

Figure 1

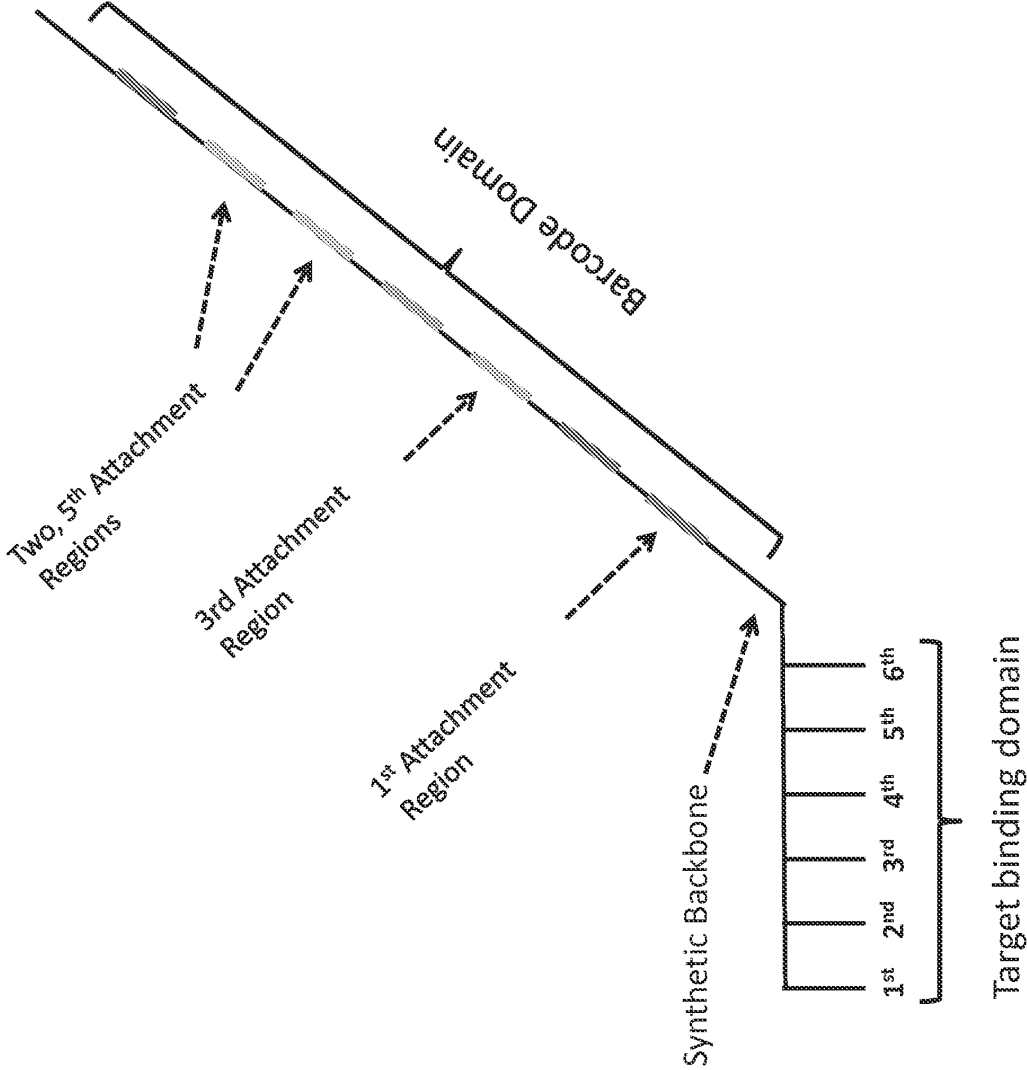


Figure 2

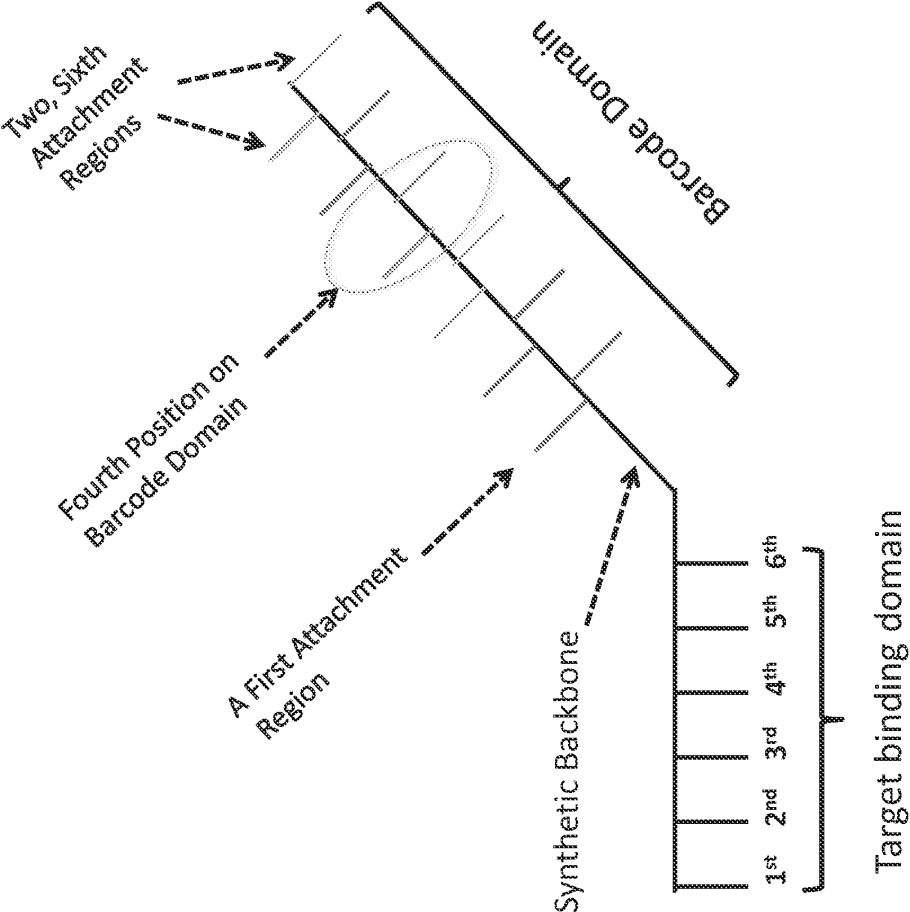


Figure 3

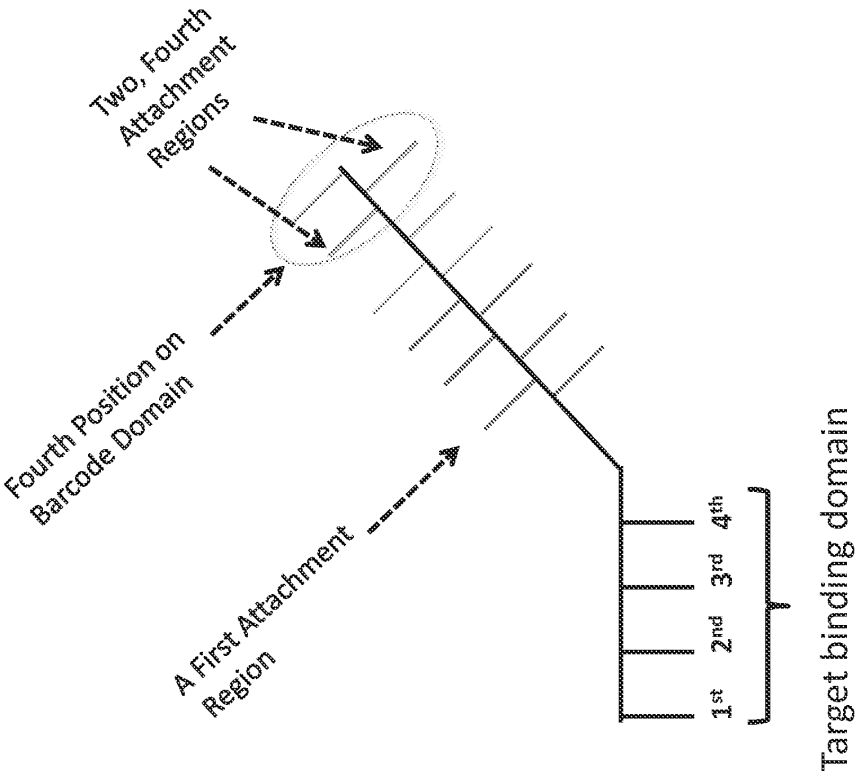
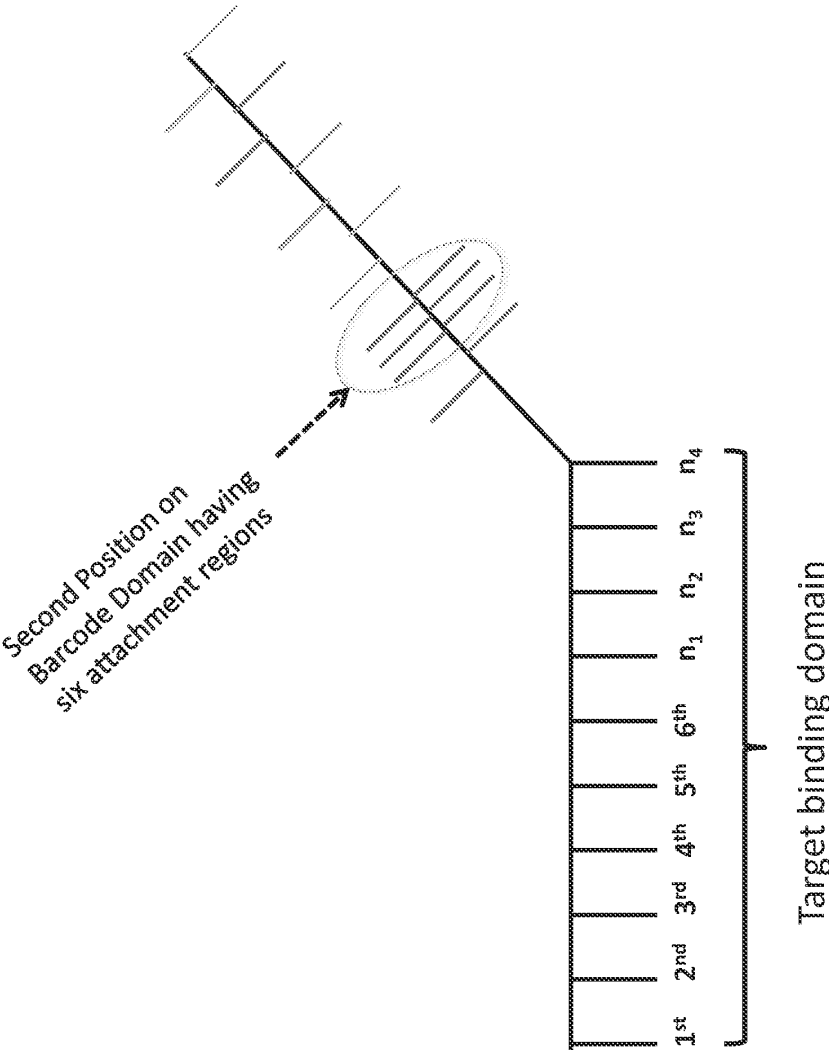


Figure 4



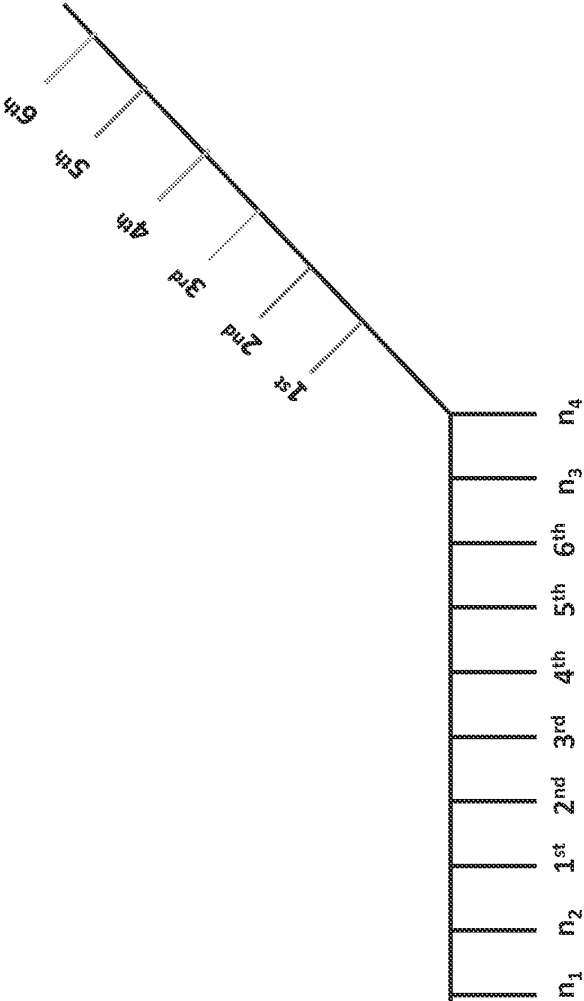


Figure 5

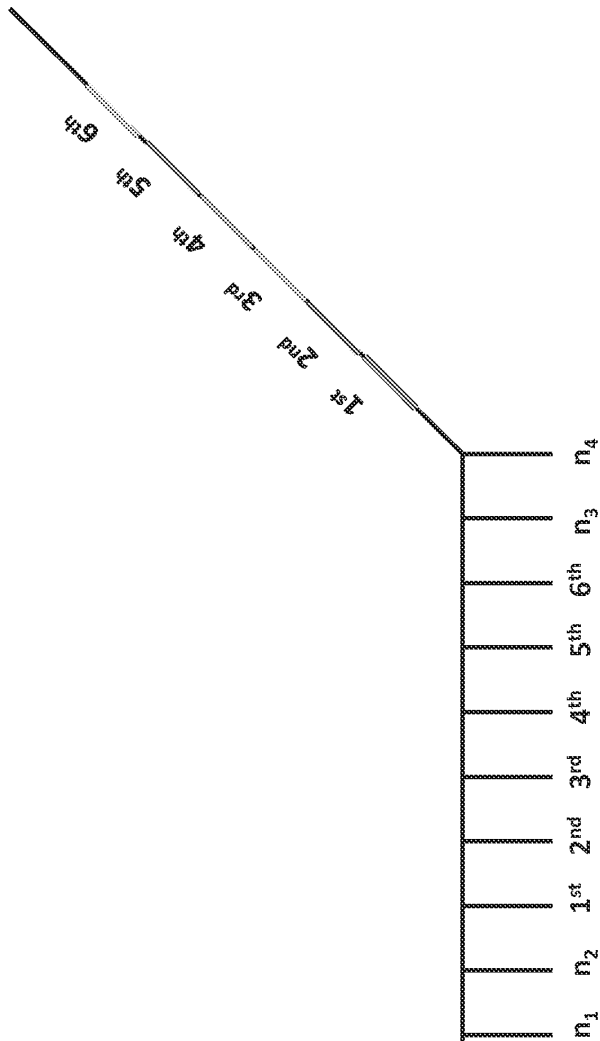


Figure 6A

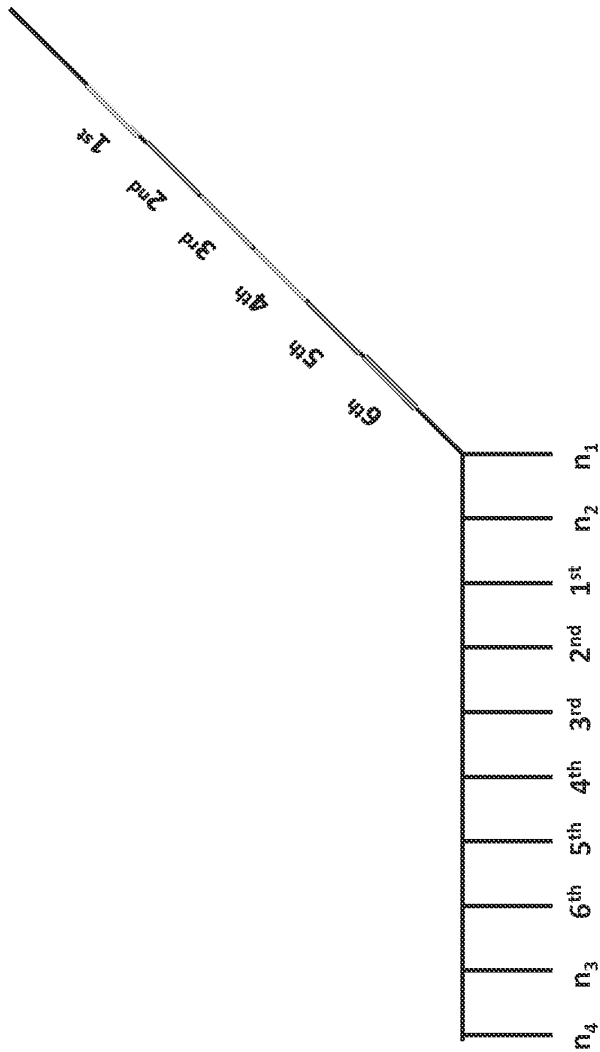


Figure 6B

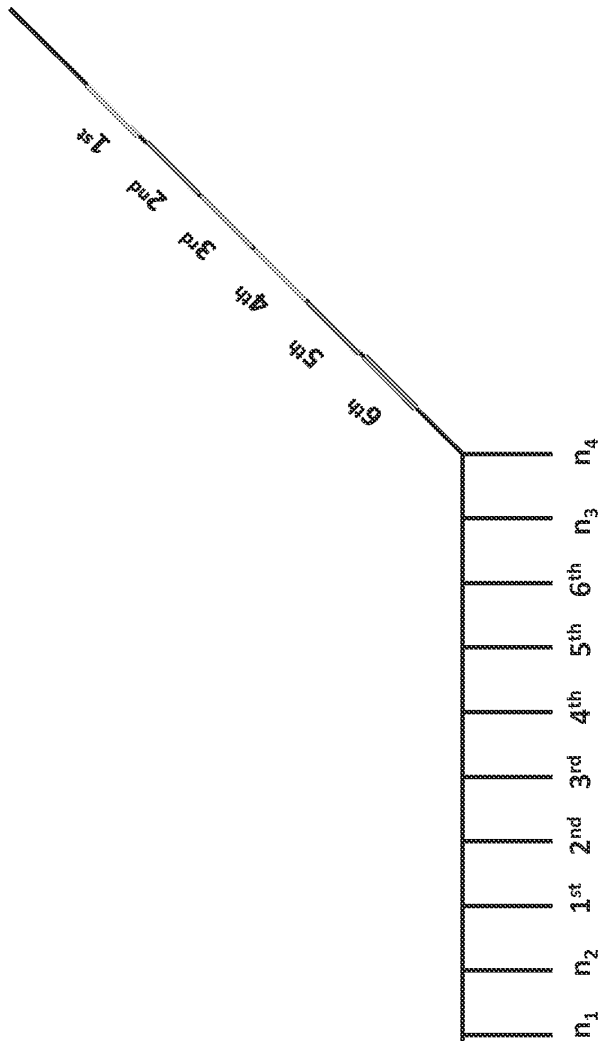


Figure 6C

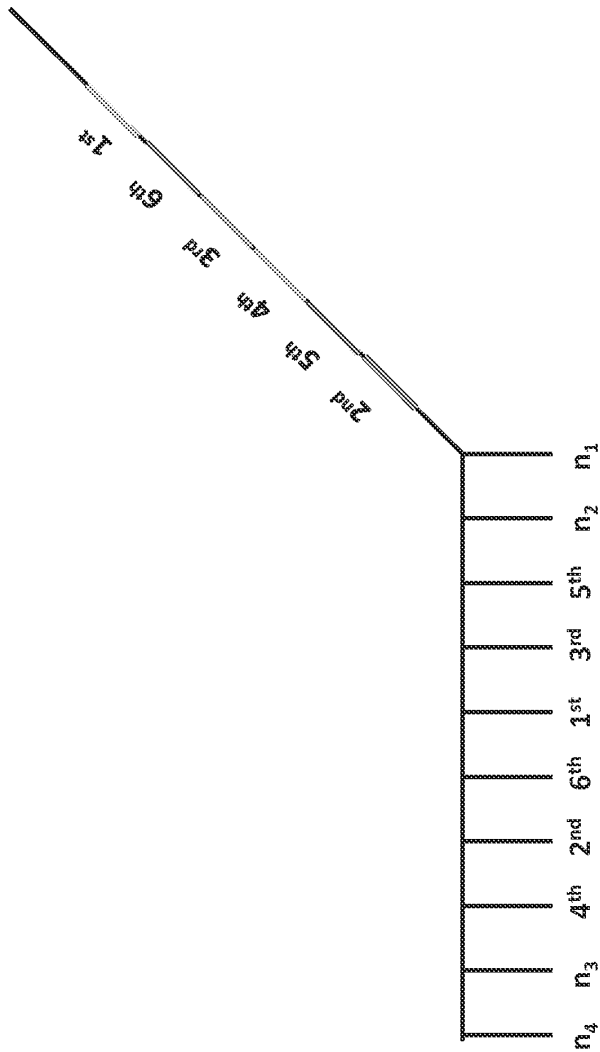


Figure 6D

Figure 7

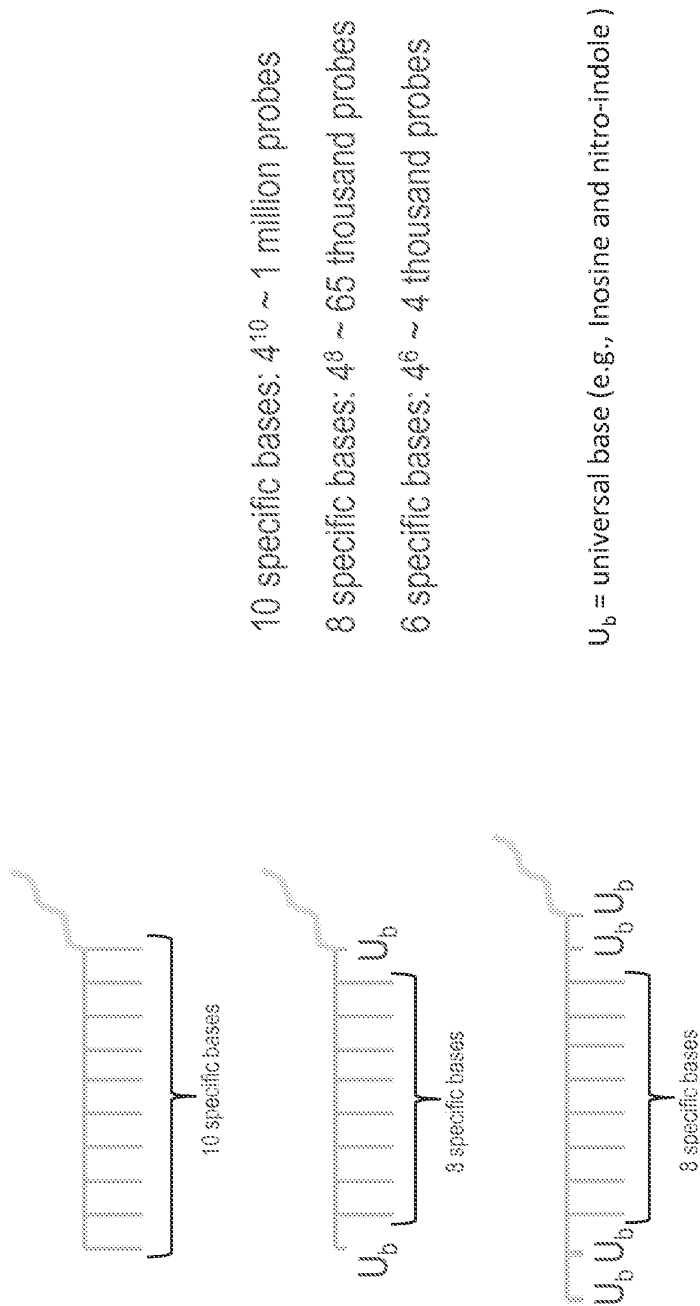


Figure 8A

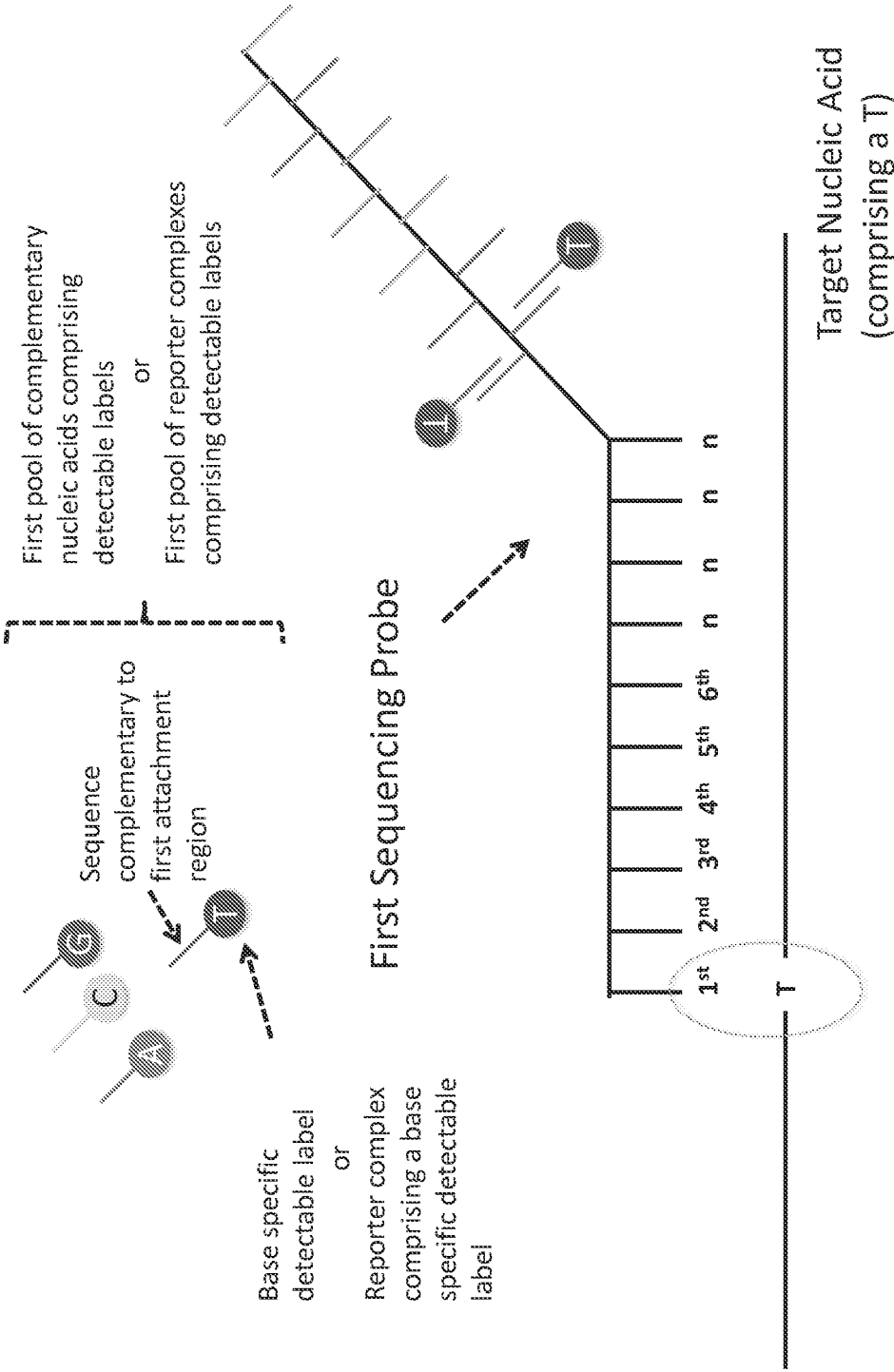


Figure 8B

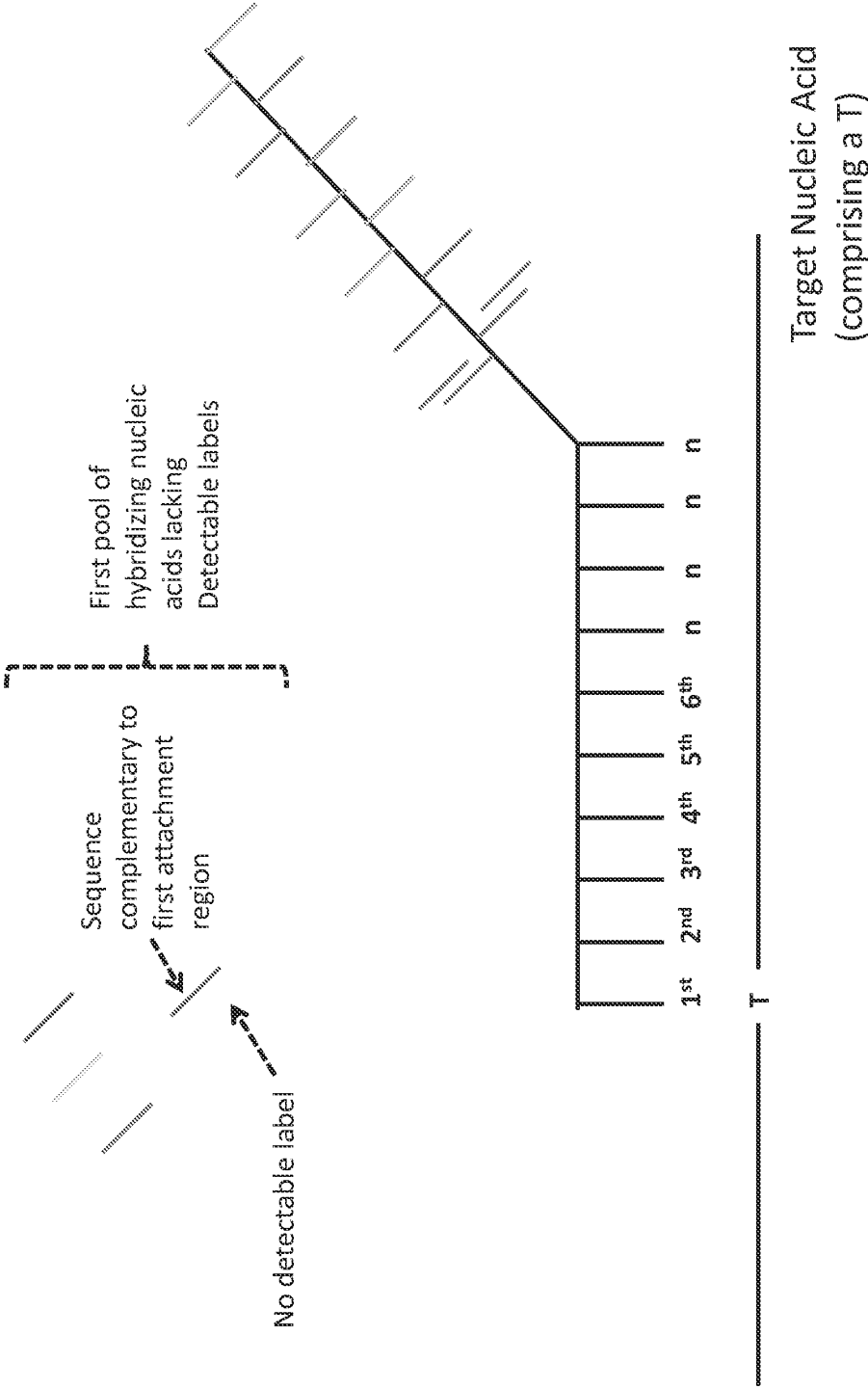


Figure 8C

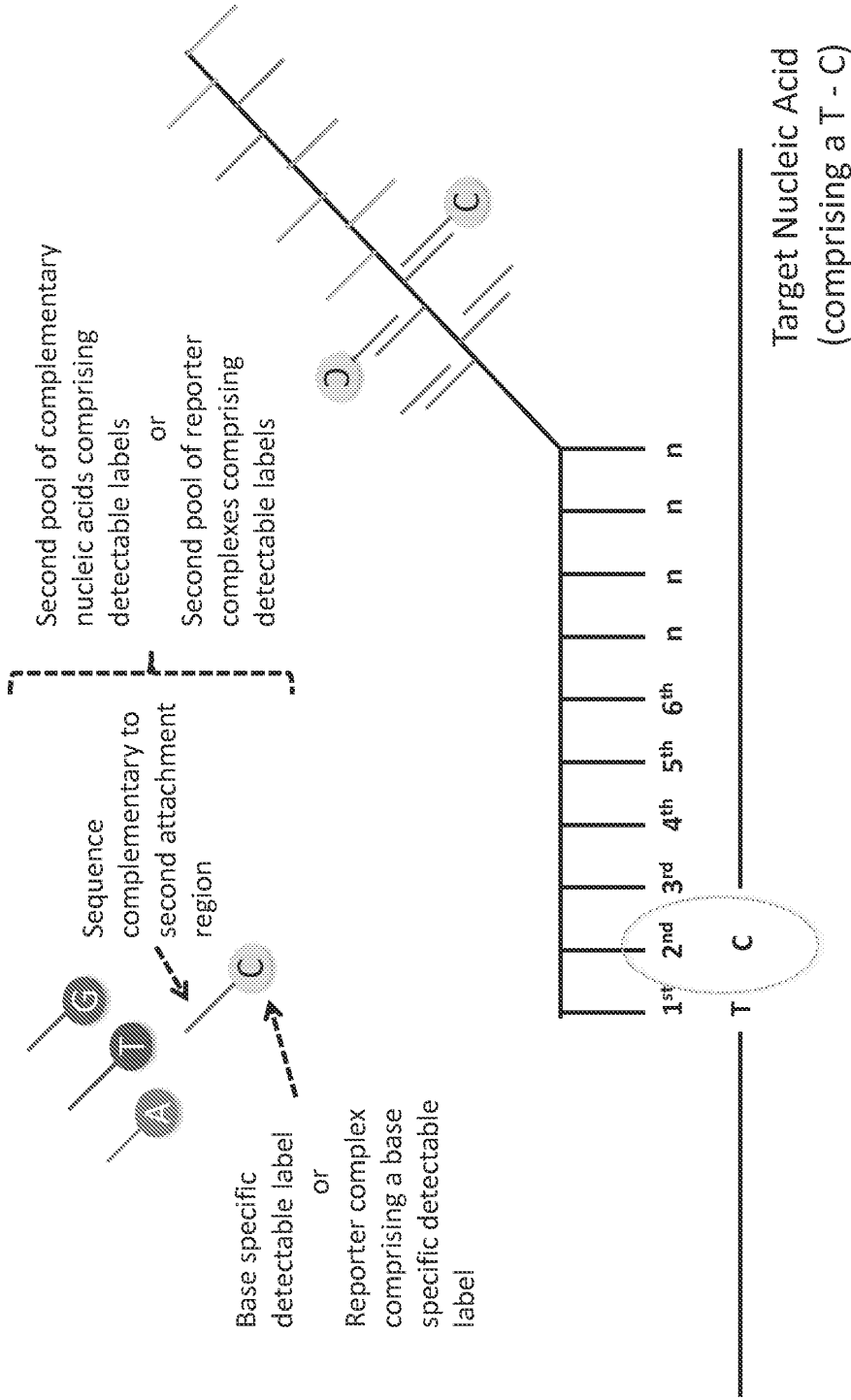


Figure 8D

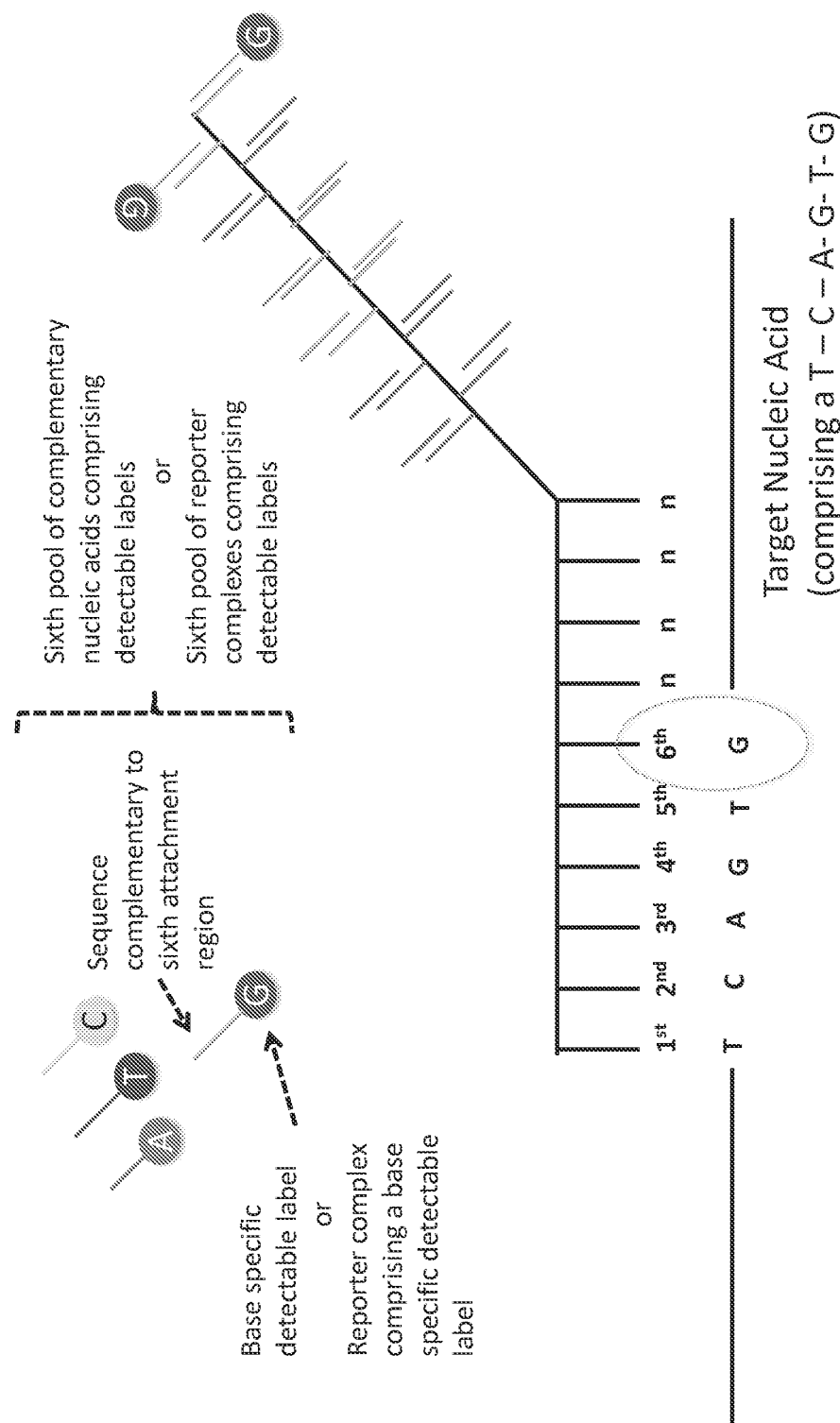


Figure 8E

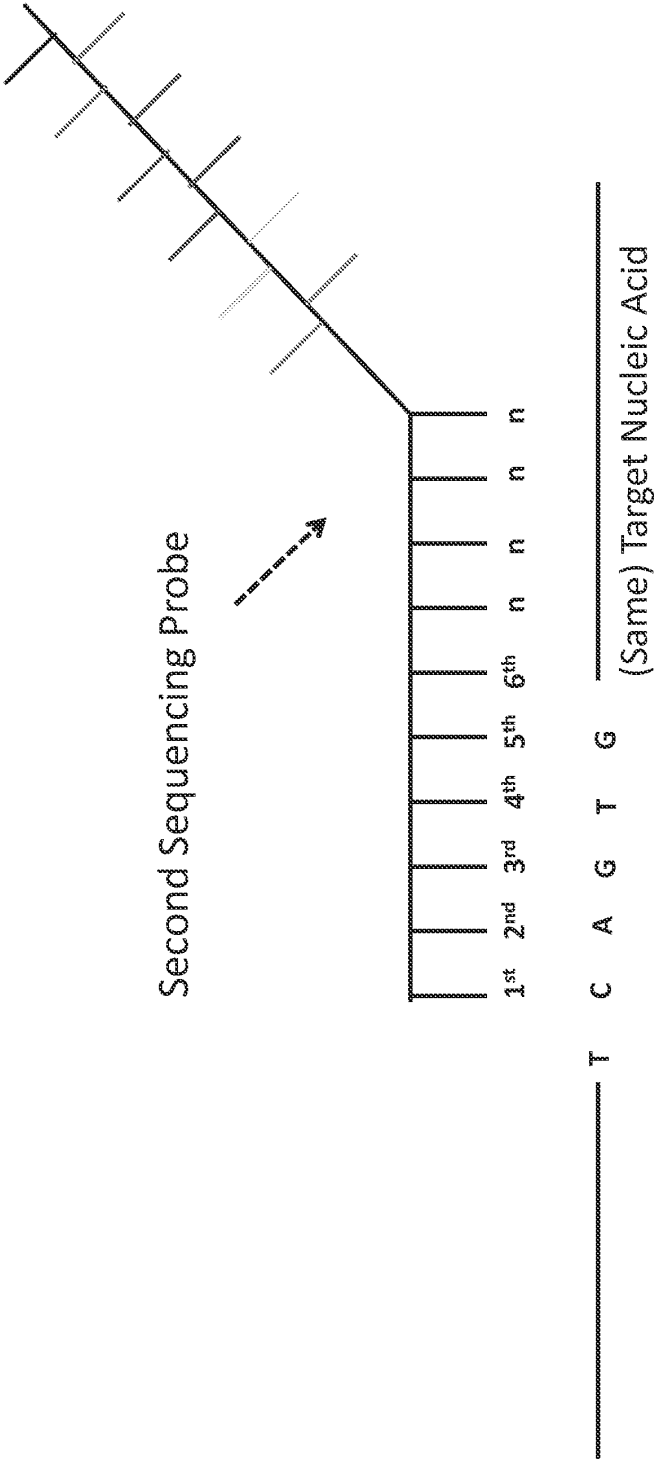


Figure 9A

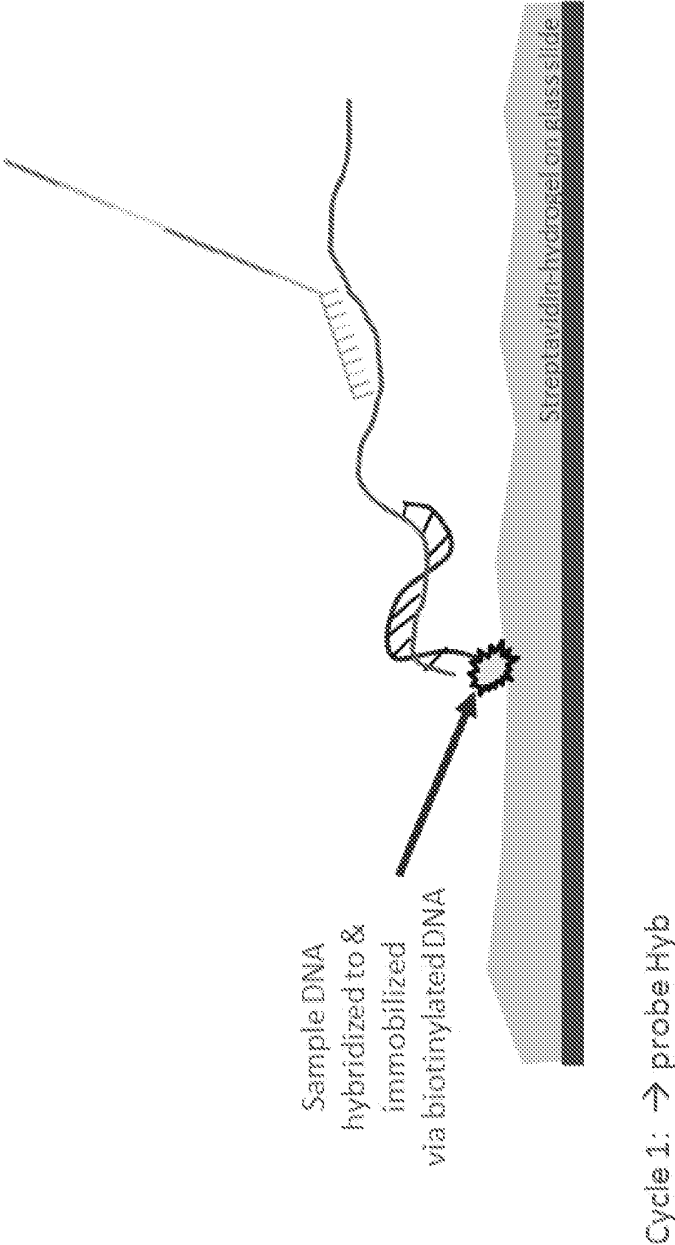
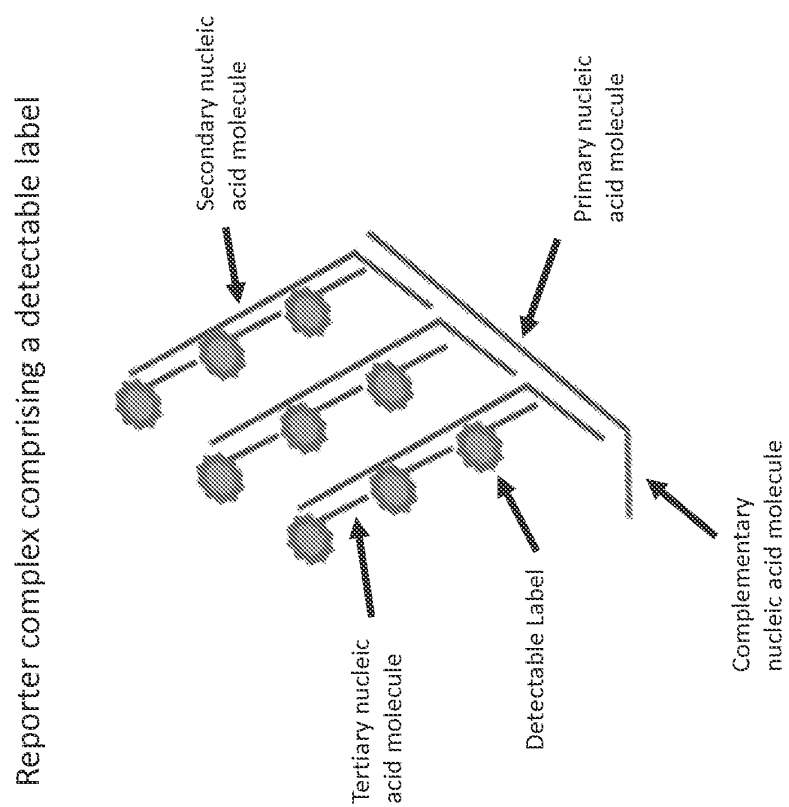


Figure 9B



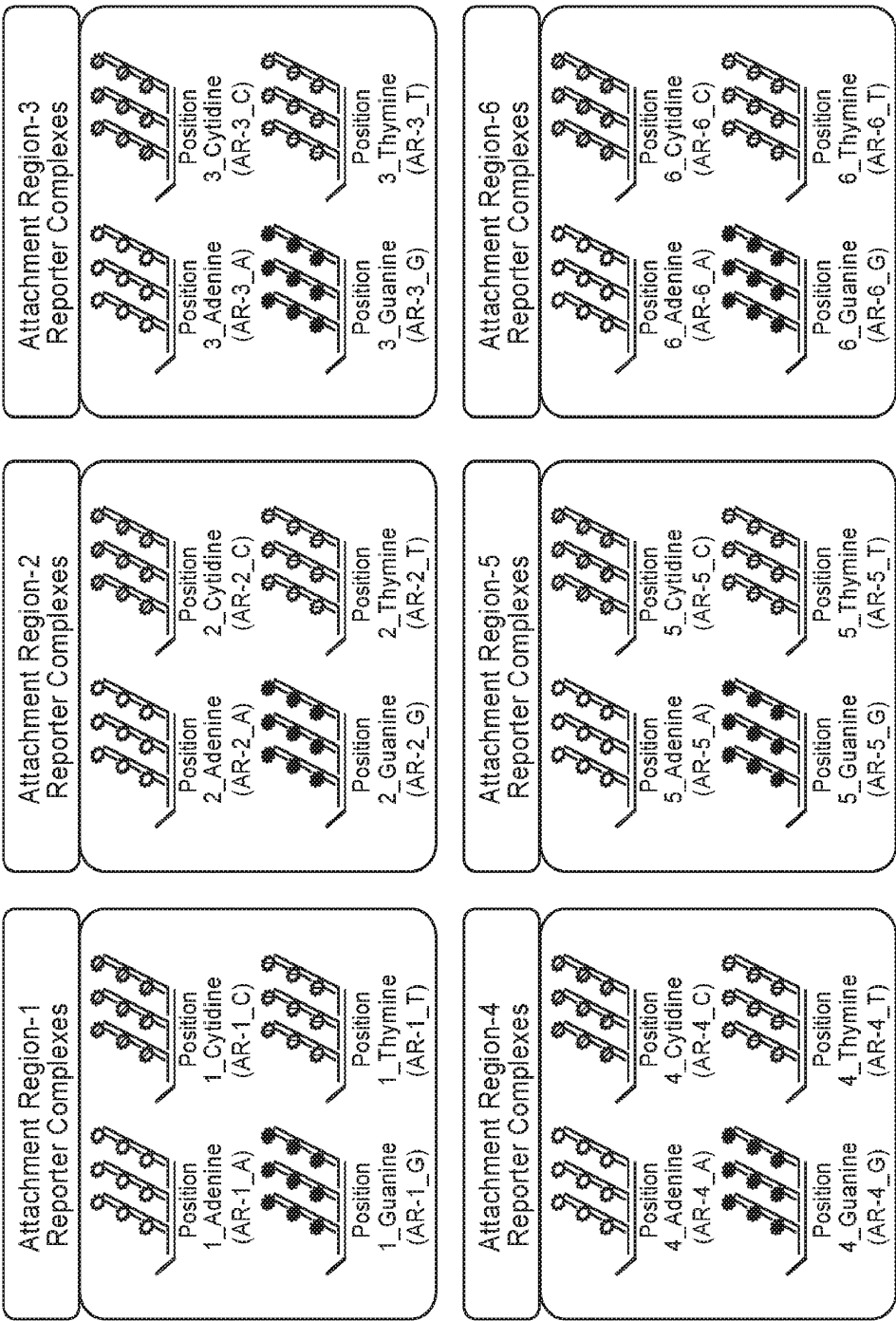
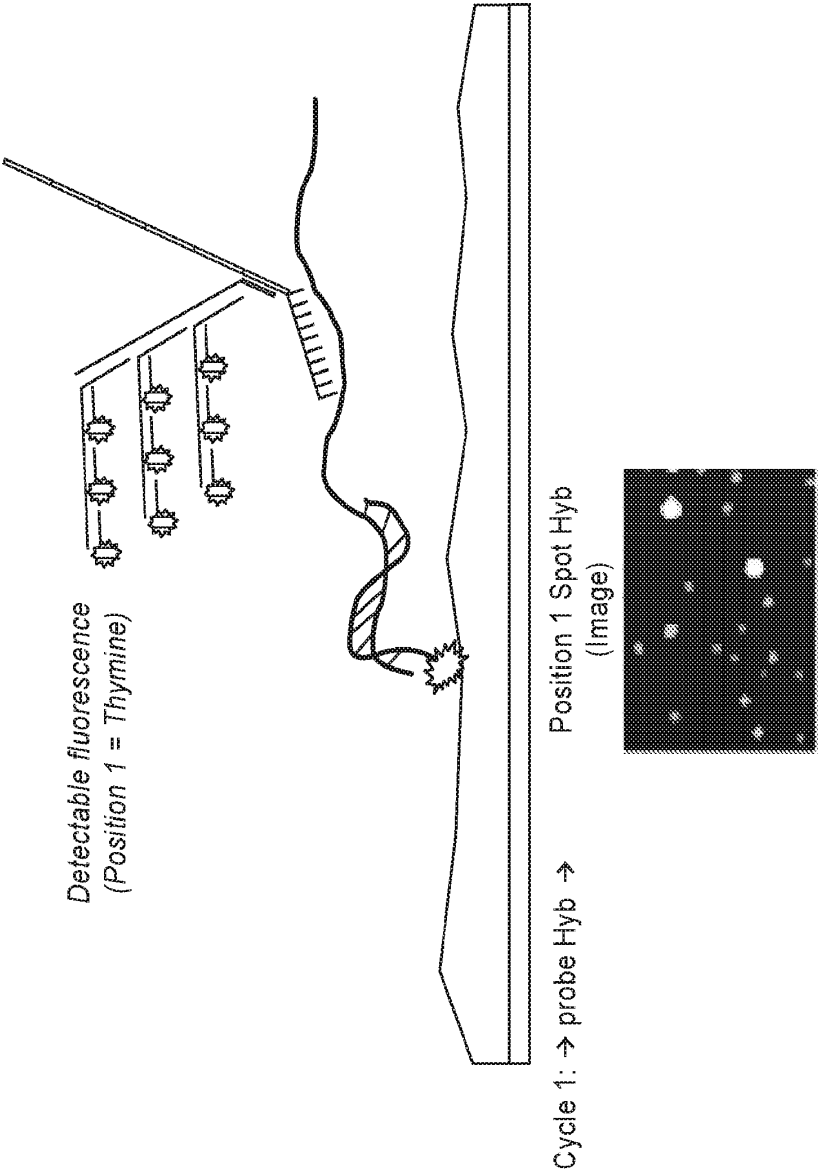
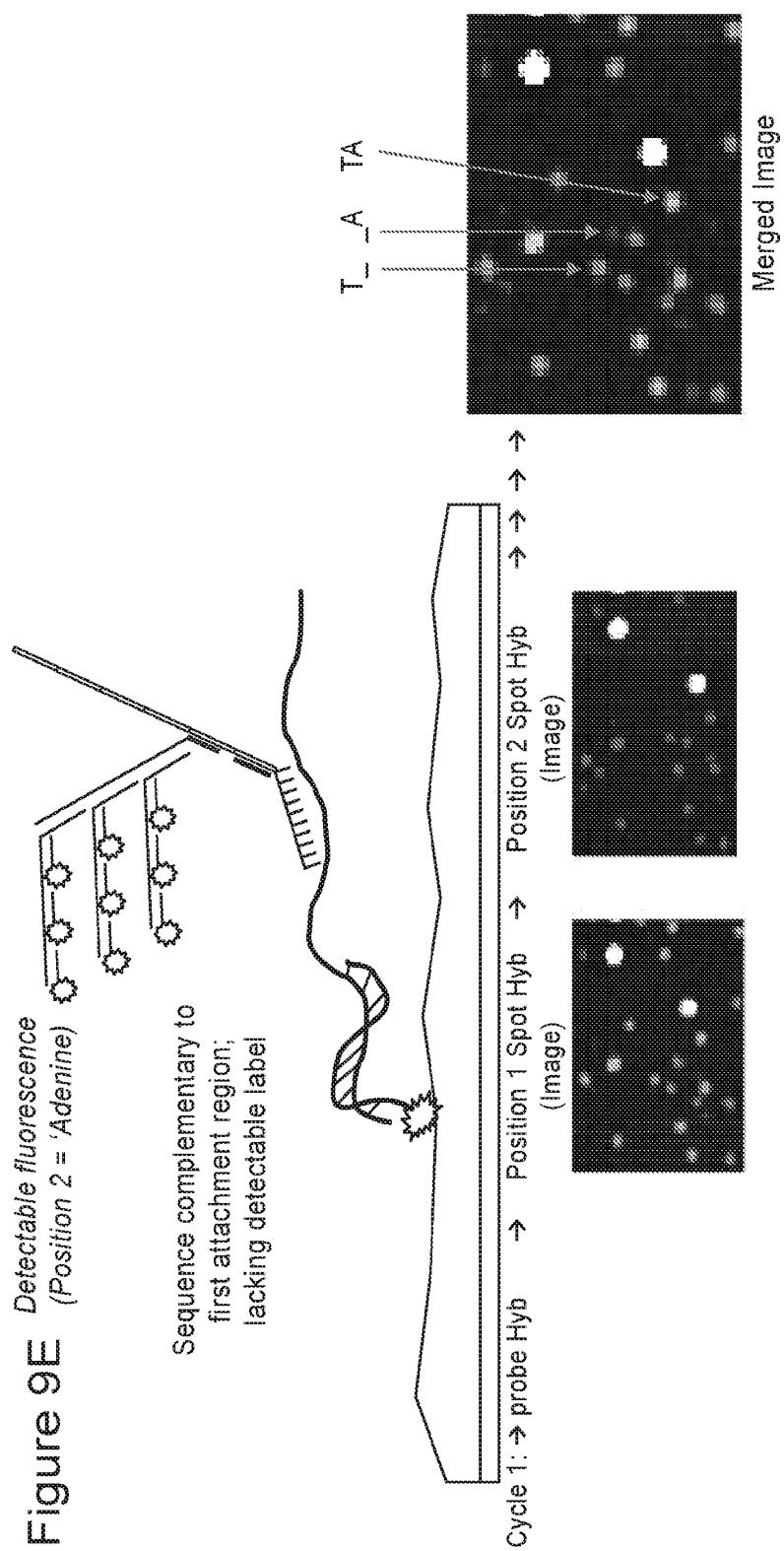


Figure 9C

Figure 9D





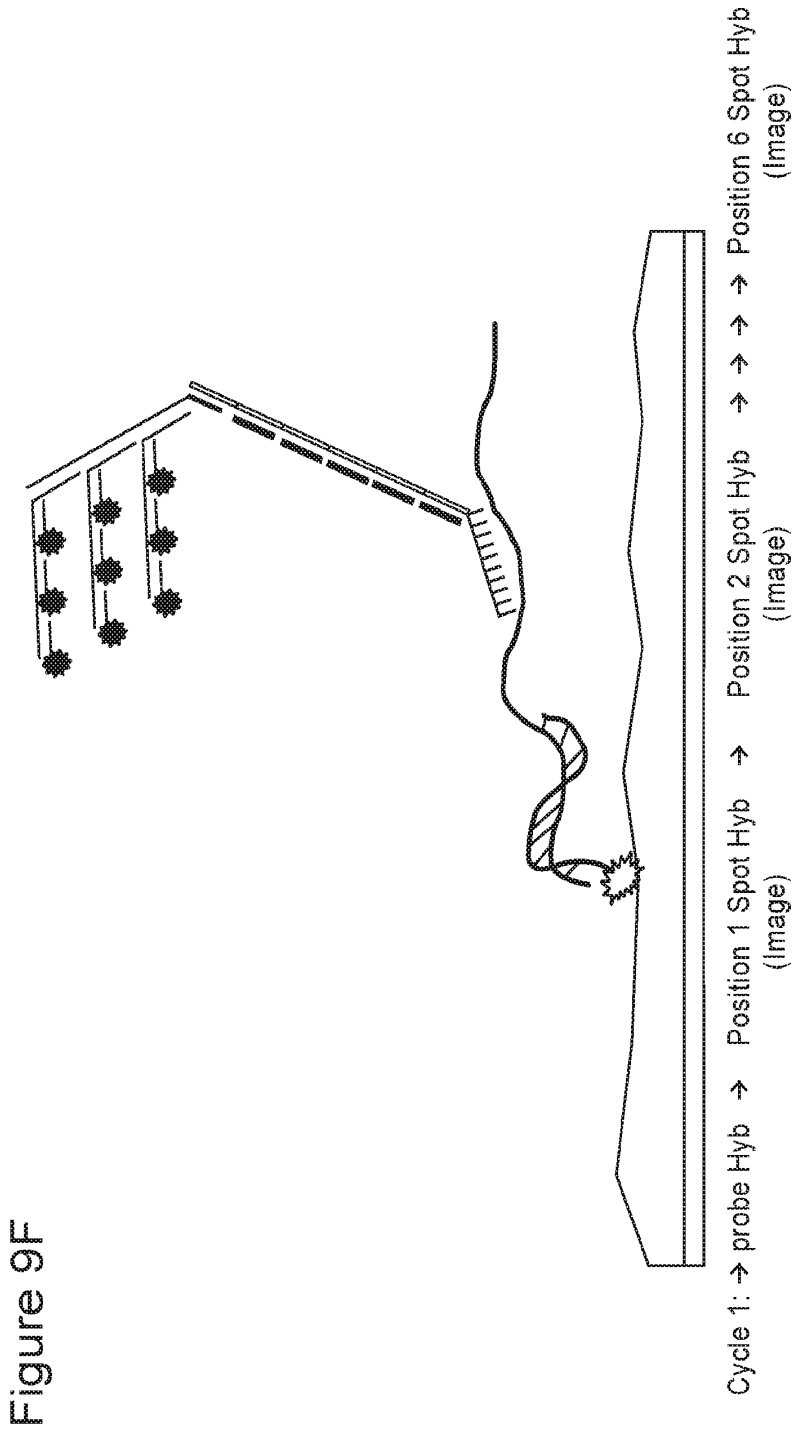


Figure 9G

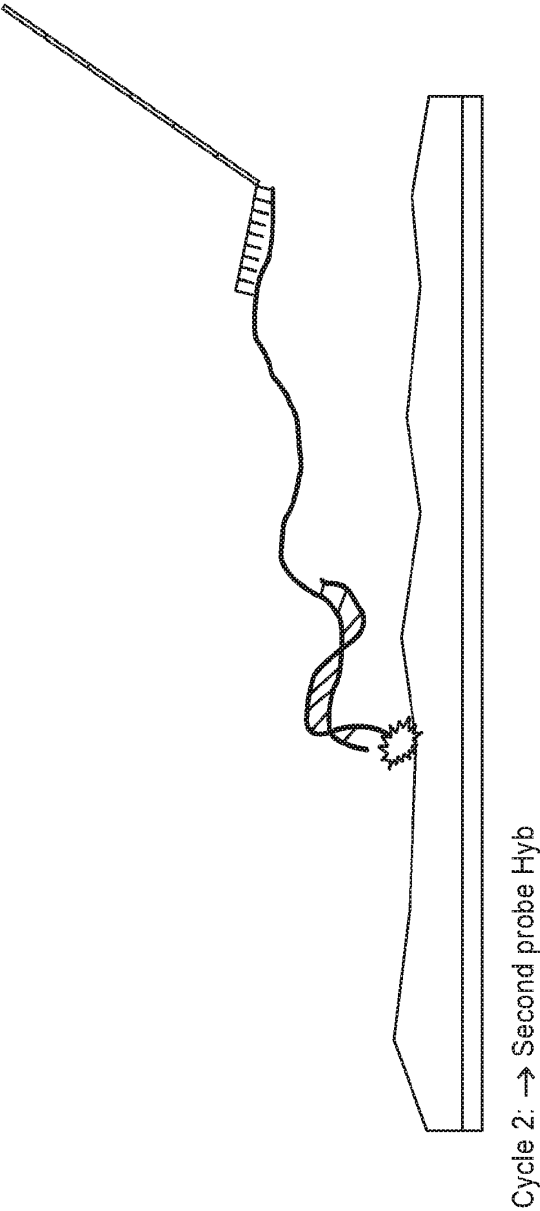


Figure 10

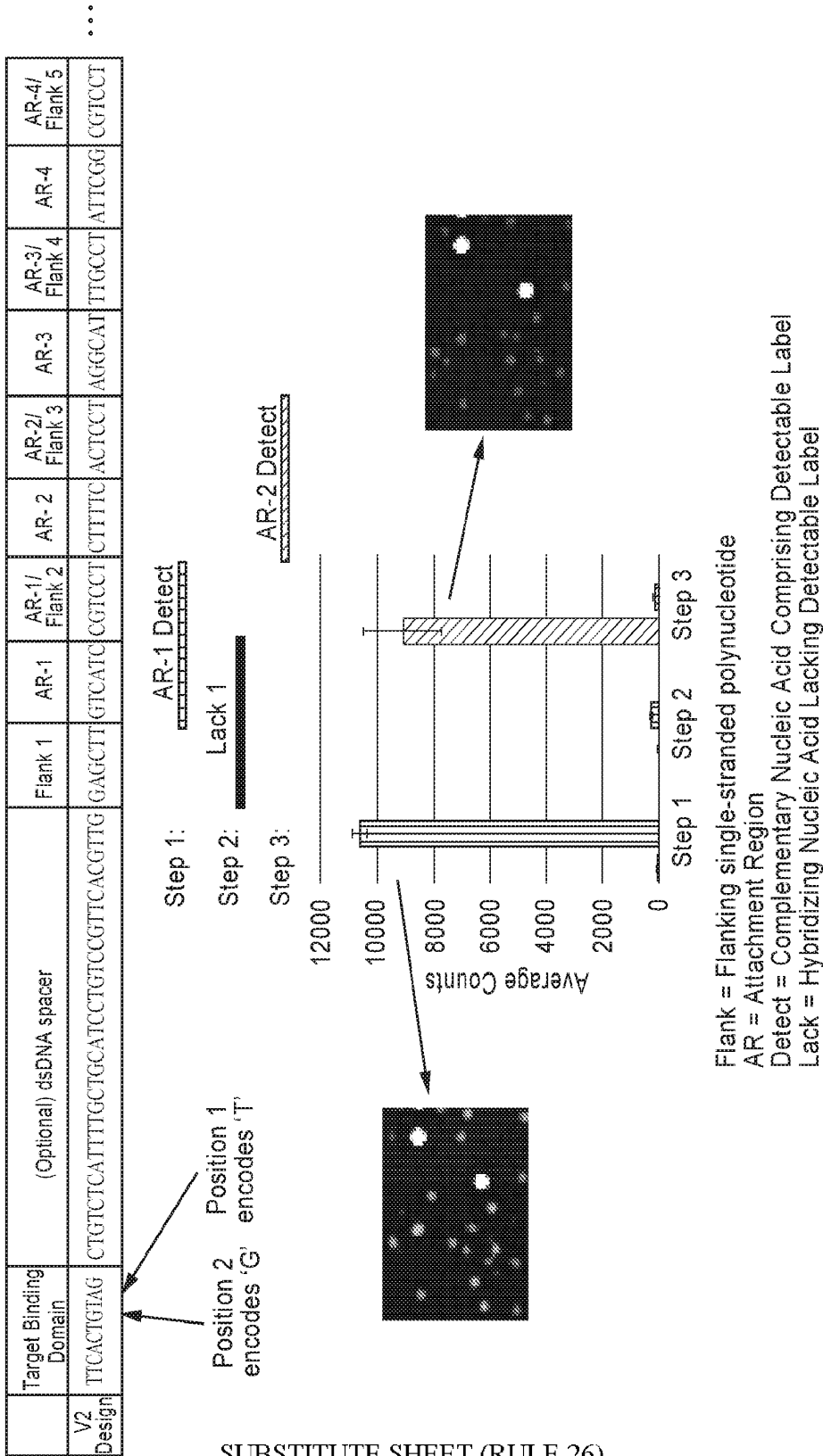
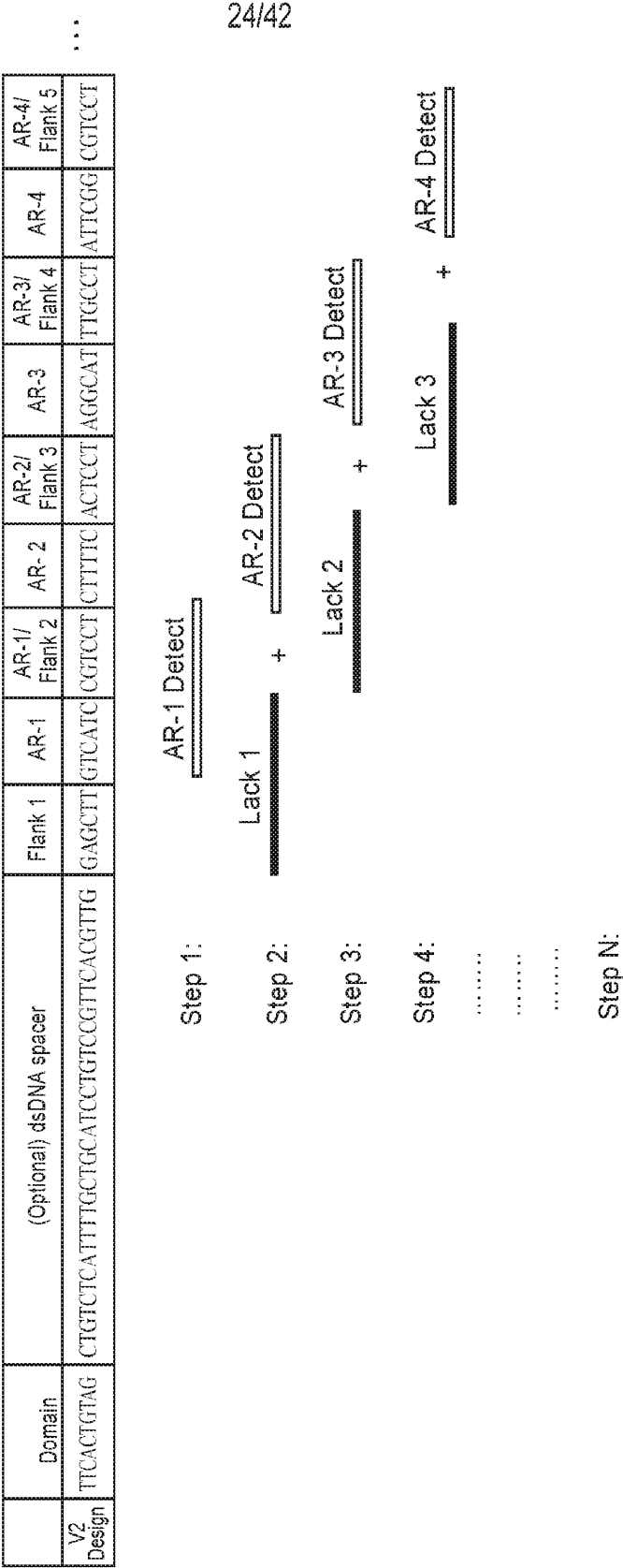


Figure 11



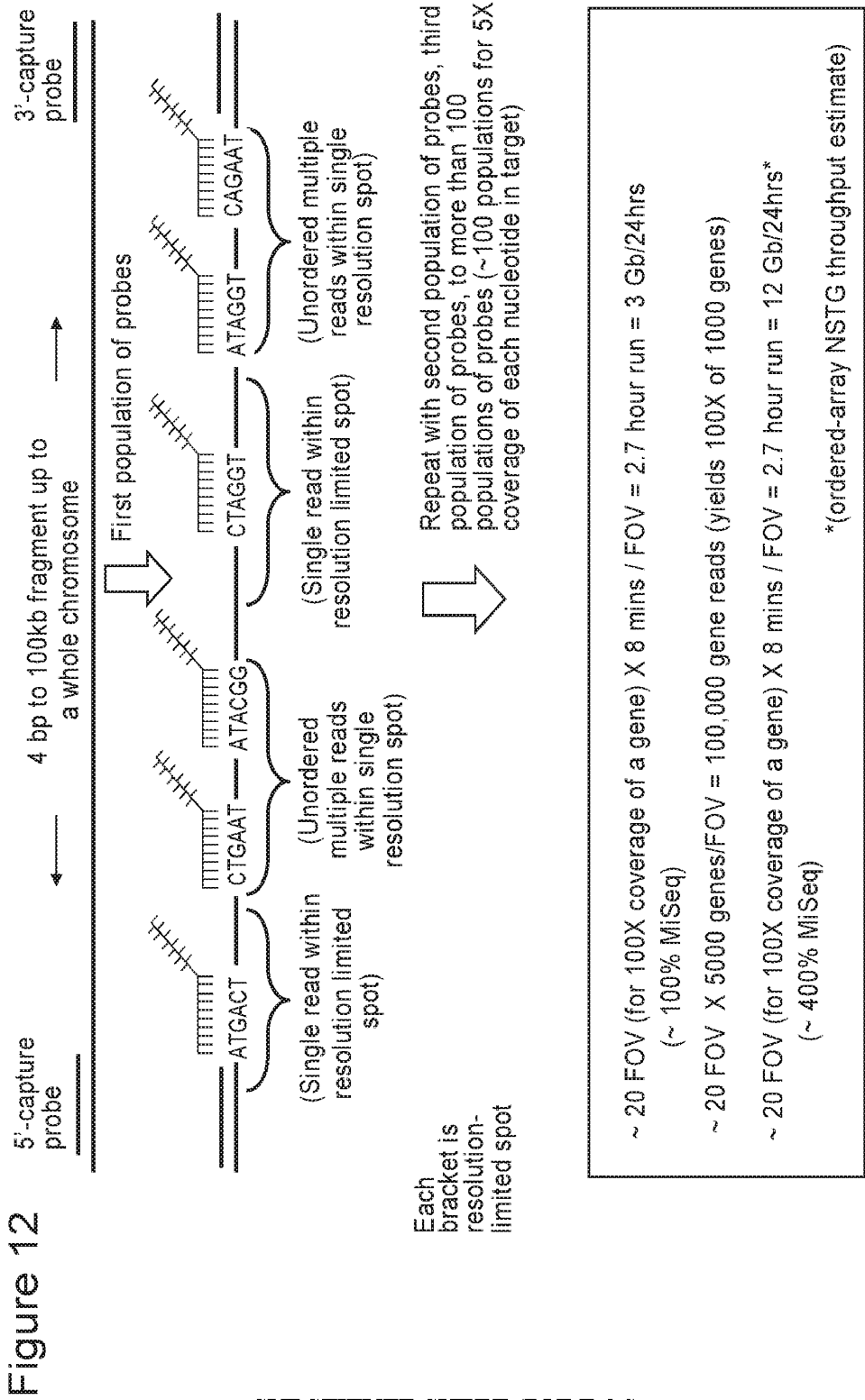
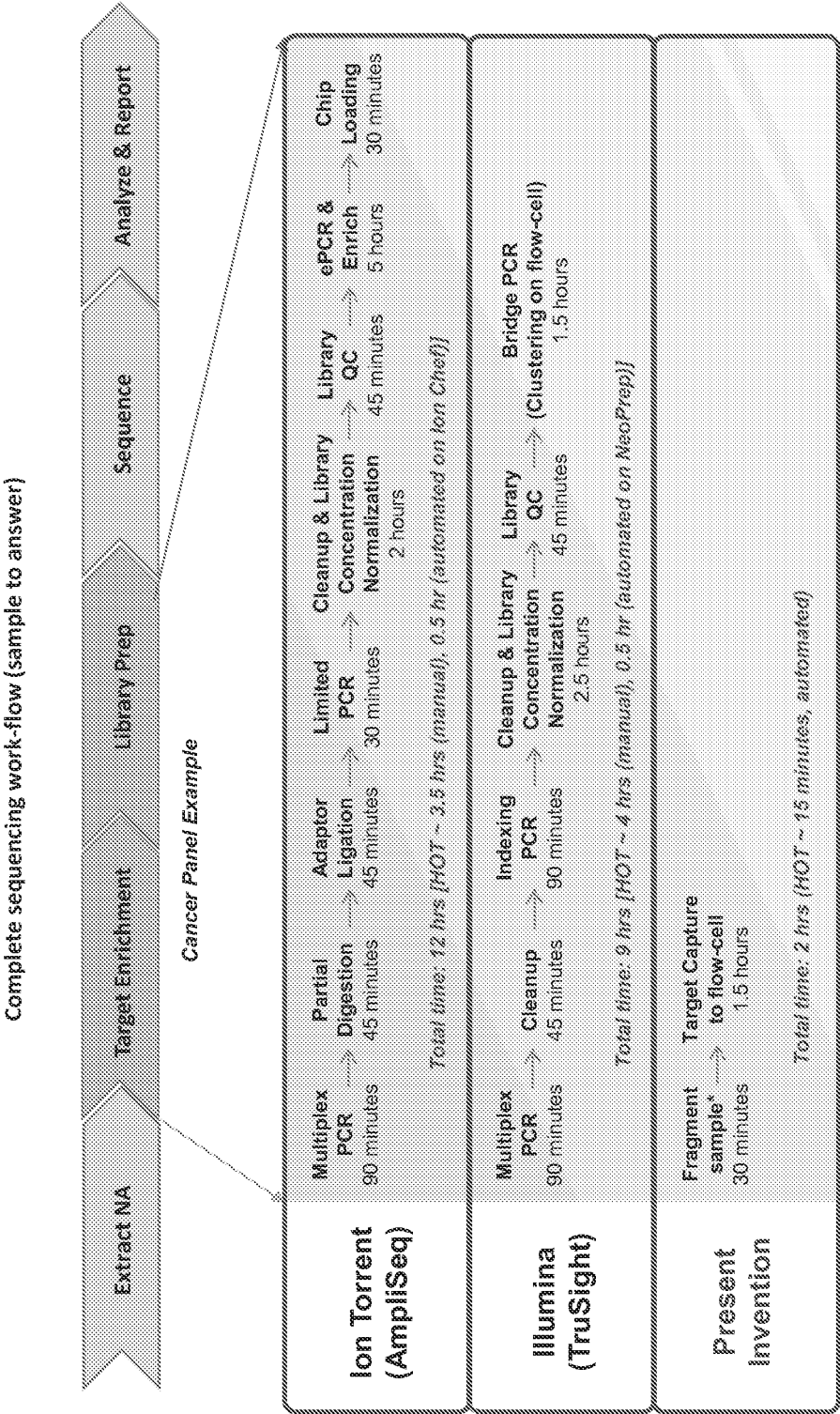
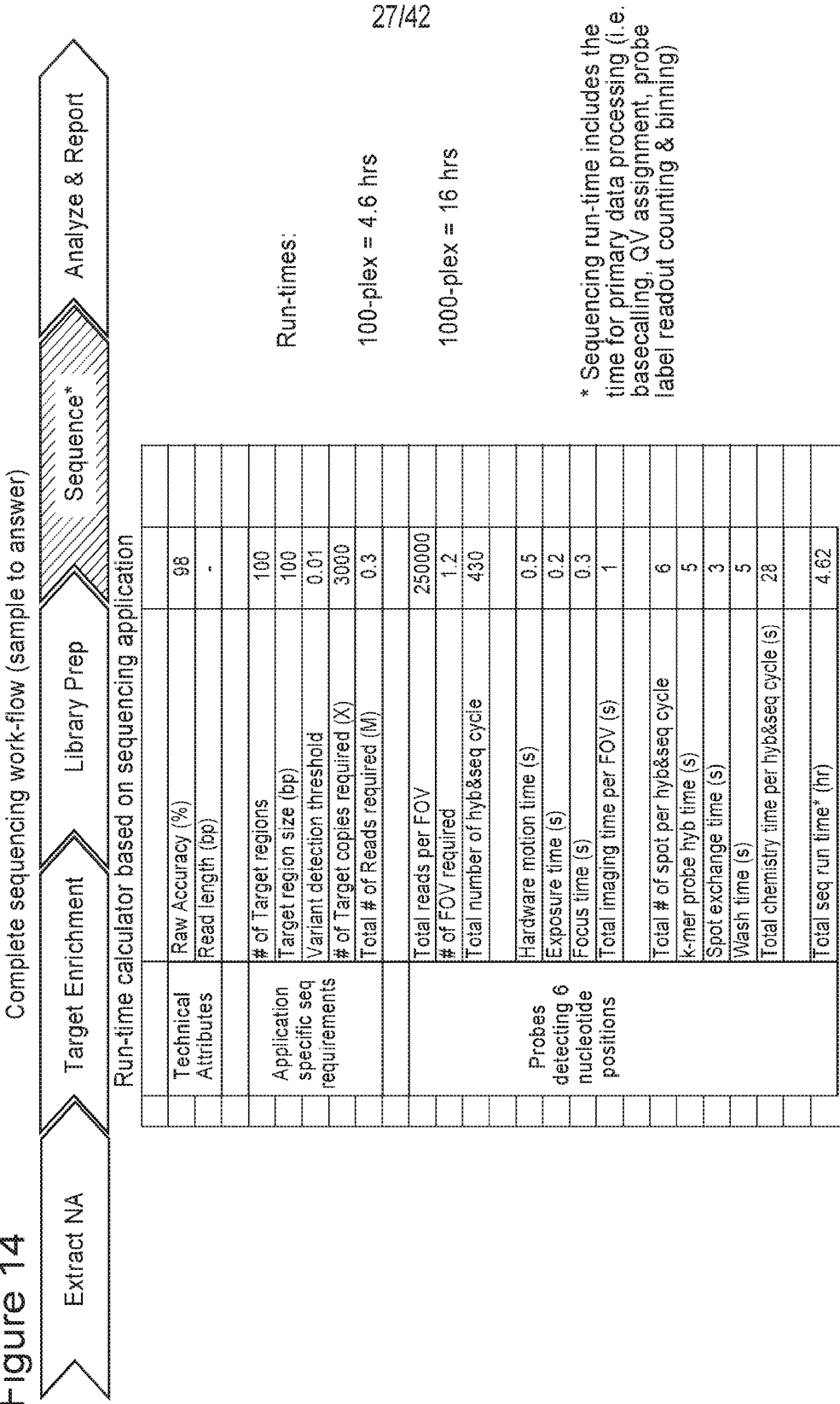


Figure 13



*Need for fragmentation will be dependent on sequencing application (i.e. long-read apps such as HLA sequencing will not require this step)

Figure 14



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Cycling calculator based on probe occupancy

Total # of single molecule targets on flow-cell	30000	
Average length of 'gapped' target (bp)	100	
Barcode occupancy (%) per seq cycle	0.75	
# of barcode cycle required to 'hit' >95% of targets on flow-cell at least once	3	
Dye occupancy (%) per position	0.8	
Total number of Dye positions	6	
Total # of 1 bp encoded read per seq cycle	6	35
Total # of 2 bp encoded read per seq cycle	23	346
Total # of 3 bp encoded read per seq cycle	92	1843
Total # of 4 bp encoded read per seq cycle	369	5530
Total # of 5 bp encoded read per seq cycle	1475	8847
Total # of 6 bp encoded read per seq cycle	5898	5898
Total number of 'usable' read per seq cycle	22499	
# of barcode cycle required to cover given single molecule target with ~3X coverage	143	
# of barcode cycle required to cover given single molecule target with ~5X coverage	238	
# of barcode cycle required to cover all single molecule target on flow-cell with ~3X coverage	430	
# of barcode cycle required to cover all single molecule target on flow-cell with ~5X coverage	713	

Figure 15

Figure 16

	Gigabases	time hrs.	# Reads	Clinical utility
HiSeq 2500	900Gb	11 days	3 Billion !	Genome sequence cancer sequence
Next Seq	120GB	27hrs	400M	Genome sequence exome Transcriptome
Proton PI	10Gb	2-4	70M	exome Transcriptome
Present Invention	3 to 12 Gb	3	0.5M	Targeted (Dx, cDx) plus multi-analyte plus sample-to-answer, long-reads
MISeq	3.5Gb	24hrs	25M	transcriptome targeted (Dx)
PGM 318	up to 2Gb	7.3/4.4	4M	Targeted
PacBio RS II	2.2Gb/day	3 hrs	50k	Niche genomes, long-reads, high error rate
PGM 314	0.1Gb	3.7/2.3	400k	Targeted

Figure 17

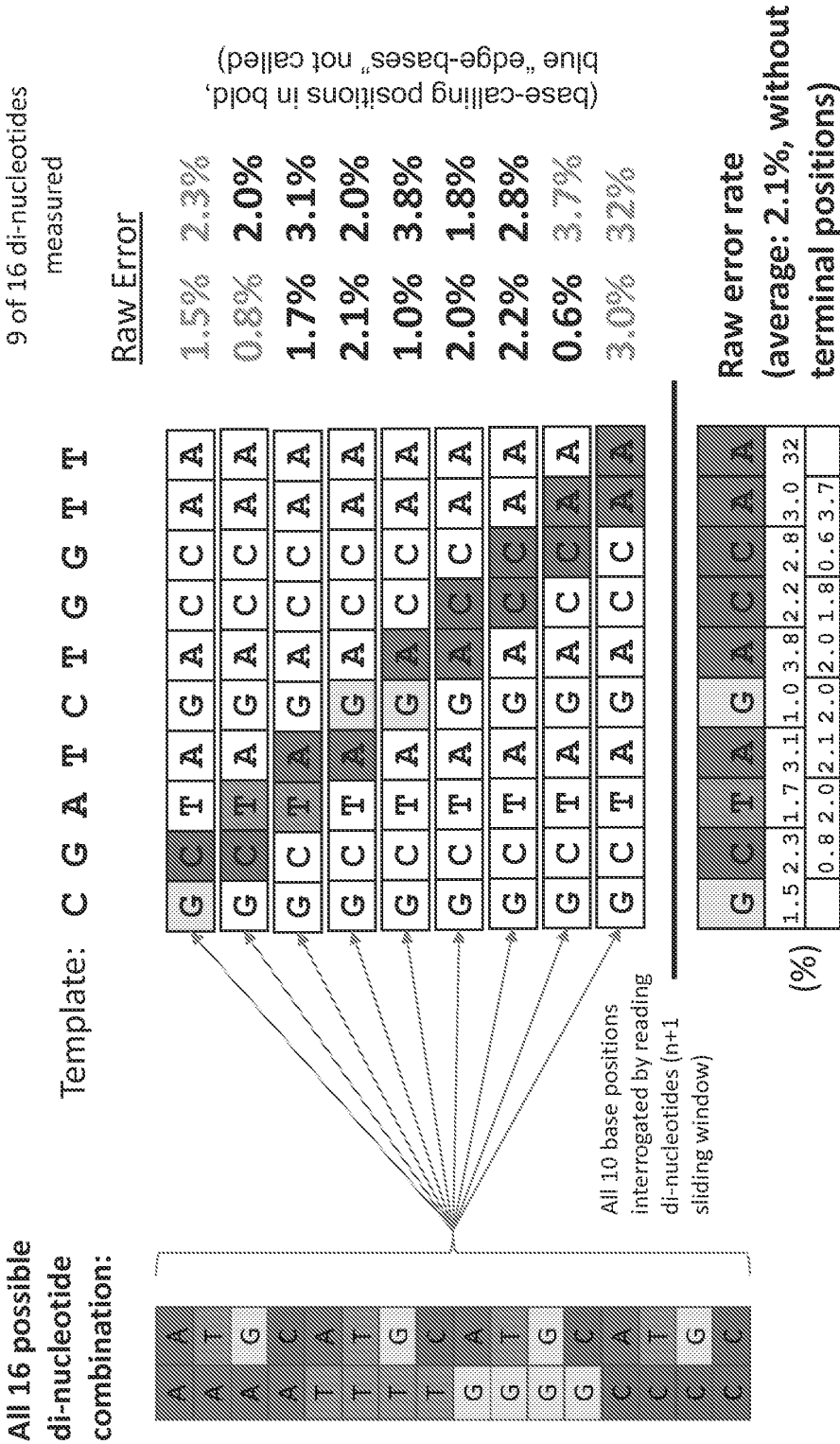


Figure 18

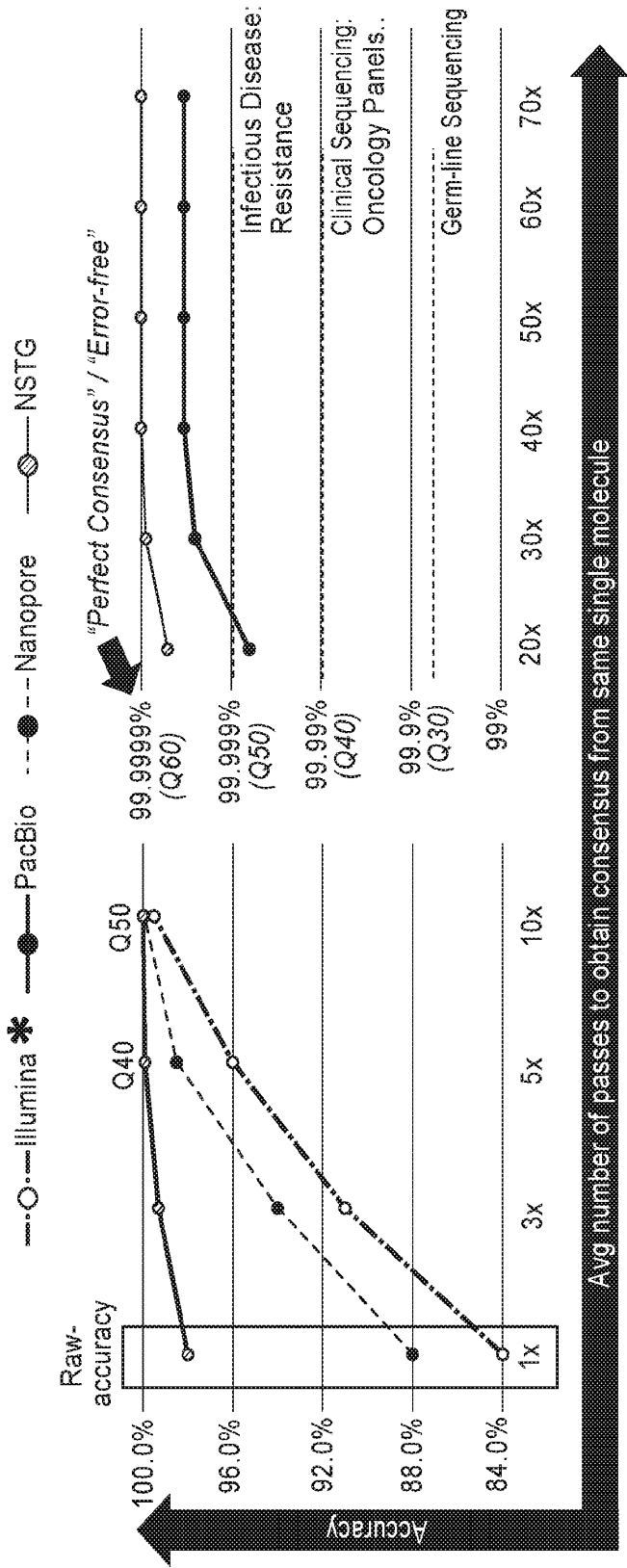
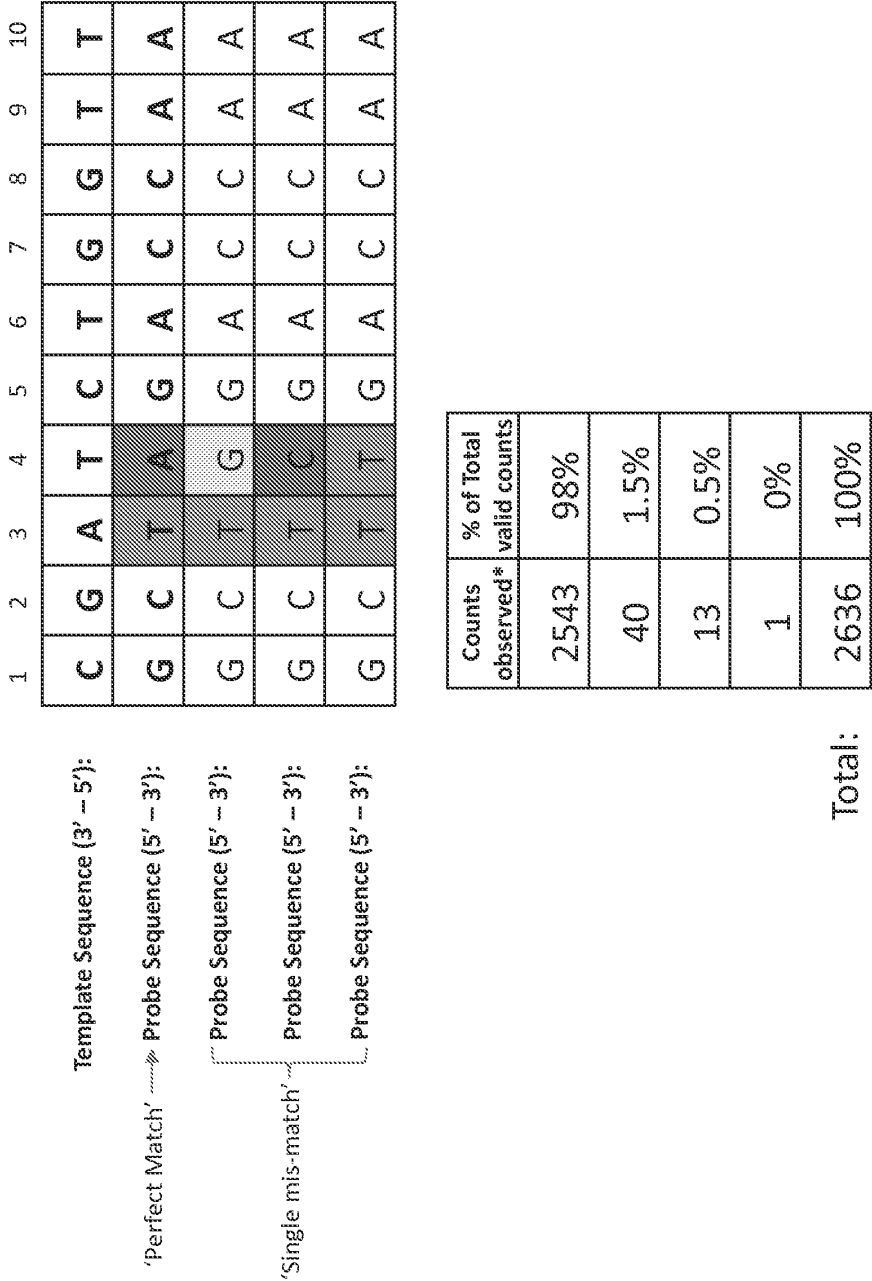


Figure 19

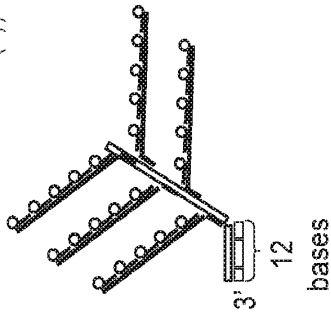


* Counts observed value represent counts after background count subtraction
** All hybridization done under buffer condition: 1xSSPE/0.1% Tween20

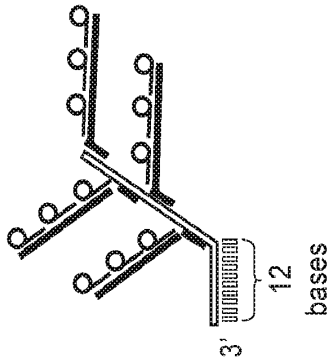
Figure 20A

5x5

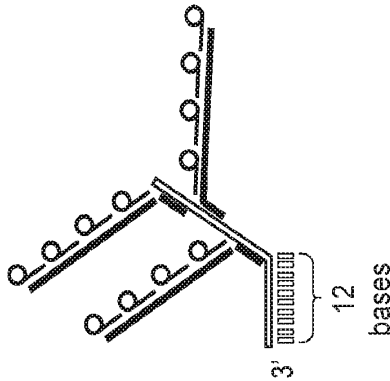
(1 primary nucleic acid molecule, 5 secondary nucleic acid molecules, 5 tertiary nucleic acid molecule with detectable label(s))



4x3



3x4



Spacer 3x4

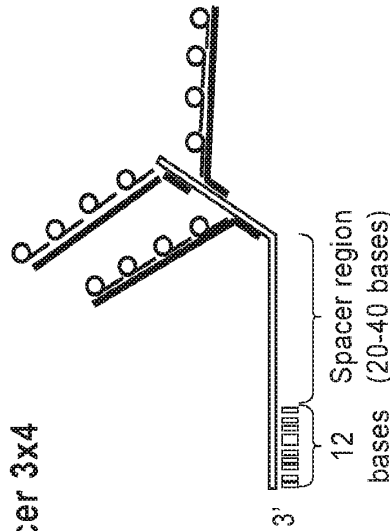


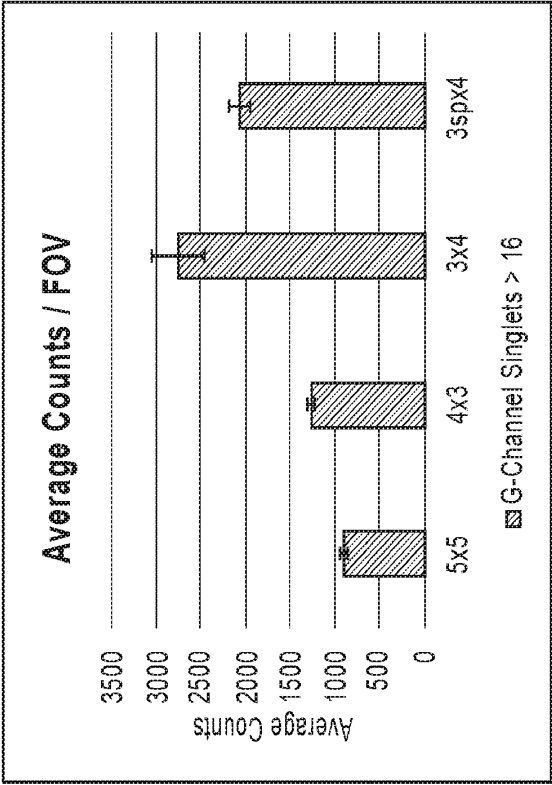
Figure 20C

Exemplary recipe for constructing reporter complexes comprising detectable labels

Volume (uL)	Primary n. a. molecule (10uM)	Secondary n. a. molecule (10uM)	Tertiary n. a. molecule (100uM)	H2O
5x4	1	4.5	2.25	92.25
5x3	1	4.5	1.8	92.7
4x4	1.28	4.5	2.25	91.97
4x3	1.28	4.5	1.8	92.42
3x4	1.8	4.5	2.25	91.45

Colors in table correspond to colors in diagrams of complexes shown at left

Figure 20B



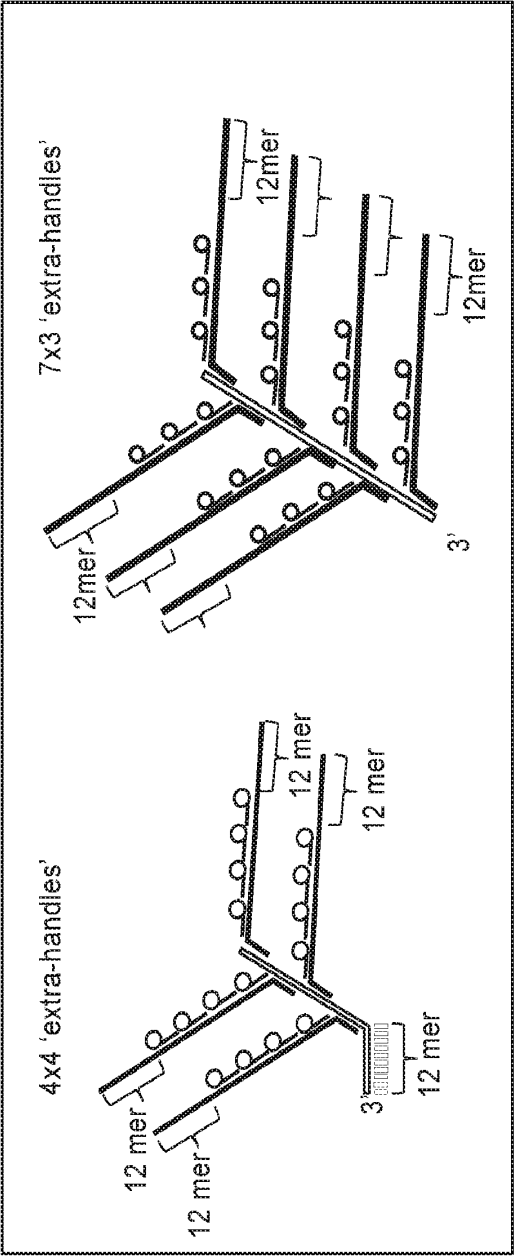


Figure 21A

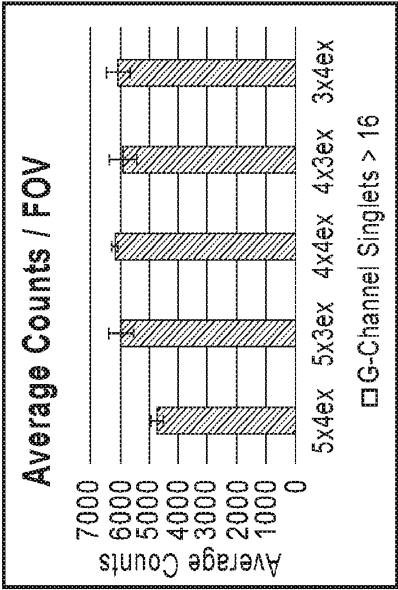


Figure 21B

Figure 22A

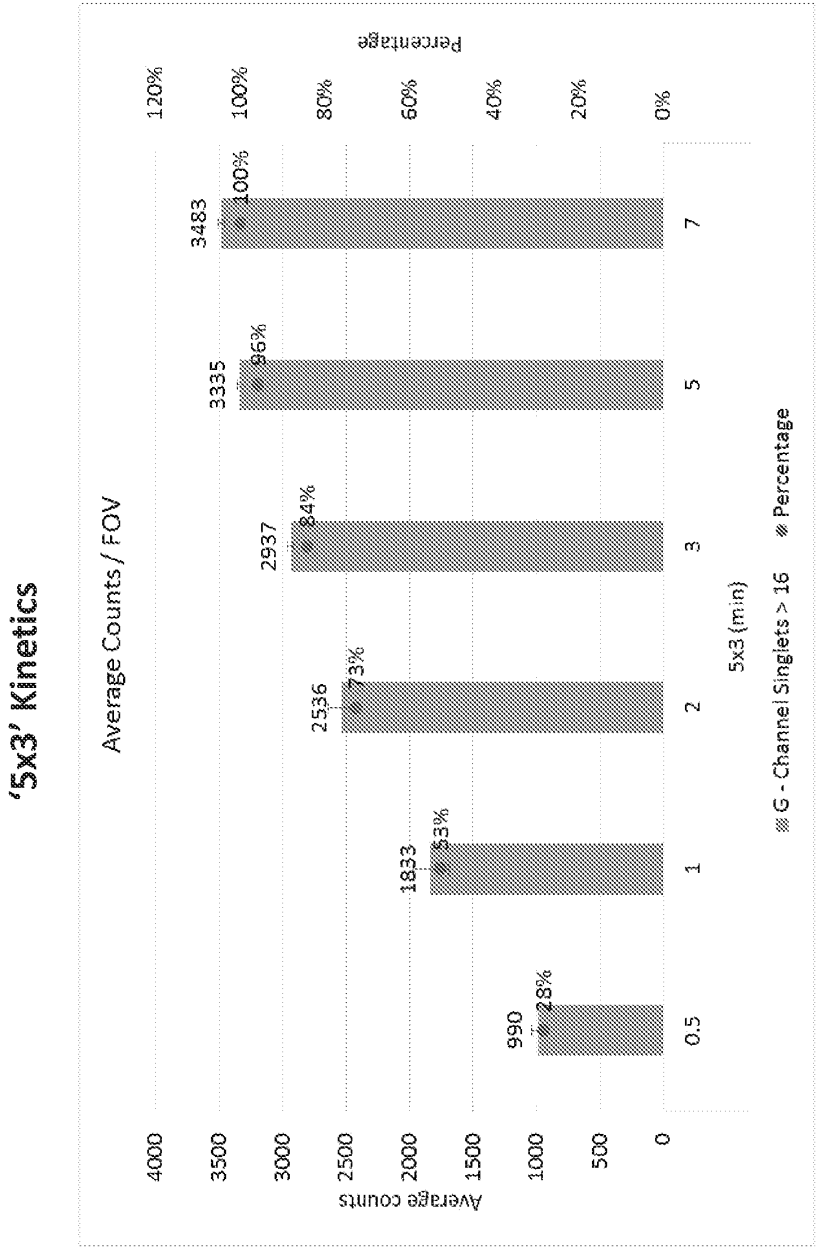


Figure 22B

'7x3 extra handles' Kinetics

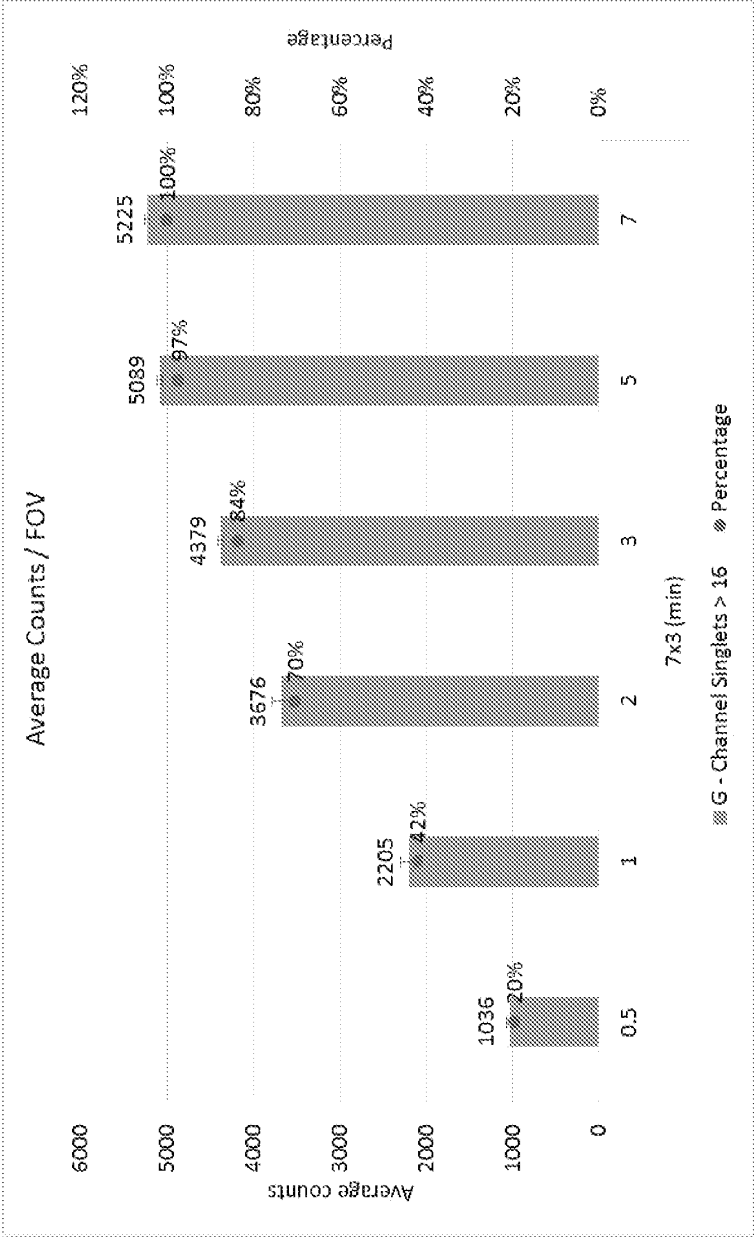
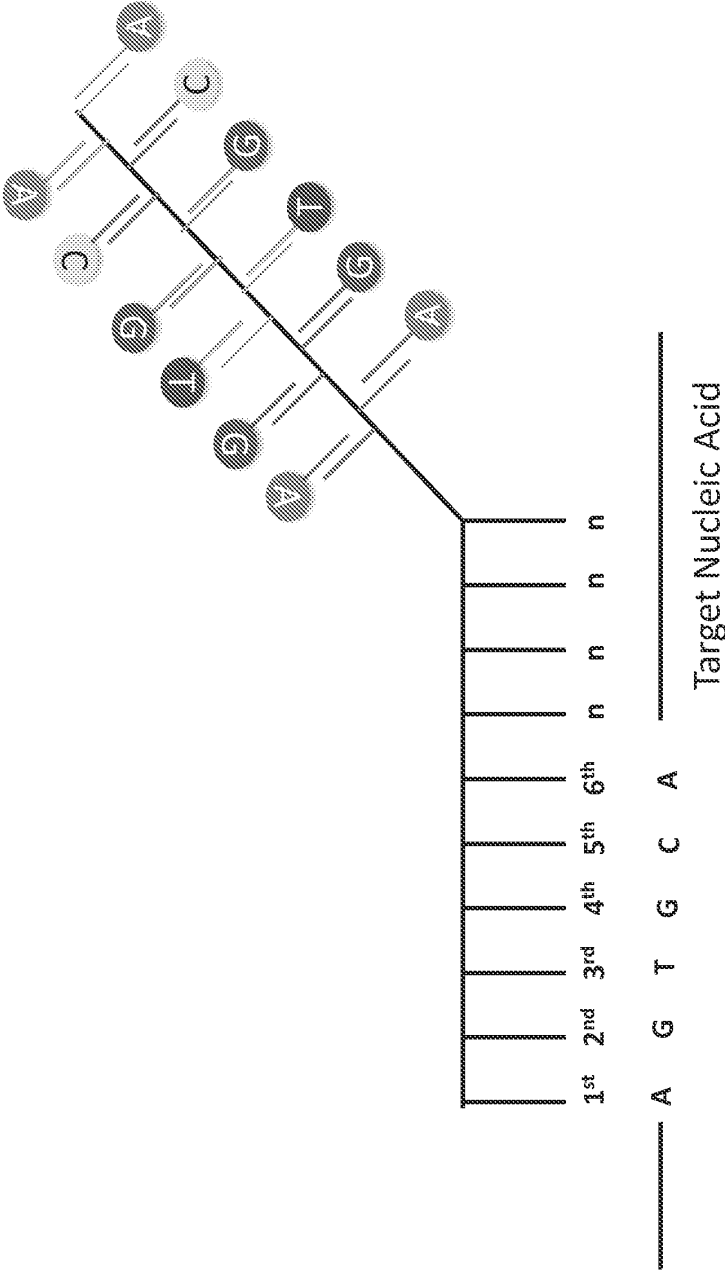


Figure 23



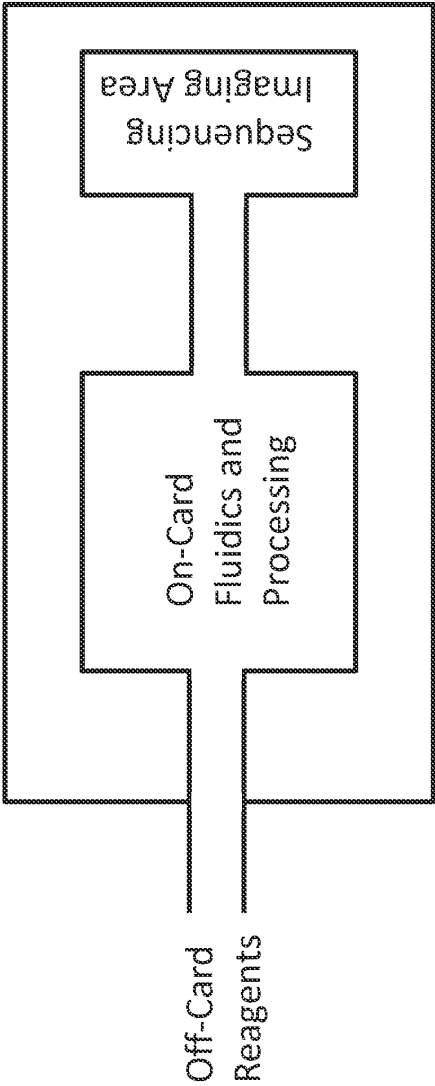


Figure 24

Figure 25

code	sequences
YGBYGR	GAGACTGTAC - original
BGYRGB	GAGACAGTAC
YBRYGB	GAGACCGTAC
RGBYGR	GAGACCGTAC
YRBGYR	GAGAGTGTAC
GRYGRB	GAGAAATGTAC
RGYBRG	GAGATTGTAC

code	counts	sequences
YGBYGR	16074	GAGACTGTAC
RGYBRG	1132	GAGATTGTAC
YBRYGB	998	GAGACCGTAC
YRBGYR	784	GAGAGTGTAC
YBRYBG	712	
YBGRBY	699	
YGRYBG	692	
YBRGBR	674	
Background Threshold	717	

Figure 26

DNA	Sequence	code	count
12 mer	CTGAGACTGTAC	RYGBRG	80845
11 mer	TGAGACTGTAC	GBRYGB	24474
10 mer	GAGACTGTAC	YGBYGR	18339
9 mer	AGACTGTAC	YRBYRG	1667
Mirror of "12 mer"	CATGTCAGAGTC	GRBGYR	1473
Mirror of "11 mer"	CATGTCAGAGT	BGYRBG	1384
8 mer	GA CTGTAC	GBRYBG	900
		Background Threshold	622

Figure 27

