

Figure 1

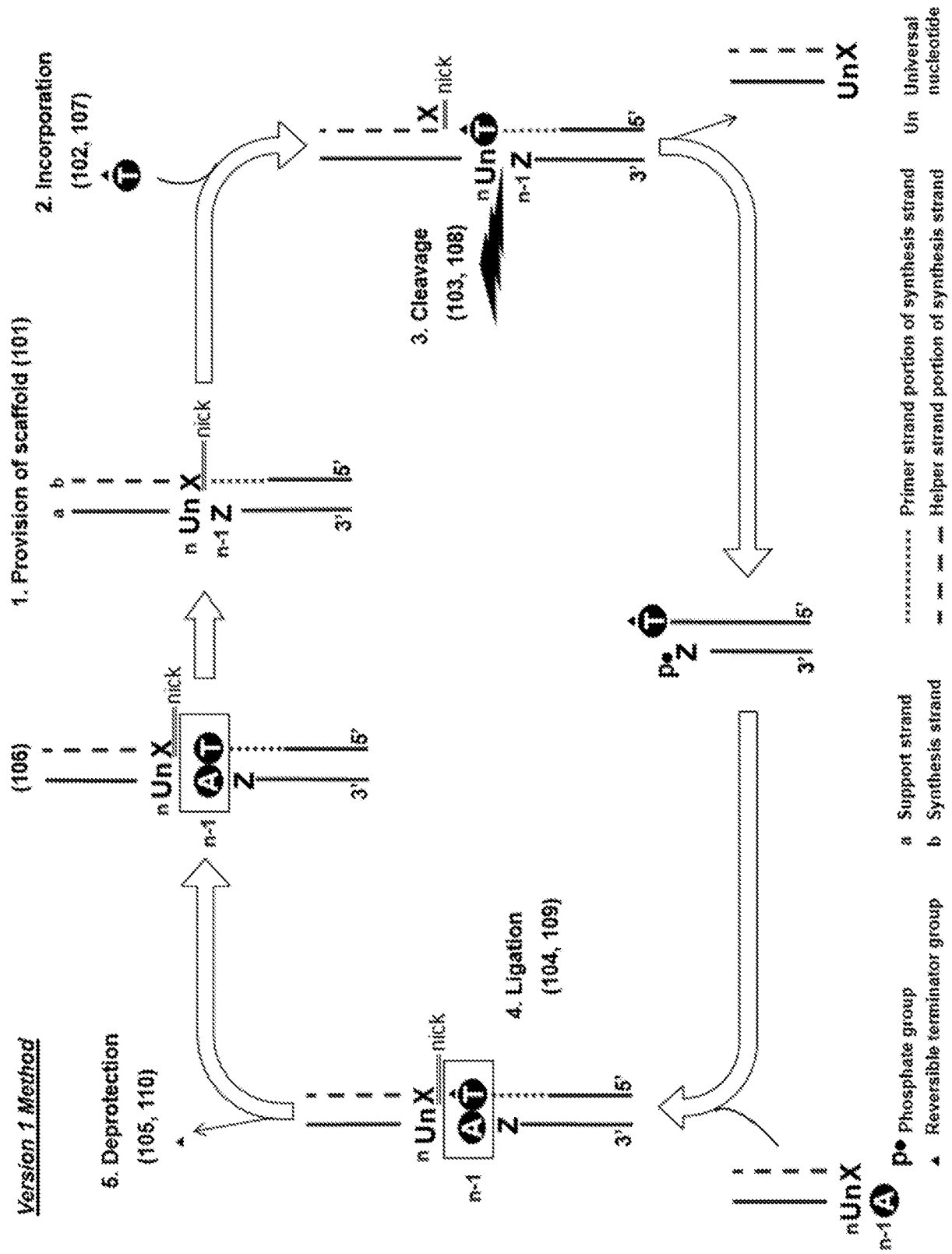


Figure 2

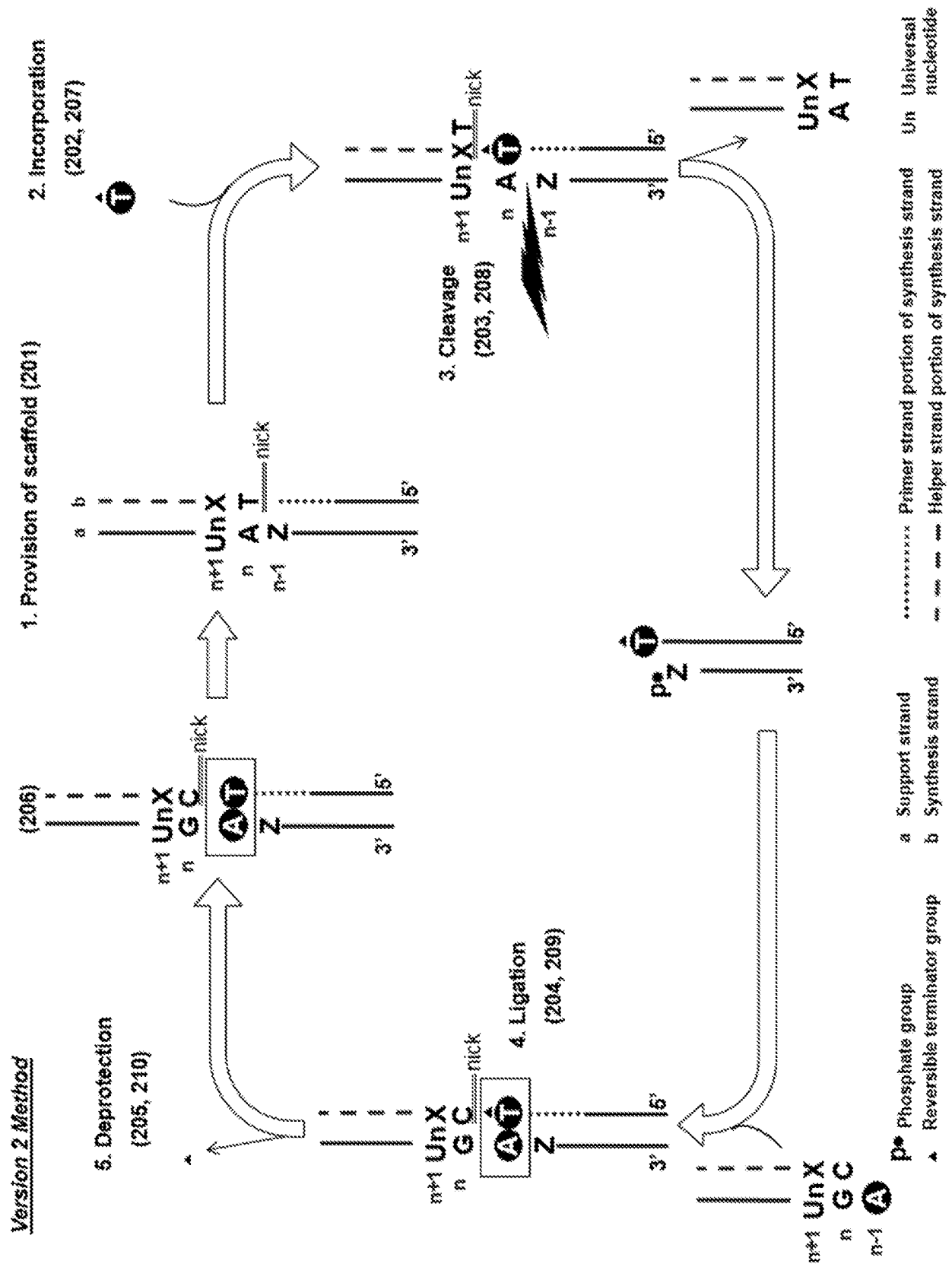


Figure 3a

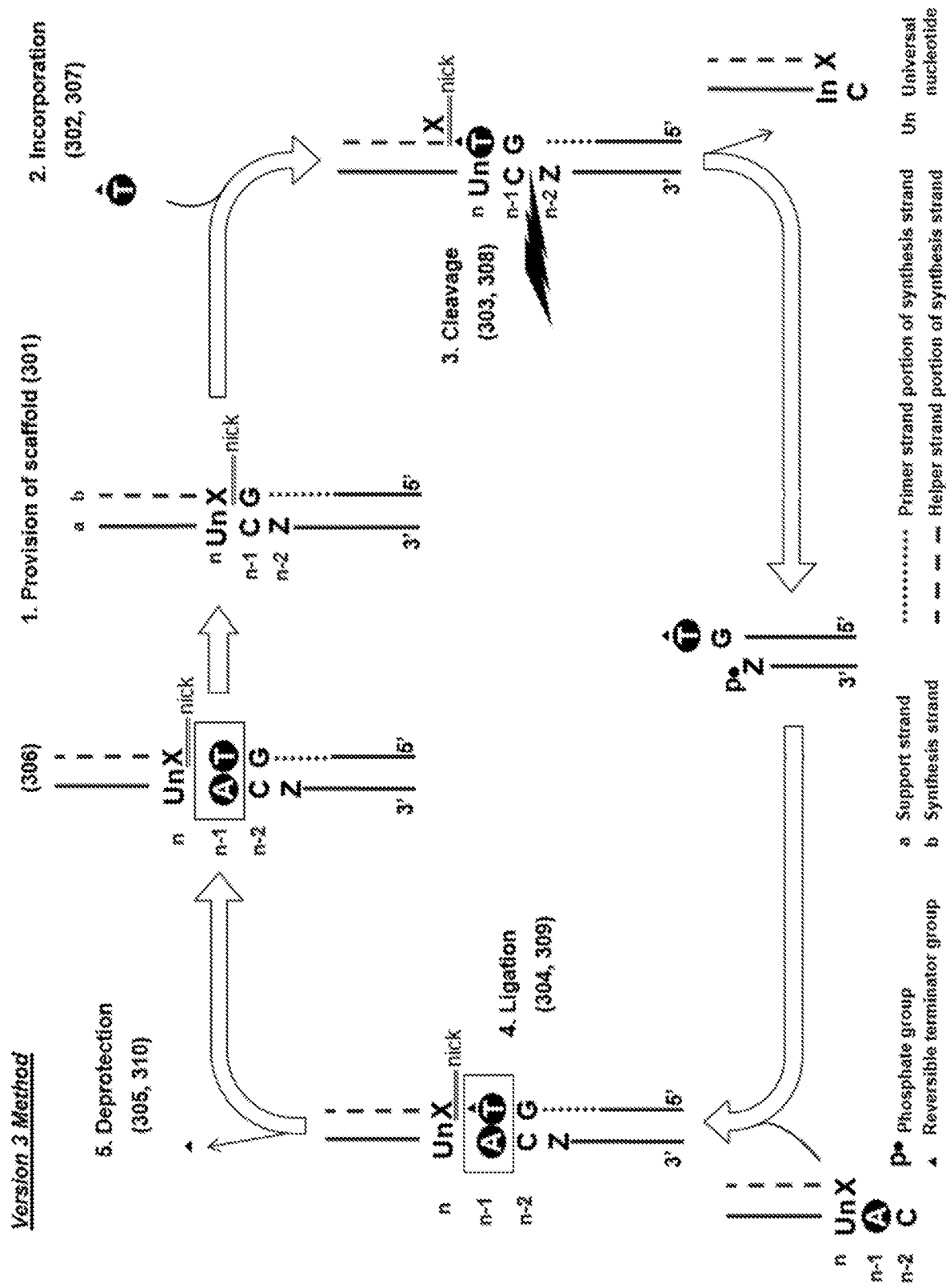


Figure 3b

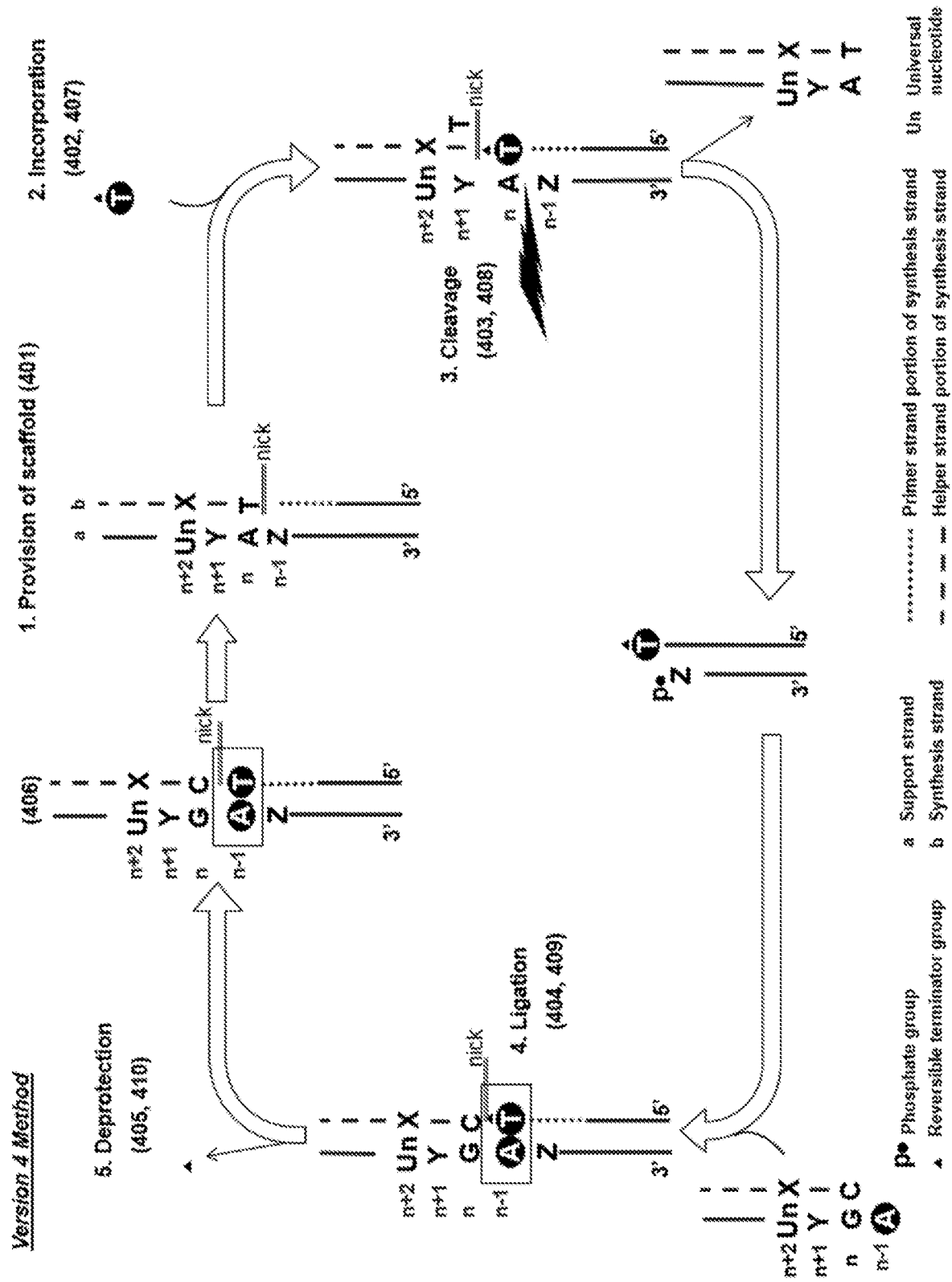


Figure 3c

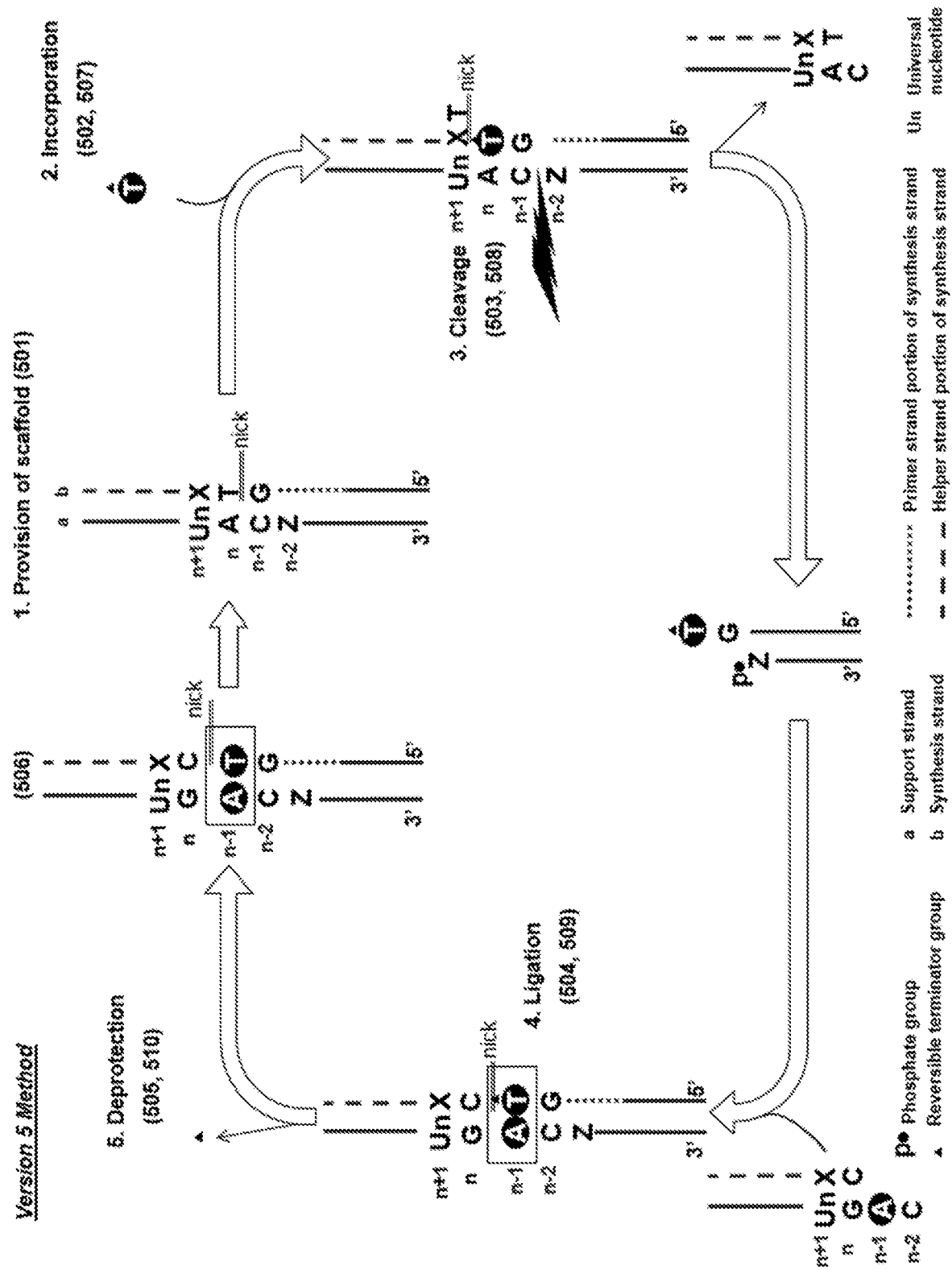
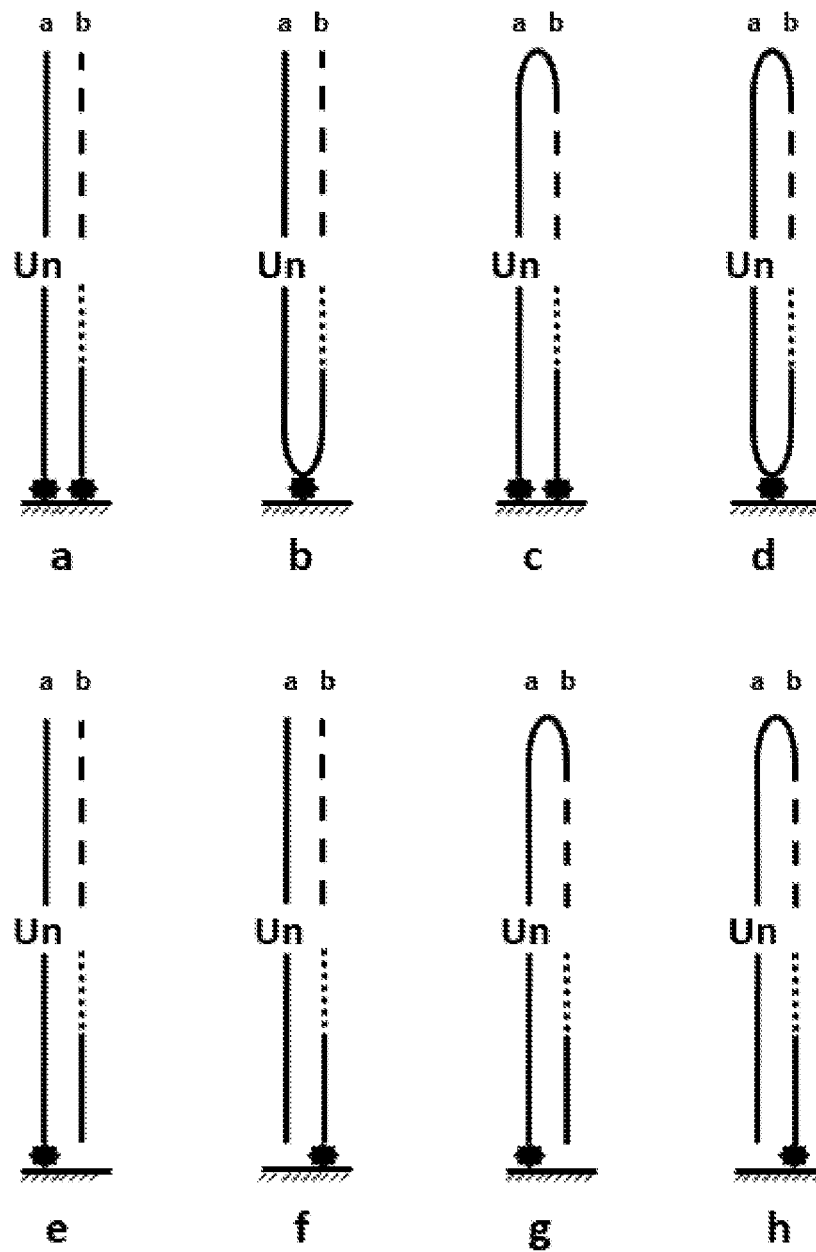


Figure 4



a Support strand
 b Synthesis strand
 Un Universal nucleotide
 Primer strand portion of synthesis strand
 - - - - - Helper strand portion of synthesis strand

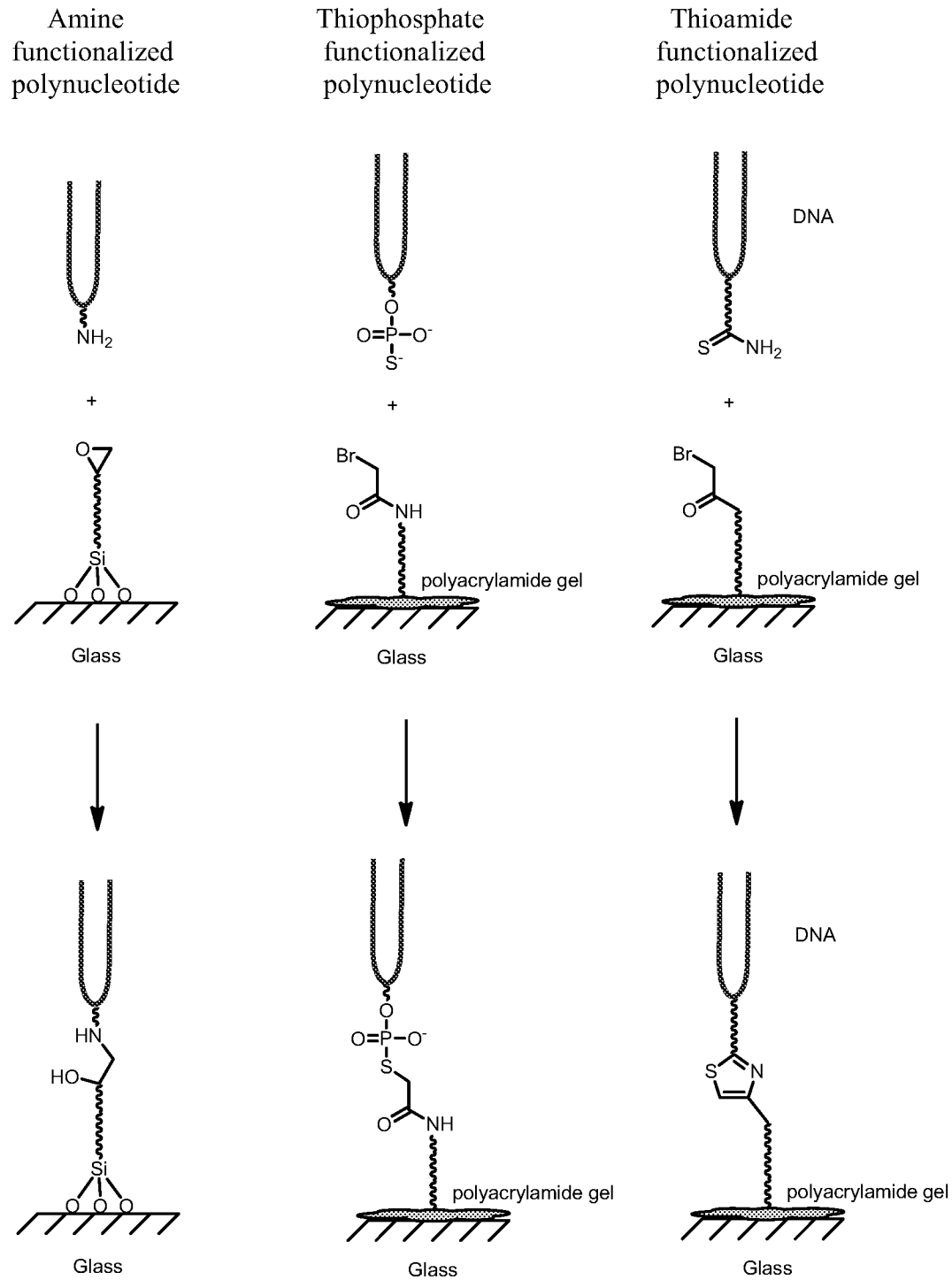
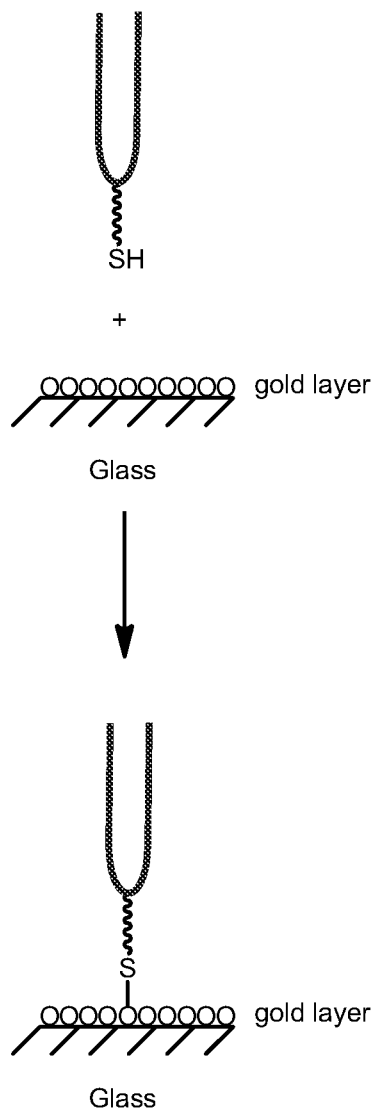
Figure 4i

Figure 4 j

Example method for
attaching polynucleotide to
gold surface:



Example method for
attaching polynucleotide by
biotin-streptavidin binding:

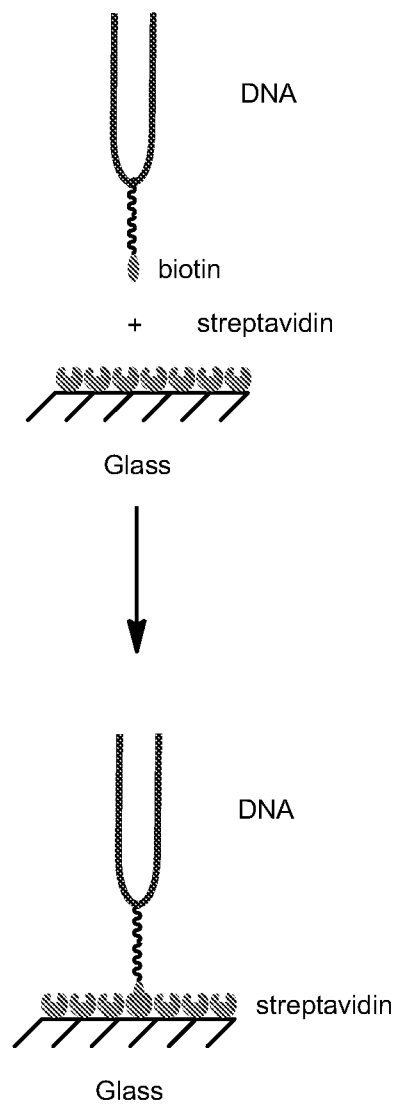


Figure 5a

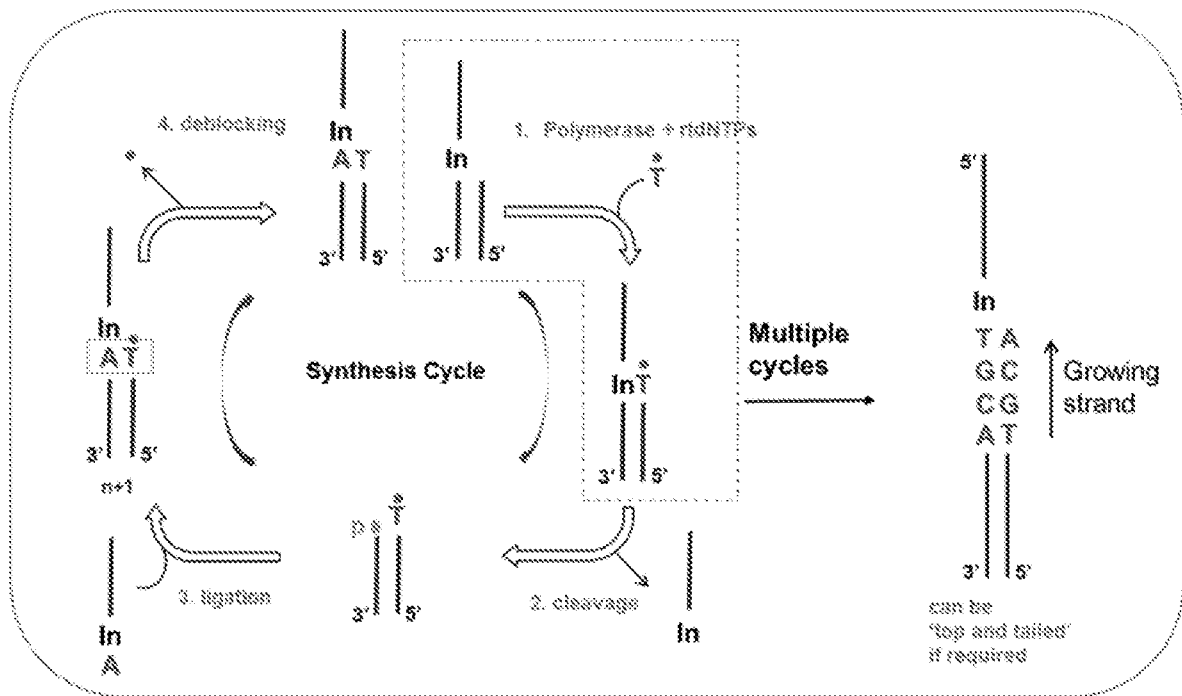


Figure 5b

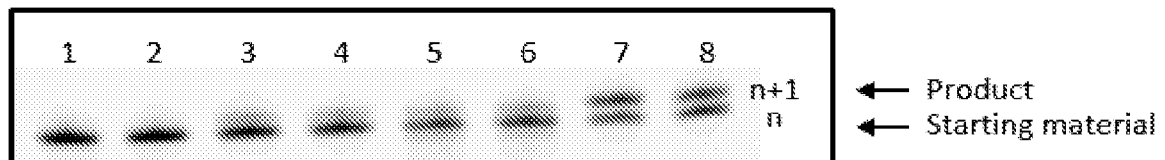
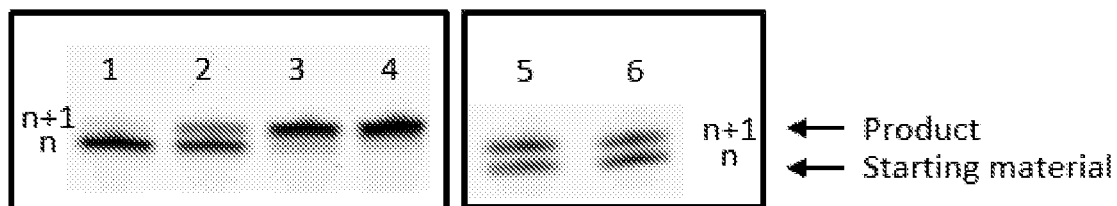
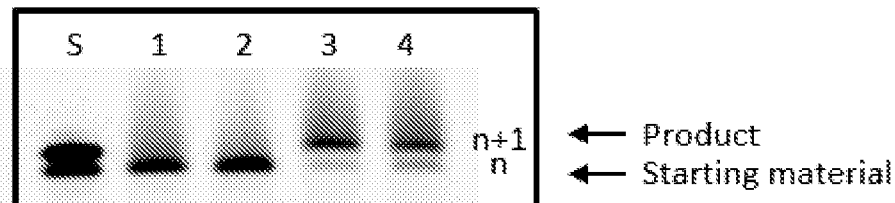


Figure 5c

Lane no.	Incorporation at 50°C	DNA polymerase	conversion
1	3'-O-allyl-dTTPs	Bst	<1%
2	3'-O-azidomethyl-dTTPs	Bst	<1%
3	3'-O-allyl-dTTPs	Deep Vent (exo-)	5%
4	3'-O-azidomethyl-dTTPs	Deep Vent (exo-)	5%
5	3'-O-allyl-dTTPs by	Therminator I	8%
6	3'-O-azidomethyl-dTTPs	Therminator I	8%
7	3'-O-allyl-dTTPs	Therminator IX	55%
8	3'-O-azidomethyl-dTTPs	Therminator IX	48%

Figure 5d**Figure 5e**

Lane No.	Incorporation	Enzyme	Temp.	Conversion
1	3'-O-allyl-dTTPs	Therminator IX	37 °C	<5%
2	3'-O-azidomethyl-dTTPs	Therminator IX	37 °C	15%
3	3'-O-allyl-dTTPs	Therminator IX	50 °C	55%
4	3'-O-azidomethyl-dTTPs	Therminator IX	50 °C	48%
5	3'-O-allyl-dTTPs	Therminator IX	65 °C	>90%
6	3'-O-azidomethyl-dTTPs	Therminator IX	65 °C	>90%

Figure 5f**Figure 5g**

Lane No.	incorporation	Enzyme	Temp	2mM Mn ²⁺	conversion
1	3'-O-allyl-dTTPs	Therminator IX	65 °C	No	<1%
2	3'-O-azidomethyl-dTTPs	Therminator IX	65 °C	No	<1%
3	3'-O-allyl-dTTPs	Therminator IX	65 °C	Yes	>90%
4	3'-O-azidomethyl-dTTPs	Therminator IX	65 °C	Yes	>90%

Figure 5h

SEQ ID NO:	Oligonucleotide name	Sequence	Modification
1	Primer	5'-GCGACAGGTGACTGCAGC-3'	TAMRA at 5'-end (attached to terminal guanine)
2	Template	5'-CACATCACGTCGTAGTCXGCTGCAG TCACCTGTCGC-3'	X = 2'- deoxyinosine

Figure 6a

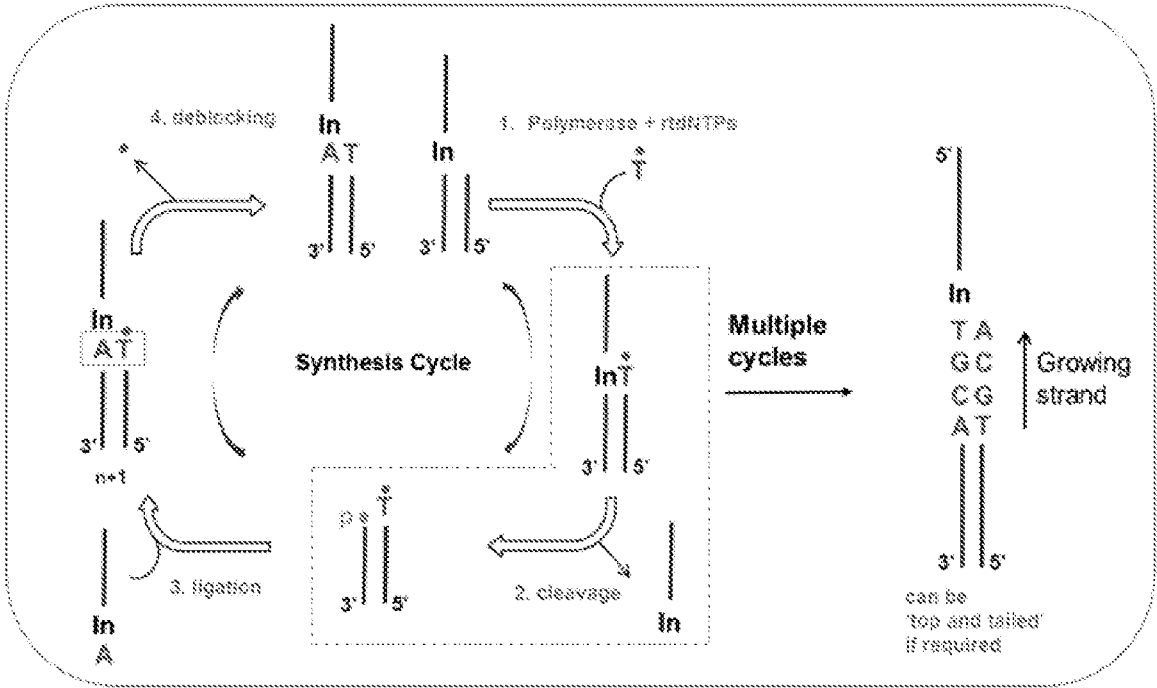


Figure 6b

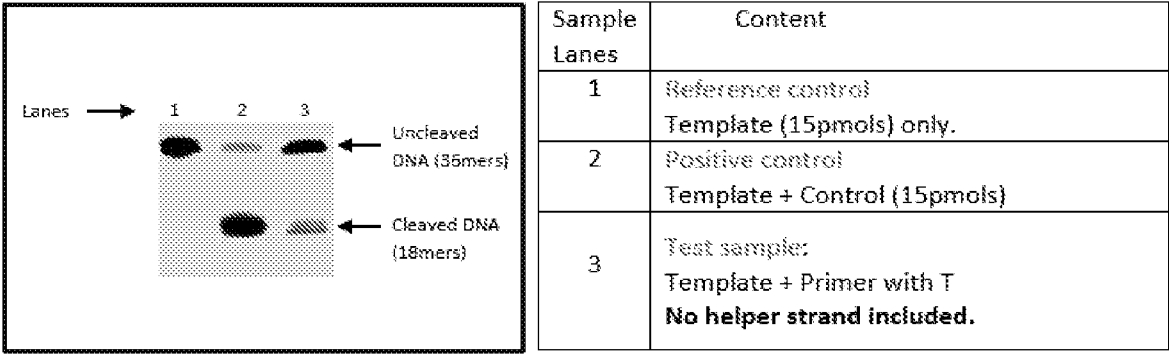
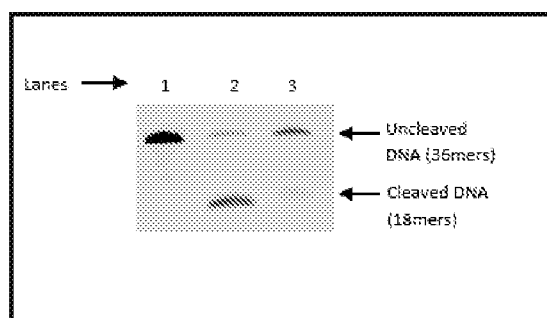


Figure 6c



Sample Lanes	Content
1	Reference control Template (15pmols) only.
2	Positive control Template + Control (15pmols)
3	Test sample: Template + Primer with T No helper strand included.

Figure 6d

Gel	Cleavage reaction combination	% Conversion of cleaved DNA without a helper strand
A	hAAG/ Chemical base	< 10
B	hAAG/Endo VIII	< 7

Figure 6e

SEQ ID NO:	Oligonucleotide name	Sequence	Modification
3	Template	5'-CACATCACGTCGTAGTCXGCT GCAGTCACCTGTCGC-3'	TAMRA at 5'-end X = 2' deoxyinosine
4	Primer with T	5'-GCGACAGGTGACTGCAGCT-3'	None
5	Control	5'-GCGACAGGTGACTGCAGCTGA CTACGACGTGATGTG-3'	None

Figure 7a

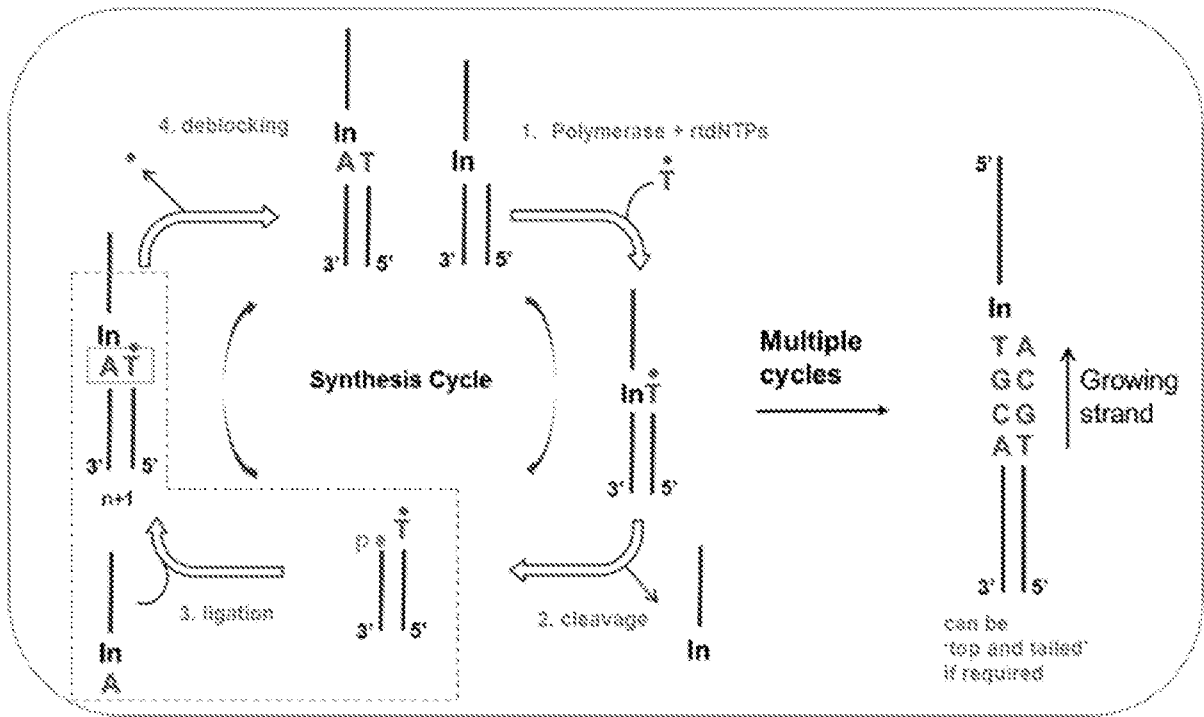


Figure 7b

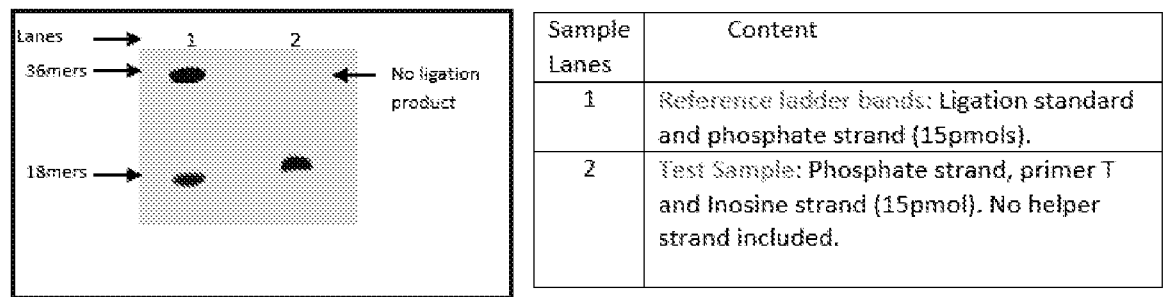


Figure 7c

SEQ ID NO:	Oligonucleotide name	Sequence	Modification
6	Ligation standard	5'-CACATCACGTCGTAGT X A GCTGCAGTCACCTGTCGC-3'	X = 2'-deoxyinosine
7	Phosphate strand	5'-GCTGCAGTCACCTGTCGC-3'	TAMRA at 3'-end (attached to terminal cytosine), phosphate at 5' end
8	Primer with T	5'-GCGACAGGTGACTGCAGCT-3'	None
9	Inosine strand	5'-CACATCACGTCGTAGT X A-3'	X = 2'-deoxyinosine

Figure 8a

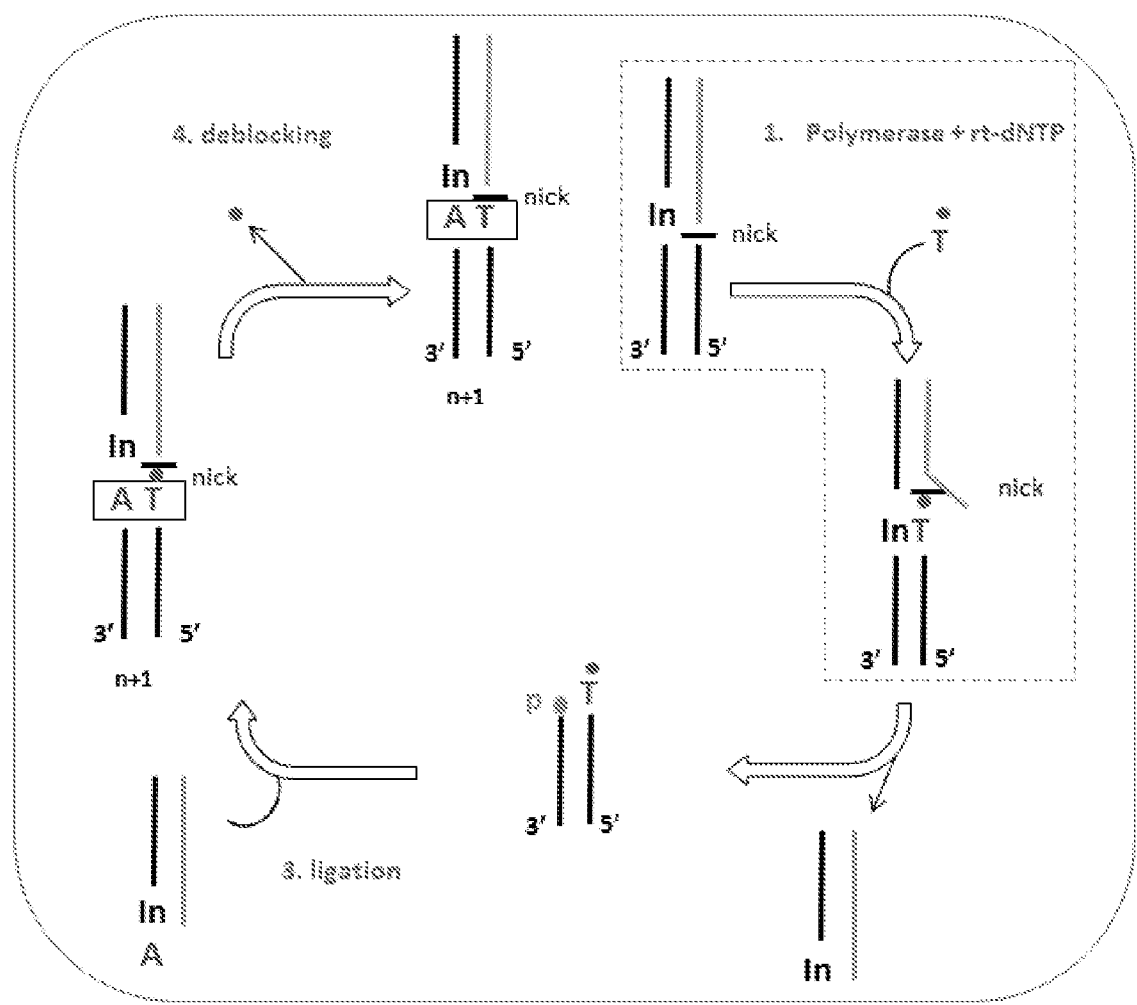
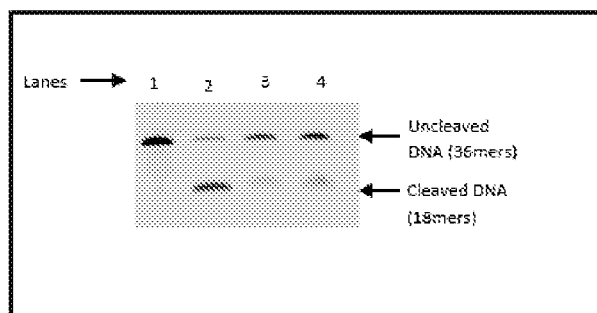


Figure 8b

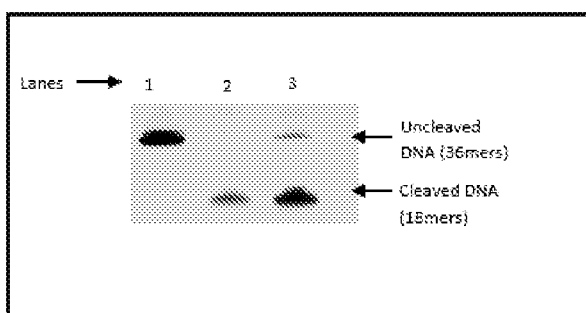
SEQ ID NO:	Oligonucleotide name	Sequence	Modification
10	Primer	5'-GCGACAGGTGACTGCAGC-3'	TAMRA at 5'-end (attached to terminal guanine)
11	Template	5'-CACATCACGTCGTAGTCXGCTGCAG TCACCTGTCGC-3'	X = 2'-deoxyinosine
12	Helper strand	5'-CGACTACGACGTGATGTG-3'	None

Figure 9c



Sample Lanes	Content
1	Reference control Template (15pmols) only.
2	Positive control Template + Control (15pmols)
3	Test sample I: Template + Primer with T No helper strand included. (15pmols)
4	Test Sample II: Template + Primer with T + Helper strand (15pmols).

Figure 9d



Sample Lanes	Content
1	Reference control Template (15pmols) only.
2	Positive control Template + Control (15pmols)
3	Test Sample I: Template + Primer with T + Helper strand (15pmols).

Figure 9e

Gel	Cleavage reaction combination	% Conversion of cleaved DNA with a helper strand
A	hAAG/ Chemical base	50
B	hAAG/Endo VIII	< 10
C	hAAG/Alternative chemical base	90*

Figure 9f

SEQ ID NO:	Oligonucleotide name	Sequence	Modification
13	Template	5'-CACATCACGTCGTAGTCXGCT GCAGTCACCTGTCGC-3'	TAMRA at 5'-end (attached to terminal cytosine), X = deoxyinosine
14	Primer with T	5'-GCGACAGGTGACTGCAGCT-3'	None
15	Control	5'-GCGACAGGTGACTGCAGCTGA CTACGACGTGATGTG-3'	None
16	Helper Strand	5'-TGACTACGACGTGATGTG-3'	None

Figure 10a

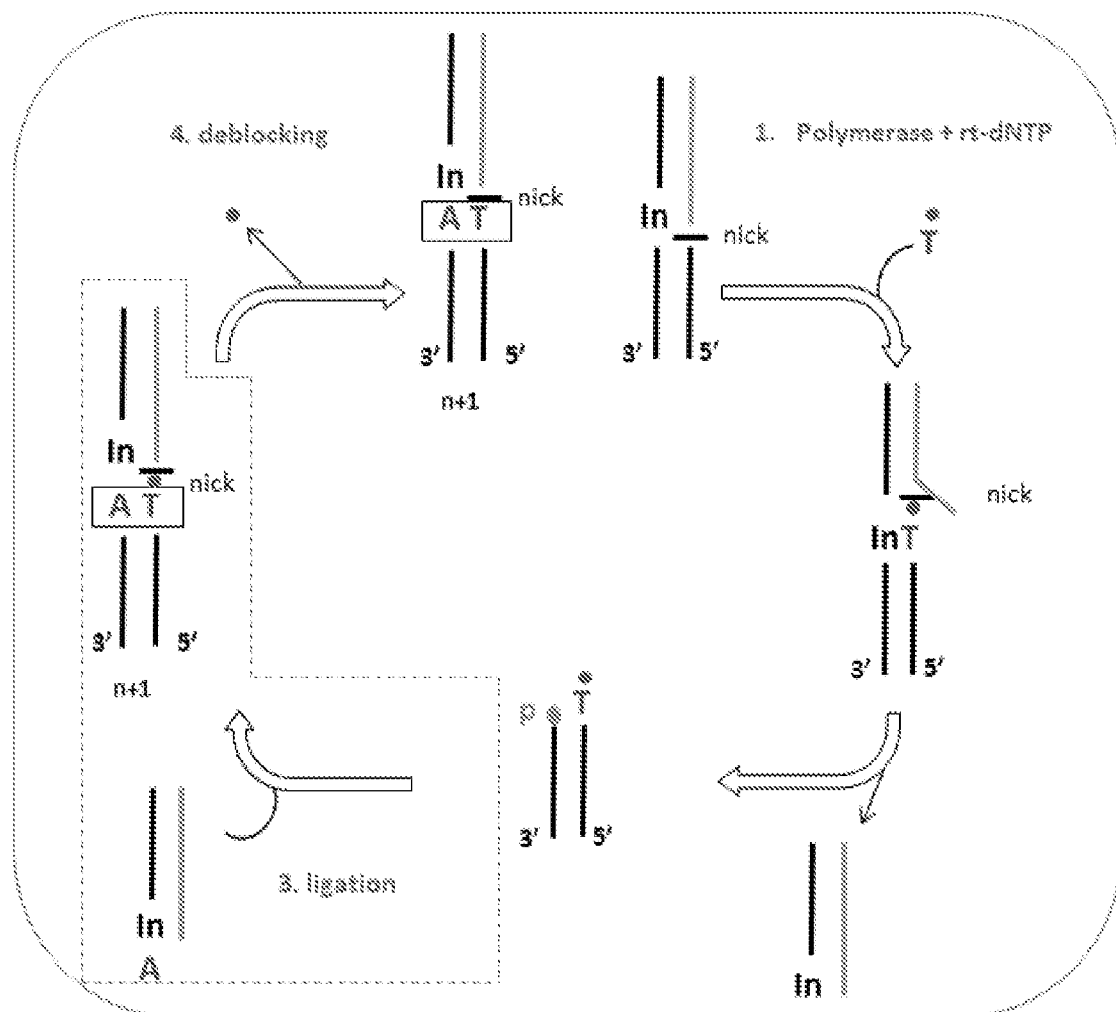


Figure 10b

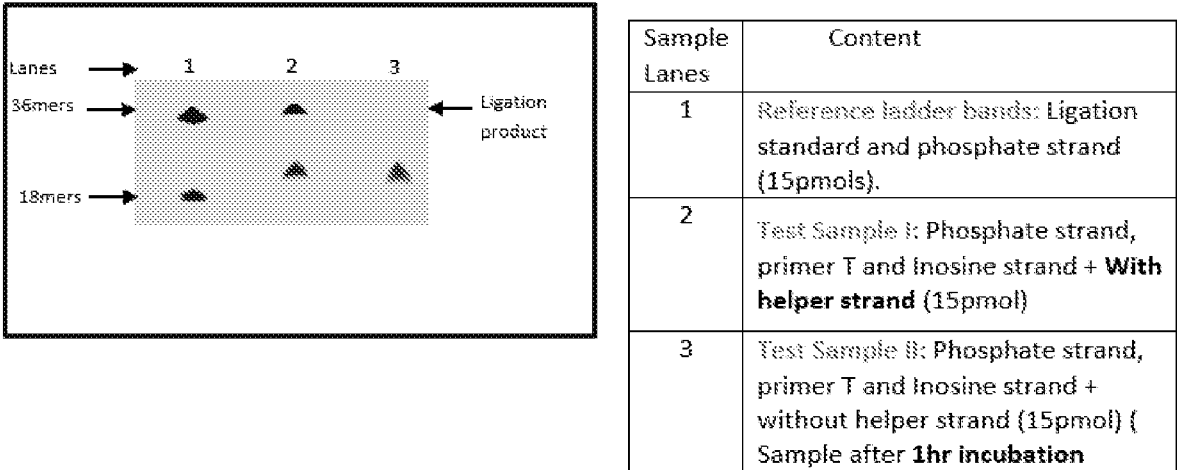


Figure 10c

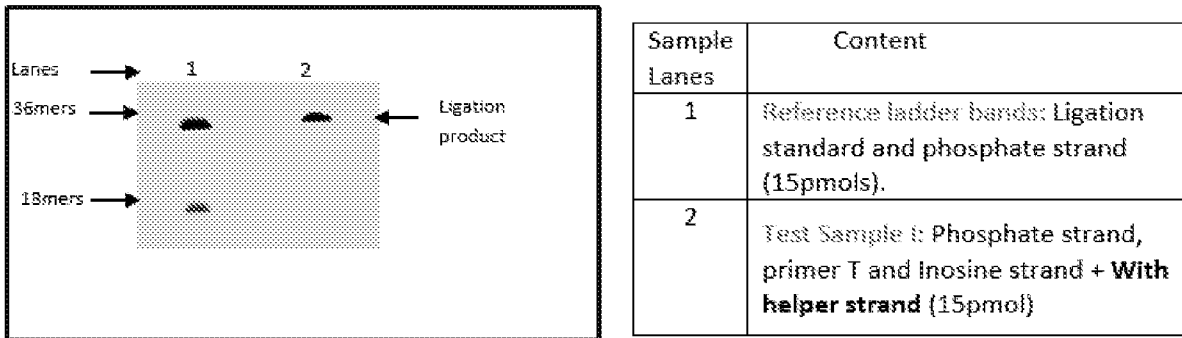


Figure 10d

SEQ ID NO:	Oligonucleotide name	Sequence	Modification
17	Ligation standard	5'-CACATCACGTCGTAGT X A GCTGCAGTCACCTGTCGC-3'	X = 2'-deoxyinosine
18	Phosphate strand	5'-GCTGCAGTCACCTGTCGC-3'	TAMRA at 3'-end, (attached to terminal cytosine), phosphate at 5' end
19	Primer with T	5'-GCGACAGGTGACTGCAGCT-3'	None
20	Inosine strand	5'-CACATCACGTCGTAGT X A-3'	X = 2'-deoxyinosine
21	Helper strand	5'-CACTACGACGTGATGTG-3'	None

Figure 11a

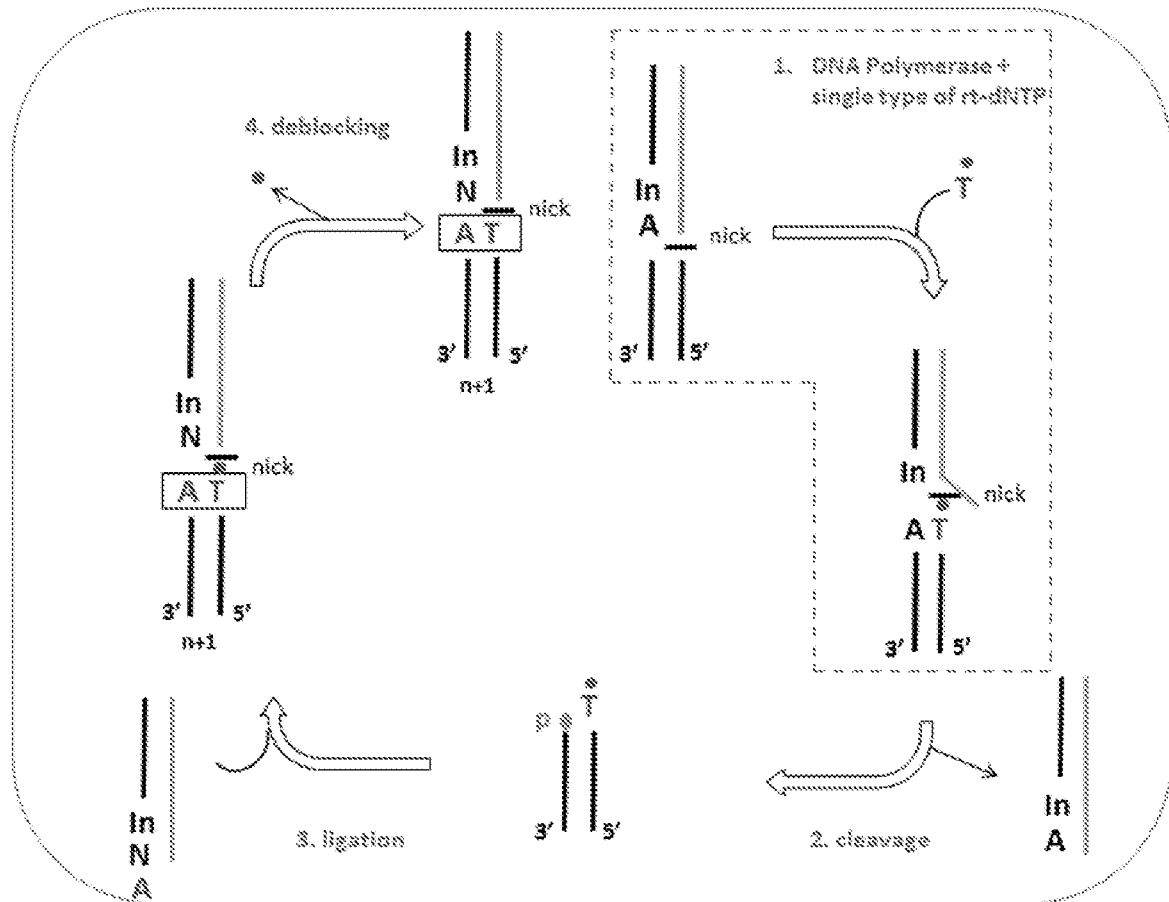


Figure 11b

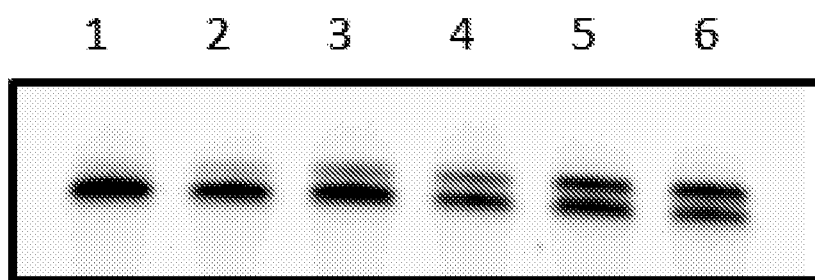


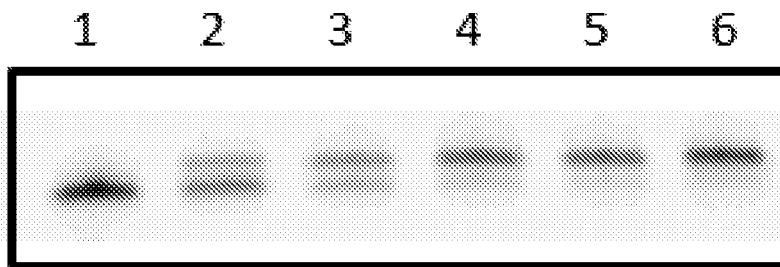
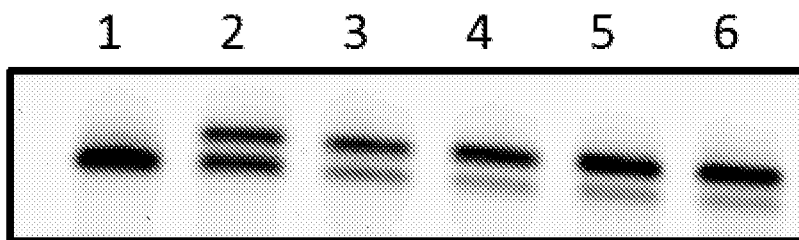
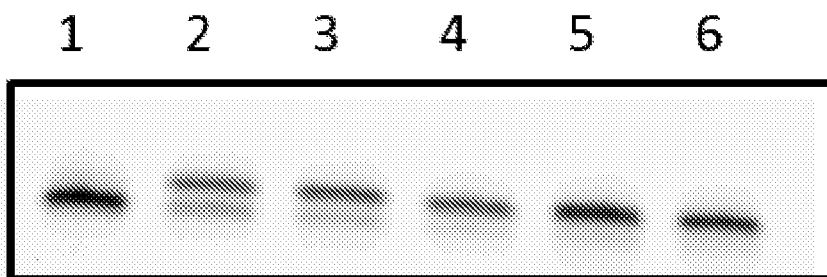
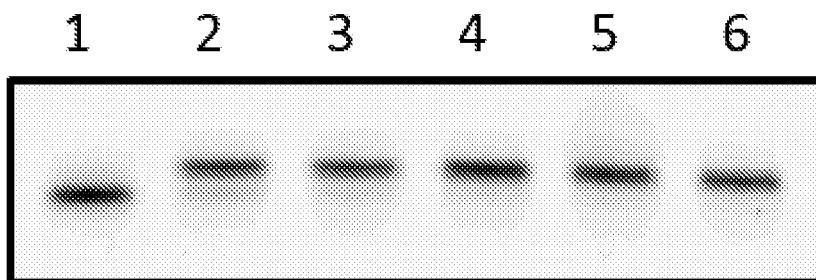
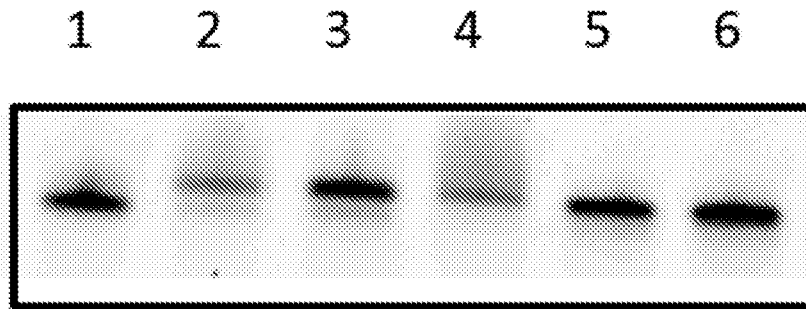
Figure 11c**Figure 11d****Figure 11e****Figure 11f**

Figure 11g**Figure 11h**

	0 min	1 min	2 min	5 min	10 min	20 min
27°C without Mn ²⁺	0	5	10	20	35	40
37°C without Mn ²⁺	0	30	58	90	90	90
47°C without Mn ²⁺	0	30	65	90	90	90
27°C with Mn ²⁺	0	70	85	92	95	95
37°C with Mn ²⁺	0	85	90	>96	>96	>96
47°C with Mn ²⁺	0	85	90	>96	>96	>96

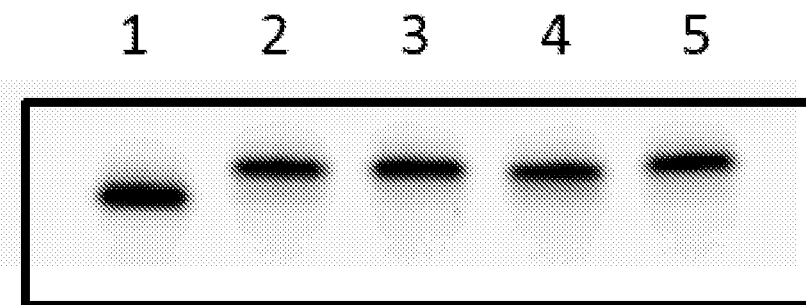
Figure 11i

Figure 11j

SEQ ID NO:	Oligonucleotide name	Sequence	Modification
22	Primer	5'-GCGACAGGTGACTGCAGC-3'	TAMRA at 5'-end (attached to terminal guanine)
23	Template-A	5'-CACATCACGTCGTAGTCXAGCTGCAG TCACCTGTCGC-3'	X = 2'- deoxyinosine
24	Template-G	5'-CACATCACGTCGTAGTCXGGCTGCAG TCACCTGTCGC-3'	X = 2'- deoxyinosine
25	Template-T	5'-CACATCACGTCGTAGTCXAGCTGCAG TCACCTGTCGC-3'	X = 2'- deoxyinosine
26	Template-C	5'-CACATCACGTCGTAGTCXAGCTGCAG TCACCTGTCGC-3'	X = 2'- deoxyinosine
27	Helper strand-T	5'-TCGACTACGACGTGATGTG-3'	None
28	Helper strand-C	5'-CCGACTACGACGTGATGTG-3'	None
29	Helper strand-A	5'-ACGACTACGACGTGATGTG-3'	None
30	Helper strand-G	5'-GCGACTACGACGTGATGTG-3'	None

Figure 12a

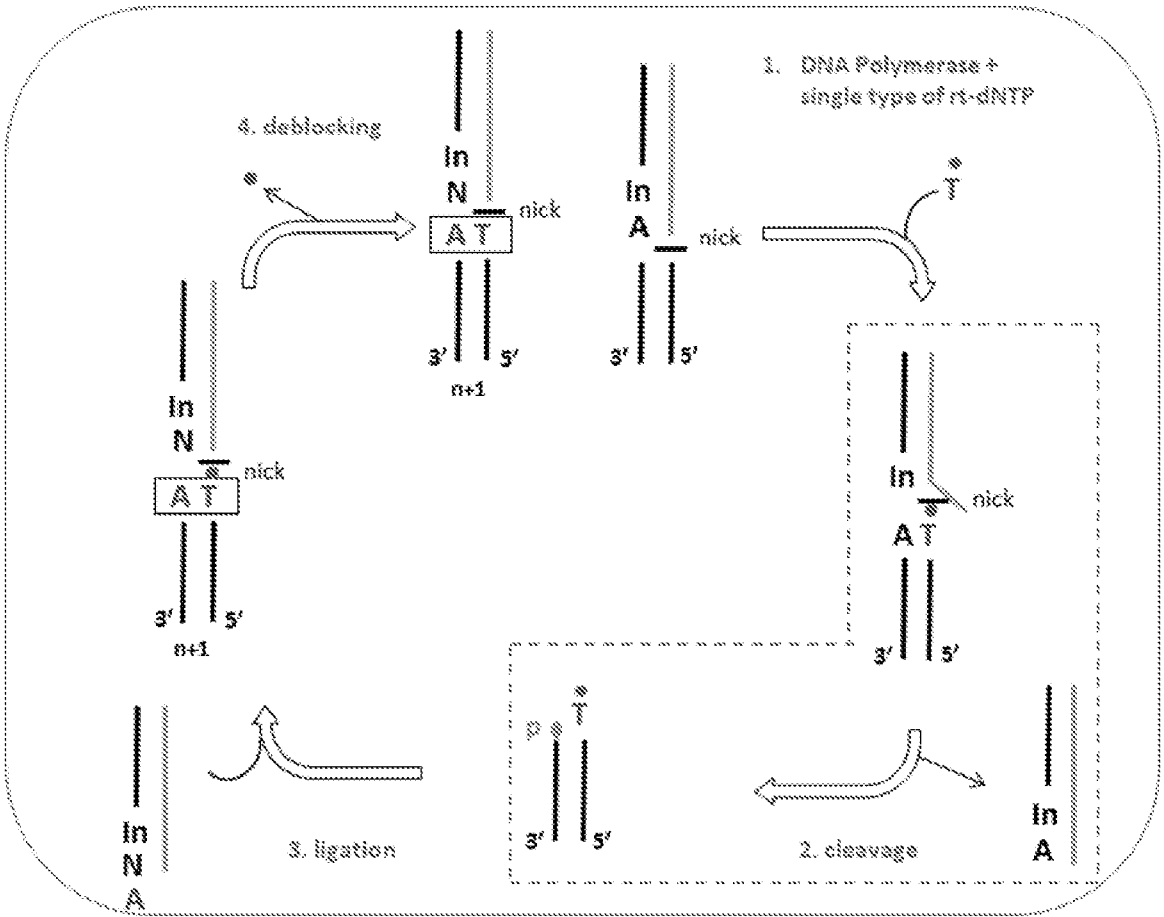
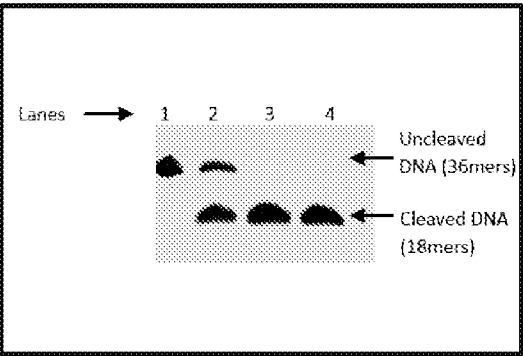


Figure 12b



Sample Lanes	Content
1	Reference control: Template (15pmols) only.
2	Positive control : Template + Control (15pmols)
3	Test sample I: Template + Primer with T (15pmols) No helper strand included.
4	Test sample II: Template + Primer with T Helper strand included (15pmols).

Figure 12c

Cleavage reaction combination	% Conversion of cleaved DNA
Full length positive control	80
Presence of helper strand	>99
Absence of helper strand	>99

Figure 12d

SEQ ID NO:	Oligonucleotide name	Sequence	Modification
31	Template	5'-CACATCACGTCGTAGTCXAGCT GCAGTCACCTGTCGC-3'	TAMRA at 5'-end (attached to terminal cytosine), X = deoxyinosine
32	Primer with T	5'-GCGACAGGTGACTGCAGCT-3'	None
33	Control	5'-GCGACAGGTGACTGCAGCTGA CTACGACGTGATGTG-3'	None
34	Helper strand	5'-TCGACTACGACGTGATGTG-3'	None

Figure 13a

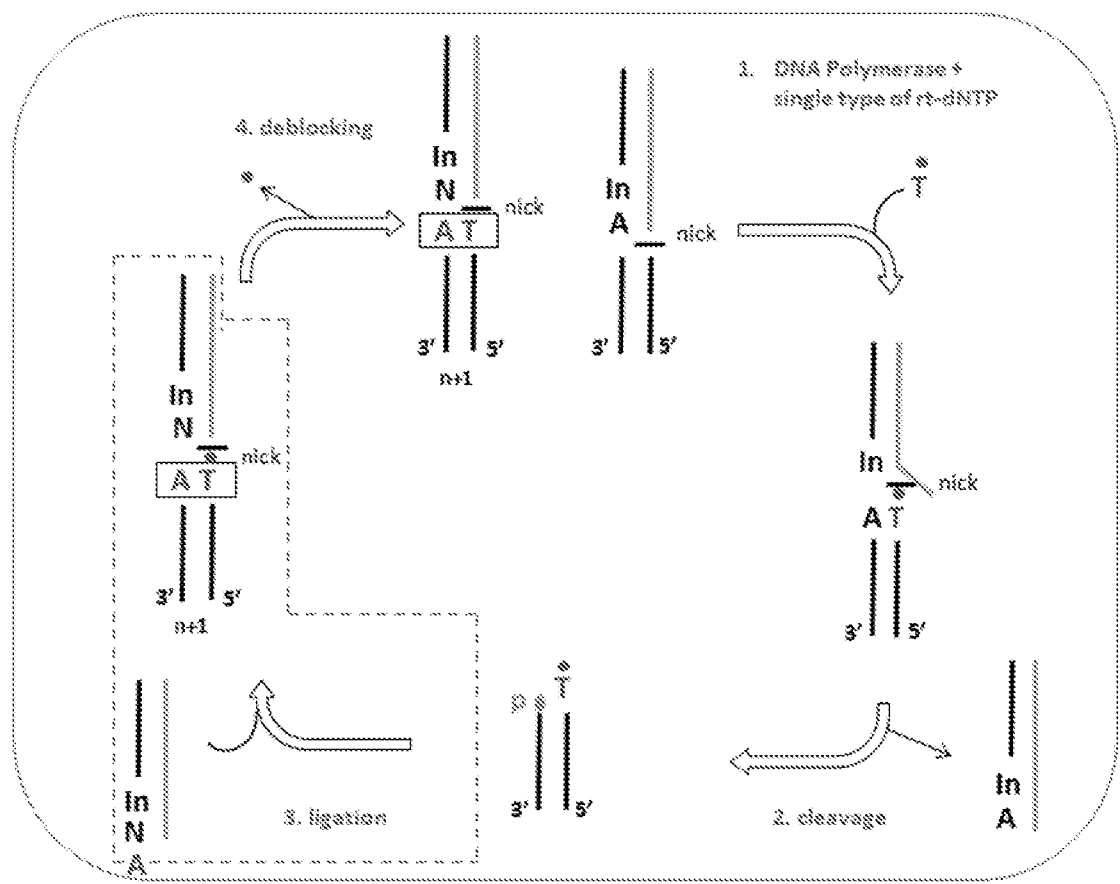


Figure 13b

SEQ ID NO:	Oligonucleotide name	Sequence	Modification
35	Phosphate strand	5'-GCTGCAGTCACCTGTCGC-3'	TAMRA at 3'-end (attached to terminal cytosine), phosphate at 5' end
36	Primer with T	5'-GCGACAGGTGACTGCAGCT-3'	None
37	Inosine strand	5'-CACATCACGTCGTAGTXGA-3'	X = 2'-deoxyinosine
38	Helper strand	5'-CCACTACGACGTGATGTG-3'	None

Figure 14a

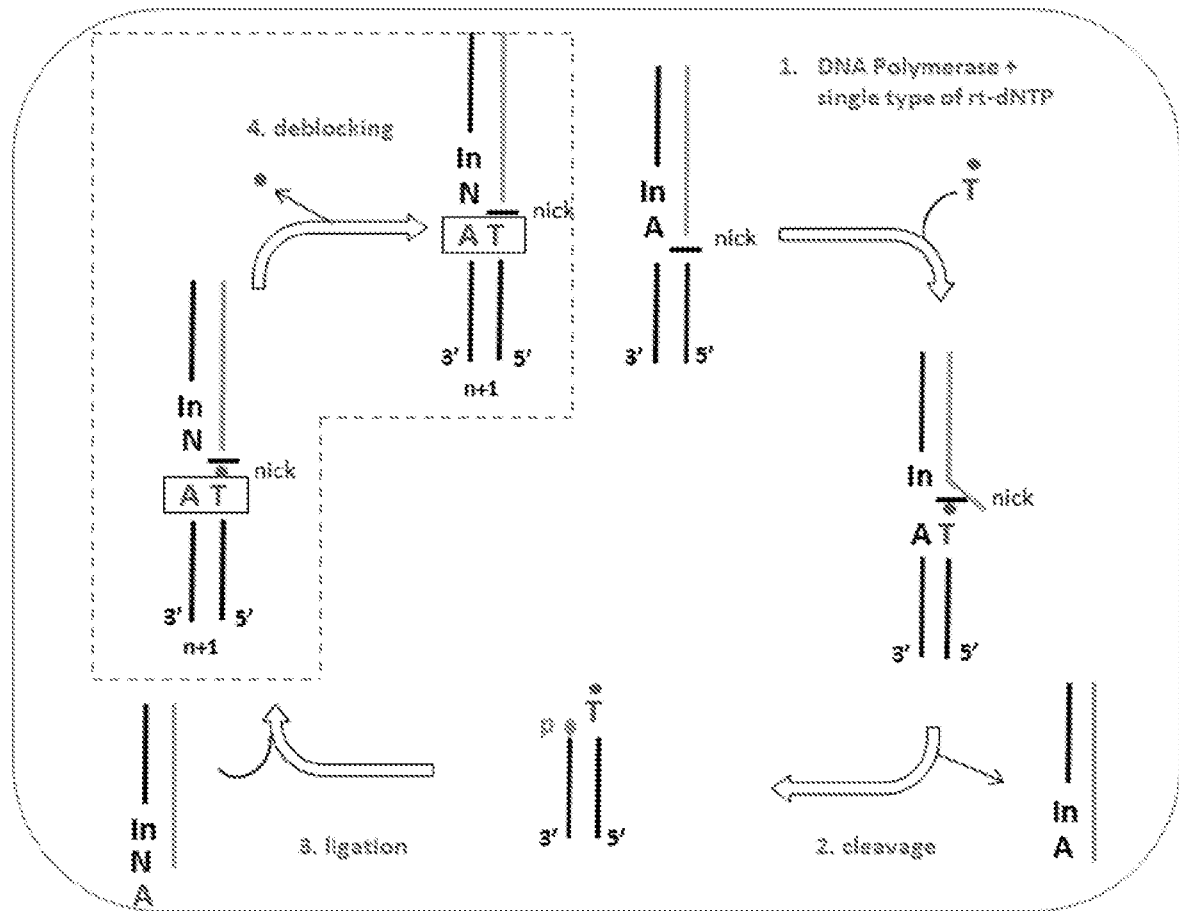


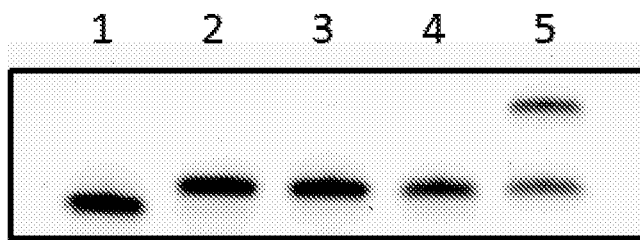
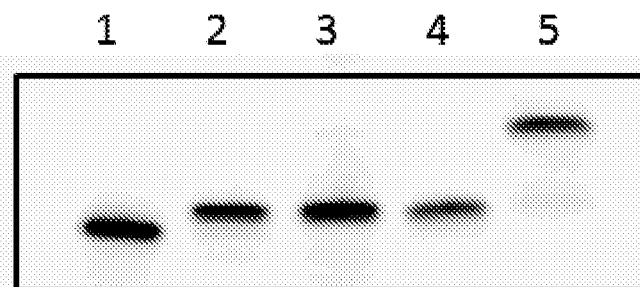
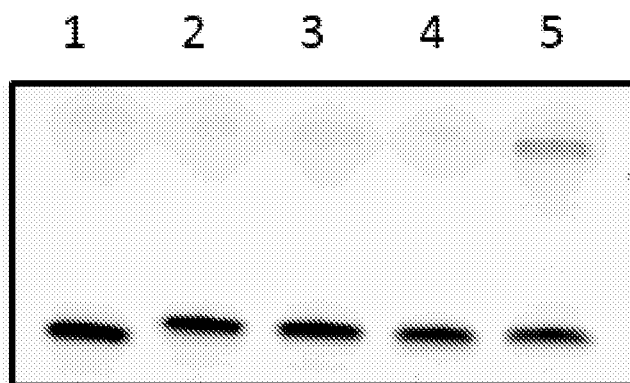
Figure 14b**Figure 14c****Figure 14d**

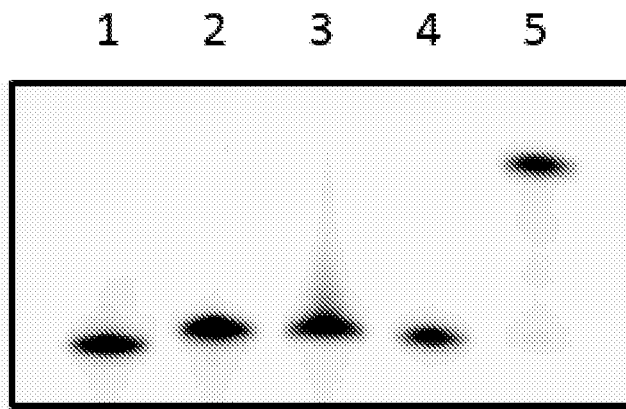
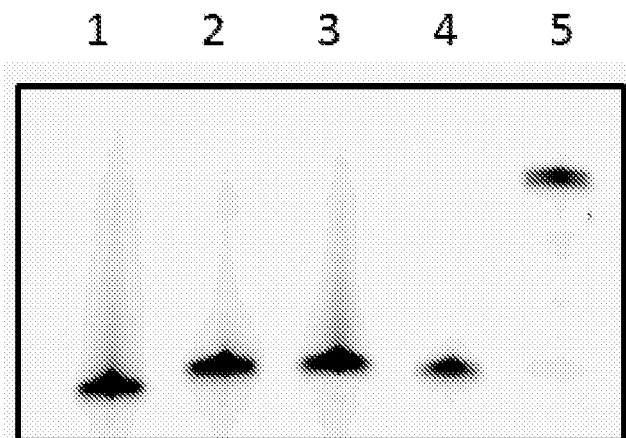
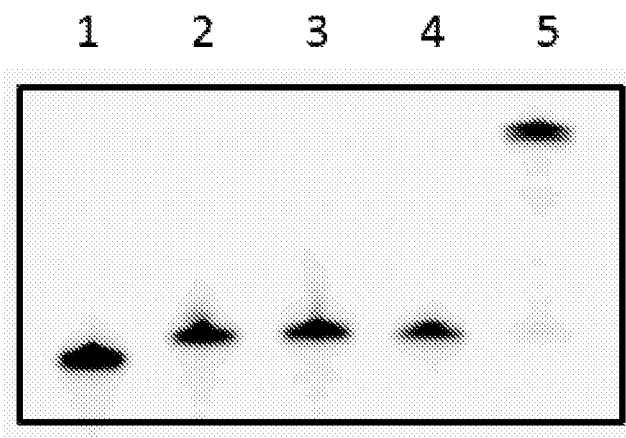
Figure 14e**Figure 14f****Figure 14g**

Figure 14h

Incorporation	Deprotection by 50 mM TCEP	Deprotection by 300 mM TCEP
3'-O-azidomethyl-dTTP	50%	95%
3'-O-azidomethyl-dCTP	20%	95%
3'-O-azidomethyl-dATP	Not tested	95%
3'-O-azidomethyl-dGTP	Not tested	95%

Figure 14i

SEQ ID NO:	Oligonucleotide name	Sequence	Modification
39	Primer	5'-GCGACAGGTGACTGCAGC-3'	TAMRA at 5'-end (attached to terminal cytosine)
40	Template-A	5'-CACATCACGTCGTCAGCT GCAGTCACCTGTCGC-3'	None
41	Template-G	5'-CACATCACGTCGTCGGCT GCAGTCACCTGTCGC-3'	None
42	Template-T	5'-CACATCACGTCGTCCTGCT GCAGTCACCTGTCGC-3'	None
43	Template-C	5'-CACATCACGTCGTCCTGCT GCAGTCACCTGTCGC-3'	None

Figure 15a

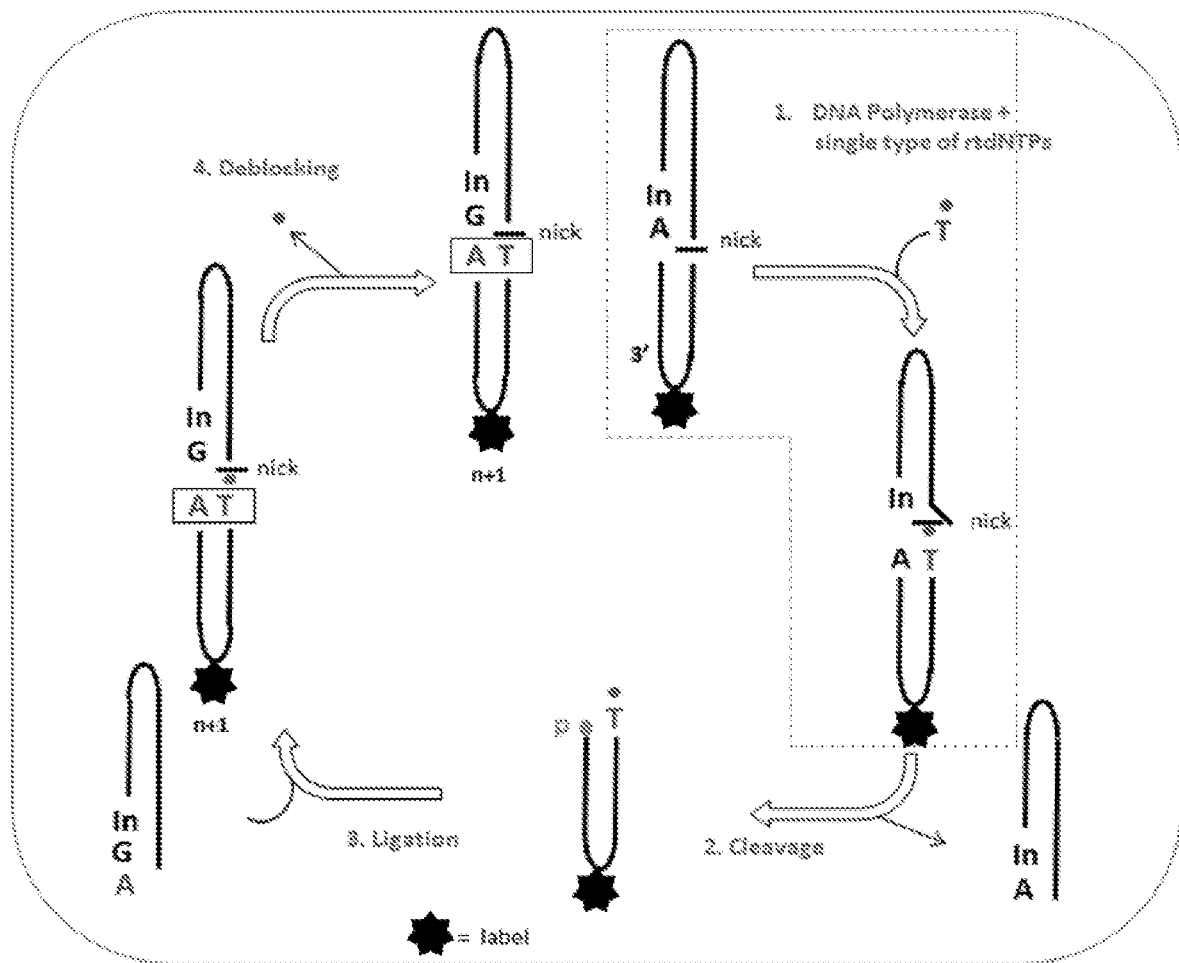


Figure 15b

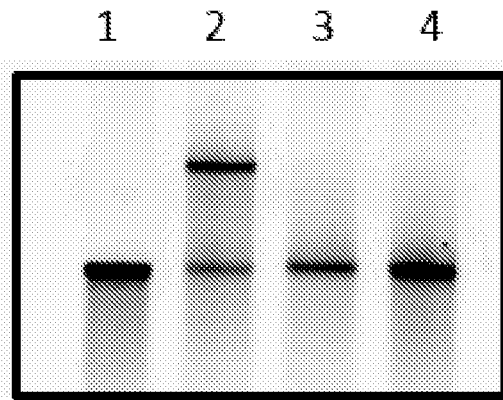


Figure 15c

SEQ ID NO:	Oligonucleotide name	Sequence	Modification
44	Dual hairpin model for incorporation	5'-TCGACTACGACGTGACTTTTAGTCACGTCGTAGTCXAGCTGCAGTCACCTGCTGCTTYTTGCAGCAGGTGACTGCAGC-3'	X = 2'-deoxyinosine Y = Tamra-dT

Figure 16a

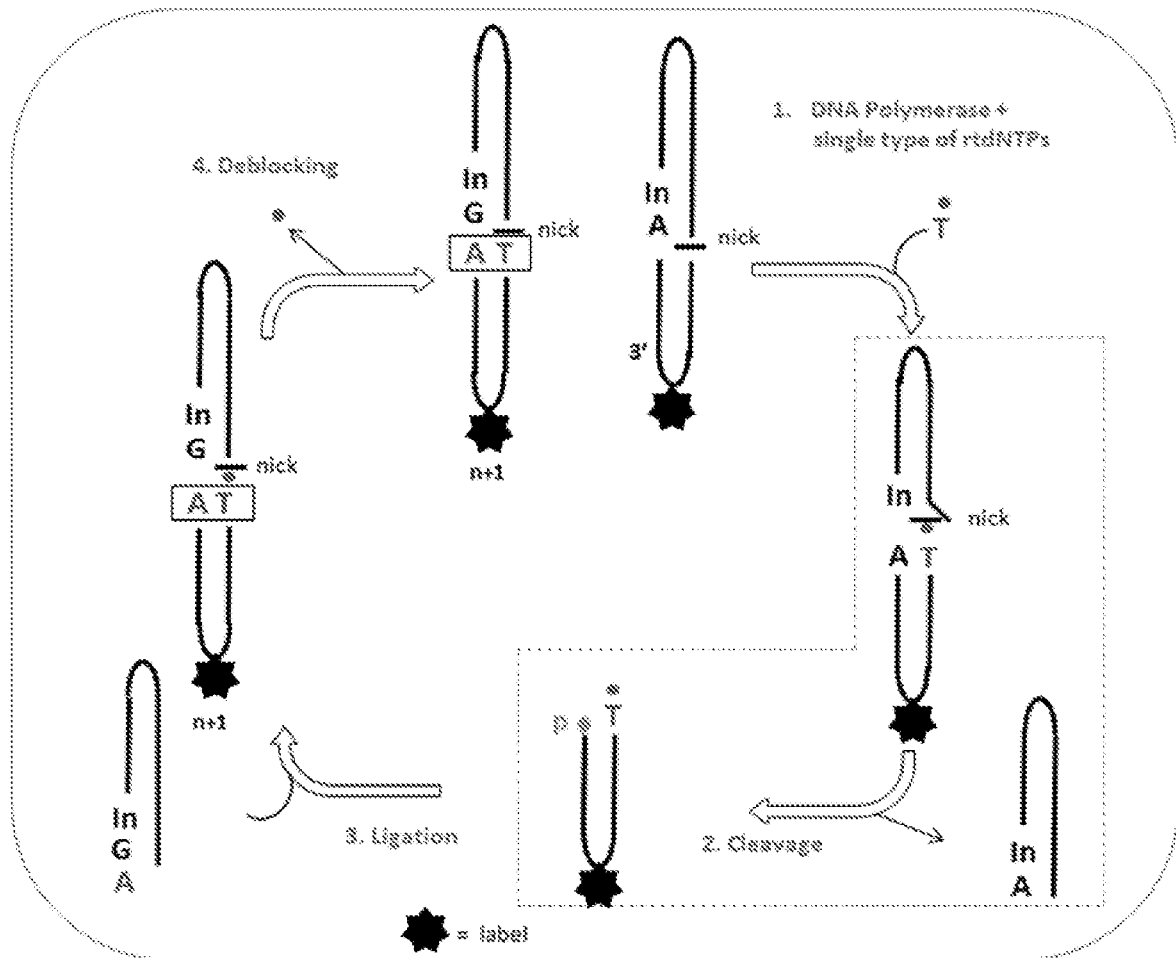


Figure 16b

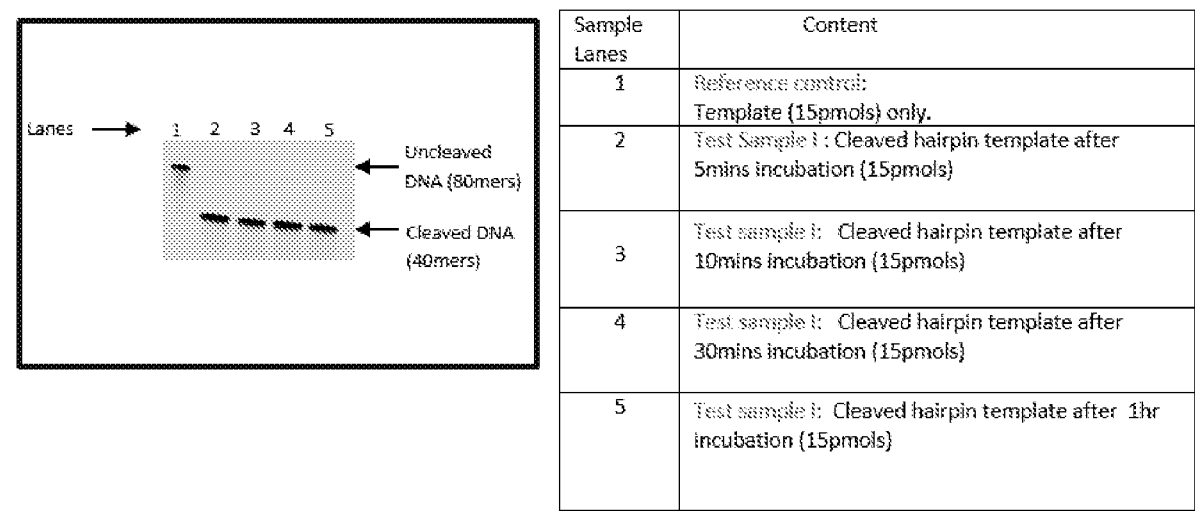


Figure 16c

SEQ ID NO:	Oligonucleotide name	Sequence	Modification
45	Dual hairpin model for incorporation	5'- TCGACTACGACGTGACTTTTAGTCAC GTCGTAGTCXAGCTGCAGTCACCTGCTG CTTYTTGCAGCAGGTGACTGCAGCT-3'	X = 2'-deoxyinosine Y = Tamra-dT

Figure 17a

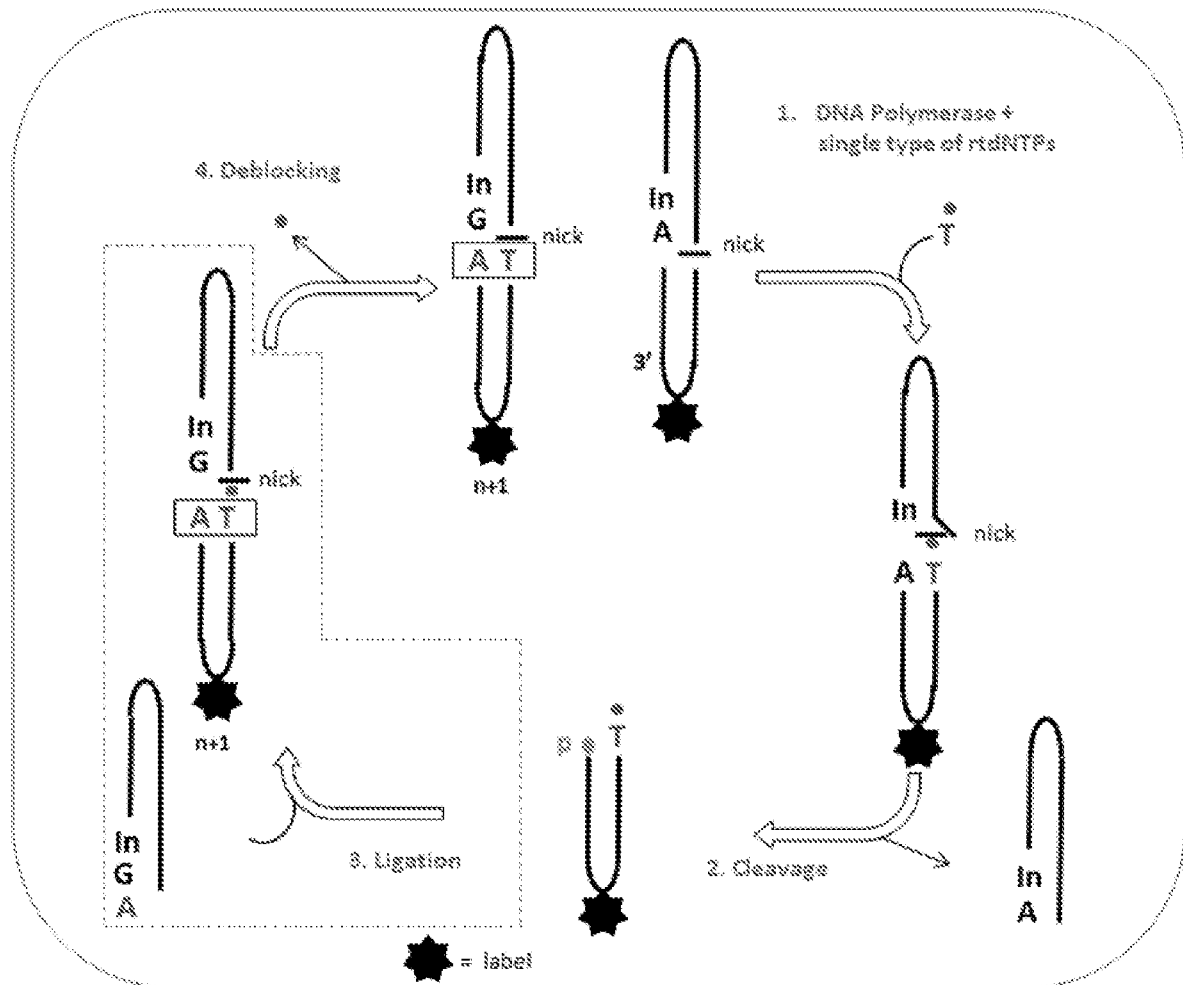


Figure 17b

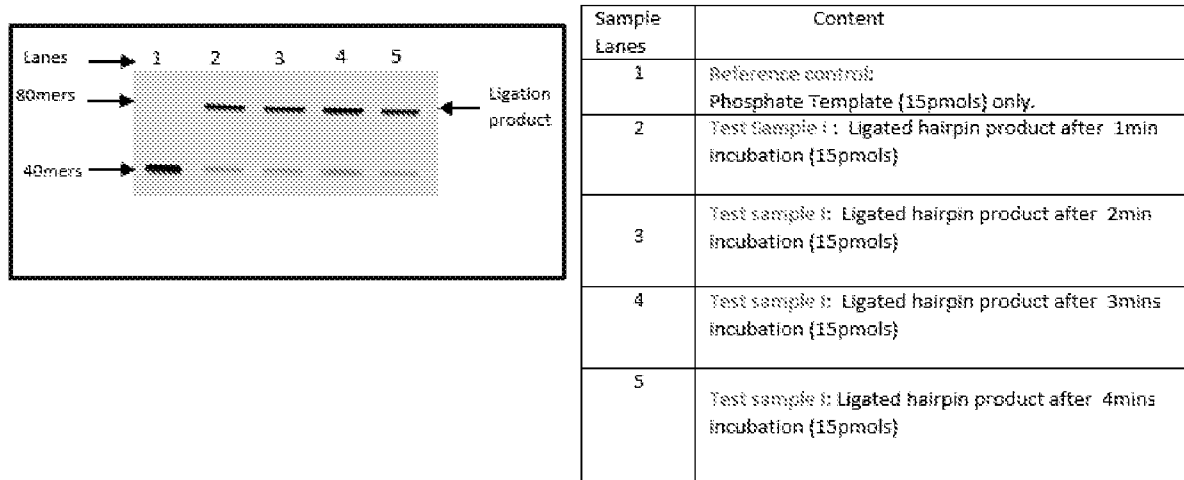


Figure 17c

SEQ ID NO:	Oligonucleotide name	Sequence	Modification
46	Phosphate hairpin	5'-GCTGCAGTCACCTGCTGCTTYT TGCAGCAGGTGACTGCAGCT-3'	Y = Tamra-dT phosphate
47	Inosine Hairpin	5'- CCGACTACGACGTGACTTTTAGT CACGTCGTAGTCXGA-3'	X = 2-deoxyinosine

Figure 18a

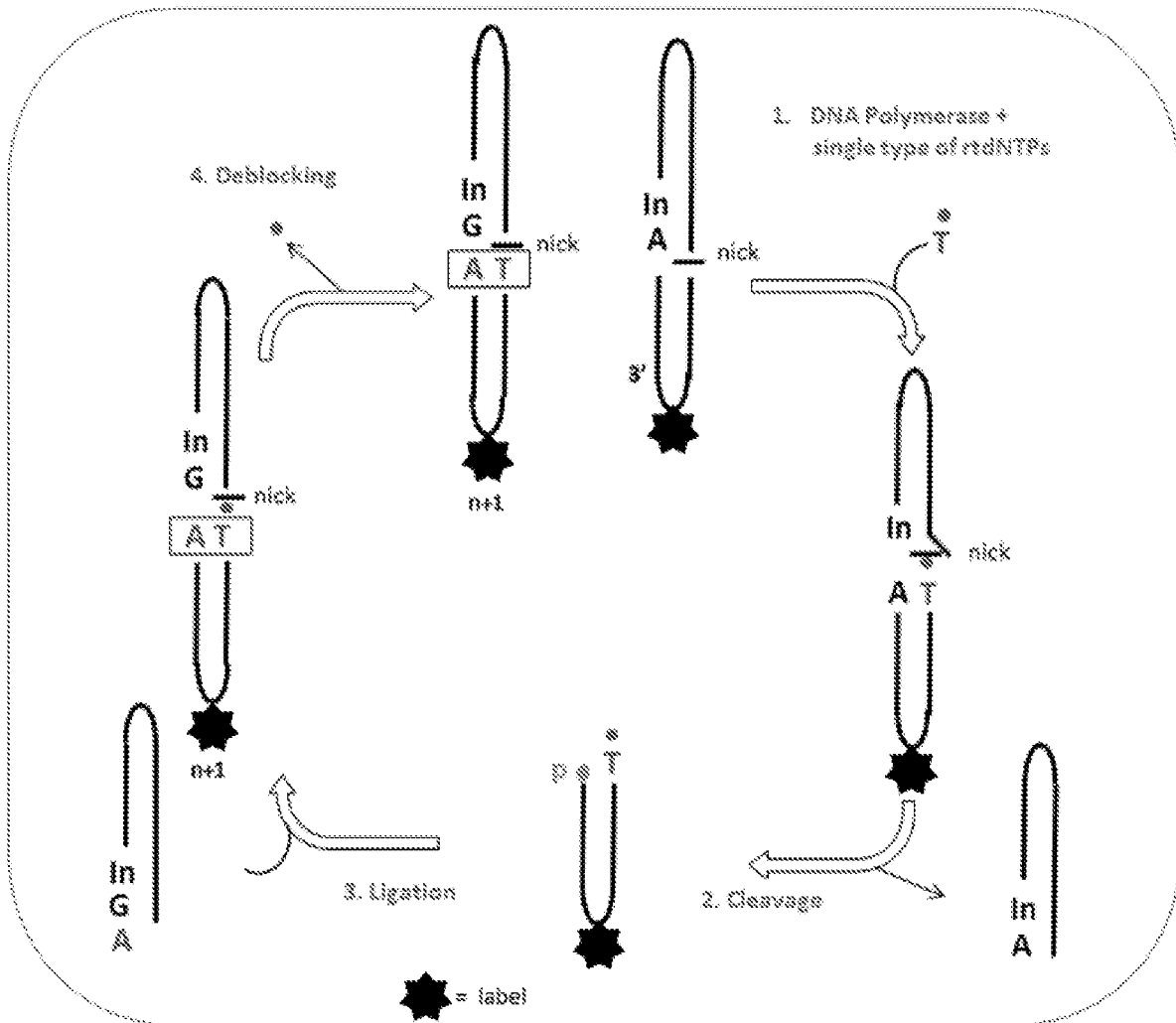


Figure 18b

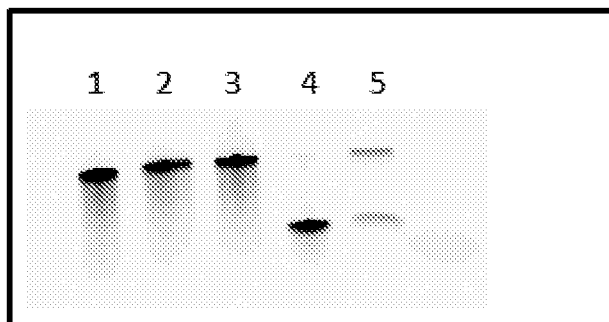


Figure 18c

SEQ ID NO:	Oligonucleotide name	Sequence	Modification
48	Double hairpin model	5'- TCGACTACGACGTGACTTTTAGTCAC GTCGTAGTCXAGCTGCAGTCACCTGCTGC TTYTTGCAGCAGGTGACTGCAGC-3'	X = 2'- deoxyinosine Y = Tamra-dT
49	Strand for ligation	5'- CCGACTACGACGTGACTTTTAGTCAC GTCGTAGTCXGA-3'	X = 2'- deoxyinosine

Figure 19a

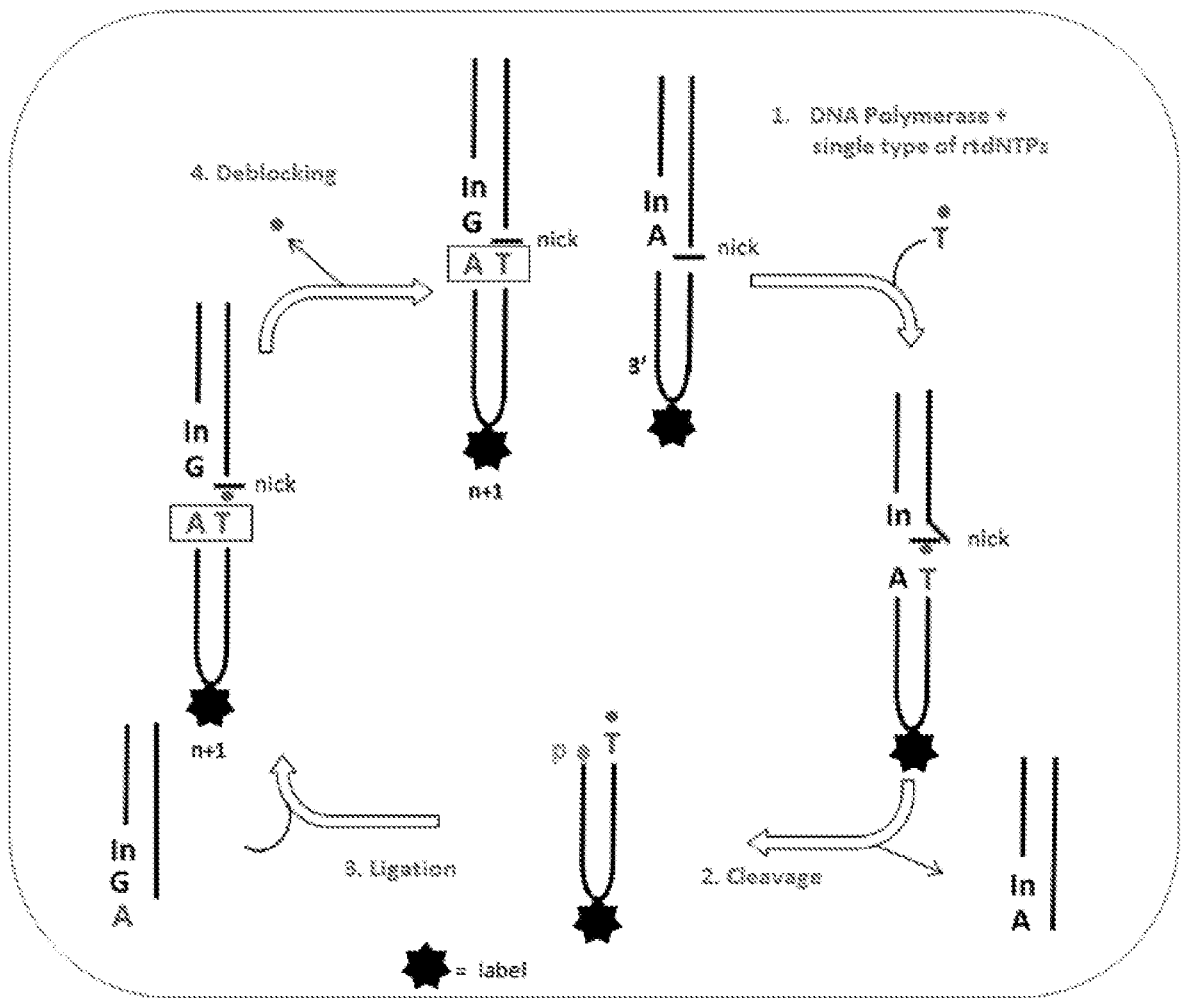


Figure 19b

SEQ ID NO:	Oligonucleotide name	Sequence	Modification
50	Single hairpin model for incorporation	5'- AGTCACGTCGTAGTCXAGCTGCAGTC ACCTGCTGCTTYTTGCAGCAGGTGACTGCAG C-3'	X = 2'-deoxyinosine Y = Tamra-dT
51	Helper strand	5'- TCGACTACGACGTGACT-3'	
52	Strand for ligation	5'- AGTCACGTCGTAGTCXGA	X = 2'-deoxyinosine
53	Helper strand for ligation	5'- CCGACTACGACGTGACT-3'	

Figure 20a

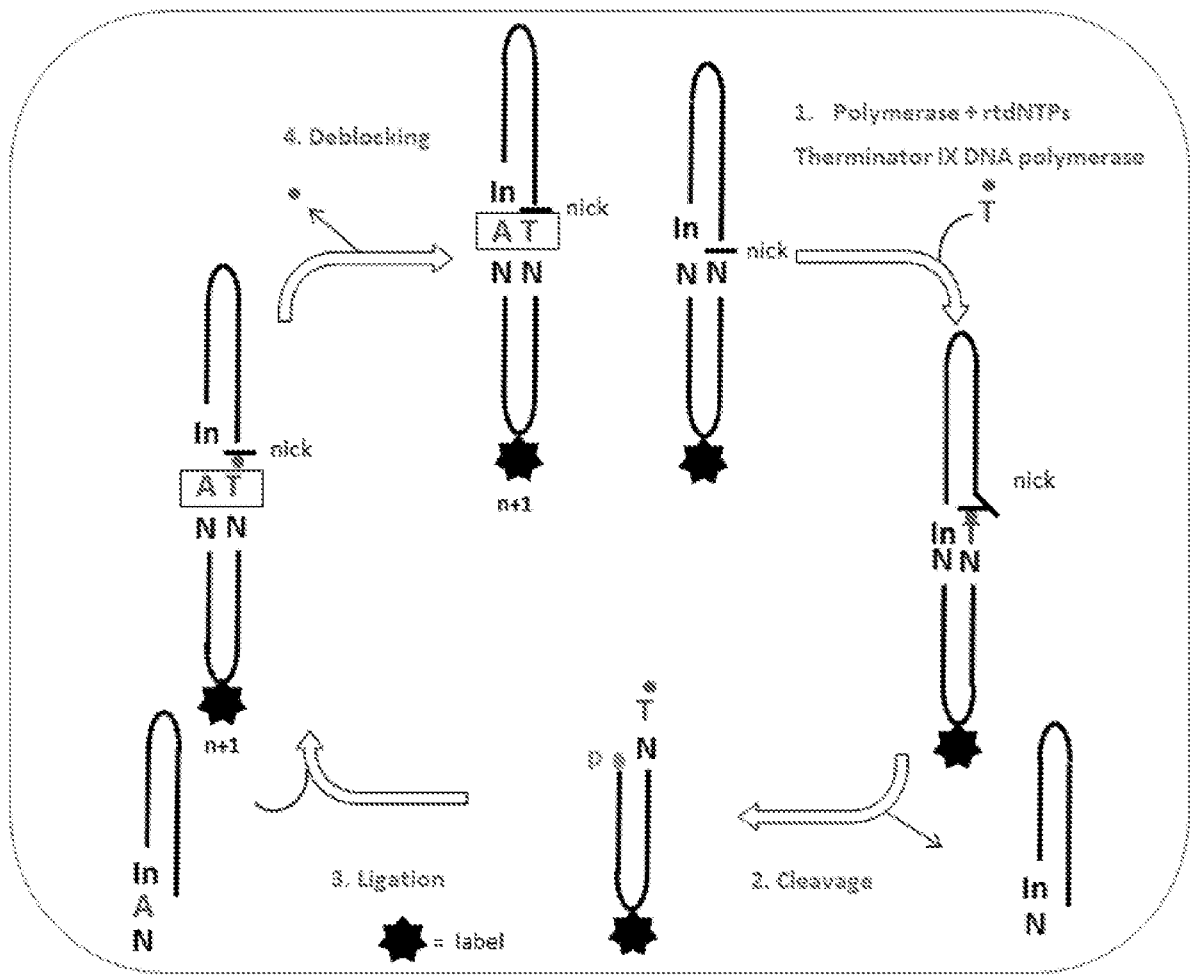


Figure 20b

SEQ ID NO:	Oligonucleotide name	Sequence	Modification
54	Double hairpin model	5'- CGACTACGACGTGACTTTTAGTCAC GTCGTAGTCXAGCTGCAGTCACCTGCTGC TTYTTGCAGCAGGTGACTGCAGCT-3'	X = 2'- deoxyinosine Y = Tamra-dT
55	Strand for ligation	5'- CGACTACGACGTGACTTTTAGTCAC GTCGTAGTCXAA-3'	X = 2'- deoxyinosine

Figure 21a

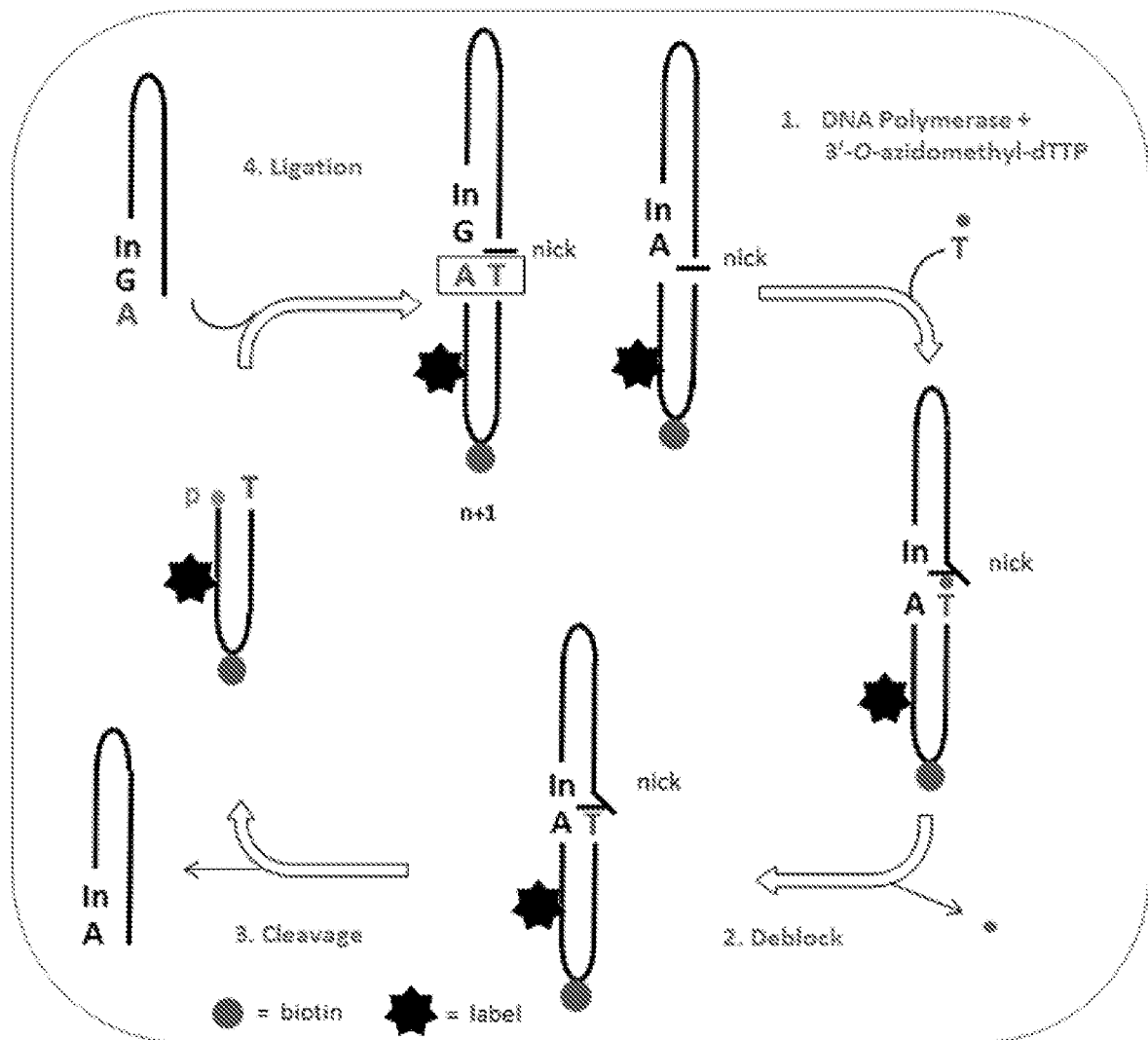


Figure 21b

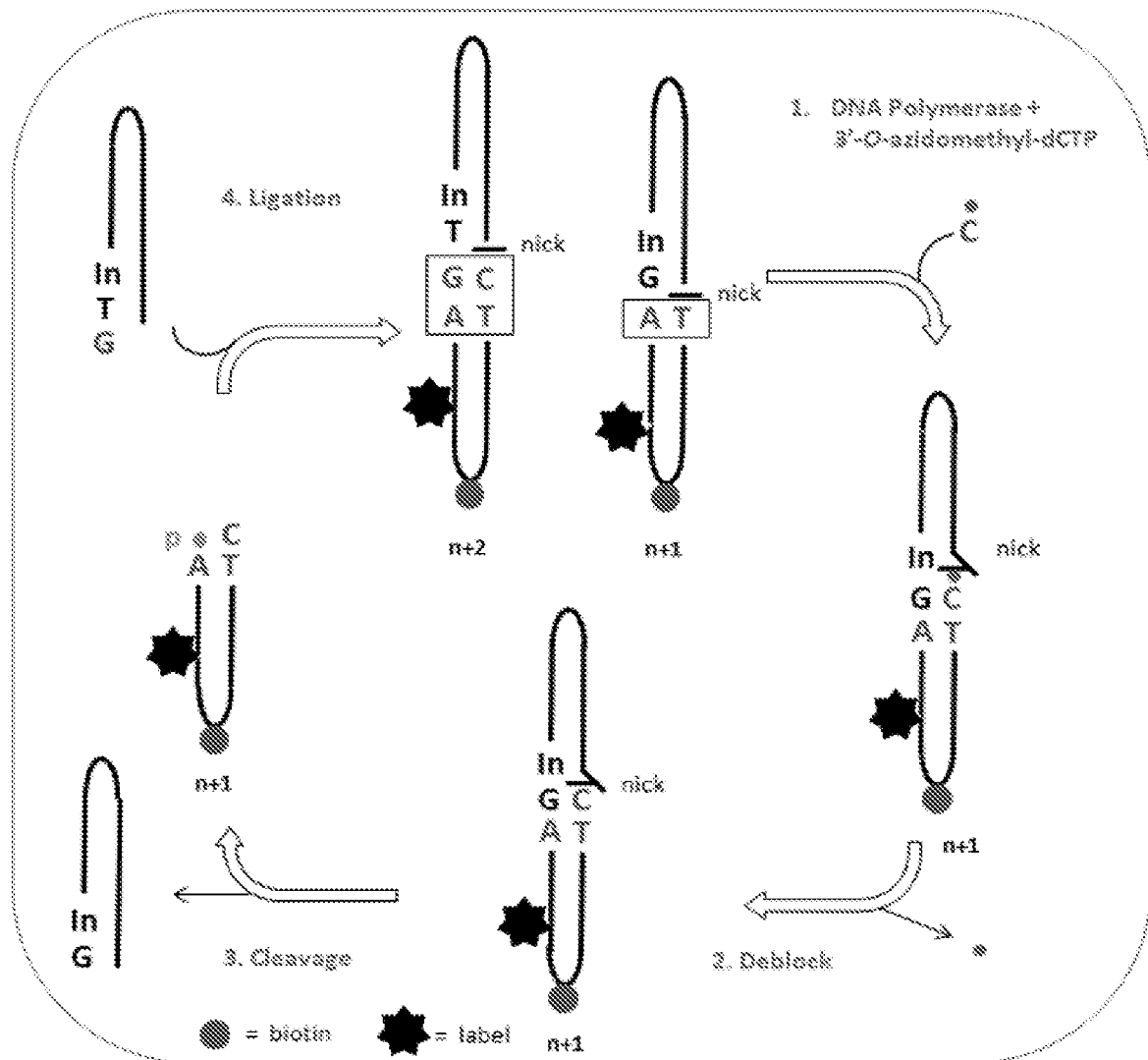


Figure 21c

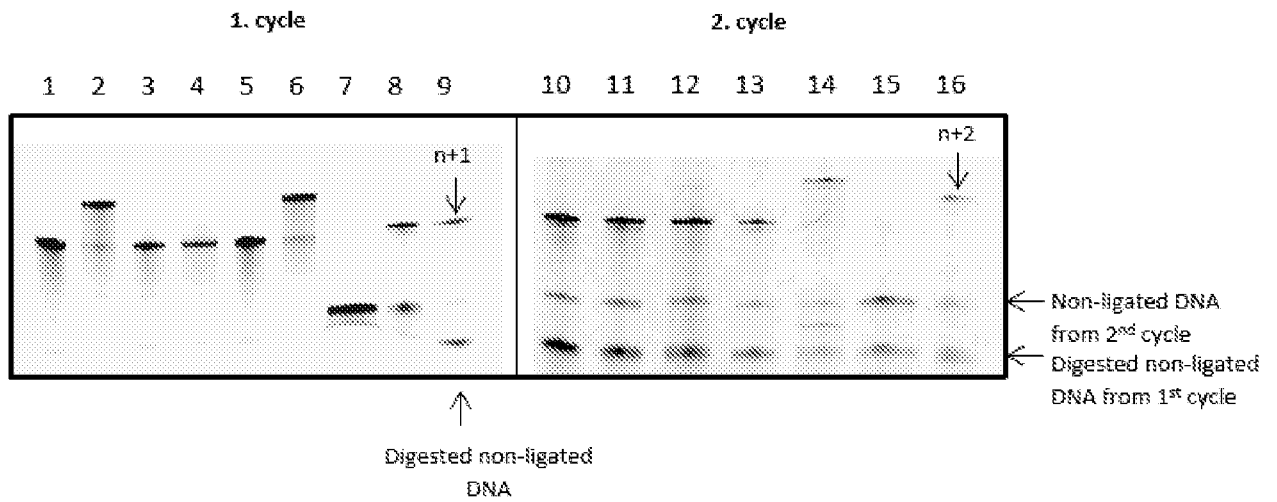


Figure 21d

SEQ ID NO:	Oligonucleotide name	Sequence	Modification
56	Double hairpin model	5'- CGACTACGACGTGACTTTTAGTCAC GTCGTAGTCXAGCTGCAGTCACCTGCGY CTTZZTTCAGCAGGTGACTGCAGCT-3'	X = 2'- deoxyinosine Y = Tamra-dT Z = Biotin-dT
57	Strand for ligation in the 1 st cycle	5'- CCGACTACGACGTGACTTTTAGTCAC GTCGTAGTCXGA-3'	X = 2'- deoxyinosine
58	Strand for ligation in the 2 nd cycle	5'- ACGAGTGACCTGGTTTTTTTTTTTTTTTT TTTTTTTTTTTTTTTTTACCAGGTCAC TCTG-3'	X = 2'- deoxyinosine

Figure 22

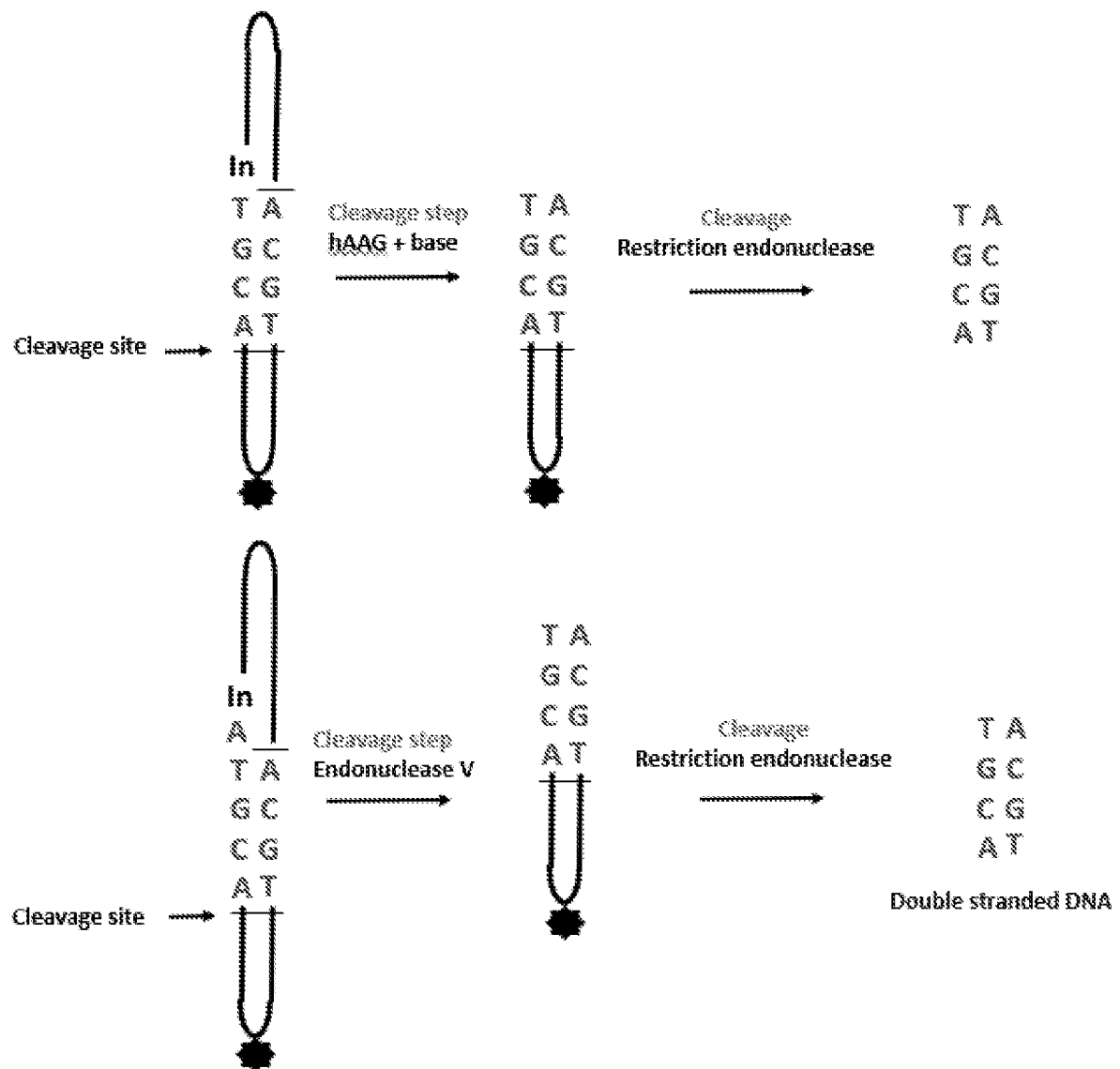


Figure 23

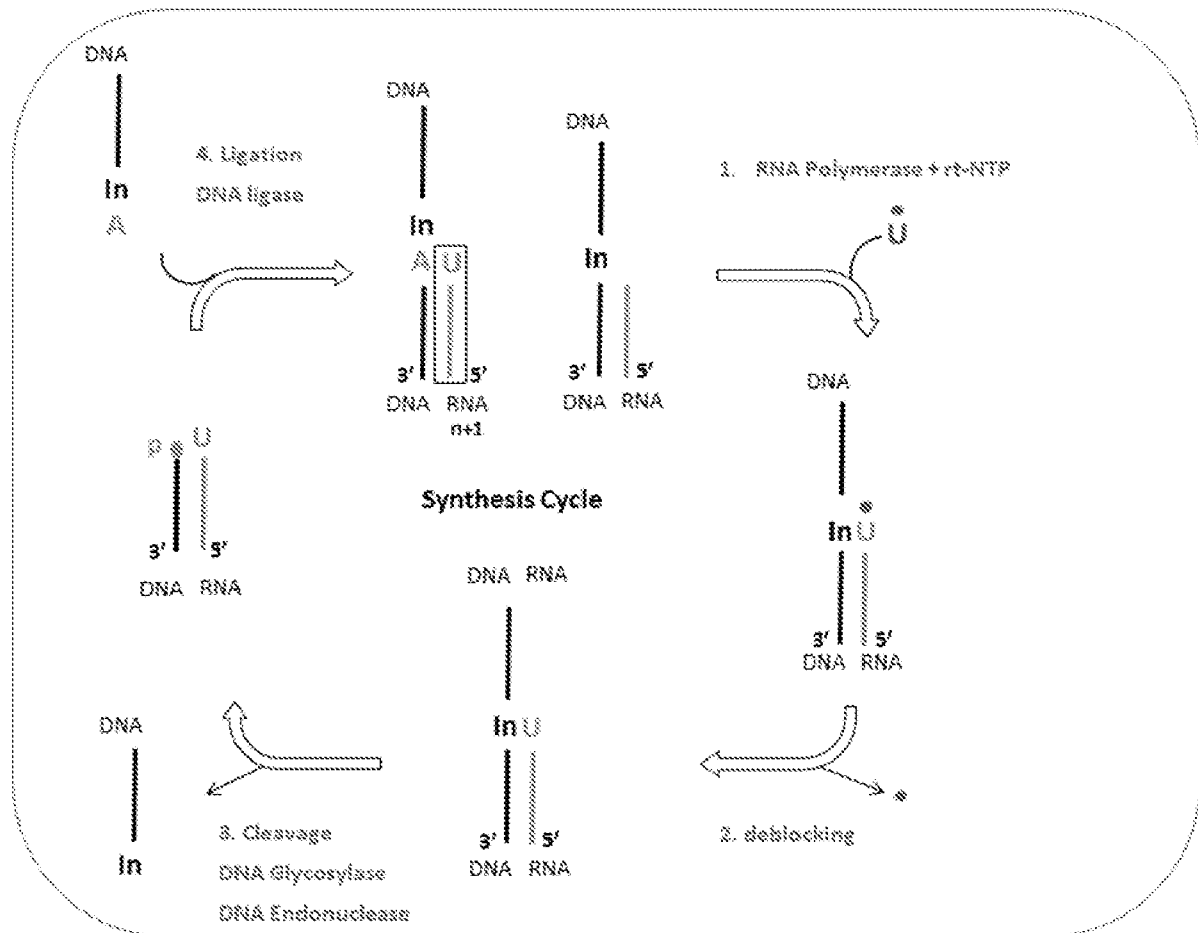


Figure 25

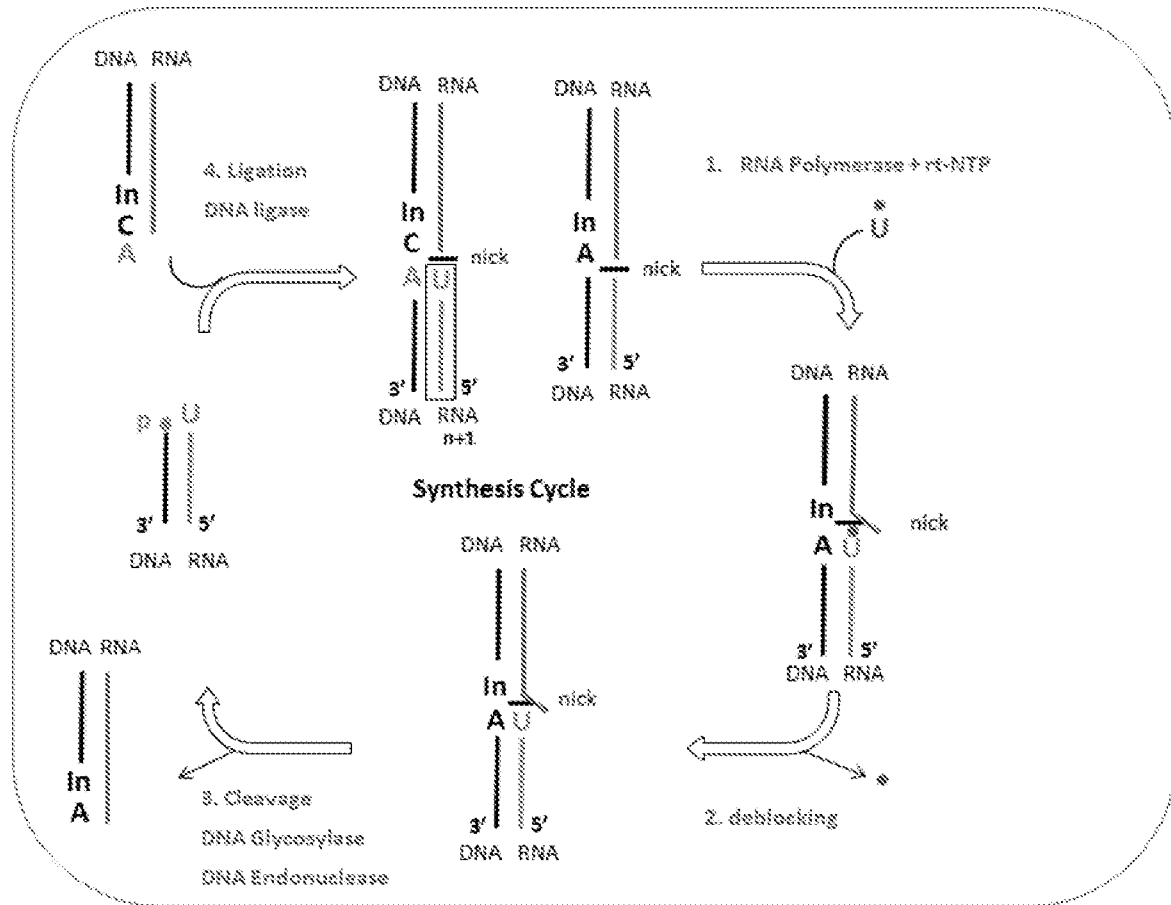


Figure 26

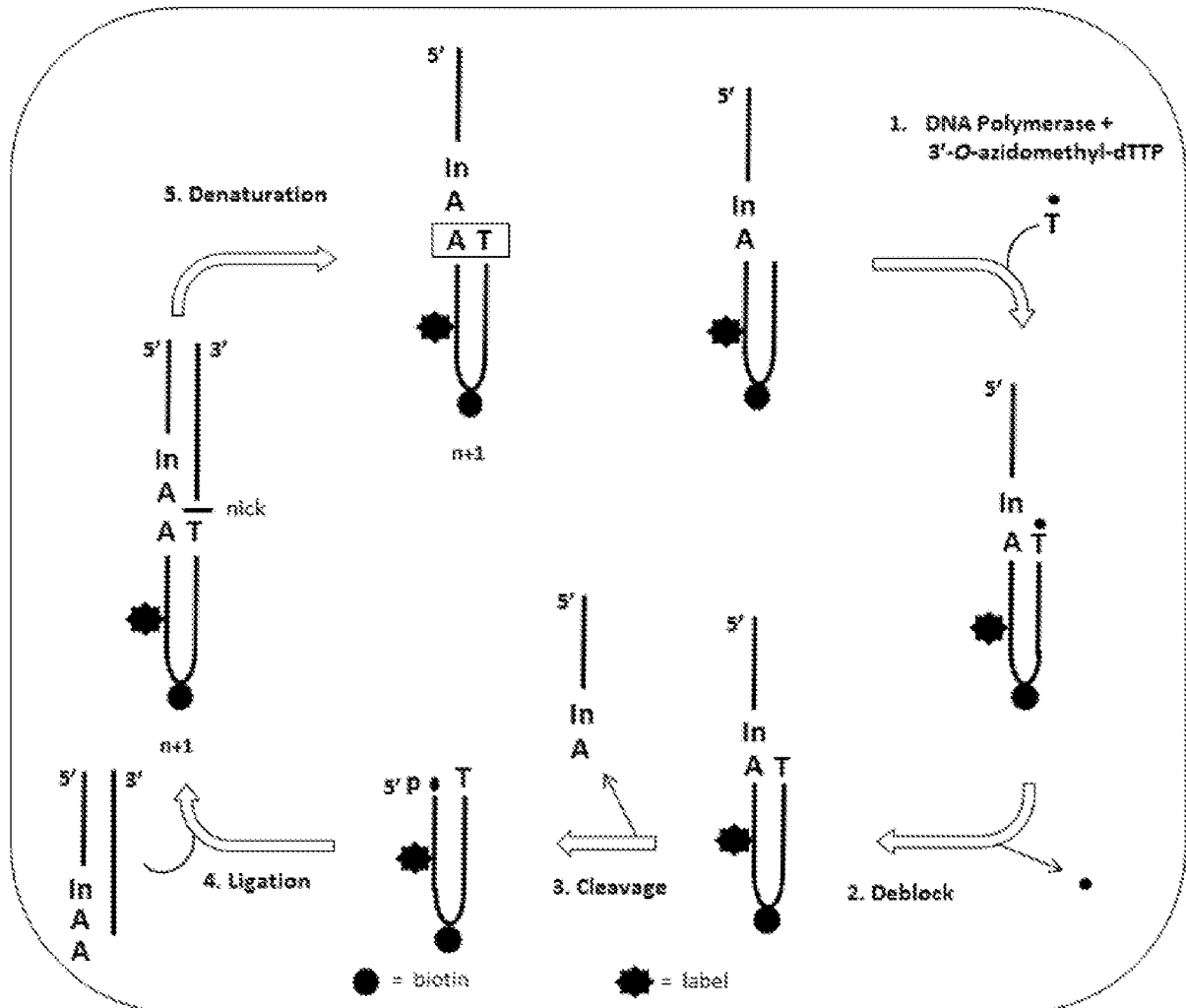


Figure 27

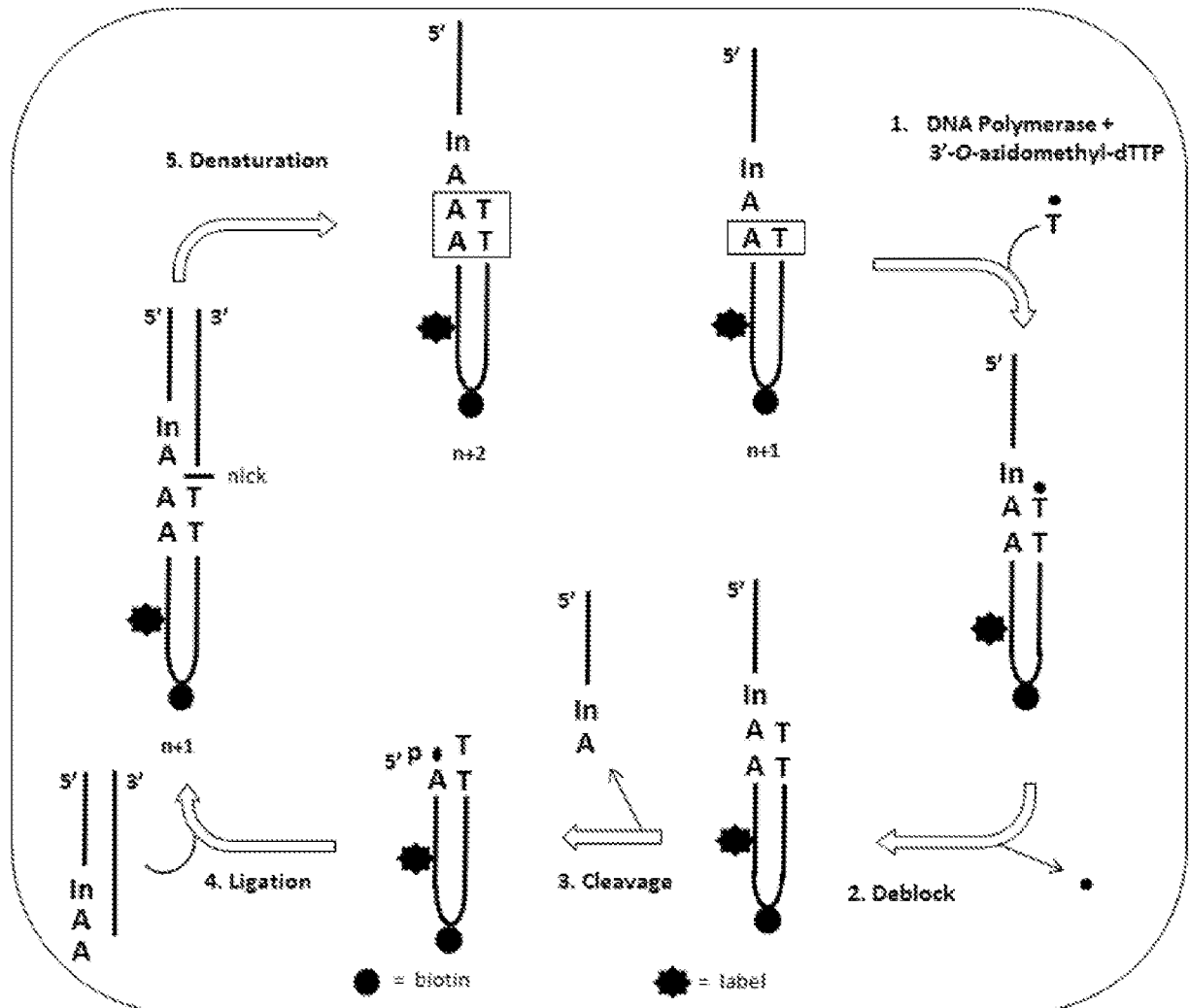


Figure 28

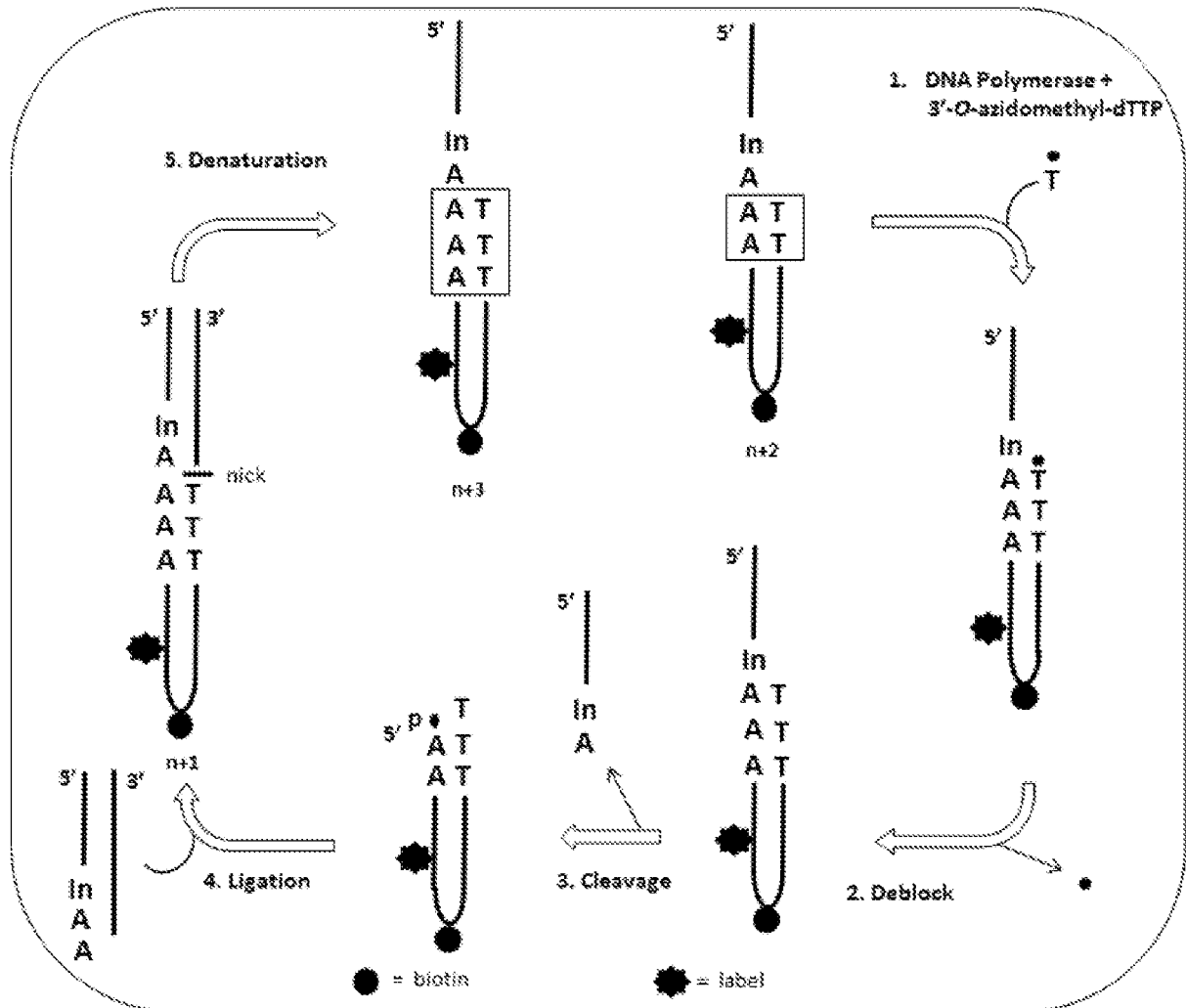


Figure 29

SEQ ID NO:	Oligonucleotide name	Sequence	Modification
59	Single hairpin model	5'- AGTCACGTCGTCAGTCXAGCTGCAGTC ACCTGCYGCTTZTTGCAGCAGGTGACTG CAGC-3'	X = 2'-deoxyinosine Y = Tamra-dT Z = Biotin-dT
60	Inosine strand for ligation	5'- AGTCACGTCGTCAGTCXAA-3'	X = 2'-deoxyinosine
51	Helper strand for ligation	5'- TCGACTACGACGTGACT-3'	

Figure 30

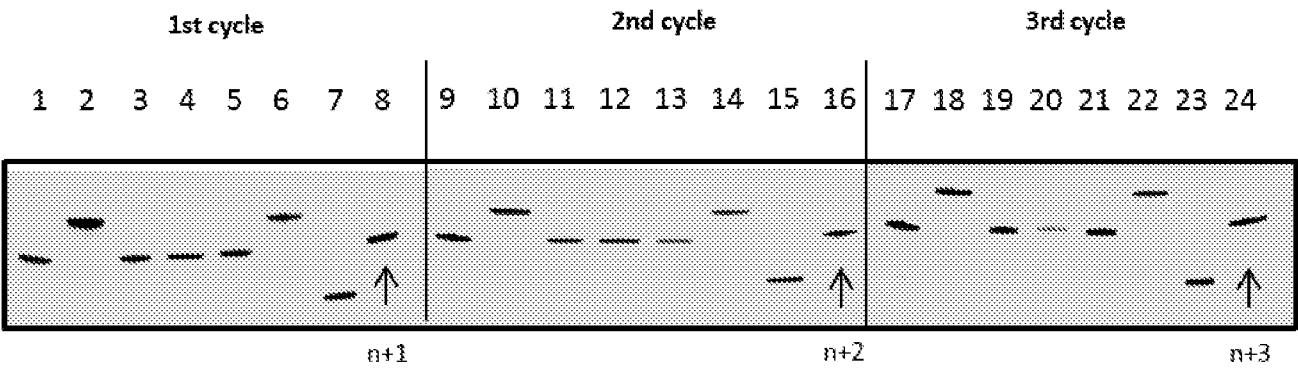


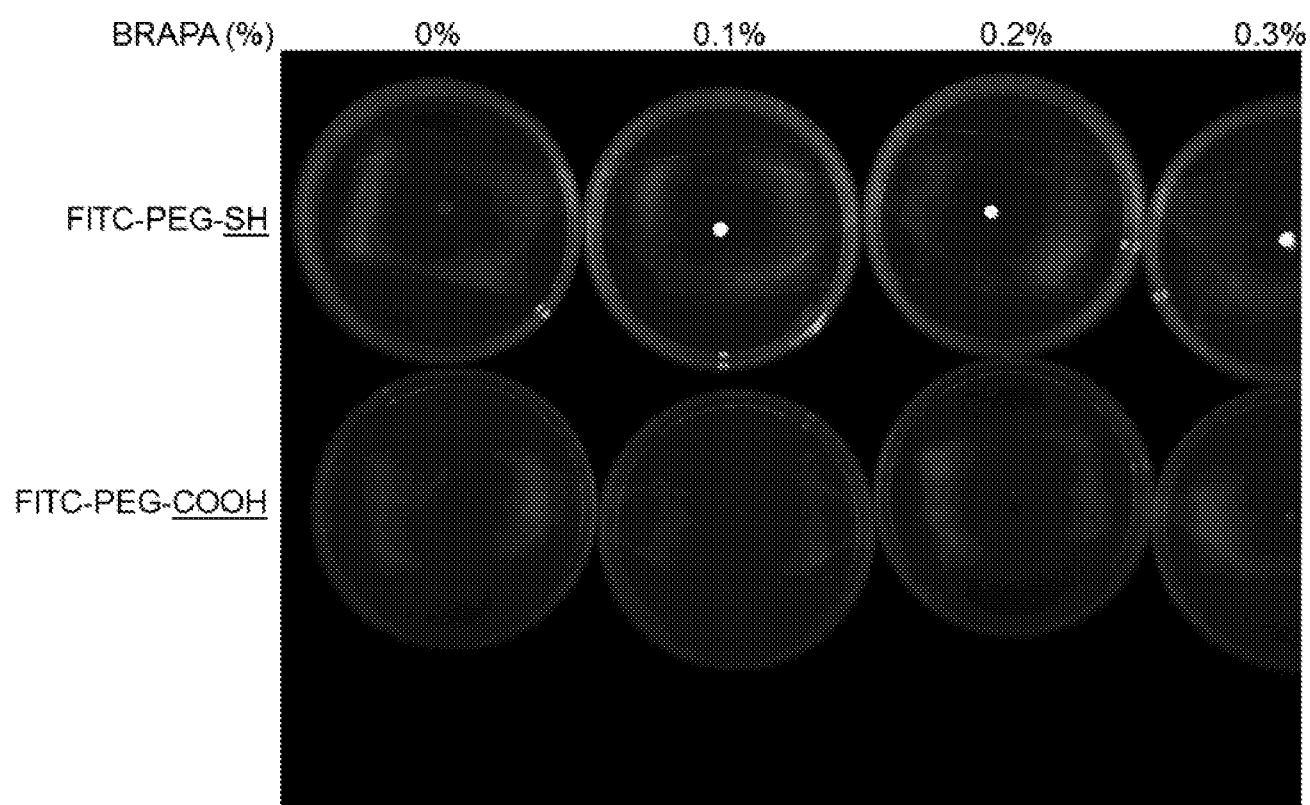
Figure 31

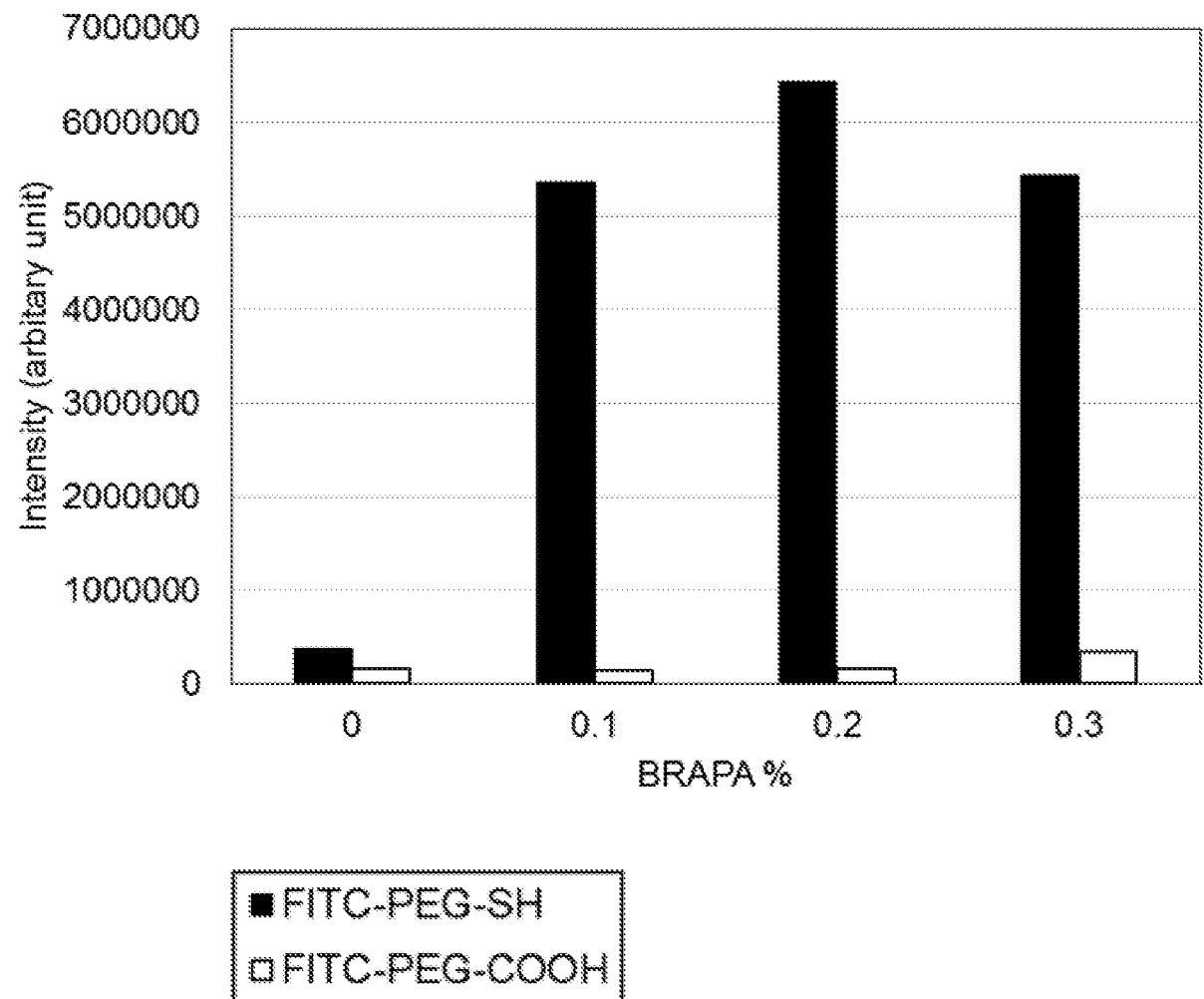
Figure 32

Figure 33a

Sample ID	Sequence	SEQ ID NO:
A (BRAPA control)	5'-Alexa647- AGTCACGTCGTAGTCIAGCTGCAGTCACCTGCTGCTTTT T*T*T*T*T*T*TTTGCAGCAGGTGACTGCAGC	SEQ ID NO: 61
B (BRAPA modified 1)	5'-Alexa647- AGTCACGTCGTAGTCIAGCTGCAGTCACCTGCTGCTTTT T*T*T*T*T*T*TTTGCAGCAGGTGACTGCAGC	SEQ ID NO: 61
C (BRAPA modified 2)	5'-Alexa647- AGTCACGTCGTAGTCIAGCTGCAGTCACCTGCTGCTTTT T*T*T*T*T*T*TTTGCAGCAGGTGACTGCAGC	SEQ ID NO: 61
D (Oligomer control)	5'-Alexa647- AGTCACGTCGTAGTCIAGCTGCAGTCACCTGCTGCTTTT TTTTTTTTTGCAGCAGGTGACTGCAGC	SEQ ID NO: 62
* = phosphorothioate bond; I = 2'-deoxyinosine		

Figure 33b

Sample ID	Sequence	SEQ ID NO:
A (BRAPA control)	5'-Alexa647- AGTCACGTCGTAGTCIAGCTGCAGTCACCTGCTG CTTZTTCAGCAGGTGACTGCAGC-3'	SEQ ID NO: 63
B (BRAPA modified 1)	5'-Alexa647- AGTCACGTCGTAGTCIAGCTGCAGTCACCTGCTG CTTZTTCAGCAGGTGACTGCAGC-3'	SEQ ID NO: 63
C (BRAPA modified 2)	5'-Alexa647- AGTCACGTCGTAGTCIAGCTGCAGTCACCTGCTG CTTZTTCAGCAGGTGACTGCAGC-3'	SEQ ID NO: 63
D (Oligomer control)	5'-Alexa647- AGTCACGTCGTAGTCIAGCTGCAGTCACCTGCTG CTTYTTCAGCAGGTGACTGCAGC-3'	SEQ ID NO: 64
Z = 5''-thiophosphate-Sp9-Sp9-Sp9-5-methylC, Y = 5''-phosphate-Sp9-Sp9-Sp9-5-methylC; Sp9 = Spacer 9; I = 2'-deoxyinosine		

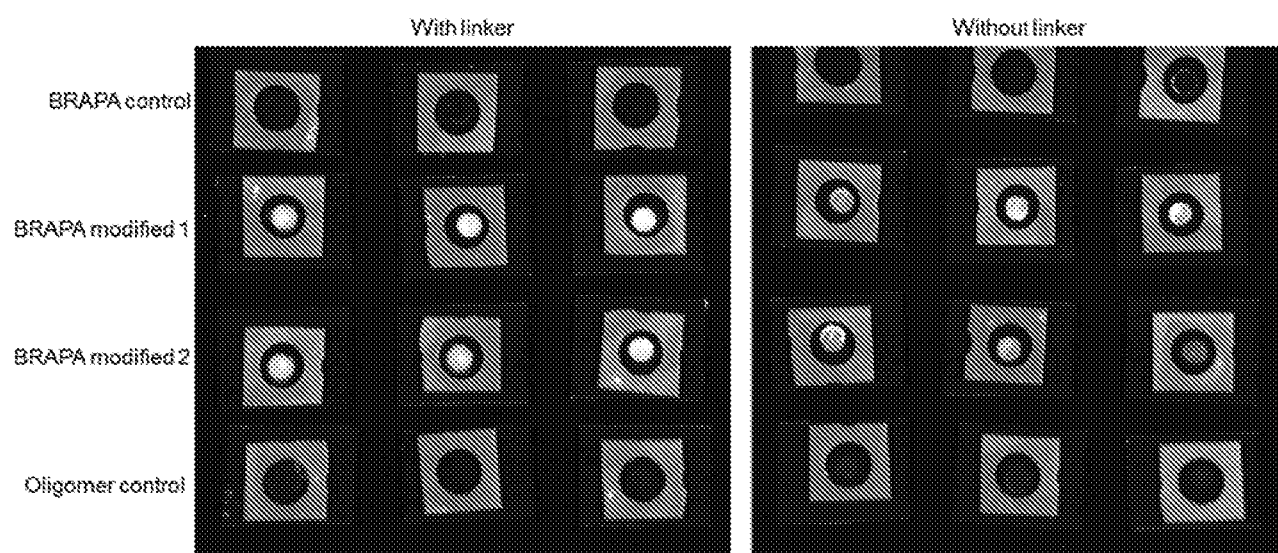
Figure 34

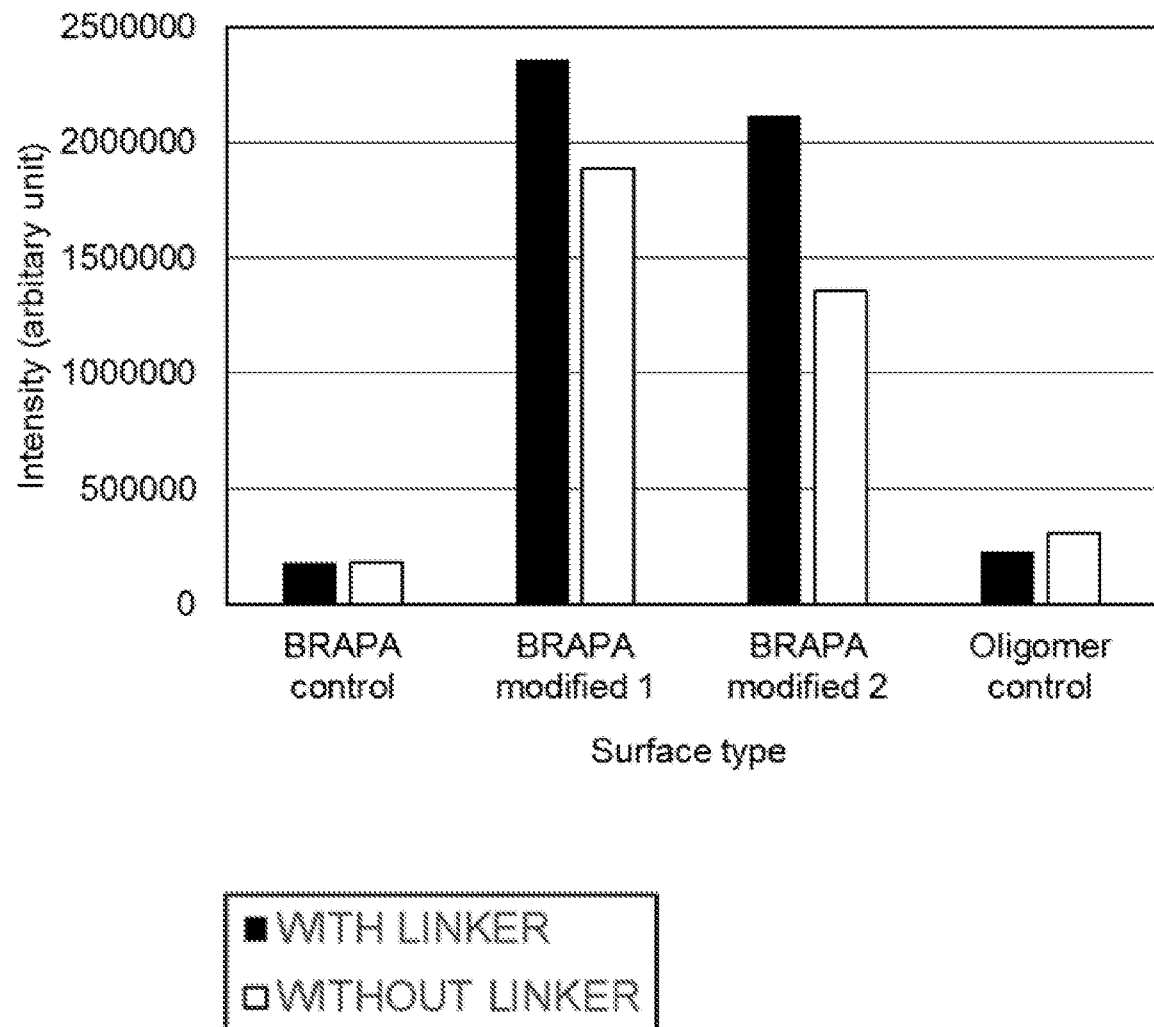
Figure 35

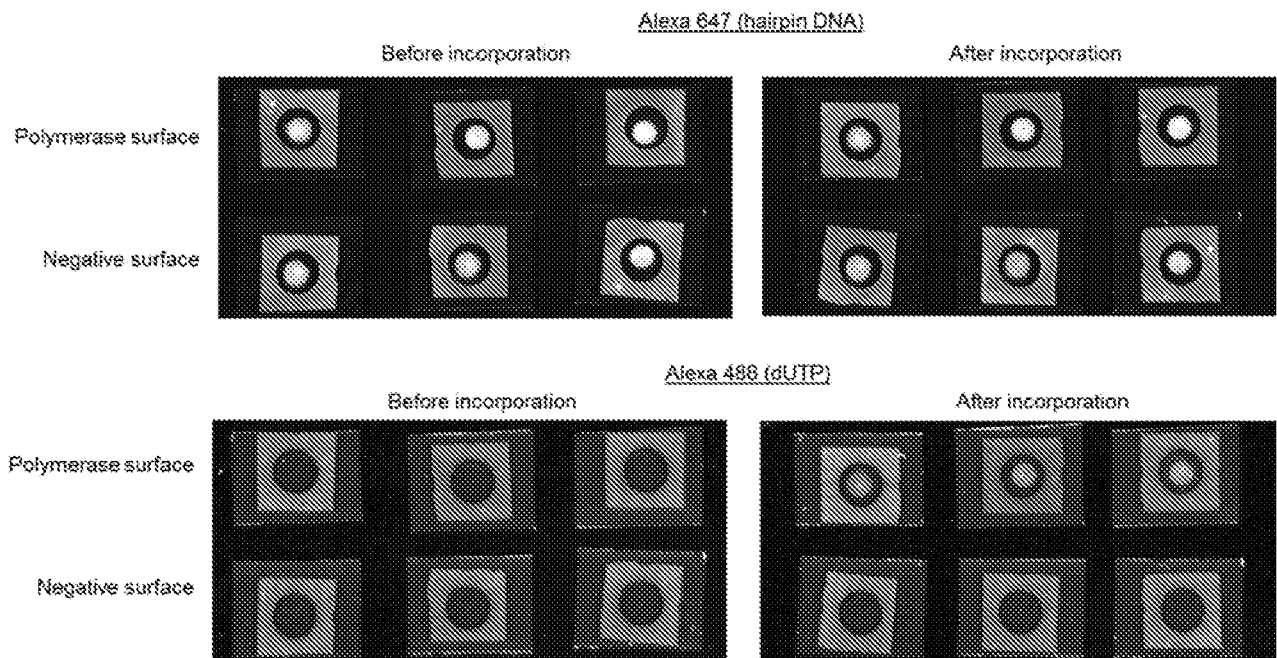
Figure 36

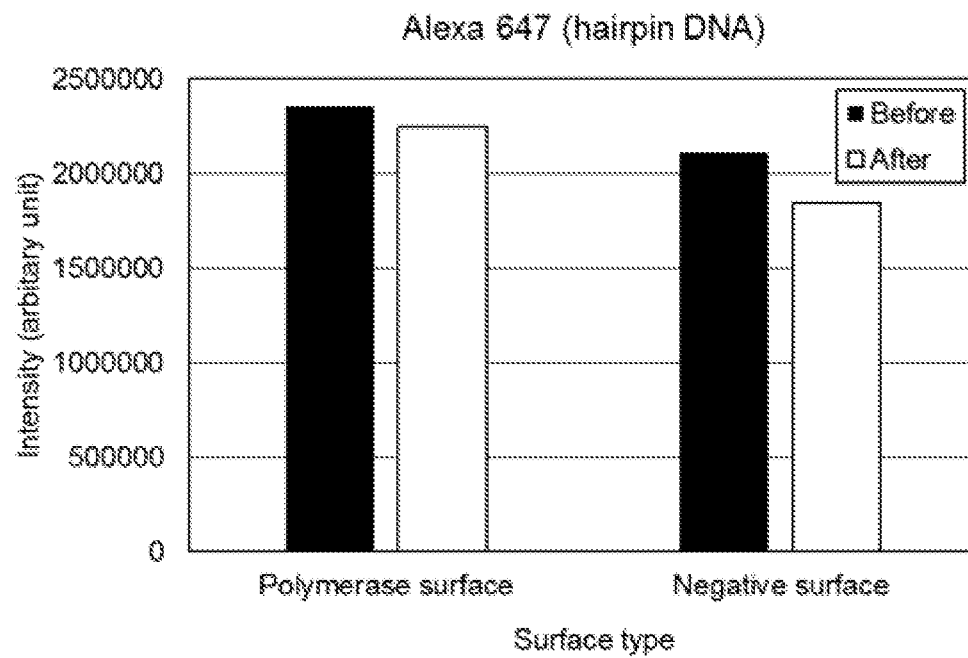
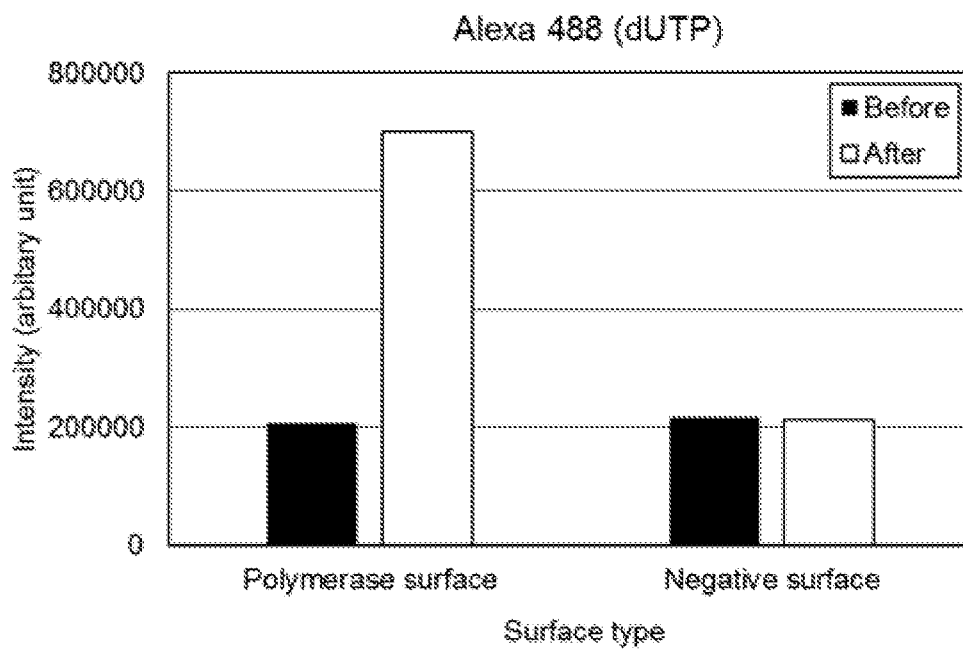
Figure 37a**Figure 37b**

Figure 38a

Sample ID	Sample description (experimental outcome)	Cleavage step	Ligation step
A (positive)	Cleavage (+) Ligation (+)	+ inosine + Endonuclease V	+ helper strand + Ligase
B (ligation negative control)	Cleavage (+) Ligation (-)	+ inosine + Endonuclease V	+ helper strand - Ligase
C (cleavage negative control 1)	Cleavage (-) Ligation (N/A)	+ inosine - Endonuclease V	N/A
D (cleavage negative control 2)	Cleavage (-) Ligation (N/A)	- inosine + Endonuclease V	N/A

Figure 38b

Sample ID	Sequence	SEQ ID NO:
A (positive samples)	5'-Alexa647-AGTCACGTCGTAGTCIAGCTGCAGTCACCTGCTG CTTZTTGCAGCAGGTGACTGCAGCT-3'	SEQ ID NO: 65
B (ligation negative control)	5'-Alexa647-AGTCACGTCGTAGTCIAGCTGCAGTCACCTGCTG CTTZTTGCAGCAGGTGACTGCAGCT -3'	SEQ ID NO: 65
C (cleavage negative control 1)	5'-Alexa647-AGTCACGTCGTAGTCIAGCTGCAGTCACCTGCTG CTTZTTGCAGCAGGTGACTGCAGCT -3'	SEQ ID NO: 65
D (cleavage negative control 2)	5'-Alexa647-AGTCACGTCGTAGTCGAGCTGCAGTCACCTGCTG CTTZTTGCAGCAGGTGACTGCAGCT-3'	SEQ ID NO: 66
Z = 5''-thiophosphate-Sp9-Sp9-Sp9-5-methylC; Sp9 = Spacer 9; I = 2'-deoxyinosine		

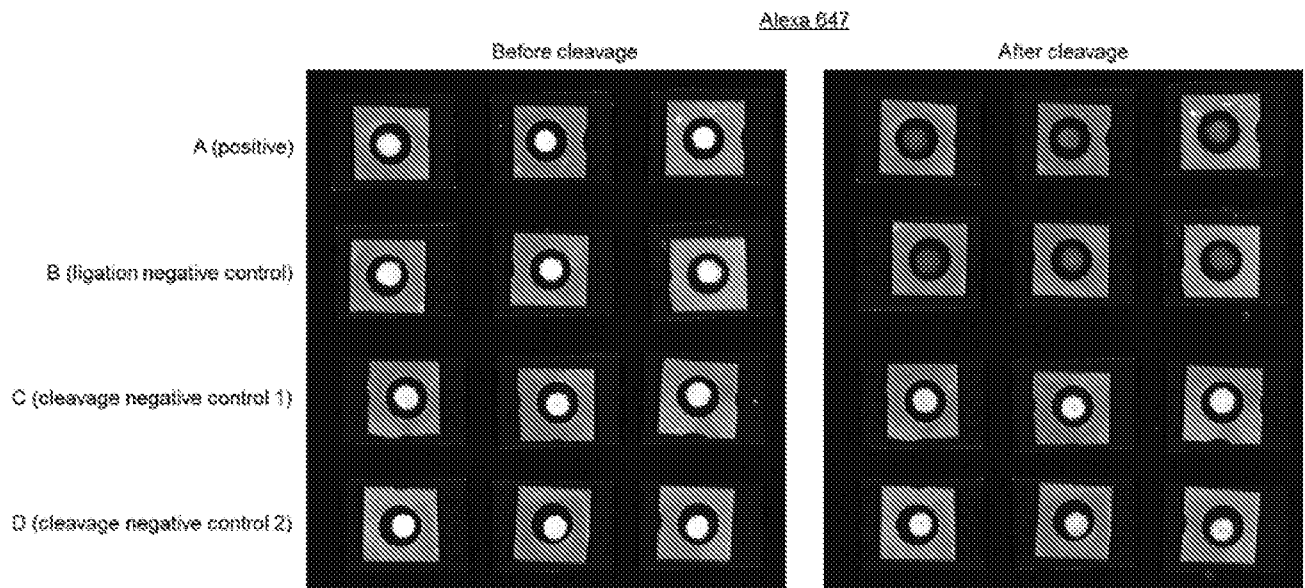
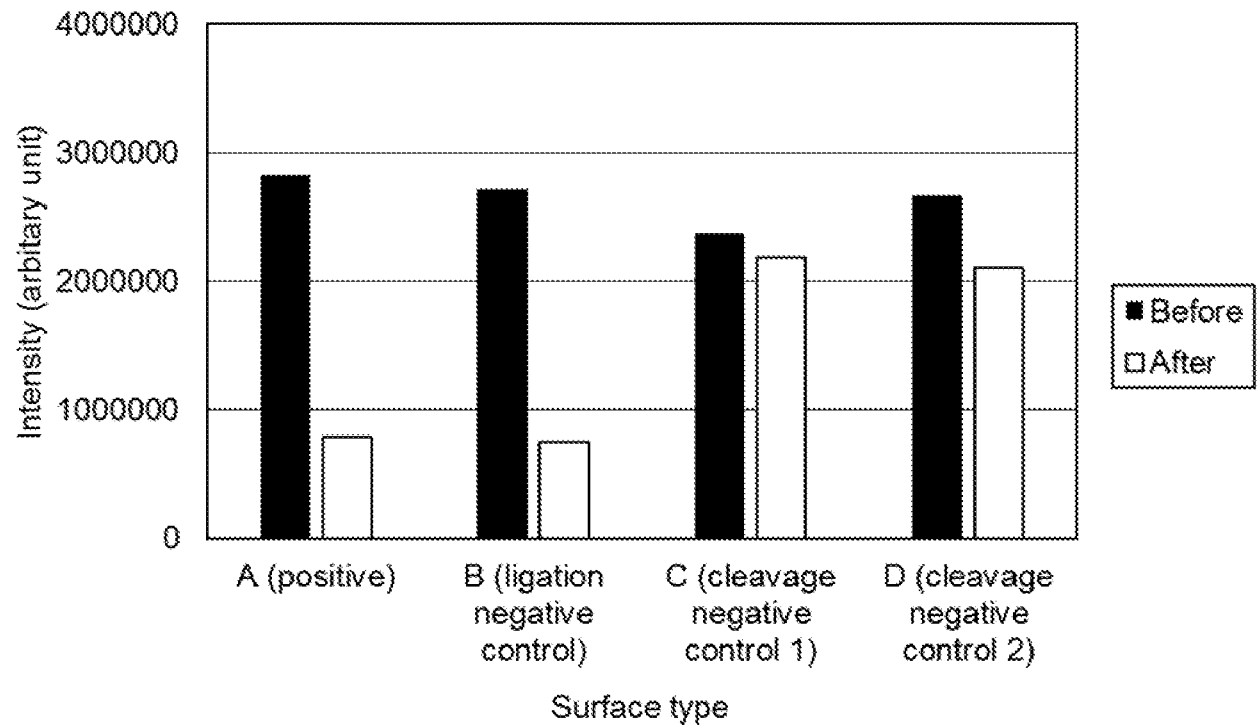
Figure 39

Figure 40**Figure 41**

DNA	Sequence	SEQ ID NO:
Inosine strand	5'-Alexa647-AGTCACGTCGTAGTCIAA-3'	SEQ ID NO: 67
Helper strand	5'-TCGACTACGACGTGACT-3'	SEQ ID NO: 51
I = 2'-deoxyinosine		

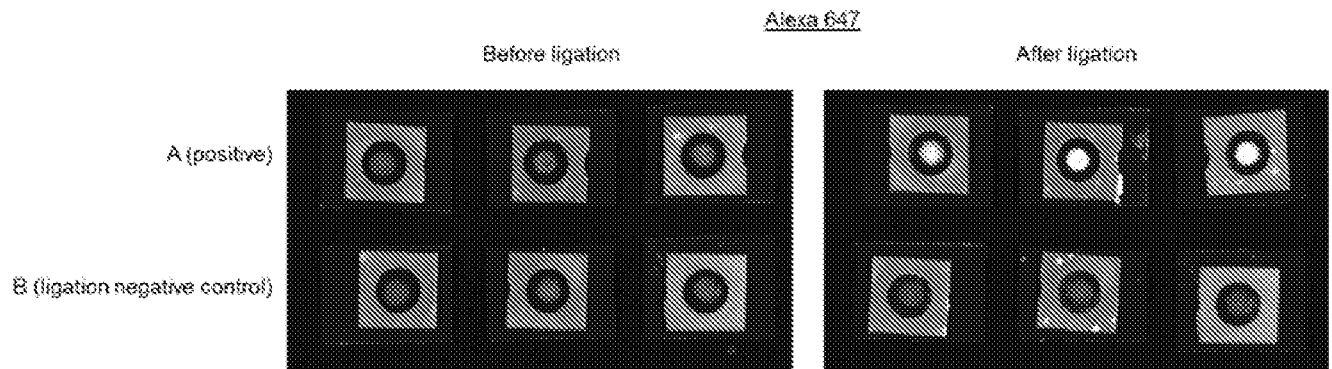
Figure 42

Figure 43

