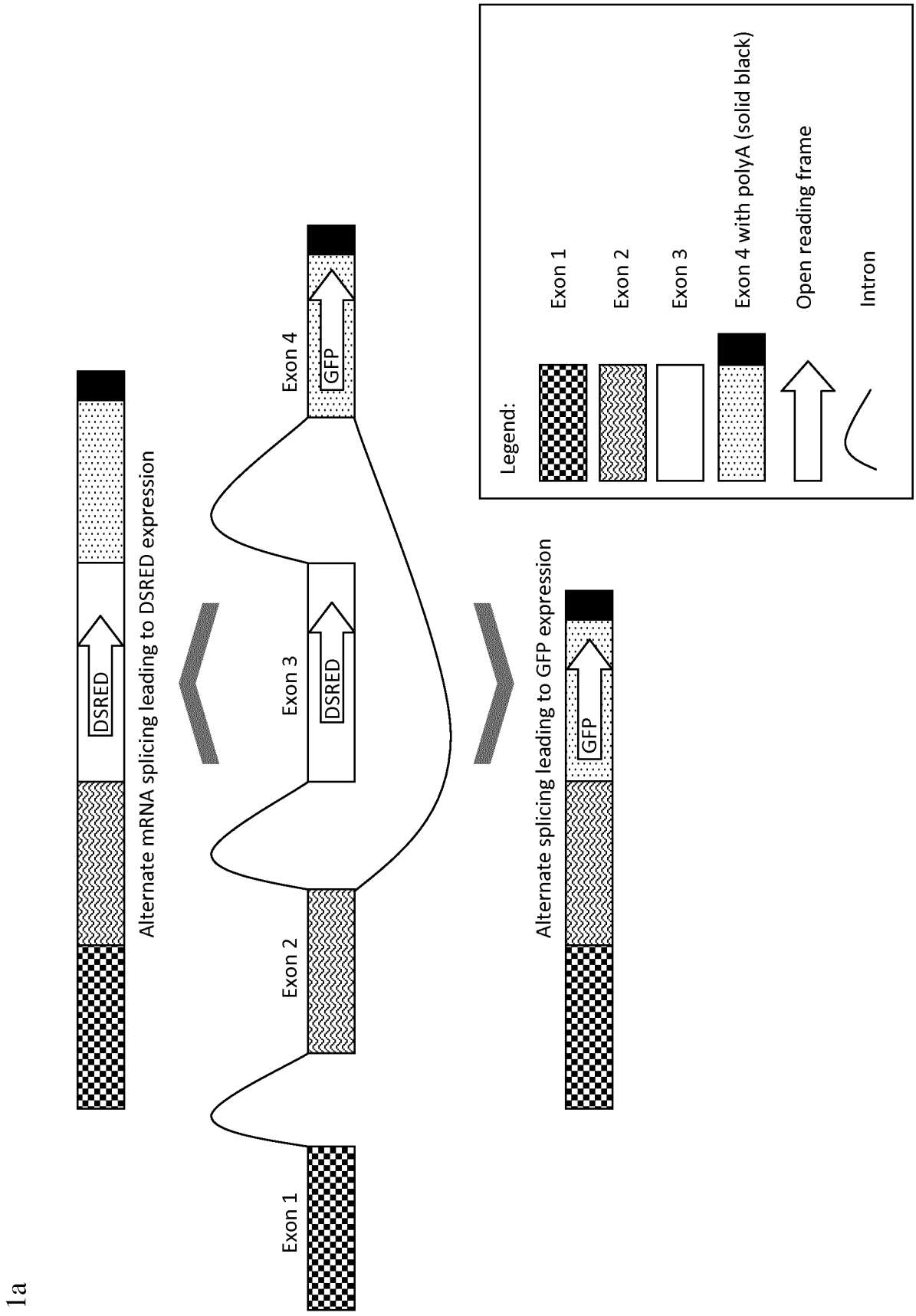

Legend:
Exon 1
Exon 2
Exon 3
Exon 4 with polyA (solid black)
Open reading frame
Intron
Exon 1
Exon 2
Exon 3
DSRED
Exon 4
GFP
Alternate mRNA splicing leading to DSRED expression
Alternate splicing leading to GFP expression
DSRED
GFP

Figure 1a

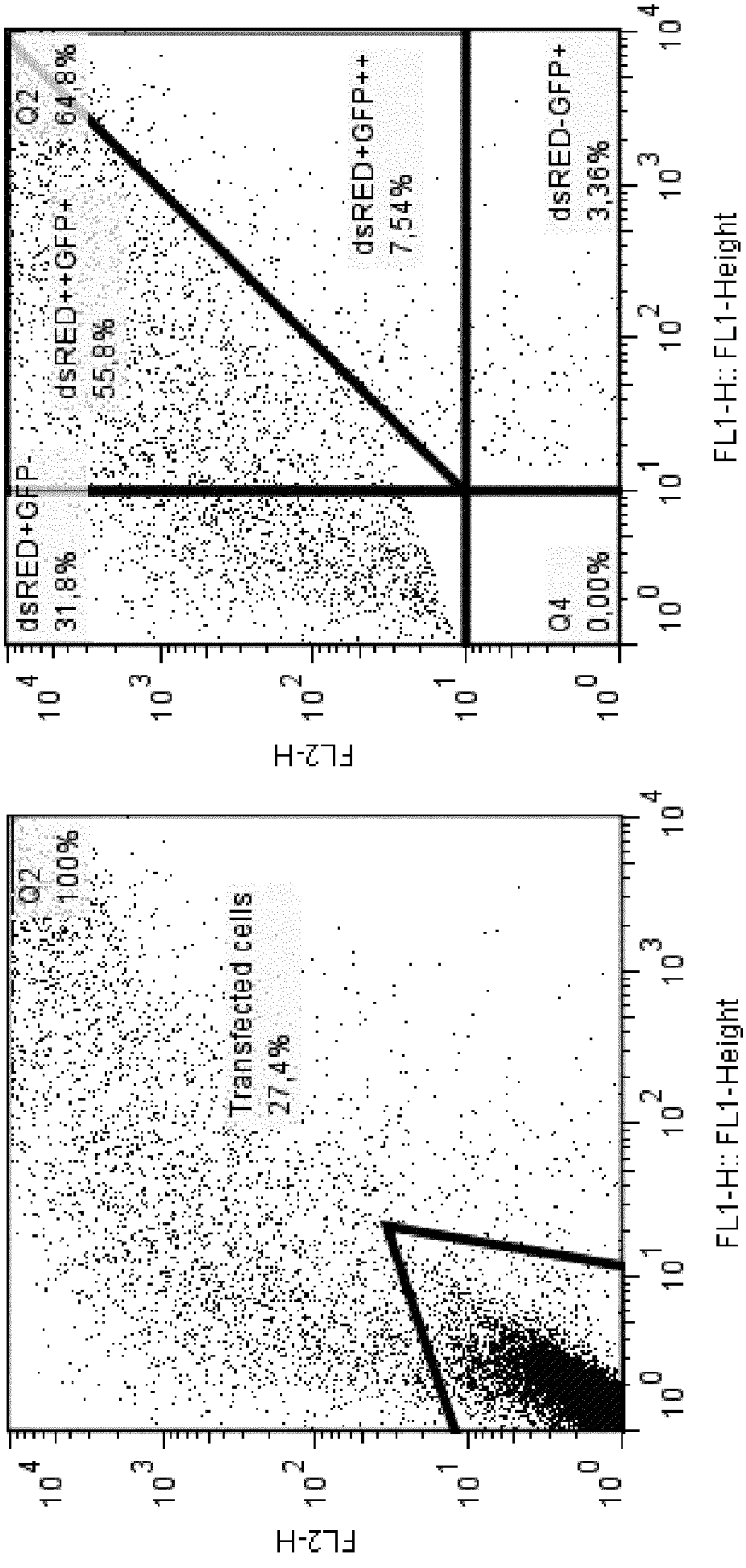

dsRED+GFP-
31,8%
dsRED++GFP+
55,8%
Q2
64,8%
dsRED+GFP++
7,54%
Q4
0,00%
dsRED-GFP+
3,36%
FL2-H
FL1-H: FL1-Height
Q2
100%
Transfected cells
27,4%
FL2-H
FL1-H: FL1-Height

Figure 1b

Figure 2a

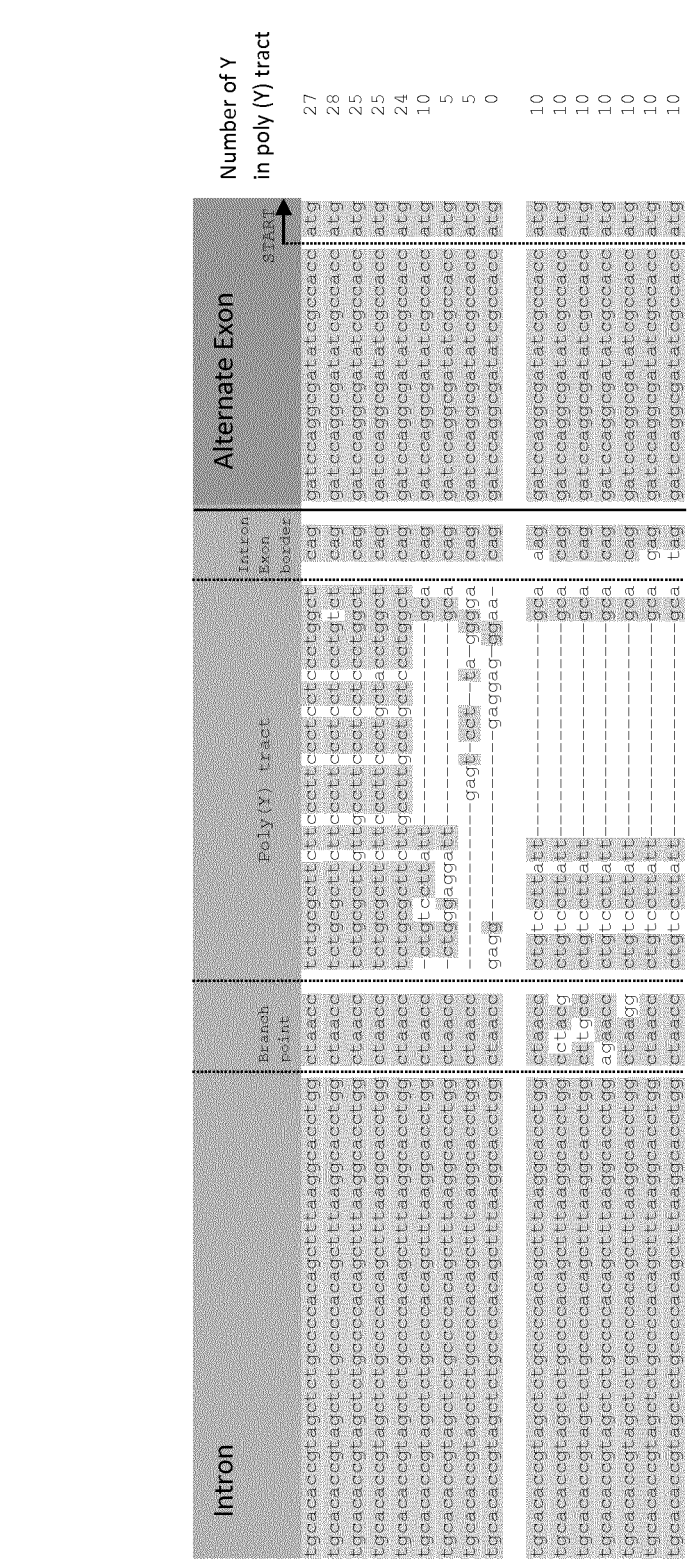
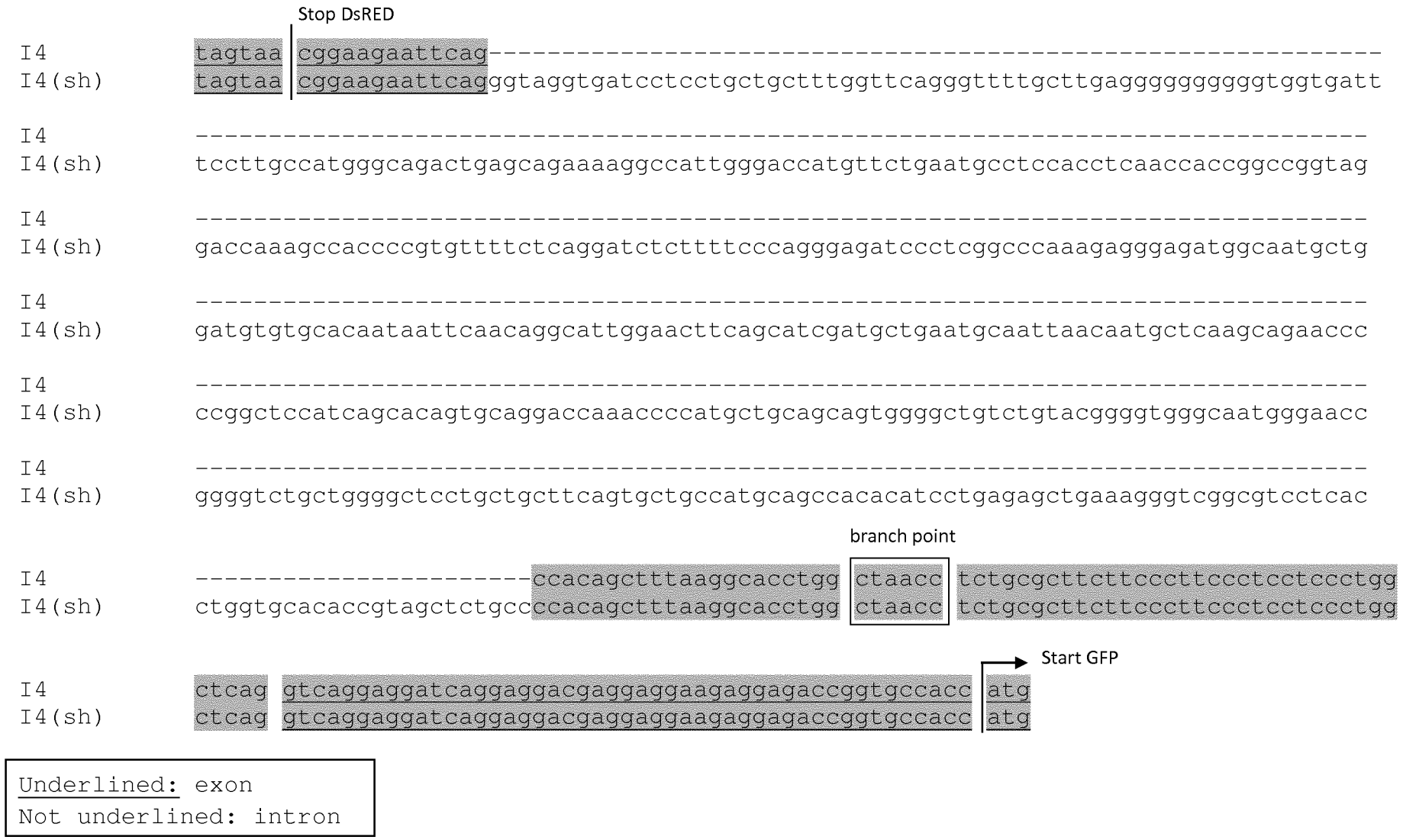


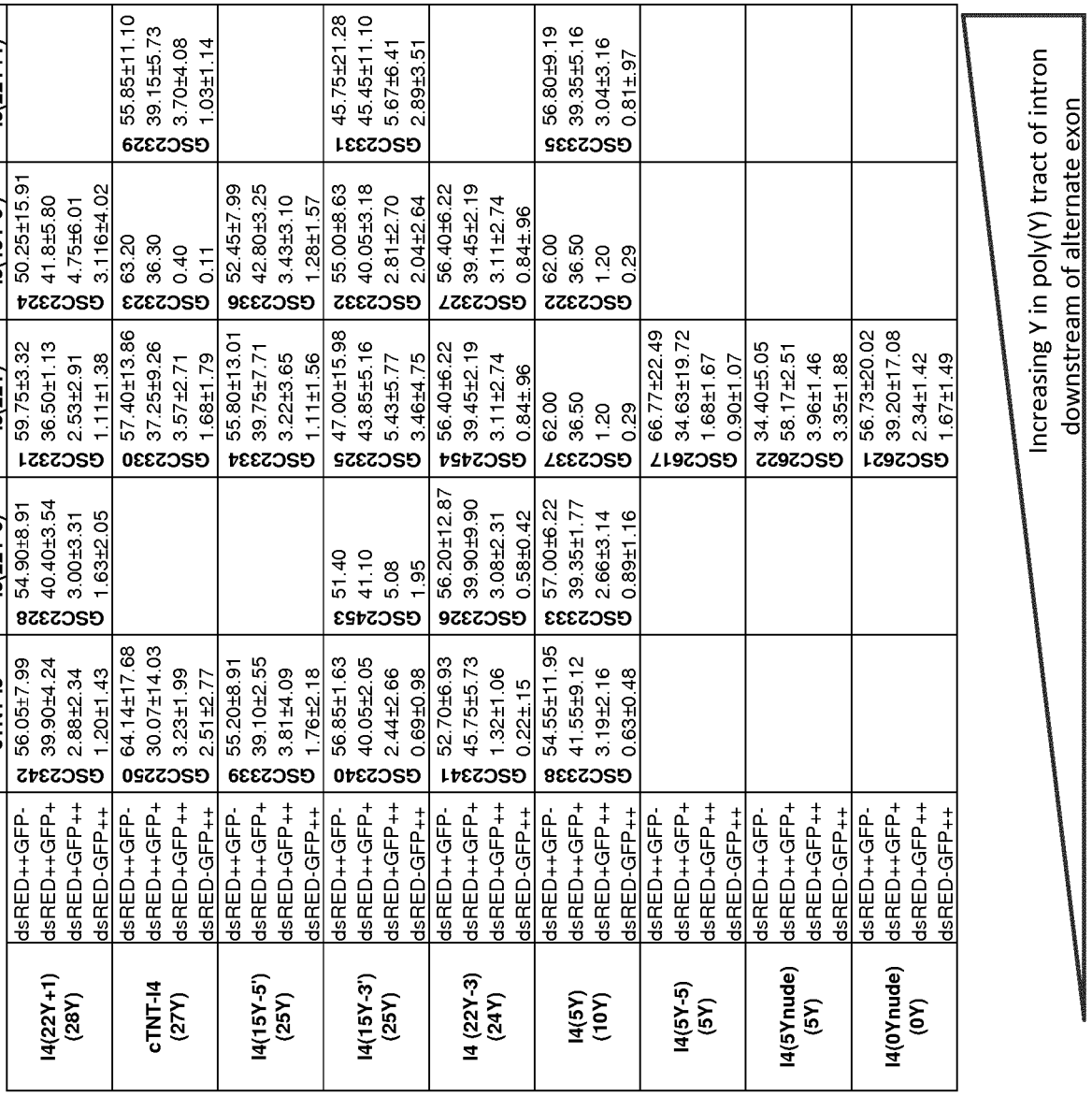
Figure 2b

Figure 2c



Underlined: exon
Not underlined: intron

Figure 3a



		cTNT-I5	I5(22Y-3)	I5(22Y)	I5(15Y-3')	I5(22Y+1)
		GSC2342	GSC2328	GSC2321	GSC2324	
I4(22Y+1) (28Y)	dsRED++GFP-	56.05±7.99	54.90±8.91	59.75±3.32	50.25±15.91	
	dsRED++GFP+	39.90±4.24	40.40±3.54	36.50±1.13	41.8±5.80	
	dsRED+GFP++	2.88±2.34	3.00±3.31	2.53±2.91	4.75±6.01	
	dsRED-GFP++	1.20±1.43	1.63±2.05	1.11±1.38	3.116±4.02	
		GSC2250		GSC2330	GSC2323	GSC2329
cTNT-I4 (27Y)	dsRED++GFP-	64.14±17.68		57.40±13.86	63.20	55.85±11.10
	dsRED++GFP+	30.07±14.03		37.25±9.26	36.30	39.15±5.73
	dsRED+GFP++	3.23±1.99		3.57±2.71	0.40	3.70±4.08
	dsRED-GFP++	2.51±2.77		1.68±1.79	0.11	1.03±1.14
		GSC2339		GSC2334	GSC2336	GSC2331
I4(15Y-5') (25Y)	dsRED++GFP-	55.20±8.91		55.80±13.01	52.45±7.99	45.75±21.28
	dsRED++GFP+	39.10±2.55		39.75±7.71	42.80±3.25	45.45±11.10
	dsRED+GFP++	3.81±4.09		3.22±3.65	3.43±3.10	5.67±6.41
	dsRED-GFP++	1.76±2.18		1.11±1.56	1.28±1.57	2.89±3.51
		GSC2340	GSC2453	GSC2325	GSC2332	
I4(15Y-3') (25Y)	dsRED++GFP-	56.85±1.63	51.40	47.00±15.98	55.00±8.63	
	dsRED++GFP+	40.05±2.05	41.10	43.85±5.16	40.05±3.18	
	dsRED+GFP++	2.44±2.66	5.08	5.43±5.77	2.81±2.70	
	dsRED-GFP++	0.69±0.98	1.95	3.46±4.75	2.04±2.64	
		GSC2341	GSC2326	GSC2454	GSC2327	
I4 (22Y-3) (24Y)	dsRED++GFP-	52.70±6.93	56.20±12.87	56.40±6.22	56.40±6.22	
	dsRED++GFP+	45.75±5.73	39.90±9.90	39.45±2.19	39.45±2.19	
	dsRED+GFP++	1.32±1.06	3.08±2.31	3.11±2.74	3.11±2.74	
	dsRED-GFP++	0.22±.15	0.58±0.42	0.84±.96	0.84±.96	
		GSC2338	GSC2333	GSC2337	GSC2322	GSC2335
I4(5Y) (10Y)	dsRED++GFP-	54.55±11.95	57.00±6.22	62.00	62.00	56.80±9.19
	dsRED++GFP+	41.55±9.12	39.35±1.77	36.50	36.50	39.35±5.16
	dsRED+GFP++	3.19±2.16	2.66±3.14	1.20	1.20	3.04±3.16
	dsRED-GFP++	0.63±0.48	0.89±1.16	0.29	0.29	0.81±.97
				GSC2617		
I4(5Y-5) (5Y)	dsRED++GFP-			66.77±22.49		
	dsRED++GFP+			34.63±19.72		
	dsRED+GFP++			1.68±1.67		
	dsRED-GFP++			0.90±1.07		
				GSC2622		
I4(5Ynude) (5Y)	dsRED++GFP-			34.40±5.05		
	dsRED++GFP+			58.17±2.51		
	dsRED+GFP++			3.96±1.46		
	dsRED-GFP++			3.35±1.88		
				GSC2621		
I4(0Ynude) (0Y)	dsRED++GFP-			56.73±20.02		
	dsRED++GFP+			39.20±17.08		
	dsRED+GFP++			2.34±1.42		
	dsRED-GFP++			1.67±1.49		

Controls	
GFP	0.00±0.00
	0.00±0.00
	2.75±4.09
	97.29±4.05
dsRED	96.46±6.10
	0.75±1.01
	0.27±0.73
	2.53±6.26

Figure 3b

		cTNT-I5	I5(22Y-3)	I5(22Y)	I5(15Y-3')	I5(22Y+1)
		GSC2342	GSC2328	GSC2321	GSC2324	
I4(22Y+1) (28Y)	dsRED++GFP-	48.50	54.30	53.40	49.90	
	dsRED++GFP+	44.20	41.30	43.40	43.60	
	dsRED+GFP++	2.92	2.72	2.10	3.67	
	dsRED-GFP++	4.74	1.63	1.08	2.82	
		GSC2250		GSC2330	GSC2323	GSC2329
I4(22Y) (27Y)	dsRED++GFP-	58.70±1.75		51.40	53.20	46.30
	dsRED++GFP+	36.43±10.05		40.20	43.50	46.80
	dsRED+GFP++	2.60±1.00		4.51	1.91	3.86
	dsRED-GFP++	1.68±1.73		3.92	1.30	2.93
		GSC2339		GSC2334	GSC2336	GSC2331
I4(15Y-5') (25Y)	dsRED++GFP-	53.60		47.00	47.80	
	dsRED++GFP+	39.10		45.90	46.20	
	dsRED+GFP++	3.86		4.20	2.83	
	dsRED-GFP++	3.43		2.55	3.14	
		GSC2340	GSC2453	GSC2325	GSC2332	GSC2331
I4(15Y-3') (25Y)	dsRED++GFP-	56.40	N/A	38.80	50.10	28.90
	dsRED++GFP+	39.40	N/A	53.30	42.30	53.00
	dsRED+GFP++	2.77	N/A	4.21	4.43	8.70
	dsRED-GFP++	1.50	N/A	4.21	2.84	9.12
		GSC2341	GSC2326	GSC2454	GSC2327	
I4 (22Y-3) (24Y)	dsRED++GFP-	46.30	46.90	N/A	55.40	
	dsRED++GFP+	44.30	47.70	N/A	37.60	
	dsRED+GFP++	4.70	2.86	N/A	3.49	
	dsRED-GFP++	4.70	2.29	N/A	3.66	
		GSC2338	GSC2333	GSC2337	GSC2322	GSC2335
I4(5Y) (10Y)	dsRED++GFP-	50.30	53.70	44.00	56.40	50.10
	dsRED++GFP+	41.90	42.20	47.40	40.90	41.70
	dsRED+GFP++	4.79	2.24	4.78	1.78	6.04
	dsRED-GFP++	2.99	1.68	3.83	0.89	2.36
				GSC2617		
I4(5Y-5) (5Y)	dsRED++GFP-			45.77±7.69		
	dsRED++GFP+			49.50±6.84		
	dsRED+GFP++			2.63±0.32		
	dsRED-GFP++			1.98±0.81		
				GSC2622		
I4(5Ynude) (5Y)	dsRED++GFP-			38.02±7.44		
	dsRED++GFP+			55.15±5.52		
	dsRED+GFP++			3.63±1.02		
	dsRED-GFP++			3.05±1.47		
				GSC2621		
I4(0Ynude) (0Y)	dsRED++GFP-			42.56±8.72		
	dsRED++GFP+			53.00±9.33		
	dsRED+GFP++			2.35±1.57		
	dsRED-GFP++			2.06±1.56		

Controls	
GFP	0.00±0.00
	0.10±0.37
	1.63±2.78
	98.13±2.92
dsRED	95.83±3.31
	2.94±2.05
	0.15±0.47
	1.07±2.43

Decreasing Y in poly(Y) tract in intron upstream of alternate exon

Increasing Y in poly(Y) tract of intron downstream of alternate exon

Branch point and intron-exon border sequence	dsRED++GFP-	95.40±1.13	95.00	94.80±1.41	
	dsRED++GFP+	4.45±1.11	4.82	4.98±1.51	
	dsRED+GFP++	0.14±0.02	0.11	0.15±0.05	
	dsRED-GFP++	0.024±0.00	0.06	0.06±0.02	
		I4(5Y,G) || cTNT-I5(22Y)	I4(5Y,b-ct) || cTNT-I5(22Y)	I4(5Y,b-y) || cTNT-I5(22Y)	
		GSC2613	GSC2615	GSC2618	
Introns arrangements	dsRED++GFP-	63.98±21.66	78.35±0.92	64.13±17.68	58.58±19.74
	dsRED++GFP+	28.30±14.09	19.30±0.42	30.07±14.03	33.52±14.50
	dsRED+GFP++	5.77±5.76	1.31±0.18	3.23±1.99	4.20±2.74
	dsRED-GFP++	1.78±1.63	0.94±0.57	2.51±2.77	3.50±3.26
		GSC2614	GSC2741	GSC2250	GSC2619
		cTNT-I5 || cTNT-I5	cTNT-I5 || cTNT-I4	cTNT-I4 || cTNT-I5	cTNT-I4 || cTNT-I4
Controls	dsRED++GFP-	0.00±0.00	96.46±6.10		
	dsRED++GFP+	0.00±0.00	0.75±1.01		
	dsRED+GFP++	2.75±4.09	0.27±0.73		
	dsRED-GFP++	97.29±4.05	2.53±6.26		
		GFP	dsRED		

Figure 4a

Branch point and intron-exon border sequence	dsRED++GFP-	75.73±3.17	71.95±2.78	76.37±1.72	
	dsRED++GFP+	23.25±3.21	26.35±2.83	22.77±2.48	
	dsRED+GFP++	0.75±0.20	1.16±0.30	0.64±0.56	
	dsRED-GFP++	0.14±0.13	0.26±0.28	0.18±0.31	
		I4(5Y,G) || cTNT-I5(22Y)	I4(5Y,b-ct) || cTNT-I5(22Y)	I4(5Y,b-y) || cTNT-I5(22Y)	
		GSC2613	GSC2615	GSC2618	
Intron arrangements	dsRED++GFP-	43.63±9.67	61.00±1.76	58.70±1.75	45.28±7.36
	dsRED++GFP+	46.12±8.92	36.00±1.57	36.43±10.05	45.90±4.81
	dsRED+GFP++	8.48±3.46	1.76±0.04	2.60±1.00	5.47±2.40
	dsRED-GFP++	1.14±0.99	0.80±0.51	1.68±1.73	1.90±1.39
		GSC2614	GSC2741	GSC2250	GSC2619
		cTNT-I5 || cTNT-I5	cTNT-I5 || cTNT-I4	cTNT-I4 || cTNT-I5	cTNT-I4 || cTNT-I4
Controls	dsRED++GFP-	0.00±0.00	95.83±3.31		
	dsRED++GFP+	0.10±0.37	2.94±2.05		
	dsRED+GFP++	1.63±2.78	0.15±0.47		
	dsRED-GFP++	98.13±2.92	1.07±2.43		
		GFP	dsRED		

Figure 4b

Reduction in poly(Y)	dsRED++GFP-	58.58±19.74	35.75±28.07	8.44±0.52		24.80±3.82
	dsRED++GFP+	33.52±14.50	49.45±12.80	55.20±3.25		60.90±4.38
	dsRED+GFP++	4.20±2.74	7.99±7.93	19.40±0.71		8.46±0.33
	dsRED-GFP++	3.50±3.26	6.67±7.25	16.40±3.11		5.61±0.43
		cTNT-I4|cTNT-I4	GSC2619	I4(5Y-5) | cTNT-I4	GSC2742 I4(5Ynude) | cTNT-I4	GSC2740 I4(0Y) | cTNT-I4
Branch Point Region	dsRED++GFP-	51.15±20.50	54.70±15.56	40.88±8.86		48.53±19.69
	dsRED++GFP+	39.13±13.38	38.65±11.81	47.30±4.14		40.73±10.84
	dsRED+GFP++	4.88±3.95	3.86±2.26	5.81±2.24		6.43±5.88
	dsRED-GFP++	4.48±4.19	2.70±1.44	5.84±3.20		4.10±4.22
		I4(5Y,b-y) | cTNT-I4	GSC2975	I4(5Y,b-2) | cTNT-I4	GSC2738 I4(5Y,b-ct) | cTNT-I4	GSC2743 I4(5Y,b-a) | cTNT-I4
Controls	dsRED++GFP-	64.13±17.68	0.00±0.00	96.46±6.10		
	dsRED++GFP+	30.07±14.03	0.00±0.00	0.75±1.01		
	dsRED+GFP++	3.23±1.99	2.75±4.09	0.27±0.73		
	dsRED-GFP++	2.51±2.77	97.29±4.05	2.53±6.26		
		cTNT-I4 | cTNT-I5	GSC2250	GFP	dsRED	

Figure 5a

Reduction in poly(Y)	dsRED++GFP-	45.28±7.36	20.35±3.53	22.13±2.39		14.87±2.78
	dsRED++GFP+	45.90±4.81	59.88±7.94	57.57±6.68		55.45±9.92
	dsRED+GFP++	5.47±2.40	12.52±4.27	12.20±3.78		17.68±5.79
	dsRED-GFP++	1.90±1.39	6.30±0.84	7.44±1.29		11.14±1.40
		cTNT-I4 | cTNT-I4	GSC2619	I4(5Y-5) | cTNT-I4	GSC2740 I4(0Y) | cTNT-I4	GSC2742 I4(5Ynude) | cTNT-I4
Controls	dsRED++GFP-	58.70±1.75	0.00±0.00	95.83±3.31		
	dsRED++GFP+	36.43±10.05	0.10±0.37	2.94±2.05		
	dsRED+GFP++	2.60±1.00	1.63±2.78	0.15±0.47		
	dsRED-GFP++	1.68±1.73	98.13±2.92	1.07±2.43		
		cTNT-I4 | cTNT-I5	GSC2250	GFP	dsRED	

Figure 5b

Shortening of second intron	dsRED++GFP-	0.18±0.13	0.09±0.08	0.01±0.01	0.06±0.06
	dsRED++GFP+	0.44±0.18	0.20±0.13	0.07±0.09	0.23±0.16
	dsRED+GFP++	53.12±2.22	48.35±2.11	38.27±3.48	47.77±2.41
	dsRED-GFP++	41.50±2.66	46.35±2.38	54.58±1.81	46.40±2.98
		GSC2781	GSC2782	GSC2784	GSC2783
		cTNT-I4 | I4(sh)	I4(5Y-5) | I4(sh)	I4(5Ynude) | I4(sh)	I4(0Y) | I4(sh)
Controls	dsRED++GFP-	45.28±7.36	58.70±1.75	0.00±0.00	95.83±3.31
	dsRED++GFP+	45.90±4.81	36.43±10.05	0.10±0.37	2.94±2.05
	dsRED+GFP++	5.47±2.40	2.60±1.00	1.63±2.78	0.15±0.47
	dsRED-GFP++	1.90±1.39	1.68±1.73	98.13±2.92	1.07±2.43
		GSC2619	GSC2250	GFP	dsRED
		cTNT-I4 | cTNT-I4	cTNT-I4 | cTNT-I5		

Figure 6

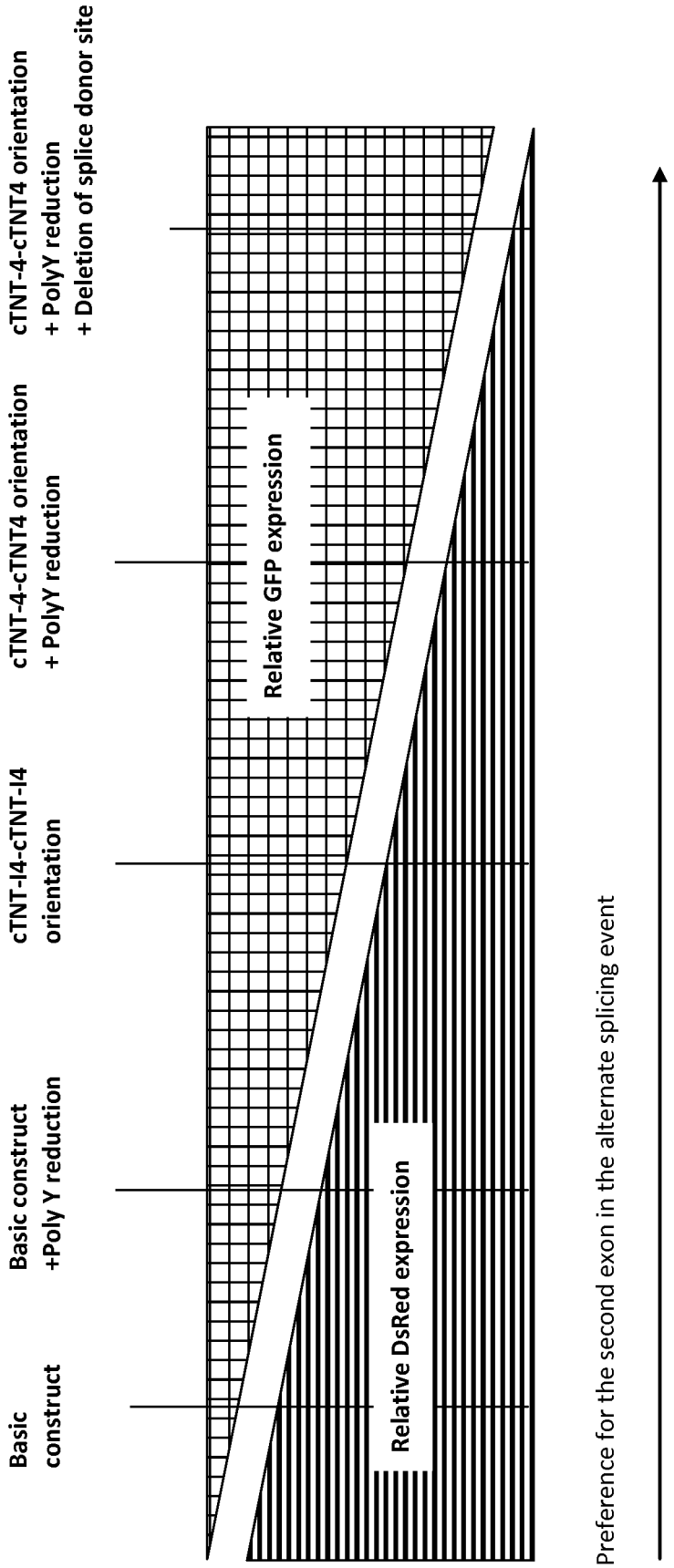

Basic construct
Basic construct +Poly Y reduction
cTNT-I4-cTNT-I4 orientation
cTNT-4-cTNT4 orientation + PolyY reduction
cTNT-4-cTNT4 orientation + PolyY reduction + Deletion of splice donor site
Relative DsRed expression
Relative GFP expression
Preference for the second exon in the alternate splicing event

Figure 7

Figure 8

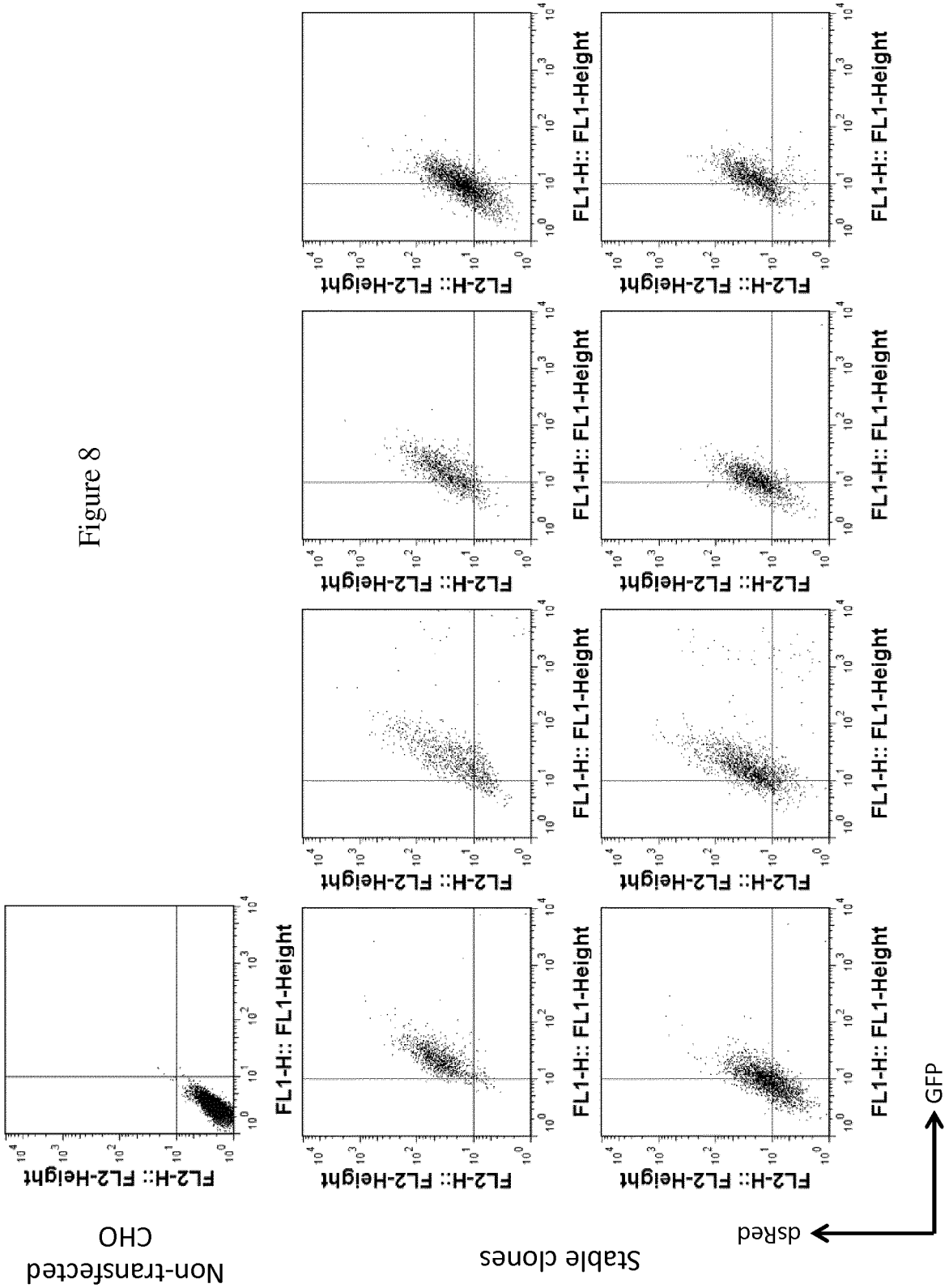

Non-transfected CHO
Stable clones
dsRed
GFP
FL1-H:: FL1-Height
FL2-H:: FL2-Height

Figure 9

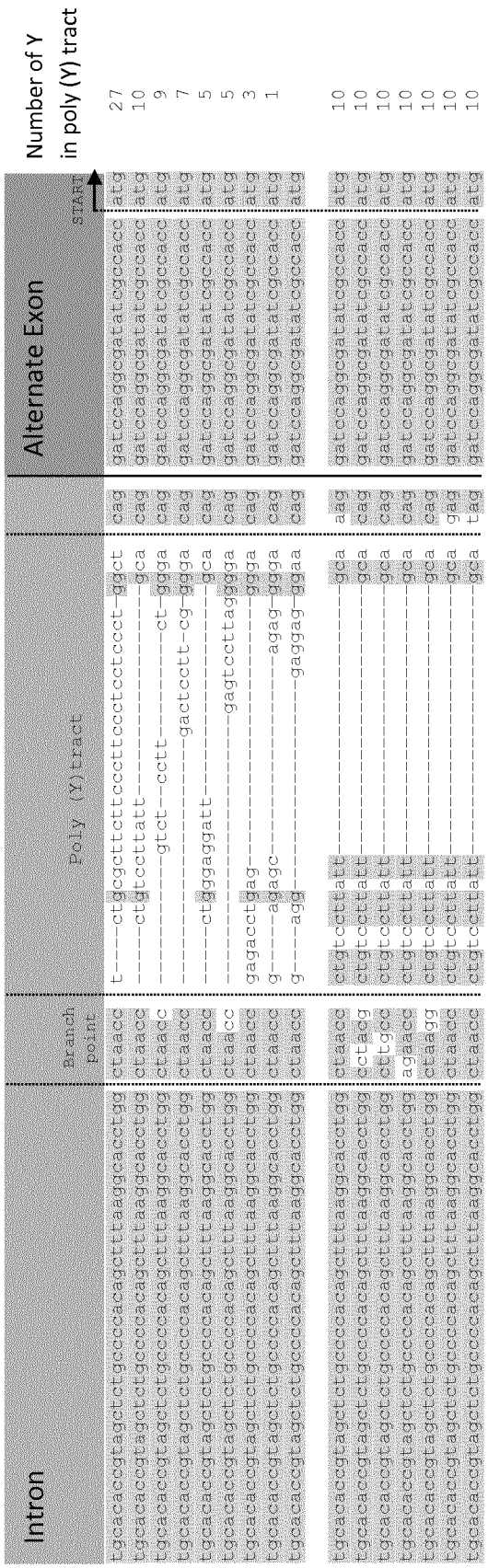
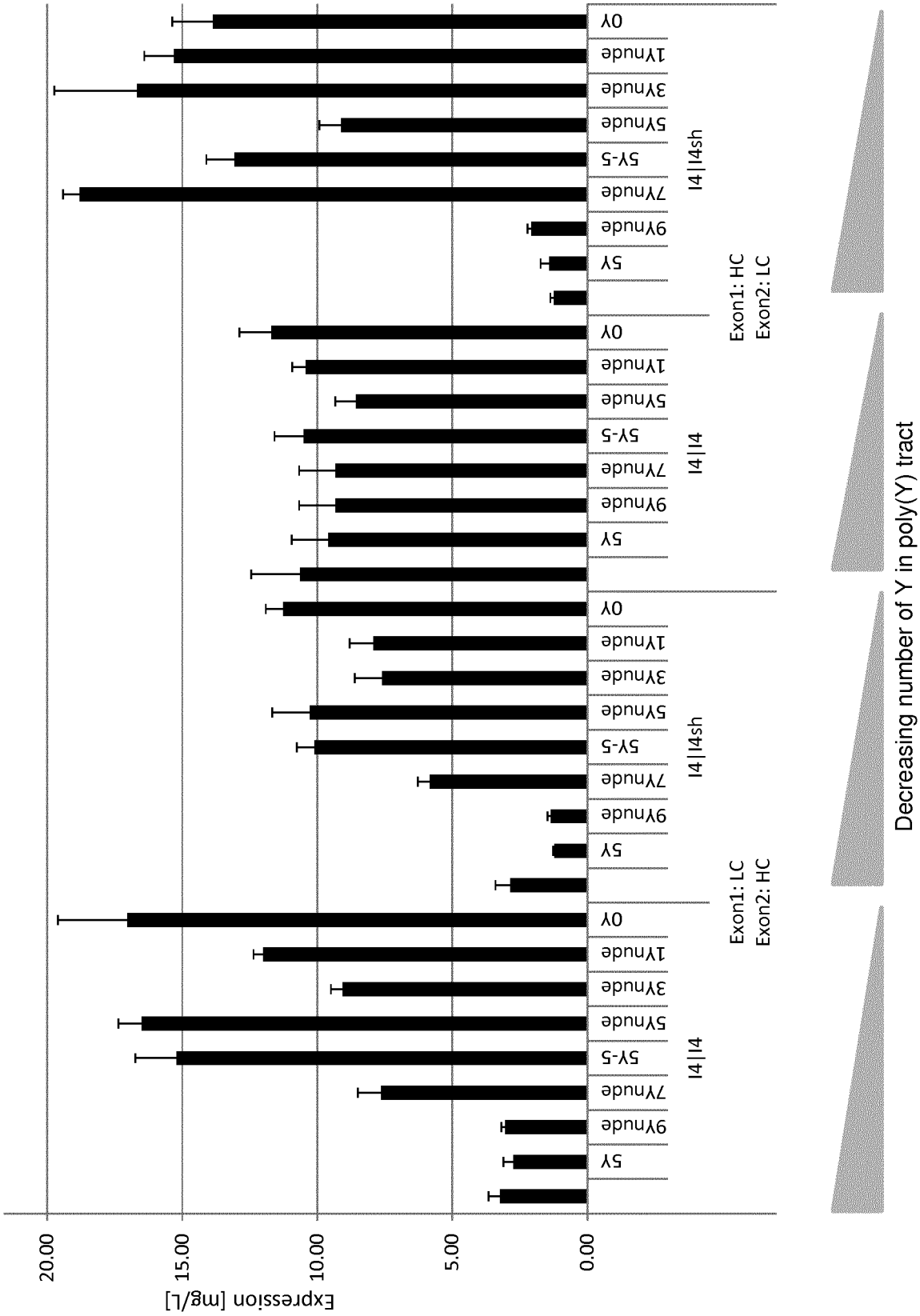

Intron
Branch point
Poly (Y)tract
Alternate Exon
START
Number of Y in poly (Y) tract
I4
I4(5Y)
I4(9Ynude)
I4(7Ynude)
I4(5Y-5)
I4(5Ynude)
I4(3Ynude)
I4(1Ynude)
I4(0Y)
I4(5Y-A)
I4(5Y-b-2)
I4(5Y-b-a)
I4(5Y-b-ct)
I4(5Y-b-y)
I4(5Y-G)
I4(5Y-T)

Figure 10


Expression [mg/L]
0.00
5.00
10.00
15.00
20.00
0Y
1Ynude
3Ynude
5Ynude
5Y-5
7Ynude
9Ynude
5Y
I4|I4
I4|I4sh
Exon1: LC
Exon2: HC
Exon1: HC
Exon2: LC
Decreasing number of Y in poly(Y) tract

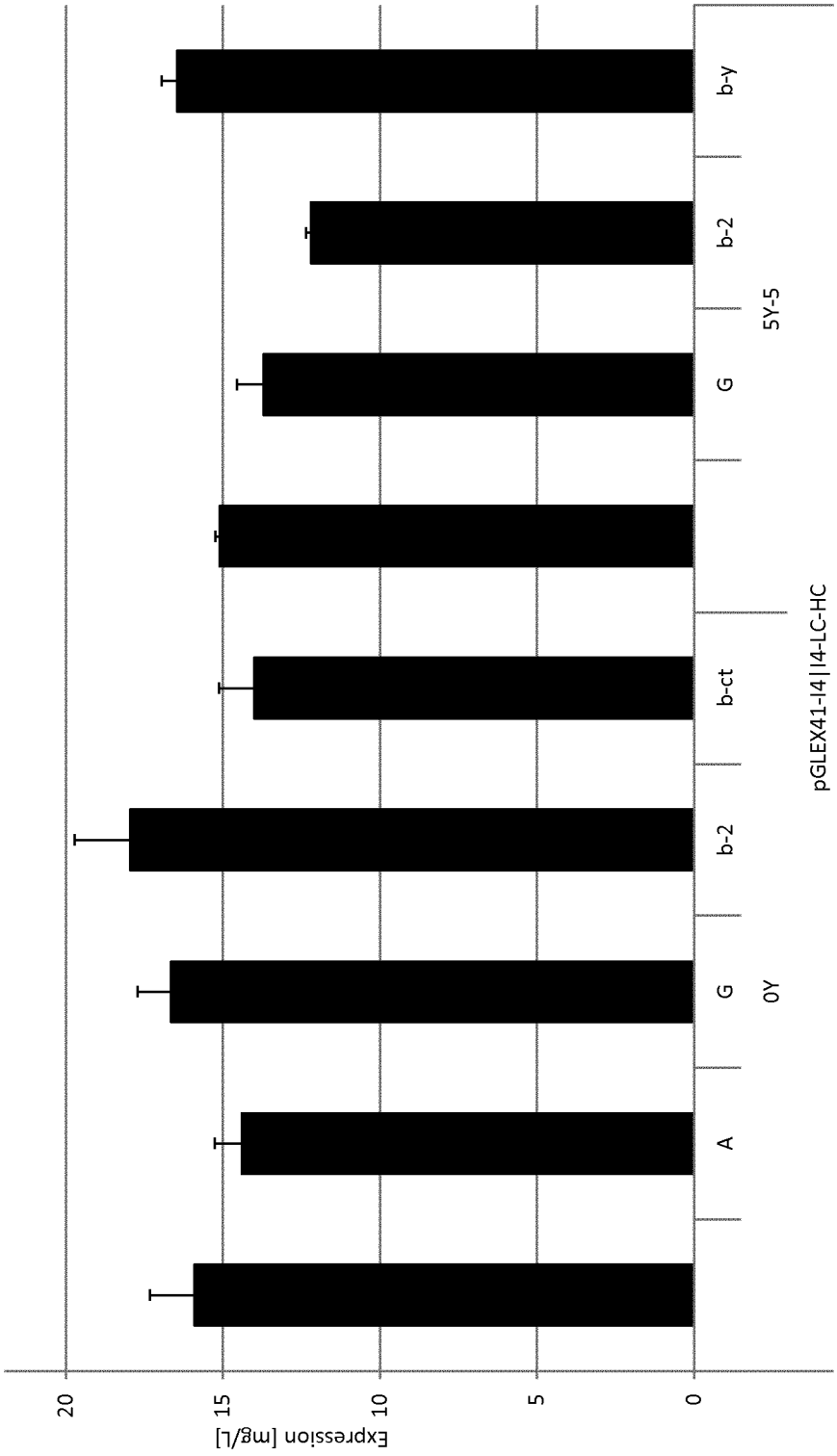
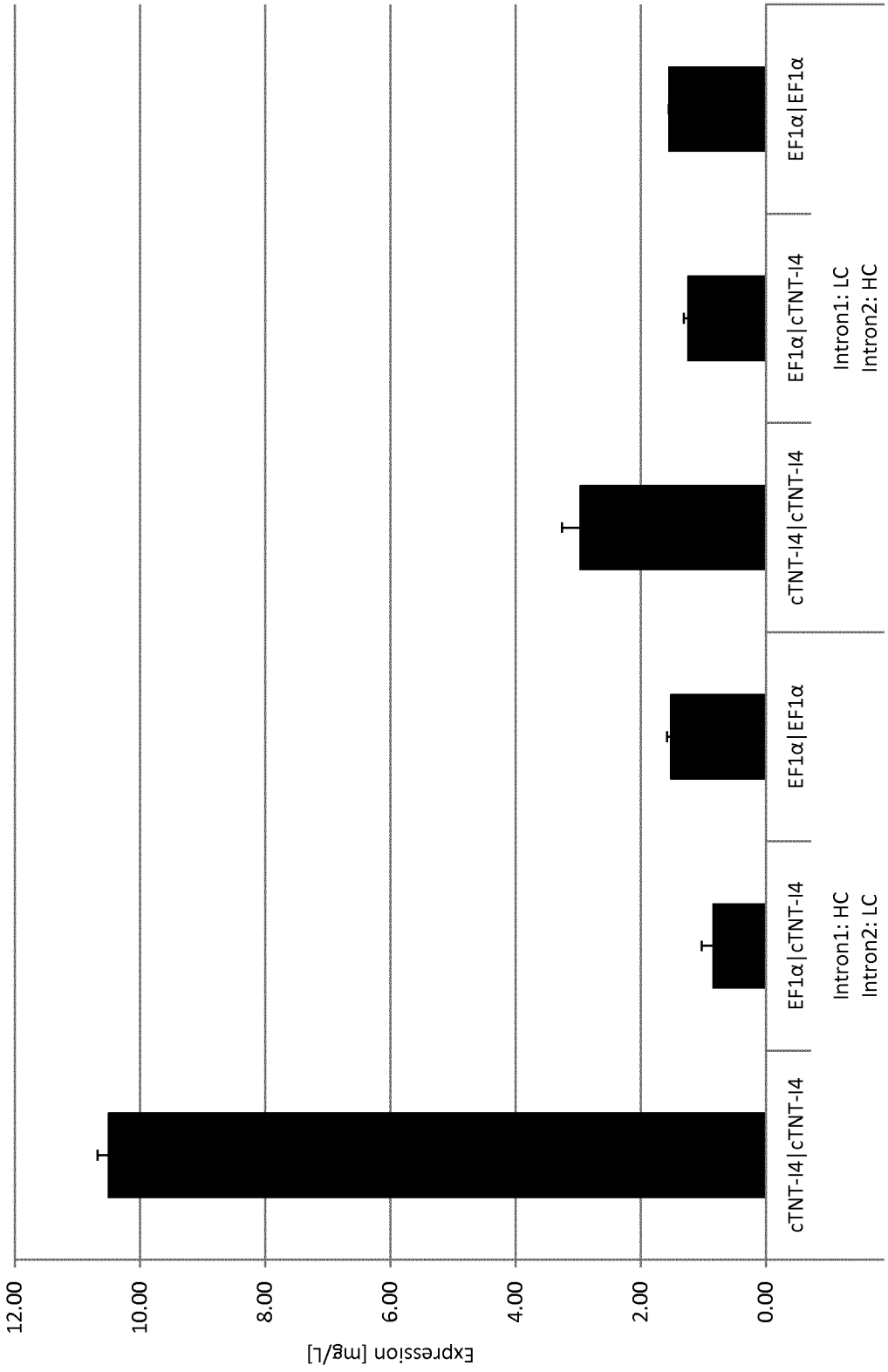

Expression [mg/L]
0
5
10
15
20
A
G
b-2
b-ct
G
b-2
b-y
0Y
5Y-5
pGLEX41-l4|l4-LC-HC

Figure 11

Figure 12


12.00
10.00
8.00
6.00
4.00
2.00
0.00
Expression [mg/L]
cTNT-I4|cTNT-I4
EF1α|cTNT-I4
EF1α|EF1α
cTNT-I4|cTNT-I4
EF1α|cTNT-I4
EF1α|EF1α
Intron1: HC
Intron2: LC
Intron1: LC
Intron2: HC

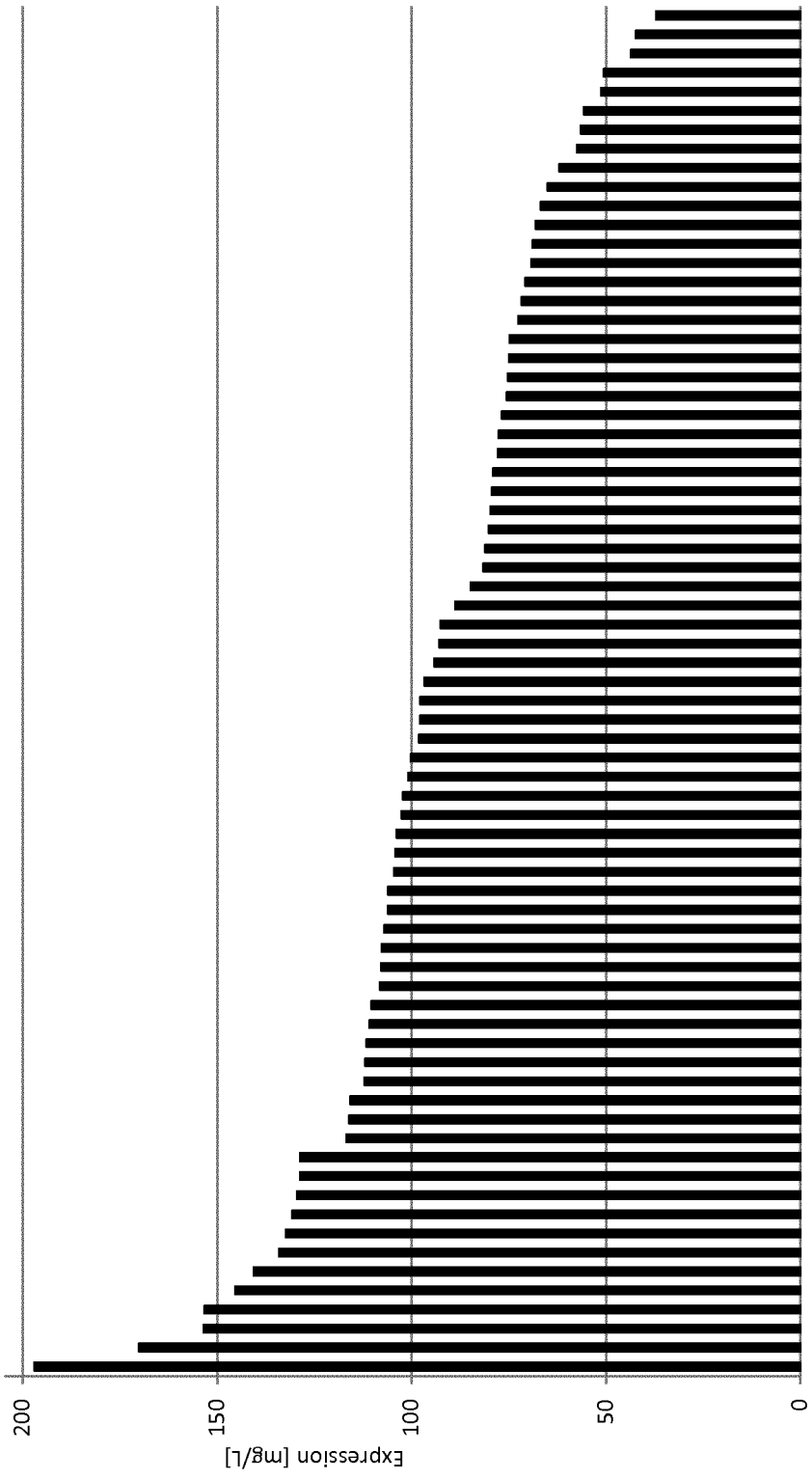


Figure 13

Figure 14

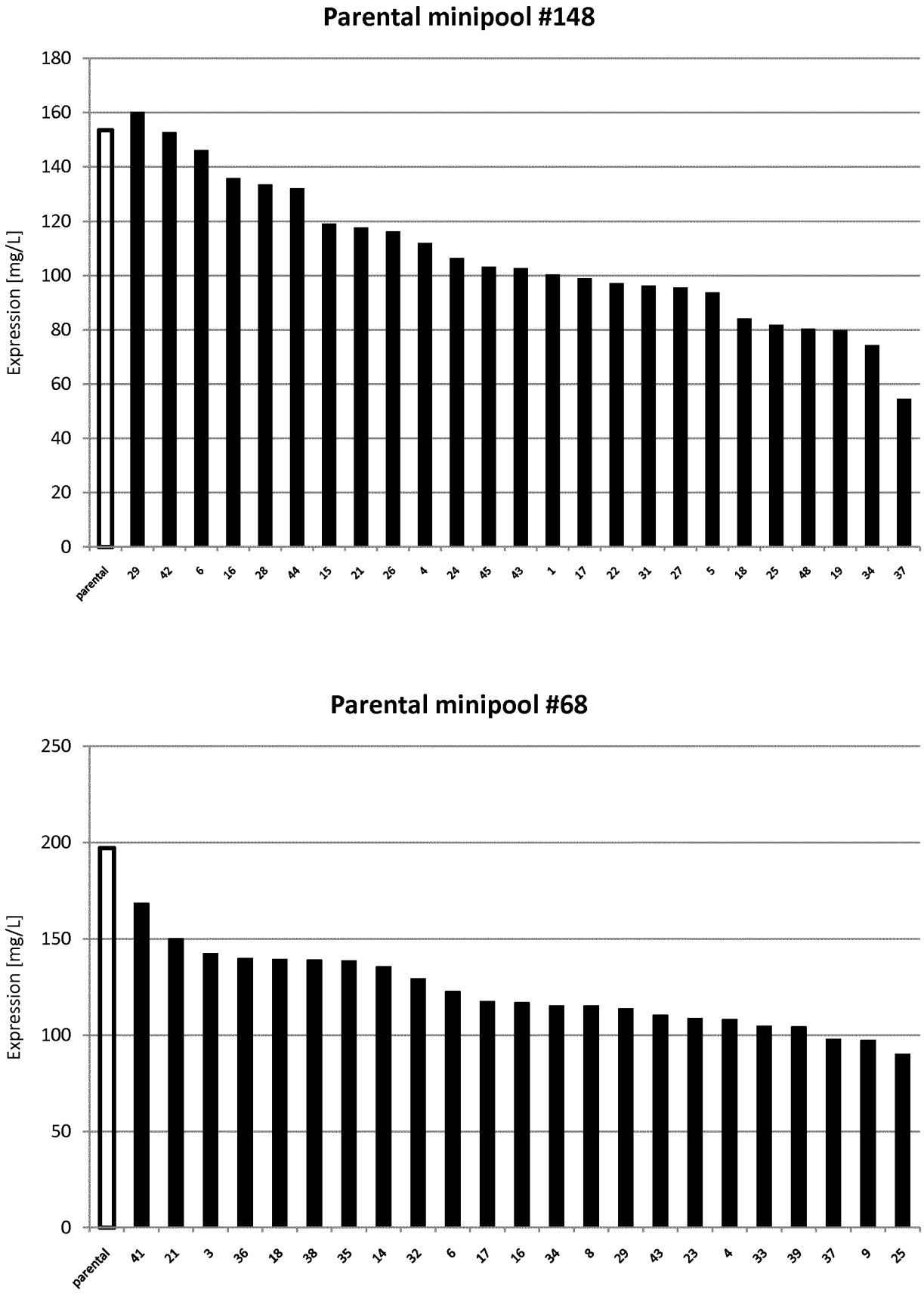
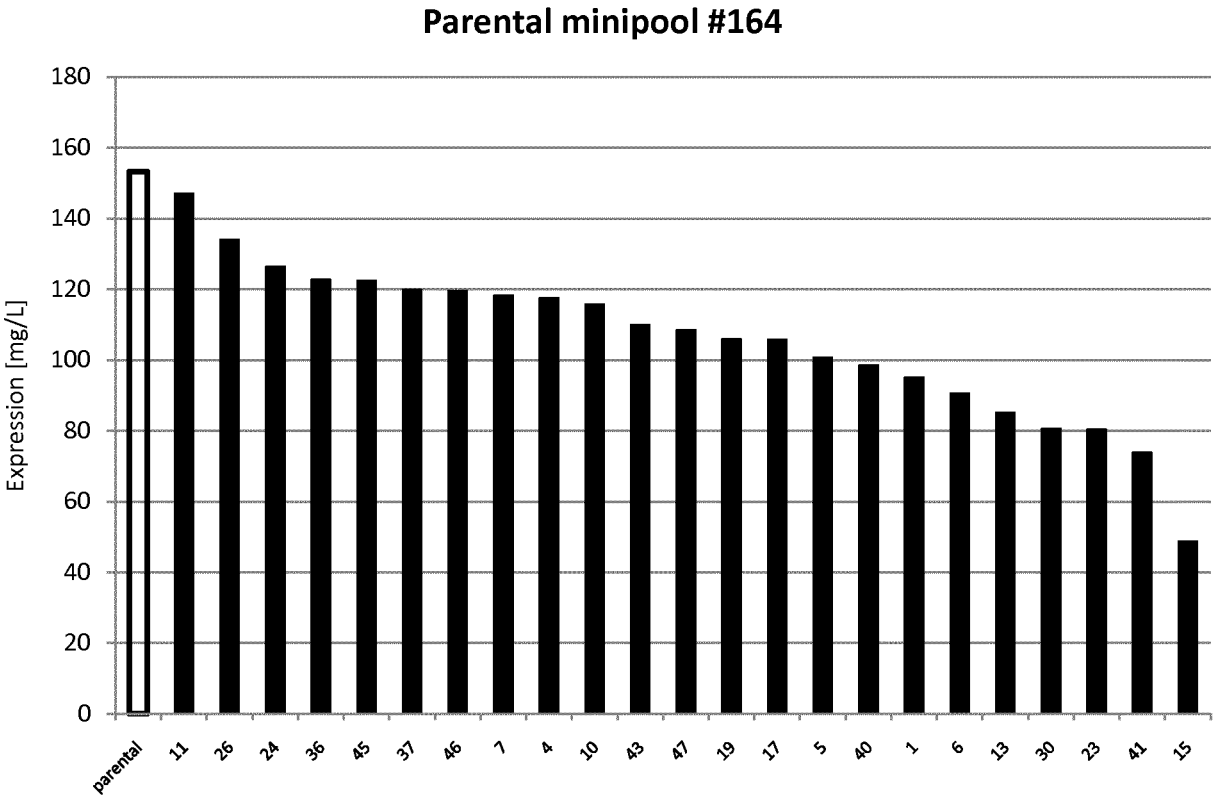
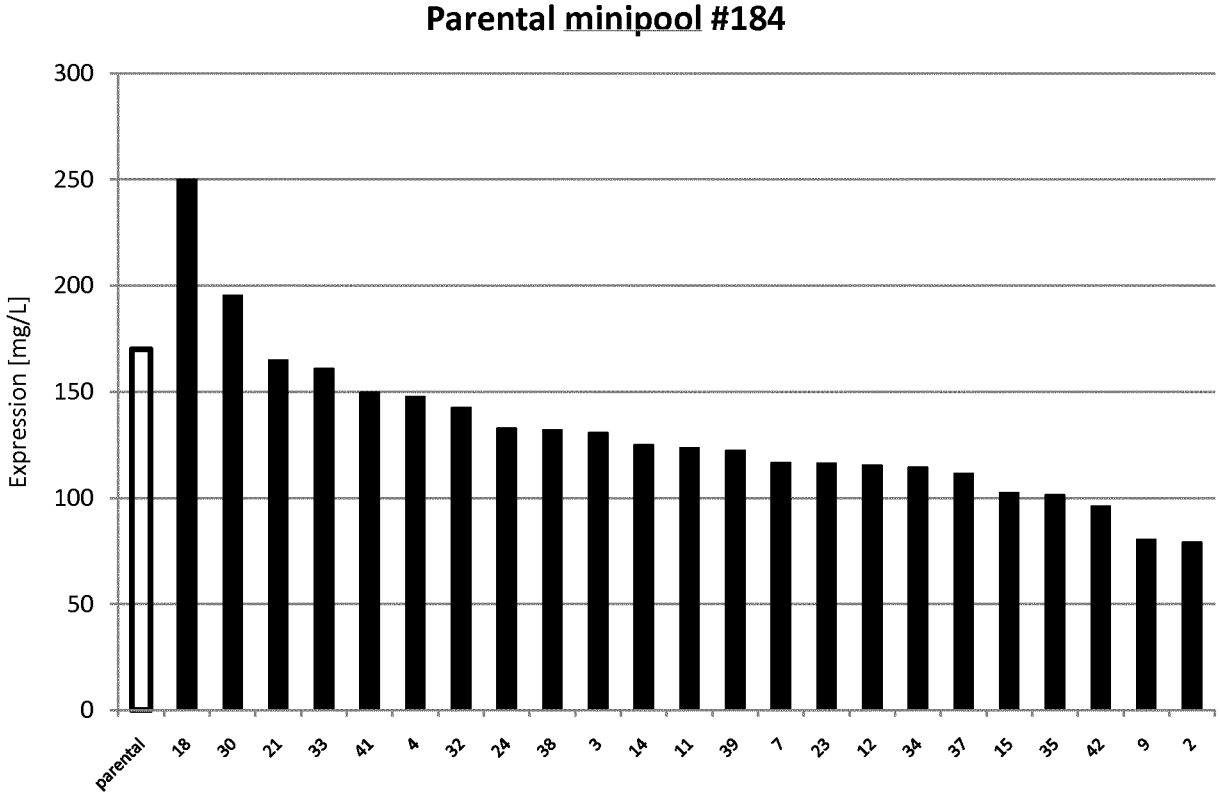

Parental minipool #148
Expression [mg/L]
180
160
140
120
100
80
60
40
20
0
parental 29 42 6 16 28 44 15 21 26 4 24 45 43 1 17 22 31 27 5 18 25 48 19 34 37
Parental minipool #68
Expression [mg/L]
250
200
150
100
50
0
parental 41 21 3 36 18 38 35 14 32 6 17 16 34 8 29 43 23 4 33 39 37 9 25

Figure 14 cont





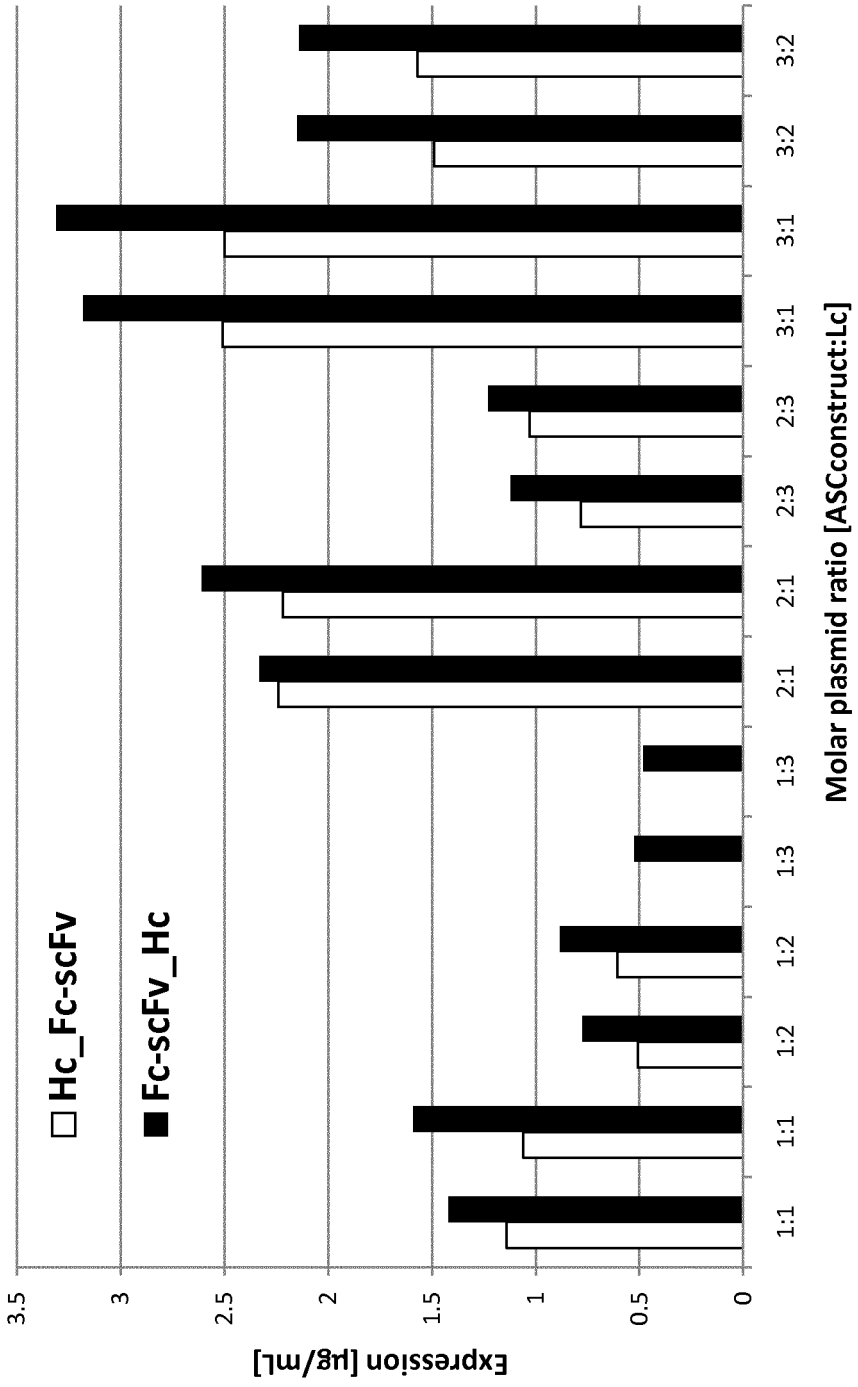

Hc_Fc-scFv
Fc-scFv_Hc
Expression [µg/mL]
0
0.5
1
1.5
2
2.5
3
3.5
1:1
1:1
1:2
1:2
1:3
1:3
1:3
2:1
2:1
2:3
2:3
3:1
3:1
3:2
3:2
Molar plasmid ratio [ASCconstruct:Lc]

Figure 15