

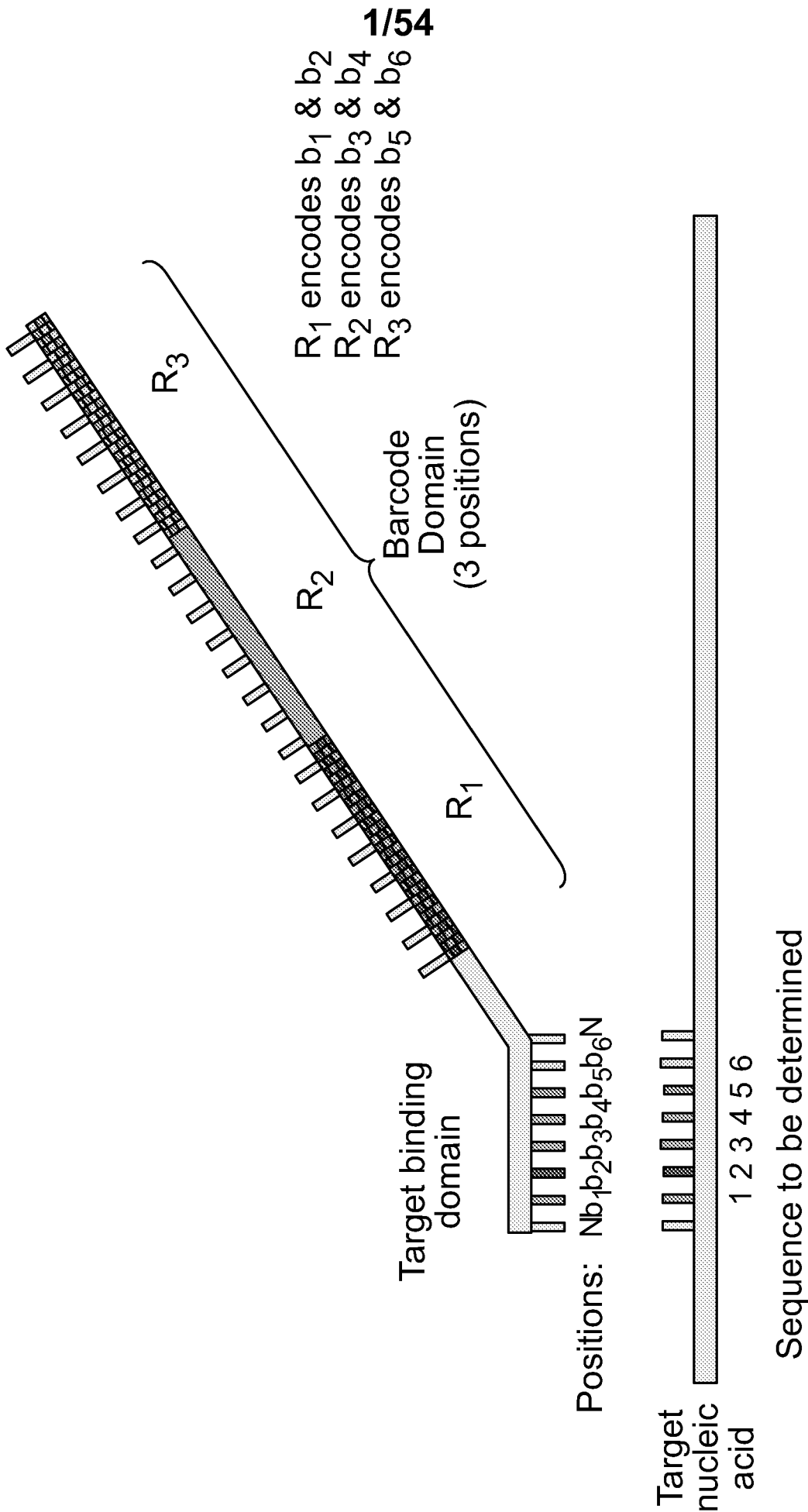


FIG. 1

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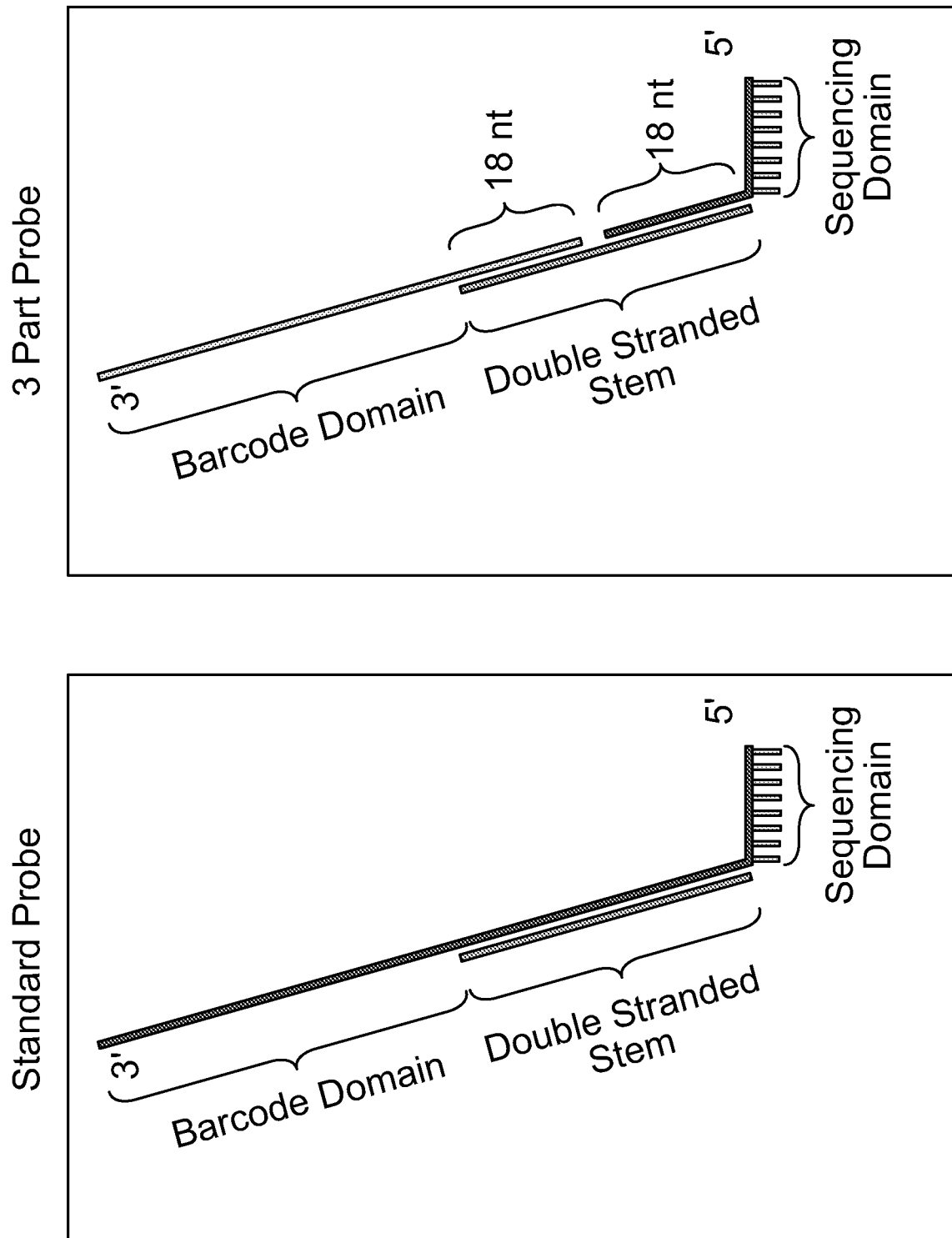


FIG. 2

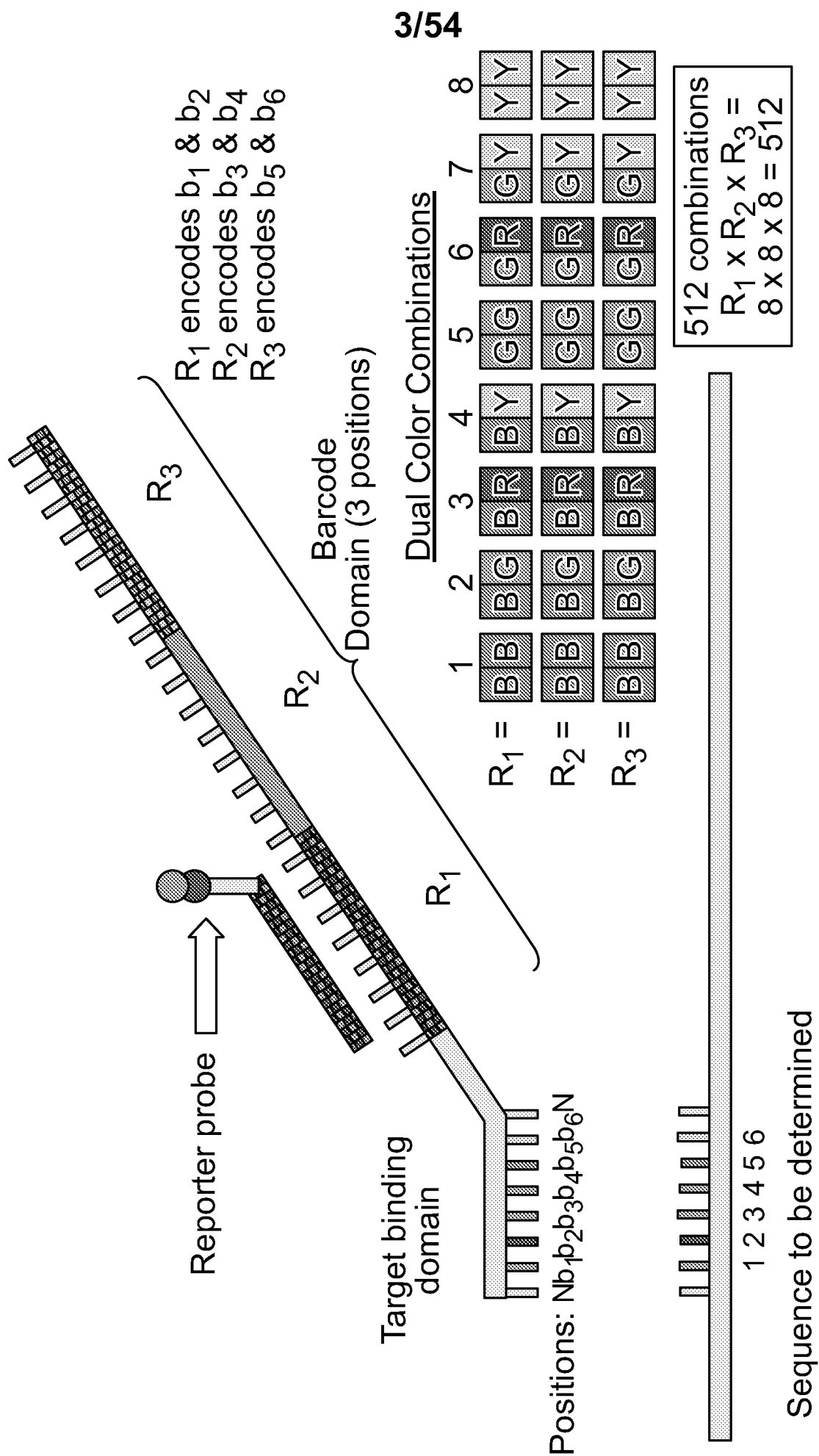


FIG. 3

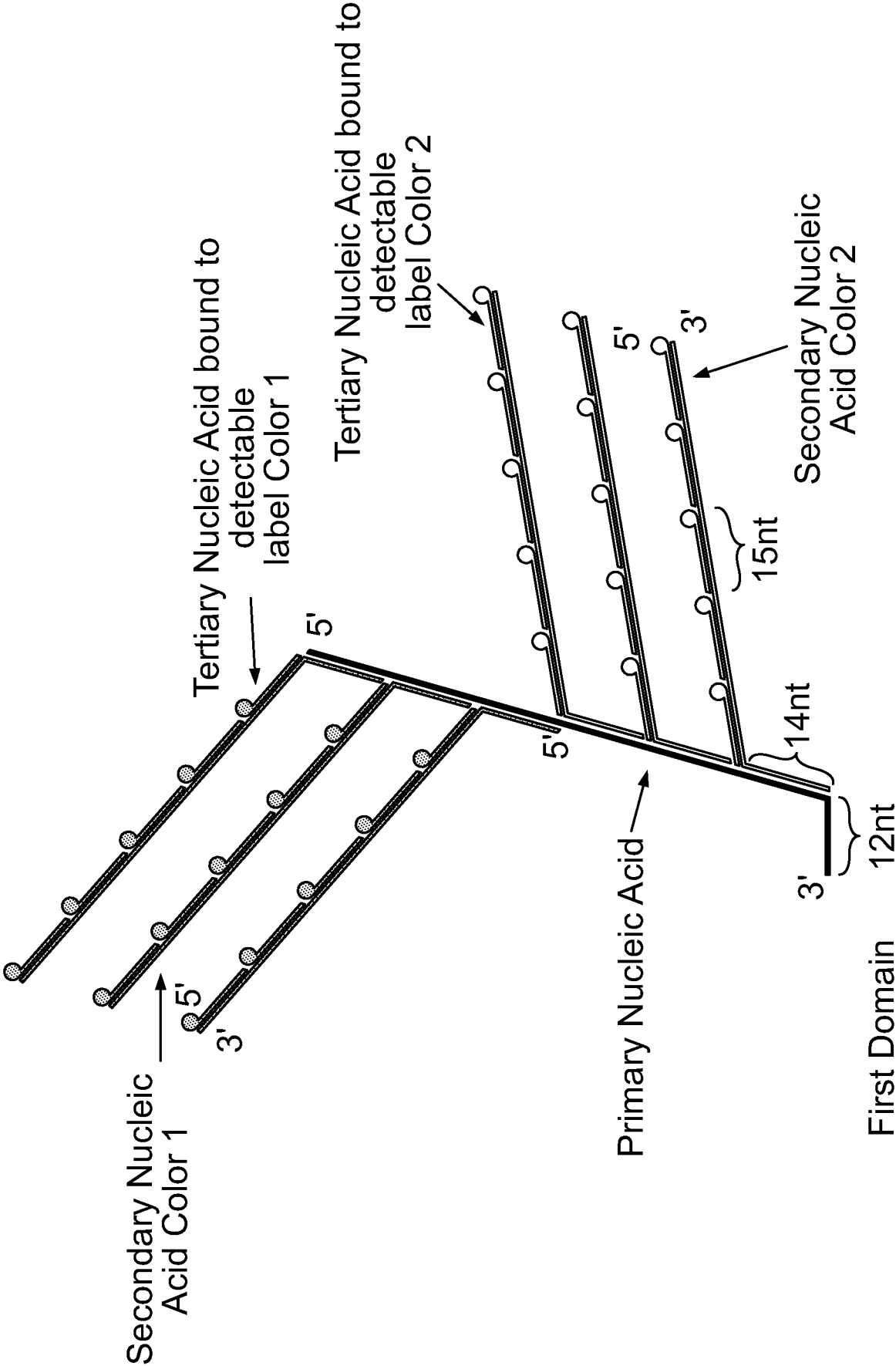


FIG. 4

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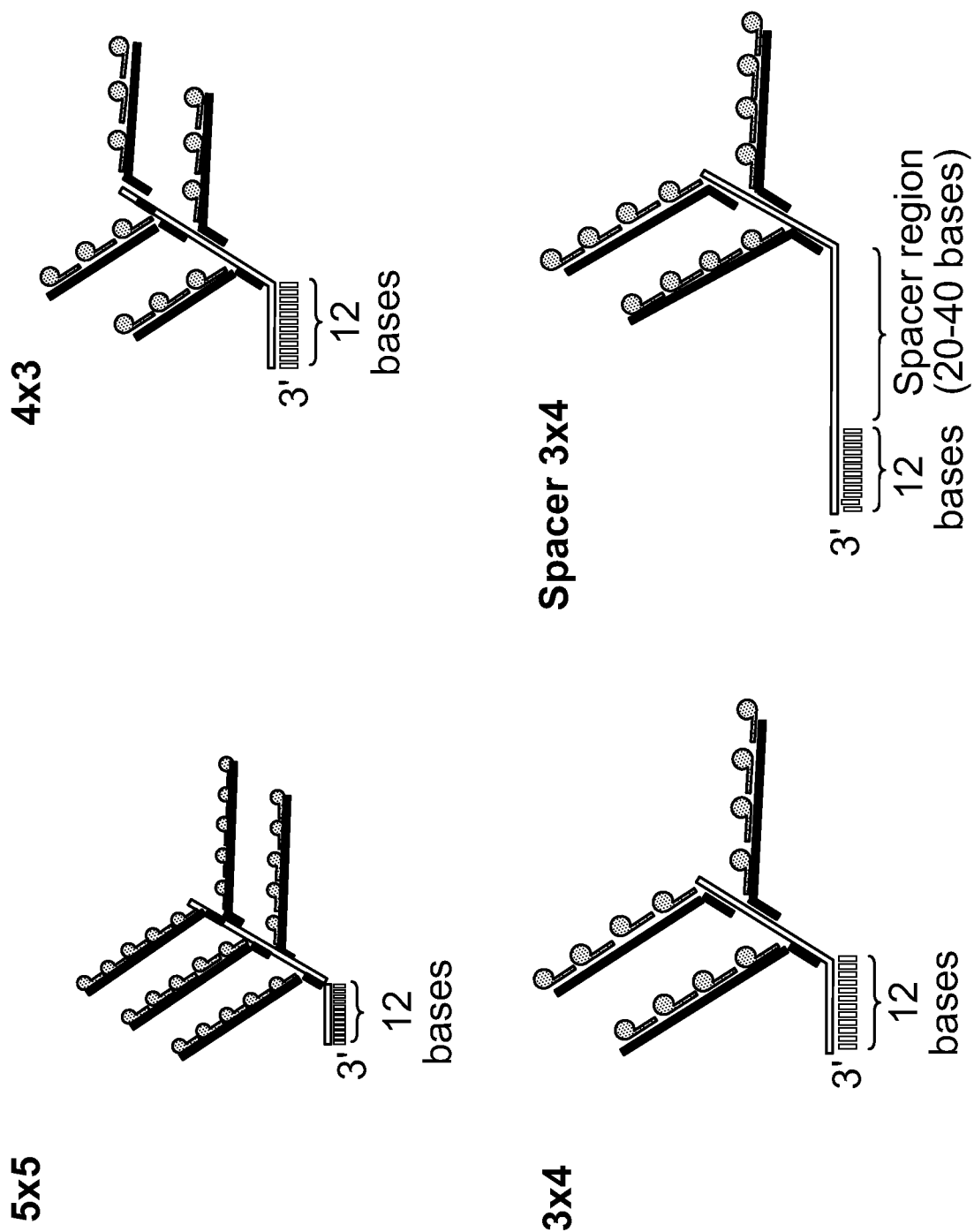


FIG. 5

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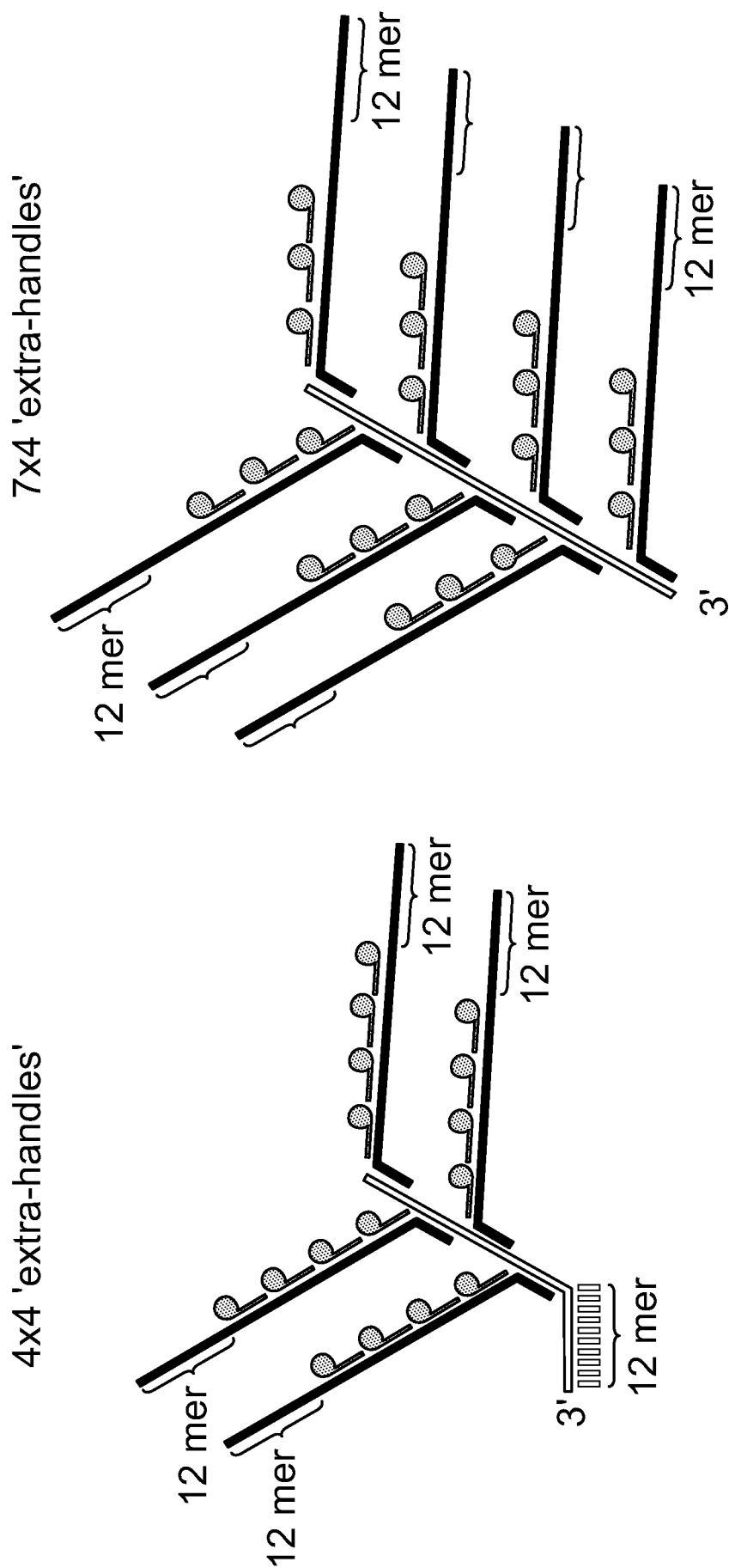


FIG. 6

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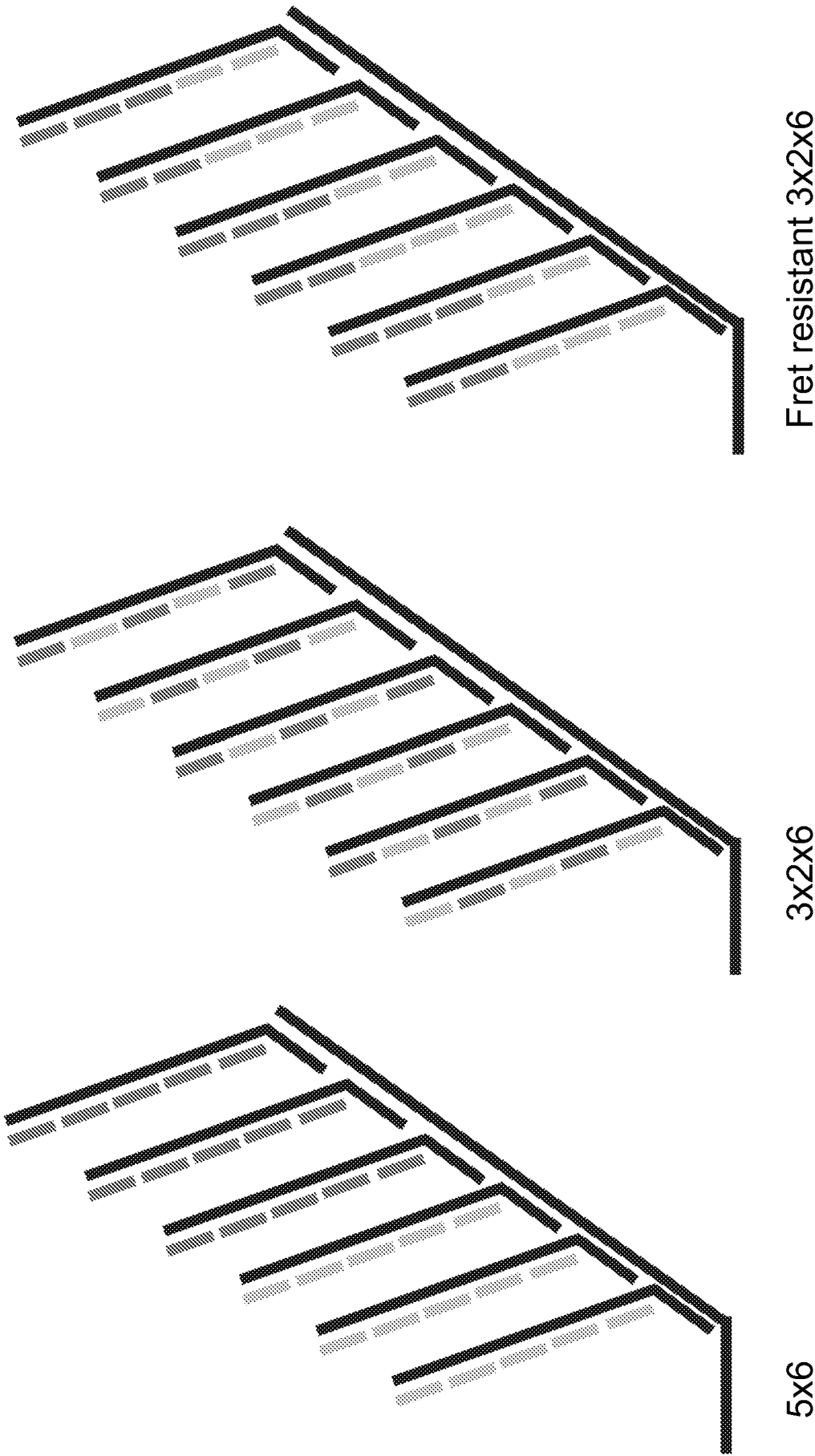


FIG. 7

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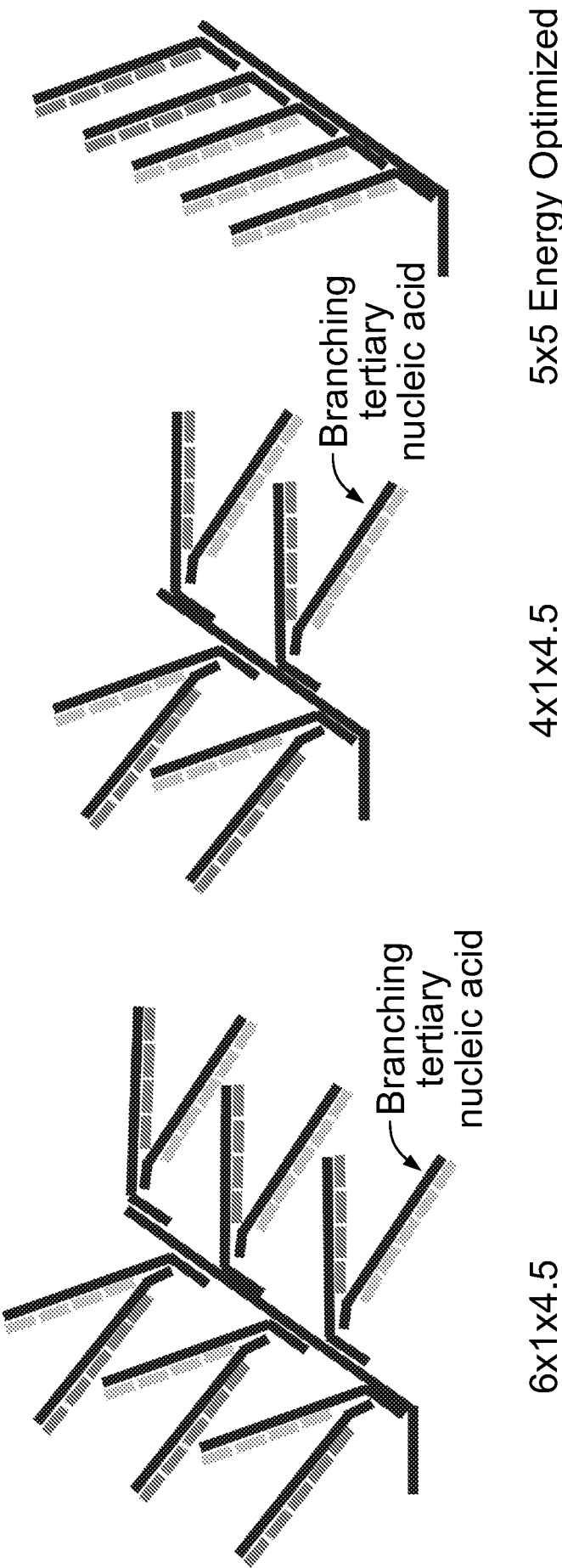


FIG. 8

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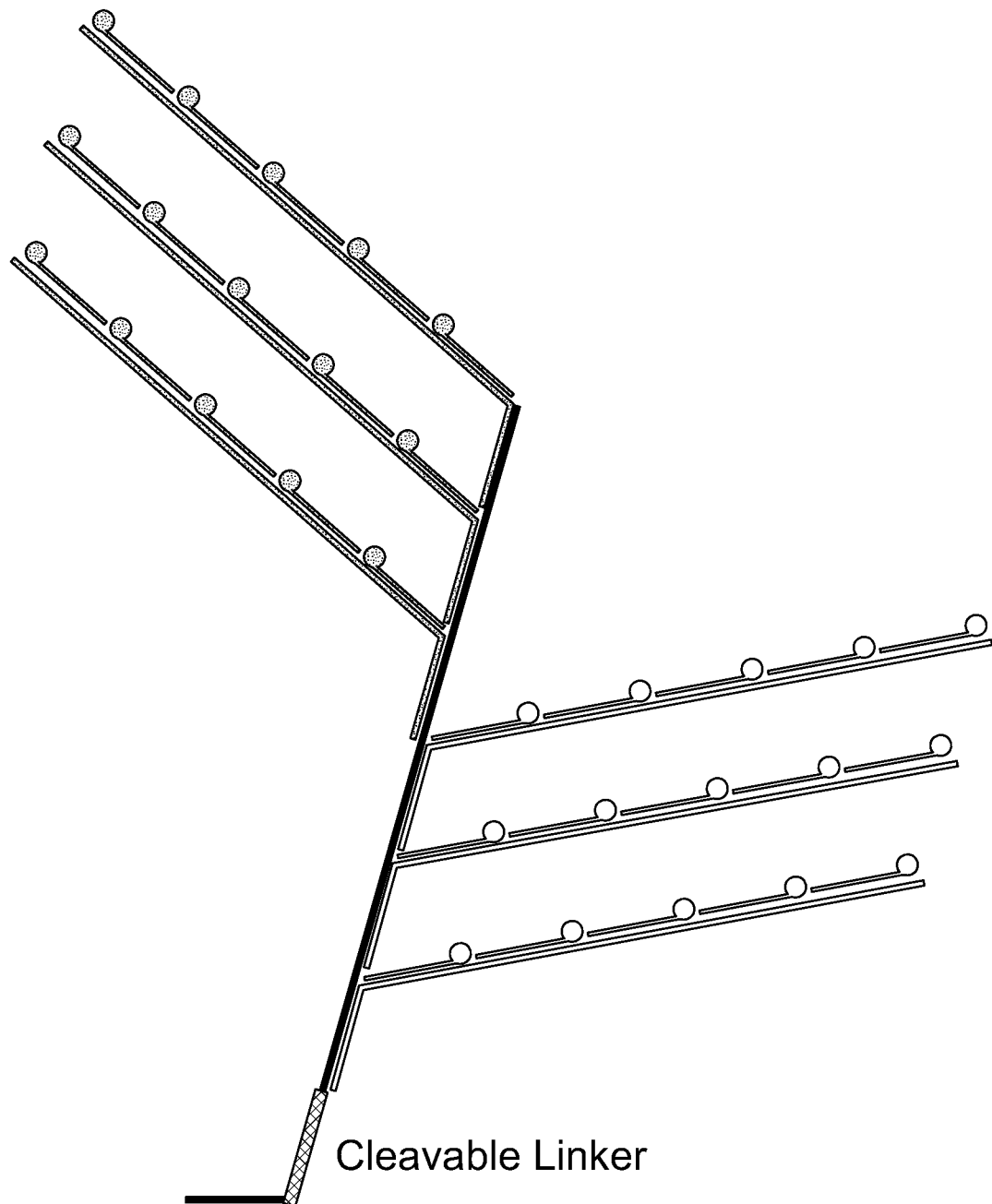


FIG. 9

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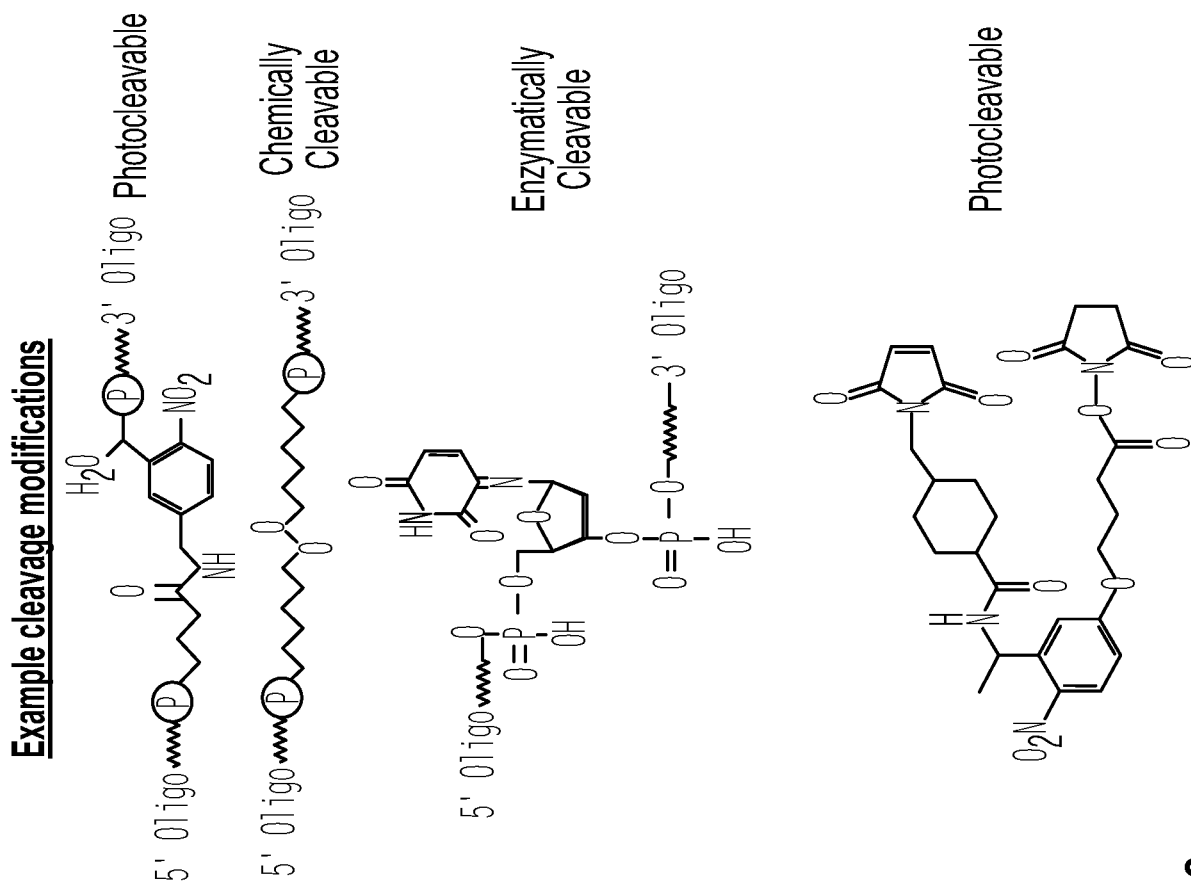


FIG. 10

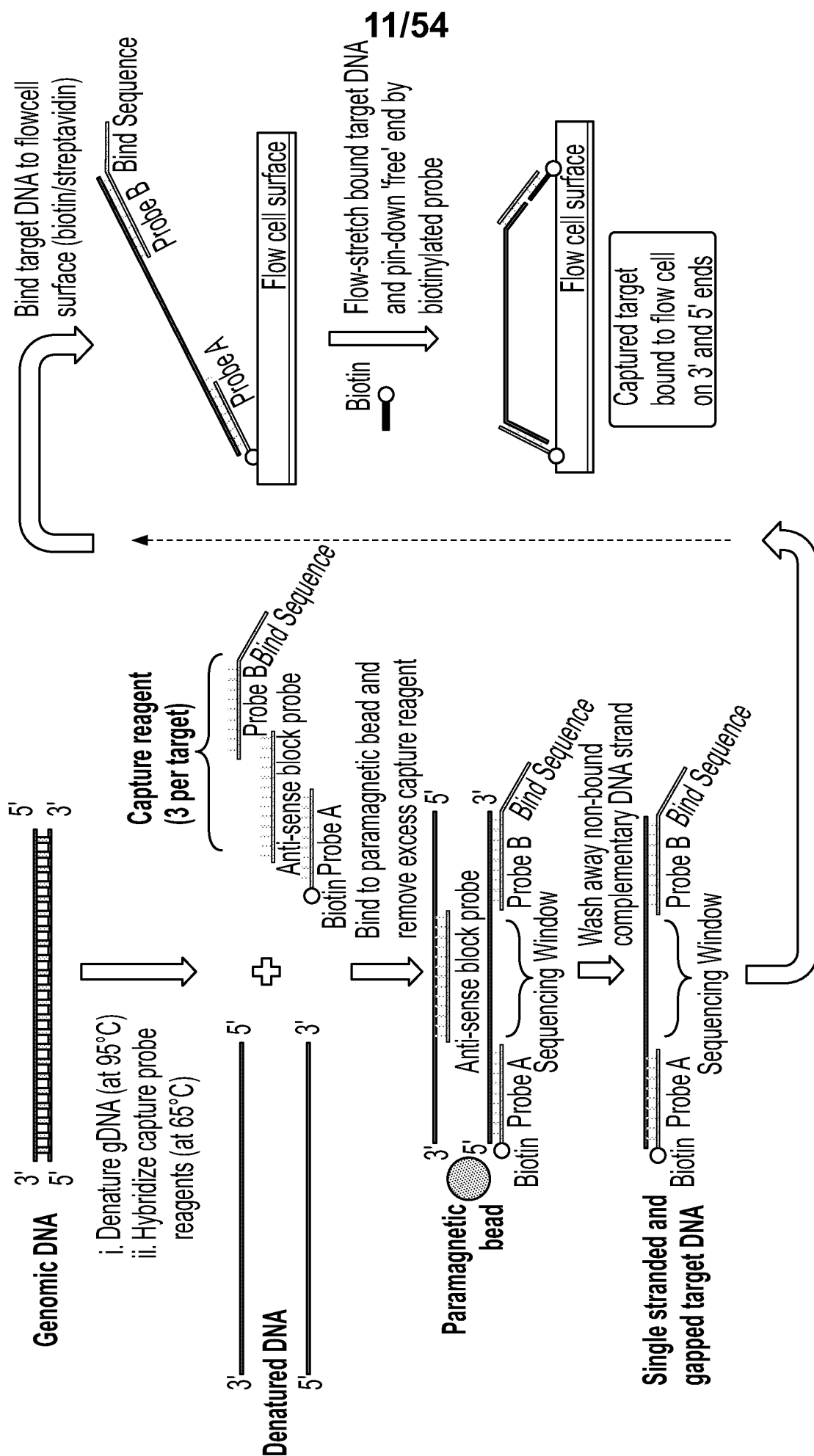
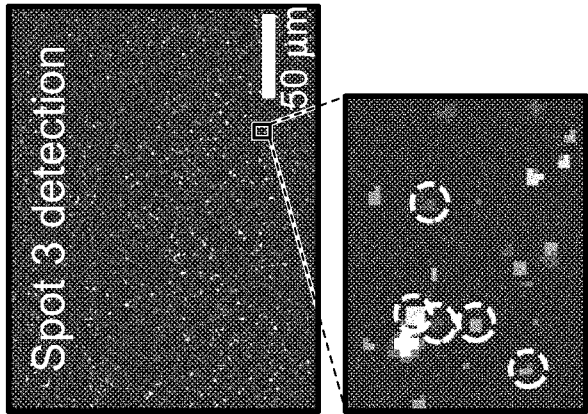
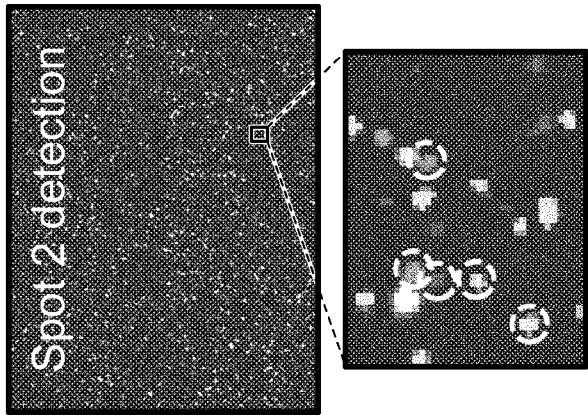
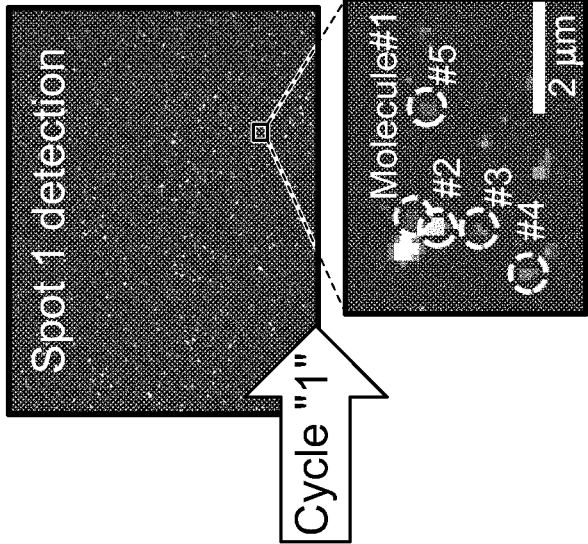


FIG. 11

Digital quantitation and identification of cancer panel (100 targets) from FFPE tissues



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Optical Barcode Pool 2

Color code	Sequence	Gene_ID	Counts
●●●●	ATC	ATM_5	1
●●●●	CGA	KIT_6	1
●●●●	GCT	PDGFRA_10	2
●●●●	ATA	PTEN_2	1

FIG. 12

FIG. 12 (Cont.)

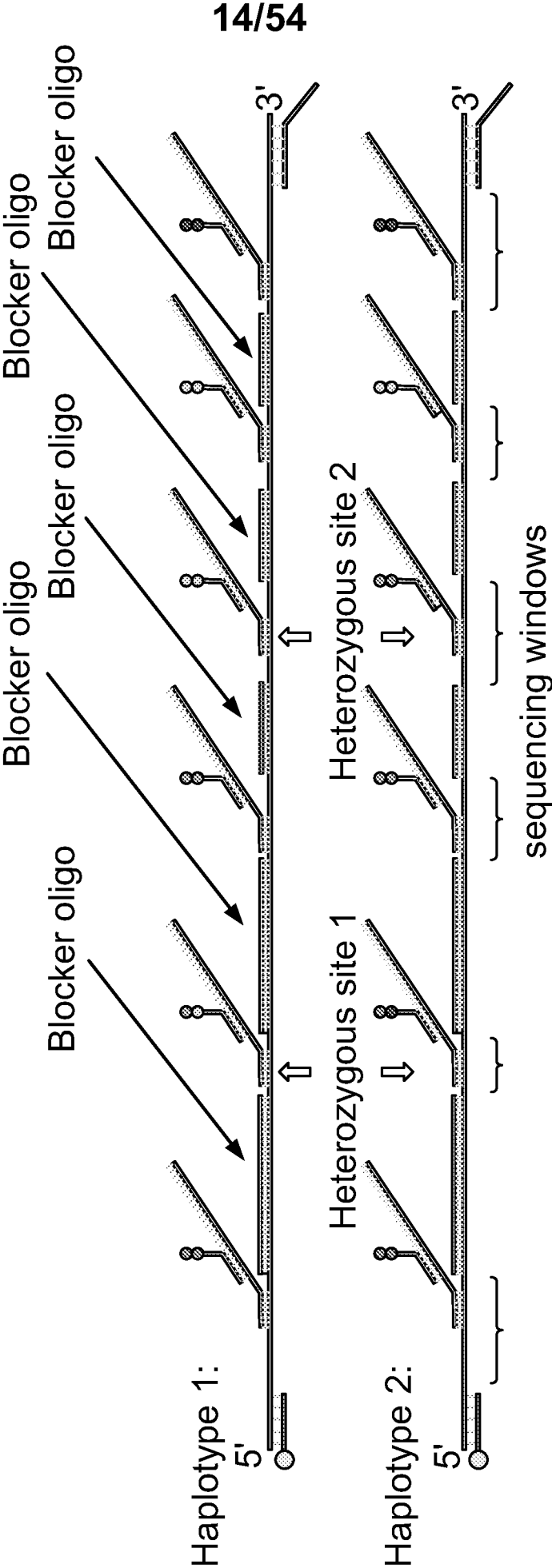


FIG. 13

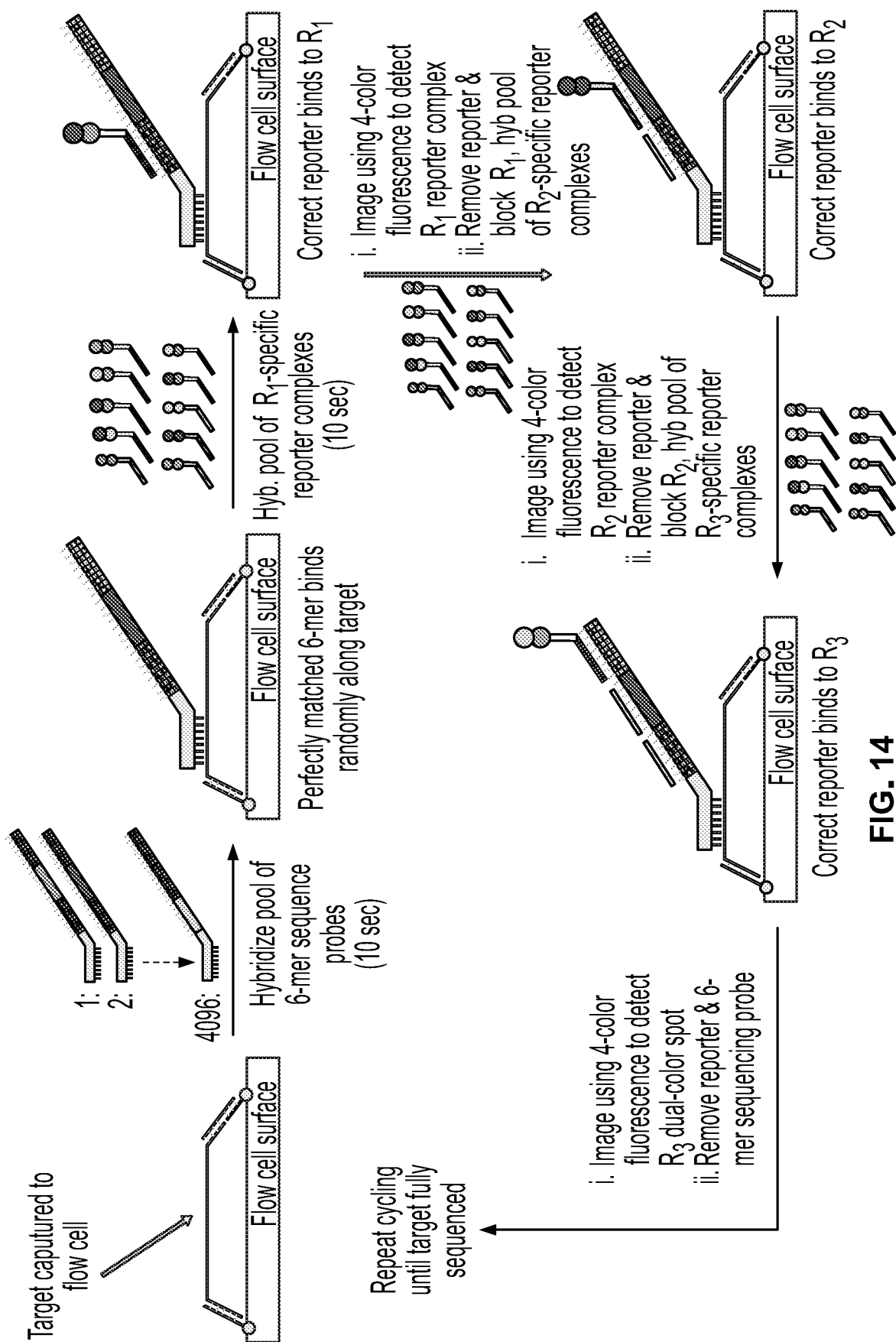


FIG. 14

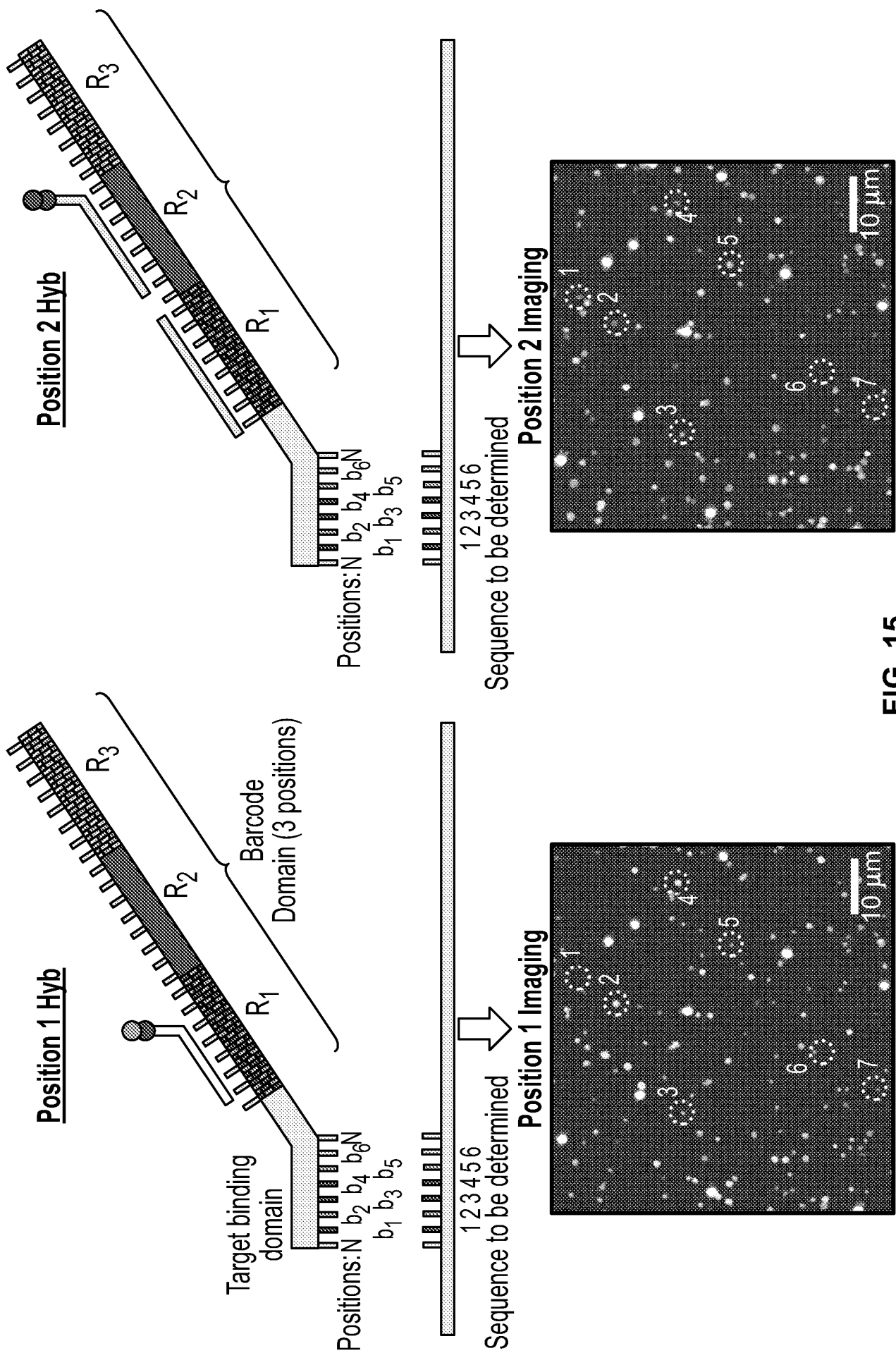


FIG. 15

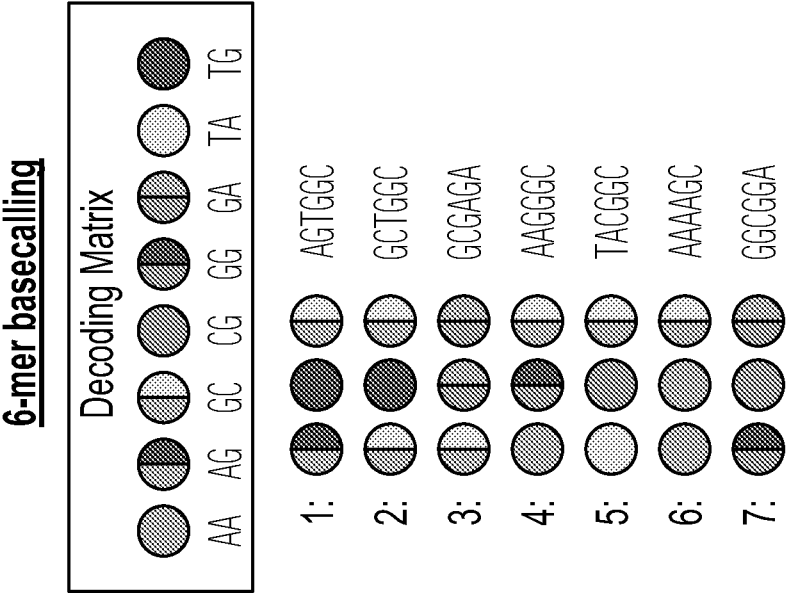
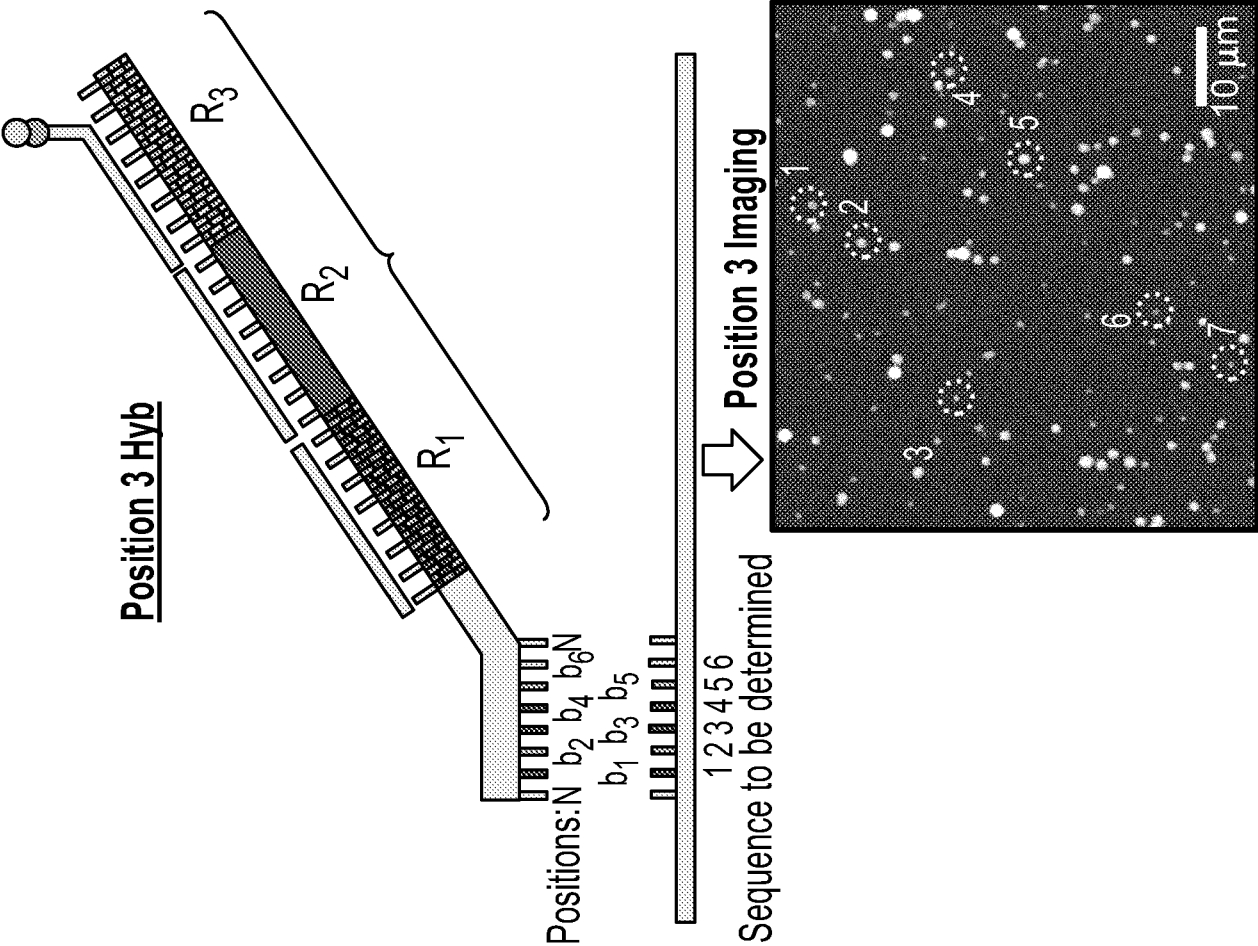


FIG. 15 (Cont.)

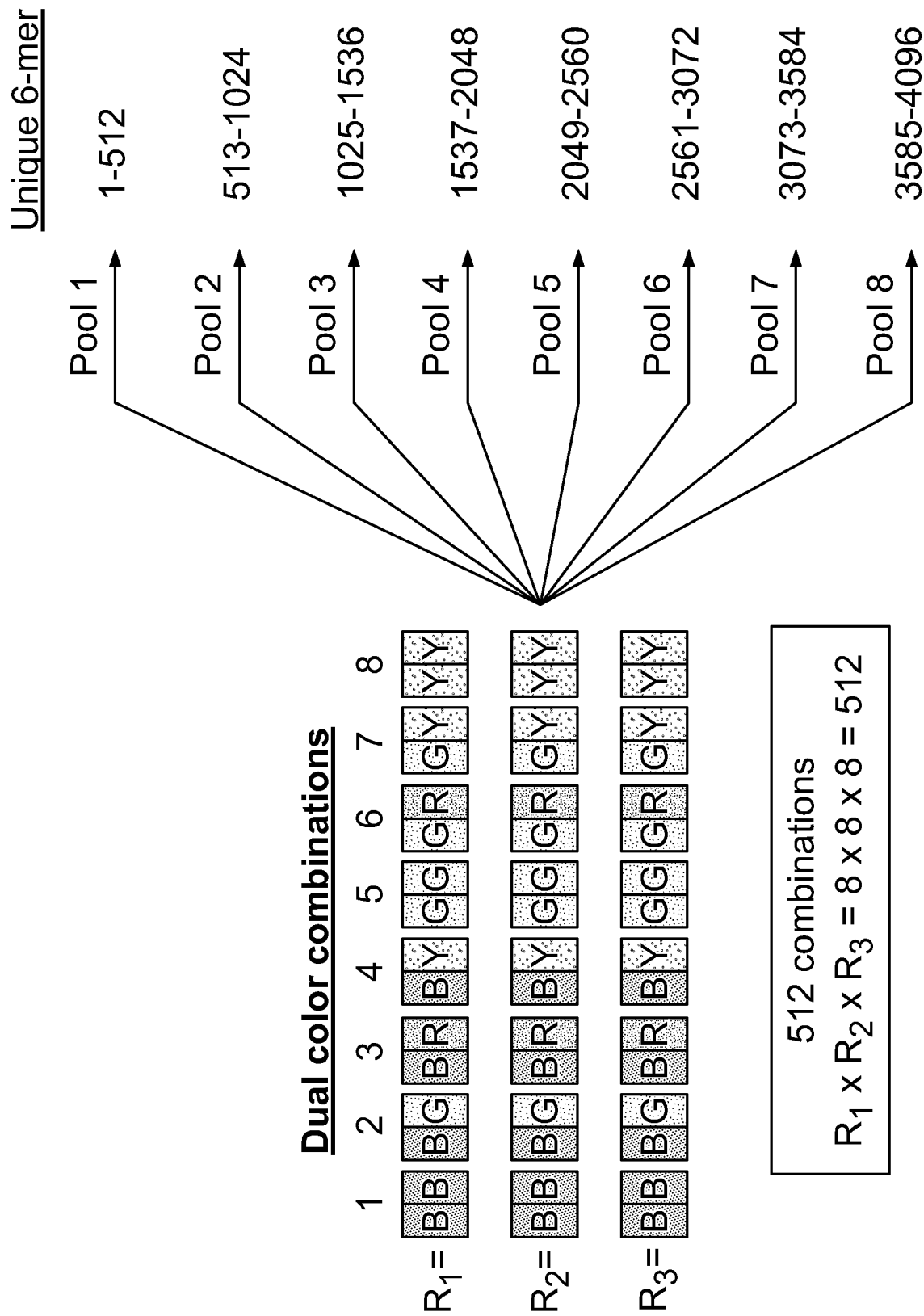


FIG. 16

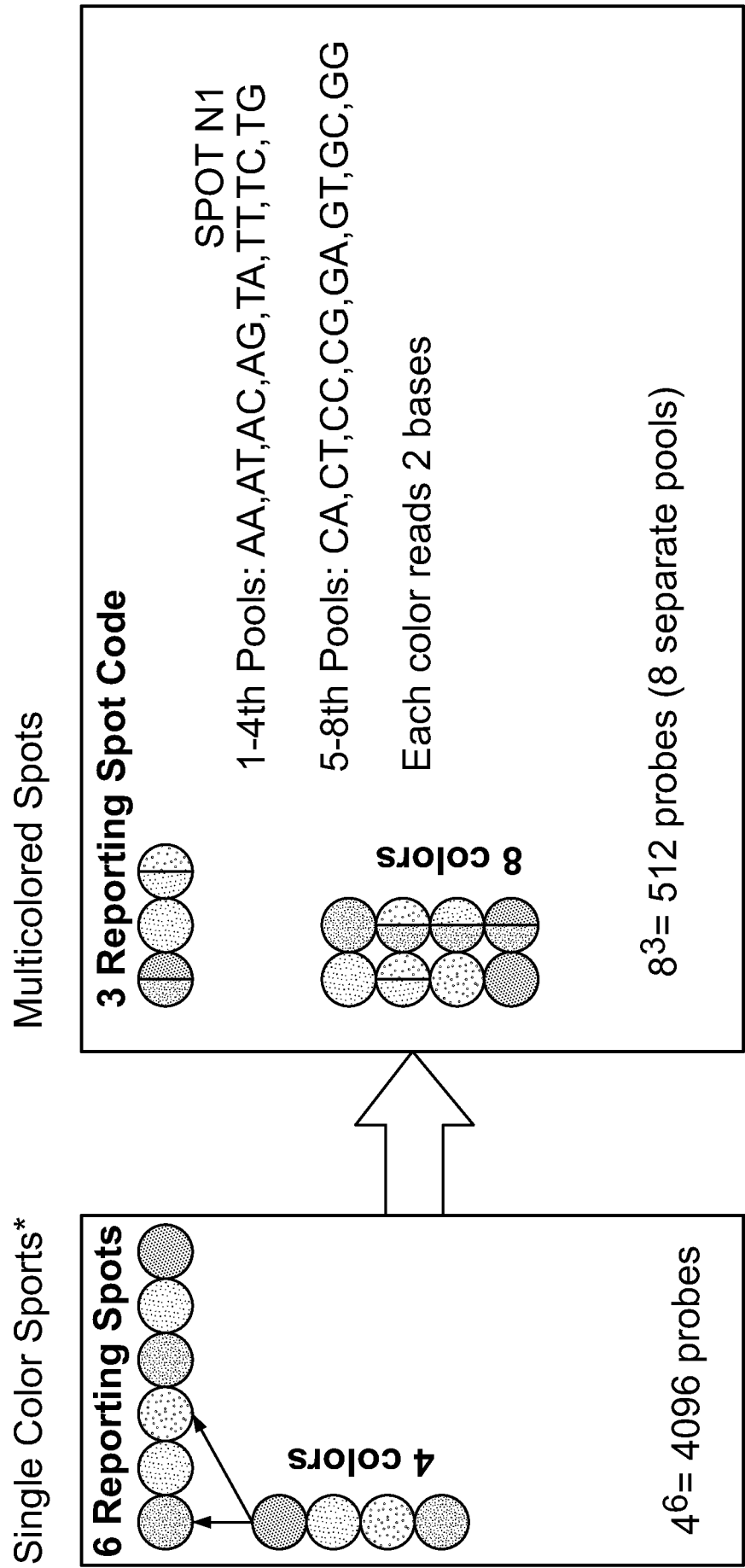


FIG. 17

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

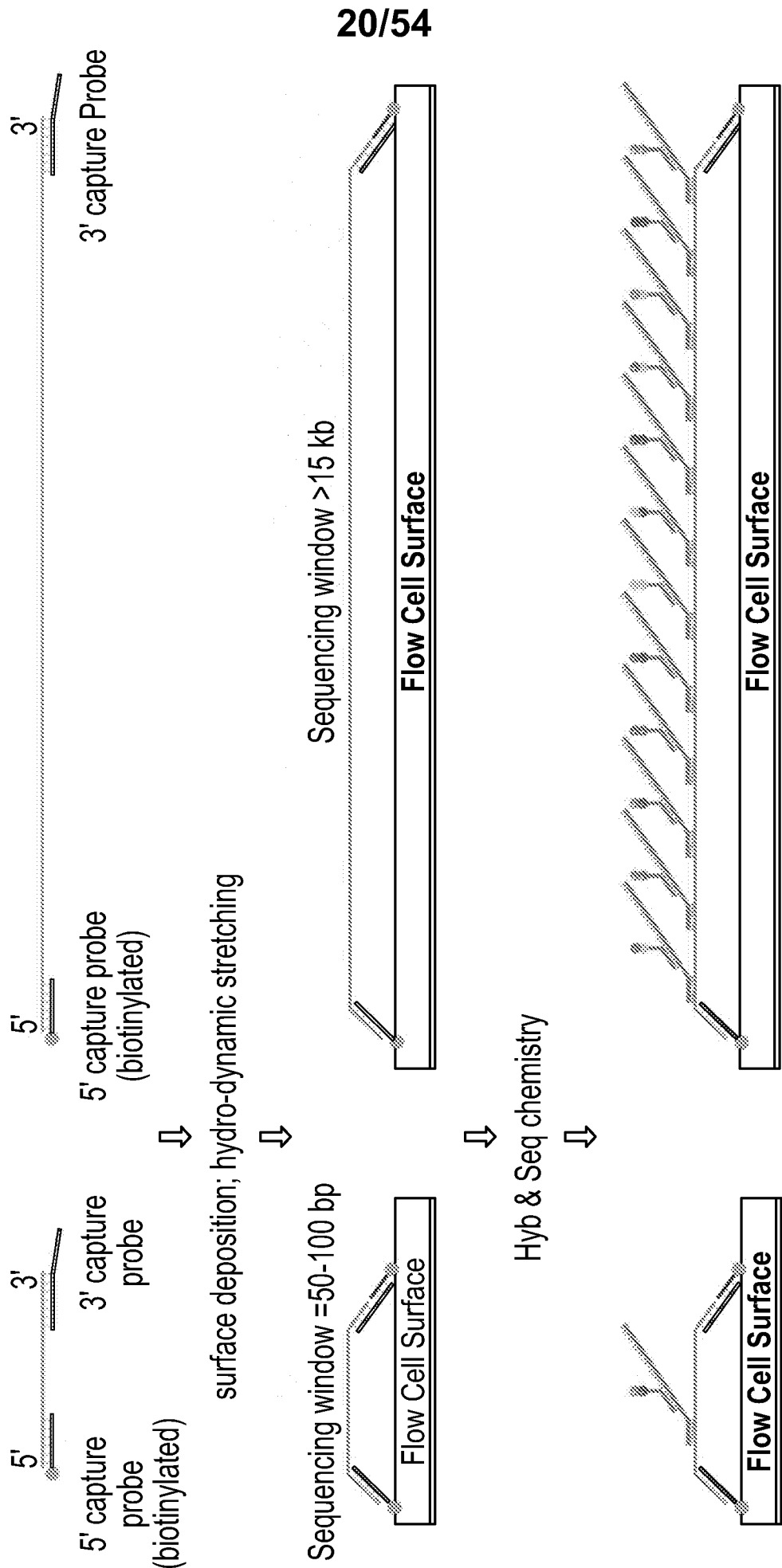


FIG. 18

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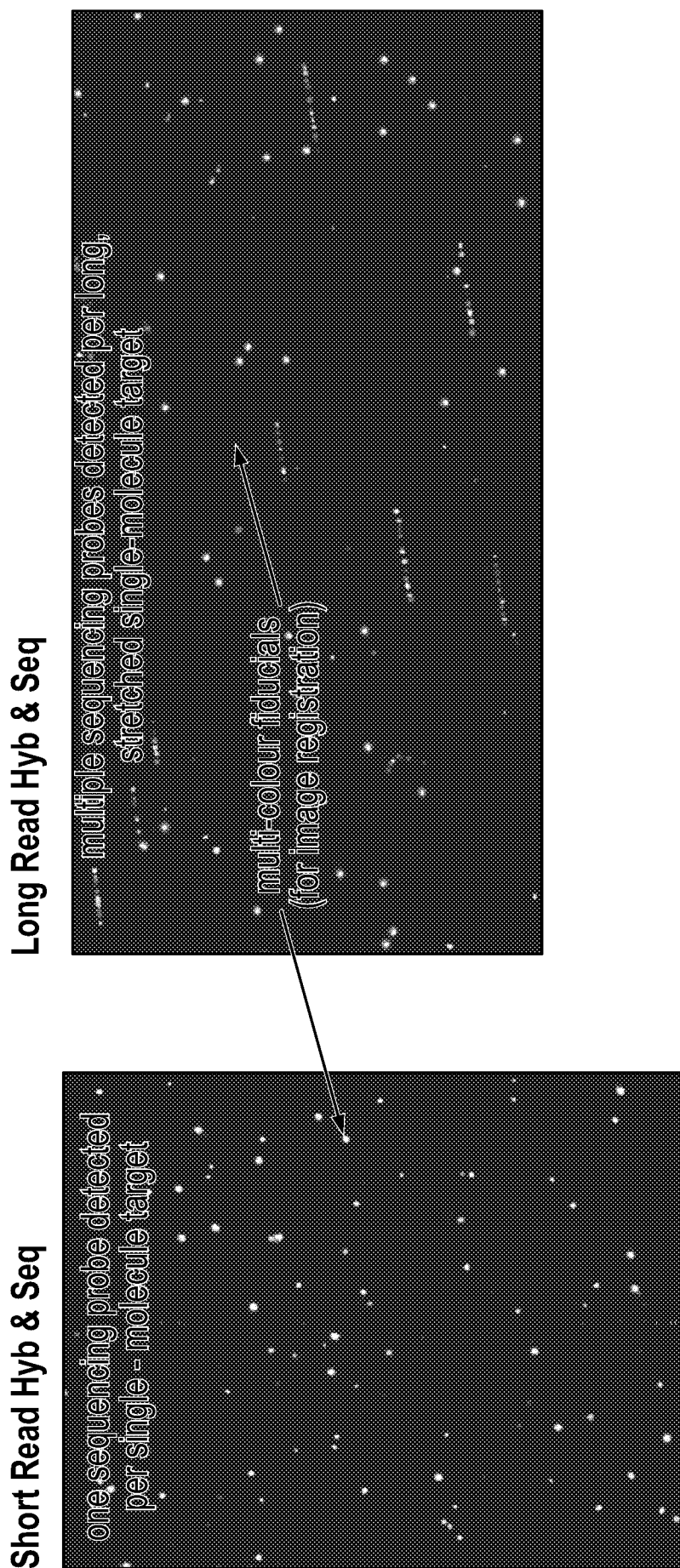


FIG. 19

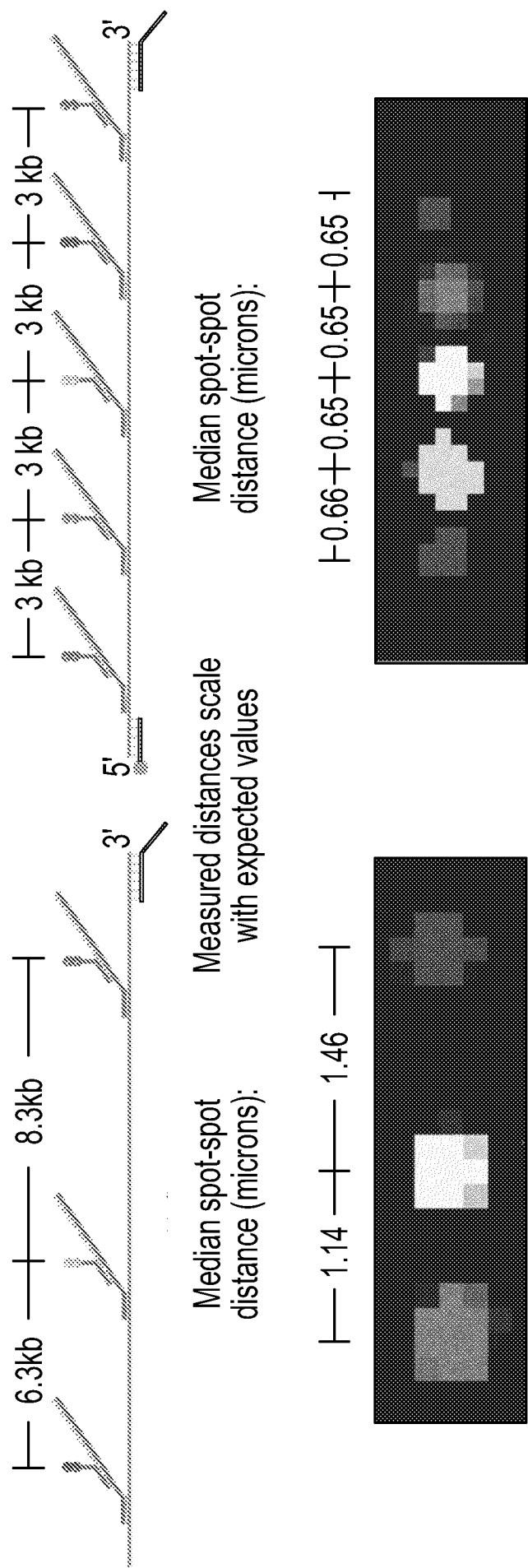
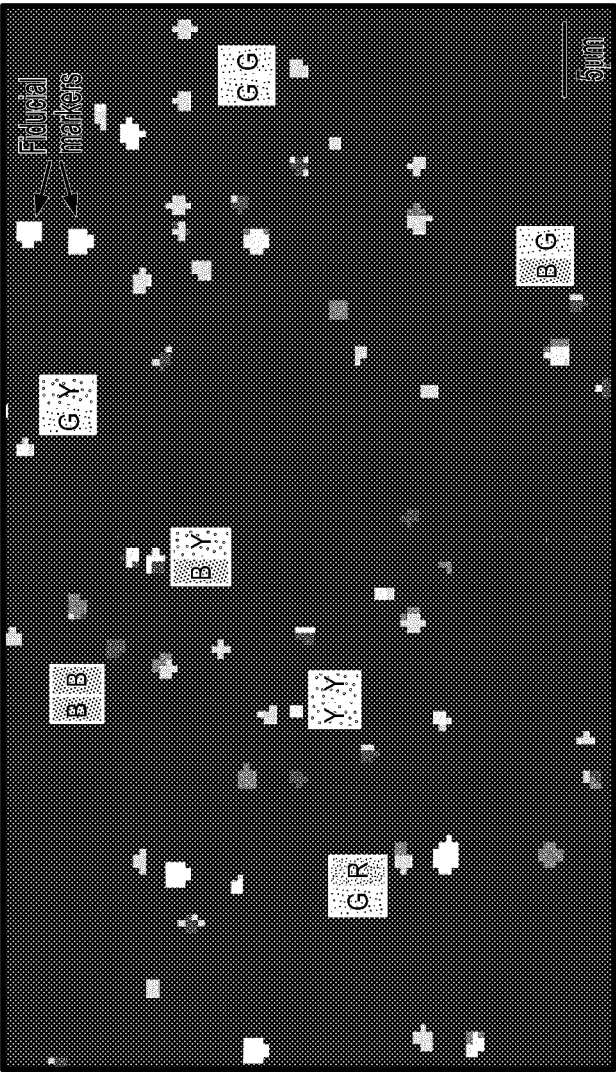
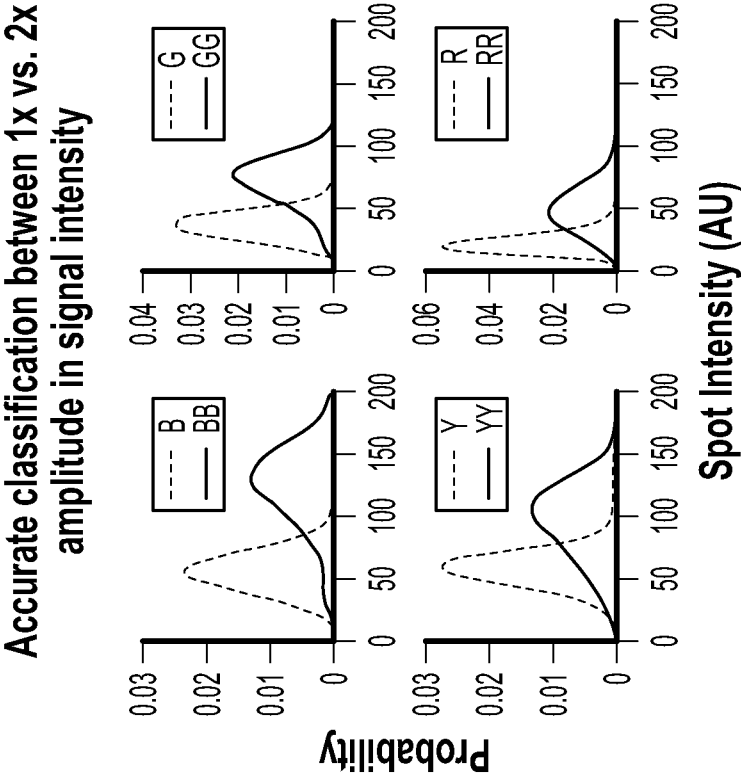


FIG. 20



Pixelated image to show dual-fluorescence signal

FIG. 21

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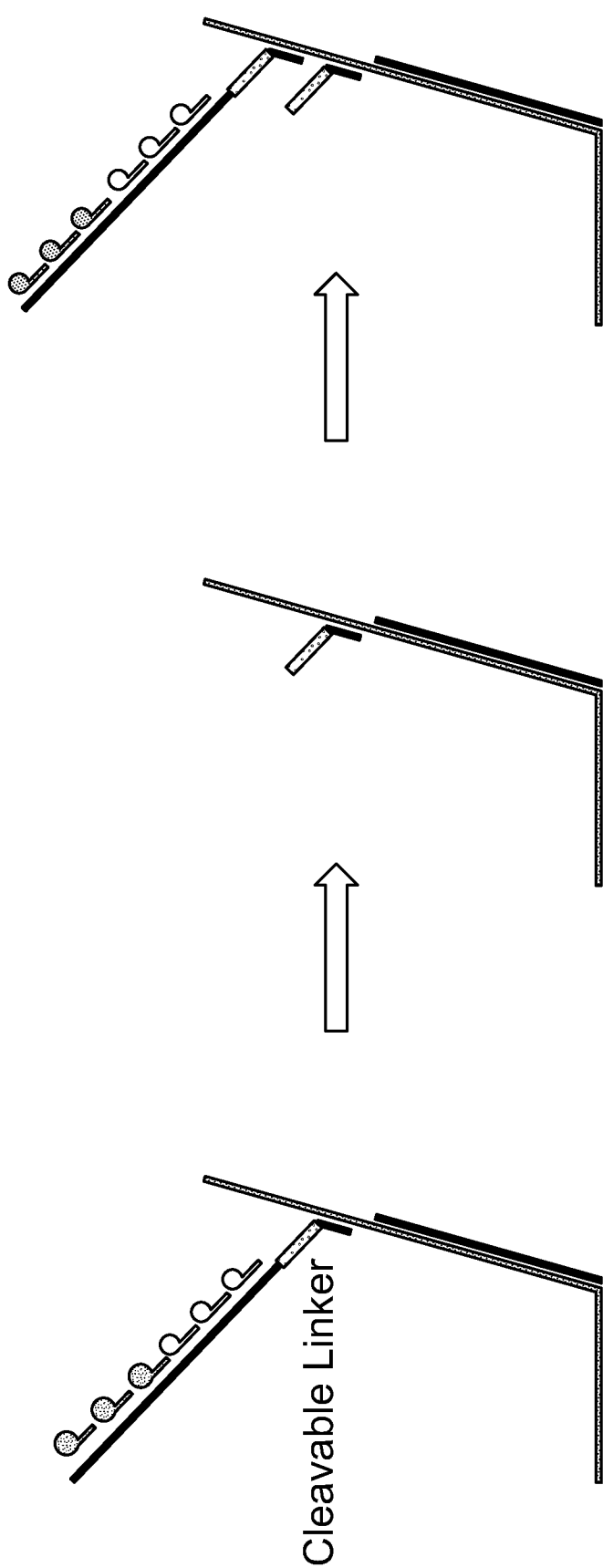


FIG. 22

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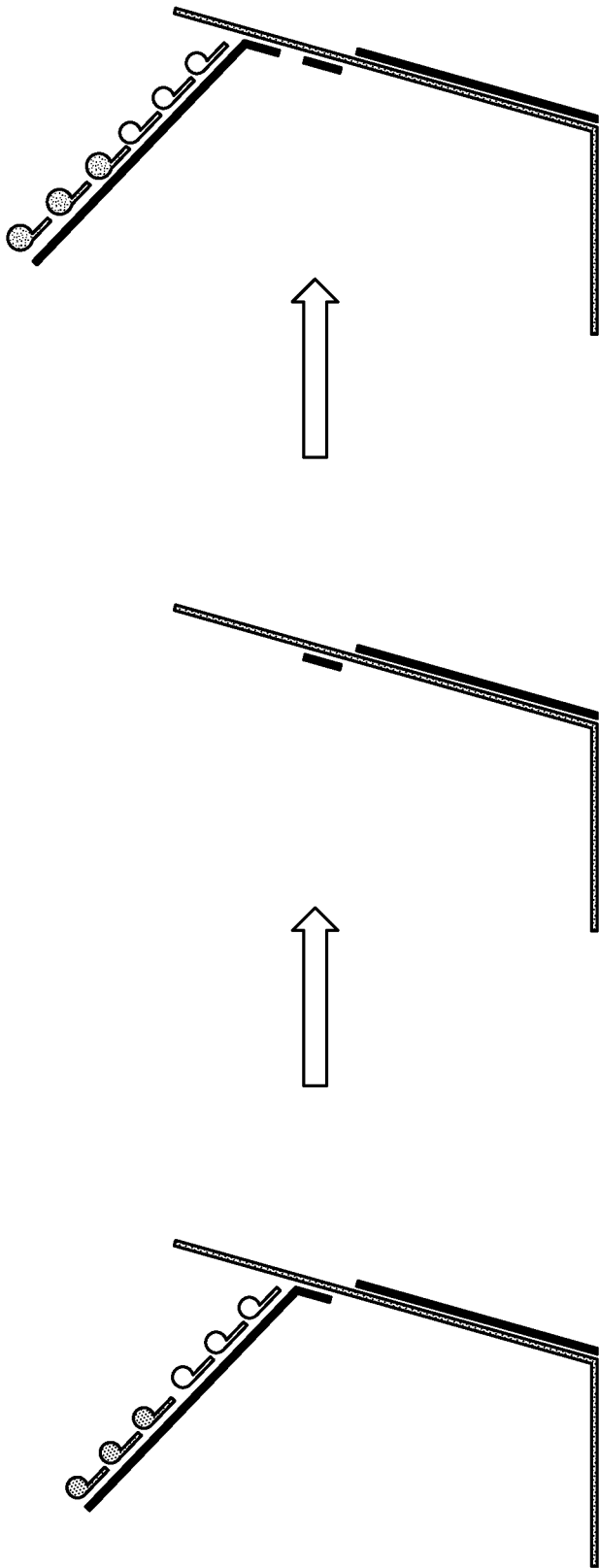


FIG. 23

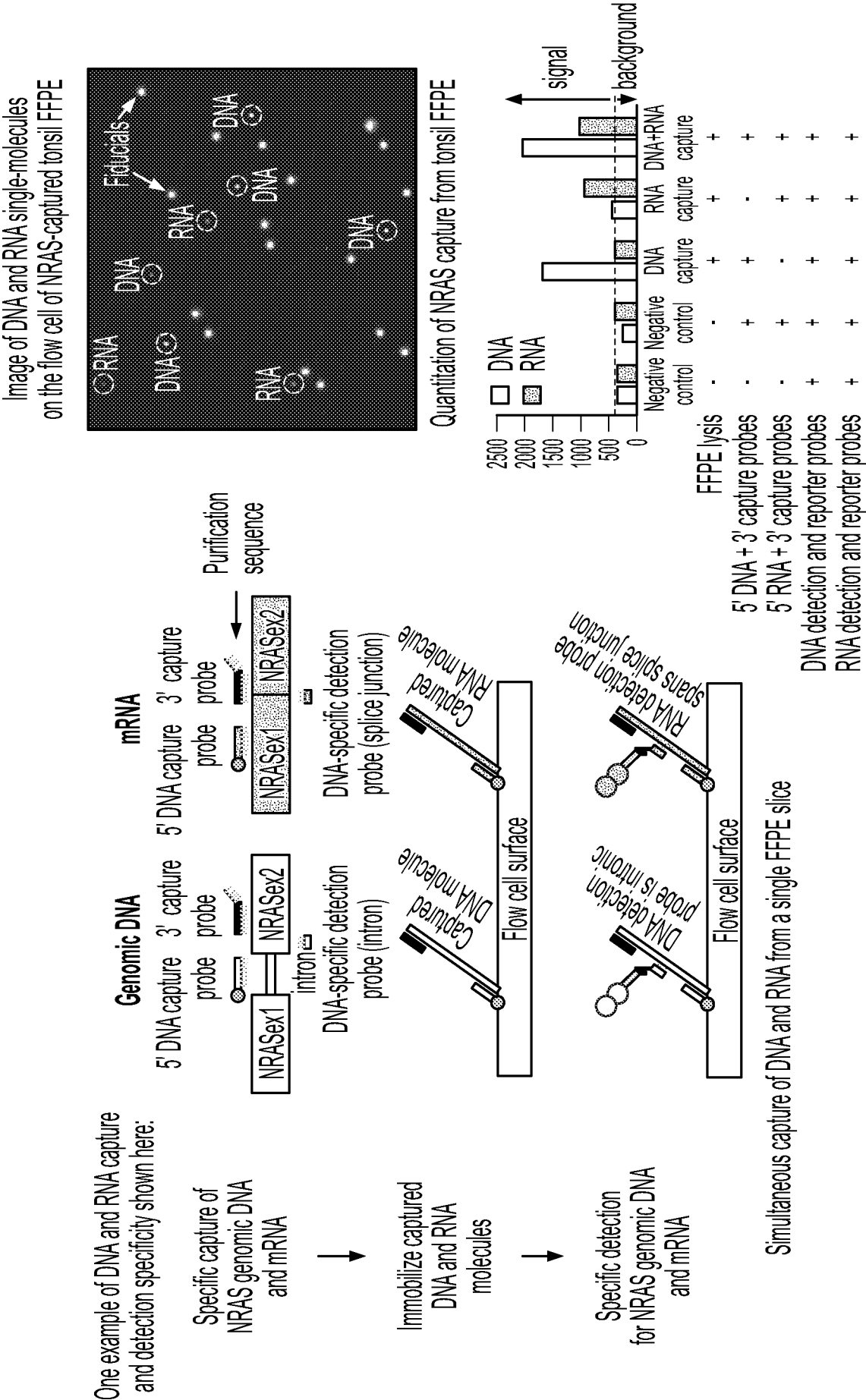


FIG. 24

Decoding Matrix									
●	⊗	⊗	○	⊗	⊗	⊗	⊗	⊗	⊗
TT CT GT CG CC TC AT CA									

				R1 R2 R3				Truth	Observed (%avg.)
Barcode 1:	C	T	T	T	A	C	C	●●●	1.6%
Barcode 2:	C	T	T	T	C	C	C	●●●	1.9%
Barcode 3:	C	T	T	T	T	C	C	●●●	1.7%
Barcode 4:	C	T	T	T	G	C	C	●●●	94.8%
NRAS exon2									
Barcode 5:	T	C	T	T	T	A	T	●●●	2.8%
Barcode 6:	T	T	C	T	T	C	C	●●●	1.7%
Barcode 7:	T	T	C	T	T	T	C	●●●	1.6%
Barcode 8:	T	T	C	T	T	G	T	●●●	93.9%
3'-ACCGTGACATGAGAAAGACAGGTCGCACATAGGTCAAC-5'									
(SEQ ID NO: 1)									

FIG. 25

Four calls of C
Two different probes observed
Voting High Quality Call

A	A	C	A	C	C	T			
A	A	C	A	C	C	T			
3'	-	A	A	C	A	C	C	T	- 5'

consensus (SEQ ID NO: 2)

Six calls of C from two different probes
One Call of A
Two different probes observed calling C
Voting High Quality Call

A	C	A	C	C	T				
C	C	A	C	C	T				
C	C	A	C	C	T				
A	A	C	A	C	C				
A	A	C	A	C	C				
A	A	C	A	C	C				
A	A	C	A	C	C				
3'	-	A	A	C	A	C	C	T	- 5'

consensus (SEQ ID NO: 2)

Three calls of C from two different Probes
Two Calls of A from a single probe
Voting Medium Quality Call

C	C	A	C	C	T				
A	C	A	C	C	T				
A	C	A	C	C	T				
C	C	A	C	C	T				
A	A	C	A	C	C				
3'	-	A	A	C	A	C	C	T	- 5'

consensus (SEQ ID NO: 2)

FIG. 26

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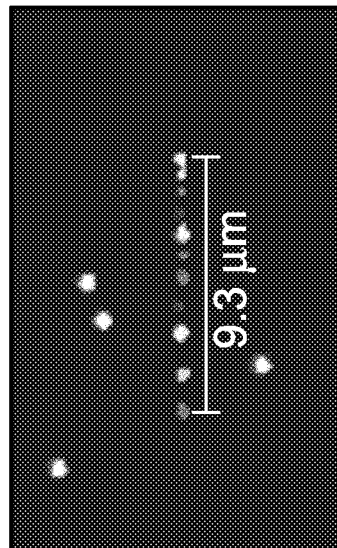
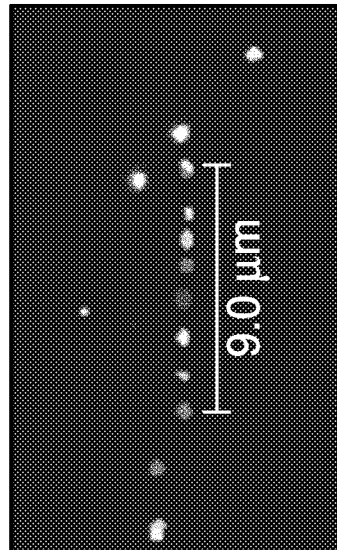
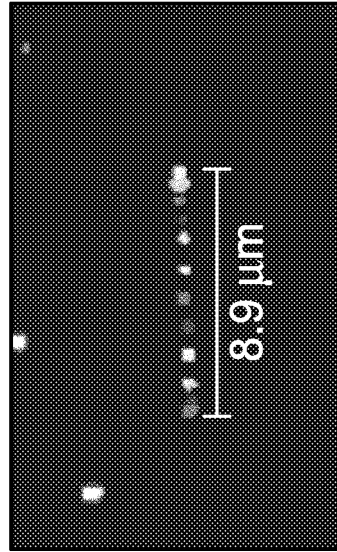


FIG. 27

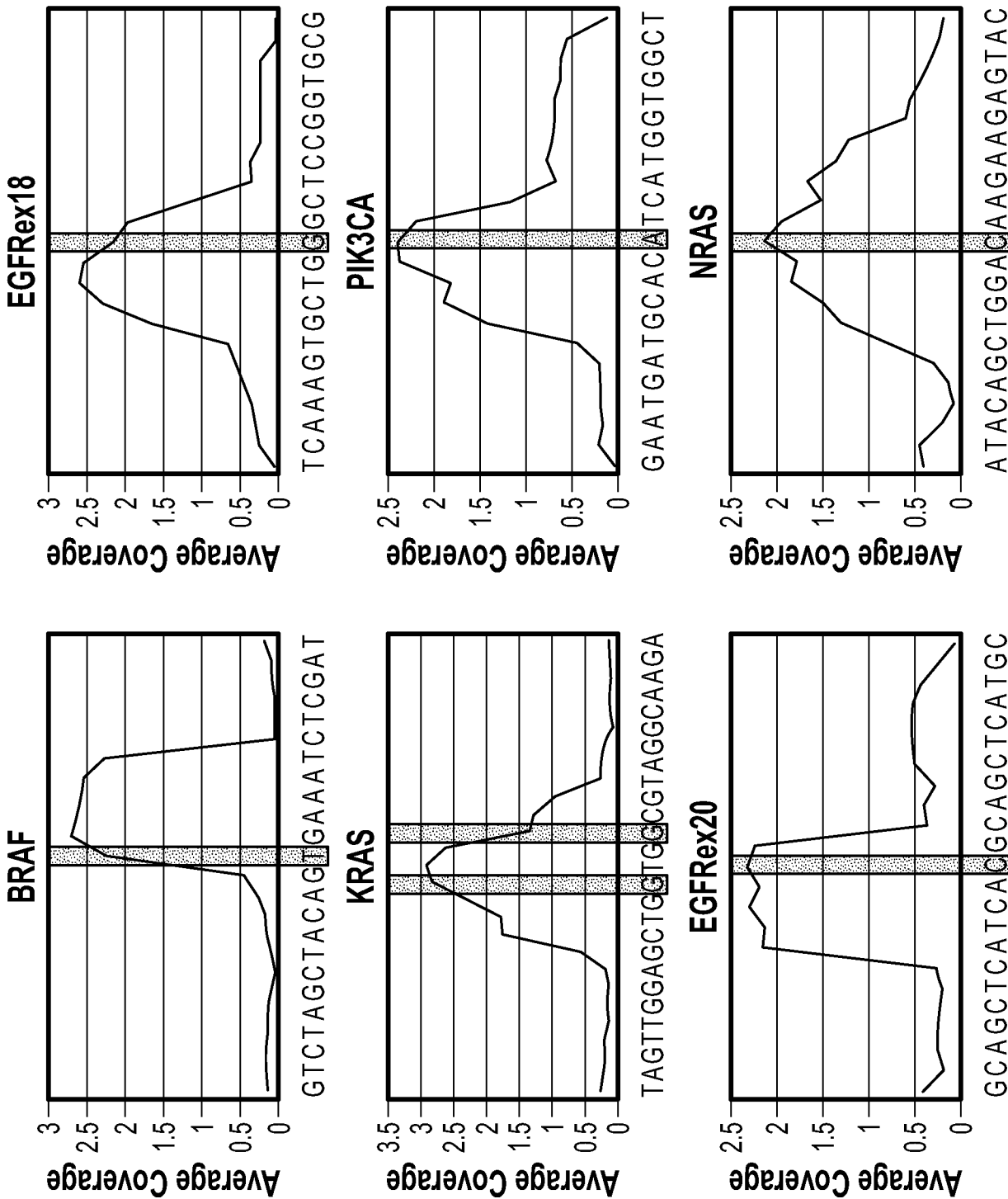
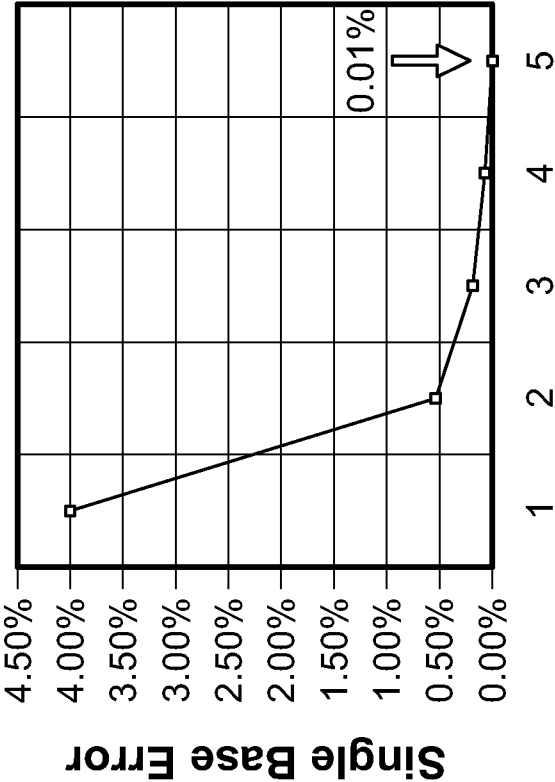


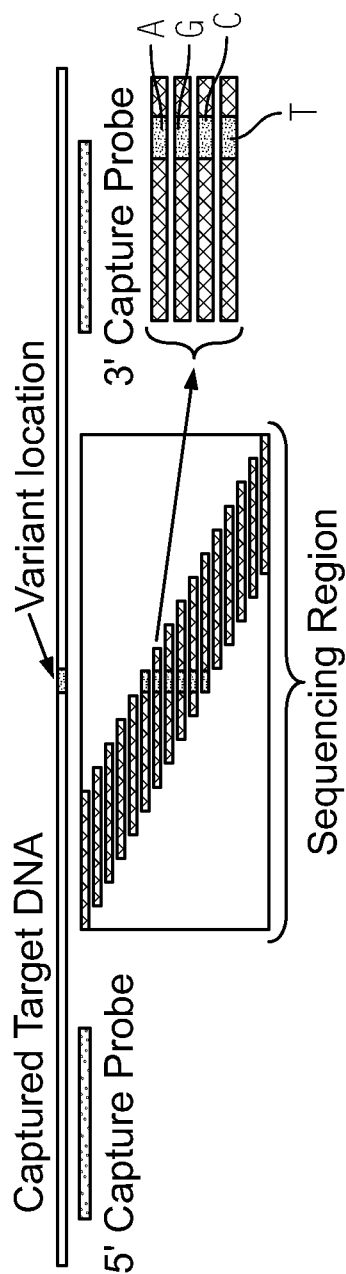
FIG. 28

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% 0.46% 0.005%	79.0% 6.0% 15.0%



Base Coverage FIG. 28 (Cont.)

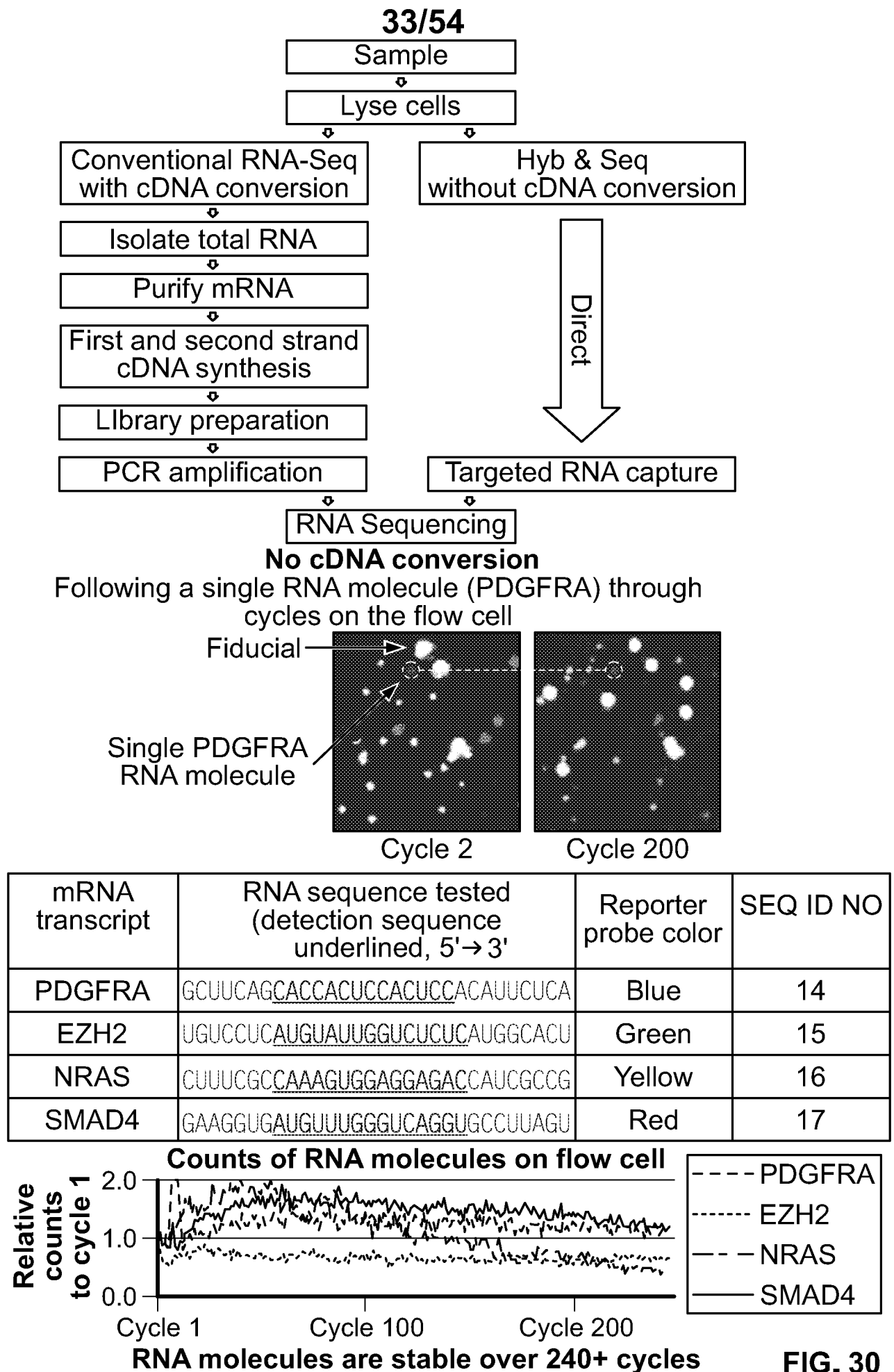
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Multiplex of 11 DNA Target are captured
Variants of interest are listed

Gene	Variant	Sequencing Region 5' → 3'	SEQ ID NO
BRAF	V600E	GTCTAGCTACAGGAAATCTCGAT	3
	V600K		
EGFR	G719S	TCAAAGTGCTGGCTCCGGTGCG	4
	G12A		
KRAS	G12R	TAGTTGGAGCTGCGTAGGCAAGA	5
	G13D		
PIK3CA	H1047R	GAATGATGCACATCATGGTGGCT	6
EGFR	T790M	GCAGCTCATCAGCAGCTCATGC	7
NRAS	Q61K	ATACAGCTGGAAGAAGAGTAC	8
FLT3	ΔI836	TGGCTCGAGATATGAGTGATTG	9
IDH1	R132C	TCATCATAGGTGTCATGCTTAT	10
JAK2	V617F	ATGGAGTATGTTCTGTGGAGAC	11
MEK1	P124L	GTGCAACTCTCGTACATCGTGG	12
NOTCH1	L1601P	CAGCCGGTGCAGCACACCAACG	13

FIG. 29



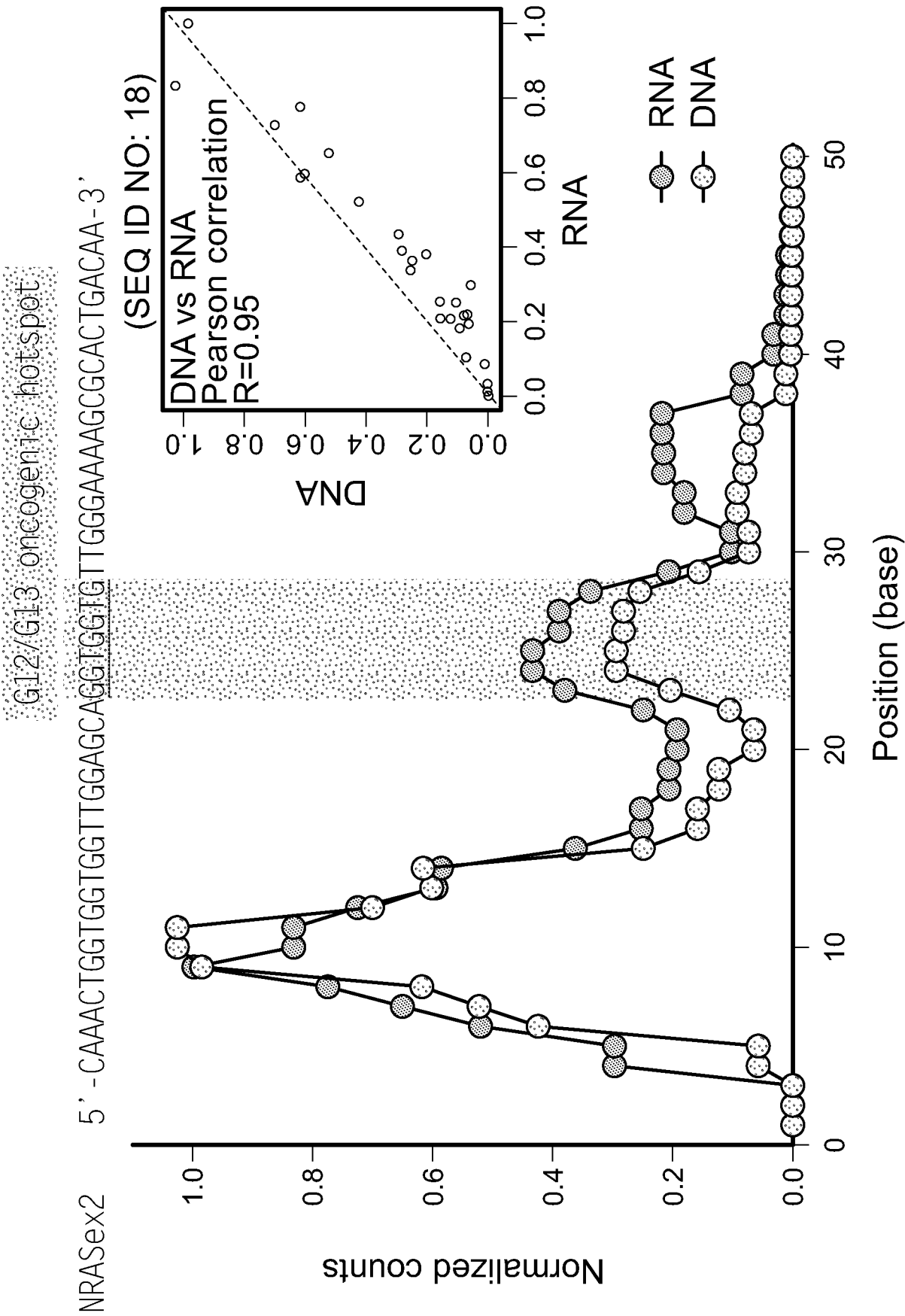
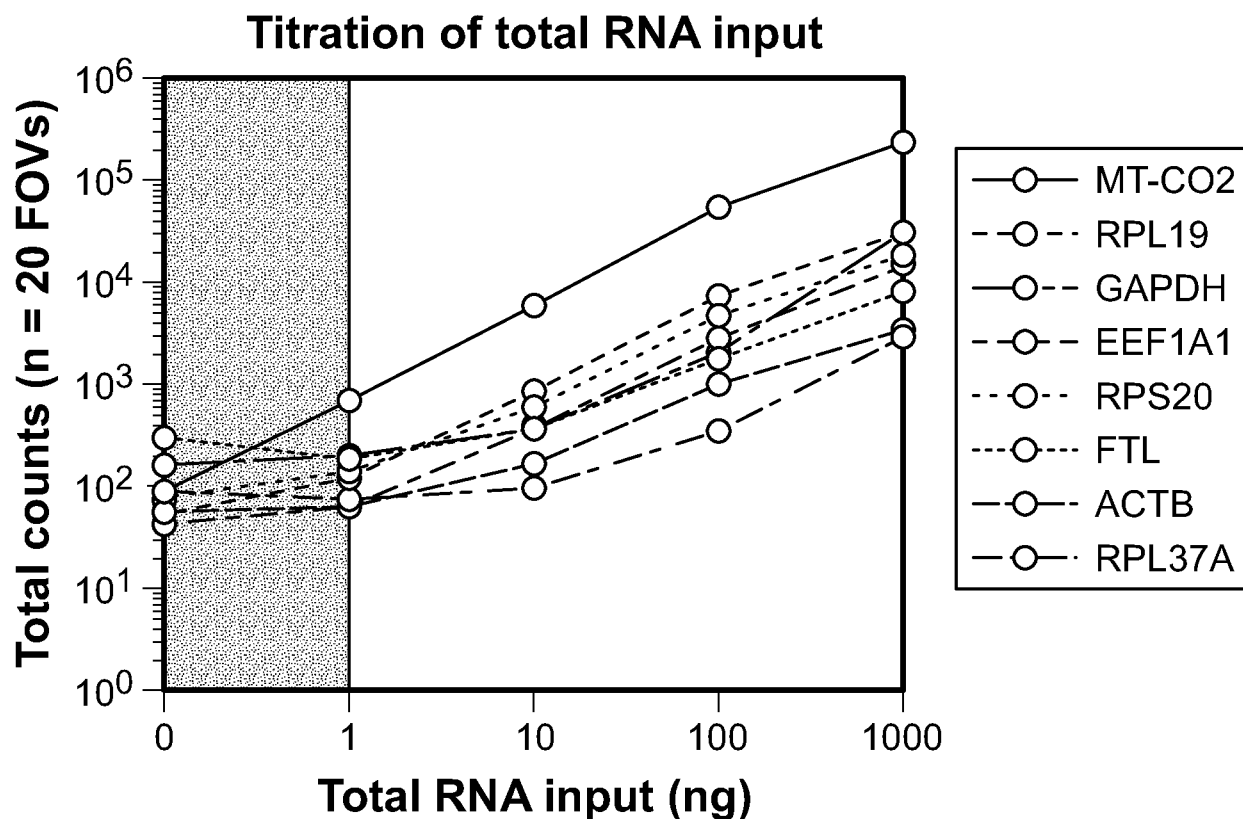


FIG. 31

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**Targeted multiplex capture with as little input
as 1-10 ng total RNA**

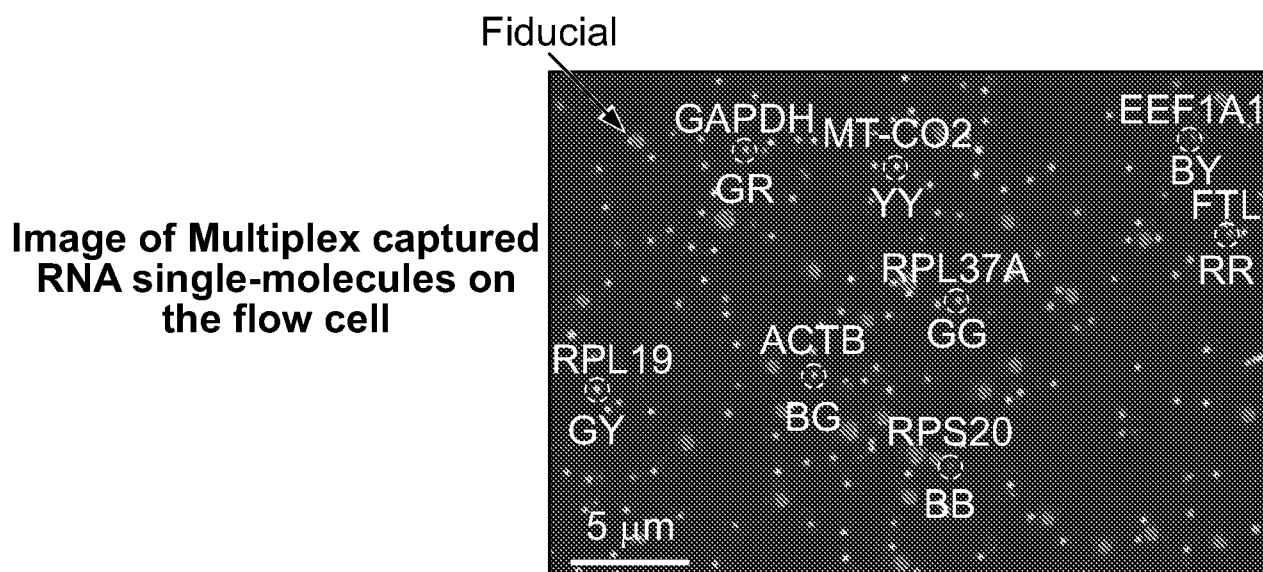


FIG. 32

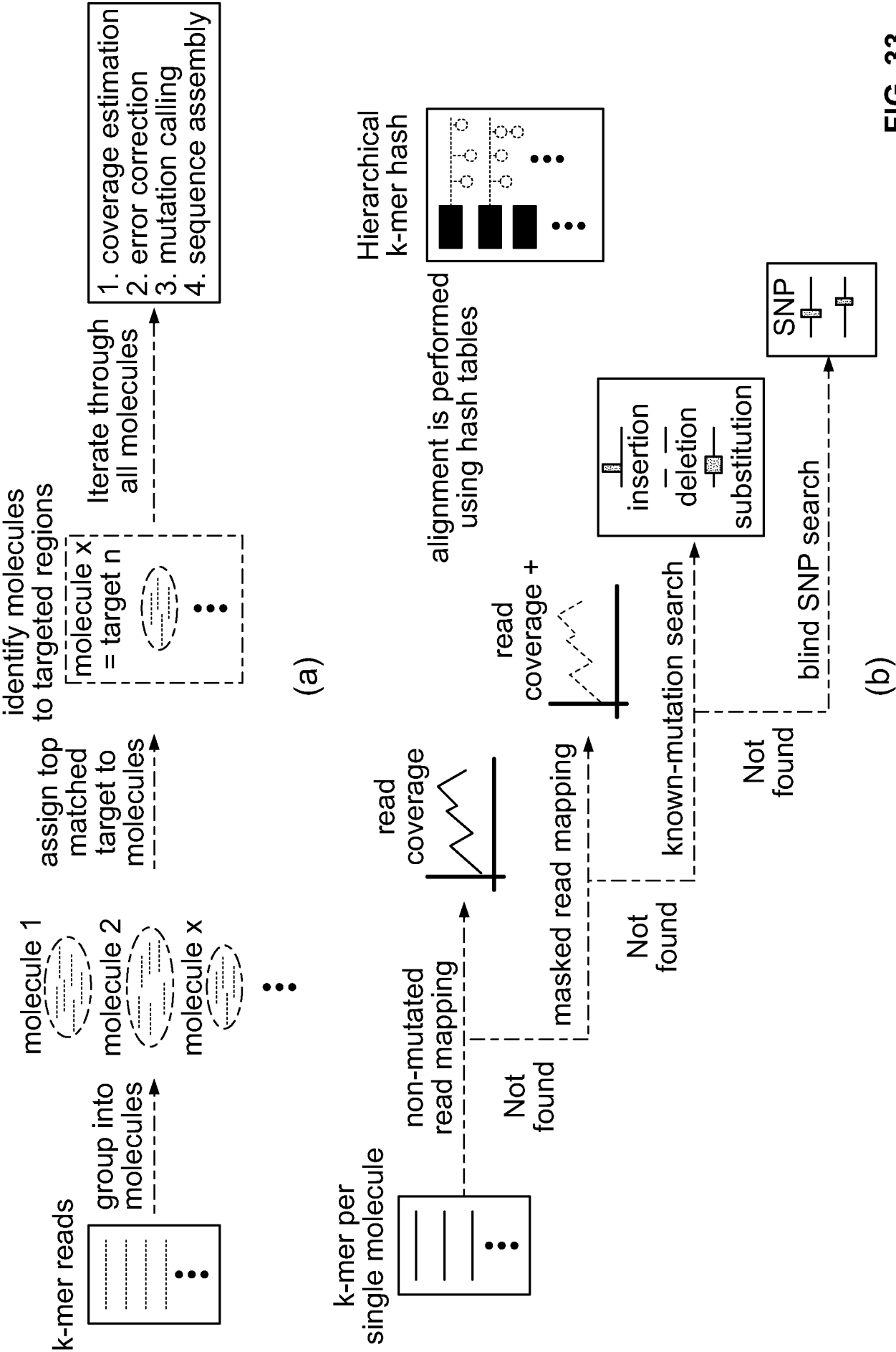
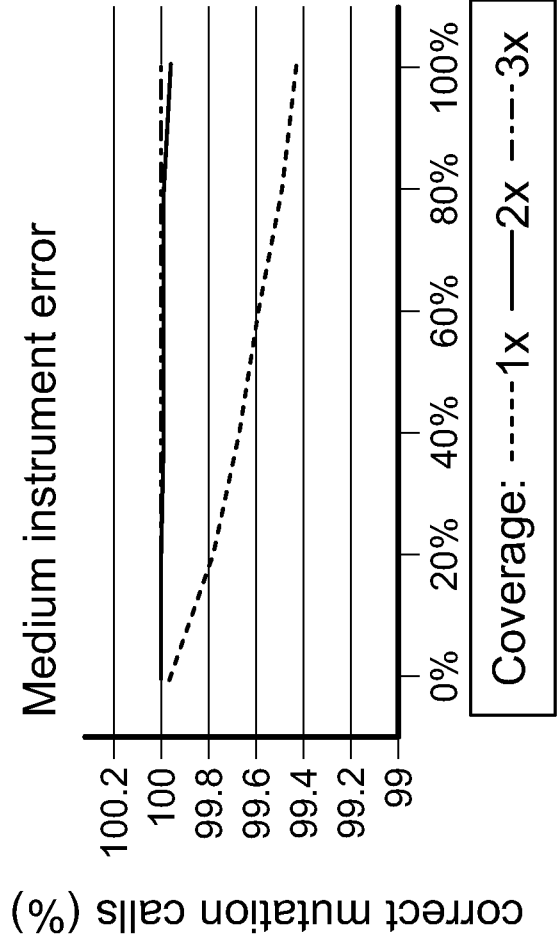
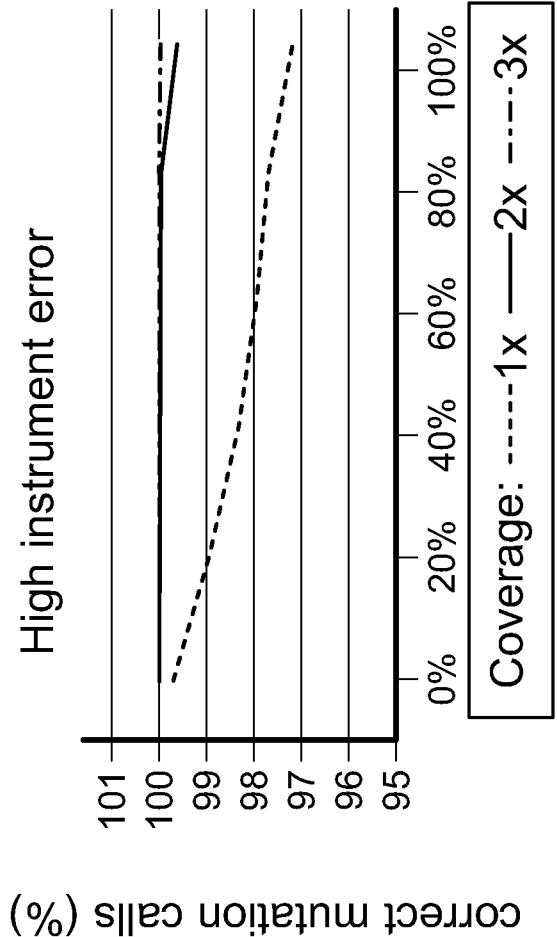


FIG. 33



(a)



(b)

FIG. 34

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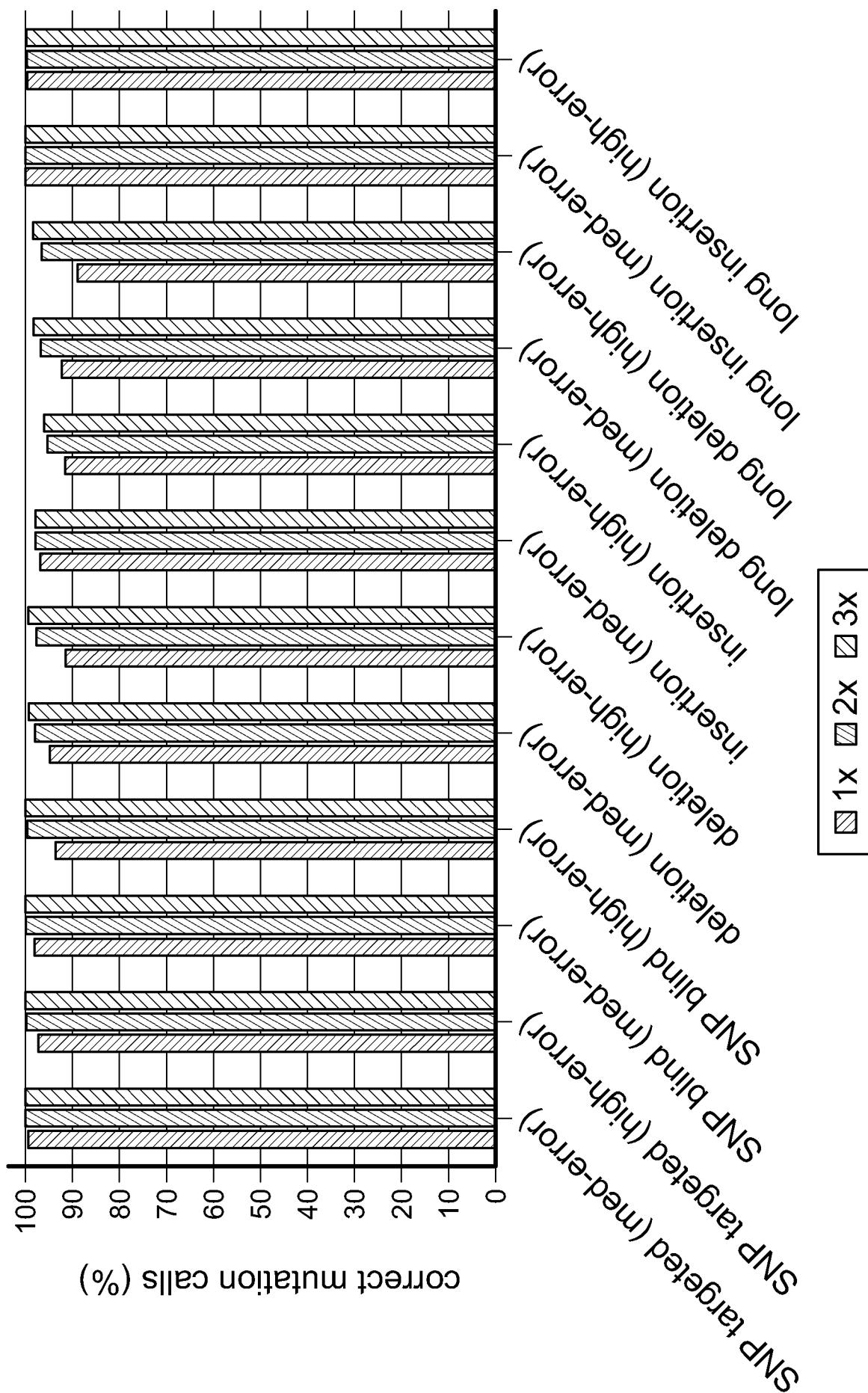


FIG. 35

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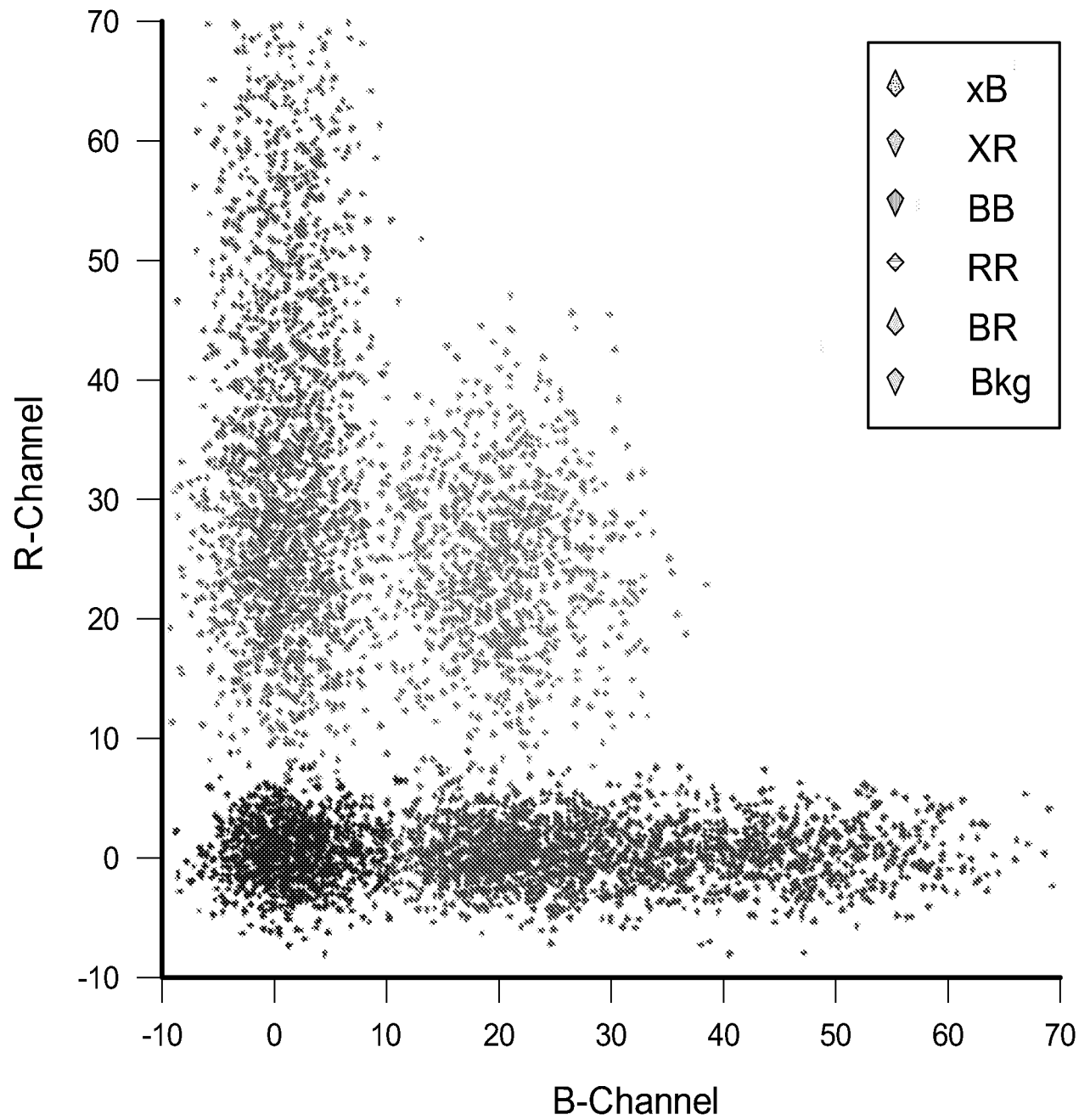


FIG. 36

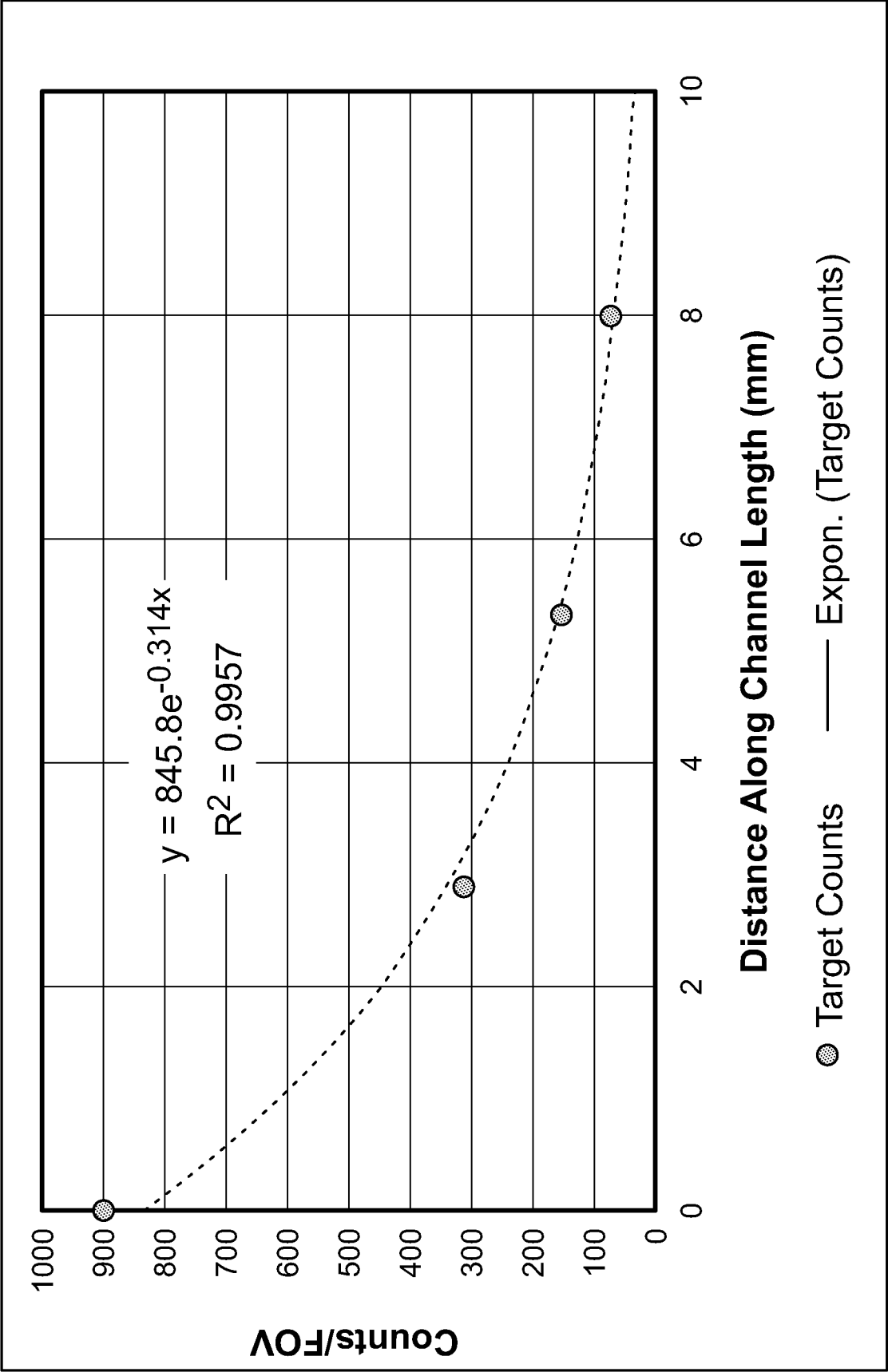


FIG. 37

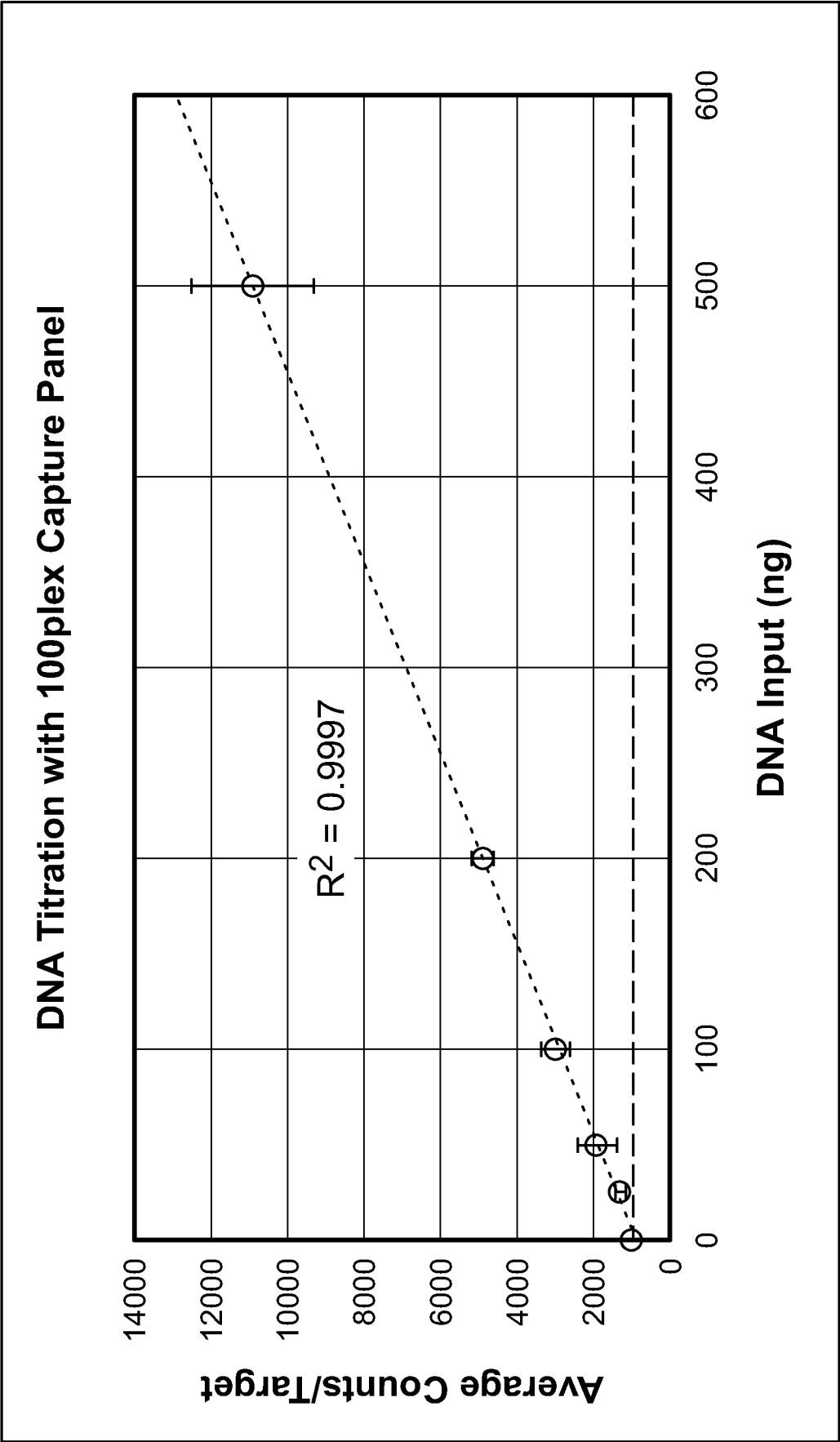
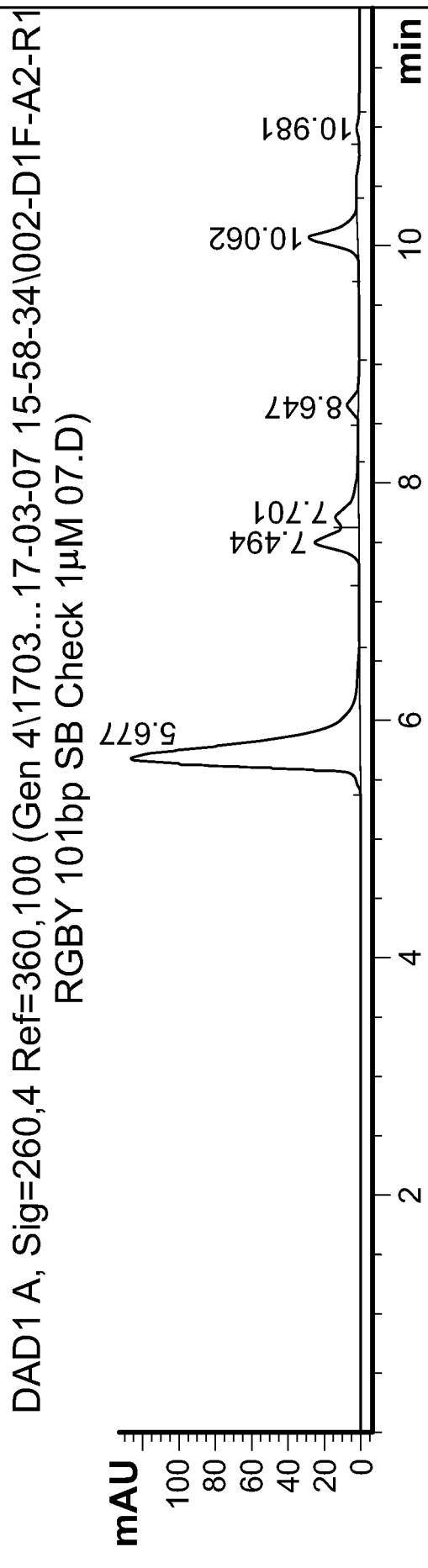


FIG. 38

Reporter Probe Unpurified Construct



Reporter Probe Purified Construct

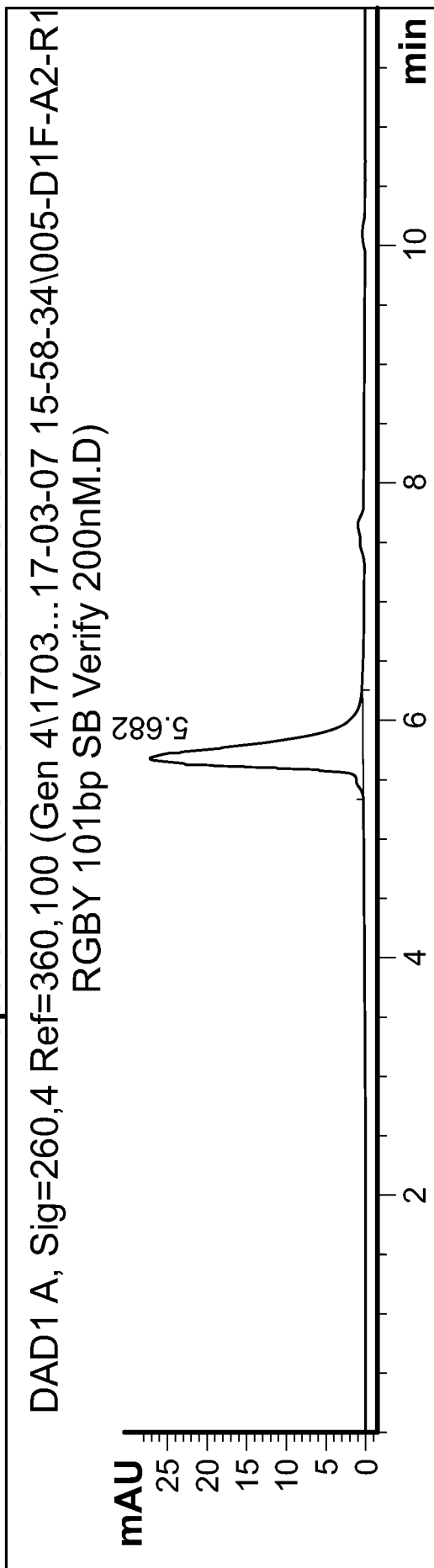


FIG. 39

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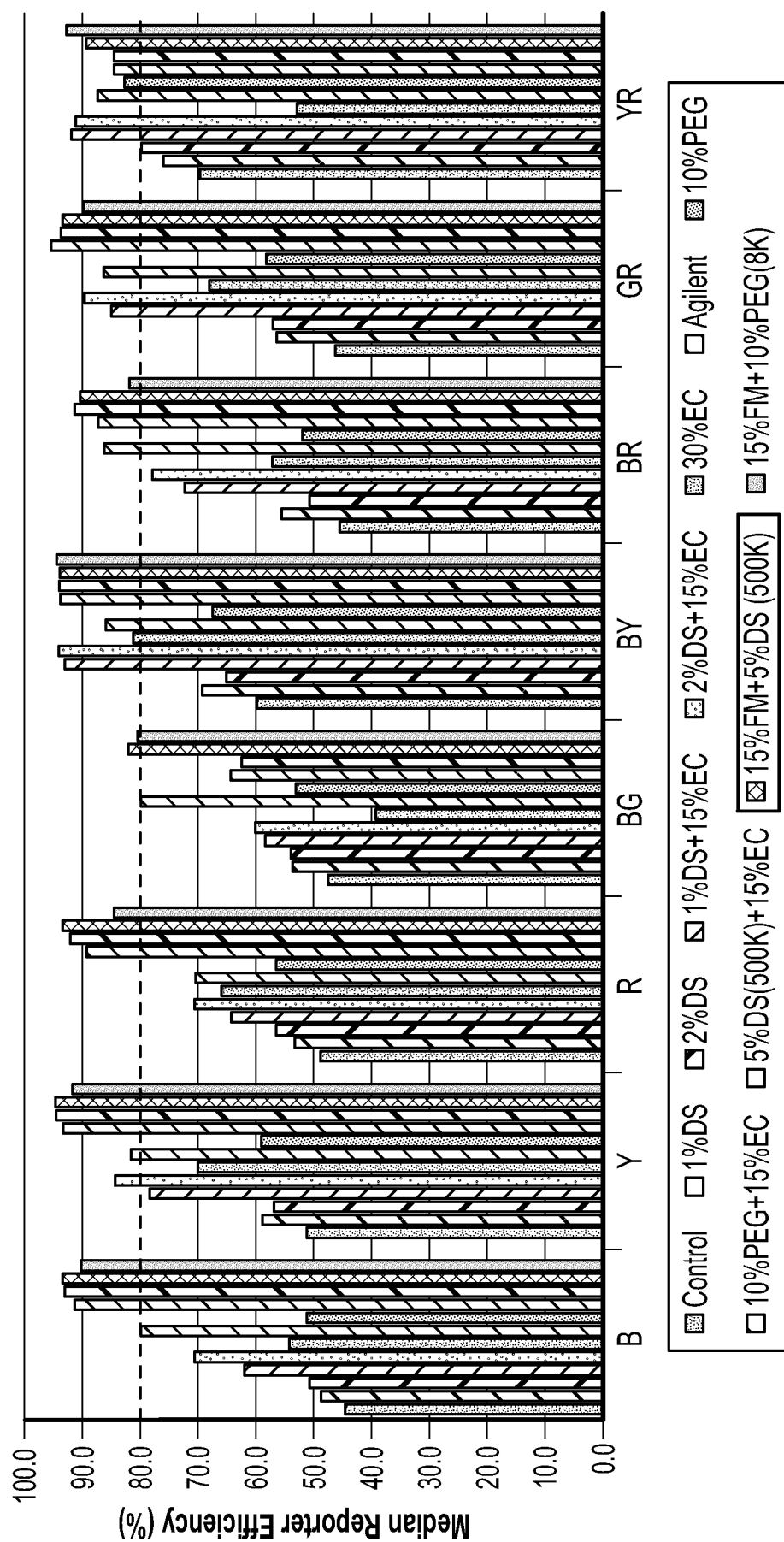


FIG. 40

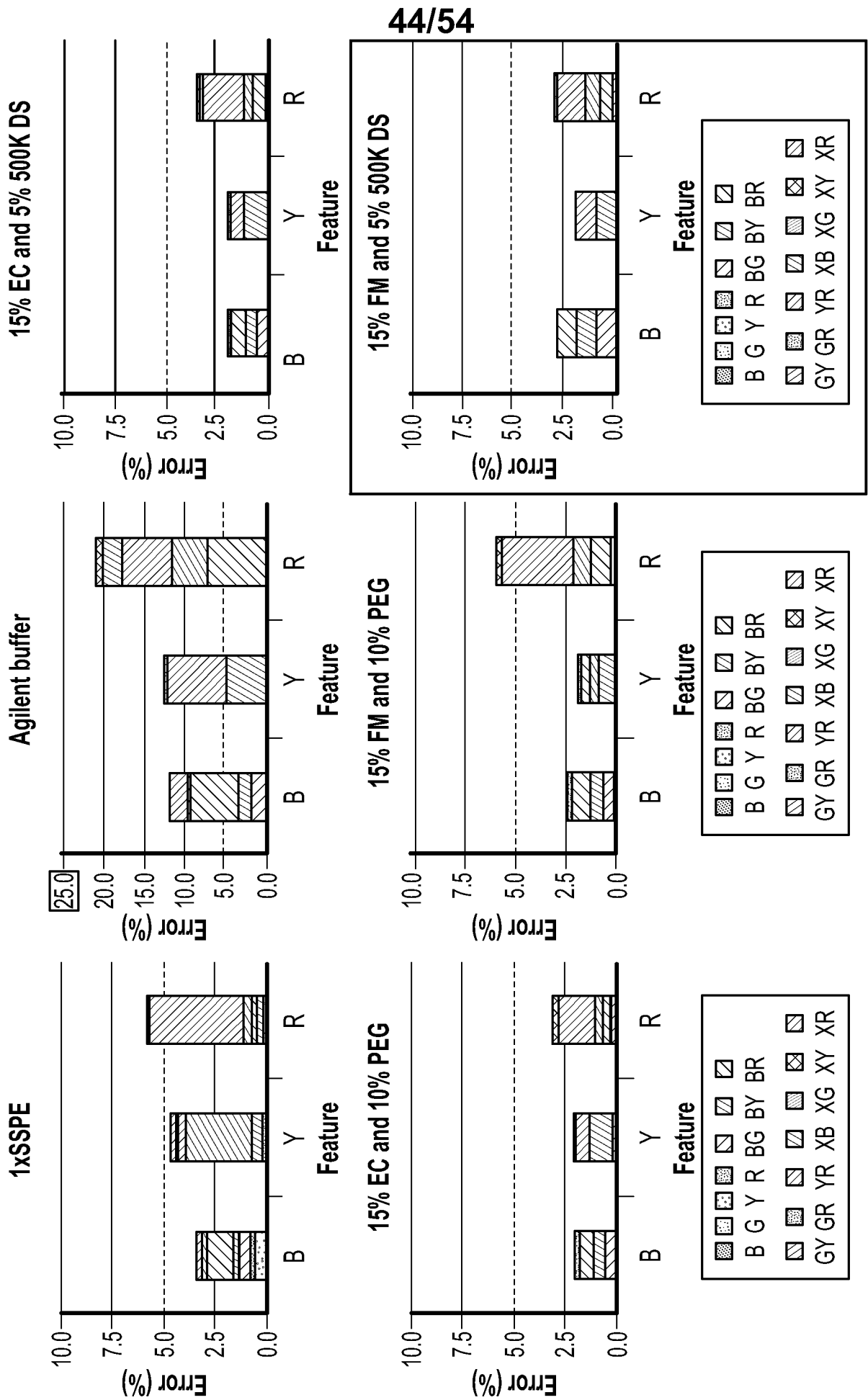
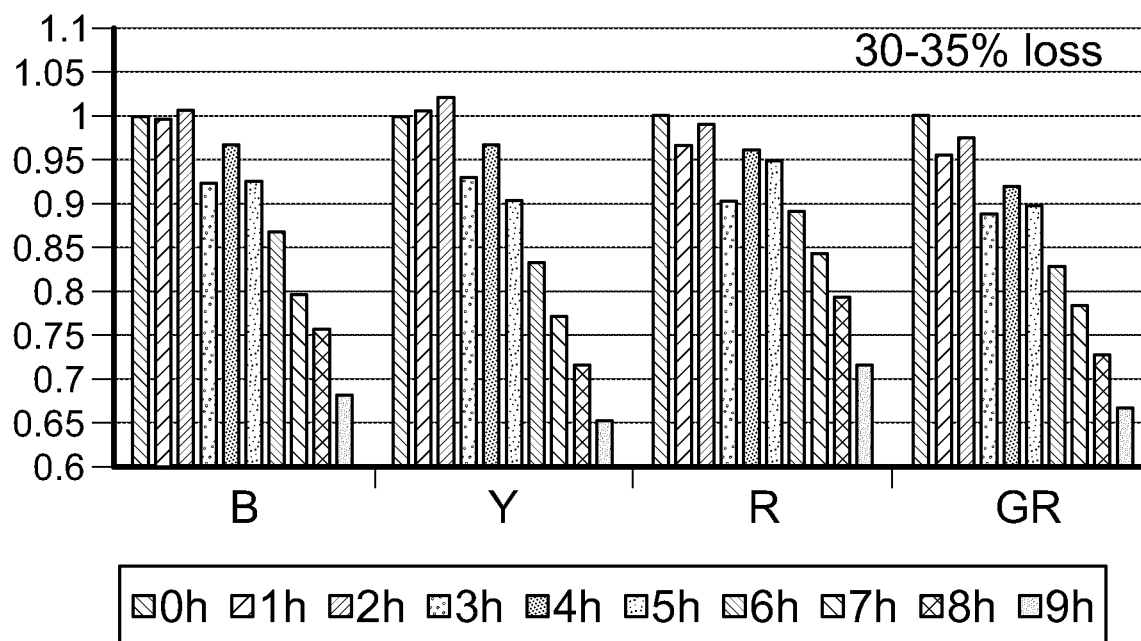


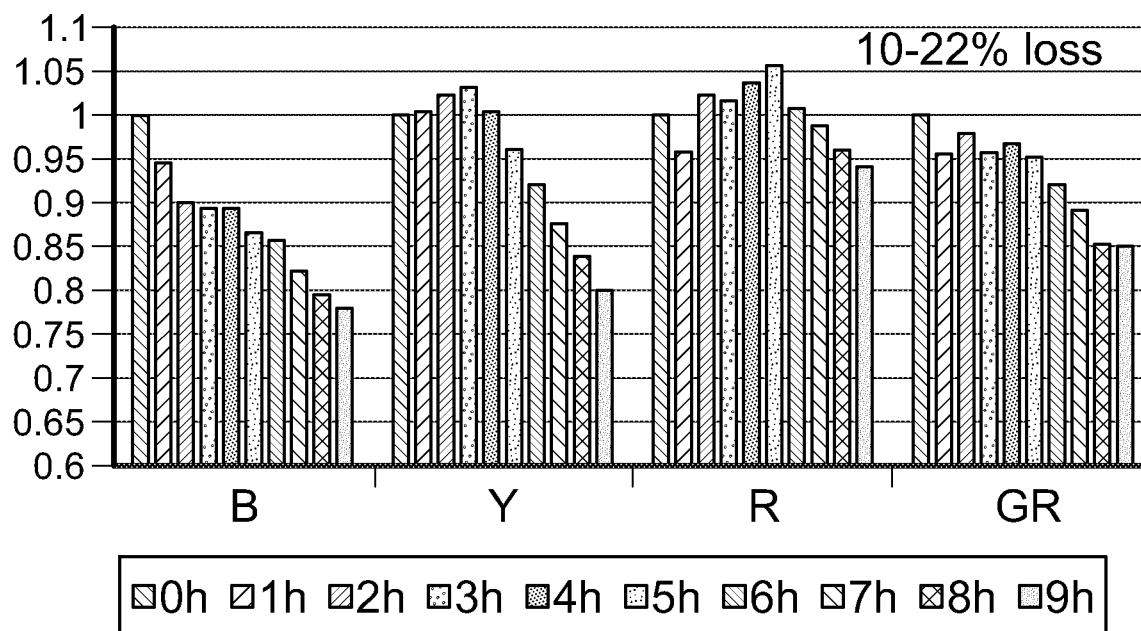
FIG. 40 (Cont.)

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15% EC at pH 6



15% formamide

**FIG. 41**

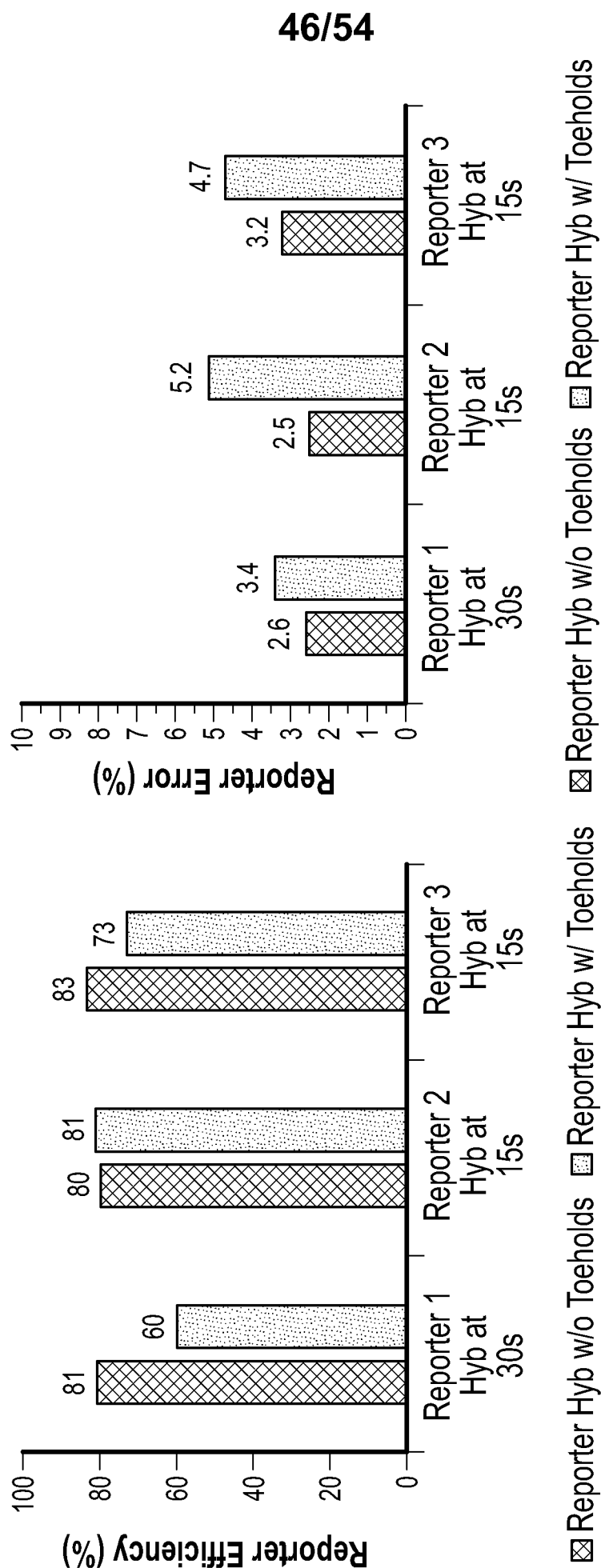


FIG. 42

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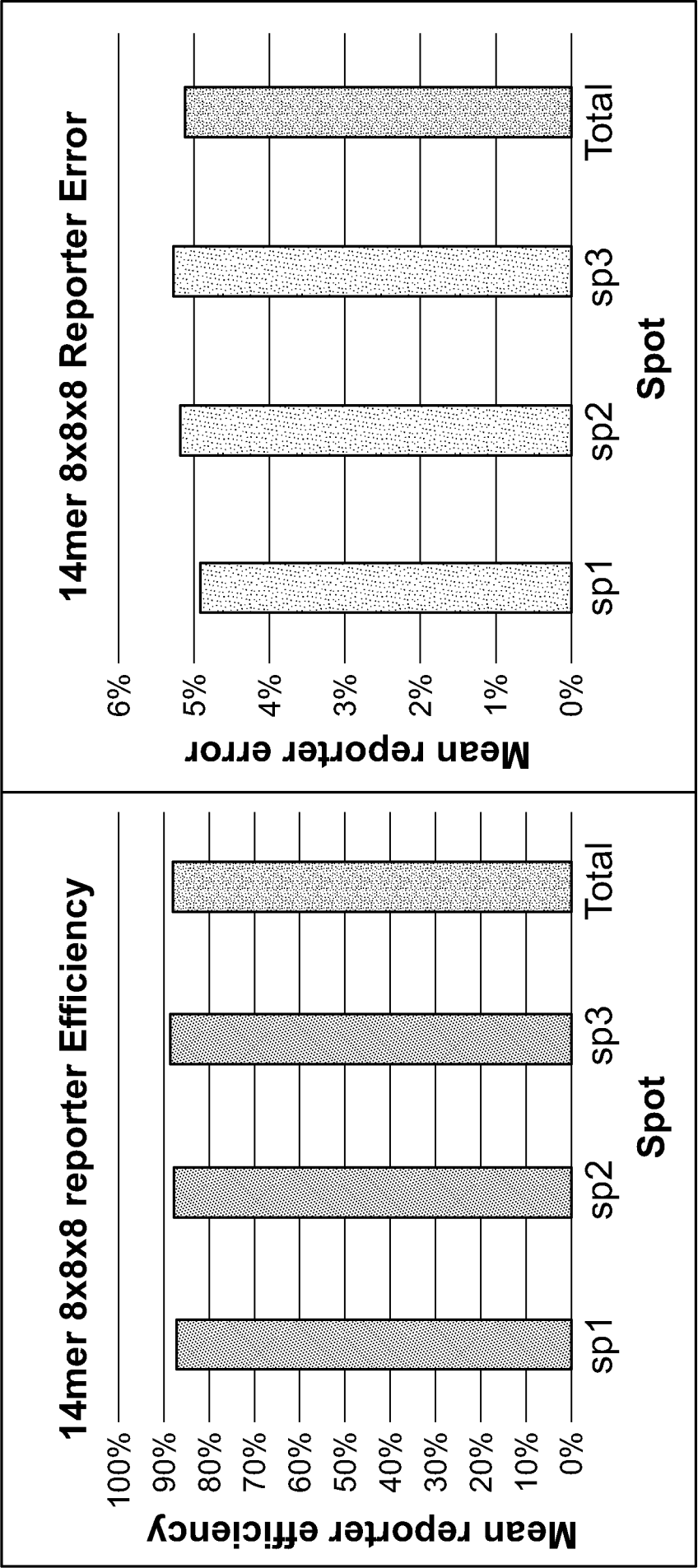


FIG. 43

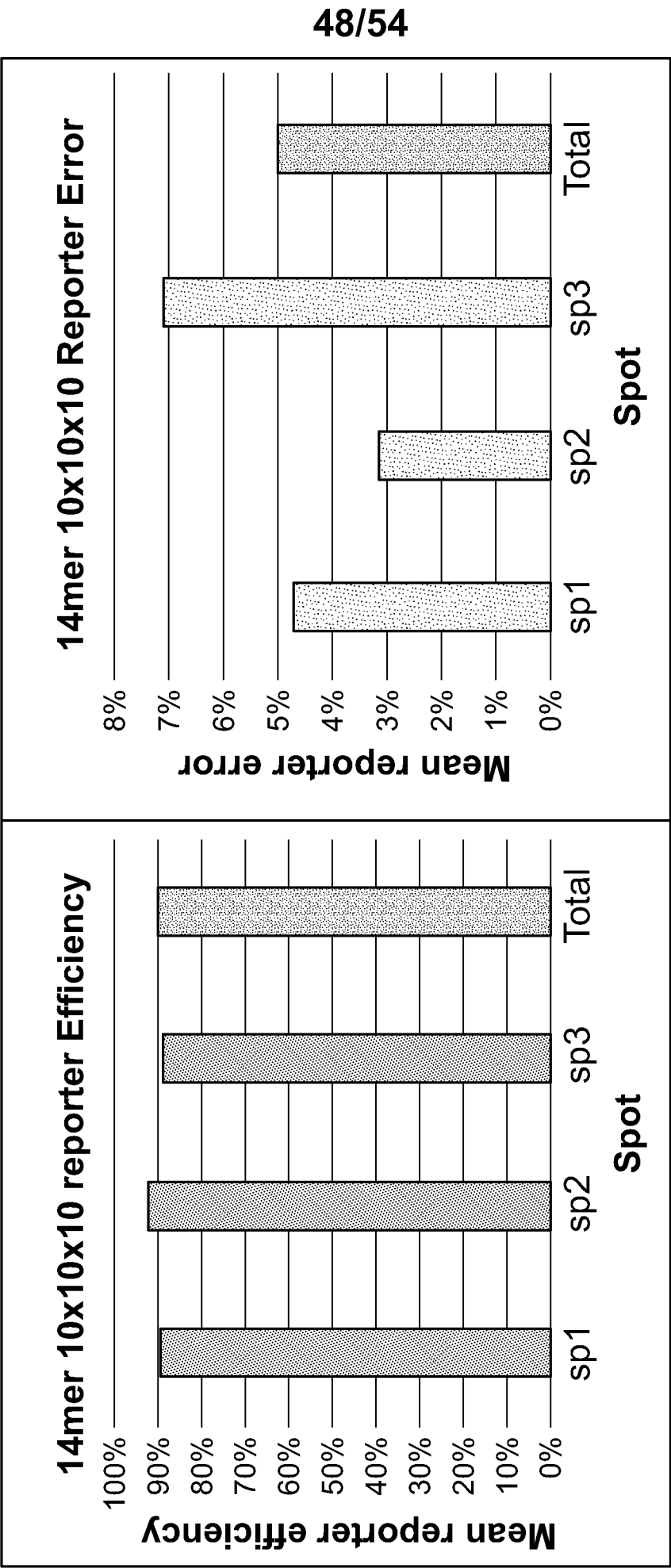


FIG. 44

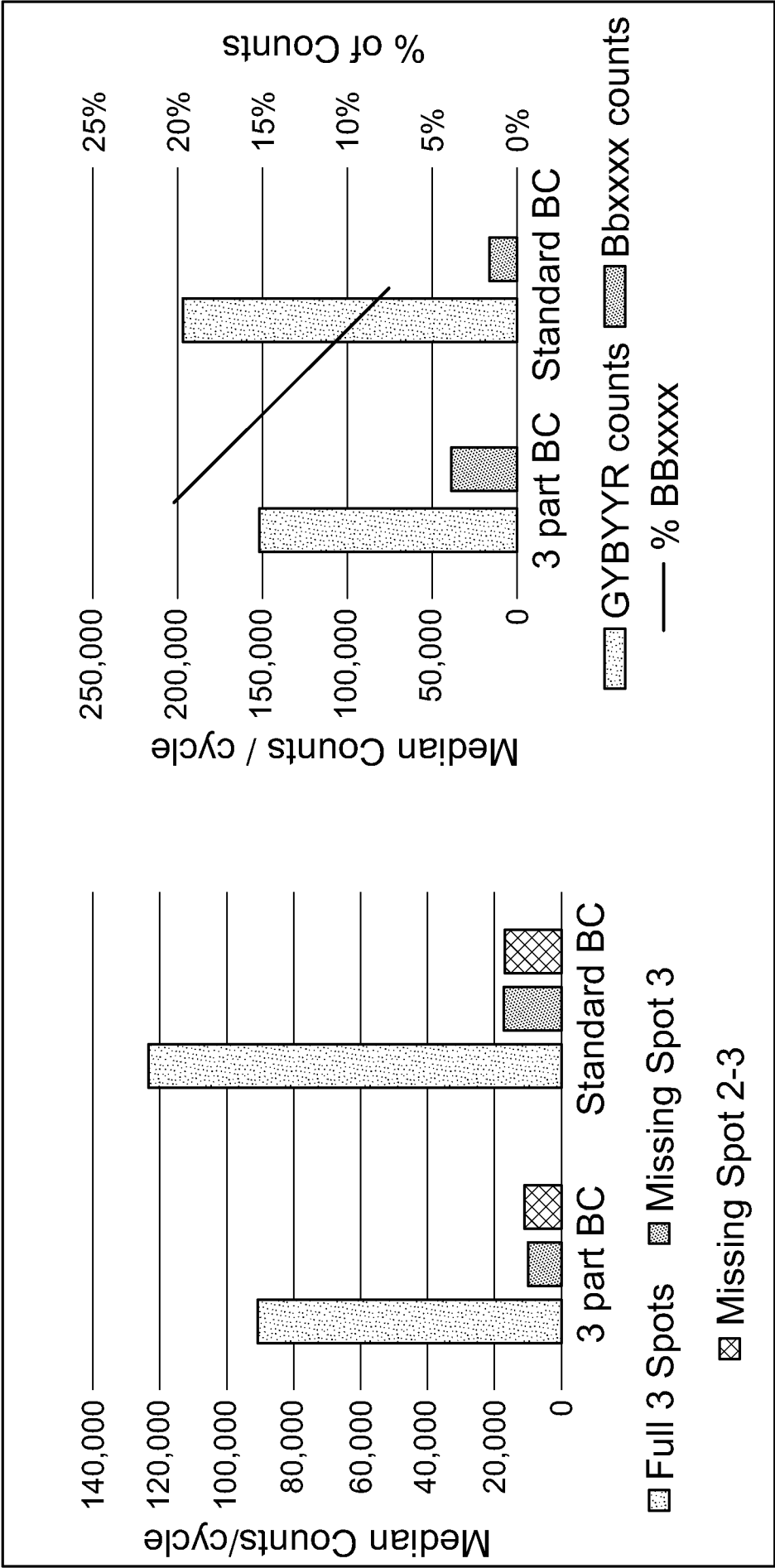


FIG. 45

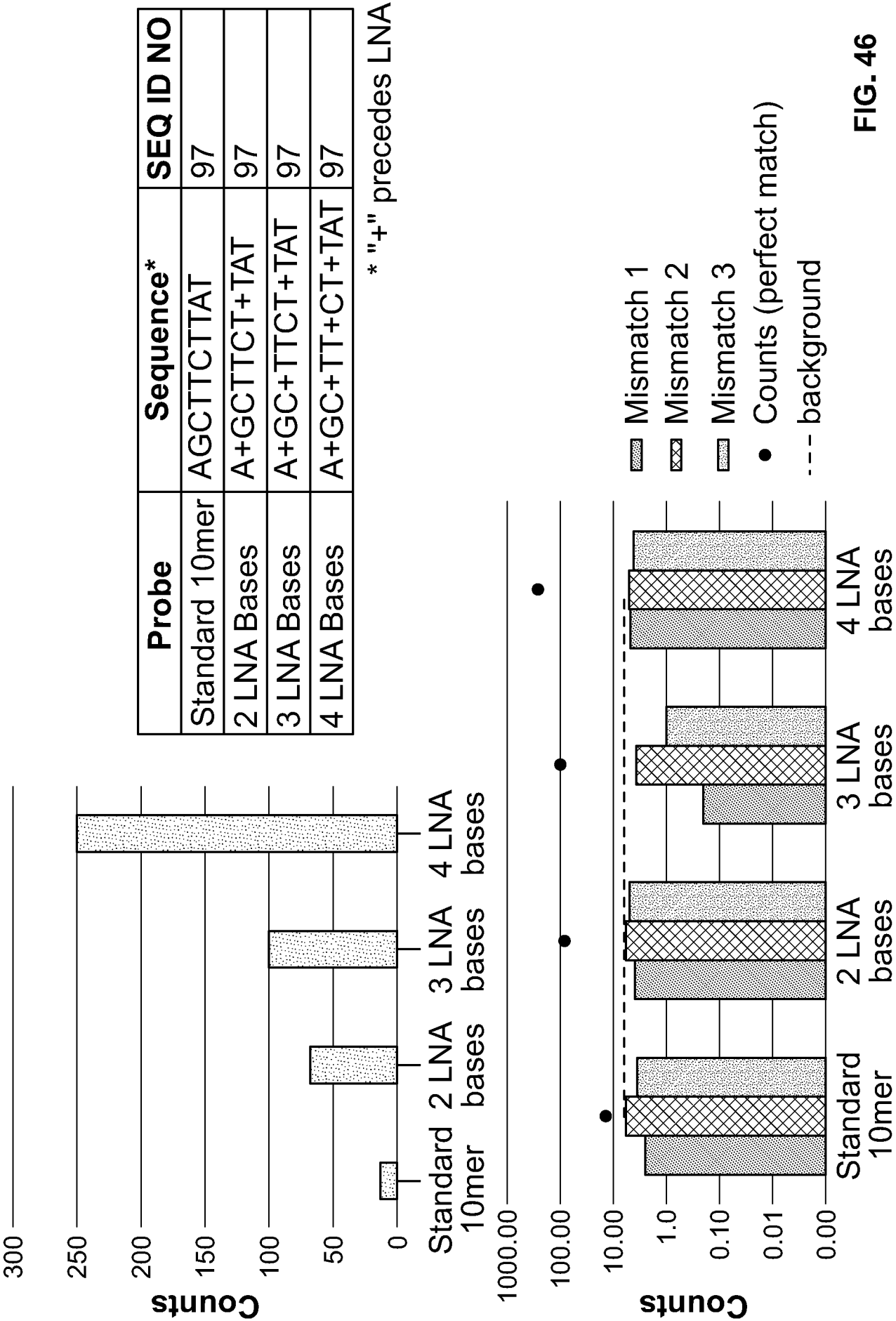


FIG. 46

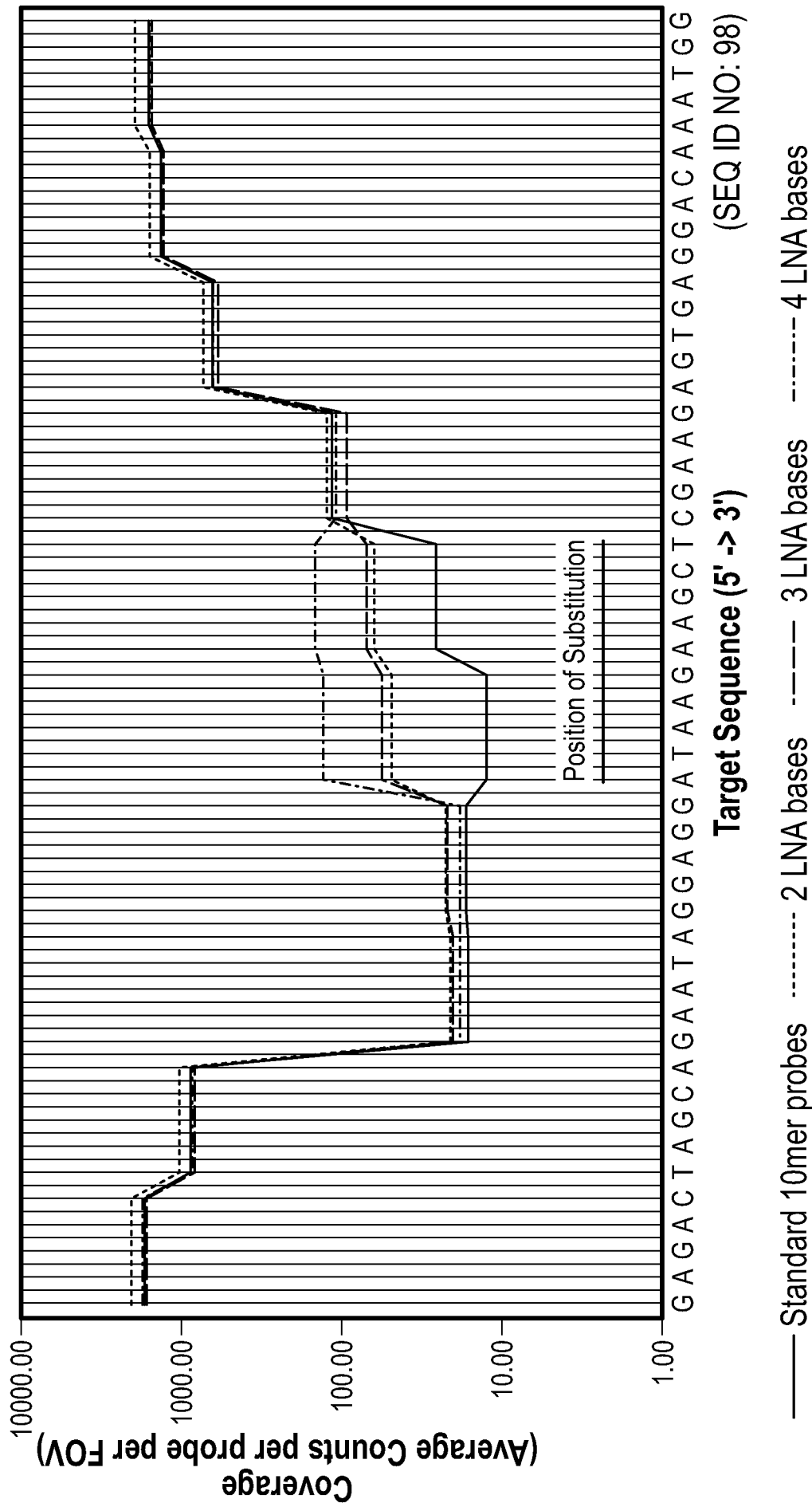


FIG. 47

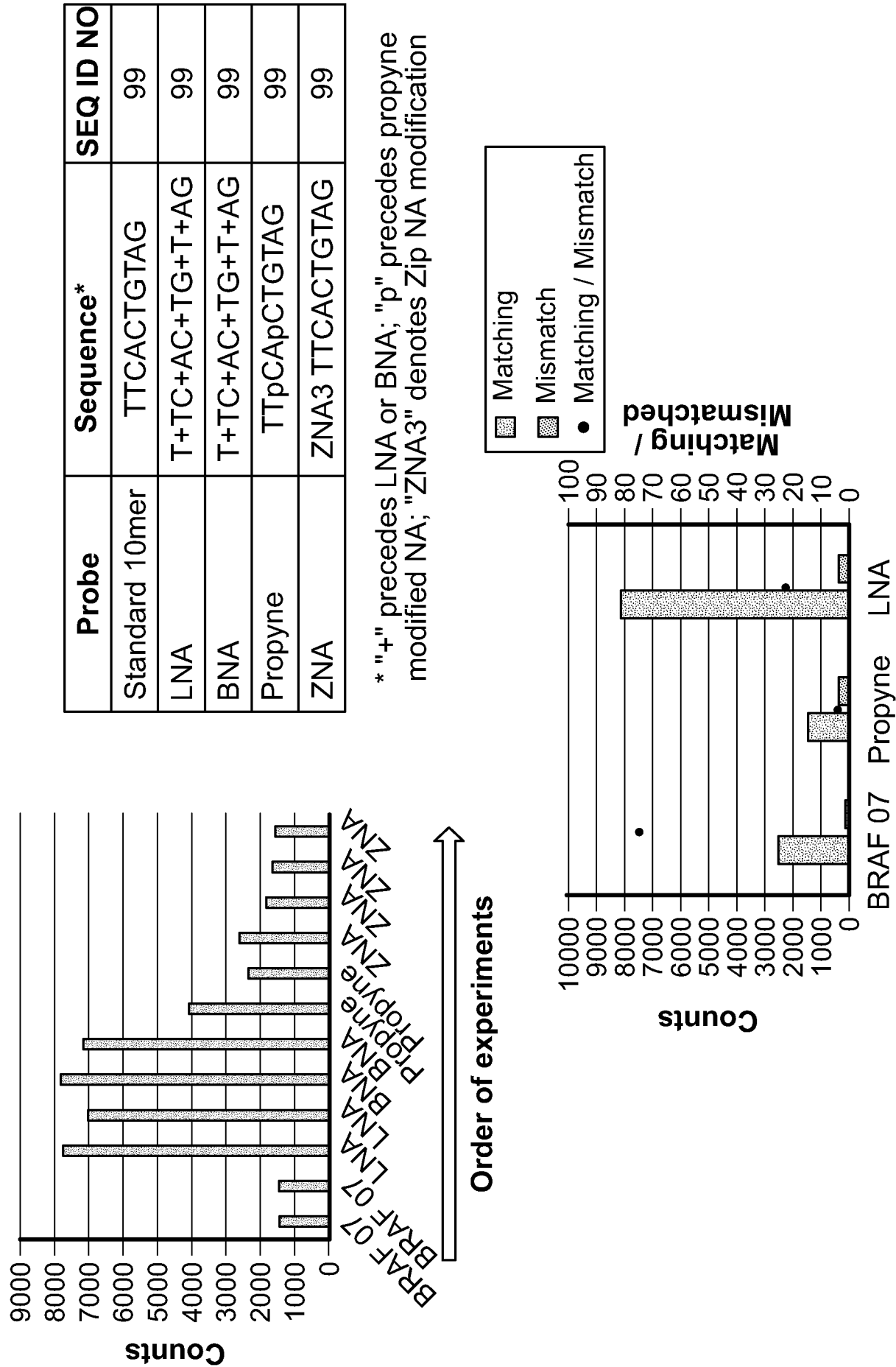


FIG. 48

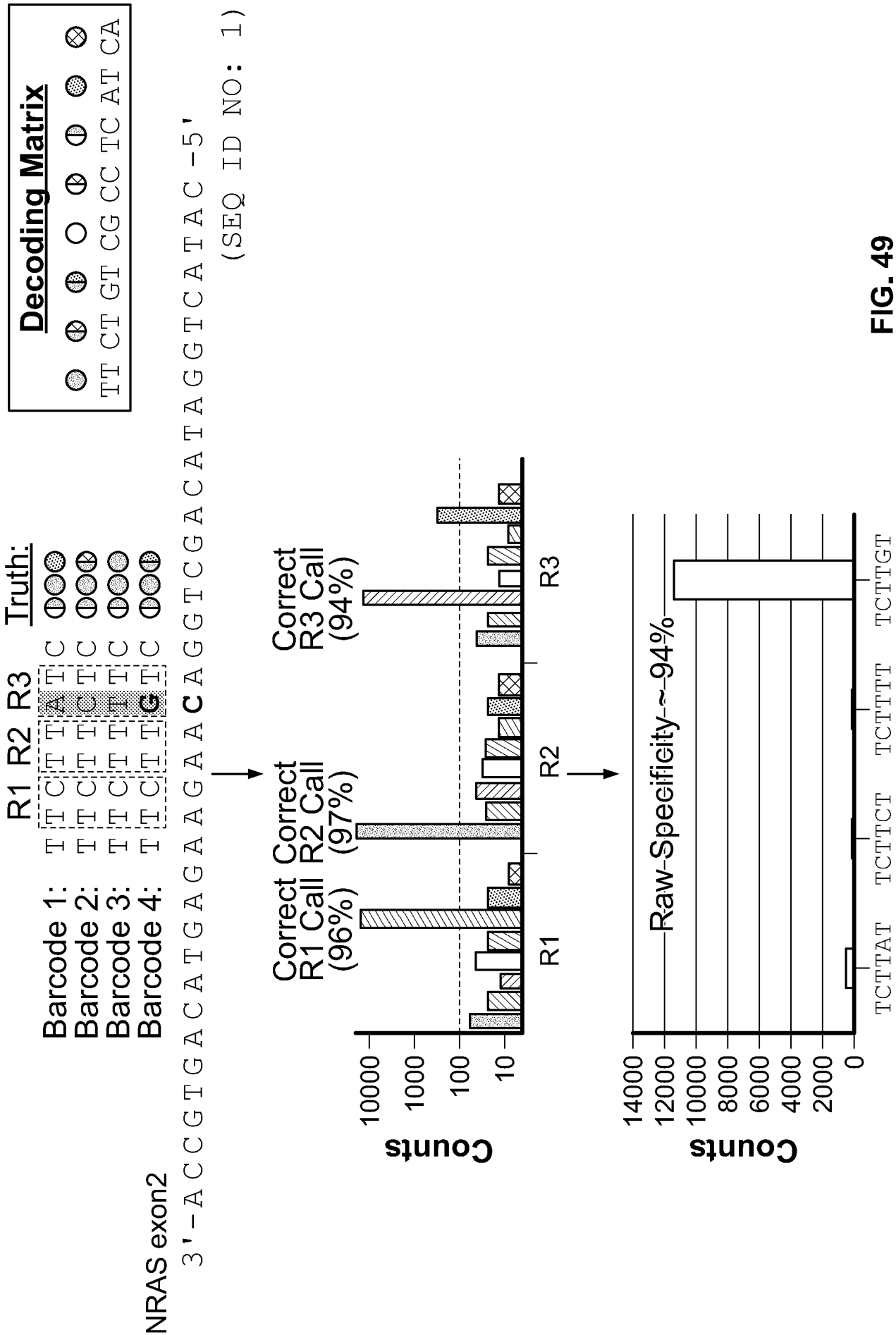


FIG. 49

Hyb & Seq Chemistry Using Dual-color Reporters has Low Raw Error Rate and the Source of Error can be Classified into Two Areas

<u>Decoding Matrix</u>							
							
TT	CT	GT	CG	CC	TC	AT	CA

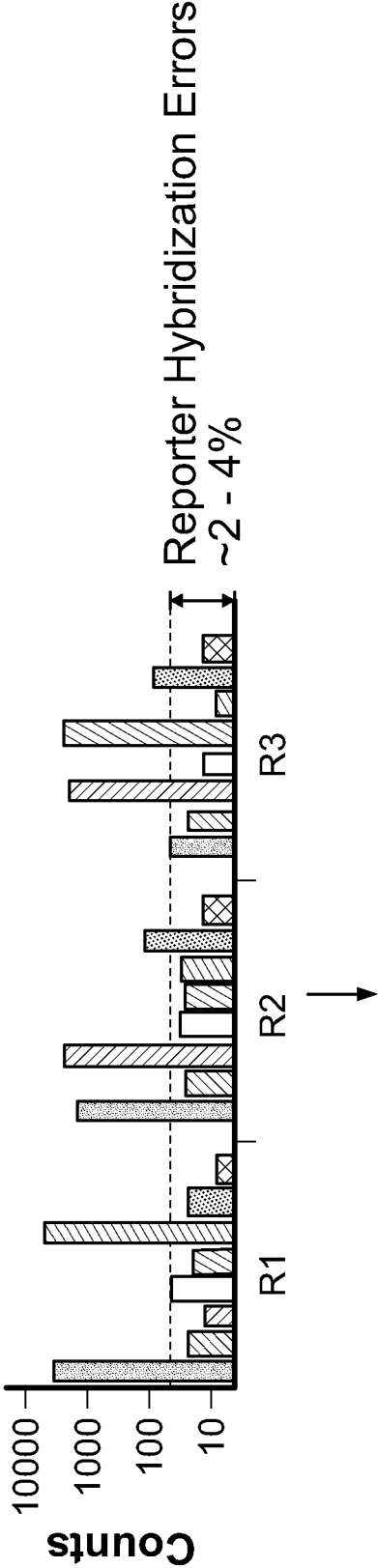
	R1	R2	R3	Truth:
Barcode 1:	C	T	T	A
Barcode 2:	C	T	T	C
Barcode 3:	C	T	T	C
Barcode 4:	C	T	T	C

	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' - ACCGTGACATGAGAGAA CAGGTCGACATAGGTCATAC -5'

(SEQ ID NO: 1)



Average ~ 99.98% accuracy (with 3-pass reads)

FIG. 50