

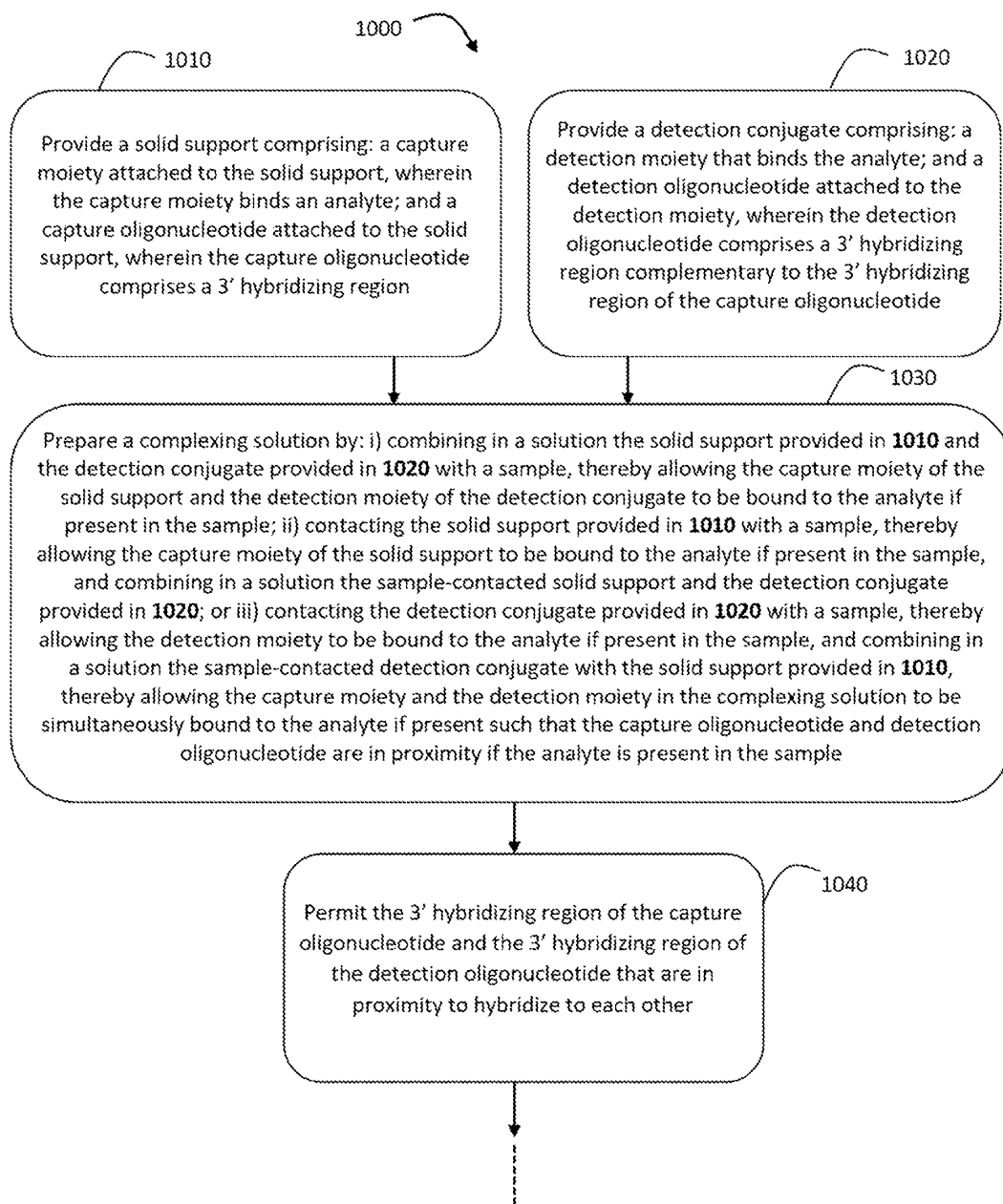


FIG. 1

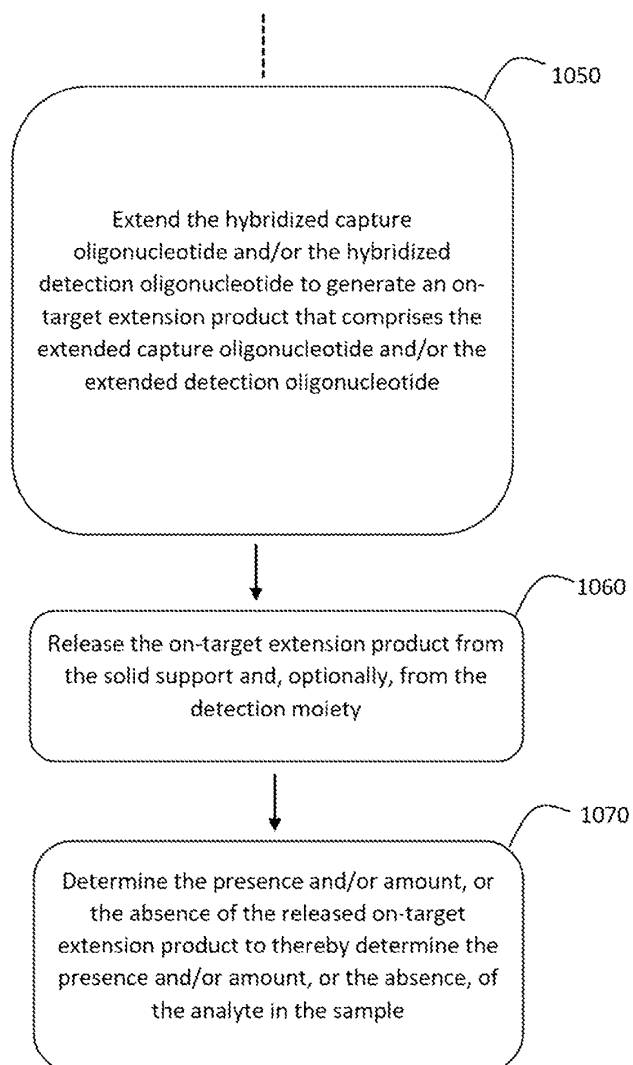


FIG. 1
CONTINUED



FIG. 2

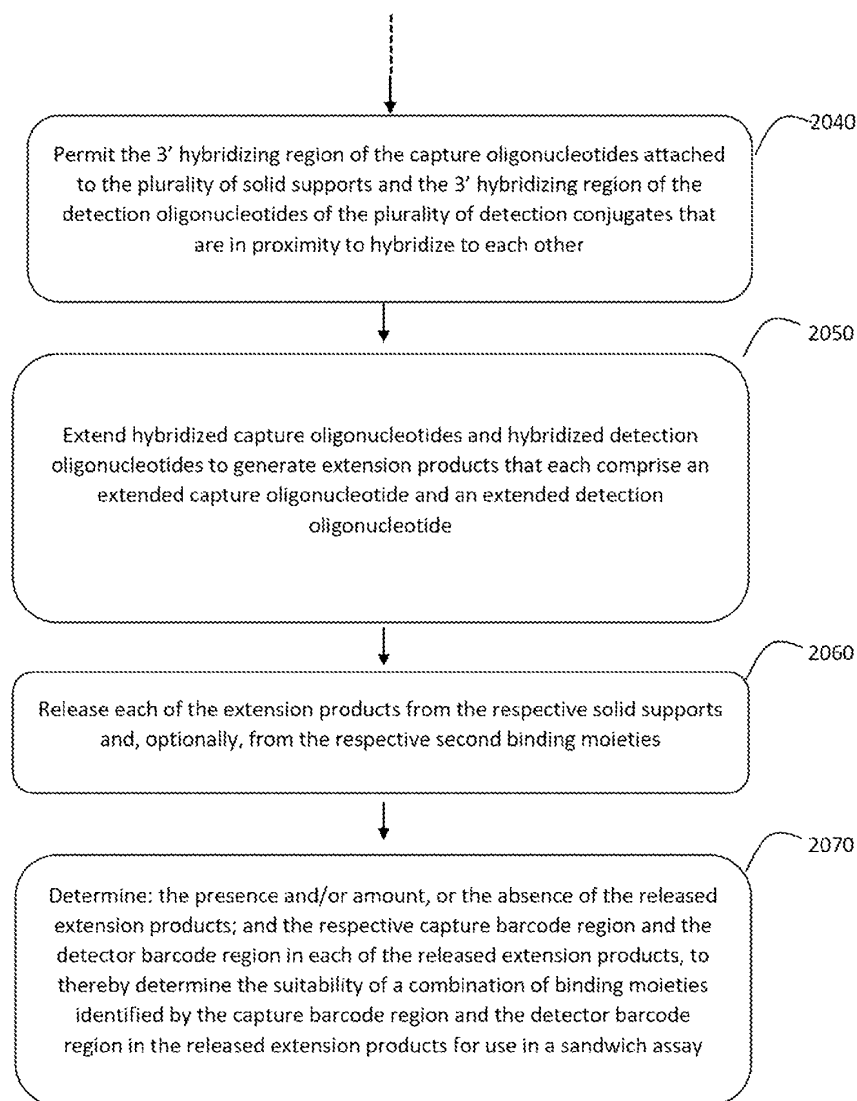


FIG. 2
CONTINUED



FIG. 3A



FIG. 3B

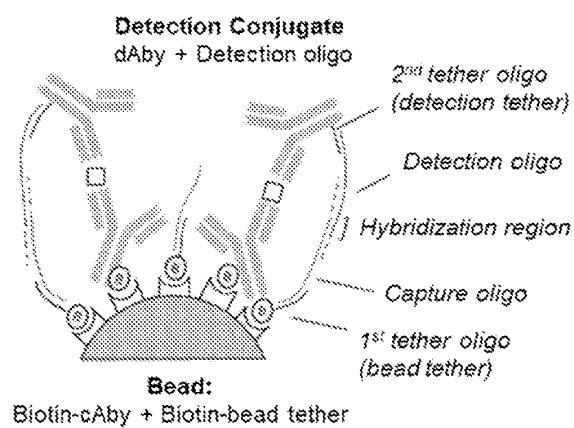


FIG. 3C

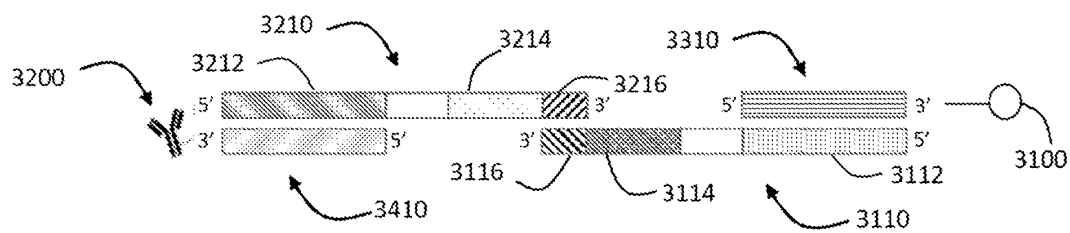


FIG. 3D

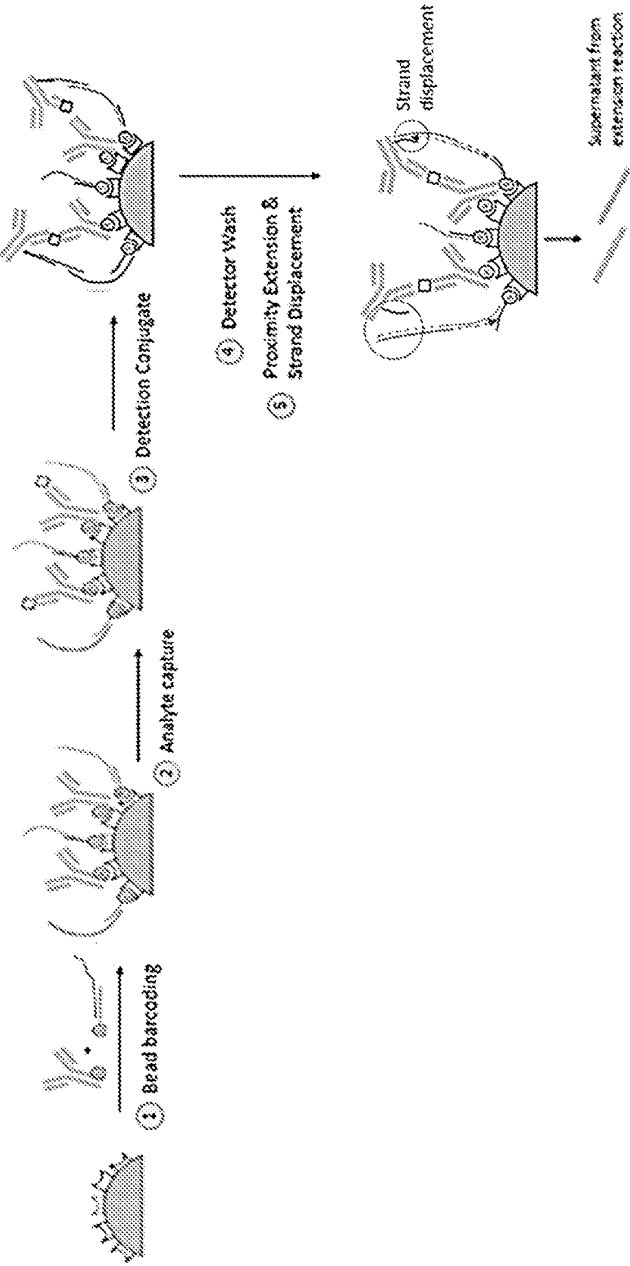


FIG. 4



FIG. 5A



FIG. 5B

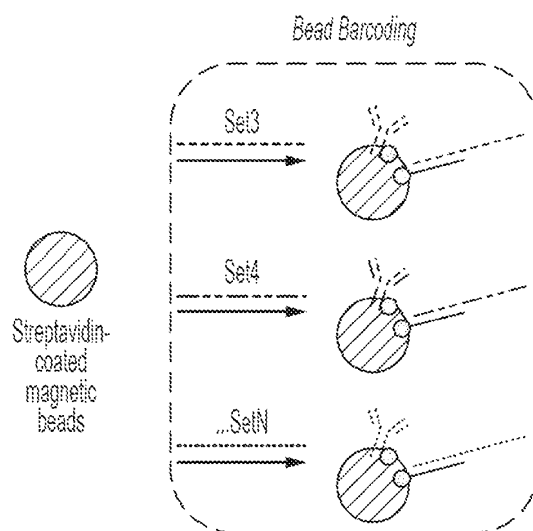


FIG. 5C



FIG. 5D

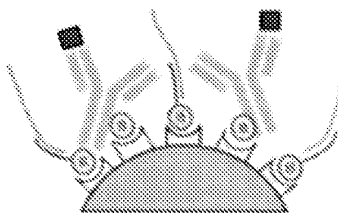


FIG. 5E

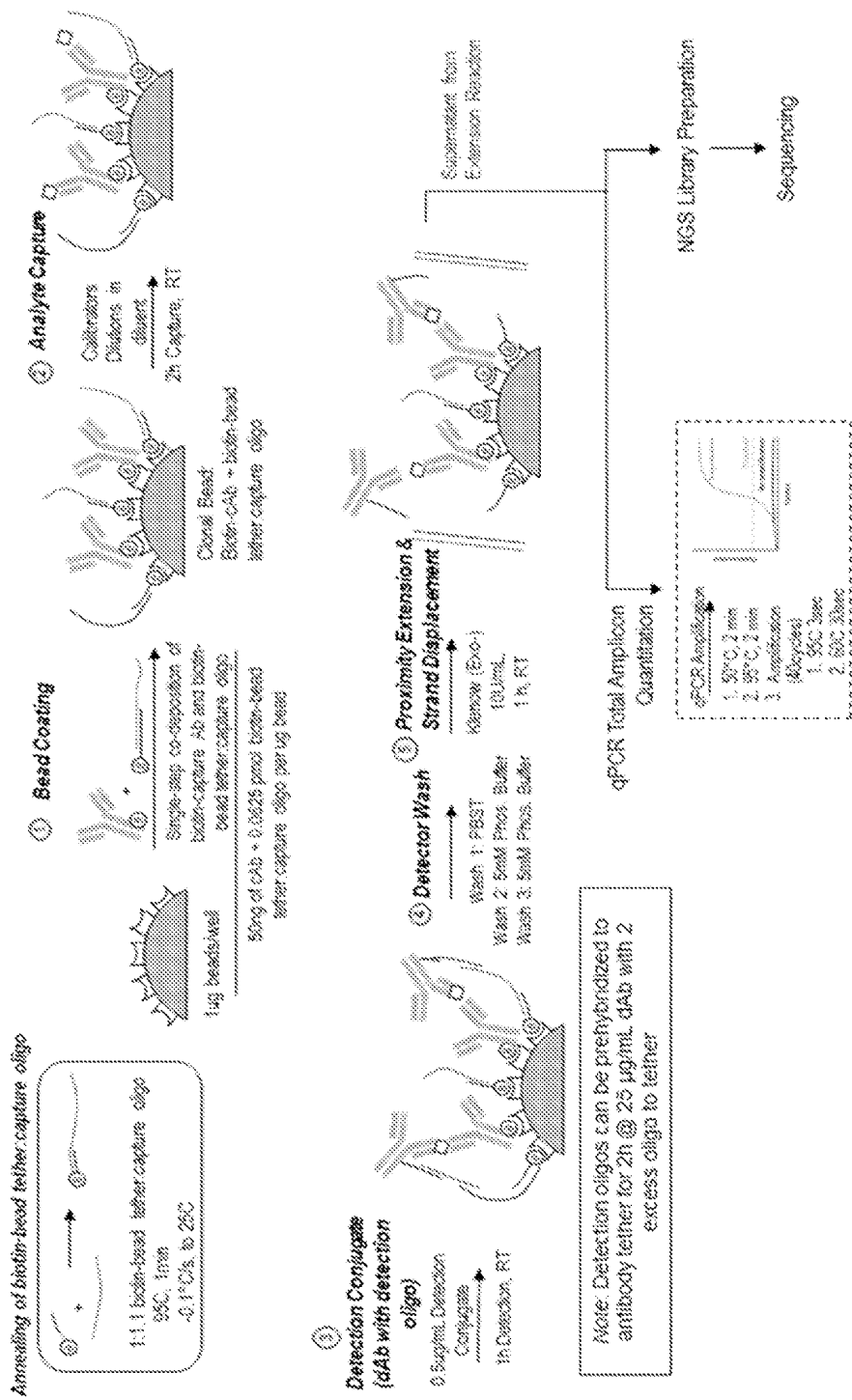


FIG. 6

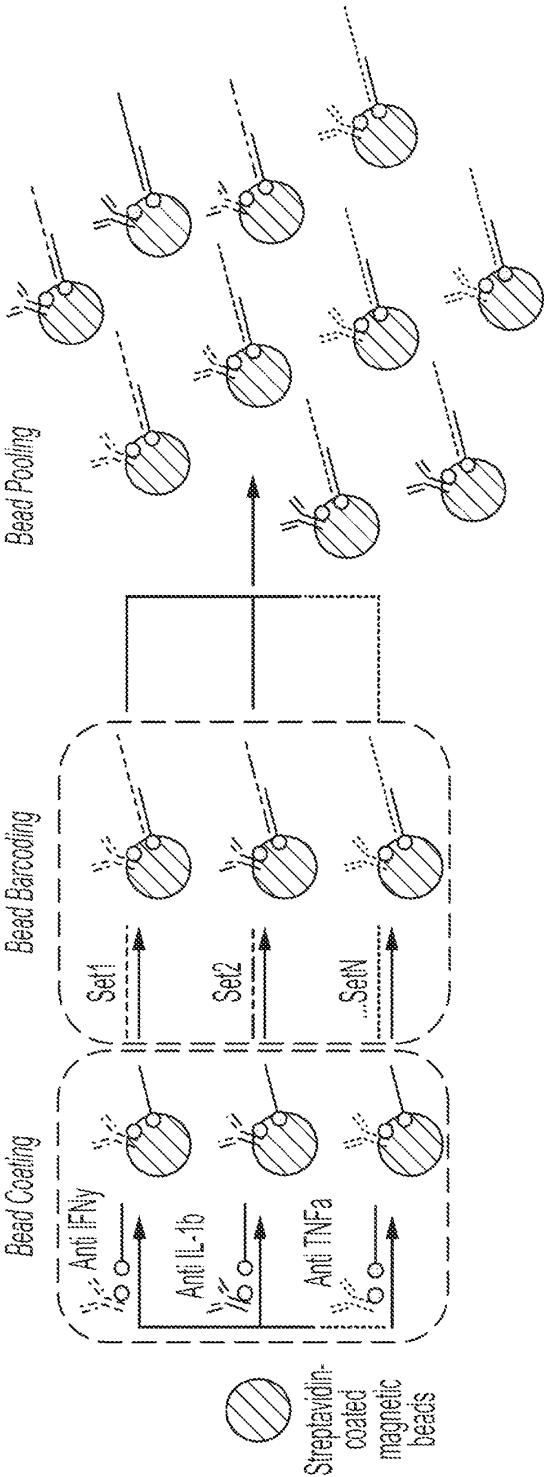


FIG. 7A

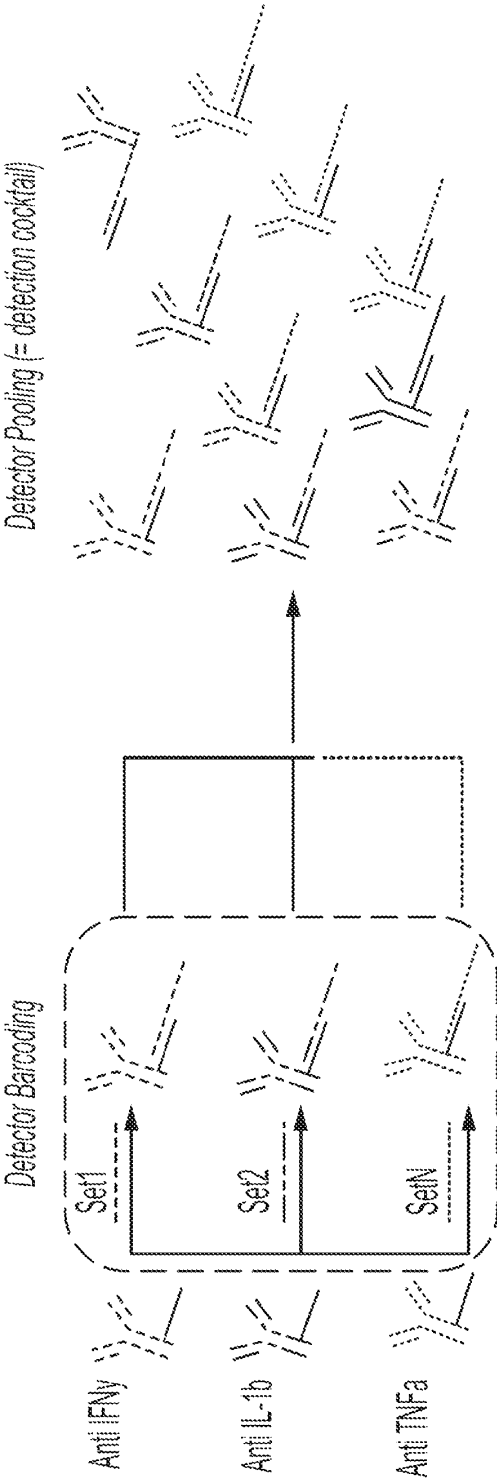


FIG. 7B

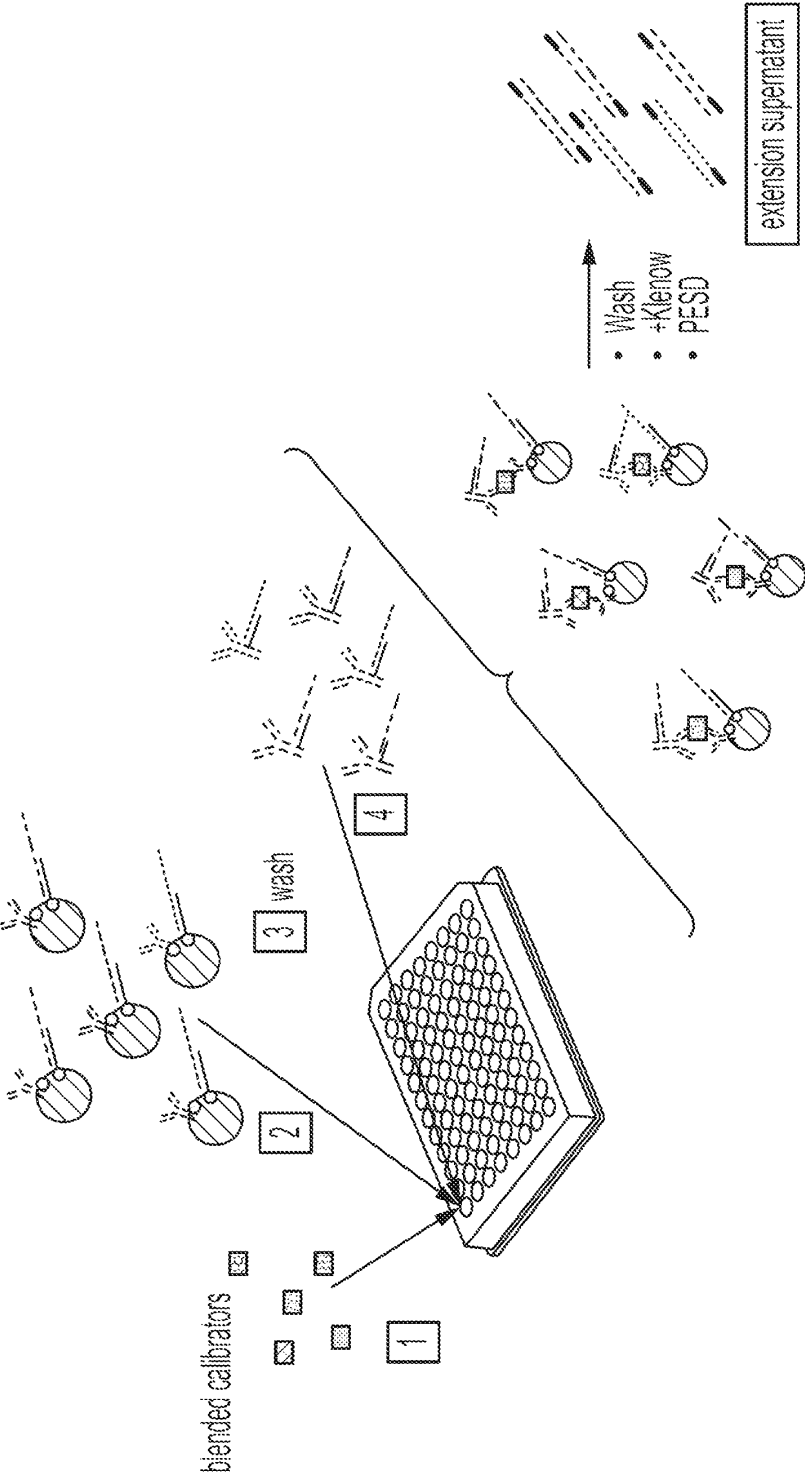


FIG. 8

Comparison of Proximity-Extension-Strand-Displacement and Proximity-Extension ¶

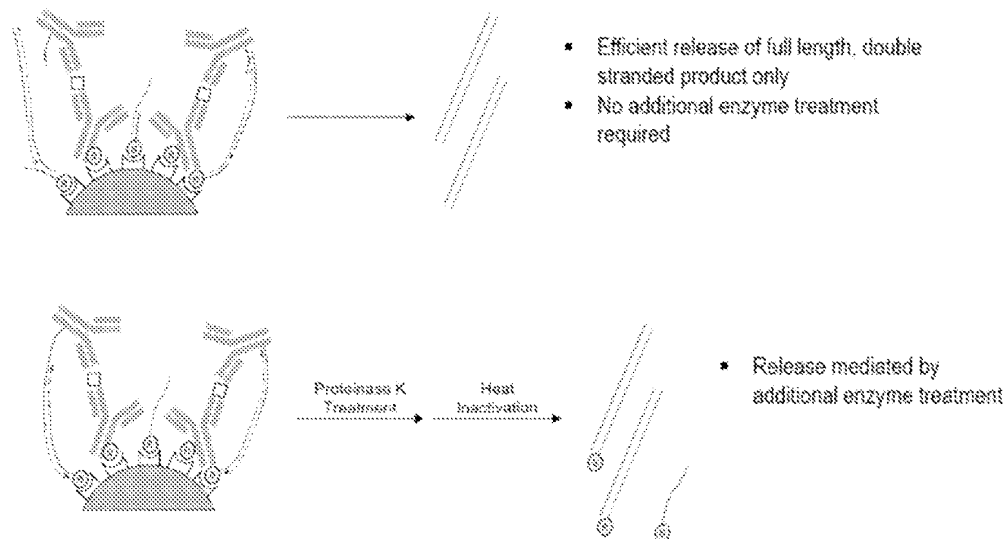


FIG. 9

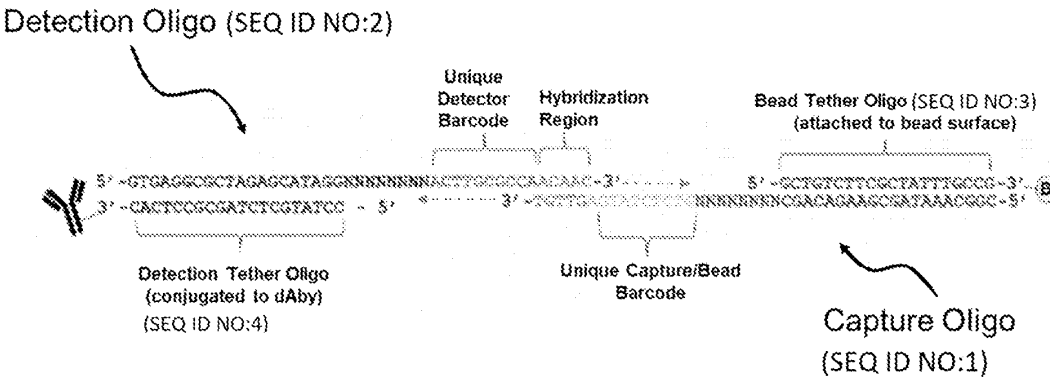


FIG. 10

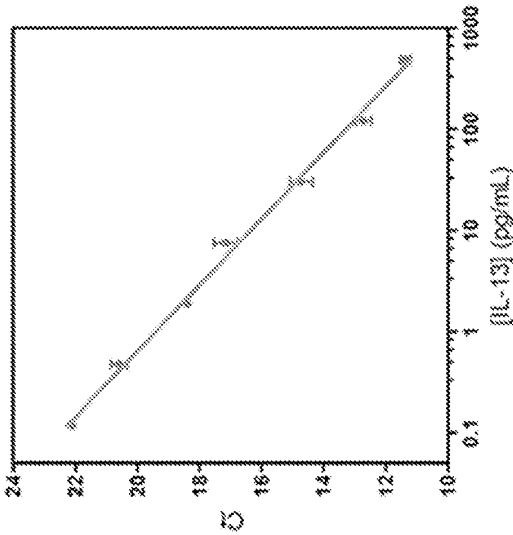


FIG. 11

IL-13 PESD Assay		
[IL-13] (pg/mL)	1	2
482	11.49	11.25
120.5	12.91	12.54
30.125	14.98	14.46
7.531	17.43	16.90
1.883	18.42	18.39
0.471	20.77	20.40
0.118	22.17	22.04
0	23.90	23.80

Calibrator #	#Ny		IL-1b		IL-2		IL-3		IL-4		IL-5		IL-6		IL-7		IL-8		IL-9		IL-10		IL-12p70		IL-13		IL-15		
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
1	5382	2719	13275	7035	19211	13615	64429	32972	174449	6424	3912	12911	6323	13534	7782	16386	76394	512	412										
2	1541	1508	3415	2442	5224	5006	1796.4	16103	72189	1678	1599	2470	2517	4000	4250	34953	22780	171	169										
3	424	306	894	717	1312	1005	4794	3491	21229	426	282	974	733	1116	930	5915	4290	47	37										
4	223	195	311	213	493	328	1690	1222	7173	143	127	501	232	373	305	1578	993	27	22										
5	156	97	107	63	161	99	626	342	2319	57	50	165	66	119	73	350	234	14	5										
6	71	68	12	22	20	42	92	192	332	16	36	20	15	25	27	46	69	15	6										
7	75	68	5	5	29	20	71	46	103	79	20	10	5	12	21	20	15	5	4										
8	20	223	9	9	7	40	27	260	8	130	10	4	14	4	26	3	23	3	3										

FIG. 12A

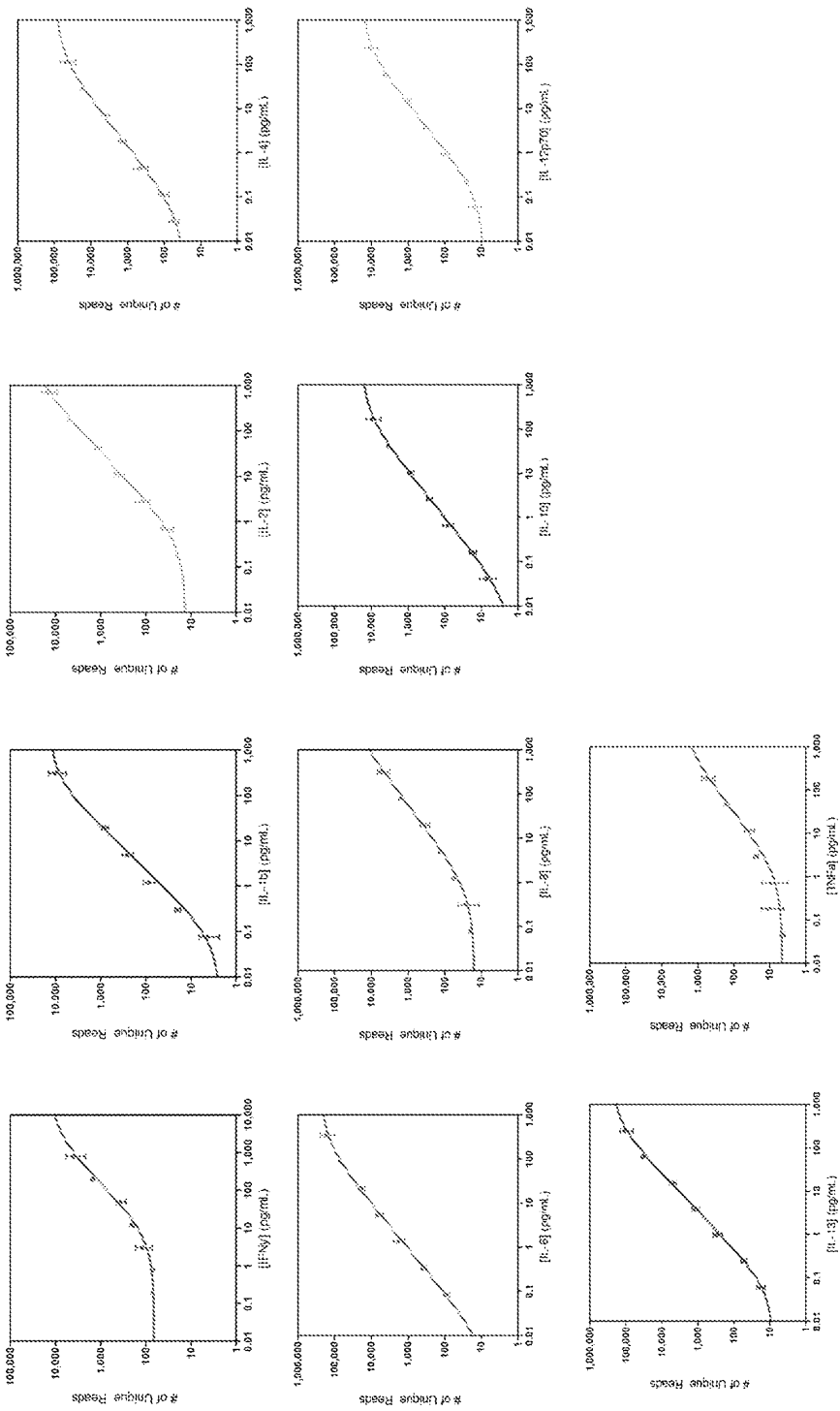


FIG. 12B

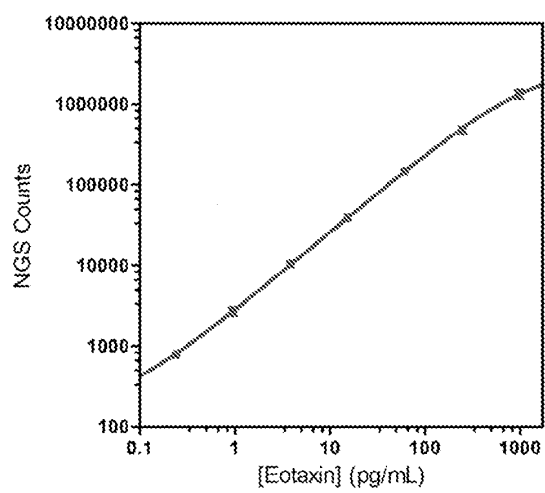


FIG. 13

Assay	Calibrator																													
	E-27	E-22	E-31	MAP-3a	Gravim	MAP-1a	MAP-1b	MCP-1	FLT-1	FLT-2	POF	VECF-D	GM-CSE	MCP-4	E-4	MDC	TARC	TNF- β	E-26a	E-5	E-15	E-7	Column 3	1a-2	E-29	E-21	E-1b	E-17a	E-10	E-2
Ex-27	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
Ex-22	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
E-31	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
MAP-3a	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
Standard A	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
MAP-1a	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
MAP-1b	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
MCP-1	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
E-4	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
MDC	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
TARC	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
TNF- β	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
E-26a	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
E-5	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
E-15	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
E-7	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
Column 3	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
1a-2	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
E-29	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
E-21	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
E-1b	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
E-17a	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
p-10	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
E-2	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%

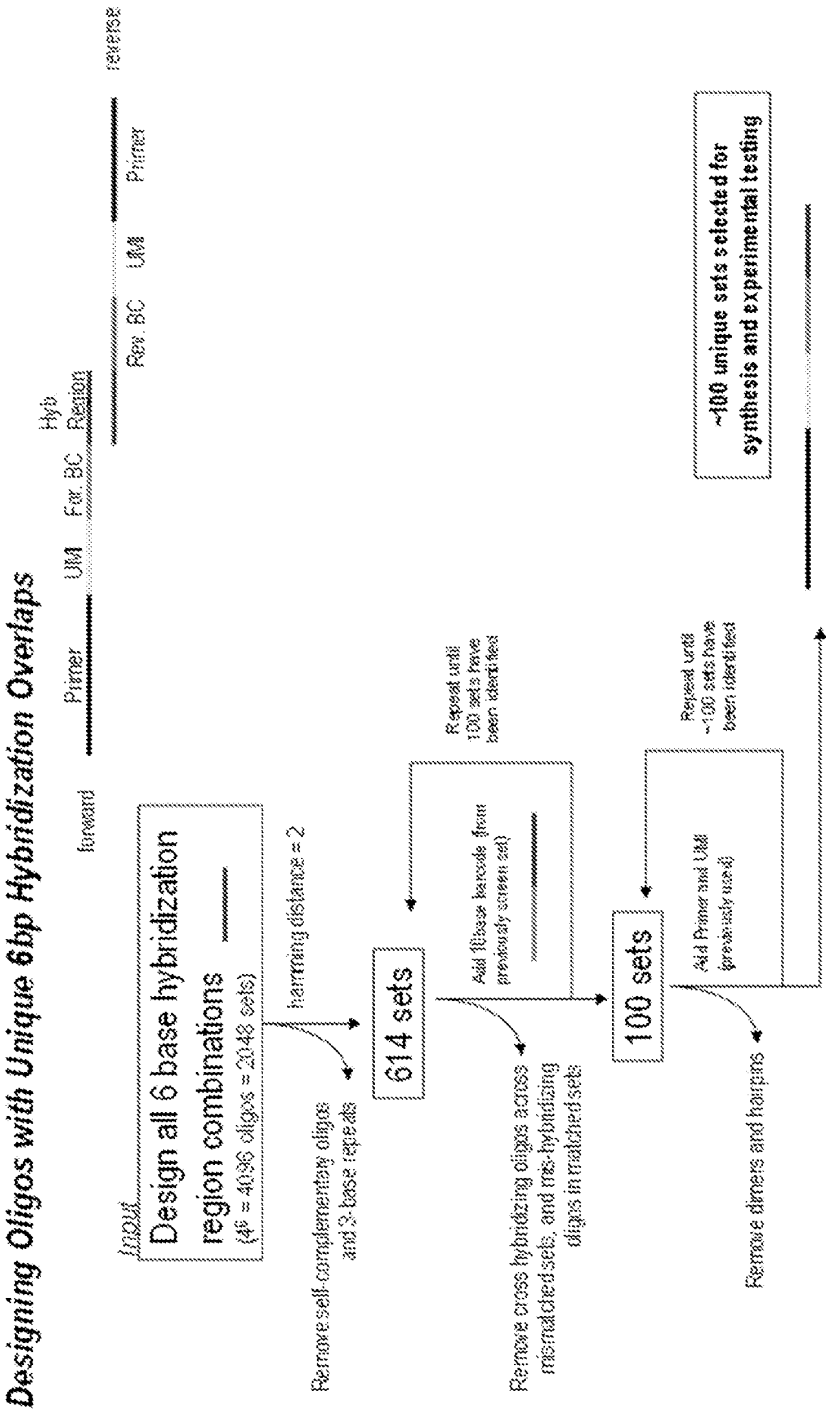


FIG. 15

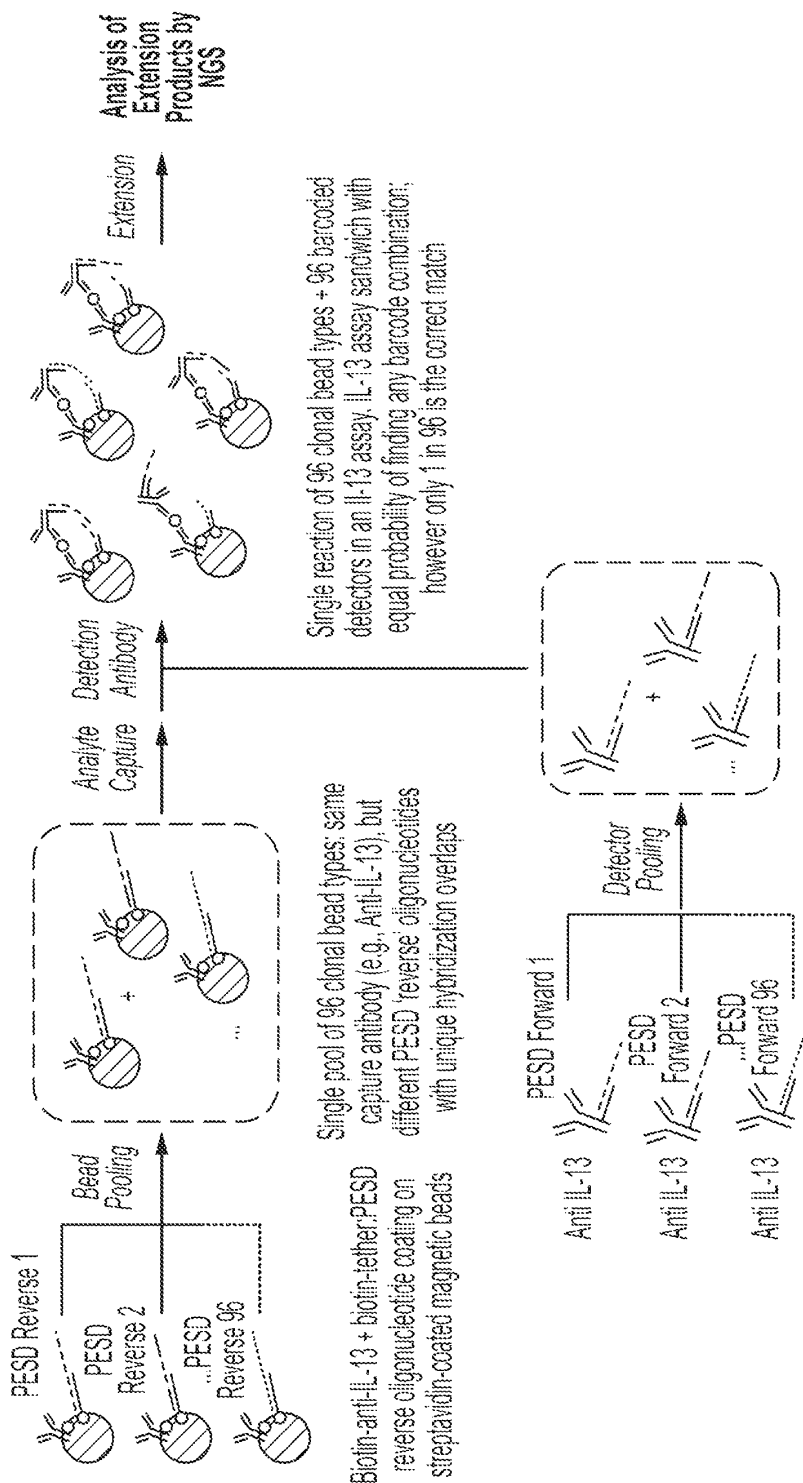
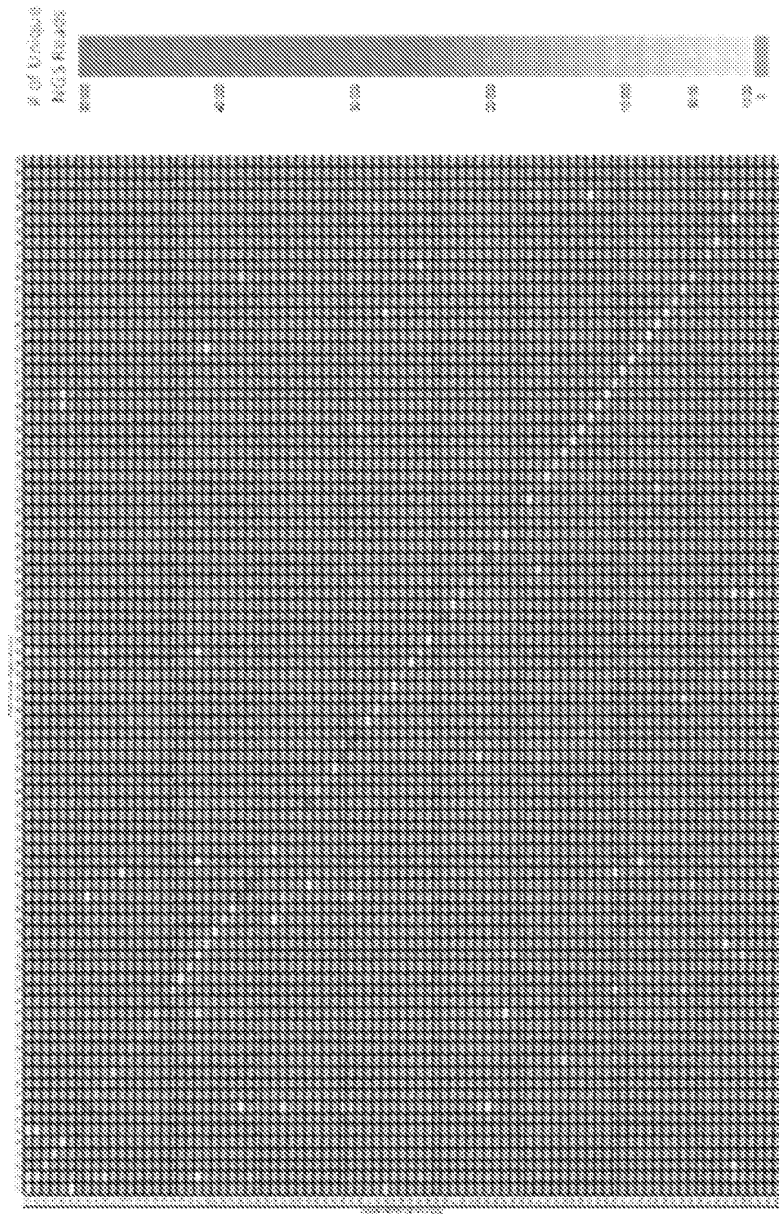


FIG. 16



The number of unique NGS reads identified for amplicons containing 2 barcodes during screening for 89 sets of PSD oligos with unique hybridization overlaps. Note that each forward oligo and reverse oligo are paired (by-design) with complementary sequences in their hybridization overlap. For visualization of on- and off-target interactions, those paired oligonucleotides were sequentially ordered in this matrix such that each complement is found at the diagonal intersection of the table from top-left to bottom-right.

FIG. 17

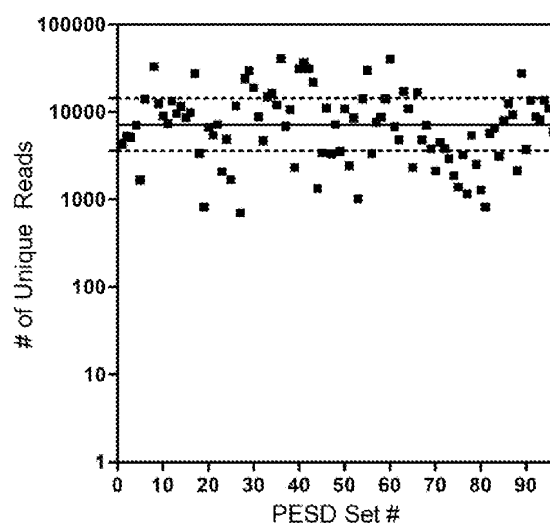


FIG. 18

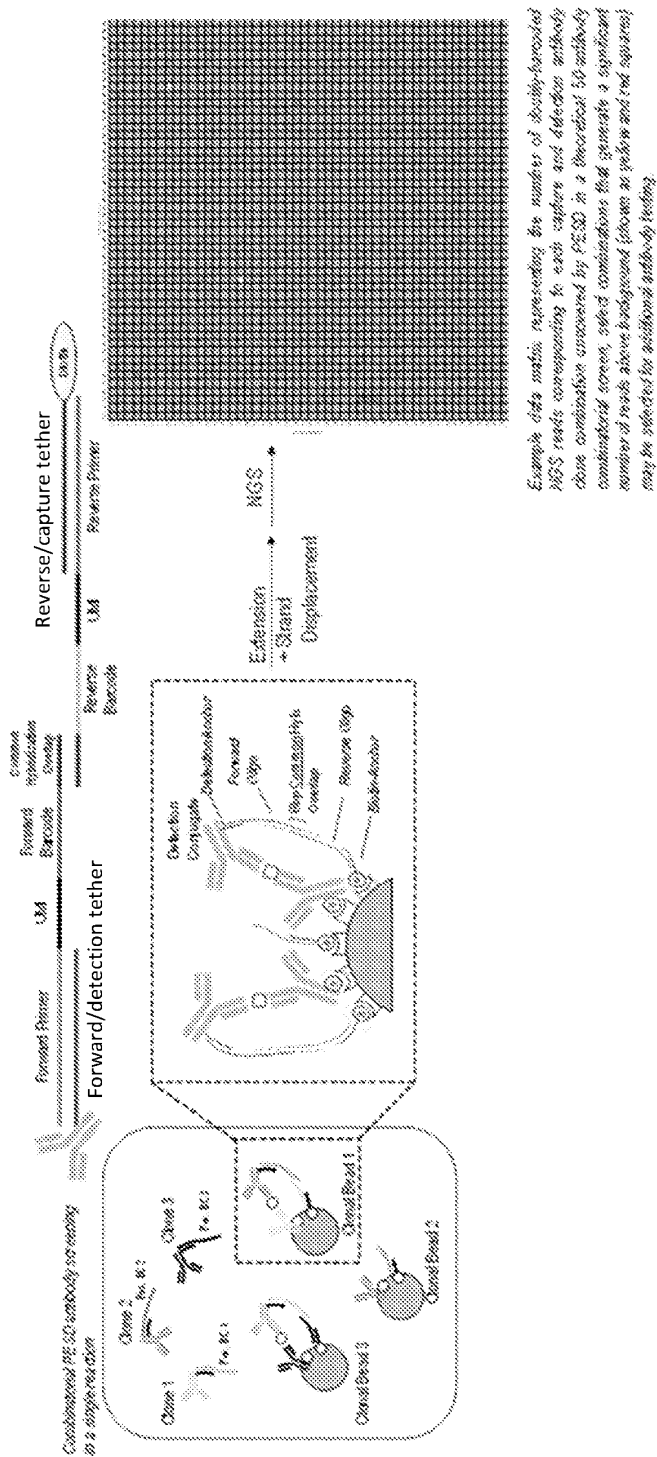


FIG. 19

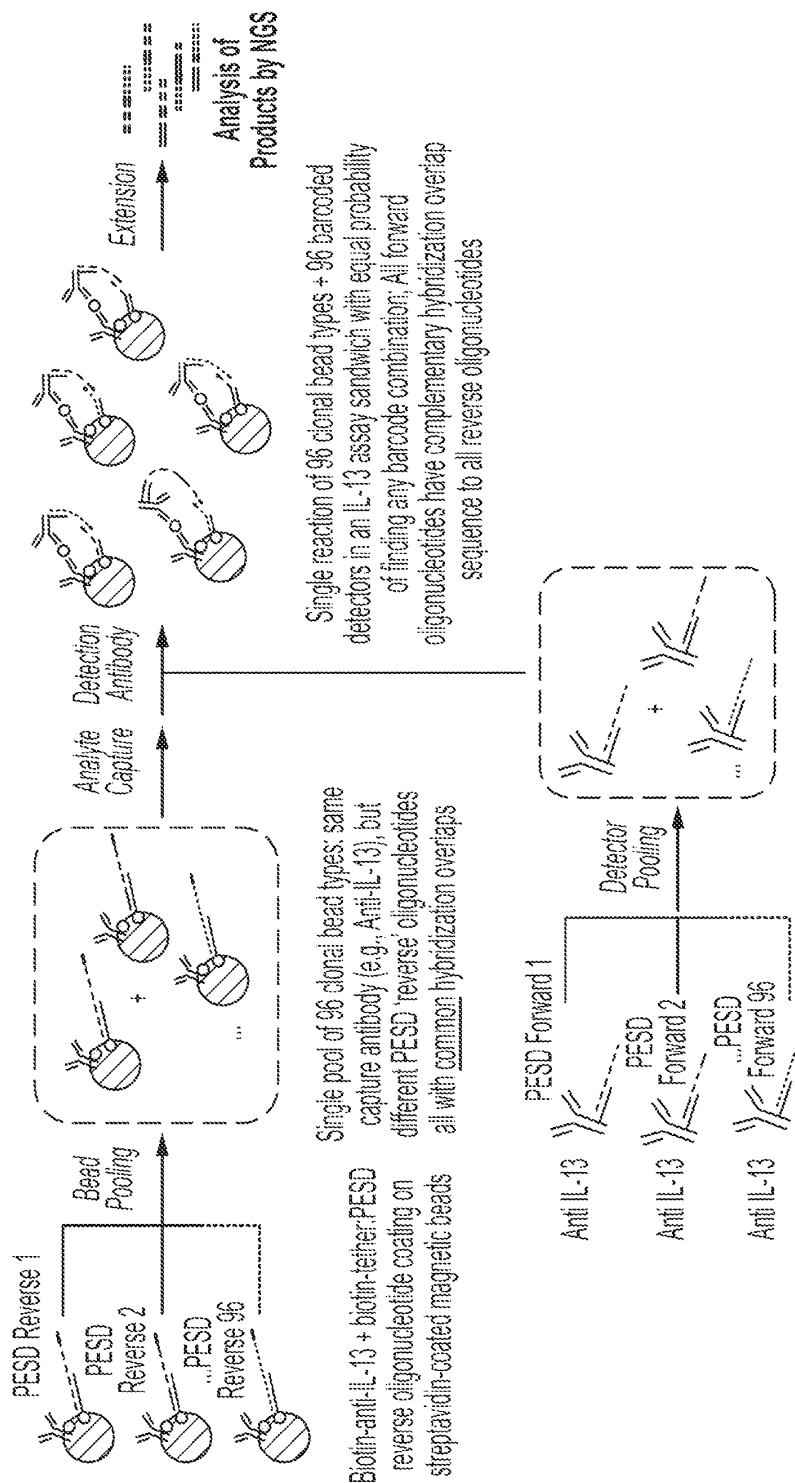


FIG. 20

Scheme for the experimental evaluation of 96 different forward/detector and reverse/capture PESD oligonucleotides, read out by NGS.

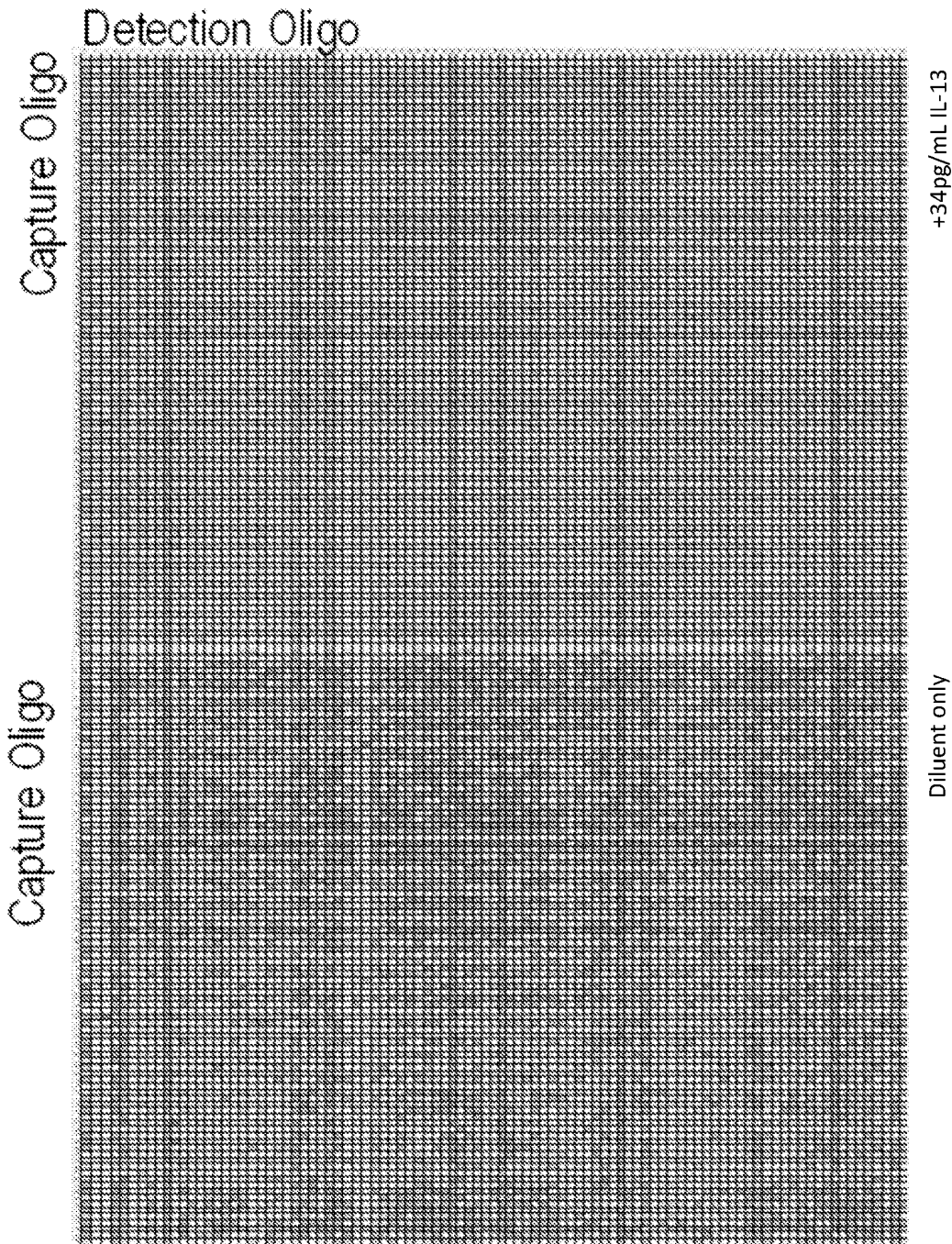


FIG. 21



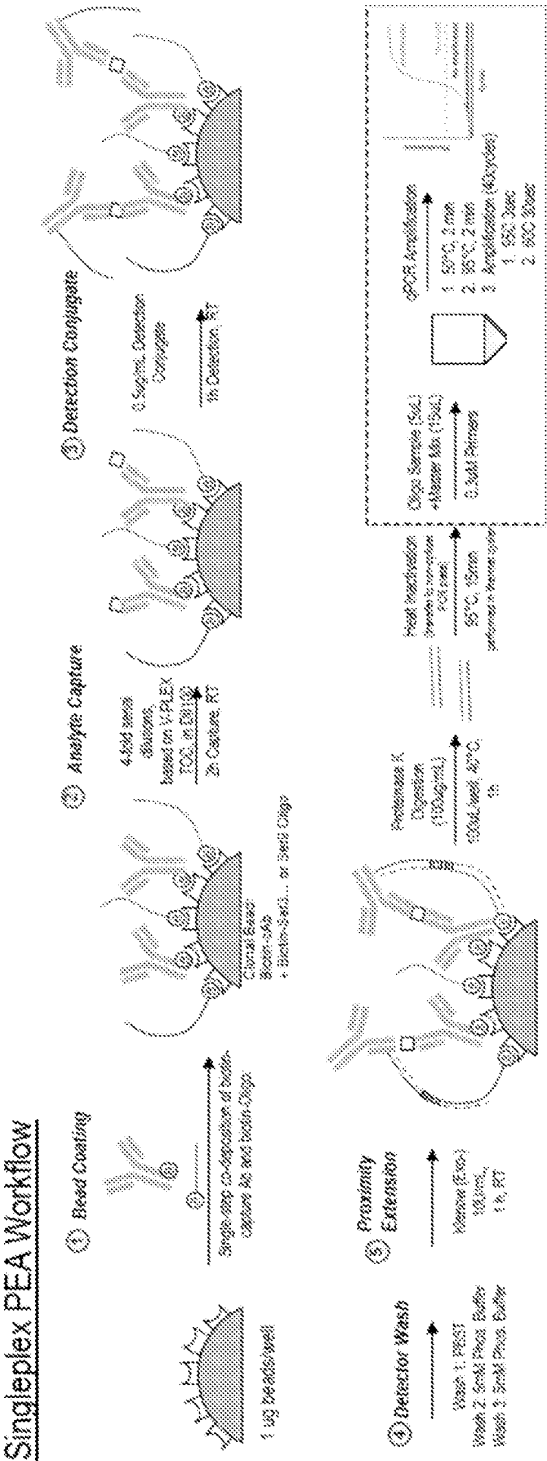


FIG. 23

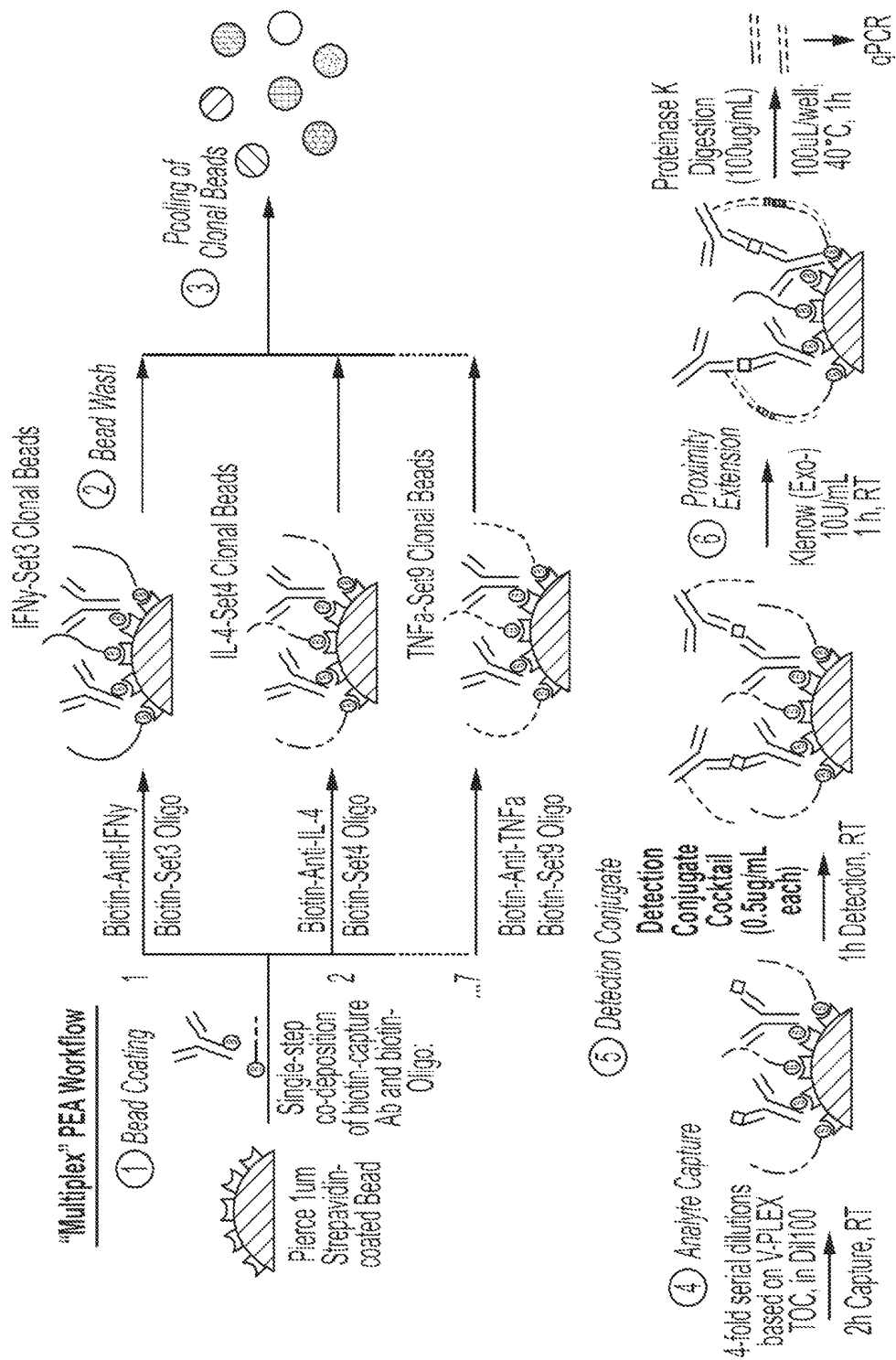


FIG. 24

R-8					
Antibody version	v1	v2	v1	v2	v2
Assay Format	Singleplex	Singleplex	Singleplex	Singleplex	Multiplex
Experiment Date	11-Jan-2022	18-Mar-2022	11-Jan-2022	18-Mar-2022	23-Mar-2022
[IL-13] (pg/mL)	1	2	1	2	1
333	17.54	17.17	14.81	15.38	15.38
95.8	19.79	19.12	19.01	19.77	19.77
23.94	21.40	21.54	17.83	17.87	17.87
5.93	23.40	23.03	19.44	19.70	19.70
1.495	25.31	25.23	21.61	21.60	21.60
0.374	27.39	27.07	23.43	22.94	22.94
0.034	28.85	28.15	24.95	23.49	23.49
0.000	29.84	29.90	26.72	23.74	23.74

R-4					
Antibody version	v1	v2	v1	v2	v2
Assay Format	Singleplex	Singleplex	Singleplex	Singleplex	Multiplex
Experiment Date	11-Jan-2022	18-Mar-2022	18-Mar-2022	23-Mar-2022	23-Mar-2022
[IL-13] (pg/mL)	1	2	1	2	1
333	16.04	15.99	15.81	15.33	15.33
95.8	18.90	18.55	17.31	17.28	17.28
23.94	20.69	20.24	19.19	18.86	18.86
5.93	22.34	22.33	21.43	21.24	21.24
1.495	24.12	24.13	23.05	23.21	23.21
0.374	25.59	25.50	25.34	25.13	25.13
0.034	26.73	27.43	27.21	26.87	26.87
0.000	28.12	28.73	28.91	28.72	28.72

R-12p72					
Antibody version	v1	v2	v1	v2	v2
Assay Format	Singleplex	Singleplex	Singleplex	Singleplex	Multiplex
Experiment Date	11-Jan-2022	21-Mar-2022	21-Mar-2022	23-Mar-2022	23-Mar-2022
[IL-13] (pg/mL)	1	2	1	2	1
333	16.04	16.55	15.35	15.44	15.16
95.8	18.24	18.32	20.30	20.28	19.50
23.94	20.04	20.51	22.06	22.34	21.19
5.93	22.16	22.14	24.32	23.58	22.75
1.495	24.12	24.45	26.06	26.21	24.45
0.374	25.47	25.65	27.59	27.03	24.99
0.034	26.96	27.13	28.59	28.82	25.06
0.000	27.45	27.51	29.65	29.51	25.59

R-13					
Antibody version	v1	v2	v1	v2	v2
Assay Format	Singleplex	Singleplex	Singleplex	Singleplex	Multiplex
Experiment Date	11-Jan-2022	18-Mar-2022	18-Mar-2022	23-Mar-2022	23-Mar-2022
[IL-13] (pg/mL)	1	2	1	2	1
333	16.77	16.77	16.77	16.73	16.73
95.8	19.67	19.67	19.67	19.73	19.73
23.94	21.69	21.69	21.69	21.69	21.69
5.93	23.69	23.69	23.69	23.69	23.69
1.495	25.69	25.69	25.69	25.69	25.69
0.374	27.69	27.69	27.69	27.69	27.69
0.034	29.69	29.69	29.69	29.69	29.69
0.000	31.69	31.69	31.69	31.69	31.69

TNF α					
Antibody version	v1	v2	v1	v2	v2
Assay Format	Singleplex	Singleplex	Singleplex	Singleplex	Multiplex
Experiment Date	11-Jan-2022	21-Mar-2022	21-Mar-2022	23-Mar-2022	23-Mar-2022
[TNF α] (pg/mL)	1	2	1	2	1
333	22.68	22.93	19.19	18.92	18.99
95.8	22.56	21.93	21.45	21.45	19.71
23.94	22.63	24.43	22.76	22.87	20.84
5.93	25.03	26.43	24.07	24.19	21.93
1.495	26.12	28.24	24.53	24.53	22.54
0.374	28.10	30.90	25.29	25.28	23.12
0.034	30.01	33.60	26.93	26.93	23.35
0.000	30.05	37.50	28.95	28.95	23.74

R-10					
Antibody version	v1	v2	v1	v2	v2
Assay Format	Singleplex	Singleplex	Singleplex	Singleplex	Multiplex
Experiment Date	11-Jan-2022	18-Mar-2022	18-Mar-2022	23-Mar-2022	23-Mar-2022
[IL-10] (pg/mL)	1	2	1	2	1
333	16.06	16.53	17.46	17.16	17.16
95.8	18.24	18.32	19.75	19.50	19.50
23.94	20.04	20.51	22.09	21.19	21.19
5.93	22.16	22.14	23.69	22.75	22.75
1.495	24.12	24.45	25.31	24.45	24.45
0.374	25.47	25.65	26.96	24.99	24.99
0.034	26.96	27.13	27.19	25.06	25.06
0.000	27.45	27.51	27.56	25.26	25.26

FIG. 25

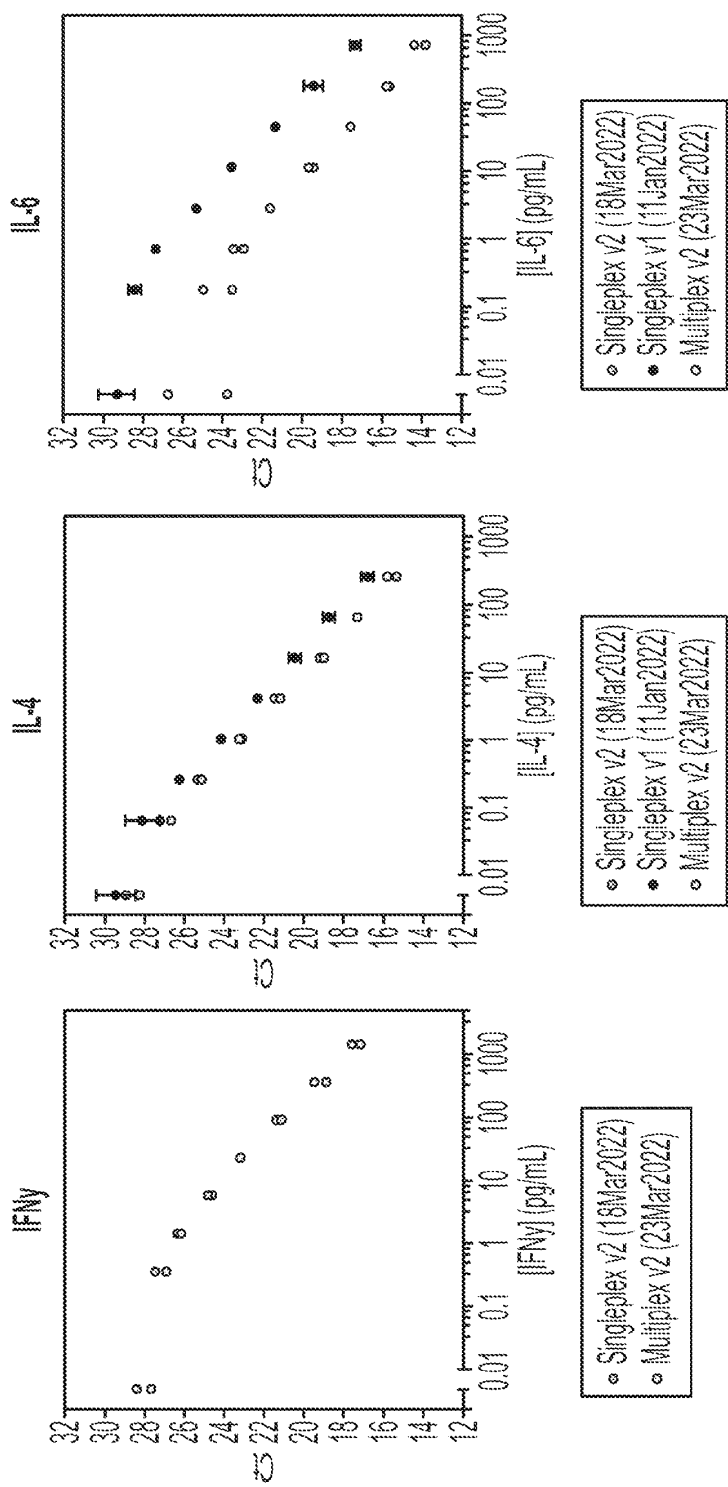


FIG. 26

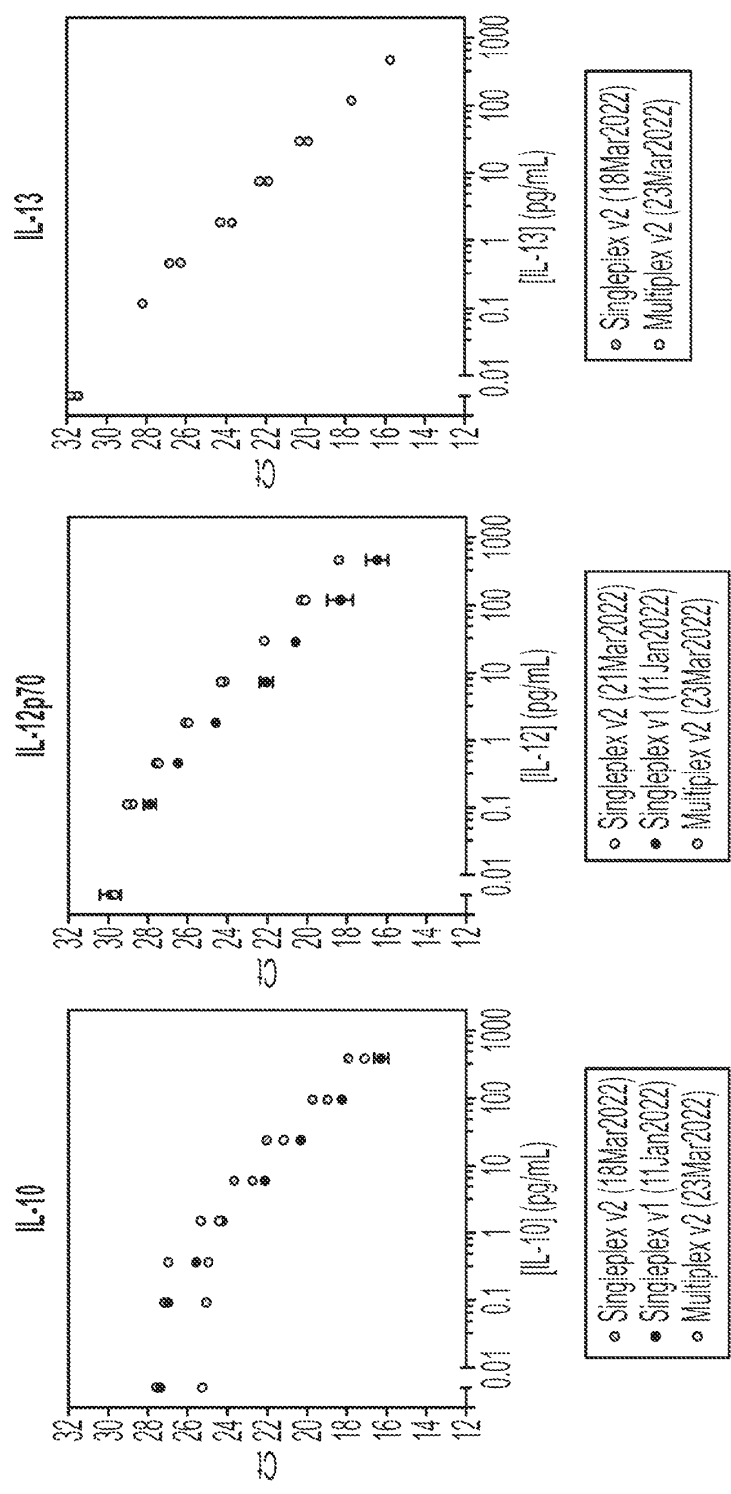


FIG. 26
CONTINUED

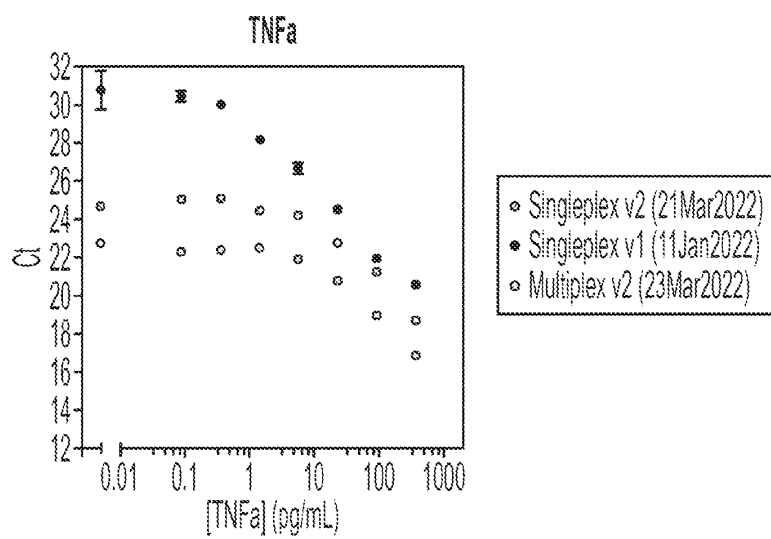


FIG. 26

CONTINUED

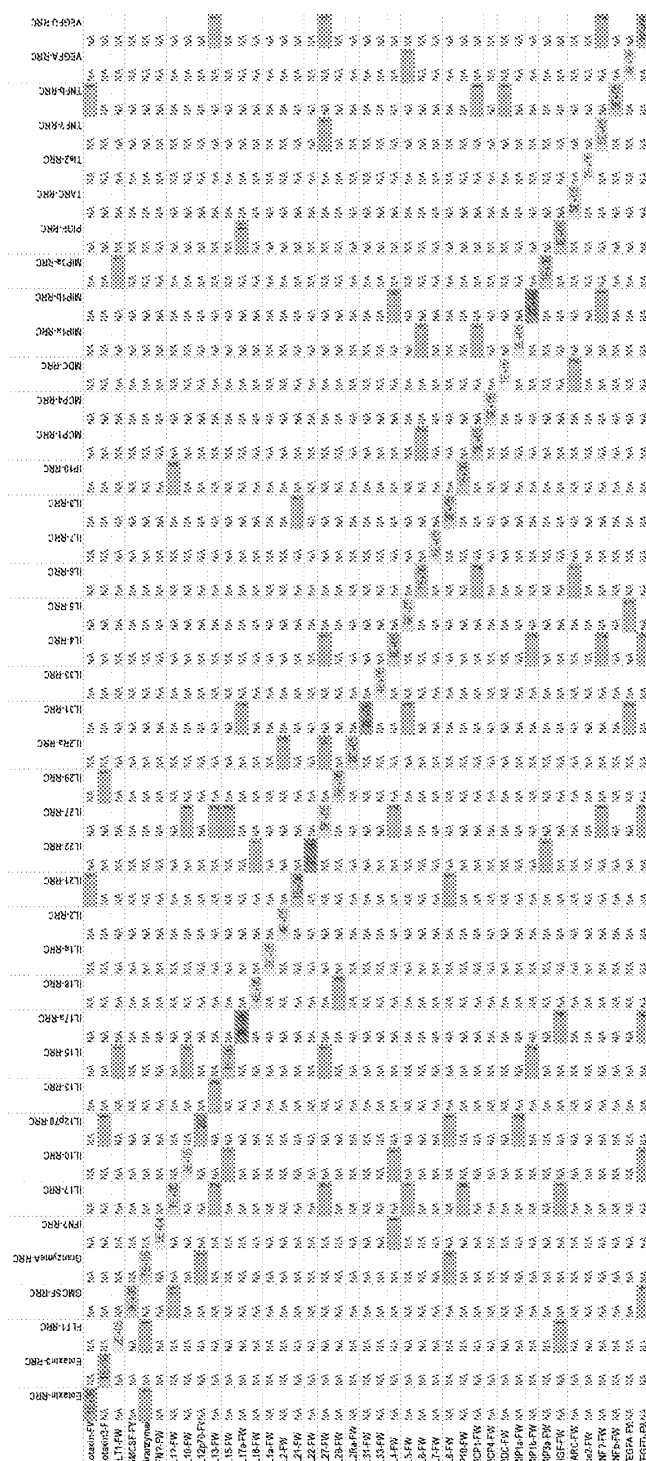


FIG. 27

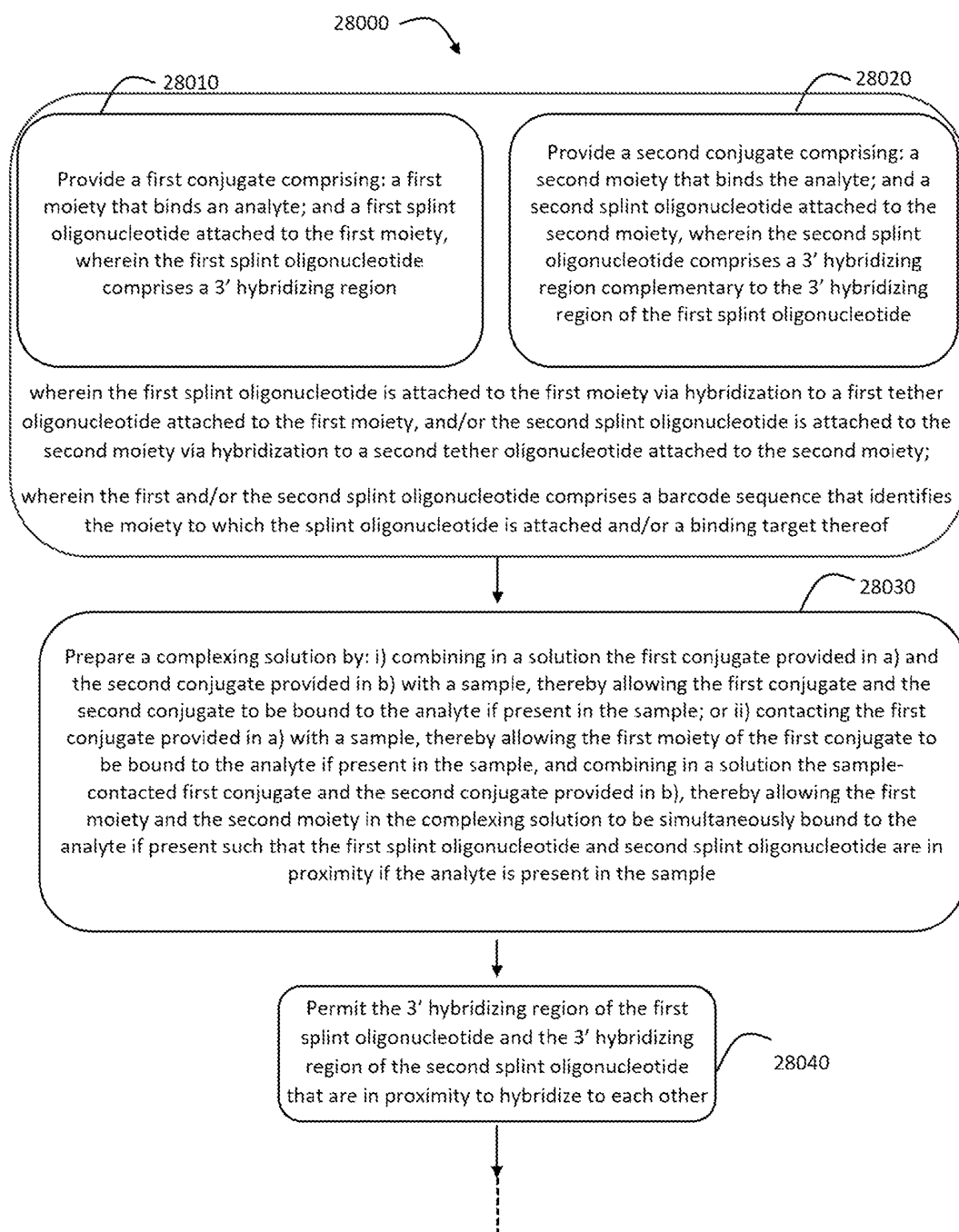


FIG. 28

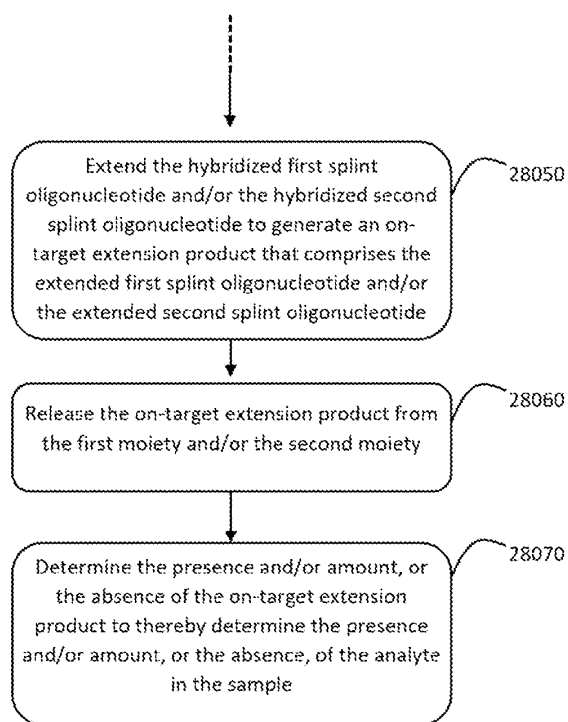


FIG. 28
CONTINUED

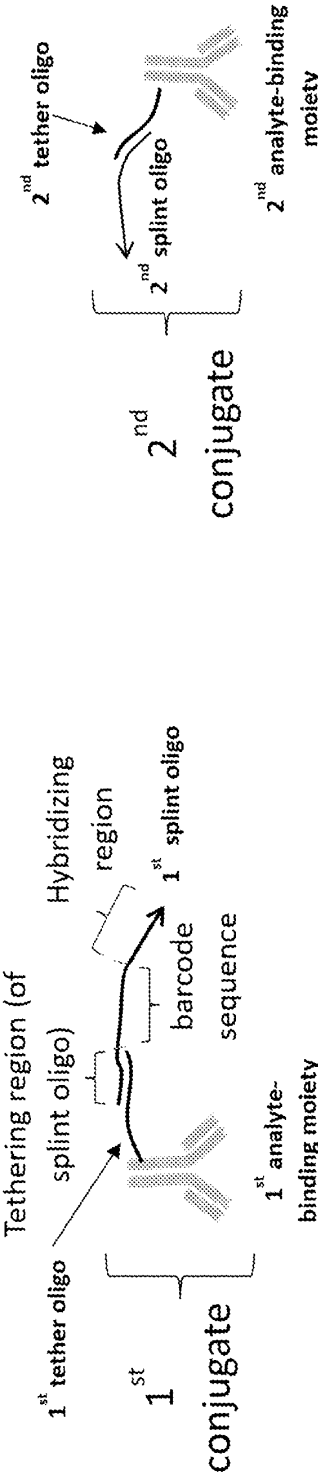


FIG. 29A

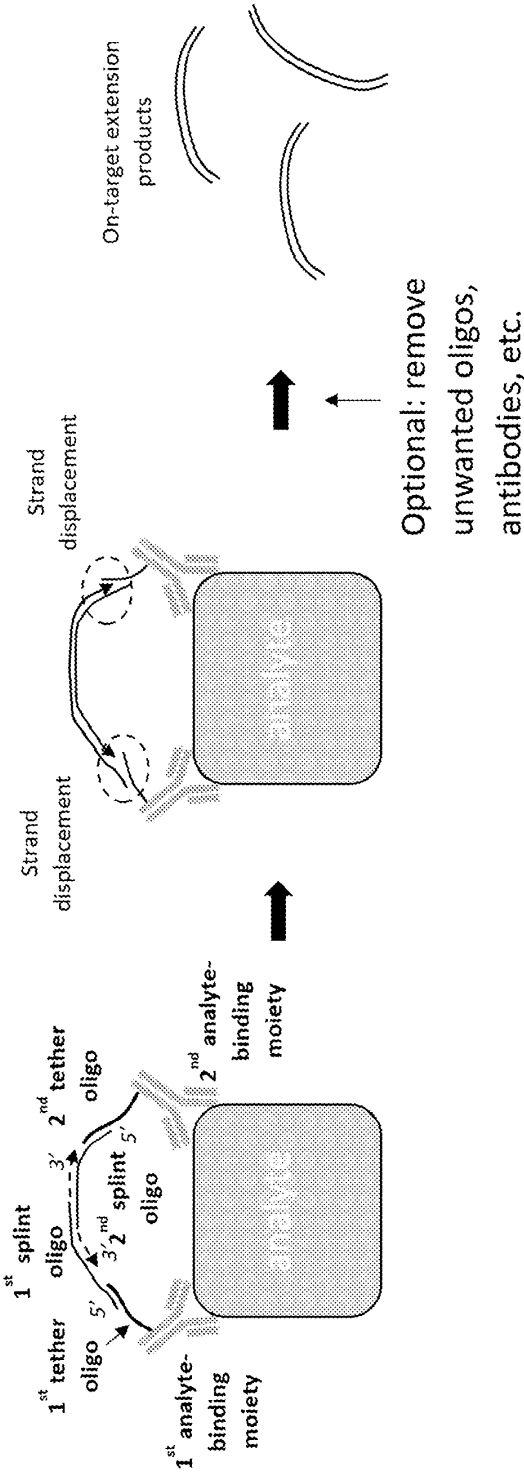


FIG. 29B

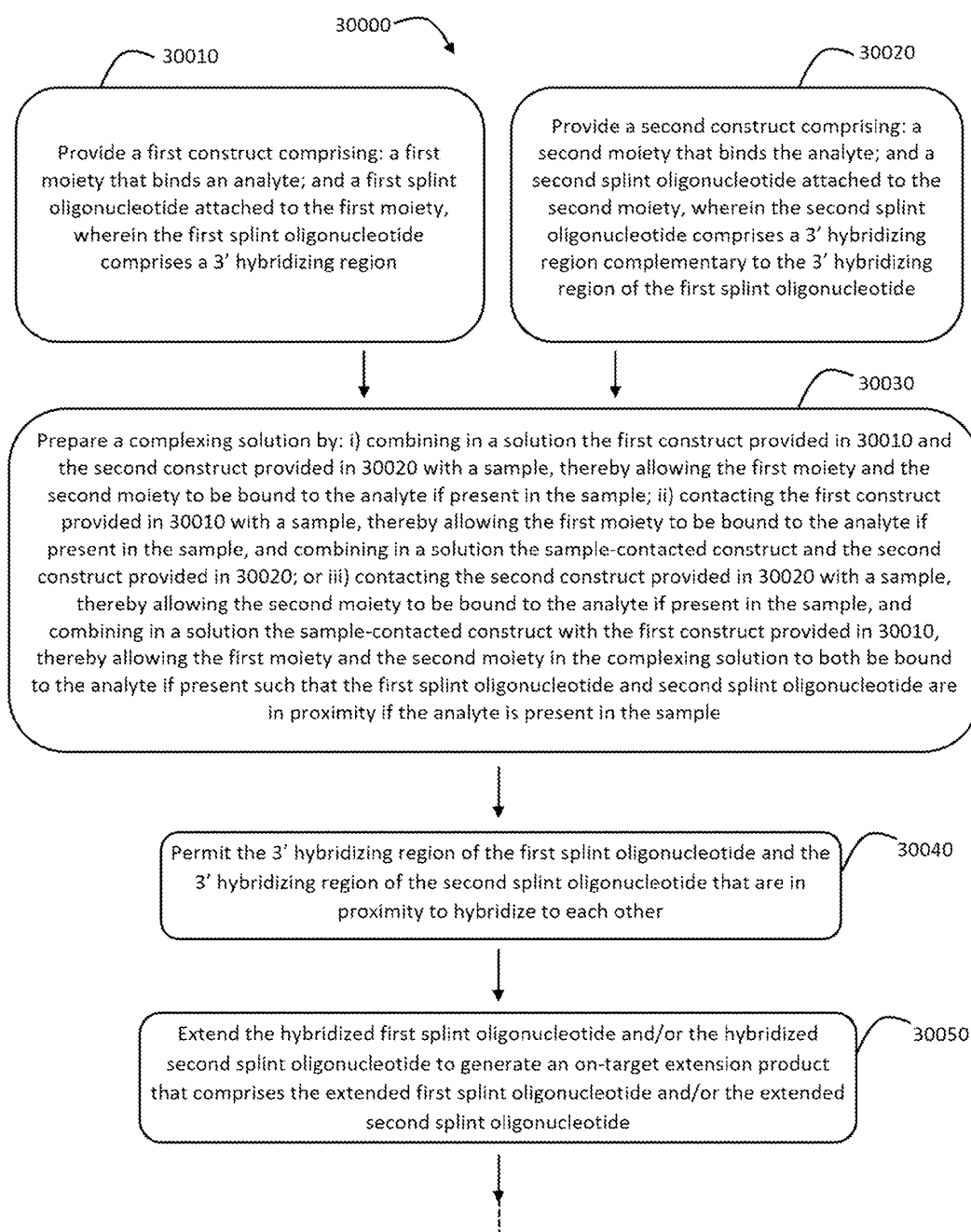


FIG. 30A

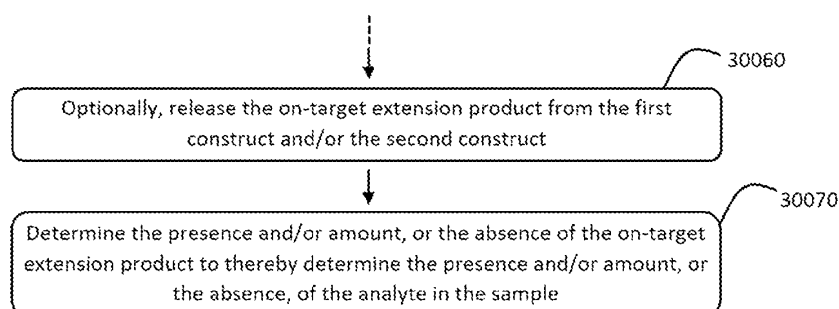


FIG. 30A
CONTINUED

30000

(I) The complexing solution comprises one or more blocker oligonucleotides, wherein each blocker oligonucleotide hybridizes to a portion of the first splint oligonucleotide and/or of the second splint oligonucleotide.

(II) Analyze the sample for a first analyte and a second analyte, wherein preparing the complexing solution in 30030 comprises: preparing the complexing solution in a plurality of subpools comprising a first subpool and second subpool, wherein the first moiety and second moiety in the prepared complexing solution of the first subpool bind the first analyte, and the first moiety and second moiety in the prepared complexing solution of the second subpool bind the second analyte; and combining the plurality of subpools before determining the presence and/or amount, or the absence of the on-target extension product in 30070.

(III) Provide a plurality of paired combinations of the first and second constructs, wherein the 3' hybridizing region of the splint oligonucleotides of one or more of the plurality of paired combinations is 1, 2, 3 or more nucleotides shorter than the 3' hybridizing region of the splint oligonucleotides of at least another one of the plurality of paired combinations, wherein the 3' hybridizing regions of the splint oligonucleotides of the at least another one of the plurality of paired combinations is different from and is not complementary to any contiguous stretch of the 3' hybridizing region of the splint oligonucleotides of the one or more of the plurality of paired combinations.

and/or

(IV) Attenuate an amount of amplification products by reducing or interfering with a binding interaction between the analyte and the first moiety or the second moiety and/or suppressing on-target interactions between the conjugate splint oligonucleotide and the first splint oligonucleotide when the first moiety and the second moiety are both bound to the analyte.

FIG. 30B

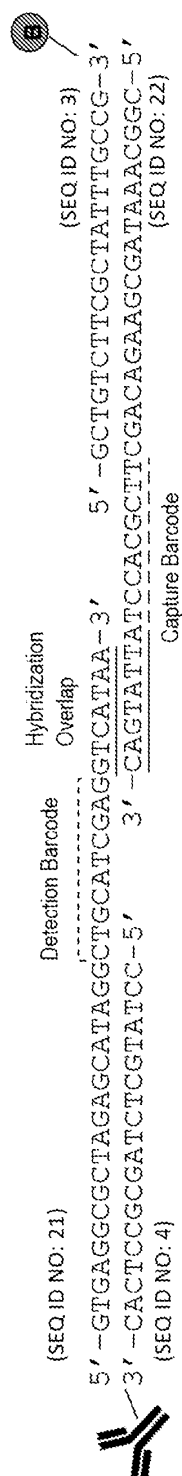


FIG. 31A

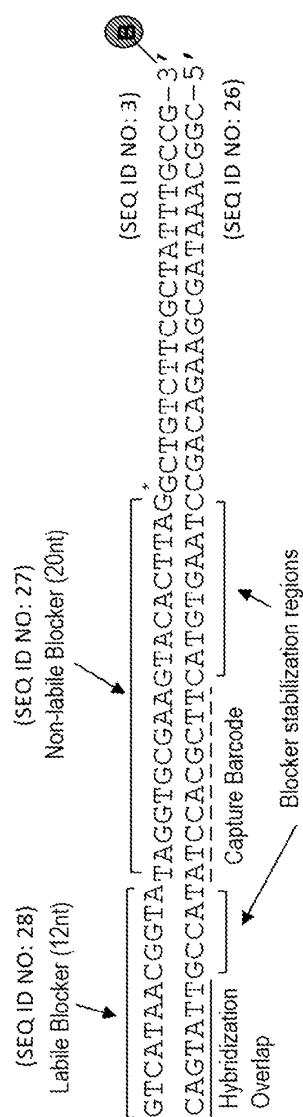
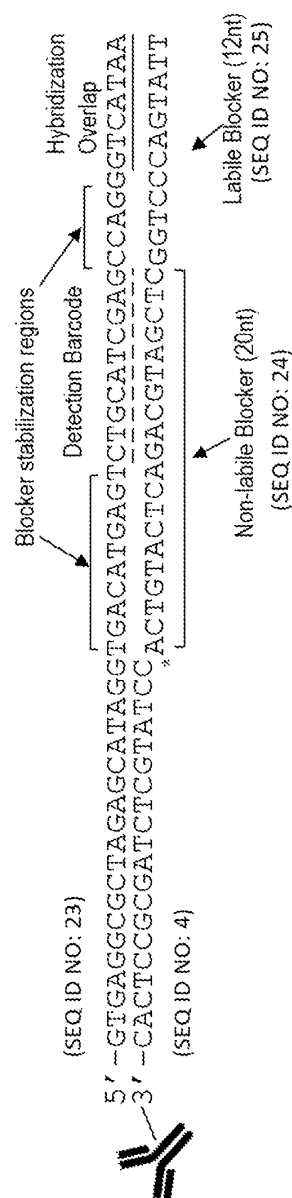


FIG. 31B

* Indicates a 3' terminator, or modified base, to inhibit 5'→3' extension from this site

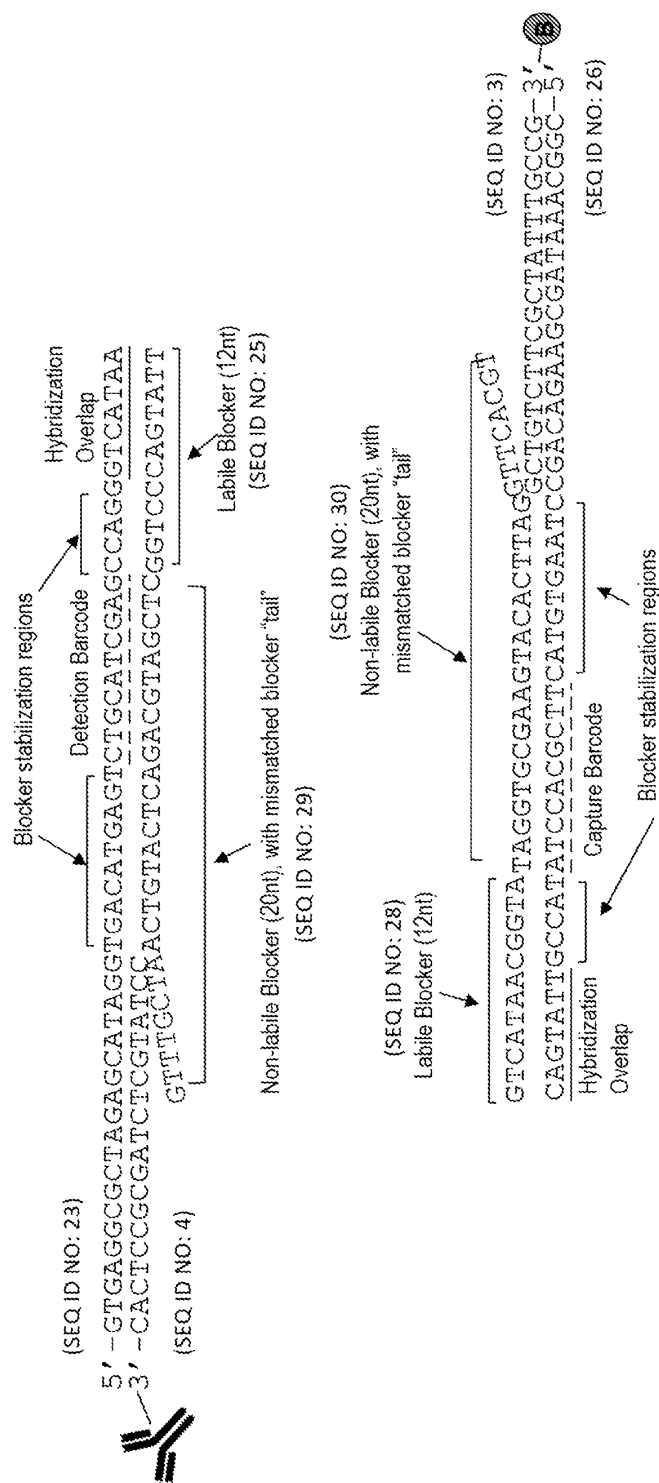


FIG. 31C

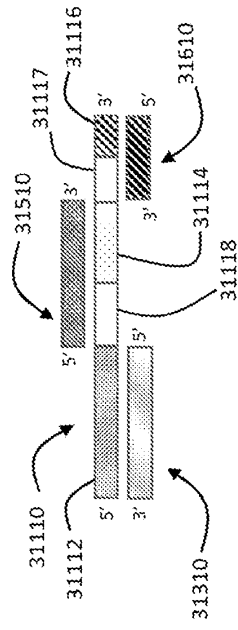


FIG. 31D

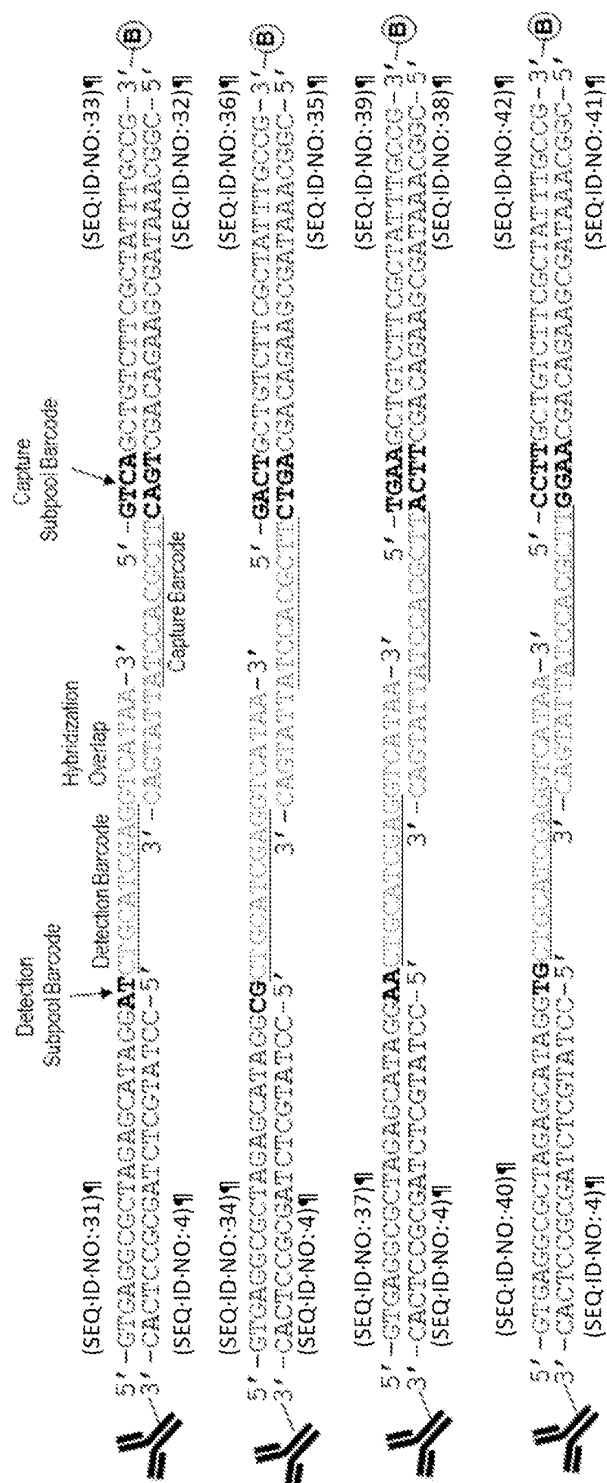
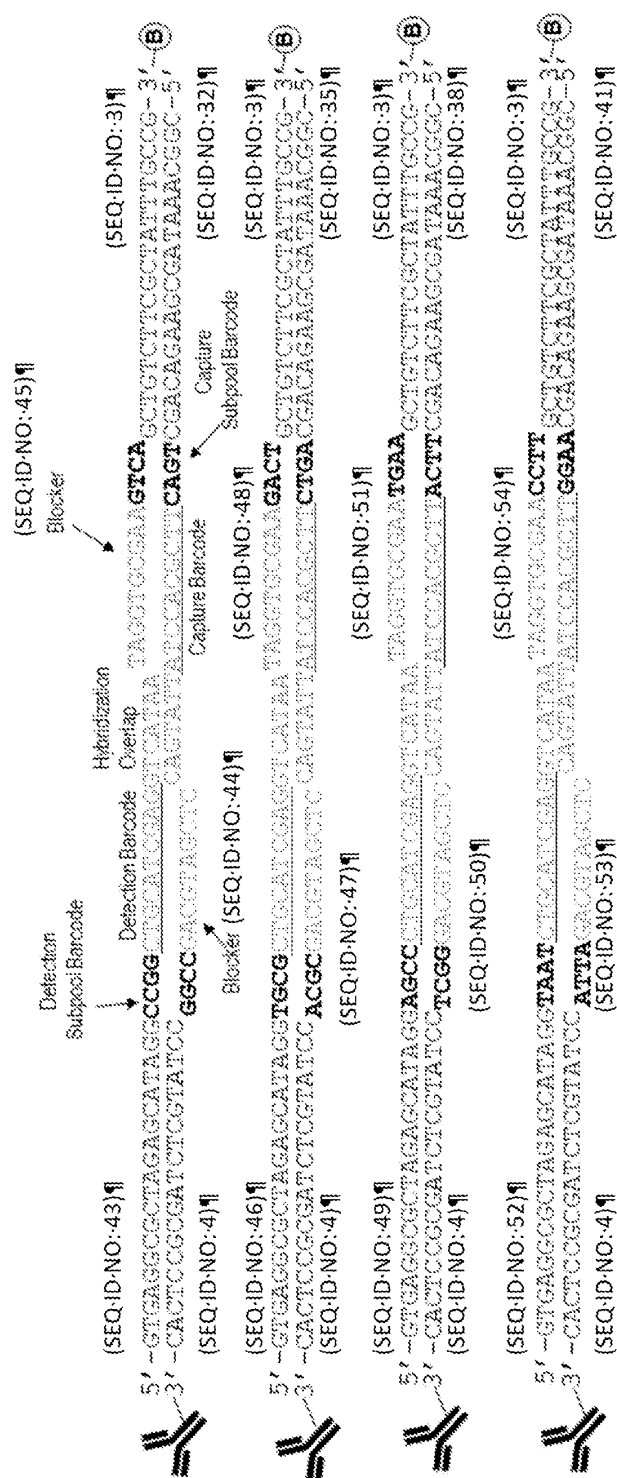


FIG. 32A



3' Terminal Base "Trimming"

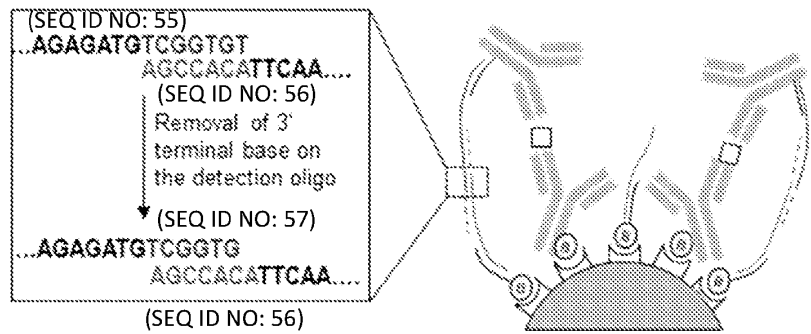


FIG. 33

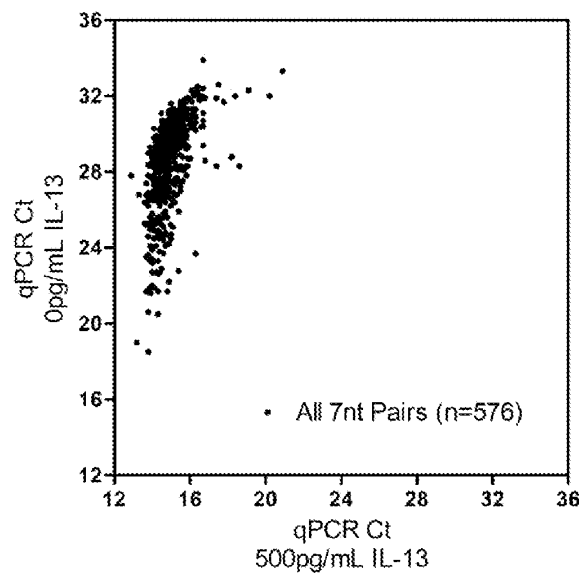


FIG. 34A

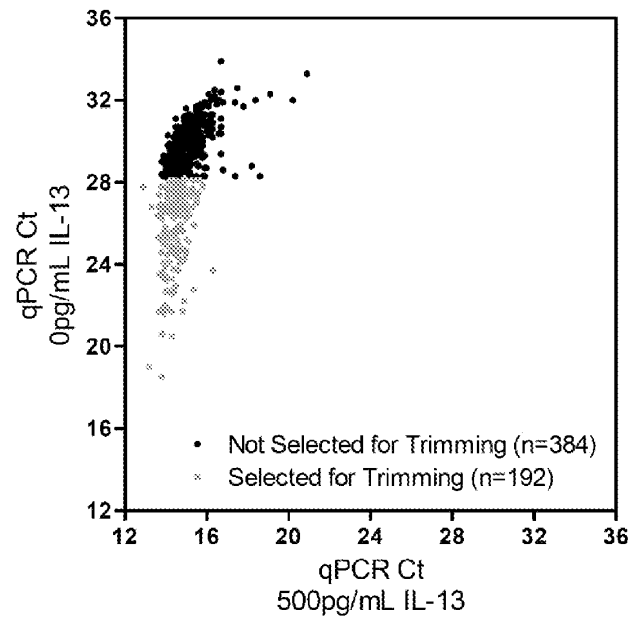


FIG. 34B

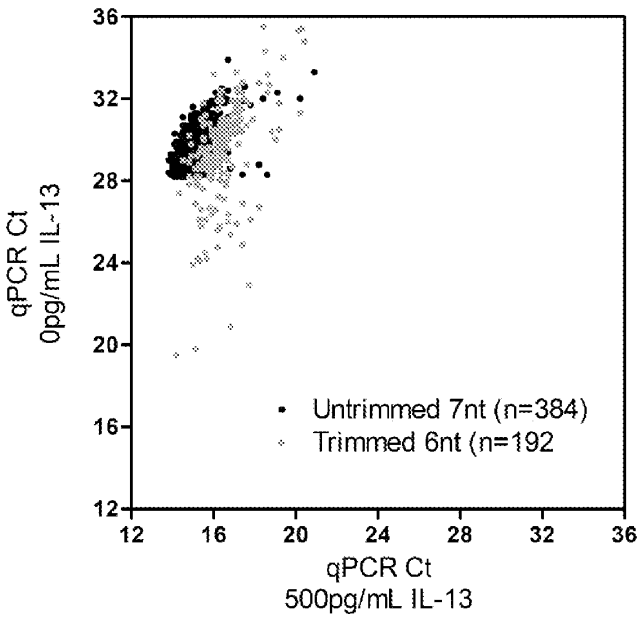


FIG. 34C

Ct Value	TOC (384 pg/mL IL-13)						NSB (Diluent 2)						Amount of Bt-Anchor Oligo (pmol Oligo/ μ g bead)	
	1	2	3	4	5	6	7	8	9	10	11	12		
A	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7	0.000	9x
B	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	0.1675	3x
C	10.7	10.5	10.9	12.3	14.0	15.9	17.5	22.4	23.6	25.9	28.3	31.0	0.0025	1x
D	11.8	11.7	11.8	13.2	14.9	17.2	20.5	26.4	26.8	28.2	30.2	32.7	0.0208	1x/3
E	13.4	13.7	13.4	14.6	16.2	18.2	20.3	28.4	26.7	29.9	31.4	33.8	0.00044	1x/9
F	15.0	14.7	15.0	15.9	17.7	19.6	20.8	30.1	29.8	31.4	32.9	33.8	0.00034	1x/27
G	16.6	16.4	16.3	17.4	19.2	21.1	21.8	32.0	31.0	32.8	36.1	34.5	0.00072	1x/61
H	18.0	18.0	18.3	19.8	21.3	23.5	22.8	35.3	33.9	35.1	43.0	43.8	0.00057	1x/243
4x	8.00	2.00	0.500	0.125	0.0313	0.00781	8.00	2.00	0.500	0.125	0.0313	0.00781		
	1x	1x	1/4x	1/16x	1/64x	1/256x	4x	1x	1/4x	1/16x	1/64x	1/256x		

Delta Ct	Delta Ct Value between TOC (384 pg/mL IL-13) and NSB (Diluent 2)																				Amount of Bt-Anchor Oligo (pmol Oligo/ μ g bead)	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20		
A	4.0	3.9	4.4	7.2	8.8	9.5							0.383	9x								
B	5.0	6.6	7.1	10.9	12.1	12.7							0.1675	3x								
C	11.7	11.9	12.7	13.8	14.3	15.1							0.0025	1x								
D	14.7	14.7	15.0	15.0	15.3	15.6							0.0208	1x/3								
E	15.0	15.2	15.3	15.2	15.2	16.4							0.00044	1x/9								
F	14.8	15.4	14.9	15.5	16.2	14.2							0.00731	1x/27								
G	15.2	15.5	15.5	15.4	16.9	15.5							0.00072	1x/61								
H	14.8	17.3	16.6	15.3	16.7	17.5							0.00057	1x/243								
4x	6.00	2.00	0.500	0.125	0.0313	0.00781							0.00057	1x/243								
	1x	1x	1/4x	1/16x	1/64x	1/256x																

FIG. 35

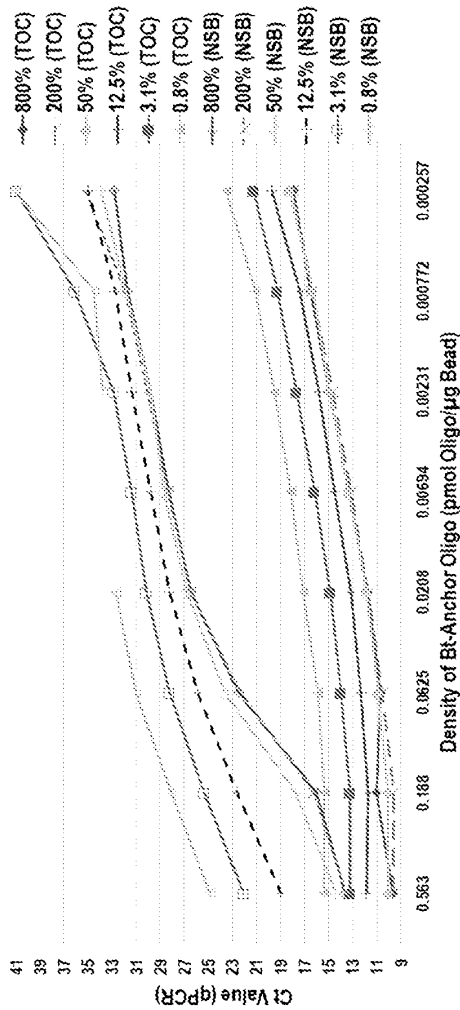


FIG. 36A

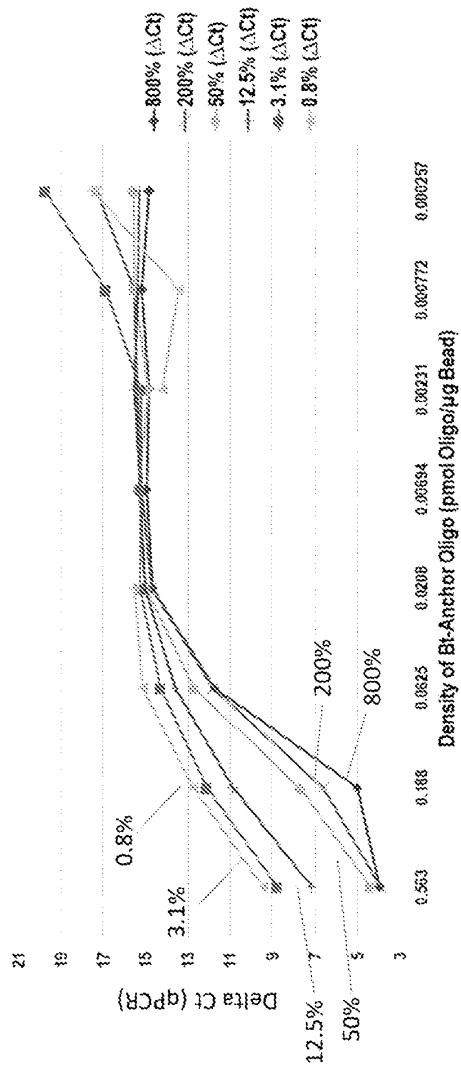


FIG. 36B

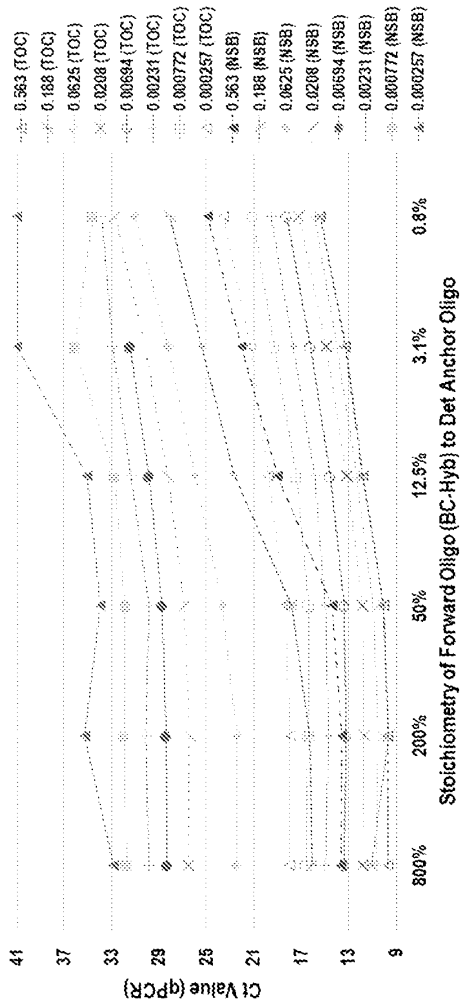


FIG. 37A

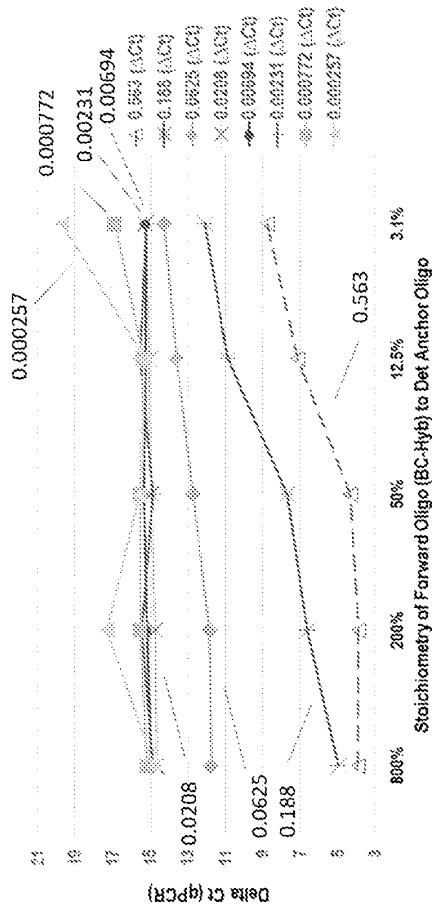


FIG. 37B

None													nM Conc in D12
3, 125 625 125 25 3, 125 625 125 25 3, 125 625 125 25													nM Conc in D13
Forward Hyb Competitor (NNT covers 7NT Hyb + 2NT BC)													Competitor Type
None													None
Reverse Hyb Competitor (NNT covers 7NT Hyb + 2NT BC)													Reverse Hyb Competitor (NNT covers 7NT Hyb + 2NT BC)
Calibrator (IL-13) Conc. in pg/mL													Calibrator (IL-13) Conc. in pg/mL
1	2	3	4	5	6	7	8	9	10	11	12	13	384
A	10.3	10.9	10.7	11.4	11.2	11.0	10.9	10.9	10.7	10.5	11.1	11.1	384
B	13.1	12.7	12.5	13.2	13.1	12.9	12.8	12.3	12.2	12.4	12.9	12.9	384
C	15.8	14.8	14.5	16.2	15.7	15.5	14.6	14.4	13.9	14.4	15.1	15.1	384
D	17.7	16.8	16.5	18.5	18.3	17.5	16.9	16.3	15.9	16.3	16.8	16.8	384
E	19.9	18.7	18.4	19.4	20.5	19.5	18.5	18.5	17.9	18.2	18.6	18.6	384
F	21.9	20.7	20.4	20.3	22.6	21.6	20.7	20.4	19.6	20.4	20.9	20.9	384
G	25.1	23.7	23.3	22.0	26.4	25.6	24.4	23.2	21.0	21.8	22.6	22.6	384
H	25.7	23.4	22.5	22.3	26.4	25.5	24.4	23.4	20.9	21.3	22.6	22.6	384
Delta Ct													Calibrator (IL-13) Conc. in pg/mL
1	2	3	4	5	6	7	8	9	10	11	12	13	384
A	14.2	13.0	11.6	11.4	15.0	14.7	13.5	12.4	10.3	11.5	11.5	11.5	384
B	12.3	11.1	9.9	9.5	13.2	13.2	12.8	11.7	8.6	9.7	9.7	9.7	384
C	8.7	9.0	7.8	7.6	10.2	10.6	9.8	8.9	7.1	7.7	7.5	7.5	384
D	7.7	7.0	5.9	5.7	7.9	8.0	7.5	7.0	5.1	5.8	5.7	5.7	384
E	5.6	5.1	3.9	3.7	5.9	6.0	6.2	5.6	4.0	3.2	3.0	3.0	384
F	3.5	3.1	2.0	1.9	3.8	4.1	4.1	3.7	2.9	1.4	1.8	1.7	384
G	0.3	0.1	0.1	0.1	0.0	0.2	0.1	0.0	0.1	0.0	0.3	0.0	384
H	0.3	0.1	0.1	0.1	0.0	0.2	0.1	0.0	0.1	0.0	0.3	0.0	384
None													None
Forward Hyb Competitor (NNT covers 7NT Hyb + 2NT BC)													nM Conc in D12
3, 125 625 125 25 3, 125 625 125 25 3, 125 625 125 25													nM Conc in D13
Reverse Hyb Competitor (NNT covers 7NT Hyb + 2NT BC)													Competitor Type
Reverse Hyb Competitor (NNT covers 7NT Hyb + 2NT BC)													Reverse Hyb Competitor (NNT covers 7NT Hyb + 2NT BC)

FIG. 38

Set 1

	SEQ ID NO:
FwPESD412-12	59
FwPESD412-11	60
FwPESD412-10	61
FwPESD412-09	62
FwPESD412-08	63
FwPESD412-07	64
FwPESD412-06	65
FwPESD412-05	66
	58
	CAAG CTG ATA GAG GCG NNN NNN NNN GAC AGA AGC GAT AAA CGG C
	Rv-PESD512

Set 2

FwPESD427-12	68
FwPESD427-11	69
FwPESD427-10	70
FwPESD427-09	71
FwPESD427-08	72
FwPESD427-07	73
FwPESD427-06	74
FwPESD427-05	75
	67
	C GCT TCG GAA GCT AAT NNN NNN NNN GAC AGA AGC GAT AAA CGG C
	Rv-PESD527

Set 3

FwPESD451-12	77
FwPESD451-11	78
FwPESD451-10	79
FwPESD451-09	80
FwPESD451-08	81
FwPESD451-07	82
FwPESD451-06	83
FwPESD451-05	84
	76
	T CCT TGG AAC AAG CAA NNN NNN NNN GAC AGA AGC GAT AAA CGG C
	Rv-PESD551

FIG. 39A

Hybridization Length (bp)	Set 1		Set 2		Set 3	
	497 pg/ml IL-13	0 pg/ml IL-13	497 pg/ml IL-13	0 pg/ml IL-13	497 pg/ml IL-13	0 pg/ml IL-13
12	7.5	8.4	9.6	17.5	8.2	11.9
11	7.3	9.7	9.7	17.9	8.1	11.9
10	8.2	13.6	9.8	19	8.5	15.4
9	8.1	14.7	10.1	21.4	8.6	17.4
8	8.4	14.6	10.3	24.3	9.4	19.8
7	9.2	15.4	10.2	24.4	9.4	23
6	9.9	21.7	10.6	24.8	10.6	25.6
5	10.9	23.6	13.3	26.7	12.6	27.9

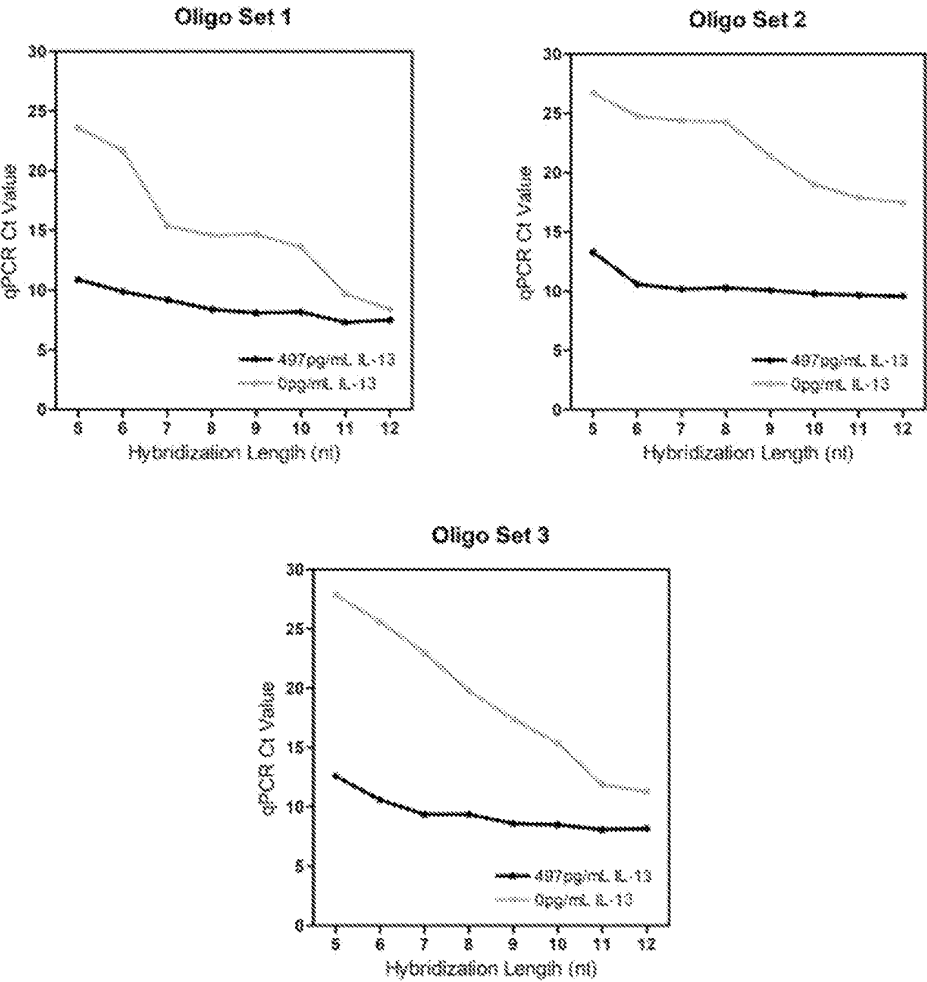


FIG. 39B

Hybridization Length (bp)	deltaCt			Dynamic Range (fold-change)		
	Set 1	Set 2	Set 3	Set 1	Set 2	Set 3
12	0.9	7.9	3.1	2	239	9
11	2.4	8.2	3.8	5	294	14
10	5.4	9.2	6.3	42	588	119
9	6.6	11.3	8.8	97	2521	446
8	6.2	14.6	10.4	74	16384	1351
7	6.2	14.2	13.6	74	18520	12417
6	11.8	14.2	15.0	9566	18520	92768
5	12.7	13.4	15.3	6654	10809	40342

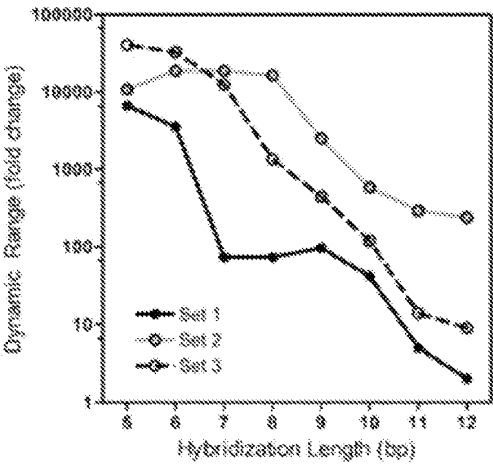


FIG. 39C

Cold cAb (ng)	0	150	450	4950
[IL-13] (pg/mL)	1X Calibration Curve			
1000	13.6	16.2	18.1	21.4
250	14.1	17.9	19.6	22.7
62.5	15.2	19.7	21.6	24.6
15.625	17.5	22.0	23.7	26.5
3.906	19.3	24.2	25.6	27.8
0.977	21.3	25.8	27.2	28.9
0.244	23.3	27.6	28.8	29.1
0	29.2	29.6	29.4	29.8
Dynamic Range	15.5	13.5	11.4	8.4

FIG. 40A

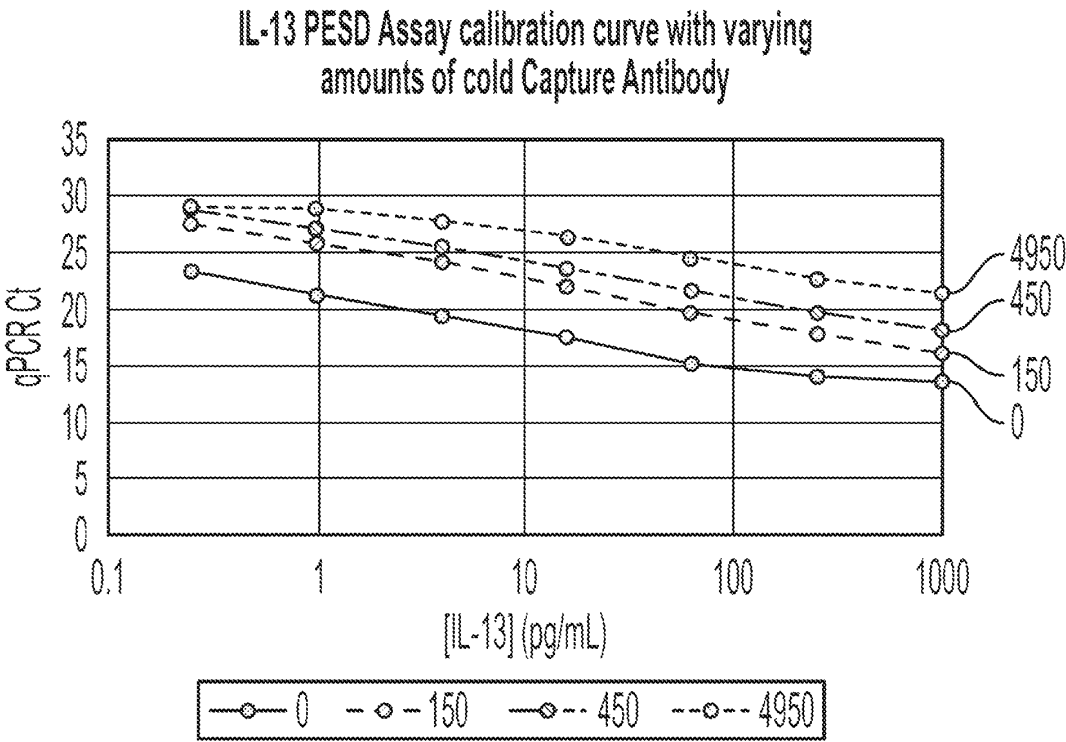


FIG. 40B

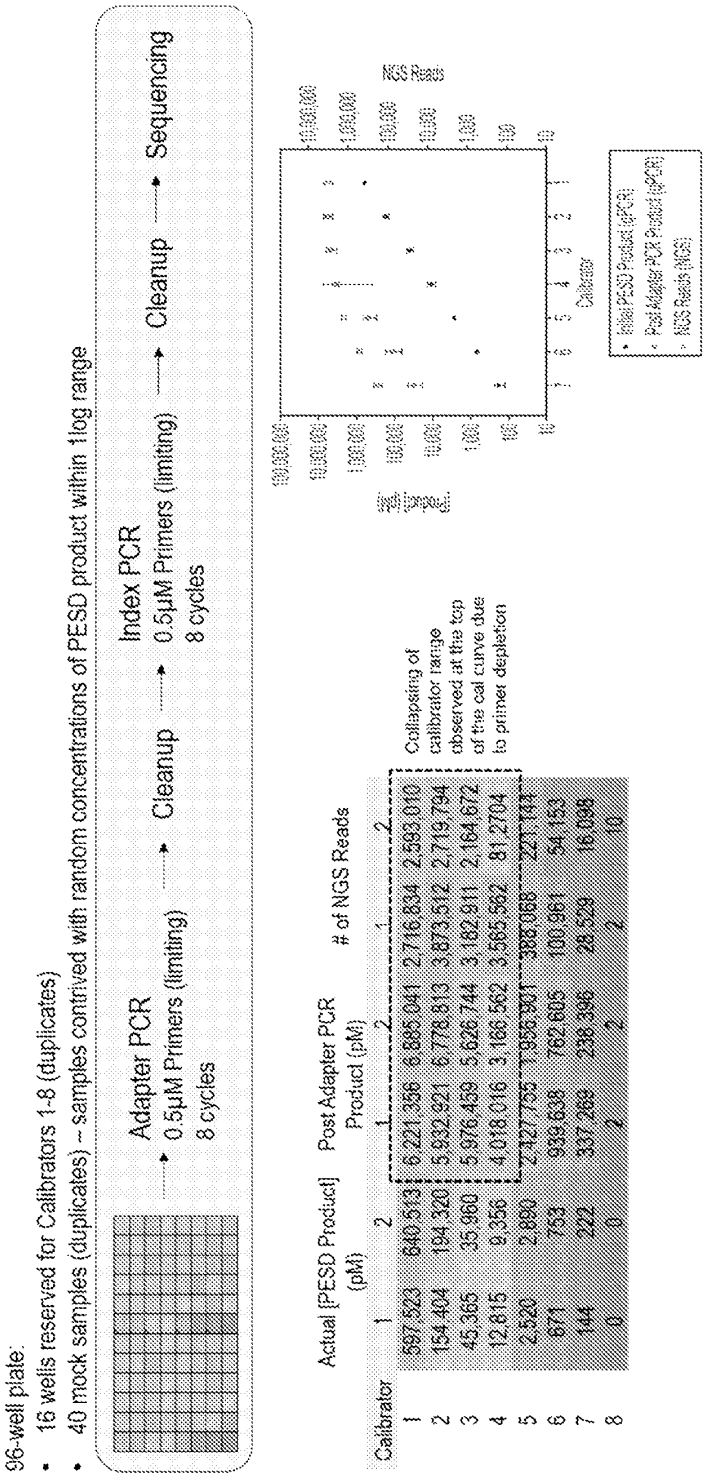


FIG. 41B

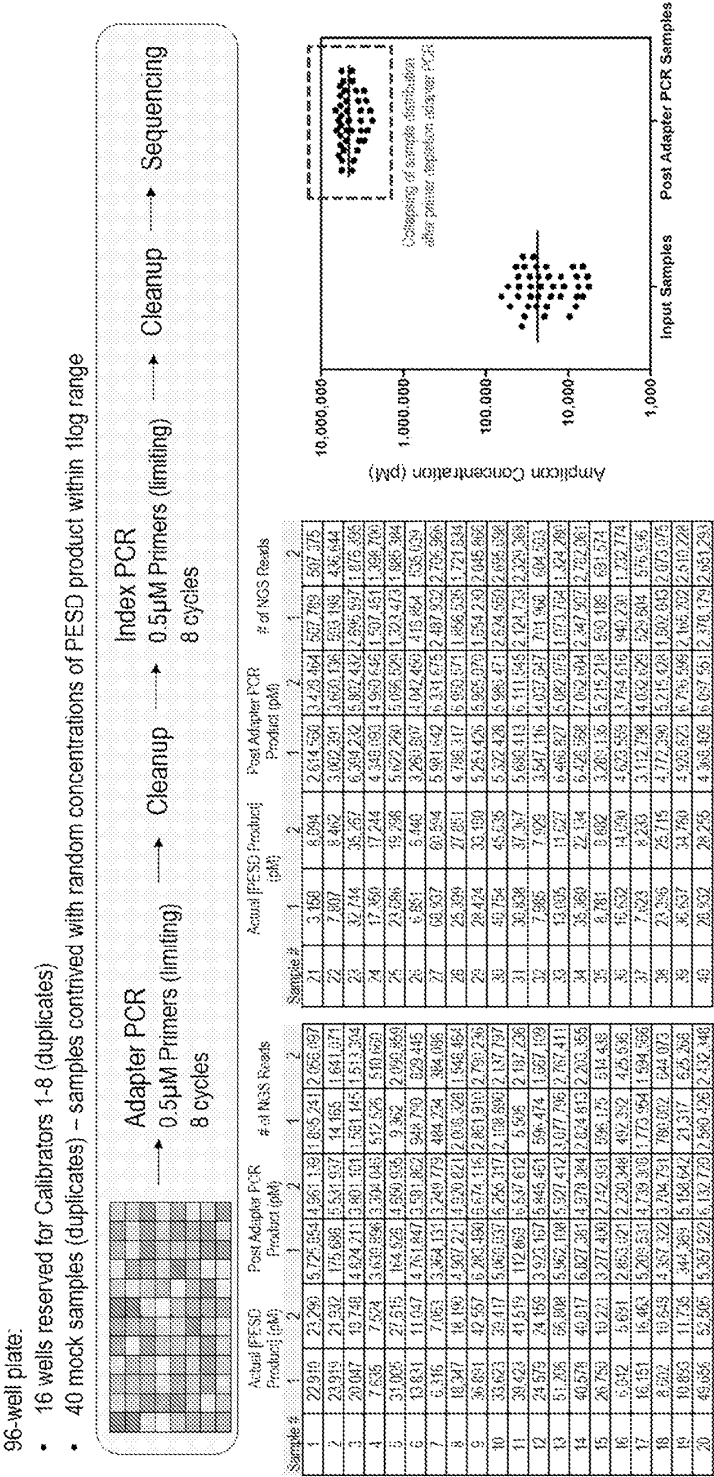


FIG. 41C

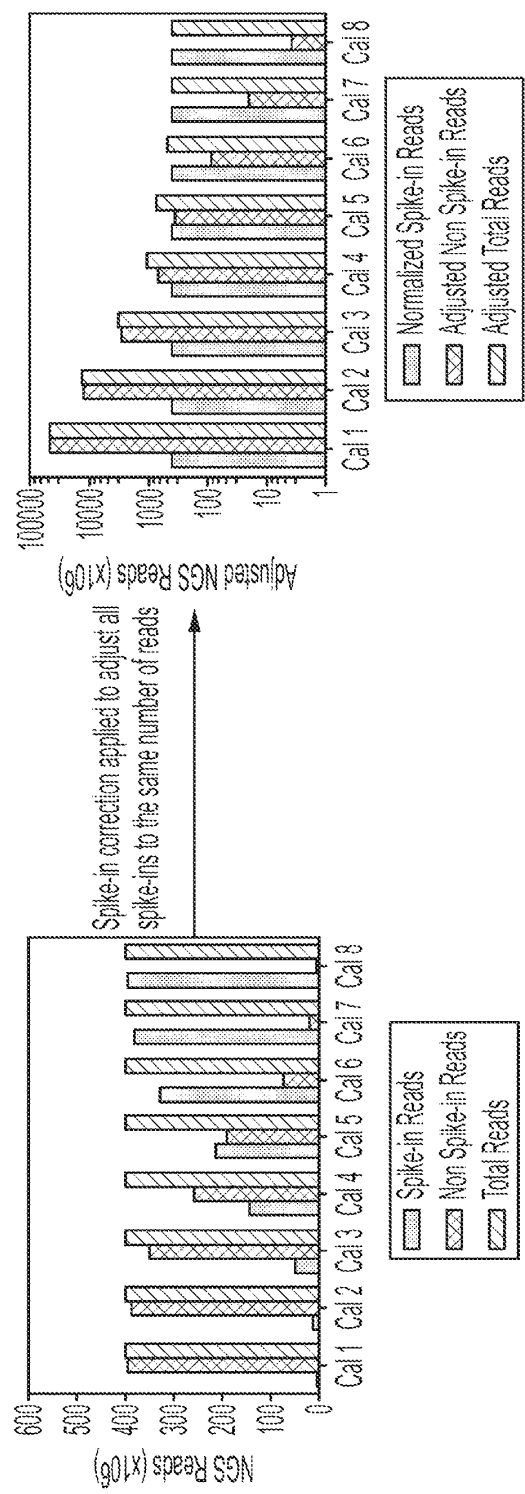
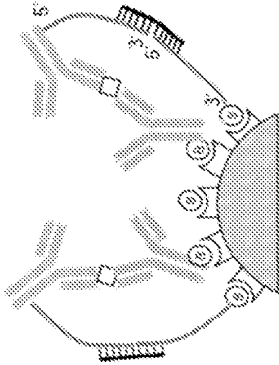


FIG. 42

Proximity Ligation Assay (PLA) Oligo Design
“Asymmetrical” Splints



Antibody-conjugated "Detector-side" Oligo (same antibody-oligo conjugate used in PEA)					Biotinylated "Capture-side" Oligo on Clonal Bead											
5' Thiol	Detector		Splint Hvb		3' 5' Phos	Capture		3' Biotin								
	Barcode	Region	Barcode	Region		Barcode	Region									
GTA TGG GAT GGC AGG TTT GAN NNN NNN GGG GTAAAT TGT GTT ACAAC (SEQ ID NO: 85)	UMI GAN NNN NNN GGG GTAAAT TGT GTT ACAAC	[3' - CA GAA TGT TG 3' - A GAA TGT TG 3' - GAA TGT TG 3' - AA TGT TG 3' - A TGT TG 3' - TGT TG 3' - GTTG 3' - TTG Asymmetrical splints for PLA	[3' - CA GAA TGT TG 3' - A GAA TGT TG 3' - GAA TGT TG 3' - AA TGT TG 3' - A TGT TG 3' - TGT TG 3' - GTTG 3' - TTG	[3' - CA GAA TGT TG 3' - A GAA TGT TG 3' - GAA TGT TG 3' - AA TGT TG 3' - A TGT TG 3' - TGT TG 3' - GTTG 3' - TTG	TCT GTT GCG GTC GTAAGA TC - 5' (SEQ ID NO: 87)	TCT GTT GCG GTC GTAAGA TC - 5' (SEQ ID NO: 88)	TCT GTT GCG GTC GTAAGA TC - 5' (SEQ ID NO: 89)	TCT GTT GCG GTC GTAAGA TC - 5' (SEQ ID NO: 90)	TCT GTT GCG GTC GTAAGA TC - 5' (SEQ ID NO: 91)	TCT GTT GCG GTC GTAAGA TC - 5' (SEQ ID NO: 92)	TCT GTT GCG GTC GTAAGA TC - 5' (SEQ ID NO: 93)	TCT GTT GCG GTC GTAAGA TC - 5' (SEQ ID NO: 94)				
					✓	Variable overhangs (10-3bp) complementary to detection conjugate oligo			✓	20bp region for hybridization to bead-coating oligo						

FIG. 43

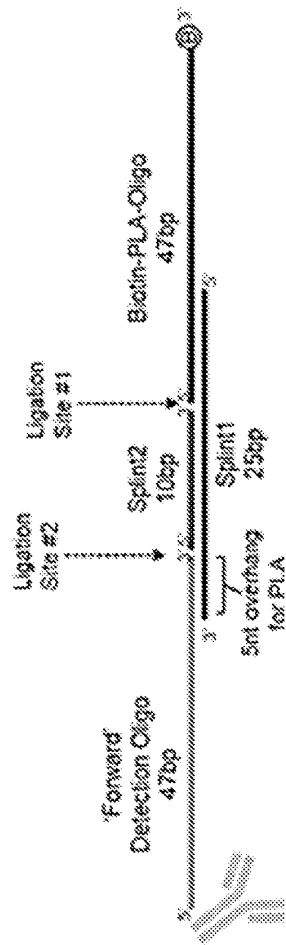


FIG. 44A

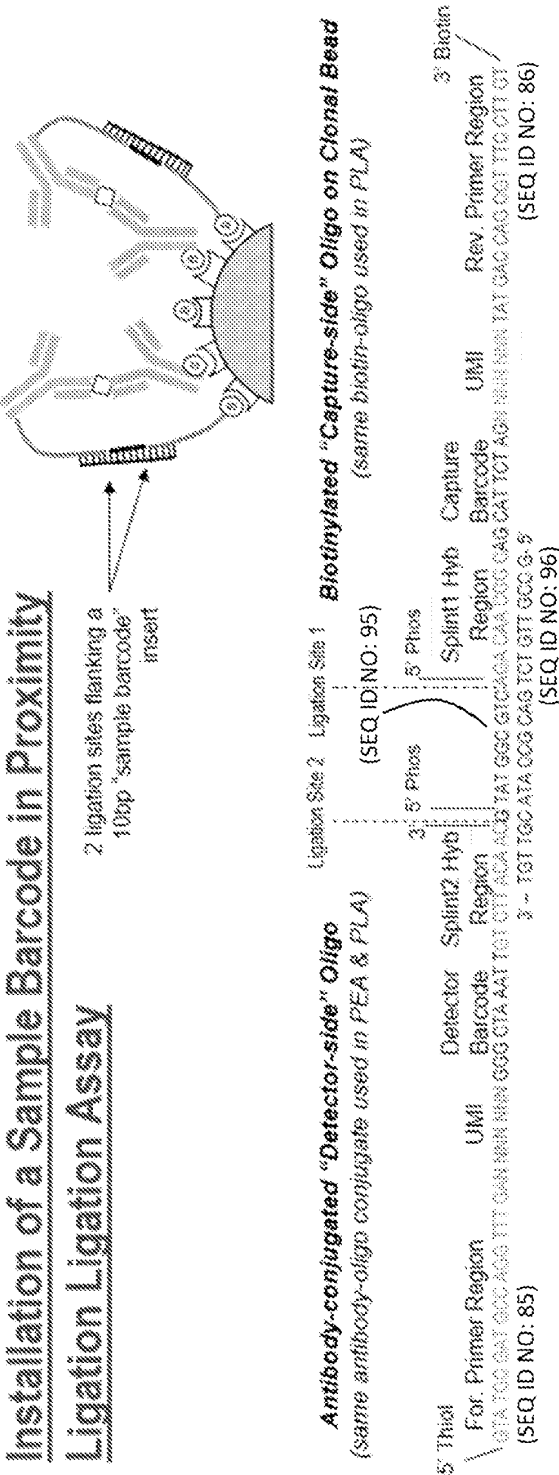


FIG. 44B

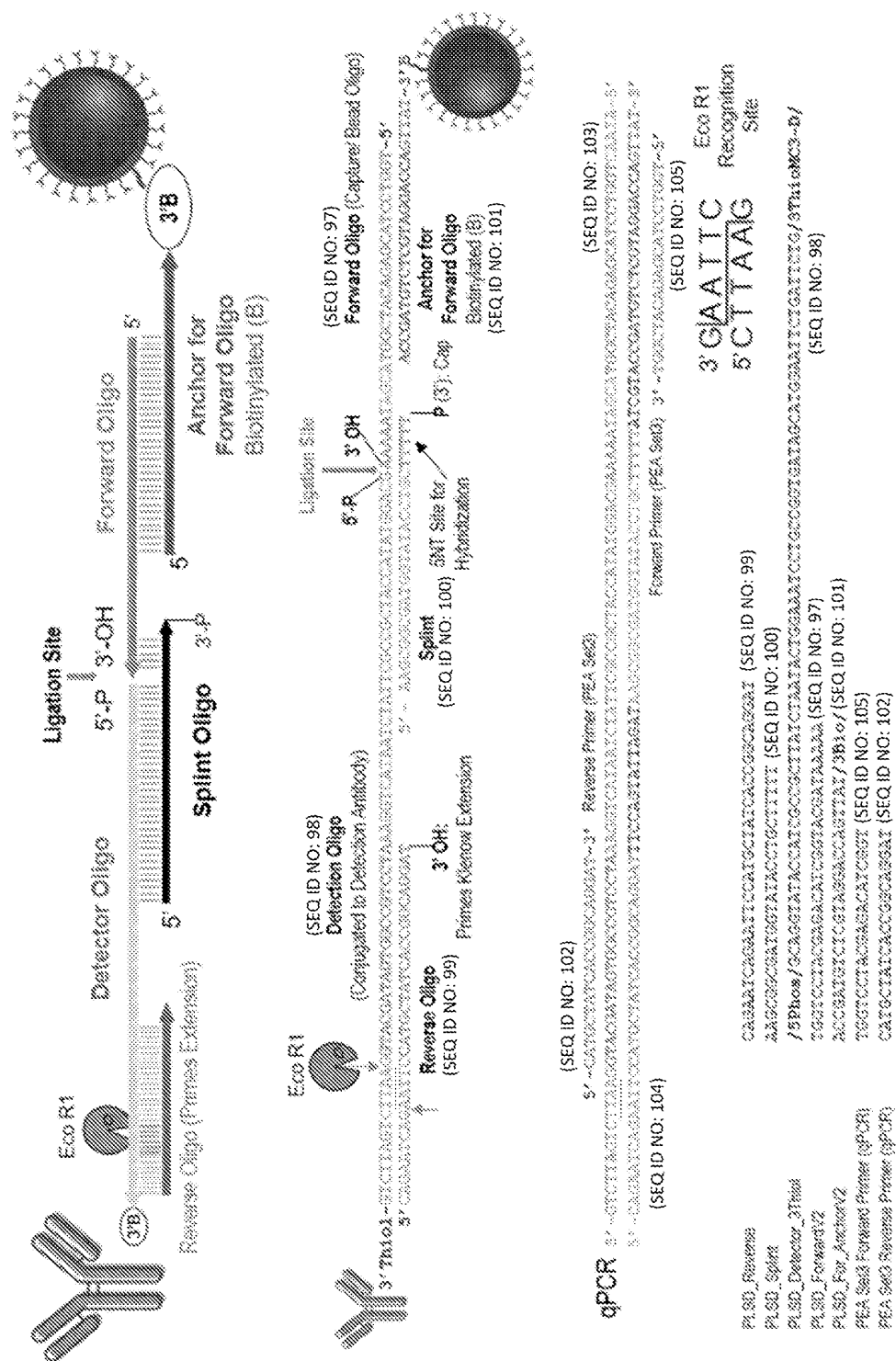


FIG. 45

[T4 Ligase] (ng/ml) [10-12] (pg/ml)		25		46.4		46.4		3		46.4		1		1	
		1	2	3	2	3	2	1	2	3	2	1	2	1	2
Splice	3	34.76	Undetermined	23.77	23.93	22.48	22.48	36.54	Undetermined	24.67	25.55	34.37	Undetermined	26.67	27.71
	4	34.46	36.06	32.39	22.48	15.12	15.12	35.04	Undetermined	24.32	24.45	36.37	30.50	26.48	26.73
	5	29.55	29.42	15.12	15.12	15.12	15.12	30.90	31.10	27.24	17.93	30.80	31.50	23.66	19.18
	6	28.34	27.51	15.11	15.10	15.11	15.10	28.03	26.67	15.09	15.10	25.75	26.95	16.20	16.80
	7	24.73	14.95	14.72	14.95	14.72	14.95	25.93	21.85	15.13	15.24	26.26	26.57	15.41	15.74
Overhang (nt)	8	22.66	24.18	14.75	14.44	14.75	14.44	23.92	23.60	14.58	15.09	23.66	23.60	15.01	15.14
	9	23.74	21.65	14.55	14.77	14.55	14.77	24.31	21.88	14.74	14.94	23.46	23.36	14.67	15.33
	10	23.60	22.32	14.74	15.55	14.74	15.55	25.06	22.49	14.60	14.80	25.60	24.52	14.65	15.55

FIG. 46A

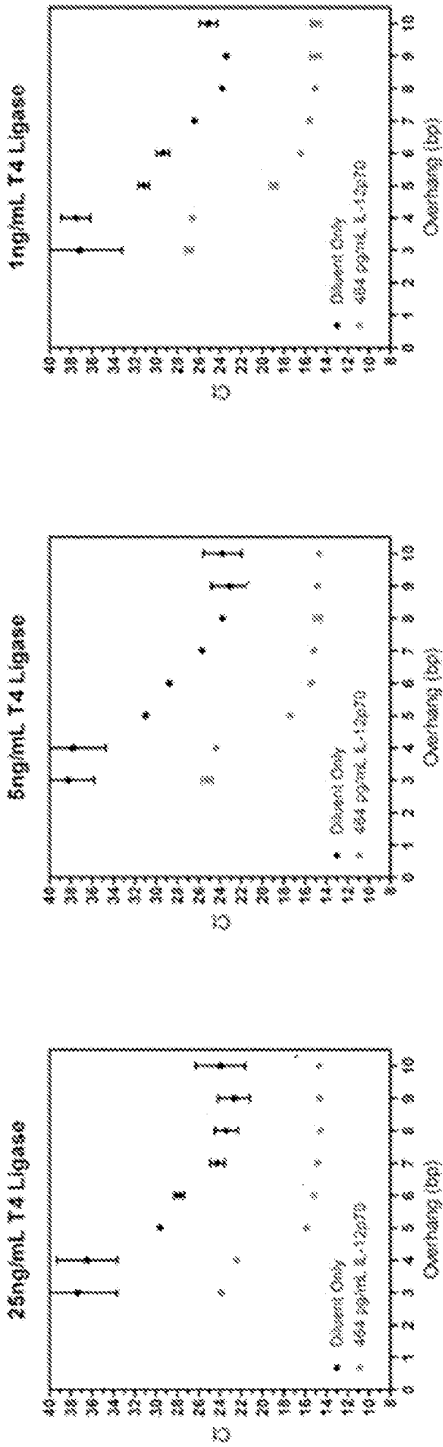


FIG. 46B

[IL-12] (pg/mL)	PLLA Singleplex IL-12		No Splint 2		No Splint 1		No Ligase (during Sample BC addition)	
	1	2	1	2	1	2	1	2
404	16.67	18.51	29.32	29.80	29.41	30.39	18.47	20.53
116	19.44	19.63	30.15	30.82	30.66	31.79	19.63	22.12
29	20.95	20.80	32.63	32.41	32.53	32.05	21.87	21.64
7.25	22.35	23.08	35.72	36.06	34.29	35.74	23.84	23.44
1.81	24.20	24.83	Undetermined	35.65	35.85	35.91	26.05	25.66
0.453	26.59	26.85	35.92	Undetermined	36.97	34.95	28.18	27.67
0.113	26.25	34.74	35.75	35.76	Undetermined	Undetermined	29.33	29.35
0.000	29.06	36.86	35.49	35.98	36.70	36.89	30.56	31.06
NTC								
							33.58	35.81

FIG. 47

NaCl	50	50	10	10	50	10	50	10	50	10	50	10	50	10	50	10	NaCl (mM)
	10	2	2	2	10	2	2	2	10	2	2	2	10	2	2	2	MgCl ₂ (mM)
	#2	#2	#5	#5	#2	#2	#5	#2	#2	#3	#5	#2	#3	#2	#3	#5	In-house Buffer

Ct	1	2	3	4	5	6	7	8	9	10	11	12	Analyte Conc.	
	A	10.9	9.9	9.9	13.4	12.6	12.7	10.5	9.8	13.2	12.6	12.5	8×TOC	
	B	13.6	12.9	12.8	16.4	15.5	15.9	13.3	12.6	16.3	15.7	15.9	1×TOC	
C	17.2	16.4	16.3	19.3	18.5	18.7	17.0	16.3	18.2	19.9	18.6	18.5	1/8×TOC	
	D	20.2	19.8	19.6	20.0	19.7	21.6	20.4	19.6	20.6	20.5	20.2	1/64×TOC	
	E	23.1	22.6	22.4	21.5	20.8	20.9	23.1	22.4	22.3	22.2	22.3	1/512×TOC	
F	24.9	24.8	24.7	20.9	21.0	20.7	25.2	24.1	24.2	22.1	21.1	21.4	1/4096×TOC	
	G	26.9	25.3	25.1	22.6	21.4	20.8	26.2	25.7	25.4	20.9	20.3	1/32768×TOC	
	H	26.0	25.9	25.9	23.2	20.4	23.9	25.9	25.8	22.7	20.2	21.4	0	
135.4												135.4	Analyte Type	

Ct (Avg)	1	2	3	4	5	6	7	8	9	10	11	12	Analyte Conc.	
	A	10.7	9.9	9.9	13.3	12.6	12.6						8×TOC	
	B	13.6	12.8	12.6	16.3	15.6	15.9						1×TOC	
C	17.1	16.4	16.2	19.2	18.5	18.6							1/8×TOC	
	D	20.3	19.6	19.5	20.3	20.1	20.9						1/64×TOC	
	E	23.1	22.5	22.3	21.9	21.5	21.8						1/512×TOC	
F	25.0	24.5	24.5	21.5	21.0	21.1							1/4096×TOC	
	G	26.1	25.5	25.4	21.7	20.2	20.9						1/32768×TOC	
	H	25.9	25.0	25.7	23.0	20.3	22.1						0	
135.4												135.4	Analyte Type	

FIG. 48

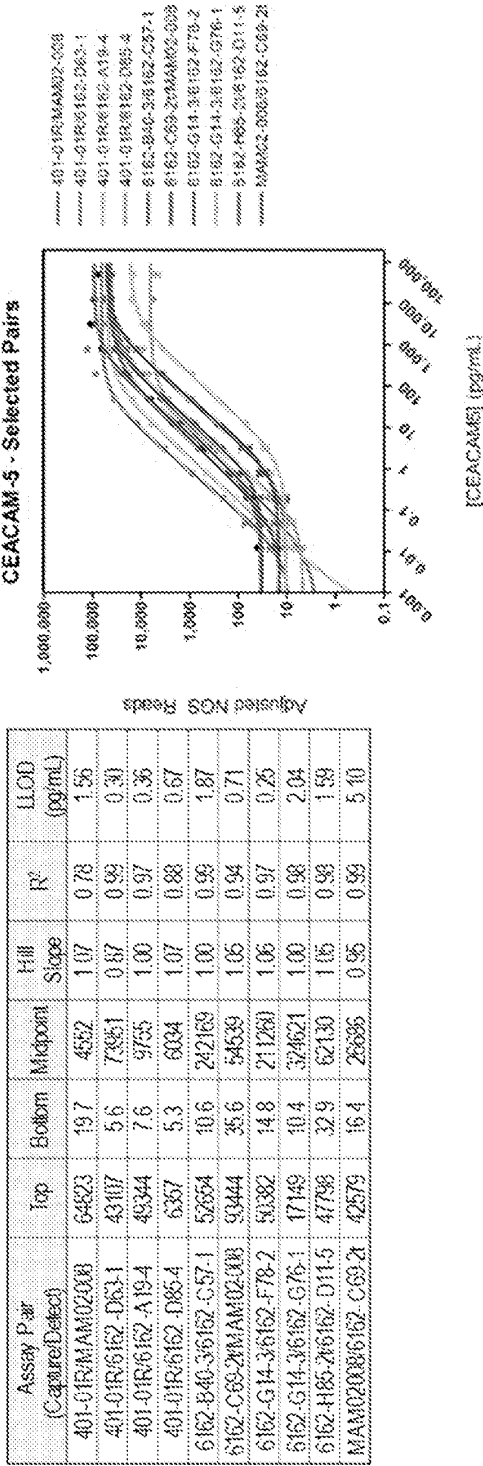


FIG. 49

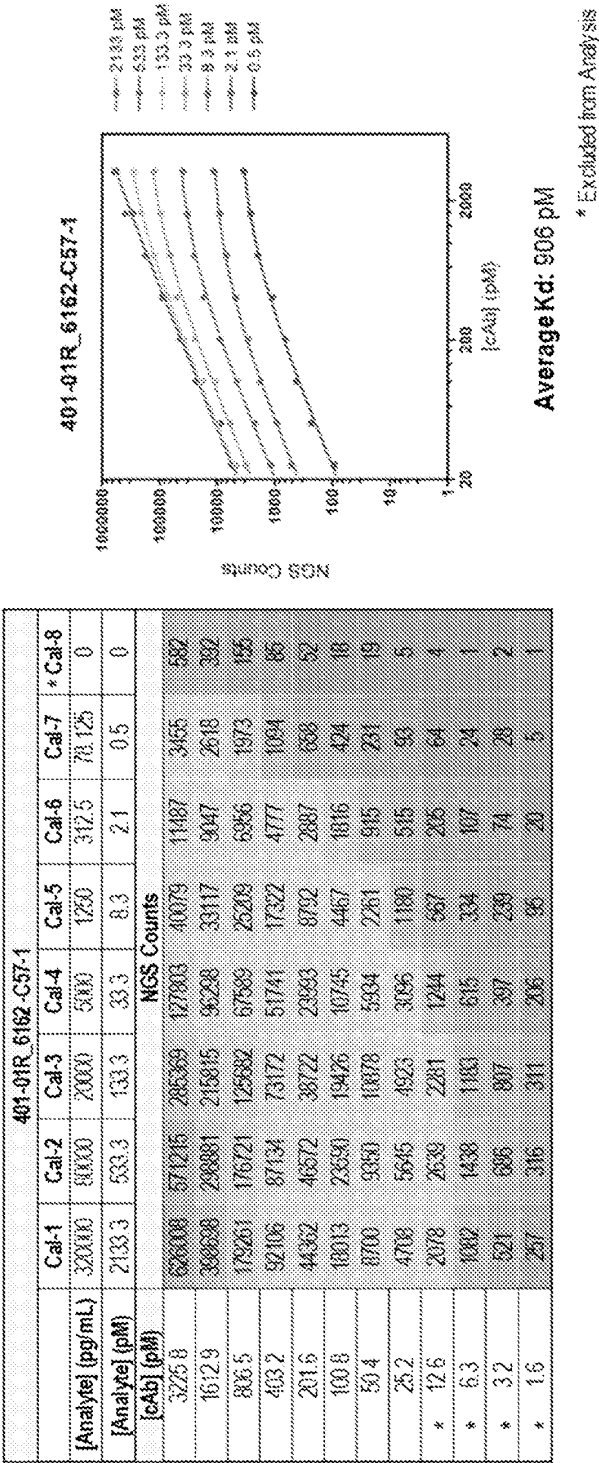
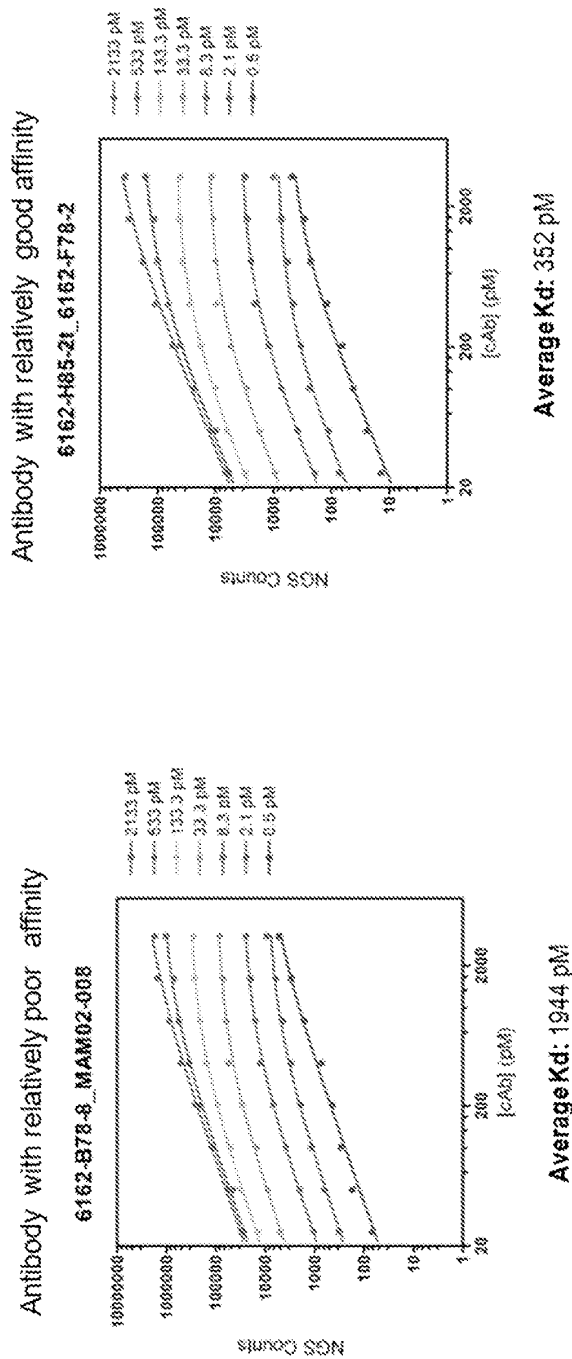


FIG. 50



* Excluded from Analysis

FIG. 51