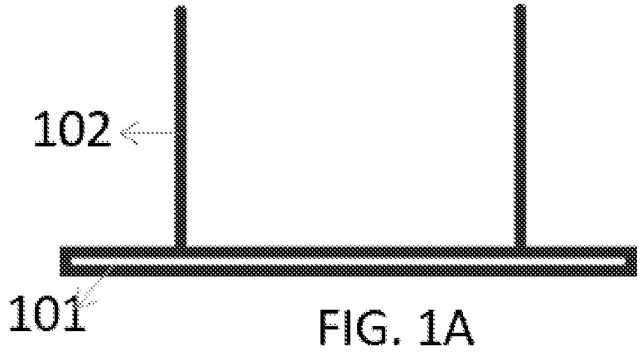


FIG. 1A

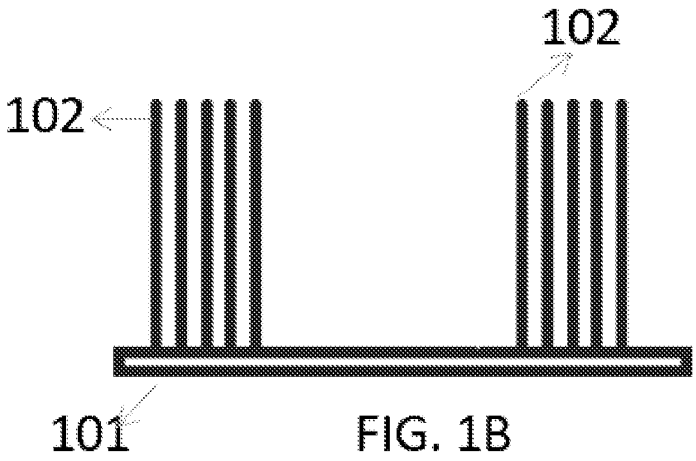


FIG. 1B

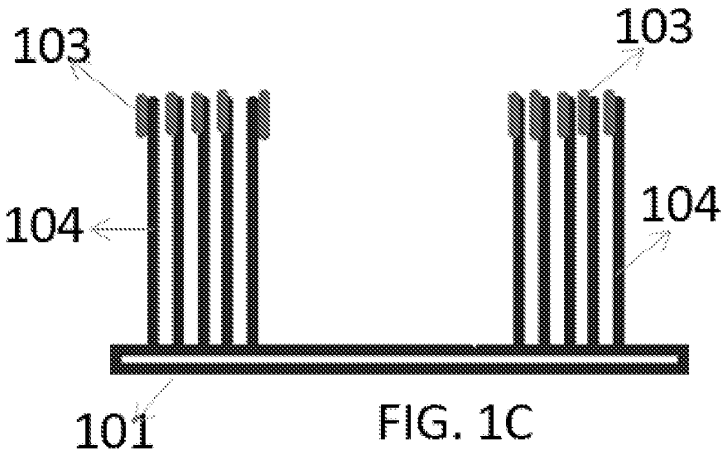


FIG. 1C

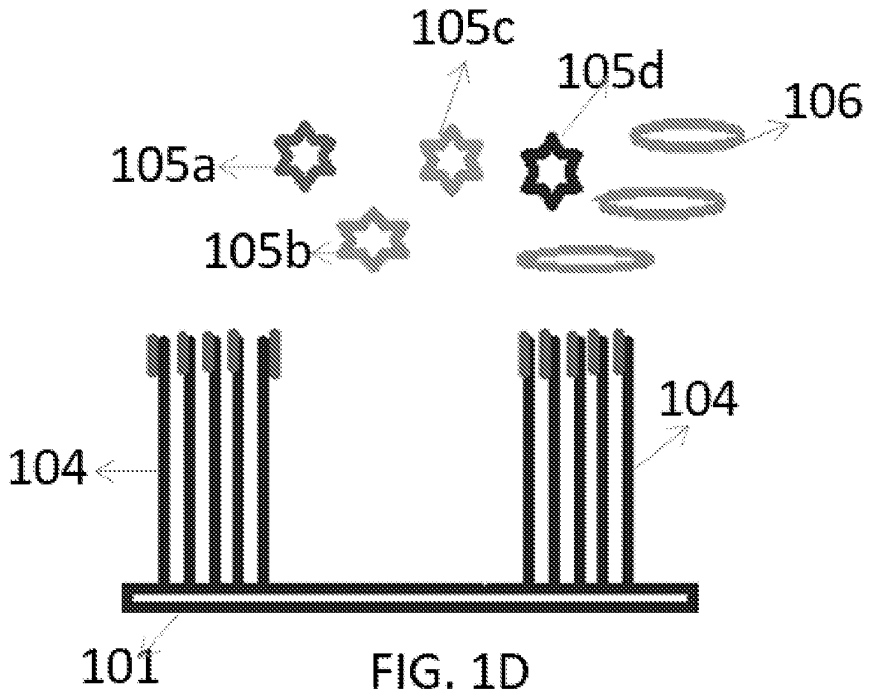


FIG. 1D

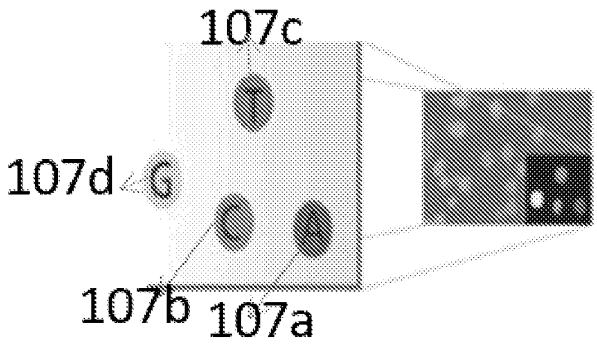


FIG. 1E

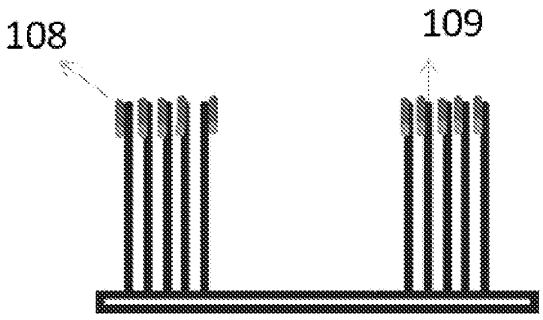


FIG. 1F

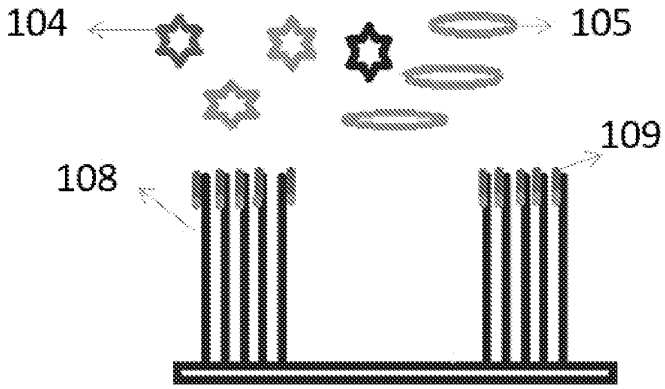


FIG. 1G

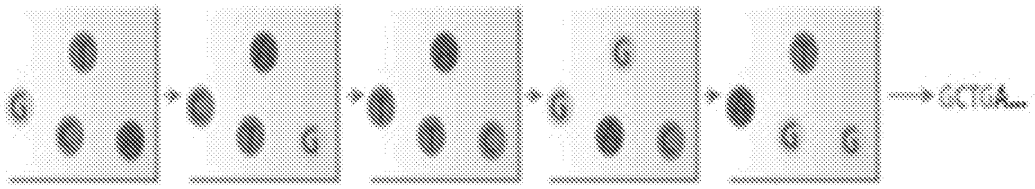


FIG. 1H

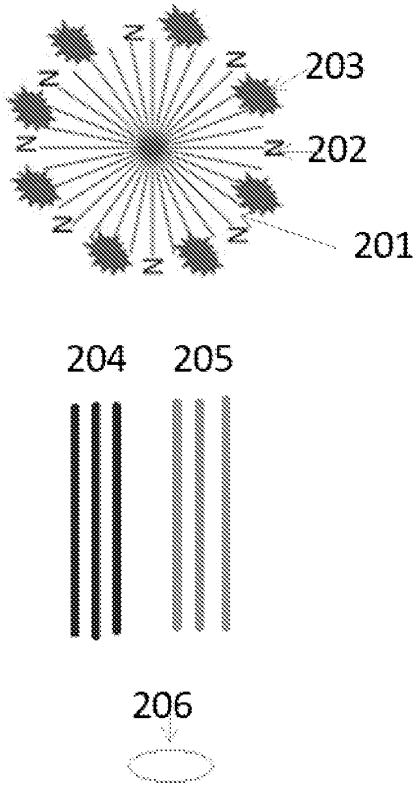


FIG. 2A

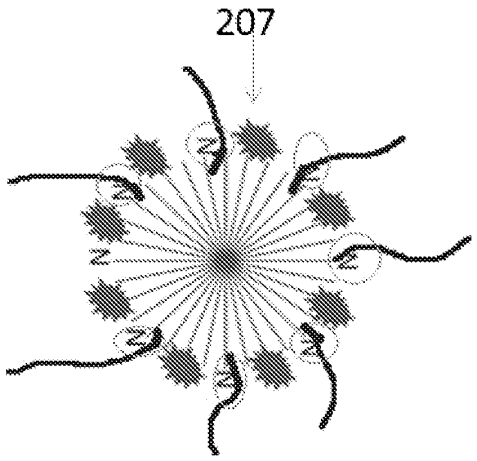


FIG. 2B

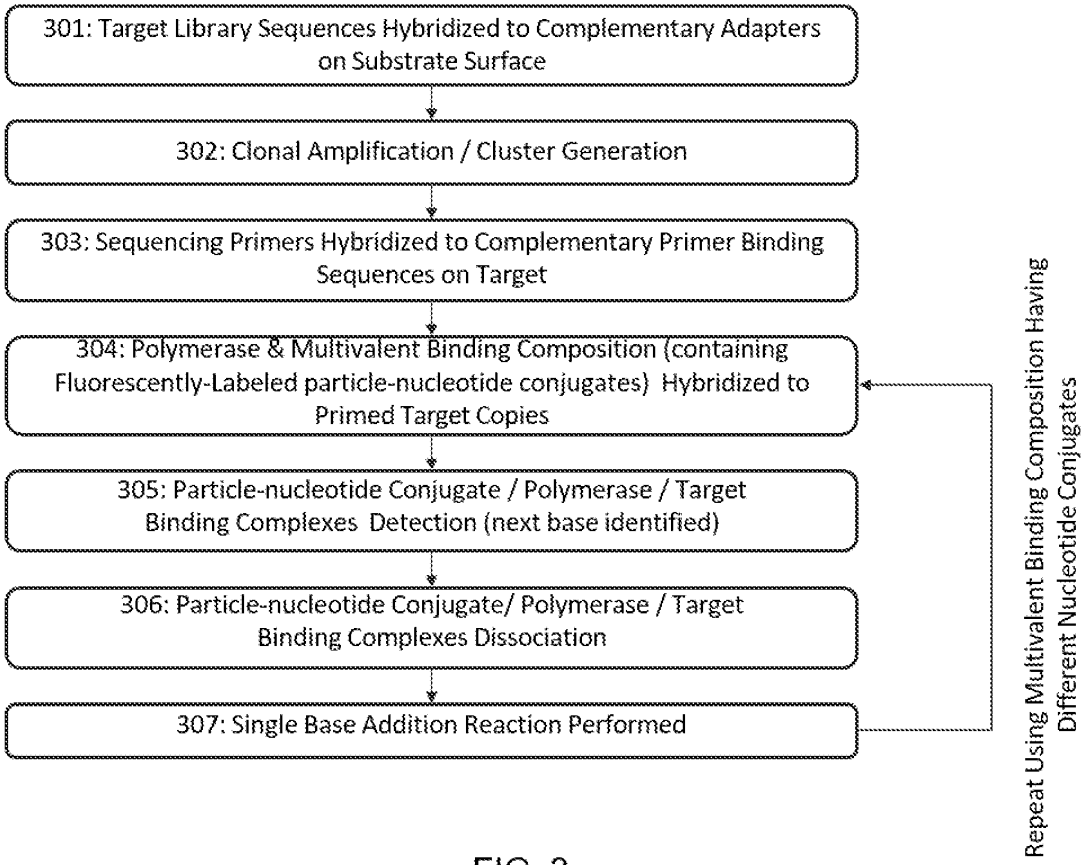

301: Target Library Sequences Hybridized to Complementary Adapters on Substrate Surface
302: Clonal Amplification / Cluster Generation
303: Sequencing Primers Hybridized to Complementary Primer Binding Sequences on Target
304: Polymerase & Multivalent Binding Composition (containing Fluorescently-Labeled particle-nucleotide conjugates) Hybridized to Primed Target Copies
305: Particle-nucleotide Conjugate / Polymerase / Target Binding Complexes Detection (next base identified)
306: Particle-nucleotide Conjugate/ Polymerase / Target Binding Complexes Dissociation
307: Single Base Addition Reaction Performed
Repeat Using Multivalent Binding Composition Having Different Nucleotide Conjugates

FIG. 3

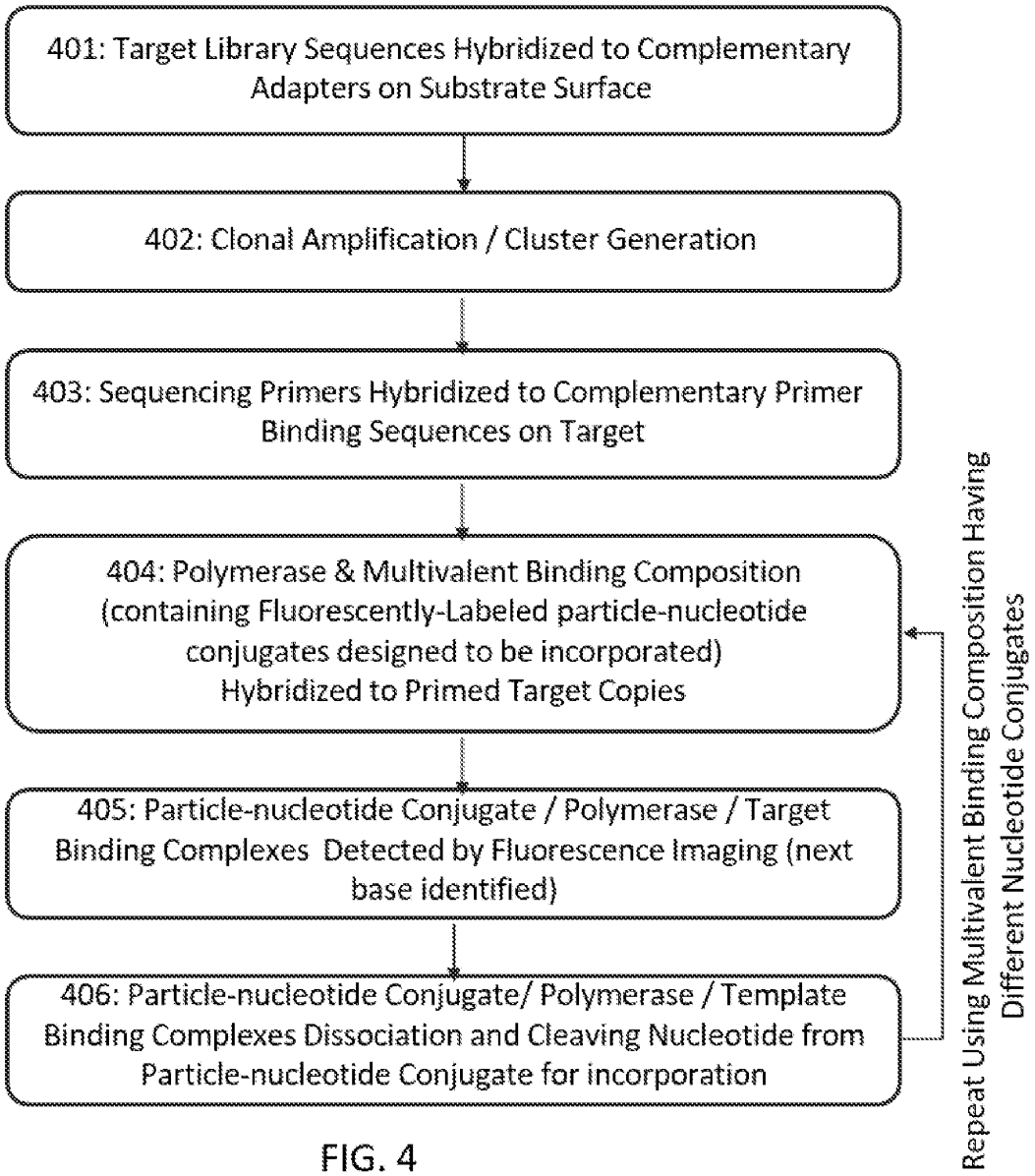

401: Target Library Sequences Hybridized to Complementary Adapters on Substrate Surface
402: Clonal Amplification / Cluster Generation
403: Sequencing Primers Hybridized to Complementary Primer Binding Sequences on Target
404: Polymerase & Multivalent Binding Composition (containing Fluorescently-Labeled particle-nucleotide conjugates designed to be incorporated) Hybridized to Primed Target Copies
405: Particle-nucleotide Conjugate / Polymerase / Target Binding Complexes Detected by Fluorescence Imaging (next base identified)
406: Particle-nucleotide Conjugate/ Polymerase / Template Binding Complexes Dissociation and Cleaving Nucleotide from Particle-nucleotide Conjugate for incorporation
Repeat Using Multivalent Binding Composition Having Different Nucleotide Conjugates

FIG. 4

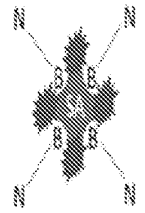
FIG. 5A

Class I

Valency = 0-4

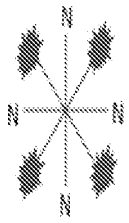
Homogenous population with saturation

"starburst"



PEG length = 1k-10k

"Helter-Skelter"



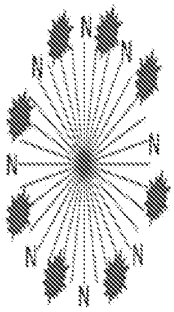
PEG length = 1.25k-5k

FIG. 5B

Class II

Valency >4

Homogenous population with Saturation



Dendrimer core 12,24,48,96 arms

PEG length = 1K-10K

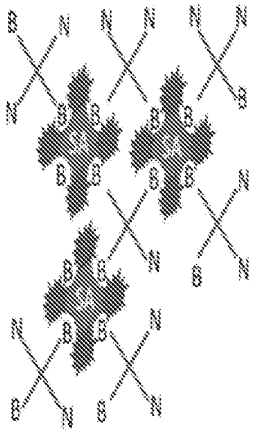
Other cores = dextrans, other polymers

FIG. 5C

Class III

Valency >4

Distribution of sizes based on construction



8arm PEG-NHS mixed with Biotin-NH2 and dNTP-NH2 then mixed with labeled streptavidin

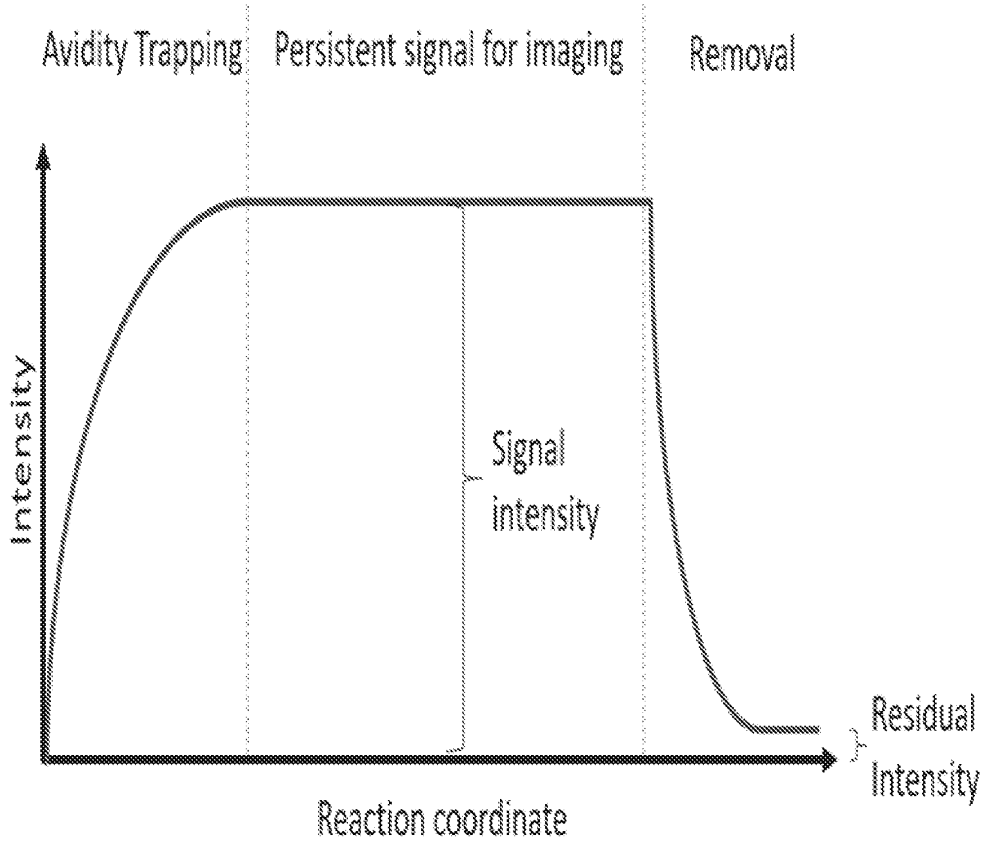

Avidity Trapping
Persistent signal for imaging
Removal
Intensity
Signal intensity
Residual Intensity
Reaction coordinate

FIG. 6

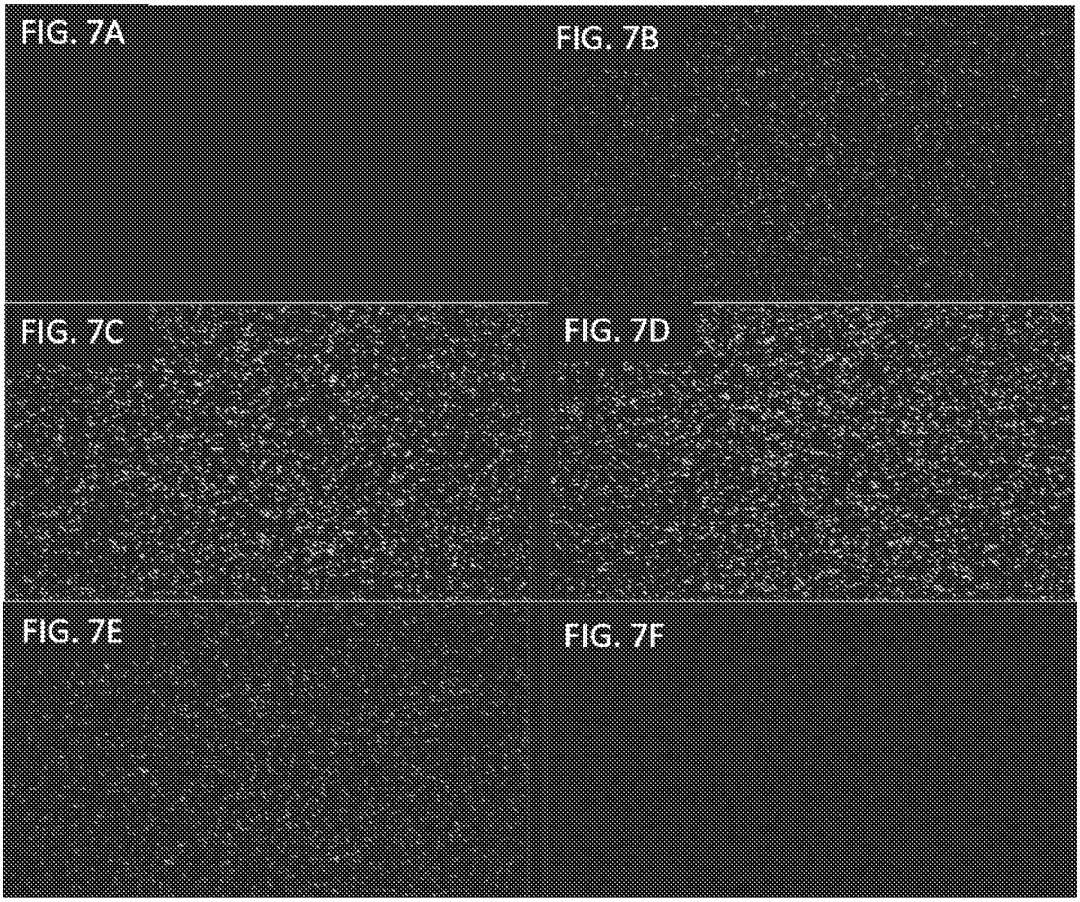
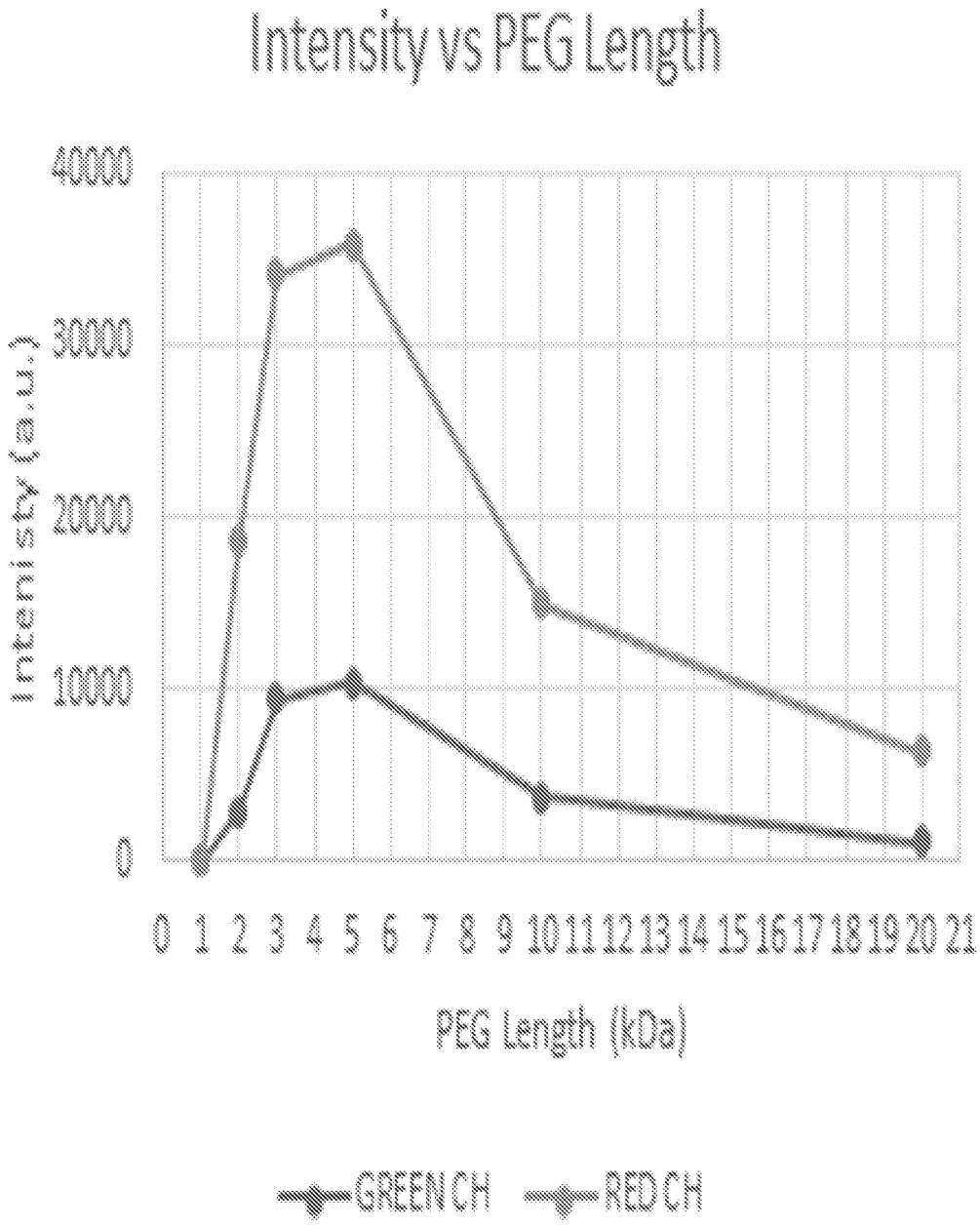
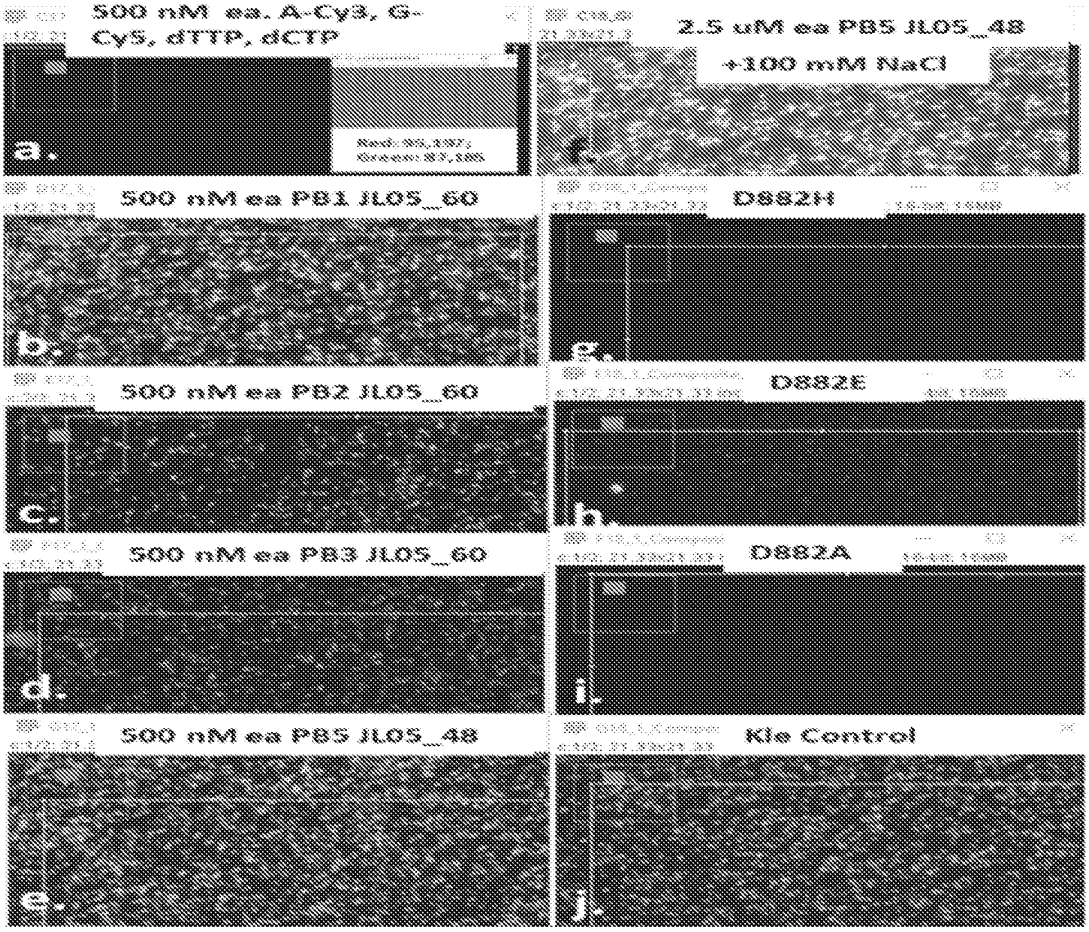

FIG. 7A
FIG. 7B
FIG. 7C
FIG. 7D
FIG. 7E
FIG. 7F



FIG. 7G


Intensity vs PEG Length
Intensity (a.u.)
40000
30000
20000
10000
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
PEG Length (kDa)
GREEN CH
RED CH

FIGS. 8


500 nM ea. A-Cy3, G-Cy5, dTTP, dCTP
a.
2.5 uM ea PB5 JL05_48
+100 mM NaCl
f.
500 nM ea PB1 JL05_60
b.
D882H
g.
500 nM ea PB2 JL05_60
c.
D882E
h.
500 nM ea PB3 JL05_60
d.
D882A
i.
500 nM ea PB5 JL05_48
e.
Kle Control
j.

FIGS. 9A-9J

Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5
NM1: TGCAT	NM1: T□CAT	NM1: TGCAT	NM1: TGCAT	NM1: TGCAT
NM2: ACTGC	NM2: ACTGC	NM2: ACTGC	NM2: ACT□C	NM2: ACTGC
NM3: CAGTG	NM3: CAGTG	NM3: CA□TG	NM3: CAGTG	NM3: CAGT□
NM4: GTACA	NM4: GTACA	NM4: GTACA	NM4: GTACA	NM4: GTACA

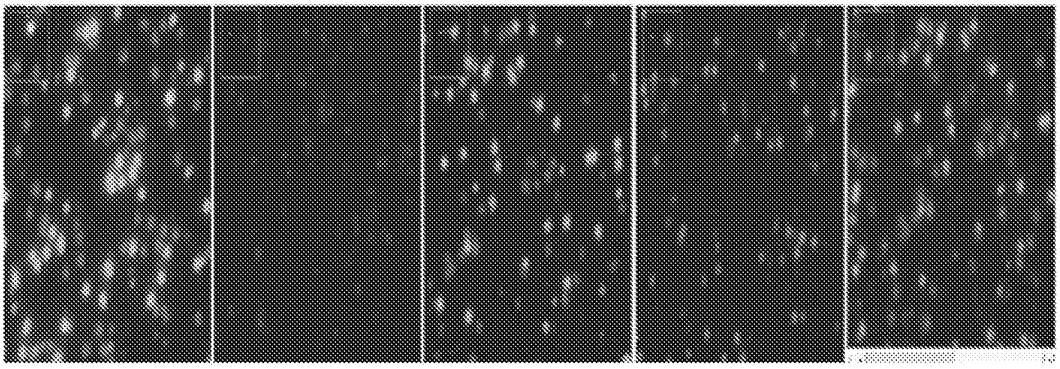
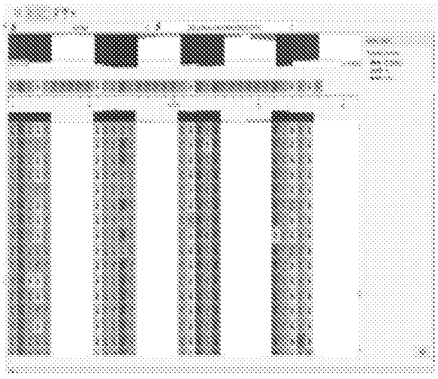


FIG. 10A

- 5 Cycles:
- G: Cy3 (Green)
- A: Cy5 (Red)
- C: dark bases (both C & T)



CGCAC	1484
ACCGC	1466
CAGCG	1518
GCACA	1320
# correct	5788
total reads	10508
#read in FOV	9238
% correct	62.6%
allow 1 error	8727
%mapped	94.4%

FIG.10B

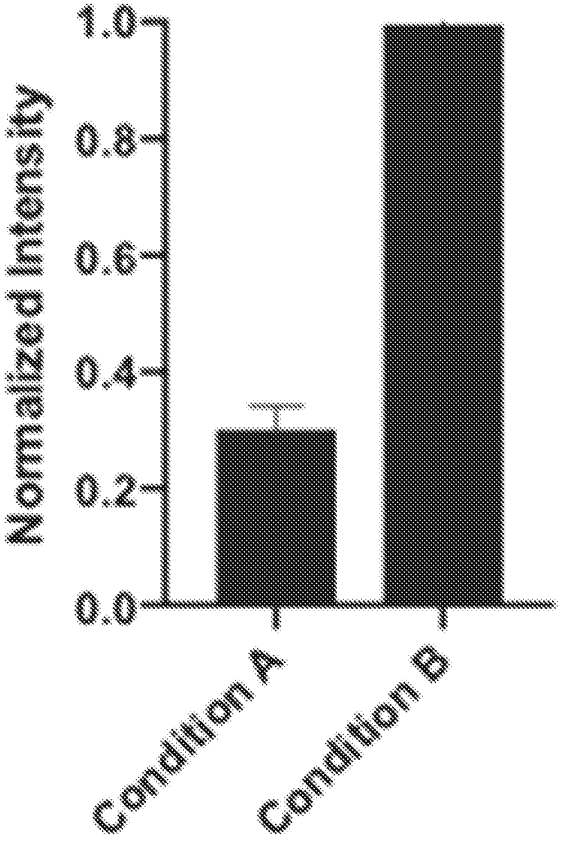

Normalized Intensity
1.0
0.8
0.6
0.4
0.2
0.0
Condition A
Condition B

FIG.11

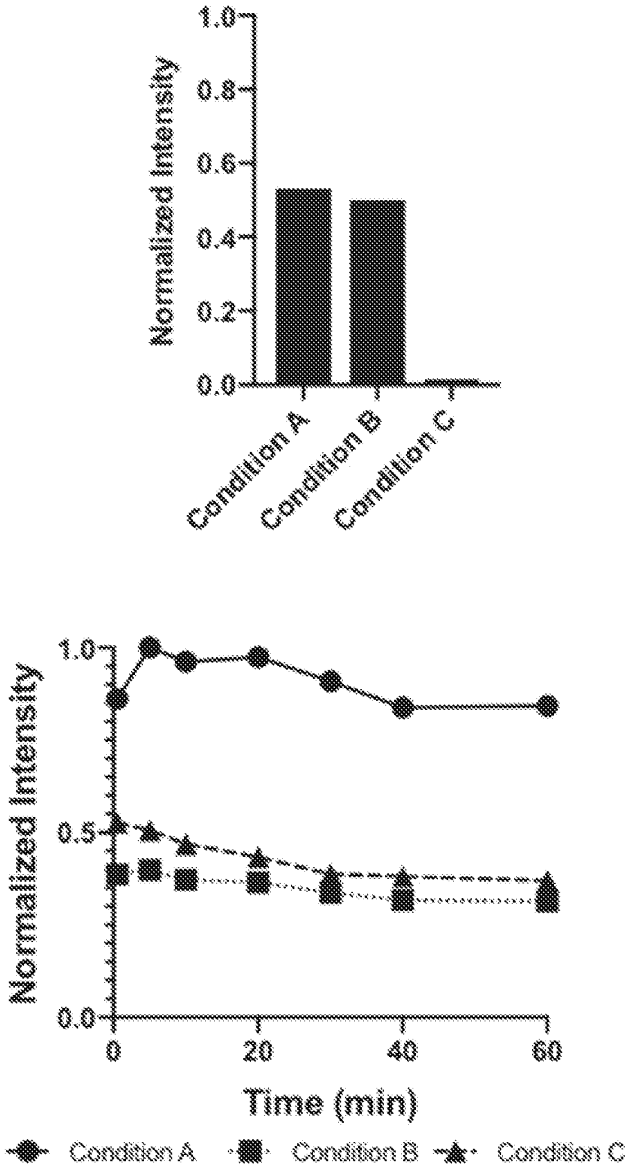

Normalized Intensity
1.0
0.8
0.6
0.4
0.2
0.0
Condition A
Condition B
Condition C
Normalized Intensity
1.0
0.5
0.0
0
20
40
60
Time (min)
Condition A
Condition B
Condition C

FIG.12

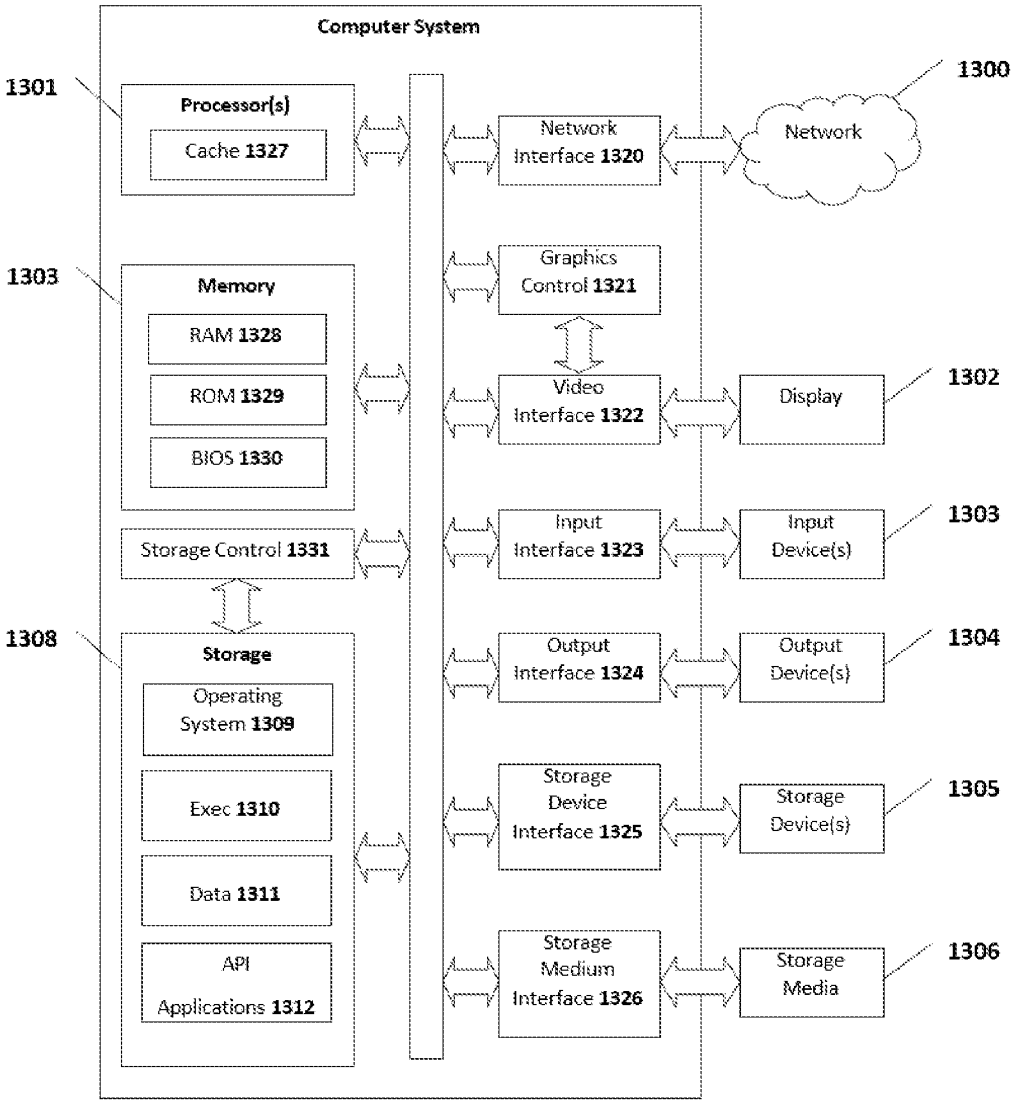

Computer System
1301
Processor(s)
Cache 1327
1303
Memory
RAM 1328
ROM 1329
BIOS 1330
Storage Control 1331
1308
Storage
Operating System 1309
Exec 1310
Data 1311
API Applications 1312
Network Interface 1320
Graphics Control 1321
Video Interface 1322
Input Interface 1323
Output Interface 1324
Storage Device Interface 1325
Storage Medium Interface 1326
1300
Network
1302
Display
1303
Input Device(s)
1304
Output Device(s)
1305
Storage Device(s)
1306
Storage Media

FIG. 13

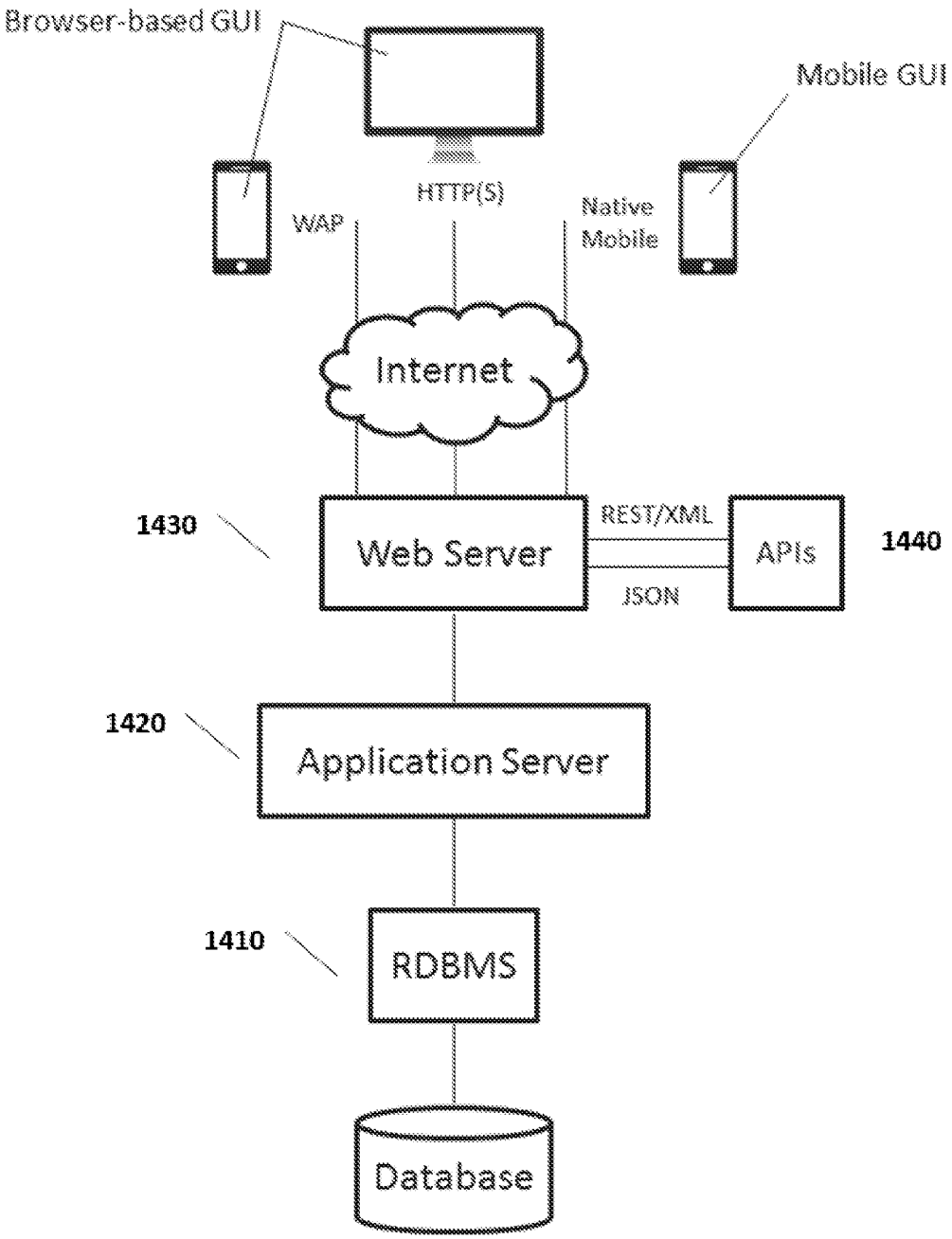

Browser-based GUI
Mobile GUI
HTTP(S)
WAP
Native Mobile
Internet
1430
Web Server
REST/XML
JSON
APIs
1440
1420
Application Server
1410
RDBMS
Database

FIG. 14

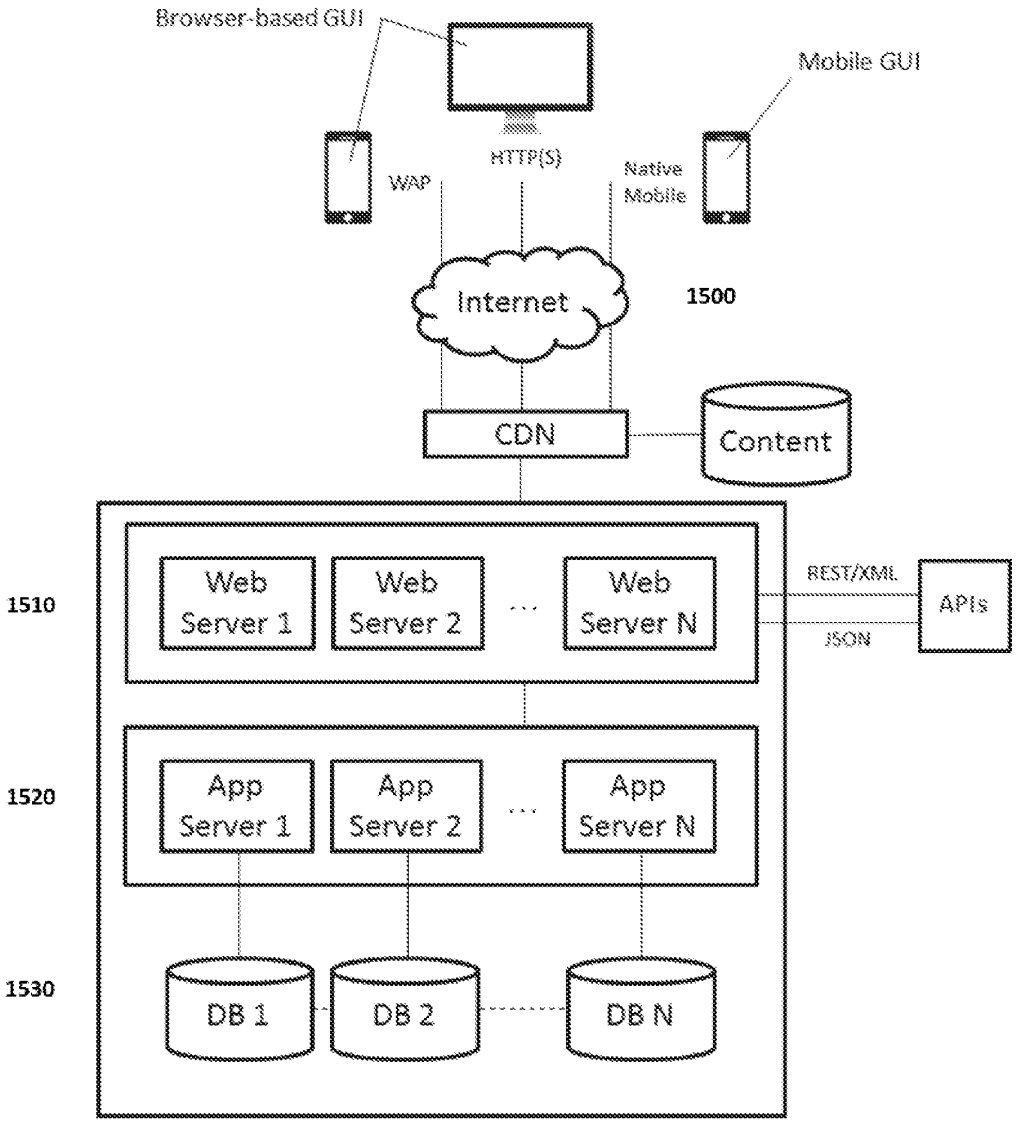

Browser-based GUI
Mobile GUI
HTTP(S)
WAP
Native Mobile
Internet
1500
CDN
Content
1510
Web Server 1
Web Server 2
Web Server N
REST/XML
JSON
APIs
1520
App Server 1
App Server 2
App Server N
1530
DB 1
DB 2
DB N

FIG. 15

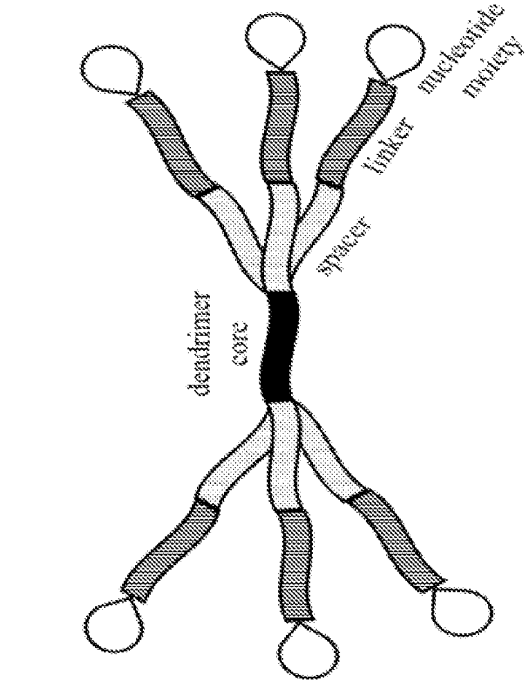

dendrimer core
spacer
linker
nucleotide moiety

FIG. 17

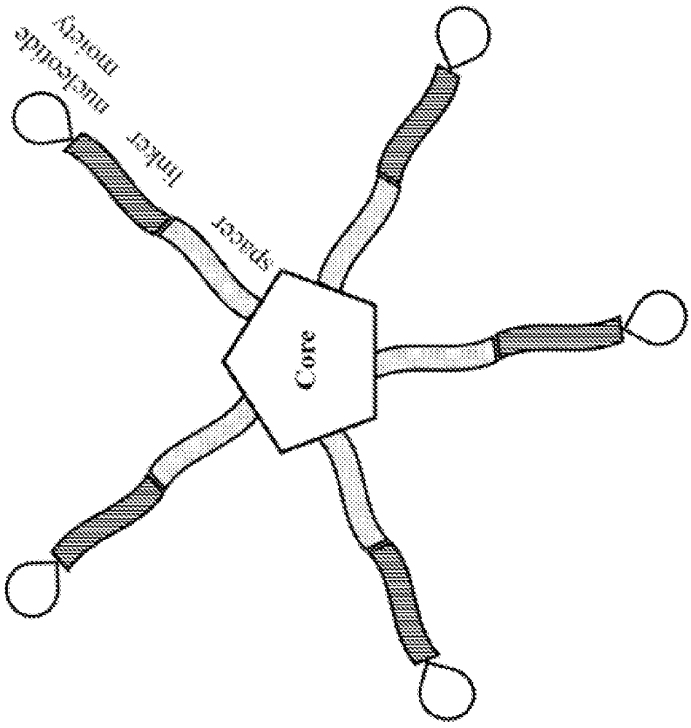

Core
spacer
linker
nucleotide moiety

FIG. 16

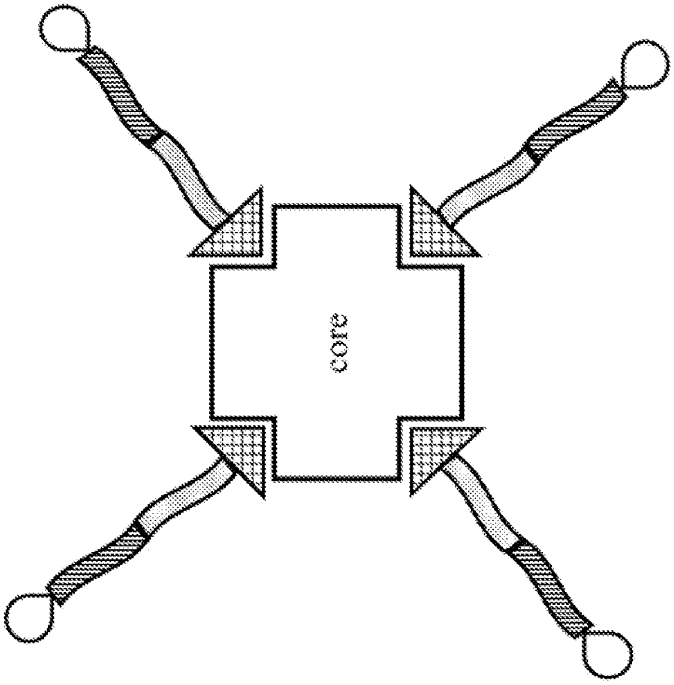

core

FIG. 18

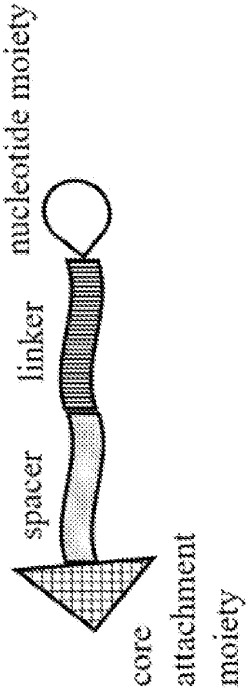

nucleotide moiety
linker
spacer
core attachment moiety

FIG. 19

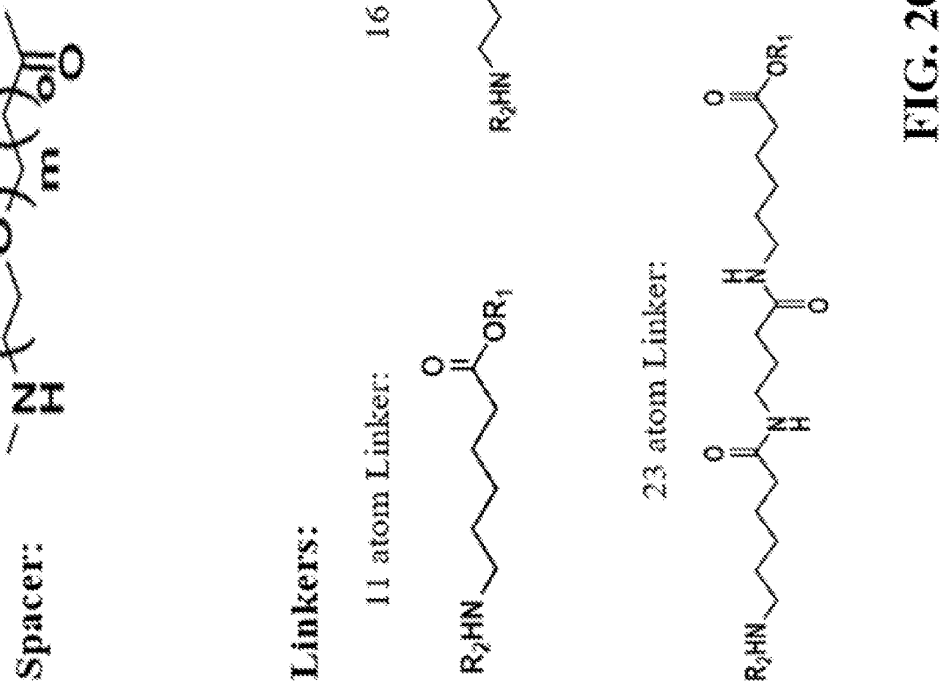

Spacer:
Linkers:
11 atom Linker:
16 atom Linker:
N3 Linker:
23 atom Linker:

FIG. 20

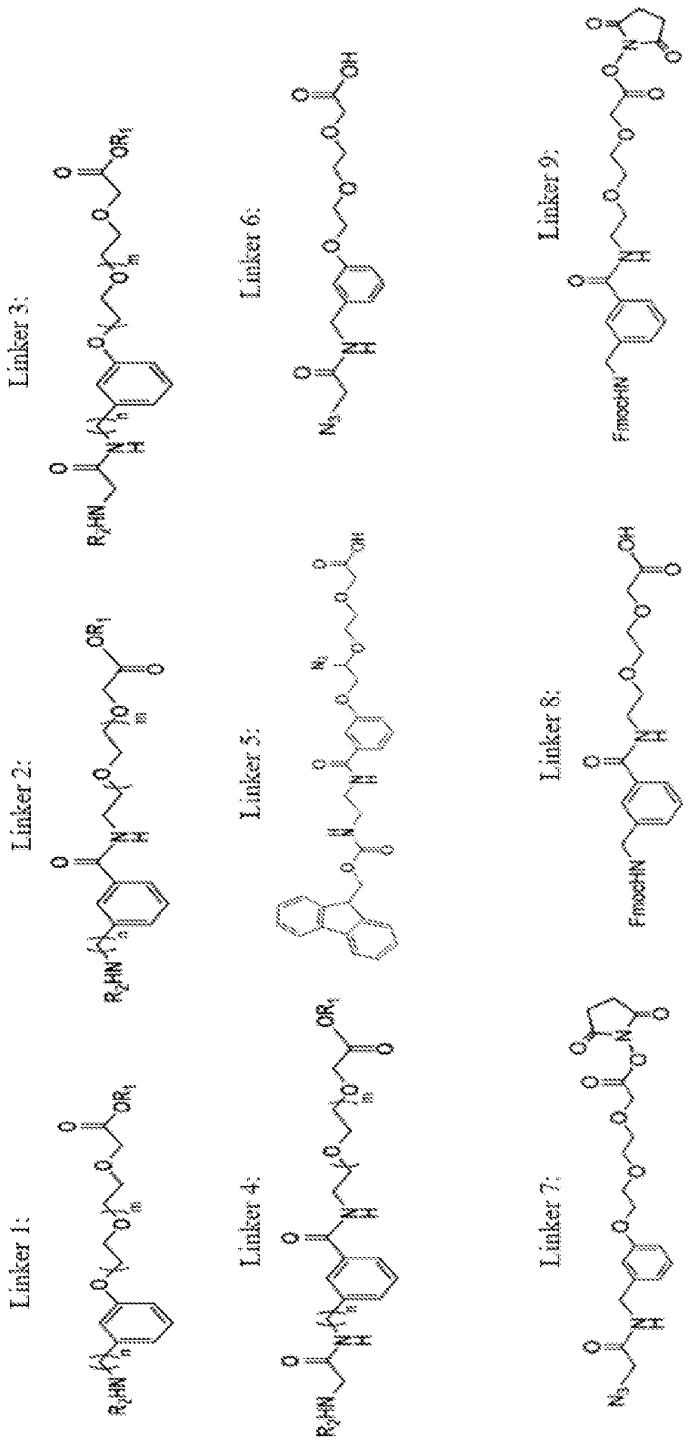

Linker 1:
Linker 2:
Linker 3:
Linker 4:
Linker 5:
Linker 6:
Linker 7:
Linker 8:
Linker 9:

FIG. 21

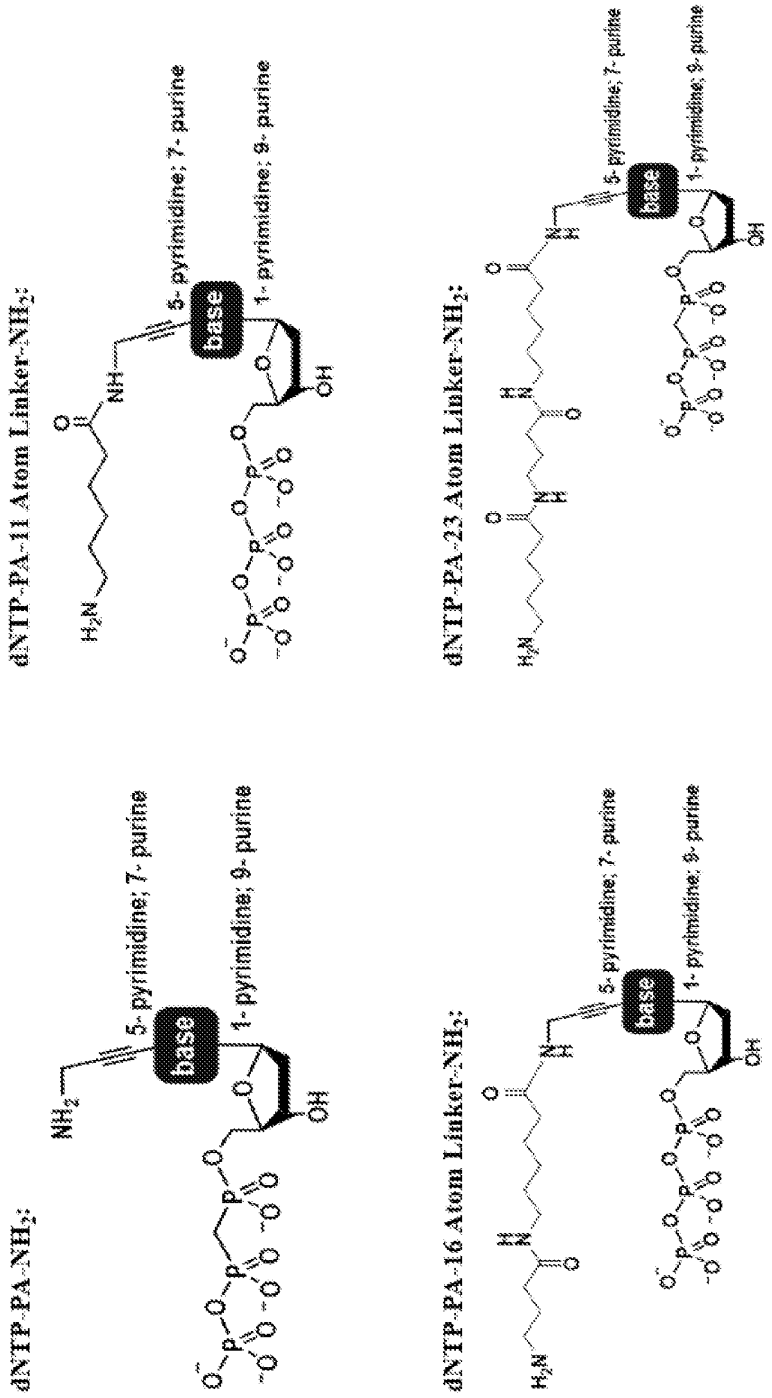

dNTP-PA-11 Atom Linker-NH2:
5- pyrimidine; 7- purine
base
1- pyrimidine; 8- purine
dNTP-PA-23 Atom Linker-NH2:
5- pyrimidine; 7- purine
base
1- pyrimidine; 8- purine
dNTP-PA-NH2:
5- pyrimidine; 7- purine
base
1- pyrimidine; 8- purine
dNTP-PA-16 Atom Linker-NH2:
5- pyrimidine; 7- purine
base
1- pyrimidine; 8- purine

FIG. 22

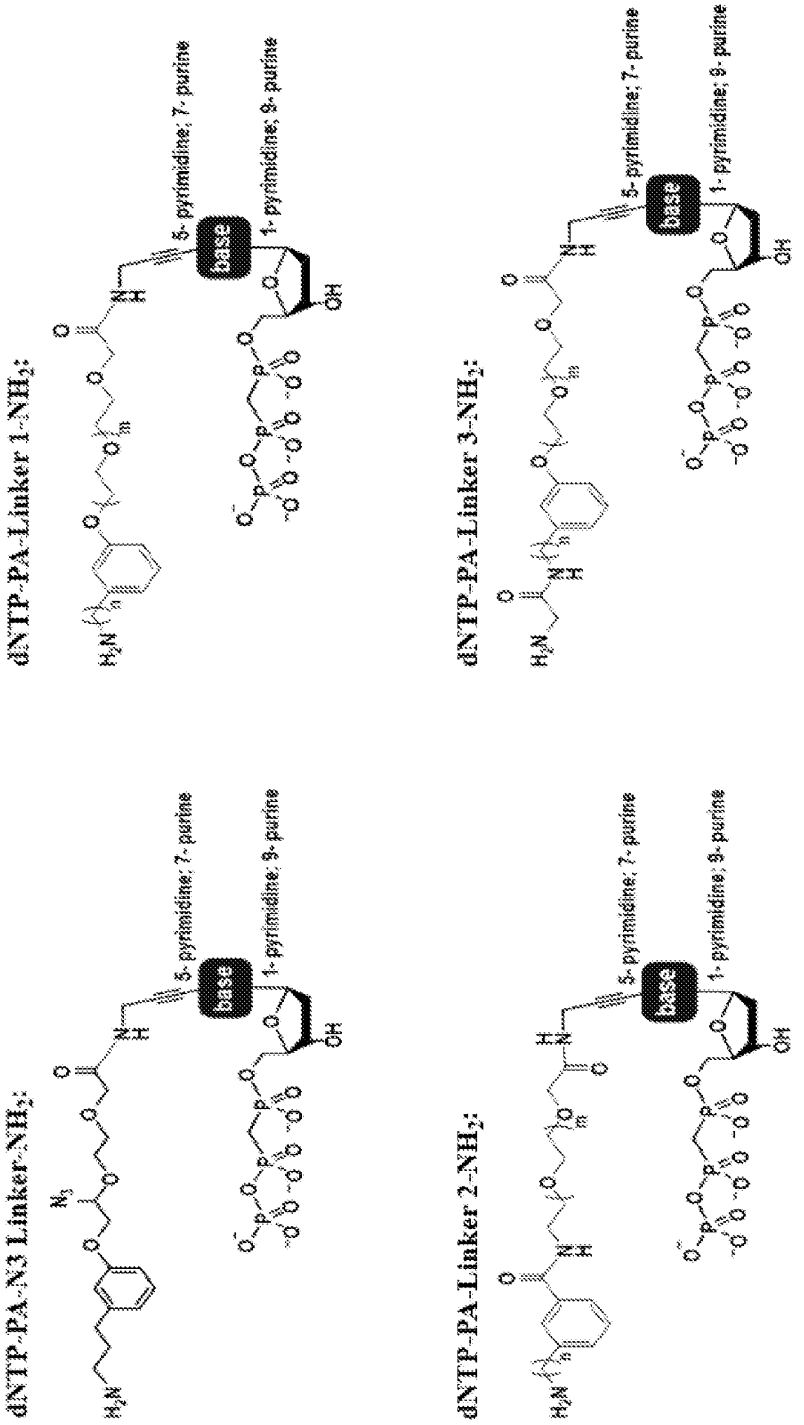

dNTP-PA-Linker 1-NH2:
dNTP-PA-Linker 3-NH2:
dNTP-PA-N3 Linker-NH2:
dNTP-PA-Linker 2-NH2:
base
5- pyrimidine; 7- purine
1- pyrimidine; 9- purine

FIG. 23

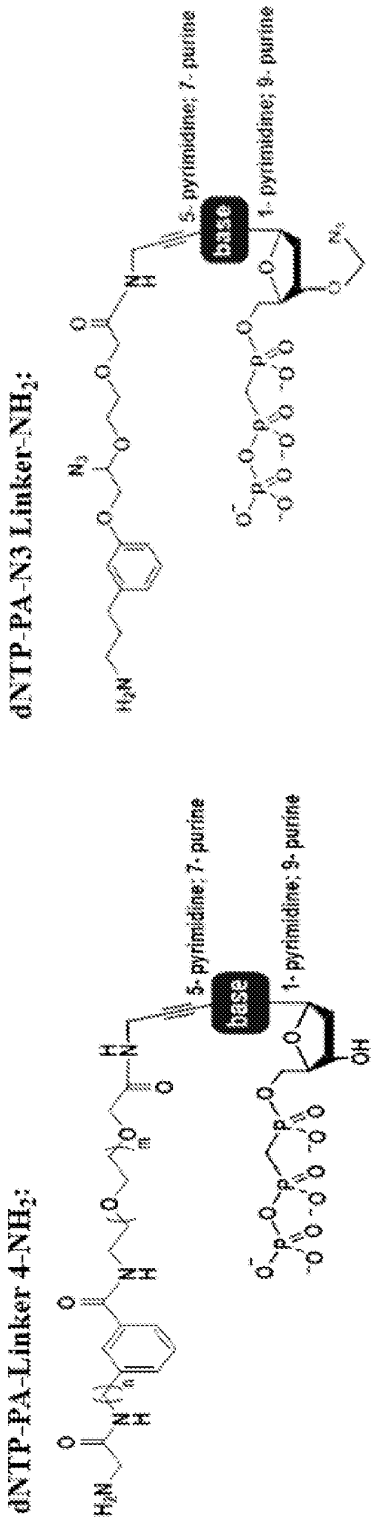

dNTP-PA-N3 Linker-NH2:
5- pyrimidine; 7- purine
base
1- pyrimidine; 9- purine
dNTP-PA-Linker 4-NH2:
5- pyrimidine; 7- purine
base
1- pyrimidine; 9- purine

FIG. 24

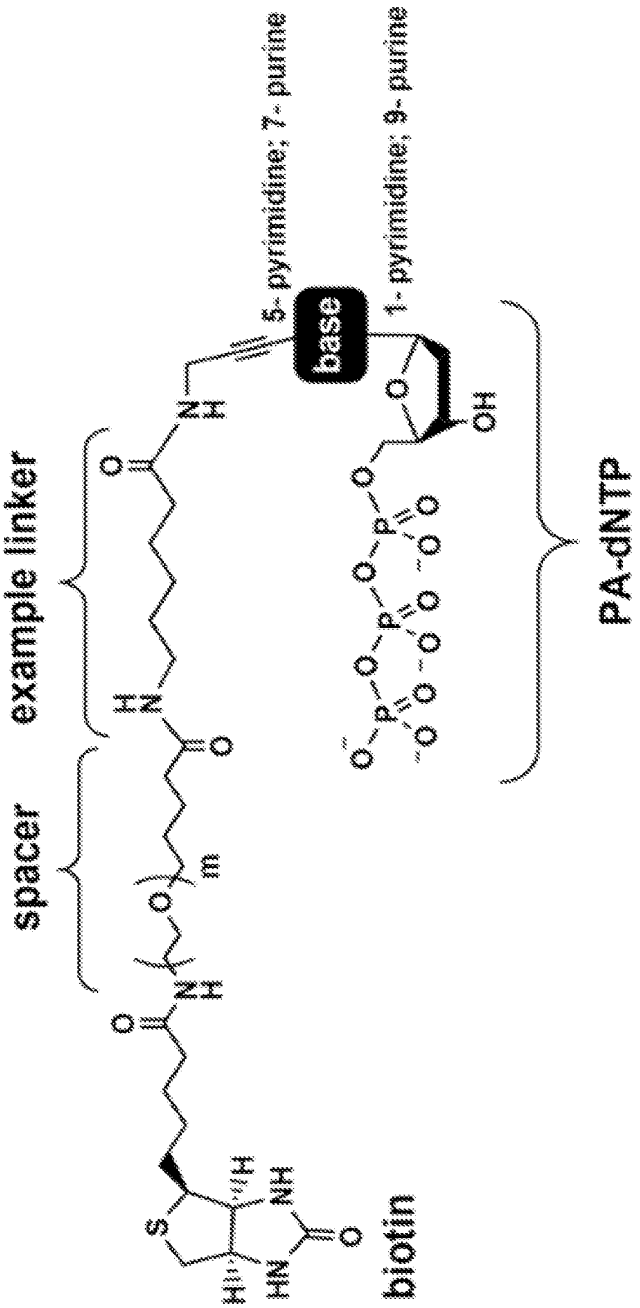

biotin
spacer
example linker
5- pyrimidine; 7- purine
base
1- pyrimidine; 9- purine
PA-dNTP

FIG. 25

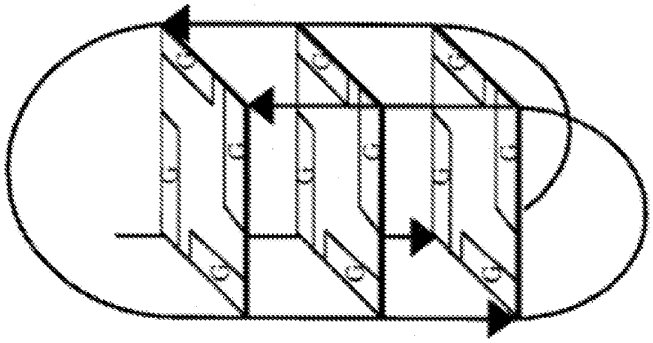


FIG. 27

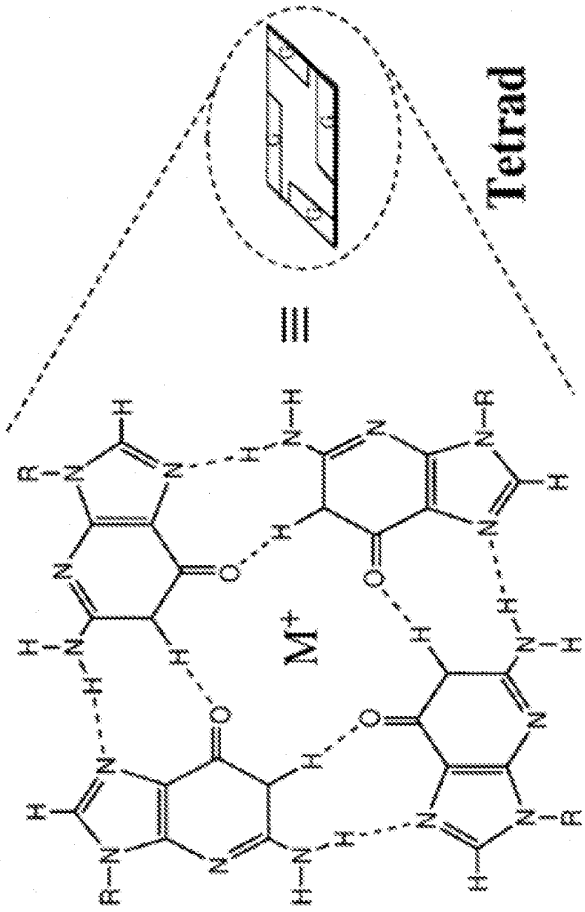


FIG. 26

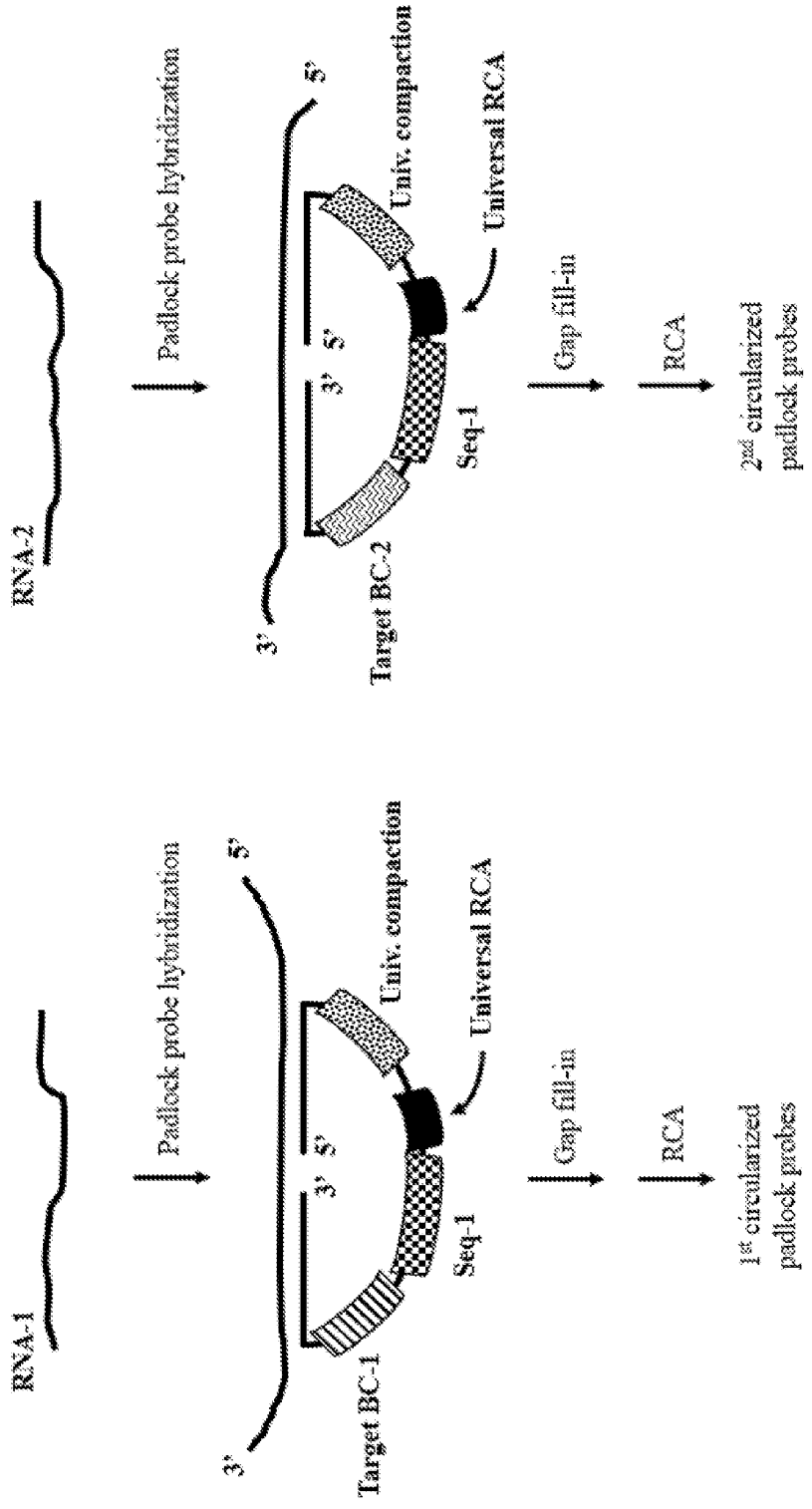

RNA-2
Padlock probe hybridization
5'
3'
3'
5'
Univ. compaction
Universal RCA
Target BC-2
Seq-1
Gap fill-in
RCA
2nd circularized padlock probes
RNA-1
Padlock probe hybridization
5'
3'
3'
5'
Univ. compaction
Universal RCA
Target BC-1
Seq-1
Gap fill-in
RCA
1st circularized padlock probes

FIG. 28

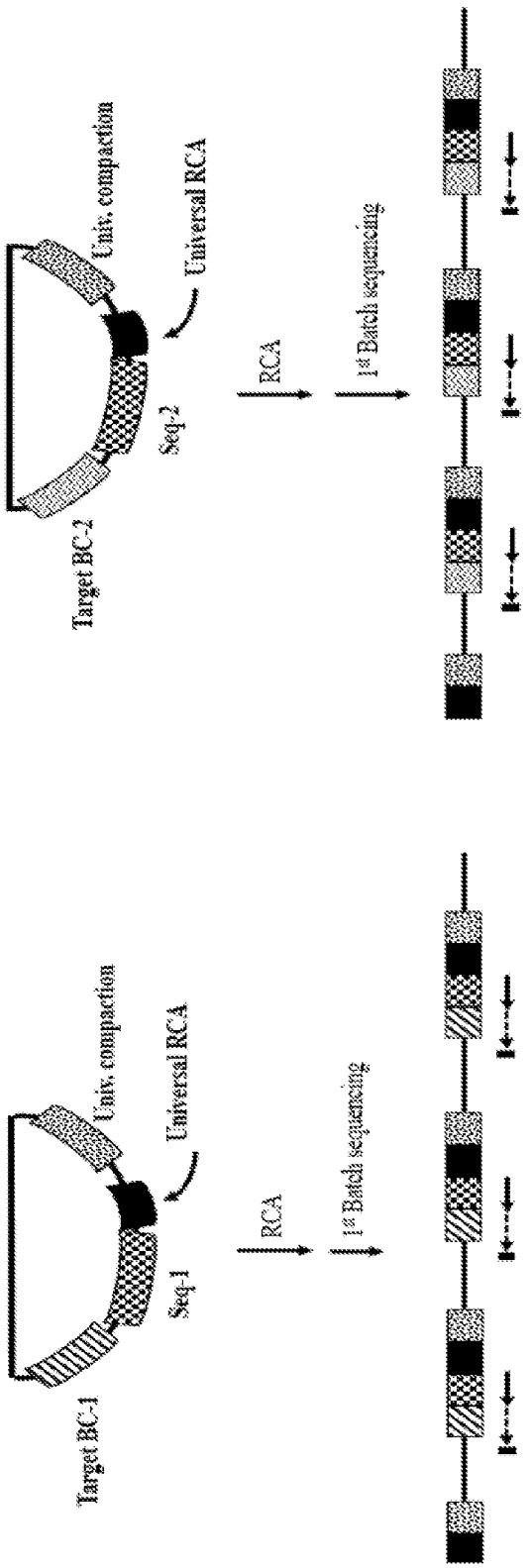

Univ. compaction
Universal RCA
Seq-2
Target BC-2
RCA
1st Batch sequencing
Univ. compaction
Universal RCA
Seq-1
Target BC-1
RCA
1st Batch sequencing

FIG. 29

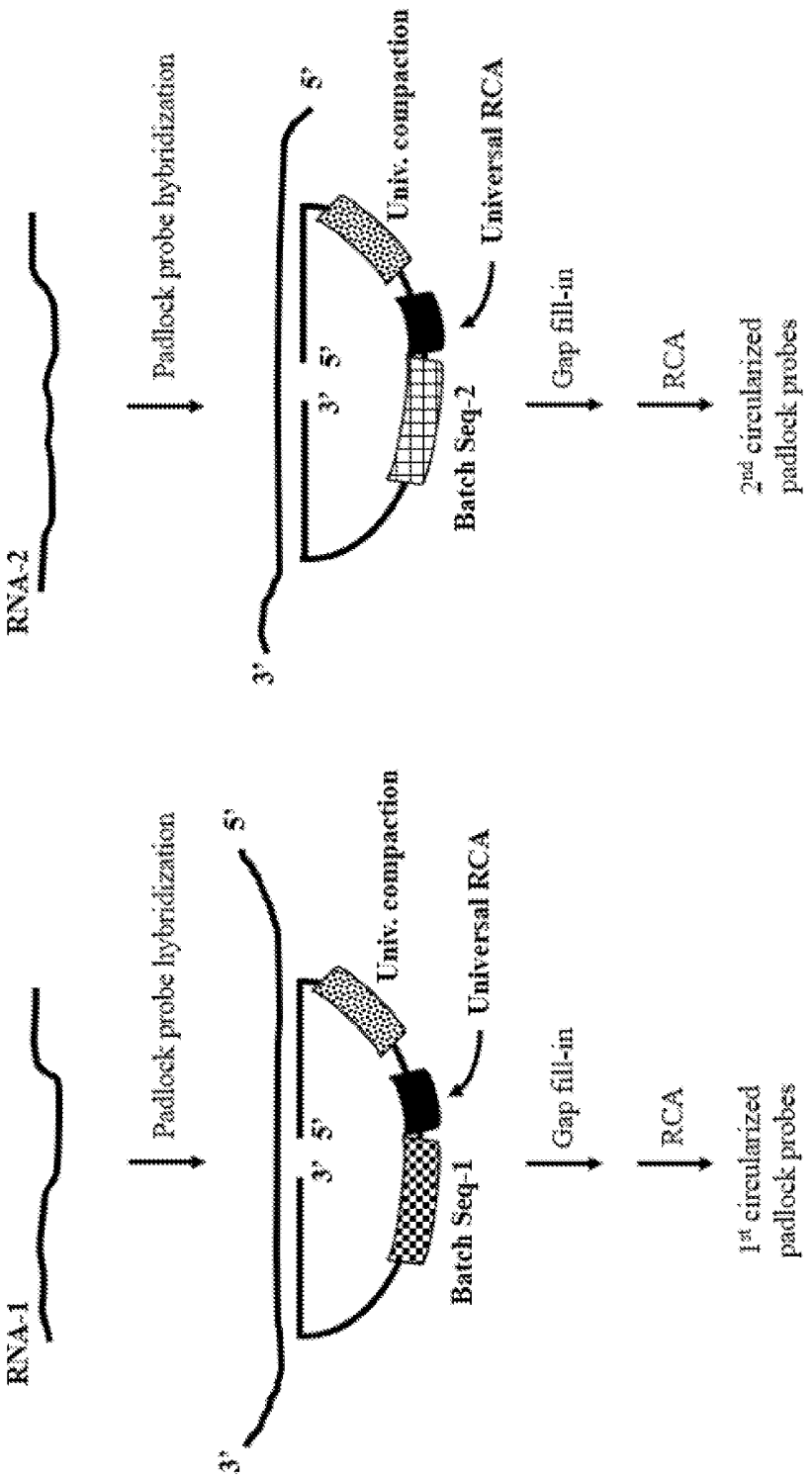

RNA-1
Padlock probe hybridization
3'
5'
3'
5'
Univ. compaction
Universal RCA
Batch Seq-1
Gap fill-in
RCA
1st circularized padlock probes
RNA-2
Padlock probe hybridization
3'
5'
3'
5'
Univ. compaction
Universal RCA
Batch Seq-2
Gap fill-in
RCA
2nd circularized padlock probes

FIG. 30

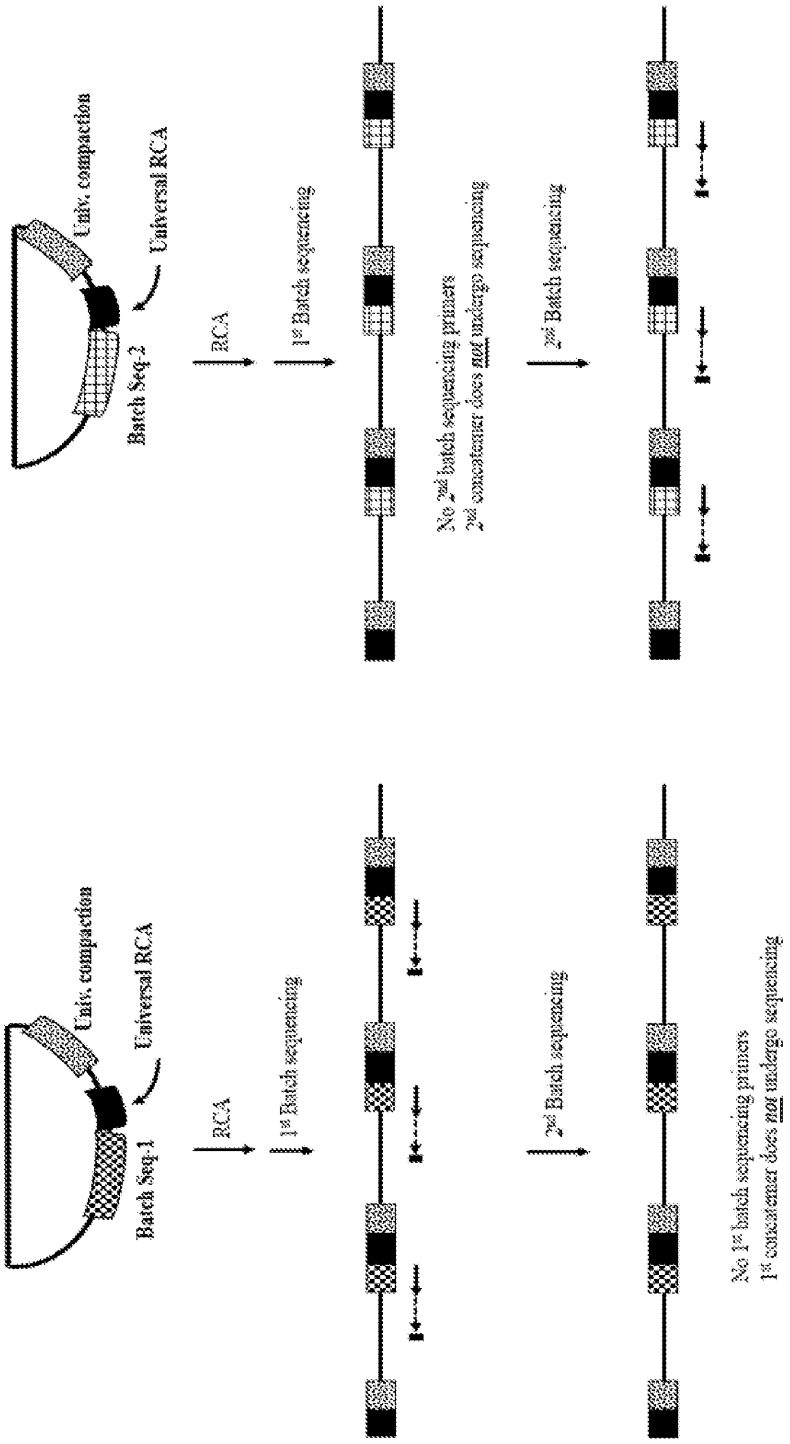

Univ. compaction
Universal RCA
Batch Seq-2
RCA
1st Batch sequencing
No 2nd batch sequencing primers
2nd concatemer does not undergo sequencing
2nd Batch sequencing
Univ. compaction
Universal RCA
Batch Seq-1
RCA
1st Batch sequencing
2nd Batch sequencing
No 1st batch sequencing primers
1st concatemer does not undergo sequencing

FIG. 31

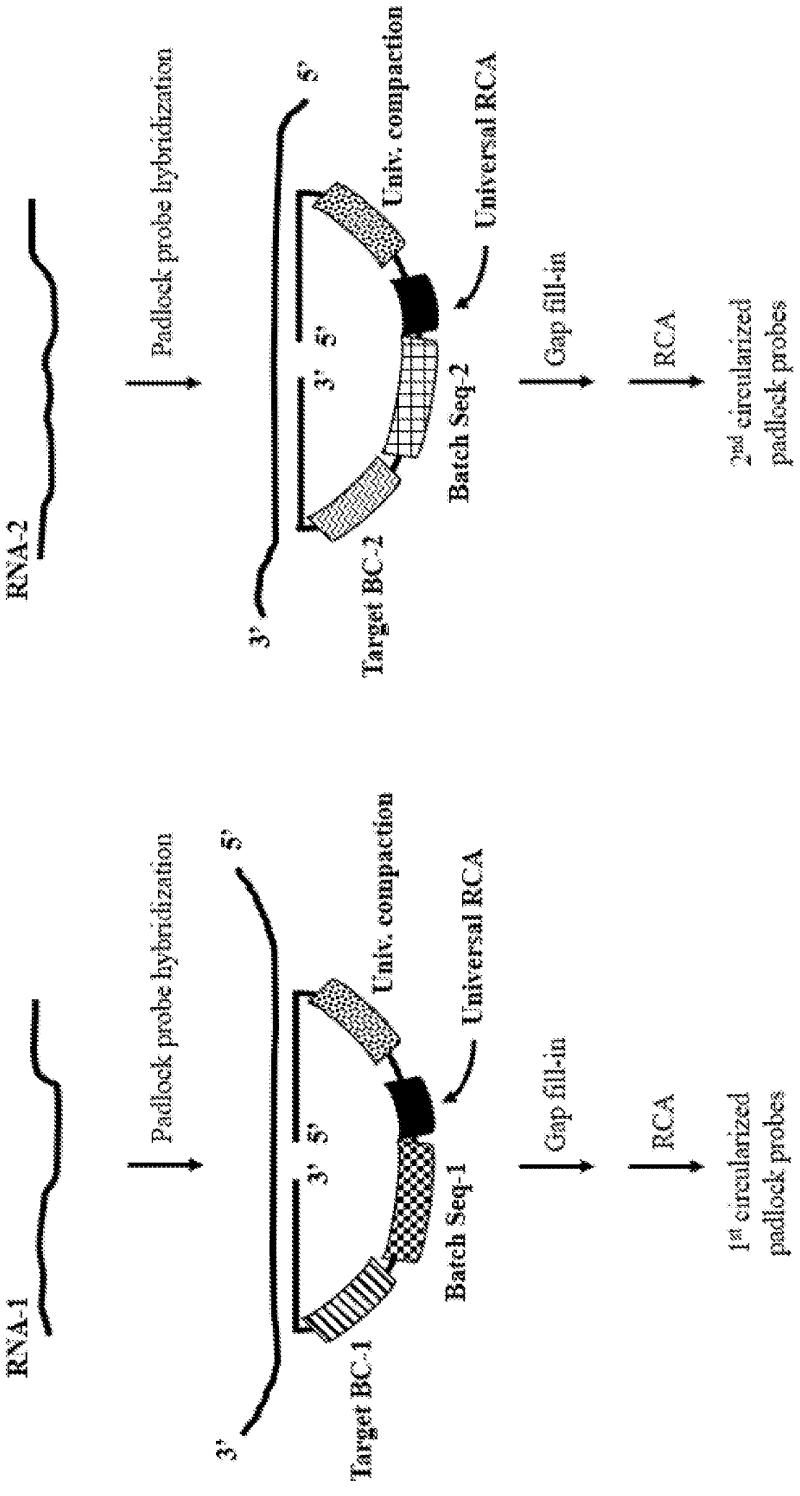

RNA-2
Padlock probe hybridization
3'
5'
3'
5'
Target BC-2
Batch Seq-2
Univ. compaction
Universal RCA
Gap fill-in
RCA
2nd circularized padlock probes
RNA-1
Padlock probe hybridization
3'
5'
3'
5'
Target BC-1
Batch Seq-1
Univ. compaction
Universal RCA
Gap fill-in
RCA
1st circularized padlock probes

FIG. 32

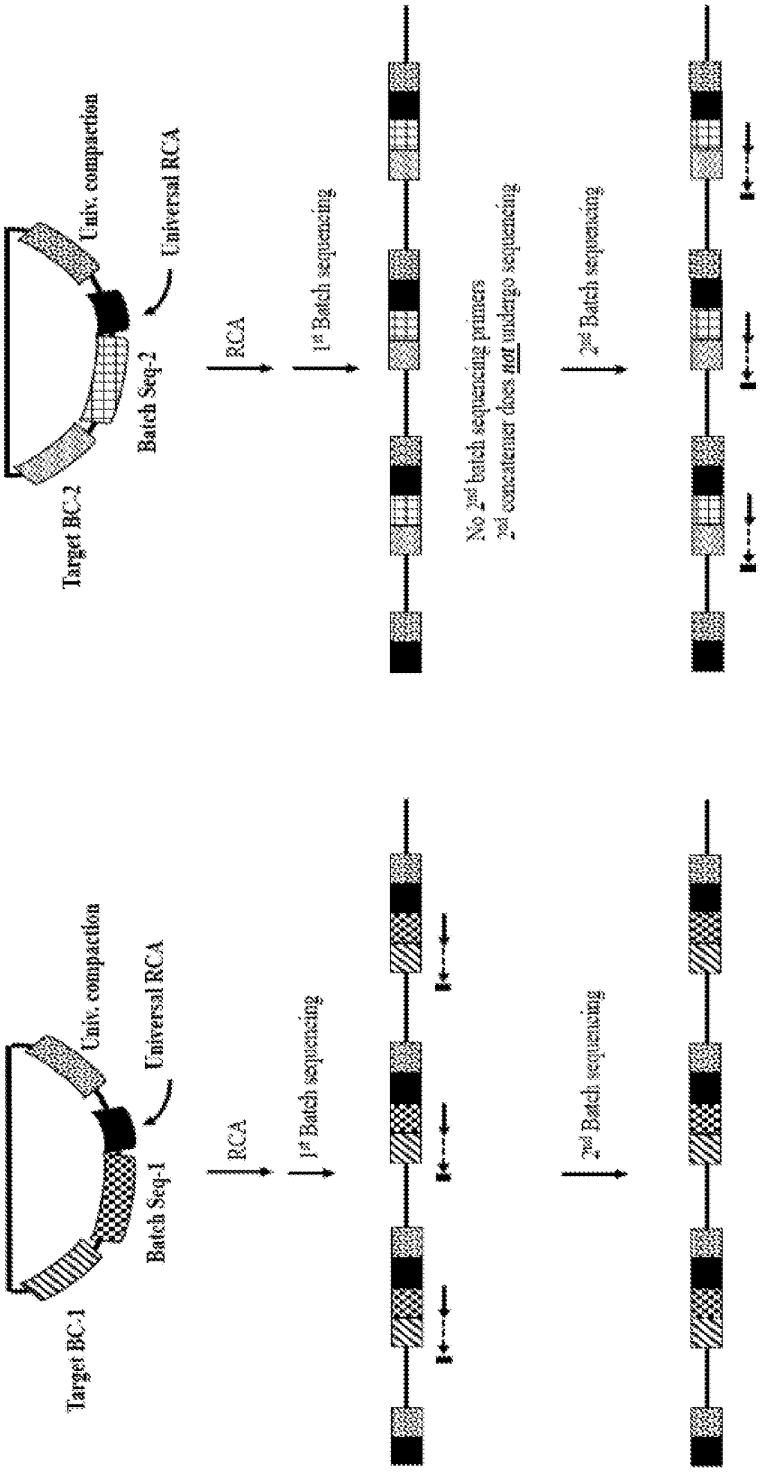

Univ. compaction
Universal RCA
Batch Seq-2
Target BC-2
RCA
1st Batch sequencing
No 2nd batch sequencing primers
2nd concatemer does not undergo sequencing
2nd Batch sequencing
Univ. compaction
Universal RCA
Batch Seq-1
Target BC-1
RCA
1st Batch sequencing
2nd Batch sequencing
No 1st batch sequencing primers
1st concatemer does not undergo sequencing

FIG. 33

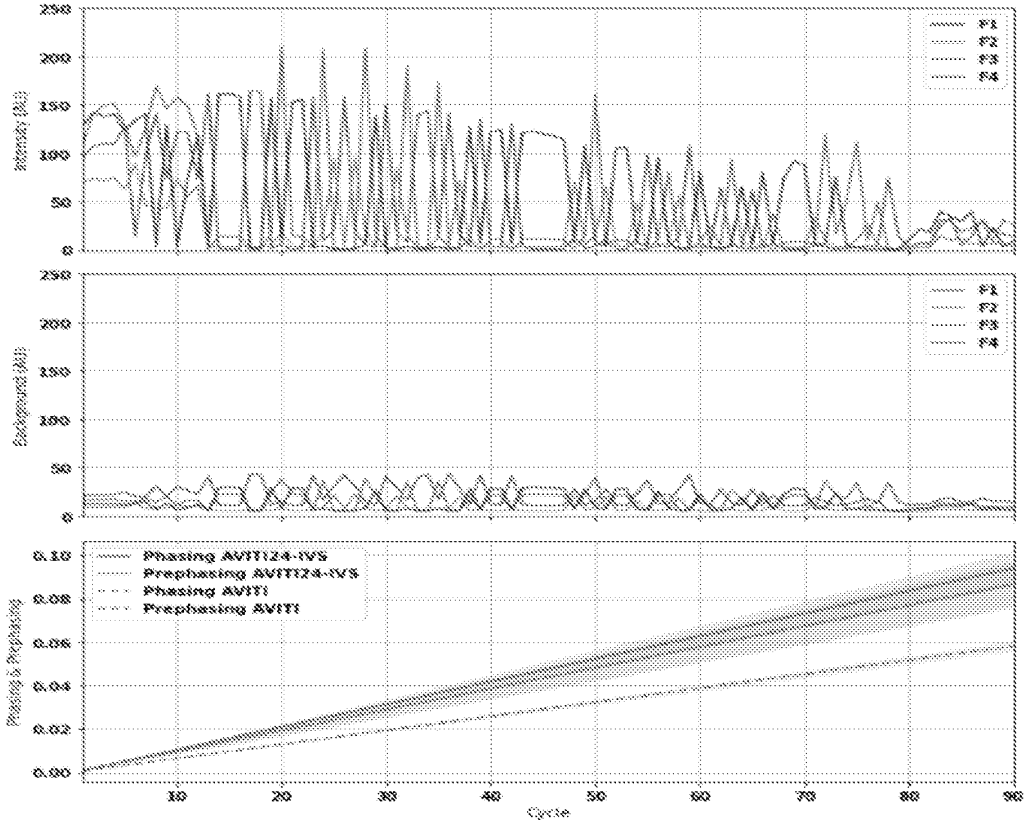

Intensity (AU)
Background (AU)
Phasing & Prephasing
Cycle
T1
T2
T3
T4
Phasing AVITI24-IVS
Prephasing AVITI24-IVS
Phasing AVITI
Prephasing AVITI

FIG. 34

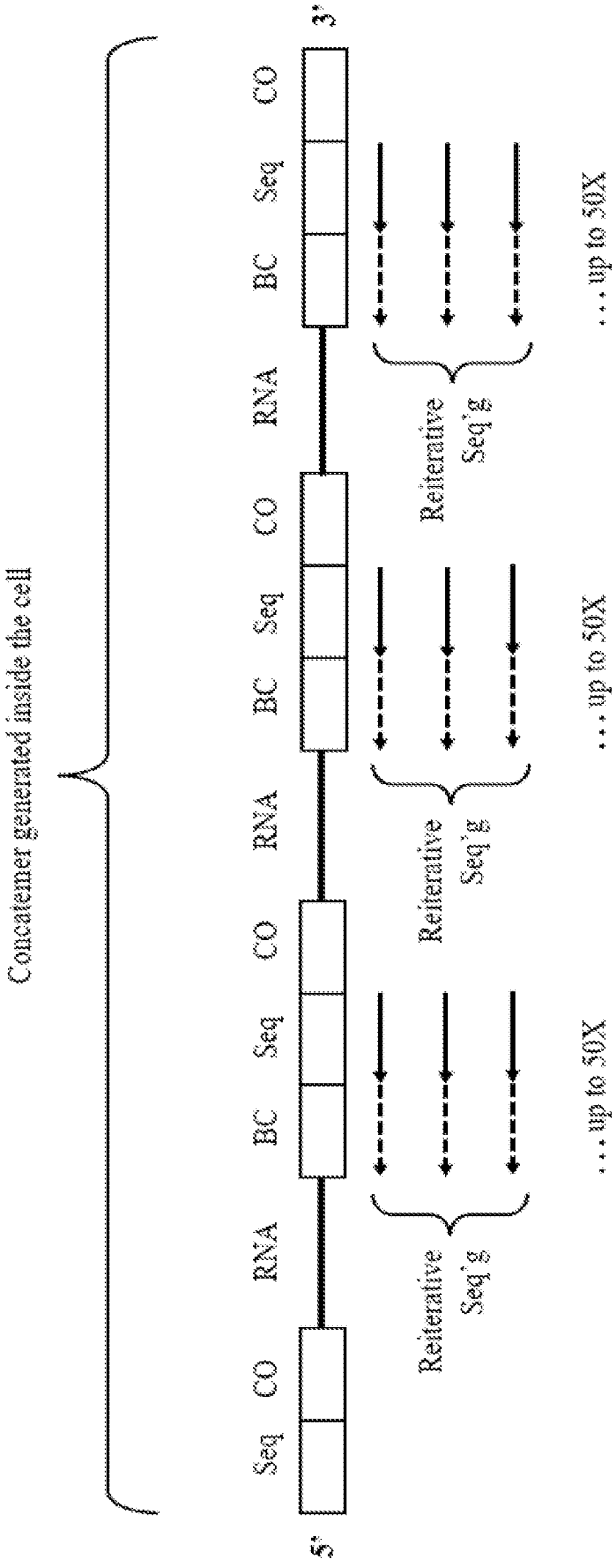

Concatemer generated inside the cell
5'
3'
Seq
CO
RNA
BC
Seq
CO
RNA
BC
Seq
CO
RNA
BC
Seq
CO
Reiterative Seq'g
Reiterative Seq'g
Reiterative Seq'g
. . . up to 50X
. . . up to 50X
. . . up to 50X

FIG. 35

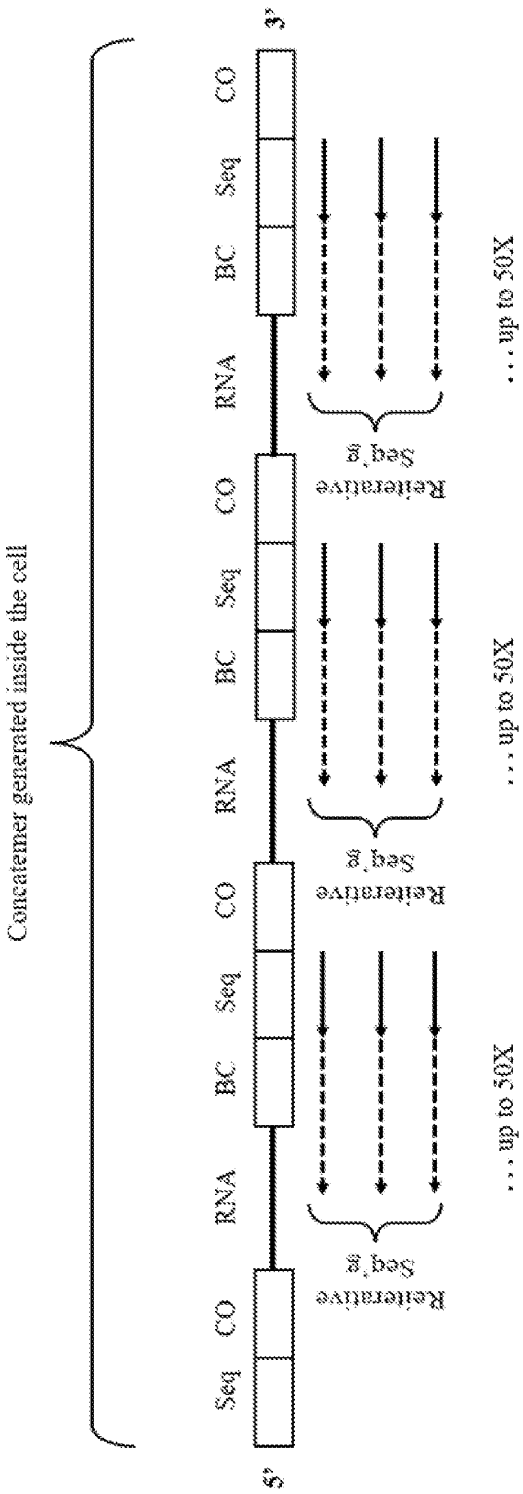

Concatemer generated inside the cell
5'
3'
Seq
CO
RNA
BC
Seq
CO
Reiterative Seq's
. . . up to 50X

FIG. 36

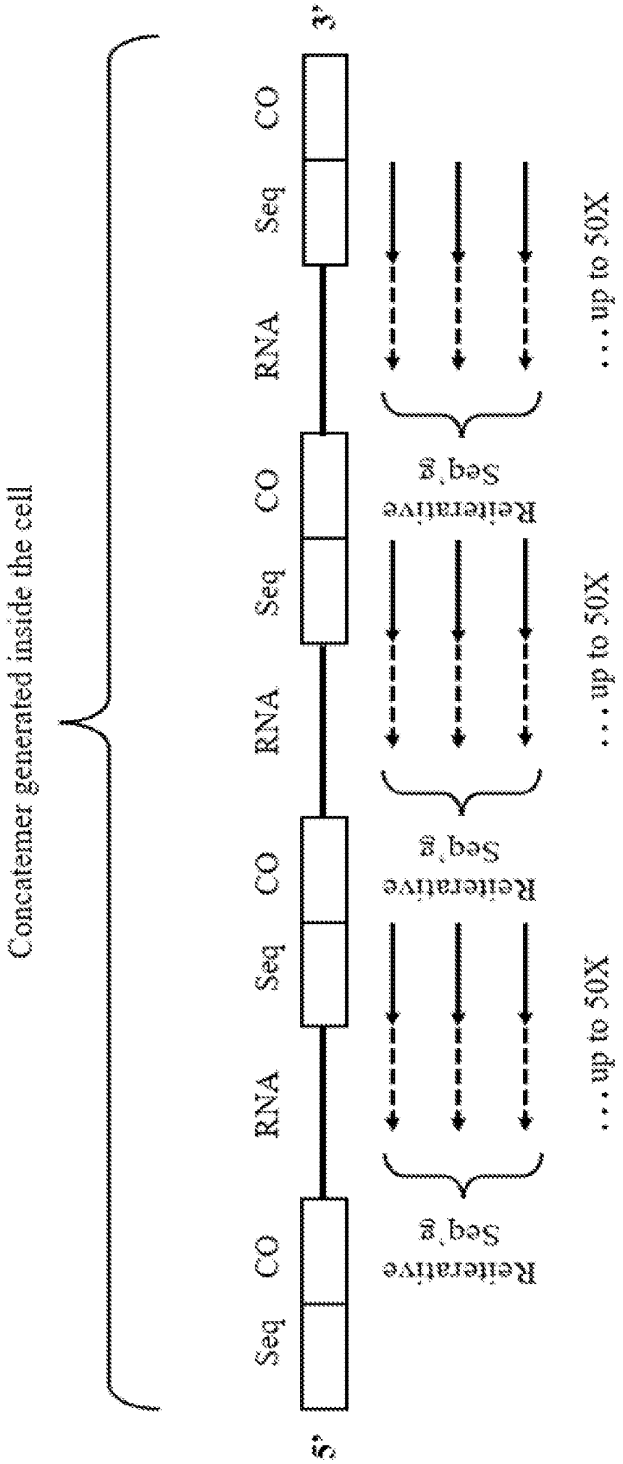

Concatemer generated inside the cell
5'
Seq
CO
RNA
Seq
CO
RNA
Seq
CO
RNA
Seq
CO
3'
Reiterative Seq'ɐ
Reiterative Seq'ɐ
Reiterative Seq'ɐ
. . . up to 50X
. . . up to 50X
. . . up to 50X

FIG. 37

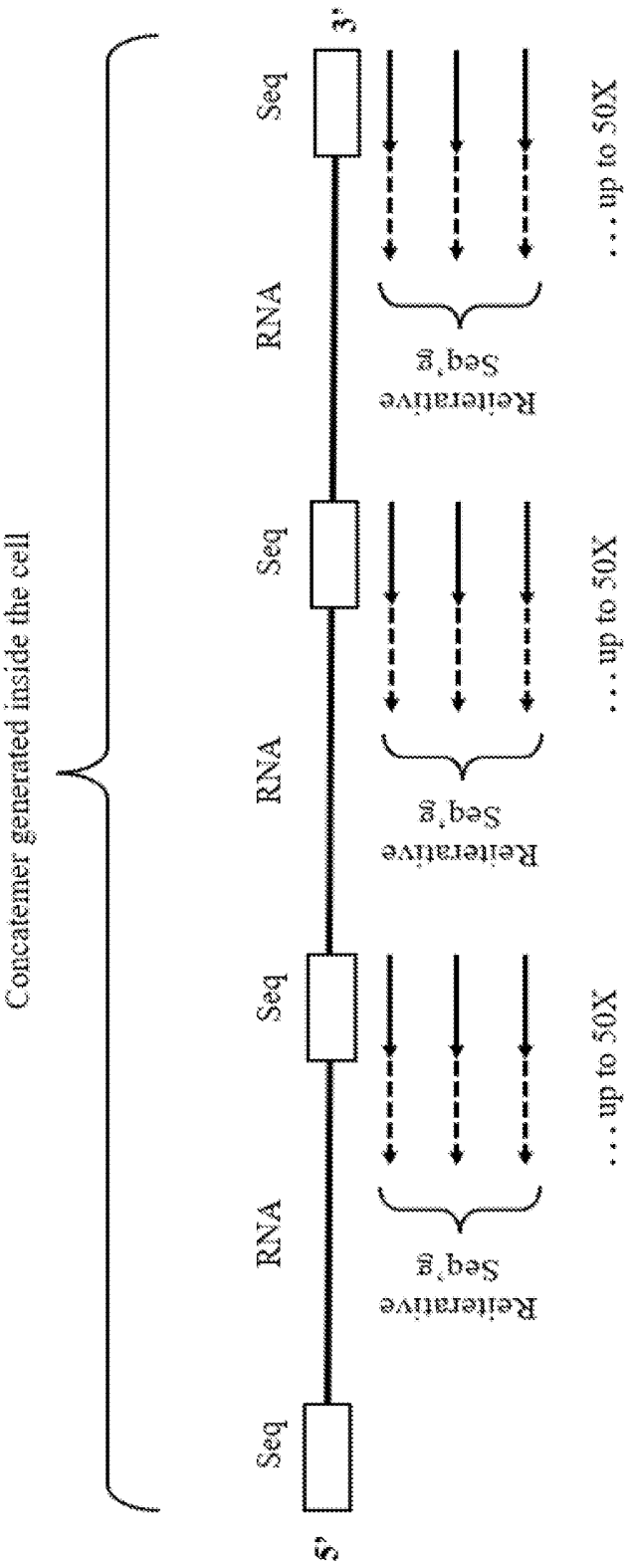

Concatemer generated inside the cell
5'
Seq
RNA
Reiterative Seq'ε
. . . up to 50X
Seq
RNA
Reiterative Seq'ε
. . . up to 50X
Seq
RNA
Reiterative Seq'ε
. . . up to 50X
Seq
3'

FIG. 38

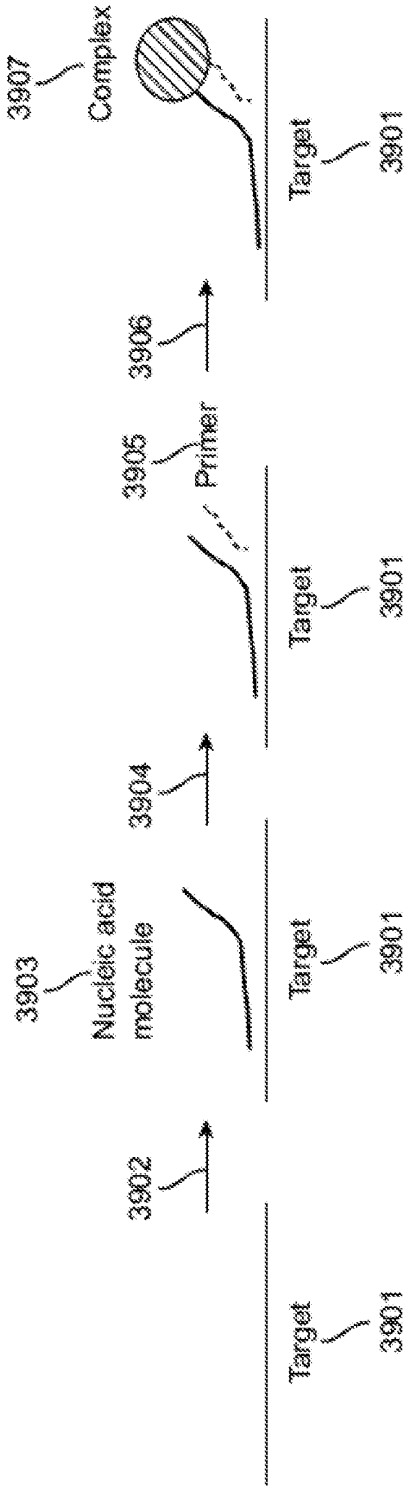

3907
Complex
3906
3905
Primer
3904
3903
Nucleic acid molecule
3902
Target
3901

FIG. 39

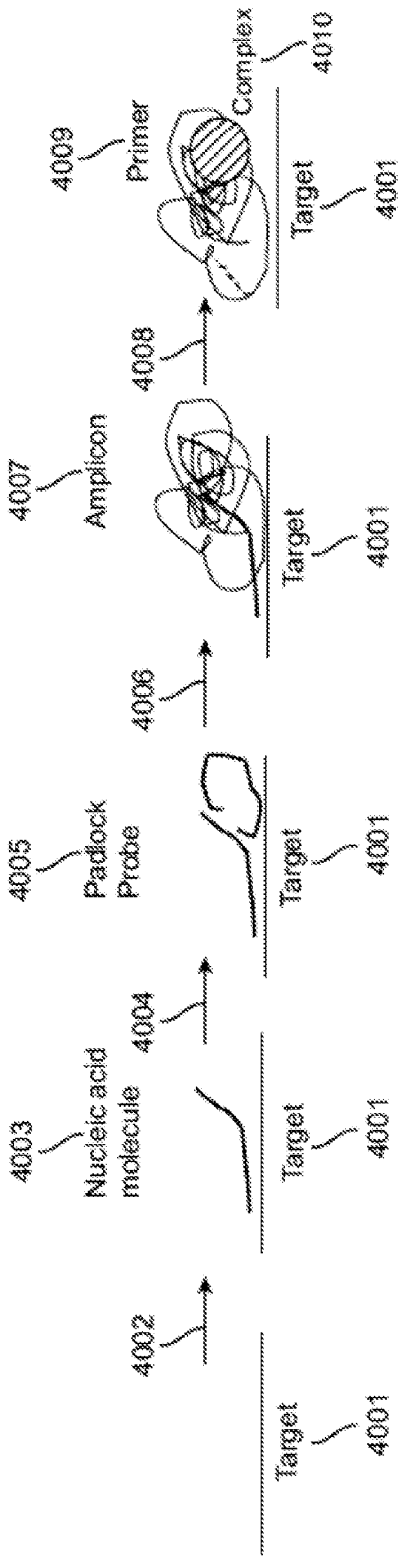

Target
4001
4002
Target
4001
4003
Nucleic acid molecule
4004
Target
4001
4005
Padlock Probe
4006
Target
4001
4007
Amplicon
4008
Target
4001
4009
Primer
Complex
4010

FIG. 40

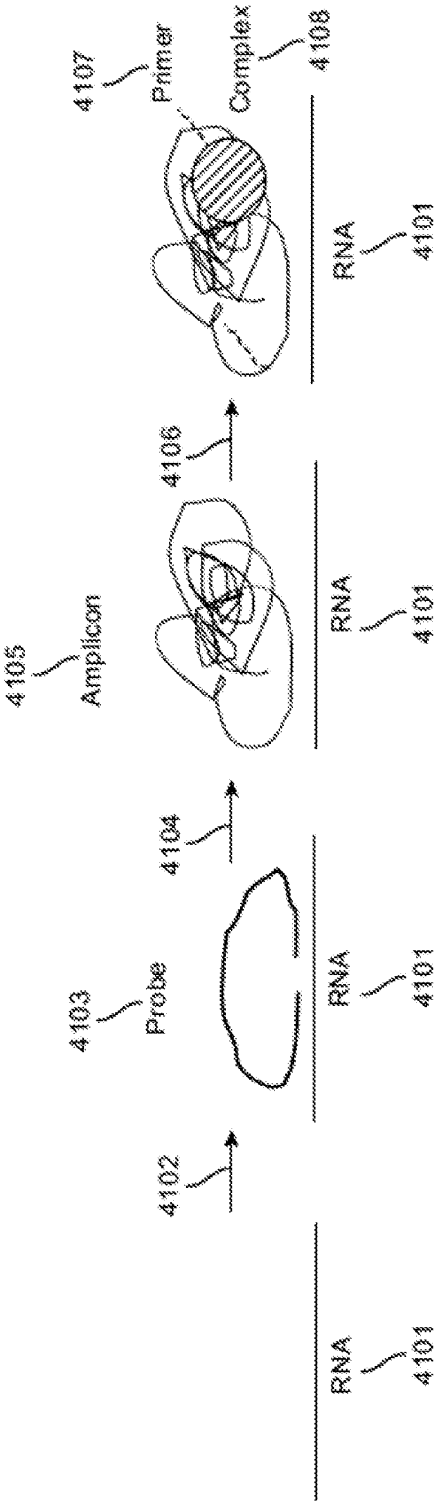

4107
Primer
Complex
4108
RNA
4101
4106
4105
Amplicon
RNA
4101
4104
4103
Probe
RNA
4101
4102
RNA
4101

FIG. 41

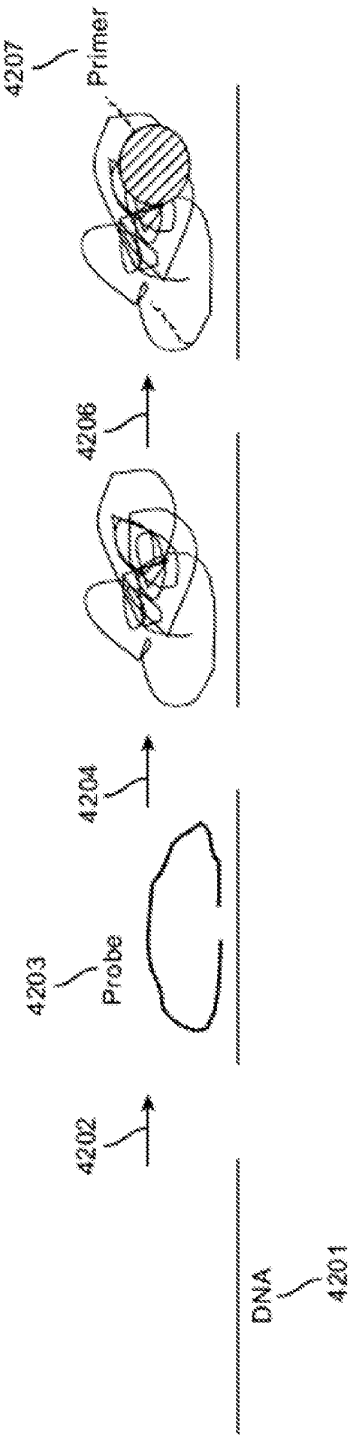


FIG. 42

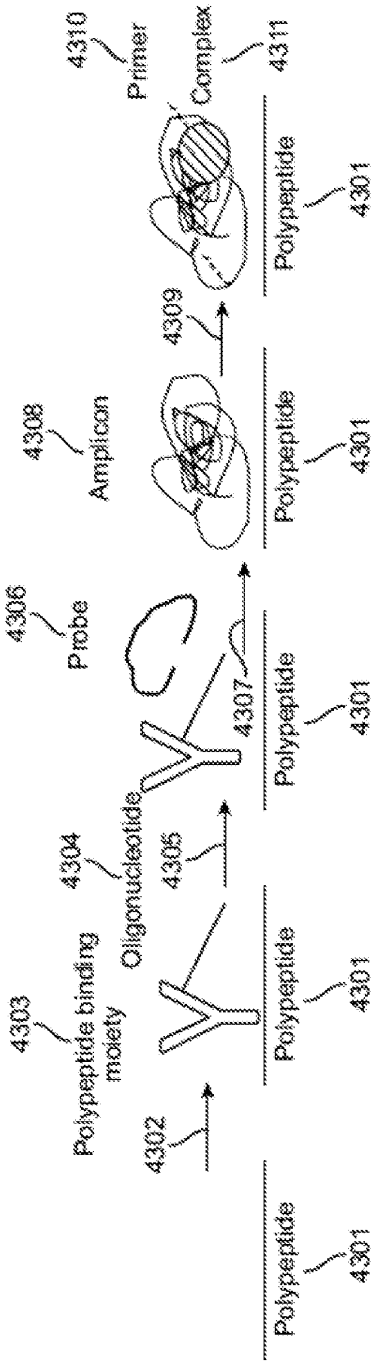

4310
Primer
Complex
4311
Polypeptide
4301
4309
4308
Amplicon
Polypeptide
4301
4306
Probe
4307
Polypeptide
4301
4304
Oligonucleotide
4305
4303
Polypeptide binding moiety
Polypeptide
4301
4302
Polypeptide
4301

FIG. 43