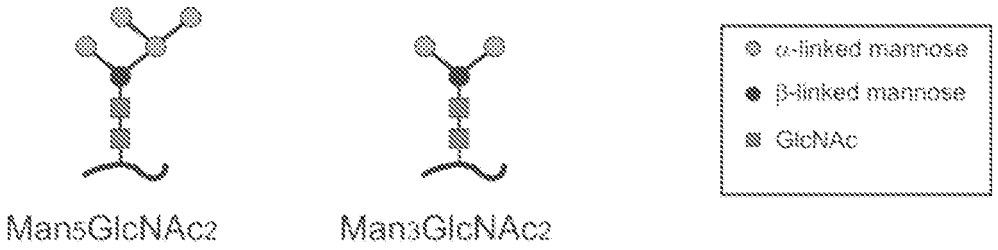
FIGURE 1

A.


α-linked mannose
β-linked mannose
GlcNAc
Man5GlcNAc2
Man3GlcNAc2

B.

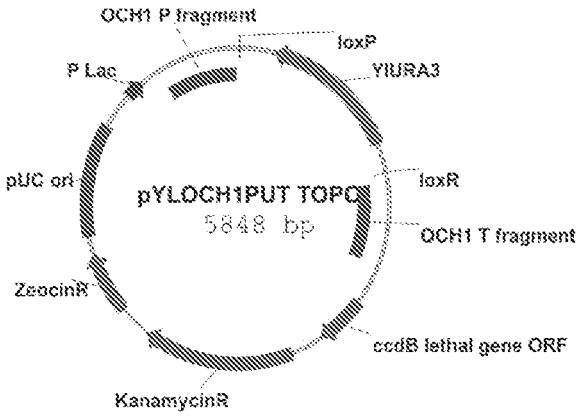

OCH1 P fragment
loxP
P Lac
YlURA3
pUC ori
pYLOCH1PUT TOPO
5848 bp
loxR
OCH1 T fragment
ZeocinR
ccdB lethal gene ORF
KanamycinR

FIGURE 2

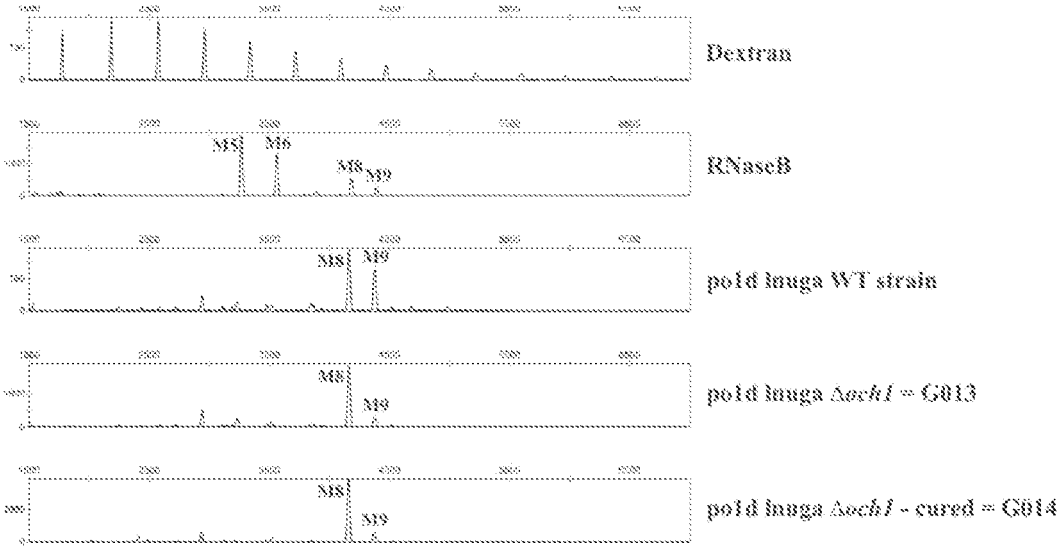
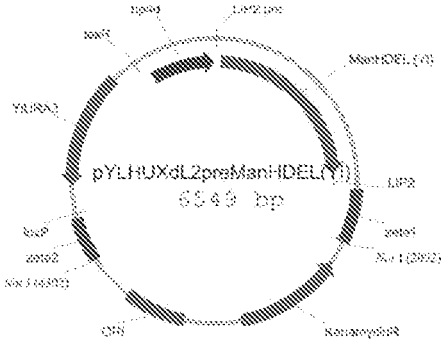

Dextran
M5
M6
M8
M9
RNaseB
M8
M9
pold Inuga WT strain
M8
M9
pold Inuga Δoch1 = G013
M8
M9
pold Inuga Δoch1 - cured = G014

FIGURE 3


pYLHUXdL2preManHDEL(Y)
6549 bp
YURA3
KanR
LEU2
CEN
KanamycinR

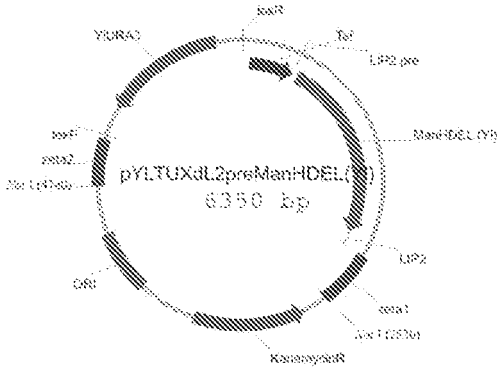
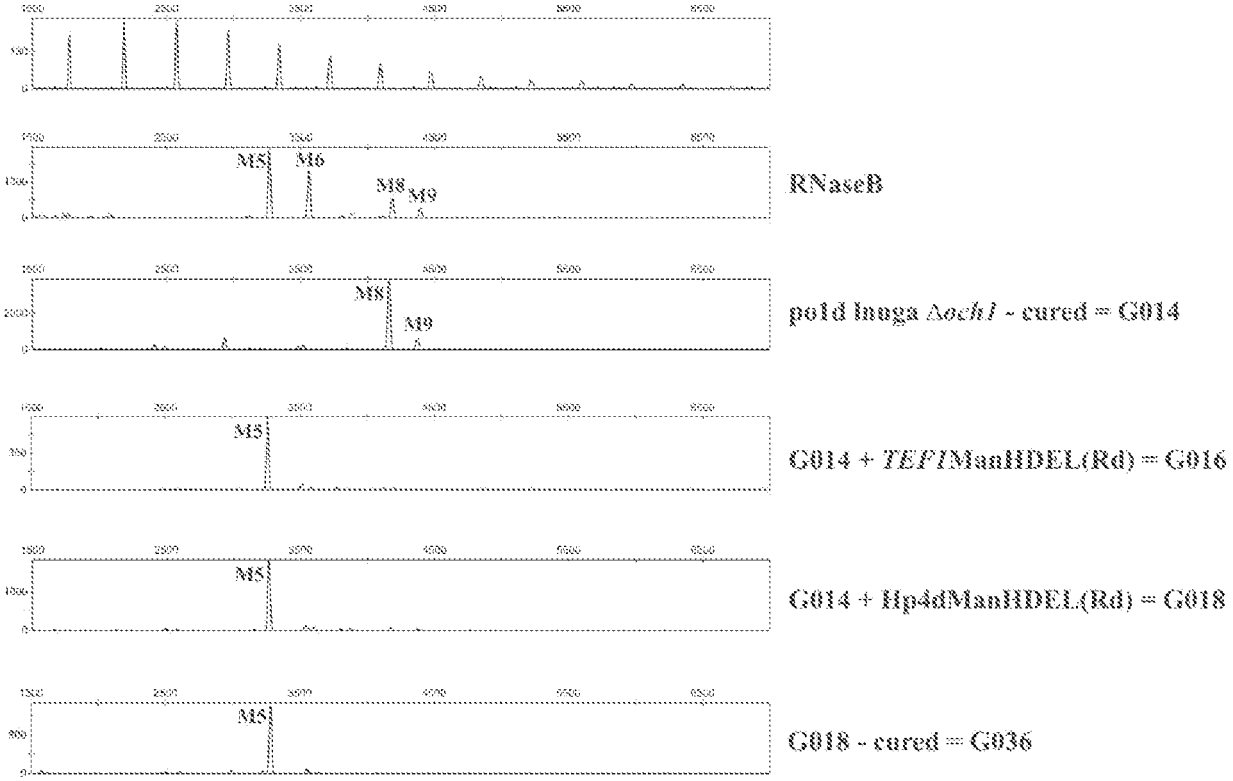

YURA3
KanR
LEU2
CEN
KanamycinR

FIGURE 4


M5
M6
M8
M9
RNaseB
M8
M9
po1d Inoga Δoch1 - cured = G014
M5
G014 + TEPIManHDEL(Rd) = G016
M5
G014 + Hp4dManHDEL(Rd) = G018
M5
G018 - cured = G036

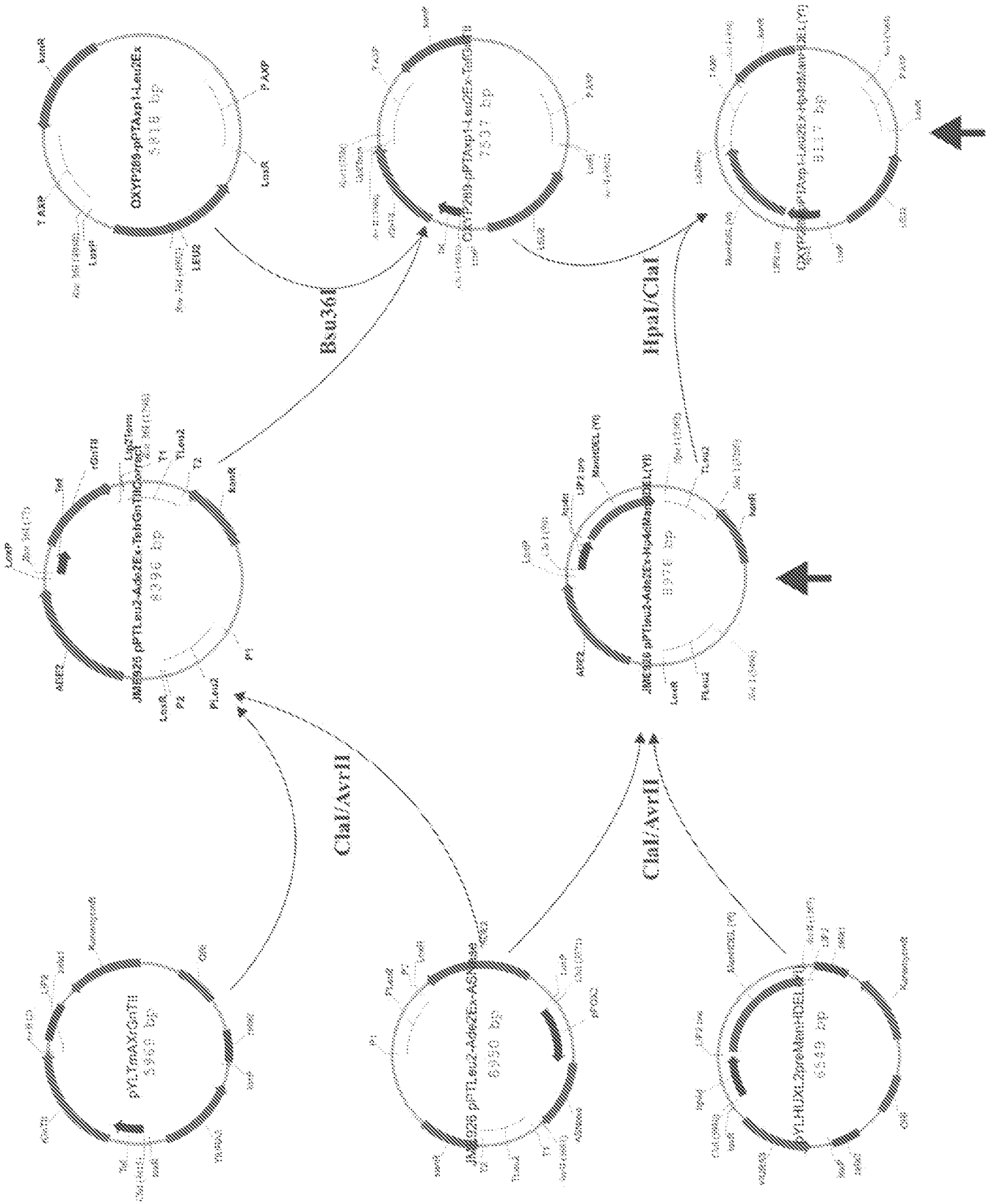


FIGURE 5

FIGURE 6

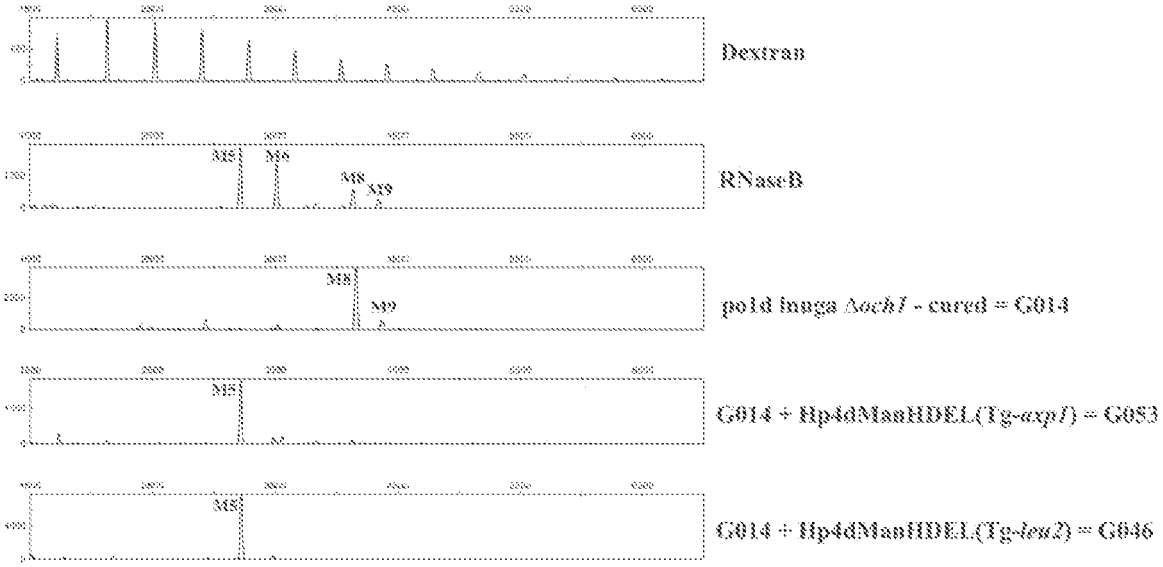

Dextran
M5
M6
M8
M9
RNaseB
M8
M9
po1d Inuga Δoch1 - cured = G014
M5
G014 + Hp4dManHDEL(Tg-axp1) = G053
M5
G014 + Hp4dManHDEL(Tg-lew2) = G046

FIGURE 7

MALETLSKRLLLRFTVIAGAVIVLLLTLANSMSRTCQYIPSSISAAFDFTSGSISPEQQVISE
ENDAKKLEQSALNSEASEDSEAMDEESKALKAAAEKADAEPAVIPILVIACDRSTVRRCL
DKLLHYRPSAELFPIIVSQDCGHEETAQAIASYGSAVTHIRQPDLSSIAVPPDHRKFQGY
YKIARHYRWALGQVFRQFRFPAAVVVEDDLEVAPDFFEYFRATYPLLKADPSLWCVSAWN
DNGKEQMVDASRPELLYRTDFFPGLGWLLLAELWAELEPKWPKAFWDDWMRRPEQRQGRA
CIRPEISRTMTFGRKGVSHGQFFDQHLKFIKLNQQFVHFTQLDLSYLQREAYDRDFLARV
YGAPQLQVEKVRTNDRKELGEVRVQYTGRDSFKAFAKALGVMDDLKSGVPRAGYRGIVTF
QFRGRRVHLAPPPTWEGYDPSWN (SEQ ID NO:3)

ATGGACCTGTTTCTGTCTAAGCGACTTATTCGATTCACCGTGATCGCCGGTGCCGTGATCGTTCTGCTGACCCTGAACGTCTTAACTCTCGAACCCAGCAGTACATCCCCCTC
TTCTATCTTCTGCCGCCTTCCACTTCACCTCTGGCTCTATCTTCCCGAGCAGCAGGAGTCATCTTCAGCAGAACGAACCCAAGAAGCTGGCAGCAGTCTGCCCTCAACTCTCAGG
CTTCTGAAGACTCCGAAGACCATGCAACGAAGTCTAAGAAACCTGAAGCCCATCTGCCGAAAAACTTACCTCCGGCTGTGATCCCCATCCTGGTCATCGCCTGTGACCGA
TCTACCGTGCGACGATGTCTGGACAAGCTGCTGCACTACCGACCCTCTGCCGAGCTGTTCCCCATCATCGTGTCTCAGGACTGTGGCCACGAGGAGACCGCCCAGGCCATTGC
CTCTTACGGCTCTGCCGTGACCCACATCCGACAGCCCGACCTGTCCTCTATCGCCGTGCCCCCCGACCACCGAAAGTTCCAGGGCTACTACAAGATCGGCCGACACTACCGAT
GGGCCCTGGGCCAGGTGTTCCGACAGTTCCCGATTCCCGCTGCCGTGGTGGAGGACGACCTGGAGGGTGGCCCCCGACTTCTTCGAGTACTTCCGAGCCACCTACGCCCCTG
CTGAAGGCCGACCCCTCTCTGTGGTGTGTCTGCCTGGAACGACAACGGCAAGGAACAGATGGTCGACGCCTCTCGACCTGAGCTGCTGTACCGAACCGACTTCTTCCCCGG
CCTGGGCTGGCTGCTGCTGGCTGAGCTGTGGGGCCGAGCTGGAGCCCAAGTGGCCCAAGGCCTTCTGGGACGACTGGATGCGACGACCCGAGCAGCAGCGACACAGGGCCGAGCCTGTA
TCCGACCCCGAGATCTCTCGAACCATGACCTTCGGCCGAAAGGGCGTGTCTCACGGCCAGTTCTTCGACCAGCACCTGAAGTTCATCAAGCTGAACCAGCAGTTCGTGCACTTC
ACCCAGCTGGACCTGTCTTACCTGCAGCGAGAGGCCTACGACCGAGACTTCCTGGCCCGAGTGTACGGCGCTCCCCAGCTGCAGGTGGAGAAGGTGCGAACCAACGACCGAAA
GGAGCTGGGCGAGGTCCGAGTGCAGTACACCGGCCGAGACTCGTTCAAGGCCTTCGCCAAGGCCCTGGGCGTGATGGACGACCTGAAGTCTGGCGTGCCCCCGAGCCCGATACC
GAGGCATCGTGACCTTCCAGTTCCGAGGCCGACGAGTGCACCTGGCCCCTCCACCCACCTGGGAGGGCTACGACCCCTCTTGGAACTAG (SEQ ID NO:4)

FIGURE 8

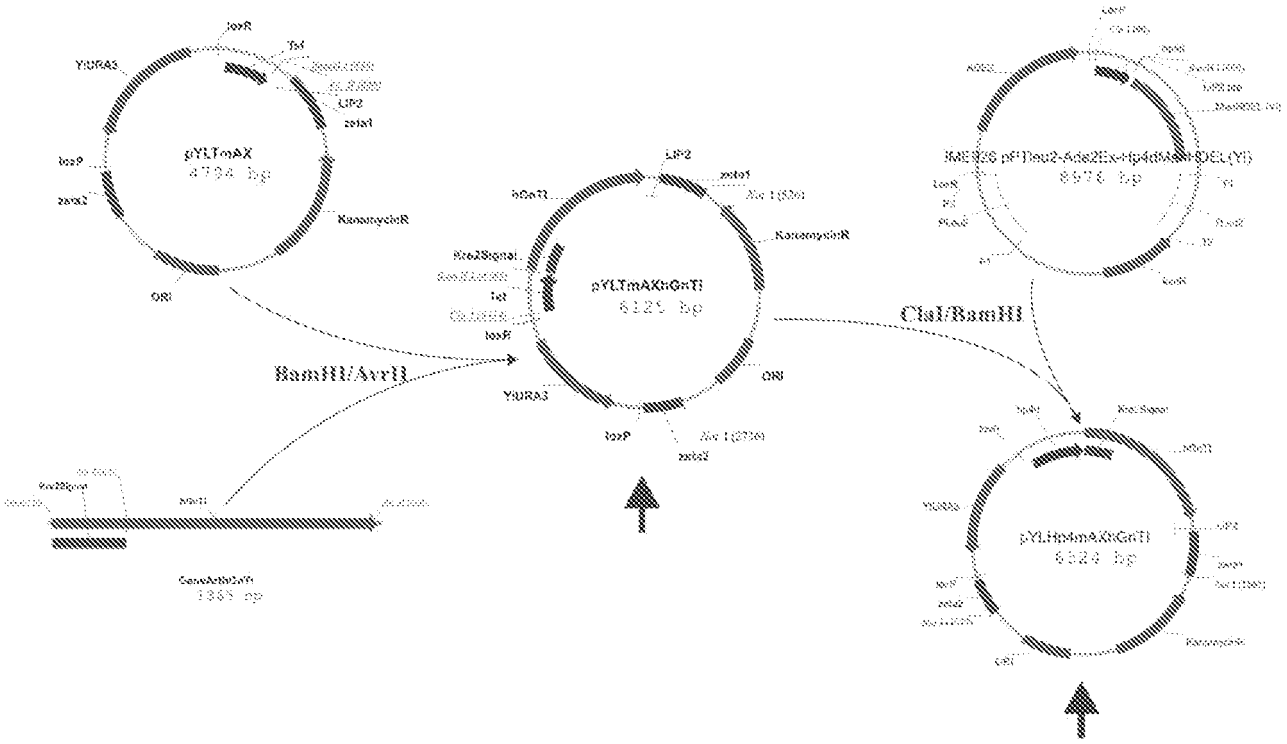

pYLTmAX
4794 bp
pYLTmAXhGnTi
6125 bp
pYLHp4mAXhGnTi
6524 bp
BamHI/AvrII
ClaI/BamHI
YlURA3
KanamycinR
ORI
LIP2
loxP
loxR

FIGURE 9

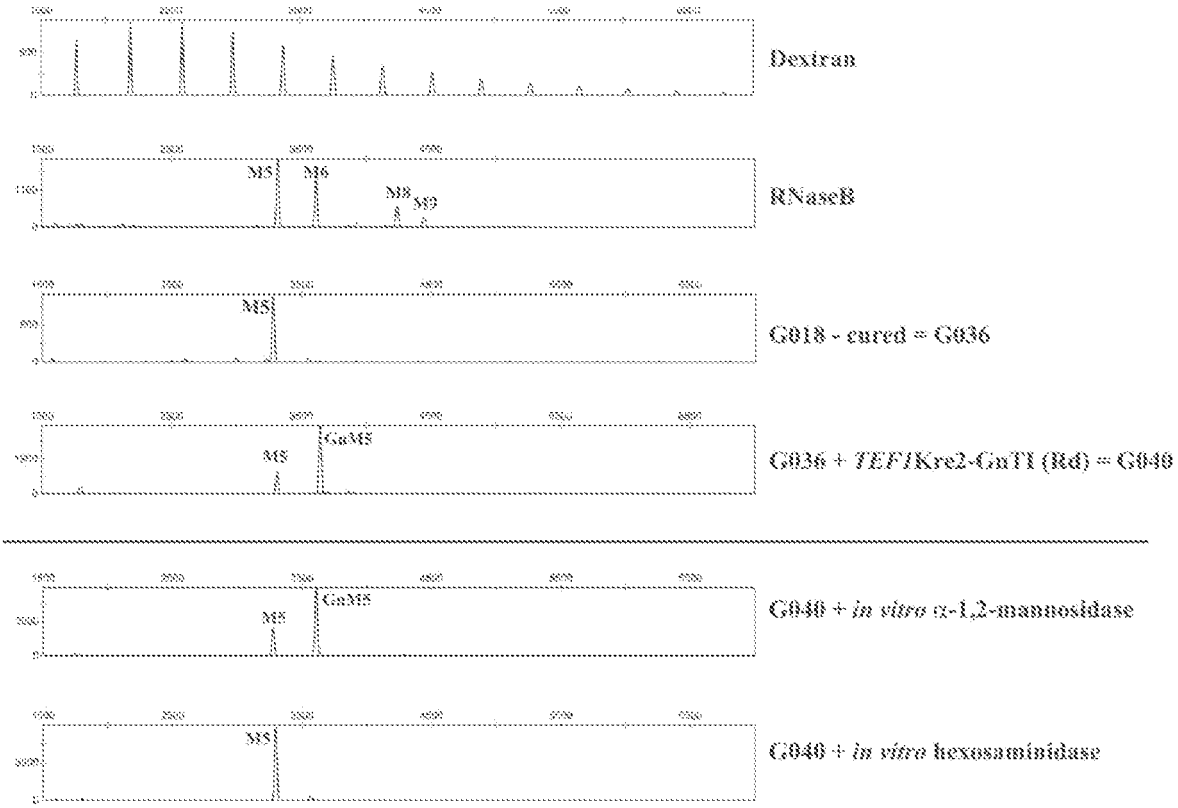

Dextran
M5
M6
M8
M9
RNaseB
M5
G018 - cured = G036
M5
GnM5
G036 + TEF1/Kre2-GnTI (Rd) = G040
M5
GnM5
G040 + in vitro α-1,2-mannosidase
M5
G040 + in vitro hexosaminidase

FIGURE 10

MLLTKRFSKLFKLTFIVLILCGLFVITNKYMDENTSRDDPIRPPLKVARSPRPGQCQDVV
QDVPNVDVQMLELYDRMSFKDIDGGVWKQGWNIKYDPLKYNAHHKLKVFVVPHSHNDPGW
IQTFEEYYQHDTKHILSNALRHLHDNPEMKFIWAEISYFARFYHDLGENKKLQMKSIVKN
GQLEFVTGGWVMPDEANSHWRNVLLQLTEGQTWLKQFMNVTPTASWAIDPFGHSPTMPYI
LQKSGFKNMLIQRTHYSVKKELAQQRQLEFLWRQIWDNKGDTALFTHMMPFYSYDIPHTC
GPDPKVCCQFDFKRMGSFGLSCPWKVPPRTISDQNVAARSDLLVDQWKKKAELYRTNVLL
IPLGDDFRFKQNTEWDVQRVNYERLFEHINSQAHFNVQAQFGTLQEYFDAVHQAERAGQA
EFPTLSGDFFTYADRSDNYWSGYYTSRPYHKRMDRVLMHYVRAAEMLSAWHSWDGMARIE
ERLEQARRELSLFQHHDGITGTAKTHVVVDYEQRMQEALKACQMVMQQSVYRLLTKPSIY
SPDFSFSYFTLDDSRWPGSGVEDSRTTIILGEDILPSKHVVMHNTLPHWREQLVDFYVSS
PFVSVTDLANNPVEAQVSPVWSWHHDTLTKTIHPQGSTTKYRIIFKARVPPMGLATYVLT
ISDSKPEHTSYASNLLLRKNPTSLPLGQYPEDVKFGDPREISLRVGNGPTLAFSEQGLLK
SIQLTQDSPHVPVHFKFLKYGVRSHGDRSGAYLFLPNGPASPVELGQPVVLVTKGKLESS
VSVGLPSVVHQTIMRGGAPEIRNLVDIGSLDNTEIVMRLETHIDSGDIFYTDLNGLQFIK
RRRLDKLPLQANYYPIPSGMFIEDANTRLTLLTGQPLGGSSLASGELEIMQDRRLASDDE
RGLGQGVLDNKPVLHIYRLVLEKVNNCVRPSKLHPAGYLTSAAHKASQSLLDPLDKFIFA
ENEWIGAQGQFGGDHPSAREDLDVSVMRRLTKSSAKTQRVGYVLHRTNLMQCGTPEEHTQ
KLDVCHLLPNVARCERTTLTFLQNLEHLDGMVAPEVCPMETAAYVSSHS (SEQ ID NO:7)

ATGCTGCTGACCAAGCGATTCTCTAAGCTGTTCAAGCTGACCTTCATCGTGCTGATCCTGTGTGGCCTGTTCGTG
ATCACCAACAAGTACATGGACGAACACCTCCCGGGATGACCCCATCCGACCCCCCCTGAAGGTGGCCCGATCT
CCCCGACCCGGCCAGTGTCAGGACGTGGTGCAGGACGTGCCCAACGTGGACGTGCAGATGCTGGAGCTGTACGAC
CGAATGTCTTTCAAGGACATCGACGGCGGCGTGTGGAAGCAGGGCTGGAACATCAAGTACGACCCCCTGAAGTAC
AACGCCCACCACAAGCTGAAGGTGTTCGTGGTGCCCCACTCTCACAACGACCCCGGCTGGATTCAGACCTTCGAG
GAGTACTACCAGCACGACACCAAGCACATCCTGTCTAACGCCCTGCGACACCTGCACGACAACCCTGAGATGAAG
TTTATCTGGGCCGAGATCTCTTACTTCGCCCGATTCTACCACGACCTGGGCGAGAACAAGAAGCTGCAGATGAAG
TCTATCGTGAAGAACGGCCAGCTGGAGTTCGTGACCGGCGGCTGGGTGATGCCCGACGAGGCCAACTCTCACTGG
CGAAACGTGCTGCTGCAGCTGACCGAGGGCCAGACCTGGCTGAAGCAGTTCATGAACGTGACCCCCACCGCCTCT
TGGGCCATCGACCCCTTCGGCCACTCTCCCACCATGCCCTACATCCTGCAGAAGTCTGGCTTCAAGAACATGCTG
ATCCAGCGAACCCACTACTCTGTGAAGAAGGAGCTGGCCCAGCAGCGACAGCTGGAGTTTCTGTGGCGACAGATC
TGGGACAACAAGGGCGACACCGCCCTGTTCACCCACATGATGCCCTTCTACTCTTACGACATCCCCCACACCTGT
GGCCCCGACCCCAAGGTGTGTTGTCAGTTCGACTTCAAGCGAATGGGCTCTTTCGGCCTGTCTTGTCCCTGGAAG
GTGCCCCCTCGAACCATCTCTGACCAGAACGTGGCCGCTCGATCTGACCTGCTGGTTGACCAGTGGAAGAAGAAG
GCCGAGCTGTACCGAACCAACGTCCTGCTGATCCCCCTGGGCGACGACTTCCGATTCAAGCAGAACACCGAGTGG
GACGTGCAGCGAGTGAACTACGAGCGACTGTTCGAGCACATCAACTCTCAGGCCCACTTCAACGTGCAGGCTCAG
TTCGGCACTCTGCAGGAGTACTTCGACGCCGTCCACCAGGCCGAGCGAGCCGGCCAGGCCGAGTTCCCCACCCTG
TCTGGCGACTTTTTCACCTACGCCGACCGATCTGACAACTACTGGTCTGGCTACTACACCTCTCGACCCTACCAC
AAGCGAATGGACCGAGTGCTGATGCACTACGTGCGAGCCGCCGAGATGCTGTCTGCCTGGCACTCTTGGGACGGC
ATGGCCCGAATCGAGGAGCGACTGGAGCAGGCCCGACGAGAGCTGTCTCTGTTCCAGCACCACGACGGCATCACC
GGCACCGCCAAGACCCACGTGGTGGTGGACTACGAGCAGCGAATGCAGGAGGCCCTGAAGGCCTGTCAGATGGTG
ATGCAGCAGTCTGTCTACCGACTCCTGACTAAGCCCTCTATCTACTCTCCCGACTTCTCTTTCTCTTACTTCACC
CTGGACGACTCTCGATGGCCCGGCTCTGGCGTGGAGGACTCTCGAACCACCATCATCCTGGGCGAGGACATCCTG
CCCTCTAAGCACGTGGTGATGCACAACACCCTGCCCCACTGGCGAGAGCAGCTGGTCGACTTCTACGTGTCCTCT
CCCTTCGTGTCTGTGACCGACCTGGCCAACAACCCCGTGGAGGCCCAGGTGTCTCCCGTGTGGTCTTGGCACCAC

GACACCCTGACCAAGACCATCCACCCCCAGGGCTCTACCACCAAGTACCGAATCATCTTCAAGGCCCGAGTGCCC
CCCATGGGCCTGGCCACCTACGTGCTGACCATCTCCGACTCTAAGCCCGAGCACACCTCTTACGCCTCTAACCTG
CTGCTGCGAAAGAACCCCACCTCTCTGCCCCTGGGCCAGTACCCCGAGGACGTGAAGTTCGGCGACCCCCGAGAG
ATCTCTCTGCGAGTGGGCAACGGCCCCACCCTGGCCTTCTCTGAGCAGGGCCTGCTGAAGTCTATCCAGCTGACC
CAGGACTCTCCCCACGTGCCCGTGCACTTCAAGTTTCTGAAGTACGGCGTGCGATCTCACGGCGACCGATCTGGC
GCCTACCTGTTCCTGCCCAACGGACCCGCCTCTCCCGTGGAGCTGGGACAGCCCGTGGTGCTGGTGACCAAGGGC
AAGCTGGAGTCCTCTGTGTCTGTGGGCCTGCCCTCTGTGGTGCACCAGACCATCATGCGAGGCGGAGCCCCCGAG
ATCCGAAACCTGGTGGACATCGGATCTCTGGACAACACCGAGATCGTGATGCGACTGGAGACCCACATCGACTCT
GGCGACATCTTCTACACCGACCTGAACGGCCTGCAGTTCATCAAGCGACGACGACTGGACAAGCTGCCCCTGCAG
GCCAACTACTACCCCATCCCCCTCTGGCATGTTCATCGAGGACGCCAACACCCGACTGACCCTGCTGACCGGCCAG
CCCCTGGGCGGATCTTCTCTGGCCTCTGGCGAGCTGGAGATCATGCAGGACCGACGACTGGCCTCTGACGACGAG
CGAGGCCTGGGCCAGGGCGTGCTGGACAACAAGCCCGTGCTGCACATCTACCGACTGGTGCTGGAGAAGGTGAAC
AACTGTGTGCGACCCTCTAAGCTGCACCCCGCTGGCTACCTGACCTCTGCCGCCCACAAGGCCTCTCAGTCTCTG
CTGGACCCCCTGGACAAGTTCATCTTCGCCGAGAACGAGTGGATCGGCGCCCAGGGCCAGTTCGGAGGCGACCAC
CCCTCTGCCCGAGAGGACCTGGACGTGTCTGTGATGCGACGACTGACCAAGTCCTCTGCCAAGACCCAGCGAGTG
GGCTACGTGCTGCACCGAACCAACCTGATGCAGTGTGGCACCCCCGAGGAGCACACCCAGAAGCTGGACGTCTGT
CACCTGCTGCCCAACGTCGCCCGATGTGAGCGAACCACCCTGACCTTTCTGCAGAACCTGGAGCACCTGGACGGC
ATGGTGGCCCCCGAGGTGTGTCCCATGGAGACCGCCGCCTACGTGTCGTCCCACTCTTCTTAG (SEQ ID NO:8)

FIGURE 11

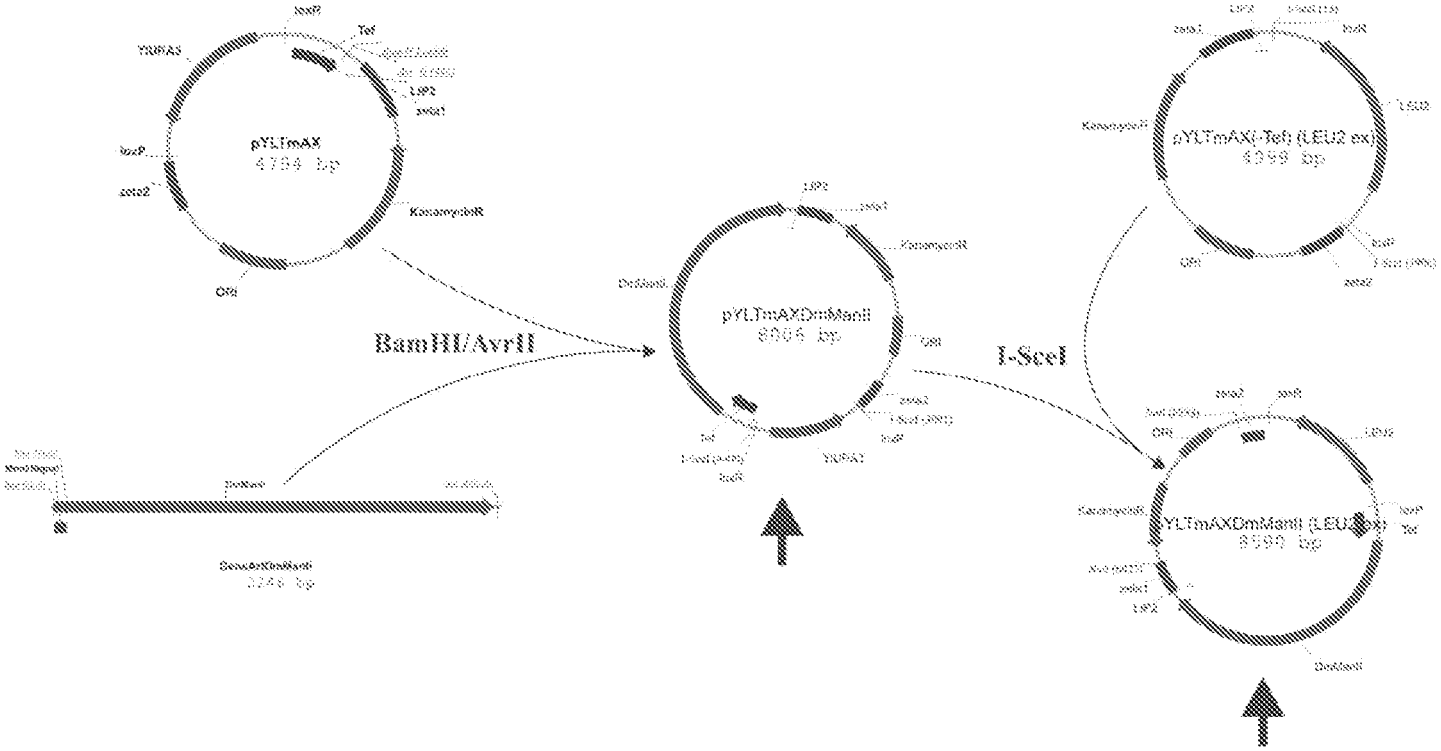


FIGURE 12

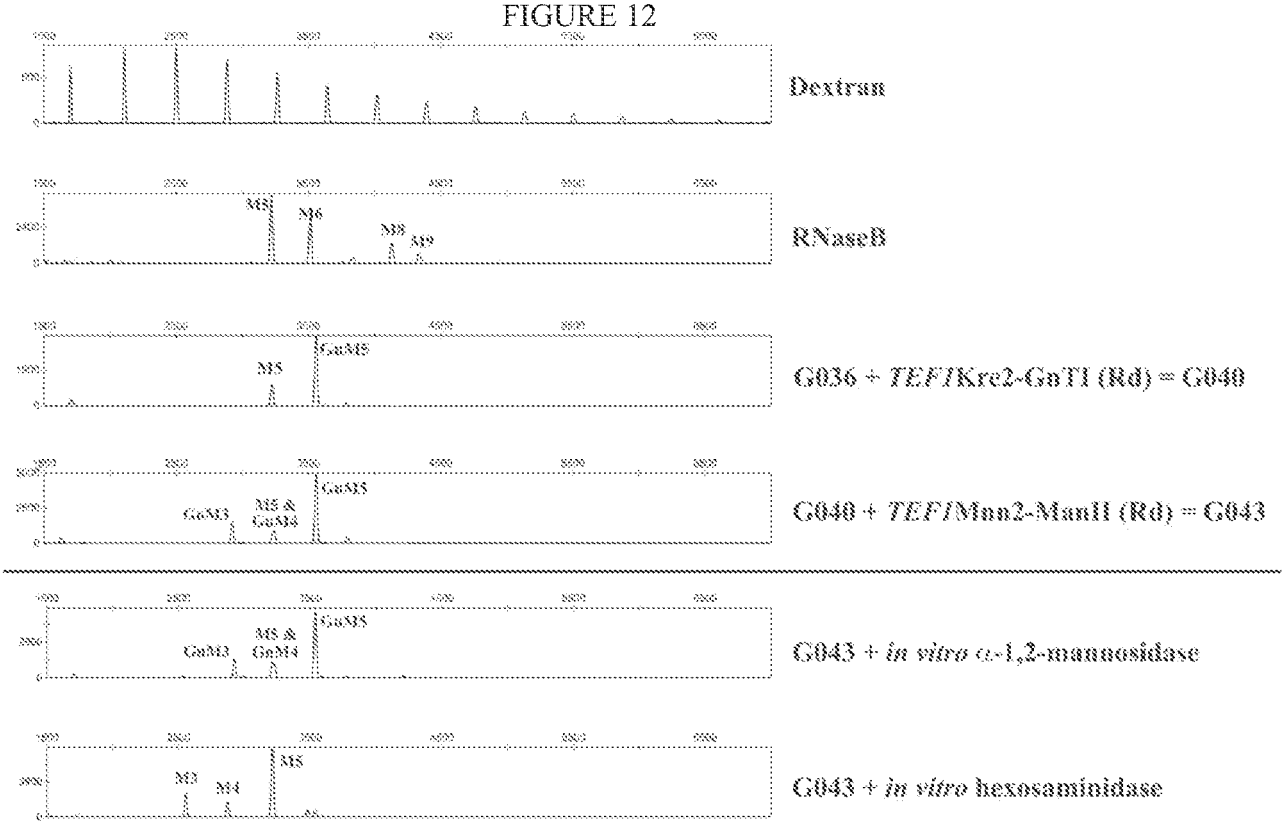

Dextran
M5
M6
M8
M9
RNaseB
GnM5
M5
G036 + TEF1Kre2-GnTI (Rd) = G040
GnM3
M5 &
GnM4
G040 + TEF1Mnn2-ManII (Rd) = G043
G043 + in vitro α-1,2-mannosidase
M3
M4
G043 + in vitro hexosaminidase

FIGURE 13

MLLTKRFSKLFKLTFIVLILCGLFVITNKYMDENTSVKEYKEYLDRCPAMTGVREGTVLV
TGGAGYIGSHTCVVLLENGYDVVIVDNLCNSRVEAVHRIEKLTGKEVIFHQVDLLDEPAL
DKVFANQNISAVIHFAGLKAVGESVQVPLSYYKNNISGTINLIECMKKYNVRDFVFSSSA
TVYGDPTRFPGGTIPIPEESCPREGTSPYGRTKLFIENLIEDETKVNKSLNAALIRYFNPGG
AHPSGELGEDPLGIPNNLLPYIAQVAVGRLDHLNVFGDDYPTSDGTPIRDYIHVCDLAEA
HVAALDYLRQHFVSCRPWNLGSGTGSTVFQVLNAFSKAVGRPLPYKVTPRRAGDVVNLTA
NPTRANEELKWKTSRSIYEICVDTWRWQQKYPYGFDLTHTKTYSGSGGGRDLSRLPQLVG
VSTPLQGGSNSAAAIGQSSGELRTGGARPPPPLGASSQPRPGGDSSPVVDSGPGPASNLT
SVPVPHTTALSLPACPEESPLLVGPMLIEFNMPVDLELVAKQNPNVKMGGRYAPRDCVSP
HKVAIIIPFRNRQEHLKYWLYYLHPVLQRQQLDYGIYVINQAGDTIFNRAKLLNVGFQEA
LKDYDYTCFVFSDVDLIPMNDHNAYRCFSQPRHISVAMDKFGFSLPYVQYFGGVSALSKQ
QFLTINGFPNNYWGWGGEDDDIFNRLVFRGMSISRPNAVVGRCRMIRHSRDKKNEPNPQR
FDRIAHTKETMLSDGLNSLTYQVLDVQRYPLYTQITVDIGTPS (SEQ ID NO:9)

ATGCTGCTGACCAAGCGATTCTCTAAGCTGTTCAAGCTGACCTTCATCGTGCTGATCCTGTGTGGCCTGTTCGTGATCACCAACAAGTACATGGACGAGAACACCTCTGTGAA
GGAATACAAGGAGTACCTGGACCGCCGCCCAGCCATGACCGGCGTGCACGAGGGCACCGTGCTGGTGACCGGCGGAGCCGGCTACATCGGCTCTCACACCTGTGTGGTGCTGC
TGGAGAAGGGCTACGACGTGGTGATCGTGGACAACCTGTGTAACTCTCGAGTGGAGGCCGTGCACCGAATCGAGAAGCTGACCGGCAAGAAGGTGATCTTCCACCAGGTGGAC
CTGCTGGACGAGCCCGCCCTGGACAAGGTGTTCGCCAACCAGAACATCTCTGCCGTGATCCACTTCGCCGGCCTGAAGGCTGTGGGCGAGTCTGTGCAGGTGGCCCTGTCTTA
CTACAAGAACAACATCTCTGGCACCATCAACCTGATCGAGTGTATGAAGAAGTACAACGTCCGAGACTTCGTGTTCTCTTCTTCTGCCACCGTGTACGGCGACCCCACCCGAC
CCGGCGGAACCATCCCCATCCCCGAGTCTTGTCCCCGAGAGGGCACCTCTCCCTACGGCCGAACCAAGCTGTTCATCGGAGACATCATCGAGGACGAAACCAAGGTGAACAAG
TCTCTGAACGCCGCCCTGCTGCGATACTTCAACCCCGGTGGCGCTCACCCCTCTGGCGAGCTGGGCGAGGACCCTCTGGGCATCCCCAACAACCTGCTGCCCTACATTGCTCA
GGTGGCTGTGGGCCGACTGGACCACCTGAAGGTGTTTGGCGACGACTACCCTACCTCTGACGGCACCCCCATCCGAGACTACATCCACGTGTGTGACCTGGCCGAGGCCCACG
TGGCCGCTCTGGACTACCTGGCAGCAGCACTTCGTGTCTTGTCGACCCTGGAACCTGGGCTCTGGCACCGGCTCTACCGTGTTCCAGGTGCTGAACGGCTTCTCTAAGGCTGTC
GGCCGAGATCTGCCCTACAAGGTGACCCCCCGACGAGCCGGCGACGTCGTGAACCTGACCGCCAACCCTACCCGAGCTAACGAGGAGCTGAAGTGGAAGACCTCTCGATCTAT
CTACGAGATCTGTGTGGACACCTGGAGATGGCAGCAGAAGTACCCCTACGGCTTCGACCTGACCCATACCAAGACCTACAAGGGCTCTGGCGGAGGACGAGATCTGTCTCGAC
TGCCTCAGCTGGTCGGCGTGTCTACCCCTCTGCAGGGCGGCTCTAACTCTGCCGCCGCTATCGGCCAGTCCTCCGGCGAGCTGCGAACCGGTGGAGCCCGACCTCCCCCTCCC
CTGGGCGCCTCTTCTCAGCCCCGACCCGGTGGCGACTCTTCTCCCGTGGTGGACTCTGGCCCCGGACCCGCCTCTAACCTGACCTCTGTGCCCGTGCCCCACACCACCGCCCT
GTCTCTGCCCGCCTGTCCCGAGGAGTCTCCCCTGCTCGTCGGCCCCATGCTGATCGAGTTCAACATGCCCGTGGACCTGGAGCTGGTCGCCAAGCAGAACCCCAACGTGAAGA
TGGGCGGACGATACGCTCCCCGAGACTGTGTGTCTCCCCACAAGGTGGCCATCATCATCCCTTTCAGAAACCGACAGGAGCACCTGAAGTACTGGCTGTACTACCTGCACCCC

GTGCTGCAGCGACAGCAGCTGGACTACGGCATCTACGTGATCAACCAGGCCGGCGACACCATCTTCAACCGAGCCAAGCTGCTGAACGTGGGCTTCCAGGAGGCCCTGAAGGA
CTACGACTACACCTGTTTCGTGTTCTCCGACGTGGACCTGATCCCCATGAACGGACCACAACGGCCTACCGATGTTTCTCCCAGCCCCGACACATCTCTGTGGCCATGGACAAGT
TCGGCTTCTCTCTGCCCTACGTGCAGTACTTCGGCGGCGTTTCTGCCCTGTCTAAGCAGCAGTTCCTGACCATCAACGGCTTCCCCAACAACTACTGGGGCTGGGGCGGAGAG
GACGACCACATTTTCAACCGACTGGTGTCTATCTCTCGACCCAACGCCGTGGTGGGCCGATGTCCGAATGATCCGACACTCTCGAGACAAGAAGAACCGAGCC
CAACCCCCAGCGATTTGACCGAATTGCTCACACTAAGGAAACCATGCTGTCTGACGGCCTGAACTCTCTGACCTACCAGGTGCTGGACGTGCAGCGATACCCCCTGTACACCC
AGATCACCGTGGACATCGGCACACCCTCTTAG (SEQ ID NO:10)

FIGURE 14

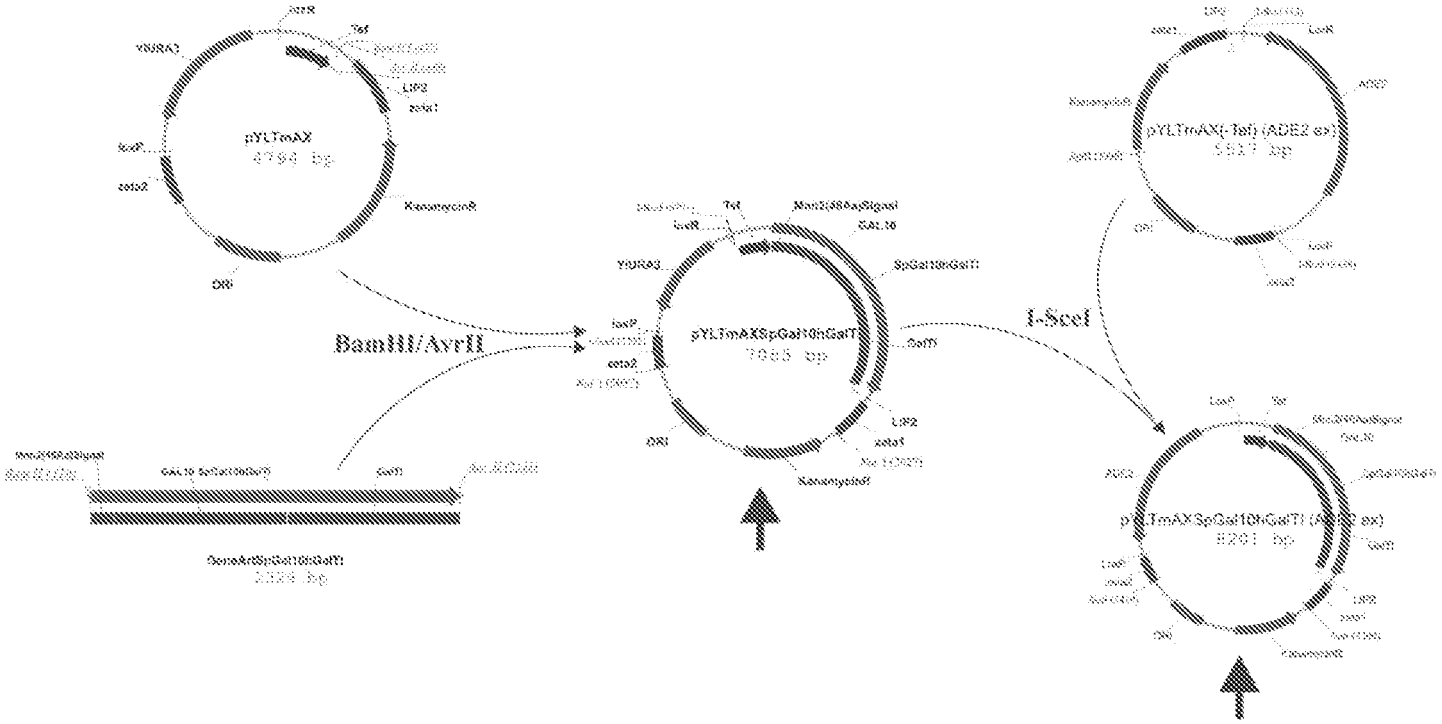


FIGURE 15

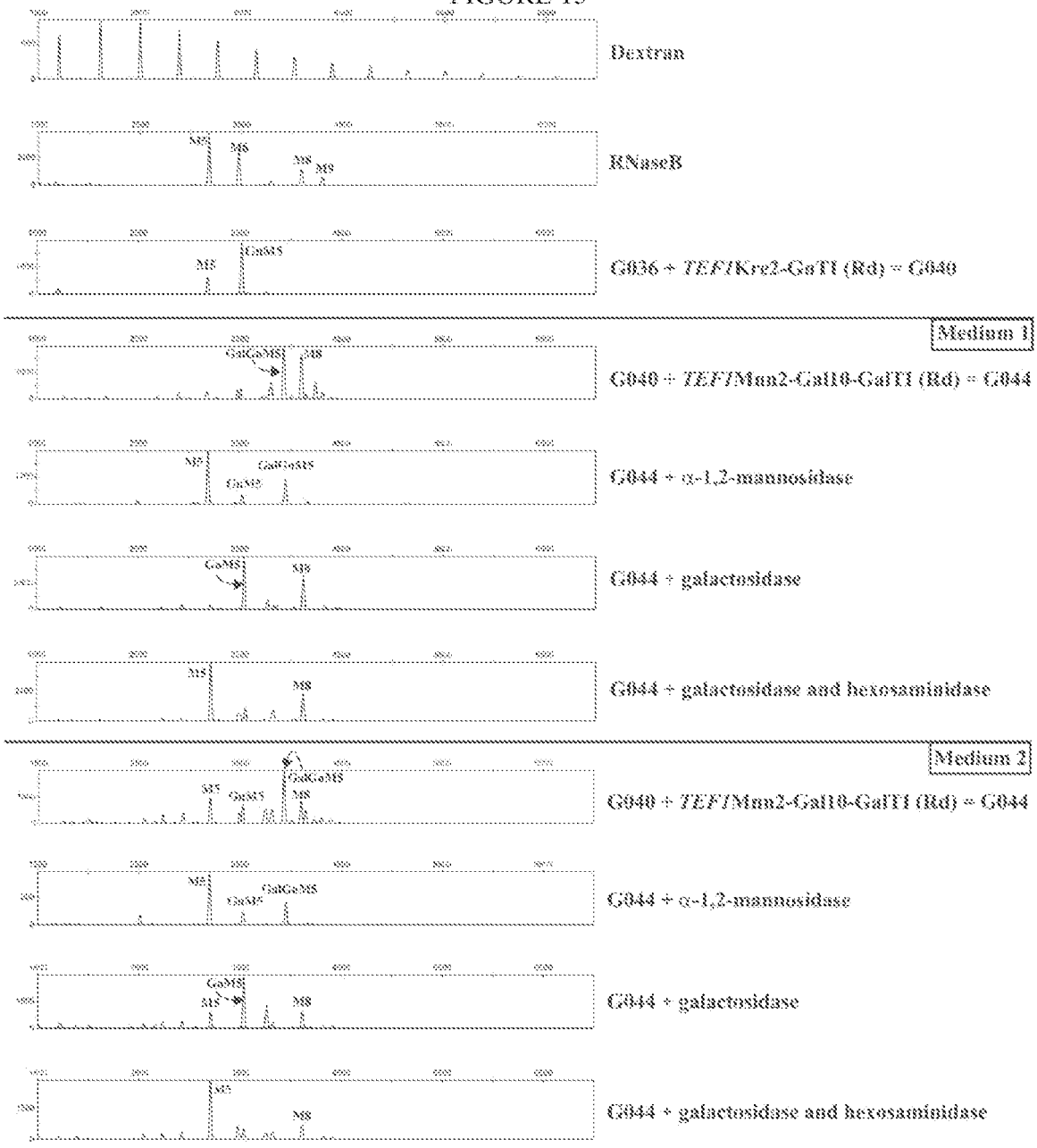

Dextran
RNaseB
G036 + TEF1Kre2-GaTI (Rd) = G040
Medium 1
G040 + TEF1Mnn2-Gal10-GaTI (Rd) = G044
G044 + α-1,2-mannosidase
G044 + galactosidase
G044 + galactosidase and hexosaminidase
Medium 2
G040 + TEF1Mnn2-Gal10-GaTI (Rd) = G044
G044 + α-1,2-mannosidase
G044 + galactosidase
G044 + galactosidase and hexosaminidase

FIGURE 16

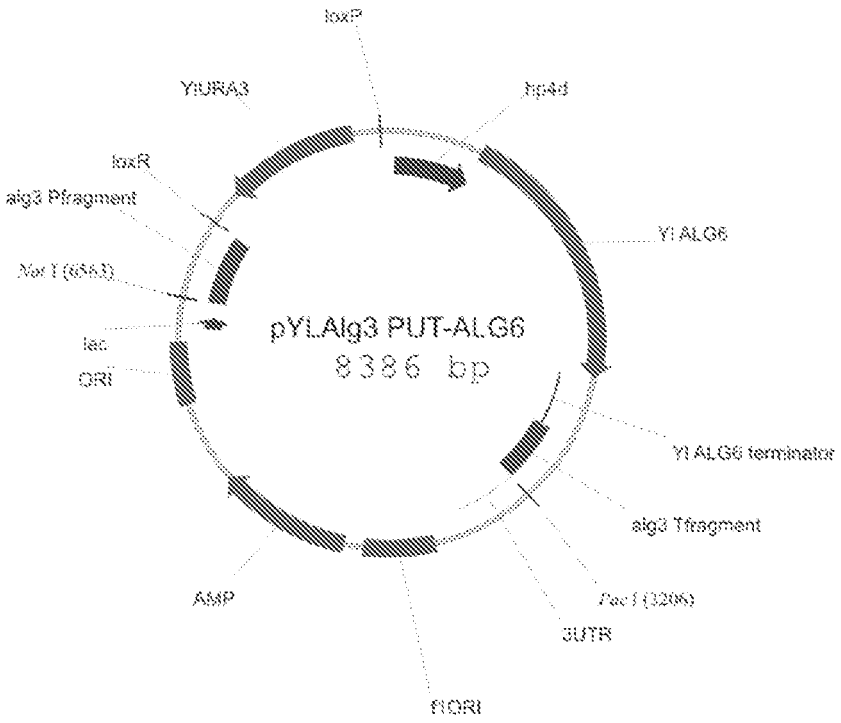

loxP
YlURA3
hp4d
loxR
alg3 Pfragment
Yl ALG6
Nar I (6543)
pYLAlg3 PUT-ALG6
8386 bp
lac
ORI
Yl ALG6 terminator
alg3 Tfragment
AMP
Pac I (3208)
3UTR
f1 ORI

FIGURE 17

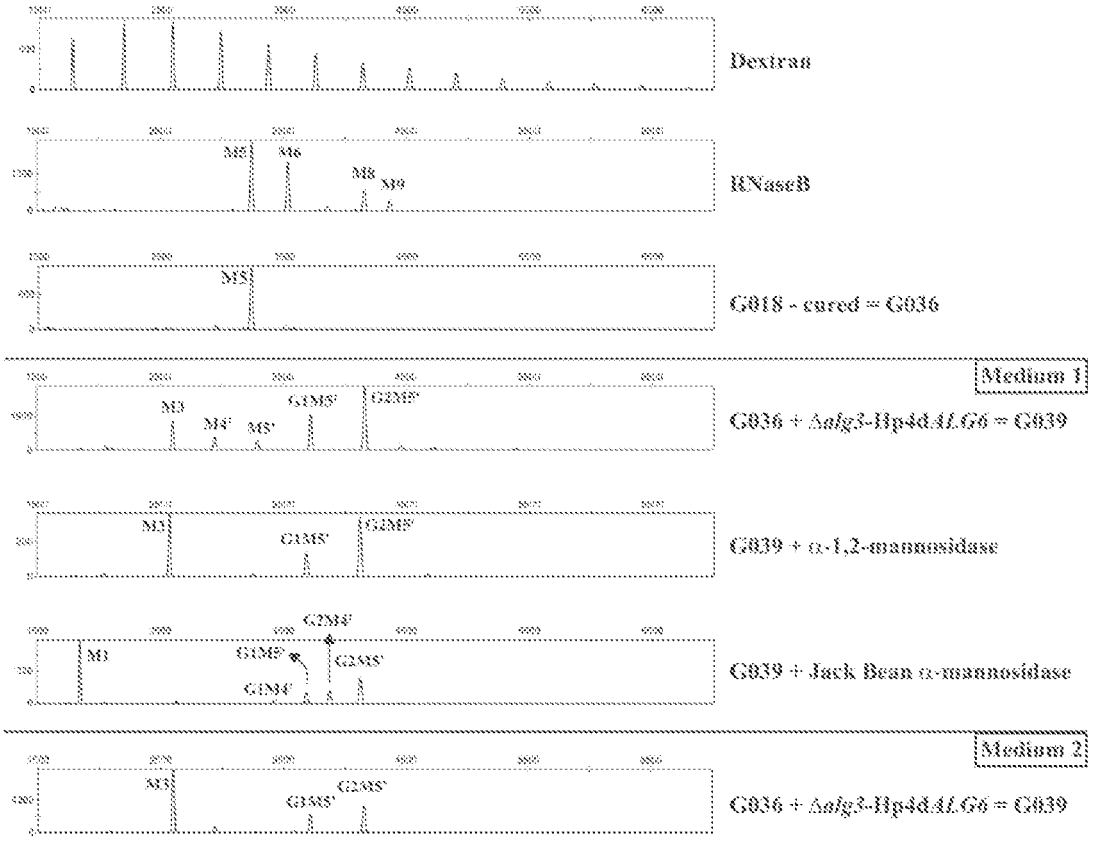

Dextran
M5
M6
M8
M9
RNaseB
M5
G018 - cured = G036
Medium 1
M3
M4'
M5'
G1M5'
G2M5'
G036 + Δalg3-Hp4d.ALG6 = G039
M3
G1M5'
G2M5'
G039 + α-1,2-mannosidase
G2M4'
M3
G1M5'
G2M5'
G1M4'
G039 + Jack Bean α-mannosidase
Medium 2
M3
G1M5'
G2M5'
G036 + Δalg3-Hp4d.ALG6 = G039

FIGURE 18

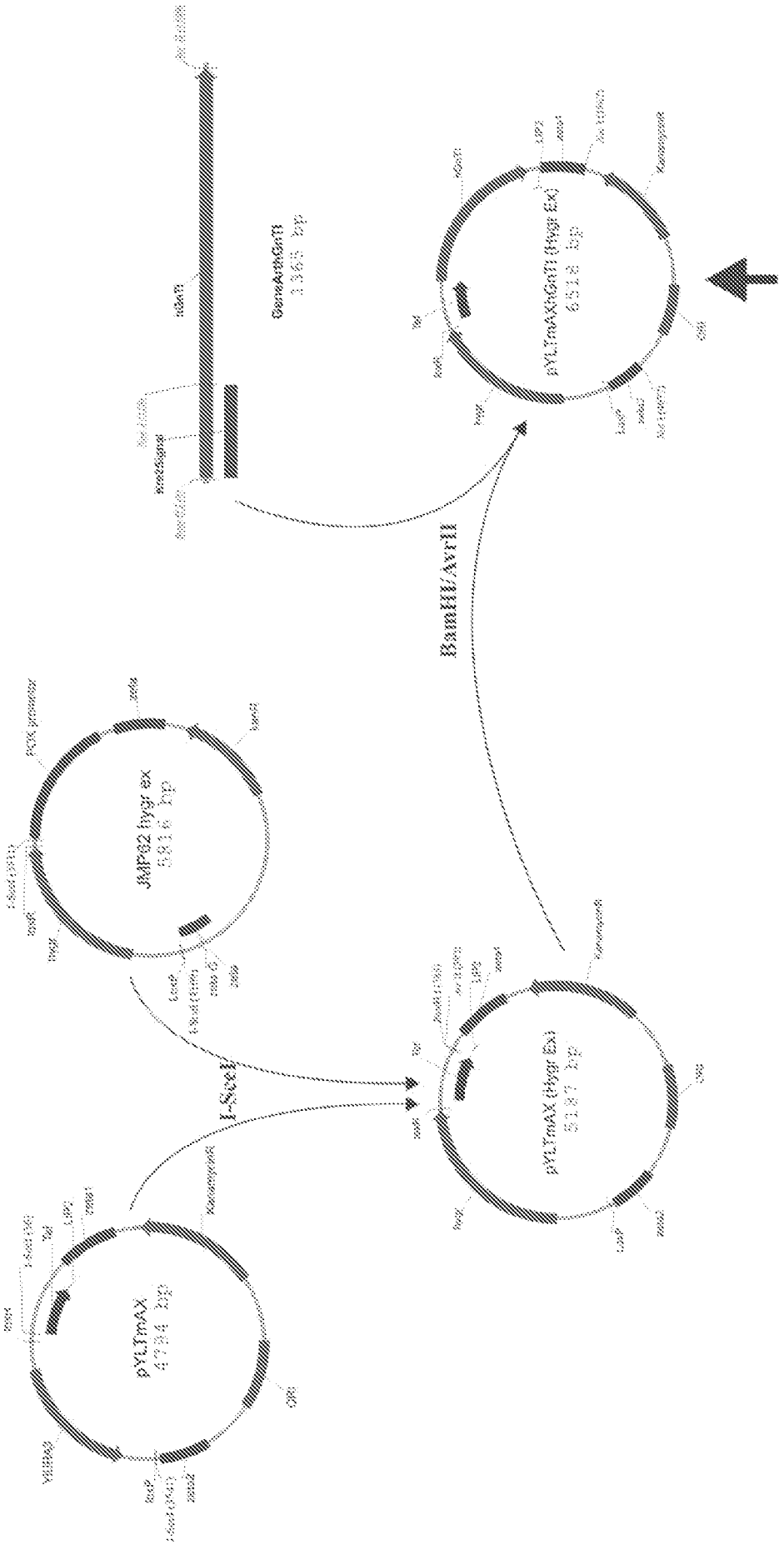

BamHI/AvrII
I-SceI
pYLTmAX
pYLTmAXnGmT1 (hygr Ex)
pYLTmAX (hygr Ex)
JMP62 hygr ex
GenAntiGmT1

FIGURE 19

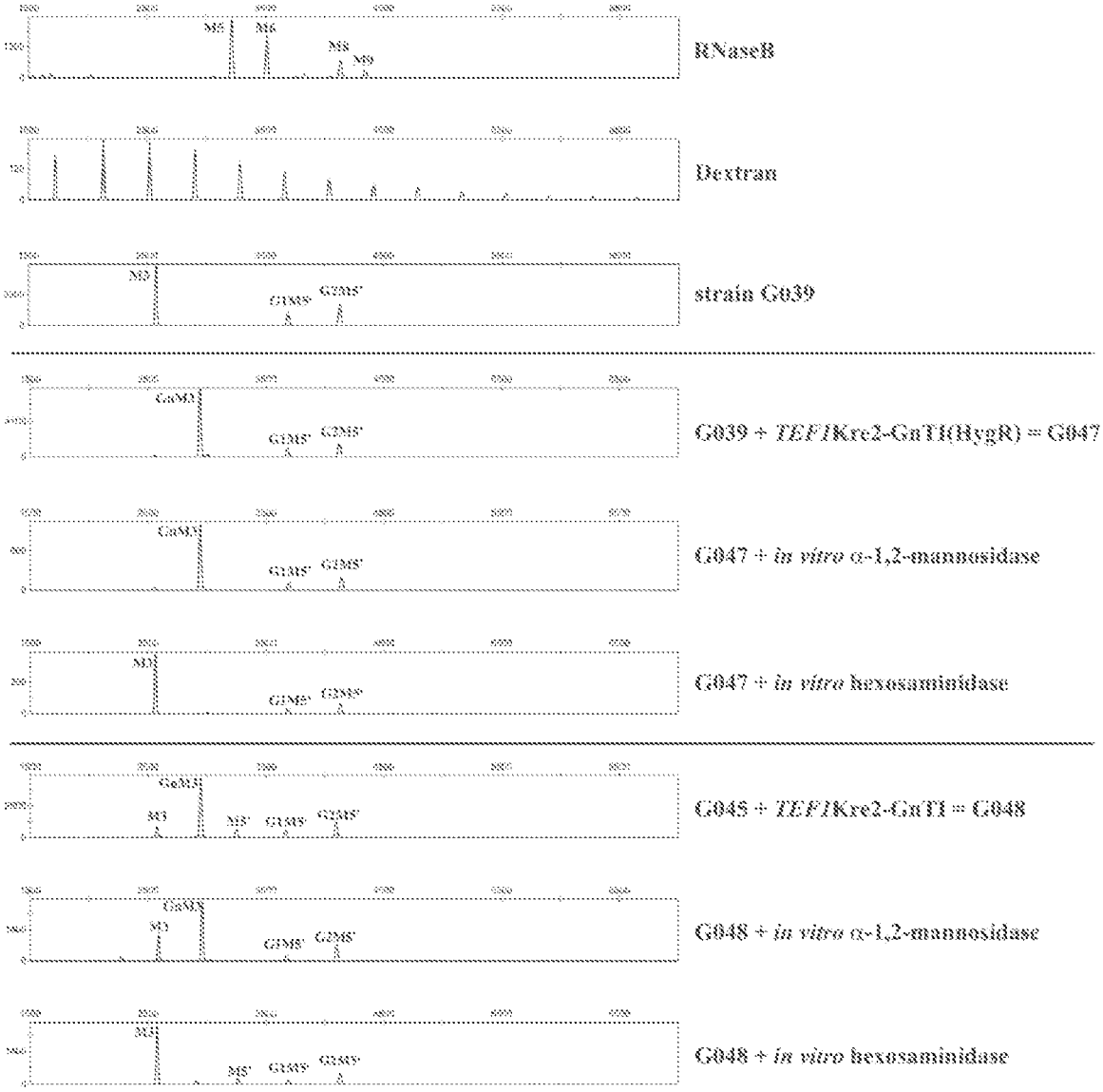

RNase8
Dextran
strain G039
G039 + TEF1Kre2-GnTI(BygR) = G047
G047 + in vitro α-1,2-mannosidase
G047 + in vitro hexosaminidase
G045 + TEF1Kre2-GnTI = G048
G048 + in vitro α-1,2-mannosidase
G048 + in vitro hexosaminidase

FIGURE 20

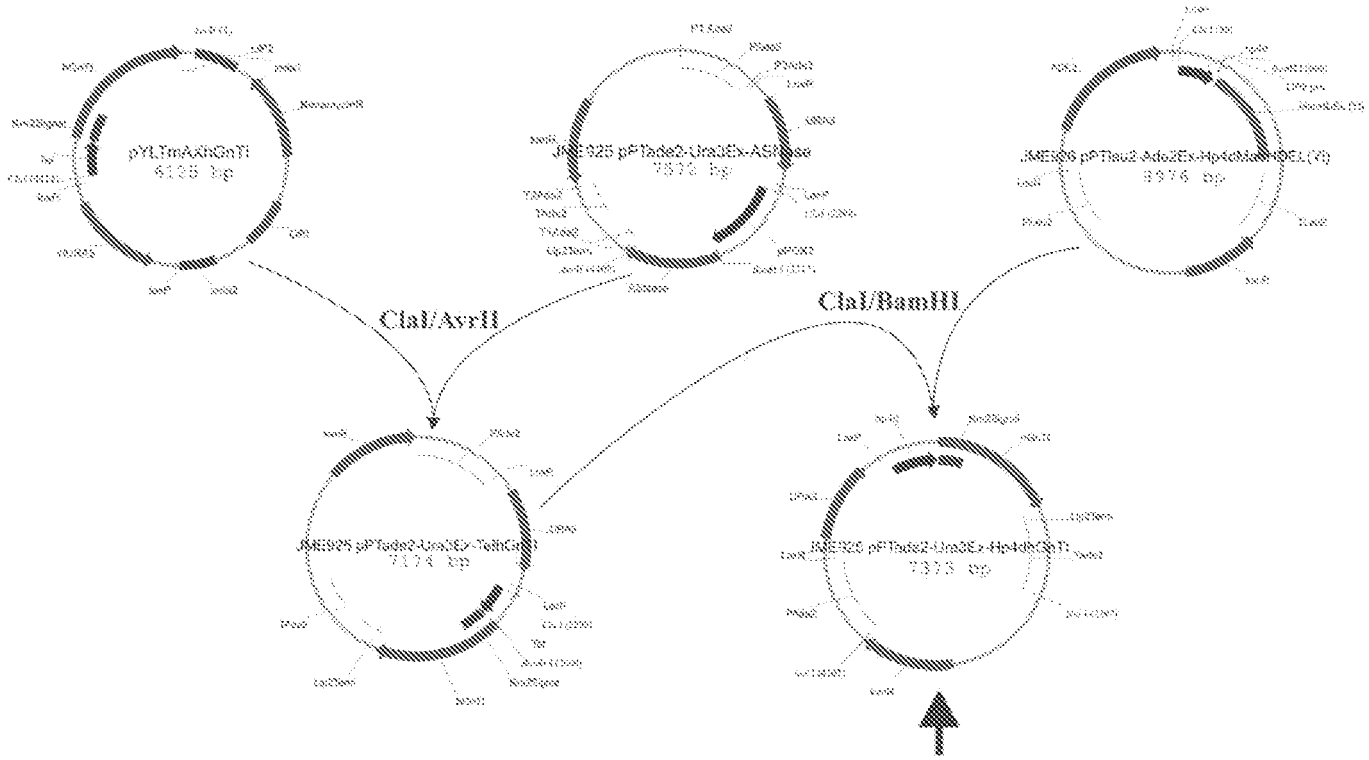


FIGURE 21

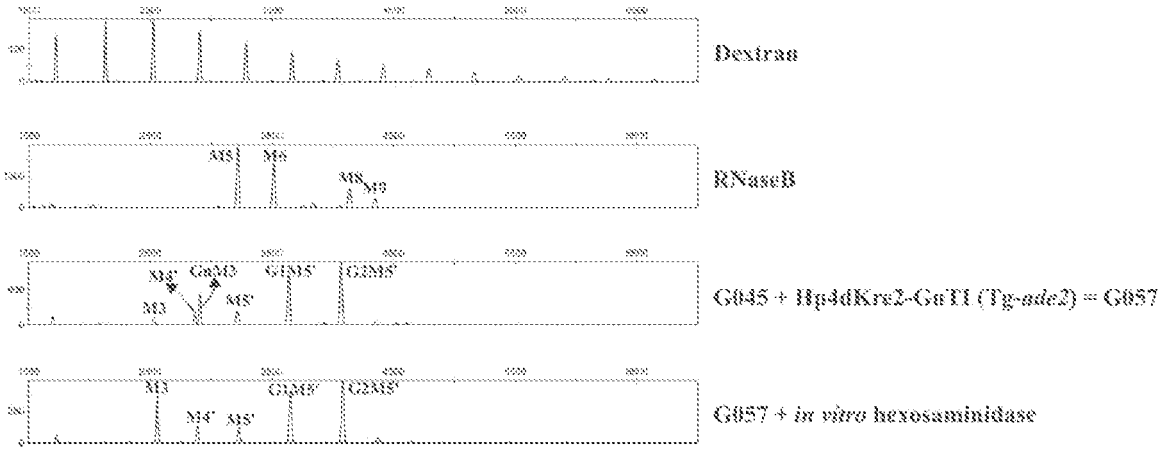

Dextran
M5
M6
M5
M9
RNaseB
M4'
GnM3
M3'
M5'
G1M5'
G2M5'
G0M5 + Hp4dKre2-GnTI (Tg-ade2) = G057
M3'
G1M5'
G2M5'
M4'
M5'
G057 + in vitro hexosaminidase

FIGURE 22

MLLTKRFSKLFKLTFIVLILCGLFVITNKYMDENTSSLVYQLNFDQMLRNVDKDGTWSPG
ELVLVVQVHNRPEYLRLLIDSLRKAQGIREVLVIFSHDFWSAEINSLISSVDFCPVLQVF
FPFSIQLYPSEFPGSDPRDCPRDLKKNAALKLGCINAEYPDSFGHYREAKFSQTKHHWWW
KLHFVWERVKVLQDYTGLILFLEEDHYLAPDFYHVFKKMWKLKQQECPGCDVLSLGTYTT
IRSFYGIADKVDVKTWKSTEHNMGLALTRDAYQKLIECTDTFCTYDDYNWDWTLQYLTLA
CLPKVWKVLVPQAPRIFHAGDCGMHHKKTCRPSTQSAQIESLLNNNKQYLFPETLVIGEK
FPMAAISPPRKNGGWGDIRDHELCKSYRRLQ (SEQ ID NO:17)

ATGCTGCTGACCAAGCGATTCTCTAAGCTGTTCAAGCTGACCTTCATCGTGCTGATCCTGTGTGGCCTGTT
CGTGATCACCAACAAGTACATGGACGAGAACACCTCGAGCCTGGTGTACCAGCTGAACTTCGACCAGAT
GCTGCGAAACGTGGACAAGGACGGCACCTGGTCTCCCGGCGAGCTGGTGCTCGTGGTGCAGGTGCACAA
CCGACCCGAGTACCTGCGACTGCTGATCGACTCTCTGCGAAAGGCCCAGGGCATCCGAGAGGTGCTGGT
GATCTTCTCTCACGACTTCTGGTCTGCCGAGATCAACTCCCTGATCTCTTCTGTGGACTTCTGTCCCGTGCT
GCAGGTGTTCTTCCCATTCAGCATCCAGCTGTACCCCTCTGAGTTCCCCGGCTCTGACCCCCGAGACTGTC
CCCGAGACCTGAAGAAGAACGCCGCCCTGAAGCTGGGCTGTATCAACGCCGAGTACCCCGACTCTTTCG
GCCACTACCGAGAGGCCAAGTTCTCTCAGACCAAGCACCACTGGTGGTGGAAGCTGCACTTCGTGTGGG
AGCGAGTGAAGGTGCTGCAGGACTACACCGGCCTGATCCTGTTCCTGGAGGAGGACCACTACCTGGCCC
CCGACTTCTACCACGTGTTCAAGAAGATGTGGAAGCTGAAGCAGCAGGAGTGTCCCGGCTGCGACGTGCT
GTCTCTGGGCACCTACACCACCATCCGATCTTTCTACGGCATCGCCGACAAGGTGGACGTCAAGACCTGG
AAGTCTACCGAGCACAACATGGGCCTGGCCCTGACCCGAGATGCCTACCAGAAGCTGATCGAGTGTACC
GACACCTTCTGTACCTACGACGACTACAACTGGGACTGGACTCTGCAGTACCTGACCCTGGCCTGTCTGC
CCAAGGTGTGGAAGGTGCTGGTGCCCCAGGCCCCTCGAATCTTCCACGCCGGCGACTGTGGCATGCACCA
CAAGAAGACCTGTCGACCCTCTACCCAGTCTGCCCAGATCGAGTCTCTGCTGAACAACAACAAGCAGTAC
CTGTTCCCTGAGACCCTGGTGATCGGCGAGAAGTTCCCCATGGCCGCCATCTCGCCTCCCCGAAAGAACG
GCGGCTGGGGCGACATCCGAGACCACGAACTCTGTAAGTCTTACCGACGACTGCAGTAG (SEQ ID
NO:18)

FIGURE 23

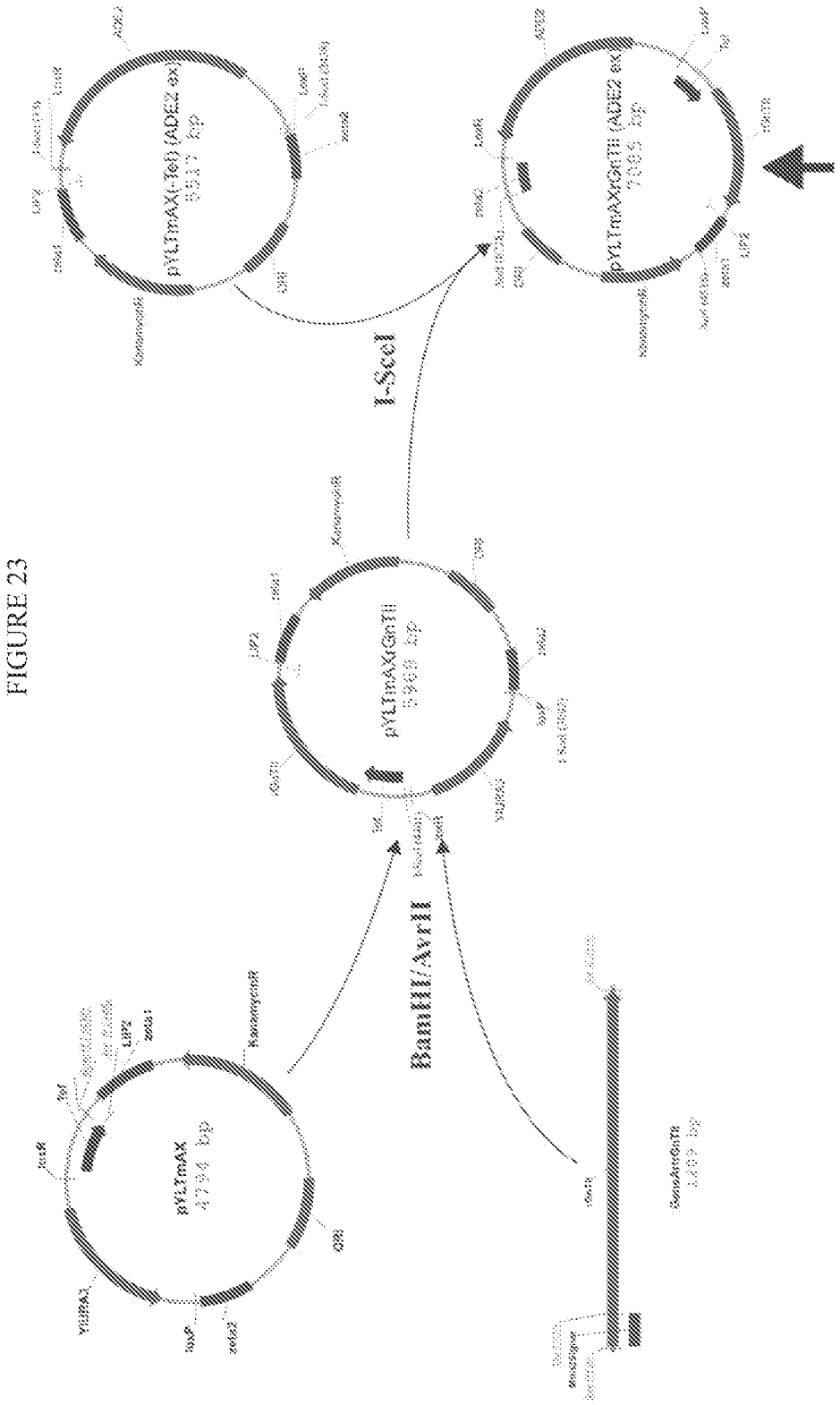


FIGURE 24

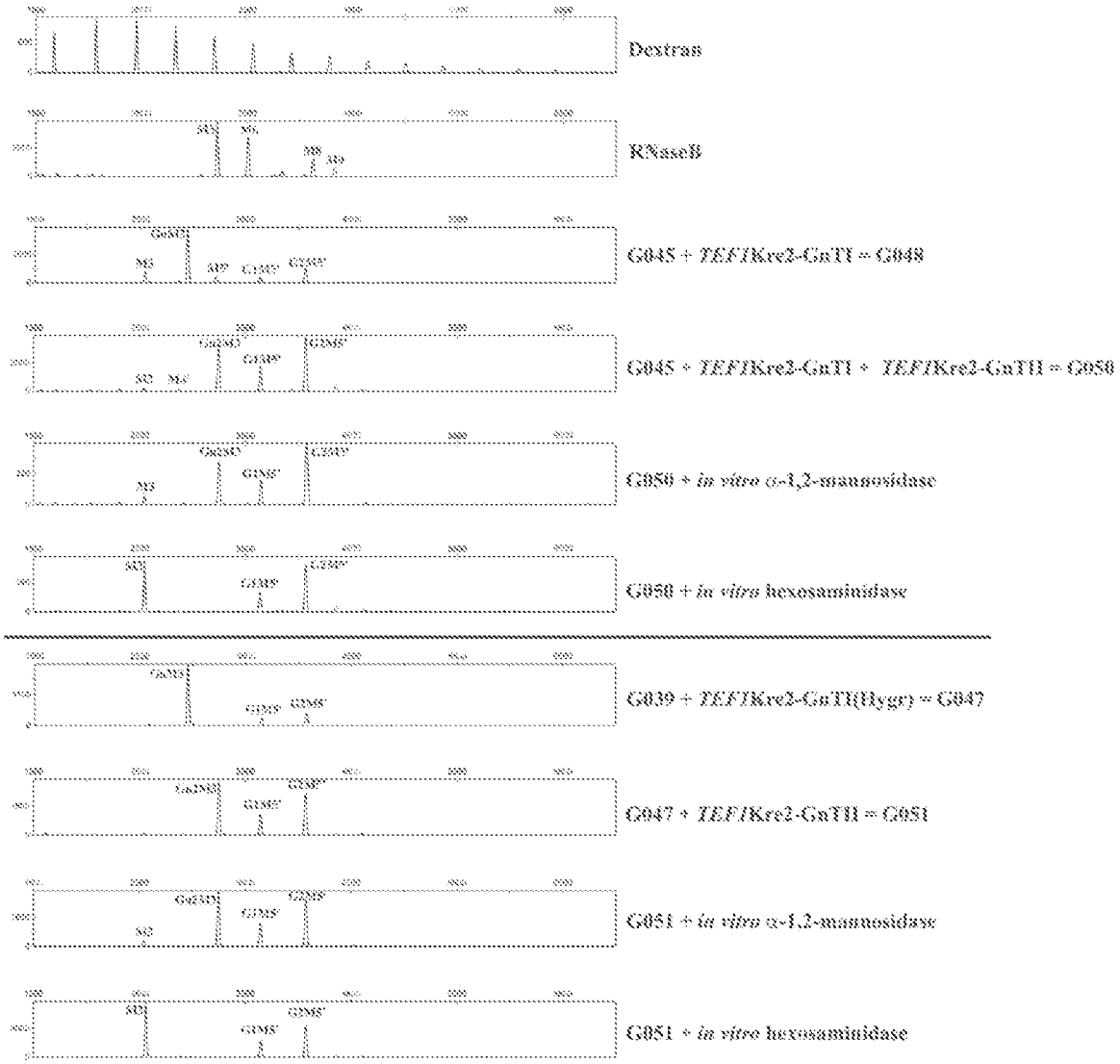

Dextran
RNaseB
G045 + TEF1Kre2-GnTI = G048
G045 + TEF1Kre2-GnTI + TEF1Kre3-GnTII = G050
G050 + in vitro α-1,2-mannosidase
G050 + in vitro hexosaminidase
G039 + TEF1Kre2-GnTI(Hygr) = G047
G047 + TEF1Kre2-GnTII = G051
G051 + in vitro α-1,2-mannosidase
G051 + in vitro hexosaminidase

FIGURE 25

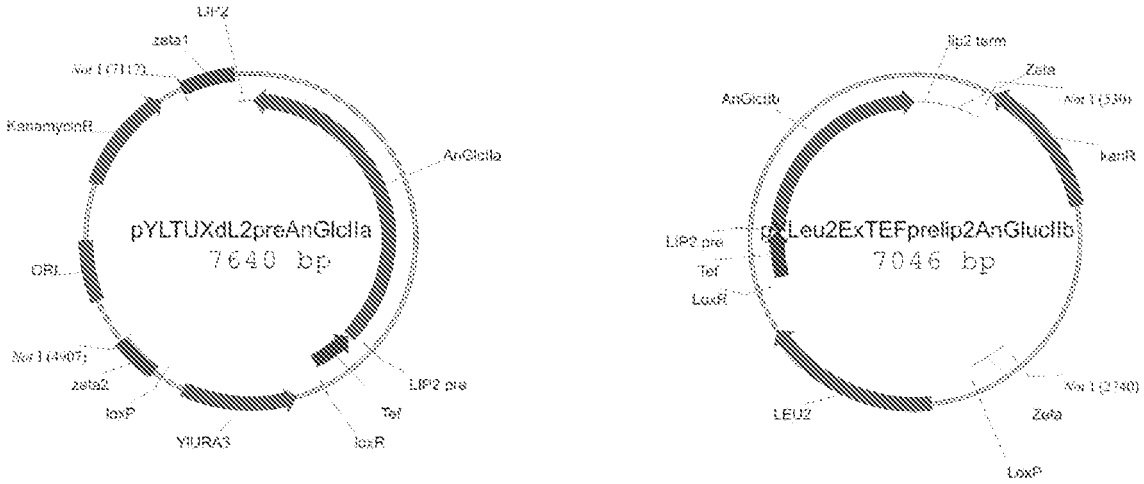

LIP2
zeta1
Xba I (7117)
Kanamycin R
AnGlcIIa
pYLTUXdL2preAnGlcIIa
7640 bp
ORI
Xba I (4907)
zeta2
loxP
YlURA3
LIP2 pre
Tef
loxR
lip2 term
Zeta
AnGlcIIb
Xba I (3350)
kanR
pYLeu2ExTEFprelip2AnGlucIIb
7046 bp
LIP2 pre
Tef
LoxR
LEU2
Xba I (2740)
Zeta
LoxP

FIGURE 26

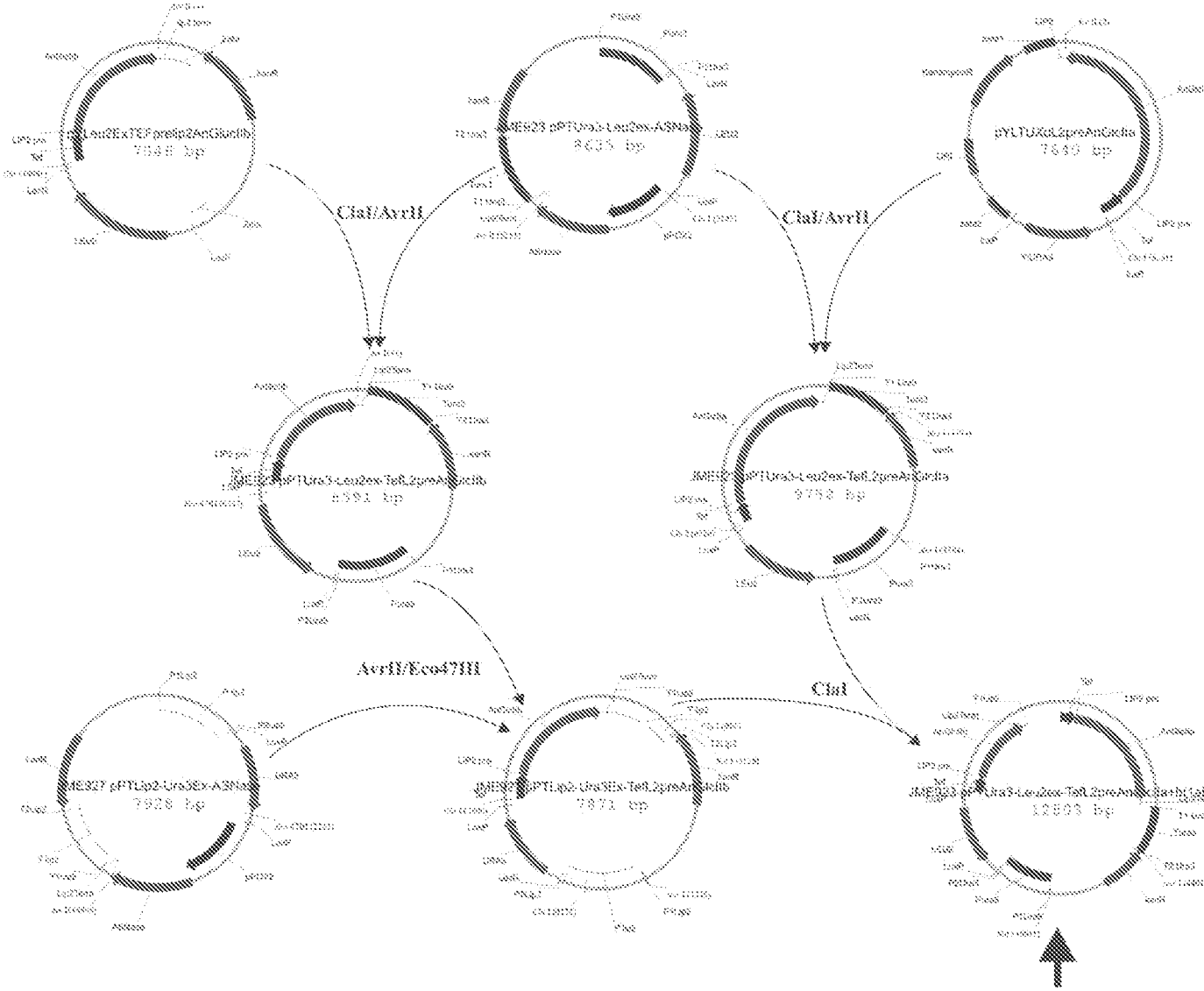


FIGURE 27

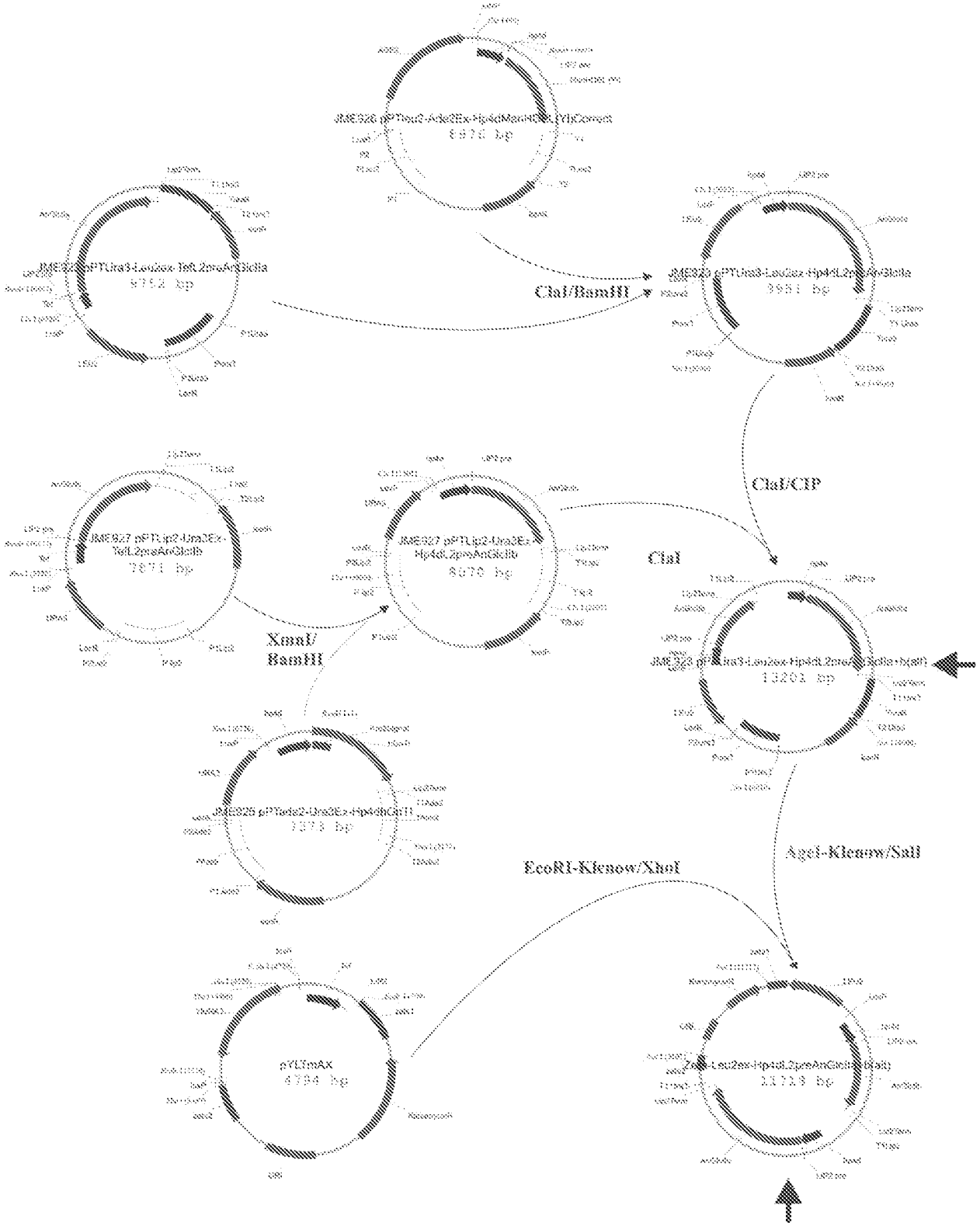
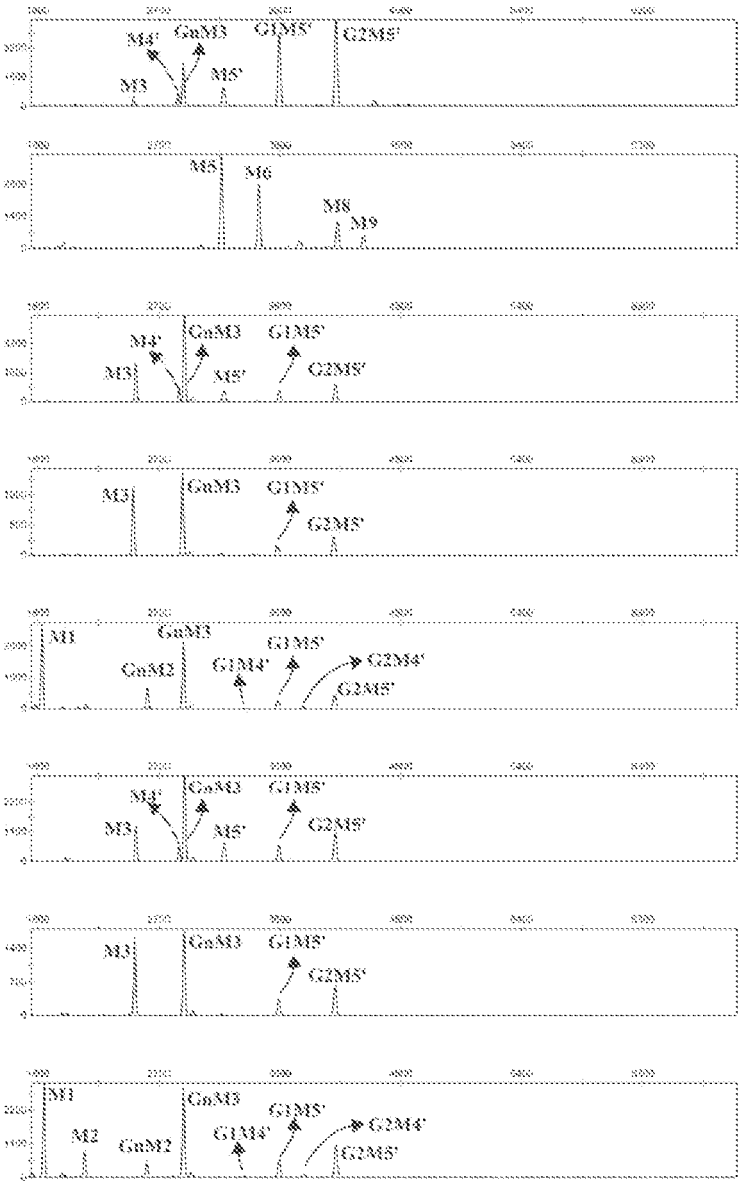

ClaI/BamHI
ClaI/CIP
ClaI
XmnI/
BamHI
AgeI-Klenow/SalI
EcoRI-Klenow/XhoI

FIGURE 28



G045 + Hp4dKre2-GnT1 (Tg-*ade2*) = G057

RNaseB

G057 + Hp4dGls2α/β (Tg-*ura3*) = G061

G061 + α-1,2-mannosidase

G061 + Jack Bean α-mannosidase

G057 + Hp4dGls2α/β (Rd) = G060

G060 + α-1,2-mannosidase

G060 + Jack Bean α-mannosidase

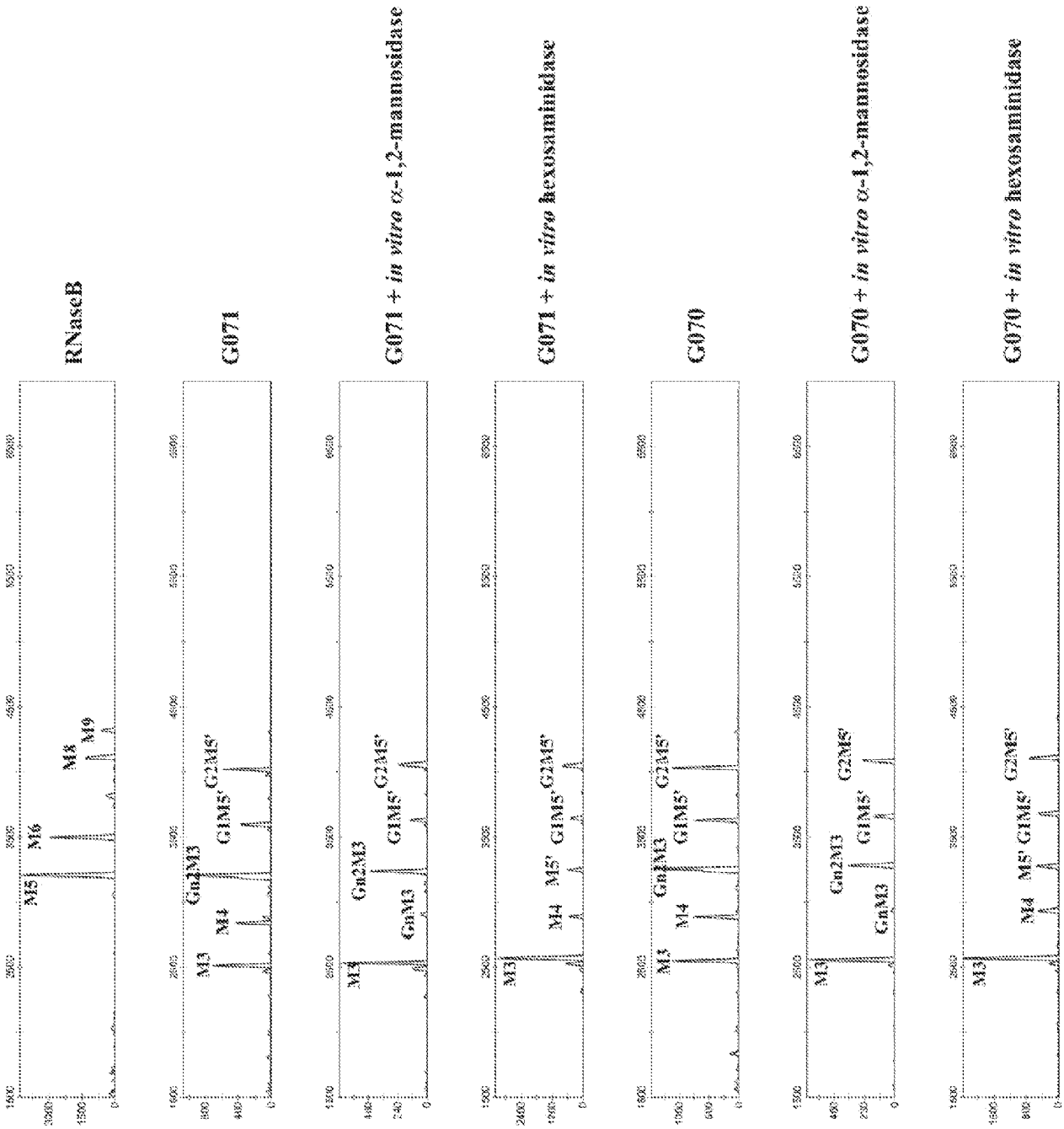

RNaseB
M5
M6
M8
M9
G071
M3
M4
Gn2M3
G1M5'
G2M5'
G071 + in vitro α-1,2-mannosidase
M3
GnM3
Gn2M3
G1M5'
G2M5'
G071 + in vitro hexosaminidase
M3
M4
M5'
G1M5'
G2M5'
G070
M3
M4
GnM3
Gn2M3
G1M5'
G2M5'
G070 + in vitro α-1,2-mannosidase
M3
GnM3
Gn2M3
G1M5'
G2M5'
G070 + in vitro hexosaminidase
M3
M4
M5'
G1M5'
G2M5'

FIGURE 29

FIGURE 30A

ATGAAGCTTTCCACCATCCTTTTCACAGCCTGCGCTACCCTGGCTGCCGCCCT
CCCTTCCCCCATCACTCCTTCTGAGGCCGCAGTTCTCCAGAAGCGAGGCGGCG
GCGACATTCAGATGACTCAGTCTCCCTCTTCTCTGTCTGCTTCTGTGGGTGAC
CGAGTGACCATTACCTGTCGAGCTTCTCAGGACGTGAACACTGCTGTTGCTTG
GTATCAGCAGAAGCCTGGAAAGGCTCCTAAGCTGCTGATCTACTCTGCCTCTT
TCCTGTACTCTGGCGTGCCTTCTCGATTTTCTGGCTCTCGATCTGGAACCGACT
TCACCCTGACCATTTCTTCTCTGCAGCCTGAGGACTTTGCTACCTACTACTGTC
AGCAGCATTACACCACCCCTCCTACTTTTGGACAGGGCACCAAGGTTGAGAT
TAAGCGAACCGTGGCTGCTCCTTCTGTGTTCATTTTCCCCCCCTCTGACGAGC
AGCTGAAGTCTGGAACTGCTTCTGTTGTGTGCCTGCTGAACAACTTTTACCCC
CGAGAGGCTAAGGTTCAGTGGAAGGTGGACAACGCTCTGCAGTCTGGAAACT
CTCAGGAGTCTGTTACTGAGCAGGACTCTAAGGACTCGACCTACTCTCTCTCT
TCTACCCTGACCCTGTCTAAGGCTGACTACGAGAAGCATAAGGTGTACGCTT
GTGAGGTTACCCATCAGGGACTGTCCTCTCCCGTGACCAAGTCTTTTAACCGA
GGCGAGTGCTAA

FIGURE 30B

MKLSTILFTACATLAAALPSPITPSEAAVLQKRGGGDIQMTQSPSSLSASVGDRVTITCR
ASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFA
TYYCQQHYTTPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA
KVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSP
VTKSFNRGEC

FIGURE 31A

ATGAAGCTTTCCACCATCCTTTTCACAGCCTGCGCTACCCTGGCTGCCGCCCT
CCCTTCCCCCATCACTCCTTCTGAGGCCGCAGTTCTCCAGAAGCGAGGCGGCG
GCGAGGTTCAGCTGGTTGAGTCTGGTGGAGGACTGGTTCAGCCTGGTGGATC
TCTGCGACTGTCTTGTGCTGCTTCTGGCTTCAACATCAAGGACACCTACATTC
ATTGGGTCCGACAGGCTCCCGGAAAGGGACTGGAGTGGGTTGCCCGAATCTA
CCCTACCAACGGCTACACTCGATACGCTGACTCTGTGAAGGGACGATTCACC
ATTTCTGCCGACACCTCTAAGAACACTGCCTACCTGCAGATGAACTCTCTGCG
AGCTGAGGACACTGCTGTGTACTACTGTTCTCGATGGGGAGGTGACGGTTTTT
ACGCCATGGACTACTGGGGACAGGGAACTCTGGTGACCGTTTCTTCTGCTTCT
ACCAAGGGACCTTCTGTGTTTCCTCTGGCCCCCTCTTCTAAGTCTACCTCTGGT
GGAACTGCTGCTCTGGGATGTCTGGTGAAGGACTACTTTCCTGAGCCTGTGAC
TGTGTCTTGGAACTCTGGCGCTCTGACTTCTGGTGTTCACACCTTCCCTGCTGT
TCTGCAGTCCTCTGGACTGTACTCTCTCTCTTCTGTGGTGACCGTGCCTTCTTC
TTCTCTGGGAACCCAGACCTACATCTGTAACGTGAACCACAAGCCCTCTAAC
ACTAAGGTGGACAAGAAGGTGGAGCCTAAGTCTTGTGACAAGACCCATACCT
GTCCCCCTTGTCCTGCTCCTGAGCTGCTGGGAGGACCCTCTGTTTTTCTGTTCC
CCCCCAAGCCTAAGGACACCCTGATGATTTCTCGAACCCCTGAGGTGACCTG
TGTTGTGGTGGACGTTTCTCATGAGGACCCTGAGGTGAAGTTTAACTGGTACG
TGGACGGTGTTGAGGTTCACAACGCTAAGACTAAGCCCCGAGAGGAGCAGTA
CAACTCTACTTACCGAGTGGTGTCTGTGCTGACTGTTCTGCATCAGGACTGGC
TGAACGGAAAGGAATACAAGTGTAAGGTCTCCAACAAGGCTCTGCCTGCTCC
TATTGAAAAGACCATCTCTAAGGCTAAGGGACAGCCCAGAGAGCCTCAGGTT
TACACTCTGCCCCCTTCCCGAGAGGAGATGACCAAGAACCAGGTGTCCCTGA
CTTGTCTGGTCAAGGGATTCTACCCCTCTGACATTGCTGTTGAGTGGGAGTCT
AACGGACAGCCTGAGAACAACTACAAGACCACCCCTCCTGTTCTGGACTCTG
ACGGCTCTTTCTTCCTGTACTCTAAGCTGACCGTGGACAAGTCTCGATGGCAG
CAGGGAAACGTGTTCTCTTGTTCCGTGATGCATGAGGCTCTGCACAACCACTA
CACCCAGAAGTCTCTGTCTCTGTCTCCCGGCAAGTAA

FIGURE 31B

MKLSTILFTACATLAAALPSPITPSEAAVLQKRGGGEVQLVESGGGLVQPGGSLRLSCAA
SGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMN
SLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAA
LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICN
VNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC
KVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW
ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPGK

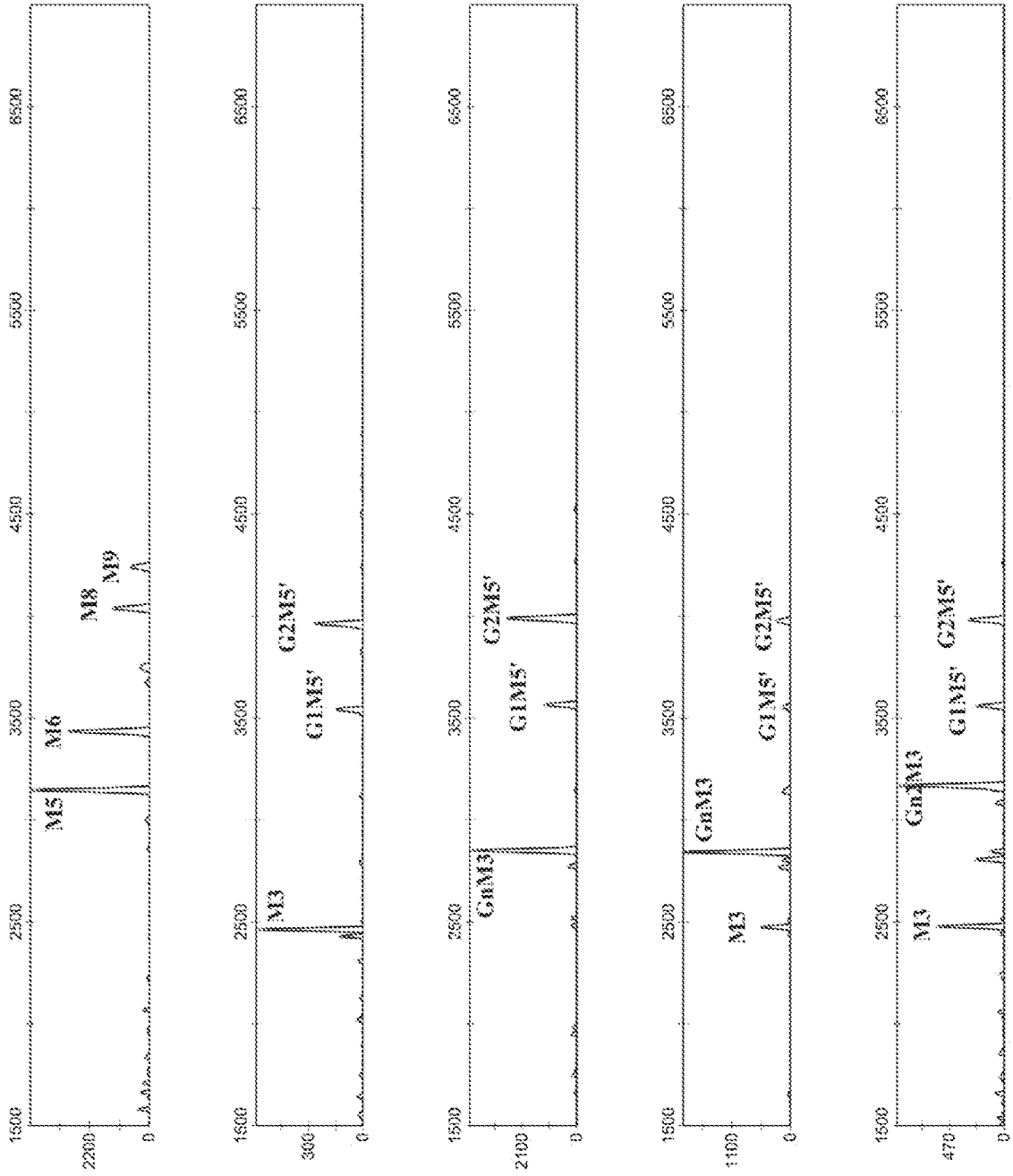

RNaseB
M5
M6
M8
M9
Man3-platform (G045)
M3
G1M5'
G2M5'
G045 + Hp4dKre2-GnTI (Tg) = G057
GnM3
G1M5'
G2M5'
G057 + Hp4dAnGls2(α+β) (Tg) = G061
M3
GnM3
G1M5'
G2M5'
G061 + Hp4dMnn2-GnTII (Tg) = G071
M3
GnM3
G1M5'
G2M5'

FIGURE 32

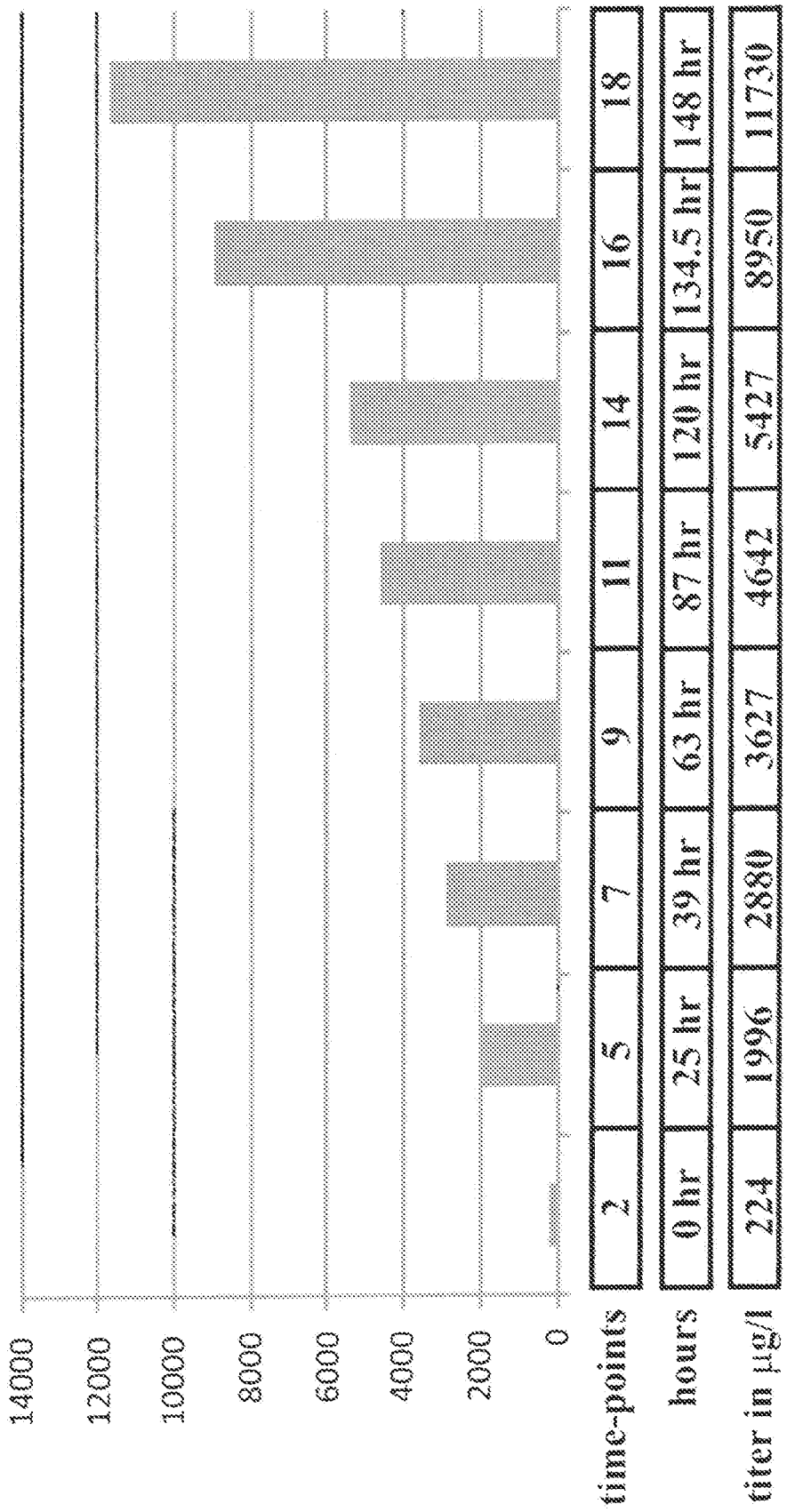

(ug/l active herceptin)
14000
12000
10000
8000
6000
4000
2000
0
time-points
2
5
7
9
11
14
16
18
hours
0 hr
25 hr
39 hr
63 hr
87 hr
120 hr
134.5 hr
148 hr
titer in µg/l
224
1996
2880
3627
4642
5427
8950
11730

FIGURE 33

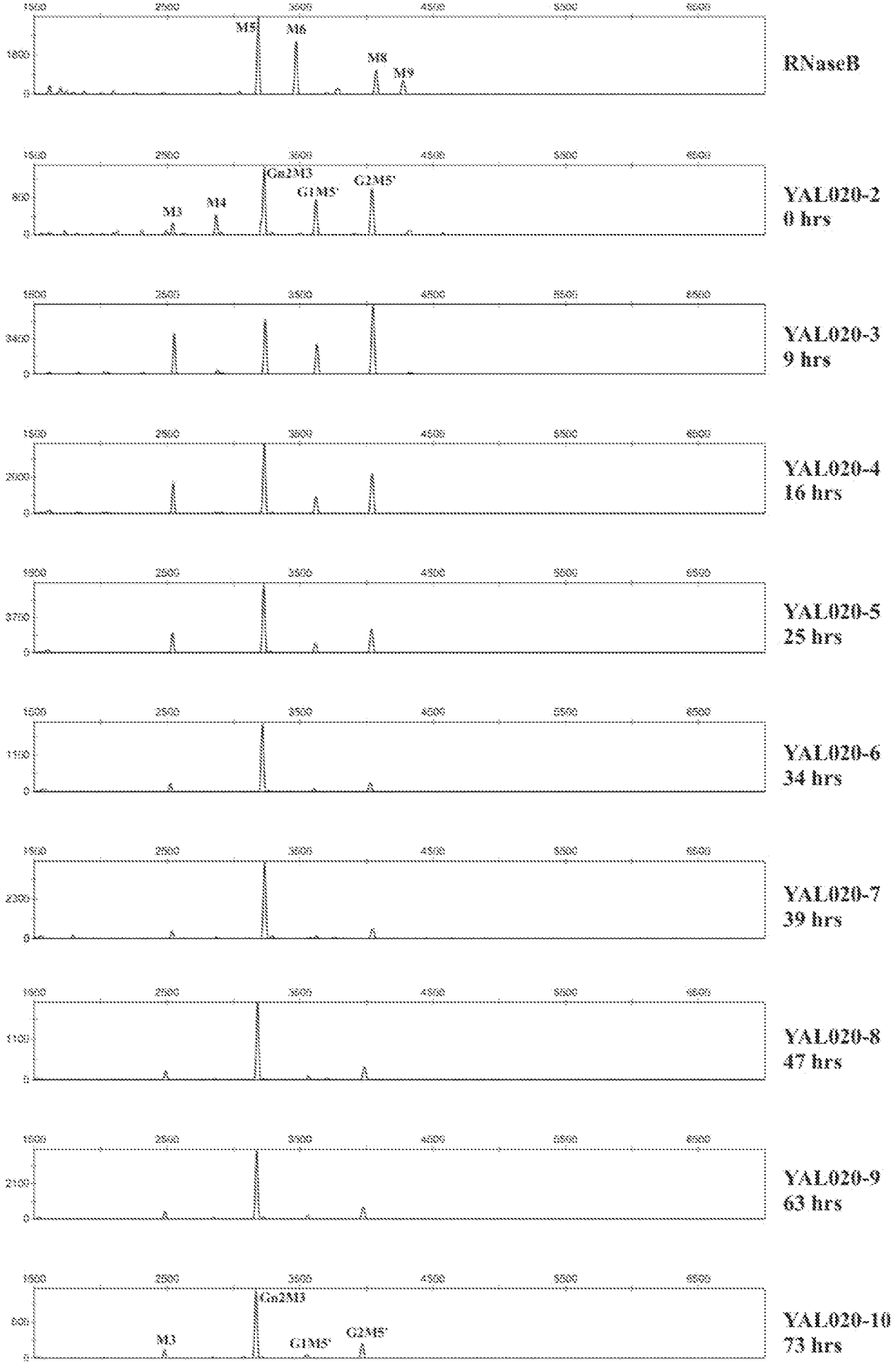

M5
M6
M8
M9
RNaseB
Gn2M3
G1M5'
G2M5'
M3
M4
YAL020-2
0 hrs
YAL020-3
9 hrs
YAL020-4
16 hrs
YAL020-5
25 hrs
YAL020-6
34 hrs
YAL020-7
39 hrs
YAL020-8
47 hrs
YAL020-9
63 hrs
YAL020-10
73 hrs

FIGURE 34A

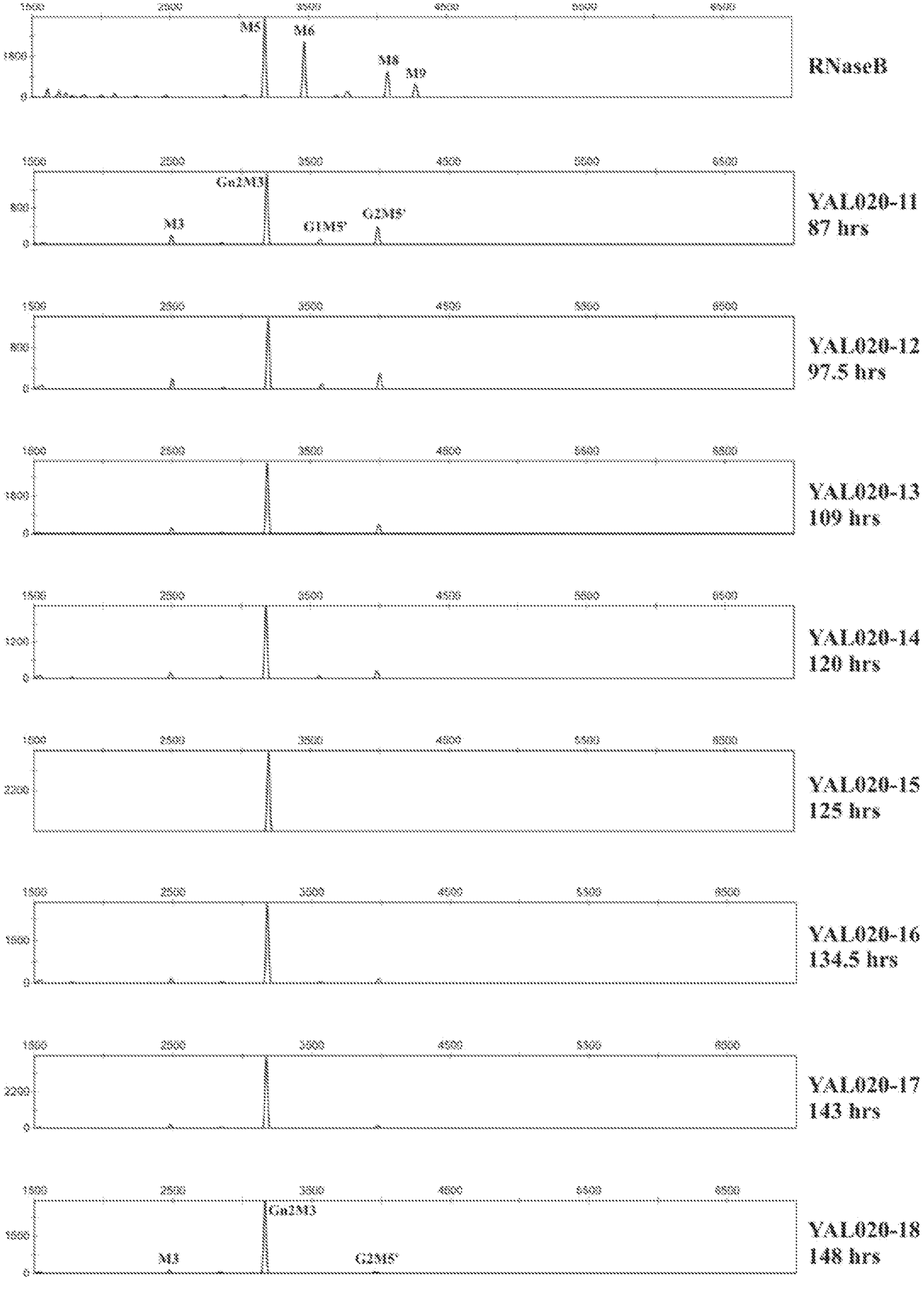

M5
M6
M8
M9
RNaseB
Ga2M3
M3
G1M5'
G2M5'
YAL020-11
87 hrs
YAL020-12
97.5 hrs
YAL020-13
109 hrs
YAL020-14
120 hrs
YAL020-15
125 hrs
YAL020-16
134.5 hrs
YAL020-17
143 hrs
Ga2M3
M3
G2M5'
YAL020-18
148 hrs

FIGURE 34B