

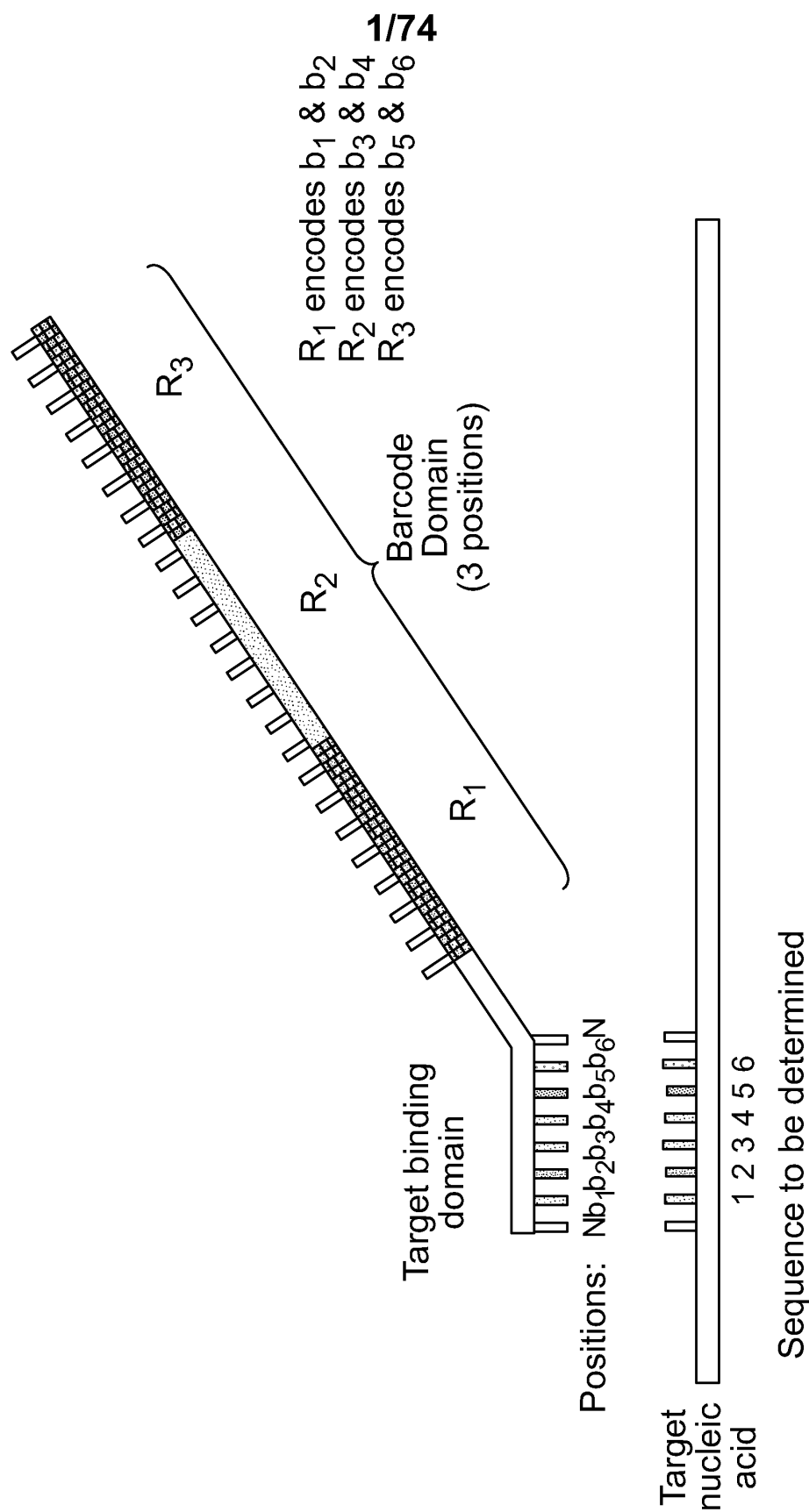


FIG. 1

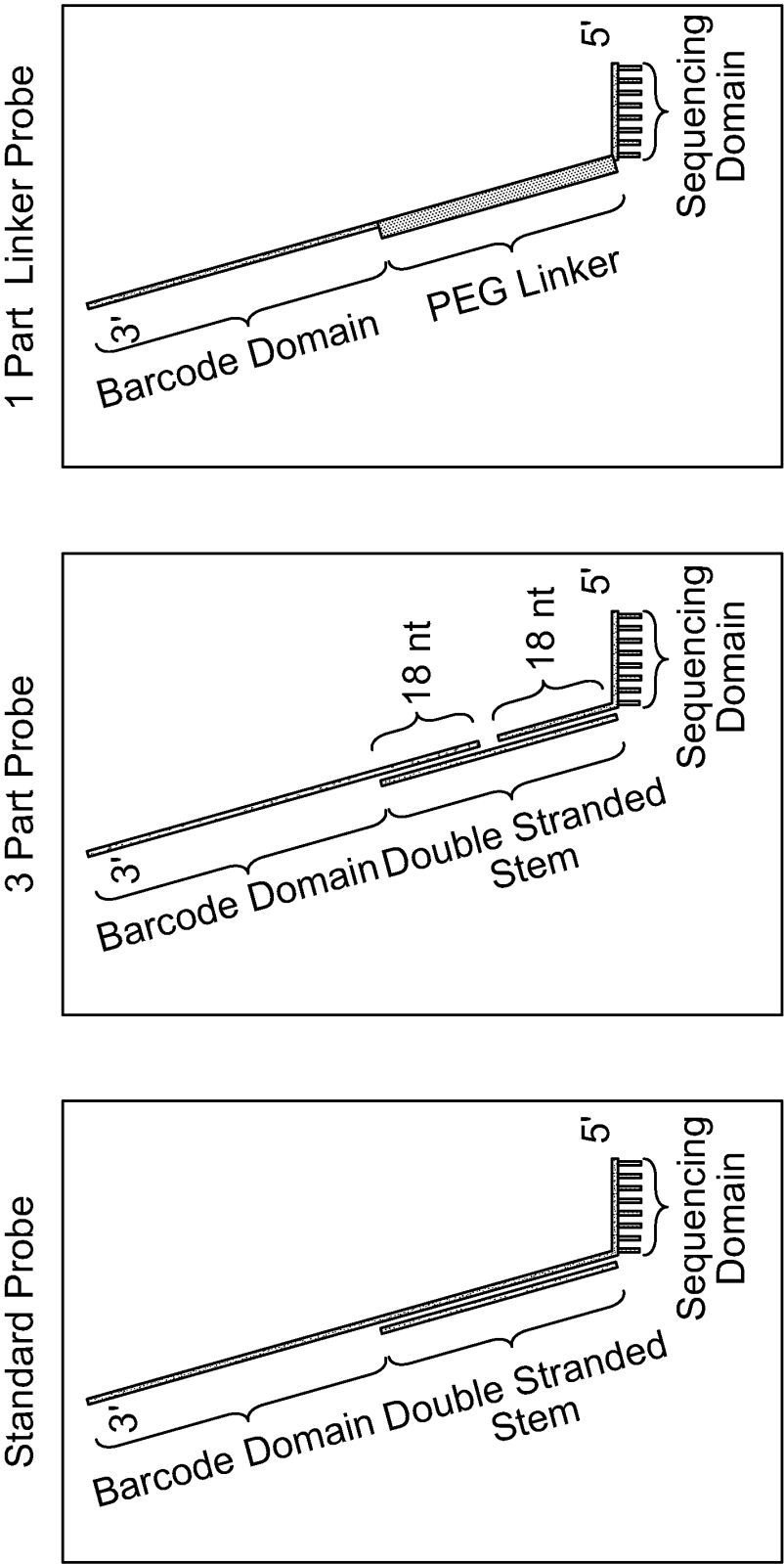


FIG. 2

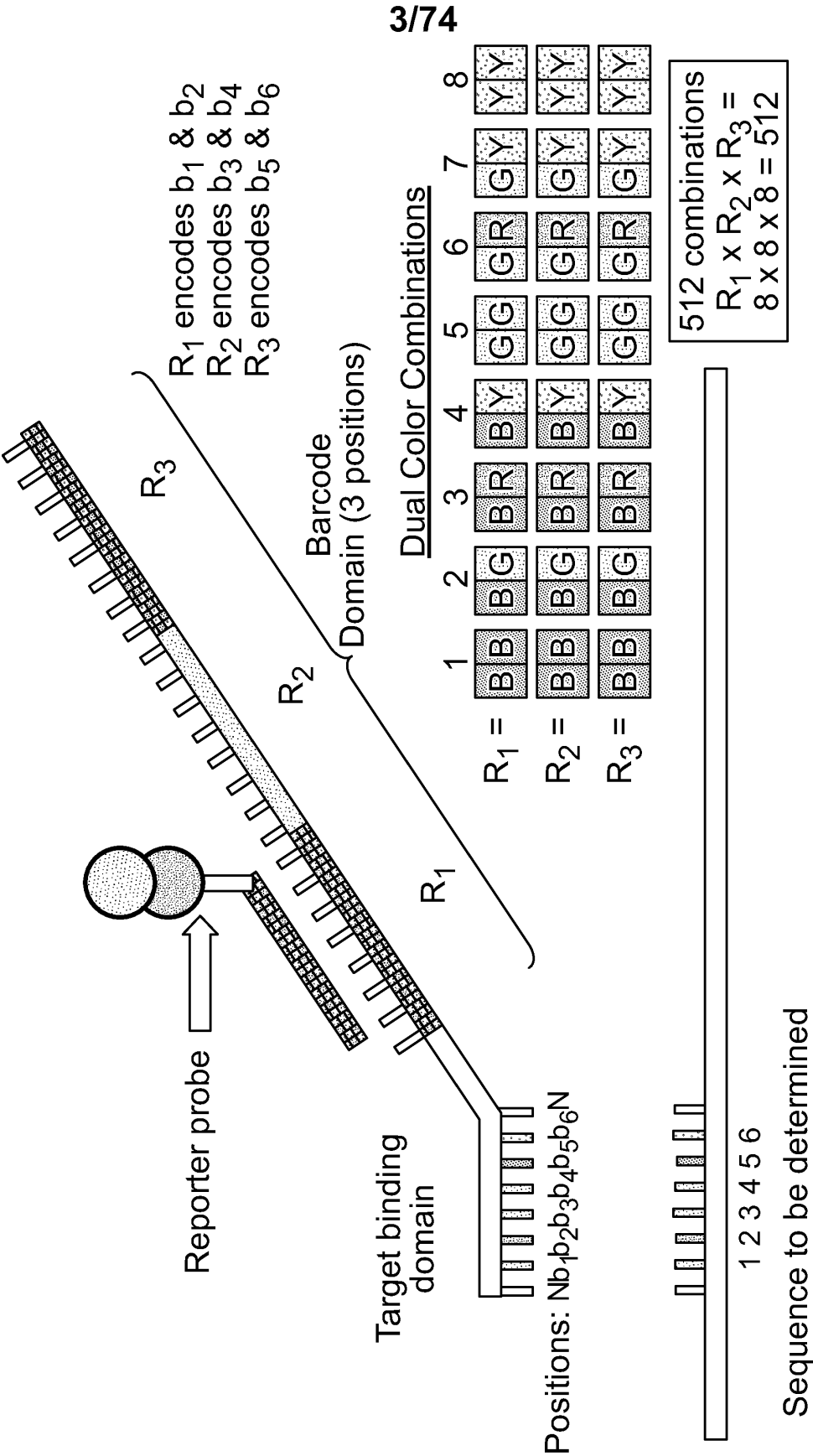


FIG. 3

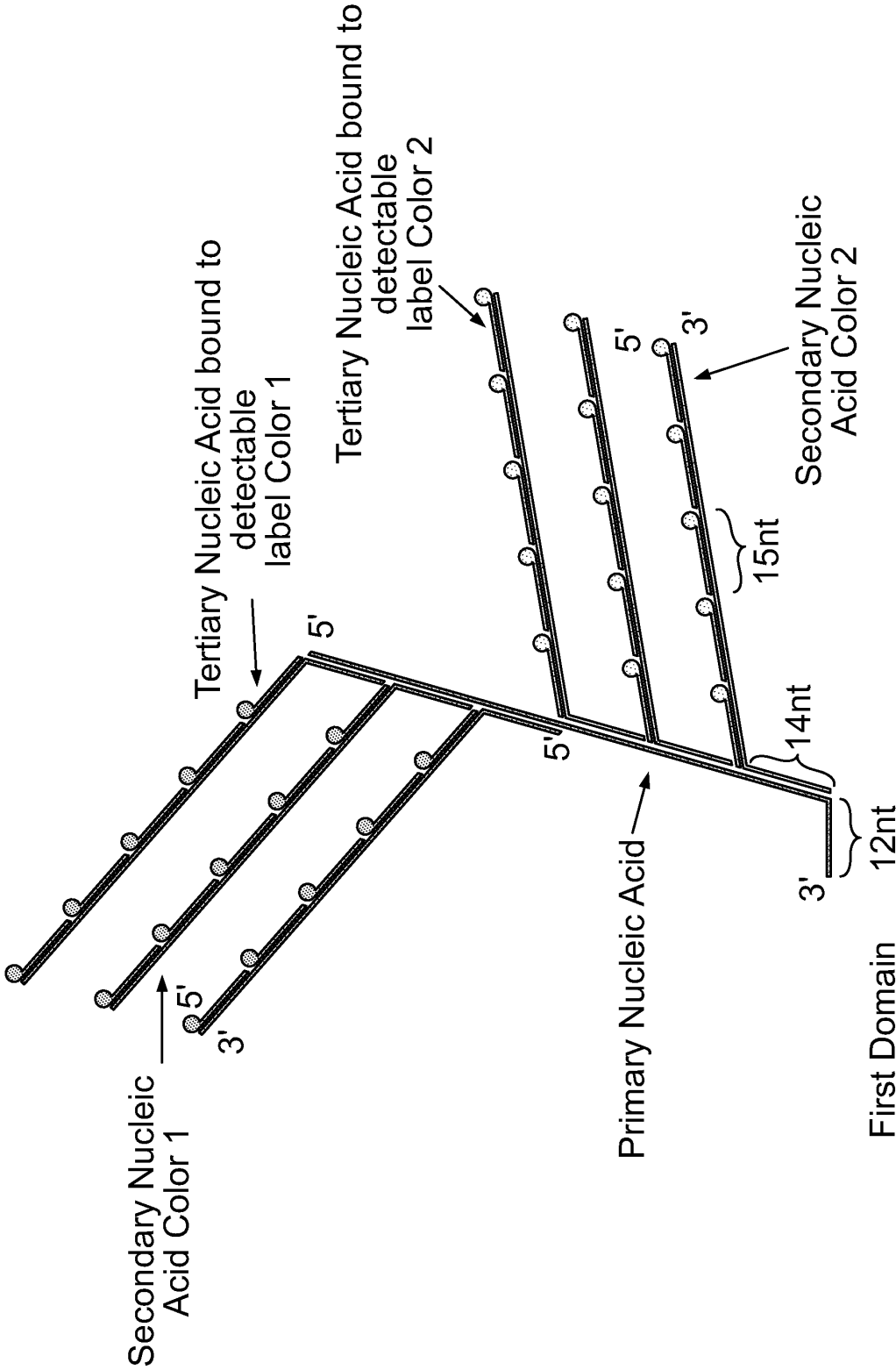
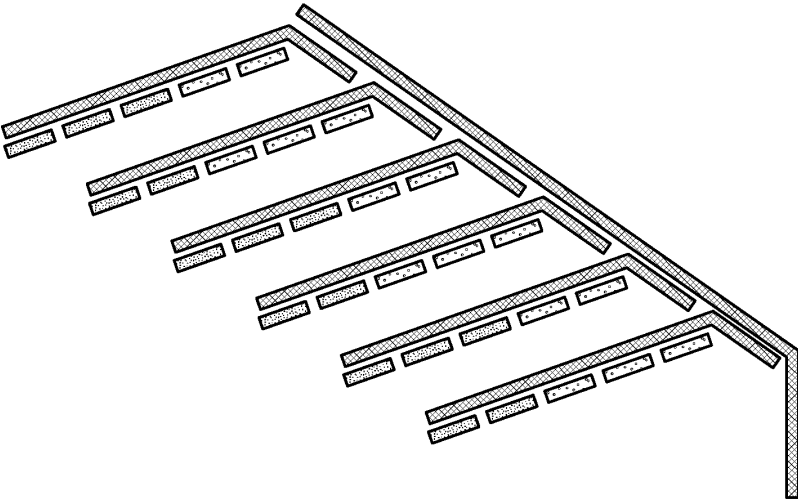
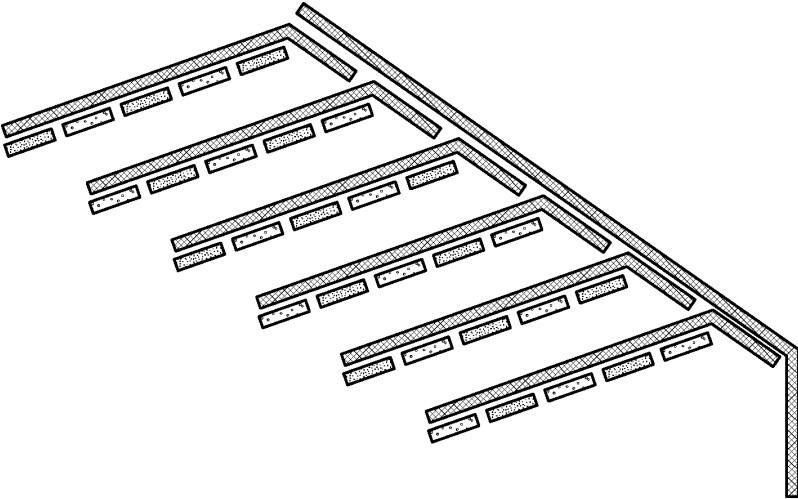


FIG. 4

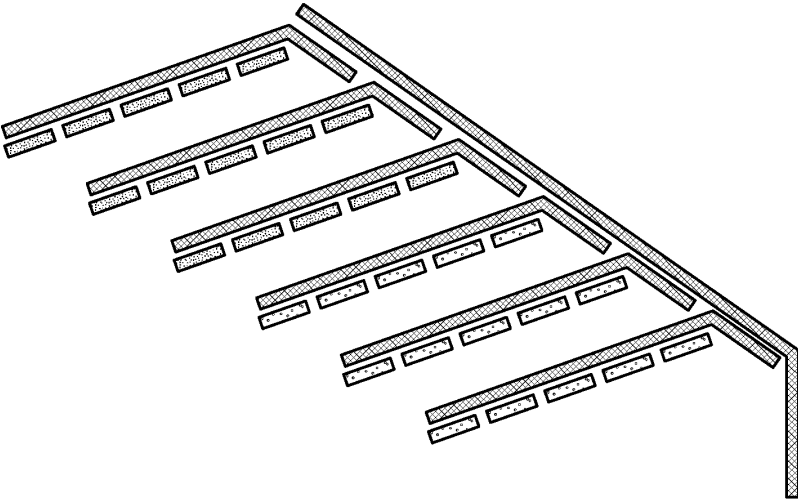
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Fret resistant 3x2x6



3x2x6



5x6

FIG. 5

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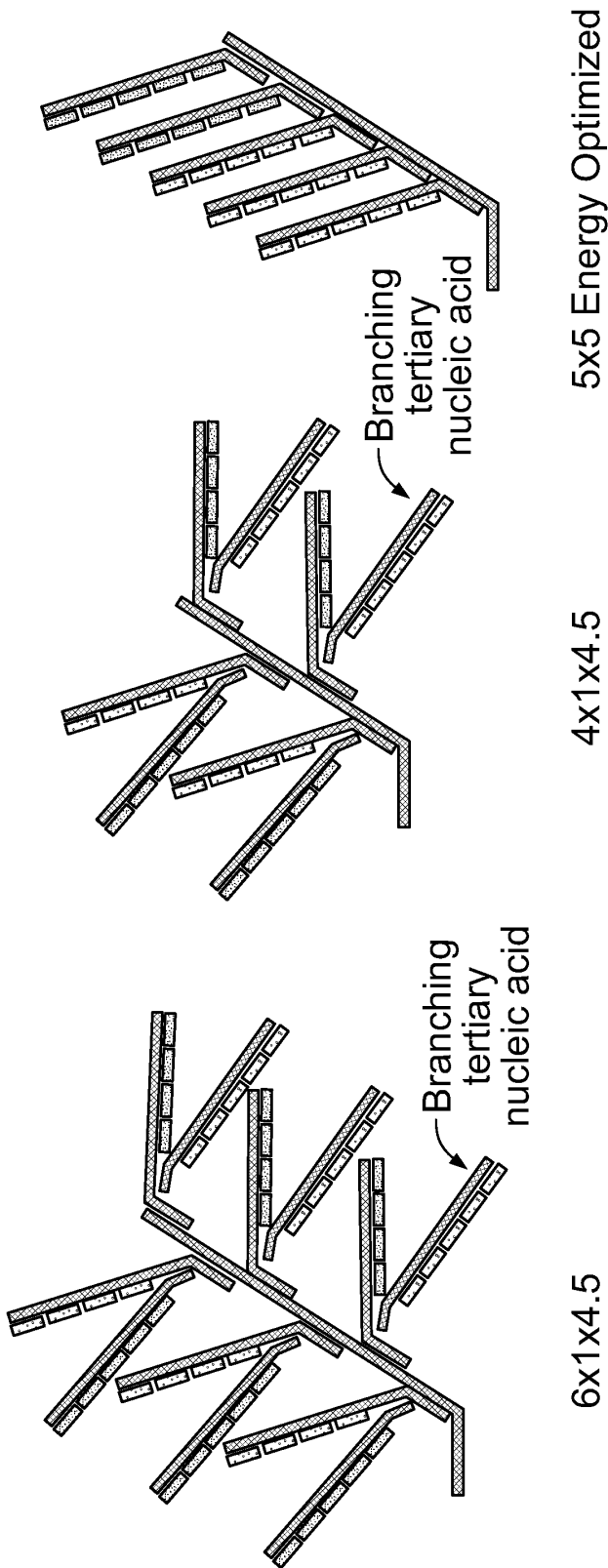


FIG. 6

Example cleavage modifications

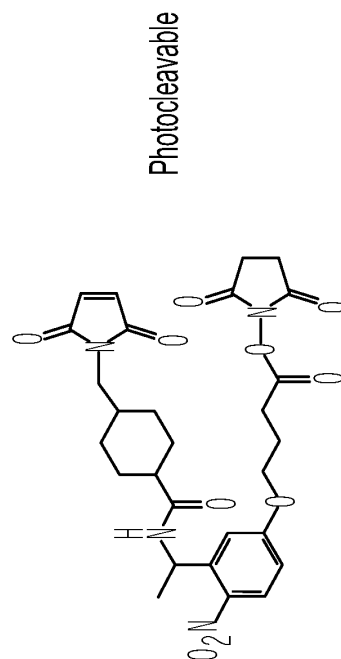
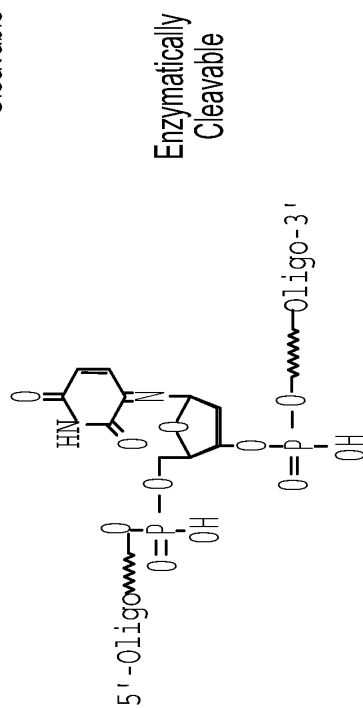
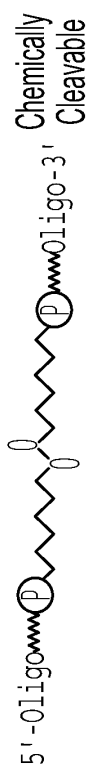
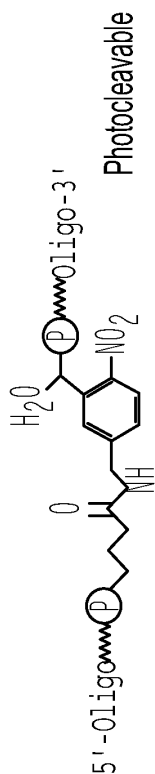
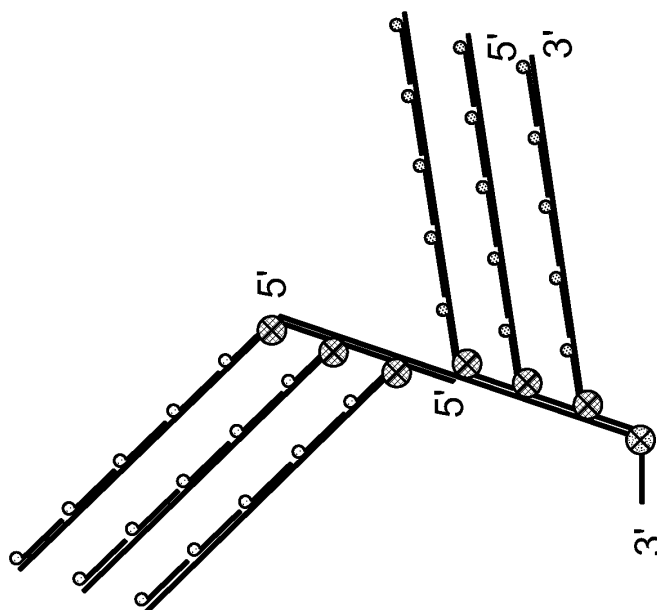


FIG. 7

Reporter design

-  = Primary nucleic acid cleavage site
 = Secondary nucleic acid cleavage site

- ⊗ = Secondary nucleic acid cleavage site



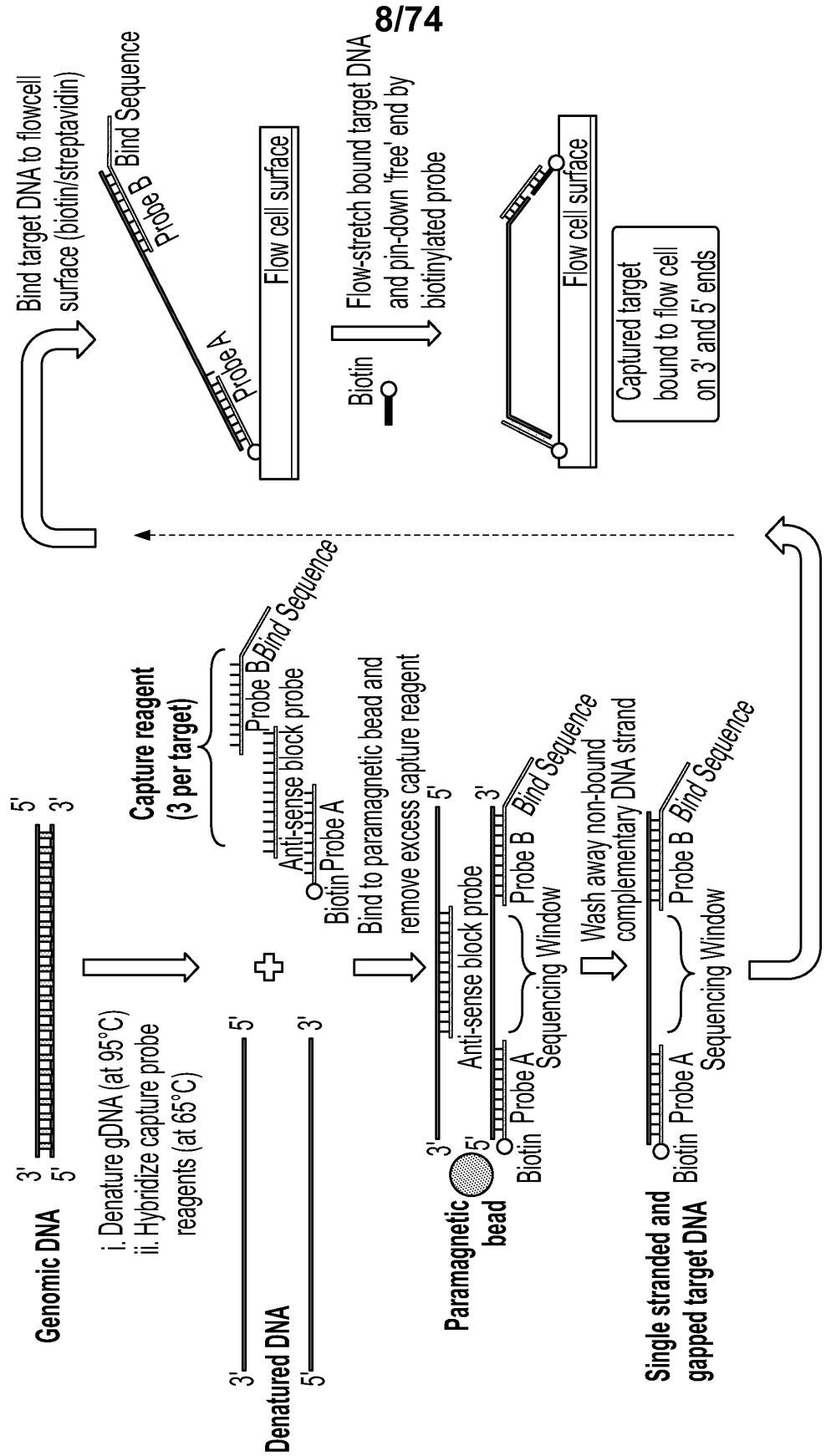


FIG. 8

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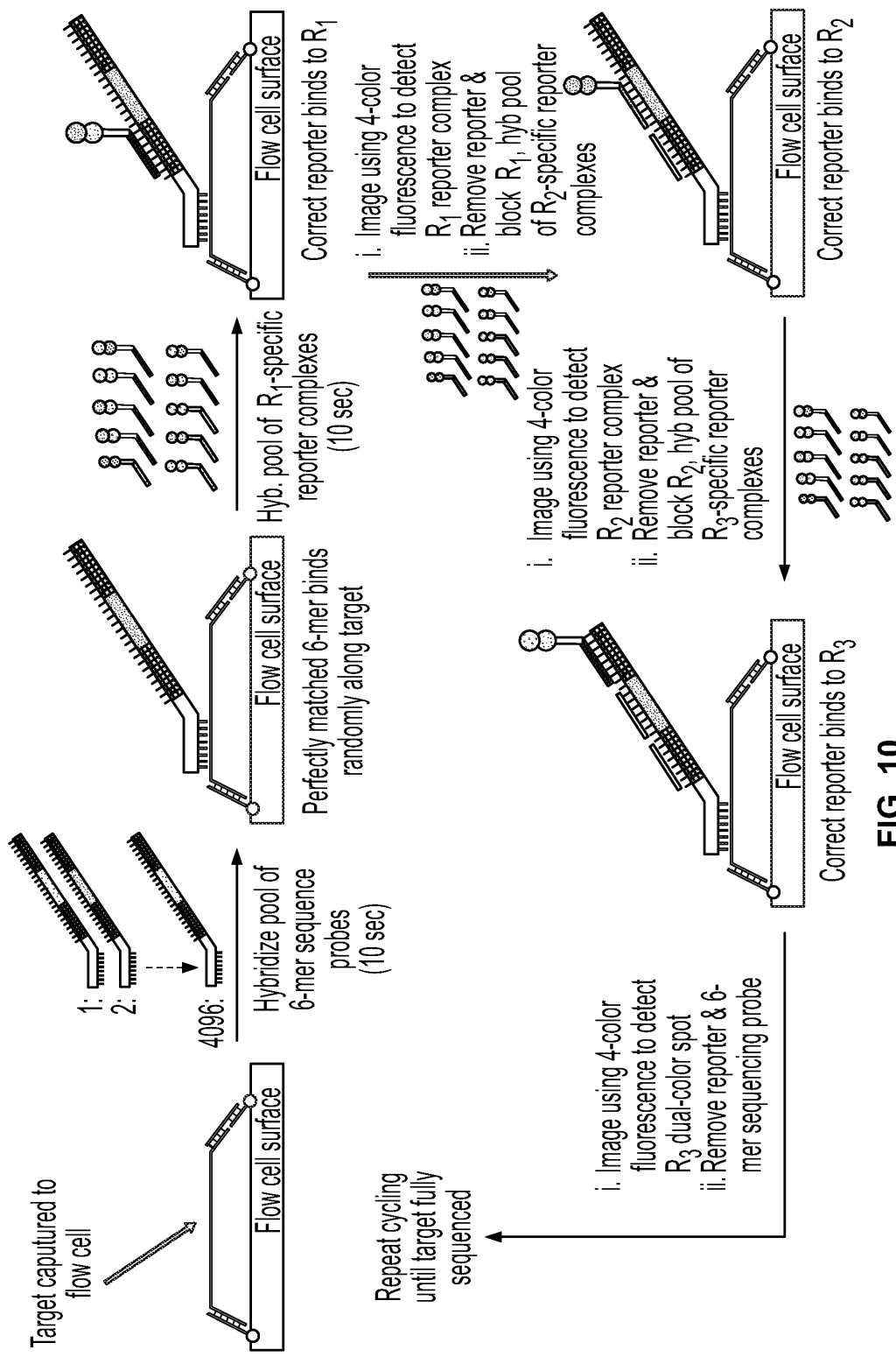


FIG. 10

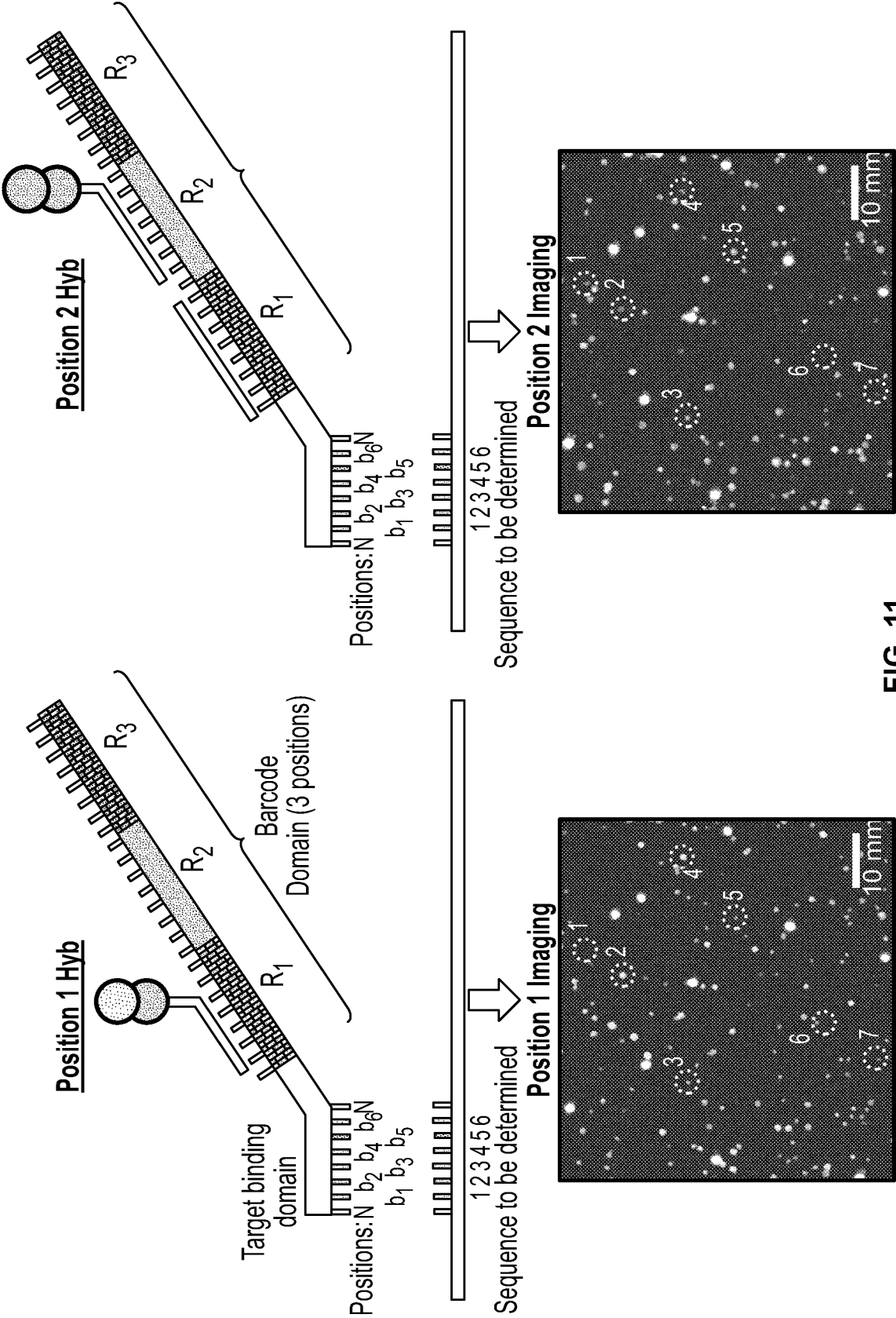


FIG. 11

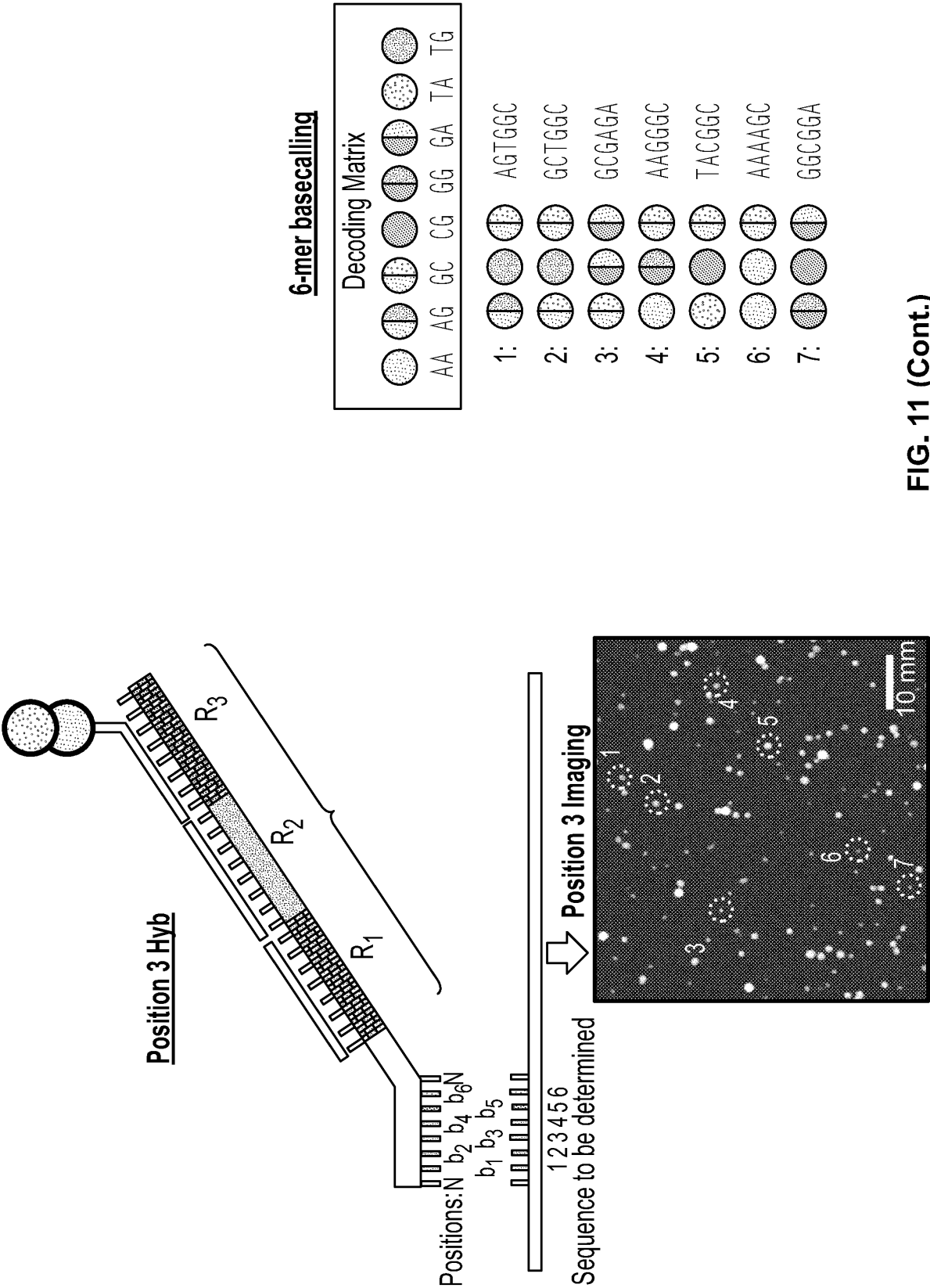


FIG. 11 (Cont.)

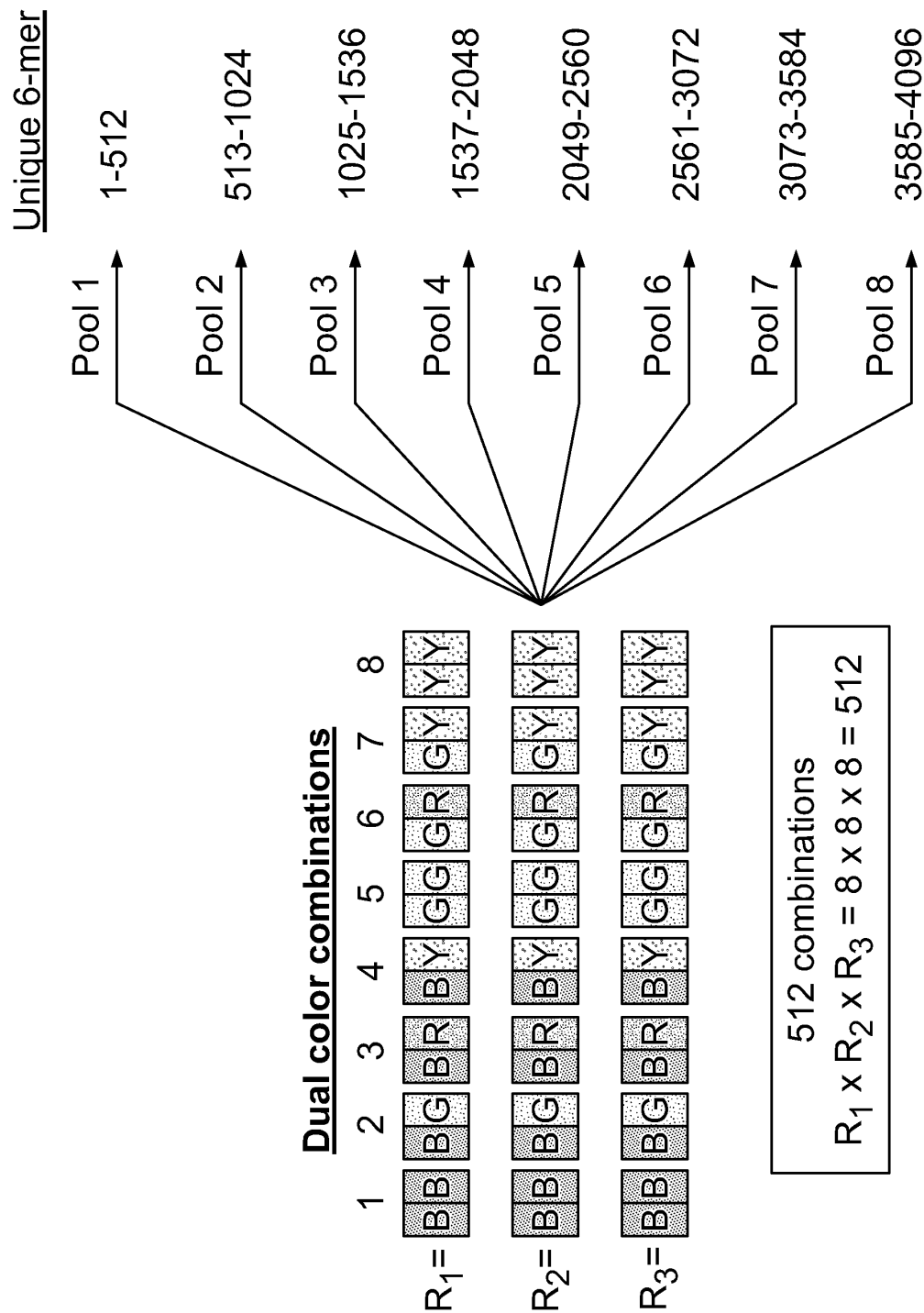


FIG. 12

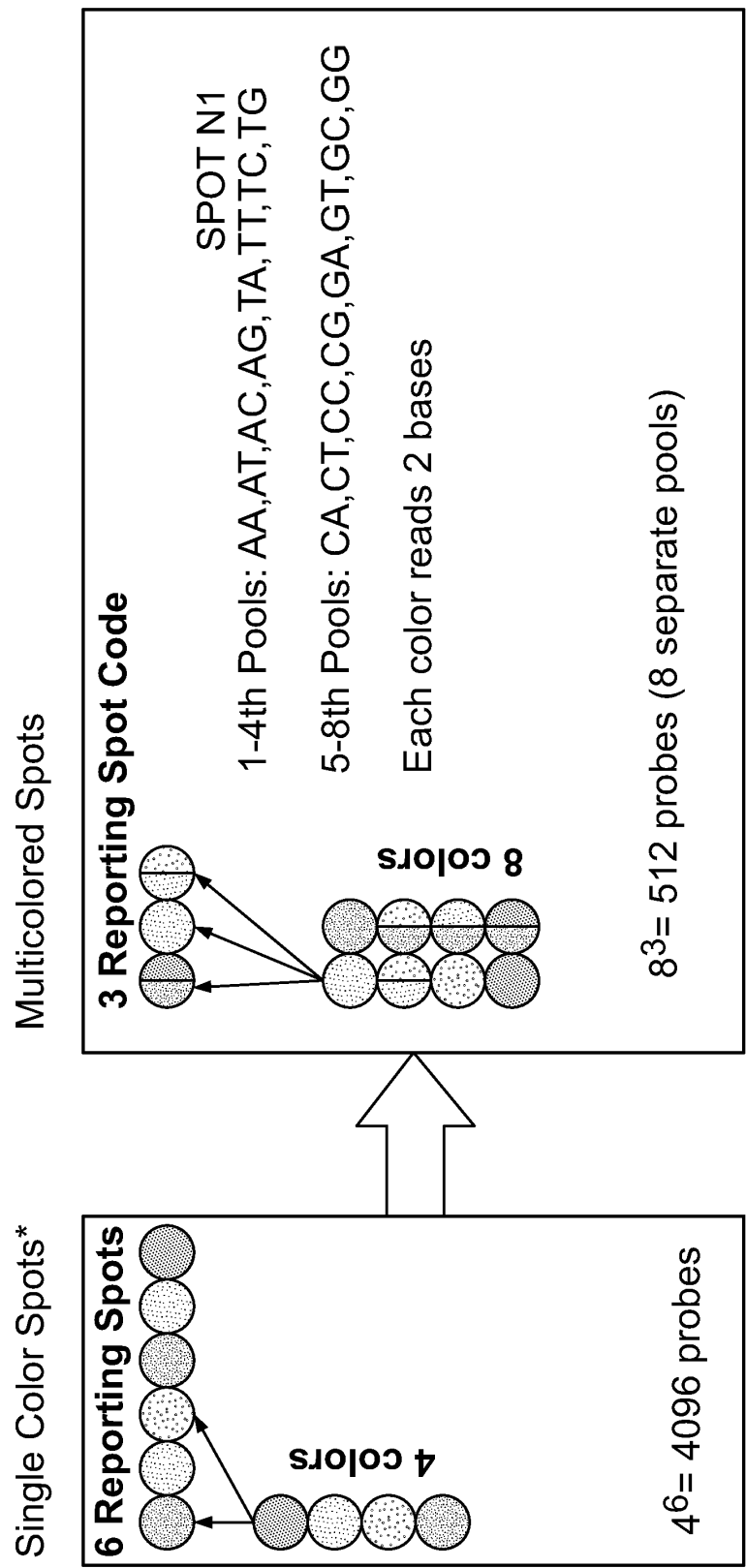


FIG. 13

*As disclosed in US 2016-0194701, which is incorporated herein by reference in its entirety

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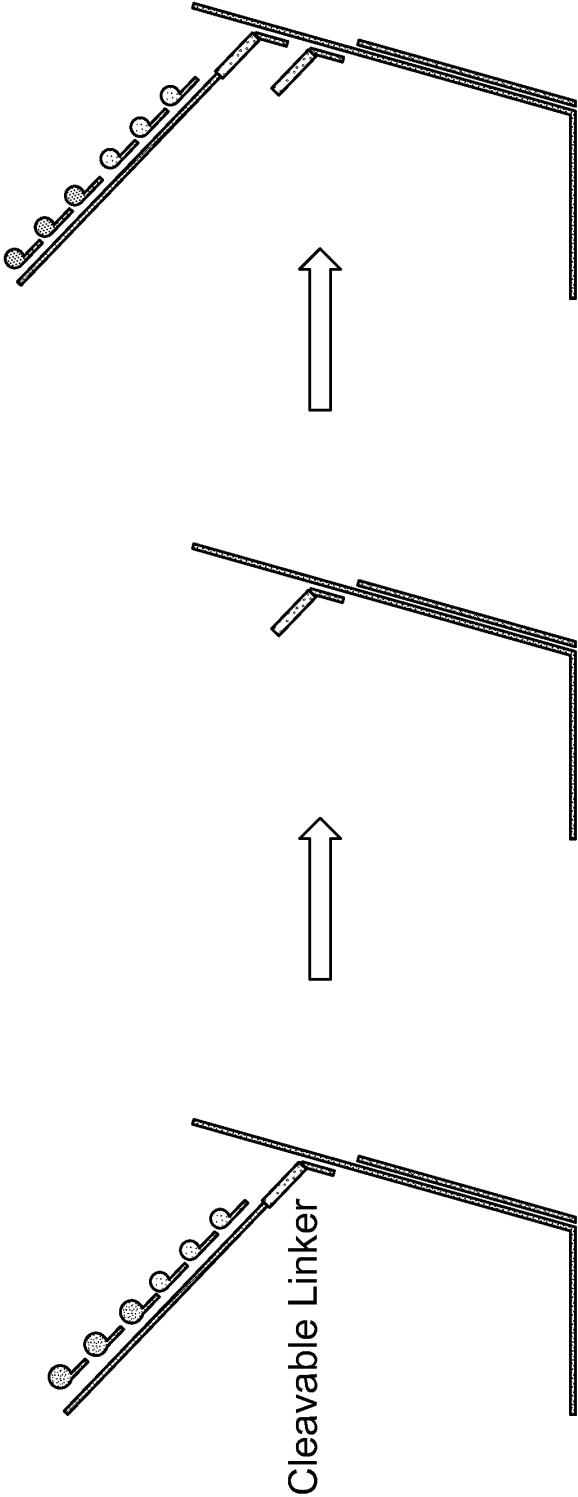


FIG. 14

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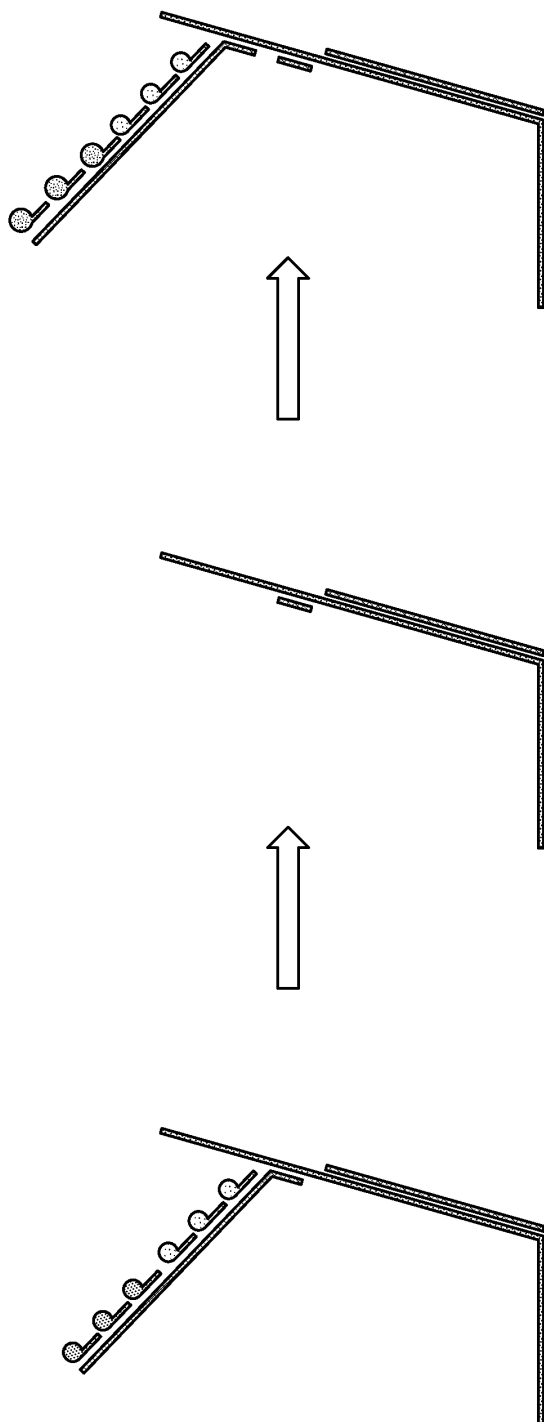
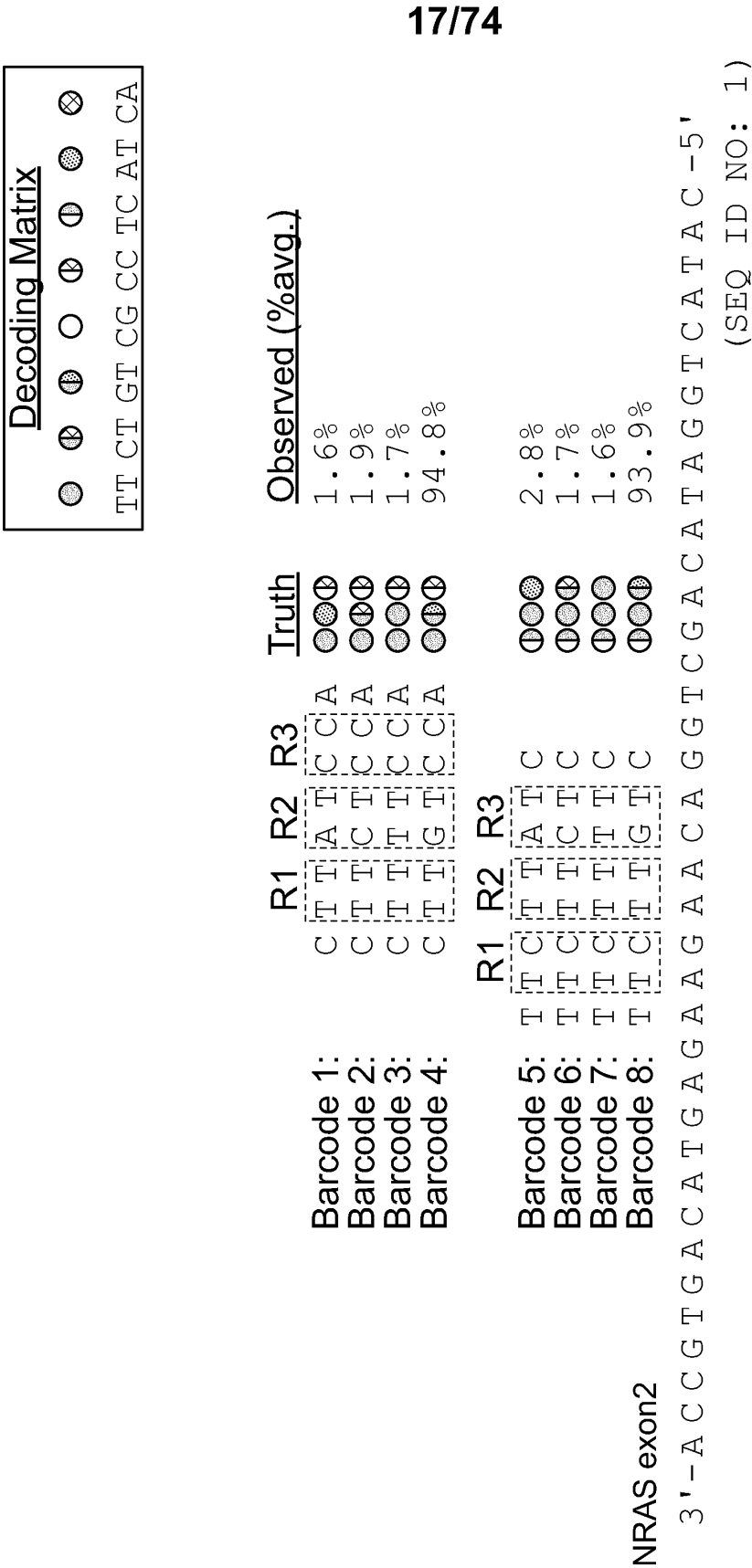


FIG. 15



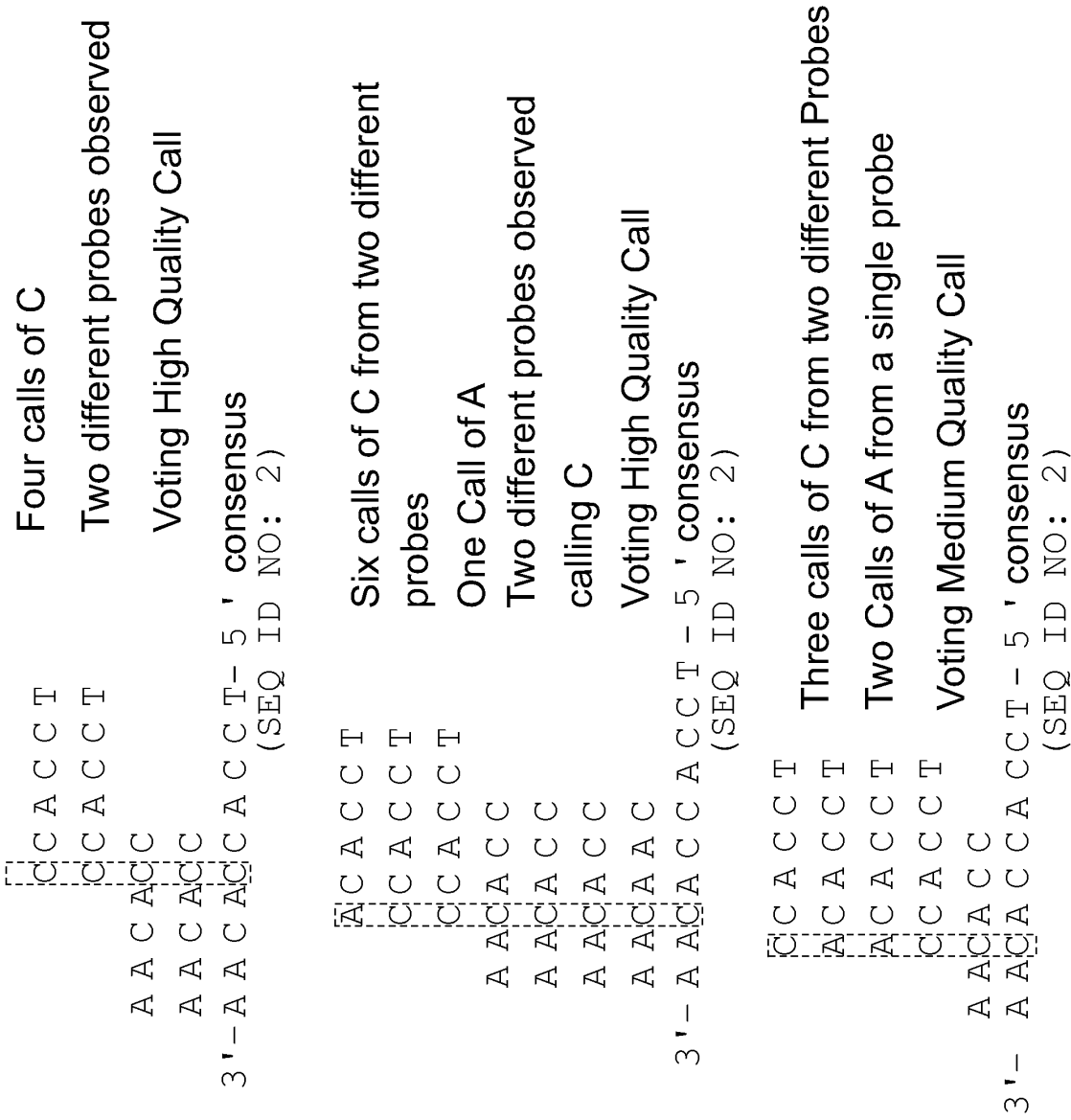


FIG. 17

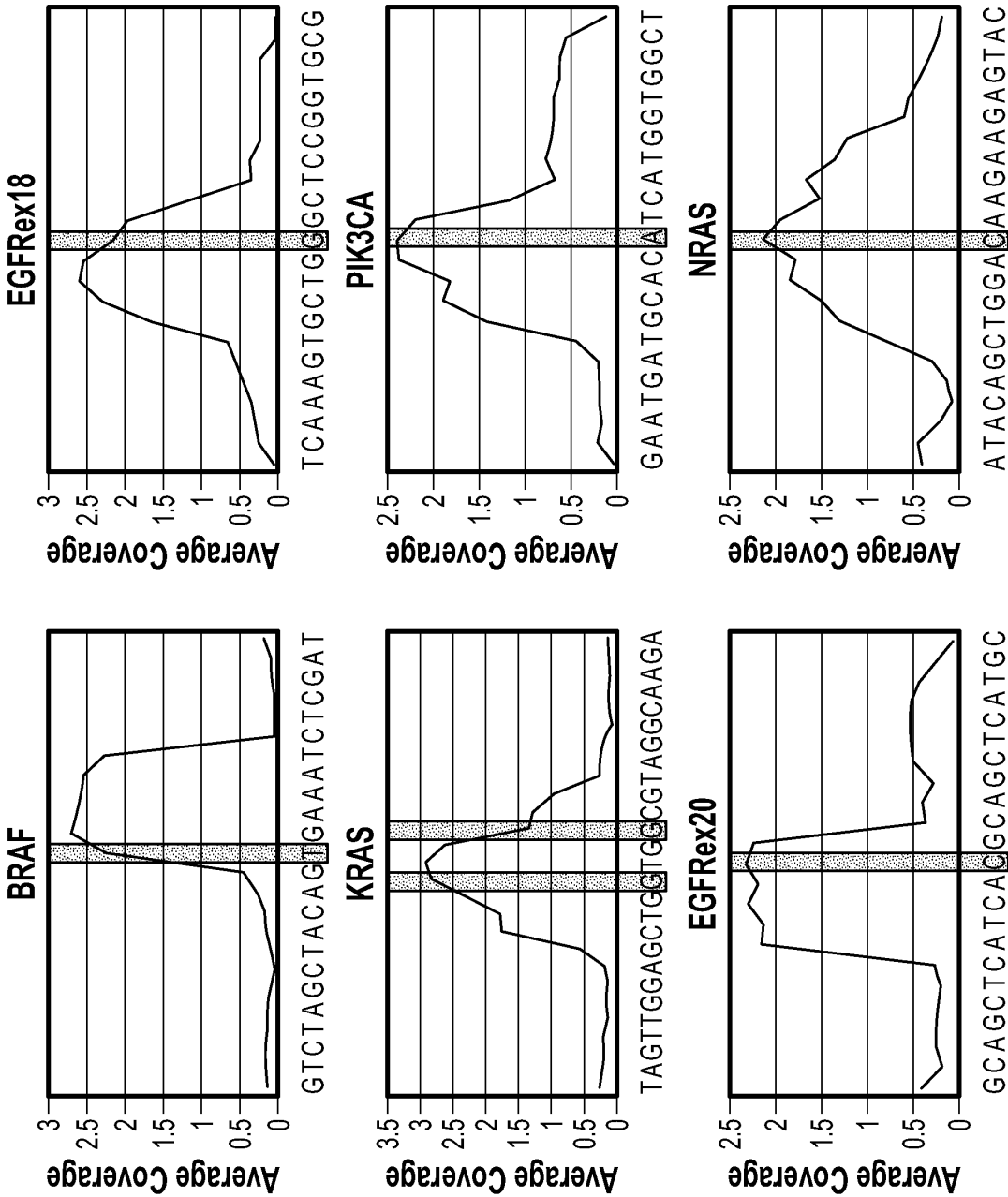
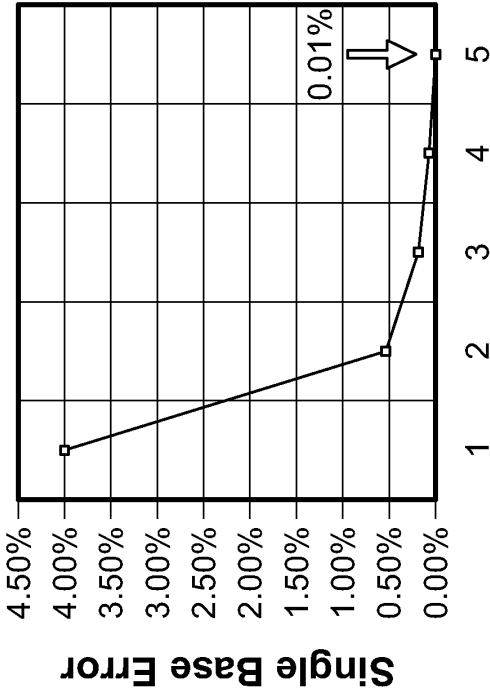


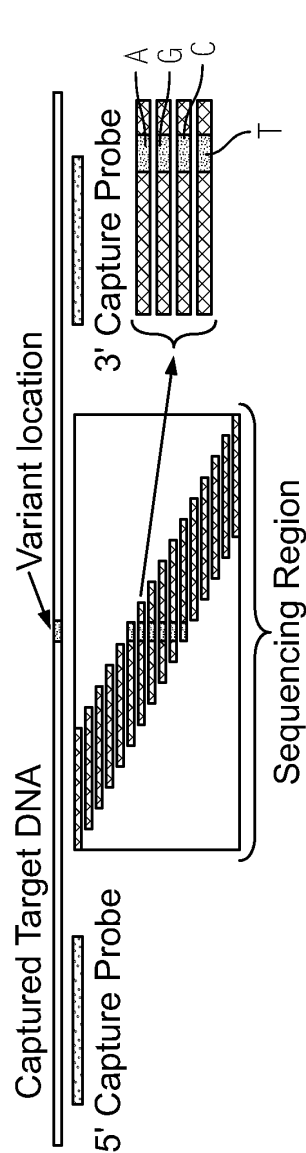
FIG. 18

Gene	Variant	Observed Freq. (%)	Expected Freq. (%)
BRAF	ref V600E	91.0% 9.0%	89.5% 10.5%
EGFR exon 18	ref G719S	88.1% 11.9%	75.5% 24.5%
EGFR exon 20	ref T790M	98.9% 1.1%	99.0% 1.0%
NRAS	ref Q61K	63.0% 37.0%	87.5% 12.5%
PICK3CA	ref H1047R	92.1% 7.9%	82.5% 17.5%
KRAS	ref G12D G13D	99.5%	79.0%
		0.46% 0.005%	6.0% 15.0%



Base Coverage

FIG. 18 (Cont.)



Multiplex of 11 DNA Target are captured
Variants of interest are listed

Gene	Variant	Sequencing Region 5' → 3'	SEQ ID NO
BRAF	V600E	GTCTAGCTACA G GAAATCTCGAT	3
	V600K		
EGFR	G719S	TCAAAGTGCT G GCTCCGGTGGC	4
	G12A		
	G12R	TAGTTGGAGCT T TCTCGTAGGCAAGA	5
KRAS	G13D		
PIK3CA	H1047R	GAATGATGCAC A TTCATGGTGGCT	6
EGFR	T790M	GCAGCTCATCA G GCAGCTCATGC	7
NRAS	Q61K	ATACAGCTGGA G AAGAAGAGTAC	8
FLT3	Δ1836	TGGCTCGAGAT A TGATGAGTGATTC	9
IDH1	R132C	TCATCATAGGT G GTCATGCTTAT	10
JAK2	V617F	ATGGAGTATGT T TCTGTGGAGAC	11
MEK1	P124L	GTGCAACTCT C GTACATCGTGG	12
NOTCH1	L1601P	CAGCCGGTGC T GCACACCAACG	13

FIG. 19

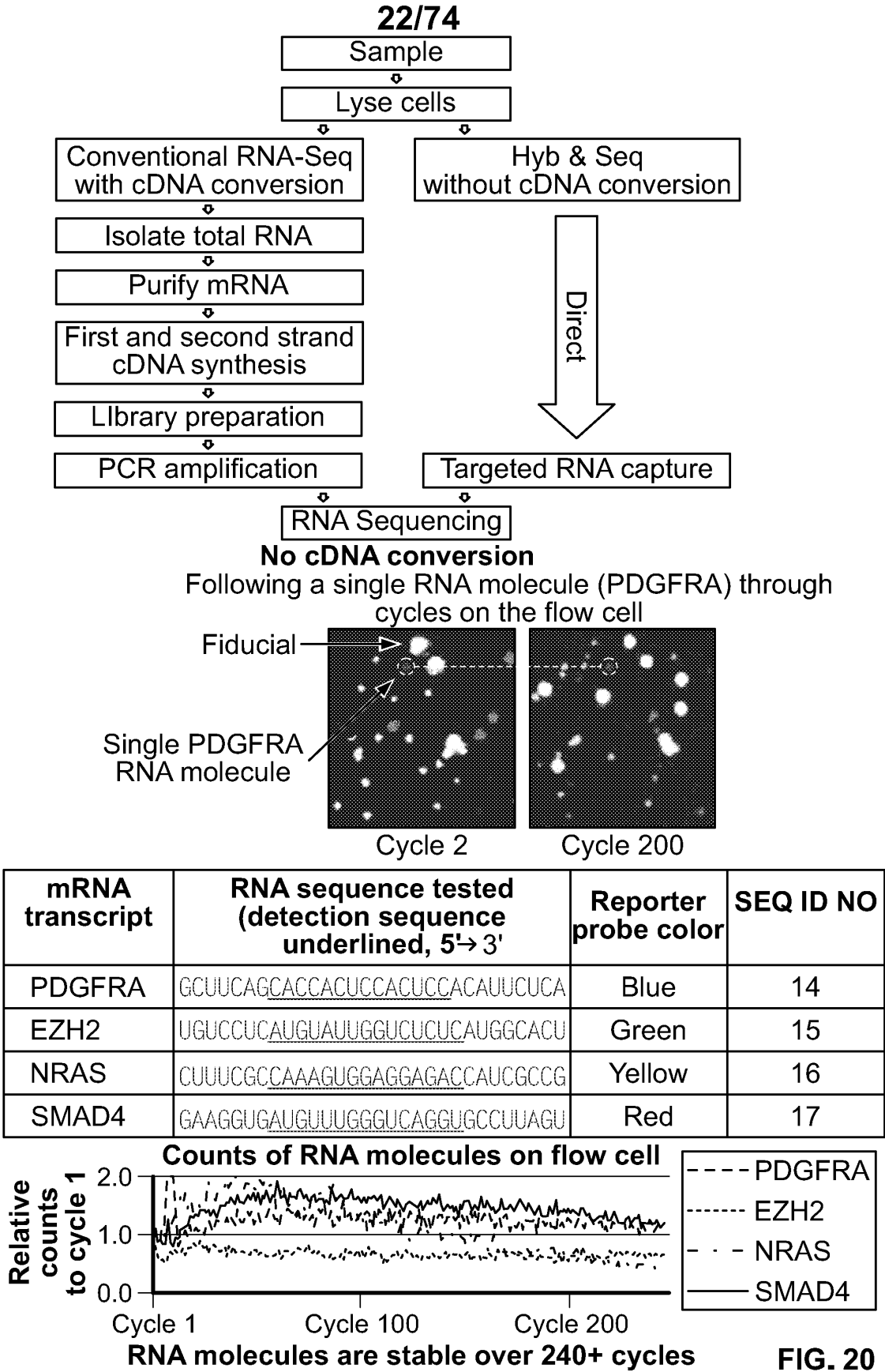


FIG. 20

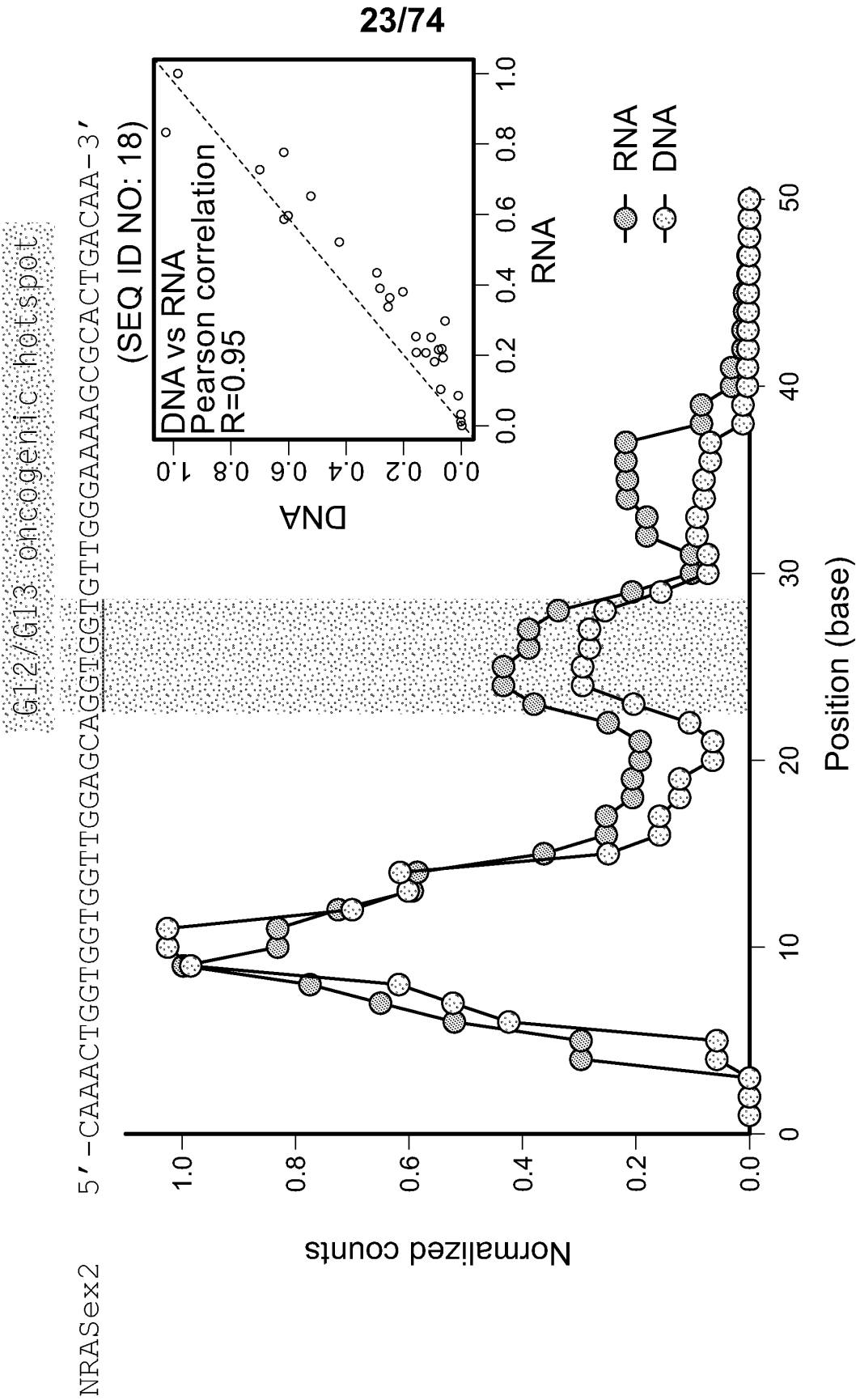


FIG. 21

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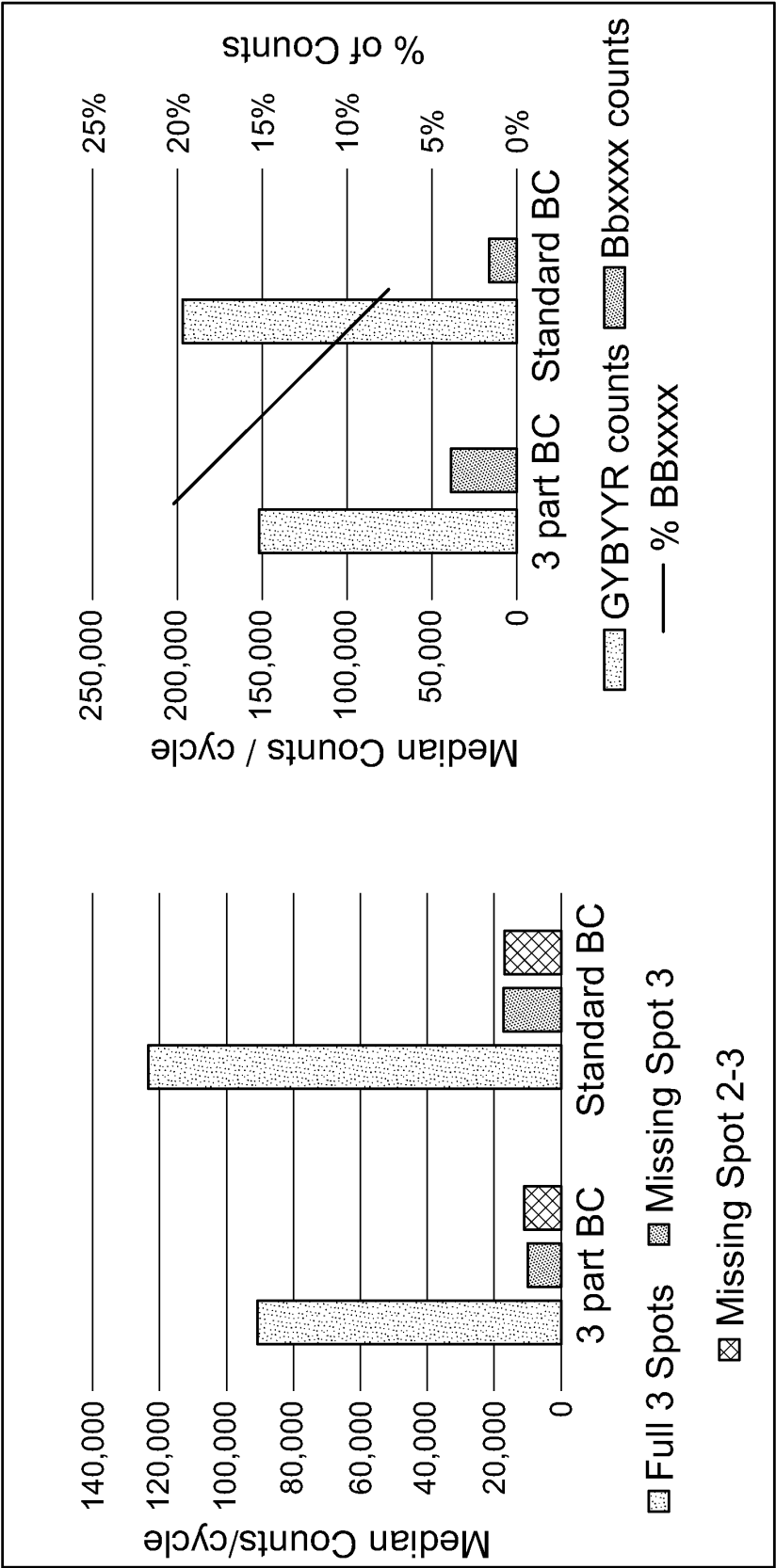


FIG. 22

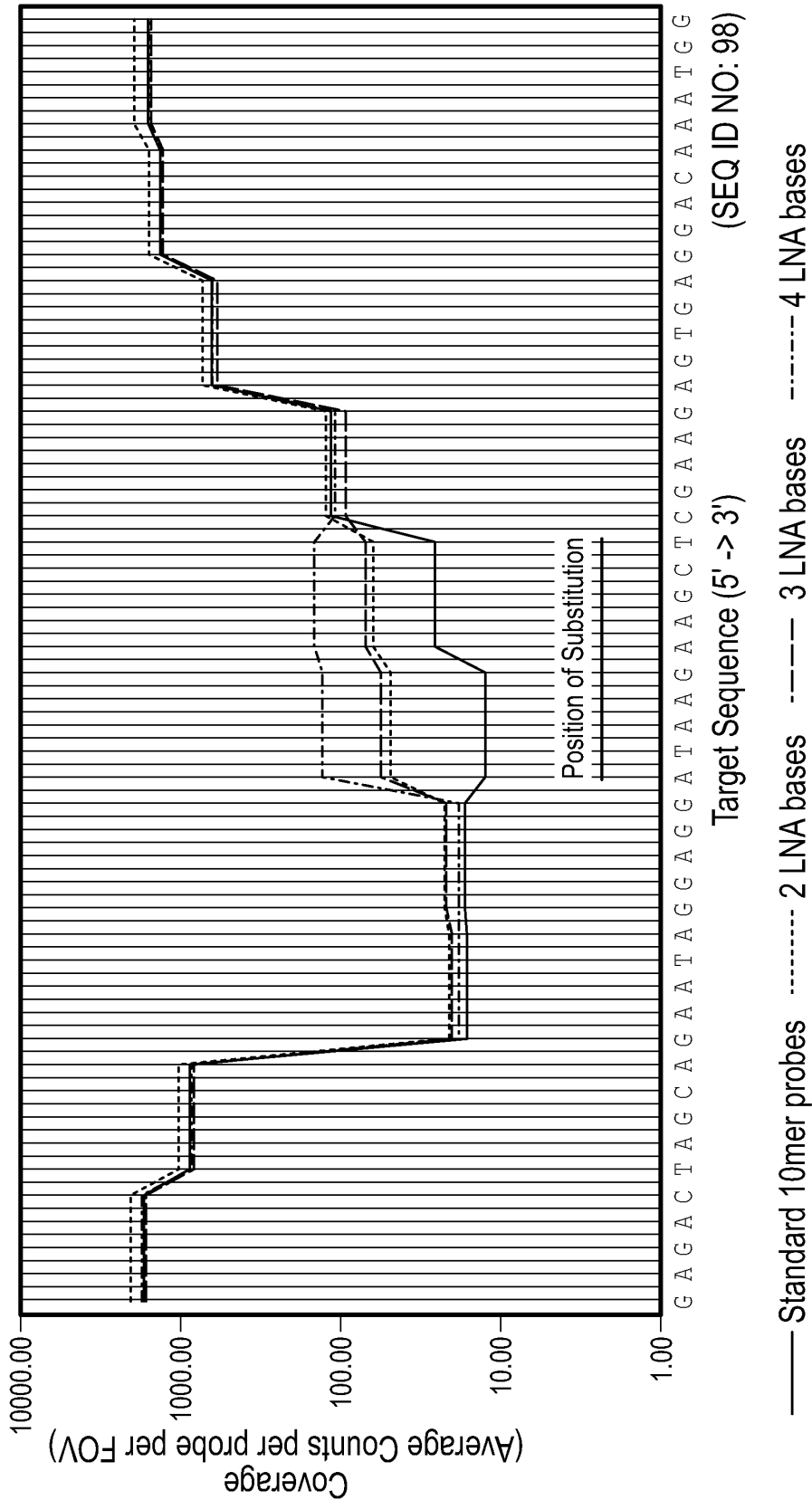


FIG. 24

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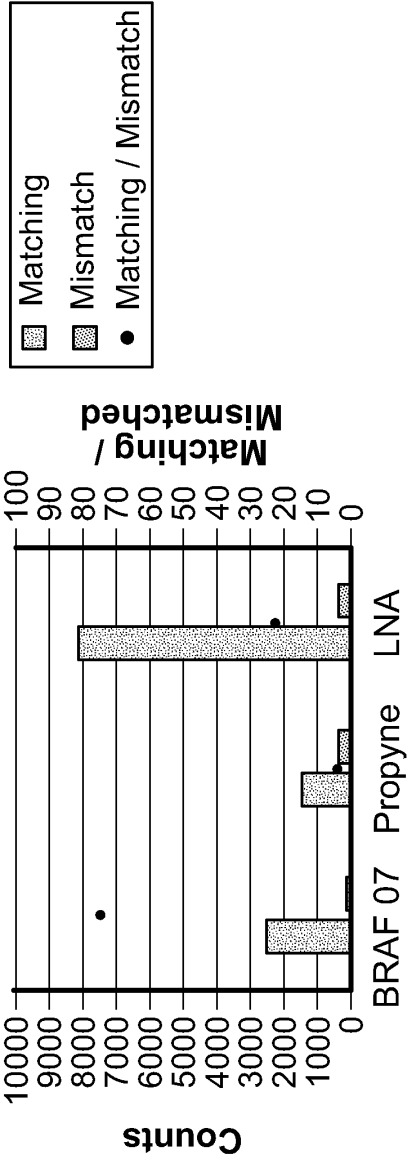
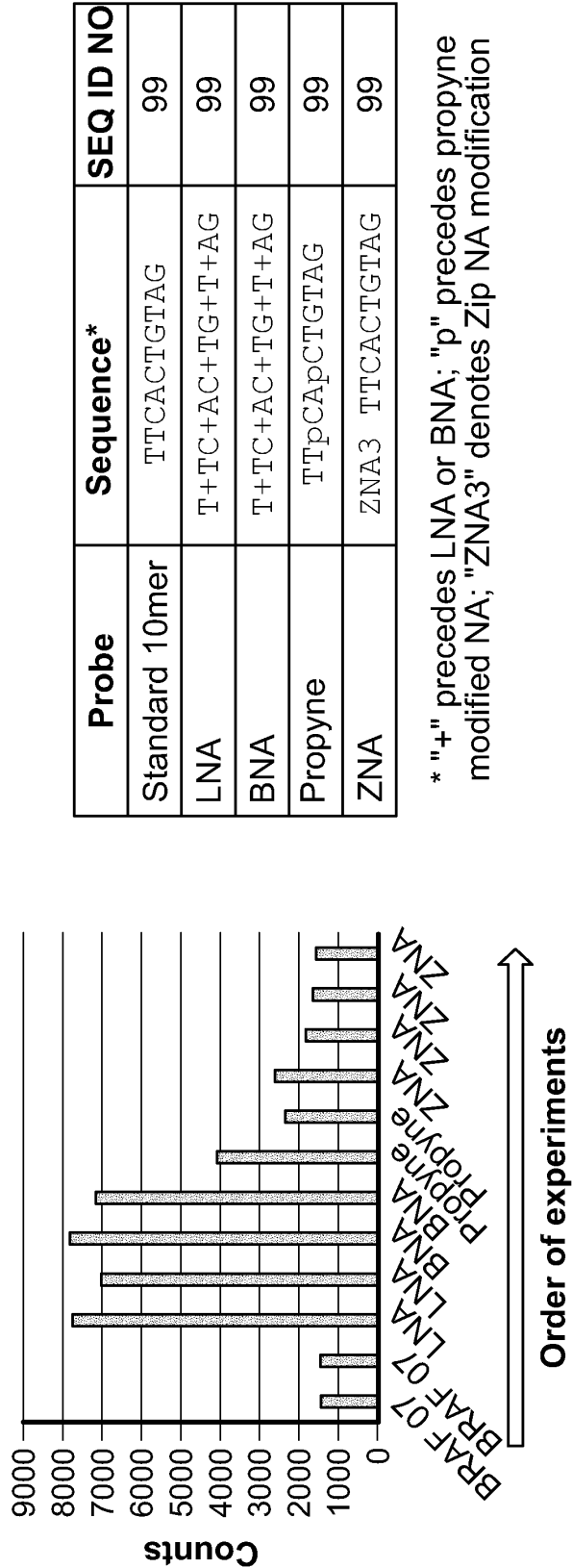
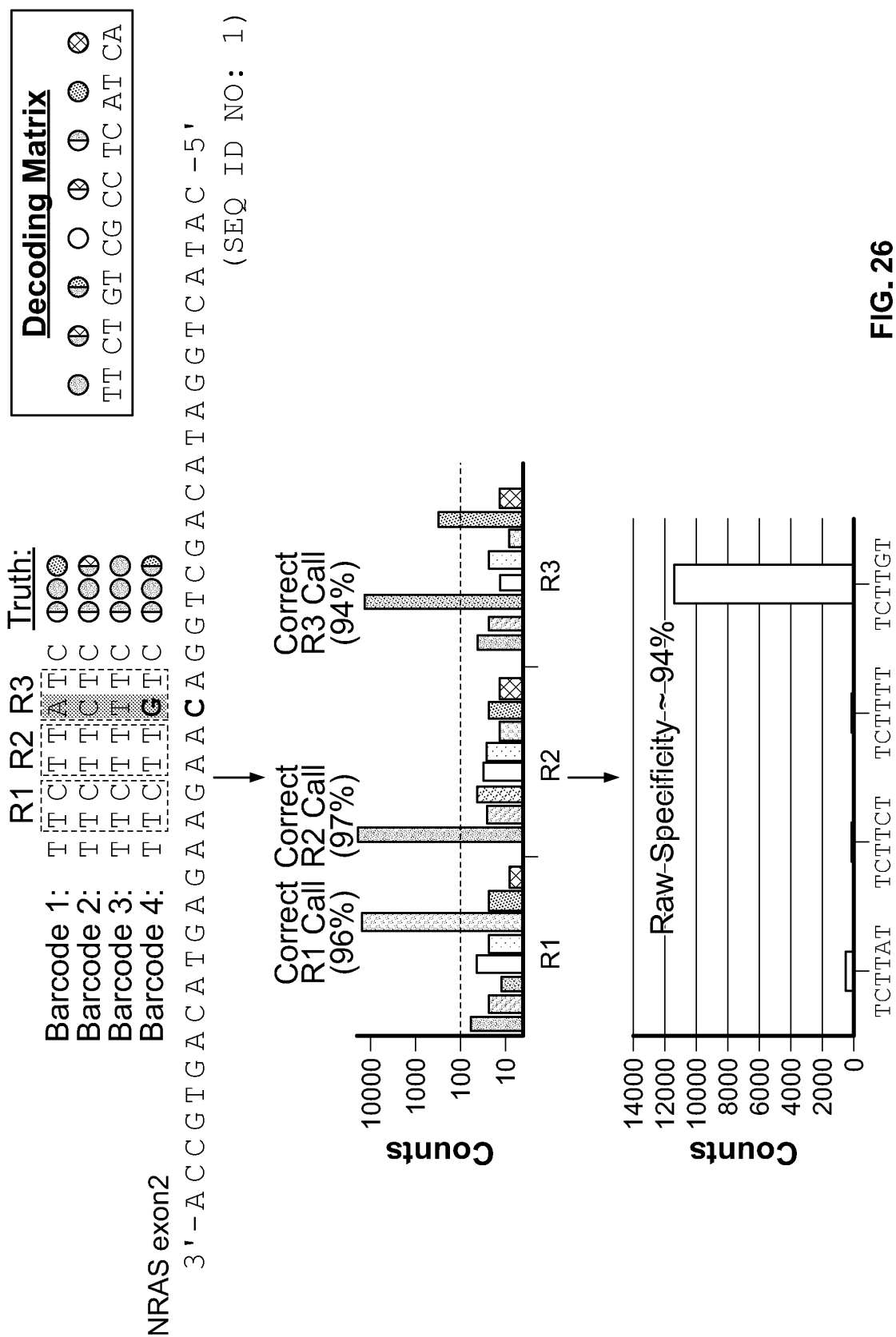


FIG. 25



Hyb & seq chemistry using dual-color reporters has low raw error rate and the source of error can be classified into two areas

Decoding Matrix			
TT	CT	GT	CG
CC	TC	AT	CA

	R1	R2	R3	Truth:
Barcode 1:	C	T	A	T
Barcode 2:	C	T	T	C
Barcode 3:	C	T	T	C
Barcode 4:	C	T	T	C

	R1	R2	R3
Barcode 5:	T	C	T
Barcode 6:	T	C	T
Barcode 7:	T	C	T
Barcode 8:	T	C	T

NRAS exon2
3' - ACCGTGACATGAGAGAGAACAGGTCGACATAGGTCATAC - 5' (SEQ ID NO: 1)

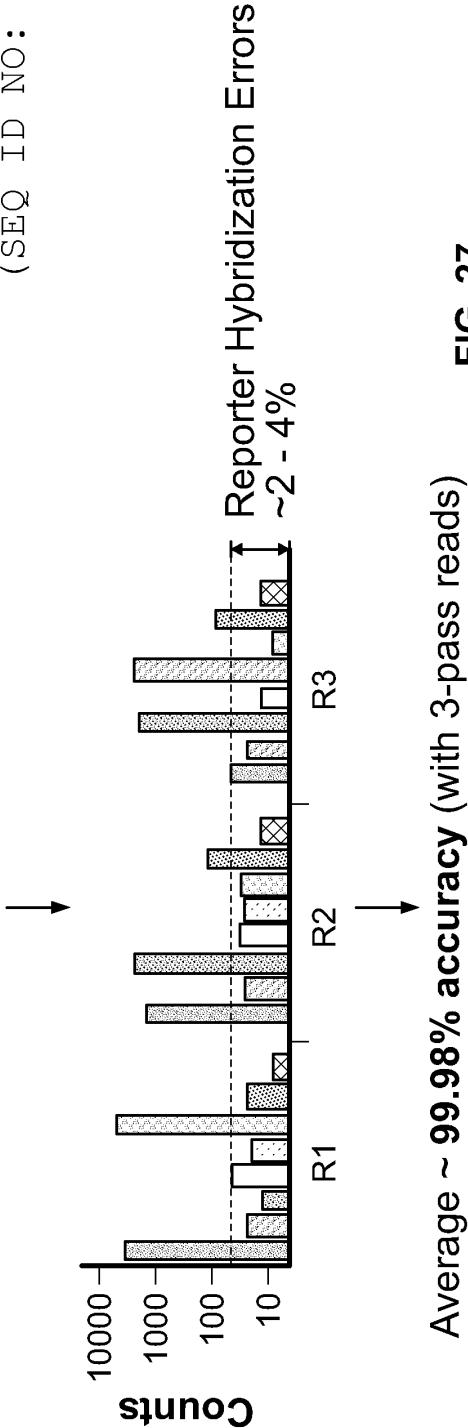


FIG. 27

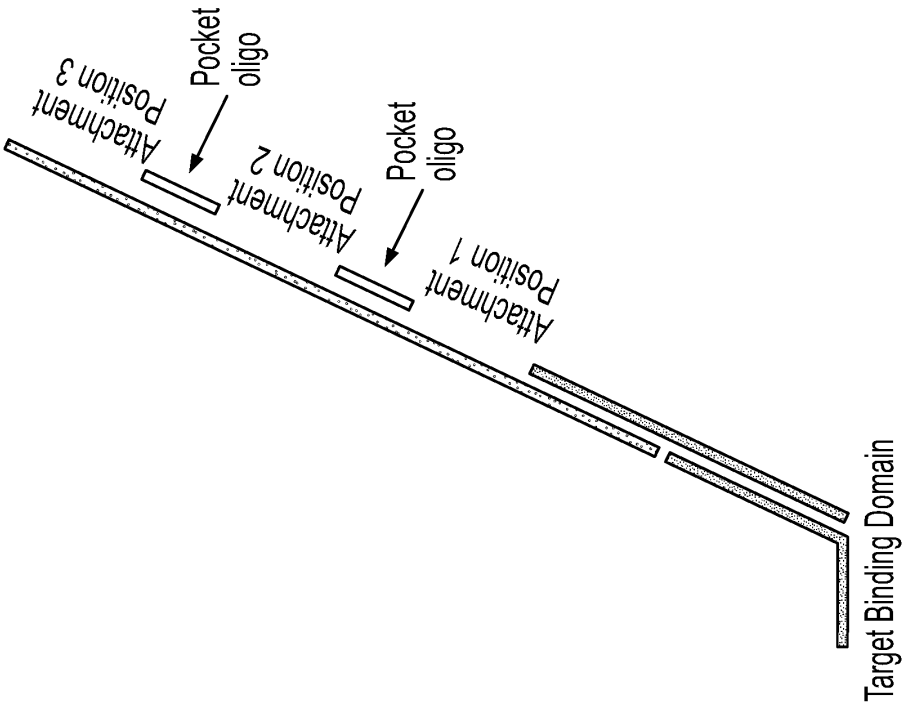


FIG. 28

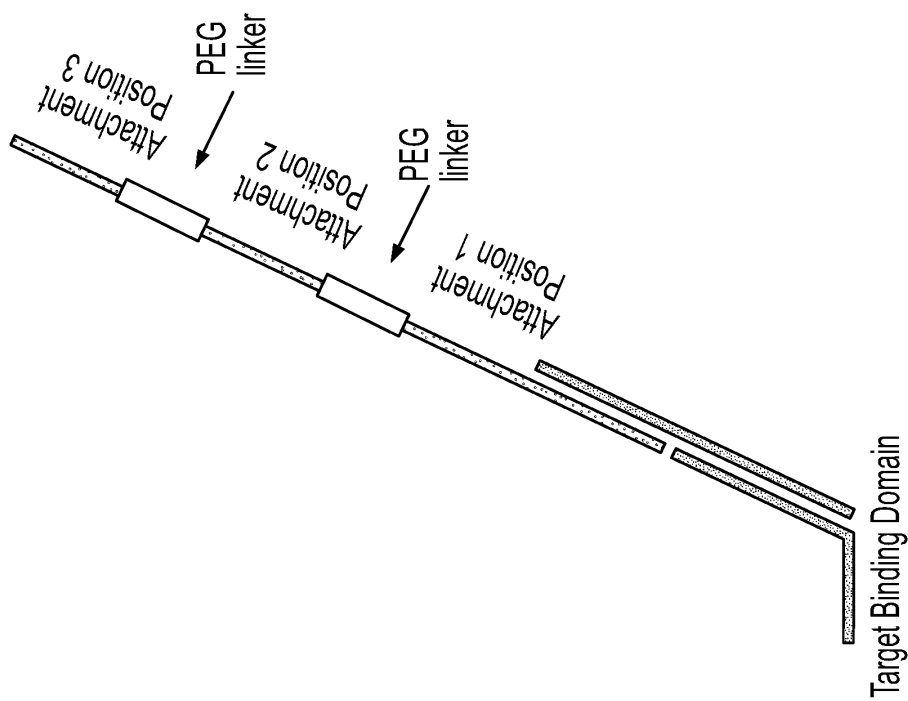


FIG. 29

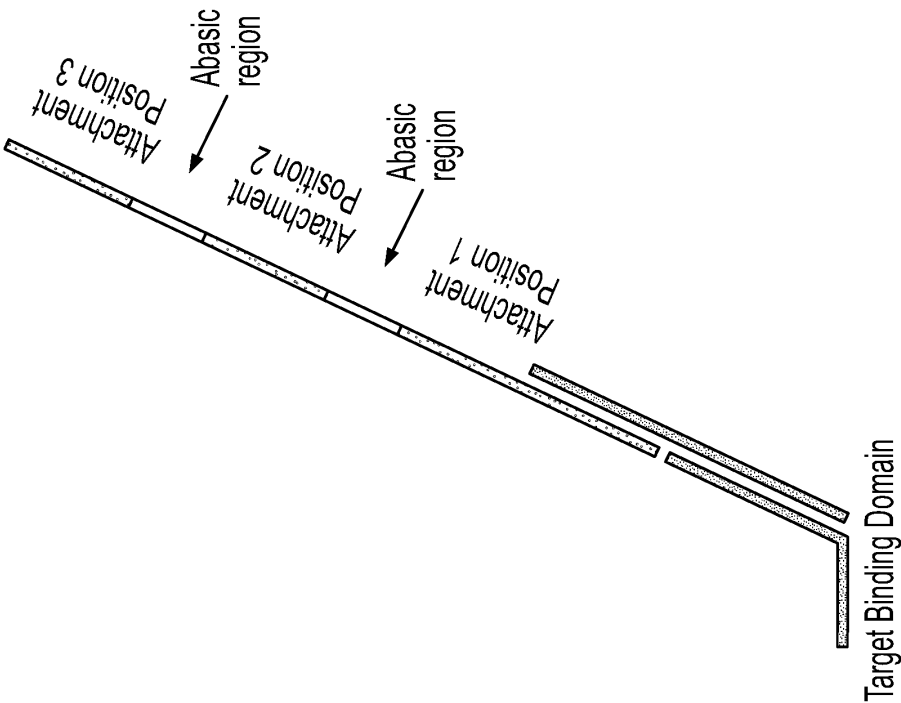


FIG. 30

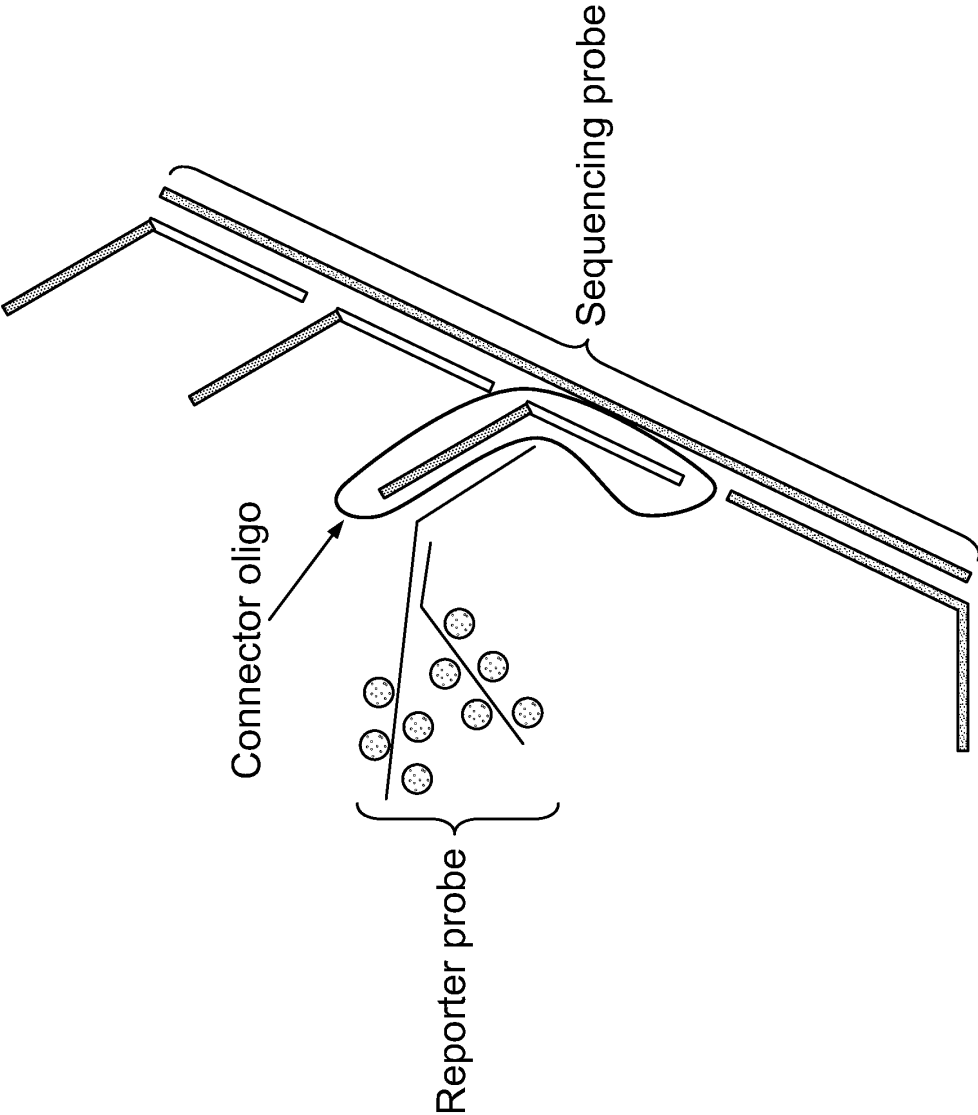


FIG. 31

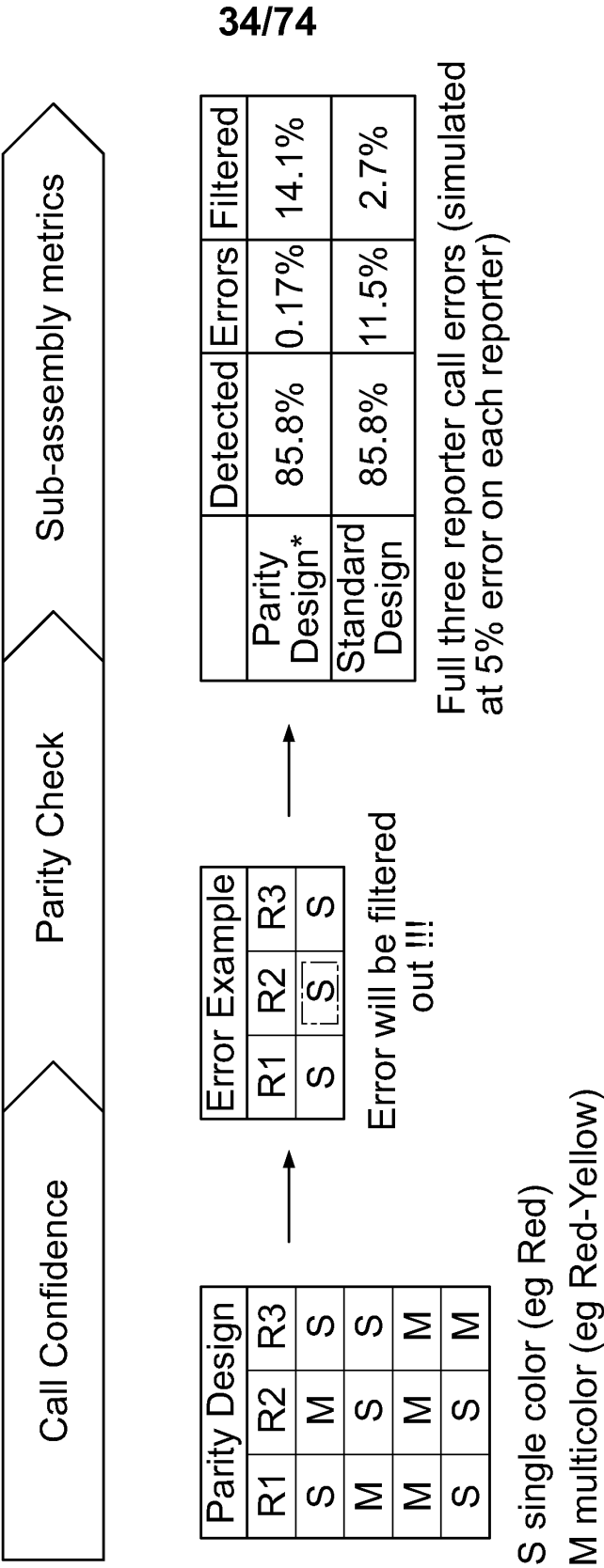


FIG. 32

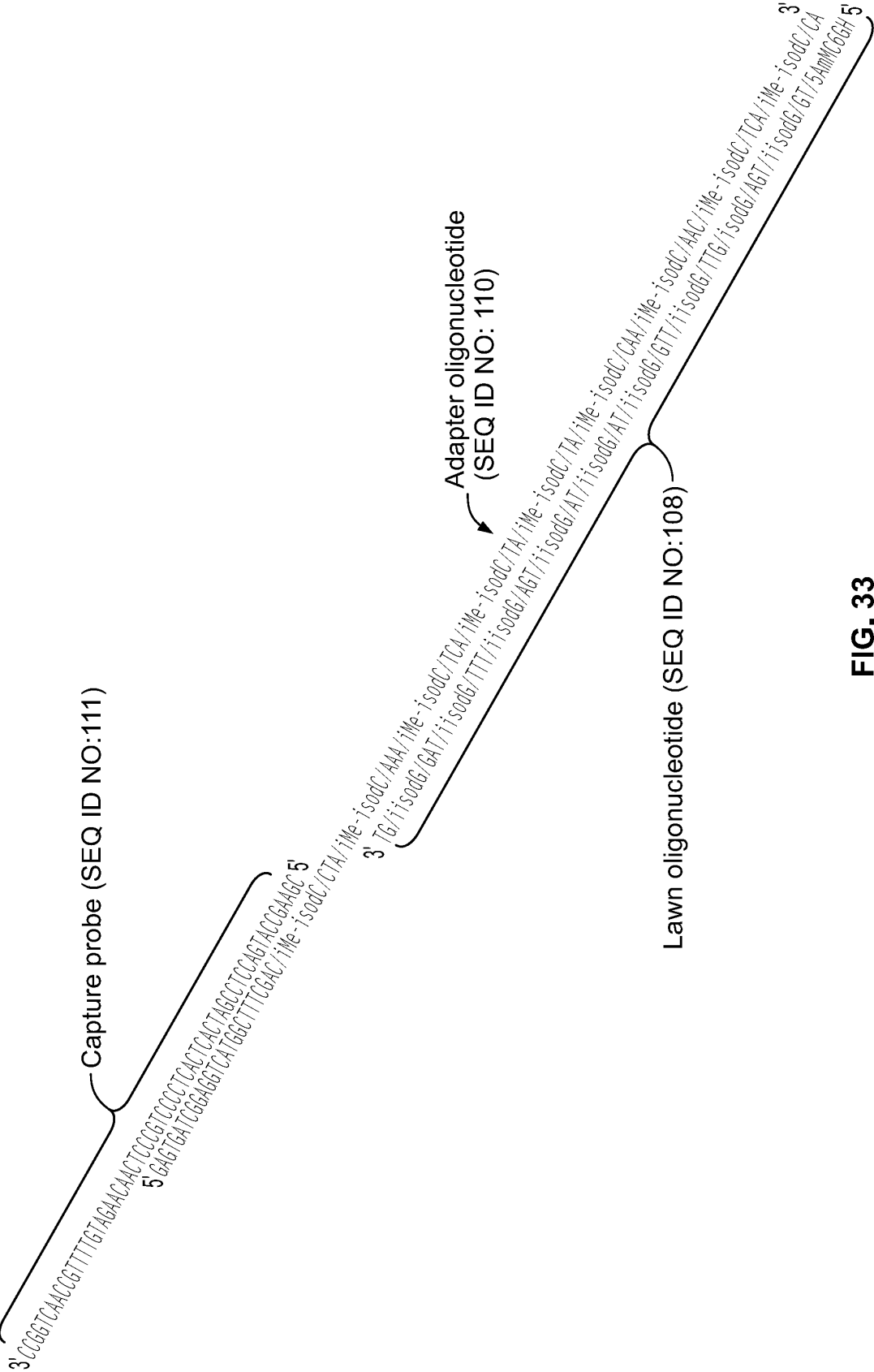


FIG. 33

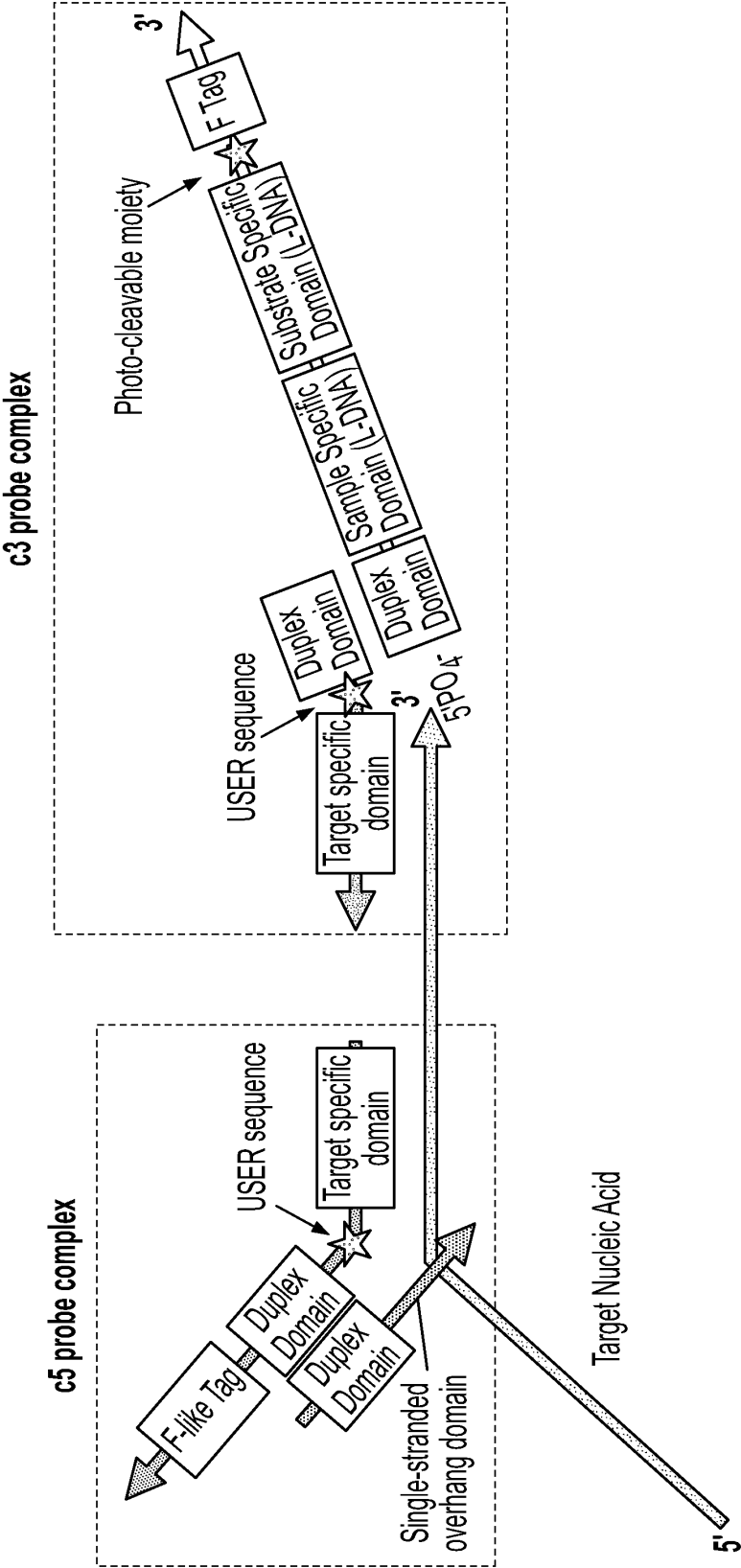


FIG. 34

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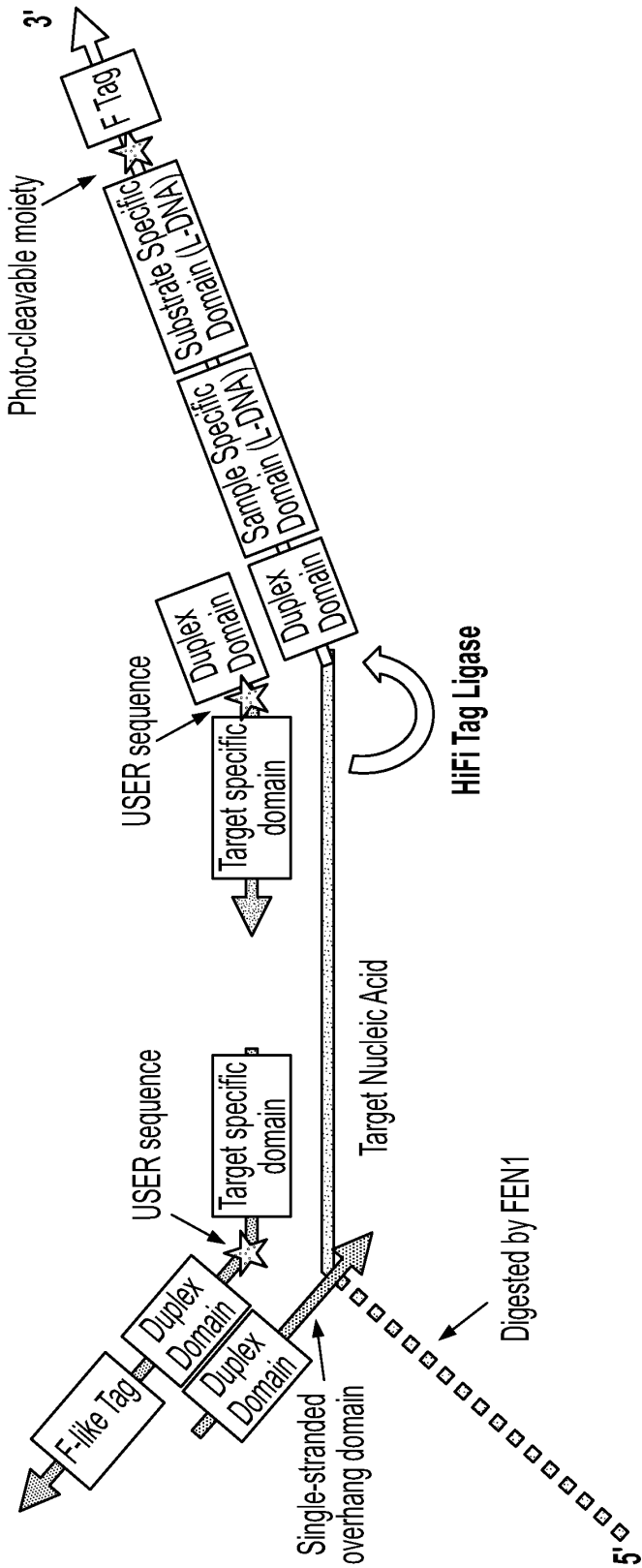


FIG. 35

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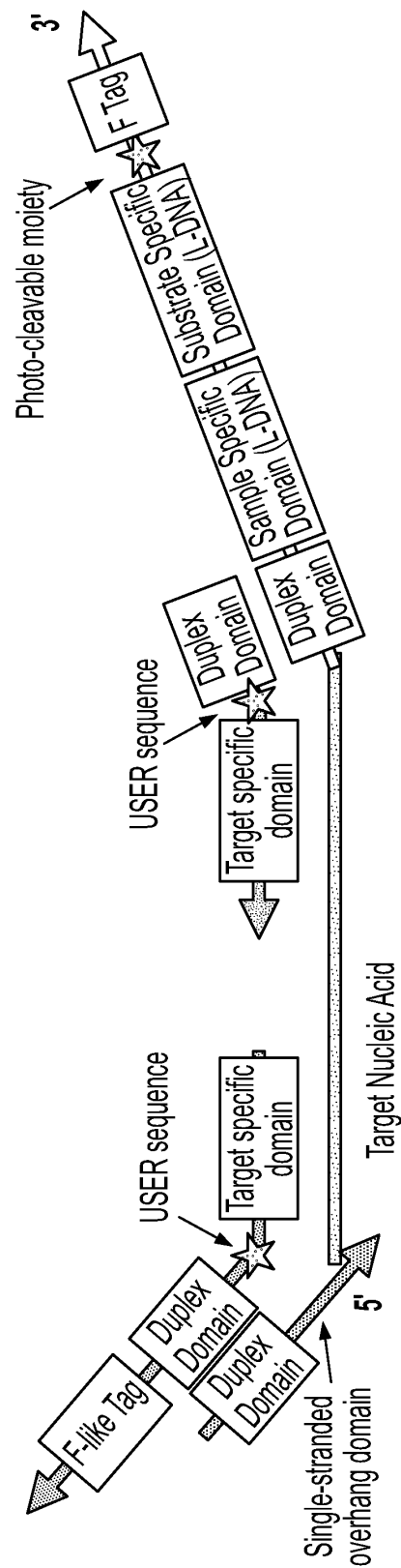


FIG. 36

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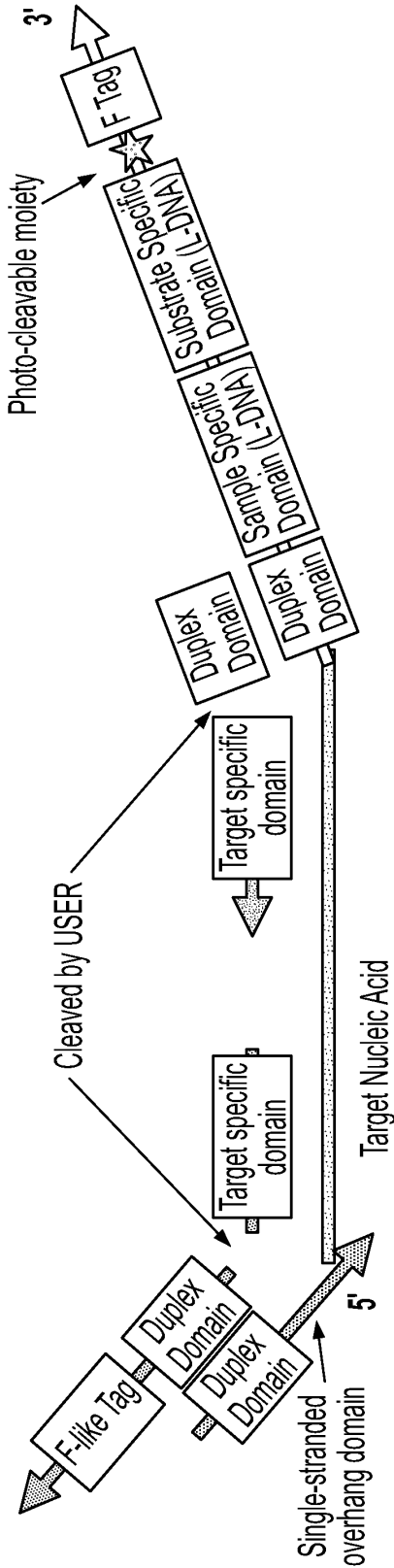


FIG. 37

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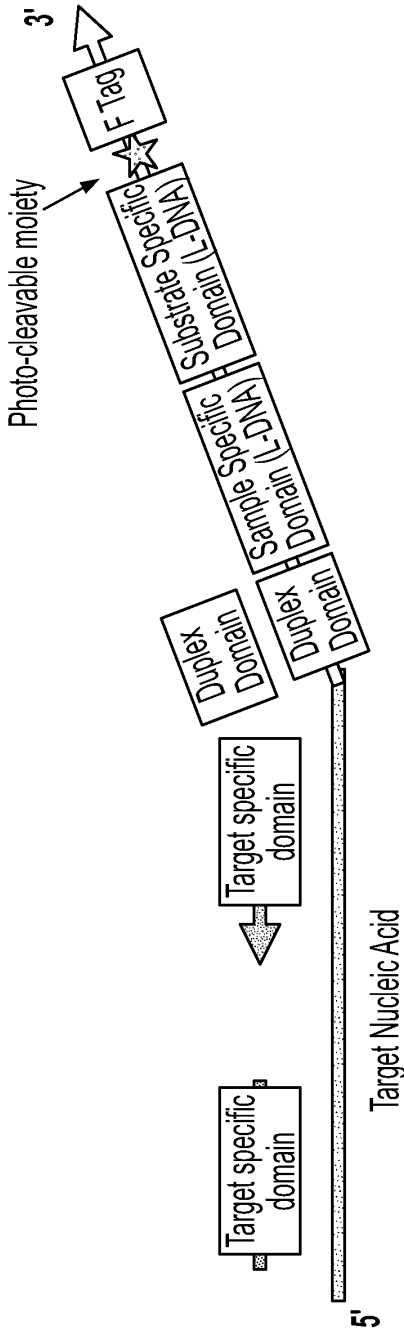


FIG. 38

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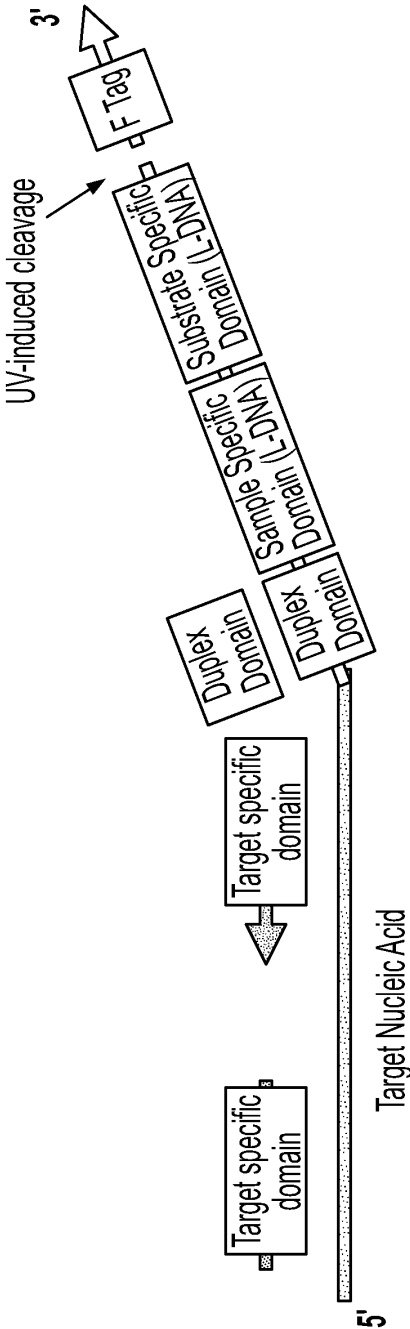


FIG. 39

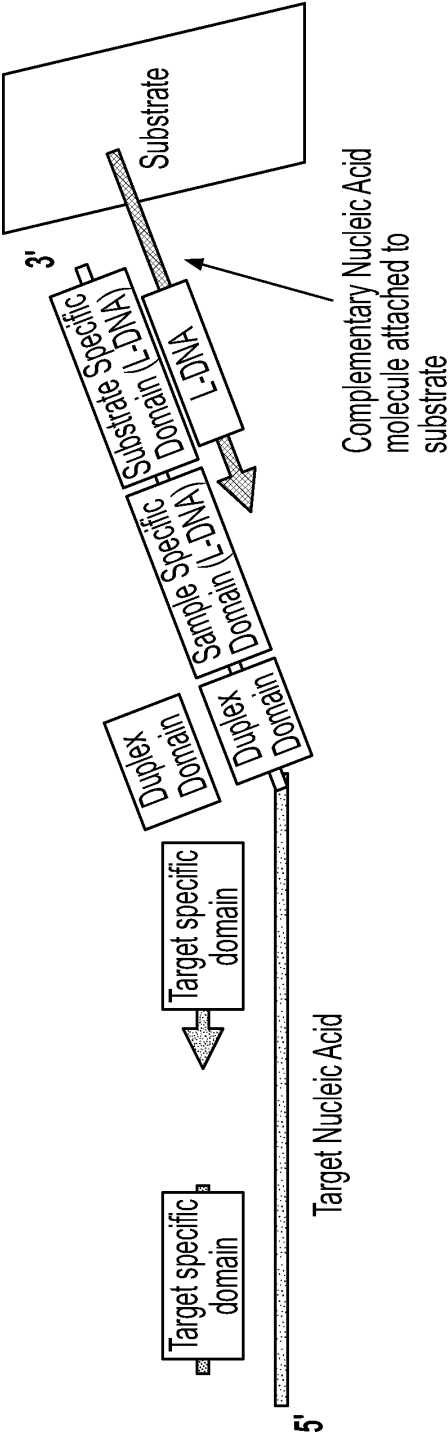


FIG. 40

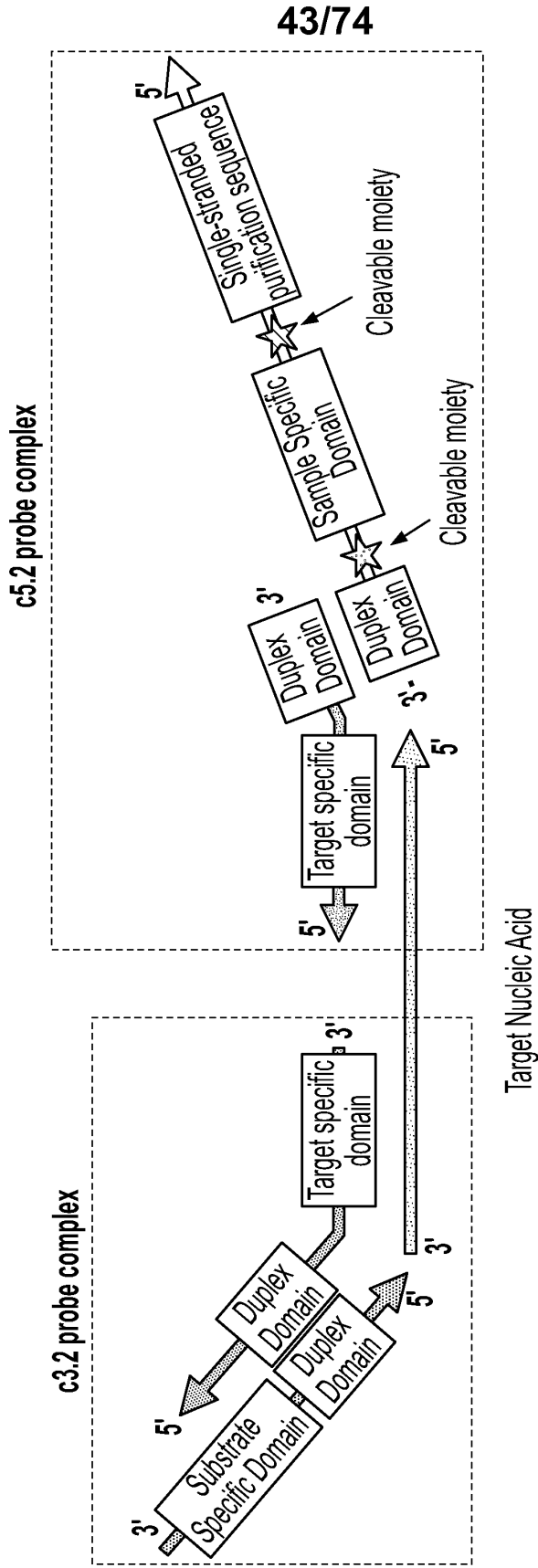


FIG. 41

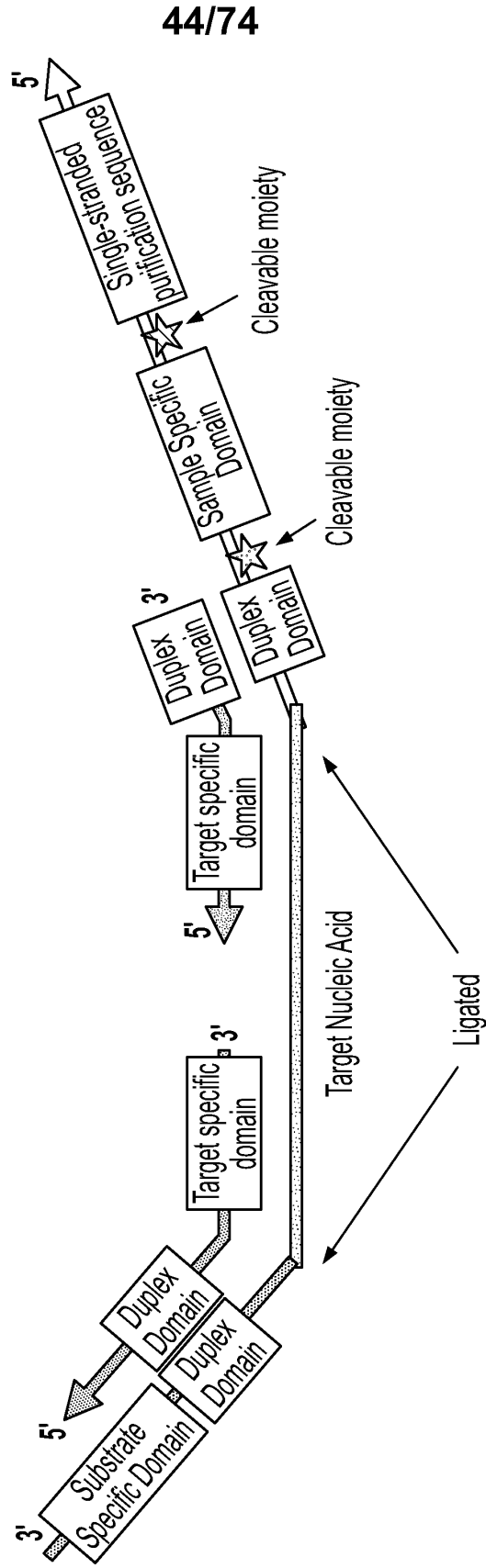


FIG. 42

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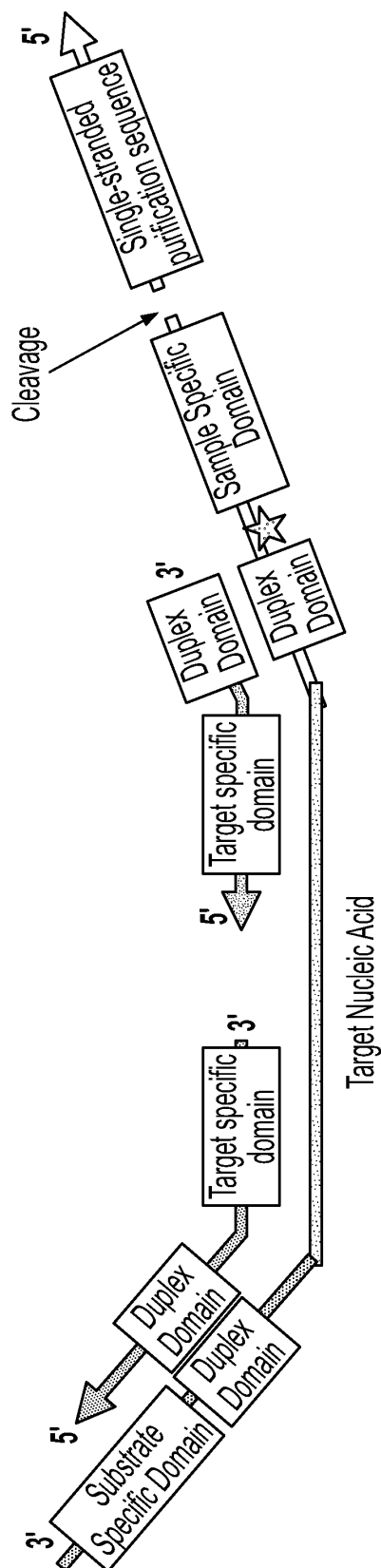


FIG. 43

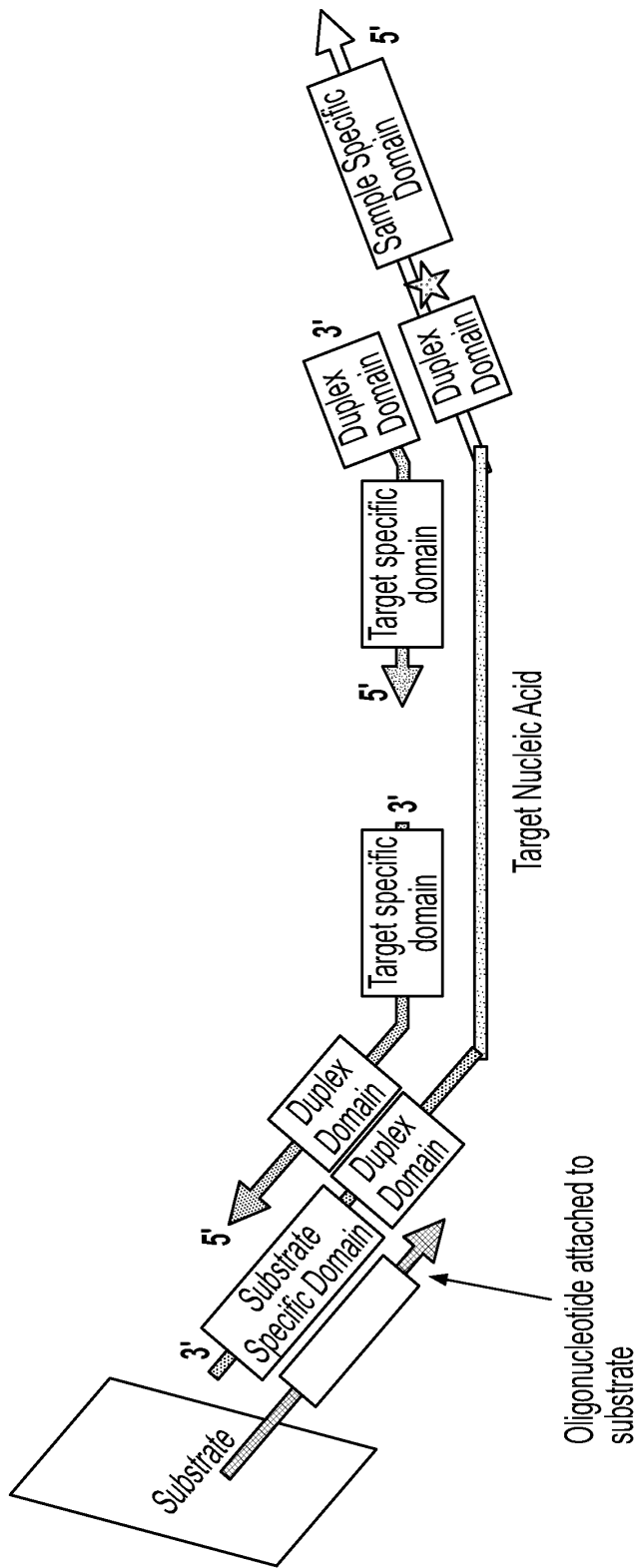


FIG. 44

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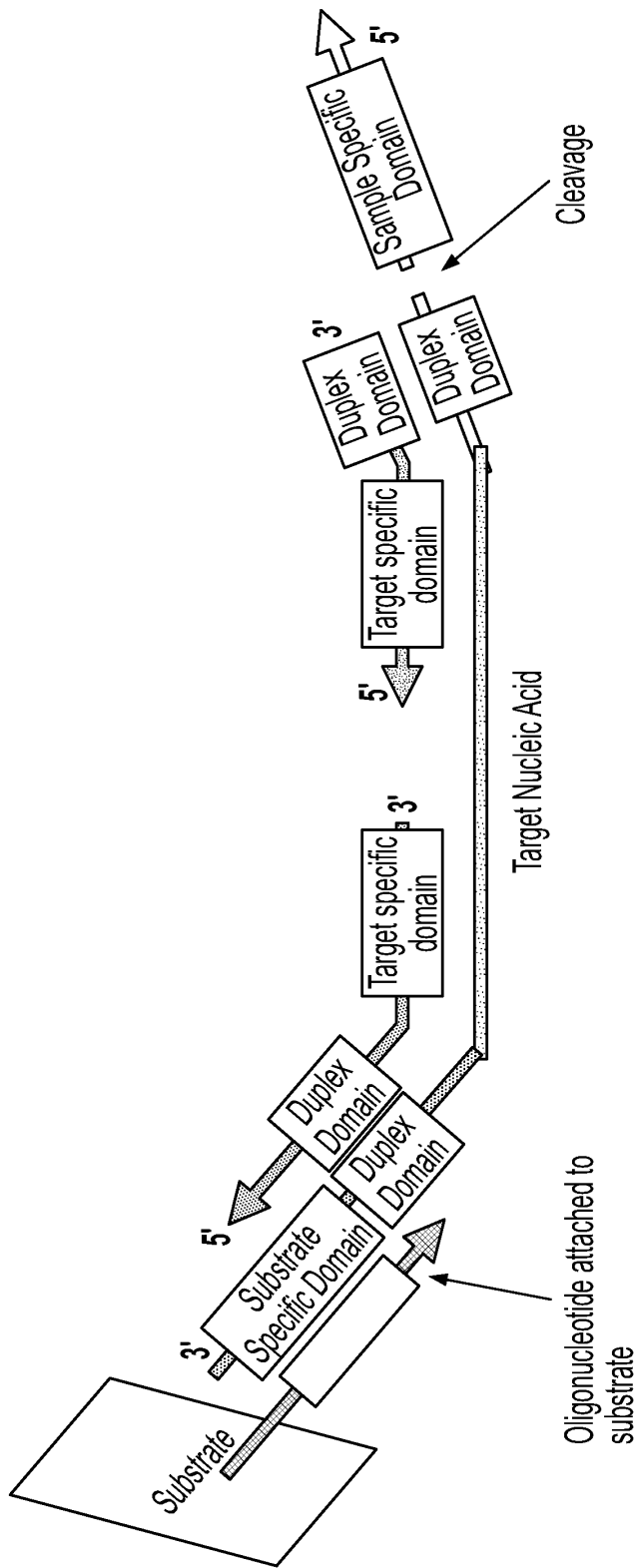


FIG. 45

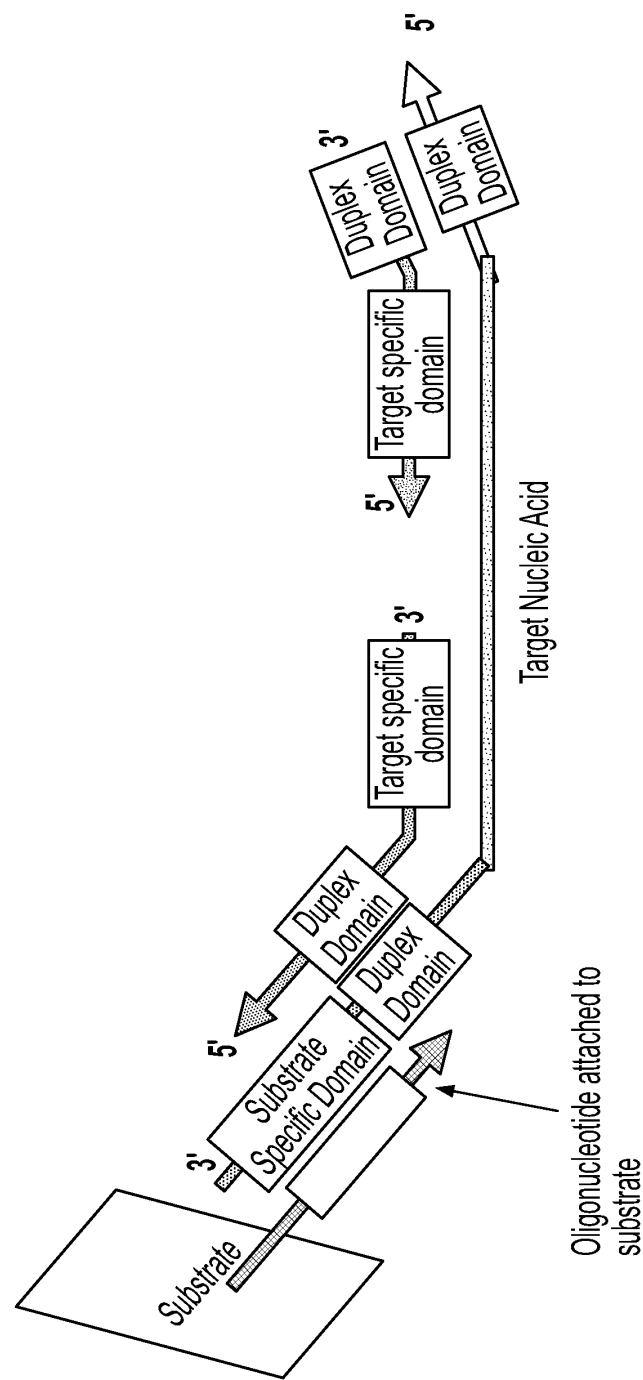


FIG. 46

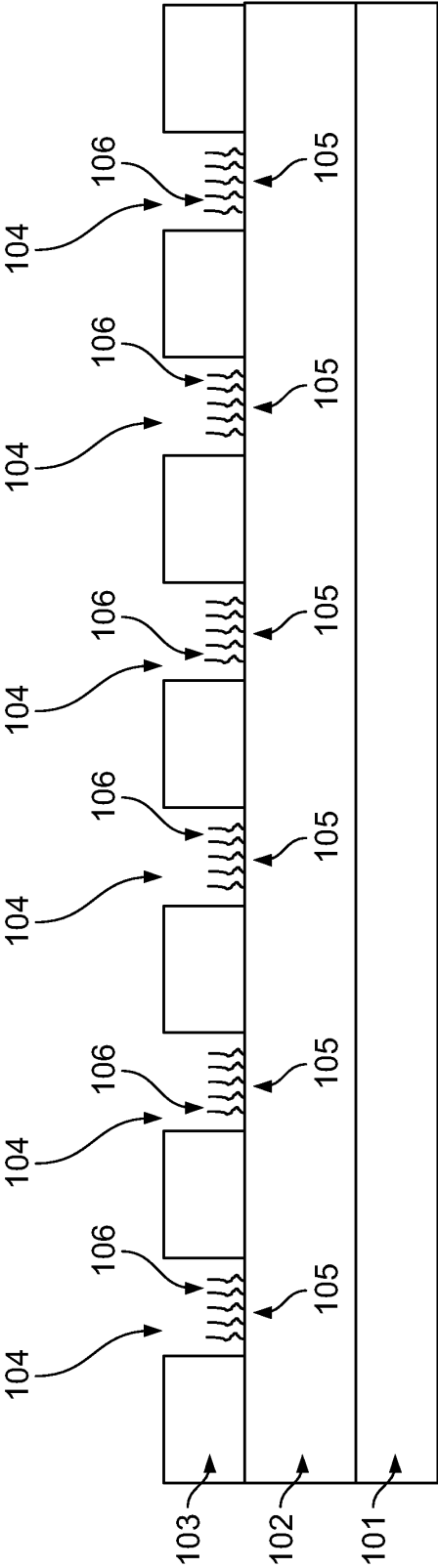


FIG. 47

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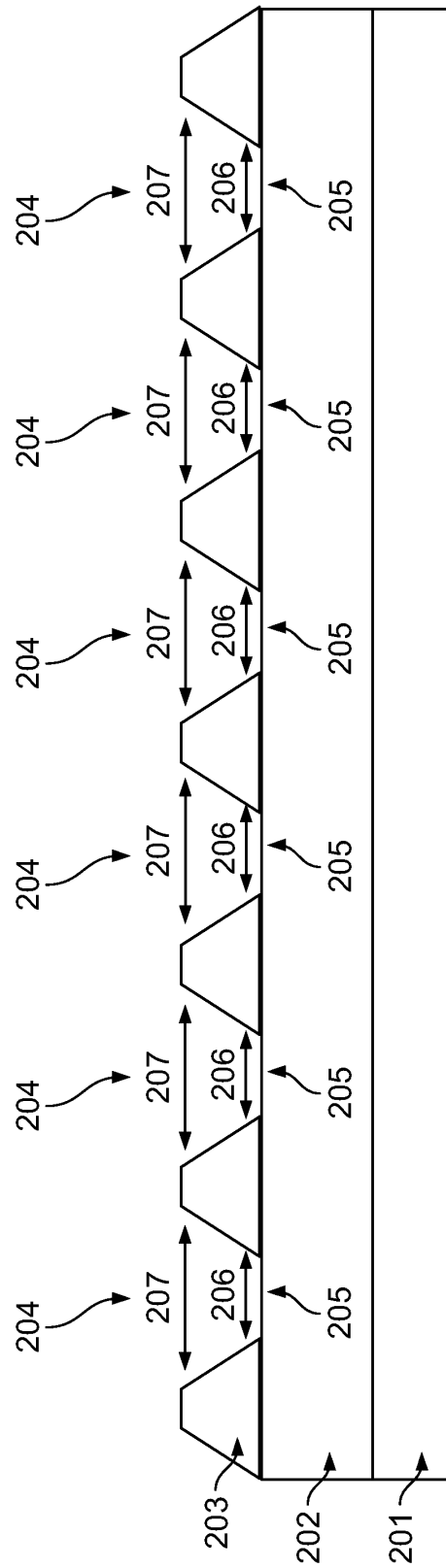
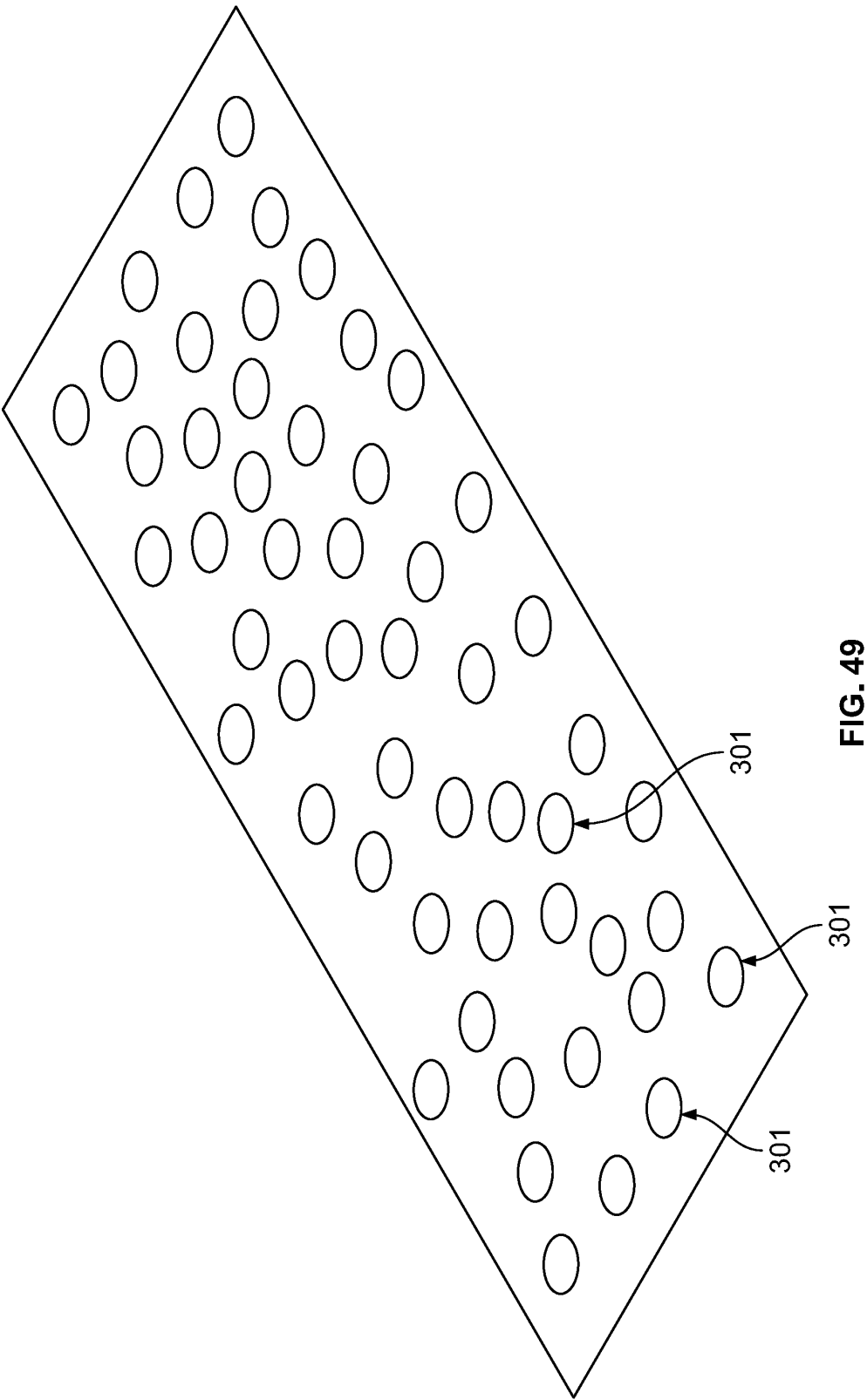


FIG. 48

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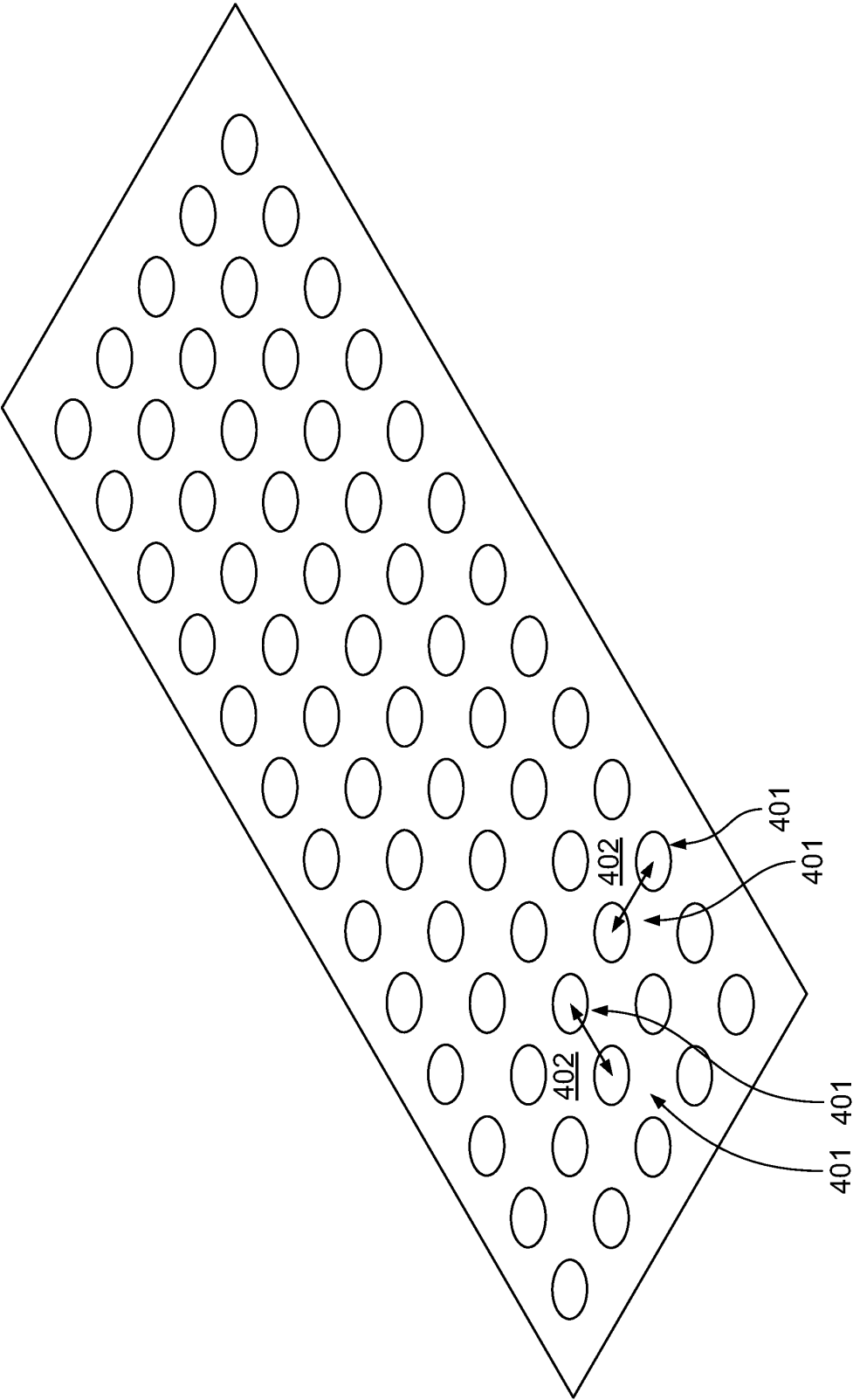


FIG. 50

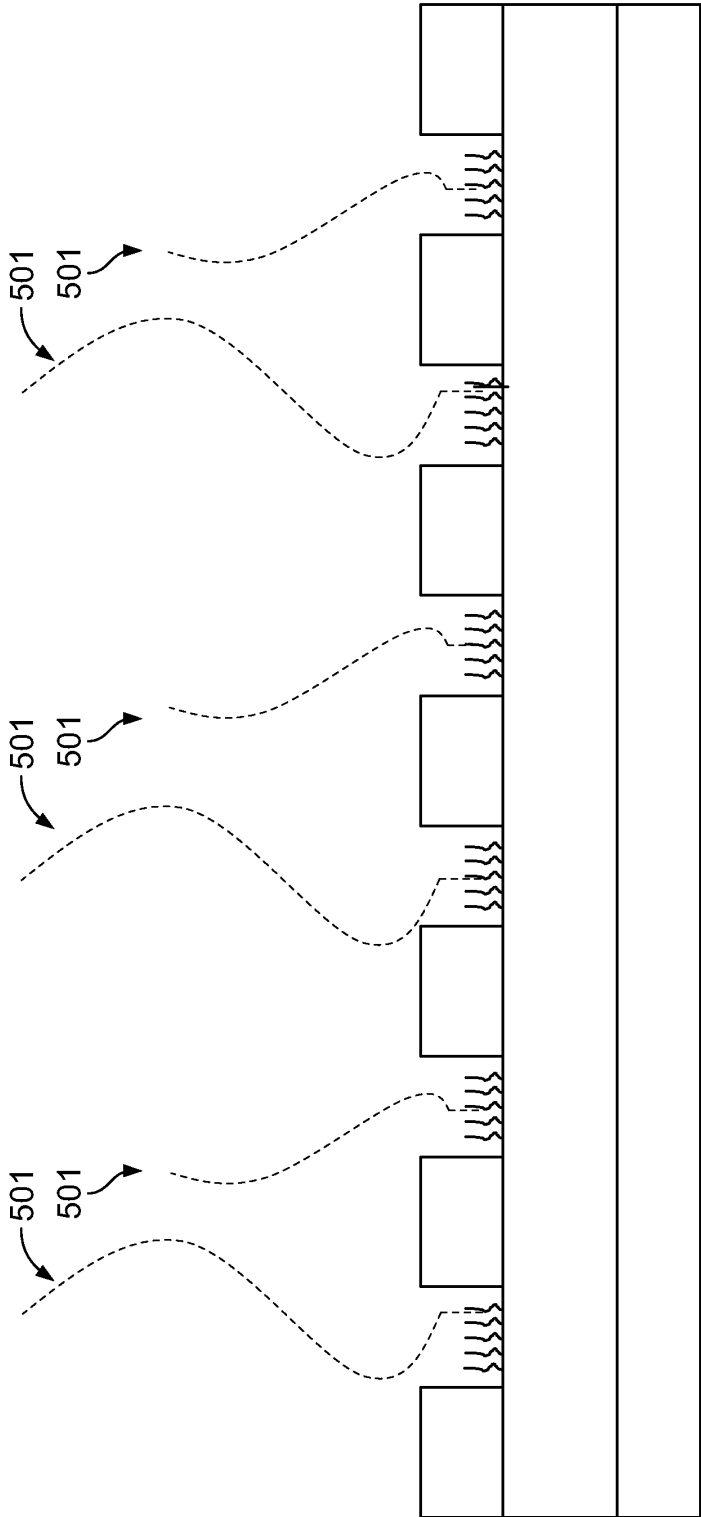


FIG. 51

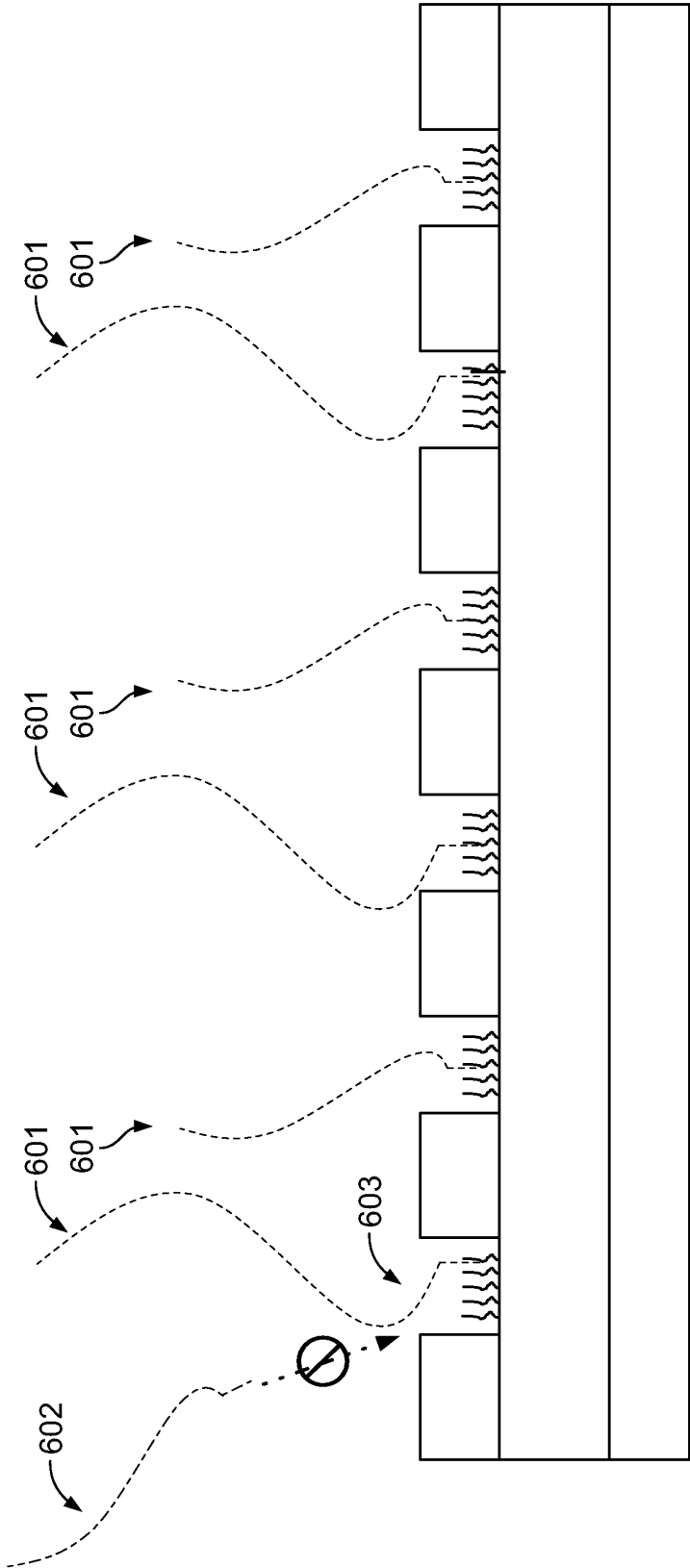


FIG. 52

FIG. 53

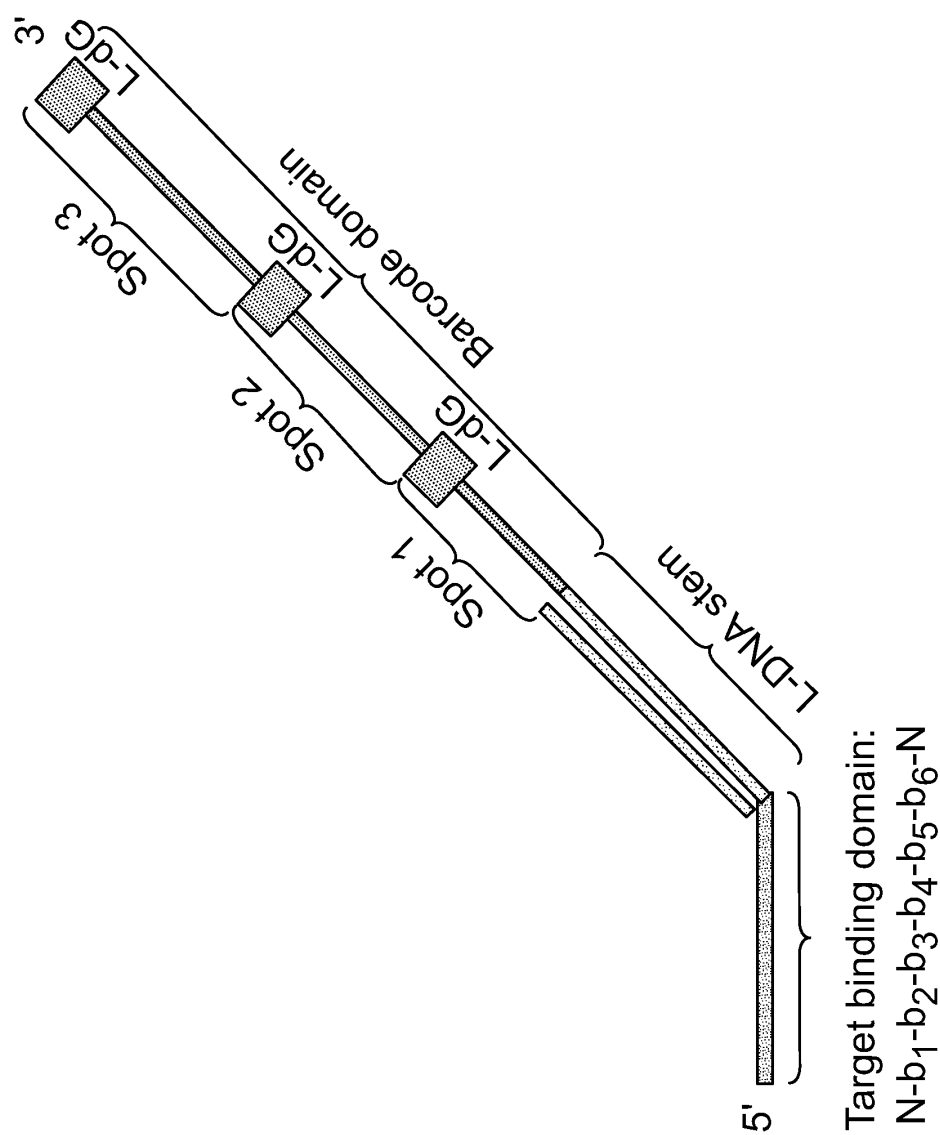
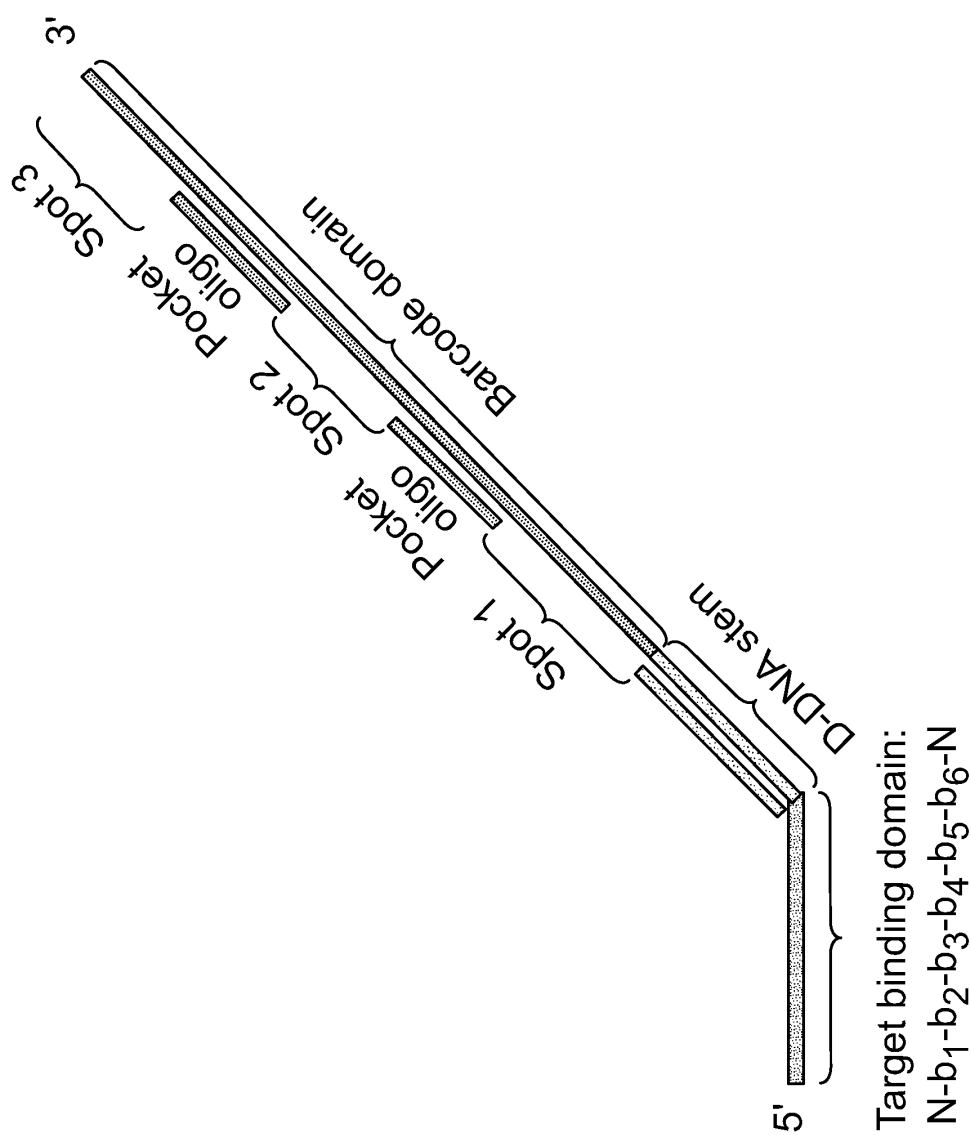


FIG. 54



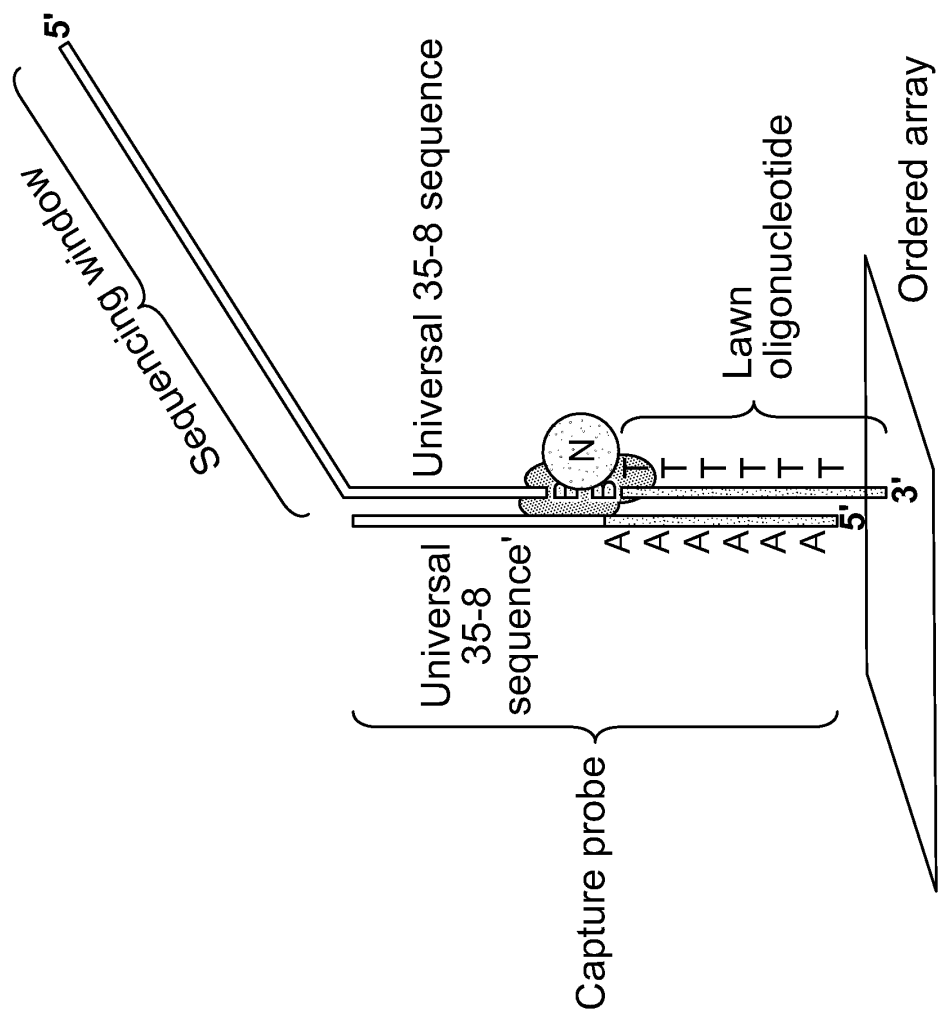


FIG. 55

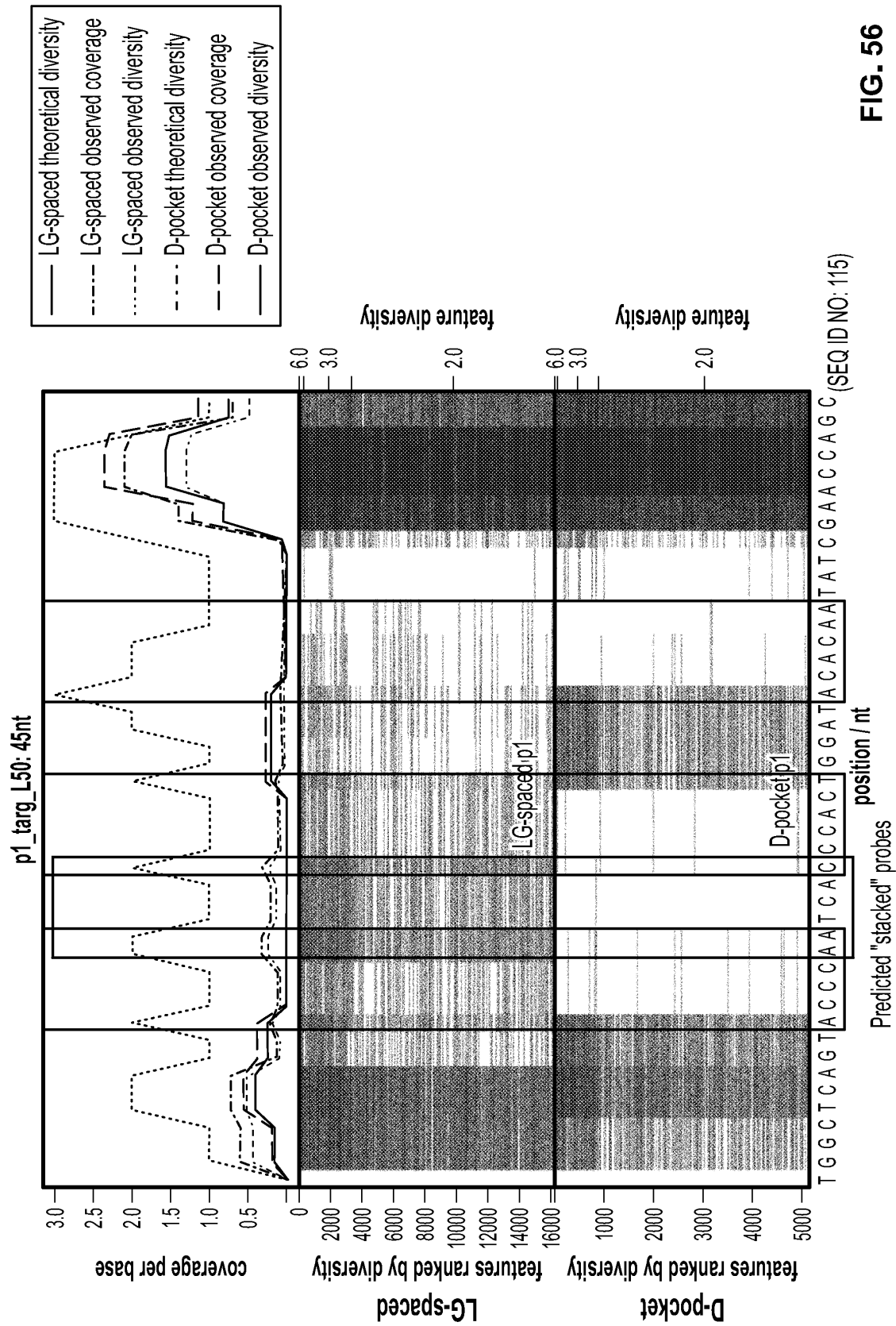
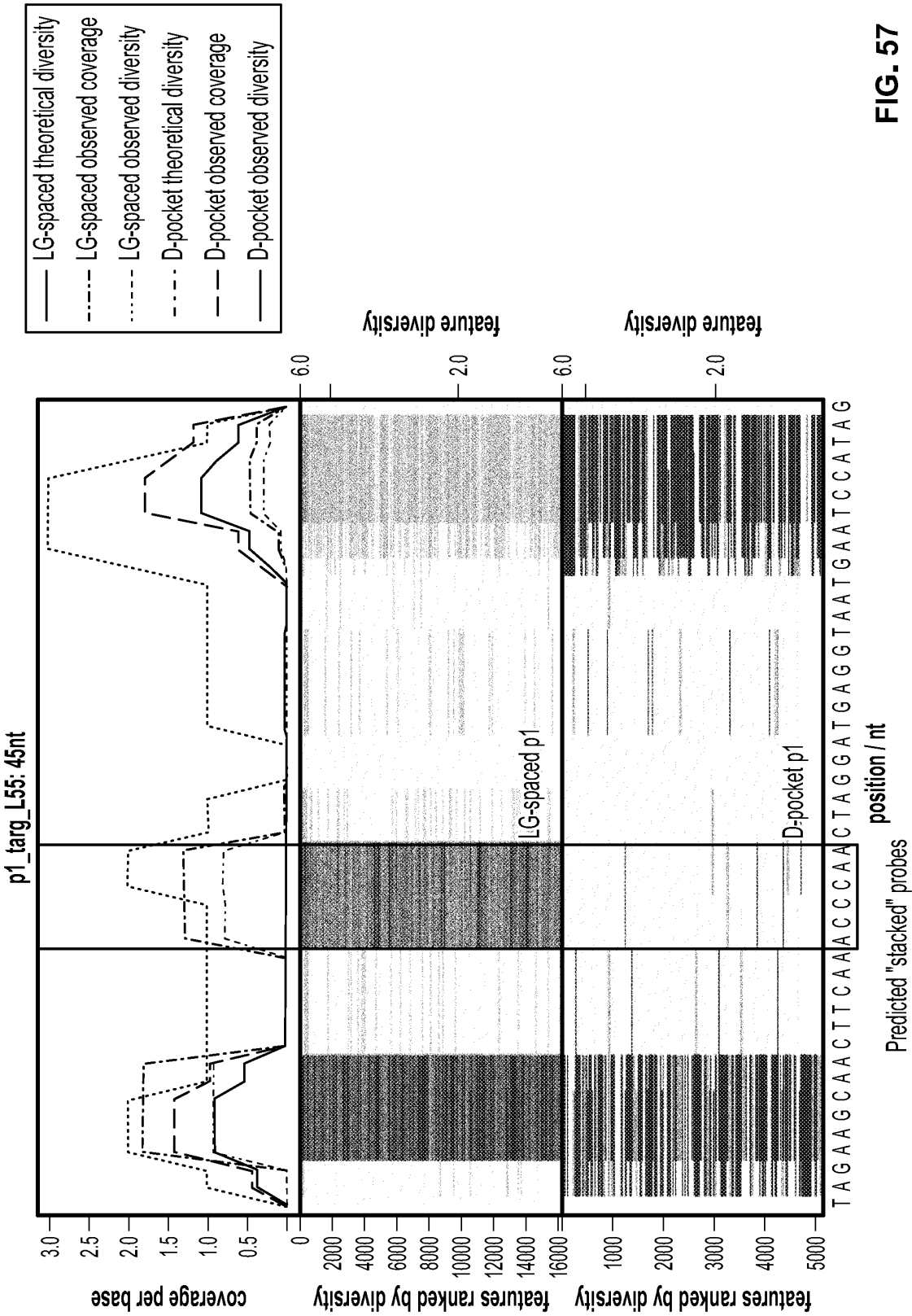


FIG. 56

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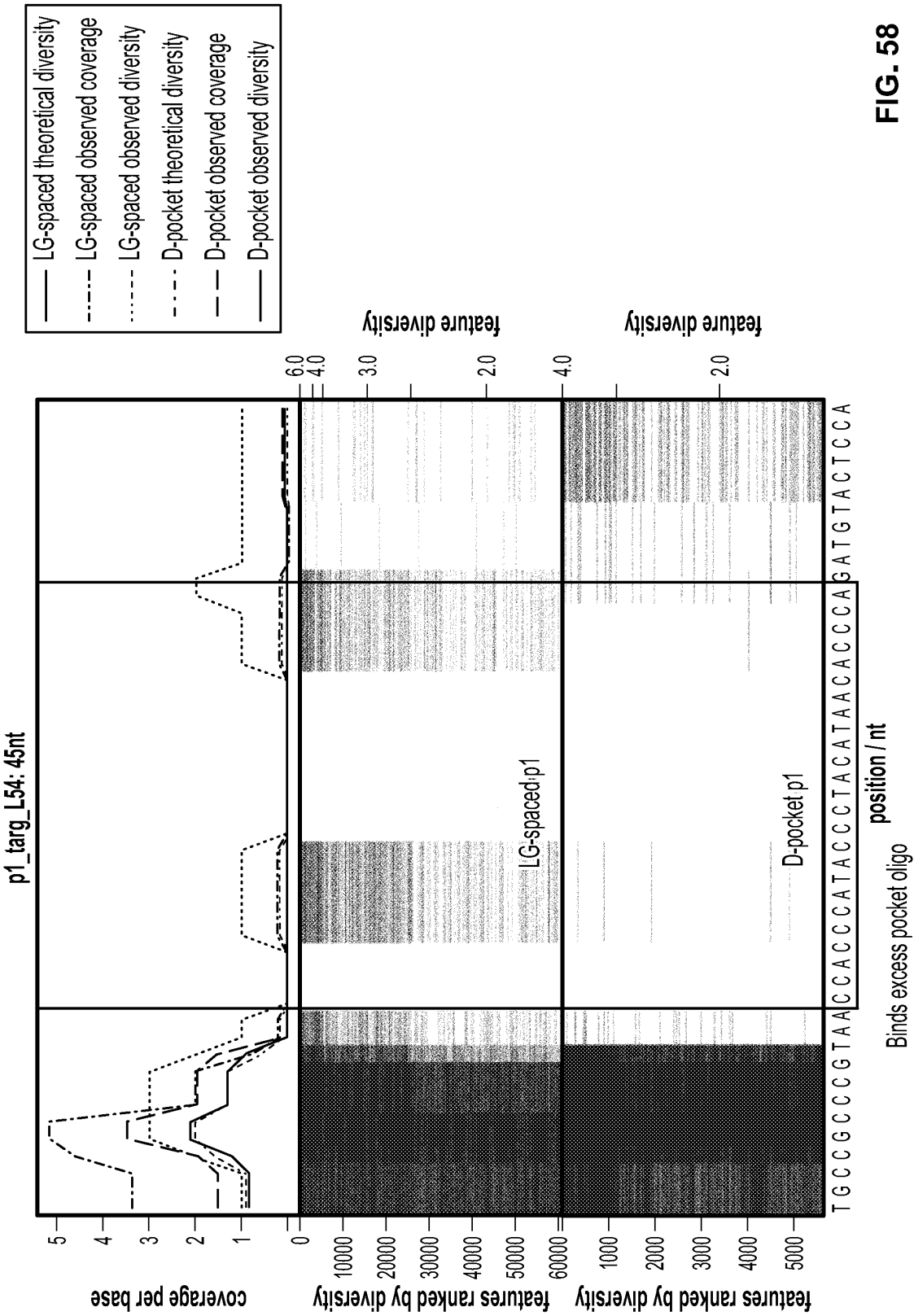


FIG- 58

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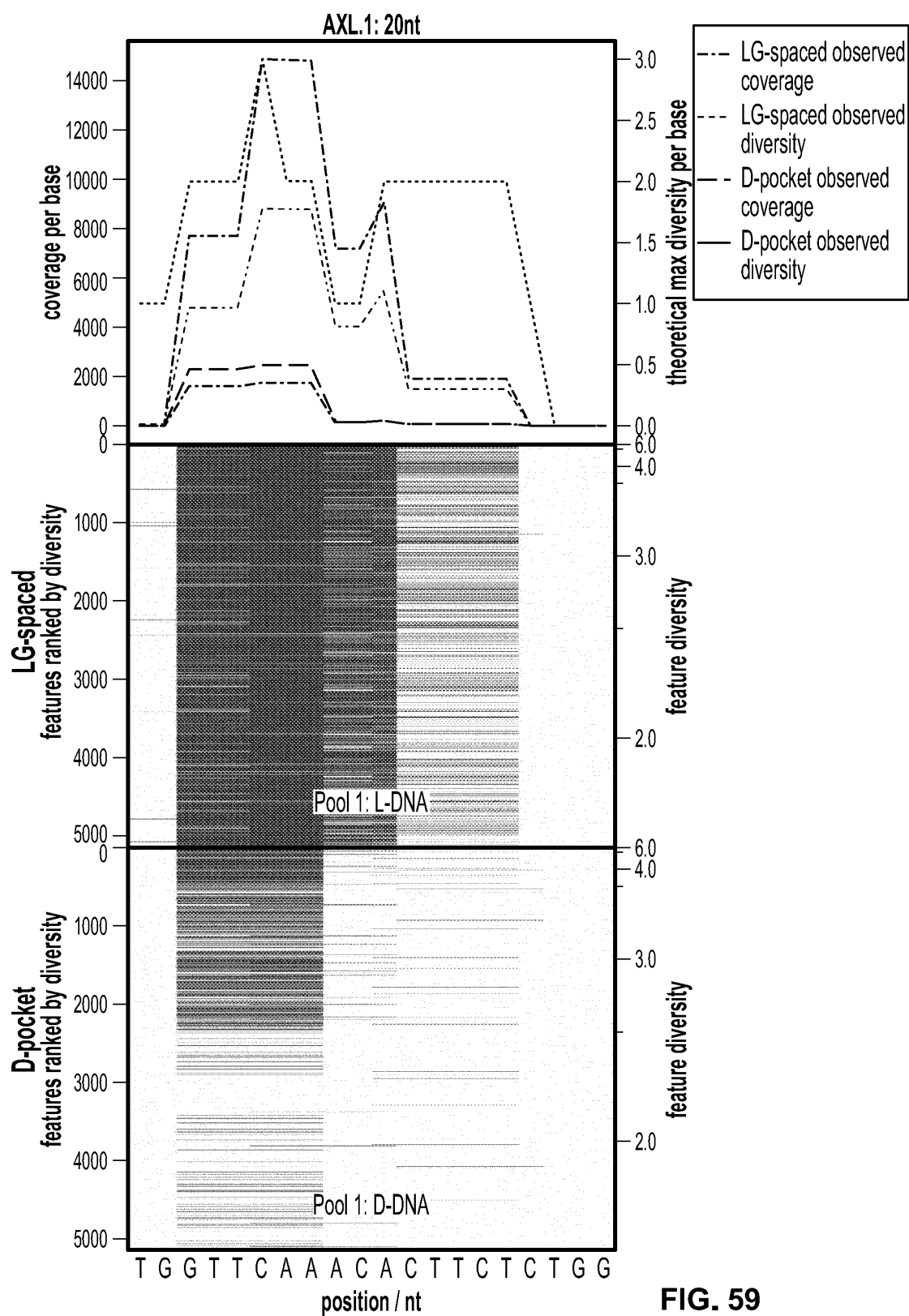


FIG. 59

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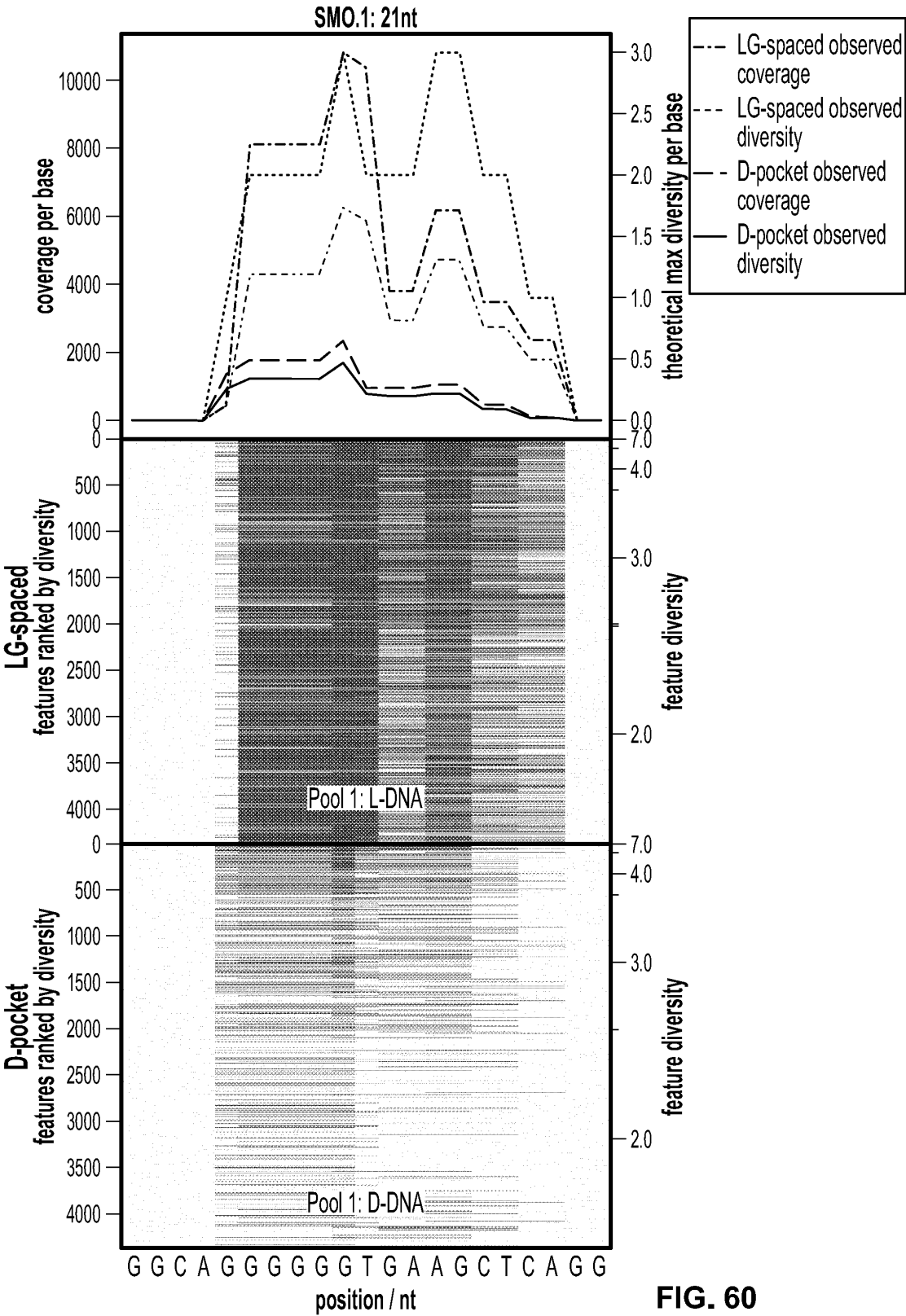


FIG. 60

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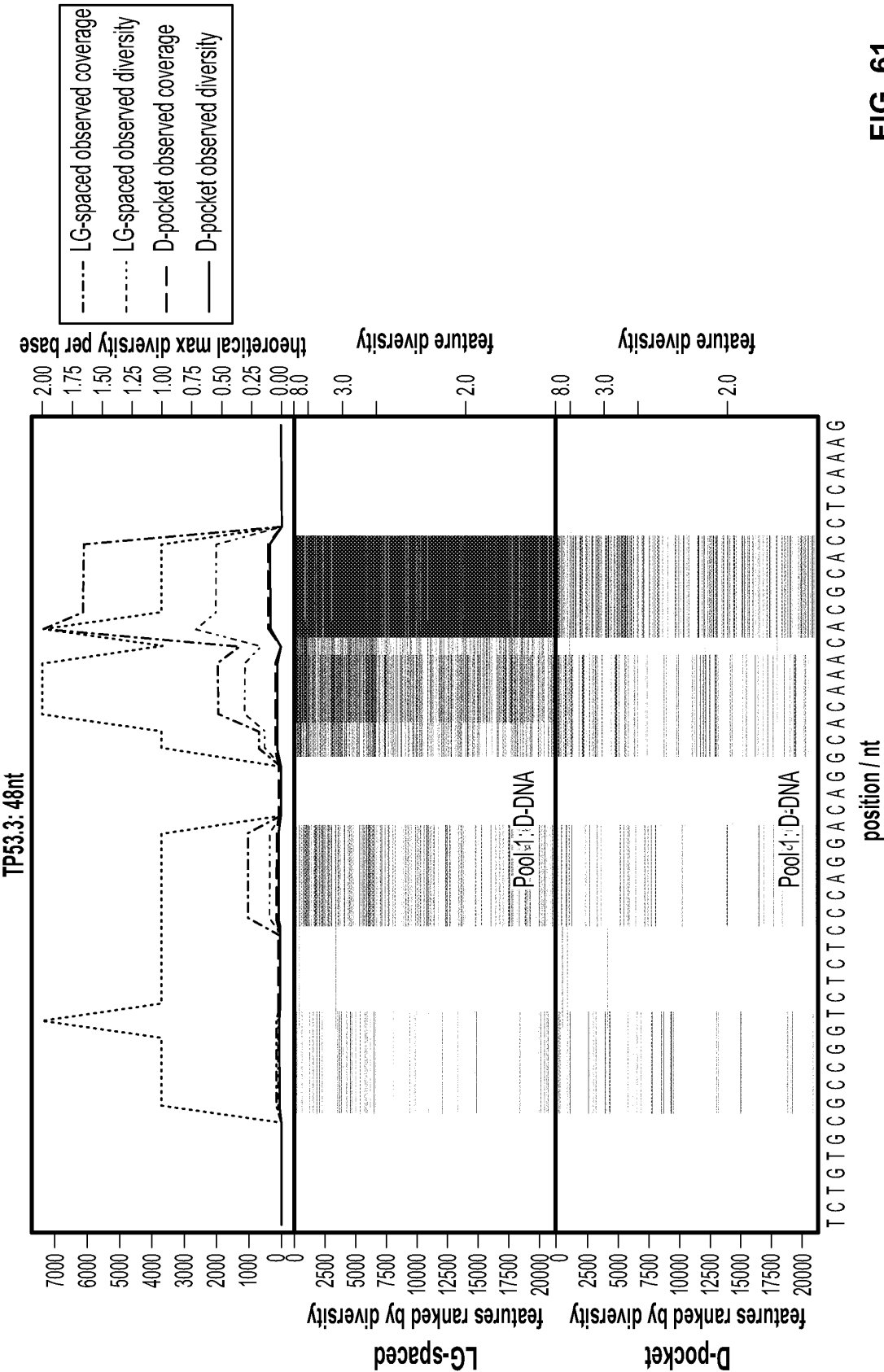


FIG. 61

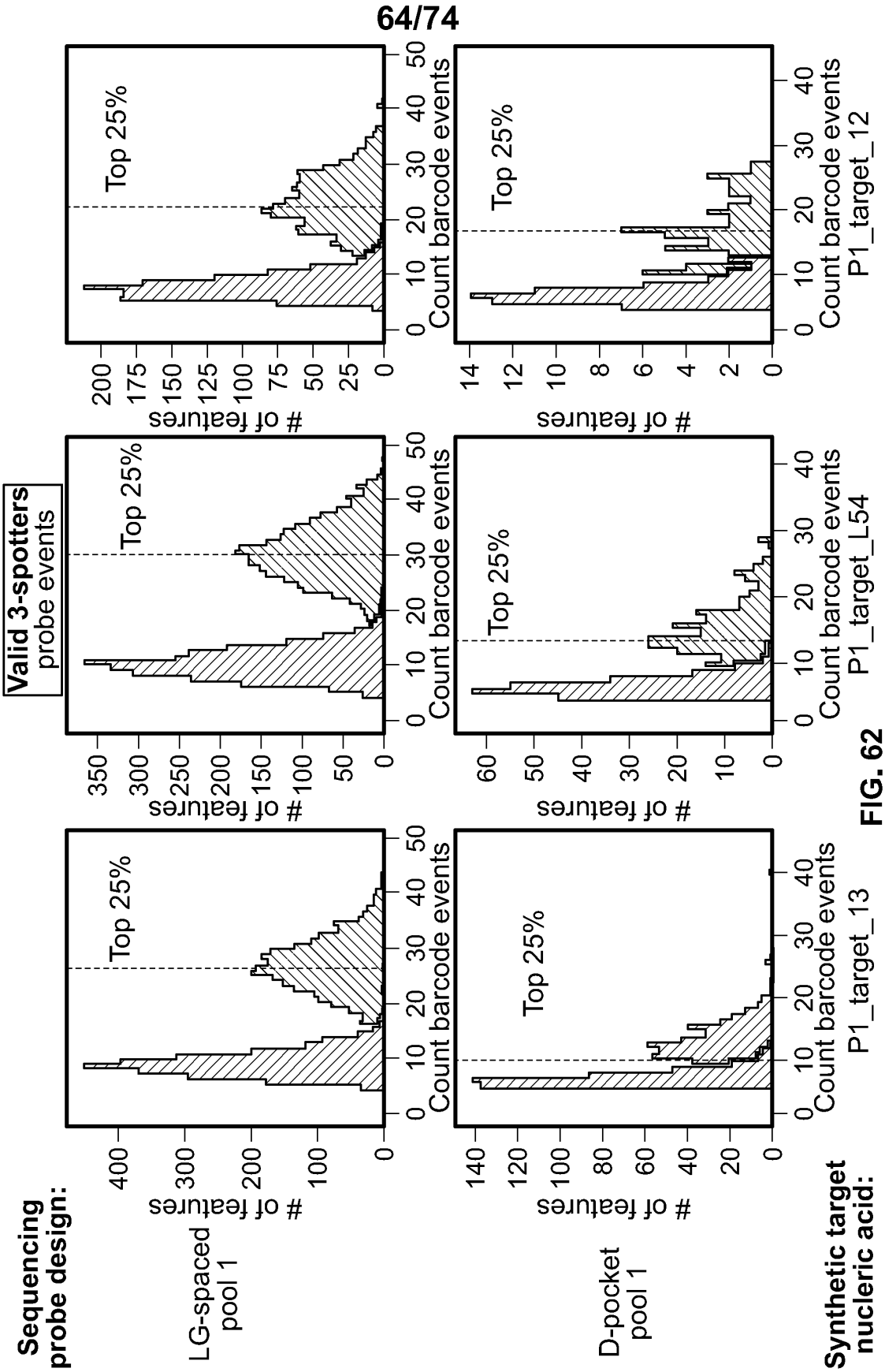


FIG. 62

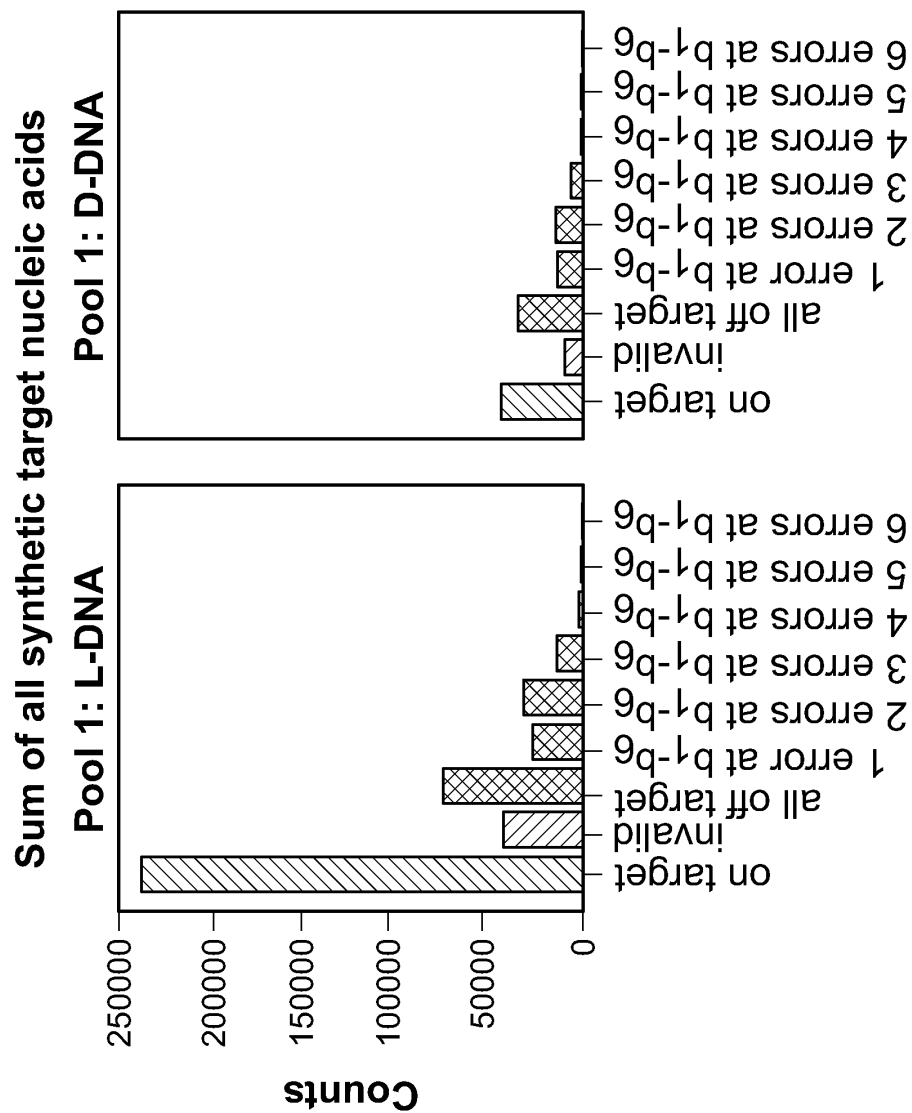


FIG. 63

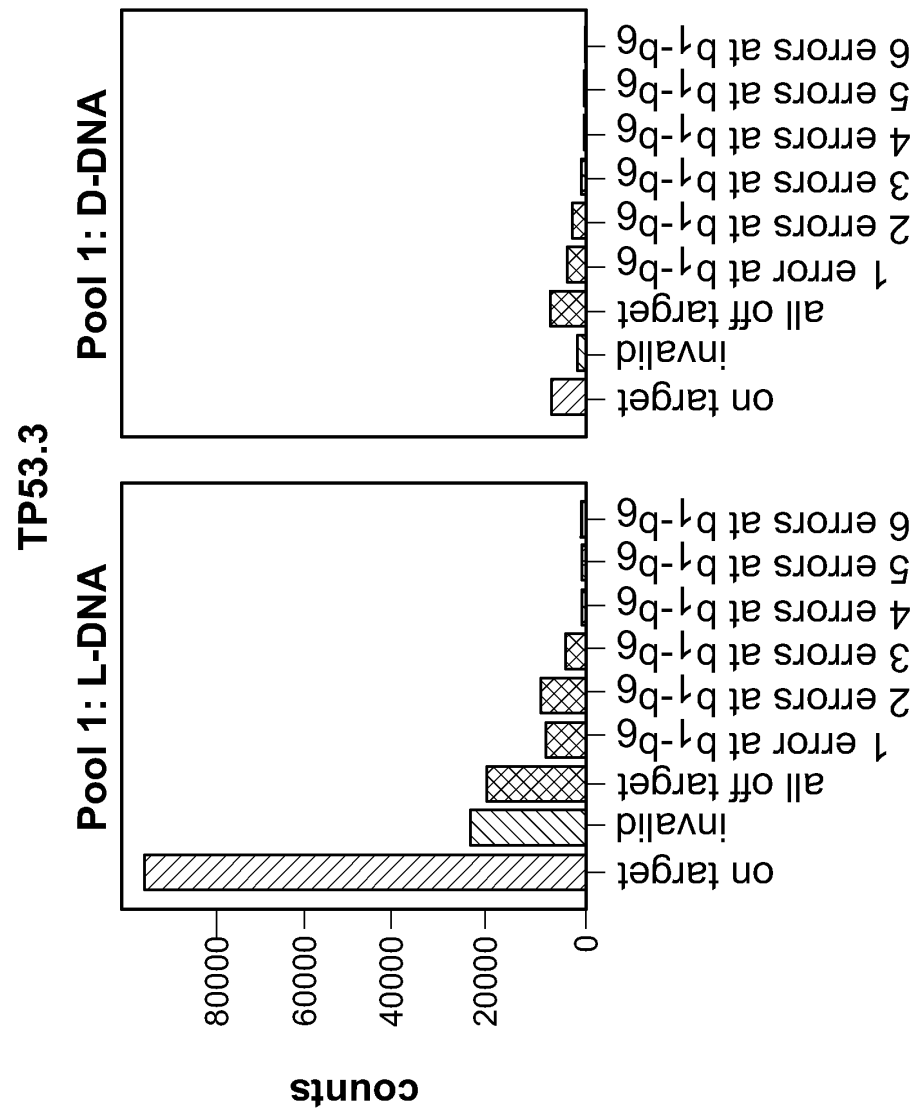


FIG. 64

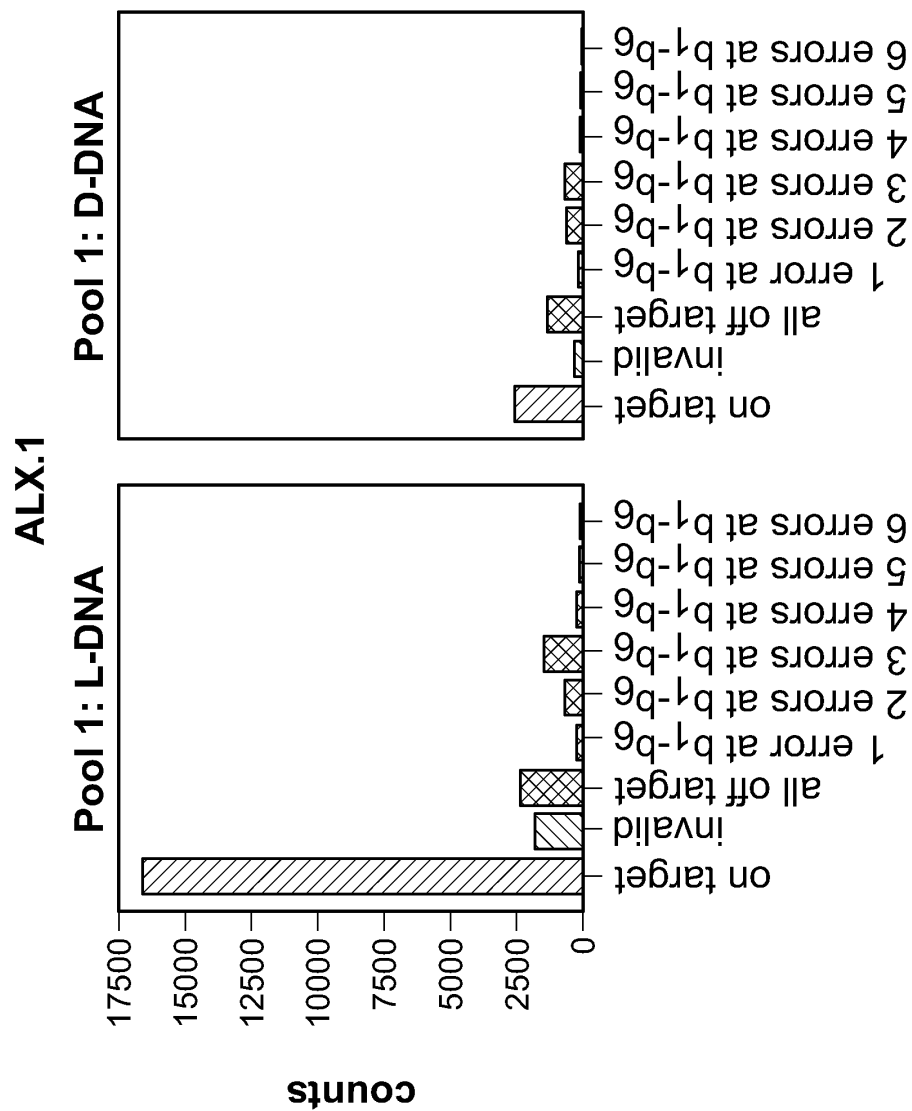


FIG. 64 (Cont.)

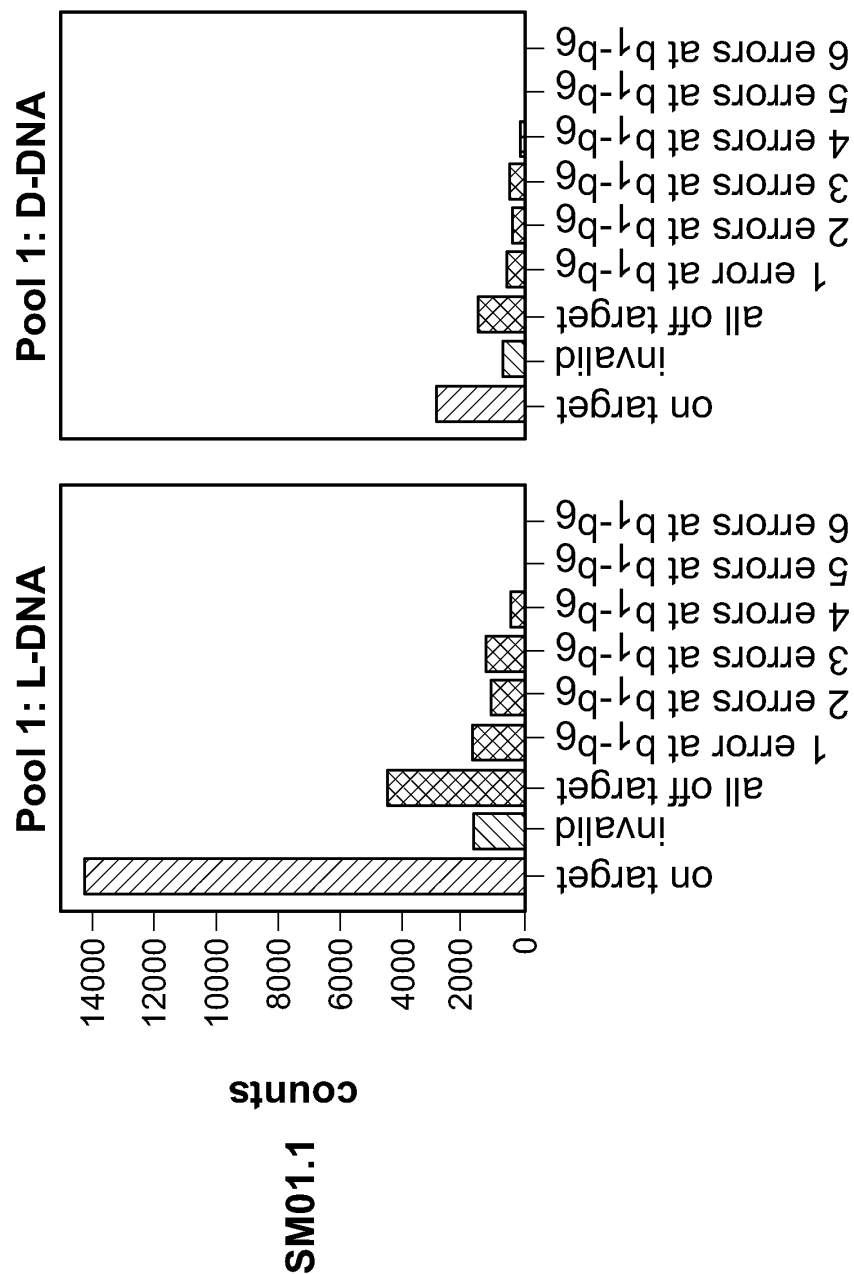


FIG. 64 (Cont.)

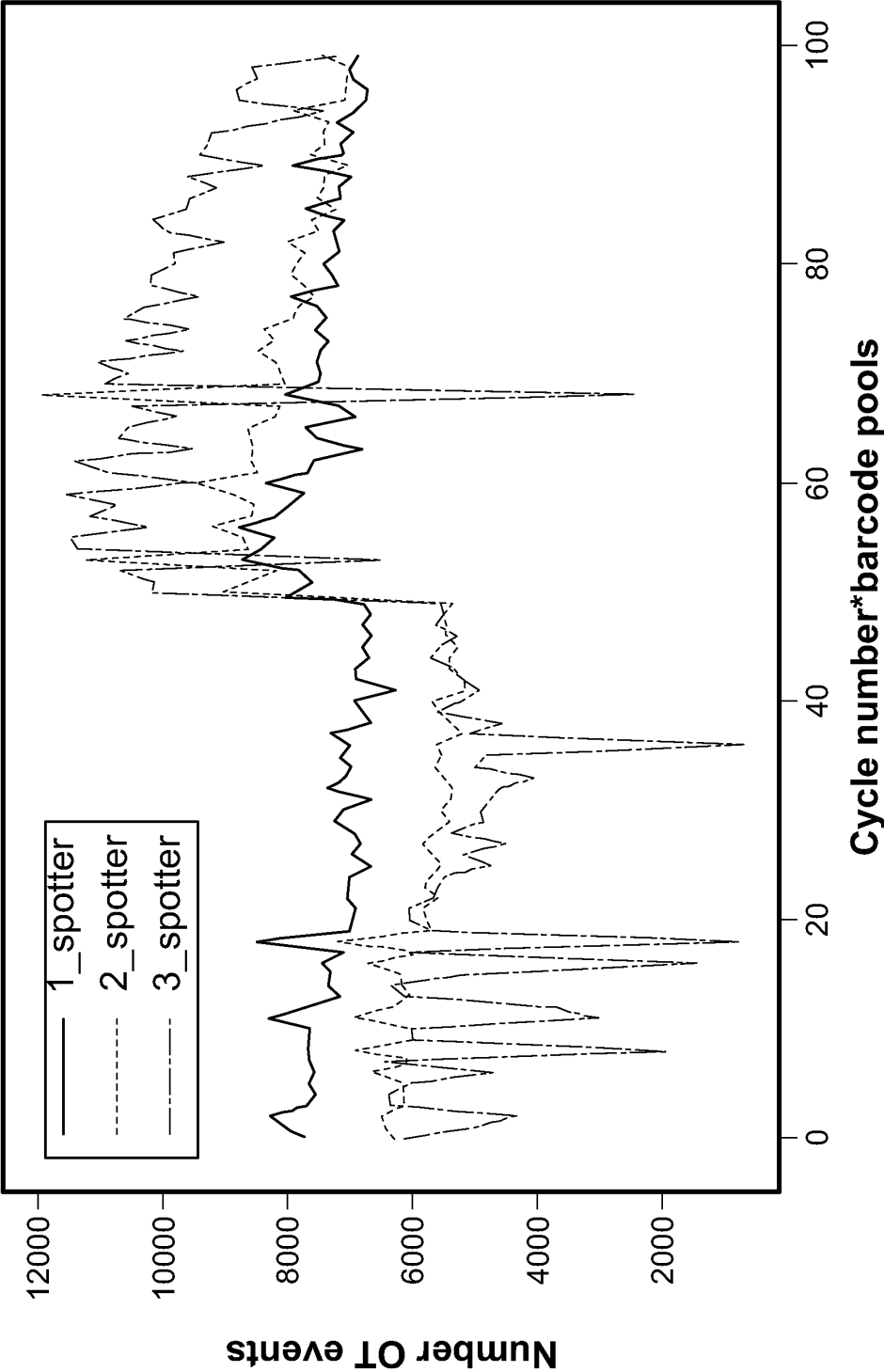


FIG. 65

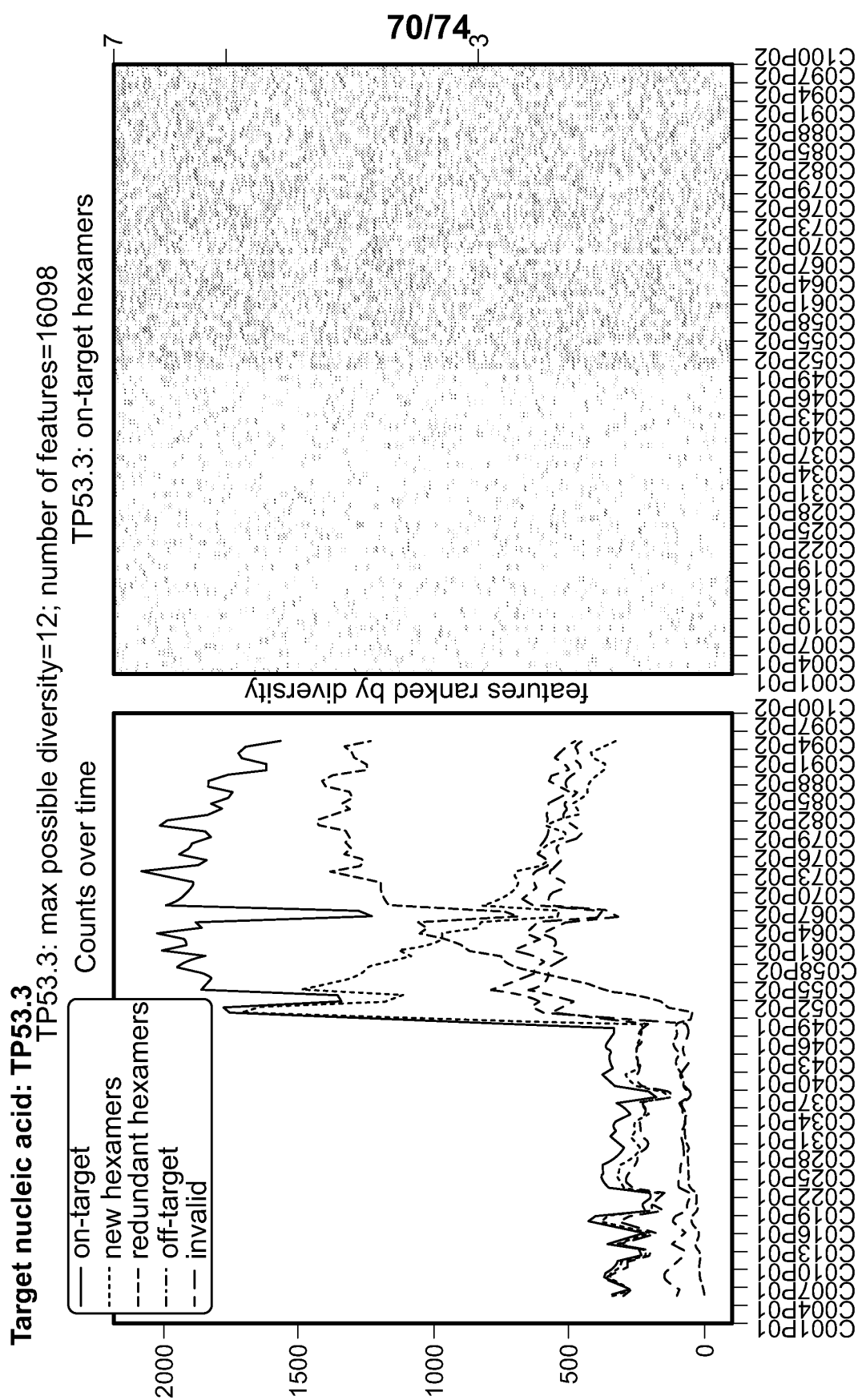


FIG. 66

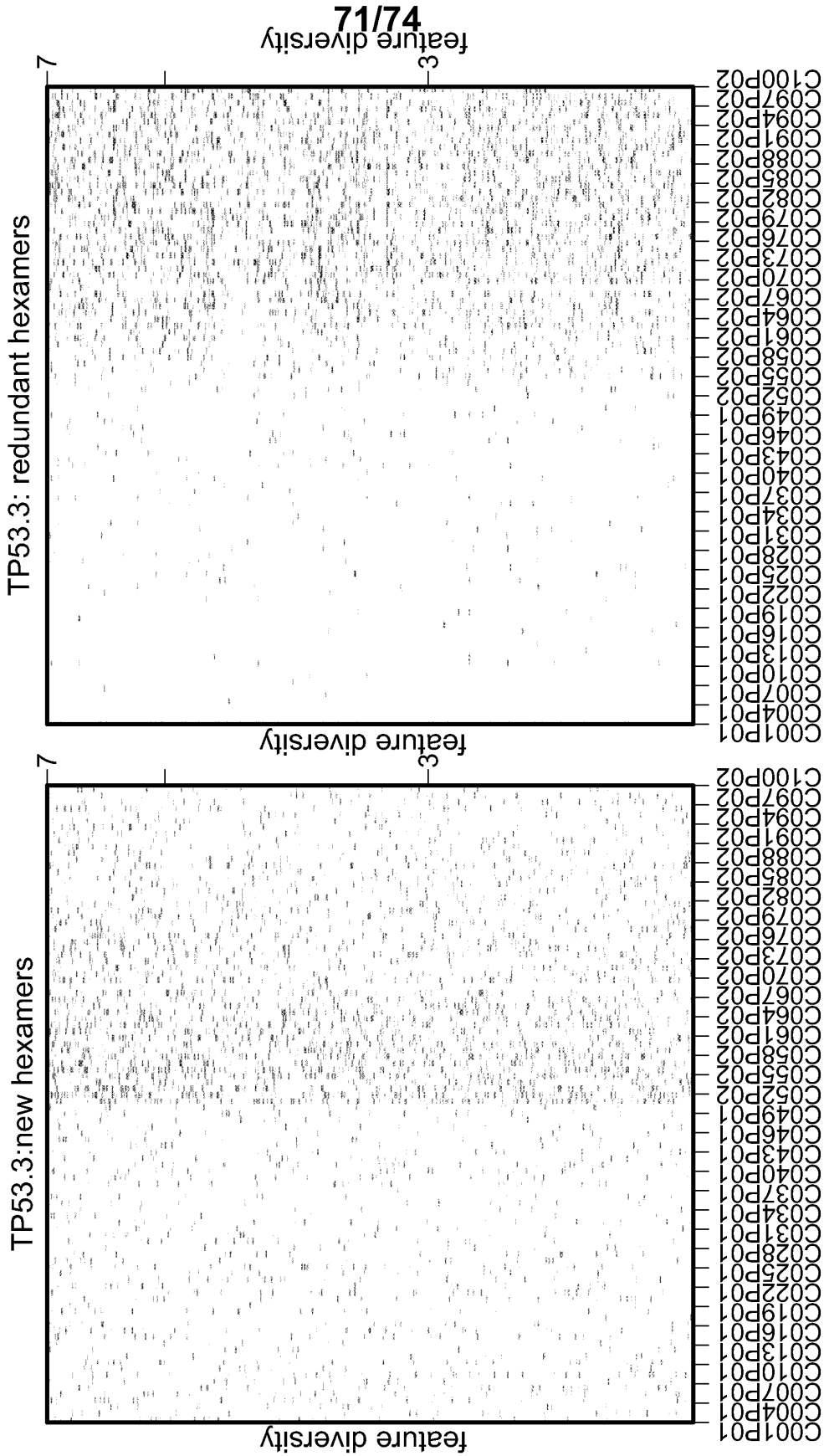


FIG. 66 (cont.)

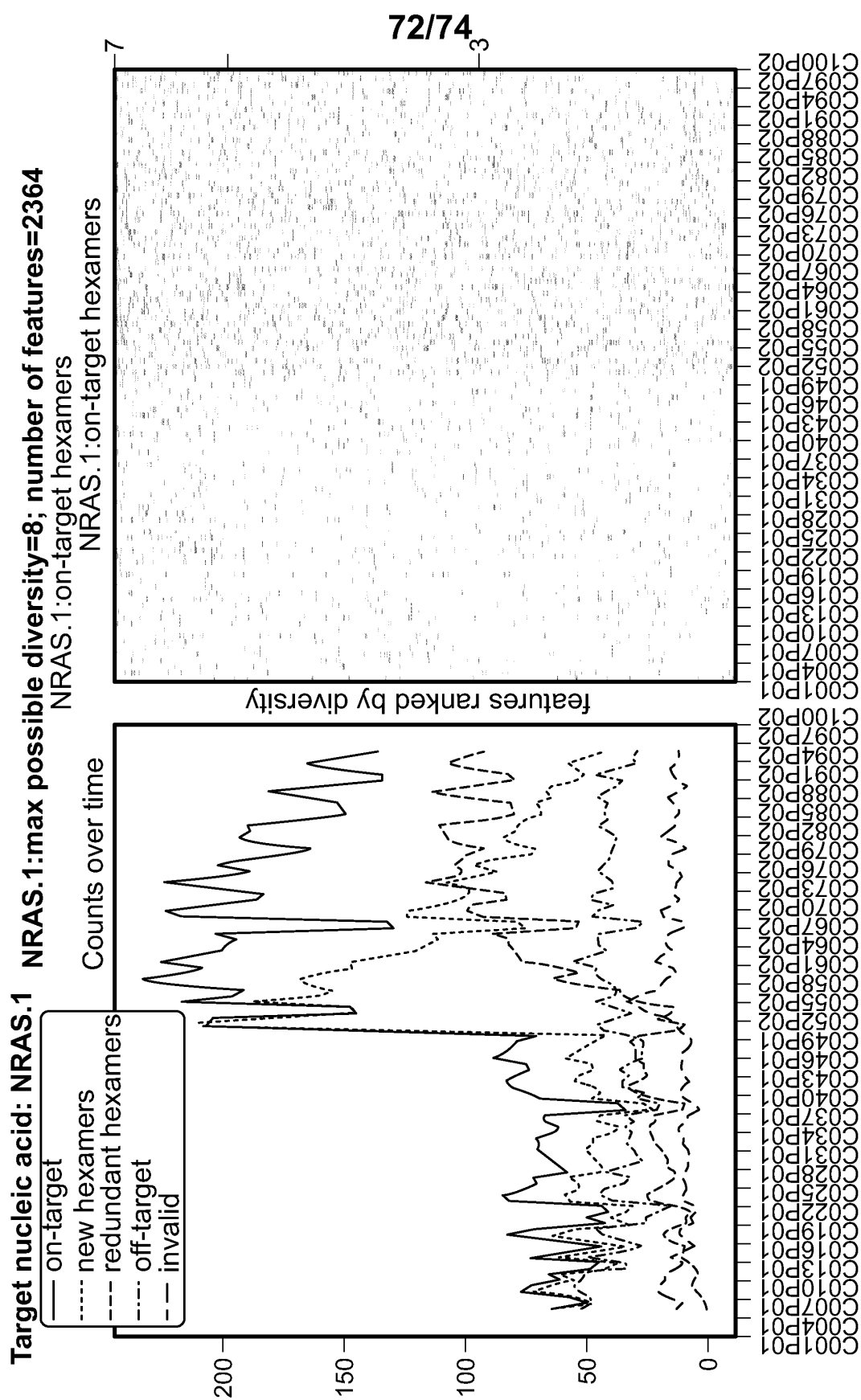


FIG. 66 (cont.)

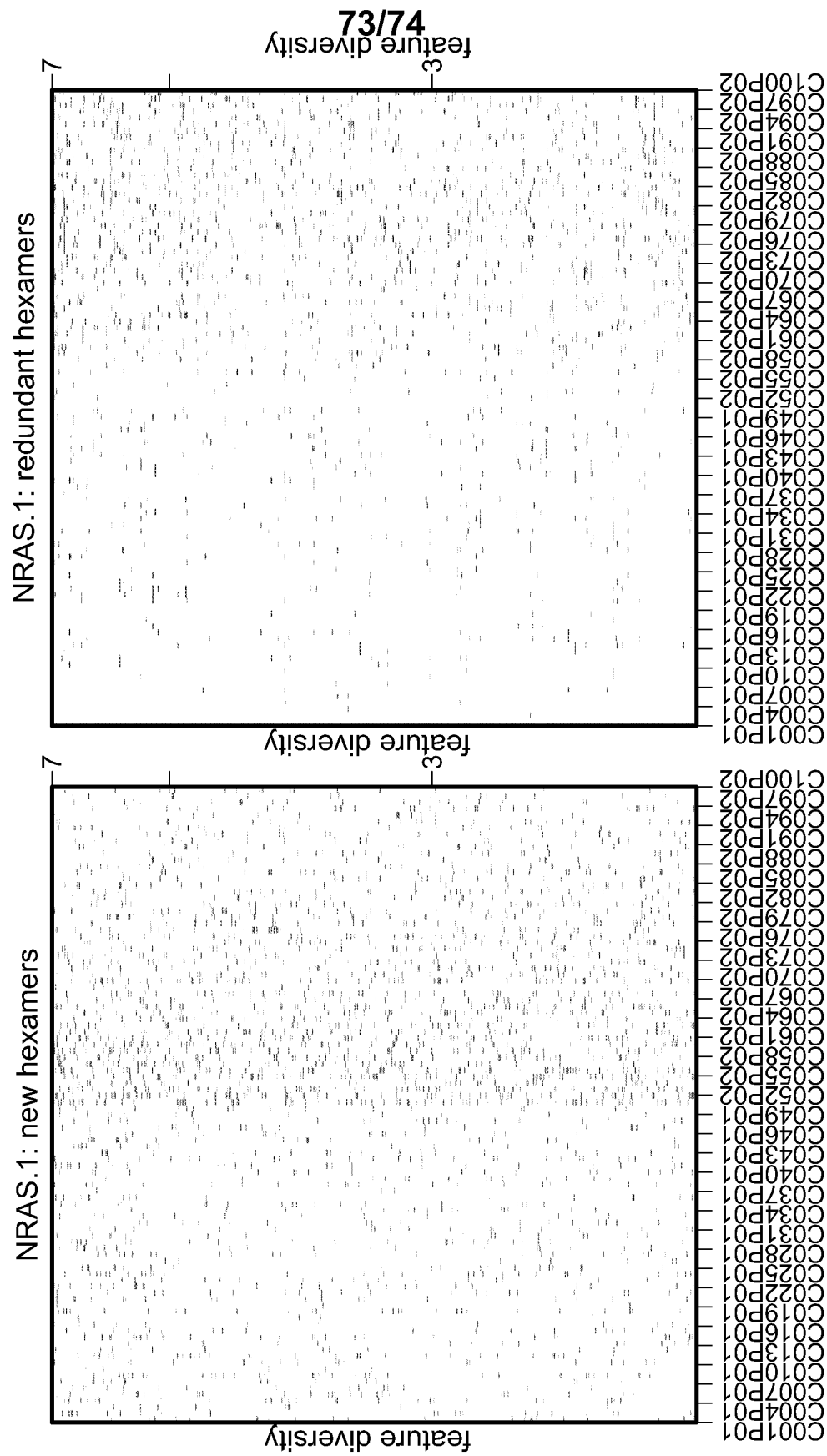


FIG. 66 (cont.)

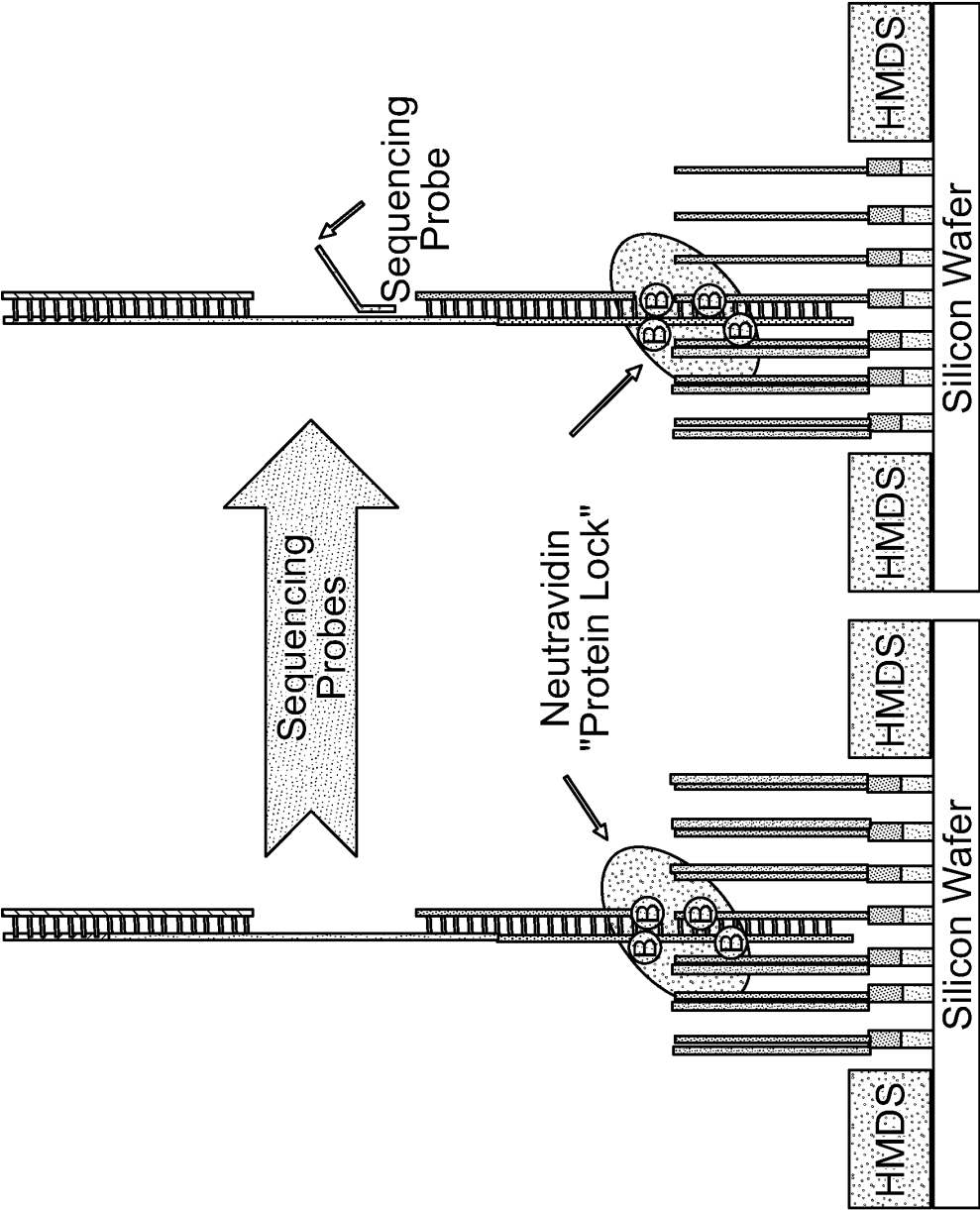


FIG. 67