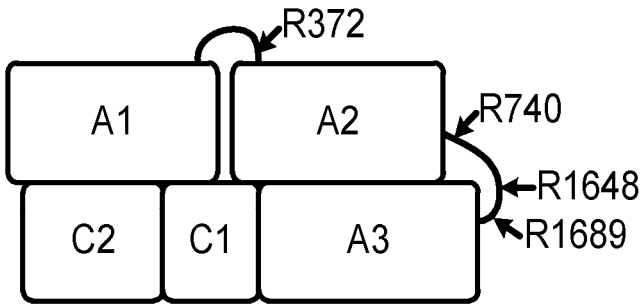
FIG. 1A

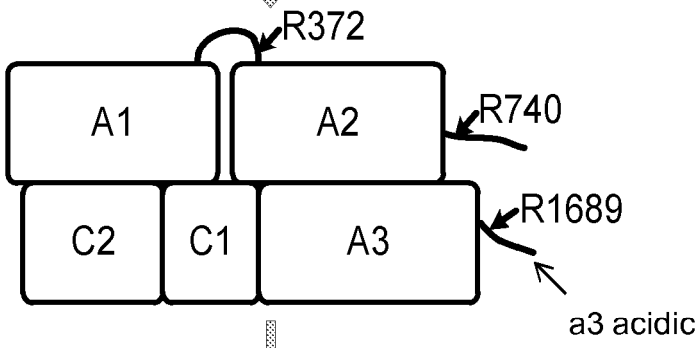
scFVIII



Intracellular processing

FIG. 1B

FVIII



Clotting event

FIG. 1C

FVIIIa

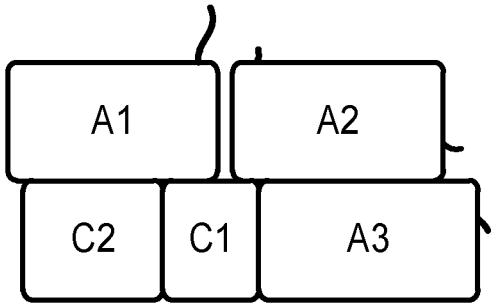


FIG. 1

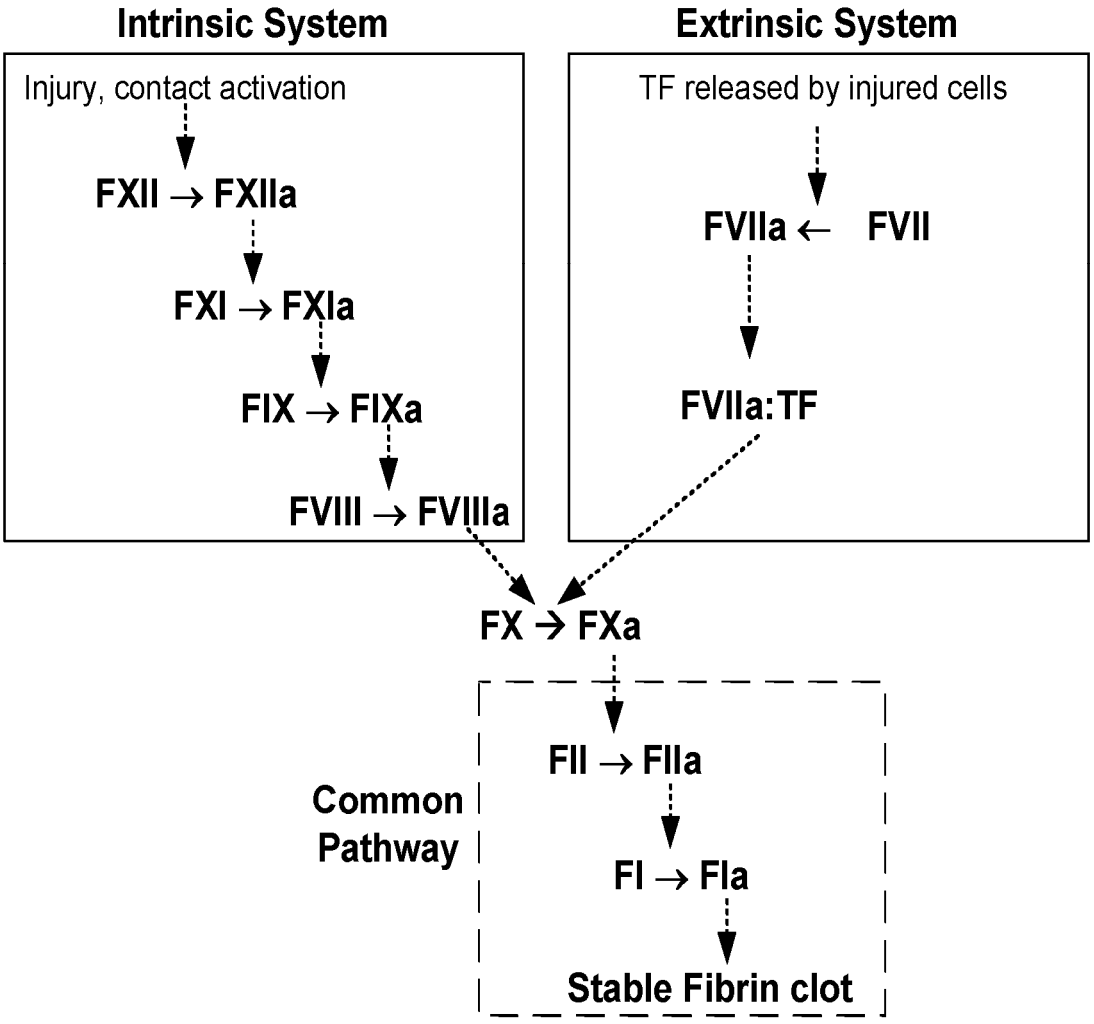

Intrinsic System
Injury, contact activation
FXII → FXIIa
FXI → FXIa
FIX → FIXa
FVIII → FVIIIa
Extrinsic System
TF released by injured cells
FVIIa ← FVII
FVIIa:TF
FX → FXa
Common Pathway
FII → FIIa
FI → FIa
Stable Fibrin clot

FIG. 2

ATRRYYLGAVELSWDYMQSDLGELPVDARFPPRVPKSFPFNTSVVYKKTLFVEFTDHLFN
IAKPRPPWMGLLGPTIQAEVYDTVVITLKNMASHPVSLHAVGVSYWKASEGAEYDDQTSQ
REKEDDKVFPGGSHTYVWQVLKENGPMASDPLCLTYSYLSHVDLVKDLNSGLIGALLVCR
EGSLAKEKTQTLHKFILLFAVFDEGKSWHSETKNSLMQDRDAASARAWPKMHTVNGYVNR
SLPGLIGCHRKSVYWHVIGMGTTPEVHSIFLEGHTFLVRNHRQASLEISPITFLTAQTLL
MDLGQFLLFCHISSHQHDGMEAYVKVDSCPEEPQLRMKNNEEAEDYDDDLTDSEMDVVRF
DDDNSPSFIQIRSVAKKHPKTWVHYIAAEEEDWDYAPLVLAPDDRSYKSQYLNNGPQRIG
RKYKKVRFMAYTDETFKTREAIQHESGILGPLLYGEVGDTLLIIFKNQASRPYNIYPHGI
TDVRPLYSRRLPKGVKHLKDFPILPGEIFKYKWTVTVEDGPTKSDPRCLTRYYSSFVNME
RDLASGLIGPLLICYKESVDQRGNQIMSDKRNVILFSVFDENRSWYLTENIQRFLPNPAG
VQLEDPEFQASNIMHSINGYVFDSLQLSVCLHEVAYWYILSIGAQTDFLSVFFSGYTFKH
KMVYEDTLTLFPFSGETVFMSMENPGLWILGCHNSDFRNRGMTALLKVSSCDKNTGDYYE
DSYEDISAYLLSKNNAIEPRSFSQNSRHPSTRQKQFNATTIPENDIEKTDPWFAHRTPMP
KIQNVSSSDLLMLLRQSPTPHGLSLSDLQEAKYETFSDDPSPGAIDSNNSLSEMTHFRPQ
LHHSGDMVFTPESGLQLRLNEKLGTTAATELKKLDFKVSSTSNNLISTIPSDNLAAGTDN
TSSLGPPSMPVHYDSQLDTTLFGKKSSPLTESGGPLSLSEENNDSKLLESGLMNSQESSW
GKNVSSTESGRLFKGKRAHGPALLTKDNALFKVSISLLKTNKTSNNSATNRKTHIDGPSL
LIENSPSVWQNILESDTEFKKVTPLIHDRMLMDKNATALRLNHMSNKTTSSKNMEMVQQK
KEGPIPPDAQNPDMSFFKMLFLPESARWIQRTHGKNSLNSGQGPSPKQLVSLGPEKSVEG
QNFLSEKNKVVVGKGEFTKDVGLKEMVFPSSRNLFLTNLDNLHENNTHNQEKKIQEEIEK
KETLIQENVVLPQIHTVTGTKNFMKNLFLLSTRQNVEGSYDGAYAPVLQDFRSLNDSTNR
TKKHTAHFSKKGEEENLEGLGNQTKQIVEKYACTTRISPNTSQQNFVTQRSKRALKQFRL
PLEETELEKRIIVDDTSTQWSKNMKHLTPSTLTQIDYNEKEKGAITQSPLSDCLTRSHSI
PQANRSPLPIAKVSSFPSIRPIYLTRVLFQDNSSHLPAASYRKKDSGVQESSHFLQGAKK
NNLSLAILTLEMTGDQREVGSLGTSATNSVTYKKVENTVLPKPDLPKTSGKVELLPKVHI
YQKDLFPTETSNGSPGHLDLVEGSLLQGTEGAIKWNEANRPGKVPFLRVATESSAKTPSK
LLDPLAWDNHYGTQIPKEEWKSQEKSPEKTAFKKKDTILSLNACESNHAIAAINEGQNKP
EIEVTWAKQGRTERLCSQNPPVLKRHQREITRTTLQSDQEEIDYDDTISVEMKKEDFDIY
DEDENQSPRSFQKKTRHYFIAAVERLWDYGMSSSPHVLRNRAQSGSVPQFKKVVFQEFTD
GSFTQPLYRGELNEHLGLLGPYIRAEVEDNIMVTFRNQASRPYSFYSSLISYEEDQRQGA
EPRKNFVKPNETKTYFWKVQHHMAPTKDEFDCKAWAYFSDVDLEKDVHSGLIGPLLVCHT
NTLNPAHGRQVTVQEFALFFTIFDETKSWYFTENMERNCRAPCNIQMEDPTFKENYRFHA
INGYIMDTLPGLVMAQDQRIRWYLLSMGSNENIHSIHFSGHVFTVRKKEEYKMALYNLYP
GVFETVEMLPSKAGIWRVECLIGEHLHAGMSTLFLVYSNKCQTPLGMASGHIRDFQITAS
GQYGQWAPKLARLHYSGSINAWSTKEPFSWIKVDLLAPMIIHGIKTQGARQKFSSLYISQ
FIIMYSLDGKKWQTYRGNSTGTLMVFFGNVDSSGIKHNIFNPPIIARYIRLHPTHYSIRS
TLRMELMGCDLNSCSMPLGMESKAISDAQITASSYFTNMFATWSPSKARLHLQGRSNAWR
PQVNNPKEWLQVDFQKTMKVTGVTTQGVKSLLTSMYVKEFLISSSQDGHQWTLFFQNGKV
KVFQGNQDSFTPVVNSLDPPLLTRYLRIHPQSWVHQIALRMEVLGCEAQDLY

FIG. 3

ATRRYYLGAVELSWDYMQSDLGELPVDARFPPRVPKSFPFNTSVVYKKTLFV
EFTVHLFNIAKPRPPWMGLLGPTIQAEVYDTVVITLKNMASHPVSLHAVGVS
YWKASEGAEYDDQTSQREKEDDKVFPGGSHTYVWQVLKENGPMASDPLCLTY
SYLSHVDLVKDLNSGLIGALLVCREGSLAKEKTQTLHKFILLFAVFDEGKSW
HSETKNSLMQDRDAASARAWPKMHTVNGYVNRSLPGLIGCHRKSVYWHVIGM
GTTPEVHSIFLEGHTFLVRNHRQASLEISPITFLTAQTLLMDLGQFLLFCHI
SSHQHDGMEAYVKVDSCPEEPQLRMKNNEEAEDYDDDLTDSEMDVVRFDDDN
SPSFIQIRSVAKKHPKTWVHYIAAEEEDWDYAPLVLAPDDRSYKSQYLNNGP
QRIGRKYKKVRFMAYTDETFKTREAIQHESGILGPLLYGEVGDTLLIIFKNQ
ASRPYNIYPHGITDVRPLYSRRLPKGVKHLKDFPILPGEIFKYKWTVTVEDG
PTKSDPRCLTRYYSSFVNMERDLASGLIGPLLICYKESVDQRGNQIMSDKRN
VILFSVFDENRSWYLTENIQRFLPNPAGVQLEDPEFQASNIMHSINGYVFDS
LQLSVCLHEVAYWYILSIGAQTDFLSVFFSGYTFKHKMVYEDTLTLFPFSGE
TVFMSMENPGLWILGCHNSDFRNRGMTALLKVSSCDKNTGDYYEDSYEDISA
YLLSKNNAIEPRSFSQNPPVLKRHQREITRTTLQSDQEEIDYDDTISVEMKK
EDFDIYDEDENQSPRSFQKKTRHYFIAAVERLWDYGMSSSPHVLRNRAQSGS
VPQFKKVVFQEFTDGSFTQPLYRGELNEHLGLLGPYIRAEVEDNIMVTFRNQ
ASRPYSFYSSLISYEEDQRQGAEPRKNFVKPNETKTYFWKVQHHMAPTKDEF
DCKAWAYFSDVDLEKDVHSGLIGPLLVCHTNTLNPAHGRQVTVQEFALFFTI
FDETKSWYFTENMERNCRAPCNIQMEDPTFKENYRFHAINGYIMDTLPGLVM
AQDQRIRWYLLSMGSNENIHSIHFSGHVFTVRKKEEYKMALYNLYPGVFETV
EMLPSKAGIWRVECLIGEHLHAGMSTLFLVYSNKCQTPLGMASGHIRDFQIT
ASGQYGQWAPKLARLHYSGSINAWSTKEPFSWIKVDLLAPMIIHGIKTQGAR
QKFSSLYISQFIIMYSLDGKKWQTYRGNSTGTLMVFFGNVDSSGIKHNIFNP
PIIARYIRLHPTHYSIRSTLRMELMGCDLNSCSMPLGMESKAISDAQITASS
YFTNMFATWSPSKARLHLQGRSNAWRPQVNNPKEWLQVDFQKTMKVTGVTTQ
GVKSLLTSMYVKEFLISSSQDGHQWTLFFQNGKVKVFQGNQDSFTPVVNSLD
PPLLTRYLRIHPQSWVHQIALRMEVLGCEAQDLY

FIG. 4

Fig. 5A

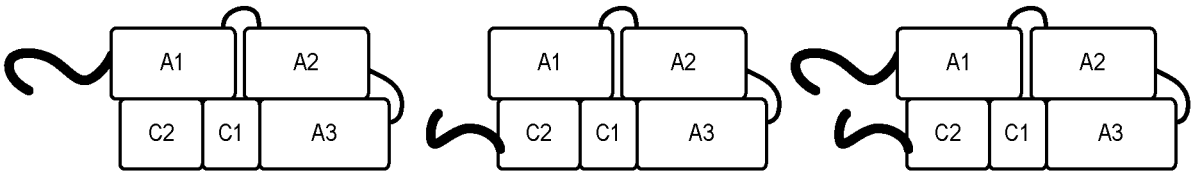


Fig. 5B

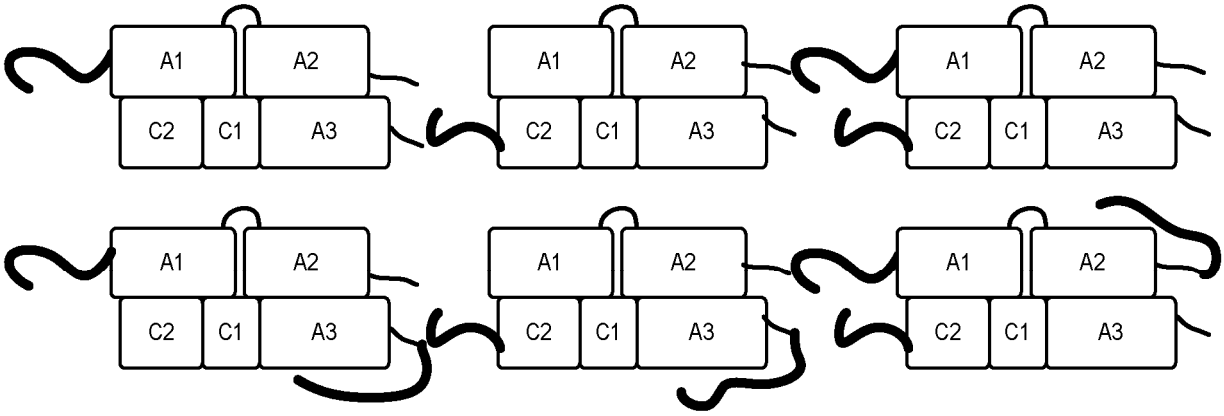


Fig. 5C

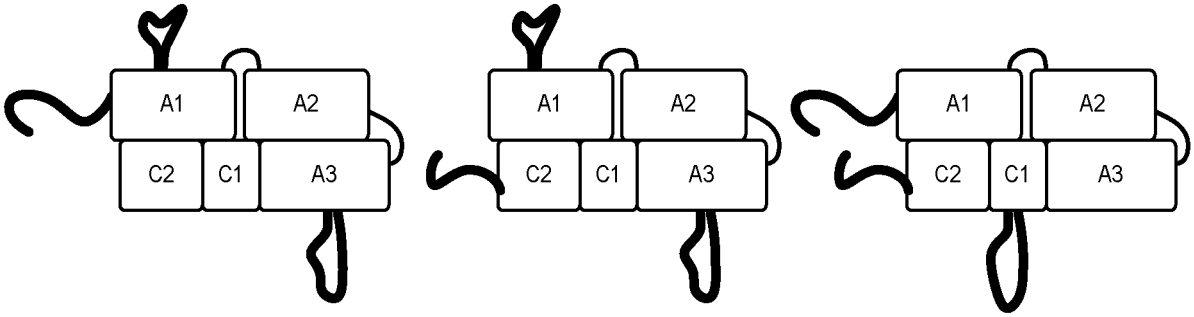


Fig. 5D

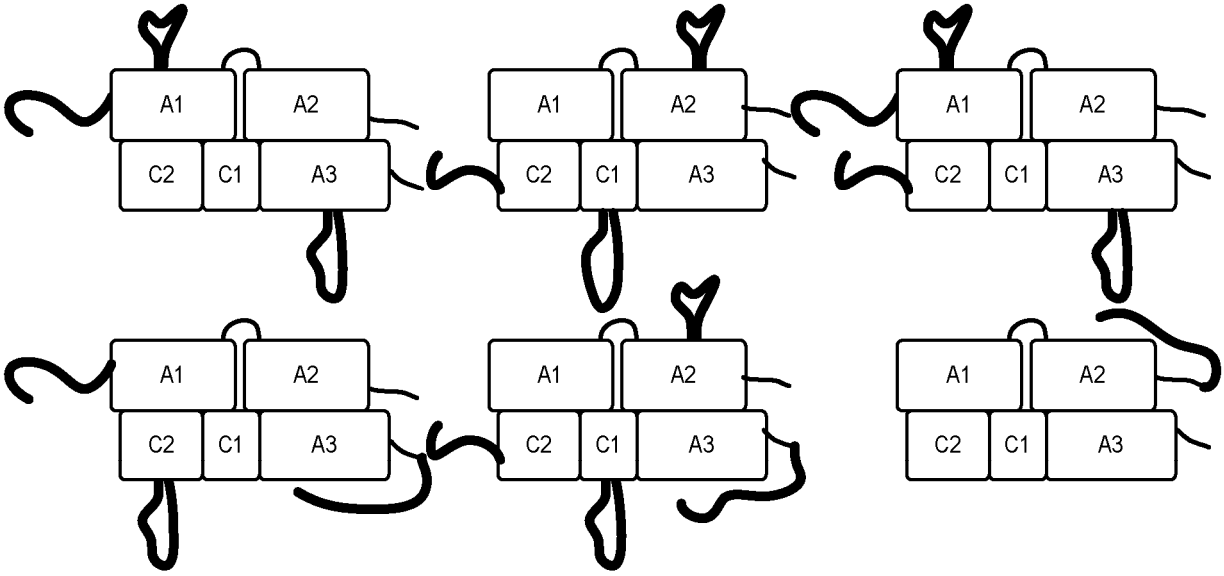


FIG. 5

Fig. 6A

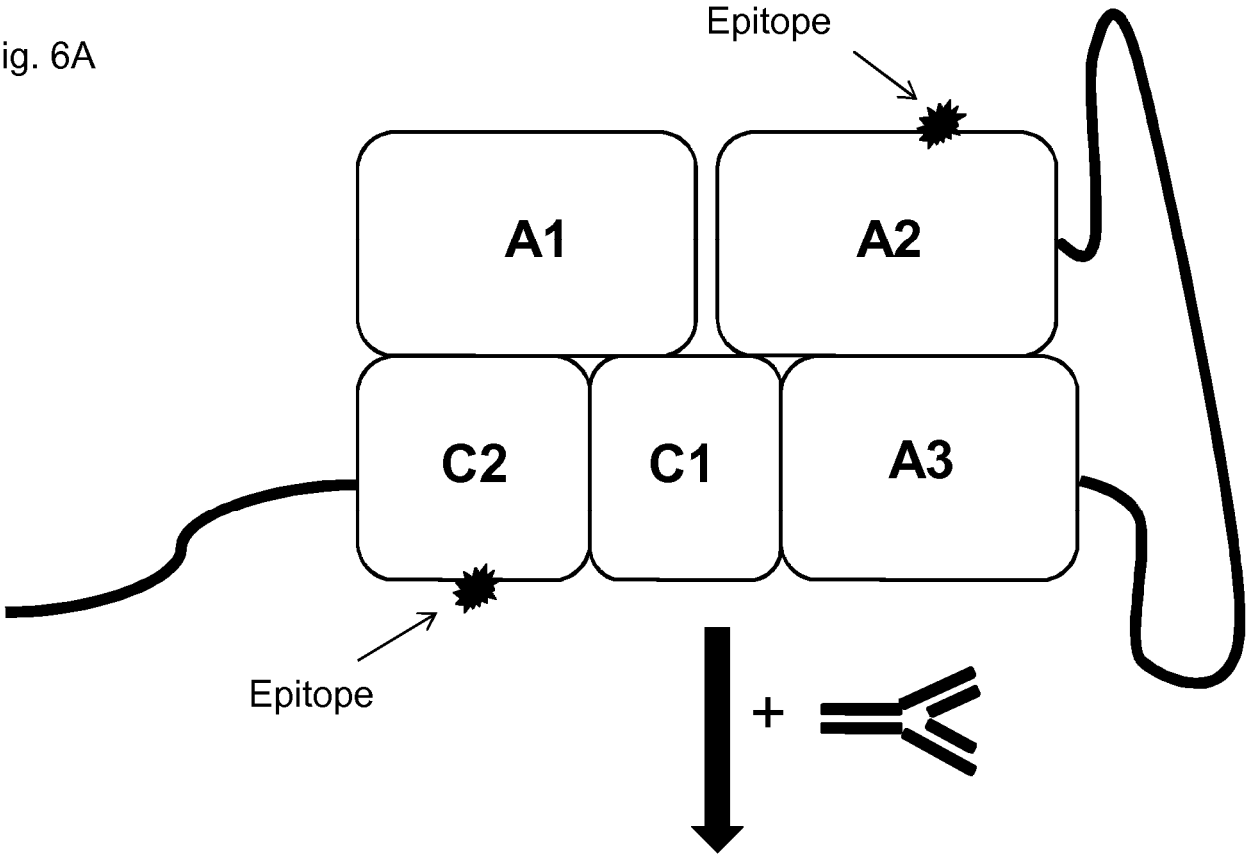


Fig. 6B

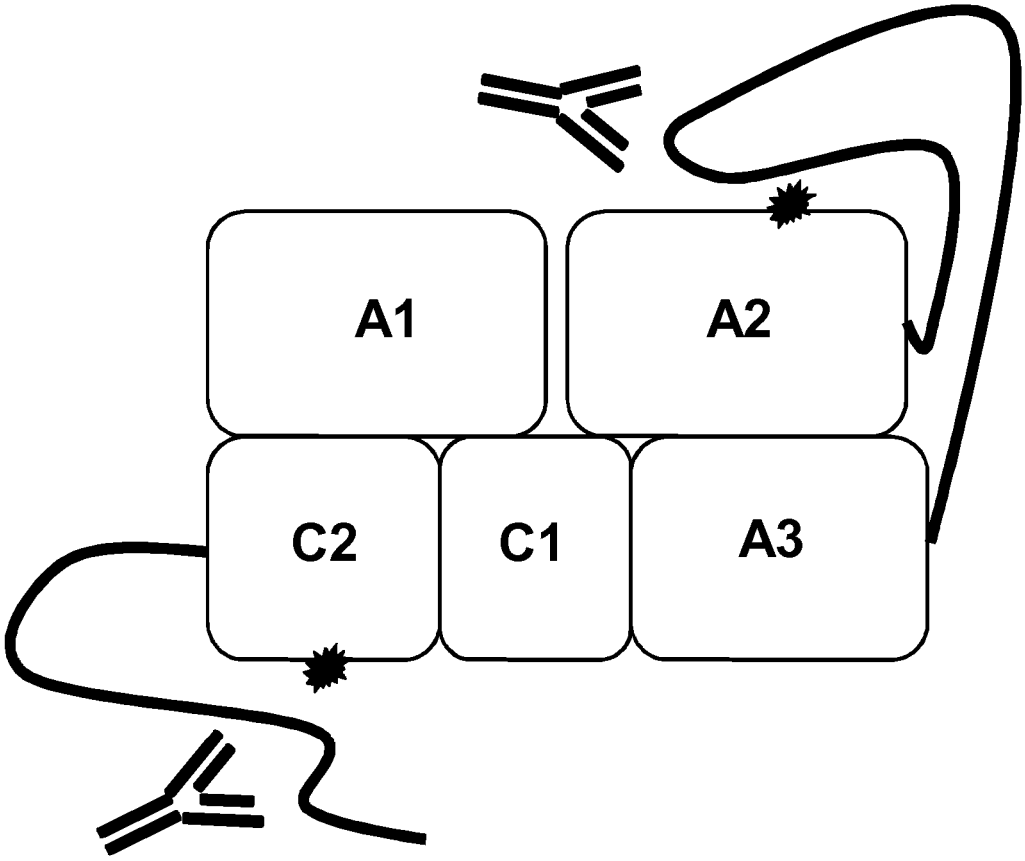


FIG. 6

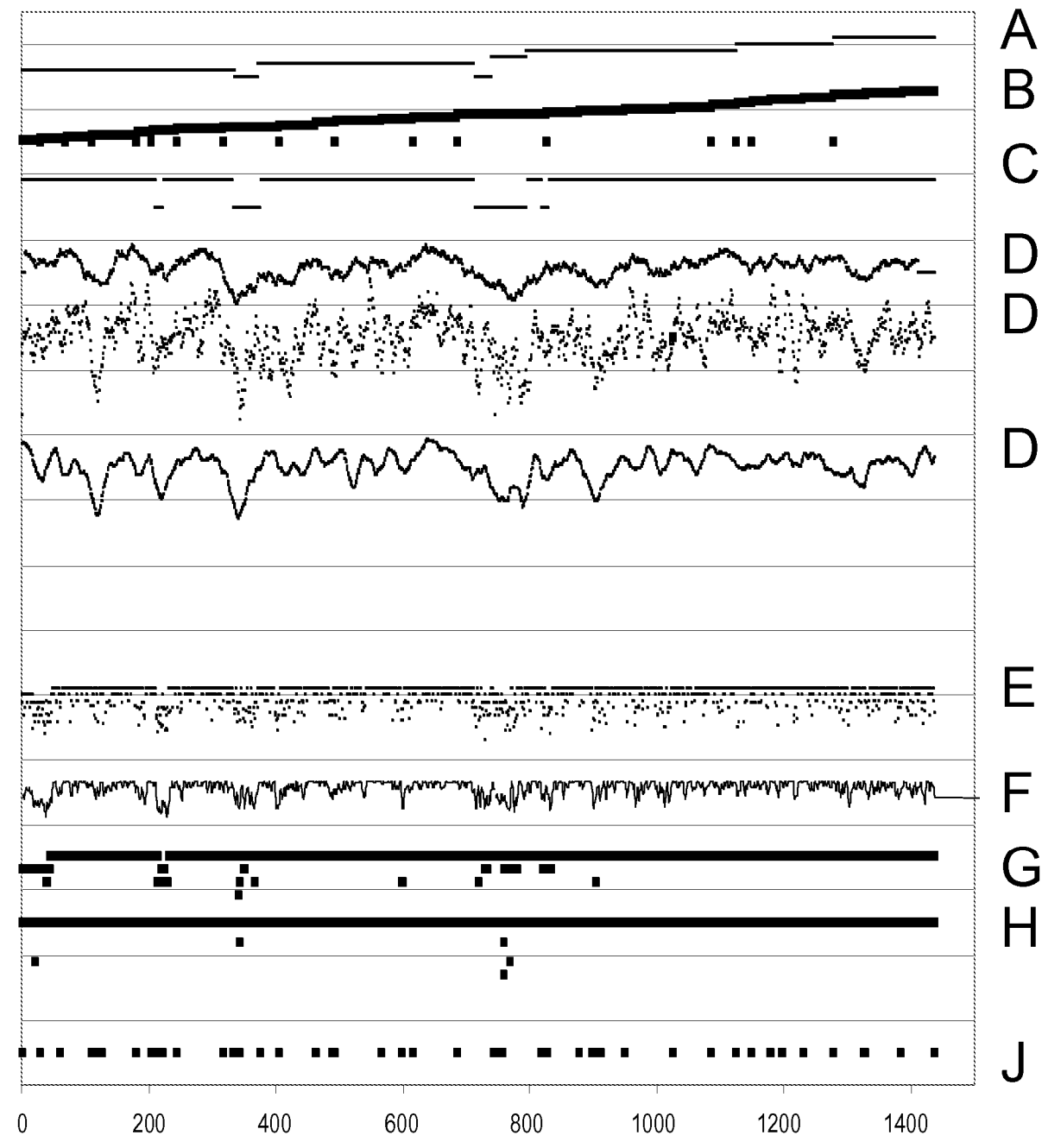


FIG. 7

ATRRYYLGAVELSWDYMQSDLGELPVDARFPPRVPKSFPFNTSVVYKKTL
FVEFTVHLFNIAKPRPPWMGLLGPTIQAEVYDTVVITLKNMASHPVSLHA
VGVSYWKASEGAEYDDQTSQREKEDDKVFPGGSHTYVWQVLKENGPMASD
PLCLTYSYLSHVDLVKDLNSGLIGALLVCREGSLAKEKTQTLHKFILLFA
VFDEGKSWHSETKNSLMQDRDAASARAWPKMHTVNGYVNRSLPGLIGCHR
KSVYWHVIGMGTTPEVHSIFLEGHTFLVRNHRQASLEISPITFLTAQTLL
MDLGQFLLFCHISSHQHDGMEAYVKVDSCPEEPQLRMKNNEEAEDYDDDL
TDSEMDVVRFDDDNSPSFIQIRSVAKKHPKTWVHYIAAEEEDWDYAPLVL
APDDRSYKSQYLNNGPQRIGRKYKKVRFMAYTDETFKTREAIQHESGILG
PLLYGEVGDTLLIIFKNQASRPYNIYPHGITDVRPLYSRRLPKGVKHLKD
FPILPGEIFKYKWTVTVEDGPTKSDPRCLTRYYSSFVNMERDLASGLIGP
LLICYKESVDQRGNQIMSDKRNVILFSVFDENRSWYLTENIQRFLPNPAG
VQLEDPEFQASNIMHSINGYVFDSLQLSVCLHEVAYWYILSIGAQTDFLS
VFFSGYTFKHKMVYEDTLTLFPFSGETVFMSMENPGLWILGCHNSDFRNR
GMTALLKVSSCDKNTGDYYEDSYEDISAYLLSKNNAIEPRSFSQNPPVLK
RHQREITRTTLQSDQEEIDYDDTISVEMKKEDFDIYDEDENQSPRSFQKK
TRHYFIAAVERLWDYGMSSSPHVLRNRAQSGSVPQFKKVVFQEFTDGSFT
QPLYRGELNEHLGLLGPYIRAEVEDNIMVTFRNQASRPYSFYSSLISYEE
DQRQGAEPRKNFVKPNETKTYFWKVQHHMAPTKDEFDCKAWAYFSDVDLE
KDVHSGLIGPLLVCHTNTLNPAHGRQVTVQEFALFFTIFDETKSWYFTEN
MERNCRAPCNIQMEDPTFKENYRFHAINGYIMDTLPGLVMAQDQRIRWYL
LSMGSNENIHSIHFSGHVFTVRKKEEYKMALYNLYPGVFETVEMLPSKAG
IWRVECLIGEHLHAGMSTLFLVYSNKCQTPLGMASGHIRDFQITASGQYG
QWAPKLARLHYSGSINAWSTKEPFSWIKVDLLAPMIIHGIKTQGARQKFS
SLYISQFIIMYSLDGKKWQTYRGNSTGTLMVFFGNVDSSGIKHNIFNPPI
IARYIRLHPTHYSIRSTLRMELMGCDLNSCSMPLGMESKAISDAQITASS
YFTNMFATWSPSKARLHLQGRSNAWRPQVNNPKEWLQVDFQKTMKVTGVT
TQGVKSLLTSMYVKEFLISSSQDGHQWTLFFQNGKVKVFQGNQDSFTPVV
NSLDPPLLTRYLRIHPQSWVHQIALRMEVLGCEAQDLY

FIG. 8

ATRRYYLGAVELSWDYMQSDLGELPVDARFPPRVPKSFPFNTSVVYKKTL
FVEFTVHLFNIAKPRPPWMGLLGPTIQAEVYDTVVITLKNMASHPVSLHA
VGVSYWKASEGAEYDDQTSQREKEDDKVFPGGSHTYVWQVLKENGPMASD
PLCLTYSYLSHVDLVKDLNSGLIGALLVCREGSLAKEKTQTLHKFILLFA
VFDEGKSWHSETKNSLMQDRDAASARAWPKMHTVNGYVNRSLPGLIGCHR
KSVYWHVIGMGTTPEVHSIFLEGHTFLVRNHRQASLEISPITFLTAQTLL
MDLGQFLLFCHISSHQHDGMEAYVKVDSCPEEPQLRMKNNEEAEDYDDDL
TDSEMDVVRFDDDNSPSFIQIRSVAKKHPKTWVHYIAAEEEDWDYAPLVL
APDDRSYKSQYLNNGPQRIGRKYKKVRFMAYTDETFKTREAIQHESGILG
PLLYGEVGDTLLIIFKNQASRPYNIYPHGITDVRPLYSRRLPKGVKHLKD
FPILPGEIFKYKWTVTVEDGPTKSDPRCLTRYYSSFVNMERDLASGLIGP
LLICYKESVDQRGNQIMSDKRNVILFSVFDENRSWYLTENIQRFLPNPAG
VQLEDPEFQASNIMHSINGYVFDSLQLSVCLHEVAYWYILSIGAQTDFLS
VFFSGYTFKHKMVYEDTLTLFPFSGETVFMSMENPGLWILGCHNSDFRNR
GMTALLKVSSCDKNTGDYYEDSYEDISAYLLSKNNAIEPRSFSQNPPVLK
RHQREITRTTLQSDQEEIDYDDTISVEMKKEDFDIYDEDENQSPRSFQKK
TRHYFIAAVERLWDYGMSSSPHVLRNRAQSGSVPQFKKVVFQEFTDGSFT
QPLYRGELNEHLGLLGPYIRAEVEDNIMVTFRNQASRPYSFYSSLISYEE
DQRQGAEPRKNFVKPNETKTYFWKVQHHMAPTKDEFDCKAWAYFSDVDLE
KDVHSGLIGPLLVCHTNTLNPAHGRQVTVQEFALFFTIFDETKSWYFTEN
MERNCRAPCNIQMEDPTFKENYRFHAINGYIMDTLPGLVMAQDQRIRWYL
LSMGSNENIHSIHFSGHVFTVRKKEEYKMALYNLYPGVFETVEMLPSKAG
IWRVECLIGEHLHAGMSTLFLVYSNKCQTPLGMASGHIRDFQITASGQYG
QWAPKLARLHYSGSINAWSTKEPFSWIKVDLLAPMIIHGIKTQGARQKFS
SLYISQFIIMYSLDGKKWQTYRGNSTGTLMVFFGNVDSSGIKHNIFNPPI
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YFTNMFATWSPSKARLHLQGRSNAWRPQVNNPKEWLQVDFQKTMKVTGVT
TQGVKSLLTSMYVKEFLISSSQDGHQWTLFFQNGKVKVFQGNQDSFTPVV
NSLDPPLLTRYLRIHPQSWVHQIALRMEVLGCEAQDLY

FIG. 9

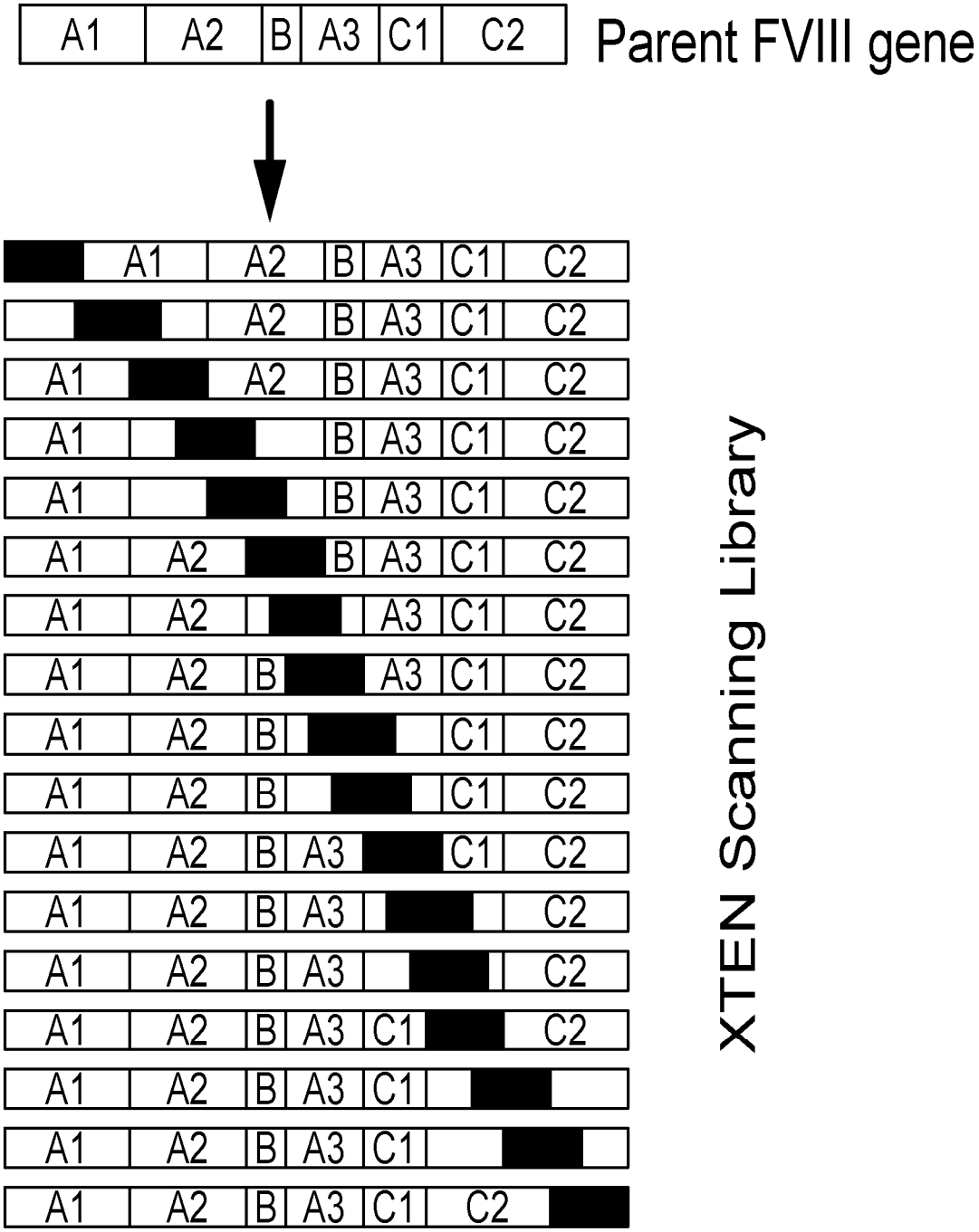


FIG. 10

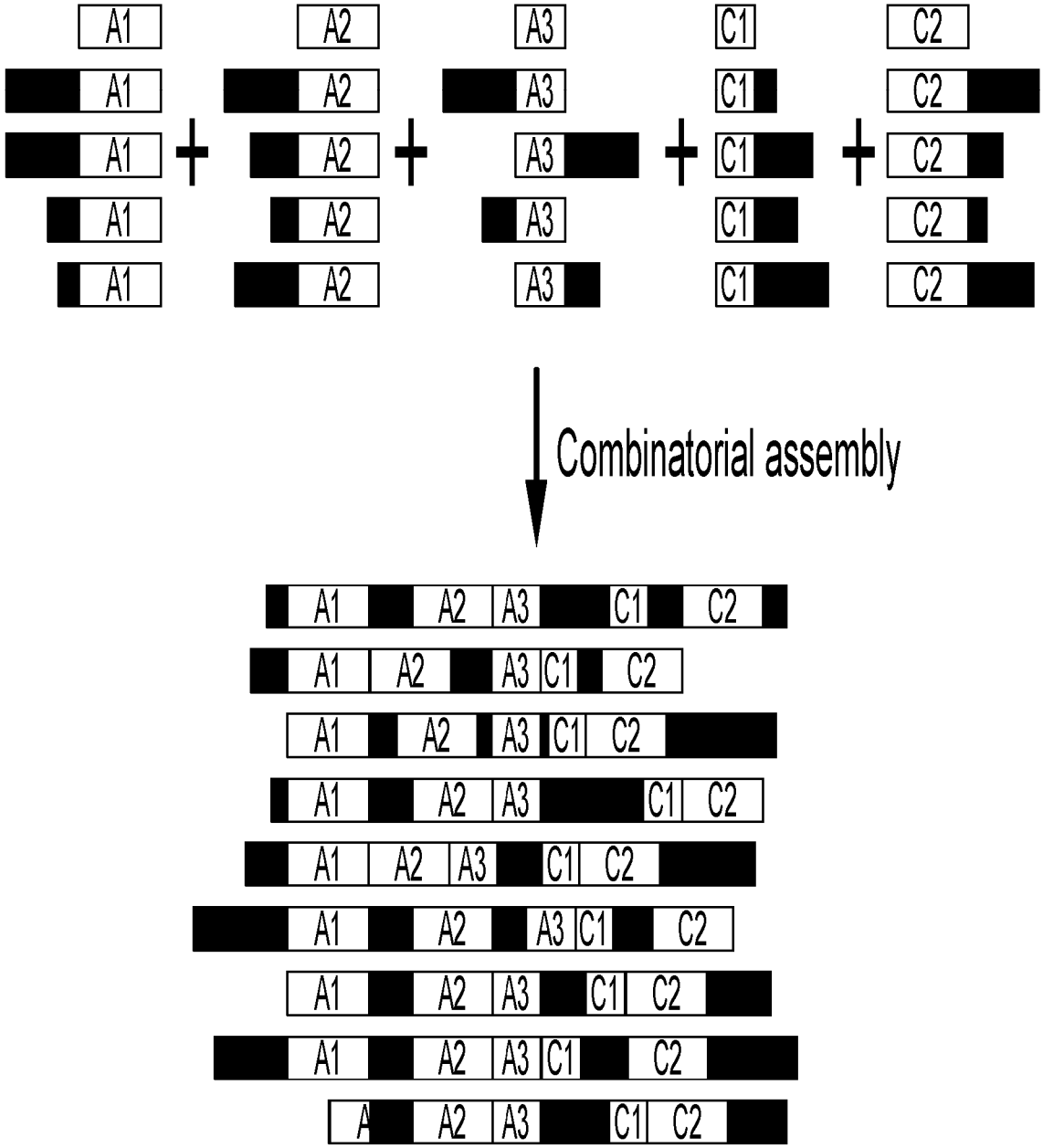


FIG. 11

FIG. 12A

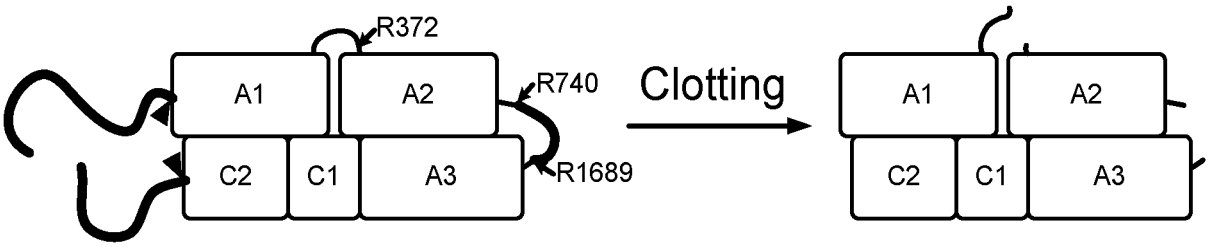


FIG. 12B

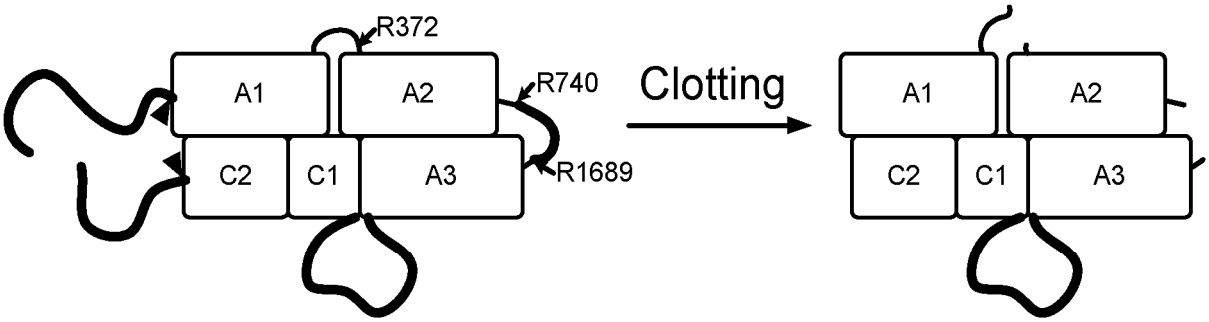


FIG. 12C

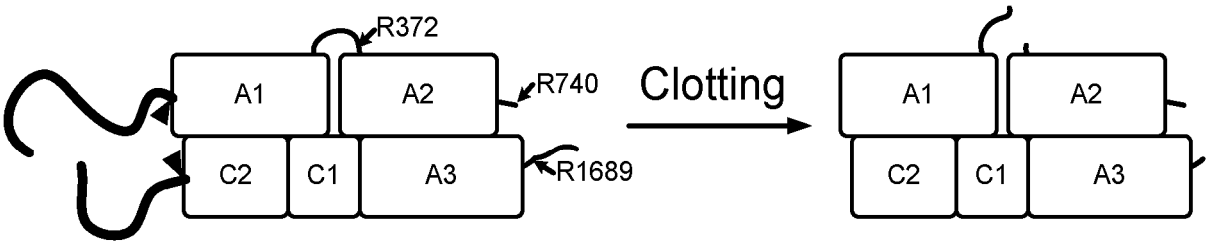


FIG. 12D

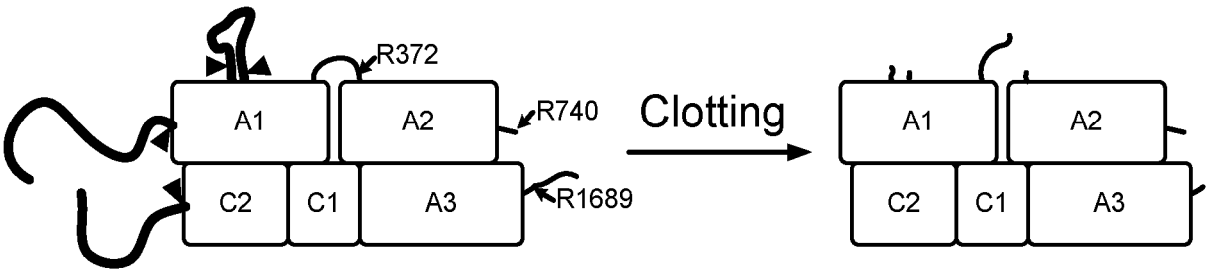


FIG. 12

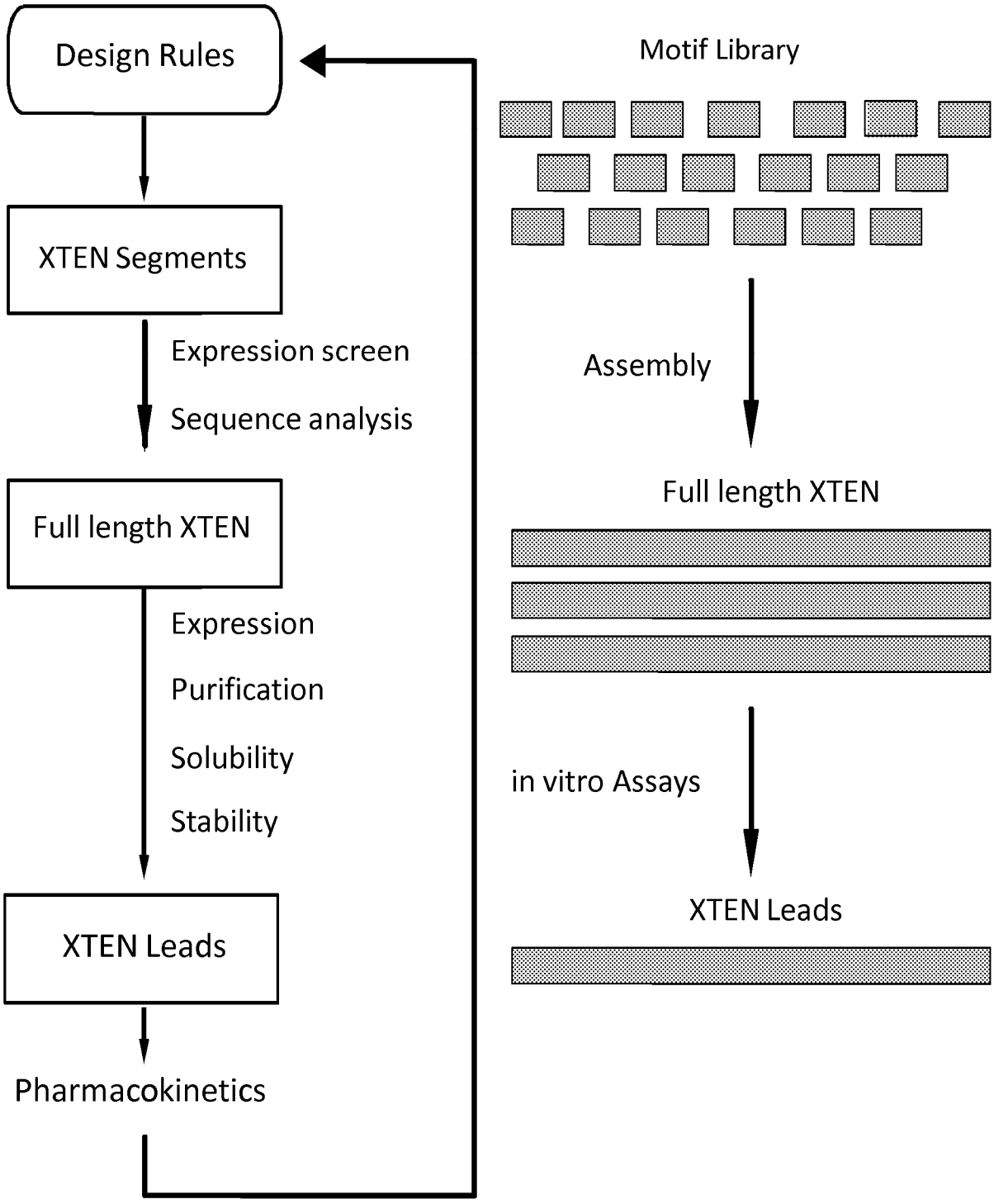

Design Rules
XTEN Segments
Expression screen
Sequence analysis
Full length XTEN
Expression
Purification
Solubility
Stability
XTEN Leads
Pharmacokinetics
Motif Library
Assembly
Full length XTEN
in vitro Assays
XTEN Leads

FIG. 13

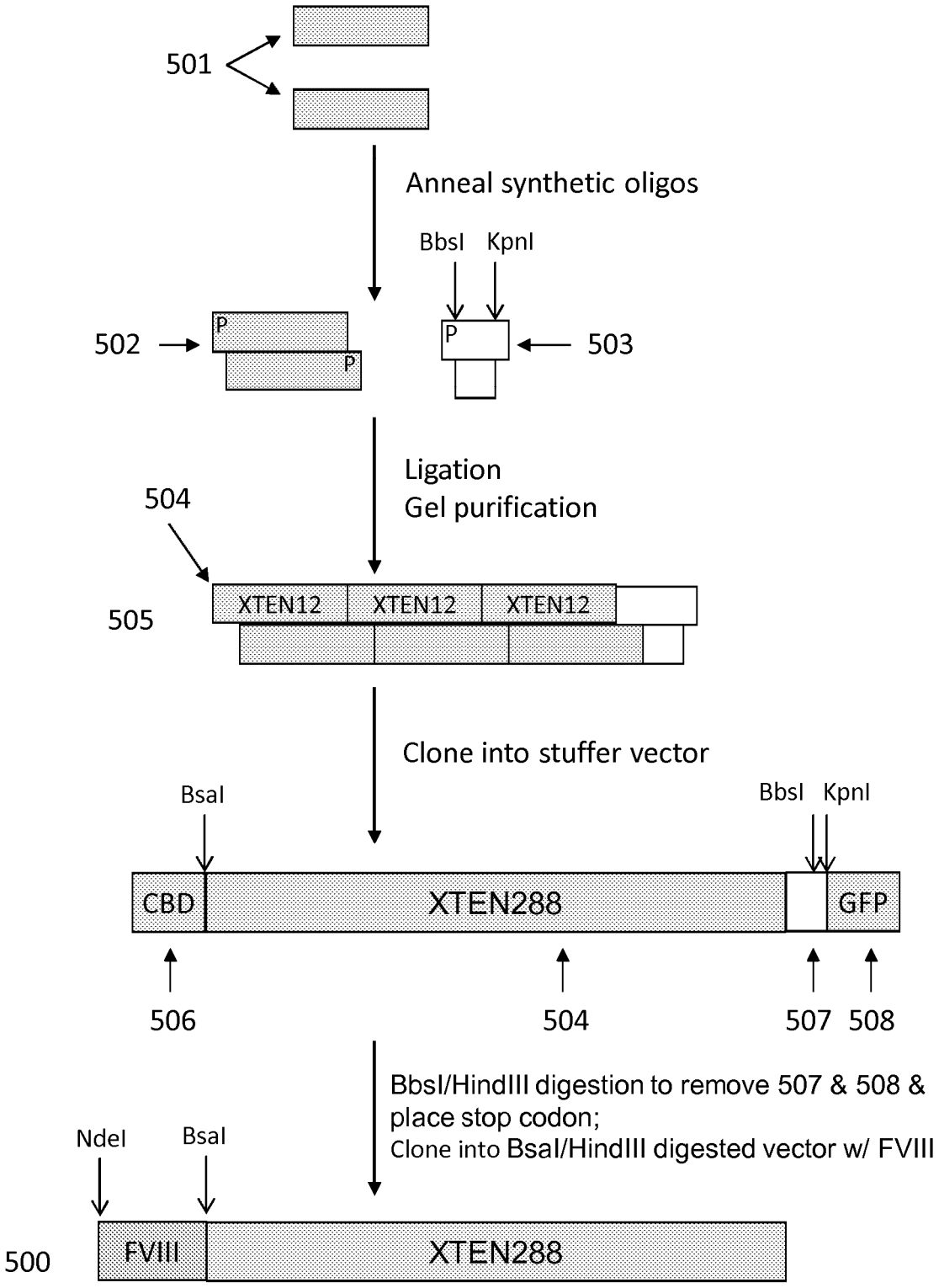

501
Anneal synthetic oligos
BbsI
KpnI
502
P
P
P
503
Ligation
Gel purification
504
505
XTEN12
XTEN12
XTEN12
Clone into stuffer vector
BsaI
BbsI
KpnI
CBD
XTEN288
GFP
506
504
507
508
BbsI/HindIII digestion to remove 507 & 508 &
place stop codon;
Clone into BsaI/HindIII digested vector w/ FVIII
NdeI
BsaI
500
FVIII
XTEN288

FIG. 14

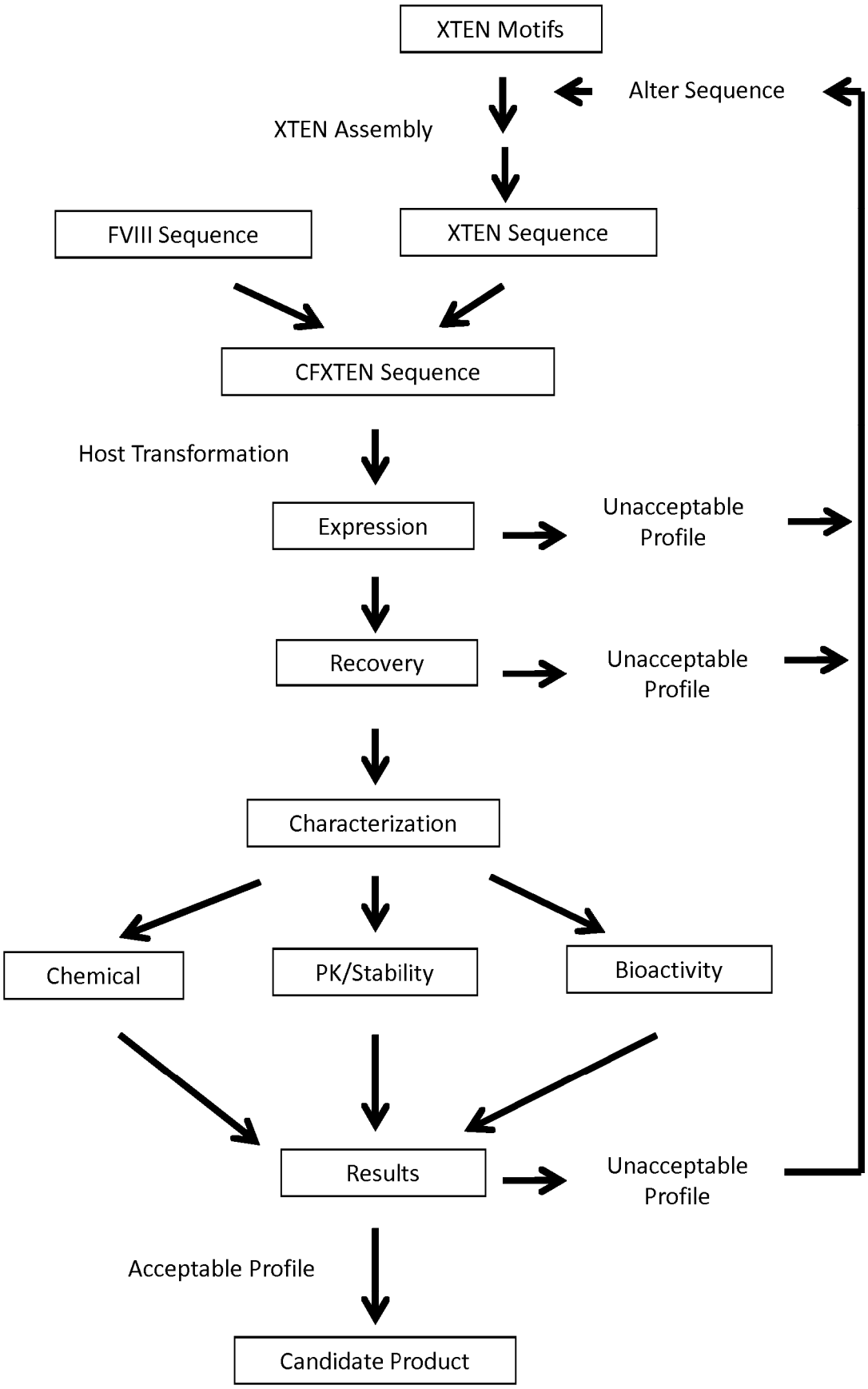

XTEN Motifs
Alter Sequence
XTEN Assembly
FVIII Sequence
XTEN Sequence
CFXTEN Sequence
Host Transformation
Expression
Unacceptable Profile
Recovery
Unacceptable Profile
Characterization
Chemical
PK/Stability
Bioactivity
Results
Unacceptable Profile
Acceptable Profile
Candidate Product

FIG. 15

AG864_2

GASPGTSSTGSPGSSPSASTGTGPGSSPSASTGTGPGTPGSGTASSSPGSSTPSGATGS
PGSSPSASTGTGPGASPGTSSTGSPGTPGSGTASSSPGSSTPSGATGSPGTPGSGTASS
SPGASPGTSSTGSPGASPGTSSTGSPGTPGSGTASSSPGSSTPSGATGSPGASPGTSST
GSPGTPGSGTASSSPGSSTPSGATGSPGSSPSASTGTGPGSSPSASTGTGPGSSTPSGA
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SPGSSTPSGATGSPGSSTPSGATGSPGASPGTSSTGSP

AG576

PGTPGSGTASSSPGSSTPSGATGSPGSSPSASTGTGPGSSPSASTGTGPGSSTPSGATG
SPGSSTPSGATGSPGASPGTSSTGSPGASPGTSSTGSPGASPGTSSTGSPGTPGSGTAS
SSPGASPGTSSTGSPGASPGTSSTGSPGASPGTSSTGSPGSSPSASTGTGPGTPGSGTA
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TSSTGSPGASPGTSSTGSPGASPGTSSTGSPGASPGTSSTGSPGTPGSGTASSSPGSST
PSGATGSPGTPGSGTASSSPGSSTPSGATGSPGTPGSGTASSSPGSSTPSGATGSPGSS
TPSGATGSPGSSPSASTGTGPGSSPSASTGTGPGASPGTSSTGSPGTPGSGTASSSPGS
STPSGATGSPGSSPSASTGTGPGSSPSASTGTGPGASPGTSSTGS

FIG. 16A

AG864_2

GASPGTSSTGSPGSSPSASTGTGPGSSPSASTGTGPGTPGSGTASSSPGSSTPSGATGS
PGSSPSASTGTGPGASPGTSSTGSPGTPGSGTASSSPGSSTPSGATGSPGTPGSGTASS
SPGASPGTSSTGSPGASPGTSSTGSPGTPGSGTASSSPGSSTPSGATGSPGASPGTSST
GSPGTPGSGTASSSPGSSTPSGATGSPGSSPSASTGTGPGSSPSASTGTGPGSSTPSGA
TGSPGSSTPSGATGSPGASPGTSSTGSPGASPGTSSTGSPGASPGTSSTGSPGTPGSGT
ASSSPGASPGTSSTGSPGASPGTSSTGSPGASPGTSSTGSPGSSPSASTGTGPGTPGSG
TASSSPGASPGTSSTGSPGASPGTSSTGSPGASPGTSSTGSPGSSTPSGATGSPGSSTP
SGATGSPGASPGTSSTGSPGTPGSGTASSSPGSSTPSGATGSPGSSTPSGATGSPGSST
PSGATGSPGSSPSASTGTGPGASPGTSSTGSPGASPGTSSTGSPGTPGSGTASSSPGAS
PGTSSTGSPGASPGTSSTGSPGASPGTSSTGSPGTPGSGTASSSPGS
STPSGATGSPGTPGSGTASSSPGSSTPSGATGSPGTPGSGTASSSPGSSTPSGATGSPG
SSTPSGATGSPGSSPSASTGTGPGSSPSASTGTGPGASPGTSSTGSPGTPGSGTASSSP
GSSTPSGATGSPGSSPSASTGTGPGSSPSASTGTGPGASPGTSSTGSPGASPGTSSTGS
PGSSTPSGATGSPGSSPSASTGTGPGASPGTSSTGSPGSSPSASTGTGPGTPGSGTASS
SPGSSTPSGATGSPGSSTPSGATGSPGASPGTSSTGSP



AG288_1

PGASPGTSSTGSPGASPGTSSTGSPGTPGSGTASSSPGSSTPSGATGSPGTPGSGTA
SSSPGSSTPSGATGSPGTPGSGTASSSPGSSTPSGATGSPGSSTPSGATGSPGSSPS
ASTGTGPGSSPSASTGTGPGASPGTSSTGSPGTPGSGTASSSPGSSTPSGATGSPGS
SPSASTGTGPGSSPSASTGTGPGASPGTSSTGSPGASPGTSSTGSPGSSTPSGATGS
PGSSPSASTGTGPGASPGTSSTGSPGSSPSASTGTGPGTPGSGTASSSPGSSTPSGA
TGS

FIG. 16B

AG864_2

GASPGTSSTGSPGSSPSASTGTGPGSSPSASTGTGPGTPGSGTASSSPGSSTPSGATGS
PGSSPSASTGTGPGASPGTSSTGSPGTPGSGTASSSPGSSTPSGATGSPGTPGSGTASS
SPGASPGTSSTGSPGASPGTSSTGSPGTPGSGTASSSPGSSTPSGATGSPGASPGTSST
GSPGTPGSGTASSSPGSSTPSGATGSPGSSPSASTGTGPGSSPSASTGTGPGSSTPSGA
TGSPGSSTPSGATGSPGASPGTSSTGSPGASPGTSSTGSPGASPGTSSTGSPGTPGSGT
ASSSPGASPGTSSTGSPGASPGTSSTGSPGASPGTSSTGSPGSSPSASTGTGPGTPGSG
TASSSPGASPGTSSTGSPGASPGTSSTGSPGASPGTSSTGSPGSSTPSGATGSPGSSTP
SGATGSPGASPGTSSTGSPGTPGSGTASSSPGSSTPSGATGSPGSSTPSGATGSPGSST
PSGATGSPGSSPSASTGTGPGASPGTSSTGSPGASPGTSSTGSPGTPGSGTASSSPGAS
PGTSSTGSPGASPGTSSTGSPGASPGTSSTGSPGASPGTSSTGSPGTPGSGTASSSPGS
STPSGATGSPGTPGSGTASSSPGSSTPSGATGSPGTPGSGTASSSPGSSTPSGATGSPG
SSTPSGATGSPGSSPSASTGTGPGSSPSASTGTGPGASPGTSSTGSPGTPGSGTASSSP
GSSTPSGATGSPGSSPSASTGTGPGSSPSASTGTGPGASPGTSSTGSPGASPGTSSTGS
PGSSTPSGATGSPGSSPSASTGTGPGASPGTSSTGSPGSSPSASTGTGPGTPGSGTASS
SPGSSTPSGATGSPGSSTPSGATGSPGASPGTSSTGSP



AG144_2

PGSSPSASTGTGPGSSPSASTGTGPGTPGSGTASSSPGSSTPSGATGSPGSSPSAST
GTGPGASPGTSSTGSPGTPGSGTASSSPGSSTPSGATGSPGTPGSGTASSSPGASPG
TSSTGSPGASPGTSSTGSPGTPGSGTASSS

FIG. 16C

AE864

GSPAGSPTSTEEGTSESATPESGPGTSTEPSEGSAPGSPAGSPTSTEEGTSTEPSEGSA
PGTSTEPSEGSAPGTSESATPESGPGSEPATSGSETPGSEPATSGSETPGSPAGSPTST
EEGTSESATPESGPGTSTEPSEGSAPGTSTEPSEGSAPGSPAGSPTSTEEGTSTEPSEG
SAPGTSTEPSEGSAPGTSESATPESGPGTSTEPSEGSAPGTSESATPESGPGSEPATSG
SETPGTSTEPSEGSAPGTSTEPSEGSAPGTSESATPESGPGTSESATPESGPGSPAGSP
TSTEEGTSESATPESGPGSEPATSGSETPGTSESATPESGPGTSTEPSEGSAPGTSTEP
SEGSAPGTSTEPSEGSAPGTSTEPSEGSAPGTSTEPSEGSAPGTSTEPSEGSAPGSPAG
SPTSTEEGTSTEPSEGSAPGTSESATPESGPGSEPATSGSETPGTSESATPESGPGSEP
ATSGSETPGTSESATPESGPGTSTEPSEGSAPGTSESATPESGPGSPAGSPTSTEEGSP
AGSPTSTEEGSPAGSPTSTEEGTSESATPESGPGTSTEPSEGSAPGTSESATPESGPGS
EPATSGSETPGTSESATPESGPGSEPATSGSETPGTSESATPESGPGTSTEPSEGSAPG
SPAGSPTSTEEGTSESATPESGPGSEPATSGSETPGTSESATPESGPGSPAGSPTSTEE
GSPAGSPTSTEEGTSTEPSEGSAPGTSESATPESGPGTSESATPESGPGTSESATPESG
PGSEPATSGSETPGSEPATSGSETPGSPAGSPTSTEEGTSTEPSEGSAPGTSTEPSEGS
APGSEPATSGSETPGTSESATPESGPGTSTEPSEGSAP

AE576

GSPAGSPTSTEEGTSESATPESGPGTSTEPSEGSAPGSPAGSPTSTEEGTSTEPSEG
SAPGTSTEPSEGSAPGTSESATPESGPGSEPATSGSETPGSEPATSGSETPGSPAGS
PTSTEEGTSESATPESGPGTSTEPSEGSAPGTSTEPSEGSAPGSPAGSPTSTEEGTS
TEPSEGSAPGTSTEPSEGSAPGTSESATPESGPGTSTEPSEGSAPGTSESATPESGP
GSEPATSGSETPGTSTEPSEGSAPGTSTEPSEGSAPGTSESATPESGPGTSESATPE
SGPGSPAGSPTSTEEGTSESATPESGPGSEPATSGSETPGTSESATPESGPGTSTEP
SEGSAPGTSTEPSEGSAPGTSTEPSEGSAPGTSTEPSEGSAPGTSTEPSEGSAPGTS
TEPSEGSAPGSPAGSPTSTEEGTSTEPSEGSAPGTSESATPESGPGSEPATSGSETP
GTSESATPESGPGSEPATSGSETPGTSESATPESGPGTSTEPSEGSAPGTSESATPE
SGPGSPAGSPTSTEEGSPAGSPTSTEEGSPAGSPTSTEEGTSESATPESGPGTSTEP
SEGSAP

FIG. 16D

AE864

GSPAGSPTSTEEGTSESATPESGPGTSTEPSEGSAPGSPAGSPTSTEEGTSTEPSEGSA
PGTSTEPSEGSAPGTSESATPESGPGSEPATSGSETPGSEPATSGSETPGSPAGSPTST
EEGTSESATPESGPGTSTEPSEGSAPGTSTEPSEGSAPGSPAGSPTSTEEGTSTEPSEG
SAPGTSTEPSEGSAPGTSESATPESGPGTSTEPSEGSAPGTSESATPESGPGSEPATSG
SETPGTSTEPSEGSAPGTSTEPSEGSAPGTSESATPESGPGTSESATPESGPGSPAGSP
TSTEEGTSESATPESGPGSEPATSGSETPGTSESATPESGPGTSTEPSEGSAPGTSTEP
SEGSAPGTSTEPSEGSAPGTSTEPSEGSAPGTSTEPSEGSAPGTSTEPSEGSAPGSPAG
SPTSTEEGTSTEPSEGSAPGTSESATPESGPGSEPATSGSETPGTSESATPESGPGSEP
ATSGSETPGTSESATPESGPGTSTEPSEGSAPGTSESATPESGPGSPAGSPTSTEEGSP
AGSPTSTEEGSPAGSPTSTEEGTSESATPESGPGTSTEPSEGSAPGTSESATPESGPGS
EPATSGSETPGTSESATPESGPGSEPATSGSETPGTSESATPESGPGTSTEPSEGSAPG
SPAGSPTSTEEGTSESATPESGPGSEPATSGSETPGTSESATPESGPGSPAGSPTSTEE
GSPAGSPTSTEEGTSTEPSEGSAPGTSESATPESGPGTSESATPESGPGTSESATPESG
PGSEPATSGSETPGSEPATSGSETPGSPAGSPTSTEEGTSTEPSEGSAPGTSTEPSEGS
APGSEPATSGSETPGTSESATPESGPGTSTEPSEGSAP

AE288_2

GTSESATPESGPGSEPATSGSETPGTSESATPESGPGSEPATSGSETPGTSESATPE
SGPGTSTEPSEGSAPGSPAGSPTSTEEGTSESATPESGPGSEPATSGSETPGTSESA
TPESGPGSPAGSPTSTEEGSPAGSPTSTEEGTSTEPSEGSAPGTSESATPESGPGTS
ESATPESGPGTSESATPESGPGSEPATSGSETPGSEPATSGSETPGSPAGSPTSTEE
GTSTEPSEGSAPGTSTEPSEGSAPGSEPATSGSETPGTSESATPESGPGTSTEPSEG
SAP

FIG. 16E

AE864

GSPAGSPTSTEEGTSESATPESGPGTSTEPSEGSAPGSPAGSPTSTEEGTSTEPSEGS
APGTSTEPSEGSAPGTSESATPESGPGSEPATSGSETPGSEPATSGSETPGSPAGSPT
STEEGTSESATPESGPGTSTEPSEGSAPGTSTEPSEGSAPGSPAGSPTSTEEGTSTEP
SEGSAPGTSTEPSEGSAPGTSESATPESGPGTSTEPSEGSAPGTSESATPESGPGSEP
ATSGSETPGTSTEPSEGSAPGTSTEPSEGSAPGTSESATPESGPGTSESATPESGPGS
PAGSPTSTEEGTSESATPESGPGSEPATSGSETPGTSESATPESGPGTSTEPSEGSAP
GTSTEPSEGSAPGTSTEPSEGSAPGTSTEPSEGSAPGTSTEPSEGSAPGTSTEPSEGS
APGSPAGSPTSTEEGTSTEPSEGSAPGTSESATPESGPGSEPATSGSETPGTSESATP
ESGPGSEPATSGSETPGTSESATPESGPGTSTEPSEGSAPGTSESATPESGPGSPAGS
PTSTEEGSPAGSPTSTEEGSPAGSPTSTEEGTSESATPESGPGTSTEPSEGSAPGTSE
SATPESGPGSEPATSGSETPGTSESATPESGPGSEPATSGSETPGTSESATPESGPGT
STEPSEGSAPGSPAGSPTSTEEGTSESATPESGPGSEPATSGSETPGTSESATPESGP
GSPAGSPTSTEEGSPAGSPTSTEEGTSTEPSEGSAPGTSESATPESGPGTSESATPES
GPGTSESATPESGPGSEPATSGSETPGSEPATSGSETPGSPAGSPTSTEEGTSTEPSE
GSAPGTSTEPSEGSAPGSEPATSGSETPGTSESATPESGPGTSTEPSEGSAP

AE144_1A

SPAGSPTSTEEGTSESATPESGPGTSTEPSEGSAPGSPAGSPTSTEEGTSTEPSEGSA
PGTSTEPSEGSAPGTSESATPESGPGSEPATSGSETPGSEPATSGSETPGSPAGSPTS
TEEGTSESATPESGPGTSTEPSEGSAPG

AE144_2B

TSTEPSEGSAPGSPAGSPTSTEEGTSTEPSEGSAPGTSTEPSEGSAPGTSESATPESG
PGTSTEPSEGSAPGTSESATPESGPGSEPATSGSETPGTSTEPSEGSAPGTSTEPSEG
SAPGTSESATPESGPGTSESATPESGPG

AE144_3A

SPAGSPTSTEEGTSESATPESGPGSEPATSGSETPGTSESATPESGPGTSTEPSEGSA
PGTSTEPSEGSAPGTSTEPSEGSAPGTSTEPSEGSAPGTSTEPSEGSAPGTSTEPSEG
SAPGSPAGSPTSTEEGTSTEPSEGSAPG

AE144_4B

TSESATPESGPGSEPATSGSETPGTSESATPESGPGSEPATSGSETPGTSESATPESG
PGTSTEPSEGSAPGTSESATPESGPGSPAGSPTSTEEGSPAGSPTSTEEGSPAGSPTS
TEEGTSESATPESGPGTSTEPSEGSAPG

FIG. 16F

FIG. 17A

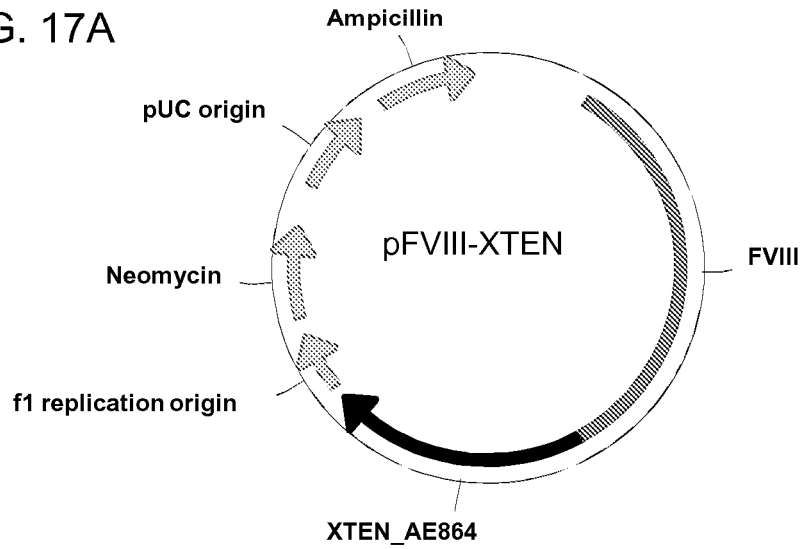


FIG. 17B

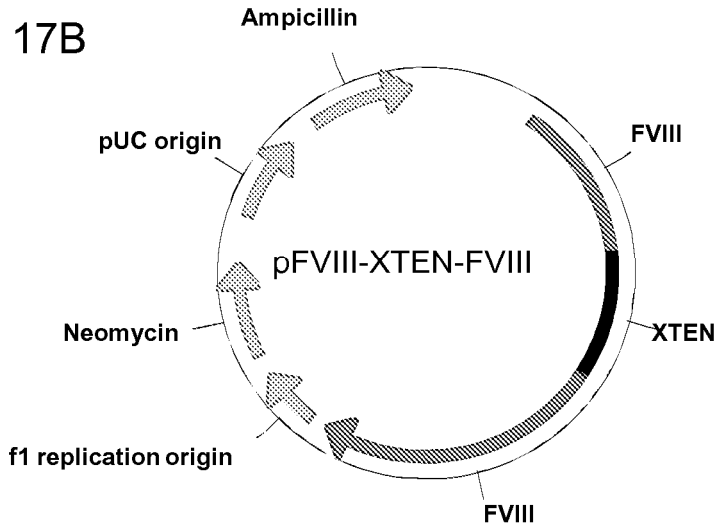


FIG. 17C

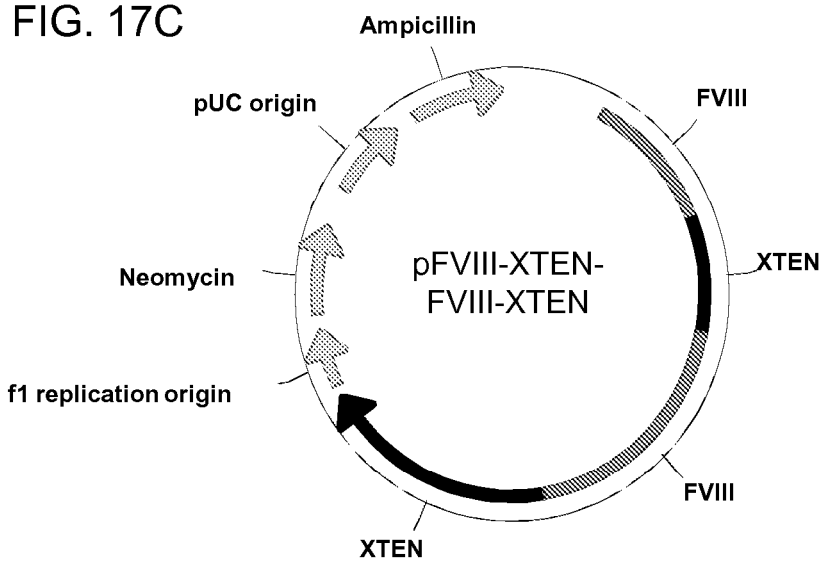


FIG. 17

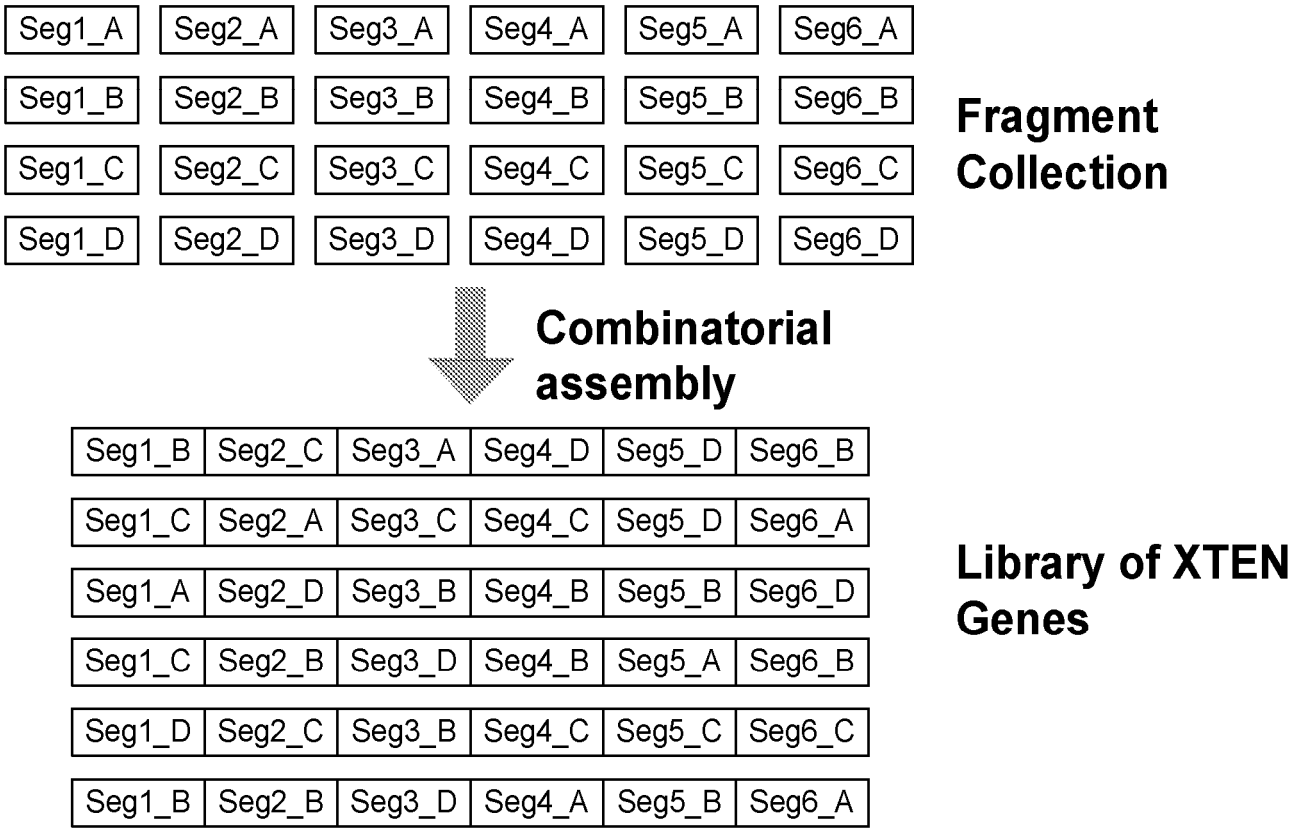

Seg1_A Seg2_A Seg3_A Seg4_A Seg5_A Seg6_A
Seg1_B Seg2_B Seg3_B Seg4_B Seg5_B Seg6_B
Seg1_C Seg2_C Seg3_C Seg4_C Seg5_C Seg6_C
Seg1_D Seg2_D Seg3_D Seg4_D Seg5_D Seg6_D
Fragment Collection
Combinatorial assembly
Seg1_B Seg2_C Seg3_A Seg4_D Seg5_D Seg6_B
Seg1_C Seg2_A Seg3_C Seg4_C Seg5_D Seg6_A
Seg1_A Seg2_D Seg3_B Seg4_B Seg5_B Seg6_D
Seg1_C Seg2_B Seg3_D Seg4_B Seg5_A Seg6_B
Seg1_D Seg2_C Seg3_B Seg4_C Seg5_C Seg6_C
Seg1_B Seg2_B Seg3_D Seg4_A Seg5_B Seg6_A
Library of XTEN Genes

FIG. 18

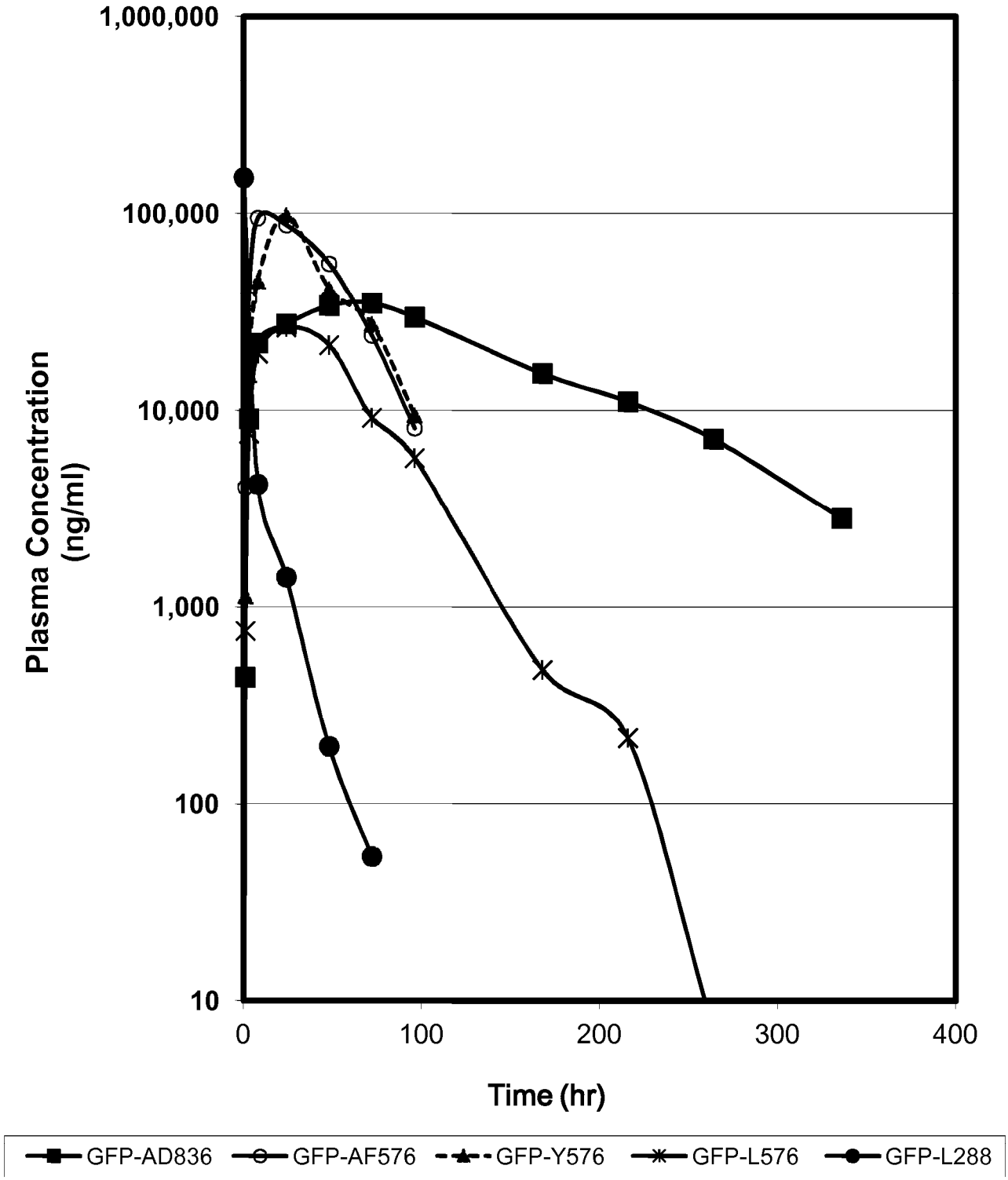

1,000,000
100,000
10,000
1,000
100
10
Plasma Concentration (ng/ml)
0
100
200
300
400
Time (hr)
GFP-AD836
GFP-AF576
GFP-Y576
GFP-L576
GFP-L288

FIG. 19

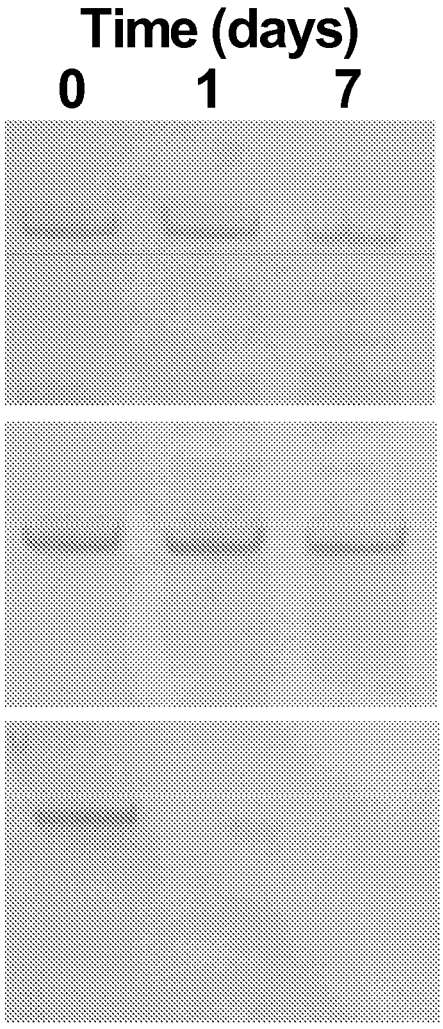


FIG. 18A

Monkey plasma

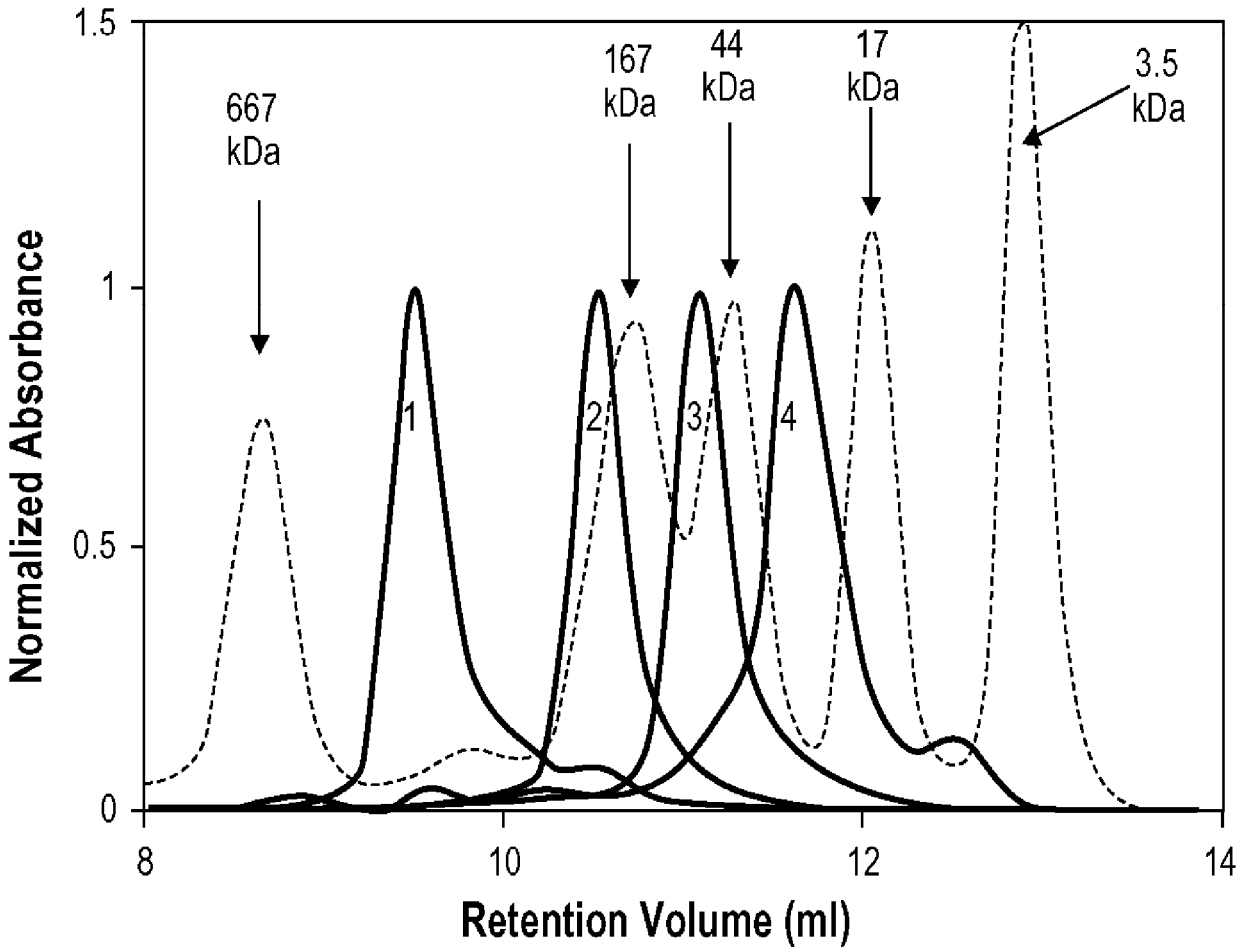
FIG. 18B

Monkey in vivo

FIG. 18C

Kidney homogenate

FIG. 20



1. Glucagon-Y288
2. Glucagon-Y144
3. Glucagon-Y72
4. Glucagon-Y36

----- = Standards

FIG. 21

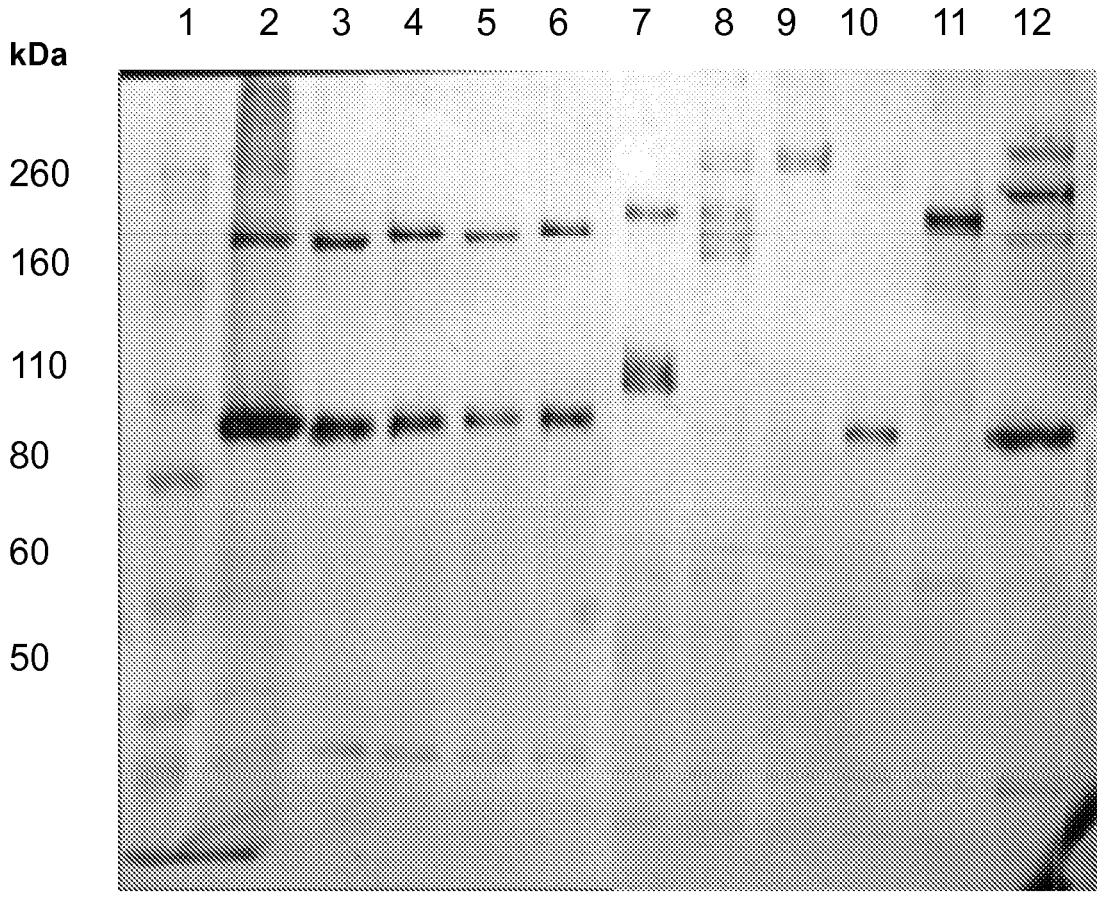


FIG. 22

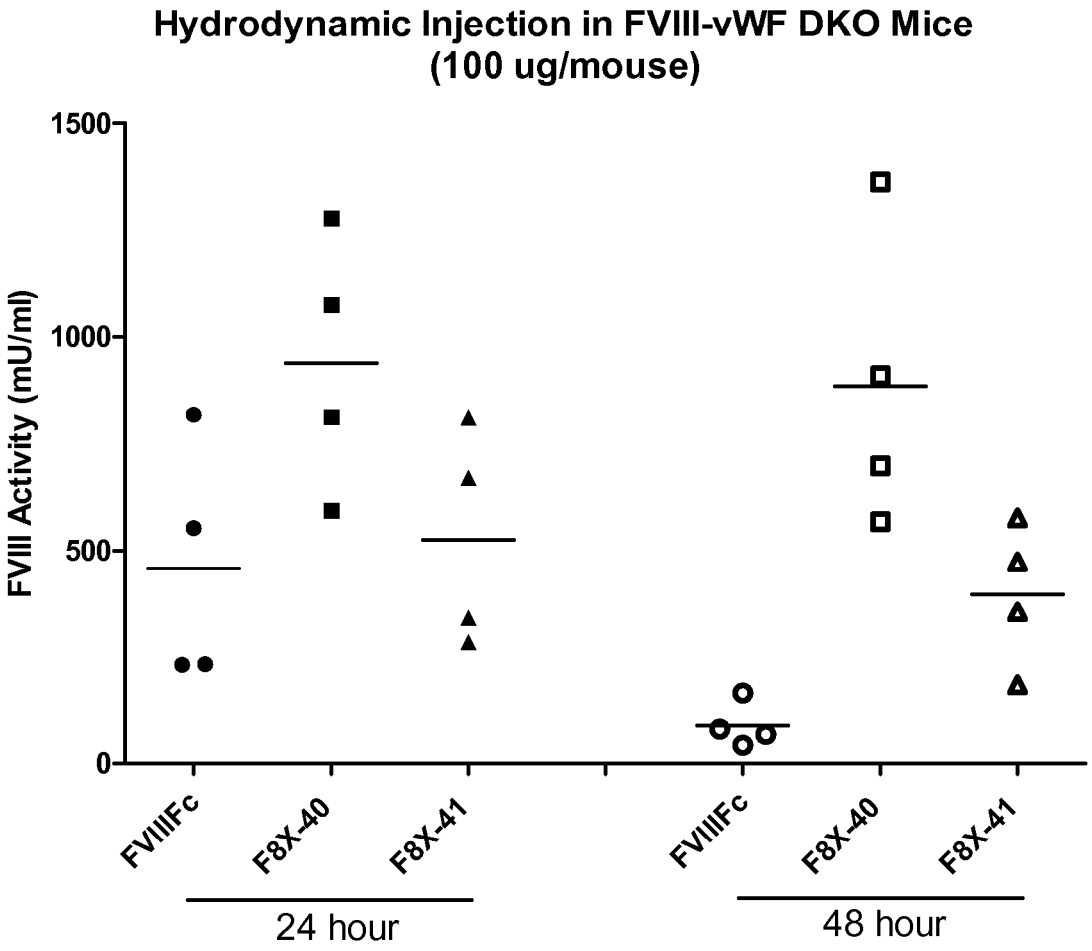

Hydrodynamic Injection in FVIII-vWF DKO Mice
(100 ug/mouse)
FVIII Activity (mU/ml)
1500
1000
500
0
FVIIIFc
F8X-40
F8X-41
FVIIIFc
F8X-40
F8X-41
24 hour
48 hour

FIG. 23

Fig. 24A

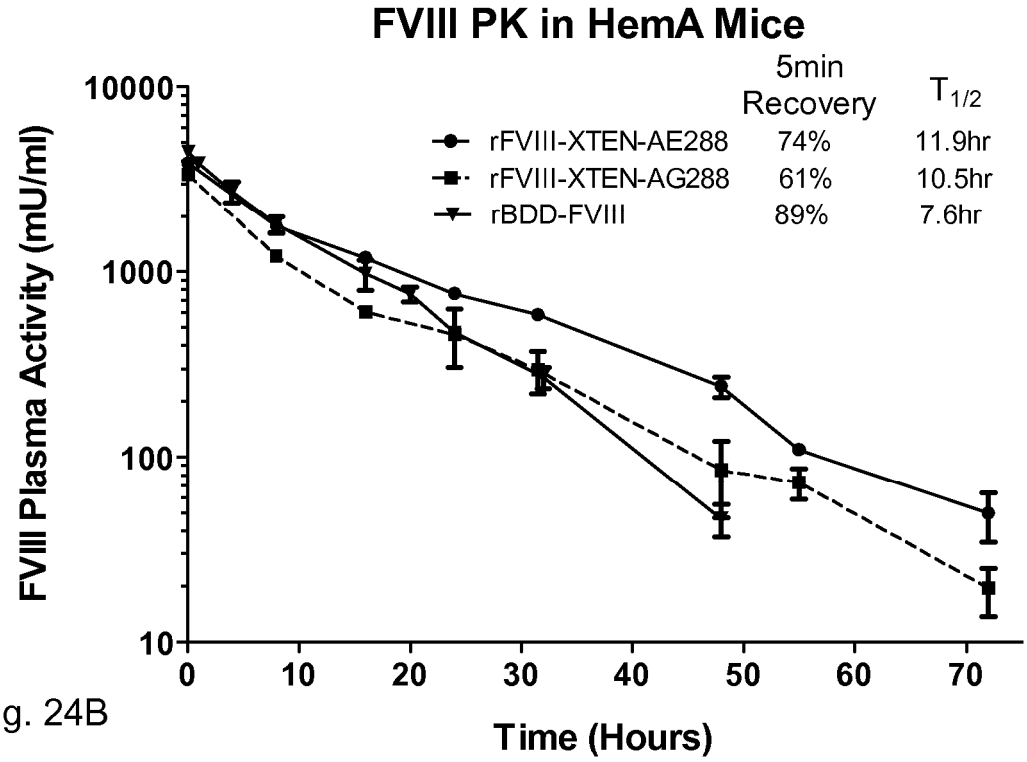


Fig. 24B

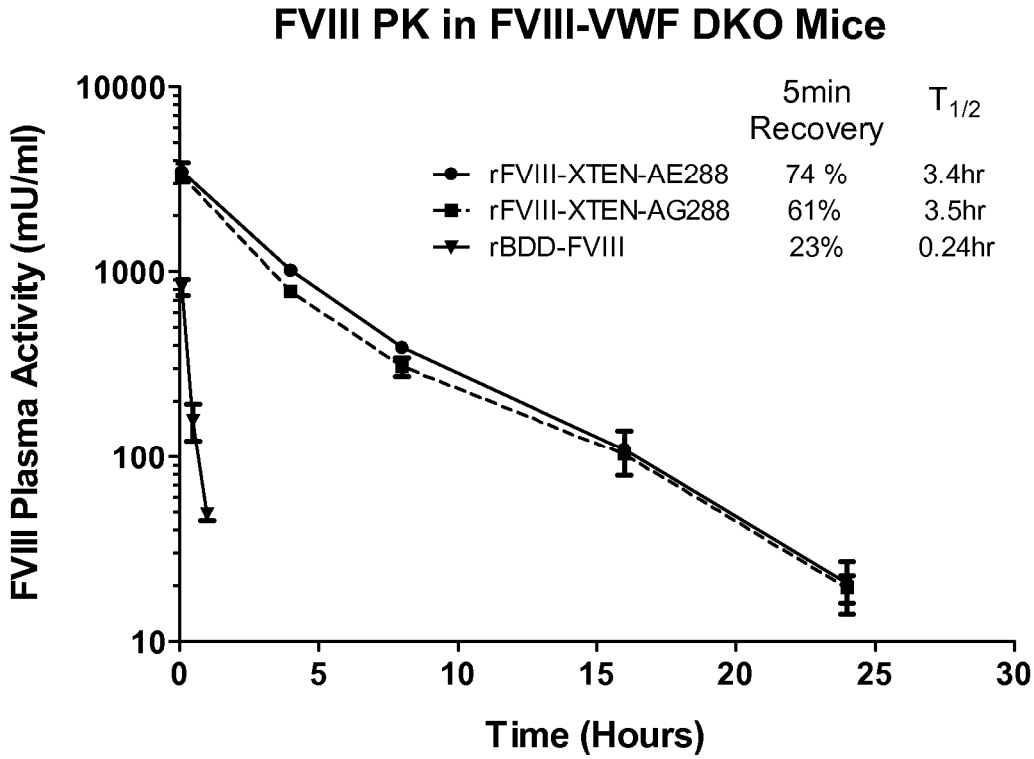


FIG. 24

Fig. 25A

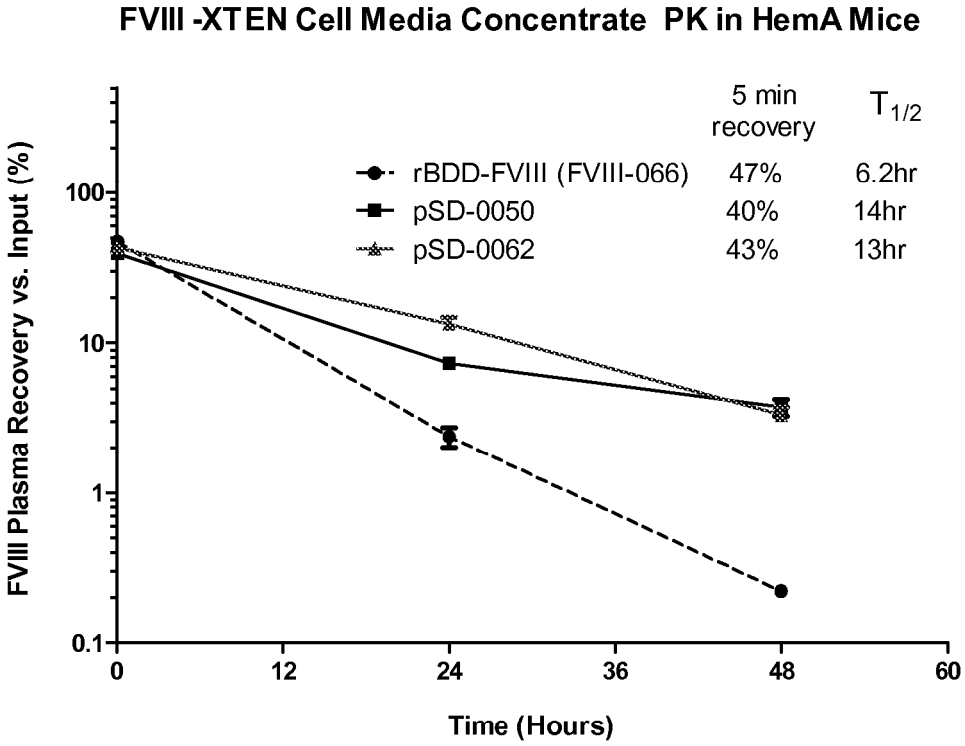


Fig. 25B

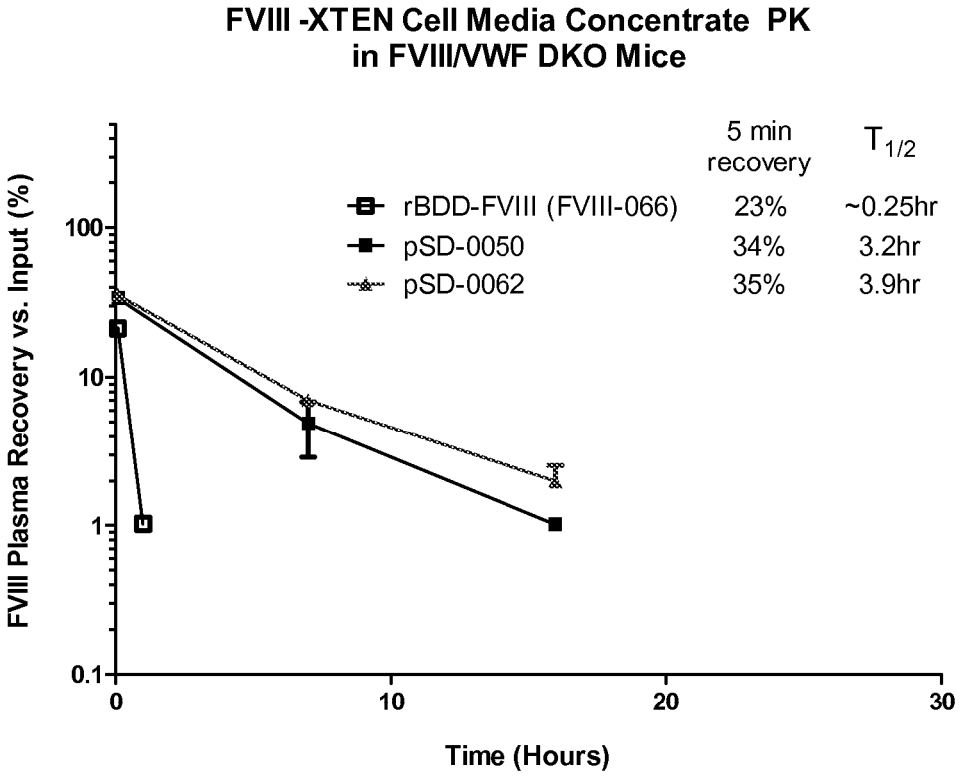


FIG. 25

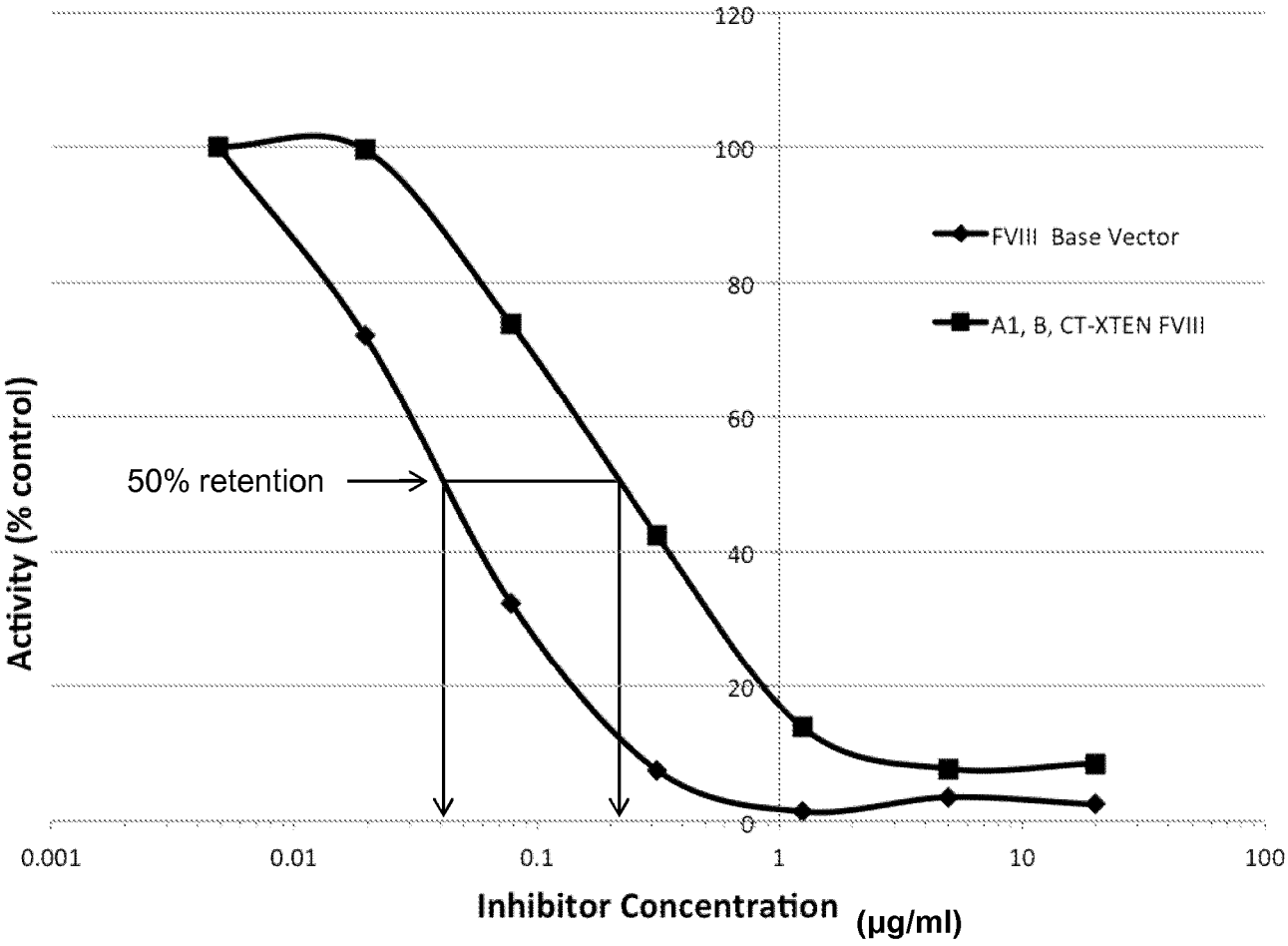

120
100
80
60
40
20
0
FVIII Base Vector
A1, B, CT-XTEN FVIII
50% retention
Activity (% control)
0.001
0.01
0.1
1
10
100
Inhibitor Concentration (µg/ml)

FIG. 26

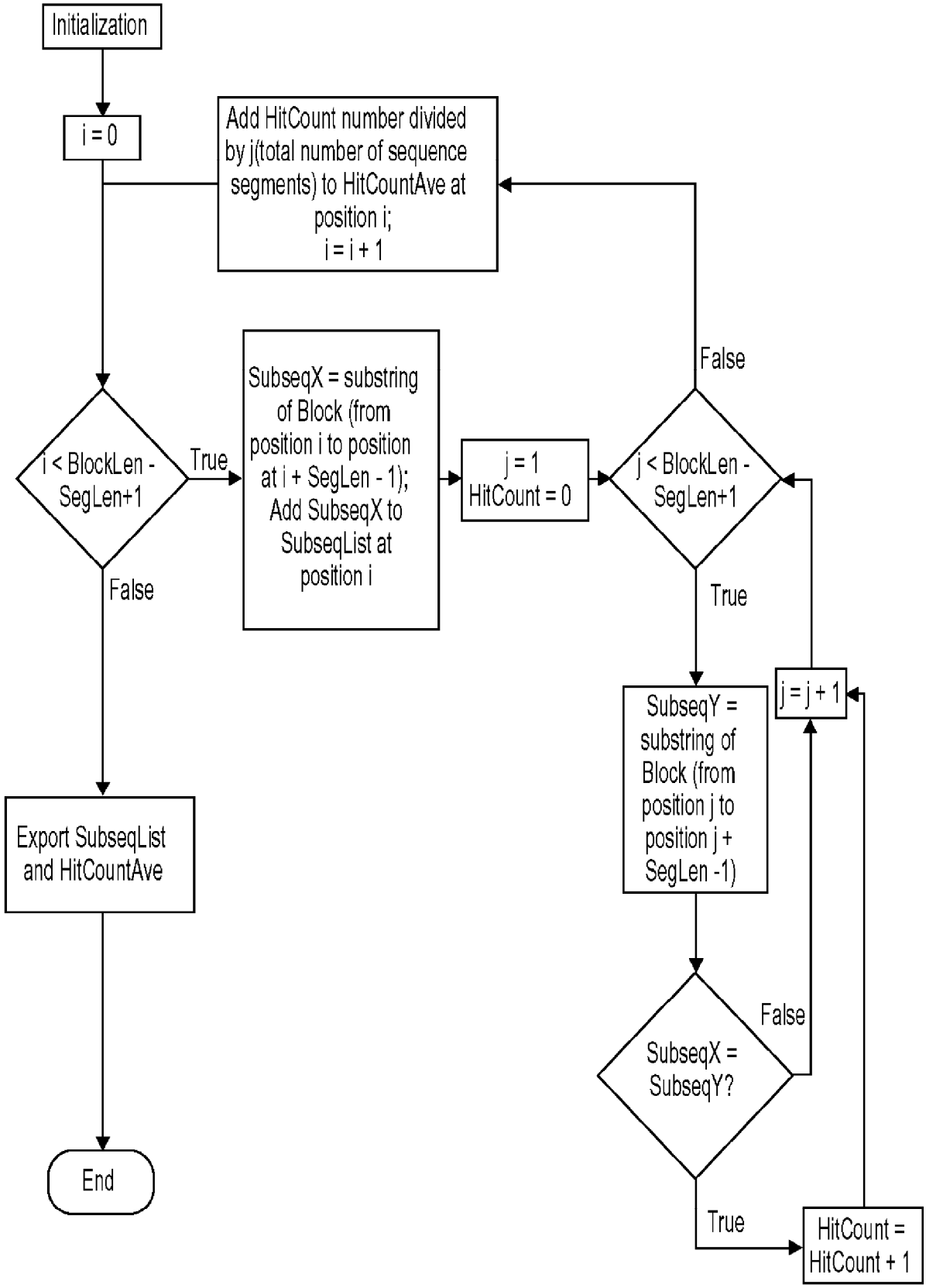

Initialization
i = 0
Add HitCount number divided by j(total number of sequence segments) to HitCountAve at position i;
i = i + 1
i < BlockLen - SegLen+1
True
False
SubseqX = substring of Block (from position i to position at i + SegLen - 1); Add SubseqX to SubseqList at position i
j = 1
HitCount = 0
j < BlockLen - SegLen+1
False
True
SubseqY = substring of Block (from position j to position j + SegLen -1)
SubseqX = SubseqY?
False
True
HitCount = HitCount + 1
j = j + 1
Export SubseqList and HitCountAve
End

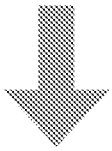
FIG. 27

XTEN Sequence

1	2	3	4	5	6	7	8	9	10	11
A	S	T	G	E	T	G	E	T	G	E

XTEN Length: N=11

Subsequence length: S=3



		Seg 1	Seg 2	Seg 3	Seg 4	Seg 5	Seg 6	Seg 7	Seg 8	Seg 9
		AST	STG	TGE	GET	ETG	TGE	GET	ETG	TGE
Seg 1	AST	x								
Seg 2	STG		x							
Seg 3	TGE			x			x			
Seg 4	GET				x			x		
Seg 5	ETG					x			x	
Seg 6	TGE			x			x			x
Seg 7	GET				x			x		
Seg 8	ETG					x			x	
Seg 9	TGE						x			x
		1	1	2	2	2	3	2	2	2

Subsequence Score = 1.89

FIG. 28

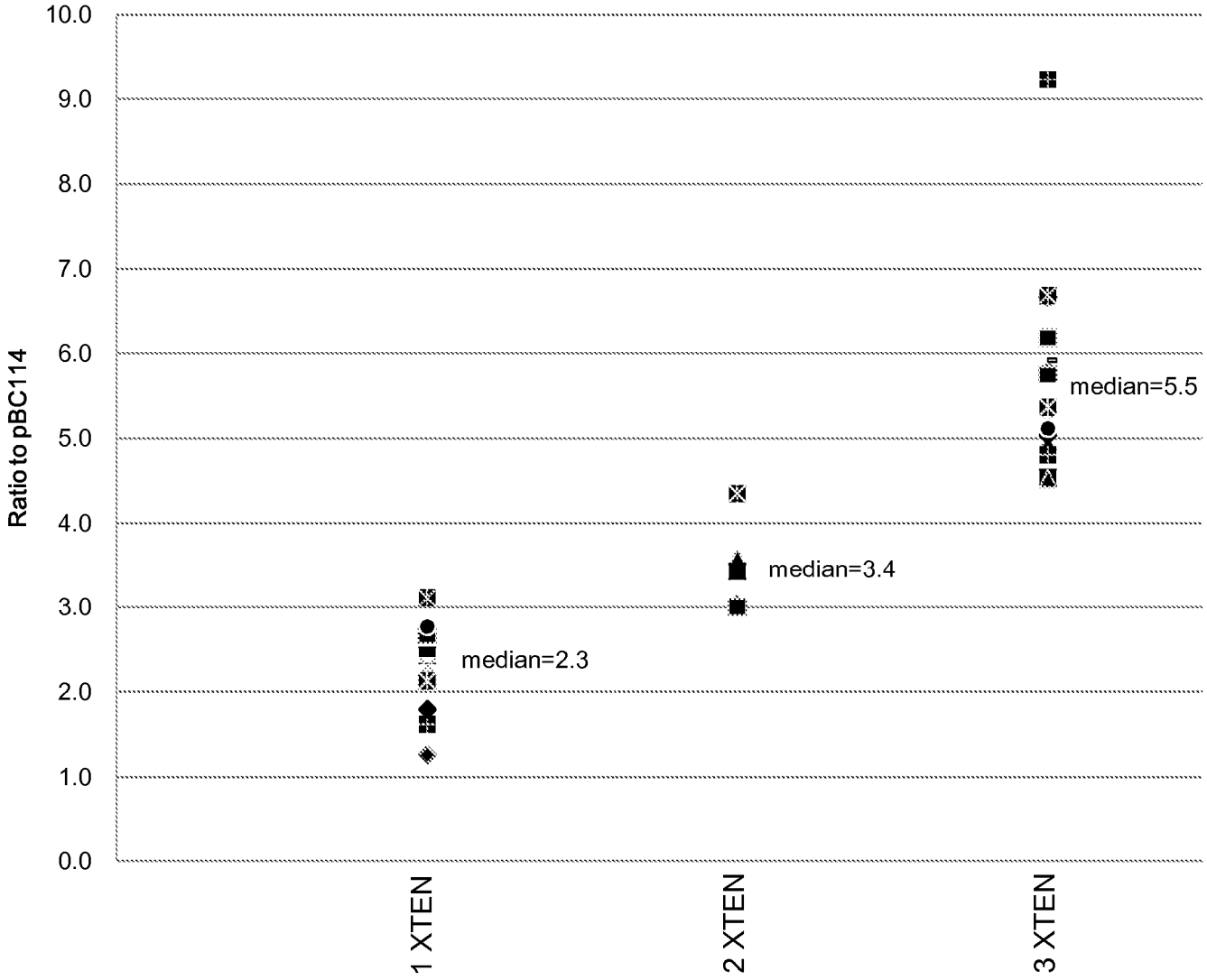

10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
Ratio to pBC114
median=2.3
median=3.4
median=5.5
1 XTEN
2 XTEN
3 XTEN

FIG. 29

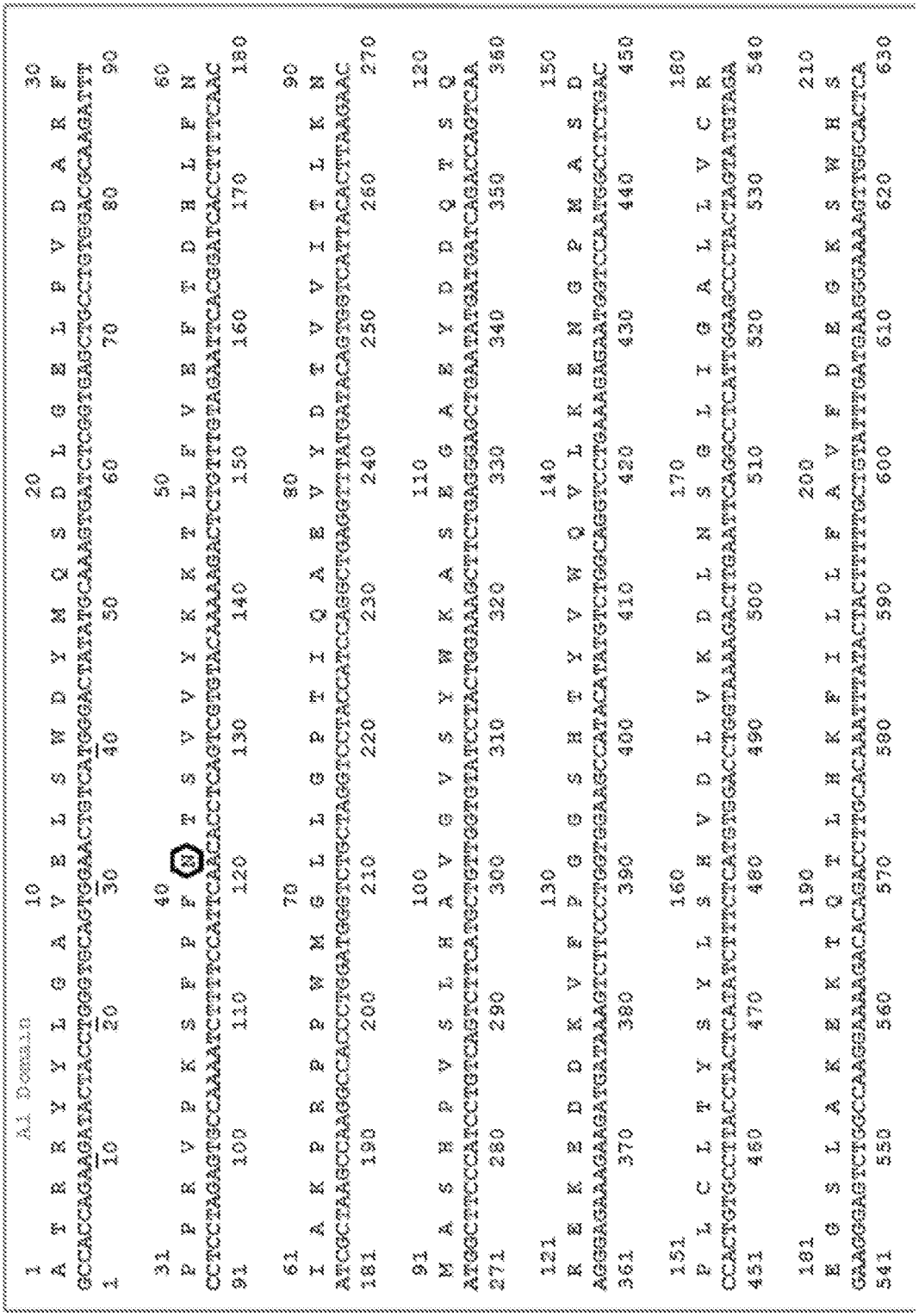

A1 Domain
1 10 20 30
A T E R Y Y L G A Y E L S W D Y M Q S D L G E L P V D A R F
GCCACCAGAAGGATACTACCTGGGTGCAGTGGAACTGTCATGGGACTATATGCAAAGTGATCTCGGTGAGCTGCCTGTGTTGGACGCAAGATTT
1 10 20 30 40 50 60 70 80 90
40
P P R V P K S F P P (N) T S V V Y K K T L F V E F T D H L F N
CCTCCTAGAGTGCCAAAATCTTTCCATTCAACCTCAGTCGTGTACAAAAAGACTCTGTTTGTAGAATTCACGGATCACCTTTTCAAC
91 100 110 120 130 140 150 160 170 180
50
I A K P R P P W M G L L G P T I Q A E V Y D T V V I T L K N
ATCGCTAAGCCAAGGCCACCCTGGATGGTCTGCTAGGTCCTACCATCCAGGCTGAAGTTATATGATACAGTGGTCATTACACTTAAGAAC
181 190 200 210 220 230 240 250 260 270
70
M A S H P V S L H A V G V S Y W K A S E G A E Y D D Q T S Q
ATGGCTTCCCATCCTGTCAGTCTTCATGCTGTTGGTGTATCCTACTGGAAAGCTTCTGAGGGAGCTGAATATGATCAGACCAGTCAA
271 280 290 300 310 320 330 340 350 360
90
K E K E D D K V F P G G S H T Y V W Q V L K E N G P M A S D
AGGGAGAAAGAAGATGATAAAGTCTTCCCTGGTGGAAGCCATACATATGTCTGGCAGGTCCTGAAAGAGAATGGTCCAATGGCCTCTGAC
361 370 380 390 400 410 420 430 440 450
110
P L C L T Y S Y L S K V D L V K D L N S G L I G A L L V C R
CCACTGTGCCTTACCTACTCATACTTTCTCATCTGTGACCTTGTAAAAGACTTGAATTCAGGCCTCATTGGAGCCCTACTAGTATGTAGA
451 460 470 480 490 500 510 520 530 540
130
E G S L A K E K T Q T L H K F I L L F A V F D E G K S W H S
GAAGGGAGTCTGGCCAAGGAAAAGACACAGACCTTGCACAAATTTATACTACTTTTTGCTGTATTTGATGAAGGGAAAAGTTGGCACTCA
541 550 560 570 580 590 600 610 620 630

FIG. 30A

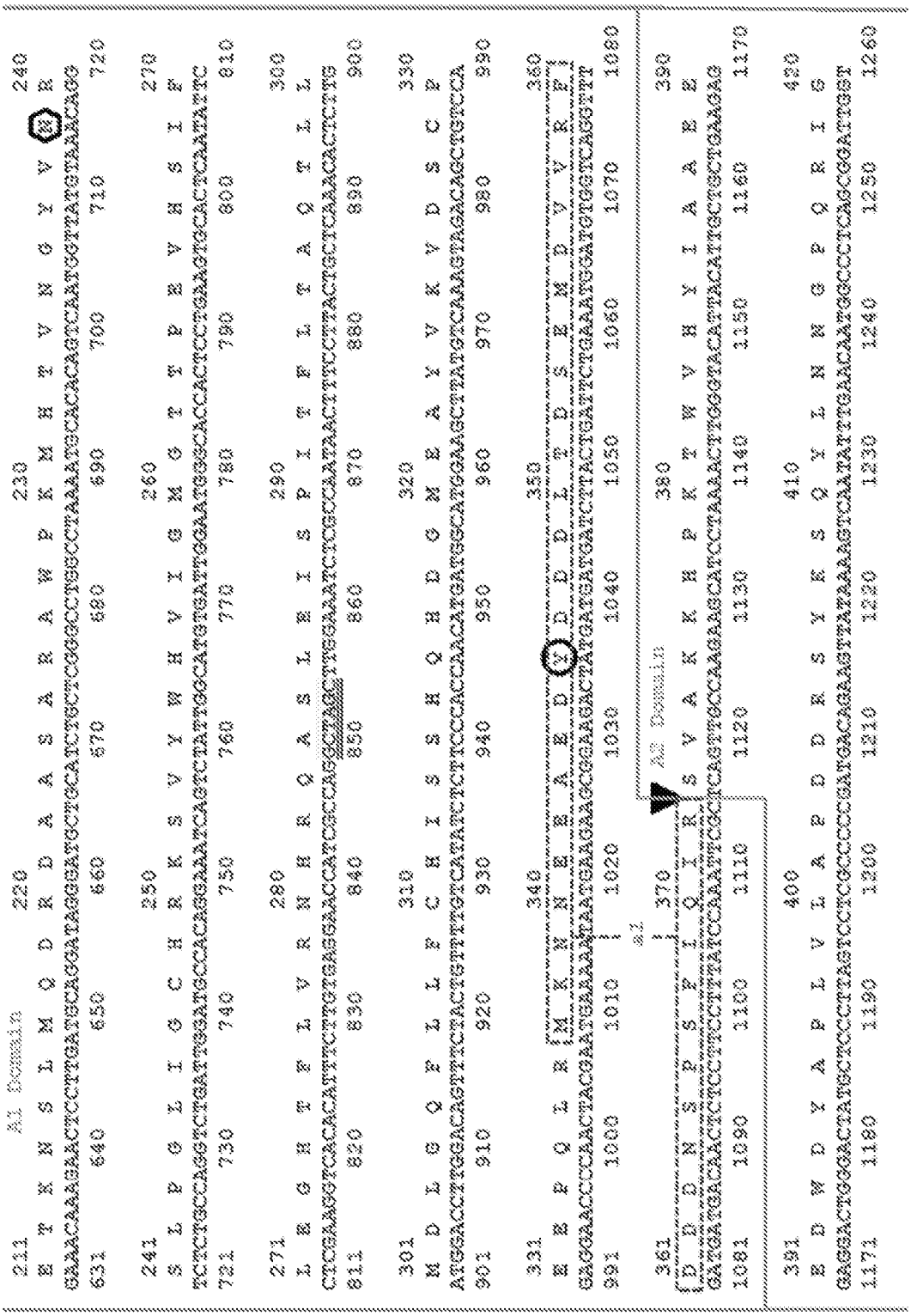


FIG. 30B

AB Domain
421 430 440 450
R K Y K K V R P M A Y T D E T F K T R E A I Q H E S G I L S
AGGAAGTACAAAAAAGTCCGATTATGGCATACACAGATGAAACCTTTAAGACTCGTGAAGCTATTCAGCATGAATCAGGAATCTTGGGA
1261 1270 1280 1290 1300 1310 1320 1330 1340 1350
451 460 470 480
P L L Y G E V G D T L L I I F K N Q A S R P Y N I Y P H G I
CCTTTACTTTATATGGGGAAGTTGGAGACACACTGTTGATTATATTTAAGAATCAAGCAAGCAGACCATATAACATCTACCCTCACGGAATC
1351 1360 1370 1380 1390 1400 1410 1420 1430 1440
481 490 500 510
T D V R P L Y S R K L P K G V K H L K D F P I L P G E I F K
ACTGATGTTCGTCCTTTGTATTCAAGGAGATTACCAAAAAGGTGTAAAACATTTGAAGGATTTTCCAATTCTGCCAGGAGAAATATTCAAA
1441 1450 1460 1470 1480 1490 1500 1510 1520 1530
511 520 530 540
Y K W T V T V E D G P T K S D P R C L T R Y Y S S F V N M E
TATAAATGGACAGTGACTGTAGAAGATGGGCCAACTAAATCAGATCCTCGGTGCCTGACCCGCTATTACTCTAGTTTCGTTAATATGGAG
1531 1540 1550 1560 1570 1580 1590 1600 1610 1620
541 550 560 570
R D L A S G L I G P L L I C Y K E S V D Q R G N Q I M S D K
AGAGATCTAGCTTCAGGACTCATTGGCCCTCTCCTCATCTGCTACAAGGAATCTGTAGATCAAGAGGAACCAGATAATGTCAGACAAG
1621 1630 1640 1650 1660 1670 1680 1690 1700 1710
571 580 590 600
R N V I L P S V P D E M R S W Y L T E N I Q R F L P N P A G
AGGAATGTCATCCTGTTTCCTGTATTTGATGAACCCGAAGCTGGTACCTCACAGAGAATATACAACGCTTTCTCCCCAATCCAGCTGGA
1711 1720 1730 1740 1750 1760 1770 1780 1790 1800
601 610 620 630
V Q L E D P E F Q A S N I M H S I N G Y V F D S L Q L S V C
GTGCAGCTTGAGGATCCAGAGTTCCAAGCCTCCAACATCATGCACAGCATCAATGGCTATGTTTTTGATAGTTTGCAGTTGTCAGTTTGT
1801 1810 1820 1830 1840 1850 1860 1870 1880 1890

FIG. 30C

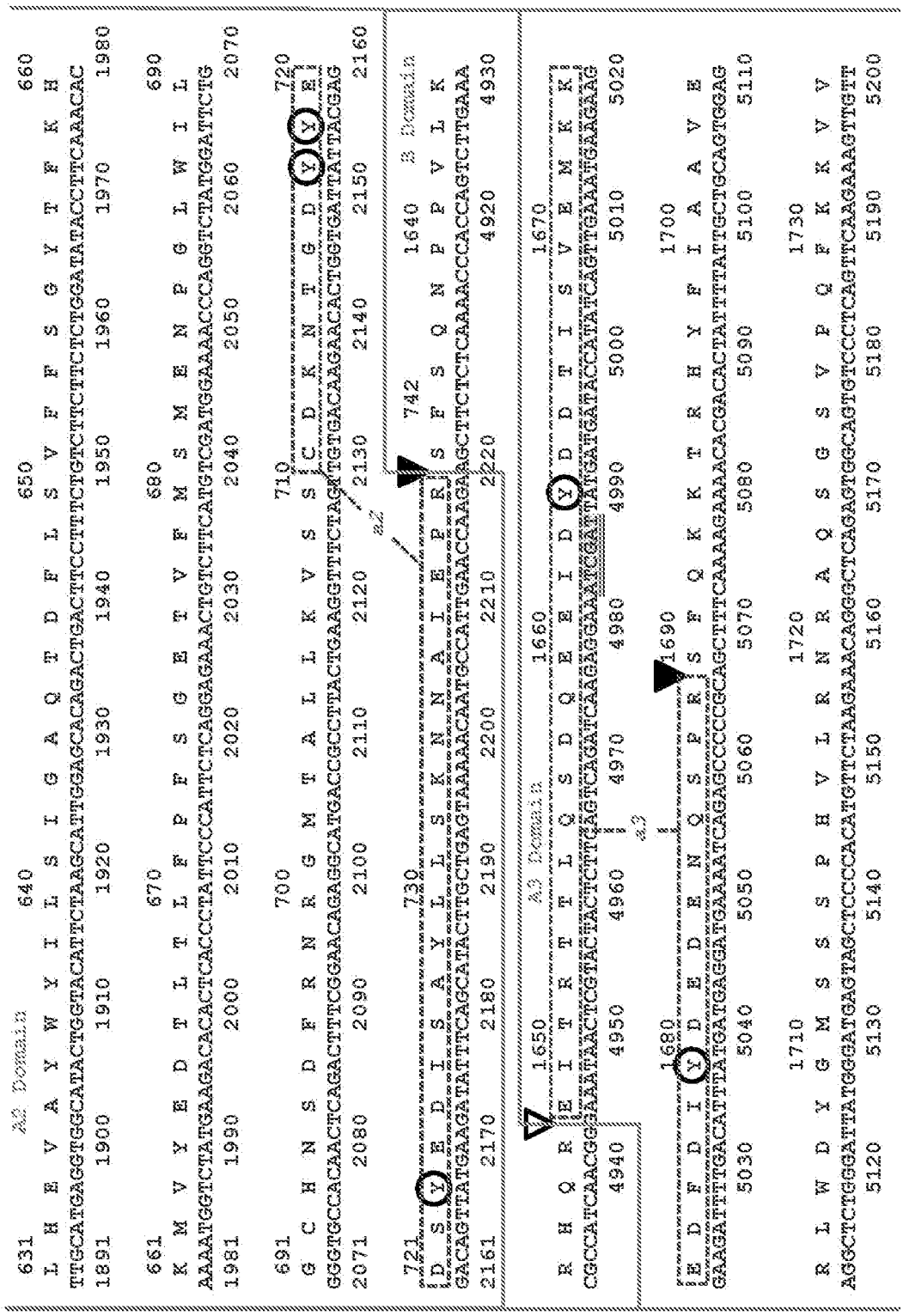


FIG. 30D

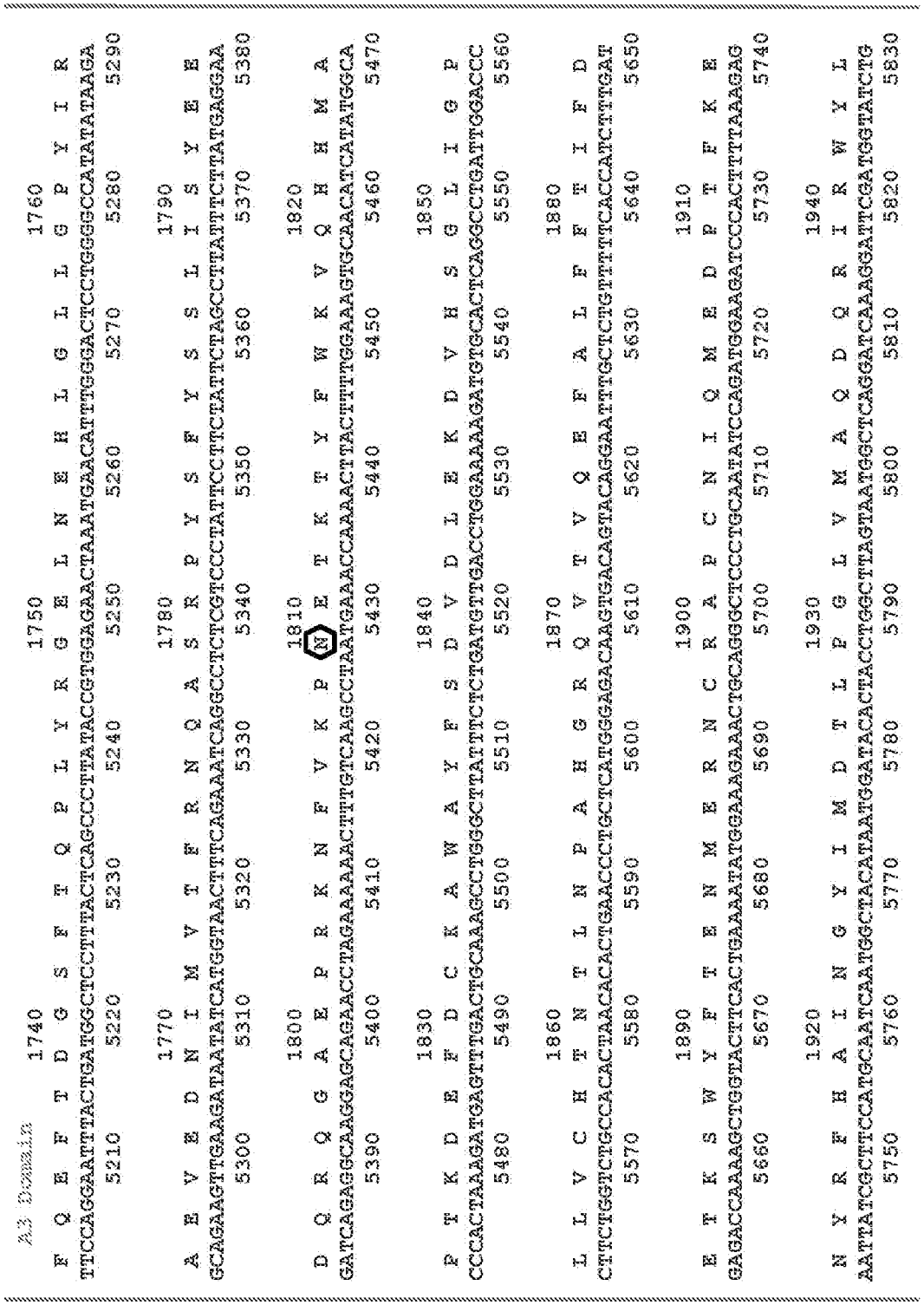

A3 Domain
1740 1750 1760
F Q E F T D G S F T Q P L Y R G E L N E H L G L L G P Y I R
TTCCAGGAATTTACTGATGGCTCCTTACTCAGCCCTATACCGTGGAGAACTAAATGAACATTTGGGACTCCTGGGGCCATATATAGA
5210 5220 5230 5240 5250 5260 5270 5280 5290
1770 1780 1790
A E V E D N I M V T F R N Q A S R P Y S F Y S S L I S Y E E
GCAGAAGTTGAAGATAATATCATGGTAACTTTCAGAAATCAGGCCTCTCGTCCCTATTCCTTCTATTCCTAGCCTTATTTCTTATGAGGAA
5300 5310 5320 5330 5340 5350 5360 5370 5380
1800 1810 1820
D Q R Q G A E P R K N F V K P N E T K T Y F W K V Q H H M A
GATCAGAGGCAAGGAGCAGAACCTAGAAAAAACTTTGTCAAGCCTAATGAAACCAAAACTACTTTTGGAAAGTGCAACATCATATGGCA
5390 5400 5410 5420 5430 5440 5450 5460 5470
1830 1840 1850
P T K D E F D C K A W A Y F S D V D L E K D V H S G L I G P
CCCACTAAAGATGAGTTTGACTGCAAAGCCTGGGCTTATTTCTCTGATGTTGACCTGGAAAAAGATGTGCACTCAGGCCTGATTGGACCC
5480 5490 5500 5510 5520 5530 5540 5550 5560
1860 1870 1880
L L V C H T N T L N P A H G R Q V T V Q E F A L F F T I F D
CTTCTGGTCTGCCACACTAACACACTGAACCCTGCTCATGGGAGACAAGTGACAGTACAGGAATTTGCTCTGTTTTTCACCATCTTTGAT
5570 5580 5590 5600 5610 5620 5630 5640 5650
1890 1900 1910
E T K S W Y F T E N M E R N C R A P C N I Q M E D P T F K E
GAGACCAAAAGCTGGTACTTCACTGAAAATATGGAAGAACTGCAGGGCTCCCTGCAATATCCAGATGGAAGATCCCACTTTTAAAGAG
5660 5670 5680 5690 5700 5710 5720 5730 5740
1920 1930 1940
N Y R F H A I N G Y I M D T L P G L V M A Q D Q R I R W Y L
AATTATCGCTTCCATGCAATCAATGGCTACATAATGGATACACTACCTGGCTTAGTAATGGCTCAGGATCAAAGGATTCGATGGTATCTG
5750 5760 5770 5780 5790 5800 5810 5820 5830

FIG. 30E

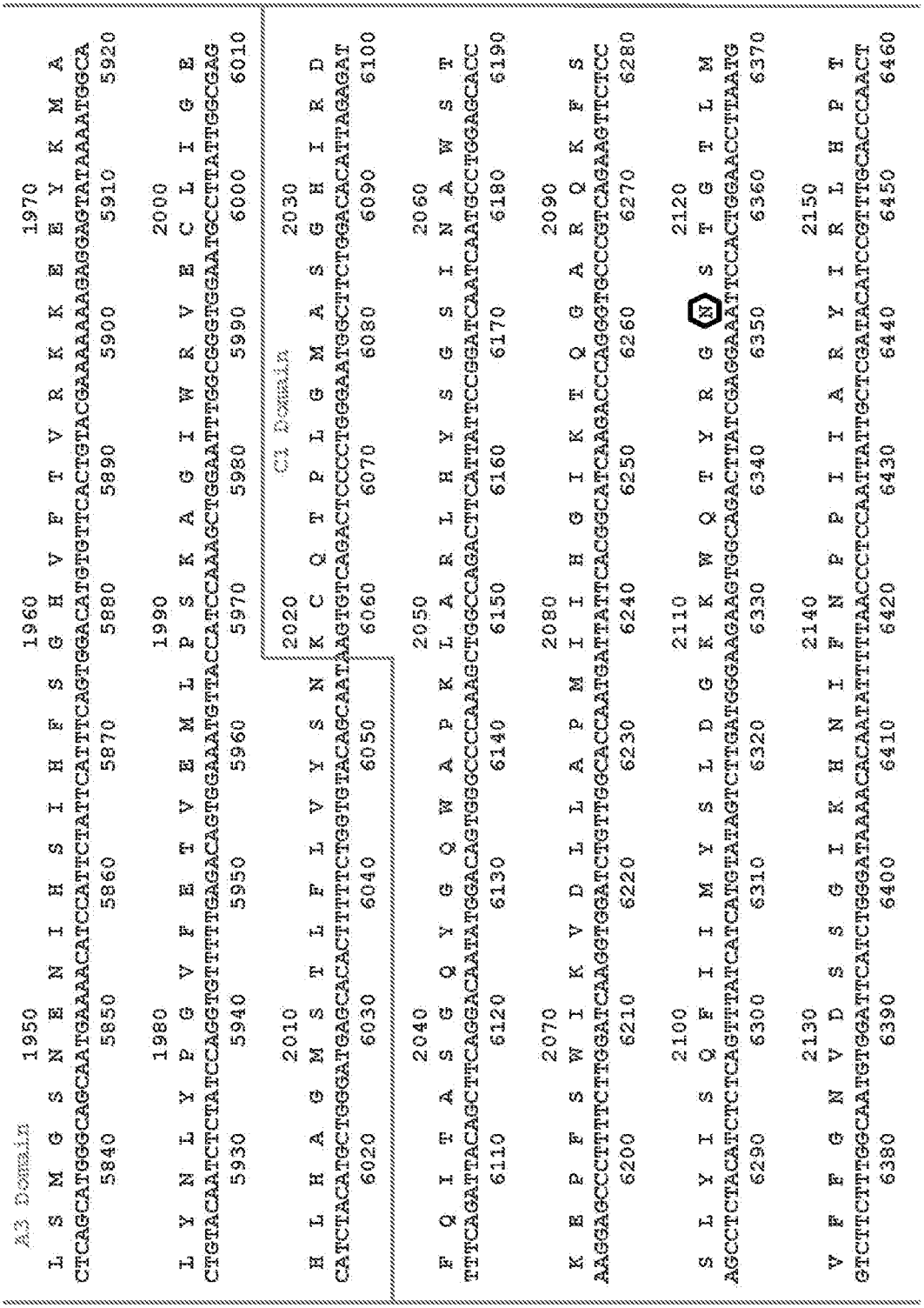


FIG. 30F

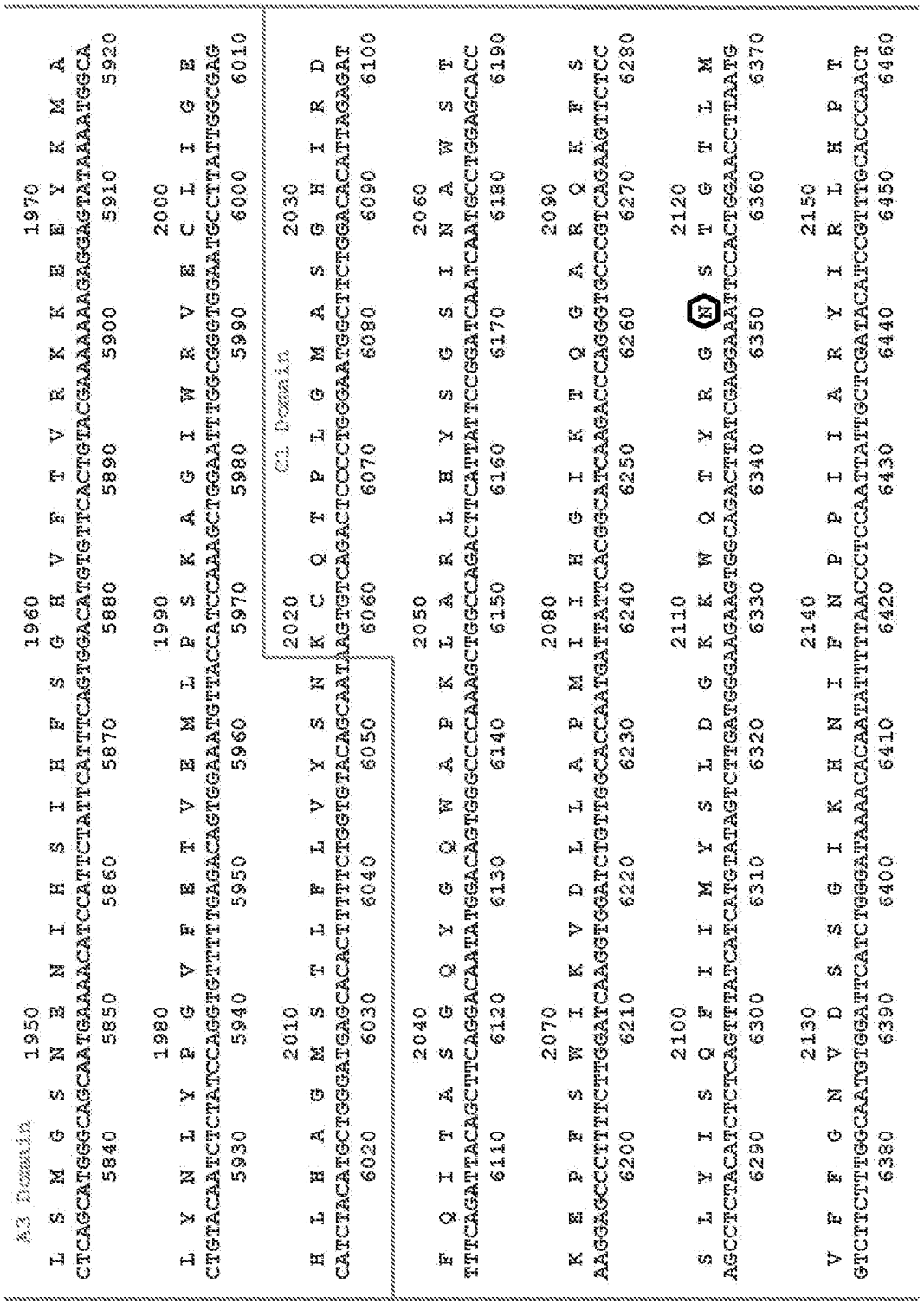

A3 Domain
C2 Domain
1950
1960
1970
1980
1990
2000
2010
2020
2030
2040
2050
2060
2070
2080
2090
2100
2110
2120
2130
2140
2150
W

FIG. 30G

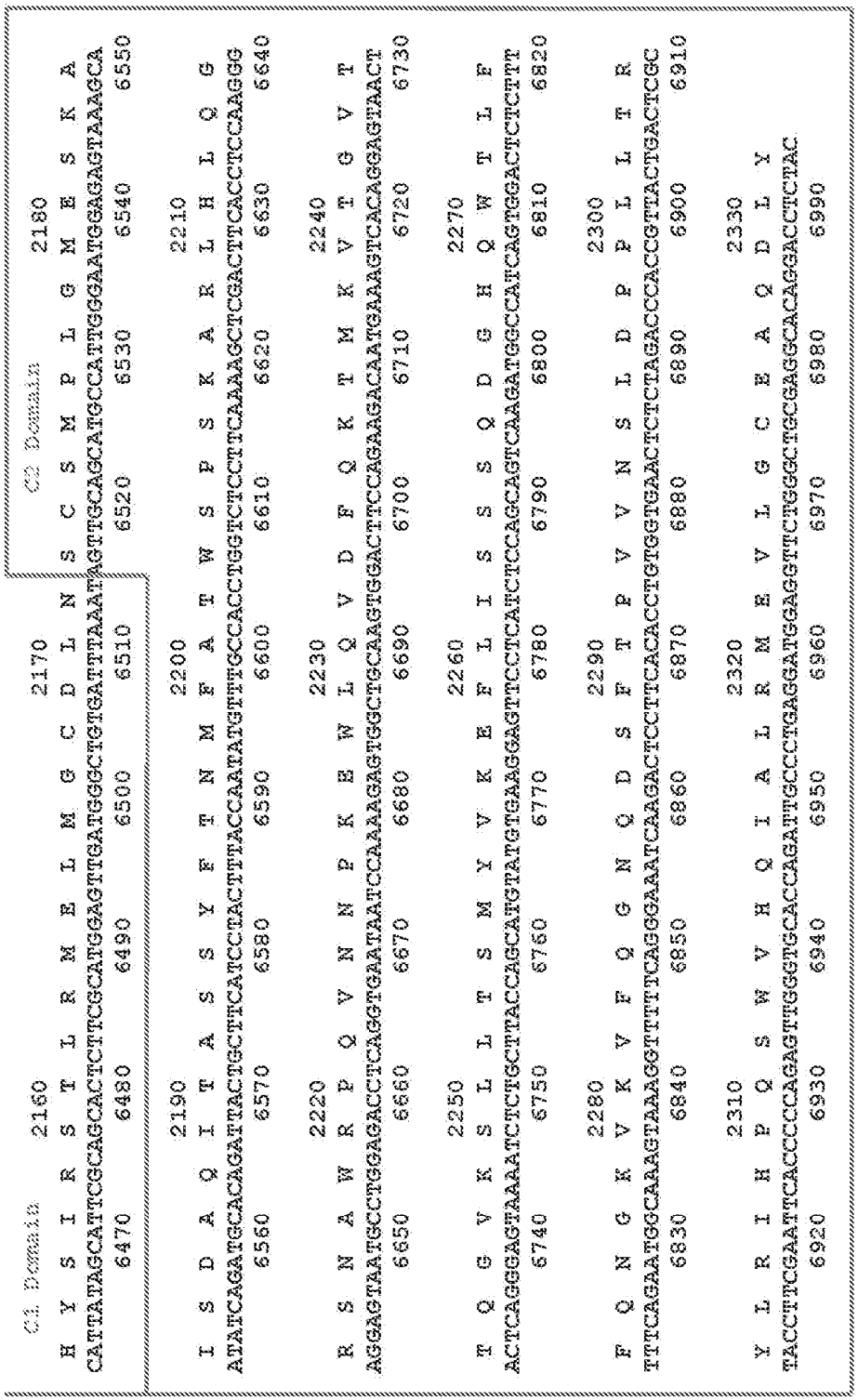

C1 Domain
C2 Domain
2160
2170
2180
H Y S I R S T L R M E L M G C D L N S C S M P L G M E S K A
CATTATAGCATTCGCAGCACTCTTCGCATGGAGTTGATGGCTGTGATTTAAATAGTTGCAGCATGCCATTGGGAATGGAGAGTAAAGCA
6470 6480 6490 6500 6510 6520 6530 6540 6550
2190
2200
2210
I S D A Q I T A S S Y F T N M F A T W S P S K A R L H L Q G
ATAICAGATGCACAGATTACTGCTTCATCCTACTTTACCAATATGTTTGCCACCTGGTCTCCTTCAAAAGCTCGACTTCACCTCCAAGGG
6560 6570 6580 6590 6600 6610 6620 6630 6640
2220
2230
2240
R S N A W R P Q V N N P K E W L Q V D F Q K T M K V T G V T
AGGAGTAATGCCTGGAGACCTCAGGTGAATAATCCAAAAGAGTGGCTGCAAGTGGACTTCCAGAAGACAATGAAAGTCACAGGAGTAACT
6650 6660 6670 6680 6690 6700 6710 6720 6730
2250
2260
2270
T Q G V K S L L T S M Y V K E F L I S S S Q D G H Q W T L F
ACTCAGGGAGTAAAAATCTCTGCTTACCAGCATGTATGTGAAGGAGTTCCTCATCTCCAGCAGTCAAGATGGCCATCAGTGGACTCTCTTT
6740 6750 6760 6770 6780 6790 6800 6810 6820
2280
2290
2300
F Q N G K V K V F Q G N Q D S F T P V V N S L D P P L L T R
TTTCAGAATGGCAAAGTAAAAGTTTTTCAGGGAAATCAAGACTCCTTCACACCTGTGGTGAACTCTCTAGACCCACCGTTACTGACTCGC
6830 6840 6850 6860 6870 6880 6890 6900 6910
2310
2320
2330
Y L R I H P Q S W V H Q I A L R M E V L G C E A Q D L Y
TACCTTCGAATTCACCCCCAGAGTTGGTGCACCAGATTGCCCTGAGGATGGAGGTTCTGGGCTGCGAGGCACAGGACCTCTAC
6920 6930 6940 6950 6960 6970 6980 6990

FIG. 30G

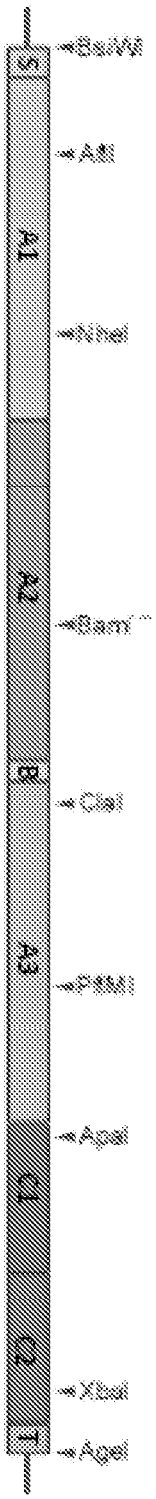


FIG. 31

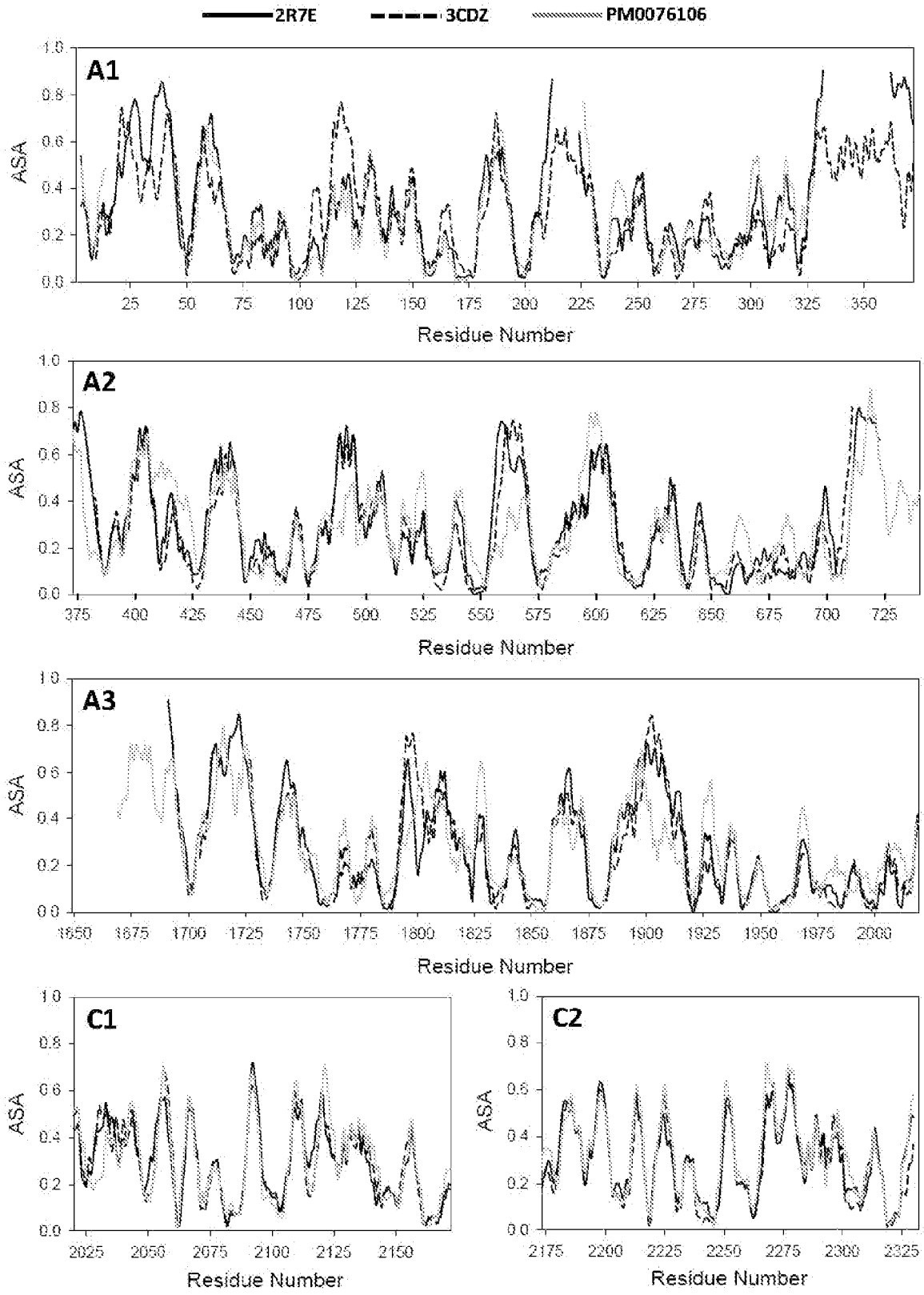


FIG. 32

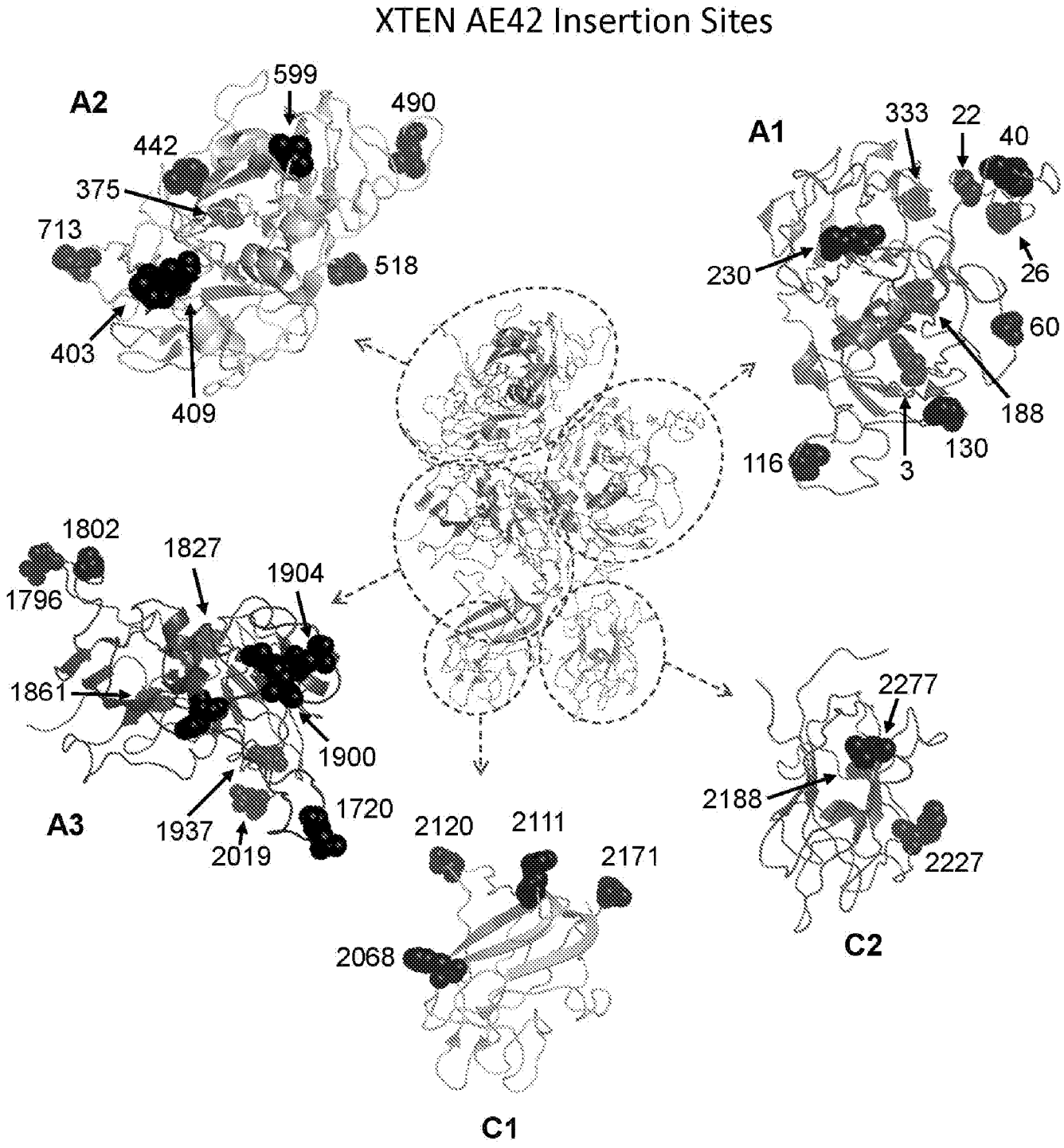

XTEN AE42 Insertion Sites
A2
599
490
442
375
713
518
403
409
A1
333
22
40
230
26
60
188
116
3
130
A3
1802
1827
1904
1796
1861
1900
1720
1937
2019
C1
2120
2111
2171
2068
C2
2277
2188
2227

FIG. 33

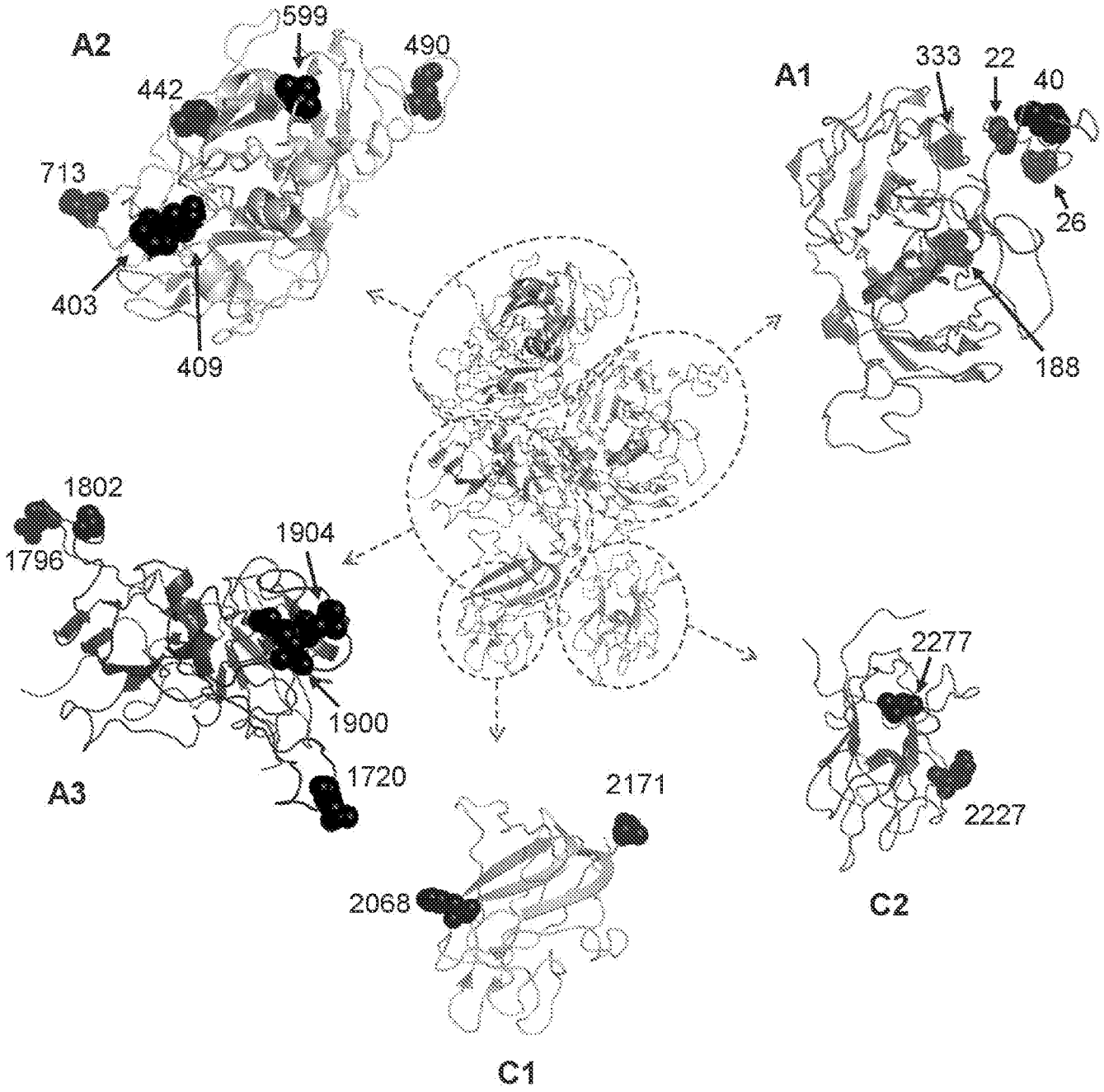

XTEN AE42 Insertion Sites with Activity
A2
599
490
442
713
403
409
A1
333
22
40
26
188
1802
1796
1904
1900
1720
A3
2277
2227
C2
2171
2068
C1

FIG. 34

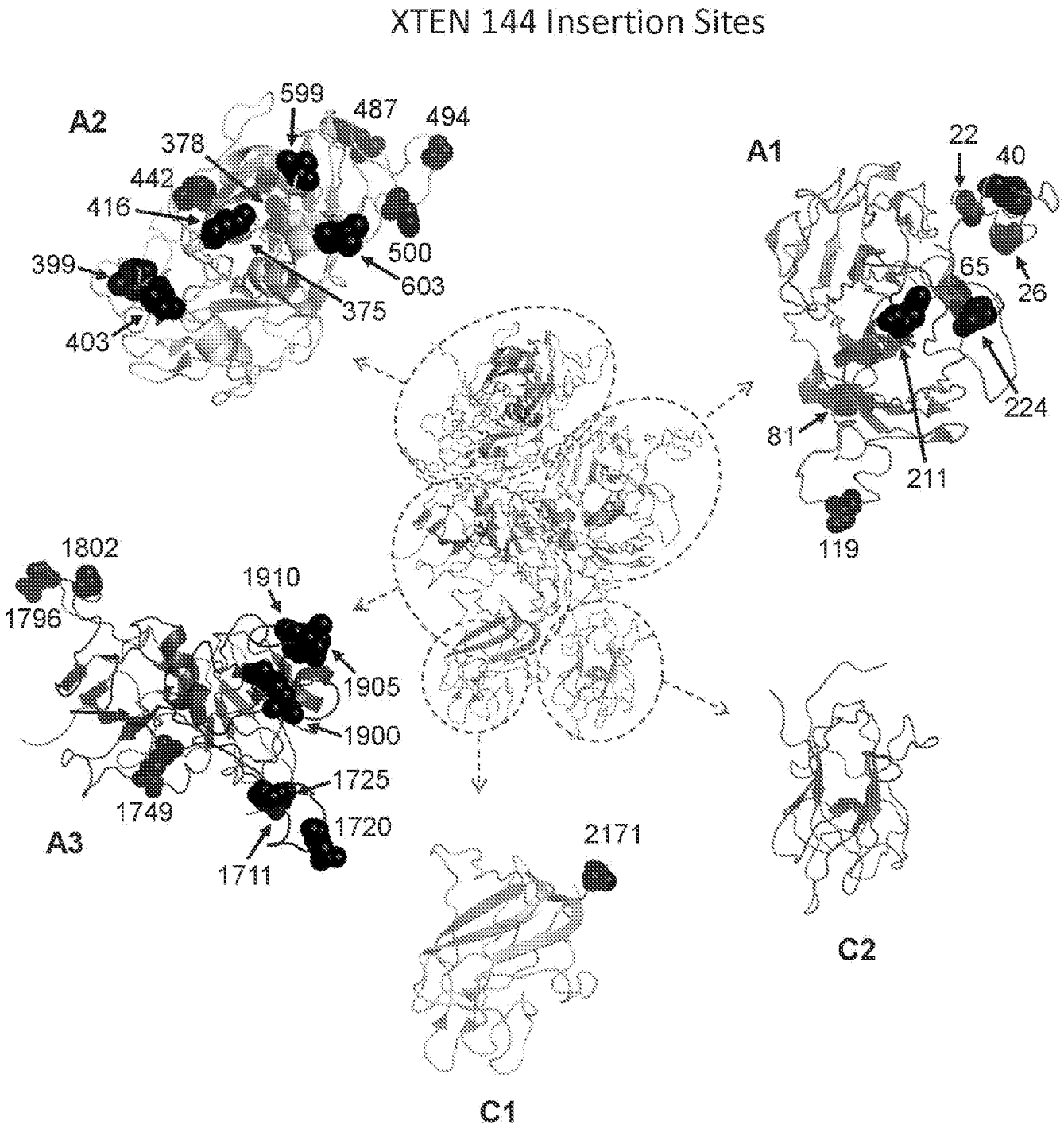

XTEN 144 Insertion Sites
A2
599
487
494
378
442
416
399
500
603
375
403
A1
22
40
65
26
224
81
211
119
1802
1796
1910
1905
1900
1725
1720
1749
1711
A3
2171
C1
C2

FIG. 35

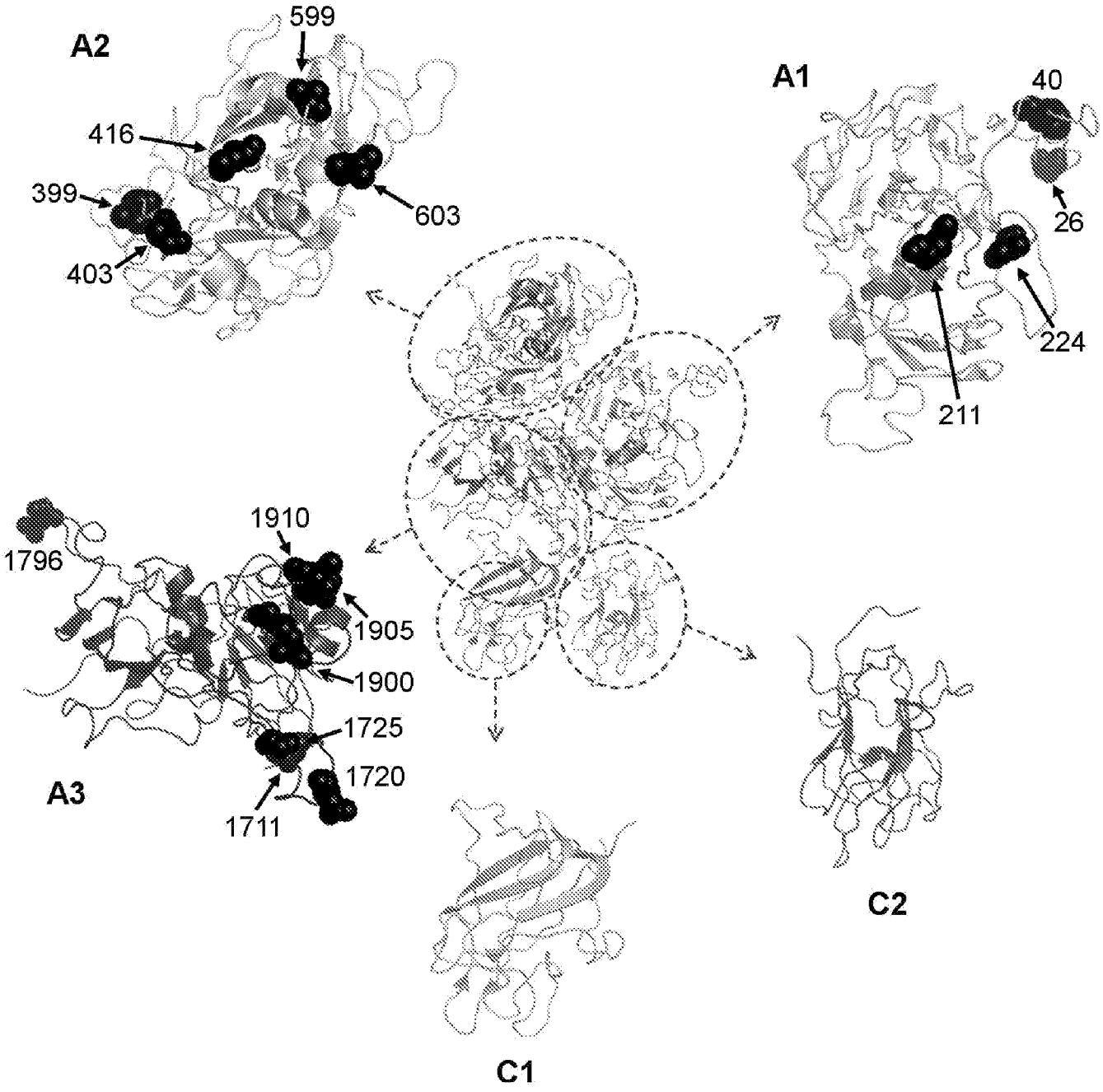

XTEN 144 Insertion Sites with Activity
A2
599
416
399
603
403
A1
40
26
224
211
1910
1796
1905
1900
1725
1720
A3
1711
C2
C1

FIG. 36

```
A1 (1)                                                          ATRRYYLGAVELSWD
A2 (373                                                      SVAKKHPKTWVHYIAAEEEDWD
A3 (1649)   EITRTTLQSDQEEIDYDDTISVEMKKEDFDIYDEDENQSPRSFQKKTRHYFIAAVERLWD
                                                                *  :::.* *  **

A1 (16)     YMQSDLGELPVDARFPPRVPKSFPFNTSVVYKKTLFVEFTDHLFNIAKPR---PPWMGLL
A2 (395)    YAPLVL--APDDRSYKSQYLNNGPQRIGRKYKKVRFMAYTDETFKTREAI---QHESGIL
A3 (1709)   YG---------MSSSPHVLRNRAQSGSVPQFKKVVFQEFTDGSFTQPLYRGELNEHLGLL
            *                    :          :**. *  :**  *.             *:*

A1 (73)     GPTIQAEVYDTVVITLKNMASHPVSLHAVGVSYWKASEGAEYDDQTSQREKEDDKVFPGG
A2 (450)    GPLLYGEVGDTLLIIFKNQASRPYNIYPHGITDVRPLY--SRRLPKGVKHLKDFPILPGE
A3 (1760)   GPYIRAEVEDNIMVTFRNQASRPYSFYSSLISYEEDQR--QGAEPR------KNFVKPNE
            ** : .** *.::: ::* **:* .::.  ::   .     .           .  : *.

A1 (133)    SHTYVWQVLKENGPMASDPLCLTYSYLSHVDLVKDLNSGLIGALLVCREGSLAK--EKTQ
A2 (508)    IFKYKWTVTVEDGPTKSDPRCLTRYYSSFVNMERDLASGLIGPLLICYKESVDQRGNQIM
A3 (1812)   TKTYFWKVQHHMAPTKDEFDCKAWAYFSDVDLEKDVHSGLIGPLLVCHTNTLNPAHGRQV
              .* * *  . .*  .:  * :  * * *:: :*: *****.**:*   ::     :

A1 (191)    TLHKFILLFAVFDEGKSWHSETKNSLMQDRDAASARAWP------KMHTVNGYVNRSLPG
A2 (568)    SDKRNVILFSVFDENRSWYLTENIQRFLPNPAGVQLEDPEFQASNIMHSINGYVFDSLQ-
A3 (1872)   TVQEFALFFTIFDETKSWYFTENMERNCRAPCNIQMEDPTFKENYRFHAINGYIMDTLPG
            : :.  ::*::*** :**:   : .     .     *       :*::***:  :*

A1 (245)    LIGCHRKSVYWHVIGMGTTPEVHSIFLEGHTFLVRN---HRQASLEISPITFLTAQTLLM
A2 (627)    LSVCLHEVAYWYILSIGAQTDFLSVFFSGYTFKHKMVYEDTLTLFPFSG---ETVFMSME
A3 (1932)   LVMAQDQRIRWYLLSMGSNENIHSIHFSGHVFTVRKKEEYKMALYNLYPGVFETVEMLPS
            *  .  :   *:::.:*:  :. *:.:.*:.*  :       :   :      *.

A1 (302)    DLGQFLLFCHISSHQHDGMEAYVKVDSCPEEPQLRMKNNEEAEDYDDDLTDSEMDVVRFD
A2 (684)    NPGLWILGCHNSDFRNRGMTALLKVSSCDKNTGDYYEDSYEDISAYLLSKNNAIEPR
A3 (1992)   KAGIWRVECLIGEHLHAGMSTLFLVYSN
            . * : : *  ... : ** : . * *

A1 (362)    DDNSPSFIQIR



C1 (2020)   KCQTPLGMASGHIRDFQITASGQYG----QWAPKLARLHYSGSINAWS--TKEPFSWIKV
C2 (2173)   SCSMPLGMESKAISDAQITASSYFTNMFATWSPSKARLHLQGRSNAWRPQVNNPKEWLQV
            .*. **** *  * * *****. :      *:*. **** .*  ***   .::* .*::*

C1 (2074)   DLLAPMIIHGIKTQGARQKFSSLYISQFIIMYSLDGKKWQTYRGNSTGTLMVFFGNVDSS
C2 (2233)   DFQKTMKVTGVTTQGVKSLLTSMYVKEFLISSSQDGHQWTLFFQN--GKVKVFQGNQDSF
            *:  .* : *:.***.:. ::*:*:.:*:*  * **::*  :  *  *.: ** ** **

C1 (2134)   GIKHNIFNPPIIARYIRLHPTHYSIRSTLRMELMGCDLN---
C2 (2291)   TPVVNSLDPPLLTRYLRIHPQSWVHQIALRMEVLGCEAQDLY
                * ::**:::**:*:**  :  : :****::**: :
```

FIG. 37

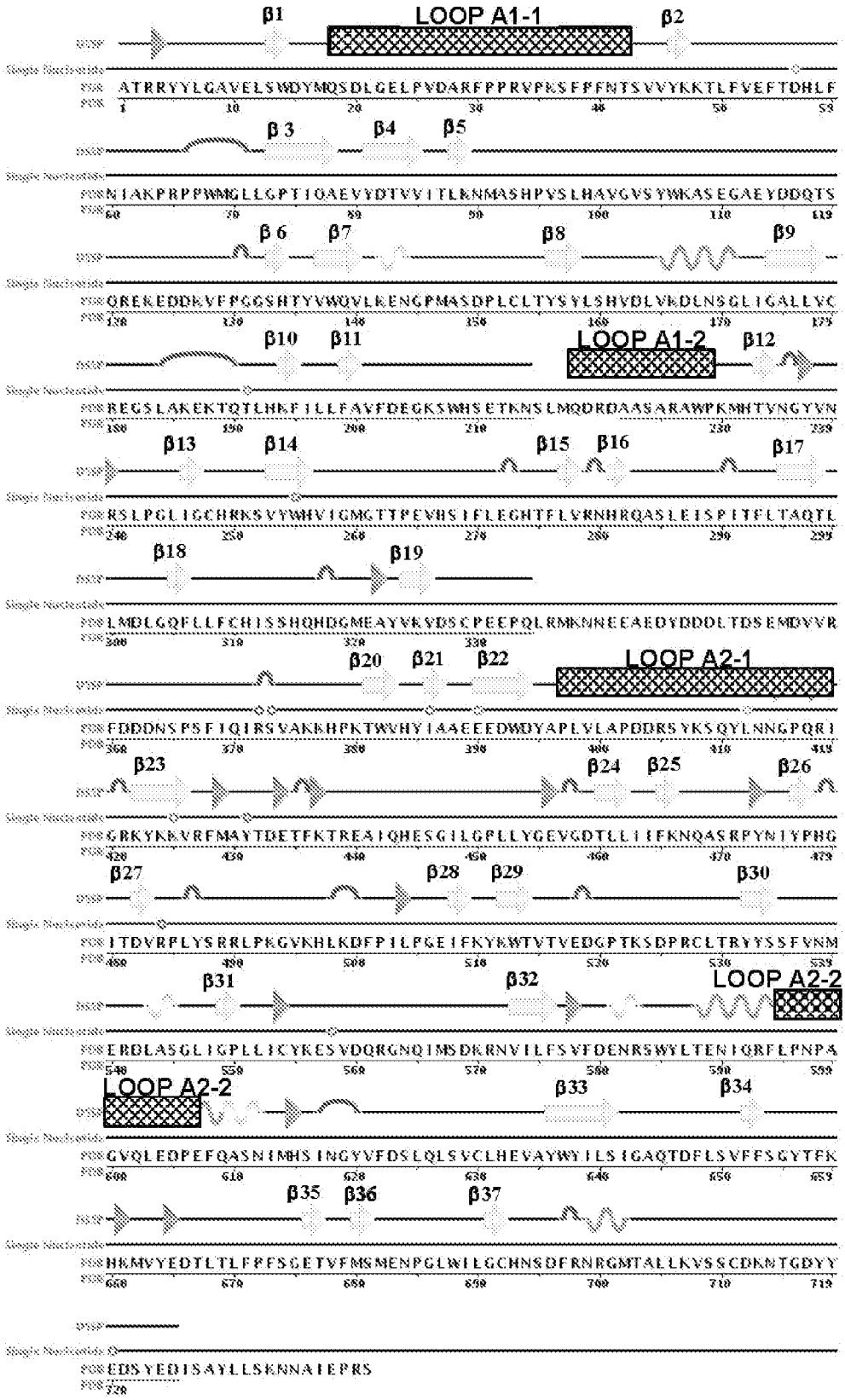

β1
LOOP A1-1
β2
β3
β4
β5
β6
β7
β8
β9
β10
β11
LOOP A1-2
β12
β13
β14
β15
β16
β17
β18
β19
β20
β21
β22
LOOP A2-1
β23
β24
β25
β26
β27
β28
β29
β30
β31
β32
LOOP A2-2
LOOP A2-2
β33
β34
β35
β36
β37

FIG. 38

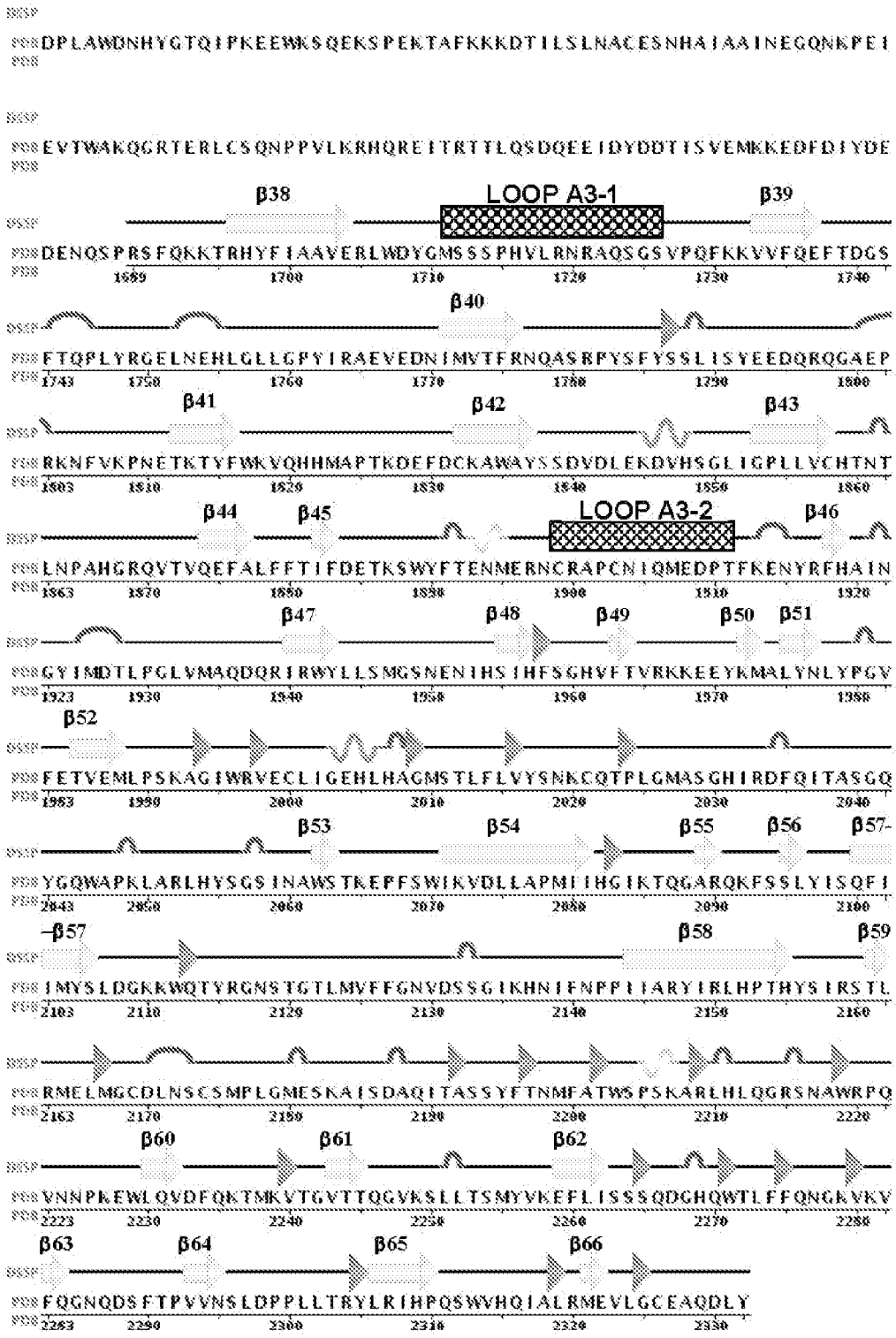

DPLAWDNHYGTQIPKEEWKSQEKSPEKTAFKKKDTILSLNACESNHAIAAINEGQNKPEI
EVTWAKQGRTERLCSQNPPVLKRHQREITRTTLQSDQEEIDYDDTISVEMKKEDFDIYDE
β38
LOOP A3-1
β39
DENQSPRSFQKKTRHYFIAAVERLWDYGMSSSPHVLRNRAQSGSVPQFKKVVFQEFTDGS
1689
1700
1710
1720
1730
1740
β40
FTQPLYRGELNEHLGLLGPYIRAEVEDNIMVTFRNQASRPYSFYSSLISYEEDQRQGAEP
1743
1750
1760
1770
1780
1790
1800
β41
β42
β43
RKNFVKPNETKTYFWKVQHHMAPTKDEFDCKAWAYFSDVDLEKDVHSGLIGPLLVCHTNT
1803
1810
1820
1830
1840
1850
1860
β44
β45
LOOP A3-2
β46
LNPAHGRQVTVQEFALFFTIFDETKSWYFTENMERNCRAPCNIQMEDPTFKENYRFHAIN
1863
1870
1880
1890
1900
1910
1920
β47
β48
β49
β50
β51
GYIMDTLPGLVMAQDQRIRWYLLSMGSNENIHSIHFSGHVFTVRKKEEYKMALYNLYPGV
1923
1930
1940
1950
1960
1970
1980
β52
FETVEMLPSKAGIWRVECLIGEHLHAGMSTLFLVYSNKCQTPLGMASGHIRDFQITASGQ
1983
1990
2000
2010
2020
2030
2040
β53
β54
β55
β56
β57-
YGQWAPKLARLHYSGSINAWSTKEPFSWIKVDLLAPMIIHGIKTQGARQKFSSLYISQFI
2043
2050
2060
2070
2080
2090
2100
β57
β58
β59
IMYSLDGKKWQTYRGNSTGTLMVFFGNVDSSGIKHNIFNPPIIARYIRLHPTHYSIRSTL
2103
2110
2120
2130
2140
2150
2160
RMELMGCDLNSCSMPLGMESKAISDAQITASSYFTNMFATWSPSKARLHLQGRSNAWRPQ
2163
2170
2180
2190
2200
2210
2220
β60
β61
β62
VNNPKEWLQVDFQKTMKVTGVTTQGVKSLLTSMYVKEFLISSSQDGHQWTLFFQNGKVKV
2223
2230
2240
2250
2260
2270
2280
β63
β64
β65
β66
FQGNQDSFTPVVNSLDPPLLTRYLRIHPQSWVHQIALRMEVLGCEAQDLY
2283
2290
2300
2310
2320
2330

FIG. 39

```
A1 (1)                                                          ATRRYYLGAVELSWD
A2 (373                                                      SVAKKHPKTWVHYIAAEEEDWD
A3 (1649)   EITRTTLQSDQEEIDYDDTISVEMKKEDFDIYDEDENQSPRSFQKKTRHYFIAAVERLWD
                                                                *  :::.* *  **

A1 (16)     YMQSDLGELPVDARFPPRVPKSFPFNTSVVYKKTLFVEFTDHLFNIAKPR---PPWMGLL
A2 (395)    YAPLVL--APDDRSYKSQYLNNGPQRIGRKYKKVRFMAYTDETFKTREAI---QHESGIL
A3 (1709)   YG---------MSSSPHVLRNRAQSGSVPQFKKVVFQEFTDGSFTQPLYRGELNEHLGLL
            *                    :          :**. *  :**  *.            *:*

A1 (73)     GPTIQAEVYDTVVITLKNMASHPVSLHAVGVSYWKASEGAEYDDQTSQREKEDDKVFPGG
A2 (450)    GPLLYGEVGDTLLIIFKNQASRPYNIYPHGITDVRPLY--SRRLPKGVKHLKDFPILPGE
A3 (1760)   GPYIRAEVEDNIMVTFRNQASRPYSFYSSLISYEEDQR--QGAEPR------KNFVKPNE
            ** : .** *.::: ::* **:* .::.  ::  .     .          .  : *.

A1 (133)    SHTYVWQVLKENGPMASDPLCLTYSYLSHVDLVKDLNSGLIGALLVCREGSLAK--EKTQ
A2 (508)    IFKYKWTVTVEDGPTKSDPRCLTRYYSSFVNMERDLASGLIGPLLICYKESVDQRGNQIM
A3 (1812)   TKTYFWKVQHHMAPTKDEFDCKAWAYFSDVDLEKDVHSGLIGPLLVCHTNTLNPAHGRQV
              .* * *  . .*  .:  * :  * * *:: :*: *****.**:*   ::     :

A1 (191)    TLHKFILLFAVFDEGKSWHSPKTKNSLMQDRDAASARAWP-L----KMHTVNGYVNRSLPG
A2 (568)    SDKRNVILFSVFDENRSWYLTENIQRFLPNPAGVQLEDPEFQASNIMHSINGYVFDSLQ-
A3 (1872)   TVQEFALFFTIFDETKSWYFTENMERNCRAPCNIQMEDPTFKENYRFHAINGYIMDTLPG
            : :.  ::*::*** :**:   : .    .      *       :*::***:  :*

A1 (245)    LIGCHRKSVYWHVIGMGTTPEVHSIFLEGHTFLVRN---HRQASLEISPITFLTAQTLLM
A2 (627)    LSVCLHEVAYWYILSIGAQTDFLSVFFSGYTFKHKMVYEDTLTLFPFSG---ETVFMSME
A3 (1932)   LVMAQDQRIRWYLLSMGSNENIHSIHFSGHVFTVRKKEEYKMALYNLYPGVFETVEMLPS
            *  .  :   *:::.:*:  :. *:.:.*:.*  :       :   :      *.

A1 (302)    DLGQFLLFCHISSHQHDGMEAYVKVDSCPEEPQLRMKNNEEAEDYDDDLTDSEMDVVRFD
A2 (684)    NPGLWILGCHNSDFRNRGMTALLKVSSCDKNTGDYYEDSYEDISAYLLSKNNAIEPR
A3 (1992)   KAGIWRVECLIGEHLHAGMSTLFLVYSN
            . * : : *  ... : ** : . * *

A1 (362)    DDNSPSFIQIR



C1 (2020)   KCQTPLGMASGHIRDFQITASGQYG----QWAPKLARLHYSGSINAWS--TKEPFSWIKV
C2 (2173)   SCSMPLGMESKAISDAQITASSYFTNMFATWSPSKARLHLQGRSNAWRPQVNNPKEWLQV
            .*. **** *  * * *****. :      *:*. **** .*  ***   .::* .*::*

C1 (2074)   DLLAPMIIHGIKTQGARQKFSSLYISQFIIMYSLDGKKWQTYRGNSTGTLMVFFGNVDSS
C2 (2233)   DFQKTMKVTGVTTQGVKSLLTSMYVKEFLISSSQDGHQWTLFFQN--GKVKVFQGNQDSF
            *:  .* : *:.***.:. ::*:*:.:*:*  * **::*  :  *  *.: ** ** **

C1 (2134)   GIKHNIFNPPIIARYIRLHPTHYSIRSTLRMELMGCDLN---
C2 (2291)   TPVVNSLDPPLLTRYLRIHPQSWVHQIALRMEVLGCEAQDLY
                * ::**:::**:*:**  :  : :******::**: :
```

FIG. 40

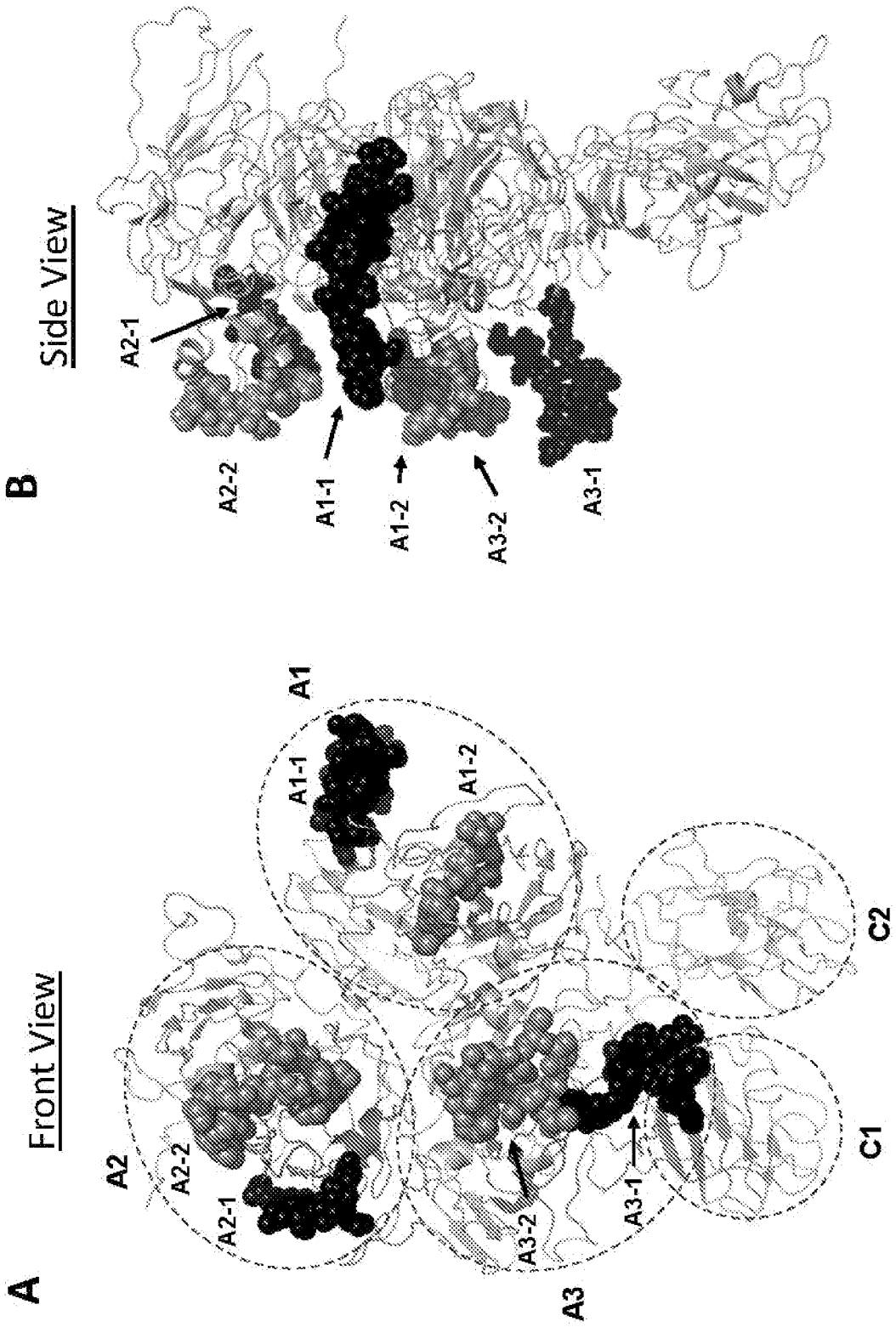

A
Front View
A1
A1-1
A1-2
A2
A2-2
A2-1
A3-2
A3-1
A3
C1
C2
B
Side View
A2-1
A2-2
A1-1
A1-2
A3-2
A3-1

FIG. 41

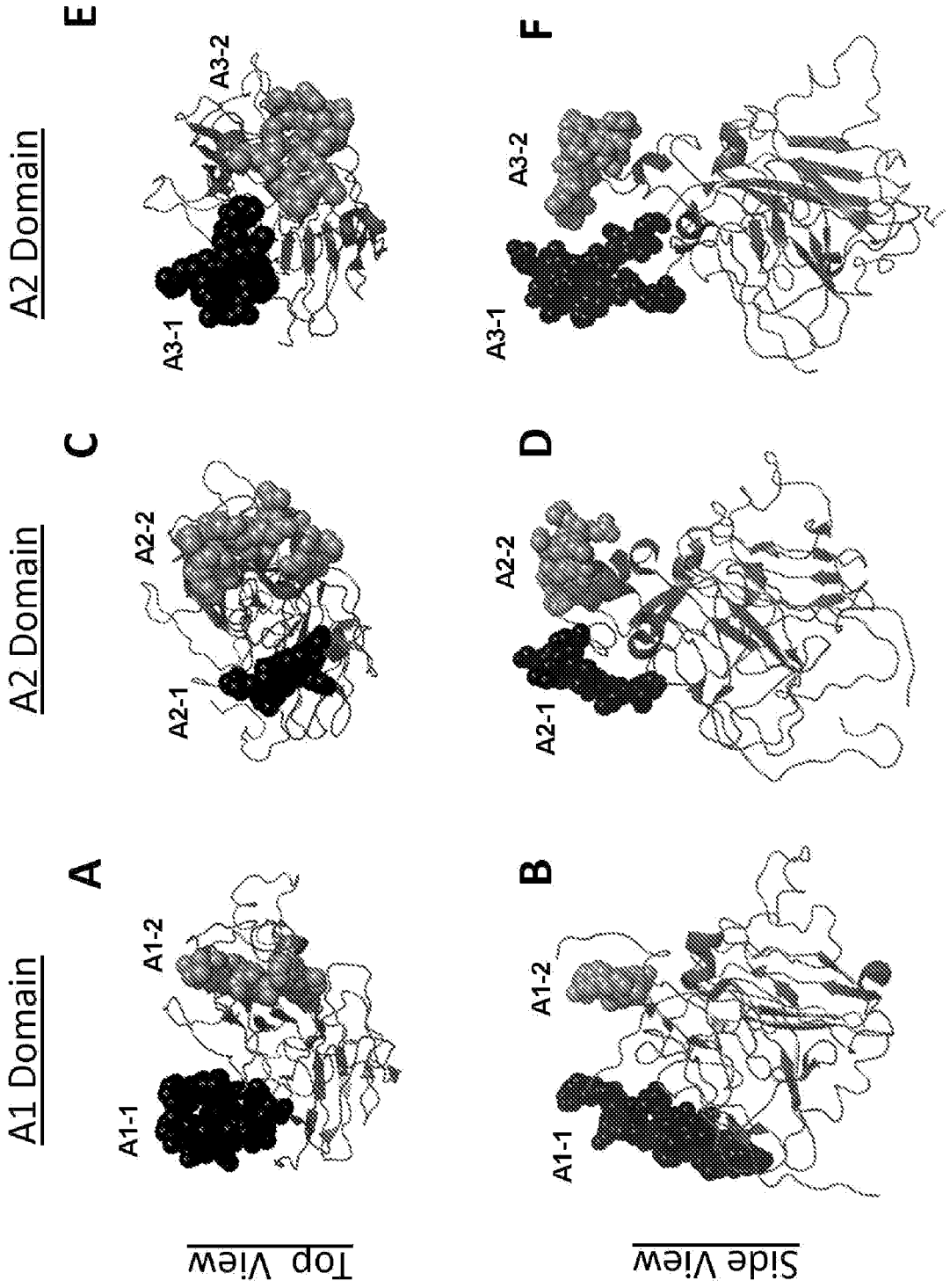

A1 Domain
A2 Domain
A2 Domain
A
C
E
A1-1
A1-2
A2-1
A2-2
A3-1
A3-2
Top View
B
D
F
A1-1
A1-2
A2-1
A2-2
A3-1
A3-2
Side View

FIG. 42

R740
R1648
Y1680
R1689
1 MNAIEPRSFSQN----------GPVLKRHQRSITRTLQSQEEIDVDTTSVEMKKLDFNVYGEMNQSERSFQKTRH FVTII_pBC0114
2 MNAIEPR----------SLQKTRH FVTII_Ref1
3 MNAIEPRSFSQNSRHST----------QSERSFQKTRH FVTII_Ref2
4 MNAIEPRSFSQXTEN_AE144PPVLKRHQRSITRTLQSQEEIDVDTTSVEMKKLDFNVYGEMNQSERSFQKTRH 0745_AE144_3B
5 MNAIEPRSFSQXTEN_AE144PPVLKRHQAIITRTLQSQEEIDVDTTSVEMKKLDFNVYGEMNQSERSFQKTRH 0745_AG144_3B/A1648a
6 MNAIEPRSFSQPPVLKRHQELITRTTLQXTEN_AG144SQEEIDVDTTSVEMKKLDFNVYGEMNQSERSFQKTRH 1656_AG144_3
7 MNAIEPRSFSQXTEN_AE144----------QSERSFQKTRH Proposal 1 pNL0006
8 MNAIEPRSFSQXTEN_AE144PPVLKRHQAIITRTLQSQEEIDVDTTSVEMKKLDFNVYGEMNQSERSFQKTRH Proposal 2 pNL0007
9 MNAIEPRSFSQXTEN_AE144PPVLKRHQRSITRTLQSQEEIDVDTTSVEMKKLDFNVYGEMNQSERSFQKTRH Proposal 3 pNL0008
10 MNAIEPRSFSQ----------XTEN_AG144SQEEIDVDTTSVEMKKLDFNVYGEMNQSERSFQKTRH Proposal 4 pNL0009/10

FIG. 43

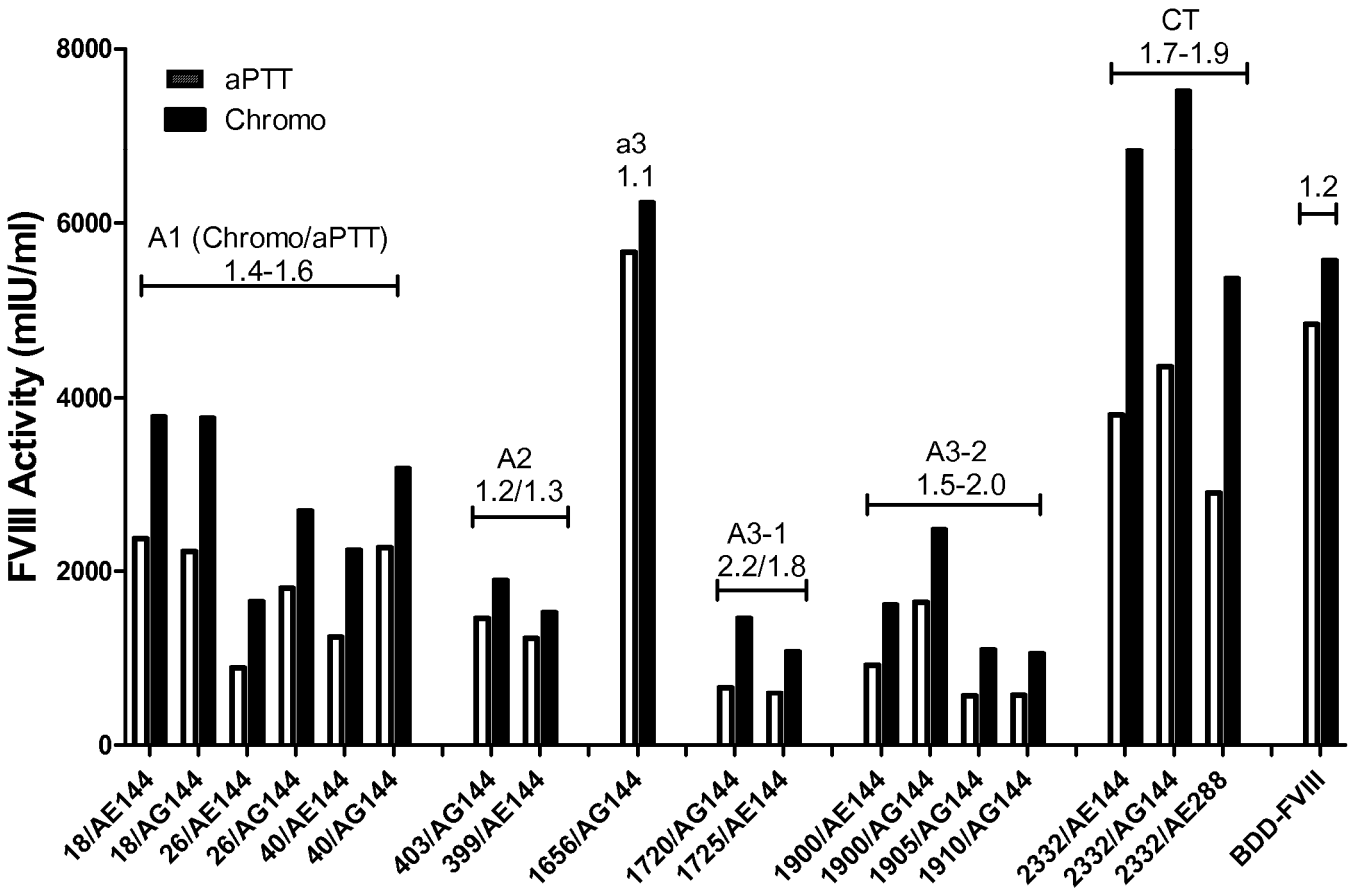

8000
6000
4000
2000
0
FVIII Activity (mIU/ml)
aPTT
Chromo
A1 (Chromo/aPTT)
1.4-1.6
A2
1.2/1.3
a3
1.1
A3-1
2.2/1.8
A3-2
1.5-2.0
CT
1.7-1.9
1.2
18/AE144
18/AG144
26/AE144
26/AG144
40/AE144
40/AG144
403/AG144
399/AE144
1656/AG144
1720/AG144
1725/AE144
1900/AE144
1900/AG144
1905/AG144
1910/AG144
2332/AE144
2332/AG144
2332/AE288
BDD-FVIII

FIG. 44

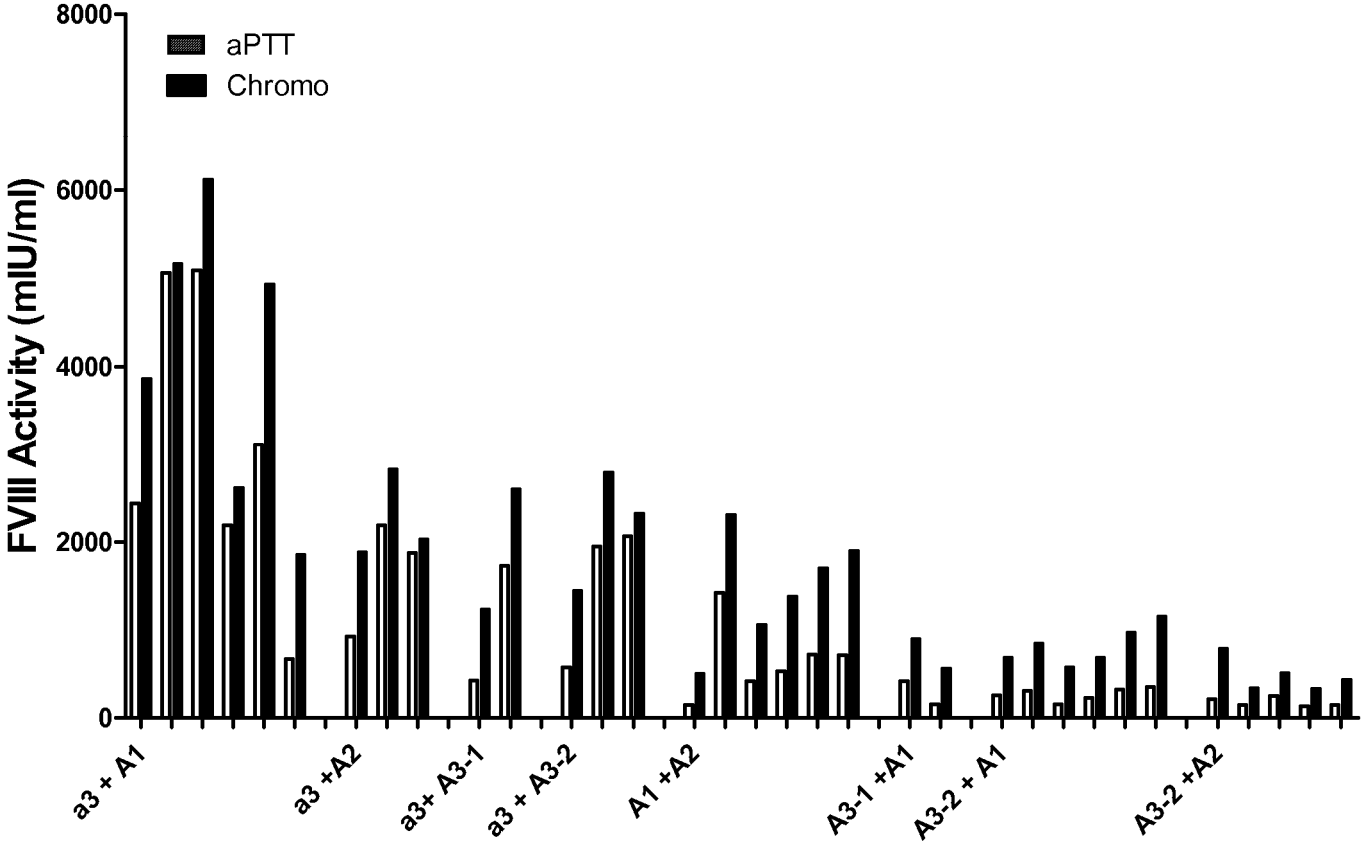

8000
6000
4000
2000
0
FVIII Activity (mIU/ml)
aPTT
Chromo
a3 + A1
a3 +A2
a3+ A3-1
a3 + A3-2
A1 +A2
A3-1 +A1
A3-2 + A1
A3-2 +A2

FIG. 45

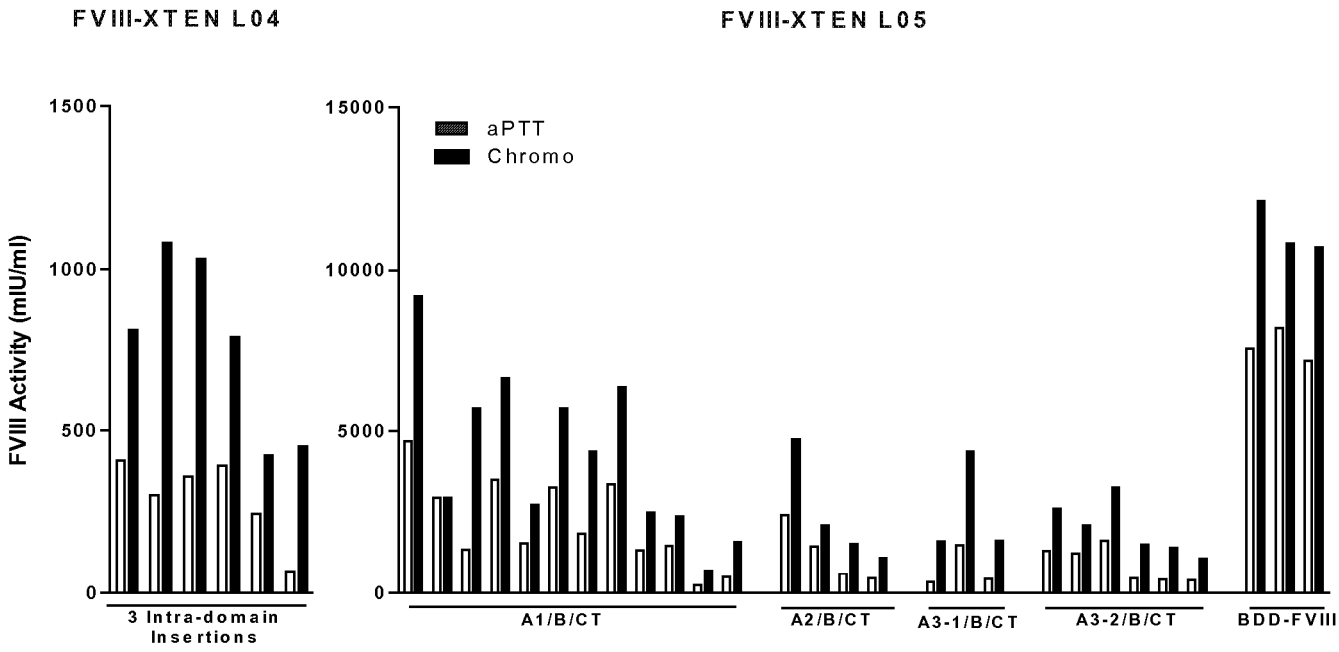

FVIII-XTEN L04
FVIII-XTEN L05
FVIII Activity (mIU/ml)
1500
1000
500
0
15000
10000
5000
0
aPTT
Chromo
3 Intra-domain Insertions
A1/B/CT
A2/B/CT
A3-1/B/CT
A3-2/B/CT
BDD-FVIII

FIG. 46

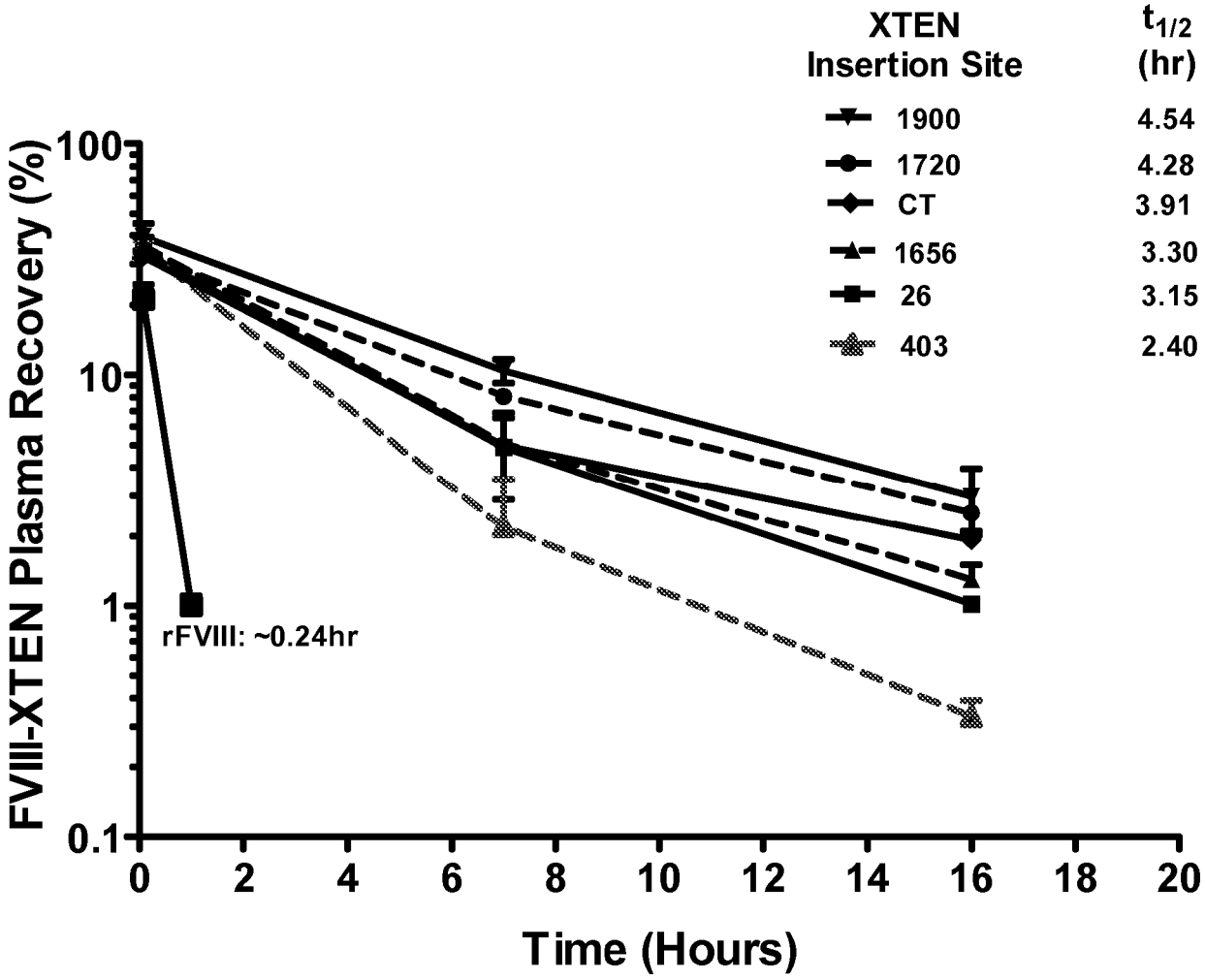

XTEN Insertion Site
$t_{1/2}$ (hr)
1900 4.54
1720 4.28
CT 3.91
1656 3.30
26 3.15
403 2.40
FVIII-XTEN Plasma Recovery (%)
100
10
1
0.1
rFVIII: ~0.24hr
0 2 4 6 8 10 12 14 16 18 20
Time (Hours)

FIG. 47

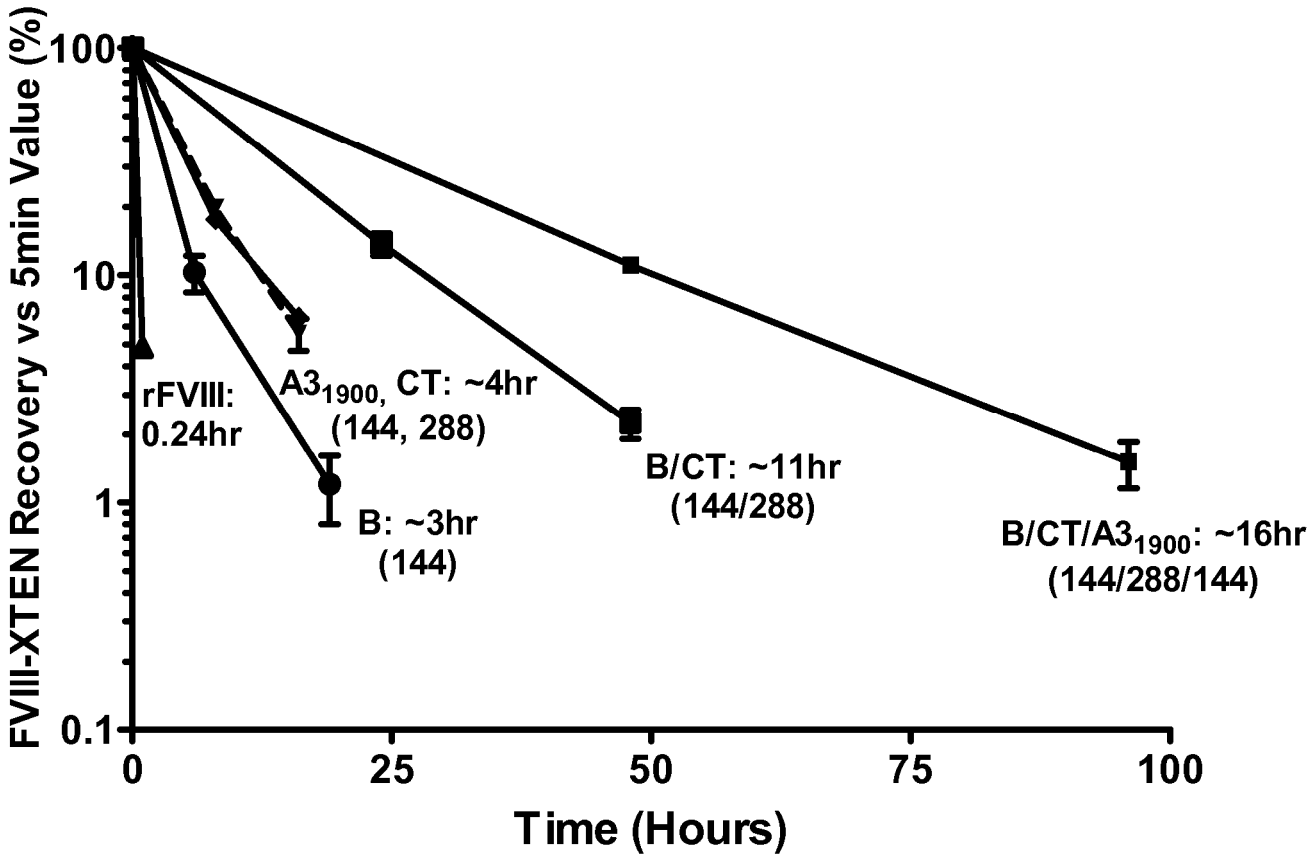
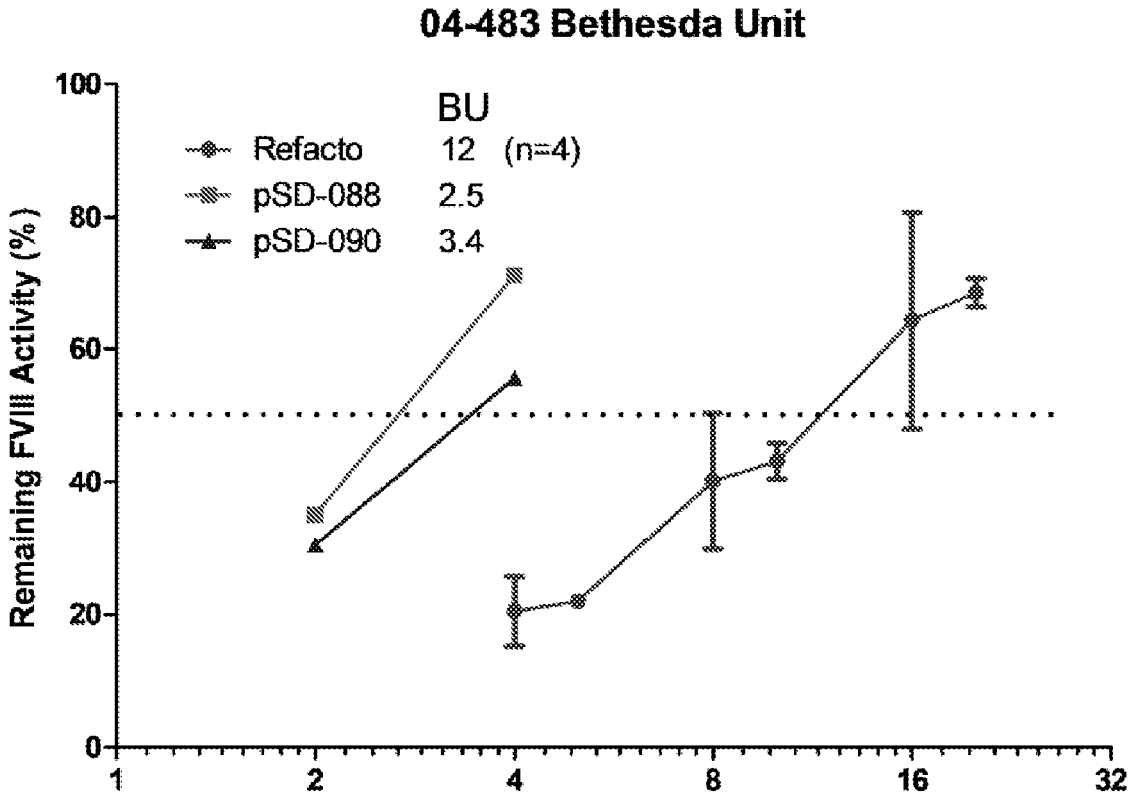

FVIII-XTEN Recovery vs 5min Value (%)
100
10
1
0.1
0
25
50
75
100
Time (Hours)
rFVIII:
0.24hr
A3$_{1900}$, CT: ~4hr
(144, 288)
B: ~3hr
(144)
B/CT: ~11hr
(144/288)
B/CT/A3$_{1900}$: ~16hr
(144/288/144)

FIG. 48

A


04-483 Bethesda Unit
BU
Refacto 12 (n=4)
pSD-088 2.5
pSD-090 3.4
Remaining FVIII Activity (%)
100
80
60
40
20
0
1
2
4
8
16
32

B

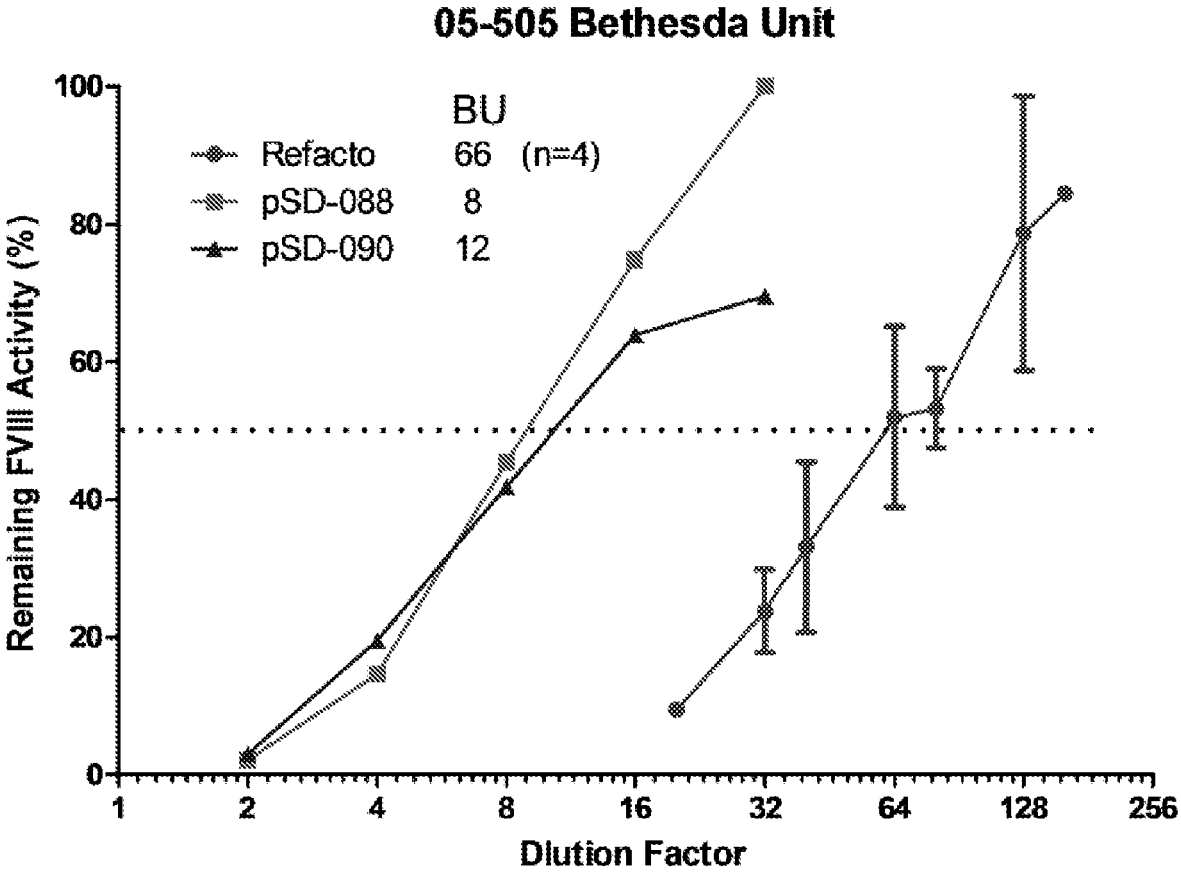
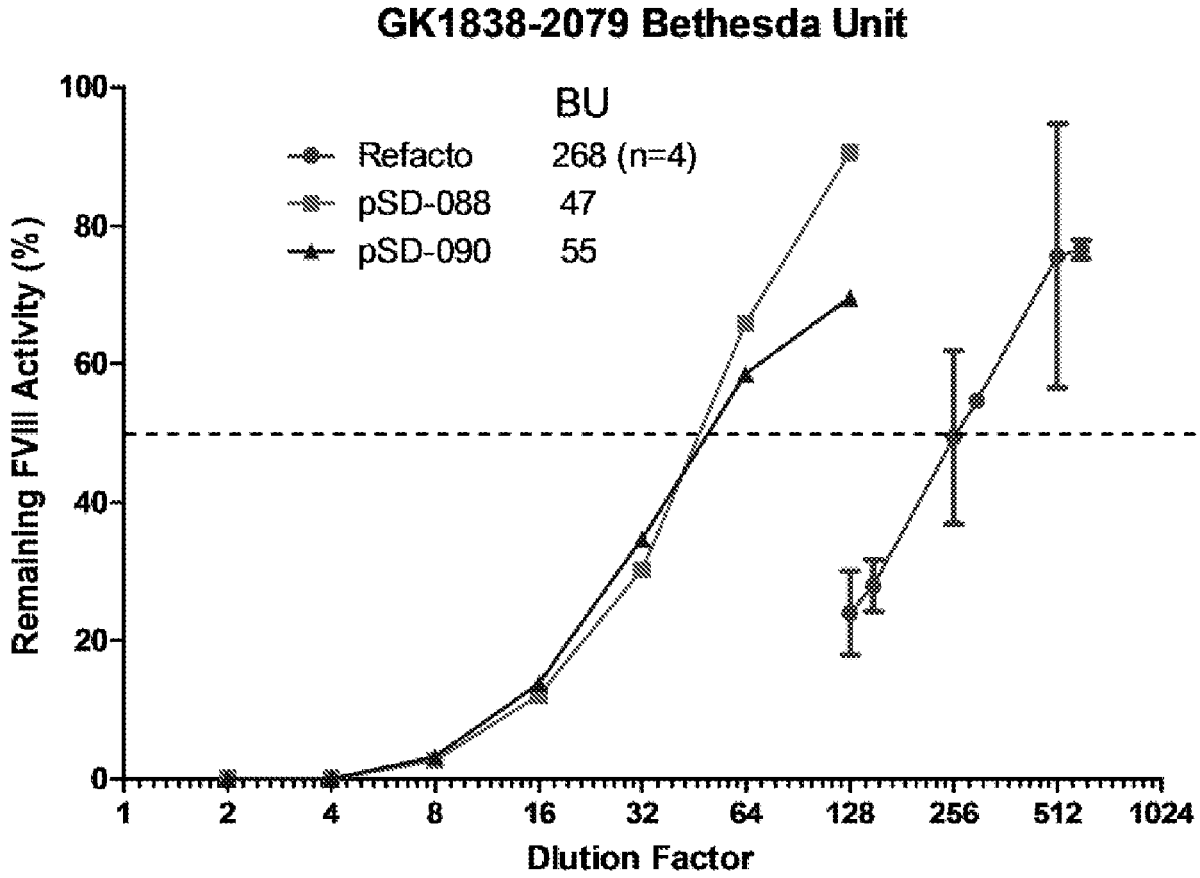

05-505 Bethesda Unit
BU
Refacto 66 (n=4)
pSD-088 8
pSD-090 12
Remaining FVIII Activity (%)
100
80
60
40
20
0
1
2
4
8
16
32
64
128
256
Dilution Factor

FIG. 49

C


GK1838-2079 Bethesda Unit
BU
Refacto 268 (n=4)
pSD-088 47
pSD-090 55
Remaining FVIII Activity (%)
100
80
60
40
20
0
1
2
4
8
16
32
64
128
256
512
1024
Dlution Factor

D

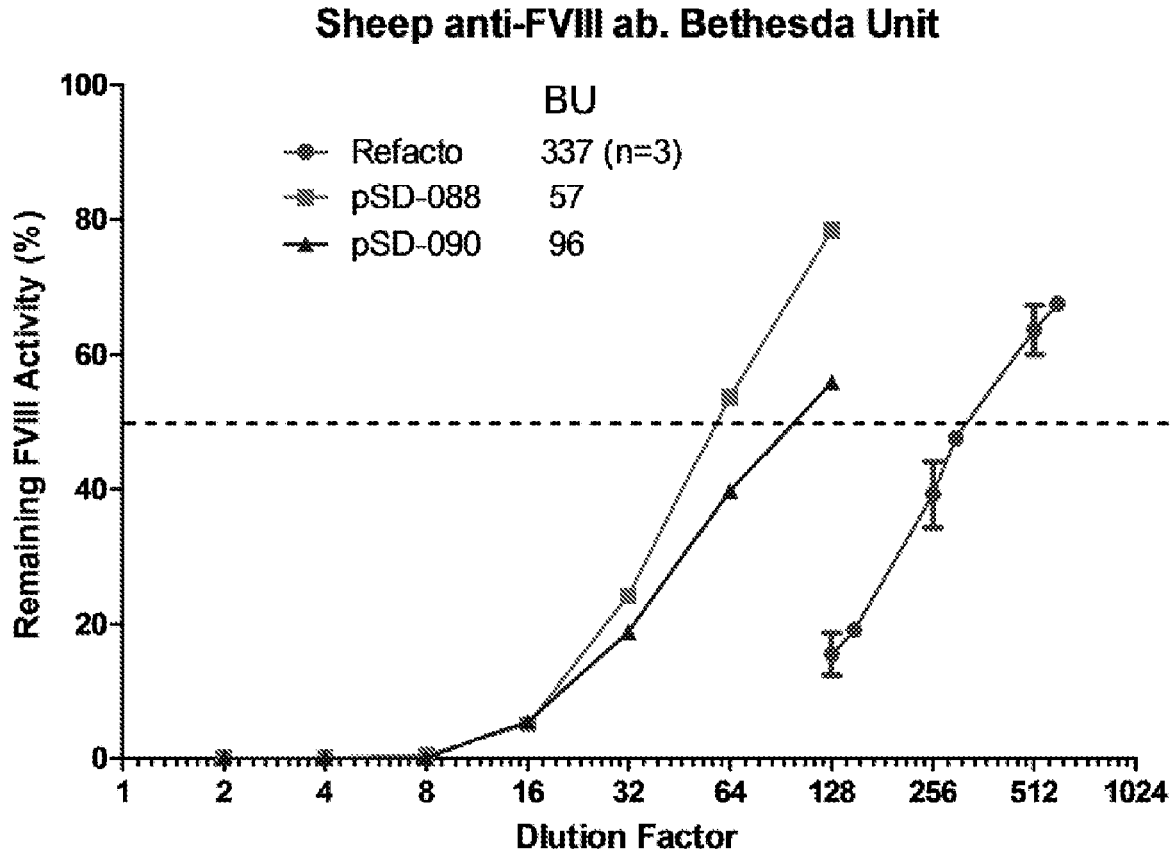

Sheep anti-FVIII ab. Bethesda Unit
BU
Refacto 337 (n=3)
pSD-088 57
pSD-090 96
Remaining FVIII Activity (%)
100
80
60
40
20
0
1
2
4
8
16
32
64
128
256
512
1024
Dlution Factor

FIG. 49