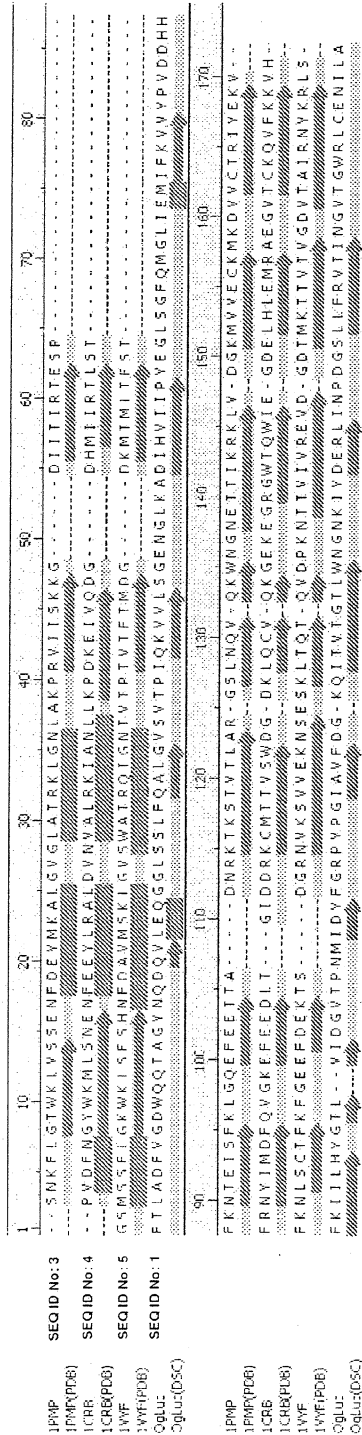
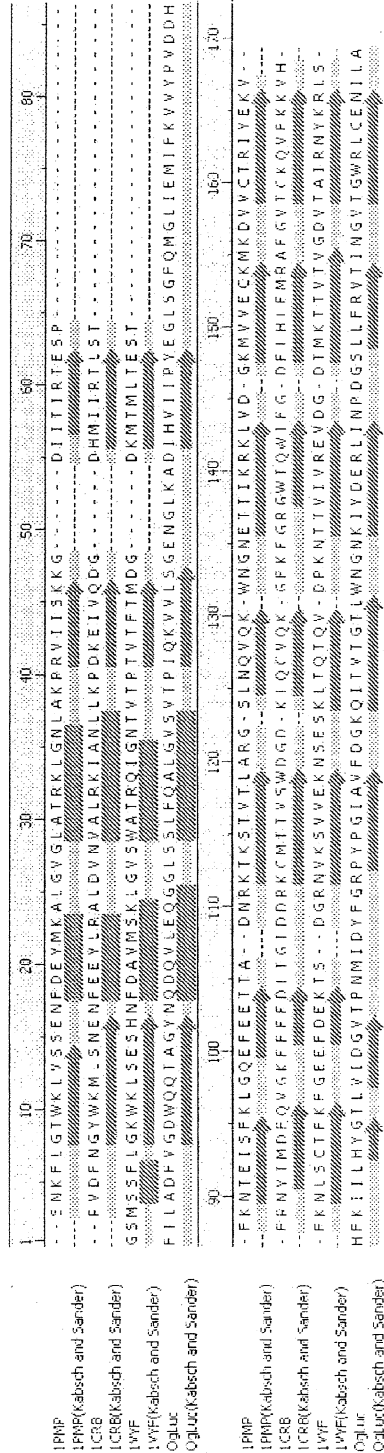


- approximate extent of helix secondary structure elements
- approximate extent of beta-sheet secondary structure elements
- secondary structure other than helix or beta-sheet

Secondary structure alignment #1:

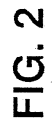


Secondary structure alignment #2:



The sequence motifs centered on the conserved residues of the calyx structural signature can be seen in the alignments. The more highly conserved N-terminal MOTIF 1 includes Ogluc residue Trp10, and the less well conserved C-terminal MOTIF 2 includes Ogluc residue N166. For alignment #2, the approximate pair-wise percent protein sequence identities are: Ogluc-1PMP 14%, Ogluc-1CRB 9%, and Ogluc-1VYF 15%.

FIG. 1



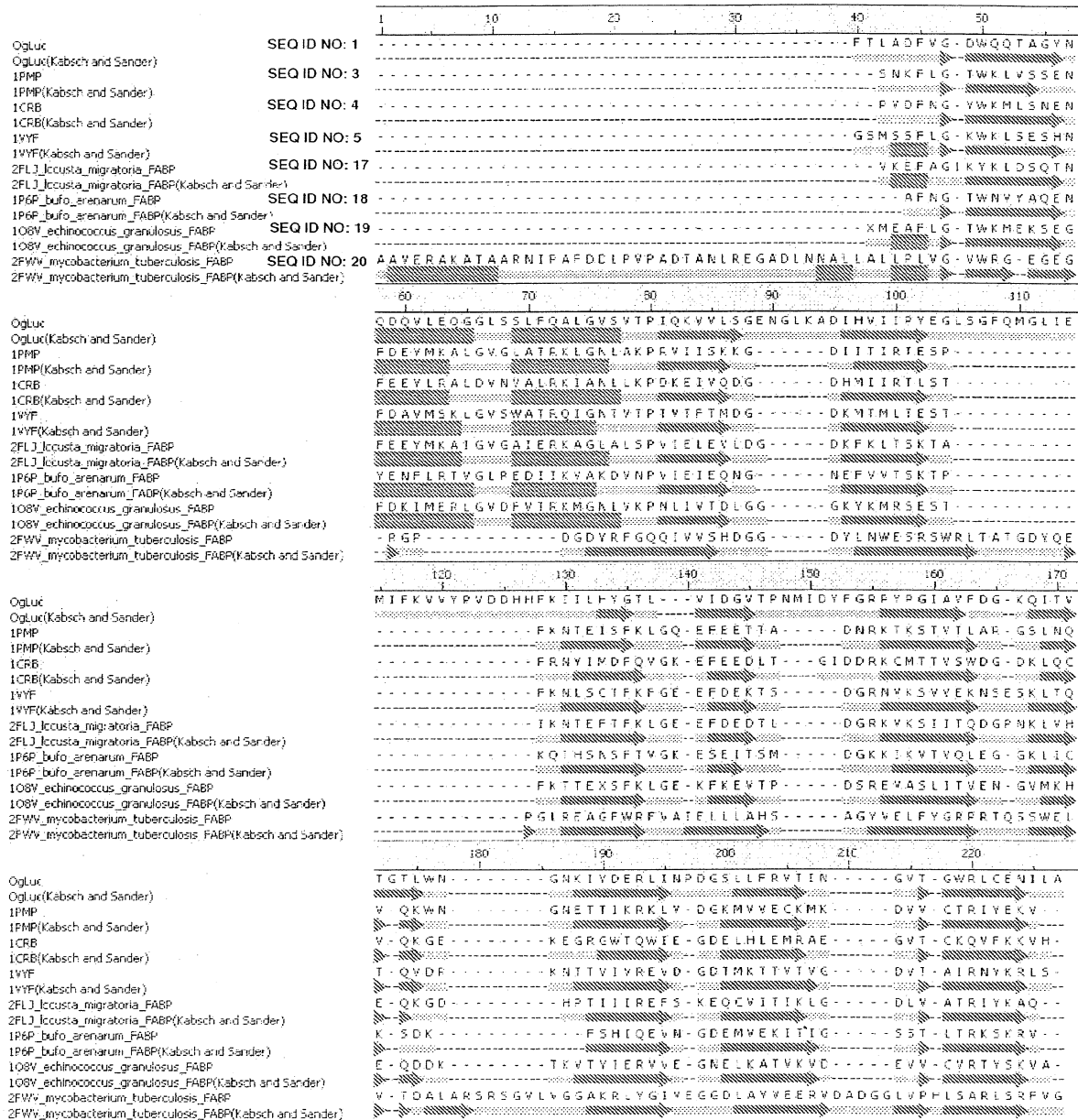
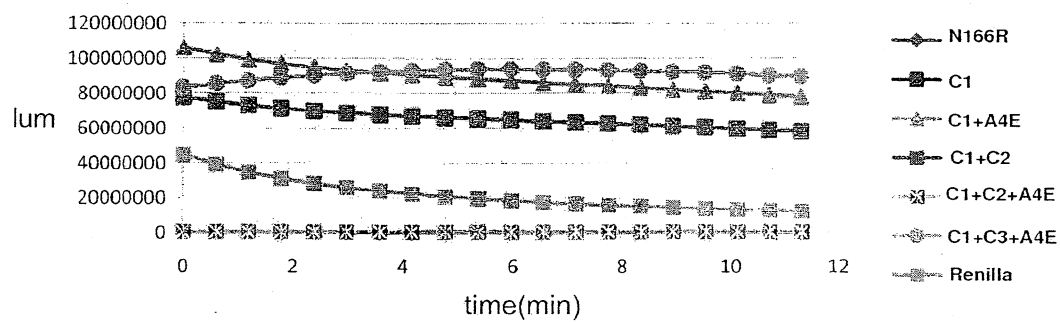


FIG. 3

4/61

Flash

A



B

Flash

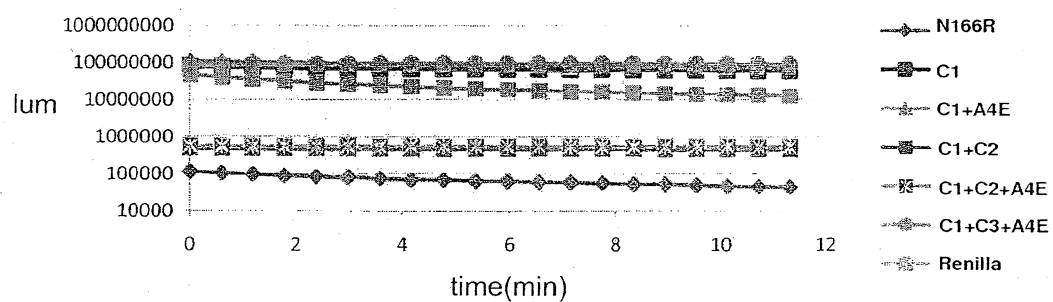
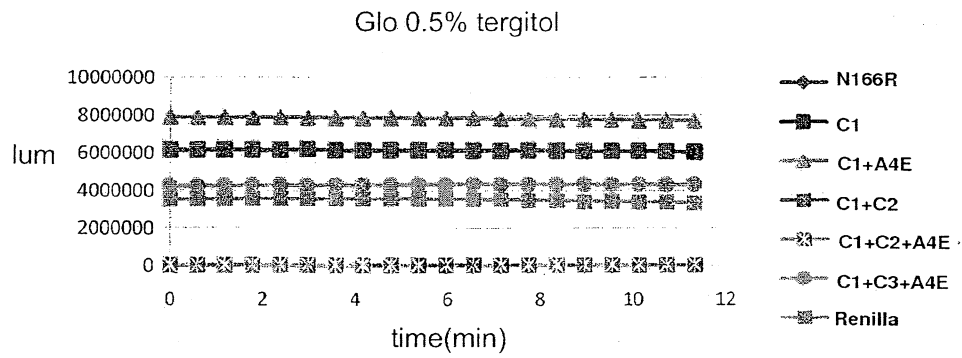
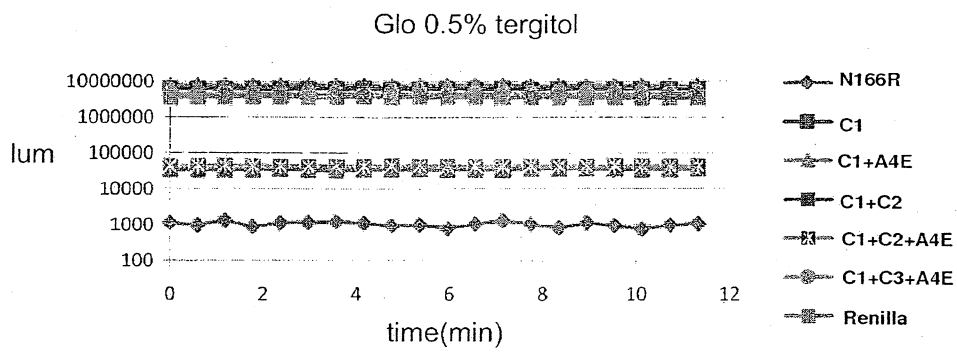


FIG. 4

C**D****FIG. 4 cont'd**

T=0 (injector) 0.5% tergitol assay buffer

S a m p l e	A v e r a g e	S t d e v	C V
W T	116,132	13,032	11.2%
N166R	160,334	18,014	11.2%
T2T	259,374	17,488	6.7%
T2T+N166R	367,468	30,406	8.3%
A4E	193,837	10,272	5.3%
Q11R	172,425	16,389	9.5%
V44I	2,155,303	231,323	10.7%
A54F	1,559,763	121,554	7.8%
A54F+N166R	8,052,686	981,637	12.2%
A54I	15,676,789	2,266,808	14.5%
P115E	406,670	18,160	4.5%
P115E+N166R	489,722	26,228	5.4%
Y138I	1,119,166	128,394	11.5%
Q124K	322,475	30,313	9.4%
Y138C+N166R	590,665	40,138	6.8%
I90V	387,765	29,162	7.5%

FIG. 5A

T=0 (injector) 0.5% tergitol buffer

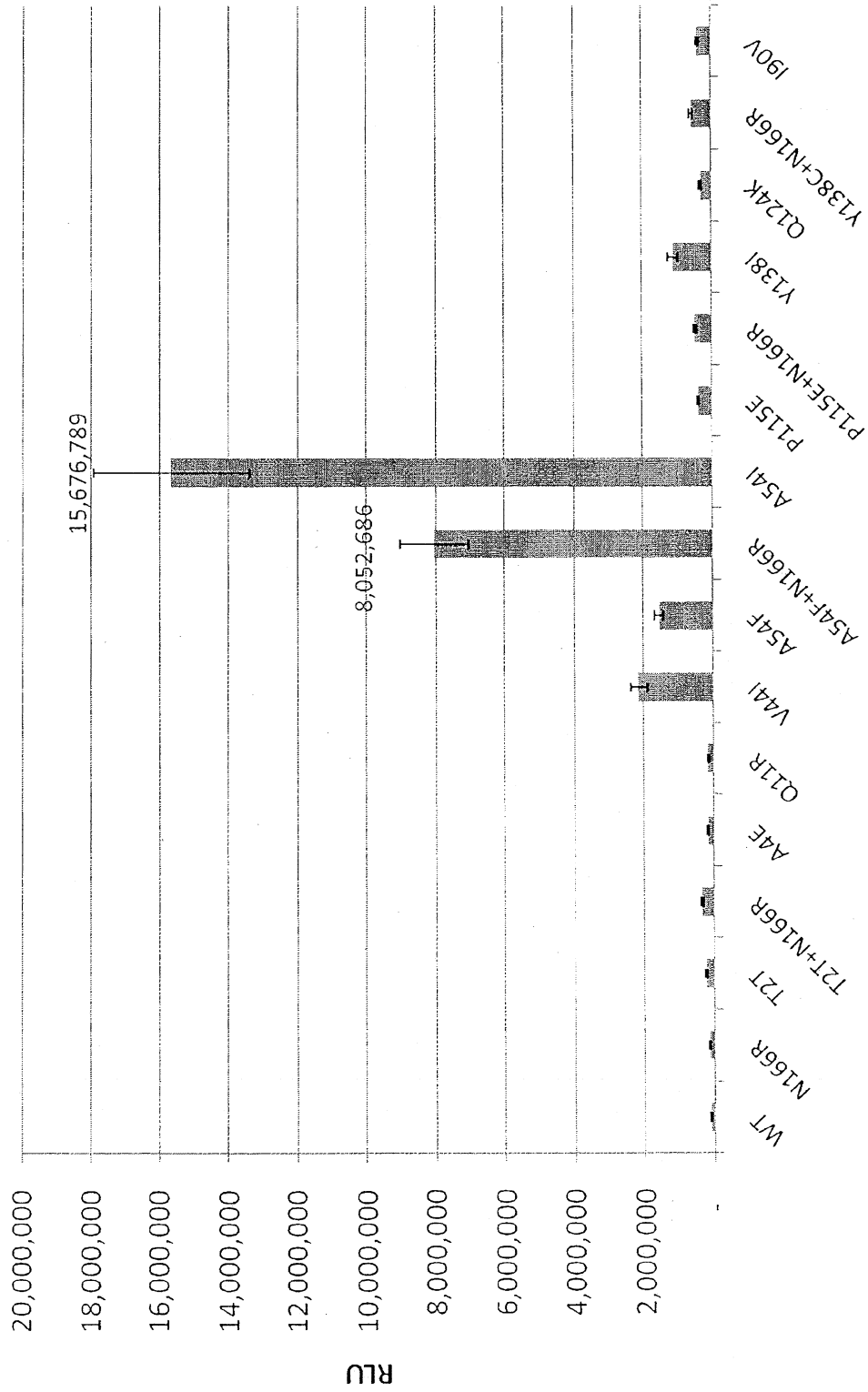


FIG. 5B

T=0 (injector) 0.5% tergitol assay buffer

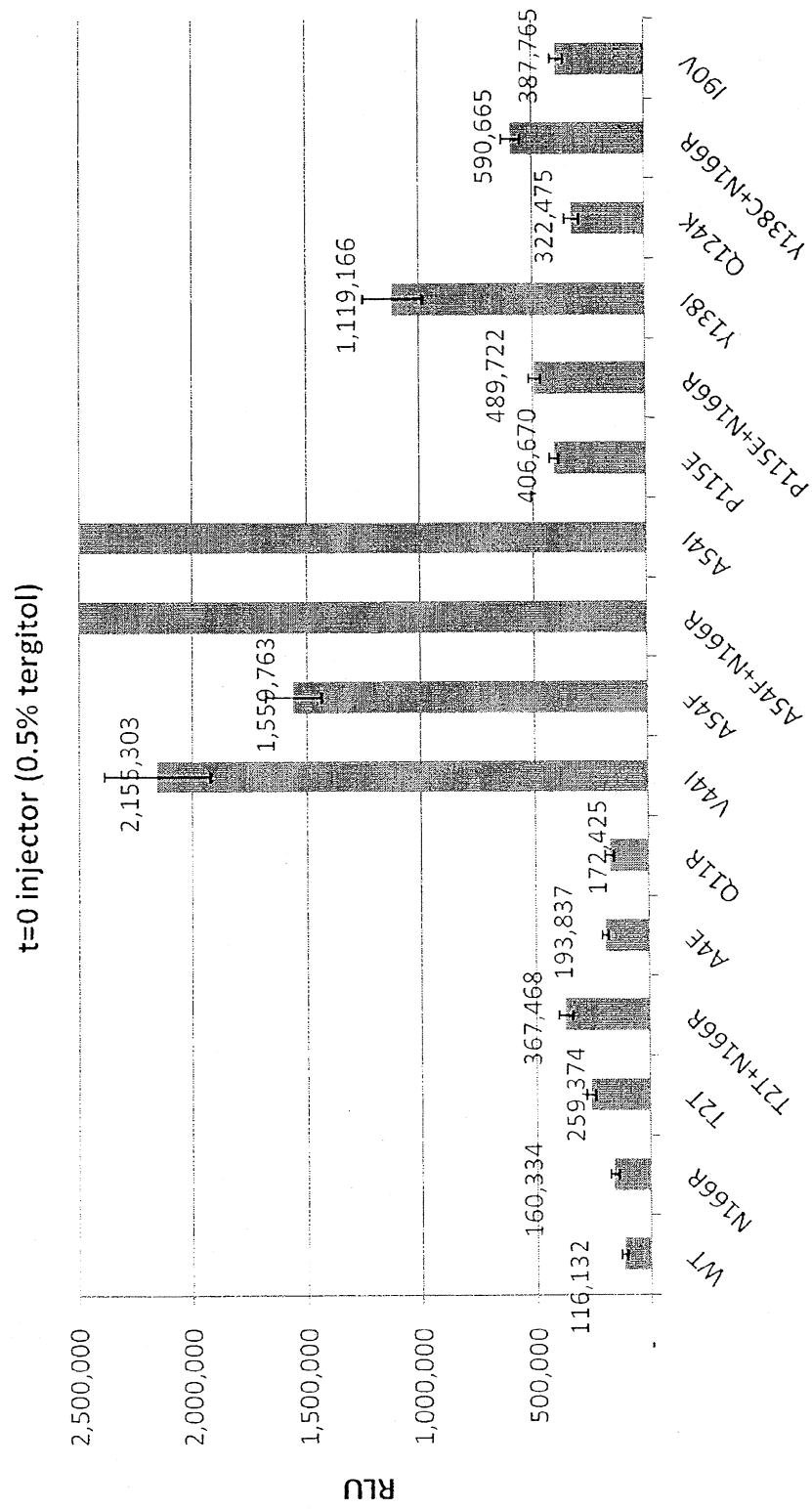


FIG. 5C

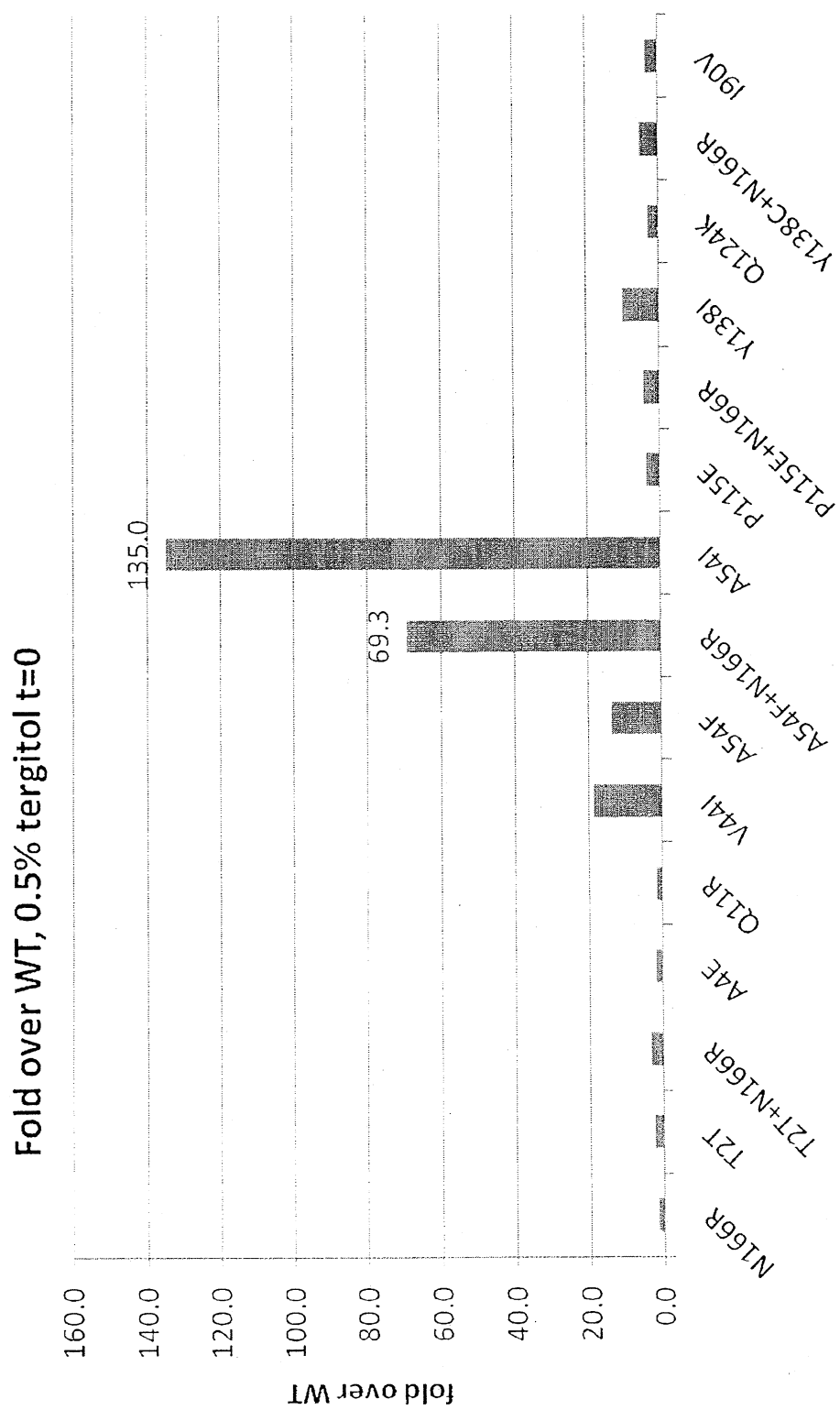


FIG. 6A

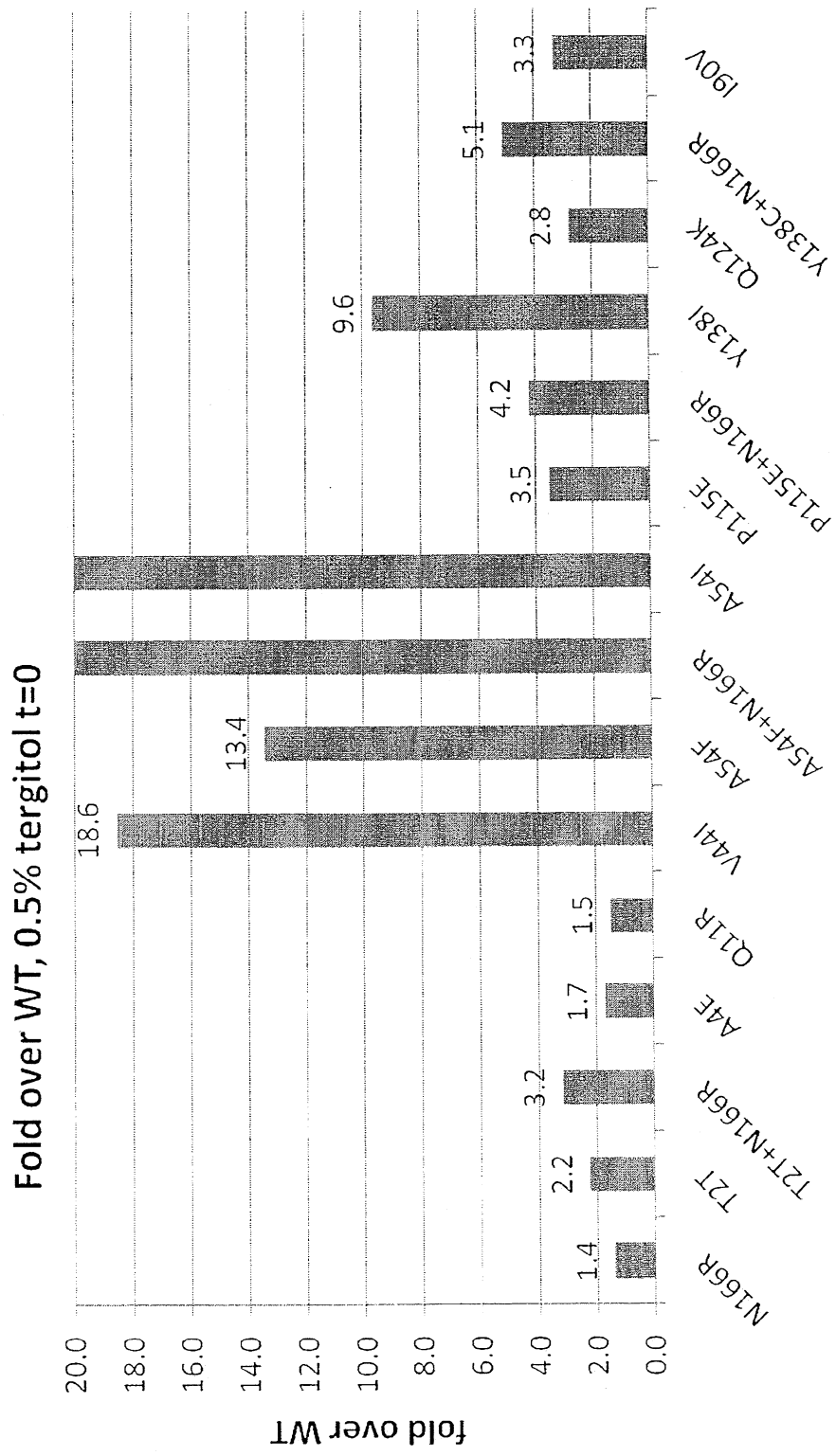


FIG. 6B

T=0 RLAB with injector

S a m p l e	A v e r a g e	S t d e v	C V
W T	2,266,822	222,850	9.8%
N166R	5,582,331	514,426	9.2%
T2T	3,599,341	244,441	6.8%
T2T+N166R	9,823,129	457,592	4.7%
A4E	2,888,005	231,055	8.0%
Q11R	4,043,391	179,037	4.4%
V44I	12,935,691	546,014	4.2%
A54F	8,572,288	464,442	5.4%
A54F+N166R	79,773,644	3,157,533	4.0%
A54I	32,309,914	2,594,059	8.0%
P115E	7,400,192	416,225	5.6%
P115E+N166R	16,167,230	481,714	3.0%
Y138I	14,573,569	619,909	4.3%
Q124K	5,473,417	517,799	9.5%
Y138C+N166R	15,516,040	1,147,103	7.4%
I90V	4,474,794	297,720	6.7%

FIG. 7A

T=0 RLAB with injector

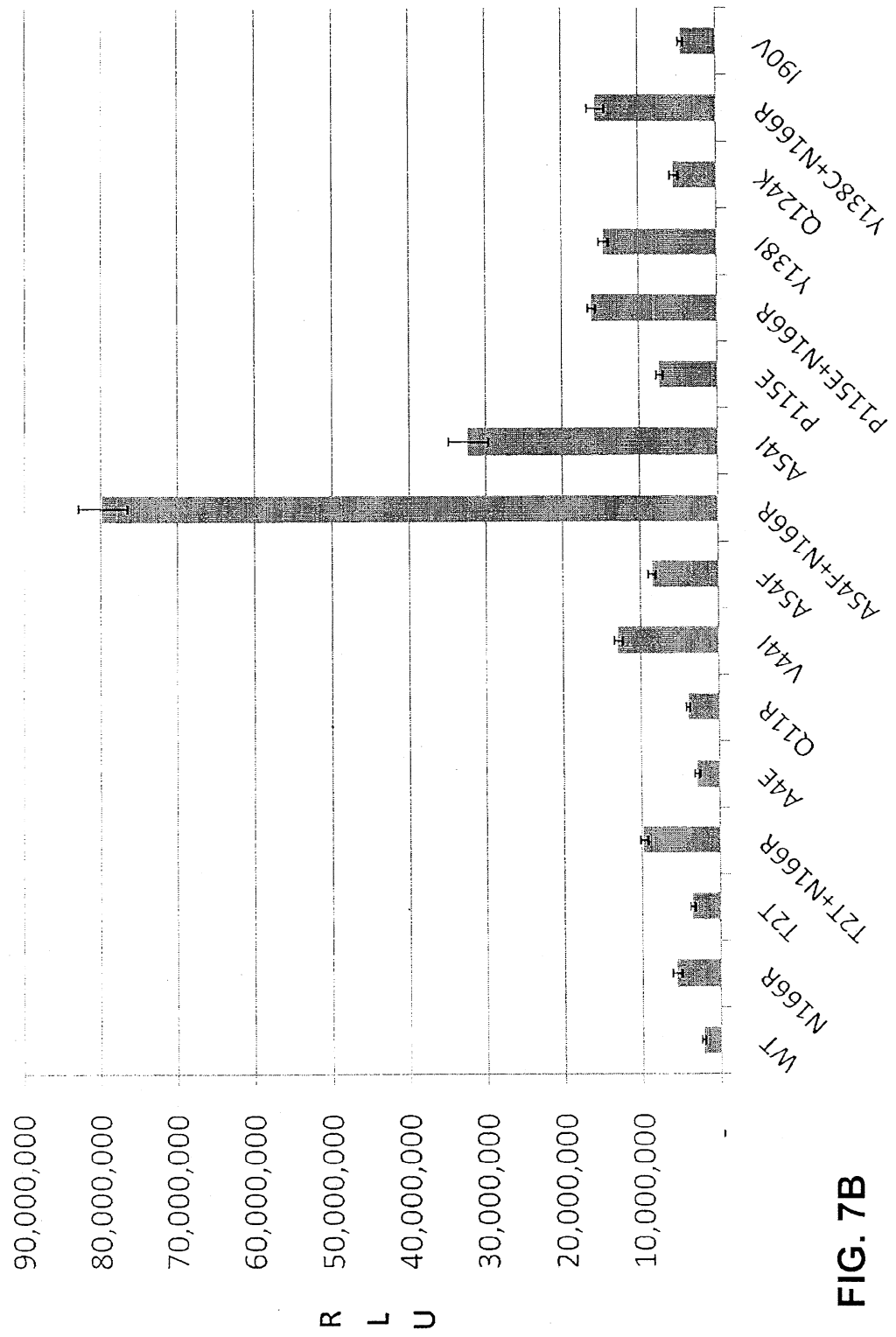


FIG. 7B

T=0 RLAB with injector

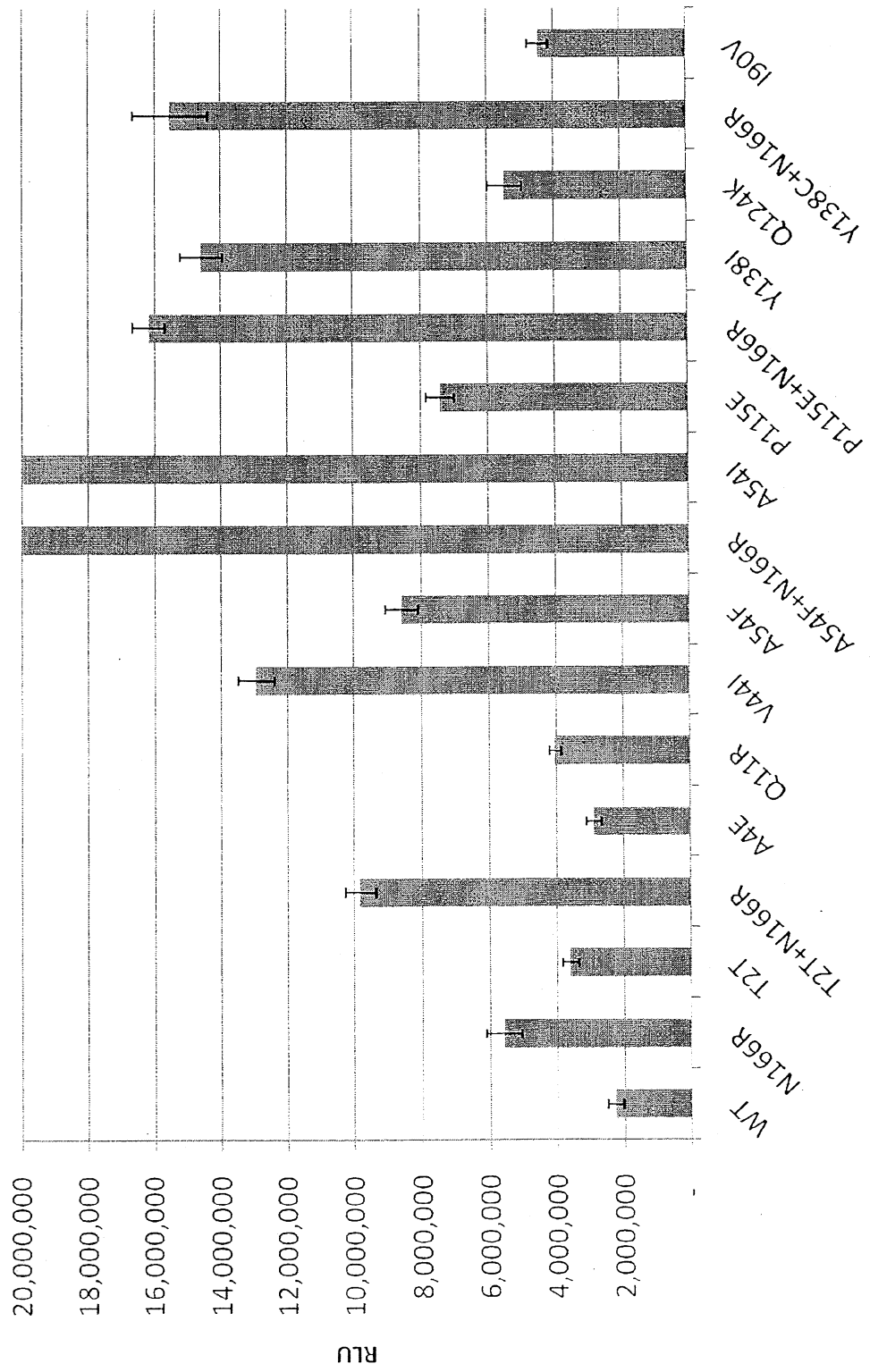


FIG. 7C

RLAB t=0 (with injector) Fold over WT

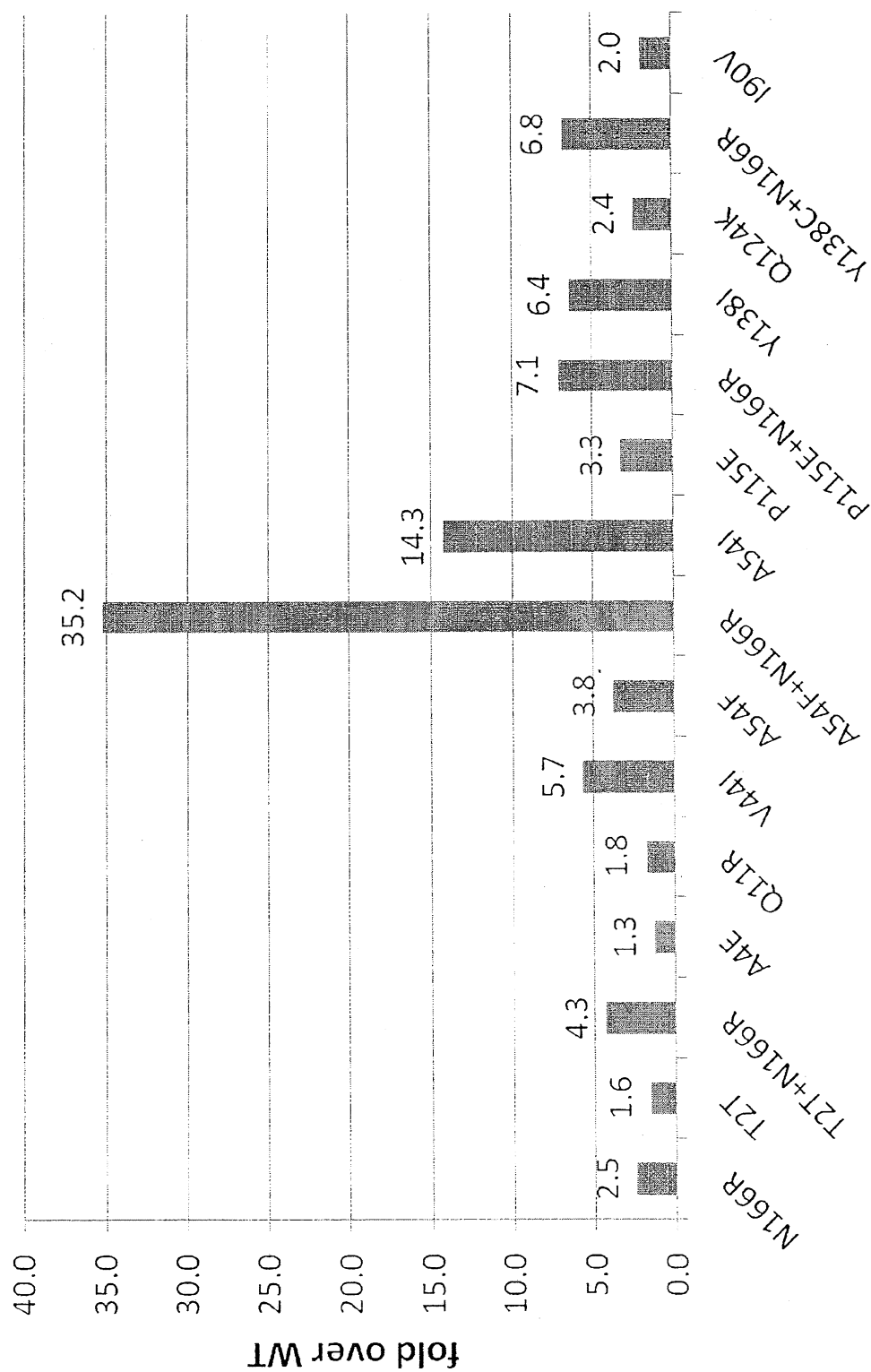


FIG. 8

Signal of Og-luc clones with 0.5% tergitol Assay buffer

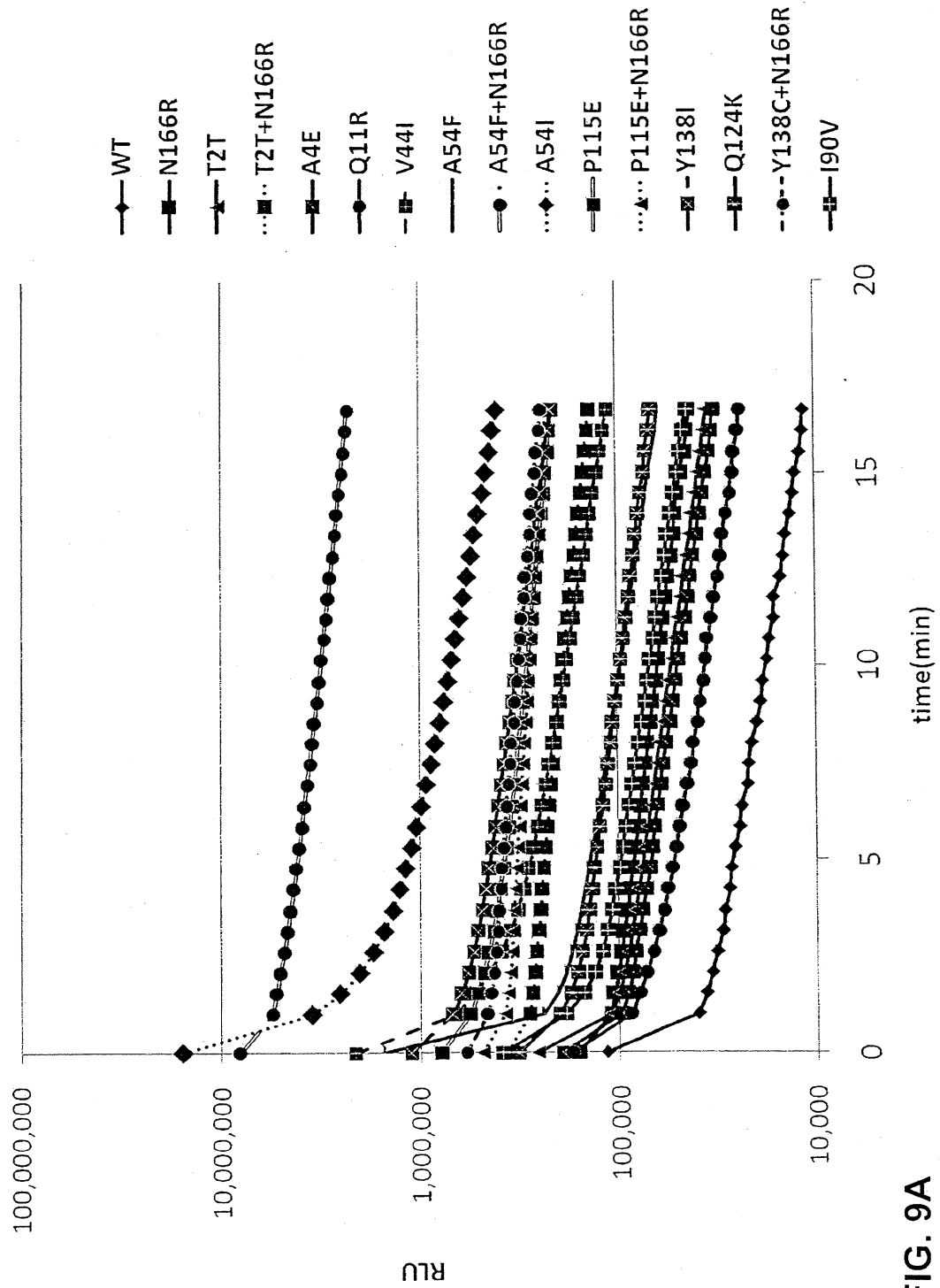


FIG. 9A

Signal Stability in 0.5% tergitol buffer

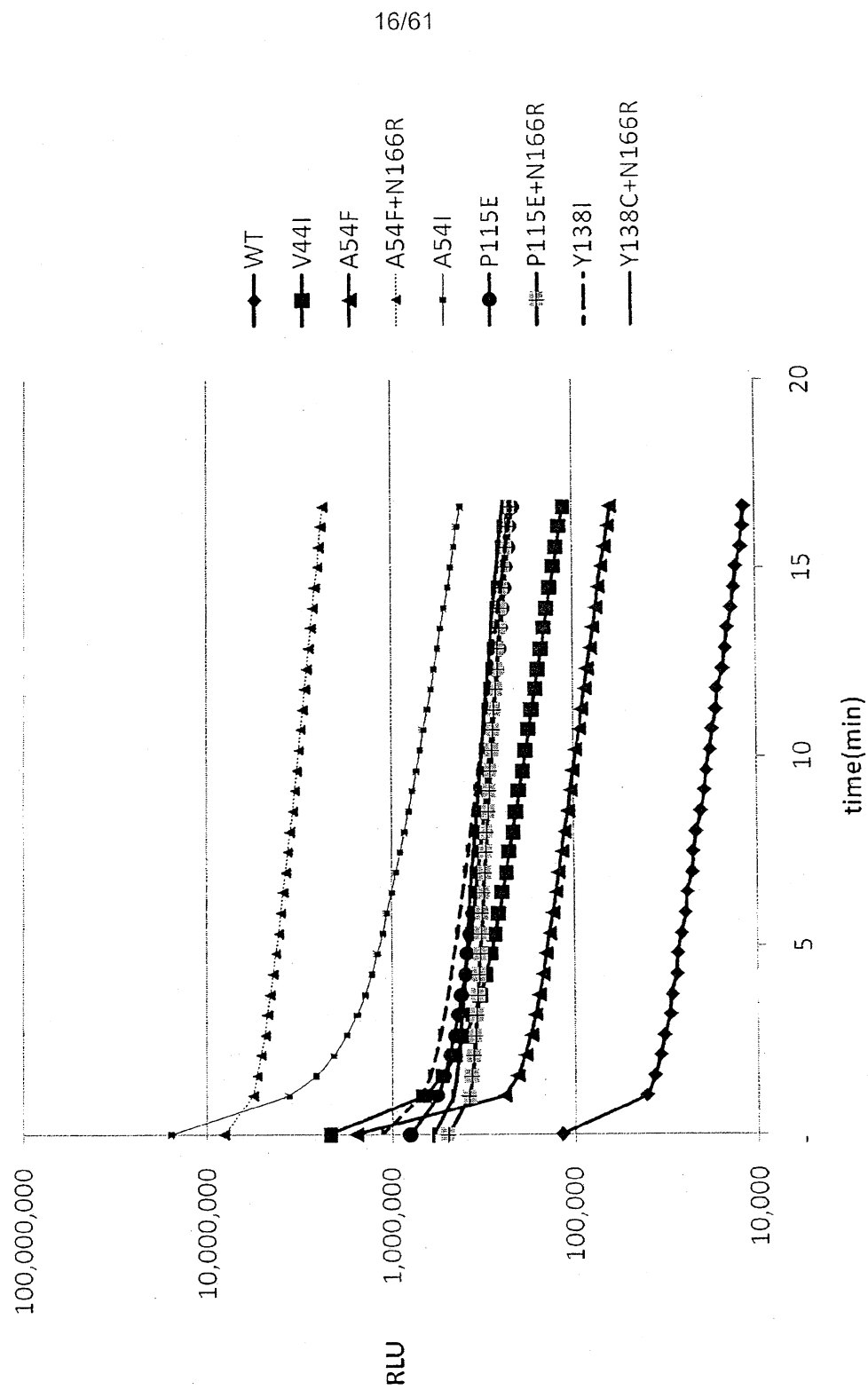


FIG. 9B

Signal stability in 0.5% tergitol buffer

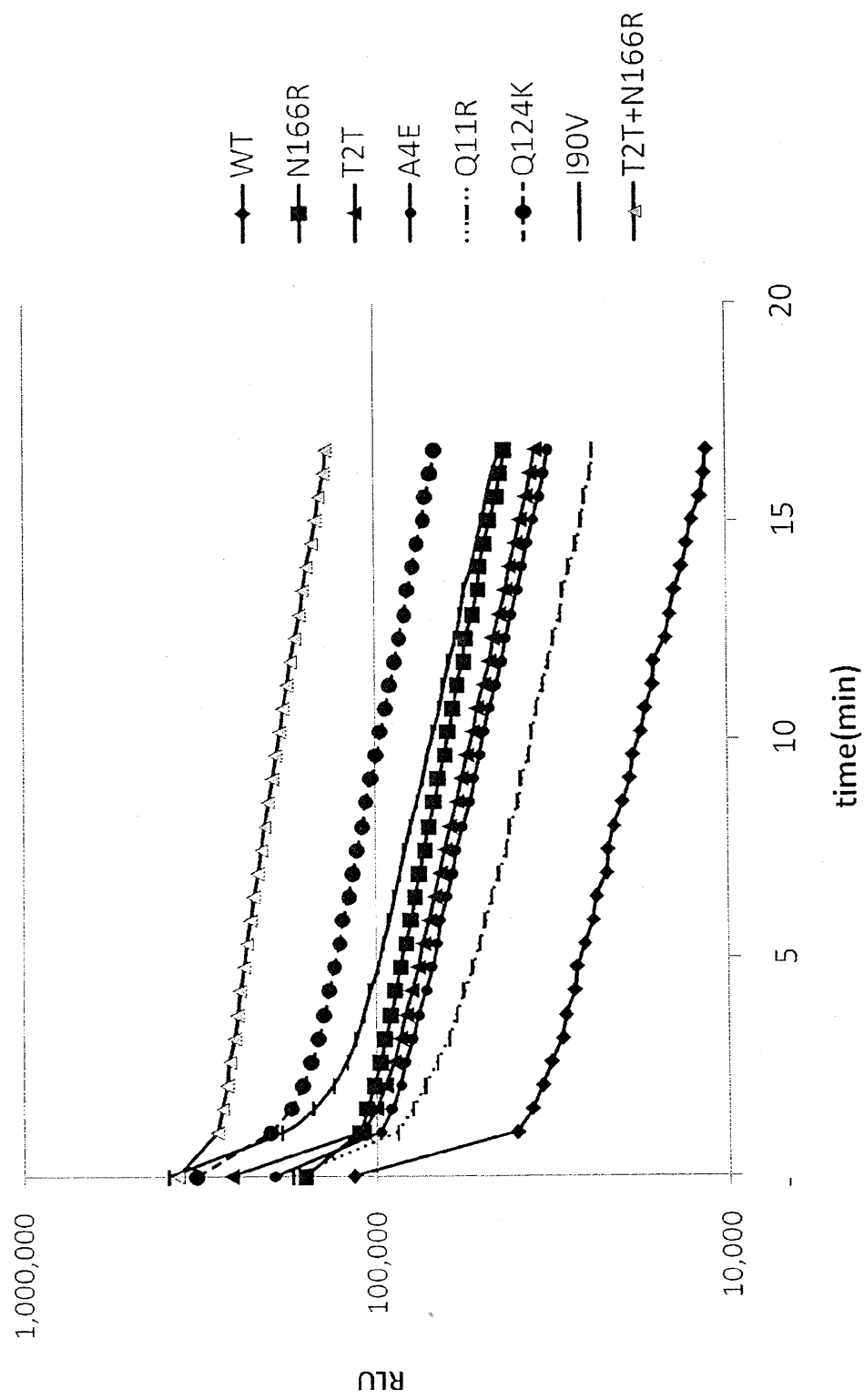


FIG. 9C

Signal half life (0.5% tergitol assay buffer)

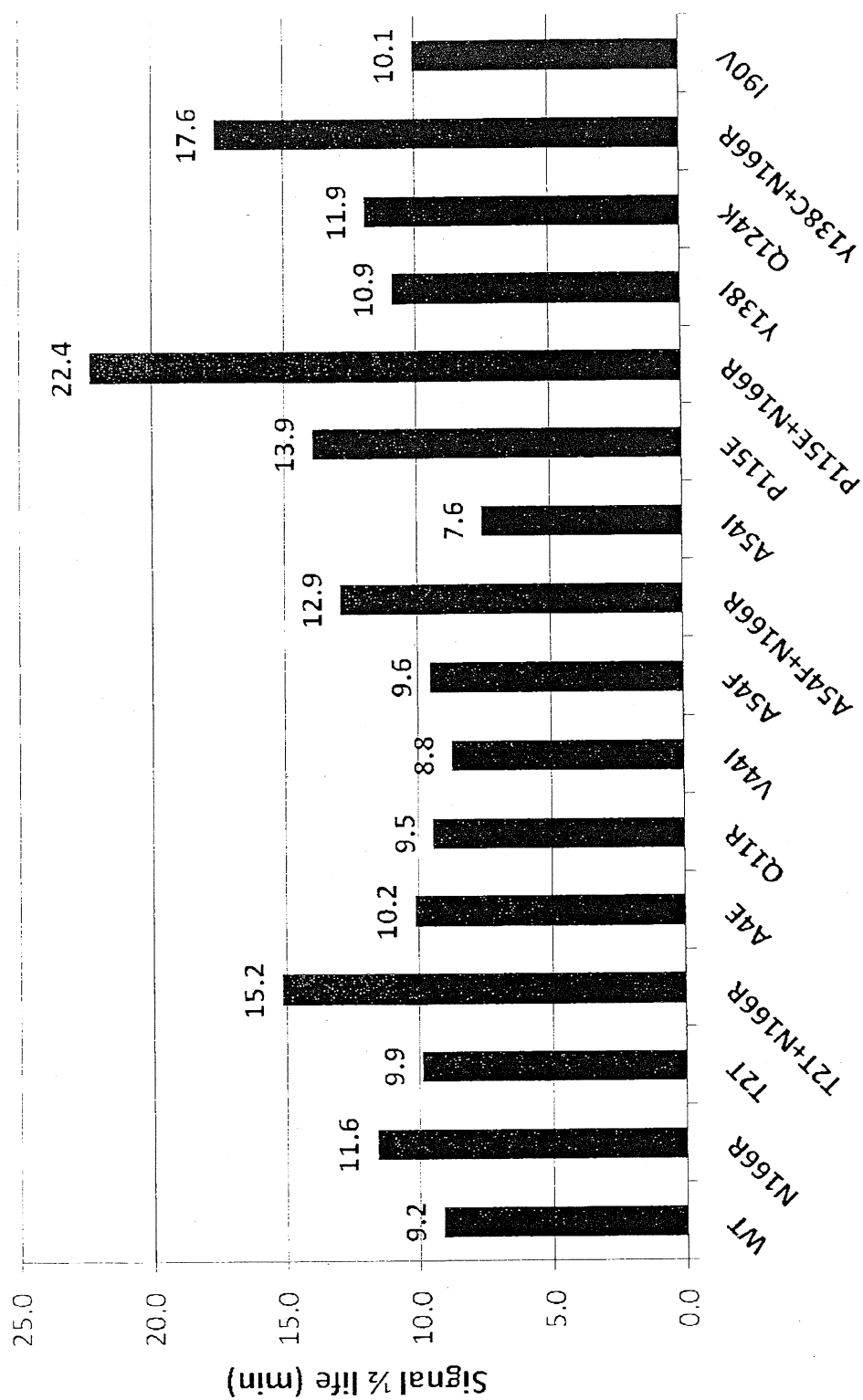


FIG. 9D

Signal stability in RLAB

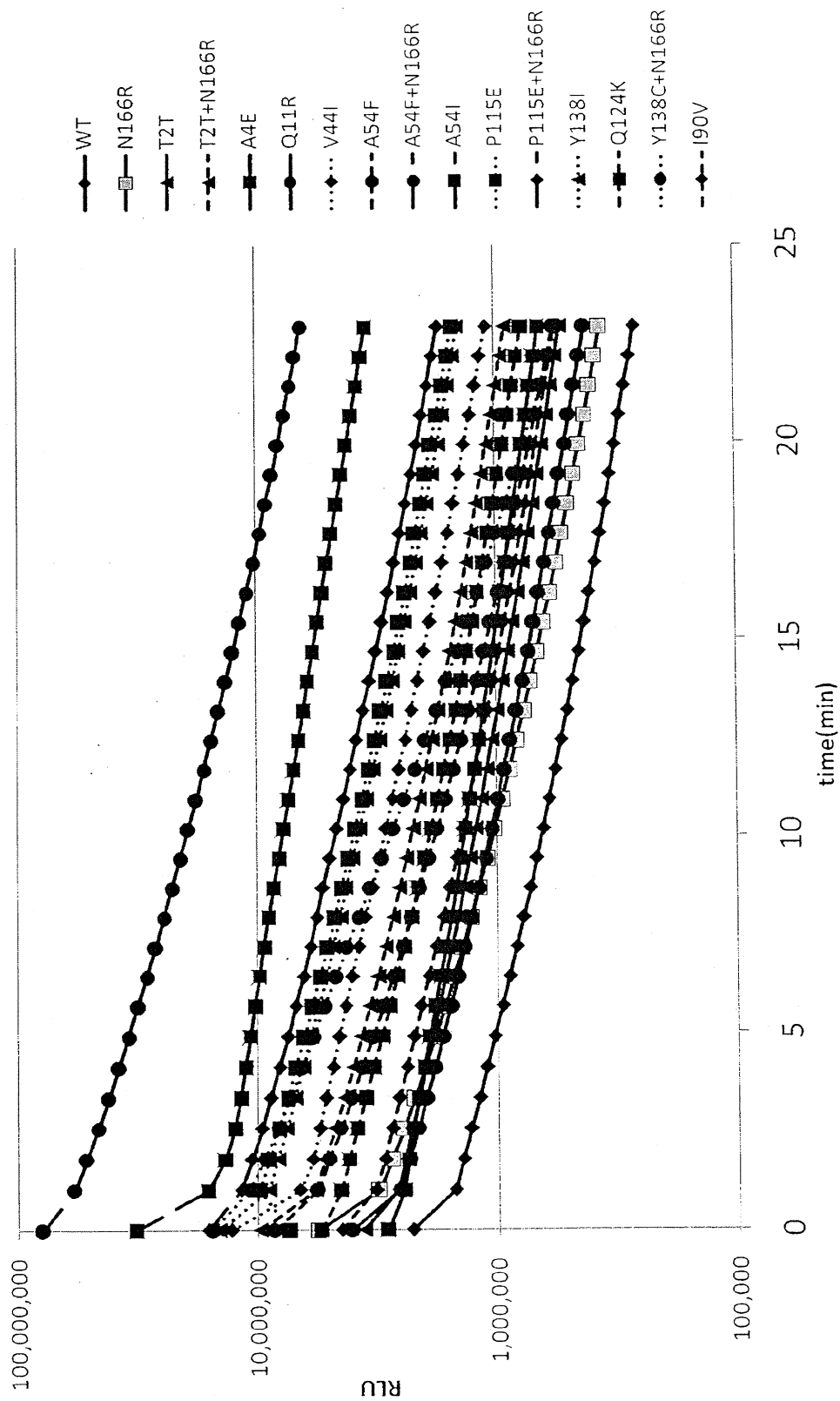


FIG. 10A

Signal stability in RLAB

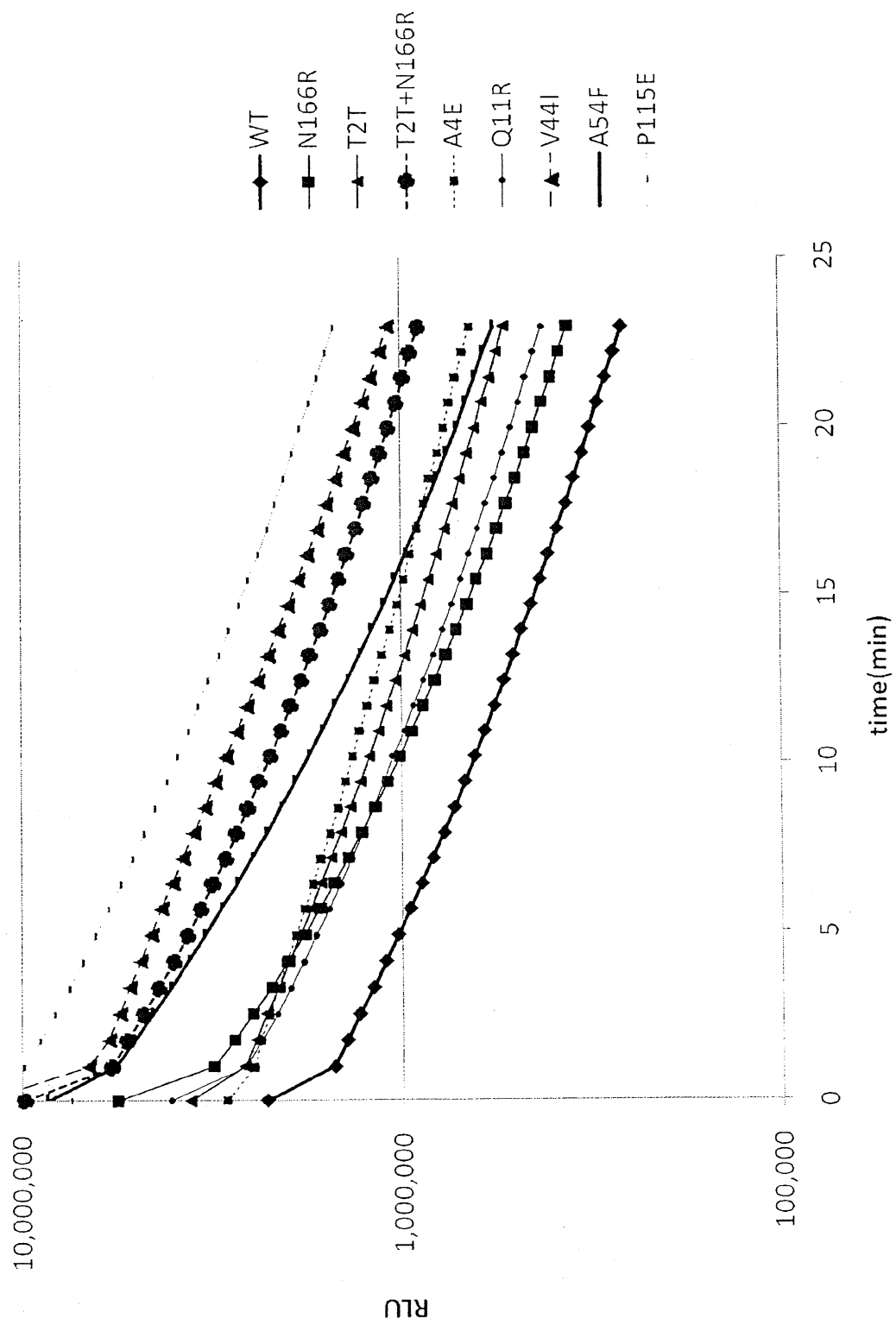


FIG. 10B

Signal stability in RLAB

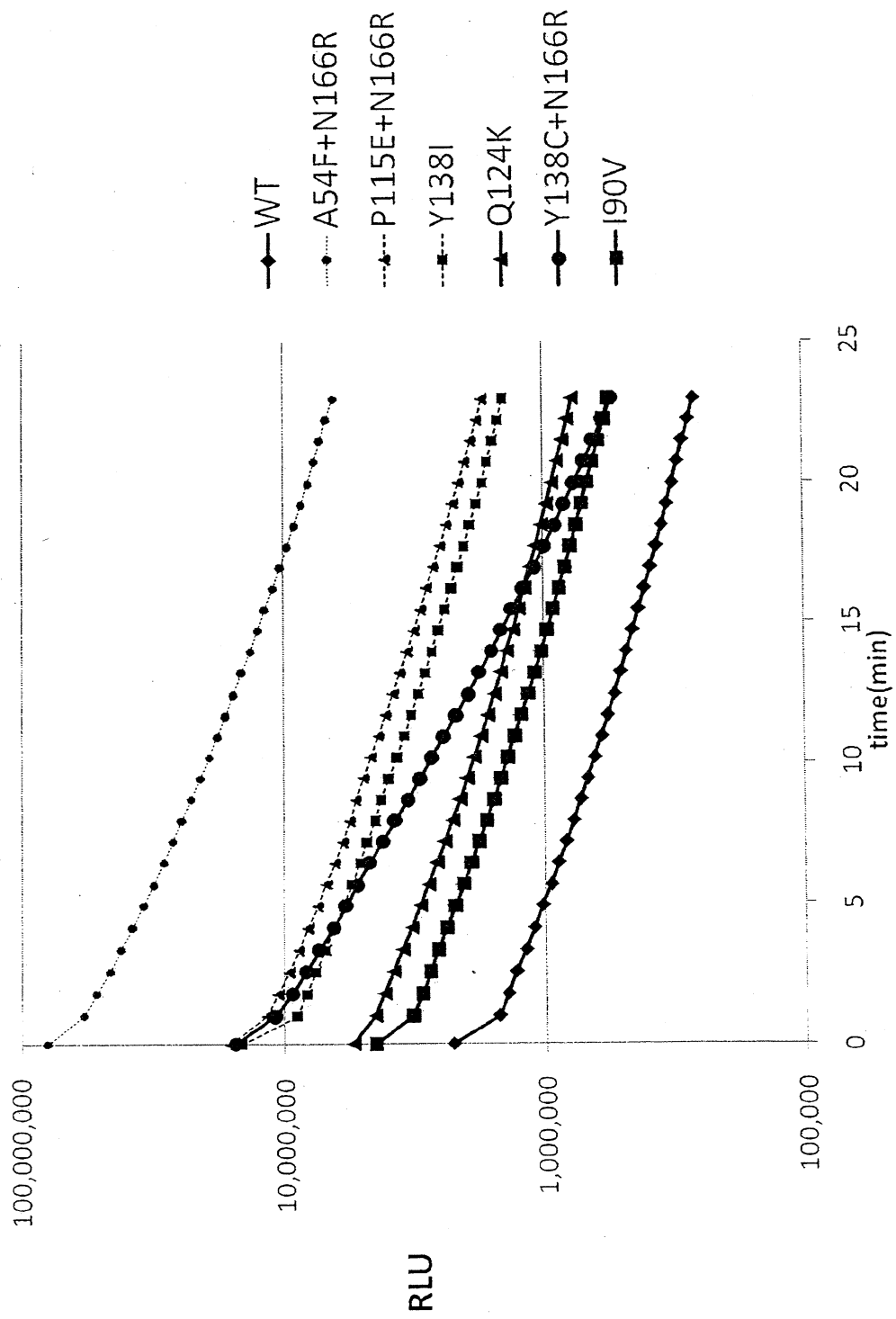


FIG. 10C

Signal ½ life (minutes) RLAB

Signal 1/2 life
(minutes) RLAB

S a m p l e	
W T	9 . 0 3
N 1 6 6 R	8 . 1 7
T 2 T	9 . 7 9
T 2 T + N 1 6 6 R	8 . 7 4
A 4 E	1 1 . 4 8
Q 1 1 R	8 . 6 9
V 4 4 I	8 . 5 4
A 5 4 F	6 . 8 0
A 5 4 F + N 1 6 6 R	7 . 3 0
A 5 4 I	1 0 . 8 8
P 1 1 5 E	8 . 3 1
P 1 1 5 E + N 1 6 6 R	8 . 1 6
Y 1 3 8 I	8 . 3 6
Q 1 2 4 K	8 . 7 7
Y 1 3 8 C + N 1 6 6 R	4 . 9 7
I 9 0 V	8 . 8 3

FIG. 11A

Signal $\frac{1}{2}$ life(minutes) RLAB

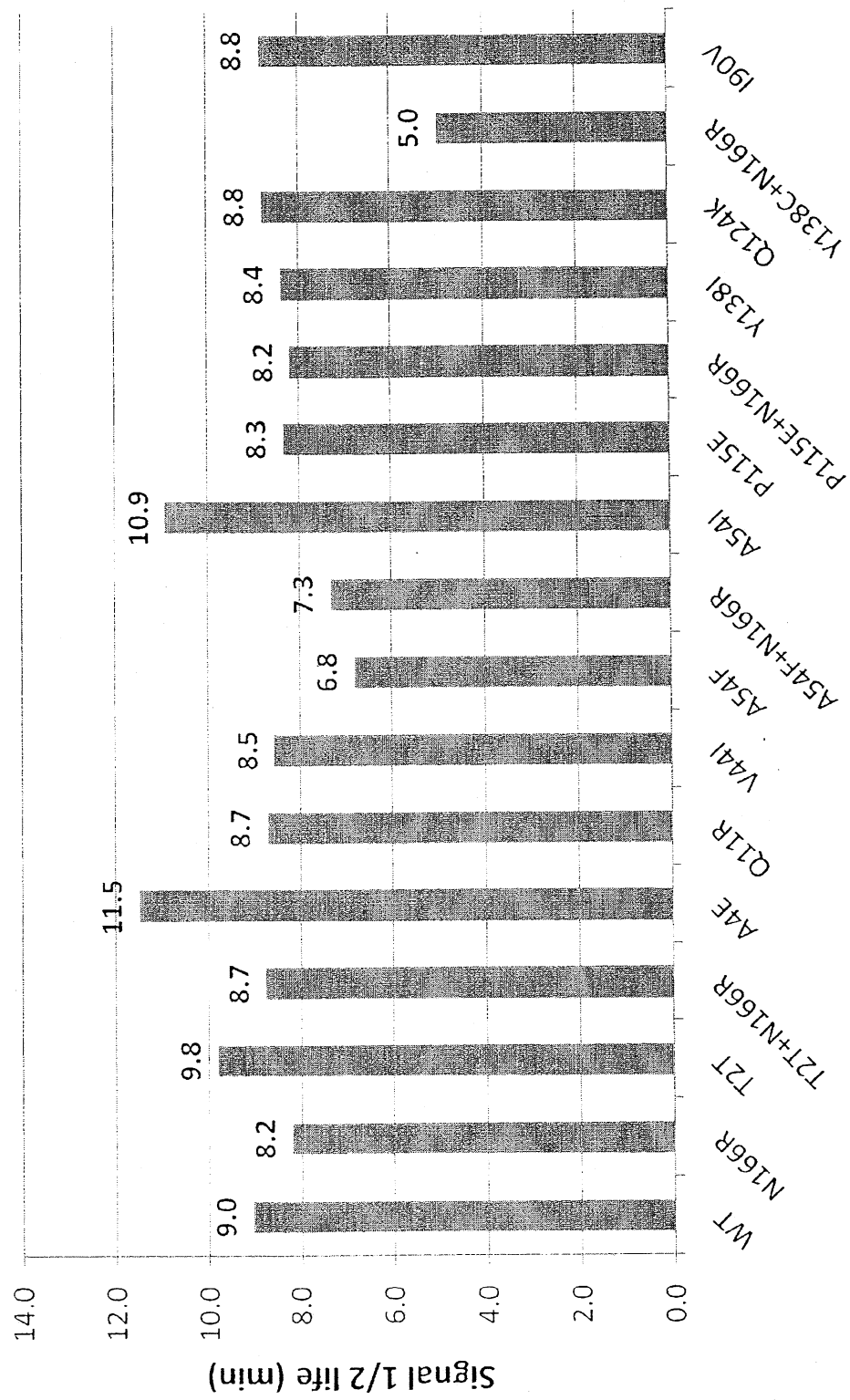


FIG. 11B

Stability at 22°C (1/2 life in minutes)

S a m p l e	s t a b i l i t y (1 / 2 l i f e a t 2 2 C)
W T	3 8 . 1
N 1 6 6 R	6 1 . 3
T 2 T	4 3 . 1
T 2 T + N 1 6 6 R	6 8 . 0
A 4 E	3 8 . 5
Q 1 1 R	4 5 . 9
V 4 4 I	4 9 . 5
A 5 4 F	5 6 . 8
A 5 4 F + N 1 6 6 R	1 7 7 . 7
A 5 4 I	6 3 . 0
P 1 1 5 E	4 9 . 9
P 1 1 5 E + N 1 6 6 R	1 1 9 . 5
Y 1 3 8 I	5 5 . 9
Q 1 2 4 K	4 3 . 9
Y 1 3 8 C + N 1 6 6 R	3 5 . 5
I 9 0 V	4 1 . 3

FIG. 12A

Stability at 22°C (1/2 life in minutes)

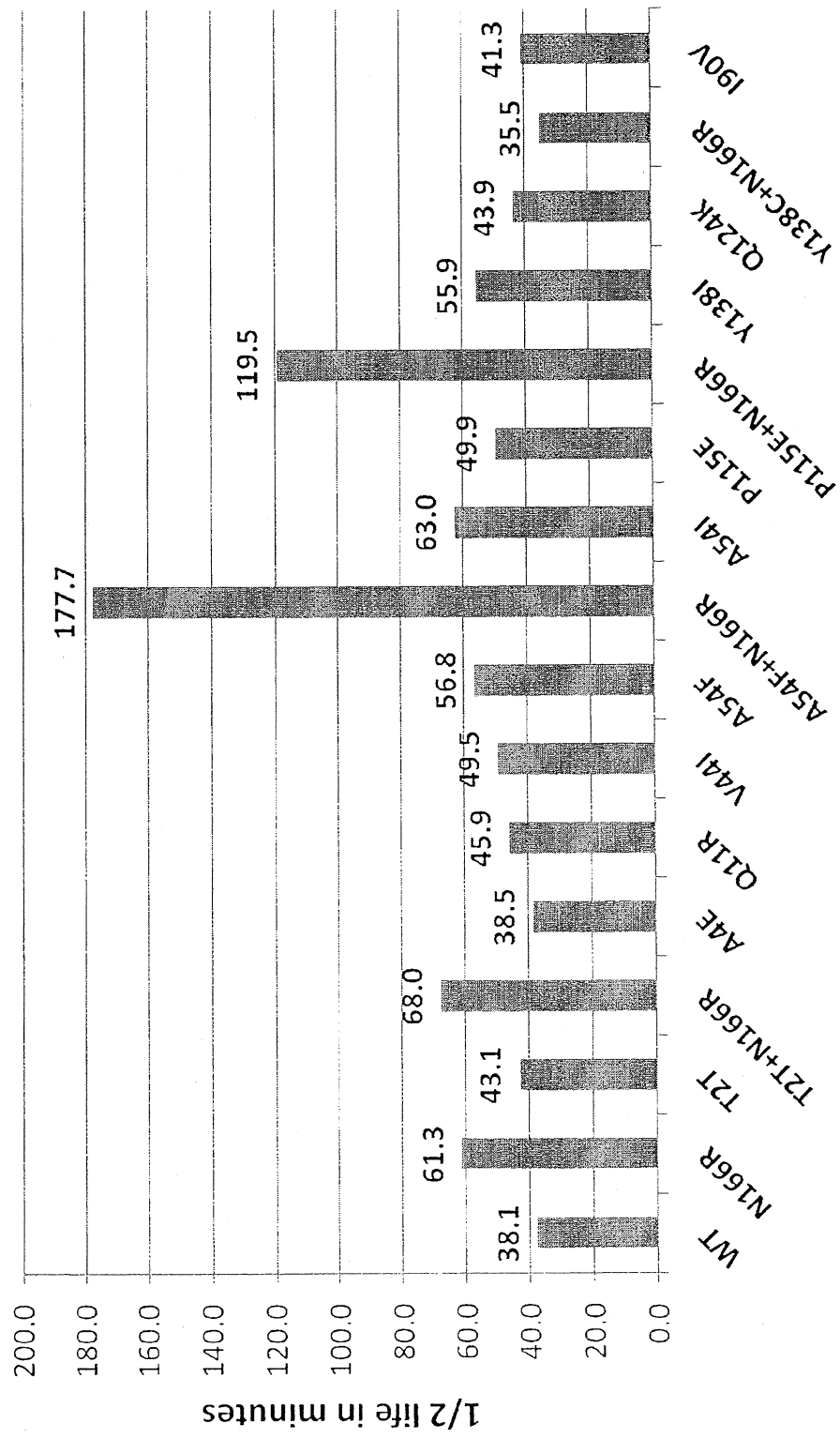


FIG. 12B

T=0 0.5% tergitol assay buffer

S a m p l e	A v e r a g e	% c v
W T	1 1 5 1 9 0 . 4	8 . 6 %
N 1 6 6 R	1 6 6 6 4 7	1 2 . 2 %
W T A 3 3 K	4 3 8 4 5 5	7 . 8 %
W T F 6 8 Y	4 4 9 0 8 3 . 3	6 . 5 %
N 1 6 6 R A 3 3 K	1 0 5 4 3 8 7	8 . 0 %
N 1 6 6 R F 6 8 Y	5 7 1 3 4 7 . 1	4 . 7 %

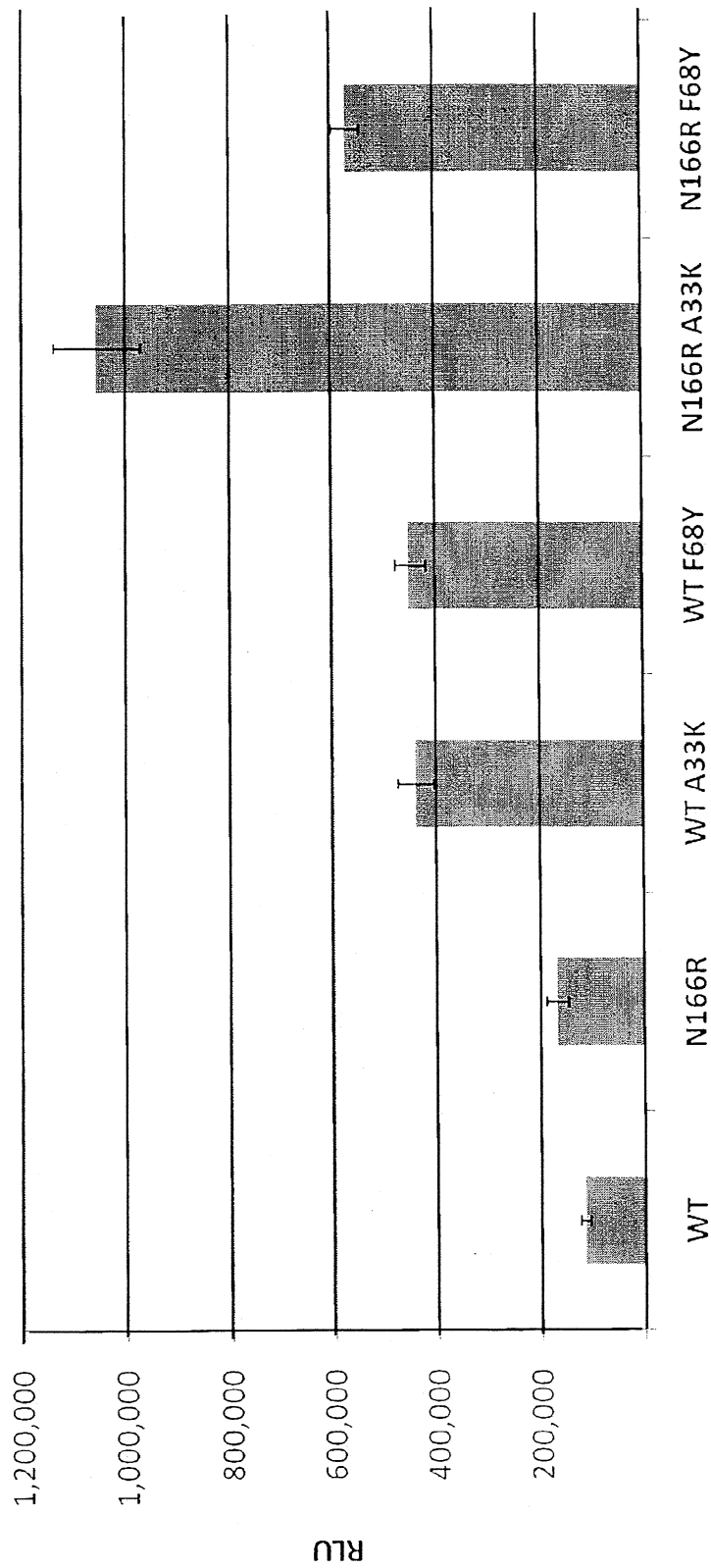


FIG. 13A

RLAB t=0 RLU

S a m p l e	A v e r a g e	c v
W T	27,306,288	8.9%
N166R	69,445,779	9.2%
W T A33K	44,140,390	14.2%
W T F68Y	45,593,349	10.3%
N166R A33K	138,736,058	8.0%
N166R F68Y	89,324,499	12.9%

RLAB t=0

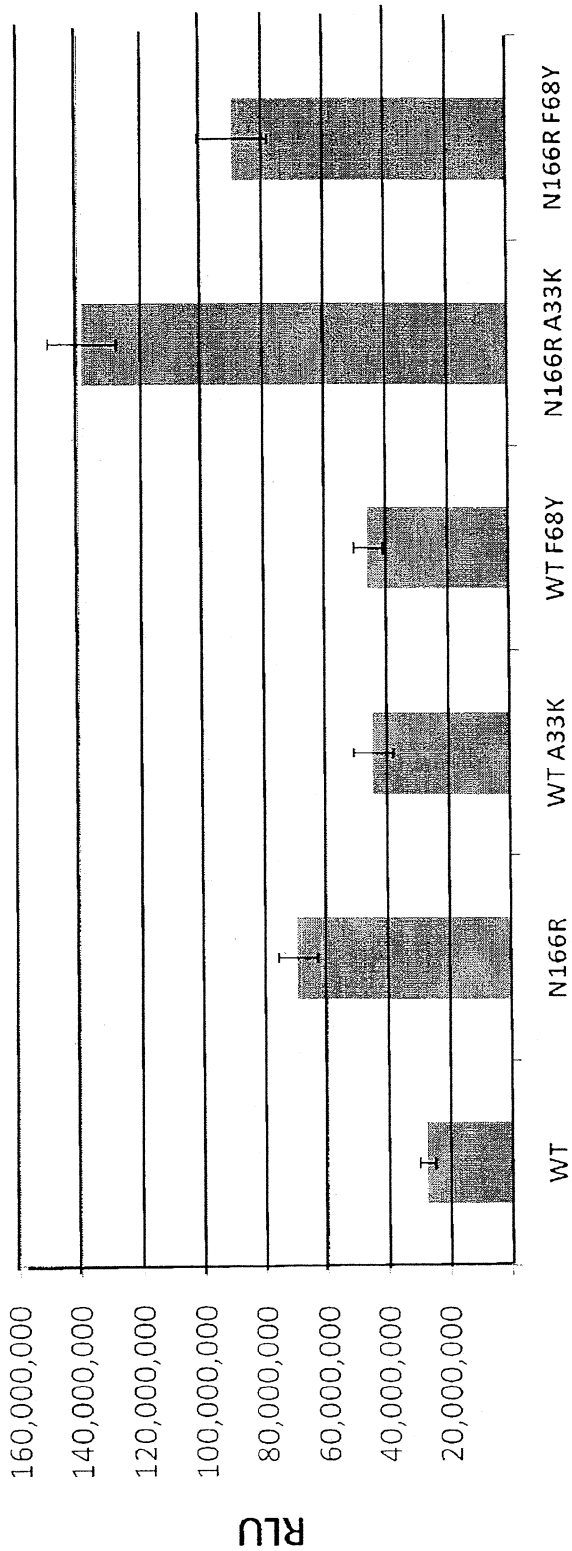


FIG. 13B

Fold over WT (0.5% tergitol assay buffer)

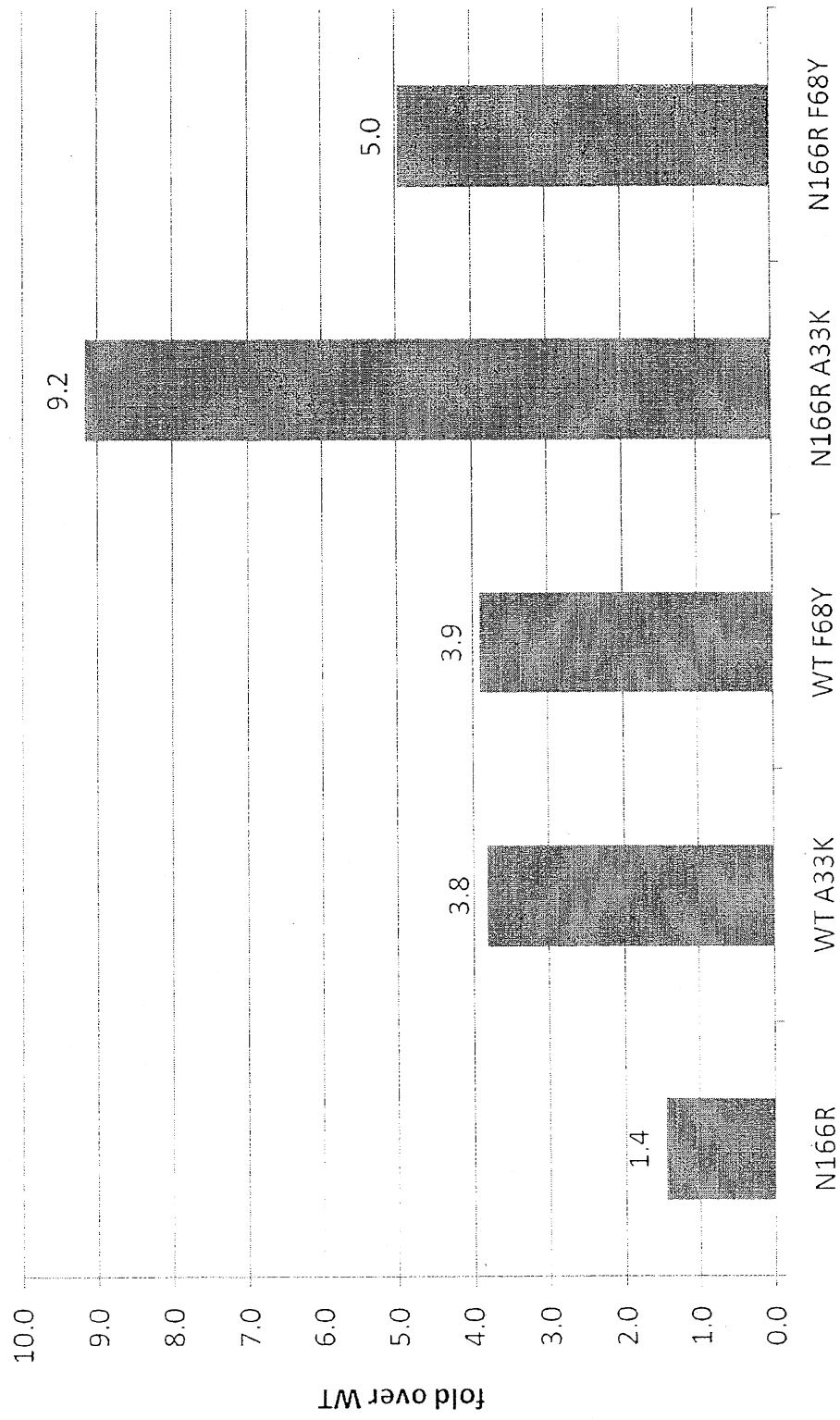


FIG. 14A

Fold over WT (RLAB)

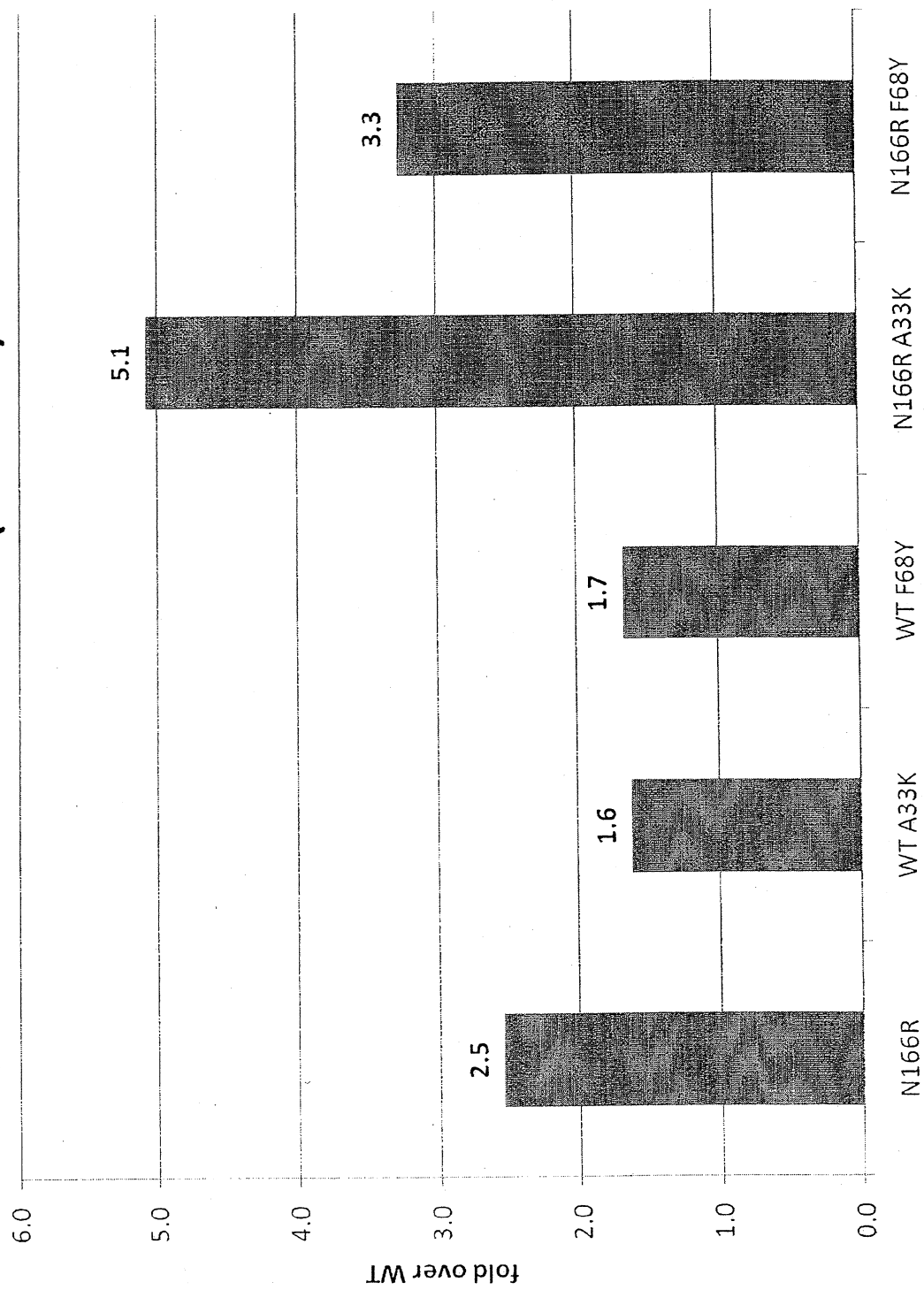


FIG. 14B

Signal kinetics (0.5% tergitol assay buffer)

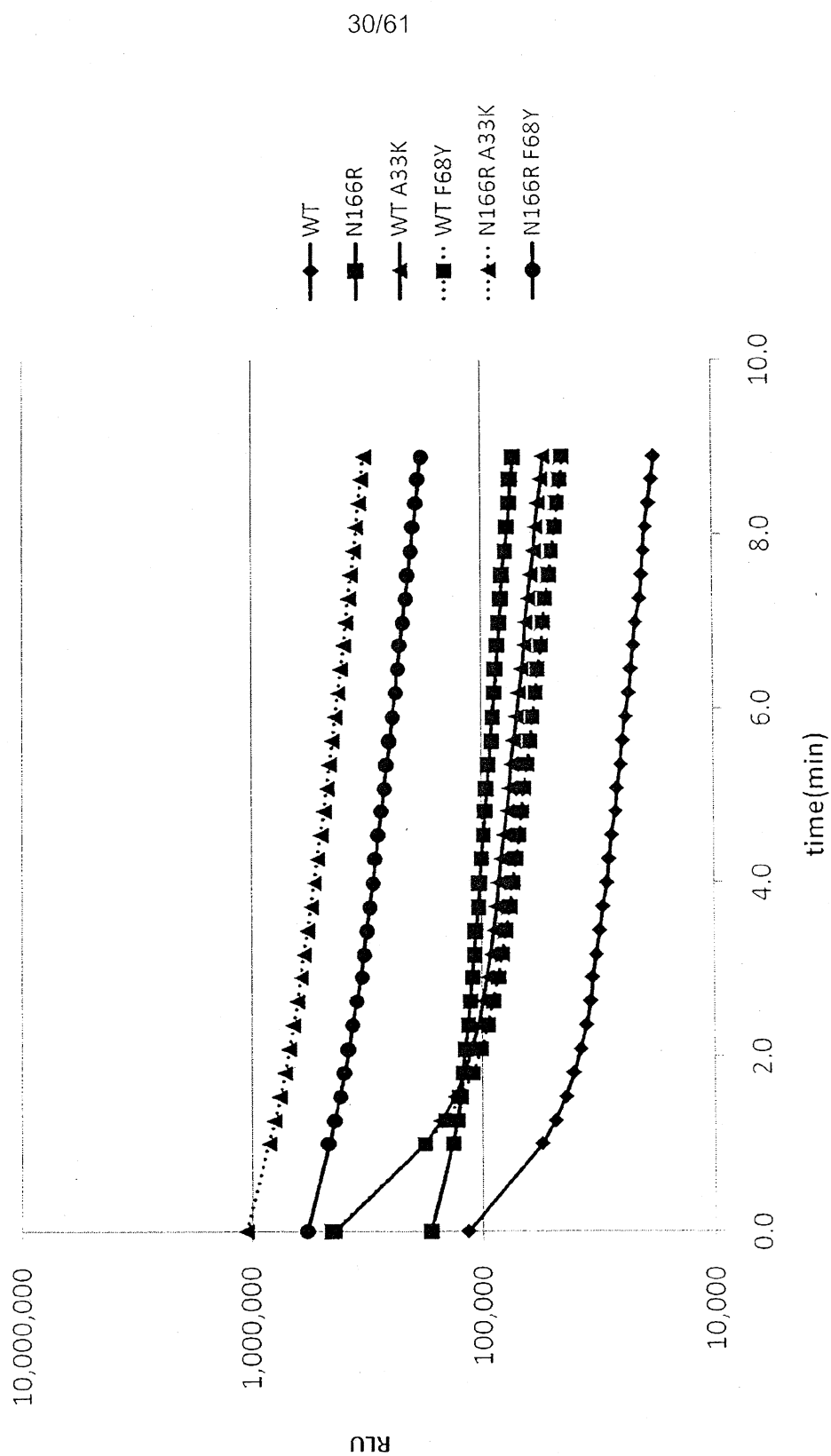


FIG. 15A

Signal stability 0.5% tergitol assay buffer

31/61

Sample	Signal 1/2 life (0.5% tergitol assay buffer)
WT	6.5
N166R	9.6
WTA33K	7.4
WTF68Y	6.3
N166R A33K	6.3
N166R F68Y	6.2

FIG. 15B

Signal kinetics (RLAB)

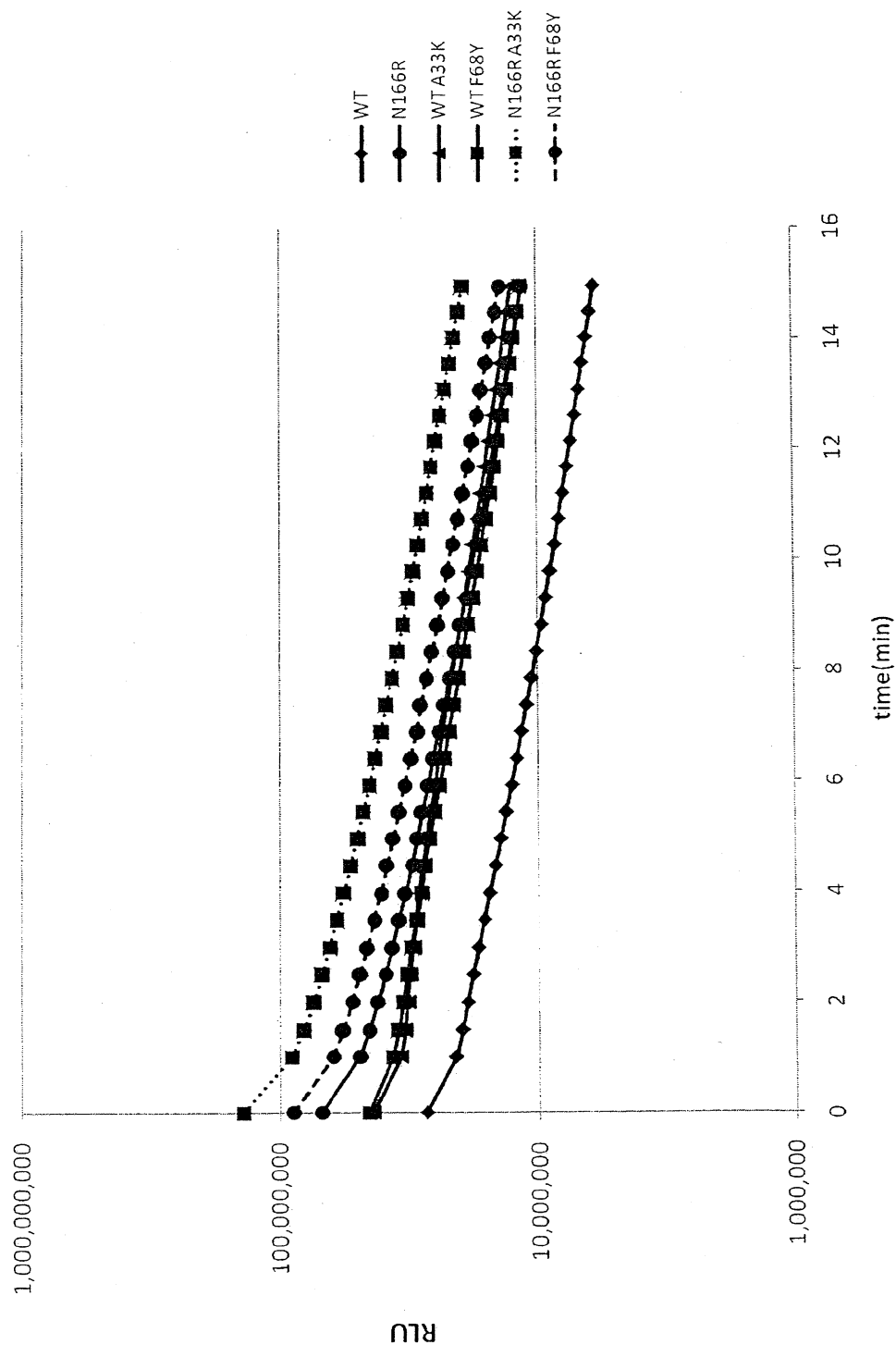


FIG. 16A

Signal stability RLAB

33/61

Sample	signal 1/2 life minutes
WT	7.64
N166R	6.73
A33K	9.22
F68Y	8.13
N166R/A33K	6.58
N166R/F68Y	6.60

FIG. 16B

Stability 1/2 life at 22°C

34/61

Sample	1/2 life (min) 22C
WT	54.6
N166R	67.3
A33K	58.2
F68Y	57.3
N166R, A33K	72.2
N166R, F68Y	77.9

FIG. 17

0.5% tergitol assay buffer

35/61

time(min)	N166R	C1+A4E	C1+A4E+F54T	C1+C2+A4E	C1+C3+A4E	C1+C3+A4E+F54T	Renilla
0.0	1,110	7,910,000	3,583,750	43,371	4,217,500	3,322,500	3,542,500
0.6	970	7,876,250	3,587,500	43,902	4,217,500	3,326,250	3,543,750
1.2	1,277	7,888,750	3,576,250	45,133	4,243,750	3,351,250	3,557,500
1.8	872	7,868,750	3,566,250	43,553	4,257,500	3,338,750	3,563,750
2.4	1,110	7,887,500	3,575,000	42,476	4,260,000	3,366,250	3,571,250
3.0	1,137	7,880,000	3,576,250	43,036	4,266,250	3,397,500	3,562,500
3.6	1,193	7,860,000	3,577,500	43,637	4,276,250	3,386,250	3,586,250
4.2	1,095	7,855,000	3,556,250	42,896	4,293,750	3,406,250	3,568,750
4.8	928	7,862,500	3,556,250	44,755	4,290,000	3,415,000	3,565,000
5.4	998	7,852,500	3,538,750	44,112	4,315,000	3,422,500	3,568,750
6.0	774	7,843,750	3,542,500	44,196	4,311,250	3,433,750	3,551,250
6.6	1,026	7,841,250	3,520,000	42,938	4,332,500	3,431,250	3,525,000
7.2	1,277	7,786,250	3,516,250	43,217	4,311,250	3,445,000	3,512,500
7.8	1,012	7,783,750	3,511,250	43,986	4,318,750	3,430,000	3,486,250
8.4	816	7,775,000	3,491,250	43,483	4,317,500	3,440,000	3,467,500
9.0	1,110	7,771,250	3,471,250	42,854	4,317,500	3,450,000	3,416,250
9.6	928	7,750,000	3,460,000	44,713	4,308,750	3,465,000	3,422,500
10.1	732	7,705,000	3,457,500	44,168	4,320,000	3,462,500	3,386,250
10.7	970	7,706,250	3,451,250	43,245	4,296,250	3,433,750	3,363,750
11.3	1,068	7,687,500	3,430,000	43,902	4,315,000	3,450,000	3,333,750

FIG. 18A

0.5% tergitol assay buffer

E. coli lysates

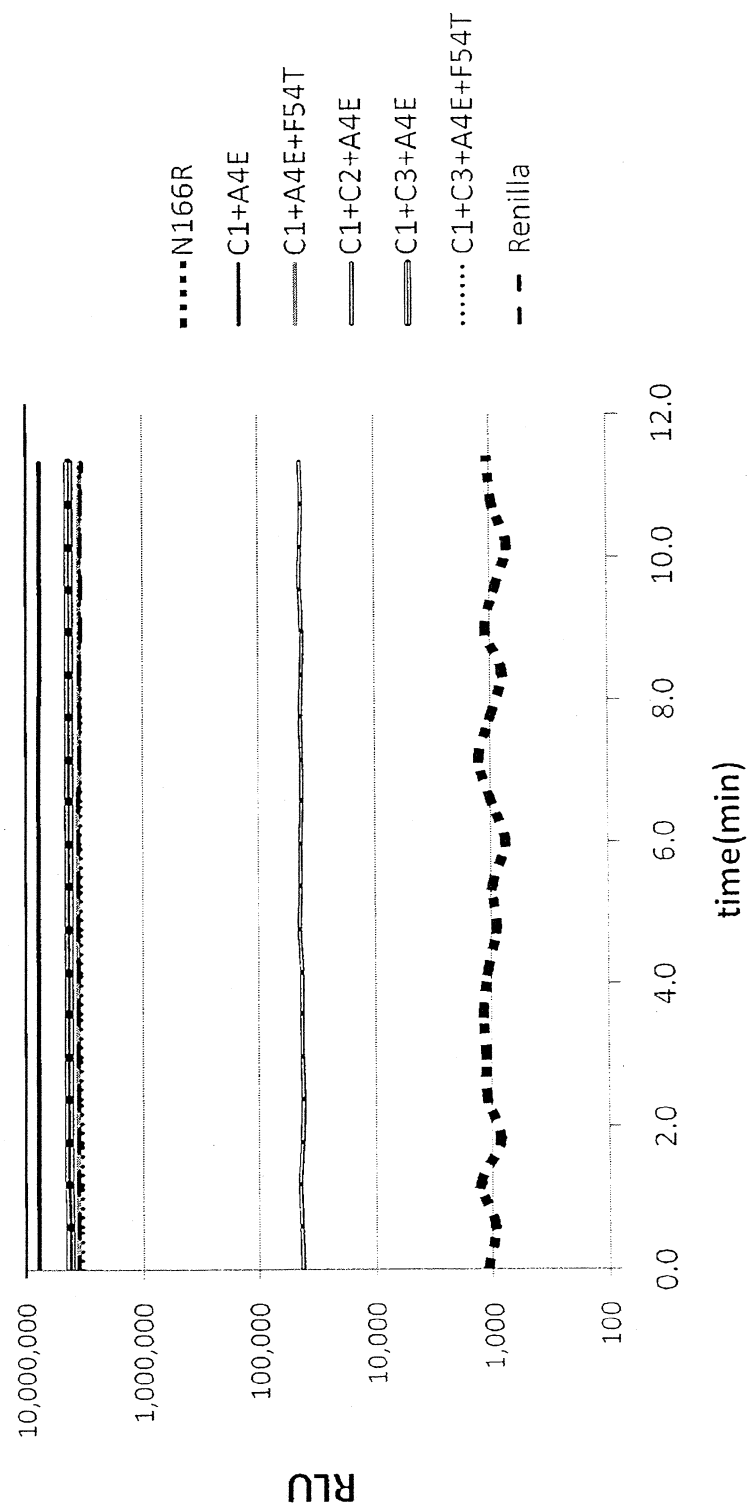


FIG. 18B

RLAB with injector *E. coli* lysates

time(min)	C1+A4E	C1+A4E+F54T	C1+C3+A4E	C1+C3+A4E+F54T	renilla	WT
0	6,288,773	10,015,723	3,998,483	19,218,247	5,721,221	10,170
1	3,880,142	1,479,839	3,501,544	6,033,827	2,111,378	3,490
2	2,515,425	242,450	2,948,188	1,422,742	827,548	1,753
3	1,739,960	99,503	2,378,597	526,400	437,314	1,326
4	1,269,359	66,080	1,926,802	273,345	277,539	966
5	957,041	51,848	1,587,709	172,383	195,944	843
6	734,186	41,485	1,320,817	120,617	147,764	729
7	573,084	34,985	1,106,277	89,734	116,523	609
8	453,838	29,690	944,146	69,148	95,257	469
9	363,570	25,892	803,433	55,316	78,655	513
10	296,648	22,868	696,959	45,239	66,302	423
11	244,349	19,838	606,222	38,464	55,581	296
12	203,980	17,906	533,878	33,612	48,518	289
13	171,296	16,269	469,833	29,523	40,784	253
14	145,640	14,579	420,908	26,163	36,657	236

RLAB injector (no tergitol) *E. coli* lysates

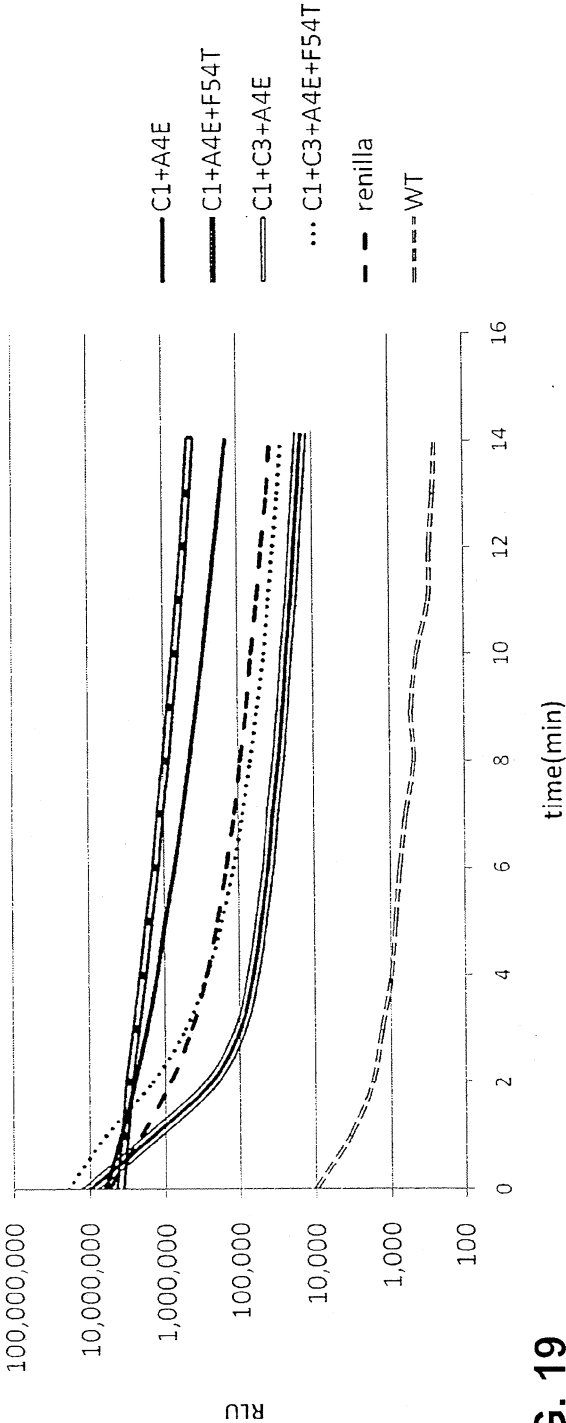


FIG. 19

Og luc composites 0.5% tergitol

time(min)	C1+C2+A4E	C1+A4	hRL	Og-Luc	T2T	A54F
0	3,283,757	705,604,545	82,499,609	40,946	129,209	3,196,722
1	3,272,774	655,089,672	81,682,809	43,269	144,141	3,053,210
2	3,263,848	607,514,297	80,390,118	39,618	141,818	3,051,850
3	3,323,641	561,553,077	78,958,229	45,923	164,050	2,972,811
4	3,251,173	520,696,293	77,087,348	29,000	165,045	2,863,212
5	3,259,501	481,737,466	75,545,993	54,218	178,318	2,773,522
6	3,332,268	443,689,477	73,595,608	38,955	168,031	2,774,186
7	3,290,426	409,149,917	71,668,815	37,296	184,622	2,630,011
8	3,389,076	375,439,634	69,682,029	44,264	180,641	2,614,748
9	3,320,986	346,313,863	67,570,182	42,936	192,586	2,575,859

Og luc (0.5% tergitol in assay buffer)

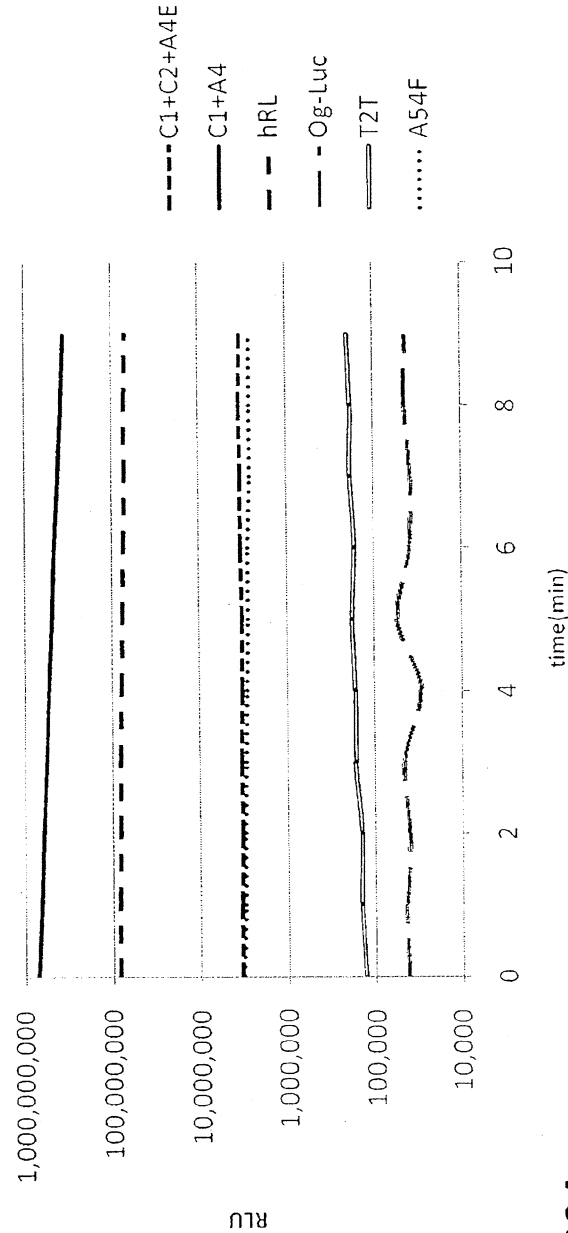


FIG. 20A

E. Coli lysates RLAB injector

Time(min)	WT	Ren	C1+A4E	C1+C2+A4E	C1+C3+A4E
0	1,516	5,224,047	7,334,767	41,049	3,886,812
1	1,146	1,400,228	4,282,362	35,602	3,162,835
2	690	628,021	2,940,985	31,649	2,451,582
3	493	362,496	2,127,271	29,607	1,842,268
4	393	243,436	1,606,017	27,491	1,412,564
5	266	176,676	1,248,100	26,635	1,117,276
6	253	137,402	998,258	25,333	906,594
7	203	111,480	820,954	24,741	751,344
8	200	90,889	681,497	23,637	634,050
9	156	78,227	573,912	23,222	542,510
10	156	66,966	487,929	22,152	469,571
11	110	58,172	422,367	21,129	413,198
12	103	51,130	368,901	21,209	363,911
13	83	44,578	321,104	21,032	327,210

E. coli lysates RLAB injector

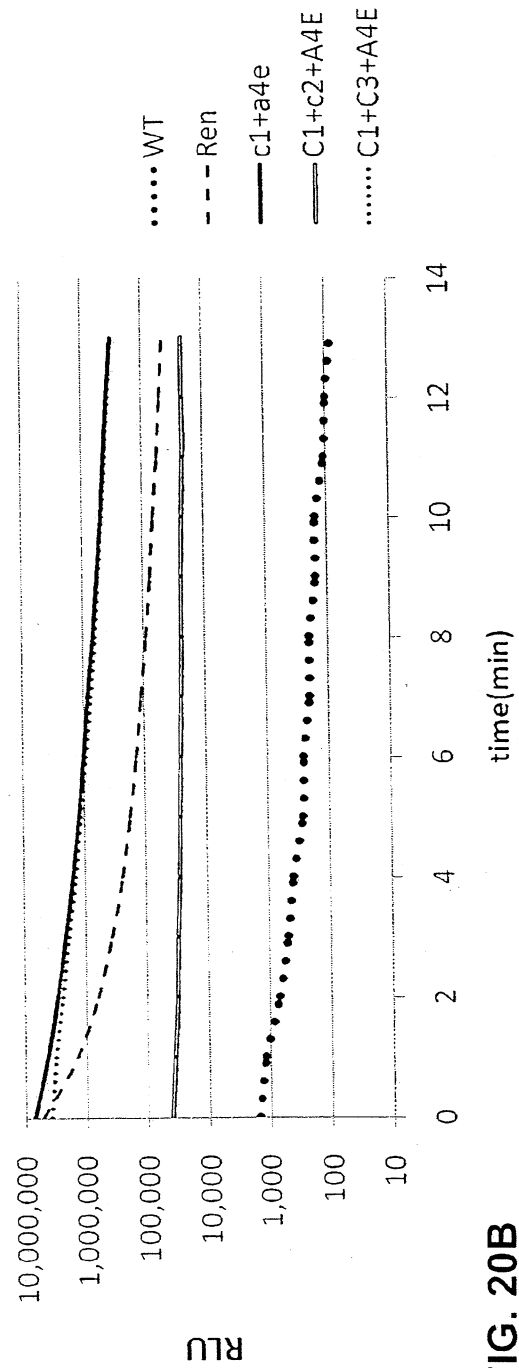


FIG. 20B

Results in *E. coli* (tergitol in assay buffer to stabilize signal)

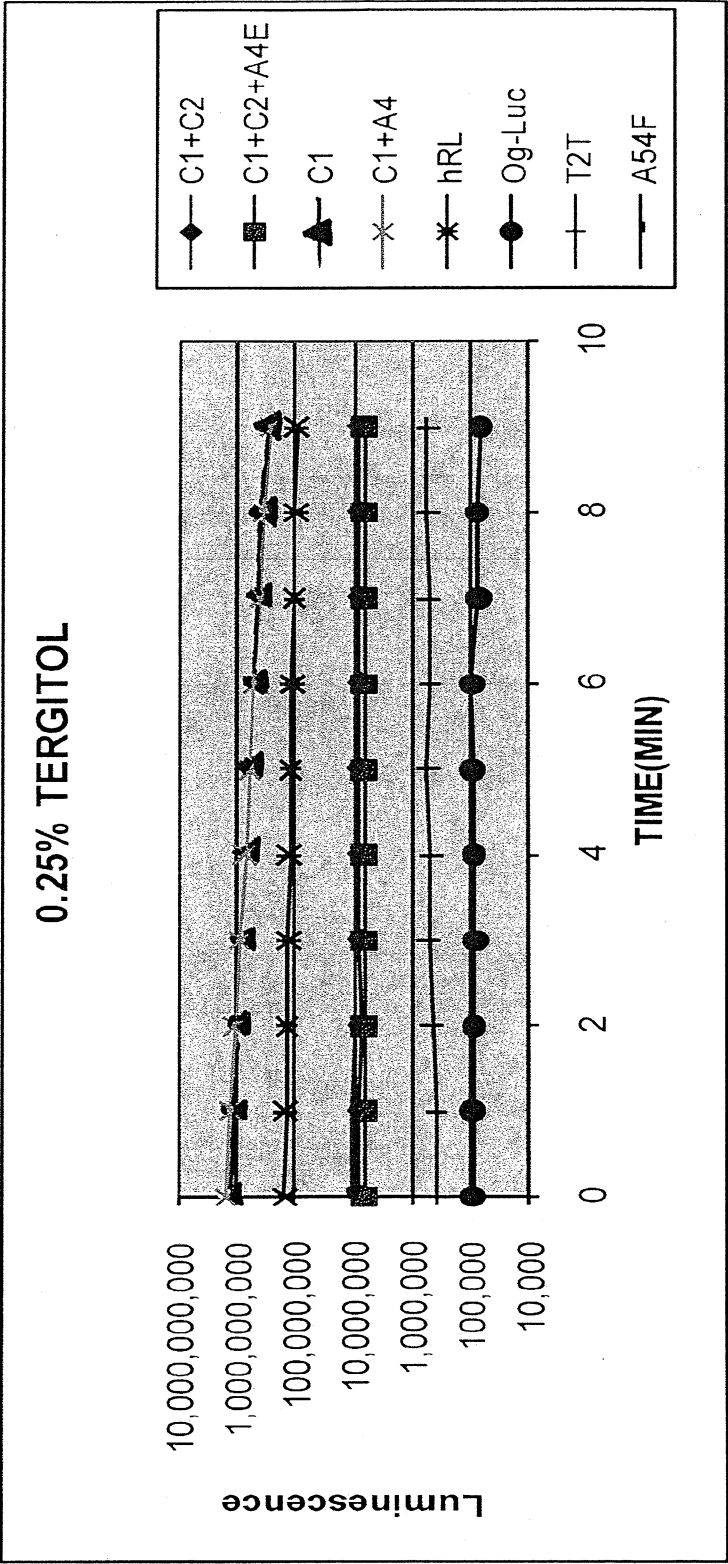


FIG. 21

Og-Luc composites in 293 cells

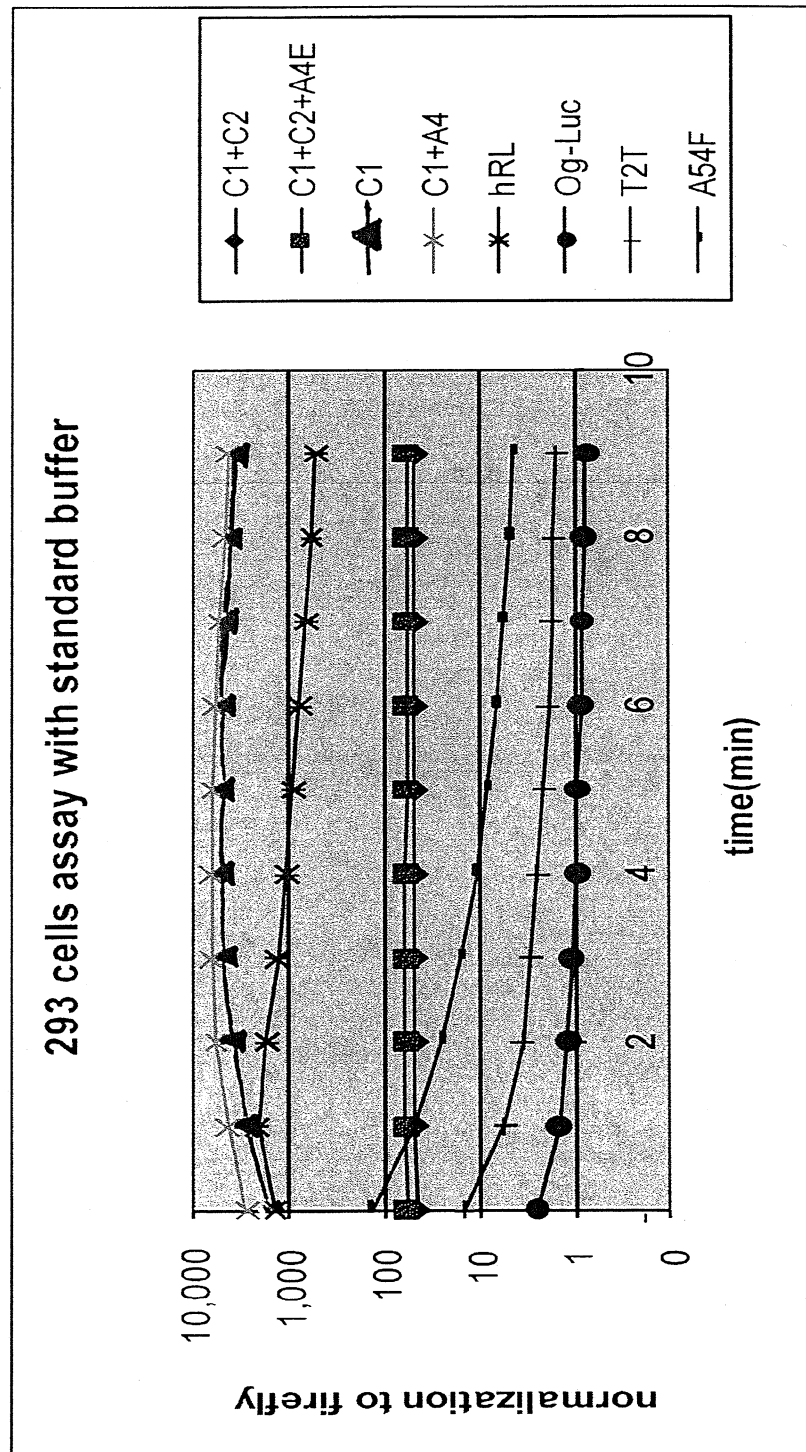


FIG. 22

293 cells normalized to firefly

0.25% tertigitol normalized to firefly
293 cells

time(min)	C1+C2+A4E	C1+A4	hRL	Og-Luc (wt)
0	2.71	741.28	344.96	0.07
1	2.78	731.25	325.17	0.07
2	2.65	722.31	306.02	0.08
3	2.90	715.06	288.69	0.05
4	2.73	706.89	269.87	0.07
5	2.92	697.22	254.60	0.05
6	2.69	693.01	239.46	0.08
7	2.61	687.04	228.25	0.07
8	2.75	676.30	215.62	0.04
9	2.76	671.85	204.51	0.04

293 cells normalized to firefly. 0.25% tertigitol

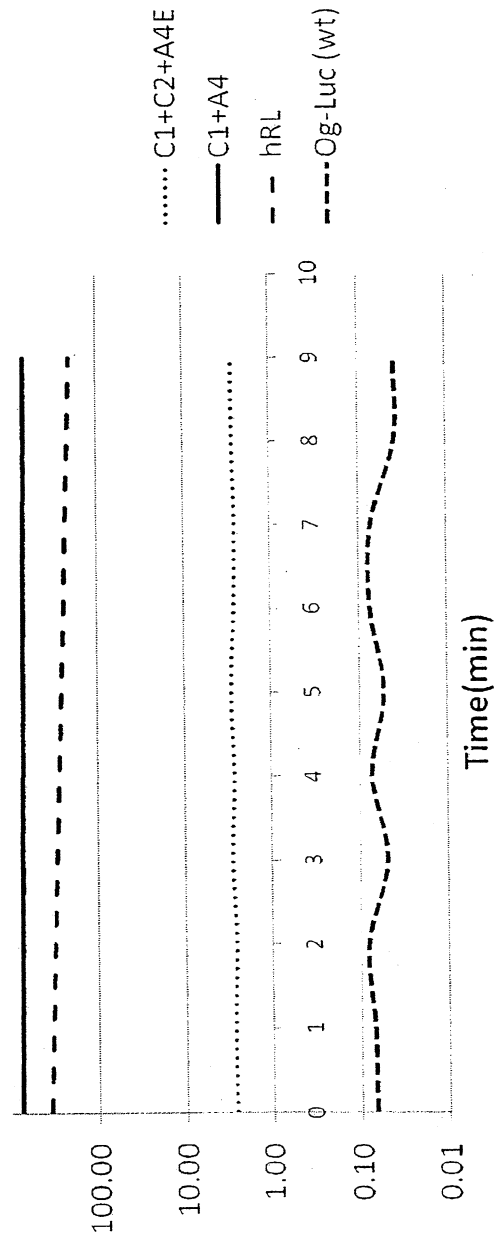


FIG. 23

Stability of og-luc clones

Temp	Clone	1/2 life
RT (22°C)	WT	1 hour
RT (22°C)	og-luc C1	9.4 hours
RT (22°C)	Renilla	~24 hours
30°C	N166R	30 minutes
30°C	og-luc C1+A4E	No decay in 6 hours
30°C	Renilla	7.9 hours
37°C	N166R	2 min
37°C	og-luc C1+A4E	no loss 3 hours
42°C	og-luc C1+A4E	6.4 hours
54°C	og-luc C1	7.1 min
54°C	og-luc C1+A4E	8 (7.1, 7.7) min
54°C	og-luc C1 +C2+A4E	128 min
54°C	og-luc C1 +C3+A4E	23.7 min

FIG. 24

Signal 1/2 life or signal stability

Sample	1/2 life (min)
WT	1.8
Ren	0.8
c1	1.7
c1+a4e	1.7
C1+c2+A4E	12.6
C1+C3+A4E	3.3

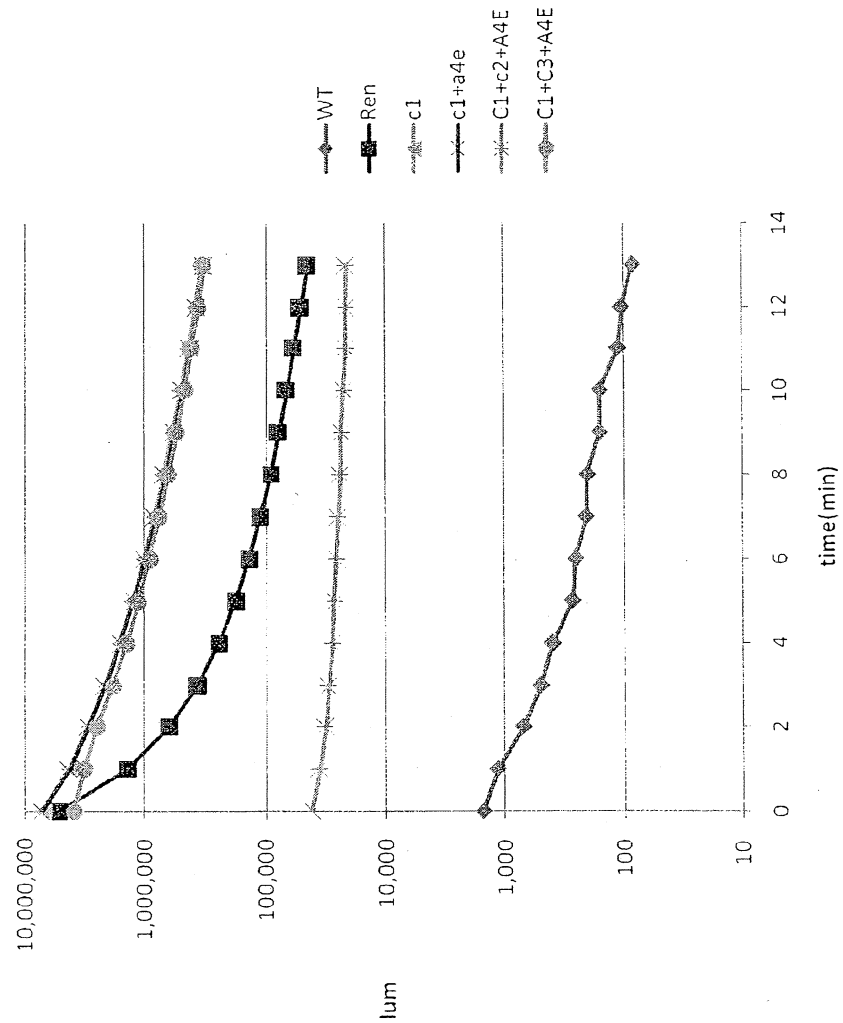


FIG. 25

Spectra of Ogluc clones

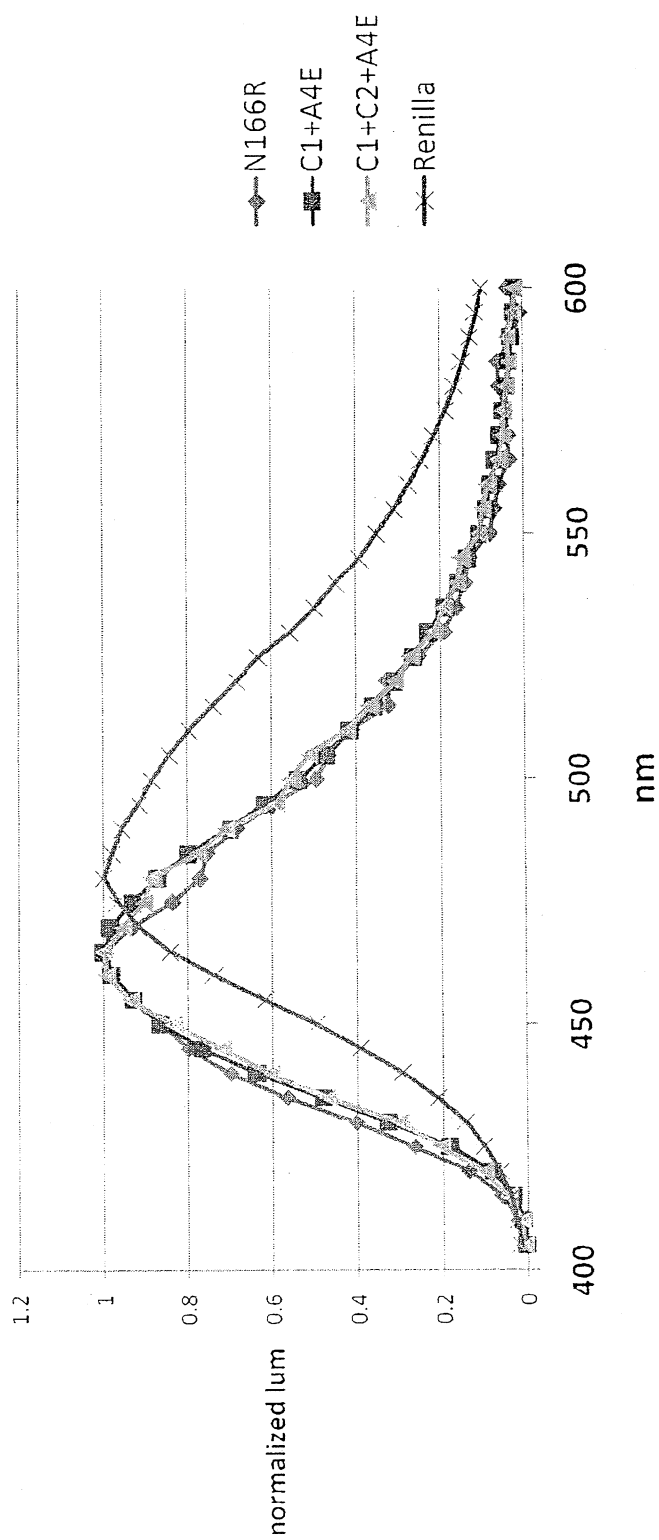


FIG. 26

Mutations: C1+A4E random mutagenesis

C1A4E random mutagenesis	AA change	Fold over C1A4E
04g7	L72Q	3
10f2	I90T	1.9
16c5	Q20R	4.1
25a1	C164S	3.1
28b2	V79I	2.9
29h7	F54I	10.3
35d11	K89E	2.2

FIG. 27A

Mutations: C1+A4E random mutagenesis

Amino Acid change	Fold over C1+A4E
I90V	1.5
F77W	2

FIG. 27B

Changes at position 92

Amino Acid change	Fold over C1A4E (20uM coelenterazine)
L92G	2.2
L92Q	2
L92S	2.9
L92A	2.5

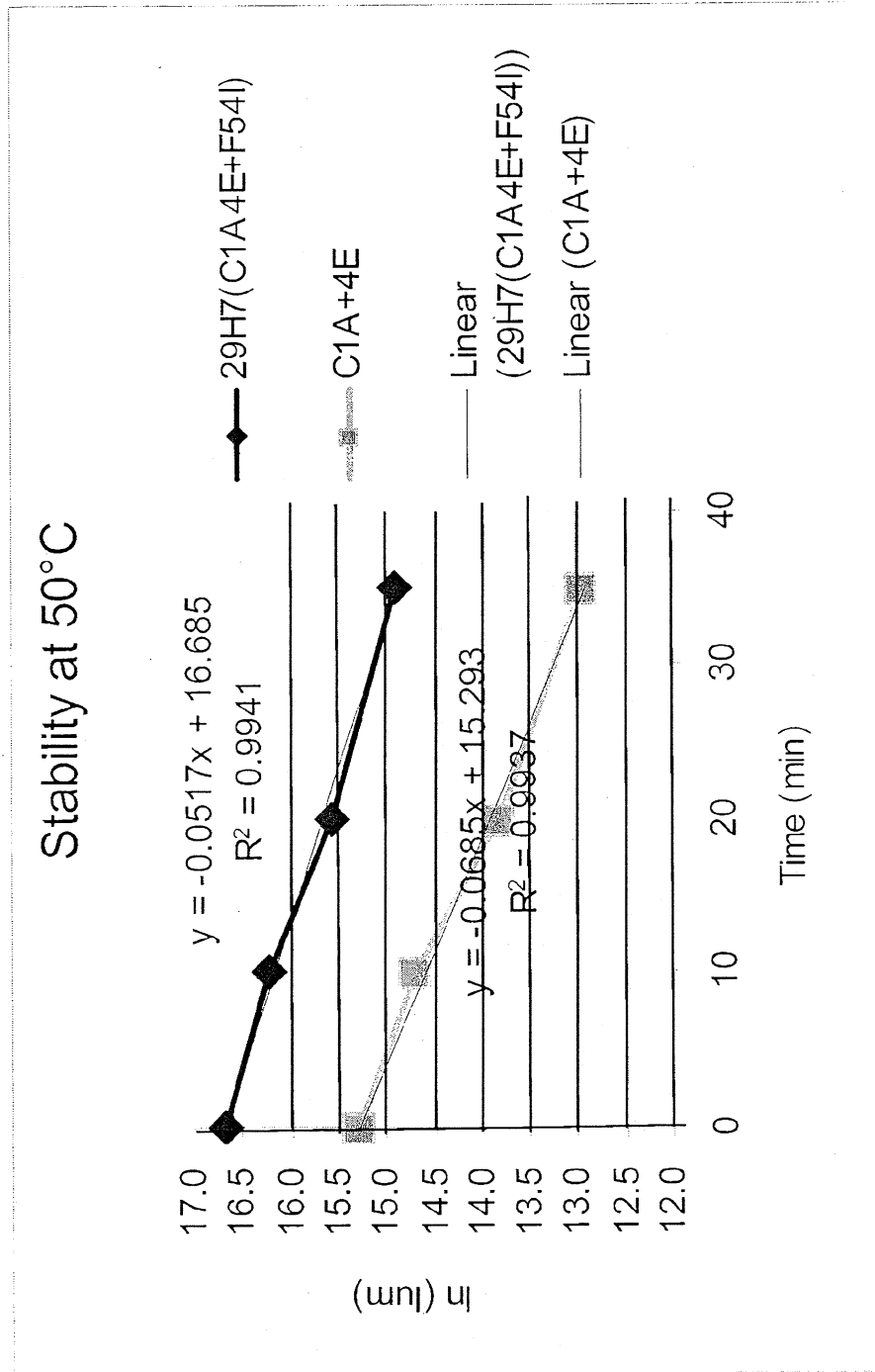
FIG. 28

Combinations that improve light output

49/61

Sample ID (Combination experiment)	Fold over C1A4E	Amino Acid change from C1A4E
17A6	19.3	F54I, F68S, I90V
14A12	17.7	F54I, M75K
1G4	17.5	F54I, M75K, I90V
3C6	17.5	F54I, F68S, M75K
14B7	17.5	F54I, I90V

FIG. 29

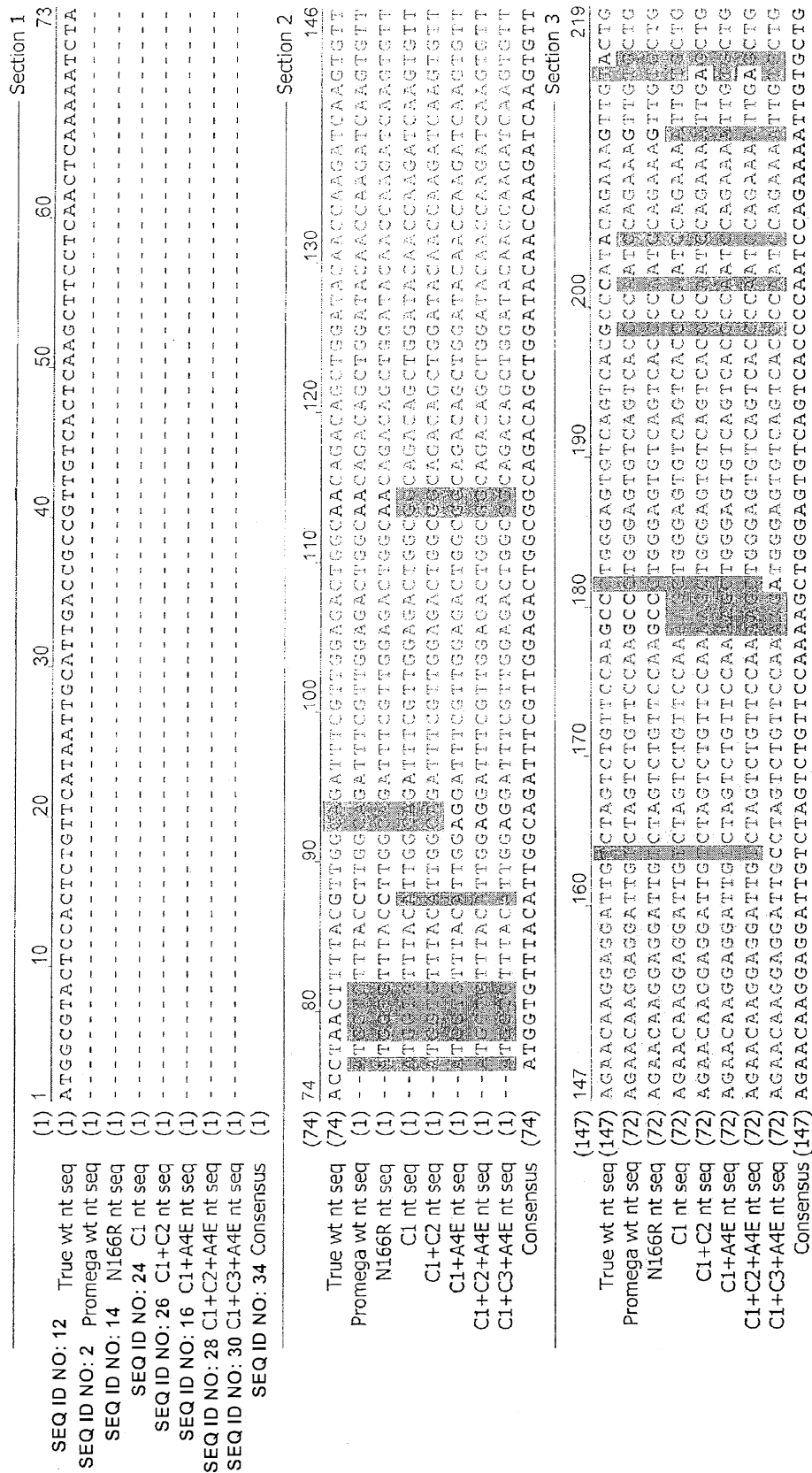


Sample	½ life 50°C (min)
C1A4E	10.1
29H7(C1A4E+F54I)	13.3

FIG. 30

		Section 1									
		1	10	20	30	40	50	60	70		
SEQ ID NO: 10	True wt protein seq	(1)	1								
SEQ ID NO: 13	Promega wt protein seq	(1)	MAYSTLFII	ALTAVV	TQAS	STQKSN	ITFTLL	DFVGDW	QQTAGYN	QDQVLE	QGGLSL
SEQ ID NO: 15	N166R protein seq	(1)	-----	-----	-----	-----	-----	-----	-----	-----	-----
SEQ ID NO: 25	C1 protein seq	(1)	-----	-----	-----	-----	-----	-----	-----	-----	-----
SEQ ID NO: 27	C1+C2 protein seq	(1)	-----	-----	-----	-----	-----	-----	-----	-----	-----
SEQ ID NO: 23	C1+A4E protein seq	(1)	-----	-----	-----	-----	-----	-----	-----	-----	-----
SEQ ID NO: 29	C1+C2+A4E protein seq	(1)	-----	-----	-----	-----	-----	-----	-----	-----	-----
SEQ ID NO: 31	C1+C3+A4E proteins seq	(1)	-----	-----	-----	-----	-----	-----	-----	-----	-----
SEQ ID NO: 33	Consensus	(1)	MVFTL	AD	FV	GD	WR	QT	AGYN	QDQVLE	QGGLSL
Section 2											
		71	80	90	100	110	120	130	140		
True wt protein seq	(71)	71	-----	-----	-----	-----	-----	-----	-----	-----	-----
Promega wt protein seq	(71)	71	LSGEN	GLK	ADIH	VIIP	YEG	LSG	FQMG	LIEM	IFK
N166R protein seq	(46)	71	LSGEN	GLK	ADIH	VIIP	YEG	LSG	FQMG	LIEM	IFK
C1 protein seq	(46)	71	LSGEN	GLK	ADIH	VIIP	YEG	LSG	FQMG	LIEM	IFK
C1+C2 protein seq	(46)	71	LSGEN	GLK	ADIH	VIIP	YEG	LSG	FQMG	LIEM	IFK
C1+A4E protein seq	(46)	71	LSGEN	GLK	ADIH	VIIP	YEG	LSG	FQMG	LIEM	IFK
C1+C2+A4E protein seq	(46)	71	LSGEN	GLK	ADIH	VIIP	YEG	LSG	FQMG	LIEM	IFK
C1+C3+A4E proteins seq	(46)	71	LSGEN	GLK	ADIH	VIIP	YEG	LSG	FQMG	LIEM	IFK
Consensus	(71)	71	IVLS	GEN	GLK	F	DIH	VIIP	YEG	LSG	FQMG
Section 3											
		141	150	160	170	180	190				
True wt protein seq	(141)	141	YPGI	AV	FD	GK	QI	IT	VT	GT	LW
Promega wt protein seq	(141)	141	YPGI	AV	FD	GK	QI	IT	VT	GT	LW
N166R protein seq	(116)	141	YPGI	AV	FD	GK	QI	IT	VT	GT	LW
C1 protein seq	(116)	141	YPGI	AV	FD	GK	QI	IT	VT	GT	LW
C1+C2 protein seq	(116)	141	YPGI	AV	FD	GK	QI	IT	VT	GT	LW
C1+A4E protein seq	(116)	141	YPGI	AV	FD	GK	QI	IT	VT	GT	LW
C1+C2+A4E protein seq	(116)	141	YPGI	AV	FD	GK	QI	IT	VT	GT	LW
C1+C3+A4E proteins seq	(116)	141	YPGI	AV	FD	GK	QI	IT	VT	GT	LW
Consensus	(141)	141	YEGI	AV	PD	GK	KI	IT	VT	GT	LW

FIG. 31



	220	230	240	250	260	270	280	290	Section 4
(220)	220	230	240	250	260	270	280	292	
True wt nt seq (220)	TCTGGGAGAGATG	GTTAAAGCTGATAT	TGATATTCATAT	TGATATTCATAT	TGATATTCATAT	TGATATTCATAT	TGATATTCATAT	TGATATTCATAT	
Promega wt nt seq (145)	TCTGGGAGAGATG	GTTAAAGCTGATAT	TGATATTCATAT	TGATATTCATAT	TGATATTCATAT	TGATATTCATAT	TGATATTCATAT	TGATATTCATAT	
N166R nt seq (145)	TCTGGGAGAGATG	GTTAAAGCTGATAT	TGATATTCATAT	TGATATTCATAT	TGATATTCATAT	TGATATTCATAT	TGATATTCATAT	TGATATTCATAT	
C1 nt seq (145)	TCTGGGAGAGATG	GTTAAAGCTGATAT	TGATATTCATAT	TGATATTCATAT	TGATATTCATAT	TGATATTCATAT	TGATATTCATAT	TGATATTCATAT	
C1+C2 nt seq (145)	TCTGGGAGAGATG	GTTAAAGCTGATAT	TGATATTCATAT	TGATATTCATAT	TGATATTCATAT	TGATATTCATAT	TGATATTCATAT	TGATATTCATAT	
C1+A4E nt seq (145)	TCTGGGAGAGATG	GTTAAAGCTGATAT	TGATATTCATAT	TGATATTCATAT	TGATATTCATAT	TGATATTCATAT	TGATATTCATAT	TGATATTCATAT	
C1+C2+A4E nt seq (145)	TCTGGGAGAGATG	GTTAAAGCTGATAT	TGATATTCATAT	TGATATTCATAT	TGATATTCATAT	TGATATTCATAT	TGATATTCATAT	TGATATTCATAT	
C1+C3+A4E nt seq (145)	TCTGGGAGAGATG	GTTAAAGCTGATAT	TGATATTCATAT	TGATATTCATAT	TGATATTCATAT	TGATATTCATAT	TGATATTCATAT	TGATATTCATAT	
Consensus (220)	TCTGGGAGAGATG	GTTAAAGCTGATAT	TGATATTCATAT	TGATATTCATAT	TGATATTCATAT	TGATATTCATAT	TGATATTCATAT	TGATATTCATAT	
(293)	293	300	310	320	330	340	350	355	Section 5
True wt nt seq (293)	GTCTAATTGAAATG	ATCTTCAAAAGT	TTGTTTACCCCGT	GGATGATCAT	CAATTTCAAGATT	ATTCCTCCATTAT	GTCTCCATTAT	GTCTCCATTAT	
Promega wt nt seq (218)	GTCTAATTGAAATG	ATCTTCAAAAGT	TTGTTTACCCCGT	GGATGATCAT	CAATTTCAAGATT	ATTCCTCCATTAT	GTCTCCATTAT	GTCTCCATTAT	
N166R nt seq (218)	GTCTAATTGAAATG	ATCTTCAAAAGT	TTGTTTACCCCGT	GGATGATCAT	CAATTTCAAGATT	ATTCCTCCATTAT	GTCTCCATTAT	GTCTCCATTAT	
C1 nt seq (218)	GTCTAATTGAAATG	ATCTTCAAAAGT	TTGTTTACCCCGT	GGATGATCAT	CAATTTCAAGATT	ATTCCTCCATTAT	GTCTCCATTAT	GTCTCCATTAT	
C1+C2 nt seq (218)	GTCTAATTGAAATG	ATCTTCAAAAGT	TTGTTTACCCCGT	GGATGATCAT	CAATTTCAAGATT	ATTCCTCCATTAT	GTCTCCATTAT	GTCTCCATTAT	
C1+A4E nt seq (218)	GTCTAATTGAAATG	ATCTTCAAAAGT	TTGTTTACCCCGT	GGATGATCAT	CAATTTCAAGATT	ATTCCTCCATTAT	GTCTCCATTAT	GTCTCCATTAT	
C1+C2+A4E nt seq (218)	GTCTAATTGAAATG	ATCTTCAAAAGT	TTGTTTACCCCGT	GGATGATCAT	CAATTTCAAGATT	ATTCCTCCATTAT	GTCTCCATTAT	GTCTCCATTAT	
C1+C3+A4E nt seq (218)	GTCTAATTGAAATG	ATCTTCAAAAGT	TTGTTTACCCCGT	GGATGATCAT	CAATTTCAAGATT	ATTCCTCCATTAT	GTCTCCATTAT	GTCTCCATTAT	
Consensus (293)	GTCTAATTGAAATG	ATCTTCAAAAGT	TTGTTTACCCCGT	GGATGATCAT	CAATTTCAAGATT	ATTCCTCCATTAT	GTCTCCATTAT	GTCTCCATTAT	
(366)	366	380	390	400	410	420	430	438	Section 6
True wt nt seq (366)	TACACTCGTTT	TTGACGGTGT	TACAC	CAACATGAT	TGACTAC	TTTGGAGA	GCCTTAC	CCCTGGAA	TTGCTGT
Promega wt nt seq (291)	TACACTCGTTT	TTGACGGTGT	TACAC	CAACATGAT	TGACTAC	TTTGGAGA	GCCTTAC	CCCTGGAA	TTGCTGT
N166R nt seq (291)	TACACTCGTTT	TTGACGGTGT	TACAC	CAACATGAT	TGACTAC	TTTGGAGA	GCCTTAC	CCCTGGAA	TTGCTGT
C1 nt seq (291)	TACACTCGTTT	TTGACGGTGT	TACAC	CAACATGAT	TGACTAC	TTTGGAGA	GCCTTAC	CCCTGGAA	TTGCTGT
C1+C2 nt seq (291)	TACACTCGTTT	TTGACGGTGT	TACAC	CAACATGAT	TGACTAC	TTTGGAGA	GCCTTAC	CCCTGGAA	TTGCTGT
C1+A4E nt seq (291)	TACACTCGTTT	TTGACGGTGT	TACAC	CAACATGAT	TGACTAC	TTTGGAGA	GCCTTAC	CCCTGGAA	TTGCTGT
C1+C2+A4E nt seq (291)	TACACTCGTTT	TTGACGGTGT	TACAC	CAACATGAT	TGACTAC	TTTGGAGA	GCCTTAC	CCCTGGAA	TTGCTGT
C1+C3+A4E nt seq (291)	TACACTCGTTT	TTGACGGTGT	TACAC	CAACATGAT	TGACTAC	TTTGGAGA	GCCTTAC	CCCTGGAA	TTGCTGT
Consensus (366)	TACACTCGTTT	TTGACGGTGT	TACAC	CAACATGAT	TGACTAC	TTTGGAGA	GCCTTAC	CCCTGGAA	TTGCTGT

FIG. 32 Cont'd

Section 7									
(439)	439	450	460	470	480	490	500	511	
True wt nt seq (439)	TTTGACGGCAAGCAGATCACAGTTACTGGAACTCTGTGGAACGGCAA	AAAGATCTATCA	GAGAGGCTATCA						
Promega wt nt seq (364)	TTTGACGGCAAGCAGATCACAGTTACTGGAACTCTGTGGAACGGCAA	AAAGATCTATCA	GAGAGGCTATCA						
N166R nt seq (364)	TTTGACGGCAAGCAGATCACAGTTACTGGAACTCTGTGGAACGGCAA	AAAGATCTATCA	GAGAGGCTATCA						
C1 nt seq (364)	TTTGACGGCAAGCAGATCACAGTTACTGGAACTCTGTGGAACGGCAA	AAAGATCTATCA	GAGAGGCTATCA						
C1+C2 nt seq (364)	TTTGACGGCAAGCAGATCACAGTTACTGGAACTCTGTGGAACGGCAA	AAAGATCTATCA	GAGAGGCTATCA						
C1+A4E nt seq (364)	TTTGACGGCAAGCAGATCACAGTTACTGGAACTCTGTGGAACGGCAA	AAAGATCTATCA	GAGAGGCTATCA						
C1+C2+A4E nt seq (364)	TTTGACGGCAAGCAGATCACAGTTACTGGAACTCTGTGGAACGGCAA	AAAGATCTATCA	GAGAGGCTATCA						
C1+C3+A4E nt seq (364)	TTTGACGGCAAGCAGATCACAGTTACTGGAACTCTGTGGAACGGCAA	AAAGATCTATCA	GAGAGGCTATCA						
Consensus (439)	TTTGACGGCAAGCAGATCACAGTTACTGGAACTCTGTGGAACGGCAA	AAAGATCTATCA	GAGAGGCTATCA						
Section 8									
(512)	512	520	530	540	550	560	570	584	
True wt nt seq (512)	ACCCGTGATGGTTCACTCCTCTCTTCAGAGTTACTCAATGAGTCAACGGGATGGAGG	CITTGCGAGAACATTTCT							
Promega wt nt seq (437)	ACCCGTGATGGTTCACTCCTCTCTTCAGAGTTACTCAATGAGTCAACGGGATGGAGG	CITTGCGAGAACATTTCT							
N166R nt seq (437)	ACCCGTGATGGTTCACTCCTCTCTTCAGAGTTACTCAATGAGTCAACGGGATGGAGG	CITTGCGAGAACATTTCT							
C1 nt seq (437)	ACCCGTGATGGTTCACTCCTCTCTTCAGAGTTACTCAATGAGTCAACGGGATGGAGG	CITTGCGAGAACATTTCT							
C1+C2 nt seq (437)	ACCCGTGATGGTTCACTCCTCTCTTCAGAGTTACTCAATGAGTCAACGGGATGGAGG	CITTGCGAGAACATTTCT							
C1+A4E nt seq (437)	ACCCGTGATGGTTCACTCCTCTCTTCAGAGTTACTCAATGAGTCAACGGGATGGAGG	CITTGCGAGAACATTTCT							
C1+C2+A4E nt seq (437)	ACCCGTGATGGTTCACTCCTCTCTTCAGAGTTACTCAATGAGTCAACGGGATGGAGG	CITTGCGAGAACATTTCT							
C1+C3+A4E nt seq (437)	ACCCGTGATGGTTCACTCCTCTCTTCAGAGTTACTCAATGAGTCAACGGGATGGAGG	CITTGCGAGAACATTTCT							
Consensus (512)	ACCCGTGATGGTTCACTCCTCTCTTCAGAGTTACTCAATGAGTCAACGGGATGGAGG	CITTGCGAGAACATTTCT							
Section 9									
(585)	585	588							
True wt nt seq (585)	TGCC								
Promega wt nt seq (510)	TGCC								
N166R nt seq (510)	TGCC								
C1 nt seq (510)	TGCC								
C1+C2 nt seq (510)	TGCC								
C1+A4E nt seq (510)	TGCC								
C1+C2+A4E nt seq (510)	TGCC								
C1+C3+A4E nt seq (510)	TGCC								
Consensus (585)	TGCC								

FIG. 32 Cont'd

Mutation	Fold over N166R
T2T	0.6
T2T+N166R	1.8
A4E	0.5
Q11R	0.7
V44I	2.3
A54F	1.5
A54F+N166R	14.3
A54I	5.8
P115E	1.3
P115E+N166R	2.9
Y138I	2.6
Q124K	1.0
Y138C+N166R	2.8
I90V	0.8
A33K	0.6
F68Y	0.7
N166R+ A33K	2.0
N166R +F68Y	1.3

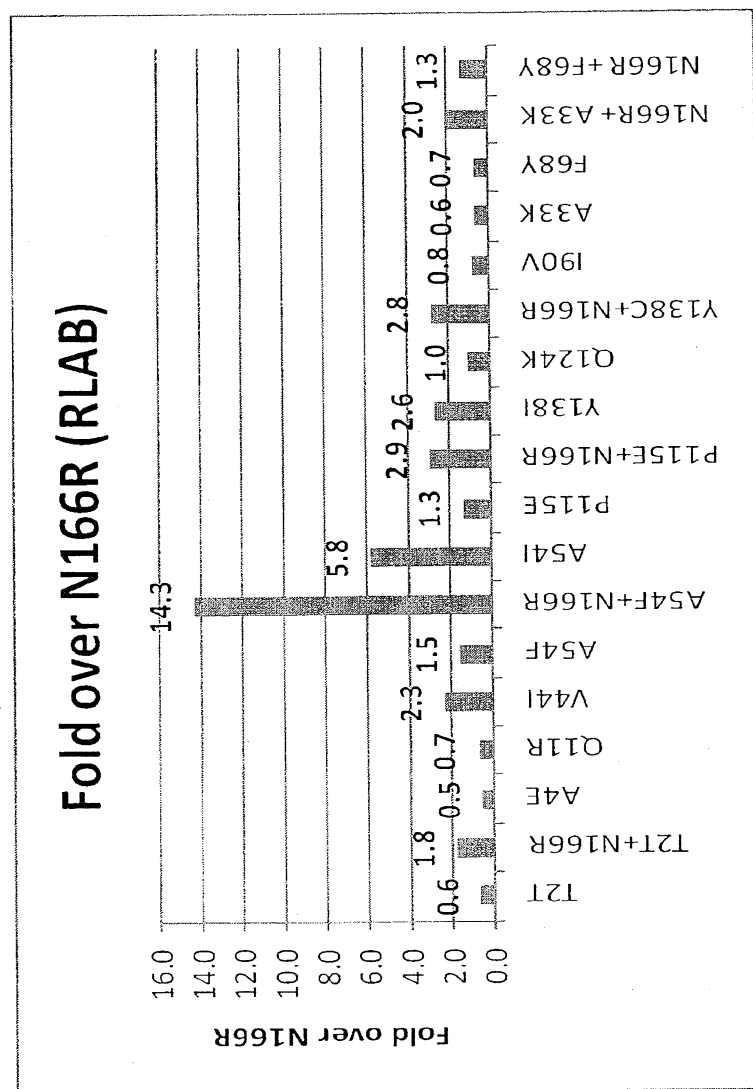


FIG. 33A

Mutation	Fold over N166R
T2T	1.6
T2T+N166R	2.3
A4E	1.2
Q11R	1.1
V44I	13.4
A54F	9.7
A54F+N166R	50.2
A54I	97.8
P115E	2.5
P115E+N166R	3.1
Y138I	7.0
Q124K	2.0
Y138C+N166R	3.7
I90V	2.4
A33K	2.6
F68Y	2.7
N166R+ A33K	6.3
N166R +F68Y	3.4

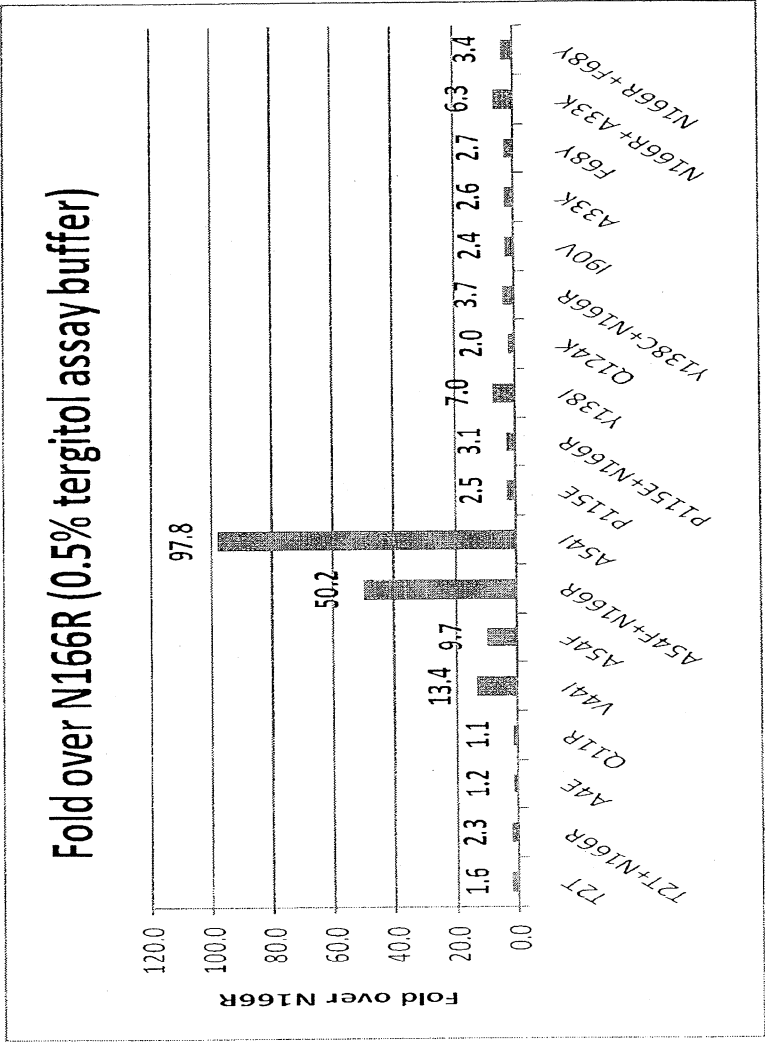


FIG. 33B

Signal 1/2 life
(minutes) RLAB
normalized by
N166R

Sample	
T2T	1.2
T2T+N166R	1.1
A4E	1.4
Q11R	1.1
V44I	1.0
A54F	0.8
A54F+N166R	0.9
A54I	1.3
P115E	1.0
P115E+N166R	1.0
Y138I	1.0
Q124K	1.1
Y138C+N166R	0.6
I90V	1.1
A33K	1.4
F68Y	1.2
N166R/A33K	1.0
N166R/F68Y	1.0

Signal stability 0.5%
tergitol buffer
normalized by N166R

Sample	
T2T	0.9
T2T+N166R	1.3
A4E	0.9
Q11R	0.8
V44I	0.8
A54F	0.8
A54F+N166R	1.1
A54I	0.7
P115E	1.2
P115E+N166R	1.9
Y138I	0.9
Q124K	1.0
Y138C+N166R	1.5
I90V	0.9
A33K	0.8
F68Y	0.9
N166R/A33K	1.0
N166R/F68Y	1.0

FIG. 33C

Stability at 22°C normalized by N166R

58/61

Sample	stability (1/2 life at 22°C) normalized to N166R
T2T	0.7
T2T+N166R	1.1
A4E	0.6
Q11R	0.7
V44I	0.8
A54F	0.9
A54F+N166R	2.9
A54I	1.0
P115E	0.8
P115E+N166R	1.9
Y138I	0.9
Q124K	0.7
Y138C+N166R	0.6
I90V	0.7

FIG. 33D

Summary

Sample	Signal 1/2		Signal 1/2 life		Fold over		stability (1/2	
	life (minutes)	0.5% tergitol buffer normalized by N166R	(minutes)	RLAB normalized by N166R	tergitol assay buffer	N166R (RLAB)	life at 22°C normalized to N166R	
T2T		0.9		1.2	1.6	0.6		0.7
T2T+N166R		1.3		1.1	2.3	1.8		1.1
A4E		0.9		1.4	1.2	0.5		0.6
Q11R		0.8		1.1	1.1	0.7		0.7
V44I		0.8		1.0	13.4	2.3		0.8
A54F		0.8		0.8	9.7	1.5		0.9
A54F+N166R		1.1		0.9	50.2	14.3		2.9
A54I		0.7		1.3	97.8	5.8		1.0
P115E		1.2		1.0	2.5	1.3		0.8
P115E+N166R		1.9		1.0	3.1	2.9		1.9
Y138I		0.9		1.0	7.0	2.6		0.9
Q124K		1.0		1.1	2.0	1.0		0.7
Y138C+N166R		1.5		0.6	3.7	2.8		0.6
I90V		0.9		1.1	2.4	0.8		0.7
A33K		0.8		1.4	2.6	0.6		0.9
F68Y		0.9		1.2	2.7	0.7		0.9
N166R+ A33K		1.0		1.0	6.3	2.0		1.1
N166R +F68Y		1.0		1.0	3.4	1.3		1.2

FIG. 33E

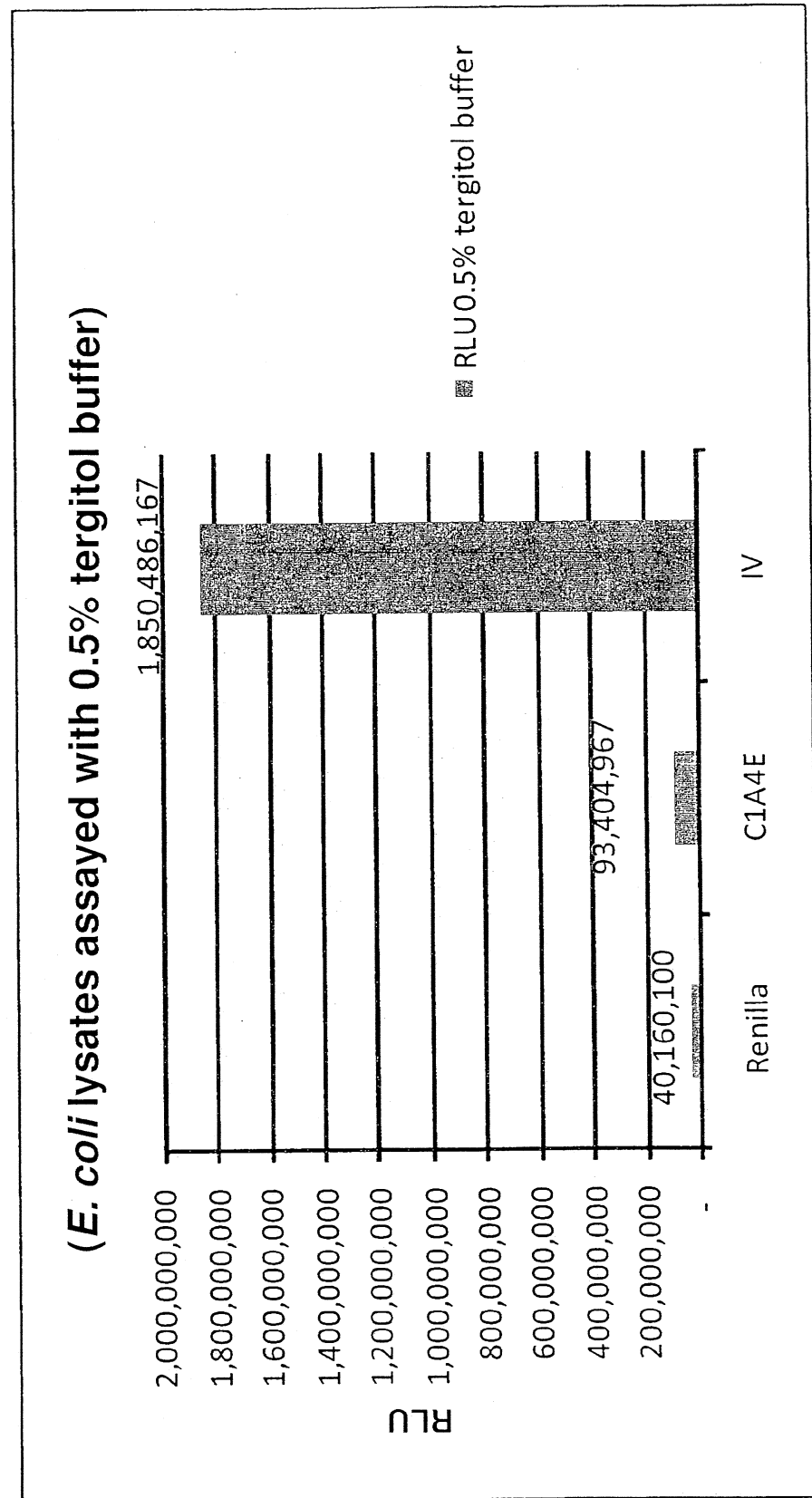
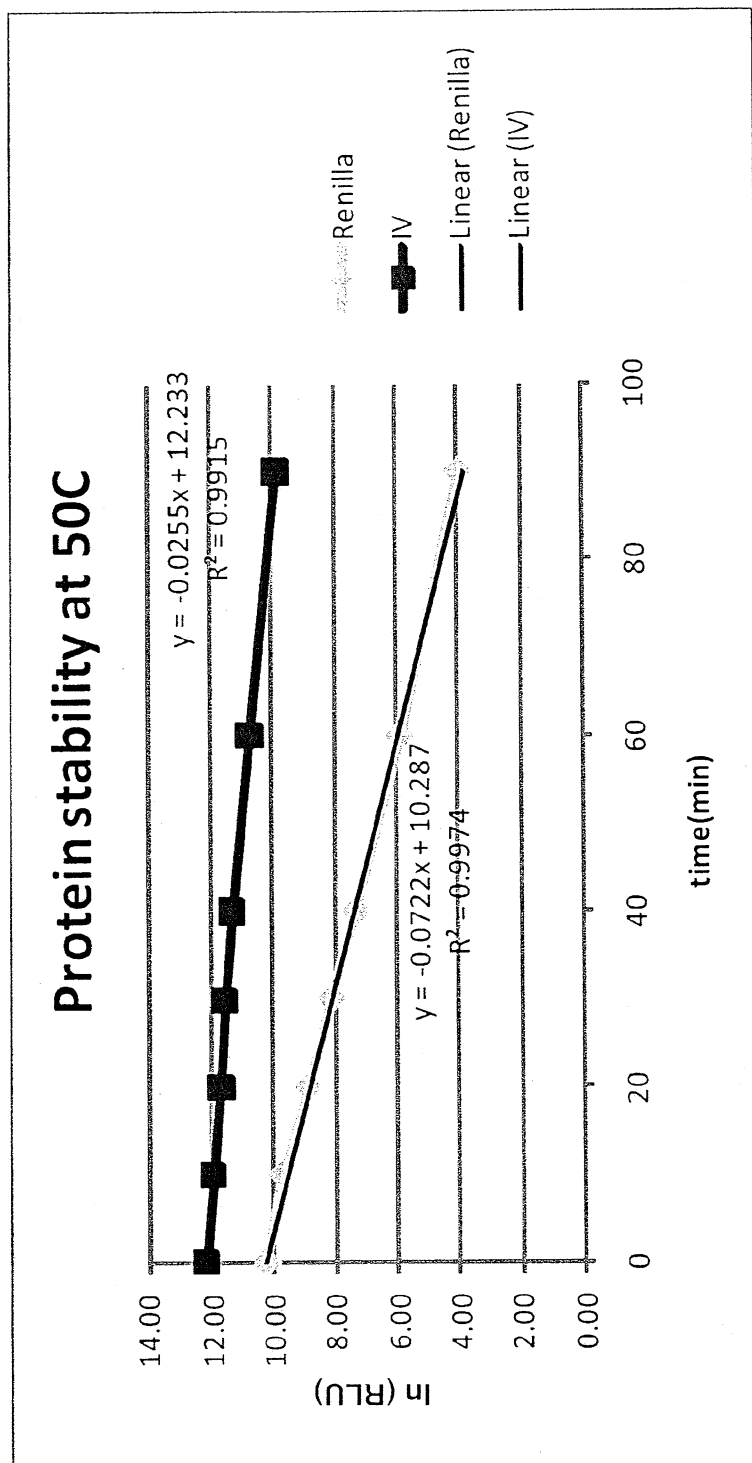


FIG. 34A



Sample 1/2 life (min)

Renilla	9.6
IV	27.2

FIG. 34B