FIG.1

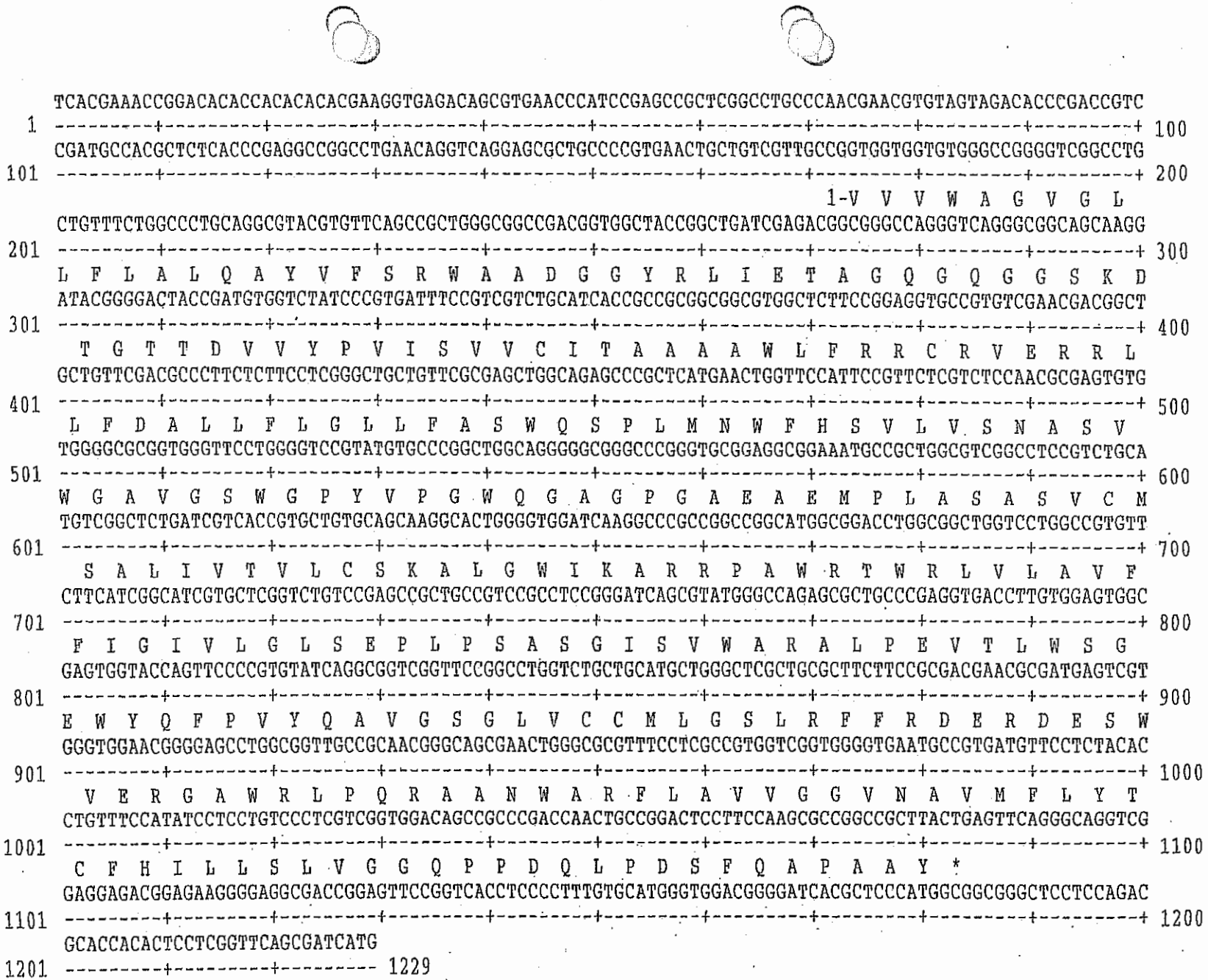

TCACGAAACCGGACACACCACACACACGAAGGTGAGACAGCGTGAACCCATCCGAGCCGCTCGGCCTGCCCAACGAACGTGTAGTAGACACCCGACCGTC
1
100
CGATGCCACGCTCTCACCCGAGGCCGGCCTGAACAGGTCAGGAGCGCTGCCCCGTGAACTGCTGTCGTTGCCGGTGGTGGTGTGGGCCGGGGTCGGCCTG
101
200
1-V V V W A G V G L
CTGTTTCTGGCCCTGCAGGCGTACGTGTTCAGCCGCTGGGCGGCCGACGGTGGCTACCGGCTGATCGAGACGGCGGGCCAGGGTCAGGGCGGCAGCAAGG
201
300
L F L A L Q A Y V F S R W A A D G G Y R L I E T A G Q G Q G G S K D
ATACGGGGACTACCGATGTGGTCTATCCCGTGATTTCCGTCGTCTGCATCACCGCCGCGGCGGCGTGGCTCTTCCGGAGGTGCCGTGTCGAACGACGGCT
301
400
T G T T D V V Y P V I S V V C I T A A A A W L F R R C R V E R R L
GCTGTTCGACGCCCTTCTCTTCCTCGGGCTGCTGTTCGCGAGCTGGCAGAGCCCGCTCATGAACTGGTTCCATTCCGTTCTCGTCTCCAACGCGAGTGTG
401
500
L F D A L L F L G L L F A S W Q S P L M N W F H S V L V S N A S V
TGGGGCGCGGTGGGTTCCTGGGGTCCGTATGTGCCCGGCTGGCAGGGGGCGGGCCCGGGTGCGGAGGCGGAAATGCCGCTGGCGTCGGCCTCCGTCTGCA
501
600
W G A V G S W G P Y V P G W Q G A G P G A E A E M P L A S A S V C M
TGTCGGCTCTGATCGTCACCGTGCTGTGCAGCAAGGCACTGGGGTGGATCAAGGCCCGCCGGCCGGCATGGCGGACCTGGCGGCTGGTCCTGGCCGTGTT
601
700
S A L I V T V L C S K A L G W I K A R R P A W R T W R L V L A V F
CTTCATCGGCATCGTGCTCGGTCTGTCCGAGCCGCTGCCGTCCGCCTCCGGGATCAGCGTATGGGCCAGAGCGCTGCCCGAGGTGACCTTGTGGAGTGGC
701
800
F I G I V L G L S E P L P S A S G I S V W A R A L P E V T L W S G
GAGTGGTACCAGTTCCCCGTGTATCAGGCGGTCGGTTCCGGCCTGGTCTGCTGCATGCTGGGCTCGCTGCGCTTCTTCCGCGACGAACGCGATGAGTCGT
801
900
E W Y Q F P V Y Q A V G S G L V C C M L G S L R F F R D E R D E S W
GGGTGGAACGGGGAGCCTGGCGGTTGCCGCAACGGGCAGCGAACTGGGCGCGTTTCCTCGCCGTGGTCGGTGGGGTGAATGCCGTGATGTTCCTCTACAC
901
1000
V E R G A W R L P Q R A A N W A R F L A V V G G V N A V M F L Y T
CTGTTTCCATATCCTCCTGTCCCTCGTCGGTGGACAGCCGCCCGACCAACTGCCGGACTCCTTCCAAGCGCCGGCCGCTTACTGAGTTCAGGGCAGGTCG
1001
1100
C F H I L L S L V G G Q P P D Q L P D S F Q A P A A Y *
GAGGAGACGGAGAAGGGGAGGCGACCGGAGTTCCGGTCACCTCCCCTTTGTGCATGGGTGGACGGGGATCACGCTCCCATGGCGGCGGGCTCCTCCAGAC
1101
1200
GCACCACACTCCTCGGTTCAGCGATCATG
1201
1229

FIG. 2

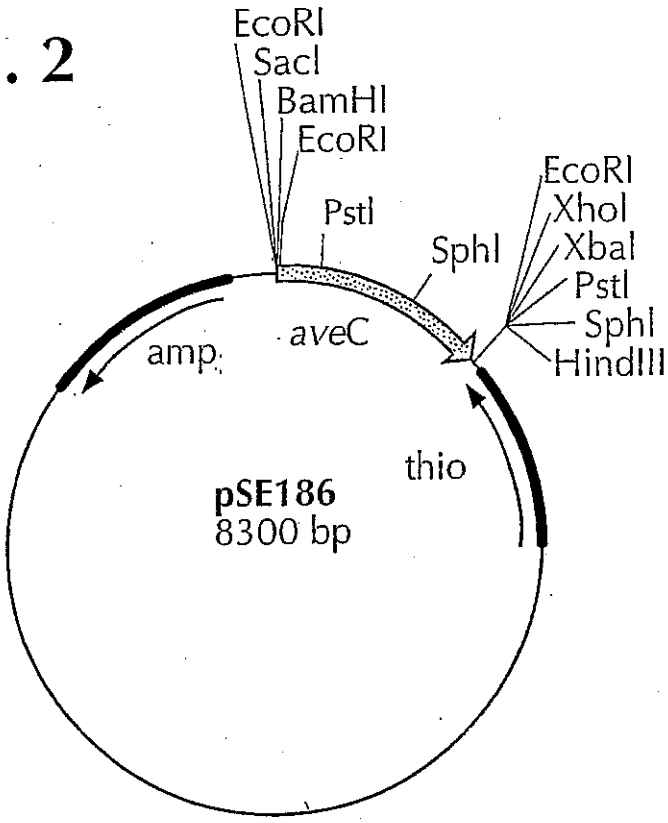
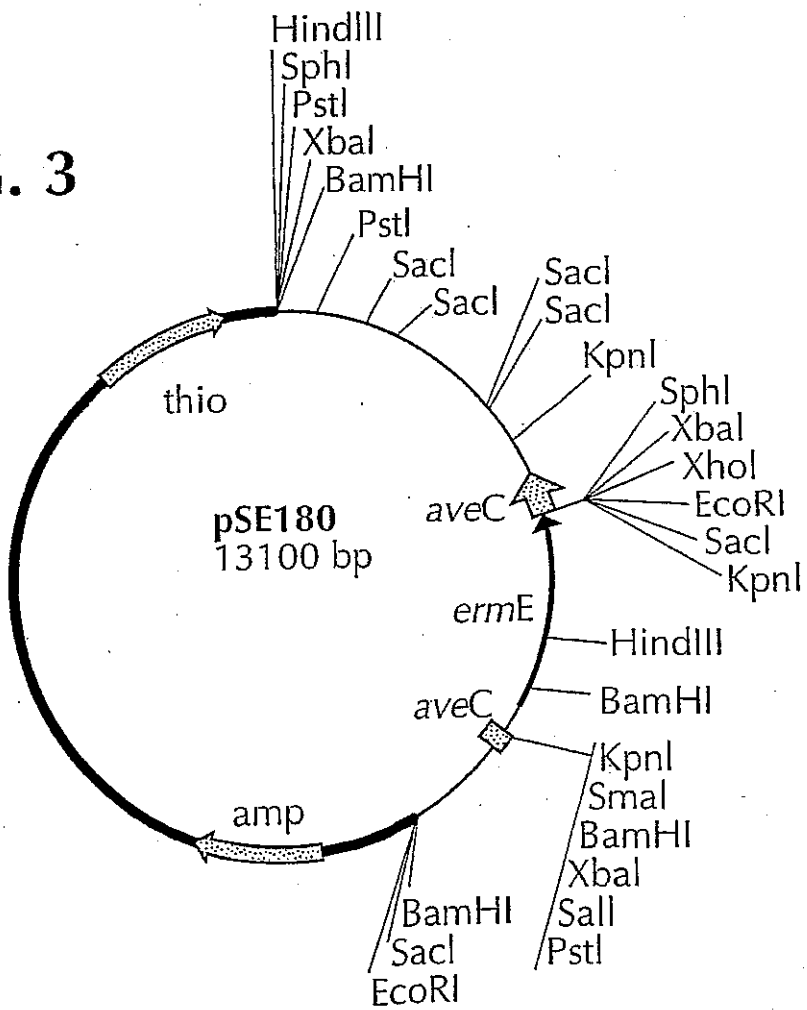

EcoRI
SacI
BamHI
EcoRI
PstI
SphI
EcoRI
XhoI
XbaI
PstI
SphI
HindIII
aveC
amp
thio
pSE186
8300 bp

FIG. 3


HindIII
SphI
PstI
XbaI
BamHI
PstI
SacI
SacI
SacI
SacI
KpnI
SphI
XbaI
XhoI
EcoRI
SacI
KpnI
thio
pSE180
13100 bp
aveC
ermE
HindIII
aveC
BamHI
KpnI
SmaI
BamHI
XbaI
SalI
PstI
amp
BamHI
SacI
EcoRI

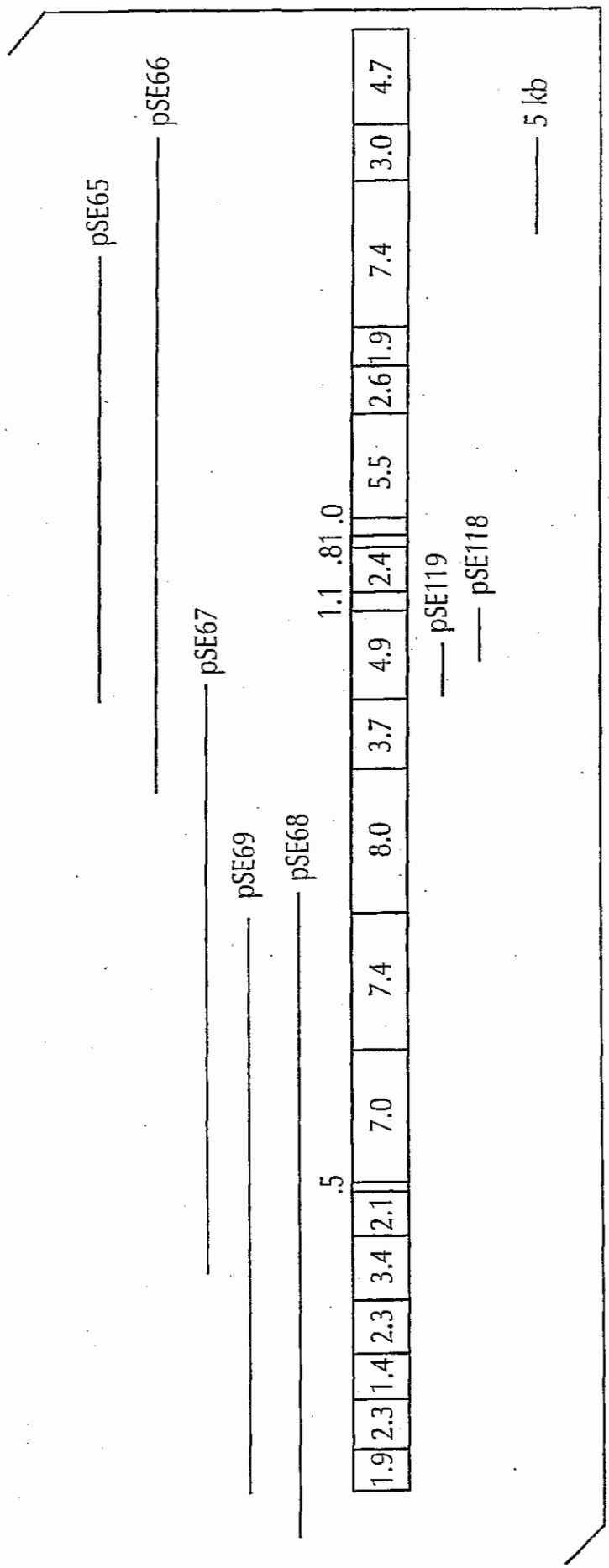

pSE65
pSE66
pSE67
pSE69
pSE68
pSE119
pSE118
5 kb
1.9
2.3
1.4
2.3
3.4
2.1
.5
7.0
7.4
8.0
3.7
4.9
2.4
1.1
.8
1.0
5.5
2.6
1.9
7.4
3.0
4.7

FIG. 4

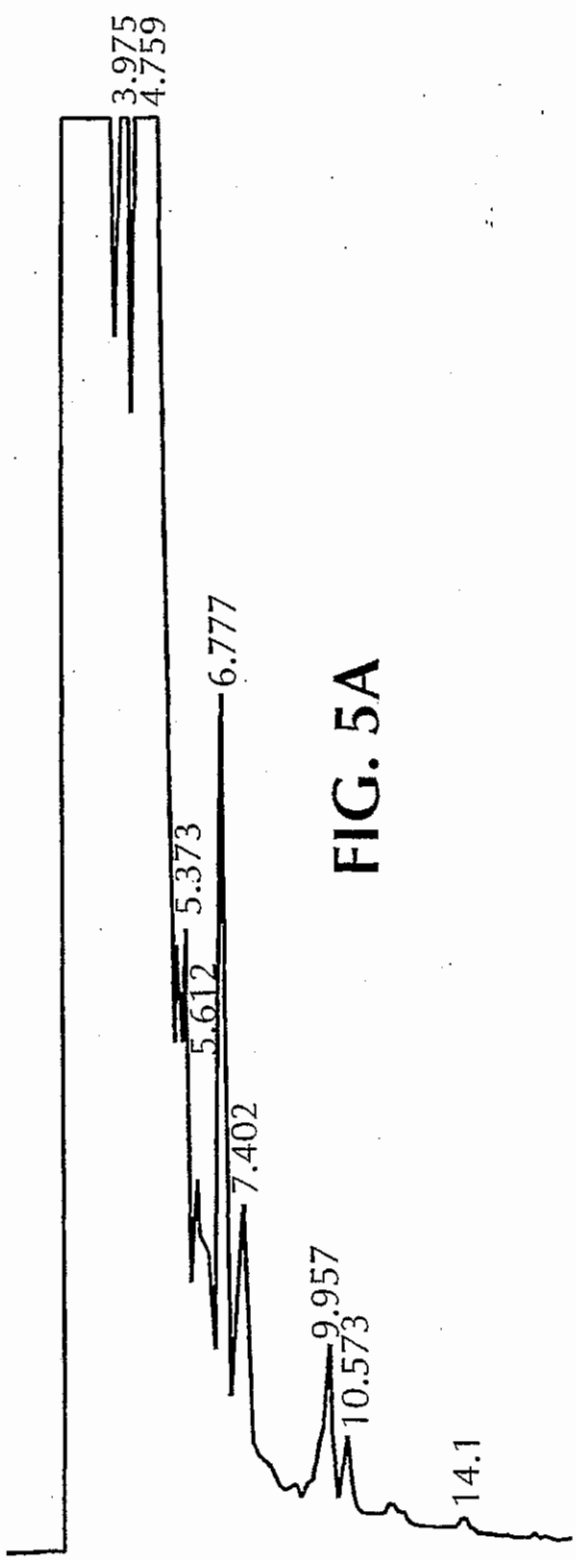

3.975
4.759
5.373
5.612
6.777
7.402
9.957
10.573
14.1

FIG. 5A

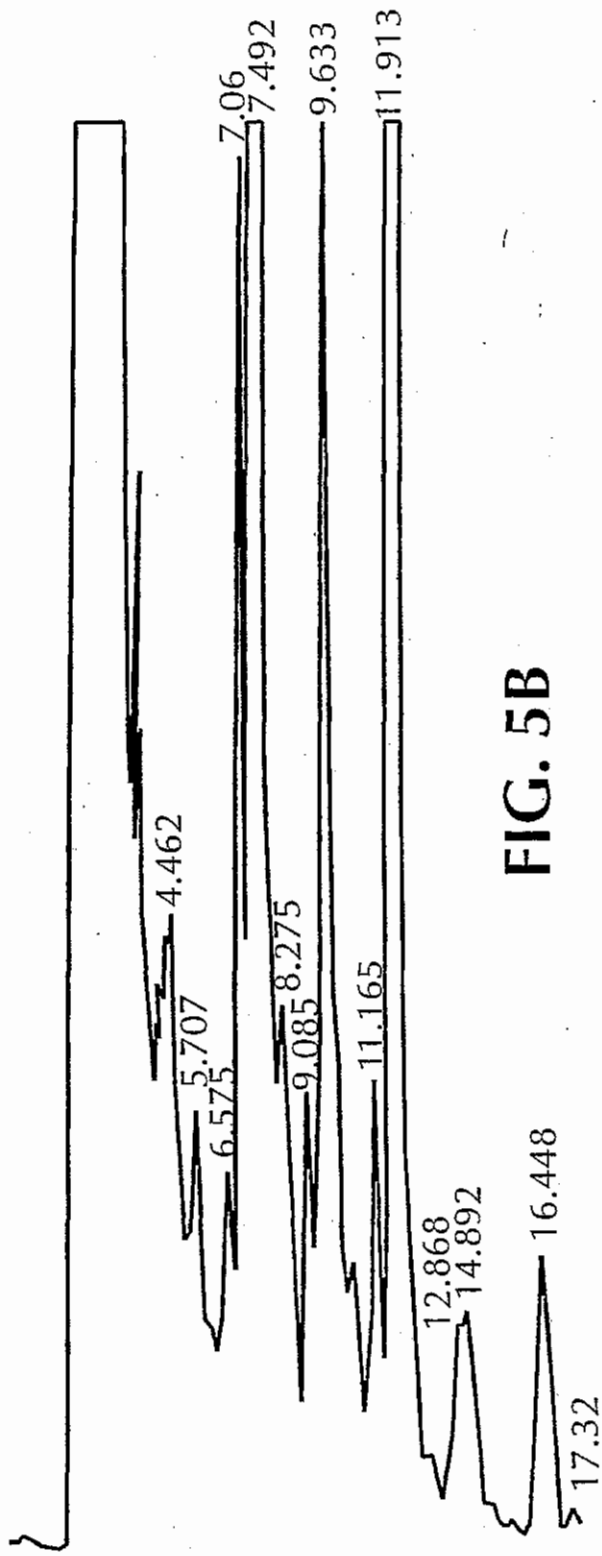

4.462
5.707
6.575
7.06
7.492
8.275
9.085
9.633
11.165
11.913
12.868
14.892
16.448
17.32

FIG. 5B

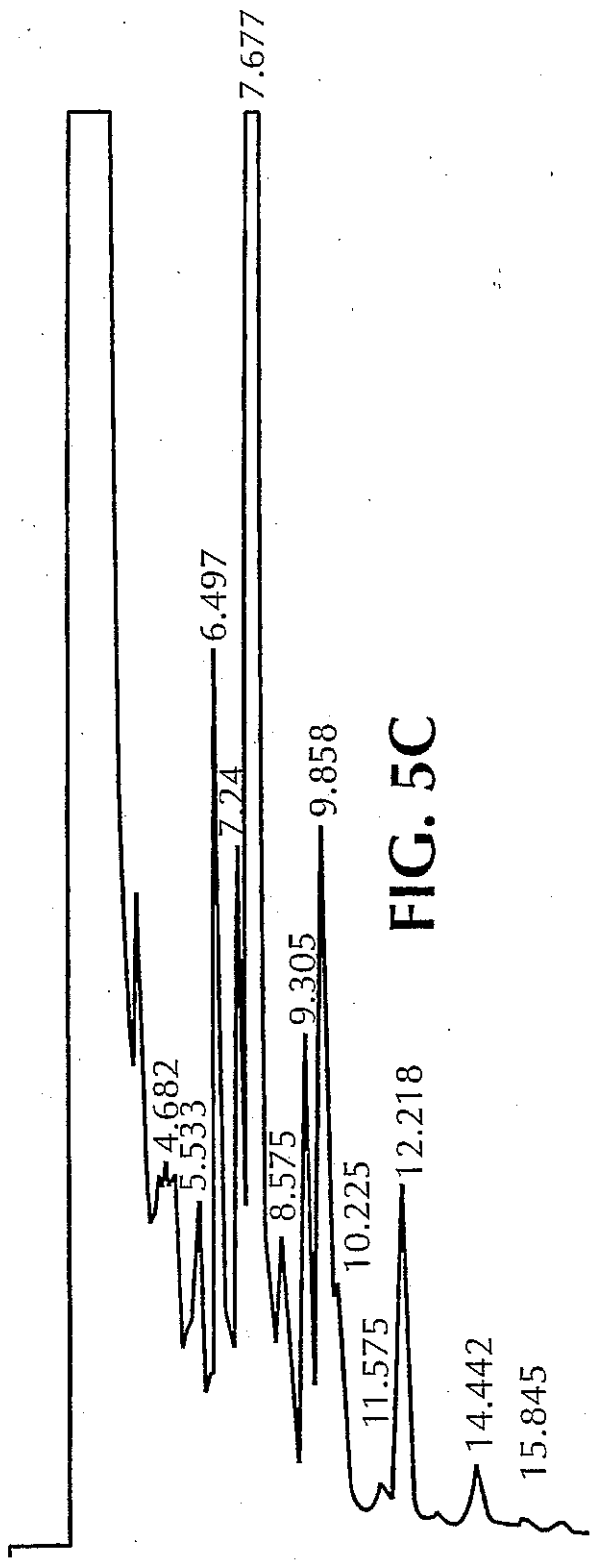

4.682
5.533
6.497
7.24
7.677
8.575
9.305
9.858
10.225
11.575
12.218
14.442
15.845

FIG. 5C

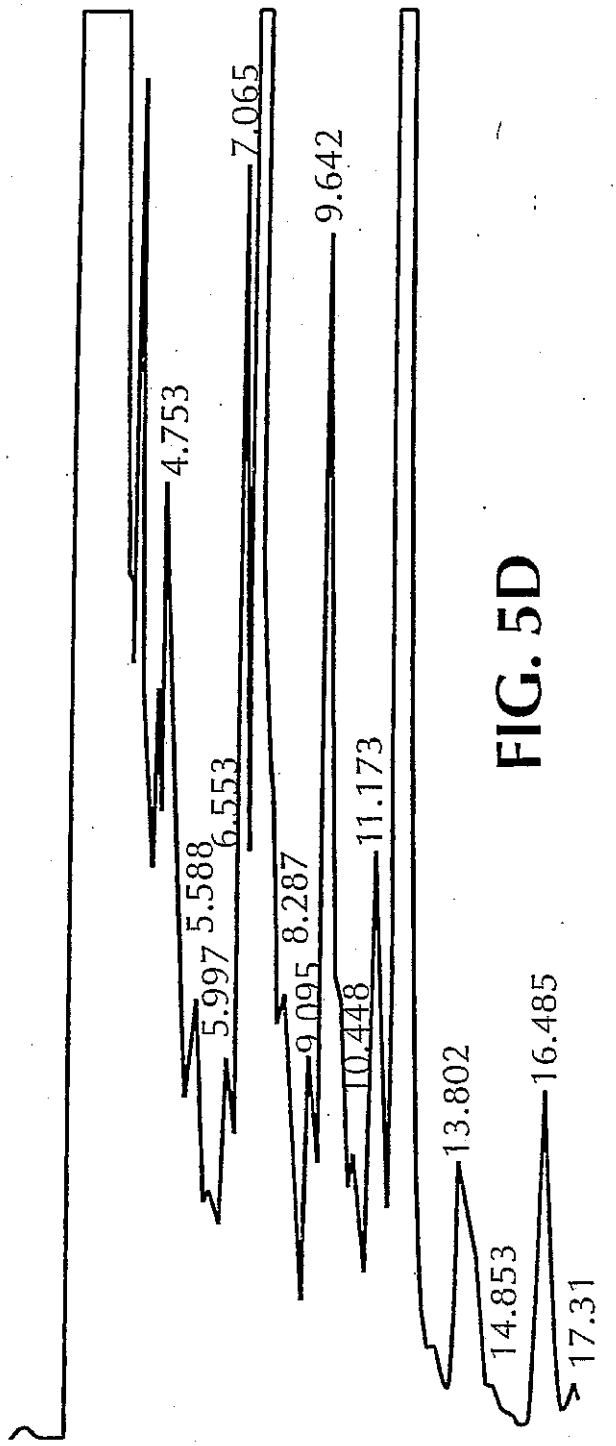

4.753
5.588
5.997
6.553
7.065
7.497
8.287
9.095
9.642
10.448
11.173
11.925
13.802
14.853
16.485
17.31

FIG. 5D

FIG. 6A

Plasmid	Mutations	[B2]	[B1]	B2/B1
pSE501	D48E, L136P, G179S, E238D (A132G), T317A, T580C, G708A, A887T, (A1124G)	52	184	0.28
PSE502	D48E, A89T, L136P, G179S, E238D T317A, G438A, T580C, G708A, A887T	42	204	0.2
pSE503	D48E, A89T, L136P, K154E, G179S,E238D T317A, G438A, (T497C), T580C, T633C, G708A, (C775T), A887T	32	172	0.19
PSE504	D25G, D48E, A89T, L136P, S138T, Al39T, Vl41A, I159T, R163Q, G179S A247G, T317A, G438A, (T497C), T580C, T585A, G588A, T595C, T649C, G661A, G708A, (C884T)	44	205	0.21
pSE505	D48E, A89T, S138T, A139T, K154R, G179S, V196A, P289L T317A, G438A, T585A, G588A, A634G, G708A, T760C, A761C, C1039T	35	178	0.2
pSE506	D48E, A89T, S138T, A139F, G179S, V196A, E238D T317A, G438A, (T497C), T585A, G588T, G589T, G708A, T760C, A761C, A887C	26	135	0.19
pSE507	D48E, A61T, A89T, L136P, G179S, V196A, A198G, P289L T317A, G354A, G438A, (T497C), T580C, G708A, T760C, A761C, C766G, C1039T	18	88	0.2

FIG. 6B

Plasmid	Mutations	[B2]	[B1]	B2/B1]
pSE508	D48E, A89T, S90G, L136P, R163Q, G179S, E238D	22	91	0.24
	T317A, G438A, A441 G, T580C, G661A, G708A, A887T, (A938G)			
pSE509	D48E, A61T, S138T, A139F, G179S,G196A,E238D,P289L	22	114	0.19
	(G136A), T317A, G354A, (T497C), T585A, G588T, C589T, G708A, T760C, A761C, A887C, C1039T			
pSE510	D48E, A61T, L136P, G179S, E238D	64	211	0.30
	T317A, G354A, (T497C), T580C, G708A, A887C			
pSE511	D48E, A61T, A89T, G111V, S138T, A139T, G179S, E238D, P289L	20	91	0.21
	T317A, G354A, (T416C), G438A, G505T, T585A, G588A, G708A, (G775T), A887C, C1039T			
pSE512	D48E, A61T, L136P, G179S	55	88	0.29
	T317A, G354A, (T497C), T580C, G708A			
pSE514	D48E, A89T, L136P, G179S	36	175	0.20
	(T33A), T317A, G438A, (T497C), T580C, G708A			
pSE515	D48E, A89T, V120A, L136P, G179S	36	189	0.19
	T317A, G438A, T532C, T580C, G708A, (T1031C)			

FIG. 6C

Plasmid	Mutations	[B2]	[Bl]	B2/B1
pSE517	D48E, A61T, A89T, S138T, A139F, G179S, V196A, A198G, E238D	20	99	0.20
	T317A, G354A, G438A, (T497C), T585A, G588T, C589T, G708A, T760C, A761C, C766G, A887C			
PSE518	D48E, A61T, A89T, G111V, S138T, A139F, G179S, V196A, E238D	11	57	0.18
	T317A, G354A, G438A, G505T, T585A, G588T, C589T, G708A, T760C, A761C, A887C			
pSE519	D48E, A89T, S138T, A139T, G179S	40	153	0.26
	T317A, G438A, T585A, G588A. G708A			
pSE520	D48E, A89T, S138T, A139T, G179S	45	177	0.25
	T317A, G438A, (T497C), T585C, G588A, G708A			
pSE523	D48E, L136P, R163Q, G179S, S231L	37	168	0.22
	T317A, T580C, G661A, G708A, (T797C), C865T			
pSE524	D48E, A89T, L136P, G179S, E238D	25	137	0.18
	T317A, G438A, (T497C), T580C, G708A, A887T			
PSE525	D48E, A89T, L136P, G179S	22	129	0.17
	T317A, G438A, (T497C), T580C, G708A			

FIG. 6D

Plasmid	Mutations	[B2]	[B1]	[B2/B1]
pSE526	D48E, A61T, L136P, G179S, A198G, P202S, E238D, P289L, T317A, G354A, (T497C), T580C, G708A, C766G, C777T, A887C, C1039T	35	135	0.26
pSE527	D48E, L136P, S138T, A139F, G179S, V196A, E238D T317A, (T497C), T580C, T585A, G588T, C589T, G708A, T760C, A761C, A887C	31	119	0.25
pSE528	D48E,A61T,L136P,S138T, A139F, G179S, E238D, P289L T317A, G354A, (T497C), T580C, T585A, G588T, C589T, G708A, A887C, C1039T	50	160	0.30
pSE529	D48E,A61T,A89T,S138T, A139T, G179S, V196A, E238D, P289L T317A, G354A, G438A, (T497C), T585A, G588A, G708A, T760C, A761C, A887C, C1039T	26	150	0.17
pSE530	D48E, L136P, G179S, A198G, E238D, P289L (C97T), T317A, T580C, G708A, C766G, A887C, C1039T	37	136	0.27
pSE531	D48E, A61T, S138T, A139F, G179S, A198G, P289L T317A, G354A, (T497C), T585A, G588T, C589T, G708A, C766G, C1039T	27	101	0.27

FIG. 6E

Plasmid	Mutations	[B2]	[B1]	B2/B1
pSE532	D48E, A61T, A89T, L136P, S138T, A139F, G179S, A198G, E238D T317A, G354A, G438A, (T497C), T580C, T585A, G588T, C589T, G708A, C766G, A887C	19	91	0.20
pSE534	D48E, L84P, G111V, S138T A139T, G179S, A198G, P289L T317A, T424C, G505T, T585A, G588A, G708A, C766G, C1039T	37	139	0.26
pSE535	Y28C, D48E, A61T, A89T, S138T, A139T, G179S, E238D A256G, T317A, G354A, G438A, T585A, G588A, G708A, A887C	34	132	0.26
pSE536	D48E, A89T, S138T, A139F, G179S,A198G,V220A (G137T), T317A, G438A, (T497C), T585A, G588T, C589T, G708A, C766G, T832C	19	106	0.17
pSE537	D48E, A61T, A89T, S138T, A139T, G179S, V196A, E238D, R239H, P289L T317A, G354A, G438A, (T497C), T585A, G588A, G708A, T760C, A761C, A887C, G889A,C1039T	34	171	0.19
pSE538	D48E, A61T, A89T, S138T A139T, G179S, A198G, P289L T317A, G354A, G438A, T585A, G588A, G708A, C766G, C1039T	13	39	0.33
pSE539	D48E, A61T, A89T, F99S, S138T, A139T, G179S, E238D T317A, G354A, G438A, G469C, (T497C), T585A, G588A, G708A, A887C, (C1094T)	43	179	0.24

FIG. 6F

Plasmid	Mutations	[B2]	[B1]	B2/B1
pSE540	G35S, D48E, A89T, S138T, A139T,G179S,P289L	42	180	0.23
	G276A, T317A, G438A, (T497C), T585A, G588A, G708A,C1039T			
pSE541	D48E, A61T, A89T, L136P, G179S, P289L	36	174	0.20
	(C266T), T317A, G354A, G438A, (T497C), T580C, G708A, C1039T			
pSE542	D48E, A61T, A107T, S108G, L136P, G179S, S192A, E238D, P289L	50	175	0.29
	T317A, G354A, G492A, A495G, (T497C), T580C, G708A, T747G, A887C, C1039T			
pSE543	D48E, A61T, A89T, G111V, S138T, A139F, G179S, E238D, P289L	17	67	0.25
	T317A, G354A, G438A, G505T, T585A, G588T, C589T, G708A, A887C,C1039T			
pSE545	D48E, L136P, G179S, R250W	37	134	0.27
	G317A, T580C, G708A, C921T			
pSE546	D48E, L136P, G179S, E238D	48	178	0.27
	G317A, T580C, G708A, A887T			
pSE547	D48E, A61T, A89T, S138T, A139T, G179S, V196A, E238D	32	142	0.23
	T317A, G354A, G438A, T585A, G588A, G708A, T760C, A761C, A887C			

FIG. 6G

Plasmid	Mutations	[B2]	[B1]	B2/B1
pSE548	D48E, A89T, S138T, A139T, R163Q, G179S	41	157	0.26
	(T114C), (T130C), T317A G438A, T585A, G588A, G661A, G708A			
pSE549	D48E, A89T, S138T, A139T G179S, V196A, E238D, P289L	24	141	0. 17
	G317A, G438A, (T497C), T585A, G588A, G708A, T760C, A761C, A887C, C1039T			
pSE550	D48E, A89T, G111V, S138T, A139T, G179S, A198G, E238D	19	76	0.25
	T317A, G438A, G505T, T585A, G588A, G708A, C766G, A887C			
pSE551	D48E, A61T, A89T, S138T, A139F, G179S, V196A, E238D	24	141	0.17
	T317A, G354A, G438A, (T497C), T585A, G588T, C589T, G708A, T760C, A761C, A887C			
pSE552	D48E, L136P, G179S, A198G, P289L	40	154	0.26
	T317A, (T497C), T580C, G708A, C766G, C1039T			
pSE553	D48E, A89T, S138T, A139T, G179S, V196A, E238D, P289L	23	123	0.18
	T317A, G438A, (T497C), T585A, G588A, G708A, T760C, A761C, A887C, C1039T			
pSE554	D48E, A61T, A89T, S138T, A139T, G179S, V196A, E238D, P289L	30	162	0.18
	T317A, G354A, G438A, (T497C), T585A, G588A, G708A, T760C, A761C, A887C, C1039T			

FIG. 6H

Plasmid	Mutations	[B2]	[B1]	B2/B1
pSE556	D48E, S138T, A139T, G179S, E238D (T169C), T317A, T585A, G588A, G708A, A887T	66	177	0.37
pSE557	D48E, F78L, A89T, L136P, G179S A317A, T405C, G438A, T580C, G708A	57	219	0.26
pSE558	S41G, D48E, A89T, L136P, G179S (G87A), A294G, T317A, G438A, T580C, G708A	51	215	0.24
pSE559	G40S, D48E, L136P, G179S, E238D G291A, T317A, T580C, G708A, A887T	81	247	0.33
pSE561	Y28C, Q38R, D48E, L136P, G179S, E238D A256G, A286G, T317A, (T497C), T580C, G708A, A887T	31	83	0.37
pSE563	D48E, A89T, L136P, R163Q, G179S, P252S T317A, G438A, T580C, G661A, G708A, C927T	43	177	0.24
pSE564	D48E,A89T,S138T,A139T, G179S, E238D, F278L T317A, G438A, (T497C), T585A, G588A, G708A, A887T, T1005C	61	216	0.28
pSE565	D48E, A89T, S138T, A139T, G179S, E238D, F278L T317A, G438A, (T497C), T585A, G588A, G708A, A887T, T1005C	59	210	0.28

FIG. 6I

Plasmid	Mutations	[B2]	[B1]	B2/B1
pSE566	D48E, A89T; L136P, G179S, F234S T317A, (C341T), G438A, (T497C), T580C, (C695T), G708A, T874C	37	154	0.24
pSE567	D48E, L136P, R163Q, G179S, T317A, T580C, G661A, G708A	60	192	0.31
pSE568	D48E, A89T, L136P, R163Q, G179S (T85C), T317A, G438A, (T497C), T580C, G661A, G708A, (C800T)	48	173	0.28
pSE569	D48E, S138T, A139T, G179S, E238D (T104C), T317A, (T497C), T585A, G588A, G708A, A887T	59	153	0.38
pSE570	D48E, L136P, R1630, G179S, E238D T317A, T580C, G661A, G708A, A887T	73	221	0.33
pSE571	D48E, L136P, R163Q, G179S, A200G, E238D (T67C), T317A, T580C, G661A, G708A, C772G, A887T	75	236	0.32
pSE572	D48E, L136P, R163Q, G179S, E238D T317A, (T497C), T580C, G661A, G708A, A887T	74	229	0.32
pSE573	D48E, A89T, L136P, R163Q, G179S, E238D T317A, G438A, T580C, G661A, G708A, A887T	39	173	0.23

FIG. 6J

Plasmid	Mutations	[B2]	[B1]	B2/B1
pSE574	Q36R, D48E, A89T, L136P, G179S, E238D	53	231	0.23
	A280G, T317A, (G359A), G438A, T580C, G708A, (T836C), A887T			
pSE575	D48E, A89T, L136P, R163Q, G179S	49	217	0.23
	T317A, G438A, T580C, G661A, (C704T), G708A, (G965A)			
pSE576	D48E, A89T, L136P, R1630, G179S	44	197	0.22
	T317A, G438A, T580C, G661A, G708A			
pSE577	D48E, A89T, S138T, G179S	36	140	0.25
	T317A, G438A, (T497C), T585A, G708A			
pSE578	D48E, A89T, L136P, R163Q, G179S, E238D	45	193	0.23
	T317A, G438A, (T497C), T580C, G661A, G708A, A887T			

FIG. 6K

Plasmid	Mutations	[B2]	[B1]	B2/B1
pSE582 ca	V3L, L136M	186	178	1.04
pSE583 cb	G26A, D48Y, R75W, S93N	94	88	1.07
pSE584 cc	R71L	314	293	1.07
pSE585 cd	T47I, W110L, A139T	168	189	0.89
pSE586 ce	V104I, S138T, V220I, F234I	150	138	1.10
pSE587 cf	G45R, A64V, R69K	182	192	0.95
pSE588 cg	S90N	251	243	1.03
pSE589 ch	G26D, W111OL, R233H	97	107	0.91
pSE590 ci	Q36R, V104I, P128S, C152W, T276A	194	171	1.13
pSE591 ci	S90N	164	147	1.12
pSE592 ck	C142Y, A302T	152	133	1.14
pSE593 cl	V2M,V56D	117	121	0.97
pSE594 cm	S41G, L87V, A139T, L206M,	122	212	0.58
pSE595 cn	A62V, A139D	150	122	1.23
pSE596 co	F176C	203	204	1.0
pSE597 cp	T149S	120	135	0.89
pSE598 cq	A64T, C142Y	105	90	1.17
pSE599 cr	A130V, C142Y, L224M, E238V, L293M	79	71	1.11
pSE600 cs	A16T, K154M, L206F	103	97	1.06

FIG. 6L

Plasmid	Mutations	[B2]	[B1]	[B2/B1]
pSE601 da	S41G, D48E, A61T, R71L, A89T, L136M, S138T, A139T, T149S. GI79S, V196A, E238D, F278L, P289L	17	137	0.12
pSE602 db	S41G, D48E, A61T, R71L, A89T, L136M, S138T, A139T, T149S. F176C, G179S, V196A, E238D, P289L	17	87	0.19
pSE603 dc	D48E, R71L, A89T, L136P, T149S. F176C, G179S, E238D, I280V	19	146	0.13
pSE604 dd	D48E, A61T, R71L, W110L, T149S, G179S, V196A, L206M, E238D, V271A, I280V	26	180	0.14
pSE605 de	D48E, A61T, R71L, A89T, L136M, S138T, A139T, T149S. G I 79S, VI196A, E238D, H279Q, P289L	22	158	0.14
pSE606 df	D48E, A61T, R71L, A89T, L136M, S138T, A139T, T149S. G179S, V196A, E238D, G287E, P289Q	16	94	0.17
pSE607 dg	D48E, A61T, R71L, A89T, L136M, S138T, A139T, T149S. G179S, V196A, E238D, P289L	21	243	0.13
PSE608 dh	D48E, A61T, R71L, A89T, A139T, T149S. F176C, G179S, V196A, E238D, V285G, P289L	18	146	0.12
pSE609 di	Q38R, D48E, A61T, R71L, L87V, A89T, L136M, S138T, A139T, T149S. G179S, V196A, E238D, P289L	20	150	0.13
pSE610 dj	D48E, A61T, L87V, A89T, W11OL,, S138T, A139T, T149S. G179S, V196A, E238D, P289L	14	110	0.13
pSE611 dk	D48E, A61T, R71L, A89T, L136M, S138T, A139T, T149S. G179S, V196A, E238D, P289L	25	184	0.14
pSE612 di	D48E, A89T, L136P, K154E, G179S, S231L, E238D	16	132	0.12

FIG. 6M

Plasmid	Mutations	[B2]	[B1]	B2/B1
pSE617 ea	D48E, A61T, R711, A89T, 1136P, T149S. F176C, G179S, V196A, E238D, 1280V	4	86	0.05
pSE620 eb	D48E, R711, A89T, 1136P, T149S. F176C, G179S, E238D, 1280V	17	179	0.08
pSE621 ec	Q36P, D48E, A61T, R71L, A89T, L136P, T149S. F176C, G179S, V196A, E238D, I280V	12	204	0.06
pSE622 ed	D48E, A61T, R711, A89T, A139T, T149S. F176C, G 179S, V196A, E238D, 1280V	16	226	0.07
pSE639 ee	V2M, D48E, A61T, R71L, A89T, L136P, T149S. F176C, G179S, V196A, E238D, I280V	15	225	0.07
pSE643 ef	D48E, A61T, R71L, A89T, L136P, T149S. F176C, G179S, V196A, E238D, I28OV, A302T	19	247	0.08
pSE646 eg	D48E, R71L, A89T, L136P, T149S. F176C, G179S, E238D, P289L	7	100	0.06
pSE655 eh	D48E, R71L, A89T, L136P, T149S. F176C, G179S, E238D, A302T	10	139	0.07
pSE657 ei	D48E, R71L, A89T, 1136P, T149S. F176C, G179S, V196A, E238D, I280V	9	157	0.06
pSE659 ej	D48E, A61T, R71L, A89T, L136P, T149S. F176C, G179S, V196A, E238D	7	105	0.06
pSE670 ek	V2M, D48E, R71L, A89T, L136P, T149S. F176C, G179S, V196A, E238D, I280V	7	104	0.07
pSE682 el	D48E, A61T, R71L, A89T, L136P, T149S. R162H, F176C, G179S, V196A, E238D, I280V	4	83	0.05
pSE683 em	D48E, R71L, A89T, V120A, L136P, T149S, K154E, G179S, S231L, E238D	8	154	0.05
pSE684 en	D48E, R71L, A89T, V120A, L136P, T149S. F176C, G179S, S231L, E238D, I280V	11	155	0.07
pSE685 eo	D48E, A61T, R71L, L87V, A89T, S90N, A139T, T149S. F176C, G179S, V196A, E238D, V285G, P289L	11	134	0.08
pSE686 ep	D48E, A61T, R71L, L87V, A89T, A139T, T149S. F176C, G179S, V196A, E238D, V285G, P289L	8	100	0.08
pSE579 eq	D48E, R71L, A89T, L136P, K154E, G179S, S231L, E238D	16	178	0.09
pSE580 er	D48E, R71L, A89T, V120A, L136P, K154E, F176C, G179S, S231L, E238D	14	149	0.09
pSE581 es	D48E, R71L, A89T, V120A, L136P, T149S, K154E, F176C, G179S, S231L, E238D	8	146	0.06