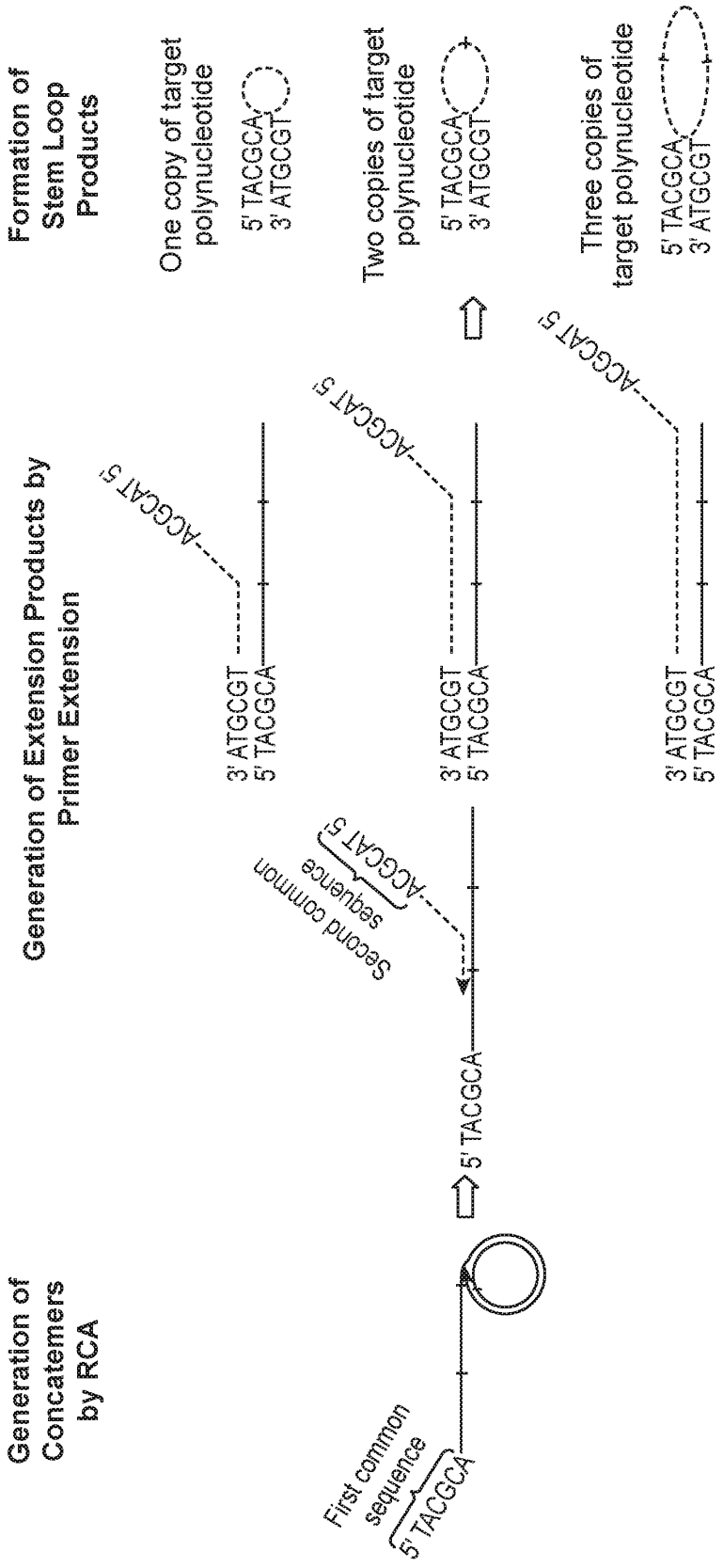

Generation of Concatemers by RCA
First common sequence
5' TACGCA
Generation of Extension Products by Primer Extension
Second common sequence
ACGCAT 5'
3' ATGCGT
5' TACGCA
Formation of Stem Loop Products
One copy of target polynucleotide
Two copies of target polynucleotide
Three copies of target polynucleotide

FIG. 1

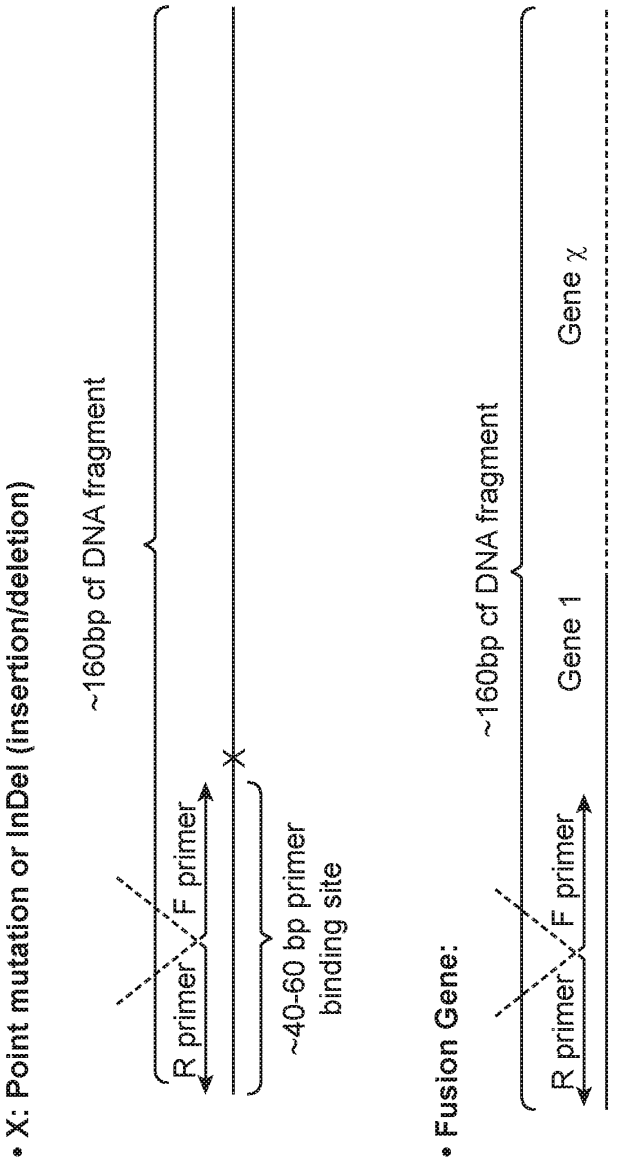

• X: Point mutation or InDel (insertion/deletion)
~160bp cf DNA fragment
R primer
F primer
~40-60 bp primer binding site
• Fusion Gene:
~160bp cf DNA fragment
Gene 1
Gene χ
R primer
F primer
~40-60 bp primer binding site

FIG. 2

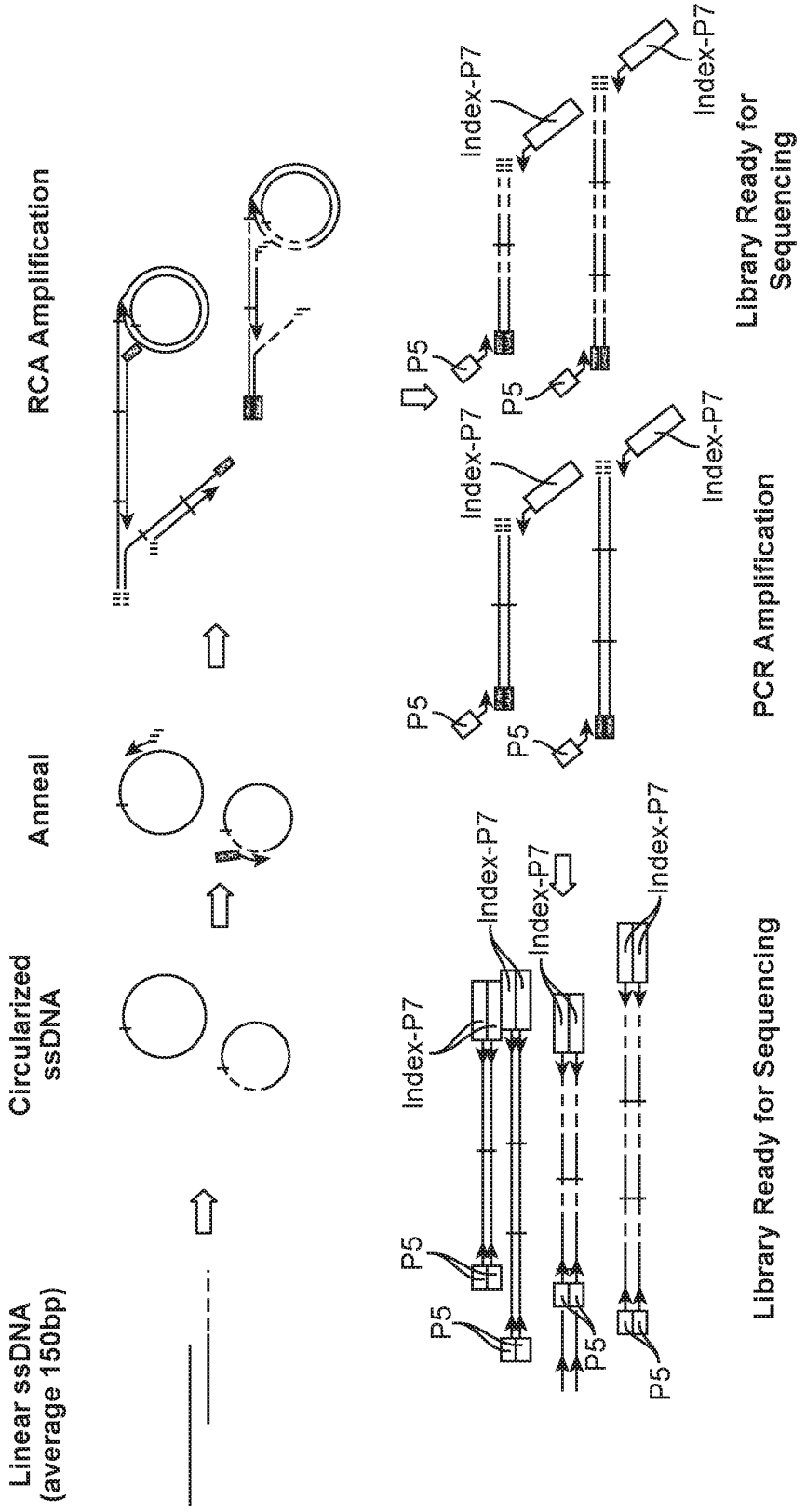

Linear ssDNA
(average 150bp)
Circularized
ssDNA
Anneal
RCA Amplification
Index-P7
P5
Library Ready for Sequencing
PCR Amplification
Library Ready for Sequencing

FIG. 3

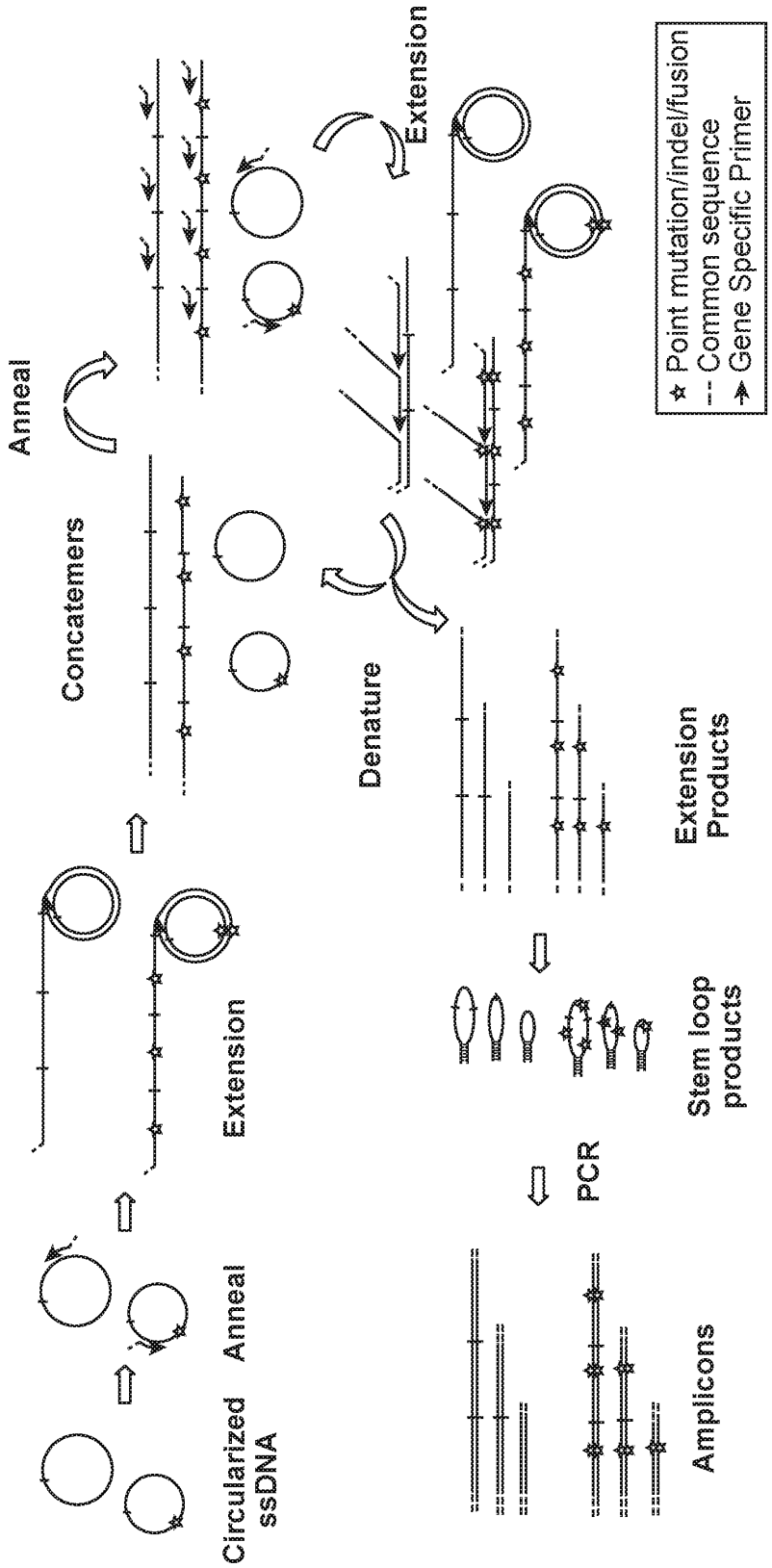

Circularized ssDNA
Anneal
Extension
PCR
Amplicons
Stem loop products
Denature
Extension Products
Concatemers
Anneal
Extension
Point mutation/indel/fusion
Common sequence
Gene Specific Primer

FIG. 4

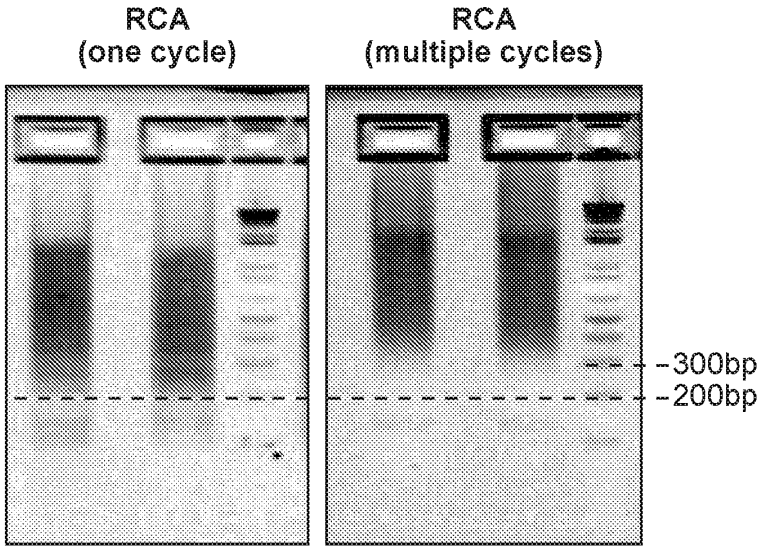


FIG. 5

Mass ratio	RCA (one cycle)	RCA (multiple cycles)
2 repeats:1 repeat	3.59	33.46
3 repeats:1 repeat	4.97	40.68

FIG. 6

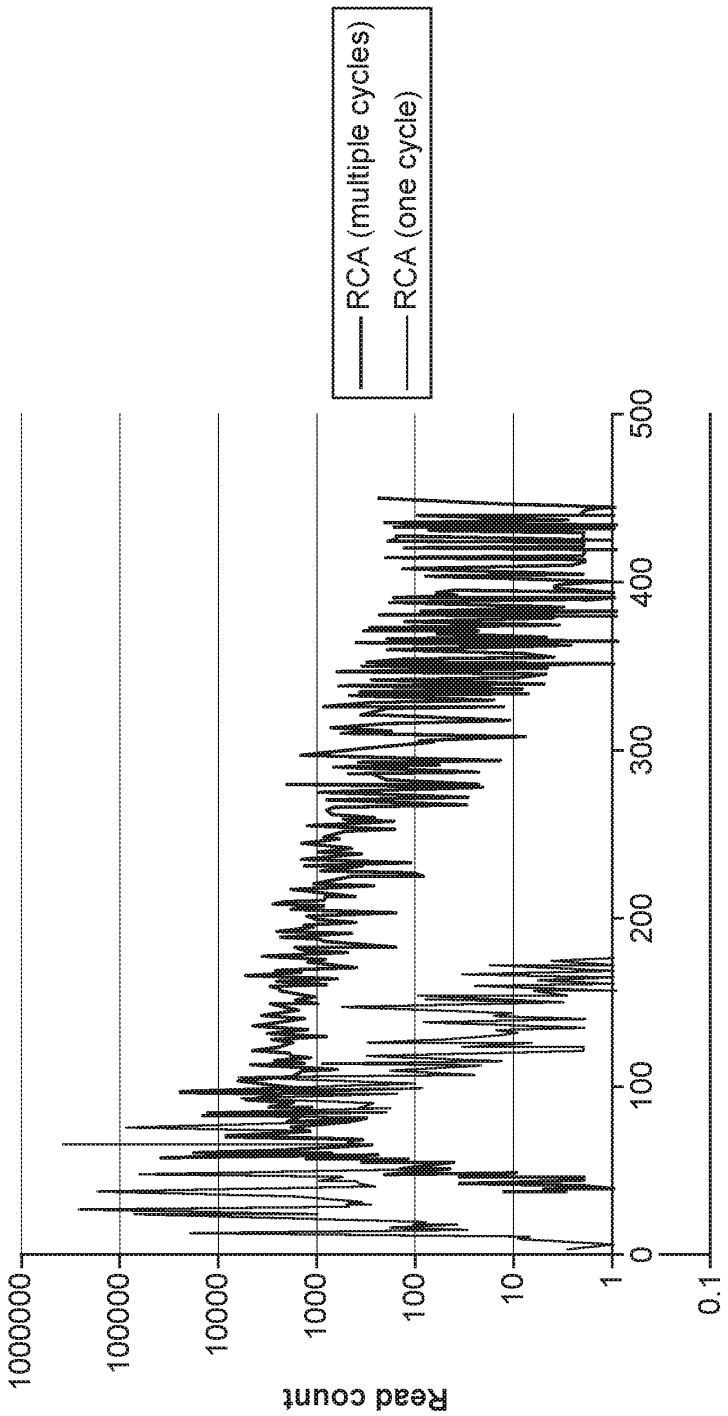

RCA (multiple cycles)
RCA (one cycle)
Read count
1000000
100000
10000
1000
100
10
1
0.1
0
100
200
300
400
500
Fragment Size

FIG. 7

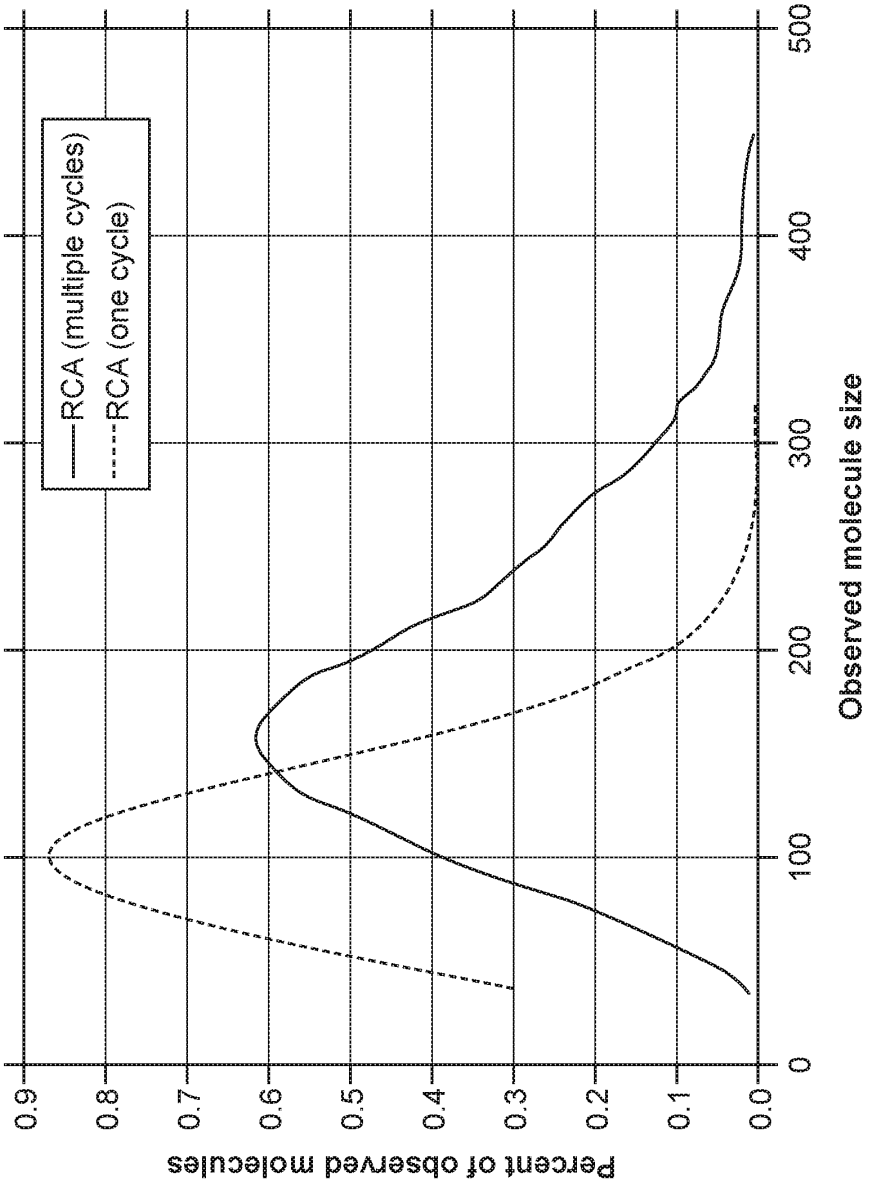

RCA (multiple cycles)
RCA (one cycle)
Percent of observed molecules
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
0
100
200
300
400
500
Observed molecule size

FIG. 8

Spike-in fusion allele frequency	2.50%	0.50%	0.05%	0.00%
ng* of Fusion DNA (HD664[a] with 50% fusion allele)	0.50	0.12	0.01	0.00
ng* of Control DNA (wild type)	9.50	9.88	9.99	10.00

*ng of circularized DNA

[a] HD664 refers to a 50% EML4/ALK DNA Reference Standard

FIG. 9

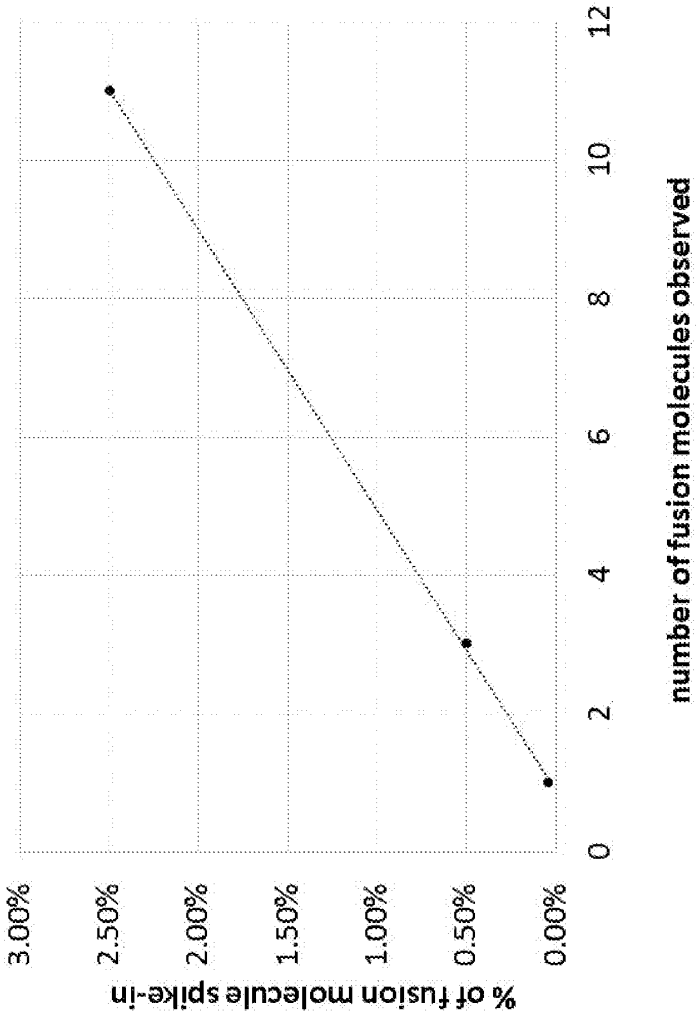
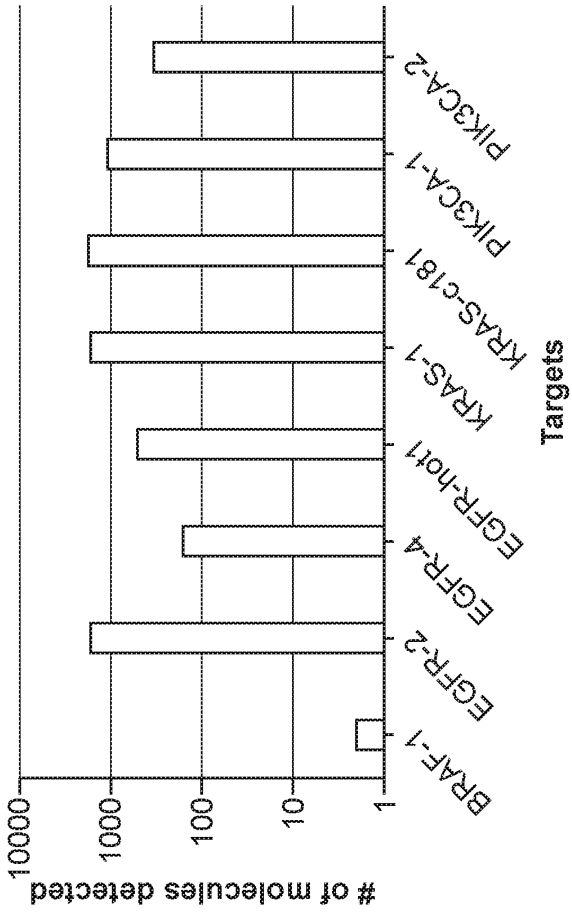

% of fusion molecule spike-in
3.00%
2.50%
2.00%
1.50%
1.00%
0.50%
0.00%
number of fusion molecules observed
0
2
4
6
8
10
12

FIG. 10



Target List
BRAF-1
EGFR-2
EGFR-4
EGFR-hot1
KRAS-1
KRAS-c181
PIK3CA-1
PIK3CA-2

FIG. 11