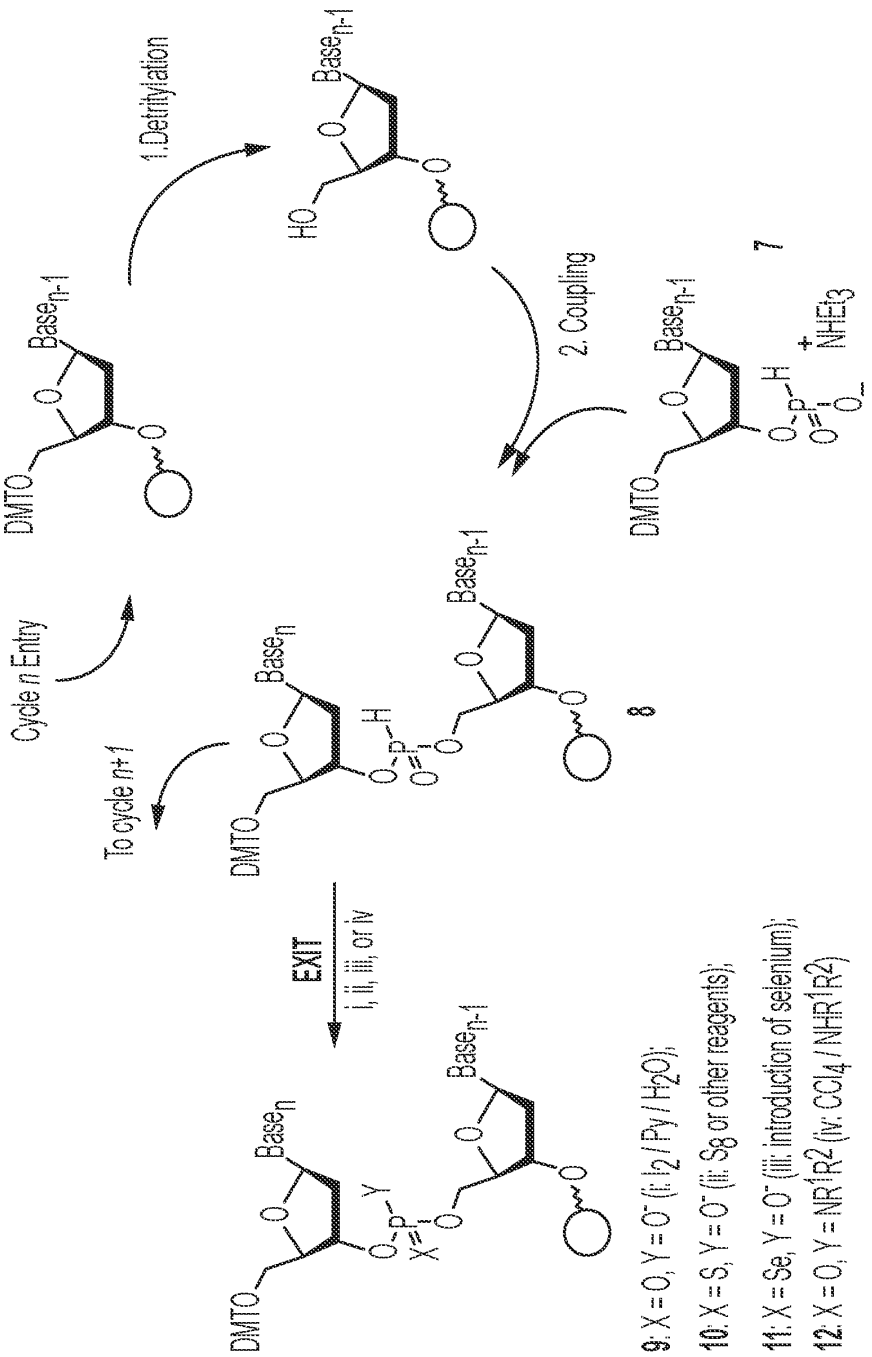

1.Detritylation
2. Coupling
Cycle n Entry
To cycle n+1
EXIT
I, II, III, or IV
DMTO
HO
Base n-1
Base n
7
8
9: X = O, Y = O⁻ (i: I2 / Py / H2O);
10: X = S, Y = O⁻ (ii: S8 or other reagents);
11: X = Se, Y = O⁻ (iii: introduction of selenium);
12: X = O, Y = NR1R2 (iv: CCl4 / NHR1R2)

FIG. 1

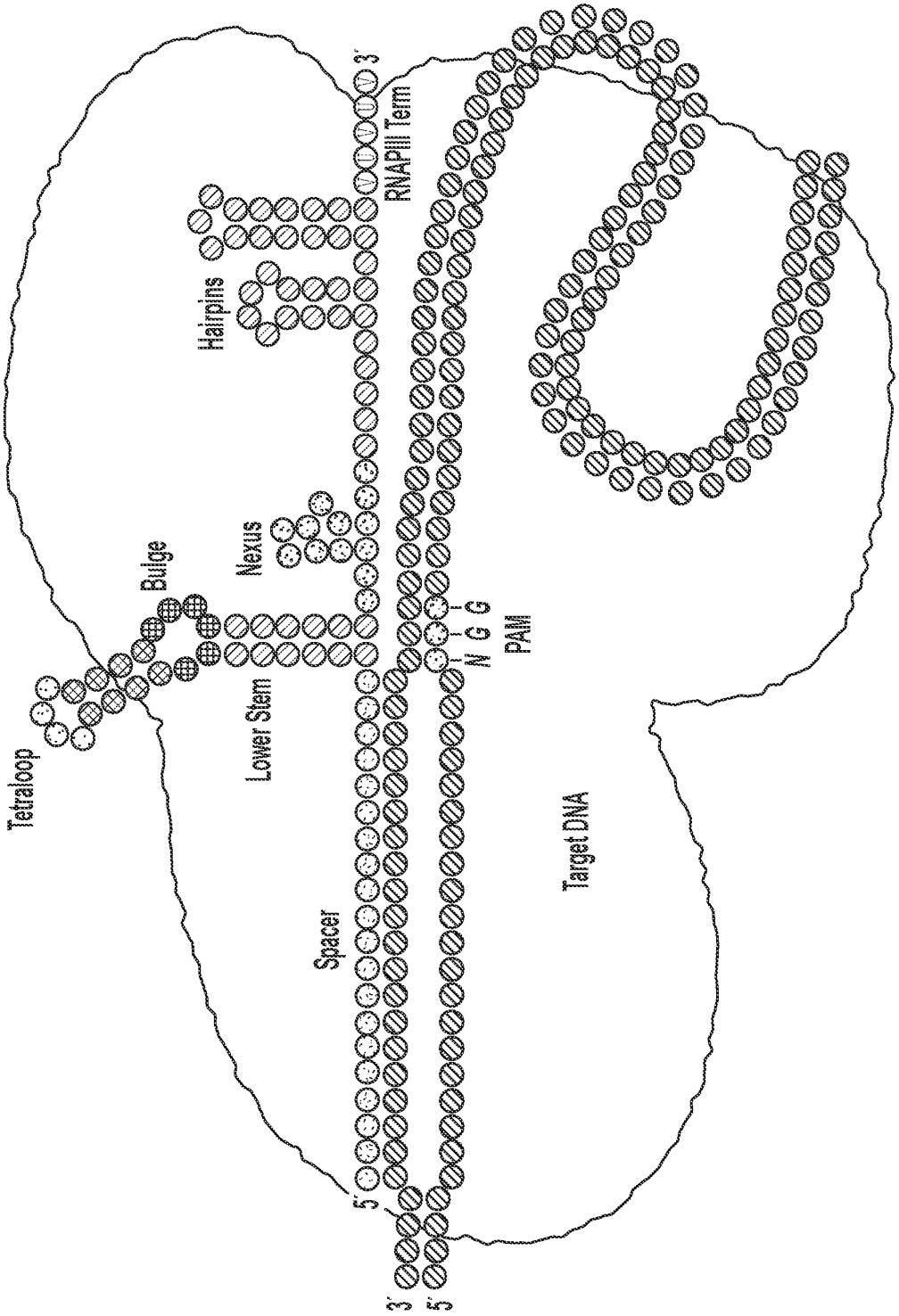

3'
RNAPIII Term
Hairpins
Nexus
Bulge
Tetraloop
Lower Stem
Spacer
N G G
PAM
Target DNA
5'
3'
5'

FIG. 2

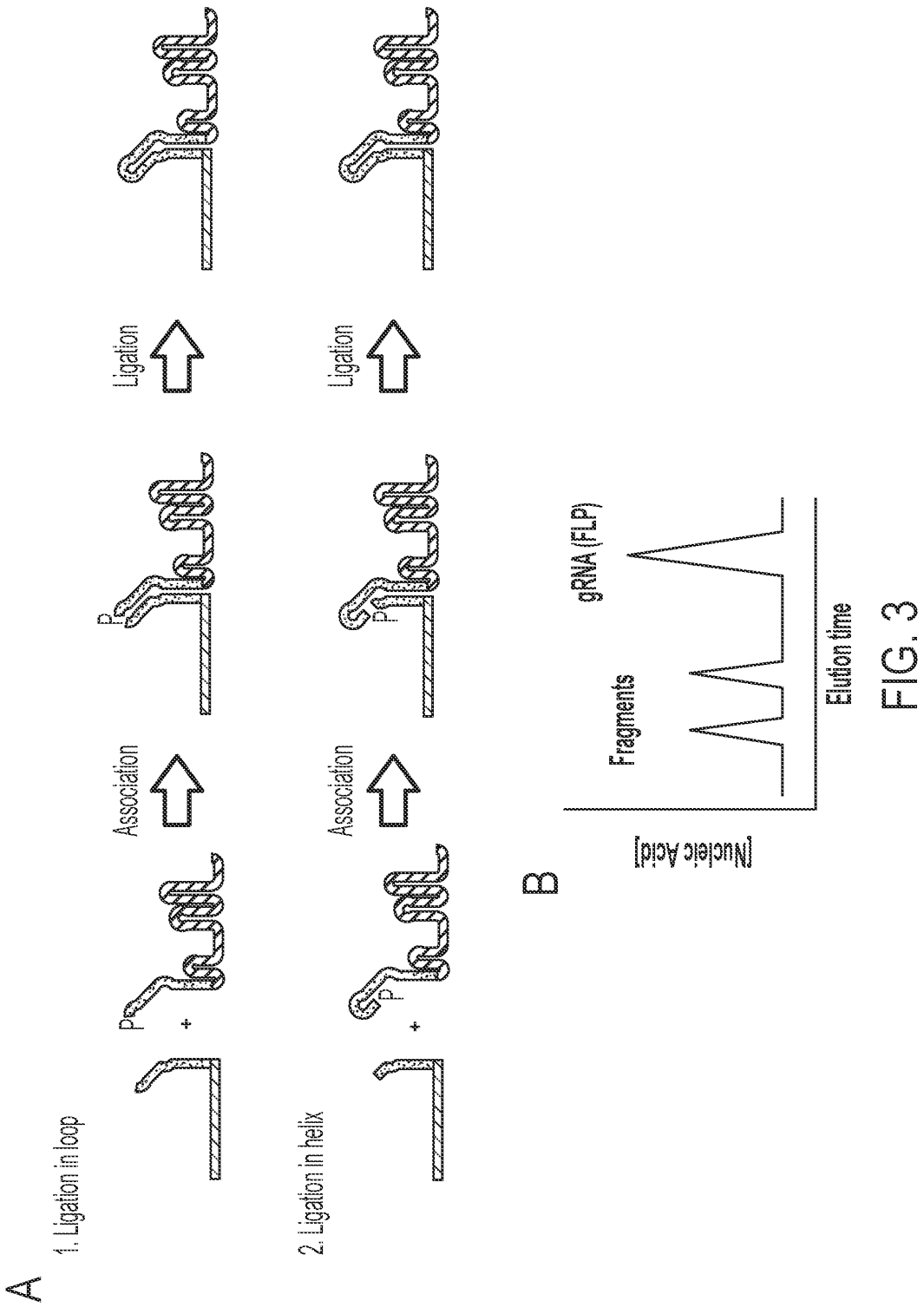

A
1. Ligation in loop
2. Ligation in helix
Association
Association
Ligation
Ligation
P
P
P
P
+
+
B
gRNA (FLP)
Fragments
[Nucleic Acid]
Elution time

FIG. 3

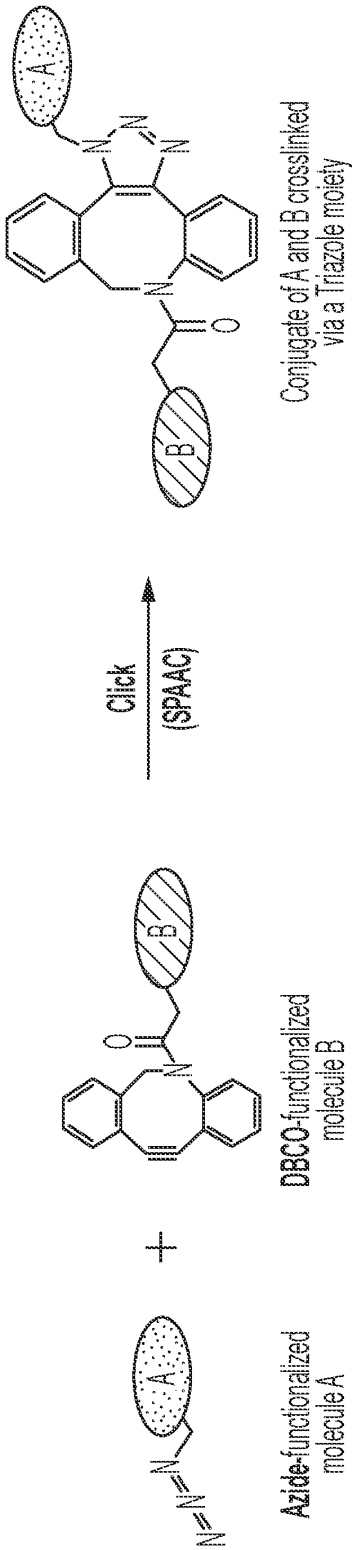

Azide-functionalized molecule A
DBCO-functionalized molecule B
Click (SPAAC)
Conjugate of A and B crosslinked via a Triazole moiety

FIG. 4

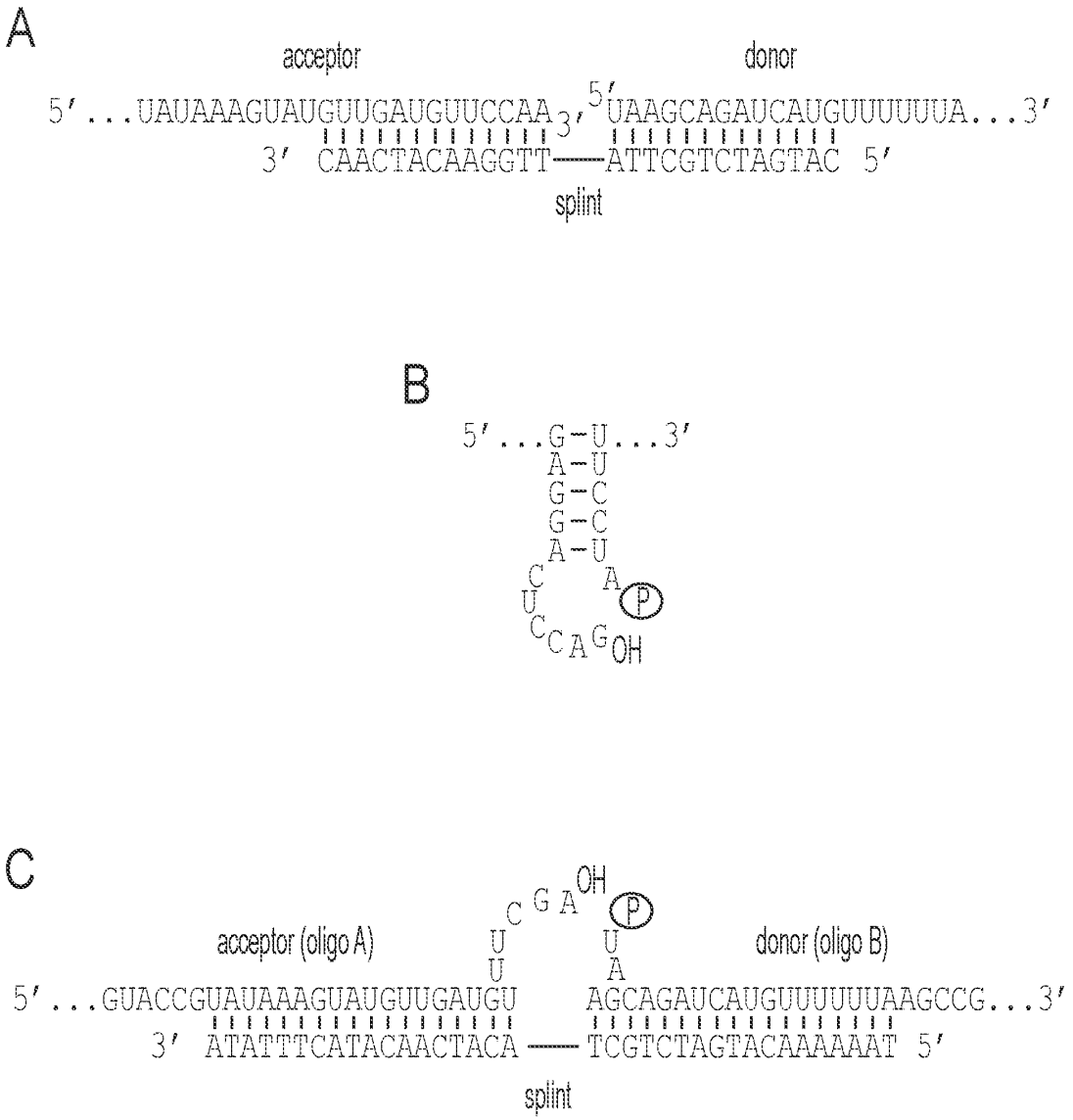

A
acceptor
donor
5′...UAUAAAGUAUGUUGAUGUUCCAA 3′ 5′UAAGCAGAUCAUGUUUUUUA...3′
3′ CAACTACAAGGTT——ATTCGTCTAGTAC 5′
splint
B
5′...G–U...3′
A–U
G–C
G–C
A–U
C A
U P
C C A G OH
C
C G A OH
P
acceptor (oligo A)
U U
U A
donor (oligo B)
5′...GUACCGUAUAAAGUAUGUUGAUGU AGCAGAUCAUGUUUUUUAAGCCG...3′
3′ ATATTTCATACAACTACA —— TCGTCTAGTACAAAAAAT 5′
splint

FIG. 5

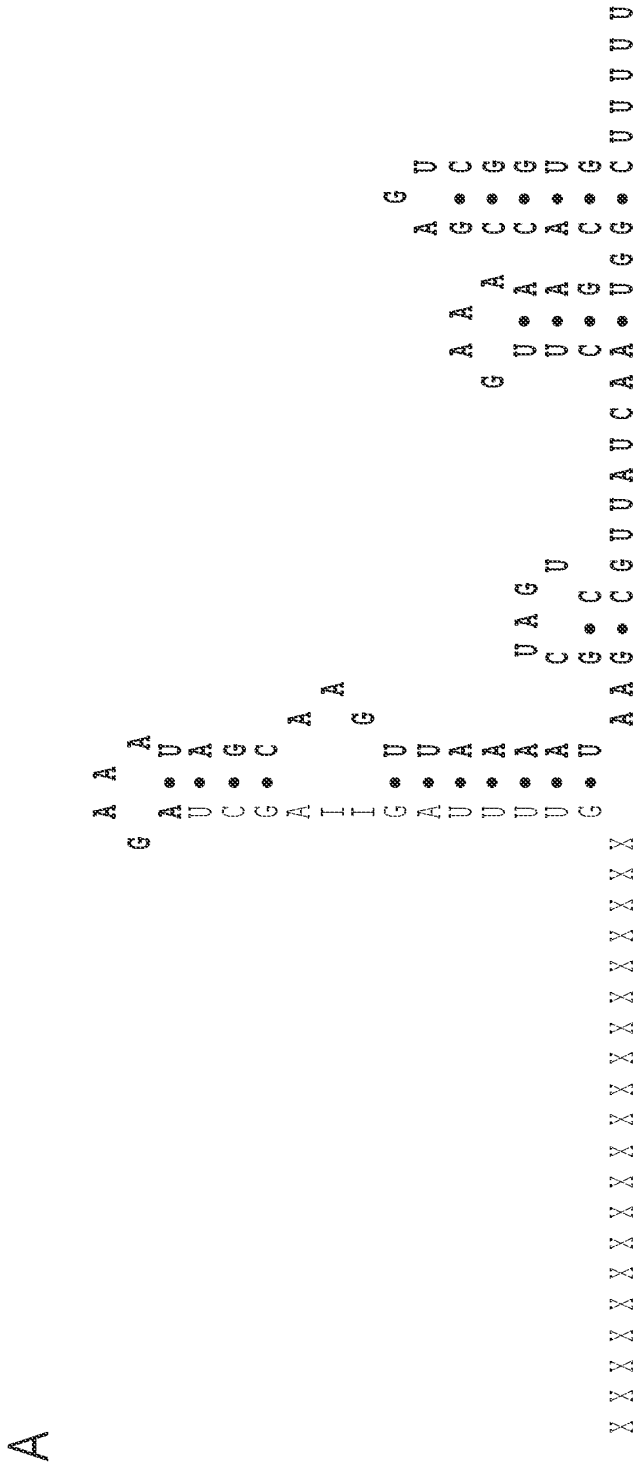


FIG. 6

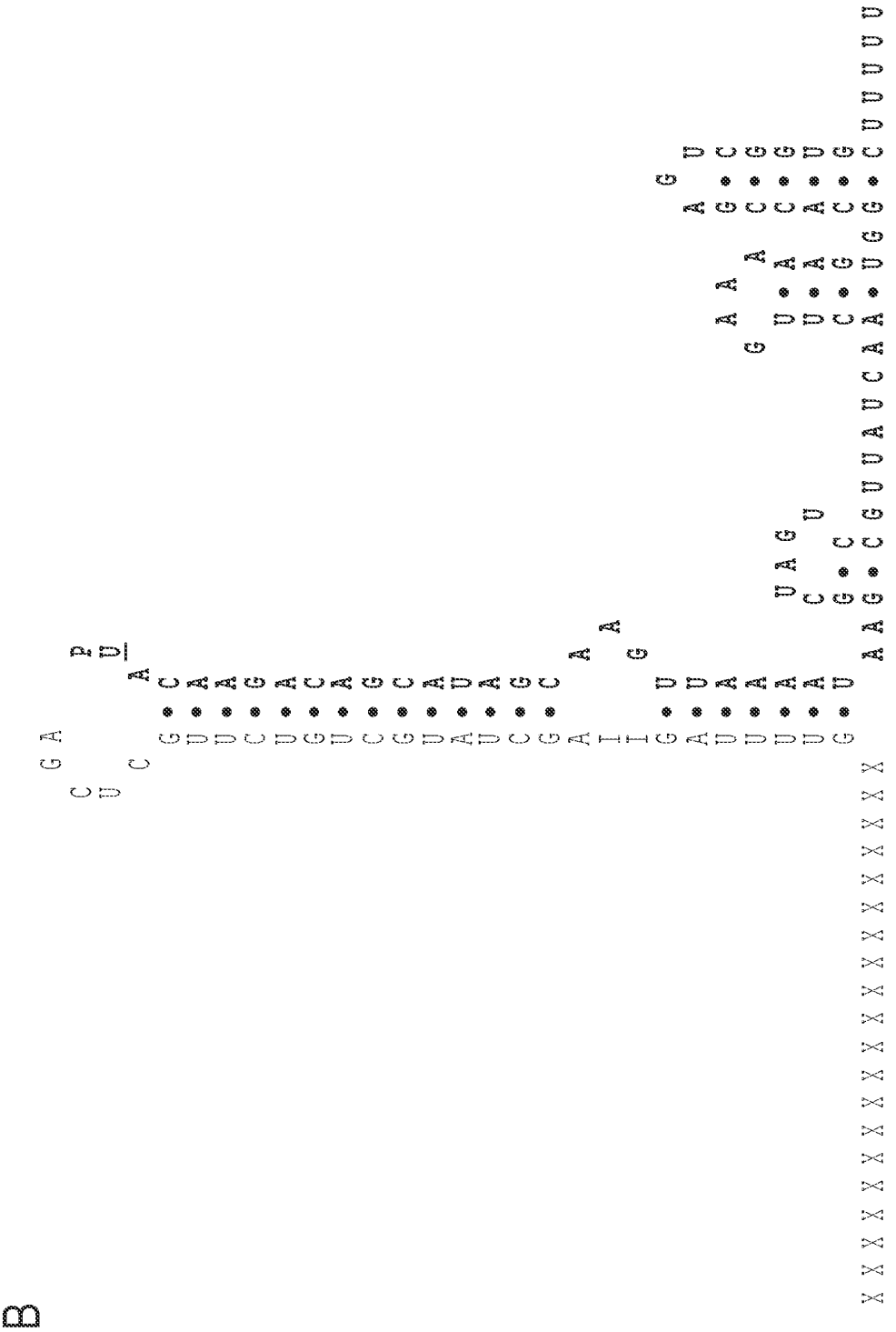


FIG. 6
CONTINUED

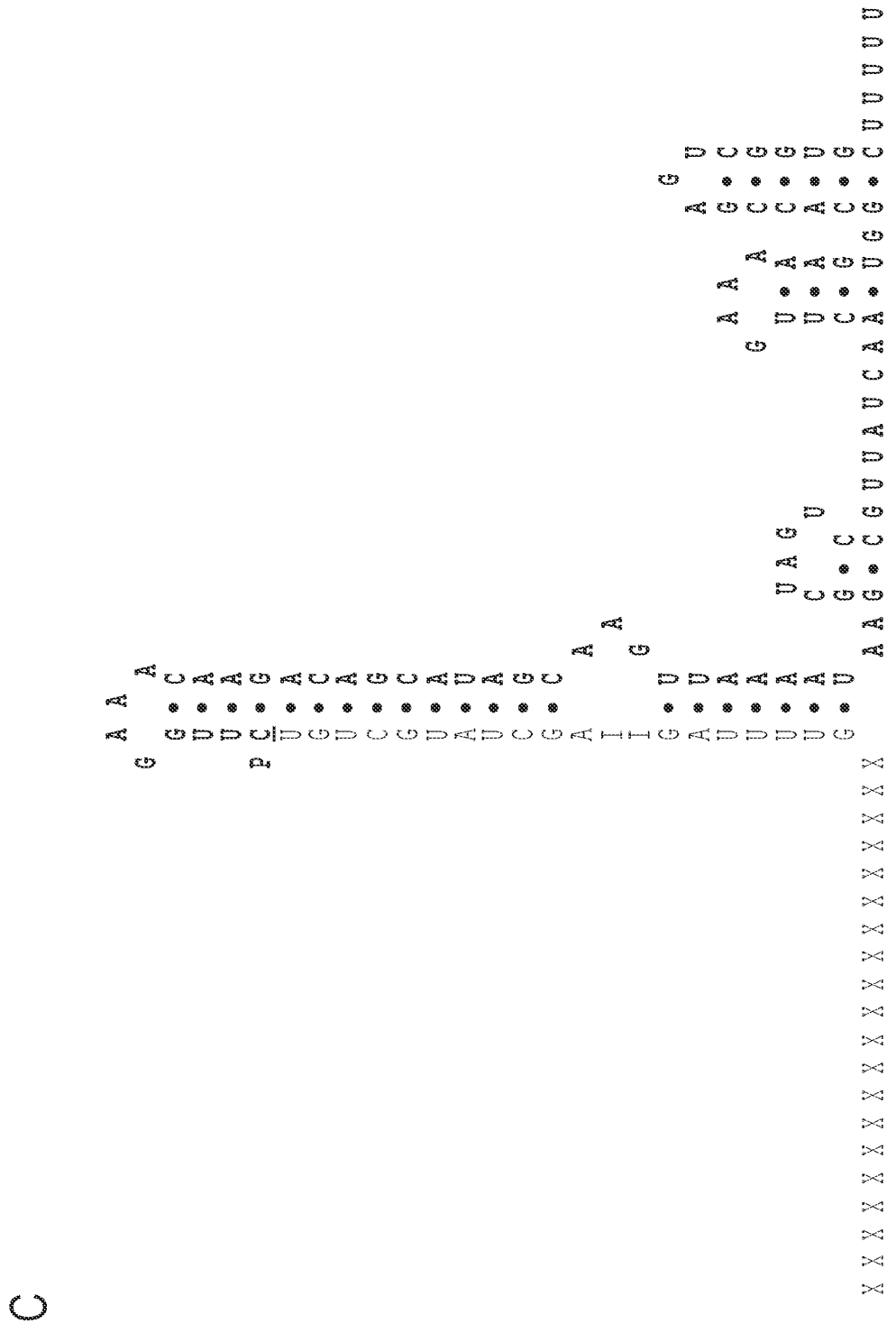


FIG. 6
CONTINUED

A

Acceptor

5′mA.mU.mC.G.A.C.A.A.G.A.A.A.G.G.G.A.C.U.G.A.G.U.U.U.U.A.G.A.G.C.U.A.U.G.C.U.G.U3′

Donor

5′pC.U.U.G.G.A.A.A.C.A.A.G.A.C.A.G.C.A.U.A.G.C.A.A.G.U.U.A.A.A.A.U.A.A.G.G.C.U.A.G.U.C.
C.G.U.U.A.U.C.A.A.C.U.U.G.A.A.A.A.A.G.U.G.G.C.A.C.C.G.A.G.U.C.G.G.U.G.C.mU.mU.mU.U3′

B

FIG. 7

C

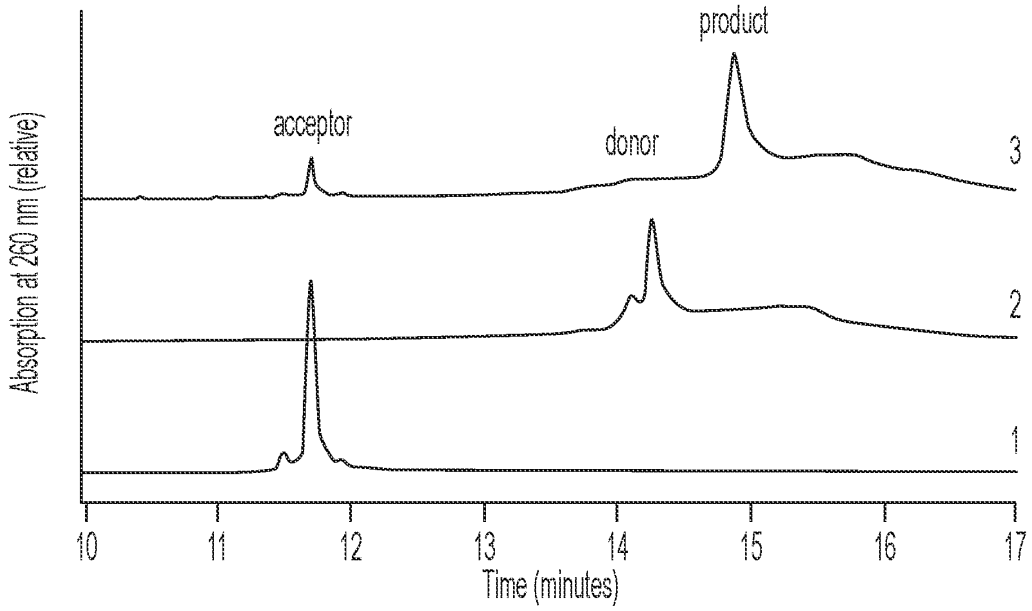
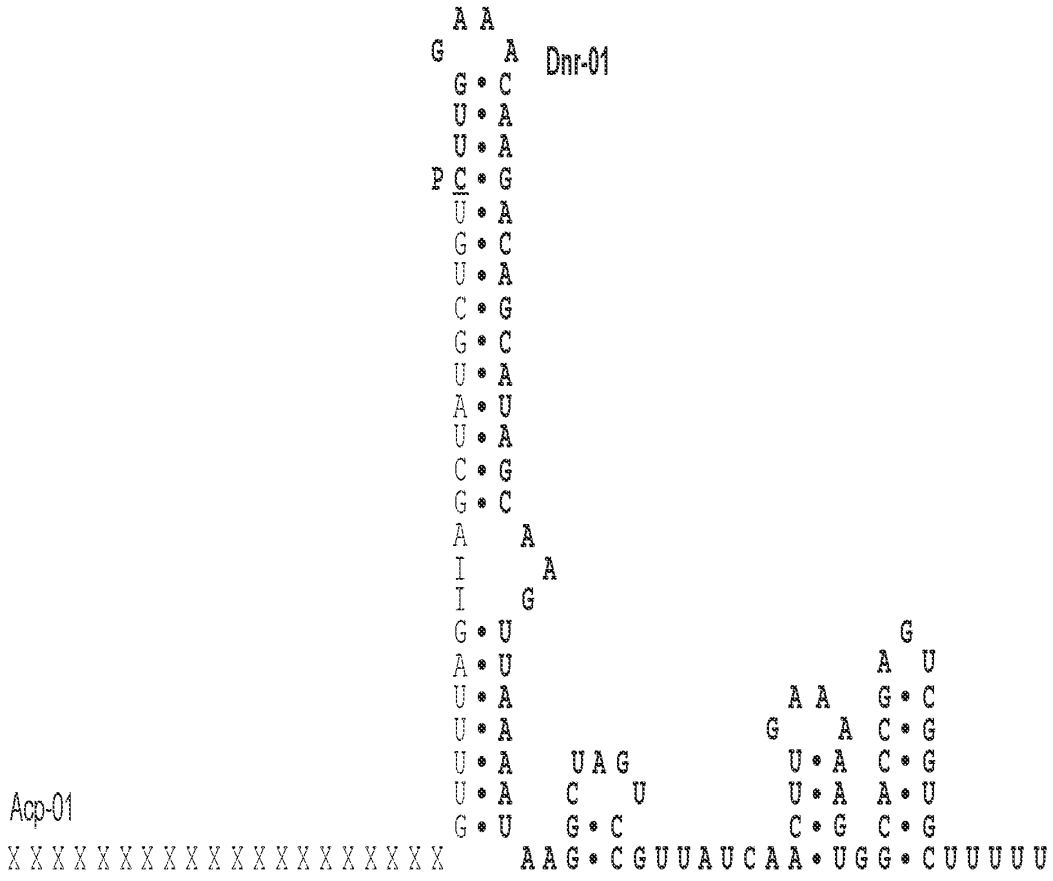


FIG. 7
CONTINUED

A


Dnr-01
Acp-01

B

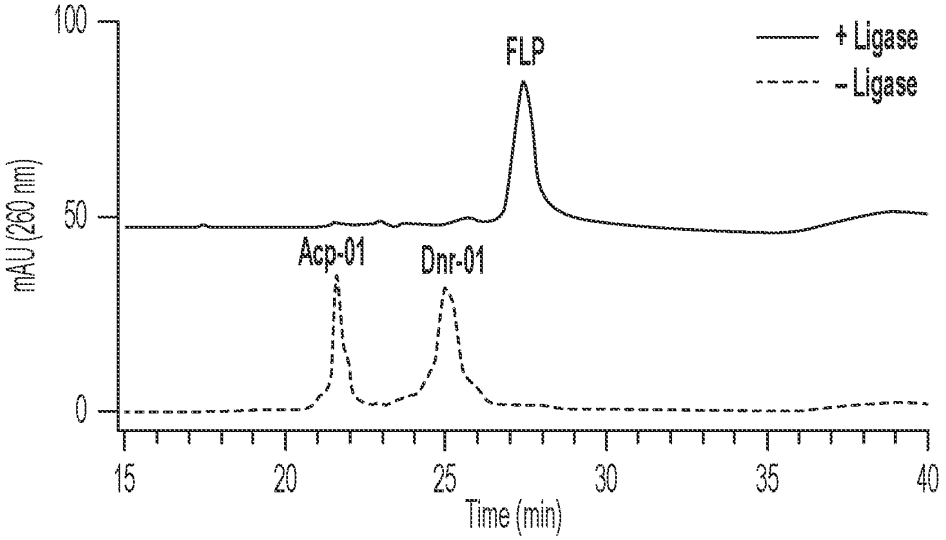

100
50
0
mAU (260 nm)
FLP
+ Ligase
- Ligase
Acp-01
Dnr-01
15
20
25
30
35
40
Time (min)

FIG. 8

A

Dnr-02
Acp-02

B

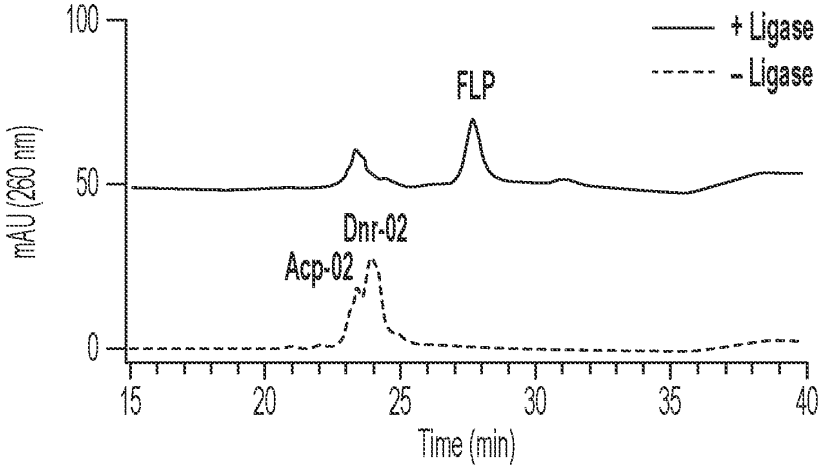
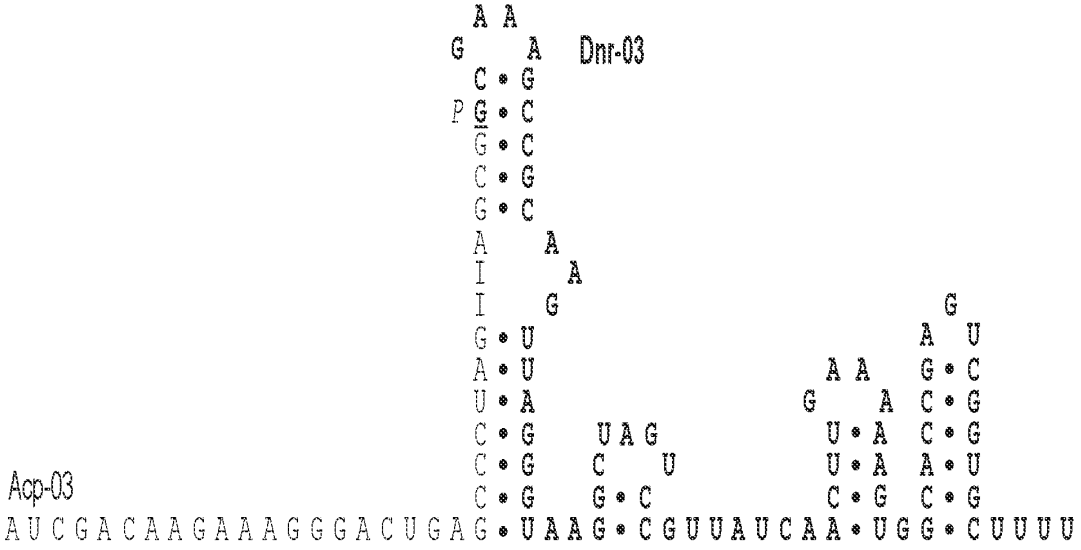

100
50
0
mAU (260 nm)
+ Ligase
- Ligase
FLP
Dnr-02
Acp-02
15
20
25
30
35
40
Time (min)

FIG. 9

A


Dnr-03
Acp-03
AUCGACAAGAAAGGGACUGAG•UAAG•CGUUAUCAA•UGG•CUUUU

B

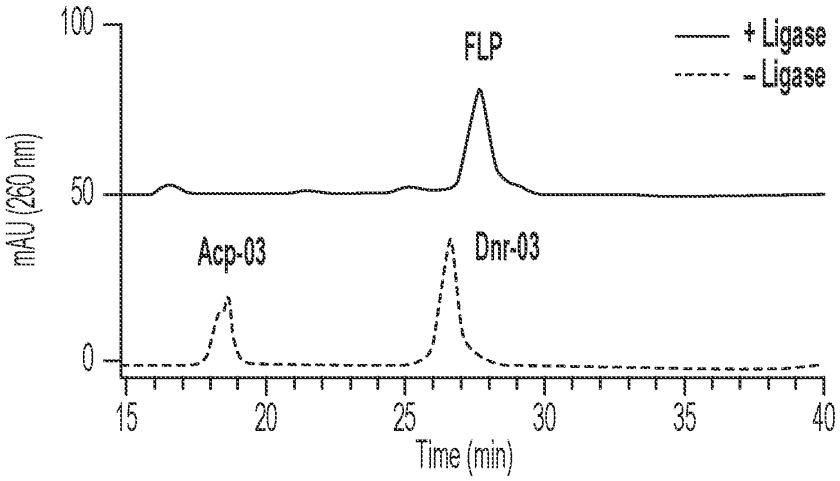

100
50
0
mAU (260 nm)
FLP
+ Ligase
– Ligase
Acp-03
Dnr-03
15
20
25
30
35
40
Time (min)

FIG. 10

C

D

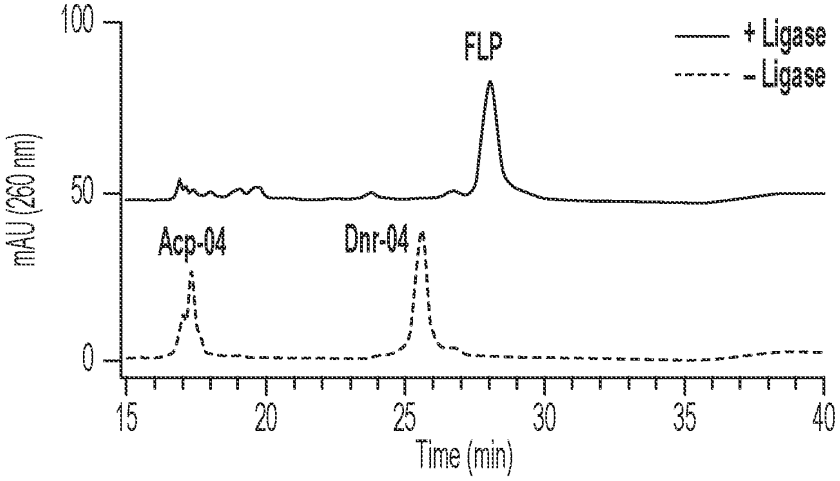


FIG. 10
CONTINUED

A

Dnr-05
Acp-05
AUCGACAAGAAAGGGACUGA
AAG•CGUUAUCAA•UGG•CUUUU

B

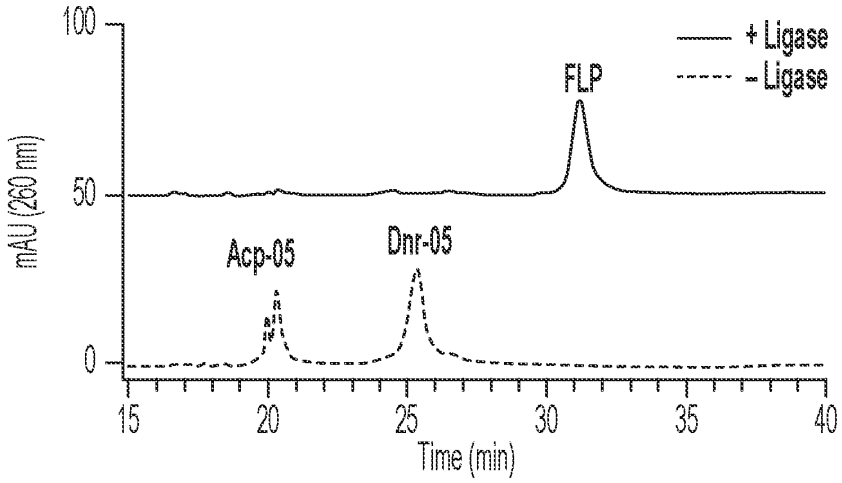
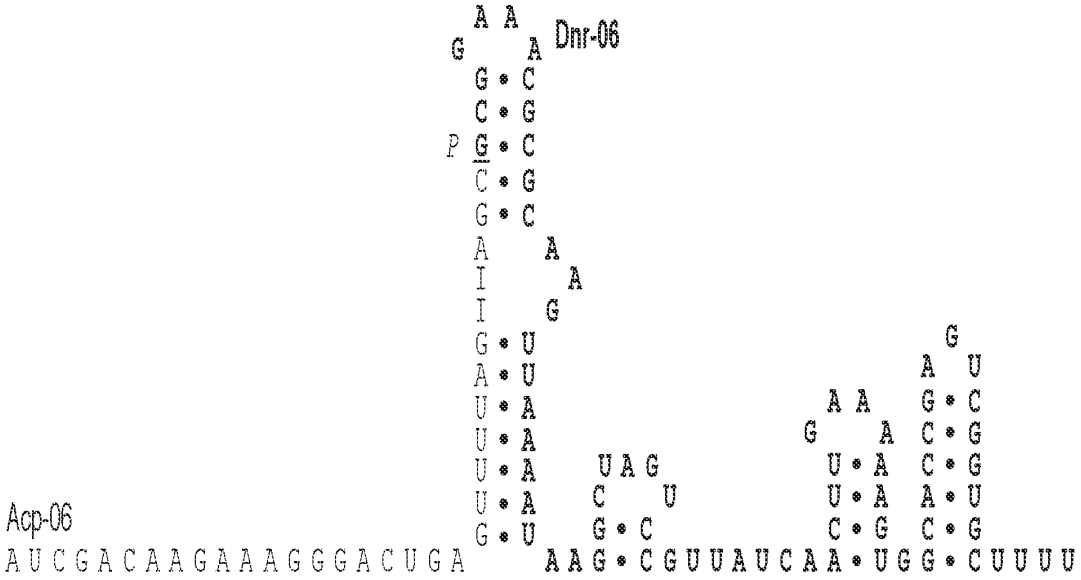

100
50
0
mAU (260 nm)
+ Ligase
– Ligase
FLP
Acp-05
Dnr-05
15
20
25
30
35
40
Time (min)

FIG. 11

C



D

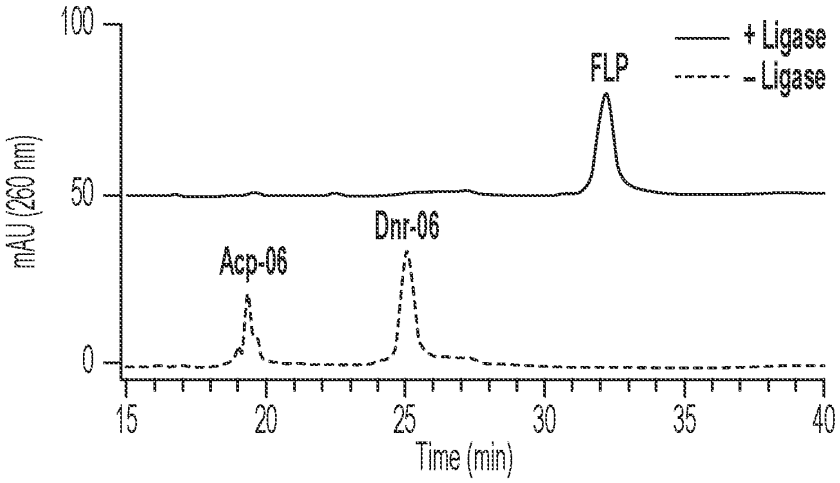
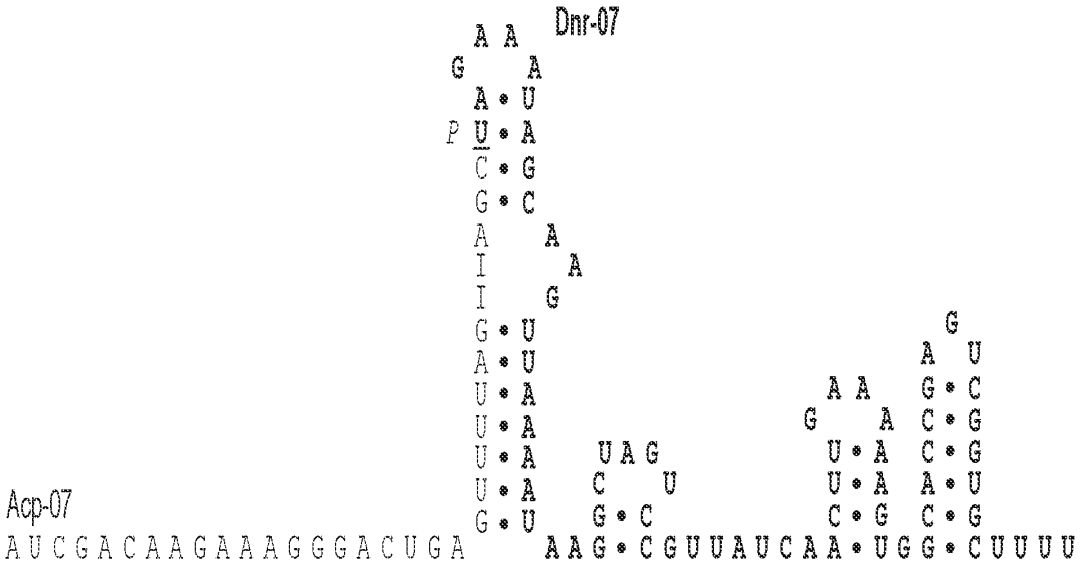


FIG. 11
CONTINUED

A


Dnr-07
Acp-07
AUCGACAAGAAAGGGACUGA
AAG•CGUUAUCAA•UGG•CUUUU

B

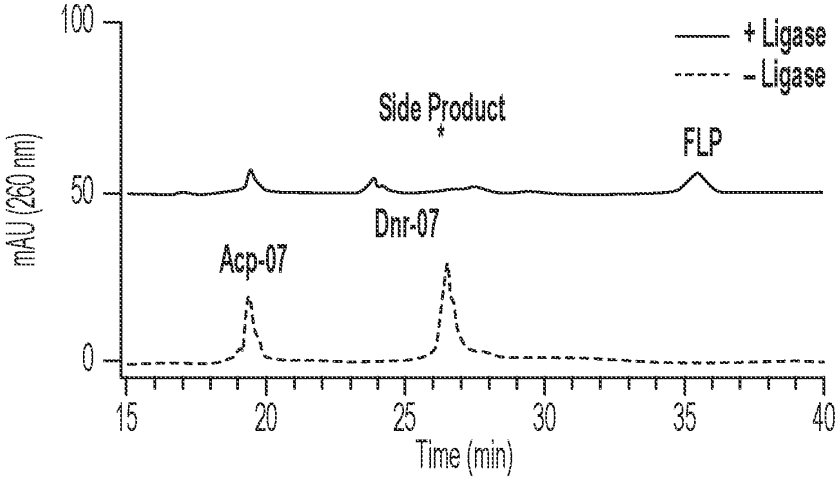

+ Ligase
– Ligase
Side Product
FLP
Dnr-07
Acp-07
mAU (260 nm)
100
50
0
15
20
25
30
35
40
Time (min)

FIG. 12

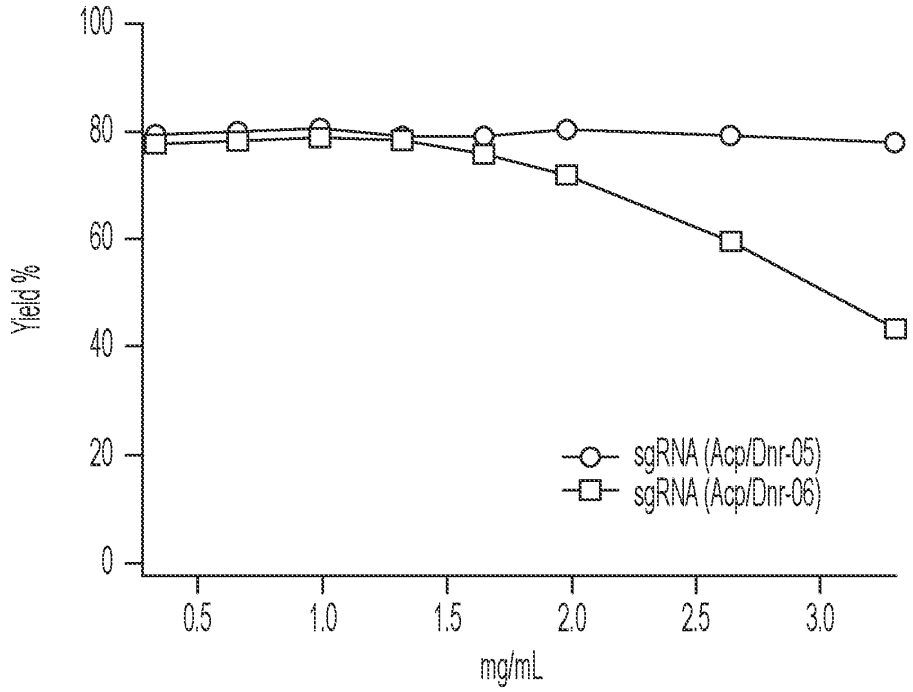
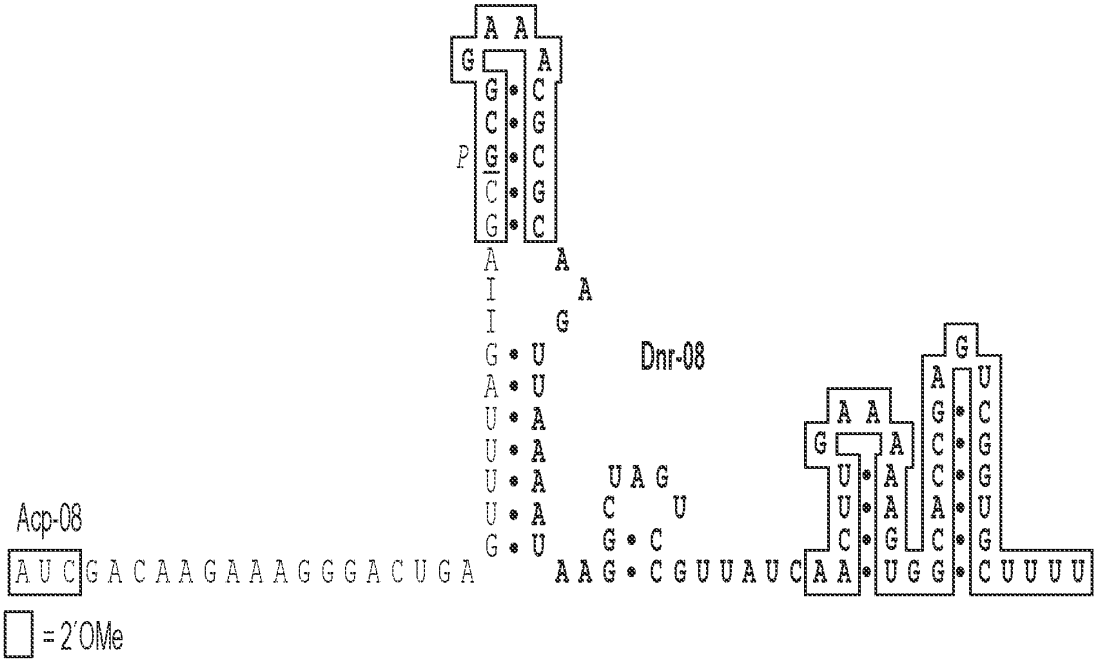

100
80
60
40
20
0
Yield %
0.5
1.0
1.5
2.0
2.5
3.0
mg/mL
sgRNA (Acp/Dnr-05)
sgRNA (Acp/Dnr-06)

FIG. 13

A


Acp-08
Dnr-08
= 2'OMe

B

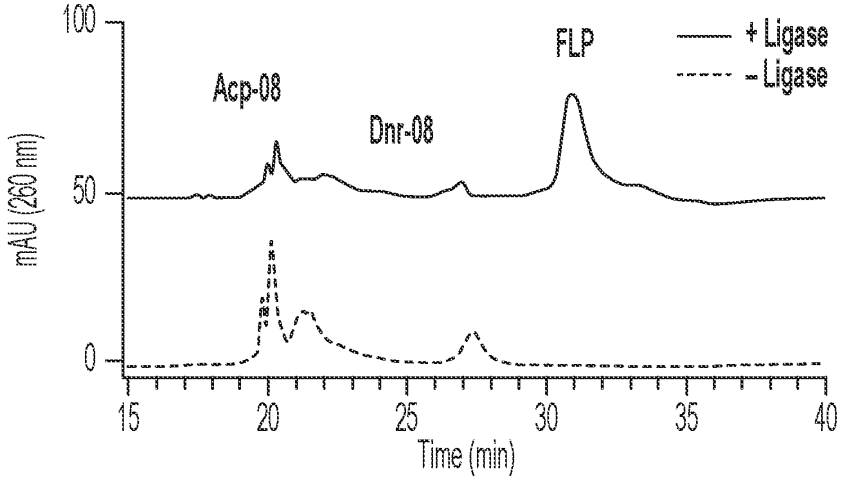

100
50
0
mAU (260 nm)
Acp-08
Dnr-08
FLP
+ Ligase
– Ligase
15
20
25
30
35
40
Time (min)

FIG. 14

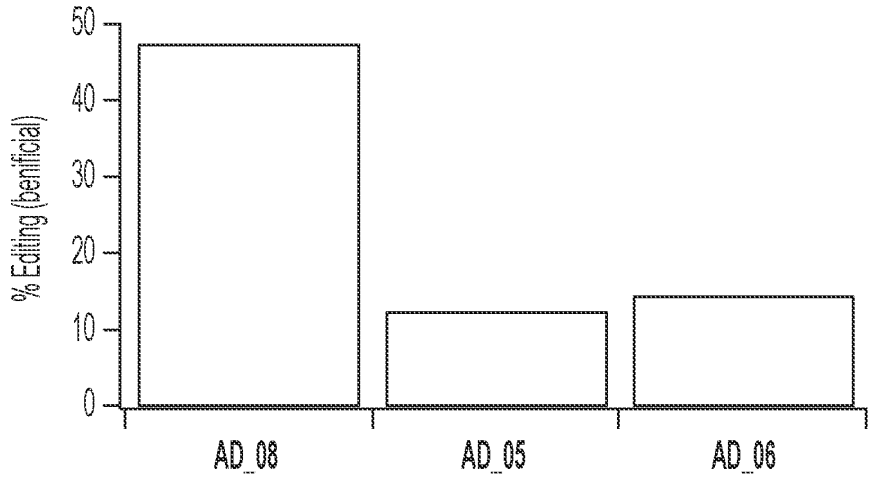


FIG. 15

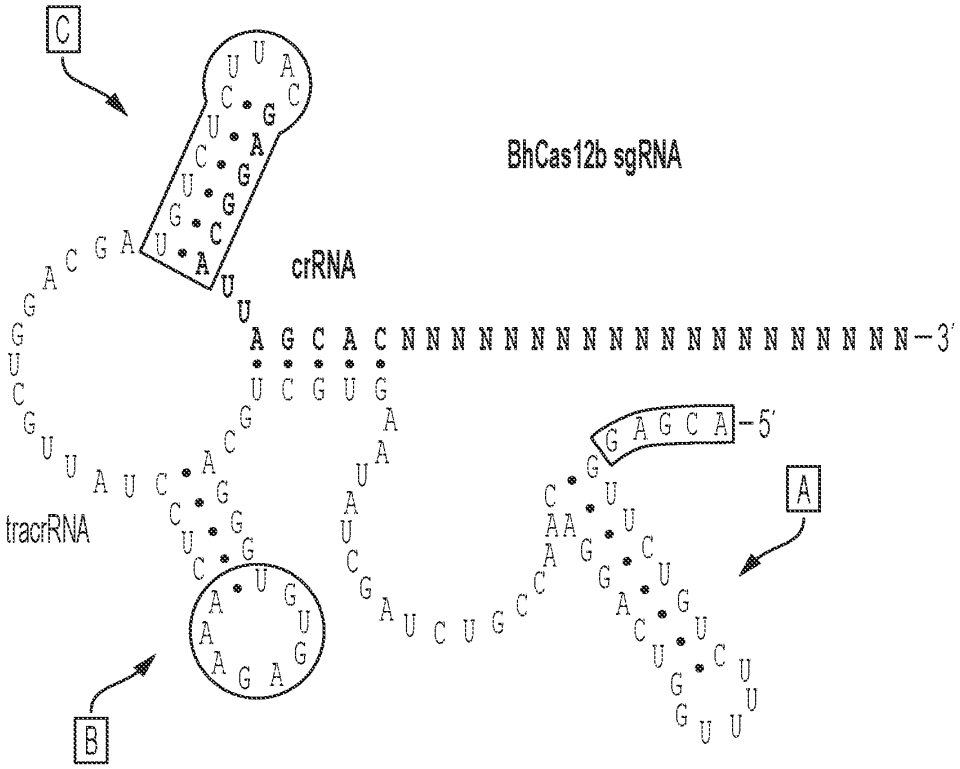


FIG. 16