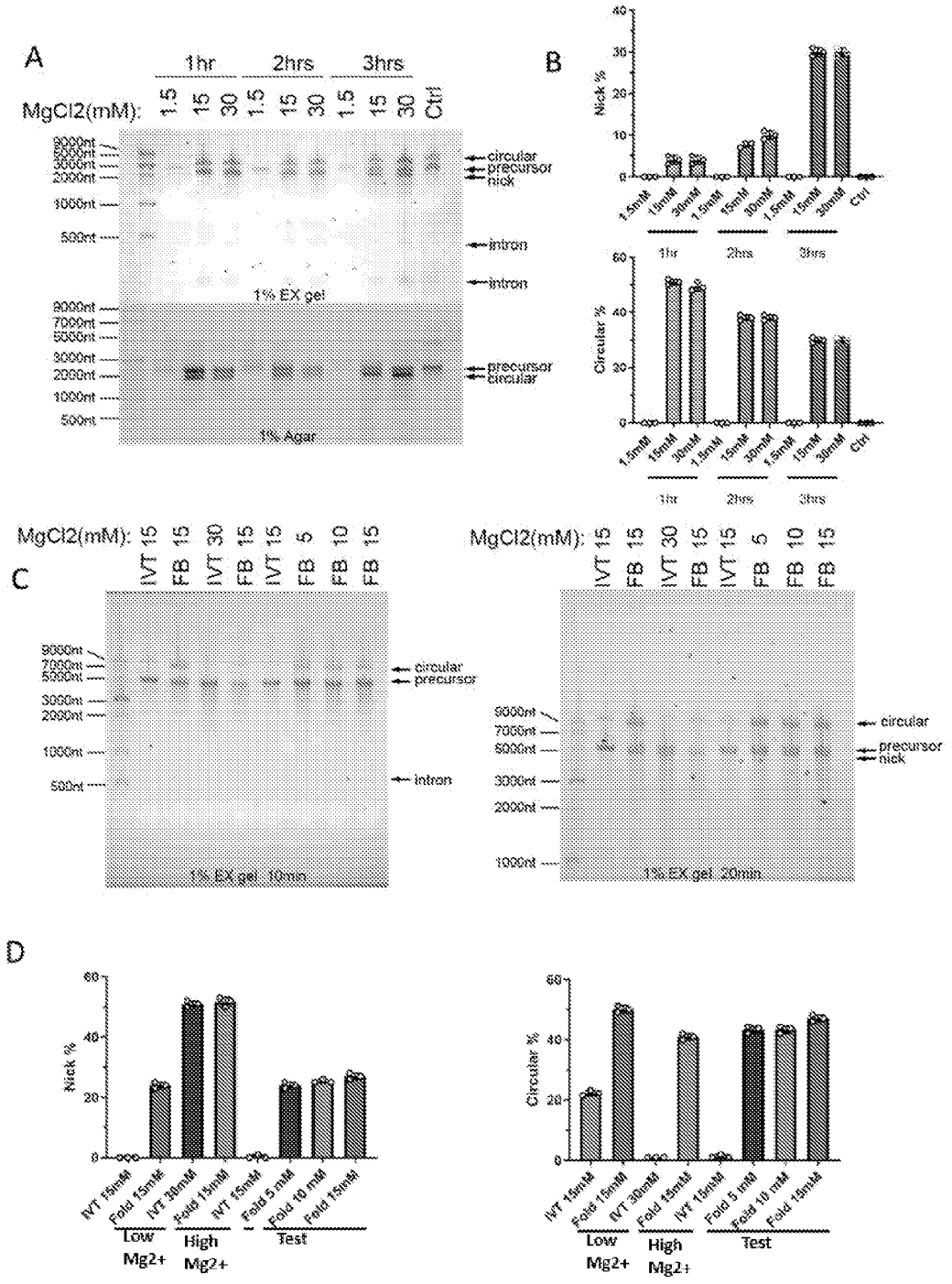

A
1hr
2hrs
3hrs
MgCl2(mM):
1.5
15
30
1.5
15
30
1.5
15
30
Ctrl
circular
precursor
nick
intron
intron
1% EX gel
precursor
circular
1% Agar
B
Nick %
Circular %
1hr
2hrs
3hrs
C
MgCl2(mM):
IVT 15
FB 15
IVT 30
FB 15
IVT 15
FB 5
FB 10
FB 15
circular
precursor
intron
1% EX gel 10min
circular
precursor
nick
1% EX gel 20min
D
Nick %
Circular %
Low Mg2+
High Mg2+
Test

**Figure 1**

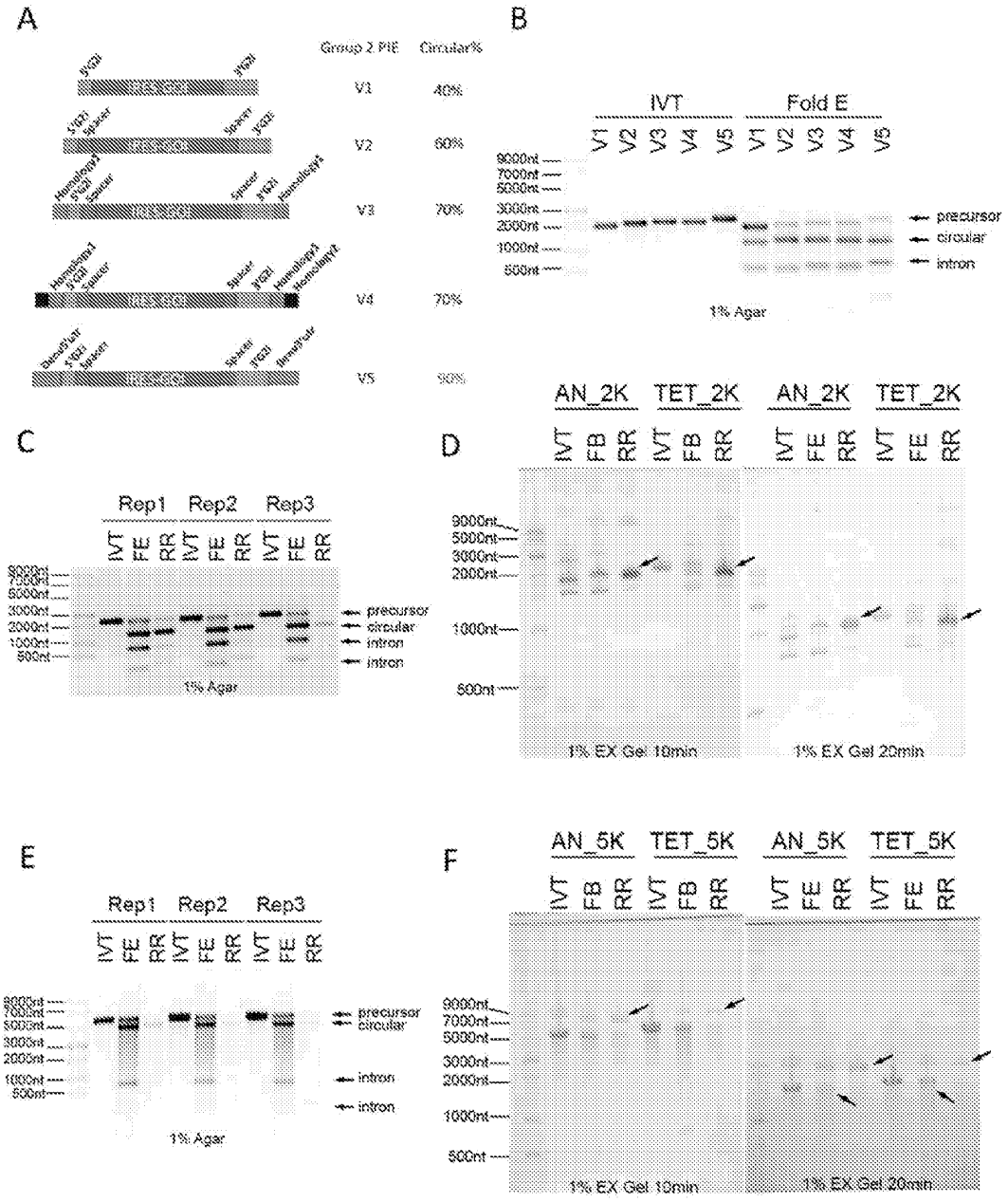

A
Group 2 PIE
Circular%
V1
40%
V2
60%
V3
70%
V4
70%
V5
90%
B
IVT
Fold E
V1 V2 V3 V4 V5 V1 V2 V3 V4 V5
9000nt
7000nt
5000nt
3000nt
2000nt
1000nt
500nt
precursor
circular
intron
1% Agar
C
Rep1 Rep2 Rep3
IVT FE RR IVT FE RR IVT FE RR
precursor
circular
intron
intron
1% Agar
D
AN_2K TET_2K AN_2K TET_2K
IVT FB RR IVT FB RR IVT FE RR IVT FE RR
1% EX Gel 10min
1% EX Gel 20min
E
Rep1 Rep2 Rep3
IVT FE RR IVT FE RR IVT FE RR
precursor
circular
intron
intron
1% Agar
F
AN_5K TET_5K AN_5K TET_5K
IVT FB RR IVT FB RR IVT FE RR IVT FE RR
1% EX Gel 10min
1% EX Gel 20min

**Figure 2**

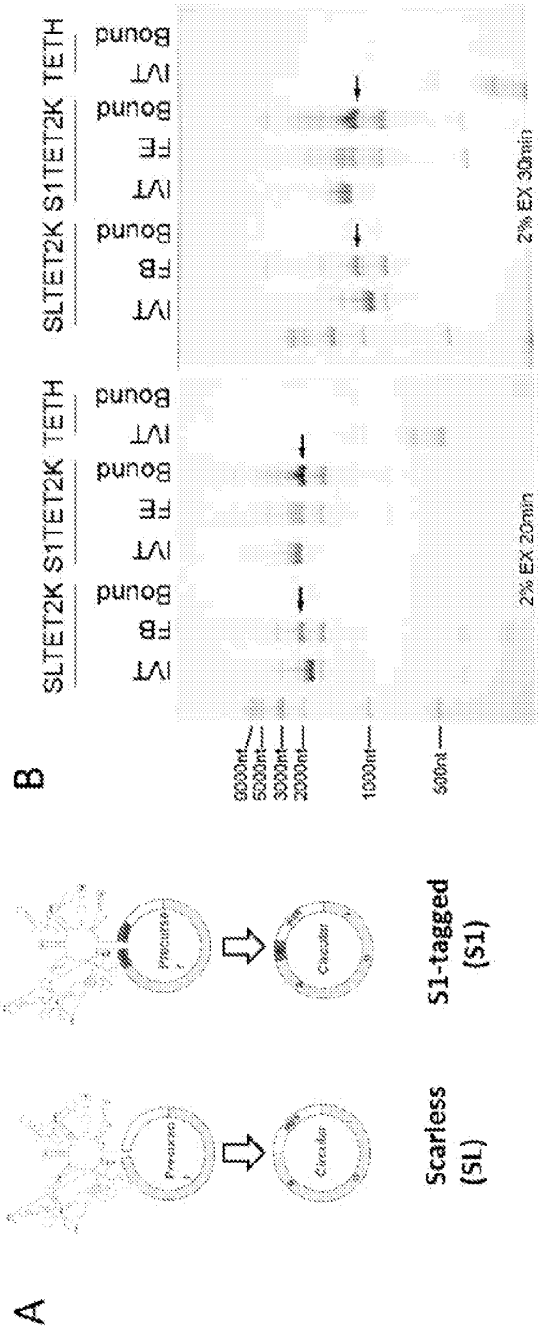



**Figure 3 (A)-(B) (continued)**

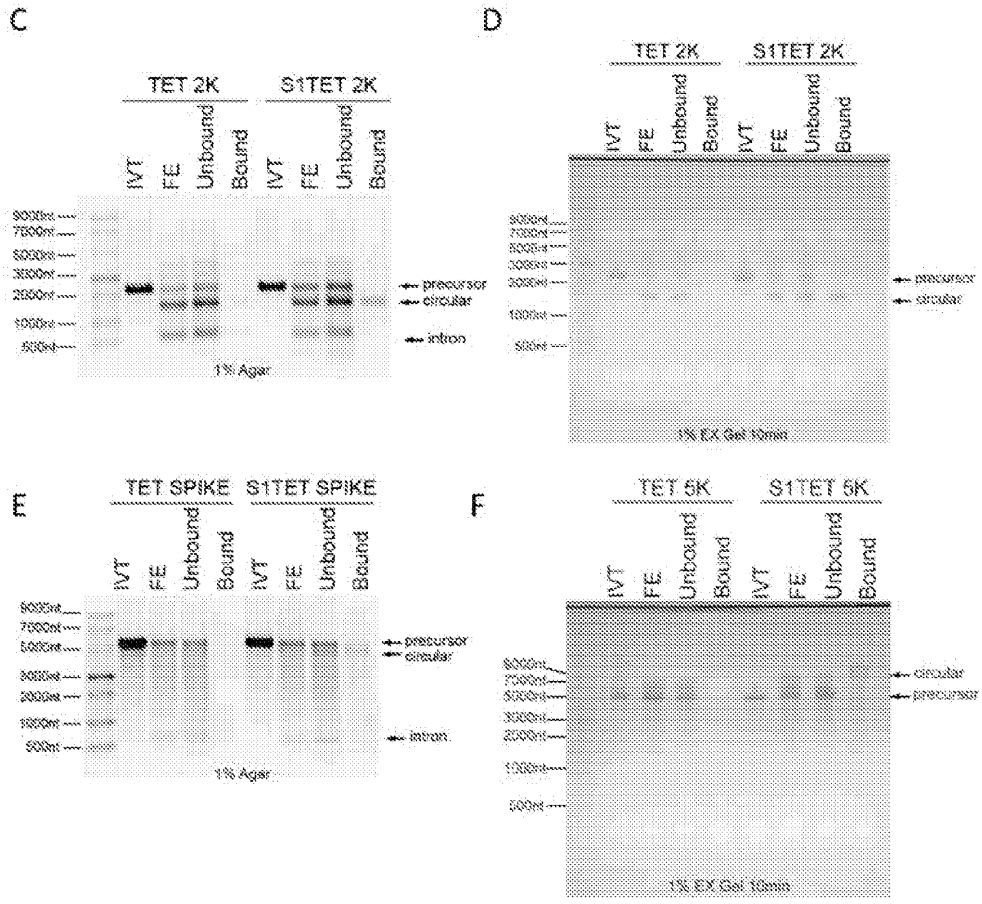



**Figure 3 (C)-(F) (end)**

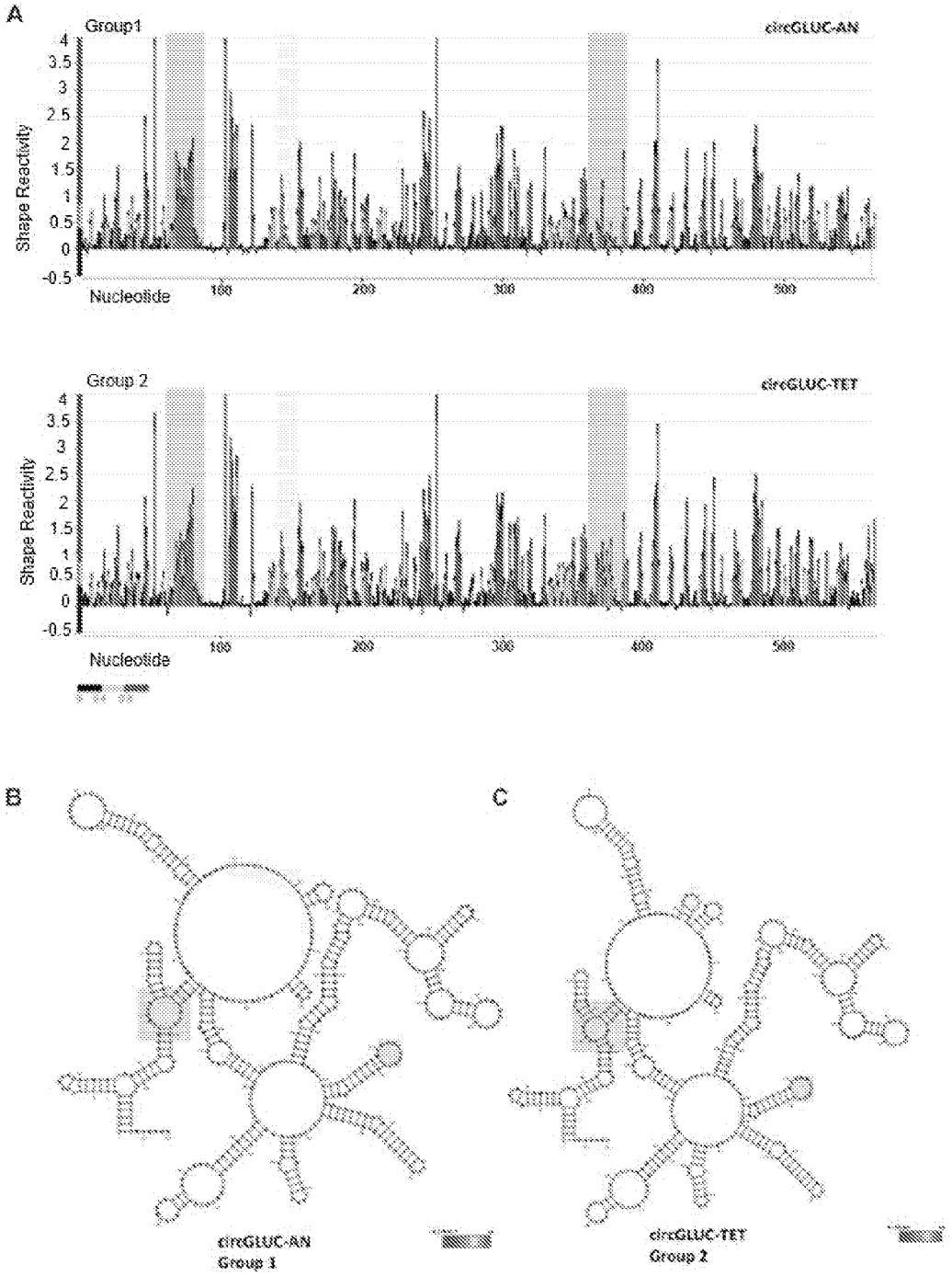

A
Group1
circGLUC-AN
Shape Reactivity
Nucleotide
Group 2
circGLUC-TET
Shape Reactivity
Nucleotide
B
C
circGLUC-AN
Group 1
circGLUC-TET
Group 2

**Figure 4**

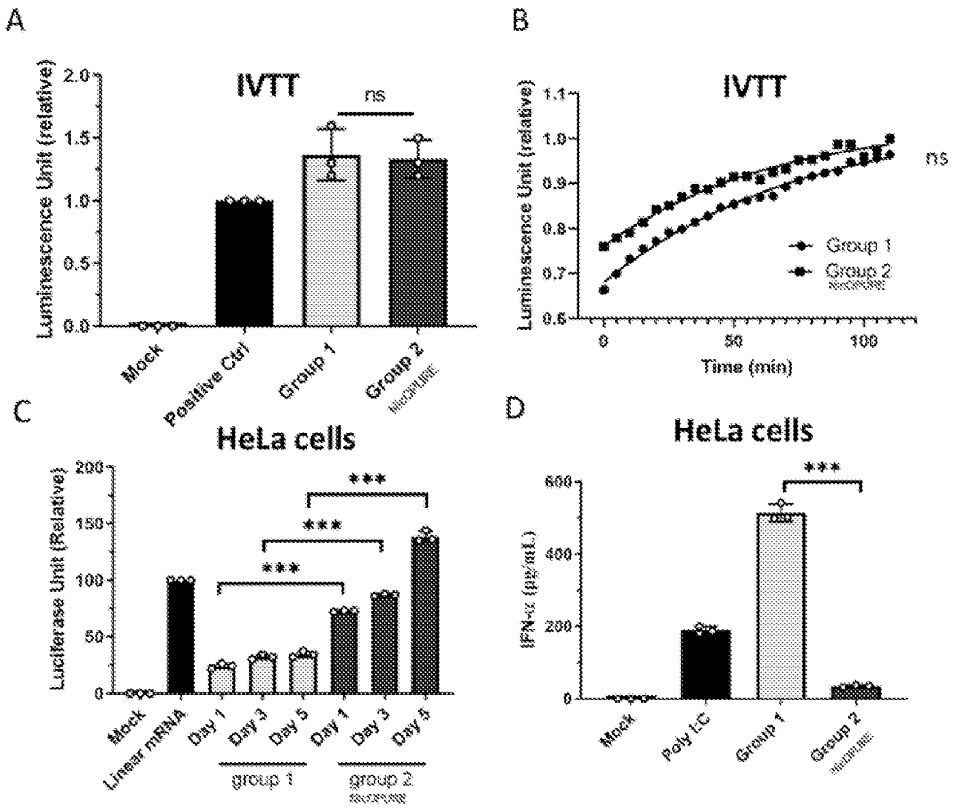

A
IVTT
ns
Luminescence Unit (relative)
2.0
1.5
1.0
0.5
0.0
Mock
Positive Ctrl
Group 1
Group 2
B
IVTT
Luminescence Unit (relative)
1.1
1.0
0.9
0.8
0.7
0.6
ns
Group 1
Group 2
0
50
100
Time (min)
C
HeLa cells
Luciferase Unit (Relative)
200
150
100
50
0
***
***
***
Mock
Linear mRNA
Day 1
Day 3
Day 5
Day 1
Day 3
Day 5
group 1
group 2
D
HeLa cells
600
400
200
0
***
Mock
Poly I:C
Group 1
Group 2

**Figure 5**

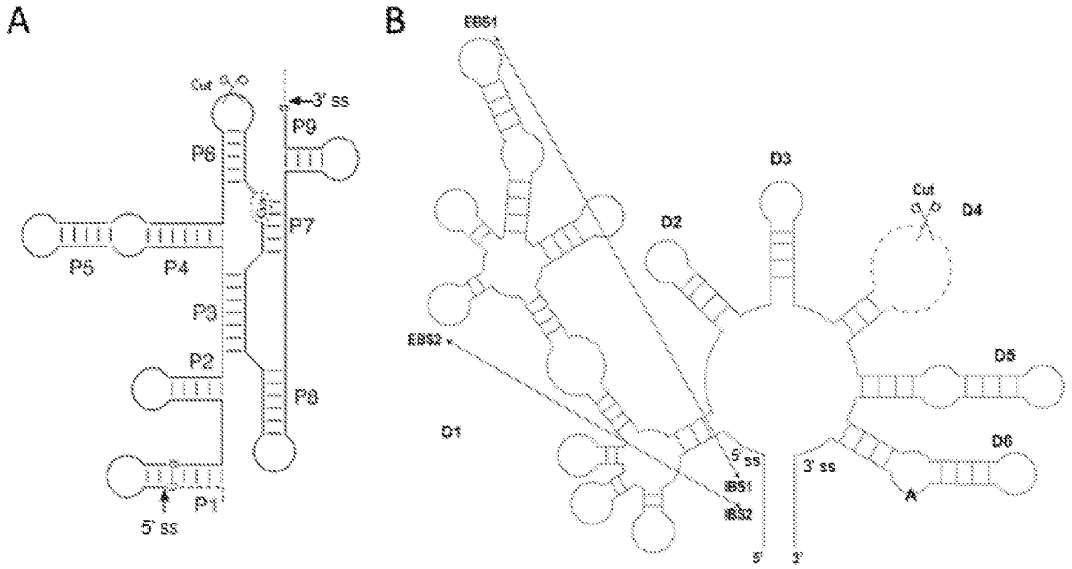
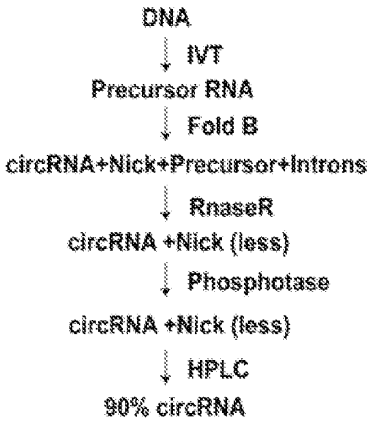
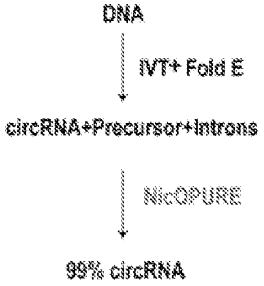

A
Cut
3' ss
P9
P6
P7
P5
P4
P3
P2
P8
P1
5' ss
B
EBS1
D3
D2
Cut
D4
EBS2
D5
D1
5' ss
IBS1
IBS2
3' ss
D6
A
5'
3'

**Figure 6**

A




B




C

| | Monovalent Salt | Divalent Salt | Buffer | Additive | Temp | Time |
|---|---|---|---|---|---|---|
| Cond A | 25mM NaCl | 15mM $MgCl_2$ | 25mM HEPES (pH7.5) | - | 37°C | 15min |
| Cond B | 25mM NaCl | 15mM $MgCl_2$ | 25mM HEPES (pH7.5) | - | 55°C | 15min |
| Cond C | 1M NaCl | 15mM $MgCl_2$ | 25mM HEPES (pH7.5) | - | 55°C | 15min |
| Cond D | 25mM NaCl | 200mM $MgCl_2$ | 25mM HEPES (pH7.5) | - | 55°C | 15min |
| Cond E | 1M $NH_4OAC$ | 100mM $Mg(OAC)_2$ | 25mM HEPES (pH7.5) | - | 45°C | 60min |
| Cond F | 0.3M KCl | 15mM $MgCl_2$ | 40mM Tris-HCL(pH7.0) | 5mM DTT, 2mM Spermidine | 55°C | 15min |
| Cond G | - | 10mM $MgCl_2$ | 50mM Tris-HCL(pH7.0) | 1mM DTT | 55°C | 15min |

**Figure 7**

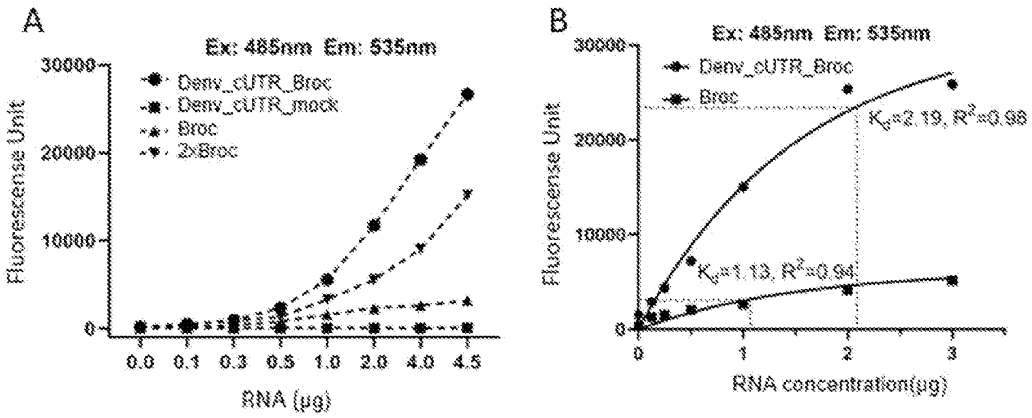

A
Ex: 485nm Em: 535nm
Fluorescense Unit
30000
20000
10000
0
Denv_cUTR_Broc
Denv_cUTR_mock
Broc
2xBroc
0.0 0.1 0.3 0.5 1.0 2.0 4.0 4.5
RNA (μg)
B
Ex: 485nm Em: 535nm
Denv_cUTR_Broc
Broc
$K_d$=2.19, $R^2$=0.98
$K_d$=1.13, $R^2$=0.94
Fluorescense Unit
30000
20000
10000
0
0 1 2 3
RNA concentration(μg)

**Figure 8**

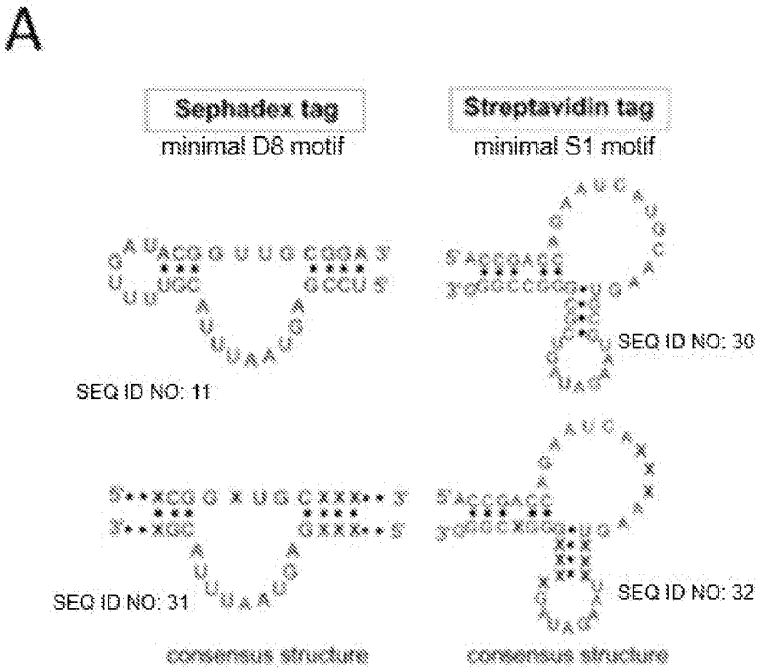



**Figure 9 (A) (continued)**

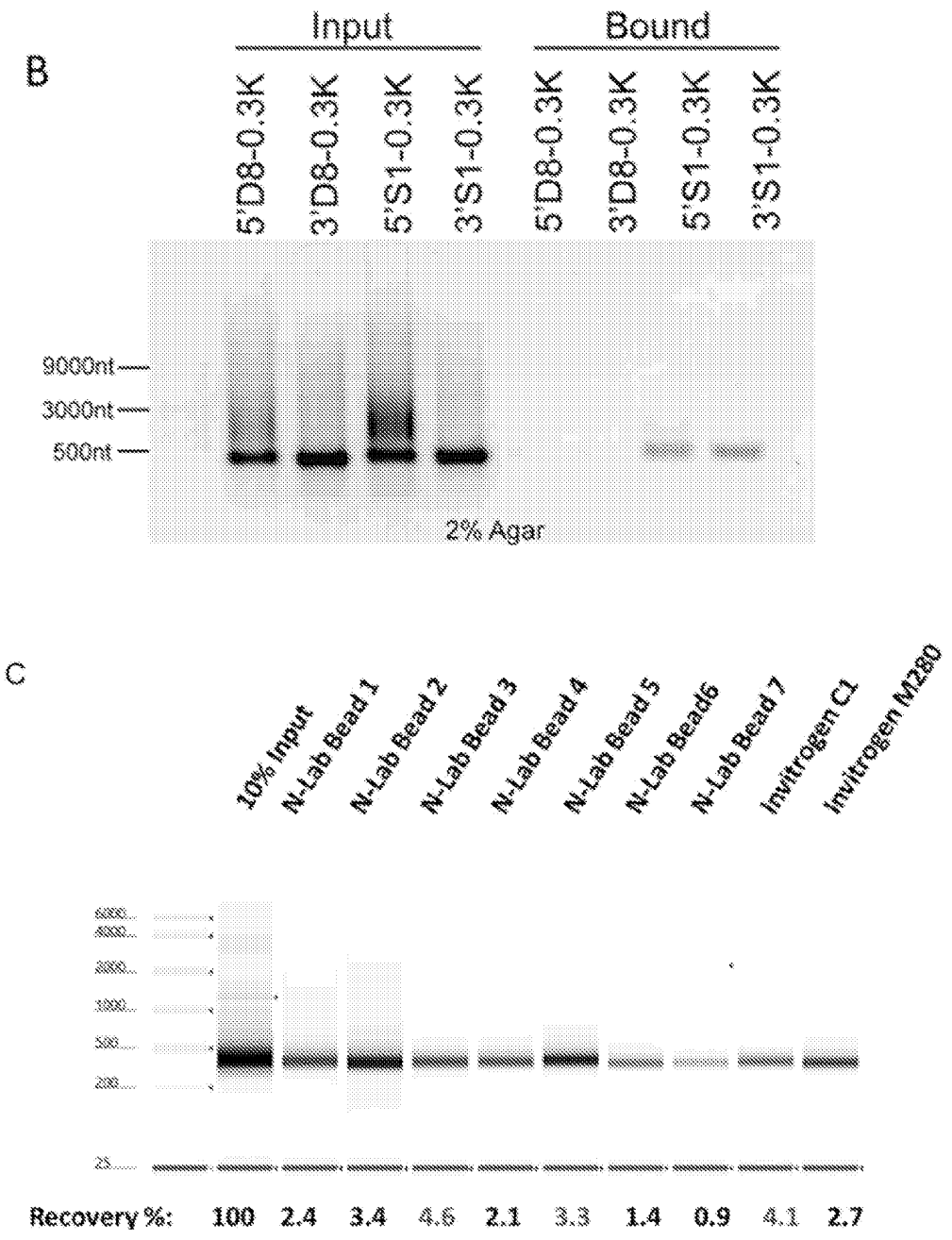



**Figure 9 (B)-(C) (end)**

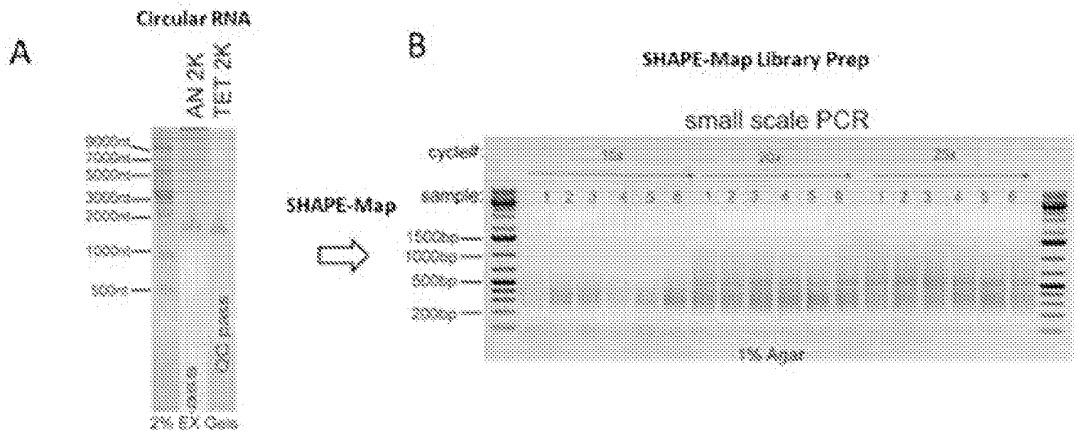



**Figure 10(A)-(B) (continued)**

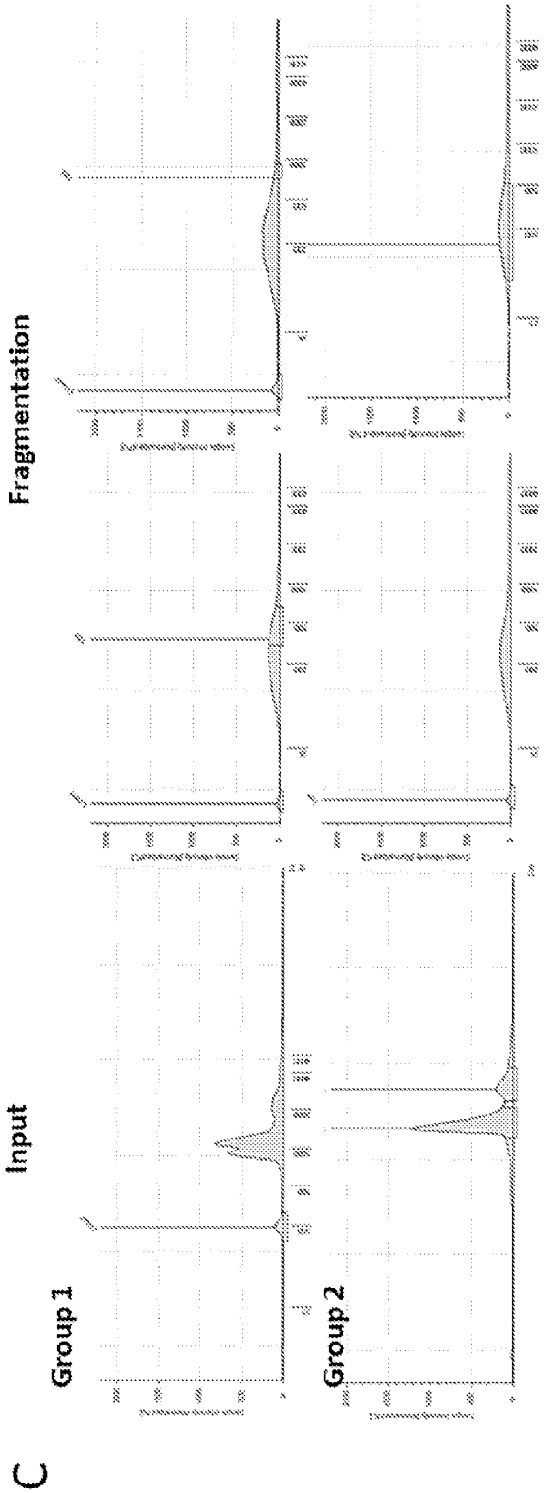



**Figure 10(C) (continued)**

D

| Sample | Index | | Cycles | ng/uL | uL | Total |
|---|---|---|---|---|---|---|
| 43 Group1_DMSO | #43 | TACAGC | 15 | 27.8 | 17 | 472.6 |
| 44 Group1_AAI | #44 | TATAAT | 15 | 19.8 | 17 | 336.6 |
| 45 Group1_Denature | #45 | TCATTC | 15 | 33.8 | 17 | 574.6 |
| 46 Group2_DMSO | #46 | TCCCGA | 15 | 3.08 | 17 | 52.36 |
| 47 Group2_AAI | #47 | TCGAAG | 15 | 30.4 | 17 | 516.8 |
| 48 Group2_Denature | #48 | TCGGCA | 15 | 38.2 | 17 | 649.4 |

**Figure 10(D) (end)**

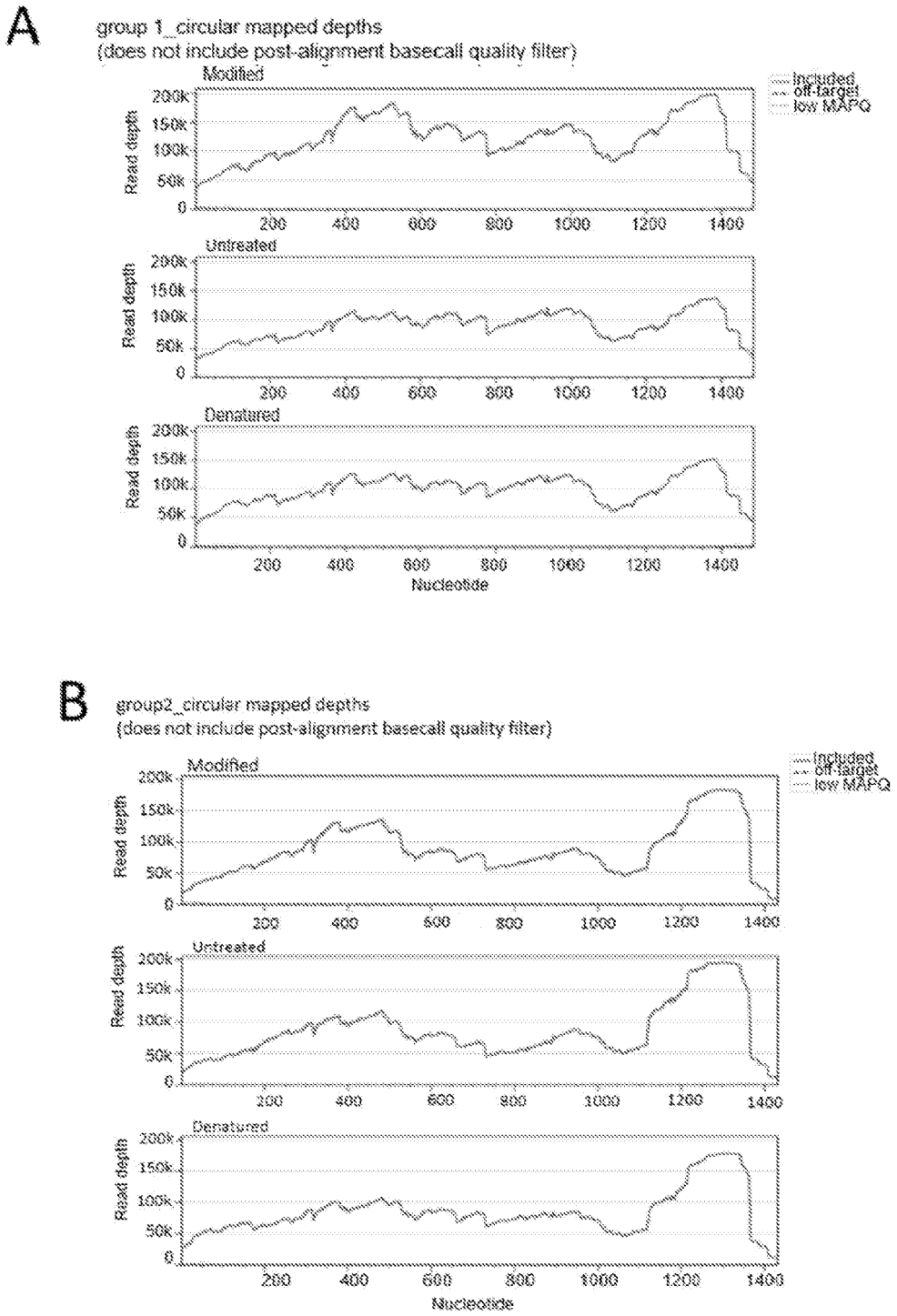



**Figure 11(A)-(B) (continued)**

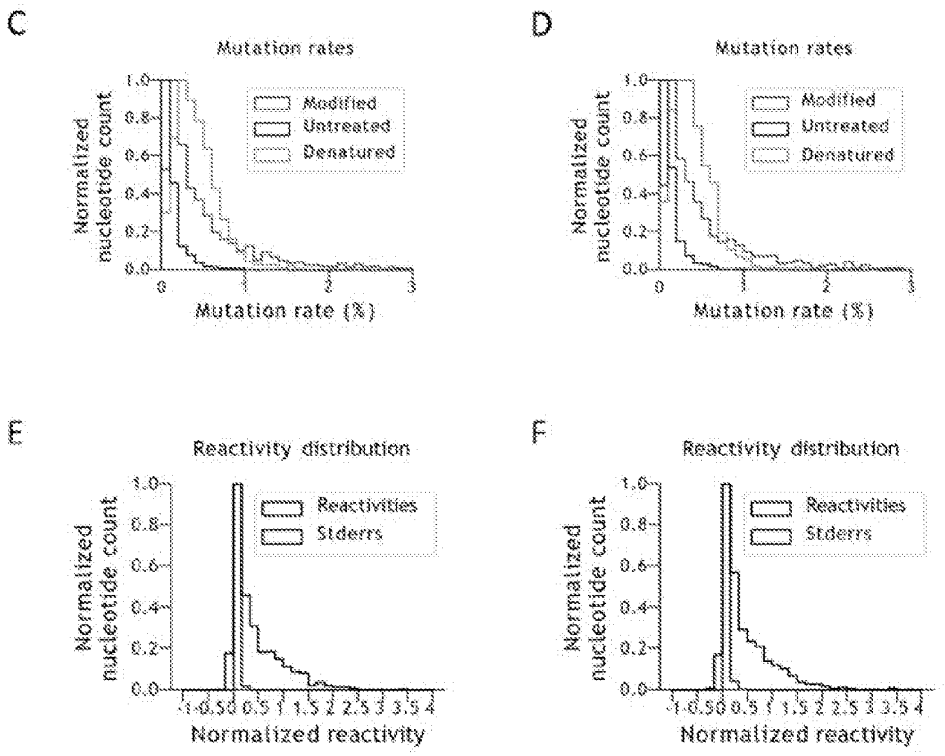



**Figure 11(C)-(F) (end)**

A

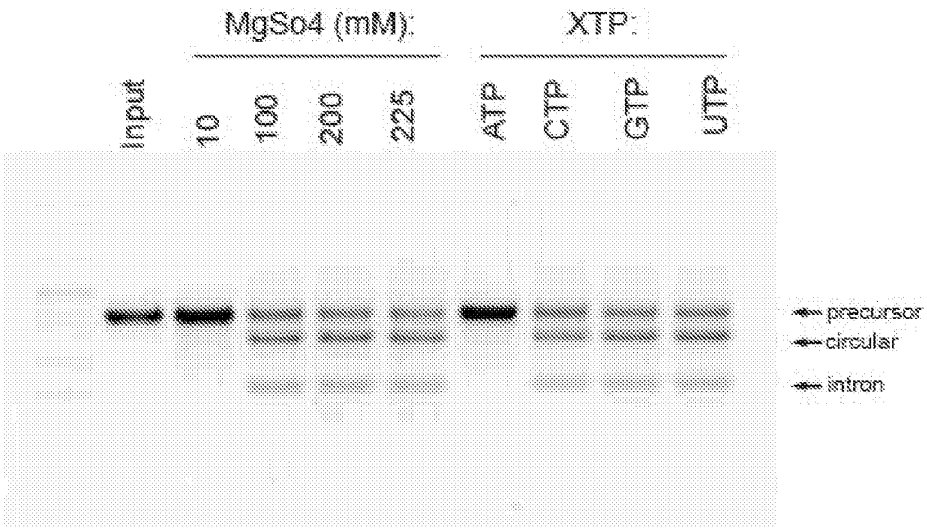

MgSo4 (mM):
XTP:
Input
10
100
200
225
ATP
CTP
GTP
UTP
precursor
circular
intron

**Figure 12**