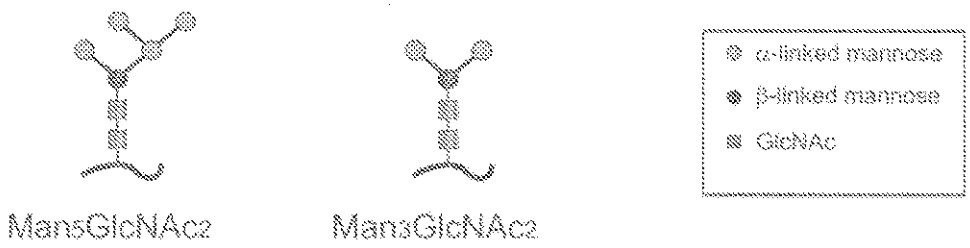
FIGURE 1

A.


Man5GlcNAc2
Man5GlcNAc2
α-linked mannose
β-linked mannose
GlcNAc

B.

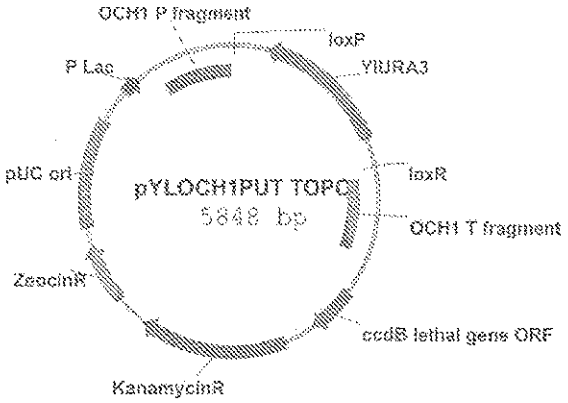

OCH1 P fragment
loxP
P Lac
YlURA3
pUC ori
pYLOCH1PUT TOPO
5848 bp
loxR
OCH1 T fragment
ZeocinR
ccdB lethal gene ORF
KanamycinR

FIGURE 2

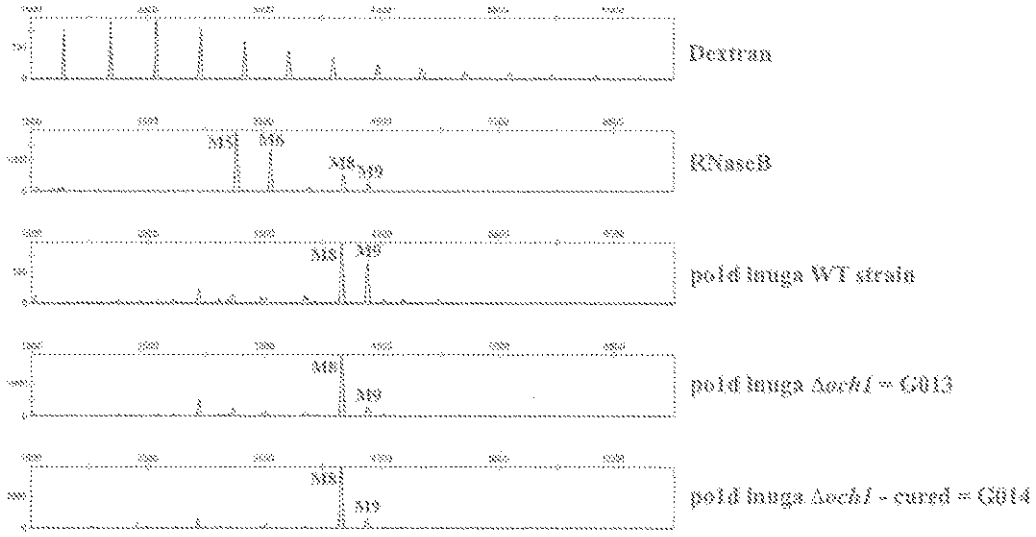
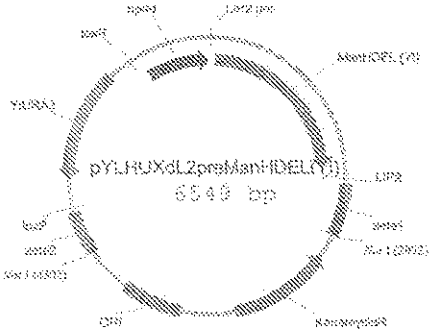

Dextran
RNaseB
N5
N6
N8
N9
pold Inuga WT strain
pold Inuga Δoch1 = G013
pold Inuga Δoch1 - cured = G014

FIGURE 3


pYLHUXdL2preMan1DELL(1)
6540 bp

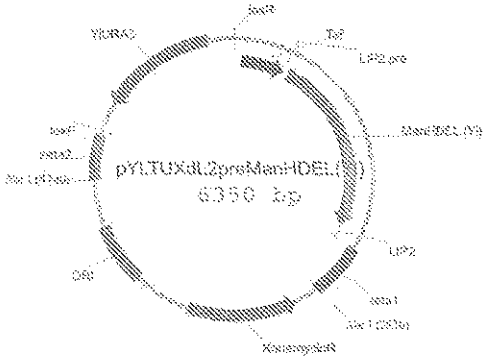
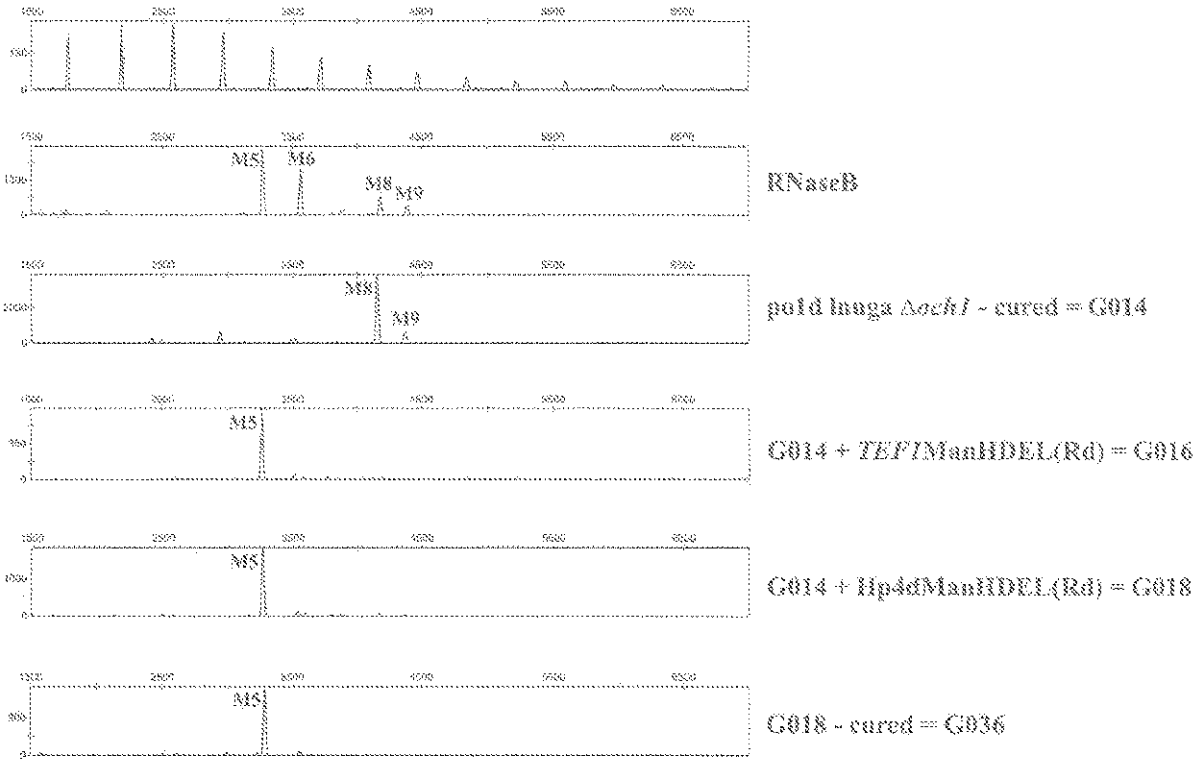

pYLTUXdL2preMan1DEL(
6350 bp

FIGURE 4


M5
M6
M8
M9
RNaseB
M5
M9
pold Inaga Δoch1 - cured = G014
M5
G014 + TEF1ManHDEL(Rd) = G016
M5
G014 + Hp4dManHDEL(Rd) = G018
M5
G018 - cured = G036

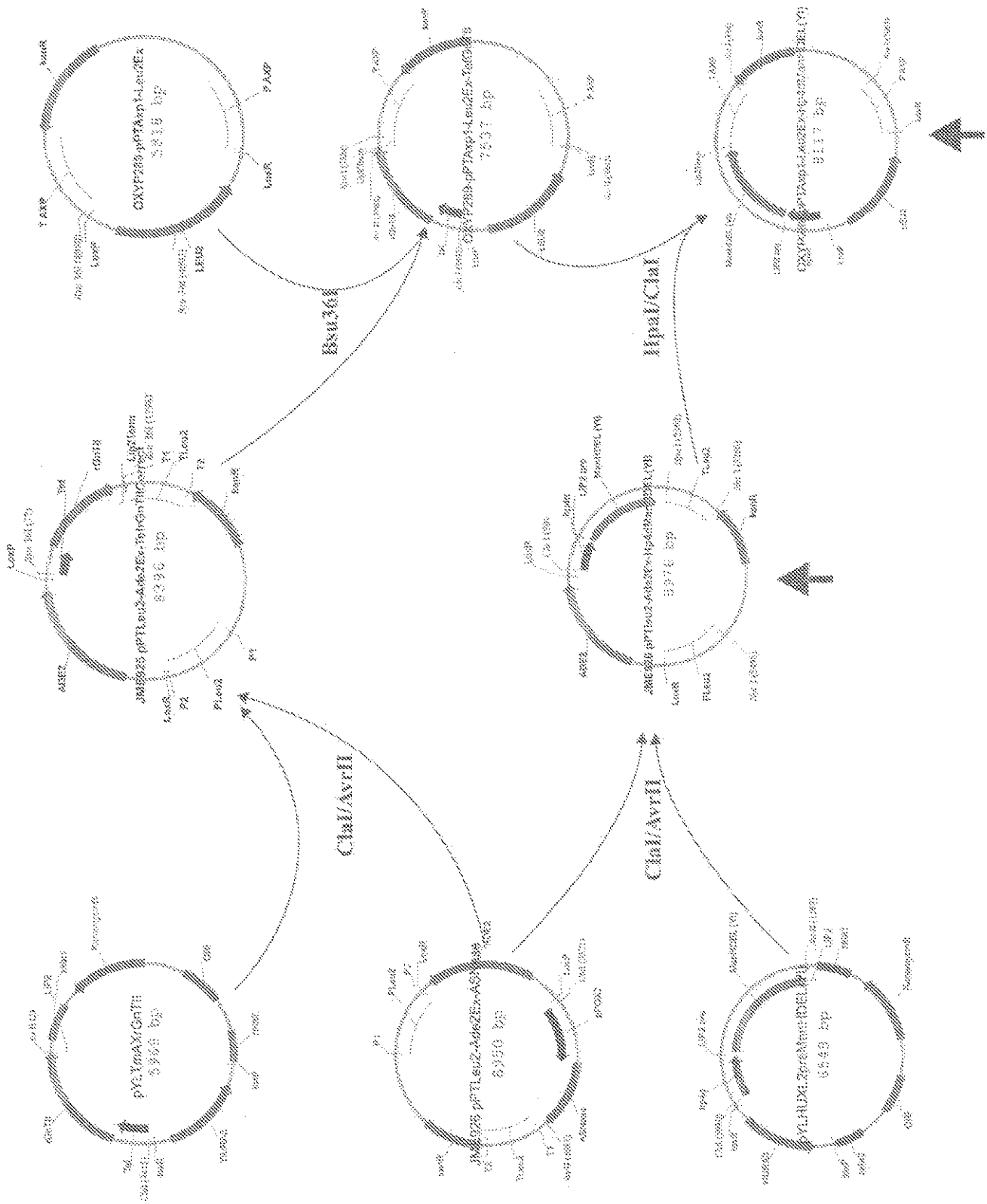


FIGURE 5

FIGURE 6

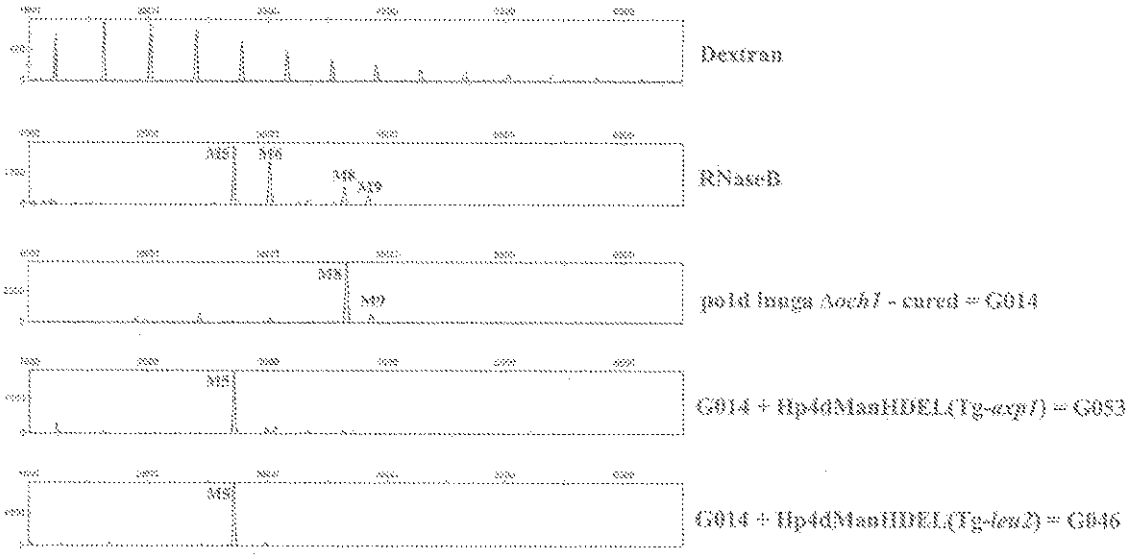

Dextran
RNaseB
M9
M8
M7
M6
M5
M9
M8
M7
M5
Hp4dMan8HDEL(Tg-exp1) = G053
M5
Hp4dMan8HDEL(Tg-lea2) = G046

FIGURE 7

MALFLSKRLLRFTVIAGAVIVLLLTLNSNSRTQQYIPSSISAAFDFTSGSISPEQQVISE
ENDAKKLEQSAINSEASEDSEAMDEESKALKAAAEKADAPPAVIPILVIACDRSTVRRCL
DKLLHYRPSAELFPIIVSQDCGHEETAQAIASYGSAVTHIRQPDLSSIAVPPDHRKFQGY
YKIARHYRWALGQVFRQFRFPAAVVVEDDLEVAPDFFEYFRATYPLLKADPSLWCVSAWN
DNGKEQMVDASRPELLYRTDFFPGLGWLLLAELWAELEPKWPKAFWDDWMRRPEQRQGRA
CIRPEISRTMTFGRKGVSHGQFFDQHLKFIKLNQQFVHFTQLDLSYLQREAYDRDFLARV
YGAPQLQVEKVRTNDRKELGEVRVQYTGRDSFKAFAKALGVMDDLKSGVPRAGYRGIVTF
QFRGRRVHLAPPPTWEGYDPSWN (SEQ ID NO:3)

ATGGCCCTGTTTCTGTCTAAGCGACTGCTGCGATTCACCGTGATCGCCGGTGCCGTGATCGTGCTGCTGCTGACCCTGAACTCTAACTCTCGAACCCAGCAGTACATCCCCTC
TTCTATCTCTGCCGCCTTCGACTTCACTCTGGCTCTATCTCTCCCGAGCAGCAGGTCATCTCTGAGGAGAACGACGCCAAGAAGCTGGAGCAGTCTGCCCTCAACTCTGAAG
CTTCTGAGGACTCCGAGGCCATGGACGAGGAGTCTAAGGCCCTGAAGGCCGCTGCCGAGAAGGCTGACGCTCCTGCCGGCTGTGATCCCCATCCTGGTCATCGCCTGTGACCGA
TCTACCGTGCGACGATGTCTGGACAAGCTGCTGCACTACCGACCCTCTGCCGAGCTGTTCCCCATCATCGTGTCTCAGGACTGTGGCCACGAGGAGACCGCCCAGGCCATTGC
CTCTTACGGCTCTGCCGTGACCCACATCCGACAGCCCGACCTGTCCTCTATCGCCGTGCCCCCCGACCACCGAAAGTTCCAGGGCTACTACAAGATCGCCCGACACTACCGAT
GGGCCCTGGGCCAGGTGTTCCGACAGTTCCGATTCCCCGCTGCCGTGGTGGTGGAGGACGACCTGGAGGTGGCCCCCGACTTCTTCGAGTACTTCCGAGCCACCTACCCCCTG
CTGAAGGCCGACCCCTCTCTGTGGTGTGTGTCTGCCTGGAACGACAACGGCAAGGAACAGATGGTCGACGCCTCTCGACCTGAGCTGCTGTACCGAACCGACTTCTTCCCCGG
CCTGGGCTGGCTGCTGCTGGCTGAGCTGTGGGCCGAGCTGGAGCCCAAGTGGCCCAAGGCCTTCTGGGACGACTGGATGCGACGACCCGAGCAGCGACAGGGCCGAGCCTGTA
TCCGACCCGAGATCTCTCGAACCATGACCTTCGGCCGAAAGGGCGTGTCTCACGGCCAGTTCTTCGACCAGCACCTGAAGTTCATCAAGCTGAACCAGCAGTTCGTGCACTTC
ACCCAGCTGGACCTGTCTTACCTGCAGCGAGAGGCCTACGACCGAGACTTCCTGGCCCGAGTGTACGGCGCTCCCCAGCTGCAGGTGGAGAAGGTGCGAACCAACGACCGAAA
GGAGCTGGGCGAGGTCCGAGTGCAGTACACCGGCCGAGACTCGTTCAAGGCCTTCGCCAAGGCCCTGGGCGTGATGGACGACCTGAAGTCTGGCGTGCCCCGAGCCGGATACC
GAGGCATCGTGACCTTCCAGTTCCGAGGCCGACGAGTGCACCTGGCCCCTCCACCCACCTGGGAGGGCTACGACCCCTCTTGGAACTAG (SEQ ID NO:4)

FIGURE 8

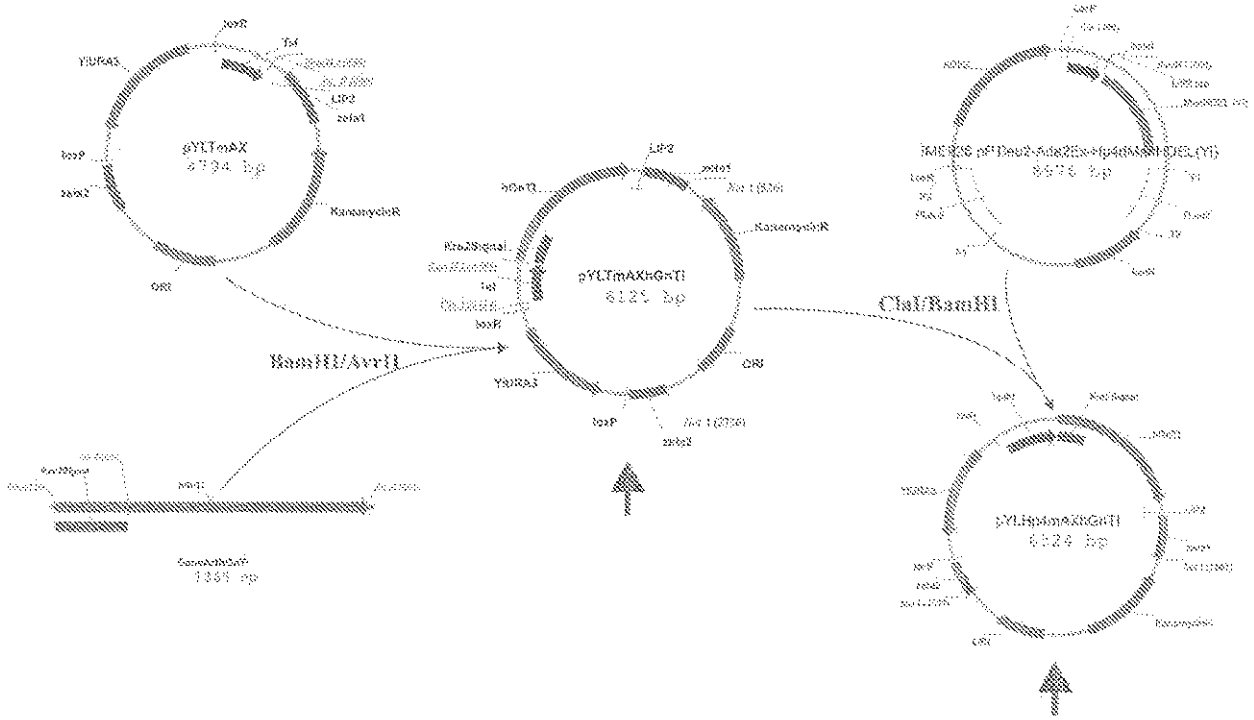

pYLTmAX
4794 bp
pYLTmAXhGhTI
6121 bp
pYLHpMmAXhGhTI
6524 bp
HindIII/AvrII
ClaI/BamHI

FIGURE 9

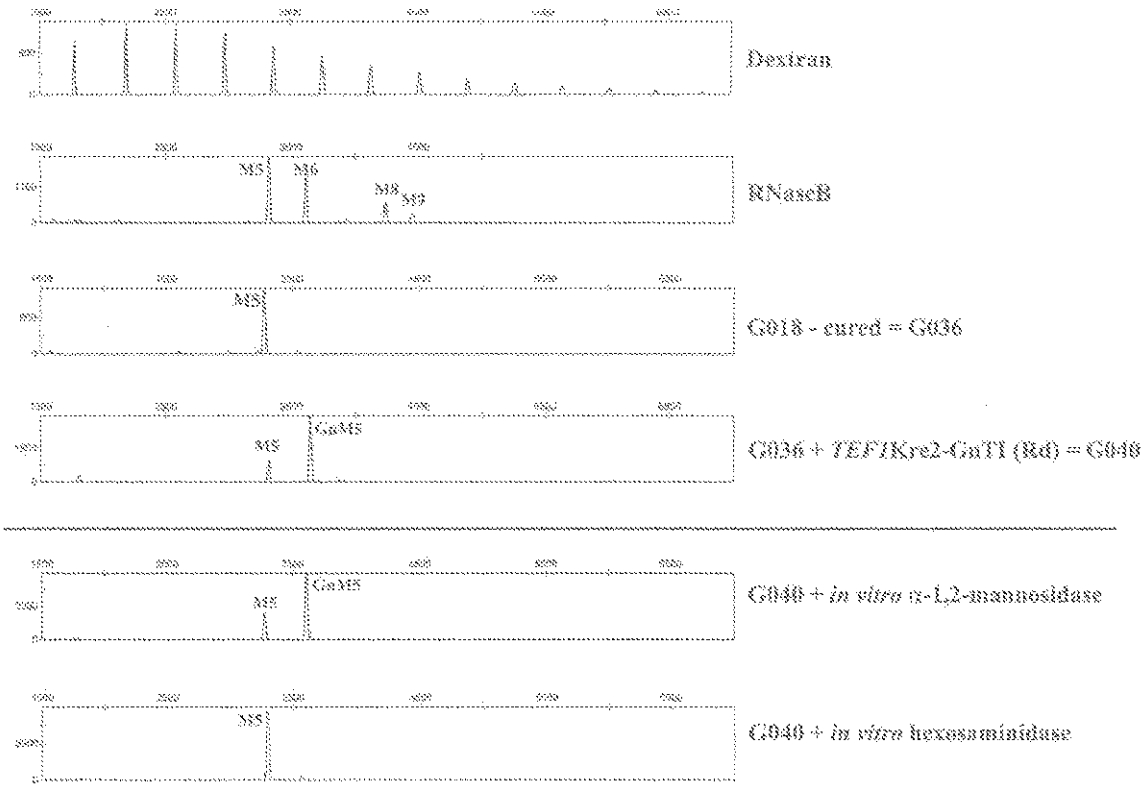

Dextran
M5
M6
M8
M9
RNaseB
M5
G0B - cured = G036
M5
GnM5
G036 + TEF1Kre2-GnTI (Rd) = G040
M5
GnM5
G040 + in vitro α-1,2-mannosidase
M5
G040 + in vitro hexosaminidase

FIGURE 10

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

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

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

FIGURE 11

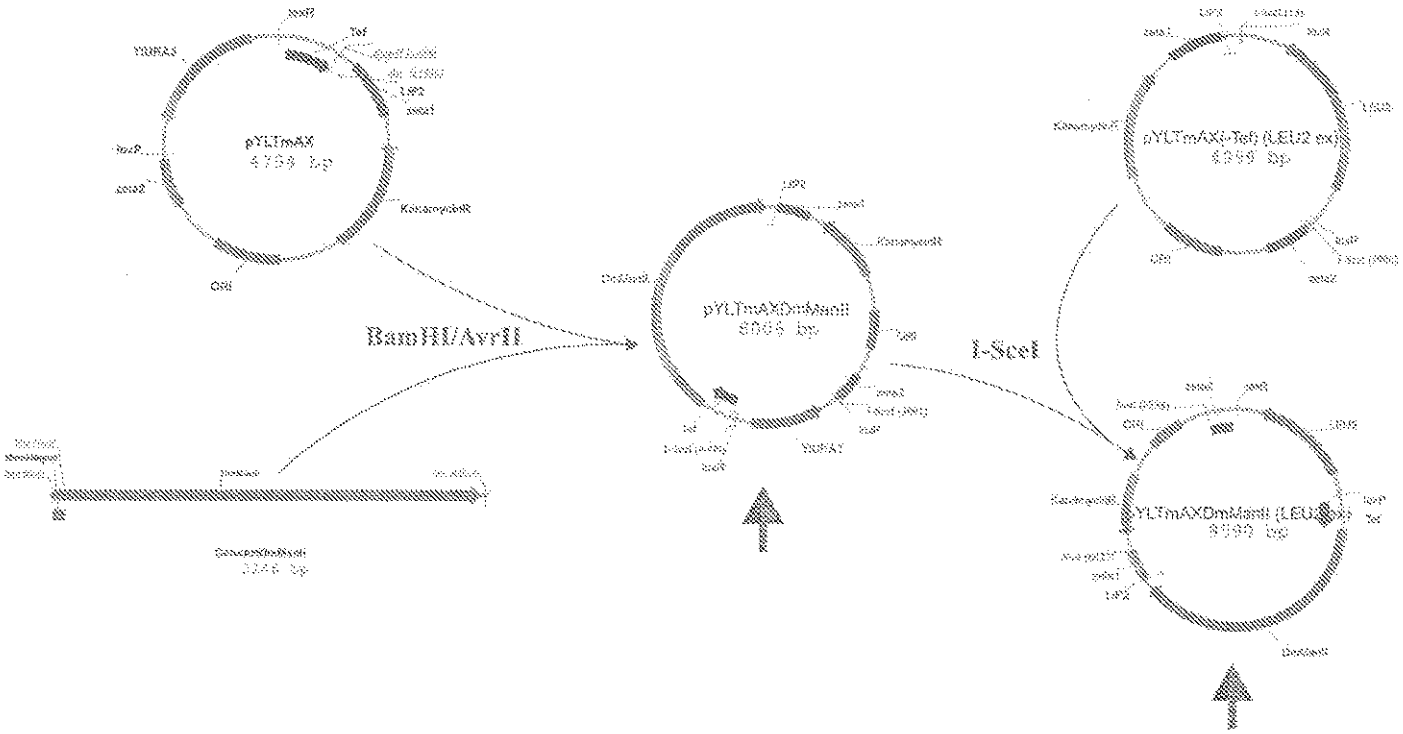


FIGURE 12

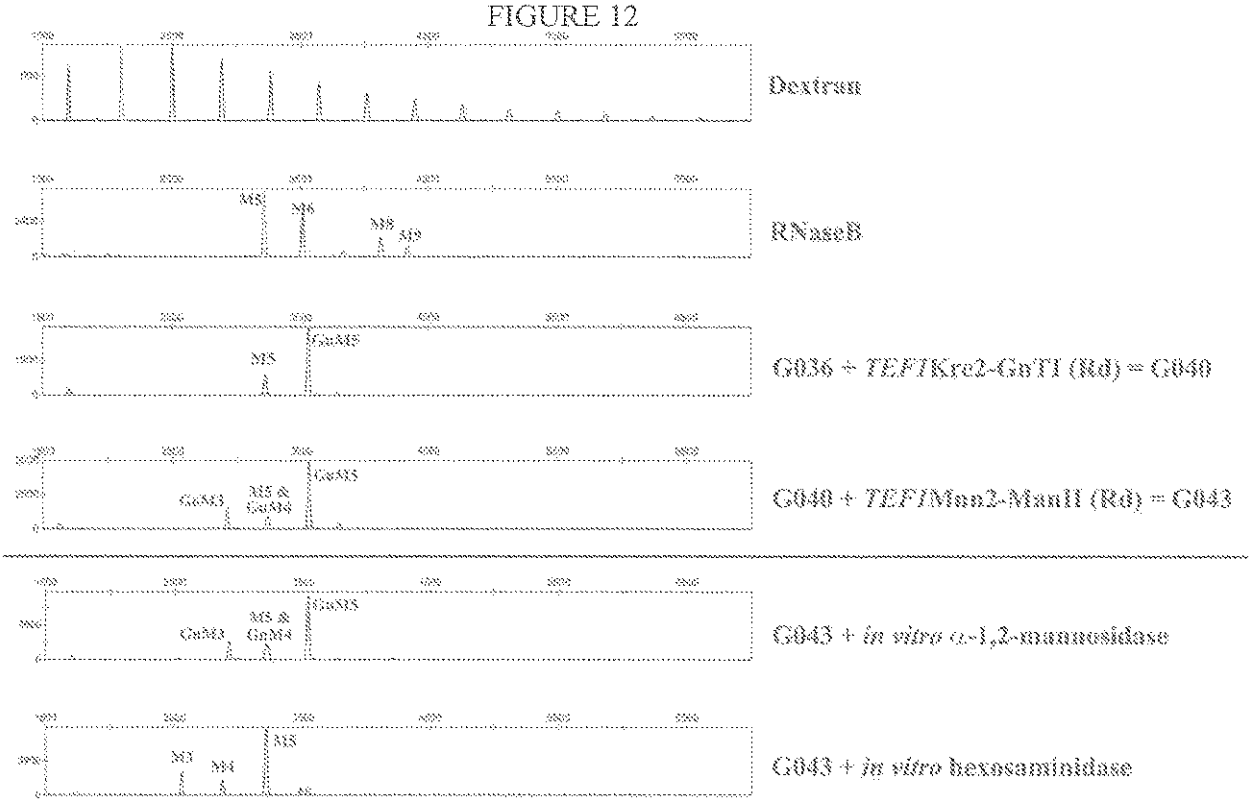

Dextran
M8
M6
M8
M9
RNase8
M5
GnM5
G036 + TEF1Kre2-GnTI (Rd) = G040
GnM3
M5 &
GnM4
GnM5
G040 + TEF1Mnn2-ManII (Rd) = G043
GnM3
M5 &
GnM4
GnM5
G043 + in vitro α-1,2-mannosidase
M3
M4
M5
G043 + in vitro hexosaminidase

FIGURE 13

MLLTKRFSKLFKLTFIVLLILCGLFVITNKYMDENTSVKEYKEYLDRGPAMTGVHEGTVLV
TGGAGYIGSHTCVVLLERGYDVVIVDNLCNSRVEAVHRIEKLTGKTVTFHQVDLLDEPAL
DKVFANQNISAVIHFAGLKAVGESVQVPLSYYKNNISGTINLIECMKKYNVRDFVFSSSA
TVYGDPTRFGGTIPIPESCPREGTSPYGRTKLFIENIIEDETKVNKSLNAALLRYFNPGG
AHPSGELGEDPLGIPNNLLPYIAQVAVGRLDHINVFGDDYPTSDGTPIRDYIHVCDLAEA
HVAALNYLRQHFVSCRPWNLGSGTGSTVFQVLNAFSKAVGRDLPYKVTPRRAGDVVNLTA
NPTRANEELKWKTSRSIYEICVDTWRWQQKYPYGFDLETKTYNGSGGRDLSRLPQLVG
VSTPLQGGSNSAAAIGQSSGELRTGGARPPPPLGASSQPRPGGDSSPVVDSGPGPASNLT
SVPVPHTTALSLPACPEESPLLVGPMLIEFNMPVDLELVAKQNPNVKMGGRYAPRDCVSP
HKVAIIIPFRNRQEHLKYWLYYLHPVLQRQQLDYGIYVINQAGDTIFNRAKLLNVGFQEA
LKDYDYTCFVFSDVDLIPMNDHNAYRCFSQPRHISVAMDKFGFSLPYVQYFGGVSALSKQ
QFLTINGFPNNYWGWGGEDDDIFNRLVFRGMSISRPNAVVGRCRMIRHSRDKKNEPNPQR
FDRIAHTKETMLSDGLNSLTYQVLDVQRYPLYTQITVDIGTPS (SEQ ID NO:9)

ATGCTGCTGACCAAGCGATTCTCTAAGCTGTTCAAGCTGACCTTCATCGTGCTGATCCTGTGTGGCCTGTTCGTGATCACCAACAAGTACATGGACGAGAACACCTCTGTGAA
GGAATACAAGGAGTACCTGGACCGCGGCCTGAGCGTATGACCGGCGTGCACGAGGGCACCGTGCTGCTGACCGACGCCGAGCCGGCTACATCGGCTCTGCACACCTGTTGTGGTGCTGC
TGGAGAAGGGCTACGACGTGGTGATCGTGGACAACCTGTCTAACTCTCCGAGTGGAGGCCGTGCACCGAATCGAGAAGCTGACCGGCAAGACCGTGACCTTCCACCAGGTGGAC
CTGCTGGACGAGCCCGCCCTGGACAAGGTGTTCGCCAACCACAACATCTCTGCCGTGATCCACTTCGCCGGCCTGAAGGCTGTGGGCGAGTCTGTGCAGGTGCCCCTGTACTTC
CTACAAGAACAACATCTCTGGCACCATCAACCTGATCGAGTGTATGAAGAAGTACAACGTCCGAGACTTCGTGTTCTCTTCTTCTGCCACCGTGTACGGCGACCCCGACCCGAC
CCGGCGGAACCATCCCCATCCCCGAGTCTTGTCCCCGAGAGGGCACCTCTCCCTACGGCCGAACCAAGCTGTTCATCGAGGAAGATCATCGAGGACGAAACCAAGGTGAACAAG
TCTCTGAACGCCGCCCTGCTGAGAATTTCAACCCTGGTGGCGCTCAGCCCTCTGGCCAGCTGGGCGAGGACCCTCTGGCCATCCCCAACAACGTGCTGCCCTACATTGCTCA
GGTGGCTGTGGCCCGACTGGACAGACTGAAGGTGTTTGGCGACGACTACCCTACCTTCTGACGGCACCCCCATCCGAGACTACATCCACGTGTGTGACCTGGCCGAGGCCCACG
TGGCCGCTCTGGACTACCTGCGCAGAGCACTTCGTGTCTTGTCGACCCTGGAACTGAATCTGGGCACCGGCTCTACCGTGTTCCAGGTGCTGCAAGCCCTTCTCTAAGGCTGTC
GCCCCAGATCTGCCCTACAAGGTGACCCCCCGACGAGCCGGCGACGTCGTGAACCTGACCGCCAACCCTACCCGAGCTAACGAGGAGCTGAAGTGGAAGACCTCTCGATCTAT
CTACCAGATCTGTGTGGACACCTGGAGATGGCAGCAGAAGTACCCCTACGGCTTCGACCTGACCCATACCAAGACCTACAAGGCCTCTGGCCGCGGACGAGATCTGTCTCGAC
TGCCTCAGCTGGTCGGCGTGTCTACCCCTCTGCAGGGCGGCTCTAACTCTGCCGCCGCTATCGGCCAGTCCTCCGGCGAGCTGCGAACCGGTGGAGCCCGACCTCCCCCTCCC
CTGGGCGCCTCTTCTCAGCCCCGACCCGGTGGCGACTCTTCTCCCGTGGTGGACTCTGGCCCCGGACCCGCCTCTAACCTGACCTCTGTGCCCGTGCCCCACACCACCGCCCT
GTCTCTGCCCGCCTGTCCCGAGGAGTCTCCCCTGCTCGTCGGCCCCATGCTGATCGAGTTCAACATGCCCGTGGACCTGGAGCTGGTCGCCAAGCAGAACCCCAACGTGAAGA
TGGGCGGACGATACGCTCCCCGAGACTGTGTGTCTCCCCACAAGGTGGCCATCATCATCCCTTTCAGAAACCGACAGGAGCACCTGAAGTACTGGCTGTACTACCTGCACCCC

GTGCTGCAGCGACAGCAGCTGGACTACGGCATCTACGTGATCAACCAGGCCGGCGACACCATCTTCAACCGAGCCAAGCTGCTGAACGTGGGCTTCCAGGAGGCCCTGAAGGA

CTACGACTACACCTGTTTCGTGTTCTCCGACGTGGACCTGAACGACCACAACGCCTACCGATGTTTCTCCCAGCCCCGACACATCTCTGTGGCCATGGACAAGT

TCGGCTTCTCTCTGCCCTACGTGCAGTACTTCGGCGGCGTTTCTTGCCCTGTCTAAGCAGCAGTTCCTGACCATCAACGGCTTCCCCAACTACTGGGGCTGGGGCGGAGAG

GACGACGACATTTTCAACCGACTGGGTGTTCCGAGGCATGTCTATCTCTCGACCCAACGCCGTGGTGGGCCCGATGTCGAATGATCCGACACTCTCGAGACAAGAAGAACGAGCCC

CAACCCCCAGCGATTTGACCGAATTGCTCACACTAAGGAAACCATGCCTGTCTGACCTACCAGGTGCTGGACGTGCAGCGATACCCCCCTGTACACCC

AGATCACCGTGGACATCGGCACACCCTCTTAG (SEQ ID NO:10)

FIGURE 14

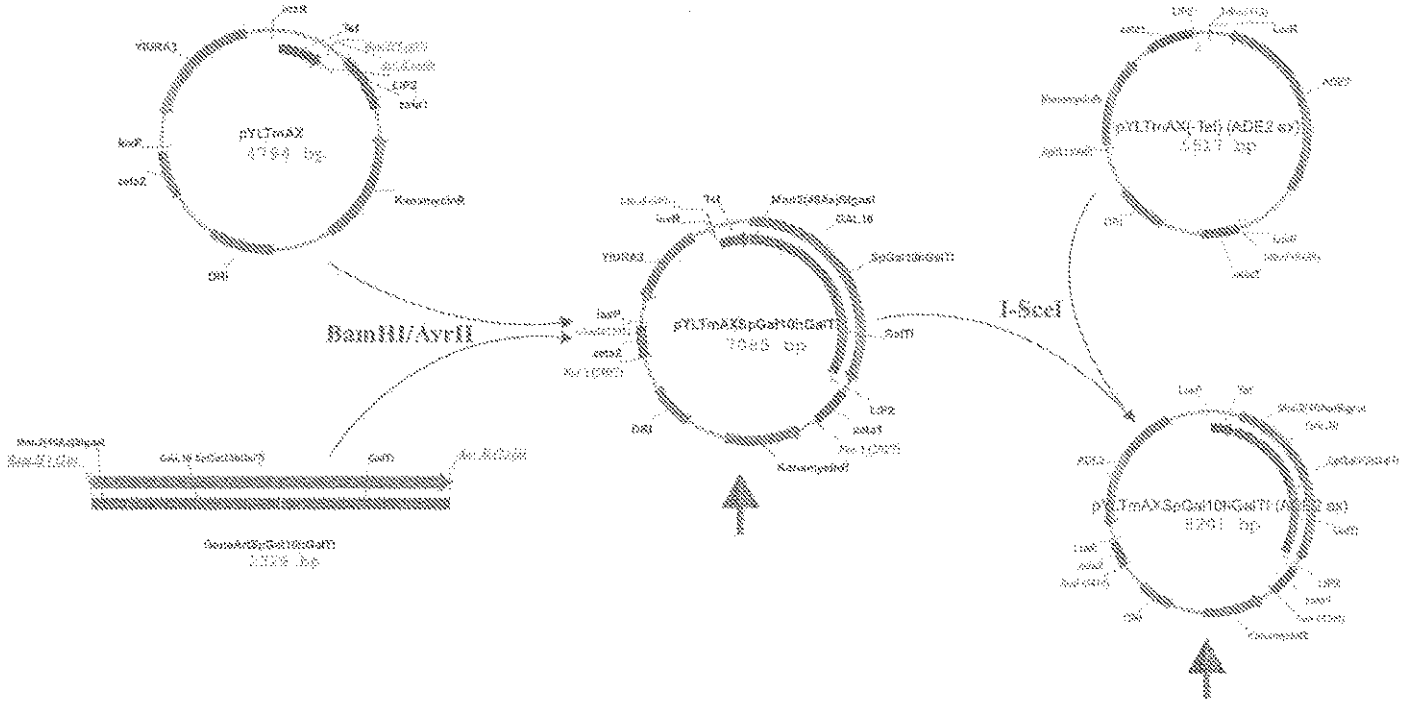


FIGURE 15

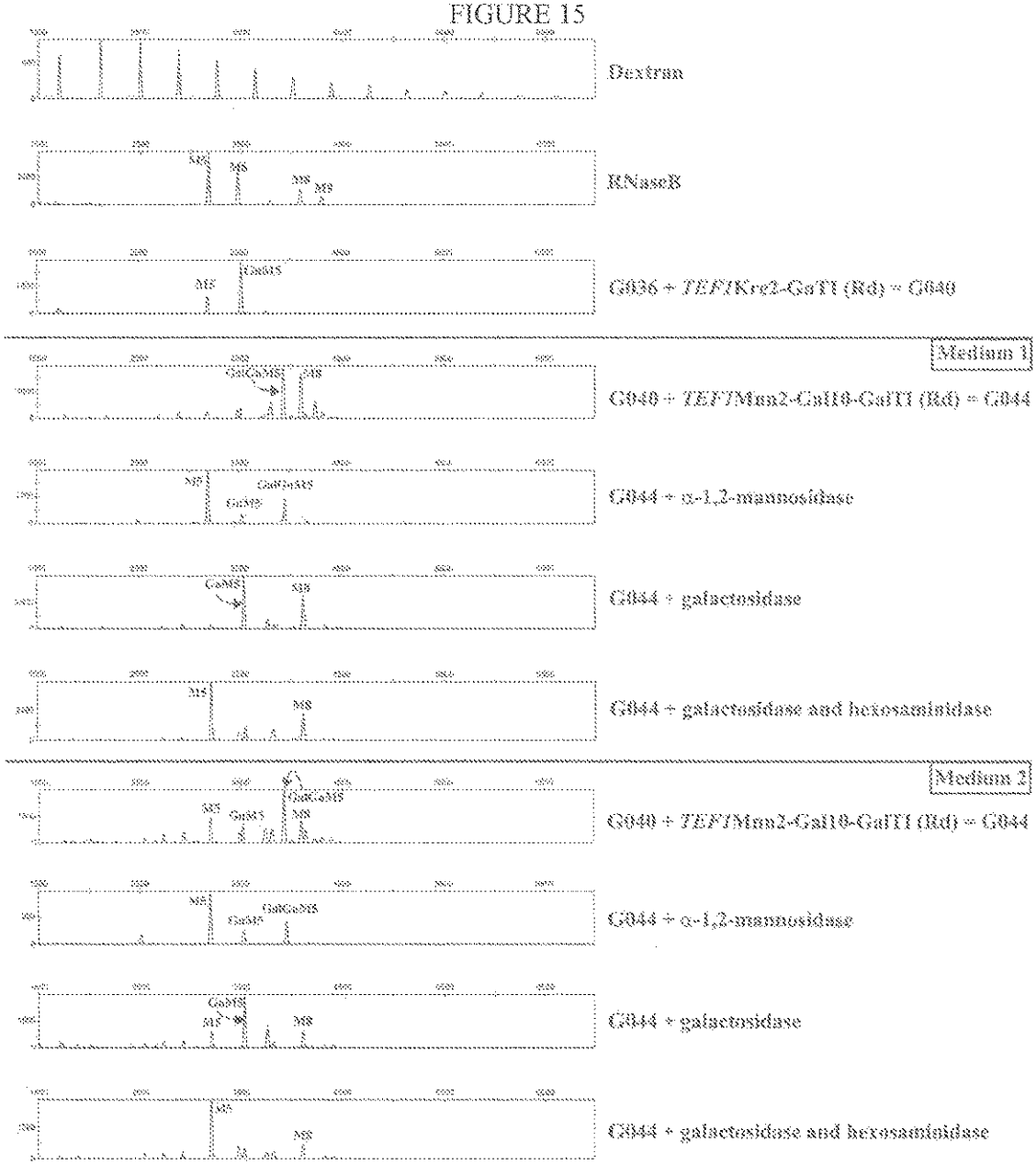

Dextran
RNaseB
G036 + TEF1Kre2-GaTI (Rd) = G040
Medium 1
G040 + TEF1Mnn2-Gal10-GaTI (Rd) = G044
G044 + α-1,2-mannosidase
G044 + galactosidase
G044 + galactosidase and hexosaminidase
Medium 2
G040 + TEF1Mnn2-Gal10-GaTI (Rd) = G044
G044 + α-1,2-mannosidase
G044 + galactosidase
G044 + galactosidase and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

FIGURE 16

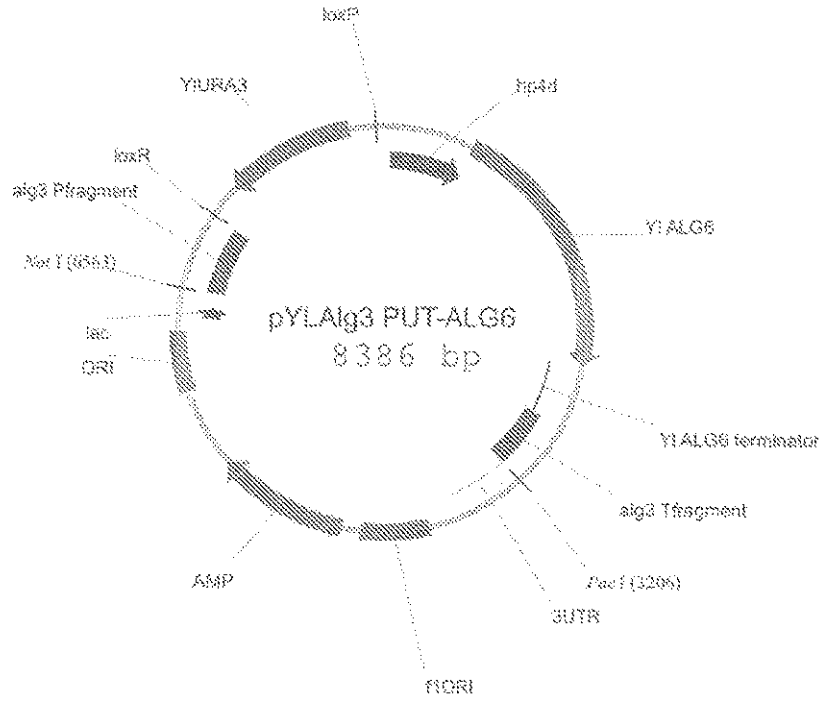

loxP
YlURA3
hp4d
loxR
alg3 Pfragment
Yl ALG6
Xba I (6561)
pYLAlg3 PUT-ALG6
8386 bp
lac
ORI
Yl ALG6 terminator
alg3 Tfragment
AMP
Pac I (320)
3UTR
f1ORI

FIGURE 17

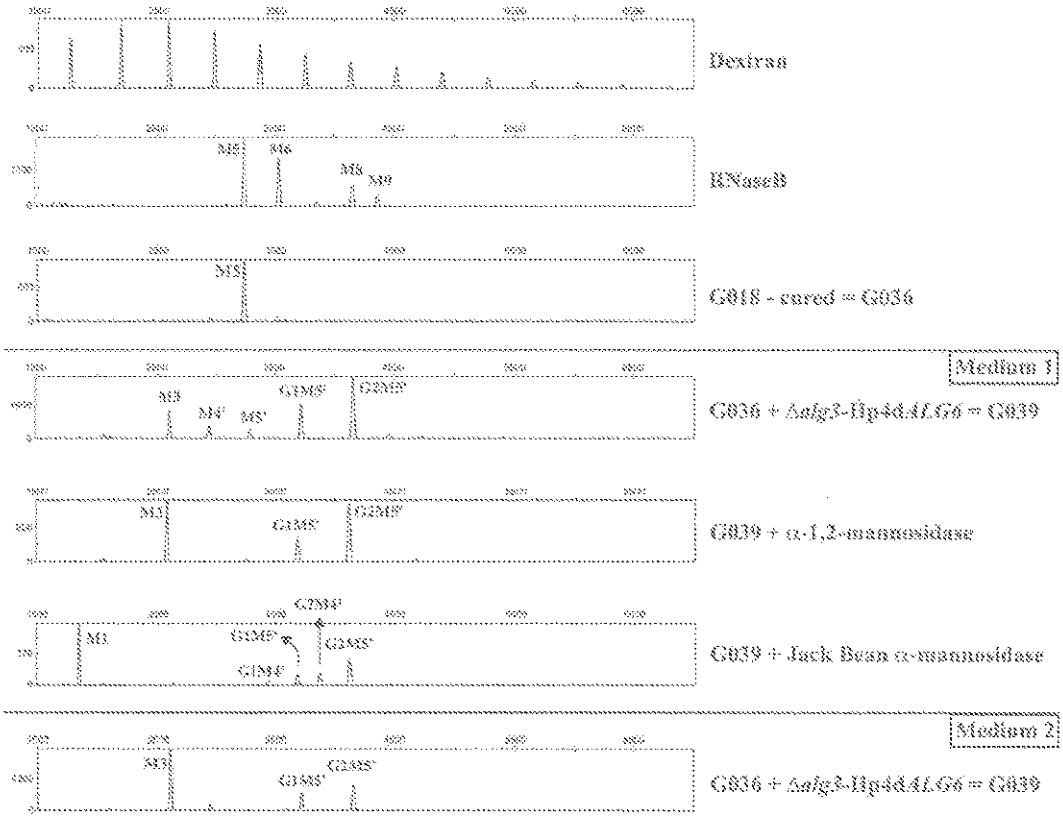

Dextran
RNaseB
G818 - cured = G036
Medium 1
G036 + Δalg3-Hp4d4LG6 = G039
G039 + α-1,2-mannosidase
G039 + Jack Bean α-mannosidase
Medium 2
G036 + Δalg3-Hp4d4LG6 = G039

FIGURE 18

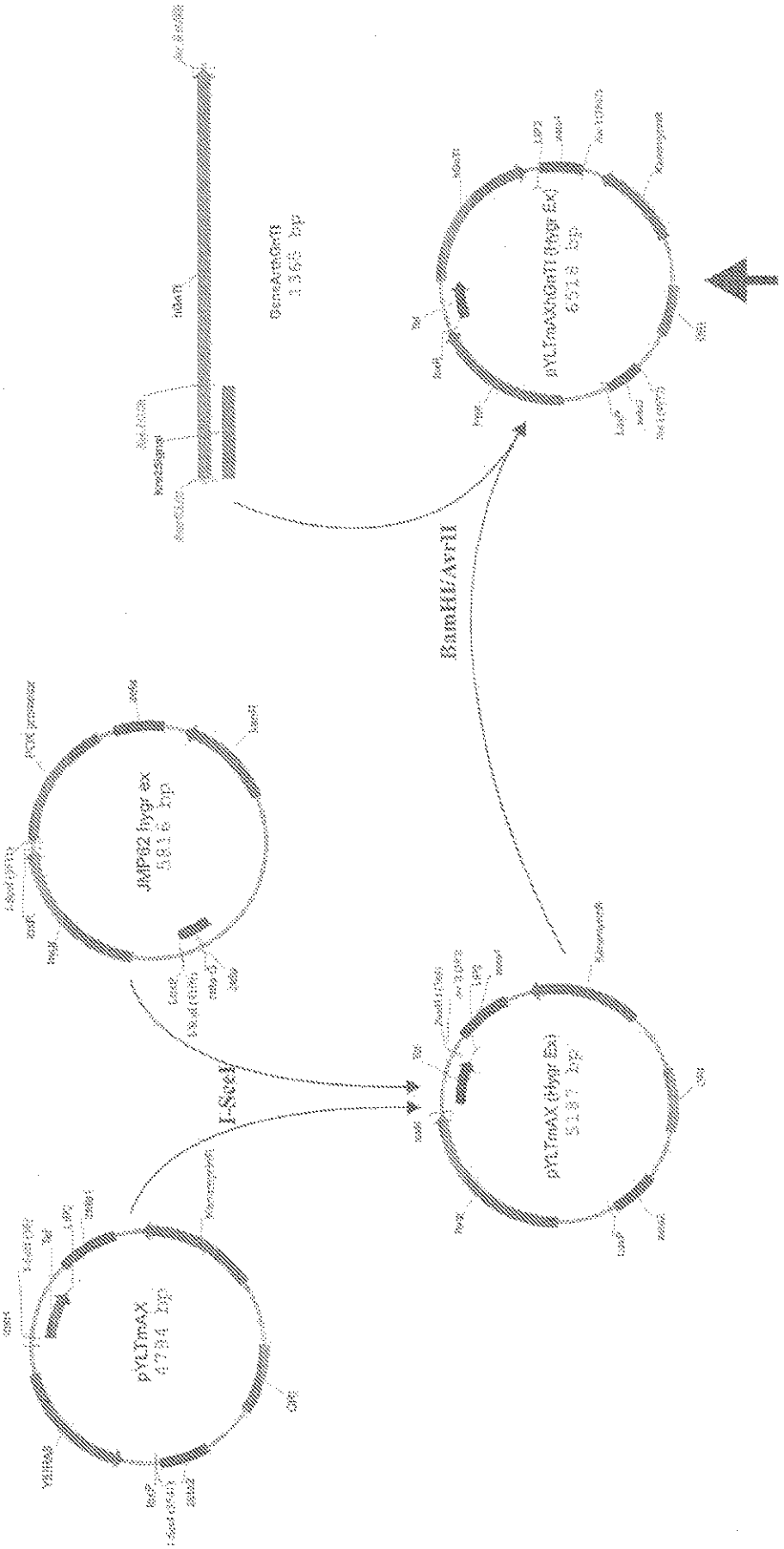


FIGURE 19

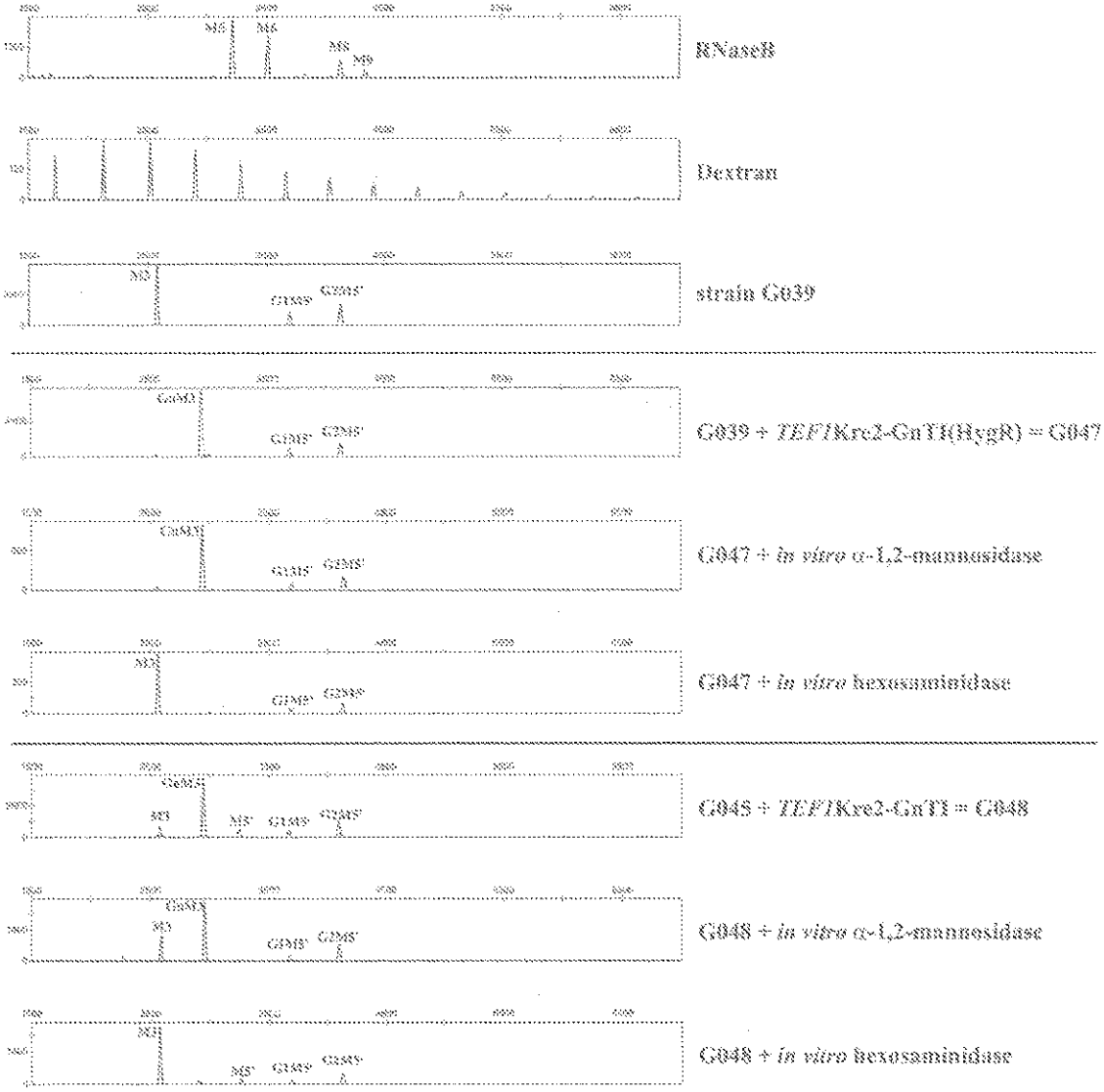

RNaseB
Dextran
strain G039
G039 + TEF1Kre2-GnTI(HygR) = G047
G047 + in vitro α-1,2-mannosidase
G047 + in vitro hexosaminidase
G048 + TEF1Kre2-GnTI = G048
G048 + in vitro α-1,2-mannosidase
G048 + in vitro hexosaminidase

FIGURE 20

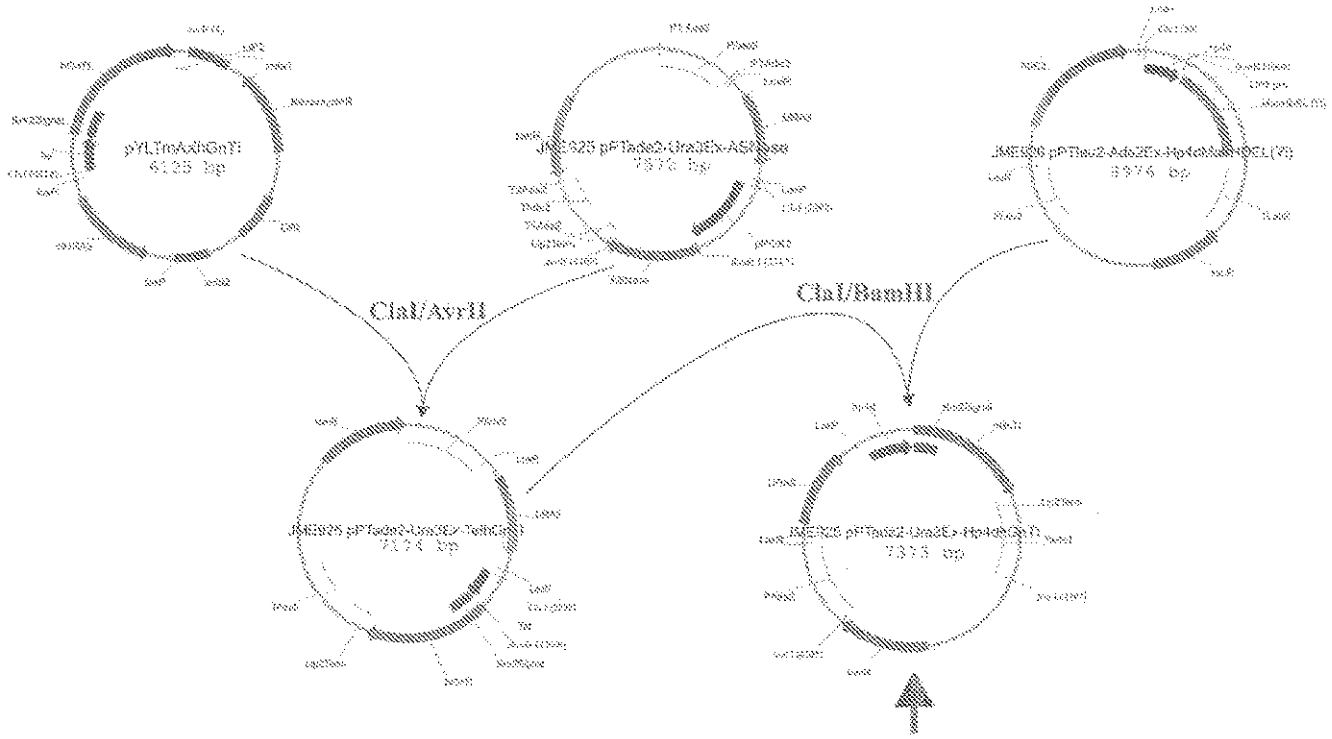


FIGURE 21

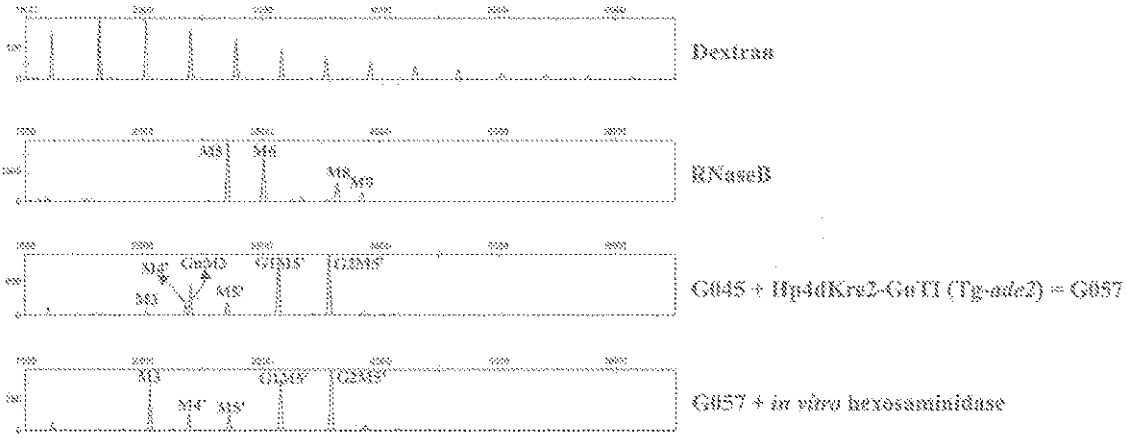

Dextran
RNaseB
G045 + Hp4dKre2-GnTI (Tg-ade2) = G057
G057 + in vitro hexosaminidase

FIGURE 22

MLLTKRFSKLFKLTFIVLLLCGLFVITNKYMDENTSSLVYQLNFDQMLRNVDKDGTWSPG
ELVLVVQVHNRPEYLRLLIDSLRKAQGIREVLVIFSHDEWSAEINSLISSVDFCPVLQVF
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

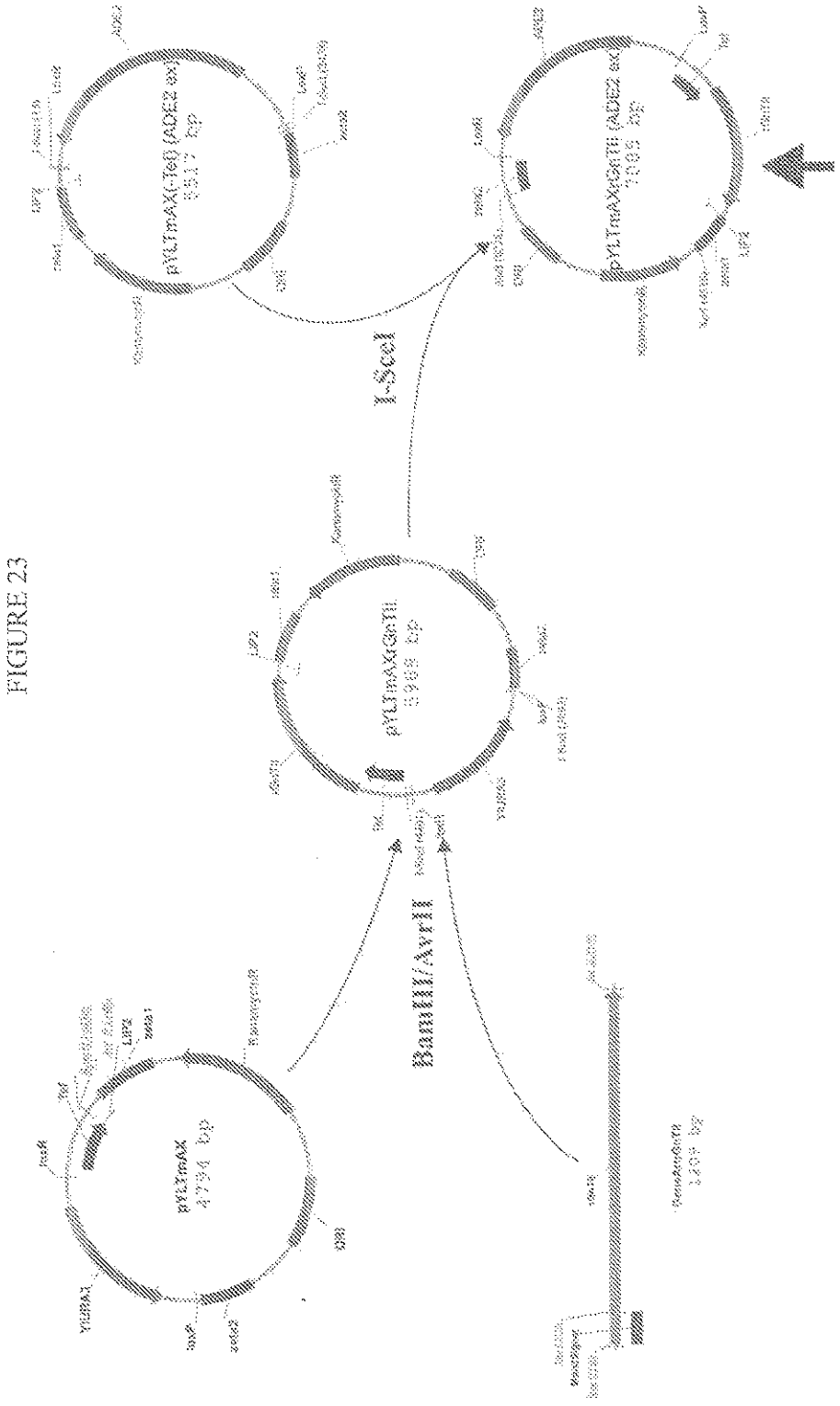


FIGURE 24

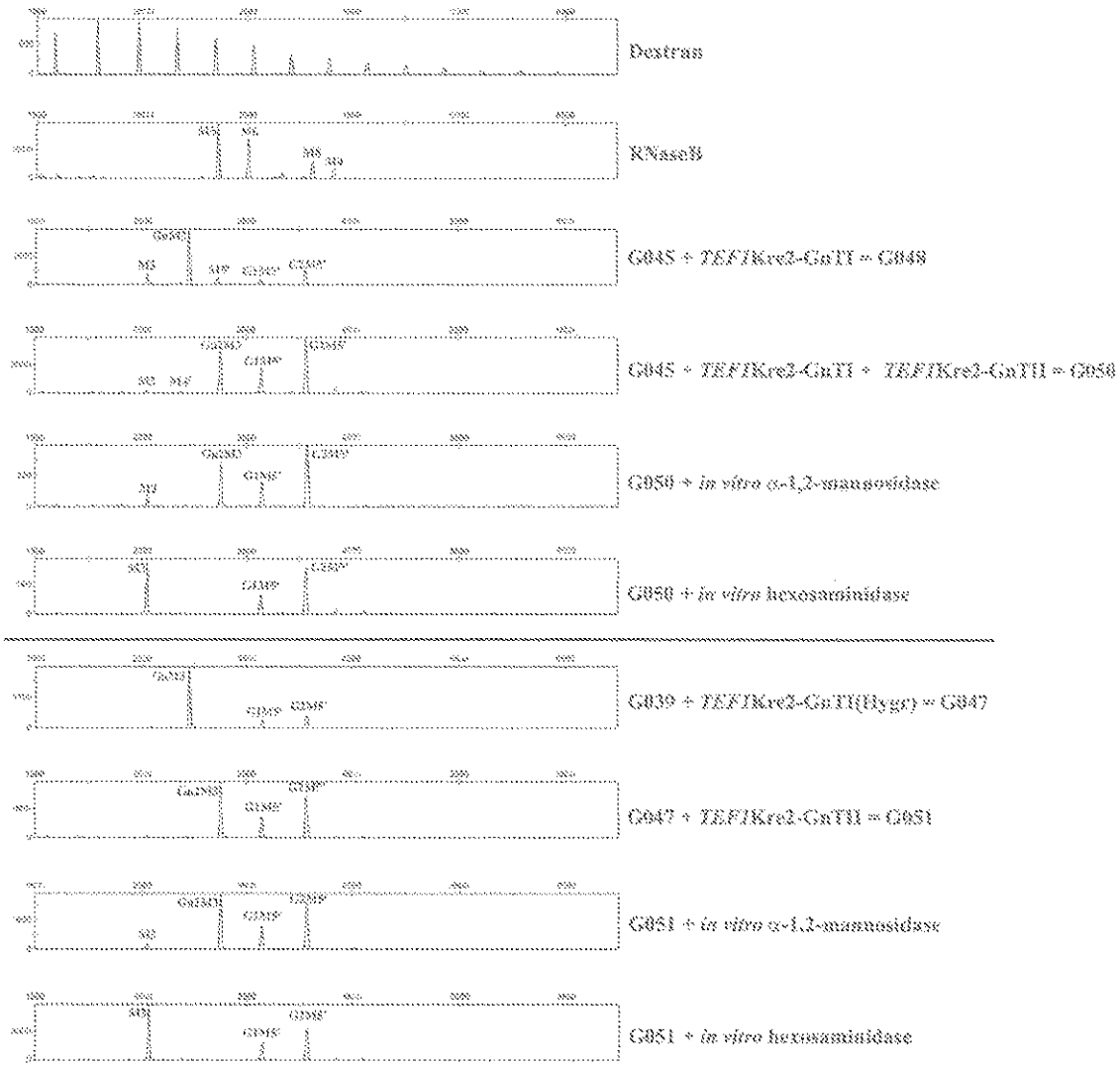

Dextran
RNaseB
G045 + TEF1Kre2-GnTI = G048
G045 + TEF1Kre2-GnTI + TEF1Kre2-GnTII = G058
G050 + in vitro α-1,2-mannosidase
G050 + in vitro hexosaminidase
G039 + TEF1Kre2-GnTI(Hygr) = G047
G047 + TEF1Kre2-GnTII = G051
G051 + in vitro α-1,2-mannosidase
G051 + in vitro hexosaminidase

FIGURE 25

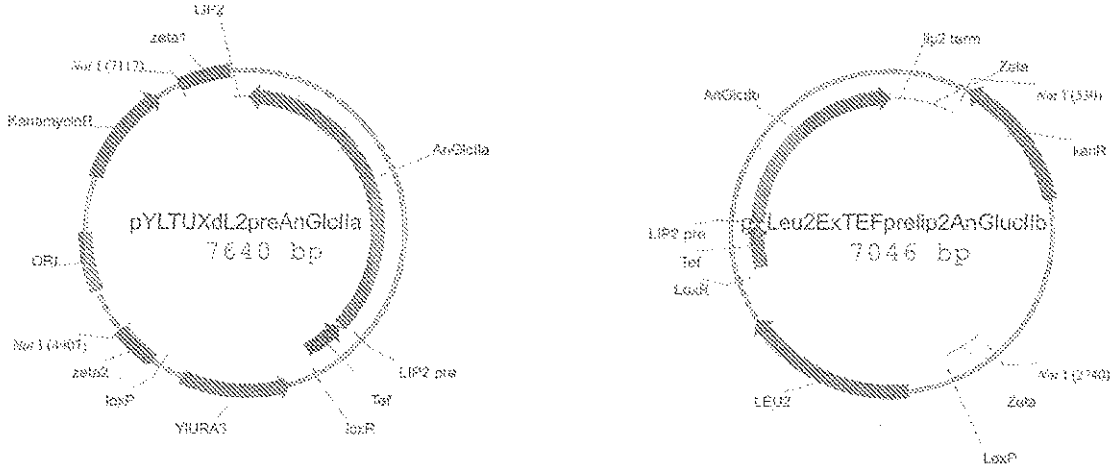

pYLTUXdL2preAnGlcIIa
7640 bp
LIP2
zeta1
Kanamycin R
AnGlcIIa
ORI
zeta2
loxP
YRURA3
loxR
TEF
LIP2 pre
pLeu2ExTEFprelip2AnGlucIIb
7046 bp
lip2 term
Zeta
AnGlcIIb
kanR
LIP2 pre
TEF
LoxP
LEU2
Zeta
LoxP

FIGURE 26

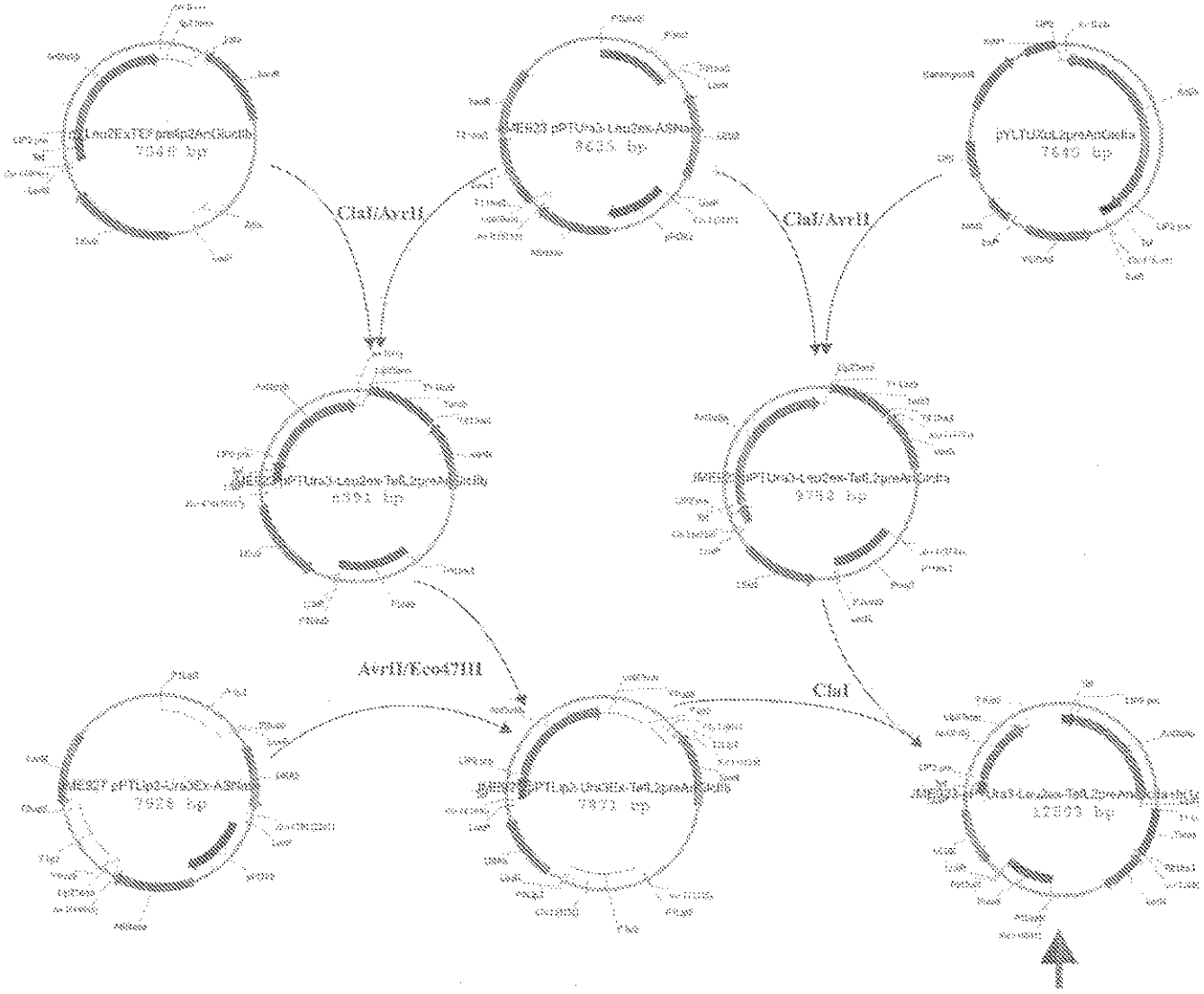


FIGURE 27

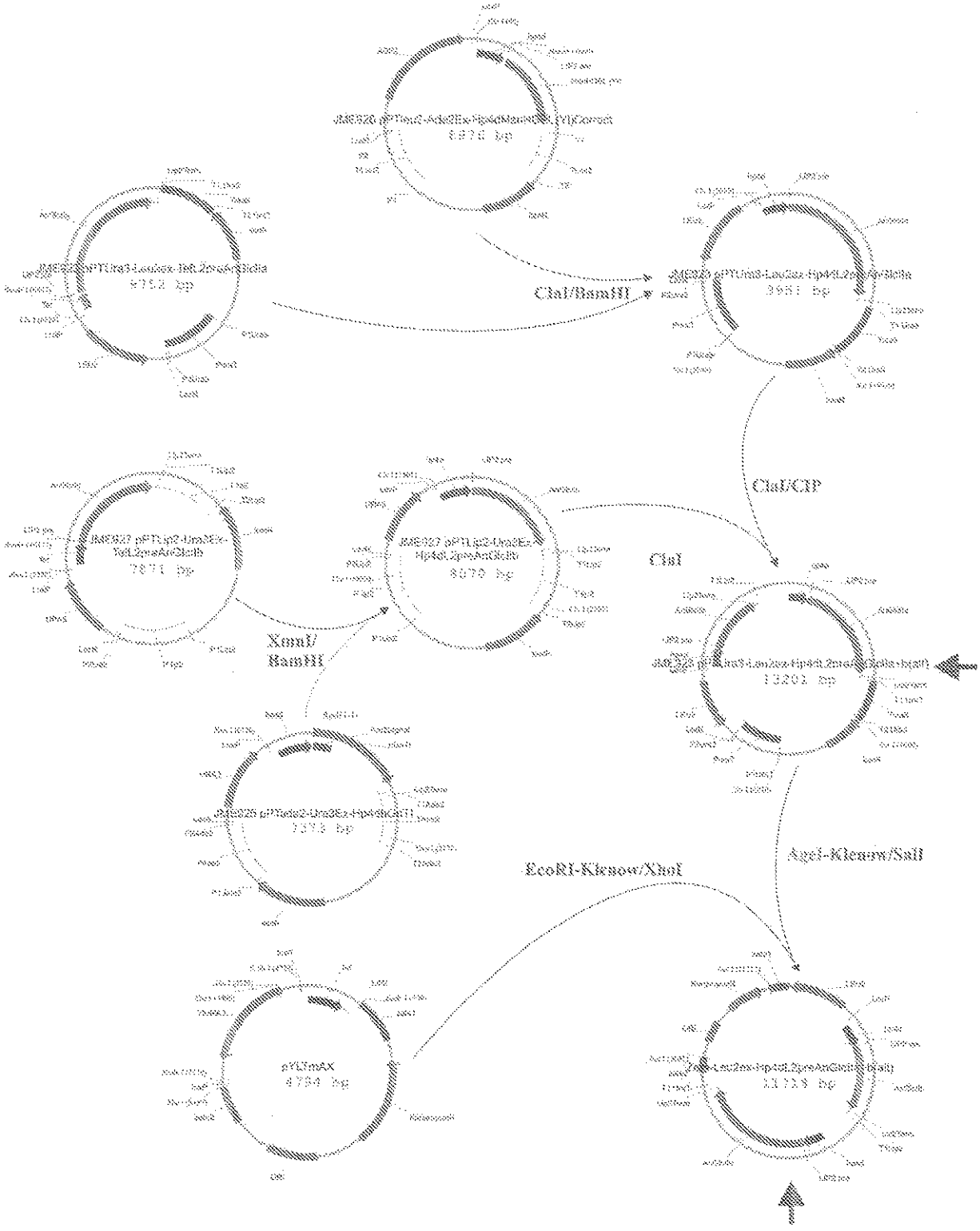
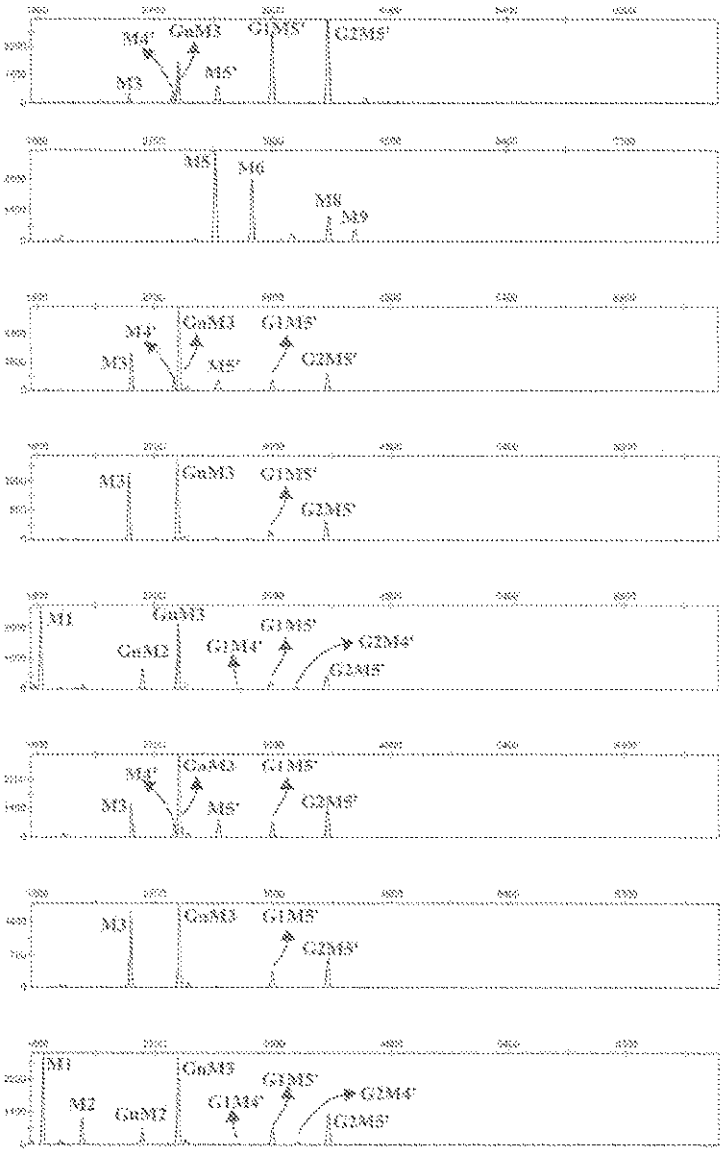

ClaI/BamHI
ClaI/CIP
ClaI
XmnI/
BamHI
EcoRI-Klenow/XhoI
AgeI-Klenow/SalI

FIGURE 28



G045 + Bp4dKre2-GnTI (*Tg-ade2*) = G057

RNaseB

G057 + Bp4dGls2α/β (*Tg-ura3*) = G061

G061 + α-1,2-mannosidase

G061 + Jack Bean α-mannosidase

G057 + Bp4dGls2α/β (Rd) = G060

G060 + α-1,2-mannosidase

G060 + Jack Bean α-mannosidase

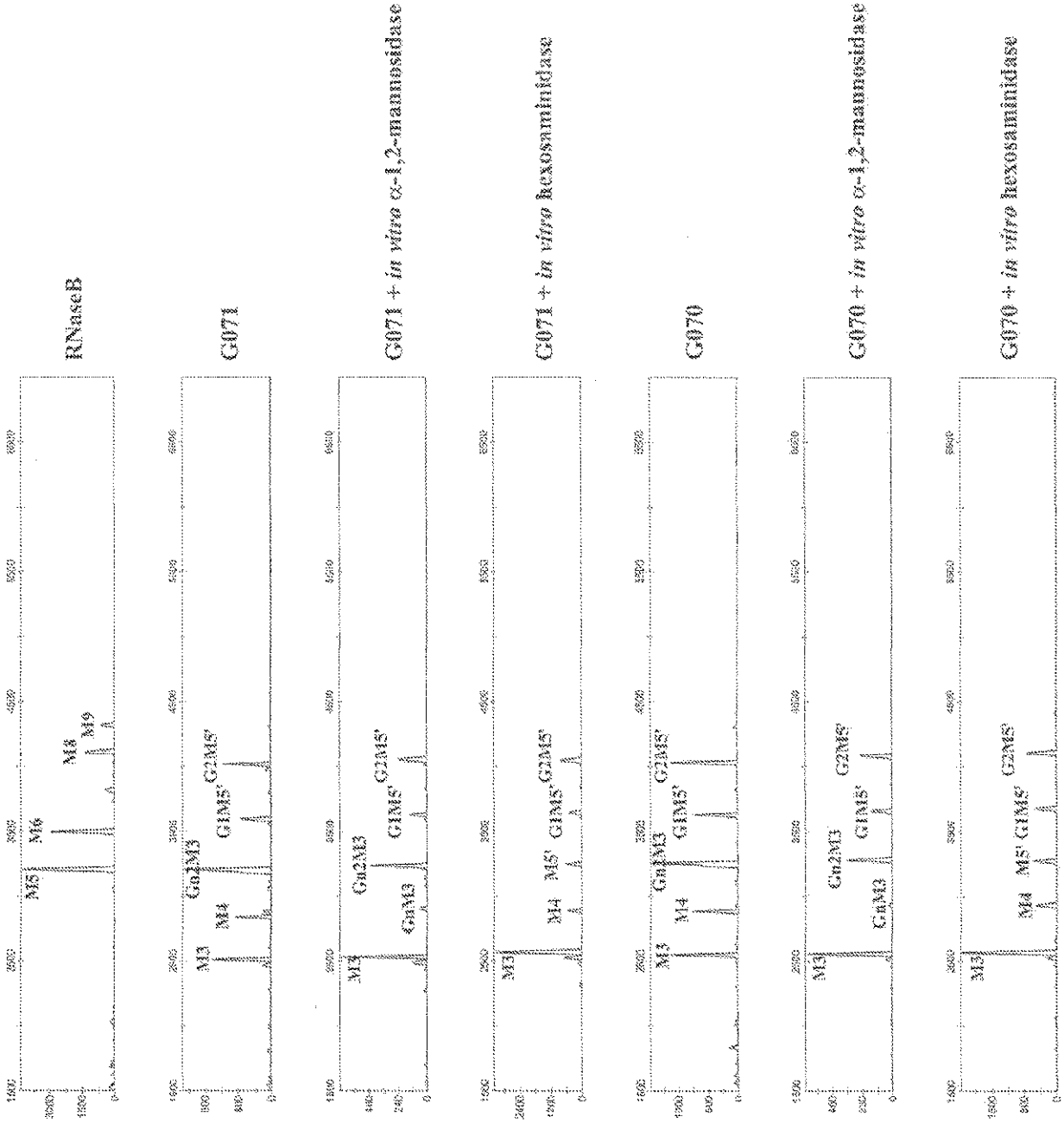

RNaseB
M5
M6
M8
M9
G071
M3
M4
Gn1M3
G1M5'
G2M5'
G071 + in vitro α-1,2-mannosidase
M3
Gn2M3
GnM3
G1M5'
G2M5'
G071 + in vitro hexosaminidase
M3
M4
M5'
G1M5'
G2M5'
G070
M3
M4
Gn1M3
G1M5'
G2M5'
G070 + in vitro α-1,2-mannosidase
M3
Gn2M3
GnM3
G1M5'
G2M5'
G070 + in vitro hexosaminidase
M3
M4
M5'
G1M5'
G2M5'

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

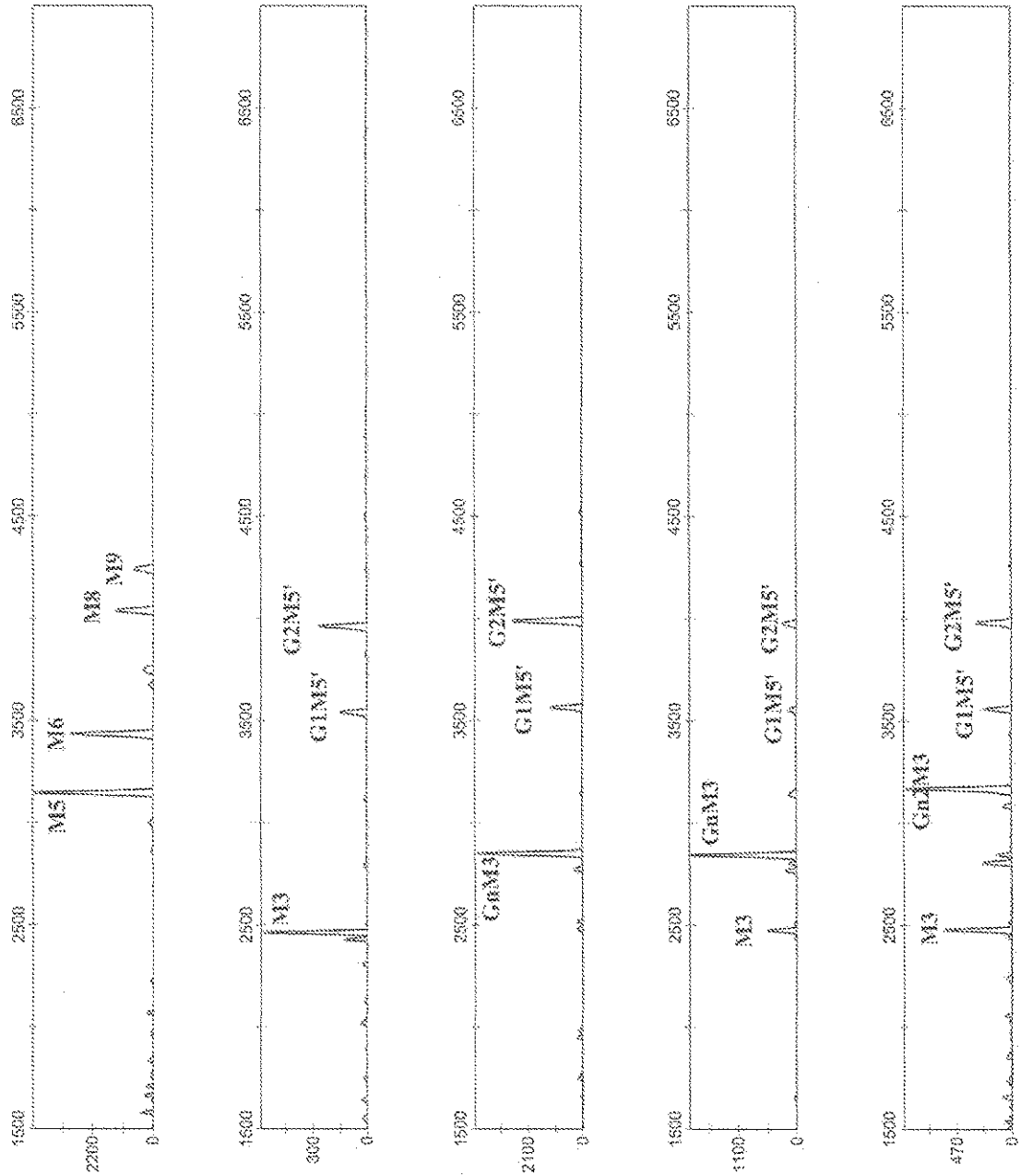

RNaseB
M5
M6
M8
M9
Man3-platform (G045)
M3
G1M5'
G2M5'
G045 + Hp4dKre2-GnTI (Tg) = G057
G0M3
G1M5'
G2M5'
G057 + Hp4dAnGls2(α+β) (Tg) = G061
G0M3
GnM3
G1M5'
G2M5'
G061 + Hp4dMan2-GnTIII (Tg) = G071

FIGURE 32

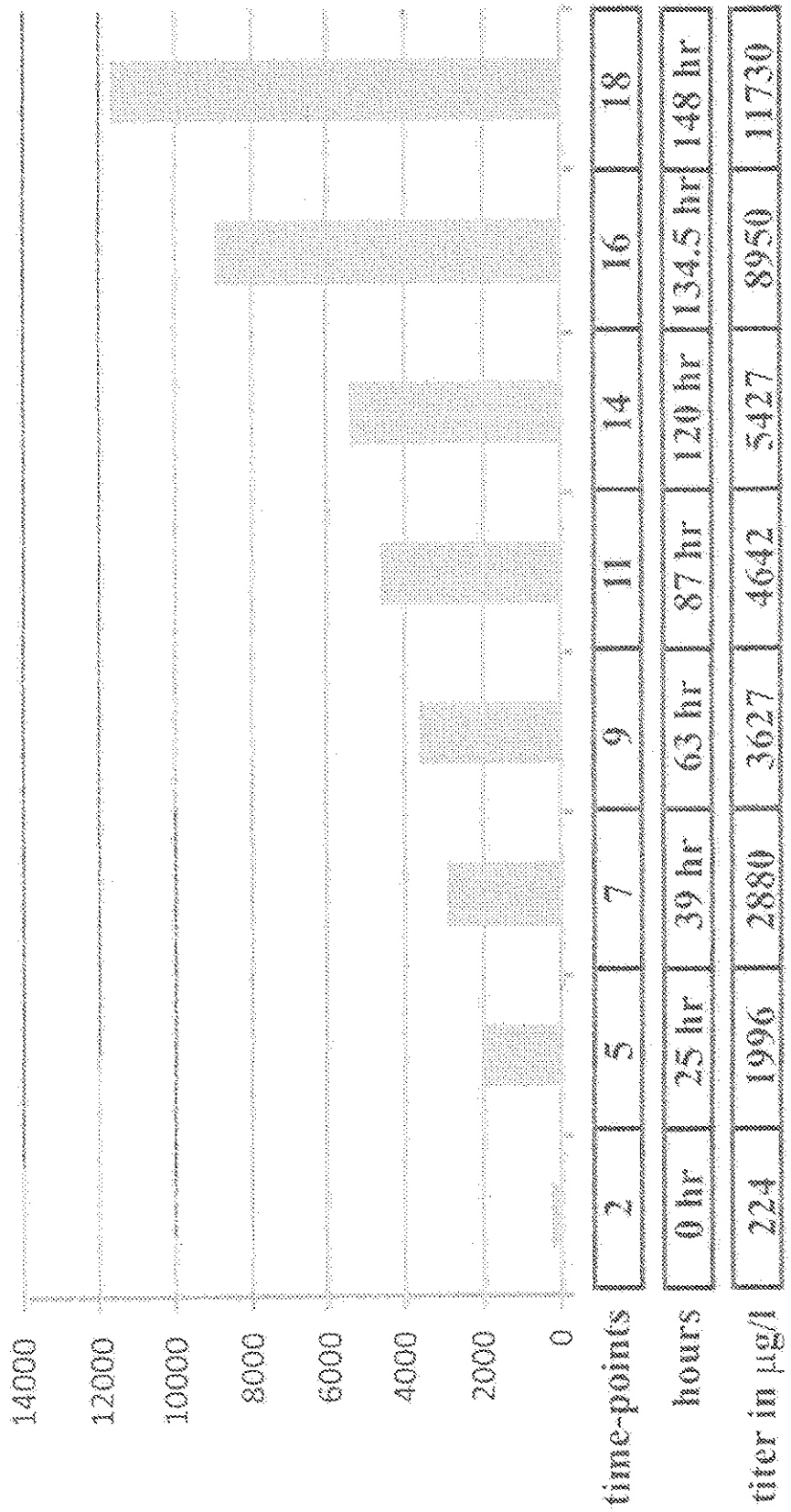

(ug/l active herceptin)
14000
12000
10000
8000
6000
4000
2000
0
time-points
2
5
7
9
11
14
16
18
hours
0 hr
25 hr
39 hr
63 hr
87 hr
120 hr
134.5 hr
148 hr
titer in µg/l
224
1996
2880
3627
4642
5427
8950
11730

FIGURE 33

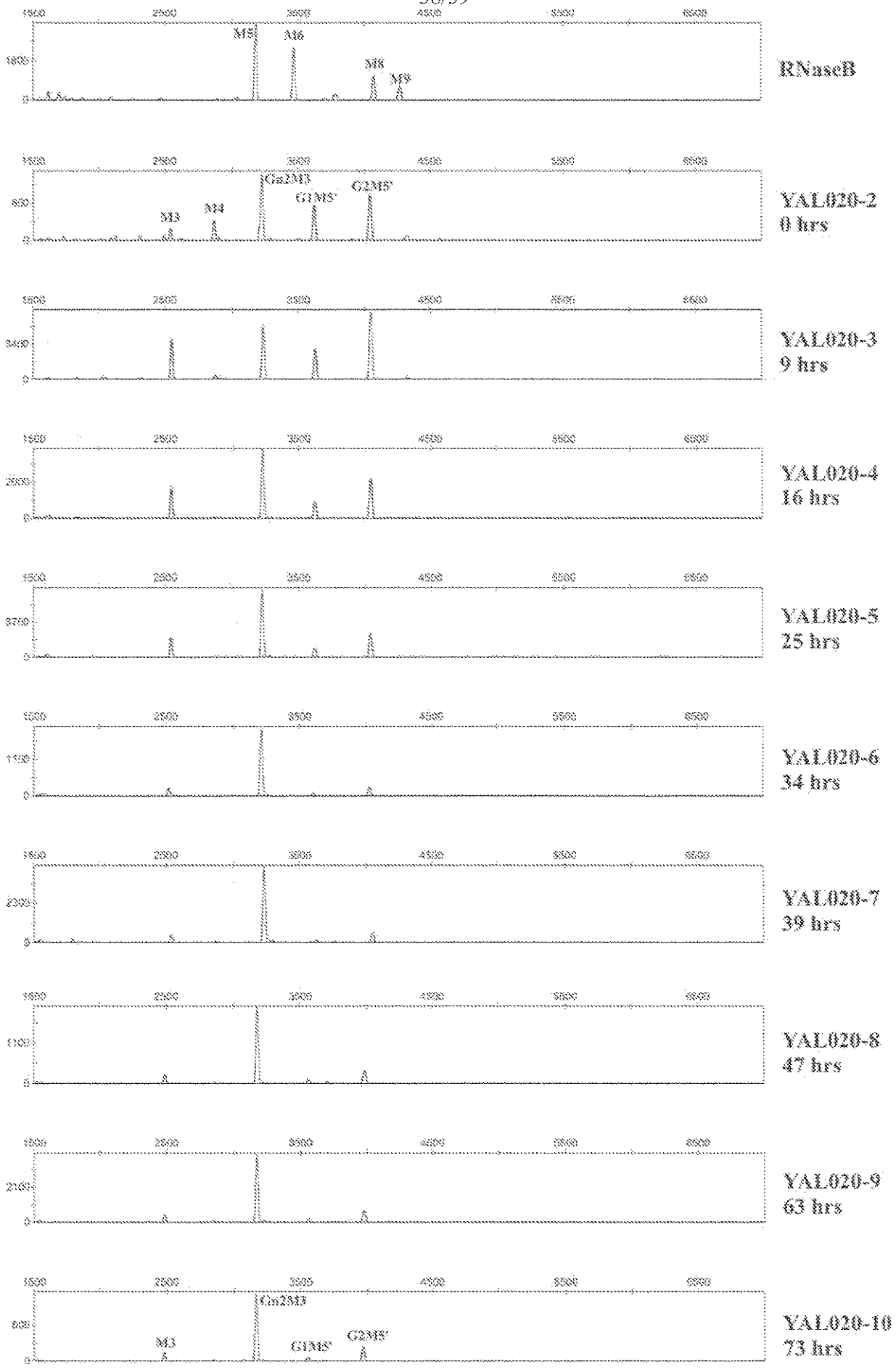

M5
M6
M8
M9
RNaseB
M3
M4
Ga2M3
G1M3'
G2M3'
YAL020-2
0 hrs
YAL020-3
9 hrs
YAL020-4
16 hrs
YAL020-5
25 hrs
YAL020-6
34 hrs
YAL020-7
39 hrs
YAL020-8
47 hrs
YAL020-9
63 hrs
Gn2M3
YAL020-10
73 hrs

FIGURE 34A

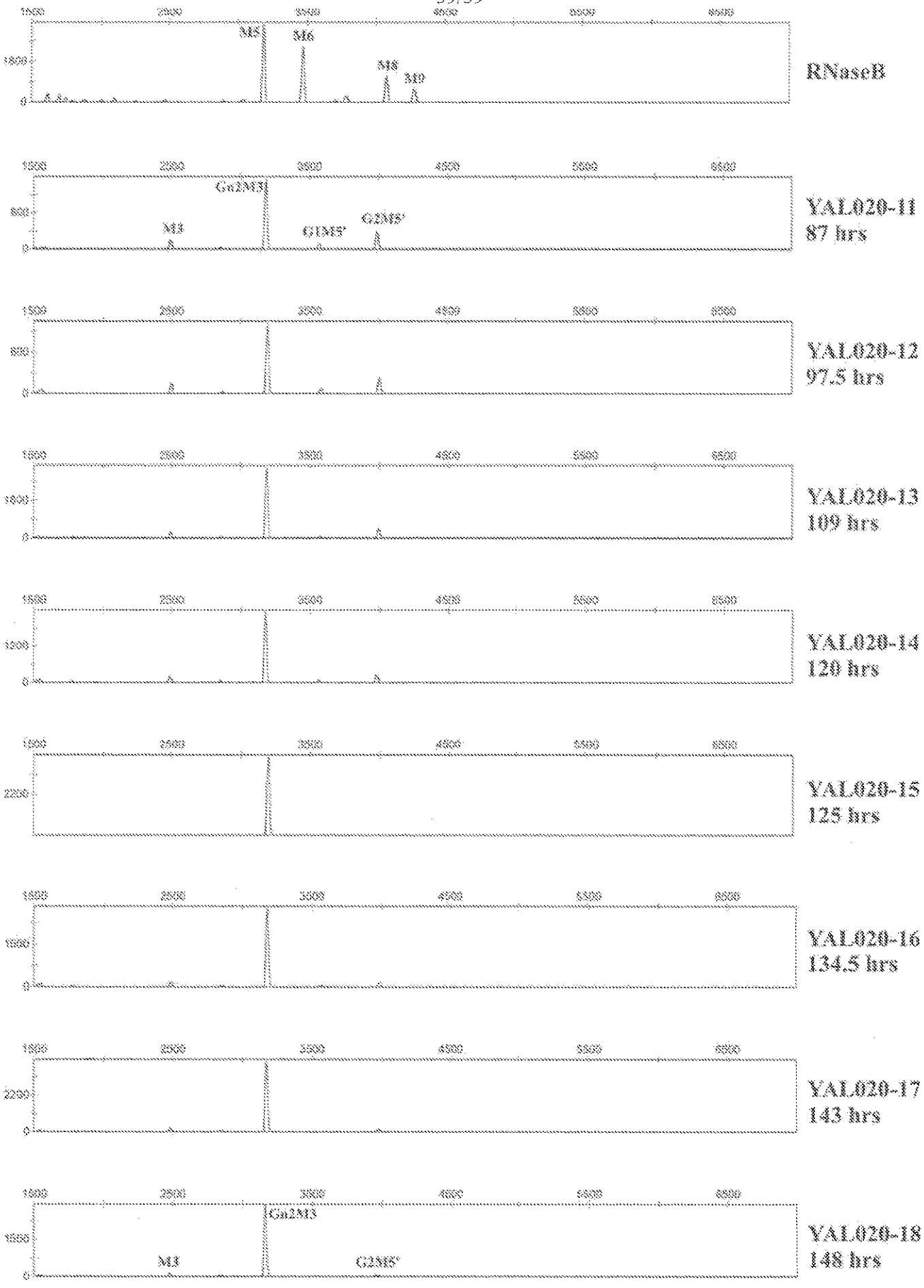

M5
M6
M8
M9
RNaseB
Gn2M3
M3
G1M5'
G2M5'
YAL020-11
87 hrs
YAL020-12
97.5 hrs
YAL020-13
109 hrs
YAL020-14
120 hrs
YAL020-15
125 hrs
YAL020-16
134.5 hrs
YAL020-17
143 hrs
Gn2M3
M3
G2M5'
YAL020-18
148 hrs

FIGURE 34B