

FIG. 1

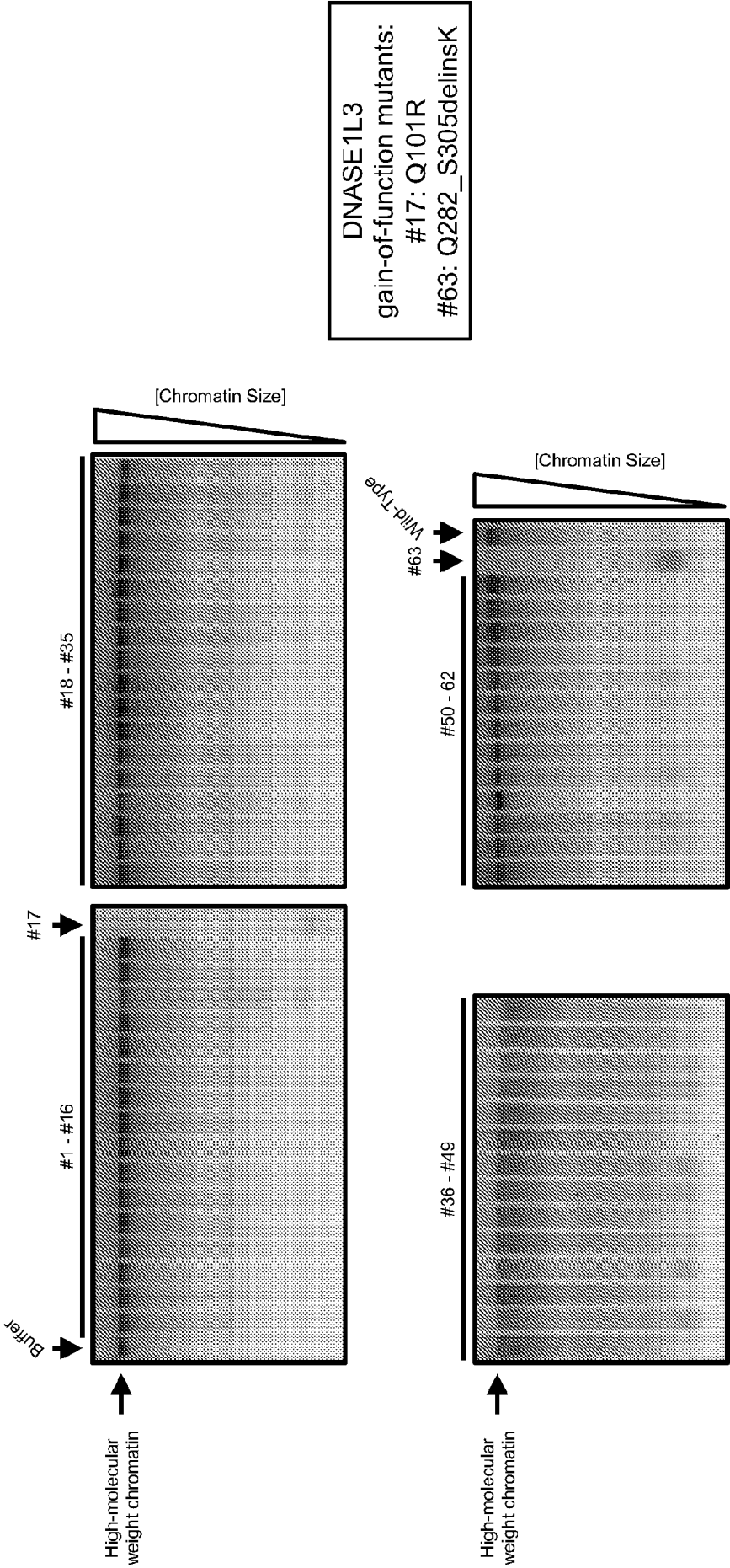


FIG. 2

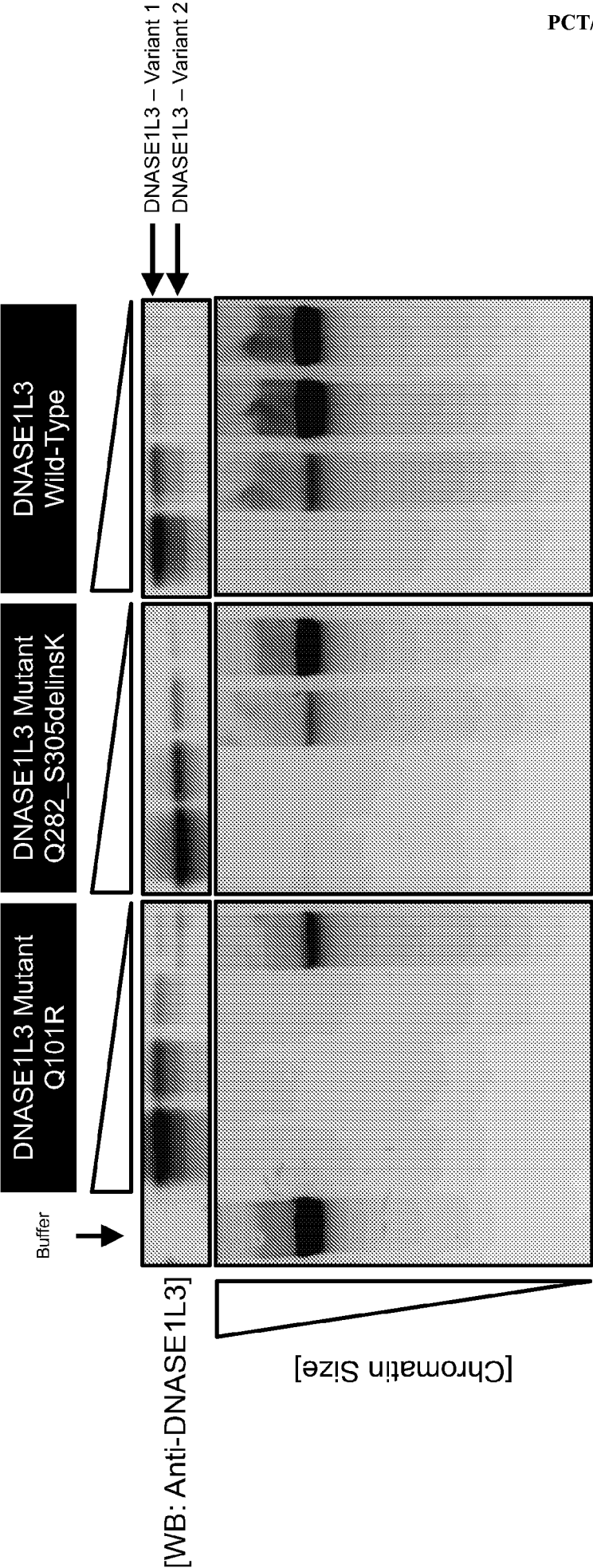


FIG. 3

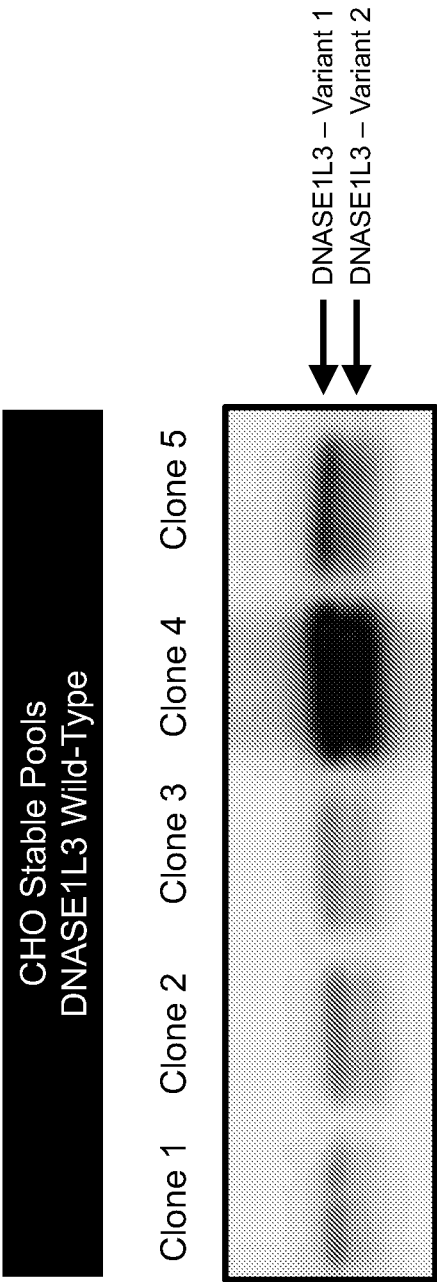
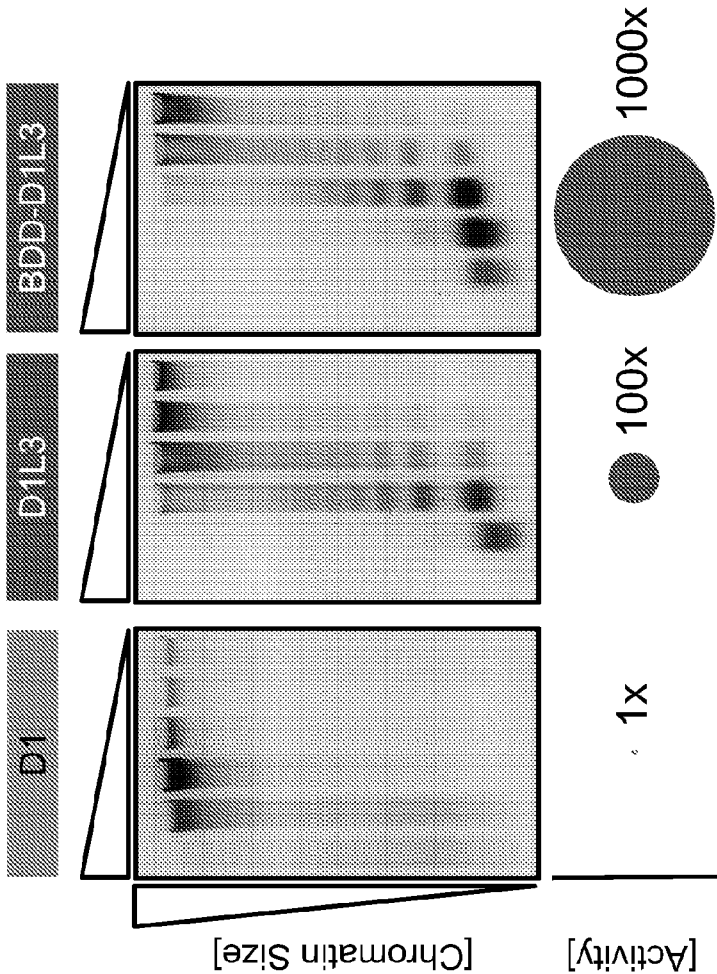
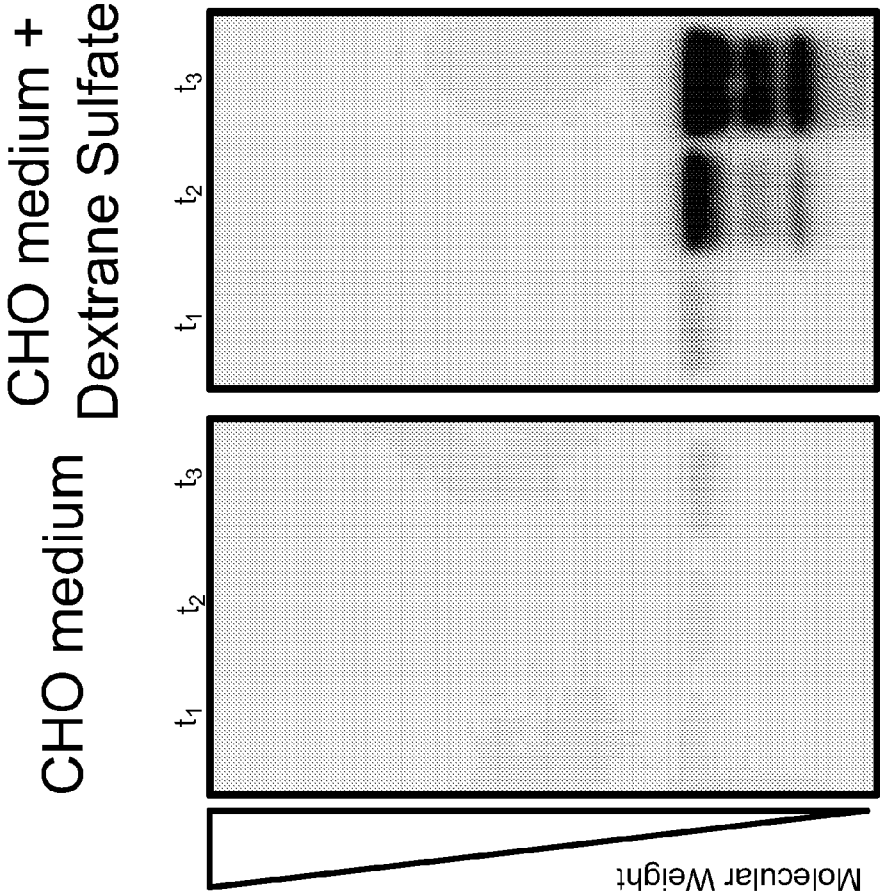


FIG. 4

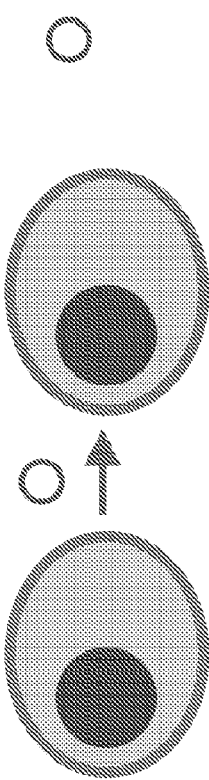


Input cDNA	C-Terminal Amino Acid Sequence	Relative Amount
	K291_S305del	54%
	K292_S305del	17%
	S293_S305del	29%
Wild-Type DNASE1L3		
Basic Domain Deleted DNASE1L3	F275Y	
	F279_K280delinsVM	
	Q282_S305delinsK	100%

FIG. 5



cGMP-Expression System:
Chinese Hamster Ovary Cells



Expression Vector:

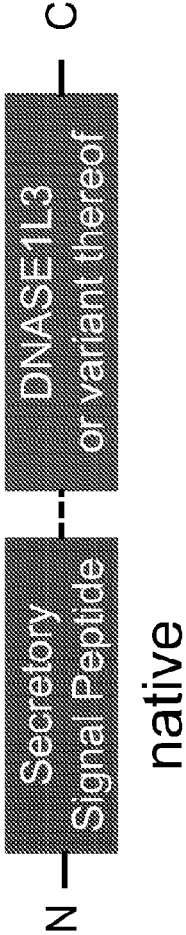


FIG. 6

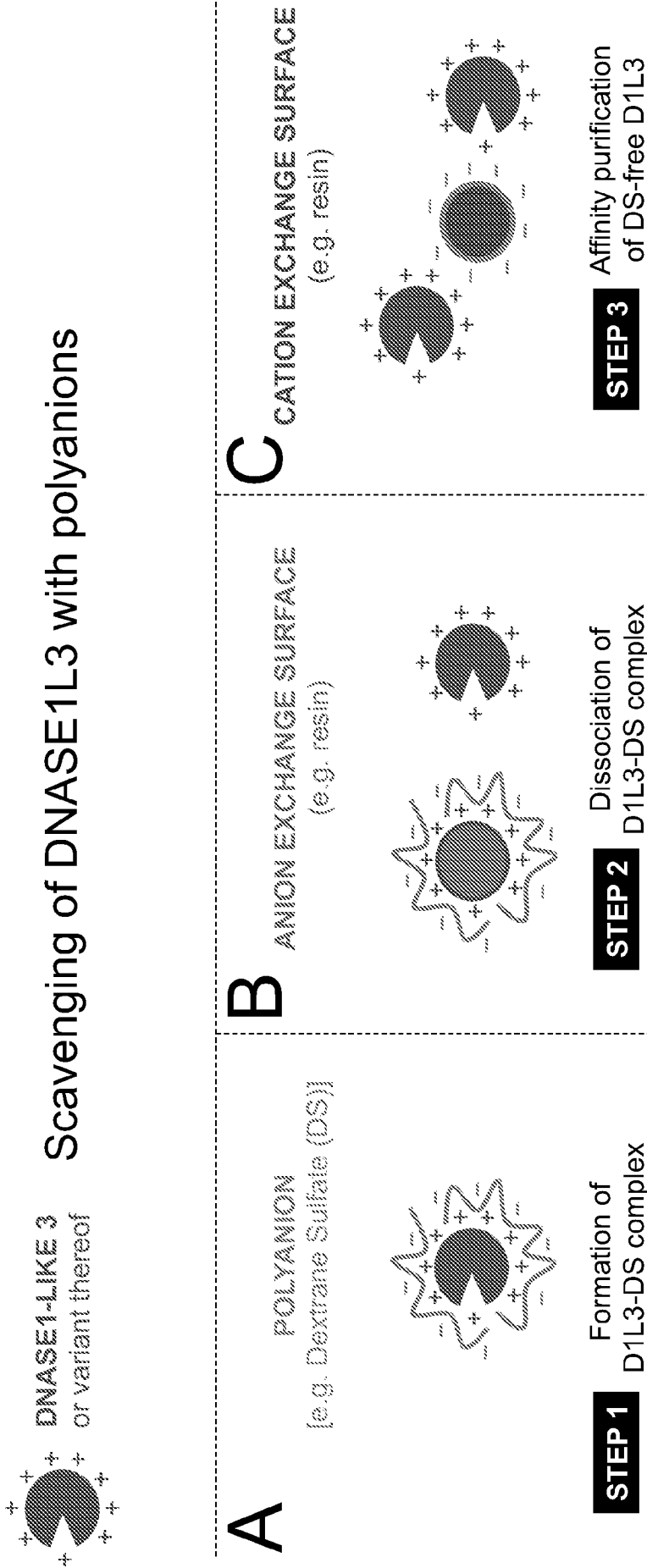


FIG. 7

Mutation of identified trypsin cleavage sites

#	AA in D1L3 (SEQ ID NO: 4)	Amino Acid Substitution	D1 (SEQ ID NO: 1) Building Block Mutation
1	R22	R22A/V/S, R22H/Q/E	M21_R22delinsLK
2	R29	R29A/V/S, R29H/Q/E	V28_S30delinsIQT
3	R51	R51A/V/S, R51H/Q/E	N/A (conserved residue)
4	R66	R66A/V/S, R66H/Q/E	N64_I70delinsHLTAVGK
5	R80	R80A/V/S, R80H/Q/E	R77_I83delinsQDAPD
6	R81	R81A/V/S, R81H/Q/E	R77_I83delinsQDAPD
7	R95	R95A/V/S, R95H/Q/E	N/A (conserved residue)
8	K99	R99A/V/S, R99H/Q/E	N/A (conserved residue)
9	R115	R115A/V/S, R115H/Q/E	V113_R115delinsAVD
10	K147	K147A/V/S, K147H/Q/E	K147_D148delinsRE
11	K163	K163A/V/S, K163H/Q/E	K163A
12	K180	K180A/V/S, K180H/Q/E	K180_A181delinsGL
11	R208	R208A/V/S, R208H/Q/E	R208W
12	R212	R212A/V/S, R212H/Q/E	R212T
13	R235	K235A/V/S, K235H/Q/E	N/A (conserved residue)
14	R239	K239A/V/S, K239H/Q/E	L238_R239delinsVA
15	K250	K250A/V/S, K250H/Q/E	K250D
16	K262	K262A/V/S, K262H/Q/E	K262G

Selection of Amino
Acid Substitution:
Grantham's distance

Arg (R) / His (H): 29

Arg (R) / Glu (E): 54

Arg (R) / Gln (Q): 53

Lys (K) / His (E): 32

Lys (K) / Gln (H): 54

Lys (K): Glu (E): 56

FIG. 8

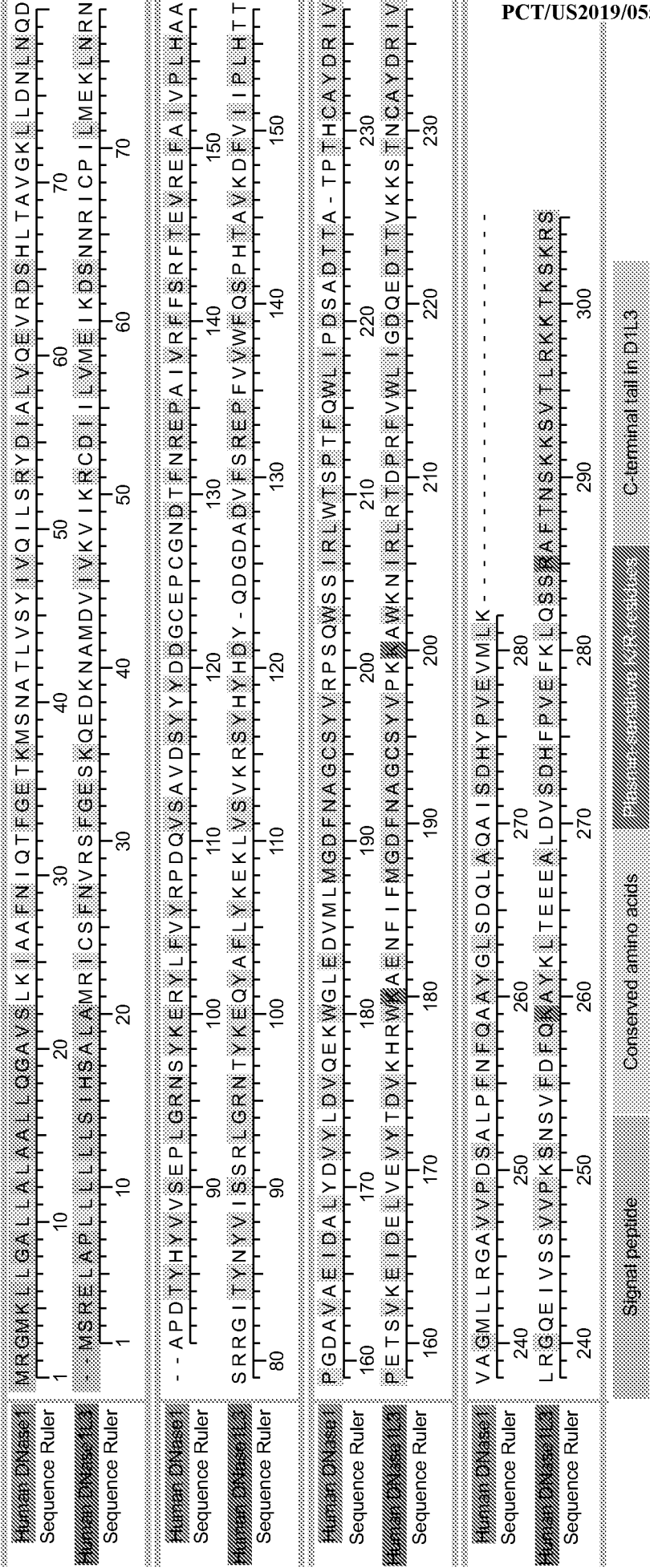


FIG. 9

Mutation of predicted plasmin cleavage sties

#	AA in D1L3 (SEQ ID NO: 4)	Amino Acid Substitution	D1 (SEQ ID NO: 1) Building Block Mutation
1	K180	K180A, K180H/Q/E	K180_A181delinsGL
2	K200	K200A, K200H/Q/E	P198_A201delinsRPSQ
3	K259	K259A, K259H/Q/E	A259A
4	R285	R285A, R285H/Q/E	N/A (absent in D1)

FIG. 10

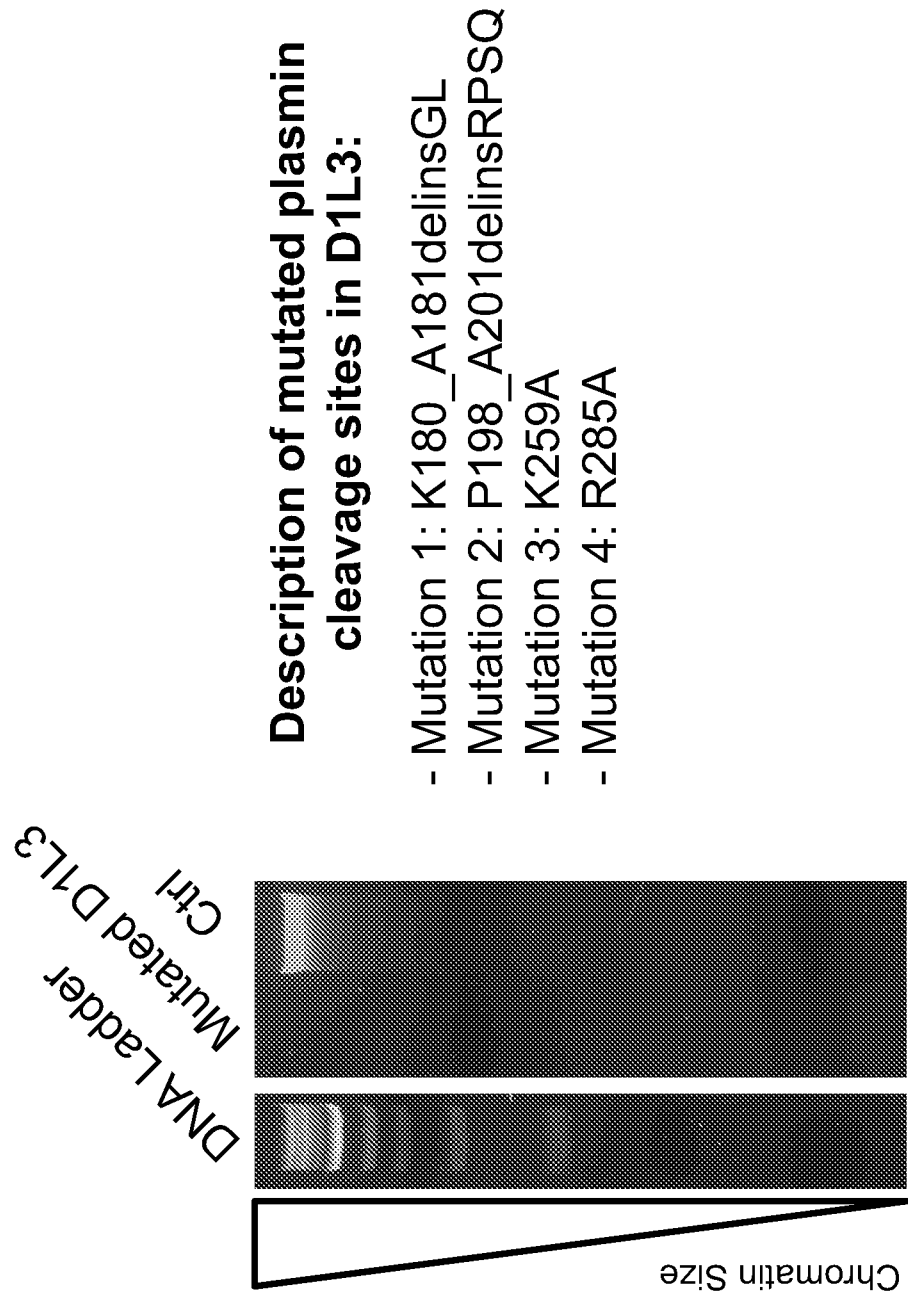


FIG. 11

Mutation of identified plasmin cleavage sites

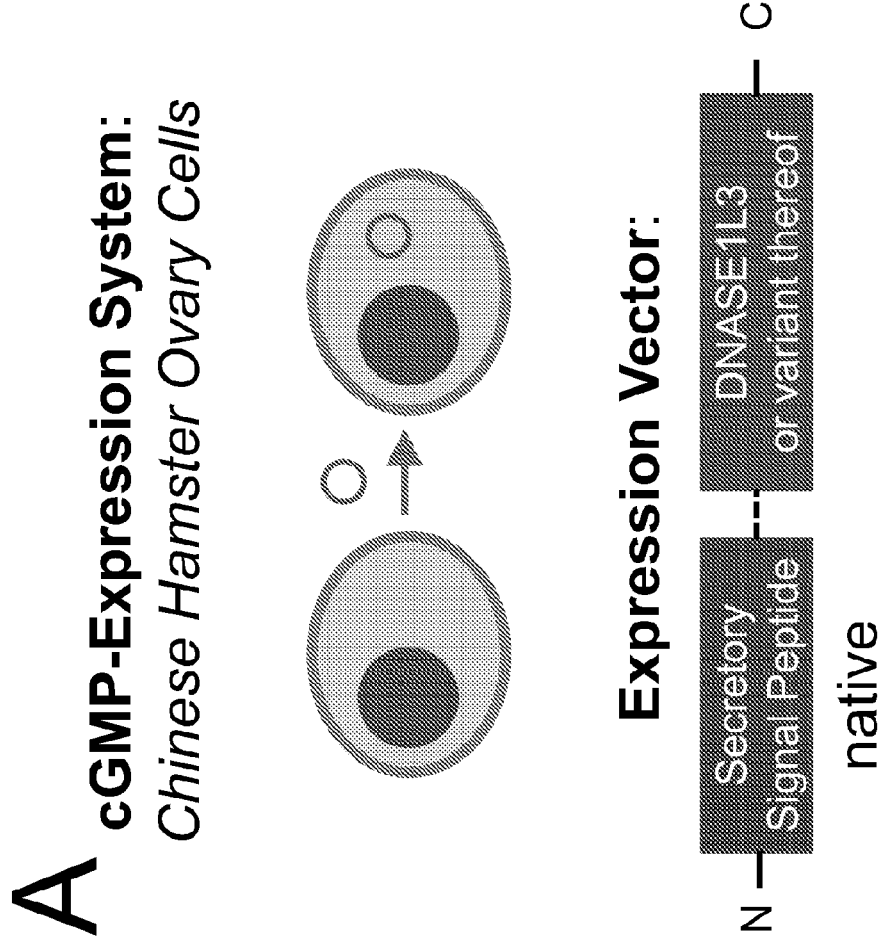
#	AA in D1L3 (SEQ ID NO: 4)	Amino Acid Substitution	D1 (SEQ ID NO: 1) Building Block Mutation
1	R22	R22H/Q/E	M21_R22delinsLK
2	R29	R29H/Q/E	V28_S30delinsQT
3	K45	K45H/Q/E	N/A (conserved residue)
4	K47	K47H/Q/E	K47_K50delinsQILS
5	K74	K74H/Q/E	M72_K74delinsLDN
6	R81	R81H/Q/E	R77_I83delinsQDAPD
7	R92	R92H/Q/E	S91_R92delinsEP
8	K107	K107H/Q/E	K107_L110delinsRPDQ
9	K176	K176H/Q/E	K176_R178delinsQEK
10	R212	R212H/Q/E	R212T
11	K226	R226H/Q/E	V225_S228delinsATP
12	K227	K227H/Q/E	V225_S228delinsATP
13	K250	K250H/Q/E	K250D
14	K259	K259H/Q/E	K259A
15	K262	K262H/Q/E	K262G

Selection of Amino
Acid Substitution:
Grantham's distance

Arg (R) / His (H): 29
Arg (R) / Glu (E): 54
Arg (R) / Gln (Q): 53

Lys (K) / His (E): 32
Lys (K) / Gln (H): 54
Lys (K): Glu (E): 56

FIG. 12



B

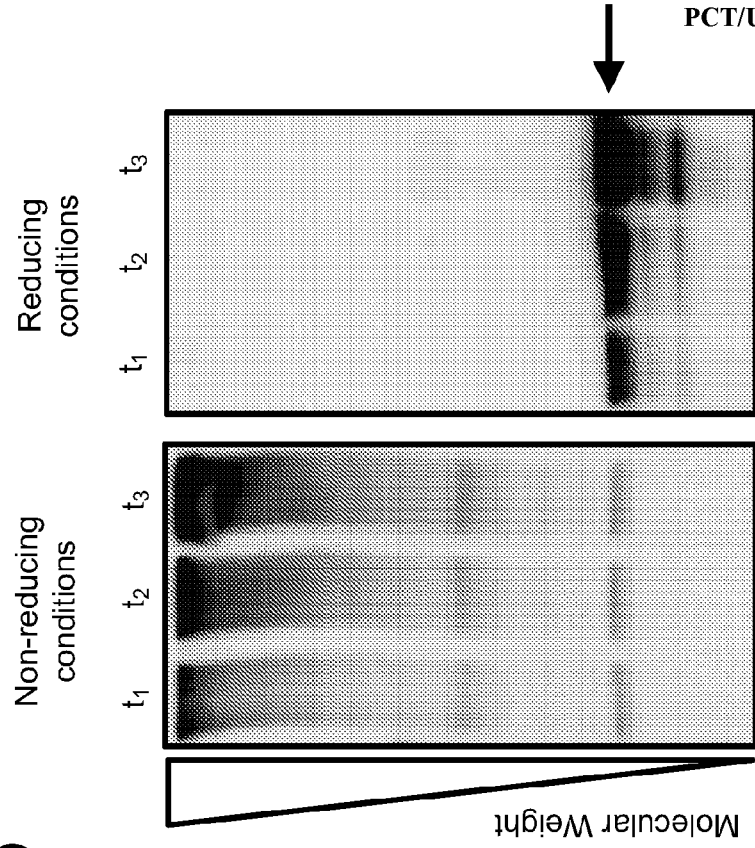


FIG. 13

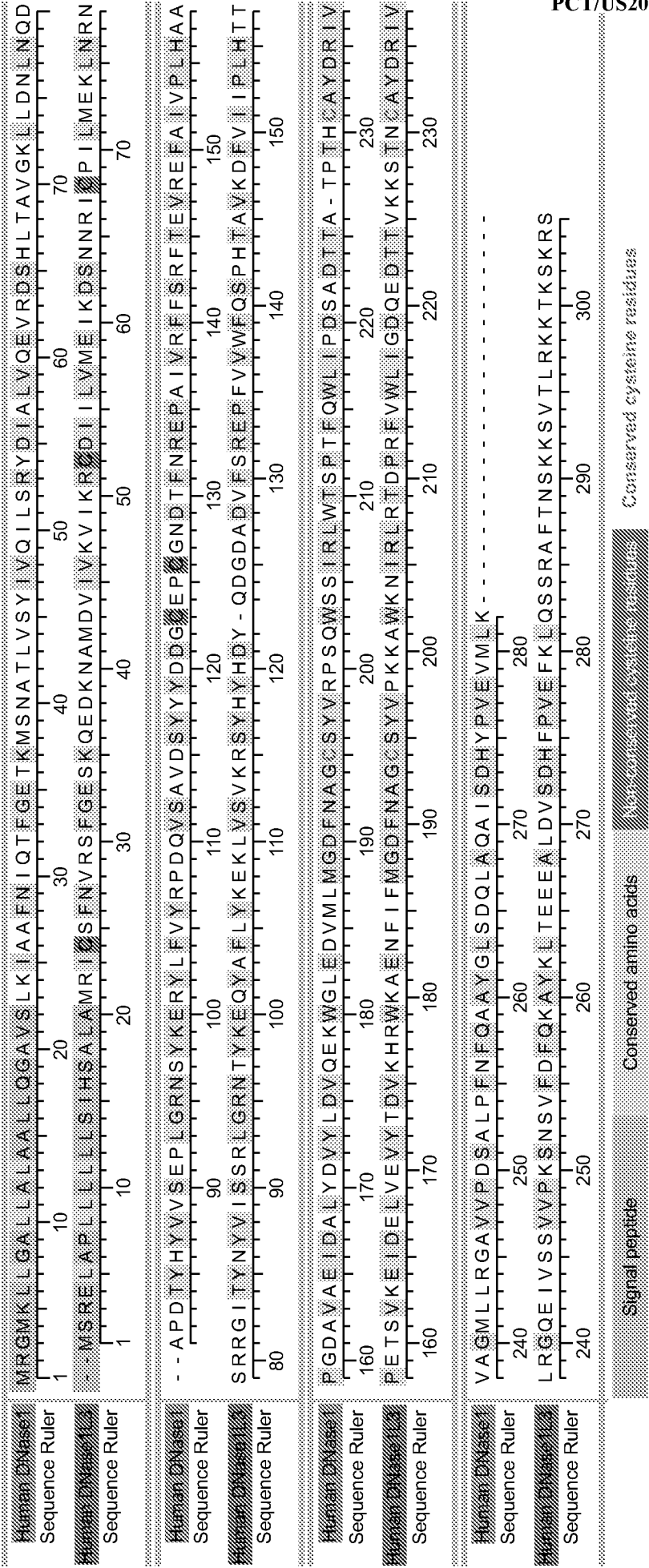


FIG. 14

Mutation of identified plasmin cleavage sites

#	AA in D1L3 (SEQ ID NO: 4)	Amino Acid Substitution	D1 (SEQ ID NO: 1) Building Block Mutation
1	C24	C24A/S/G	C24_S25delinsAA
2	C52	C52A/S/G	C52Y
3	C68	C68A/S/G	N64_I70delinsHLTAVGK
4	C194	C194A/S/G	N/A (conserved residue)
5	C231	C231A/S/G	N/A (conserved residue)

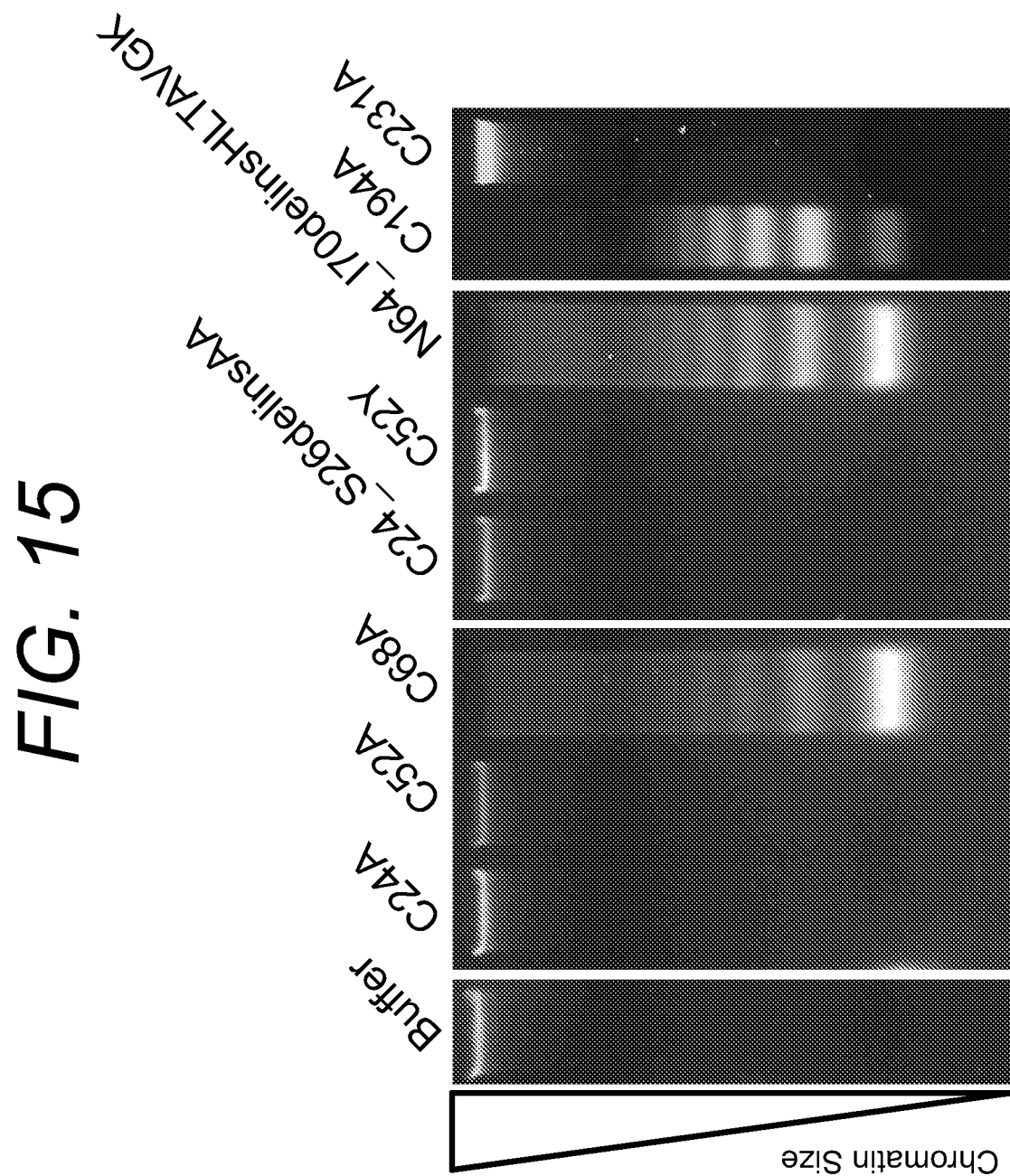


FIG. 16

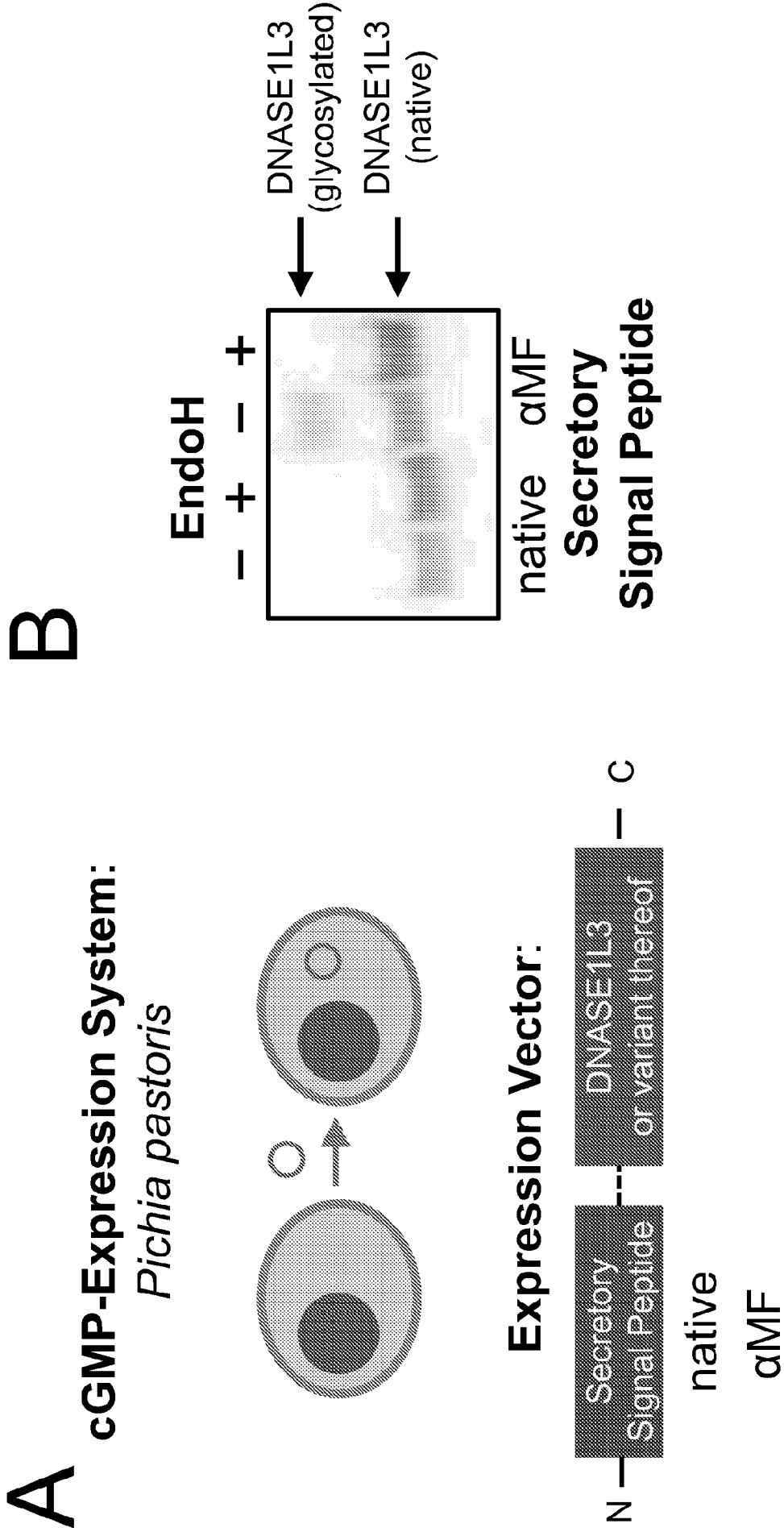


FIG. 17

A **cGMP-Expression System:**
Pichia pastoris

Expression Vector:

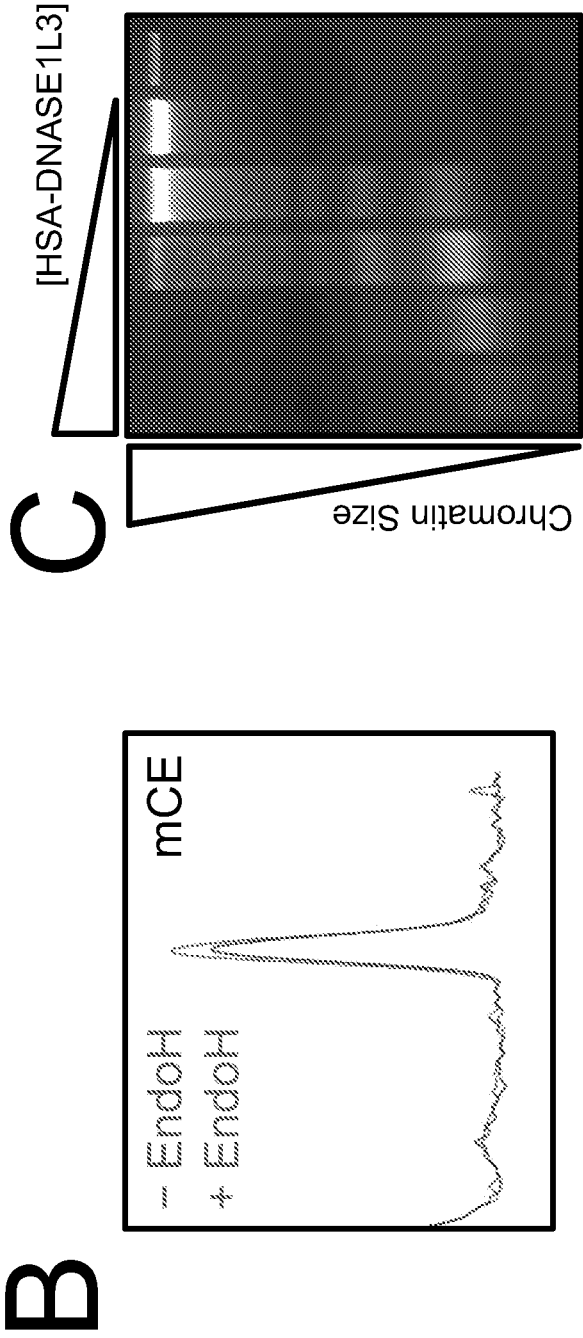
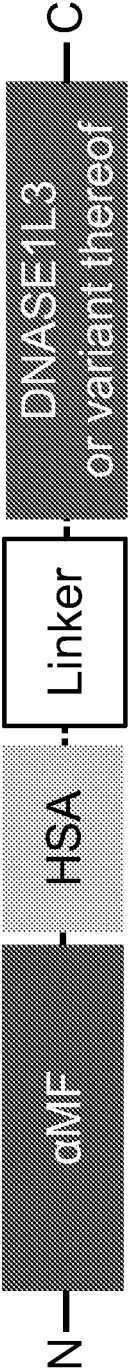


FIG. 18

Expression Vector		Expression Level (rel. Units)
SEQ ID NO: 14	BDD-D1L3	4.3±0.5
SEQ ID NO: 17	HSA -	4.3±0.4
SEQ ID NO: 18	HSA L1	12±1.9
SEQ ID NO: 19	HSA L2	32±3.2
SEQ ID NO: 20	BDD-D1L3 L1	<1
SEQ ID NO: 21	BDD-D1L3 L2	1.8±0.2
SEQ ID NO: 4	D1L3	1.1±0.1
SEQ ID NO: 22	HSA L2	22±1.3

Linker	Sequence	Length
SEQ ID NO: 31	GGGGS	5 AA
SEQ ID NO: 32	(GGGGS) ₃	15 AA

FIG. 19

SEQ ID NO: 22	Expression Vector			Expression Level (rel. Units)
	HSA	L2	D1L3	
	HSA	L3	D1L3	
	HSA	L4	D1L3	
SEQ ID NO: 23				17±5.5
SEQ ID NO: 24				22±6.9
SEQ ID NO: 24				11±4.9

SEQ ID NO: 32	Linker	Sequence	Length	Property
	L2	(GGGGS) ₃	15	flexible
	L3	(AP) ₇	14	rigid
	L4	A(EAAAK) ₂ A	12	rigid

FIG. 20

Expression Vector		Linker		Sequence	Length
SEQ ID NO: 25	HSA	L5	SEQ ID NO: 35	SGSGSS	7
SEQ ID NO: 26	HSA	L6	SEQ ID NO: 36	S(GGS) ₄ GSS	16
SEQ ID NO: 27	HSA	L7	SEQ ID NO: 37	S(GGS) ₉ GSS	31

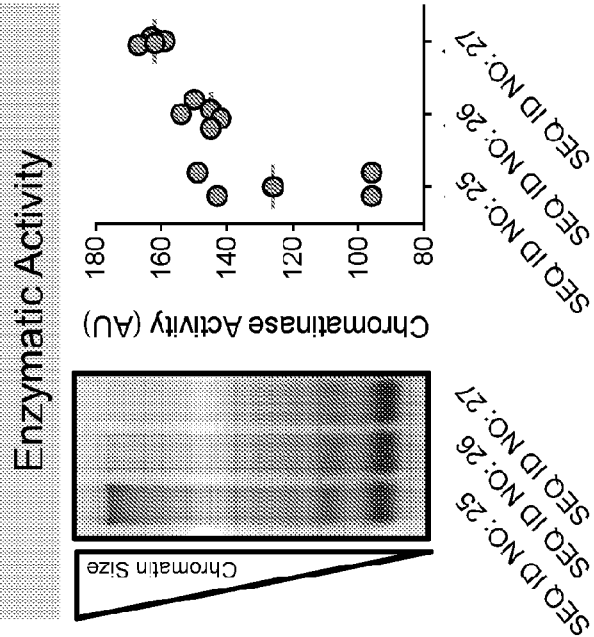
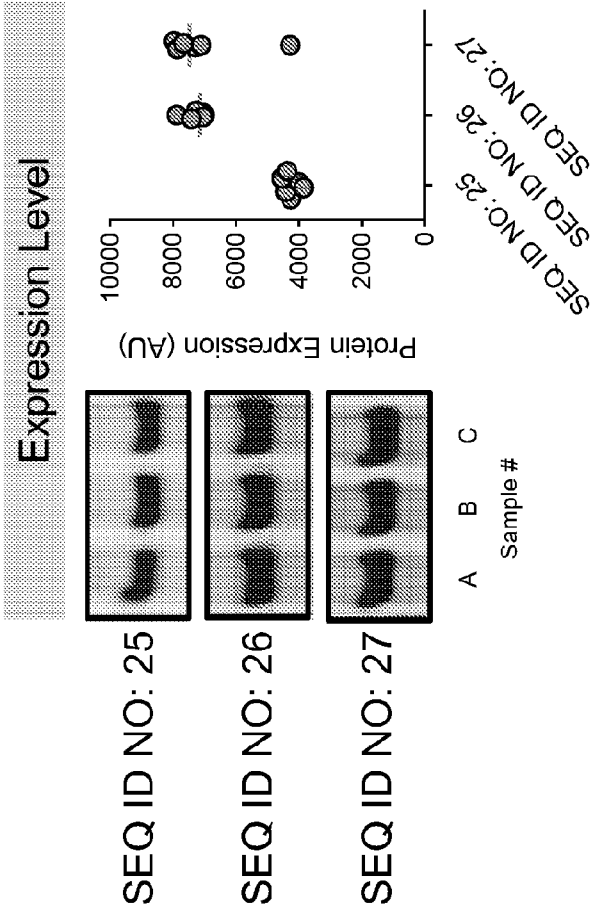
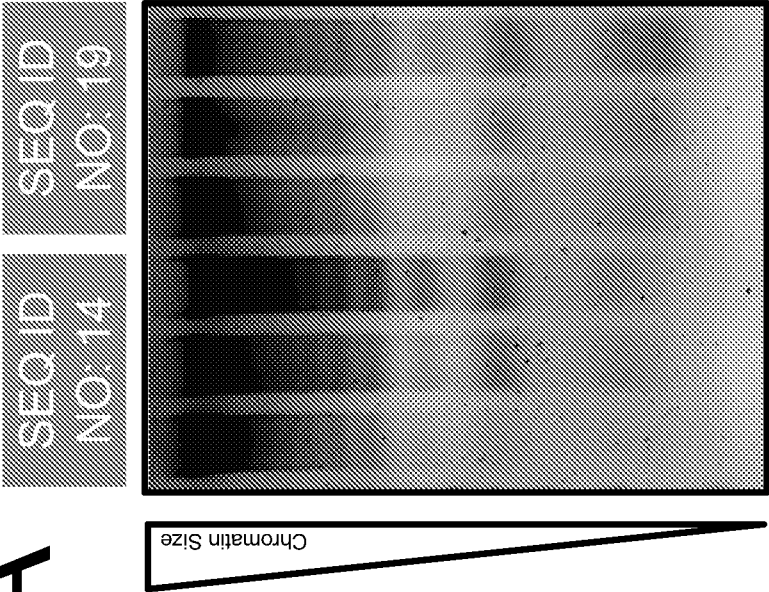
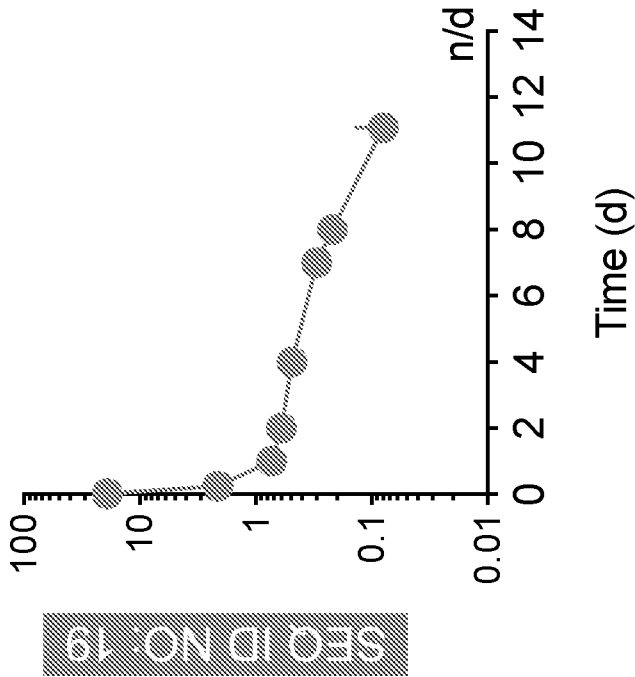


FIG. 21

A



B

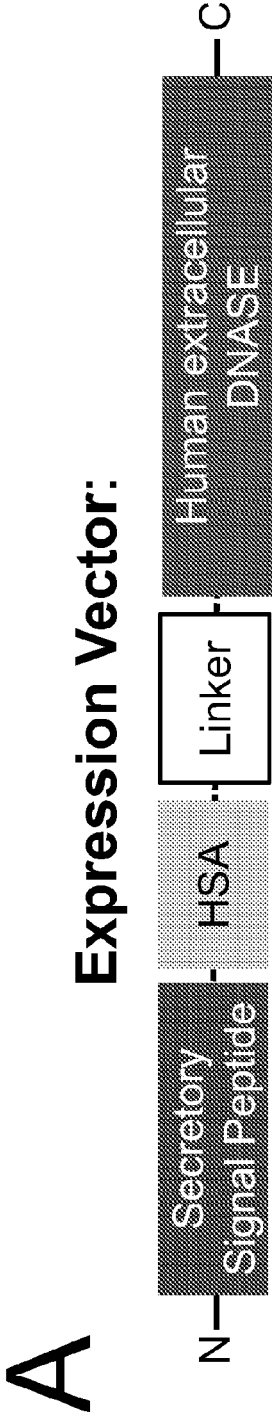


$t_{\max} = 5 \text{ Minutes}$
 $C_{\max} = 19 \text{ } \mu\text{g/ml}$
 $t_{1/2} = 3.3 \text{ Days}$

FIG. 22

		Expression Vector		Expression Level (rel. Units)
SEQ ID NO: 27	HSA	L7	BDD-D1L3	32±5.9
SEQ ID NO: 28	HSA	L7	BDD-D1L3	30±8.3
			L8	
			HSA	
Linker		Sequence	Length	
SEQ ID NO: 37	L7	S(GGS) ₉ GSS	31	
SEQ ID NO: 38	L8	(GGS) ₉ GS	29	

FIG. 23



B

Factor Xlla Cleavable Linker:

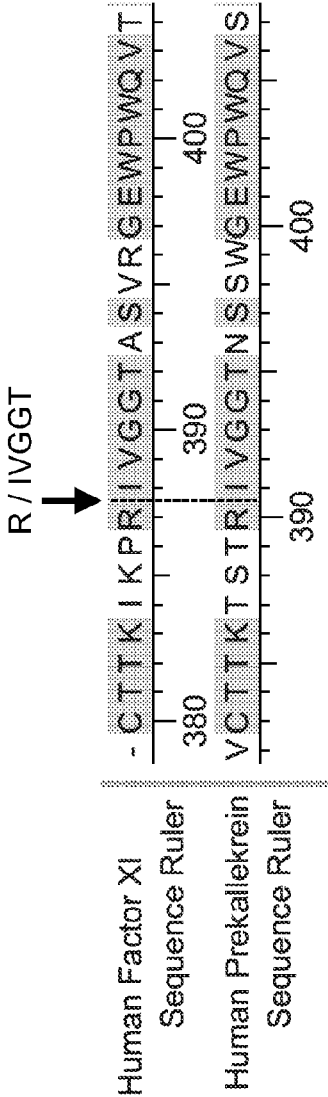


FIG. 24

Expression Vector:

