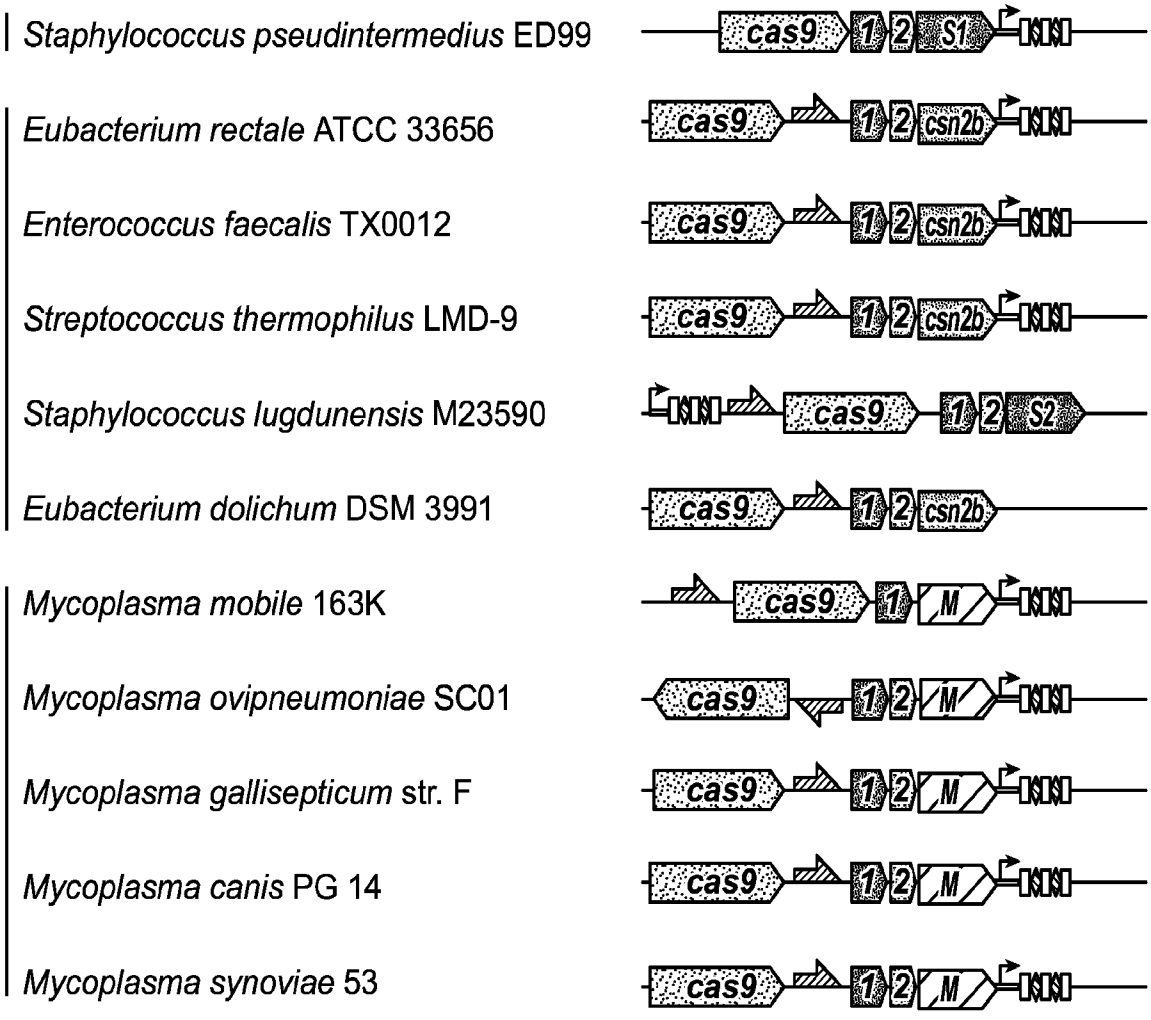


FIG. 33 (Cont. 2)

D Type II-C

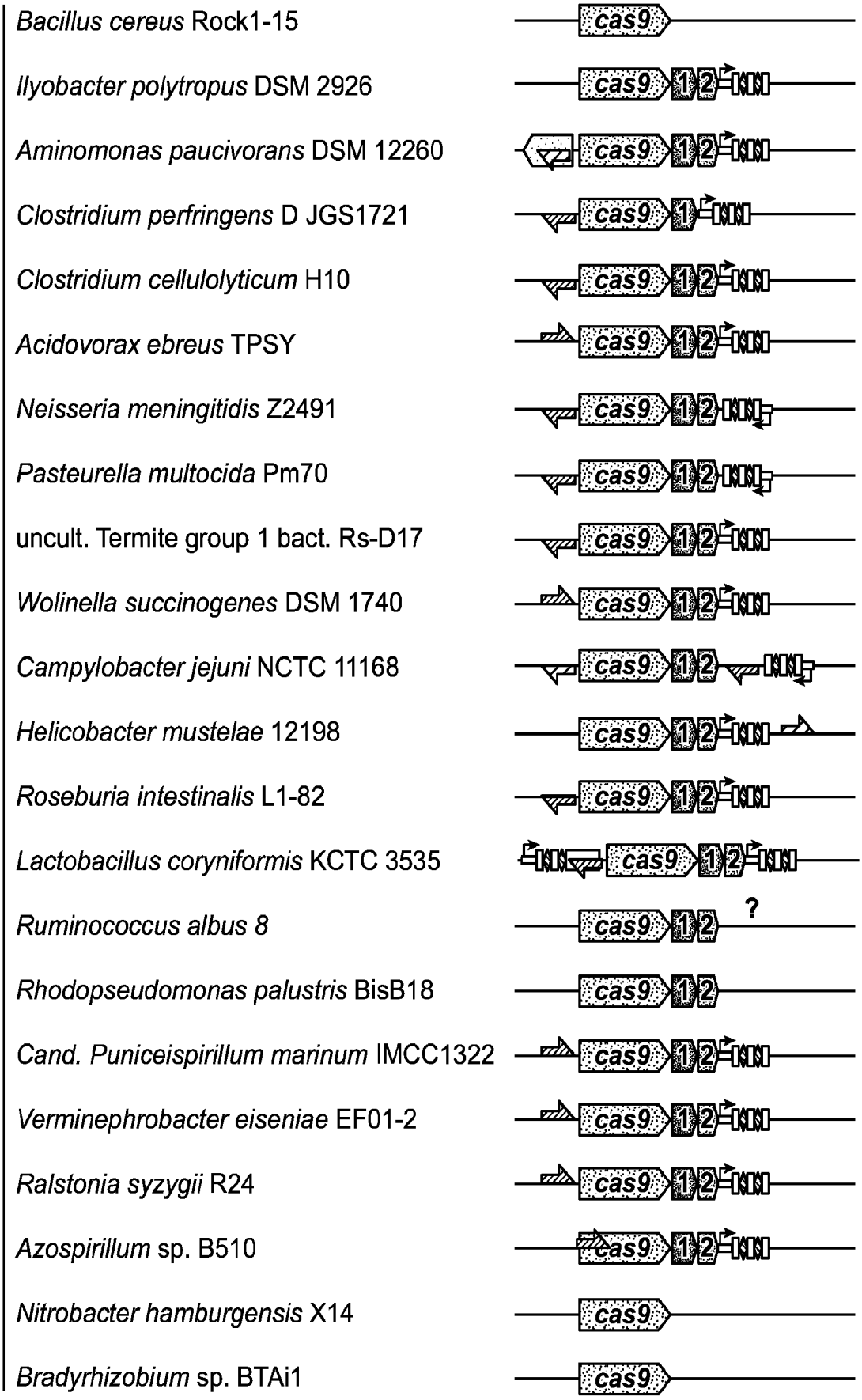


FIG. 33 (Cont. 3)

E

Type II-C (Continued)

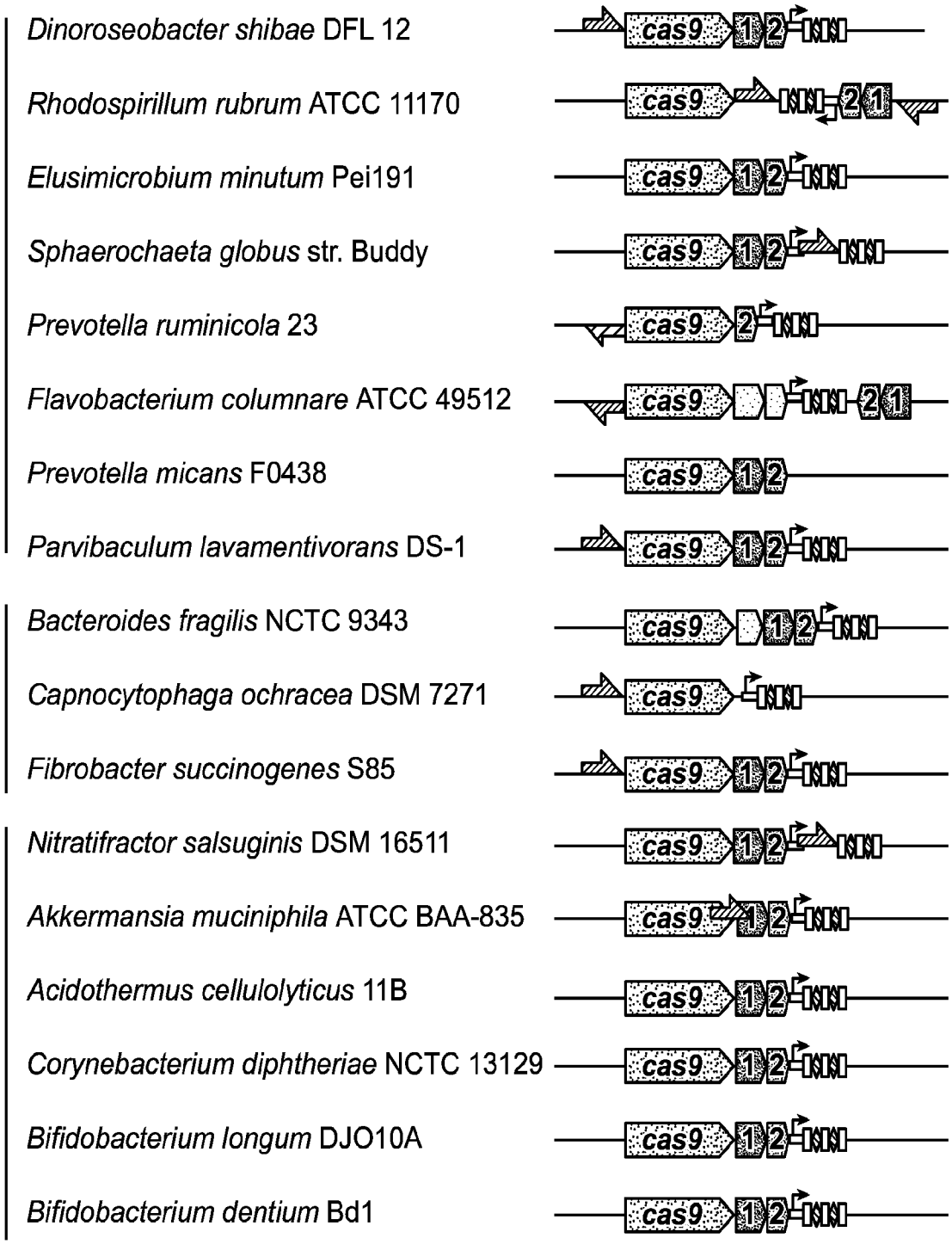


FIG. 33 (Cont. 4)

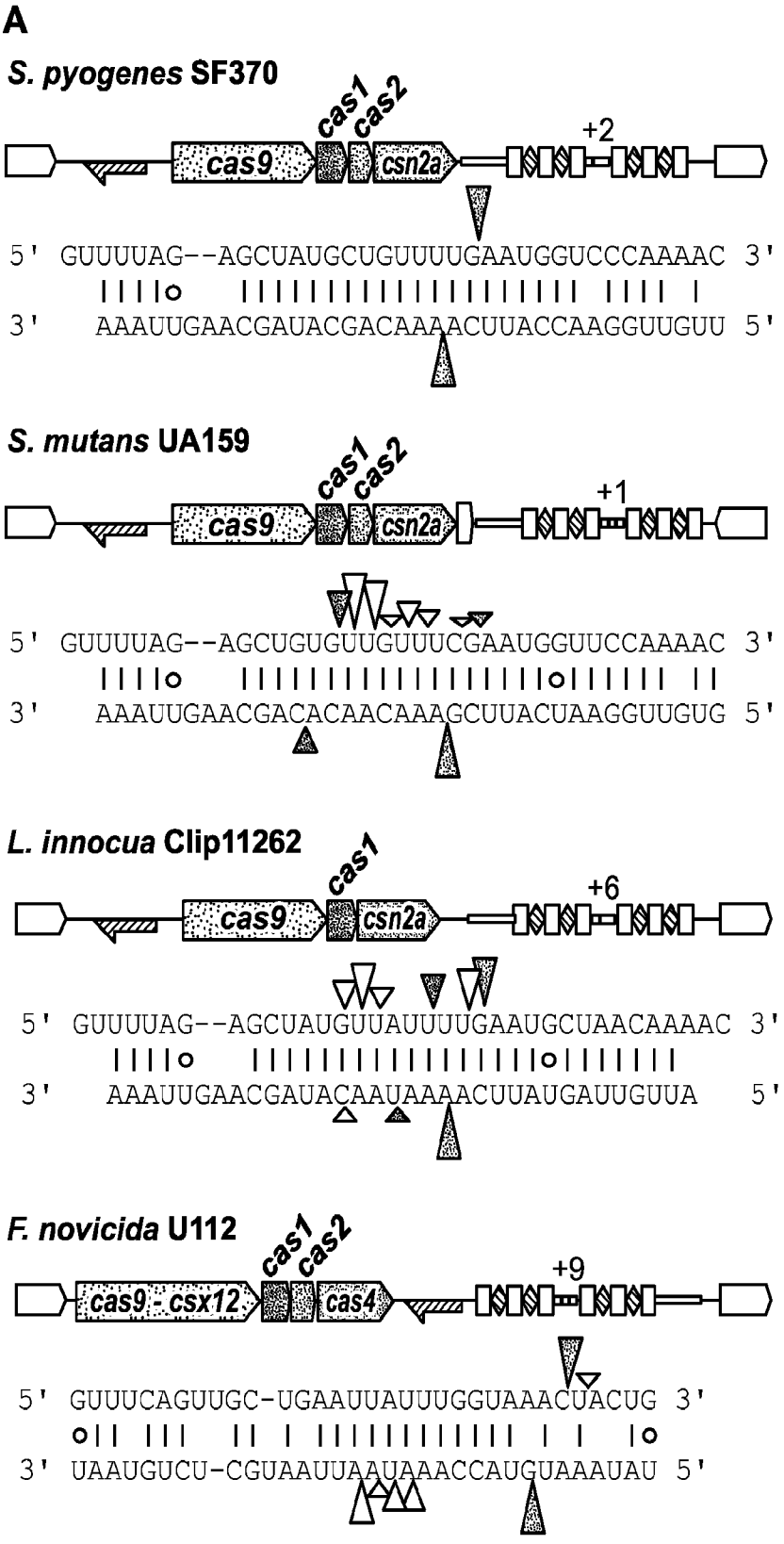

A
S. pyogenes SF370
cas1
cas2
cas9
csn2a
+2
5' GUUUUAG--AGCUAUGCUGUUUUGAAUGGUCCCAAAAC 3'
3' AAAUUGAACGAUACGACAAAACUUACCAAGGUUGUU 5'
S. mutans UA159
cas1
cas2
cas9
csn2a
+1
5' GUUUUAG--AGCUGUGUUGUUUCGAAUGGUUCCAAAAC 3'
3' AAAUUGAACGACACAACAAAGCUUACUAAGGUUGUG 5'
L. innocua Clip11262
cas1
cas9
csn2a
+6
5' GUUUUAG--AGCUAUGUUAUUUUGAAUGCUAACAAAAC 3'
3' AAAUUGAACGAUACAAUAAAACUUAUGAUUGUUA 5'
F. novicida U112
cas1
cas2
cas9 - csx12
cas4
+9
5' GUUUCAGUUGC-UGAAUUAUUUGGUAAACUACUG 3'
3' UAAUGUCU-CGUAAUUAAUAAACCAUGUAAAUAU 5'

FIG. 34

B

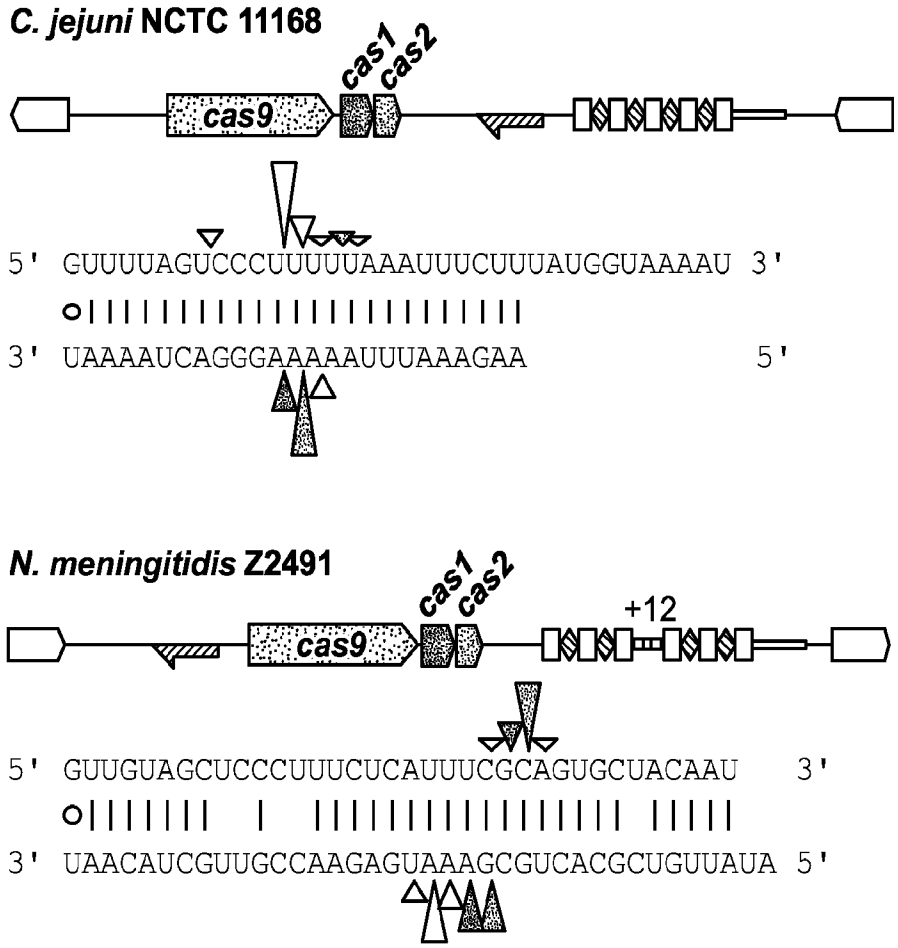

C. jejuni NCTC 11168
cas1
cas2
cas9
5' GUUUUAGUCCCUUUUUAAAUUUCUUUAUGGUAAAAU 3'
3' UAAAAUCAGGGAAAAAUUUAAAGAA 5'
N. meningitidis Z2491
cas1
cas2
cas9
+12
5' GUUGUAGCUCCCUUUCUCAUUUCGCAGUGCUACAAU 3'
3' UAACAUCGUUGCCAAGAGUAAAGCGUCACGCUGUUAUA 5'

FIG. 34 (Cont.)

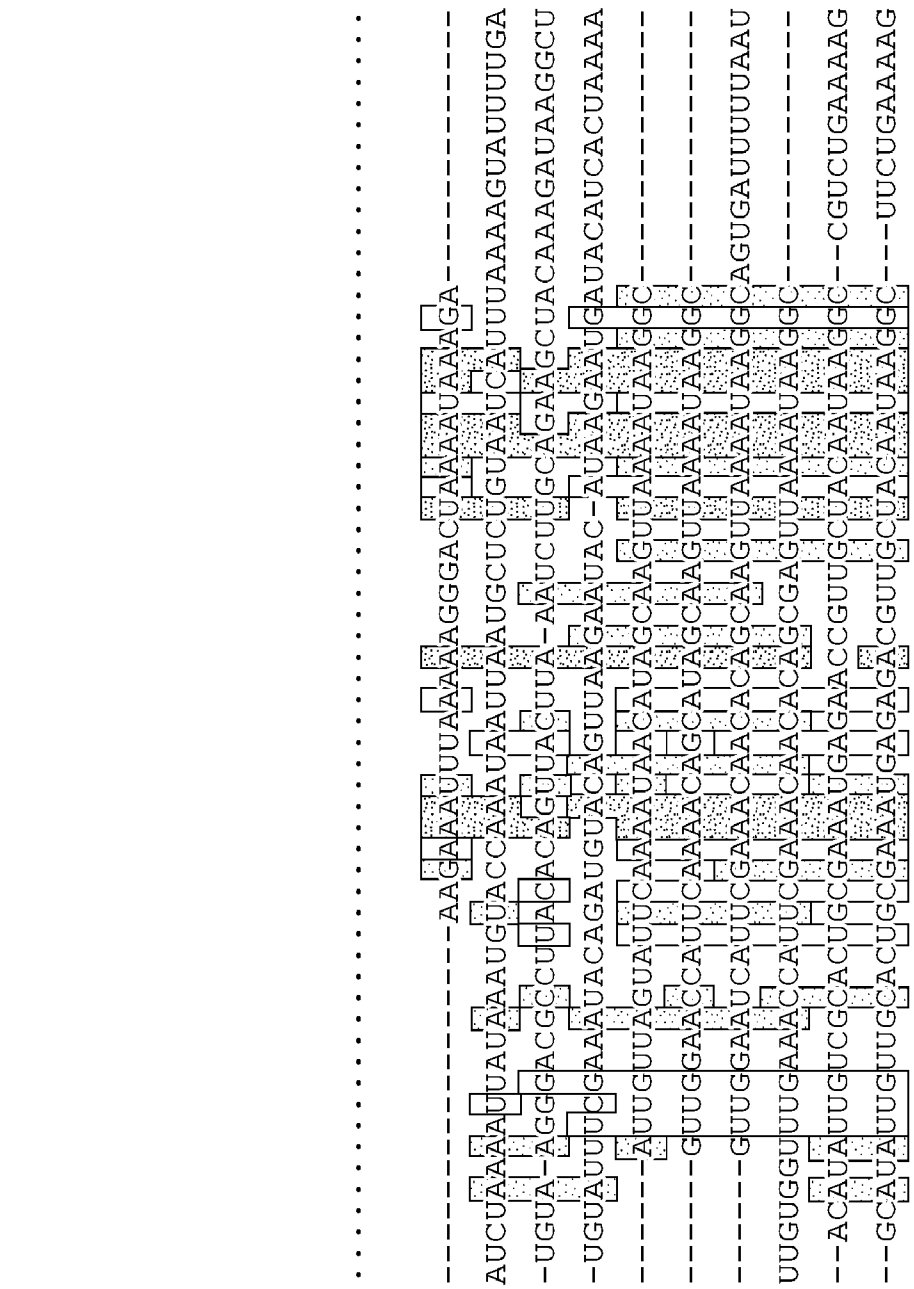

Consensus
structure
C. jejuni
F. novicida
S. thermophilus2
M. mobile
L. innocua
S. pyogenes
S. mutans
S. thermophilus
N. meningitidis
P. multocida

FIG. 35

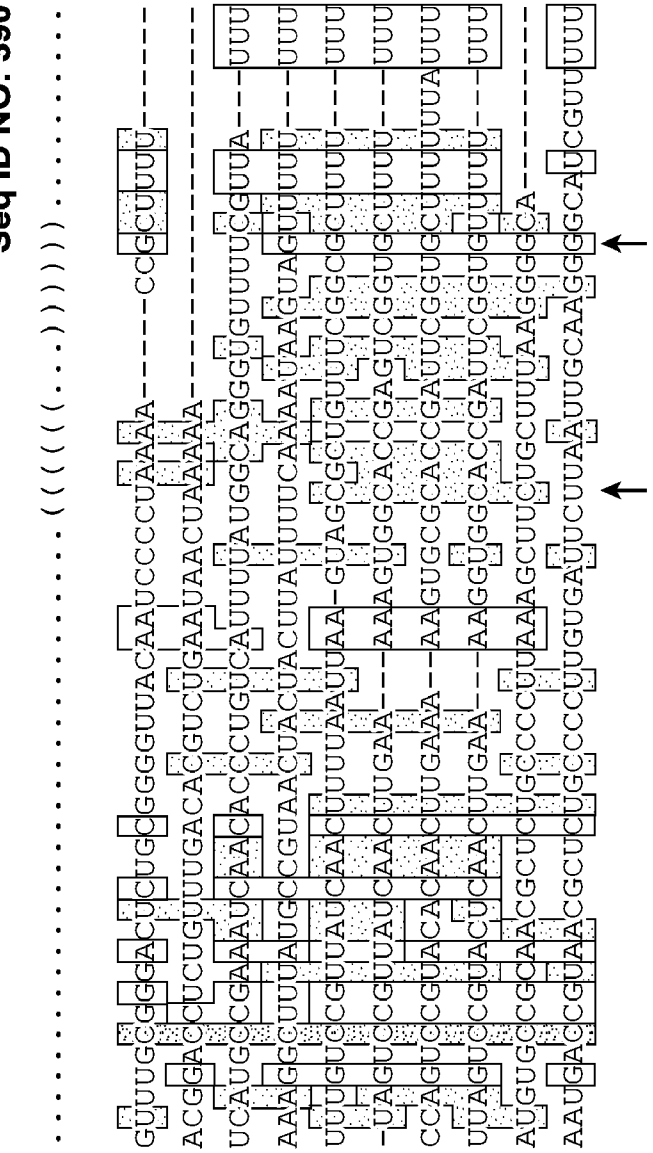

Seq ID NO: 390
Consensus
structure
C. jejuni
F. novicida
S. thermophilus2
M. mobile
L. innocua
s. pyogenes
s. mutans
s. thermophilus
N. meningitidis
P. multocida

FIG. 35 (Cont.)

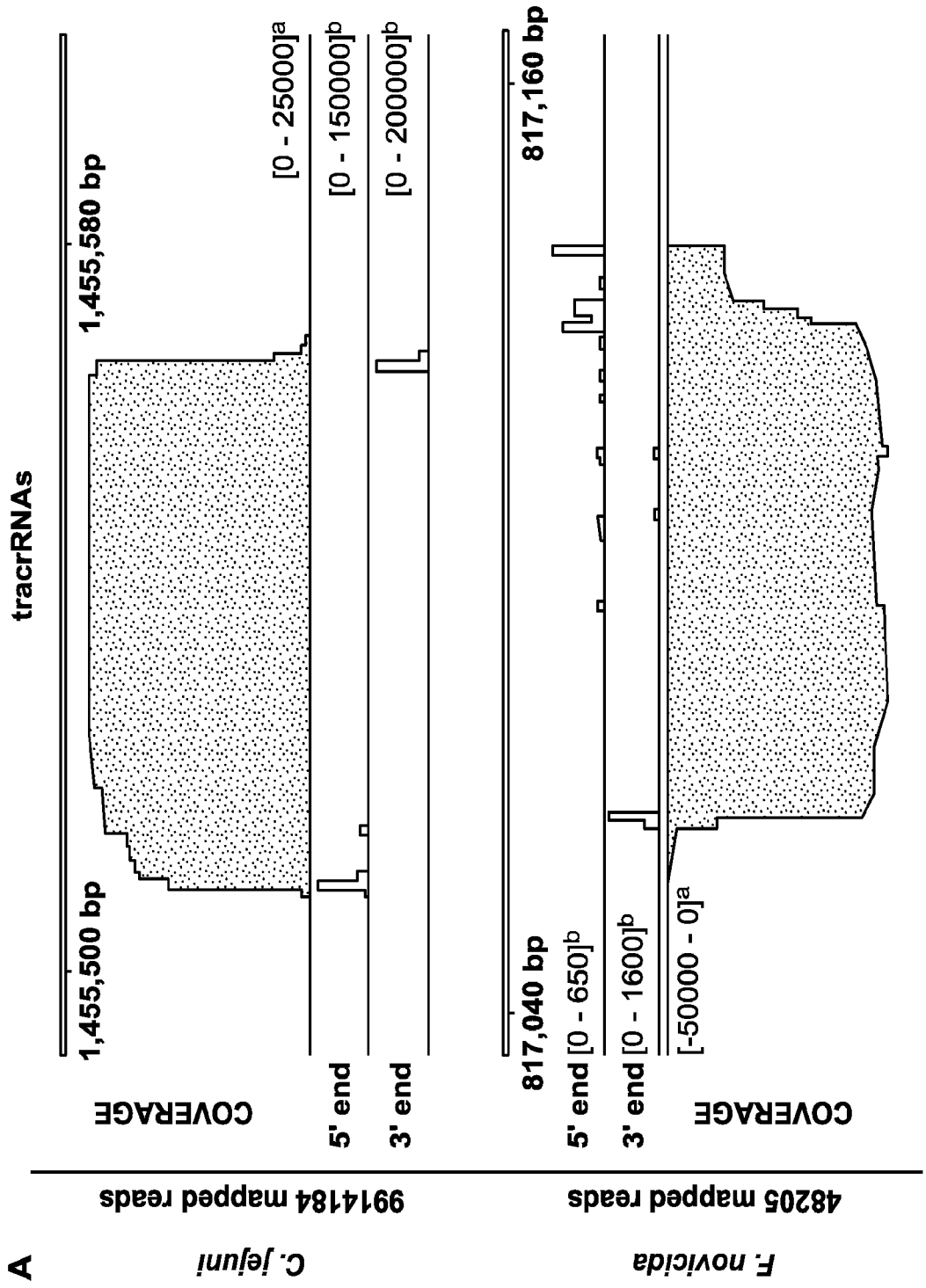

A
C. jejuni
F. novicida
tracrRNAs
9914184 mapped reads
48205 mapped reads
COVERAGE
5' end
3' end
1,455,500 bp
1,455,580 bp
817,040 bp
817,160 bp
[0 - 25000]a
[0 - 150000]b
[0 - 200000]b
[0 - 650]b
[0 - 1600]b
[-50000 - 0]a

FIG. 36

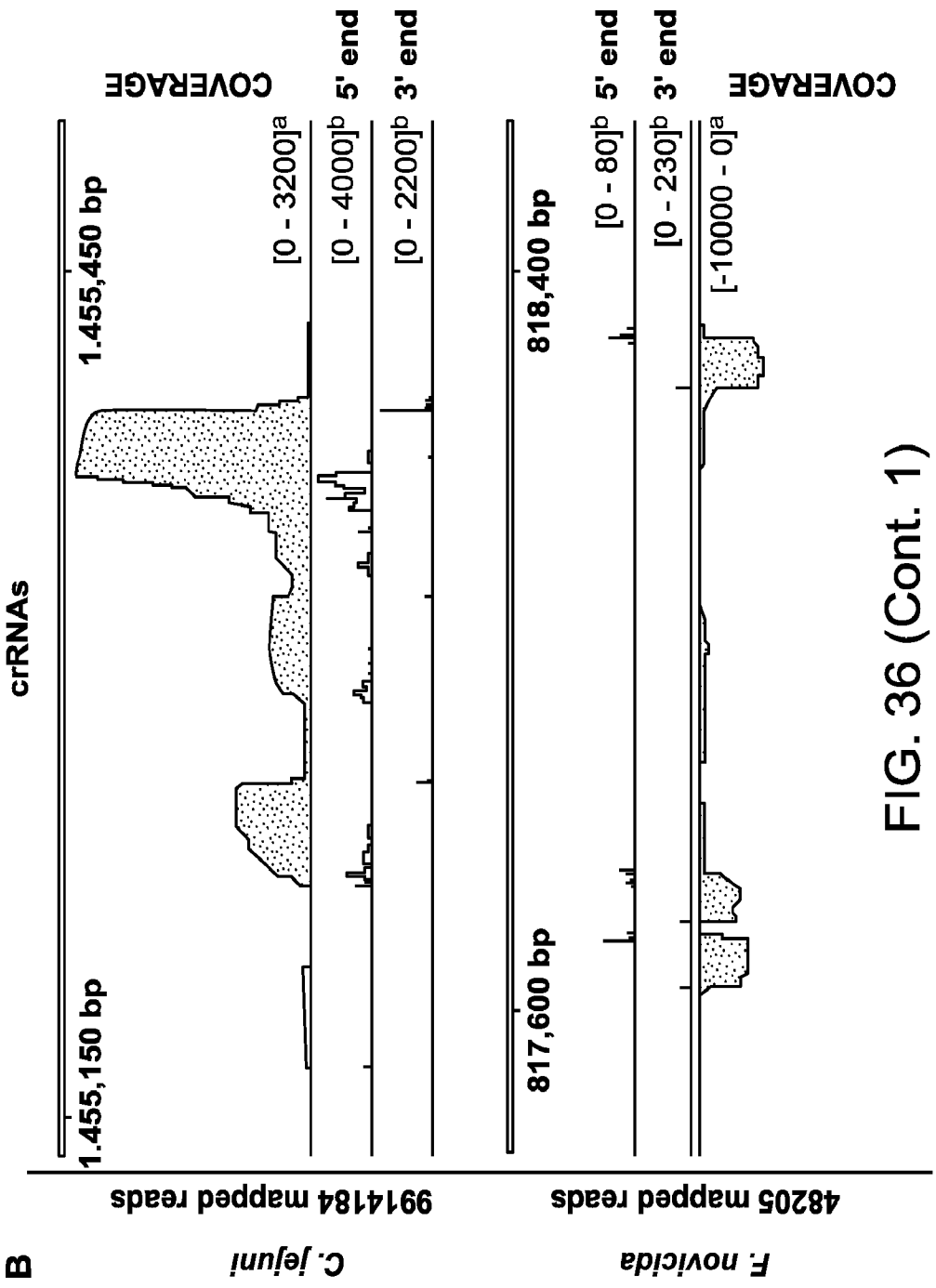

B
C. jejuni
991184 mapped reads
crRNAs
1,455,150 bp
1,455,450 bp
COVERAGE
[0 - 3200]a
5' end
[0 - 4000]b
3' end
[0 - 2200]b
F. novicida
48205 mapped reads
817,600 bp
818,400 bp
COVERAGE
[0 - 80]b
5' end
[0 - 230]b
3' end
[-10000 - 0]a

FIG. 36 (Cont. 1)

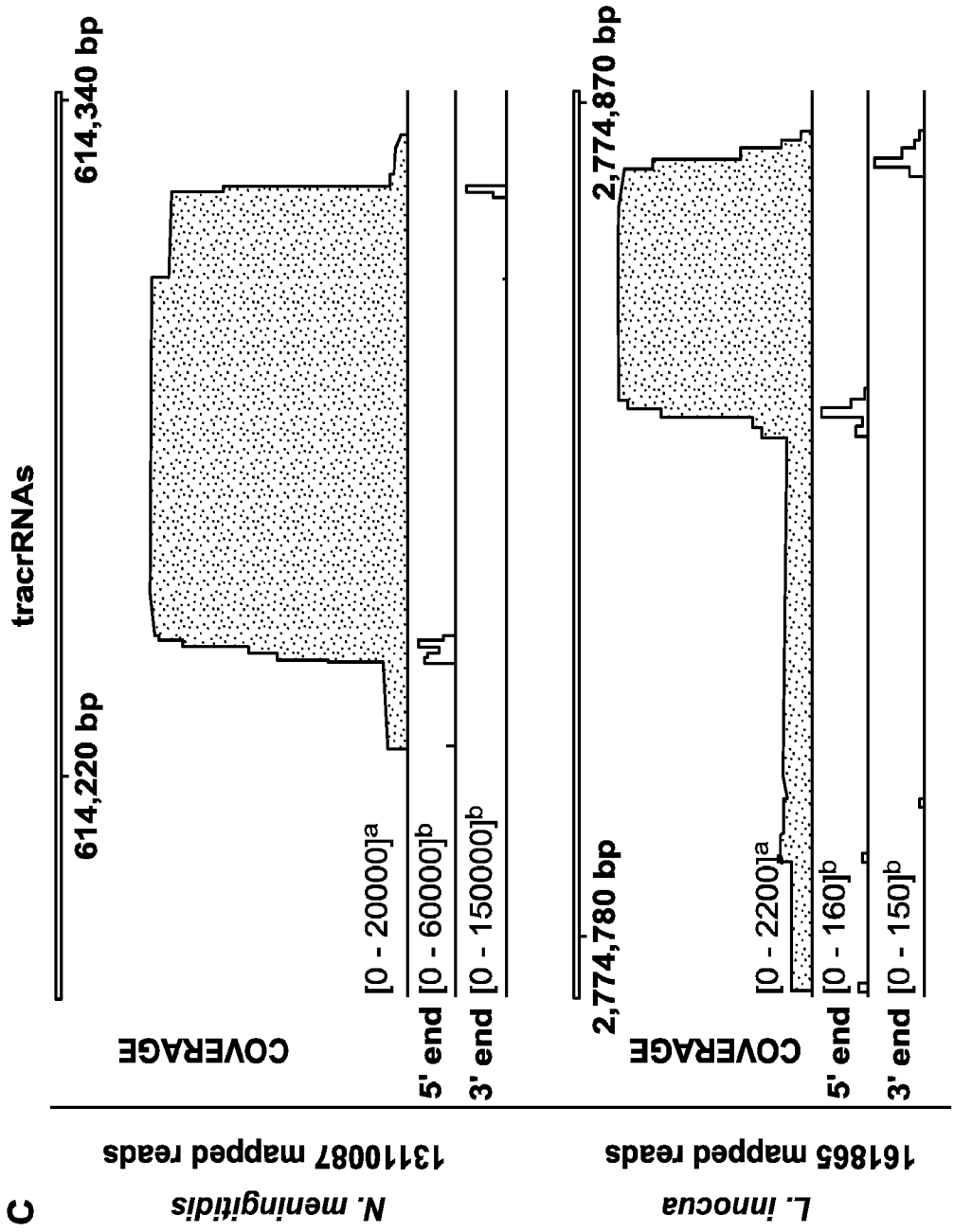

C
N. meningitidis
1311008? mapped reads
tracrRNAs
614,220 bp
614,340 bp
COVERAGE
[0 - 20000]a
5' end
[0 - 60000]b
3' end
[0 - 150000]b
L. innocua
161865 mapped reads
2,774,780 bp
2,774,870 bp
COVERAGE
[0 - 2200]a
5' end
[0 - 160]b
3' end
[0 - 150]b

FIG. 36 (Cont. 2)

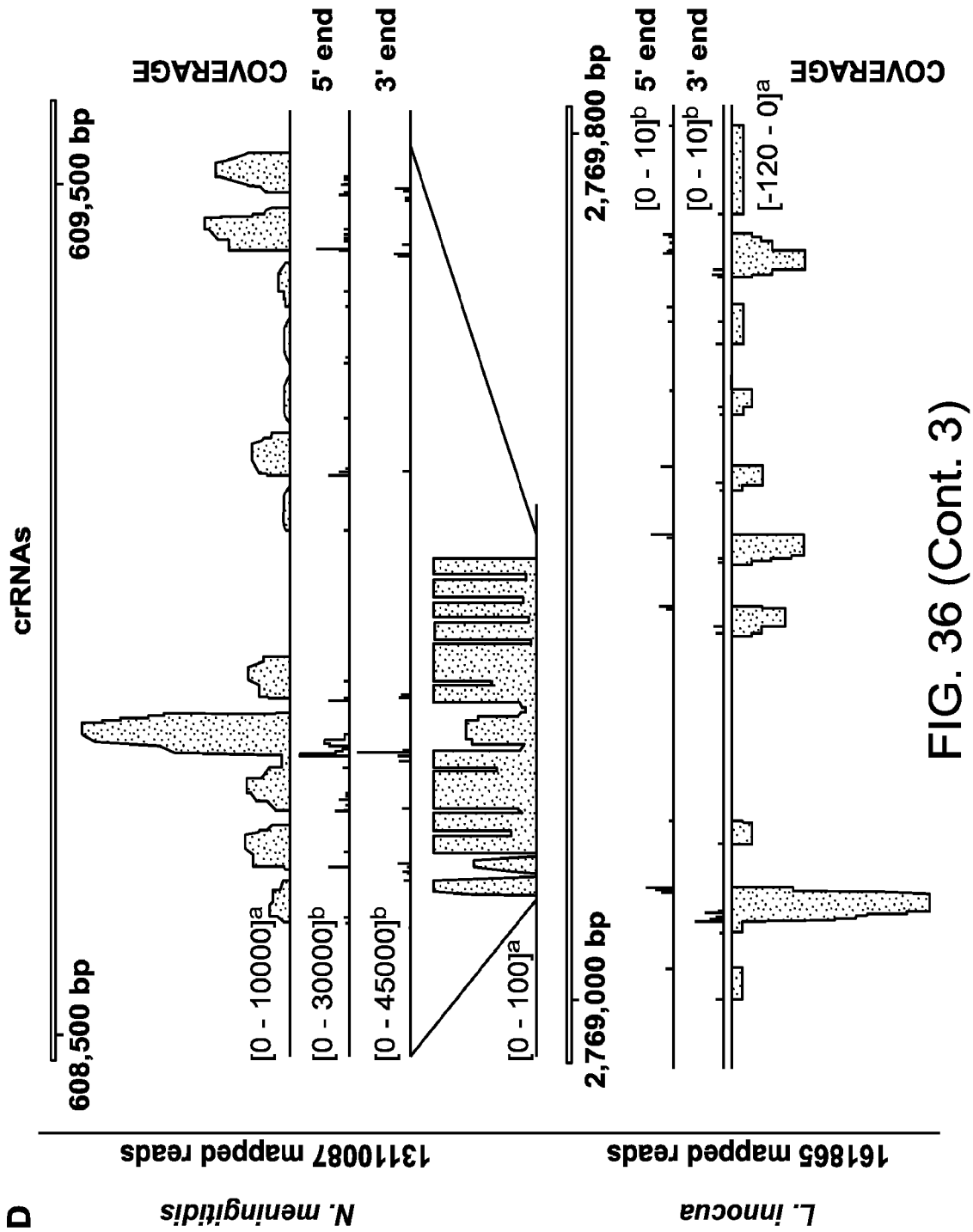

D
N. meningitidis
1311008? mapped reads
608,500 bp
609,500 bp
crRNAs
COVERAGE
[0 - 10000]a
5' end
[0 - 30000]b
3' end
[0 - 45000]b
[0 - 100]a
L. innocua
161865 mapped reads
2,769,000 bp
2,769,800 bp
COVERAGE
[0 - 10]b
5' end
[0 - 10]b
3' end
[-120 - 0]a

FIG. 36 (Cont. 3)

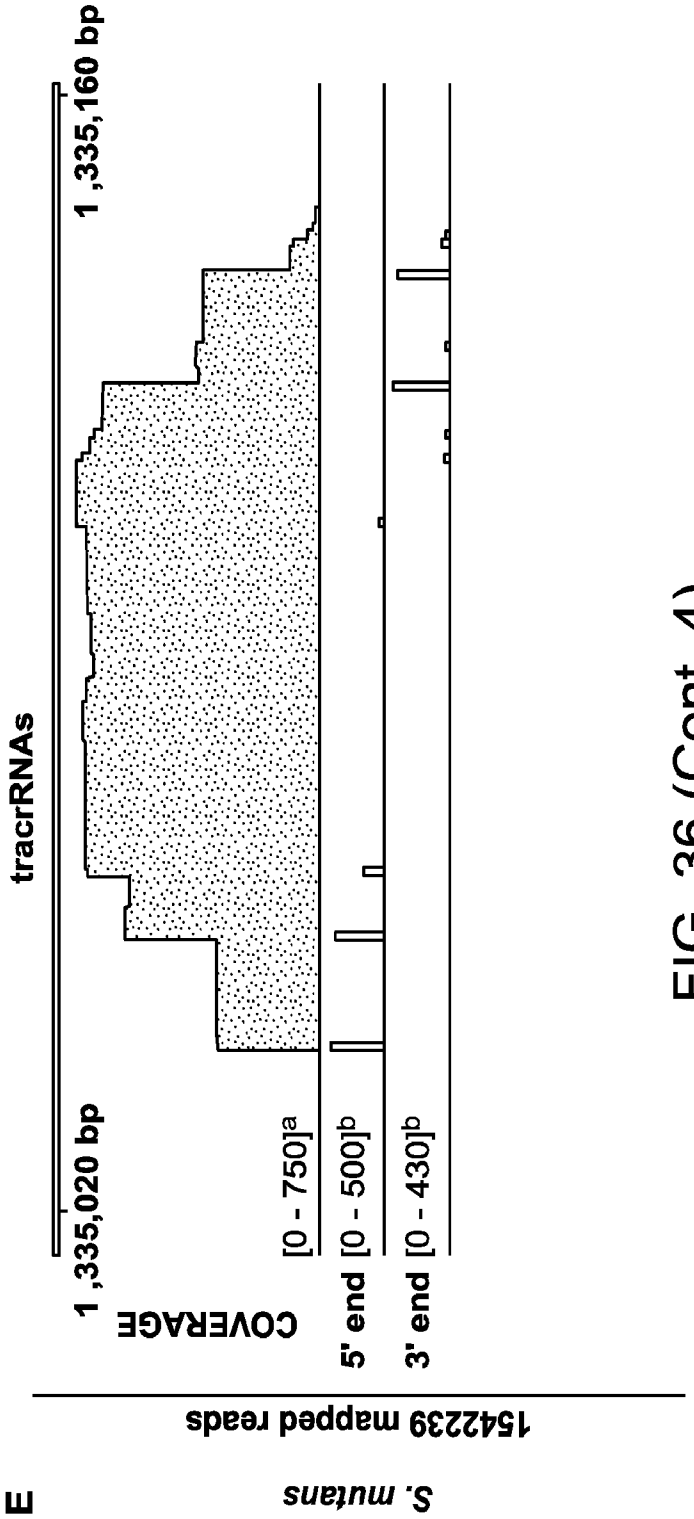

E
S. mutans
1542239 mapped reads
tracrRNAs
1 ,335,020 bp
1 ,335,160 bp
COVERAGE
[0 - 750]a
5' end [0 - 500]b
3' end [0 - 430]b

FIG. 36 (Cont. 4)

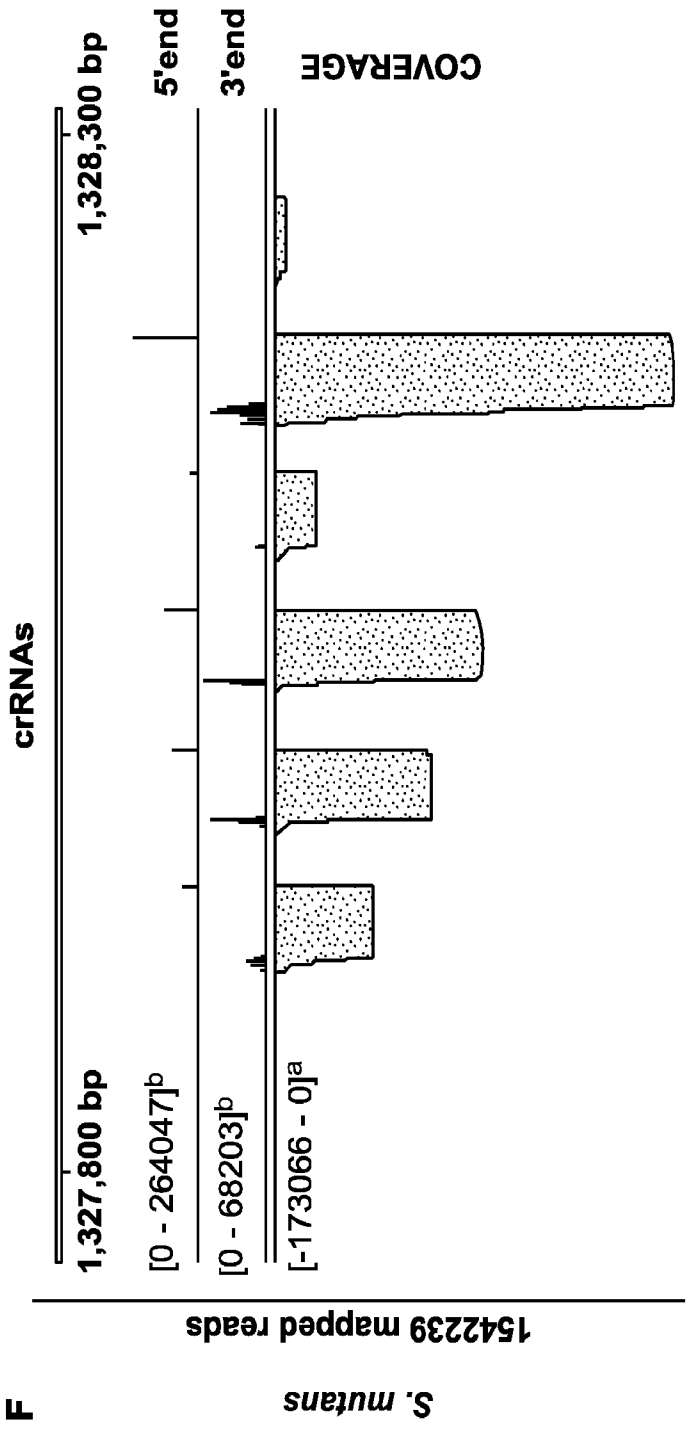

F
S. mutans
1542239 mapped reads
crRNAs
1,327,800 bp
1,328,300 bp
5'end
3'end
COVERAGE
[0 - 264047]b
[0 - 68203]b
[-173066 - 0]a

FIG. 36 (Cont. 5)

A

sRNA	Strand	Size mature form	Region of interest		Reads	Coverage (%)	Sequence	5' end read number		3' end read number	
C. jejuni NCTC 11168 (NC_002163.1), total mapped reads: 9914184											
crRNA 4 spacers	→	crRNA1	36	1455167 1455202	781	0,0079	AGTTTTTAAAAGAGCTTGGCGGTTGTTTTAGT CCCTTTTT	1455162	A	1455198	T
								1455165	G	1455199	C
								1455166	C	1455200	C
								1455167	A	1455201	C
								1455168	G	1455202	T
								1455169	T	1455203	T
								1455170	T	1455204	T
								1455171	T	1455205	T
								1455172	T	1455206	T
								1455173	T		
								1455174	A		
		crRNA2	38	1455231 1455268	2658	0,0268	CAAAGTTTCATTAGTTGAATTTAACTGTTTTA GTCCCTTTTT	1455227	T	1455264	T
			29	1455240 1455268				1455228	T	1455265	C
								1455230	C	1455266	C
								1455231	C	1455267	C
								1455232	A	1455268	T
								1455233	A	1455269	T
								1455234	A	1455270	T
								1455235	G	1455271	T
								1455236	T	1455272	T
								1455237	T	1455273	A
								1455238	T		
								1455239	C		
								1455240	A		
								1455241	T		
								1455242	T		
								1455243	A		
								1455244	G		

								1455162	A	1	1455198 T 5
								1455165	G	2	1455199 C 5
								1455166	C	3	1455200 C 16
								1455167	A	419	1455201 C 72
								1455168	G	19	1455202 T 385
								1455169	T	7	1455203 T 82
								1455170	T	75	1455204 T 30
								1455171	T	24	1455205 T 112
								1455172	T	9	1455206 T 5
								1455173	T	11	
								1455174	A	4	
		crRNA2	38	1455231 1455268	2658	0,0268	CAAAGTTTCATTAGTTGAATTTAACTGTTTTA GTCCCTTTTT	1455227	T	4	1455264 T 3
			29	1455240 1455268				1455228	T	1	1455265 C 14
								1455230	C	37	1455266 C 60
								1455231	C	206	1455267 C 240
								1455232	A	72	1455268 T 1364
								1455233	A	34	1455269 T 348
								1455234	A	59	1455270 T 29
								1455235	G	280	1455271 T 44
								1455236	T	168	1455272 T 15
								1455237	T	130	1455273 A 2
								1455238	T	36	
								1455239	C	259	
								1455240	A	317	
								1455241	T	148	
								1455242	T	298	
								1455243	A	157	
								1455244	G	40	

FIG. 37

B

sRNA	Strand	Size mature form	Region of interest	Reads	Coverage (%)	Sequence	5' end read number			3' end read number		
***C. jejuni* NCTC 11168 (NC_002163.1), total mapped reads: 9914184**												
crRNA 4 spacers		crRNA3	35 1455300 1455334	4729	0,0477	AAGAATGAGGATGATGATATTTTACAGTTTTA GTCCCTTTTT	1455292	T	16	1455329	G	21
							1455293	C	62	1455330	T	479
							1455294	T	5	1455331	C	23
							1455295	A	12	1455332	C	48
							1455296	C	23	1455333	C	179
							1455297	A	367	1455334	T	1226
							1455298	A	143	1455335	T	220
							1455299	G	563	1455336	T	54
							1455300	A	1014	1455337	T	49
							1455301	A	415	1455338	T	55
							1455302	T	242	1455339	A	1
							1455303	G	589			
							1455304	A	55			
							1455305	G	2			
		crRNA4	26 1455376 1455401	17404	0,1755	GTGTGCTAAAAAAAATGGACTTAAATGTTTTA GTCCCTTTTT	1455360	T	6	1455398	C	63
							1455361	G	2	1455399	C	645
							1455362	A	9	1455400	T	9844
							1455363	G	261	1455401	T	1518
							1455364	T	51	1455402	T	481
							1455365	G	598	1455403	T	763
							1455366	T	77	1455404	T	578
							1455367	G	552	1455405	A	4
							1455368	C	579			
							1455369	T	901			
							1455370	A	816			
							1455371	A	227			
							1455372	A	262			
							1455373	A	1072			
							1455374	A	1269			
							1455375	A	1991			
							1455376	A	2204			
							1455377	A	1978			
							1455378	T	371			

FIG. 37 (Cont. 1)

SUBSTITUTE SHEET (RULE 26)

C

sRNA	Strand	Size mature form	Region of interest	Reads	Coverage (%)	Sequence	5' end read number			3' end read number	
C. jejuni NCTC 11168 (NC_002163.1), total mapped reads: 9914184											
tracrRNA →	tracrRNA1	65	1455502 1455566	833829	8,4105	AAGAAATTTAAAAAGGGACTAAAATAAAGAGT	1455496	T	1	1455565	G 12371
	tracrRNA2	58	1455509 1455566			TTGCGGGACTCTGCGGGGTTACAATCCCCTAA	1455497	A	31	1455566	C 713292
						AACCGCTTTT	1455498	A	27	1455567	T 74594
							1455499	G	24	1455568	T 10412
							1455500	A	19	1455569	T 9580
							1455501	A	232	1455570	T 426
							1455502	A	435	1455571	A 91
							1455503	T	369	1455572	A 97
							1455504	T	253	1455573	A 542
							1455505	T	85		
							1455506	A	65		
							1455507	A	193		
							1455508	A	33472		
							1455509	A	615001		
							1455510	A	131879		
							1455511	G	16444		
							1455512	G	9390		
							1455513	G	1053		

FIG. 37 (Cont. 2)

D

sRNA	Strand	Size mature form	Region of interest	Reads	Coverage (%)	Sequence	5' end read number			3' end read number		
F. novicida U112 (NC_008601.1), total mapped reads: 48205												
crRNA 13 spacers	←	crRNA5	52 817556 817607	117	0,2427	ATAACTCGACCAATATTTGACAAAGTTTCAGT TGCTGAATTATTTGGTAAACCT	817612	T	10	817557	A	3
							817611	T	10	817556	C	55
							817607	A	53	817555	T	13
							817606	T	7	817553	C	12
										817552	T	1
		crRNA6	56 817627 817682	116	0,2406	GGCAGGTTTTGTATGGTCATATAGGAGTGTTT CAGTTGCTGAATTATTTGGTAAACCT	817685	A	4	817629	A	4
							817682	G	23	817628	A	3
							817681	G	13	817627	C	53
							817679	A	2	817626	T	20
							817677	G	4			
		crRNA7	66 817699 817764	11	0,0228	AGCTATAGGGTTACCTATCTTTTGAGTGTTGG CAAATAAGTTTCAGTTGCTGAATTATTTGGTA AACCT	817764	A	4	817699	A	2
							817763	G	1			
							817754	T	4			
							817745	T	2			
		crRNA9	53 817845 817897	24	0,0498	ATGCTTTTAAACTACTGATATATACGTTTCAG TTGCTGAATTATTTGGTAAACT	817897	A	3	817845	C	7
tracrRNA	←	tracrRNA1	74 817065 817138	2808	5,8251	GTACCAAATAATTAATGCTCTGTAATCATTTA AAAGTATTTTGAACGGACCTCTGTTTGACACG TCTGAATAACTAAAAA	817140	A	2	817066	A	28
		tracrRNA2	64 817065 817128				817139	T	2	817065	C	1523
							817138	G	615	817064	T	440
							817136	A	9	817063	A	7
							817135	C	2	817062	A	10
							817134	C	37	817061	A	19
							817133	A	26	817060	A	14
							817132	A	7	817059	A	6
							817131	A	328	817058	G	5
							817130	T	355	817057	C	32
							817129	A	165	817055	A	10
							817128	A	484			
							817127	T	24			
							817126	T	55			
							817125	A	32			
							817124	A	19			

FIG. 37 (Cont. 3)

SUBSTITUTE SHEET (RULE 26)

E

sRNA	Strand	Size mature form	Region of interest		Reads	Coverage (%)	Sequence	5' end read number		3' end read number	
N. meningitidis AZ2491 (NC_003116.1), total mapped reads: 13110087											
crRNA → 16 spacers	crRNA1	48	608456	608503	1346	0,0103	TATCCATTCCCAGCCGGAAATTAAGTTGTAGC TCCCTTTCTCATTTCGCAGT	608453	T	30 608500	T 25
								608454	G	105 608501	C 77
								608455	T	64 608502	G 175
								608456	A	259 608503	C 293
								608457	T	4 608504	A 78
								608458	C	21 608505	G 505
								608459	C	175 608507	T 1
								608460	A	118	
								608461	T	39	
	crRNA2	50	608520	608569	685	0,0052	GCCTTTTTACAAGCTCGCTTTCTTTGTTGTAG CTCCCTTTCTCATTTCGCAGT	608517	T	5 608564	T 8
								608518	C	41 608565	T 16
								608519	T	7 608566	T 8
								608520	G	61 608567	C 31
								608521	C	21 608568	G 101
								608522	C	44 608569	C 173
								608523	T	21 608570	A 43
								608524	T	31 608571	G 6
								608525	T	17 608572	T 2

FIG. 37 (Cont. 4)

F

sRNA	Strand	Size mature form	Region of interest		Reads	Coverage (%)	Sequence	5' end read number		3' end read number	
N. meningitidis AZ2491 (NC_003116.1), total mapped reads: 13110087											
	crRNA3	50	608586	608635	12402	0,0946	TAAAGGTTTCTGTTGCGACCCGAATGTTGTAG CTCCCTTTCTCATTTCGCAGT	608583	T	5 608631	T 75
								608584	G	3 608632	T 114
								608585	G	513 608633	C 414
								608586	T	2243 608634	G 1510
								608587	A	2091 608635	C 2219
								608588	A	188 608636	A 297
								608589	A	233 608637	G 52
								608590	G	744 608638	T 8
								608591	G	1152	
	crRNA4	49	608653	608701	26361	0,2011	TAACTTTGACCGTGTGCAATCCAGTTAGTTGT AGCTCCCTTTCTCATTTCGCAGT	608646	T	7 608697	T 295
								608647	C	127 608698	T 569
								608648	T	203 608699	C 1167
								608649	T	377 608700	G 4910
								608650	T	2751 608701	C 4968
								608651	A	477 608702	A 764
								608652	A	1334 608703	G 62
								608653	C	11694 608704	T 4
								608654	T	3665	
								608655	T	382	

FIG. 37 (Cont. 5)

G

sRNA	Strand	Size mature form	Region of interest		Reads	Coverage (%)	Sequence	5' end read number			3' end read number	
N. meningitidis AZ2491 (NC_003116.1), total mapped reads: 13110087												
	crRNA5	49	608719	608767	28747	0,2193	AACCCACTAAATTTTGCAAATGCGGTTGTAGC TCCCTTTCTCATTTCGCAGT	608717	G	20	608764	T 426
								608718	C	1171	608765	C 1316
								608719	A	8128	608766	G 3040
								608720	A	116	608767	C 6431
								608721	C	59	608768	A 1049
								608722	C	420	608769	G 102
								608723	C	1089	608770	T 11
								608724	A	557		
	crRNA6	50	608784	608833	121014	0,9231	TTTTTTTGTACTGTTGTTTGAACGAGTTGTAG CTCCCTTTCTCATTTCGCAGT	608781	G	42	608830	T 2295
								608782	C	630	608831	C 7060
								608783	C	10039	608832	G 13864
								608784	T	26026	608833	C 44472
								608785	T	11430	608834	A 6853
								608786	T	8248	608835	G 802
								608787	T	3648	608836	T 69
	crRNA7	52	608848	608899	24611	0,1877	TTCGTTTCAGATAGCAAACGCAGTAGTGTTGT AGCTCCCTTTCTCATTTCGCAGT	608846	A	28	608896	T 369
								608847	A	631	608897	C 1114
								608848	T	11039	608898	G 3441
								608849	T	753	608899	C 9015
								608850	C	239	608900	A 1558
								608851	G	2205	608901	G 137
								608852	T	1910	608902	T 8
								608853	T	826		
		84	608916	608999	901	0,0069	ATATGACGGTGGGCAACTGGTACAGGTTGTAG CTCCCTTTCTCATTTCGCAGTGCTACAATGCC GGATATGACGGTGGGCAACT	608913	C	6	608996	A 6
								608914	G	3	608997	A 2
								608915	G	2	608998	C 2
								608916	A	266	608999	T 259
								608917	T	17	609000	G 171
								608918	A	96	609001	G 24
								608919	T	26	609004	T 16

FIG. 37 (Cont. 6)

H

sRNA	Strand	Size mature form	Region of interest		Reads	Coverage (%)	Sequence	5' end read number			3' end read number	
N. meningitidis AZ2491 (NC_003116.1), total mapped reads: 13110087												
	crRNA10	49	609049	609097	5027	0,0383	CTTTTGTATTGATTCAAGGTGCTTGTTGTAGC TCCCTTTCTCATTTCGCAGT	609046	T	89	609094	T 81
								609047	C	37	609095	C 218
								609048	G	647	609096	G 845
								609049	C	1614	609097	C 1237
								609050	T	583	609098	A 219
								609051	T	208	609099	G 36
								609052	T	104	609101	G 7
								609053	T	18		
	crRNA11	52	609112	609163	22711	0,1732	ATTCGTCGATGATGGAAACTCGAGCATGTTGT AGCTCCCTTTCTCATTTCGCAGT	609109	A	58	609160	T 540
								609110	G	331	609161	C 1263
								609111	T	99	609162	G 2850
								609112	A	10627	609163	C 4882
								609113	T	1234	609164	A 552
								609114	T	66	609165	G 52
								609115	C	191	609166	T 15
								609116	G	4906		
								609117	T	136		
	crRNA12	52	609178	609229	5067	0,0386	TAGCCAGTGCTAAAACCGCACCCGCTTGTTGT AGCTCCCTTTCTCATTTCGCAGT	609175	G	4	609226	T 57
								609176	G	3	609227	C 189
								609177	G	42	609228	G 576
								609178	T	897	609229	C 1348
								609179	A	72	609230	A 321
								609180	G	584	609231	G 33
								609181	C	154	609232	T 2
								609182	C	314		
								609183	A	38		

FIG. 37 (Cont. 7)

I

sRNA	Strand	Size mature form	Region of interest		Reads	Coverage (%)	Sequence	5' end read number			3' end read number	
N. meningitidis AZ2491 (NC_003116.1), total mapped reads: 13110087												
	crRNA13	51	609245	609295	4666	0,0356	ATAGAAATACATACGCCGAGTAATTAGTTGTA GCTCCCTTTCTCATTTCGCAGT	609243	A	6	609292	T 65
								609244	A	62	609293	C 201
								609245	A	1311	609294	G 475
								609246	T	141	609295	C 1039
								609247	A	116	609296	A 229
								609248	G	44	609297	G 25
											609298	T 1
	crRNA14	51	609311	609361	7147	0,0545	TTTTTGTAATTGTTCTGCCTTTTTTAGTTGTA GCTCCCTTTCTCATTTCGCAGT	609308	T	12	609358	T 136
								609309	T	22	609359	C 442
								609310	C	207	609360	G 858
								609311	T	1190	609361	C 2335
								609312	T	774	609362	A 540
								609313	T	577	609363	G 69
								609314	T	195	609364	T 5
								609315	T	37		
	crRNA15	50	609378	609427	49818	0,3800	ACGGCGGAAACCATTGCCACAAAACGTTGTAG CTCCCTTTCTCATTTCGCAGT	609375	C	319	609424	T 532
								609376	C	7253	609425	C 1414
								609377	C	7249	609426	G 2553
								609378	A	19015	609427	C 7448
								609379	C	547	609428	A 1854
								609380	G	307	609429	G 210
								609381	G	190	609430	T 10

FIG. 37 (Cont. 8)

J

sRNA	Strand	Size mature form	Region of interest	Reads	Coverage (%)	Sequence	5' end read number	3' end read number
N. meningitidis AZ2491 (NC_003116.1), total mapped reads: 13110087								
	crRNA16	50 609444	609493	42398	0,3234	AATAATAACCCAATACACGATGTTAAGTTGTA GCTCCCTTTCTCATTTCGCAGT	609441 A 8 609490 T 579 609442 A 548 609491 C 2197 609443 A 3686 609492 G 6466 609444 A 4638 609493 C 13217 609445 T 1428 609494 A 2438 609446 A 2861 609495 G 200 609447 A 1322 609496 T 12 609448 T 287	
tracrRNA →	tracrRNA1 163 614162		614324	208318	1,5890	TTGTCTTATTTATATACAAATAGATTATTGAC	614158 C 1 614319 G 311	
	tracrRNA2 100 614225		614324			TTATCATCTCACAACGGCTAGAATCCCAAACA	614161 A 1 614320 G 109	
	tracrRNA3 82 614243		614324			TATTGTCGCACTGCGAAATGAGAACCGTTGCT	614162 T 2 614321 G 258	
						ACAATAAGGCCGTCTGAAAAGATGTGCCGCAA	614164 G 1 614322 G 150	
						CGCTCTGCCCCTTAAAGCTTCTGCTTTAAGGG	614223 A 596 614323 C 41244	
						GCATCGTTTAT	614224 C 39 614324 A 128531	
							614225 A 14761 614325 T 167	
							614226 T 169 614326 C 4197	
							614227 A 276 614327 G 36	
							614239 C 208 614328 T 122	
							614240 G 42037 614329 T 865	
							614241 A 39965 614330 T 1132	
							614242 A 22890 614331 A 1061	
							614243 A 51186 614332 T 2788	
							614244 T 17711 614333 T 3758	
							614245 G 4192 614334 T 77	
							614246 A 488 614335 C 18	
							614336 G 3	
							614337 G 3	
							614338 T 1	

FIG. 37 (Cont. 9)

K

sRNA	Strand	Size mature form	Region of interest	Reads	Coverage (%)	Sequence	5' end read number			3' end read number		
L. innocua Clip11262 (NC_003212.1), total mapped reads: 161865 (Note: low quality of the RNA library)												
crRNA ← 10 spacers		crRNA1	35 2769606 2769640	2	0,0012	GGTAACTTTGCCTAGGATAGTTTTAGAGCTATGTT	2769640	G	1	2769606	T	1
		crRNA2	22 2769540 2769561	2	0,0012	CATTATGTTTTAGAGCTATGTT	2769561 2769560	C A	1 1	2769540	T	1
		crRNA3	24 2769468 2769491	3	0,0019	GAGTTTTAGAGCTATGTTATTTTG	2769491	G	3	2769468	G	1
		crRNA4	27 2769402 2769428	7	0,0043	TTATAGTTTTAGAGCTATGTTATTTTG	2769428 2769427	T T	5 2	2769407 2769406 2769405 2769403 2769402	A T T T G	1 1 1 1 1
		crRNA5	26 2769337 2769362	5	0,0031	TAAATGTTTTAGAGCTATGTTATTTT	2769362 2769360	T A	3 2	2769339 2769337	T T	1 2
		crRNA8	23 2769142 2769164	2	0,0012	TACAAGTTTTAGAGCTATGTTAT	2769164 3769163	T A	1 1	2769143 2769142	A T	1 1
		crRNA9	30 2769072 2769101	19	0,0017	TTCATGTTGTTTTAGAGCTATGTTATTTTG	2769101 2769100 2769099 2769098 2769097 2769096	T T C A T G	6 1 4 3 3 2	2769079 2769078 2769075 2769073 2769072	T T T T G	4 1 2 3 6
		crRNA10	28 2769000 2769027	19	0,0017	GTTTTAGAGCTATGCTATTTCGAATACT	2769027	G	1	2769000	T	1

FIG. 37 (Cont. 10)

SUBSTITUTE SHEET (RULE 26)

L

sRNA	Strand	Size mature form	Region of interest	Reads	Coverage (%)	Sequence	5' end read number		3' end read number	
L. innocua Clip11262 (NC_003212.1), total mapped reads: 161865 (Note: low quality of the RNA library)										
tracrRNA →	tracrRNA1	90	2774774 2774863	367	0,2267	ATTGTTAGTATTCAAAATAACATAGCAAGTTA	2774774	A 34	2774861	T 2
	tracrRNA2	76	2774788 2774863			AAATAAGGCTTTGTCCGTTATCAACTTTTAAT	2774783	A 1	2774862	T 47
	tracrRNA3	68	2774796 2774863			TAAGTAGCGCTGTTTCGGCGCTTTTTTT	2774786	C 1	2774863	T 150
							2774787	A 1	2774864	T 67
							2774788	A 22	2774865	T 30
							2774794	C 1	2774866	G 15
							2774795	A 1	2774867	T 6
							2774796	T 5		
							2774797	A 2		
							2774799	C 5		
							2774801	A 1		

FIG. 37 (Cont. 11)

M

sRNA	Strand	Size mature form	Region of interest	Reads	Coverage (%)	Sequence	5' end read number		3' end read number	
S. mutans UA159 (NC_004350.2), total mapped reads: 1542239										
crRNA 5 spacers	←	crRNA1	38 1328162 1328199	267104	17,3192	GCCATTAATTAATATGGTGAGTTTTAGAGCTG TGTTGTTTCGA	1328201	A 8	1328166	G 18547
							1328200	C 178	1328165	T 41345
							1328199	G 264047	1328164	T 53386
							1328198	C 191	1328163	G 9084
							1328197	C 167	1328162	T 59197
							1328196	A 117	1328161	T 28333
									1328160	T 4240
									1328159	C 18236
									1328158	G 26742
									1328157	A 5573
									1328156	A 17
		crRNA2	36 1328098 1328133	26578	1,7233	GCTAGCGCAGTTAGTGCTCTGTTTTAGAGCTG TGTTGTTTCGA	1328135	C 4	1328101	T 37
							1328134	A 13	1328100	G 800
							1328133	G 25656	1328099	T 6395
							1328132	C 212	1328098	T 11256
							1328131	T 25	1328097	G 301
							1328130	A 62	1328096	T 1453
							1328129	G 25	1328095	T 1755
									1328094	T 447
									1328093	C 1302
									1328092	G 1996
									1328091	A 670
									1328090	A 5

FIG. 37 (Cont. 12)

N

sRNA	Strand	Size mature form	Region of interest	Reads	Coverage (%)	Sequence	5' end read number			3' end read number	
S. mutans UA159 (NC_004350.2), total mapped reads: 1542239											
	crRNA3	34	1328034 1328134	138067	8,9567	TGTTGTGTCATCATAGTTAGGTTTTAGAGCTG TGTTGTTTCGA	1328069	G	4	1328036	G 2880
							1328068	C	66	1328035	T 733
							1328067	T	134361	1328034	G 68203
							1328066	G	609	1328033	T 37212
							1328065	T	321	1328032	T 24528
										1328031	G 1003
										1328030	T 889
										1328029	T 610
										1328028	T 124
										1328027	C 371
										1328026	G 546
										1328025	A 145
	crRNA4	35	1327967 1328001	104705	6,7892	CAATTAGACAATAGACAAACGTTTTAGAGCTG TGTTGTTTCGA	1328003	T	8	1328969	T 348
							1328002	T	36	1328968	G 8606
							1328001	C	101017	1328967	T 59863
							1328000	A	681	1328966	T 25845
							1327999	A	216	1328965	G 756
										1328964	T 3053
										1328963	T 1433
										1328962	T 1602
										1328961	C 864
										1328960	G 839
										1328959	A 322

FIG. 37 (Cont. 13)

O

sRNA	Strand	Size mature form	Region of interest		Reads	Coverage (%)	Sequence	5' end read number			3' end read number		
S. mutans UA159 (NC_004350.2), total mapped reads: 1542239													
	crRNA5	37	1327899	1327935	63999	4,1497	TTCGGACATGACTTGCCACAGTTTTAGAGCTG TGTTGTTATCGA	1327940	A	4	1327902	G	902
								1327937	T	6	1327901	T	3296
								1327936	A	17	1327900	T	12381
								1327935	T	62587	1327899	G	19843
								1327934	T	1029	1327898	T	2864
								1327933	C	108	1327897	T	14180
								1327932	G	19	1327896	T	2061
								1327931	G	36	1327895	C	2079
											1327894	G	5259
											1327893	A	630
tracrRNA →	tracrRNA1	102	1335040	1335141	1299	0,0842	GTTGGAATCATTCGAAACAACACAGCAAGTTA	1335038	G	1	1335140	T	13
	tracrRNA2	88	1335054	1335141			AAATAAGGCAGTGATTTTTAATCCAGTCCGTA	1335040	G	466	1335141	T	64
	tracrRNA3	80	1335062	1335141			CACAACTTGAAAAAGTGCGCACCGATTCGGTG	1335041	T	6	1335142	T	29
							CTTTTTTATTT	1335042	T	1	1335143	A	13
								1335051	T	4	1335144	T	6
								1335053	G	3	1335145	T	13
								1335054	A	415	1335146	T	1
								1335055	A	2	1335149	T	1
								1335057	C	1			
								1335058	A	1			
								1335062	C	186			
								1335063	A	15			

FIG. 37 (Cont. 14)

A

Cluster	SEQ ID NO:
1	2, 3, 4, 5, 6, 7, 8, 15, 23, 24, 25, 36, 37, 38, 39, 41, 71, 74, 105, 116, 136, 138, 166, 177, 180, 183, 193, 204, and 232
2	83, 75, 156, 96, 121, 235, 208, 127, 182, 134, 119, 246, 153, 202
3	101, 168, 48, 226, 216, 210, 120, 102, 176, 57, 108, 79, 1, 245
4	219, 135, 53, 62, 240, 165, 217, 82, 212, 19, 40, 18, 194
5	84, 21, 150, 221, 111, 76, 47, 59, 77, 112, 198, 147
6	90, 91, 214, 92, 152, 98, 243, 197, 32, 227, 162
7	103, 187, 223, 151, 158, 126
8	88, 167, 13, 164, 184, 123
9	58, 73, 195, 148, 31, 33
10	206, 188, 211, 161, 205, 44

Cluster	SEQ ID NO:
11	50, 54, 78, 106, 174
12	209, 220, 146, 157
13	70, 154, 100, 117
14	128, 144, 118, 129
15	131, 66, 149, 145
16	89, 169, 163
17	141, 49, 72
18	196, 114, 86

Cluster	SEQ ID NO:
19	55, 27, 215
20	228, 234
21	160, 213
22	207, 237
23	230, 94
24	200, 247
25	133, 143
26	64, 68
27	20, 45
28	60, 56
29	99, 52

FIG. 38

B

Cluster	SEQ ID NO:
30	244, 185
31	43
32	189
33	170
34	11
35	107
36	14
37	236
38	12
39	17
40	239
41	61
42	85
43	191
44	22

Cluster	SEQ ID NO:
45	233
46	122
47	16
48	242
49	203
50	26
51	137
52	199
53	34
54	201
55	178
56	42
57	190
58	81

Cluster	SEQ ID NO:
59	218
60	65
61	171
62	97
63	63
64	46
65	225
66	10
67	173
68	51
69	142
70	69
71	28
72	139
73	80
74	172
75	115
76	229
77	175
78	181

FIG. 38 (Cont.)

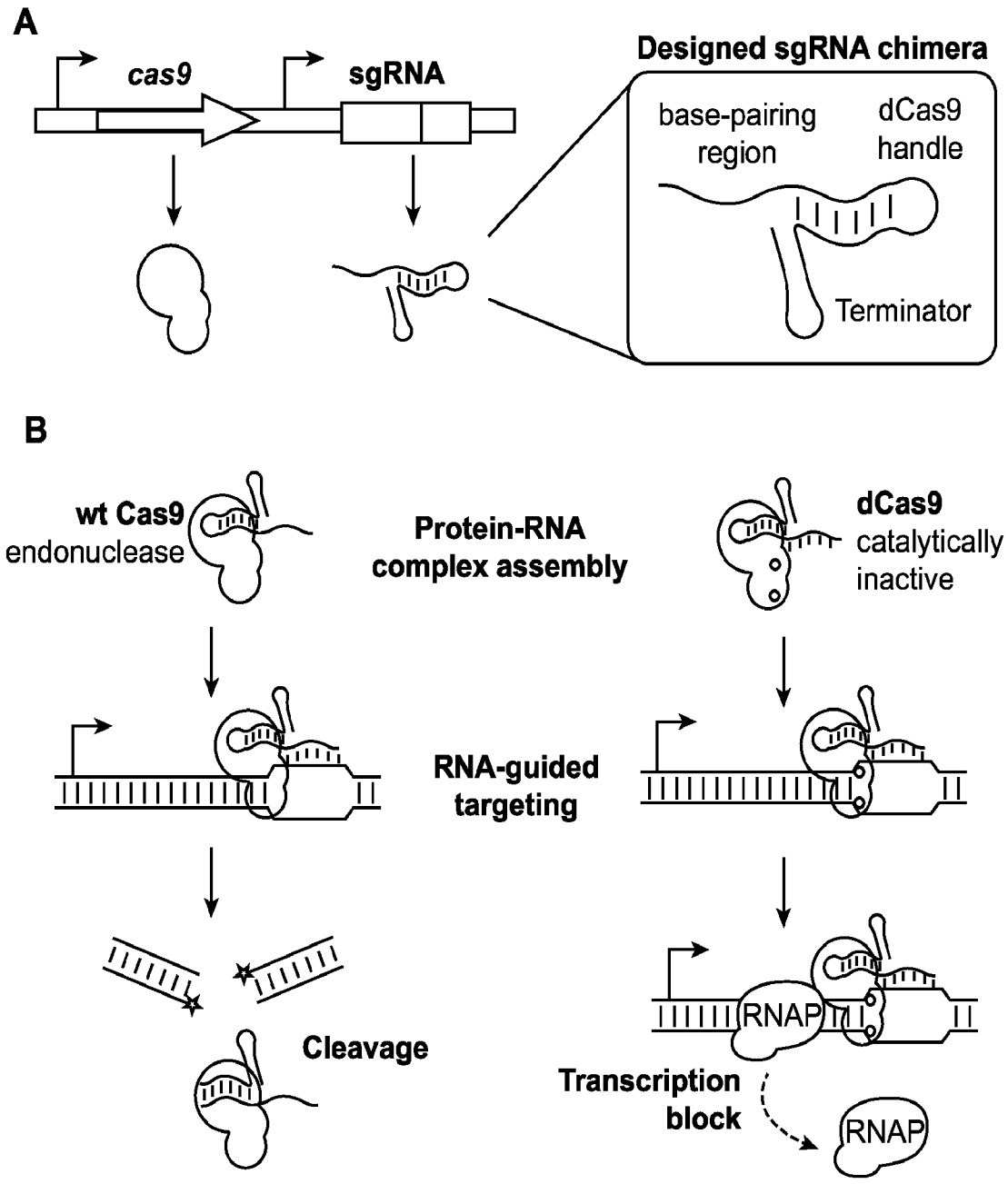

A
cas9
sgRNA
Designed sgRNA chimera
base-pairing region
dCas9 handle
Terminator
B
wt Cas9 endonuclease
Protein-RNA complex assembly
dCas9 catalytically inactive
RNA-guided targeting
Cleavage
RNAP
Transcription block
RNAP

FIG. 39

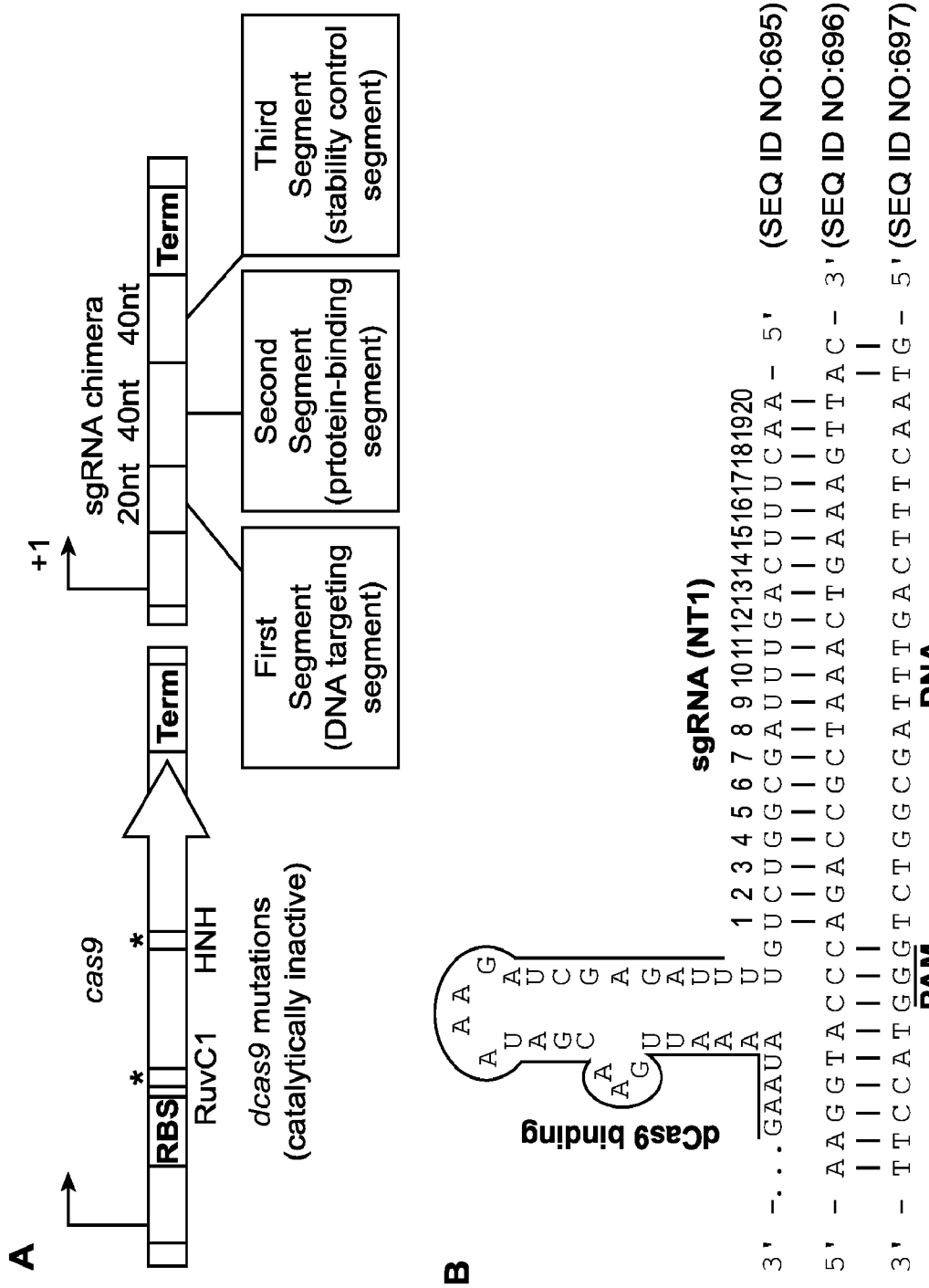

A
cas9
RBS
RuvC1
HNH
dcas9 mutations
(catalytically inactive)
Term
Term
+1
sgRNA chimera
20nt 40nt 40nt
First Segment (DNA targeting segment)
Second Segment (prtotein-binding segment)
Third Segment (stability control segment)
B
dCas9 binding
sgRNA (NT1)
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
3' -. . . GAAUA U GUCCUGGCGAUUUGACUUUUCAA - 5' (SEQ ID NO:695)
5' - AAGGTACCCAGACCGCTAAAACTGAAAAGTTAC - 3' (SEQ ID NO:696)
3' - TTCCATGGGTCTGGCGATTTGACTTTTCAATG - 5' (SEQ ID NO:697)
PAM
DNA

FIG. 40

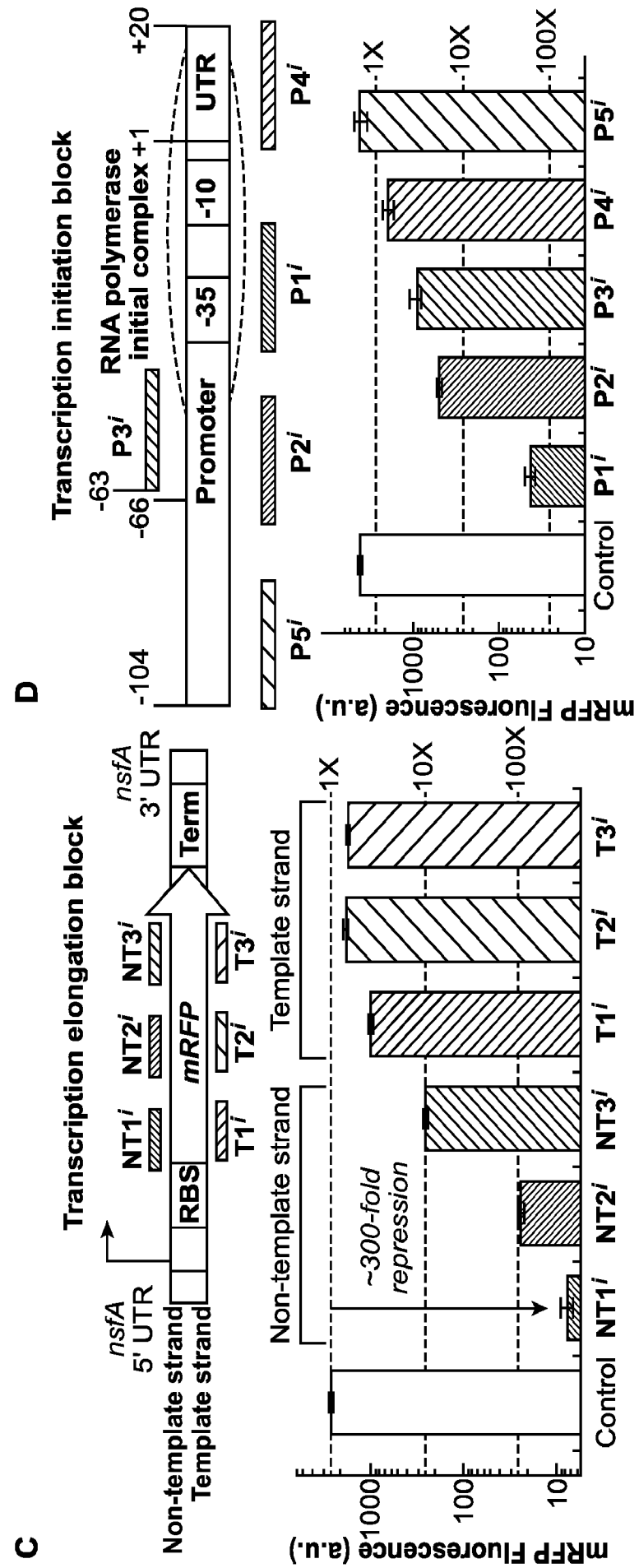

C
Transcription elongation block
nsfA 5' UTR
Non-template strand
Template strand
RBS
mRFP
nsfA 3' UTR
Term
NT1i
NT2i
NT3i
T1i
T2i
T3i
Non-template strand
Template strand
1X
10X
100X
~300-fold repression
Control
mRFP Fluorescence (a.u.)
1000
100
10
D
Transcription initiation block
-63
-66
-104
+1
+20
P3i
RNA polymerase initial complex
Promoter
-35
-10
UTR
P1i
P2i
P4i
P5i
Control
1X
10X
100X

FIG. 40 (Cont. 1)

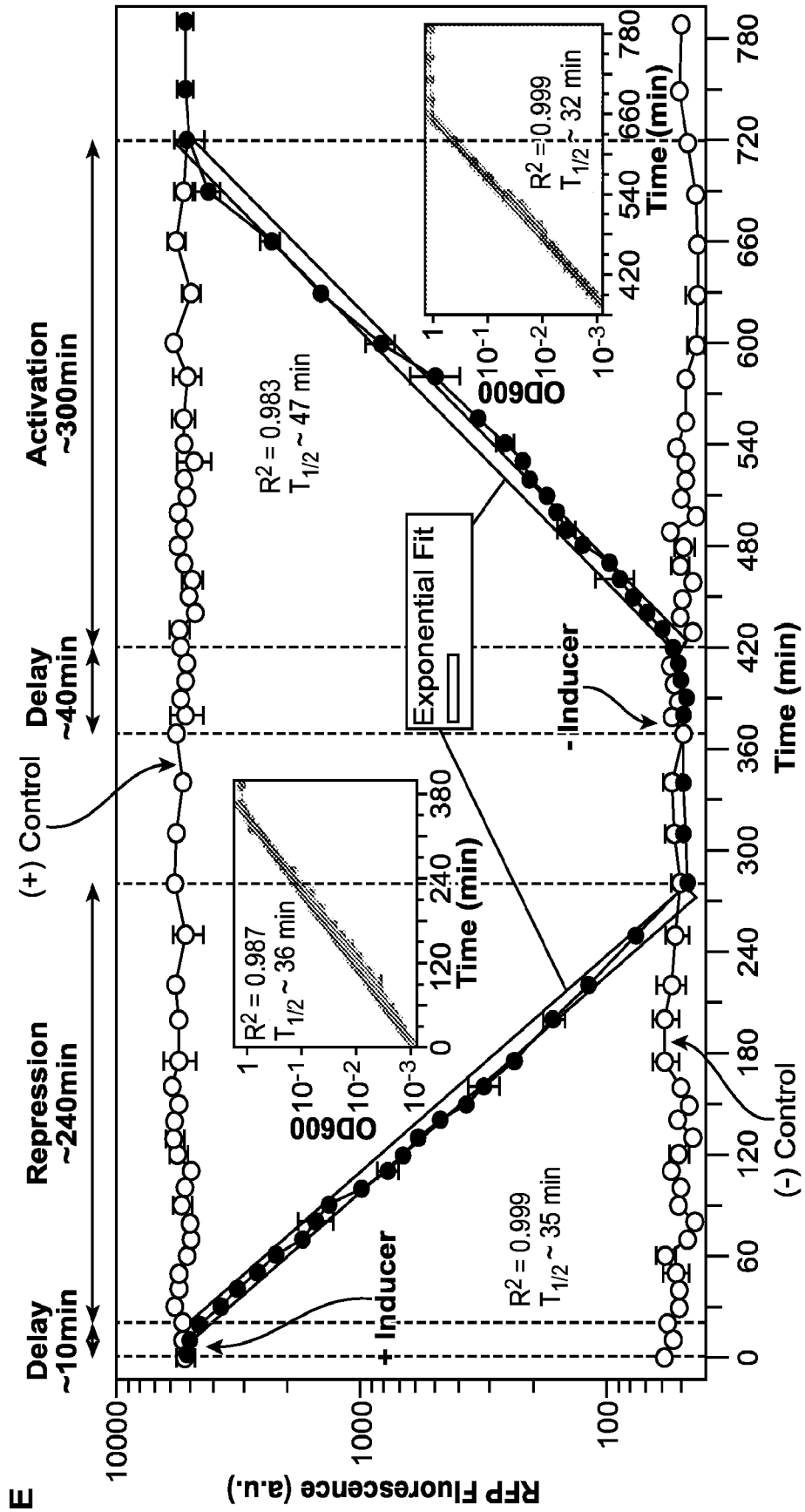
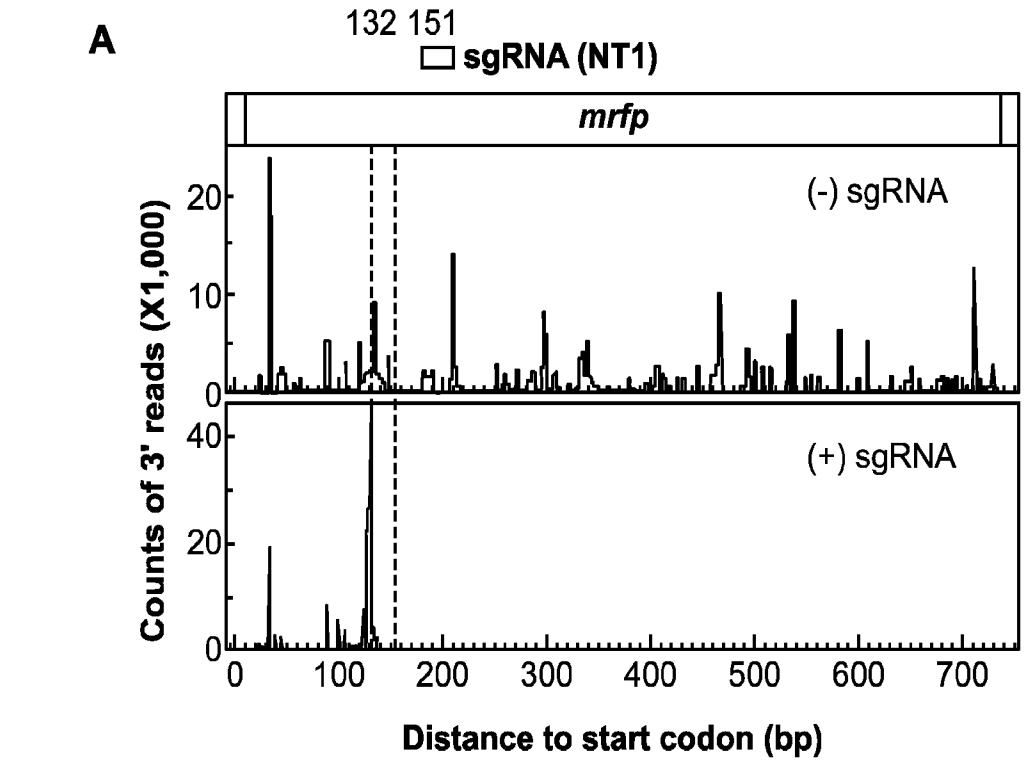

E
RFP Fluorescence (a.u.)
10000
1000
100
Time (min)
0
60
120
180
240
300
360
420
480
540
600
660
720
780
Delay ~10min
Repression ~240min
Delay ~40min
Activation ~300min
(+) Control
(-) Control
+ Inducer
- Inducer
Exponential Fit
R2 = 0.983
T1/2 ~ 47 min
R2 = 0.999
T1/2 ~ 35 min
R2 = 0.987
T1/2 ~ 36 min
R2 = 0.999
T1/2 ~ 32 min
OD600
1
10-1
10-2
10-3
Time (min)
0
120
240
380
420
540
660
780

FIG. 40 (Cont. 2)


A
132 151
sgRNA (NT1)
mrfp
(-) sgRNA
(+) sgRNA
Counts of 3' reads (X1,000)
20
10
0
40
20
0
0
100
200
300
400
500
600
700
Distance to start codon (bp)

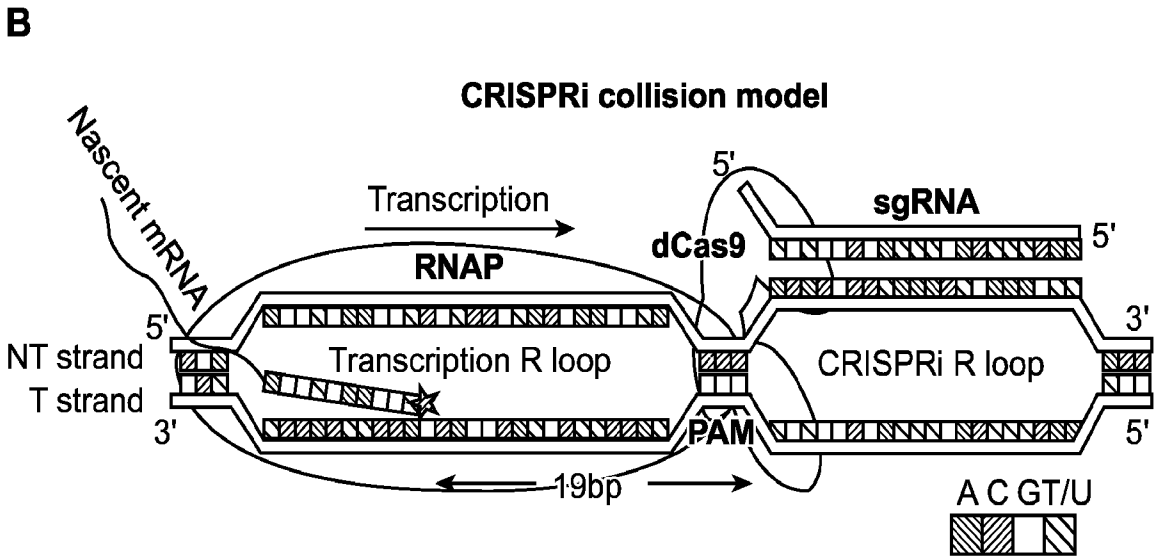

B
CRISPRi collision model
Nascent mRNA
Transcription
RNAP
dCas9
sgRNA
5'
3'
NT strand
T strand
Transcription R loop
CRISPRi R loop
PAM
19bp
A C GT/U

FIG. 41

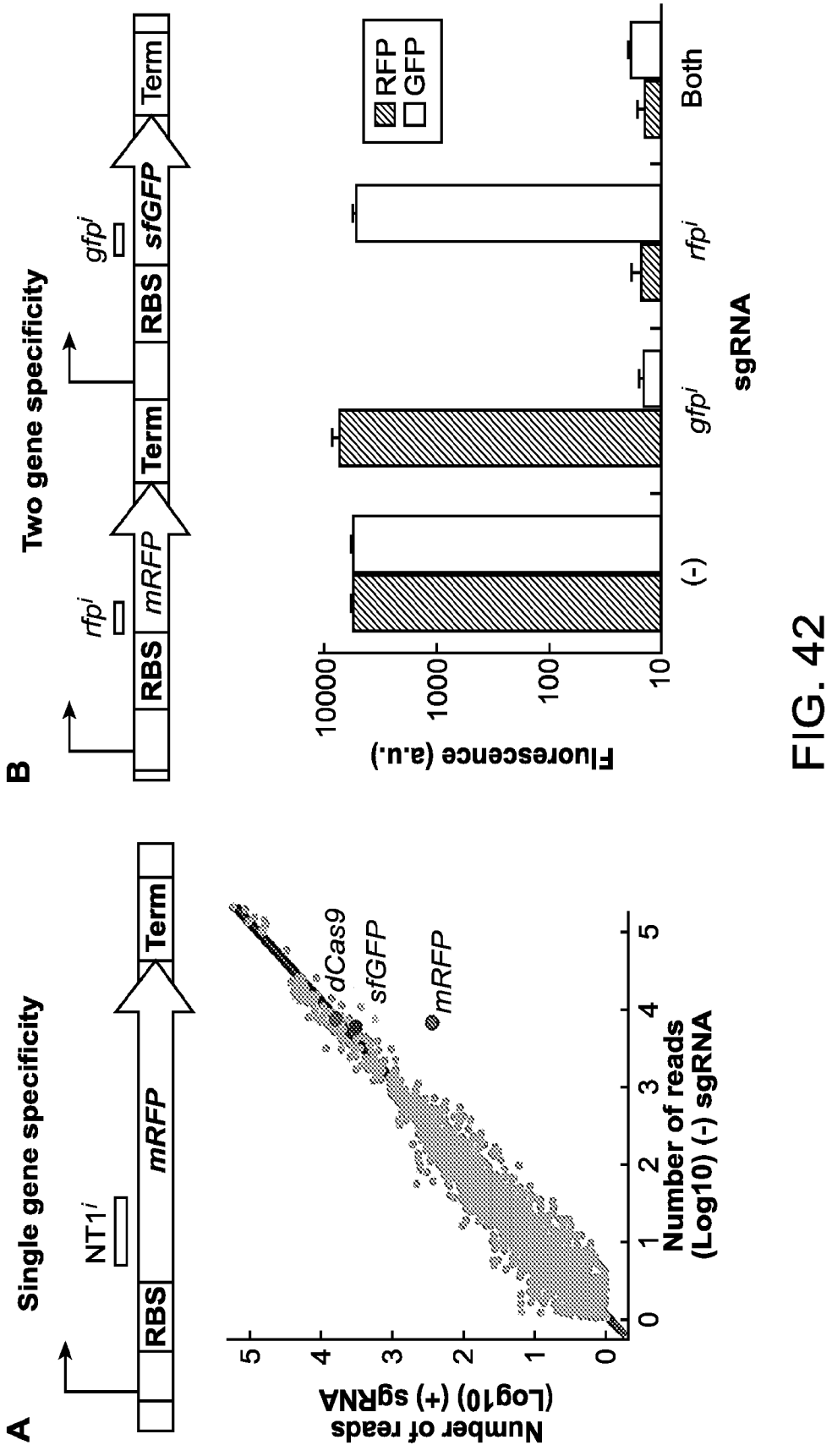

A
Single gene specificity
NT1i
RBS
mRFP
Term
Number of reads (Log10) (+) sgRNA
Number of reads (Log10) (-) sgRNA
dCas9
sfGFP
mRFP
B
Two gene specificity
rfpi
gfpi
RBS
mRFP
Term
RBS
sfGFP
Term
Fluorescence (a.u.)
10000
1000
100
10
RFP
GFP
(-)
gfpi
rfpi
Both
sgRNA

FIG. 42

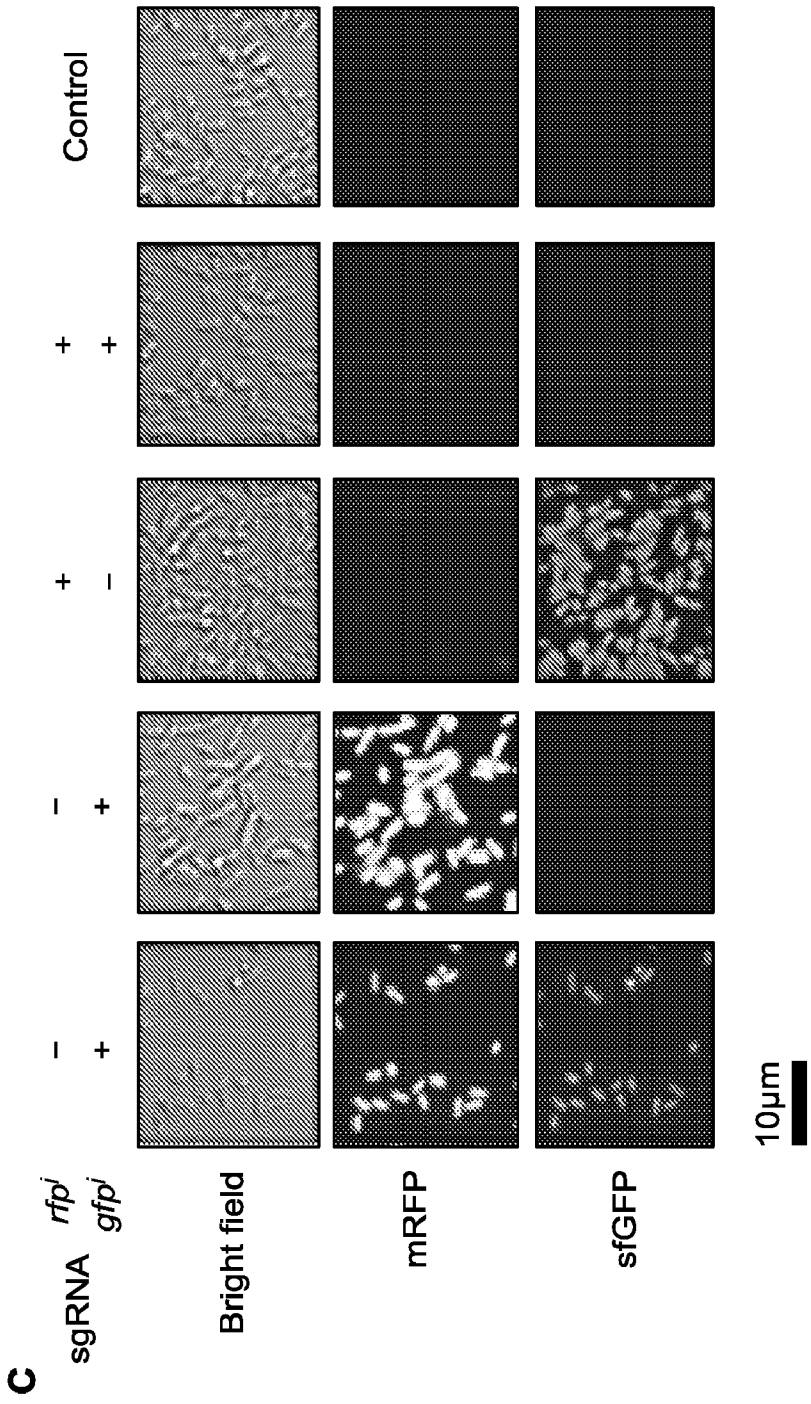


FIG. 42 (Cont.)

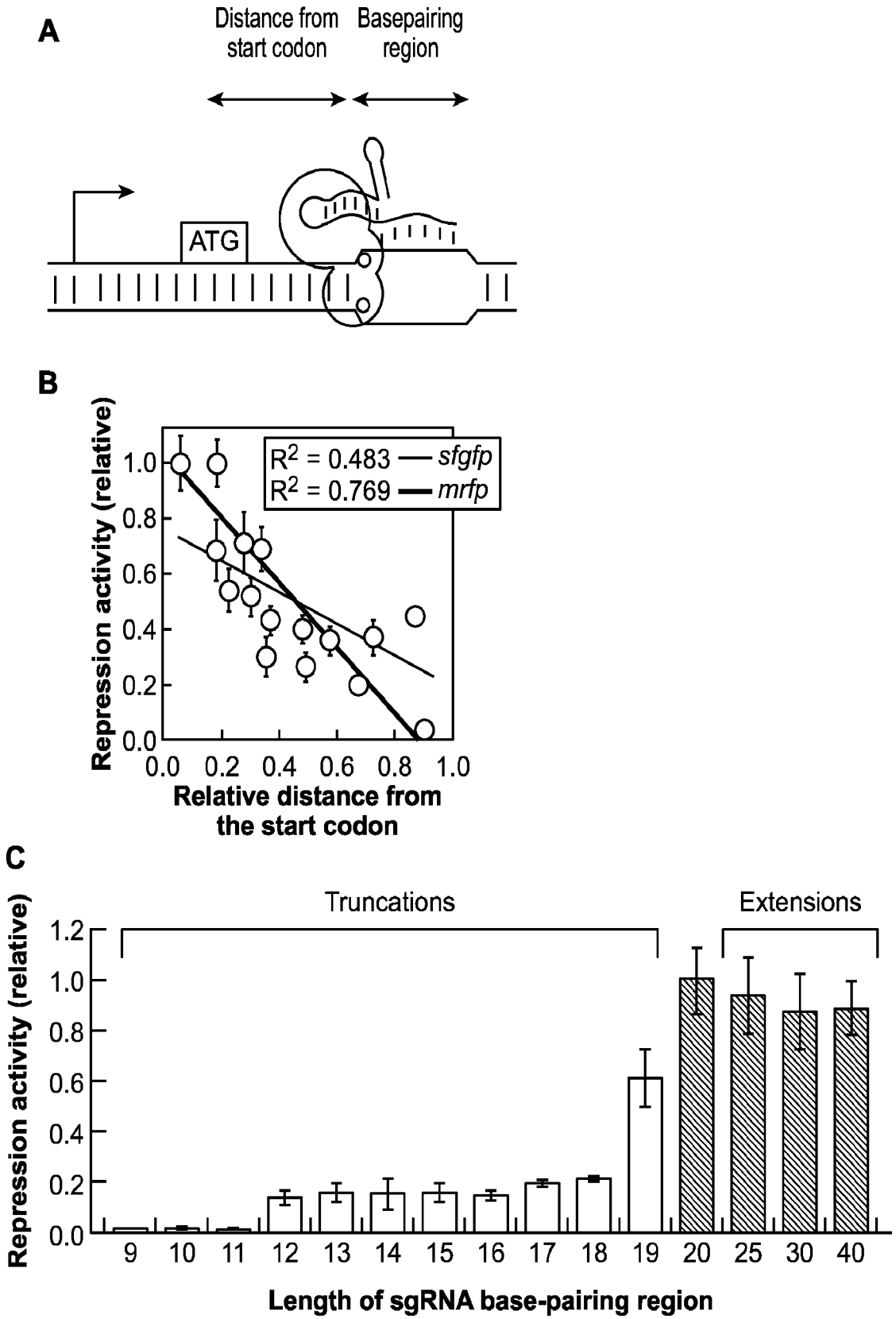

A
Distance from start codon
Basepairing region
ATG
B
Repression activity (relative)
R2 = 0.483 sfgfp
R2 = 0.769 mrfp
Relative distance from the start codon
C
Truncations
Extensions
Repression activity (relative)
Length of sgRNA base-pairing region

FIG. 43

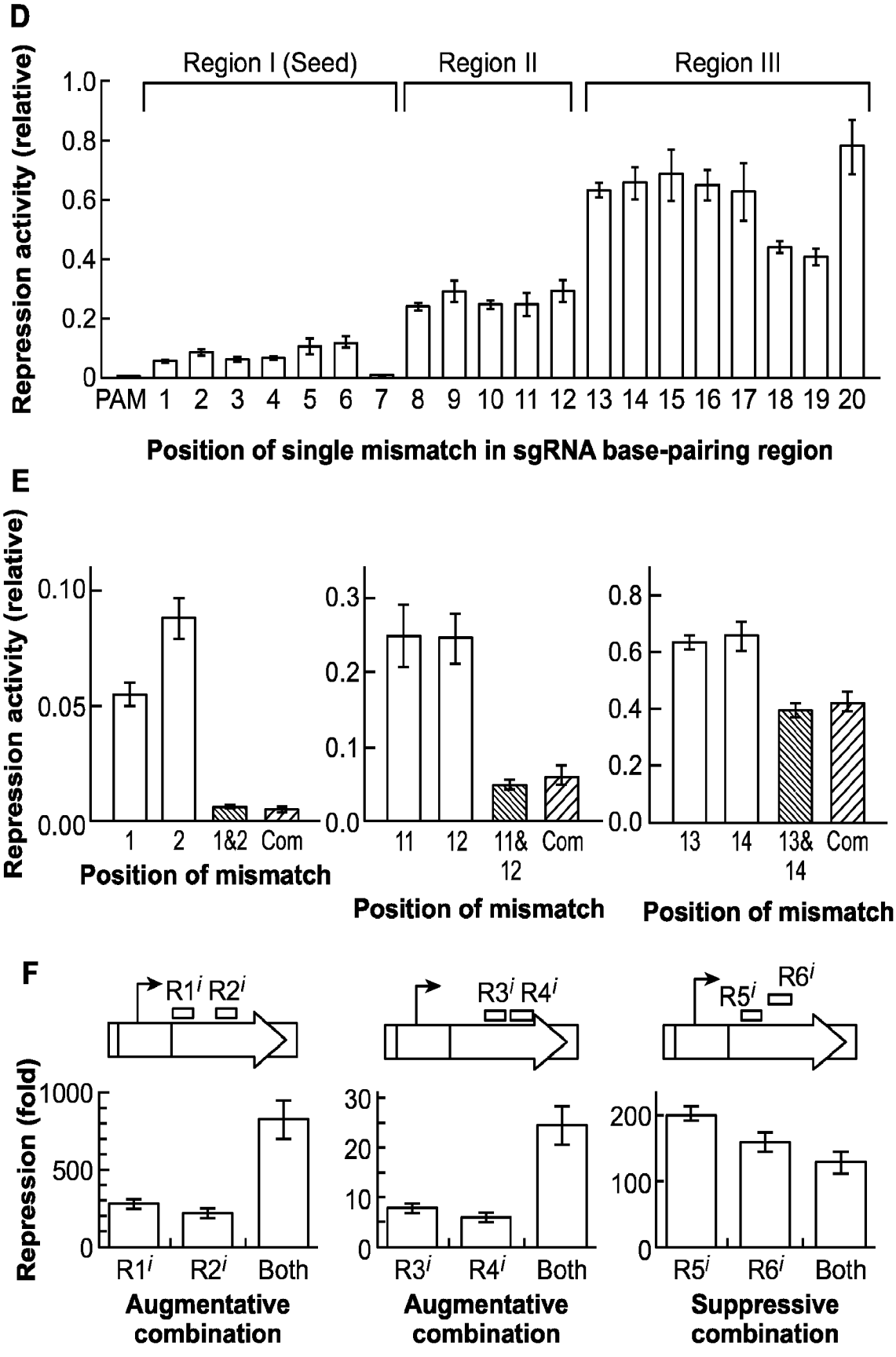

D
Repression activity (relative)
Region I (Seed)
Region II
Region III
1.0
0.8
0.6
0.4
0.2
0
PAM 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Position of single mismatch in sgRNA base-pairing region
E
Repression activity (relative)
0.10
0.05
0.00
1 2 1&2 Com
Position of mismatch
0.3
0.2
0.1
0.0
11 12 11& Com
12
Position of mismatch
0.8
0.6
0.4
0.2
0.0
13 14 13& Com
14
Position of mismatch
F
R1i R2i
R3i R4i
R5i R6i
Repression (fold)
1000
500
0
R1i R2i Both
Augmentative combination
30
20
10
0
R3i R4i Both
Augmentative combination
200
100
0
R5i R6i Both
Suppressive combination

FIG. 43 (Cont.)

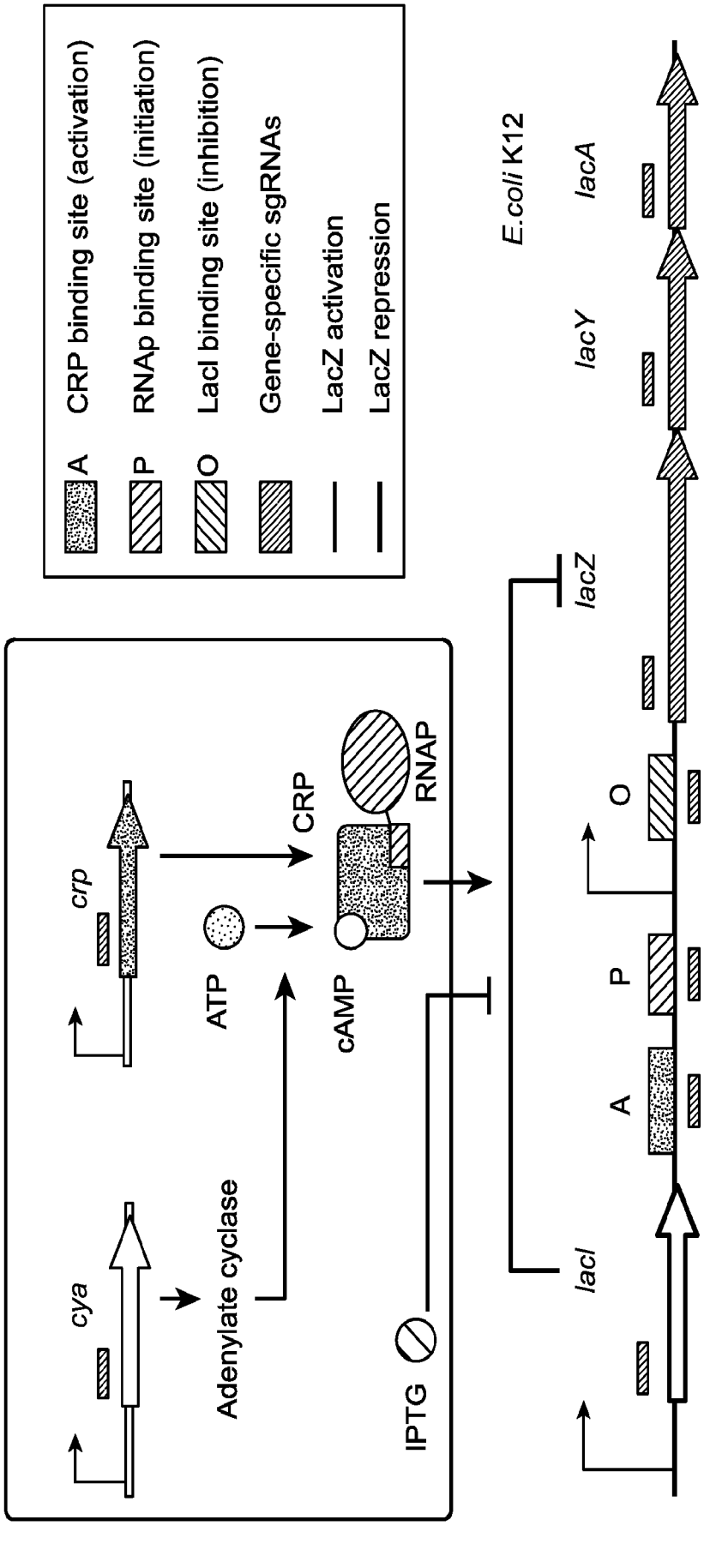

A
CRP binding site (activation)
RNAp binding site (initiation)
LacI binding site (inhibition)
Gene-specific sgRNAs
LacZ activation
LacZ repression
E.coli K12
lacI
A
P
O
lacZ
lacY
lacA
cya
Adenylate cyclase
crp
ATP
cAMP
CRP
RNAP
IPTG

FIG. 44

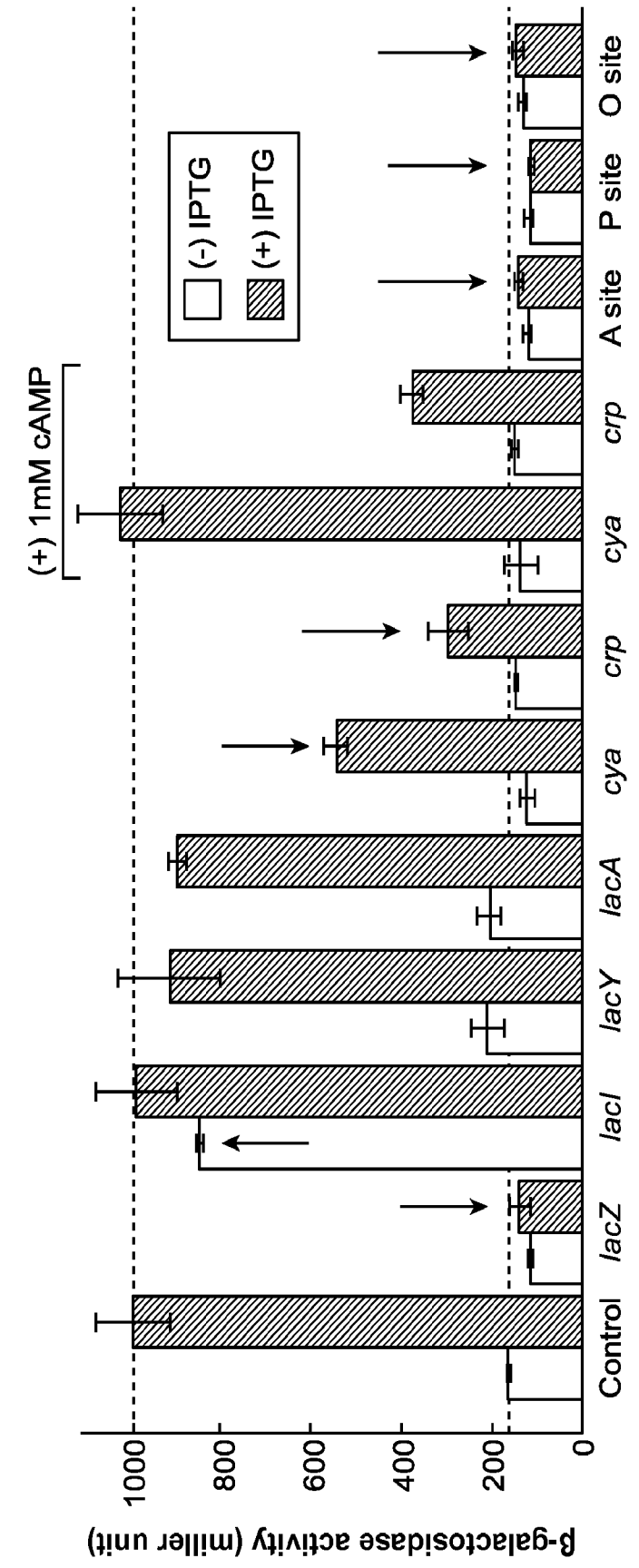
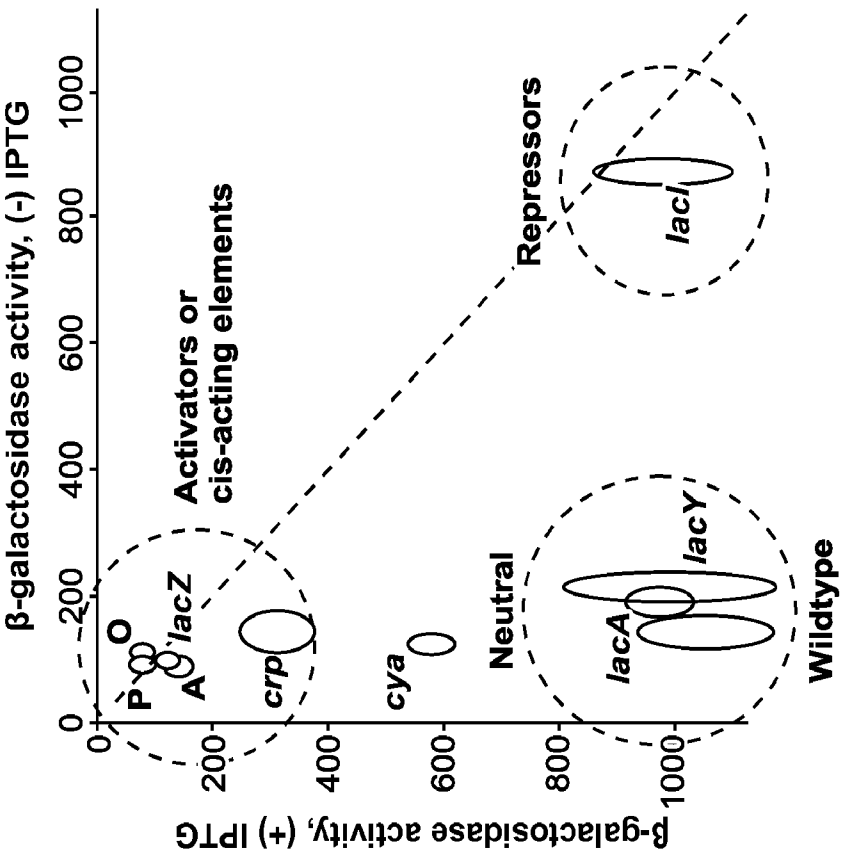

B
β-galactosidase activity (miller unit)
0
200
400
600
800
1000
(-) IPTG
(+) IPTG
(+) 1mM cAMP
Control
lacZ
lacI
lacY
lacA
cya
crp
cya
crp
A site
P site
O site
Gene/locus perturbed

FIG. 44 (Cont. 1)


β-galactosidase activity, (-) IPTG
0
200
400
600
800
1000
P
O
A
lacZ
Activators or
cis-acting elements
crp
cya
Neutral
lacA
lacY
Wildtype
Repressors
lacI
β-galactosidase activity, (+) IPTG
0
200
400
600
800
1000

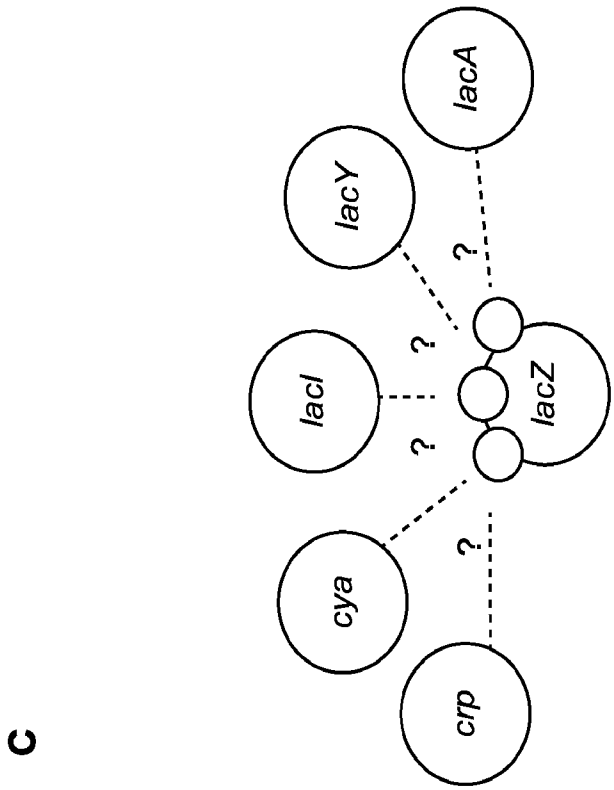
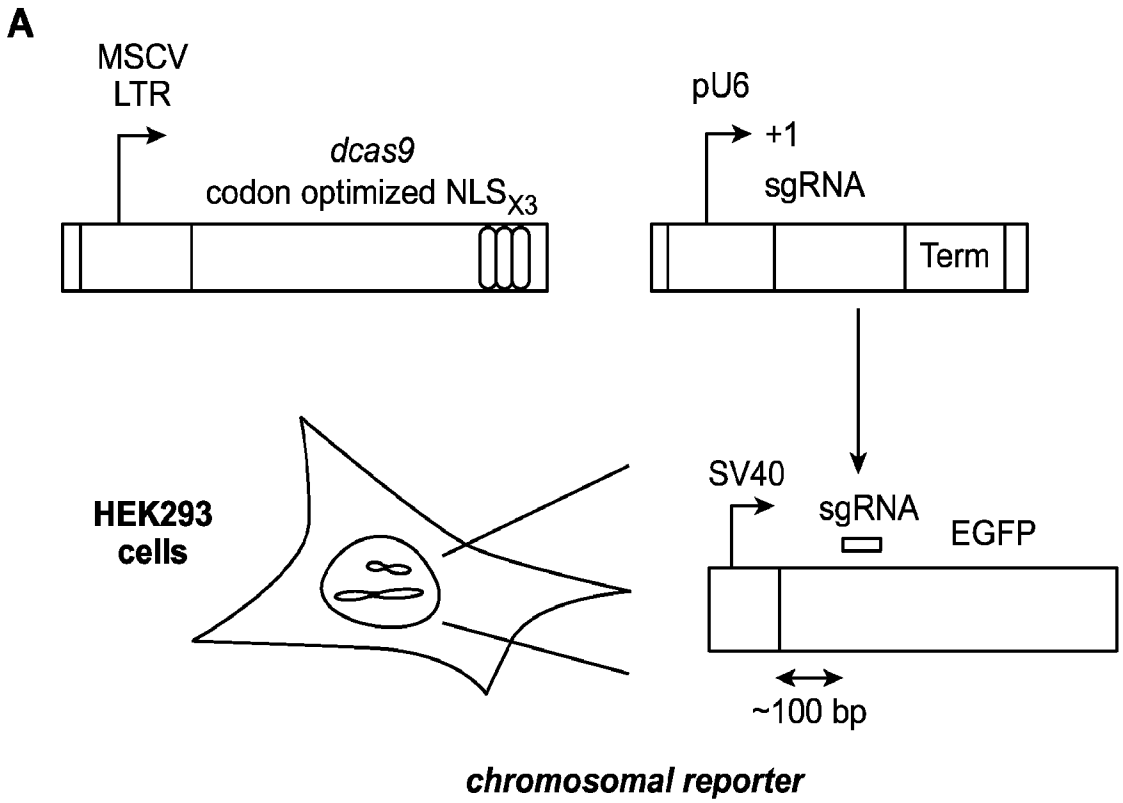

C
crp
cya
lacI
lacZ
lacY
lacA
?
?
?
?

FIG. 44 (Cont. 2)


A
MSCV
LTR
dcas9
codon optimized NLS$_{X3}$
pU6
+1
sgRNA
Term
HEK293
cells
SV40
sgRNA
EGFP
~100 bp
chromosomal reporter

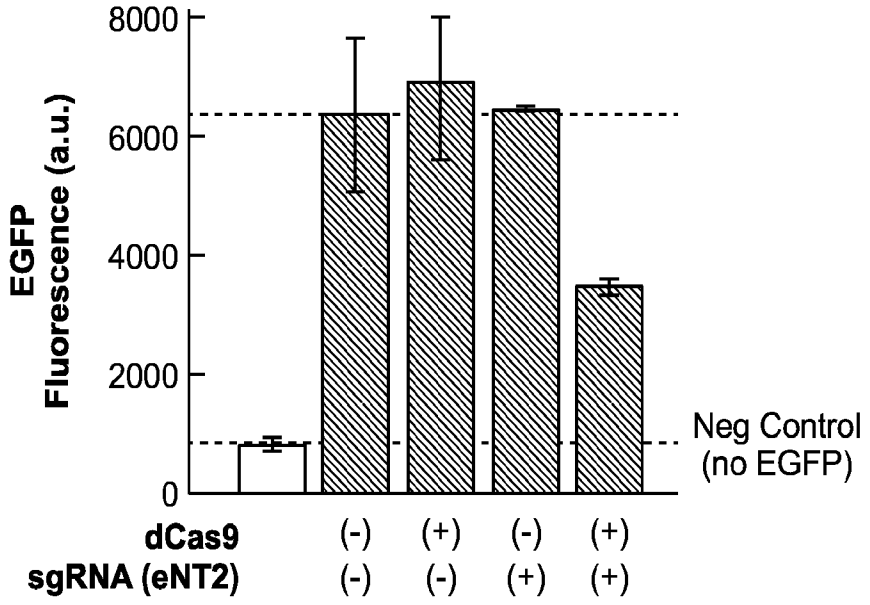

8000
6000
4000
2000
0
EGFP
Fluorescence (a.u.)
Neg Control
(no EGFP)
dCas9
sgRNA (eNT2)
(-) (+) (-) (+)
(-) (-) (+) (+)

FIG. 45

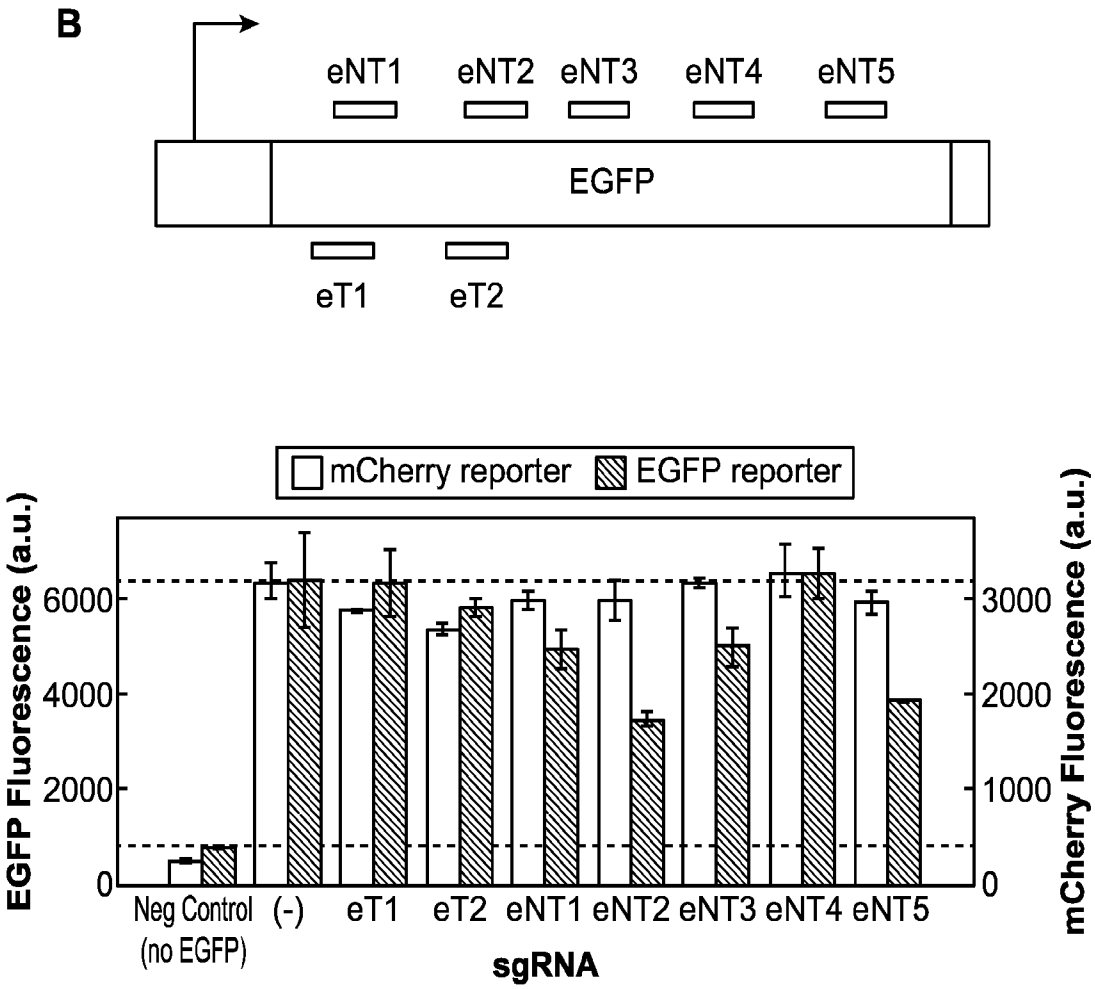

B
eNT1
eNT2
eNT3
eNT4
eNT5
EGFP
eT1
eT2
mCherry reporter
EGFP reporter
EGFP Fluorescence (a.u.)
mCherry Fluorescence (a.u.)
6000
4000
2000
0
3000
2000
1000
0
Neg Control (no EGFP)
(-)
eT1
eT2
eNT1
eNT2
eNT3
eNT4
eNT5
sgRNA

FIG. 45 (Cont.)

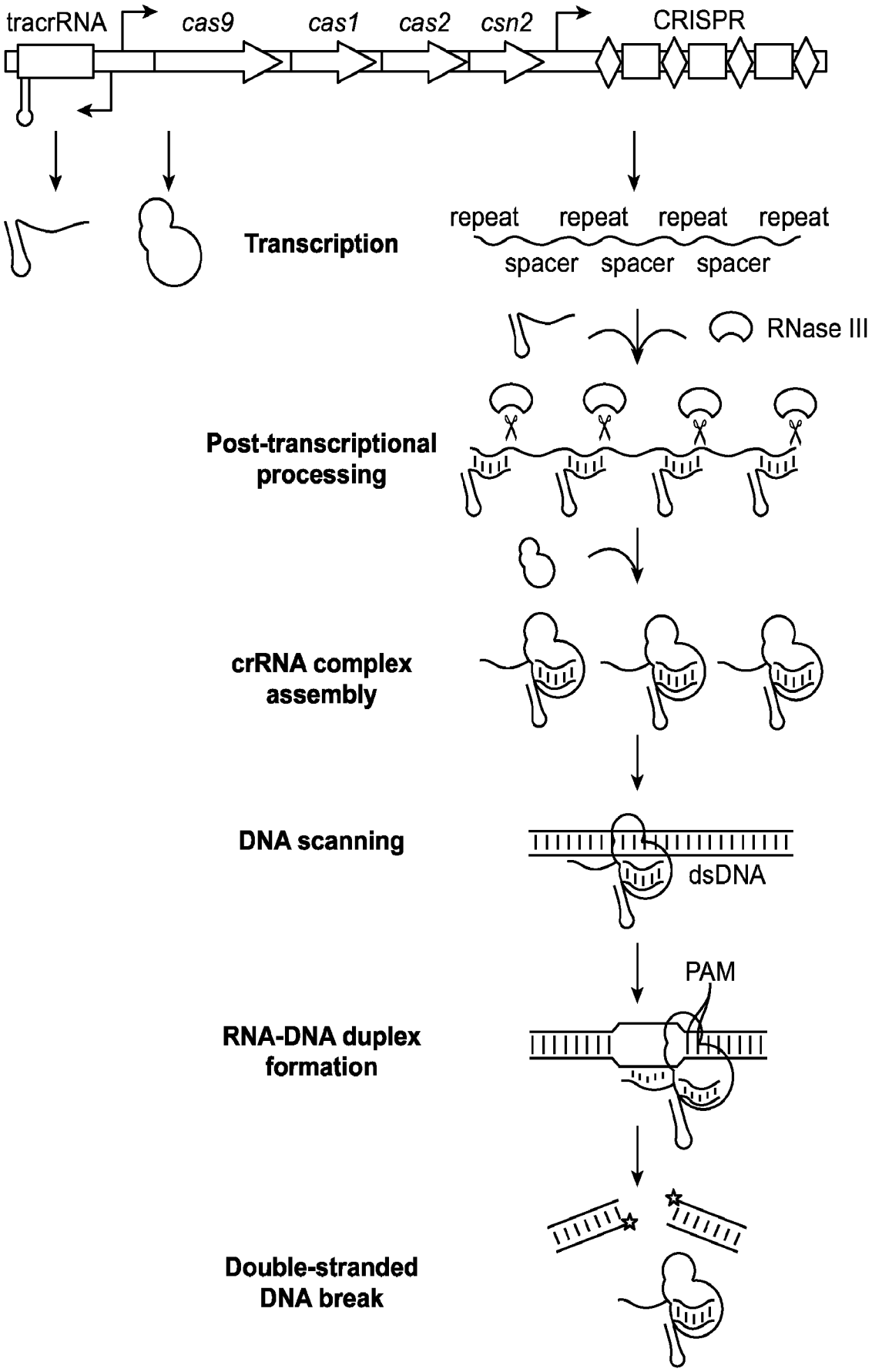

tracrRNA
cas9
cas1
cas2
csn2
CRISPR
Transcription
repeat
repeat
repeat
repeat
spacer
spacer
spacer
RNase III
Post-transcriptional processing
crRNA complex assembly
DNA scanning
dsDNA
PAM
RNA-DNA duplex formation
Double-stranded DNA break

FIG. 46

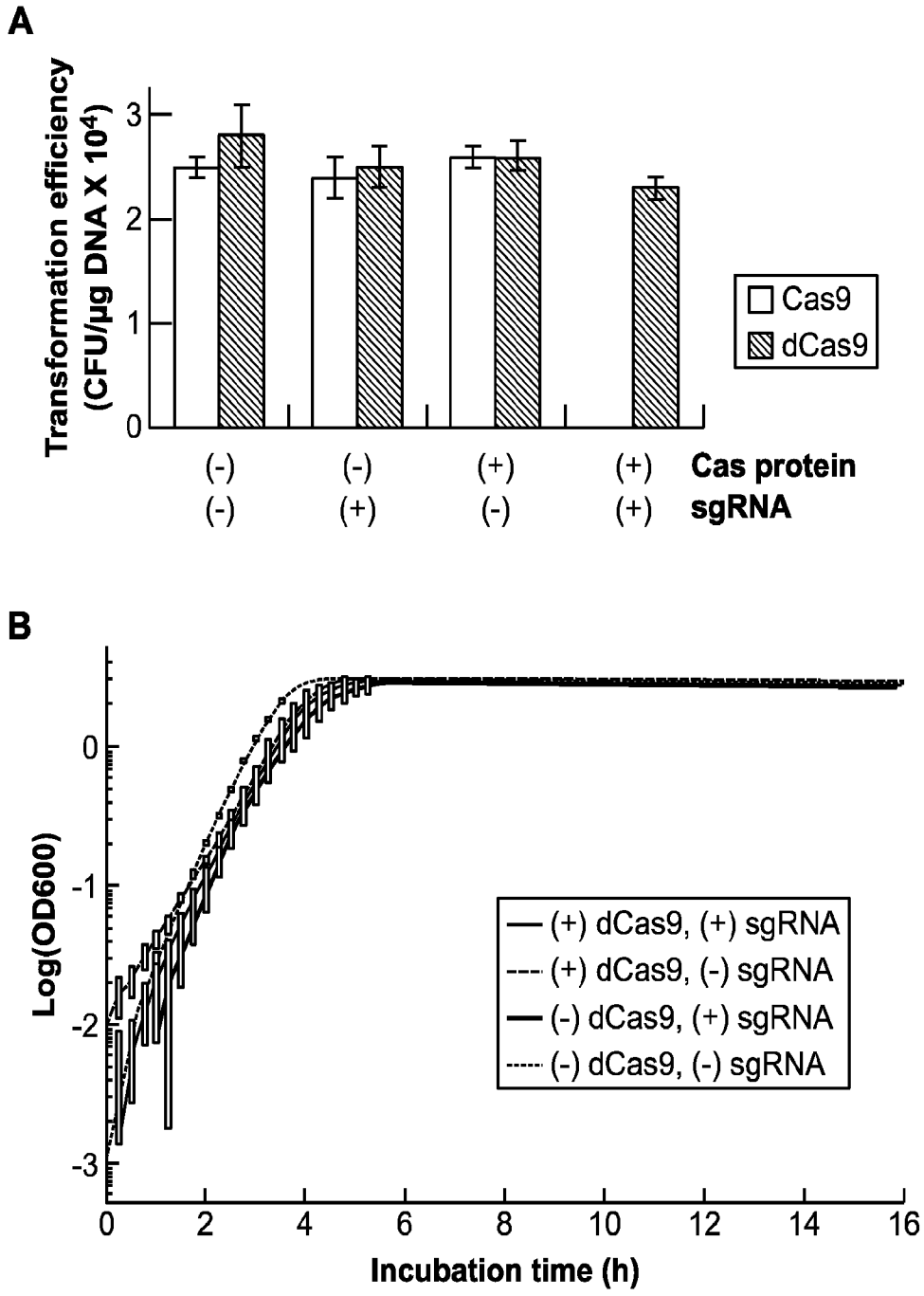

A
Transformation efficiency (CFU/μg DNA X 10^4)
3
2
1
0
Cas9
dCas9
(-) (-) (+) (+) Cas protein
(-) (+) (-) (+) sgRNA
B
Log(OD600)
0
-1
-2
-3
0 2 4 6 8 10 12 14 16
Incubation time (h)
(+) dCas9, (+) sgRNA
(+) dCas9, (-) sgRNA
(-) dCas9, (+) sgRNA
(-) dCas9, (-) sgRNA

FIG. 47

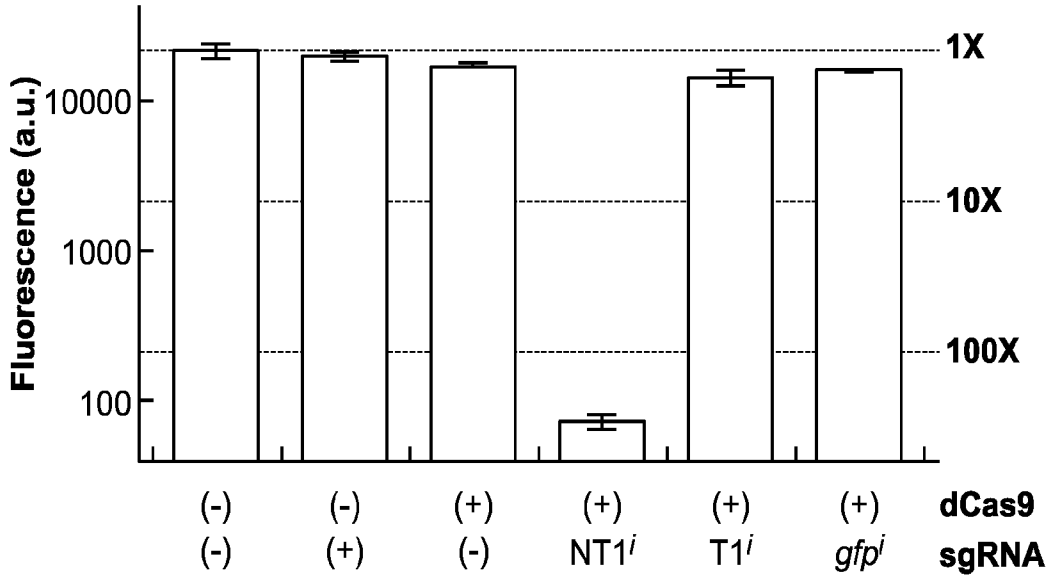

Fluorescence (a.u.)
10000
1000
100
1X
10X
100X
(-) (-) (+) (+) (+) (+) dCas9
(-) (+) (-) NT1i T1i gfpi sgRNA

FIG. 48

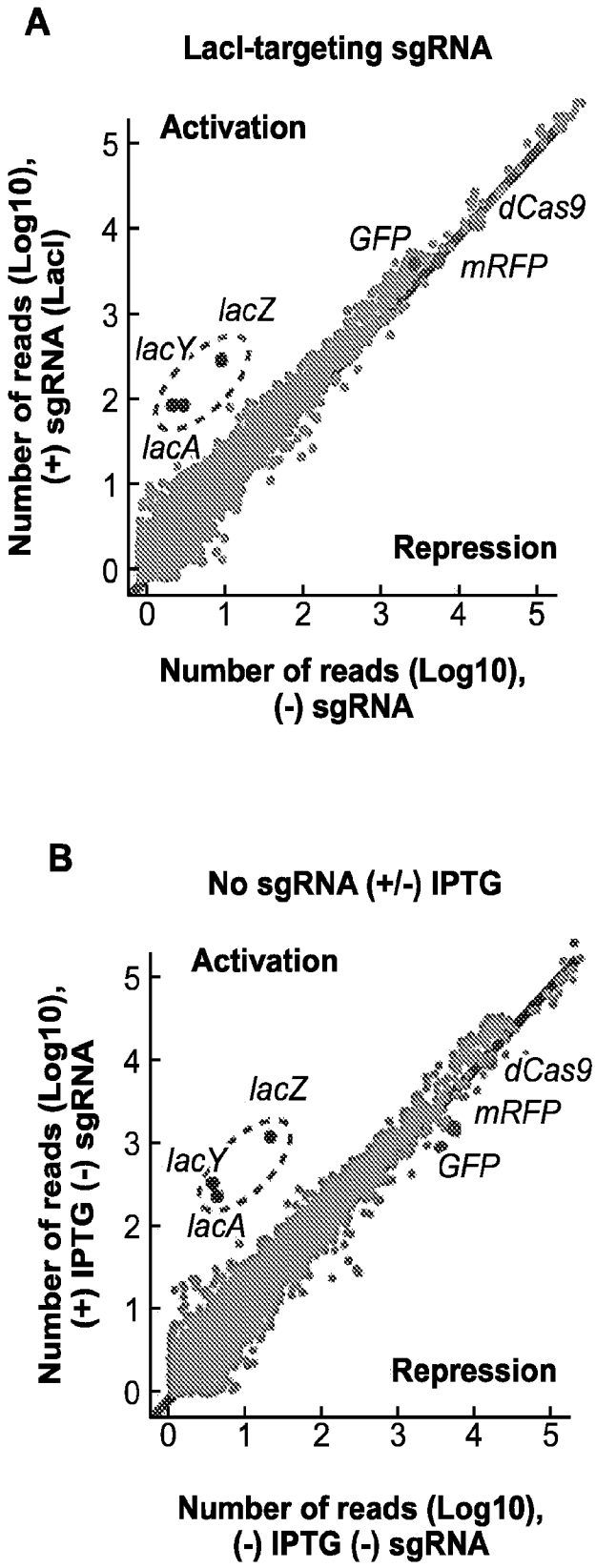

A
LacI-targeting sgRNA
Activation
Repression
Number of reads (Log10), (+) sgRNA (LacI)
Number of reads (Log10), (-) sgRNA
dCas9
GFP
mRFP
lacZ
lacY
lacA
0
1
2
3
4
5
B
No sgRNA (+/-) IPTG
Activation
Repression
Number of reads (Log10), (+) IPTG (-) sgRNA
Number of reads (Log10), (-) IPTG (-) sgRNA
dCas9
mRFP
GFP
lacZ
lacY
lacA

FIG. 49

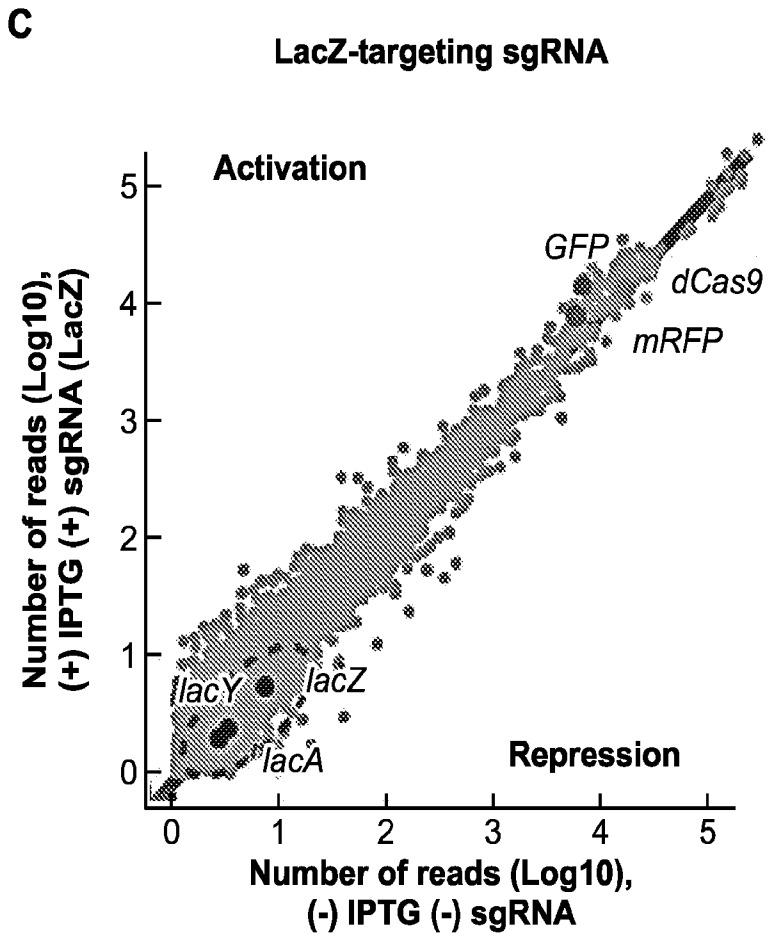

C
LacZ-targeting sgRNA
Activation
Repression
GFP
dCas9
mRFP
lacY
lacZ
lacA
Number of reads (Log10), (+) IPTG (+) sgRNA (LacZ)
Number of reads (Log10), (-) IPTG (-) sgRNA
0
1
2
3
4
5

FIG. 49 (Cont.)

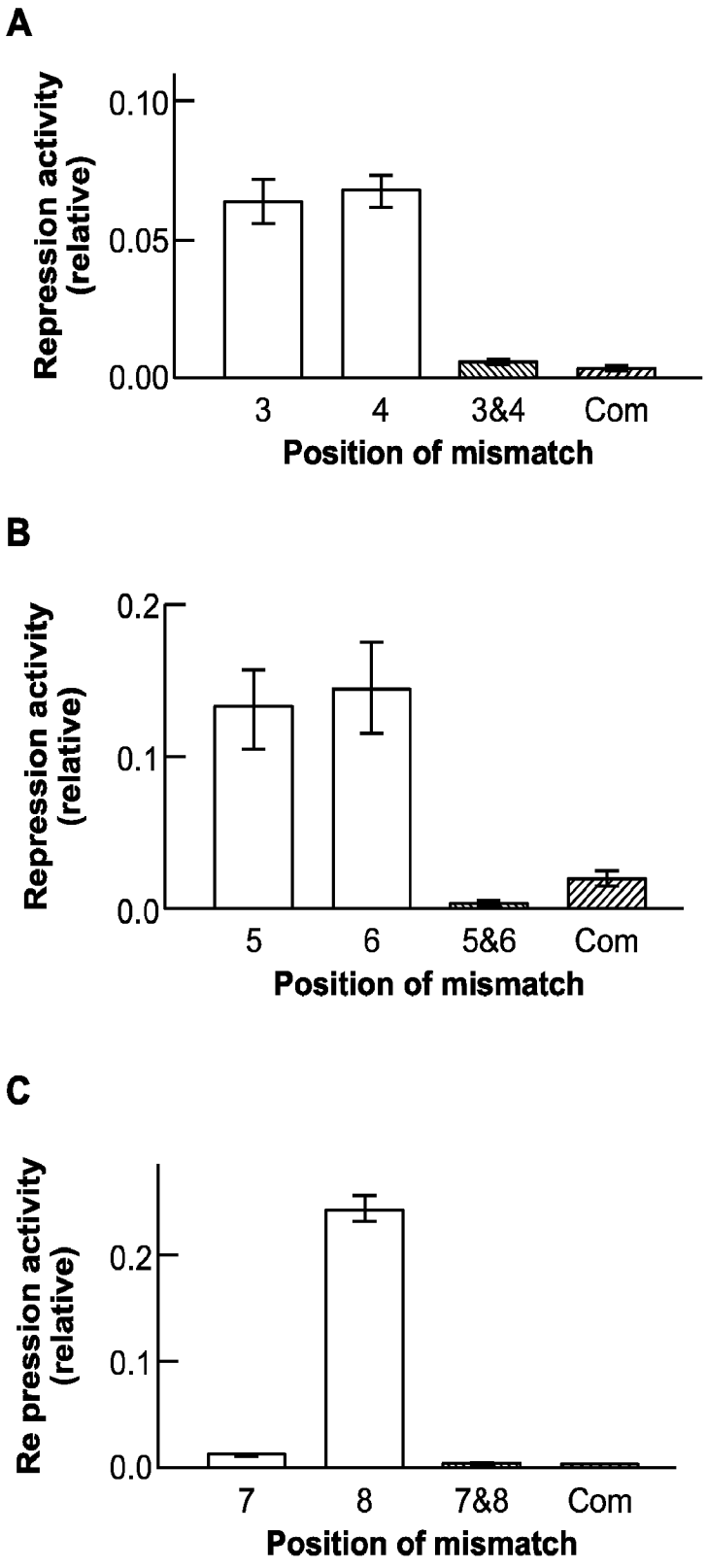
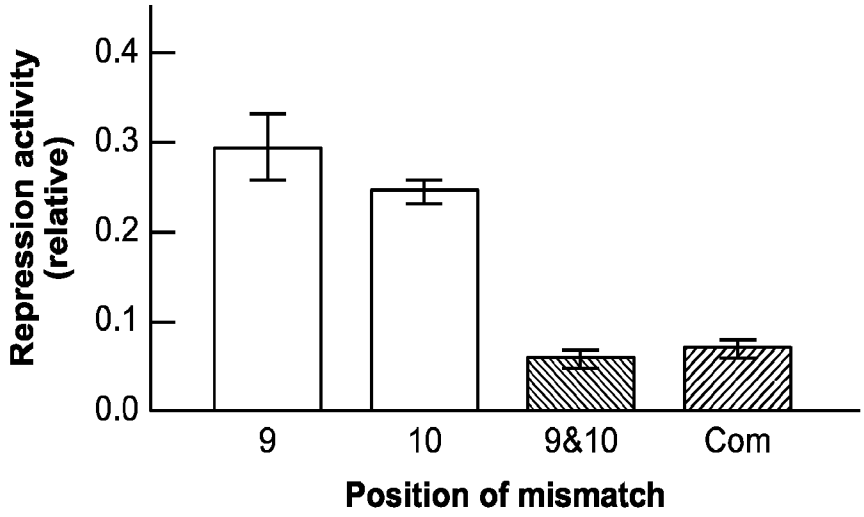

A
Repression activity (relative)
0.10
0.05
0.00
3
4
3&4
Com
Position of mismatch
B
Repression activity (relative)
0.2
0.1
0.0
5
6
5&6
Com
Position of mismatch
C
Re pression activity (relative)
0.2
0.1
0.0
7
8
7&8
Com
Position of mismatch

FIG. 50

D



E

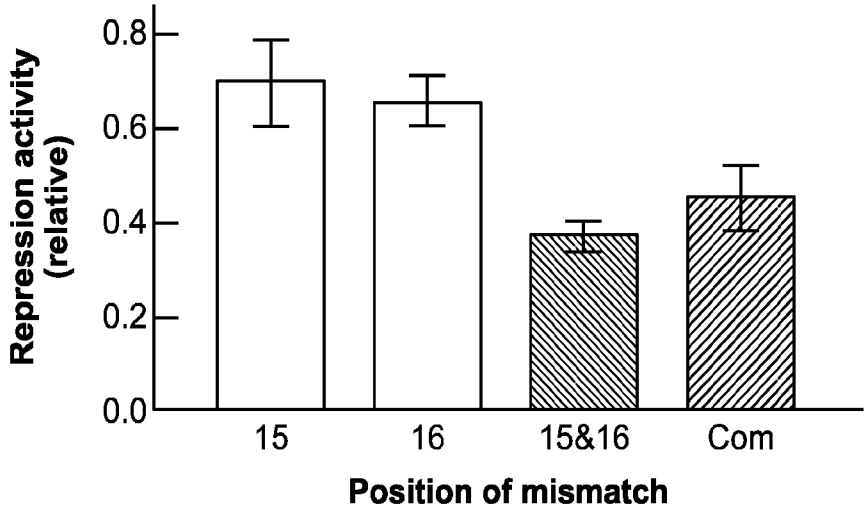


FIG. 50 (Cont.)

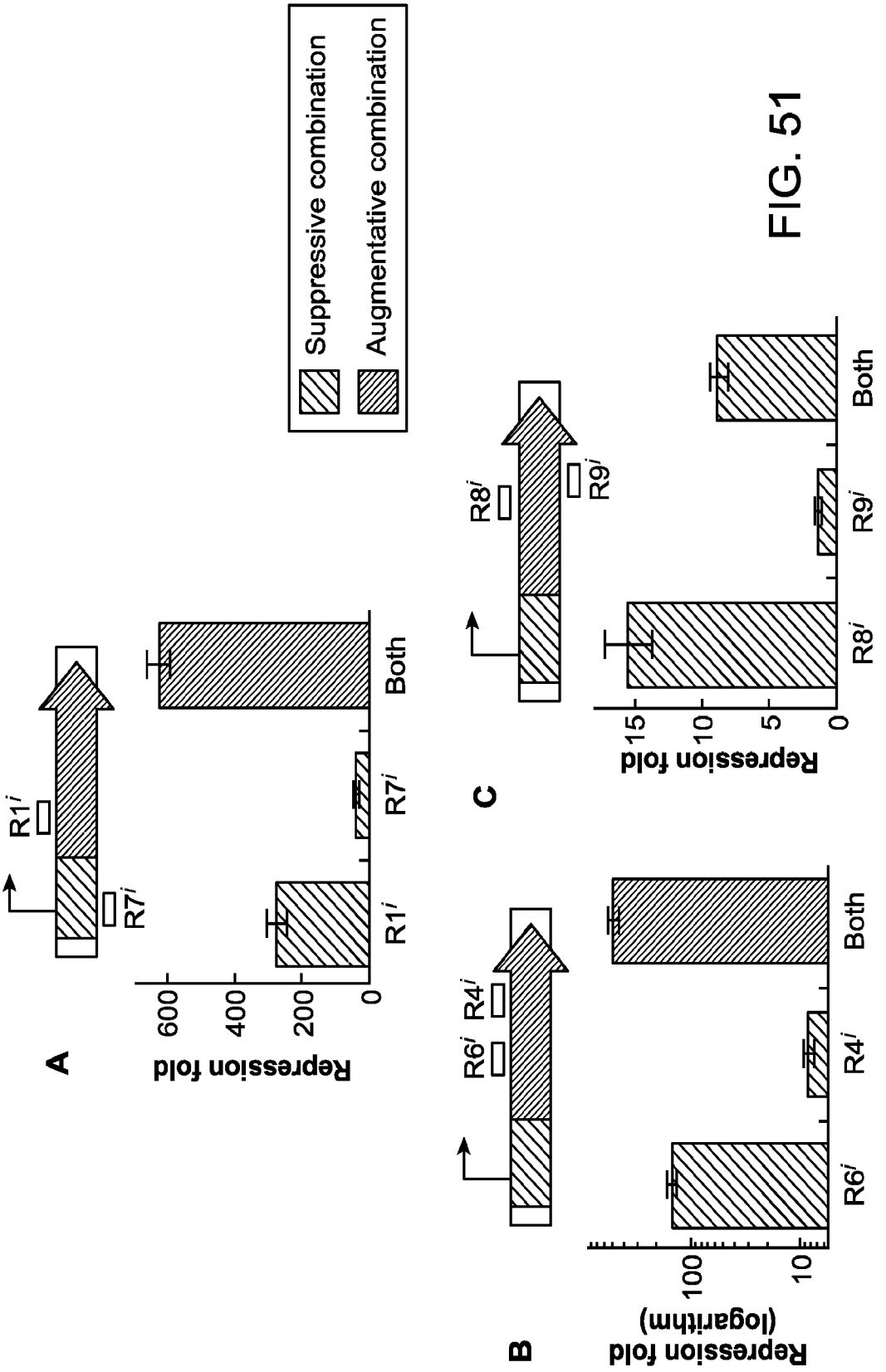
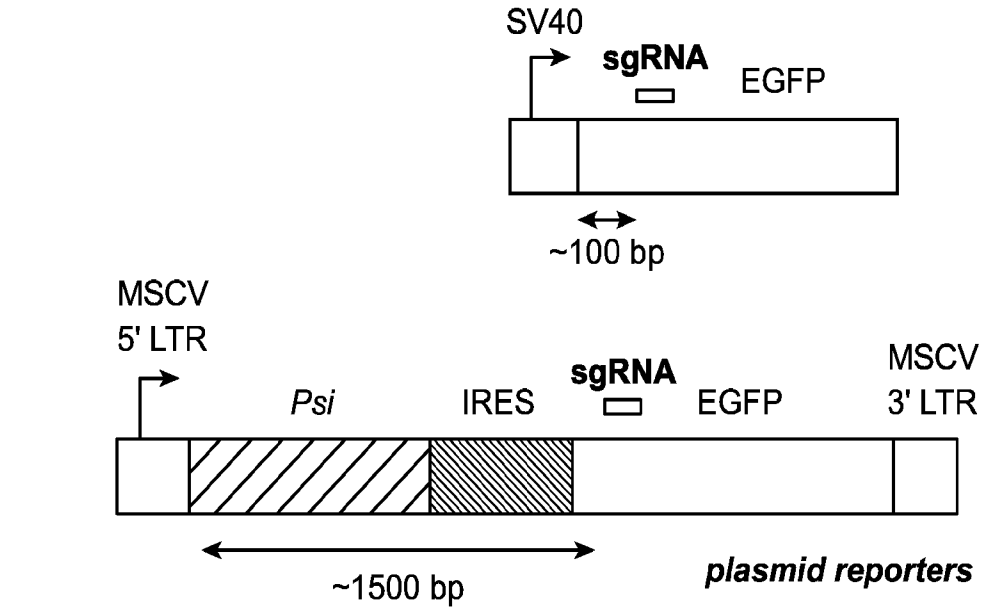

A
Repression fold
600
400
200
0
R1i
R7i
Both
Suppressive combination
Augmentative combination
B
Repression fold (logarithm)
100
10
R6i
R4i
Both
C
Repression fold
15
10
5
0
R8i
R9i
Both

FIG. 51


SV40
sgRNA
EGFP
~100 bp
MSCV
5' LTR
Psi
IRES
sgRNA
EGFP
MSCV
3' LTR
~1500 bp
plasmid reporters

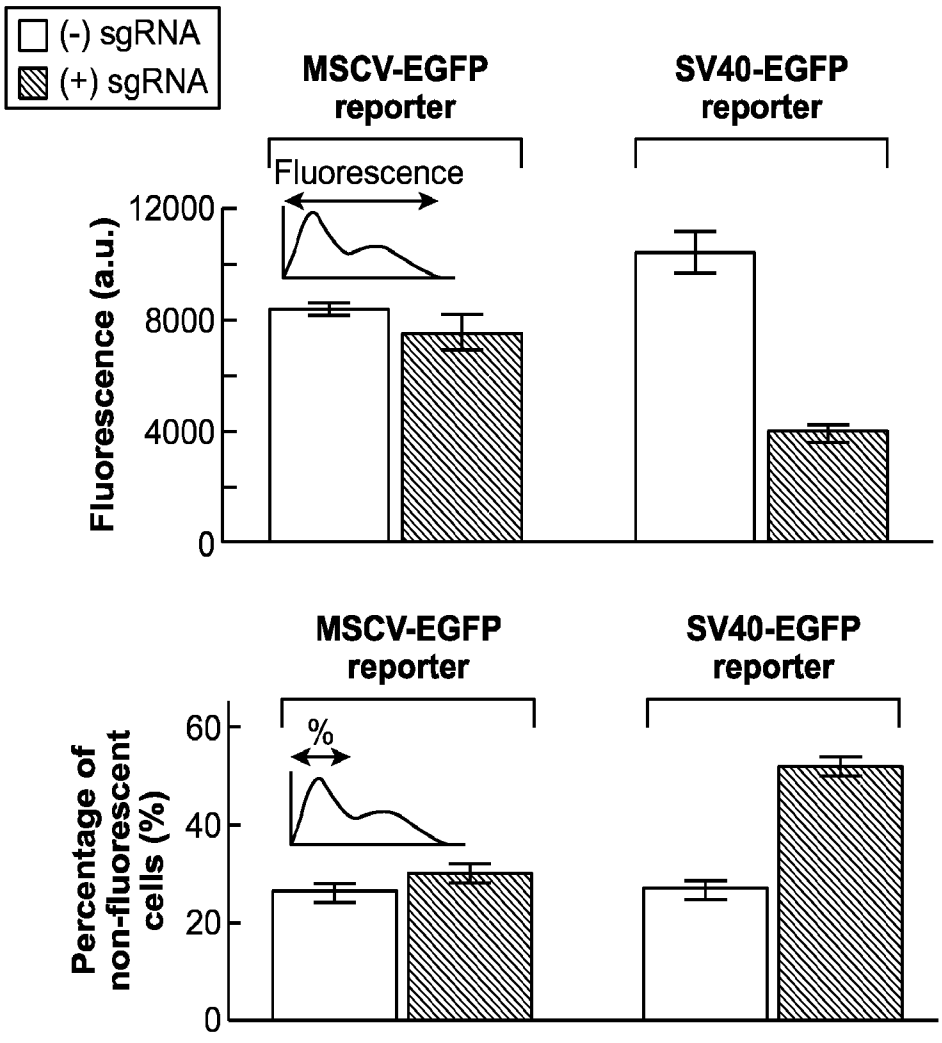

(-) sgRNA
(+) sgRNA
MSCV-EGFP reporter
SV40-EGFP reporter
Fluorescence
Fluorescence (a.u.)
12000
8000
4000
0
MSCV-EGFP reporter
SV40-EGFP reporter
%
Percentage of non-fluorescent cells (%)
60
40
20
0

FIG. 52

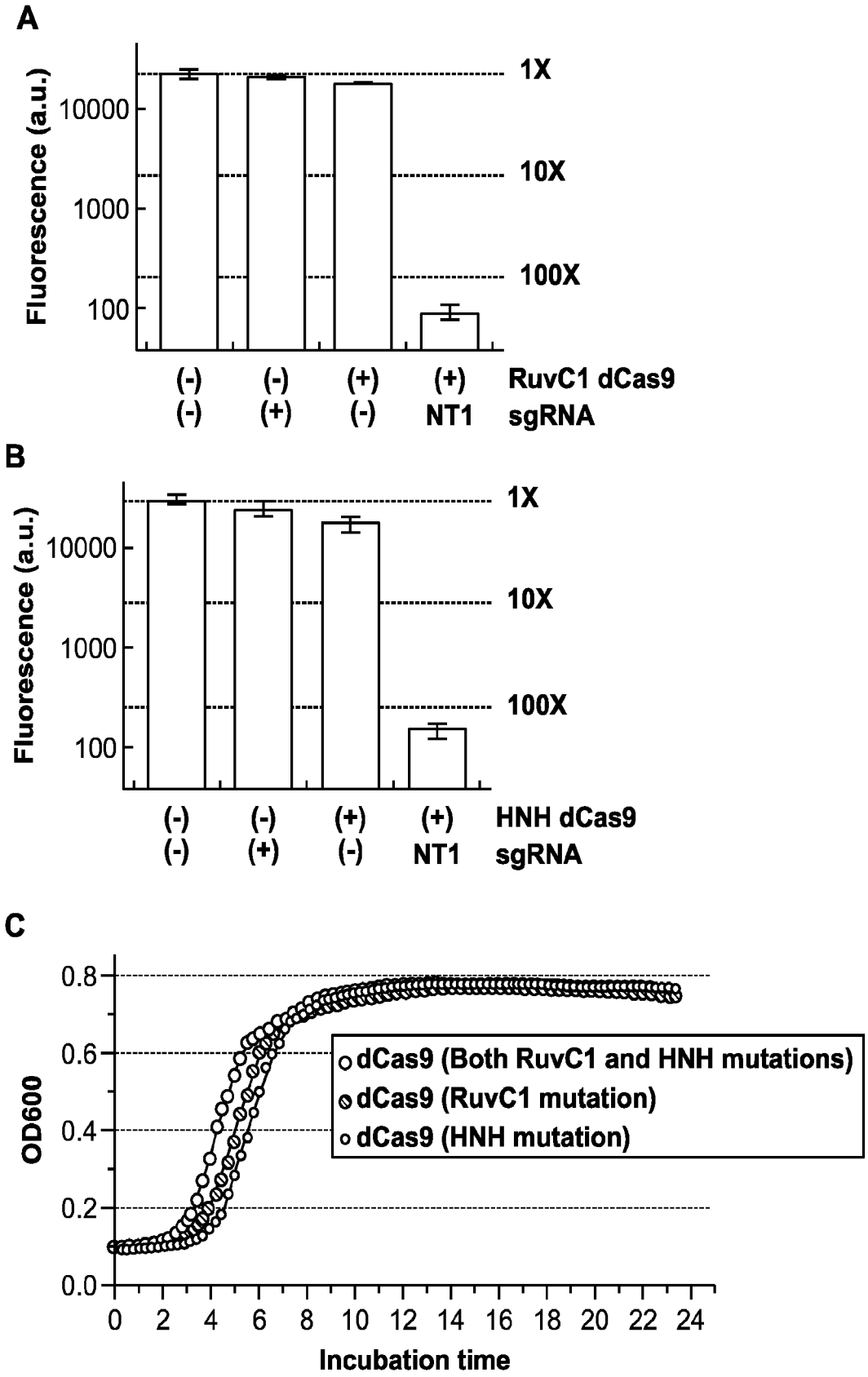

A
Fluorescence (a.u.)
10000
1000
100
1X
10X
100X
(-) (-) (+) (+) RuvC1 dCas9
(-) (+) (-) NT1 sgRNA
B
Fluorescence (a.u.)
10000
1000
100
1X
10X
100X
(-) (-) (+) (+) HNH dCas9
(-) (+) (-) NT1 sgRNA
C
OD600
0.8
0.6
0.4
0.2
0.0
0 2 4 6 8 10 12 14 16 18 20 22 24
Incubation time
dCas9 (Both RuvC1 and HNH mutations)
dCas9 (RuvC1 mutation)
dCas9 (HNH mutation)

FIG. 53

A

Protein name	Function
Transcriptional Activators	
GAL4	Transcription activation
VP16	Transcription activation
VP64	Transcription activation
p65 subdomain (NFkB)	Transcription activation
Transcriptional repressors	
KRAB	Transcription repression
Mad mSIN3 interaction domain (SID)	Transcription repression
the ERF repressor domain (ERD)	Transcription repression
Histone lysine methyltransferases (KMT)	
KMT1 family: SUV39H1, SUV39H2, G9A, ESET/SETDB1, and homologs (Clr4, Su(var)3-9)	Heterochromatin formation/ transcription repression
KMT2 family: hSET1A, hSET1B, MLL1 to 5, ASH1, and homologs (Trx, Trr, Ash1)	Transcription activation
KMT3 family: SYMD2, NSD1	Transcription activation
KMT4: DOT1L and homologs	Transcription activation
KMT5 family: Pr-SET7/8, SUV4-20H1, and homologs (PR-set7, Suv4-20, Set9)	DNA damage response, transcription repression
KMT6: EZH2	Polycomb silencing
KMT8: RIZ1	Transcription repression
Histone lysine demethylates (KDM)	
KDM1: LSD1/BHC110 and homologs (SpLsd1/Swm1/Saf110, Su(var)3-3)	Transcription activation and repression, heterochromatin formation
KDM3 family: JHDM2a/b	Androgen receptor gene activation, spermatogenesis
KDM4 family: JMJD2A/JHDM3A, JMJD2B, JMJD2C/GASC1, JMJD2D, and homologs (Rph1)	Transcription elongation, transcription repression, heterochromatin formation, genome integrity
KDM5 family: JARID1A/RBP2, JARID1B/PLU-1, JARID1C/SMCX, JARID1D/SMCY, and homologs (Lid, Jhn2, Jmj2)	Transcription repression
KDM6 family: UTX, JMJD3	Transcription activation

FIG. 54

B

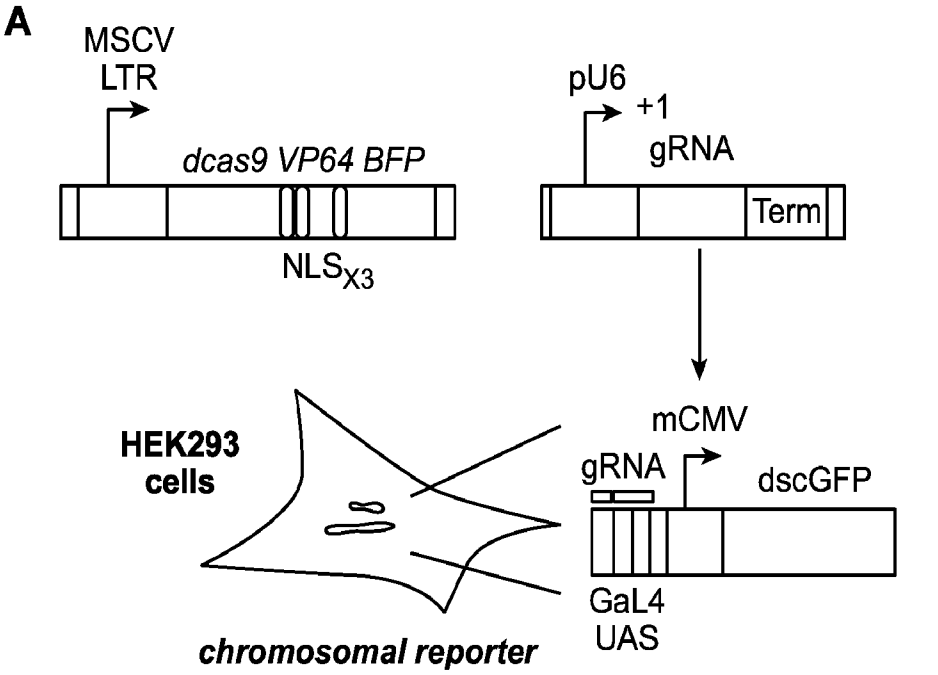
Protein name	Function
Histone lysine acetyltransferases (KAT)	
KAT2 family: hGCN5, PCAF, and homologs (dGCN5/PCAF, Gcn5)	Transcription activation, DNA repair
KAT3 family: CBP, p300, and homologs (dCBP/NEJ)	Transcription activation, DNA repair
KAT4: TAF1 and homologs (dTAF1)	Transcription activation
KAT5: TIP60/PLIP, and homologs	Transcription activation, DNA repair
KAT6: MOZ/MYST3, MORF/MYST4, and homologs (Mst2, Sas3, CG1894)	Transcription activation and elongation, DNA replication
KAT7: HBO1/MYST2, and homologs (CHM, Mst2)	Transcription, DNA replication
KAT8: HMOF/MYST1, and homologs (dMOF, CG1894, Sas2, Mst2)	Chromatin boundaries, dosage compensation, DNA repair
KAT13 family: SRC1, ACTR, P160, CLOCK, and homologs	Transcription activation
Histone lysine deacetylases	
Class I: HDAC1, HDAC2, HDAC3, HDAC8, and its homologs (Rpd3, Hos1 , Cir6)	Transcription repression, heterochromatin formation
Class IIa: HDAC4, HDAC5, HDAC7, HDAC9, and its homologs (Hda1, Cir3 etc.)	Transcription repression, heterochromatin formation
Class III: SIRT1, SIRT2, and its homologs (Sir2, Hst1, Hst2, Hst3, Hst4)	Transcription repression, heterochromatin formation
Class IV: HDAC11	Transcription repression
DNA methylases(adenosine or cytosine modification)	
Dam (E. coli)	Restriction system
Dcm (E. coli)	Restriction system
M. SssI (Spiroplasma sp)	Restriction system
DNMT1	Transcription repression. imprinting, heterochromatin formation
DNMT3a/DNMT3b, METI, DRM3 (plants), and homologs	Transcription repression. imprinting, heterochromatin formation
Chromomethylases e.g. ZMET2, CMT1, CMT2 (plants)	Transcription repression. imprinting, heterochromatin formation
DNA demethylases	
AID/Apobec deaminase family: AID	Transcription activation, genome integrity
TET dioxygenase family: TET1	Transcription activation, genome integrity
DEMETER glycosylase family: DME, DML1, DML2, ROS1	Transcription activation, genome integrity

FIG. 54 (Cont. 1)

C

Protein name	Function
Boundary elements	
CTCF	Chromatin insulation, heterochromatin spreading suppression
Periphery recruitment elements	
Lamin A	Transcription repression
Lamin B	Transcription repression
Protein docking elements	
FKBP/FRB (S. pombe)	rapamycin dependent recruitment
Pil1/Aby1 (E. coli)	ABA dependent recruitment

FIG. 54 (Cont. 2)


A
MSCV
LTR
dcas9 VP64 BFP
NLS$_{X3}$
pU6
+1
gRNA
Term
HEK293
cells
mCMV
gRNA
dscGFP
GaL4
UAS
chromosomal reporter

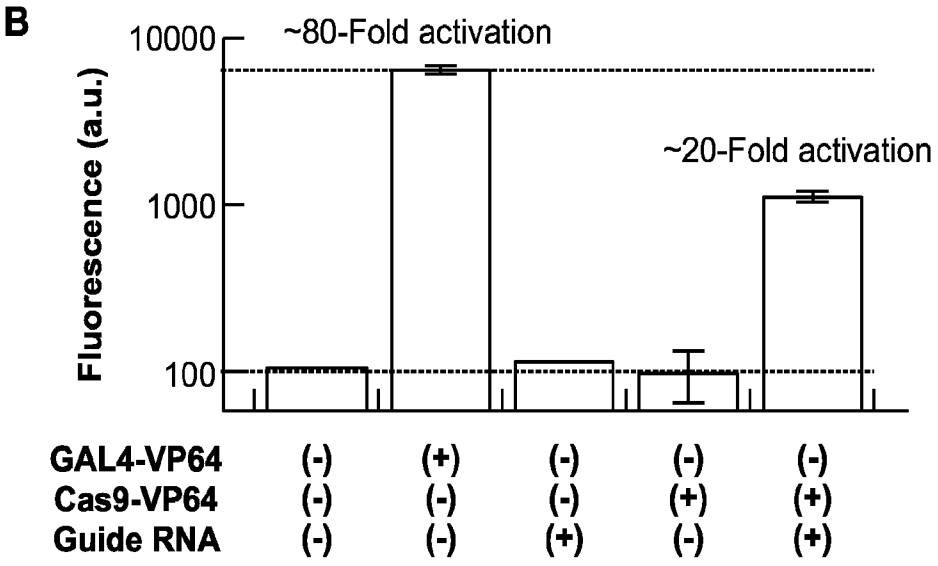

B
10000
1000
100
Fluorescence (a.u.)
~80-Fold activation
~20-Fold activation
GAL4-VP64 (-) (+) (-) (-) (-)
Cas9-VP64 (-) (-) (-) (+) (+)
Guide RNA (-) (-) (+) (-) (+)

FIG. 55

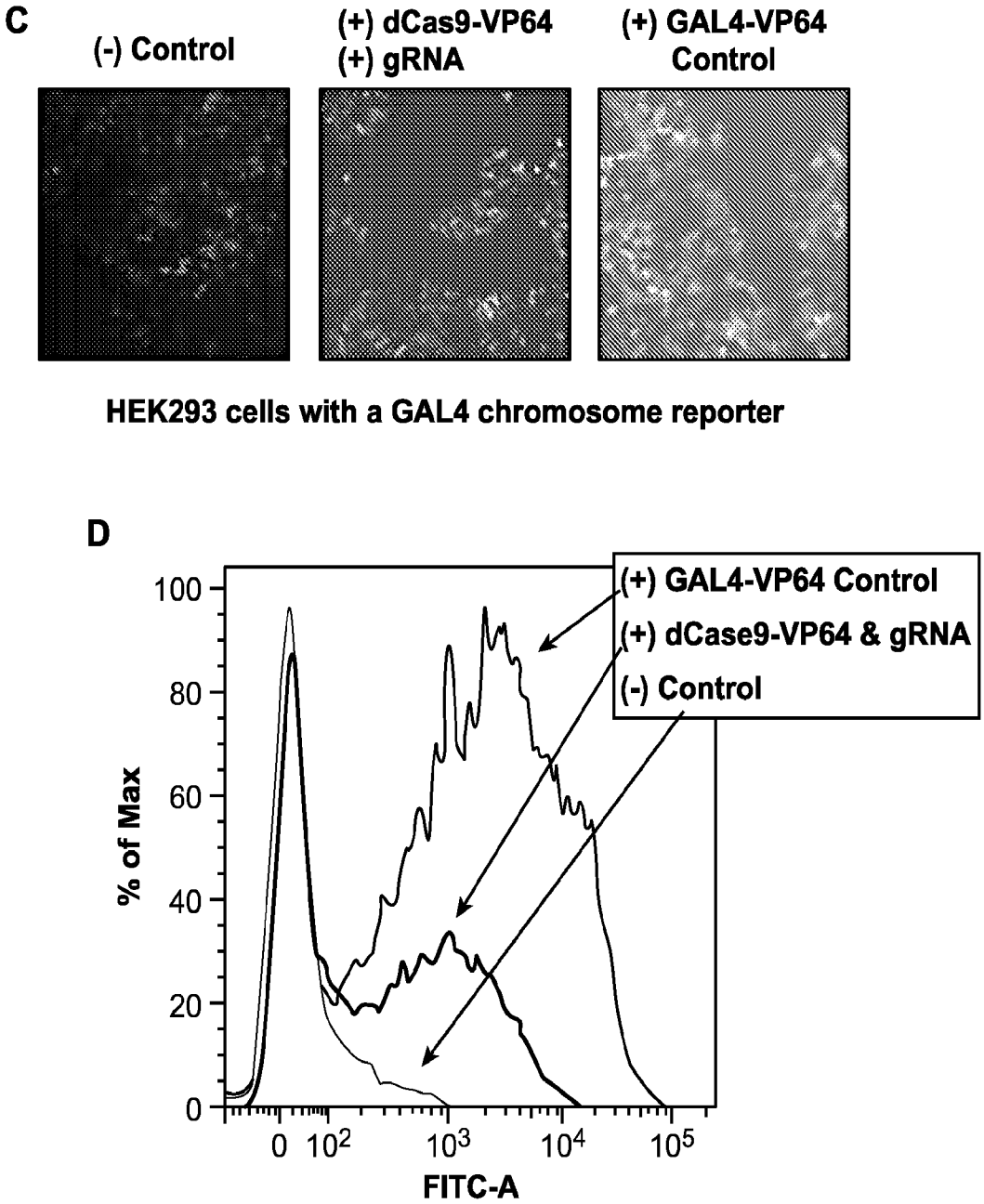

C
(-) Control
(+) dCas9-VP64
(+) gRNA
(+) GAL4-VP64
Control
HEK293 cells with a GAL4 chromosome reporter
D
(+) GAL4-VP64 Control
(+) dCase9-VP64 & gRNA
(-) Control
% of Max
100
80
60
40
20
0
0
10^2
10^3
10^4
10^5
FITC-A

FIG. 55 (Cont.)

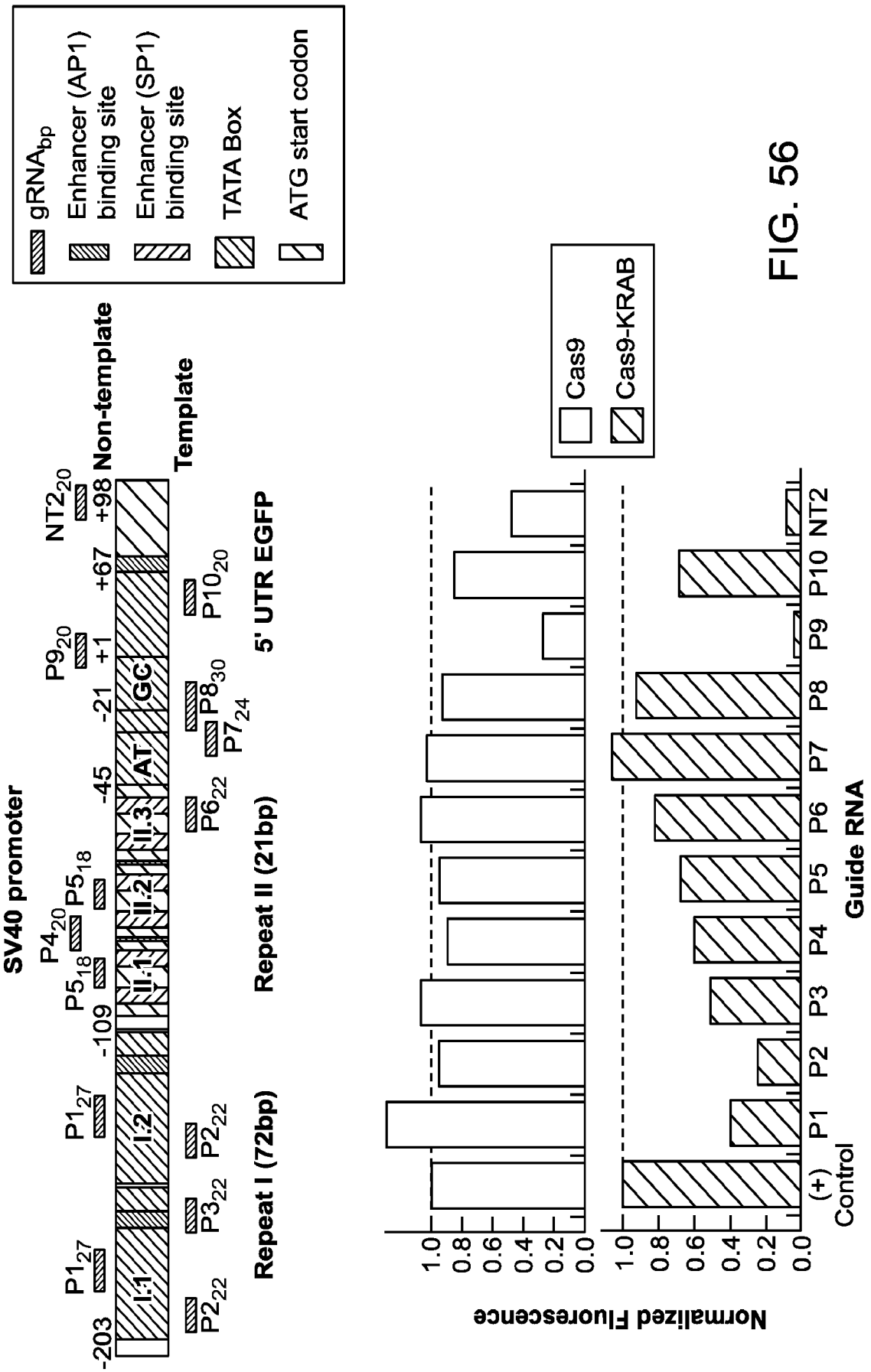

gRNA$_{bp}$
Enhancer (AP1) binding site
Enhancer (SP1) binding site
TATA Box
ATG start codon
SV40 promoter
Repeat I (72bp)
Repeat II (21bp)
5' UTR EGFP
Template
Non-template
-203
-109
-45
-21
+1
+67
+98
I.1
I.2
II.1
II.2
II.3
AT
GC
P1$_{27}$
P2$_{22}$
P3$_{22}$
P4$_{20}$
P5$_{18}$
P6$_{22}$
P7$_{24}$
P8$_{30}$
P9$_{20}$
P10$_{20}$
NT2$_{20}$
Cas9
Cas9-KRAB
Normalized Fluorescence
1.0
0.8
0.6
0.4
0.2
0.0
(+) Control
P1
P2
P3
P4
P5
P6
P7
P8
P9
P10
NT2
Guide RNA

FIG. 56

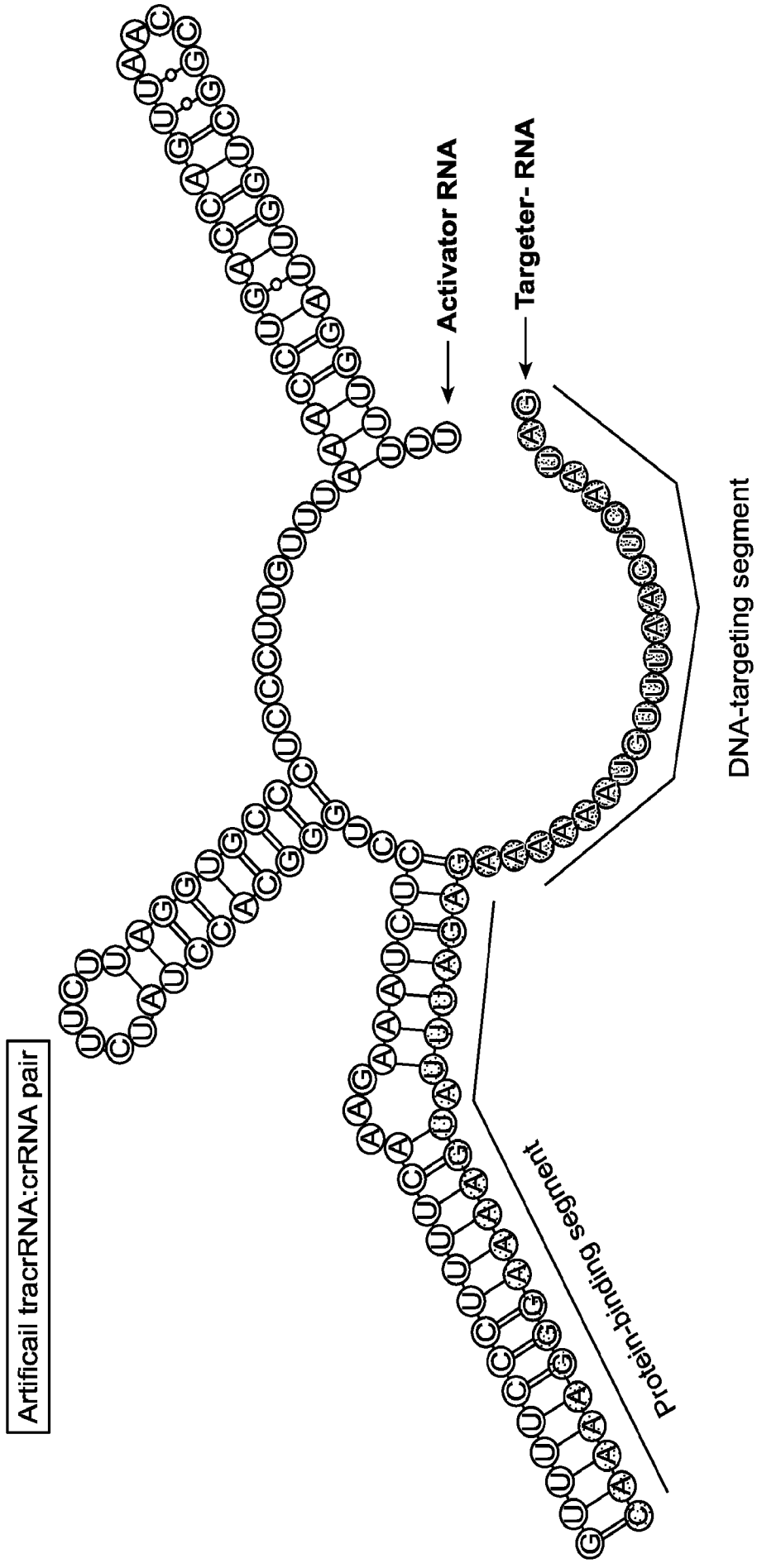

A
Artificail tracrRNA:crRNA pair
Activator RNA
Targeter- RNA
Protein-binding segment
DNA-targeting segment

FIG. 57

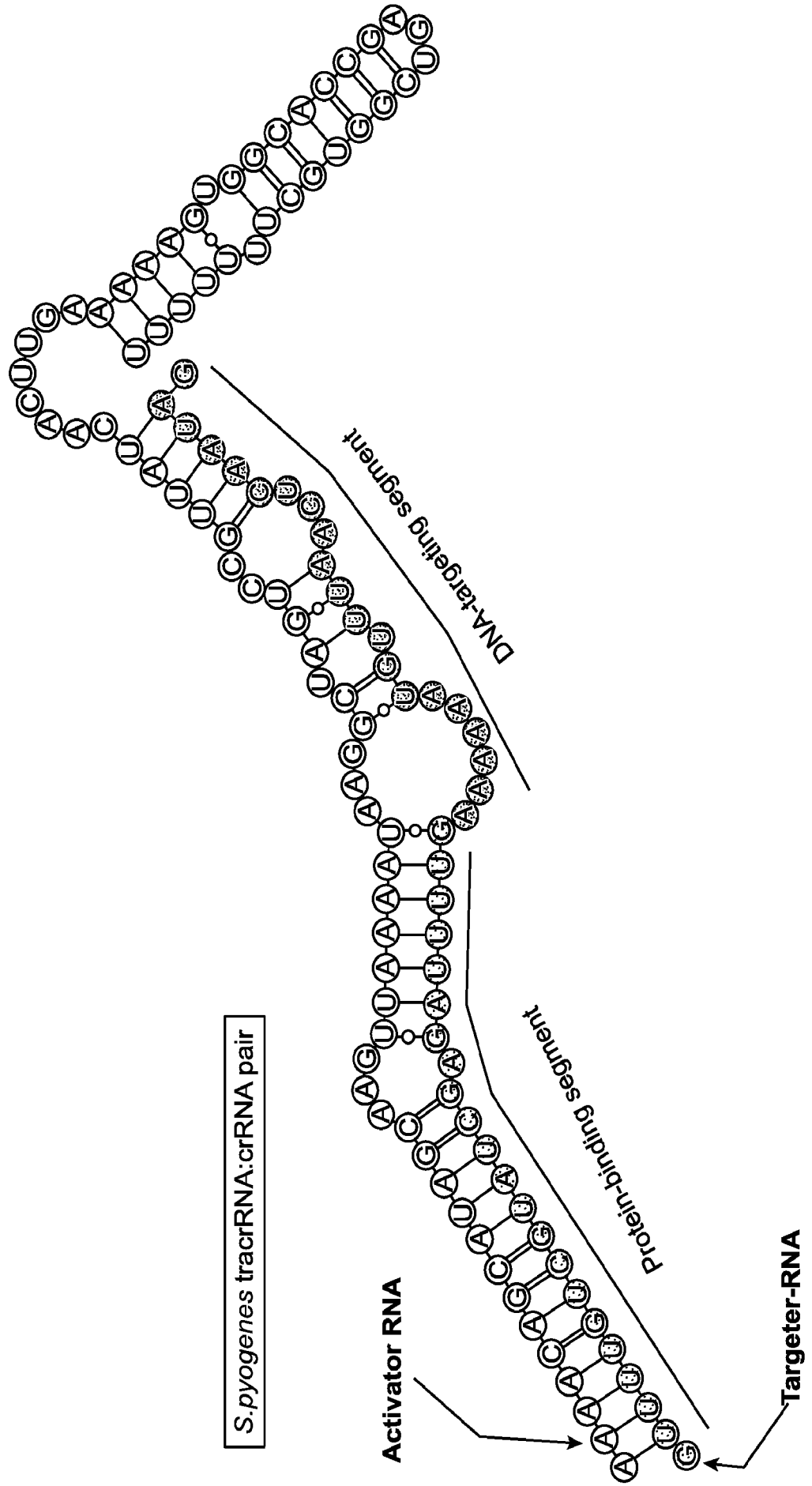
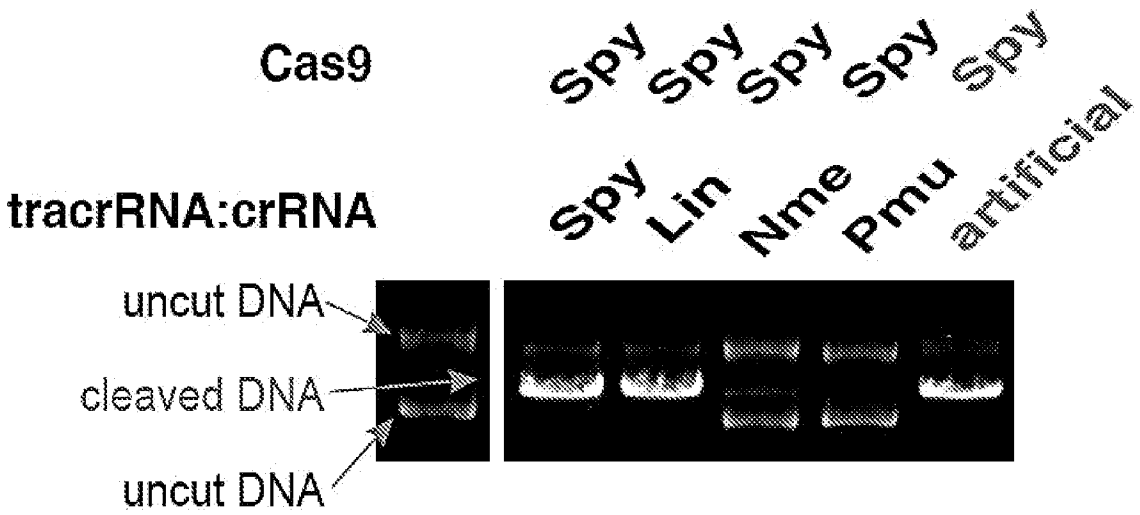

S.pyogenes tracrRNA:crRNA pair
Activator RNA
Protein-binding segment
DNA-targeting segment
Targeter-RNA

FIG. 57 (Cont. 1)

FIGURE 57 B


Cas9
Spy
Spy
Spy
Spy
Spy
tracrRNA:crRNA
Spy
Lin
Nme
Pmu
artificial
uncut DNA
cleaved DNA
uncut DNA