FIGURE 1

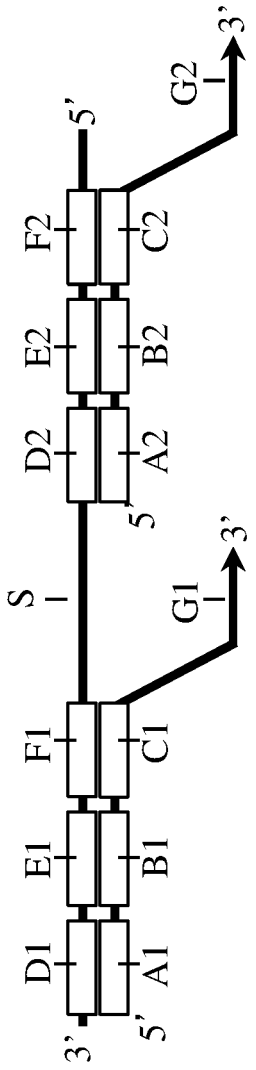


FIGURE 2

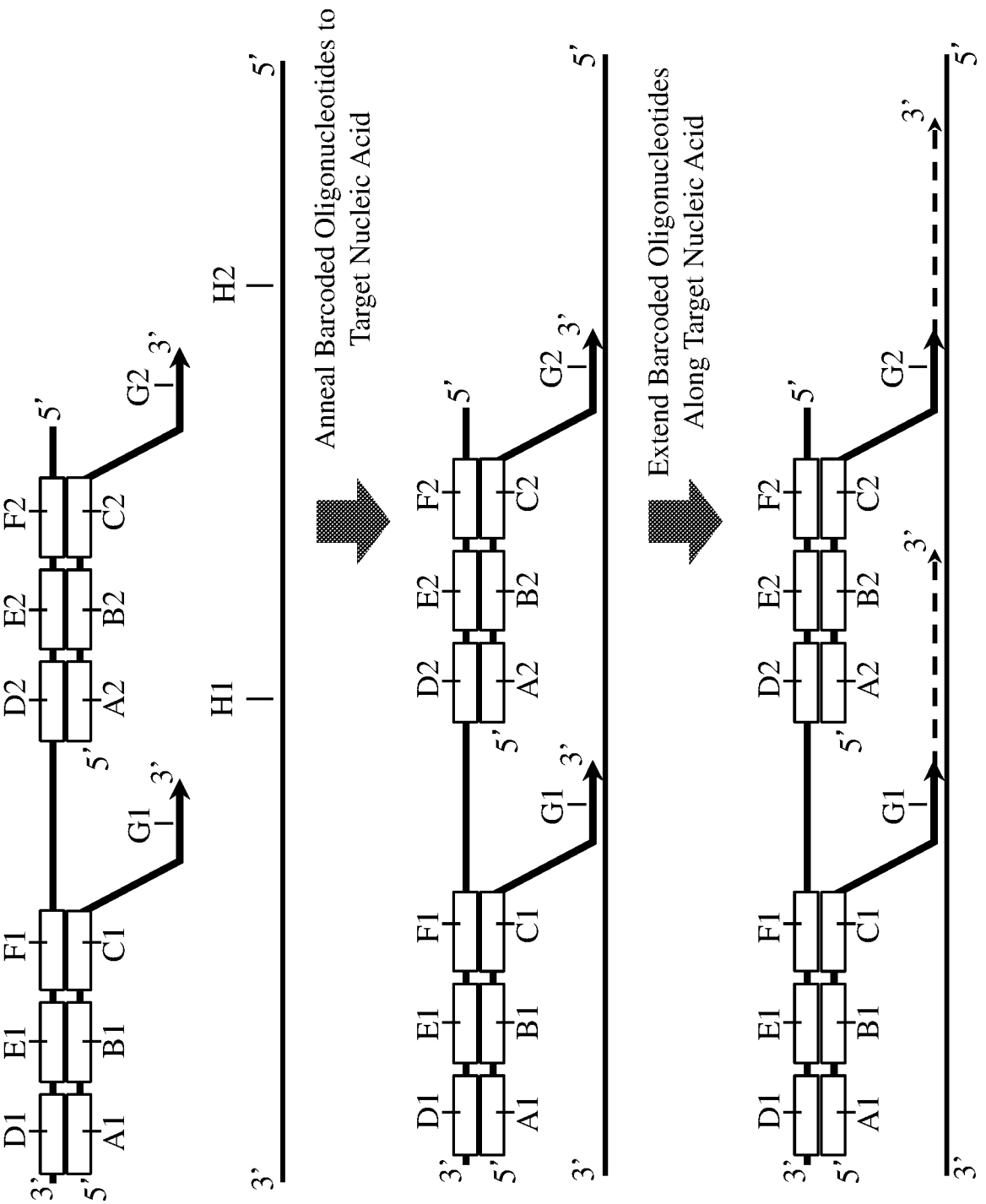

D1
E1
F1
A1
B1
C1
3'
5'
G1
3'
D2
E2
F2
5'
A2
B2
C2
G2
3'
H1
H2
3'
5'
Anneal Barcoded Oligonucleotides to Target Nucleic Acid
Extend Barcoded Oligonucleotides Along Target Nucleic Acid

FIGURE 3

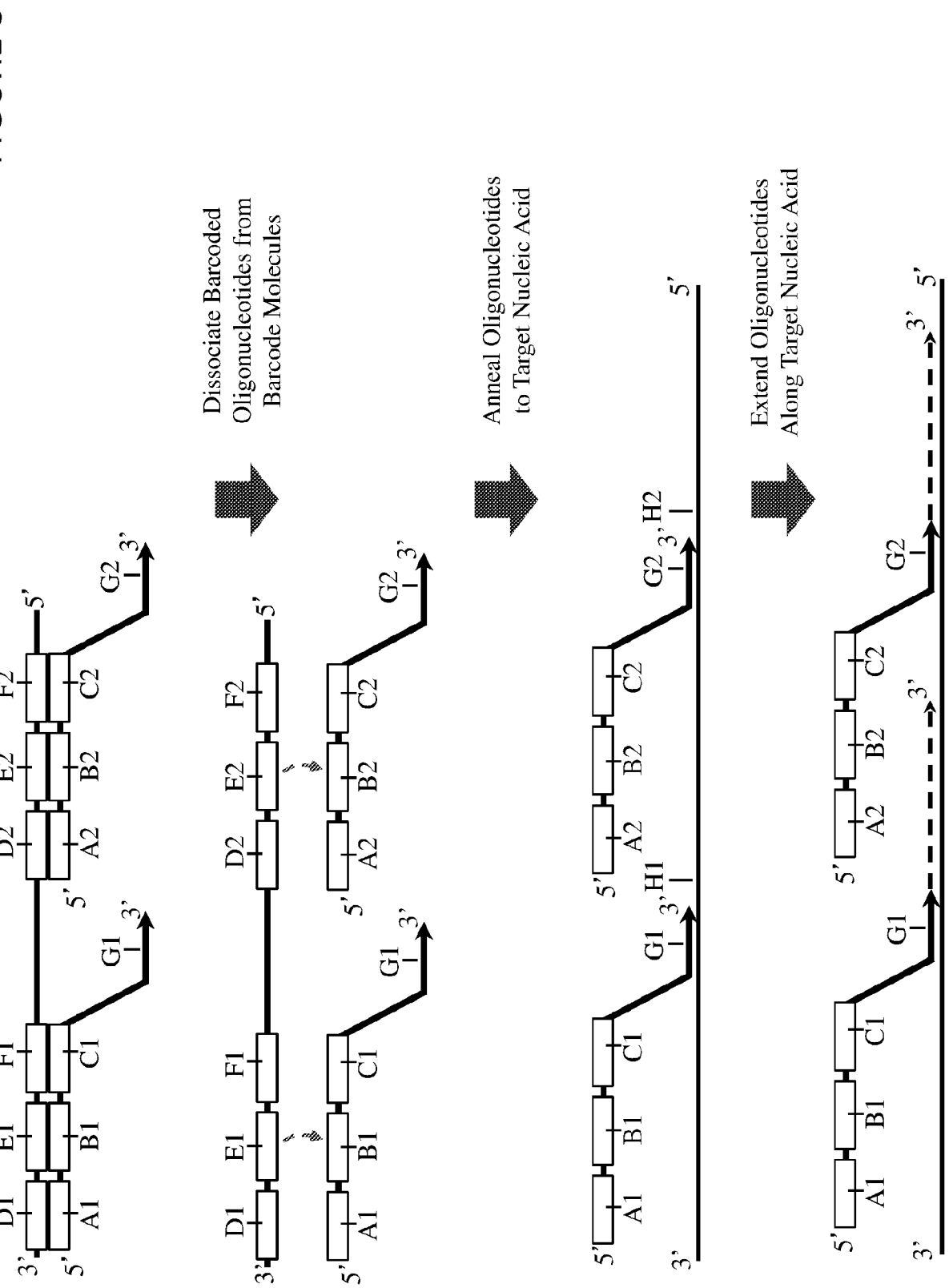

Dissociate Barcoded Oligonucleotides from Barcode Molecules
Anneal Oligonucleotides to Target Nucleic Acid
Extend Oligonucleotides Along Target Nucleic Acid
D1
E1
F1
A1
B1
C1
D2
E2
F2
A2
B2
C2
G1
G2
H1
H2
5'
3'

FIGURE 4

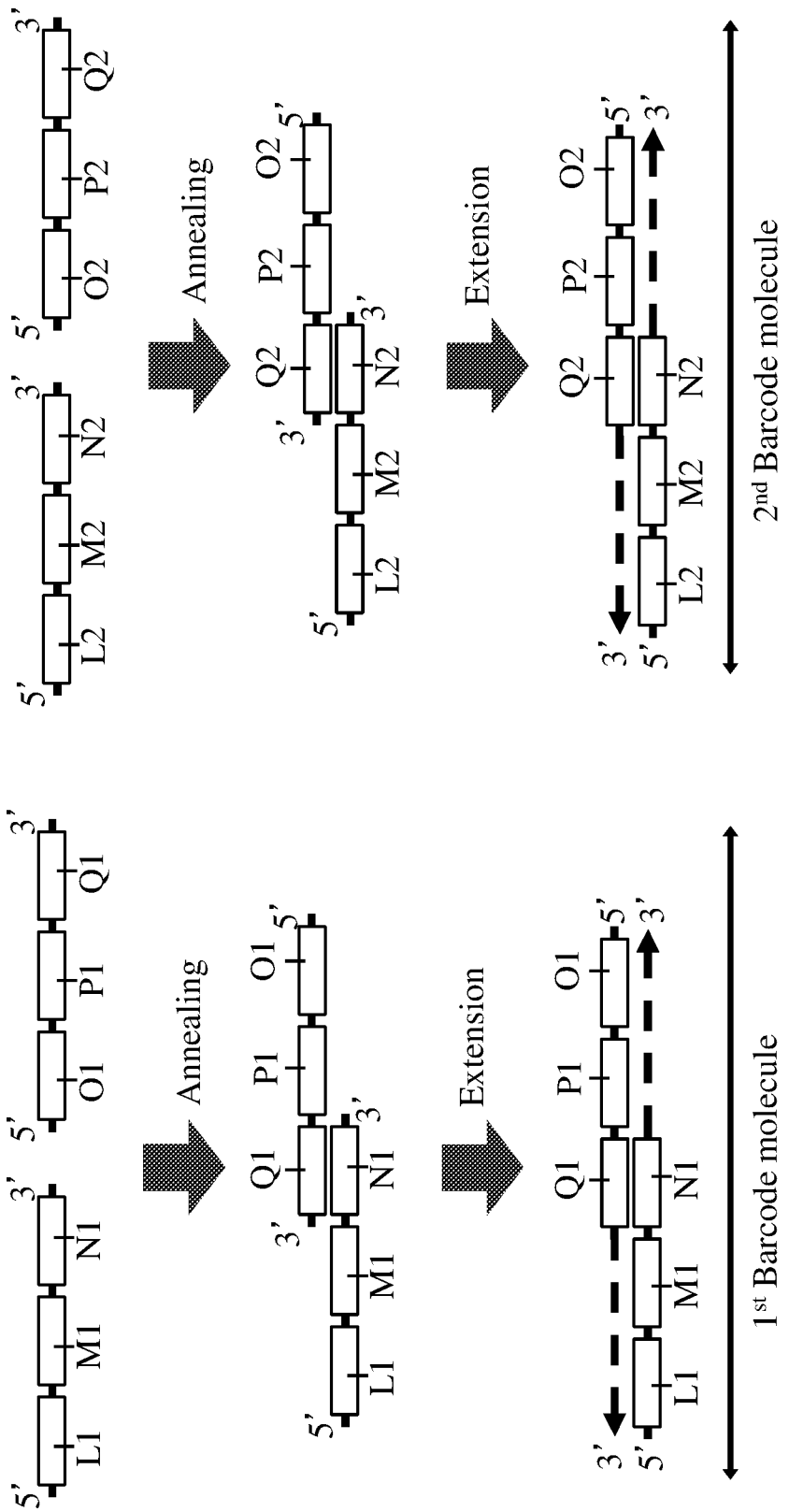

5'
3'
L1
M1
N1
O1
P1
Q1
L2
M2
N2
O2
P2
Q2
Annealing
Extension
1st Barcode molecule
2nd Barcode molecule

FIGURE 5

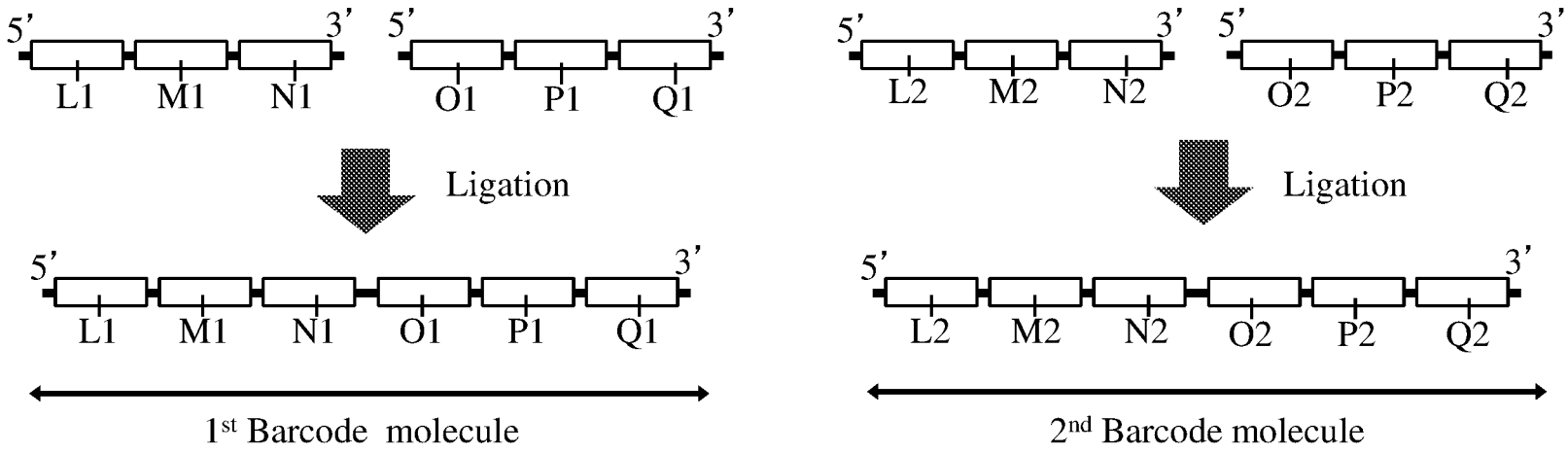

5'
3'
L1
M1
N1
O1
P1
Q1
Ligation
1st Barcode molecule
L2
M2
N2
O2
P2
Q2
2nd Barcode molecule

FIGURE 6

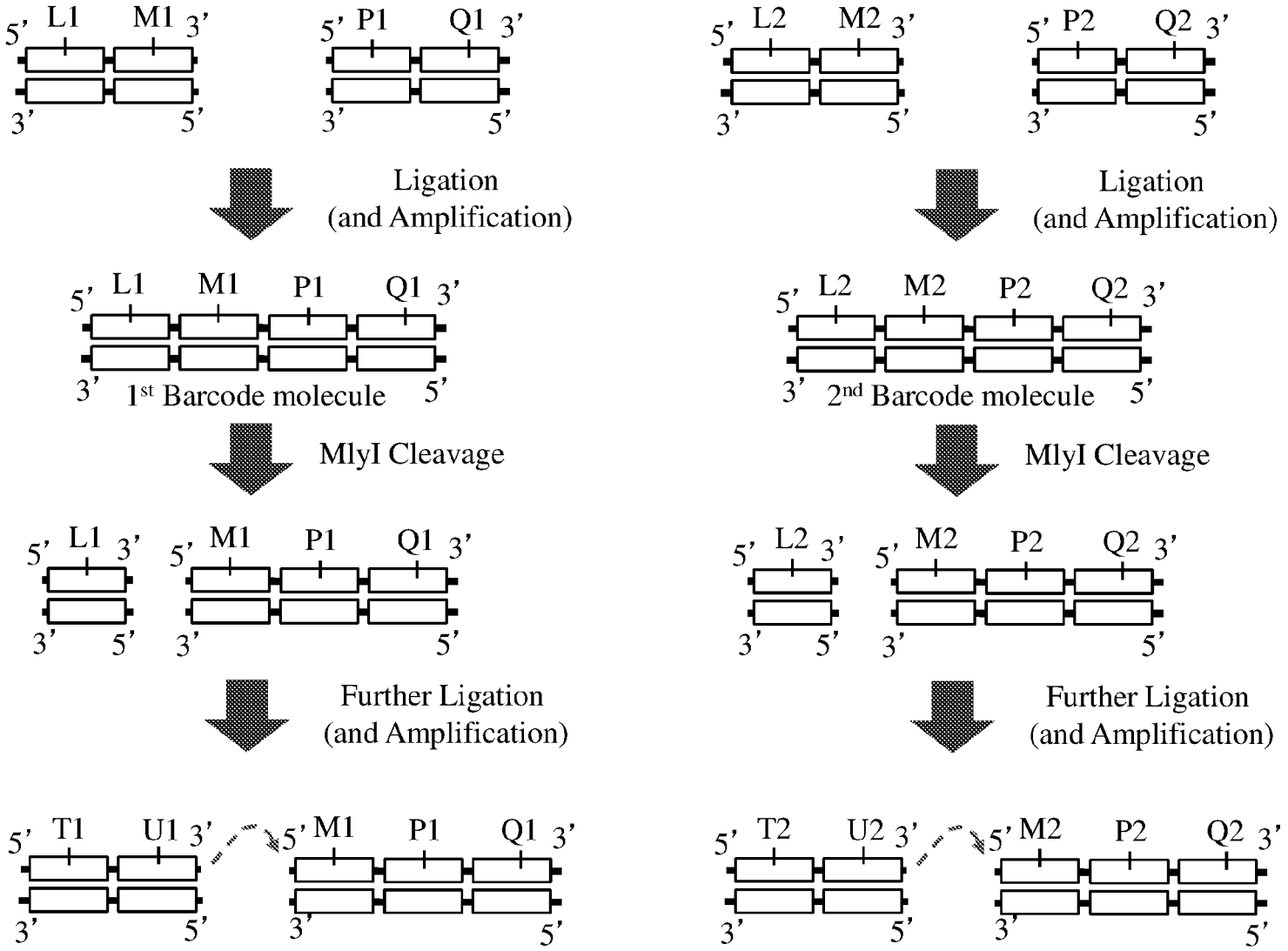
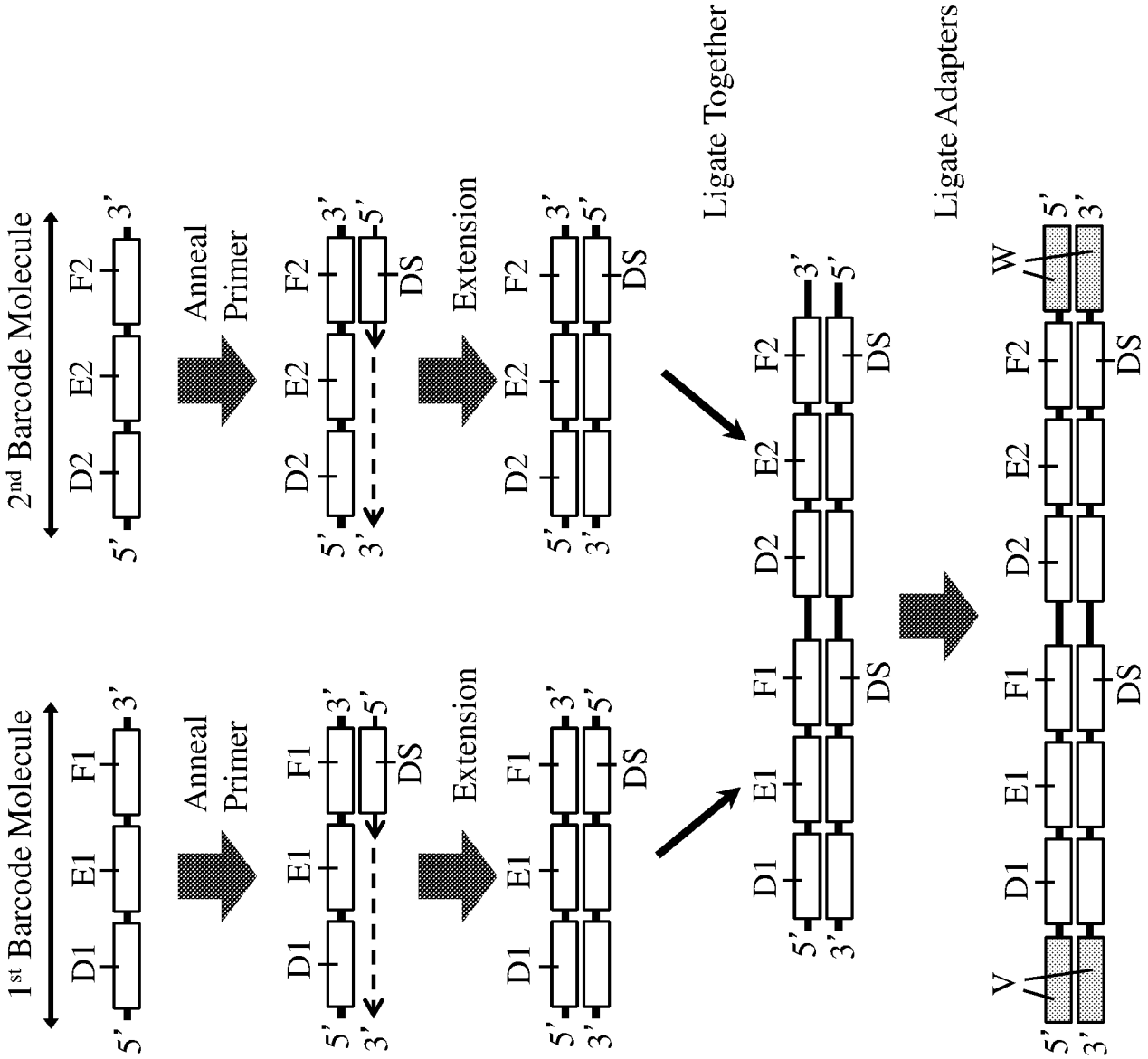

5' L1 M1 3'
3' 5'
5' P1 Q1 3'
3' 5'
Ligation
(and Amplification)
5' L1 M1 P1 Q1 3'
3' 1st Barcode molecule 5'
MlyI Cleavage
5' L1 3'
3' 5'
5' M1 P1 Q1 3'
3' 5'
Further Ligation
(and Amplification)
5' T1 U1 3'
3' 5'
5' M1 P1 Q1 3'
3' 5'
5' L2 M2 3'
3' 5'
5' P2 Q2 3'
3' 5'
Ligation
(and Amplification)
5' L2 M2 P2 Q2 3'
3' 2nd Barcode molecule 5'
MlyI Cleavage
5' L2 3'
3' 5'
5' M2 P2 Q2 3'
3' 5'
Further Ligation
(and Amplification)
5' T2 U2 3'
3' 5'
5' M2 P2 Q2 3'
3' 5'

FIGURE 7


1st Barcode Molecule
2nd Barcode Molecule
D1
E1
F1
D2
E2
F2
5'
3'
Anneal Primer
Extension
DS
Ligate Together
Ligate Adapters
V
W

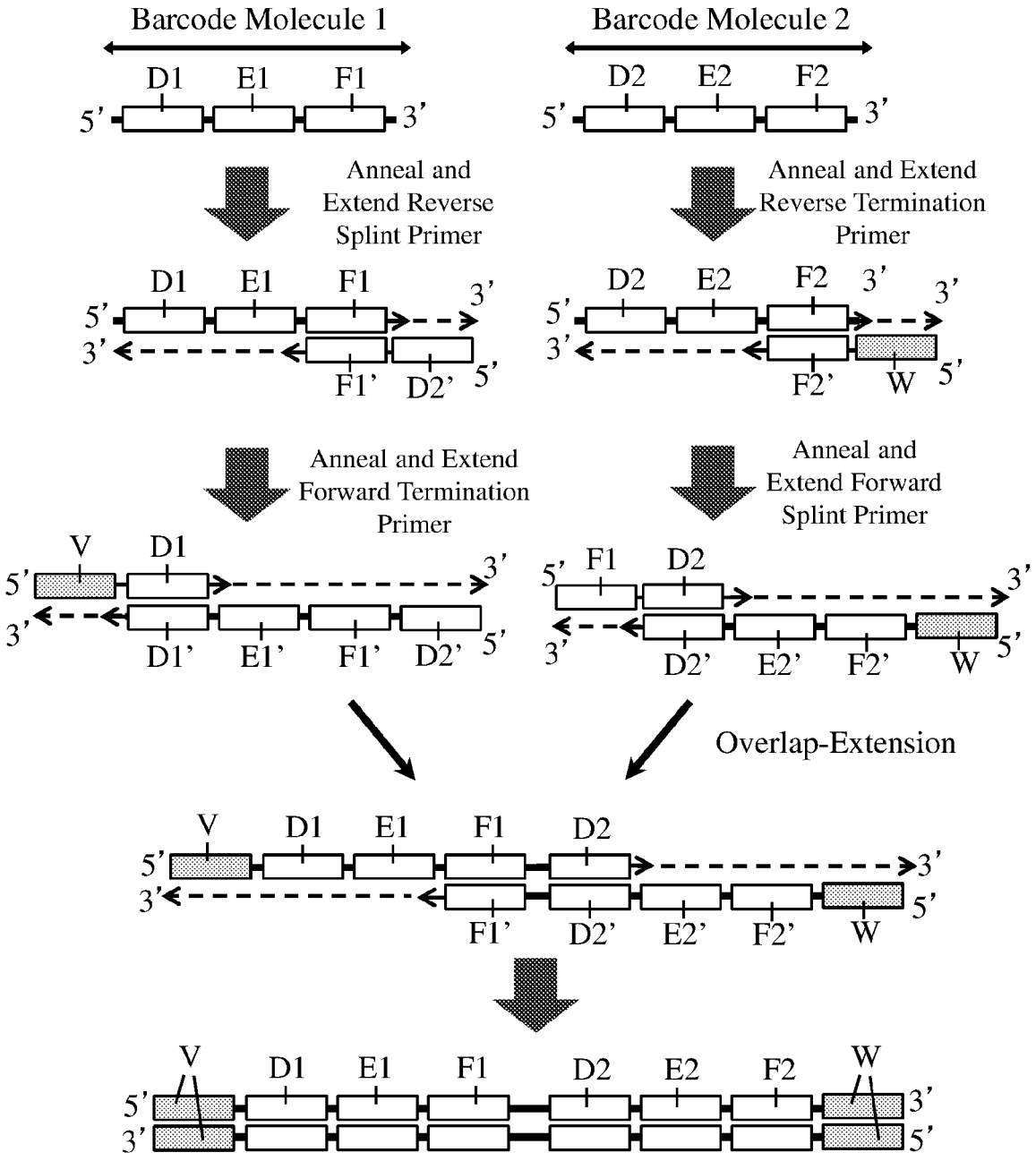

Barcode Molecule 1
D1 E1 F1
5' 3'
Barcode Molecule 2
D2 E2 F2
5' 3'
Anneal and Extend Reverse Splint Primer
Anneal and Extend Reverse Termination Primer
F1' D2'
F2' W
Anneal and Extend Forward Termination Primer
Anneal and Extend Forward Splint Primer
V D1
D1' E1' F1' D2'
F1 D2
D2' E2' F2' W
Overlap-Extension
V D1 E1 F1 D2
F1' D2' E2' F2' W
V D1 E1 F1 D2 E2 F2 W

FIGURE 8

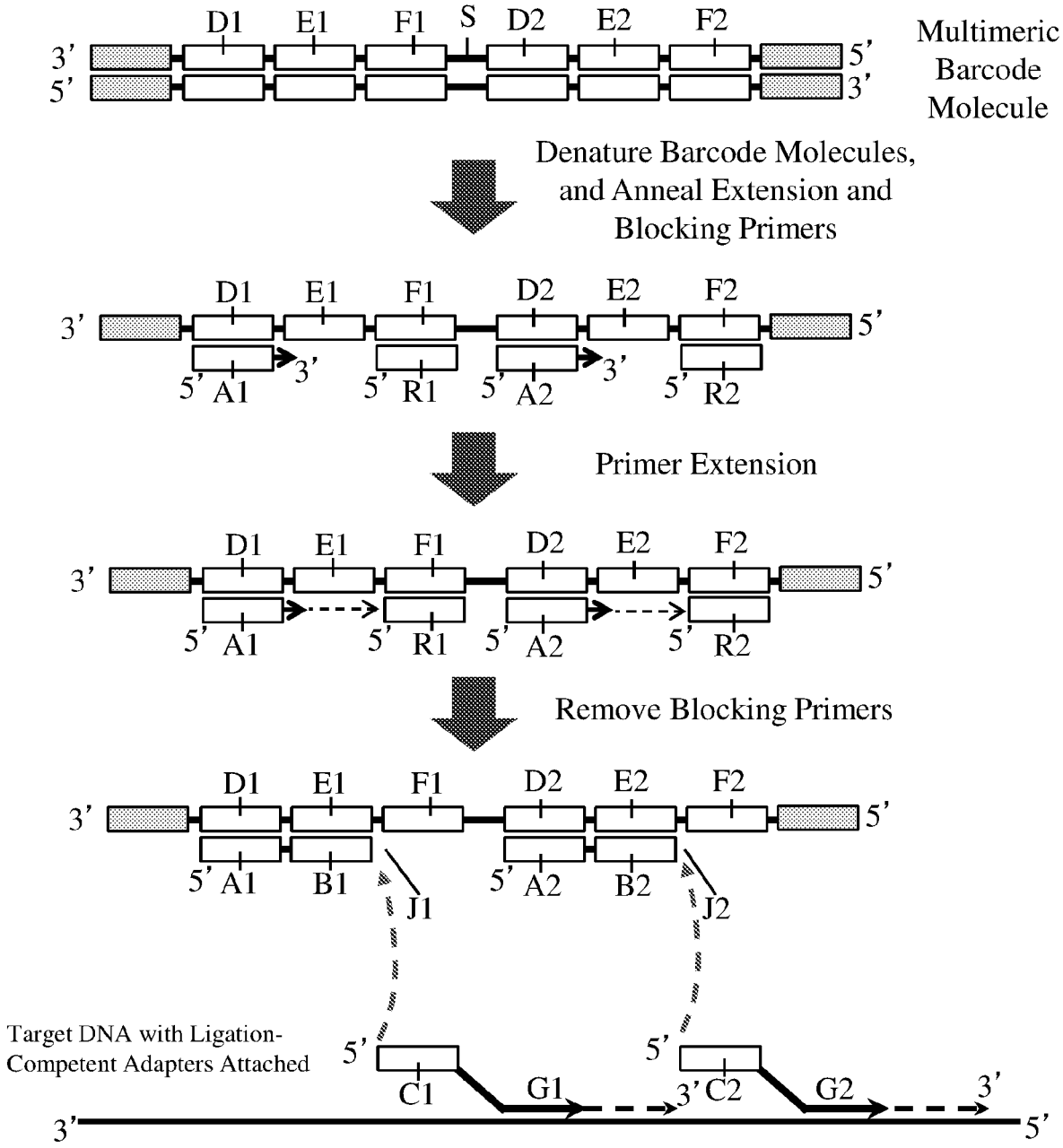

D1
E1
F1
S
D2
E2
F2
3'
5'
5'
3'
Multimeric Barcode Molecule
Denature Barcode Molecules, and Anneal Extension and Blocking Primers
A1
R1
A2
R2
Primer Extension
Remove Blocking Primers
B1
B2
J1
J2
Target DNA with Ligation-Competent Adapters Attached
C1
G1
C2
G2

FIGURE 9

FIGURE 10

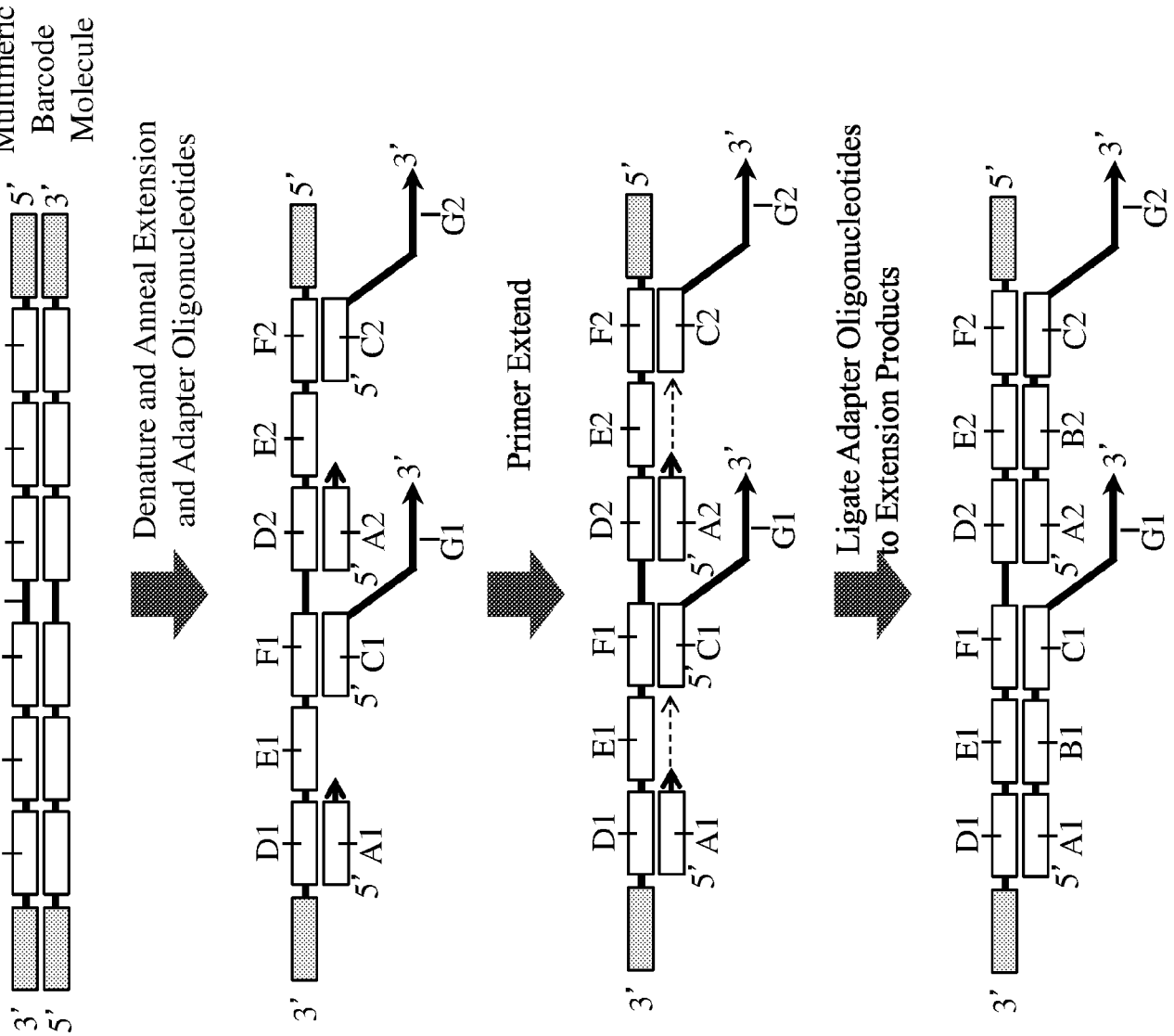

Multimeric Barcode Molecule
Denature and Anneal Extension and Adapter Oligonucleotides
Primer Extend
Ligate Adapter Oligonucleotides to Extension Products
D1
E1
F1
S
D2
E2
F2
A1
A2
B1
B2
C1
C2
G1
G2

FIGURE 11

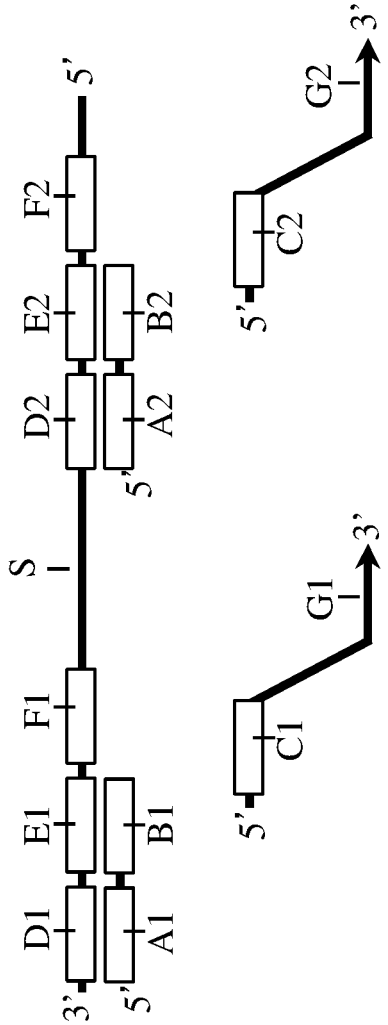


FIGURE 12

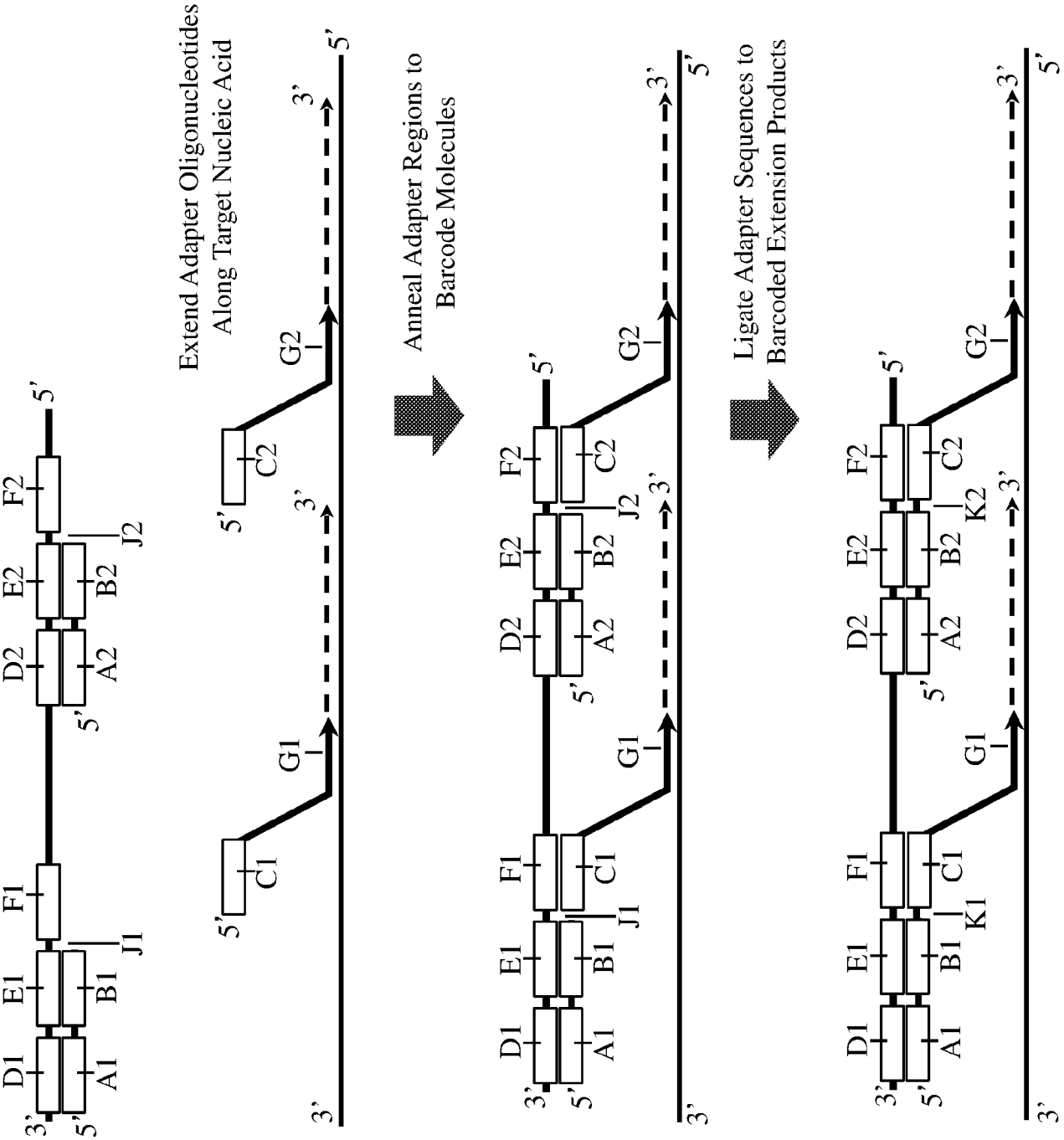

Extend Adapter Oligonucleotides
Along Target Nucleic Acid
Anneal Adapter Regions to
Barcode Molecules
Ligate Adapter Sequences to
Barcoded Extension Products
D1
E1
F1
D2
E2
F2
A1
B1
J1
A2
B2
J2
C1
G1
C2
G2
K1
K2
5'
3'

FIGURE 13

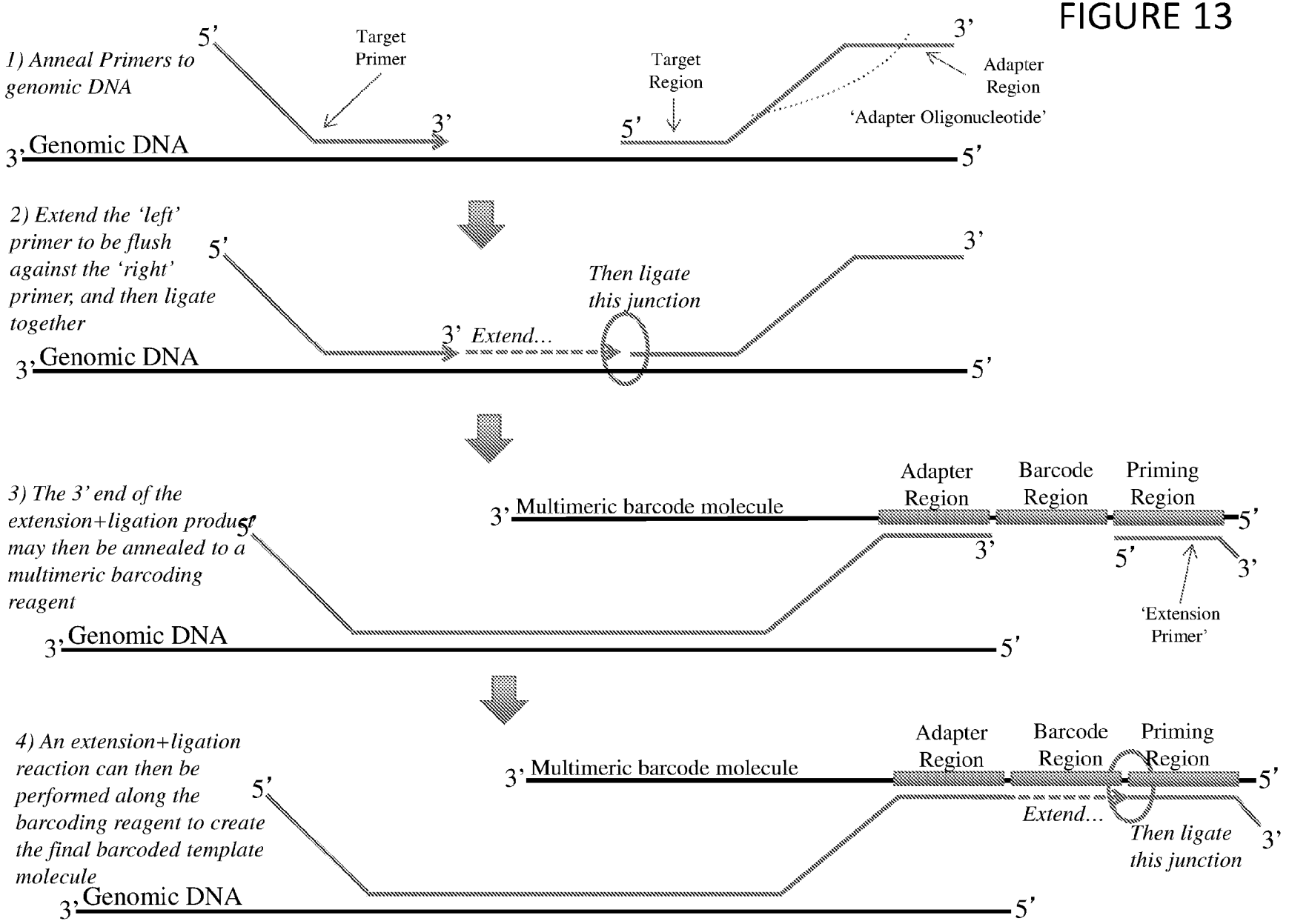

1) Anneal Primers to genomic DNA
5'
Target Primer
3'
3'
Genomic DNA
5'
Target Region
5'
3'
Adapter Region
'Adapter Oligonucleotide'
2) Extend the 'left' primer to be flush against the 'right' primer, and then ligate together
5'
3'
Then ligate this junction
3'
Extend...
3'
Genomic DNA
5'
3) The 3' end of the extension+ligation product may then be annealed to a multimeric barcoding reagent
3'
Multimeric barcode molecule
Adapter Region
Barcode Region
Priming Region
5'
5'
3'
5'
3'
'Extension Primer'
3'
Genomic DNA
5'
4) An extension+ligation reaction can then be performed along the barcoding reagent to create the final barcoded template molecule
5'
3'
Multimeric barcode molecule
Adapter Region
Barcode Region
Priming Region
5'
Extend...
Then ligate this junction
3'
3'
Genomic DNA
5'

FIGURE 14

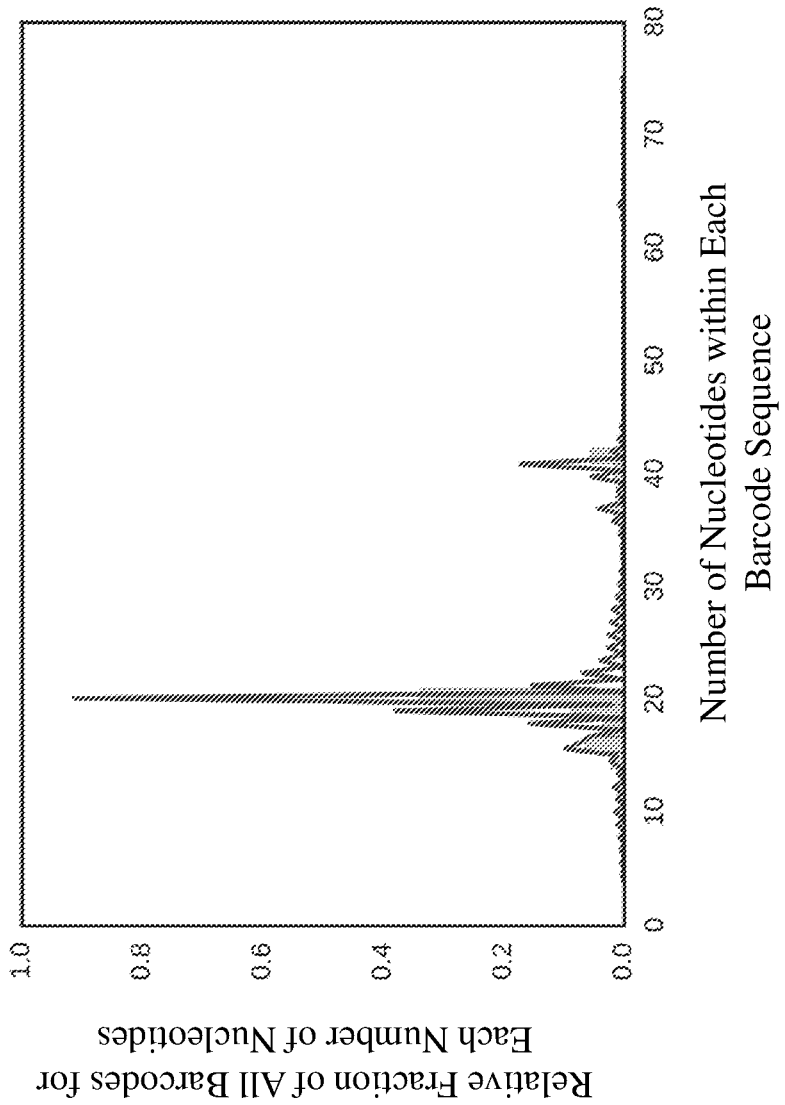

Relative Fraction of All Barcodes for Each Number of Nucleotides
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
40
50
60
70
80
Number of Nucleotides within Each Barcode Sequence

FIGURE 15

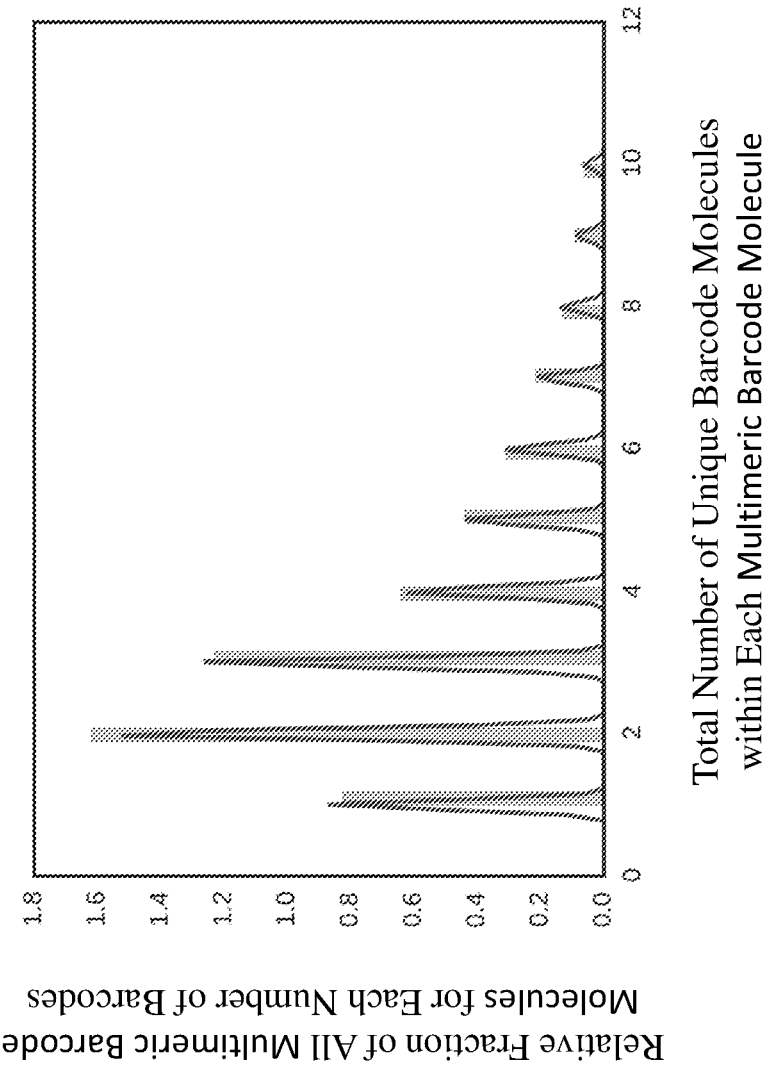

Relative Fraction of All Multimeric Barcode Molecules for Each Number of Barcodes
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
2
4
6
8
10
12
Total Number of Unique Barcode Molecules within Each Multimeric Barcode Molecule

FIGURE 16

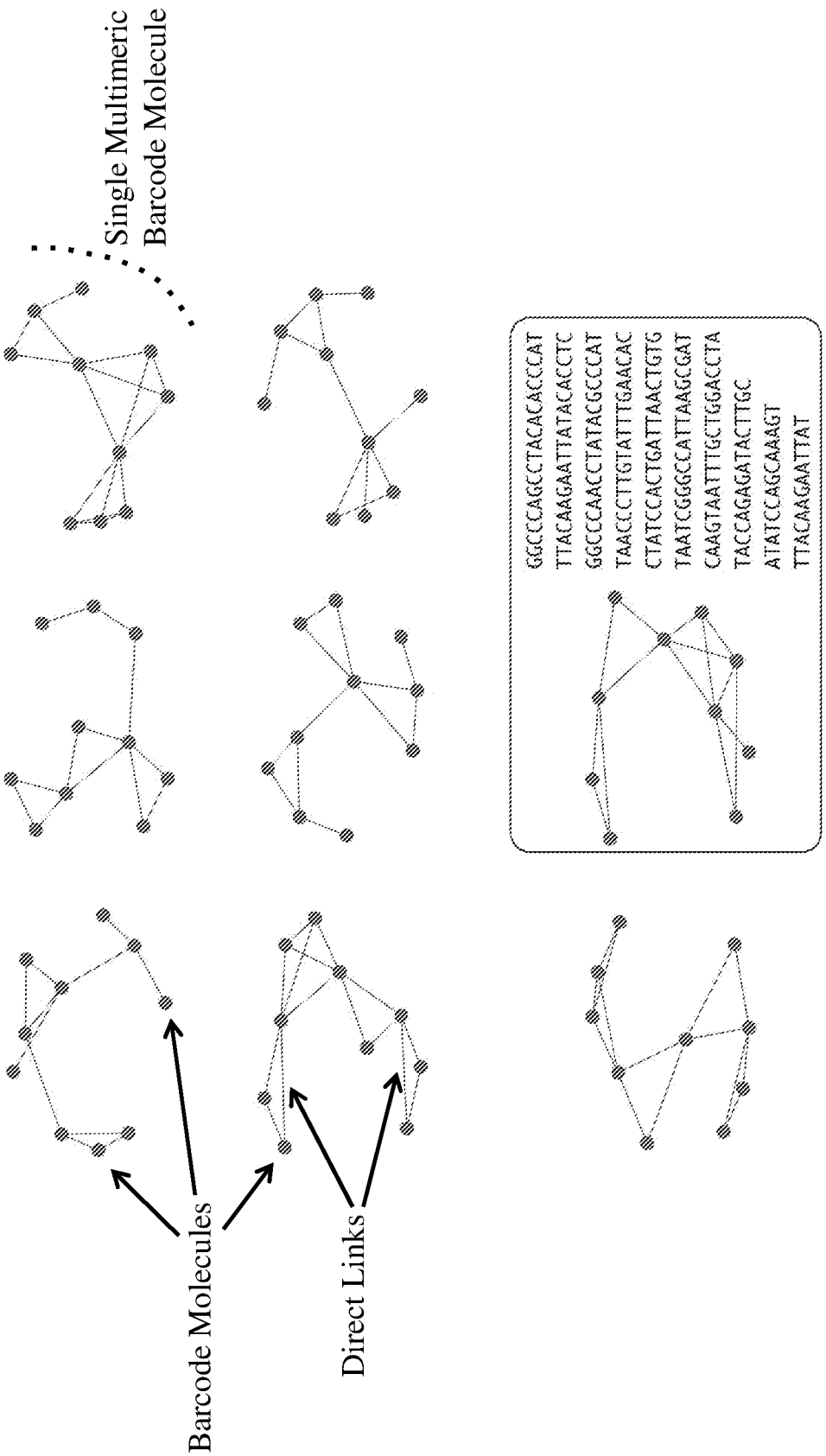

Single Multimeric Barcode Molecule
Barcode Molecules
Direct Links
GGCCCAGCCTACACACCCAT
TTACAAGAATTATATACACCTC
GGCCCAACCTATACGCCCAT
TAACCCTTGTATTTGAACAC
CTATCCACTGATTAACTGTG
TAATCGGGCCATTAAGCGAT
CAAGTAATTTGCTGGACCTA
TACCAGAGATACTTGC
ATATCCAGCAAAGT
TTACAAGAATTAT

FIGURE 17

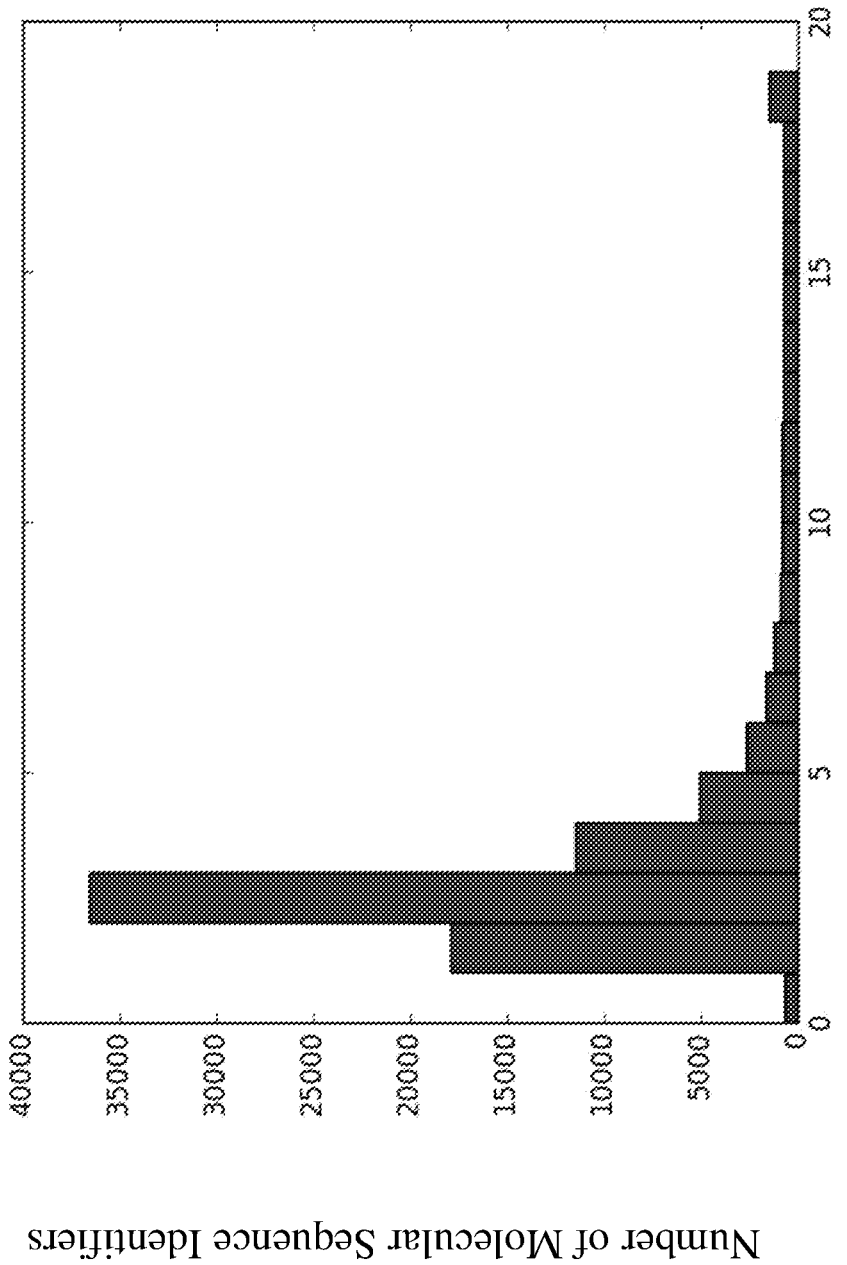

Number of Molecular Sequence Identifiers
40000
35000
30000
25000
20000
15000
10000
5000
0
0
5
10
15
20
Number of Unique Barcodes per Molecular Sequence Identifier

FIGURE 18

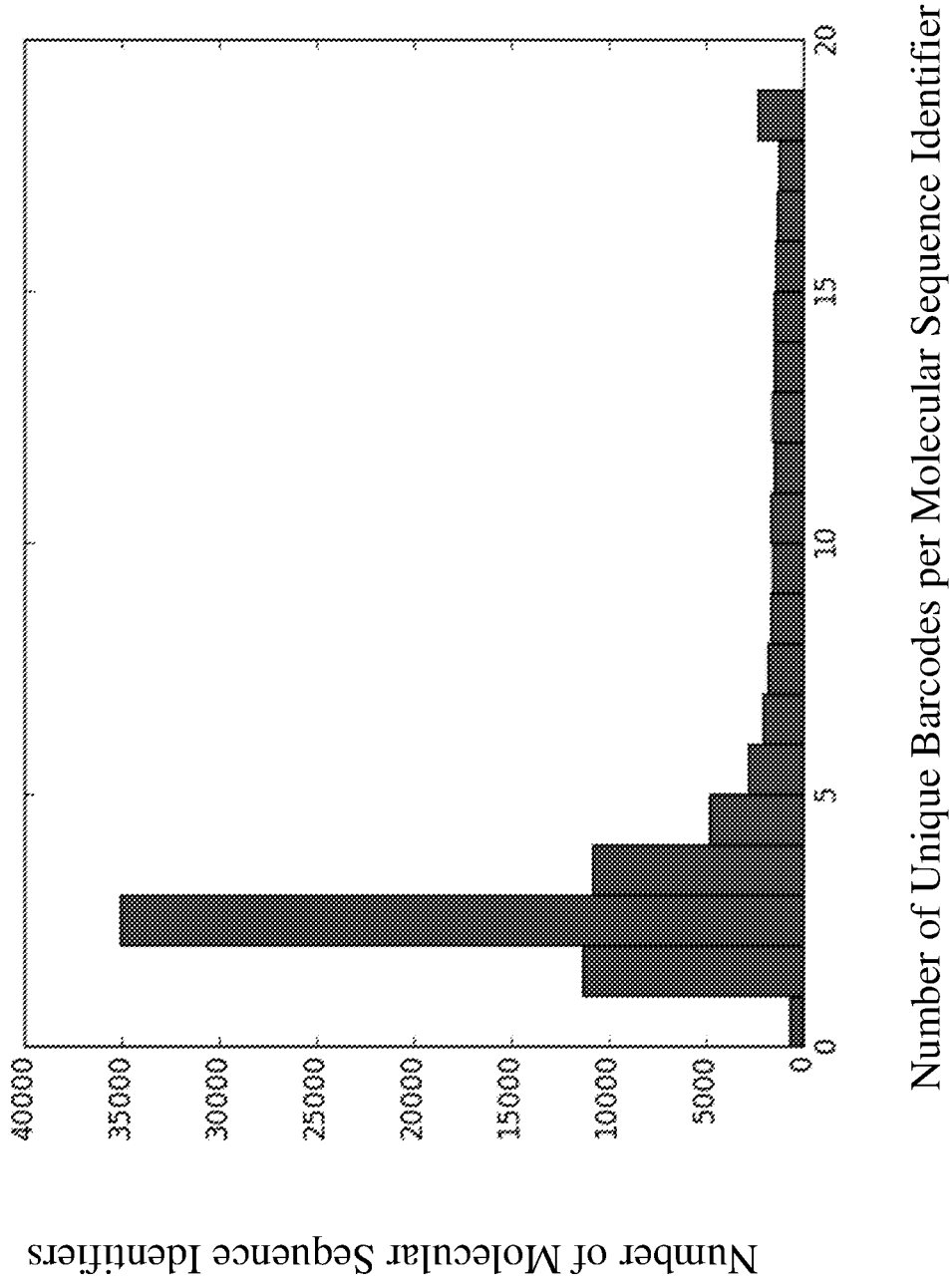
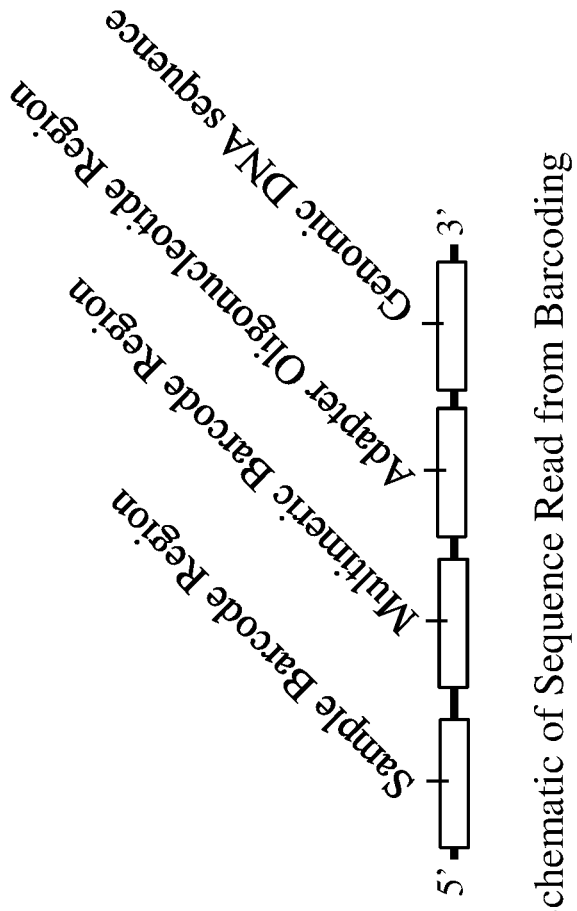

Number of Molecular Sequence Identifiers
40000
35000
30000
25000
20000
15000
10000
5000
0
0
5
10
15
20
Number of Unique Barcodes per Molecular Sequence Identifier

FIGURE 19

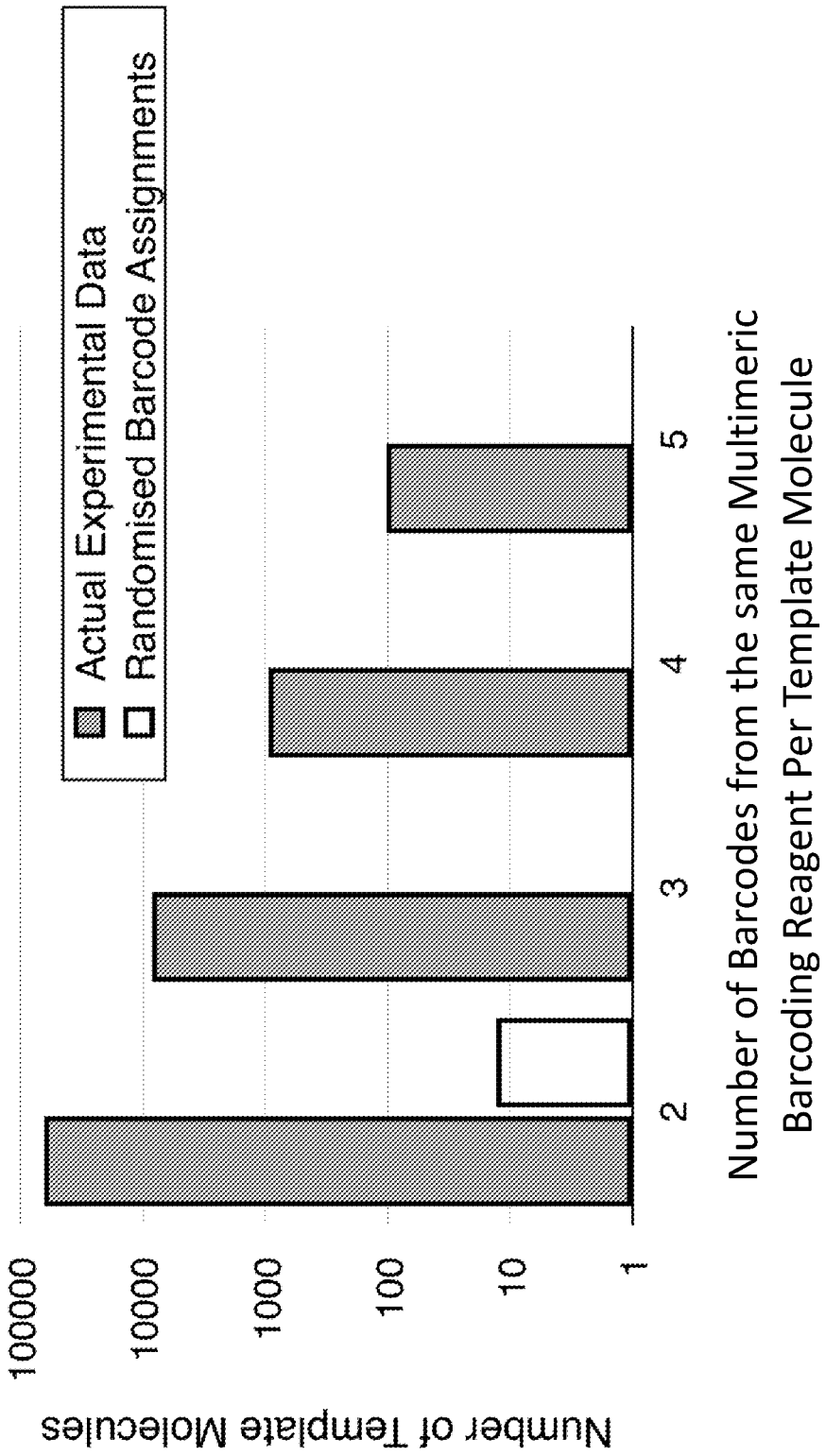
	Annealed to Genomic DNA Overnight	Annealed to Genomic DNA Overnight; Barcoding Reagents Denatured First	Annealed to Genomic DNA for 3 Hours	Annealed to Genomic DNA for 3 Hours; Barcoding Reagents Denatured First
HLA-A_Exon2	153	62	103	50
HLA-A_Exon3	2548	341	818	106
HLA-A_Exon4	489	235	400	108
DQB1_Exon2	557	627	285	635
DQB1_Exon3	2186	1614	1085	221
BRCA1_US1	1771	941	1047	544
BRCA1_SNP7	2524	1261	90	43
BRCA1_SNP6	731	537	245	339
BRCA1_SNP1	10033	5787	2905	1813
BRCA1_DS1	4	103	5	0
BRCA_AMP6	81	69	37	24
BRCA_AMP7	731	391	40	472
On-Target Fraction:	81 %	76 %	82 %	70 %

FIGURE 20



Schematic of Sequence Read from Barcoding of genomic DNA loci
(In 5' to 3' Direction)

FIGURE 21


Actual Experimental Data
Randomised Barcode Assignments
100000
10000
1000
100
10
1
Number of Template Molecules
2
3
4
5
Number of Barcodes from the same Multimeric Barcoding Reagent Per Template Molecule