FIG. 1

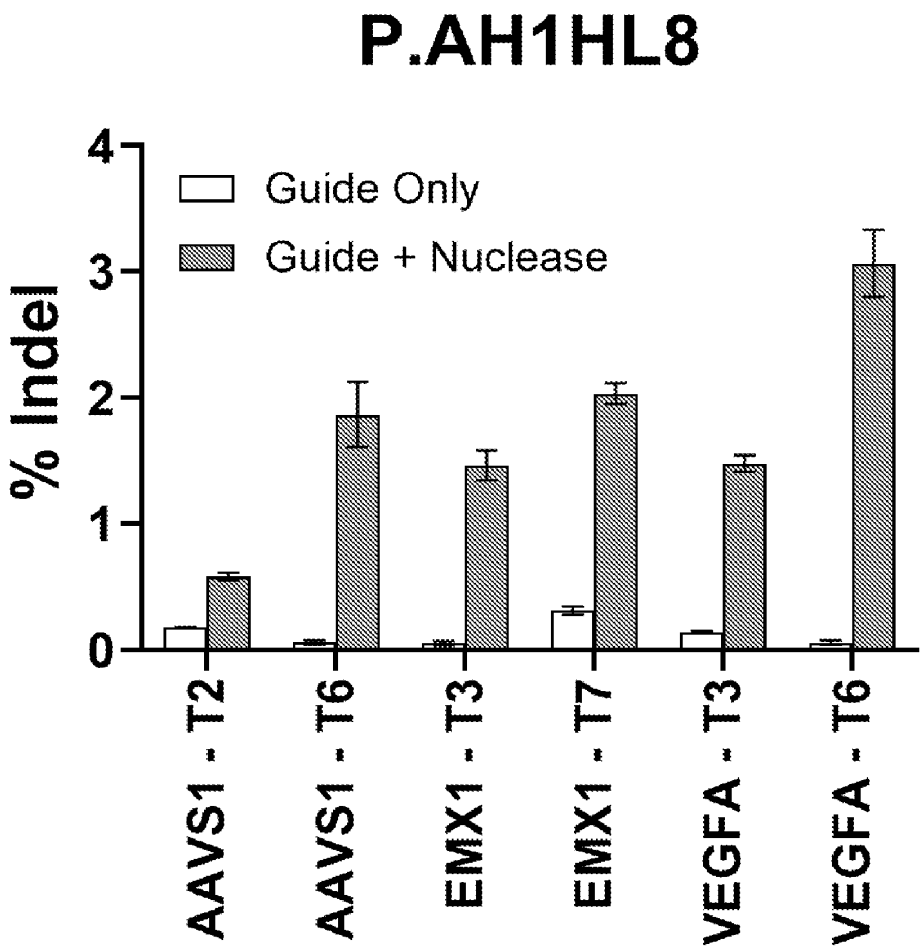

P.AH1HL8
% Indel
0
1
2
3
4
Guide Only
Guide + Nuclease
AAVS1 - T2
AAVS1 - T6
EMX1 - T3
EMX1 - T7
VEGFA - T3
VEGFA - T6

FIG. 2A

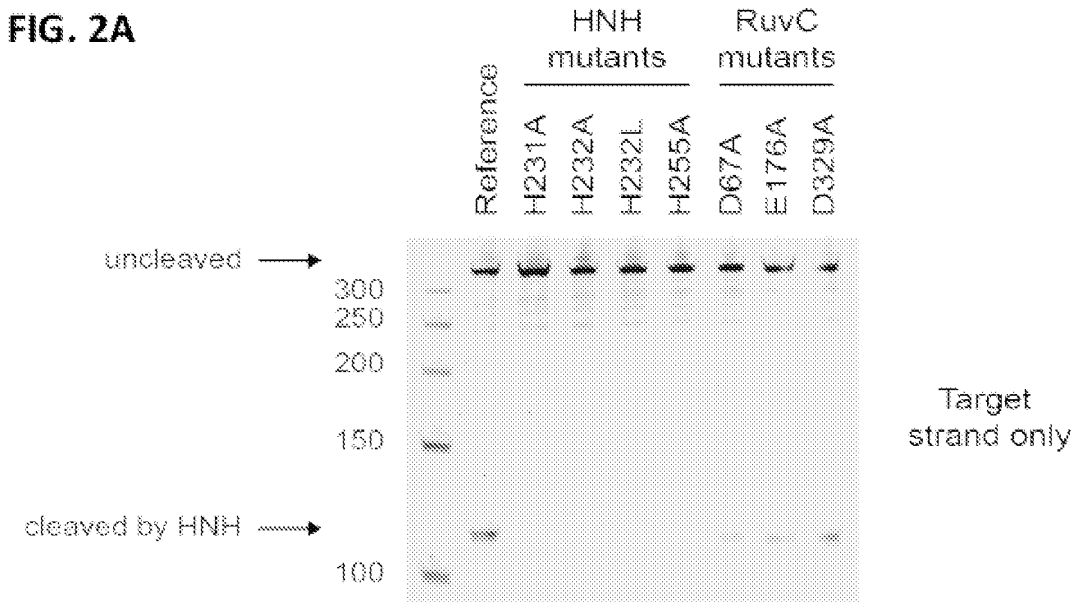

HNH mutants
RuvC mutants
Reference
H231A
H232A
H232L
H255A
D67A
E176A
D329A
uncleaved
cleaved by HNH
300
250
200
150
100
Target strand only

FIG. 2B

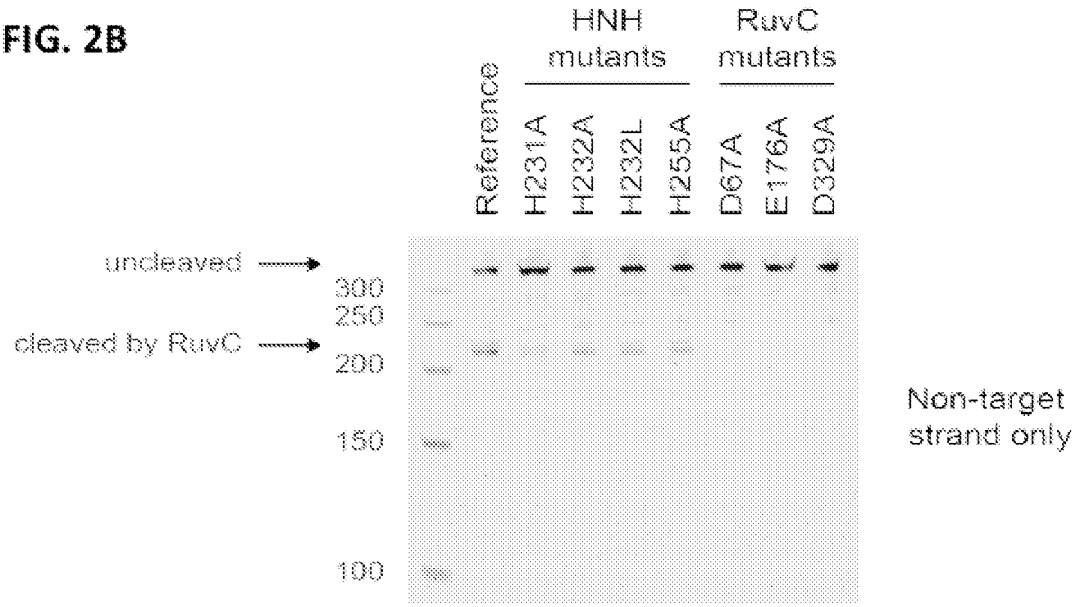

HNH mutants
RuvC mutants
Reference
H231A
H232A
H232L
H255A
D67A
E176A
D329A
uncleaved
cleaved by RuvC
300
250
200
150
100
Non-target strand only

FIG. 2C

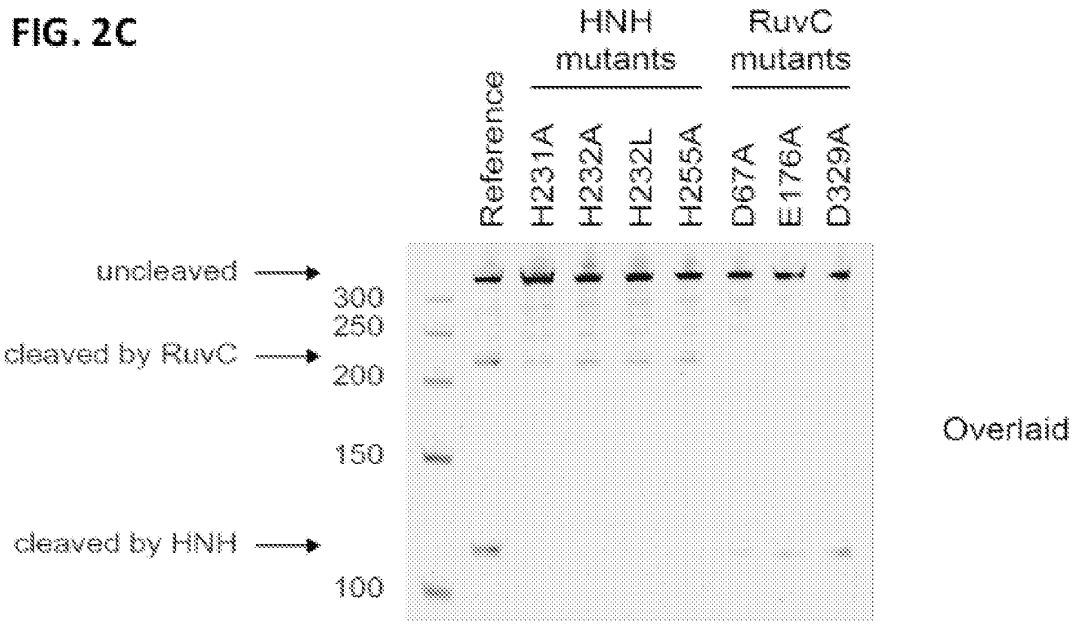
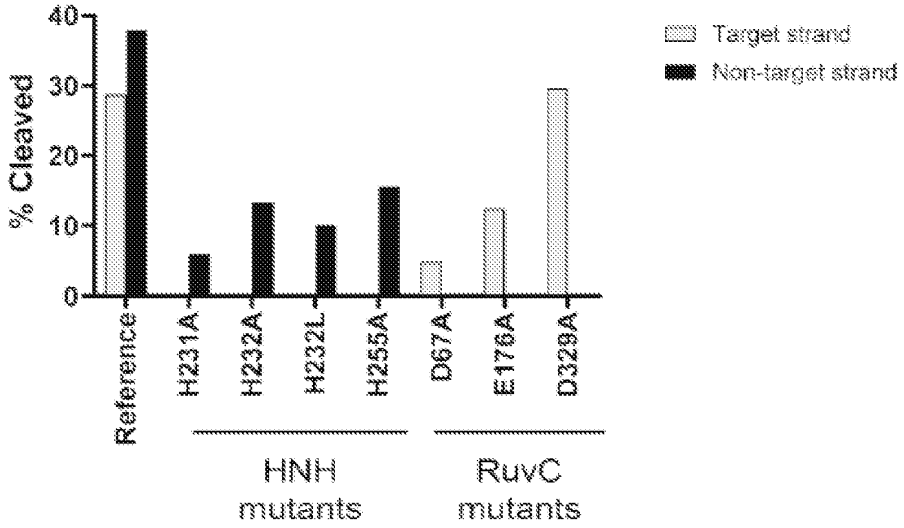

HNH mutants
RuvC mutants
Reference
H231A
H232A
H232L
H255A
D67A
E176A
D329A
uncleaved
cleaved by RuvC
cleaved by HNH
300
250
200
150
100
Overlaid

FIG. 2D


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

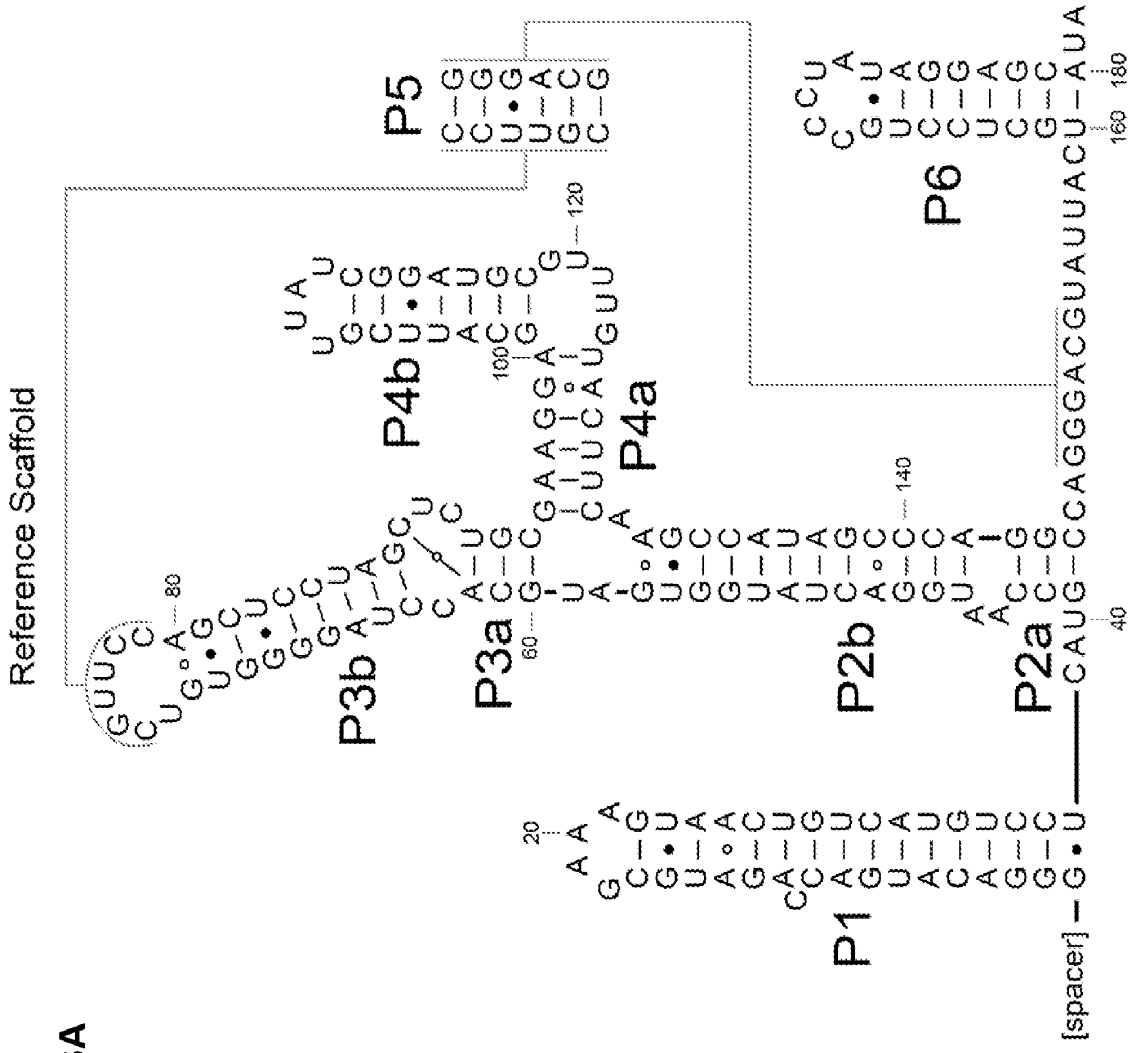


FIG. 3A

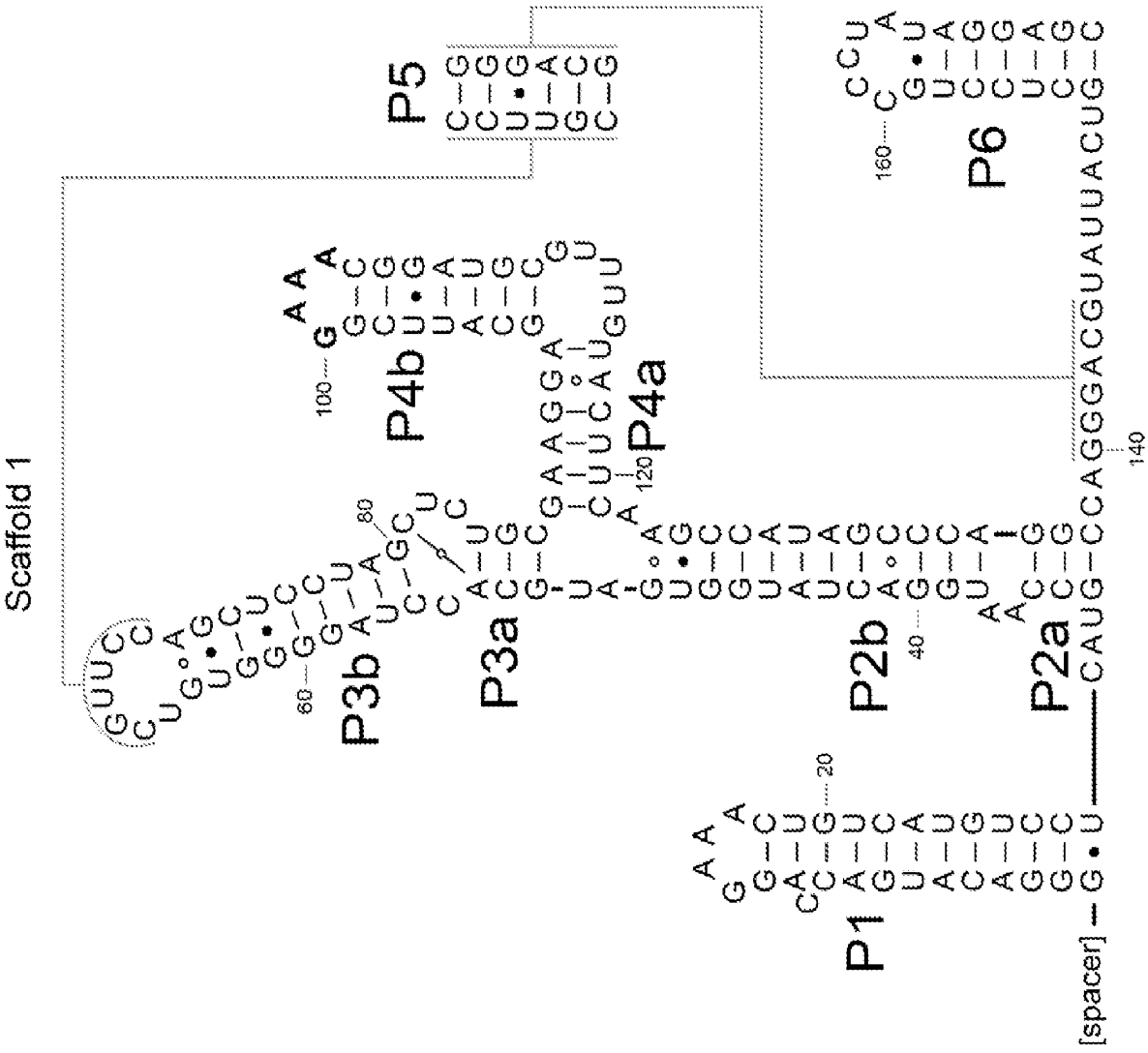


FIG. 3B

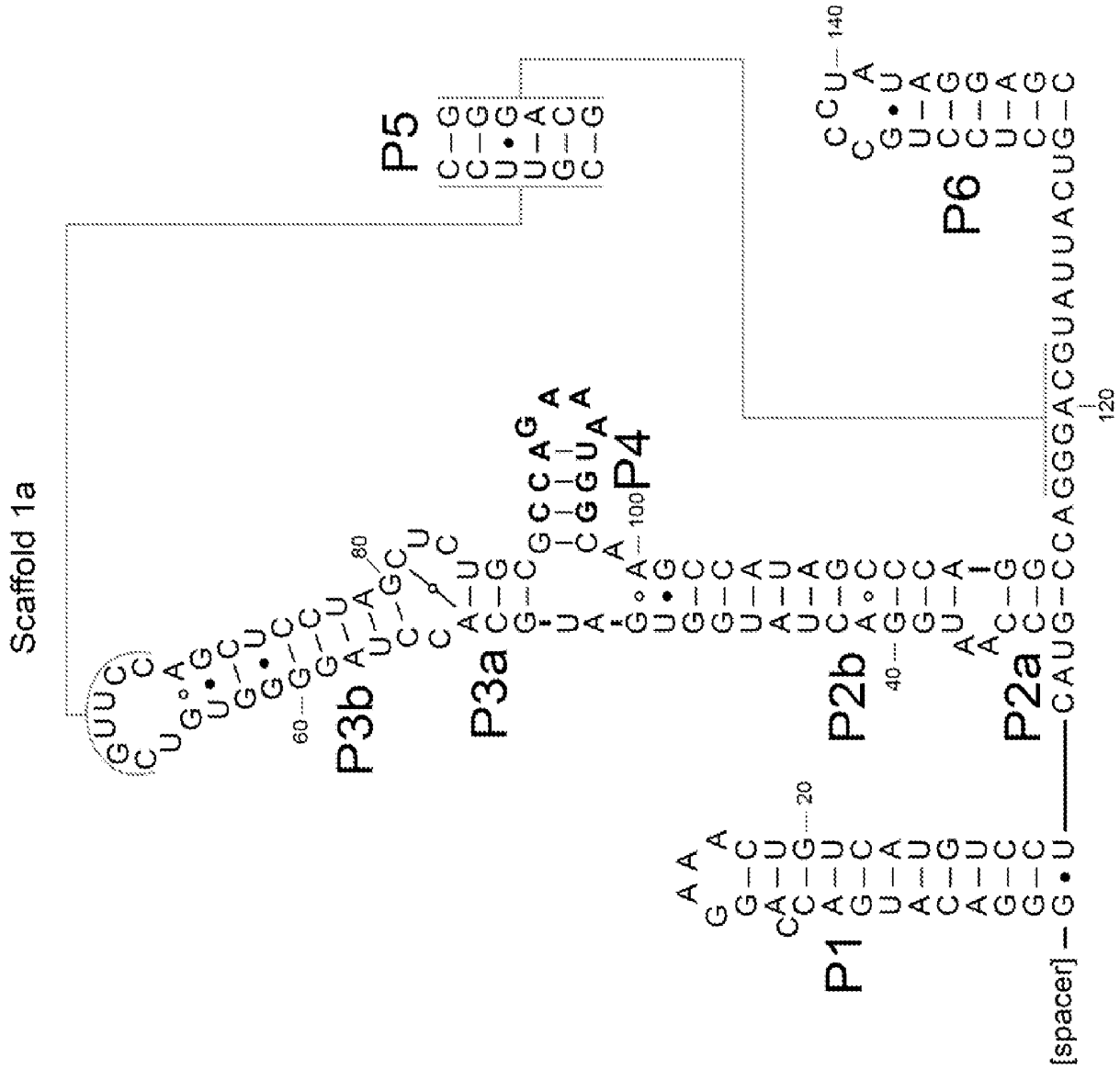

Scaffold 1a
P5
P6
P3b
P3a
P4
P2b
P2a
P1
[spacer]
20
40
60
80
100
120
140

FIG. 3C

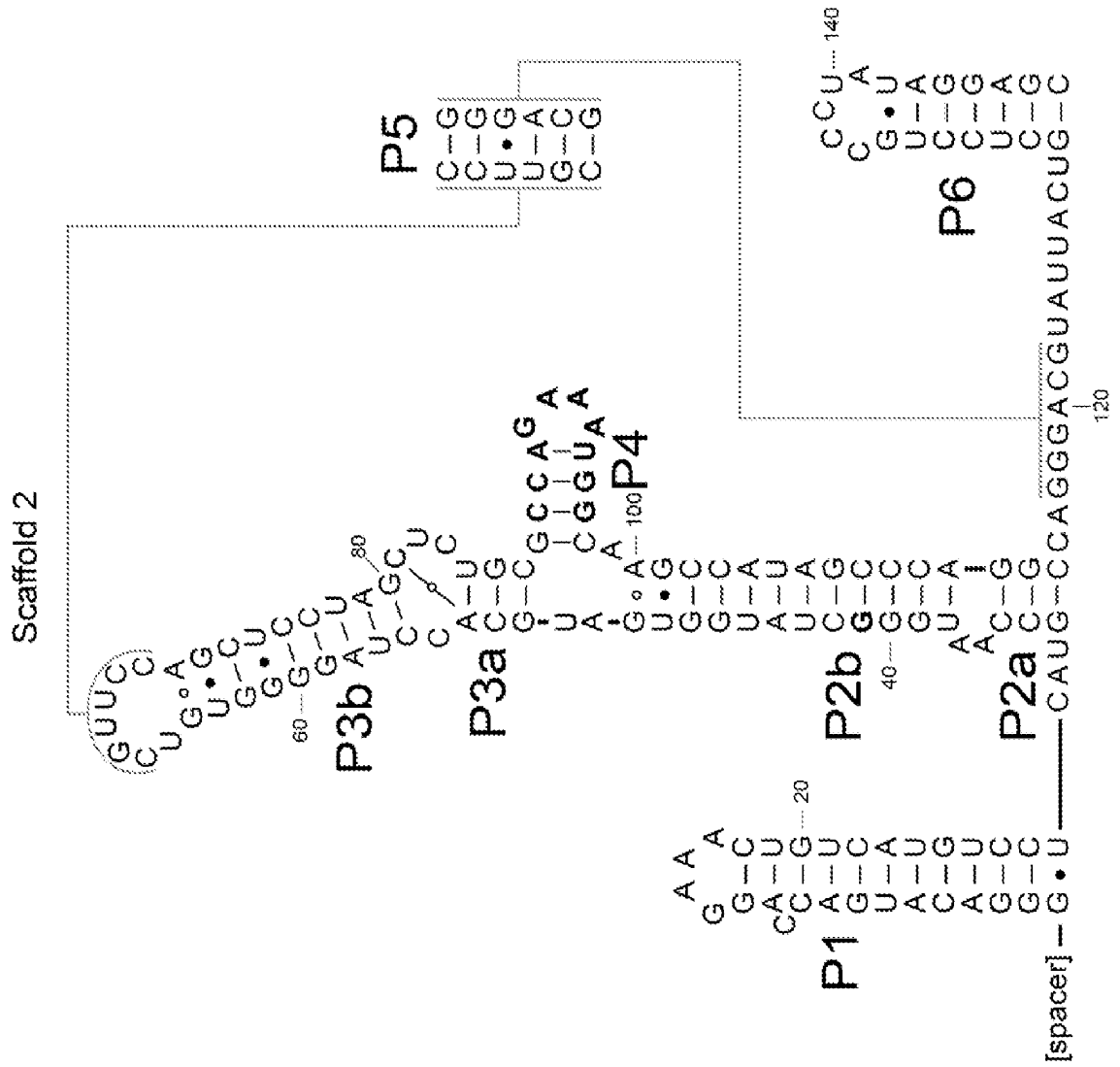

Scaffold 2
P5
P6
P3b
P3a
P4
P2b
P2a
P1
[spacer]
20
40
60
80
100
120
140

FIG. 3D

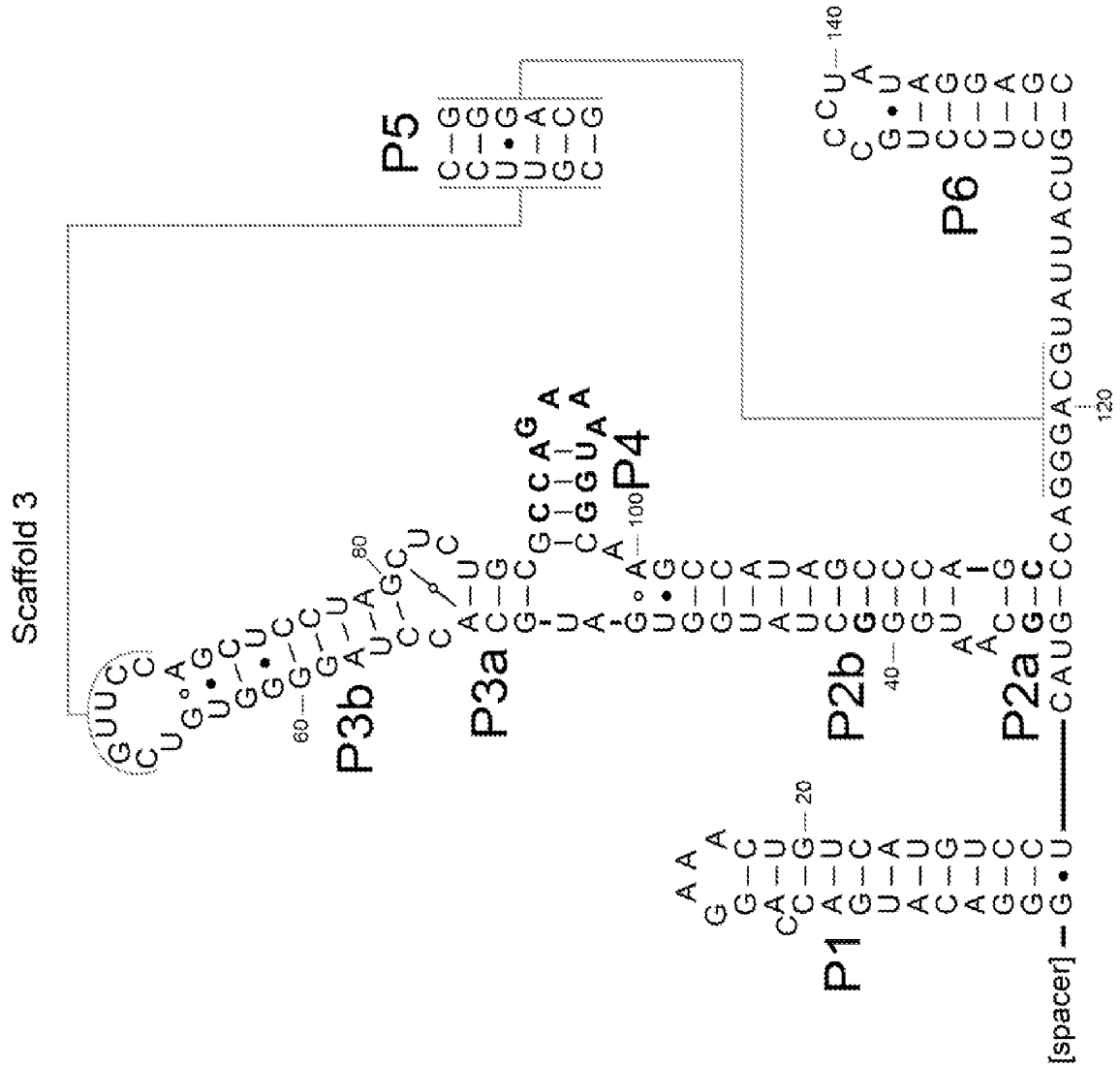

Scaffold 3
P1
P2a
P2b
P3a
P3b
P4
P5
P6
[spacer]
20
40
60
80
100
120
140

FIG. 3E

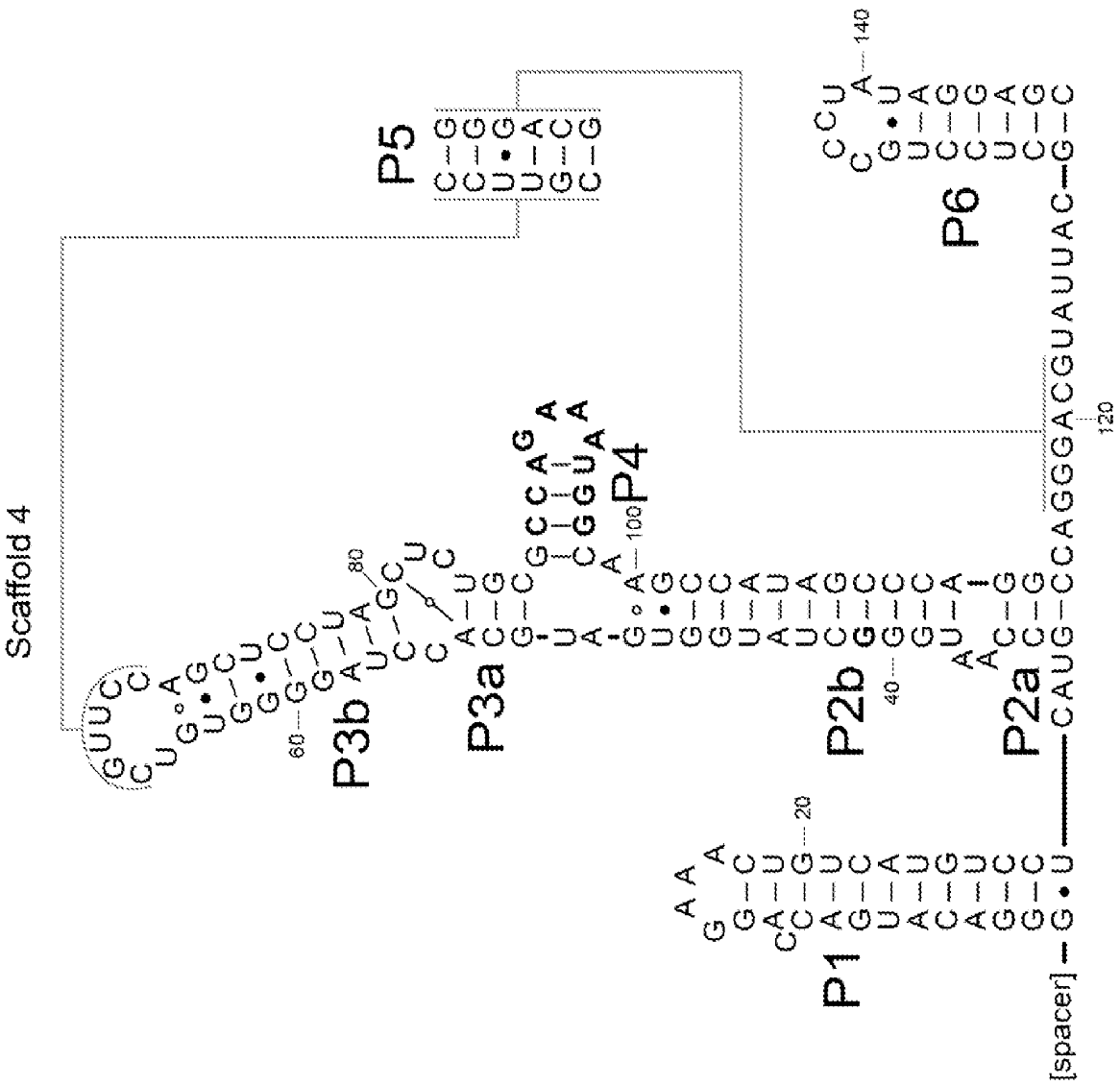

Scaffold 4
P1
P2a
P2b
P3a
P3b
P4
P5
P6
[spacer]
20
40
60
80
100
120
140

FIG. 3F

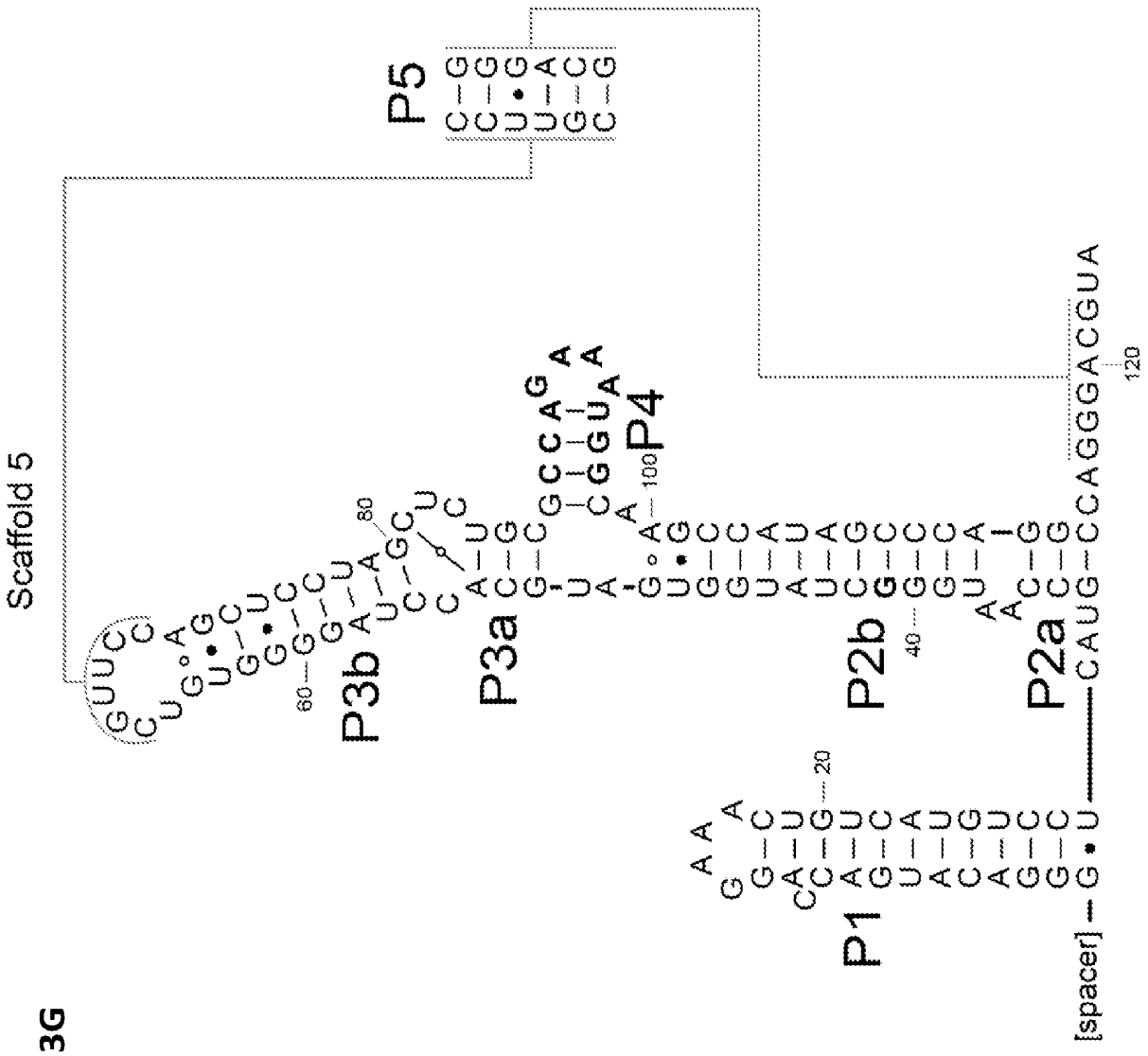


FIG. 3G

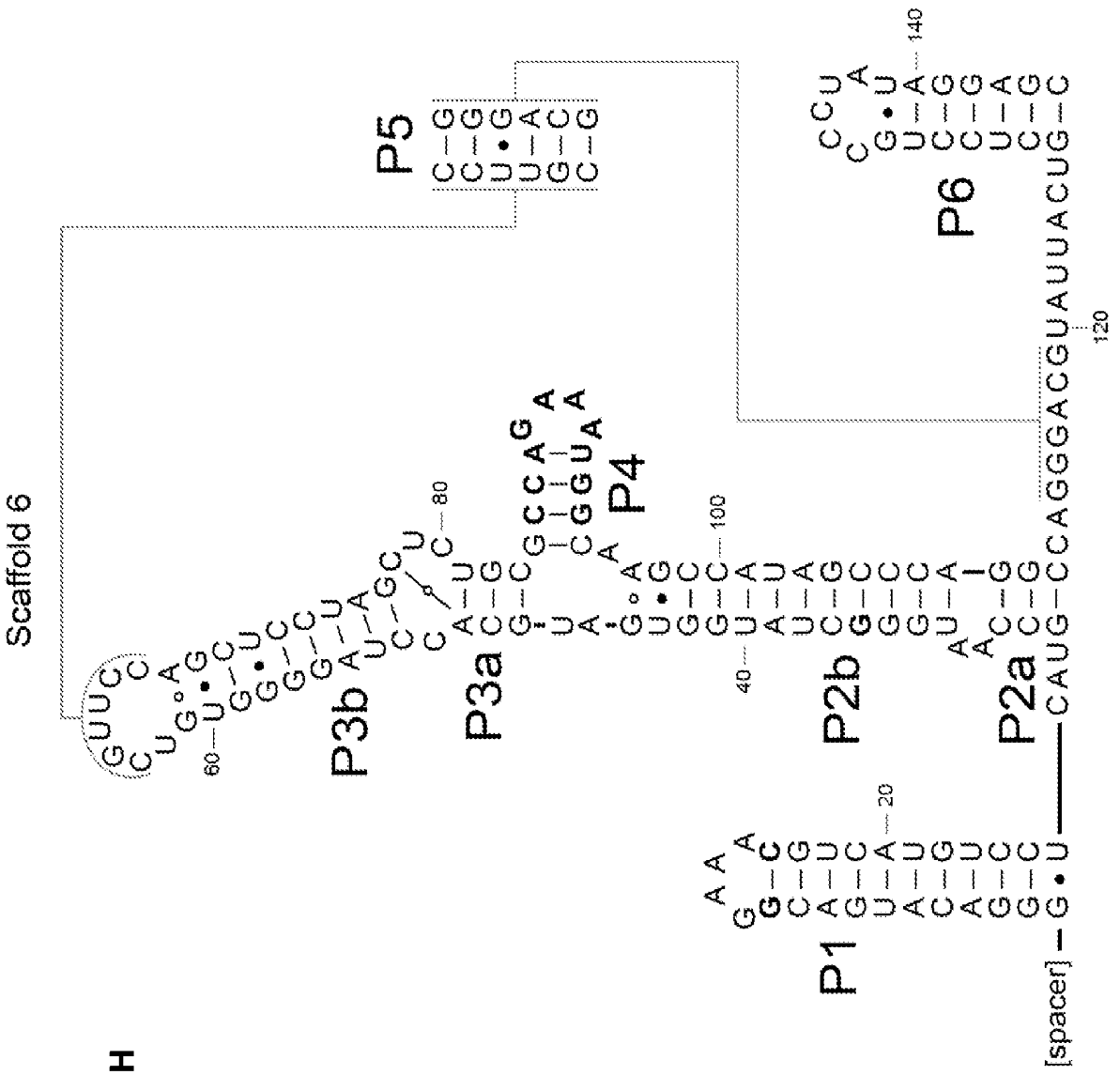

Scaffold 6
P5
P6
P4
P3b
P3a
P2b
P2a
P1
[spacer]
20
40
60
80
100
120
140

FIG. 3H

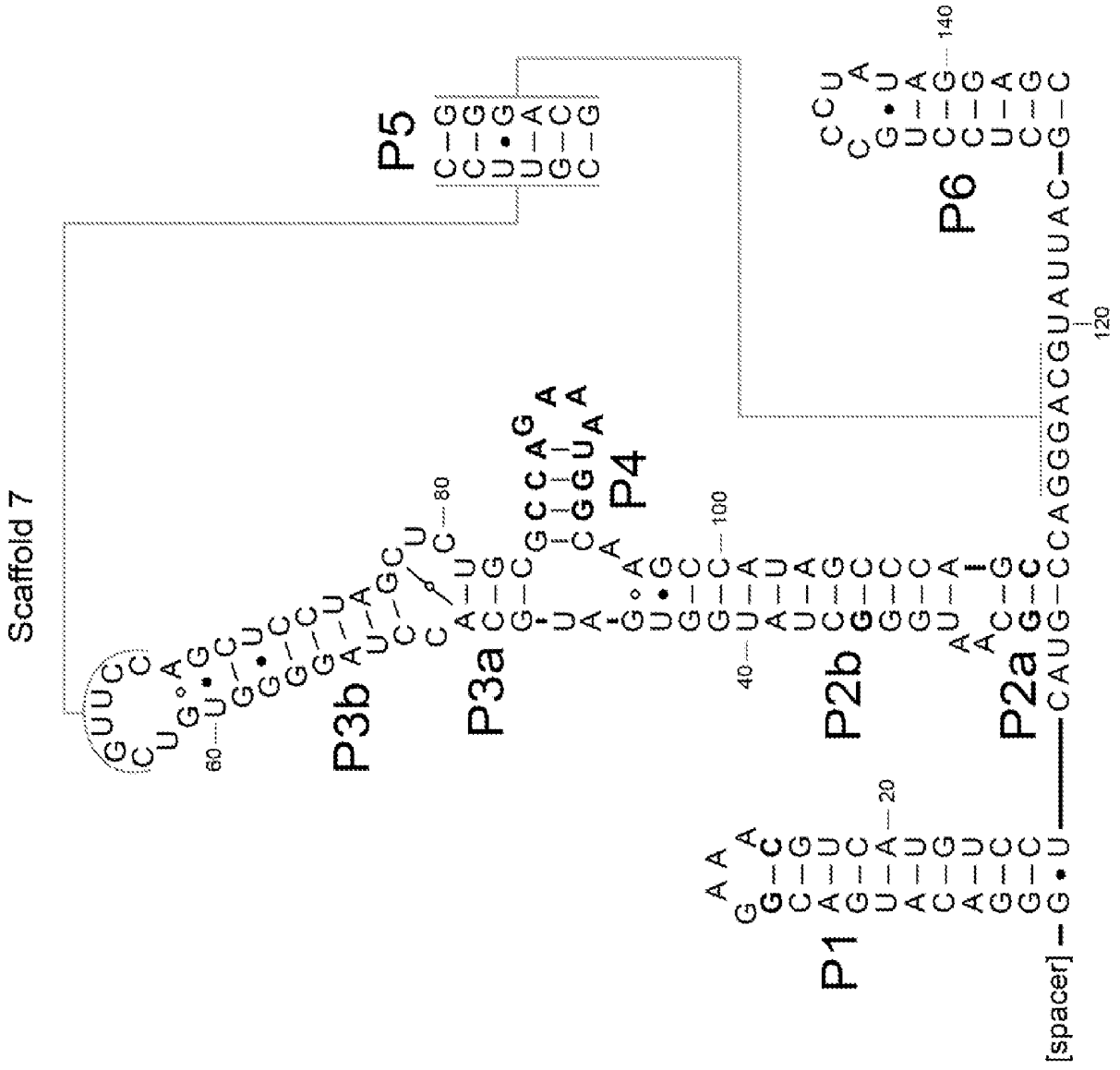

Scaffold 7
P1
P2a
P2b
P3a
P3b
P4
P5
P6
[spacer]
20
40
60
80
100
120
140

FIG. 3I

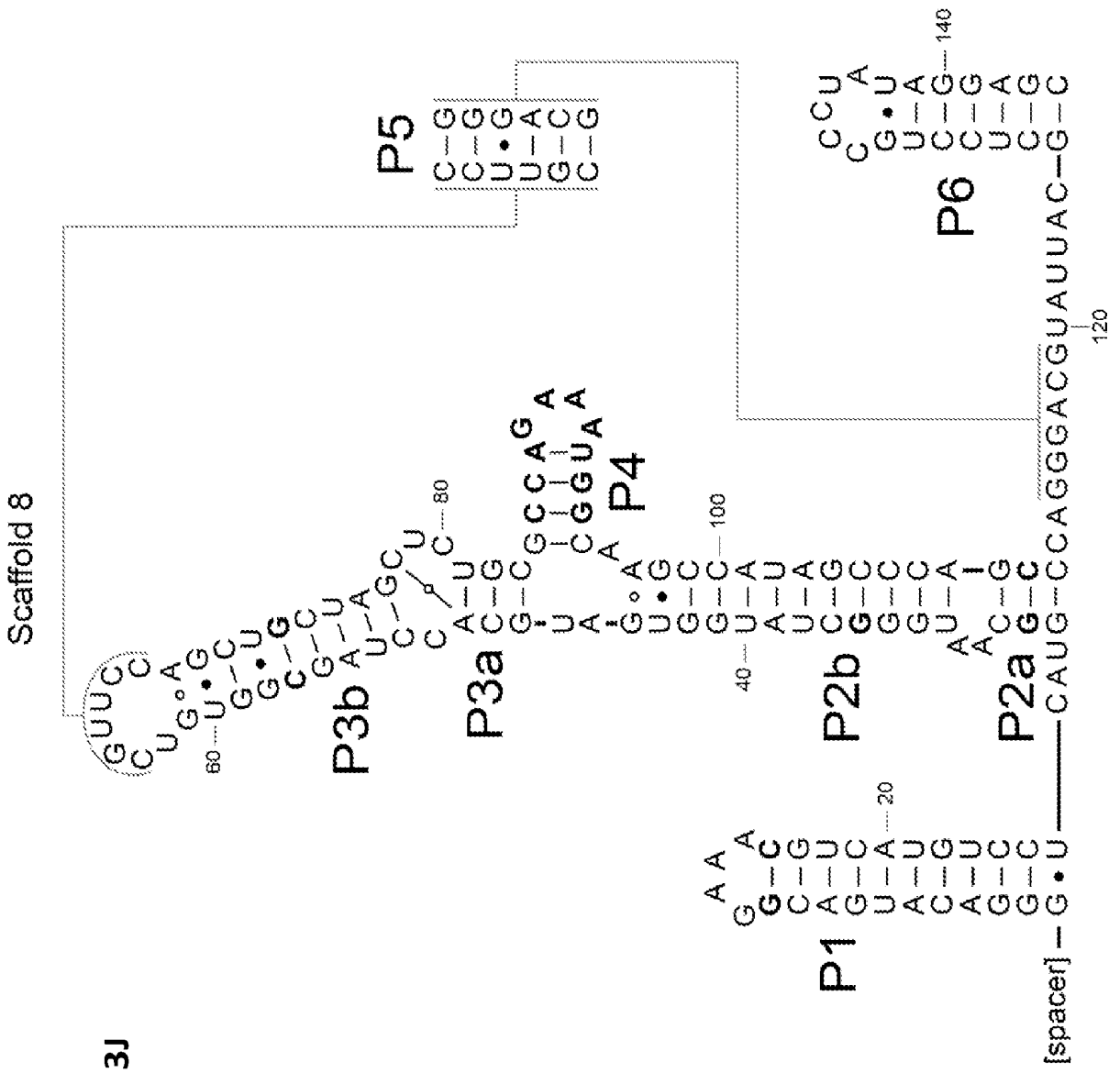

Scaffold 8
P5
P6
P3b
P3a
P4
P2b
P2a
P1
[spacer]
20
40
60
80
100
120
140

FIG. 3J