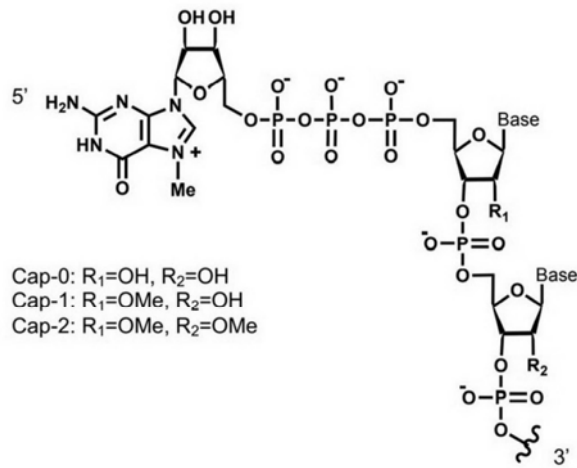


Fig. 1

A) 5'-capped structure



B) Capped RNA

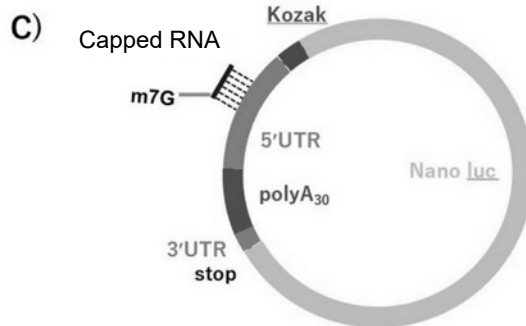
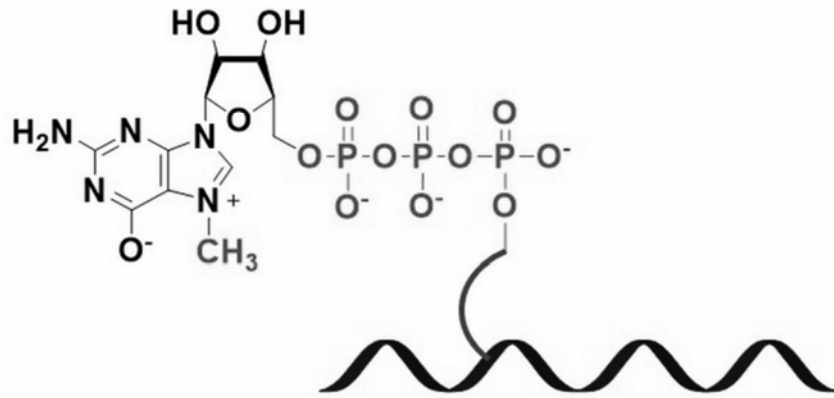


Fig. 2

(a)



(b)

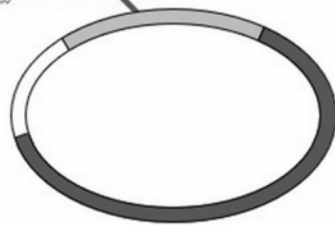
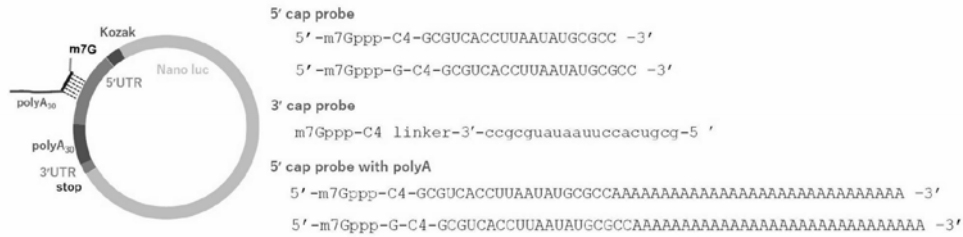
5' m^7G -ppp- G_m G_m AGCG

Fig. 3

Circular mRNA hybridized with capped short RNA



5' -m7Gppp-C4-GCGUCACCUAAUAUGCGCCAAAAAAAAAAAAAAAAAAAAAAAAAAAA -3'
5' -m7Gppp-G-C4-GCGUCACCUAAUAUGCGCCAAAAAAAAAAAAAAAAAAAAAAAAAAAA -3'

ggcgcauauuaggugagcgugugggccucgaacaccgagcgaccugcagcgaccgcuuaaaagcuuggca
auccgguacuuguguaaagccaccaugguuucacacucgaagauuucguugggacuggcgacagacagcc
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cuccgauccaaaggauuuccugagcgguuaaaugggcugaagaucgacauccaugucaucauccgguaga
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uuuaggugauccugcacuauggcacacugguuauucgacgggguaucggcgaacauagcagacuaauucggac
ggccguagaaggcaucgcccugugucgagcgcaaaaagaucaacuguaacagggaaccuguggaacggcaca
auuuuacgacgagcgccugaucaaccccgacggcuuccugugucgaguaaccaucaacggagugacgggc
uggcgcgugugcgaaacgcauucugcggaauucuaagAAAAAAAAAAAAAAAAAAAAAAAAAAAA

Fig. 4

cccgatccTAAACGACTCACTATAgcgcatattaaggtgacgctgtgtgcctcgaacaccgagcgaccctgcagcgacccgcttaaaagct
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Fig. 5

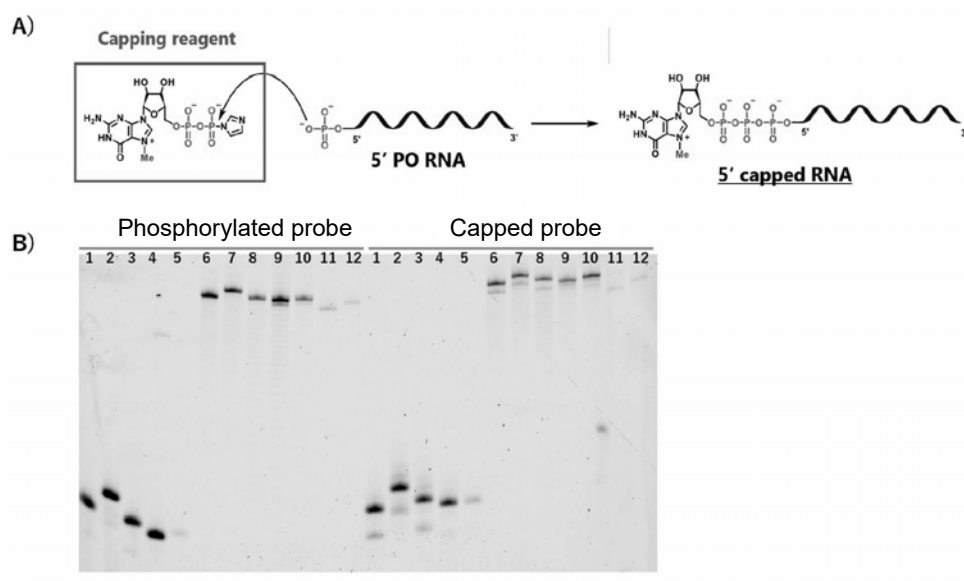


Fig. 6

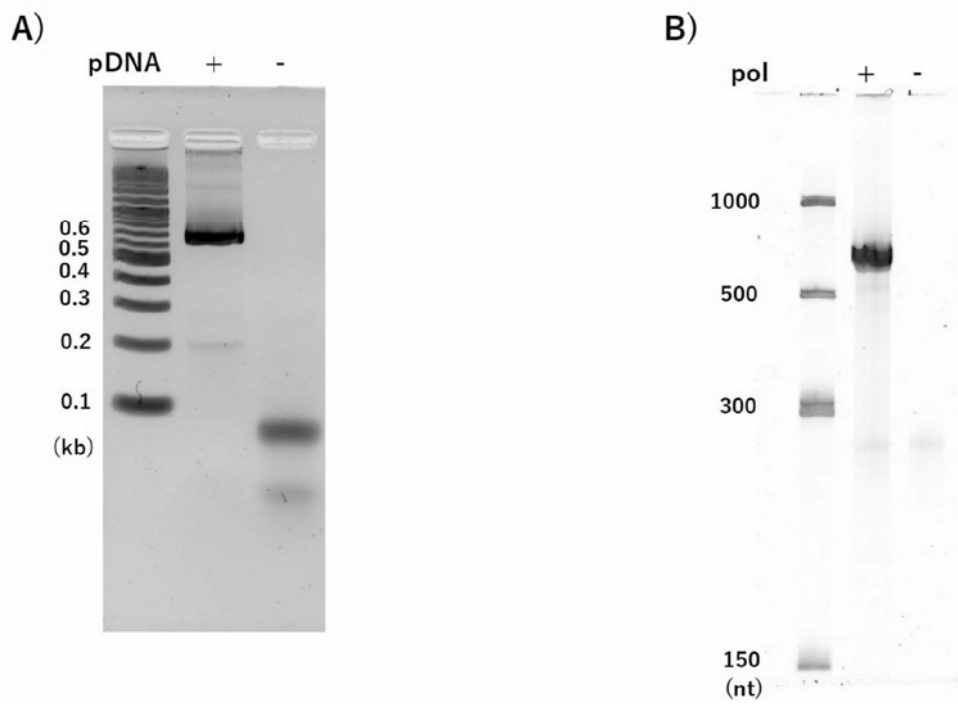


Fig. 7

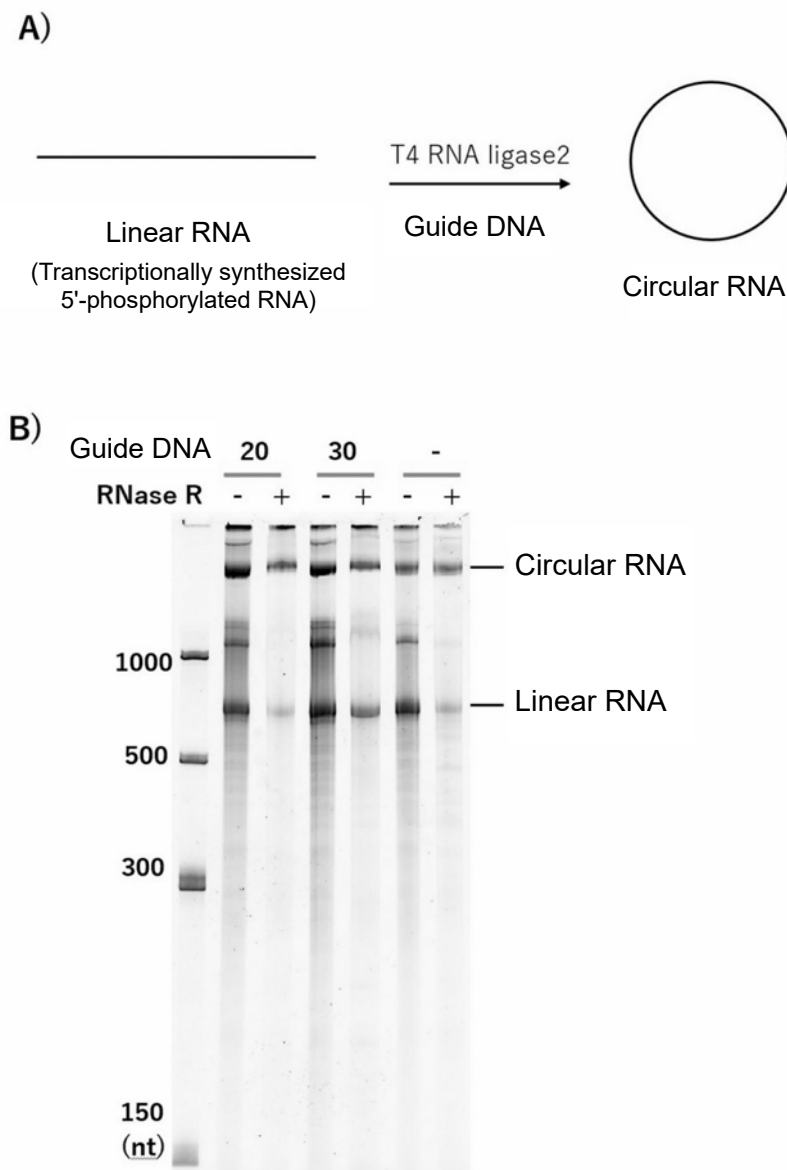


Fig. 8

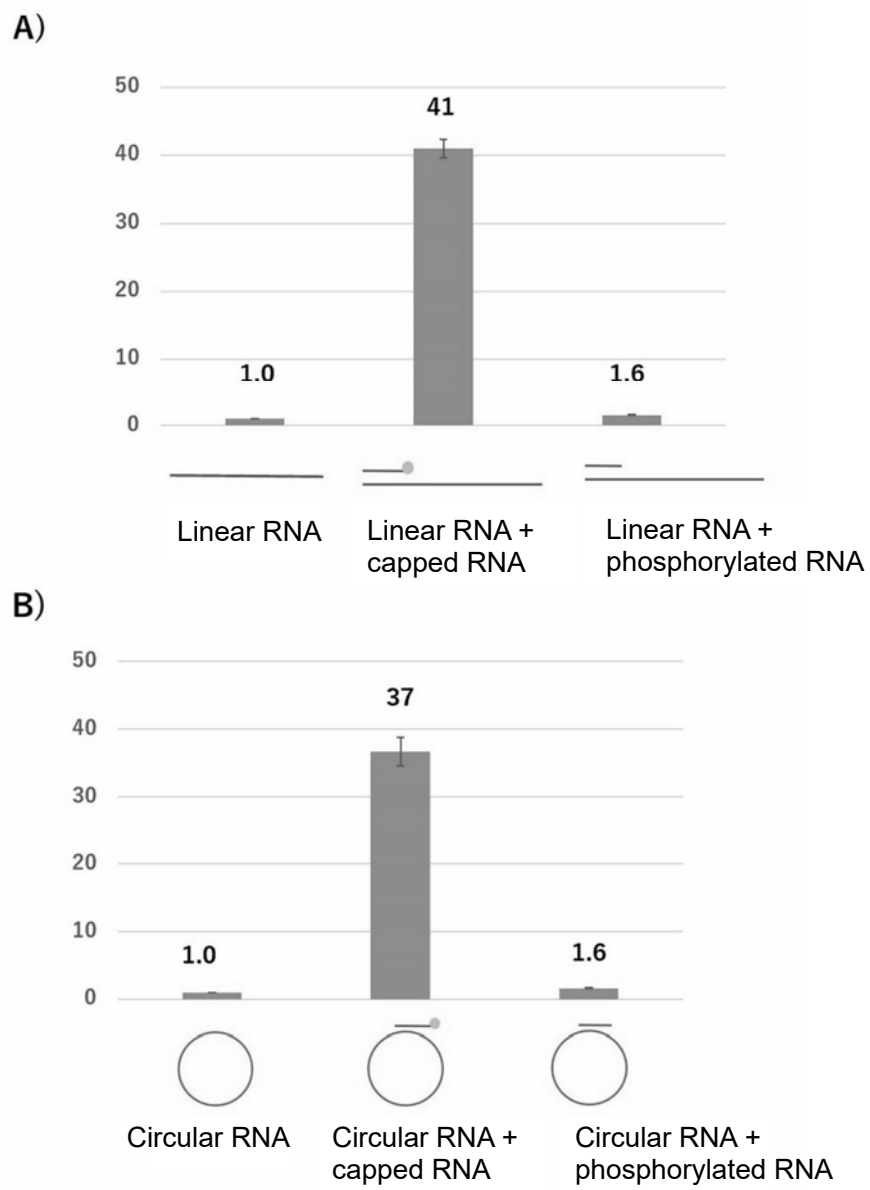


Fig. 9

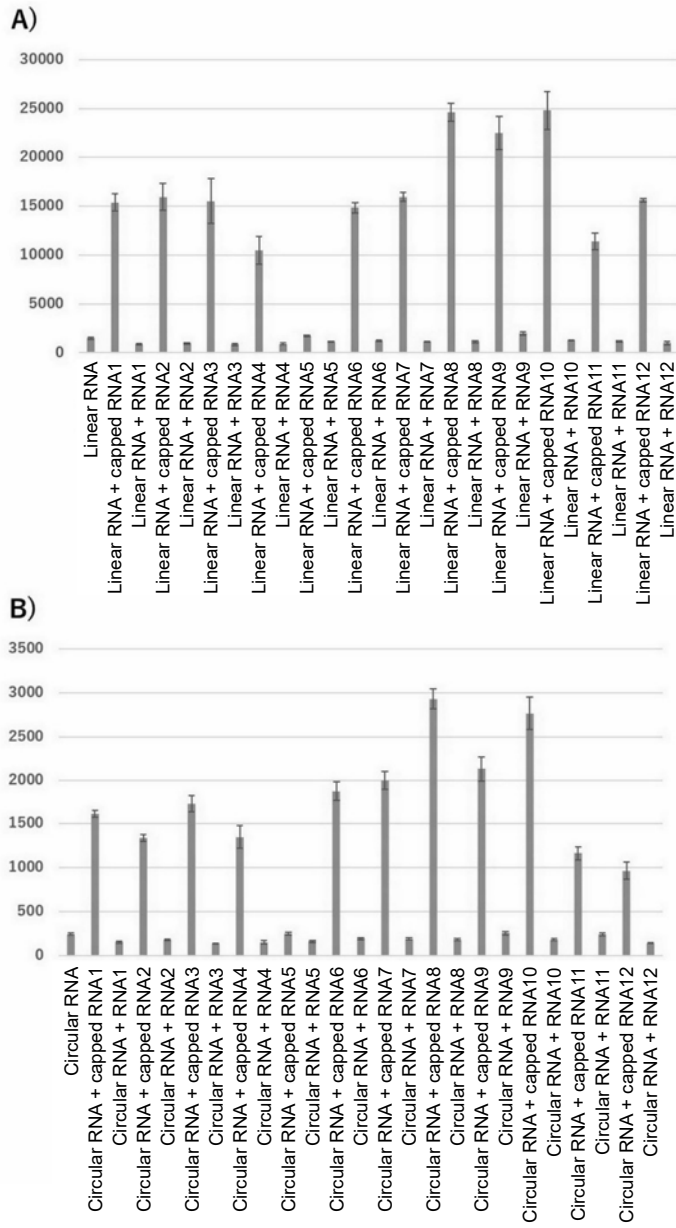


Fig. 10

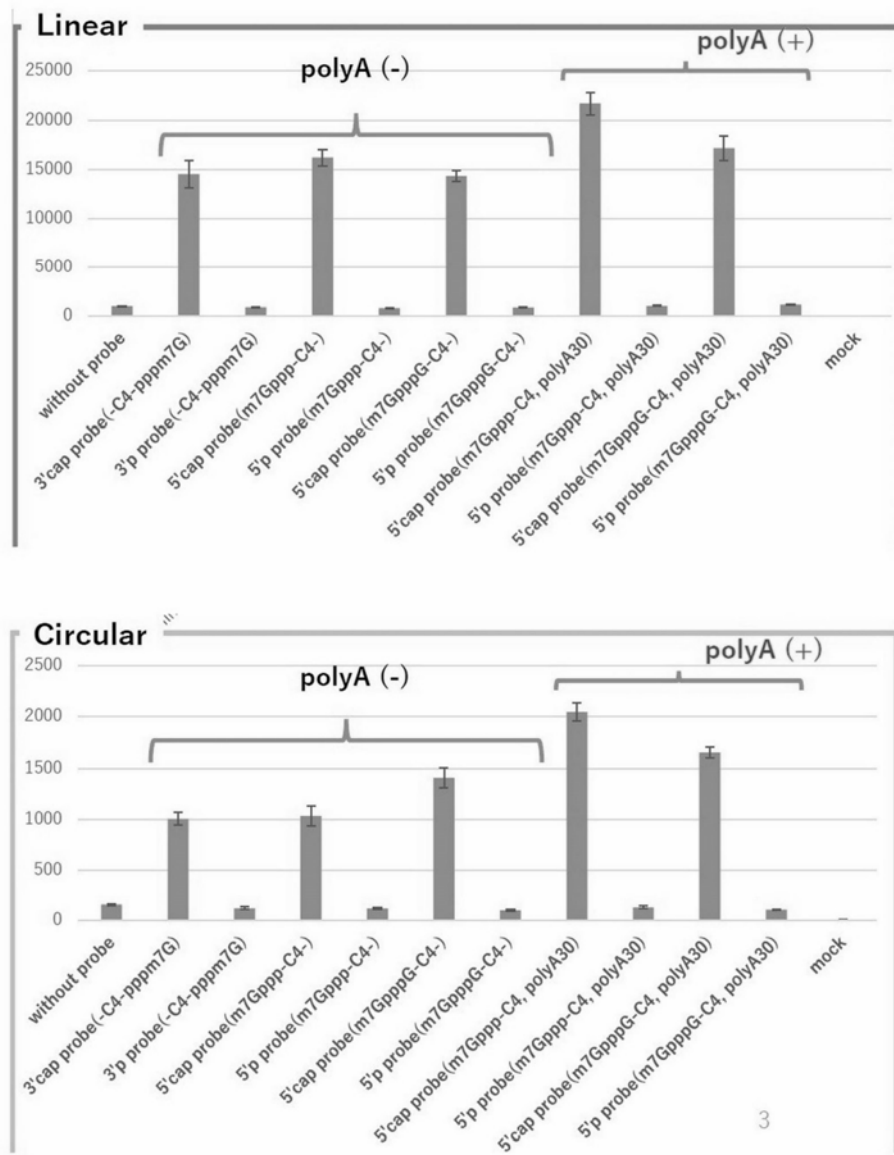


Fig. 11

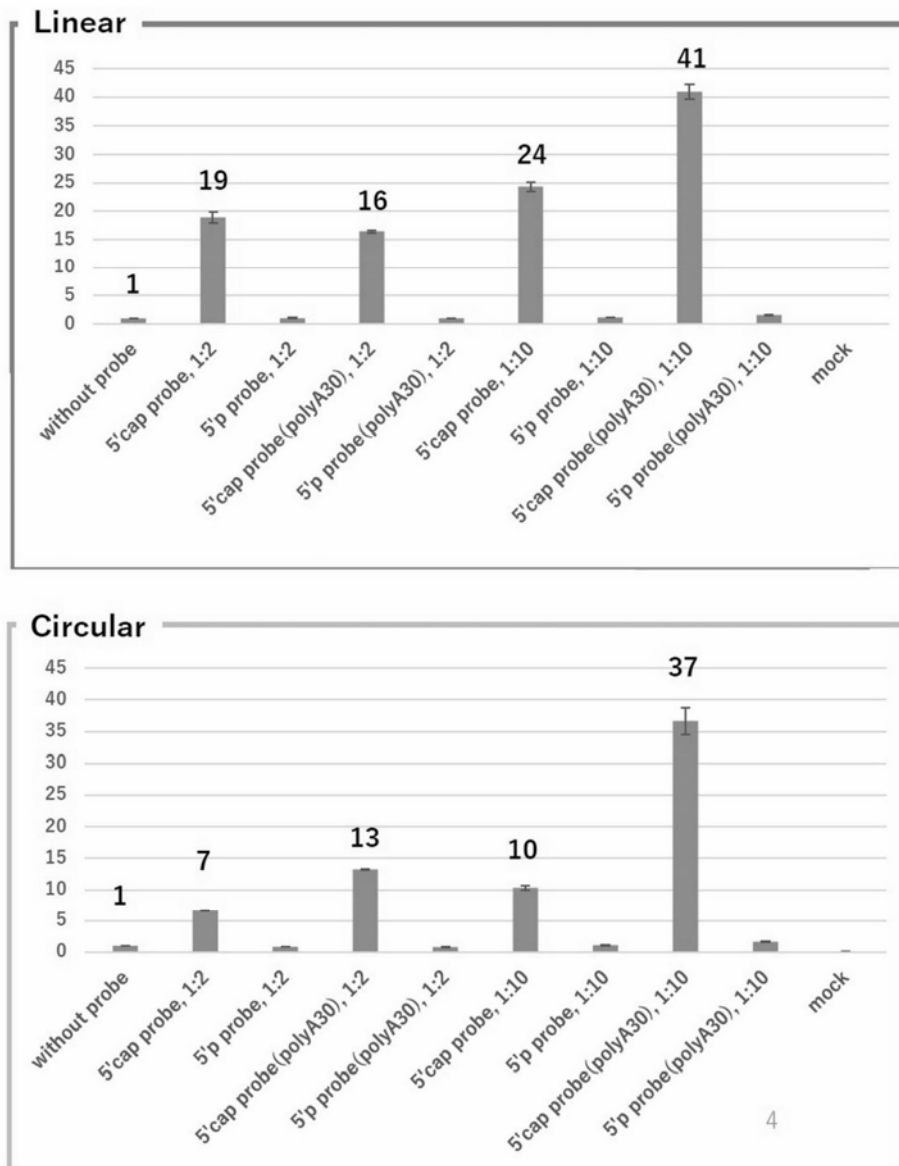


Fig. 12

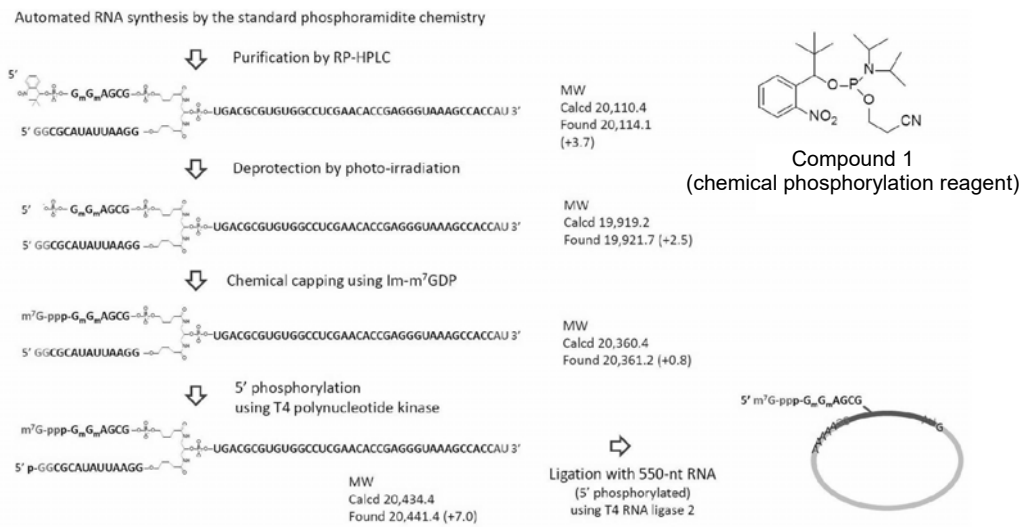


Fig. 13

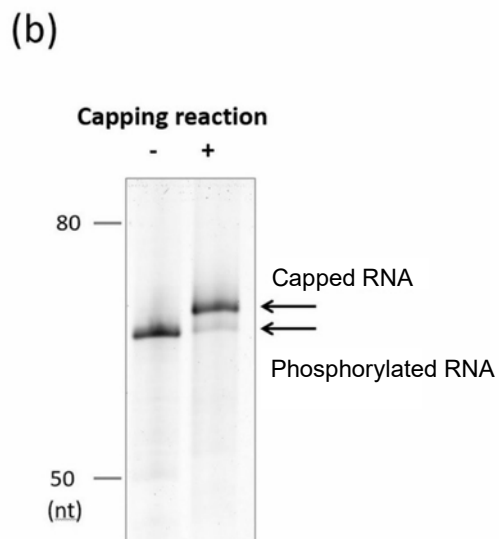
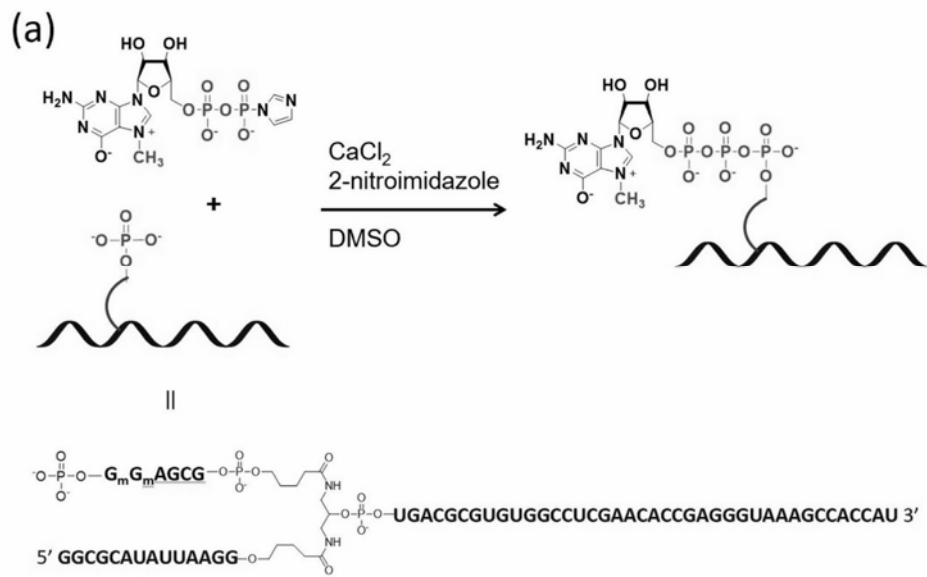


Fig. 14

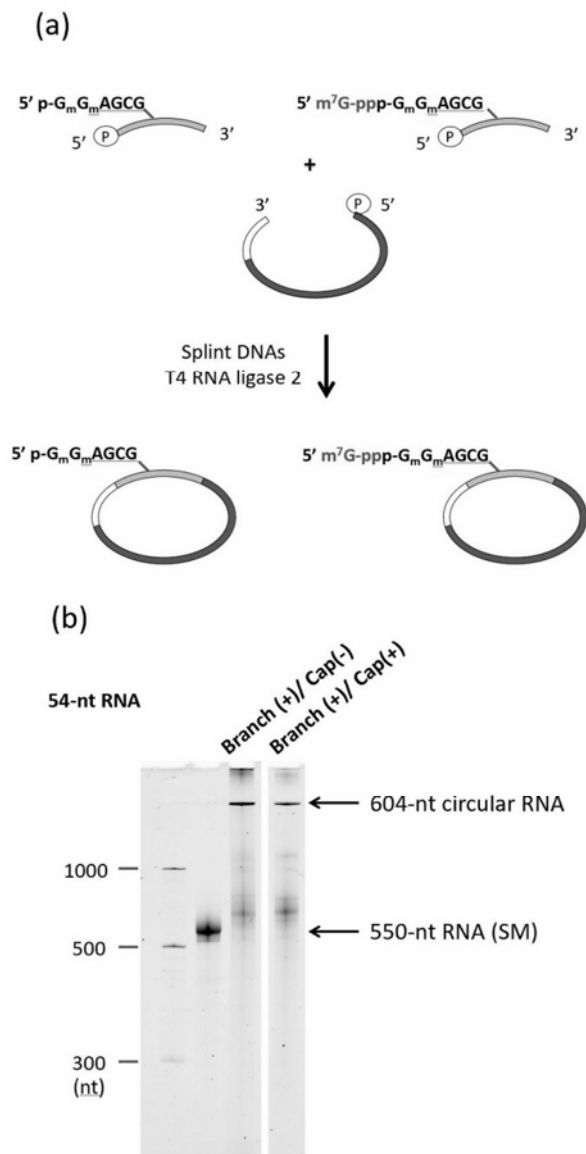


Fig. 15

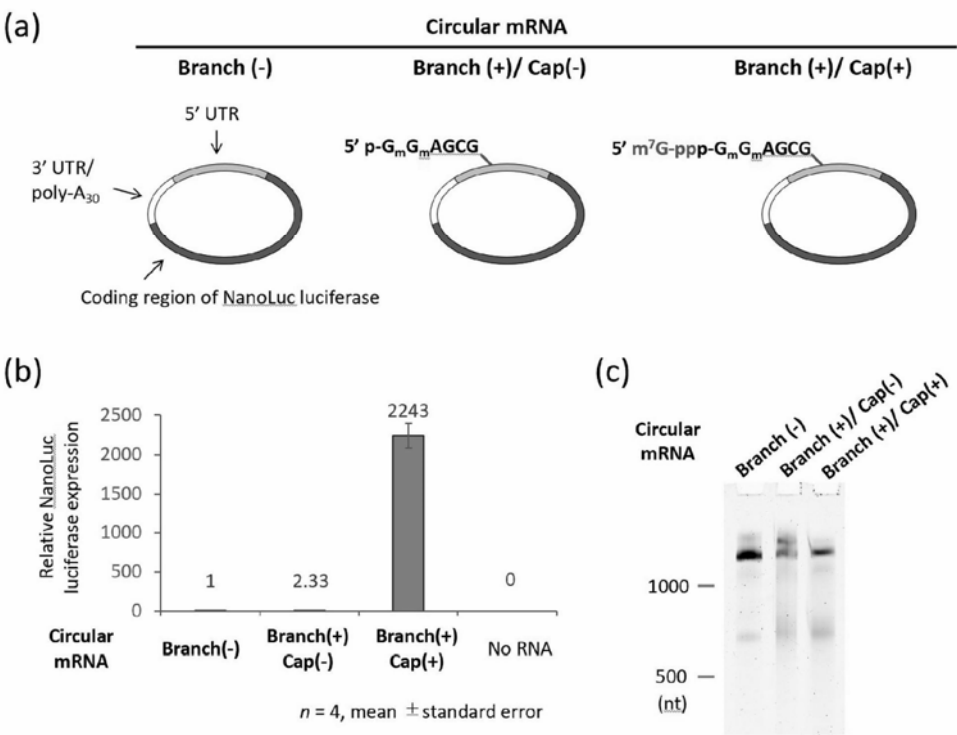


Fig. 16

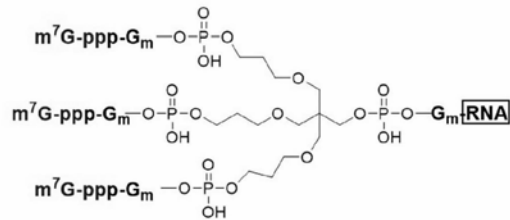
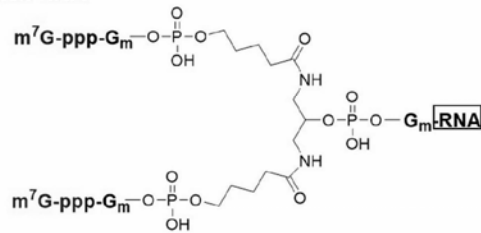
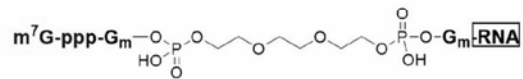
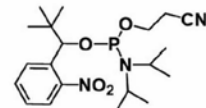
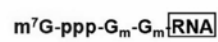
3-Branch RNA**2-Branch RNA****Linker w no branch RNA****No linker RNA**Chemical capping reagent
Nb-CPR

Fig. 17

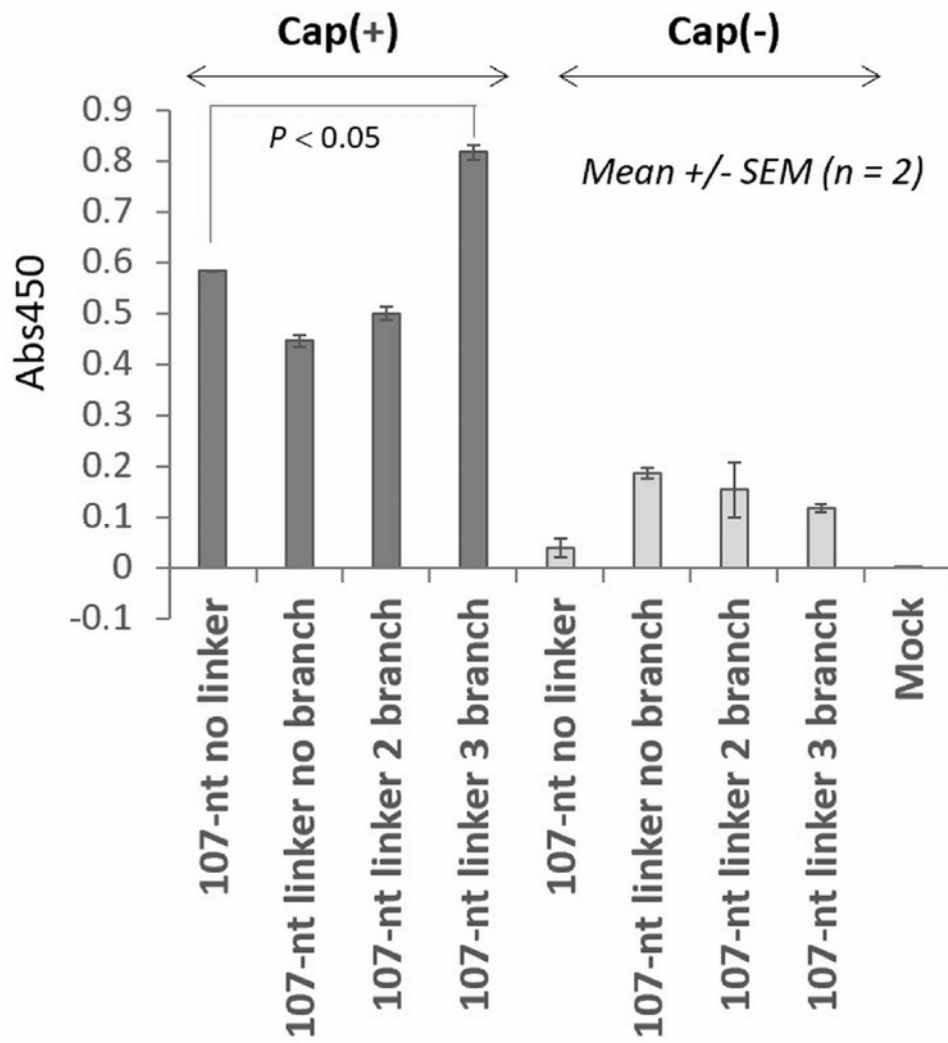


Fig. 18

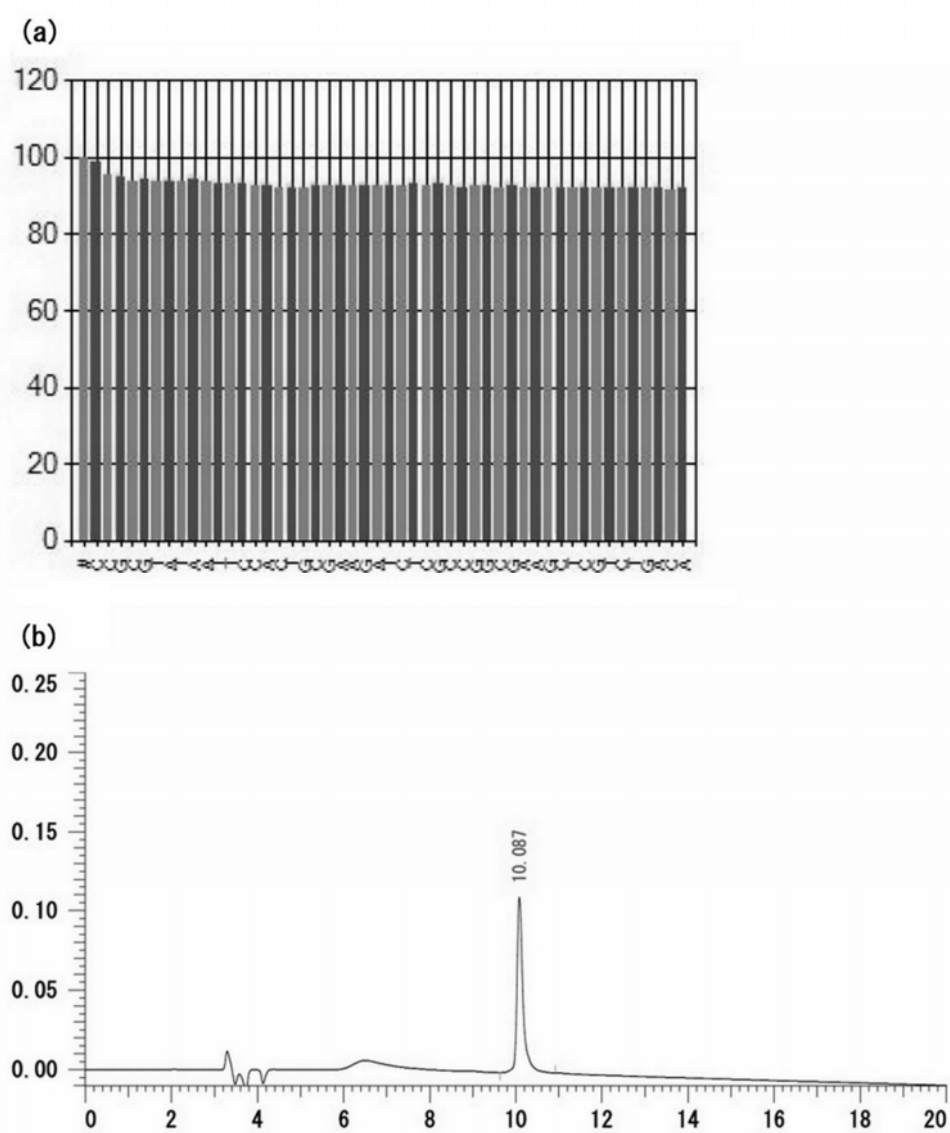


Fig. 19

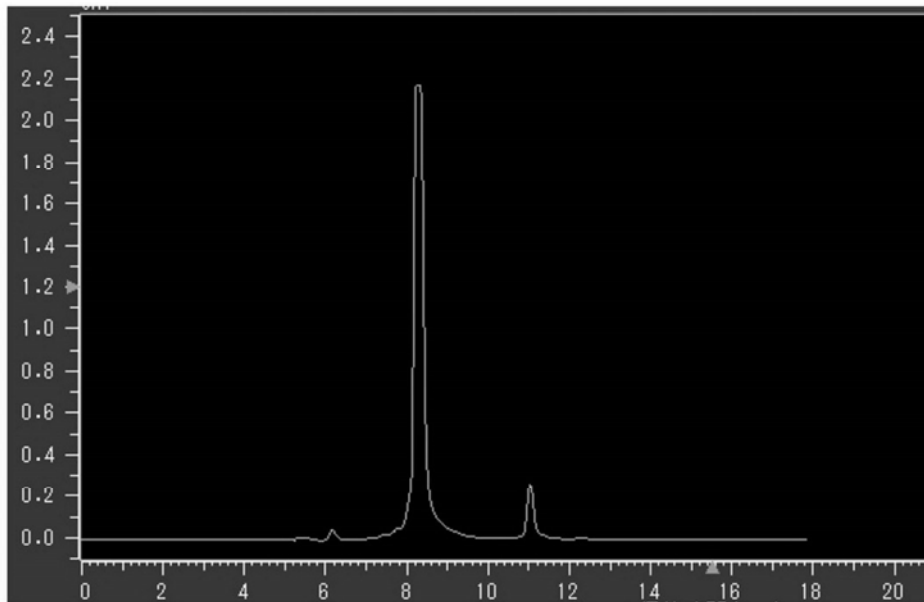
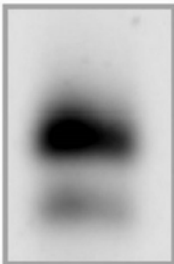
(a)**(b)**

Fig. 20

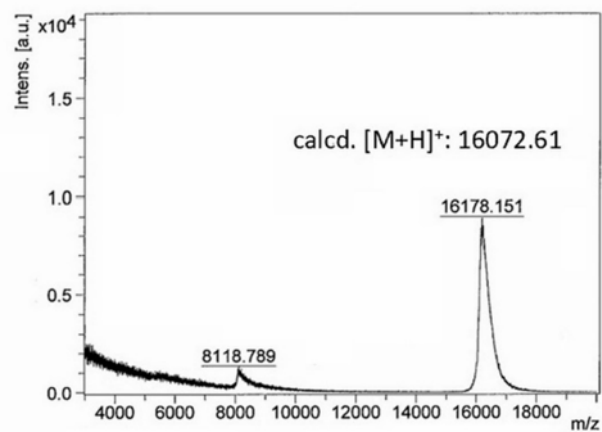
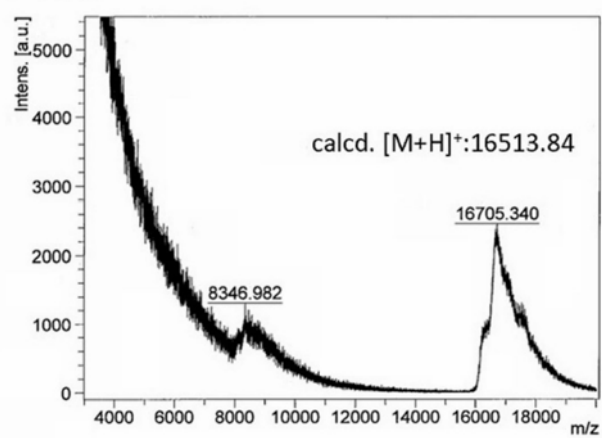
(a)cap(-)**(b)**cap(+)

Fig. 21

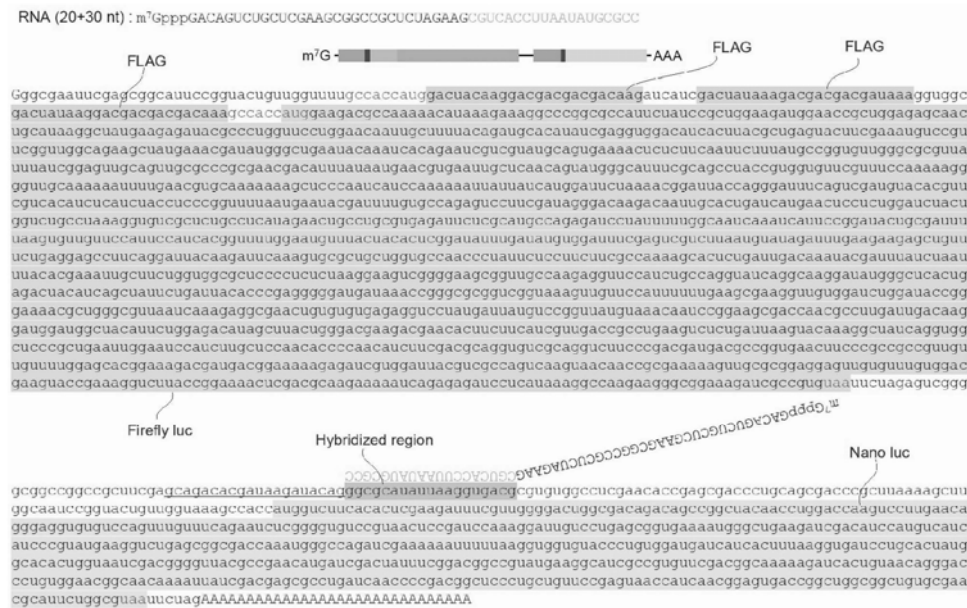


Fig. 22

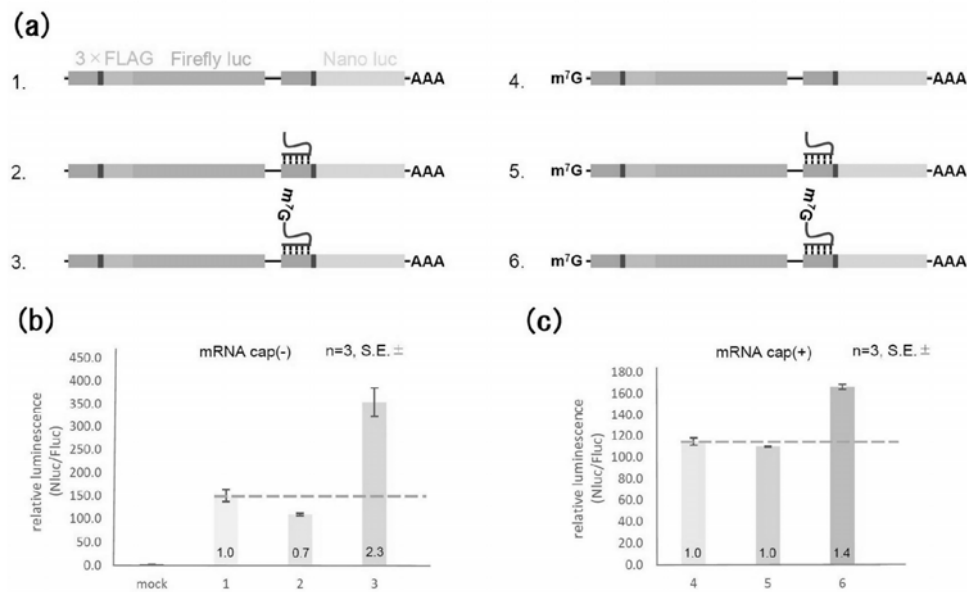


Fig. 23

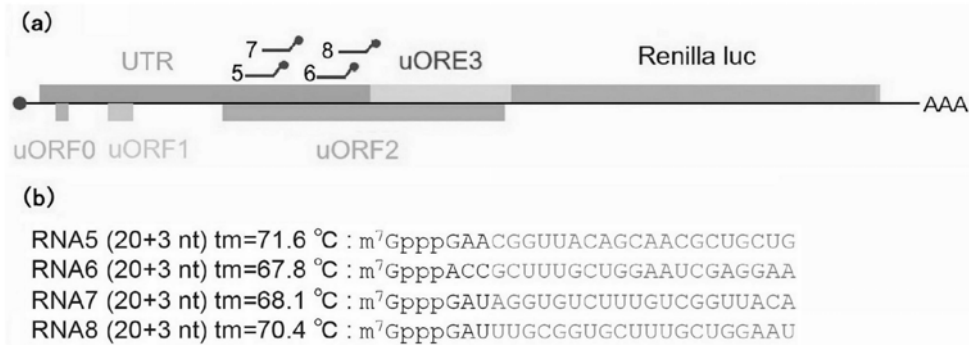


Fig. 24

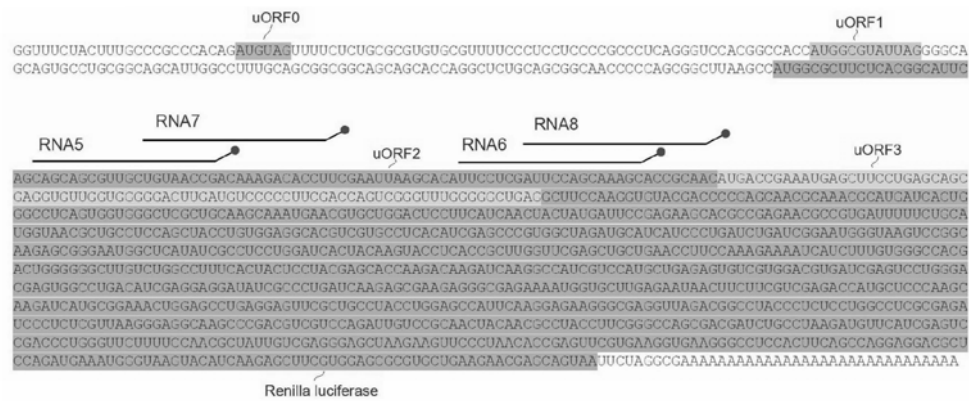
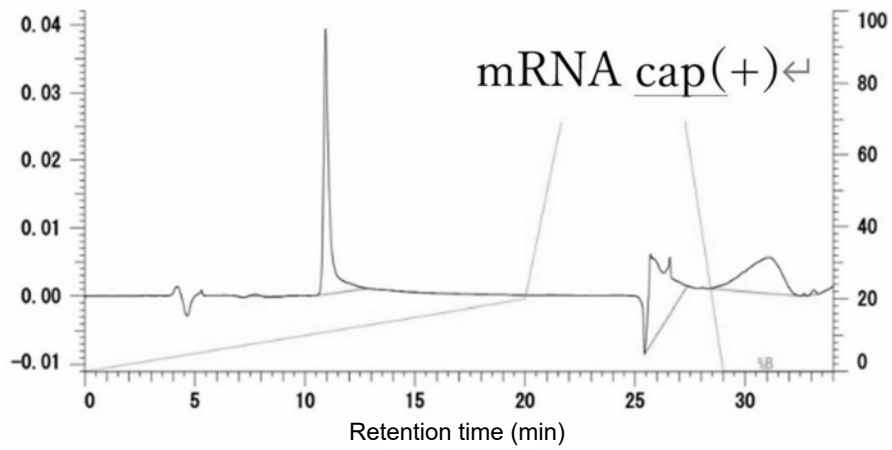


Fig. 25

(a)



(b)

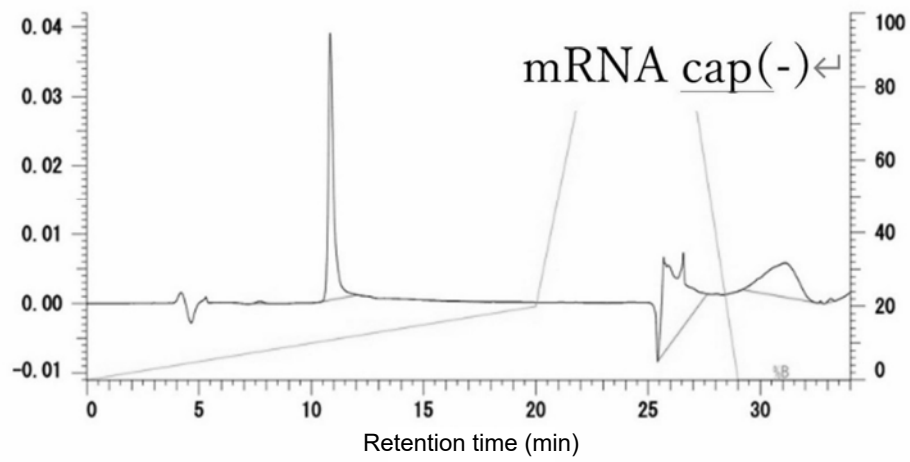


Fig. 26

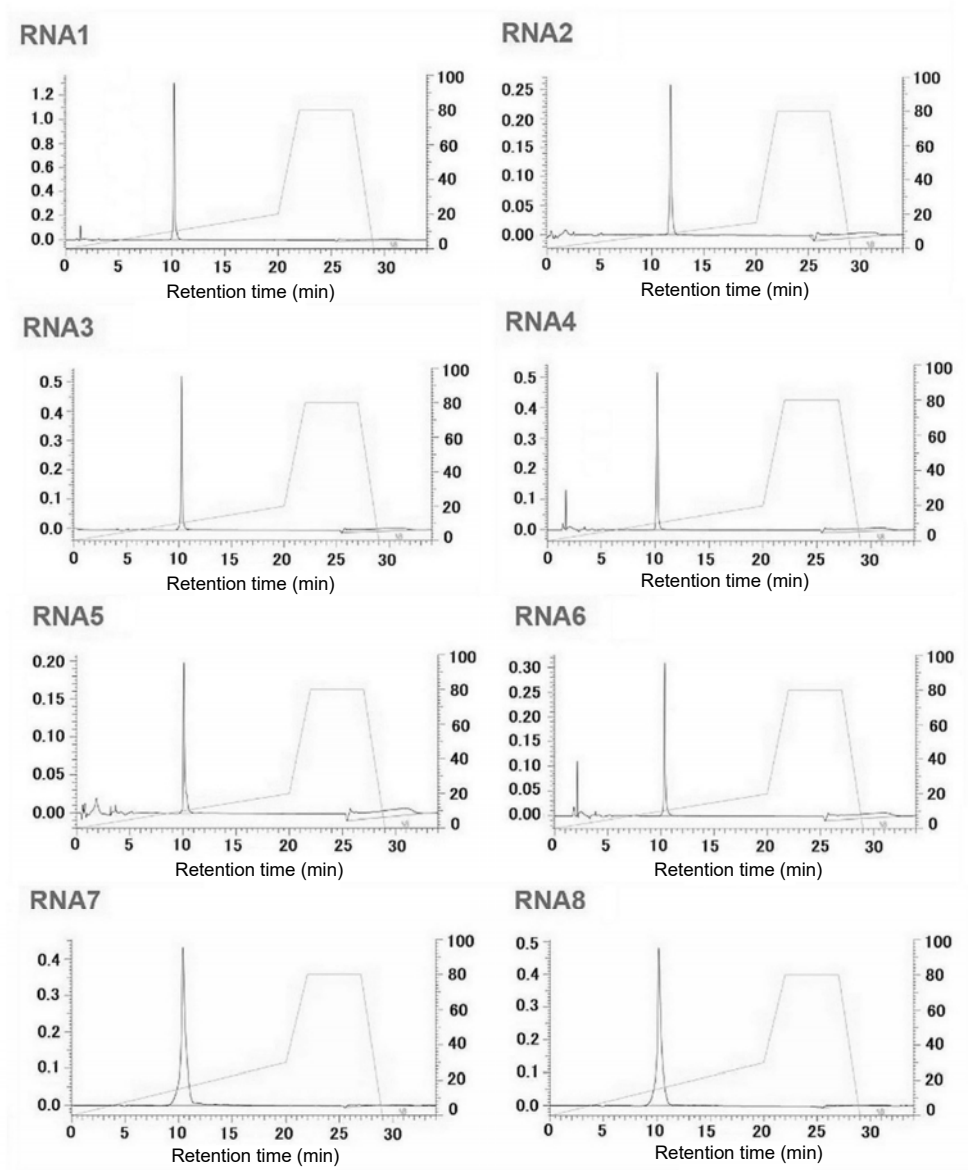


Fig. 27

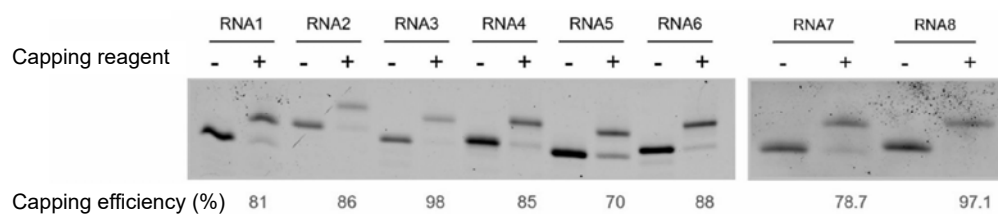


Fig. 28

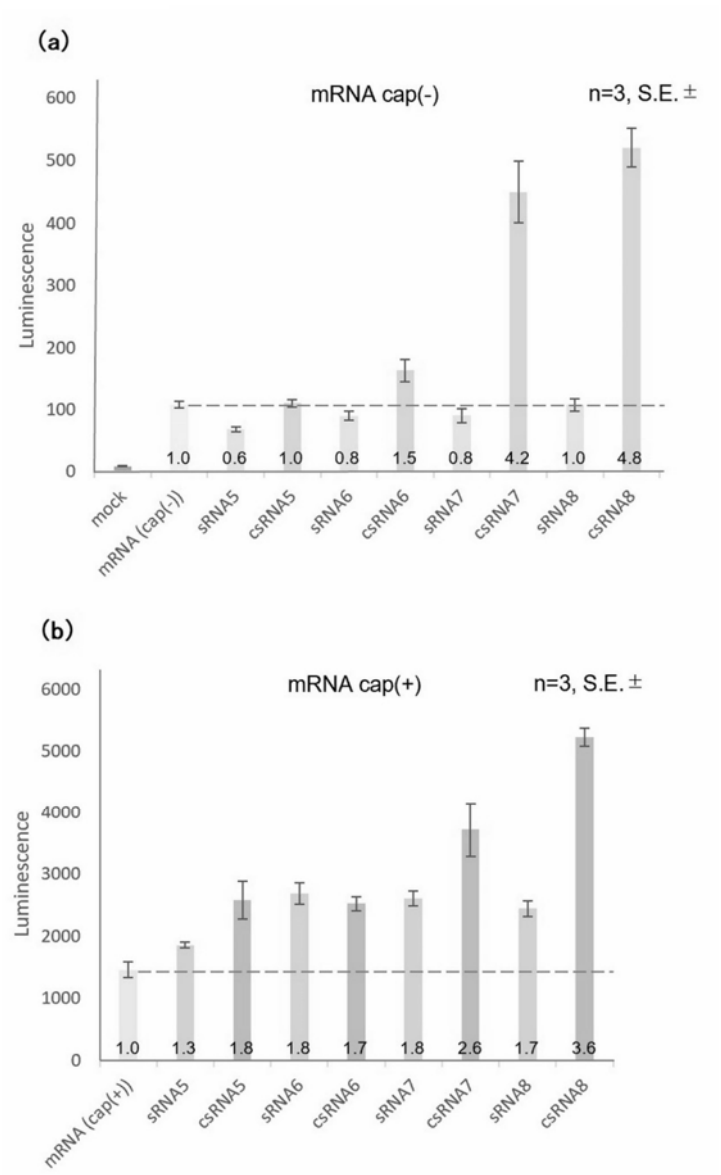


Fig. 29

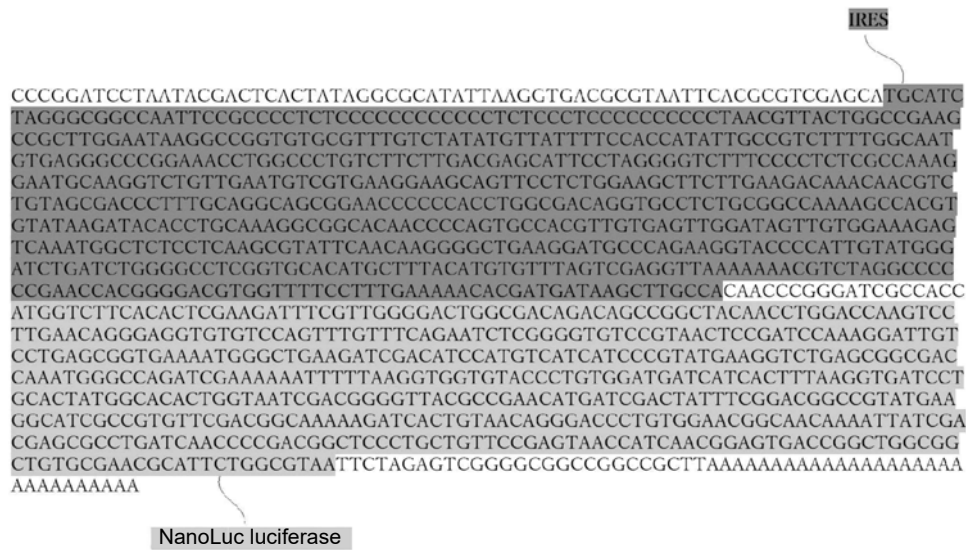


Fig. 30

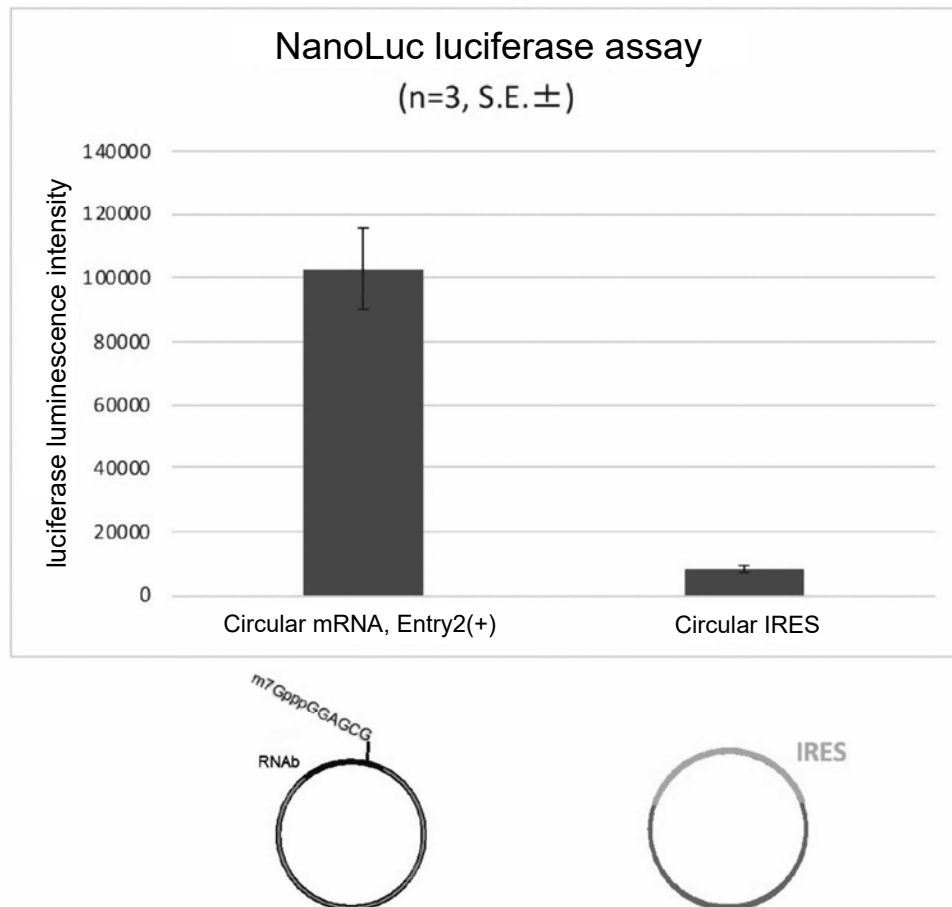


Fig. 31

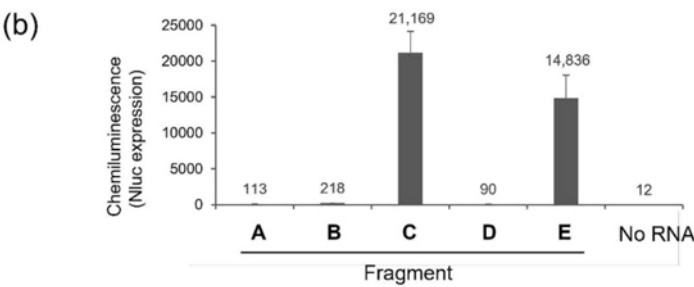
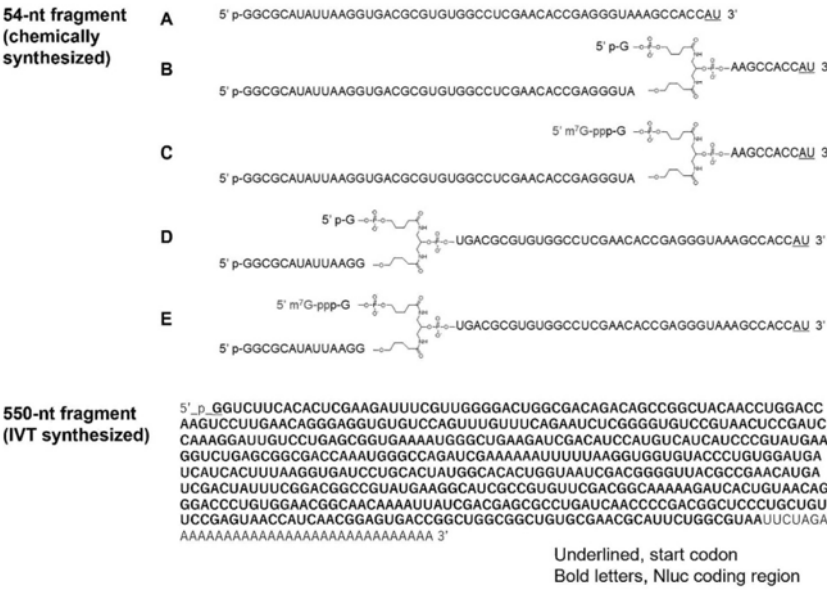
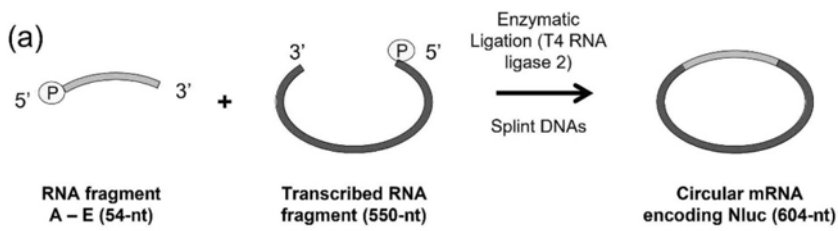


Fig. 32

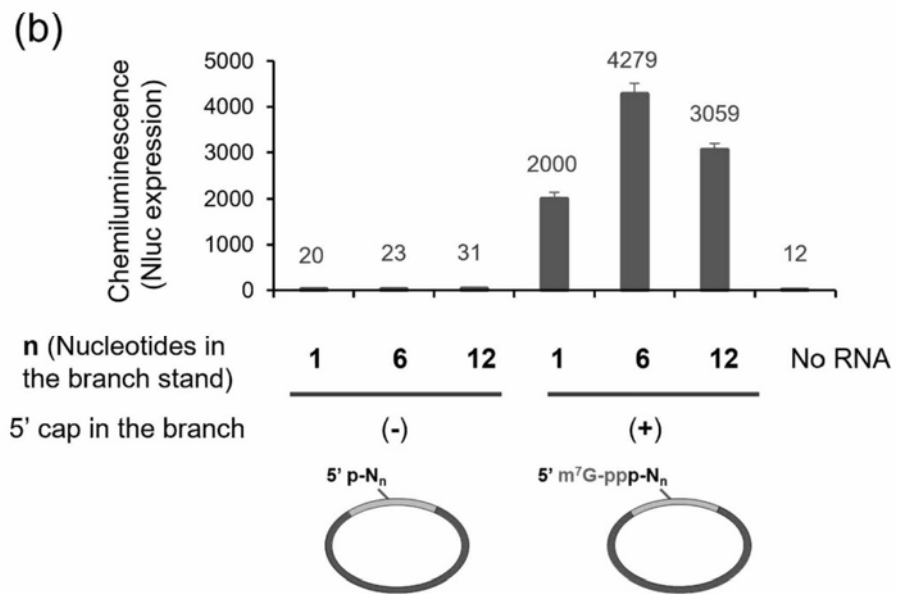
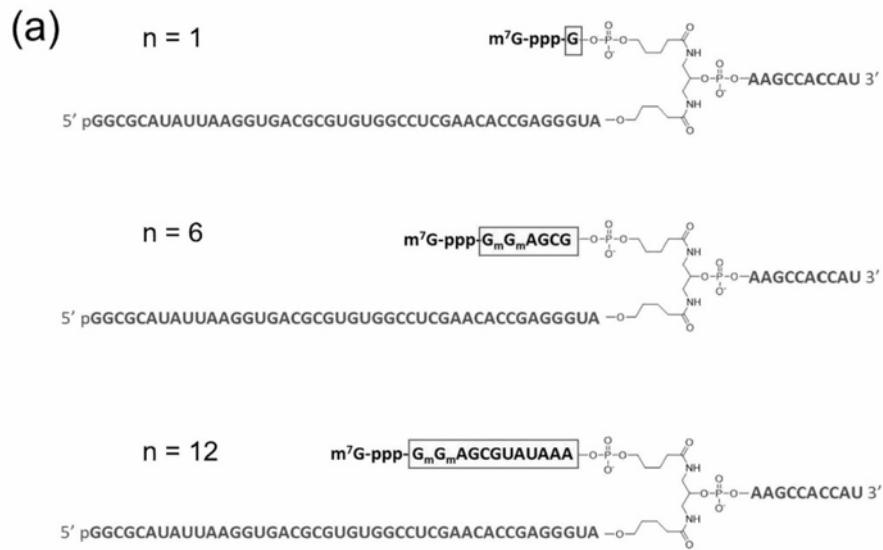


Fig. 33

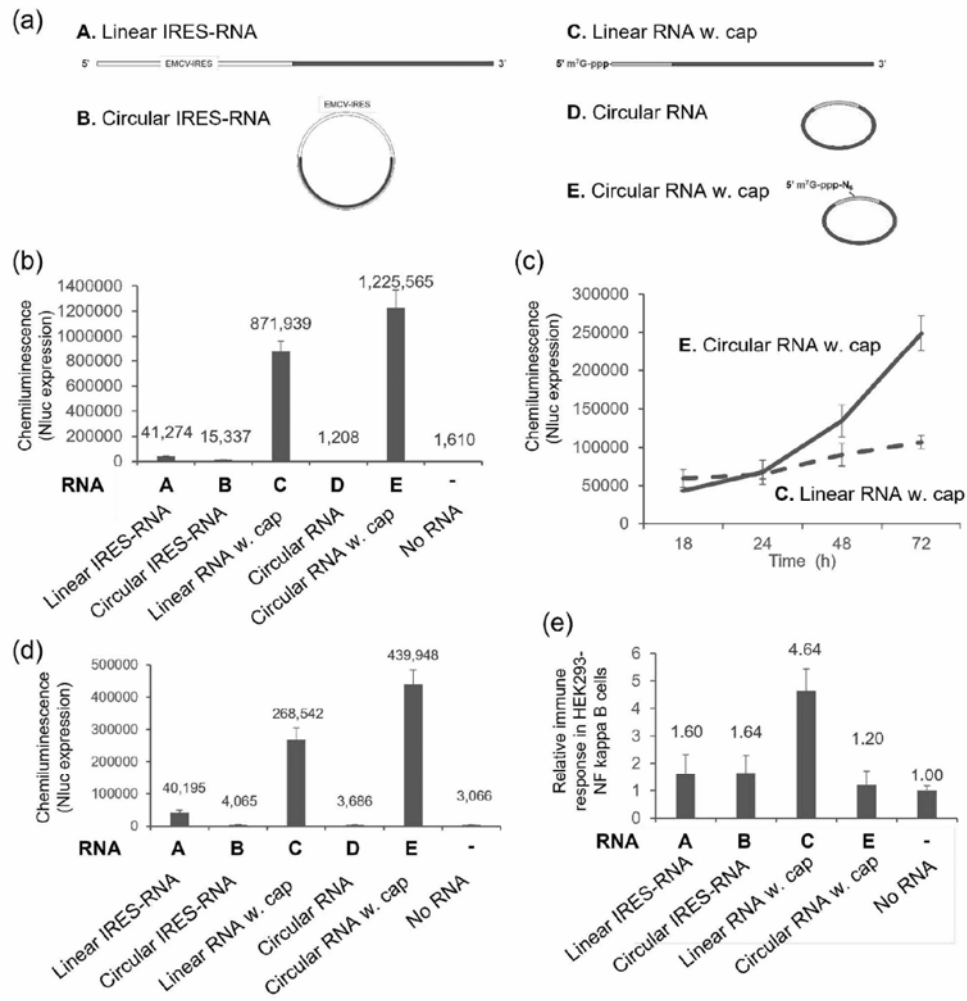
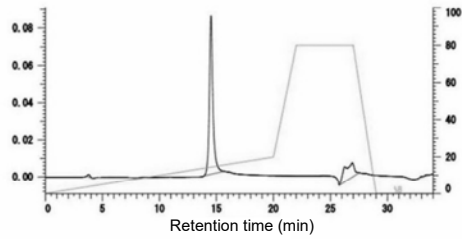
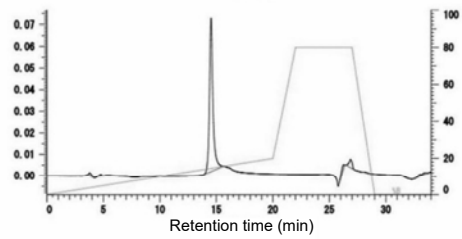


Fig. 34

Analytical HPLC after isolationATF4-Renilla mRNA cap (+)

HPLC: HITACHI NEW/NEW2
Loop size: 1 mL
HPLC column: Hydrosphere C18 (HS12S05-2546WT, serial#112YA90228) 250 × 4.6 mmI.D., S-5 µm, 12 nm
Solvent: A: 50mM TEAA (pH 7.0), 5% CH₃CN; B: CH₃CN
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 mmI.D., S-5 µm, 12 nm
Solvent: A: 50mM TEAA (pH 7.0), 5% CH₃CN; B: CH₃CN
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

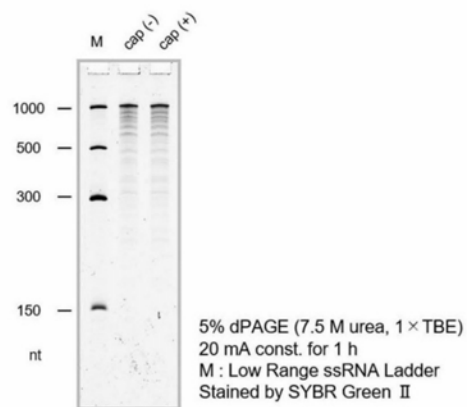
denaturing PAGE

Fig. 35

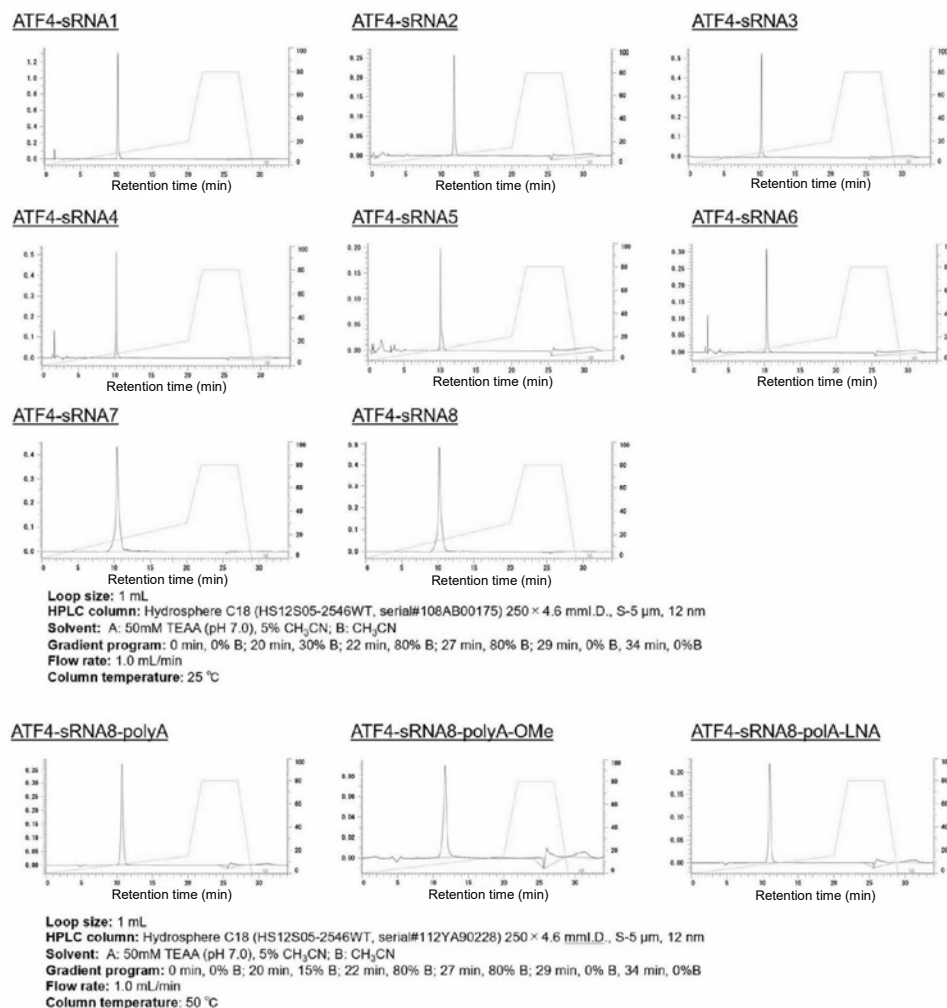
Analytical HPLC after isolation

Fig. 36

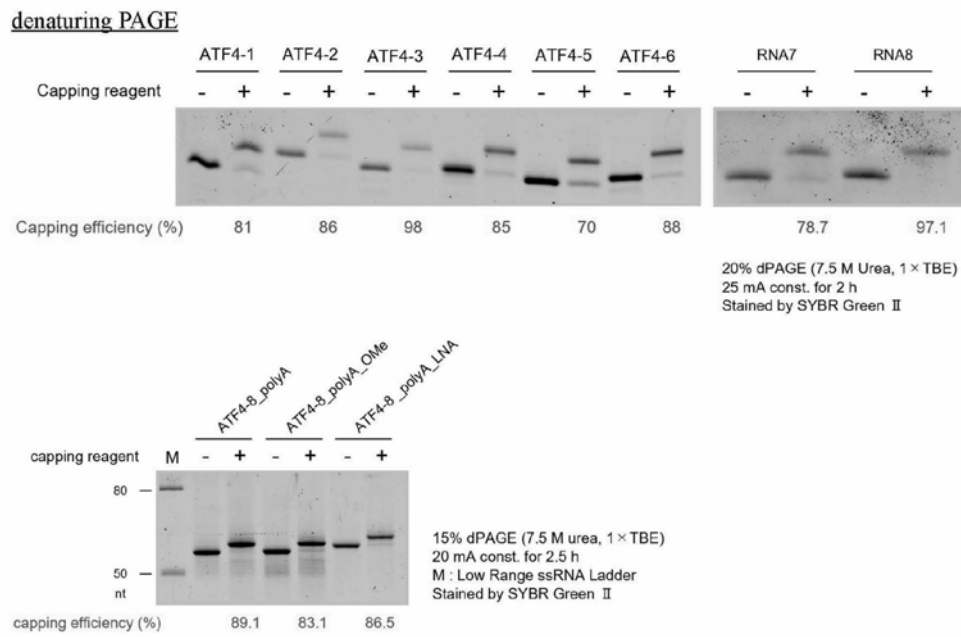
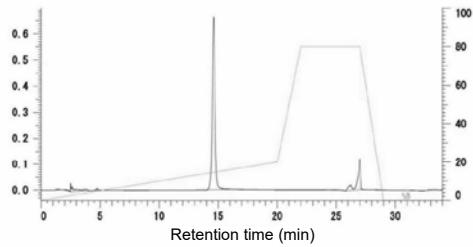


Fig. 37

Analytical HPLC after isolation

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% CH₃CN; B: CH₃CN
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

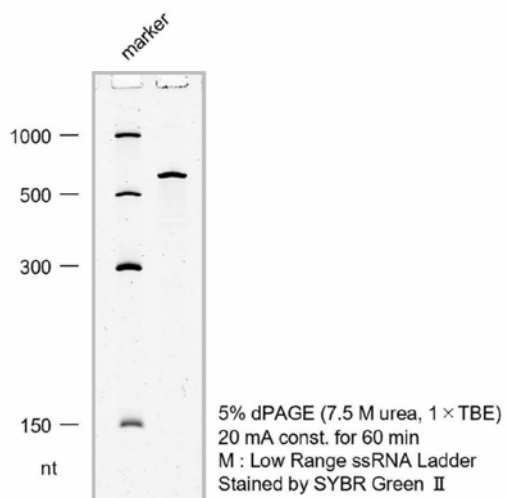
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Fig. 38

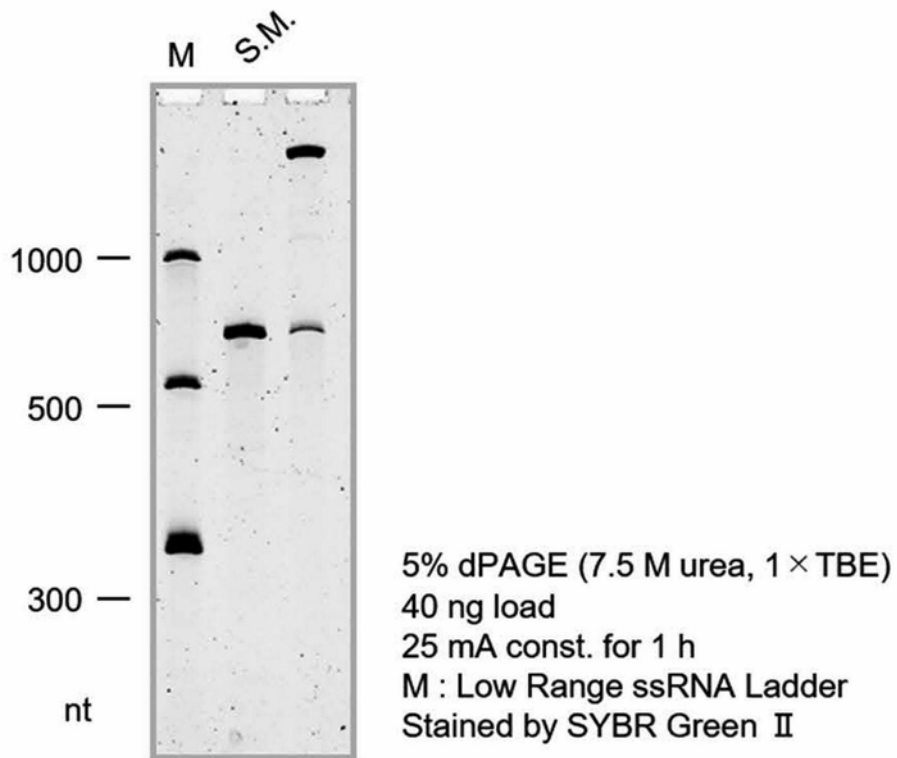
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Fig. 39

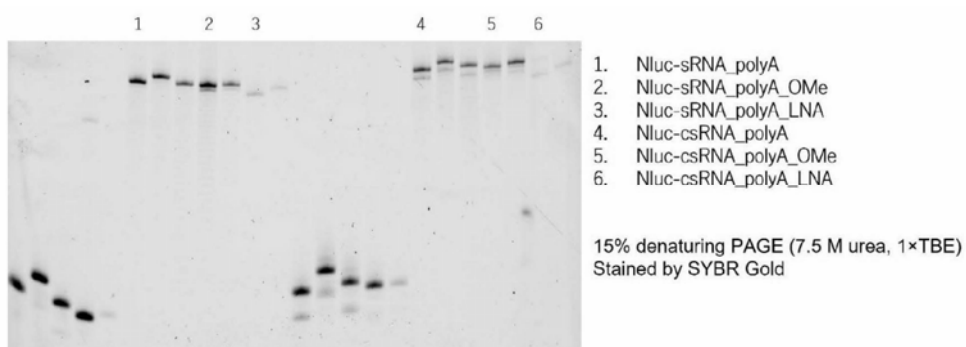
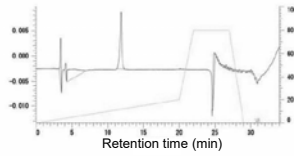


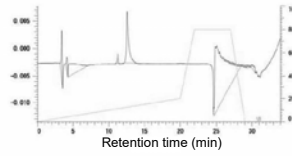
Fig. 40

Analytical HPLC after isolation

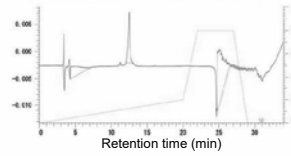
Nluc-sRNA_natural



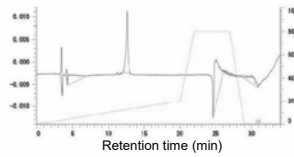
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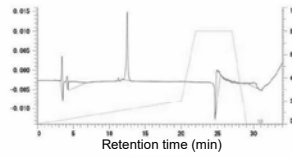
Nluc-sRNA_LNA_5/20_3' type



Nluc-sRNA_LNA_10/20 type



Nluc-sRNA_LNA_5/20 type



Nluc-sRNA_LNA_6/20 type

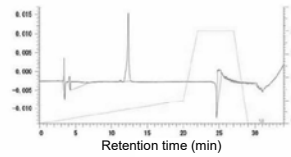
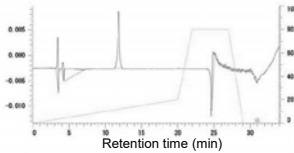
**HPLC:** HITACH NEW2 Loop size: 1 mL**HPLC column:** Hydrosphere C18 (HS12S05-2546WT, serial#125XB20166) 250 × 4.6 mmI.D., S-5 μm, 12 nm**Solvent:** A: 50mM TEAA (pH 7.0), 5% CH₃CN; B: CH₃CN**Gradient program:** 0 min, 0% B; 20 min, 20% B; 22 min, 80% B; 27 min, 80% B; 29 min, 0% B; 34 min, 0% B**Flow rate:** 1.0 mL/min **Column temperature:** 50 °C**Injection volume and solution condition:** 40 μL (MQ) **Injection amount:** 16 pmol

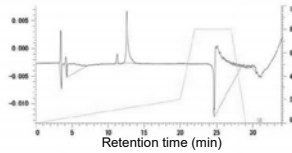
Fig. 41

Analytical HPLC after isolation

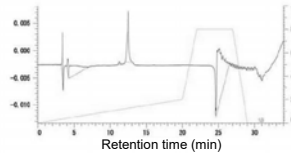
Nluc-csRNA_natural



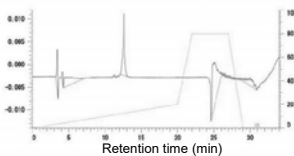
Nluc-csRNA_LNA_10/20_3' type



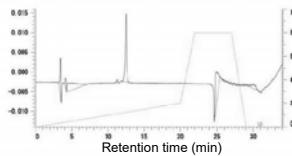
Nluc-csRNA_LNA_5/20_3' type



Nluc-csRNA_LNA_10/20 type



Nluc-csRNA_LNA_5/20 type



Nluc-csRNA_LNA_6/20 type

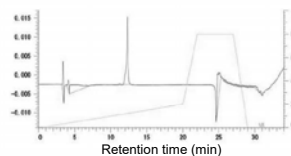
**HPLC:** HITACH NEW2 Loop size: 1 mL**HPLC column:** Hydrosphere C18 (HS12S05-2546WT, serial#125XB20166) 250 × 4.6 mmI.D., S-5 μm, 12 nm**Solvent:** A: 50mM TEAA (pH 7.0), 5% CH₃CN; B: CH₃CN**Gradient program:** 0 min, 0% B; 20 min, 20% B; 22 min, 80% B; 27 min, 80% B; 29 min, 0% B; 34 min, 0% B**Flow rate:** 1.0 mL/min **Column temperature:** 50 °C**Injection volume and solution condition:** 40 μL (MQ) **Injection amount:** 16 pmol

Fig. 42

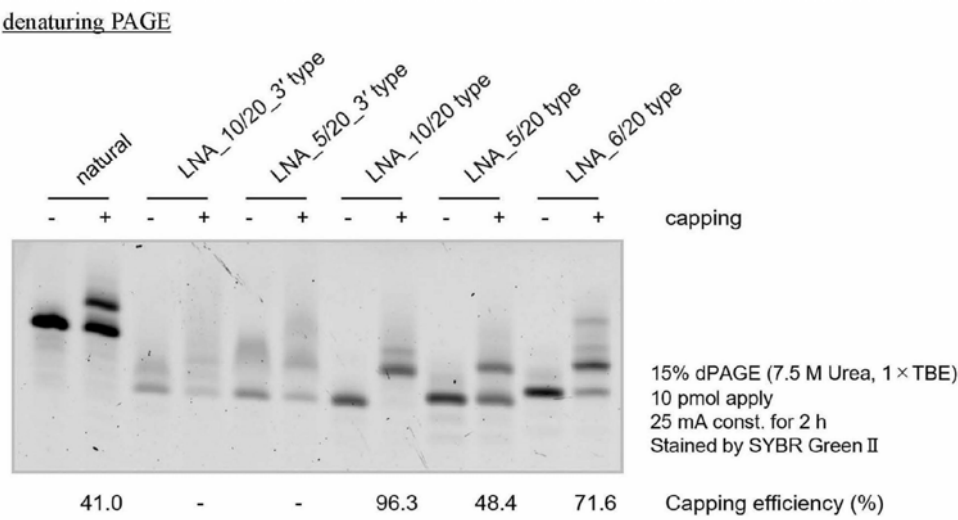


Fig. 43

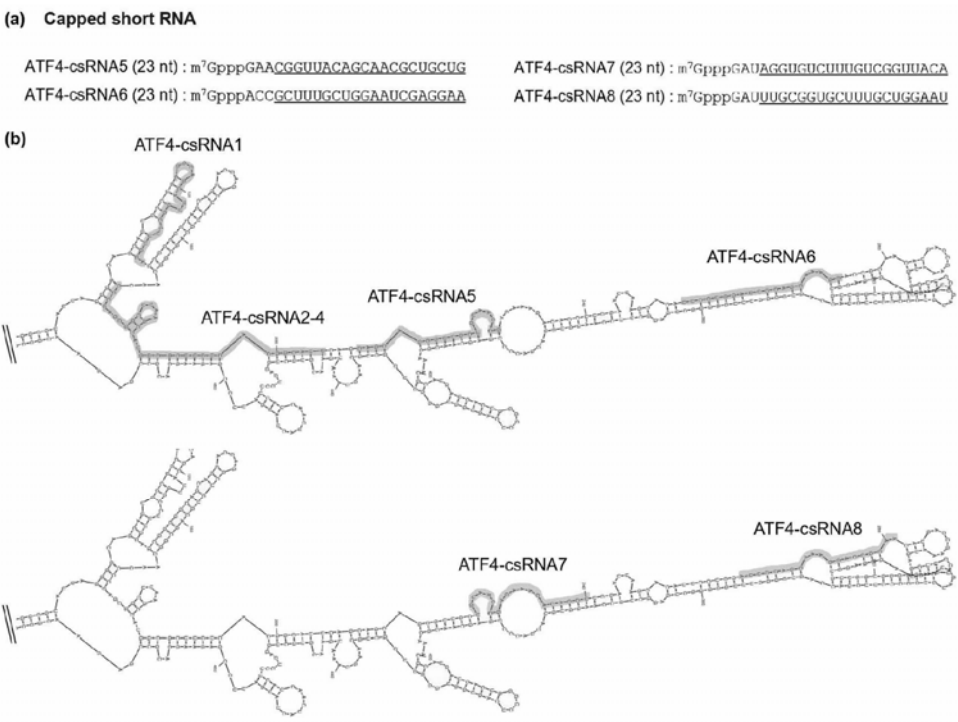


Fig. 44

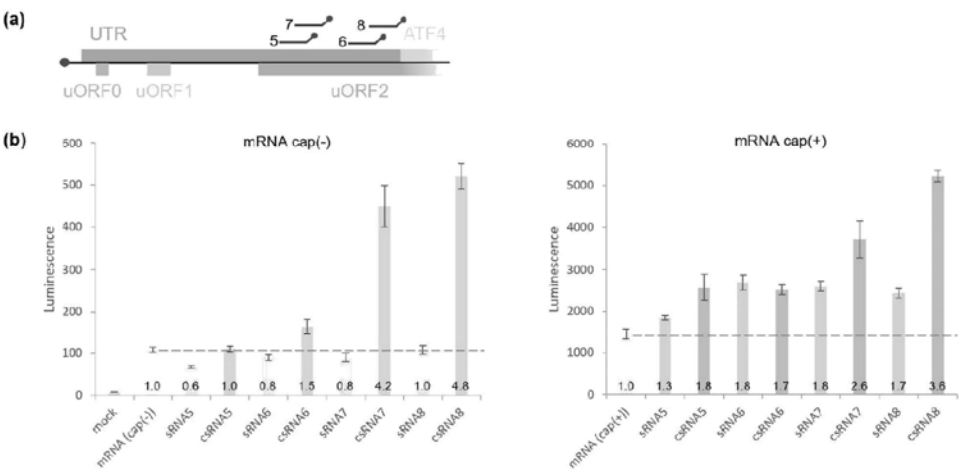


Fig. 45

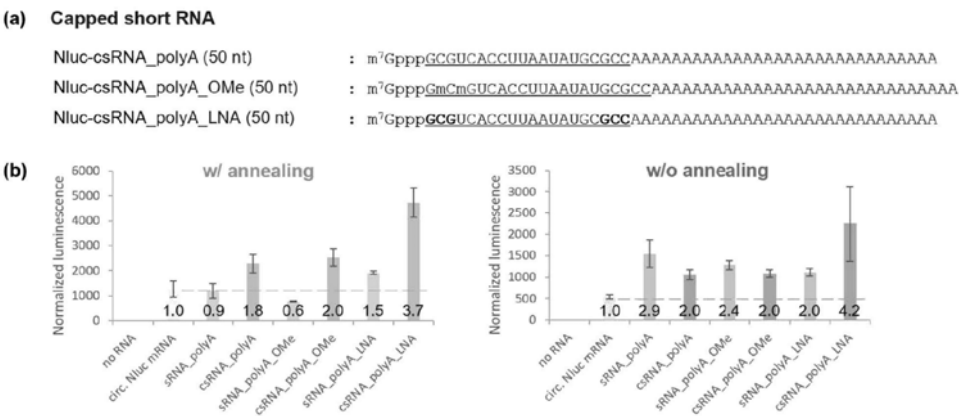


Fig. 46

(a) Capped short RNA

natural (25 nt)	: m ⁷ Gppp-GAGCGGCGUCACCUUAAUAUGCGCC
Nluc-csRNA_LNA_10/20_3' type (25 nt)	: m ⁷ Gppp-GAGCGGCGUCACCUUAAUAUGCGCC
Nluc-csRNA_LNA_5/20_3' type (25 nt)	: m ⁷ Gppp-GAGCGGCGUCACCUUAAUAUGCGCC
Nluc-csRNA_LNA_10/20 type (25 nt)	: m ⁷ Gppp-GAGCGGCGUCACCUUAAUAUGCGCC
Nluc-csRNA_LNA_5/20 type (25 nt)	: m ⁷ Gppp-GAGCGGCGUCACCUUAAUAUGCGCC
Nluc-csRNA_LNA_6/20 type (25 nt)	: m ⁷ Gppp-GAGCGGCGUCACCUUAAUAUGCGCC

(b)