FIG. 1

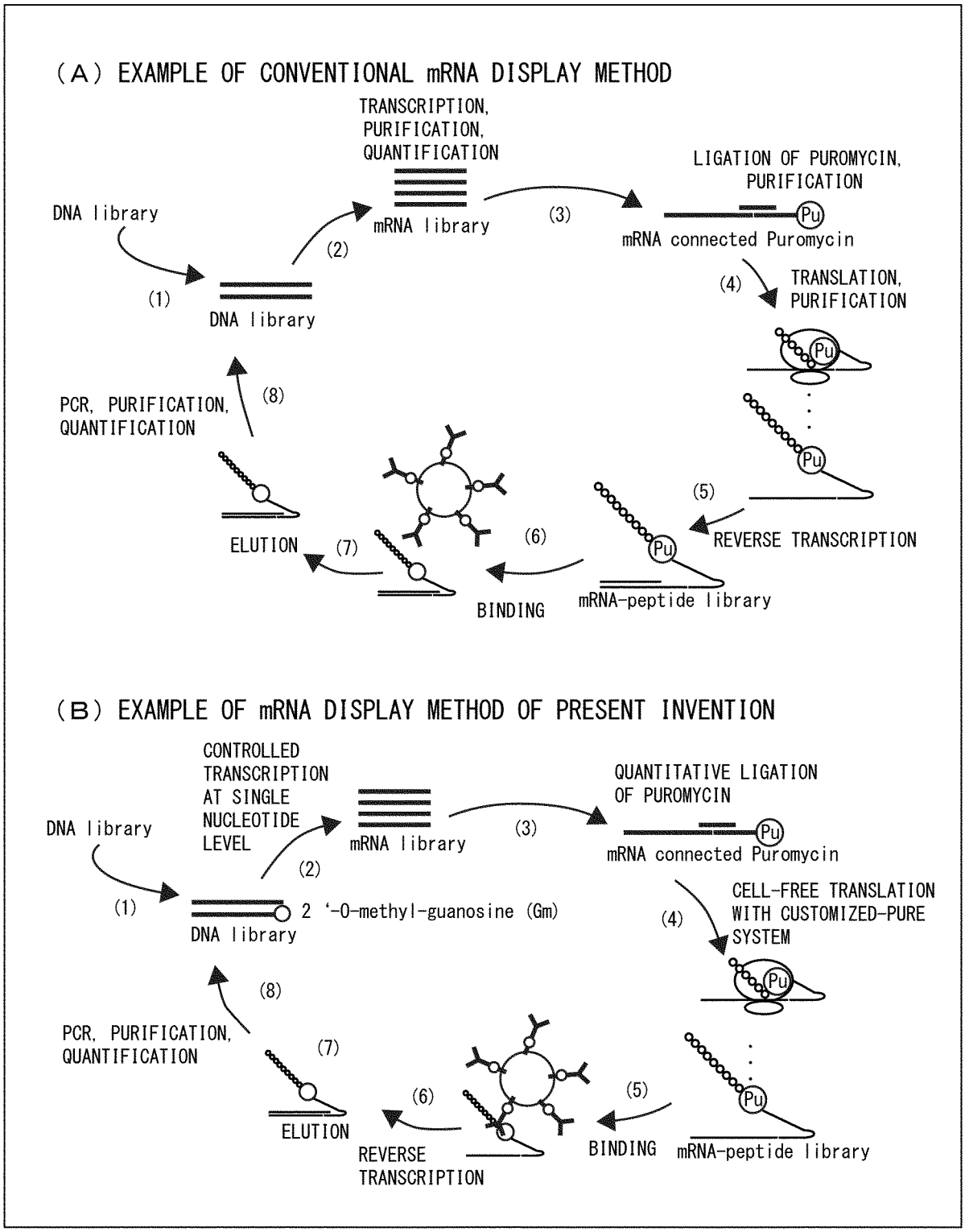
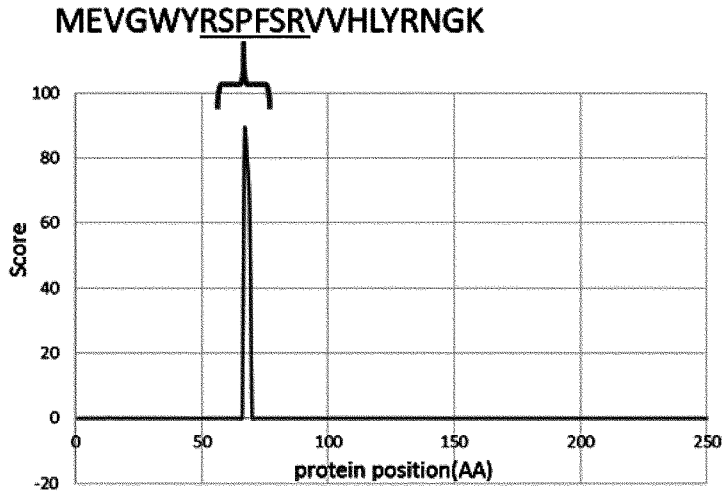

(A) EXAMPLE OF CONVENTIONAL mRNA DISPLAY METHOD
TRANSCRIPTION, PURIFICATION, QUANTIFICATION
LIGATION OF PUROMYCIN, PURIFICATION
DNA library
mRNA library
mRNA connected Puromycin
(1)
(2)
(3)
(4)
TRANSLATION, PURIFICATION
DNA library
Pu
PCR, PURIFICATION, QUANTIFICATION
(8)
(5)
REVERSE TRANSCRIPTION
ELUTION
(7)
(6)
BINDING
mRNA-peptide library
(B) EXAMPLE OF mRNA DISPLAY METHOD OF PRESENT INVENTION
CONTROLLED TRANSCRIPTION AT SINGLE NUCLEOTIDE LEVEL
QUANTITATIVE LIGATION OF PUROMYCIN
DNA library
mRNA library
mRNA connected Puromycin
(1)
(2)
(3)
2 '-O-methyl-guanosine (Gm)
DNA library
CELL-FREE TRANSLATION WITH CUSTOMIZED-PURE SYSTEM
(4)
Pu
(8)
PCR, PURIFICATION, QUANTIFICATION
(7)
(6)
(5)
ELUTION
REVERSE TRANSCRIPTION
BINDING
mRNA-peptide library

FIG. 2

(A)

| Rank | Sequence | Position | Score |
|---|---|---|---|
| 1 | K S P F S R L T | 68 | 63.646 |
| 2 | R S P F N R _ T | 68 | 12.659 |
| 3 | Y S S P F N R K | 67 | 8.382 |
| 4 | H R S P F N R K | 67 | 1.947 |
| 5 | I S E Y K L R L | 103 | 0.789 |
| 6 | K S P F S R L T | 68 | 0.252 |
| 7 | S P F S R L T C | 69 | 0.252 |
| 8 | Y R S P F N R K | 67 | 0.163 |
| 9 | K S P F S R L T | 68 | 0.126 |
| 10 | S P F S R L T S | 69 | 0.126 |

(B)




(C)



FIG. 3

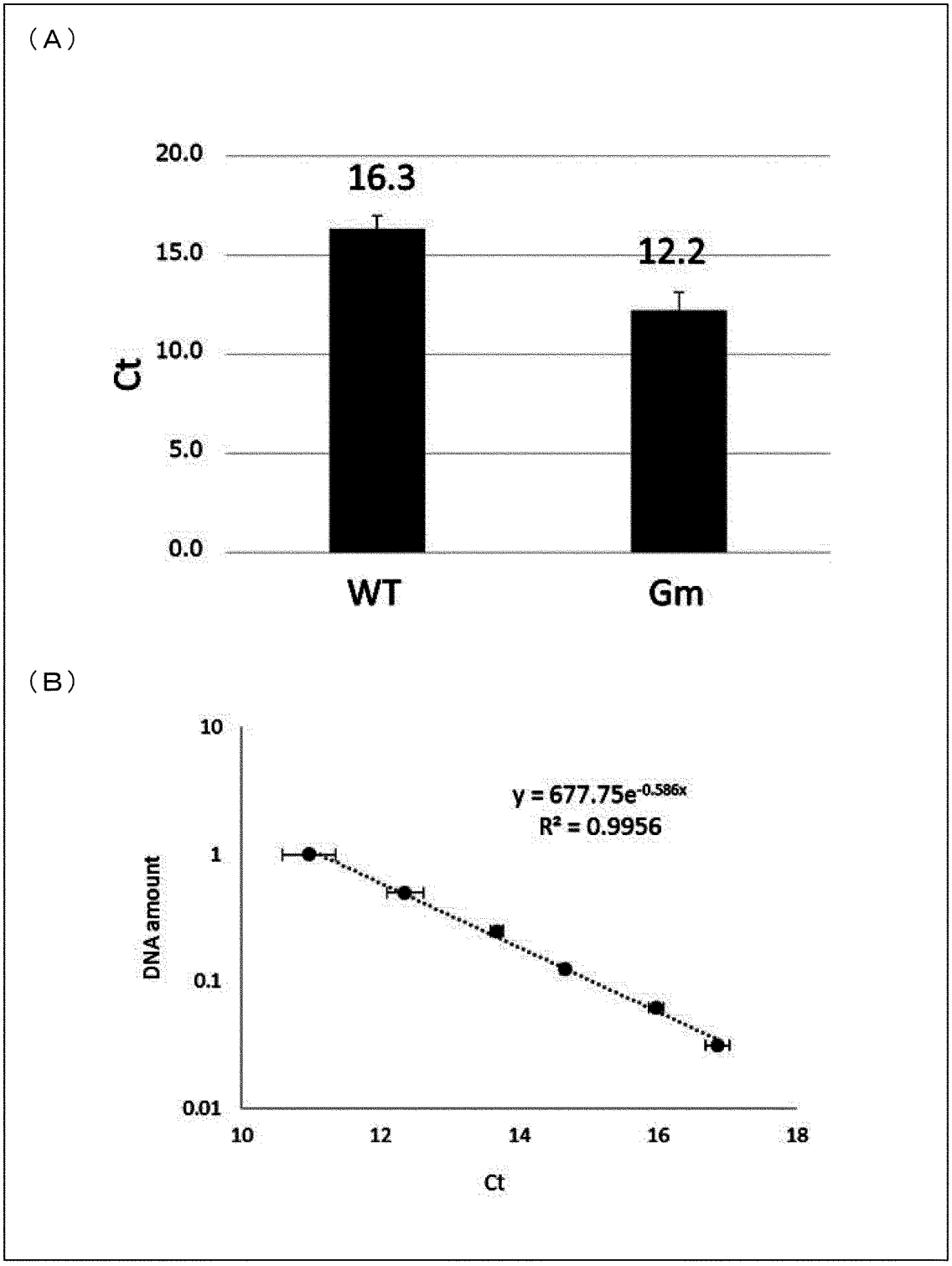

(A)
20.0
15.0
10.0
5.0
0.0
Ct
16.3
12.2
WT
Gm
(B)
10
1
0.1
0.01
DNA amount
y = 677.75e-0.586x
R² = 0.9956
10
12
14
16
18
Ct

FIG. 4

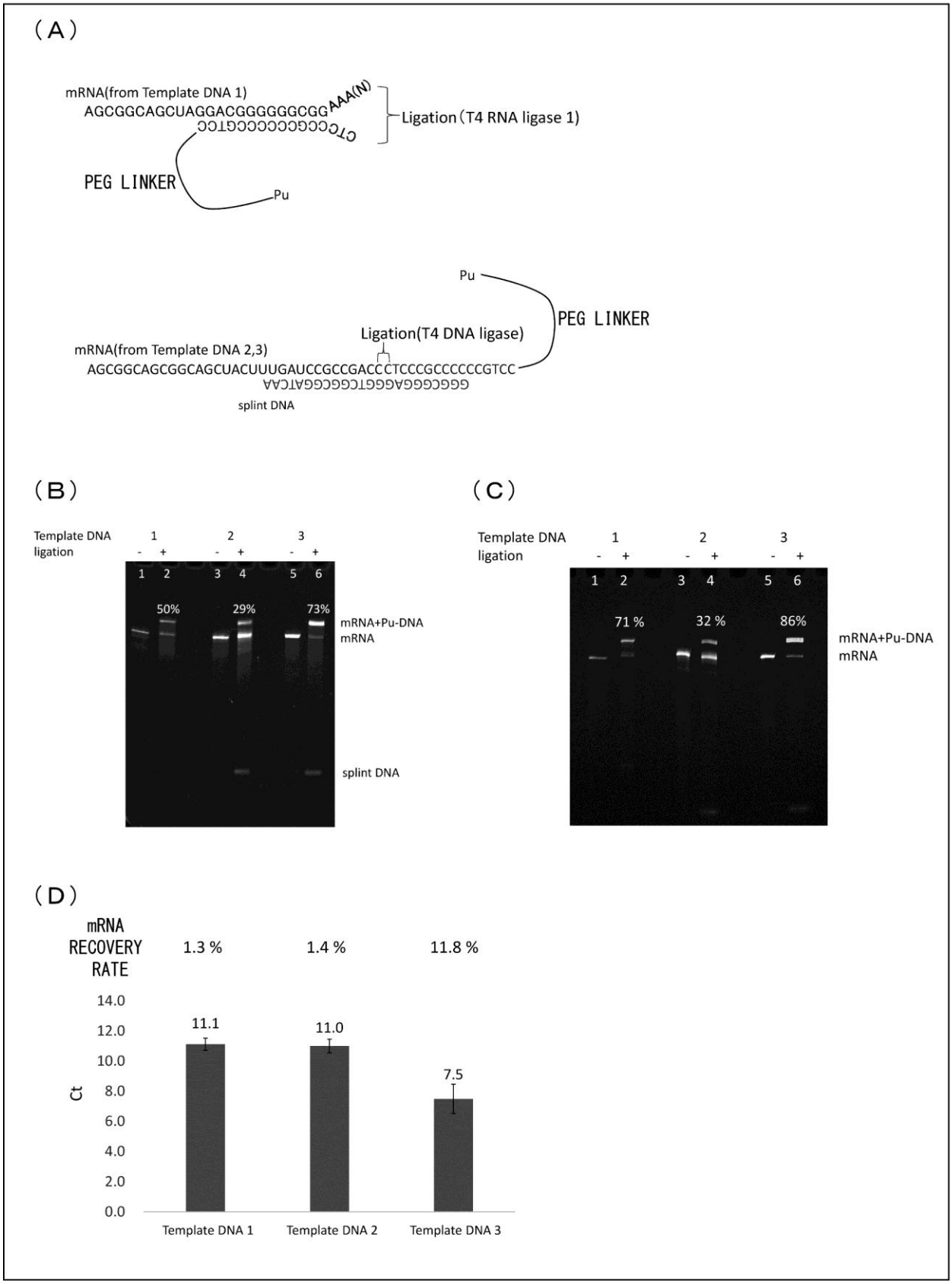

(A)
mRNA(from Template DNA 1)
AGCGGCAGCUAGGACGGGGGGCGGAAA(N)
CTCCGCCCCCCGTCC
Ligation (T4 RNA ligase 1)
PEG LINKER
Pu
Pu
PEG LINKER
Ligation(T4 DNA ligase)
mRNA(from Template DNA 2,3)
AGCGGCAGCGGCAGCUACUUUGAUCCGCCGACCCTCCCGCCCCCCGTCC
GGGCGGGAGGGTCGGCGGATCAA
splint DNA
(B)
Template DNA
ligation
1 2 3
- + - + - +
1 2 3 4 5 6
50% 29% 73%
mRNA+Pu-DNA
mRNA
splint DNA
(C)
Template DNA
ligation
1 2 3
- + - + - +
1 2 3 4 5 6
71 % 32 % 86%
mRNA+Pu-DNA
mRNA
(D)
mRNA
RECOVERY
RATE
1.3 % 1.4 % 11.8 %
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
Ct
11.1
11.0
7.5
Template DNA 1
Template DNA 2
Template DNA 3

FIG. 5

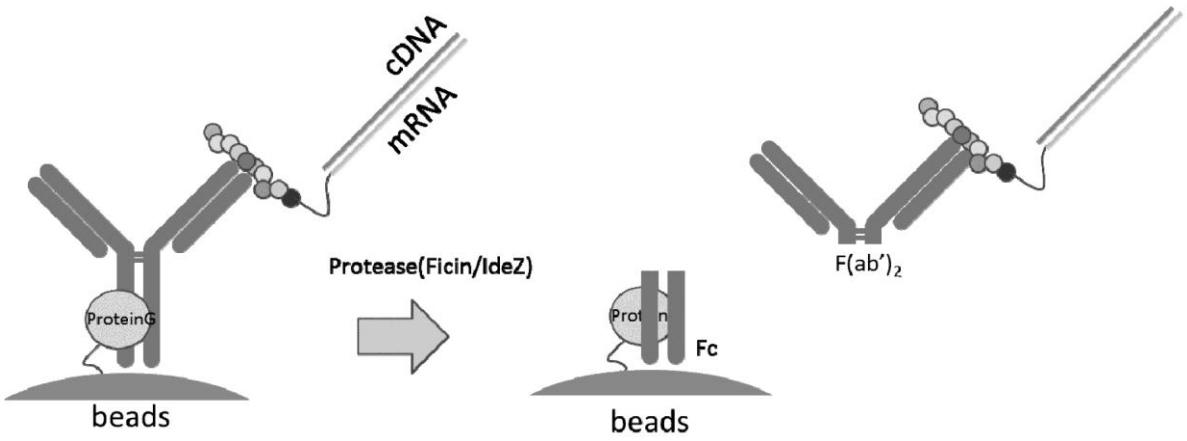

cDNA
mRNA
ProteinG
beads
Protease(Ficin/IdeZ)
Fc
beads
F(ab')2

FIG. 6

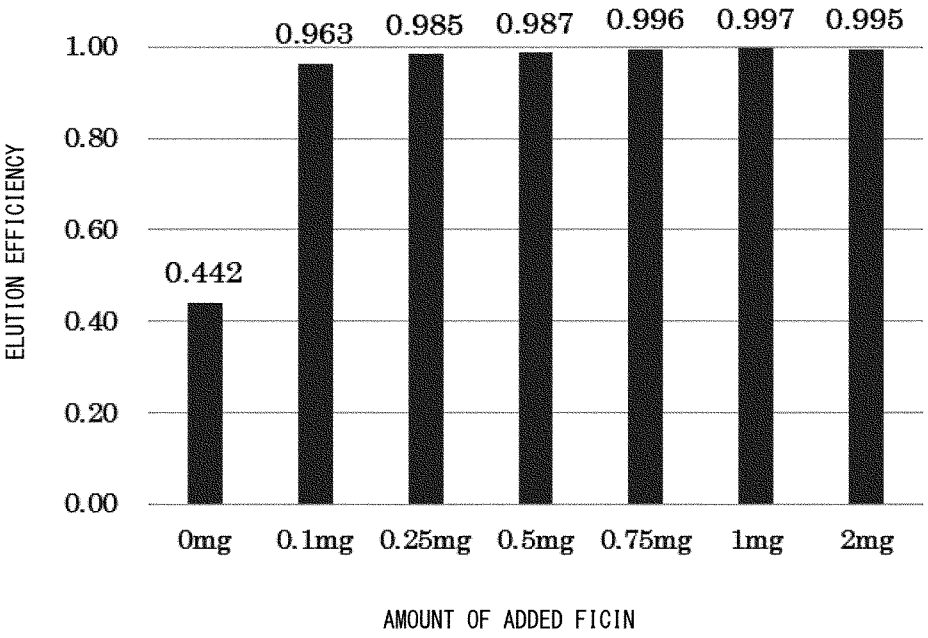

ELUTION EFFICIENCY
1.00
0.80
0.60
0.40
0.20
0.00
0.442
0.963
0.985
0.987
0.996
0.997
0.995
0mg
0.1mg
0.25mg
0.5mg
0.75mg
1mg
2mg
AMOUNT OF ADDED FICIN

FIG. 7

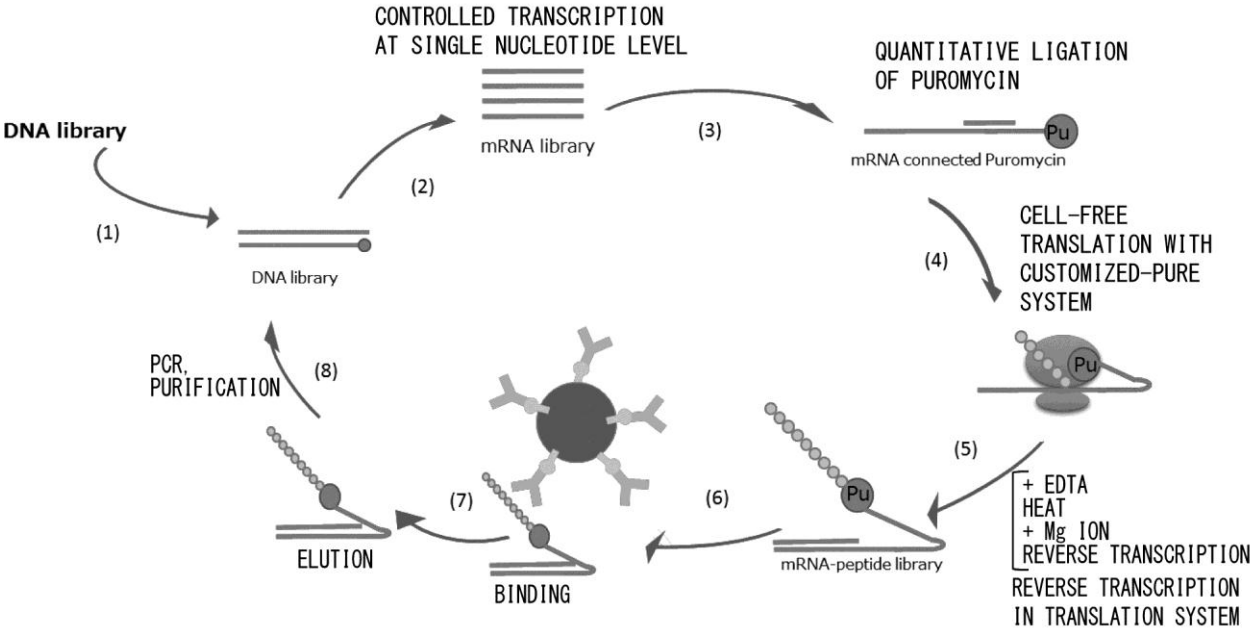
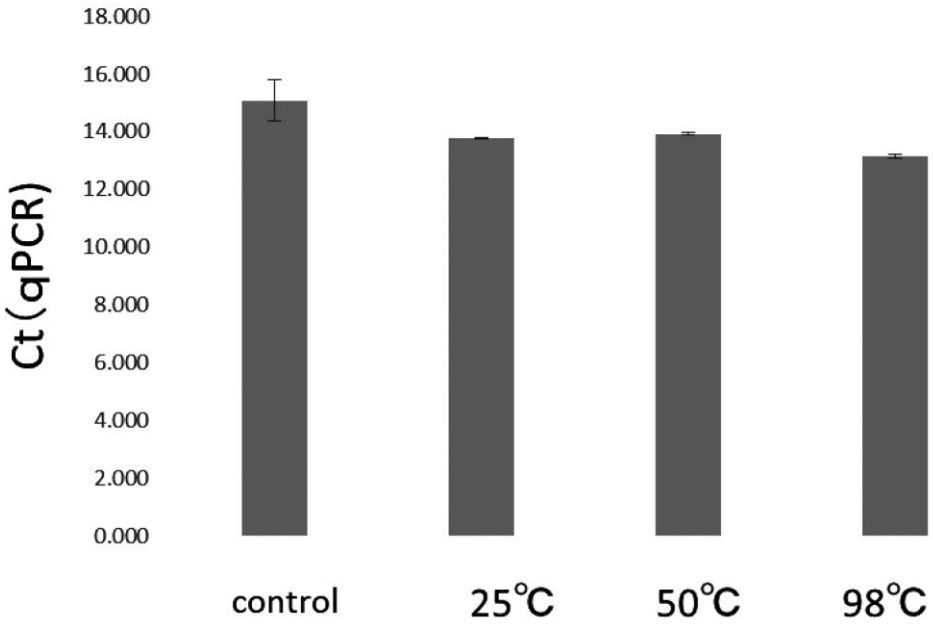

CONTROLLED TRANSCRIPTION
AT SINGLE NUCLEOTIDE LEVEL
QUANTITATIVE LIGATION
OF PUROMYCIN
DNA library
mRNA library
mRNA connected Puromycin
Pu
(1)
(2)
(3)
(4)
DNA library
CELL-FREE
TRANSLATION WITH
CUSTOMIZED-PURE
SYSTEM
PCR,
PURIFICATION
(8)
(5)
+ EDTA
HEAT
+ Mg ION
REVERSE TRANSCRIPTION
REVERSE TRANSCRIPTION
IN TRANSLATION SYSTEM
(7)
(6)
ELUTION
BINDING
mRNA-peptide library

FIG. 8


18.000
16.000
14.000
12.000
10.000
8.000
6.000
4.000
2.000
0.000
Ct(qPCR)
control
25℃
50℃
98℃