Fig. 1A

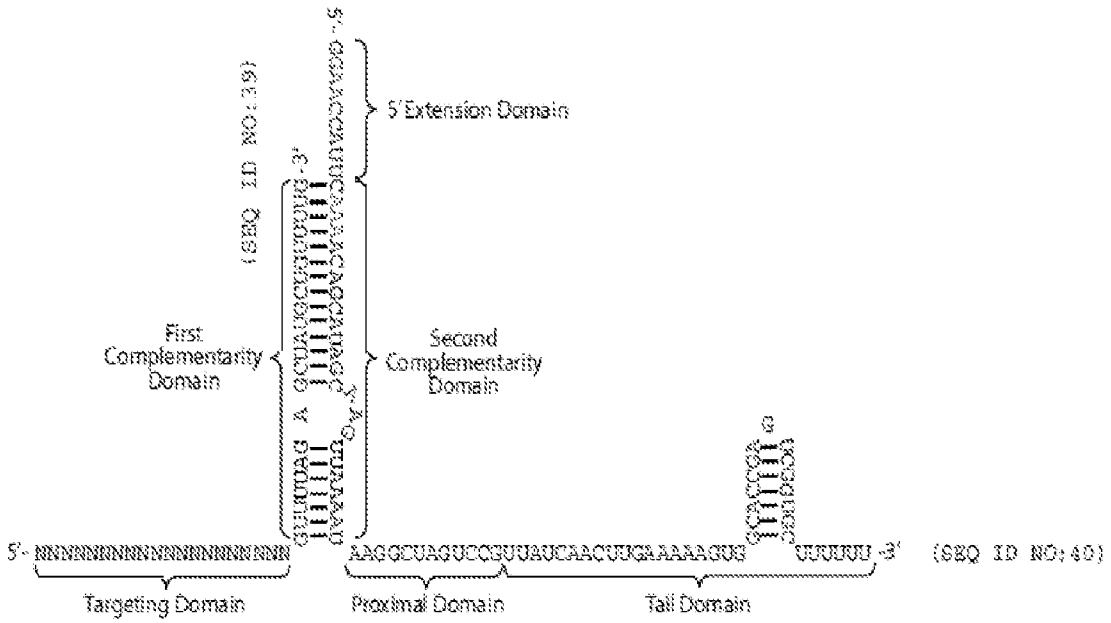

(SEQ ID NO:39)
5'Extension Domain
First Complementarity Domain
Second Complementarity Domain
5'-
(SEQ ID NO:40)
Targeting Domain
Proximal Domain
Tail Domain

Fig. 1B

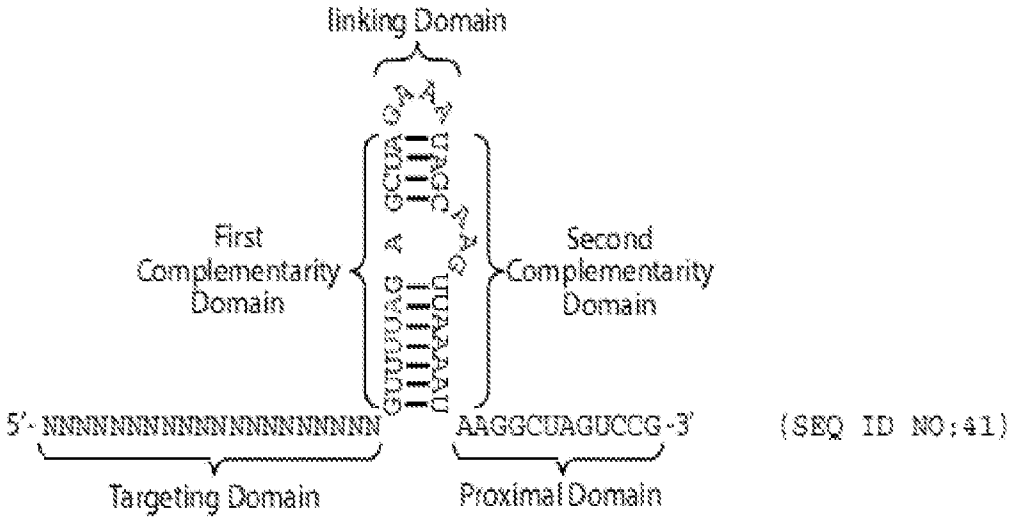

linking Domain
First Complementarity Domain
Second Complementarity Domain
5'-NNNNNNNNNNNNNNNNNNNN
AAGGCUAGUCCG-3'
(SEQ ID NO:41)
Targeting Domain
Proximal Domain

Fig. 1C

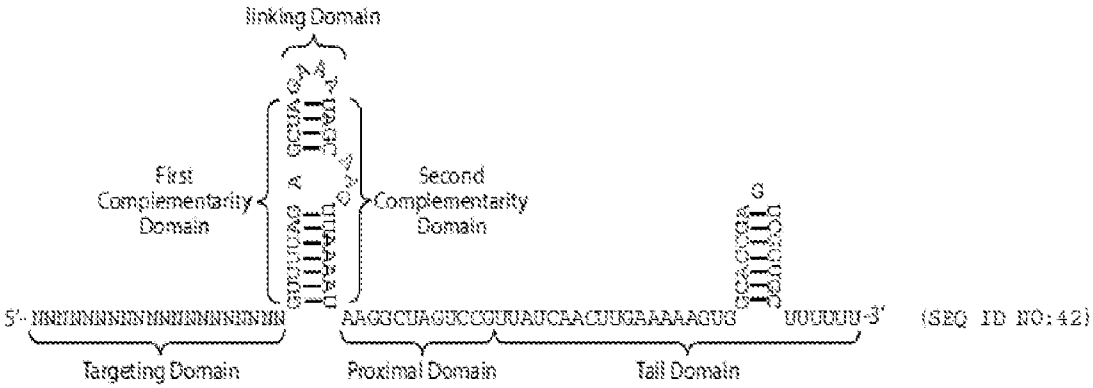

linking Domain
First Complementarity Domain
Second Complementarity Domain
5'-
AAGGCUAGUCCGUUAUCAACUUGAAAAAGUG
UUUUUU-3'
(SEQ ID NO:42)
Targeting Domain
Proximal Domain
Tail Domain

Fig. 1D

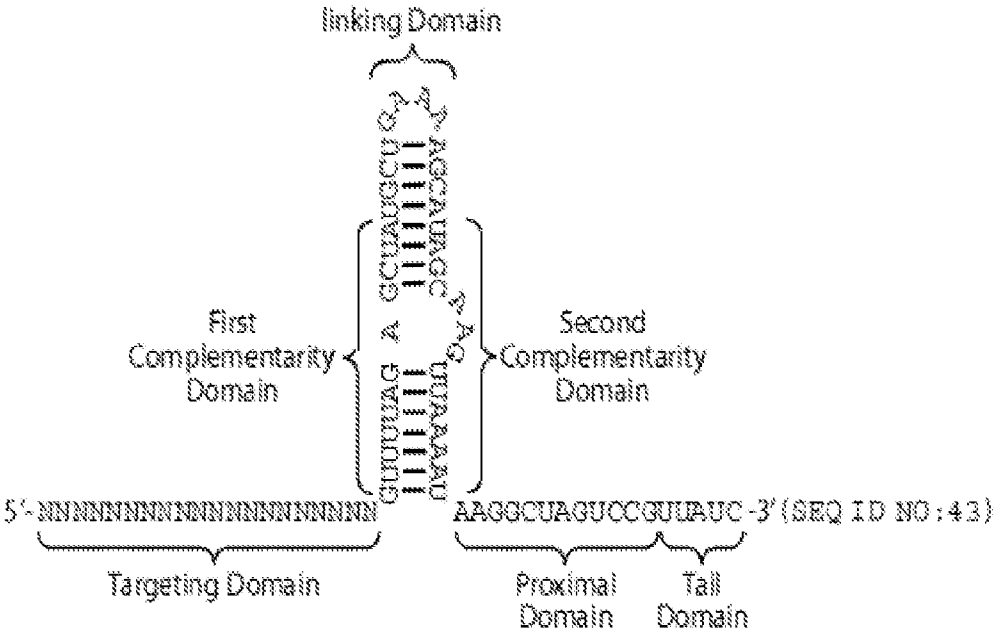

linking Domain
First Complementarity Domain
Second Complementarity Domain
5'-NNNNNNNNNNNNNNNNNNNN
AAGGCUAGUCCGUUAUC-3' (SEQ ID NO:43)
Targeting Domain
Proximal Domain
Tail Domain

Fig. 1E

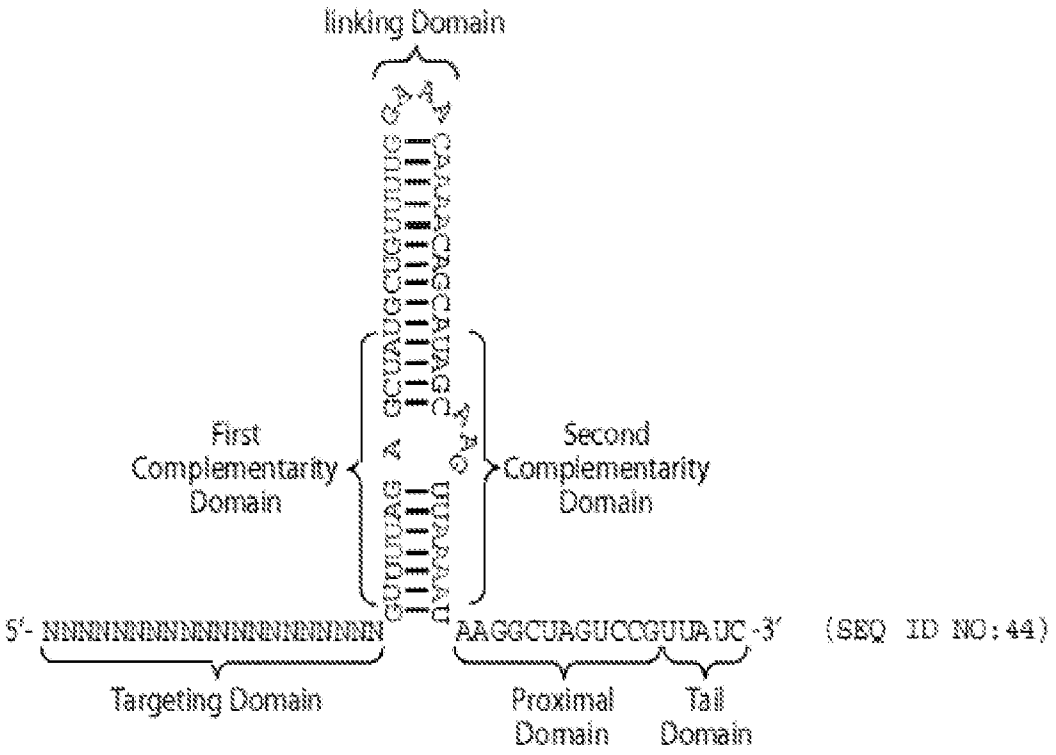

linking Domain
First Complementarity Domain
Second Complementarity Domain
5'- NNNNNNNNNNNNNNNNNNNN
AAGGCUAGUCCGUUAUC-3'
(SEQ ID NO:44)
Targeting Domain
Proximal Domain
Tail Domain

Fig. 1F

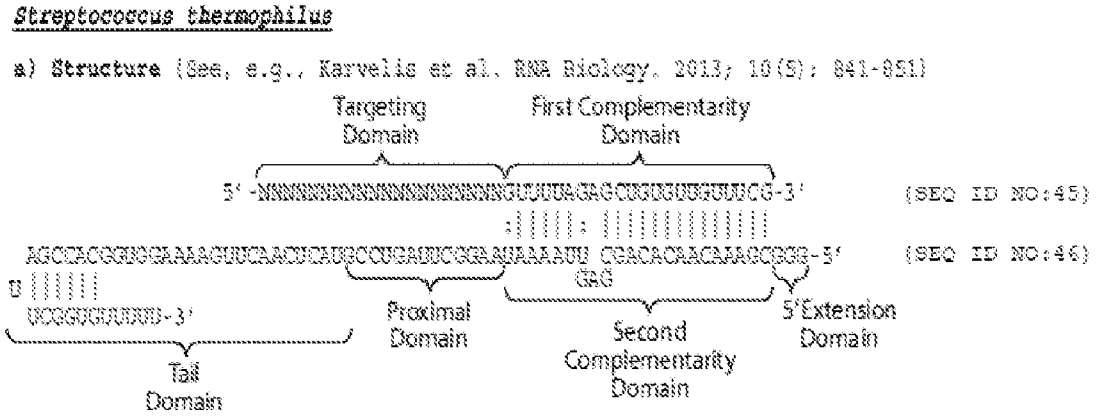

Streptococcus thermophilus
a) Structure (See, e.g., Karvelis et al. RNA Biology. 2013; 10(5): 841-851)
Targeting Domain
First Complementarity Domain
5'-NNNNNNNNNNNNNNNNNNNNGUUUUAGAGCUGGUGUUGUUUCG-3' (SEQ ID NO:45)
AGCCACGGGUGAAAACUUCAACUCAUGCCUGAUUCGGAAUAAAAUU CGACACAACAAAGCGCG-3' (SEQ ID NO:46)
GAG
U
UCGGUGUUUU-3'
Proximal Domain
5'Extension Domain
Second Complementarity Domain
Tail Domain

Fig. 1G

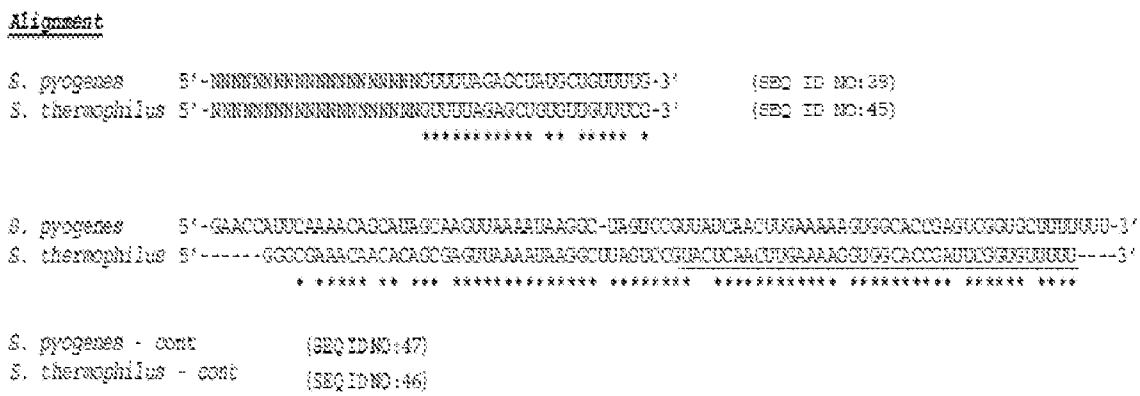

Alignment
S. pyogenes 5'-NNNNNNNNNNNNNNNNNNNNGUUUUAGAGCUAUGCUGUUUUG-3' (SEQ ID NO:39)
S. thermophilus 5'-NNNNNNNNNNNNNNNNNNNNGUUUUAGAGCUGUGUUGUUUUC-3' (SEQ ID NO:45)
S. pyogenes 5'-GAACCAUUCAAAACAGCAUAGCAAGUUAAAAUAAGGC-UAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUUUU-3'
S. thermophilus 5'------GCCCCRAACAACACAGCRGUUAAAAURAGCUURGUCGUACUCAACUUGAAAAGGUGGCACCGAUUCGGUGUUUU----3'
S. pyogenes - cont (SEQ ID NO:47)
S. thermophilus - cont (SEQ ID NO:46)

Fig. 1H

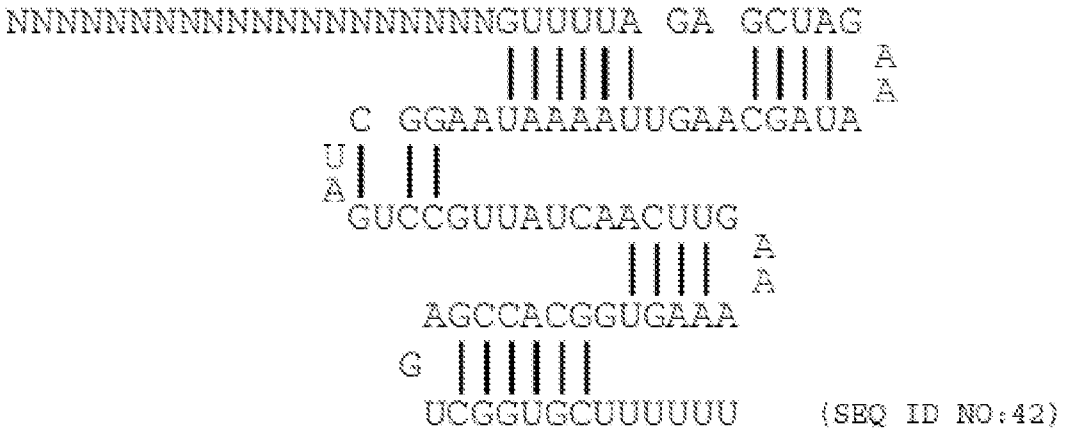

NNNNNNNNNNNNNNNNNNNNGUUUUA GA GCUAG
A
A
C GGAAUAAAAUUGAACGAUA
U
A
GUCCGUUAUCAACUUG
A
A
AGCCACGGUGAAA
G
UCGGUGCUUUUUU
(SEQ ID NO:42)

Fig. 1I

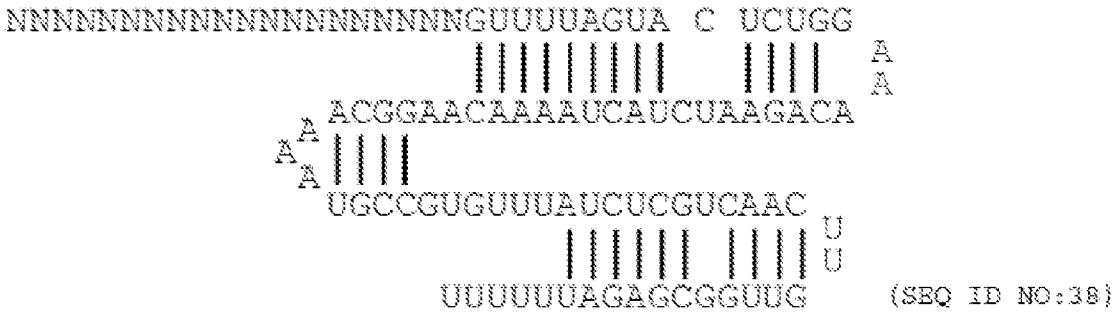

NNNNNNNNNNNNNNNNNNNNGUUUUAGUA C UCUGG
A
A
ACGGAACAAAAUCAUCUAAGACA
A
A
A
UGCCGUGUUUAUCUCGUCAAC
U
U
UUUUUUAGAGCGGUUG
(SEQ ID NO:38)

Fig. 2B


SM QRLFQEFLAVYDNTFENSS-------LQEQNVQVEEILTDKISKSAKKDRVLKLFPNEKSN
SP DKLFIQLVQTYNQLFEENP-------INASGVDAKAILSARLSKSRRLENLIAQLPGEKKN
ST QKNFQDFLDTYNAIFESDL-------SLENSKQLEEIVKDKISKLEKKDRILKLFPGEKNS
LI DGIYKQFIQTYNQVFASGIEDGSLKKLEDNEDVAKILVEKVTRKEKLERILKLYPGEKSA
Motif: *--*-***--Y*--f----------*---I*--****--*-*-**---P-EK--
SM GRFAEFLKLIVGNQADFKKHFELEEKAPLQSSKDTYEEELEVLLAQIGDNYAELFLSAKK
SP GLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKN
ST GIFSEFLKLIVGNQADFRKCFNLDEKASLNFSKESYDEDLETLLGYIGDDYSDVFLKAKK
LI GMFAQFISLIVGSKGNFQKPFDLIEKSDIECAKDSYEEDLESLLALIGDEYAELFVAAKN
Motif: G-F-***-L-*G---*F*--F*L-E-*-*--*K**Y***L*-LL--IGD*Y***F*-AK*
SM LYDSILLSGILTVTDVGTKAPLSASMIQRYNEHQNDLAQLKQFTRQKLSDKYNEVFSDVS
SP LSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQS
ST LYDAILLSGFLTVTDNETEAPLSSAMIKRYNEHKEDLALLKEYIRNISLKTYNEVFKDDT
LI AYSAVVLSSIITVAETETNAKLSASMIERFDTHEEDLGELKAFTKLHLPKHYEEIFSNTE
Motif: ---***LS-**-V----T*A-LS**MI*R**-H--DL--LK--**-------Y*E*F-*--
SM KDGYAGYIDGKTNQEAFYKYLKGLLNKIEGSGYFLDKIERKDFLRKQRTFDNGSIPHQIH
SP KNGYAGYIDGGASQEEFYKFIKPILEKMEGTEELLVKLNREDLLRKQRTFDNGSIPHQIH
ST KNGYAGYIDGKTNQEDFYVYLKKLLAEFEGADYFLEKIDREDFLRKQRTFDNGSIPYQIH
LI KHGYAGYIDGKTKQADFYKYMKMTLENIEGADYFIAKIEKENFLRKQRTFDNGAIPHQLH
Motif: K-GYAGYIDG-*-Q--FY-**K--L-***G*--**-K***E**LRKQRTFDNG*IP*Q*H

Fig. 2A

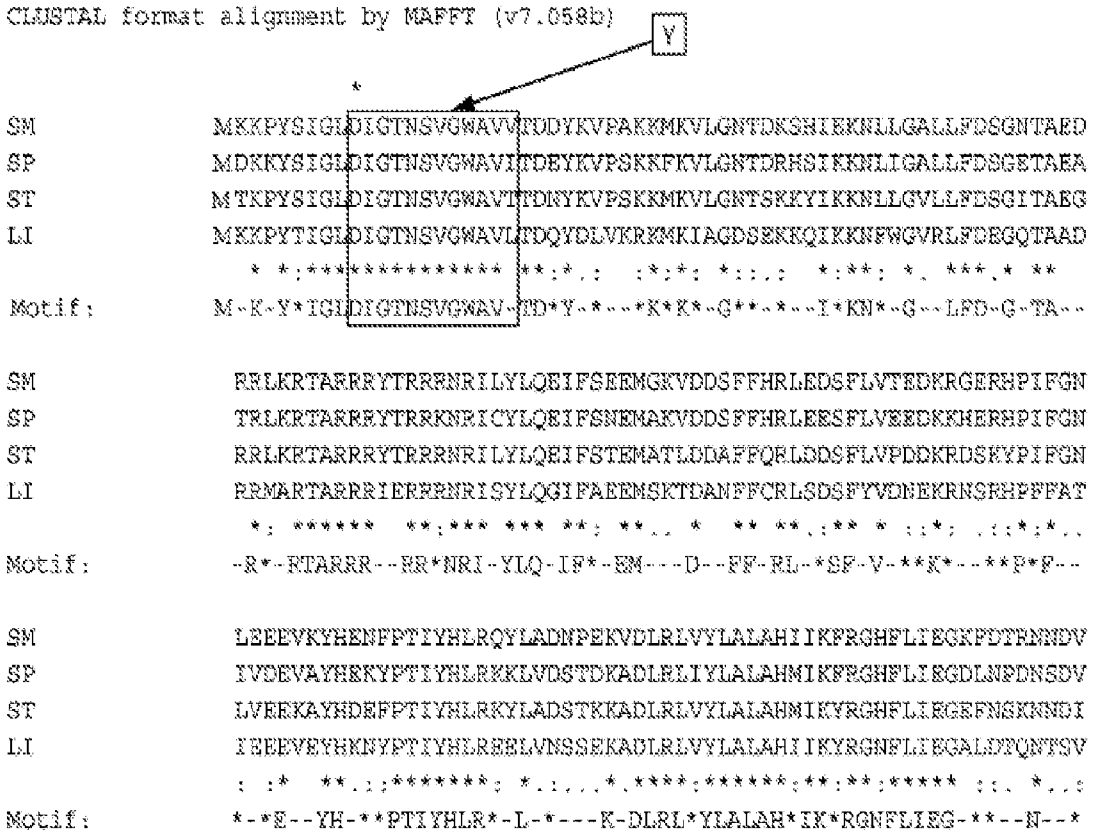

CLUSTAL format alignment by MAFFT (v7.058b)
Y
SM MKKPYSIGLDIGTNSVGWAVVTDDYKVPAKKMKVLGNTDKSHIEKNLLGALLFDSGNTAED
SP MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEA
ST MTKPYSIGLDIGTNSVGWAVTTDNYKVPSKKMKVLGNTSKKYIKKNLLGVLLFDSGITAEG
LI MKKPYTIGLDIGTNSVGWAVLTDQYDLVKRKMKIAGDSEKKQIKKNFWGVRLFDEGQTAAD
Motif: M-K-Y*IGLDIGTNSVGWAV-TD*Y-*---*K*K*-G**-*--I*KN*-G--LFD-G-TA--
SM RRLKRTARRRYTRRRNRILYLQEIFSEEMGKVDDSFFHRLEDSFLVTEDKRGERHPIFGN
SP TRLKRTARREYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGN
ST RRLKRTARRRYTRRRNRILYLQEIFSTEMATLDDAFFQRLDDSFLVPDDKRDSKYPIFGN
LI RRMARTARRRIERRRNRISYLQGIFAEEMSKTDANFFCRLSDSFYVDNEKRNSRHPFFAT
Motif: -R*-RTARRR--RR*NRI-YLQ-IF*-EM---D--FF-RL-*SF-V-**K*---**P*F--
SM LEEEVKYHENFPTIYHLRQYLADNPEKVDLRLVYLALAHIIKFRGHFLIEGKFDTRNNDV
SP IVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDV
ST LVEEKAYHDEFPTIYHLRKYLADSTKKADLRLVYLALAHMIKYRGHFLIEGEFNSKNNDI
LI IEEEVEYHKNYPTIYHLREELVNSSEKADLRLVYLALAHIIKYRGNFLIEGALDTQNTSV
Motif: *-*E--YH-**PTIYHLR*-L-*---K-DLRL*YLALAH*IK*RGNFLIEG-**--N--*

Fig. 2C

```
SM          LQEMRAIIRRQAEFYPFLADNQDRIEKLLTFRIPYYVGPLARGKSDFAWLSRKSADKITP
SP          LGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITP
ST          LQEMRAILDKQAKFYPFLAKNKERIEKILTFRIPYYVGPLARGNSDFAWSIRKRNEKITP
LI          LEELEAILHQQAKYYPFLKENYDKIKSLVTFRIPYFVGPLANGQSEFAWLTRKADGEIEP
            * *:.**: :* .:**** .* ::*:.:******:*****.*:* ***  **    * *
Motif:      L-E*-AI*-*Q--*YPFL--N-**I*-**TFRIPY*VGPLA-G*S-FAW--RK----I-P

SM          WNFDEIVDKESSAEAFINRMTNYDLYLPNQKVLPKHSLLYEKFTVYNELTKVKYKTE-QG
SP          WNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMR
ST          WNFEDVIDKESSAEAFINRMTSFDLYLPEEKVLFKHSLLYETFNVYNELTKVRFIAESMR
LI          WNIEEKVDFGKSAVDFIEKMTNKDTYLPKENVLPKHSLCYQKYLVYNELTKVRYIND-QG
            **::: :*   **  **::**, *  **:::******** *: : ********::  :
Motif:      WN****-*D---SA--PI**MT--D--LP***VLPKHSL-Y*-*-VYNELTKV**--*---

SM          KTAFFDAMMKQEIFDGVFKVYRKVTKDKLMDFLEKEFDEFRIVDLTGLDKENKVFNASYG
SP          KPAFLSGEQKNAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDR---FNASLG
ST          DYQFLDSKQKKDIVRLYFKDKRKVTDKDIIEYL-HAIYGYDGIELKGIEKQ---FNSSLS
LI          KTSYFSGQEKEQIFNDLFKQKRKVKKKDLELFL-RNMSHVESPTIEGLEDS---FNSSYS
            ,  ::.:: *: *,   **  ***, ..:     : :       : *::.    **:* .
Motif:      ---**--*-K*-I----FK--RKV----*-----*-*--------*-G**-----FN*S--

SM          TYHDLCKIL-DKDFLDNSKNEKILEDIVLTLTLFEDREMIRKRLENYSDLLTKEQVKKLE
SP          TYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLK
ST          TYHDLLMIINDKEFLDDSSNEAIIEEIIHTLTIFEDREMIKQRLSKFENIFDKSVLKKLS
LI          TYHDLLKVGIKQEILDNPVNTEMLENIVKILTVFEDKRMIKEQLQQFSDVLDGVVLKKLE
            ***** ::  .:::**;  *  ::*:*;  **;***:.**.::*. : .::    :*:*.
Motif:      TYHDL-**---***LD*--N--**E*I*--LT*FED*-MI-**L--*--**----*K*L-
```

Fig. 2D

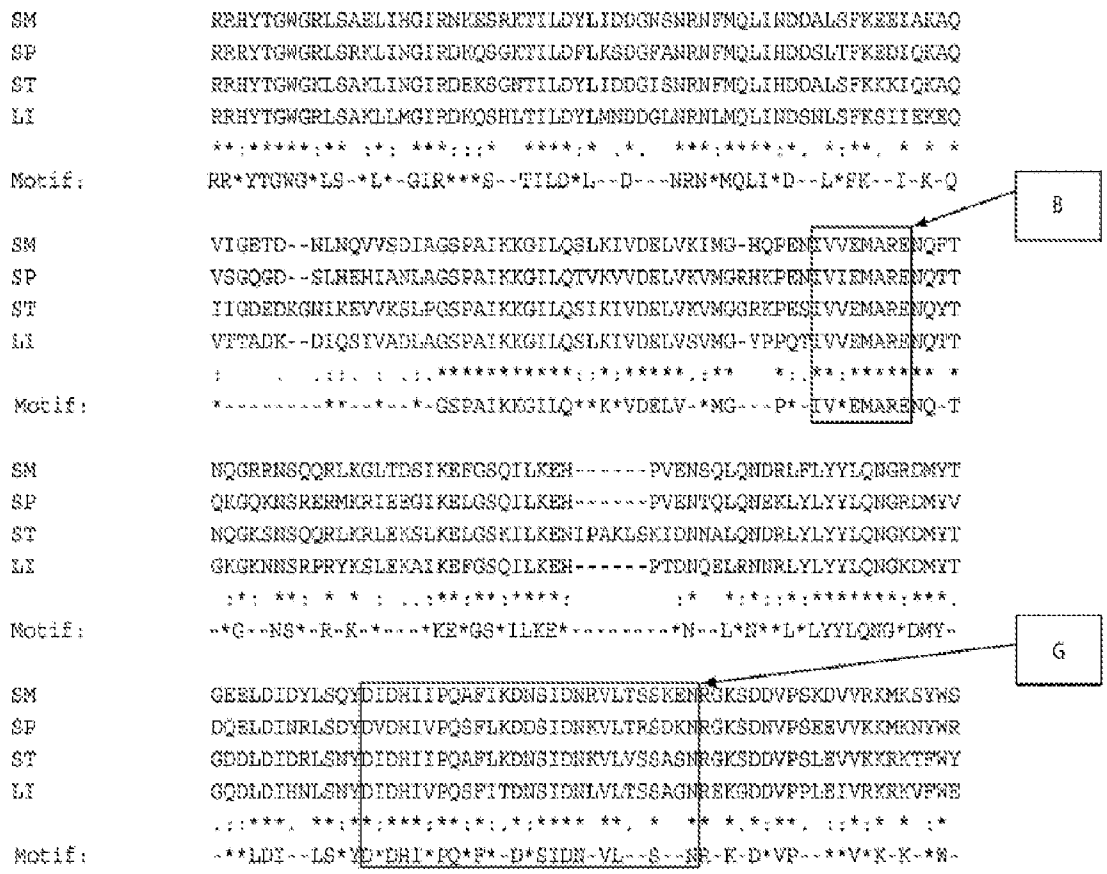

SM RRHYTGWGRLSAELINGIRNKESSRETILDYLIDDGNSNRNFMQLIHDDALSFKEEIAKAQ
SP RERYTGWGRLSRELINGIRDMQSGETILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQ
ST RRHYTGWGKLSAELINGIRDEKSGNTILDYLIDDGISNRNFMQLIHDDALSFKEKIQKAQ
LI RRHYTGWGRLSAKLLMGIRDKQSHLTILDYLMNDDGLNRNLMQLINDSNLSFKSIIEKEQ
Motif: RR*YTGWG*LS-*L*-GIR***S--TILD*L--D---NRN*MQLI*D--L*FK--I-K-Q
B
SM VIGETD--NLNQVVSDIAGSPAIKKGILQSLKIVDELVKIMG-HQPENEVVEMARENQFT
SP VSGQGD--SLNEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENEVIEMARENQTT
ST IIQDEDKGNIKEVVKSLPQSPAIKKGILQSIKIVDELVKVMGGRKPESEVVEMARENQYT
LI VTTADK--DIQSIVADLAGSPAIKKGILQSLKIVDELVSVMG-VPPQTEVVEMARENQTT
Motif: *---------**--*--*-GSPAIKKGILQ**K*VDELV-*MG---P*-EV*EMARENQ-T
SM NQGRRNSQQRLKGLTDSIKEFGSQILKEH------PVENSQLQNDRLFLYYLQNGRDMYT
SP QKGQKNSRERMKRIEEGIKELGSQILKEH------PVENTQLQNEKLYLYYLQNGRDMYV
ST NQGKSNSQQRLKGLEKSLKELGSKILKENIPAKLSKIDNNALQNDRLYLYYLQNGKDMYT
LI GKGKNNSRPRYKSLEKAIKEFGSQILKEH------PTDNQELRNNRLYLYYLQNGKDMYT
Motif: -*G--NS*-R-K-*---*KE*GS*ILKE*---------*N--L*N**L*LYYLQNG*DMY-
G
SM GEELDIDYLSQYDIDHIIPQAFIKDNSIDNRVLTSSKENRGKSDDVPSKDVVRKMKSYWS
SP DQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWR
ST GDDLDIDRLSNYDIDHIIPQAFLKDNSIDNKVLVSSASNRGKSDDVPSLEVVKKRKTFWY
LI GQDLDIHNLSNYDIDHIVPQSFITDNSIDNLVLTSSAGNREKGDDVPPLEIVRKRKVFWE
Motif: -**LDI--LS*YD*DHI*PQ*F*-D*SIDN-VL--S--NR-K-D*VP--**V*K-K-**W-

Fig. 2E

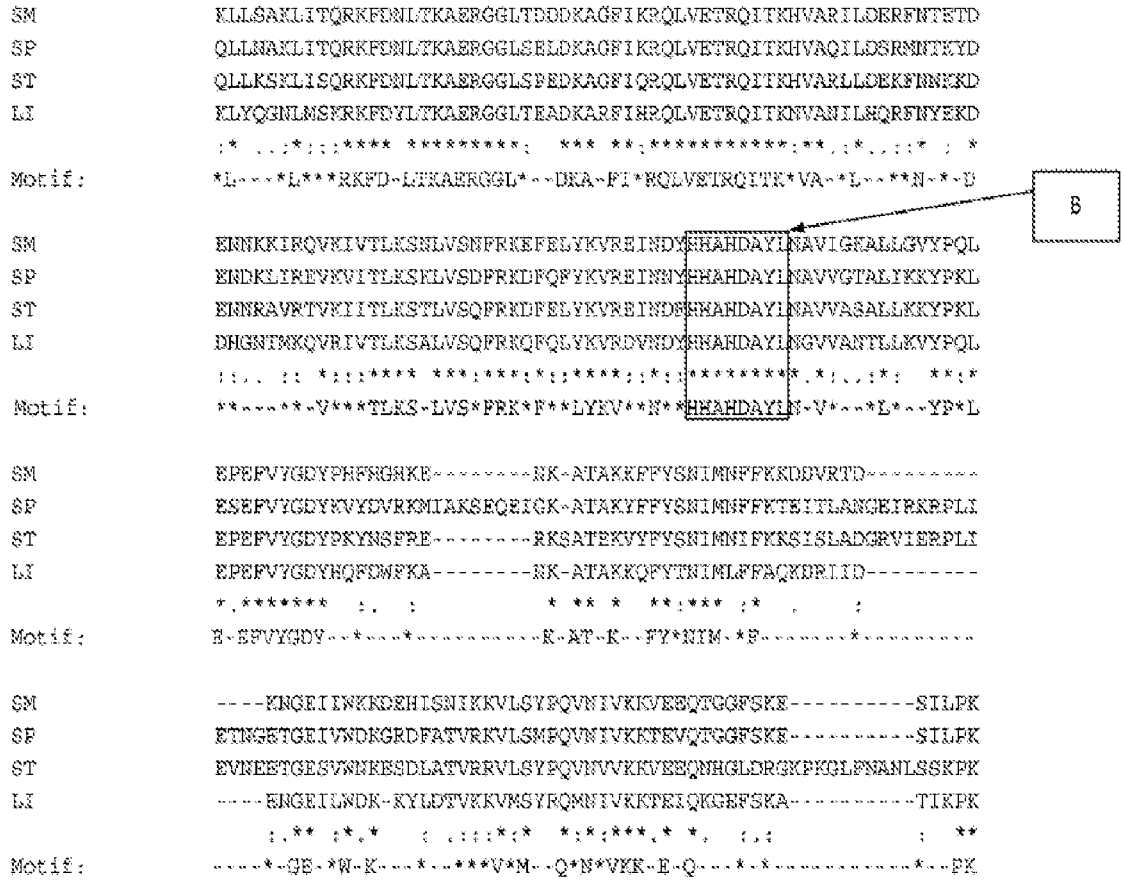

SM KLLSAKLITQRKFDNLTKAERGGLTDDDKAGFIKRQLVETRQITKHVARILDERFNTETD
SP QLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYD
ST QLLKSKLISQRKFDNLTKAERGGLSFEDKAGFIQRQLVETRQITKHVARLLDEKFMNKKD
LI KLYQGNLMSFRKFDYLTKAERGGLTEADKARFIHRQLVETRQITKNVANILHQRFNYEKD
Motif: *L----*L***RKFD-LTKAERGGL*--DKA-FI*RQLVETRQITK*VA--*L--**N-*-D
SM EMNKKIRQVKIVTLKSNLVSNFRKEFELYKVREINDYHHAHDAYLNAVIGKALLGVYPQL
SP ENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKL
ST EMNRAVETVKIITLKSTLVSQFRKDFELYKVREINDSHHAHDAYLNAVVASALLKKYPKL
LI DHGNTMKQVRIVTLKSALVSQFRKQFQLYKVRDVNDYHHAHDAYLNGVVANTLLKVYPQL
Motif: **----**-V***TLKS-LVS*FRK*F**LYKV**R**HHAHDAYLN-V*--*L*--YP*L
SM EPEFVYGDYPHFNGNKE--------NK-ATAKKFFYSNIMNFFKKDDVRTD----------
SP ESEFVYGDYKVYDVRKMIAKSEQEIGK-ATAKYFFYSNIMNFFKTEITLANGEIRKRPLI
ST EPEFVYGDYPKYNSFRE--------RKSATEKVYFYSNIMNIFKKSISLADGRVIERPLI
LI EPEFVYGDYHQFDMFKA--------NK-ATAKKQFYTNIMLFFAQKDRIID---------
Motif: E-EFVYGDY--*---*----------R-AT-K--FY*NIM-*F--------*----------
SM ----ENGEIINKKDEHISNIKKVLGYFQVNIVKKVEEQTGGFSKE----------SILPK
SP ETNGETGEIVNDKGRDFATVRKVLSMFQVNIVKKTEVQTGGFSKE----------SILPK
ST EVNEETGESVNNKESDLATVRRVLSYFQVNVVKKVEEQNHGLDRGKPKGLFNANLSSKPK
LI ----ENGEILNDK-KYLDTVKKVMSYFQMNIVKKTEIQKGEFSKA----------TIKPK
Motif: ----*-GE-*W-K---*--***V*M--Q*N*VKK-E-Q---*-*--------------*--FK
B

Fig. 2F


SM GNSDK-LIPRKTKKFYWDTKKYGGFDSPIVAYSILVIADIEKGKSKKLKTVKALVGVTIM
SP RNSDK-LIARKKD---WDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIM
ST PNSNENLVGAKEY---LDPKKYGGYAGISNSFTVLVKGTIEKGAKKKITNVLEFQGISIL
LI GNSSK-LIPRKTN---WDPMKYGGLDSPNMAYAVVI--EYAKGKN-KLVFEKKIIEVTIM
Motif: -NS-*-L*--K------D--EYGG------*****------EG---K*------*---**I*
SM EKMTFERDPVAFLEKKGYRNVQEENIIKLPKYSLFKLENGRKELLAS-------ARELQK
SP ERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLAS-------AGELQK
ST DRINYRKDKLNFLLEKGYKDI--ELIIELPKYSLFELSDGSRRMLASILSTNNKRGEIHK
LI ERKAFEKDEKAFLEEQGYRQP--EVLAKLPKYTLYECEEGRRRMLAS-------ANEAQK
Motif: **--*-**---FL--*GY**------*-*LPRY*L**--*G-*R*LAS----------E-*K
SM GNEIVLPNHLGTLLYHAKNIHKV-------DEPKHLDYVDKHKDEFKELLDVVSNFSKKYT
SP GNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQL-FVEQHKHYLDEIIEQISEFSKRVI
ST GNQIFLSQKFVKLLYHAKRISNF------INENHRKYVENHKKEFEELFYYILEFNENYV
LI GNQQVLPNHLVTLLHHAANCEVS-------DGKSLDYIESNREMFAELLAHVSEFAKRYT
Motif: GN*--L--*---*L*-A---------------*-*---***-**---*-E*---*-*F-*---
SM LAEGNLEKIKELYAQNNGEDLKELASSFI---------NLLTFTAIGAPATFKFFDKNIDR
SP LADANLDKVLSAYNKHRDKPIREQAENII---------HLFTLTNLGAPAAFKYFDTTIDR
ST GAKKNGKLLNSAFQSWQNHSIDELCSSFIGPTGSERKGLFELTSRGSAADFEFLGVKIPR
LI LAEANLNKINQLFEQNKEGDIKAIAQSFV---------DLMAFNAMGAPASFKFFETTIER
Motif: -A--N---*---*---*--------*-------**----------L*-*---G*-A-F***---I-R

Fig. 2G

```
SM          KR-YTSTTEILNATLIHQSITGLYETRIDLNKLGGD   (SEQ ID NO:1)
SP          KR-YTSTKEVLDATLIHQSITGLYETRIDLSQLGGD   (SEQ ID NO:2)
ST          YRDYTPSSLLKDATLIHQSVTGLYETRIDLAKLGEG   (SEQ ID NO:4)
LI          KR-YNNLKELLNSTIIYQSITGLYESRKRLD----D   (SEQ ID NO:5)
             * *.  . : ::*:*:**:*****:*  *     .
Motif:      -R-Y------*-**T*I*QS*TGLYE*R--L-------  (SEQ ID NO:14)
```

Fig. 3A

Alignment of the N terminal RuvC-like Domains disclosed in Chylinski et al. (excluding sequence outliers).
(CLUSTAL format alignment by MAFFT (v7.058b))

1,12	DIGTNSVGWAVT	(SEQ ID NO:120)
3,20	DVGTNSVGWAVT	(SEQ ID NO:121)
15	DMGTNSVGWAVT	(SEQ ID NO:122)
4	DVGTSSVGWAVT	(SEQ ID NO:123)
7	DIGTASVGWAVT	(SEQ ID NO:52)
6	DVGTGSVGWAVT	(SEQ ID NO:53)
9	DIGTNSVGWAVV	(SEQ ID NO:54)
10	DIGTNSVGWAVI	(SEQ ID NO:55)
11	DIGTNSVGWAVL	(SEQ ID NO:56)
42	DLGTNSIGWAVV	(SEQ ID NO:57)
48	DLGTNSIGWAI-	(SEQ ID NO:58)
43	DLGTNSIGWALV	(SEQ ID NO:59)
2	DIGTNSVGWCVT	(SEQ ID NO:60)
14	DIGTNSVGYAVT	(SEQ ID NO:61)
5	DMGTGSLGWAVT	(SEQ ID NO:62)
16	DIGTSSVGWAAI	(SEQ ID NO:63)
8	DLGTGSVGWAVV	(SEQ ID NO:64)
22	DLGVGSVGWAIV	(SEQ ID NO:65)
23	DLGIASIGWAII	(SEQ ID NO:66)
24	DLGIASVGWAIV	(SEQ ID NO:67)
25	DLGVASVGWSIV	(SEQ ID NO:68)
26	DIGIASVGWAIL	(SEQ ID NO:69)
28	DLGISSVGWSVI	(SEQ ID NO:70)
32	DIGIASVGWSVI	(SEQ ID NO:71)
33	DVGIGSIGWAVI	(SEQ ID NO:72)
39	DLGVGSIGFAIV	(SEQ ID NO:73)
34	DIGYASIGWAVI	(SEQ ID NO:74)
47	DTGTNSLGWAIV	(SEQ ID NO:75)
50	DLGTNSIGWCLL	(SEQ ID NO:76)
49	DIGTDSLGWAVF	(SEQ ID NO:77)
18	DIGSNSIGFAVV	(SEQ ID NO:78)
41	DLGVGSIGVAVA	(SEQ ID NO:79)
45	DLGIASCGWGVV	(SEQ ID NO:80)

Fig. 3B

21	DLGIASVGWCLT	(SEQ ID NO:81)
27	DIGIGSVGVGIL	(SEQ ID NO:82)
29	DIGITSVGYGLI	(SEQ ID NO:83)
30	DIGITSVGFGII	(SEQ ID NO:84)
31	DVGITSTGYAVL	(SEQ ID NO:85)
40	DLGITSFGYAIL	(SEQ ID NO:86)
17	DIGNASVGWSAF	(SEQ ID NO:87)
19	DVGTNSCGWVAM	(SEQ ID NO:88)
35	DVGERSIGLAAV	(SEQ ID NO:89)
36	DVGLNSVGLAAV	(SEQ ID NO:90)
37	DVGLMSVGLAAI	(SEQ ID NO:91)
38	DVGTFSVGLAAI	(SEQ ID NO:92)
13	DIGTGSVGYACM	(SEQ ID NO:92)
44	DLGTTSIGFAHI	(SEQ ID NO:94)
46	DLGTNSIGSSVR	(SEQ ID NO:95)
	* * * *	

Fig. 4A

Alignment of the N terminal RuvC-like Domains disclosed in Chylinski *et al.* (CLUSTAL format alignment by MAFFT (v7.058b))

1,12	D----IGTNSVGWAVT	(SEQ ID NO:120)
3,20	D----VGTNSVGWAVT	(SEQ ID NO:121)
15	D----MGTNSVGWAVT	(SEQ ID NO:122)
4	D----VGTSSVGWAVT	(SEQ ID NO:123)
7	D----IGTASVGWAVT	(SEQ ID NO:52)
6	D----VGTGSVGWAVT	(SEQ ID NO:53)
9	D----IGTNSVGWAVV	(SEQ ID NO:54)
10	D----IGTNSVGWAVI	(SEQ ID NO:55)
52	D----IGTNSIGWAVI	(SEQ ID NO:96)
11	D----IGTNSVGWAVL	(SEQ ID NO:56)
42	D----LGTNSIGWAVV	(SEQ ID NO:57)
48	D----LGTNSIGWAI-	(SEQ ID NO:58)
43	D----LGTNSIGWALV	(SEQ ID NO:59)
2	D----IGTNSVGWCVT	(SEQ ID NO:60)
14	D----IGTNSVGYAVT	(SEQ ID NO:61)
5	D----MGTGSLGWAVT	(SEQ ID NO:62)
16	D----IGTSSVGWAAI	(SEQ ID NO:63)
8	D----LGTGSVGWAVV	(SEQ ID NO:64)
22	D----LGVGSVGWAIV	(SEQ ID NO:65)
23	D----LGIASIGWAII	(SEQ ID NO:66)
24	D----LGIASVGWAIV	(SEQ ID NO:67)
68	D----LGIASVGWAVV	(SEQ ID NO:97)
25	D----LGVASVGWSIV	(SEQ ID NO:68)
26	D----IGIASVGWAIL	(SEQ ID NO:69)
58	D----IGIASVGWAVL	(SEQ ID NO:98)
59	D----IGIASIGWAVI	(SEQ ID NO:99)
61	D----IGIASVGWAII	(SEQ ID NO:100)
64	D----VGIASVGWAVI	(SEQ ID NO:101)
62	D----IGIASVGWAL-	(SEQ ID NO:102)
67	D----IGIASVGWAMV	(SEQ ID NO:103)
32	D----IGIASVGWSVI	(SEQ ID NO:71)
28	D----LGISSVGWSVI	(SEQ ID NO:70)
63	D----IGITSVGWAVI	(SEQ ID NO:104)

Fig. 4B

33	D----VGIGSIGWAVI	(SEQ ID NO:72)
57	D----LGISSLGWAIV	(SEQ ID NO:109)
39	D----LGVGSIGFAIV	(SEQ ID NO:73)
34	D----IGYASIGWAVI	(SEQ ID NO:74)
50	D----LGTNSIGWCLL	(SEQ ID NO:76)
54	D----LGTNSIGWGLL	(SEQ ID NO:106)
47	D----TGTNSLGWAIV	(SEQ ID NO:75)
49	D----IGTDSLGWAVF	(SEQ ID NO:77)
51	D----LGSTSLGWAIF	(SEQ ID NO:107)
58	D----IGISSIGWAFS	(SEQ ID NO:108)
21	D----LGIASVGWCLT	(SEQ ID NO:81)
45	D----LGIASCGWGVV	(SEQ ID NO:80)
18	D----IGSNSIGFAVV	(SEQ ID NO:78)
65	D----IGTTSIGFSVI	(SEQ ID NO:109)
29	D----IGITSVGYGLI	(SEQ ID NO:83)
30	D----IGITSVGFGII	(SEQ ID NO:84)
44	D----LGTTSIGFAHI	(SEQ ID NO:94)
27	D----IGIGSVGVGIL	(SEQ ID NO:82)
41	D----LGVGSIGVAVA	(SEQ ID NO:79)
31	D----VGITSTGYAVL	(SEQ ID NO:85)
40	D----LGITSPGYAIL	(SEQ ID NO:86)
53	D----IGTSSIGWWLY	(SEQ ID NO:110)
55	D----LGSNSLGWFVT	(SEQ ID NO:111)
56	D----LGANSLGWFVV	(SEQ ID NO:112)
17	D----IGNASVGWSAF	(SEQ ID NO:87)
19	D----VGTNSCGWVAM	(SEQ ID NO:88)
35	D----VGERSIGLAAV	(SEQ ID NO:89)
36	D----VGLNSVGLAAV	(SEQ ID NO:90)
37	D----VGLMSVGLAAI	(SEQ ID NO:91)
38	D----VGTFSVGLAAI	(SEQ ID NO:92)
13	D----IGTGSVGYACM	(SEQ ID NO:93)
46	D----LGTNSIGSSVR	(SEQ ID NO:95)
60	DIGLRIGITSCGWSI-	(SEQ ID NO:113)
69	D----MGAKYTGVFYA	(SEQ ID NO:114)
73	D----LGGKNTGFFSF	(SEQ ID NO:115)
74	D----LGVKNTGVFSA	(SEQ ID NO:116)
70	D----LGAKFTGVALY	(SEQ ID NO:117)
71	D----LGGKFTGVCLS	(SEQ ID NO:118)
72	D----LGGTYTGTFIT	(SEQ ID NO:119)

Fig. 5A

Alignment of the HNH-like Domains disclosed in Chylinski et al.
(CLUSTAL format alignment by MAFFT (v7.058b))

```
1              YDIDHIYPRS-LTKD------D&F-DNLVLCERTAN      (SEQ ID NO:196)
2              -DIDHIYPRSKVIKD------DSF-DNLVLVLKNEN      (SEQ ID NO:197)
3              -DRDHIYPQS-KIKD------DSI-DNLVLVNKTYN      (SEQ ID NO:198)
4              -DIDHIYPRS-KIKD------DSI-TNRVLVEKDIN      (SEQ ID NO:195)
6              -DIDHIYPQS-KIKD------DSI-SNRVLVCSSCN      (SEQ ID NO:134)
5              -DIDHIYPQS-KTMD------DSL-NNRVLVKKNYN      (SEQ ID NO:135)
7              -DQDHIYPKS-KIYD------DSL-ENRVLVKKNLN      (SEQ ID NO:126)
8              -QIDHIVPQS-LVKD------DSF-DNRVLVVPSEN      (SEQ ID NO:127)
9              -DIDHIIPQA-FIKD------NSI-DNRVLTSSKEN      (SEQ ID NO:128)
12             -DIDHIIPQA-FLKD------NSI-DNKVLVSSASN      (SEQ ID NO:129)
16             -DIDHIIPQA-YTKD------NSL-DNRVLVSNITN      (SEQ ID NO:130)
11             -DIDHIVPQS-FITD------NSI-DNLVLTSSAQN      (SEQ ID NO:131)
10             -DVDHIVPQS-FLKD------DSI-DNKVLTRSDKN      (SEQ ID NO:132)
14             -NIDHIYPQS-MVKD------DSL-DNKVLVQSEIN      (SEQ ID NO:133)
18             -DIDHILPQS-LIKD------DSL-DNRVLVNATIN      (SEQ ID NO:134)
19             -DIDHILPQS-FIKD------DSL-ENRVLVKKAVN      (SEQ ID NO:135)
13             -EVDHIFPRS-FIKD------DSI-DNKVLVIKKMN      (SEQ ID NO:136)
15             -EVDHIIPRS-YIKD------DSF-ENKVLVYREEN      (SEQ ID NO:137)
17             -DIDHIIPQA-VTQN------DSI-DNRVLVARAEN      (SEQ ID NO:138)
22             -EIDHIIPYS-ISFD------DSS-SNKLLVLAESN      (SEQ ID NO:139)
24             -EIDHIIPYS-LCFD------DSS-ANKVLVHKQSN      (SEQ ID NO:140)
32             -DIDHIIPYS-RSMD------DSY-SNKVLVLSGEN      (SEQ ID NO:141)
63             -DIDHIIPYS-KSMD------DSF-NNKVLCLAEEN      (SEQ ID NO:142)
59             -EIDHIYPYS-RSFD------DSY-MNKVLVFTKQN      (SEQ ID NO:143)
65             -QIDHIYPYS-RSMD------DSY-MNKVLVLTDEN      (SEQ ID NO:144)
64             -EIDHIIPFS-RSFD------DSL-SNKILVLGSEN      (SEQ ID NO:145)
68             -EIDHALPFS-RTWD------DSF-NNKVLVLGSEN      (SEQ ID NO:146)
69             -EIDHALPFS-RTWD------DSF-NNKVLVLASEN      (SEQ ID NO:147)
28             -EIDHIIPIS-ISLD------DSI-NNKVLVLSKAN      (SEQ ID NO:148)
30             -EVDHIIPIS-ISLD------DSI-TNKVLVTHREN      (SEQ ID NO:149)
62             -QVDHALPYS-RSYD------DSK-NNKVLVLTHEN      (SEQ ID NO:150)
27             -EVDHILPLS-ITFD------DSL-ANKVLVYATAN      (SEQ ID NO:151)
26             -EIDHIIPRS-ISFD------DAR-SNKVLVYRSEN      (SEQ ID NO:152)
```

Fig. 5B

29	-EVDHIIPRS-VSFD------NSY-HNKVLVKQSEN	(SEQ ID NO:153)
67	-DIDHILPYS-ITFD------DSF-RNKVLVTSQEN	(SEQ ID NO:154)
58	-EIDHILPRS-RSAD------DSF-ANKVLCLARAN	(SEQ ID NO:155)
51	-EIEHLLPFS-LTLD------DSM-ANKTVCFRQAN	(SEQ ID NO:156)
55	-DIDHILPFS-VSLD------DSA-ANKVVCLREAN	(SEQ ID NO:157)
57	-DIDHLIPFS-ISWD------DSA-ANKVVCMRYAN	(SEQ ID NO:158)
56	-DIDHILPVA-MTLD------DSP-ANKIICMRYAN	(SEQ ID NO:159)
54	-DVDHILPYS-RTLD------DSF-PNRTLCLREAN	(SEQ ID NO:160)
52	-EIEHILPFS-RTLD------DSL-NNRTVAMRRAN	(SEQ ID NO:161)
31	-EVDHIIPYS-ISWD------DSY-TNKVLTSAKCN	(SEQ ID NO:162)
45	-QVDHILPWS-RFGD------DSY-LNKTLCTARSN	(SEQ ID NO:163)
53	-QVDHILPFS-KTLD------DSF-ANKVLAQHDAN	(SEQ ID NO:164)
60	-QIDHAFPLS-RSLD------DSQ-SNKVLCLTSSN	(SEQ ID NO:165)
21	-DIDHIVPRS-ISFD------DSF-SNLVIVNKLDN	(SEQ ID NO:166)
23	-EIEHIIPYS-MSYD------NSQ-ANKILTEKAEN	(SEQ ID NO:167)
25	-EIDHVIPYS-KSAD------DSW-FNKLLVKKSTN	(SEQ ID NO:168)
49	-EMDHILPYS-RSLD------NGW-HNRVLVHGKDN	(SEQ ID NO:169)
33	-EVDHIVPYS-LILD------NTI-NNKALVYAEEN	(SEQ ID NO:170)
42	-EIEHVIPQS-LYFD------DSF-SNKVICEAEVN	(SEQ ID NO:171)
43	-DIEHIIPQA-RLFD------DSF-SNKTLEARSVN	(SEQ ID NO:172)
44	-EIEHIVPKA-RVFD------DSF-SNKTLTFHRIN	(SEQ ID NO:173)
20	-DKDHIIPQS-MKKD------DSIINNLVLVNKNAN	(SEQ ID NO:174)
66	-EVEHIWPRS-RSFD------NSP-RNKTLCRKDVN	(SEQ ID NO:175)
61	-IVNHIIPYN-RSFD------DTY-HNRVLTLTETK	(SEQ ID NO:176)
46	-DMEHTIPKS-ISFD------NSD-QNLTLCESYYN	(SEQ ID NO:177)
47	-DIEHTIPRS-AGGD------STK-MNLTLCSSRFN	(SEQ ID NO:178)
48	-DIEHTIPRS-ISQD------NSQ-MNKTLCSLKFN	(SEQ ID NO:179)
50	-DIDHVIPLA-RGGR------DSL-DNMVLCQSDAN	(SEQ ID NO:180)
39	-DIEHLFPIA-ESED------NGR-NNLVISHSACN	(SEQ ID NO:181)
41	-DVDHIFPRD-DTAD------NSY-GNKVVAHRQCN	(SEQ ID NO:182)
40	-DIEHIVPQS-LGGL------STD-YNTIVTLKSVN	(SEQ ID NO:183)
35	-ELDHIVPRT-DGGS------NRH-ENLAITCGACN	(SEQ ID NO:184)
36	-EMDHIVPRKGVGST------NTR-TNFAAVCAECN	(SEQ ID NO:185)
37	-EMDHIVPRKGVGST------NTR-VNLAAACAACN	(SEQ ID NO:186)
38	-EMDHIVPRAGQGST------NTR-ENLVAVCHRCN	(SEQ ID NO:187)
70	-EIDHILPRS-LIKDARGIVFNAE-PNLIYASSRGN	(SEQ ID NO:188)
71	-EIDHIIPRS-LTGRTKKTVFNSE-ANLIYCSSKGN	(SEQ ID NO:189)
73	-EIDHIIPRS-LTLKKSESIYNSE-VNLIFVSAQGN	(SEQ ID NO:190)

Fig. 5C

```
72          -EIDHIYPRS-LSKKHPGVIFNSE-VNLIYCSSQGN      (SEQ ID NO:191)
74          -EIDHILPRS-HTLKIYGTVFNPE-GNLIYVHQNDN      (SEQ ID NO:192)
75          -ELDHIIPRS-HKKY---GTLNDE-ANLICVTRGDN      (SEQ ID NO:193)
34          -ELEHIVPHS-FRQS------NAL-SSLVLTWPGVN      (SEQ ID NO:194)
               :*  *              .    .        :
```

Fig. 6A

Alignment of the HNH-like Domains disclosed in Chylinski et al. (excluding sequence outliers). (CLUSTAL format alignment by MAFFT (v7.058b))

1	YDIDHIYPRS-LTKDDS-FDNLVLCERTAN	(SEQ ID NO:196)
2	-DIDHIYPRSKVIKDDS-FDNLVLVLKNEN	(SEQ ID NO:197)
3	-DRDHIYPQS-KIKDDS-IDNLVLVNKTYN	(SEQ ID NO:198)
4	-DIDHIYPRS-KIKDDS-ITNRVLVEKDIN	(SEQ ID NO:195)
6	-DIDHIYPQS-KIKDDS-ISNRVLVCSSCN	(SEQ ID NO:124)
5	-DIDHIYPQS-KIMDDS-LNNRVLVKKNYN	(SEQ ID NO:125)
7	-DQDHIYPKS-KIYDDS-LENRVLVKKNLN	(SEQ ID NO:126)
8	-QIDHIVPQS-LVKDDS-FDNRVLVVPSEN	(SEQ ID NO:127)
9	-DIDHIIPQA-FIKDNS-IDNRVLTSSKEN	(SEQ ID NO:128)
12	-DIDHIIPQA-FLKDNS-IDNKVLVSSASN	(SEQ ID NO:129)
16	-DIDHIIPQA-YTKDNS-LDNRVLVSNITN	(SEQ ID NO:130)
11	-DIDHIVPQS-FITDNS-IDNLVLTSSACN	(SEQ ID NO:131)
10	-DVDHIVPQS-FLKDDS-IDNKVLTRSDKN	(SEQ ID NO:132)
14	-NIDHIVPQS-MVKDDS-LDNKVLVQSEIN	(SEQ ID NO:133)
18	-DIDHILPQS-LIKDDS-LDNRVLVNATIN	(SEQ ID NO:134)
19	-DIDHILPQS-FIKDDS-LENRVLVKKAVN	(SEQ ID NO:135)
13	-EVDHIFPRS-FIKDDS-IDNKVLVIKKMN	(SEQ ID NO:136)
15	-EVDHIIPRS-YIKDDS-FENKVLVYREEN	(SEQ ID NO:137)
17	-DIDHIIPQA-VTQNDS-IDNRVLVARAEN	(SEQ ID NO:138)
21	-DIDHIVPRS-ISFDDS-FSNLVIVNKLDN	(SEQ ID NO:166)
22	-EIDHIIPYS-ISFDDS-SSNKLLVLAESN	(SEQ ID NO:139)
24	-EIDHIIPYS-LCFDDS-SANKVLVHKQSN	(SEQ ID NO:140)
28	-EIDHIIPIS-ISLDDS-INNKVLVLSKAN	(SEQ ID NO:148)
30	-EVDHIIPIS-ISLDDS-ITNKVLVTHREN	(SEQ ID NO:149)
27	-EVDHILPLS-ITFDDS-LANKVLVYATAN	(SEQ ID NO:151)
26	-EIDHIIPRS-ISFDDA-RSNKVLVYRSEN	(SEQ ID NO:152)
29	-EVDHIIPRS-VSFDNS-YHNKVLVKQSEN	(SEQ ID NO:153)
31	-EVDHIIPYS-ISWDDS-YTNKVLTSAKCN	(SEQ ID NO:162)
32	-DIDHIIPYS-RSMDDS-YSNKVLVLSGEN	(SEQ ID NO:141)
23	-EIEHIIPYS-MSYDNS-QANKILTEKAEN	(SEQ ID NO:167)
33	-EVDHIVPYS-LILDNT-INNKALVYAEEN	(SEQ ID NO:170)
25	-EIDHVIPYS-KSADDS-WFNKLLVKKSTN	(SEQ ID NO:168)
49	-EMDHILPYS-RSLDNG-WHNRVLVHGKDN	(SEQ ID NO:169)
42	-EIEHVIPQS-LYFDDS-FSNKVICEAEVN	(SEQ ID NO:171)
43	-DIEHIIPQA-RLFDDS-FSNKTLEARSVN	(SEQ ID NO:172)

Fig. 6B

```
44    -EIEHIVPKA-RVFDDS-FSNKTLTPHRIN    (SEQ ID NO:173)
20    -DKDHIIPQS-MKKDDSIINNLVLVNKNAN    (SEQ ID NO:174)
45    -QVDHILPWS-RFGDDS-YLNKTLCTARSN    (SEQ ID NO:163)
50    -DIDHVIPLA-RGGRDS-LDNMVLCQSDAN    (SEQ ID NO:180)
46    -DMEHTIPKS-ISFDNS-DQNLTLCESYYN    (SEQ ID NO:177)
47    -DIEHTIPRS-AGGDST-KMNLTLCSSRFN    (SEQ ID NO:178)
48    -DIEHTIPRS-ISQDNS-QMNKTLCSLKFN    (SEQ ID NO:179)
39    -DIEHLFPIA-ESEDNG-RNNLVISHSACN    (SEQ ID NO:181)
41    -DVDHIFPRD-DTADNS-YGNKVVAHRQCN    (SEQ ID NO:182)
40    -DIEHIVPQS-LGGLST-DYNTIVTLKSVN    (SEQ ID NO:183)
35    -ELDHIVPRT-DGGSNR-HENLAITCGACN    (SEQ ID NO:184)
36    -EMDHIVPRKGVGSTNT-RTNFAAVCAECN    (SEQ ID NO:185)
37    -EMDHIVPRKGVGSTNT-RVNLAAACAACN    (SEQ ID NO:186)
38    -EMDHIVPRAGQGSTNT-RENLVAVCHRCN    (SEQ ID NO:187)
34    -ELEHIVPHS-FRQSNA-LSSLVLTWPGVN    (SEQ ID NO:194)
```

Fig. 7

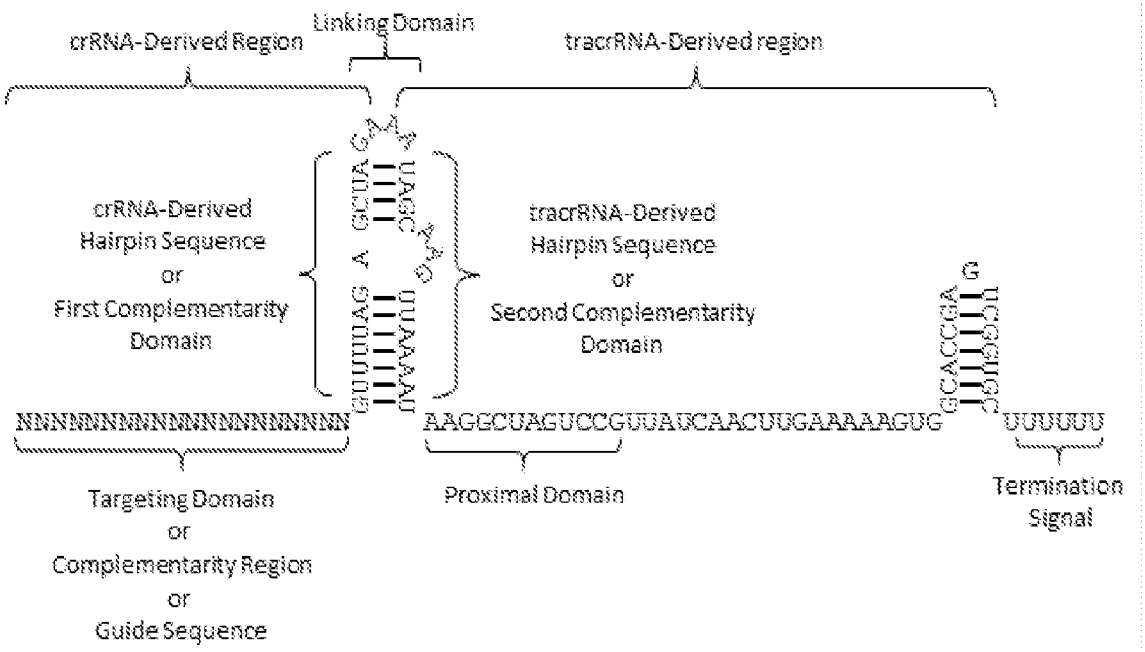

crRNA-Derived Region
Linking Domain
tracrRNA-Derived region
crRNA-Derived Hairpin Sequence or First Complementarity Domain
tracrRNA-Derived Hairpin Sequence or Second Complementarity Domain
NNNNNNNNNNNNNNNNNNNN
AAGGCUAGUCCGUUAUCAACUUGAAAAAGUG
UUUUUU
Targeting Domain or Complementarity Region or Guide Sequence
Proximal Domain
Termination Signal

Fig. 8A

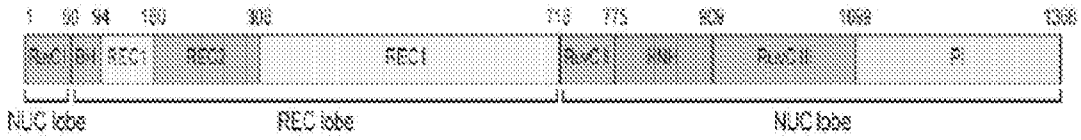

1
90
94
180
308
719
775
909
1098
1368
REC1
REC2
REC3
NUC lobe
REC lobe
NUC lobe

Fig. 8B

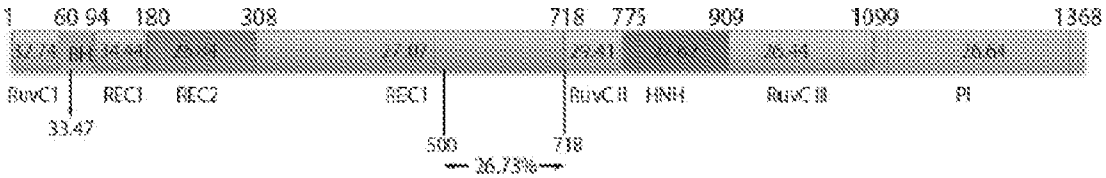
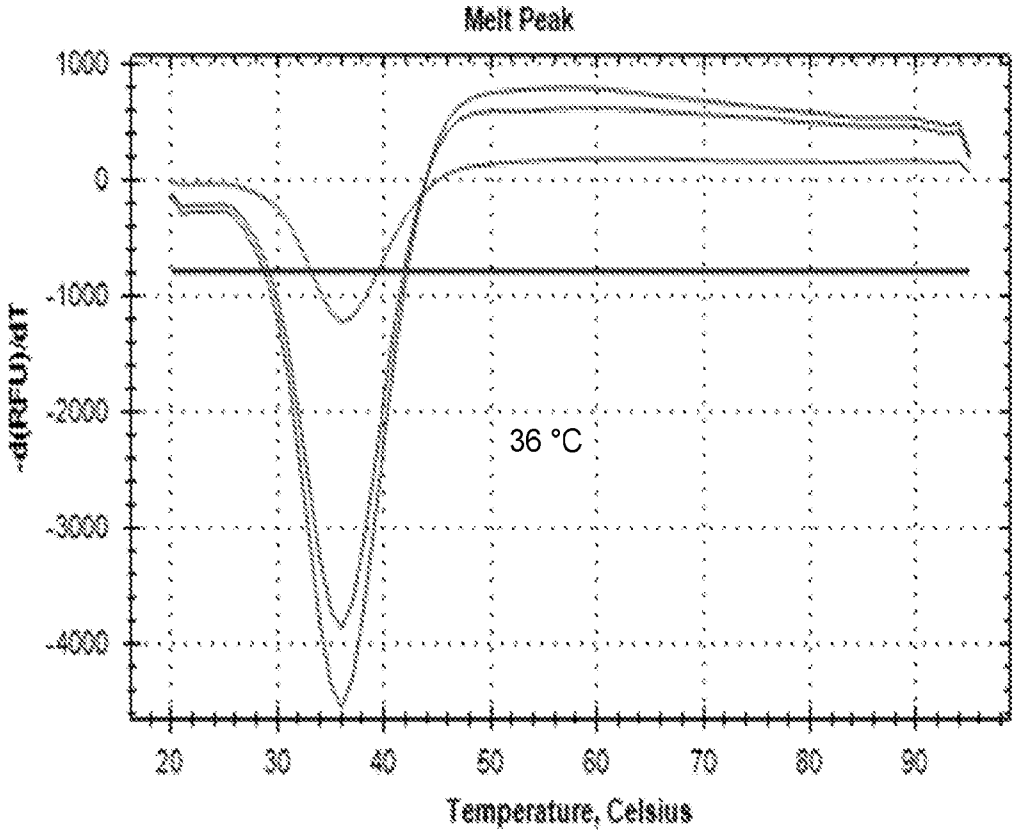
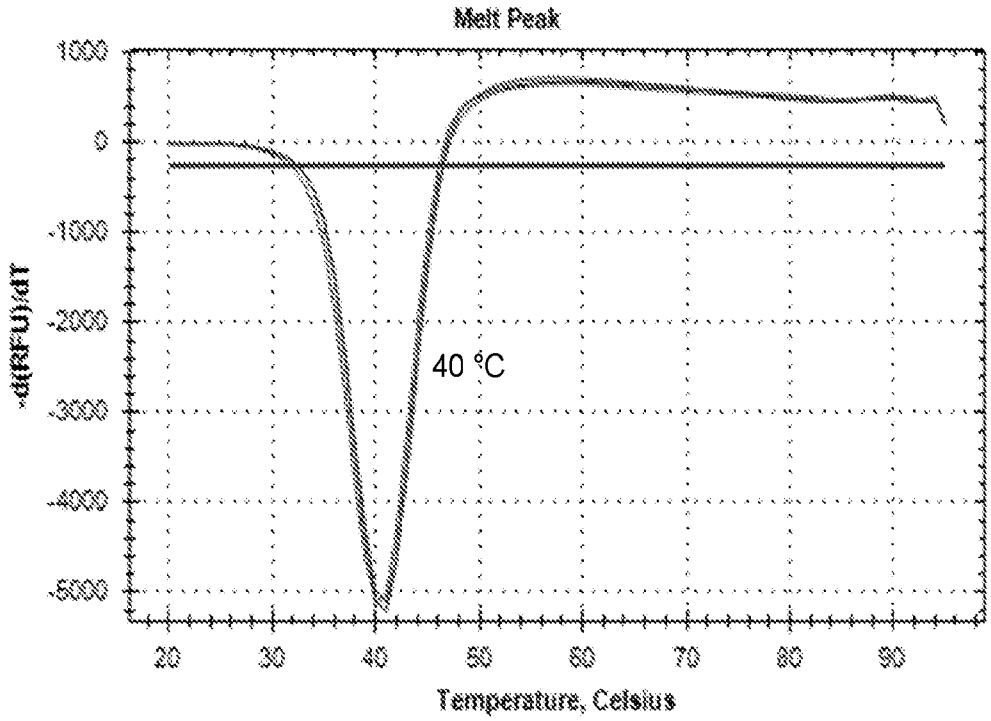

1
60 94
180
308
718
775
909
1099
1368
RuvC I
REC3
REC2
REC1
RuvC II
HNH
RuvC III
PI
33 47
503
738
26.73%

Fig. 9A



S. aureus Cas9

Fig. 9B



S. pyogenes Cas9

Fig. 10

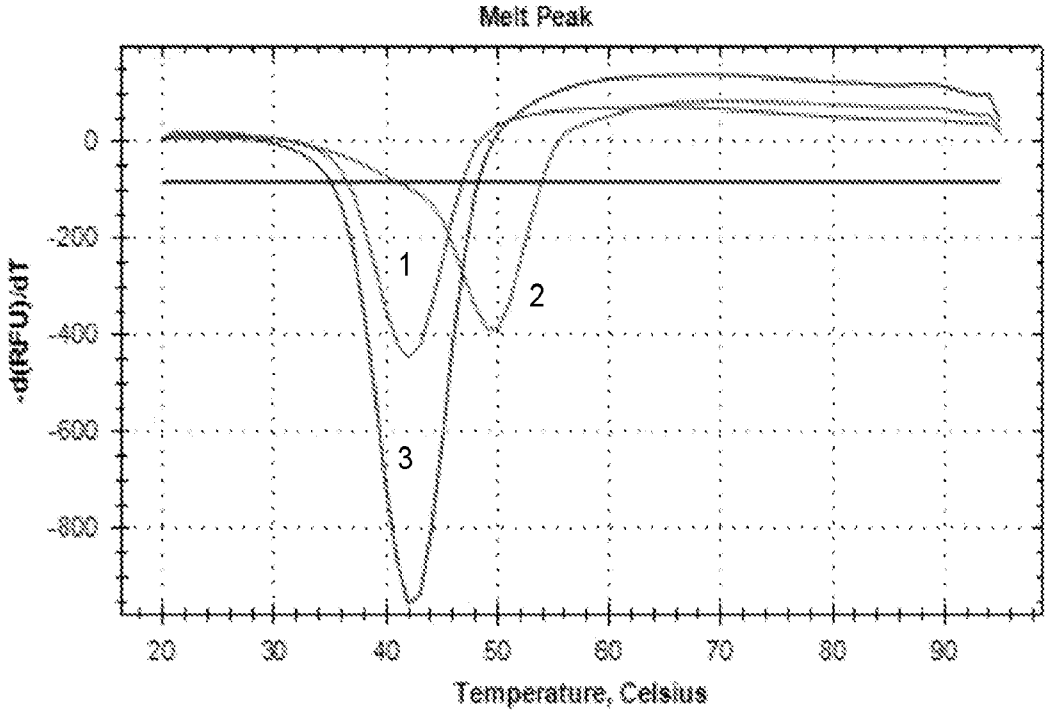

Melt Peak
0
-200
-400
-600
-800
dH(J/g)/dT
1
2
3
20
30
40
50
60
70
80
90
Temperature, Celsius

Fig. 11A

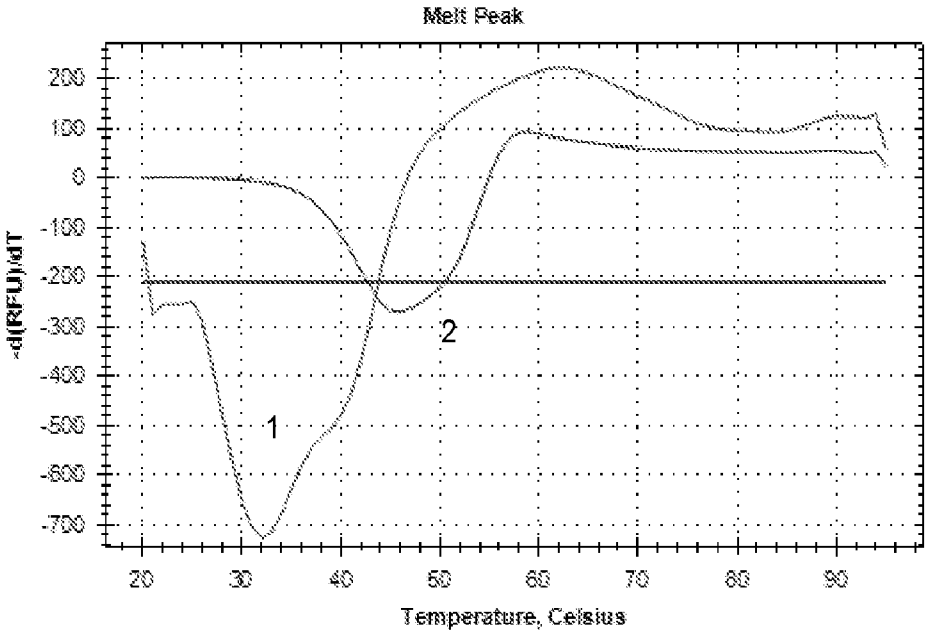

Melt Peak
200
100
0
-100
-200
-300
-400
-500
-600
-700
-d(RFU)/dT
1
2
20
30
40
50
60
70
80
90
Temperature, Celsius

Fig. 11B

