Figure 1

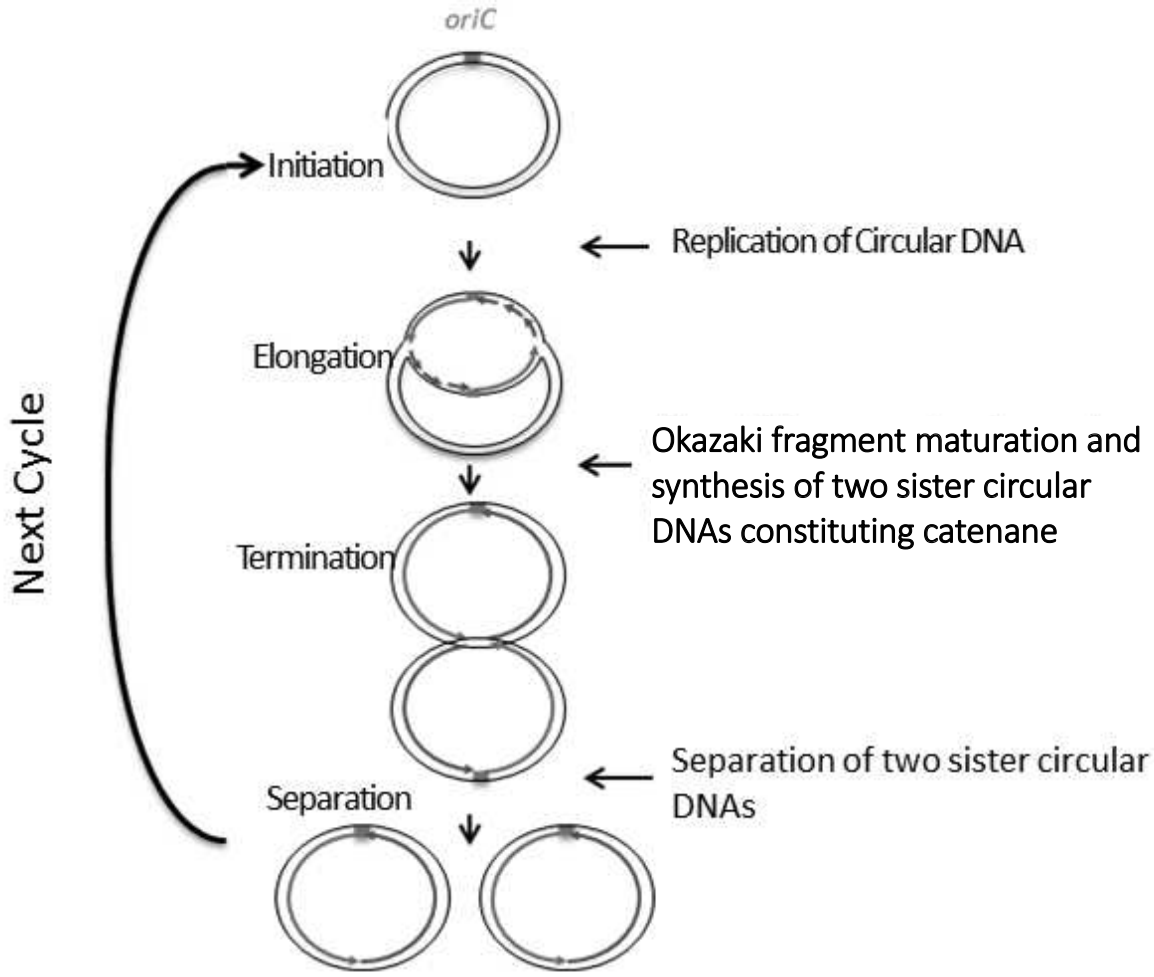

oriC
Initiation
Replication of Circular DNA
Elongation
Okazaki fragment maturation and synthesis of two sister circular DNAs constituting catenane
Termination
Separation of two sister circular DNAs
Separation
Next Cycle

Figure 2

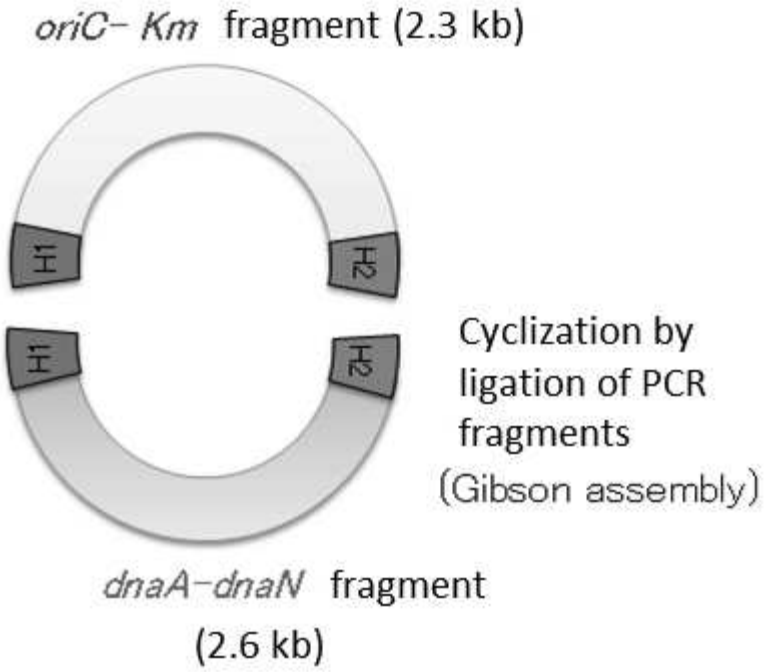


Figure 3

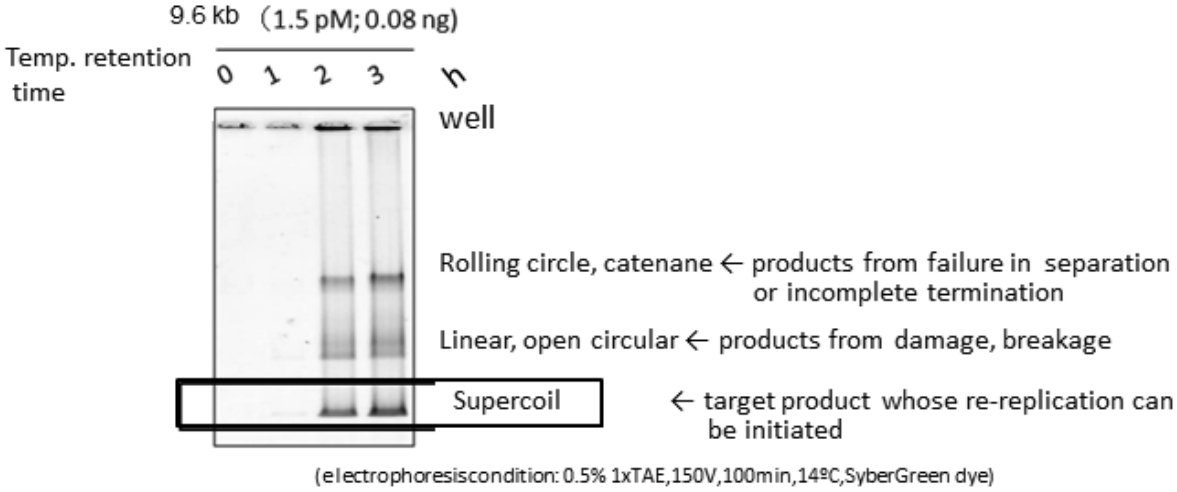
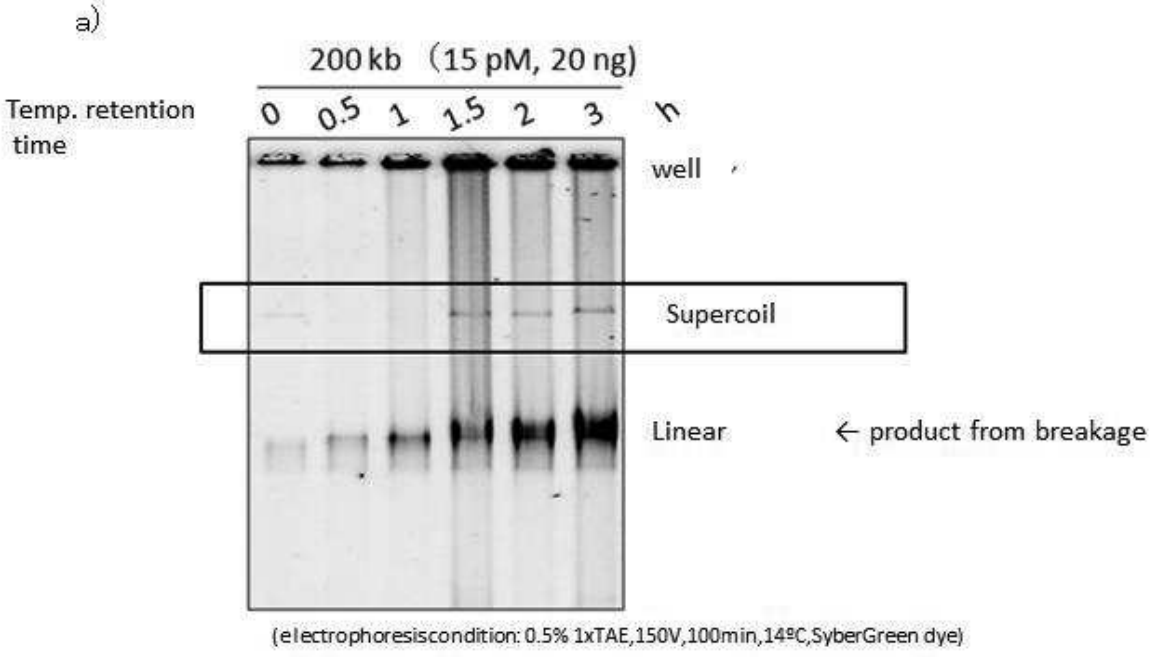


Figure 4


a)
200 kb (15 pM, 20 ng)
Temp. retention time
0 0.5 1 1.5 2 3 h
well
Supercoil
Linear
← product from breakage
(electrophoresiscondition: 0.5% 1xTAE,150V,100min,14ºC,SyberGreen dye)

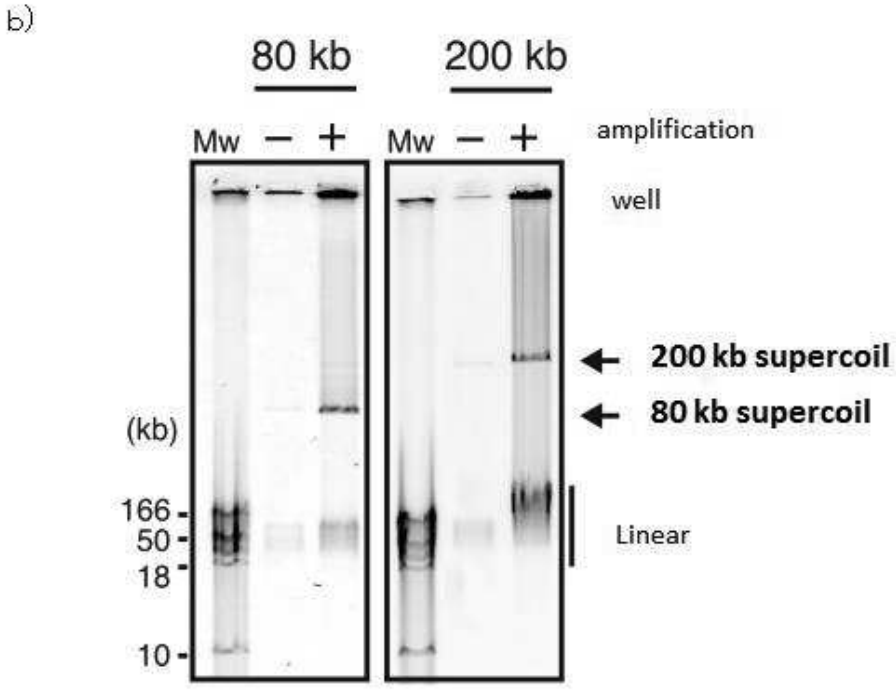

b)
80 kb
200 kb
Mw − + Mw − +
amplification
well
200 kb supercoil
80 kb supercoil
(kb)
166
50
18
10
Linear

Figure 5

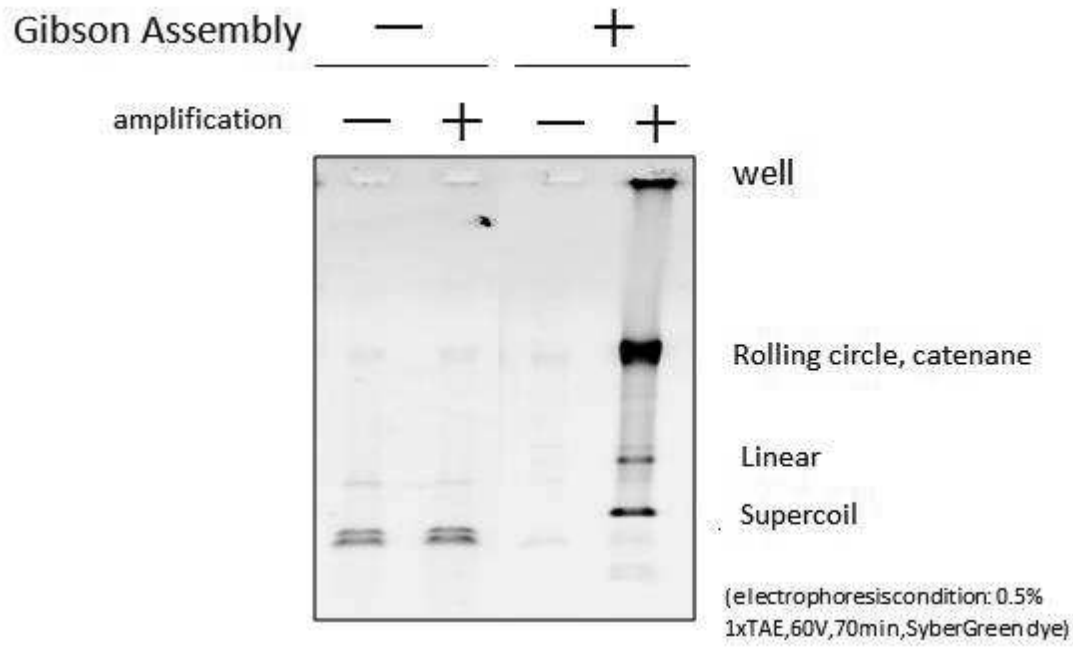

Gibson Assembly
—
+
amplification
—
+
—
+
well
Rolling circle, catenane
Linear
Supercoil
(electrophoresiscondition: 0.5%
1xTAE,60V,70min,SyberGreen dye)

Figure 6

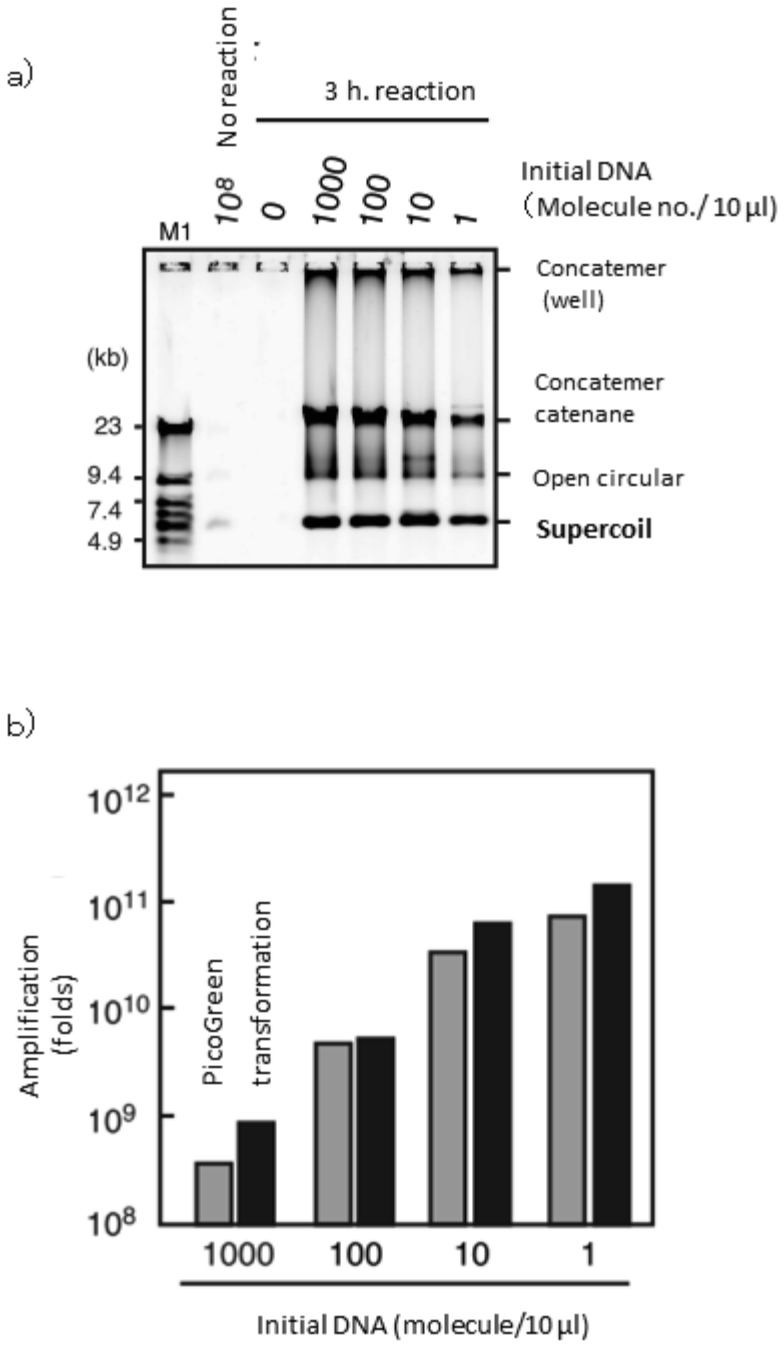

a)
No reaction
3 h. reaction
Initial DNA
(Molecule no./10 µl)
M1
10^8
0
1000
100
10
1
(kb)
23
9.4
7.4
4.9
Concatemer
(well)
Concatemer
catenane
Open circular
Supercoil
b)
Amplification
(folds)
10^12
10^11
10^10
10^9
10^8
PicoGreen
transformation
1000
100
10
1
Initial DNA (molecule/10 µl)

Figure 7

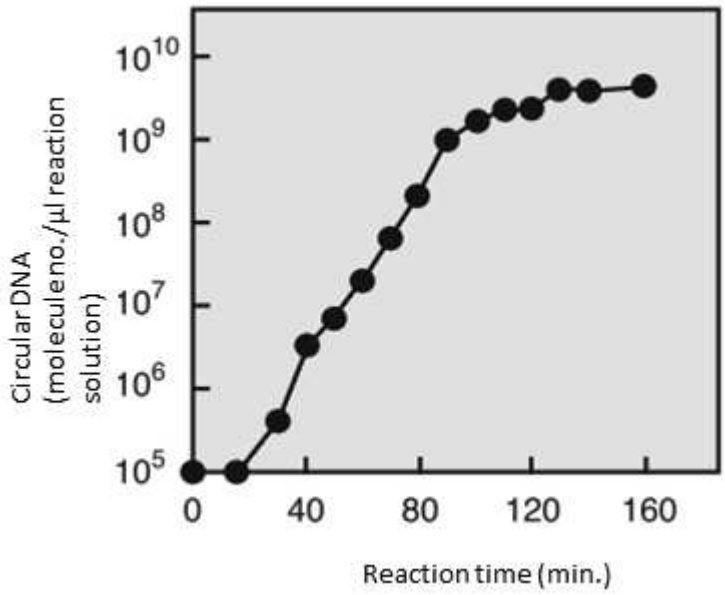

Circular DNA
(molecule no./μl reaction
solution)
10^10
10^9
10^8
10^7
10^6
10^5
0
40
80
120
160
Reaction time (min.)

Figure 8

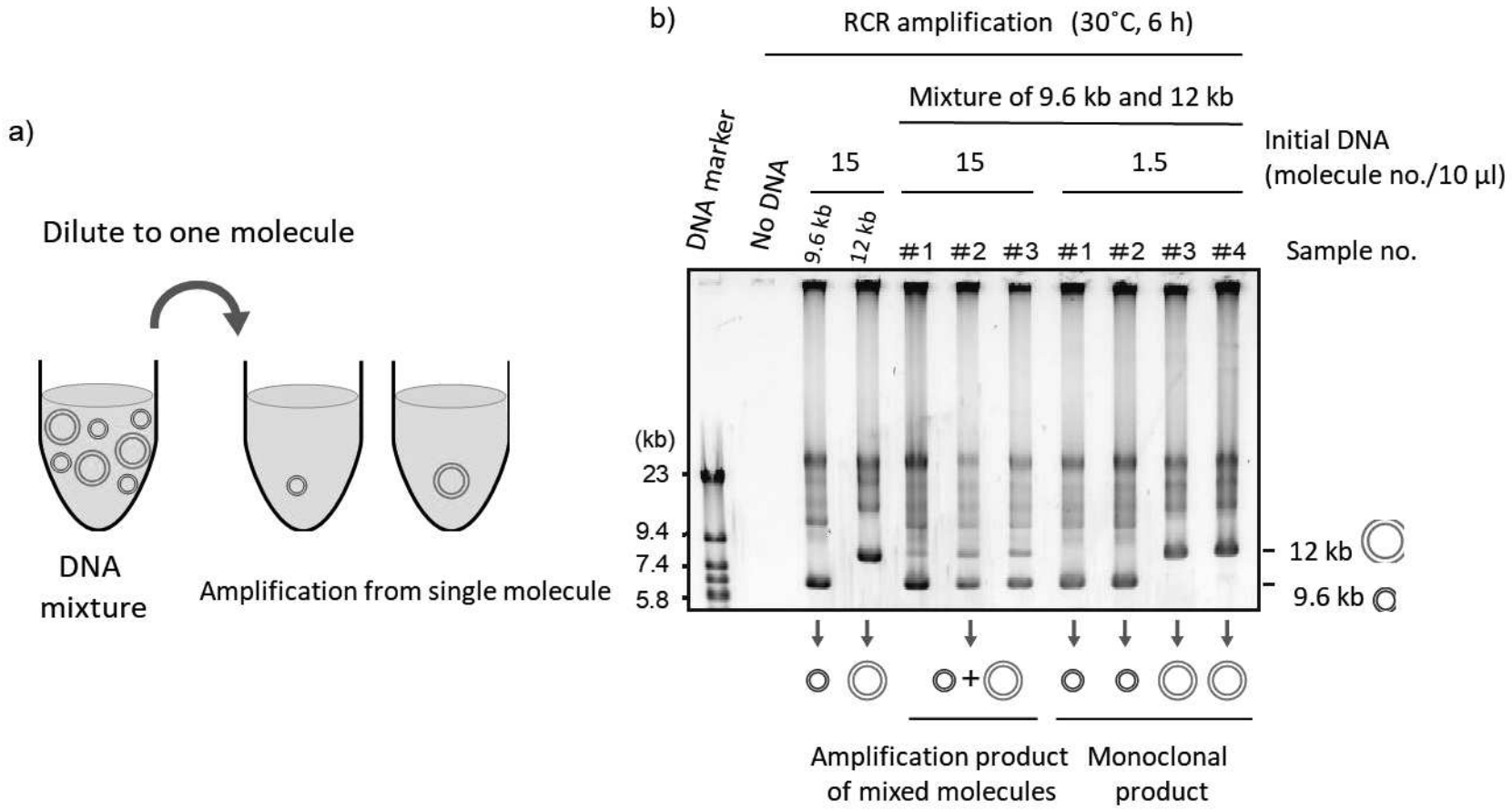

a)
Dilute to one molecule
DNA mixture
Amplification from single molecule
b)
RCR amplification (30˚C, 6 h)
Mixture of 9.6 kb and 12 kb
Initial DNA (molecule no./10 µl)
DNA marker
No DNA
9.6 kb
12 kb
15
15
1.5
#1 #2 #3 #1 #2 #3 #4
Sample no.
(kb)
23
9.4
7.4
5.8
12 kb
9.6 kb
Amplification product of mixed molecules
Monoclonal product

Figure 9

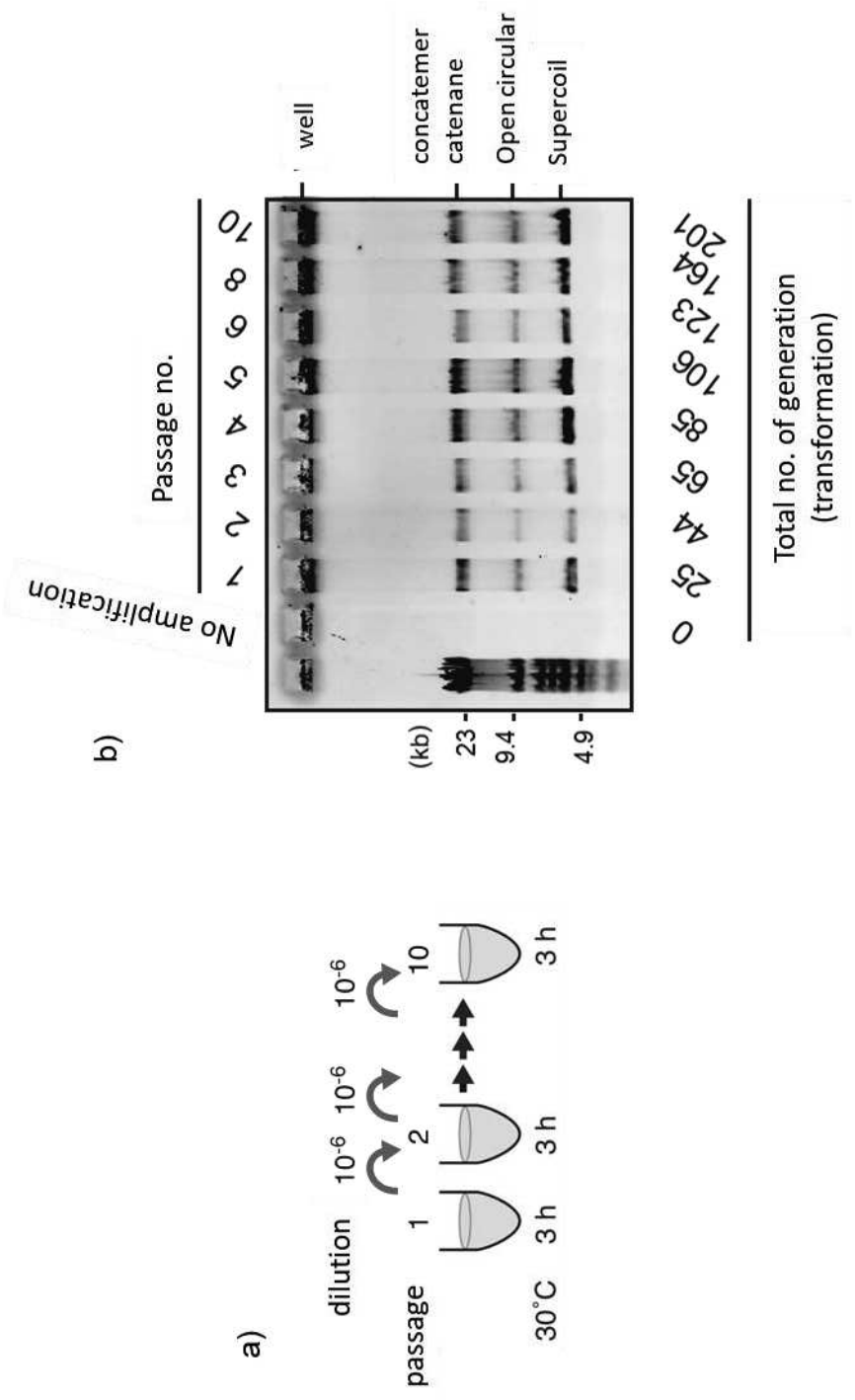

a)
dilution
passage
30°C
1
2
10
3 h
3 h
3 h
10-6
10-6
10-6
b)
No amplification
Passage no.
1
2
3
4
5
6
8
10
well
concatemer
catenane
Open circular
Supercoil
(kb)
23
9.4
4.9
0
25
44
65
85
106
123
164
201
Total no. of generation
(transformation)

Figure 10

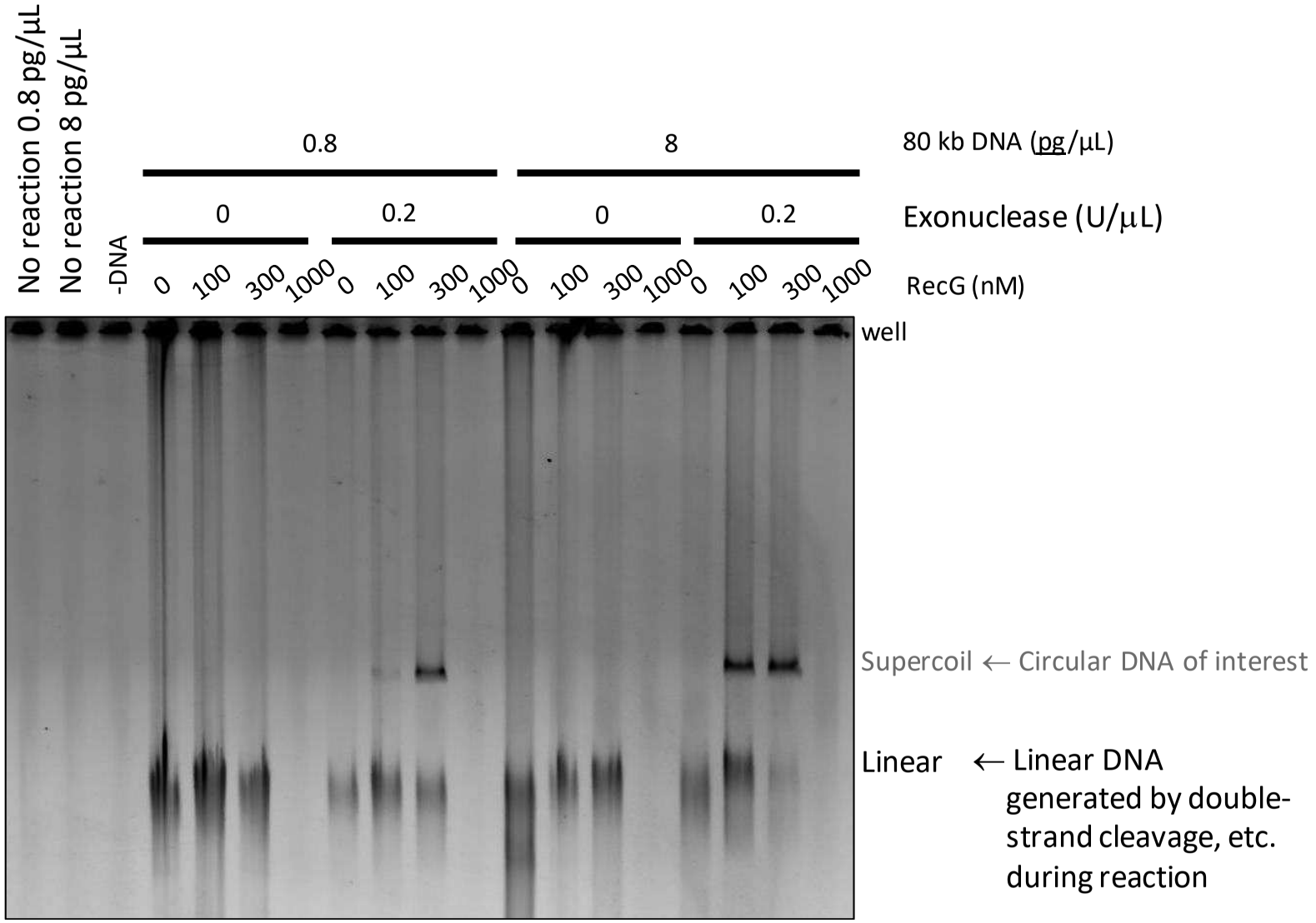

No reaction 0.8 pg/µL
No reaction 8 pg/µL
-DNA
0.8
8
80 kb DNA (pg/µL)
0
0.2
0
0.2
Exonuclease (U/µL)
0 100 300 1000 0 100 300 1000 0 100 300 1000 0 100 300 1000
RecG (nM)
well
Supercoil ← Circular DNA of interest
Linear ← Linear DNA generated by double-strand cleavage, etc. during reaction

Figure 11

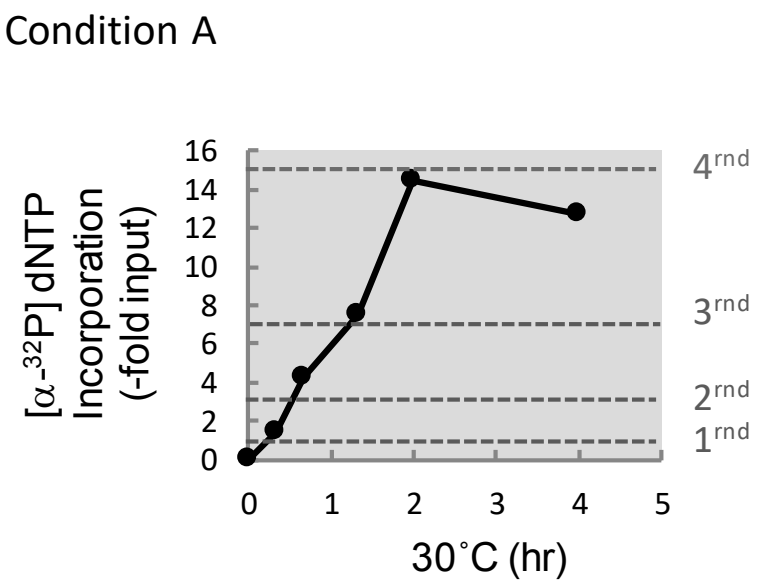
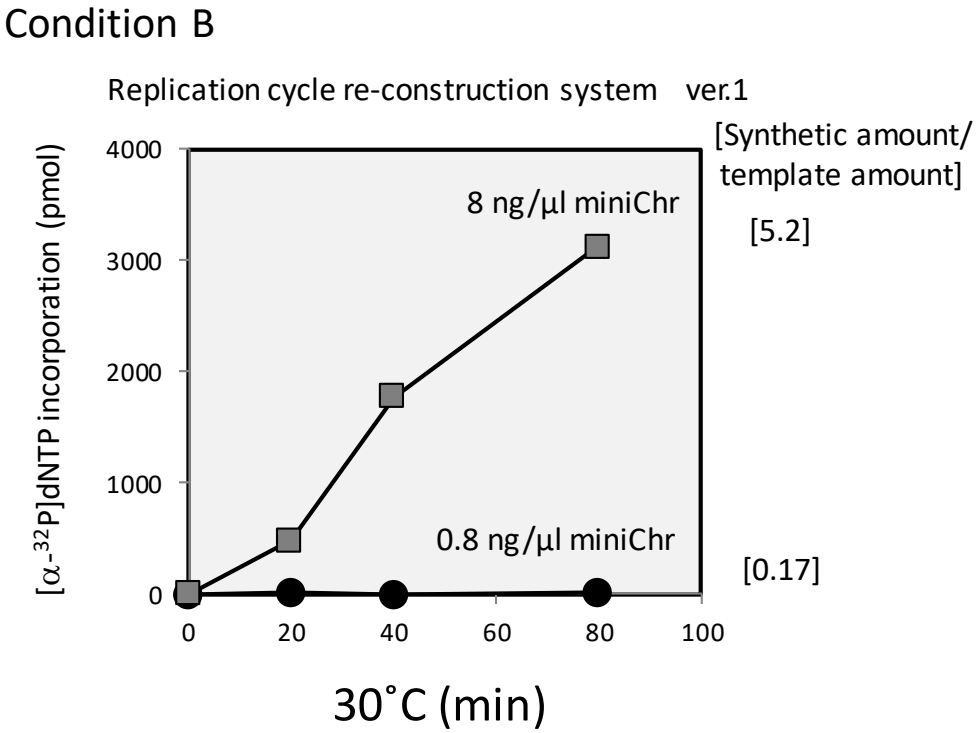


Figure 12



miniChr: minichromosome (8-kb circular DNA)

Figure 13

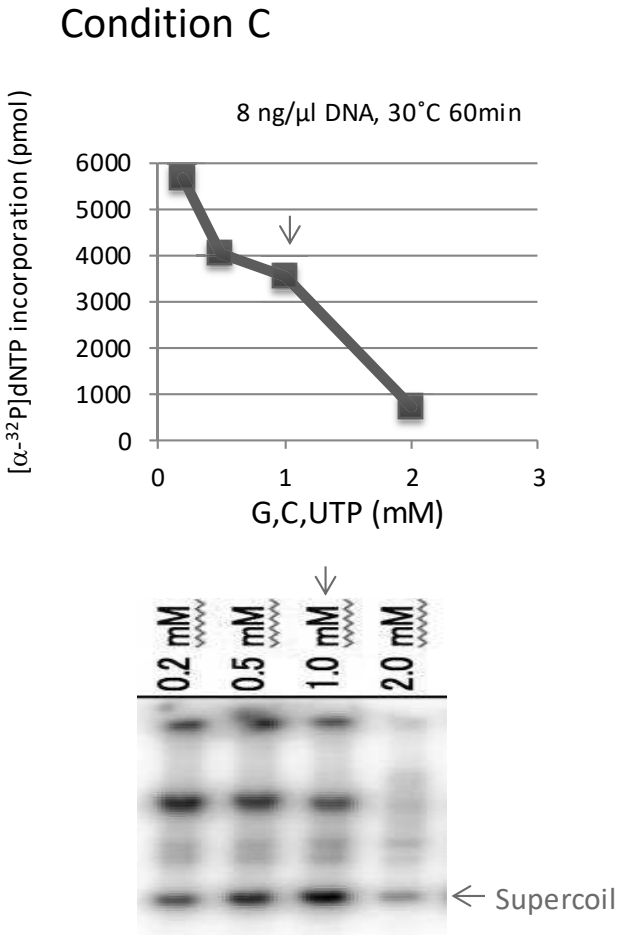


Figure 14

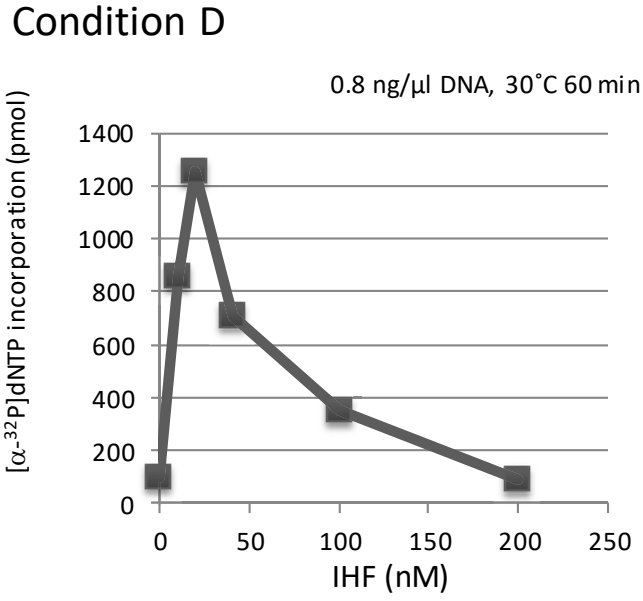


Figure 15

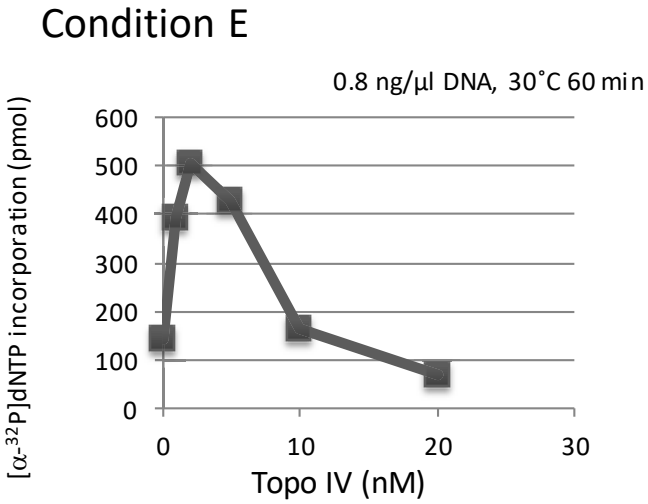


Figure 16

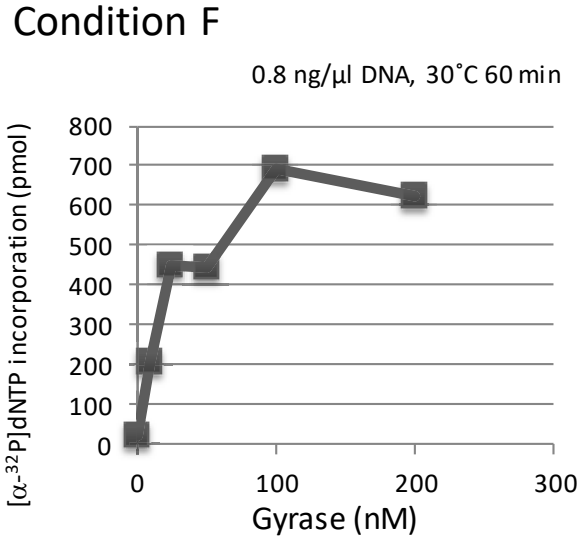


Figure 17

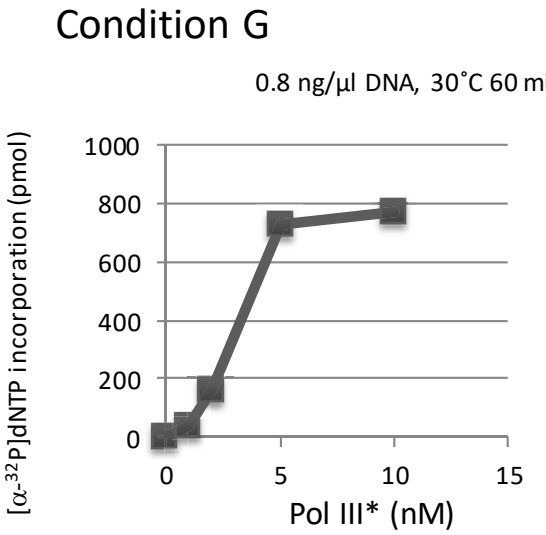


Figure 18

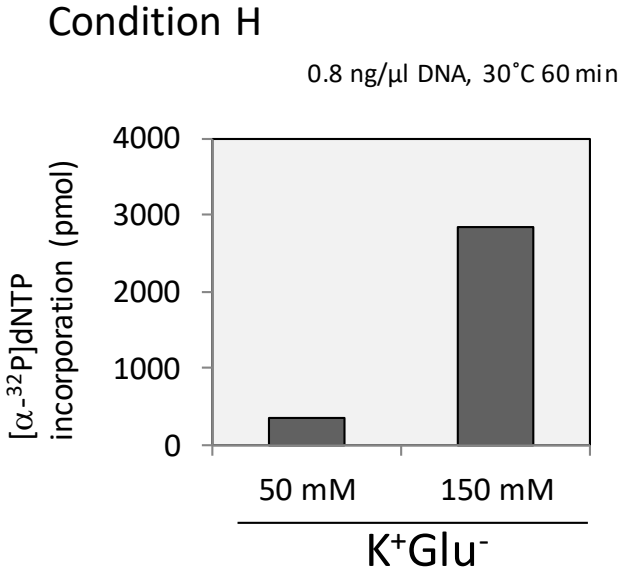


Figure 19

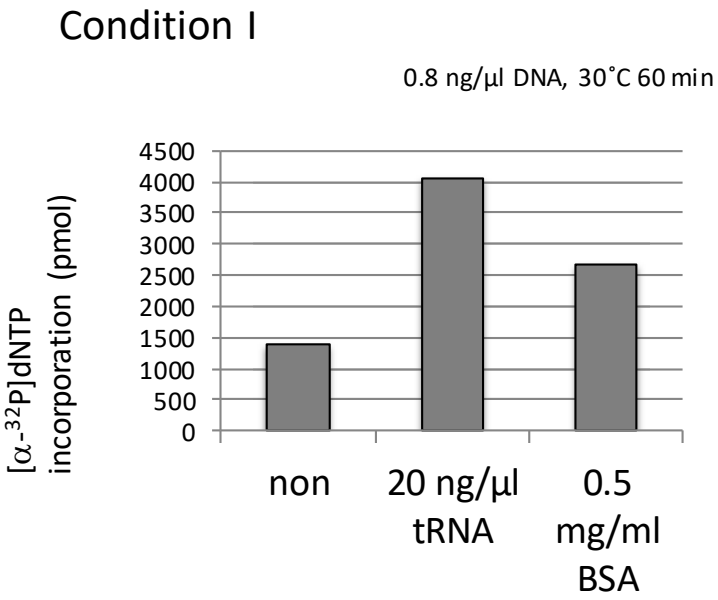


Figure 20

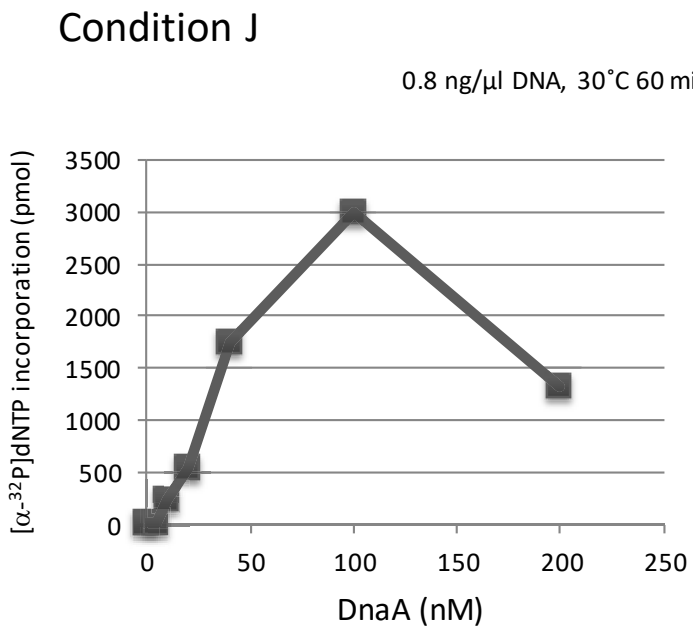


Figure 21

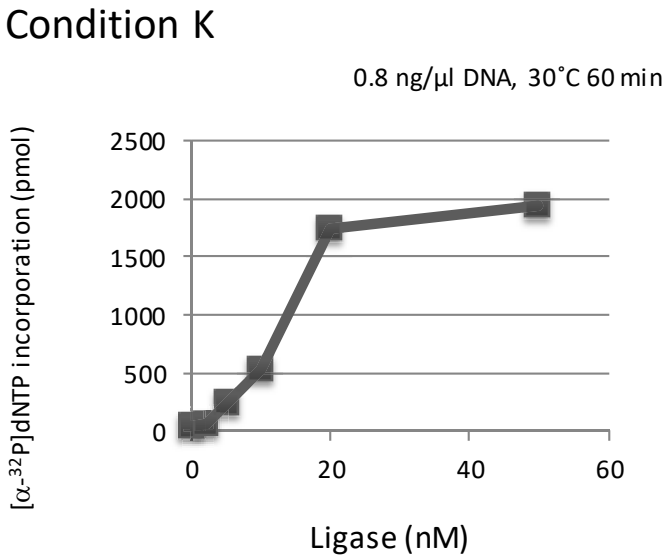


Figure 22

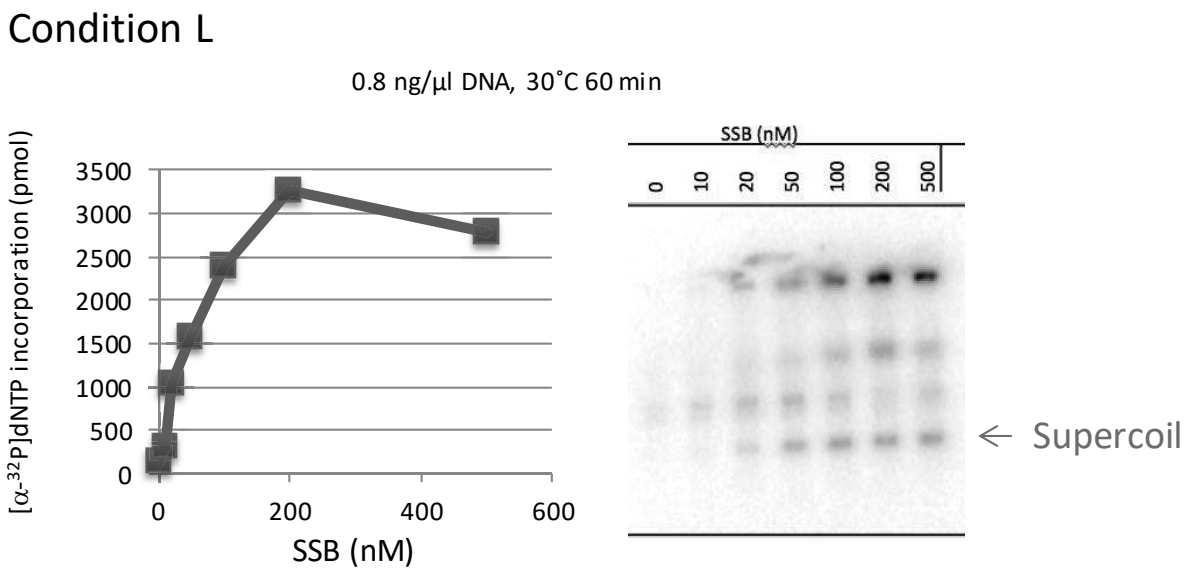


Figure 23

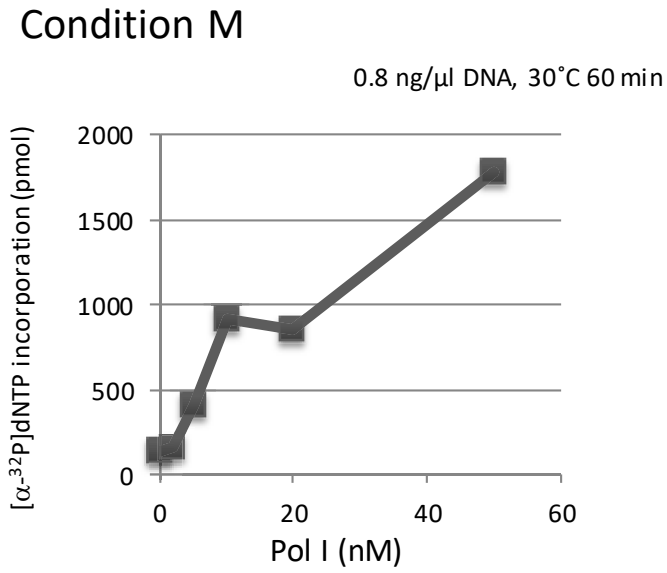


Figure 24

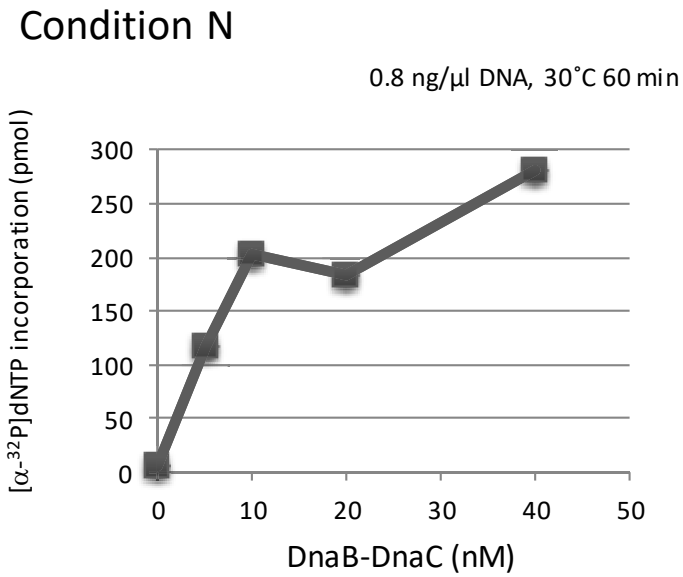


Figure 25

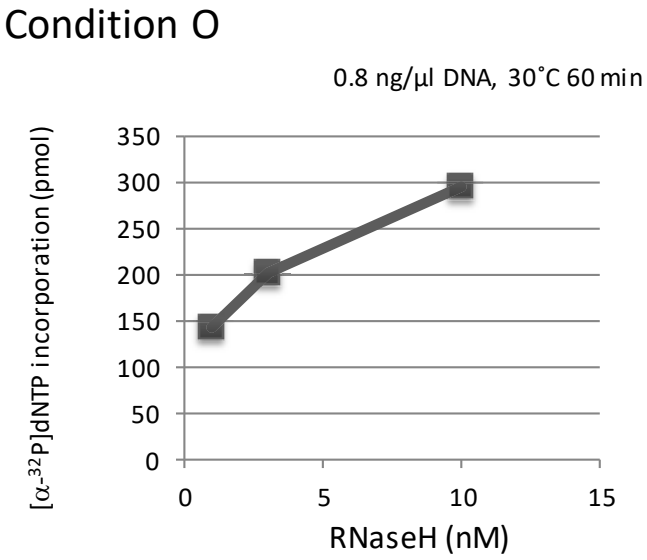


Figure 26

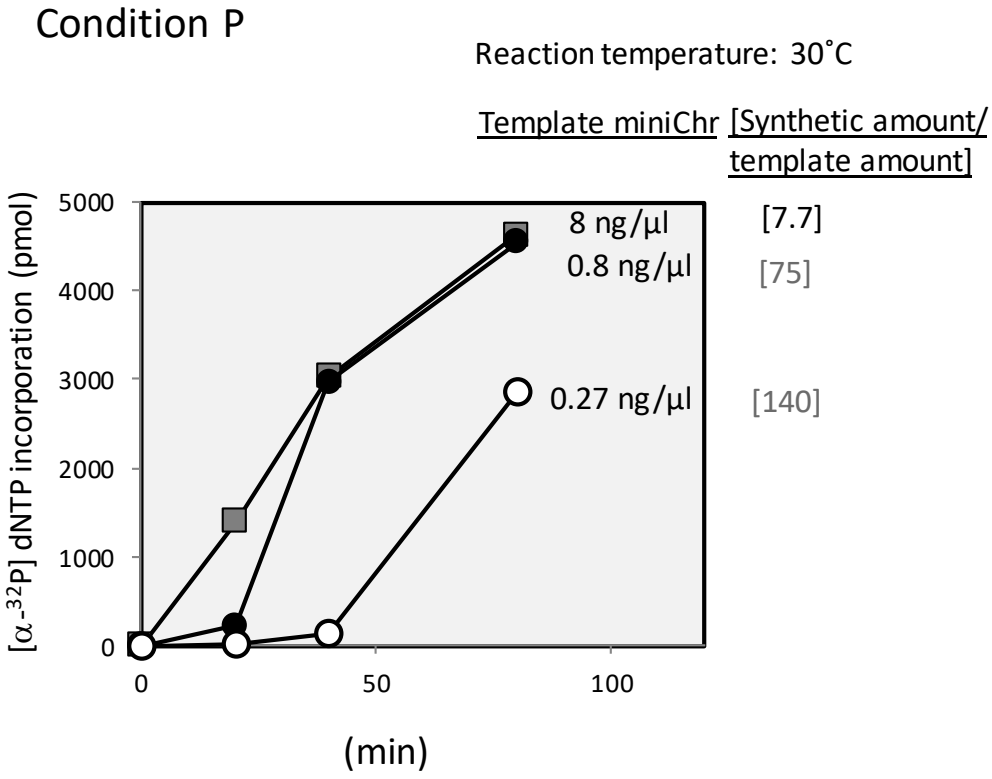


Figure 27

Condition Q

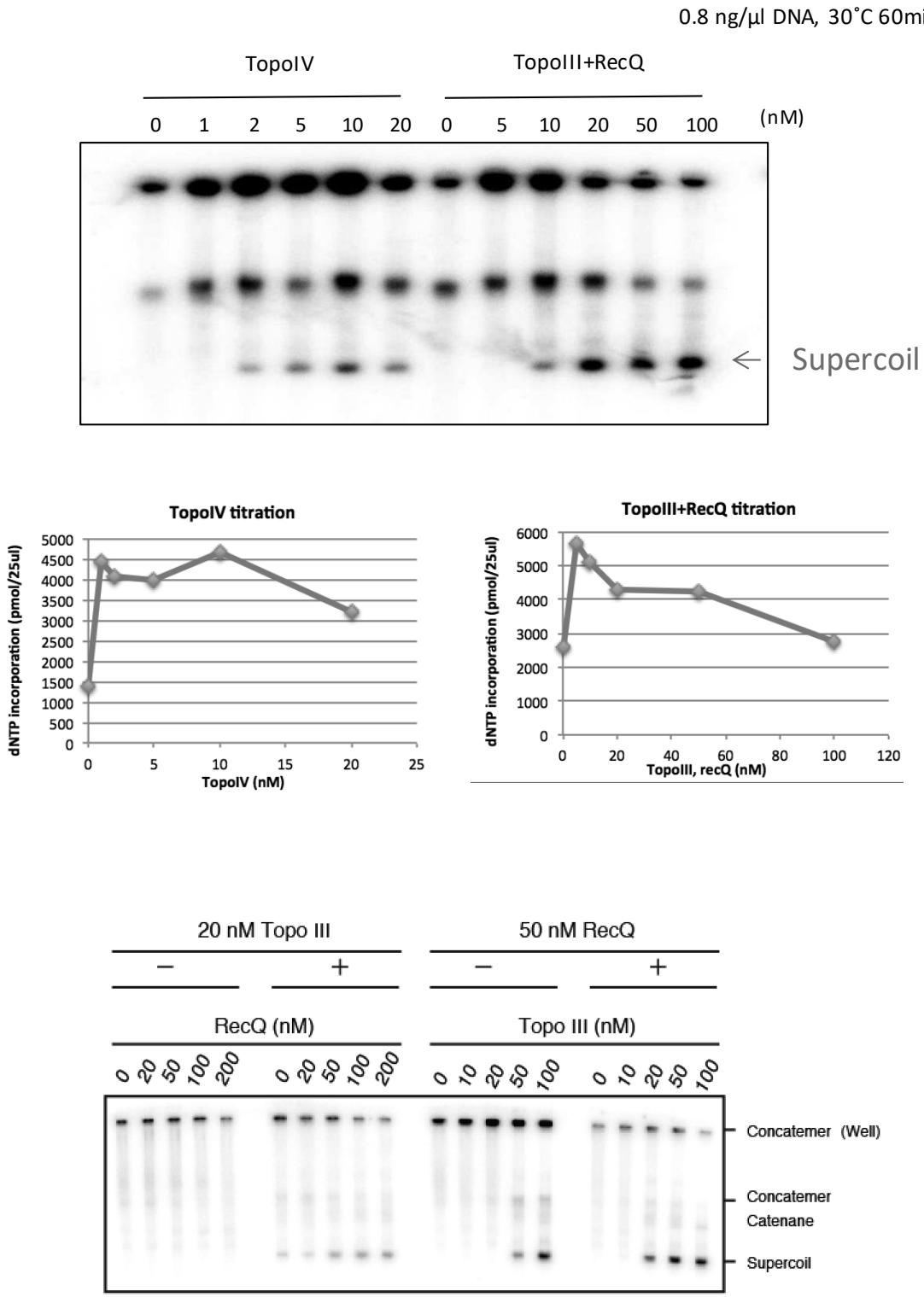


Figure 28

Condition R

0.8 ng/µl DNA, 30˚C 60min

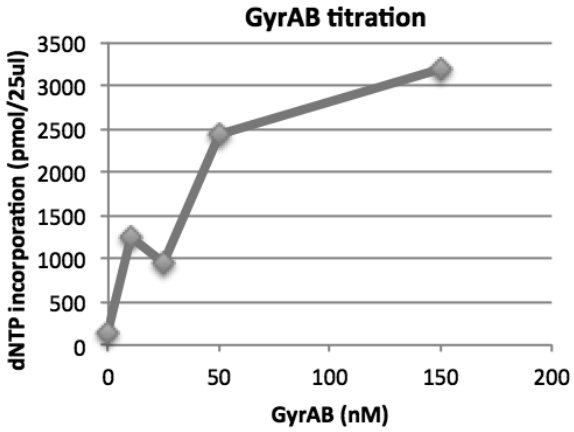


Figure 29

Condition S

80 kb DNA (8 ng/µl) , 30˚C 120min

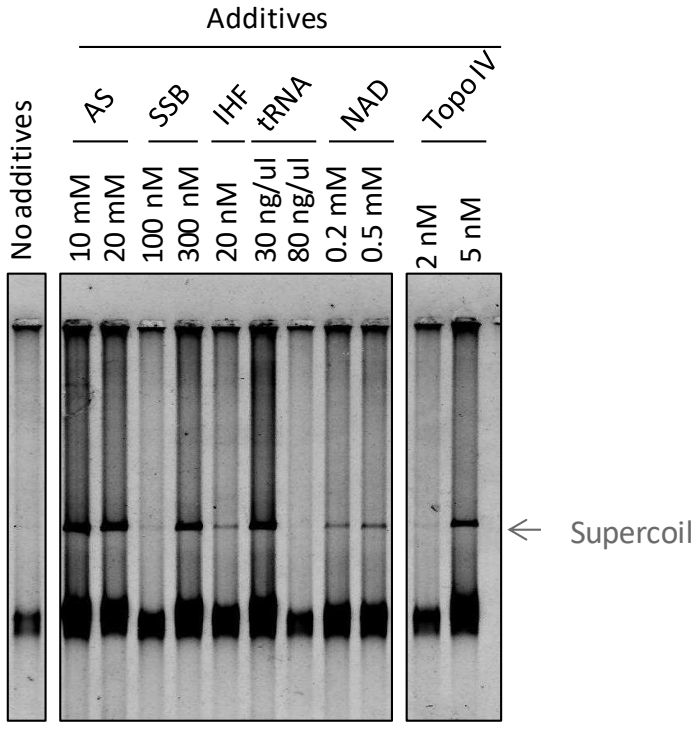


Figure 30

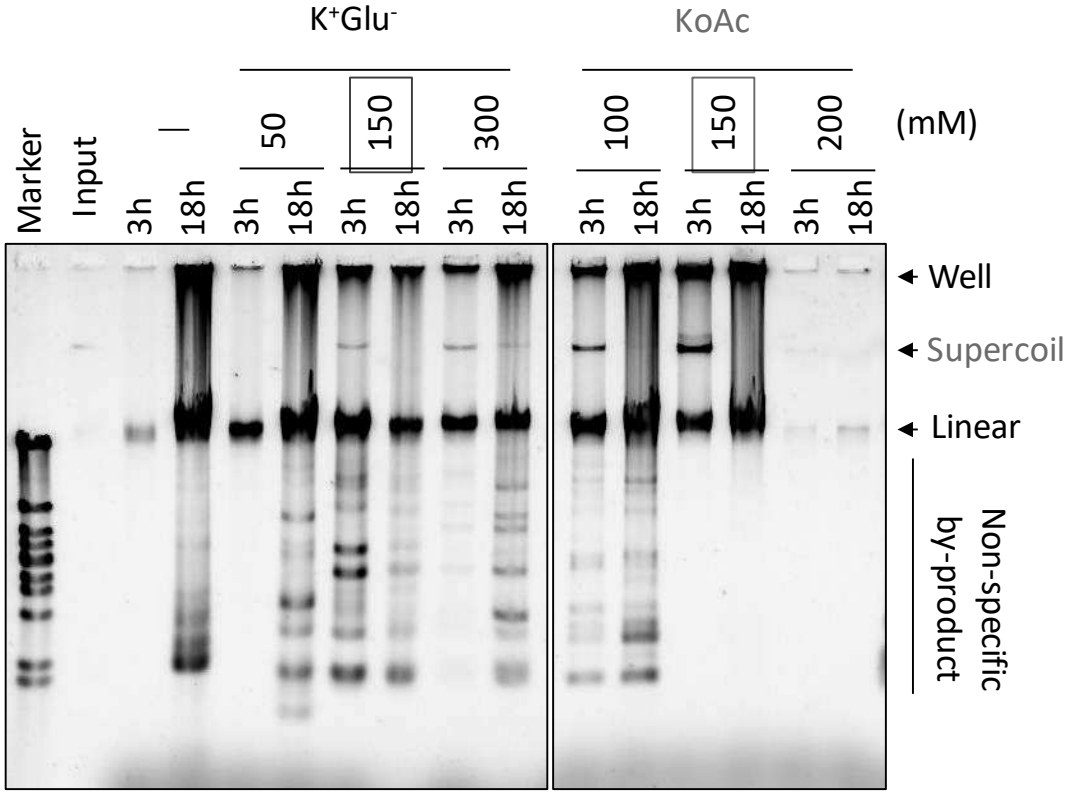


Figure 31

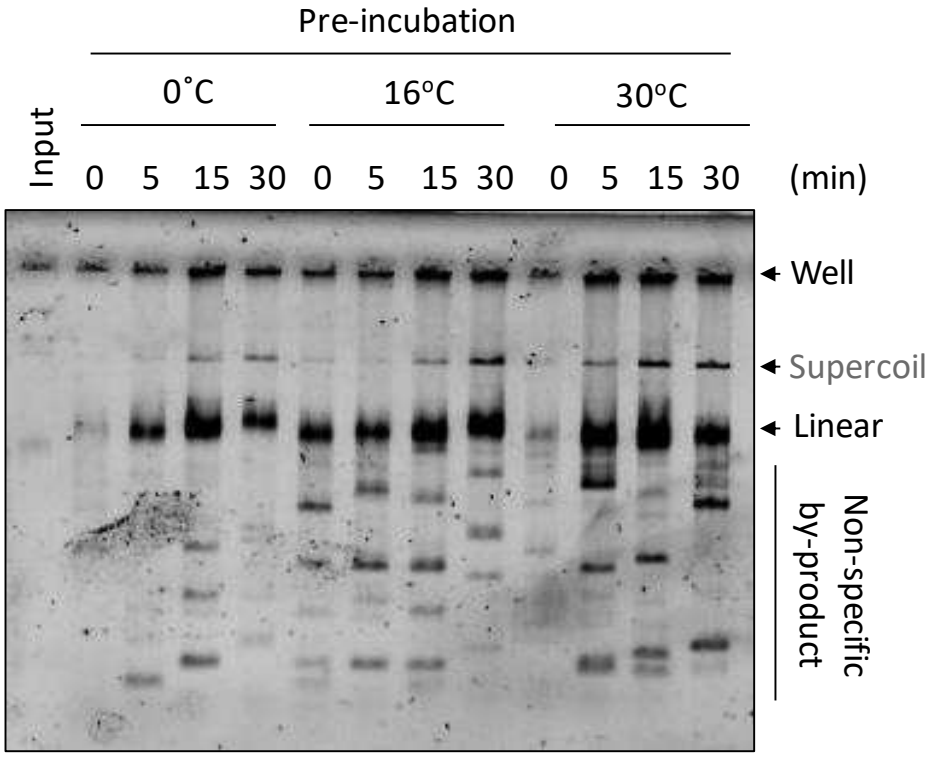


Figure 32

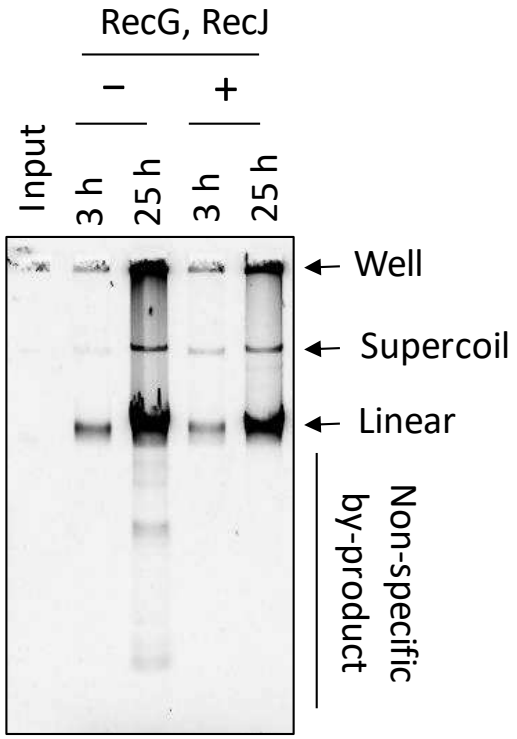


Figure 33

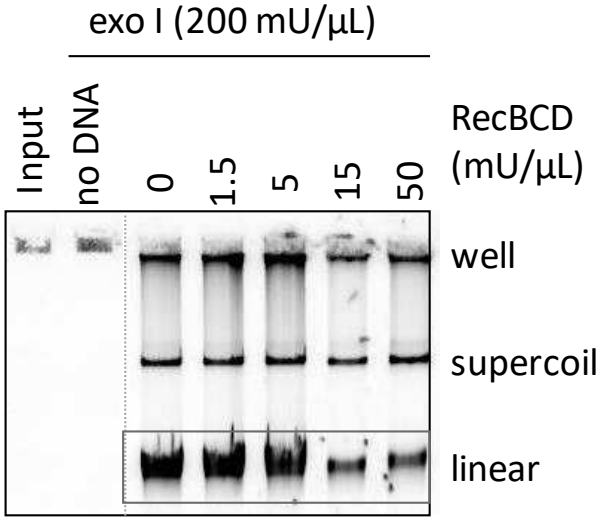


Figure 34

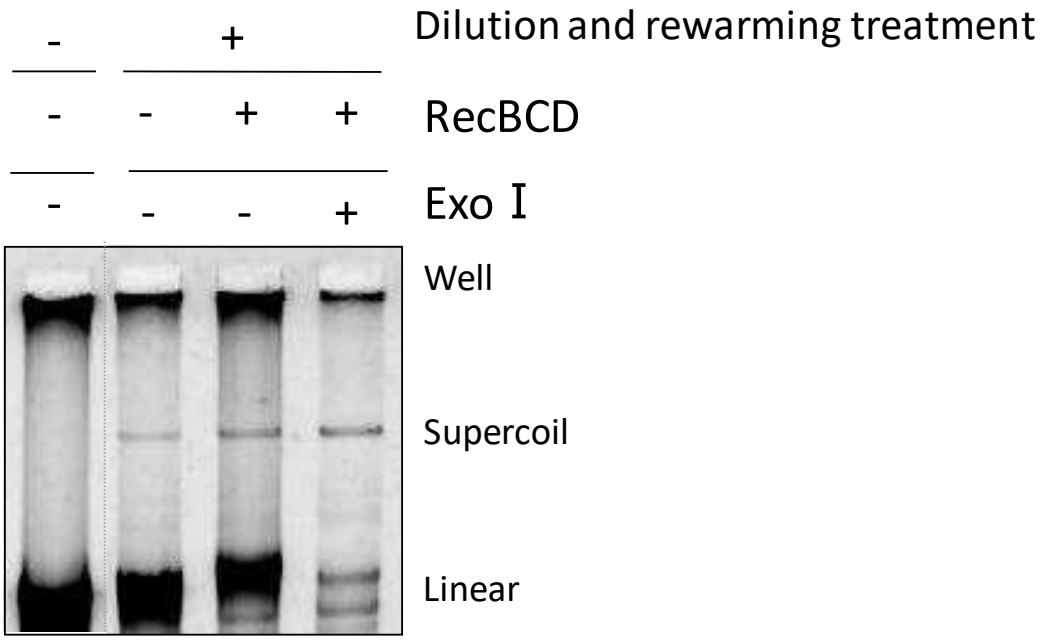


Figure 35

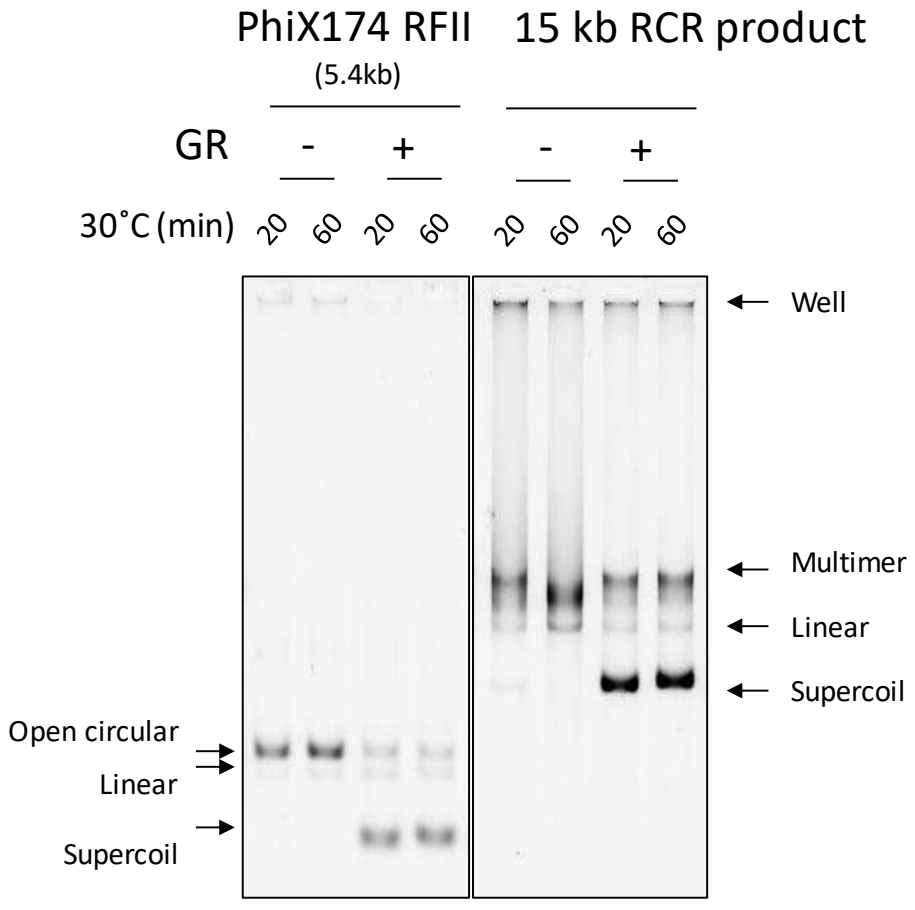


Figure 36

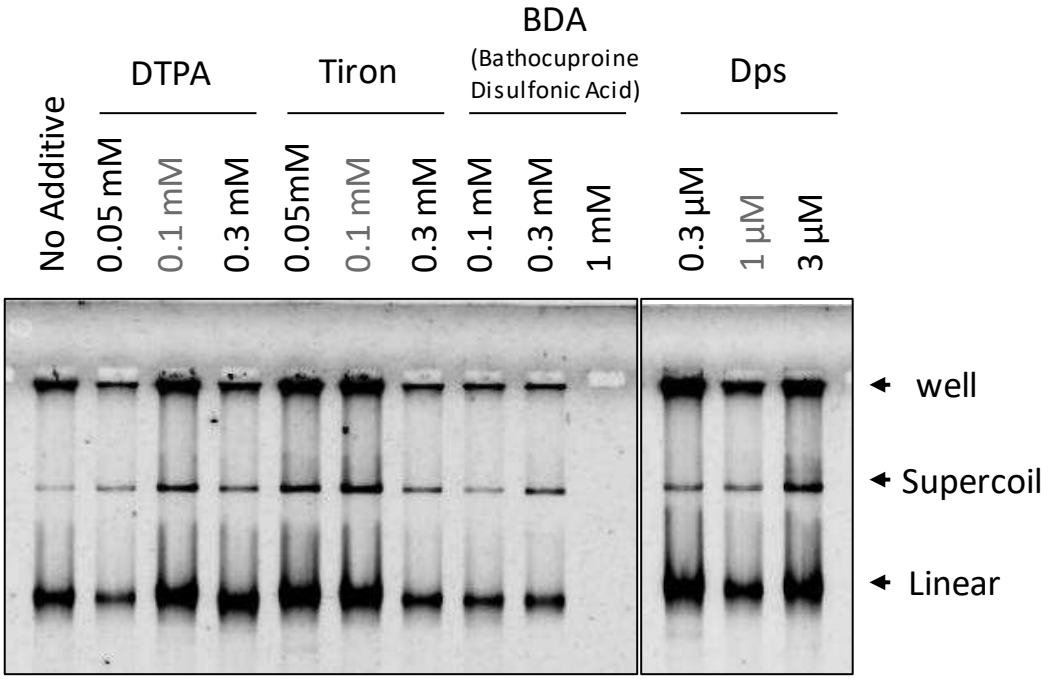


Figure 37

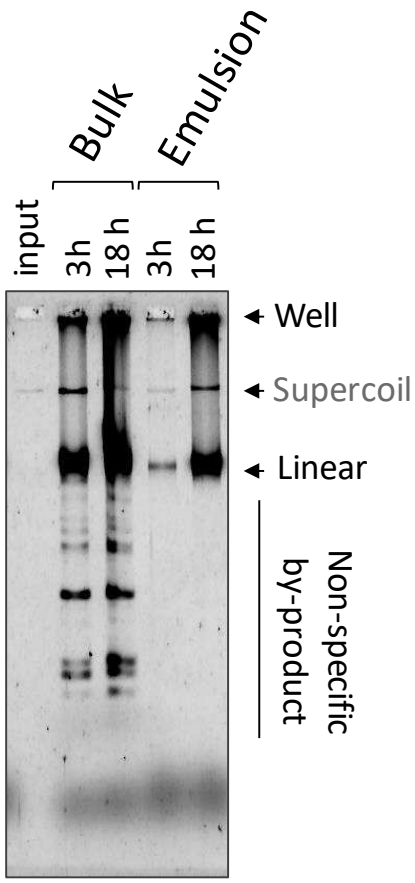
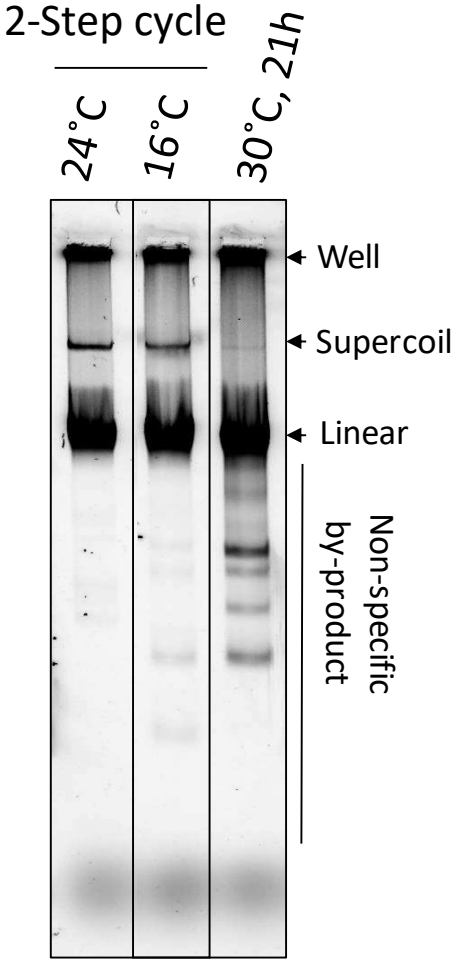


Figure 38

2-Step cycle

37˚C, 1 min
16 or 24˚C, 30 min] × 40 cycle