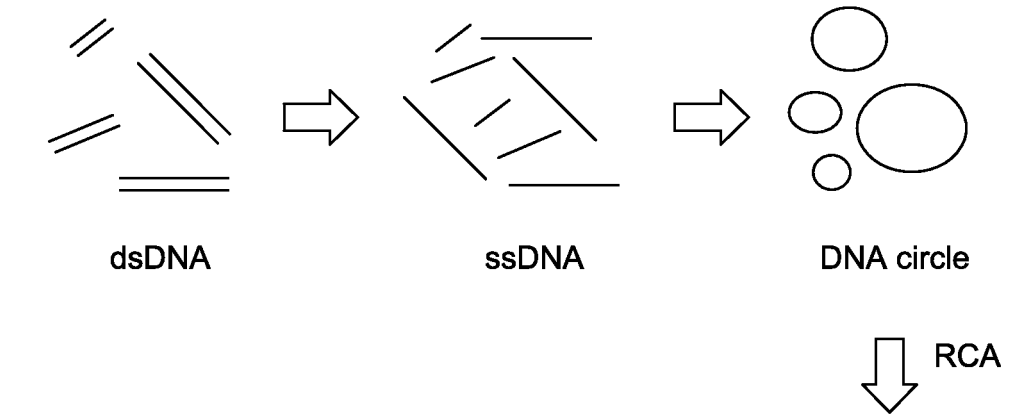

FIG. 1

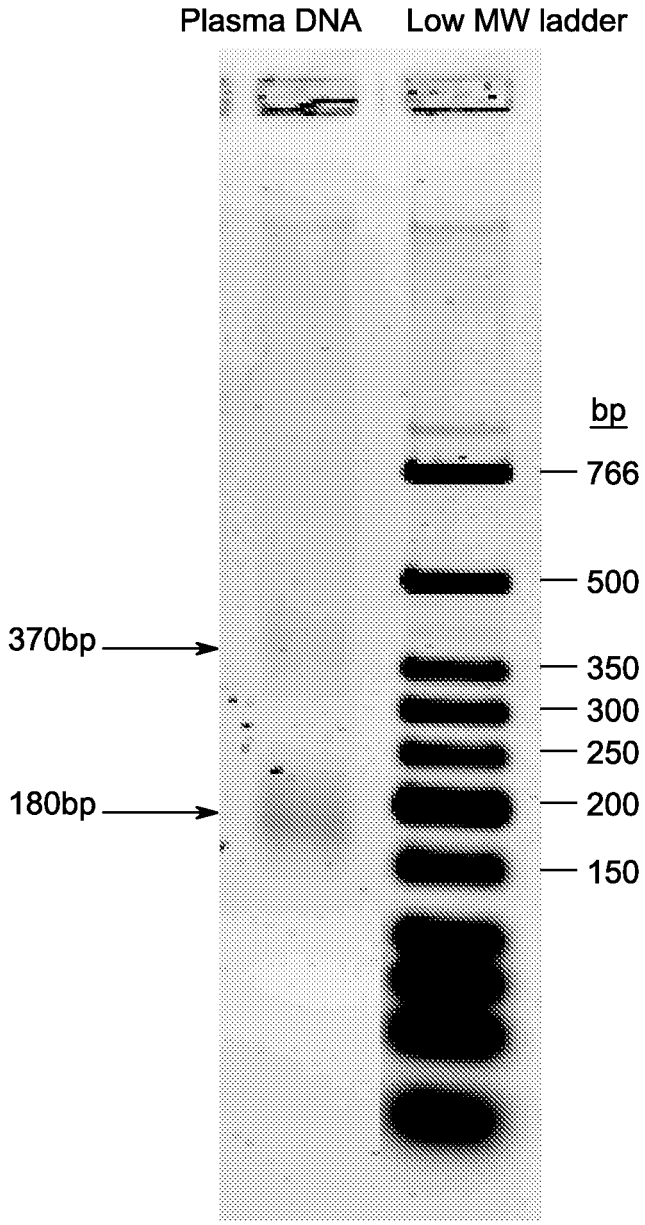
Plasma DNA
Low MW ladder
bp
766
500
370bp
350
300
250
180bp
200
150

FIG. 2

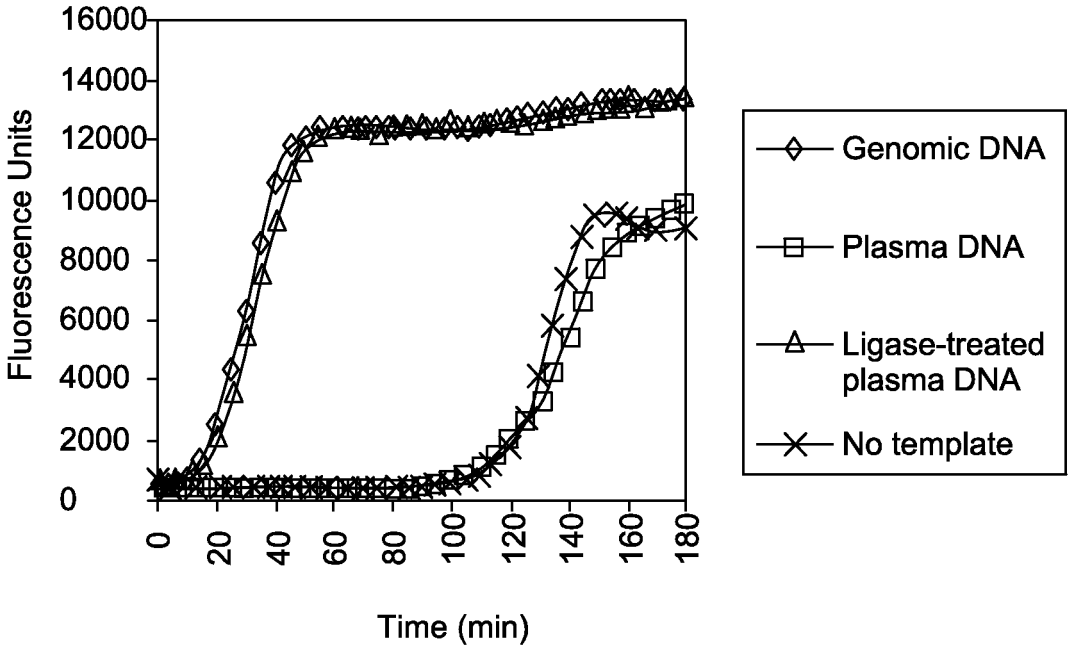
16000
14000
12000
10000
8000
6000
4000
2000
0
Fluorescence Units
0
20
40
60
80
100
120
140
160
180
Time (min)
Genomic DNA
Plasma DNA
Ligase-treated plasma DNA
No template

FIG. 3A

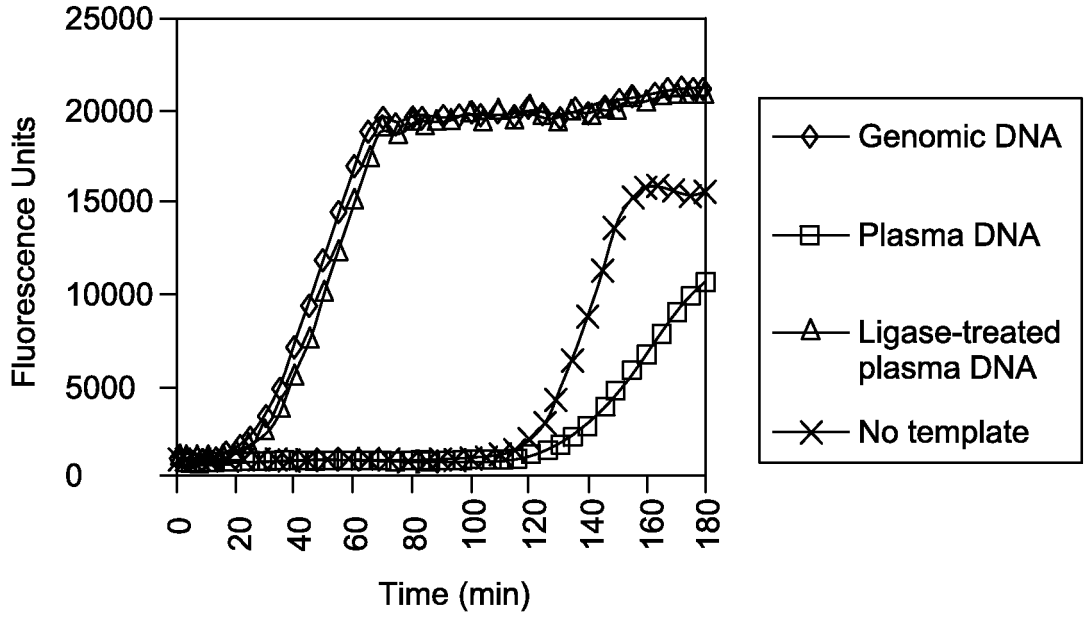
25000
20000
15000
10000
5000
0
Fluorescence Units
0
20
40
60
80
100
120
140
160
180
Time (min)
Genomic DNA
Plasma DNA
Ligase-treated plasma DNA
No template

FIG. 3B

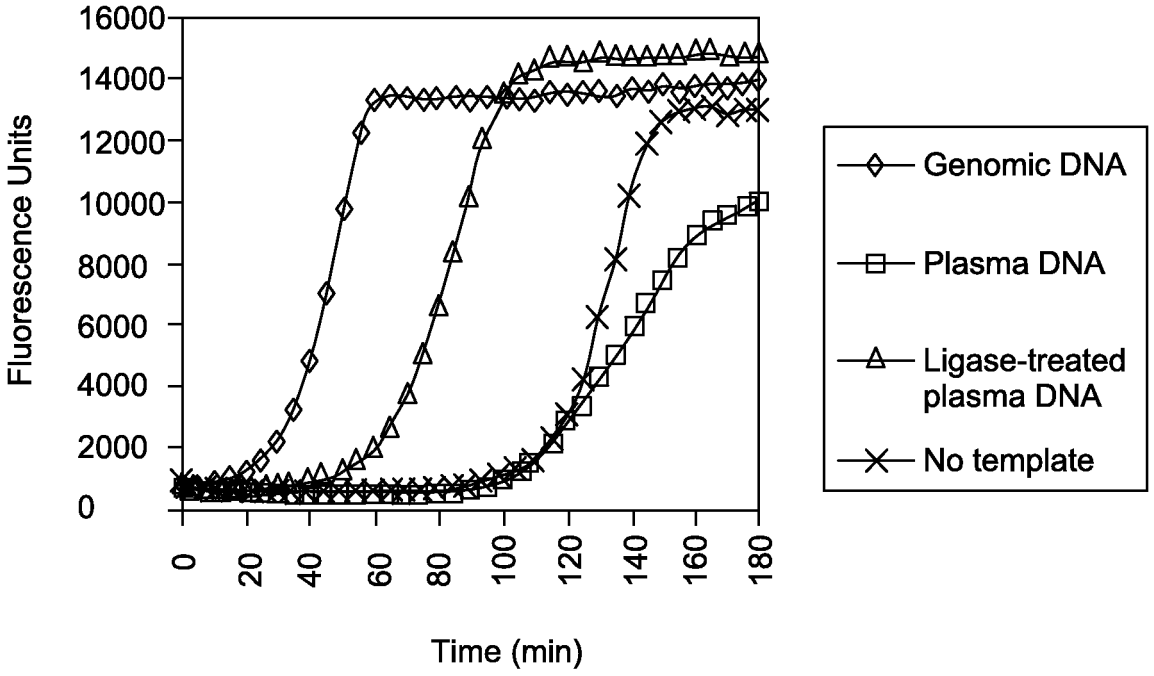
16000
14000
12000
10000
8000
6000
4000
2000
0
Fluorescence Units
0
20
40
60
80
100
120
140
160
180
Time (min)
Genomic DNA
Plasma DNA
Ligase-treated plasma DNA
No template

FIG. 3C

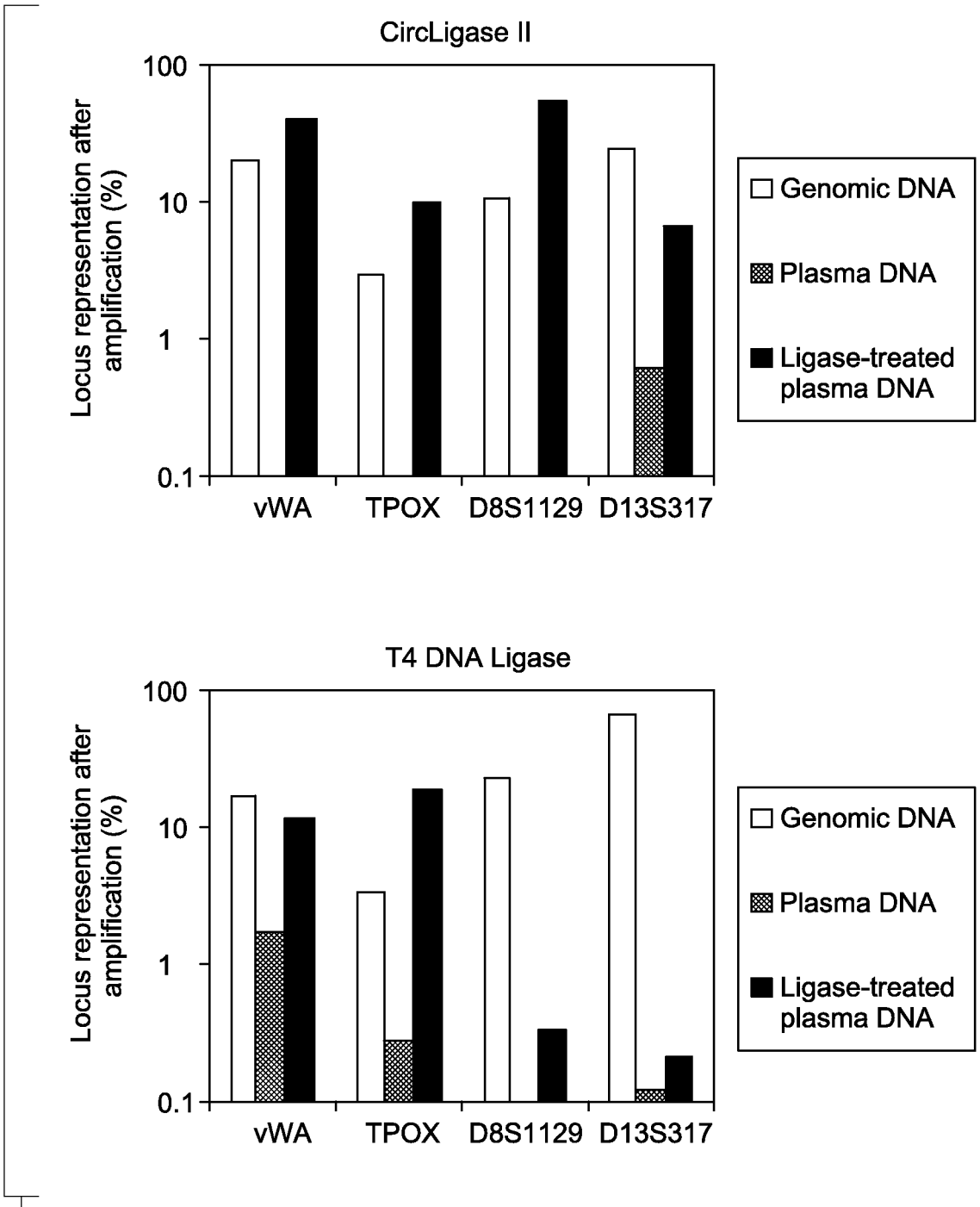
CircLigase II
Locus representation after amplification (%)
100
10
1
0.1
vWA
TPOX
D8S1129
D13S317
Genomic DNA
Plasma DNA
Ligase-treated plasma DNA
T4 DNA Ligase
Locus representation after amplification (%)
100
10
1
0.1
vWA
TPOX
D8S1129
D13S317
Genomic DNA
Plasma DNA
Ligase-treated plasma DNA

FIG. 4

Locus	Amplicon size (bp)	Genomic DNA	Plasma DNA	CircLigase II - treated plasma DNA
mini CSF1PO	89-129	22.26	X	25.55
mini TH01	51-98	23.43	X	25.24
mini TPOX	65-101	24.62	X	23.92
mini vWA	88-148	21.29	X	20.66
mini D5S818	81-117	22.14	X	26.19
mini D7S820	136-176	23.46	X	27.11
mini D8S1129	86-134	22.72	X	20.26
mini D13S317	88-132	23.03	28.73	25.24
mini D16S539	88-121	20.74	20.40	20.83
mini D18S51	113-193	23.84	X	X
Amelogenin	106, 112	20.72	29.67	22.27
D3S1358	99-147	20.13	20.77	20.85
Total		12/12 loci	4/12 loci	11/12 loci

FIG. 5

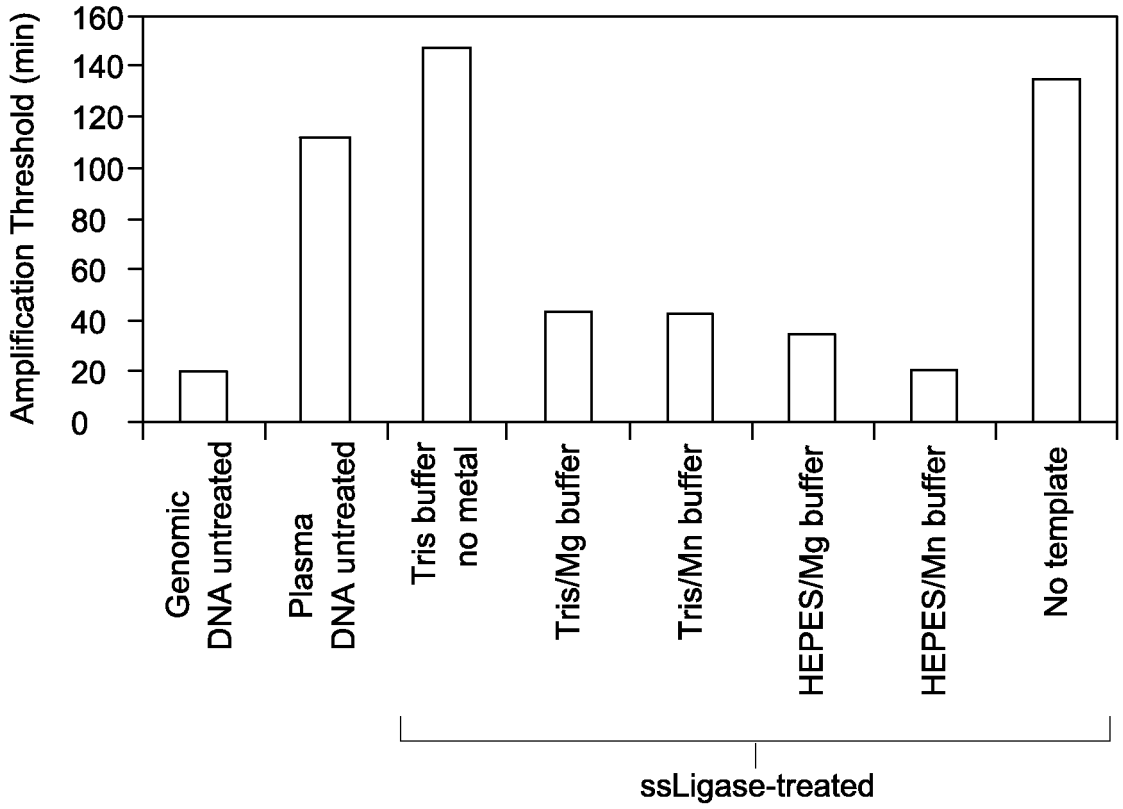
Amplification Threshold (min)
160
140
120
100
80
60
40
20
0
Genomic DNA untreated
Plasma DNA untreated
Tris buffer no metal
Tris/Mg buffer
Tris/Mn buffer
HEPES/Mg buffer
HEPES/Mn buffer
No template
ssLigase-treated

FIG. 6

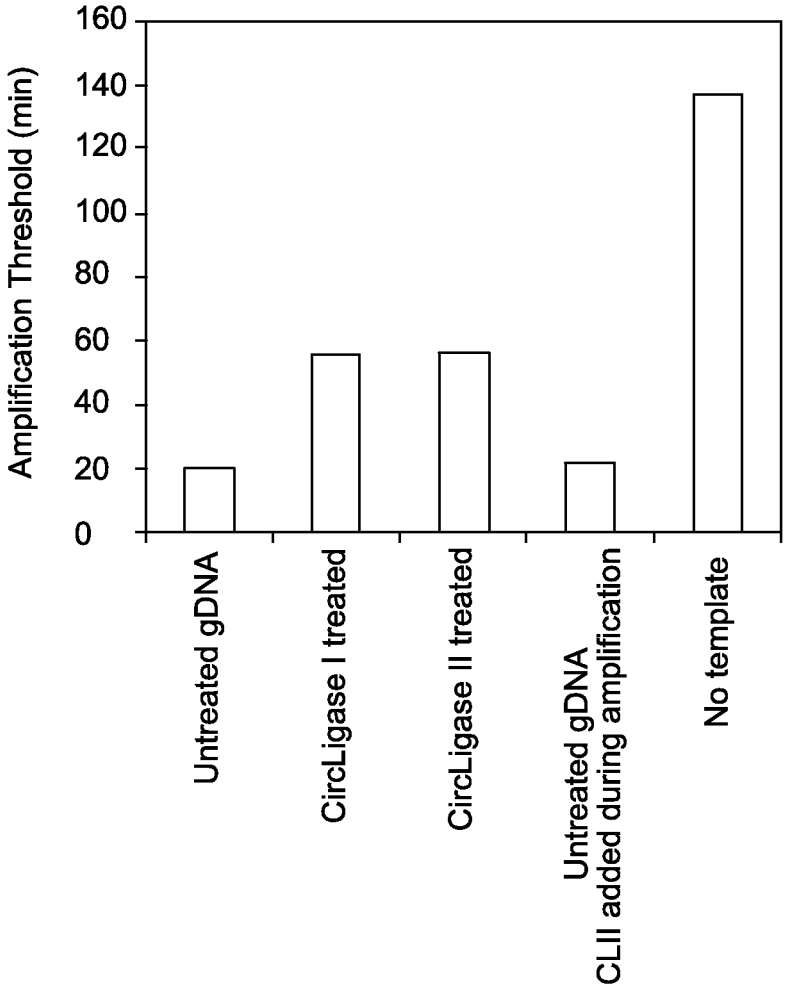
Amplification Threshold (min)
160
140
120
100
80
60
40
20
0
Untreated gDNA
CircLigase I treated
CircLigase II treated
Untreated gDNA
CLII added during amplification
No template

FIG. 7

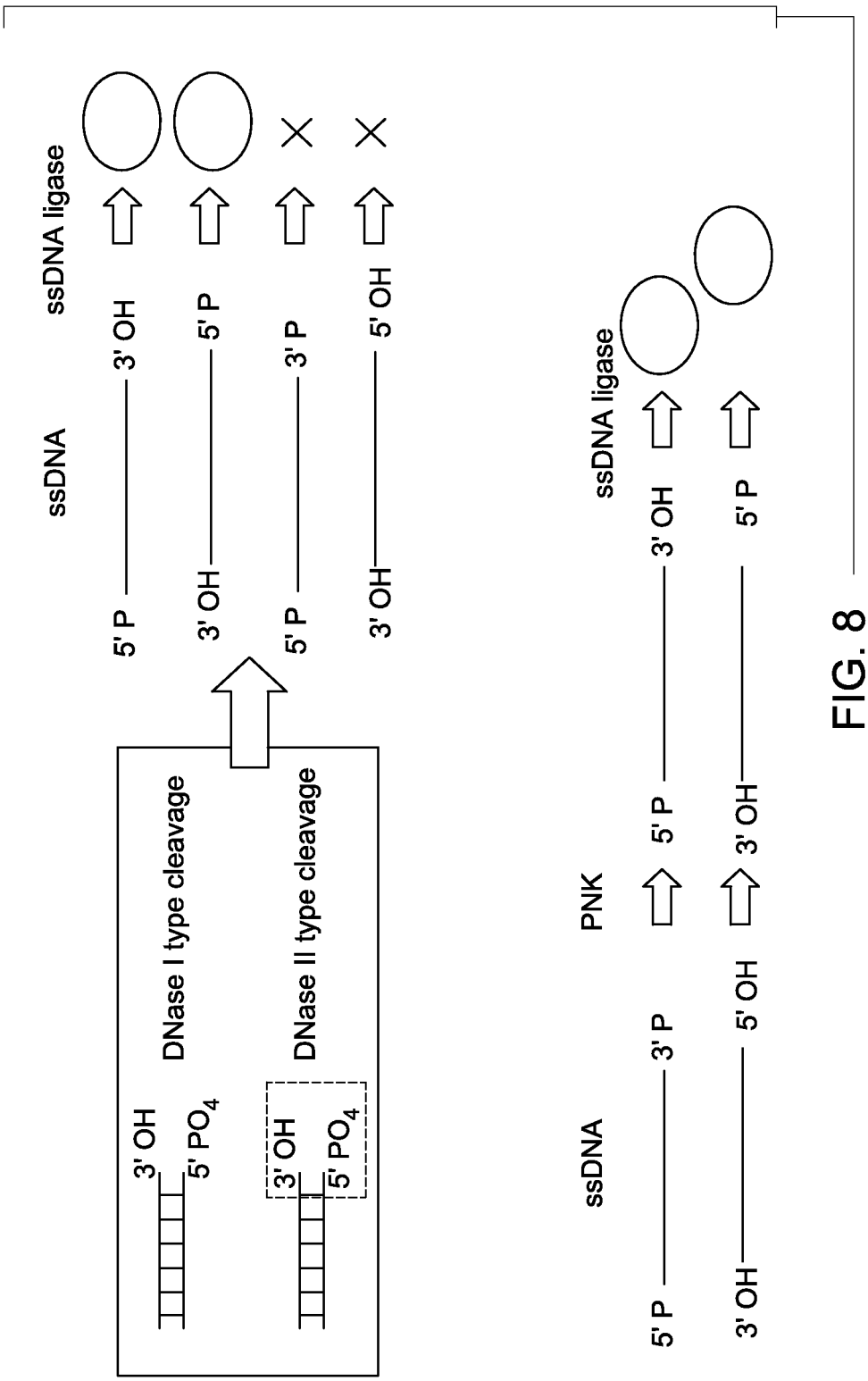
ssDNA
ssDNA ligase
5' P
3' OH
3' OH
5' P
5' P
3' P
3' OH
5' OH
DNase I type cleavage
DNase II type cleavage
3' OH
5' PO4
3' OH
5' PO4
ssDNA
PNK
ssDNA ligase
5' P
3' P
3' OH
5' OH
5' P
3' OH

FIG. 8

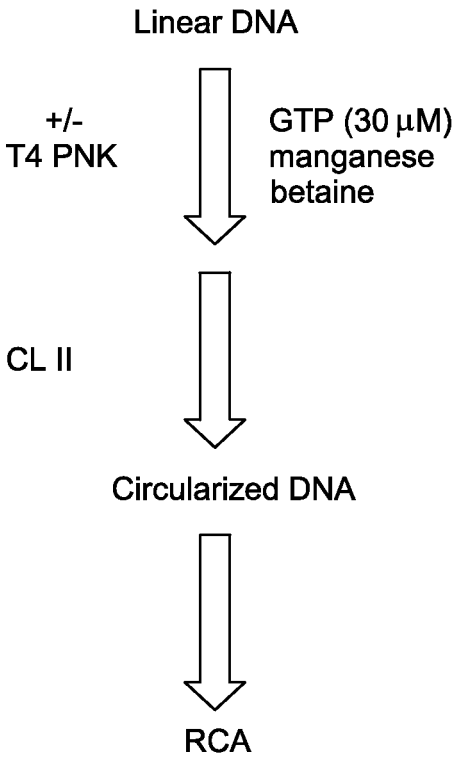
Linear DNA
+/-
T4 PNK
GTP (30 μM)
manganese
betaine
CL II
Circularized DNA
RCA

FIG. 9

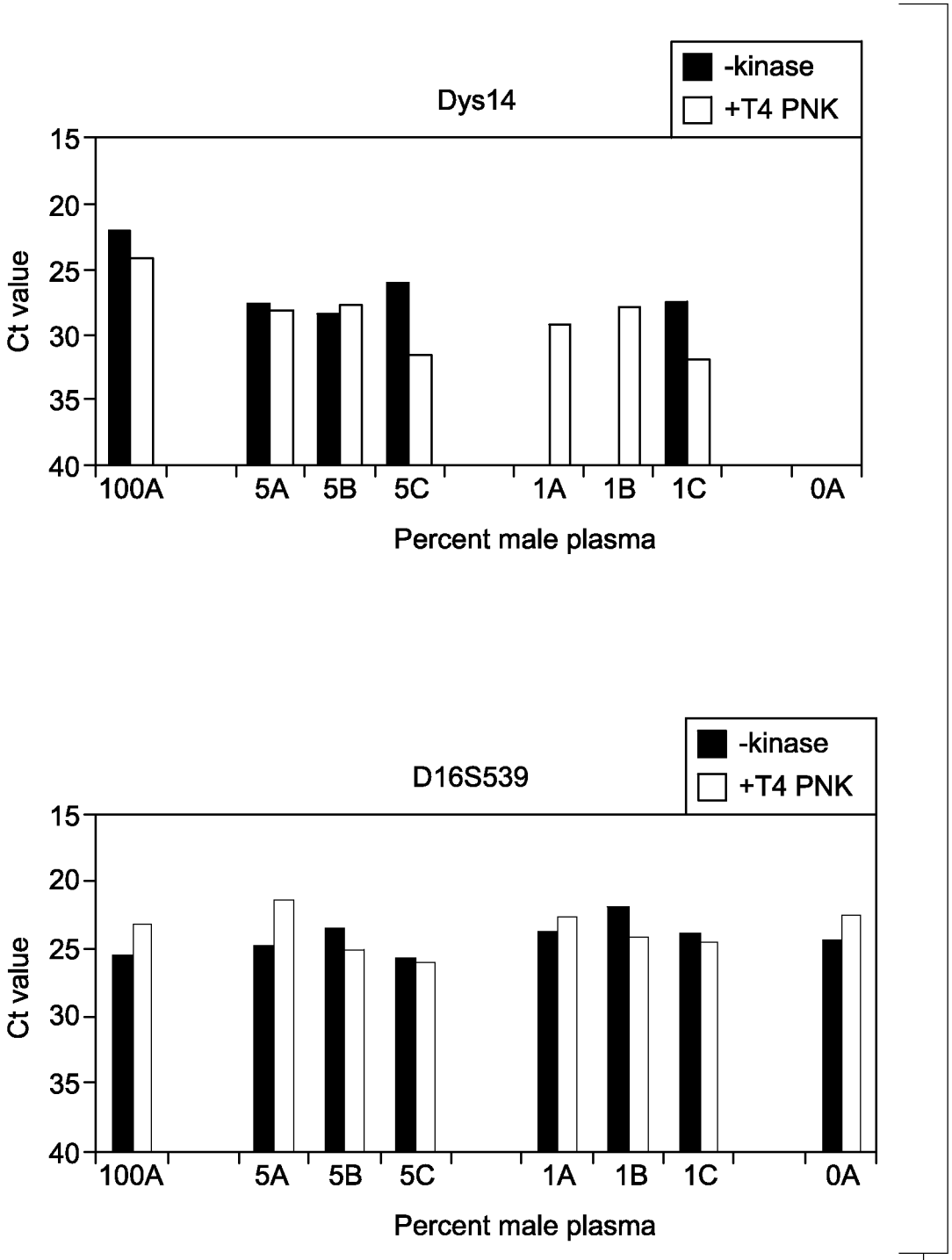
Dys14
-kinase
+T4 PNK
Ct value
15
20
25
30
35
40
100A
5A
5B
5C
1A
1B
1C
0A
Percent male plasma
D16S539
-kinase
+T4 PNK
Ct value
15
20
25
30
35
40
100A
5A
5B
5C
1A
1B
1C
0A
Percent male plasma

FIG. 10

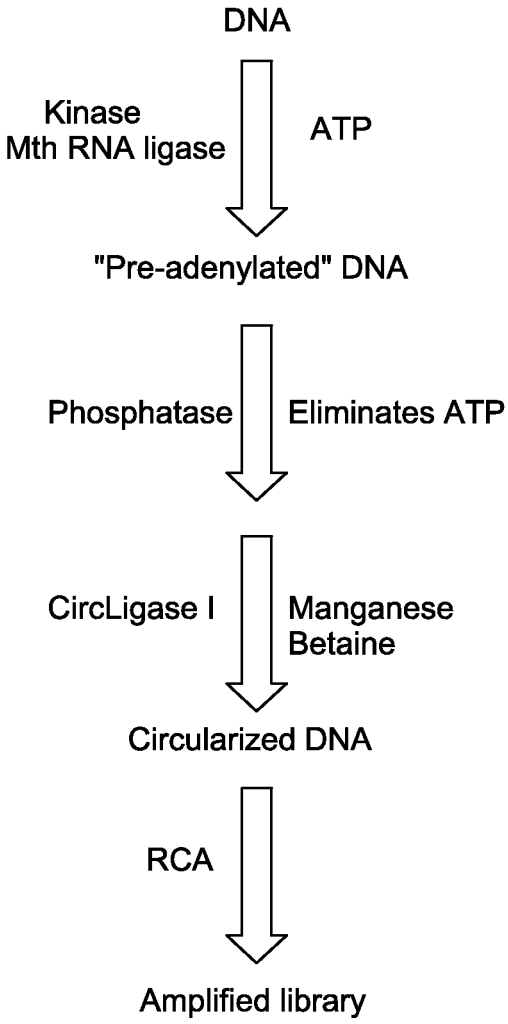
DNA
Kinase
Mth RNA ligase
ATP
"Pre-adenylated" DNA
Phosphatase
Eliminates ATP
CircLigase I
Manganese
Betaine
Circularized DNA
RCA
Amplified library

FIG. 11

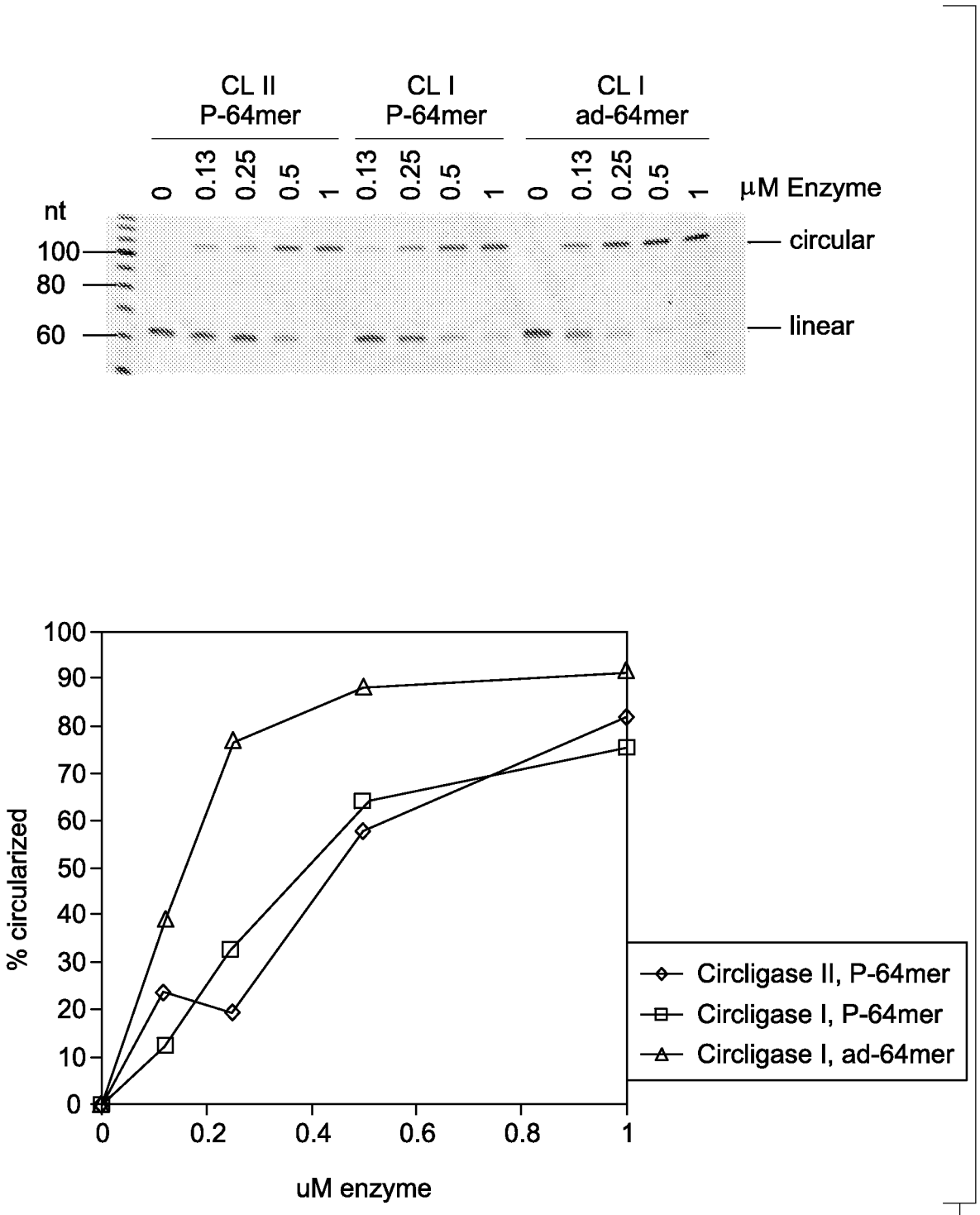
CL II
P-64mer
CL I
P-64mer
CL I
ad-64mer
0
0.13
0.25
0.5
1
0.13
0.25
0.5
1
0
0.13
0.25
0.5
1
µM Enzyme
nt
100
80
60
circular
linear
% circularized
100
90
80
70
60
50
40
30
20
10
0
0
0.2
0.4
0.6
0.8
1
uM enzyme
Circligase II, P-64mer
Circligase I, P-64mer
Circligase I, ad-64mer

FIG. 12

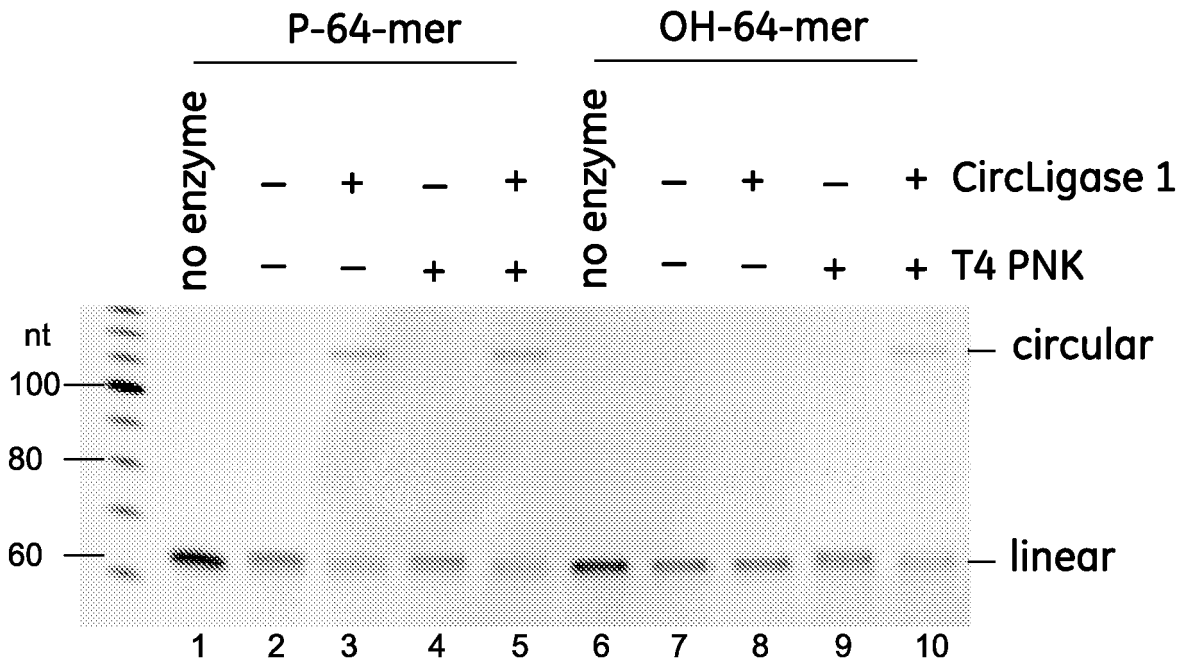
P-64-mer
OH-64-mer
no enzyme
no enzyme
– + – + – + – + CircLigase 1
– – + + – – + + T4 PNK
nt
100
80
60
circular
linear
1 2 3 4 5 6 7 8 9 10

FIG. 13