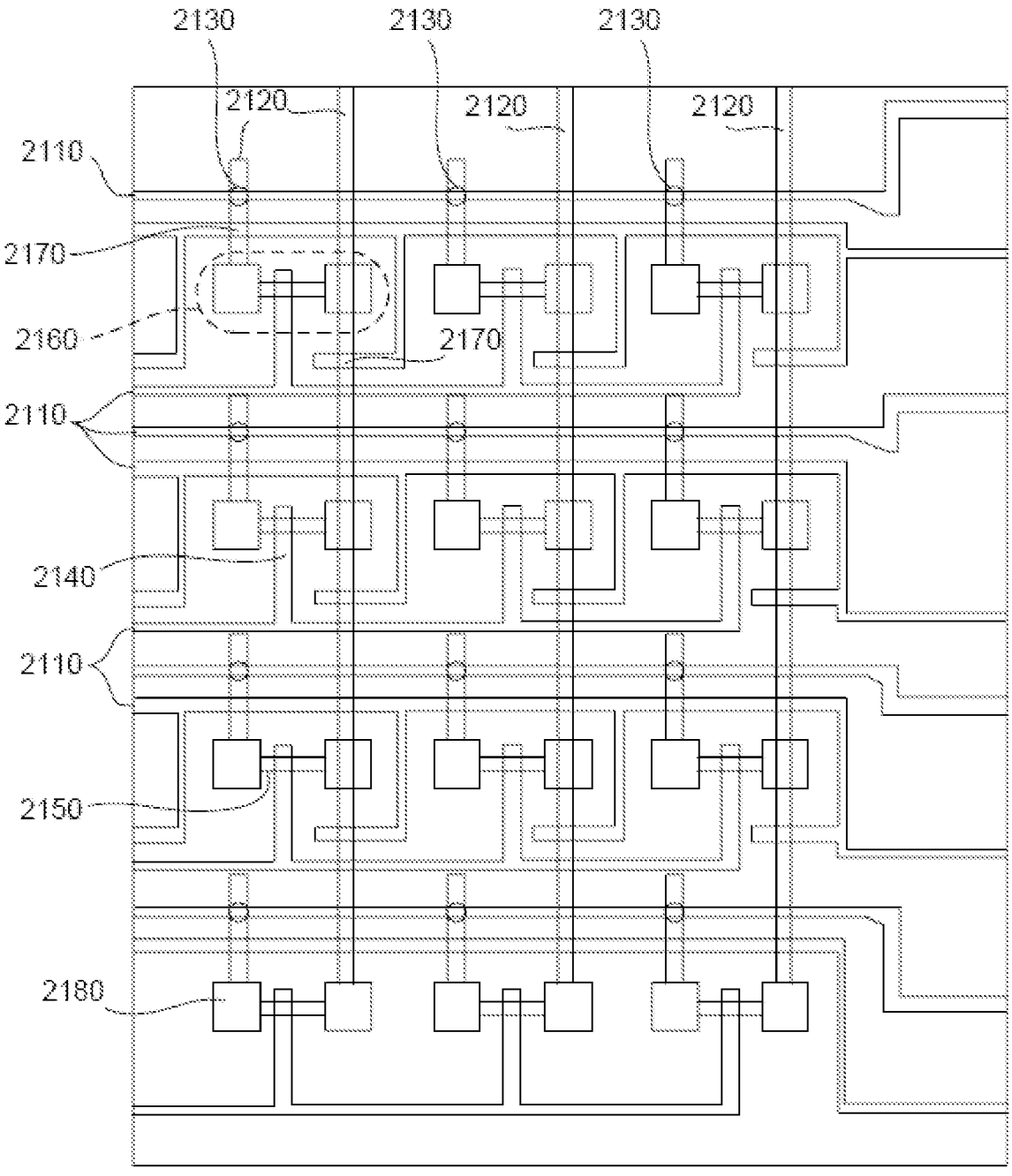

2130
2130
2130
2120
2120
2120
2110
2170
2160
2170
2110
2140
2110
2150
2180

Fig. 1

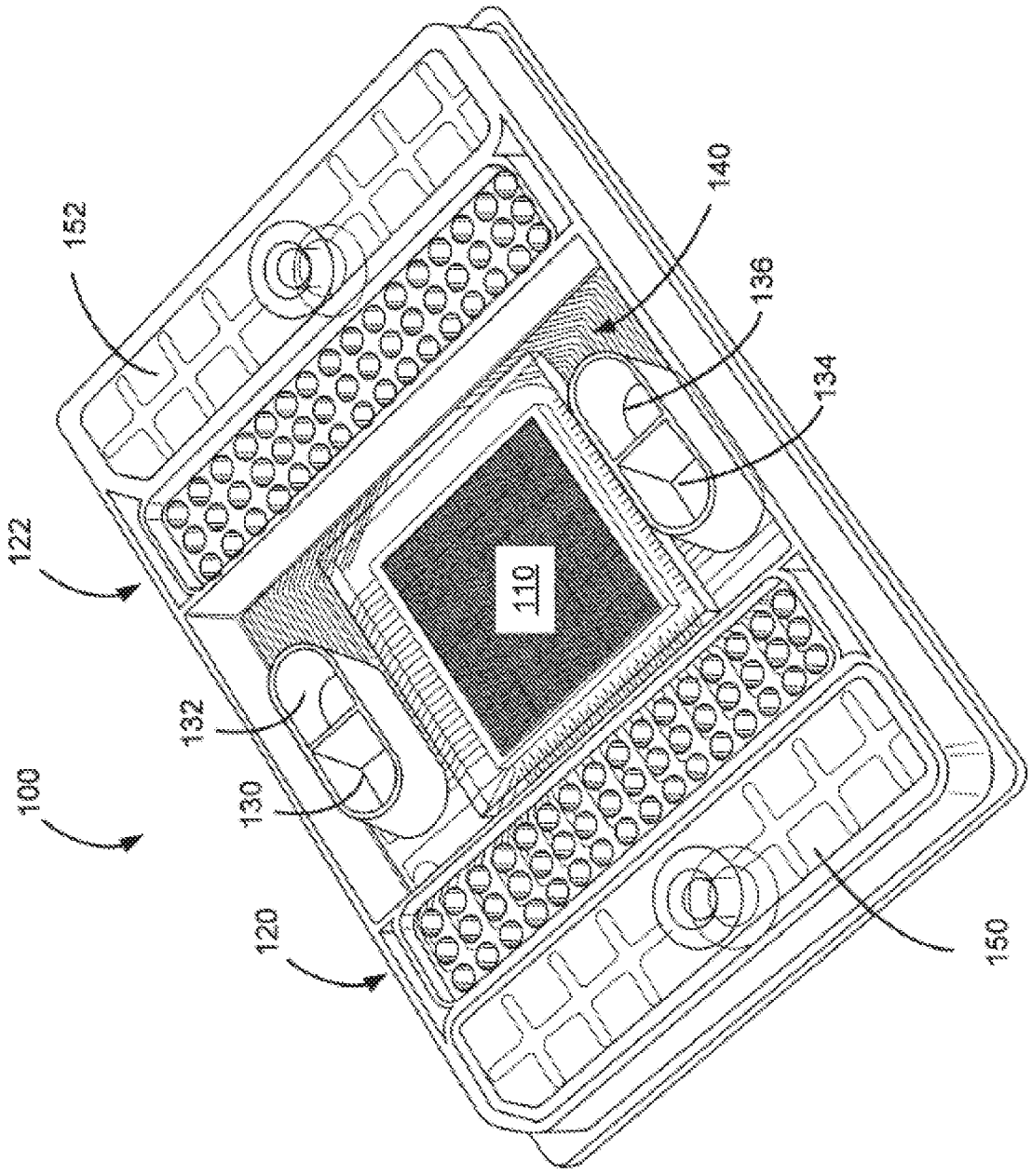


Fig. 2

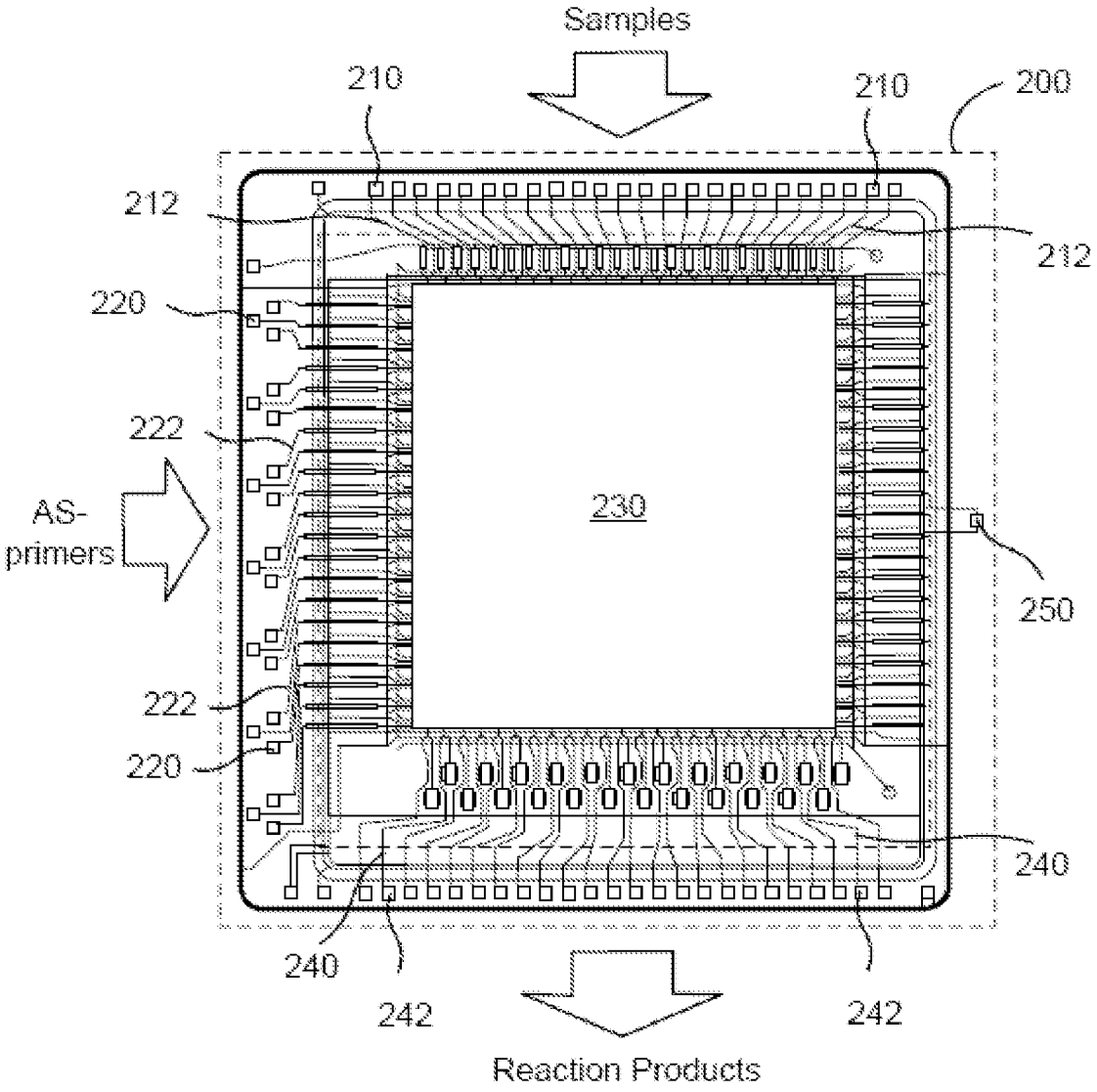

Samples
210
210
200
212
212
220
222
AS-
primers
230
250
222
220
240
240
242
242
Reaction Products

Fig. 3

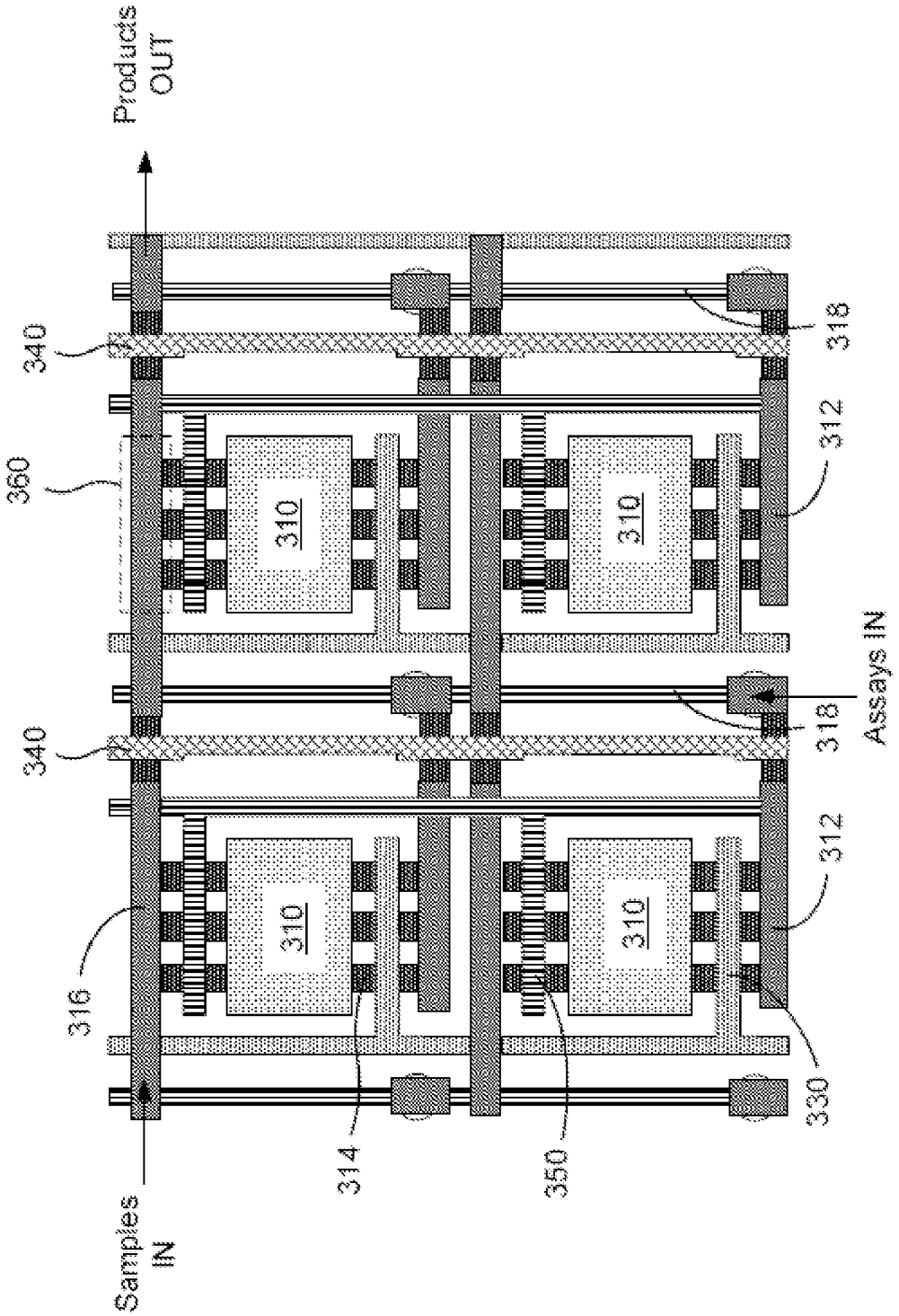

Products OUT
340
360
310
312
318
Assays IN
316
314
350
330
Samples IN

Fig. 4

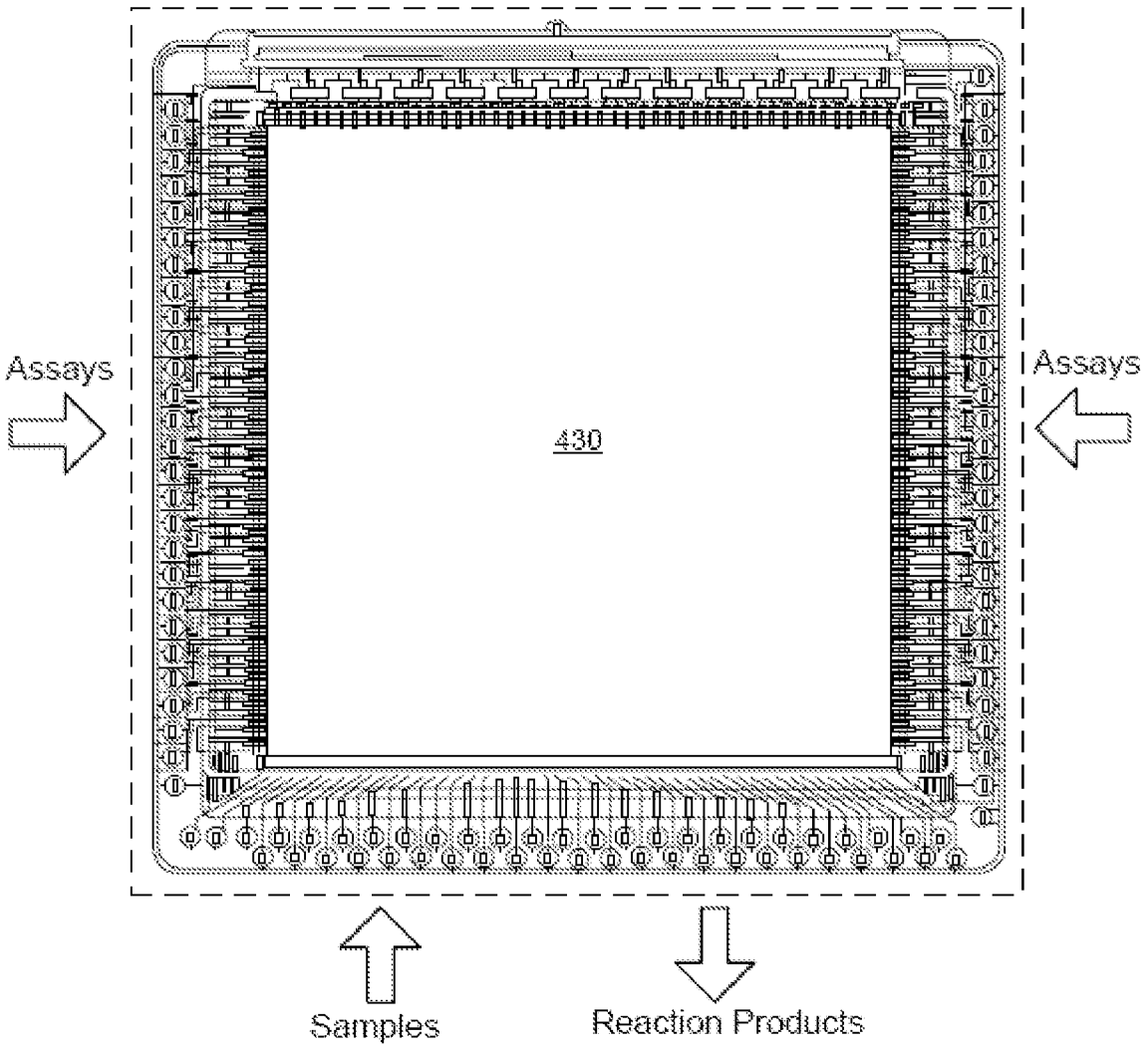


Fig. 5A

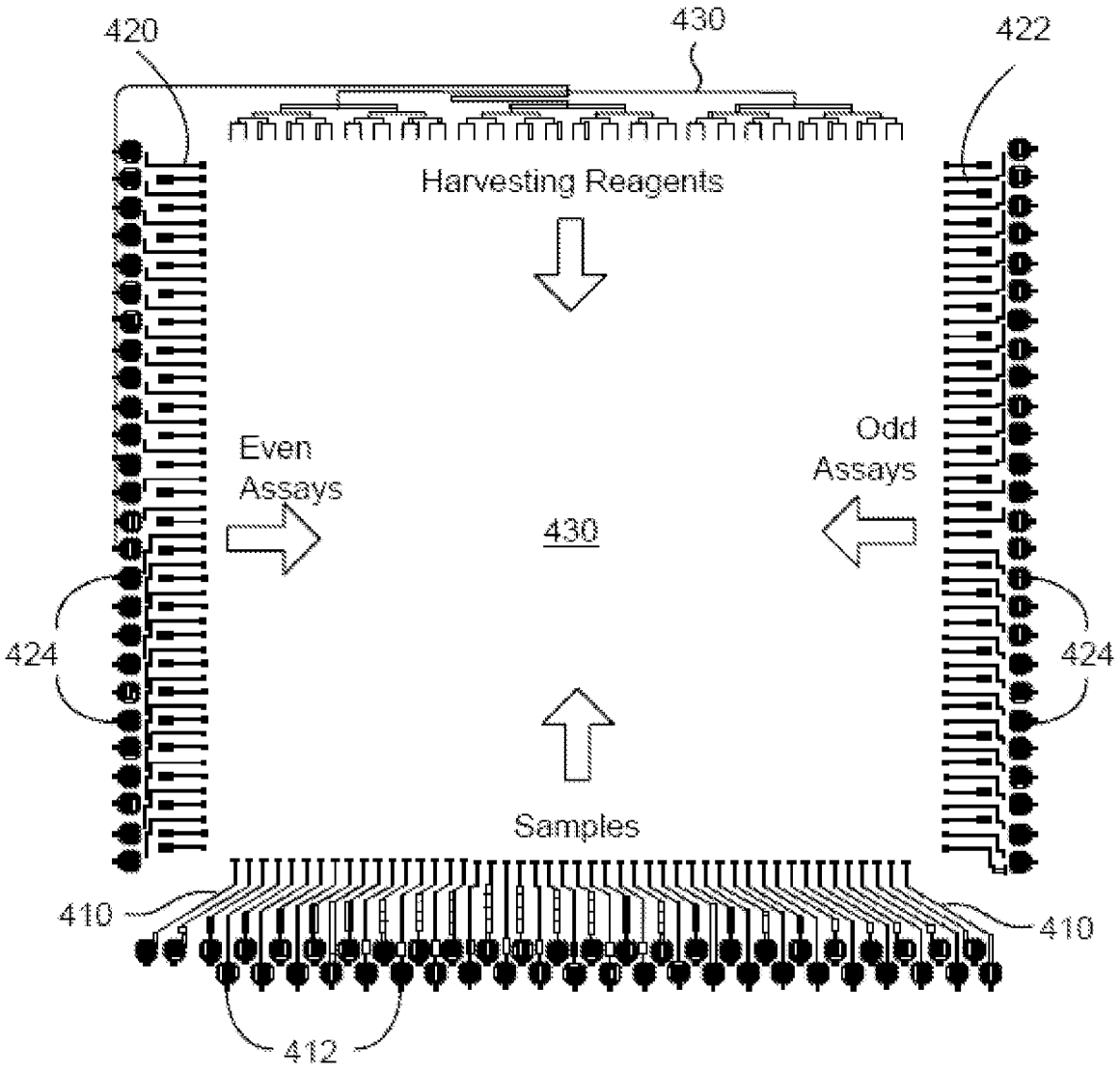

420
430
422
Harvesting Reagents
Even
Assays
Odd
Assays
430
424
424
Samples
410
410
412

Fig. 5B

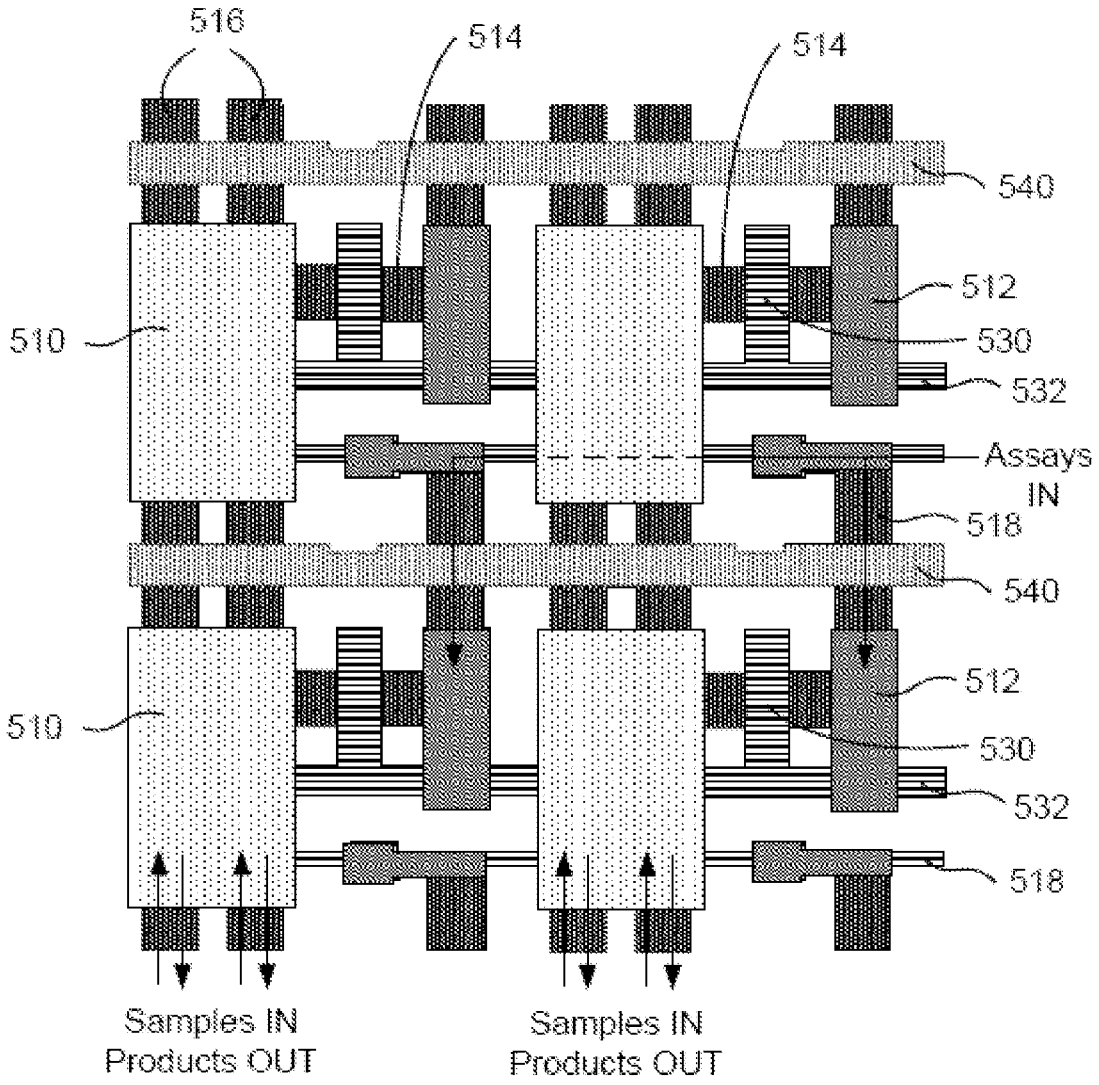

516
514
514
540
512
510
530
532
Assays IN
518
540
512
510
530
532
518
Samples IN
Products OUT
Samples IN
Products OUT

Fig. 6

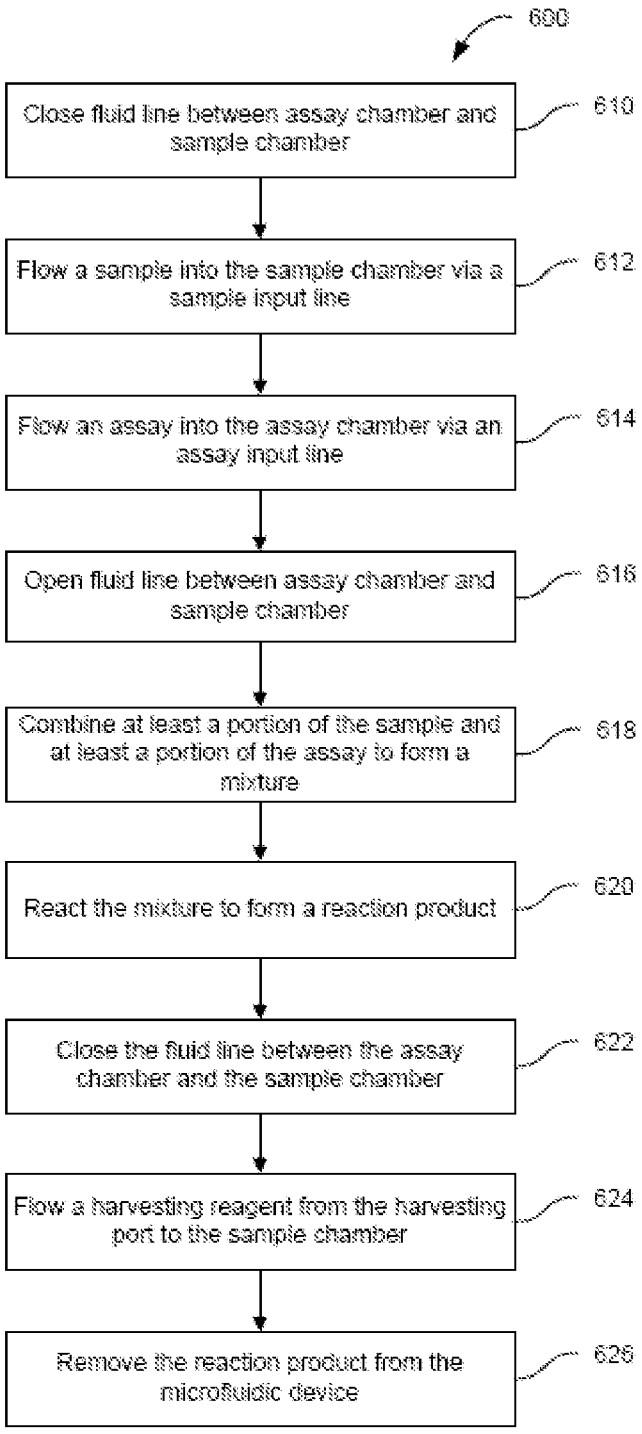

600
Close fluid line between assay chamber and sample chamber
610
Flow a sample into the sample chamber via a sample input line
612
Flow an assay into the assay chamber via an assay input line
614
Open fluid line between assay chamber and sample chamber
616
Combine at least a portion of the sample and at least a portion of the assay to form a mixture
618
React the mixture to form a reaction product
620
Close the fluid line between the assay chamber and the sample chamber
622
Flow a harvesting reagent from the harvesting port to the sample chamber
624
Remove the reaction product from the microfluidic device
626

Fig. 7

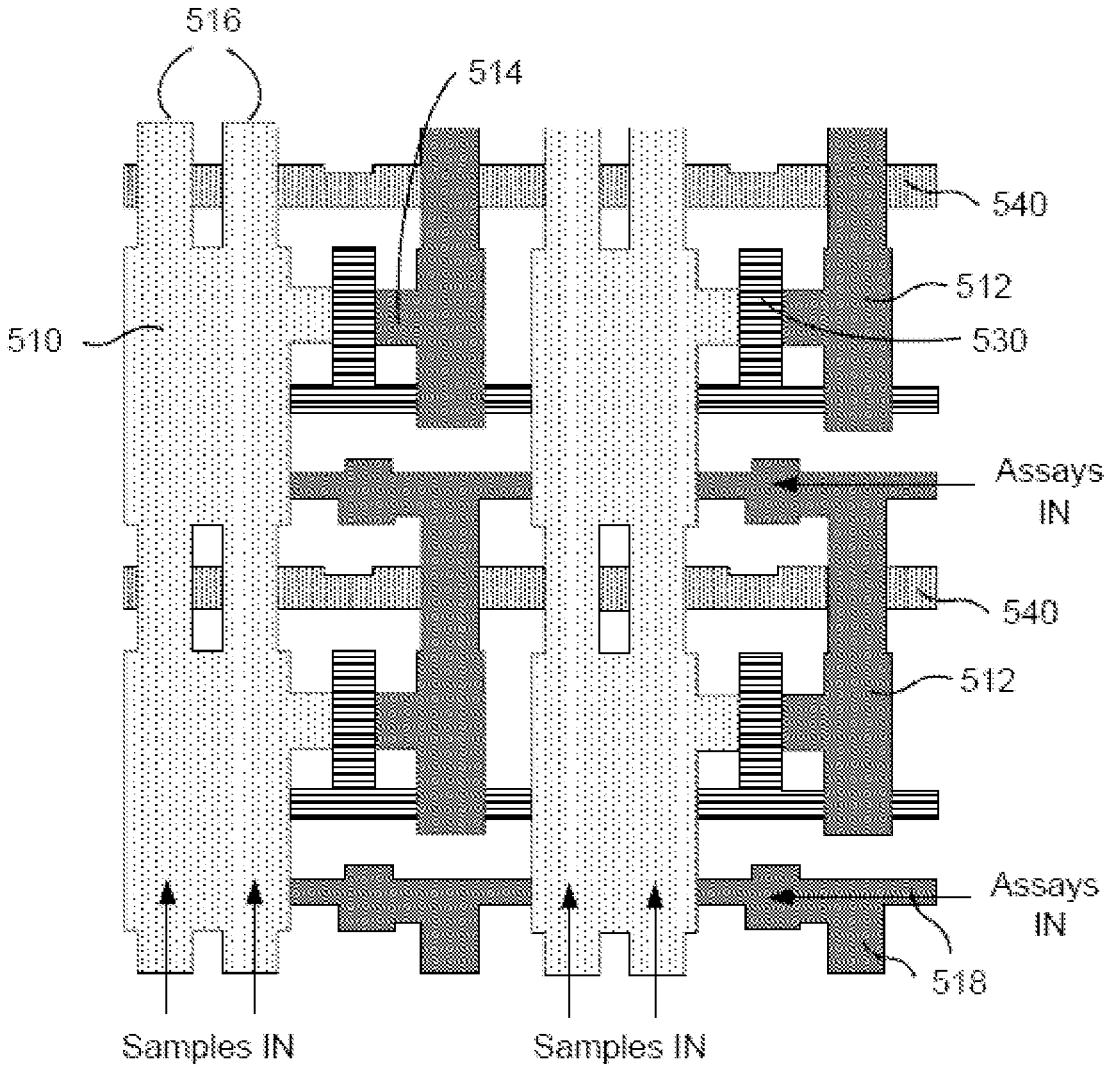

516
514
540
512
510
530
Assays IN
540
512
Assays IN
518
Samples IN
Samples IN

Fig. 8A

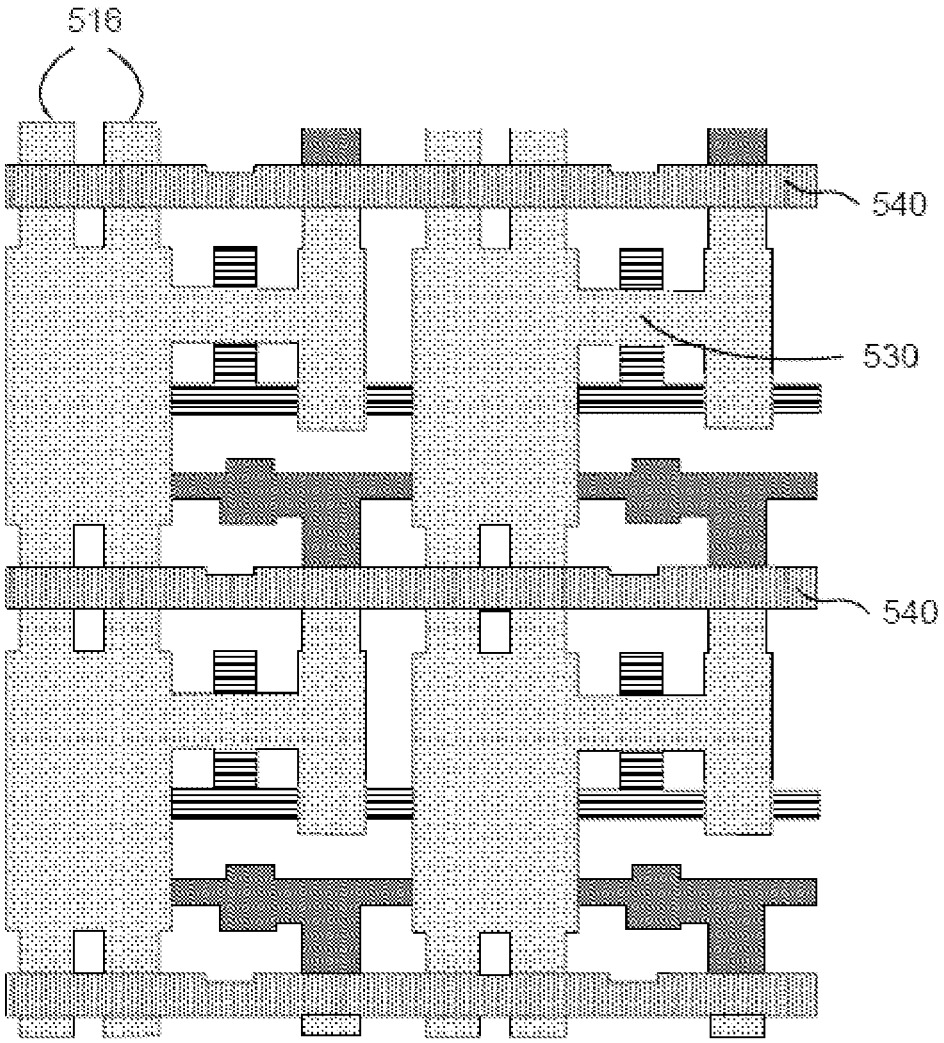


Fig. 8B

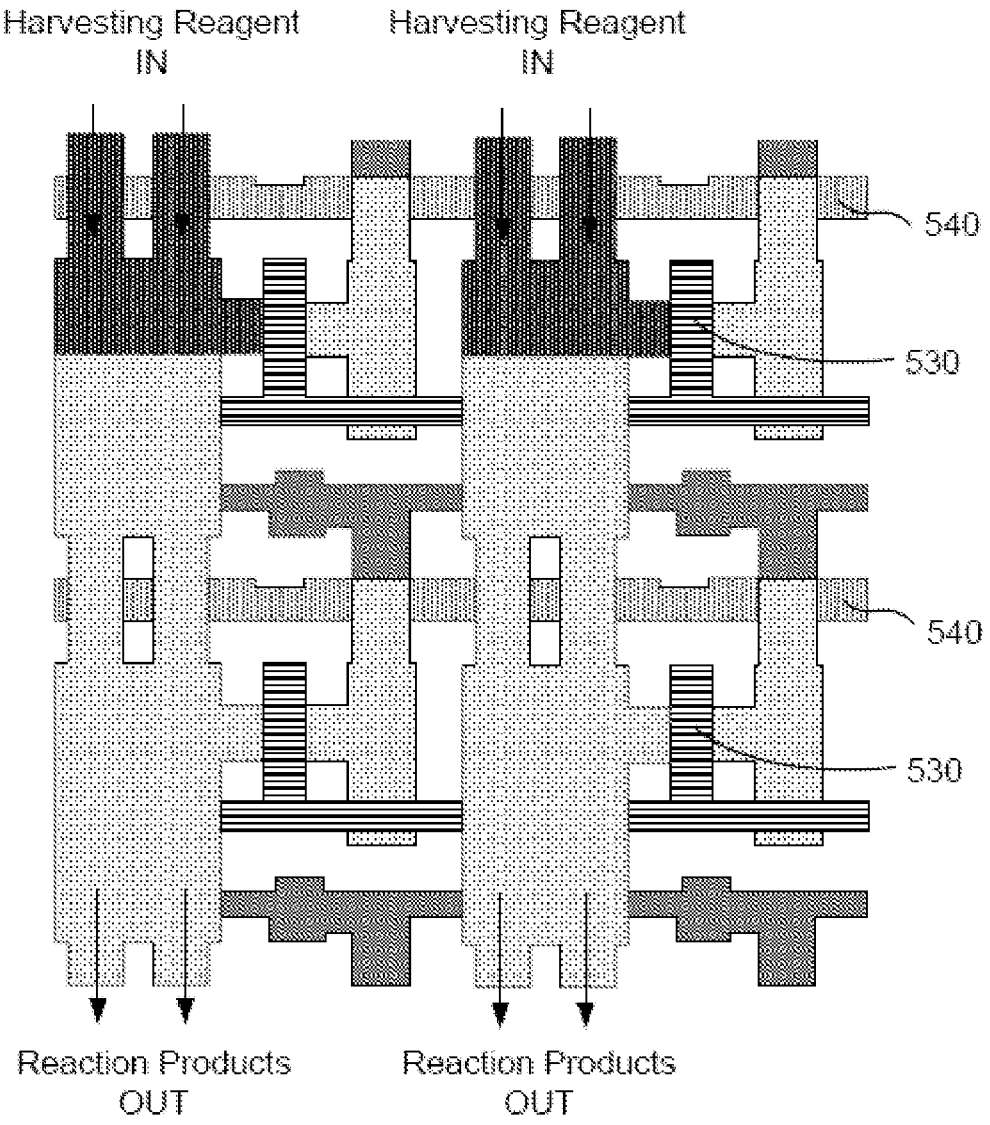

Harvesting Reagent IN
Harvesting Reagent IN
540
530
540
530
Reaction Products OUT
Reaction Products OUT

Fig. 8C

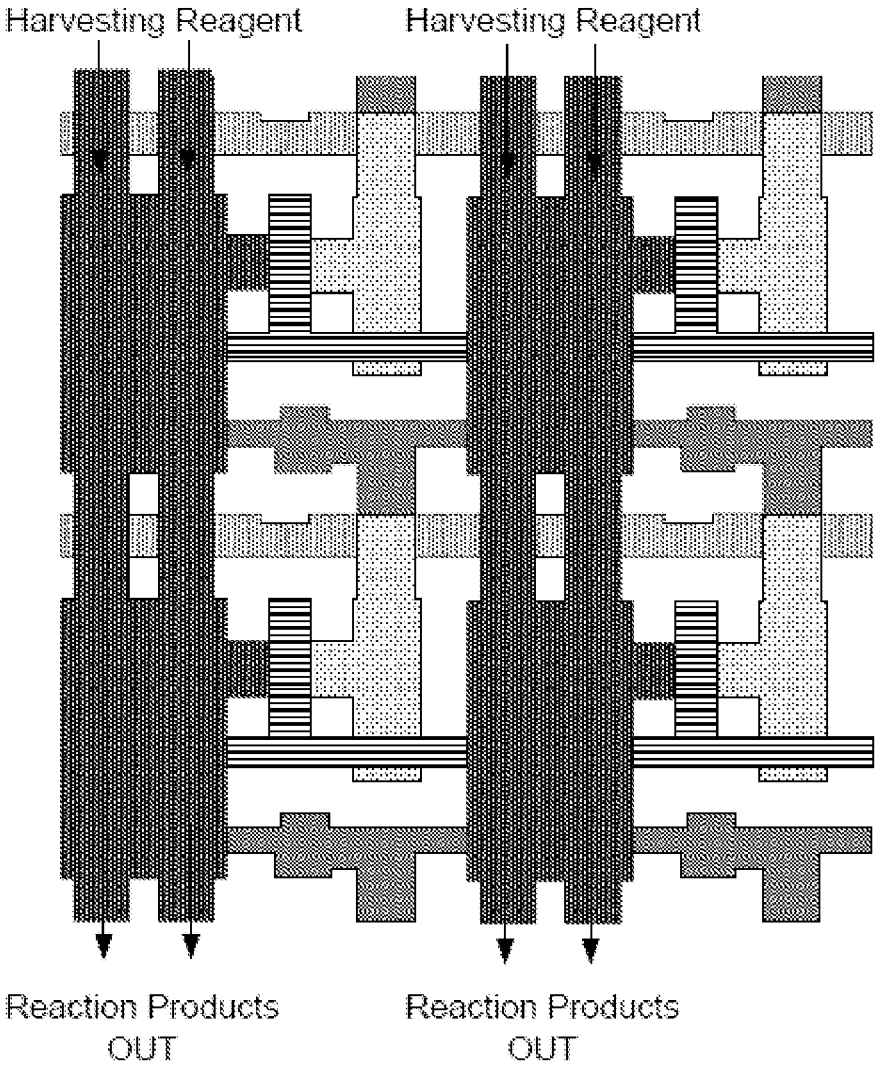

Harvesting Reagent
Harvesting Reagent
Reaction Products OUT
Reaction Products OUT

Fig. 8D

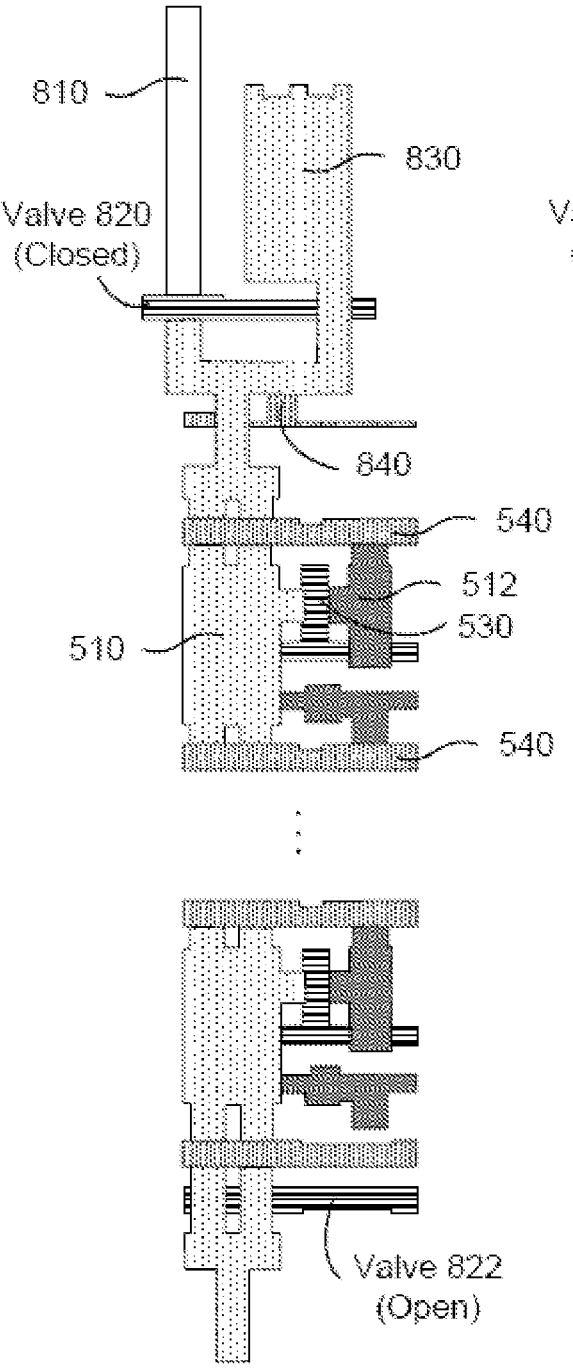

810
830
Valve 820
(Closed)
840
540
512
530
510
540
Valve 822
(Open)

Fig. 9A

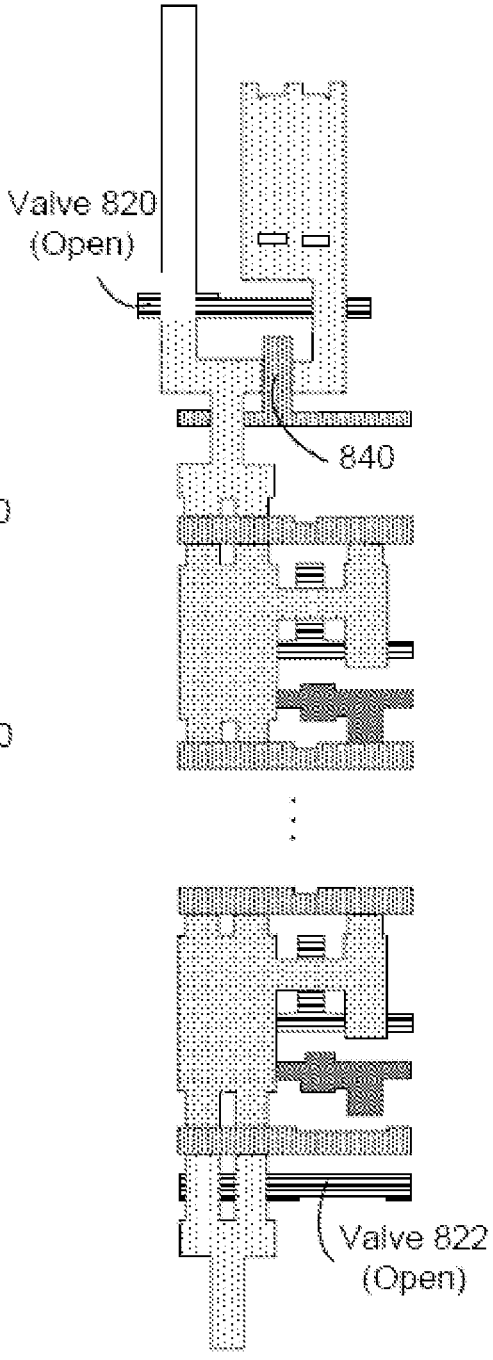

Valve 820
(Open)
840
Valve 822
(Open)

Fig. 9B

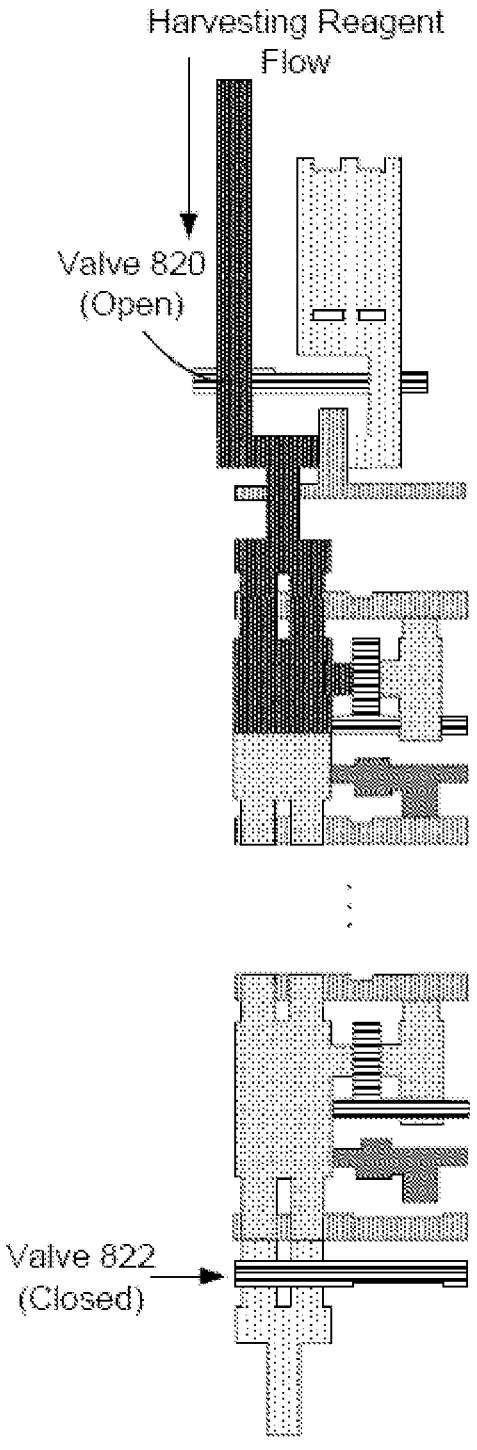

Harvesting Reagent Flow
Valve 820 (Open)
Valve 822 (Closed)

Fig. 9C

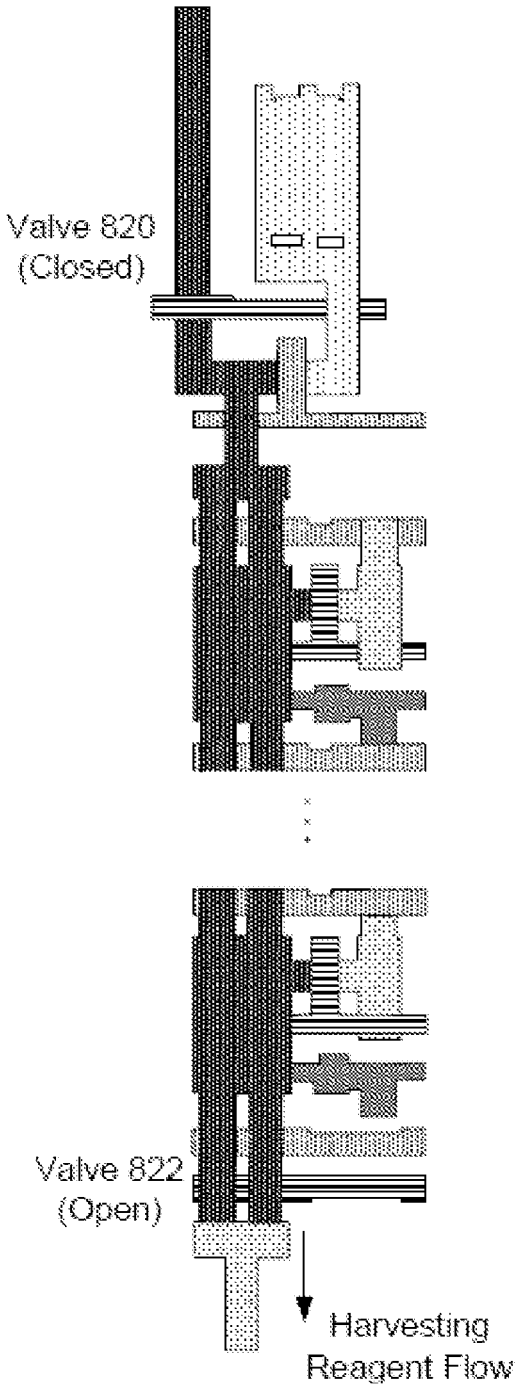

Valve 820 (Closed)
Valve 822 (Open)
Harvesting Reagent Flow

Fig. 9D

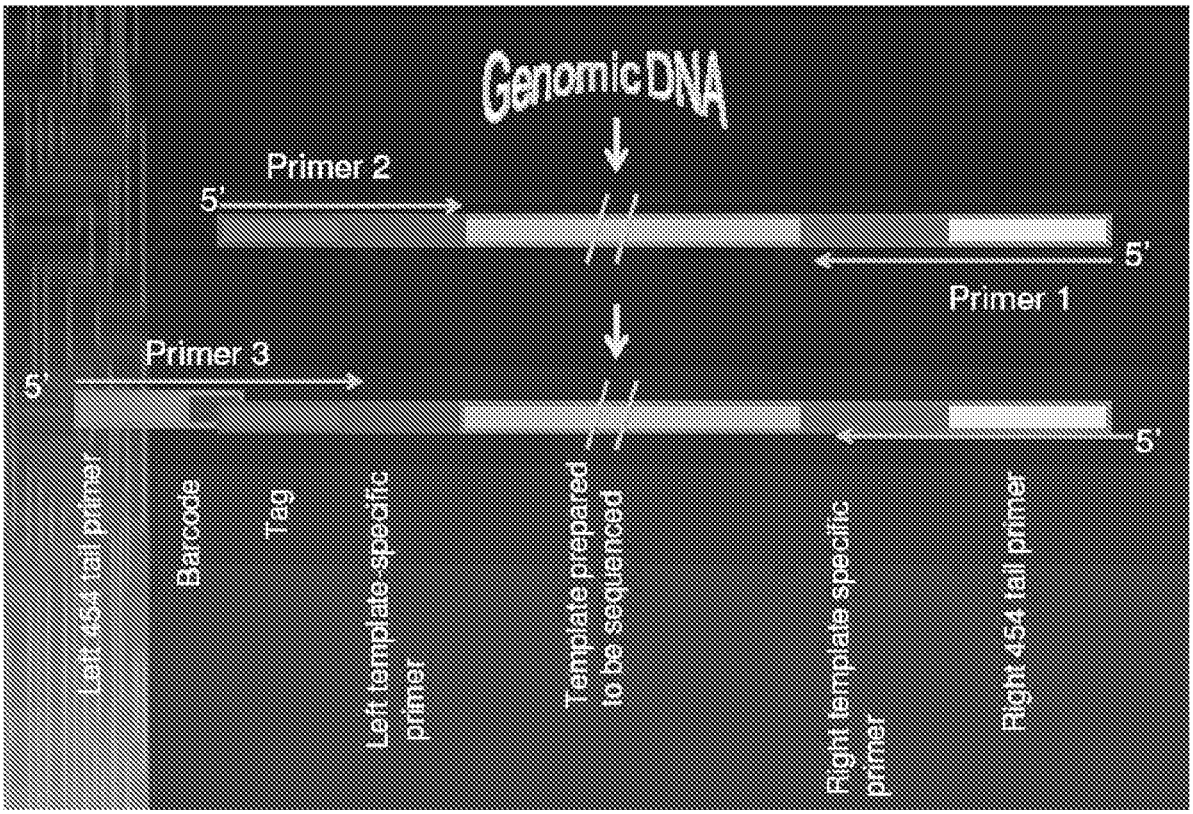

Genomic DNA
Primer 2
5'
5'
Primer 1
Primer 3
5'
5'
Left 454 tail primer
Barcode
Tag
Left template specific primer
Template prepared to be sequenced
Right template specific primer
Right 454 tail primer

Fig. 10

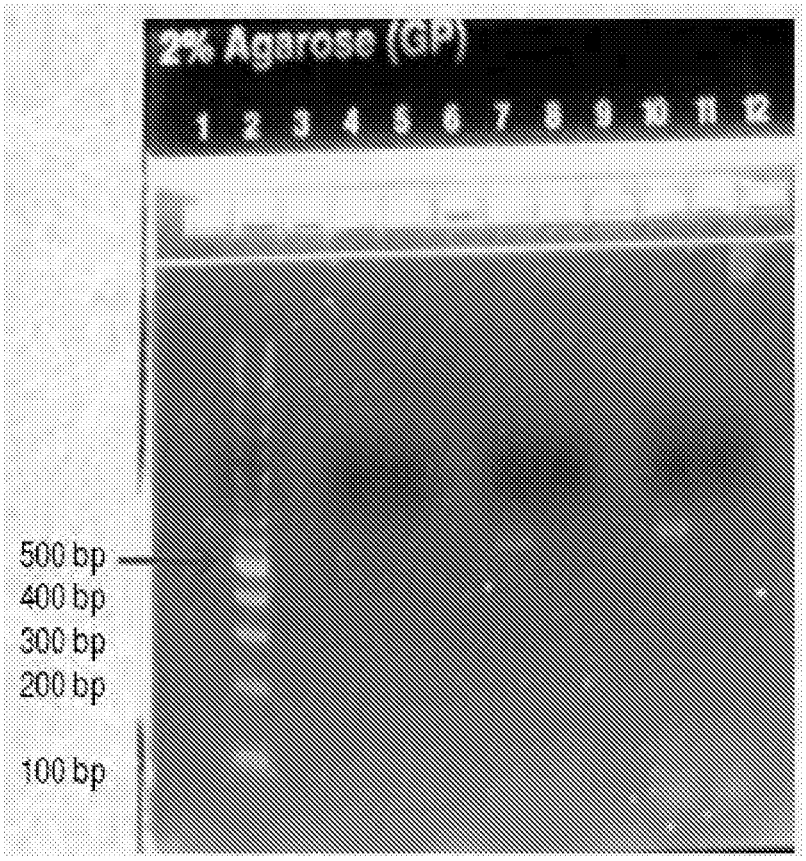

2% Agarose (dP)
1 2 3 4 5 6 7 8 9 10 11 12
500 bp
400 bp
300 bp
200 bp
100 bp

Fig. 11

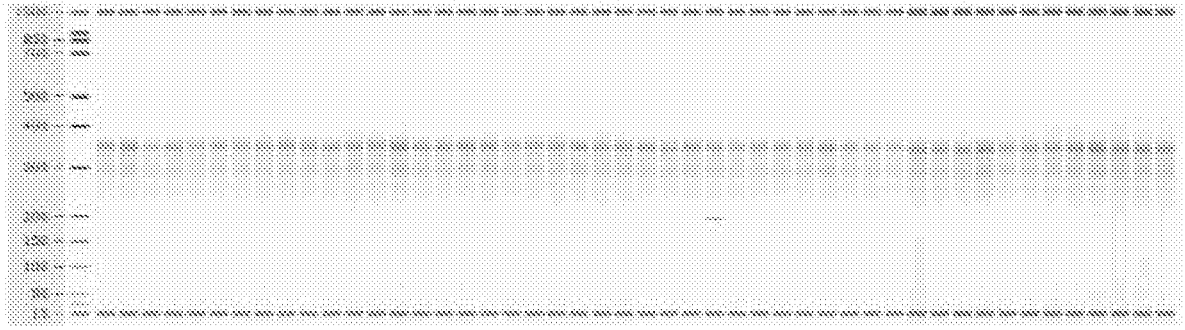
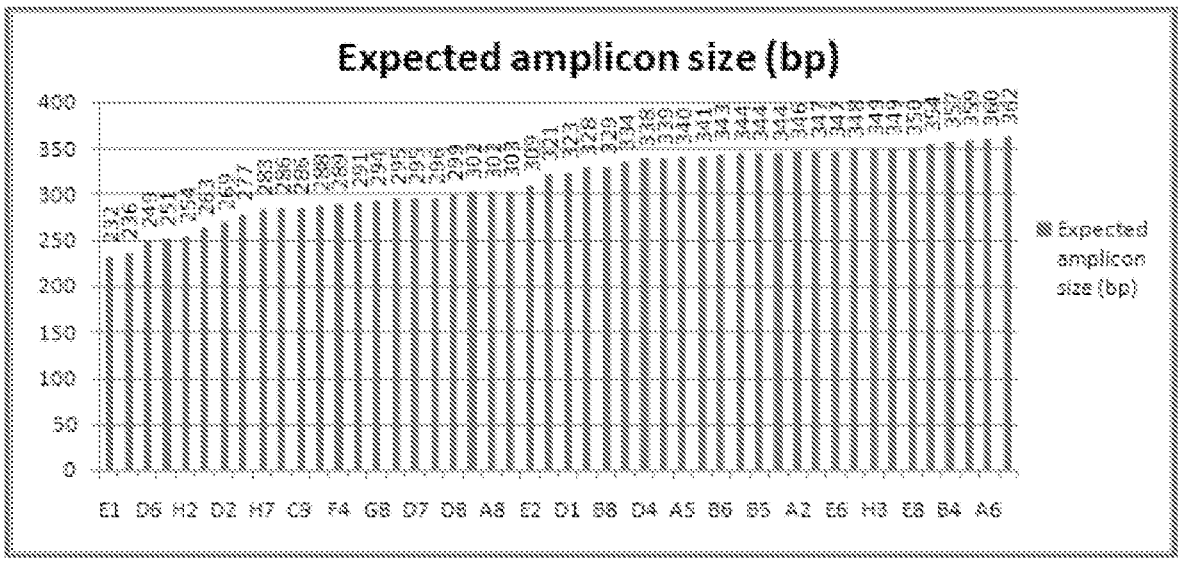


Fig. 12

A


Expected amplicon size (bp)
400
350
300
250
200
150
100
50
0
E1 D6 H2 D2 H7 C9 F4 G8 D7 D8 A8 E2 D1 B8 D4 A5 B6 B5 A2 E6 H3 E8 B4 A6
Expected amplicon size (bp)

B

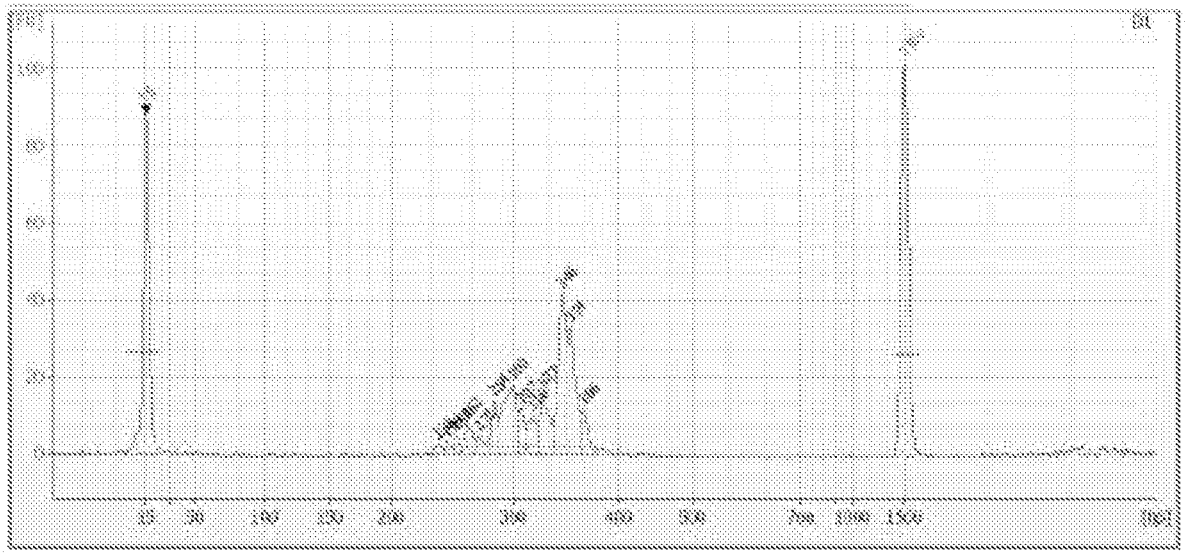
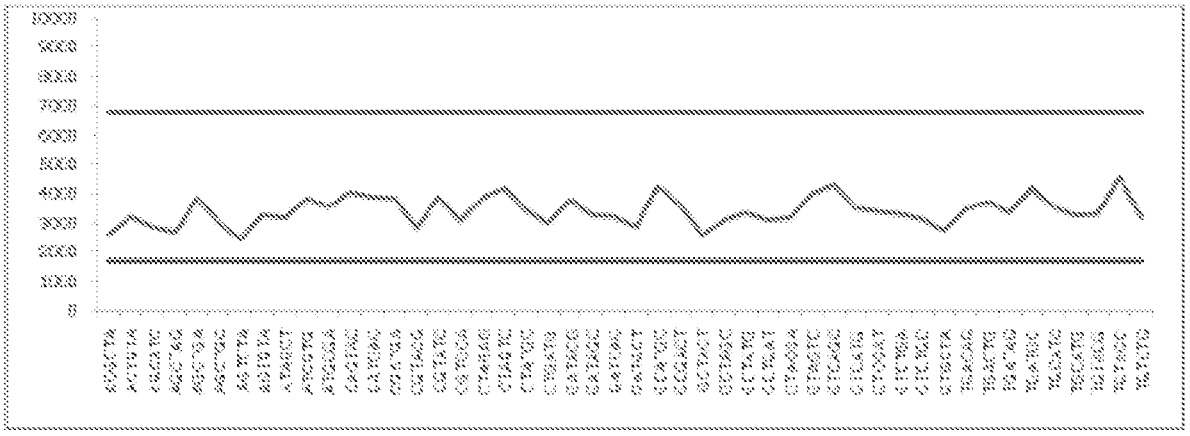


Fig. 13A-B

A



B

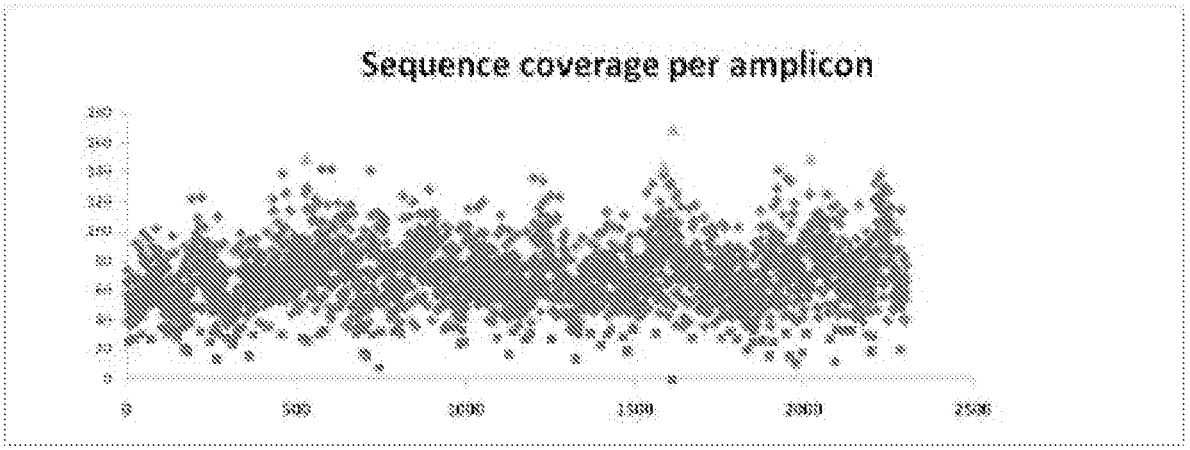


Fig. 14A-B

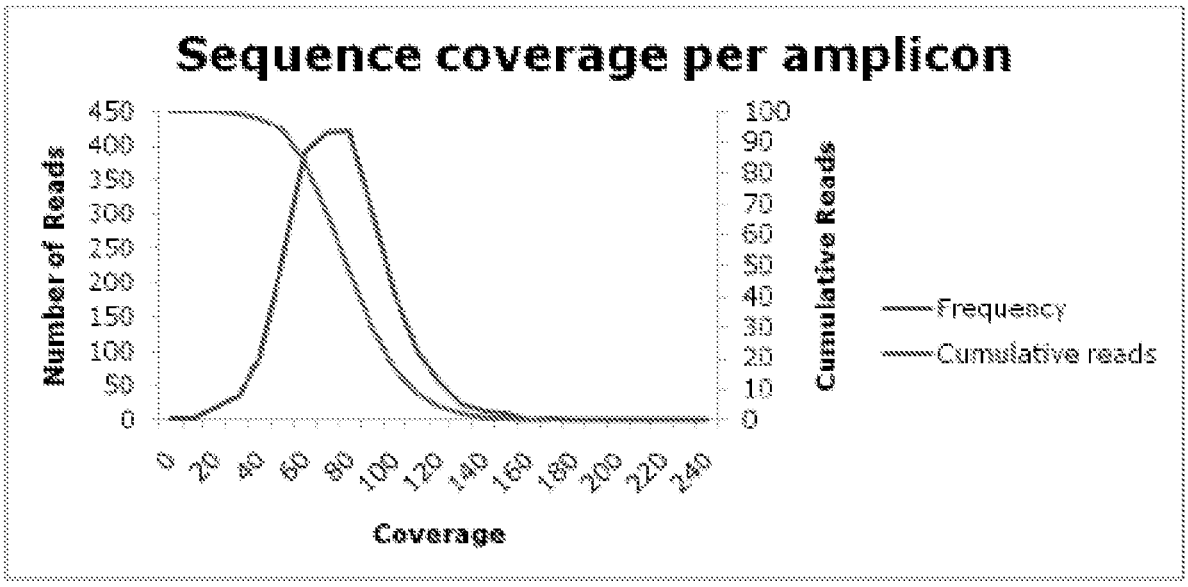
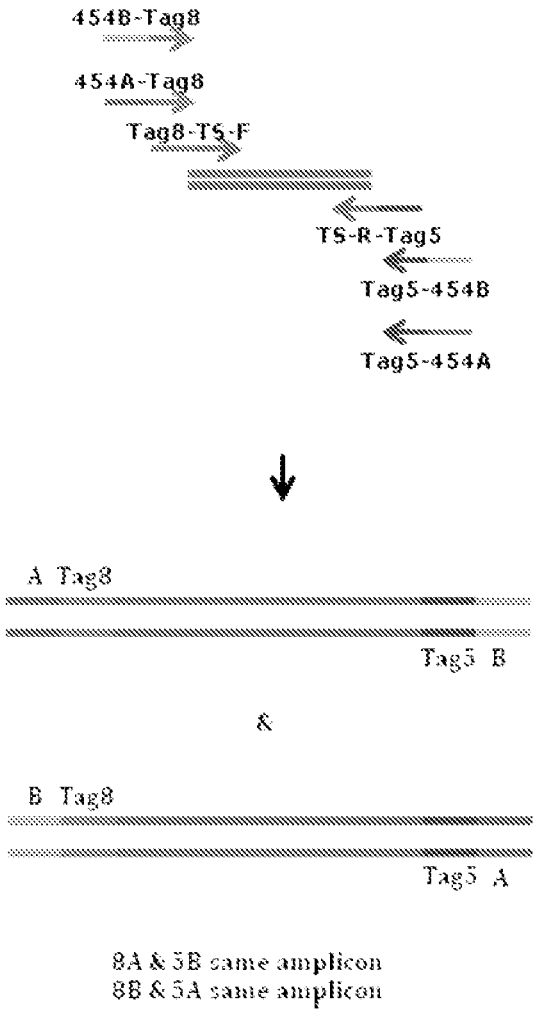

Sequence coverage per amplicon
Number of Reads
Cumulative Reads
Coverage
Frequency
Cumulative reads
450
400
350
300
250
200
150
100
50
0
100
90
80
70
60
50
40
30
20
10
0
0
20
40
60
80
100
120
140
160
180
200
220
240

Fig. 14C

A


454B-Tag8
454A-Tag8
Tag8-TS-F
TS-R-Tag5
Tag5-454B
Tag5-454A
A Tag8
Tag5 B
&
B Tag8
Tag5 A
8A & 5B same amplicon
8B & 5A same amplicon

B

Fig. 15A-B

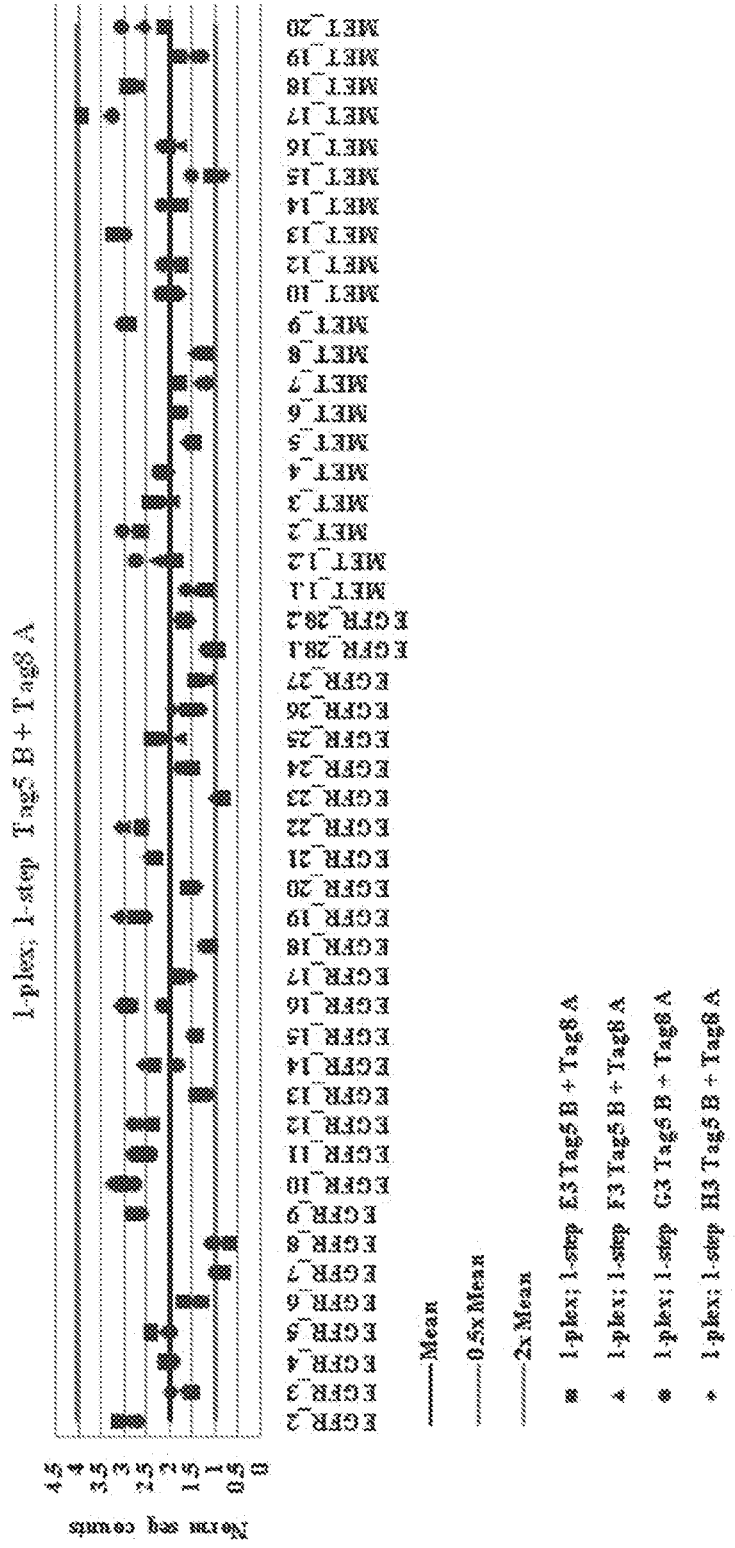

1-plex; 1-step Tag5 B + Tag8 A
Norm eq counts
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
EGFR_2
EGFR_3
EGFR_4
EGFR_5
EGFR_6
EGFR_7
EGFR_8
EGFR_9
EGFR_10
EGFR_11
EGFR_12
EGFR_13
EGFR_14
EGFR_15
EGFR_16
EGFR_17
EGFR_18
EGFR_19
EGFR_20
EGFR_21
EGFR_22
EGFR_23
EGFR_24
EGFR_25
EGFR_26
EGFR_27
EGFR_28.1
EGFR_28.2
MET_1.1
MET_1.2
MET_2
MET_3
MET_4
MET_5
MET_6
MET_7
MET_8
MET_9
MET_10
MET_12
MET_13
MET_14
MET_15
MET_16
MET_17
MET_18
MET_19
MET_20
Mean
0.5x Mean
2x Mean
1-plex; 1-step E3 Tag5 B + Tag8 A
1-plex; 1-step F3 Tag5 B + Tag8 A
1-plex; 1-step G3 Tag5 B + Tag8 A
1-plex; 1-step H3 Tag5 B + Tag8 A

Fig. 16A

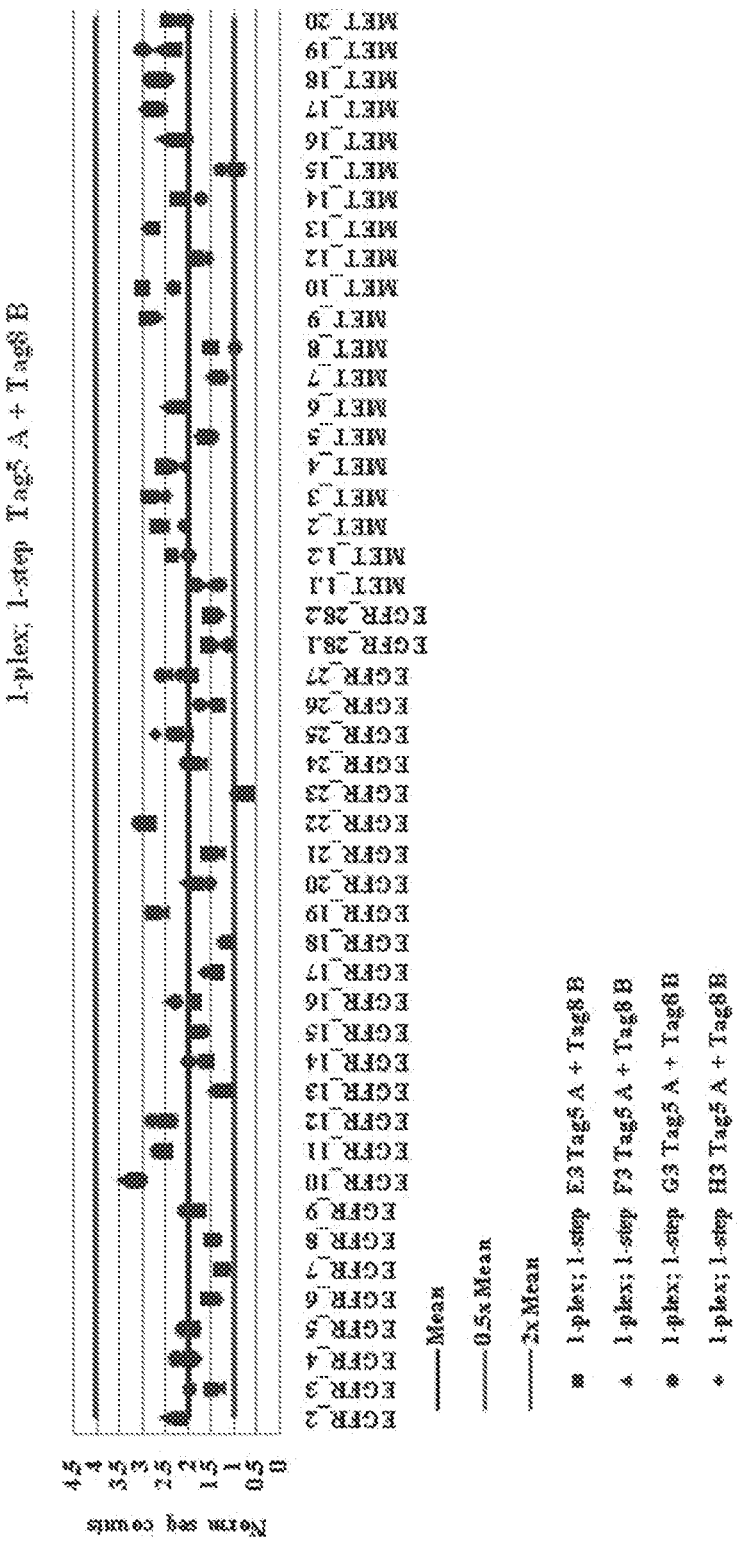

1-plex; 1-step Tag5 A + Tag6 B
Norm seq counts
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
EGFR_2
EGFR_3
EGFR_4
EGFR_5
EGFR_6
EGFR_7
EGFR_8
EGFR_9
EGFR_10
EGFR_11
EGFR_12
EGFR_13
EGFR_14
EGFR_15
EGFR_16
EGFR_17
EGFR_18
EGFR_19
EGFR_20
EGFR_21
EGFR_22
EGFR_23
EGFR_24
EGFR_25
EGFR_26
EGFR_27
EGFR_28.1
EGFR_28.2
MET_1.1
MET_1.2
MET_2
MET_3
MET_4
MET_5
MET_6
MET_7
MET_8
MET_9
MET_10
MET_12
MET_13
MET_14
MET_15
MET_16
MET_17
MET_18
MET_19
MET_20
Mean
0.5x Mean
2x Mean
1-plex; 1-step E3 Tag5 A + Tag6 B
1-plex; 1-step F3 Tag5 A + Tag6 B
1-plex; 1-step G3 Tag5 A + Tag6 B
1-plex; 1-step H3 Tag5 A + Tag6 B

Fig. 16B

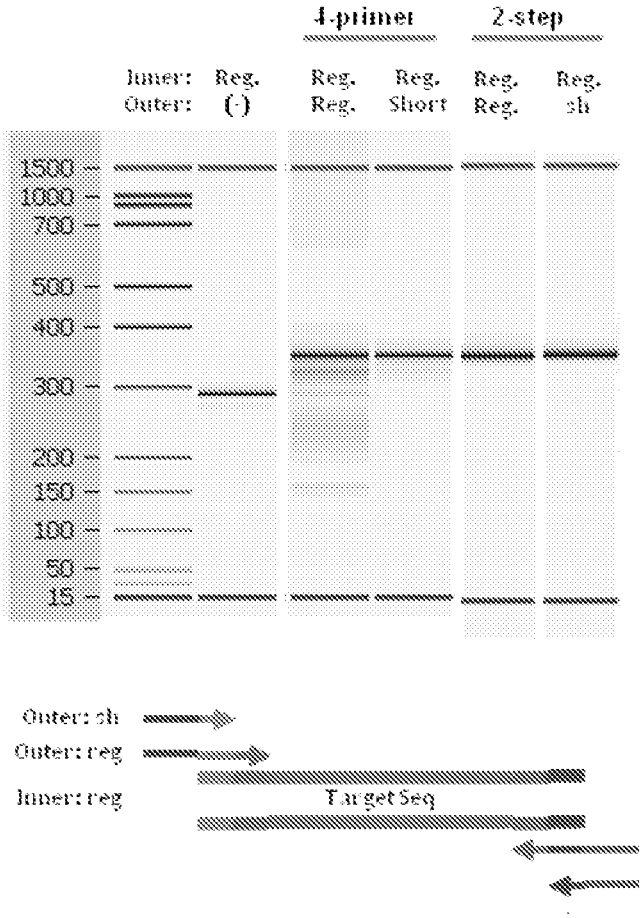

4-primer
2-step
Inner: Reg.
Outer: (-)
Reg. Reg.
Reg. Short
Reg. Reg.
Reg. sh
1500
1000
700
500
400
300
200
150
100
50
15
Outer: sh
Outer: reg
Inner: reg
Target Seq

Fig. 17

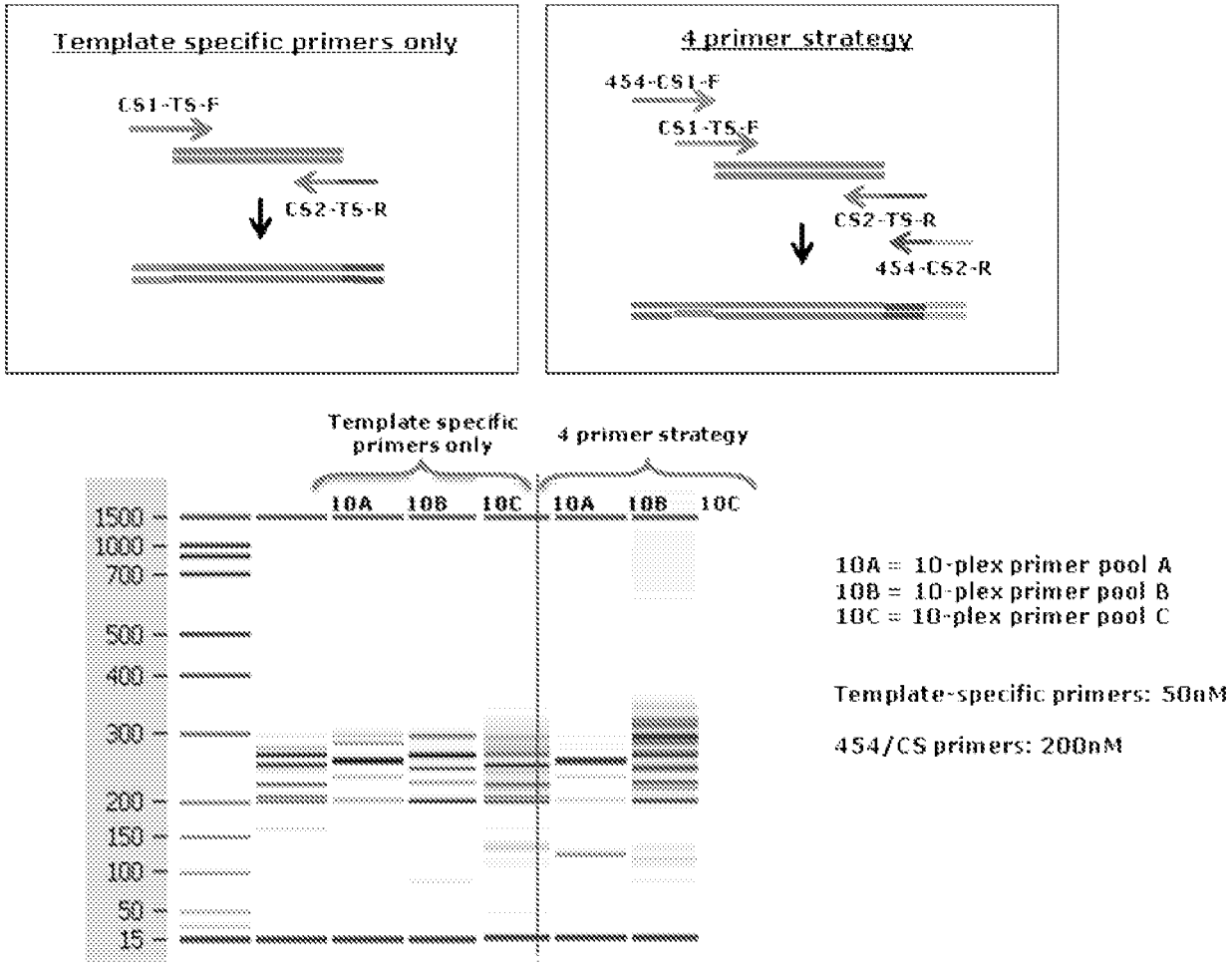

Template specific primers only
CS1-TS-F
CS2-TS-R
4 primer strategy
454-CS1-F
CS1-TS-F
CS2-TS-R
454-CS2-R
Template specific primers only
4 primer strategy
10A
10B
10C
10A
10B
10C
1500
1000
700
500
400
300
200
150
100
50
15
10A = 10-plex primer pool A
10B = 10-plex primer pool B
10C = 10-plex primer pool C
Template-specific primers: 50nM
454/CS primers: 200nM

Fig. 18

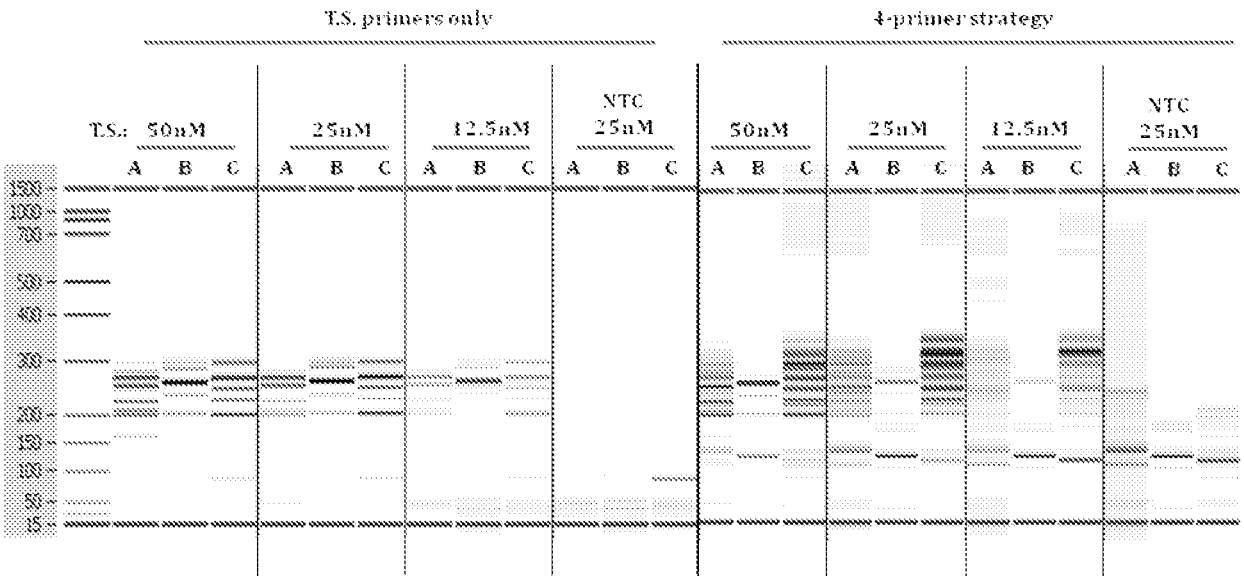

T.S. primers only
4-primer strategy
T.S.: 50nM
25nM
12.5nM
NTC 25nM
50nM
25nM
12.5nM
NTC 25nM
A B C
150
100
70
60
50
40
30
25
20
15
10
5

Fig. 19