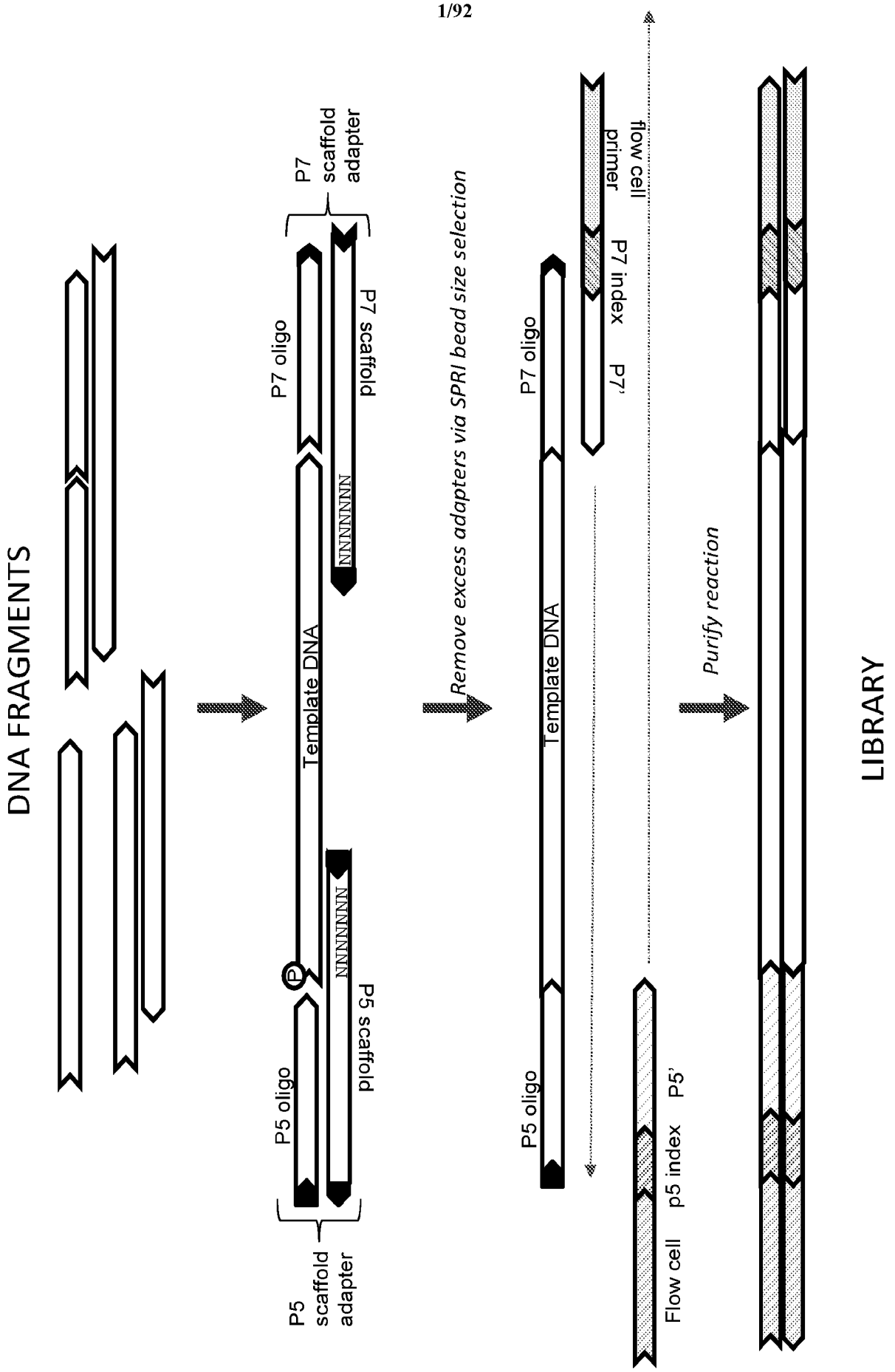

DNA FRAGMENTS
P7 scaffold adapter
P7 oligo
P7 scaffold
NNNNNNN
Template DNA
P
NNNNNNN
P5 scaffold
P5 oligo
P5 scaffold adapter
Remove excess adapters via SPRI bead size selection
P7 oligo
Template DNA
P5 oligo
flow cell
primer
P7 index
P7'
Flow cell
p5 index
P5'
Purify reaction
LIBRARY

Fig. 1

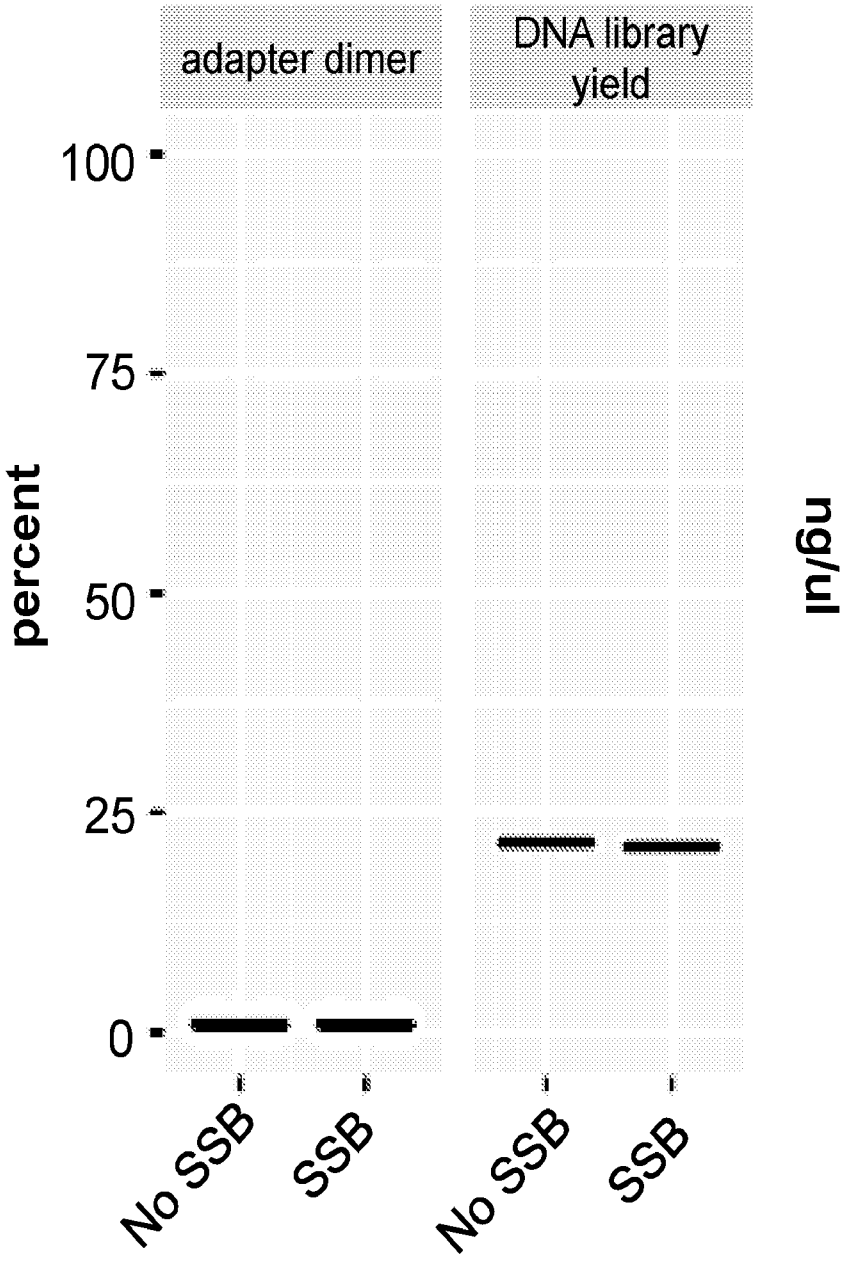

adapter dimer
DNA library yield
100
75
50
25
0
percent
ng/ul
No SSB
SSB
No SSB
SSB

Fig. 2A

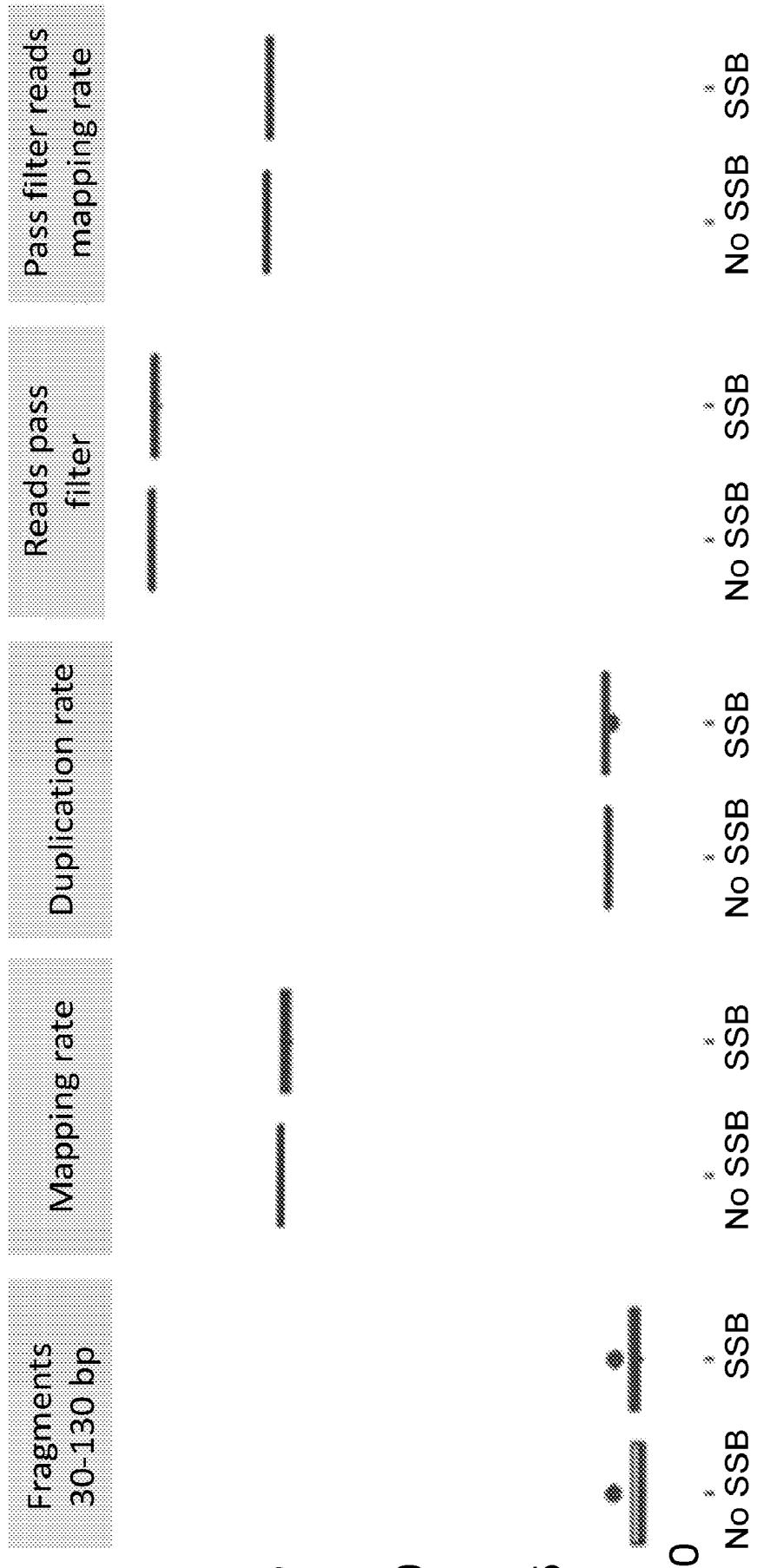

Fragments 30-130 bp
Mapping rate
Duplication rate
Reads pass filter
Pass filter reads mapping rate
Percent
100
75
50
25
0
No SSB
SSB

Fig. 2B

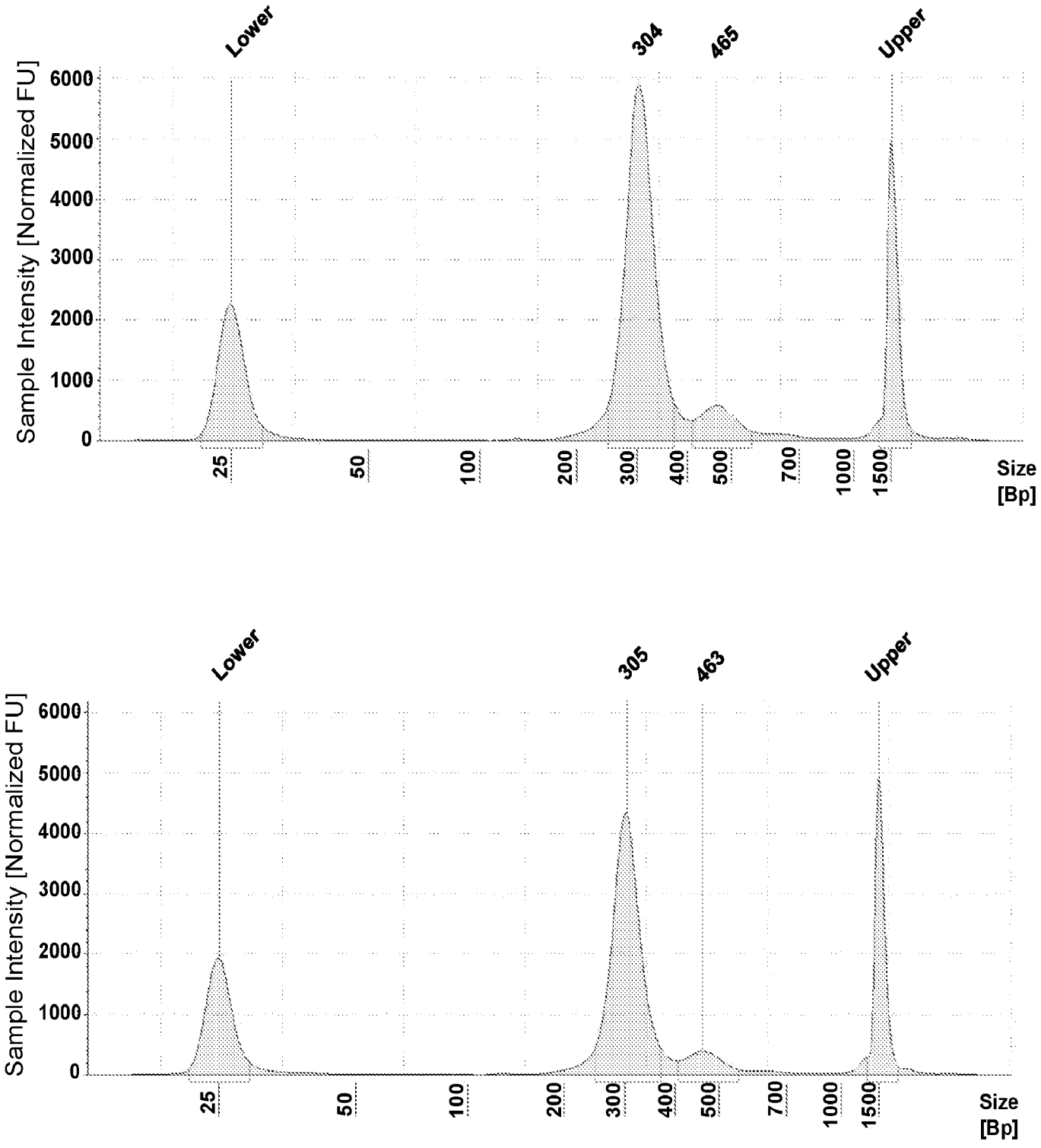

Lower
304
465
Upper
Sample Intensity [Normalized FU]
6000
5000
4000
3000
2000
1000
0
25
50
100
200
300
400
500
700
1000
1500
Size [Bp]
Lower
305
463
Upper
Sample Intensity [Normalized FU]
6000
5000
4000
3000
2000
1000
0
25
50
100
200
300
400
500
700
1000
1500
Size [Bp]

Fig. 3A

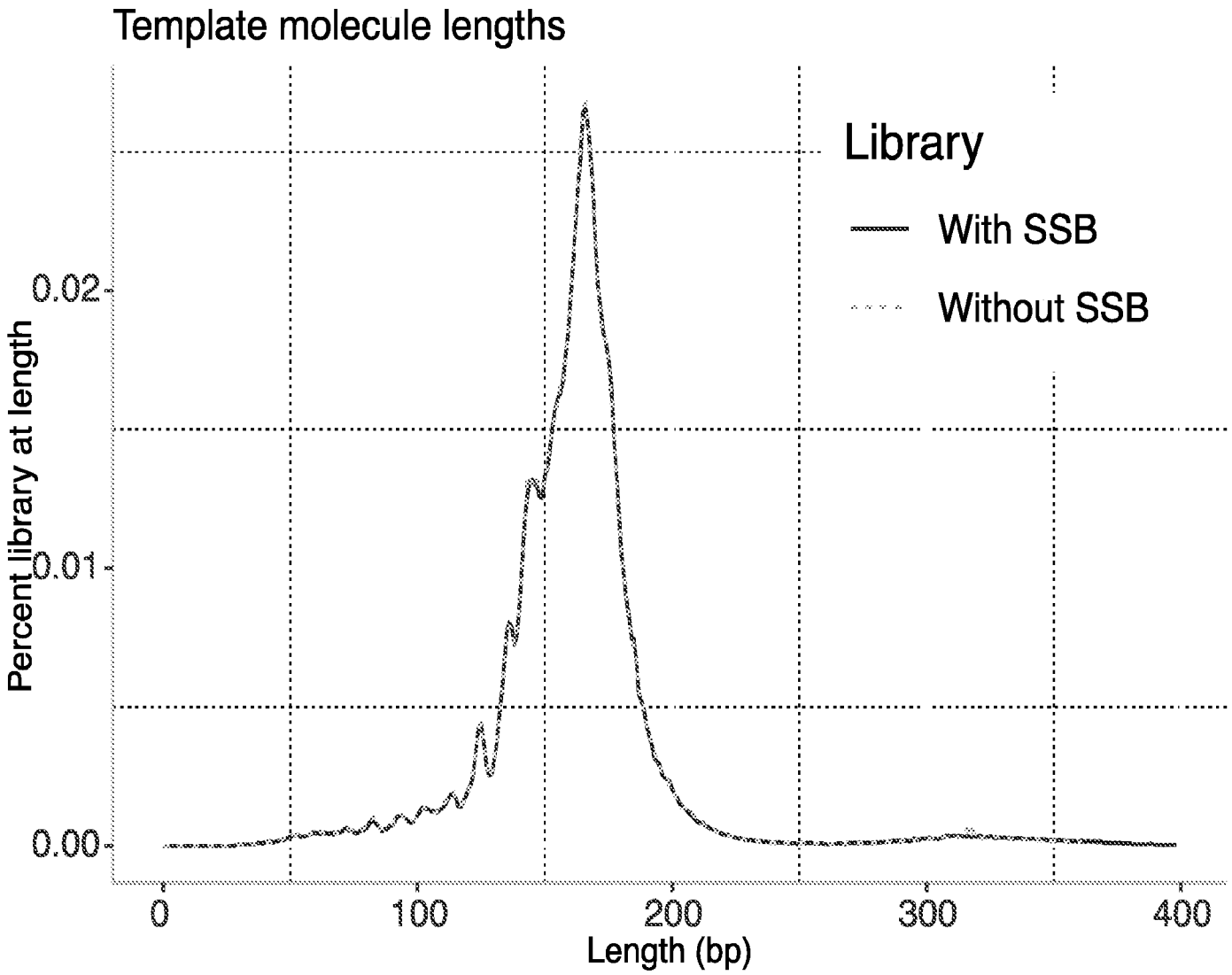

Template molecule lengths
Library
With SSB
Without SSB
Percent library at length
0.02
0.01
0.00
0
100
200
300
400
Length (bp)

Fig. 3B

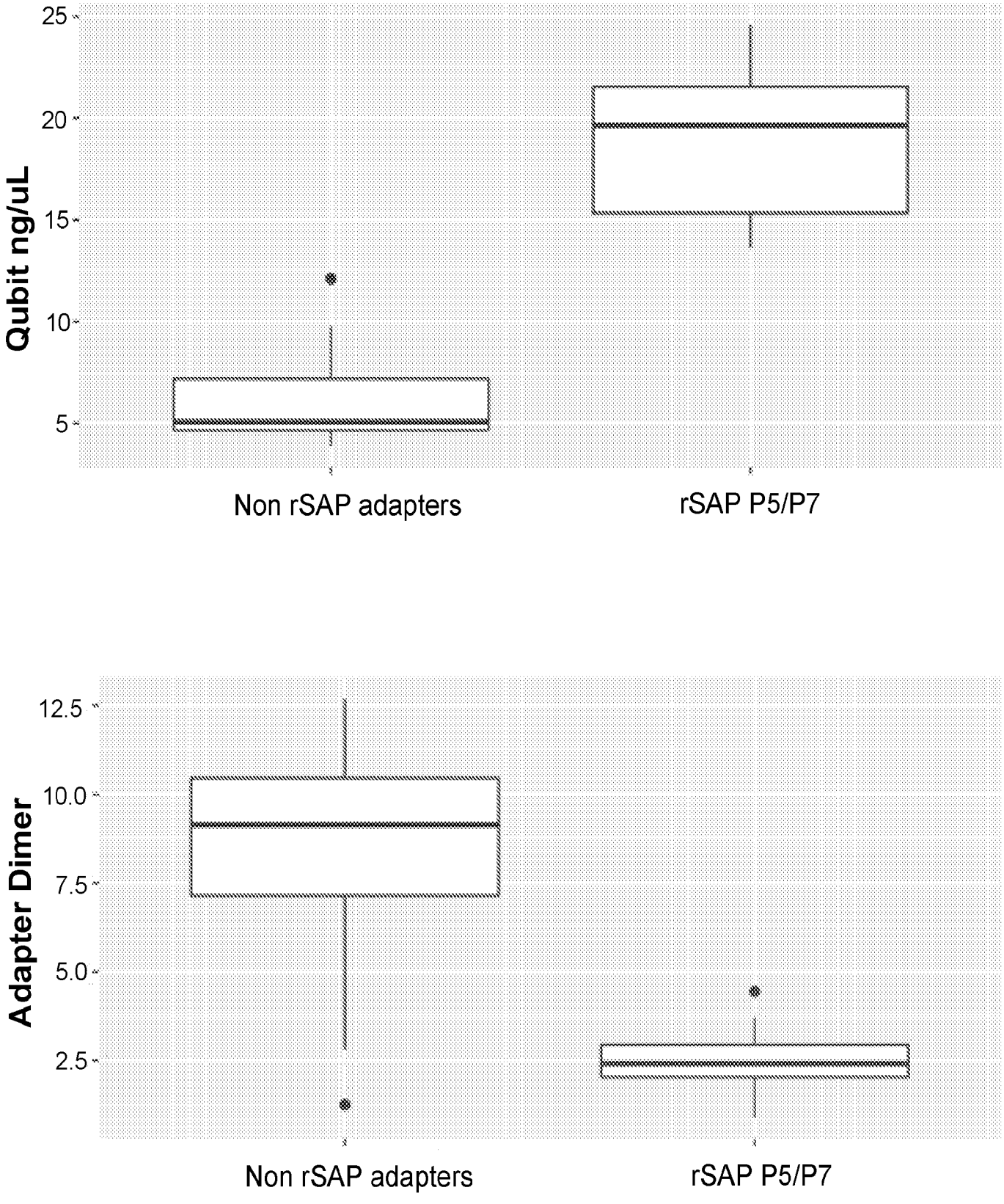

Qubit ng/uL
25
20
15
10
5
Non rSAP adapters
rSAP P5/P7
Adapter Dimer
12.5
10.0
7.5
5.0
2.5
Non rSAP adapters
rSAP P5/P7
Adapter treatment

Fig. 4

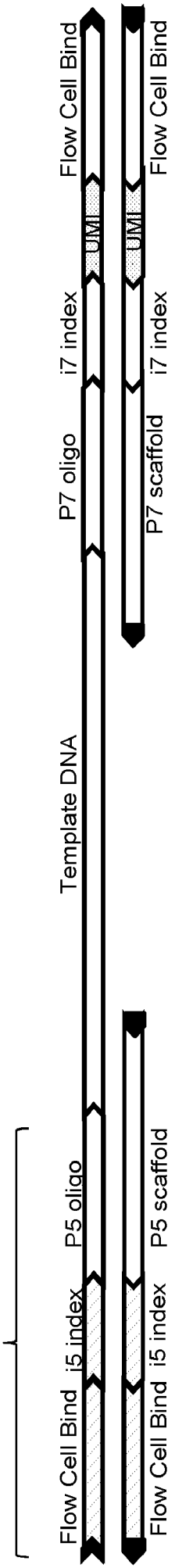

Flow Cell Bind
i7 index
UMI
P7 oligo
P7 scaffold
Template DNA
P5 oligo
P5 scaffold
i5 index
Flow Cell Bind

Fig. 5A

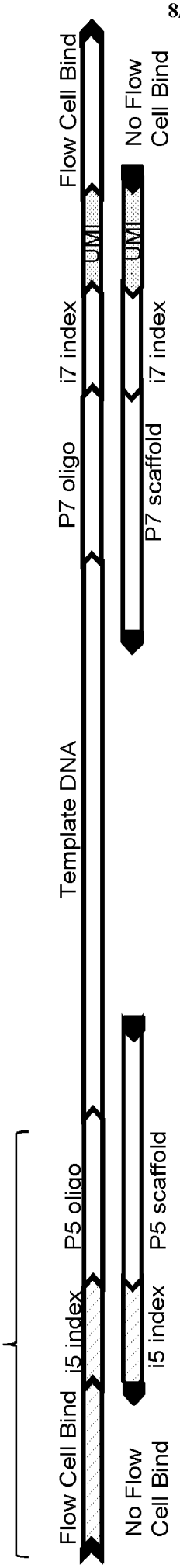

Flow Cell Bind
i5 index
P5 oligo
Template DNA
P7 oligo
i7 index
UMI
Flow Cell Bind
No Flow Cell Bind
i5 index
P5 scaffold
No Flow Cell Bind
UMI
i7 index
P7 scaffold

Fig. 5B

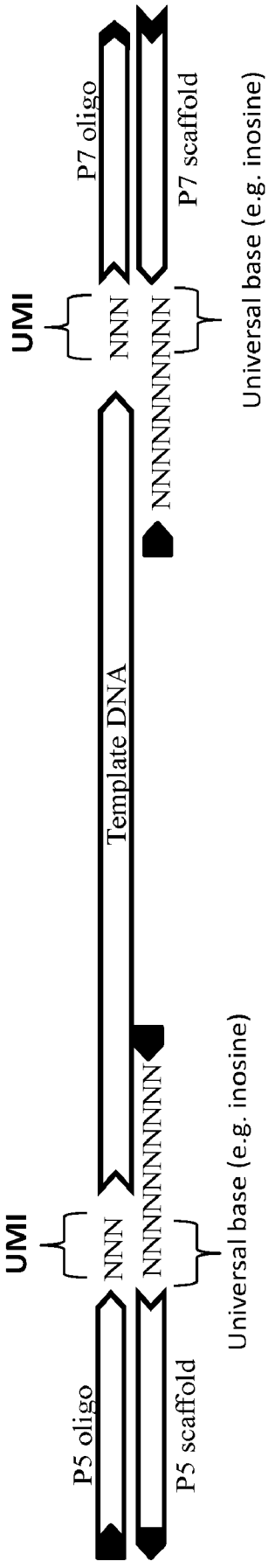

P7 oligo
P7 scaffold
UMI
NNN
NNNNNNNNNNN
Universal base (e.g. inosine)
Template DNA
UMI
NNN
NNNNNNNNNNN
Universal base (e.g. inosine)
P5 oligo
P5 scaffold

Fig. 6A

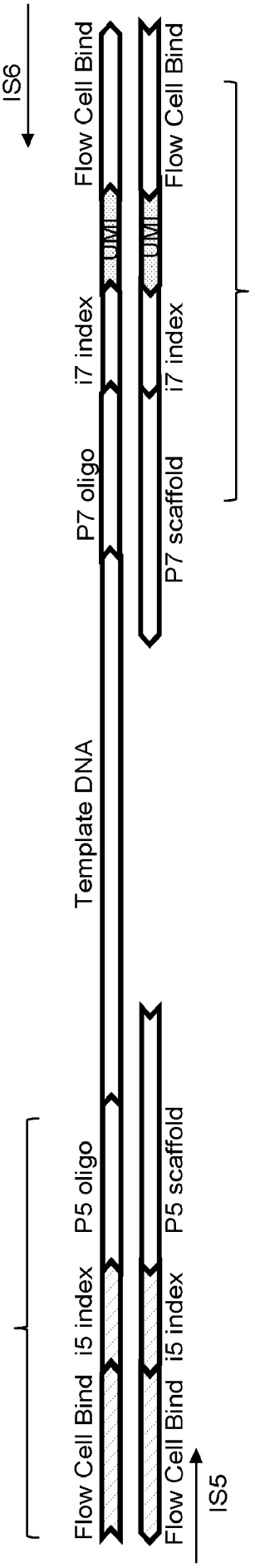

IS6
Flow Cell Bind
i7 index
UMI
P7 oligo
P7 scaffold
Template DNA
P5 oligo
P5 scaffold
i5 index
Flow Cell Bind
IS5

Fig. 6B

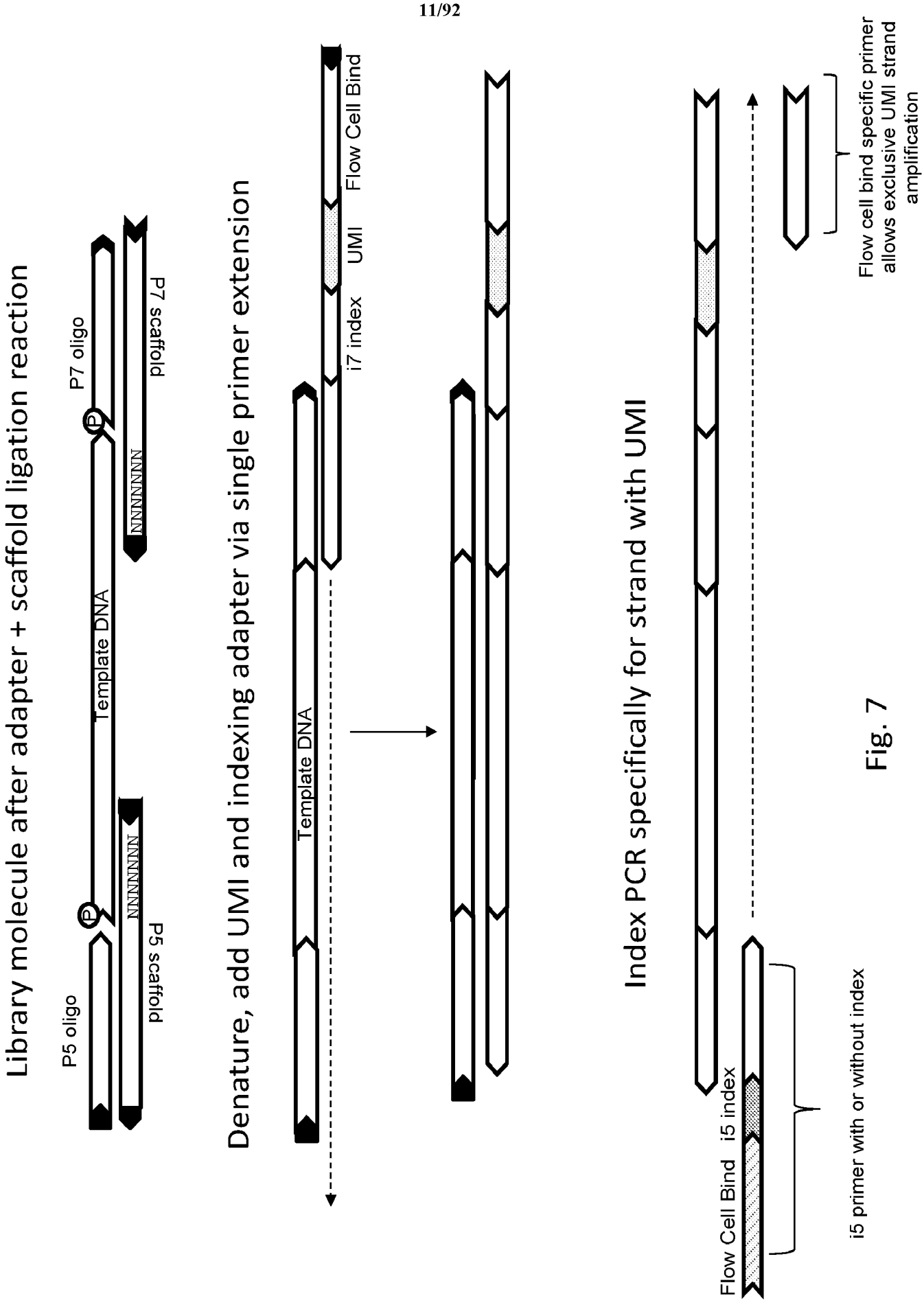

Library molecule after adapter + scaffold ligation reaction
P5 oligo
P7 oligo
Template DNA
P5 scaffold
P7 scaffold
NNNNNNN
Denature, add UMI and indexing adapter via single primer extension
Template DNA
i7 index
UMI
Flow Cell Bind
Index PCR specifically for strand with UMI
Flow Cell Bind
i5 index
i5 primer with or without index
Flow cell bind specific primer allows exclusive UMI strand amplification

Fig. 7

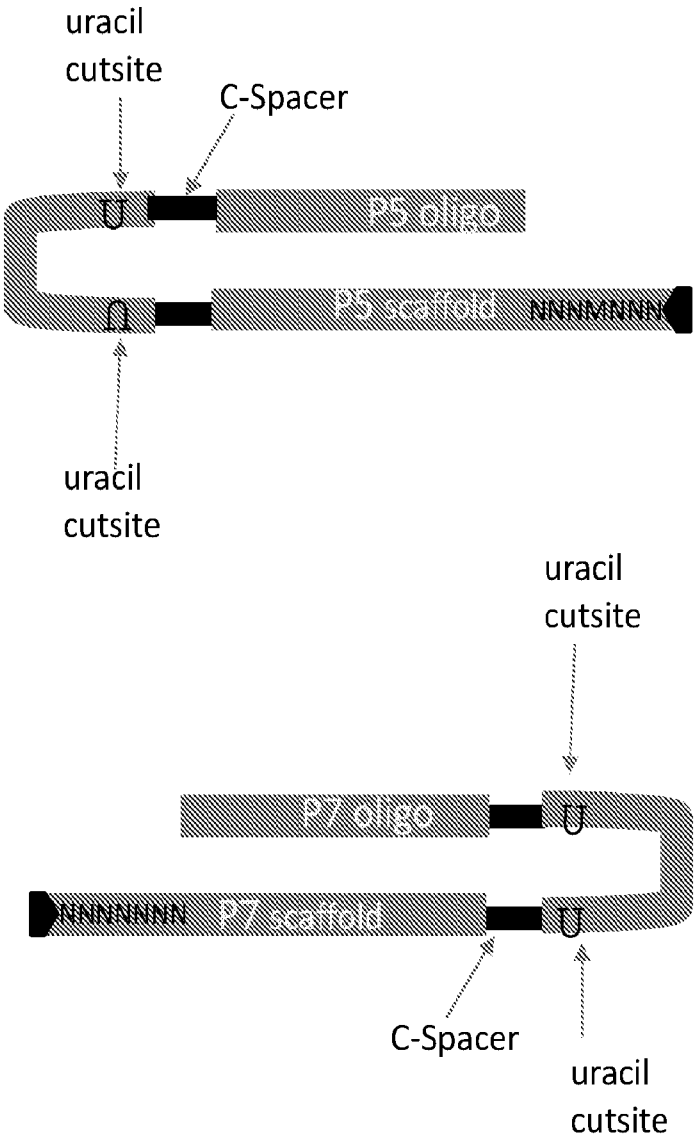

uracil
cutsite
C-Spacer
U
P5 oligo
U
P5 scaffold
NNNMNNN
uracil
cutsite
uracil
cutsite
P7 oligo
U
NNNNNNN
P7 scaffold
U
C-Spacer
uracil
cutsite

Fig. 8

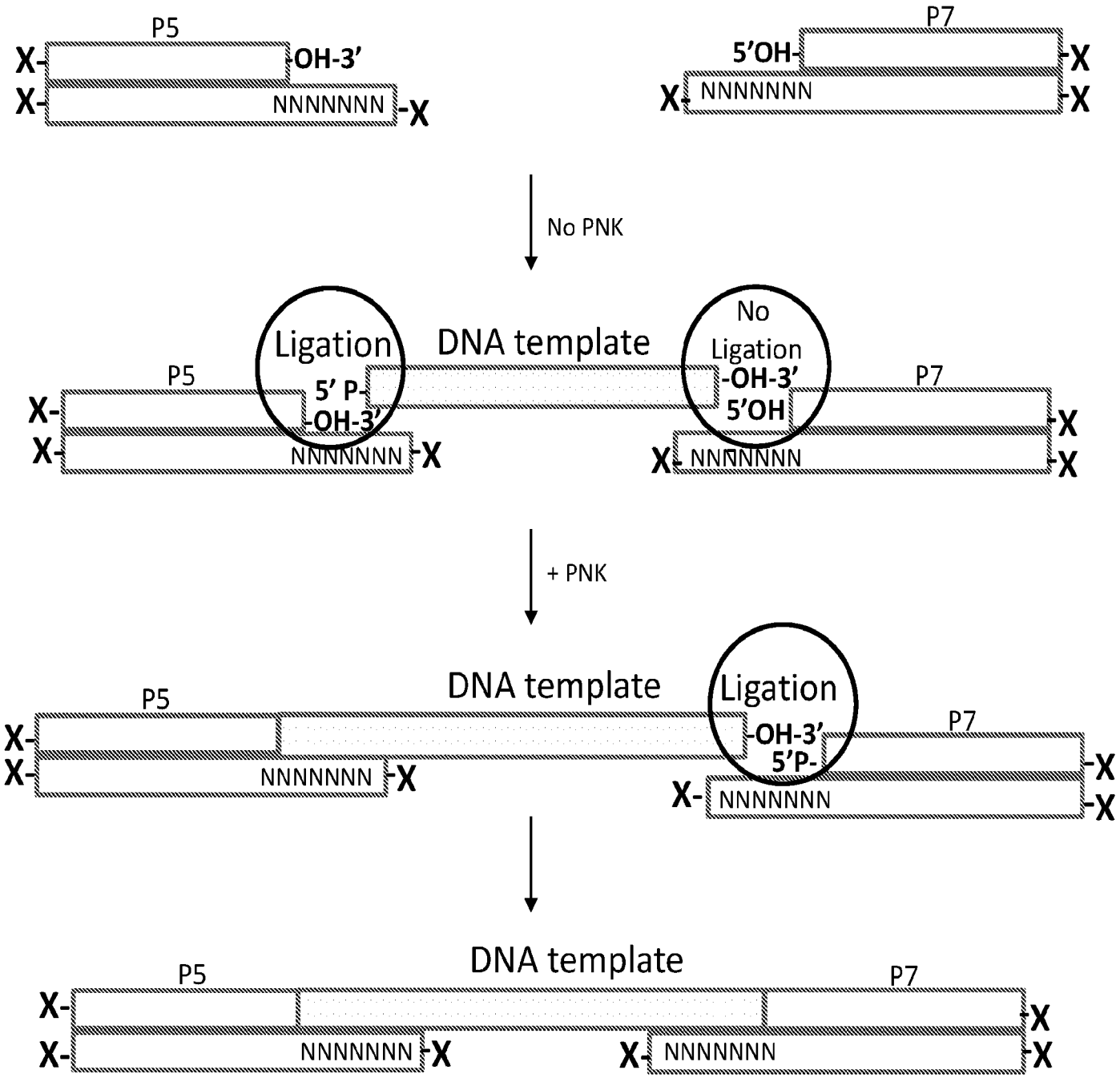

P5
X-
-OH-3'
X-
NNNNNNN
-X
P7
5'OH-
-X
X-
NNNNNNN
-X
No PNK
Ligation
DNA template
No
Ligation
P5
5' P-
-OH-3'
-OH-3'
5'OH
P7
X-
X-
NNNNNNN
-X
X-
NNNNNNN
-X
+ PNK
DNA template
Ligation
P5
-OH-3'
5'P-
P7
X-
X-
NNNNNNN
-X
X-
NNNNNNN
-X
DNA template
P5
P7
X-
X-
NNNNNNN
-X
X-
NNNNNNN
-X

Fig. 9

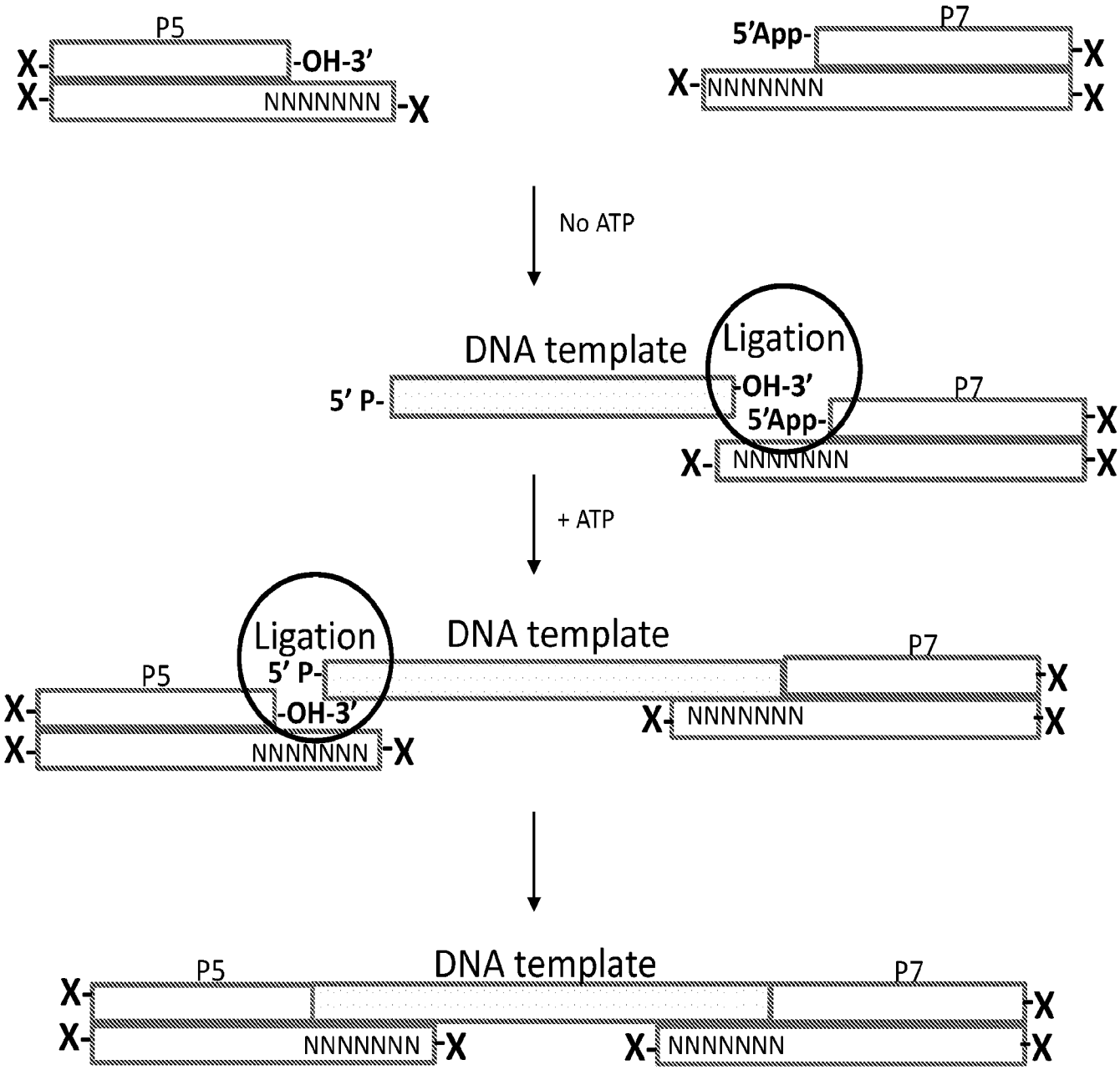

P5
X-
-OH-3'
X-
NNNNNNN
-X
5'App-
P7
-X
X-
NNNNNNN
-X
No ATP
DNA template
Ligation
5' P-
-OH-3'
5'App-
P7
X-
NNNNNNN
-X
+ ATP
Ligation
DNA template
P5
5' P-
P7
X-
-OH-3'
X-
NNNNNNN
-X
X-
NNNNNNN
-X
DNA template
P5
P7
X-
X-
NNNNNNN
-X
X-
NNNNNNN
-X

Fig. 10

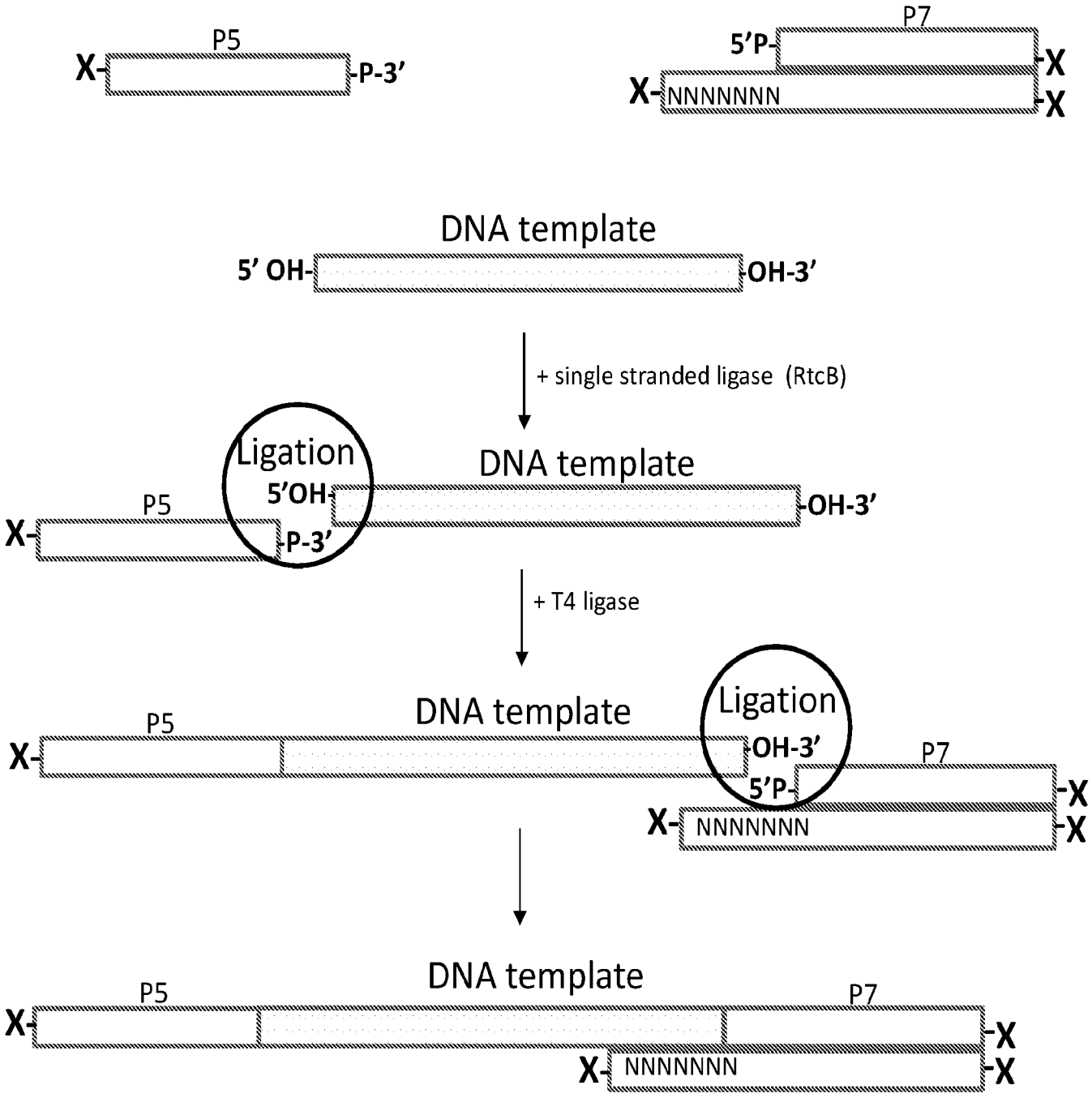

P5
X-
-P-3'
P7
5'P-
-X
X-
NNNNNNN
-X
DNA template
5' OH-
-OH-3'
+ single stranded ligase (RtcB)
Ligation
5'OH-
DNA template
P5
X-
-P-3'
-OH-3'
+ T4 ligase
DNA template
Ligation
P5
X-
-OH-3'
P7
5'P-
-X
X-
NNNNNNN
-X
DNA template
P5
P7
X-
-X
X-
NNNNNNN
-X

Fig. 11

i

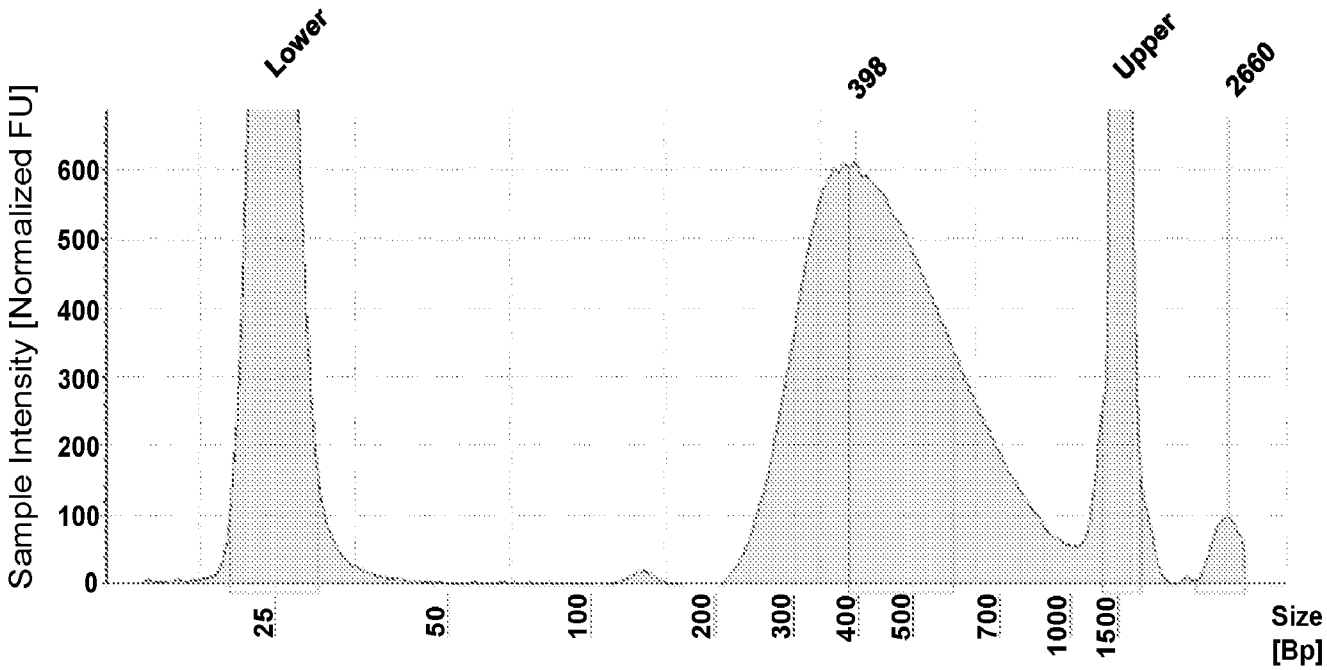

Lower
398
Upper
2660
Sample Intensity [Normalized FU]
600
500
400
300
200
100
0
25
50
100
200
300
400
500
700
1000
1500
Size
[Bp]

Fig. 12

ii

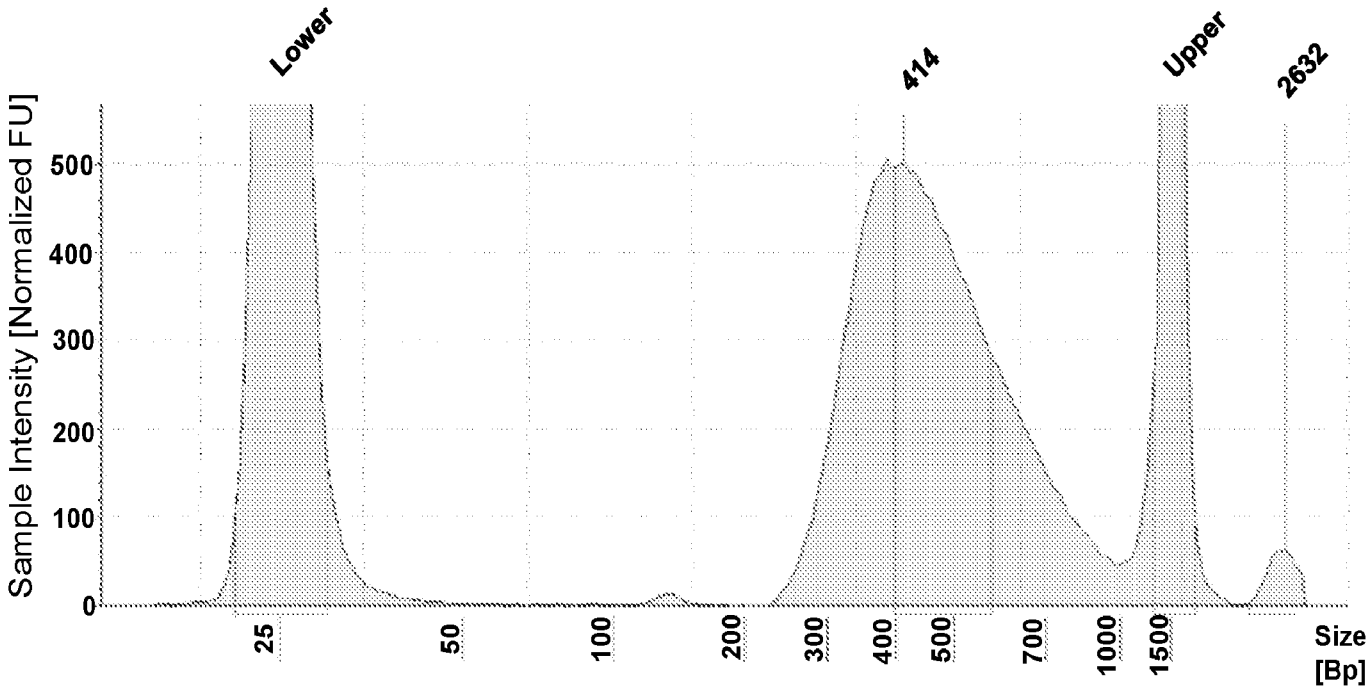


Fig. 12 (cont.)

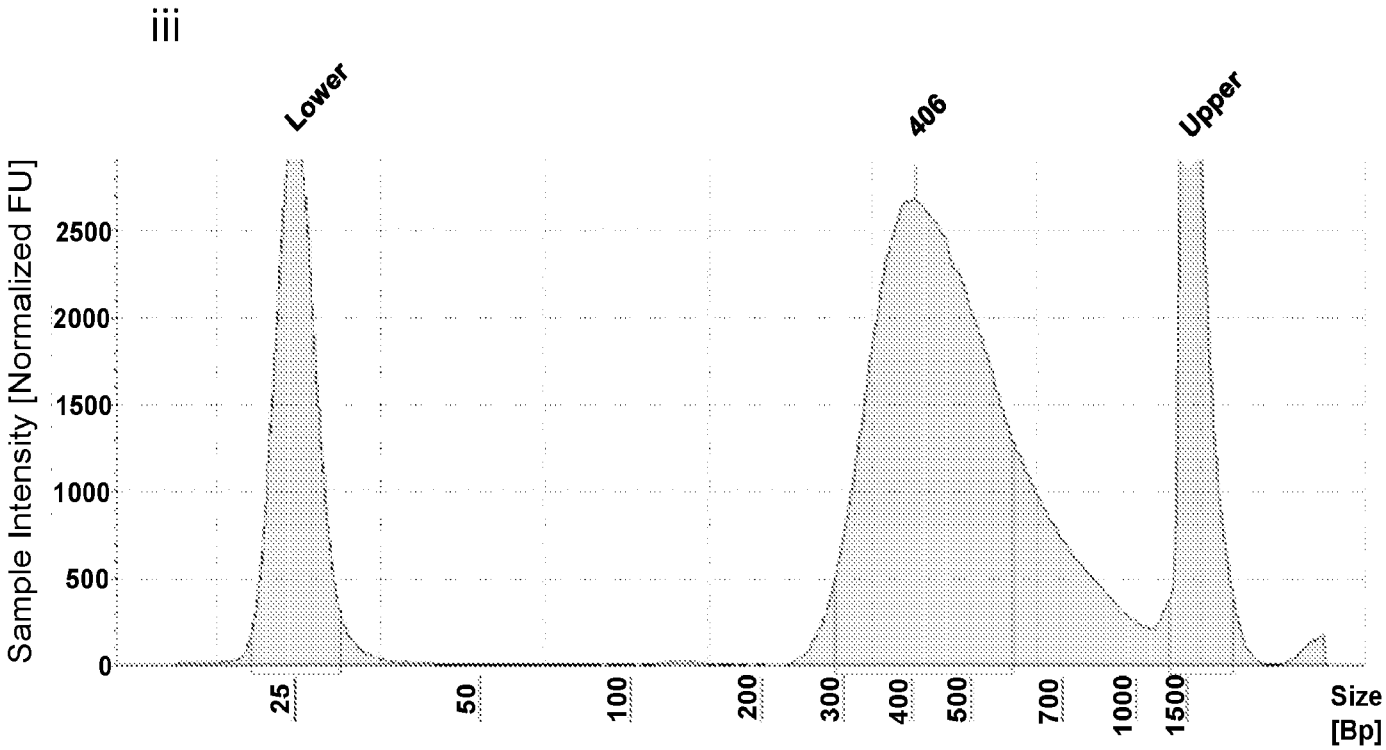

iii
Lower
406
Upper
Sample Intensity [Normalized FU]
2500
2000
1500
1000
500
0
25
50
100
200
300
400
500
700
1000
1500
Size
[Bp]

Fig. 12 (cont.)

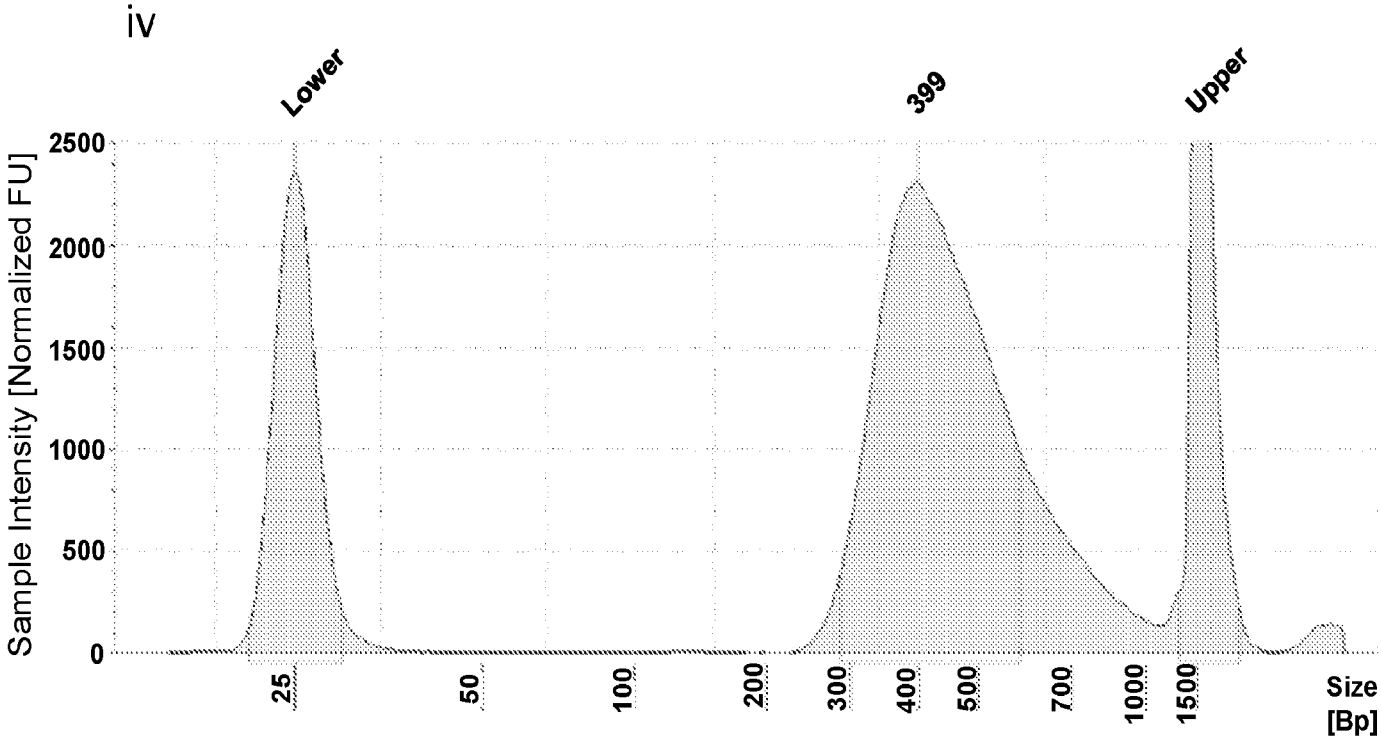
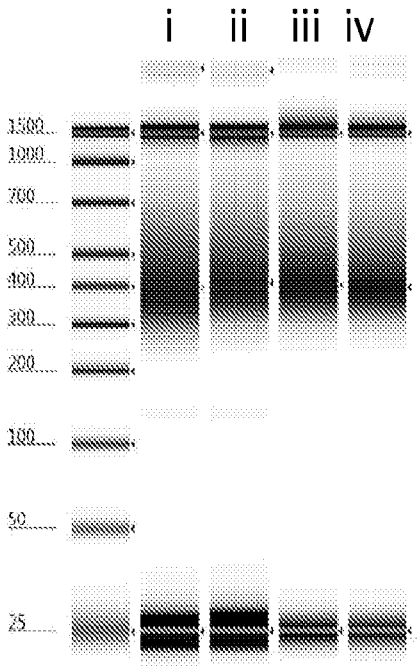

iv
Lower
399
Upper
Sample Intensity [Normalized FU]
2500
2000
1500
1000
500
0
25
50
100
200
300
400
500
700
1000
1500
Size
[Bp]

Fig. 12 (cont.)



	RNA input (ng)	Quantity (ng/µl)	Tapestation (bp)
i	10	12	398
ii	10	11.2	414
iii	10	28.2	406
iv	10	28.5	399

Fig. 13

	i	ii	iii	iv
Number of input reads	441589	272701	250203	250592
Average input read length	202	202	202	202
Uniquely mapped reads number	337546	205866	208486	213879
Uniquely mapped reads %	76.44%	75.49%	83.33%	85.35%
Average mapped length	196.76	196.85	200.6	200.63
Number of reads mapped to multiple loci	22877	14759	7711	7877
% of reads mapped to multiple loci	5.18%	5.41%	3.08%	3.14%
Number of reads mapped to too many loci	445	286	110	86
% of reads mapped to too many loci	0.10%	0.10%	0.04%	0.03%
% of reads unmapped: too many mismatches	0.00%	0.00%	0.00%	0.00%
% of reads unmapped: too short	18.26%	18.97%	13.52%	11.45%
% of reads unmapped: other	0.02%	0.02%	0.02%	0.02%
Number of chimeric reads	0	0	0	0
% of chimeric reads	0.00%	0.00%	0.00%	0.00%

Fig. 14

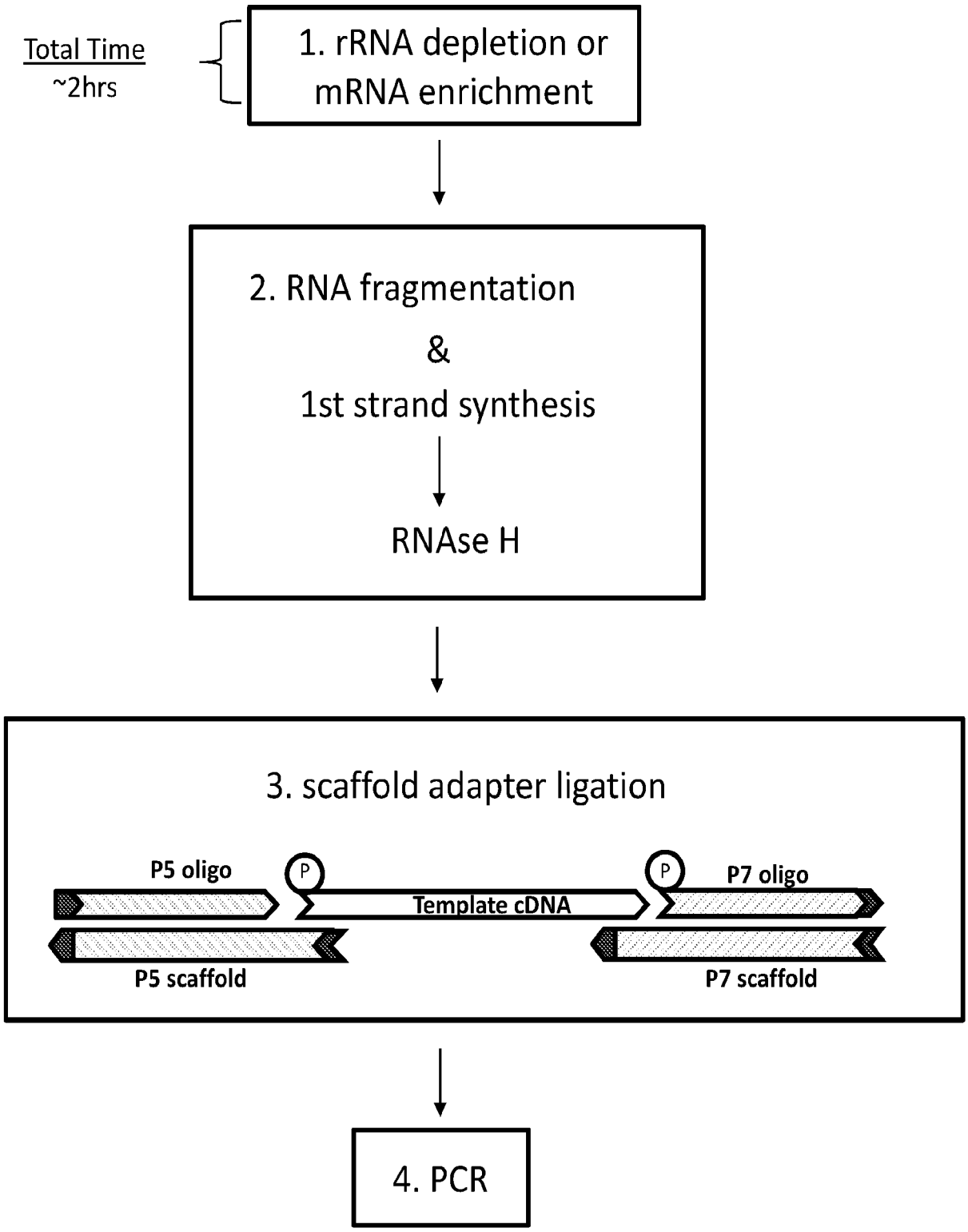

Total Time
~2hrs
1. rRNA depletion or mRNA enrichment
2. RNA fragmentation
&
1st strand synthesis
RNAse H
3. scaffold adapter ligation
P5 oligo
P
Template cDNA
P
P7 oligo
P5 scaffold
P7 scaffold
4. PCR

Fig. 15

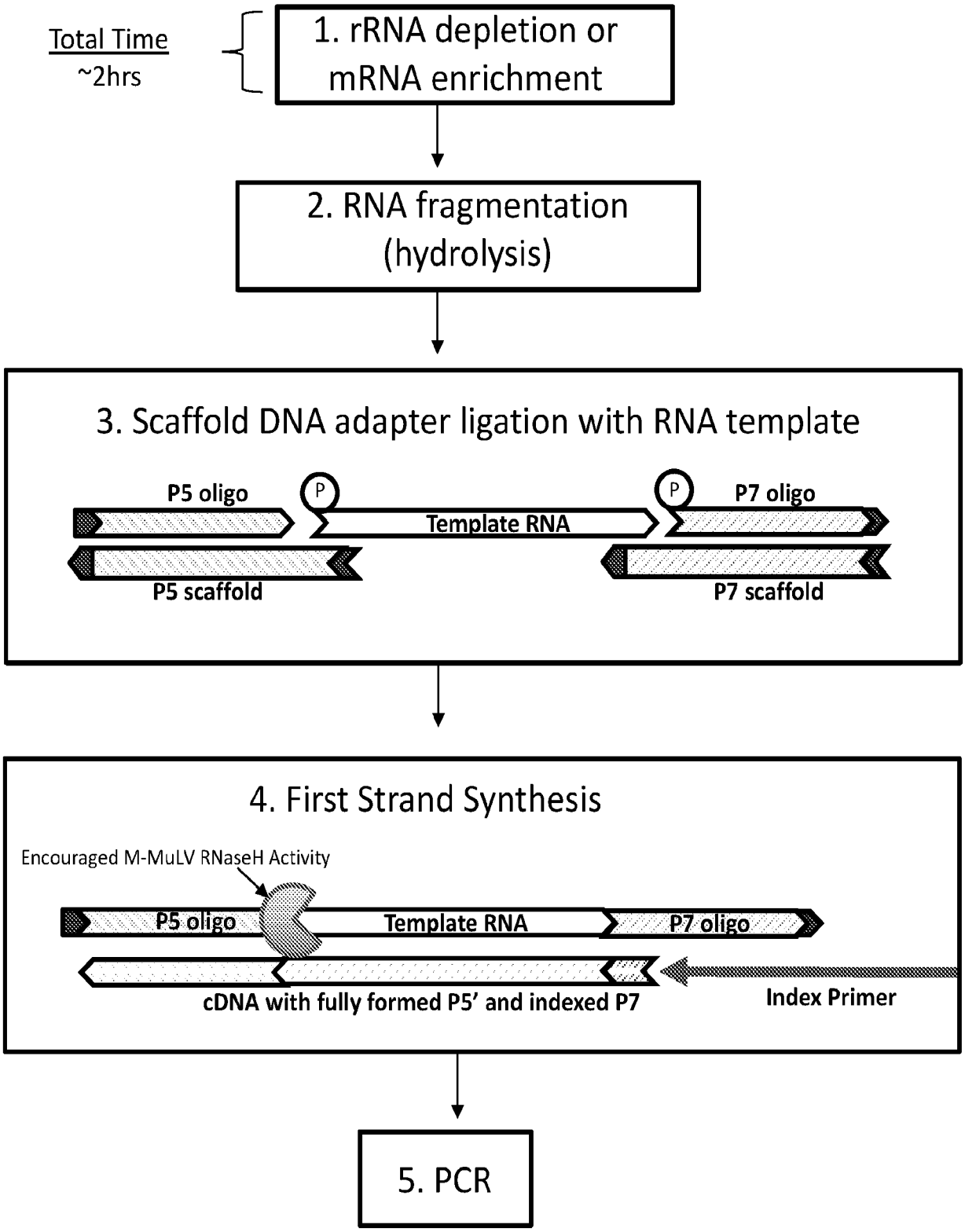

Total Time
~2hrs
1. rRNA depletion or mRNA enrichment
2. RNA fragmentation (hydrolysis)
3. Scaffold DNA adapter ligation with RNA template
P5 oligo
P
Template RNA
P
P7 oligo
P5 scaffold
P7 scaffold
4. First Strand Synthesis
Encouraged M-MuLV RNaseH Activity
P5 oligo
Template RNA
P7 oligo
cDNA with fully formed P5' and indexed P7
Index Primer
5. PCR

Fig. 16

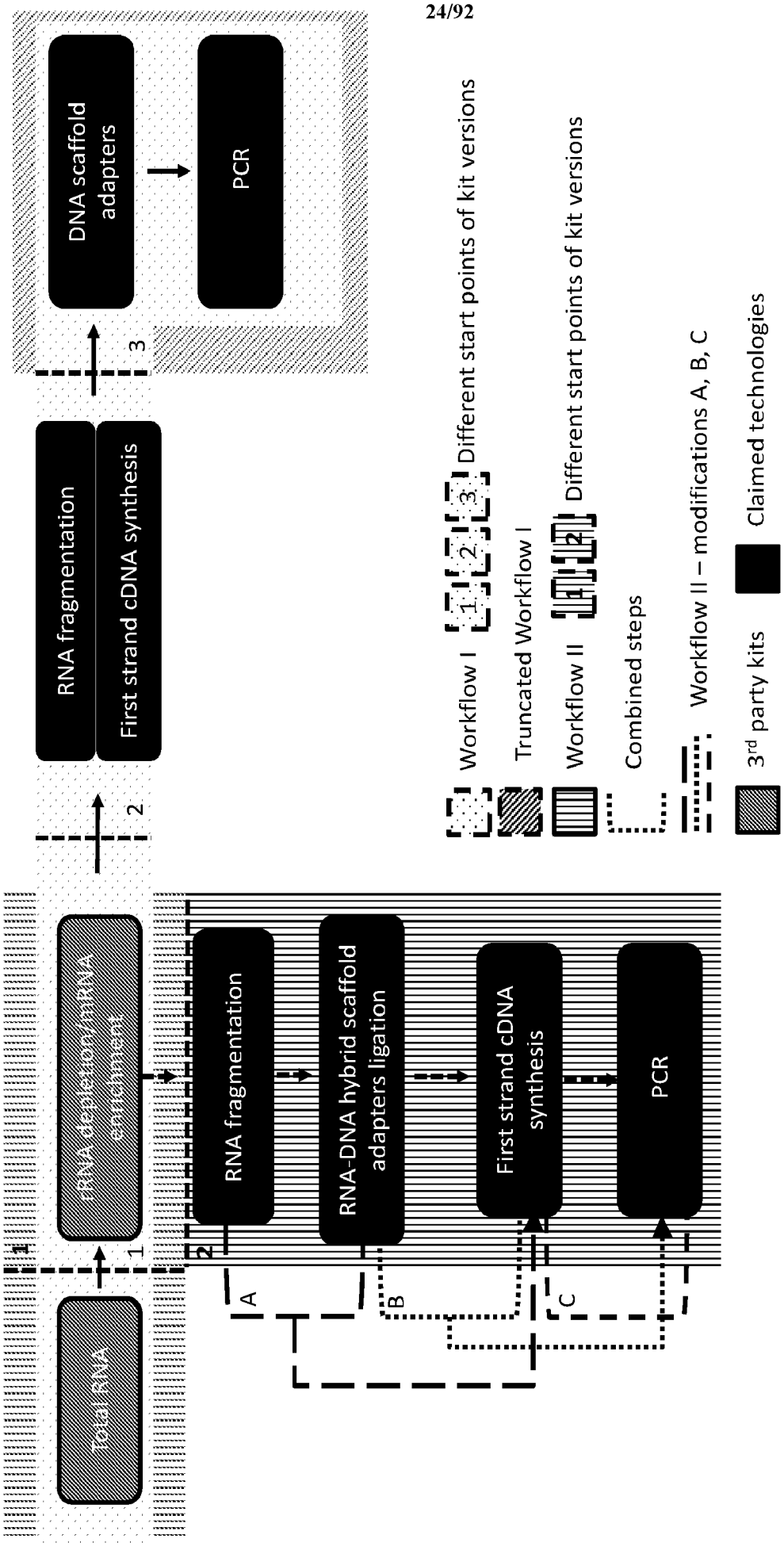
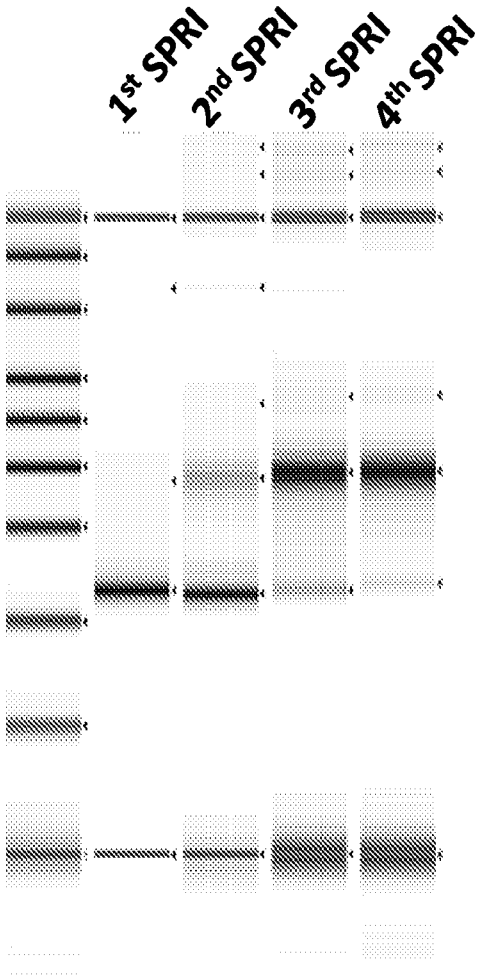

Total RNA
rRNA depletion/mRNA enrichment
RNA fragmentation
RNA-DNA hybrid scaffold adapters ligation
First strand cDNA synthesis
PCR
A
B
C
1
2
RNA fragmentation
First strand cDNA synthesis
DNA scaffold adapters
PCR
3
Workflow I
Different start points of kit versions
Truncated Workflow I
Workflow II
Different start points of kit versions
Combined steps
Workflow II – modifications A, B, C
3rd party kits
Claimed technologies

Fig. 17



Purpose	Input cfDNA	cfDNA Purification Kit	Original Tapestation Dimer %	1st Serial 1.2x SPRI Dimer %	2nd Serial 1.2x SPRI Dimer %	3rd Serial 1.2x SPRI Dimer %
1.2x Serial SPRI Clean x3	s0072	Zymo	87.55	52.13	12.97	5.48

Fig. 18

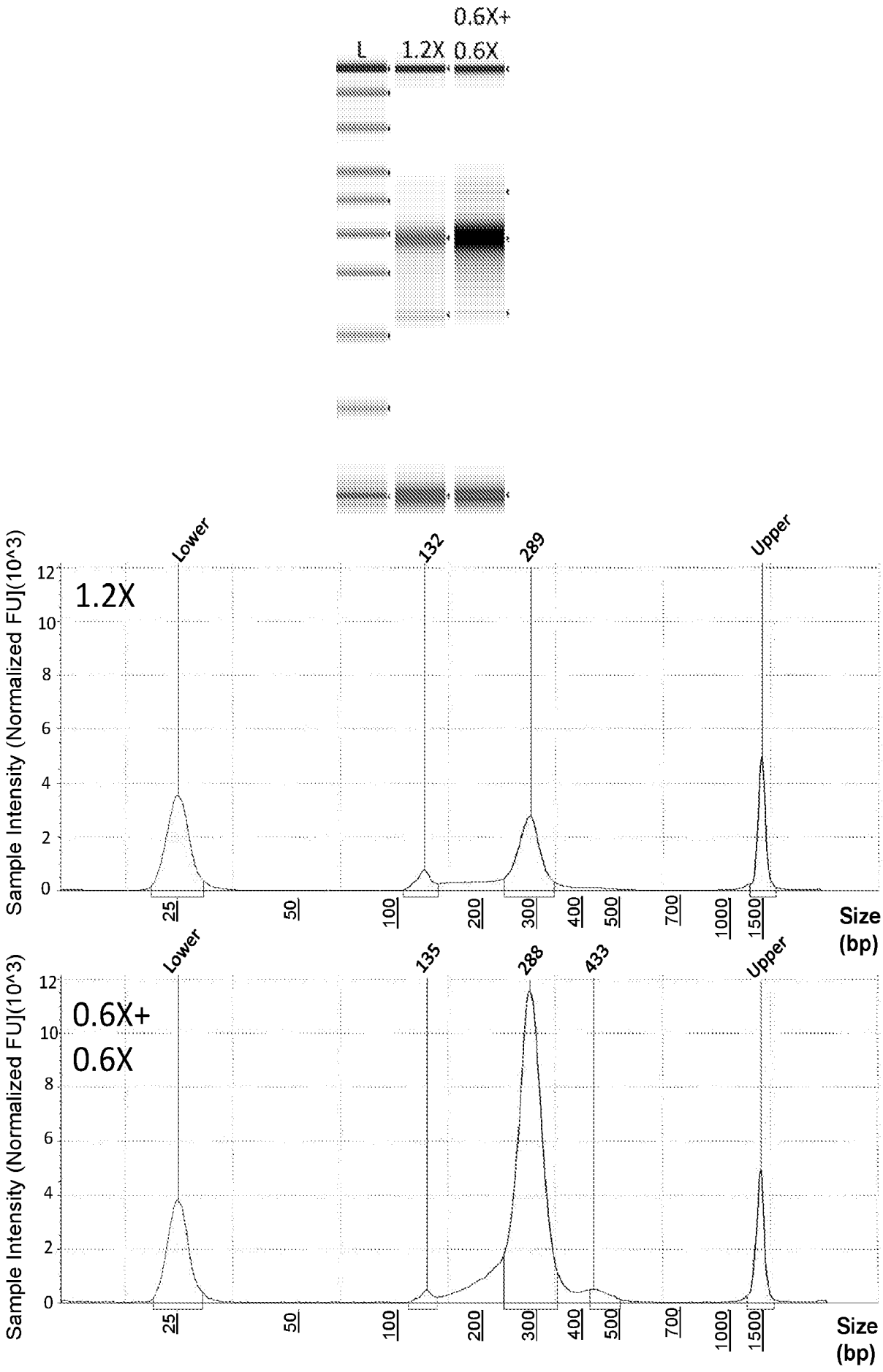

0.6X+
L
1.2X
0.6X
Lower
132
289
Upper
1.2X
Sample Intensity (Normalized FU](10^3)
Size (bp)
Lower
135
288
433
Upper
0.6X+
0.6X
Sample Intensity (Normalized FU](10^3)
Size (bp)

Fig. 19

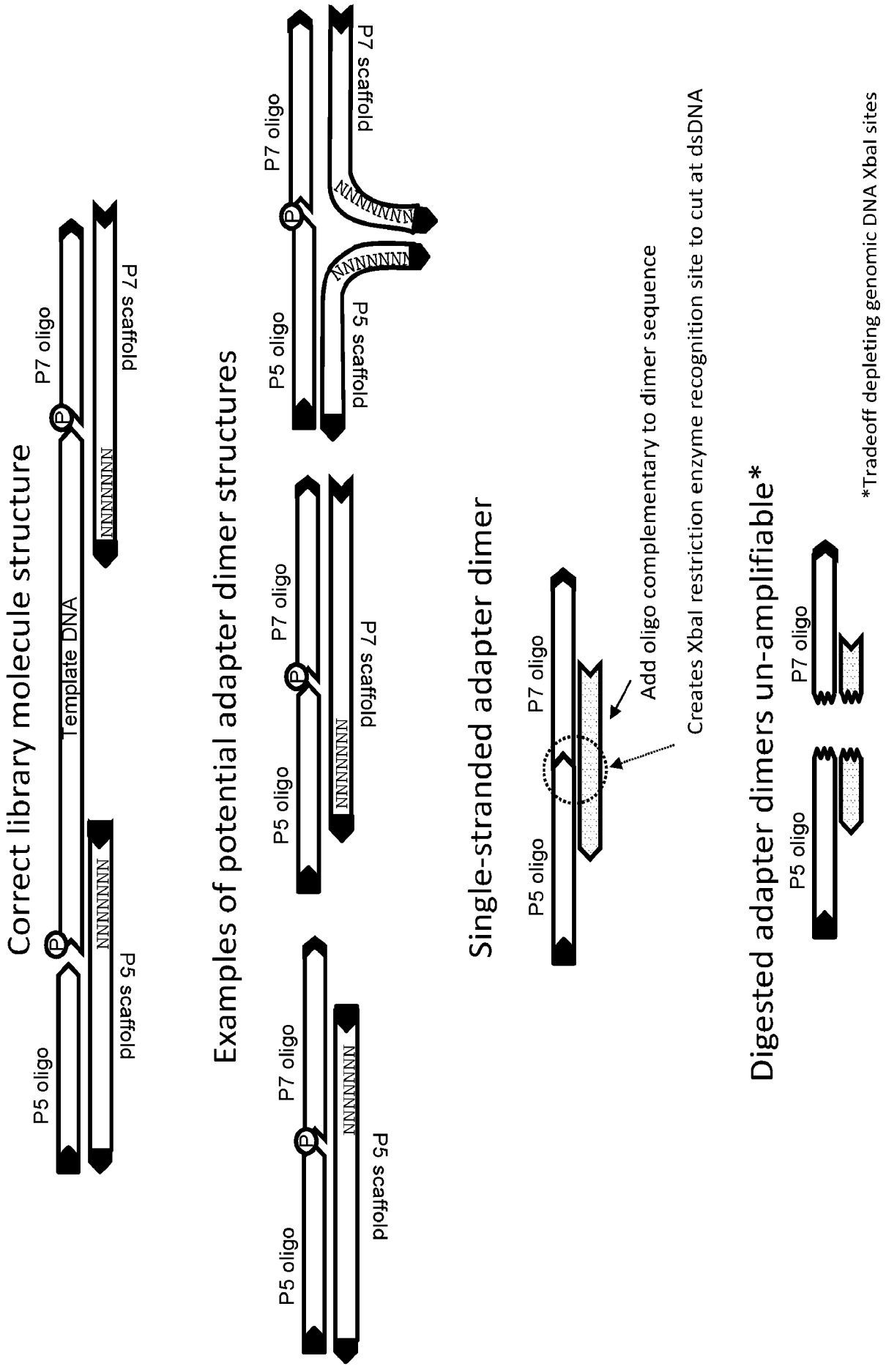

Correct library molecule structure
P7 oligo
P
Template DNA
P7 scaffold
NNNNNNN
P5 oligo
P5 scaffold
Examples of potential adapter dimer structures
P7 oligo
P7 scaffold
P5 oligo
P5 scaffold
Single-stranded adapter dimer
P7 oligo
P5 oligo
Add oligo complementary to dimer sequence
Creates XbaI restriction enzyme recognition site to cut at dsDNA
Digested adapter dimers un-amplifiable*
P7 oligo
P5 oligo
*Tradeoff depleting genomic DNA XbaI sites

Fig. 20

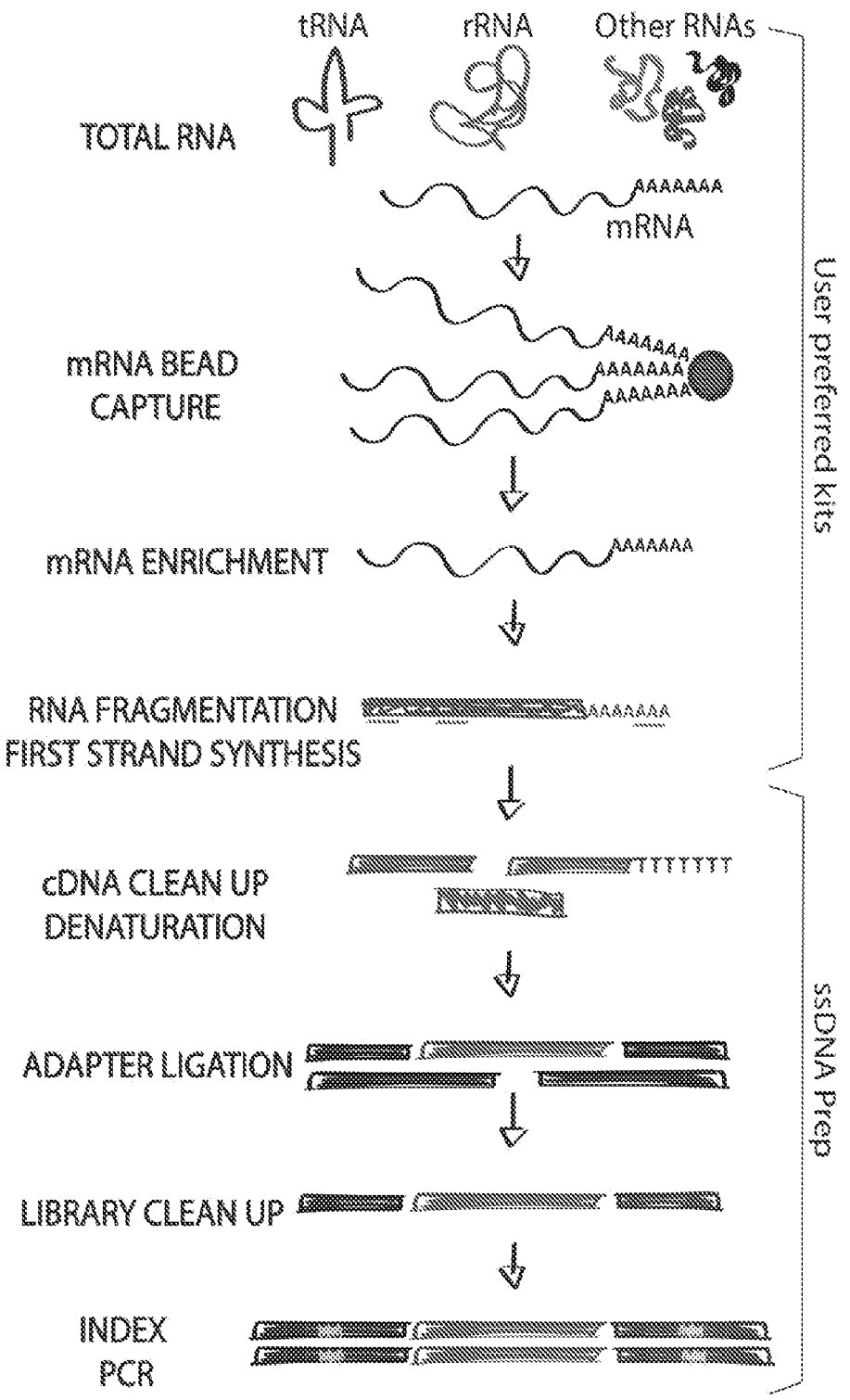

tRNA
rRNA
Other RNAs
TOTAL RNA
mRNA
mRNA BEAD CAPTURE
mRNA ENRICHMENT
RNA FRAGMENTATION
FIRST STRAND SYNTHESIS
cDNA CLEAN UP
DENATURATION
ADAPTER LIGATION
LIBRARY CLEAN UP
INDEX PCR
User preferred kits
ssDNA Prep

Fig. 21

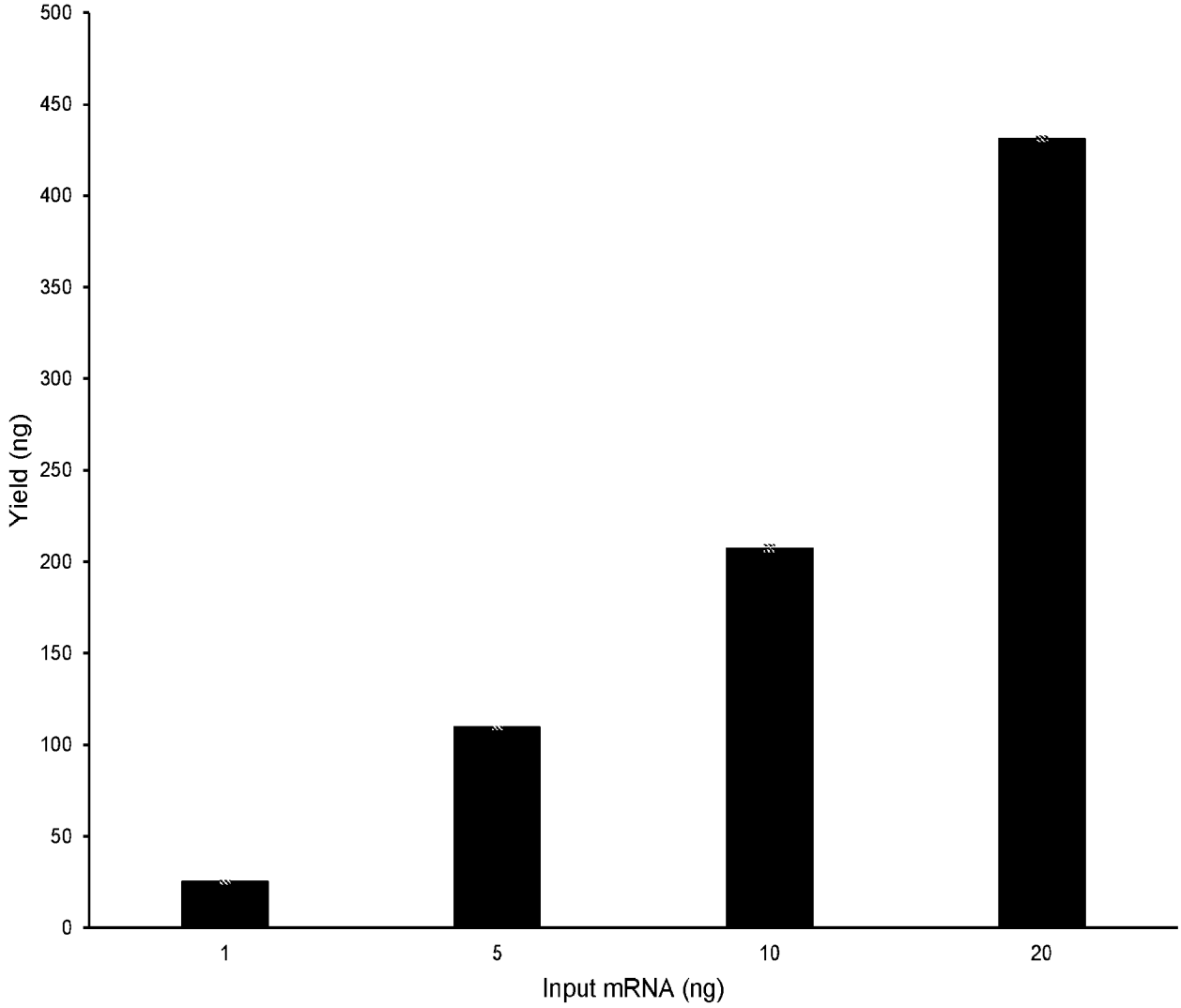

500
450
400
350
300
250
200
150
100
50
0
Yield (ng)
1
5
10
20
Input mRNA (ng)

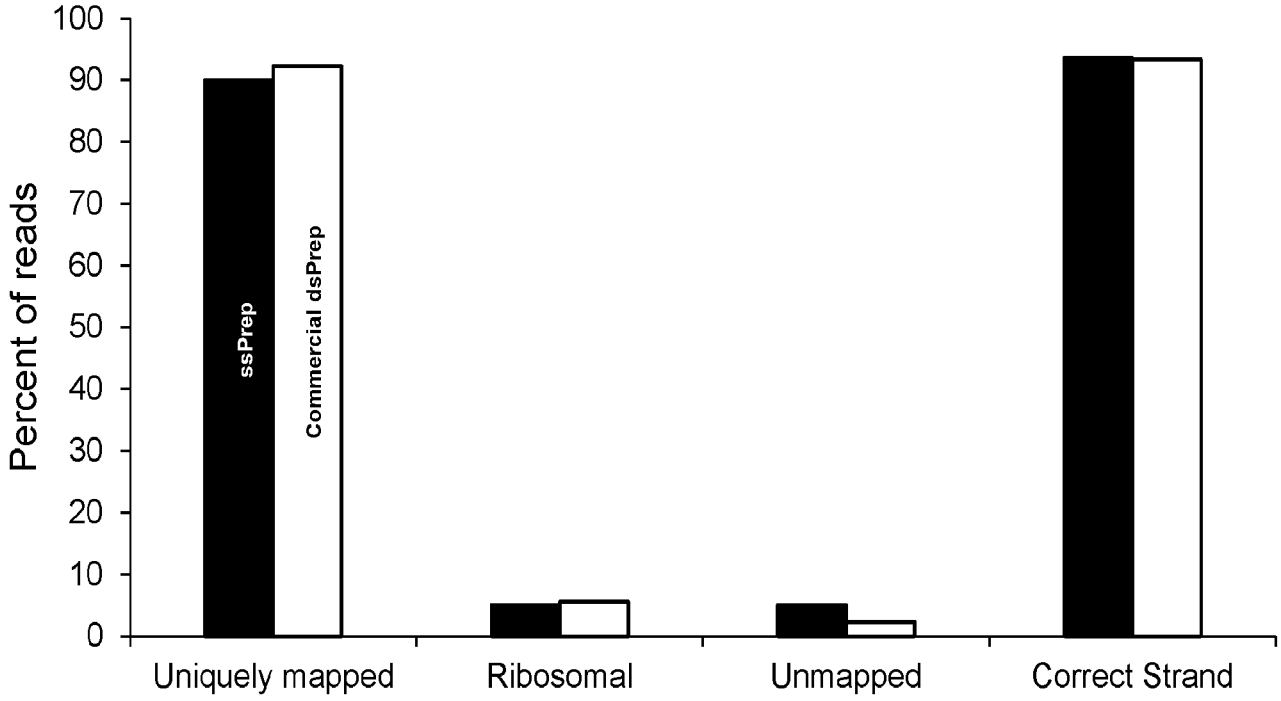
Fig. 22

Directional RNA-Seq Kits	Method Type	Input mRNA	Protocol time (hours)*	PCR cycles for 10ng input
ssPrep	Single-stranded	1-20ng	**~2**	**9**
commercial kit 1	Double-stranded	1-100ng	**~5**	**9-10**
commercial kit 2	Double-stranded	Up to 100ng	**~5**	**>15**
commercial kit 3	Double-stranded	10-100ng	**~6.5**	**15**

Fig. 23

Kit	Type of Prep Method	Sample	Yield	Input Reads	Uniquely mapped reads	Average mapped length
ssPrep	Single-stranded	ssPrep-1	378 ng	12,821,116	11,558,853	145
		ssPrep-2	370 ng	13,332,866	11,963,648	145
Commercial kit	Double-stranded	dsPrep-1	386 ng	15,247,791	14,059,492	149
		dsPrep-2	432 ng	15,716,512	14,488,921	149

Fig. 24


Percent of reads
100
90
80
70
60
50
40
30
20
10
0
ssPrep
Commercial dsPrep
Uniquely mapped
Ribosomal
Unmapped
Correct Strand

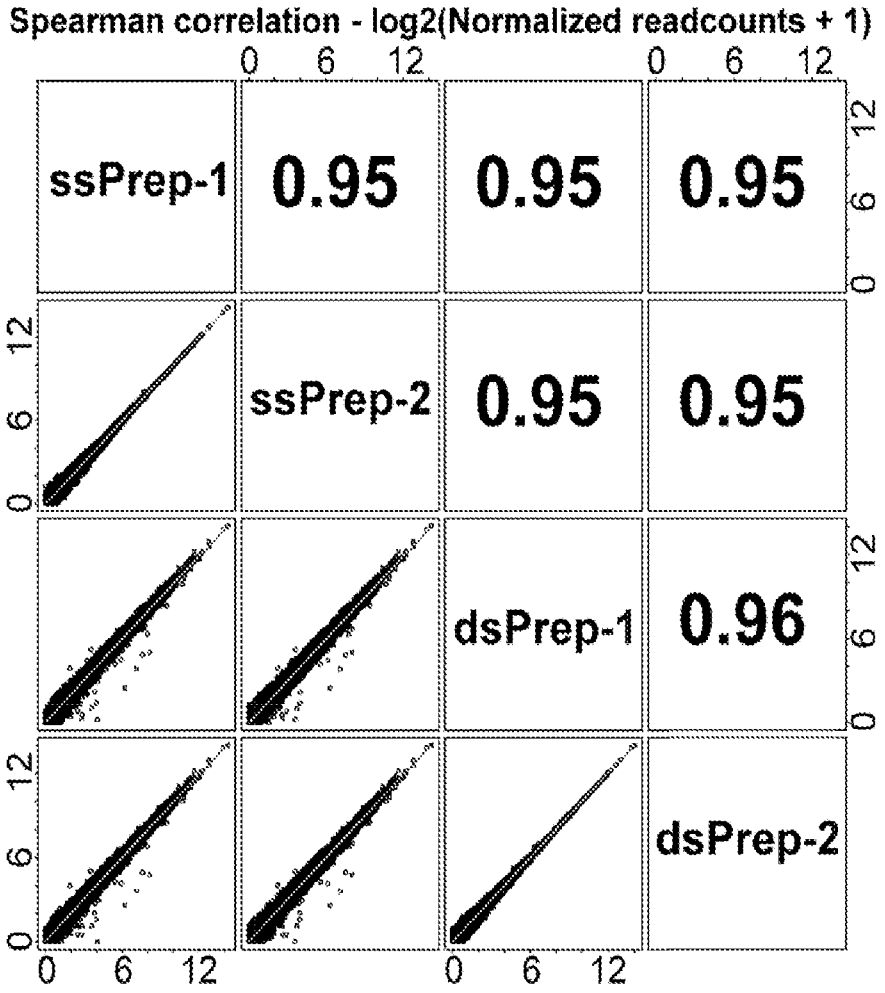

Spearman correlation - log2(Normalized readcounts + 1)
ssPrep-1
ssPrep-2
dsPrep-1
dsPrep-2
0.95
0.95
0.95
0.95
0.95
0.96
0
6
12

Fig. 25

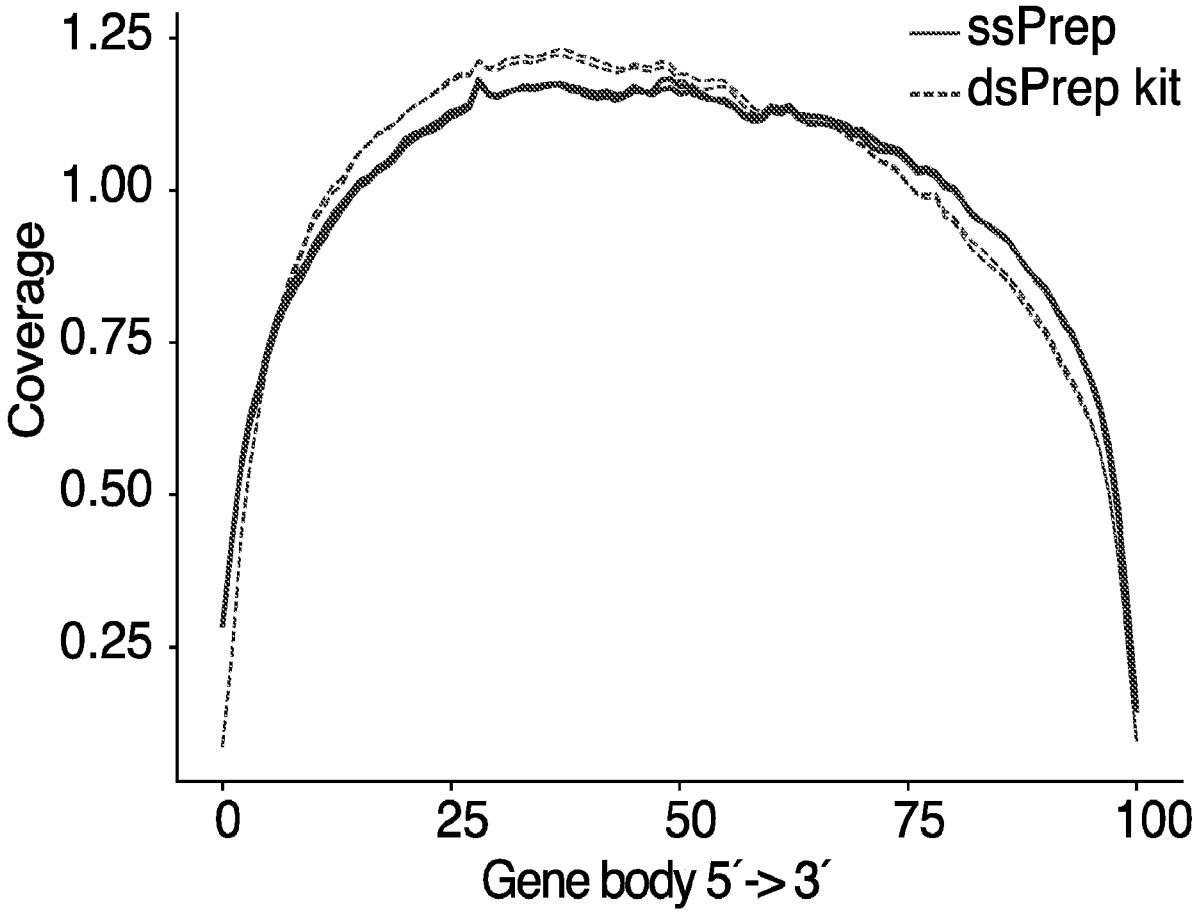

ssPrep
dsPrep kit
Coverage
1.25
1.00
0.75
0.50
0.25
0
25
50
75
100
Gene body 5´-> 3´

Fig. 26

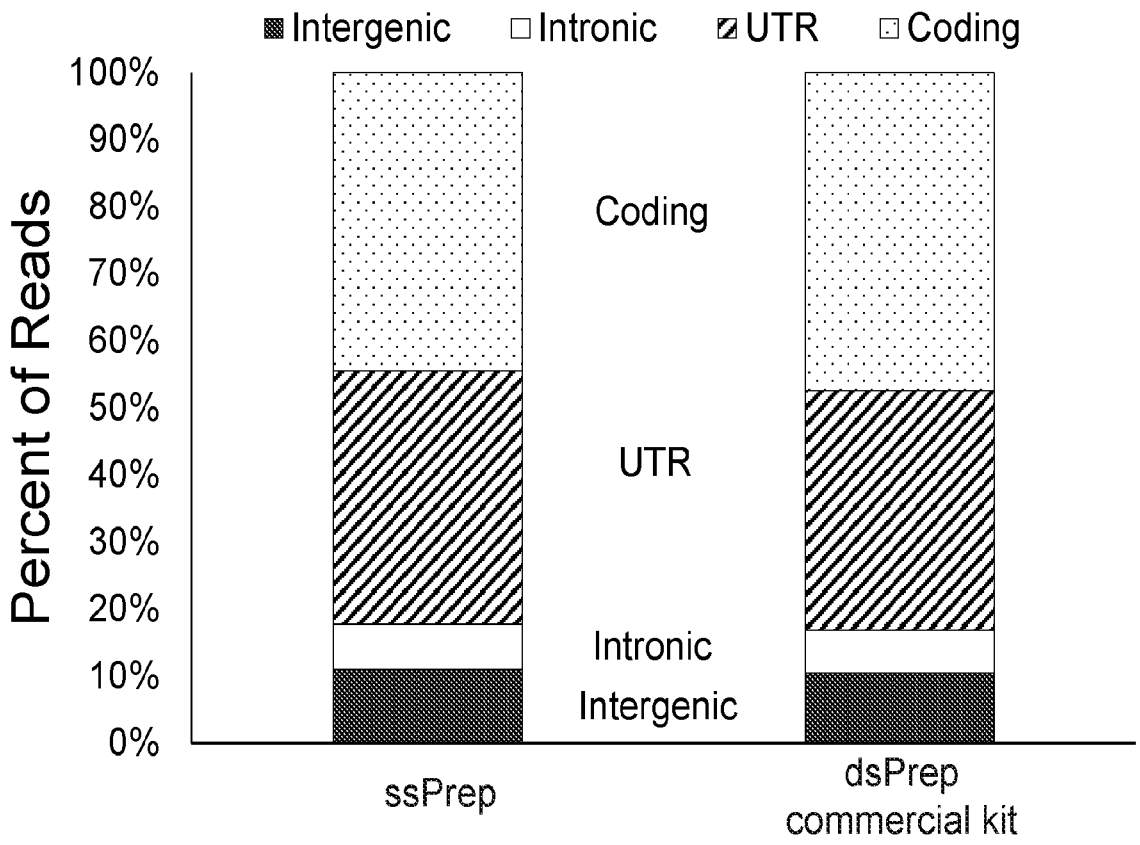

Intergenic
Intronic
UTR
Coding
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Percent of Reads
Coding
UTR
Intronic
Intergenic
ssPrep
dsPrep
commercial kit

Fig. 26 (cont.)

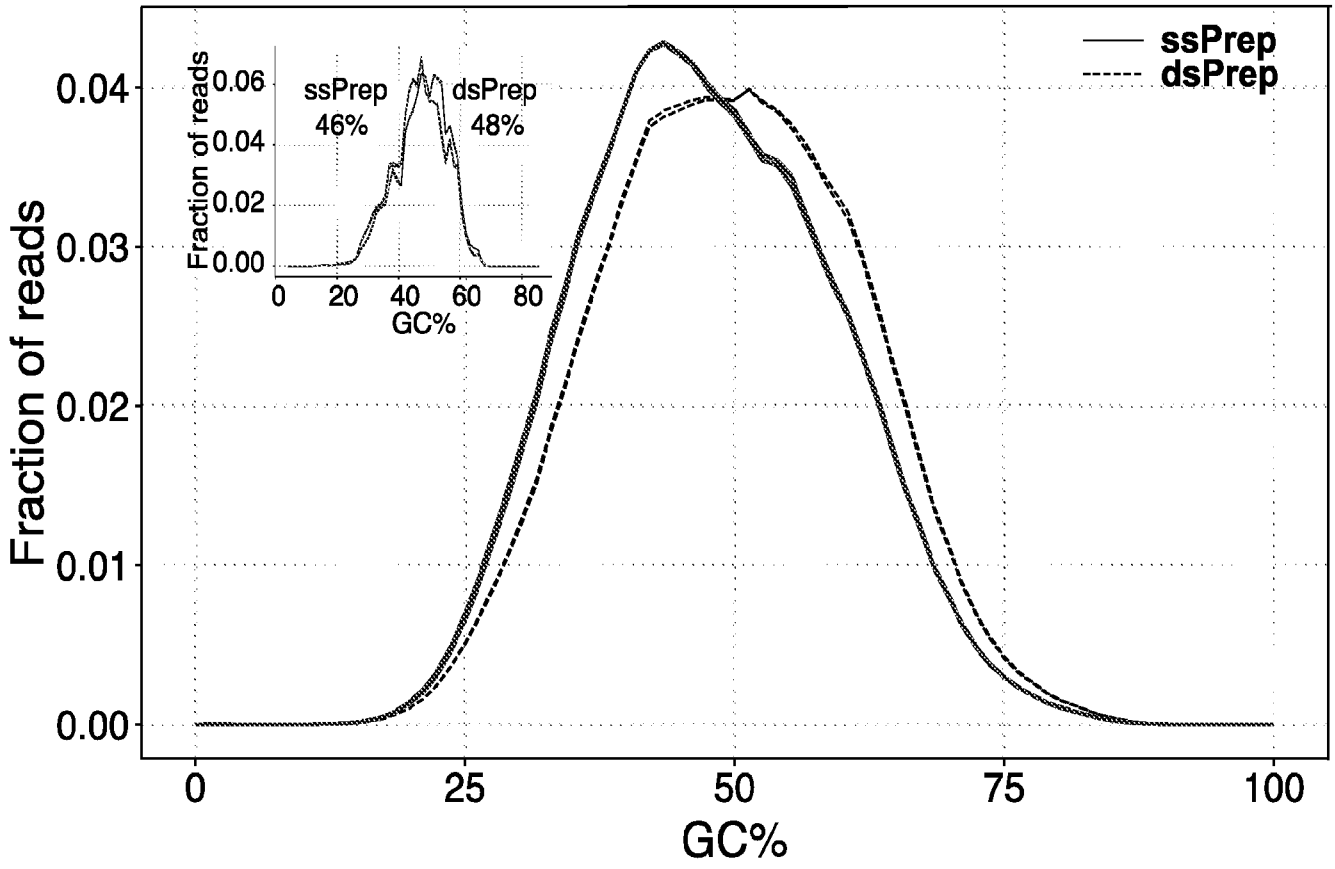

ssPrep
dsPrep
Fraction of reads
0.04
0.03
0.02
0.01
0.00
0
25
50
75
100
GC%
Fraction of reads
0.06
0.04
0.02
0.00
ssPrep
46%
dsPrep
48%
0
20
40
60
80
GC%

Fig. 26 (cont.)

Library	Total ERCC reads	Incorrect Strand	Correct Strand	Percent Correct Strand
ssPrep	349499	1845	347654	99.47
ssPrep	348261	2199	346062	99.37
dsPrep	384339	858	383481	99.78
dsPrep	389578	764	388814	99.80

Fig. 27

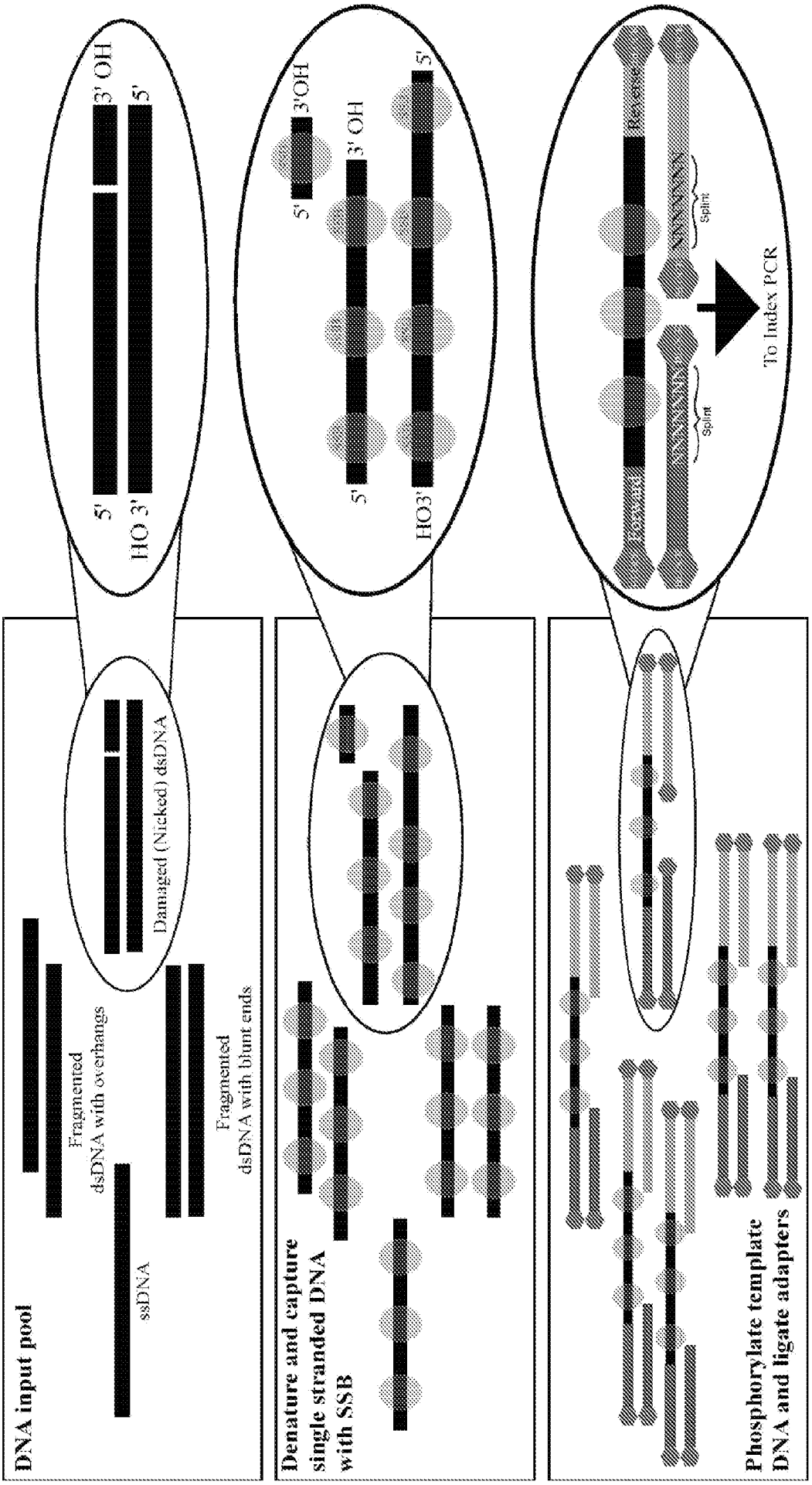

DNA input pool
ssDNA
Fragmented dsDNA with overhangs
Damaged (Nicked) dsDNA
Fragmented dsDNA with blunt ends
3' OH
5'
5'
HO 3'
Denature and capture single stranded DNA with SSB
3'OH
5'
3' OH
5'
5'
HO3'
Phosphorylate template DNA and ligate adapters
Reverse
Splint
Splint
To Index PCR

Fig. 28

A

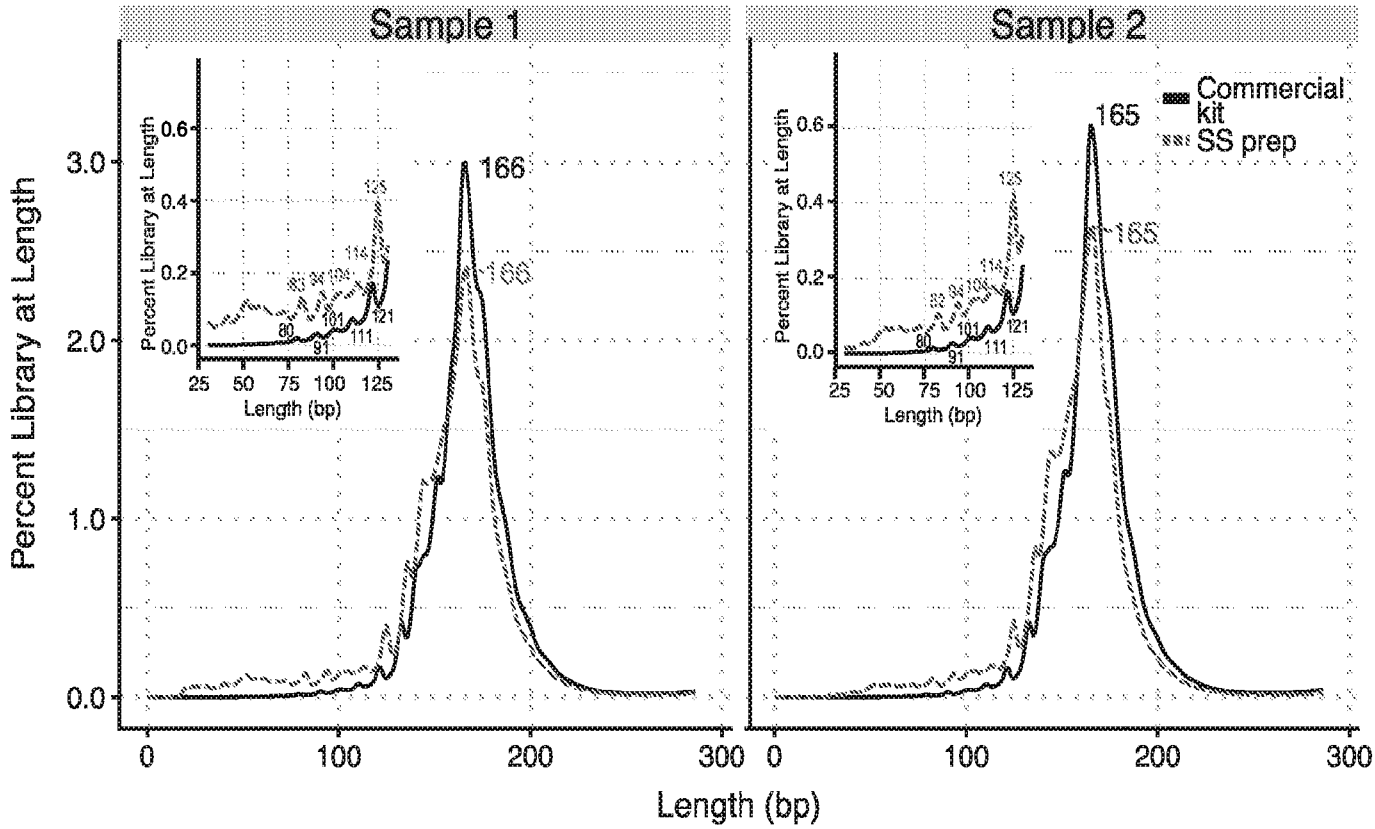

Sample 1
Sample 2
Commercial kit
SS prep
Percent Library at Length
Length (bp)
166
165
80
91
101
111
121
125

Fig. 29

B

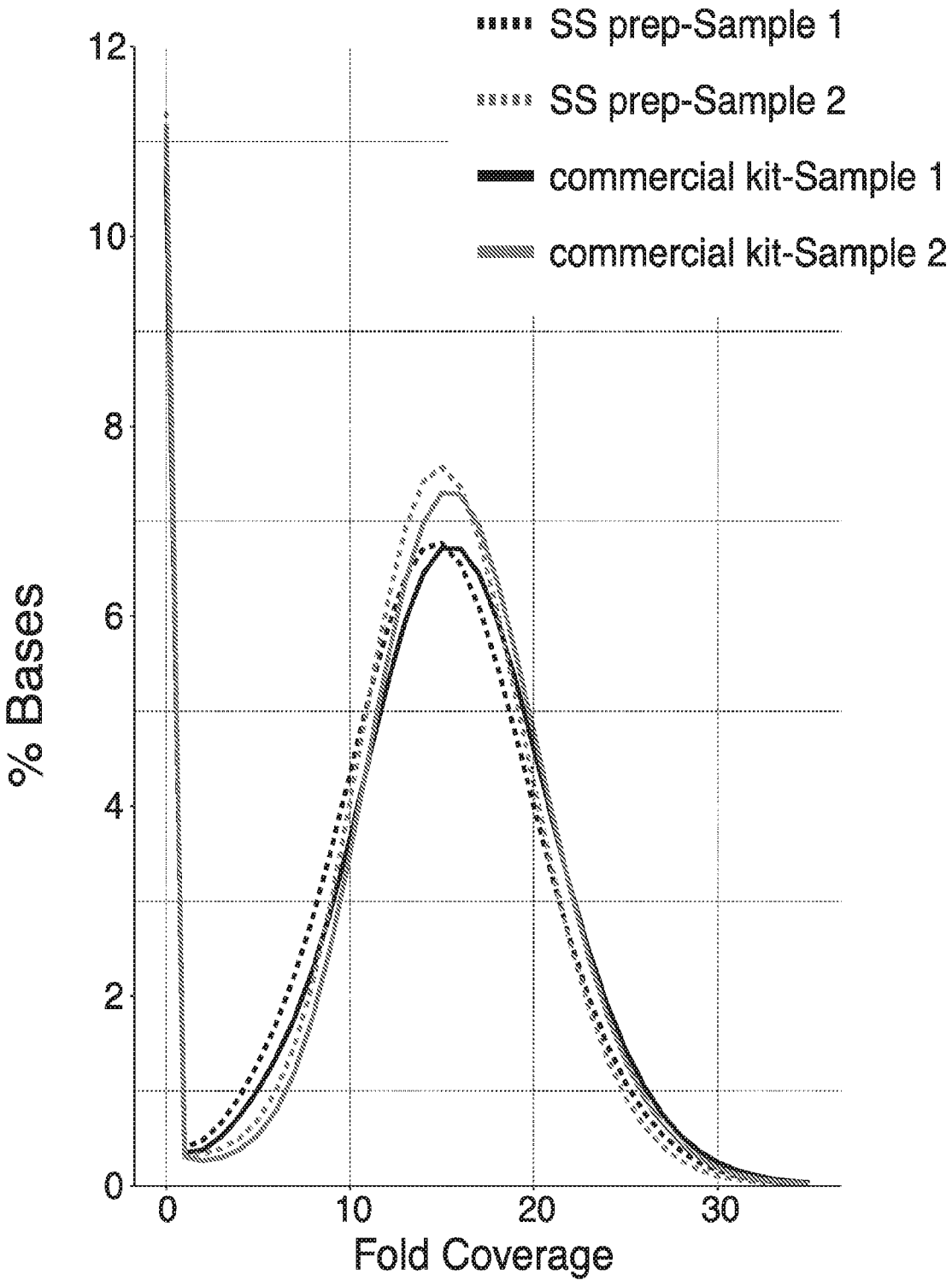
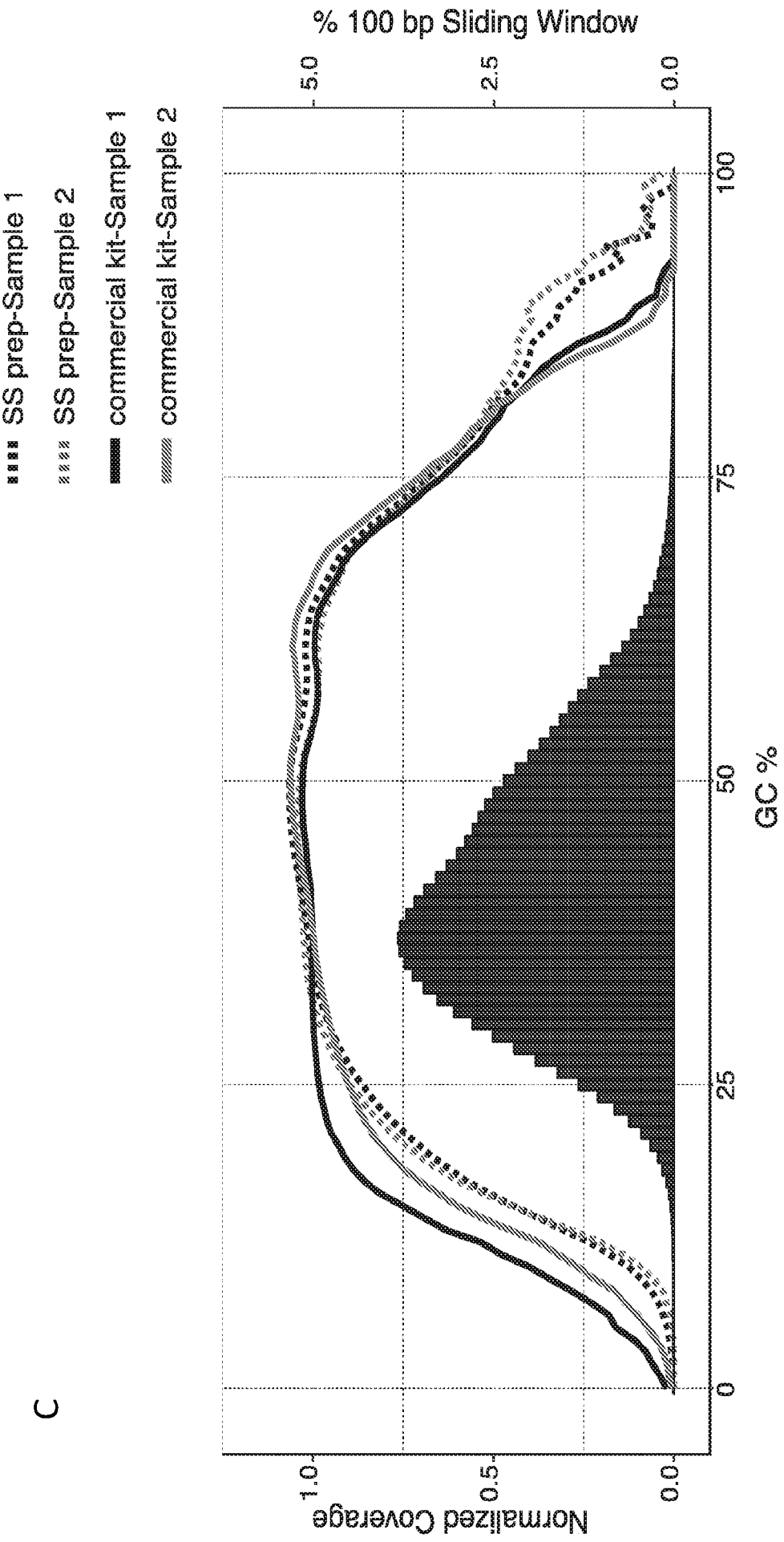

SS prep-Sample 1
SS prep-Sample 2
commercial kit-Sample 1
commercial kit-Sample 2
% Bases
Fold Coverage
0
2
4
6
8
10
12
0
10
20
30

Fig. 29 (cont.)


% 100 bp Sliding Window
5.0
2.5
0.0
SS prep-Sample 1
SS prep-Sample 2
commercial kit-Sample 1
commercial kit-Sample 2
Normalized Coverage
1.0
0.5
0.0
0
25
50
75
100
GC %

C

Fig. 29 (cont.)

D

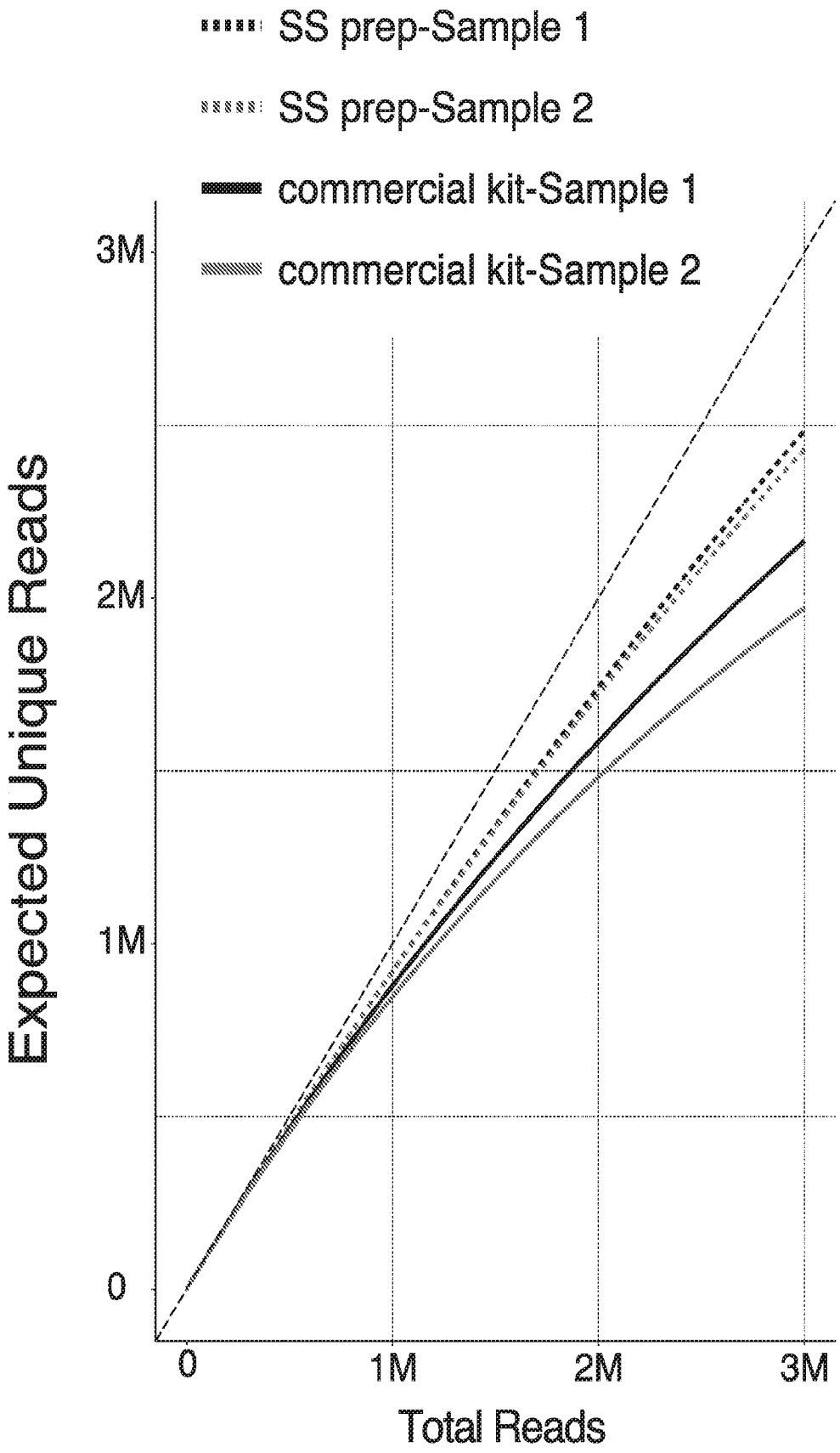

SS prep-Sample 1
SS prep-Sample 2
commercial kit-Sample 1
commercial kit-Sample 2
Expected Unique Reads
3M
2M
1M
0
0
1M
2M
3M
Total Reads

Fig. 29 (cont.)

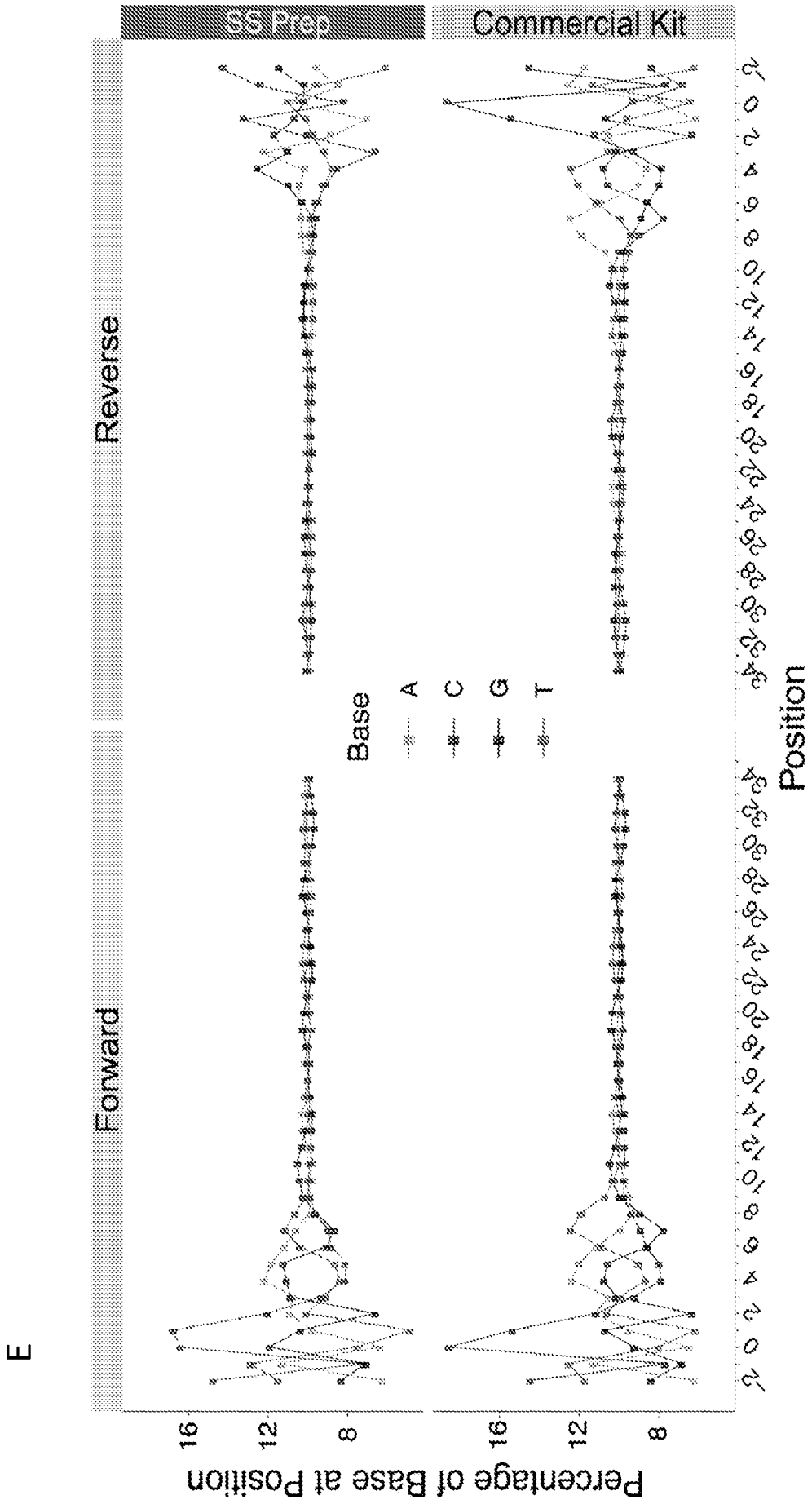

E
SS Prep
Commercial Kit
Reverse
Forward
Base
A
C
G
T
Position
Percentage of Base at Position
16
12
8

Fig. 29 (cont.)

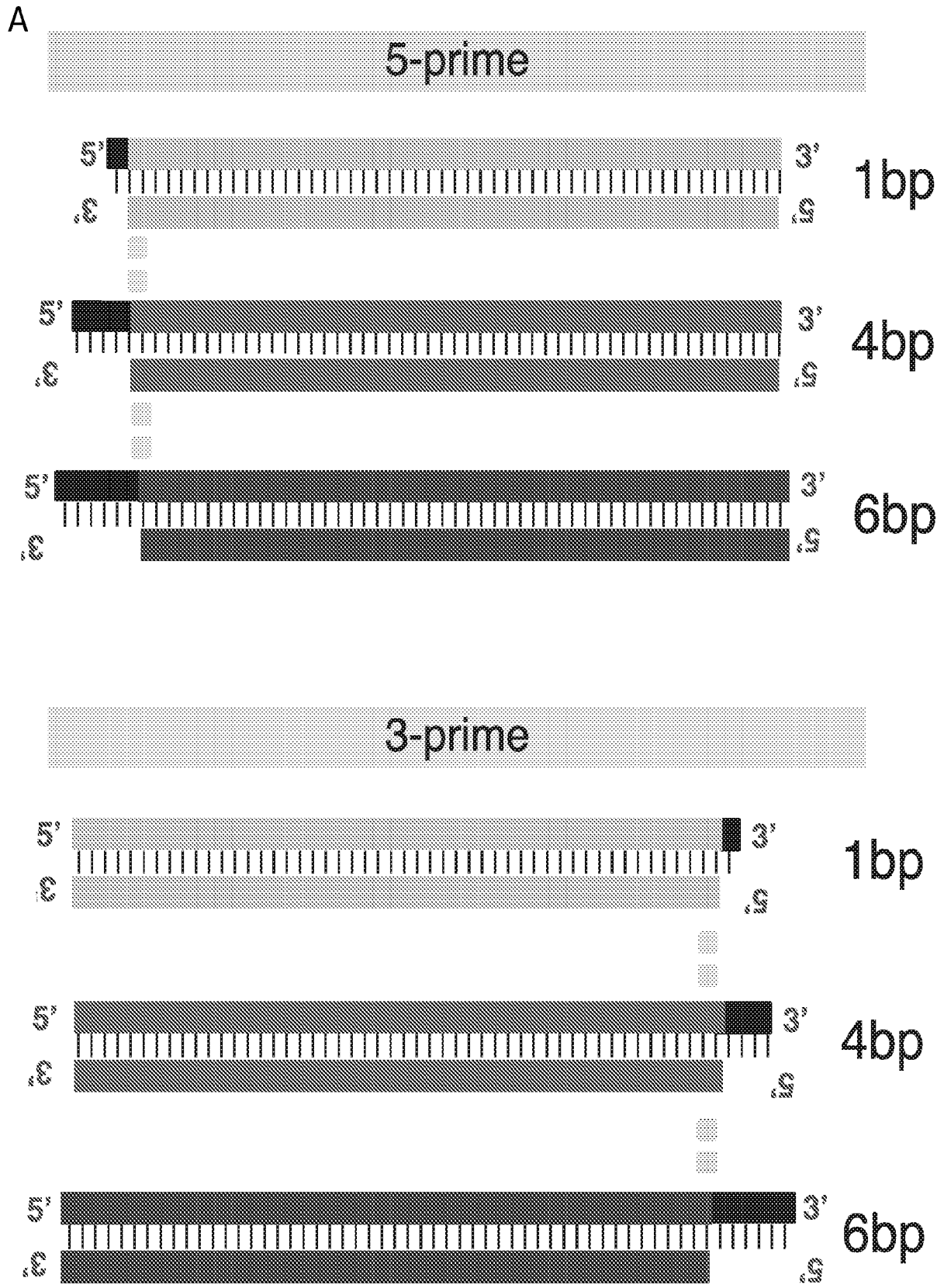


Fig. 30

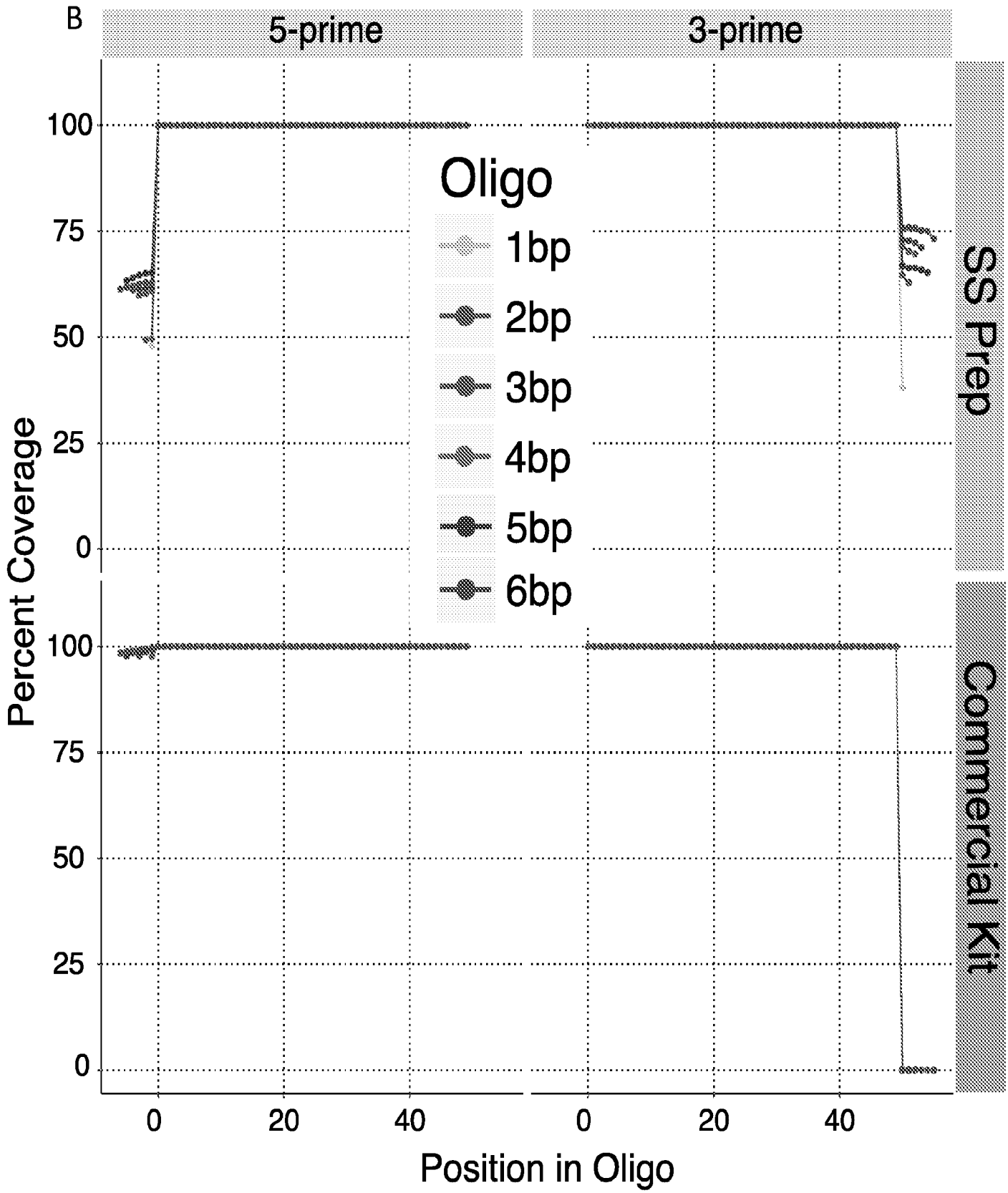

B
5-prime
3-prime
SS Prep
Commercial Kit
Oligo
1bp
2bp
3bp
4bp
5bp
6bp
Percent Coverage
100
75
50
25
0
0
20
40
Position in Oligo

Fig. 30 (cont.)

A

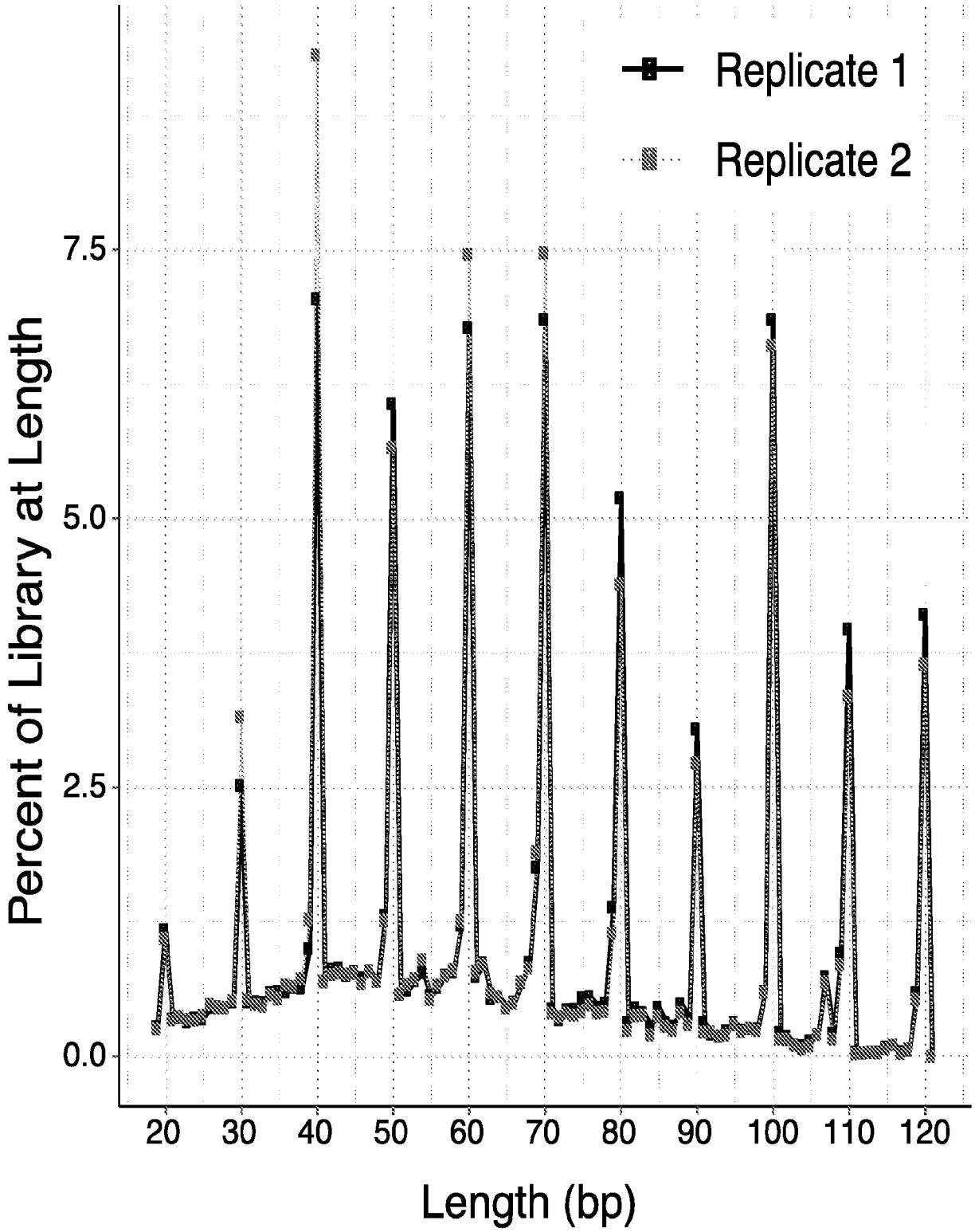

Replicate 1
Replicate 2
Percent of Library at Length
7.5
5.0
2.5
0.0
20
30
40
50
60
70
80
90
100
110
120
Length (bp)

Fig. 31

B

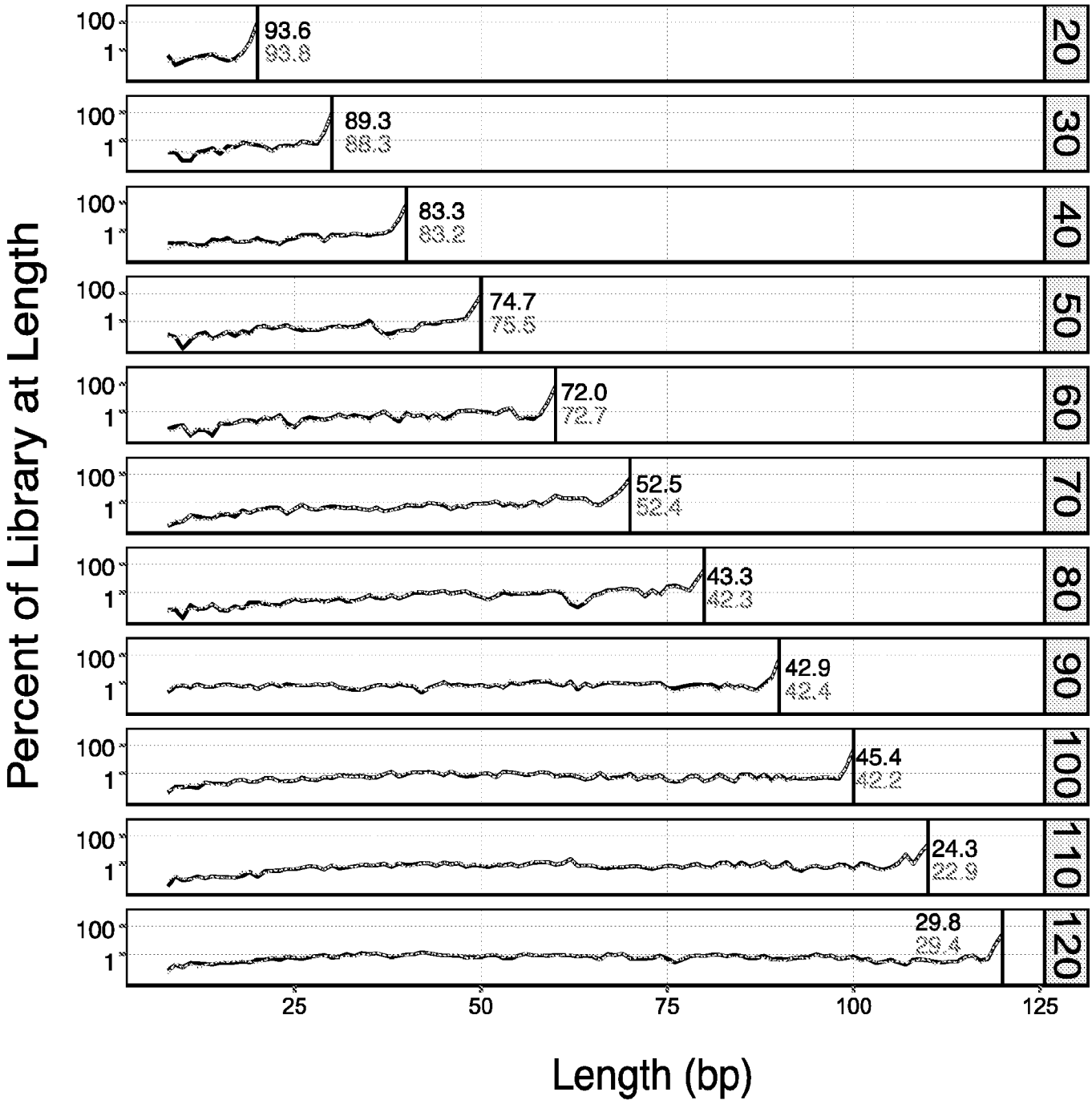

Percent of Library at Length
100
1
93.6
93.8
20
89.3
88.3
30
83.3
83.2
40
74.7
75.5
50
72.0
72.7
60
52.5
52.4
70
43.3
42.3
80
42.9
42.4
90
45.4
42.2
100
24.3
22.3
110
29.8
29.4
120
25
50
75
100
125
Length (bp)

Fig. 31 (cont.)

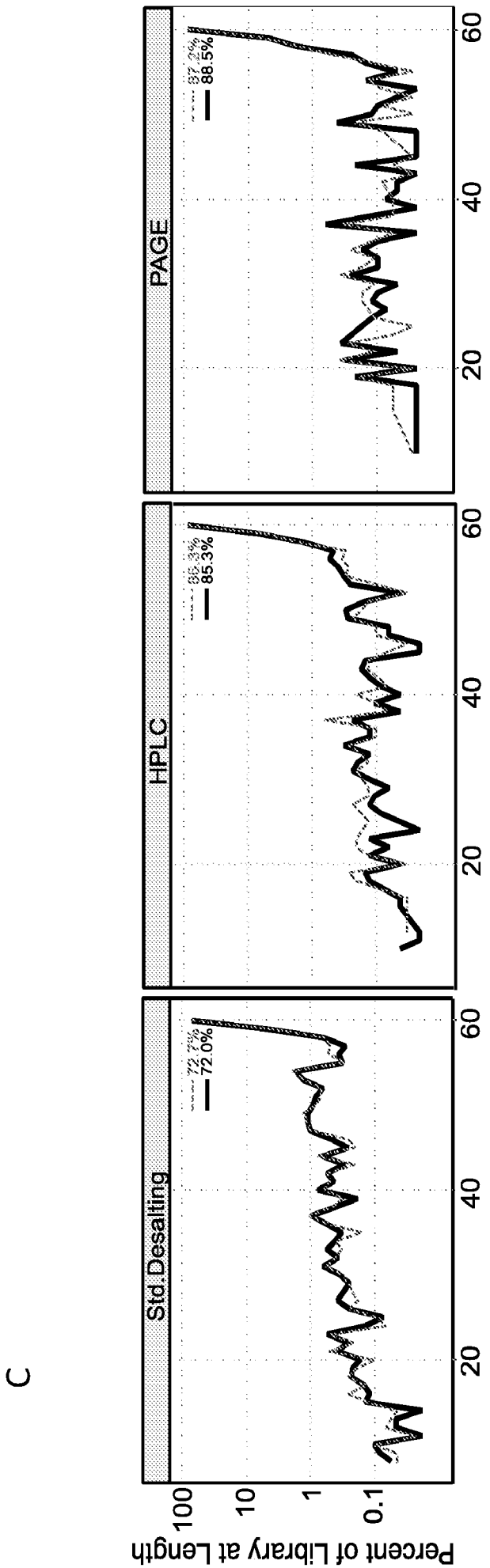

C
Std Desalting
HPLC
PAGE
72.7%
72.0%
86.3%
85.3%
87.2%
88.5%
Percent of Library at Length
100
10
1
0.1
20
40
60
Length (bp)

Fig. 31 (cont.)

A

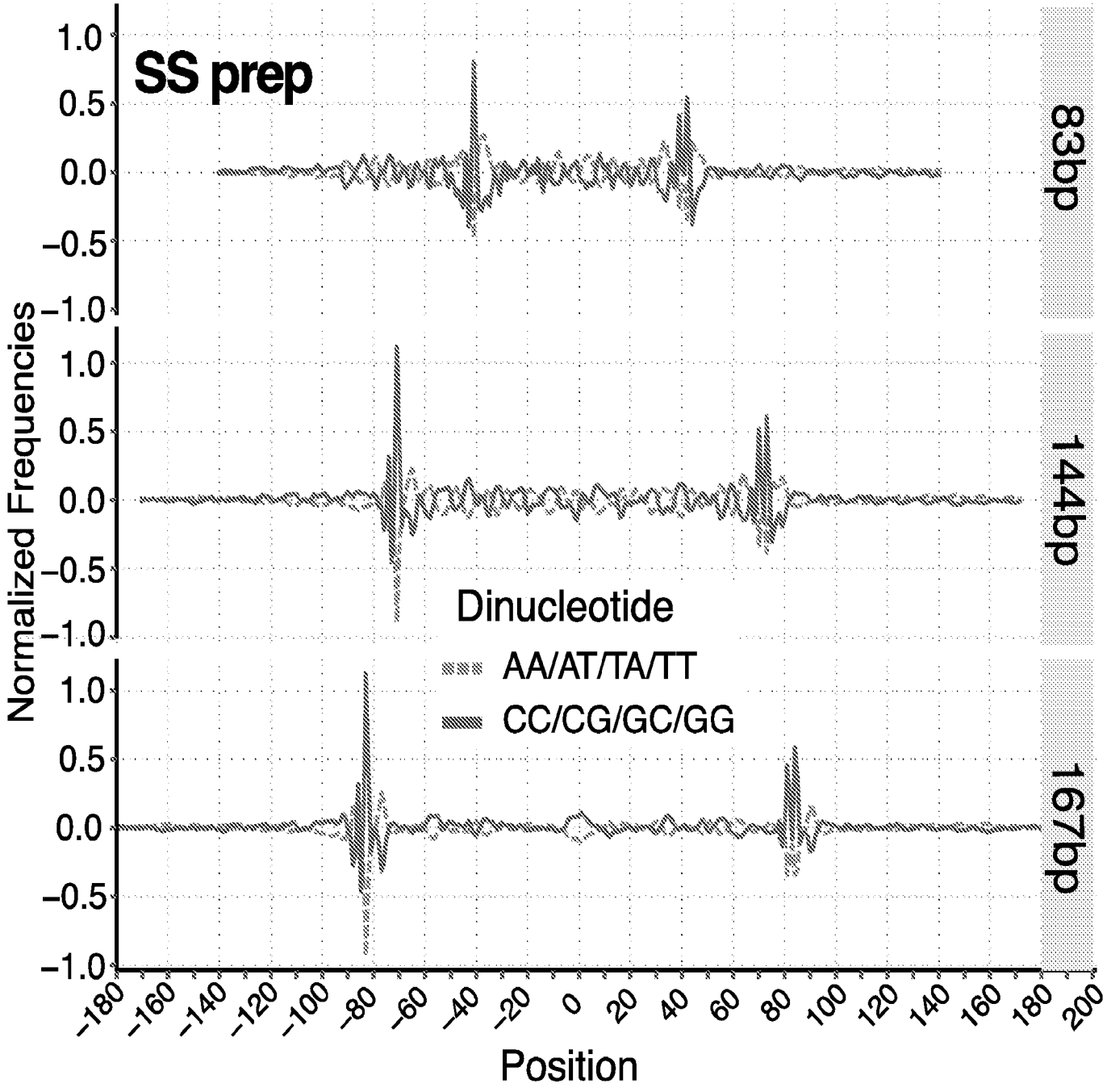

SS prep
83bp
144bp
167bp
Normalized Frequencies
1.0
0.5
0.0
−0.5
−1.0
Dinucleotide
AA/AT/TA/TT
CC/CG/GC/GG
−180
−160
−140
−120
−100
−80
−60
−40
−20
0
20
40
60
80
100
120
140
160
180
200
Position

Fig. 32

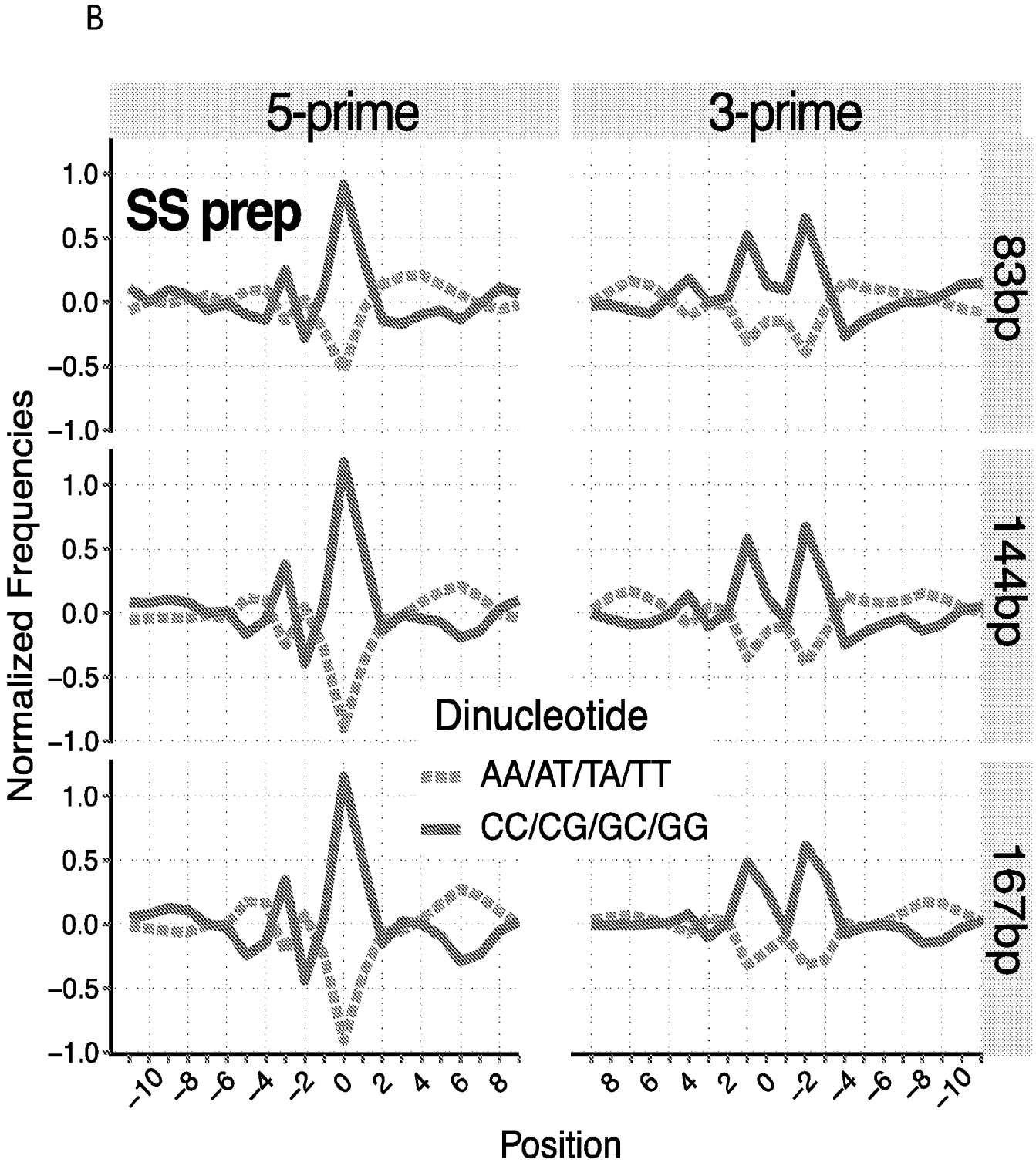

B
5-prime
3-prime
SS prep
83bp
144bp
167bp
Normalized Frequencies
1.0
0.5
0.0
−0.5
−1.0
Dinucleotide
AA/AT/TA/TT
CC/CG/GC/GG
−10 −8 −6 −4 −2 0 2 4 6 8
8 6 4 2 0 −2 −4 −6 −8 −10
Position

Fig. 32 (cont.)

C

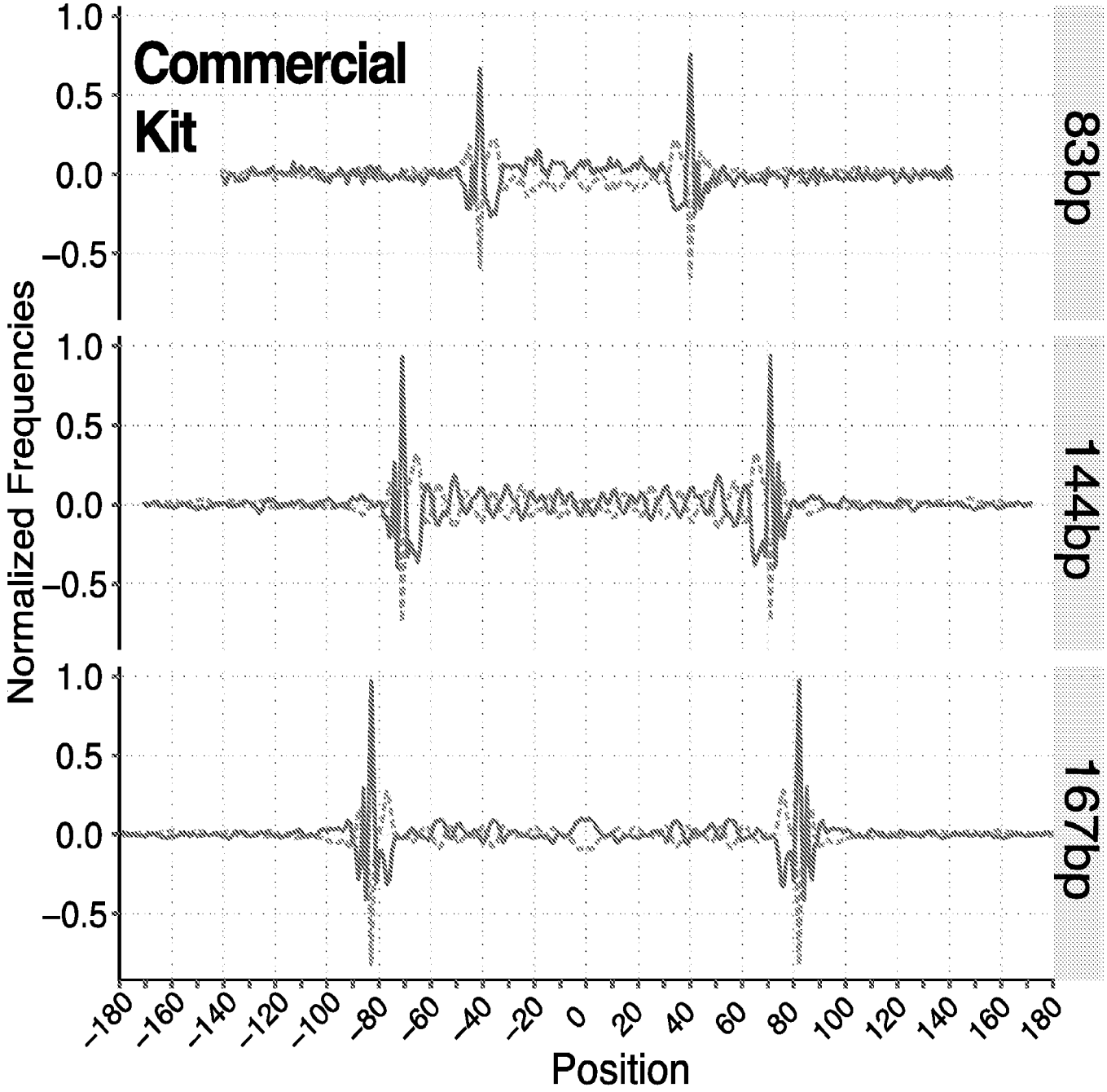

Commercial Kit
Normalized Frequencies
1.0
0.5
0.0
−0.5
83bp
144bp
167bp
−180 −160 −140 −120 −100 −80 −60 −40 −20 0 20 40 60 80 100 120 140 160 180
Position

Fig. 32 (cont.)

D

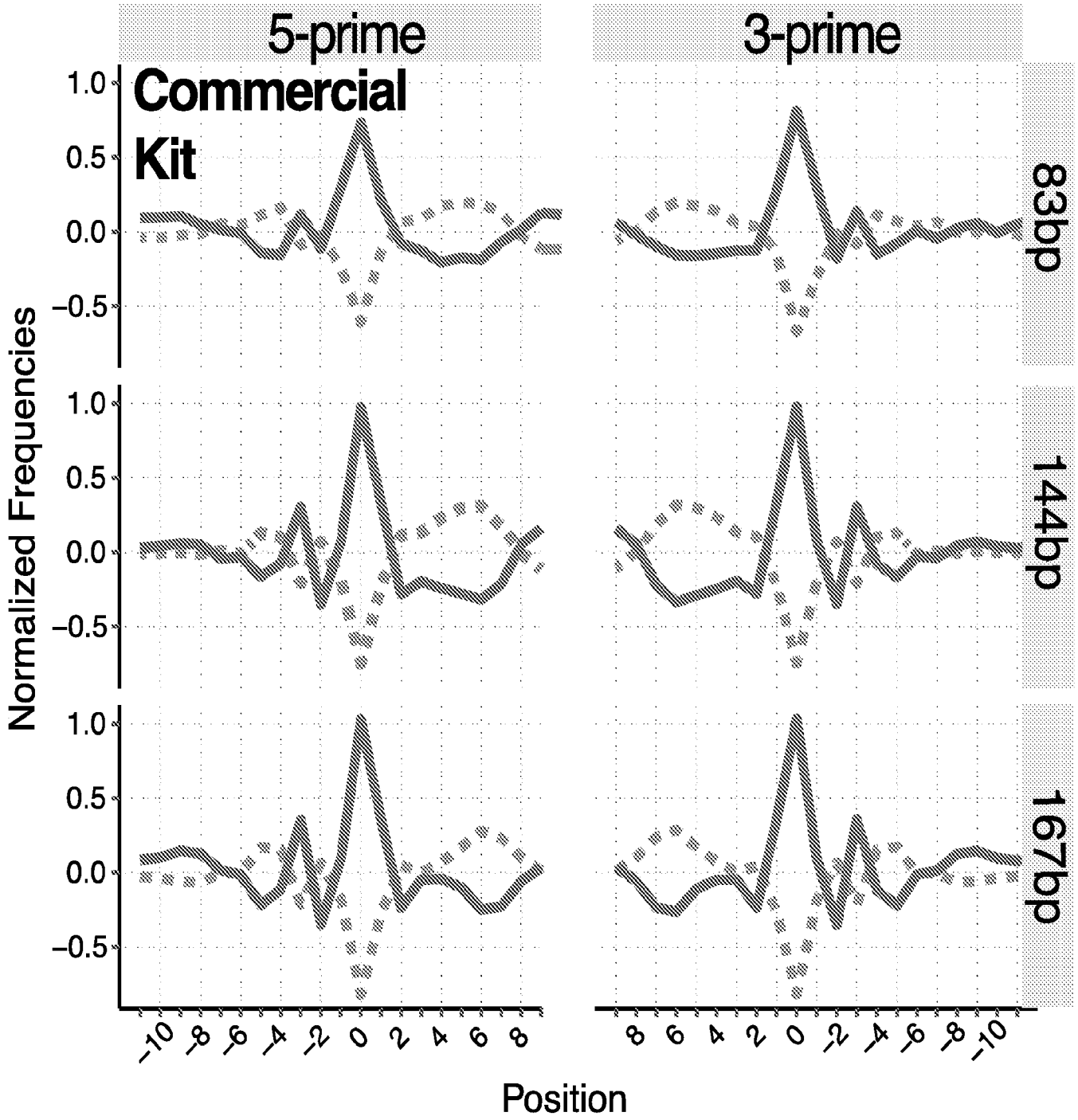

5-prime
3-prime
Commercial Kit
83bp
144bp
167bp
Normalized Frequencies
1.0
0.5
0.0
−0.5
−10 −8 −6 −4 −2 0 2 4 6 8
8 6 4 2 0 −2 −4 −6 −8 −10
Position

Fig. 32 (cont.)

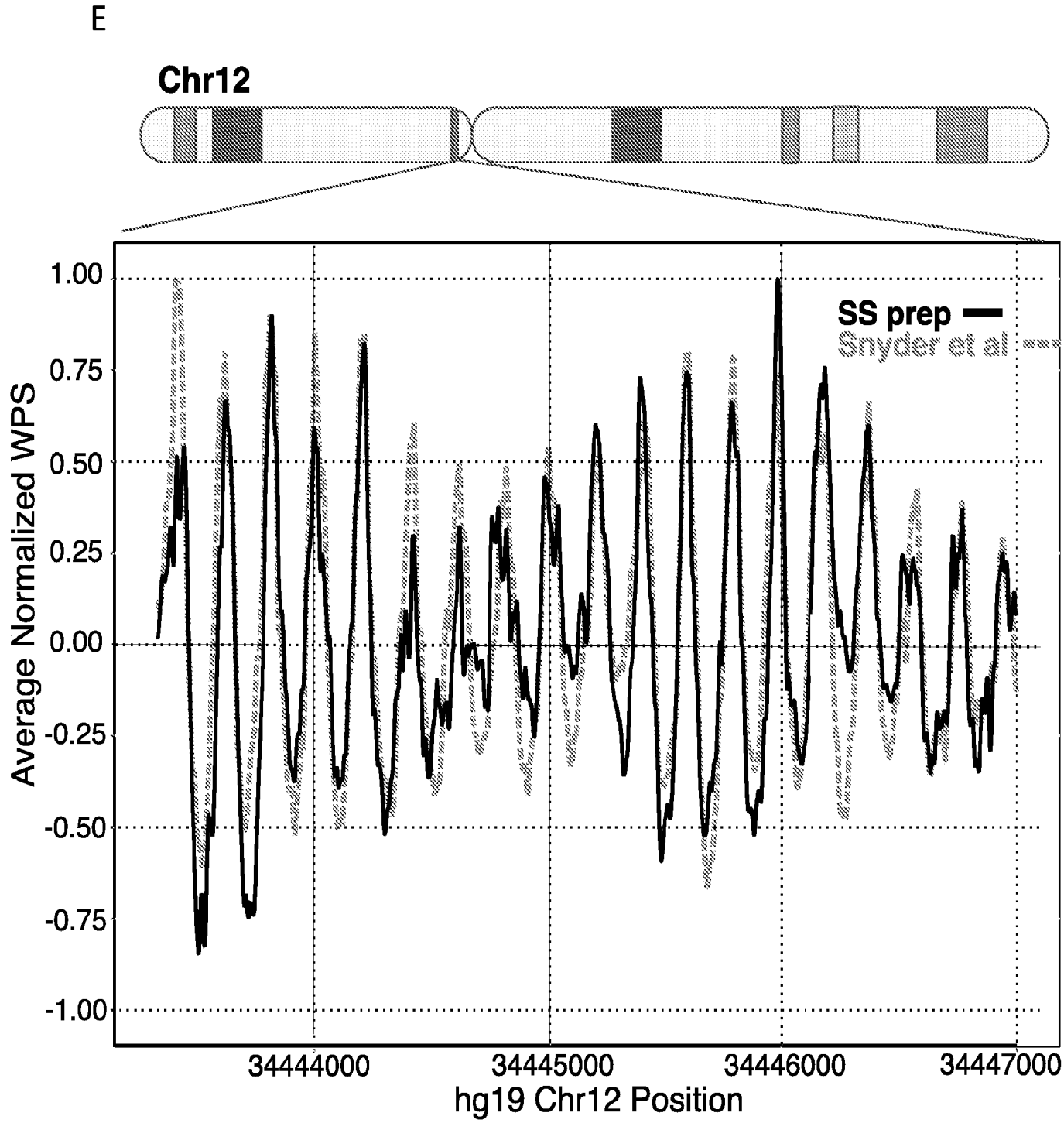

E
Chr12
1.00
0.75
0.50
0.25
0.00
-0.25
-0.50
-0.75
-1.00
Average Normalized WPS
SS prep
Snyder et al
34444000
34445000
34446000
34447000
hg19 Chr12 Position

Fig. 32 (cont.)

F

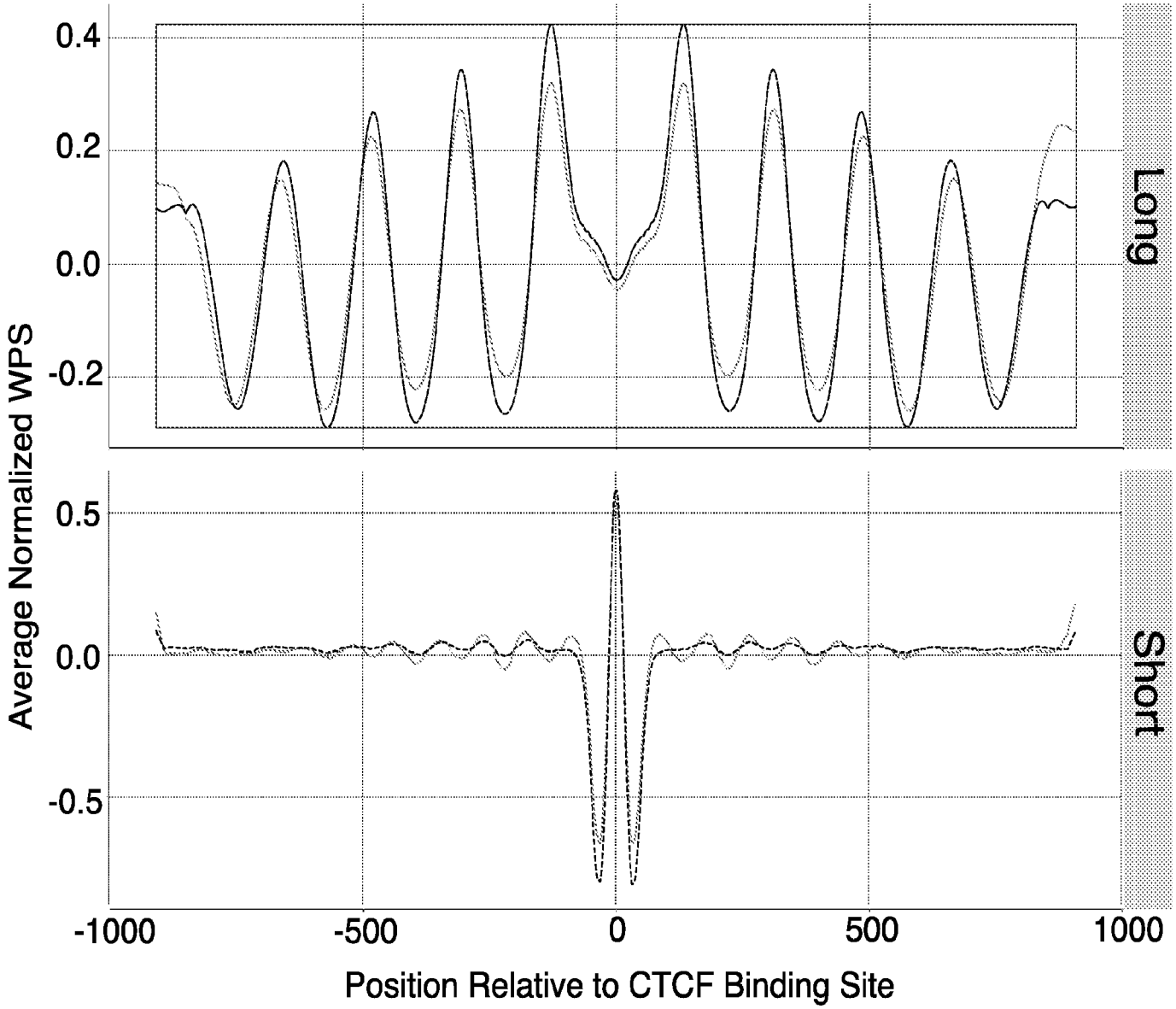

0.4
0.2
0.0
-0.2
Long
0.5
0.0
-0.5
Short
Average Normalized WPS
-1000
-500
0
500
1000
Position Relative to CTCF Binding Site

Fig. 32 (cont.)

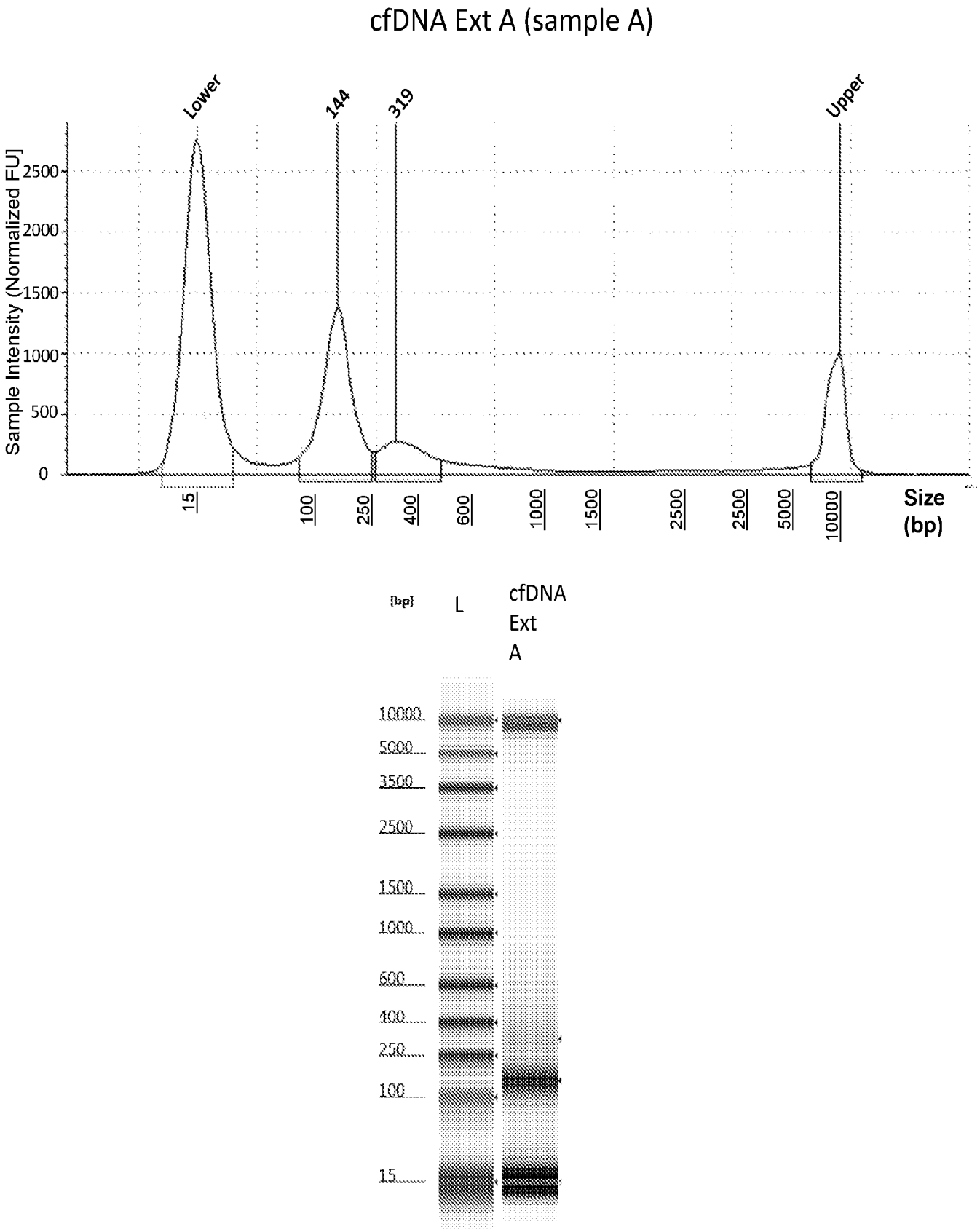
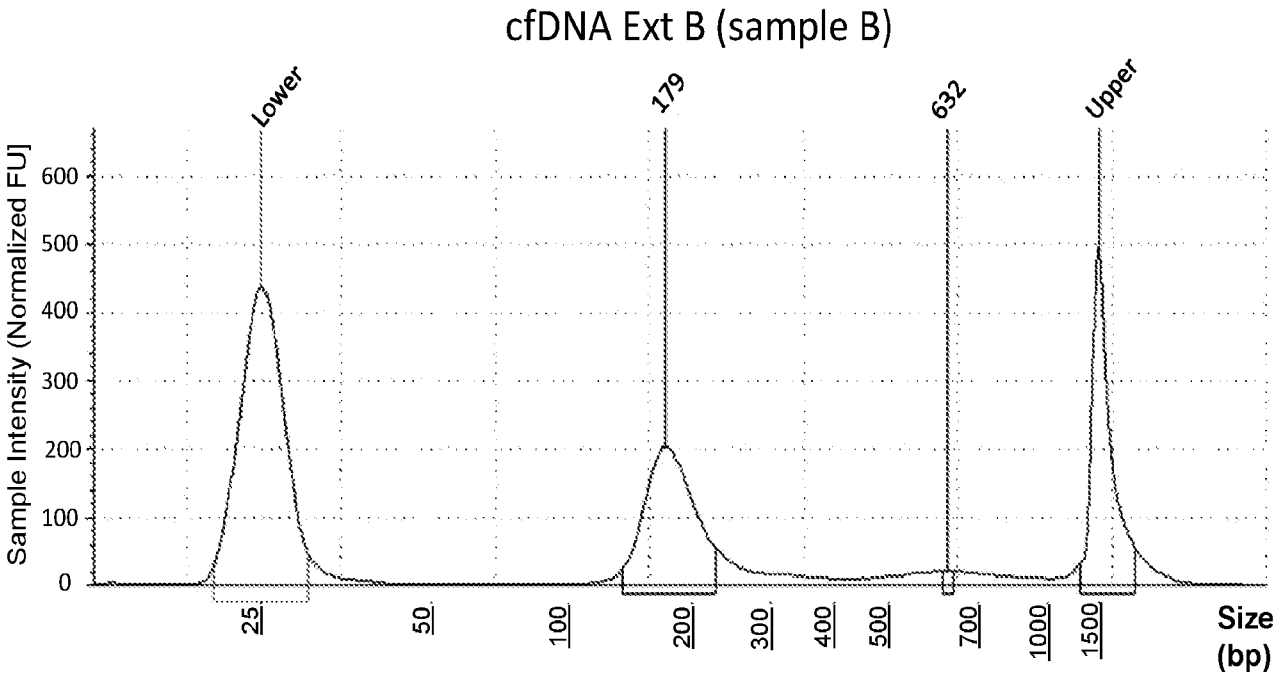

cfDNA Ext A (sample A)
Lower
144
319
Upper
Sample Intensity (Normalized FU)
2500
2000
1500
1000
500
0
15
100
250
400
600
1000
1500
2500
2500
5000
10000
Size (bp)
[bp]
L
cfDNA Ext A
10000
5000
3500
2500
1500
1000
600
400
250
100
15

Fig. 33A


cfDNA Ext B (sample B)
Lower
179
632
Upper
Sample Intensity (Normalized FU)
600
500
400
300
200
100
0
25
50
100
200
300
400
500
700
1000
1500
Size (bp)

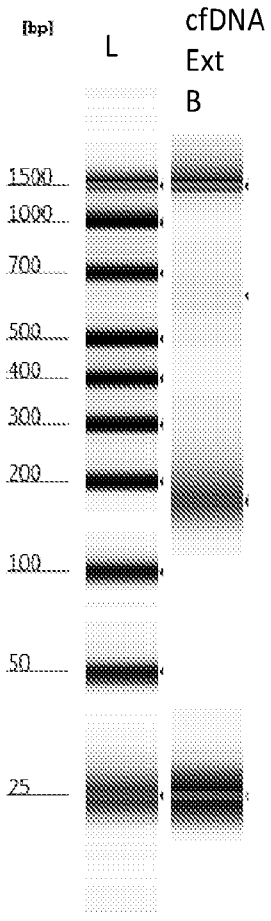

[bp]
L
cfDNA Ext B
1500
1000
700
500
400
300
200
100
50
25

Fig. 33A (cont.)

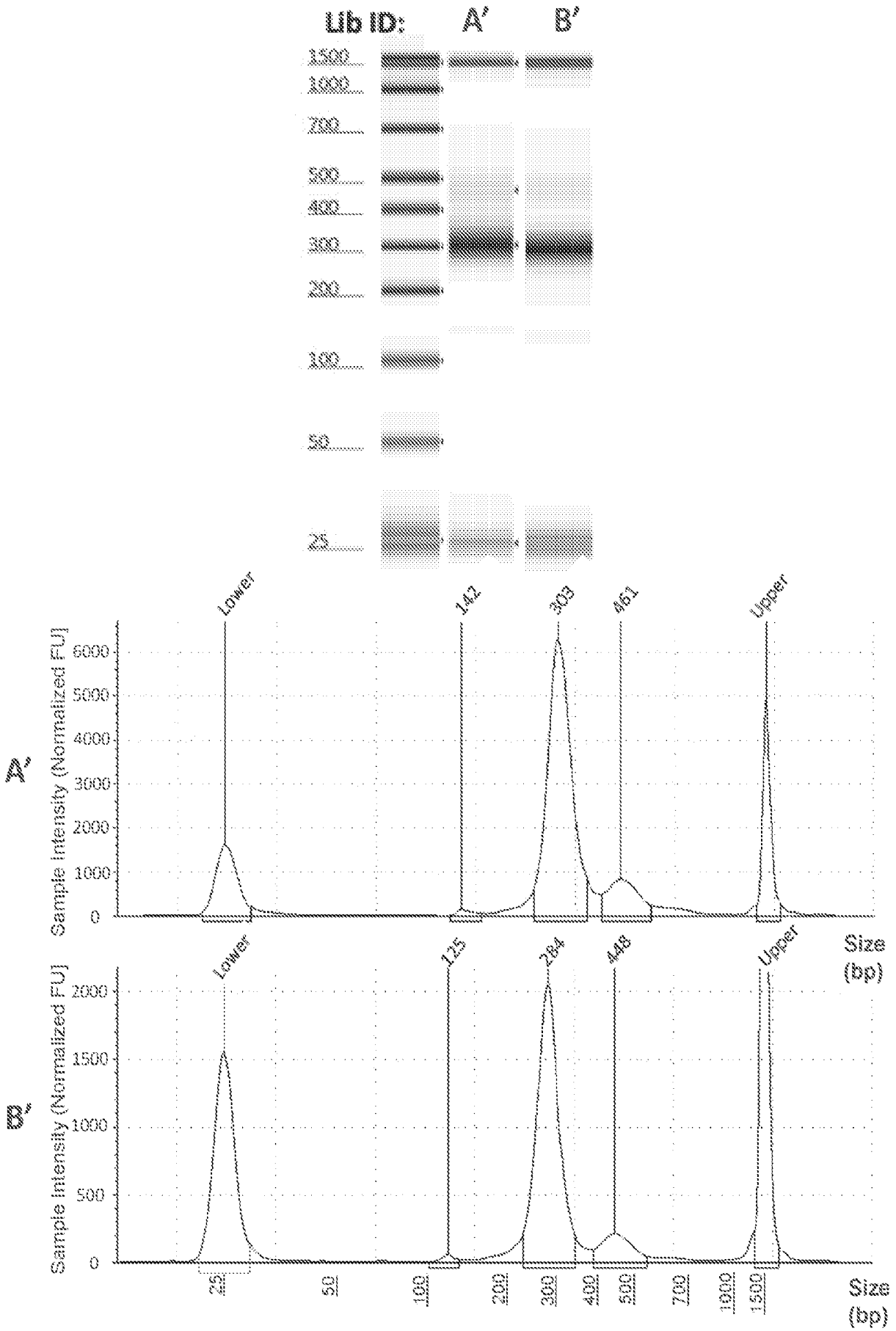

Ub ID:
A'
B'
1500
1000
700
500
400
300
200
100
50
25
A'
Sample Intensity (Normalized FU)
6000
5000
4000
3000
2000
1000
0
Lower
142
303
461
Upper
B'
Sample Intensity (Normalized FU)
2000
1500
1000
500
0
Lower
125
284
448
Upper
Size (bp)
25
50
100
200
300
400
500
700
1000
1500
Size (bp)

Fig. 33B

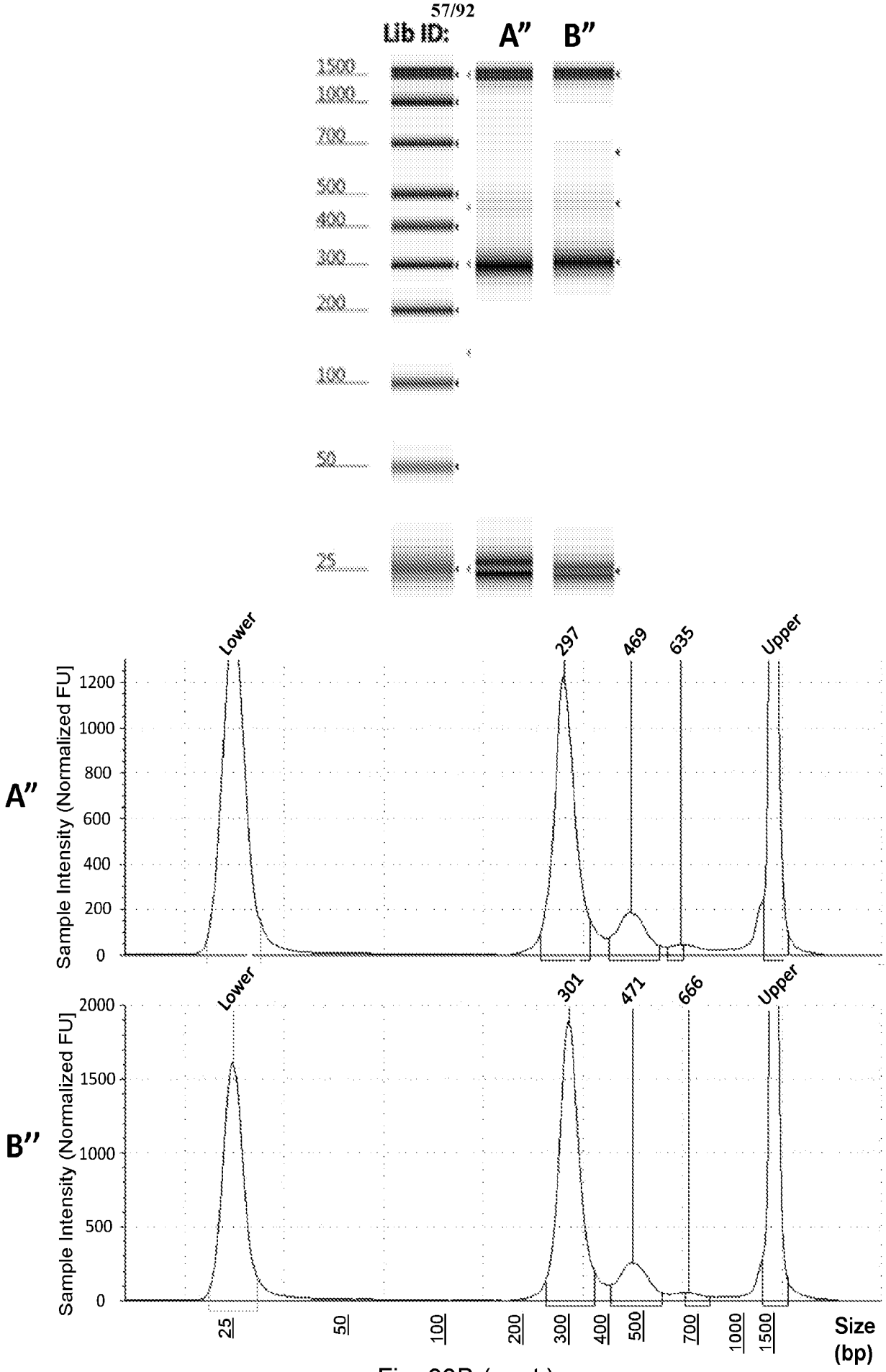

Lib ID:
A"
B"
1500
1000
700
500
400
300
200
100
50
25
A"
B"
Sample Intensity (Normalized FU)
Lower
297
469
635
Upper
301
471
666
25
50
100
200
300
400
500
700
1000
1500
Size (bp)

Fig. 33B (cont.)

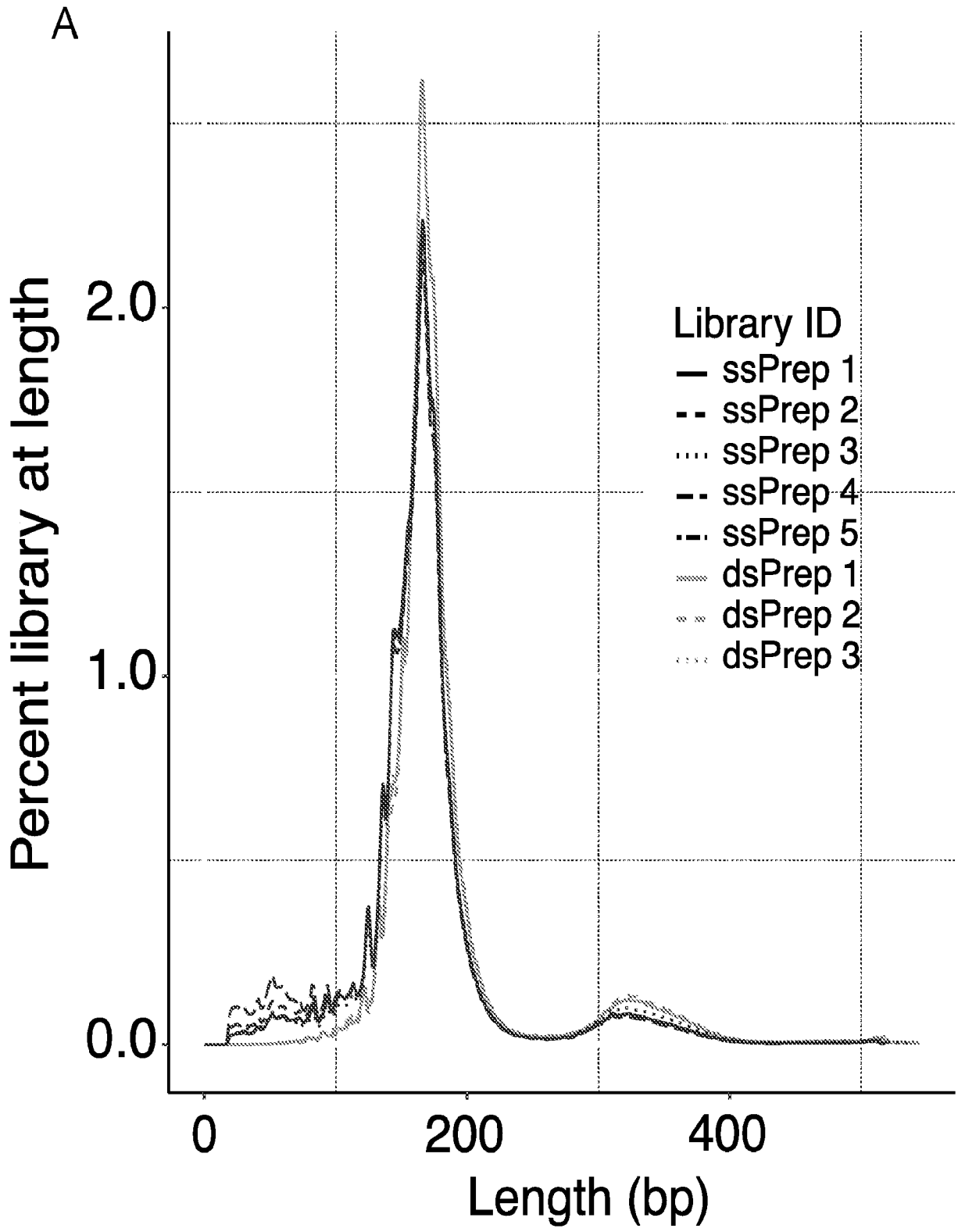

A
Percent library at length
2.0
1.0
0.0
0
200
400
Length (bp)
Library ID
ssPrep 1
ssPrep 2
ssPrep 3
ssPrep 4
ssPrep 5
dsPrep 1
dsPrep 2
dsPrep 3

Fig. 34

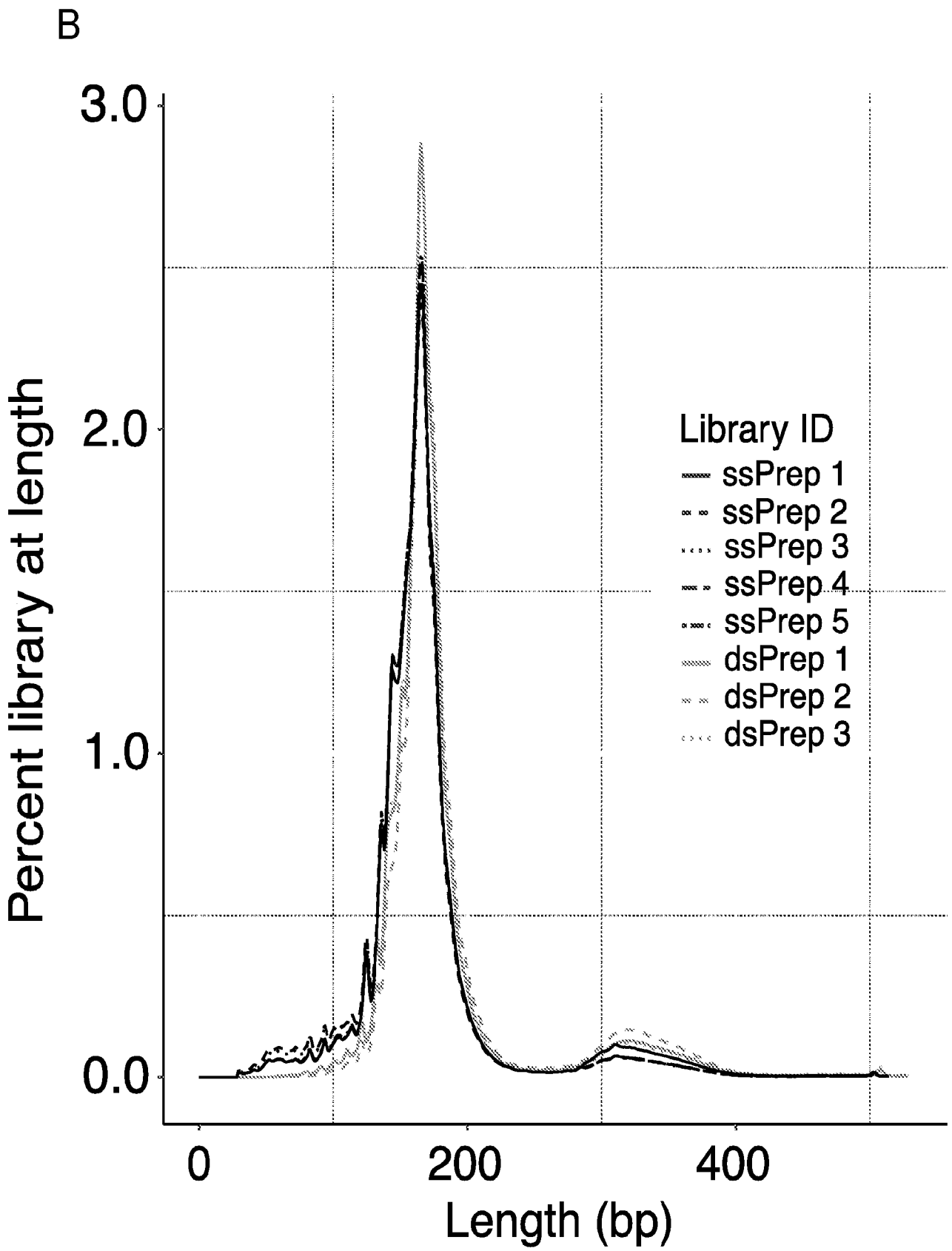

B
3.0
2.0
1.0
0.0
Percent library at length
0
200
400
Length (bp)
Library ID
ssPrep 1
ssPrep 2
ssPrep 3
ssPrep 4
ssPrep 5
dsPrep 1
dsPrep 2
dsPrep 3

Fig. 34 (cont.)

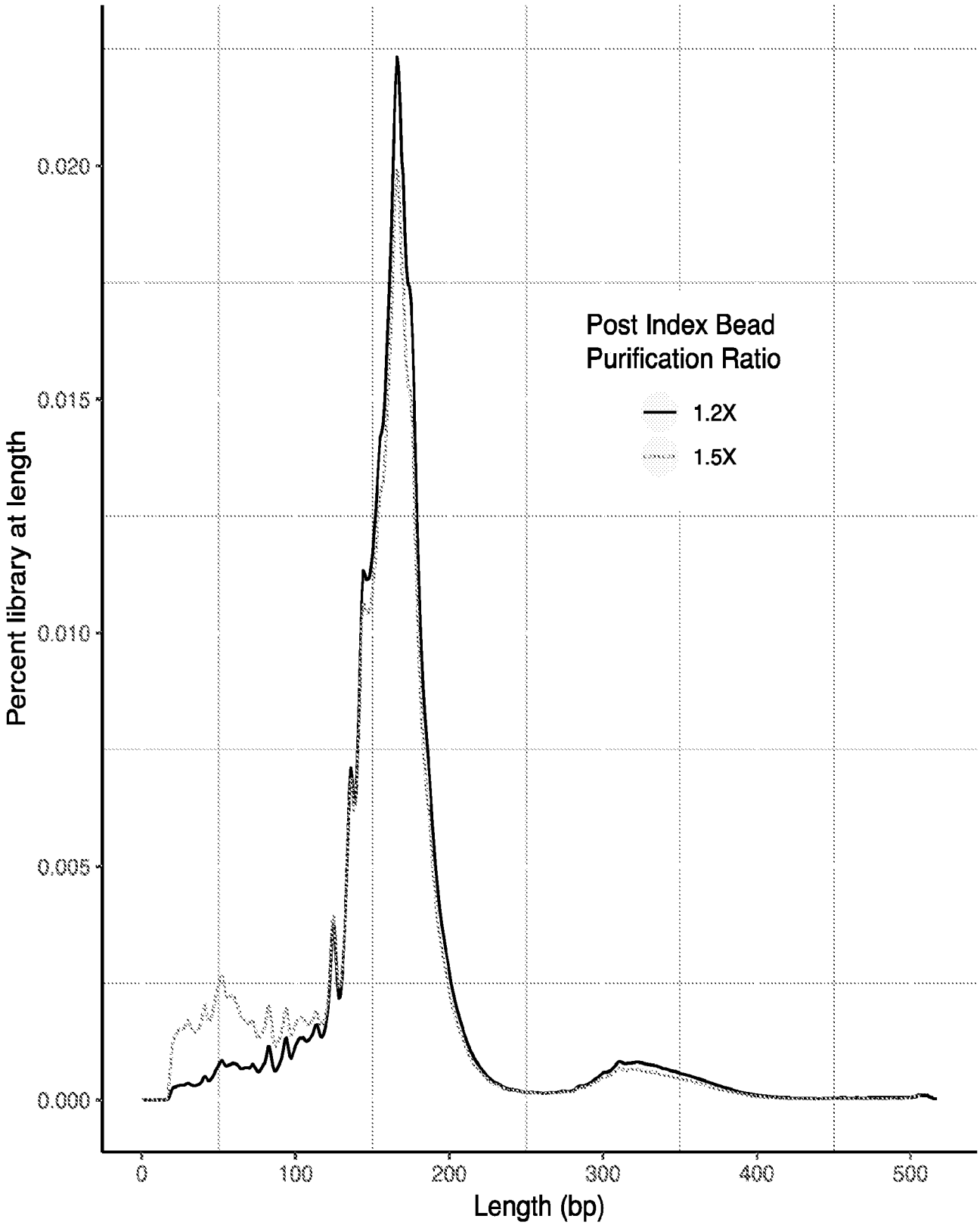

Post Index Bead
Purification Ratio
1.2X
1.5X
Percent library at length
0.020
0.015
0.010
0.005
0.000
0
100
200
300
400
500
Length (bp)

Fig. 35

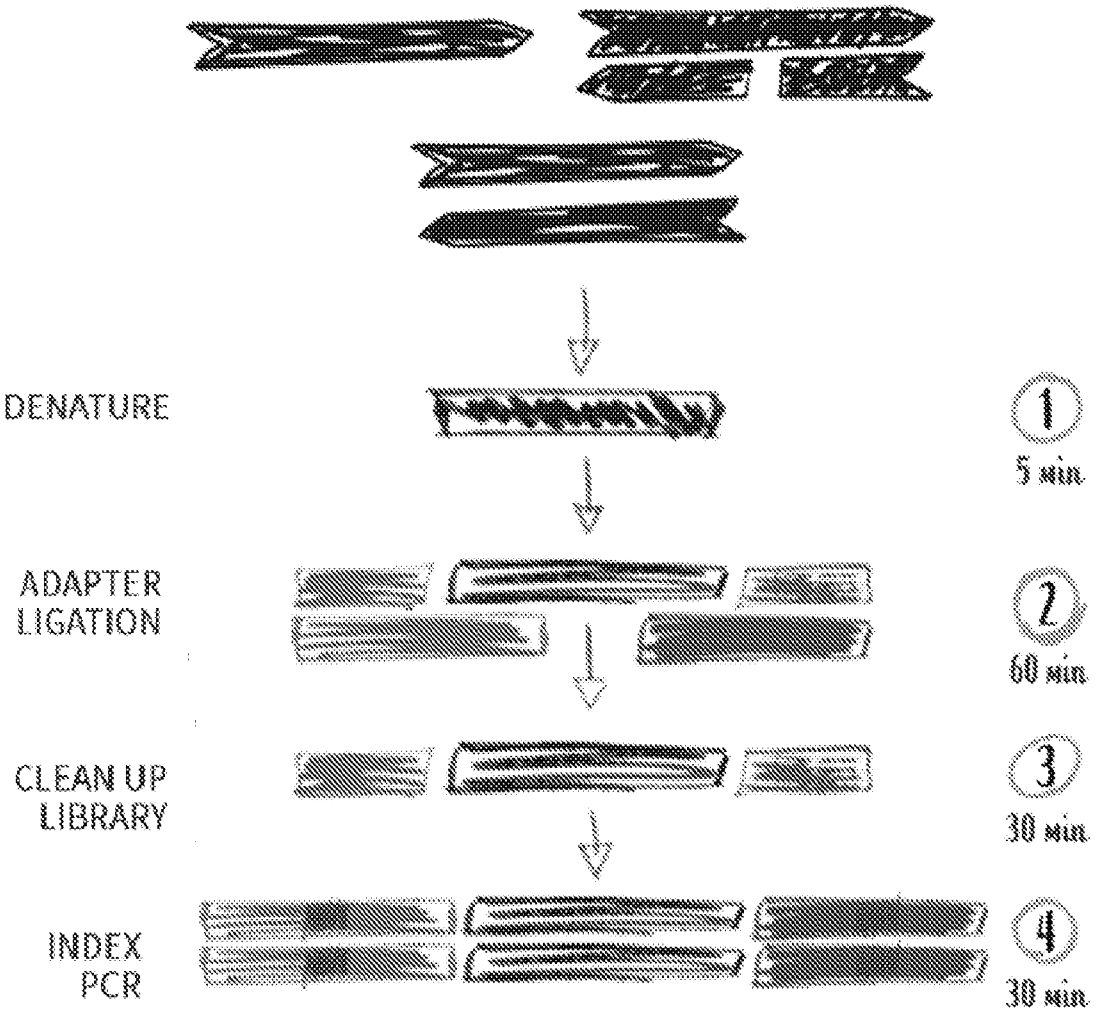

DNA Fragments
DENATURE
1
5 min
ADAPTER LIGATION
2
60 min
CLEAN UP LIBRARY
3
30 min
INDEX PCR
4
30 min

Fig. 36

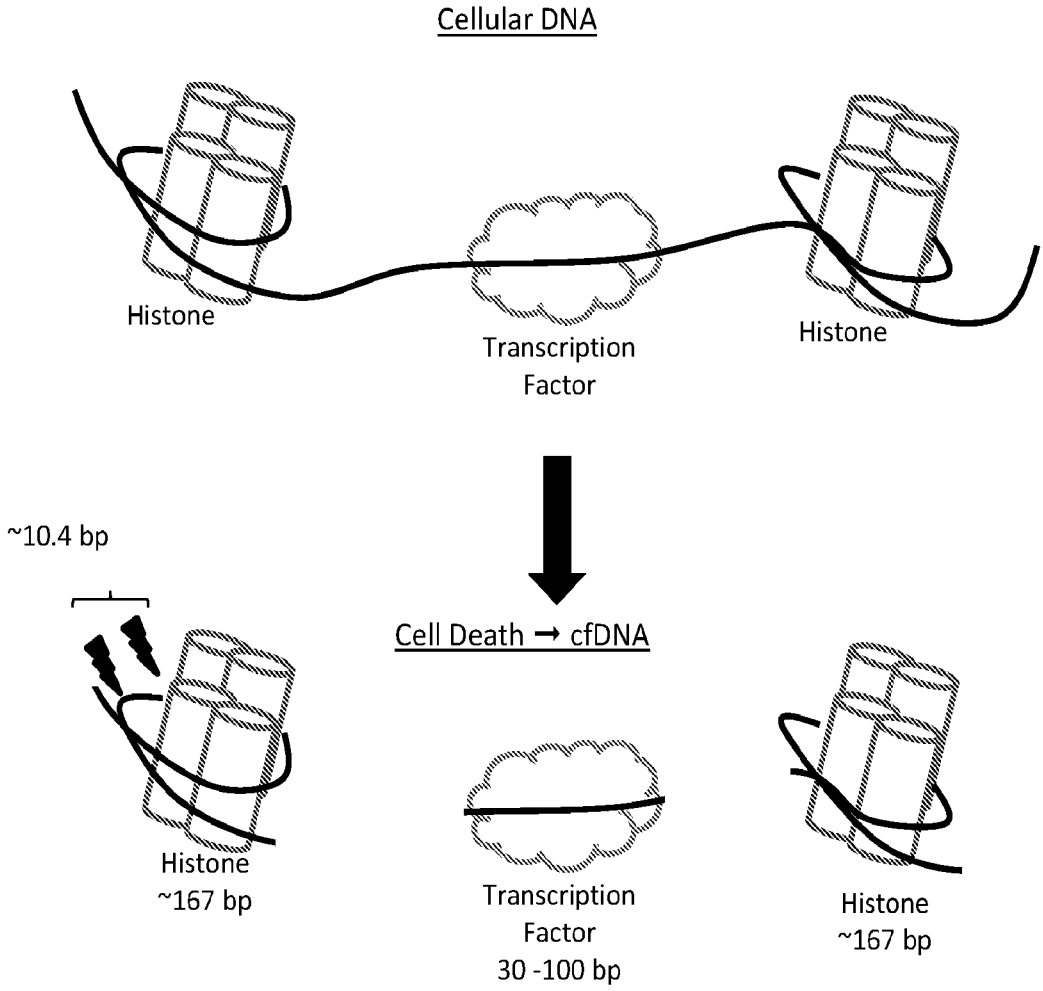

Cellular DNA
Histone
Transcription Factor
Histone
~10.4 bp
Cell Death → cfDNA
Histone ~167 bp
Transcription Factor 30 -100 bp
Histone ~167 bp

Fig. 37

Kit	Type of Prep Method	Input Amount	Total Time thru Index PCR
ssPrep	Single-stranded	1ng	2.5hrs
Commercial kit 1	Double-stranded	1ng	3hrs
Commercial kit 2	Double-stranded	1ng	2.5hrs
Commercial kit 3	Single-stranded	1ng	3.5hrs

Fig. 38

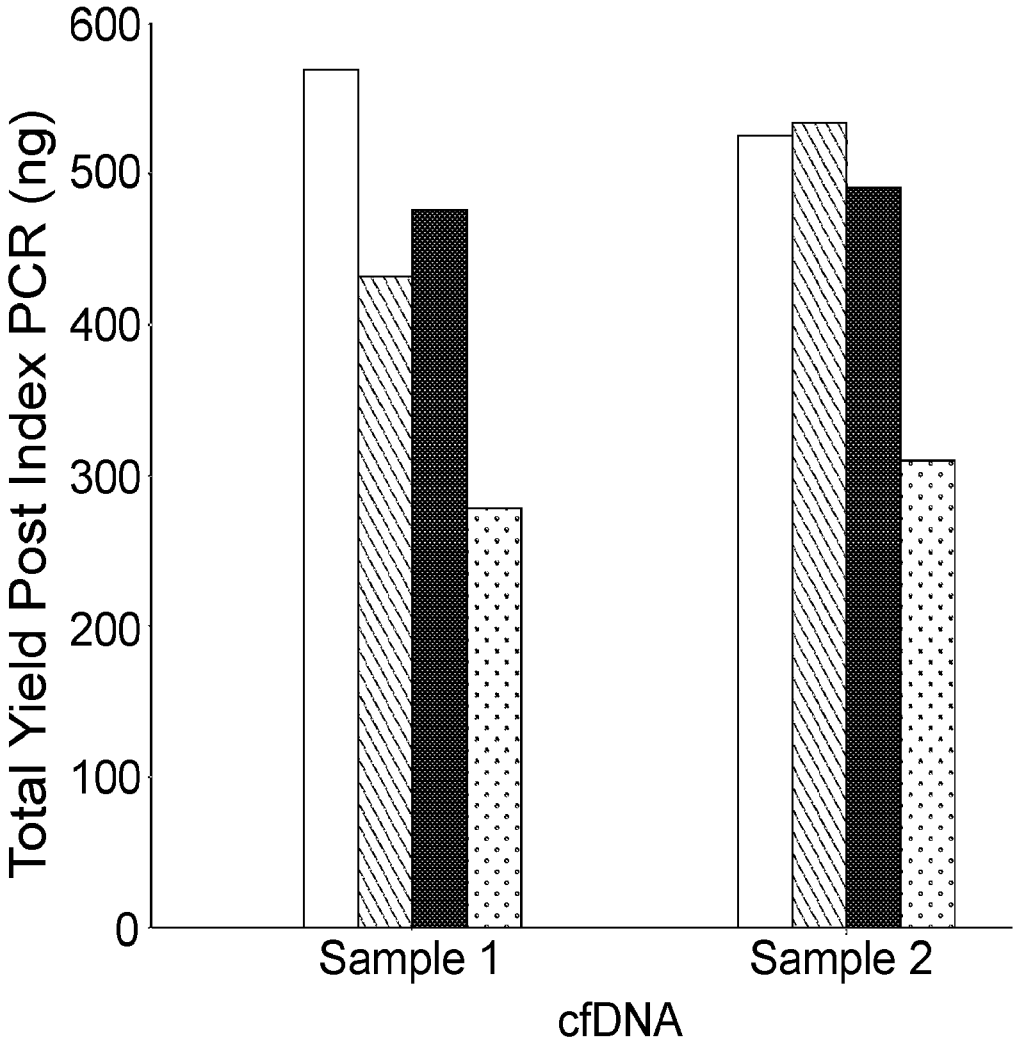

600
500
400
300
200
100
0
Total Yield Post Index PCR (ng)
Sample 1
Sample 2
cfDNA

Fig. 39

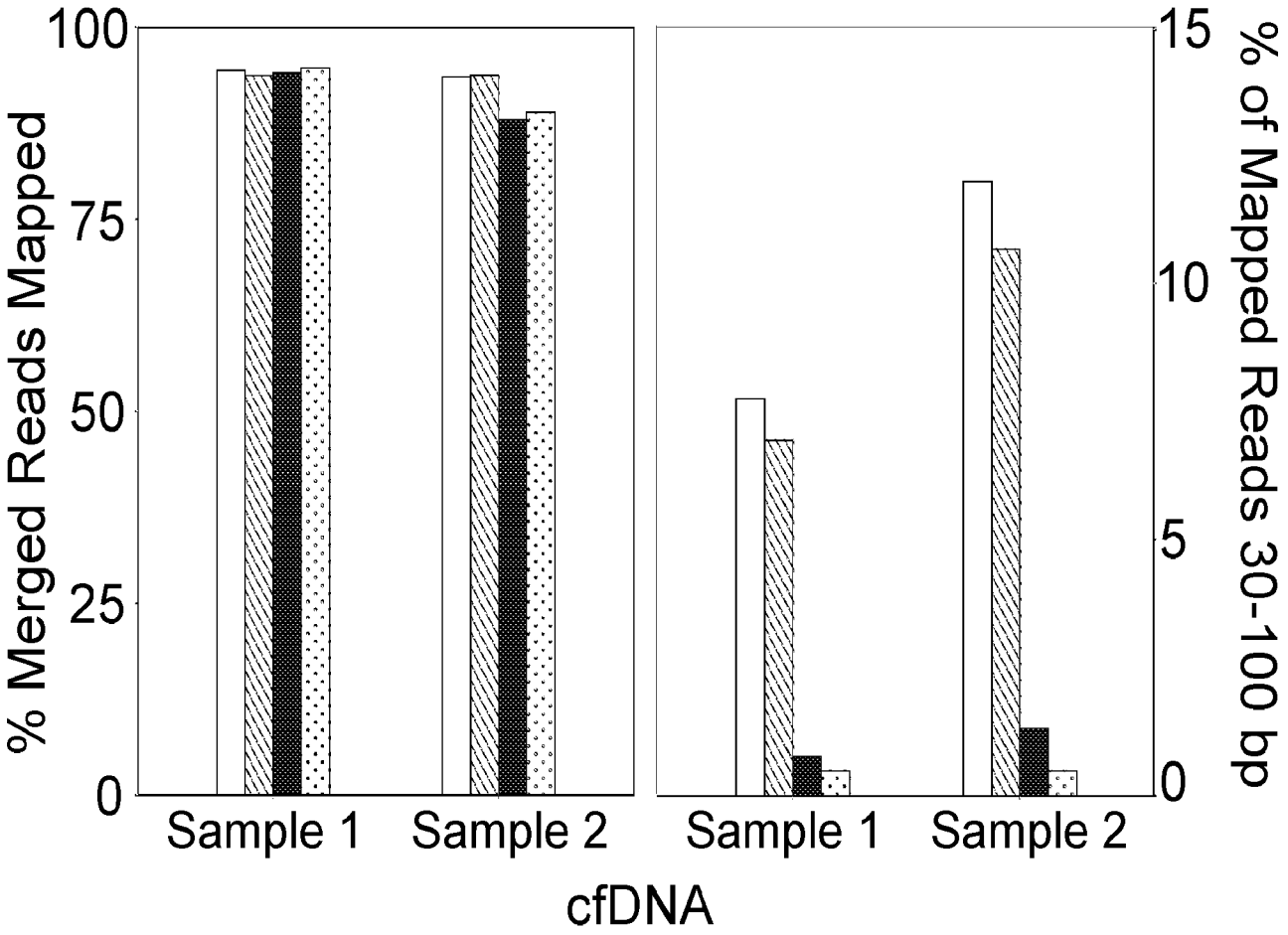
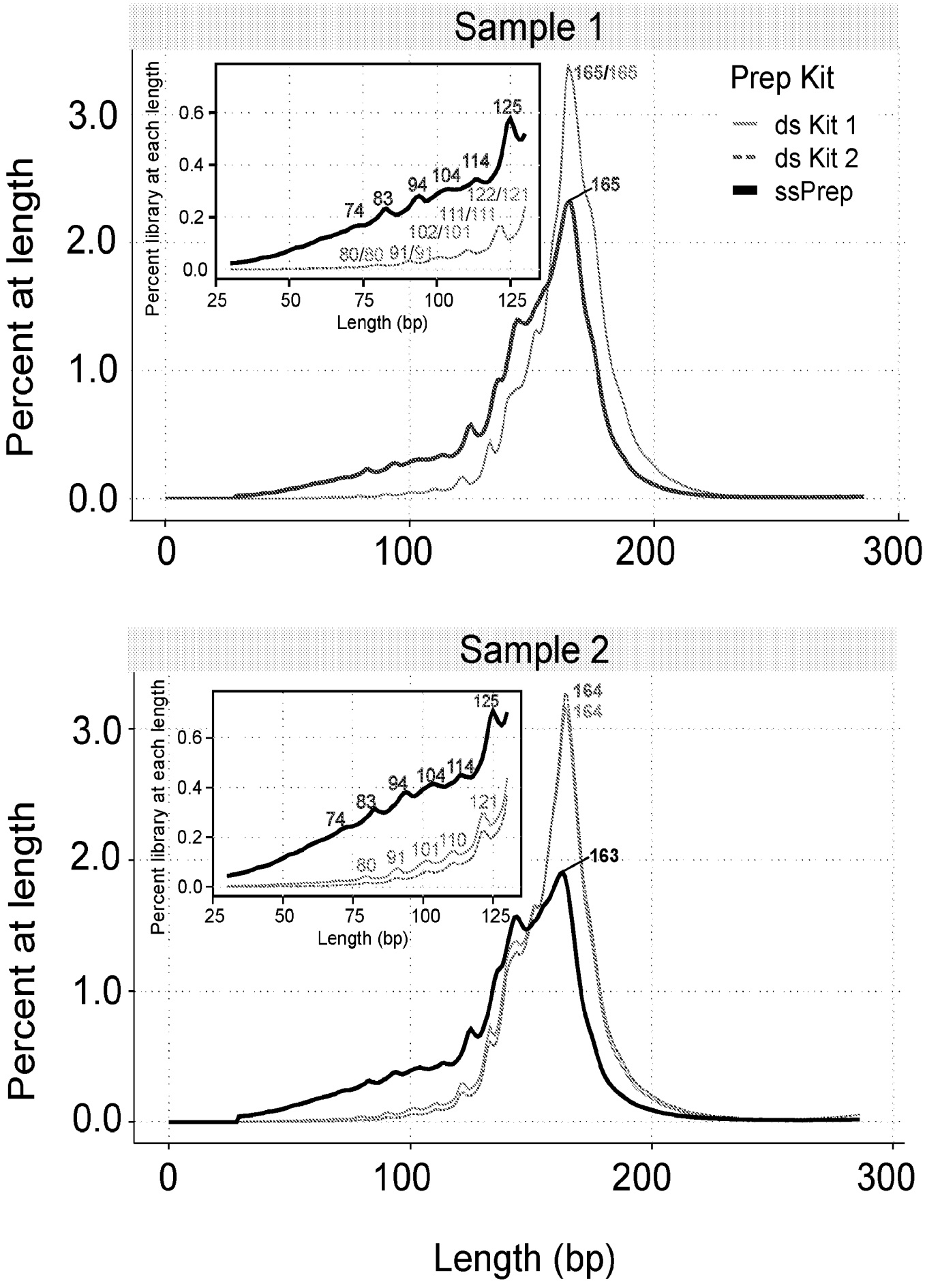

100
75
50
25
0
% Merged Reads Mapped
15
10
5
0
% of Mapped Reads 30-100 bp
Sample 1
Sample 2
Sample 1
Sample 2
cfDNA

Fig. 40

Kit	Type of Prep Method	Input cfDNA Extract ID	Yield Post 10c Index PCR (total)	Merged Read Pairs Sequenced	Mapping Rate*	Mapped Reads in 30-100bp* Bin
ssPrep	Single-stranded	Sample 1	569ng	73,077,812	94.35%	7.77%
		Sample 2	525ng	63,449,167	93.52%	12.0%
Commercial kit 1	Double-stranded	Sample 1	278ng	78,705,033	94.66%	0.49%
		Sample 2	310ng	54,734,560	88.88%	0.49%
Commercial kit 2	Double-stranded	Sample 1	476ng	74,429,274	94.01%	0.77%
		Sample 2	470ng	71,294,525	87.97%	1.32%
Commercial kit 3	Single-stranded	Sample 1	432ng	78,018,897	93.66%	6.94%
		Sample 2	534ng	69,959,246	93.73%	10.68%

Fig. 41



Length (bp)

Fig. 42A

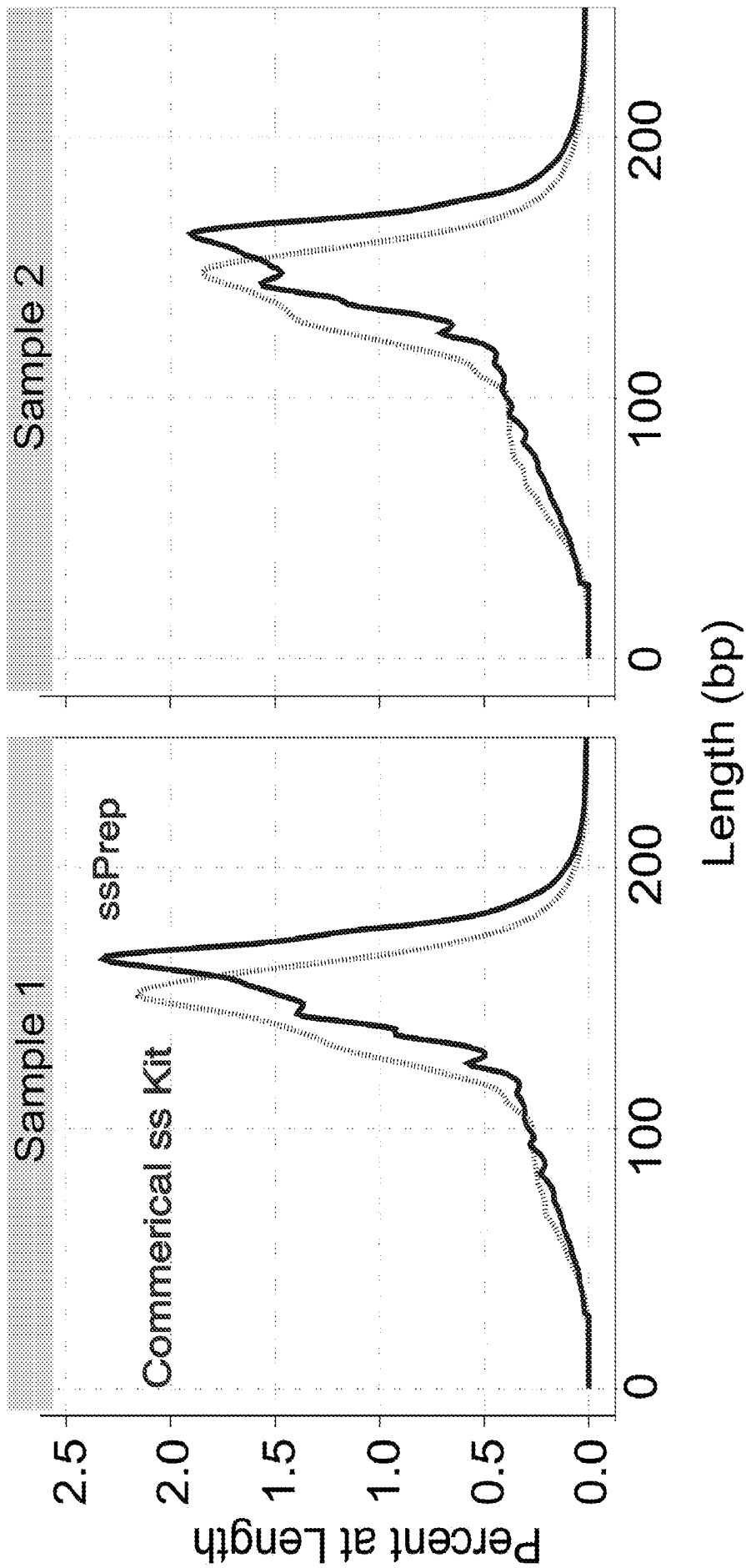

Sample 1
Sample 2
ssPrep
Commerical ss Kit
Percent at Length
Length (bp)
0.0
0.5
1.0
1.5
2.0
2.5
0
100
200

Fig. 42B

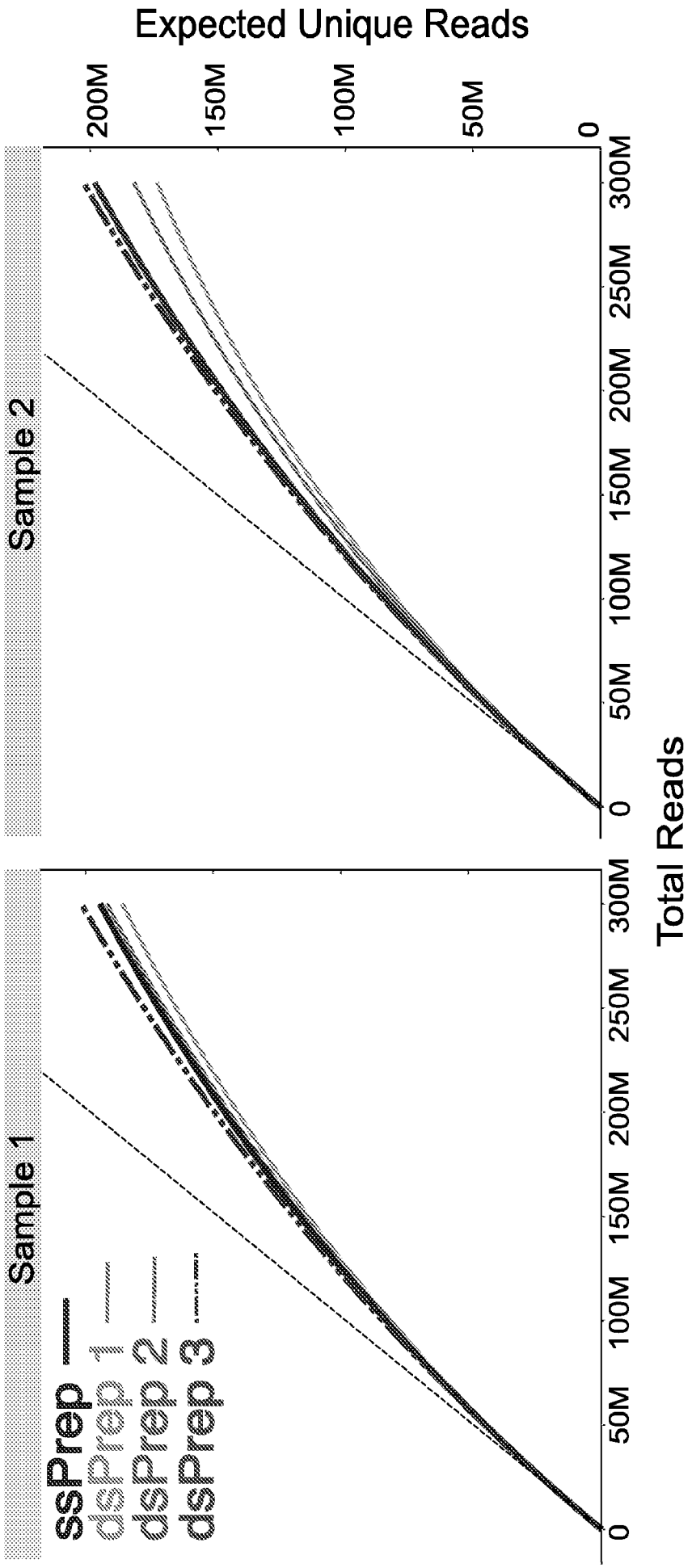

Expected Unique Reads
200M
150M
100M
50M
0
Sample 2
Sample 1
ssPrep
dsPrep 1
dsPrep 2
dsPrep 3
0
50M
100M
150M
200M
250M
300M
Total Reads

Fig. 43

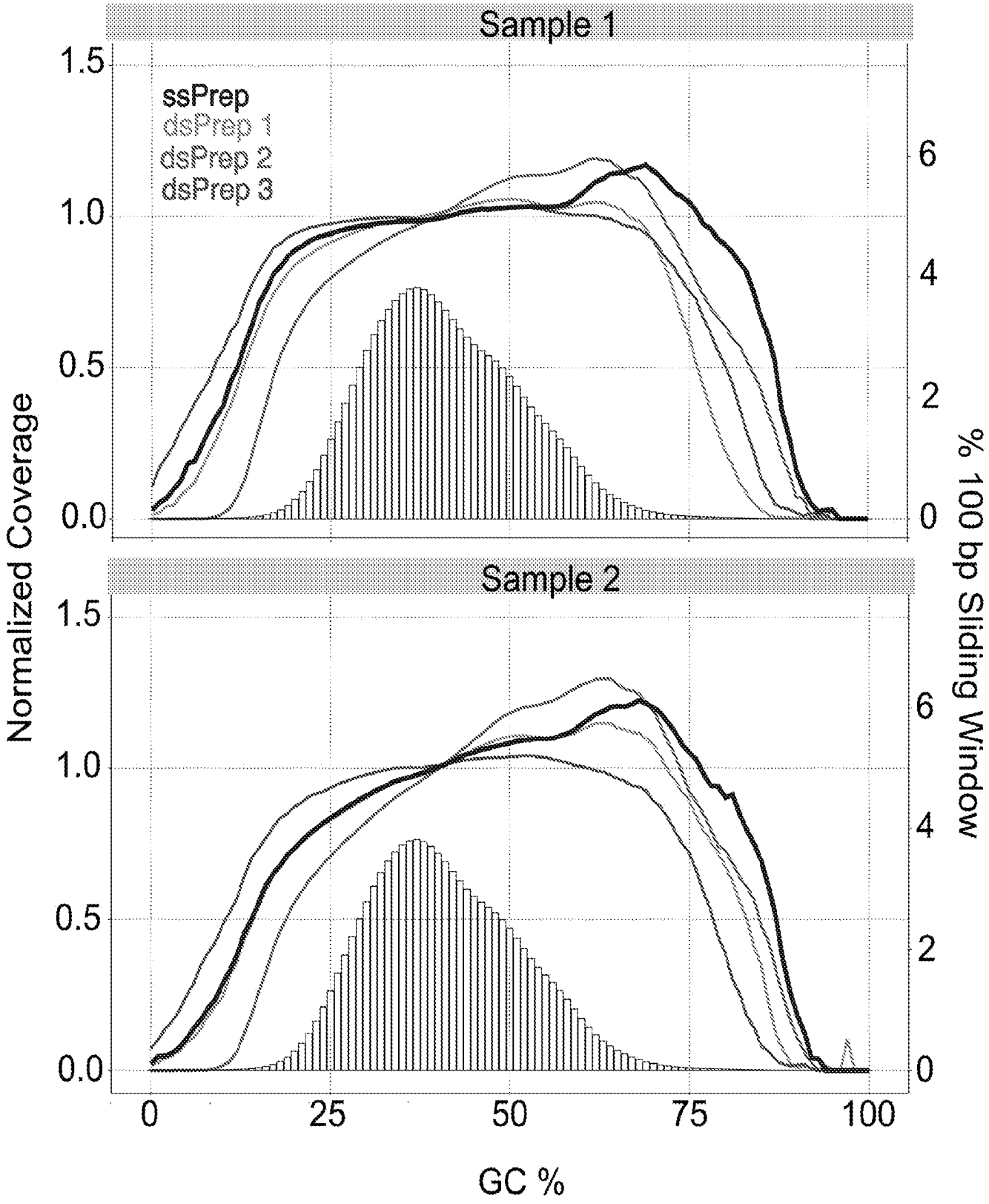

Sample 1
ssPrep
dsPrep 1
dsPrep 2
dsPrep 3
Sample 2
Normalized Coverage
% 100 bp Sliding Window
GC %
0.0
0.5
1.0
1.5
0
2
4
6
0
25
50
75
100

Fig. 44

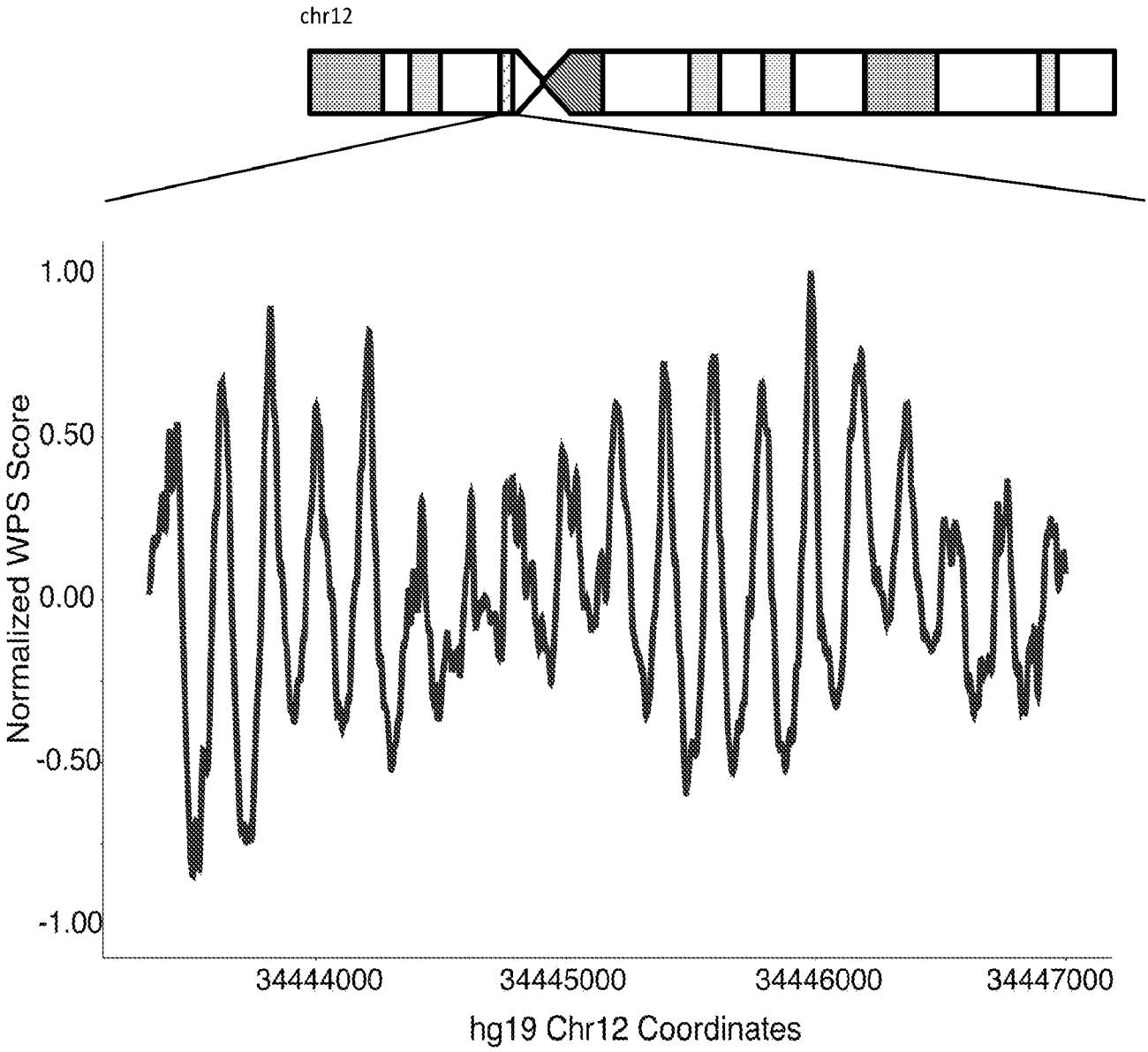

chr12
1.00
0.50
0.00
-0.50
-1.00
Normalized WPS Score
34444000
34445000
34446000
34447000
hg19 Chr12 Coordinates

Fig. 45

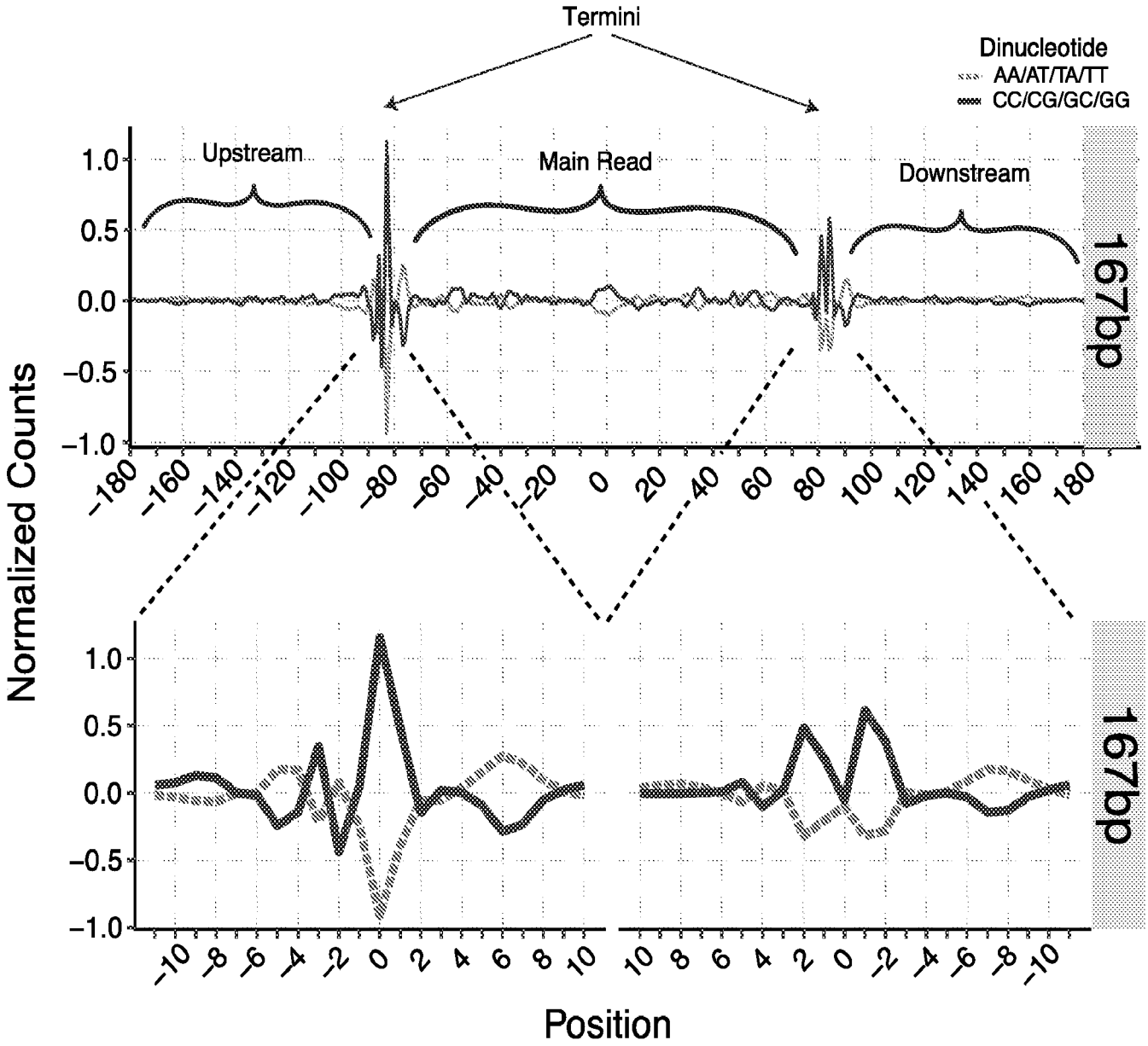

Termini
Dinucleotide
AA/AT/TA/TT
CC/CG/GC/GG
Upstream
Main Read
Downstream
1.0
0.5
0.0
−0.5
−1.0
−180 −160 −140 −120 −100 −80 −60 −40 −20 0 20 40 60 80 100 120 140 160 180
167bp
1.0
0.5
0.0
−0.5
−1.0
−10 −8 −6 −4 −2 0 2 4 6 8 10
10 8 6 4 2 0 −2 −4 −6 −8 −10
167bp
Normalized Counts
Position

Fig. 46

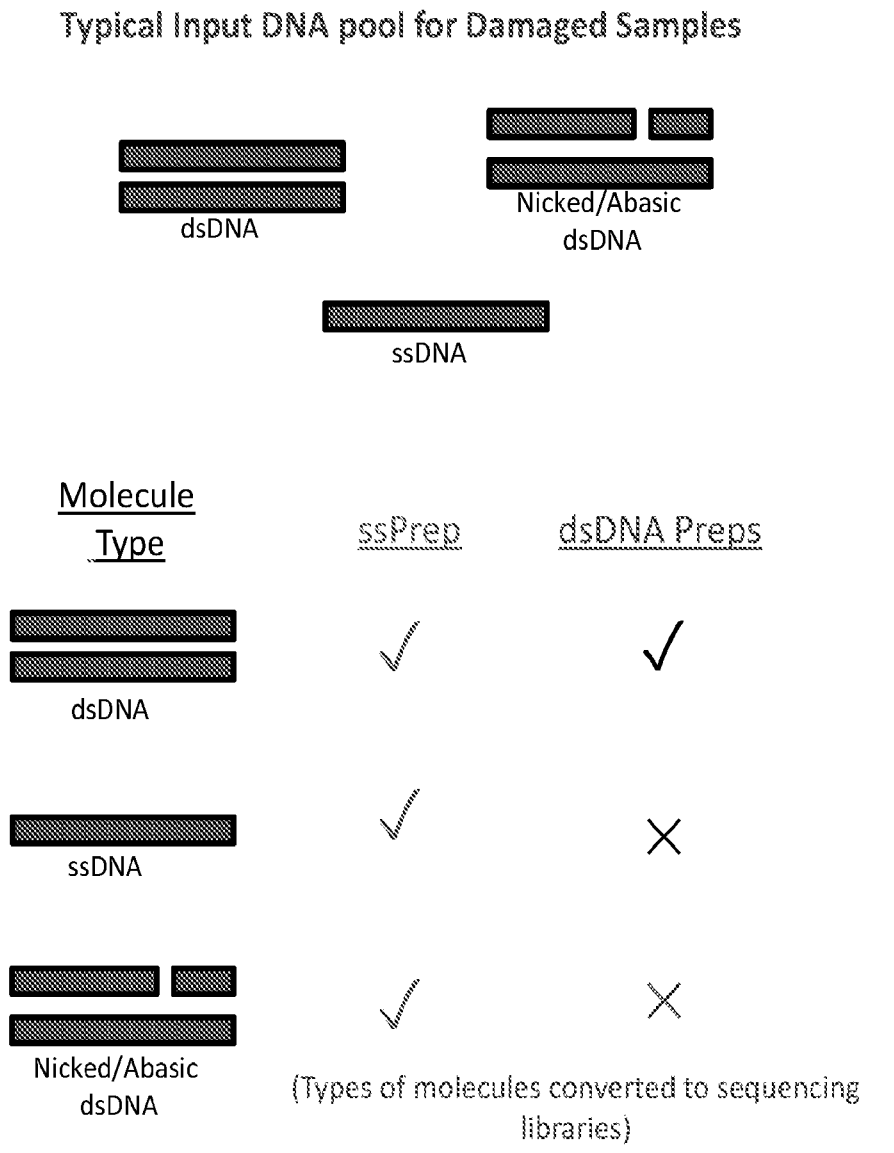

Typical Input DNA pool for Damaged Samples
dsDNA
Nicked/Abasic dsDNA
ssDNA
Molecule Type
ssPrep
dsDNA Preps
dsDNA
ssDNA
Nicked/Abasic dsDNA
(Types of molecules converted to sequencing libraries)

Fig. 47

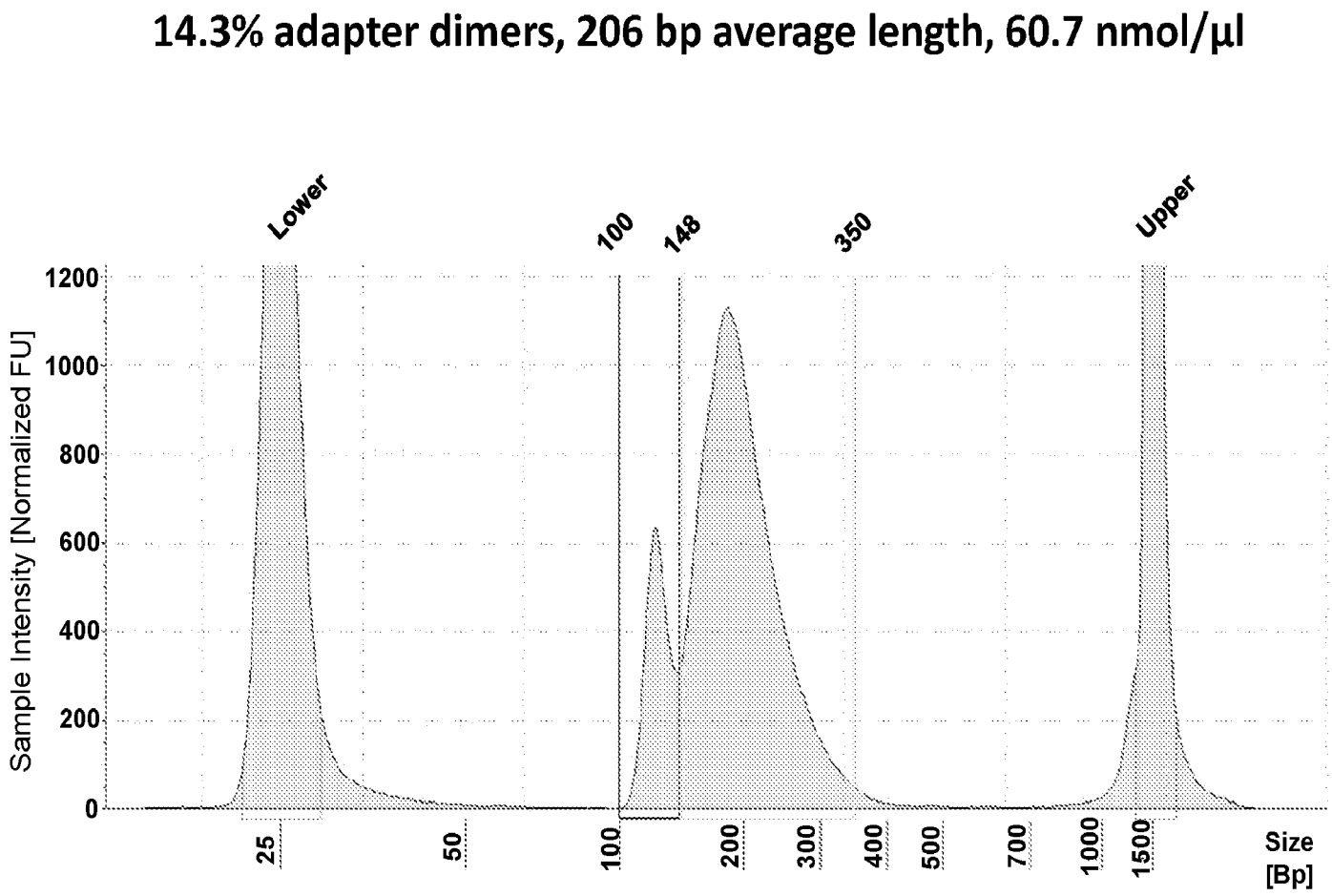

14.3% adapter dimers, 206 bp average length, 60.7 nmol/µl
Lower
100
148
350
Upper
Sample Intensity [Normalized FU]
1200
1000
800
600
400
200
0
25
50
100
200
300
400
500
700
1000
1500
Size [Bp]

Fig. 48A

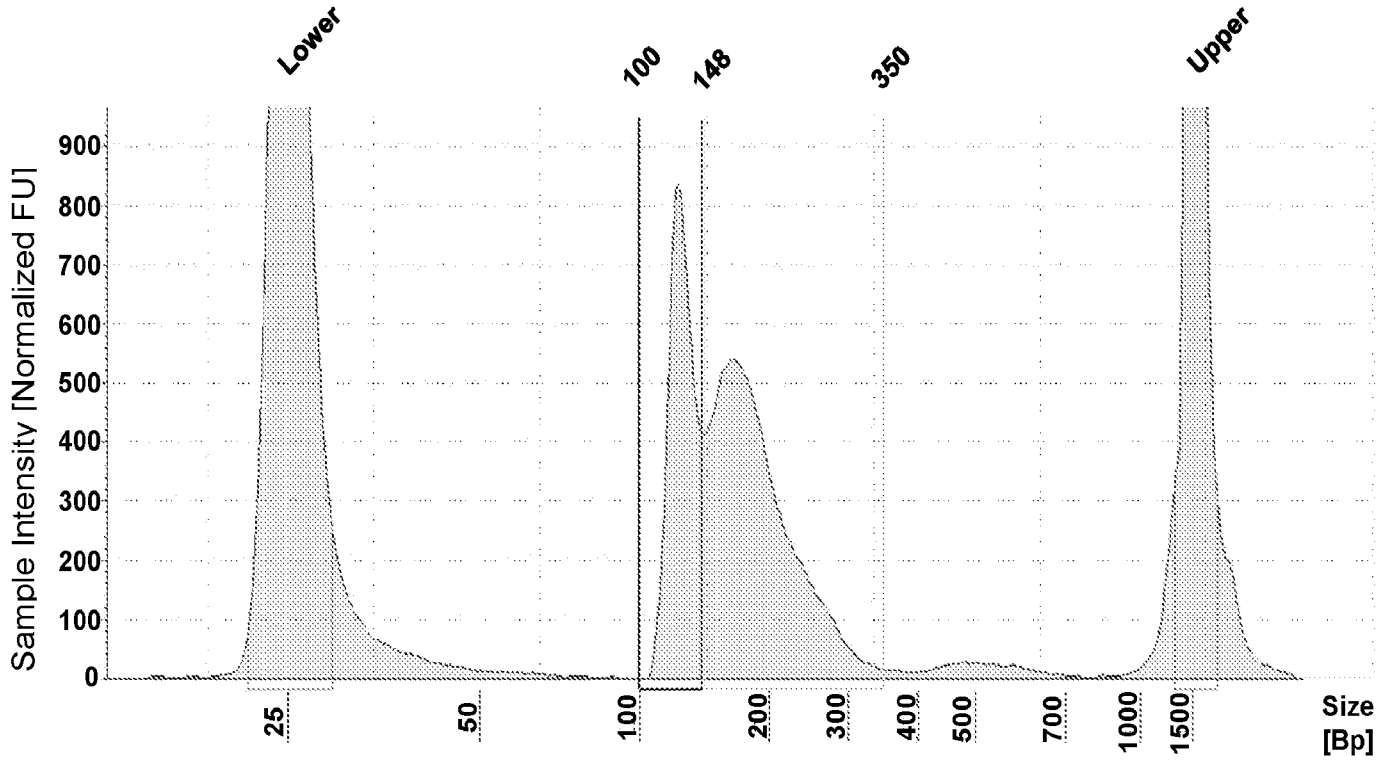

26.4% adapter dimers, 197 bp average length, 28.5 nmol/µl
Lower
100
148
350
Upper
Sample Intensity [Normalized FU]
900
800
700
600
500
400
300
200
100
0
25
50
100
200
300
400
500
700
1000
1500
Size [Bp]

Fig. 48B

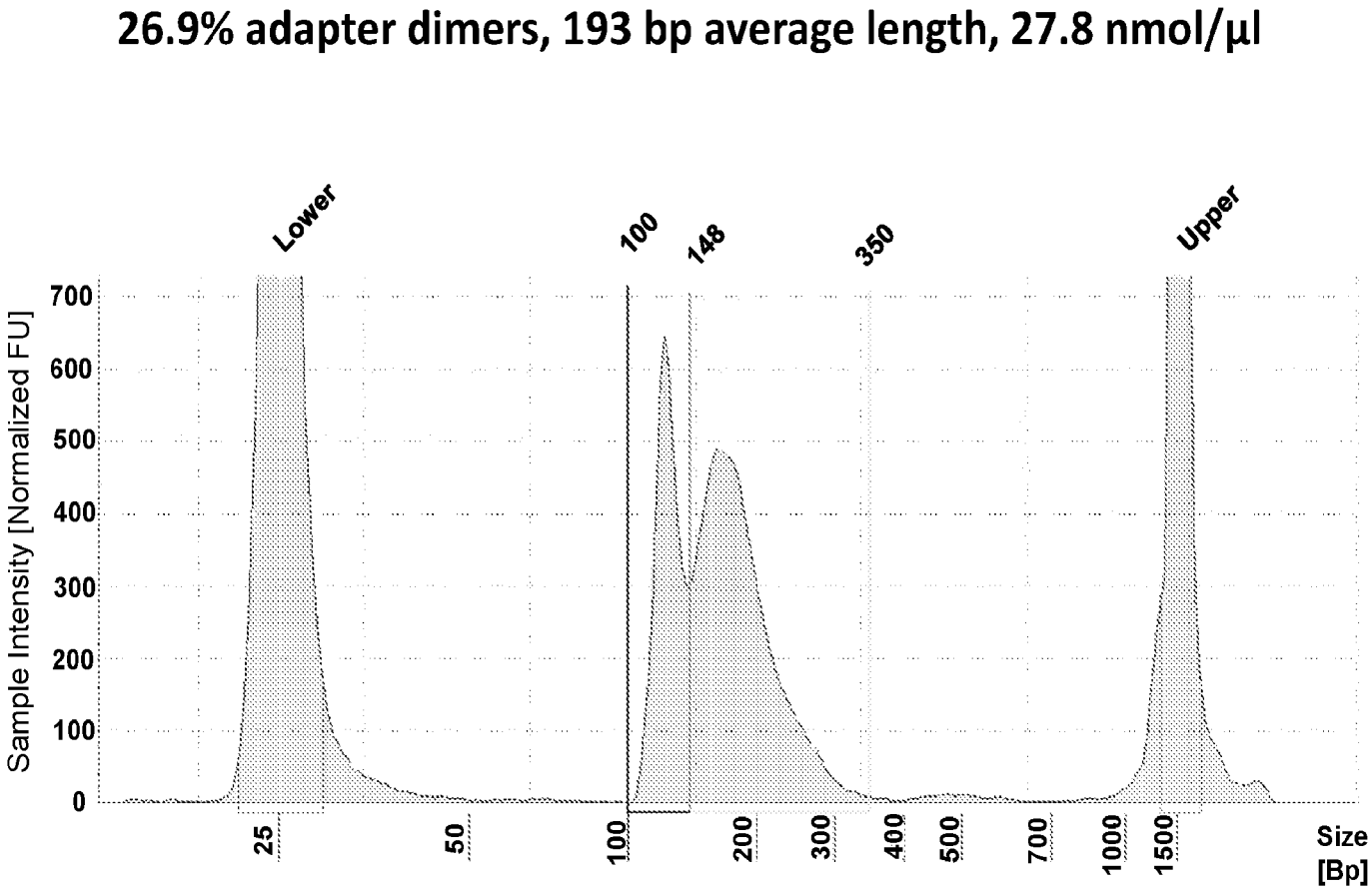

26.9% adapter dimers, 193 bp average length, 27.8 nmol/µl
Lower
100
148
350
Upper
Sample Intensity [Normalized FU]
700
600
500
400
300
200
100
0
25
50
100
200
300
400
500
700
1000
1500
Size
[Bp]

Fig. 48C

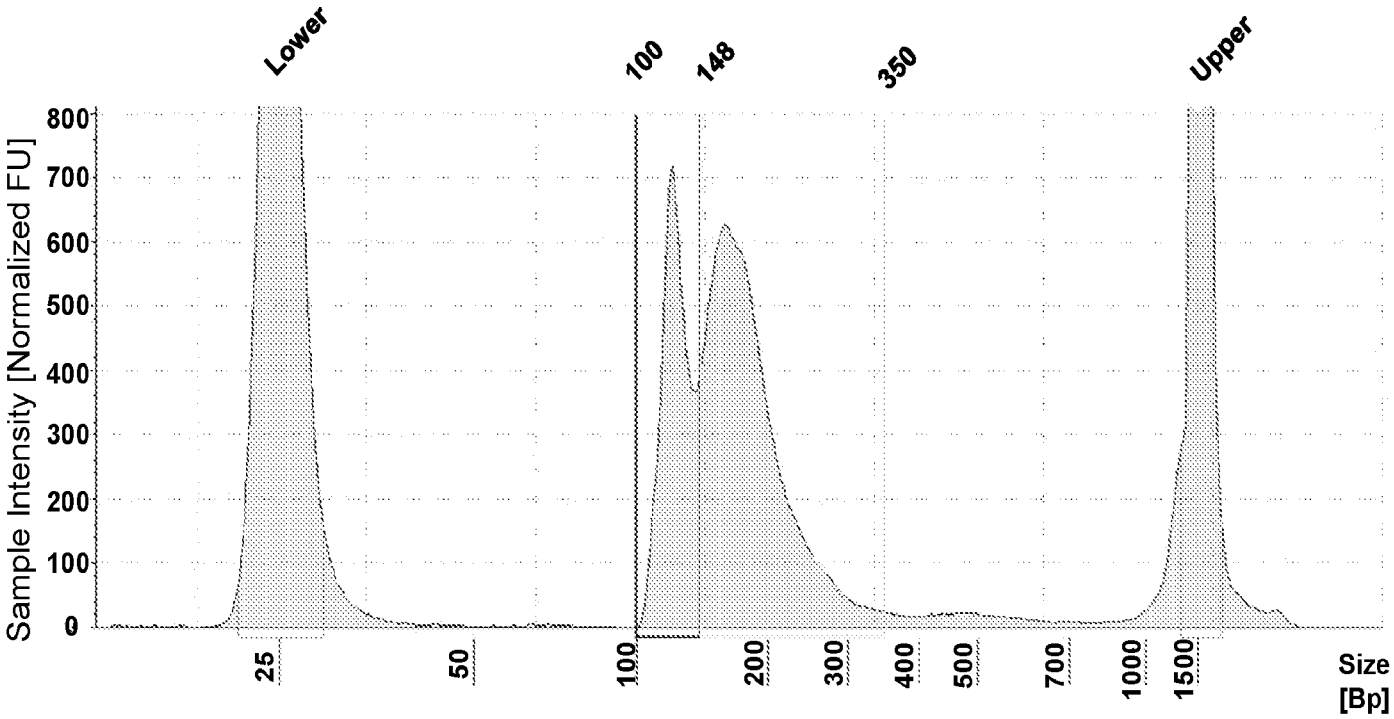

27.8% adapter dimers, 192 bp average length, 32.8 nmol/µl
Lower
100
148
350
Upper
Sample Intensity [Normalized FU]
800
700
600
500
400
300
200
100
0
25
50
100
200
300
400
500
700
1000
1500
Size
[Bp]

Fig. 48D

50 µl EB - SPRI

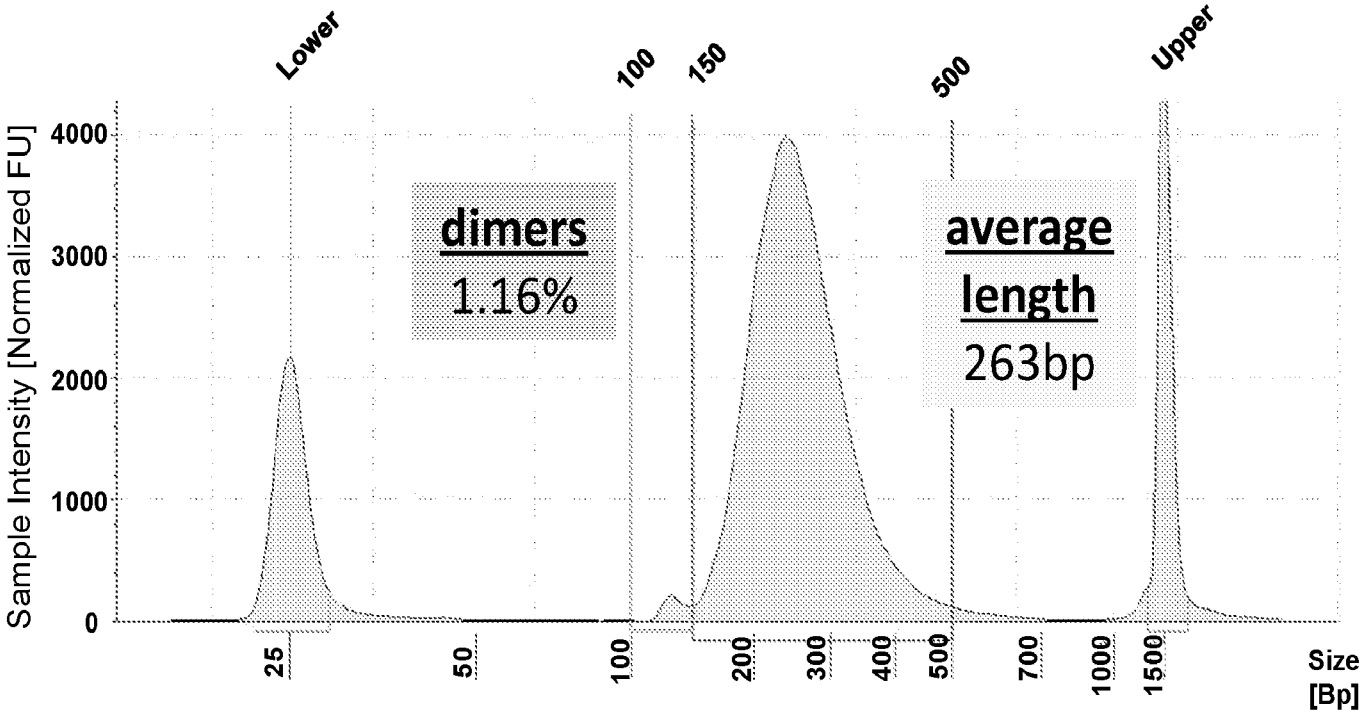


Fig. 49A

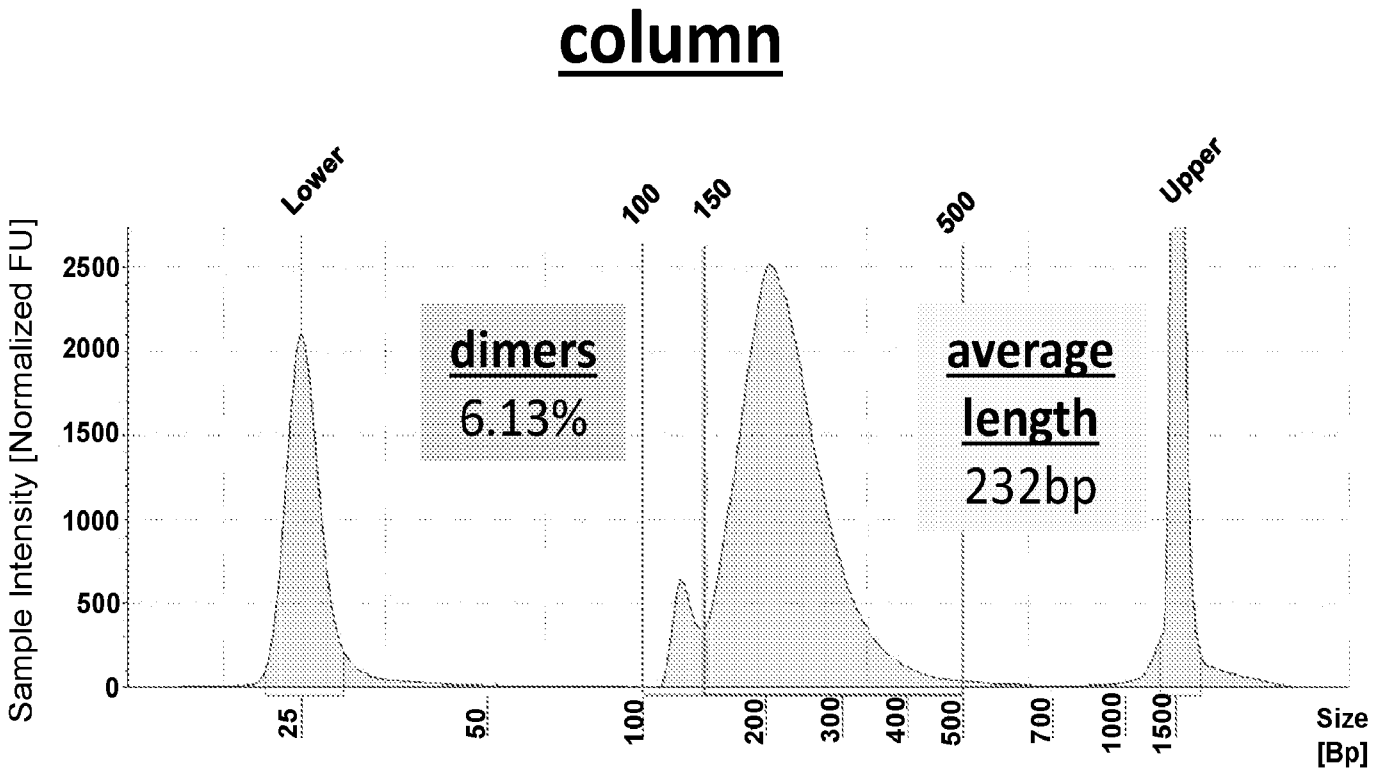

column
Lower
100
150
500
Upper
Sample Intensity [Normalized FU]
2500
2000
1500
1000
500
0
dimers
6.13%
average
length
232bp
25
50
100
200
300
400
500
700
1000
1500
Size
[Bp]

Fig. 49B

5 µl isopropanol, 45 µl EB - SPRI

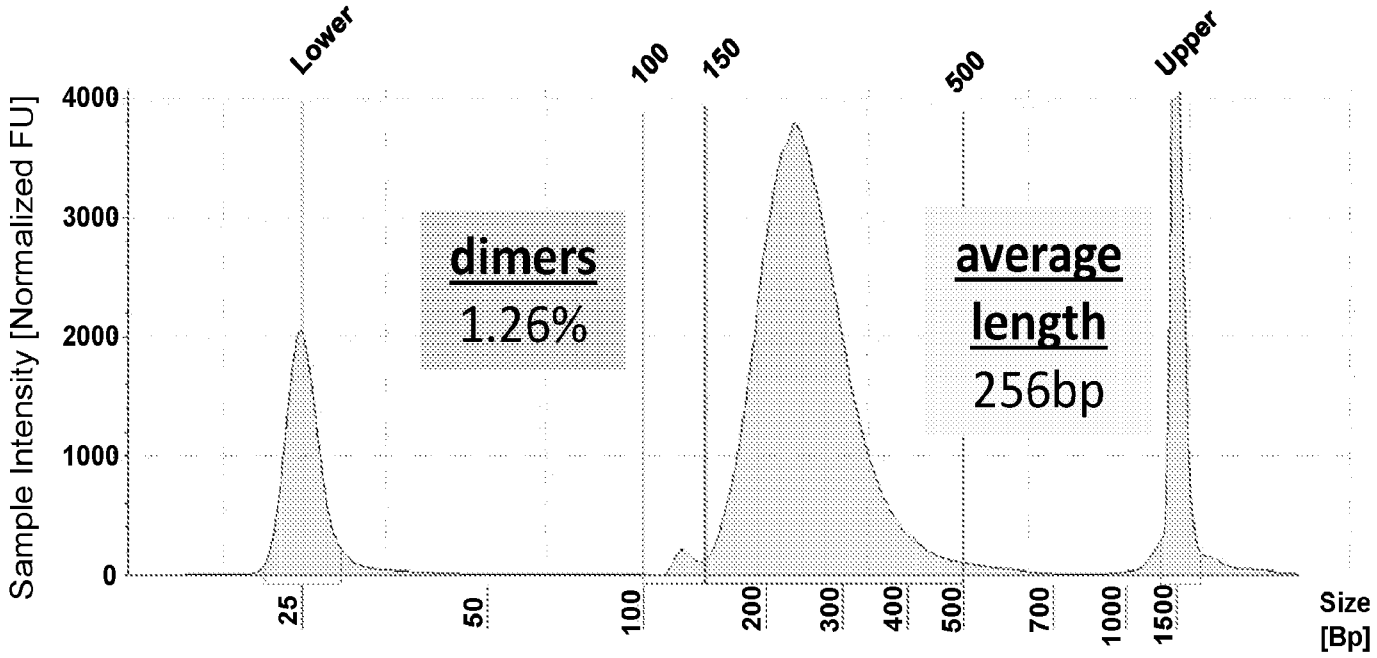


Fig. 49C

10 µl isopropanol, 40 µl EB - SPRI

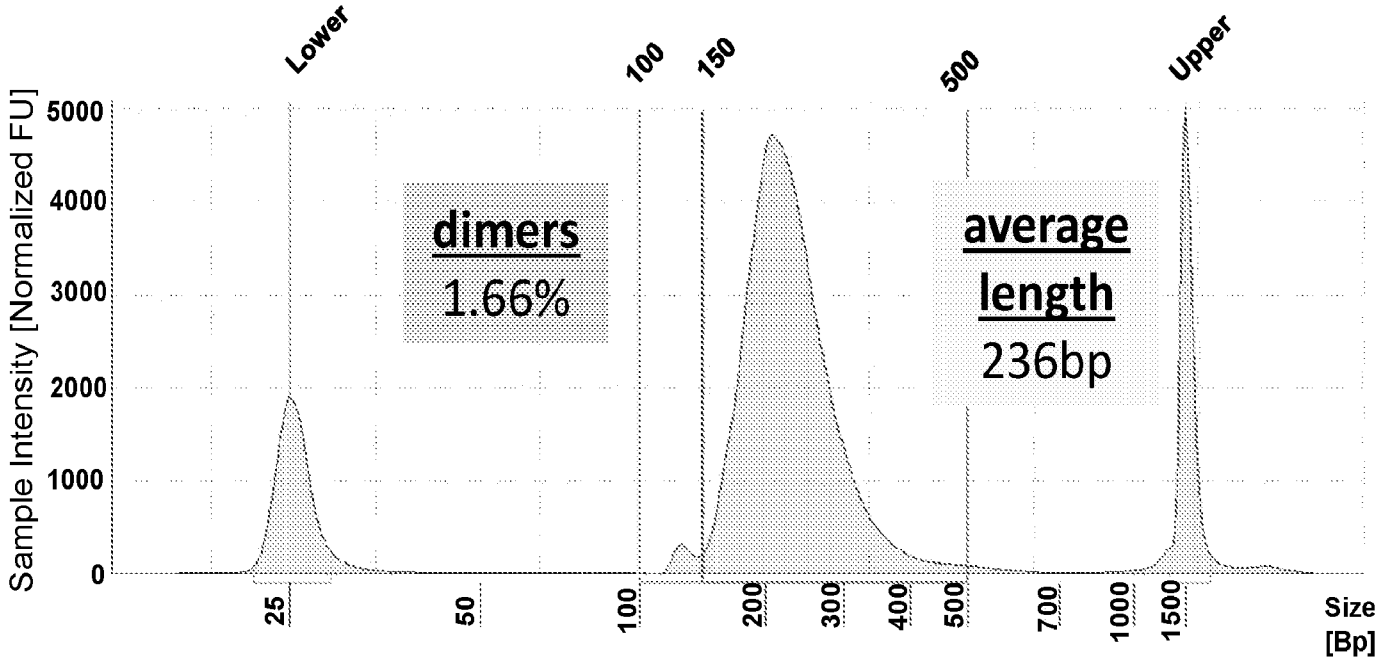


Fig. 49D

20 µl isopropanol, 30 µl EB - SPRI

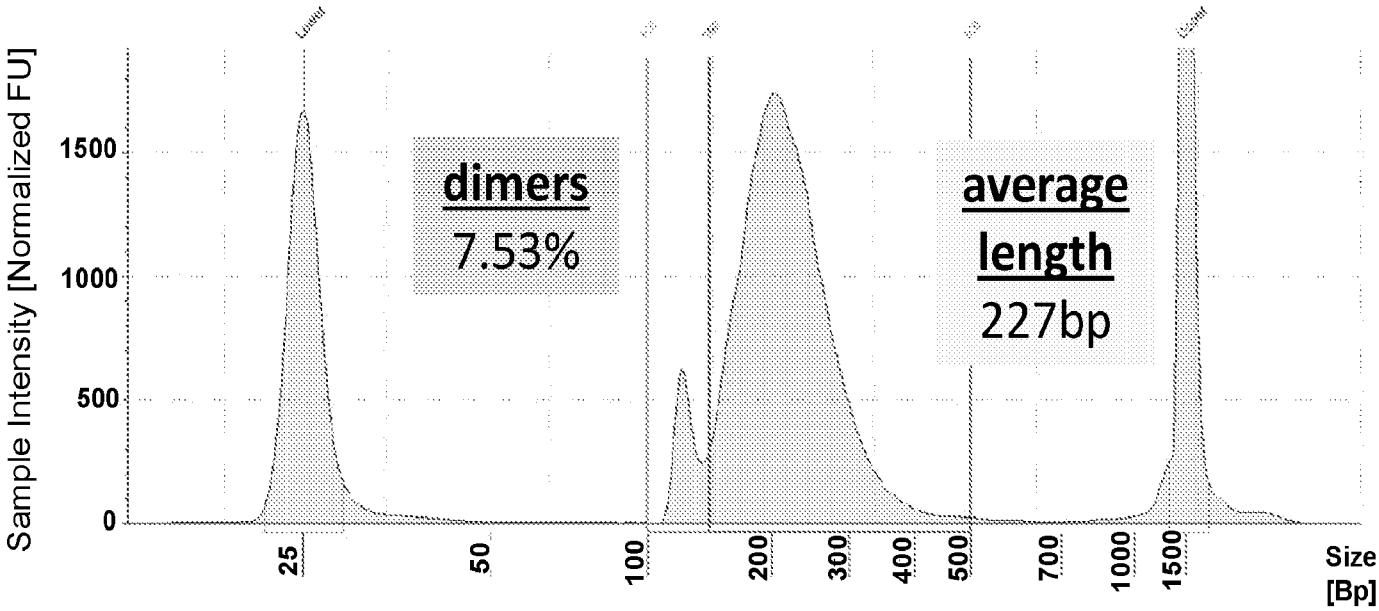


Fig. 49E

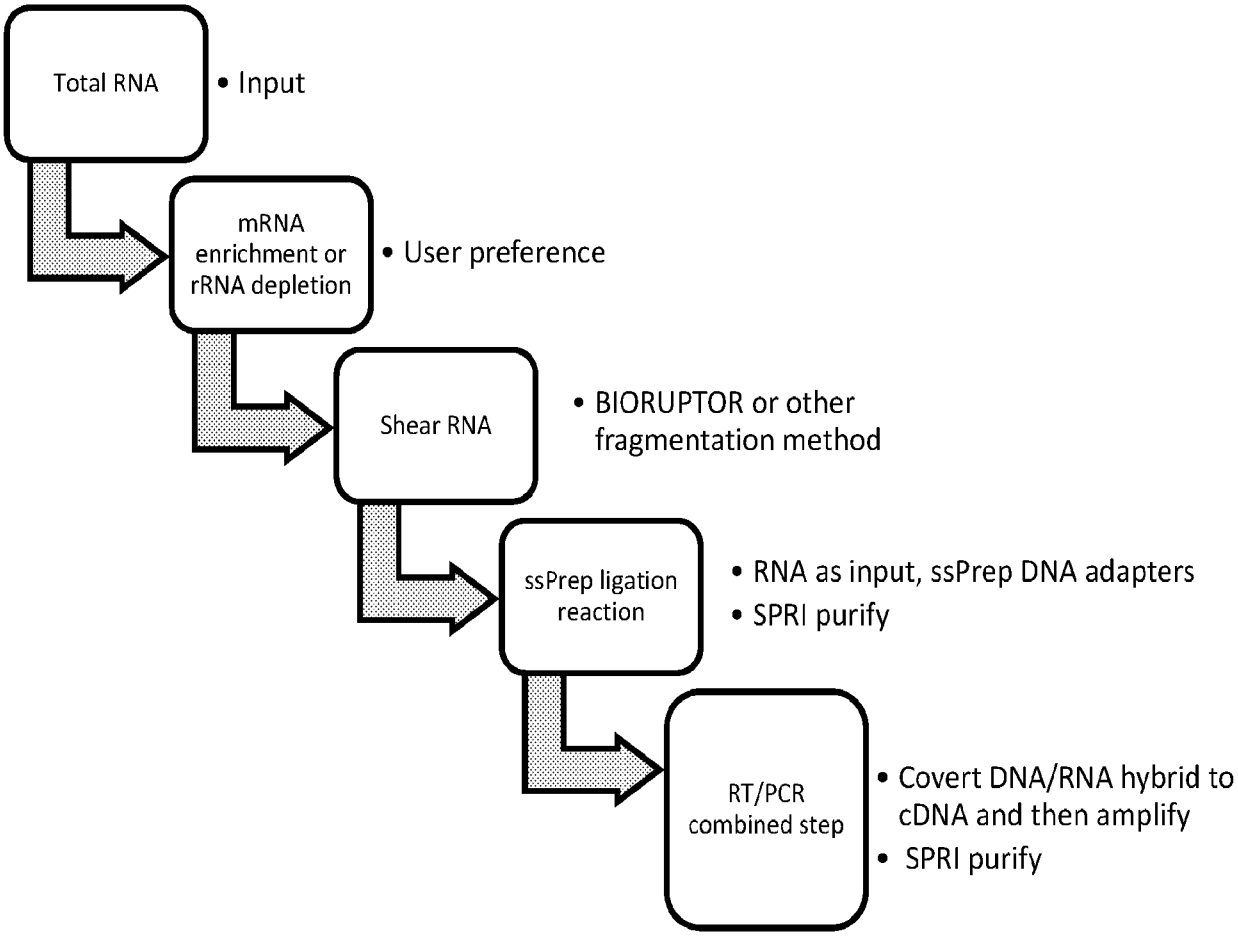
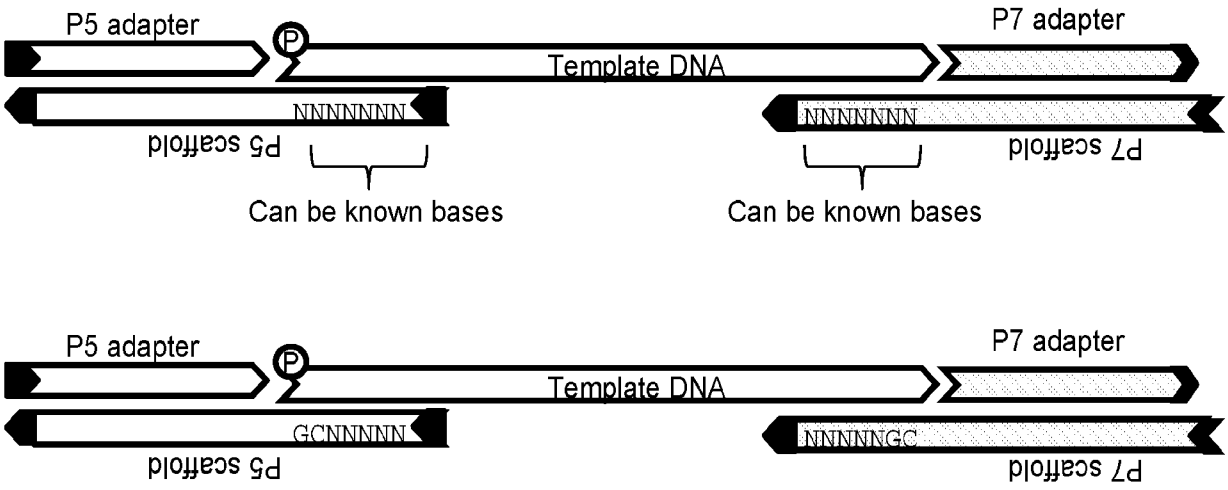

Total RNA
• Input
mRNA enrichment or rRNA depletion
• User preference
Shear RNA
• BIORUPTOR or other fragmentation method
ssPrep ligation reaction
• RNA as input, ssPrep DNA adapters
• SPRI purify
RT/PCR combined step
• Covert DNA/RNA hybrid to cDNA and then amplify
• SPRI purify

Fig. 50



Library Information	Percent of Reads that Start with CG	Percent of Reads that End with CG
No Enriched Scaffolds	1.7	1.1
CG Dinucleotide Enriched Scafolds	5.2	19.6

Fig. 51

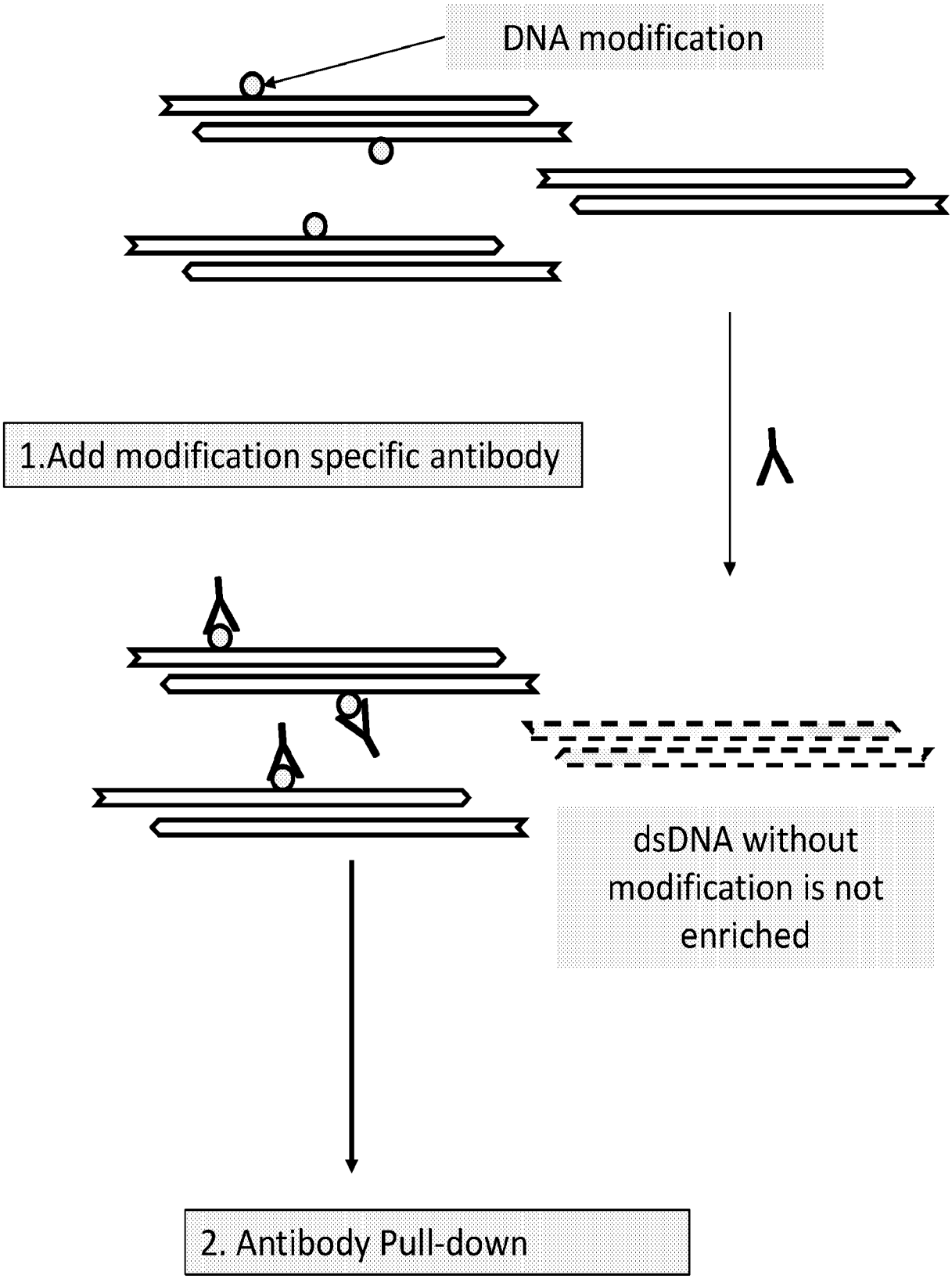

DNA modification
1.Add modification specific antibody
dsDNA without modification is not enriched
2. Antibody Pull-down

Fig. 52A

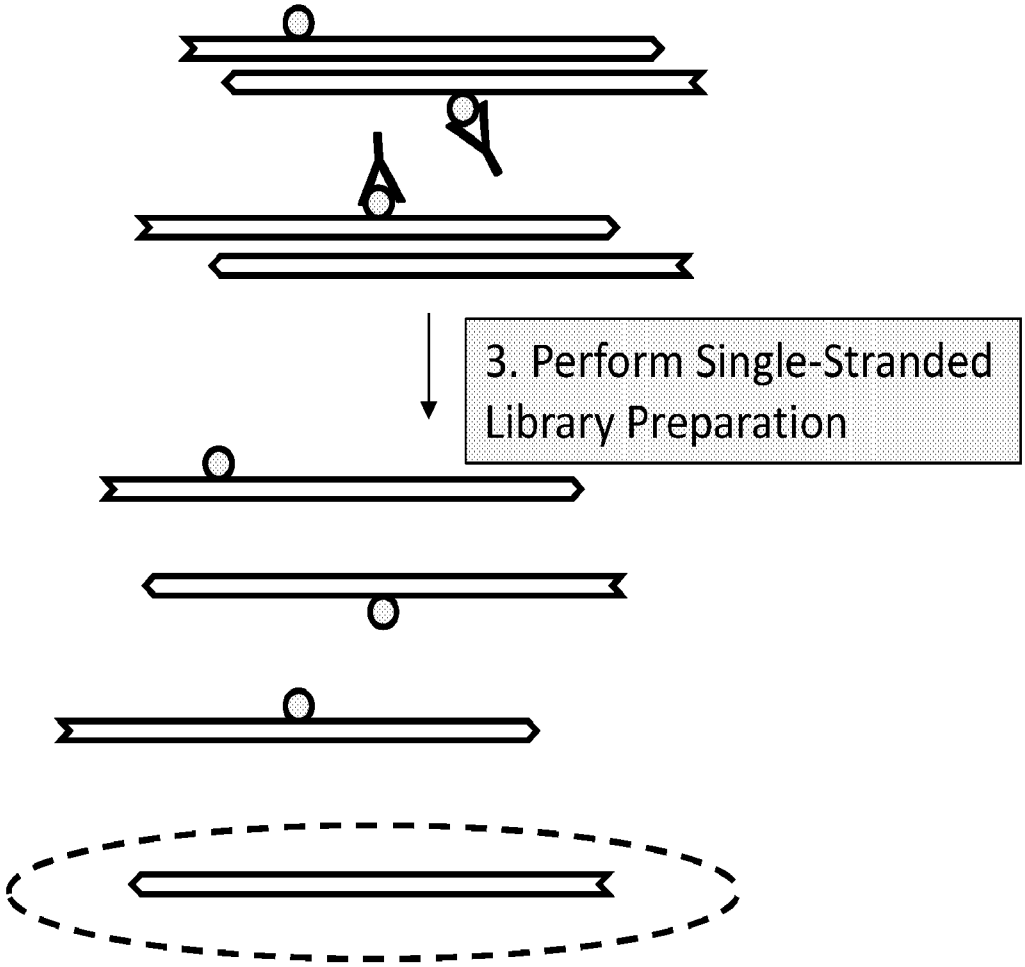


Fig. 52B

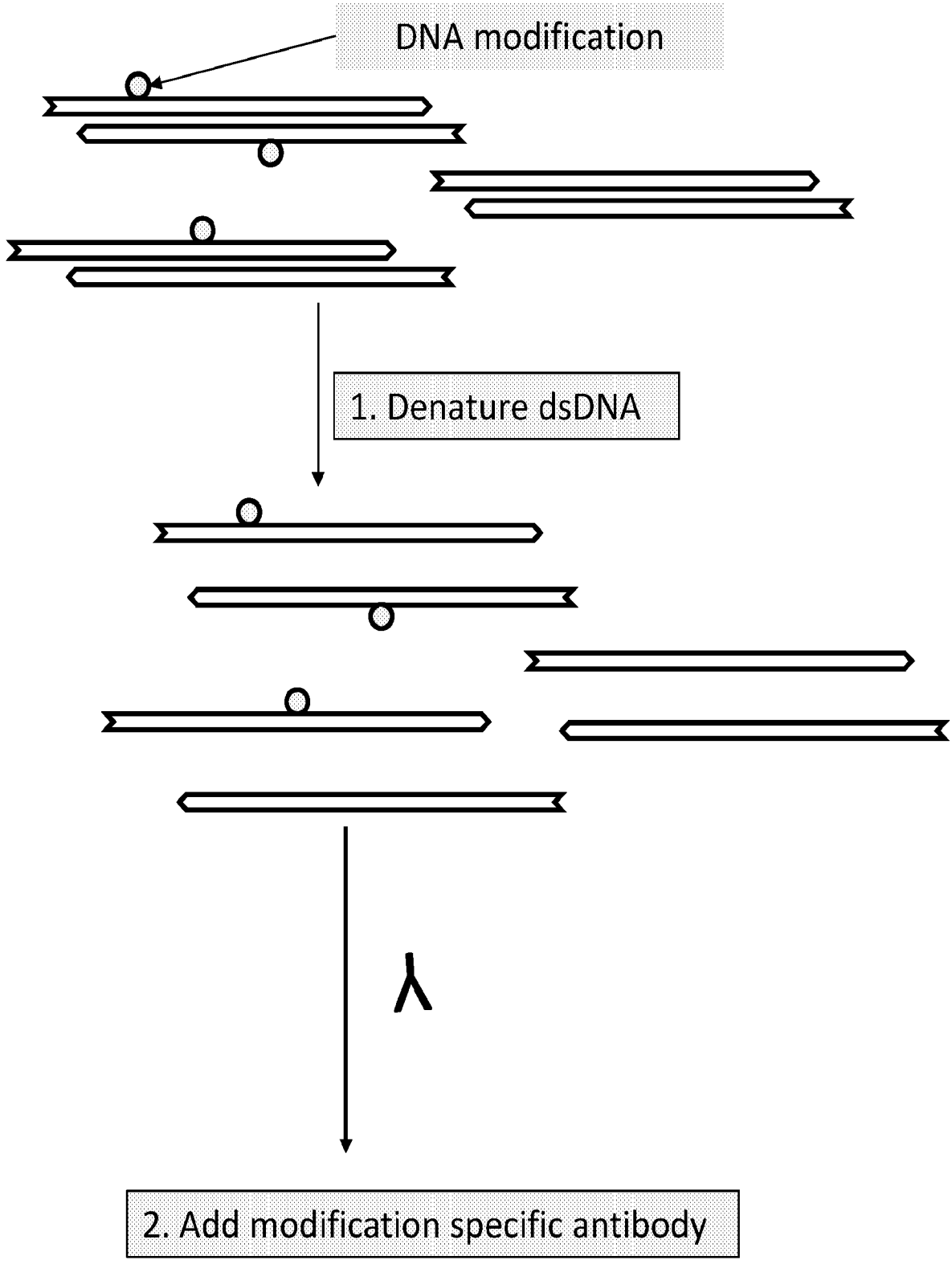

DNA modification
1. Denature dsDNA
2. Add modification specific antibody

Fig. 52C

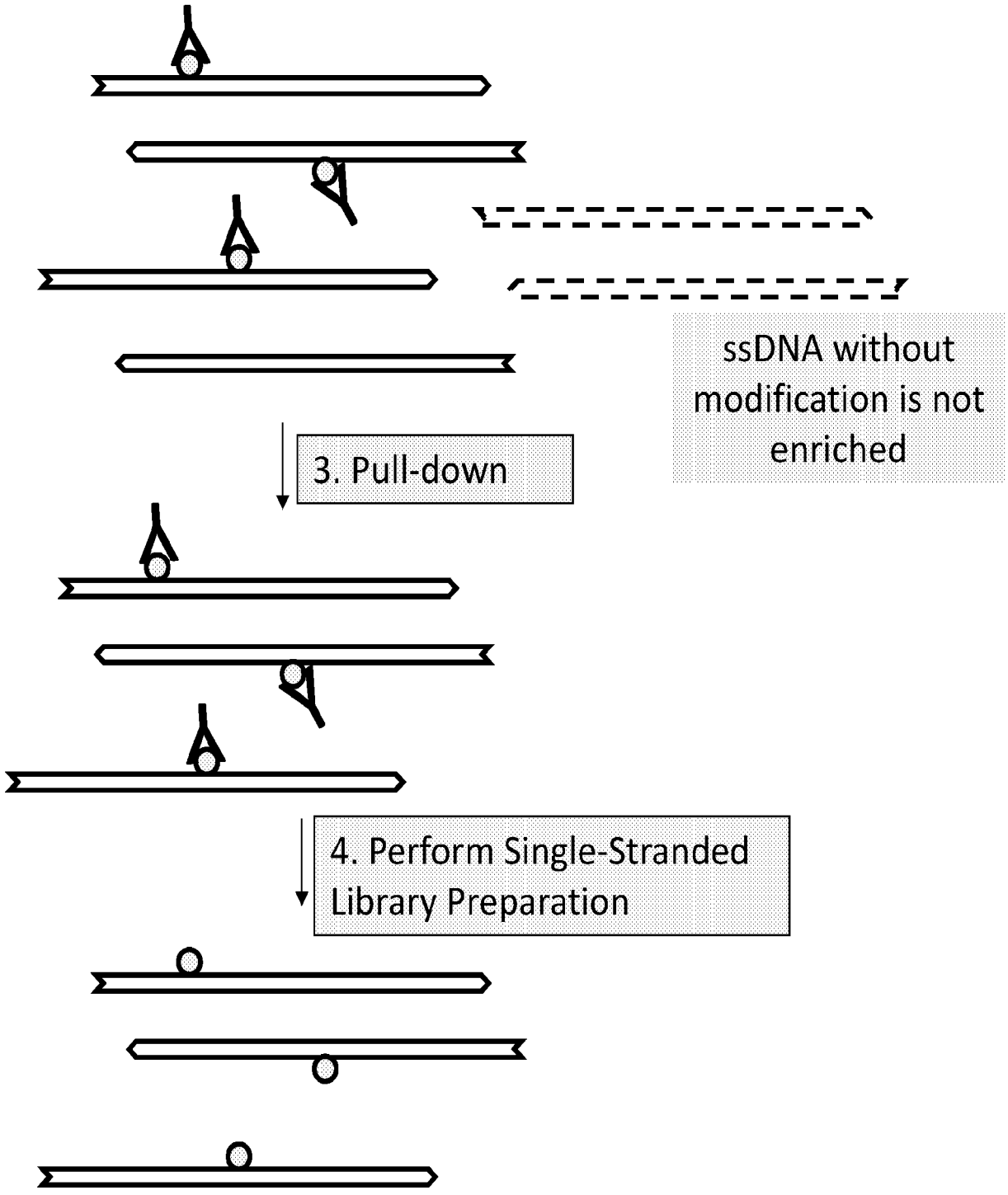
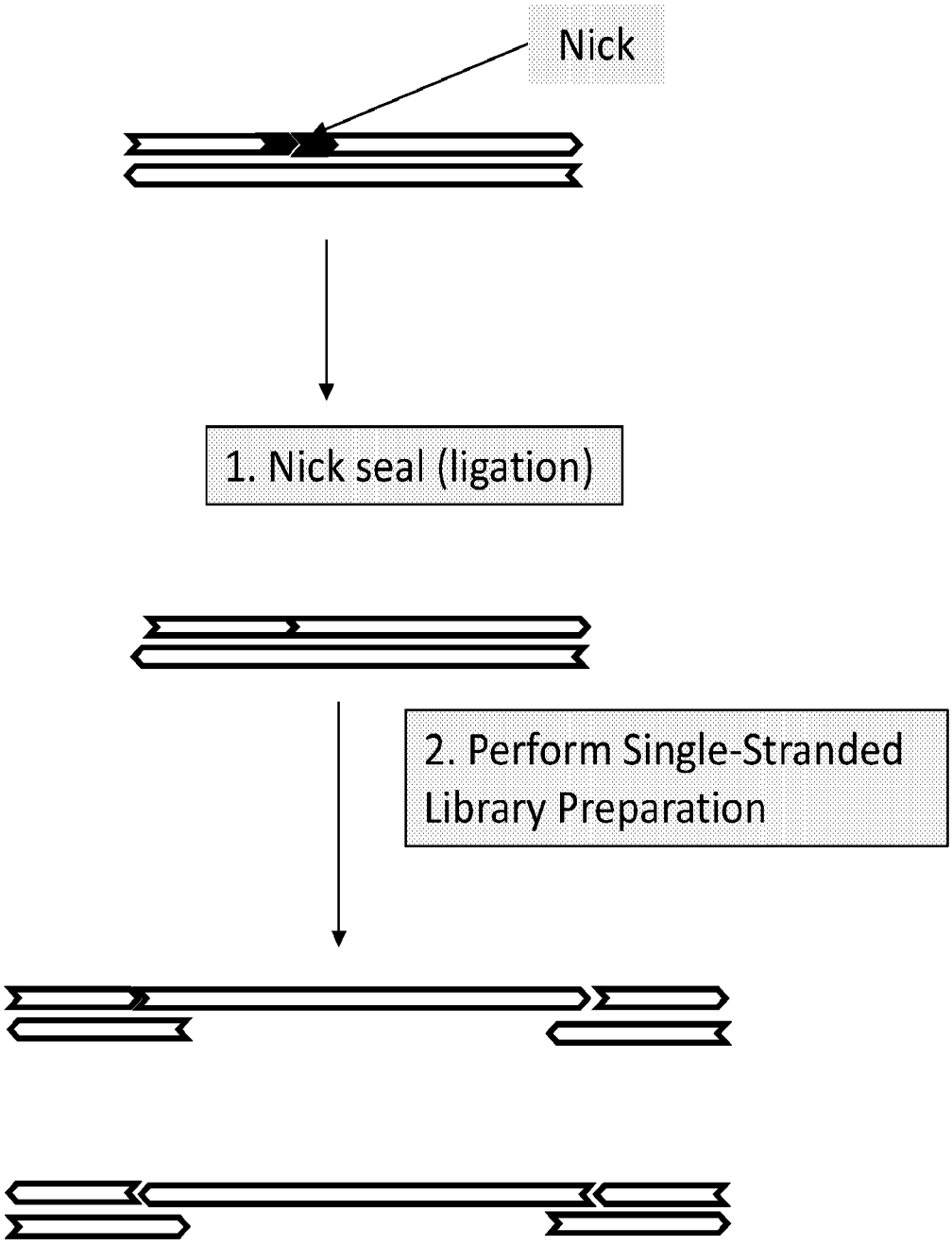
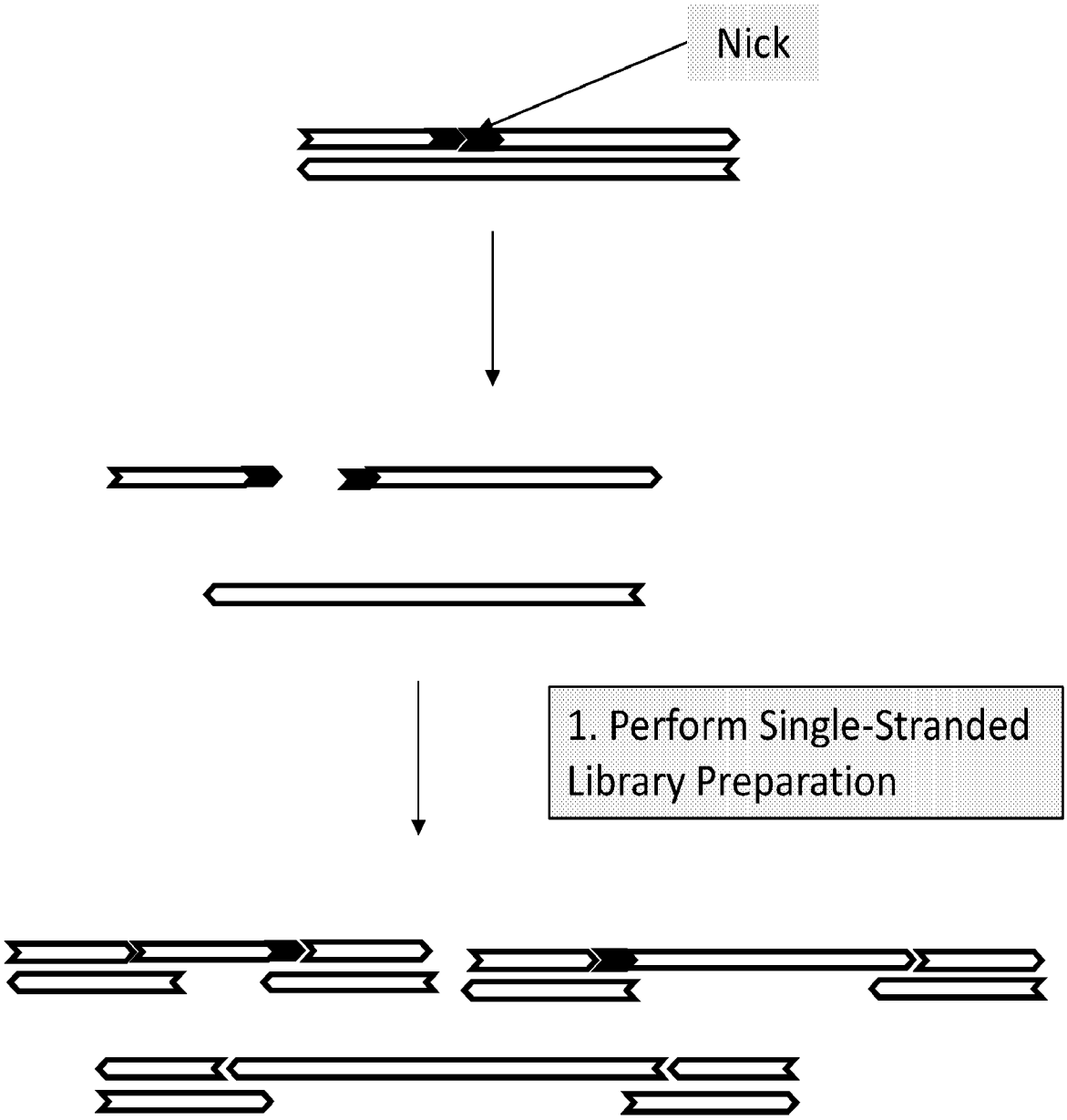

ssDNA without modification is not enriched
3. Pull-down
4. Perform Single-Stranded Library Preparation

Fig. 52D



Library A- Loses nick end information

Fig. 53A



Library B -Retains nick end information

Fig. 53B

Oligonucleotide DNA and
Scaffold Polynucleotide DNA:

Oligonucleotide RNA and
Scaffold Polynucleotide DNA:

Oligonucleotide RNA and
Scaffold Polynucleotide RNA:

Fig. 54

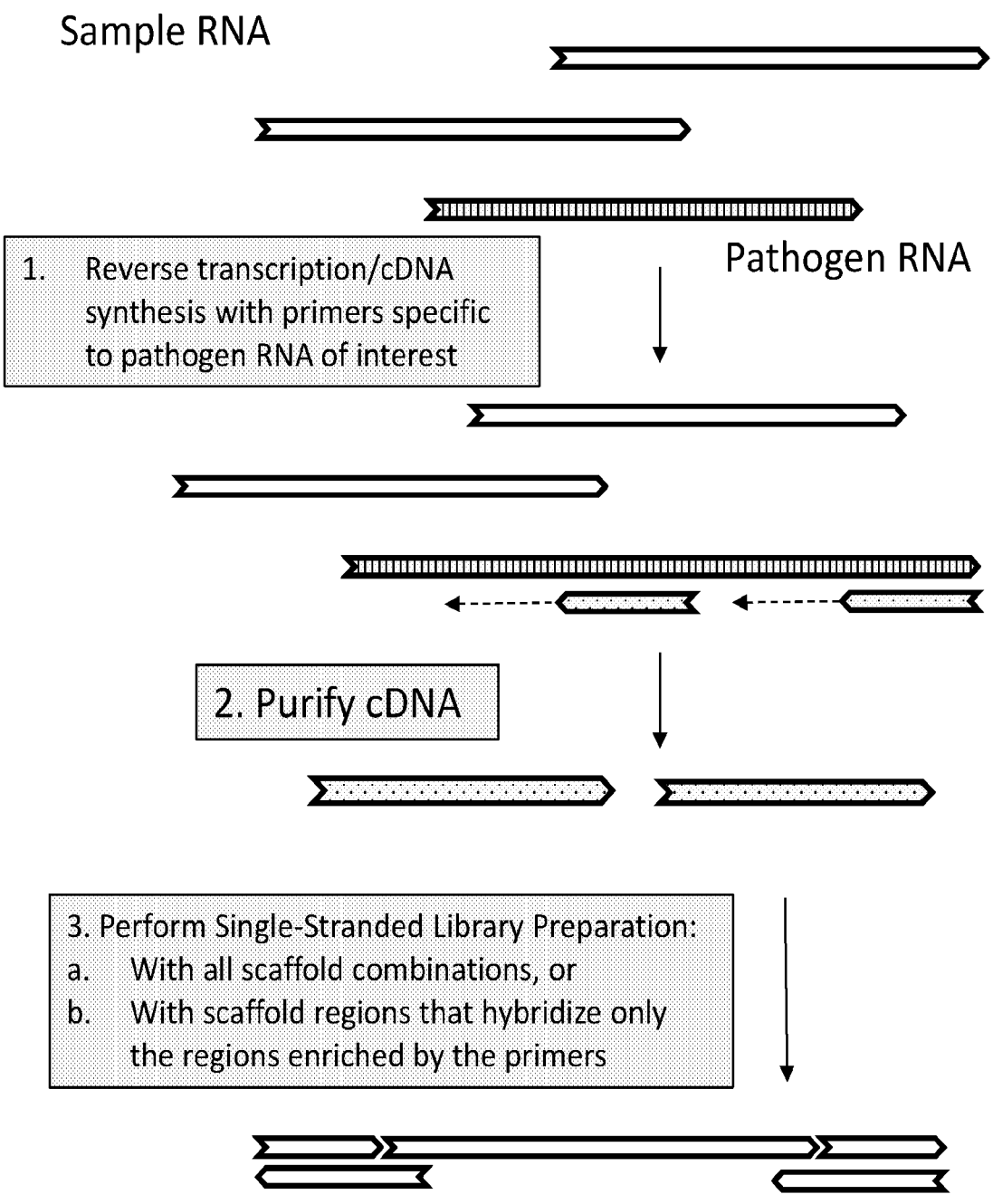

Sample RNA
Pathogen RNA
1. Reverse transcription/cDNA synthesis with primers specific to pathogen RNA of interest
2. Purify cDNA
3. Perform Single-Stranded Library Preparation:
a. With all scaffold combinations, or
b. With scaffold regions that hybridize only the regions enriched by the primers

Fig. 55