FIG. 1

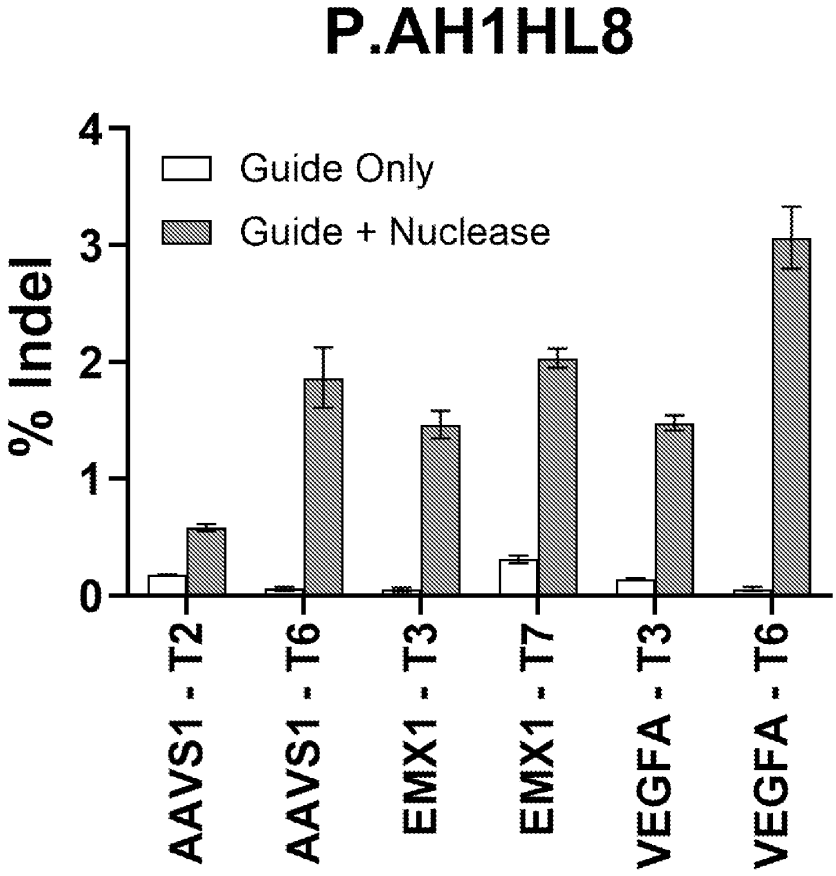

P.AH1HL8
% Indel
0
1
2
3
4
Guide Only
Guide + Nuclease
AAVS1 - T2
AAVS1 - T6
EMX1 - T3
EMX1 - T7
VEGFA - T3
VEGFA - T6

FIG. 2A

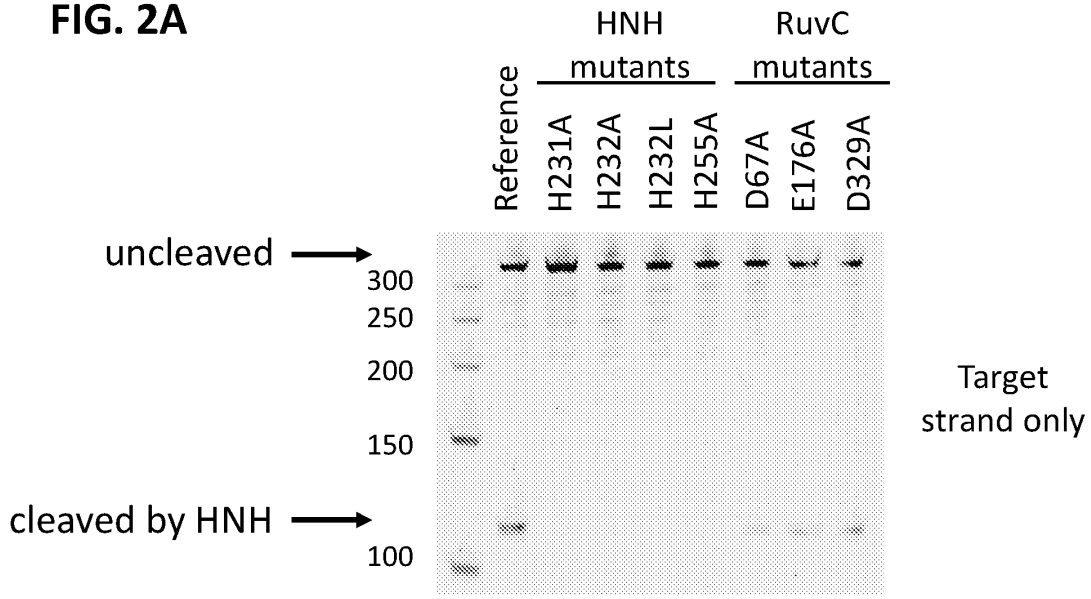

HNH mutants
RuvC mutants
Reference
H231A
H232A
H232L
H255A
D67A
E176A
D329A
uncleaved
cleaved by HNH
300
250
200
150
100
Target strand only

FIG. 2B

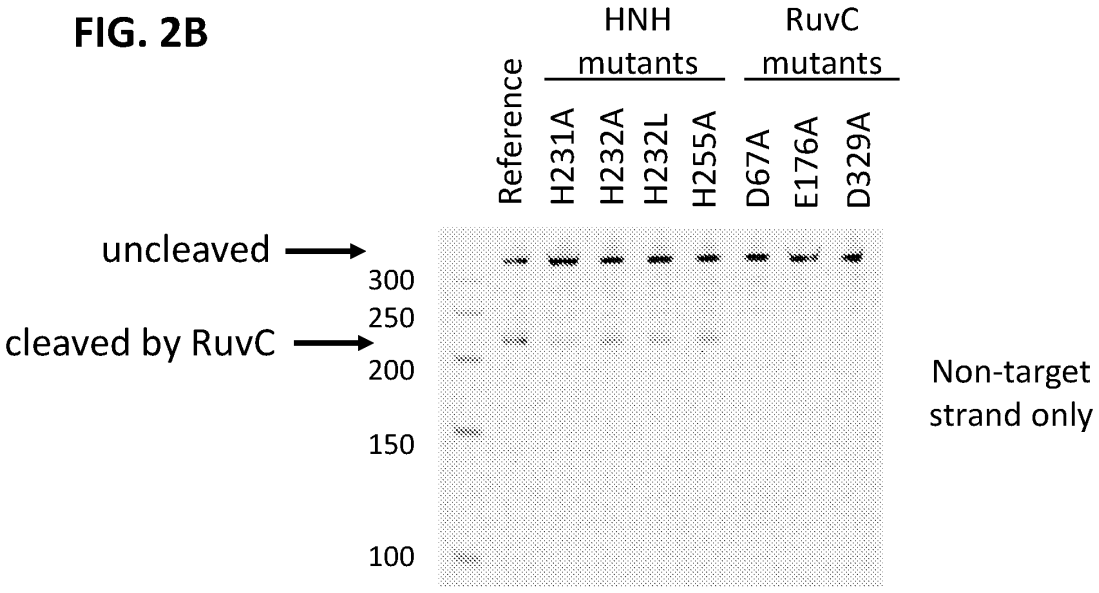

HNH mutants
RuvC mutants
Reference
H231A
H232A
H232L
H255A
D67A
E176A
D329A
uncleaved
cleaved by RuvC
300
250
200
150
100
Non-target strand only

FIG. 2C

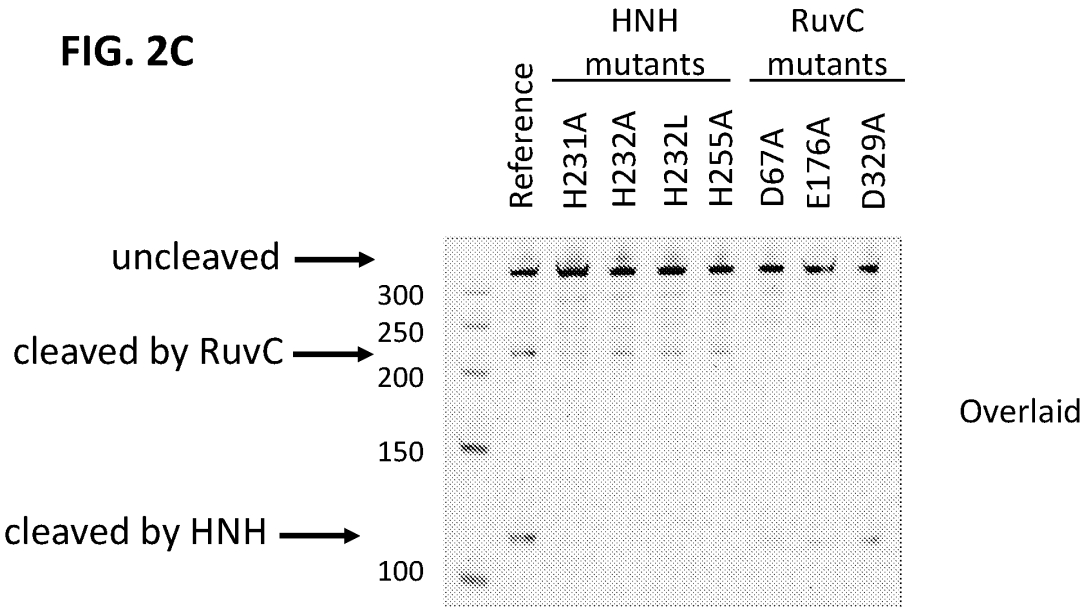
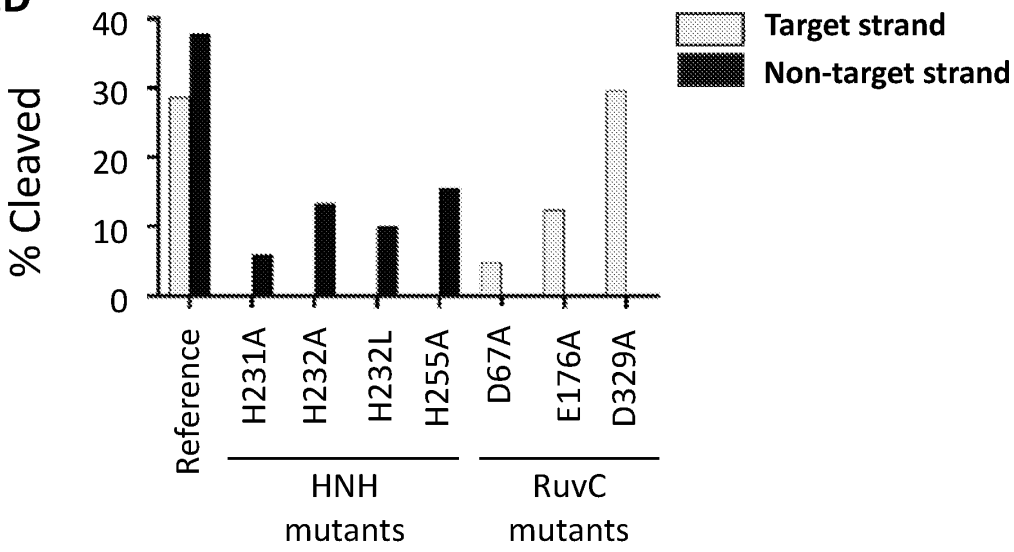

HNH mutants
RuvC mutants
Reference
H231A
H232A
H232L
H255A
D67A
E176A
D329A
uncleaved
cleaved by RuvC
cleaved by HNH
300
250
200
150
100
Overlaid

FIG. 2D


Target strand
Non-target strand
% Cleaved
40
30
20
10
0
Reference
H231A
H232A
H232L
H255A
D67A
E176A
D329A
HNH mutants
RuvC mutants

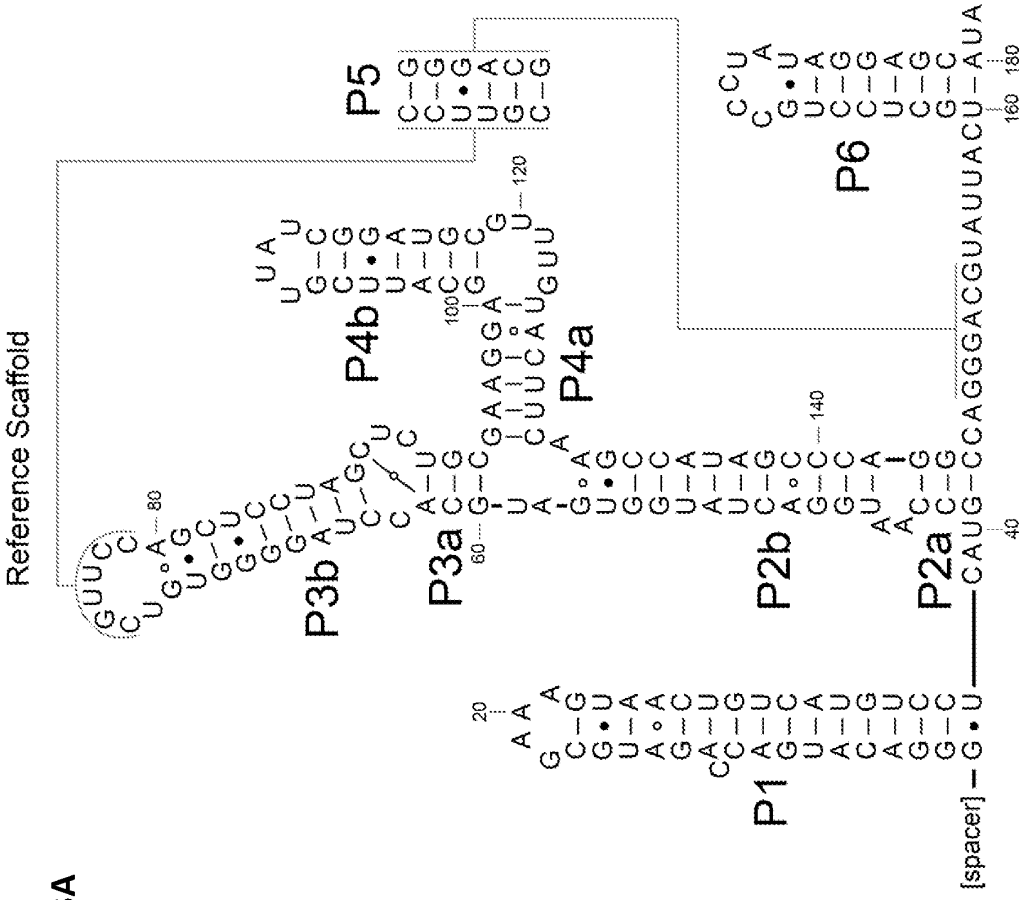

Reference Scaffold
P5
P4b
P4a
P6
P3b
P3a
P2b
P2a
P1
[spacer]
20
40
60
80
100
120
140
160
180

FIG. 3A

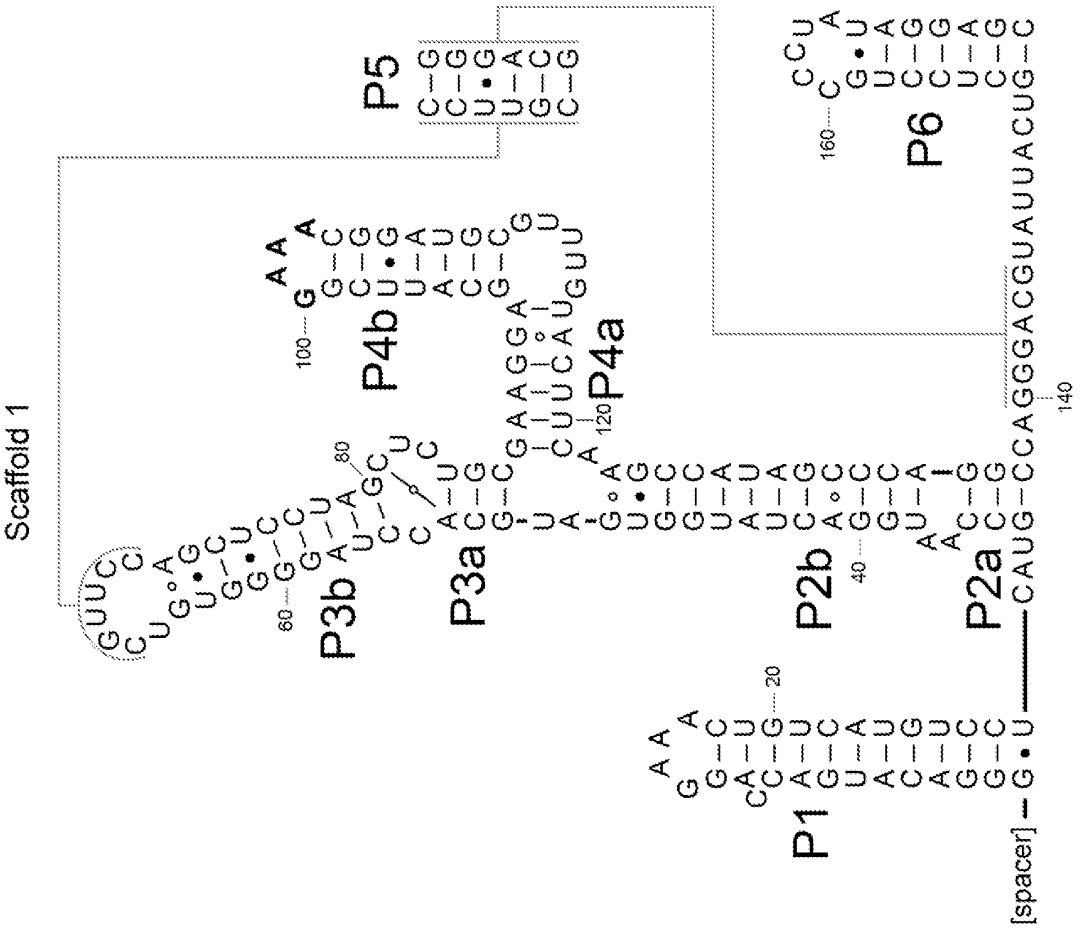

Scaffold 1
P1
P2a
P2b
P3a
P3b
P4a
P4b
P5
P6
[spacer]
20
40
60
80
100
120
140
160

FIG. 3B

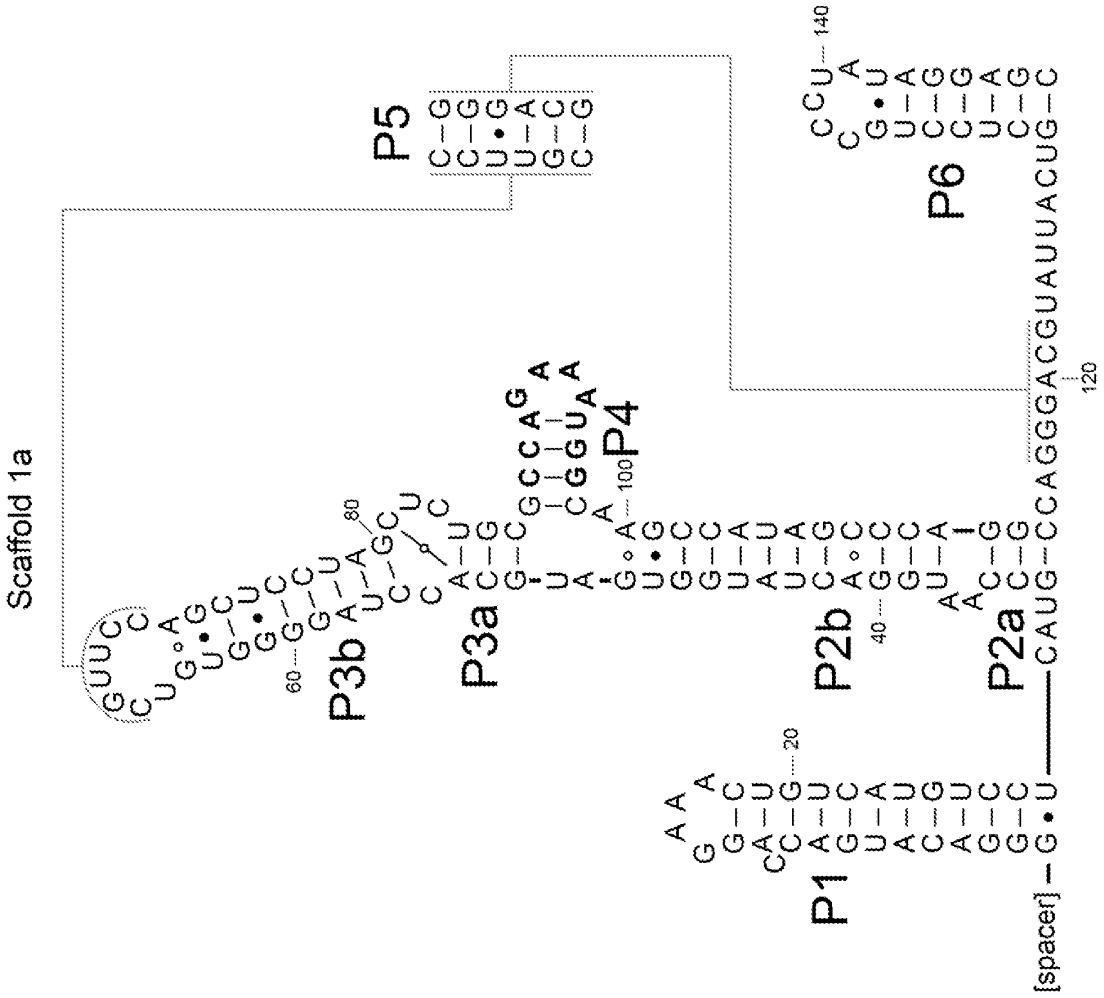

Scaffold 1a
P5
P6
P4
P3a
P3b
P2b
P2a
P1
[spacer]
20
40
60
80
100
120
140

FIG. 3C

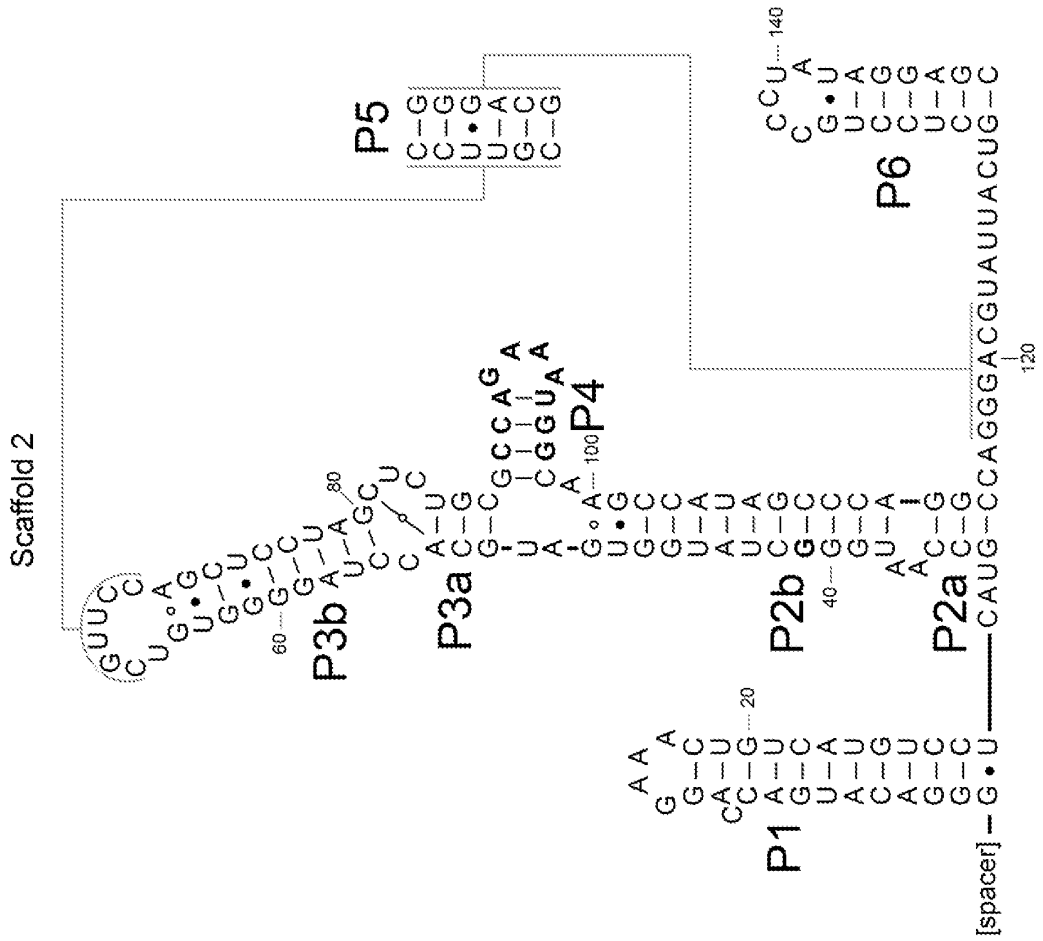

Scaffold 2
P1
P2a
P2b
P3a
P3b
P4
P5
P6
[spacer]
20
40
60
80
100
120
140

FIG. 3D

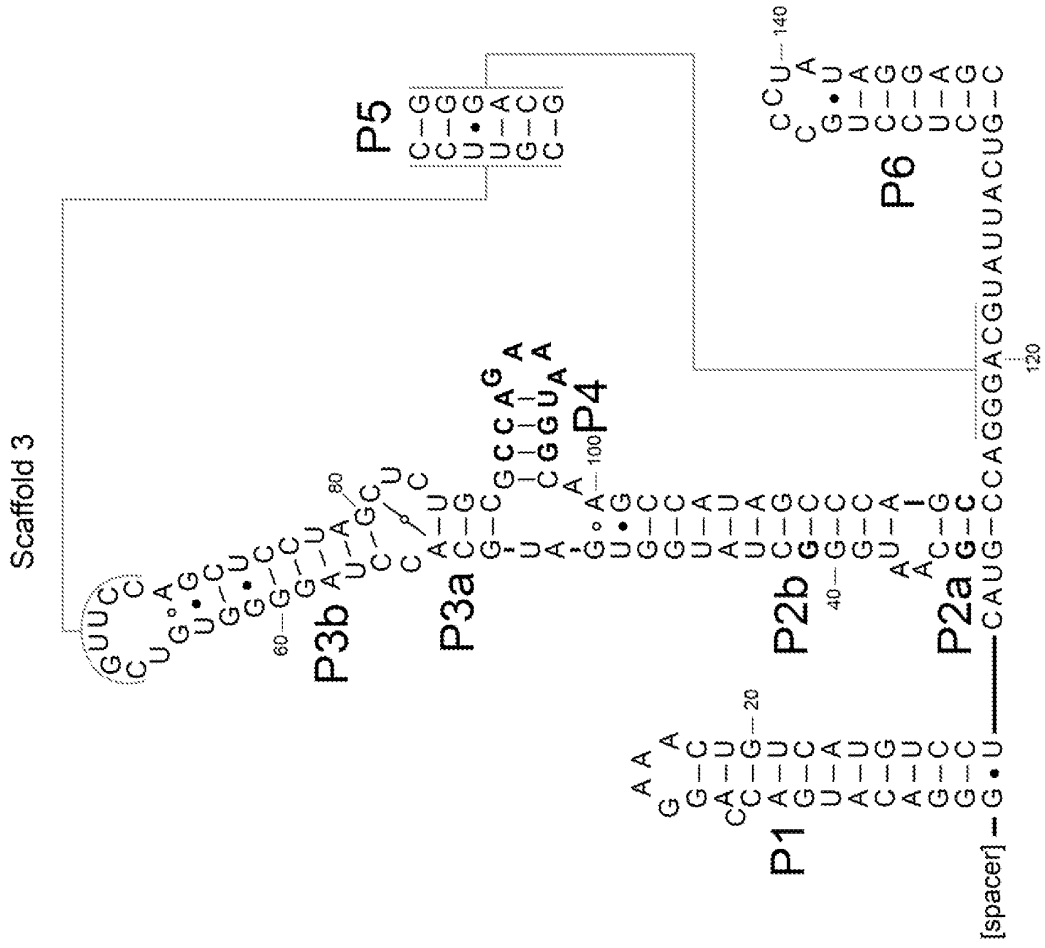

Scaffold 3
P5
P6
P3b
P3a
P4
P2b
P2a
P1
[spacer]
20
40
60
80
100
120
140

FIG. 3E

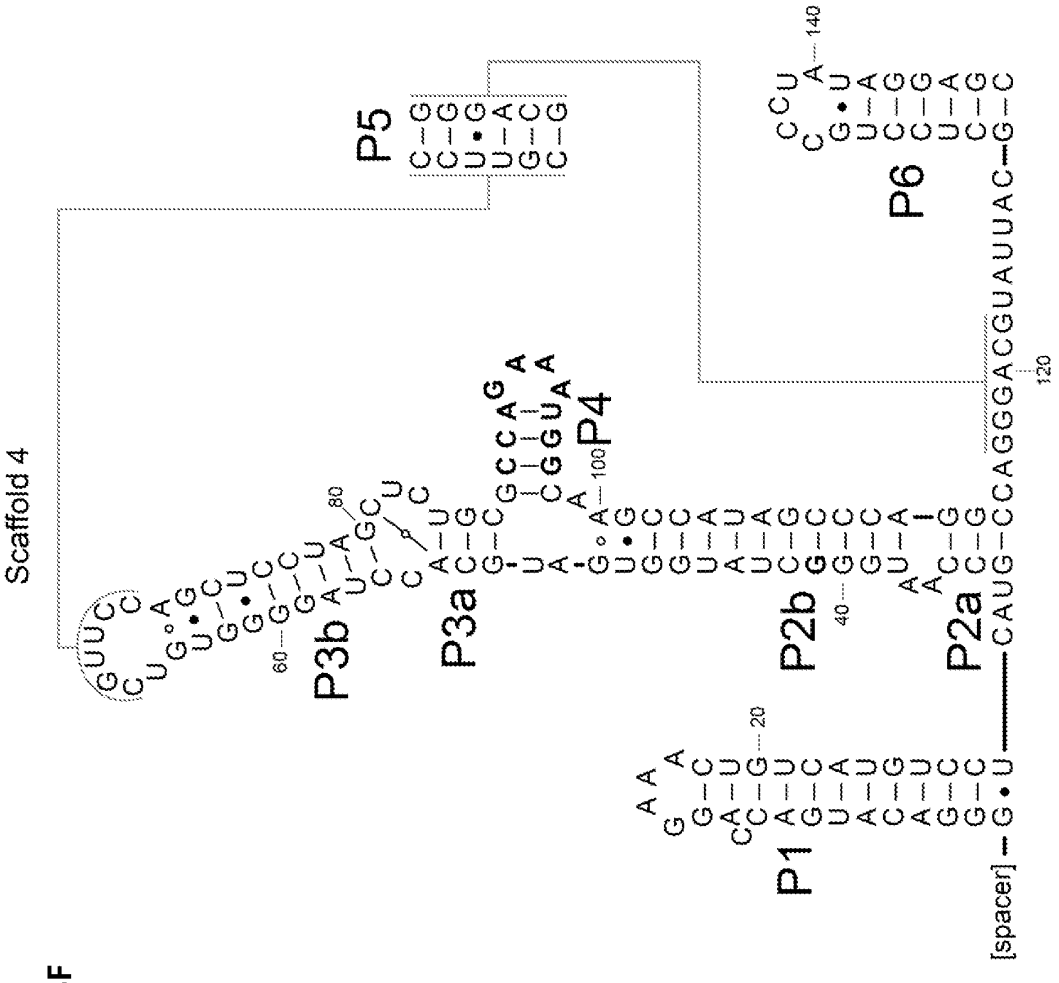

Scaffold 4
P1
P2a
P2b
P3a
P3b
P4
P5
P6
[spacer]
20
40
60
80
100
120
140

FIG. 3F

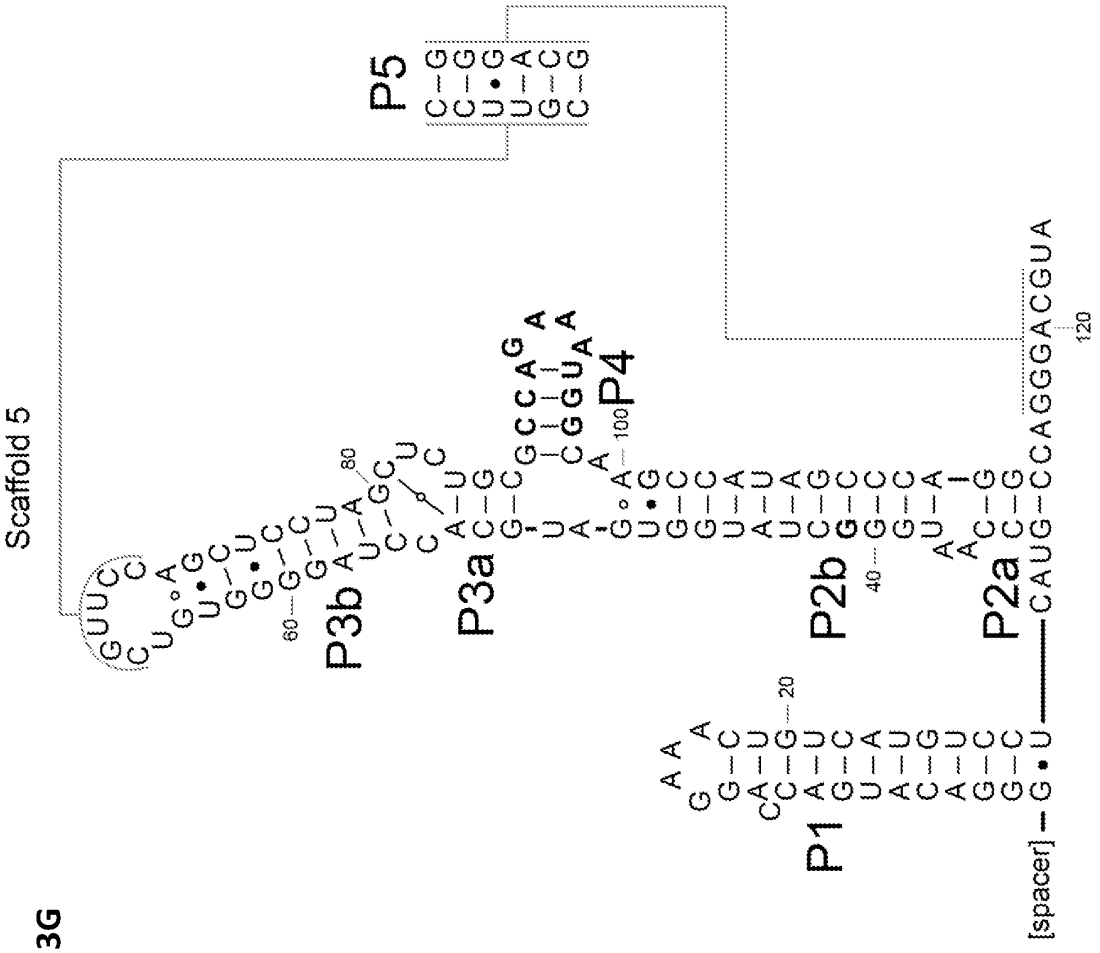


FIG. 3G

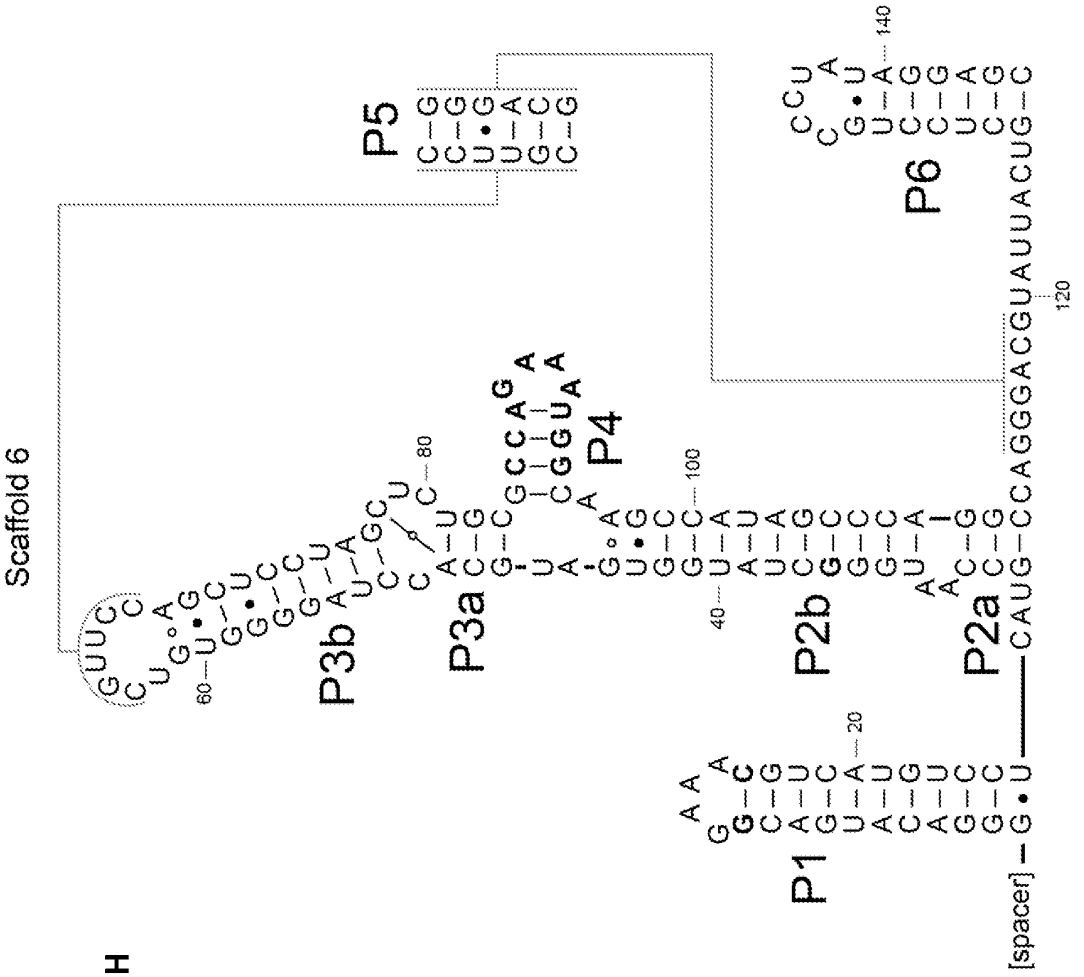

Scaffold 6
P5
P6
P3b
P3a
P4
P2b
P2a
P1
[spacer]
20
40
60
80
100
120
140

FIG. 3H

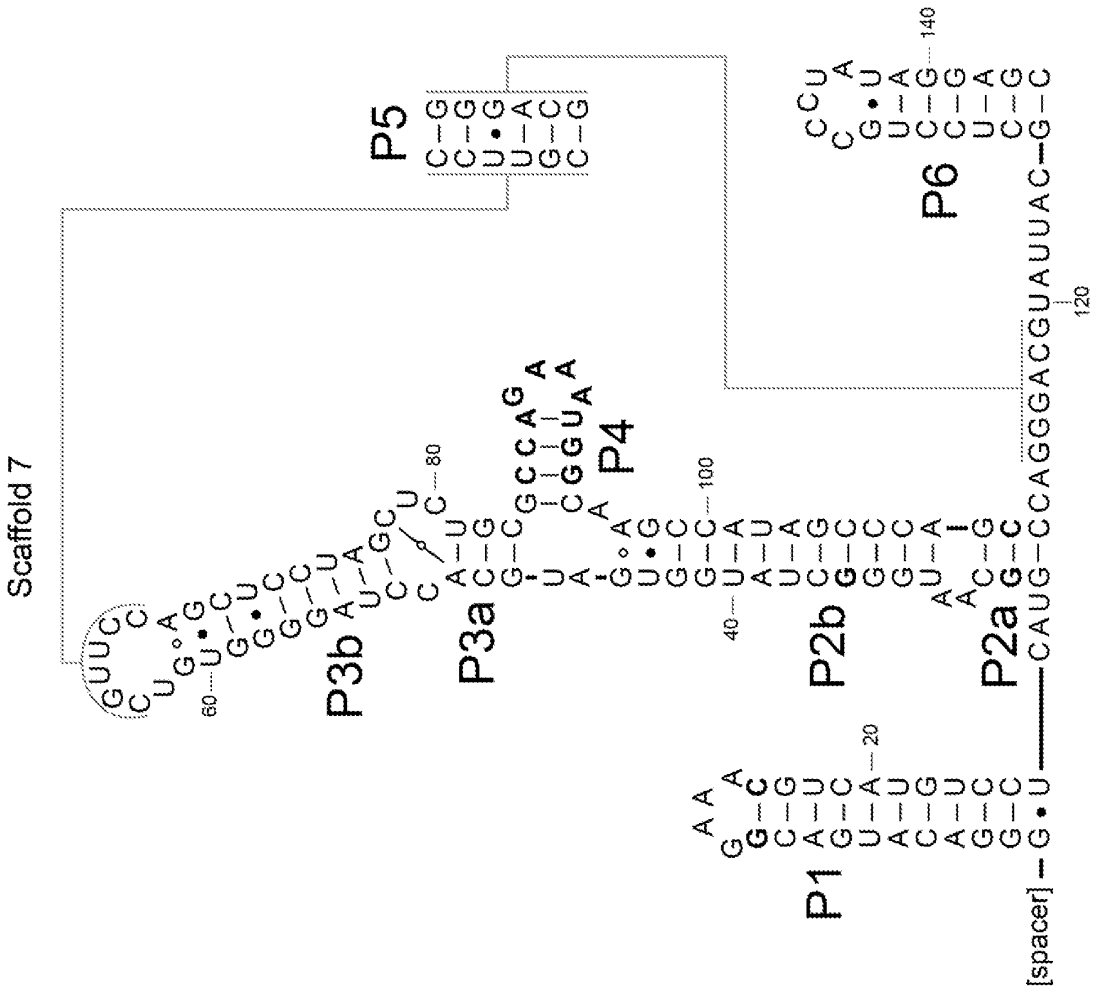

Scaffold 7
P1
P2a
P2b
P3a
P3b
P4
P5
P6
[spacer]
CAUG
CCAGGGACGUAUUAC
20
40
60
80
100
120
140

FIG. 3I

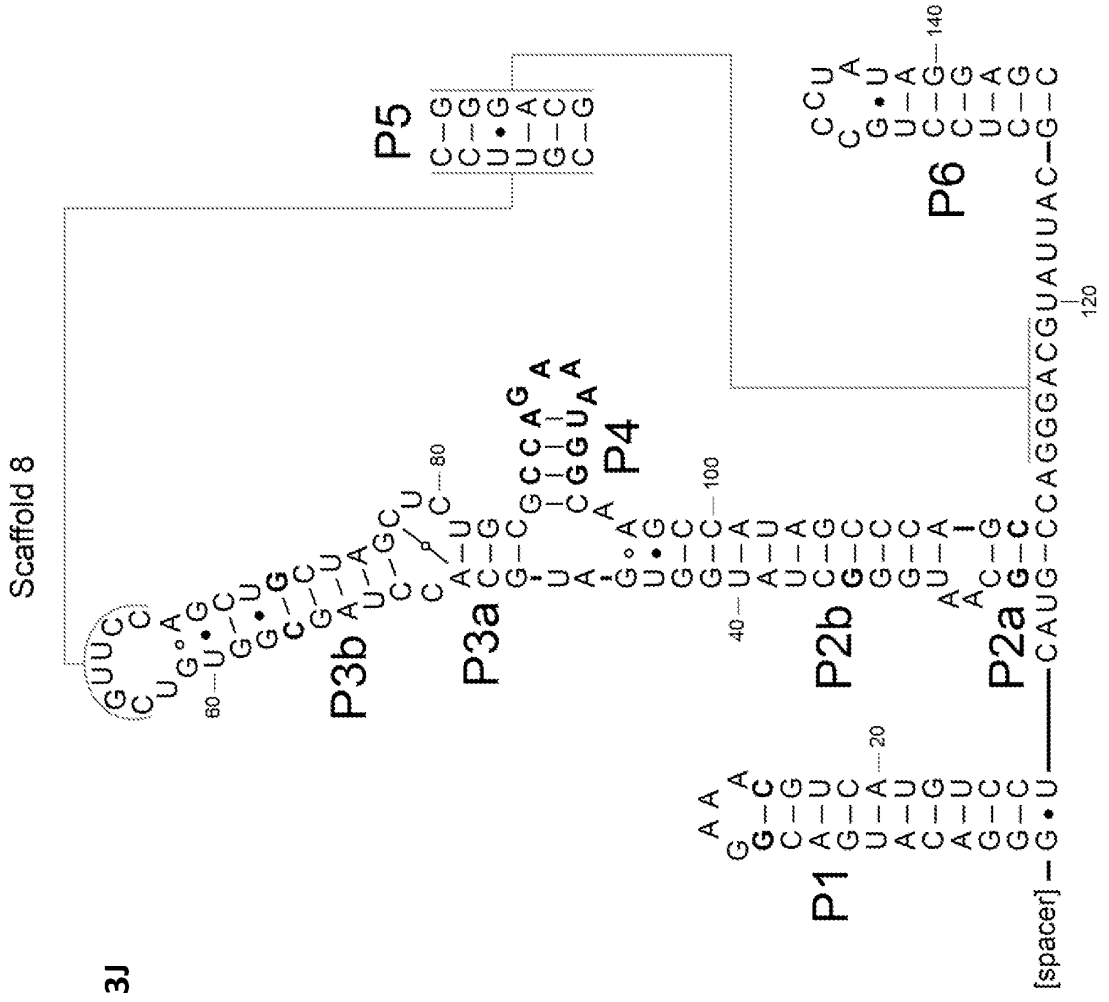

Scaffold 8
P1
P2a
P2b
P3a
P3b
P4
P5
P6
[spacer]
20
40
60
80
100
120
140

FIG. 3J