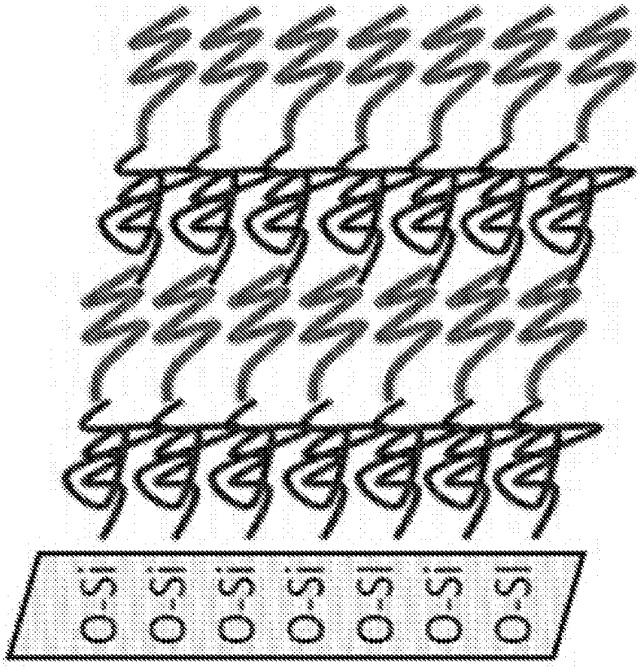
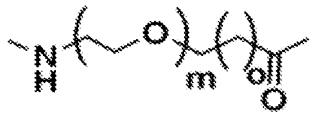
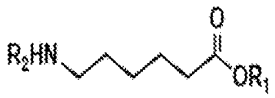
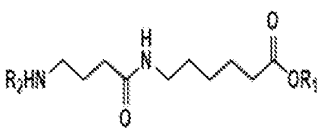


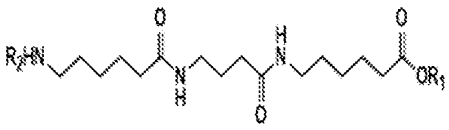
FIG. 1

Spacer: 

Linkers:

11 atom Linker: 

16 atom Linker: 

23 atom Linker: 

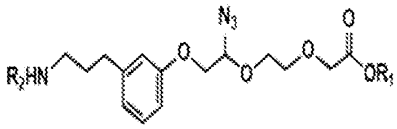
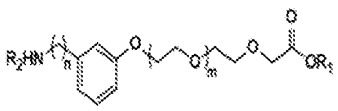
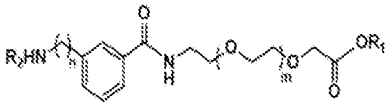
N3 Linker: 

FIG. 2

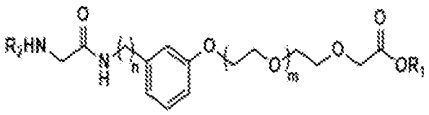
Linker 1:



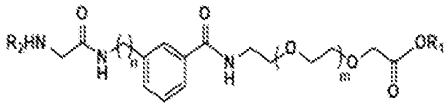
Linker 2:



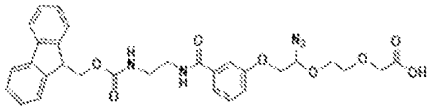
Linker 3:



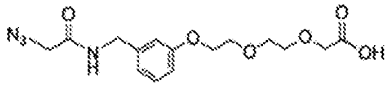
Linker 4:



Linker 5:

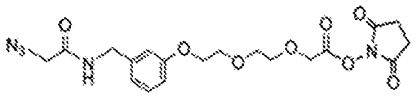


Linker 6:



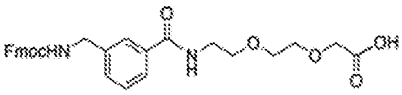
Molecular Weight: 352.35

Linker 7:



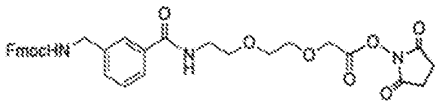
Molecular Weight: 449.42

Linker 8:



Molecular Weight: 518.57

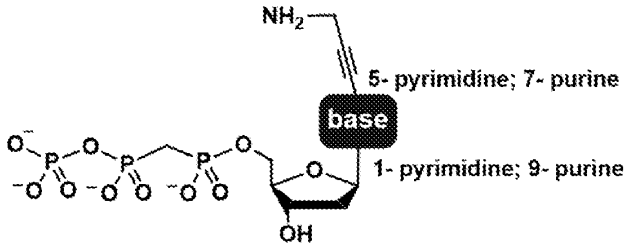
Linker 9:



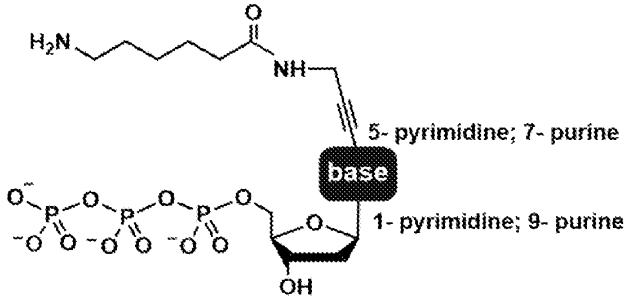
Molecular Weight: 615.64

FIG. 3

dNTP-PA-NH_2:



dNTP-PA-11 Atom Linker-NH_2:



dNTP-PA-16 Atom Linker-NH_2:

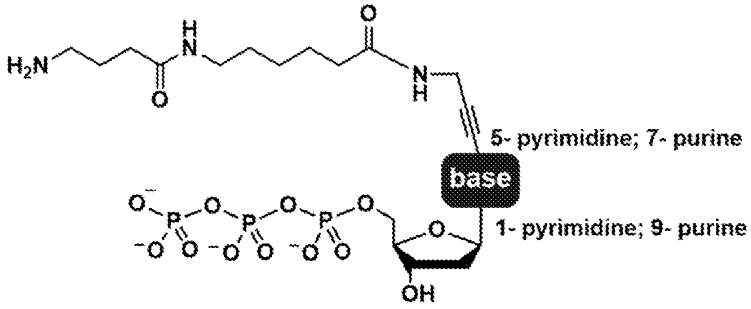
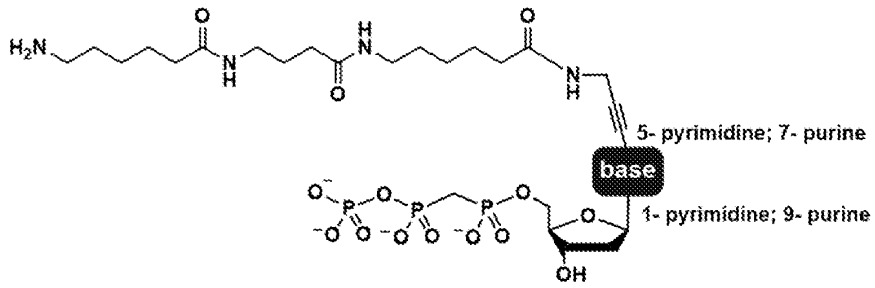
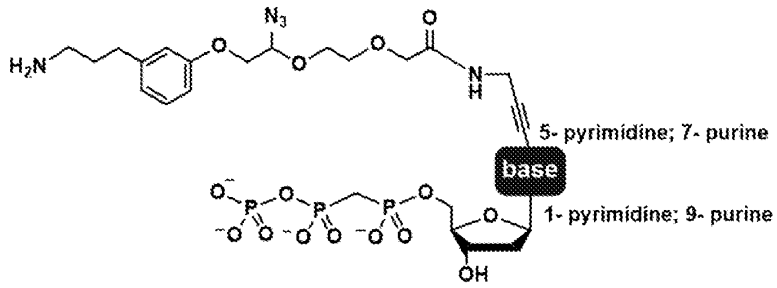


FIG. 4A

dNTP-PA-23 Atom Linker-NH_2:



dNTP-PA-N3 Linker-NH_2:



dNTP-PA-Linker 1-NH_2:

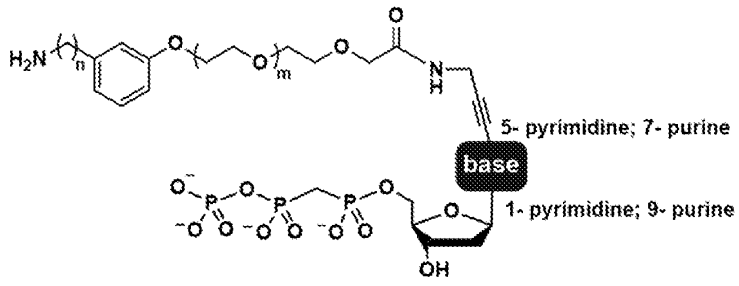
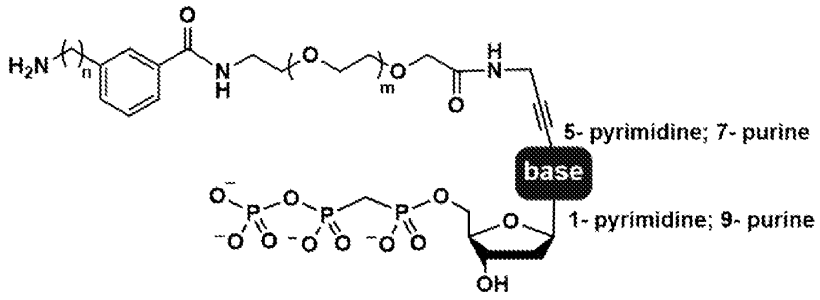


FIG. 4B

dNTP-PA-Linker 2-NH_2:



dNTP-PA-Linker 3-NH_2:

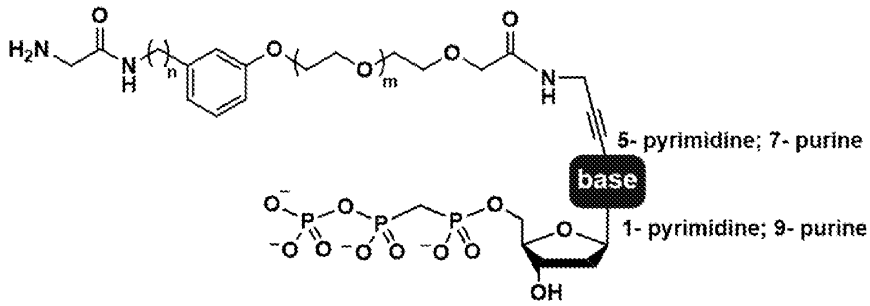
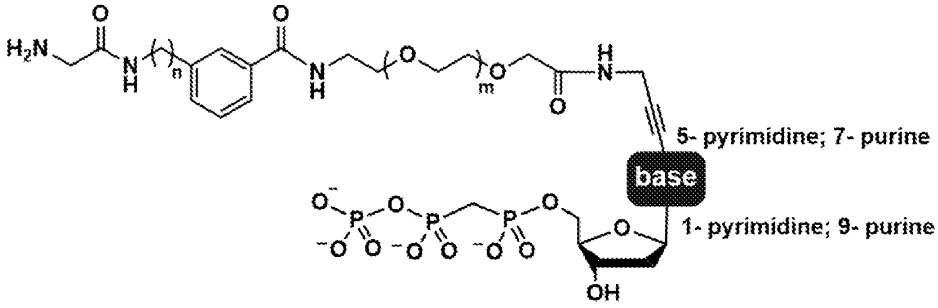


FIG. 4C

dNTP-PA-Linker 4-NH_2:



dNTP-PA-N3 Linker-NH_2:

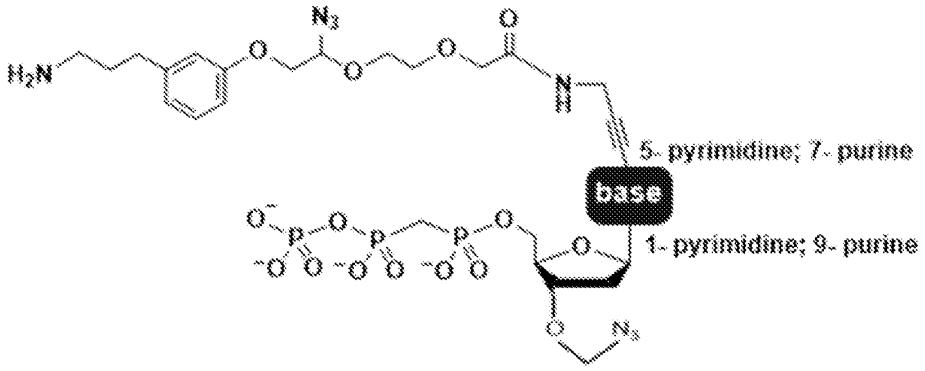


FIG. 4D

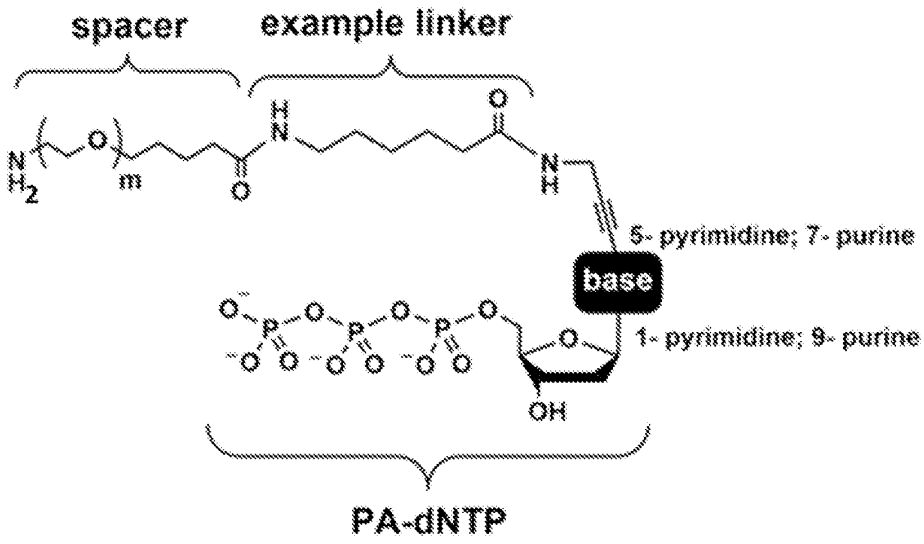

spacer
example linker
5- pyrimidine; 7- purine
base
1- pyrimidine; 9- purine
PA-dNTP

FIG. 5

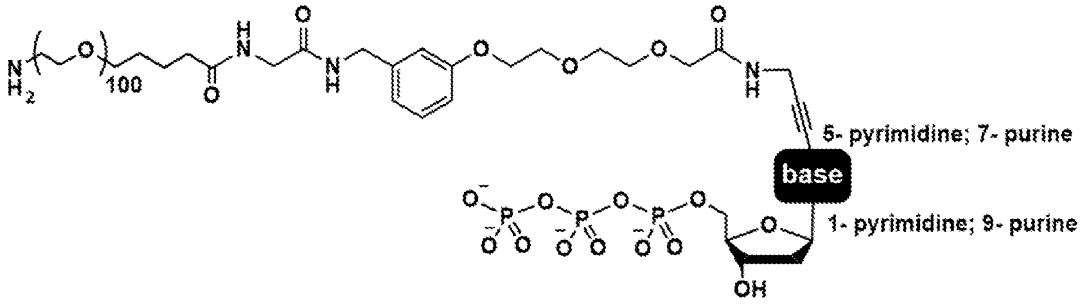

100
5- pyrimidine; 7- purine
base
1- pyrimidine; 9- purine

FIG. 6

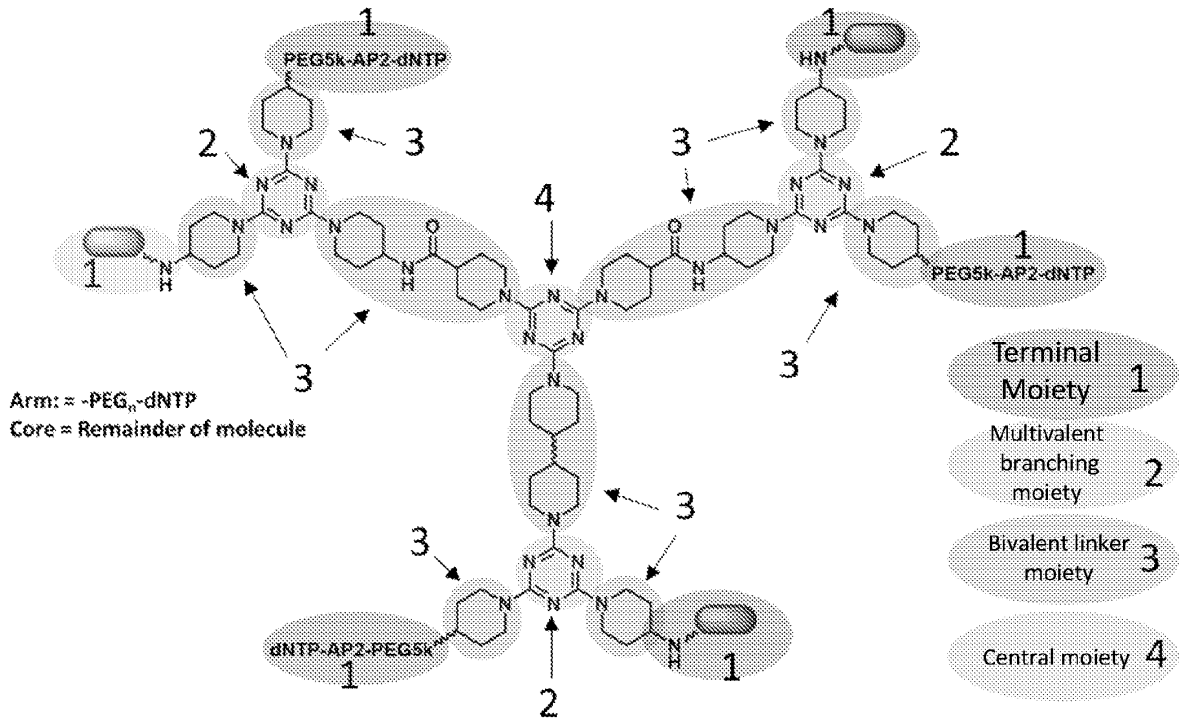

PEG5k-AP2-dNTP
HN
PEG5k-AP2-dNTP
dNTP-AP2-PEG5k
Arm: = -PEG$_n$-dNTP
Core = Remainder of molecule
Terminal Moiety 1
Multivalent branching moiety 2
Bivalent linker moiety 3
Central moiety 4

FIG. 7

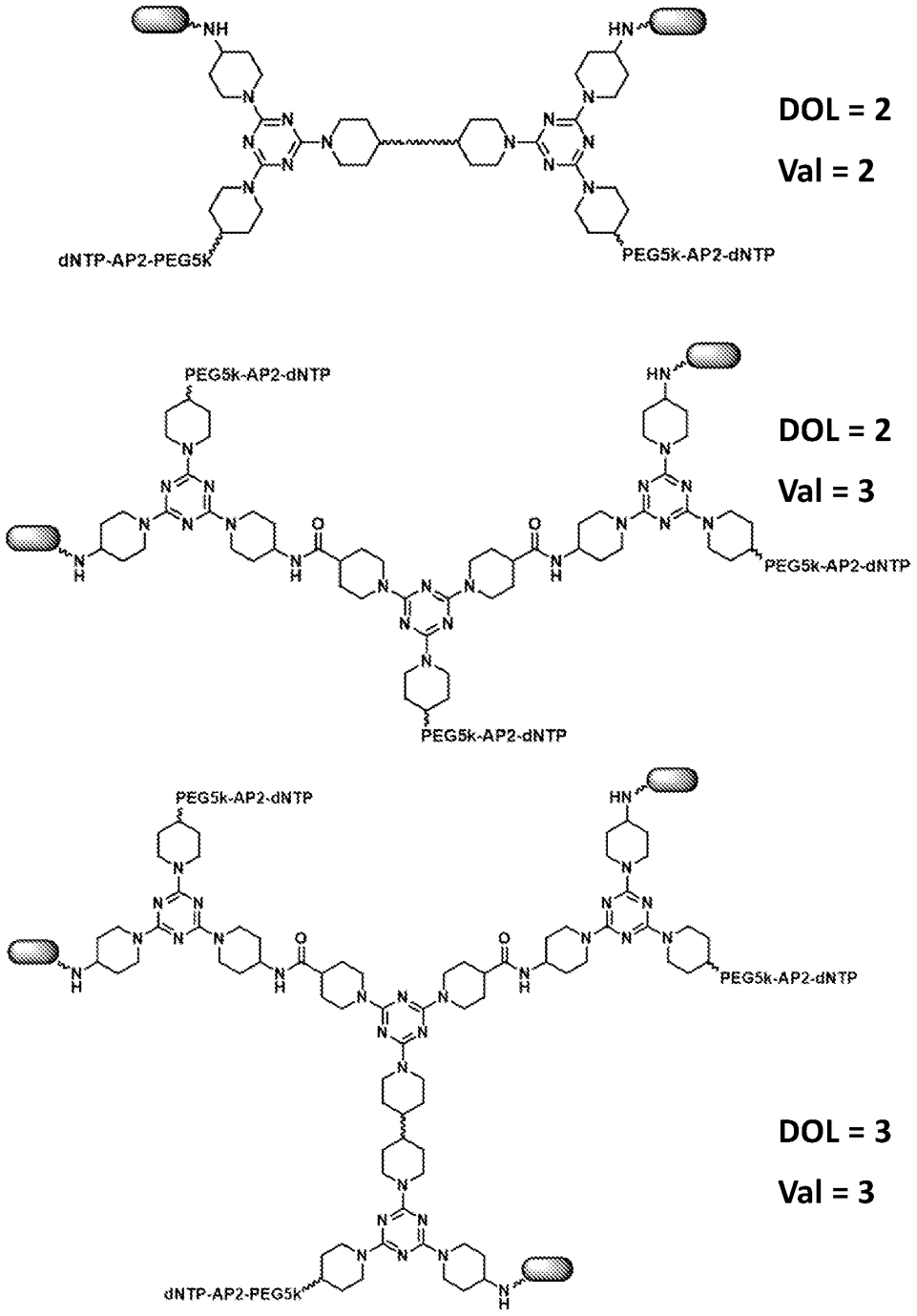
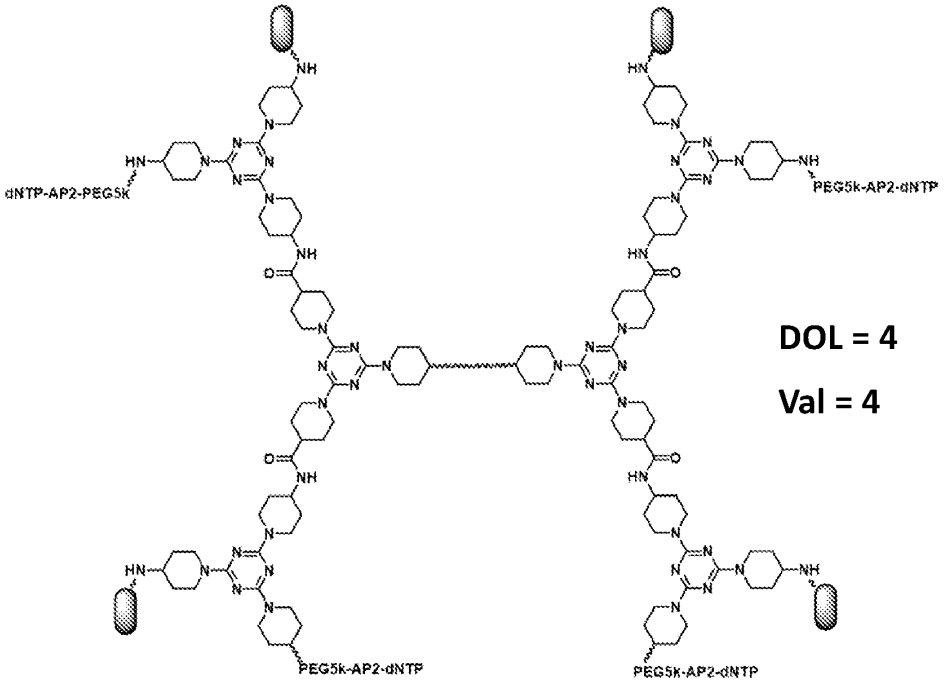

NH
HN
dNTP-AP2-PEG5K
PEG5k-AP2-dNTP
DOL = 2
Val = 2
PEG5k-AP2-dNTP
HN
PEG5k-AP2-dNTP
PEG5k-AP2-dNTP
DOL = 2
Val = 3
PEG5k-AP2-dNTP
HN
PEG5k-AP2-dNTP
dNTP-AP2-PEG5k
DOL = 3
Val = 3

FIG. 8A


dNTP-AP2-PEG5k
PEG5k-AP2-dNTP
PEG5k-AP2-dNTP
PEG5k-AP2-dNTP
DOL = 4
Val = 4

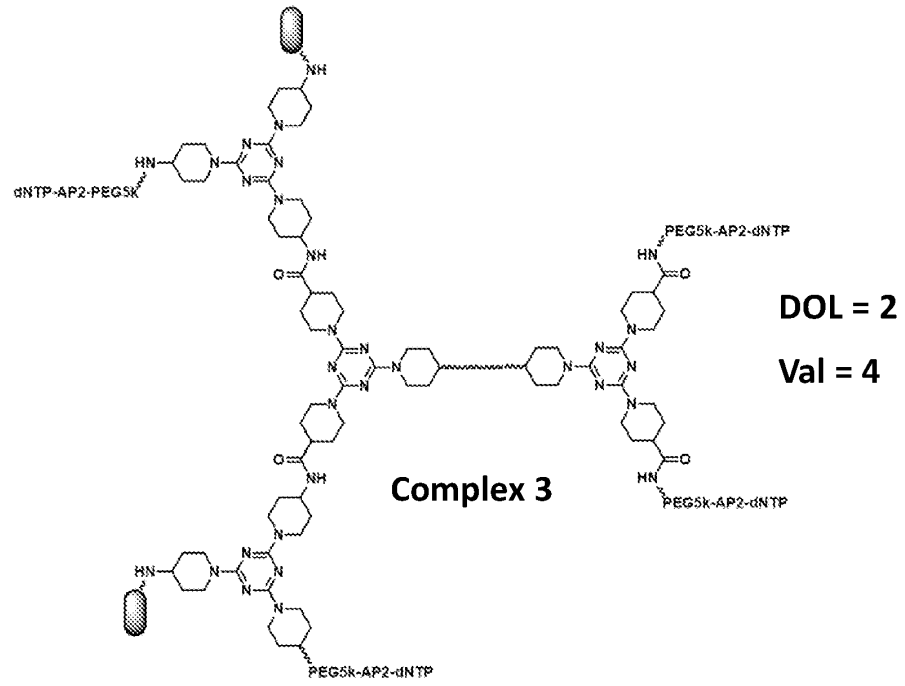

dNTP-AP2-PEG5k
PEG5k-AP2-dNTP
PEG5k-AP2-dNTP
PEG5k-AP2-dNTP
DOL = 2
Val = 4
Complex 3

FIG. 8B

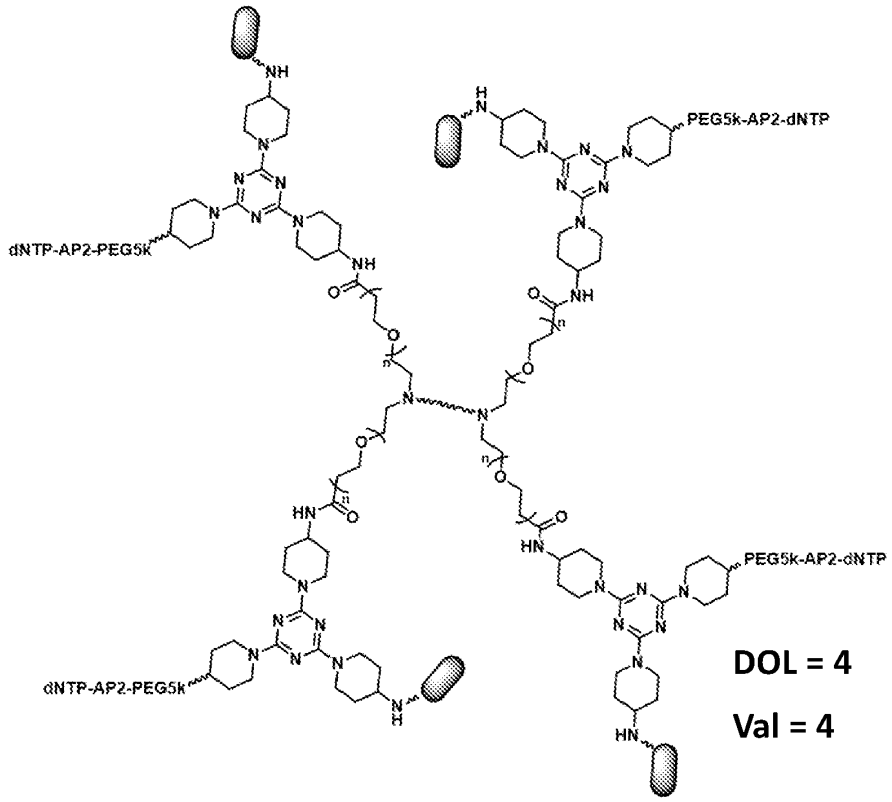

dNTP-AP2-PEG5k
PEG5k-AP2-dNTP
dNTP-AP2-PEG5k
PEG5k-AP2-dNTP
DOL = 4
Val = 4

FIG. 8C

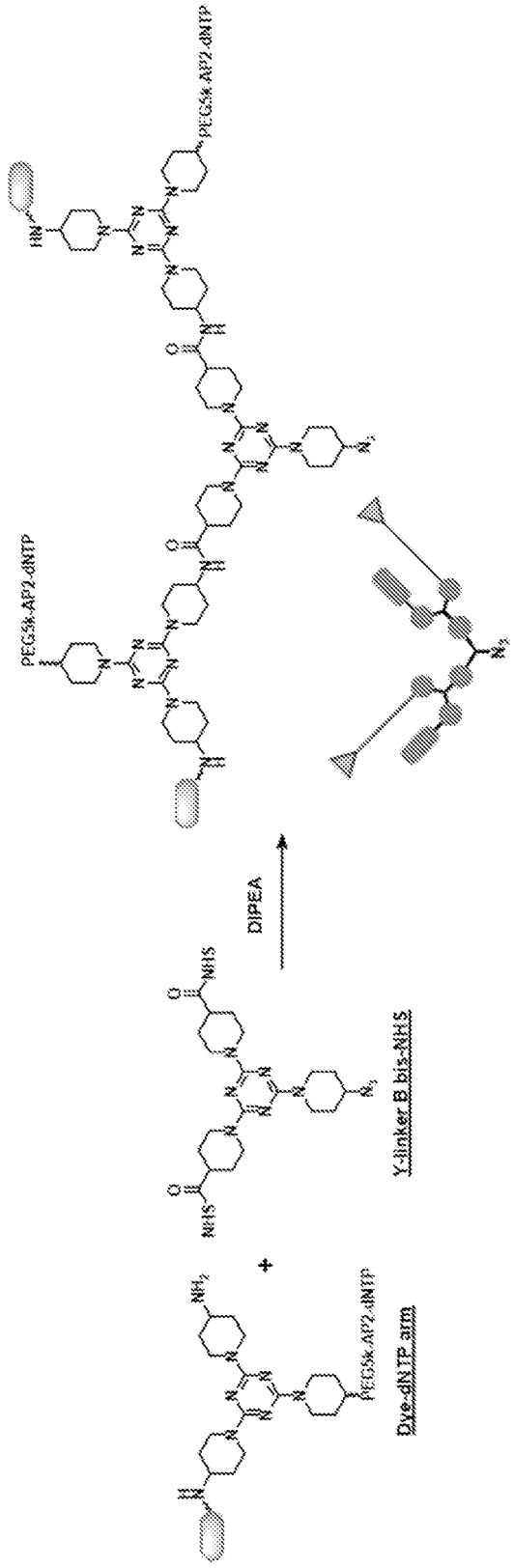

PEG5k AP2 dNTP
PEG5k AP2 dNTP
PEG5k AP2 dNTP
DIPEA
Y-linker B bis-NHS
Dye-dNTP arm

FIG. 9

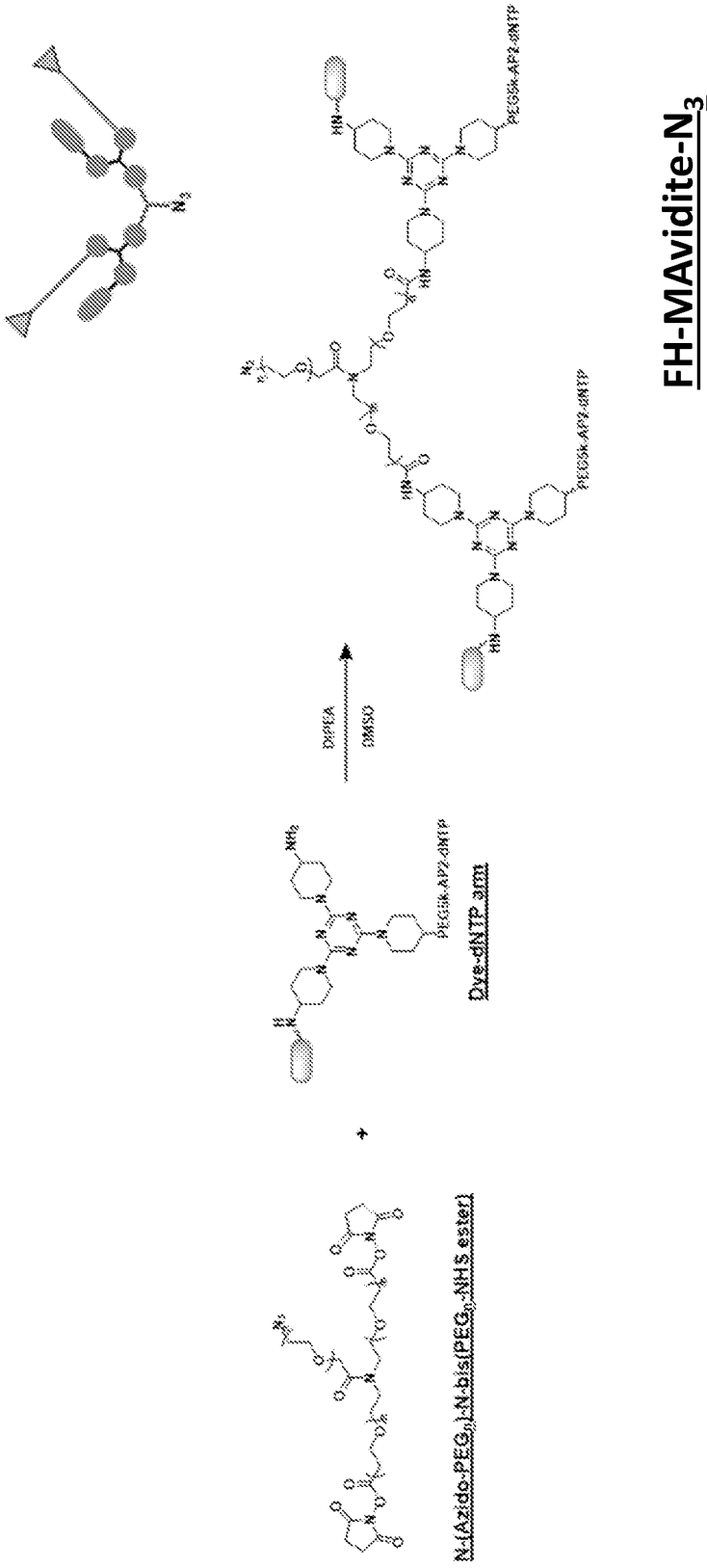
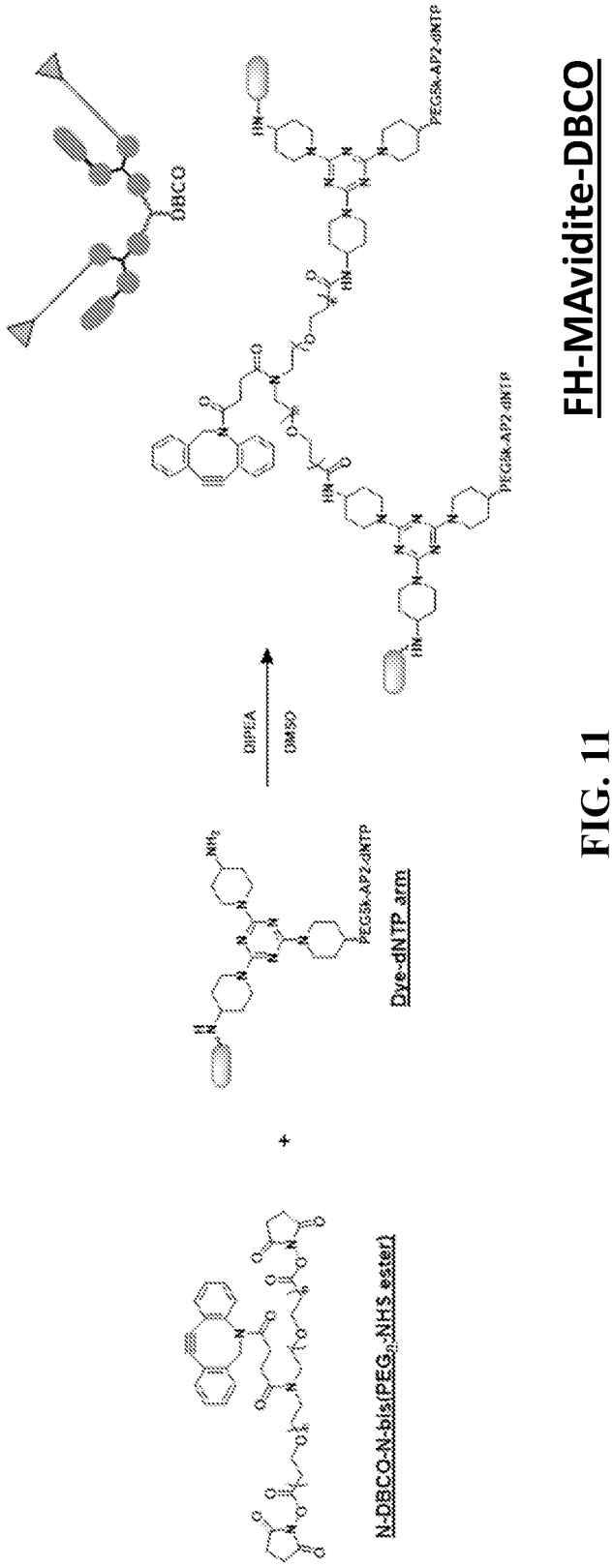

N-(Azido-PEG3)-N-bis(PEG3-NHS ester)
Dye-dNTP arm
DIPEA
DMSO
FH-MAvidite-N3

FIG. 10



FH-MAvidite-DBCO

FIG. 11

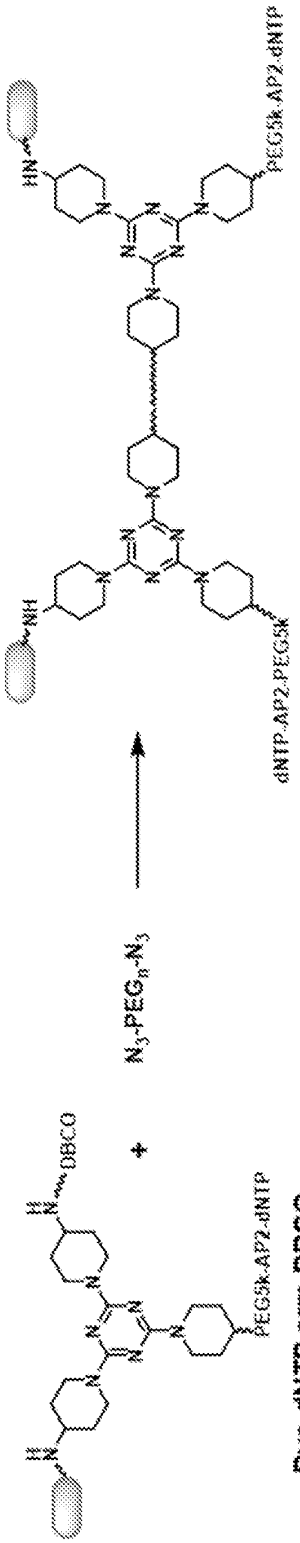
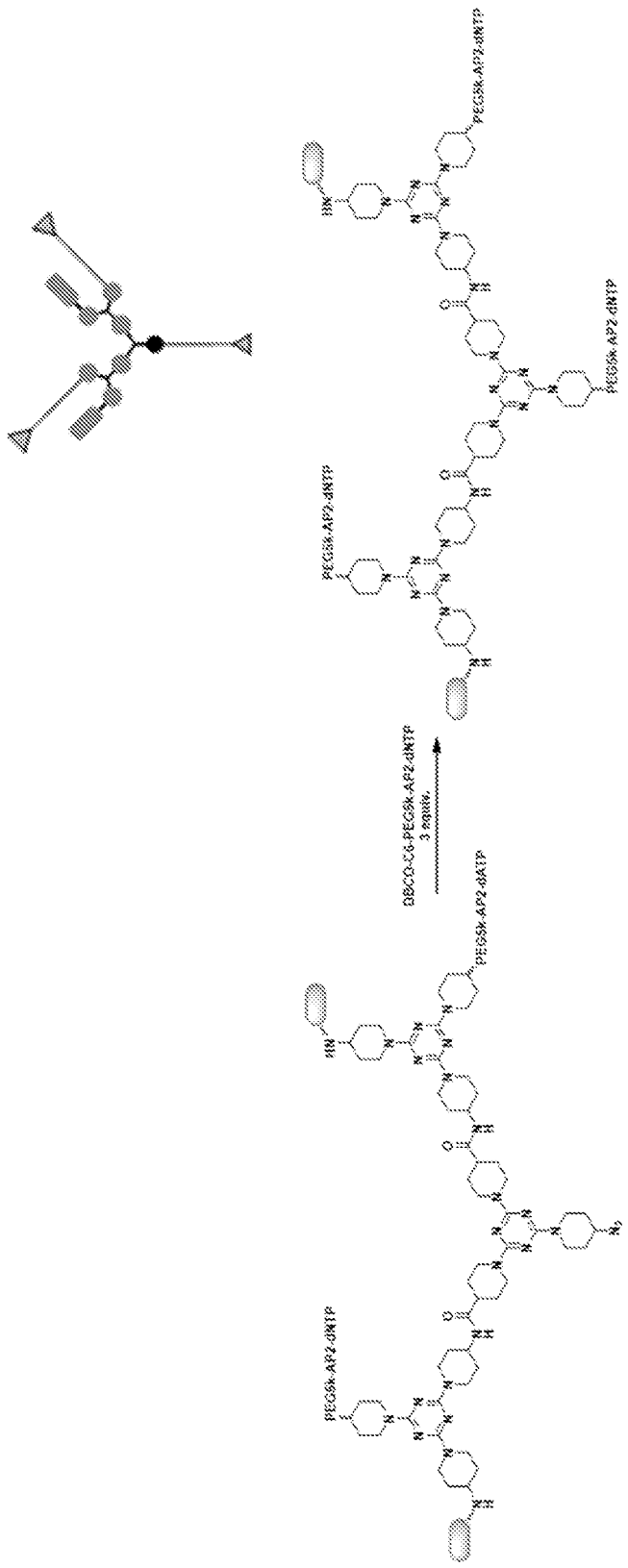

Dye-dNTP arm-DBCO
DBCO
PEG5k-AP2-dNTP
+
N_3-PEG$_n$-N_3
dNTP-AP2-PEG5k
PEG5k-AP2-dNTP
Dye$_2$-dNTP$_2$ MAvidite
DOL = 2
Val = 2

FIG. 12A



Dye$_2$-dNTP$_3$ MAvidite

DOL = 2

Valency = 3

FIG. 12B

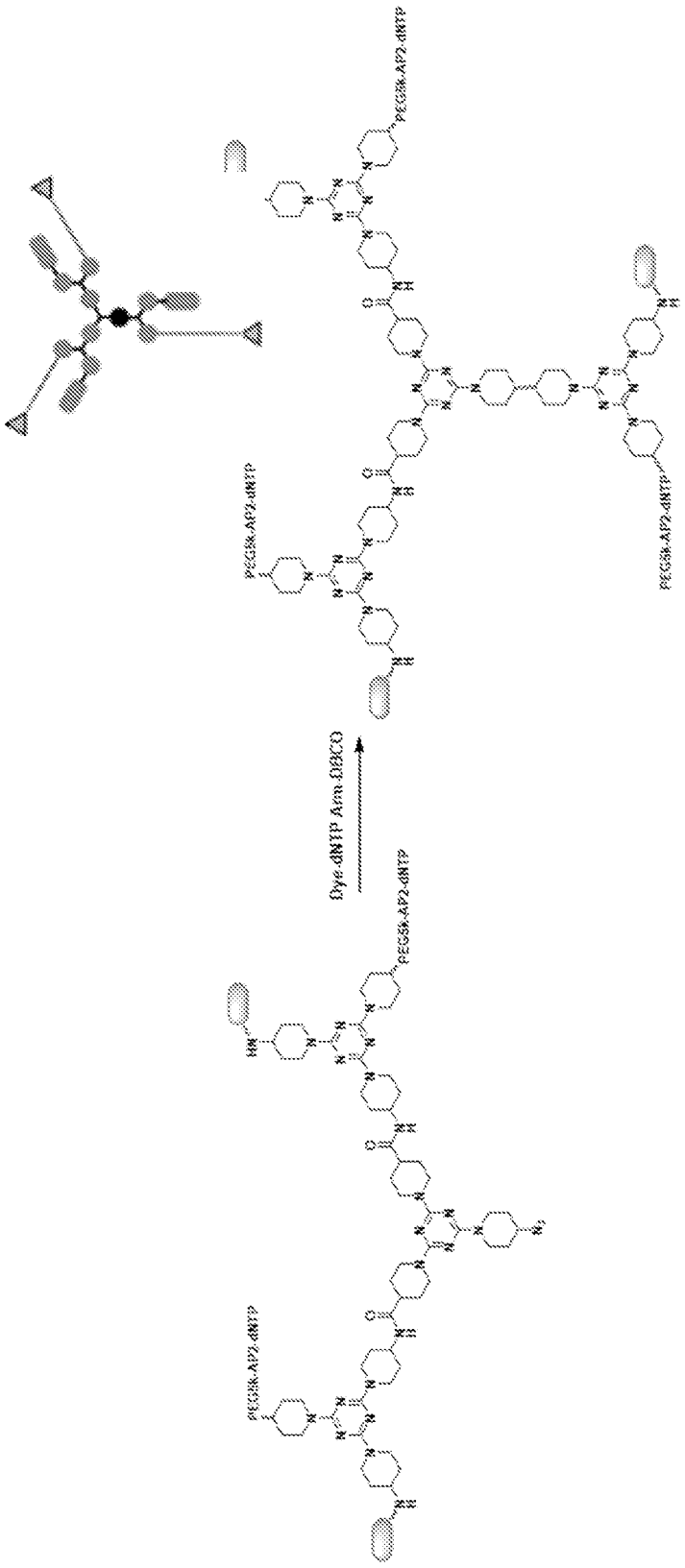
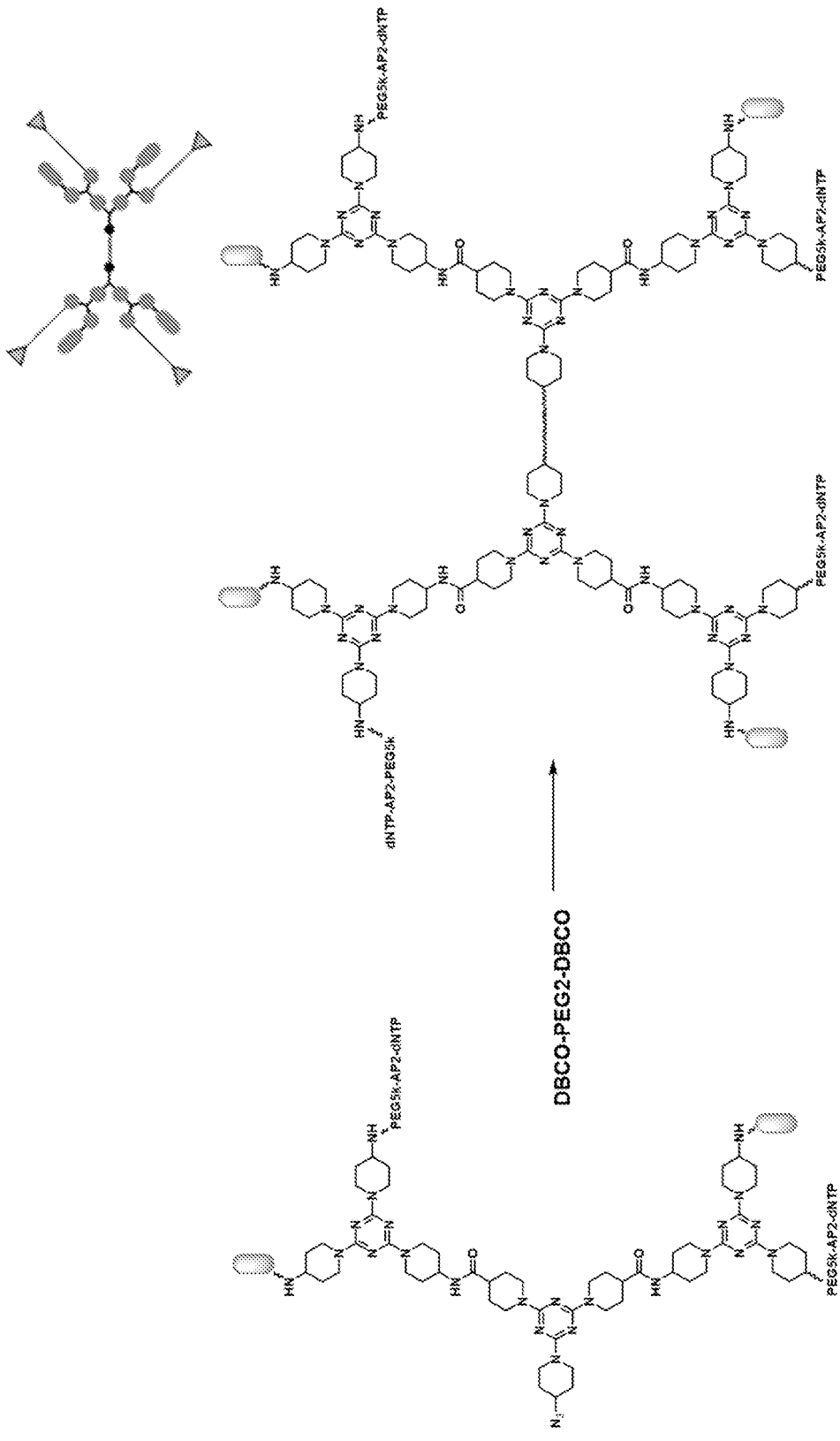


FIG. 12C

Dye$_3$-dNTP$_3$ MAvidite

DOL = 3

Valency = 3



Rigid half MAvidite-N_3 (RH-Mavidite-N_3)

Dye_4-$dNTP_4$ MAvidite

DOL = 4

Valency = 4

FIG. 12D

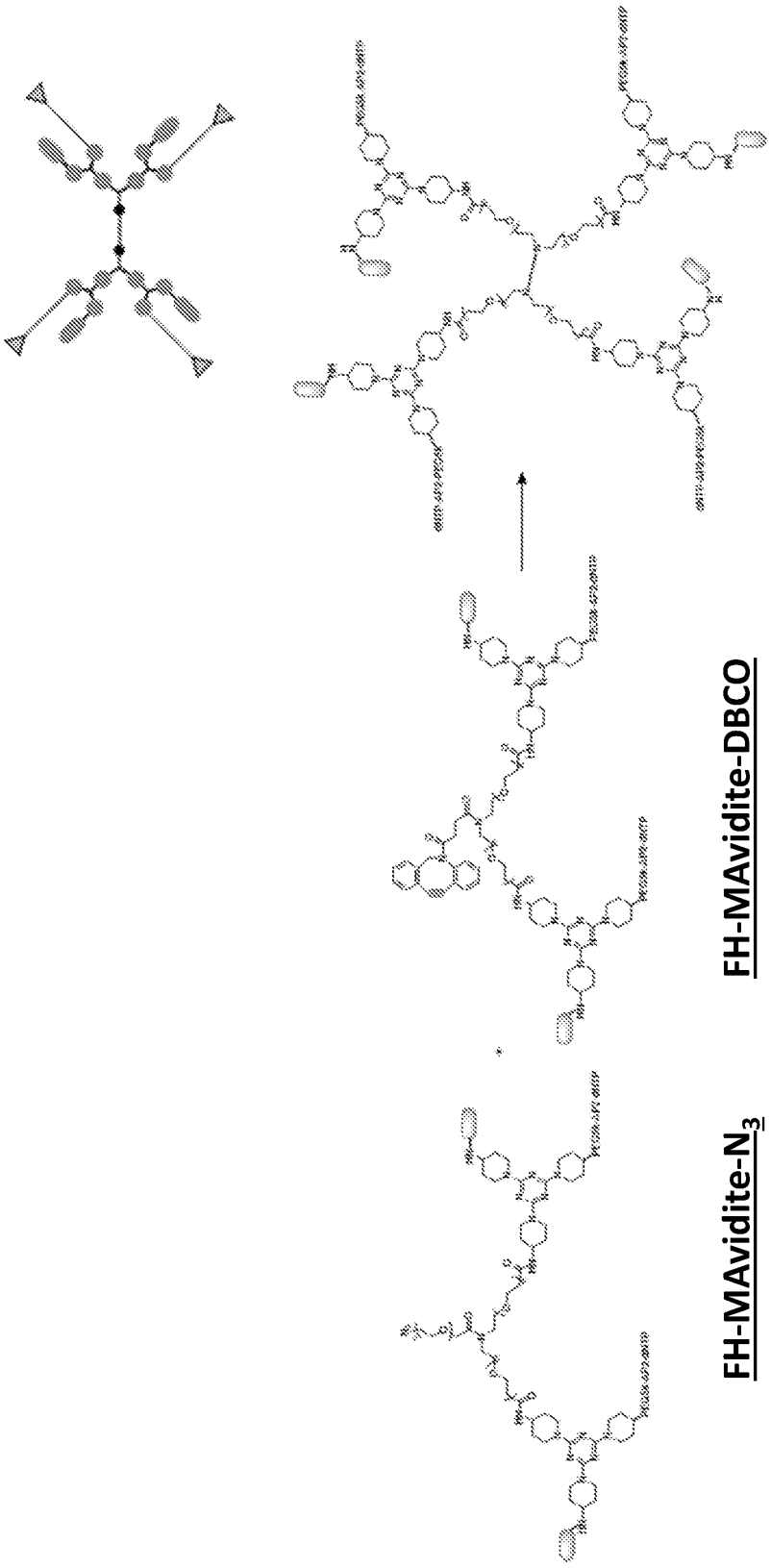
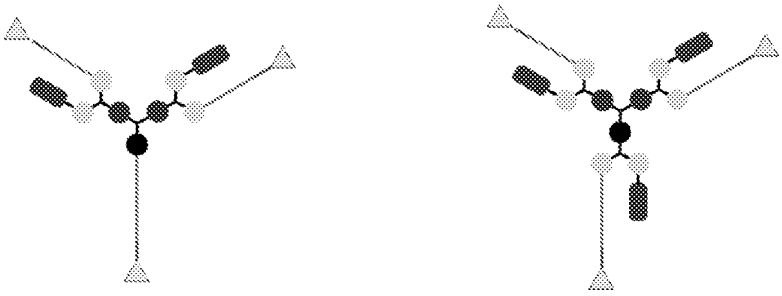

FH-MAvidite-N3
FH-MAvidite-DBCO
Dye4-dNTP4 MAvidite
DOL = 4
Val = 4

FIG. 12E

CF647-dA Macro-Avidite **<u>110 cycles</u>**	DOL = 2, Val = 3 Complex 1	DOL = 3, Val = 3 Complex 2
Q30 (%)	97.8	97.6
Q40 (%)	92.6	91.8
Error rate (%)	0.17	0.19
Phasing (%)	11.1	14.1
Prephasing (%)	8.2	11.7
Residual (@ cycle 90)	0.0037	0.0077

FIG. 13A



DOL = 2
Val = 3
Complex 1

DOL = 3
Val = 3
Complex 2

FIG. 13B

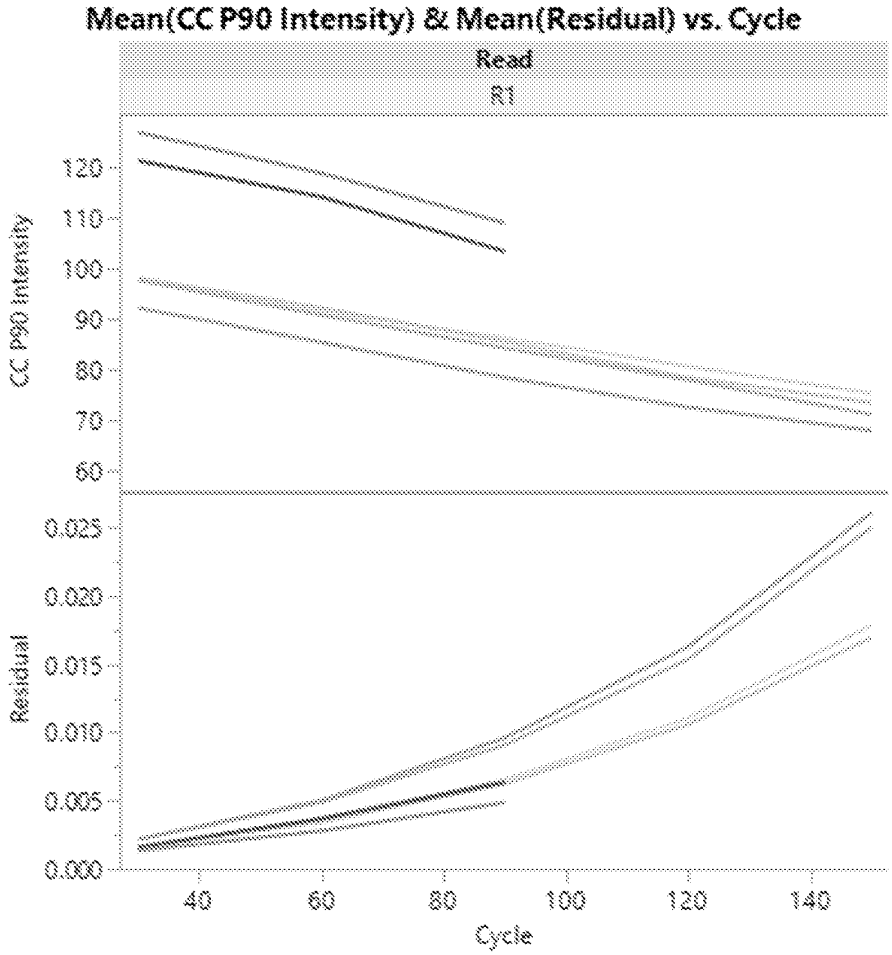

Mean(CC P90 Intensity) & Mean(Residual) vs. Cycle
Read
R1
CC P90 Intensity
120
110
100
90
80
70
60
Residual
0.025
0.020
0.015
0.010
0.005
0.000
40
60
80
100
120
140
Cycle

FIG. 14

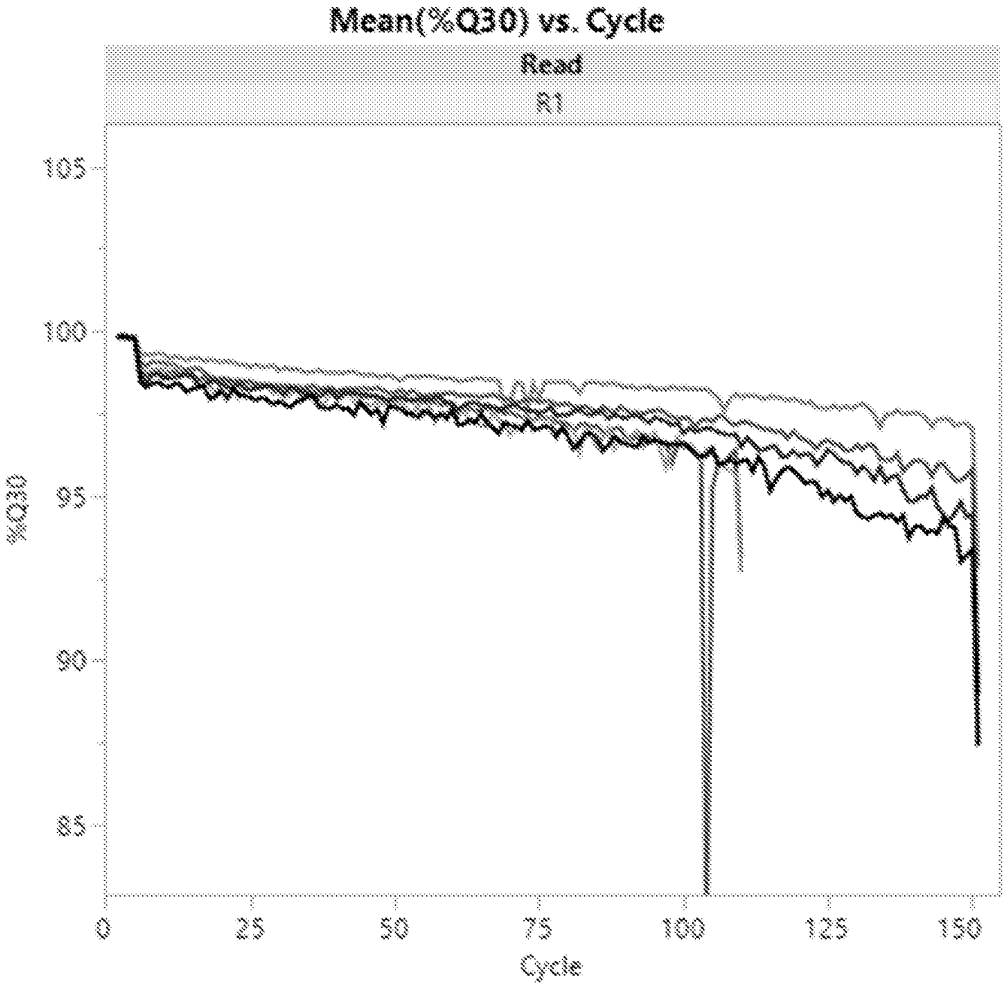

Mean(%Q30) vs. Cycle
Read
R1
105
100
95
90
85
%Q30
0
25
50
75
100
125
150
Cycle

FIG. 15

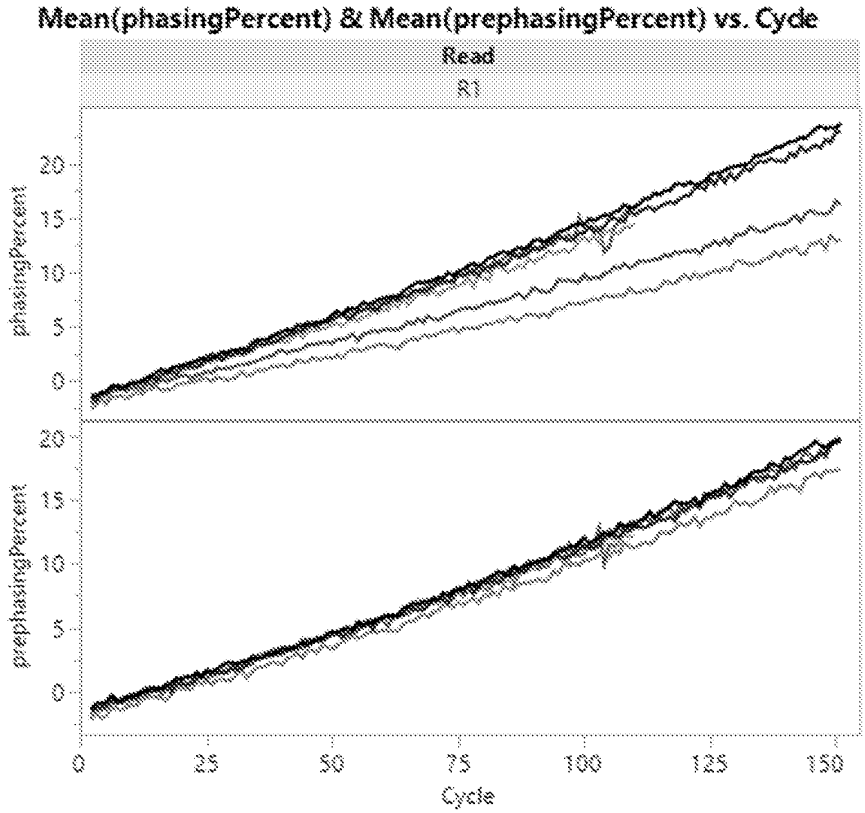

Mean(phasingPercent) & Mean(prephasingPercent) vs. Cycle
Read
R1
phasingPercent
prephasingPercent
0
5
10
15
20
0
25
50
75
100
125
150
Cycle

FIG. 16

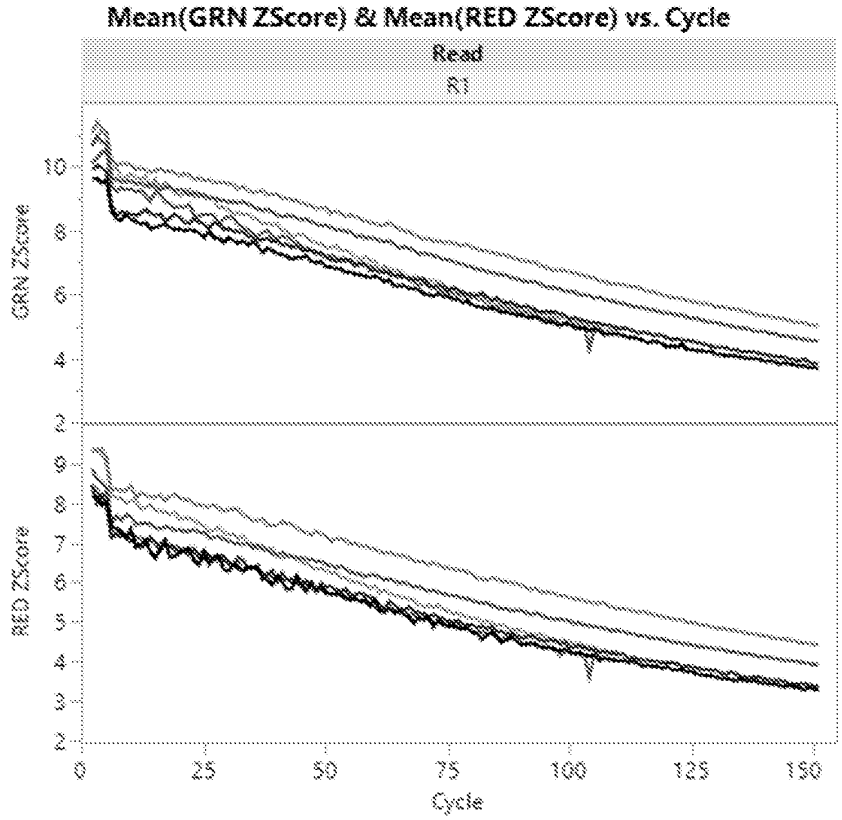

Mean(GRN ZScore) & Mean(RED ZScore) vs. Cycle
Read
R1
GRN ZScore
RED ZScore
Cycle
0
25
50
75
100
125
150

FIG. 17

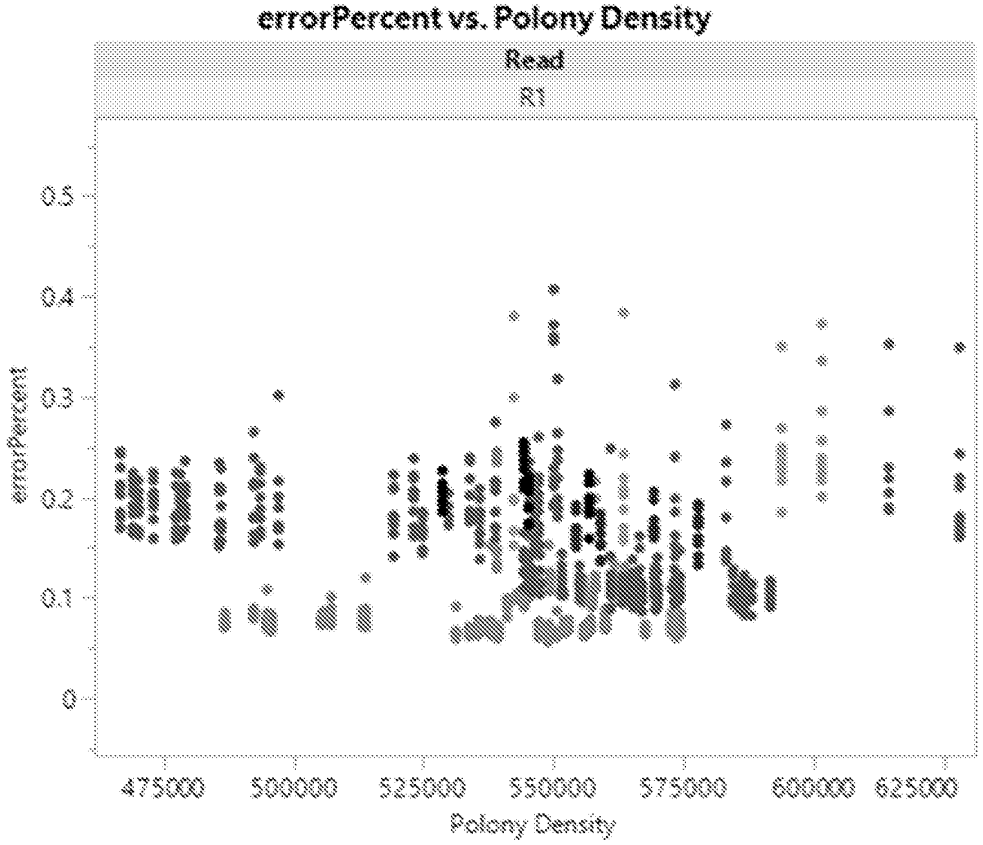

errorPercent vs. Polony Density
Read
R1
errorPercent
0.5
0.4
0.3
0.2
0.1
0
475000
500000
525000
550000
575000
600000
625000
Polony Density

FIG. 18A

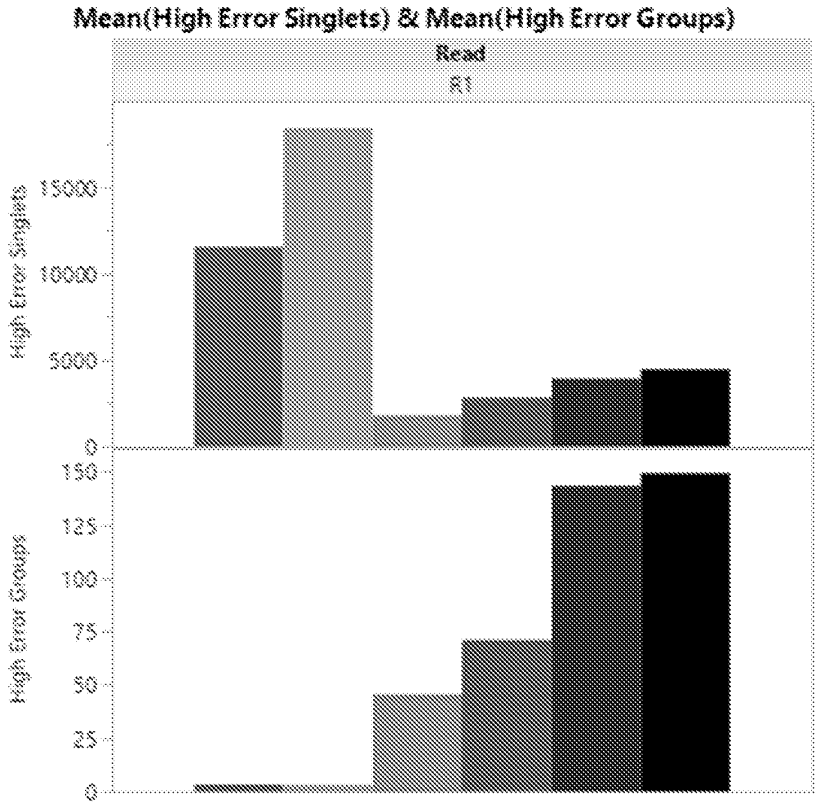

Mean(High Error Singlets) & Mean(High Error Groups)
Read
R1
High Error Singlets
15000
10000
5000
0
High Error Groups
150
125
100
75
50
25
0

FIG. 18B

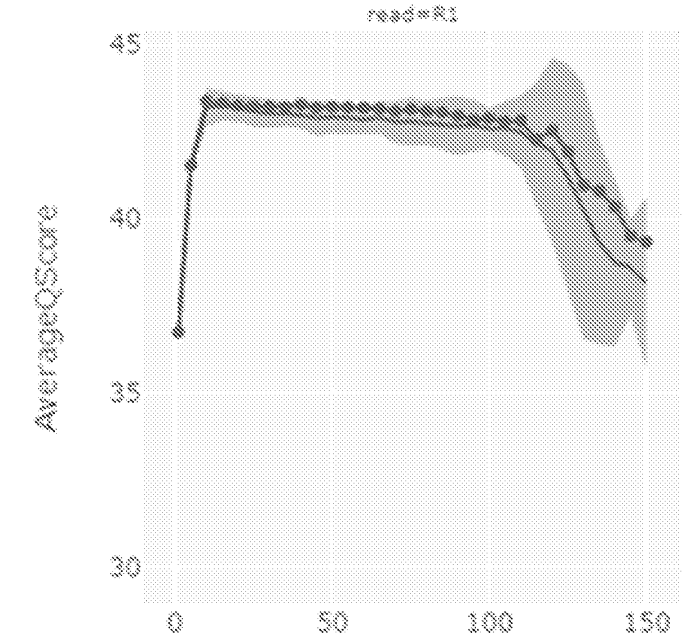


FIG. 19A

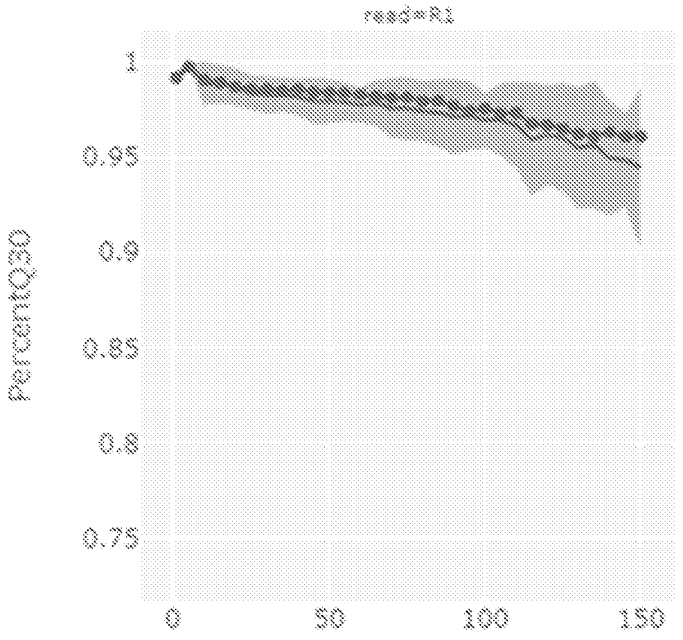


FIG. 19B

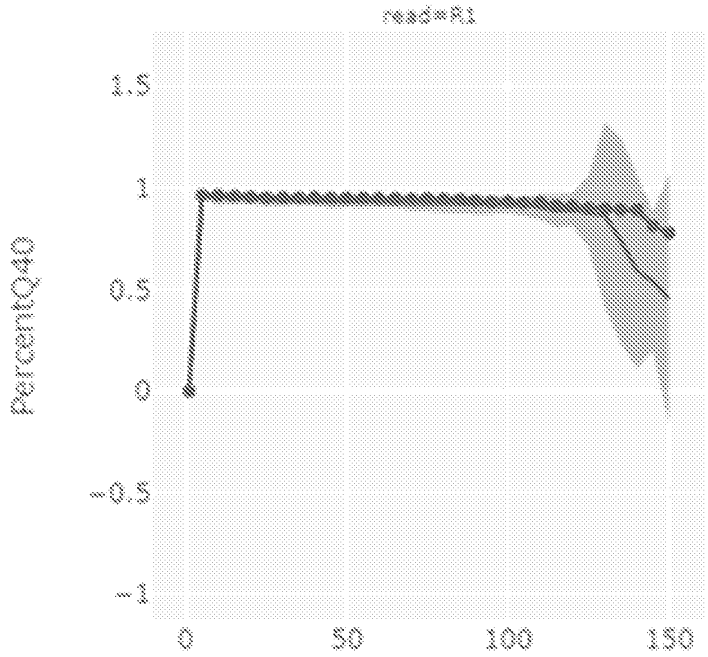


FIG. 19C

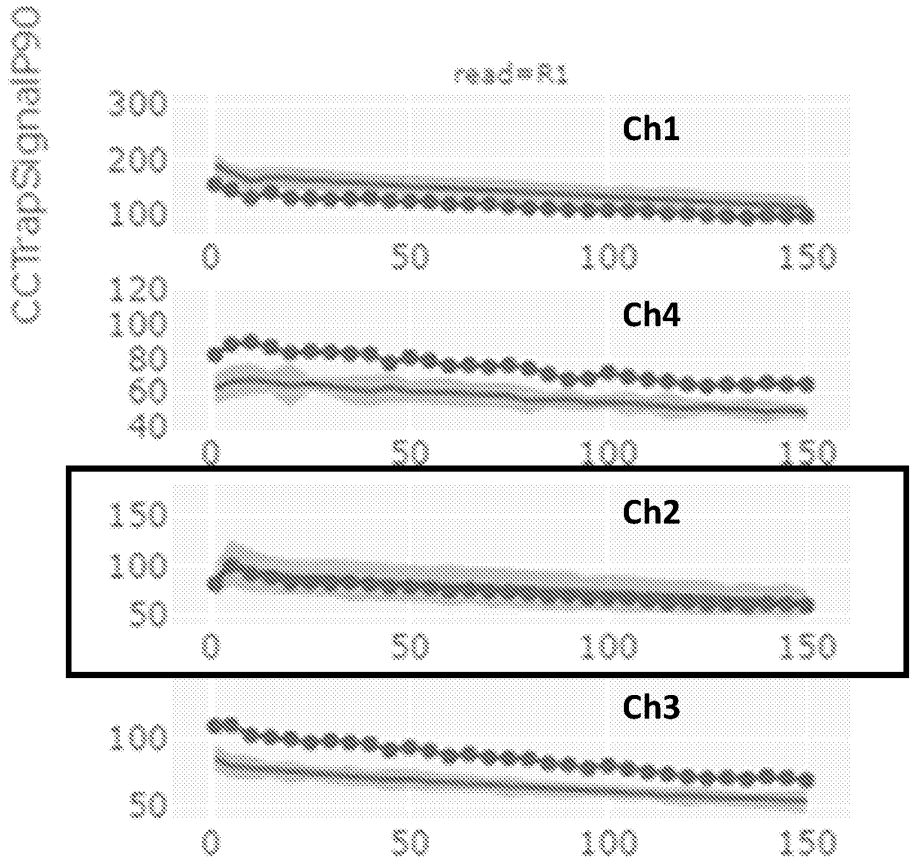

CCTrapSignalPeo
read=R1
Ch1
300
200
100
0
50
100
150
Ch4
120
100
80
60
40
Ch2
150
100
50
Ch3

FIG. 20A

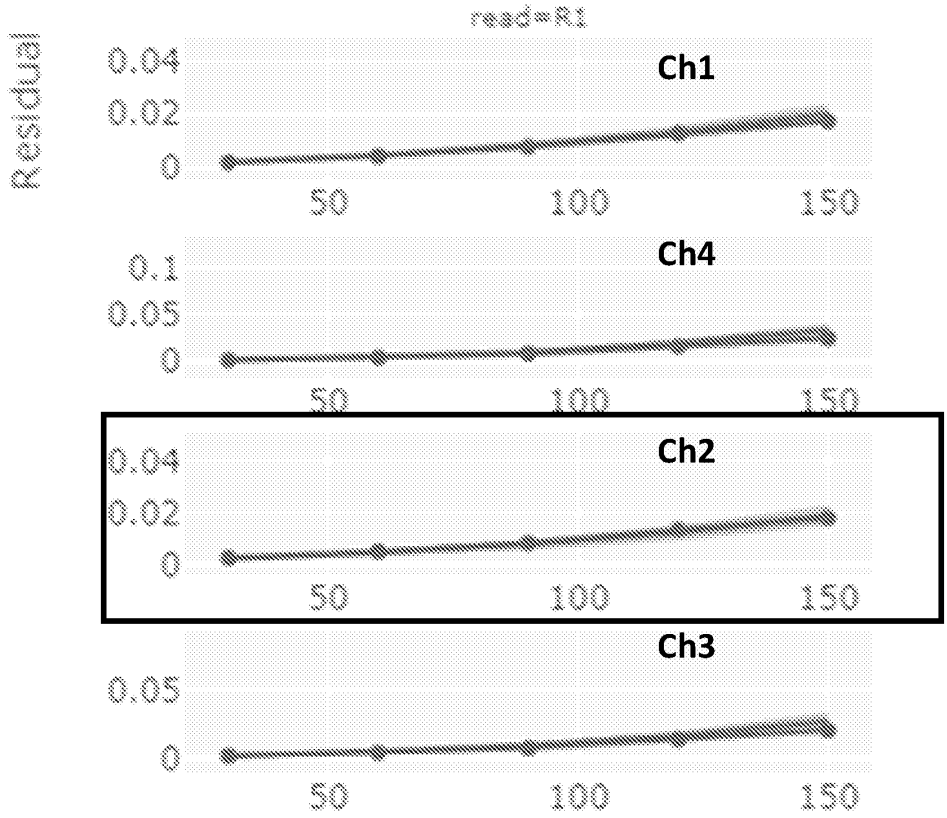


FIG. 20B

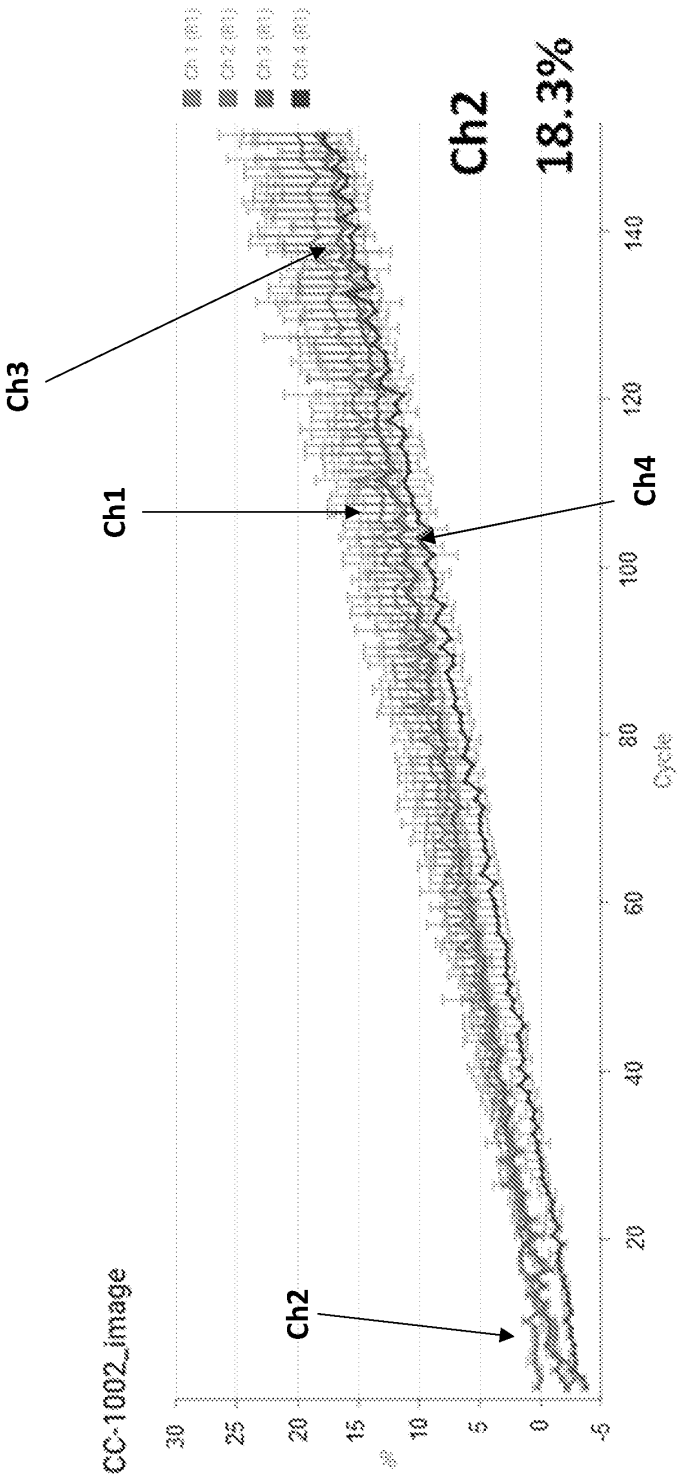


FIG. 21

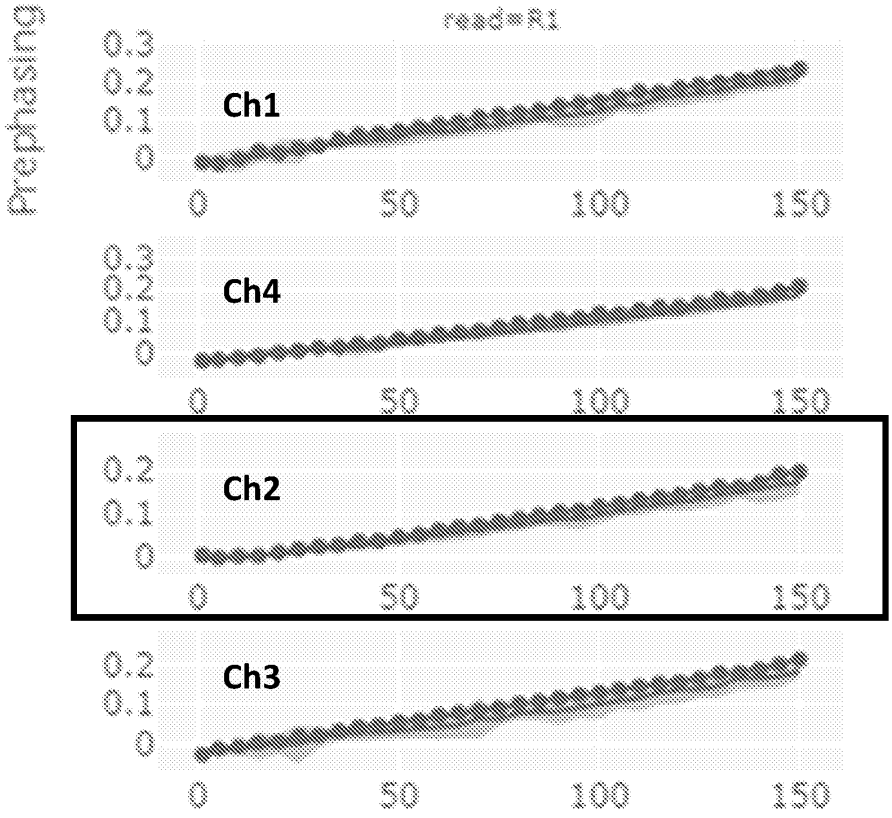


FIG. 22

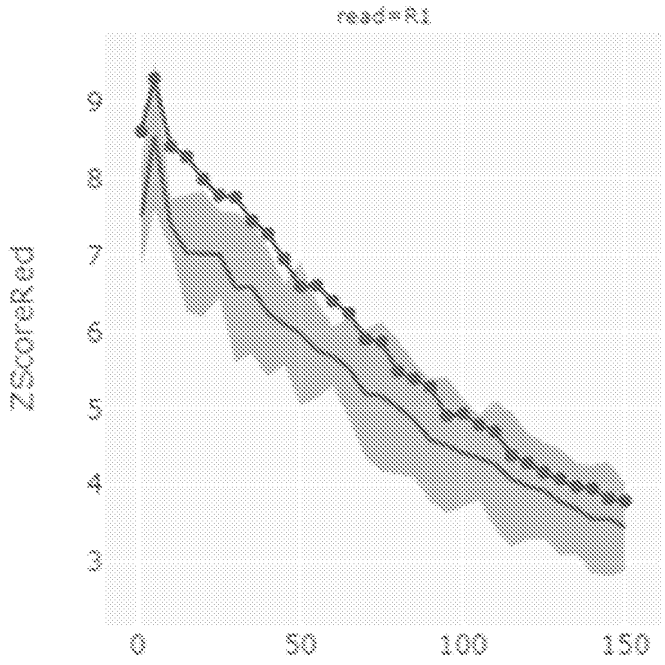


FIG. 23A

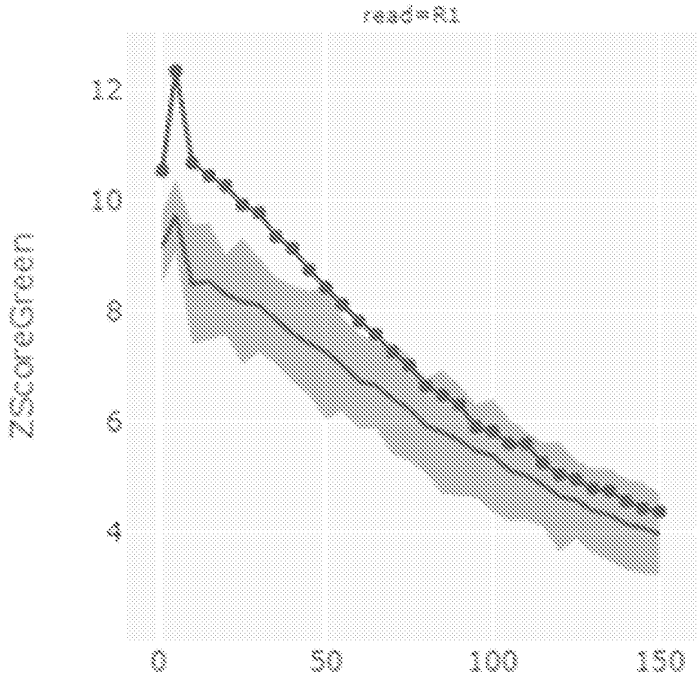


FIG. 23B

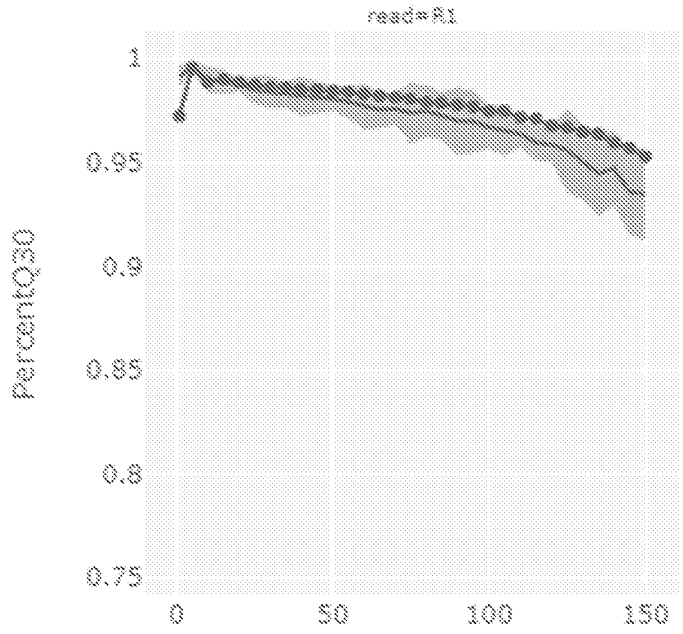


FIG. 24A

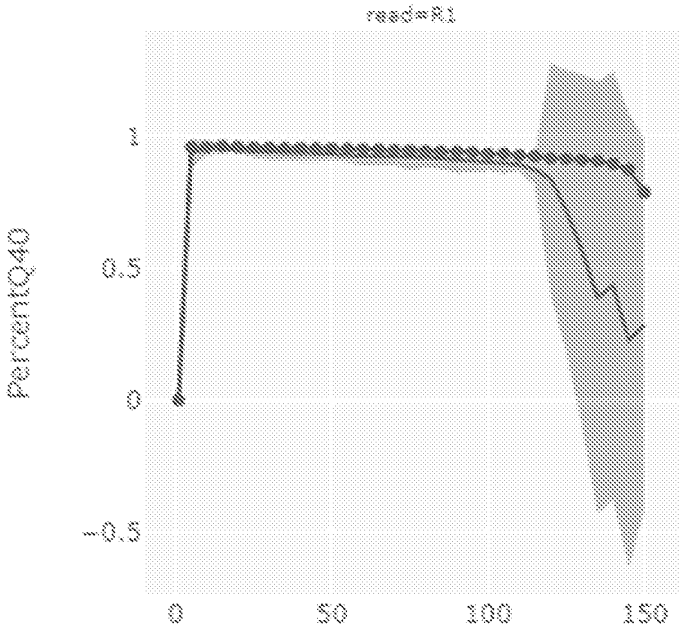


FIG. 24B

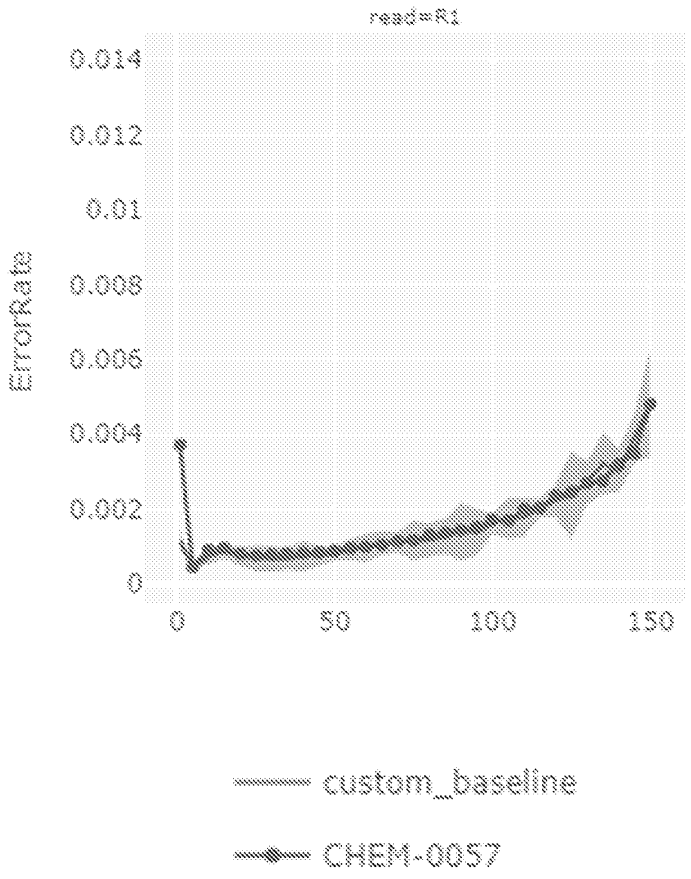

read=R1
0.014
0.012
0.01
0.008
0.006
0.004
0.002
0
ErrorRate
0
50
100
150
custom_baseline
CHEM-0057

FIG. 24C

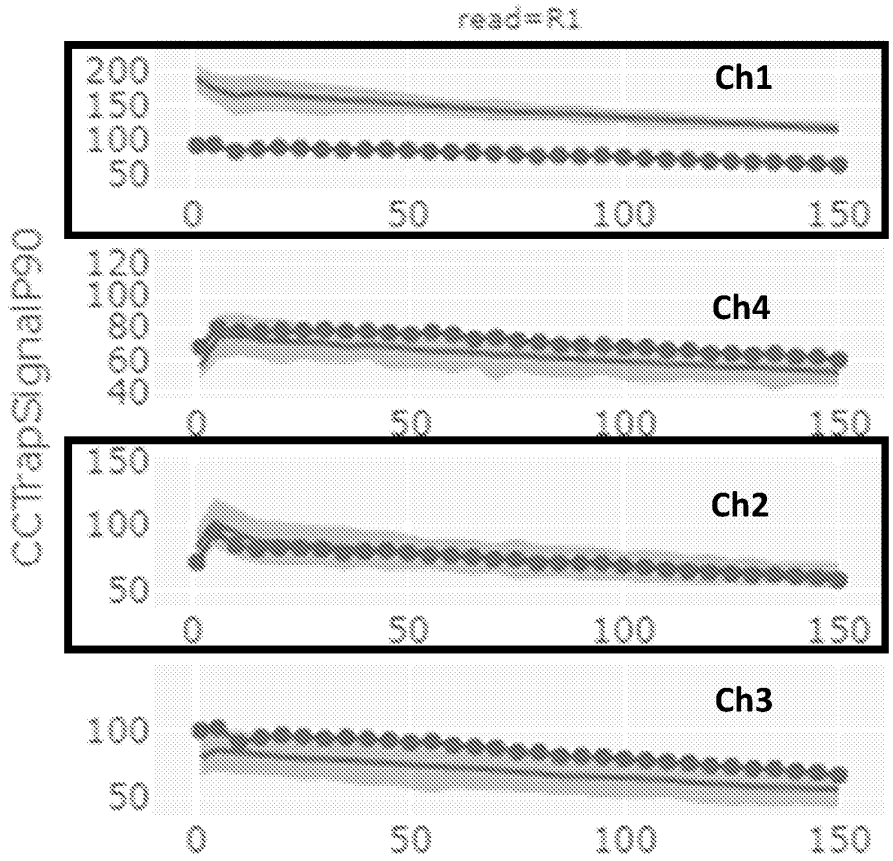

read=R1
Ch1
Ch4
Ch2
Ch3
CCTrapSignalP90
200
150
100
50
120
100
80
60
40
0
50
100
150

FIG. 25A

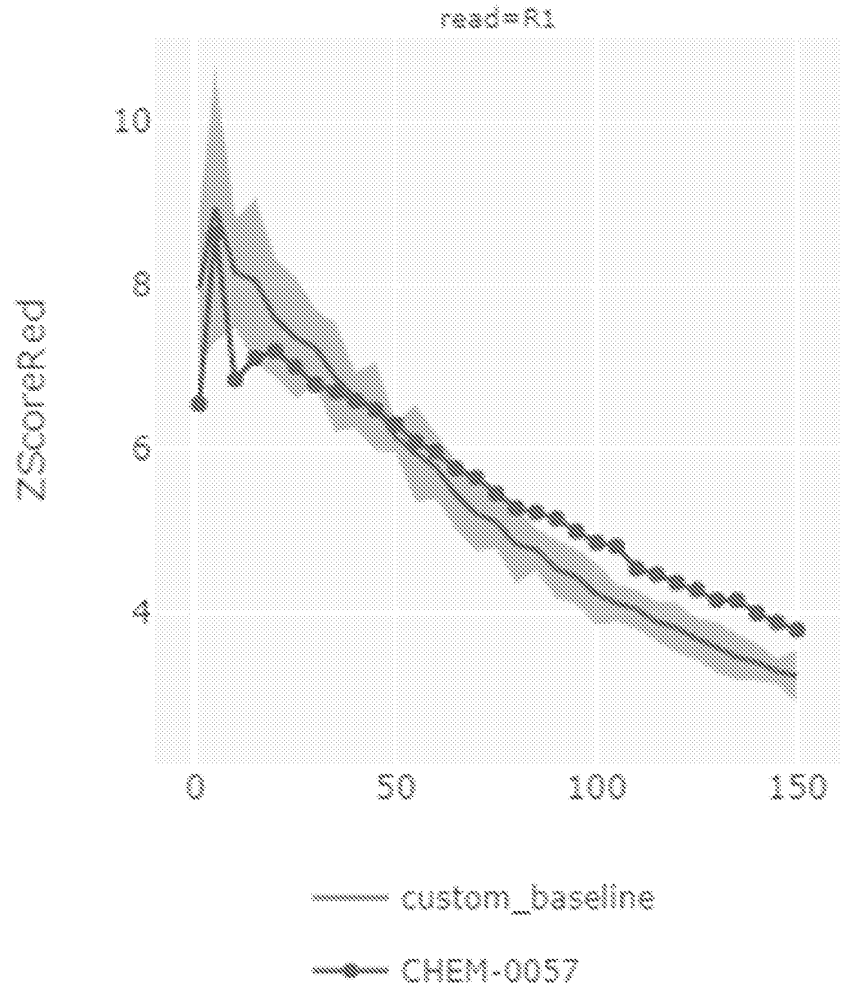

read=R1
ZScoreRed
10
8
6
4
0
50
100
150
custom_baseline
CHEM-0057

FIG. 25B

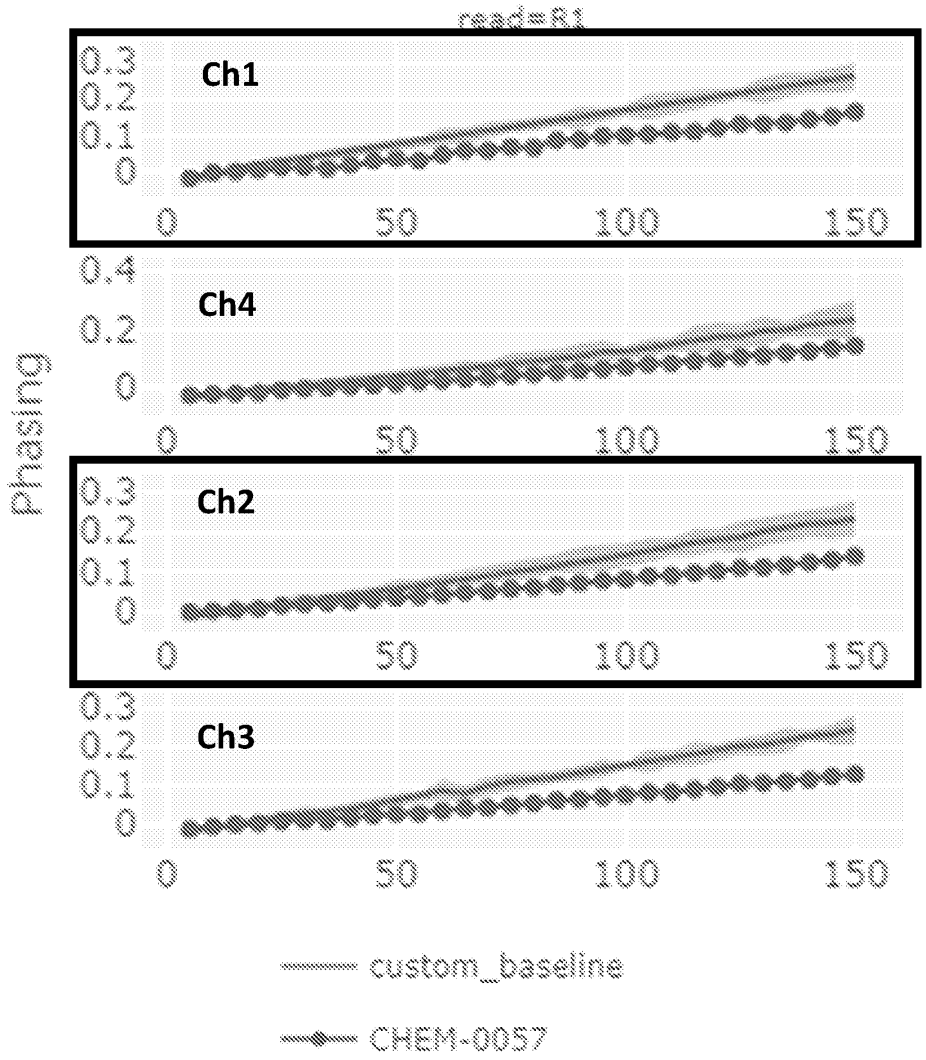

read=81
Ch1
Ch4
Ch2
Ch3
phasing
0
50
100
150
custom_baseline
CHEM-0057

FIG. 26A

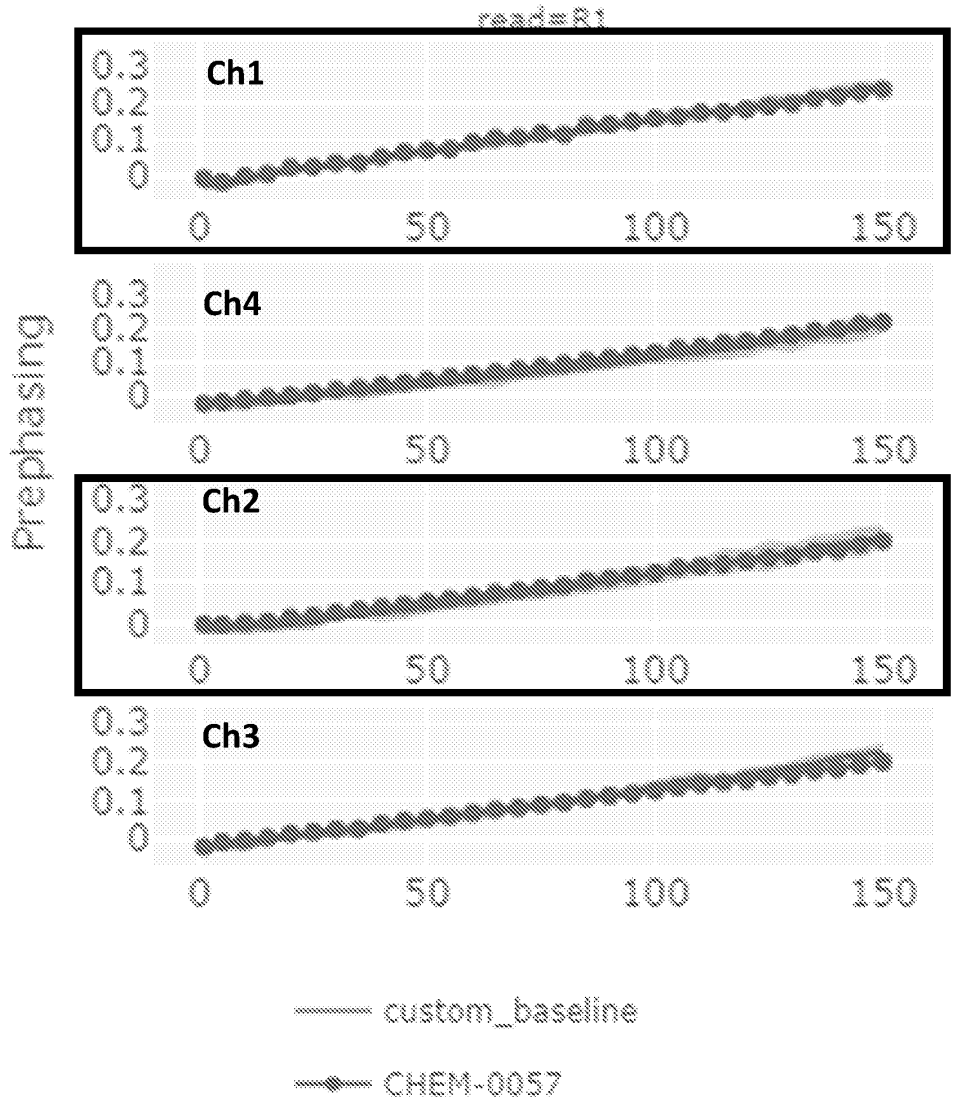

read=R1
Ch1
Ch4
Ch2
Ch3
Prephasing
0.3
0.2
0.1
0
0
50
100
150
custom_baseline
CHEM-0057

FIG. 26B

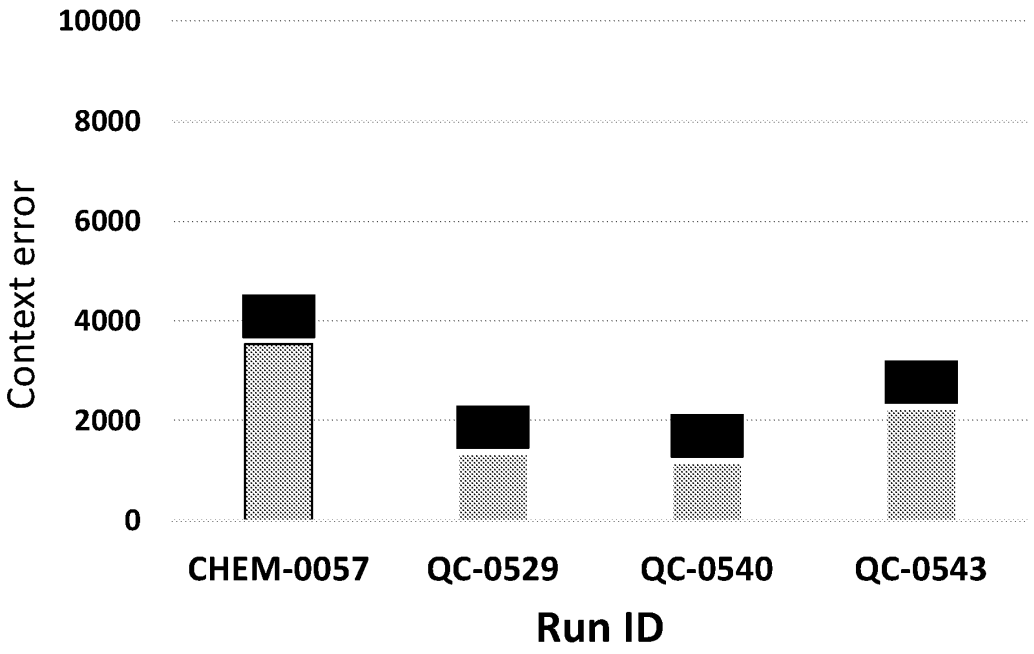

10000
8000
6000
4000
2000
0
Context error
CHEM-0057
QC-0529
QC-0540
QC-0543
Run ID

FIG. 27

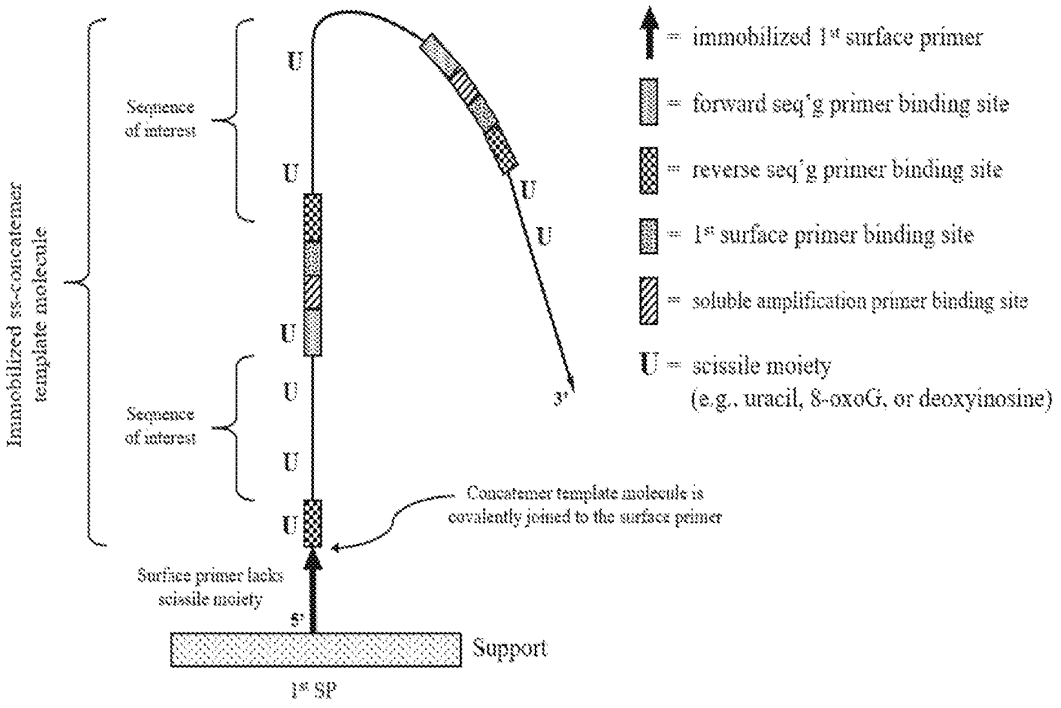

Immobilized ss-concatemer template molecule
Sequence of interest
Sequence of interest
U
U
U
U
U
U
U
U
3'
Concatemer template molecule is covalently joined to the surface primer
Surface primer lacks scissile moiety
5'
Support
1st SP
= immobilized 1st surface primer
= forward seq'g primer binding site
= reverse seq'g primer binding site
= 1st surface primer binding site
= soluble amplification primer binding site
U = scissile moiety
(e.g., uracil, 8-oxoG, or deoxyinosine)

FIG. 28

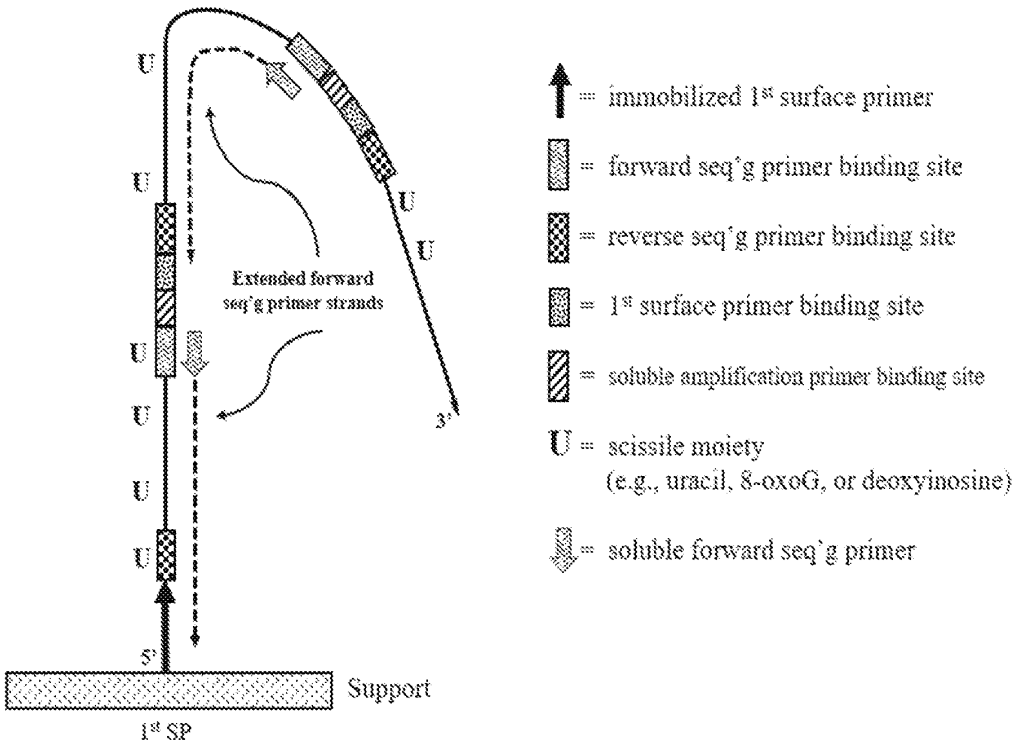

U
U
U
U
U
U
U
U
Extended forward seq'g primer strands
3'
5'
Support
1st SP
= immobilized 1st surface primer
= forward seq'g primer binding site
= reverse seq'g primer binding site
= 1st surface primer binding site
= soluble amplification primer binding site
U = scissile moiety (e.g., uracil, 8-oxoG, or deoxyinosine)
= soluble forward seq'g primer

FIG. 29

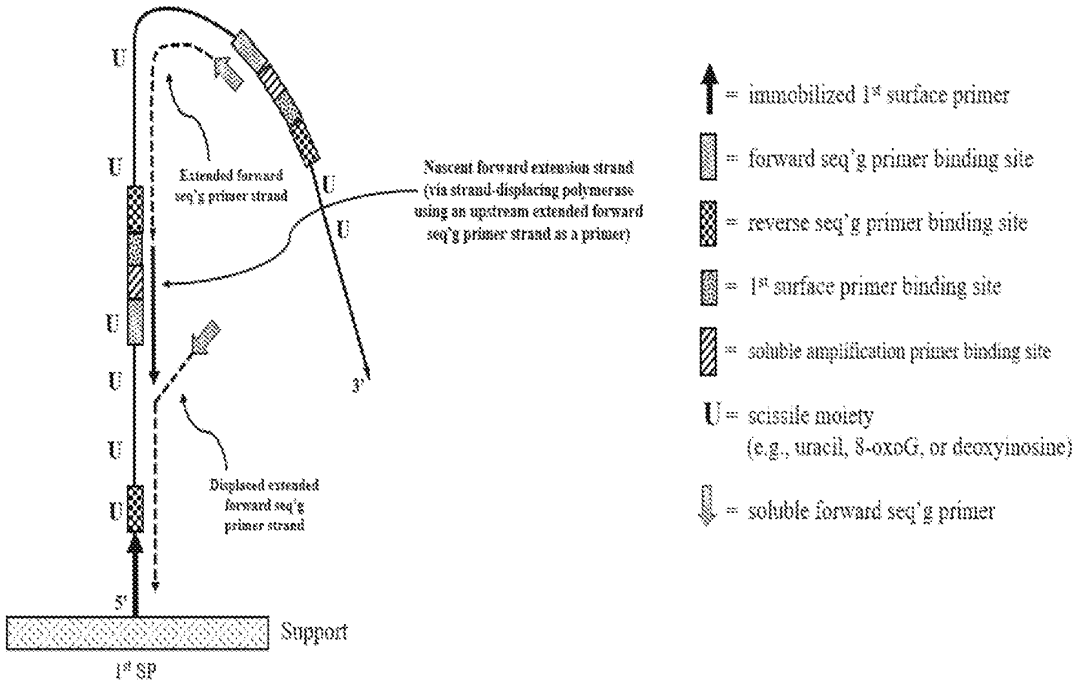

U
U
U
U
U
U
Extended forward seq'g primer strand
Nascent forward extension strand (via strand-displacing polymerase using an upstream extended forward seq'g primer strand as a primer)
Displaced extended forward seq'g primer strand
5'
3'
Support
1st SP
= immobilized 1st surface primer
= forward seq'g primer binding site
= reverse seq'g primer binding site
= 1st surface primer binding site
= soluble amplification primer binding site
U = scissile moiety (e.g., uracil, 8-oxoG, or deoxyinosine)
= soluble forward seq'g primer

FIG. 30

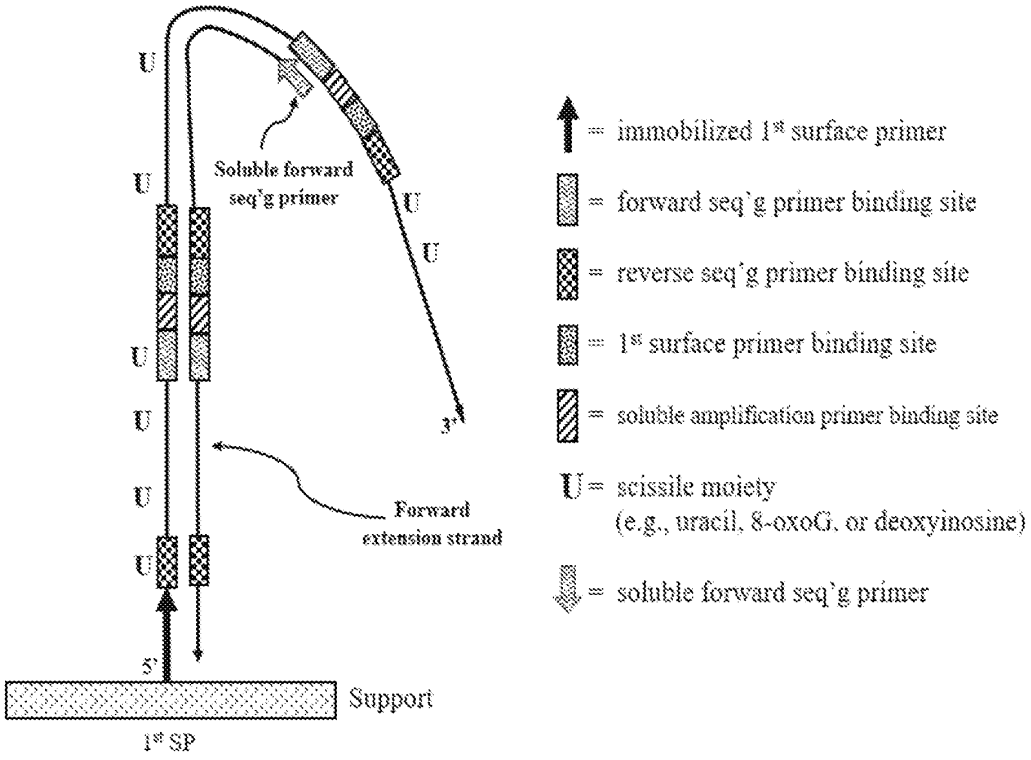

U
U
U
U
U
U
Soluble forward seq'g primer
U
U
3'
Forward extension strand
5'
Support
1st SP
= immobilized 1st surface primer
= forward seq'g primer binding site
= reverse seq'g primer binding site
= 1st surface primer binding site
= soluble amplification primer binding site
U = scissile moiety (e.g., uracil, 8-oxoG, or deoxyinosine)
= soluble forward seq'g primer

FIG. 31

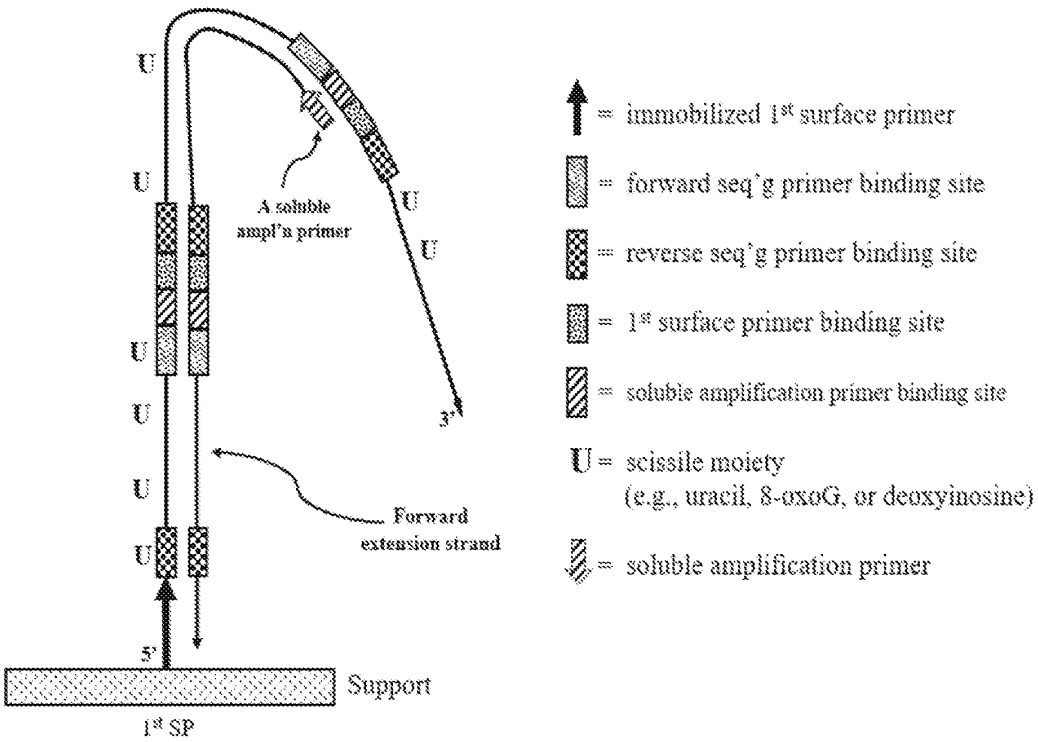

U
U
U
U
U
U
U
A soluble
ampl'n primer
3'
Forward
extension strand
5'
Support
1st SP
= immobilized 1st surface primer
= forward seq'g primer binding site
= reverse seq'g primer binding site
= 1st surface primer binding site
= soluble amplification primer binding site
U = scissile moiety
(e.g., uracil, 8-oxoG, or deoxyinosine)
= soluble amplification primer

FIG. 32

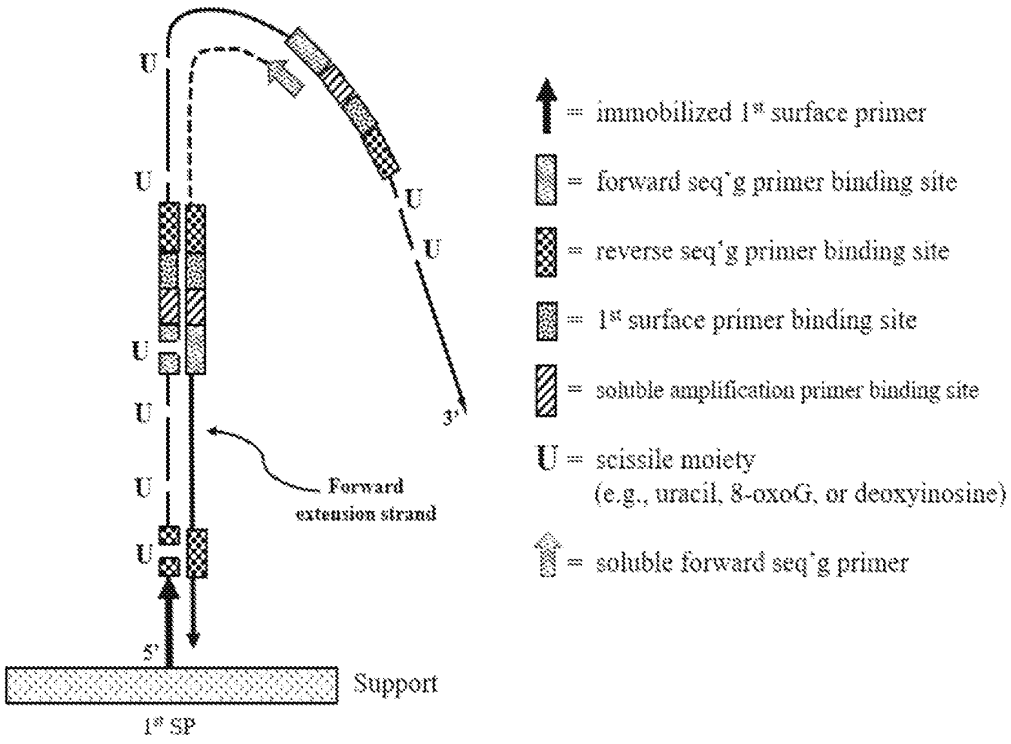

U
U
U
U
U
U
U
3'
5'
Forward extension strand
Support
1st SP
= immobilized 1st surface primer
= forward seq'g primer binding site
= reverse seq'g primer binding site
= 1st surface primer binding site
= soluble amplification primer binding site
U = scissile moiety (e.g., uracil, 8-oxoG, or deoxyinosine)
= soluble forward seq'g primer

FIG. 33

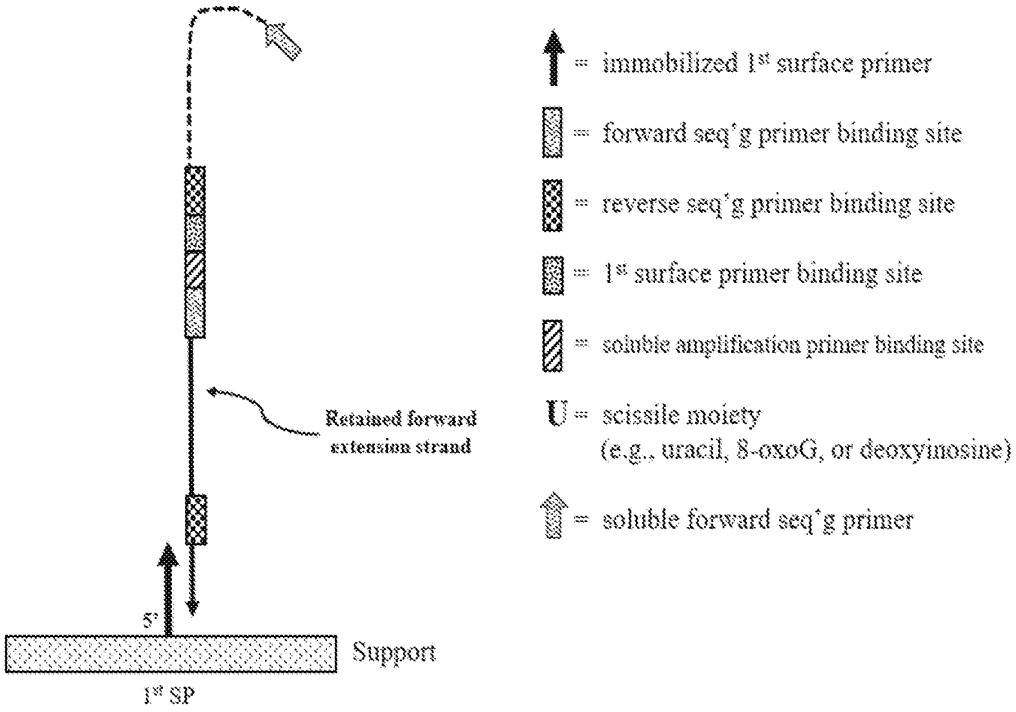

= immobilized 1st surface primer
= forward seq'g primer binding site
= reverse seq'g primer binding site
= 1st surface primer binding site
= soluble amplification primer binding site
U = scissile moiety
(e.g., uracil, 8-oxoG, or deoxyinosine)
= soluble forward seq'g primer
Retained forward extension strand
5'
Support
1st SP

FIG. 34

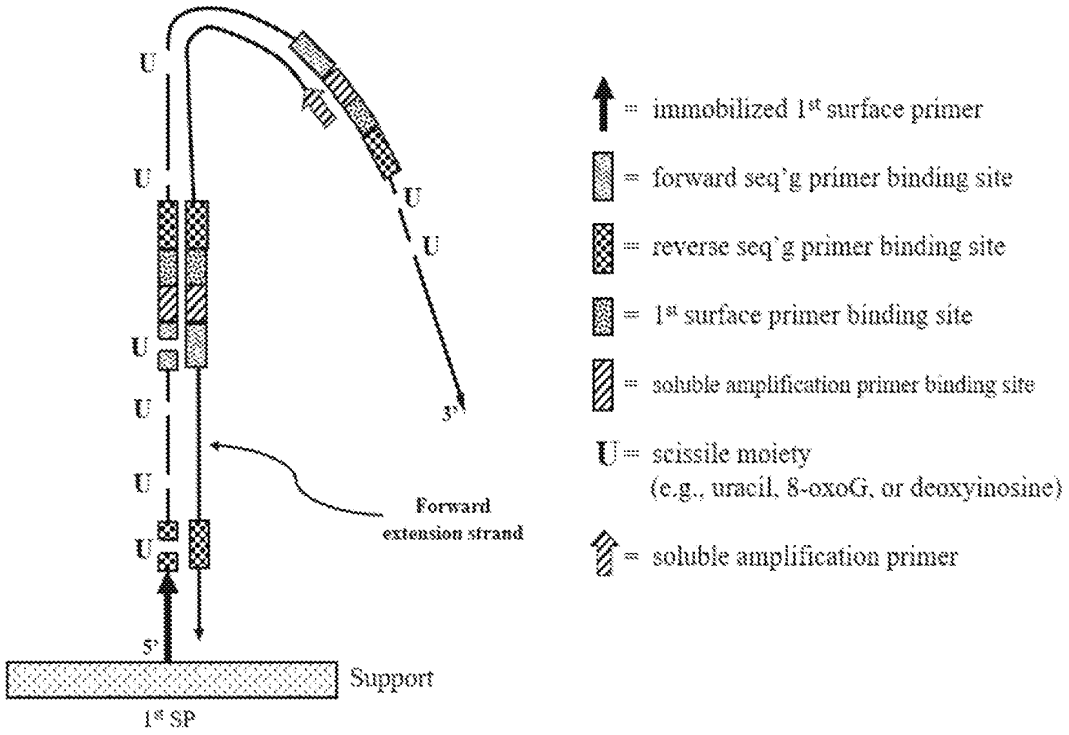

U
U
U
U
U
U
U
U
5'
Forward
extension strand
5'
Support
1st SP
= immobilized 1st surface primer
= forward seq'g primer binding site
= reverse seq'g primer binding site
= 1st surface primer binding site
= soluble amplification primer binding site
U = scissile moiety
(e.g., uracil, 8-oxoG, or deoxyinosine)
= soluble amplification primer

FIG. 35

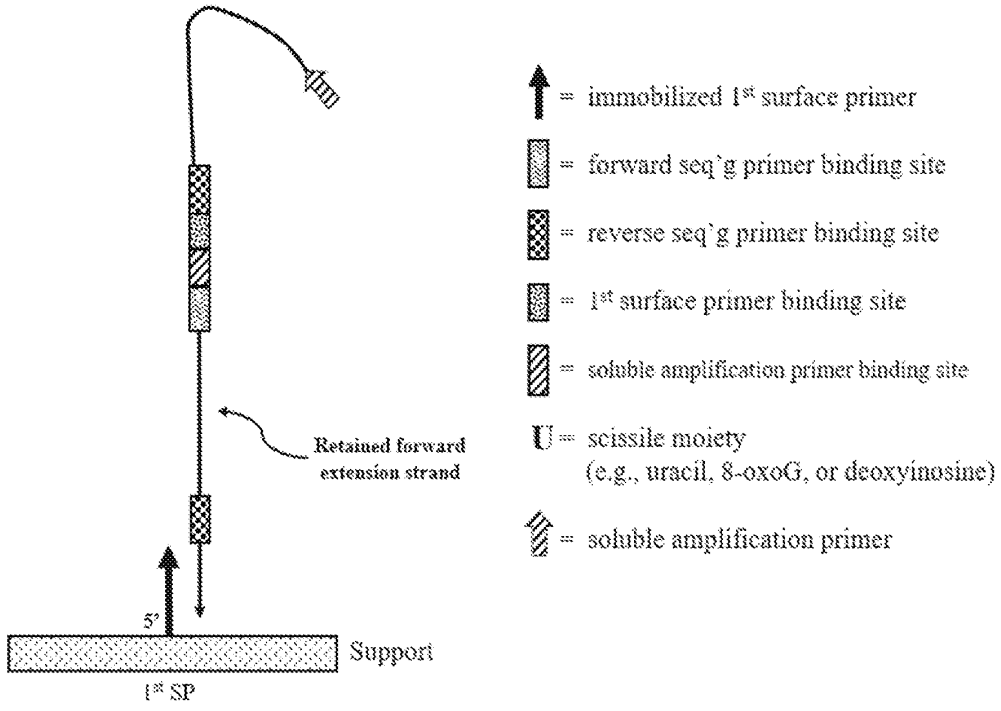

= immobilized 1st surface primer
= forward seq`g primer binding site
= reverse seq`g primer binding site
= 1st surface primer binding site
= soluble amplification primer binding site
U = scissile moiety
(e.g., uracil, 8-oxoG, or deoxyinosine)
= soluble amplification primer
Retained forward extension strand
5'
Support
1st SP

FIG. 36

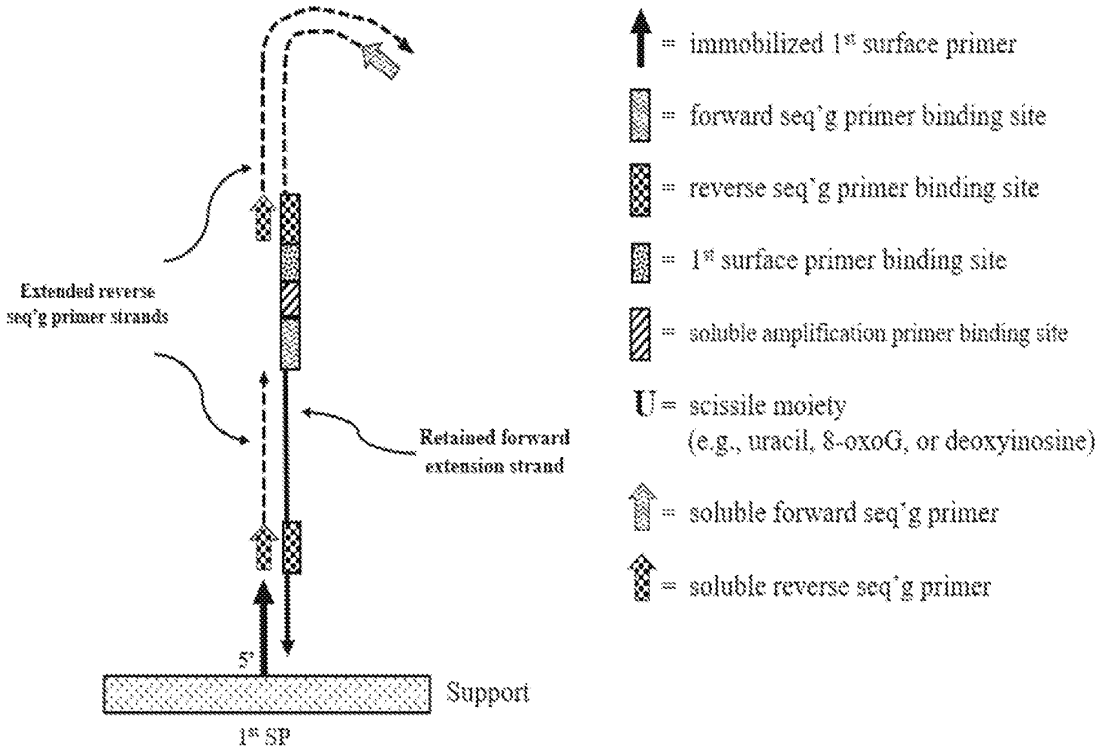

Extended reverse seq'g primer strands
Retained forward extension strand
5'
Support
1st SP
= immobilized 1st surface primer
= forward seq'g primer binding site
= reverse seq'g primer binding site
= 1st surface primer binding site
= soluble amplification primer binding site
U = scissile moiety (e.g., uracil, 8-oxoG, or deoxyinosine)
= soluble forward seq'g primer
= soluble reverse seq'g primer

FIG. 37

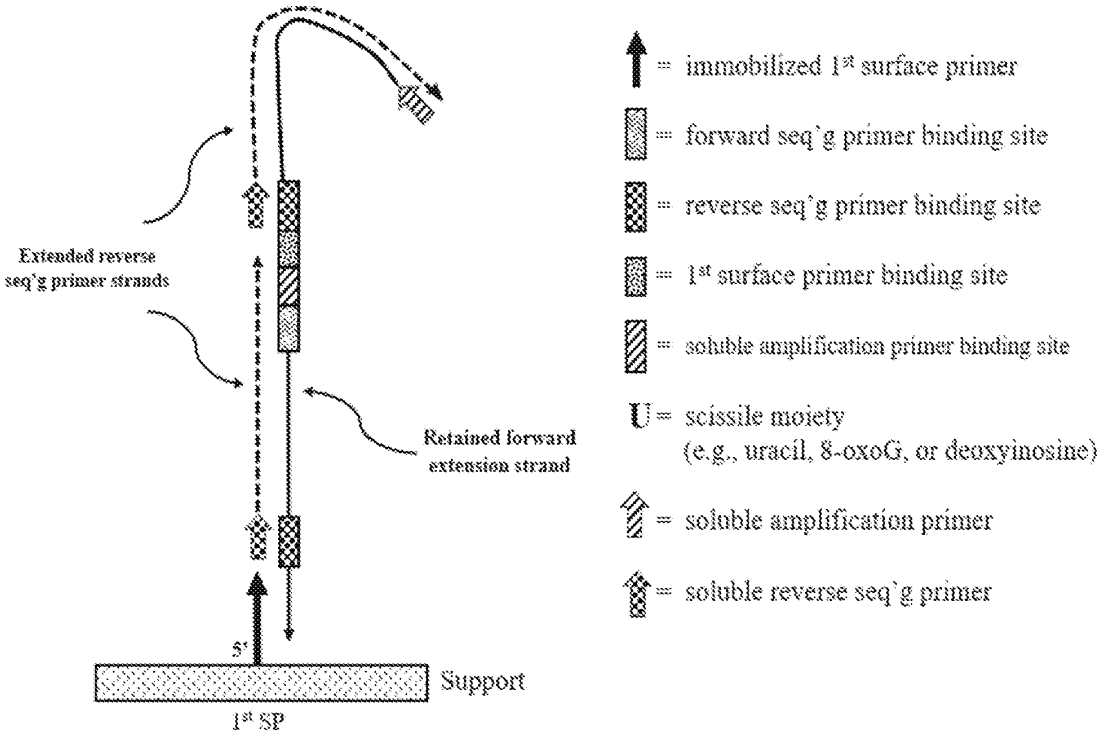

Extended reverse seq'g primer strands
Retained forward extension strand
5'
Support
1st SP
= immobilized 1st surface primer
= forward seq'g primer binding site
= reverse seq'g primer binding site
= 1st surface primer binding site
= soluble amplification primer binding site
U = scissile moiety (e.g., uracil, 8-oxoG, or deoxyinosine)
= soluble amplification primer
= soluble reverse seq'g primer

FIG. 38

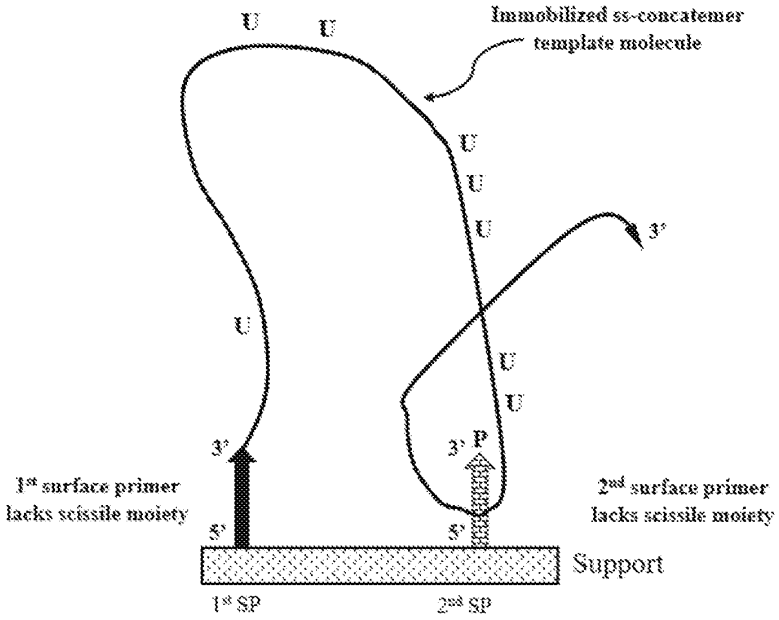

U
U
Immobilized ss-concatemer template molecule
U
U
U
3'
U
U
U
3'
P
3'
1st surface primer lacks scissile moiety
2nd surface primer lacks scissile moiety
5'
5'
Support
1st SP
2nd SP

FIG. 39