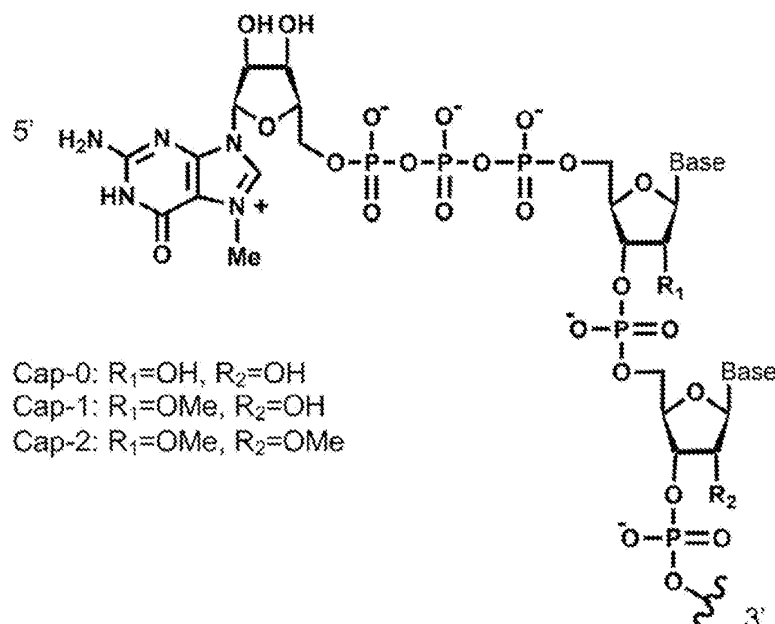
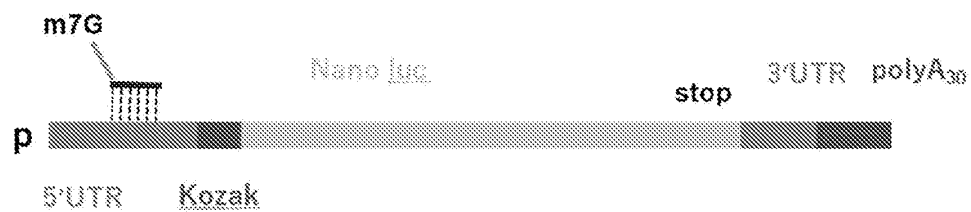


[図1]

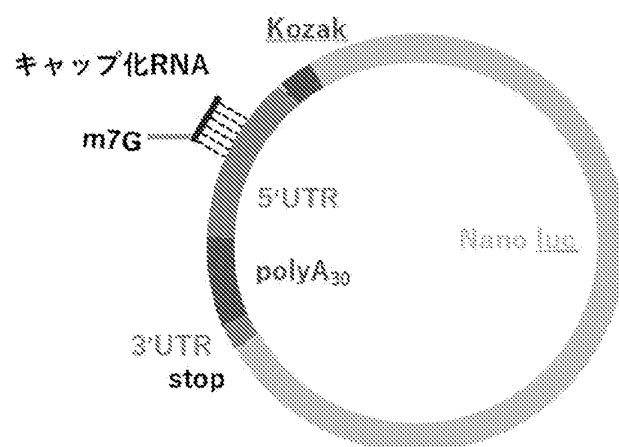
## A) 5' キャップ構造



## B) キャップ化RNA

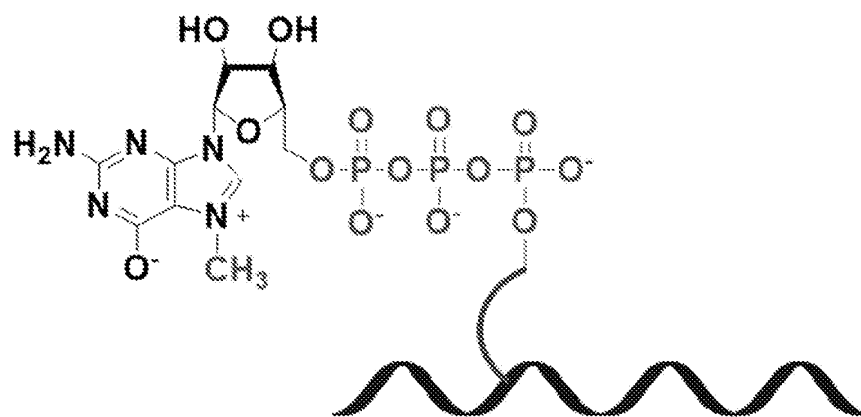


## C)

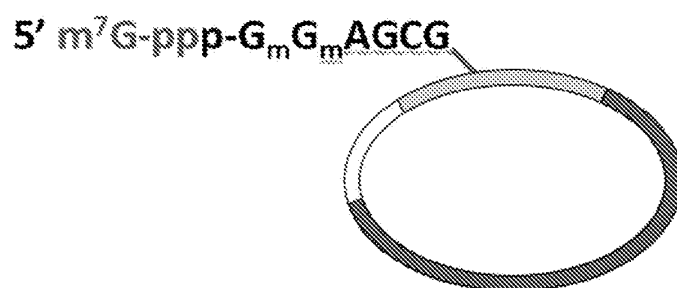


[図2]

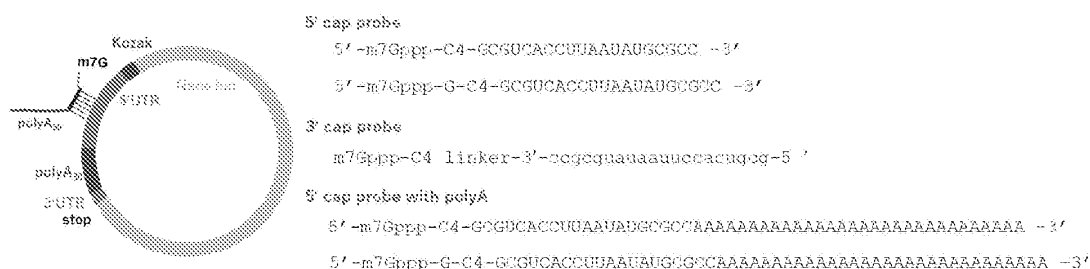
(a)



(b)



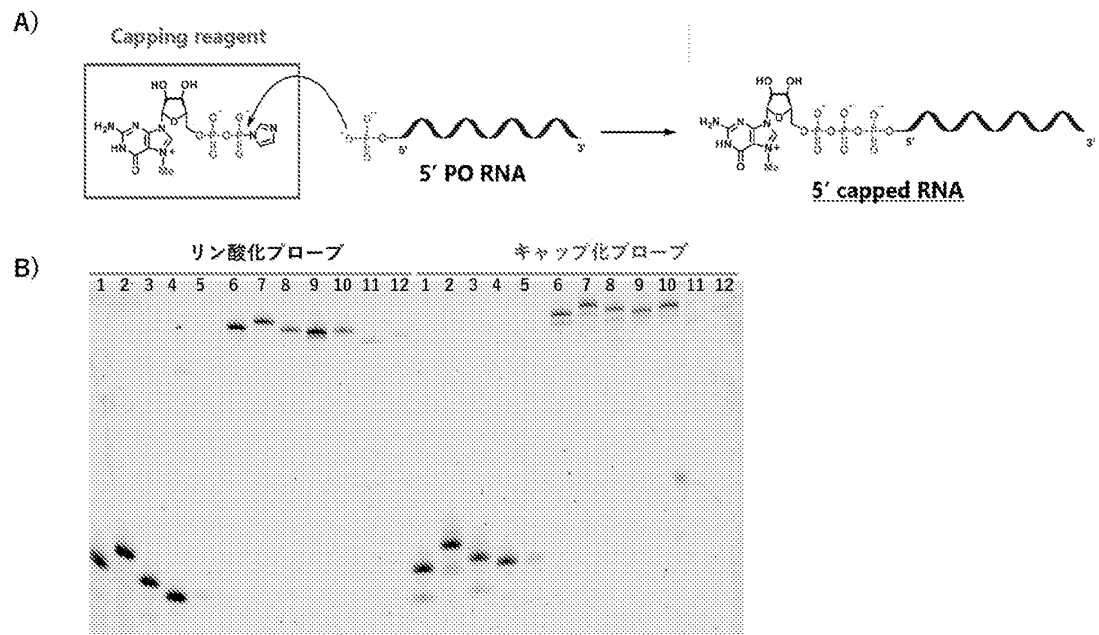
Circular mRNA hybridized with capped short RNA

[illegible]

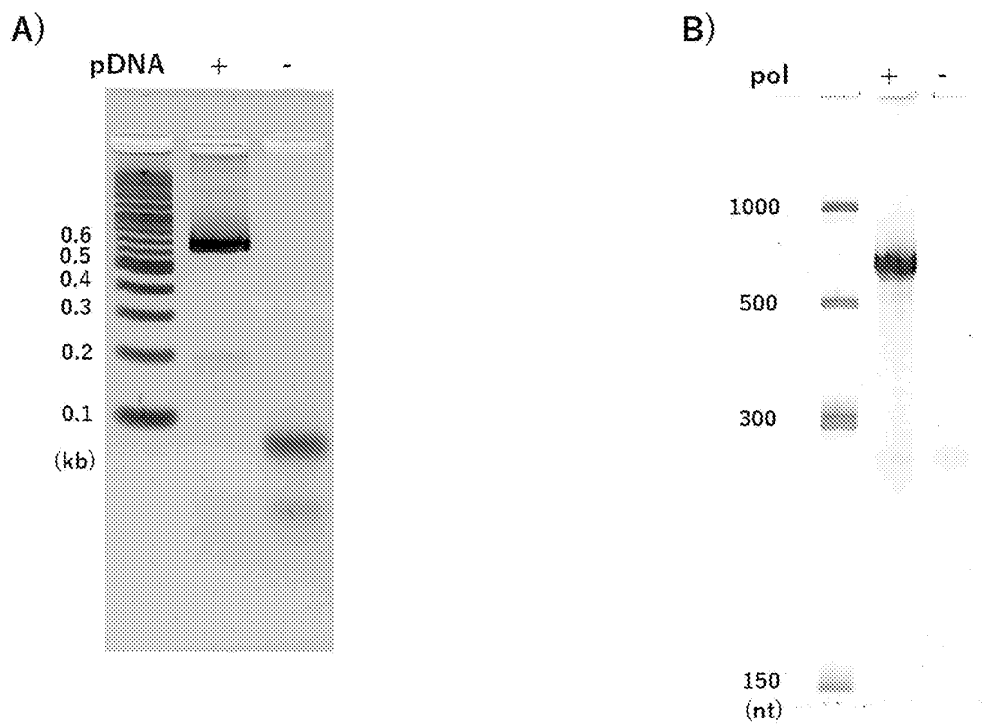
cccgcatccTATACGAGCTCACATggcgccatataaggtgacggcgtgaggcctcgagacacggggcgacccctgacgggacccgctataagct  
tggcaatccgggtaactgttggttaagccacacatggttttccacattcgagatttttgttggggatctgggacagacacggcgttccacacctggaa  
aactcccttaaacagagagagctgtccagctgtgtttcaaatctctccggctgtccctaatccatccagagatttgccttaagcgttcaaatgag  
gttgaagttgcacattccatgtaacacgtttagtgggttgcctgagggcgagacaaatggcgagatcgagaaatttttaaggttggtttacct  
gtgagtgatcatcacttaaggtgatactgcaatttggcaacacitggtaattgcacggggttaagccgcaacatgatactatttgggcggcgt  
atgcaggatcggcgtgttgcgcaggaaataagattccatgttaaacgggacccctgtggaaagggaacaaatatcgagcagagcctgatcaaaa  
cagggcgccacatgctgttcggagcaacacacaaaggggcgagcggctgggggtttgtgcgaacacatctgggttaattctagTTTTTTTTTTTT  
TTTTTTTTTTTTTTTT

[illegible]

[図5]

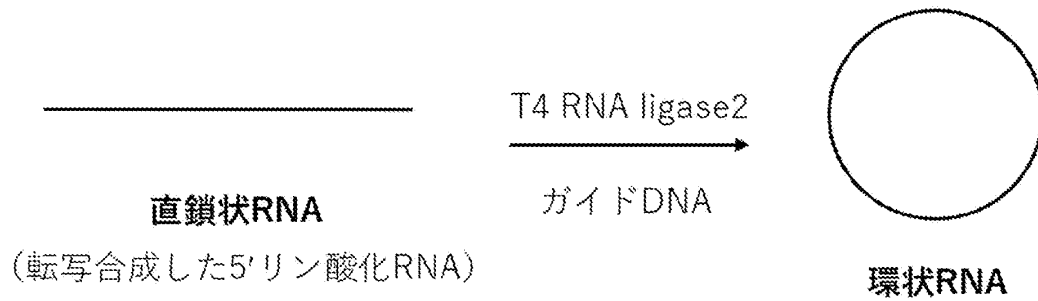


[図6]

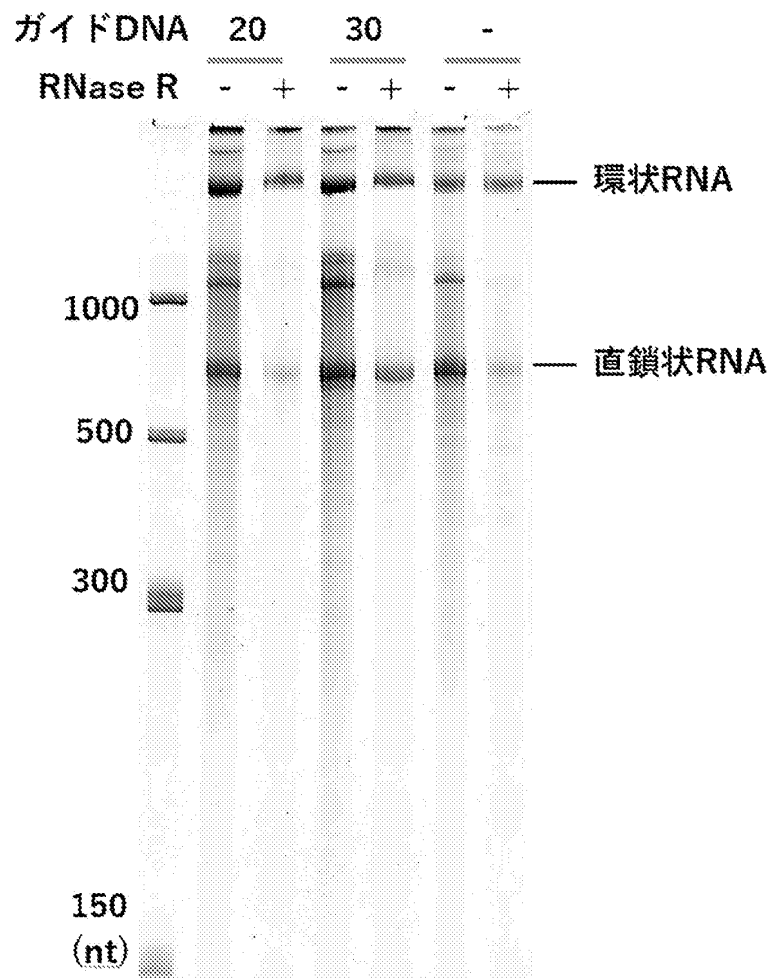


[図7]

A)

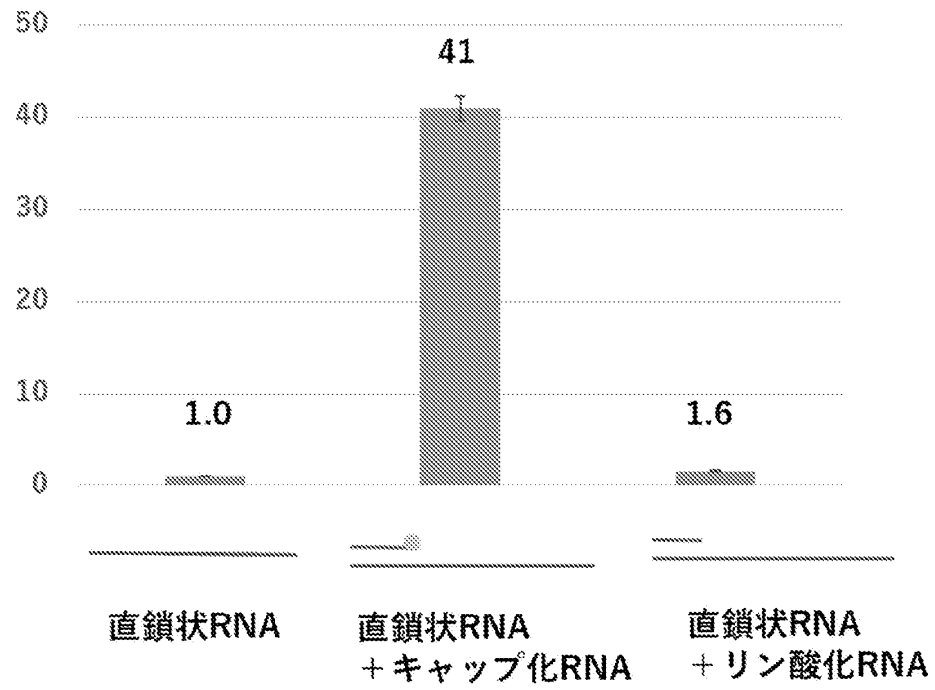


B)

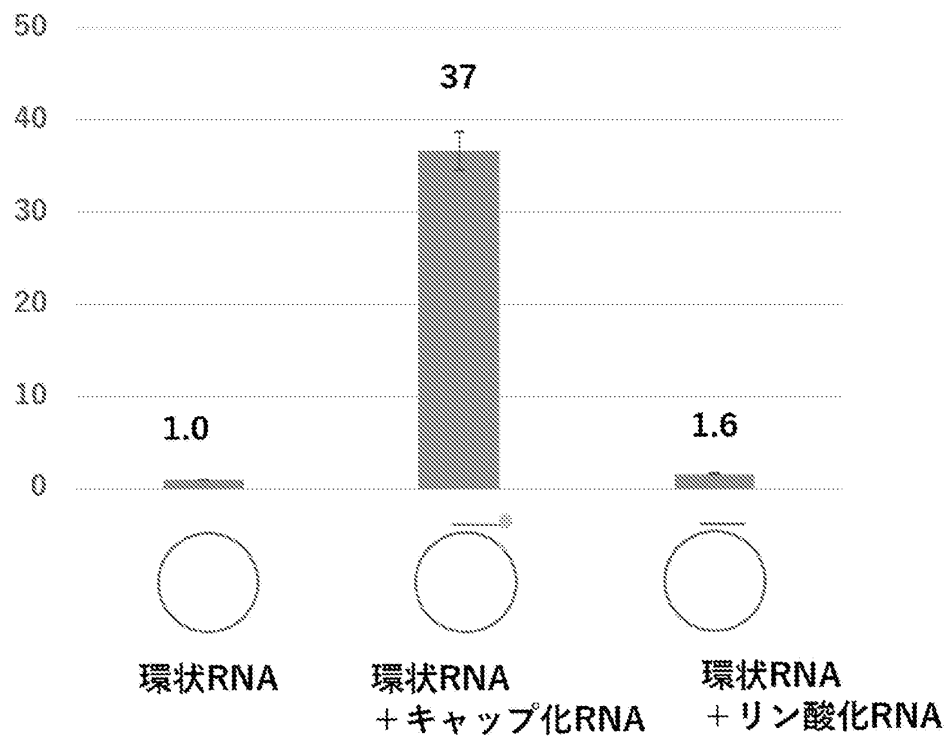


[図8]

A)

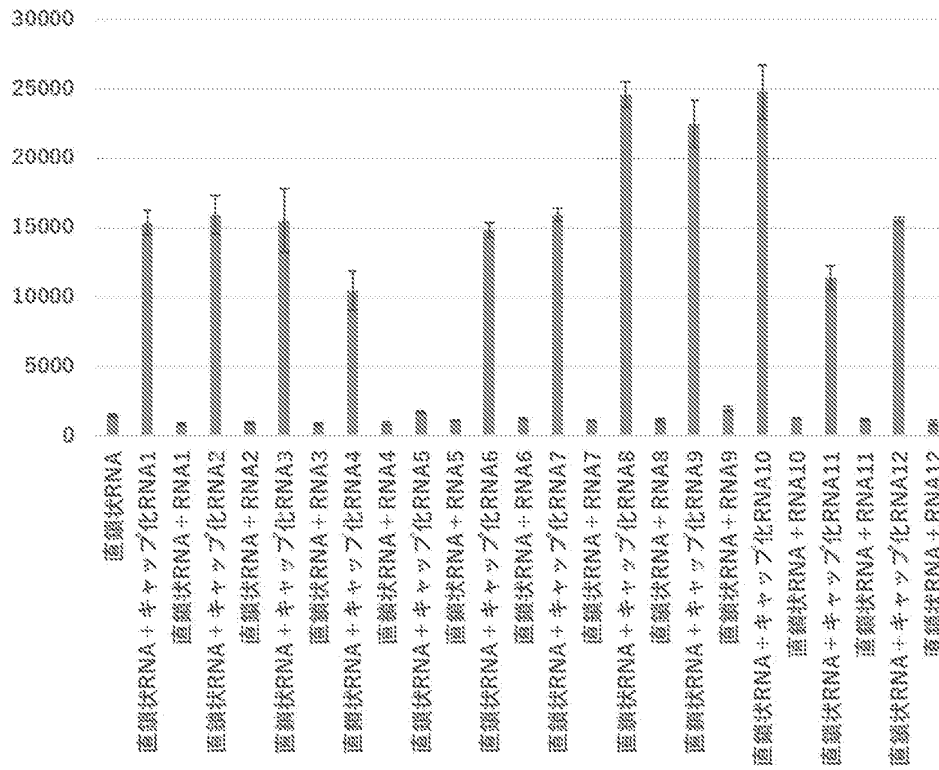


B)

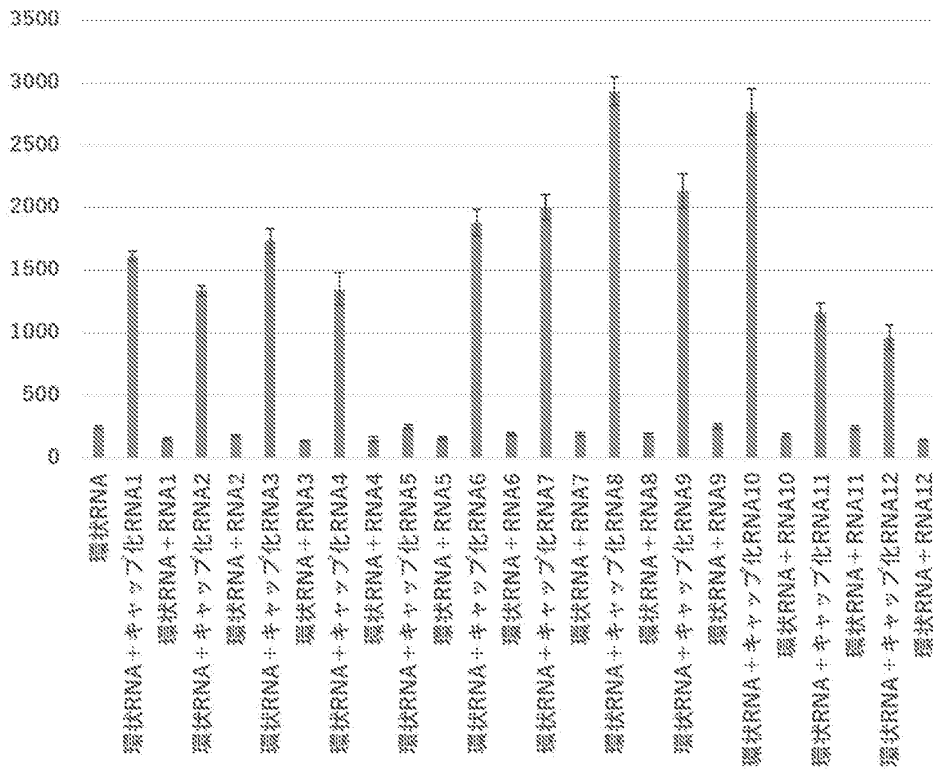


[図9]

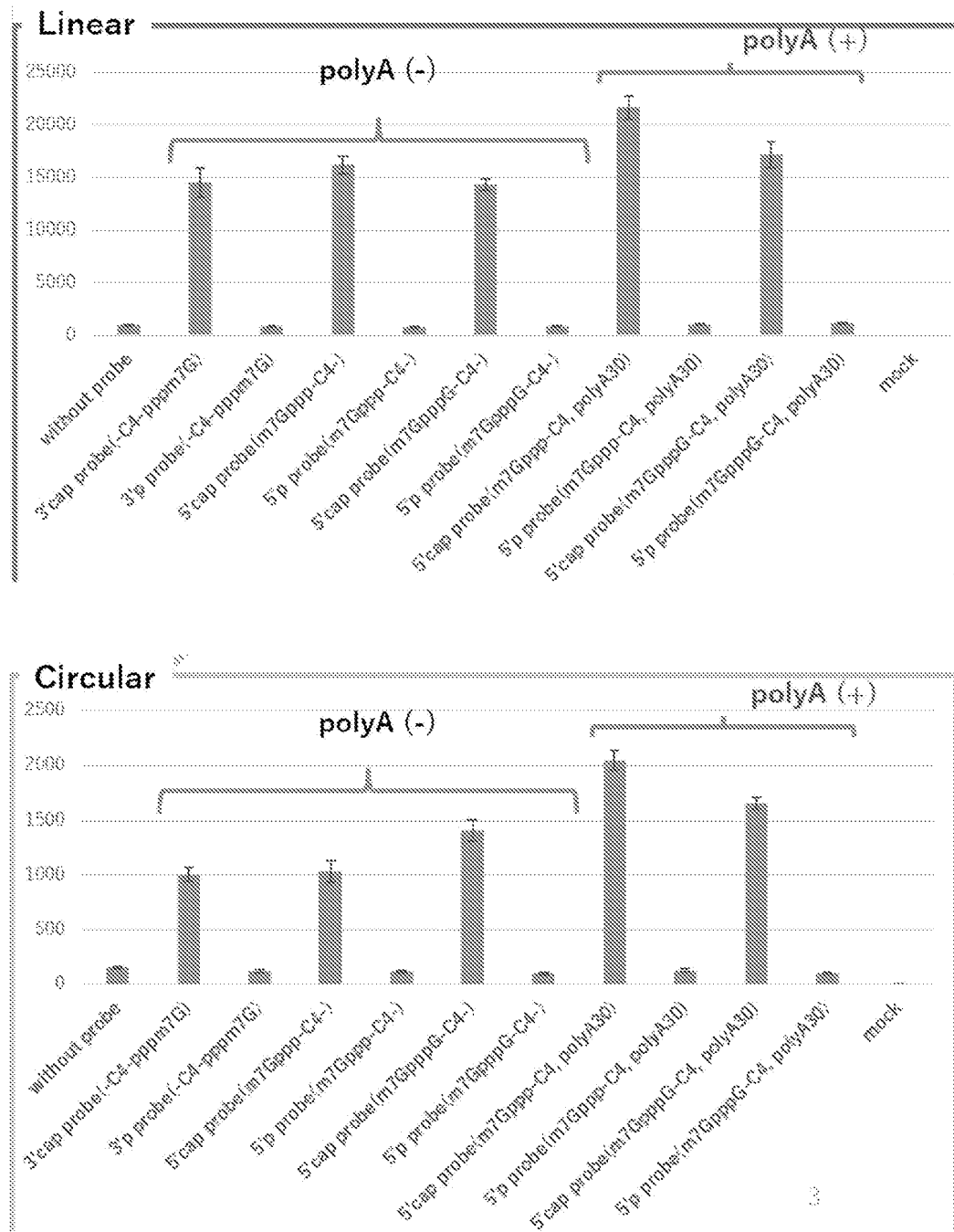
A)



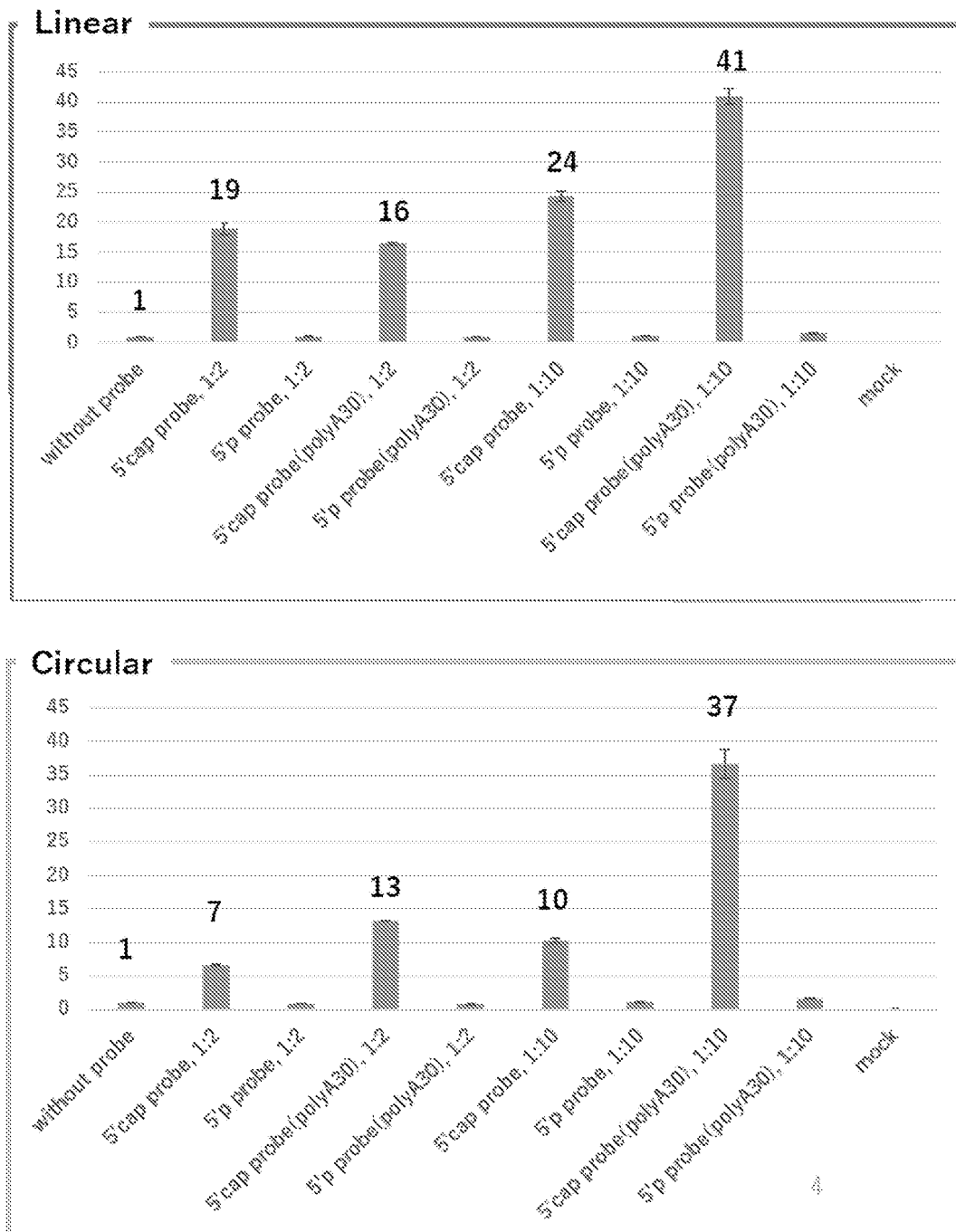
B)



[図10]

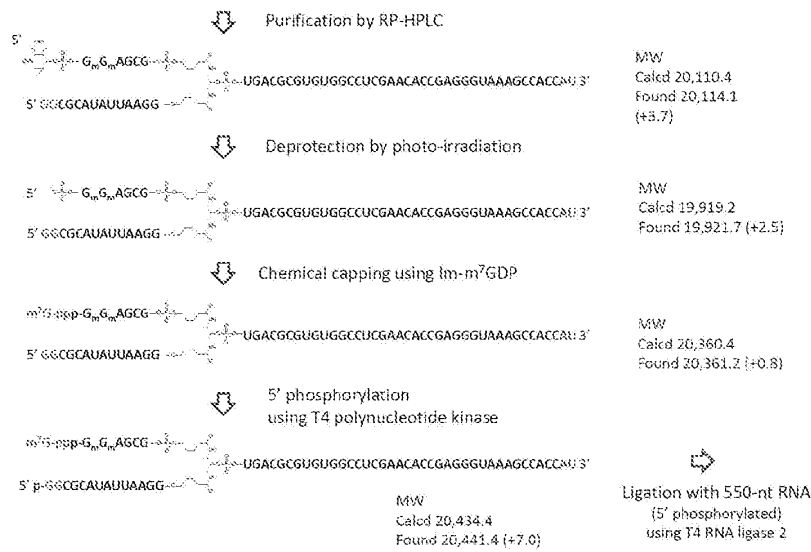


[図11]

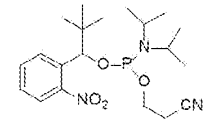


## [図12]

Automated RNA synthesis by the standard phosphoramidite chemistry



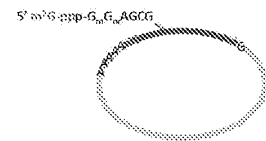
MW  
Calcd 20,110.4  
Found 20,114.1  
(+3.7)



化合物1  
(化学リン酸化試薬)

MW  
Calcd 19,919.2  
Found 19,921.7 (+2.5)

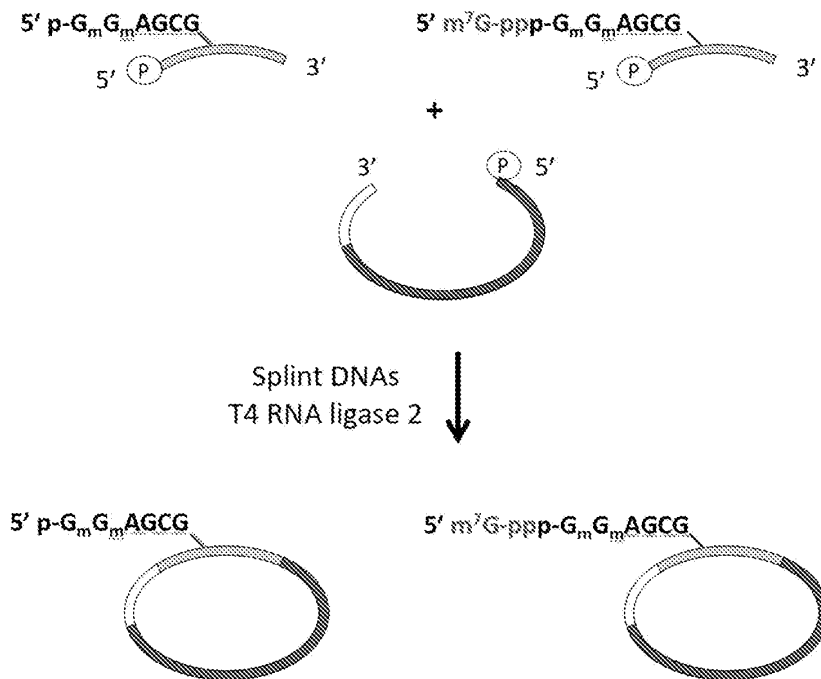
MW  
Calcd 20,360.4  
Found 20,361.2 (+0.8)



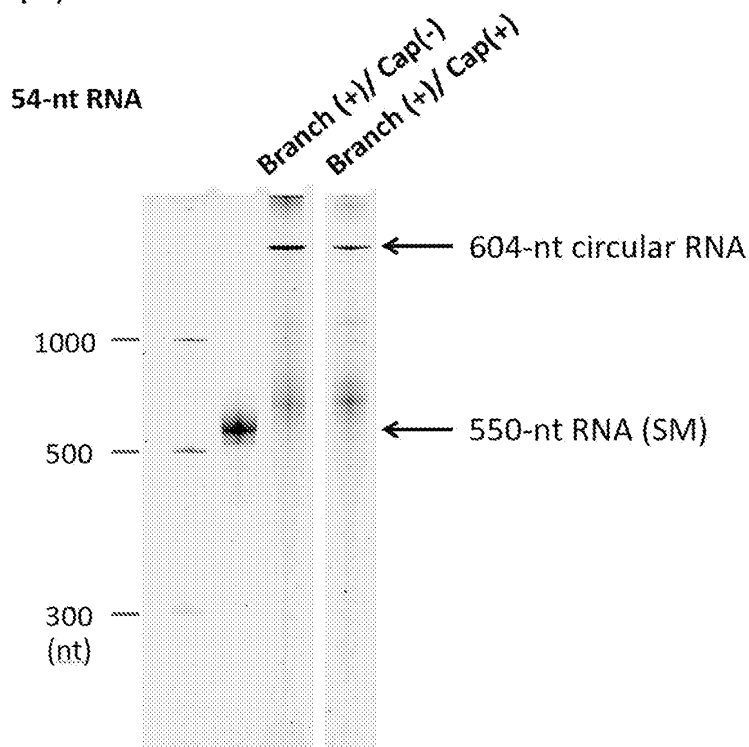


[図14]

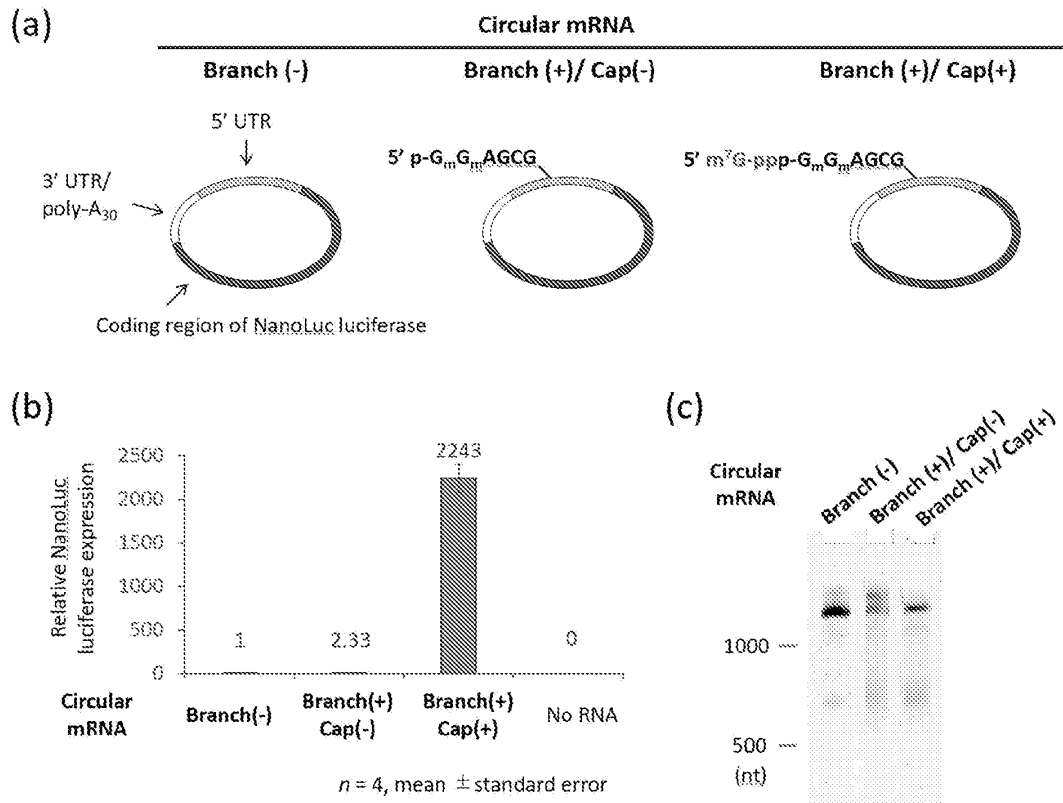
(a)



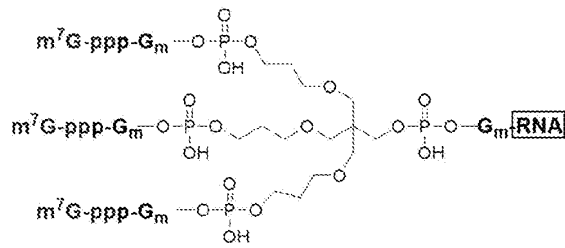
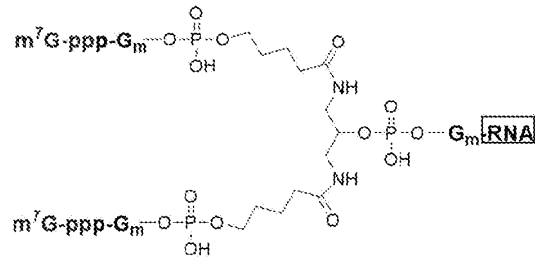
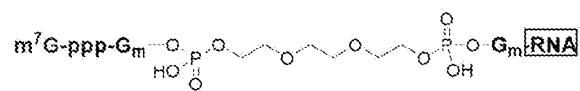
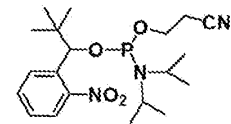
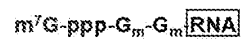
(b)



[図15]

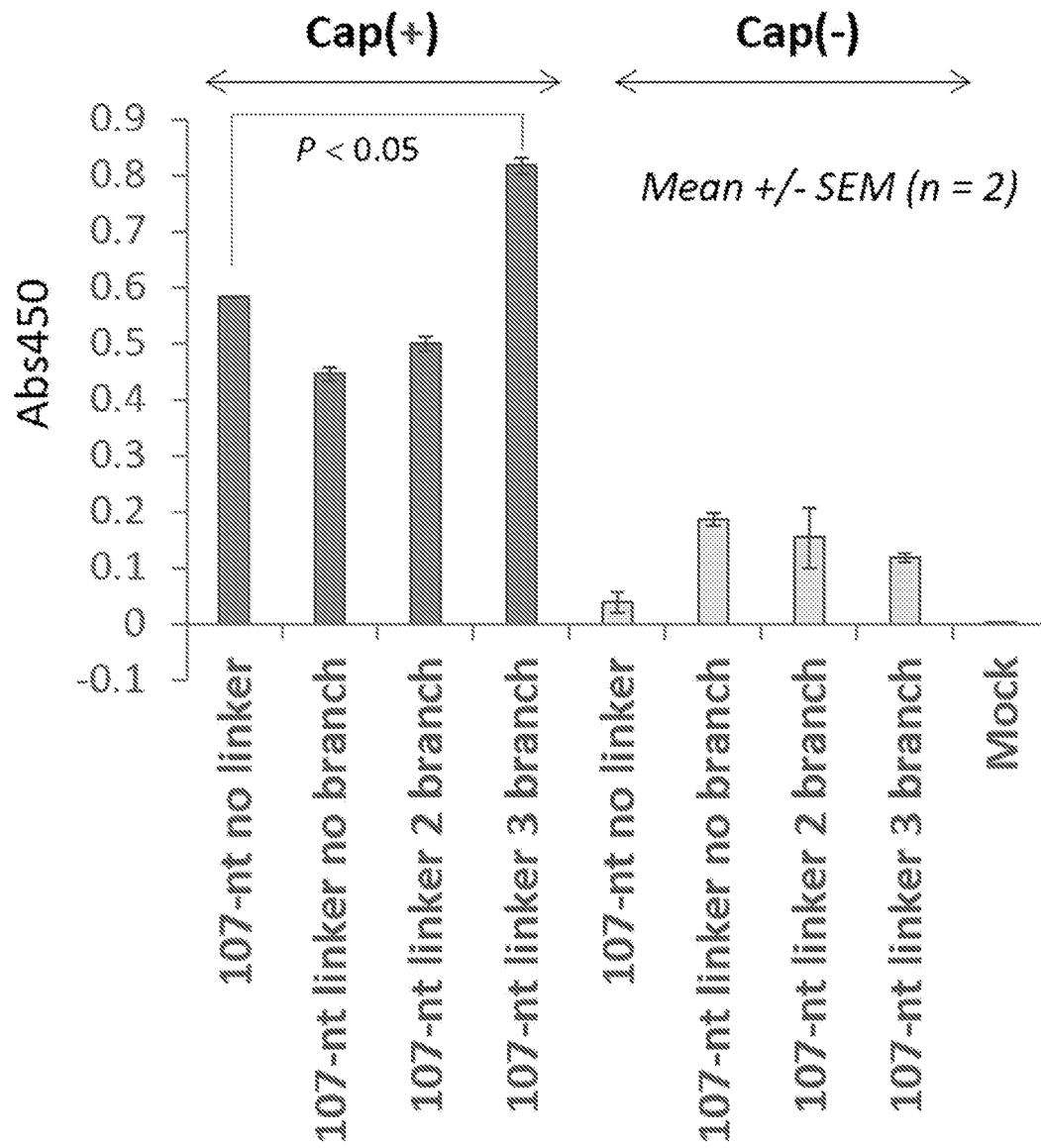


[図16]

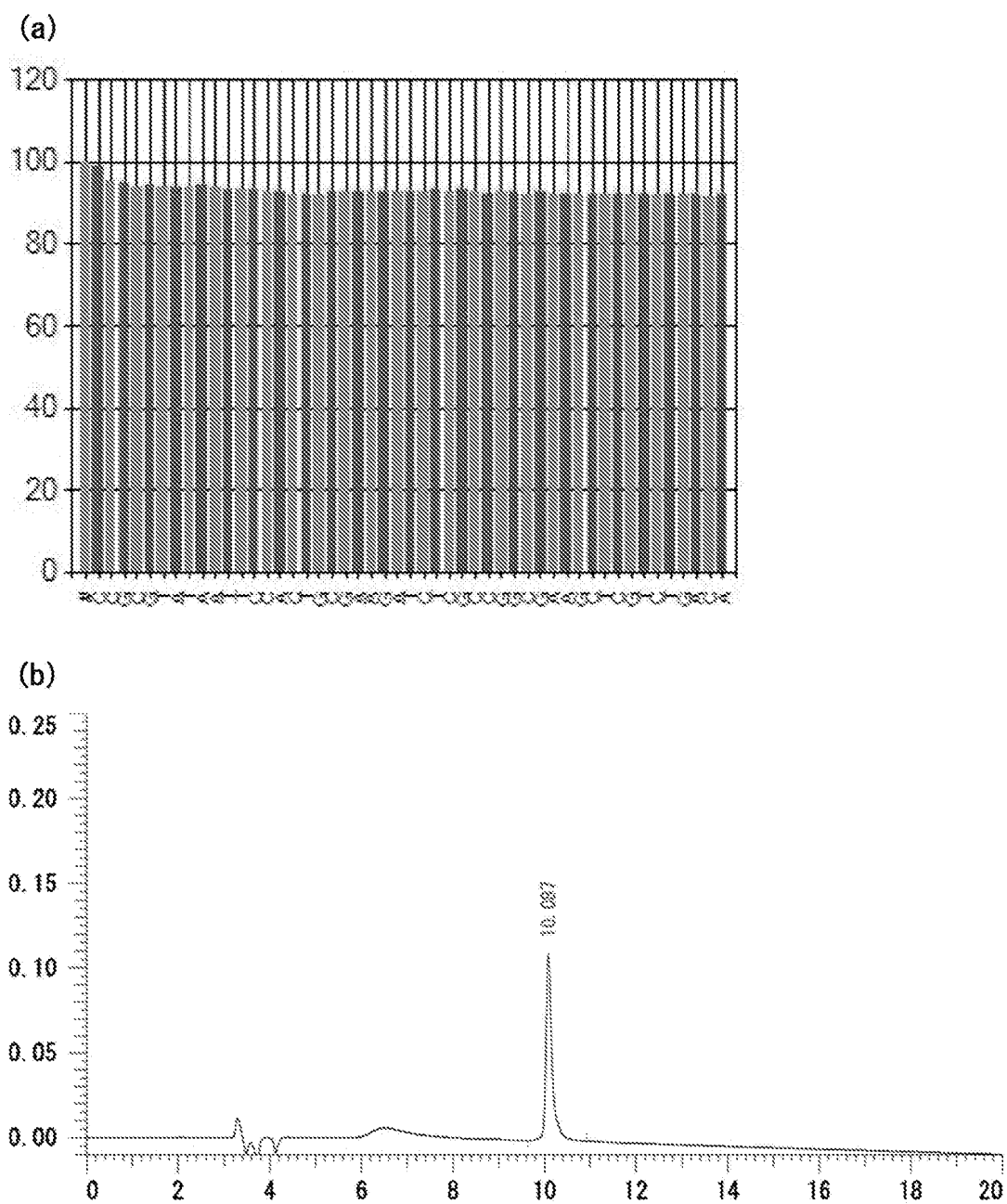
**3-Branch RNA****2-Branch RNA****Linker w no branch RNA****No linker RNA**

化学キャップ化試薬 Nb-CPR

[図17]

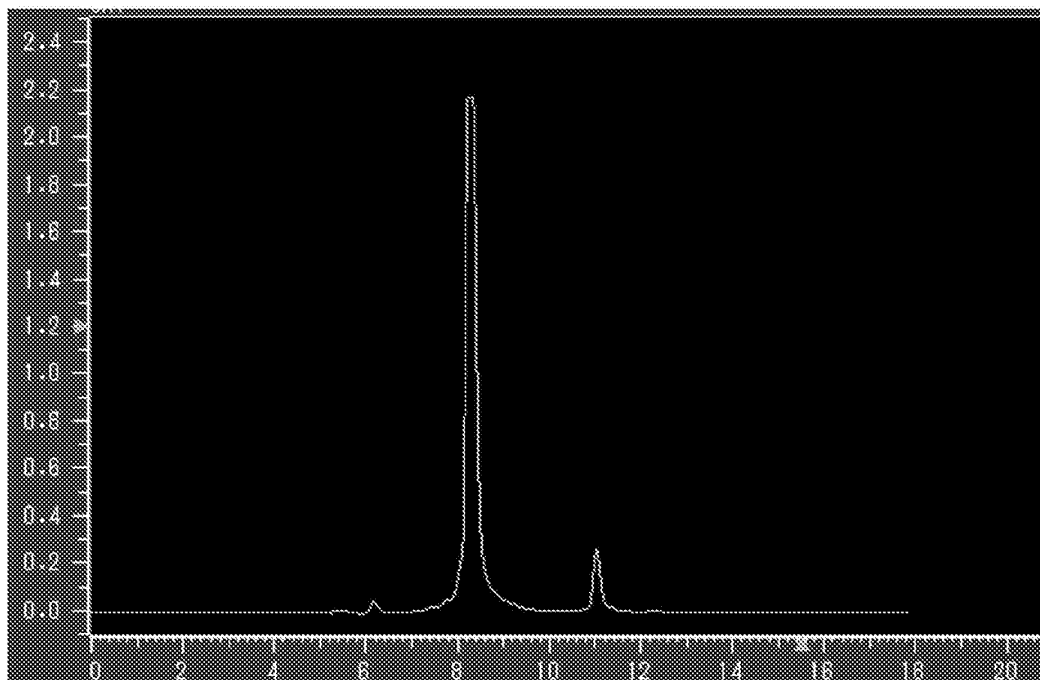


[図18]

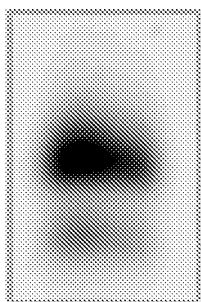


[図19]

(a)



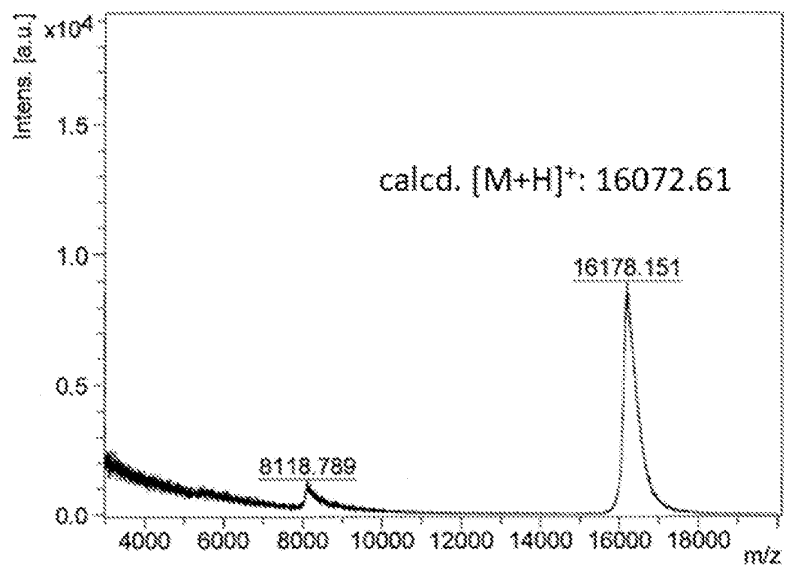
(b)



[図20]

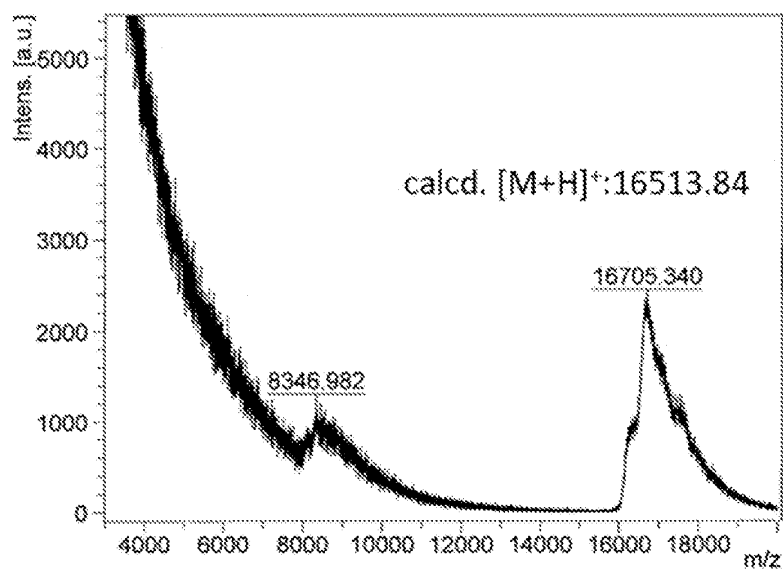
(a)

cap(-)

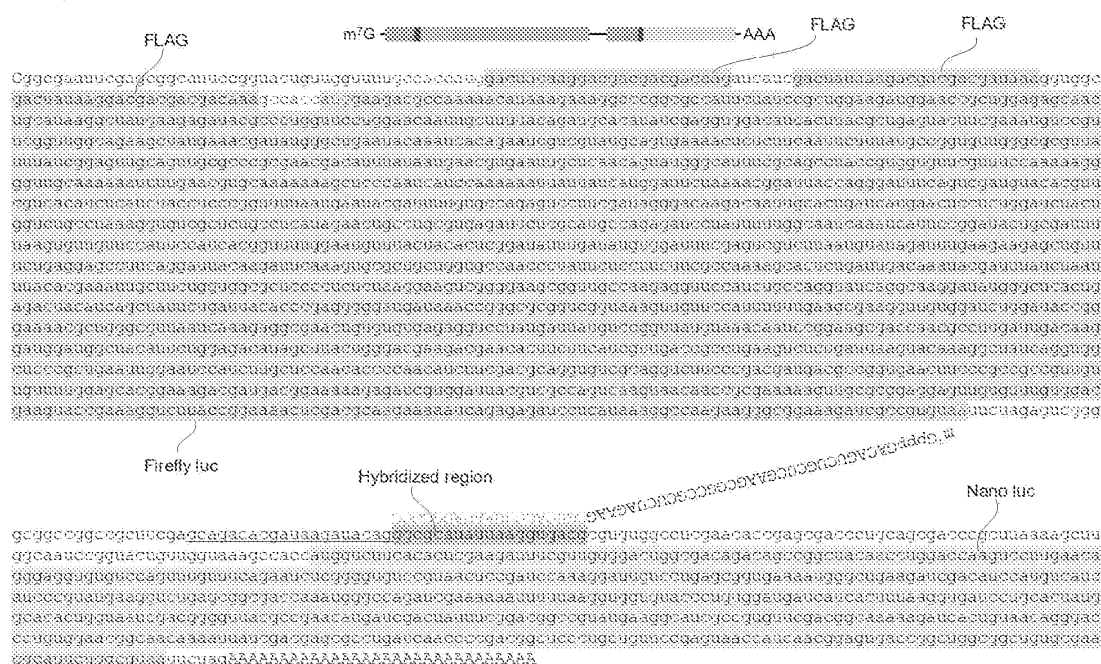


(b)

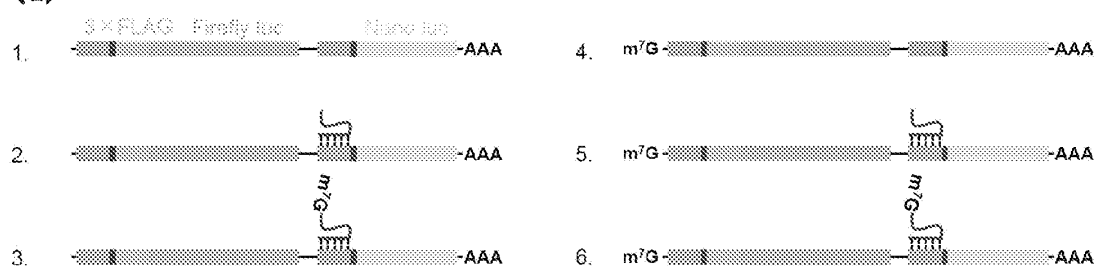
cap(+)



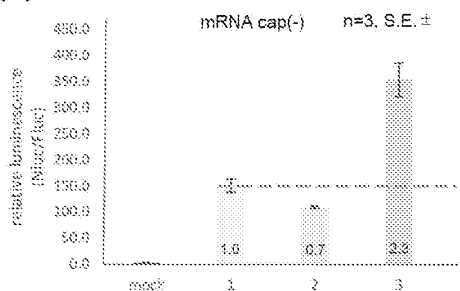
RNA (20+30 nt) : m<sup>7</sup>GpppGACAGUCUGCGUAGCGGGCCGUUAGAAG-3'UTR/CUUAAGAGCUGCA



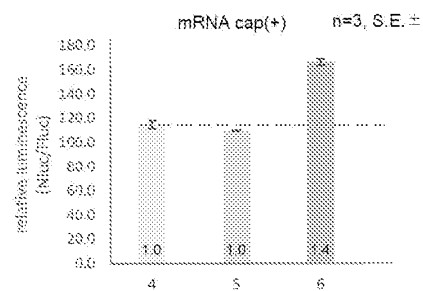
(a)



(b)



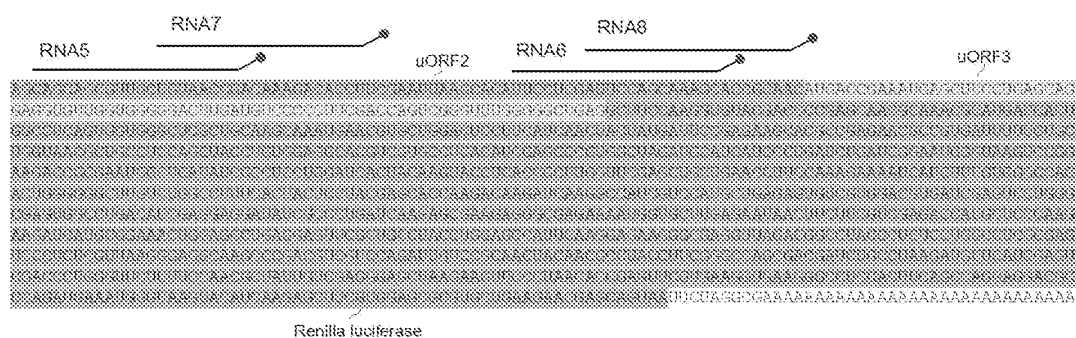
(c)



(a)

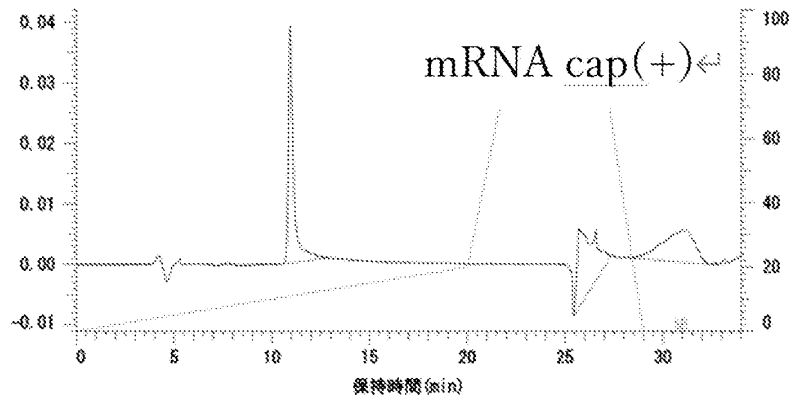


RNA8 (20+3 nt)  $t_m=70.4^\circ\text{C}$ : m<sup>7</sup>GpppGAUUUGCGGUGCUUUGCUGGAU

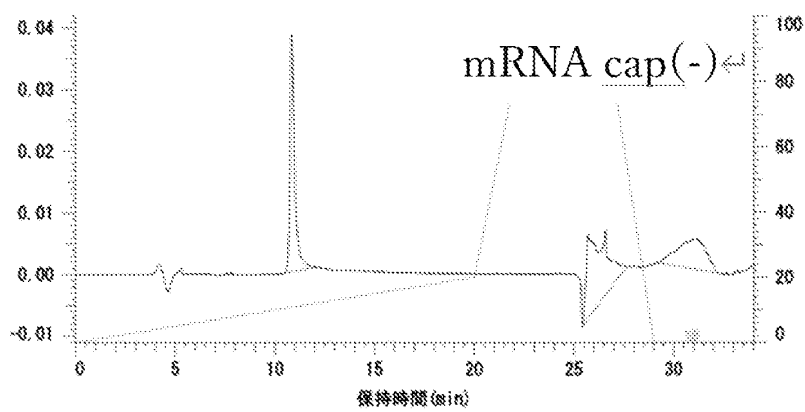


[図25]

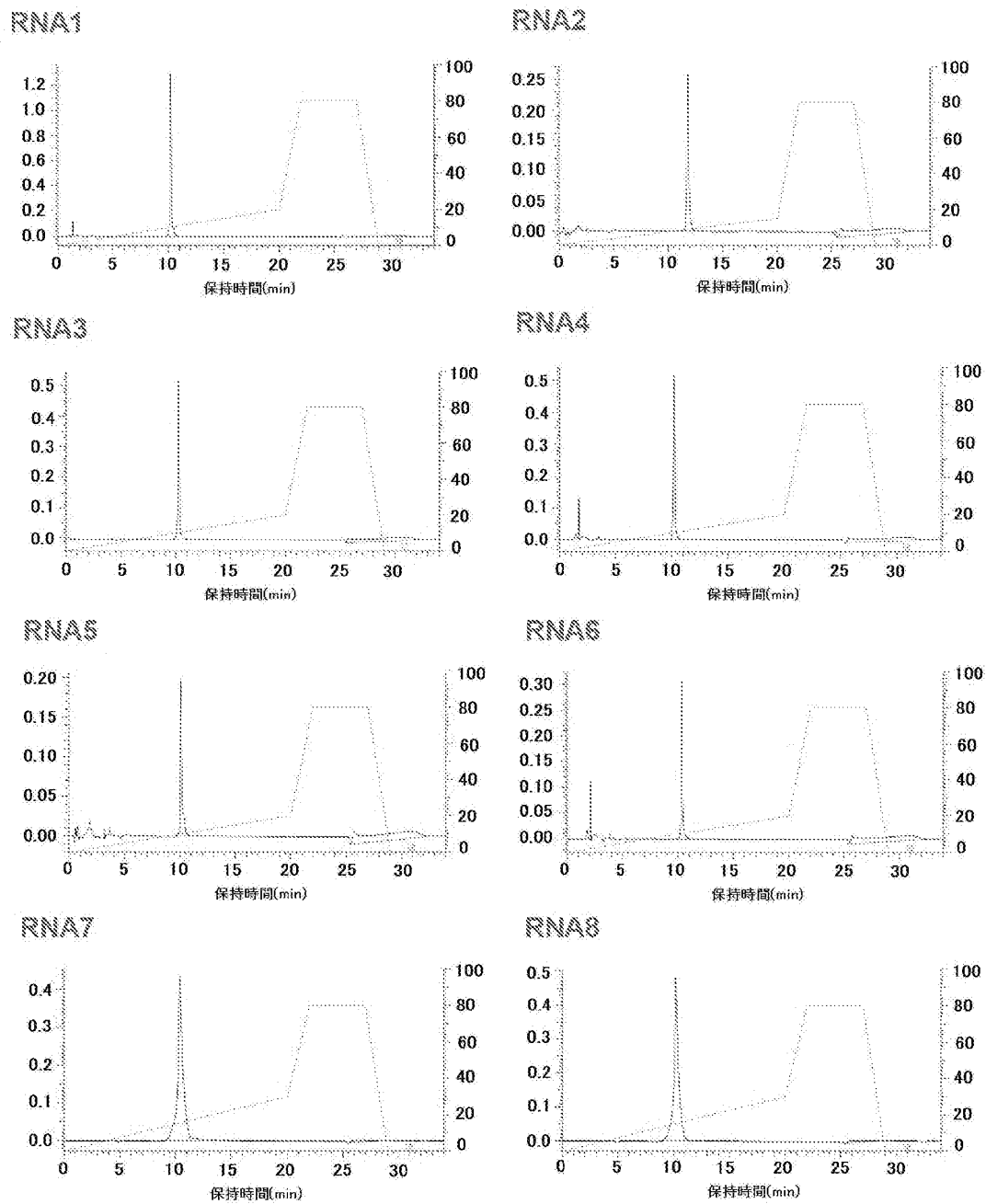
(a)



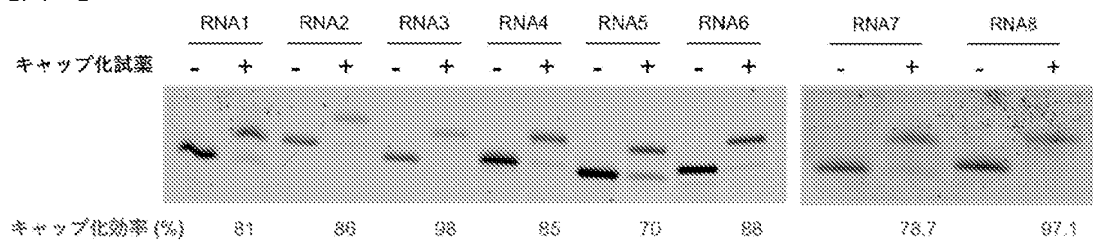
(b)



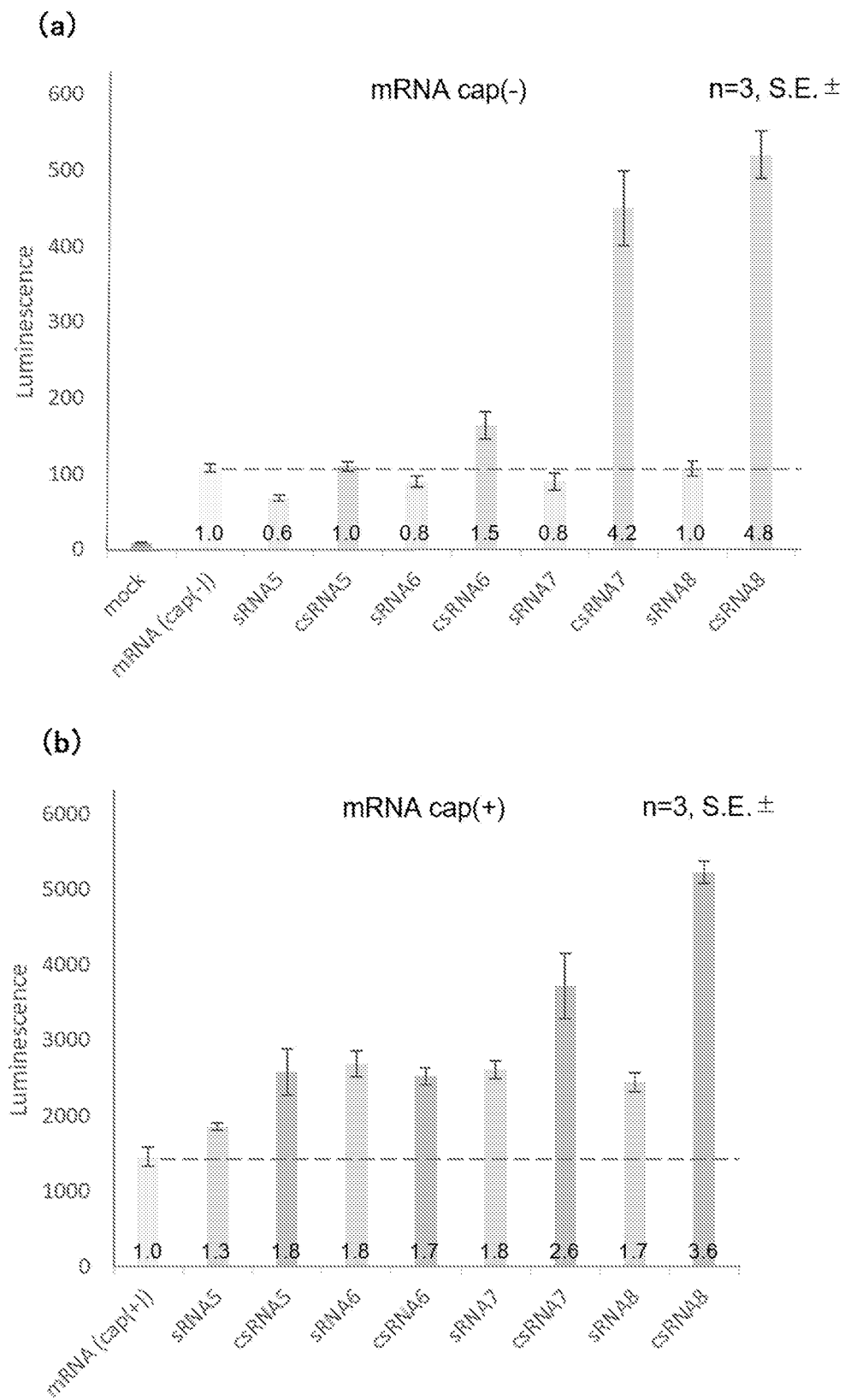
[図26]



[図27]



[図28]



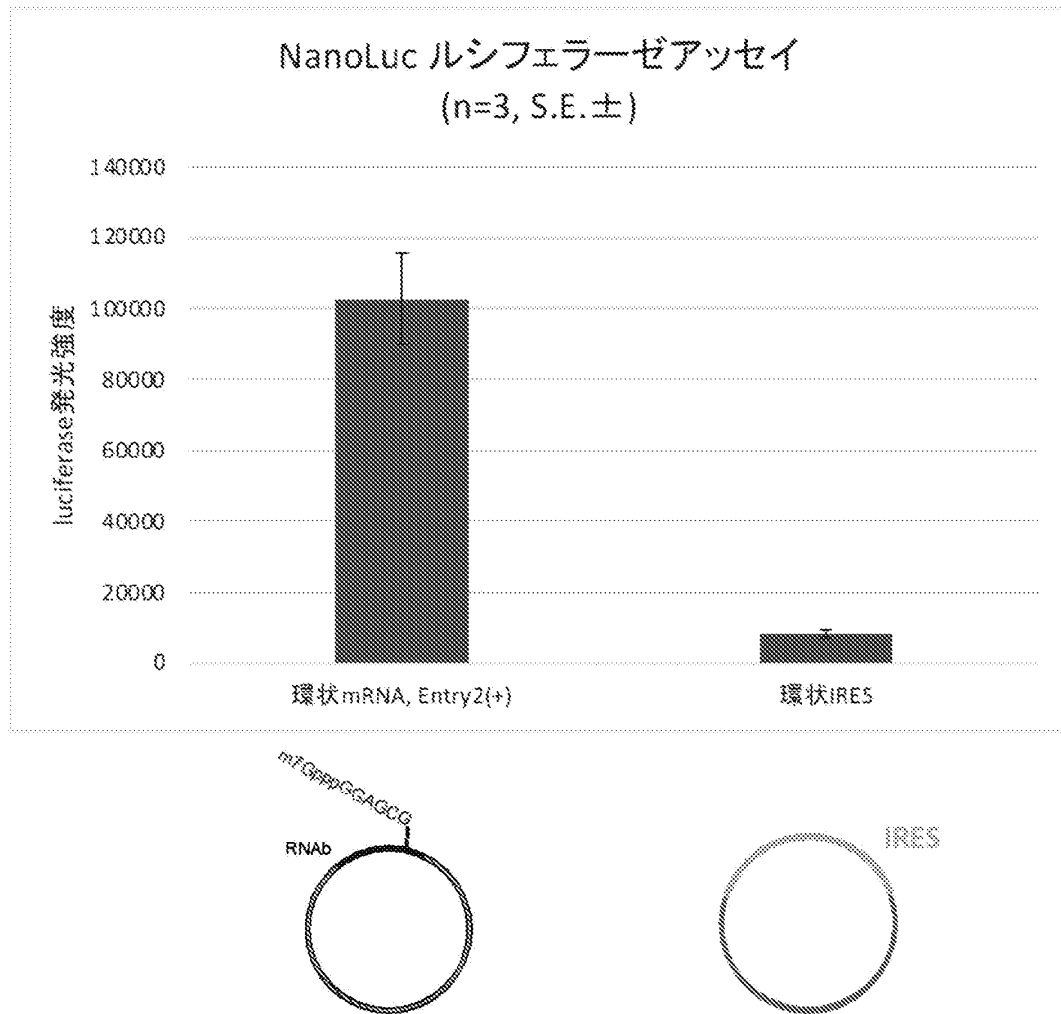
[図29]

IRFS

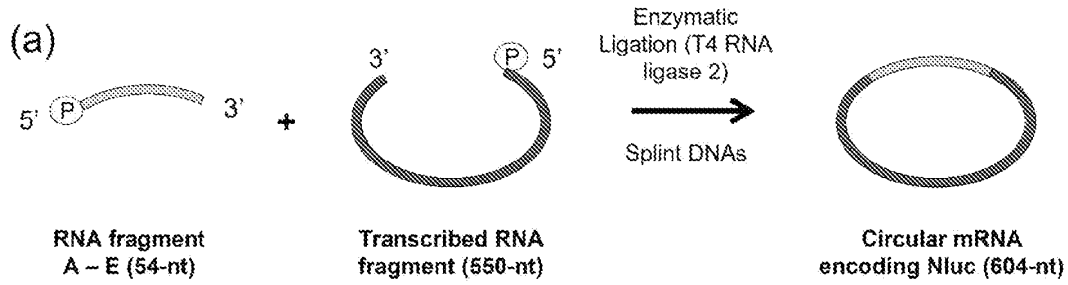
CCCCGATCCTAATACGACTCACTATAGGCGCATATTAAGGTGACGCGTAATTCACGCGTCGAGCAATGCATC  
TAGGGGCGGCCAATTGCGGCCCTCTCCCCCGCCCTCTGCTTCCCCCCCCCTAACGTACGCGCGGAAG  
CCGCTTGGAATAGGCGCGGTGCGGCTTGCTATATGTTATTTCCACCAATATGCGGTCTTTGGCAAT  
GTGAGGGGCGGGAAGCTGGGCTGTCTTTGACGAGCATTCCTAGCGGTCCTTCCCTCTCGGCAAG  
GAATGCAAGCTCTGTGGAATGTCTCAAGGAAGCAGTTCTCTGGAACTTCTTGAAGACAAACAAGCTT  
TGTAGCGACCTTTGCGAGGCAGCGGAACCCCTTGGCGACAGGTCTCTGCGGCCAAAAGCCACCT  
GTATAAGATACACTGCAAAGGCGGCACAACCCCACTGCCACGTTGTGAGTTGGATAGTTGTGGAAGAG  
TCAATGGCTCTCTCAAGCGTATCAACAAGGGGCTGAAGGATGCCAGAAAGGTACCCATTGTATGGG  
ATCTGATCTGGGGCTCGGTGACATGCTTACATGTTTATGTCGAGGTAAAAAAGCTTACGACCT  
CCGAACACCGGGGACCTGGTTTCTCTTGA AAAACACGATGATAAGCTTCCACAACCCGGGATCGCCACC  
ATGGTCTTCACACTCGAAGATTTCTTTGGGGACTGGCGACAGACAGCCGGCTACAACCTGGACCAAGTCC  
TTGAACACGGAGGTGTGTCCAGTTTGTTCAGAACTCTCGGCGTGTCCGTAACCTCCGATCCAAAGGATTGT  
CCTGAGCGGTGAAAAATGGGCTGAAGATCGACATCCATGTCATCATCCCGTATGAAGTCTGAGCGGGCAG  
CAATGGGCGCATCGAAAAAATTTTAAAGGTGGTGTACCCCTGTGGATGATCATCACTTTAAGGTGATCCT  
GCACATGGCACACTGGTAATCGACGGGTTACGCCGAACATGATCGACTATTTGGGACGGCGTATGAA  
GGCATCGCCGTGTTGACGGCAAAAAGATCACTGTAAACAGGGACCTGTGGAACGGCAACAAAATTATCGA  
EGAGCGCTGATCAACCCCGACGGCTCCCTGCTGTTCGAGTAACCATCAACGGAGTGACCGGCTGGCGG  
CTGTGCGAAGCATCTGGCGTAATCTAGAGTCGGGGCGGCCGGCCGCTTAAAAAAAAAAAAAAAAAAAA  
AAAAAAAAAA

NanoLuc ルシフェラーゼ

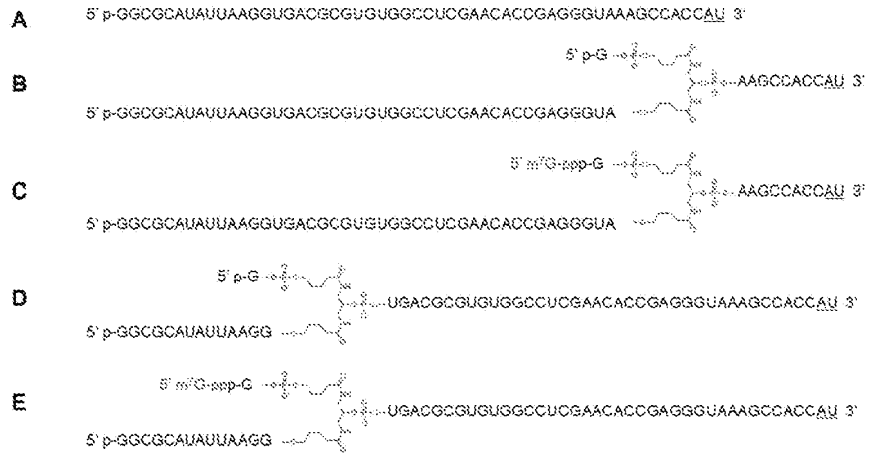
[図30]



[図31]



54-nt fragment  
(chemically  
synthesized)

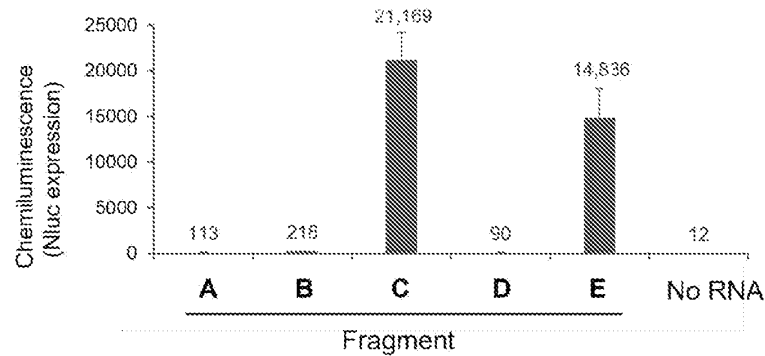


550-nt fragment  
(IVT synthesized)

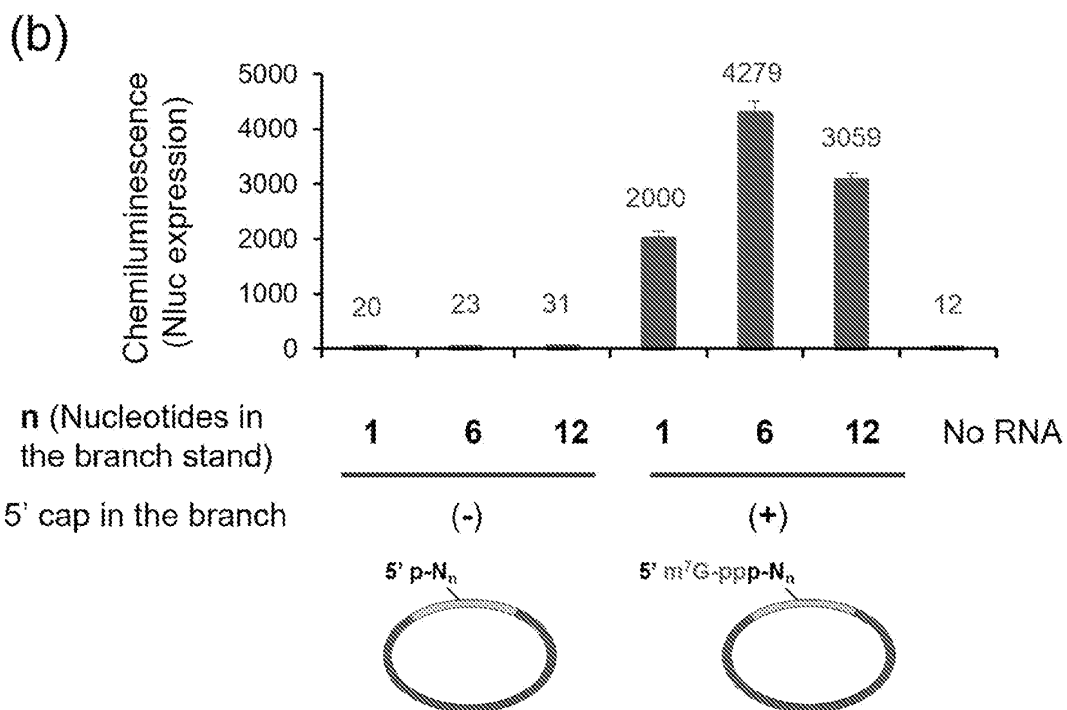
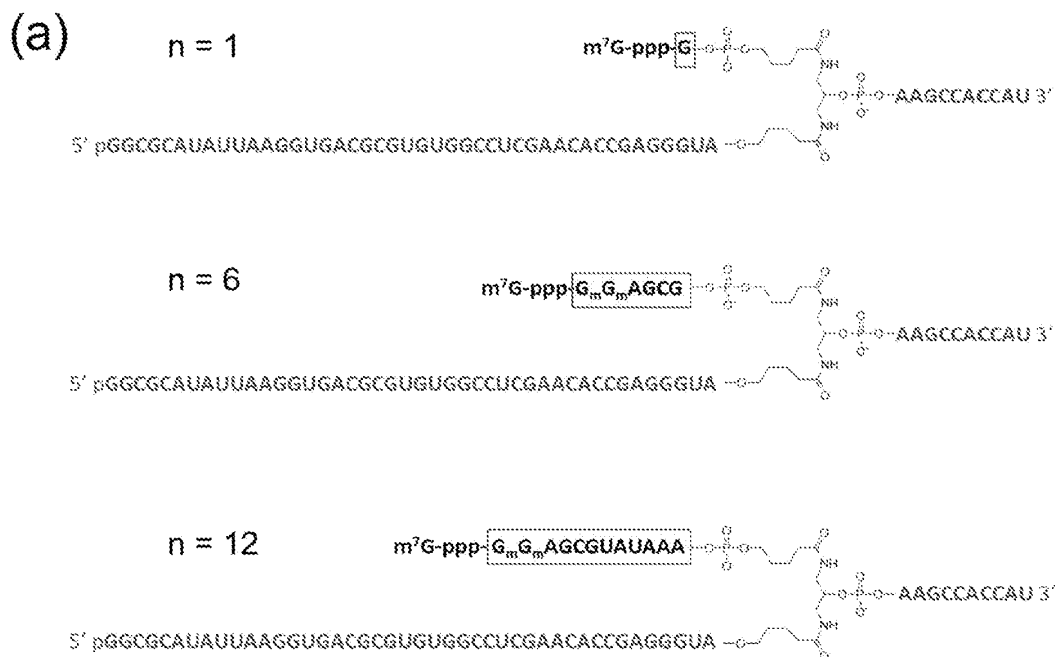
5' p\_GGUCUUCACACUCGAAGAUUUCGUUGGGGACUGGCGACAGACAGCCGGCUACAACCUGGACC  
AAGUCCUUGAACAGGGAGGUGUGUCCAGUUUGUUUCAGAAUCUCGGGGUGUCCGUAAACUCCGAUC  
CAAAGGAUUGUCCUGAGCGGUGAAAAUGGGCUGAAGAUUCGACAUCCAUGUCAUACCCGUAUGAA  
GGUCUGAGCGGCGACCAAUGGGCCAGAUUCGAAAAAUUUUAAAGGUGGUGUACCCUGUGGAUGA  
UCAUCACUUUAAGGUGAUCCUGCACUAUGGCACACUGGUAUUCGACGGGUUACGCCGAACAUGA  
UCGACUAUUUCGGACGGCCGUAUGAAGGCAUCGCCGUGUUCGACGGCAAAAAGAUACUGUAACAG  
GGACCCUGUGGAACGGCAACAAAUUAUCGACGAGCGCCUGAUCAACCCGACGGCUCCUUGCUGU  
UCCGAGUAACCAUCAACGGAGUGACCGGCUGGCGGUGUGCGAACGCAUUCUGGCGUAAUUCUAGA  
AAAAAAAAAAAAAAAAAAAAAAAAAAAA 3'

Underlined, start codon  
Bold letters, Nluc coding region

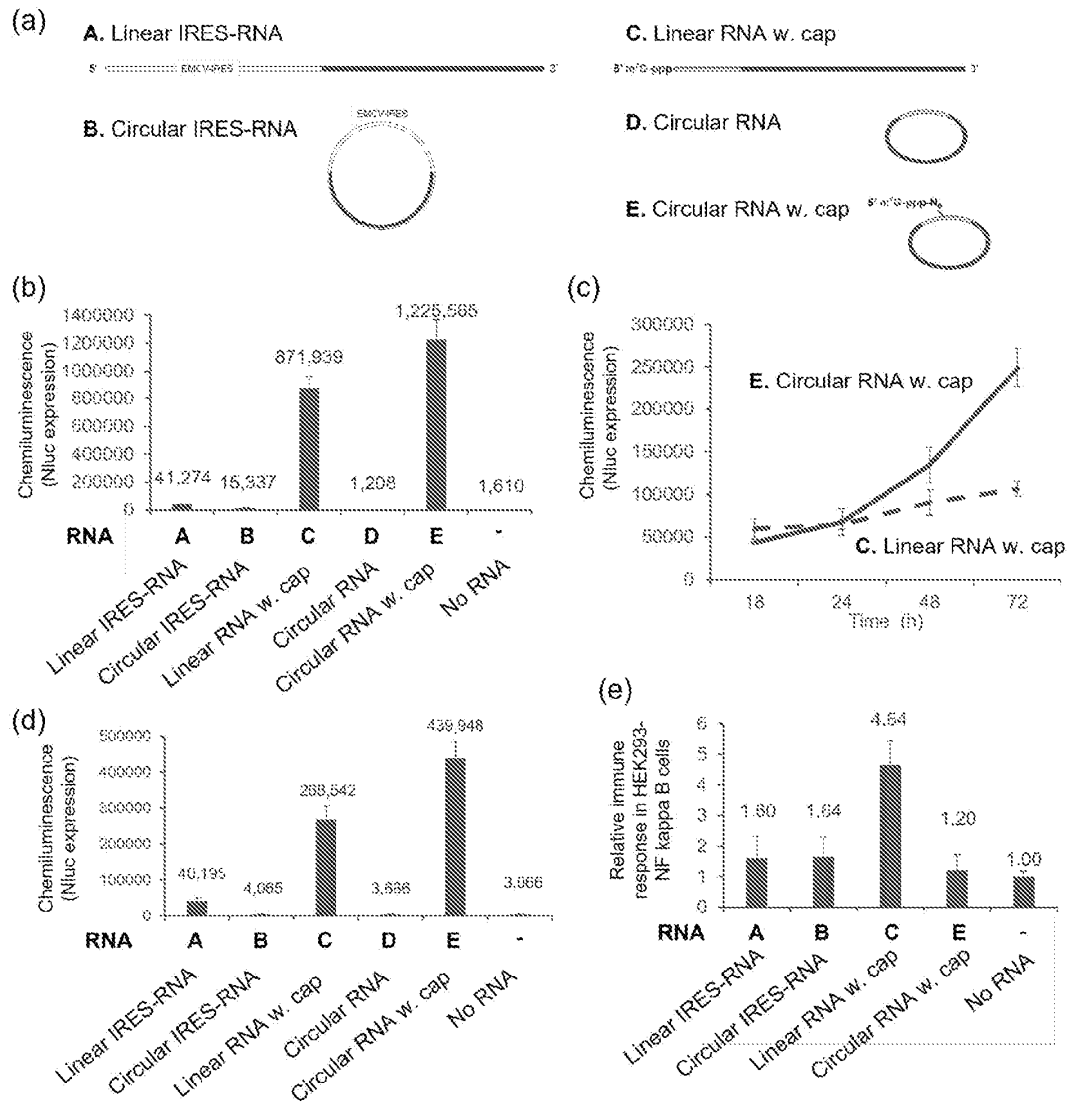
(b)



[図32]



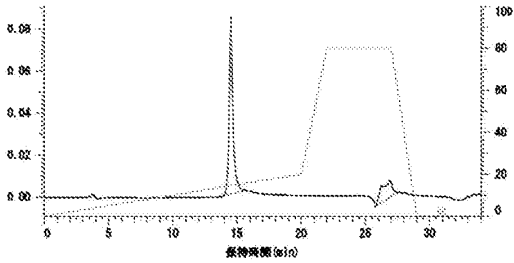
[図33]



[図34]

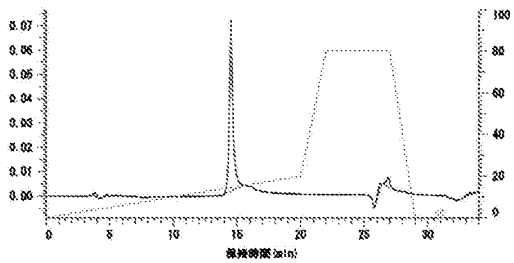
Analytical HPLC after isolation

ATF4-Renilla mRNA cap (+)



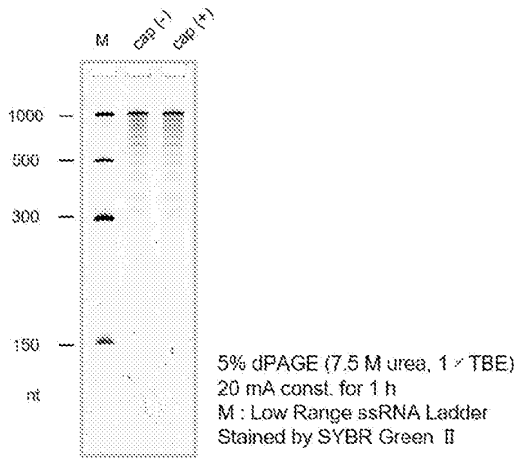
HPLC: HITACHI NEW/NEW2  
Loop size: 1 mL  
HPLC column: Hydrosphere C18 (HS12S05-2546WT, serial#112YA90228) 250 × 4.6 mm I.D., 5-μm, 12 nm  
Solvent: A: 50mM TEAA (pH 7.0), 5% CH<sub>3</sub>CN; B: CH<sub>3</sub>CN  
Gradient program: 0 min, 0% B; 20 min, 20% B; 22 min, 80% B; 27 min, 80% B; 29 min, 0% B; 34 min, 0% B  
Flow rate: 1.0 mL/min  
Column temperature: 50 °C

ATF4-Renilla mRNA cap (-)

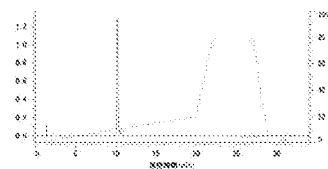
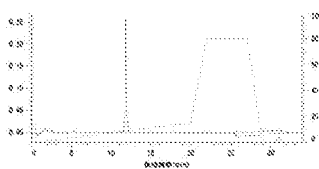
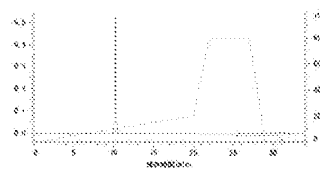
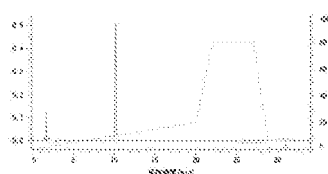
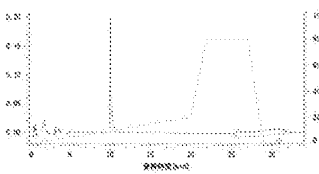
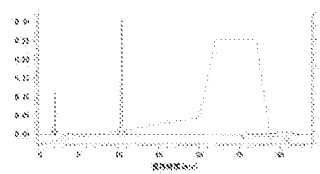
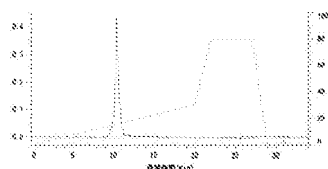
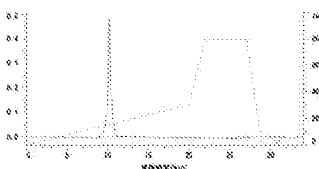


HPLC: HITACHI NEW/NEW2  
Loop size: 1 mL  
HPLC column: Hydrosphere C18 (HS12S05-2546WT, serial#112YA90228) 250 × 4.6 mm I.D., 5-μm, 12 nm  
Solvent: A: 50mM TEAA (pH 7.0), 5% CH<sub>3</sub>CN; B: CH<sub>3</sub>CN  
Gradient program: 0 min, 0% B; 20 min, 20% B; 22 min, 80% B; 27 min, 80% B; 29 min, 0% B; 34 min, 0% B  
Flow rate: 1.0 mL/min  
Column temperature: 50 °C

denaturing PAGE



## [図35]

Analytical HPLC after isolationATF4-sRNA1ATF4-sRNA2ATF4-sRNA3ATF4-sRNA4ATF4-sRNA5ATF4-sRNA6ATF4-sRNA7ATF4-sRNA8

Loop size: 1 mL

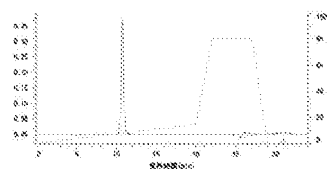
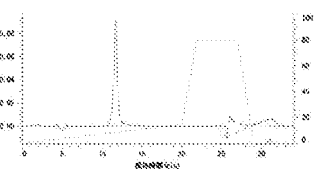
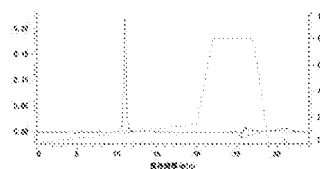
HPLC column: Hydrosphere C18 (HS12S05-2546WT, serial#108AB00175) 250 × 4.6 mm i.d., 5-5 μm, 12 nm

Solvent: A: 50mM TEAA (pH 7.0), 5% CH<sub>3</sub>CN; B: CH<sub>3</sub>CN

Gradient program: 0 min, 0% B; 20 min, 30% B; 22 min, 80% B; 27 min, 80% B; 29 min, 0% B; 34 min, 0% B

Flow rate: 1.0 mL/min

Column temperature: 25 °C

ATF4-sRNA8-polyAATF4-sRNA8-polyA-OMeATF4-sRNA8-polyA-LNA

Loop size: 1 mL

HPLC column: Hydrosphere C18 (HS12S05-2546WT, serial#112YA90228) 250 × 4.6 mm i.d., 5-5 μm, 12 nm

Solvent: A: 50mM TEAA (pH 7.0), 5% CH<sub>3</sub>CN; B: CH<sub>3</sub>CN

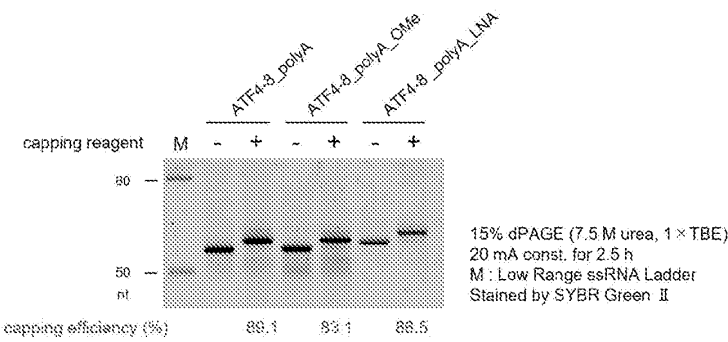
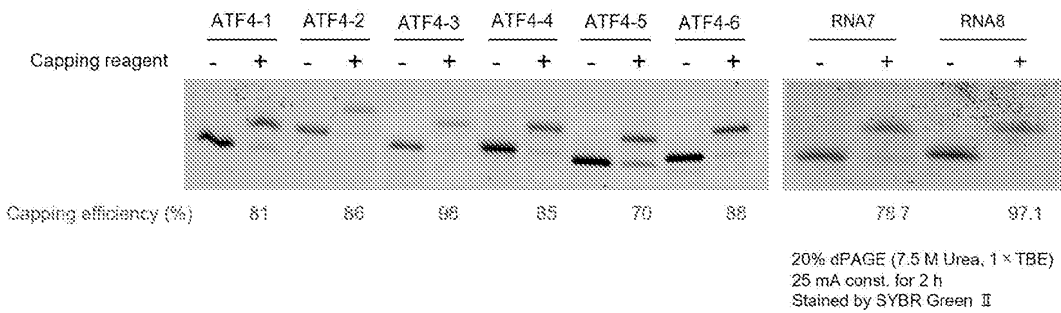
Gradient program: 0 min, 0% B; 20 min, 15% B; 22 min, 80% B; 27 min, 80% B; 29 min, 0% B; 34 min, 0% B

Flow rate: 1.0 mL/min

Column temperature: 50 °C

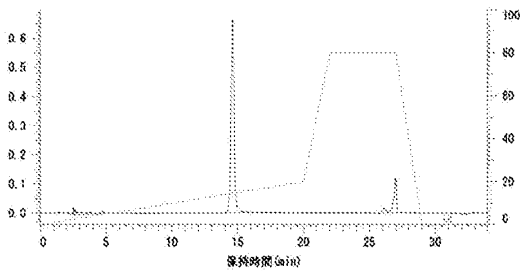
[図36]

denaturing PAGE



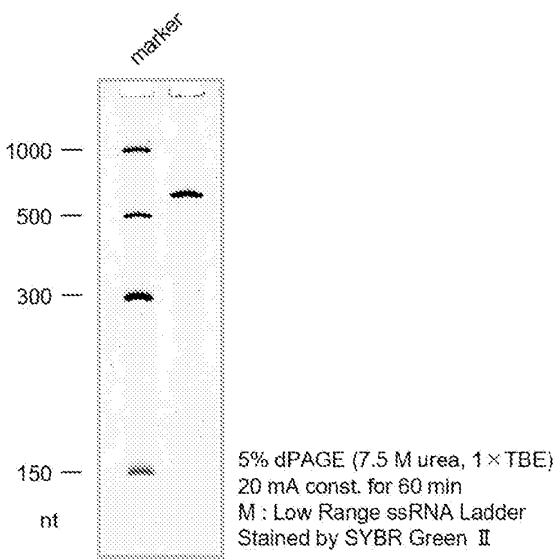
[図37]

Analytical HPLC after isolation

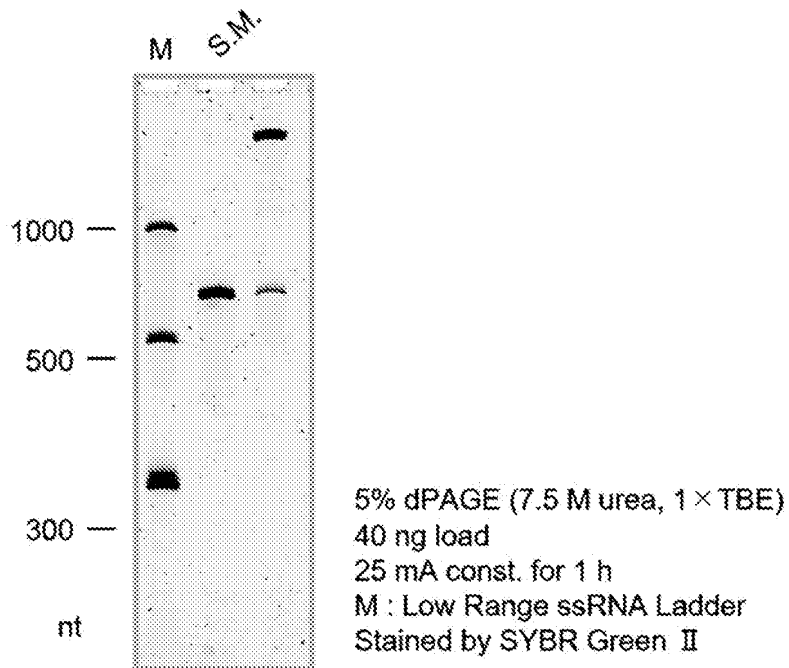


Loop size: 1 mL  
HPLC column: Hydrosphere C18 (HS12S05-2546WT, serial#112YA90228) 250 × 4.6 mm I.D., 5-5 μm, 12 nm  
Solvent: A: 50mM TEAA (pH 7.0), 5% CH<sub>3</sub>CN; B: CH<sub>3</sub>CN  
Gradient program: 0 min, 0% B; 20 min, 20% B; 22 min, 60% B; 27 min, 80% B; 29 min, 0% B; 34 min, 0%B  
Flow rate: 1.0 mL/min  
Column temperature: 50 °C

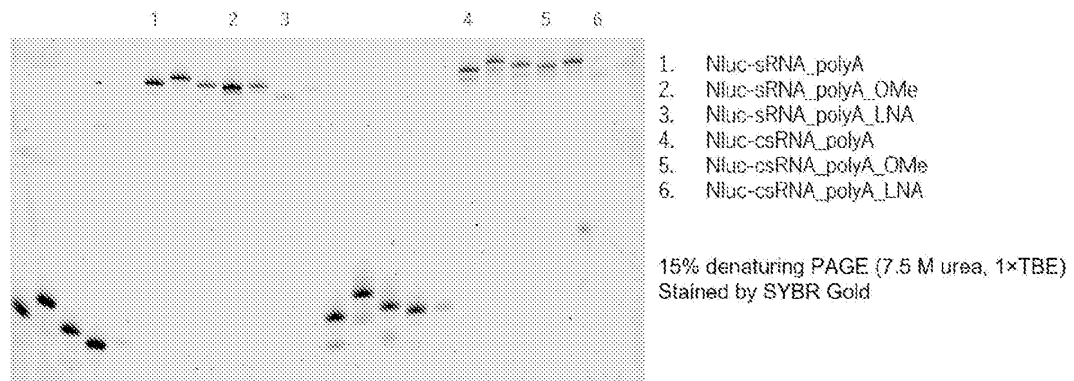
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[図38]

denaturing PAGE

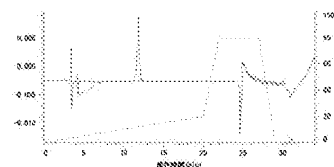
[図39]



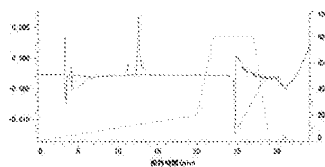
## [図40]

Analytical HPLC after isolation

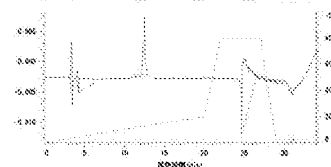
Nluc-sRNA\_natural



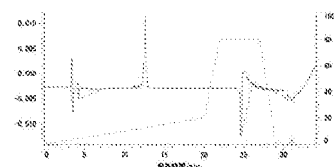
Nluc-sRNA\_LNA\_10/20\_3' type



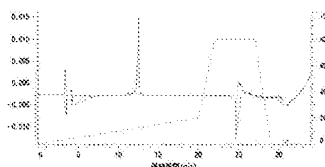
Nluc-sRNA\_LNA\_5/20\_3' type



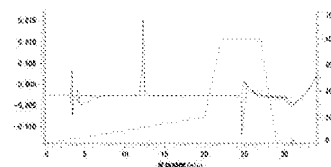
Nluc-sRNA\_LNA\_10/20 type



Nluc-sRNA\_LNA\_5/20 type



Nluc-sRNA\_LNA\_6/20 type



HPLC: HITACH NEW2 Loop size: 1 mL

HPLC column: Hydrosphere C18 (HS12S05-2546WT, serial#125XB20166) 250 × 4.6 mm I.D., S-5 μm, 12 nm

Solvent: A: 50mM TEAA (pH 7.0), 5% CH<sub>3</sub>CN; B: CH<sub>3</sub>CN

Gradient program: 0 min, 0% B; 20 min, 20% B; 22 min, 80% B; 27 min, 80% B; 29 min, 0% B; 34 min, 0% B

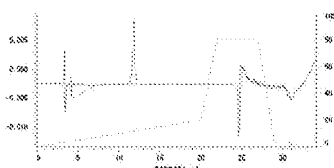
Flow rate: 1.0 mL/min Column temperature: 50 °C

Injection volume and solution condition: 40 μL (MQ) Injection amount: 16 pmol

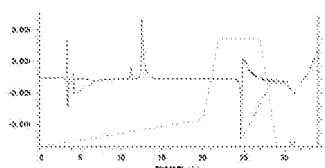
## [図41]

Analytical HPLC after isolation

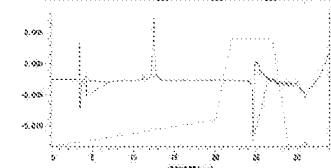
Nluc-csRNA\_natural



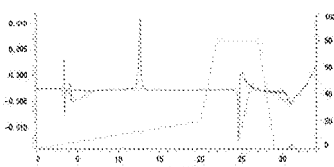
Nluc-csRNA\_LNA\_10/20\_3' type



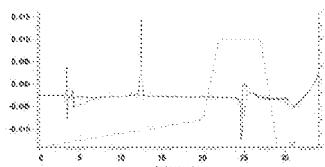
Nluc-csRNA\_LNA\_5/20\_3' type



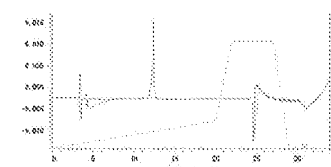
Nluc-csRNA\_LNA\_10/20 type



Nluc-csRNA\_LNA\_5/20 type



Nluc-csRNA\_LNA\_6/20 type



HPLC: HITACH NEW2 Loop size: 1 mL

HPLC column: Hydrosphere C18 (HS12S05-2546WT, serial#125XB20166) 250 × 4.6 mm I.D., S-5 μm, 12 nm

Solvent: A: 50mM TEAA (pH 7.0), 5% CH<sub>3</sub>CN; B: CH<sub>3</sub>CN

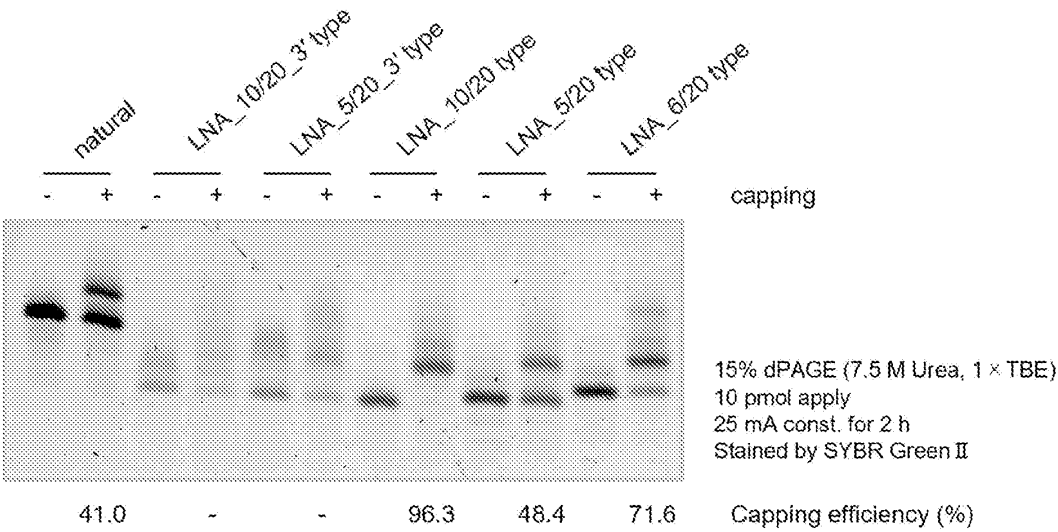
Gradient program: 0 min, 0% B; 20 min, 20% B; 22 min, 80% B; 27 min, 80% B; 29 min, 0% B; 34 min, 0% B

Flow rate: 1.0 mL/min Column temperature: 50 °C

Injection volume and solution condition: 40 μL (MQ) Injection amount: 16 pmol

[図42]

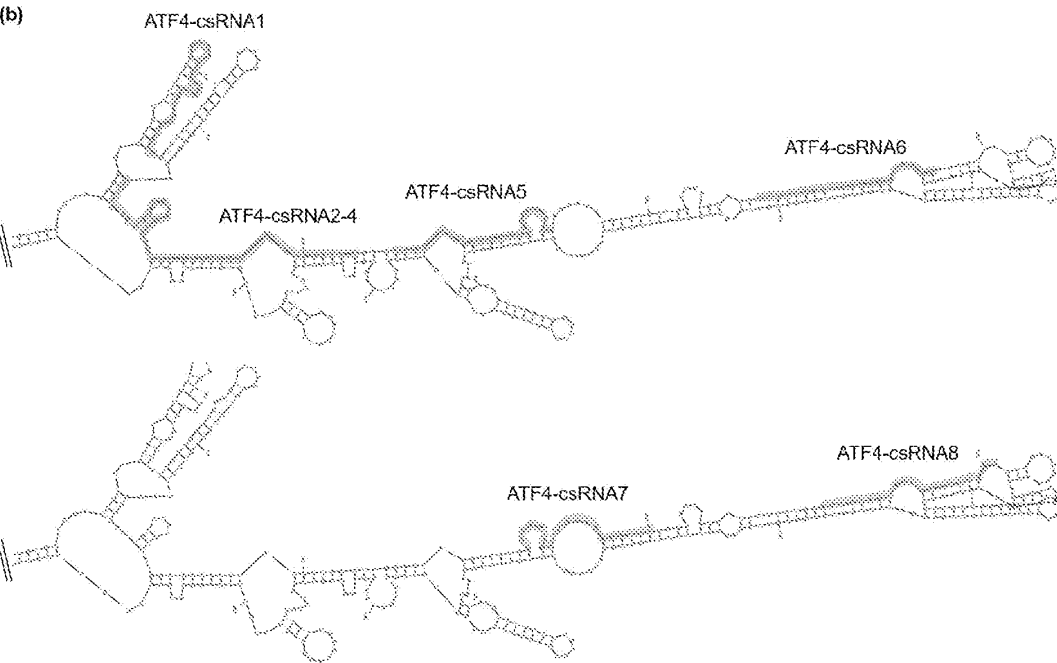
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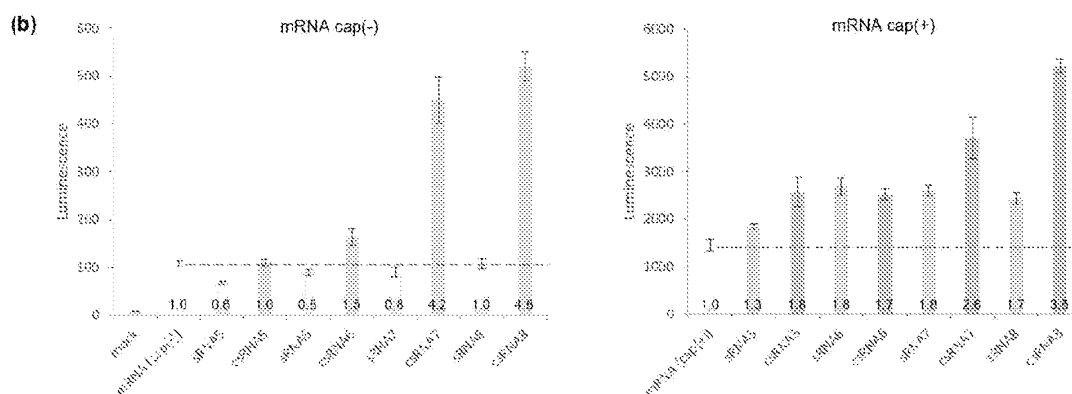
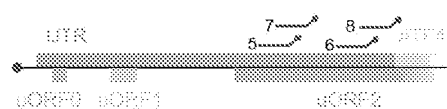
[図43]

(a) Capped short RNA

ATF4-csRNA5 (23 nt) : m<sup>7</sup>GpppGAACGGUDACAGCAACGCUGCUG  
ATF4-csRNA6 (23 nt) : m<sup>7</sup>GpppACCGCUGUGCUGGAADCGAGGA  
ATF4-csRNA7 (23 nt) : m<sup>7</sup>GpppGAUAGUGUCUUUGUCGGUDACA  
ATF4-csRNA8 (23 nt) : m<sup>7</sup>GpppGAUUECGUGCUUUGCUGGAU



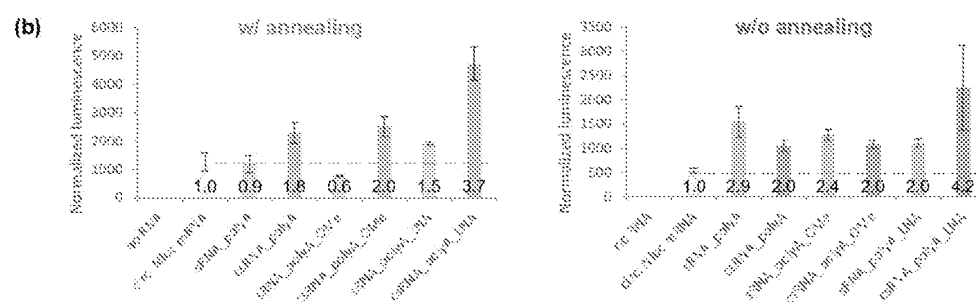
(a)



(a)

(a) Capped short RNA

Nluc-csRNA\_polyA (50 nt) : m<sup>7</sup>GpppGCGUCACCUUAUAUAGCGGCCAAAAAAAAAAAAAAAAAAAAAAAAAAAAA



## [図46]

## (a) Capped short RNA

|                                         |                                                  |
|-----------------------------------------|--------------------------------------------------|
| natural (25 nt)                         | : m <sup>7</sup> Gppp-GAGCGGCGGUCACCUUAAUAUGCGCC |
| Nluc-csRNA__LNA__10/20__3' type (25 nt) | : m <sup>7</sup> Gppp-GAGCGGCGGUCACCUUAAUAUGCGCC |
| Nluc-csRNA__LNA__5/20__3' type (25 nt)  | : m <sup>7</sup> Gppp-GAGCGGCGGUCACCUUAAUAUGCGCC |
| Nluc-csRNA__LNA__10/20 type (25 nt)     | : m <sup>7</sup> Gppp-GAGCGGCGGUCACCUUAAUAUGCGCC |
| Nluc-csRNA__LNA__5/20 type (25 nt)      | : m <sup>7</sup> Gppp-GAGCGGCGGUCACCUUAAUAUGCGCC |
| Nluc-csRNA__LNA__6/20 type (25 nt)      | : m <sup>7</sup> Gppp-GAGCGGCGGUCACCUUAAUAUGCGCC |

## (b)

