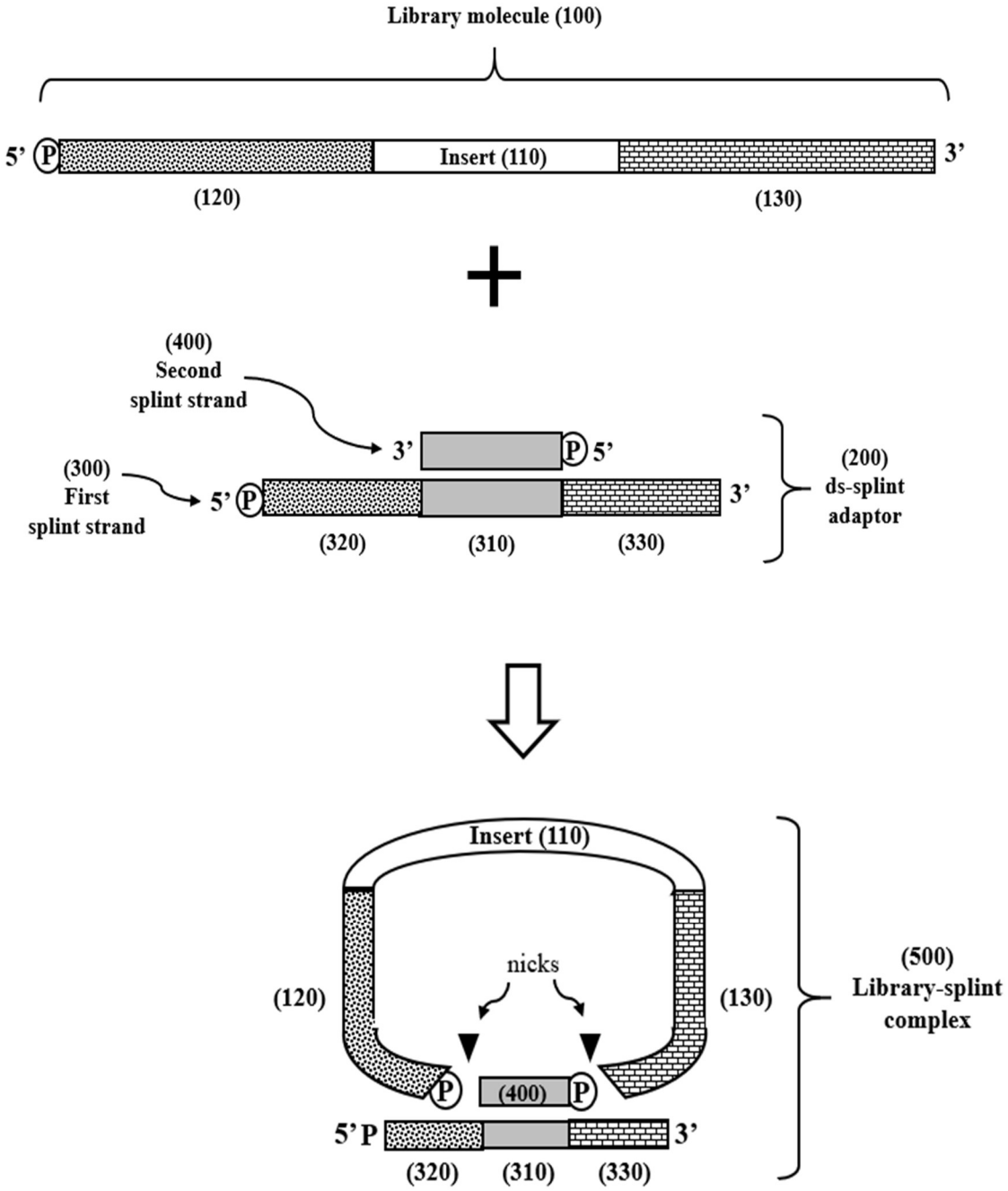

Library molecule (100)
5'
P
Insert (110)
3'
(120)
(130)
(400)
Second
splint strand
3'
P
5'
(300)
First
splint strand
5'
P
3'
(200)
ds-splint
adaptor
(320)
(310)
(330)
Insert (110)
nicks
(120)
(130)
(500)
Library-splint
complex
P
(400)
P
5' P
3'
(320)
(310)
(330)

FIG. 1

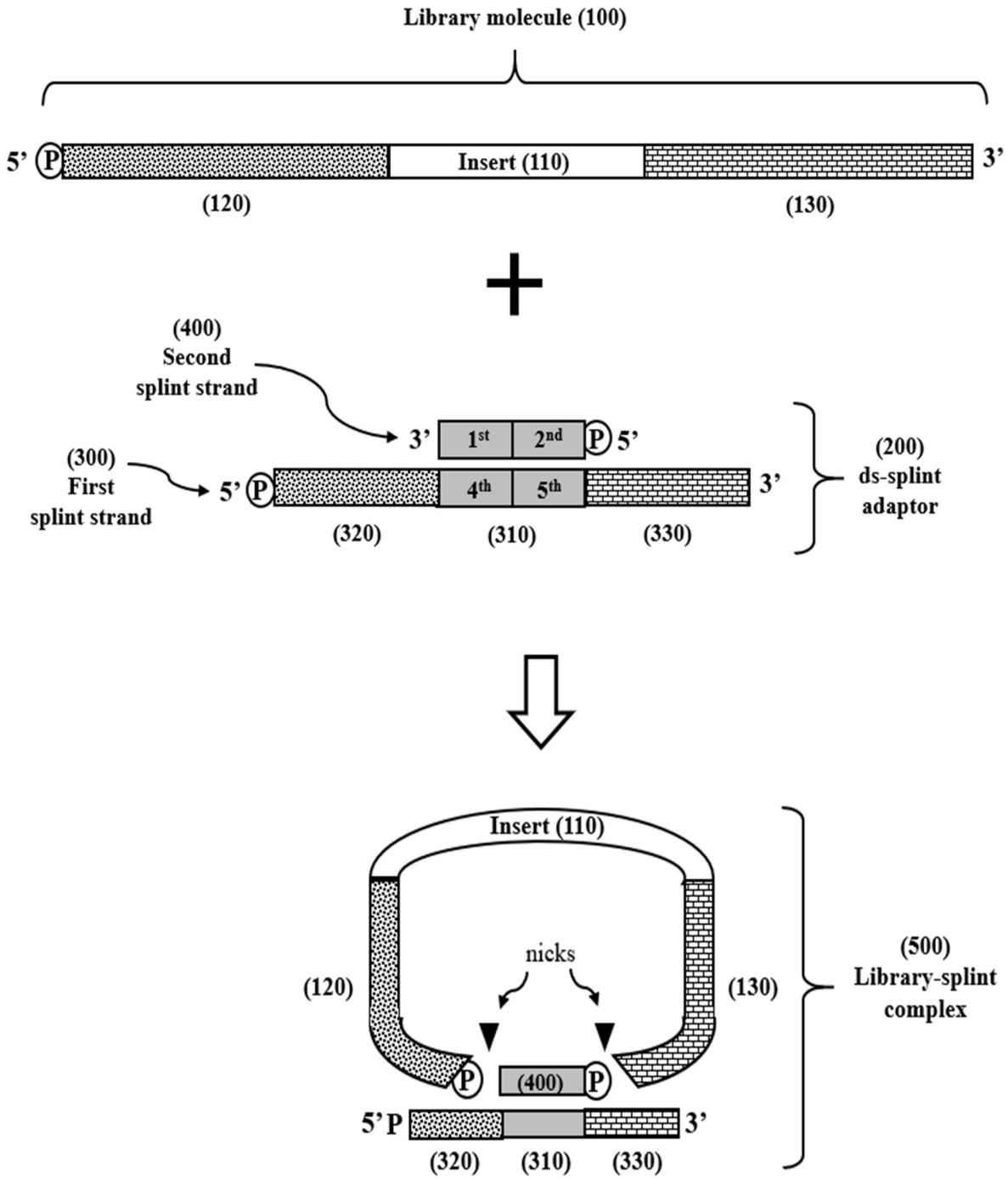

Library molecule (100)
5'
P
Insert (110)
3'
(120)
(130)
(400)
Second
splint strand
3'
1st
2nd
P
5'
(300)
First
splint strand
5'
P
4th
5th
3'
(200)
ds-splint
adaptor
(320)
(310)
(330)
Insert (110)
nicks
(120)
(130)
(500)
Library-splint
complex
P
(400)
P
5' P
3'
(320)
(310)
(330)

FIG. 2

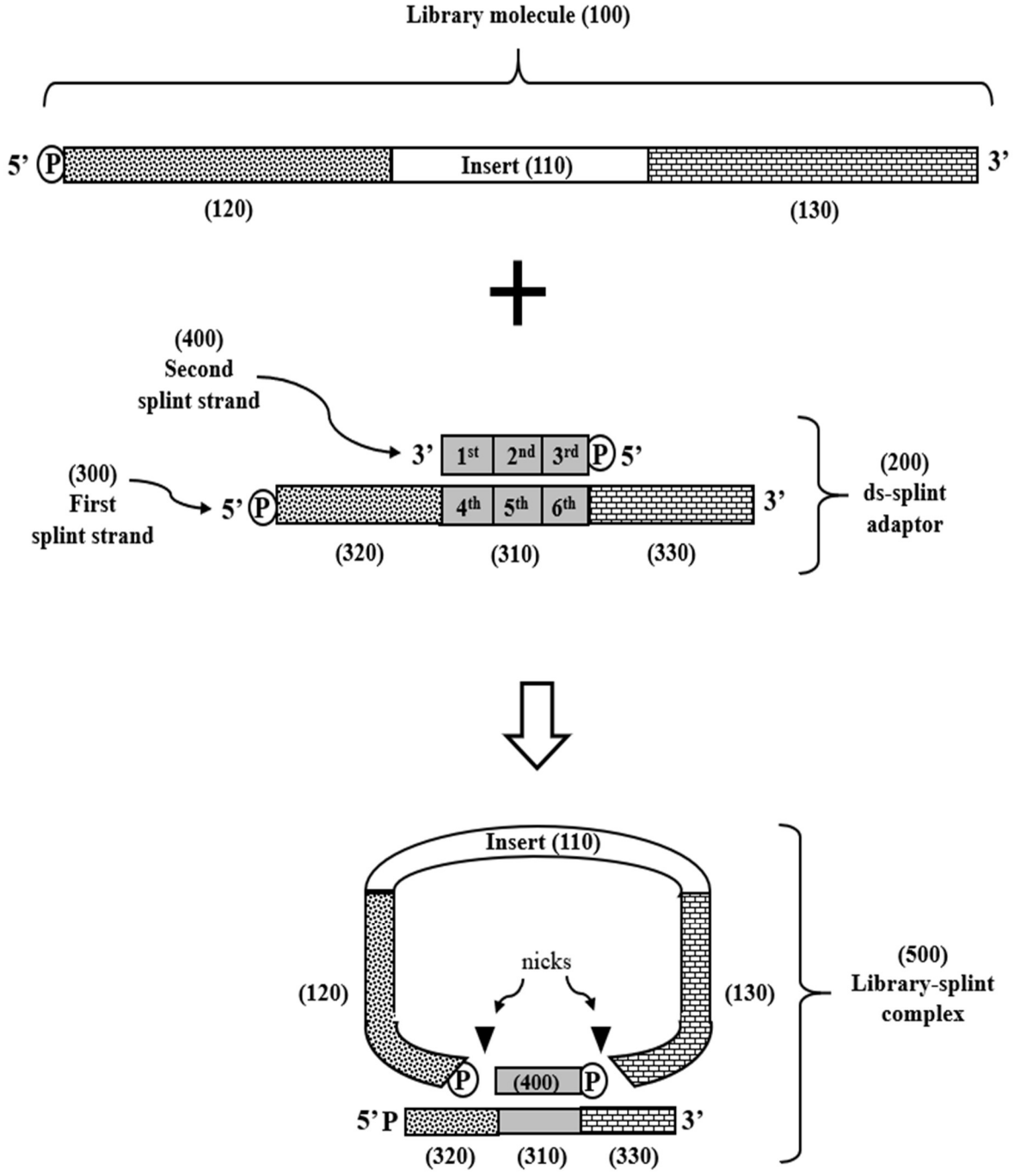

Library molecule (100)
5'
P
Insert (110)
3'
(120)
(130)
(400)
Second
splint strand
3'
1st
2nd
3rd
P
5'
(300)
First
splint strand
5'
P
4th
5th
6th
3'
(200)
ds-splint
adaptor
(320)
(310)
(330)
Insert (110)
nicks
(120)
(130)
(500)
Library-splint
complex
P
(400)
P
5' P
3'
(320)
(310)
(330)

FIG. 3

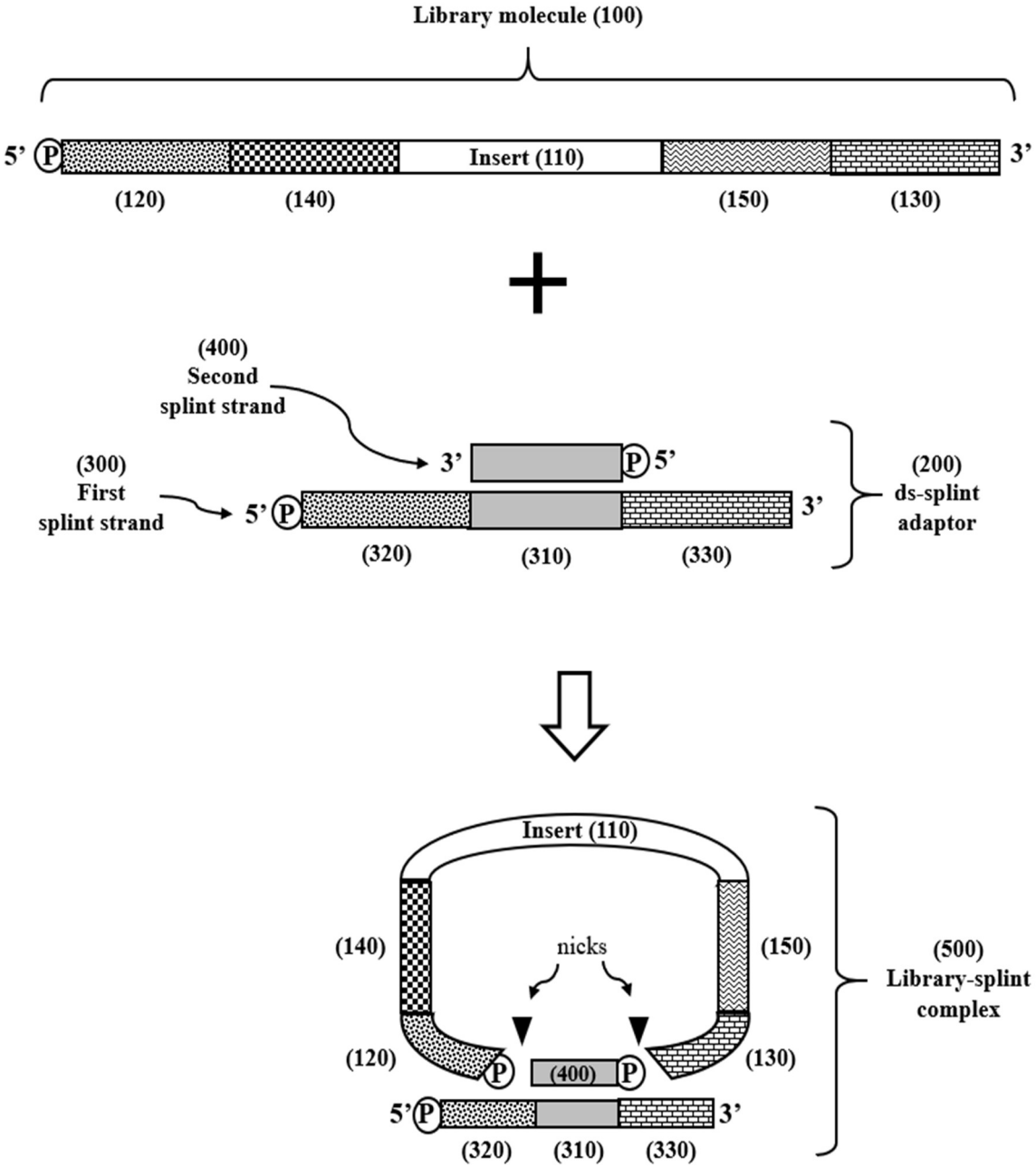

Library molecule (100)
5'
P
Insert (110)
3'
(120)
(140)
(150)
(130)
(400)
Second
splint strand
(300)
First
splint strand
3'
P
5'
5'
P
3'
(200)
ds-splint
adaptor
(320)
(310)
(330)
Insert (110)
(140)
nicks
(150)
(500)
Library-splint
complex
(120)
P
(400)
P
(130)
5'
P
3'
(320)
(310)
(330)

FIG. 4

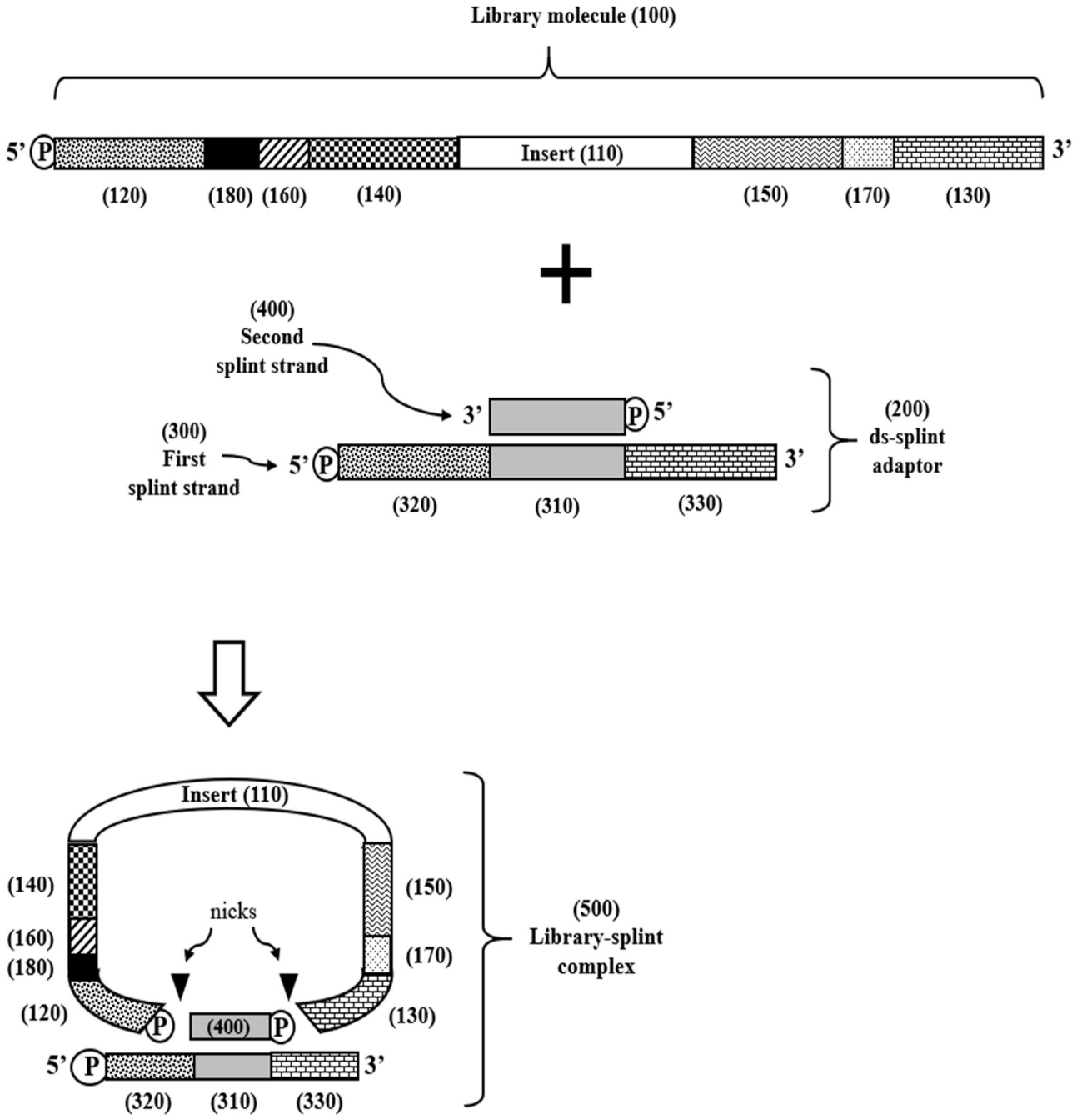

Library molecule (100)
5'
P
Insert (110)
3'
(120)
(180)
(160)
(140)
(150)
(170)
(130)
(400)
Second
splint strand
3'
P
5'
(300)
First
splint strand
5'
P
3'
(200)
ds-splint
adaptor
(320)
(310)
(330)
Insert (110)
(140)
(150)
nicks
(160)
(180)
(170)
(120)
P
(400)
P
(130)
5'
P
3'
(320)
(310)
(330)
(500)
Library-splint
complex

FIG. 5

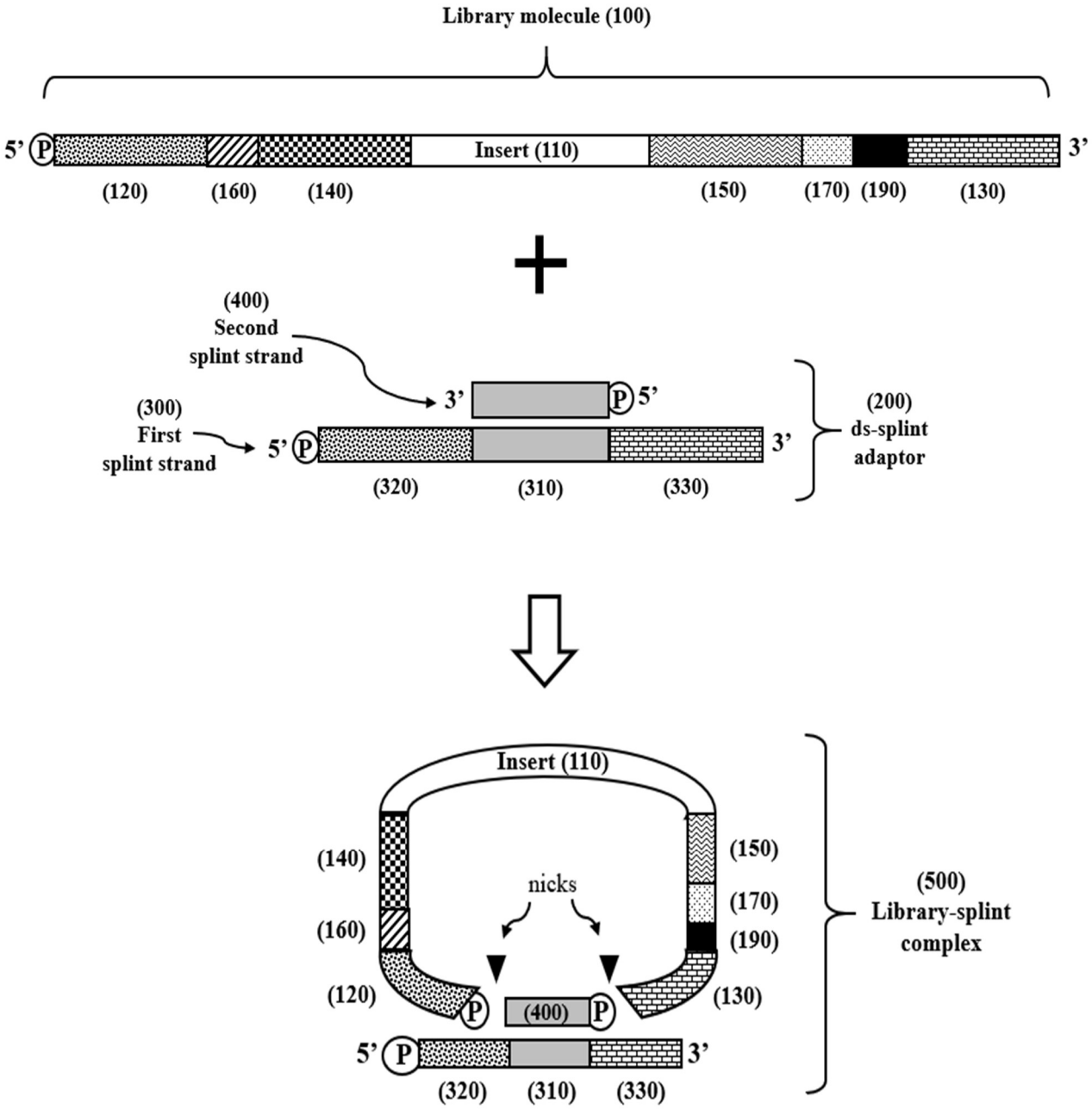

Library molecule (100)
5'
P
Insert (110)
3'
(120)
(160)
(140)
(150)
(170)
(190)
(130)
(400)
Second
splint strand
3'
P
5'
(300)
First
splint strand
5'
P
3'
(200)
ds-splint
adaptor
(320)
(310)
(330)
Insert (110)
(140)
(150)
nicks
(160)
(170)
(190)
(120)
P
(400)
P
(130)
5'
P
3'
(320)
(310)
(330)
(500)
Library-splint
complex

FIG. 6

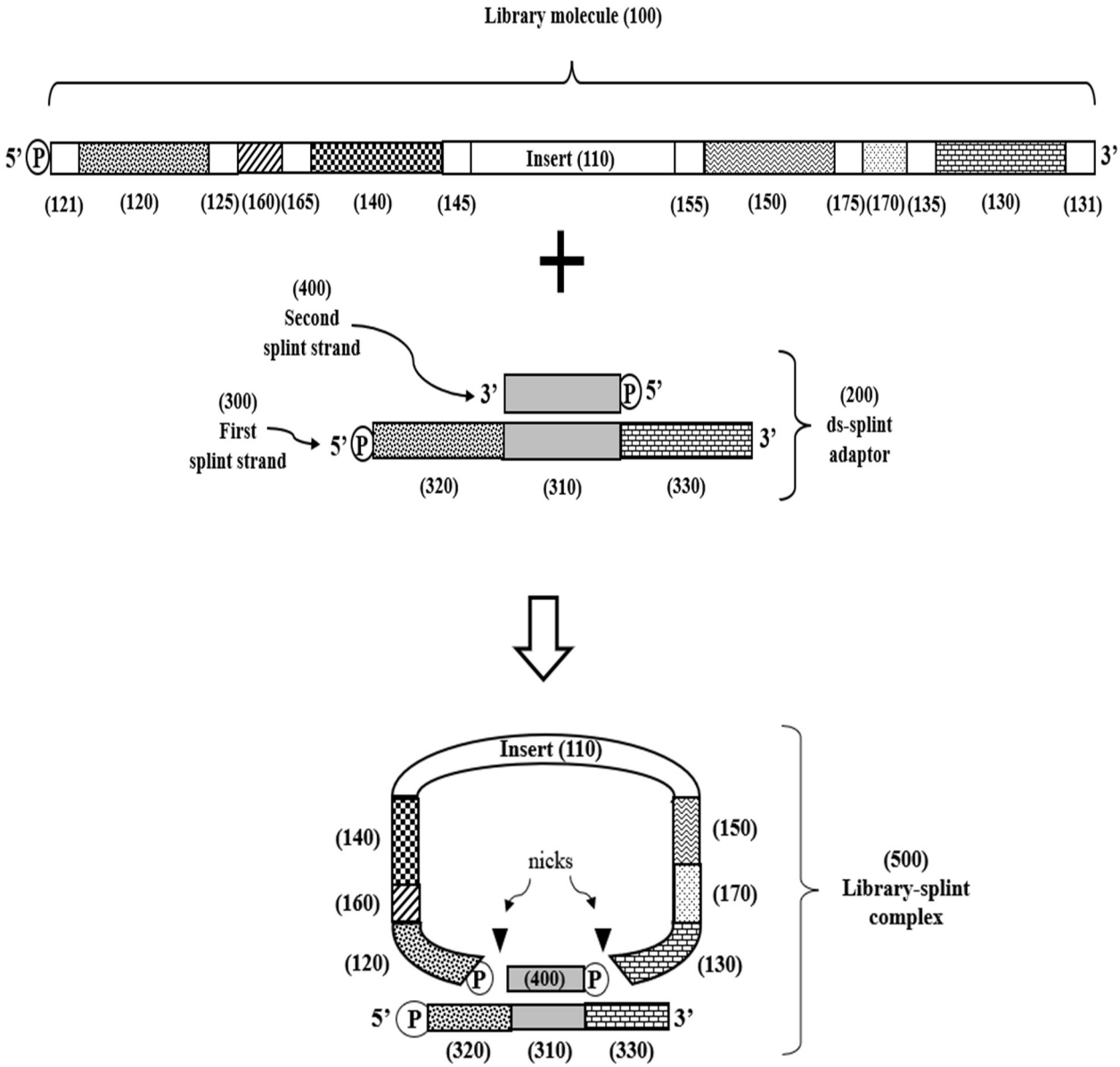

Library molecule (100)
5'
P
Insert (110)
3'
(121)
(120)
(125)
(160)
(165)
(140)
(145)
(155)
(150)
(175)
(170)
(135)
(130)
(131)
(400)
Second
splint strand
3'
P
5'
(300)
First
splint strand
5'
P
3'
(200)
ds-splint
adaptor
(320)
(310)
(330)
Insert (110)
(140)
(150)
nicks
(160)
(170)
(120)
P
(400)
P
(130)
5'
P
3'
(320)
(310)
(330)
(500)
Library-splint
complex

FIG. 7

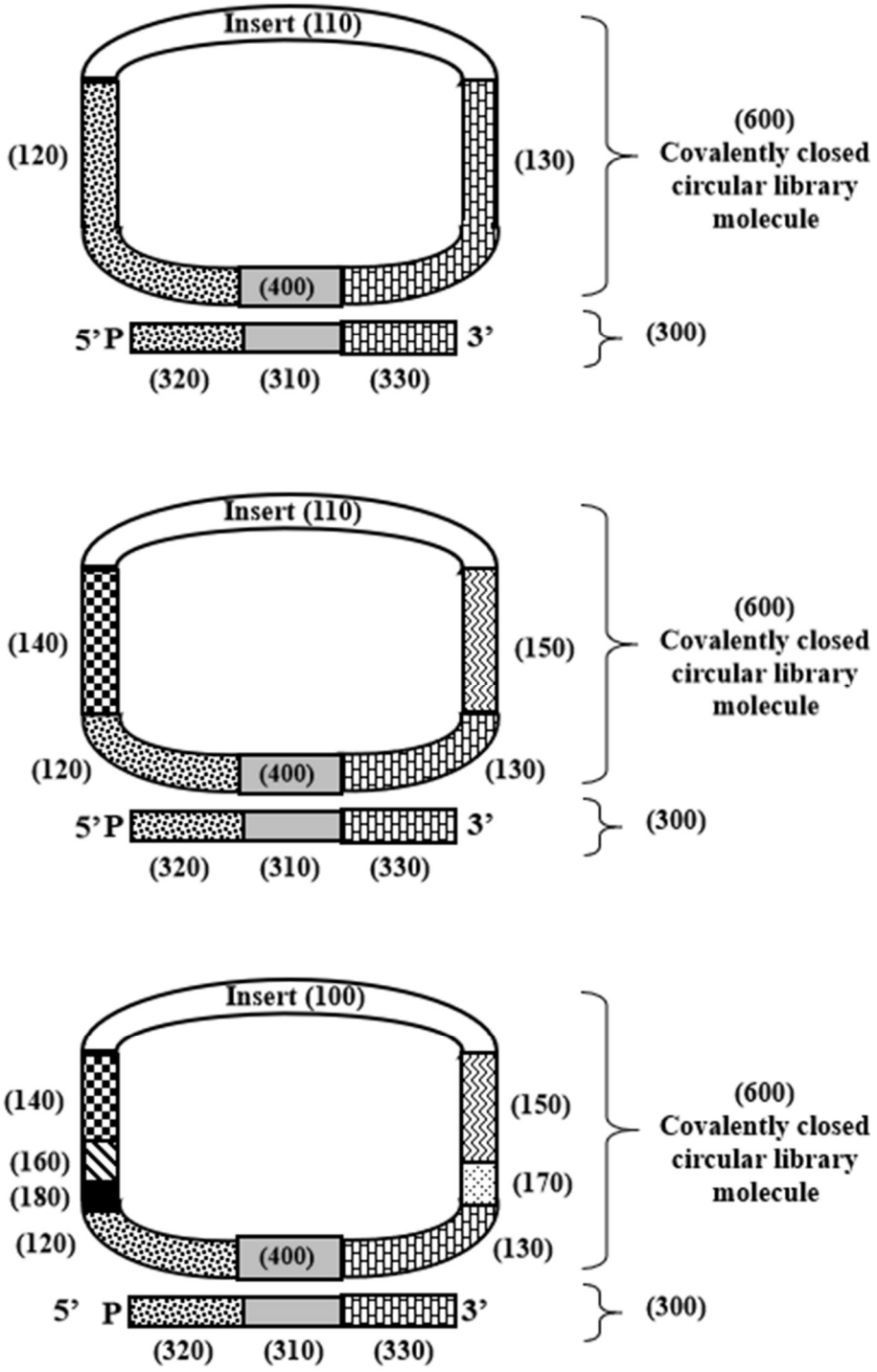

Insert (110)
(120)
(130)
(400)
(600)
Covalently closed
circular library
molecule
5'P
3'
(300)
(320)
(310)
(330)
Insert (110)
(140)
(150)
(120)
(400)
(130)
(600)
Covalently closed
circular library
molecule
5'P
3'
(300)
(320)
(310)
(330)
Insert (100)
(140)
(150)
(160)
(180)
(170)
(120)
(400)
(130)
(600)
Covalently closed
circular library
molecule
5'
P
3'
(300)
(320)
(310)
(330)

FIG. 8

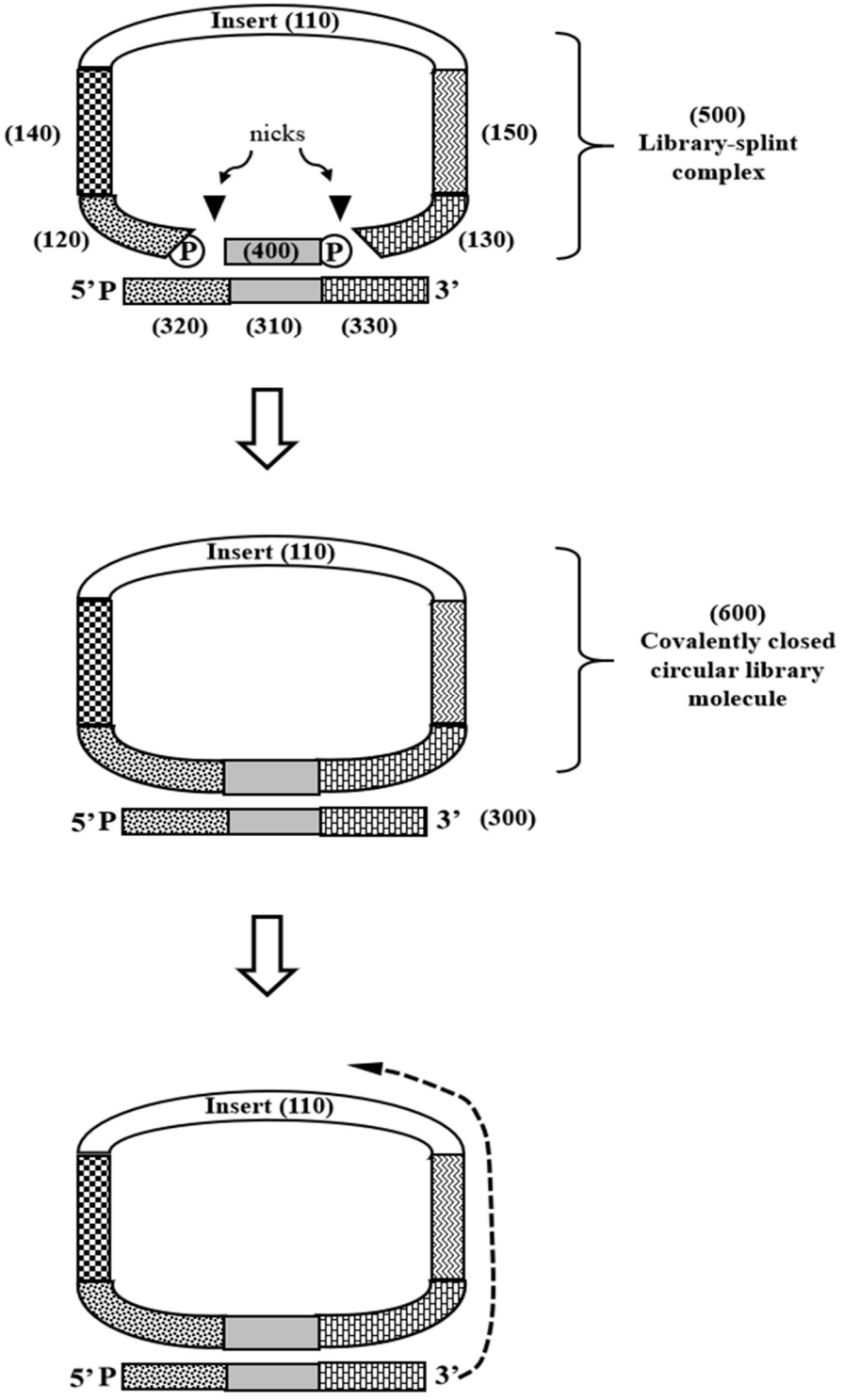

Insert (110)
(140)
nicks
(150)
(500)
Library-splint
complex
(120)
P
(400)
P
(130)
5' P
3'
(320)
(310)
(330)
Insert (110)
(600)
Covalently closed
circular library
molecule
5' P
3'
(300)
Insert (110)
5' P
3'

FIG. 9

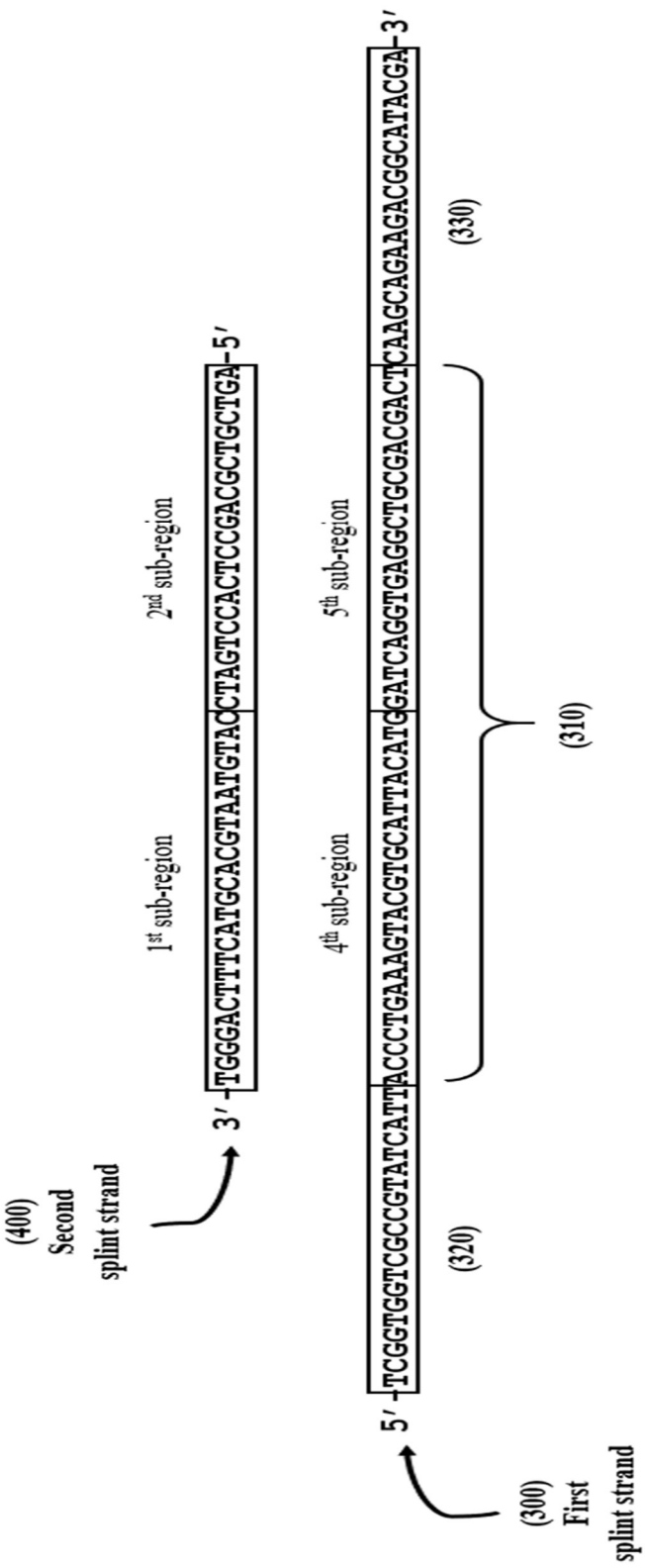

(400)
Second
splint strand
3′
TGGGACTTTCATGCACGTAATGTACCTAGTCCACTCCGACGCTGCTGA
5′
1st sub-region
2nd sub-region
4th sub-region
5th sub-region
5′
TCGGTGGTCGCCGTATCATTTACCCTGAAAGTACGTGCATTACATGGATCAGGTGAGGCTGCGACGACTCAAGCAGAAGACGGCATACGA
3′
(320)
(310)
(330)
(300)
First
splint strand

FIG. 10

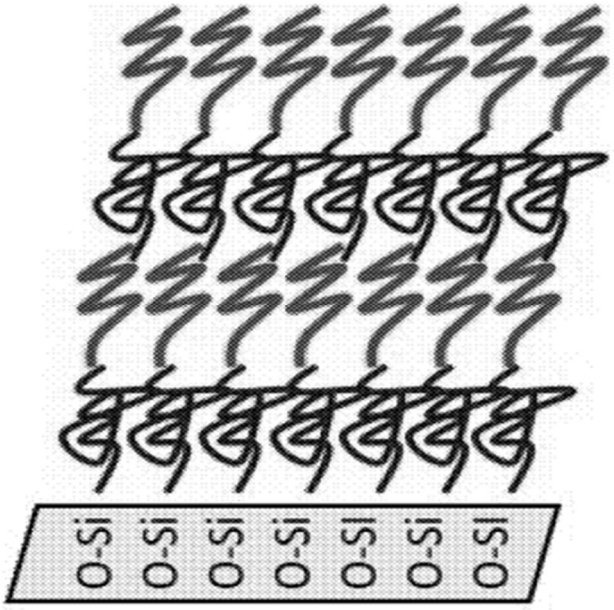


FIG. 11

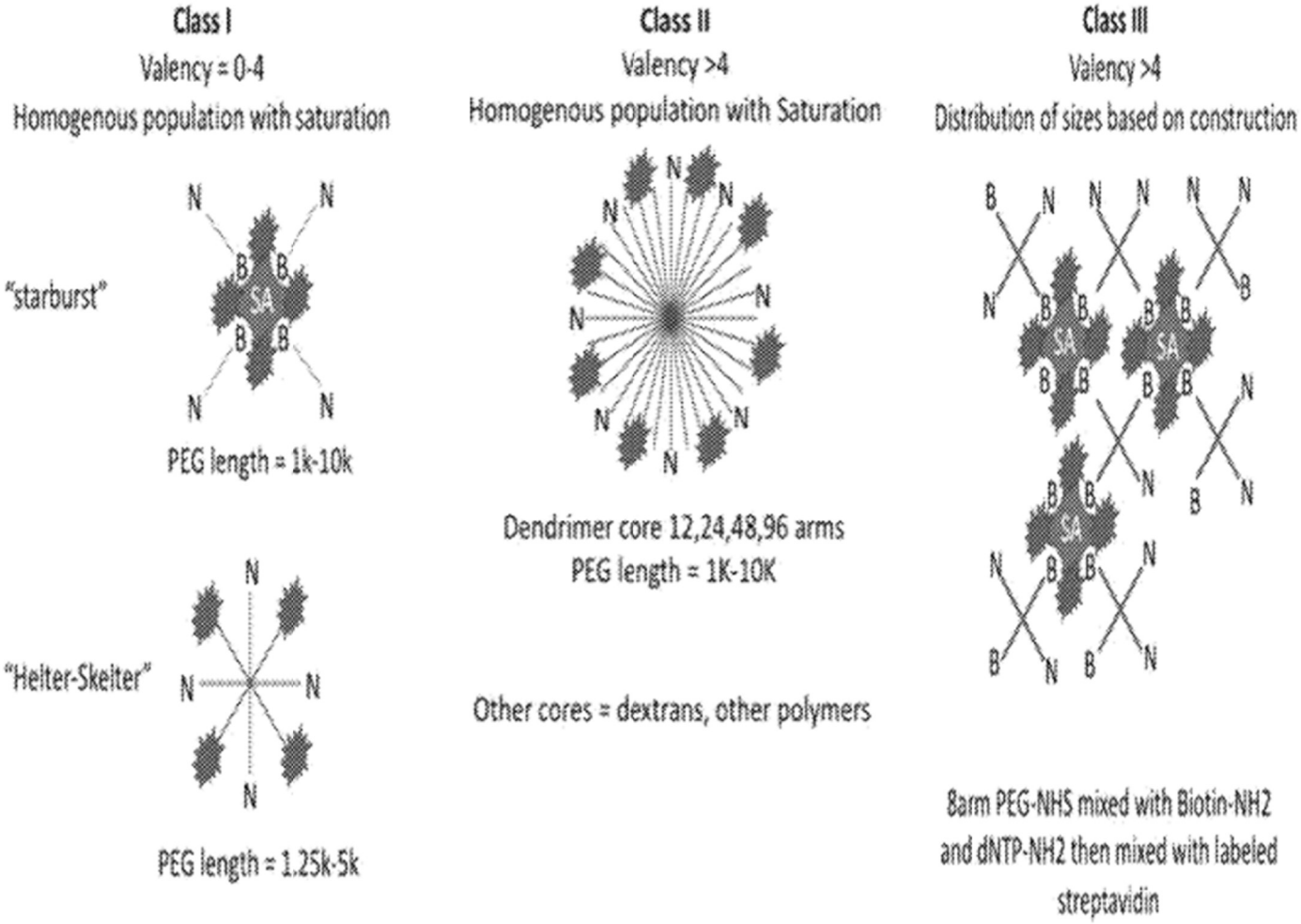

Class I
Valency = 0-4
Homogenous population with saturation
"starburst"
PEG length = 1k-10k
"Helter-Skelter"
PEG length = 1.25k-5k
Class II
Valency >4
Homogenous population with Saturation
Dendrimer core 12,24,48,96 arms
PEG length = 1K-10K
Other cores = dextrans, other polymers
Class III
Valency >4
Distribution of sizes based on construction
8arm PEG-NHS mixed with Biotin-NH2
and dNTP-NH2 then mixed with labeled
streptavidin

FIG. 12

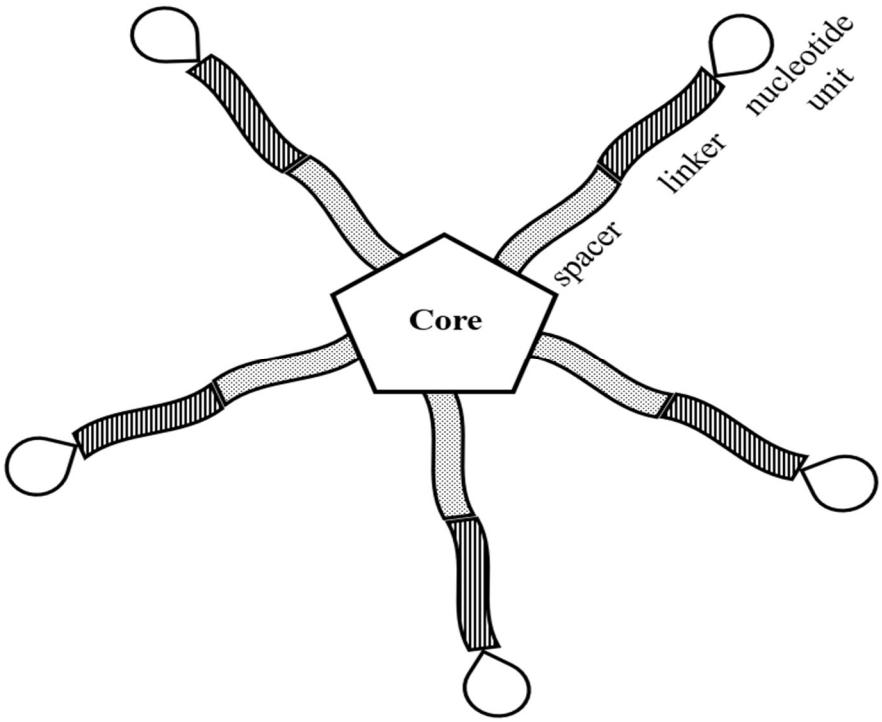

Core
spacer
linker
nucleotide unit

FIG. 13

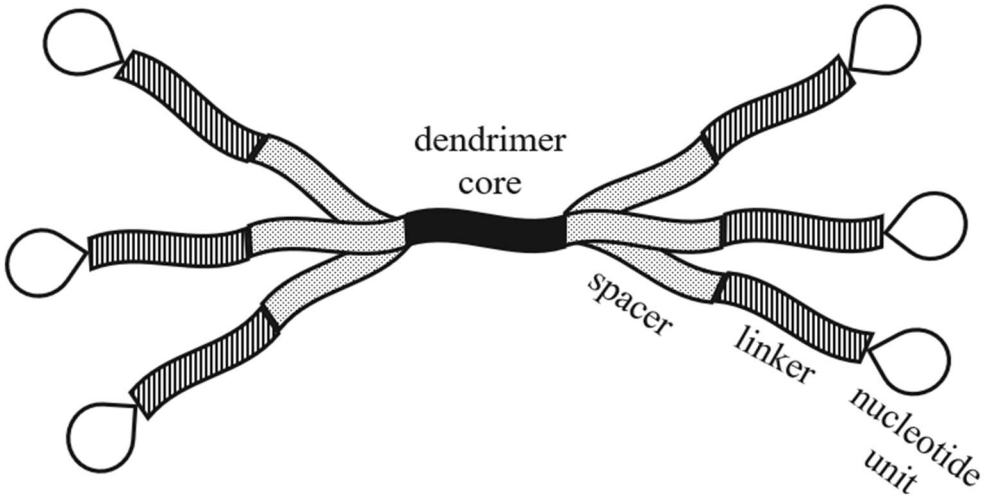

dendrimer core
spacer
linker
nucleotide unit

FIG. 14

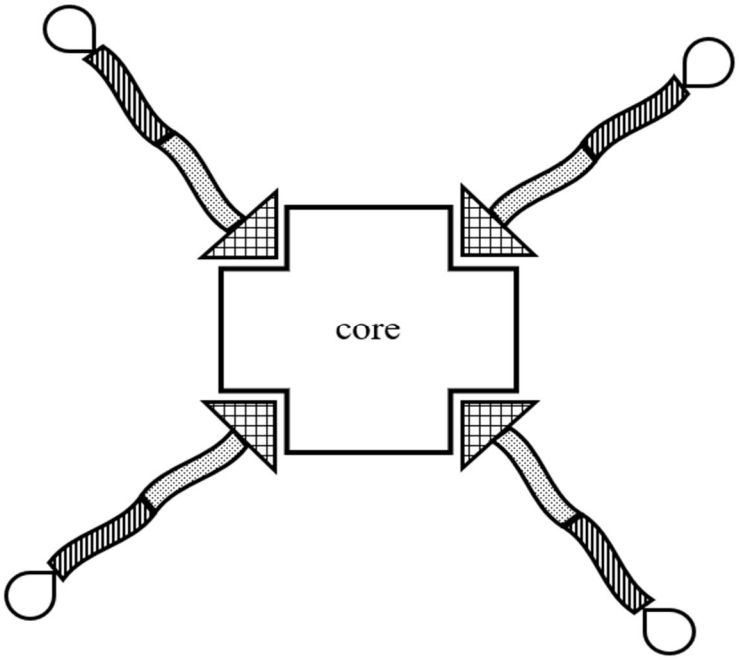

core

FIG. 15

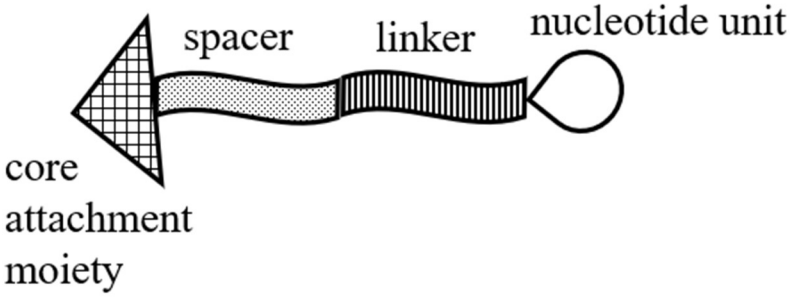
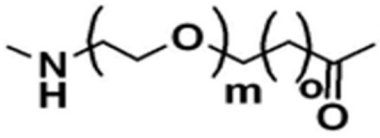
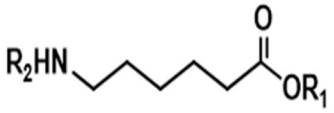

spacer
linker
nucleotide unit
core
attachment
moiety

FIG. 16

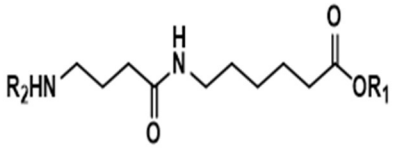
Spacer: 

Linkers:

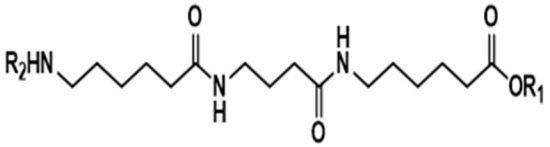
11 atom Linker:



16 atom Linker:



23 atom Linker:



N3 Linker:

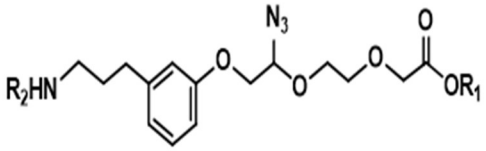
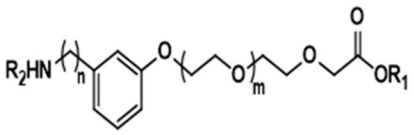
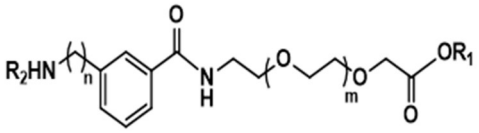


FIG. 17

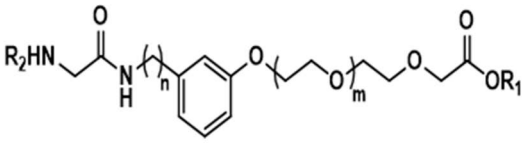
Linker 1:



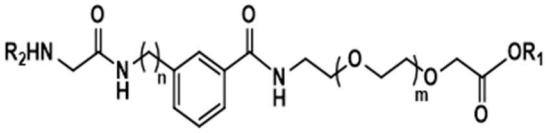
Linker 2:



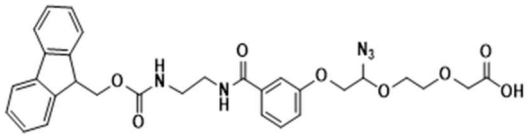
Linker 3:



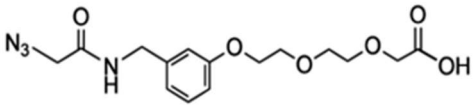
Linker 4:



Linker 5:

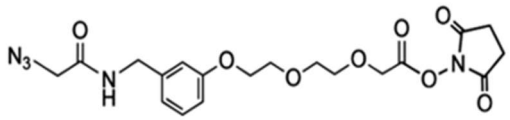


Linker 6:



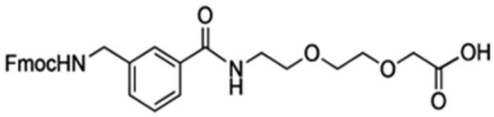
Molecular Weight: 352.35

Linker 7:



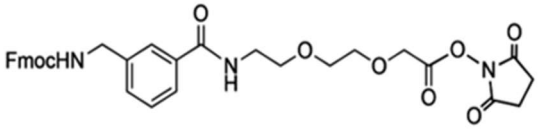
Molecular Weight: 449.42

Linker 8:



Molecular Weight: 518.57

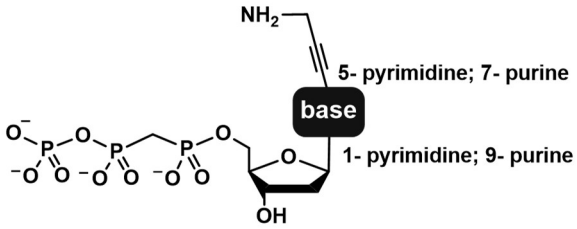
Linker 9:



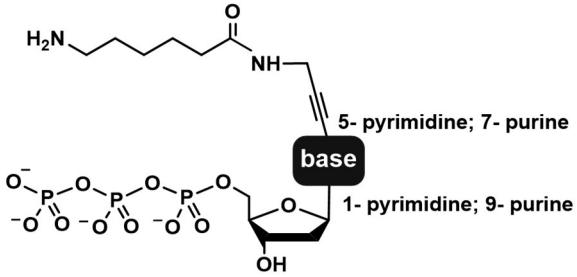
Molecular Weight: 615.64

FIG. 18

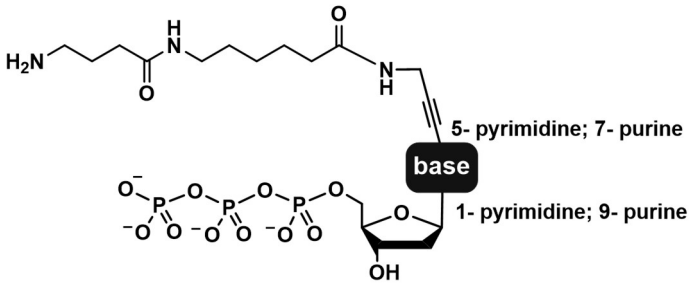
dNTP-PA-NH$_2$:



dNTP-PA-11 Atom Linker-NH$_2$:



dNTP-PA-16 Atom Linker-NH$_2$:



dNTP-PA-23 Atom Linker-NH$_2$:

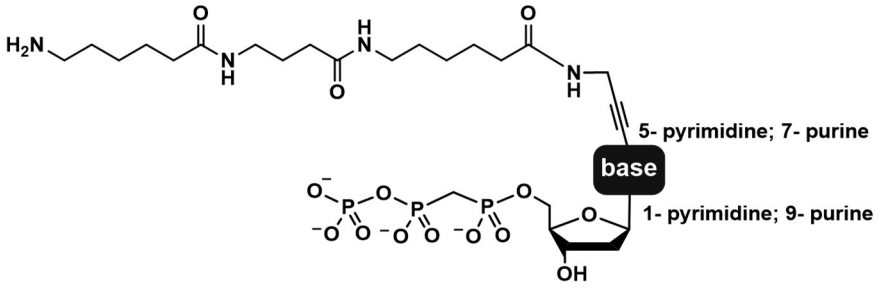
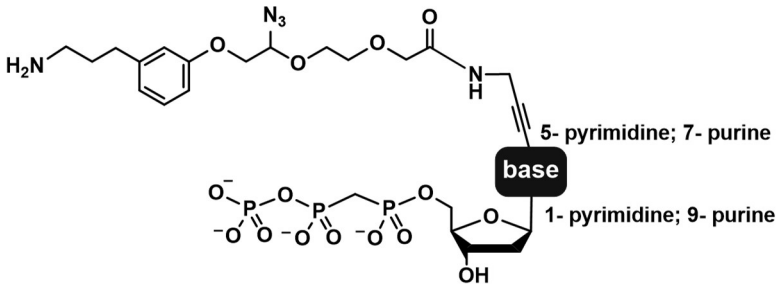
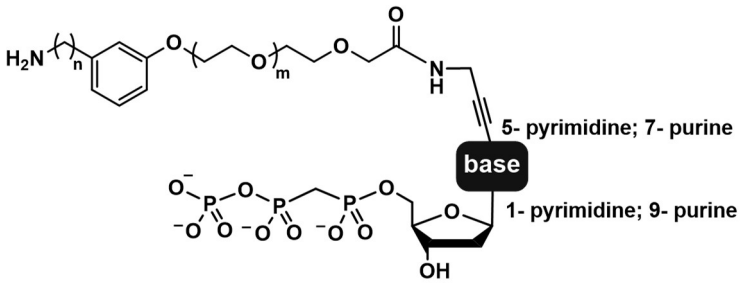


FIG. 19

dNTP-PA-N3 Linker-NH_2:



dNTP-PA-Linker 1-NH_2:



dNTP-PA-Linker 2-NH_2:

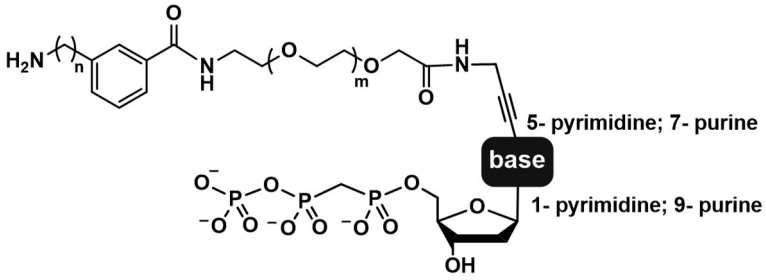
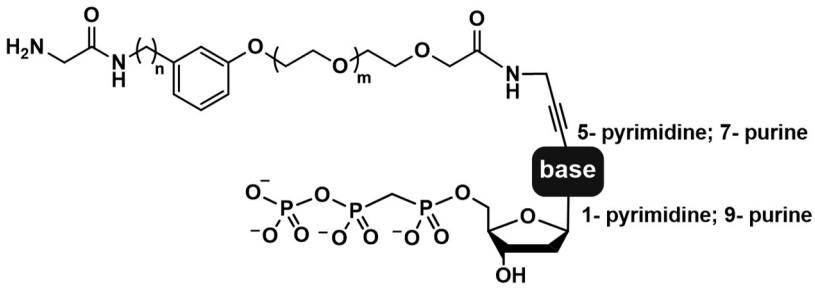
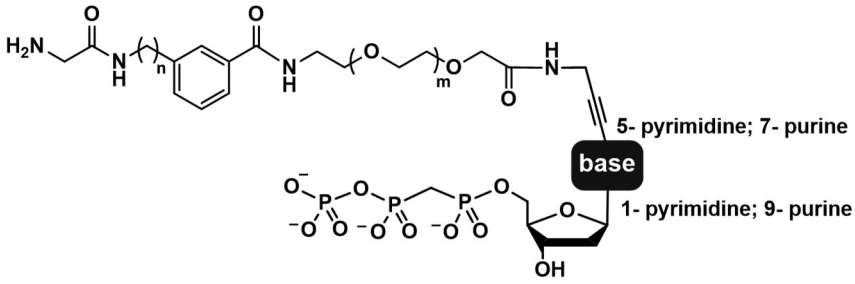


FIG. 20

dNTP-PA-Linker 3-NH_2:



dNTP-PA-Linker 4-NH_2:



dNTP-PA-N3 Linker-NH_2:

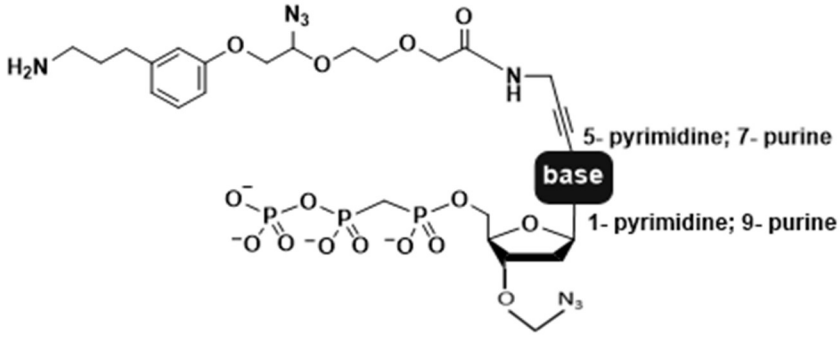


FIG. 21

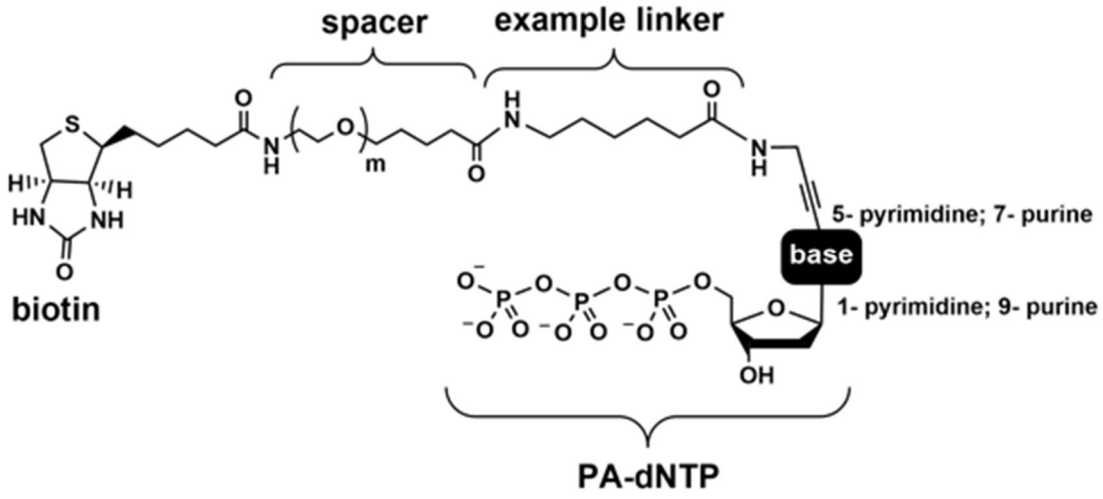

spacer
example linker
biotin
5- pyrimidine; 7- purine
base
1- pyrimidine; 9- purine
PA-dNTP

FIG. 22

Table 1:

First left index sequences (160)		
Index Name	**Sequence (orientation: 5' to 3')**	**SEQ ID NO:**
007	CCACTCATT	1
008	AACTAACAC	2
037	TTATGGAGA	3
022	GAGTTCGCT	4
002	TTGTTGTTC	5
035	CCATATTCT	6
001	AATCATGCG	7
014	GGACAGCTC	8
004	CCAACACGA	9
034	TGTAAGCGG	10
012	ATAACTACC	11
019	CGCACTGTG	12
020	TTACACTCA	13
045	ATTGACCTG	14
011	CACCAGTGT	15
038	GGTGCTCCT	16
033	ATCCGCGTC	17
026	CGGTCCTAA	18
046	TGGTATGCC	19
036	AAGATAGAG	20
015	CCGGTATCG	21
042	GGTCCGAGA	22
023	CGTGATAGC	23
081	TATGTGGCC	24
024	GTACCAGAG	25
016	TACATCAGA	26
028	AGGCGACCT	27
039	CAGCACAAG	28
031	CTCTGTCCA	29
013	TCTTGTGAT	30
043	CCAGGAGTC	31
025	ACCGTTCGT	32
044	GACACTTAT	33

FIG. 23A

Table 1 (continued):

First left index sequences (160)		
Index Name	**Sequence (orientation: 5' to 3')**	**SEQ ID NO:**
005	TTGGCTGGA	34
017	ATTGCGTAT	35
080	CTCGAAGAT	36
094	TGAACGGAT	37
049	AATACCGGT	38
058	GGCCTTCAA	39
067	TGAGAACAA	40
079	AGATCTCTA	41
054	GGCAACGCA	42
086	CGAATGACA	43
051	CAAGTGCAG	44
059	CAAGCAACT	45
065	TTCAGGTCT	46
064	CAGTAGGTA	47
060	ACGAAGGCT	48
053	TTCTATTGG	49
062	GTGTCTCAC	50
077	GCTGAATGT	51
071	GAAGATGTT	52
088	ATTCGTTGA	53
095	TCGCATAGT	54
057	TCATGCTTG	55
050	TGGAGTATA	56
055	AAGGTGATT	57
063	AGAGGAAGG	58
069	GATCGCGAA	59
056	CTTCGACAC	60
066	GCTATTATG	61
082	GACTGATTA	62
085	ACGGCCAAC	63
075	CTTATCGCG	64
061	TCTCTCTGC	65
073	CAGCGTTCC	66

FIG. 23B

Table 1 (continued):

First left index sequences (160)		
Index Name	**Sequence (orientation: 5' to 3')**	**SEQ ID NO:**
092	GATTCACGG	67
074	TCCTCGCAA	68
072	ACCACAATT	69
097	CGTTGAATT	70
106	CAGTTGACC	71
111	CCACGAACG	72
112	AGCACCTAG	73
113	TAGGCCGTC	74
114	GTTCTGTAA	75
115	CACTGACGT	76
116	TCCAATACC	77
117	ATATTGGTG	78
119	CCGACCACT	79
120	GAACAACGA	80
121	ATTGTAACC	81
123	TCCACCTGT	82
124	CTACATGAA	83
127	CCAATGTTG	84
132	GTTACCAAC	85
133	CAATTGTGA	86
134	AGGCGTAAG	87
135	ACAGCCGTT	88
136	CACCAACCG	89
137	TCTAGCCTC	90
138	GTGTTAGGA	91
139	CGCGAGACT	92
140	TCAACTTAG	93
141	AAGAGCGCC	94
143	CGACCTTGA	95
144	TACGTACTC	96

FIG. 23C

Table 1 (continued):

First right index sequences (170)		
Index Name	**Sequence (orientation: 5' to 3')**	**SEQ ID NO:**
001	NNNGTAGGAGCC	97
003	NNNCCGCTGCTA	98
004	NNNAACAACAAG	99
006	NNNGGTGGTCTA	100
008	NNNTTGGCCAAC	101
011	NNNTCCATCGTT	102
013	NNNATTCTAGAG	103
014	NNNGGATGCAAT	104
015	NNNCAGGAGTGC	105
016	NNNATCACACTA	106
018	NNNGCGGATGAT	107
019	NNNCGAATCTGG	108
021	NNNATGCGTCCT	109
022	NNNGGTCTGAGC	110
023	NNNGCCTCATAG	111
024	NNNCTATACCTC	112
025	NNNAAGATTGGA	113
026	NNNTGCCAGCAT	114
027	NNNACTAGCTCA	115
028	NNNTAAGTACTG	116
029	NNNCGTGCAACG	117
030	NNNGTCCACGGA	118
034	NNNAGGTCCGTG	119
035	NNNCTCGTTAGT	120
036	NNNACACAATCC	121
037	NNNTCTTCGCAA	122
038	NNNGGTCACTTG	123
039	NNNCCGATATAT	124
040	NNNACAGGTCGC	125
042	NNNGTGCGGTTC	126
043	NNNCACGCACAT	127
044	NNNTGGATCACA	128
045	NNNATATTCGGT	129

FIG. 23D

Table 1 (continued):

First right index sequences (170)		
Index Name	**Sequence (orientation: 5' to 3')**	**SEQ ID NO:**
047	NNNCCAGAGATG	130
048	NNNTACCGTTGA	131
049	NNNATTGATACC	132
051	NNNCCAAGACCG	133
052	NNNAAGCCGGAC	134
054	NNNGGATCTCCG	135
055	NNNCCTGGAGAA	136
056	NNNATCCTCATC	137
057	NNNTATGTCTCT	138
059	NNNACGTAGAGT	139
060	NNNGTACTTCAA	140
061	NNNCATTCCAGC	141
062	NNNTGGCGTGAG	142
063	NNNTCAACGTCT	143
064	NNNAACGAAGTC	144
065	NNNCTTAATCGA	145
066	NNNGCGGCCTTA	146
068	NNNTTCCGAACG	147
071	NNNCGCACCGAA	148
073	NNNTAGTGACCA	149
074	NNNGTTATGCTG	150
075	NNNCTCCGCTAT	151
076	NNNTGAGATGGA	152
078	NNNGATAGATGT	153
079	NNNCCTCATGTC	154
081	NNNGACTTAACC	155
082	NNNCAAGGCGTT	156
083	NNNAGGAAGCCG	157
084	NNNTCACTCAAG	158
085	NNNTCCACTAGC	159
087	NNNCTGTCATGA	160
088	NNNAGCGTTCAG	161
089	NNNGCATAGGCA	162

FIG. 23E

Table 1 (continued):

First right index sequences (170)		
Index Name	**Sequence (orientation: 5' to 3')**	**SEQ ID NO:**
091	NNNTATCAACGC	163
092	NNNACAGTGTAA	164
096	NNNTGCGGATAC	165
097	NNNGCTATTACT	166
106	NNNGCTCAATAA	167
111	NNNCAGAGCTCC	168
112	NNNACGGCACGA	169
113	NNNTTCCTTGGC	170
114	NNNGGTTGGATG	171
115	NNNCCACACCAT	172
116	NNNTACATATCG	173
117	NNNATTGCCATA	174
119	NNNCCATCTTGC	175
120	NNNTGGAATGCT	176
121	NNNAATTCGTCT	177
123	NNNCACCGACTC	178
124	NNNACAATCAGC	179
127	NNNCCGCGAACA	180
132	NNNGAGCATCTA	181
133	NNNCTATTAGAC	182
134	NNNTGTGGCAGT	183
135	NNNCAACCGCAG	184
136	NNNACCTATTCT	185
137	NNNTGTAGGCCA	186
138	NNNGTGGTTATC	187
139	NNNCACCAATGG	188
140	NNNACAACCGAT	189
141	NNNTGTCTGTAA	190
143	NNNTCCGCACTC	191
144	NNNGAGAGCAGA	192

FIG. 23F

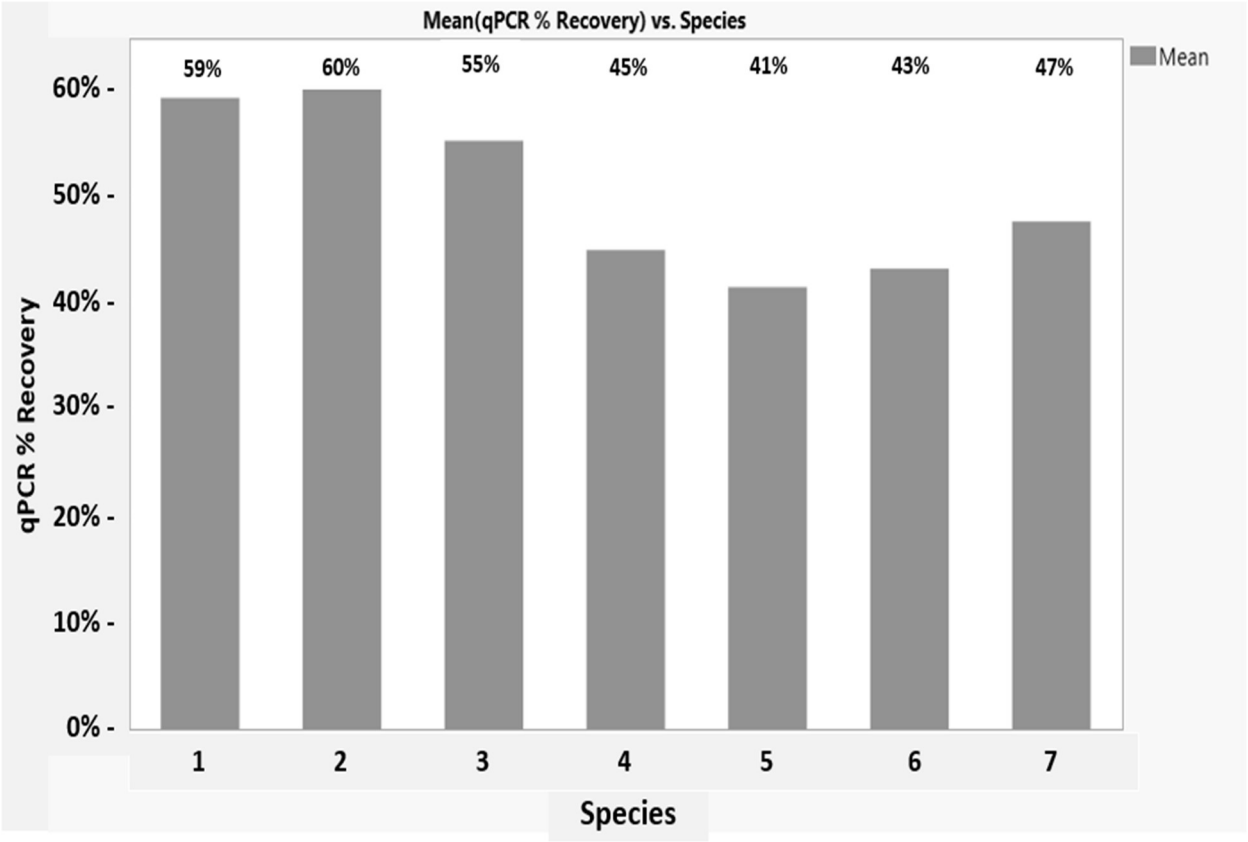

Mean(qPCR % Recovery) vs. Species
Mean
59%
60%
55%
45%
41%
43%
47%
60% -
50% -
40% -
30% -
20% -
10% -
0% -
qPCR % Recovery
1
2
3
4
5
6
7
Species

FIG. 24

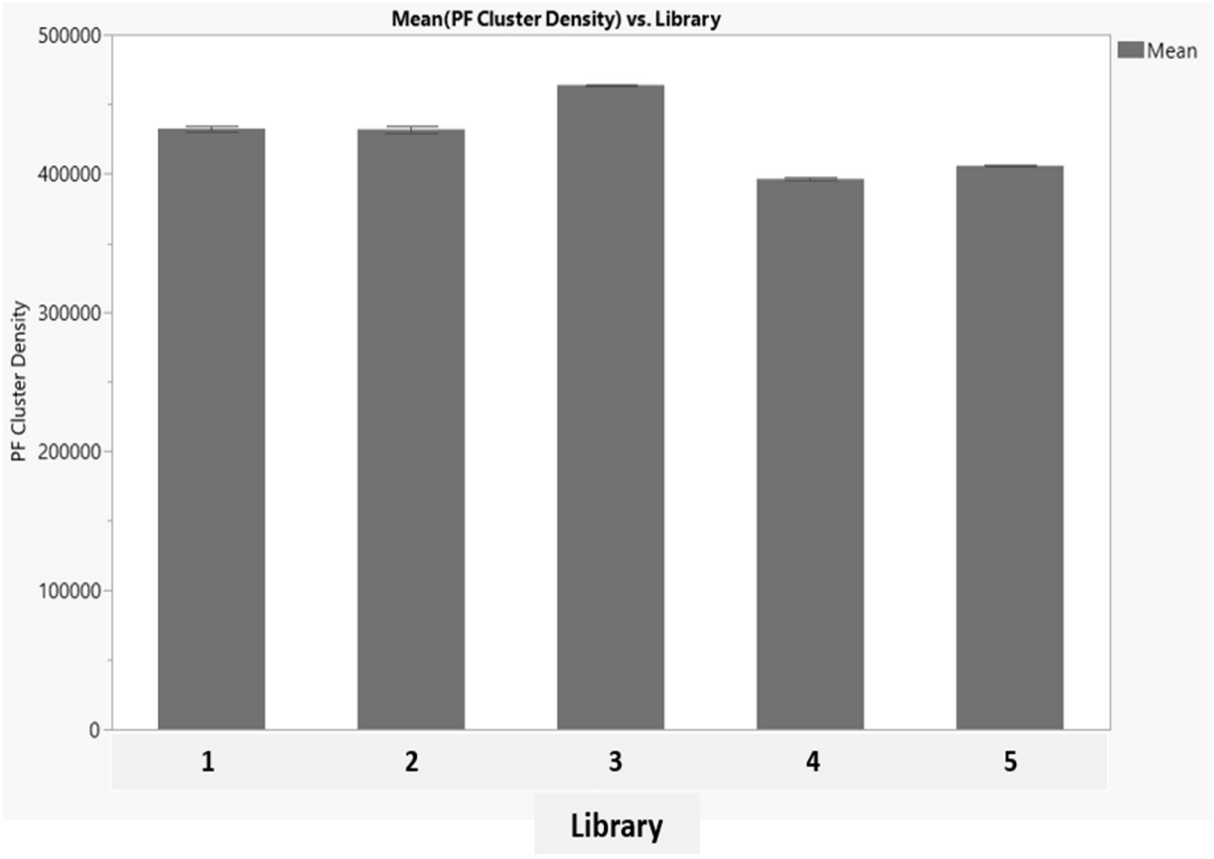

Mean(PF Cluster Density) vs. Library
Mean
PF Cluster Density
500000
400000
300000
200000
100000
0
1
2
3
4
5
Library

FIG. 25

FIG. 26

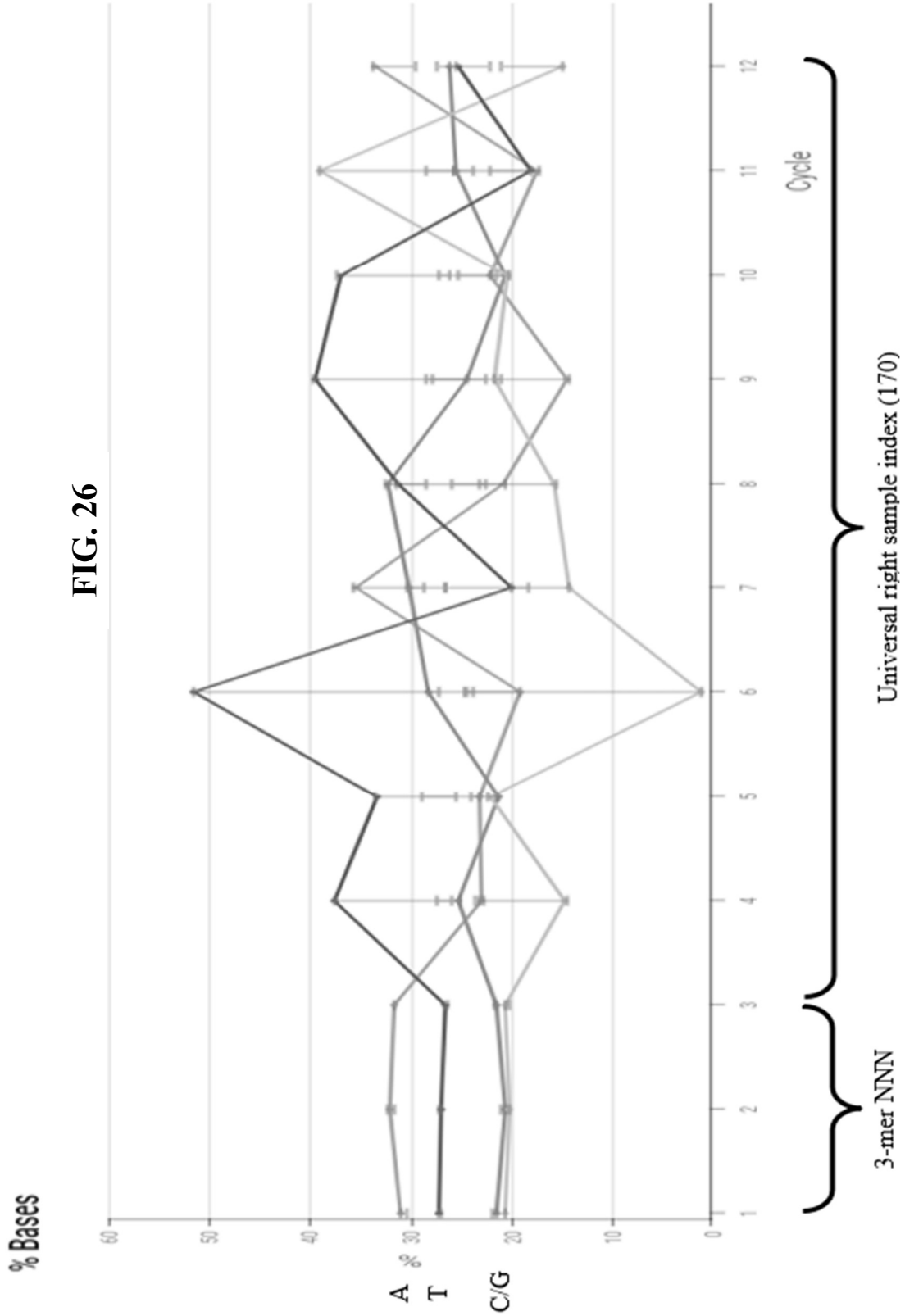
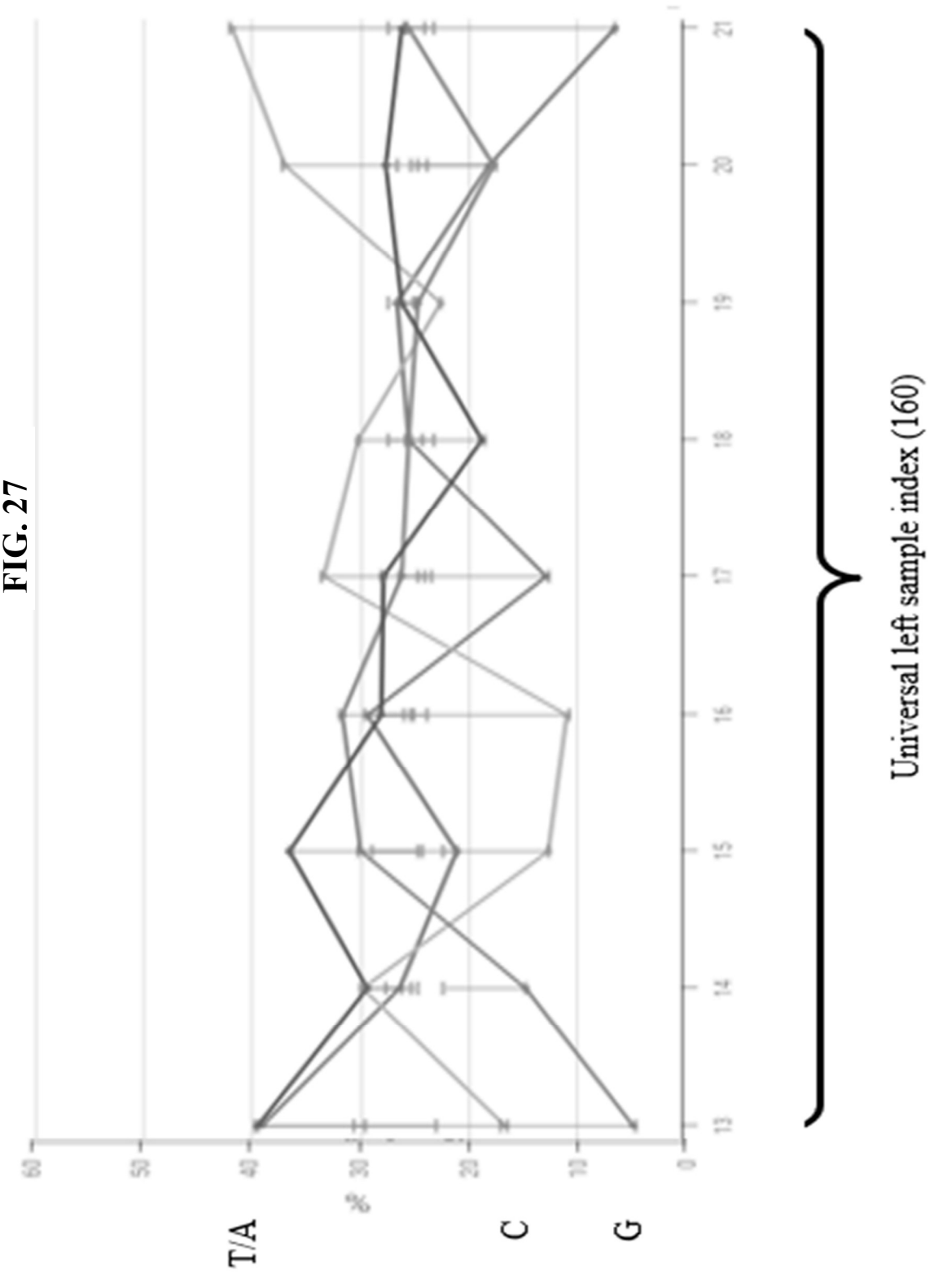


FIG. 27


T/A
C
G
%
60
50
40
30
20
10
0
13
14
15
16
17
18
19
20
21
Universal left sample index (160)