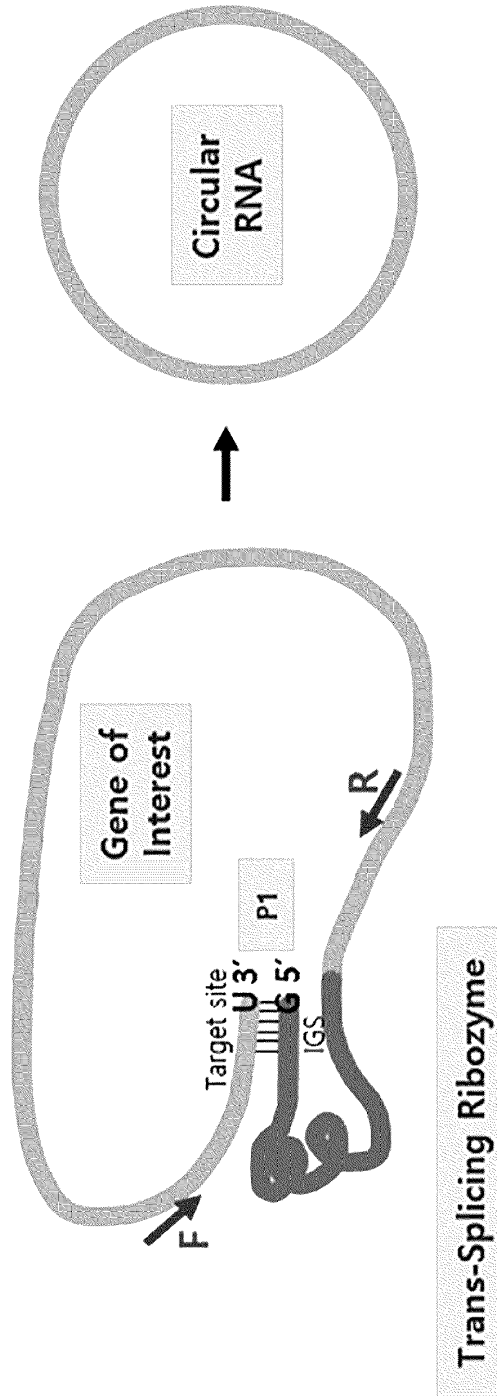
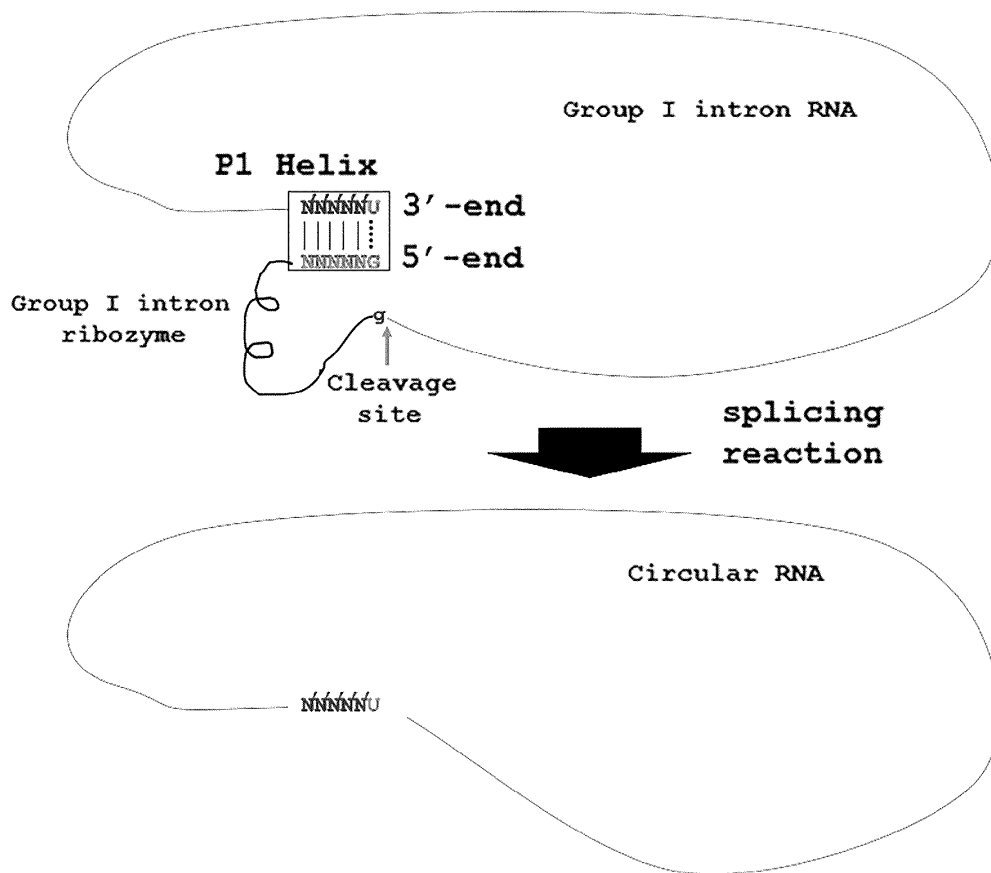


[Figure 1a]

New Strategy for Circular RNA Preparation : Circularization by Trans-Splicing Reaction

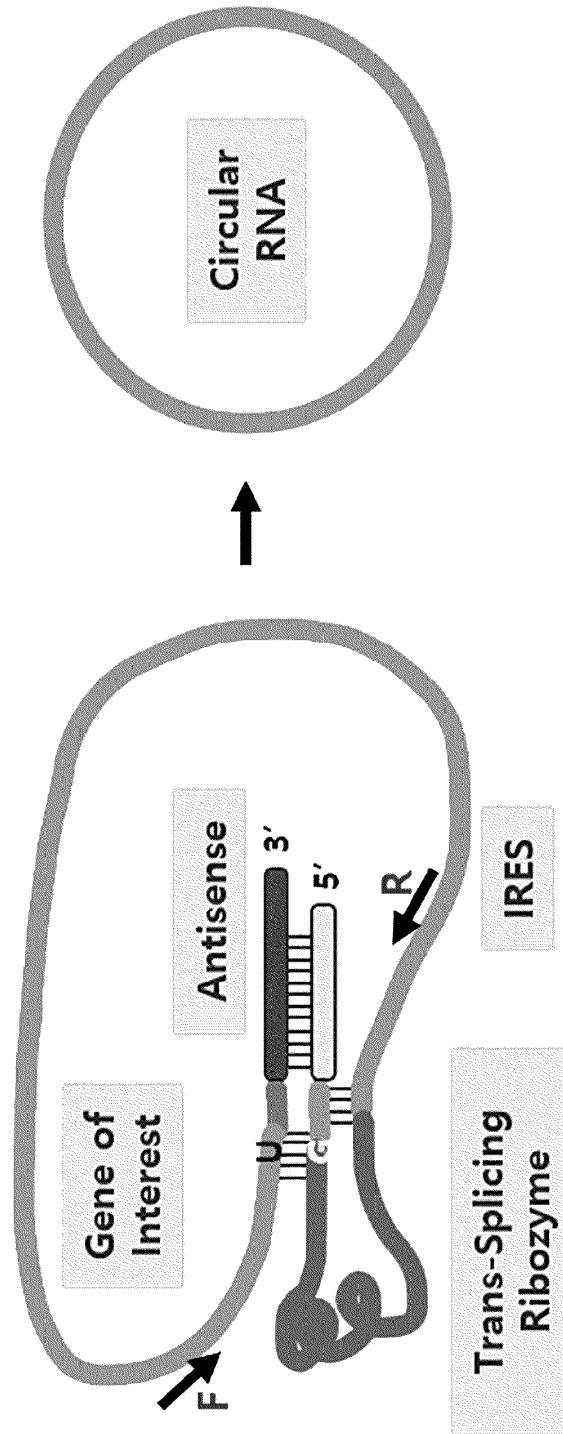


[Figure 1b]

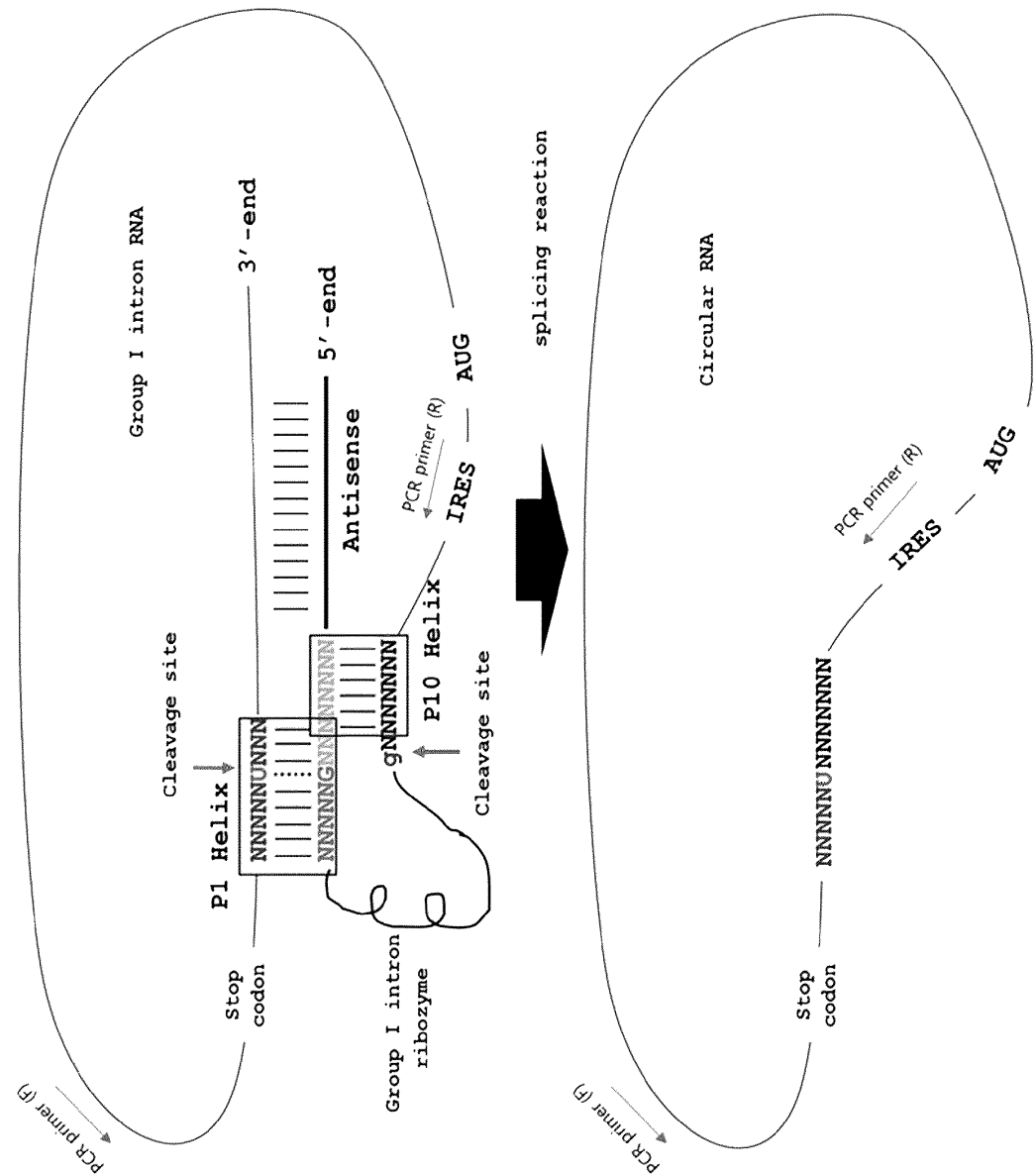


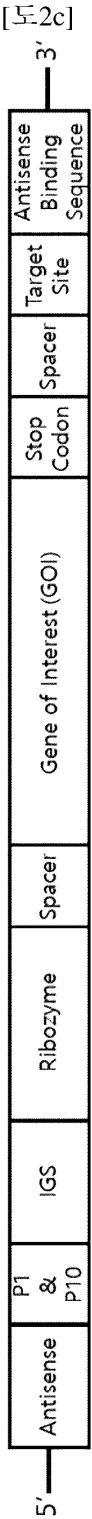
[도2a]

New Strategy for Circular RNA Preparation : Circularization by Self-Targeting & Splicing (STS) Reaction



[도2b]





[2d]

T7 Promoter

Antisense 150

GGGATTGGAACATCGATTAAATAGGACTACATATAGGGGATCGATTGAATTGTGACGAATTGCGGCCAGCATGGAGAACTGCCATGGCTCAGCCAGGTAGTactgtggtggtactcgaagTg
gctgcgtaccacaccctgtgcalttgagaagggcacgtagaagttaggccctctgtgcccattcatggctgtgcccctgtggtccacccGAAATTCggtactccGcccaAATAAGGTTATCAGGCATGCACCTGGTAGCTAGCTCT

P1-P10 IGS

TTAAACCAATAGATTGCATCGGTTTAAAGGCAAGACCCTCAAAATTGCGGGAAGGGGTCAACAGCGTTTCAGTACCAAGTCTCAGGGGAAACTTTGAGATGGCTTGCCAAAGGGTATG

Trans-Splicing Ribozyme

GTAATAAGCTGACGGACATGGTCTTAACACCGCAGCCAAAGTCCTAAGTCAACAGATCTCTGTGATGGAATGCAGATCAACAGACTAAATGTCGGTGGGGAAGATGATTTCTTCAT
AAGATATAGTCGGACCTCTCCTTAATGGAGCTAGCGGATGAAGTATGCAACACTGGAGCGCTGGGAACCTAATTTGTATGCGAAAGTATATTGATTAGTTTTTGGAGTACTCGcgagtacg

EMCV IRES

CTTAAGAAAAAAGagggcccgaaacctggccctgtctcttgacgagcattcctaggggtctttccctctcgccaaaggaaatgcaaggctgtgtaatgtctggaaggagcagttcctctggaagctcttgaagacaacaa
cgctgtagcgacctttgcaggcagcggaaacccccaccctggcagcaggtgctctgcggccaaaagccacgtataaagatacacctgcaaaaggcgcacaacccccagtgccacgttggtgagttgtggaaagagctcaaatgg
ctctctcaagcgattcaacaagggtgaaggatgccagaaggtacccccattgtatgggctgcatctggggccctgggtgcacatgtcttaacatgtgttttagtcgaggttaaaaaaacgtctagggccccccgggacgtggttttc

Gene of Interest (GOI, Gaussia Luciferase)

ctttgaaaaacacgataaaGcttgcacaaccCTTAAGaccggtAtggagtcgaagttctgtttccctgtatctgcatcgtgtgcccagggccaaagcccccagagaaacacgaagacttaacatcgtggccgtggccagcaacttgc
cgaccaedgatctcatgctgaccggtggaagttgcccggcaagaagctgcccgtgcaggtgctcaaaagagatggaagcccaatgcccgaagctggtctgcaccaggggtgtctgcatctgctgtccacatcaagtgcaagcccaagat
gaagaagttcatccaggatgctgcacacatcaggaagcgataaagagfctccacagggtggcatalaggcaggcgatcgtgacatctctgagattccttggttcaaggacttggagcccatggagcagttcatcgcacaggctgactgt

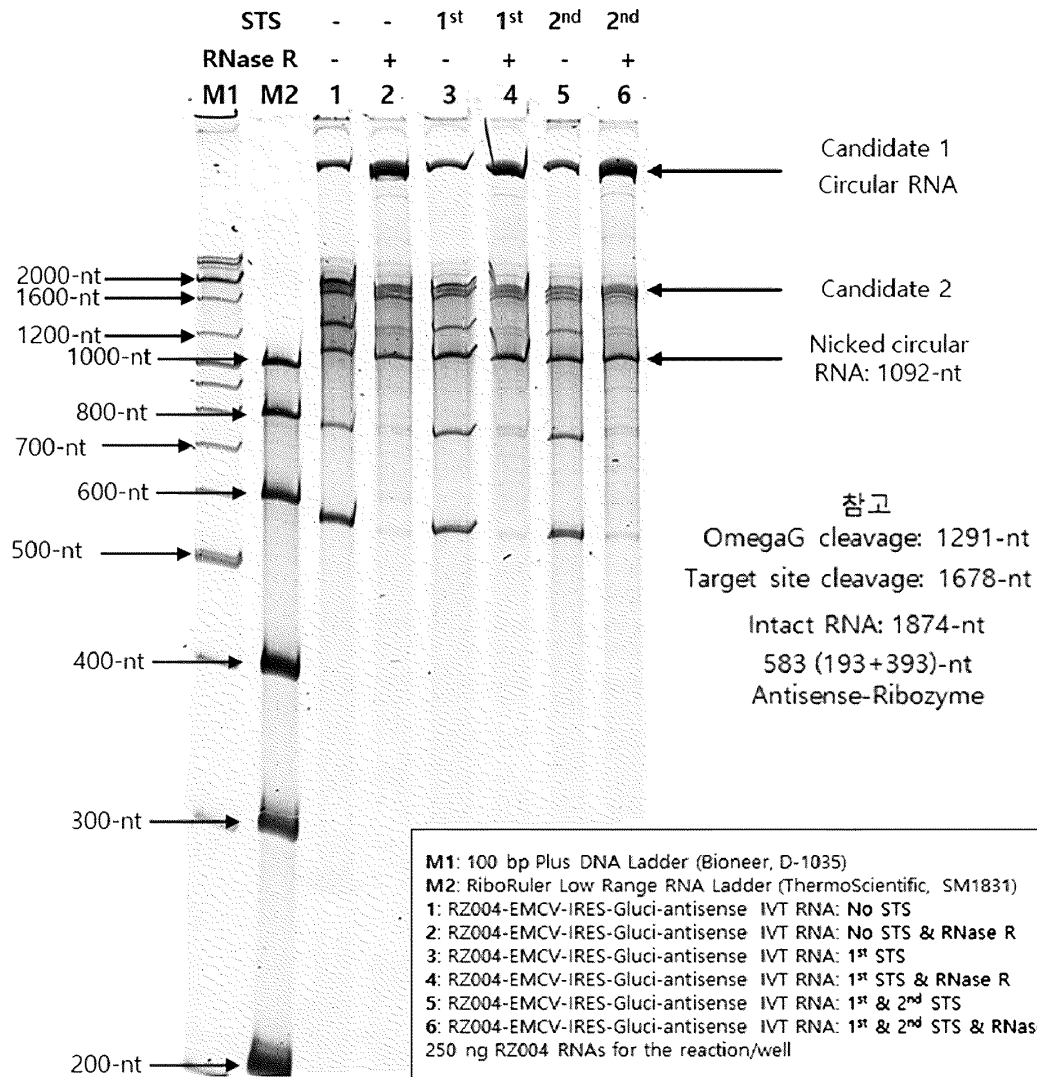
Target Site

Antisense Binding Sequence

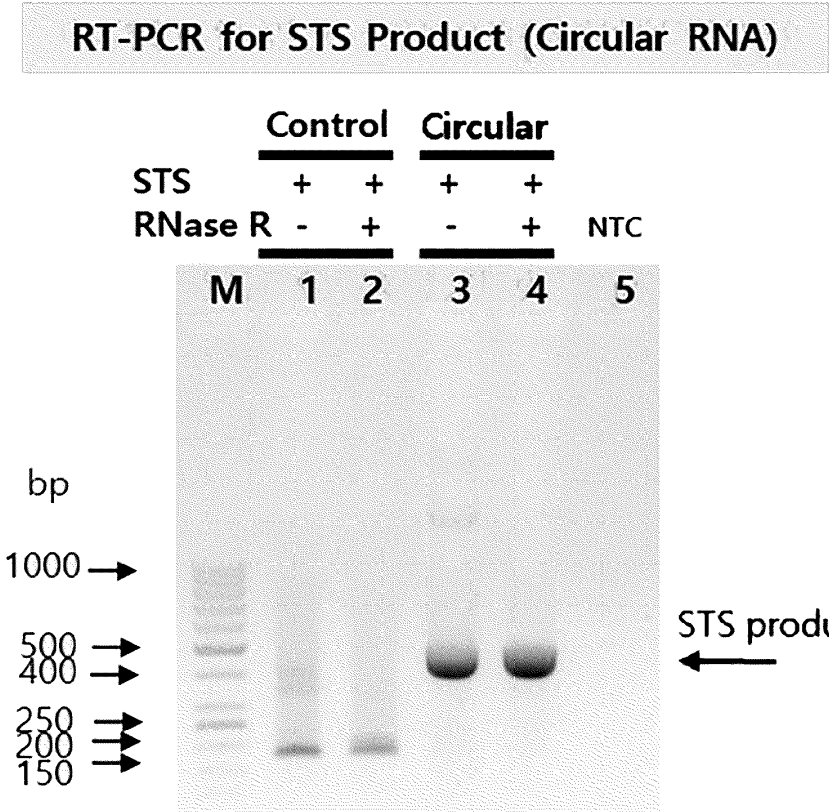
gtgtggactgcacaactggtgctctaaagggtttgccacgtgcagtgcttctgaactgtctaaagaagtggctccgcgaacgtgtgcgaactttgccgaagcaggtggaacaagatacaaggggggcggtggtgactaaAA
AAAAAAAaccggtTTGGTGGGagcagccacgggtcagccacaagggtccacagccatgaatggcacagaagggtccctaactctacgtgacctctctcaatgcgacgggtgtgtgtacgcagccActtcgagfaccacacagTACTAC

CTGGCTGAGCCATGGCAGTTCTCCATGCTGGGCCGCCAAGTTGCCCTCGAGCAGCGCTGCTCGAGAGATCT

[도3]



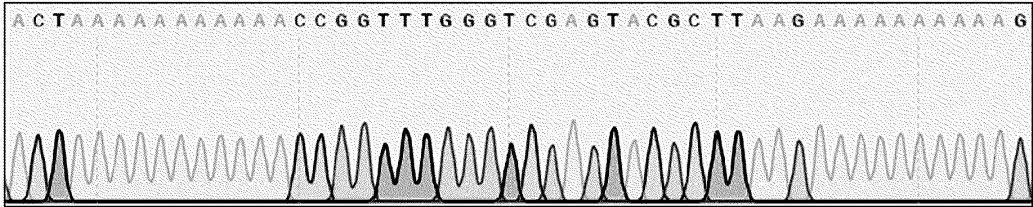
[도4a]



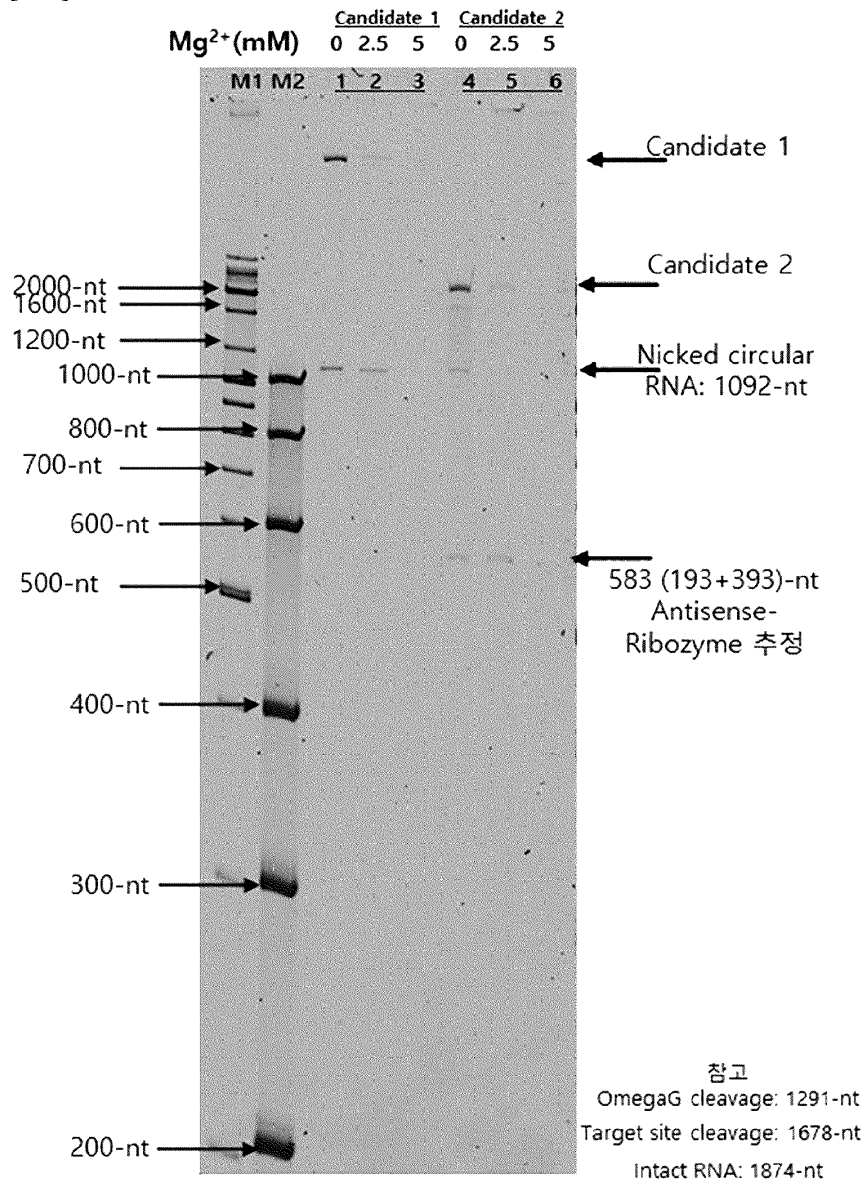
M: GenRuler 50 bp DNA Ladder (Thermo Scientific, SM0373)
1: EMCV-IRES-Gluc1-antisense IVT RNA Control + STS
2: EMCV-IRES-Gluc1-antisense IVT RNA Control + STS & RNase R
3: RZ004-EMCV-IRES-Gluc1-antisense IVT RNA: Direct STS
4: RZ004-EMCV-IRES-Gluc1-antisense IVT RNA: Direct STS & RNase R
5: NTC

[도4b]

G. Luci 3' end ↓ IRES 5' end

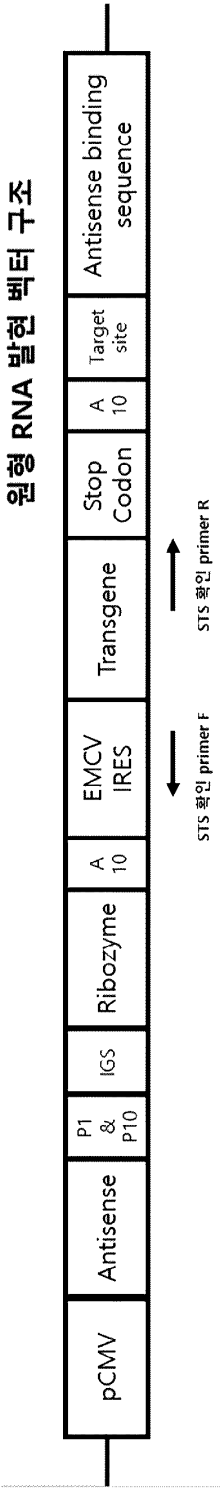


[도5]



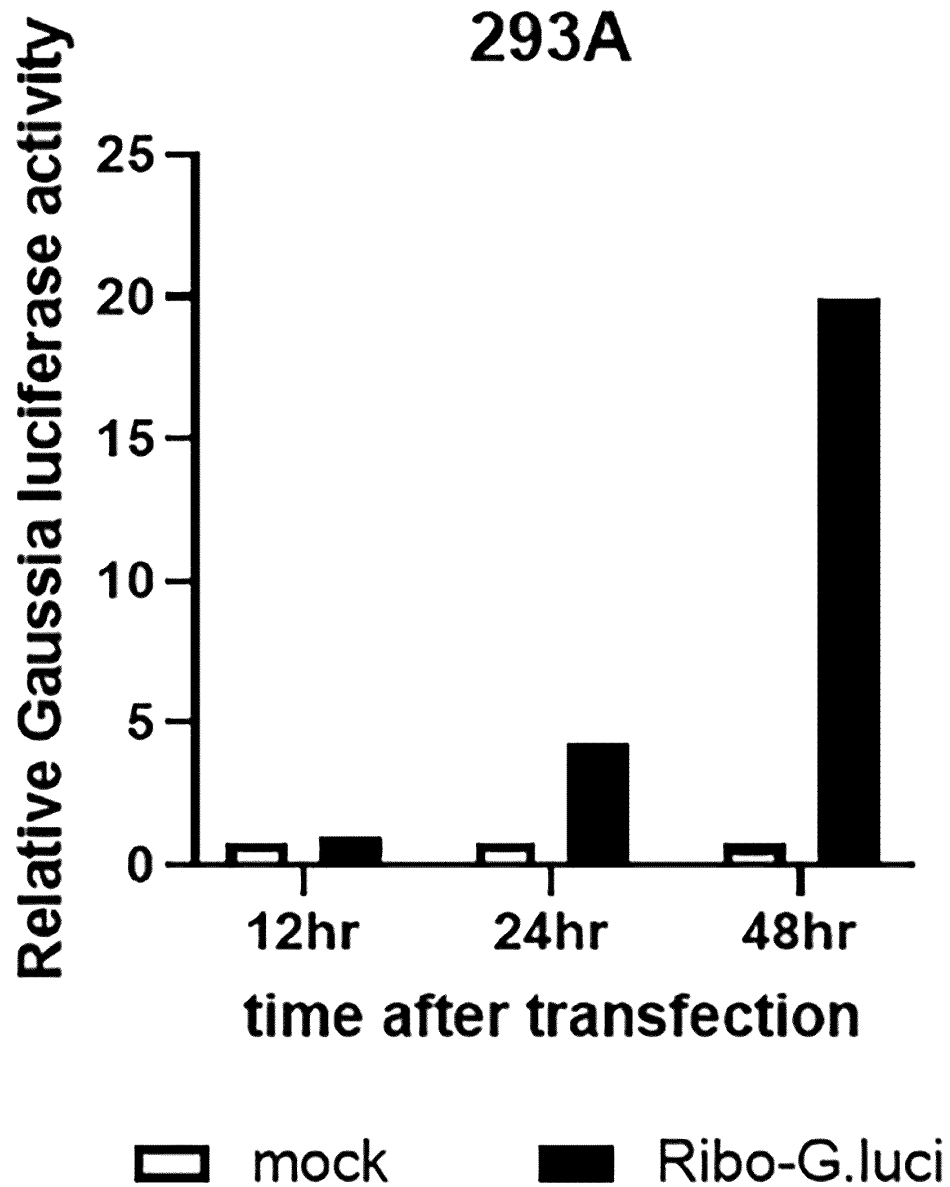
M1: 100 bp Plus DNA Ladder (Bioneer, D-1035)
M2: RiboRuler Low Range RNA Ladder (ThermoScientific, SM1831)
1: Circular RNA candidate 1 elu
2: Circular RNA candidate 1 elu (2.5 mM MgCl₂, 65°C 30 min)
3: Circular RNA candidate 1 elu (5 mM MgCl₂, 65°C 30 min)
4: Circular RNA candidate 2 elu
5: Circular RNA candidate 2 elu (2.5 mM MgCl₂, 65°C 30 min)
6: Circular RNA candidate 2 elu (5 mM MgCl₂, 65°C 30 min)
 100 ng RNA for the reaction/well

[도 6a]



[도6b]

Report gene expression



[도6c]

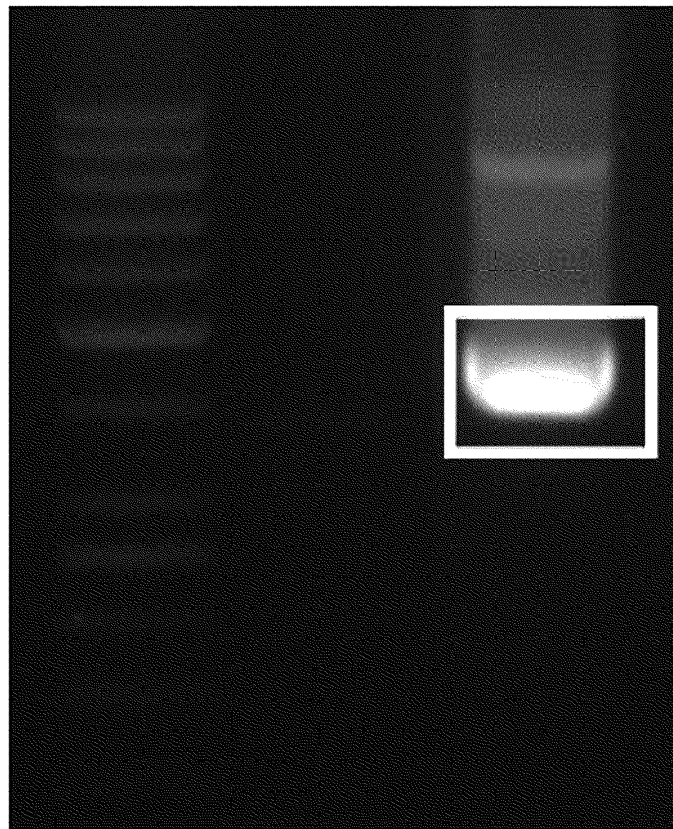
Splicing product RT-PCR

Mock

Ribo-G.luci

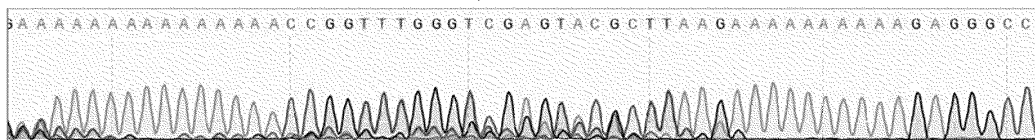
500bp →

400bp →



[도6d]

G.Luci 3' end ↓ IRES 5' end



[도7a]

분석조건

- ✓ HPLC system: Agilent 1290 Infinity II Bio UHPLC
- ✓ Analytical column: Agilent PLRP-S 4000A 8um, 2.1 x 50mm
- ✓ Injection volume: 2 μ L (Prep 10 – 20uL)
- ✓ Flow rate: 0.5 mL/min
- ✓ Column oven temperature: 60 °C
- ✓ Detector: UV 260nm
- ✓ Mobile phase A: 100mM Hexylamine acetate pH7.0
- ✓ Mobile phase B: 100mM Hexylamine acetate in acetonitrile
- ✓ Gradient

- Fraction collection

Time(min)	%A	%B
0	70	30
2	70	30
15	45	55
16	10	90
17	10	90
17.1	70	30
20	70	30

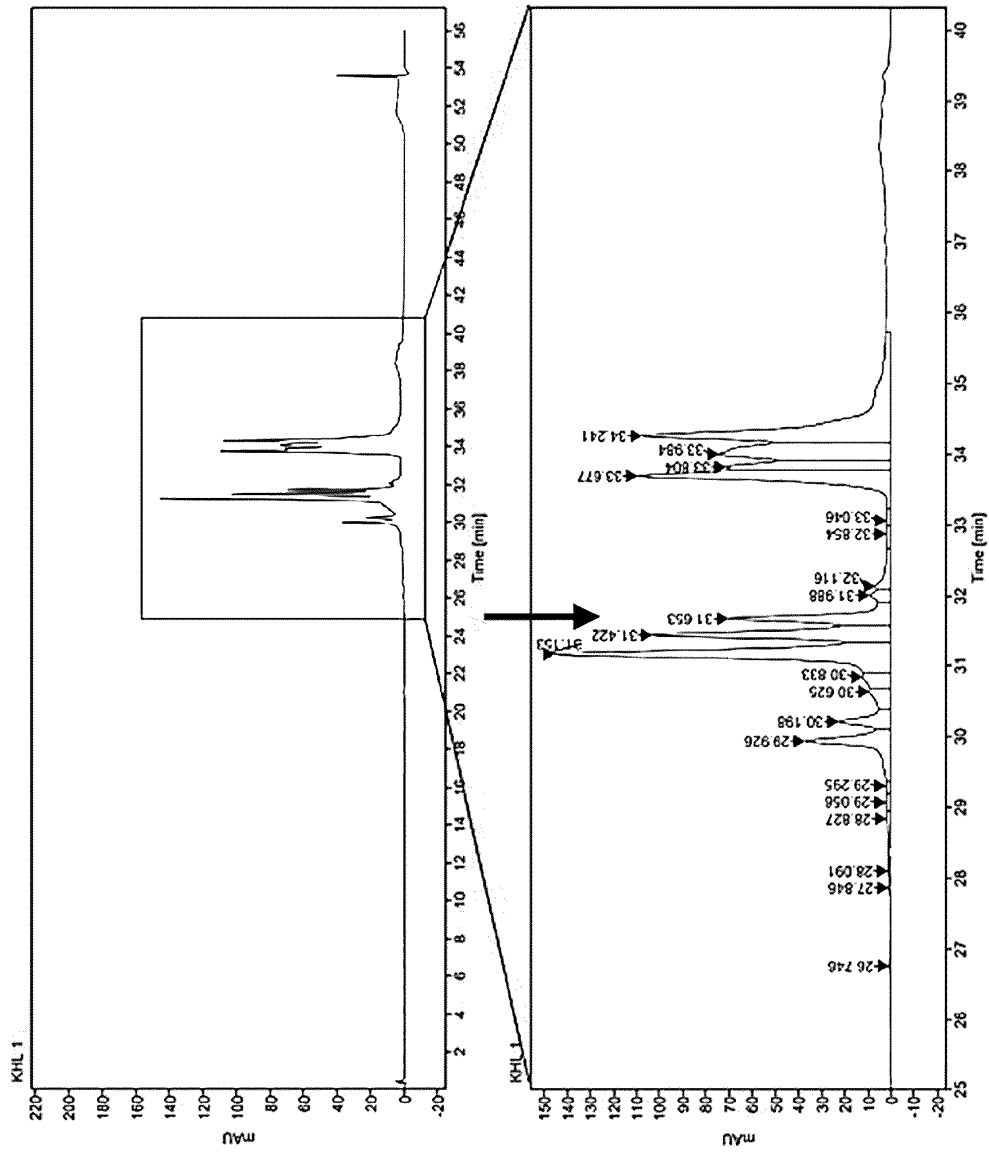
- Analytical

Time(min)	%A	%B
0	80	20
2	80	20
50	40	60
51	10	90
53	10	90
53.1	80	20
56	80	20

- ✓ Fraction collection 9 min ~ 12 min 범위에서 0.1min 간격으로 2 position / 6port valve position increase 하여 96 wellplate(PP, 250uL)에 수집

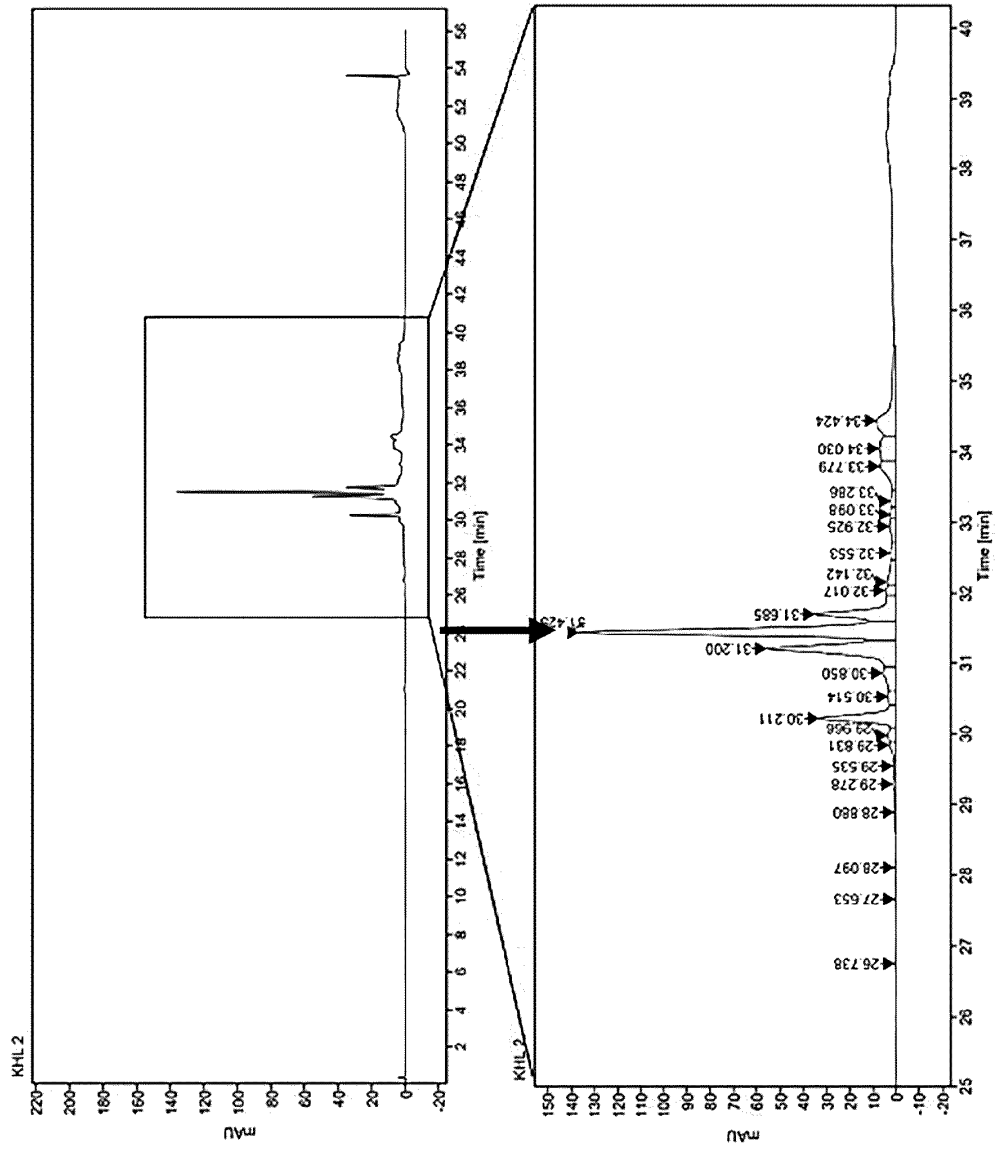
[도 7b]

2) KHL 1 IVT crude sample (Spin-column purification)

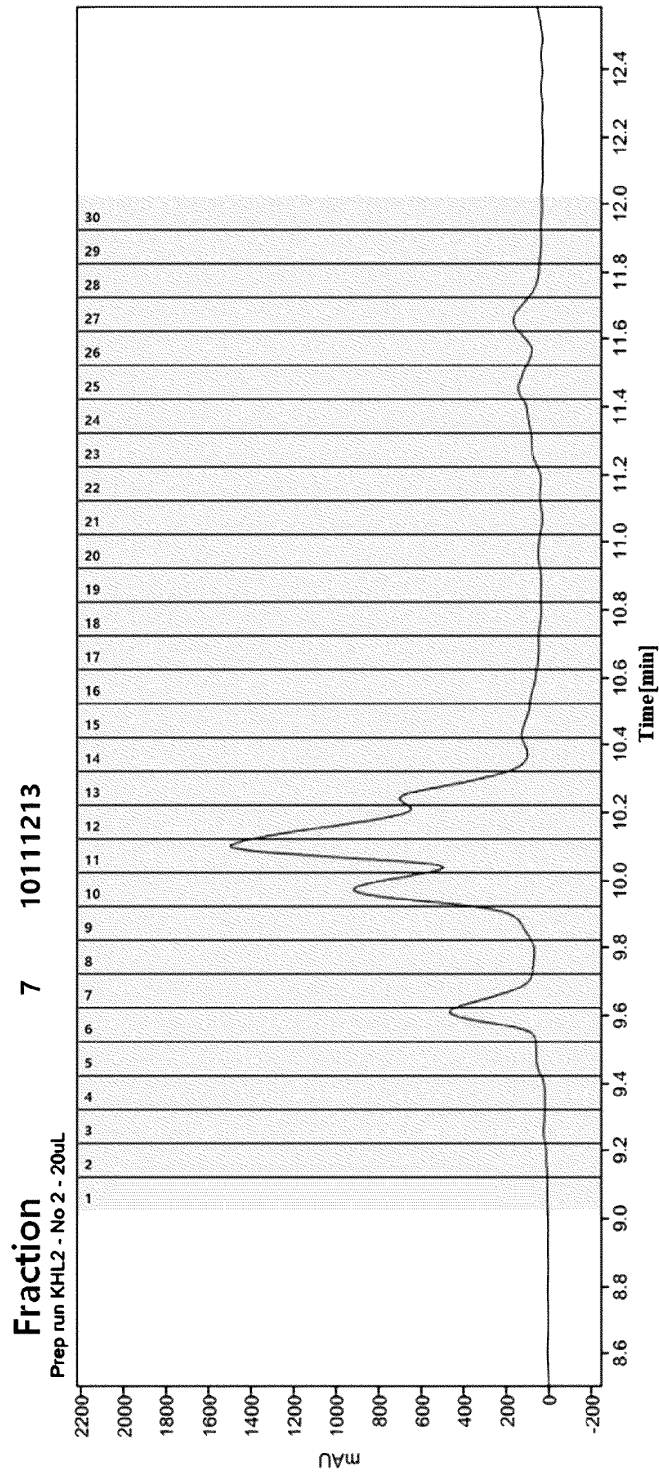


[圖7c]

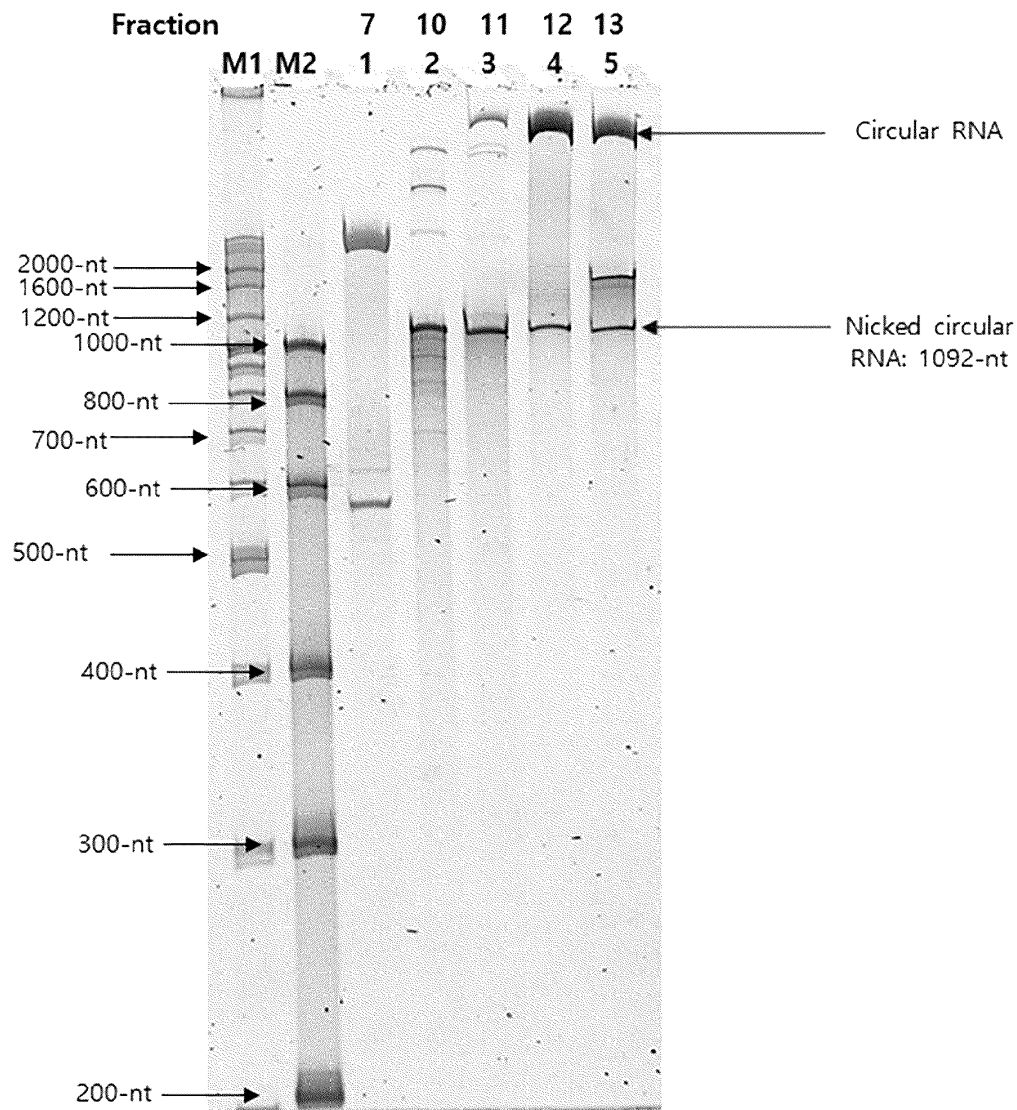
3) KHL 2 RNase R-treated IVT sample (Spin-column purification)



[도8a]

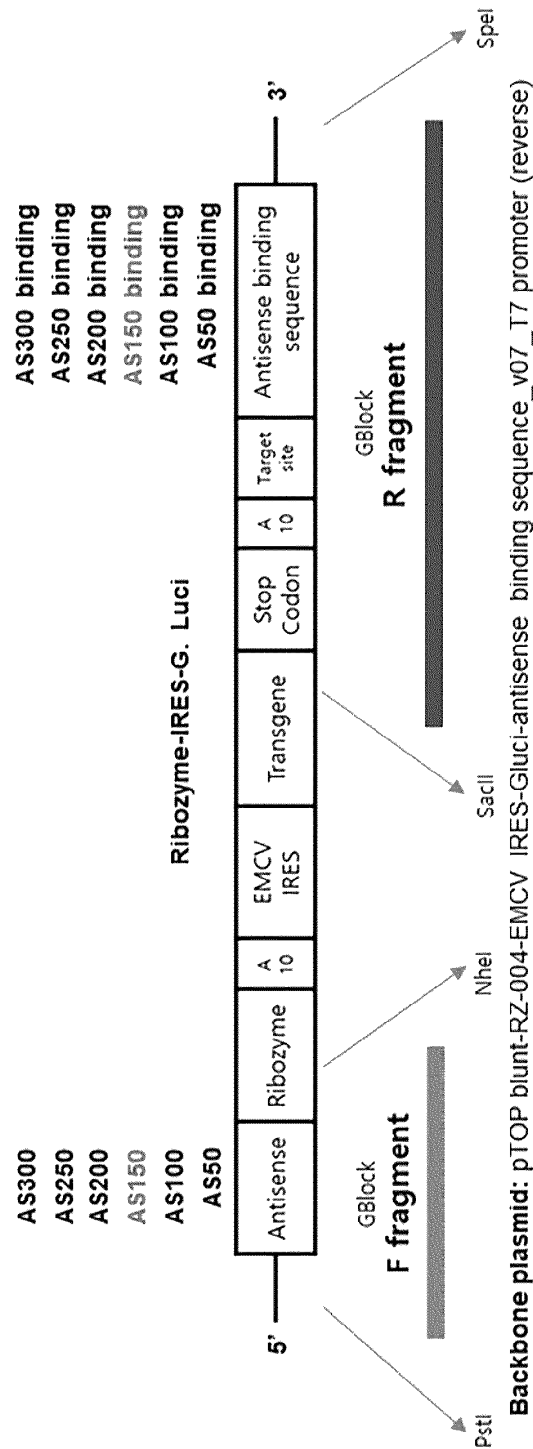


[도8b]

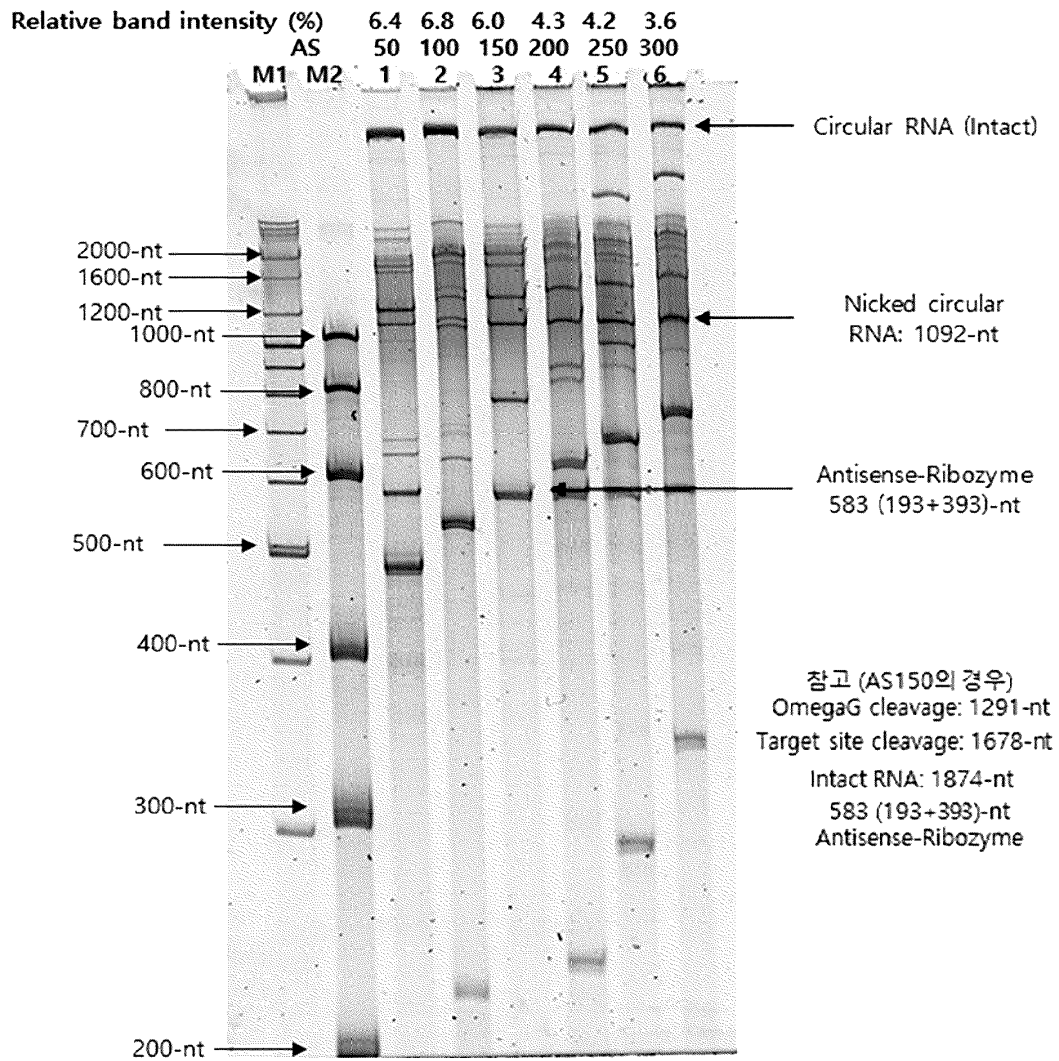


M1: 100 bp Plus DNA Ladder (Bioneer, D-1035)
M2: RiboRuler Low Range RNA Ladder (ThermoScientific, SM1831)
1: RZ004-EMCV-IRES-Gluc1-antisense IVT RNA: Fraction 7
2: RZ004-EMCV-IRES-Gluc1-antisense IVT RNA: Fraction 10
3: RZ004-EMCV-IRES-Gluc1-antisense IVT RNA: Fraction 11
4: RZ004-EMCV-IRES-Gluc1-antisense IVT RNA: Fraction 12
5: RZ004-EMCV-IRES-Gluc1-antisense IVT RNA: Fraction 13
 200 ng/well

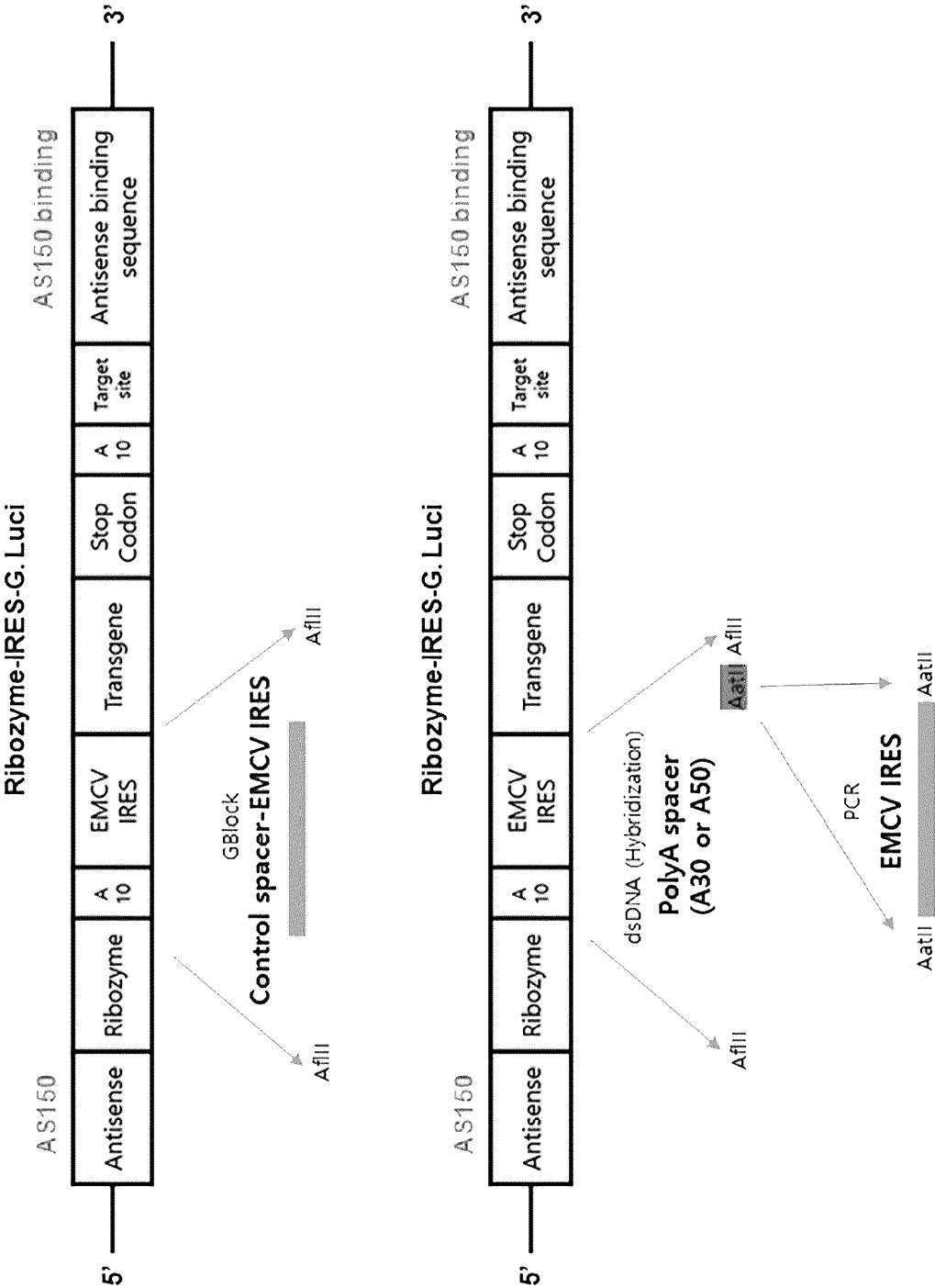
[도9]



[도 10]

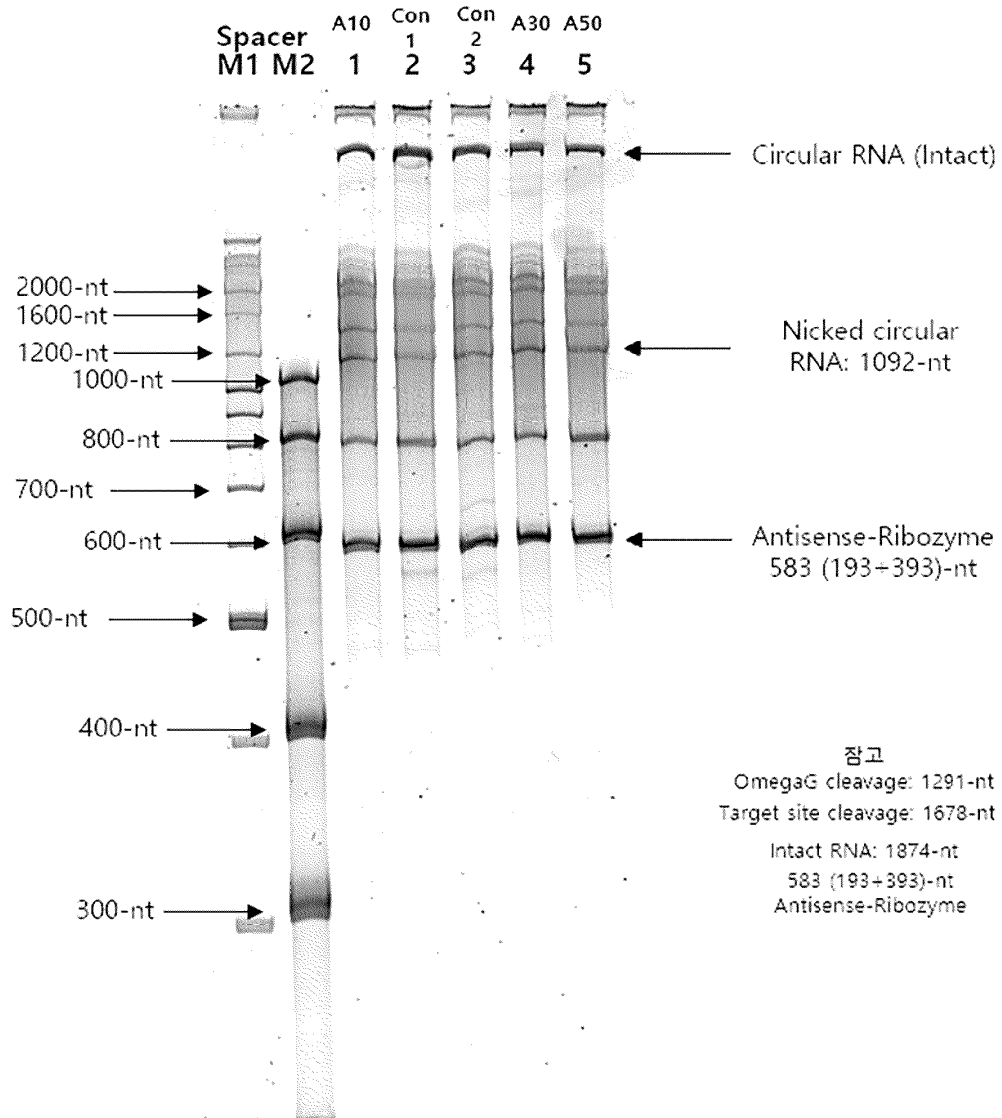


[511]

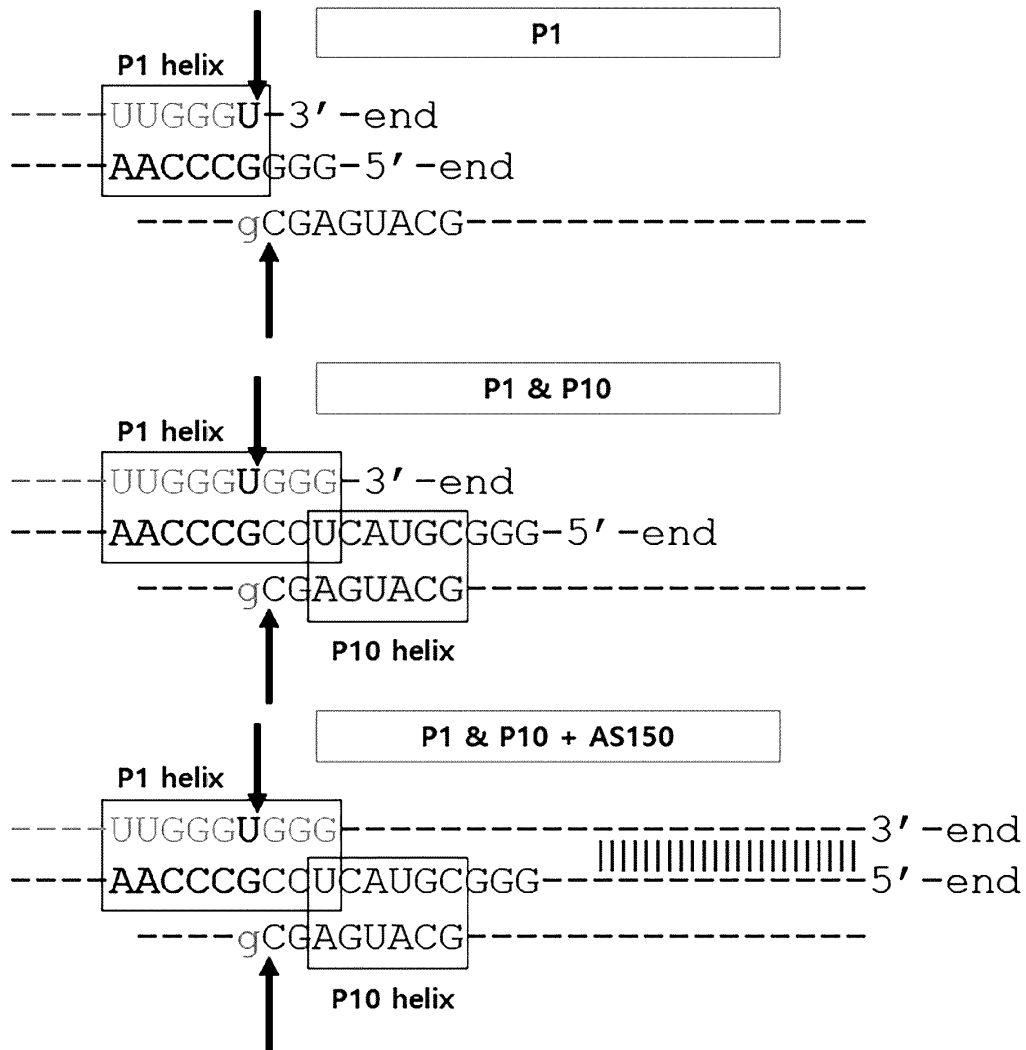


[도 12]

Relative band intensity (%) 5.6 6.7 6.2 6.5 5.7



[도 13]



T7 Promoter IGS

GGGGATTCGAACATCGATTAAATACGACTCACTATAGGGGGcccaaaAAAGTTATCAGGCATGCACCTGGTAGCTAGTCTTTAAACCAATAGATTGCATCGGTTAAAGGCAAGACCGTCAAA

TTTGGGGAAAGGGGTCAACAGCGGTTCAGTACCAAGTCTCAGGGGAACTTTGAGATGGCCTTGCAAAGGGTATGGTAATAAGCTGACGGGACATGGTCCTAACCCAGCGAGCCAAAGTCCT

AAAGTCAACAGATCTTCTGTTGATATGGATGCAGTTCACAGACTAAATGTTCGGTCGGGAAGATGTATTCTTCATAGAATATAGTCGGACCTCTCCTTAATGGGAGCTAGCGGATGAAGT

Trans-Splicing Ribozyme

GATGCAACACTGGAGCCGCTGGGAAGTAATTGTATGCGAAAGTATTGATTAGTTTGGAGTACTCGgagtagcCTTAAGAAAAAAGAgggcccgaaacctggccctgtcttcttgacgag

aatctctagggtcttccctctcgccaaggaaatgcaaggtgttgaaatgctgtagaagcagttctctggaagcttctggaagacaaacaacgctgtagcgacctttgcaggcagcggaacccccaccctggcgacagggtgcctct

gcgggccaaagccagtgataagatacacctgcaaggcggcacacccccagtgccacgttgtagttggatagttggaaagagtc aaatggctctctcaagcggtattcaacaagggtgaaggatgcccaaggtagccccattgta

EMCV IRES

gggatctgctgggctcggtgcacatgctttacatgttttagtcgaggttaaaaaaacgtctagggccccgaaccacgggacgtgttttcttttgaaaaaacacagatgataaGcttgccacaacCCTTAGaccgggAtgtgggagtc

aaagttctgttgcctgattcgtctgttgccgaggccaagcccaccgagaacaacgaagactcaatcgtggccgtggccagcaattcgaccgatctgatctgaccgcggaagtgcgccgggcaagaagctgcgcgtg

gagggtctcaagagatggaaagcaatgcccggaagctggctgcaccagggtctctgatctgctgtccacatcaagtgcacgcccaagatgaagaagttcatccaggacgtgccacacctacgaagggaacaaagaggtccgcac

Gene of interest (GOI, Gaussia Luciferase)

aggggcgcataggcgaggcagatcgacattcctgagattctgggttcaggactggagccatggagcagttatcgacagttatcgacacactggctgctcccaagggttgcacacgtgcagtgcttgacct

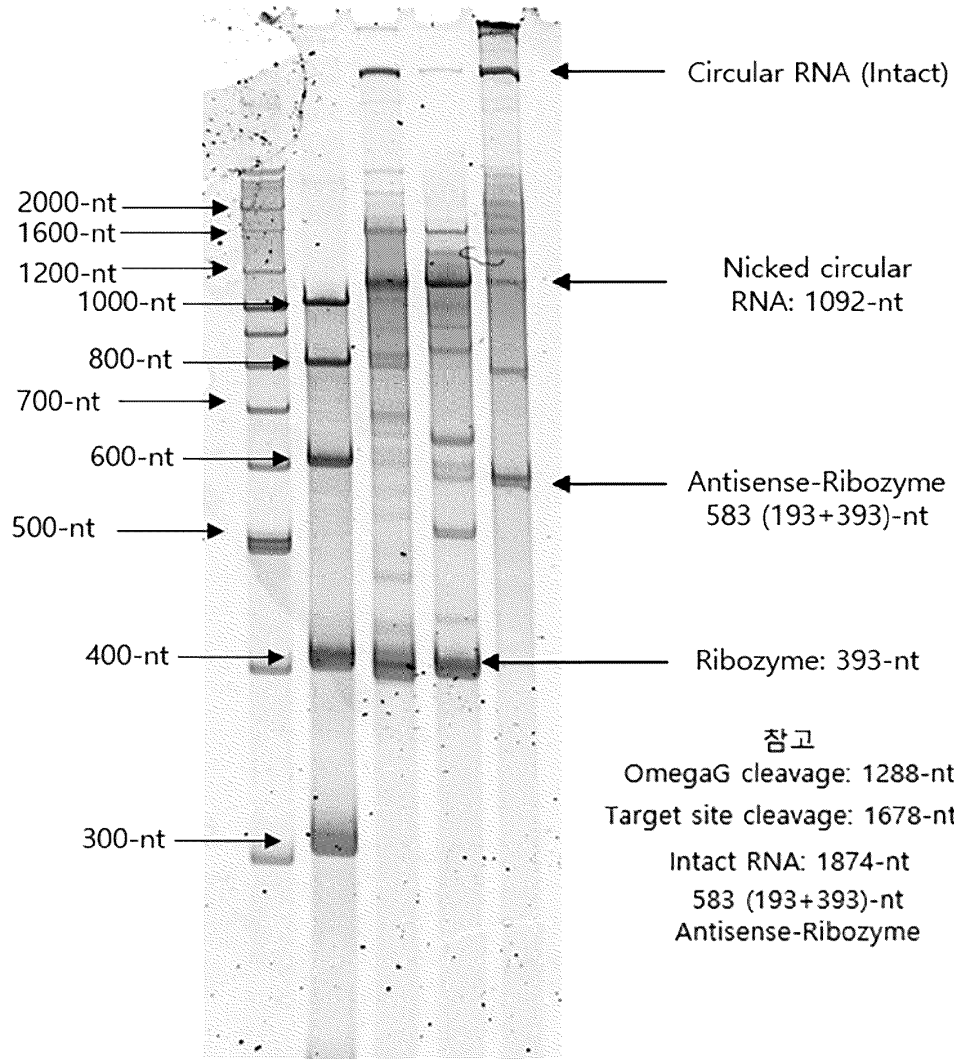
Target Site

gctcaagaagggtgctgcaacgtgtggagaccttggcagcaagatccaggccagggtggacagatcaaggggccgggtgggtgactaaAAAAAAaccggtTGGGT

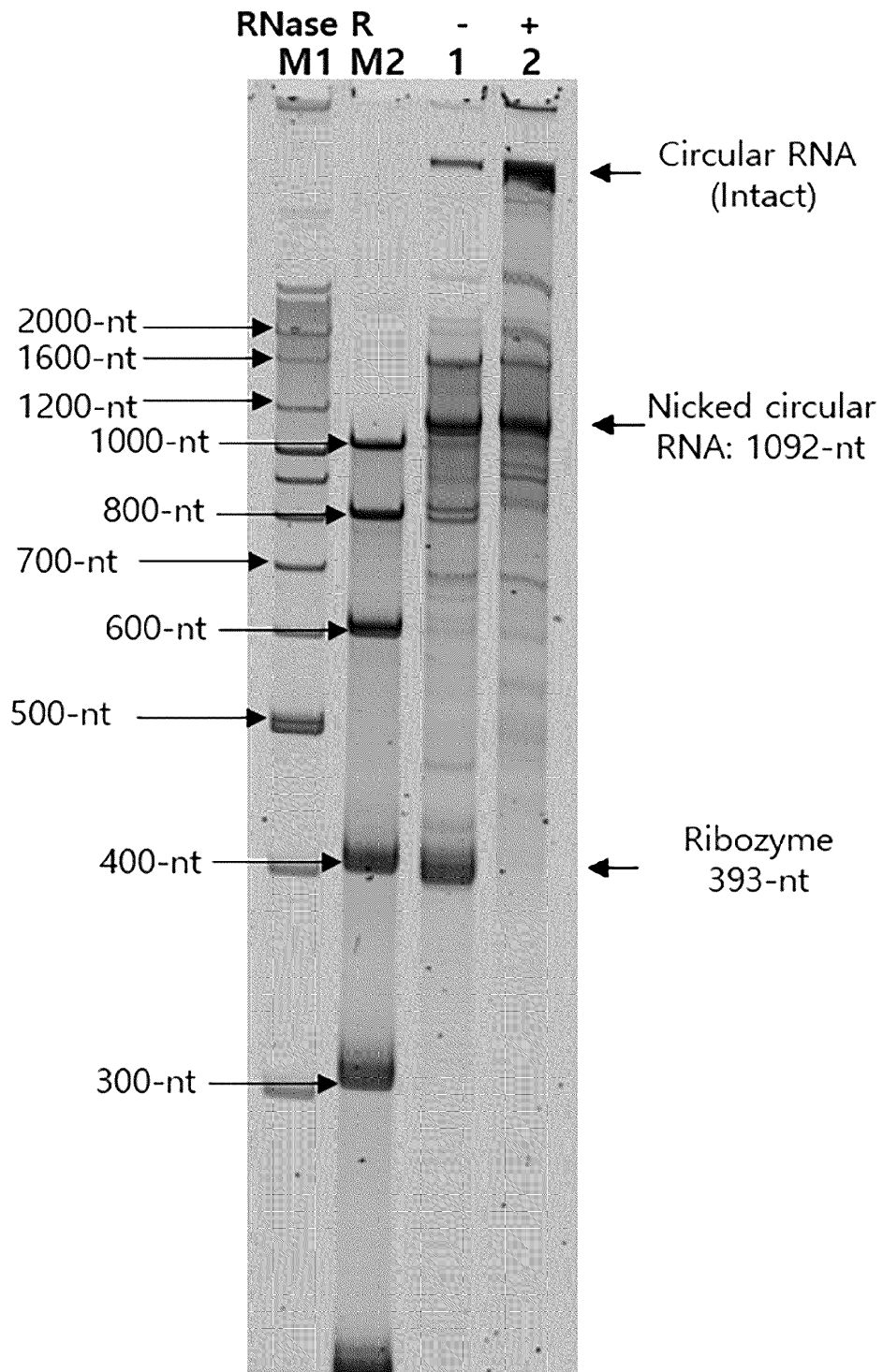
[도15]

Relative band intensity (%) 3.2 0.5 5.1

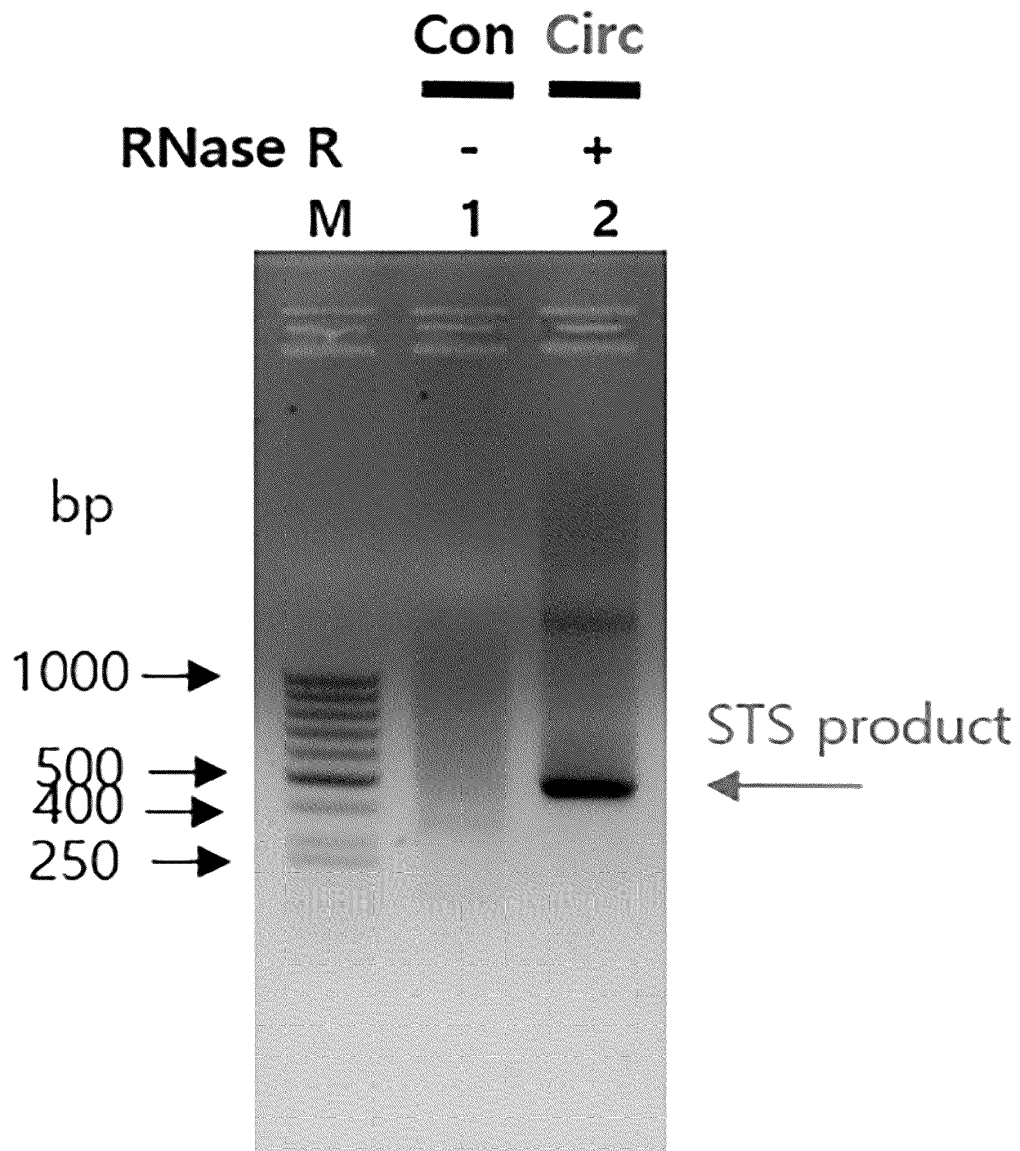
	AS150	P10	P1	M1	M2	1	2	3
AS150	-	-	+					
P10	-	+	+					
P1	+	+	+					



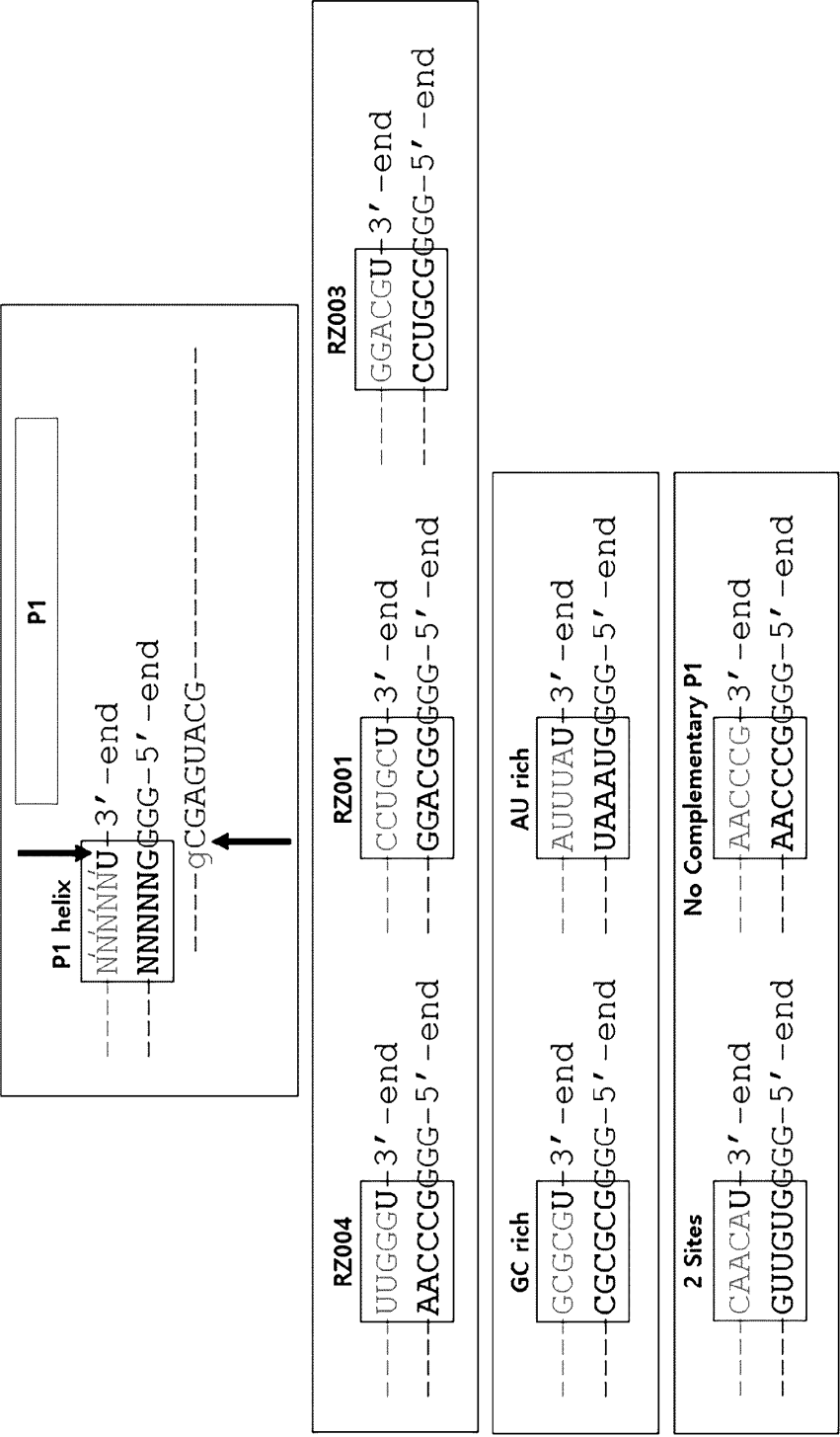
[Figure 16a]



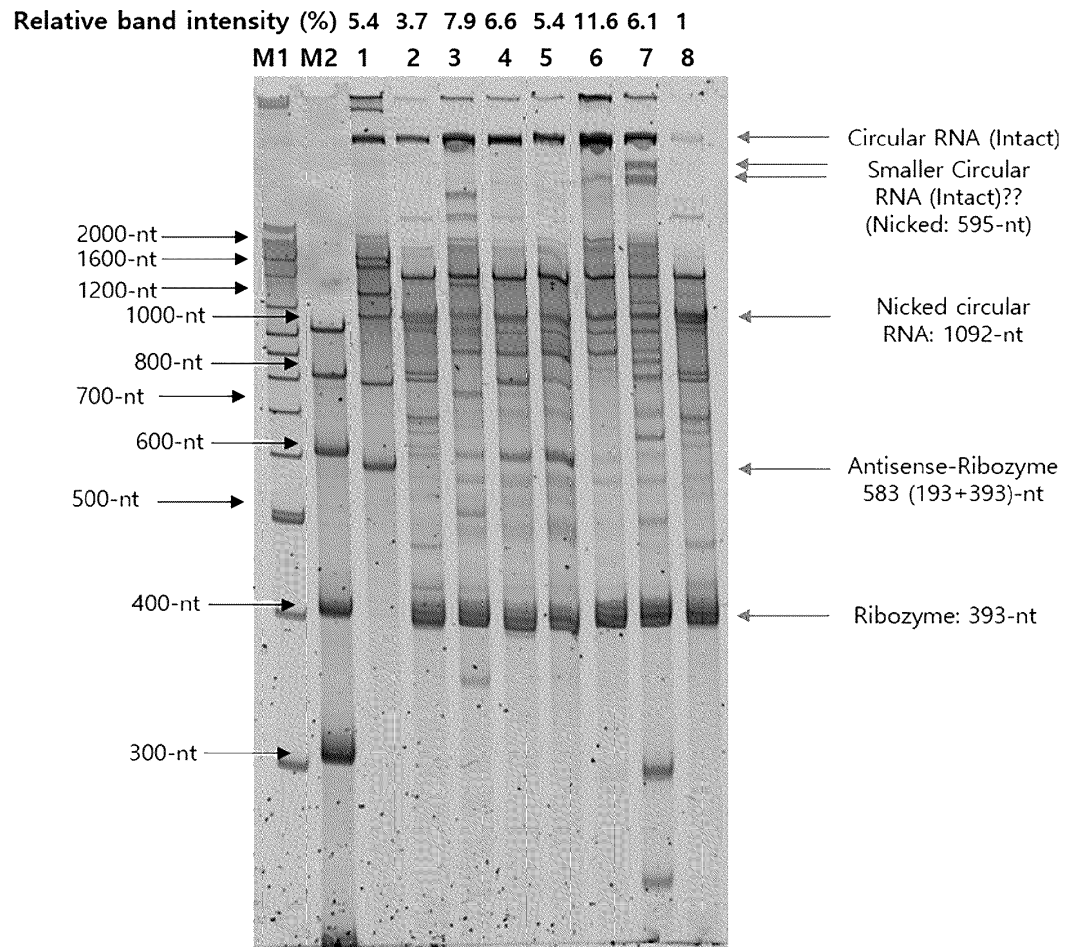
[도 16b]



[도17]



[도 18]



M1: 100 bp Plus DNA Ladder (Bioneer, D-1035)

M2: RiboRuler Low Range RNA Ladder (ThermoScientific, SM1831)

1: RZ004-EMCV-IRES-Gluc1-AS150 (P1, P10, AS150) IVT RNA

2: RZ004-EMCV-IRES-Gluc1 (P1, No AS) IVT RNA

3: RZ001-EMCV-IRES-Gluc1 (P1, No AS) IVT RNA

4: RZ003-EMCV-IRES-Gluc1 (P1, No AS) IVT RNA

5: GC-rich-EMCV-IRES-Gluc1 (P1, No AS) IVT RNA

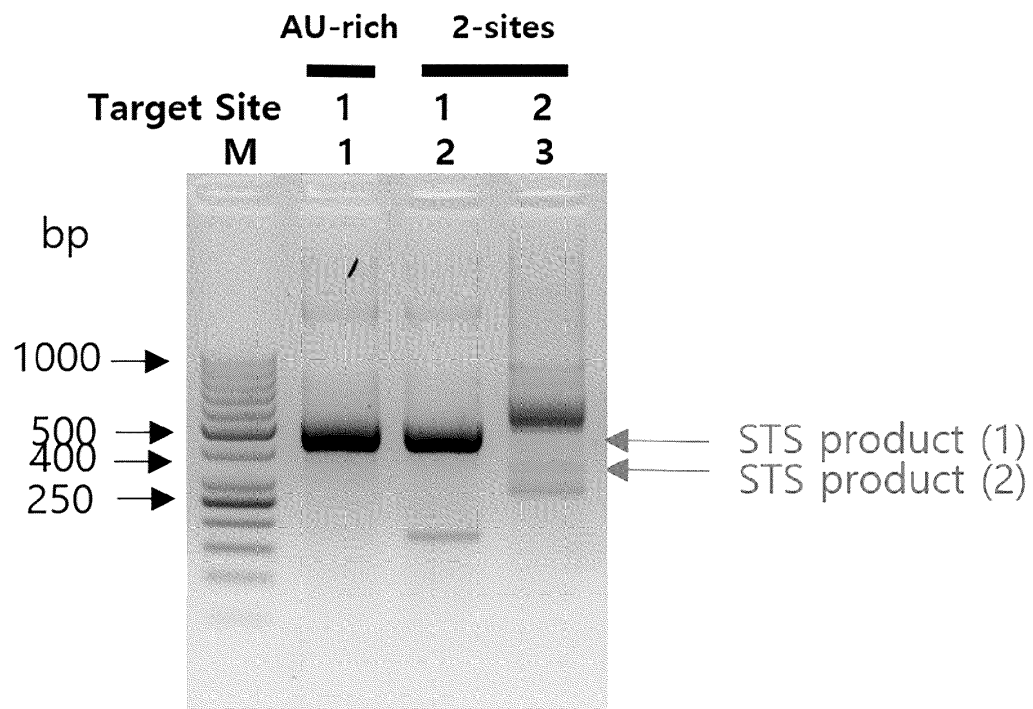
6: AU-rich-EMCV-IRES-Gluc1 (P1, No AS) IVT RNA

7: 2 sites-EMCV-IRES-Gluc1 (P1, No AS) IVT RNA

8: No complementary-EMCV-IRES-Gluc1 (P1, No AS) IVT RNA

250 ng IVT RNAs/well

[도19]



[도20a]



[Figure 20b]

