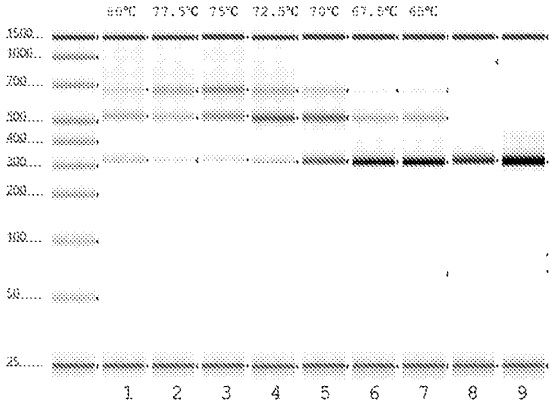
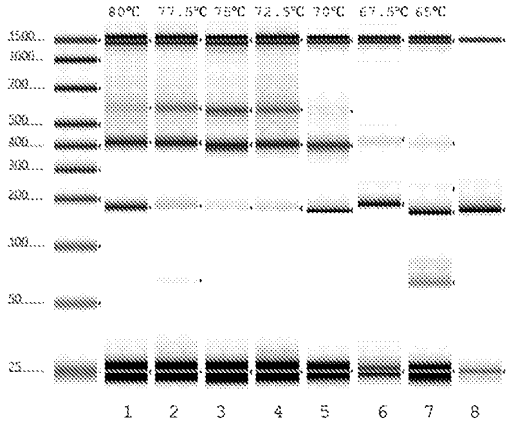
FIGURE 1

A.


80°C 77.5°C 75°C 72.5°C 70°C 67.5°C 65°C
1500
1000
700
500
400
300
200
100
50
25
1 2 3 4 5 6 7 8 9

B.


80°C 77.5°C 75°C 72.5°C 70°C 67.5°C 65°C
1500
1000
700
500
400
300
200
100
50
25
1 2 3 4 5 6 7 8

C.

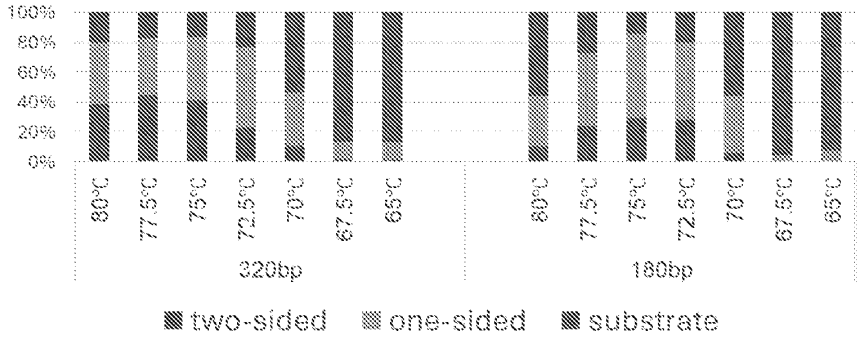
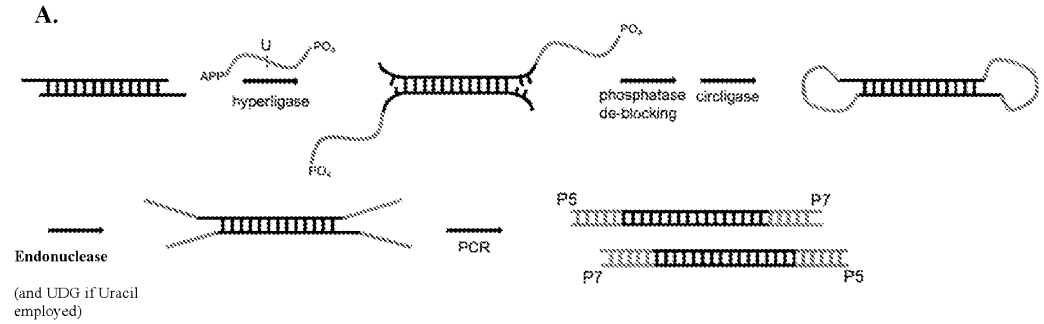
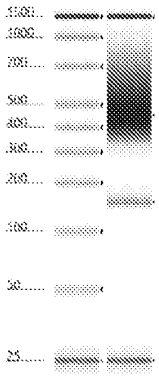

100%
80%
60%
40%
20%
0%
80°C
77.5°C
75°C
72.5°C
70°C
67.5°C
65°C
320bp
180bp
two-sided
one-sided
substrate

FIGURE 2


A.
U
APP
hyperligase
phosphatase
de-blocking
circligase
Endonuclease
(and UDG if Uracil
employed)
PCR
P5
P7
P7
P5


B.

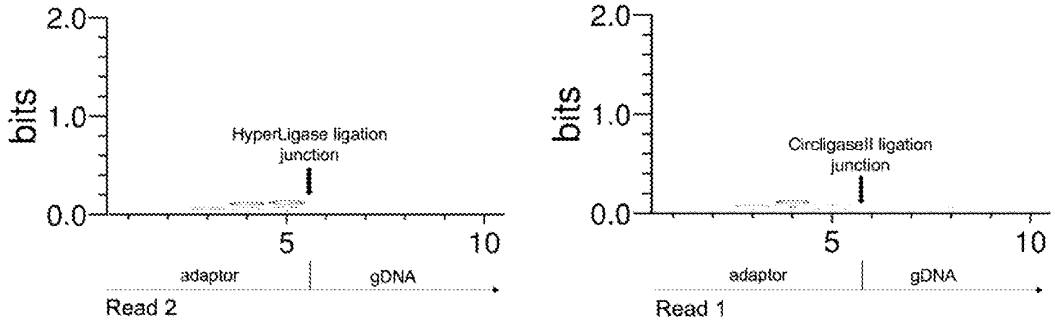
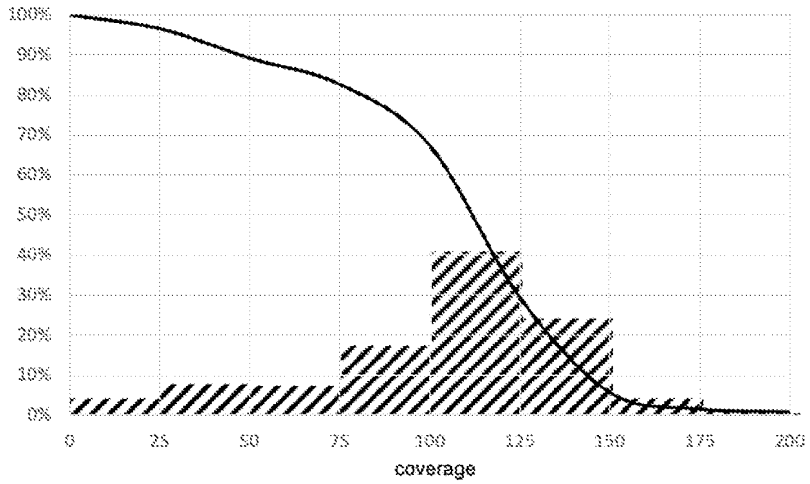

C.
2.0
1.0
0.0
bits
HyperLigase ligation
junction
5
10
adaptor
gDNA
Read 2
2.0
1.0
0.0
bits
CircligaseII ligation
junction
5
10
adaptor
gDNA
Read 1

FIGURE 2 (cont.)

D.



E.

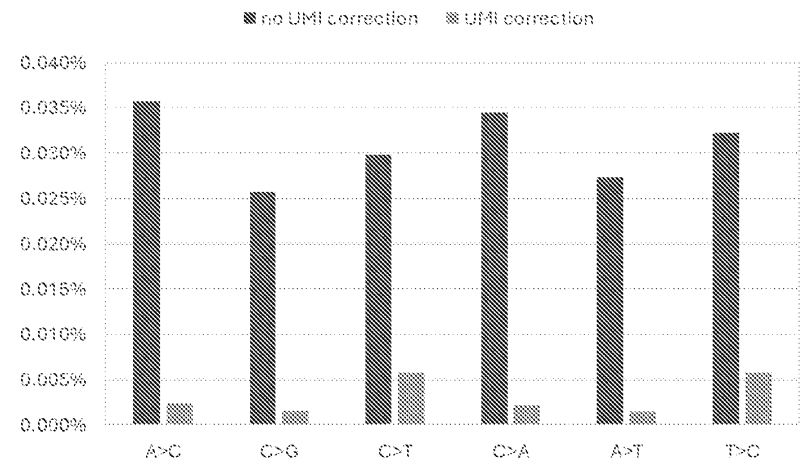


FIGURE 3

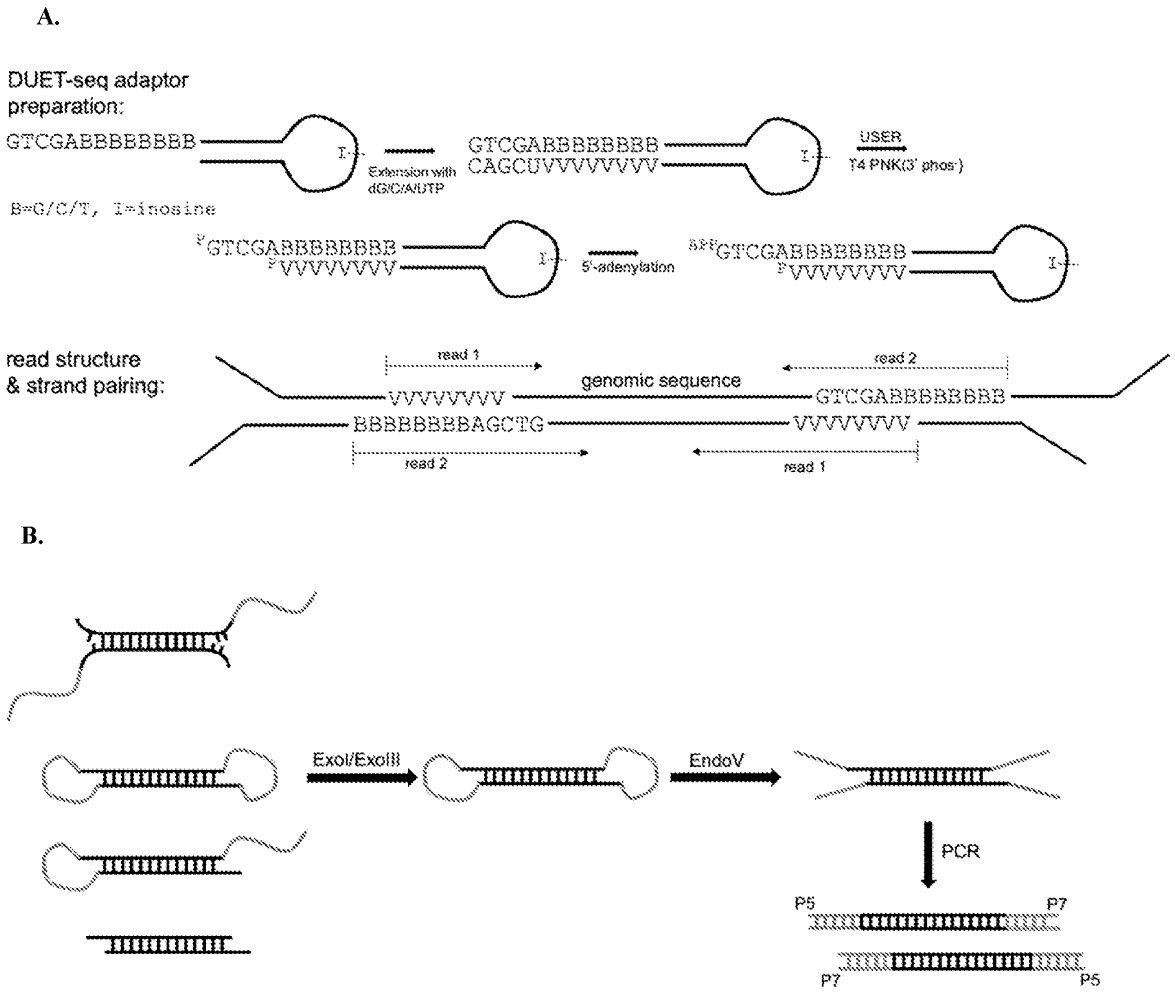
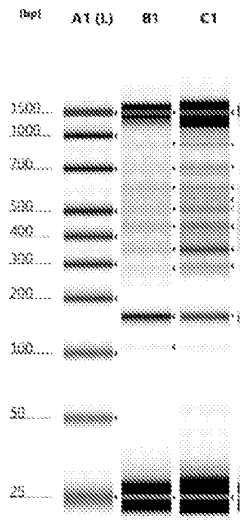

A.
DUET-seq adaptor preparation:
GTCGABBBBBBBB
Extension with dG/C/A/dTTP
GTCGABBBBBBBB
CAGCUVVVVVVVV
USER
T4 PNK(3' phos-)
B=G/C/T, I=inosine
5'-adenylation
read structure & strand pairing:
read 1
read 2
genomic sequence
VVVVVVVV
GTCGABBBBBBBB
BBBBBBBBAGCTG
VVVVVVVV
read 2
read 1
B.
ExoI/ExoIII
EndoV
PCR
P5
P7
P7
P5

FIGURE 3 (cont.)

C.



D.

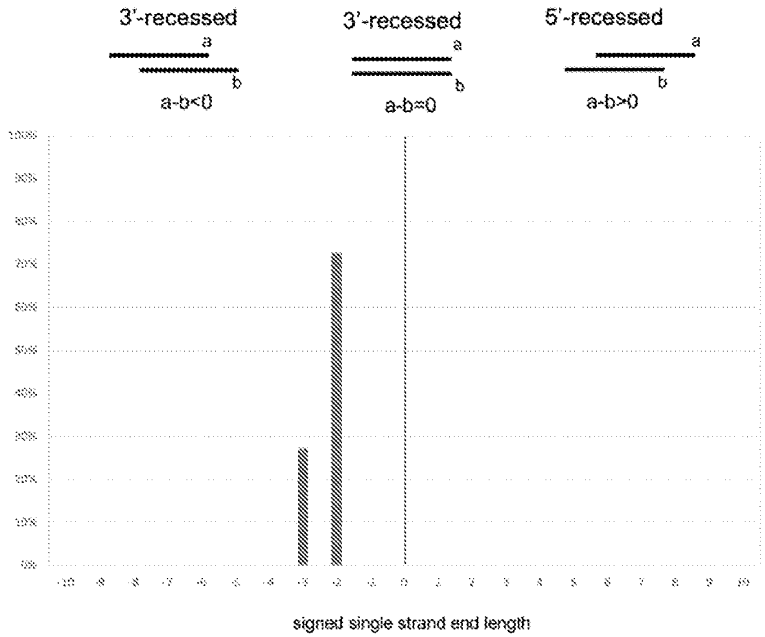
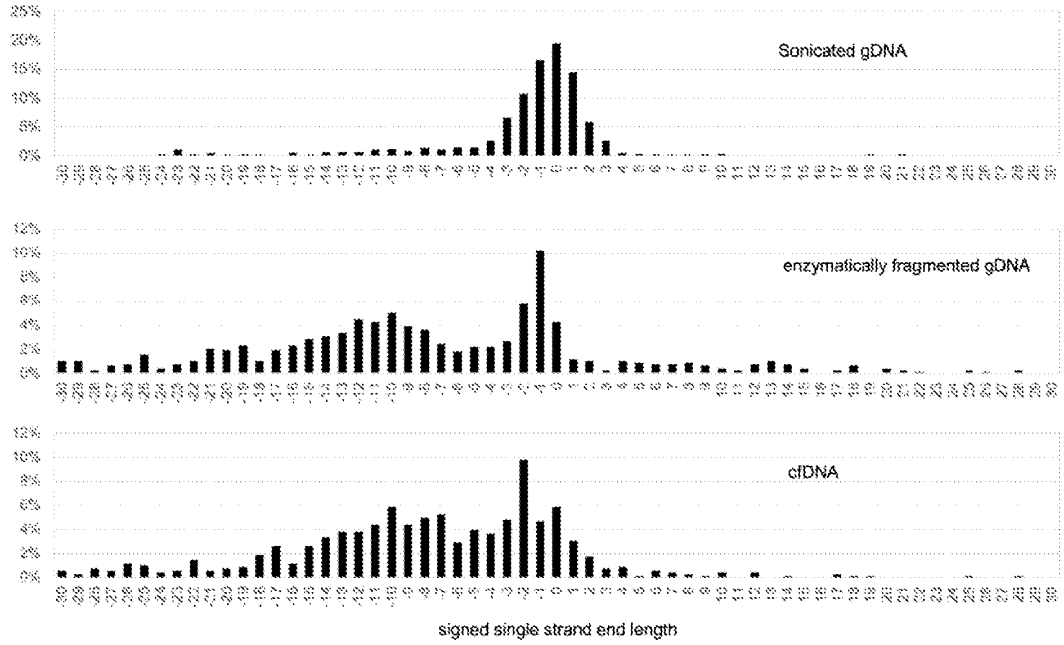


FIGURE 4

A.


Sonicated gDNA
enzymatically fragmented gDNA
cfDNA
signed single strand end length

B.

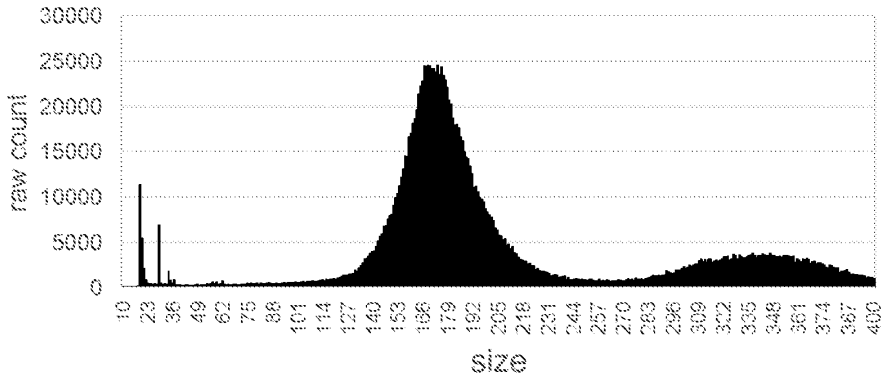

raw count
size

FIGURE 5

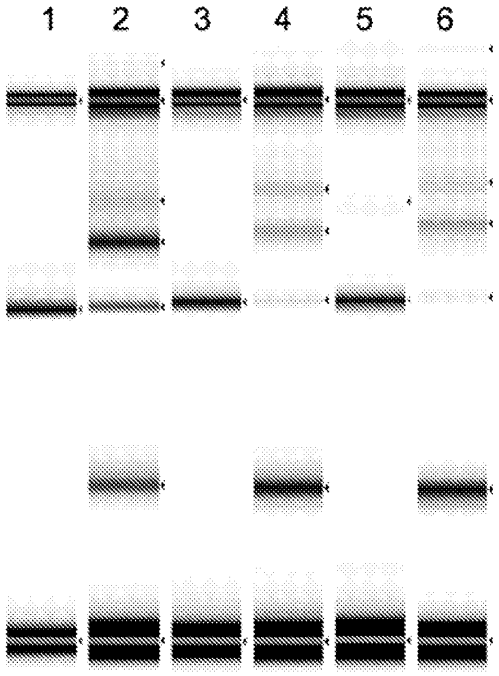


FIGURE 6

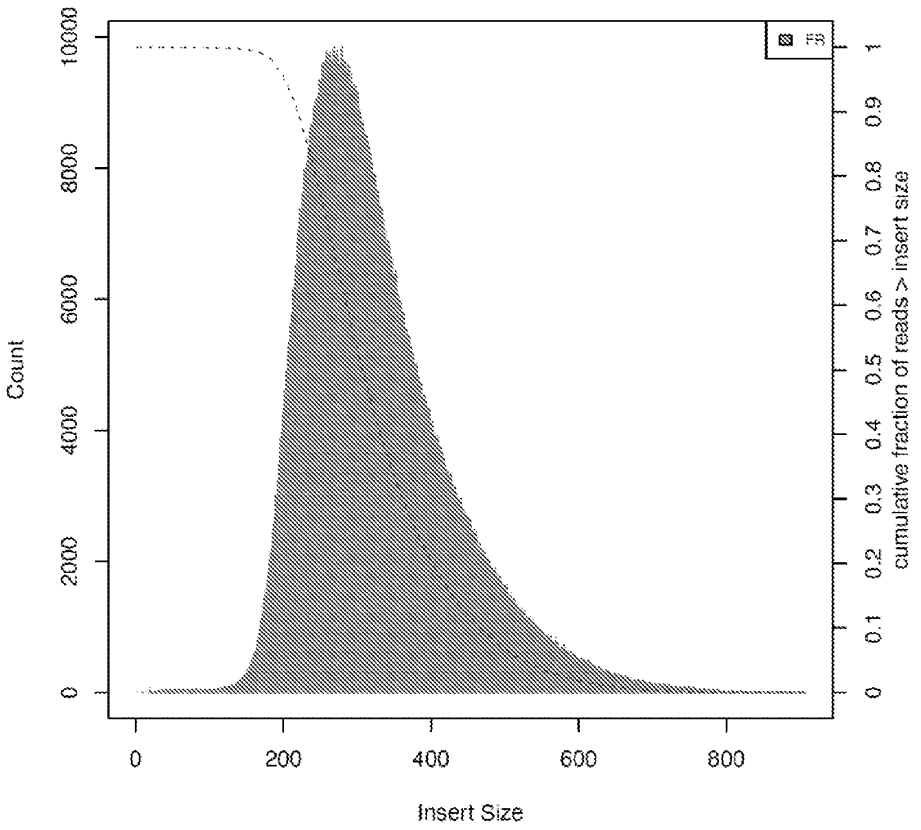

Count
0
2000
4000
6000
8000
10000
0
200
400
600
800
Insert Size
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
cumulative fraction of reads > insert size

FIGURE 7

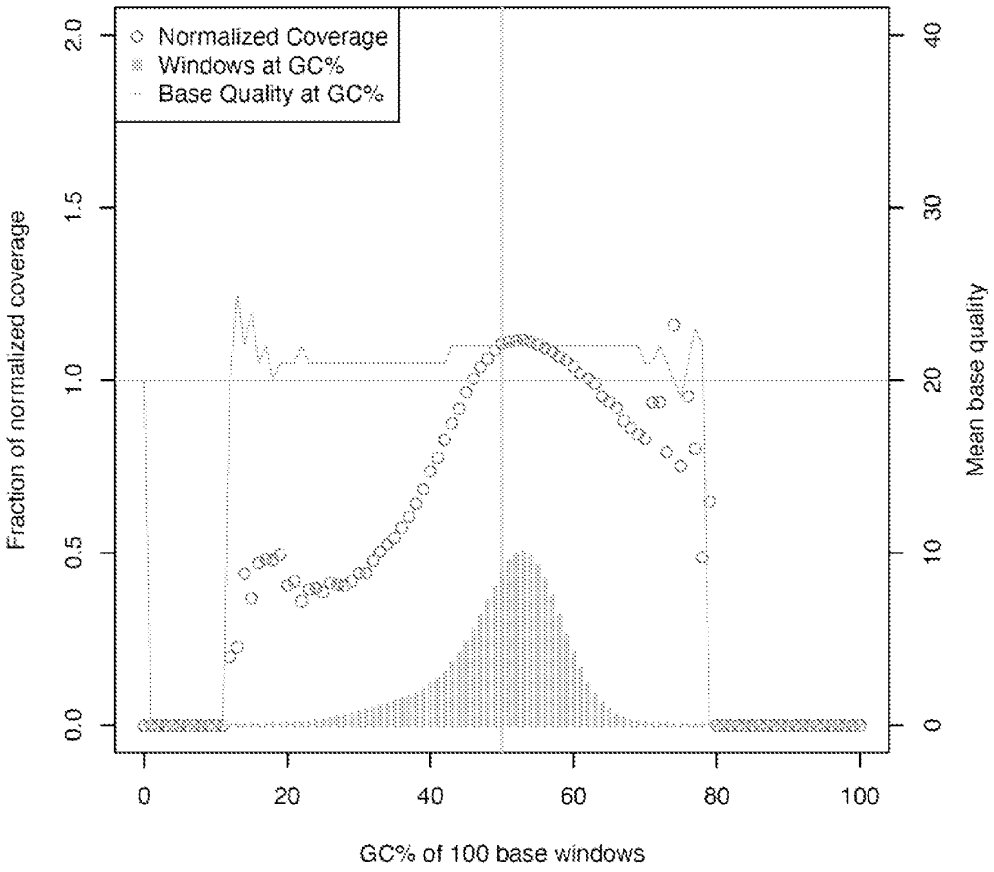

Normalized Coverage
Windows at GC%
Base Quality at GC%
Fraction of normalized coverage
Mean base quality
GC% of 100 base windows
0.0
0.5
1.0
1.5
2.0
0
10
20
30
40
0
20
40
60
80
100

FIGURE 8

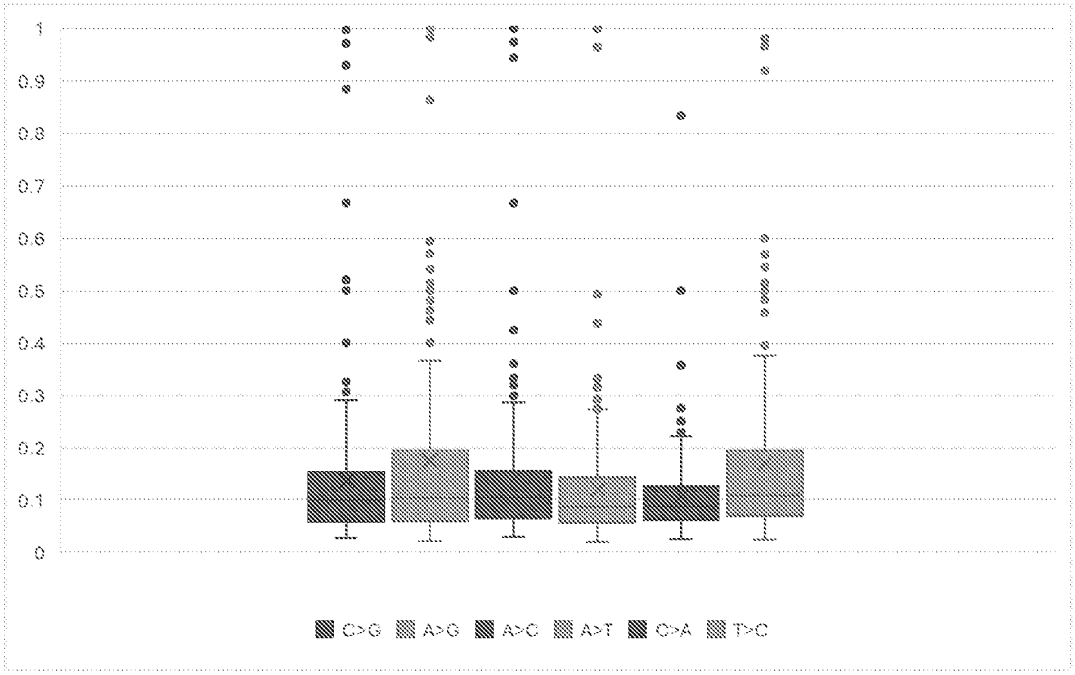


FIGURE 9

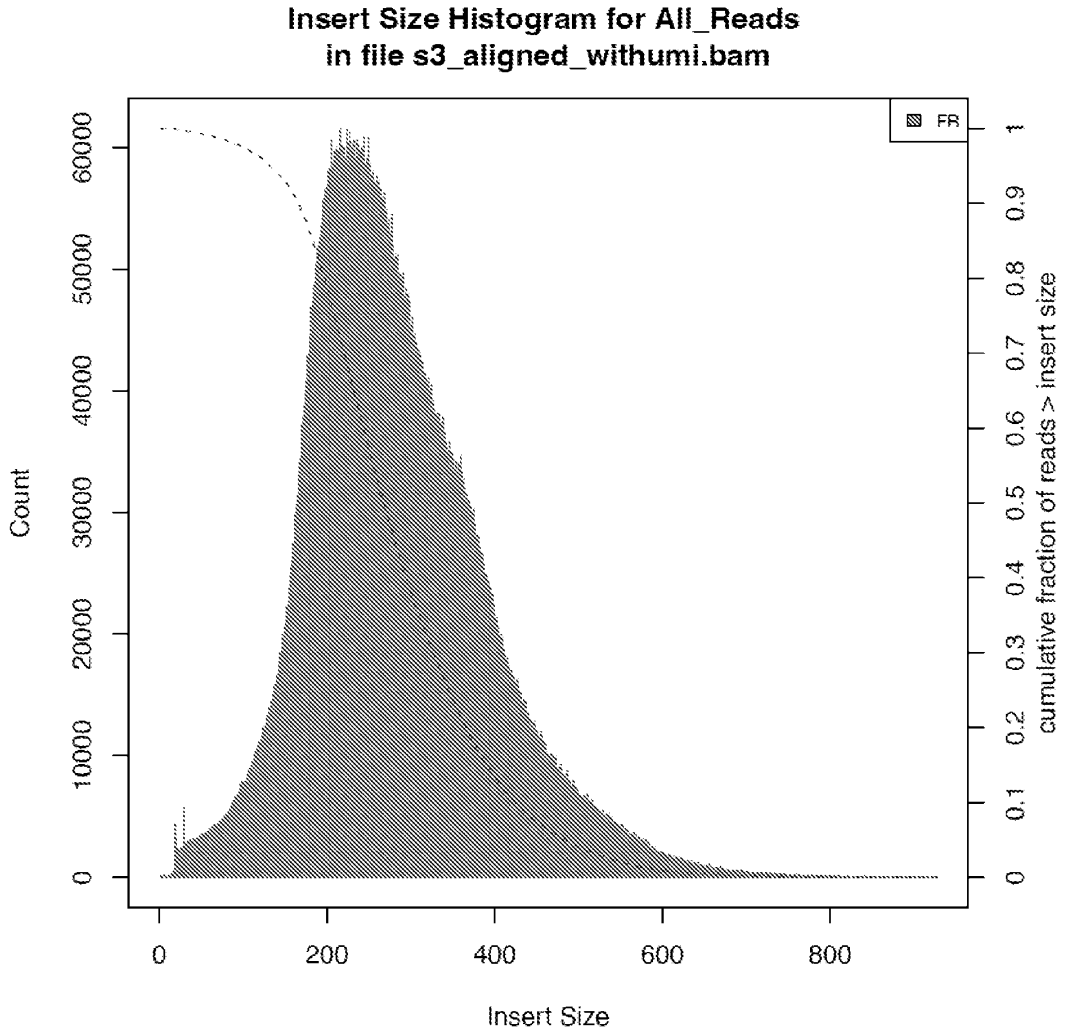

Insert Size Histogram for All_Reads
in file s3_aligned_withumi.bam
FR
Count
0
10000
20000
30000
40000
50000
60000
0
200
400
600
800
Insert Size
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
cumulative fraction of reads > insert size

FIGURE 10

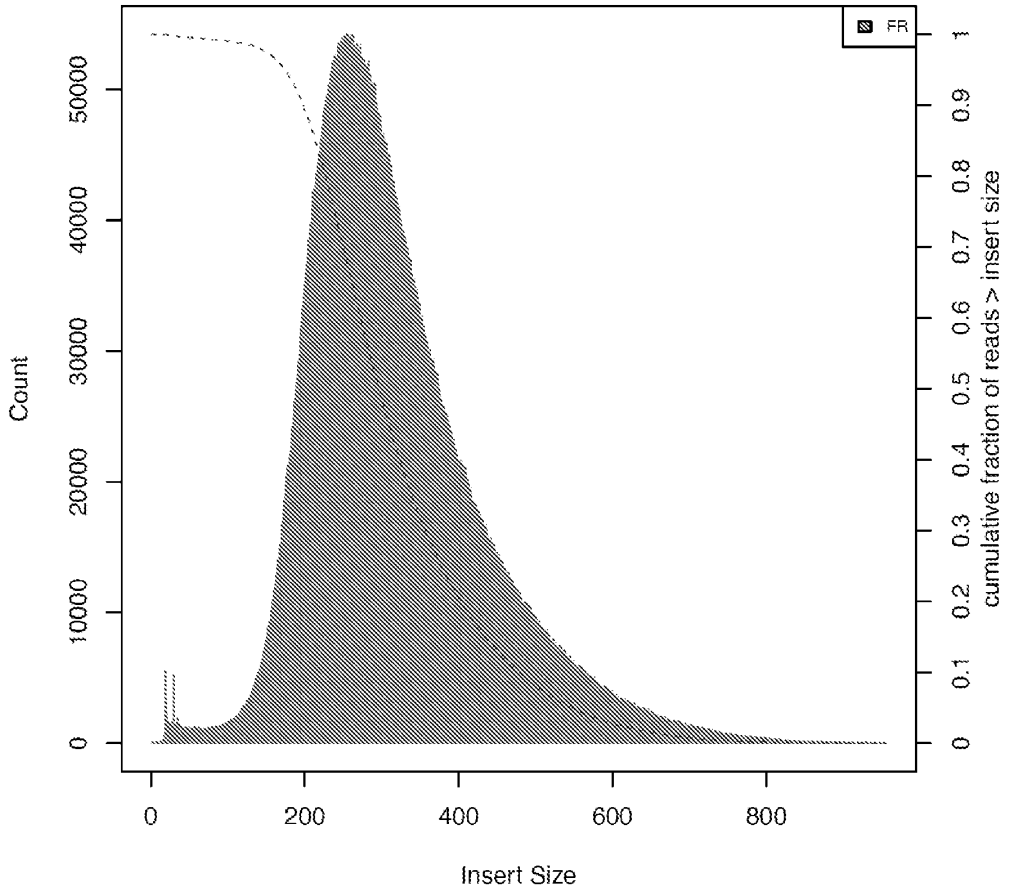
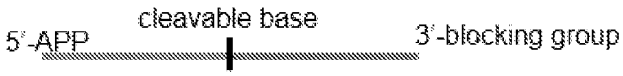

FR
Count
0
10000
20000
30000
40000
50000
0
200
400
600
800
Insert Size
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
cumulative fraction of reads > insert size

FIGURE 11

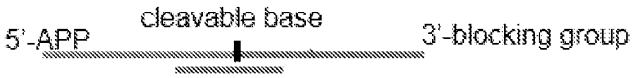
A.



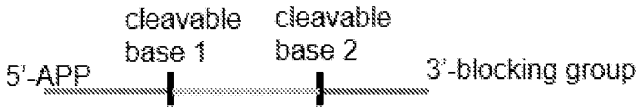
B.



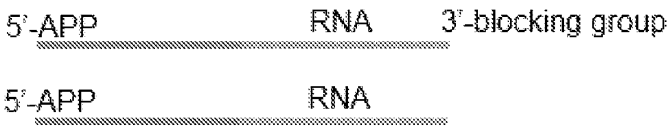
C.



D.



E.



F.

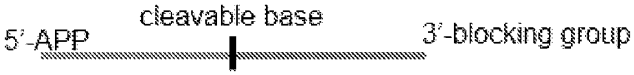
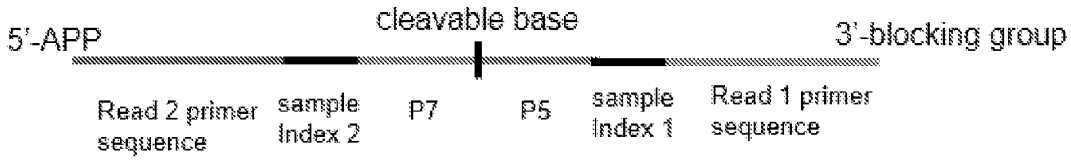


FIGURE 12

A.


5'-APP
cleavable base
3'-blocking group
Read 2 primer sequence
sample Index 2
P7
P5
sample Index 1
Read 1 primer sequence

B.

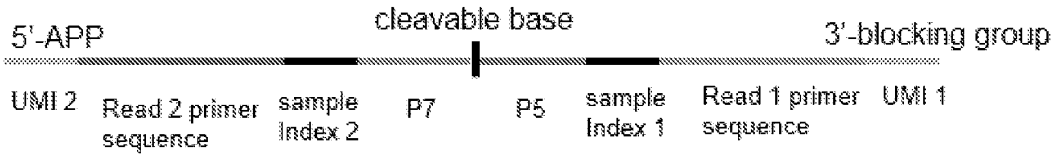

5'-APP
cleavable base
3'-blocking group
UMI 2
Read 2 primer sequence
sample Index 2
P7
P5
sample Index 1
Read 1 primer sequence
UMI 1

FIGURE 13

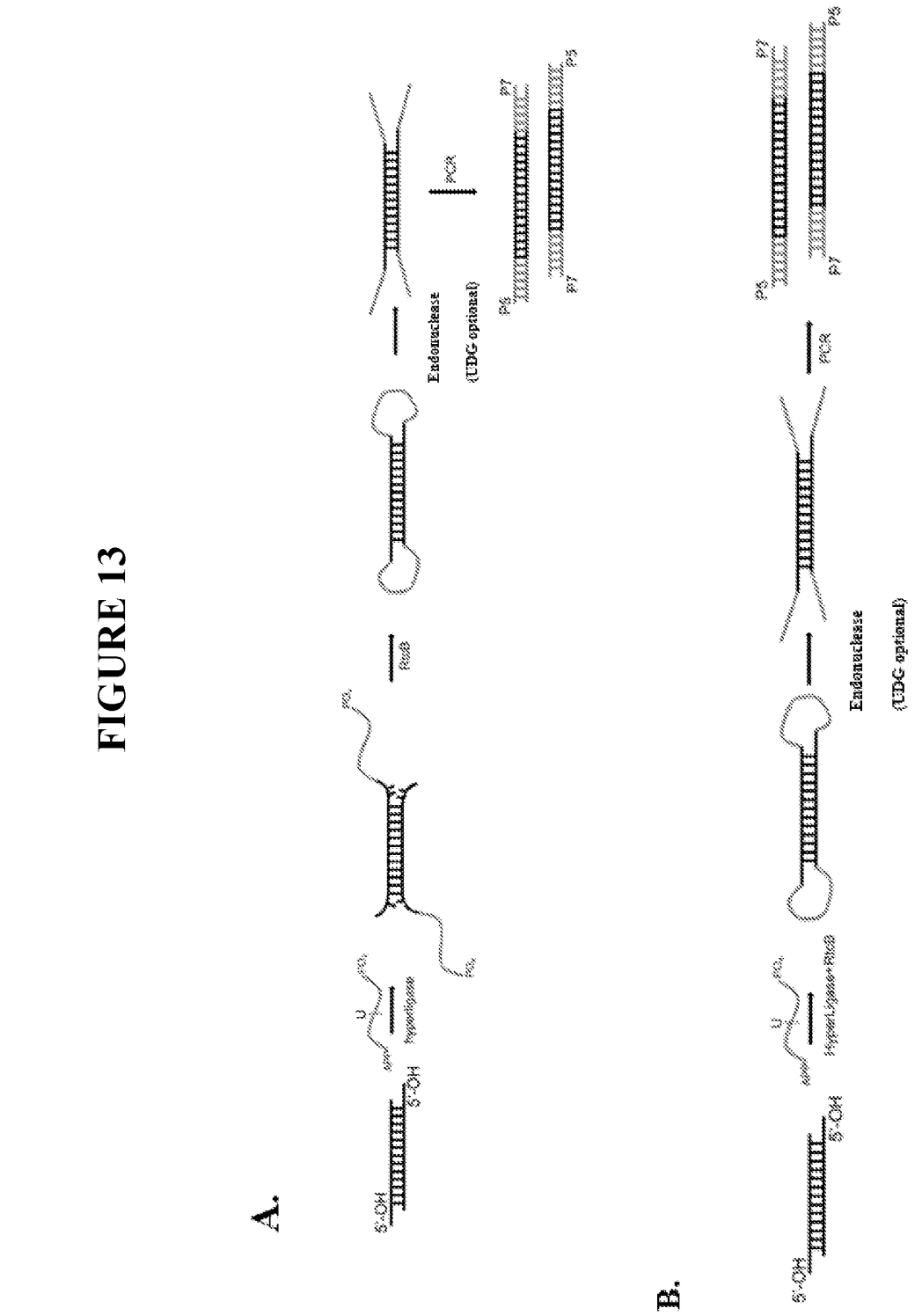

A.
5'-OH
3'-OH
U
Hybridization
RNaseH
Endonuclease
(UDG optional)
PCR
P5
P7
B.
5'-OH
3'-OH
U
Hybridization-RNaseH
Endonuclease
(UDG optional)
PCR
P5
P7

FIGURE 14A

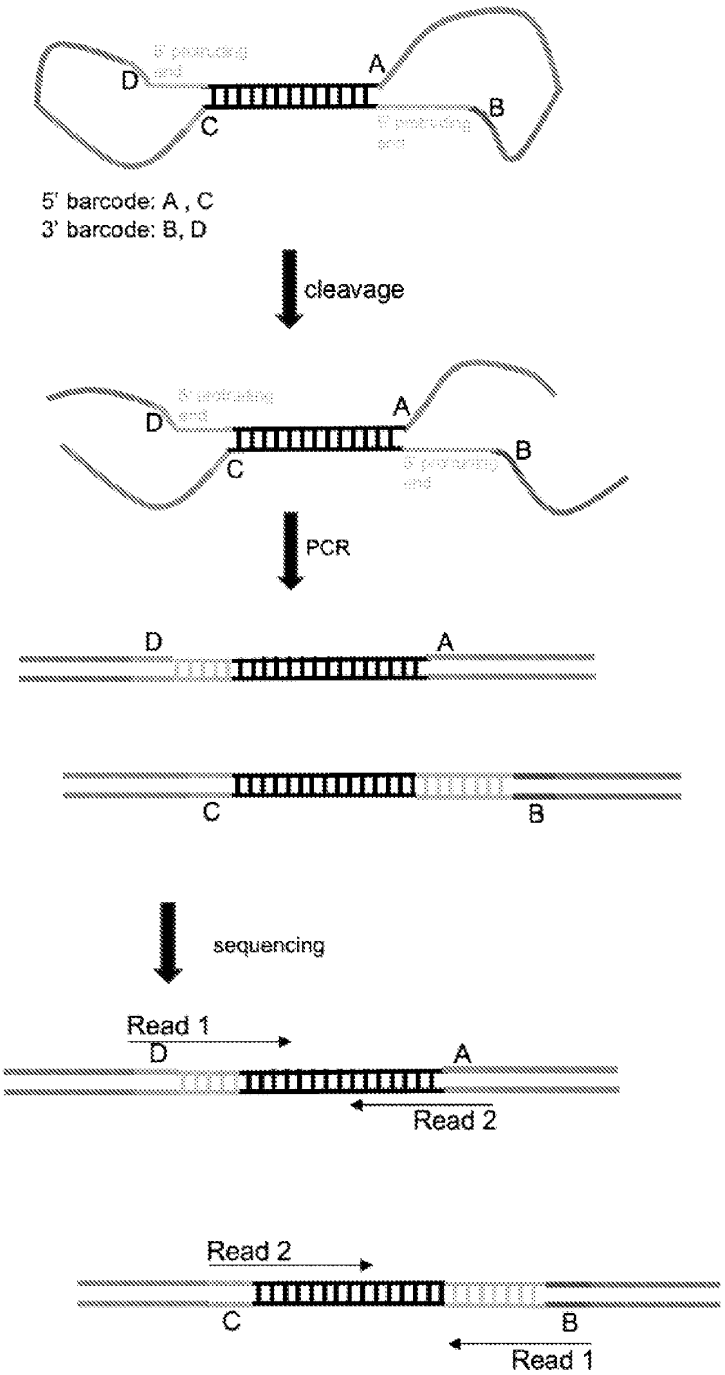

5' barcode: A , C
3' barcode: B, D
cleavage
PCR
sequencing
Read 1
Read 2
Read 2
Read 1
A
B
C
D

FIGURE 14B

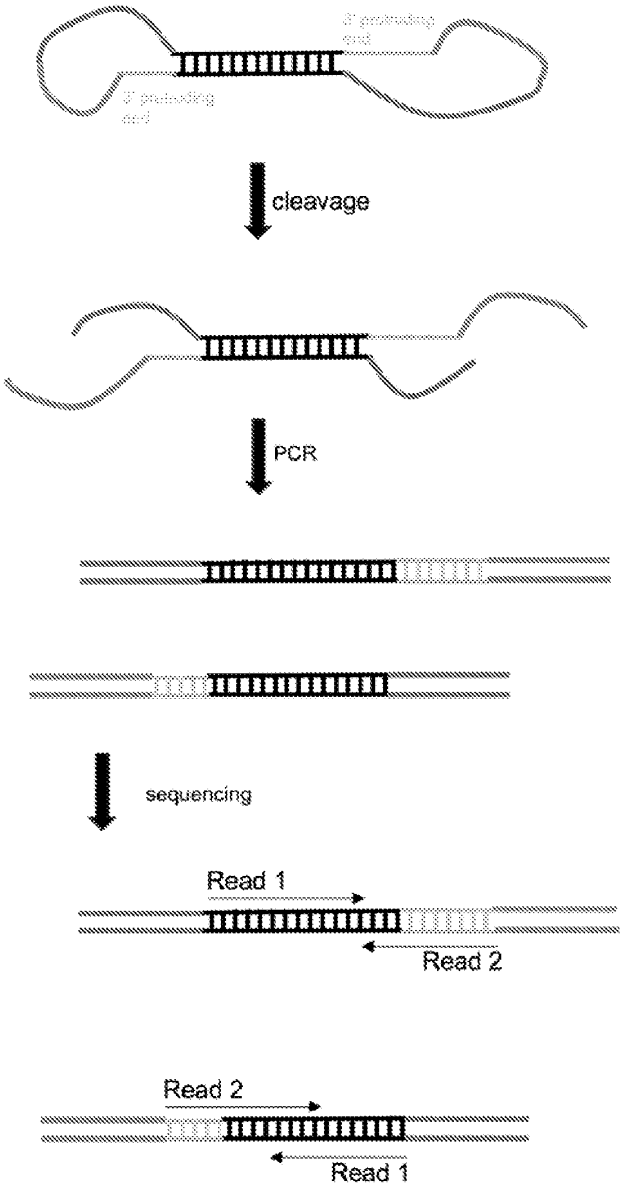


FIGURE 14C

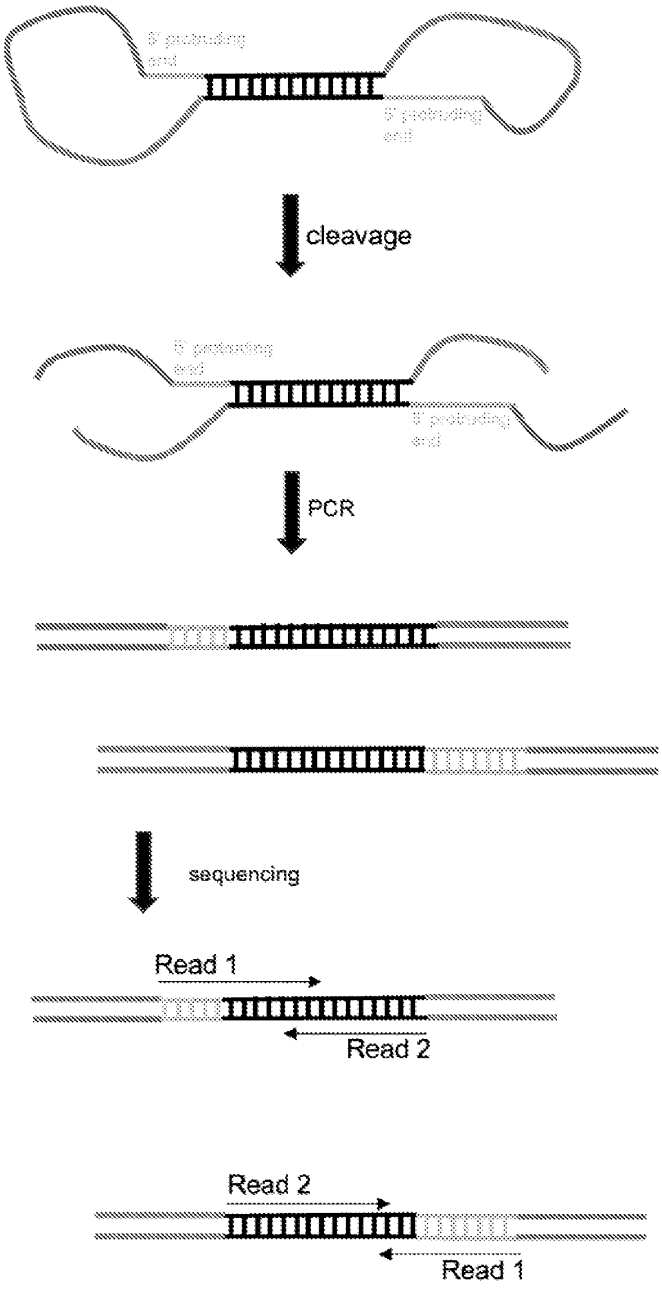
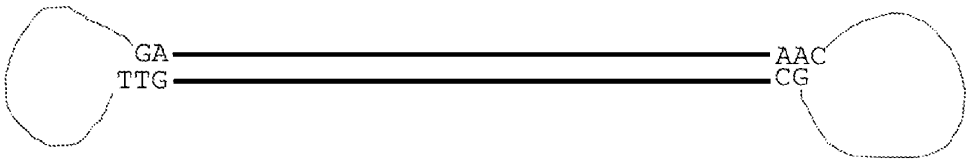

5' protruding end
5' protruding end
cleavage
5' protruding end
5' protruding end
PCR
sequencing
Read 1
Read 2
Read 2
Read 1

FIGURE 15

A.



B.

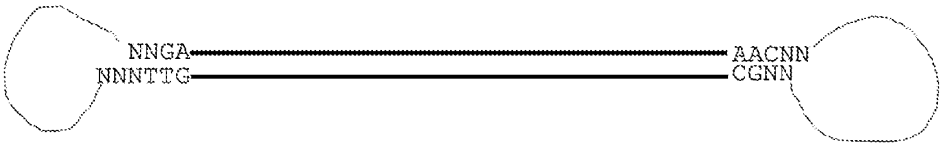
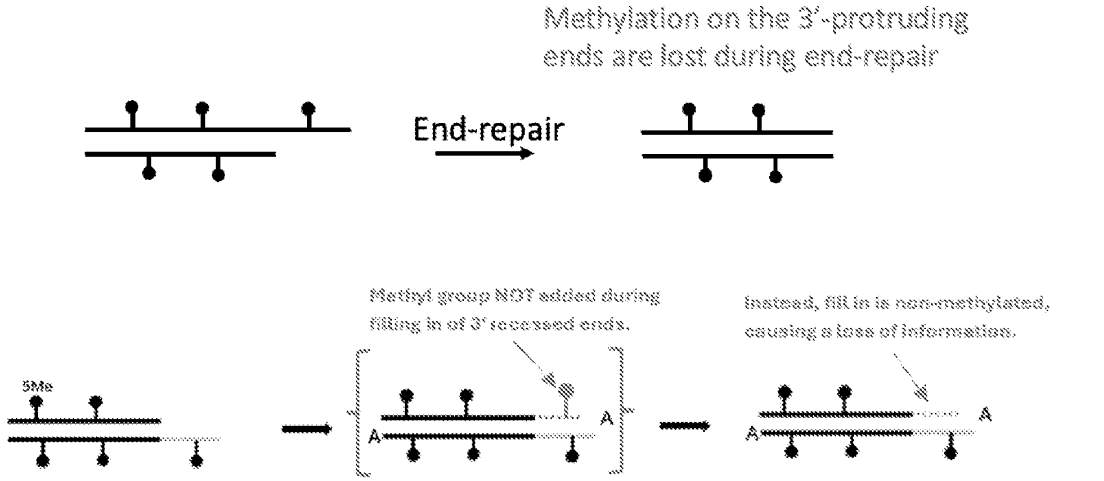


FIGURE 16

A.


Methylation on the 3'-protruding ends are lost during end-repair
End-repair
Methyl group NOT added during filling in of 3' recessed ends.
Instead, fill in is non-methylated, causing a loss of information.
5Me
A
A
A
A

B.

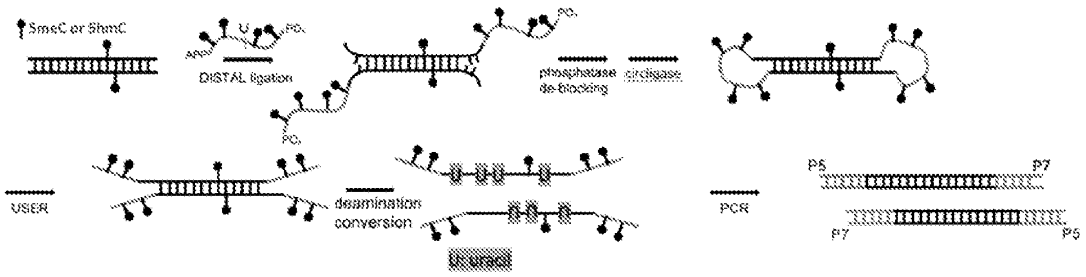

5mC or 5hmC
DISTAL ligation
phosphatase de-blocking
circligase
USER
deamination conversion
PCR
P5
P7
P7
P5

FIGURE 17

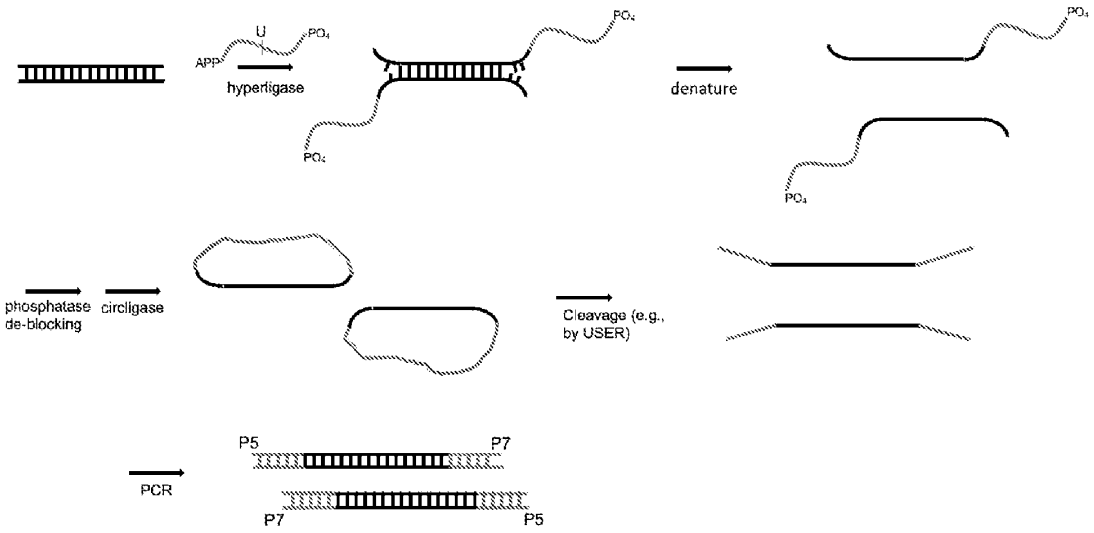
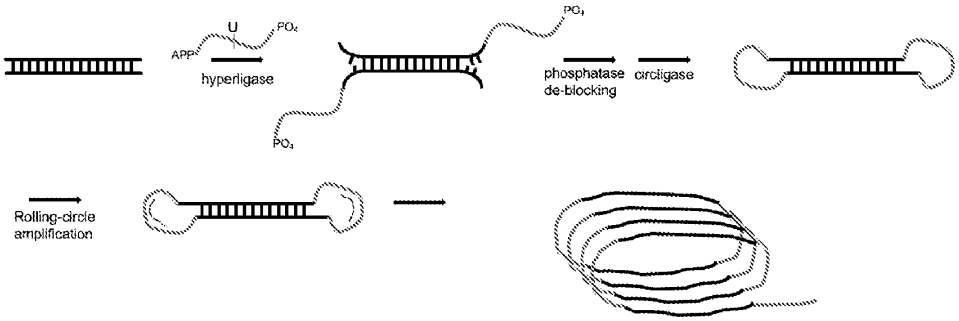

U
APP
hyperligase
denature
phosphatase
de-blocking
circligase
Cleavage (e.g.,
by USER)
P5
P7
PCR
P7
P5

FIGURE 18

A.


U
APP
hyperligase
phosphatase
de-blocking
circligase
Rolling-circle
amplification

B.

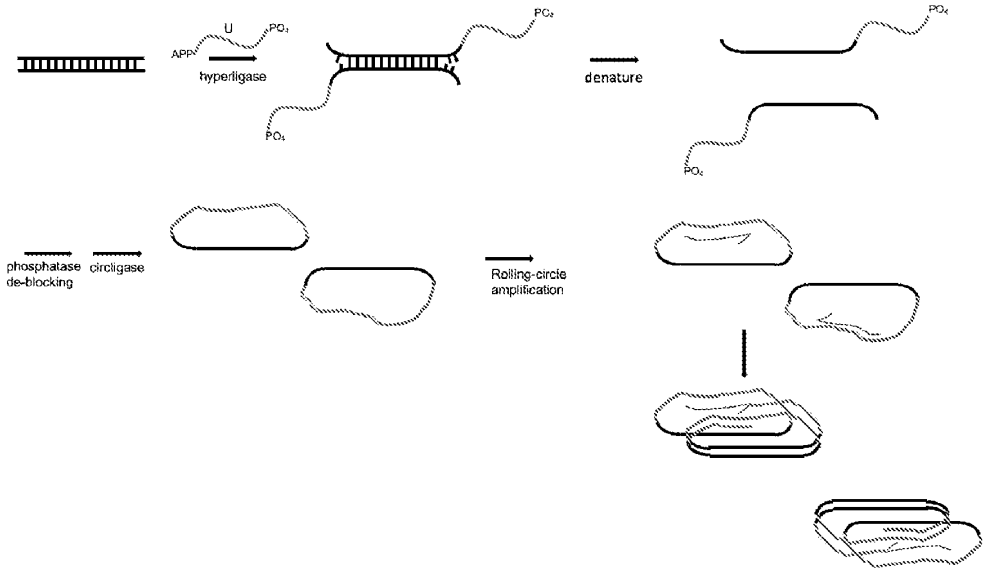

U
APP
hyperligase
denature
phosphatase
de-blocking
circligase
Rolling-circle
amplification

FIGURE 19

A. (5APP)AGATCGGAAGAGCACACGTCTGAACTCCAGTCAC/ACACTCTTTCCCTACACGACGCTCTTCCGATCT(3P) SEQ ID NO:54

Read 1 sequencing primer

Read 2 sequencing primer

B. (5APP)NNNNNAGATCGGAAGAGCACACGTCTGAACTCCAGTCAC/ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNN(3P) SEQ ID NO:55

C. (5APP)(BARCODE1)AGATCGGAAGAGCACACGTCTGAACTCCAGTCAC/ACACTCTTTCCCTACACGACGCTCTTCCGATCT(BARCODE2)(3P) SEQ ID NO:54

D. (5APP)AGATCGGAAGAGCACACGTCTGAACTCCAGTCACATCTCGTATGCCGTCTTCTGCTTG/AATGATACGGCGACCACCGAGATCTACACACACTCTTTCCCTACACGACGCTCTTCCGATCT(3P)

Read 2 sequencing primer | P7 | P5 | Read 1 sequencing primer

SEQ ID NO:58

E. (5APP)AGATCGGAAGAGCACACGTCTGAACTCCAGTCACXXXXXXXXATCTCGTATGCCGTCTTCTGCTTG/AATGATACGGCGACCACCGAGATCTACACXXXXXXXXACACTCTTTCCCTACACGACGCTCTTCCGATCT(3P)

Read 2 sequencing primer | i7 index | P7 | P5 | i5 index | Read 1 sequencing primer

SEQ ID NO:61

5APP=5' adenylation

3P=3' phosphate