A role in 15-deacetylcalonectrin acetylation in the non-enzymatic cyclization of an earlier bicyclic intermediate in *Fusarium* trichothecene biosynthesis

Yoshiaki Koizumi[1], Yuichi Nakajima[2], Yuya Tanaka[2], Kosuke Matsui[2], Masato Sakabe[3], Kazuyuki Maeda[2], Masayuki Sato[4], Hiroyuki Koshino[5], Soichi Sato[1,3], Makoto Kimura[2,4], Naoko Takahashi-Ando[1,3,4]

[1]Graduate School of Science and Engineering, Toyo University, 2100 Kujirai, Kawagoe, Saitama 350-8585, Japan

[2]Graduate School of Bioagricultural Sciences, Nagoya University, Furo-cho, Chikusa-ku, Nagoya, Aichi 464-8601, Japan

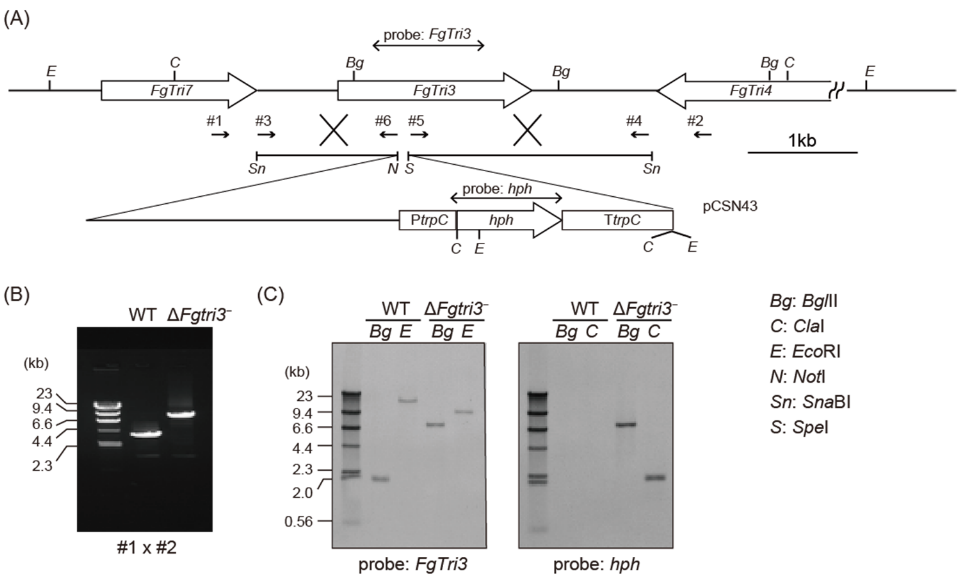
[3]Faculty of Science and Engineering, Toyo University, 2100 Kujirai, Kawagoe, Saitama 350-8585, Japan

[4]Plant & Microbial Engineering Research Unit, Discovery Research Institute (DRI) RIKEN, 2-1, Hirosawa, Wako, Saitama 351-0198, Japan

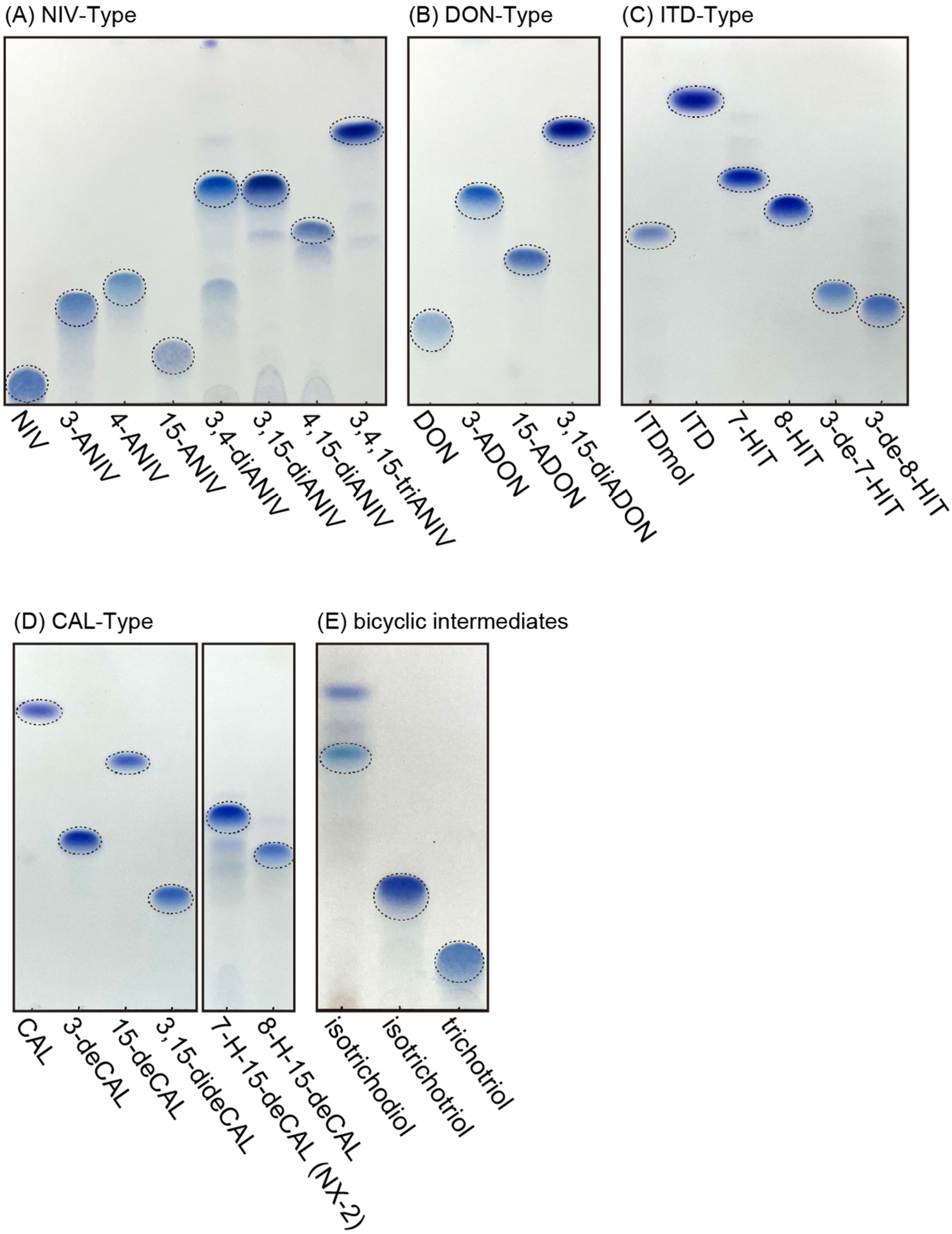
[5]Molecular Structure Characterization Unit, Technology Platform Division, Center for Sustainable Resource Science (CSRS) RIKEN, 2-1, Hirosawa, Wako, Saitama 351-0198, Japan

Table S1. Primers used for generation of the Δ*Fgtri3* (FGD3) and Δ*Fgtri5*Δ*Fgtri11* (FGD5/11) mutants

No.	Primer name	Primer sequence	Comments
#1	MFTri3-LAU	5′-TATCTTTACCACAACAGCCGATTGCCCC-3′	Primer for screening of the *FgTri3* gene disruption mutant (used in combination with #2)
#2	MFTri3-LAD	5′-GCACTCTCTGATTGGAGGAGAACACTTG-3′	Primer for screening of the *FgTri3* gene disruption mutant (used in combination with #1)
#3	MFTri3U-Sna	5′-GTACGTAGTCATTGTCTCCATTATAACAAGGC-3′	Inward primer containing a *Sna*BI site (underlined) used for the construction of the *FgTri3* gene disruption vector (used in combination with #4)
#4	MFTri3D-Sna	5′-TTTACGTATGCAGGAATGGAAGTTCATTGTAG-3′	Inward primer containing a *Sna*BI site (underlined) used for the construction of the *FgTri3* gene disruption vector (used in combination with #3)
#5	MFTri3U-inv/Spe	5′-AACTAGTATGGGGCCAAGAGATCAAGAACCTG-3′	Outward primer containing a *Spe*I site (underlined) used for the construction of the *FgTri3* gene disruption vector (used in combination with #6)
#6	MFTri3D-inv/Not	5′-AGCGGCCGCTTGAGGGATTGGCGTCGAGTCTG-3′	Outward primer containing a *Not*I site (underlined) used for the construction of the *FgTri3* gene disruption vector (used in combination with #5)
#7	MFTri11-LAU2	5′-TCAAGGCTCTCCCTGGGATG-3′	Primer for PCR confirmation of *FgTri11* gene disruption (used in combination with #8)
#8	MFTri11-LAD	5′- ATGAGTCGATCAATTTAGCGGCTGCGAG-3′	Primer for PCR confirmation of *FgTri11* gene disruption (used in combination with #7)



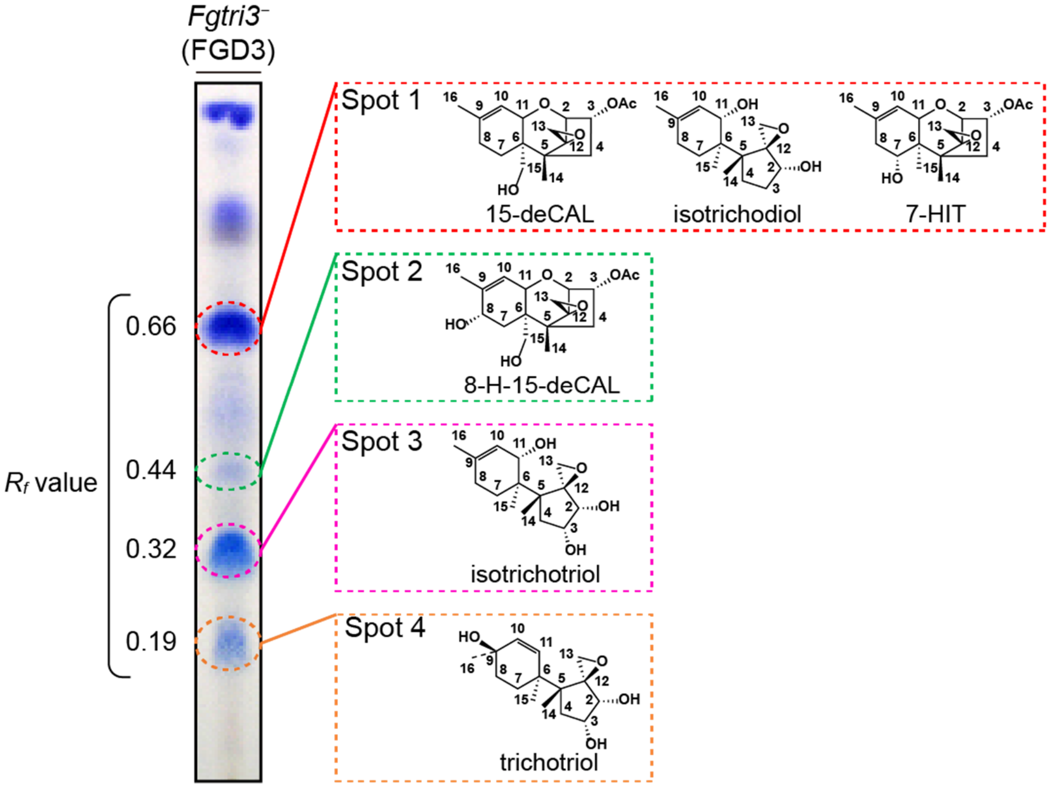
Supplementary Figure S1. Generation of a Δ*Fgtri3* mutant used in this study. (A) The gene disruption vector pΔFgTri3-hph was constructed by replacing a portion of an *FgTri3* coding region with pCSN43 [1] that contains the *hph* (hygromycin B phosphotransferase gene) cassette by the inverse polymerase chain reaction (IPCR) method [2] as follows: (1) *FgTri3* and its flanking regions were amplified by long PCR with inward primers #3 and #4 (Table S1), both of which had a *Sna*BI recognition site (Table S1); (2) the amplified product was self-ligated after digestion with *Sna*BI; (3) the ligated flanking regions were amplified by IPCR with the outward primers #5 and #6 (Table S1) that had *Spe*I and *Not*I sites, respectively (Table S1); and (4) the IPCR product was cloned between *Spe*I and *Not*I sites downstream of the *trpC* terminator. After transformation, the colonies were selected with 300 μg/ml of hygromycin B. (B) PCR analysis of genomic DNA isolated from the wild-type (WT) and Δ*Fgtri3* mutant strains. Two primers #1 and #2 that amplify the region encompassing *FgTri3* were used. An amplicon from the disruption mutant is 4.4 kb larger than the one from the WT strain. (C) Southern blot analysis of genomic DNA isolated from the WT and Δ*Fgtri3* mutant strains. The probes were synthesized with a PCR DIG probe synthesis kit [3] and detected with a DIG Luminescent Detection kit (Roche Diagnostics GmbH, Mannheim); standard hybridization, washing, and detection conditions provided by the manufacture were used for detection. Either blot showed expected banding patterns with probes that correspond to *FgTri3* and *hph*.



Supplementary Figure S2. Trichothecene standards on TLC. (A) NIV-type trichothecenes. NIV was produced by the deacetylation of 4-acetylnivalenol (4-ANIV). 4-ANIV and 4,15-diacetylnivalenol (4,15-diANIV) were produced through the incubation of MAFF 111233 WT culture. 3-Acetylnivalenol (3-ANIV) was produced through the incubation of the culture of

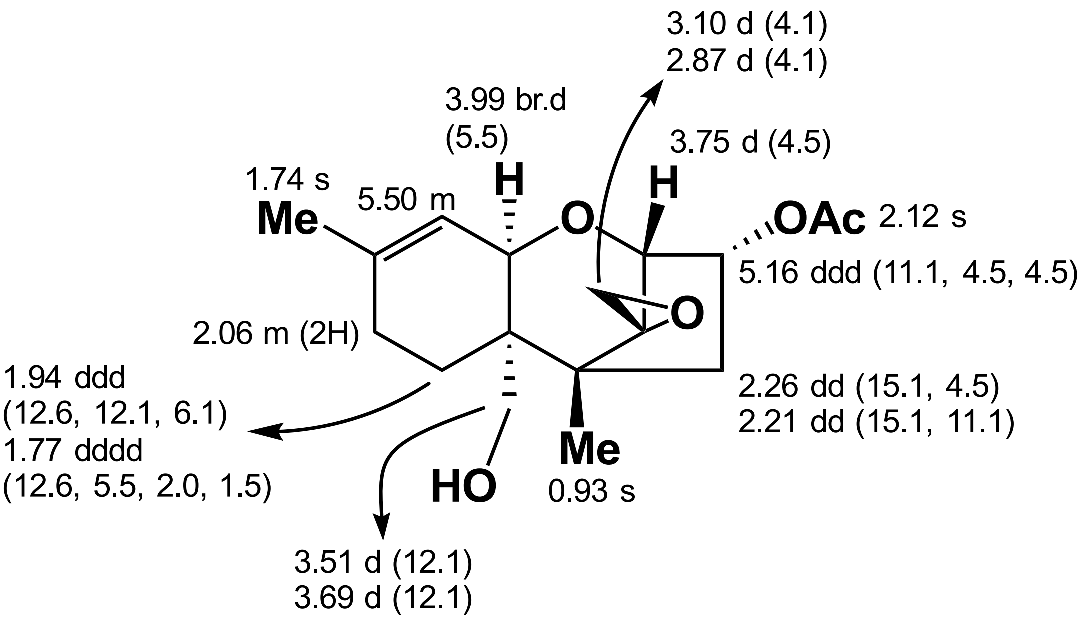
NBRC 113183, an *Fgtri13*_o/e strain of *F. graminearum* MAFF 101551 [4]. 15-Acetylnivalenol (15-ANIV) and 3,15-diacetylnivalenol (3,15-diANIV) were produced through the incubation of the culture of NBRC 113182, an *Fgtri7*$^{-}$ #1 s1* strain of *F. graminearum* MAFF 111233 [4]. 3,4,15-Triacetylnivalenol (3,4,15-triANIV) was produced through the incubation of the culture of NBRC 114123, an *FgTri8* disruptant of *F. graminearum* MAFF 111233 [5]. 3,4-Diacetylnivalenol (3,4-diANIV) was obtained by 3-*O*-acetylation of 4-ANIV by Tri101p. (B) DON-type trichothecenes. 3,15-diADON was produced through the incubation of the culture of YN_149. (C) ITD-type trichothecenes. 3-Deacetyl-7-hydroxyisotrichodermin (3-de-7-HIT) and 3-deacetyl-8-hydroxyisotrichodermin (3-de-8-HIT) were obtained through the alkaline deacetylation of 7-HIT and 8-HIT, respectively. (D) CAL-type trichothecenes. 3,15-dideCAL is the abbreviation for 3,15-dideacetylcalonectrin. 7-H-15-deCAL was obtained through the incubation of the culture of *F. graminearum* Δ*Fgtri1*/*FgTri1*$_{\text{NX chemotype}}$ [6]. Another name for 7-H-15-deCAL is NX-2. 8-H-15-deCAL was obtained through the incubation of FGD3 culture, and its structure was confirmed by NMR analysis (Supplementary Figure S3, spot 3). (E) Bicyclic trichothecene intermediates. Isotrichodiol, isotrichotriol and trichotriol were produced through the incubation of FGD3 culture, and their structures were confirmed by NMR analysis (Supplementary Figure S3, spots 1, 3, and 4).

(A)



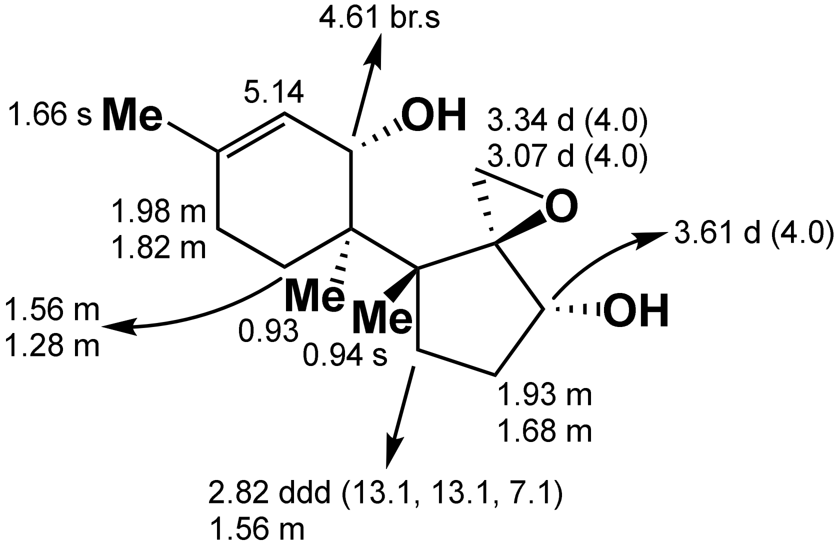
(B)

Spot 1：15-deCAL [^{1}H NMR Data of 15-deCAL (600 MHz, $CDCl_3$)]



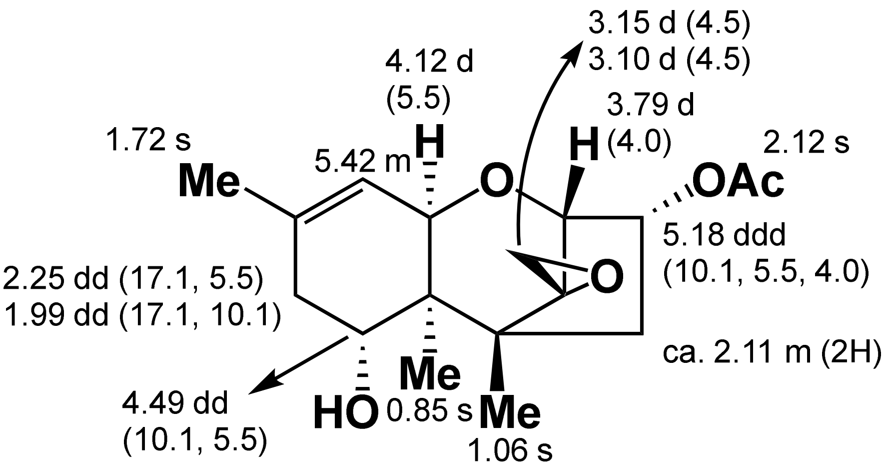
^{13}C NMR Data of 15-deCAL (151 MHz, $CDCl_3$) 78.1 (C-2), 71.4 (C-3), 39.3 (C-4), 45.3 (C-5), 44.2 (C-6), 20.8 (C-7), 28.4 (C-8), 140.5 (C-9), 119.3 (C-10), 68.3 (C-11), 65.2 (C-12), 48.6 (C-13), 12.5 (C-14), 62.8 (C-15), 23.3 (C-16), 21.0, and 170.5 (3-OAc) ppm.

Spot 1 : isotrichodiol [^{1}H NMR Data of isotrichodiol (600 MHz, $CDCl_3$)]



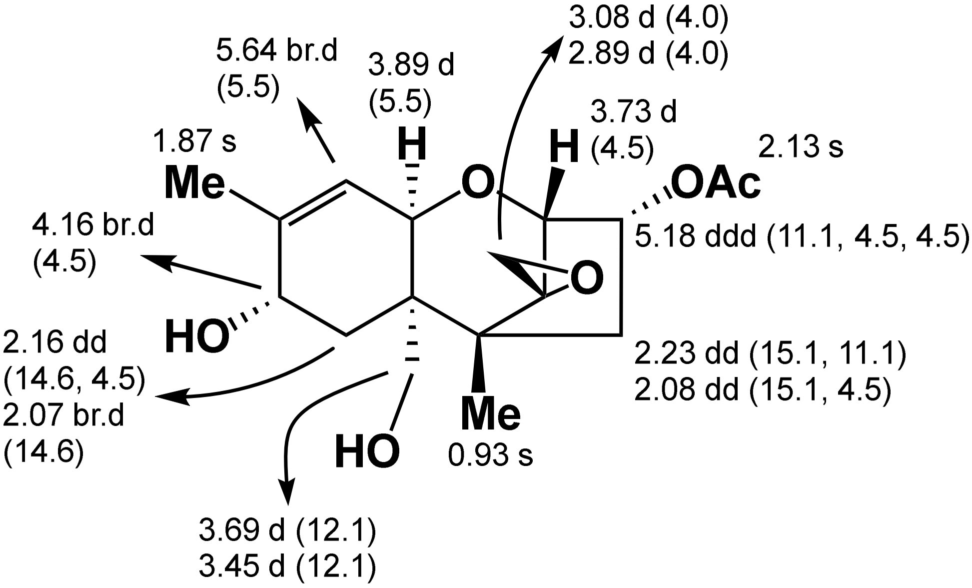
^{13}C NMR Data of isotrichodiol (151 MHz, $CDCl_3$) 81.7 (C-2), 30.3 (C-3), 37.1 (C-4), 46.8 (C-5), 41.0 (C-6), 29.2 (C-7), 27.8 (C-8), 130.5 (C-9), 125.5 (C-10), 72.0 (C-11), 69.3 (C-12), 49.5 (C-13), 20.2 (C-14), 13.0 (C-15), and 22.3 (C-16) ppm.

Spot 1 : 7-HIT [^{1}H NMR Data of 7-HIT (600 MHz, $CDCl_3$)]



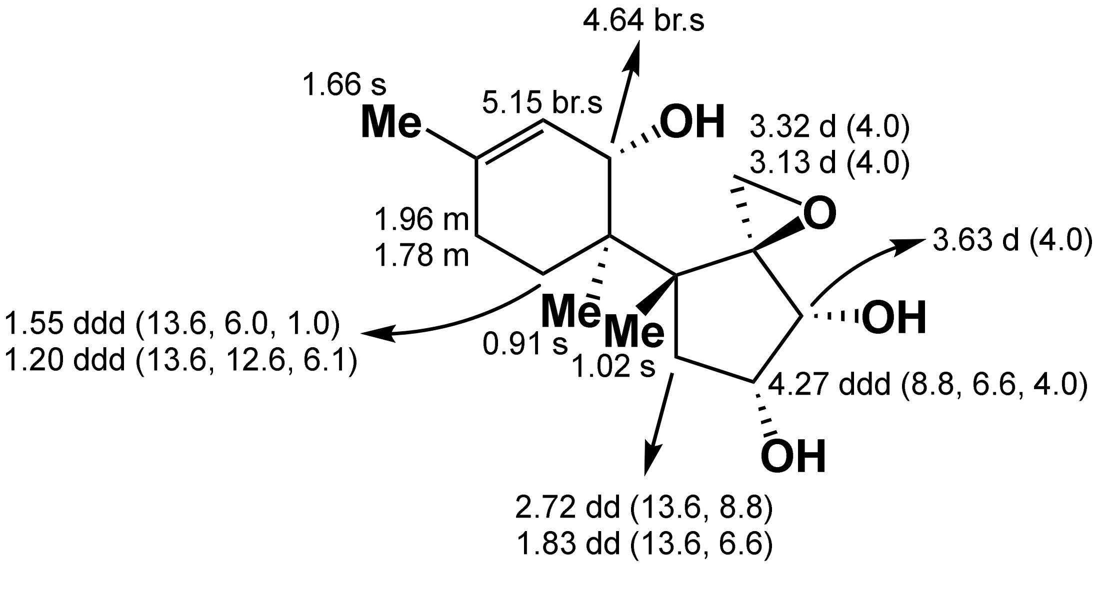
^{13}C NMR Data of 7-HIT (151 MHz, $CDCl_3$) 79.0 (C-2), 71.4 (C-3), 41.5 (C-4), 46.2 (C-5), 44.7 (C-6), 69.1 (C-7), 39.6 (C-8), 137.9 (C-9), 119.5 (C-10), 74.7 (C-11), 65.0 (C-12), 47.7 (C-13), 15.1 (C-14), 10.3 (C-15), and 22.5 (C-16) ppm.

Spot 2：8-H-15-deCAL [^{1}H NMR Data of 8-H-15-deCAL (600 MHz, $CDCl_3$)]



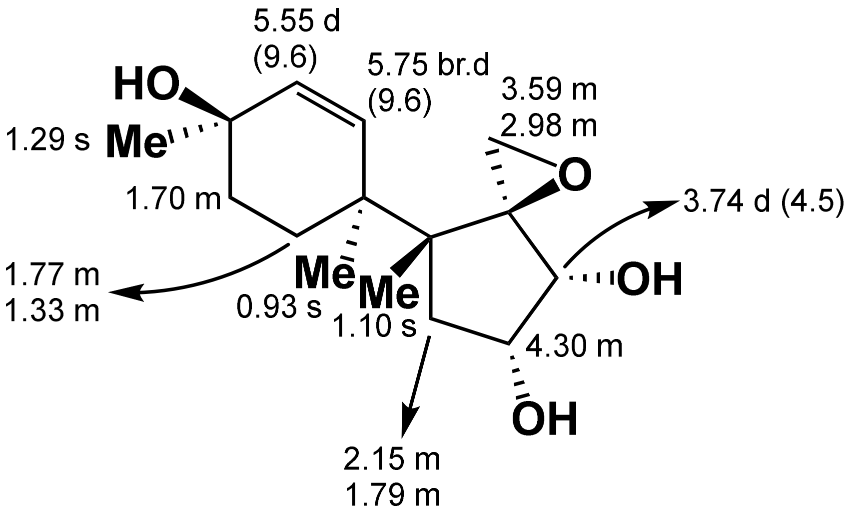
^{13}C NMR Data of 8-H 15-deCAL (151 MHz, $CDCl_3$) 77.9 (C-2), 71.3 (C-3), 39.5 (C-4), 45.7 (C-5), 44.0 (C-6), 28.3 (C-7), 67.3 (C-8), 138.8 (C-9), 122.8 (C-10), 68.6 (C-11), 67.0 (C-12), 48.5 (C-13), 12.6 (C-14), 62.7 (C-15), 20.6 (C-16), 21.1, and 170.7 (3-OAc) ppm.

Spot 3：isotrichotriol [^{1}H NMR Data of isotrichotriol (600 MHz, $CDCl_3$)]



^{13}C NMR Data of isotrichotriol (151 MHz, $CDCl_3$) 79.6 (C-2), 70.0 (C-3), 43.5 (C-4), 45.2 (C-5), 41.2 (C-6), 29.1 (C-7), 27.6 (C-8), 135.3 (C-9), 125.3 (C-10), 71.5 (C-11), 67.5 (C-12), 49.3 (C-13), 22.0 (C-14), 13.1 (C-15), and 22.5 (C-16) ppm.

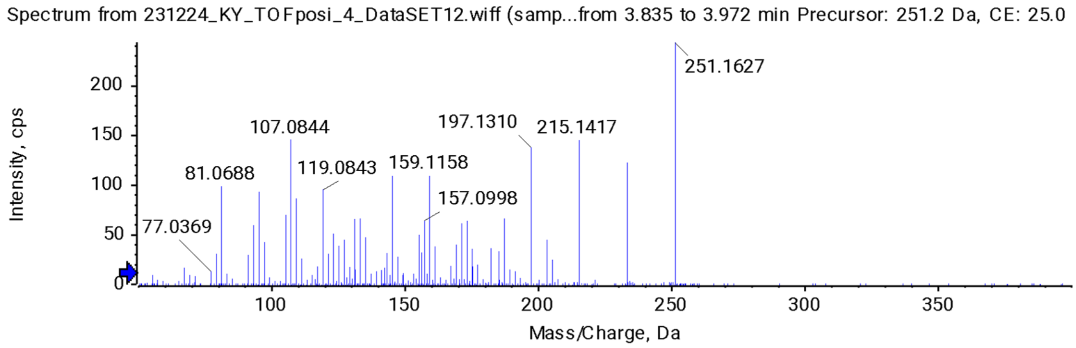
Spot 4：trichotriol [^{1}H NMR Data of trichotriol (600 MHz, $CDCl_3$)]



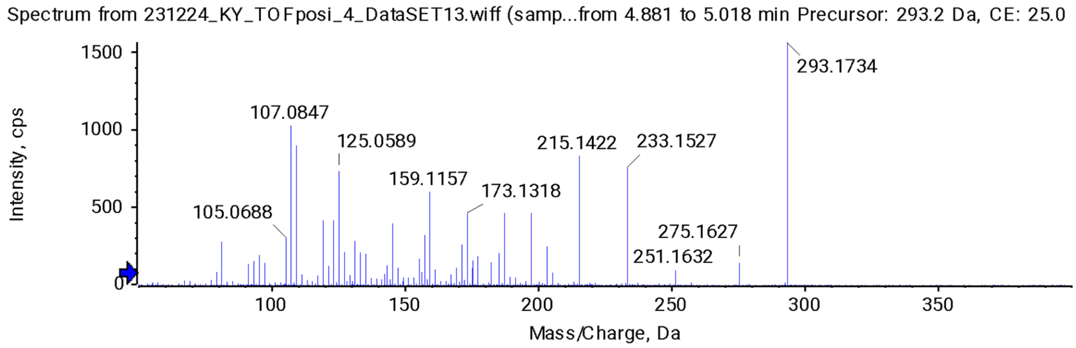
^{13}C NMR Data of trichotriol (151 MHz, $CDCl_3$) 77.5 (C-2), 69.0 (C-3), 41.5 (C-4), 43.9 (C-5), 39.5 (C-6), 27.0 (C-7), 34.0 (C-8), 66.5 (C-9), 131.3 (C-10), 136.3 (C-11), 69.2 (C-12), 49.9 (C-13), 20.4 (C-14), 21.3 (C-15), and 30.7 (C-16) ppm.

Supplementary Figure S3. Bicyclic precursors and tricyclic trichothecene intermediates of FGD3. (A) TLC analysis of trichothecene metabolites. Spot 1 contained 15-deCAL, isotrichodiol, and 7-HIT. Spots 2, 3, and 4 contained 8-H-15-deCAL, isotrichotriol, and trichotriol, respectively. (B) The compounds in spots 1–4 were confirmed by NMR analyses.

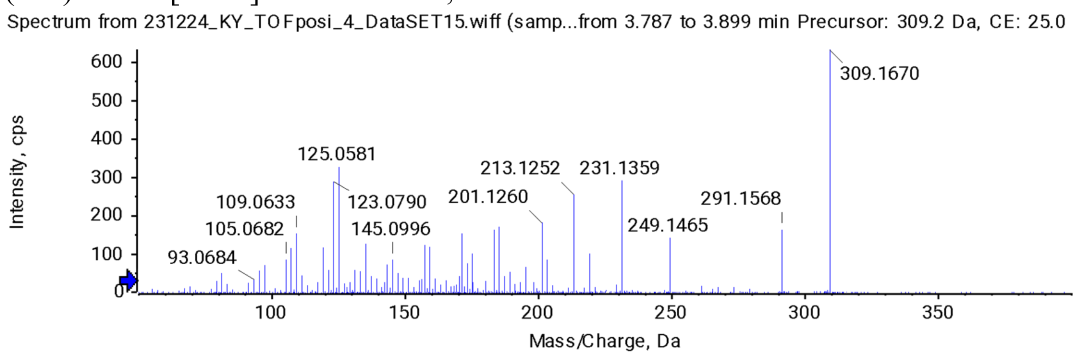
(A) ITDmol $[M+H]^+$ *m/z*: 251.164, RT: 3.88 min



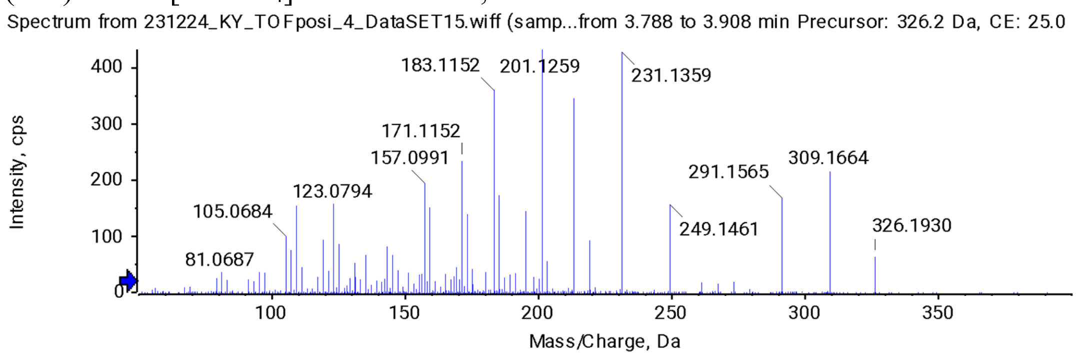
(B) ITD $[M+H]^+$ *m/z*: 293.175, RT: 4.95 min



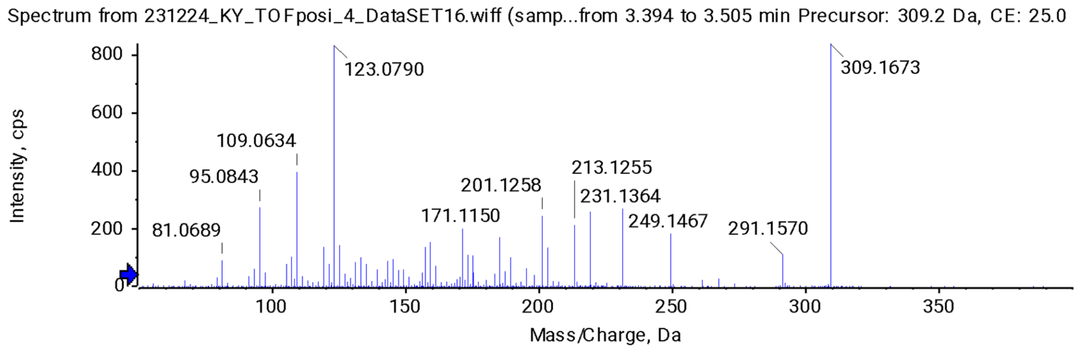
(C-1) 7-HIT $[M+H]^+$ *m/z*: 309.170, RT: 3.84 min



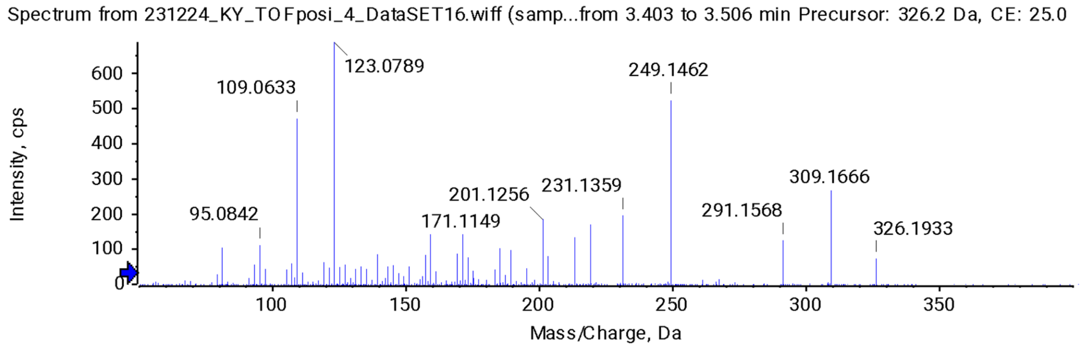
(C-2) 7-HIT $[M+NH_4]^+$ *m/z*: 326.196, RT: 3.84 min



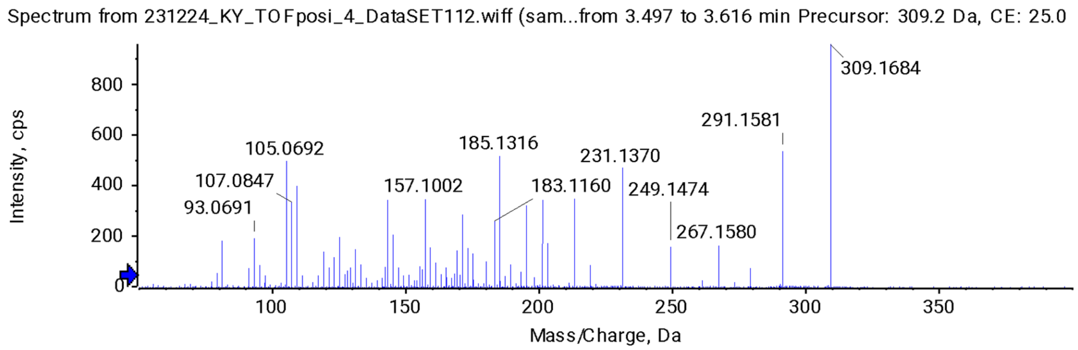
(D-1) 8-HIT $[M+H]^+$ *m/z*: 309.170, RT: 3.45 min



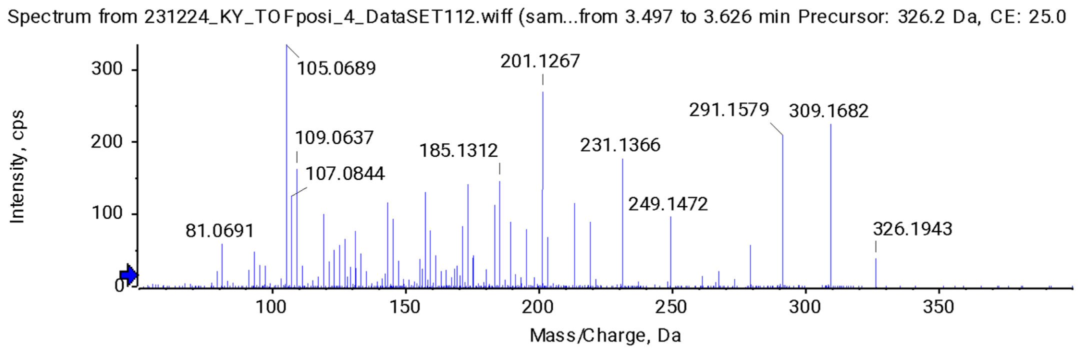
(D-2) 8-HIT $[M+NH_4]^+$ *m/z*: 326.196, RT: 3.44 min



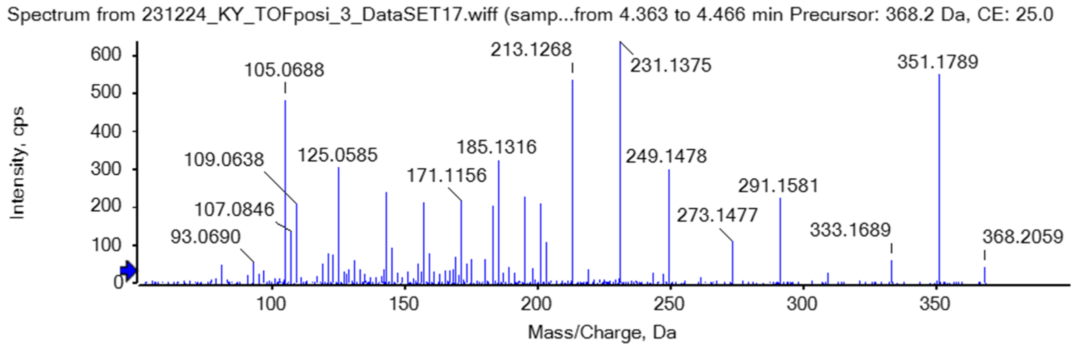
(E-1) 15-deCAL $[M+H]^+$ *m/z*: 309.170, RT: 3.55 min



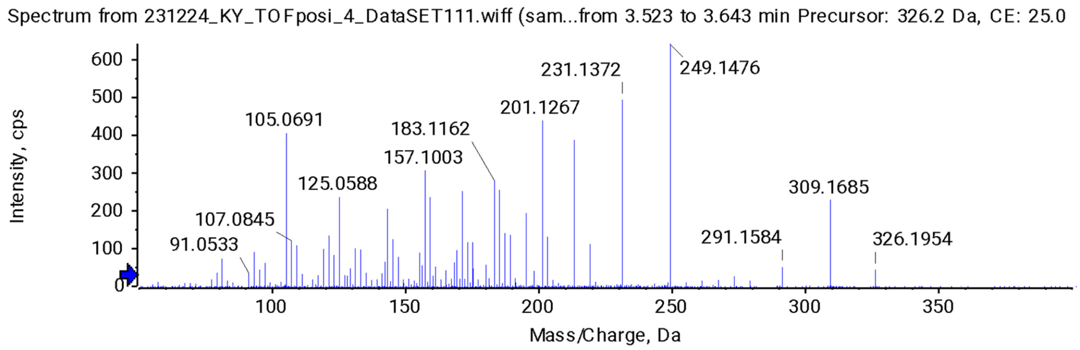
(E-2) 15-deCAL $[M+NH_4]^+$ *m/z*: 326.196, RT: 3.54 min



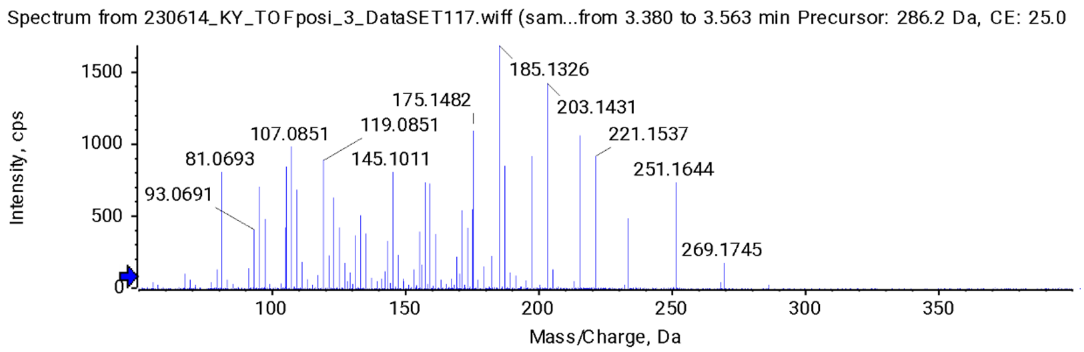
(F) CAL $[M+NH_4]^+$ *m/z*: 368.207, RT: 4.41 min



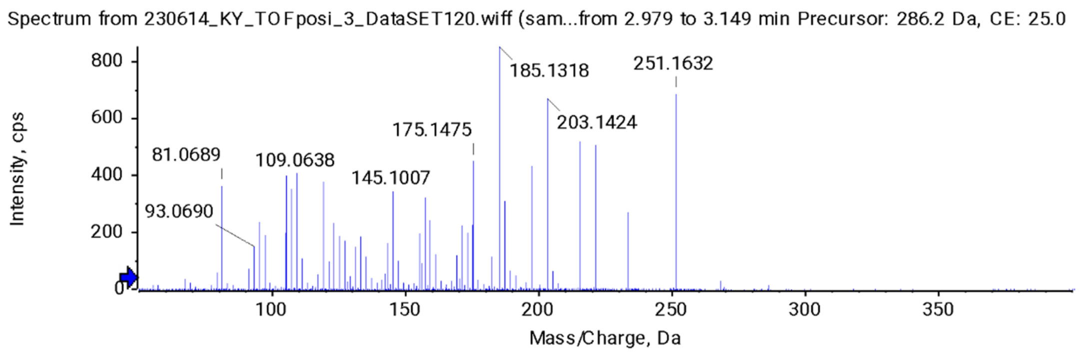
(G) 3-deCAL $[M+NH_4]^+$ *m/z*: 326.196, RT: 3.58 min



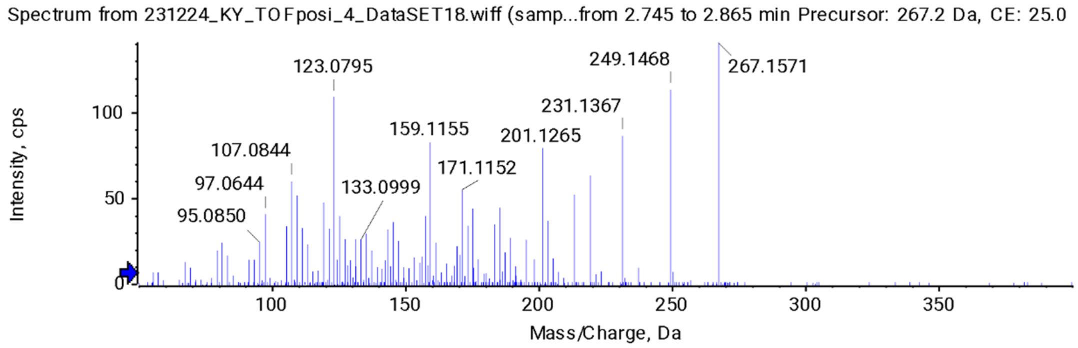
(H) isotrichotriol $[M+NH_4]^+$ *m/z*: 286.201, RT: 3.45 min



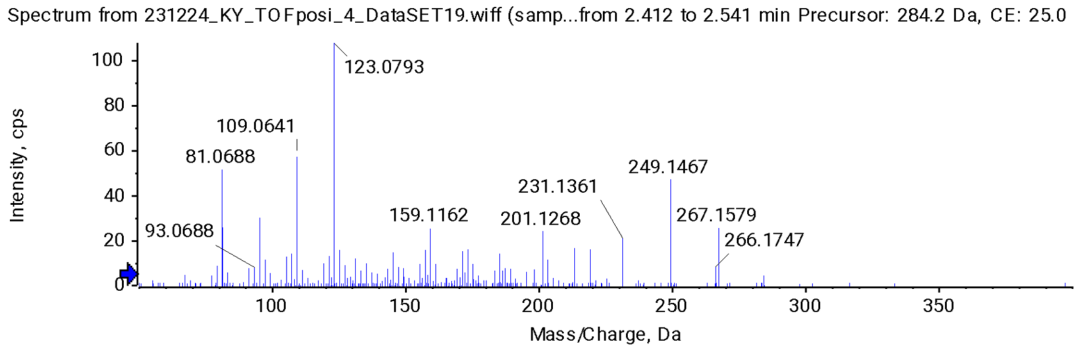
(I) trichotriol $[M+NH_4]^+$ *m/z*: 286.201, RT: 3.04 min



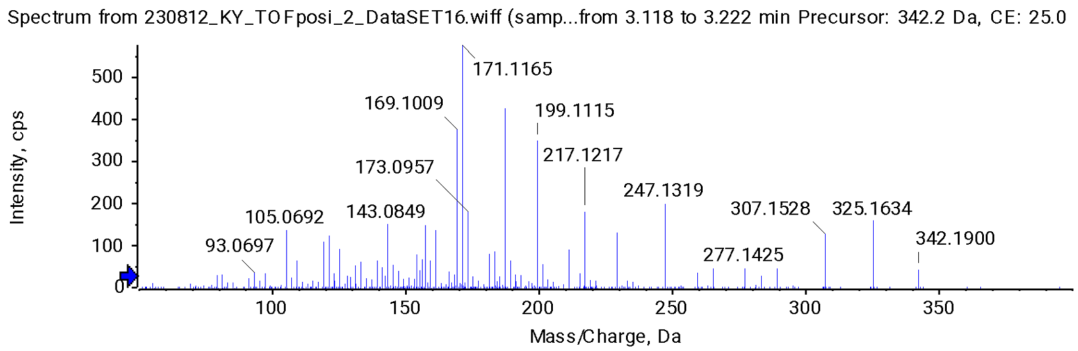
(J) 3-deacetyl-7-hydroxyisotrichodermin (3-de-7-HIT) $[M+H]^+$ *m/z*: 267.159, RT: 2.80 min



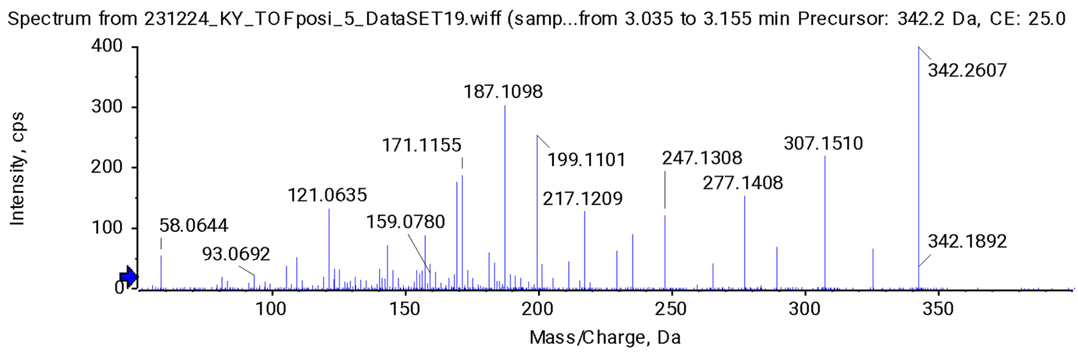
(K) 3-deacetyl-8-hydroxyisotrichodermin (3-de-8-HIT) $[M+NH_4]^+$ *m/z*: 284.186, RT: 2.48 min



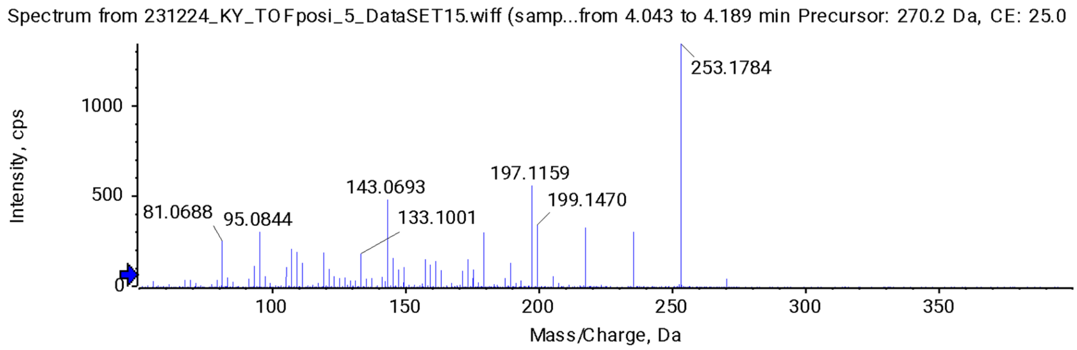
(L) 7-H-15-deCAL (NX-2) $[M+NH_4]^+$ *m/z*: 342.191, RT: 3.15 min



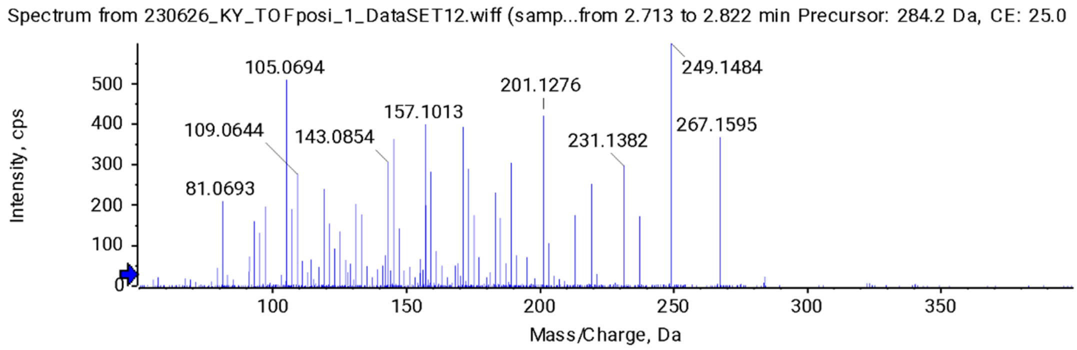
(M) 8-H-15-deCAL $[M+NH_4]^+$ *m/z*: 342.191, RT: 3.10 min



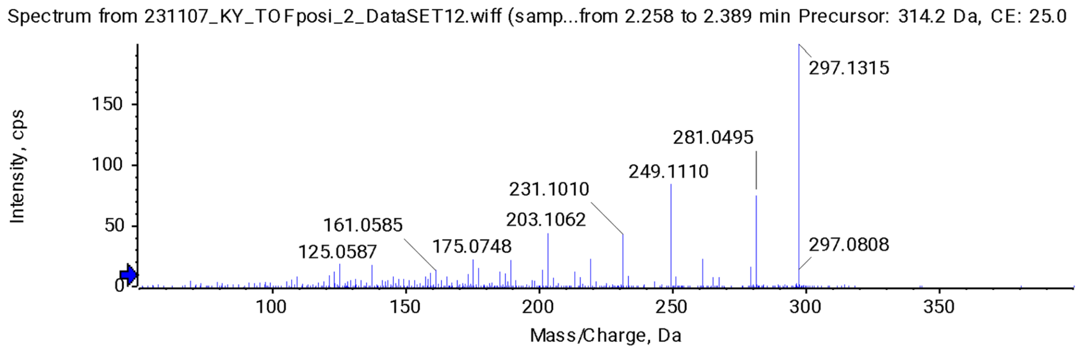
(N) isotrichodiol [M+NH4]$^+$ *m/z*: 270.206, RT: 4.11 min



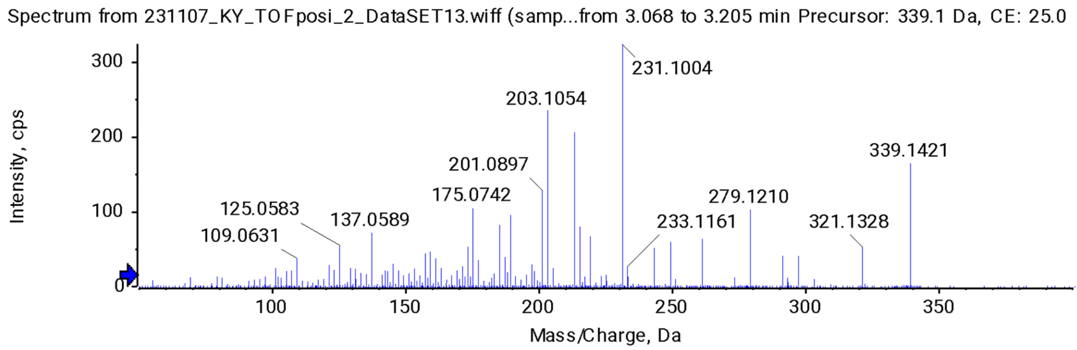
(O) 3,15-dideacetylcalonectrin (3,15-dideCAL) [M+NH4]$^+$ *m/z*: 284.186, RT: 2.77 min



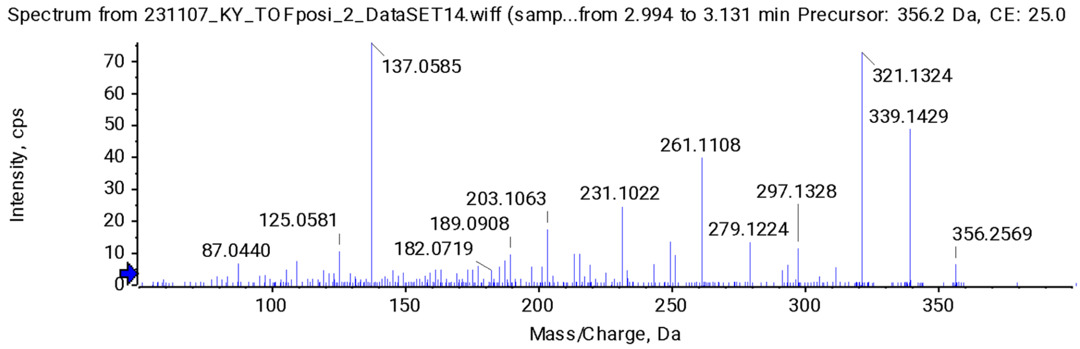
(P) DON [M+NH4]$^+$ *m/z*: 314.160, RT: 2.29 min



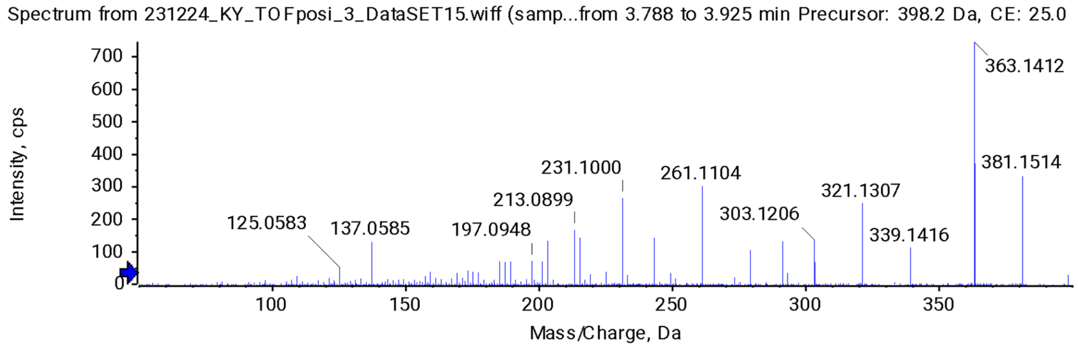
(Q) 3-ADON [M+H]$^+$ *m/z*: 339.144, RT: 3.10 min



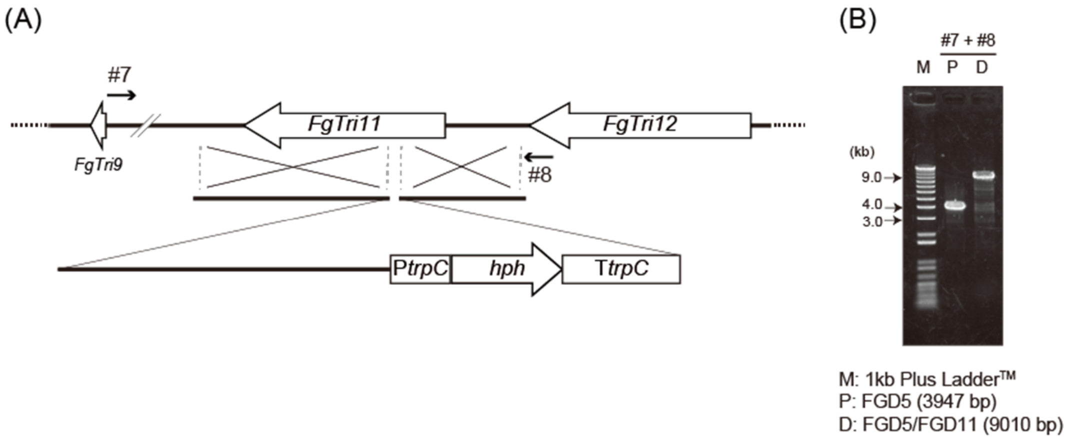
(R) 15-ADON $[M+NH_4]^+$ *m/z*: 356.170, RT: 3.05 min



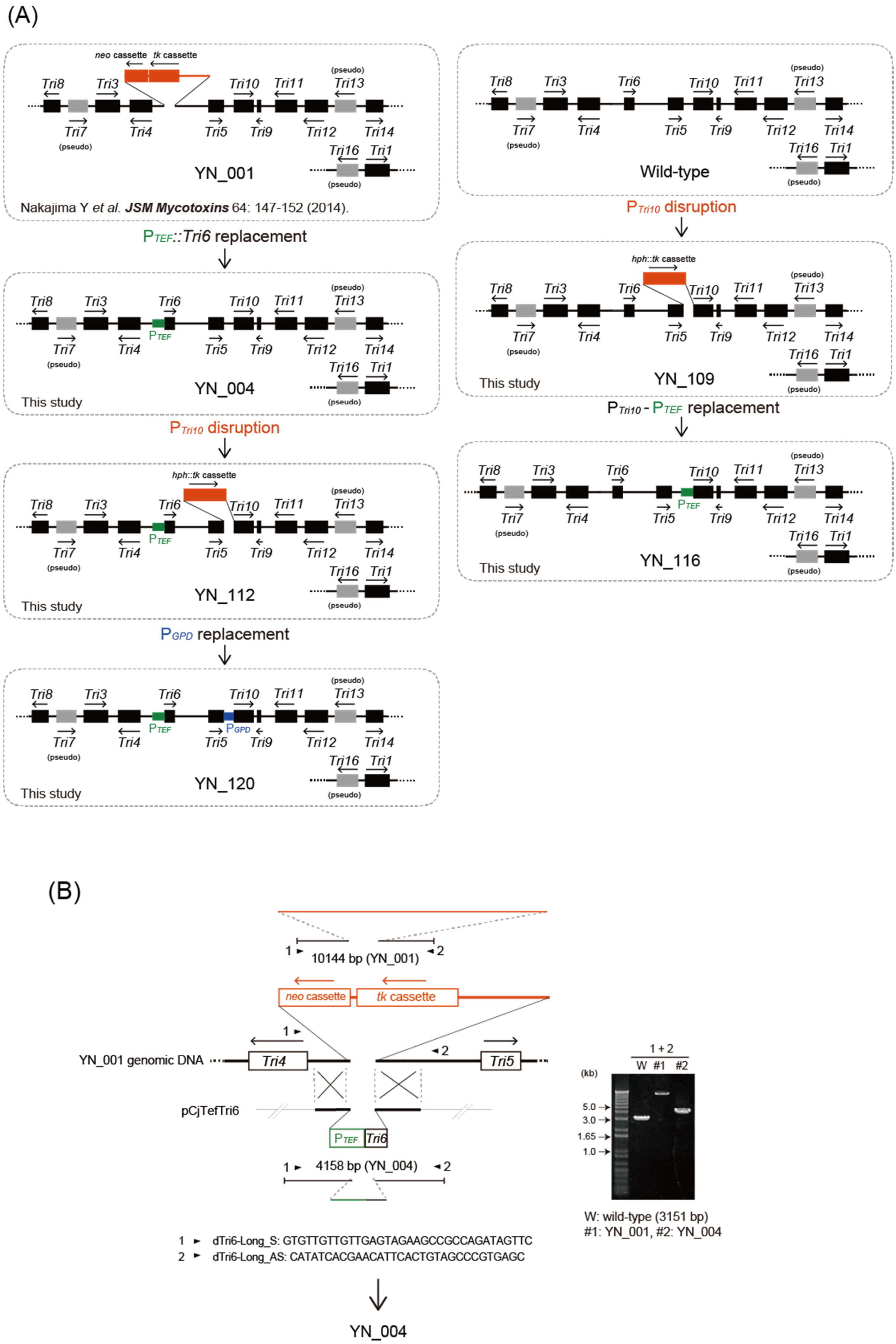
(S) 3,15-diADON $[M+NH_4]^+$ *m/z*: 398.181, RT: 3.84 min

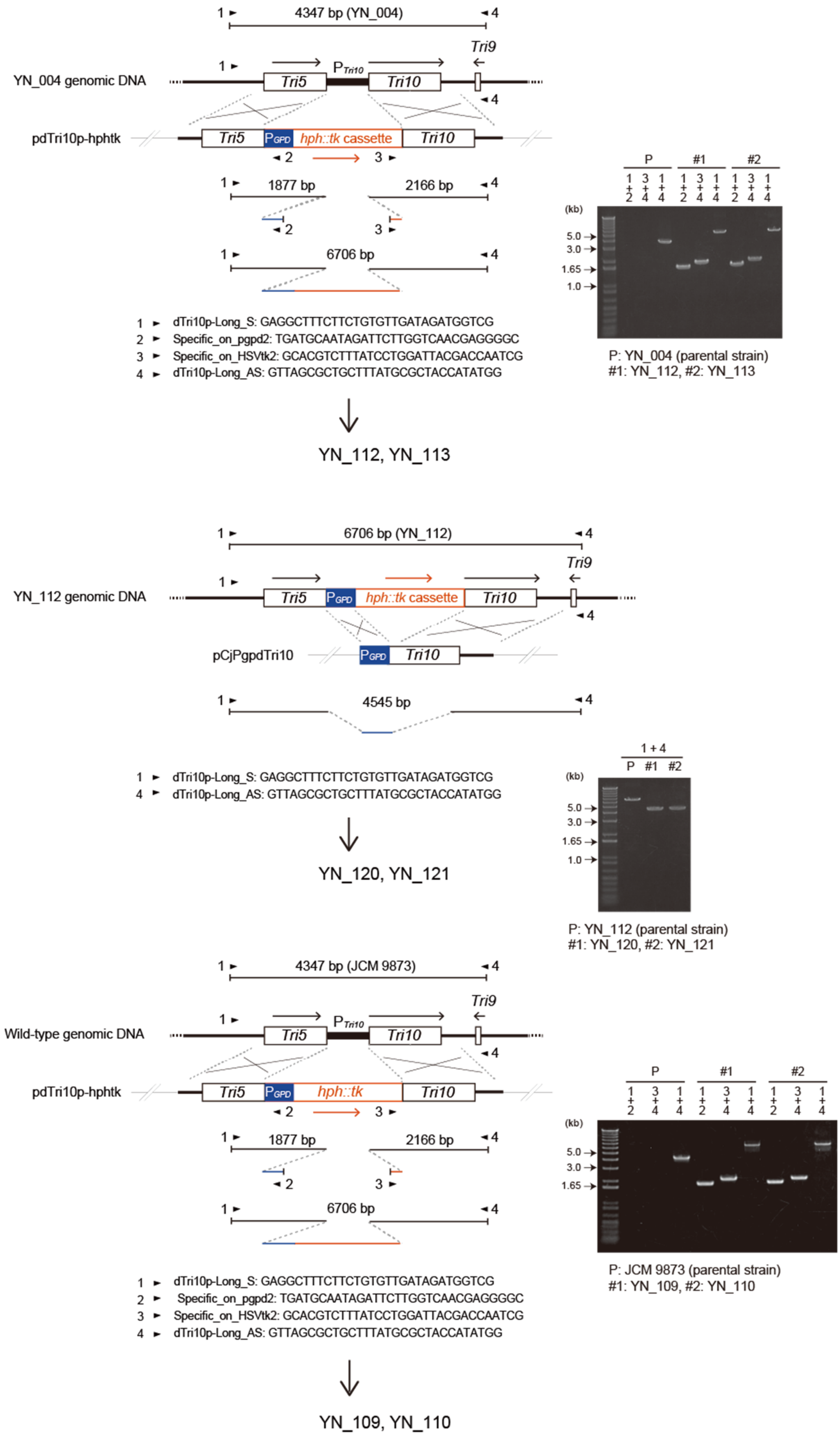


Supplementary Figure S4. MS/MS spectra of 16 tricyclic and 3 bicyclic trichothecenes and shunts. The purified samples were applied to LC-MS, and their MS/MS spectra were obtained in the positive ion mode, using the TOF–MS methods.

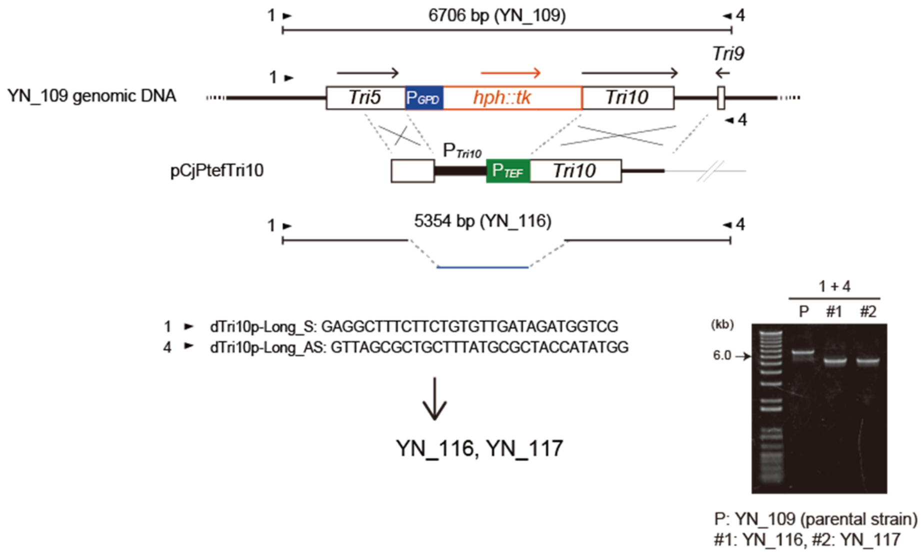


Supplementary Figure S5. Generation of a Δ*Fgtri5*Δ*Fgtri11* (FGD5/11) disruption mutant used in this study. (A) The *FgTri11* gene disruption vector [7] that contains the *hph* (hygromycin B phosphotransferase gene) cassette was transformed into FGD5 mutant strain [8]. After transformation, colonies were selected with 300 µg/ml of hygromycin B. (B) PCR confirmation of genomic DNA isolated from the FGD5 and FGD5/FGD11 mutant strains. Primers #7 and #8 were used to amplify the region encompassing *FgTri11*. An amplicon from the double disruption mutant is 5.0 kb larger than that from its parent FGD5.


(A)
neo cassette
tk cassette
Tri8 Tri3 Tri10 Tri11 Tri13 (pseudo)
Tri7 (pseudo) Tri4 Tri5 Tri9 Tri12 Tri14
Tri16 Tri1 (pseudo)
YN_001
Nakajima Y et al. JSM Mycotoxins 64: 147-152 (2014).
Wild-type
PTEF::Tri6 replacement
YN_004
This study
PTri10 disruption
hph::tk cassette
YN_109
YN_112
PTri10 - PTEF replacement
YN_116
PGPD replacement
YN_120
(B)
10144 bp (YN_001)
neo cassette
tk cassette
YN_001 genomic DNA
Tri4
Tri5
pCjTefTri6
PTEF Tri6
4158 bp (YN_004)
1 ► dTri6-Long_S: GTGTTGTTGTTGAGTAGAAGCCGCCAGATAGTTC
2 ► dTri6-Long_AS: CATATCACGAACATTCACTGTAGCCCGTGAGC
YN_004
1 + 2
W #1 #2
(kb)
5.0
3.0
1.65
1.0
W: wild-type (3151 bp)
#1: YN_001, #2: YN_004

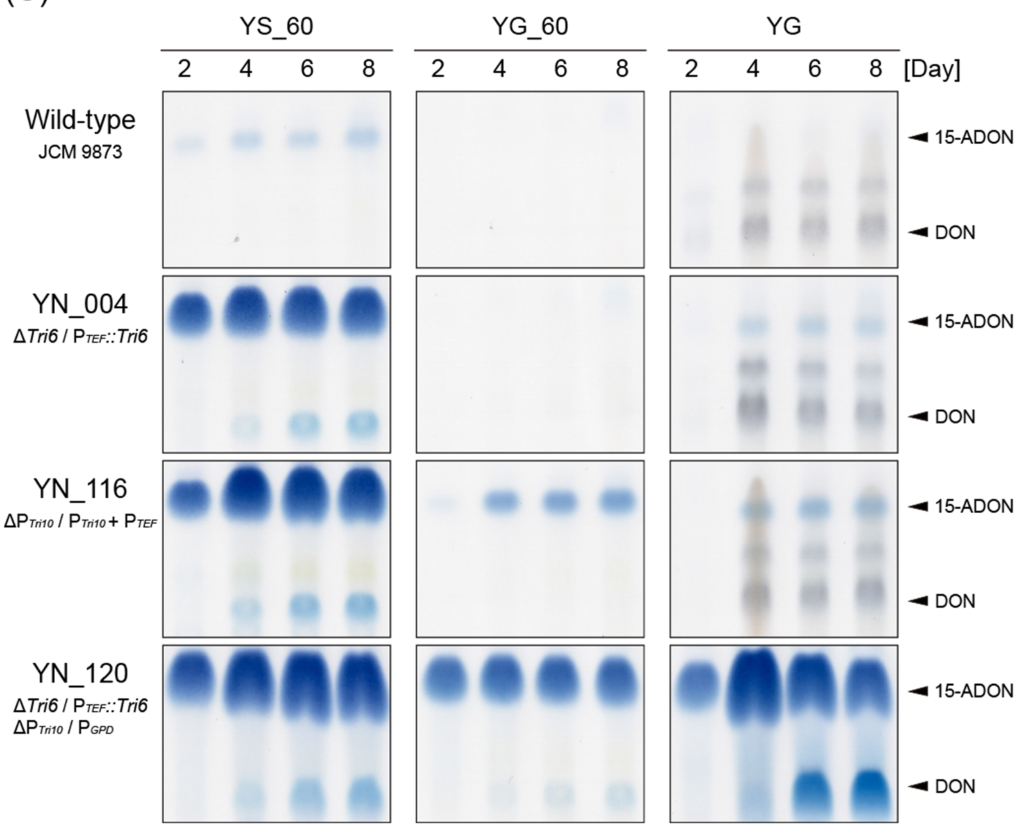


4347 bp (YN_004)
YN_004 genomic DNA
Tri5
PTri10
Tri10
Tri9
pdTri10p-hphtk
PGPD
hph::tk cassette
1877 bp
2166 bp
6706 bp
1 ► dTri10p-Long_S: GAGGCTTTCTTCTGTGTTGATAGATGGTCG
2 ► Specific_on_pgpd2: TGATGCAATAGATTCTTGGTCAACGAGGGGC
3 ► Specific_on_HSVtk2: GCACGTCTTTATCCTGGATTACGACCAATCG
4 ► dTri10p-Long_AS: GTTAGCGCTGCTTTATGCGCTACCATATGG
(kb)
5.0
3.0
1.65
1.0
P
#1
#2
P: YN_004 (parental strain)
#1: YN_112, #2: YN_113
YN_112, YN_113
6706 bp (YN_112)
YN_112 genomic DNA
pCjPgpdTri10
4545 bp
1 ► dTri10p-Long_S: GAGGCTTTCTTCTGTGTTGATAGATGGTCG
4 ► dTri10p-Long_AS: GTTAGCGCTGCTTTATGCGCTACCATATGG
1 + 4
P: YN_112 (parental strain)
#1: YN_120, #2: YN_121
YN_120, YN_121
4347 bp (JCM 9873)
Wild-type genomic DNA
hph::tk
P: JCM 9873 (parental strain)
#1: YN_109, #2: YN_110
YN_109, YN_110



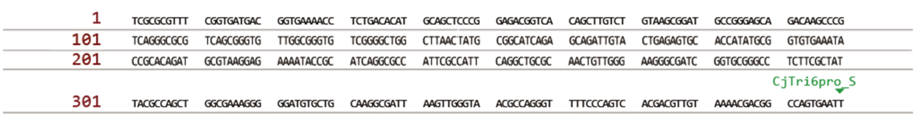
1 ► 6706 bp (YN_109) ◄ 4
Tri9
YN_109 genomic DNA
Tri5
PGPD
hph::tk
Tri10
PTri10
pCjPtefTri10
PTEF
Tri10
1 ► 5354 bp (YN_116) ◄ 4
1 ► dTri10p-Long_S: GAGGCTTTCTTCTGTGTTGATAGATGGTCG
4 ► dTri10p-Long_AS: GTTAGCGCTGCTTTATGCGCTACCATATGG
YN_116, YN_117
1 + 4
P #1 #2
(kb)
6.0
P: YN_109 (parental strain)
#1: YN_116, #2: YN_117

(C)



YS_60
YG_60
YG
2 4 6 8
[Day]
Wild-type
JCM 9873
YN_004
ΔTri6 / PTEF::Tri6
YN_116
ΔPTri10 / PTri10 + PTEF
YN_120
ΔTri6 / PTEF::Tri6
ΔPTri10 / PGPD
15-ADON
DON

(D)



1 TCGCGCGTTT CGGTGATGAC GGTGAAAACC TCTGACACAT GCAGCTCCCG GAGACGGTCA CAGCTTGTCT GTAAGCGGAT GCCGGGAGCA GACAAGCCCG
101 TCAGGGCGCG TCAGCGGGTG TTGGCGGGTG TCGGGGCTGG CTTAACTATG CGGCATCAGA GCAGATTGTA CTGAGAGTGC ACCATATGCG GTGTGAAATA
201 CCGCACAGAT GCGTAAGGAG AAAATACCGC ATCAGGCGCC ATTCGCCATT CAGGCTGCGC AACTGTTGGG AAGGGCGATC GGTGCGGGCC TCTTCGCTAT
CjTri6pro_S
301 TACGCCAGCT GGCGAAAGGG GGATGTGCTG CAAGGCGATT AAGTTGGGTA ACGCCAGGGT TTTCCCAGTC ACGACGTTGT AAAACGACGG CCAGTGAATT

CjTri6pro_S

Tri6_upstream region

401 CGAGCTCGGT ACCCAGGTAC CTTGTCTATC GCGTCTCTAG AGTGCTTTGC ATGCGTTGTG GCCGTAAACG CTCACAACTC TGAAGTTGTC CTCAGTATCG

Tri6_upstream region

501 CCGTGTCAGA TAAGCTTCGC AAGAGTGTCC GGTCGGAAAC TCACCAATCA ACTCAGCAGG ATGAACAAGG GGTCTGAAAG GCCTGGCAGG CCTGACAGGA

Tri6_upstream region

601 GTGATAAAAT GTGAGAAGAG ATATGCCGAT ACAACCGTGT AACTTGTGAA ACGGGGCATG GAATCCCATG GCAAGTTATG GGGTCAGCAG CAACTGAATT

Tri6_upstream region

701 GCCTACGAGT CAAGAAGTGC ATCCTTTCAC CGGCGGCTTA TCCGAAGTTG CTGCCGATCA GATACAGACA TGCATGCAGA GTGGTACGAC TGCGCGGAAG

Tri6_upstream region

801 AATAAGAATC ATCAGTGCGC CGCAATGTTA AAAACTGATG TGCGGAAGCA ACATTAAGCT TTGGAGGCAT GCCAGGGTCT TGTCTCGAAA TATCTTTGTC

Tri6_upstream region

901 TACCGAGACC CATGCATGGT CAAAAGTATG TACATGGATG GTCTTGCACA GAAGACAGCC TCGAGTGTTA TGCAGACTGT CACGGCTGCA GTAAGTTGCC

Tri6_upstream region

1001 ACAGACTCGA ATCGATTATC ATTGACCGTT CGGAAGCGCT CTGTTAGGAA TCTTTCTAGA CCACAACTAC CACTTTGGCA TCTGCATACT AACACTAGTA

Tri6_upstream region

1101 GCCACATAGT AAACCTTCAA CTGCCGCCGC ATCAAACTGT AAACAGGTAC CGGCCGACGC GTCTCGGATA AGAATACCTT TTAAACTGCC GTAGCAAACT

Tri6_upstream region

1201 GTAATTGTCG GTACTTCTCG GACAATATTT TCATGGCTTT CAGAAGCTTT CACTTTTAAT AAAACTTGAT CTGAATAAGA AACTTTATCA ATCGTATCCC

TEF promoter

CTefjTri6_S

Tri6_upstream region

1301 ATCCCATCAA GGCTCAAGCC ATCTTTTATT TTTTATTTTT TGCATCGCCA ACCAATATAT TGAACATCTA TTTTGACTAC CCTCGAATAG CGACTTAGAG

jT6Cpro_AS

CTefjTri6_S

TEF promoter

1401 TGCACGCTCA AAAGATACCC CTAACTTCTG ATTTGATAGC ATTTGTATAT GGCATATAGT ACGCCTGAAG CATAAAAAAA AAAACAGAAT TATTATAGTT

TEF promoter

1501 TATTAATACG AGACAGCAGA ATCACCGCCC AAGTTAAGCC TTTGTGCTGA TCATGCTCTC GAACGGGCCA AGTTCGGGAA AAGCAAAGGA GCGTTTAGTG

TEF promoter

1601 AGGGGCAATT TGACTCACCT CCCAGGCAAC AGATGAGGGG GGCAAAAAGA AAGAAATTTT CGTGAGTCAA TATGGATTCC GAGCATCATT TTCTTGCGGT

TEF promoter

1701 CTATCTTGCT ACGTATGTTG ATCTTGACGC TGTGGATCAA GCAACGCCAC TCGCTCGCTC CATCGCAGGC TGGTCGCAGA CAAATTAAAA GGCGGCAAAC

TEF promoter

1801 TCGTACAGCC GCGGGGTTGT CCGCTGCAAA GTACAGAGTG ATAAAAGCCG CCATGCGACC ATCAACGCGT TGATGCCCAG CTTTTTCGAT CCGAGAATCC

TEF promoter

1901 ACCGTAGAGG CGATAGCAAG TAAAGAAAAG CTAAACAAAA AAAAATTTCT GCCCCTAAGC CATGAAAACG AGATGGGGTG GAGCAGAACC AAGGAAAGAG

TEF promoter

2001 TCGCGCTGGG CTGCCGTTCC GGAAGGTGTT GTAAAGGCTC GACGCCCAAG GTGGGAGTCT AGGAGAAGAA TTTGCATCGG GAGTGGGGCG GGTTACCCCT

TEF promoter

2101 CCATATCCAA TGACAGATAT CTACCAGCCA AGGGTTTGAG CCCGCCCGCT TAGTCGTCGT CCTCGCTTGC CCCTCCATAA AAGGATTTCC CCTCCCCCTC

TEF promoter

2201 CCACAAAATT TTCTTTCCCT TCCTCTCCTT GTCCGCTTCA GTACGTATAT CTTCCCTTCC CTCGCTTCTC TCCTCCATCC TTCTTTCATC CATCTCCTGC

Tri6

jT6C_S

TEF promoter

+1 M I

2301 TAACTTCTCT GCTCAGCACC TCTACGCATT ACTAGCCGTA GTATCTGAGC ACTTCTCCCT TTTATATTCC ACAAAACATA ACACAACCTT CACCATGATT

CTefjTri6_AS

jT6C_S

Tri6

+1 Y M E A E S H Y E S W S A L P L F D R V A S P D P A K D F V P D L N

2401 TACATGGAGG CCGAATCTCA CTACGAATCT TGGAGCGCCT TGCCCCTCTT TGATCGAGTT GCGTCTCCCG ATCCTGCCAA GGACTTTGTC CCAGATCTAA

CTefjTri6_AS

Tri6

+1 · N D Y E S P T F E I D L L S E T Y D F D N F P T Y S L P T V D S T K

2501 ACGACTATGA ATCACCAACA TTCGAAATAG ATCTTCTCTC AGAAACTTAT GACTTTGACA ACTTCCCCAC ATACTCTCTA CCAACGGTGG ATTCAACCAA

Tri6

+1 · K T L Y S E E P L V C F D F D F A N P A I E N Y I T T S S G L L D A

2601 GACTTTGTAC TCCGAAGAAC CACTTGTTTG CTTCGACTTT GACTTCGCGA ACCCGGCTAT CGAAAATTAT ATAACCACAT CGTCGGGACT GTTGGACGCA

Tri6

+1 V P S Q L I A L P T F T R P S K C P F P S C K S A T V F E S G R D F

2701 GTGCCAAGCC AGCTTATCGC CCTTCCCACC TTCACACGGC CAAGCAAATG CCCATTCCCT AGTTGCAAGT CGGCCACAGT CTTTGAAAGC GGACGGGACT

Tri6

+1 · F R R H Y R Q H F K R F F C R Y S E C P Q S A Q D L Q E V G T K G F

2801 TTAGGCGGCA TTACCGGCAA CACTTCAAGC GCTTTTTCTG TCGCTACTCA GAATGCCCTC AGTCAGCTCA AGACCTGCAA GAAGTCGGCA CCAAAGGCTT

Tri6

+1 · F A T R K D R A R H E S K H K P T V R C P W Q D K E G Q Q C L R V F

2901 TGCGACTCGC AAGGACCGTG CTCGGCATGA GTCTAAGCAC AAACCAACAG TGCGGTGCCC TTGGCAAGAC AAGGAAGGAC AACAATGTCT GAGGGTCTTT

Tri6

Tri6 downstream region

+1 S R V D N M R D H Y R R I H K C *

3001 AGCAGGGTGG ATAACATGCG AGATCACTAT AGGCGGATAC ATAAGTGTTG ACGAGGGATC GGTGTGCAAA ACTGCAGATA GTTACTCATA AAAGGCAACA

Tri6 downstream region

3101 TTTGCGAAAA TCAATAACAA TATCTCCATA AATATCCCAT TAGACTTTTT GAGTTTCATA AATGATGACG AGTTTGGCGC CGATCGATGC TGTTTCCAGC

Tri6 downstream region

3201 ACCAATTCAT AATATACTCC ATAGTAGCCG AGACCCTGCA GCTCATTGGT ACCAGCCCTG TTTTTATTGC ACAATTAATG ACACTCTATT CTGCACGCGA

Tri6 downstream region

3301 CTCAACGGCT TTCAACACTT ACATATTGCC TCTACGCGTG GCTGTGTCTA AGCGAGATCA GCGCGGTCAT TGTTATTGCT GCGTTGCTTT GGCCCCGTAC

Tri6 downstream region

3401 GTTCCACAAC AATCTGGCCA CTGCAAGGTA TTTGCGGCCG CTGGTTAAAT TCTCTTAGTA GTCTGCCTCA TTTGGCAGGT GCCAATAATA TATTTTACCC

Tri6 downstream region

3501 GCGTTTGGCT GGCCATGCTA CCCAAAAGGC TTCATACATA TCTGGCCCAT ATCGTTTACT CGGTTCCACG TTTACGAGCA TACGCCTCTC GTATCAATGT

Tri6 downstream region
3601 CGCAAACAAA CATACGTCAT TGGTTACTAT GCCGGGTTTC CGCGAACCAT TGCTGTGCCG CAAAAGGATG CATCGGCACT CTAAAGATTG CGATCCTGTG

Tri6 downstream region
3701 TTAGAGGGTG ACTTGAGTCA AGAAGTAACG TAACAAGTGA GATAATGAAA TTAATTACCT GAGGGCAATT TAAGGTTTCA ACCTCCGAGG AAAGCCATTC

Tri6 downstream region
3801 GTCGTGGGAT ATAATCTGCA ATAGGGTAGG CTTTGCTGTT TTTTTCGGAG CATTTGTGAC TGTTTTGGAT TGTGTCTTTG GGGTCCTTTT GCTTTCAAGG

Tri6 downstream region
3901 CTGGTGAGTT TGCAAGGAAC GAGTCAGTCA CGGCAGGCAC TGAGTCAAAC ACTGTTTCGT AATAATCATG ATTTGTTGTT GCAAAATTCC AACGAAGCTT

Tri6 downstream region
4001 TGCGCGGTCA GTCAAATTCG CACACGTCGA TACTTTTTGC TGGCATTGGC CCAGTATTCA TCCATGAGTG GCTGAACCGT AGTTGATTCT CAAGTCCAAC

Tri6 downstream region
4101 CCTAATAGTG CCCGGCGGAA TGAGACGTTT TCGGGTGTCT GTAGCCGAGA TGTGGATAGT AACGGTAACC CTAGTCAAAT GAGACGTGGG CAGGGTTCAT

Tri6 downstream region
4201 GGTTGTTGAA CCTTGTTCAT CAGAATGTTG ATGCCGTTGA CCTACGGAAT ACCATCTTTC ACATGTATTT GTTCCCAACC CACGTGGCTA TACCAACATC

Tri6 downstream region BamHI
4301 CGCTGTATAT TCATTGGTTG GCTTTATATA TTGATACAGG TAATTTCAAG ATCGCGAGCG GGAATTTCCT TGTCGGATCA GAGAGATGCT CTGGGGATCC
jT6Cter_AS

4401 TCTAGAGTCG ACCTGCAGGC ATGCAAGCTT GGCGTAATCA TGGTCATAGC TGTTTCCTGT GTGAAATTGT TATCCGCTCA CAATTCCACA CAACATACGA
jT6Cter_AS

4501 GCCGGAAGCA TAAAGTGTAA AGCCTGGGGT GCCTAATGAG TGAGCTAACT CACATTAATT GCGTTGCGCT CACTGCCCGC TTTCCAGTCG GGAAACCTGT
4601 CGTGCCAGCT GCATTAATGA ATCGGCCAAC GCGCGGGGAG AGGCGGTTTG CGTATTGGGC GCTCTTCCGC TTCCTCGCTC ACTGACTCGC TGCGCTCGGT
4701 CGTTCGGCTG CGGCGAGCGG TATCAGCTCA CTCAAAGGCG GTAATACGGT TATCCACAGA ATCAGGGGAT AACGCAGGAA AGAACATGTG AGCAAAAGGC
4801 CAGCAAAAGG CCAGGAACCG TAAAAAGGCC GCGTTGCTGG CGTTTTTCCA TAGGCTCCGC CCCCCTGACG AGCATCACAA AAATCGACGC TCAAGTCAGA
4901 GGTGGCGAAA CCCGACAGGA CTATAAAGAT ACCAGGCGTT TCCCCCTGGA AGCTCCCTCG TGCGCTCTCC TGTTCCGACC CTGCCGCTTA CCGGATACCT
5001 GTCCGCCTTT CTCCCTTCGG GAAGCGTGGC GCTTTCTCAA TGCTCACGCT GTAGGTATCT CAGTTCGGTG TAGGTCGTTC GCTCCAAGCT GGGCTGTGTG
5101 CACGAACCCC CCGTTCAGCC CGACCGCTGC GCCTTATCCG GTAACTATCG TCTTGAGTCC AACCCGGTAA GACACGACTT ATCGCCACTG GCAGCAGCCA
5201 CTGGTAACAG GATTAGCAGA GCGAGGTATG TAGGCGGTGC TACAGAGTTC TTGAAGTGGT GGCCTAACTA CGGCTACACT AGAAGGACAG TATTTGGTAT
5301 CTGCGCTCTG CTGAAGCCAG TTACCTTCGG AAAAAGAGTT GGTAGCTCTT GATCCGGCAA ACAAACCACC GCTGGTAGCG GTGGTTTTTT TGTTTGCAAG
5401 CAGCAGATTA CGCGCAGAAA AAAAGGATCT CAAGAAGATC CTTTGATCTT TTCTACGGGG TCTGACGCTC AGTGGAACGA AAACTCACGT TAAGGGATTT
5501 TGGTCATGAG ATTATCAAAA AGGATCTTCA CCTAGATCCT TTTAAATTAA AAATGAAGTT TTAAATCAAT CTAAAGTATA TATGAGTAAA CTTGGTCTGA
5601 CAGTTACCAA TGCTTAATCA GTGAGGCACC TATCTCAGCG ATCTGTCTAT TTCGTTCATC CATAGTTGCC TGACTCCCCG TCGTGTAGAT AACTACGATA
bla

5701 CGGGAGGGCT TACCATCTGG CCCCAGTGCT GCAATGATAC CGCGAGACCC ACGCTCACCG GCTCCAGATT TATCAGCAAT AAACCAGCCA GCCGGAAGGG
bla

5801 CCGAGCGCAG AAGTGGTCCT GCAACTTTAT CCGCCTCCAT CCAGTCTATT AATTGTTGCC GGGAAGCTAG AGTAAGTAGT TCGCCAGTTA ATAGTTTGCG
bla

5901 CAACGTTGTT GCCATTGCTA CAGGCATCGT GGTGTCACGC TCGTCGTTTG GTATGGCTTC ATTCAGCTCC GGTTCCCAAC GATCAAGGCG AGTTACATGA
bla

6001 TCCCCCATGT TGTGCAAAAA AGCGGTTAGC TCCTTCGGTC CTCCGATCGT TGTCAGAAGT AAGTTGGCCG CAGTGTTATC ACTCATGGTT ATGGCAGCAC
bla

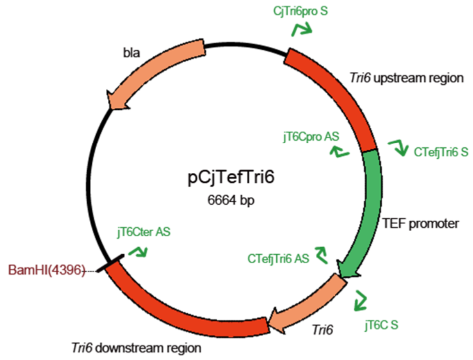
6101 TGCATAATTC TCTTACTGTC ATGCCATCCG TAAGATGCTT TTCTGTGACT GGTGAGTACT CAACCAAGTC ATTCTGAGAA TAGTGTATGC GGCGACCGAG
bla

6201 TTGCTCTTGC CCGGCGTCAA TACGGGATAA TACCGCGCCA CATAGCAGAA CTTTAAAAGT GCTCATCATT GGAAAACGTT CTTCGGGGCG AAAACTCTCA
bla

6301 AGGATCTTAC CGCTGTTGAG ATCCAGTTCG ATGTAACCCA CTCGTGCACC CAACTGATCT TCAGCATCTT TTACTTTCAC CAGCGTTTCT GGGTGAGCAA
bla

6401 AAACAGGAAG GCAAAATGCC GCAAAAAAGG GAATAAGGGC GACACGGAAA TGTTGAATAC TCATACTCTT CCTTTTTCAA TATTATTGAA GCATTTATCA
bla

6501 GGGTTATTGT CTCATGAGCG GATACATATT TGAATGTATT TAGAAAAATA AACAAATAGG GGTTCCGCGC ACATTTCCCC GAAAAGTGCC ACCTGACGTC
6601 TAAGAAACCA TTATTATCAT GACATTAACC TATAAAAATA GGCGTATCAC GAGGCCCTTT CGTC



Upstream HR
dTri10P_U_S
1 GGGTAACGCC AGGGTTTTCC CAGTCACGAC GTTGTAAAAC GACGGCCAGT GCCAAGCTTG CATGCCTGCA GGTCGACTCT AGAGTTTGTT CCAAGAGCCG
Vb_AS

Upstream HR
dTri10P_U_S
101 GATGTTTTCT GATATCTGTA GCCTTGCAGG GAATGAGAGA GCACGTCCAT ACGTCAAGGT CTCTCTTCAC GACTGTCTGG TTGGGGACGC TATTCGCATT

Upstream HR
201 GACTTTGGAT CAGTCTTTAG GCCTAACAAT ACAATCTTGA CTAACAACTG CATACGGGTC AAGATATTTG CCATCTAATG ATATATAGTT AATAGCAACA

Tri5
Upstream HR
301 GAACTTTGTA ATTAGAGAAT GACAACGACT TGACTTGTTA ATTTATTGAA TAACTGTTAC CAGTACAACC TTGCCATCAT GGAAAACTTT CCCACCGAGT

Tri5
Upstream HR
401 ATTTTCTCAA CACTAGCGTG CGCCTTCTCG AGTATATTCG ATACCGAGAC AGCAATTACA CCCGAGAGGA GCGCATCGAG AATTTGCACT ATGCTTACAA

Tri5
Upstream HR
501 CAAGGCTGCC CACCACTTTG CTCAGCCTCG CCAACAGCAG ATGCTCAAGG TAGACCCTAA GCGACTACAG GCTTCCCTCC AAACAATCGT TGGCATGGTT

Tri5
Upstream HR
601 GTATACAGCT GGGCAAAGGT GTCCAAAGAG TGCATGGCGG ATCTATCTAT TCACTACACC TATACTCTCG TTTTGGATGA CAGCAGCGAT GATCCCCATC

Tri5
Upstream HR
701 CTGCCATGTT GAACTATTTT GACGACCTTC AAGCCGGACG AGAGCAGGCC CATCCATGGT GGGCACTTGT CAACGAGCAC TTTCCCAACG TCCTTCGCCA

Tri5
Upstream HR
801 TTTTGGACCT TTCTGCTCAT TGAACCTTAT CCGTAGCACT ATGGACTGTA AGATACCGAT TCTATTATTC ACCCGTATGA AGAAGCTAAC GGCGGTGGAA

Tri5
Upstream HR
901 CAACAGTTTT TGAGGGATGT TGGATTGAGC AGTACAACTT TGGAGGATTC CCAGGATCTG ATGACTACCC TCAATTCCTT CGTCGTATGA ATGGTTTGGG

Tri5
Upstream HR
1001 TCATTGTGTT GGGGCTTCTC TATGGCCCAA GGACCTGTTT GATGAGCGGA AGCATTTCCT TGAAATCACG TCAGCCGTTG CTCAGATGGA GAACTGGATG

Tri5
Upstream HR
1101 GTTTGGGTCA ATGATCTCAT GTCATTCTAC AAGGAATTCG ACGATGAGCG TGACCAAATC AGTCTGGTCA AGAACTTTGT CACCTGCCAT GAGATCACTC

Tri5
Upstream HR
1201 TGGATGAAGC TTTGGAGAAG CTCACCCAGG AAACCCTACA CTCGTCTAAG CAGATGGTTG CTGTCTTCTC GGACAAGGAC CCTCAGGTGA TGGACACGAT

Tri5
Upstream HR
1301 TGAGTGTTTC ATGCATGGCT ACGTCACGTG GCACTTGTGC GACGCTCGAT ACCGCCTCCA TGAGATTTAT GAAAAGGTCA AGGATCAGGA TACAGAGGAC

Tri5
Upstream HR
1401 GCCAAGAAGT TCTGCAAGTT CTTTGAGCAG GCGGCCAATG TCGGCGCCGT TGCACCCTCG GAGTGGGCTT ATCCACAAGT TGCACAACTG GCAAACGTTC

hphtkC2_S (marker bar over the seventh to tenth blocks)
Tri5 (marker bar over the first six blocks)
Upstream HR (marker bar over the first six blocks); GPD promoter (marker bar over the seventh to tenth blocks)
1501 GGGCCAAGGA CGATGTGAAG GAGGCTCAGA AGCCCATCCT AAGTTCAATT GAGCTAGTGG AGTAACCCCC GGTGACTCTT TCTGGCATGC GGAGAGACGG
dTri10P-U_AS (marker bar under the fifth to eighth blocks)

GPD promoter
1601 ACGGACGCAG AGAGAAGGGC TGAGTAATAA GCGCCACTGC GCCAGACAGC TCTGGCGGCT CTGAGGTGCA GTGGATGATT ATTAATCCGG GACCGGCCGC

GPD promoter
1701 CCCTCCGCCC CGAAGTGGAA AGGCTGGTGT GCCCCTCGTT GACCAAGAAT CTATTGCATC ATCGGAGAAT ATGGAGCTTC ATCGAATCAC CGGCAGTAAG

GPD promoter
1801 CGAAGGAGAA TGTGAAGCCA GGGGTGTATA GCCGTCGGCG AAATAGCATG CCATTAACCT AGGTACAGAA GTCCAATTGC TTCCGATCTG GTAAAAGATT

GPD promoter
1901 CACGAGATAG TACCTTCTCC GAAGTAGGTA GAGCGAGTAC CCGGCGCGTA AGCTCCCTAA TTGGCCCATC CGGCATCTGT AGGGCGTCCA AATATCGTGC

GPD promoter
2001 CTCTCCTGCT TTGCCCGGTG TATGAAACCG GAAAGGCCGC TCAGGAGCTG GCCAGCGGCG CAGACCGGGA ACACAAGCTG GCAGTCGACC CATCCGGTGC

GPD promoter
2101 TCTGCACTCG ACCTGCTGAG GTCCCTCAGT CCCTGGTAGG CAGCTTTGCC CCGTCTGTCC GCCCGGTGTG TCGGCGGGGT TGACAAGGTC GTTGCGTCAG

GPD promoter
2201 TCCAACATTT GTTGCCATAT TTTCCTGCTC TCCCCACCAG CTGCTCTTTT CTTTTCTCTT TCTTTTCCCA TCTTCAGTAT ATTCATCTTC CCATCCAAGA

GPD promoter
2301 ACCTTTATTT CCCCTAAGTA AGTACTTTGC TACATCCATA CTCCATCCTT CCCATCCCTT ATTCCTTTGA ACCTTTCAGT TCGAGCTTTC CCACTTCATC

GPD promoter (marker bar over the first three blocks); hph (marker bar from the fourth block to the end of the row)
2401 GCAGCTTGAC TAACAGCTAC CCCGCTTGAG ATCGATATGA AAAAGCCTGA ACTCACCGCG ACGTCTGTCG AGAAGTTTCT GATCGAAAAG TTCGACAGCG

hph
2501 TCTCCGACCT GATGCAGCTC TCGGAGGGCG AAGAATCTCG TGCTTTCAGC TTCGATGTAG GAGGGCGTGG ATATGTCCTG CGGGTAAATA GCTGCGCCGA

hph
2601 TGGTTTCTAC AAAGATCGTT ATGTTTATCG GCACTTTGCA TCGGCCGCGC TCCCGATTCC GGAAGTGCTT GACATTGGGG AGTTCAGCGA GAGCCTGACC

hph
2701 TATTGCATCT CCCGCCGTGC ACAGGGTGTC ACGTTGCAAG ACCTGCCTGA AACCGAACTG CCCGCTGTTC TCCAGCCGGT CGCGGAGGCC ATGGATGCGA

hph
2801 TCGCTGCGGC CGATCTTAGC CAGACGAGCG GGTTCGGCCC ATTCGGACCG CAAGGAATCG GTCAATACAC TACATGGCGT GATTTCATAT GCGCGATTGC

hph
2901 TGATCCCCAT GTGTATCACT GGCAAACTGT GATGGACGAC ACCGTCAGTG CGTCCGTCGC GCAGGCTCTC GATGAGCTGA TGCTTTGGGC CGAGGACTGC

hph
3001 CCCGAAGTCC GGCACCTCGT GCATGCGGAT TTCGGCTCCA ACAATGTCCT GACGGACAAT GGCCGCATAA CAGCGGTCAT TGACTGGAGC GAGGCGATGT

hph
3101 TCGGGGATTC CCAATACGAG GTCGCCAACA TCCTCTTCTG GAGGCCGTGG TTGGCTTGTA TGGAGCAGCA GACGCGCTAC TTCGAGCGGA GGCATCCGGA

hph
3201 GCTTGCAGGA TCGCCGCGCC TCCGGGCGTA TATGCTCCGC ATTGGTCTTG ACCAACTCTA TCAGAGCTTG GTTGACGGCA ATTTCGATGA TGCAGCTTGG

hph
3301 GCGCAGGGTC GATGCGACGC AATCGTCCGA TCCGGAGCCG GGACTGTCGG GCGTACACAA ATCGCCCGCA GAAGCGCGGC CGTCTGGACC GATGGCTGTG

HSVtk (marker bar from the seventh block to the end of the row)
hph (marker bar over the first six blocks)
3401 TAGAAGTACT CGCCGATAGT GGAAACCGAC GCCCCAGCAC TCGTCCGAGG GCAAAGGAAA TGGCTTCGTA CCCCGGCCAT CAACACGCGT CTGCGTTCGA

HSVtk
3501 CCAGGCTGCG CGTTCTCGCG GCCATAGCAA CCGACGTACG GCGTTGCGCC CTCGCCGGCA GCAAGAAGCC ACGGAAGTCC GCCCGGAGCA GAAAATGCCC

HSVtk
3601 ACGCTACTGC GGGTTTATAT AGACGGTCCC CACGGGATGG GGAAAACCAC CACCACGCAA CTGCTGGTGG CCCTGGGTTC GCGCGACGAT ATCGTCTACG

HSVtk
3701 TACCCGAGCC GATGACTTAC TGGCGGGTGC TGGGGGCTTC CGAGACAATC GCGAACATCT ACACCACACA ACACCGCCTC GACCAGGGTG AGATATCGGC

HSVtk
3801 CGGGGACGCG GCGGTGGTAA TGACAAGCGC CCAGATAACA ATGGGCATGC CTTATGCCGT GACCGACGCC GTTCTGGCTC CTCATATCGG GGGGGAGGCT

HSVtk
3901 GGGAGCTCAC ATGCCCCGCC CCCGGCCCTC ACCCTCATCT TCGACCGCCA TCCCATCGCC GCCCTCCTGT GCTACCCGGC CGCGCGGTAC CTTATGGGCA

HSVtk
4001 GCATGACCCC CCAGGCCGTG CTGGCGTTCG TGGCCCTCAT CCCGCCGACC TTGCCCGGCA CCAACATCGT GCTTGGGGCC CTTCCGGAGG ACAGACACAT

HSVtk
4101 CGACCGCCTG GCCAAACGCC AGCGCCCCGG CGAGCGGCTG GACCTGGCTA TGCTGGCTGC GATTCGCCGC GTTTACGGGC TACTTGCCAA TACGGTGCGG

HSVtk
4201 TATCTGCAGT GCGGCGGGTC GTGGCGGGAG GACTGGGGAC AGCTTTCGGG GACGGCCGTG CCGCCCCAGG GTGCCGAGCC CCAGAGCAAC GCGGGCCCAC

HSVtk
4301 GACCCCATAT CGGGGACACG TTATTTACCC TGTTTCGGGC CCCCGAGTTG CTGGCCCCCA ACGGCGACCT GTATAACGTG TTTGCCTGGG CCTTGGACGT

HSVtk
4401 CTTGGCCAAA CGCCTCCGTT CCATGCACGT CTTTATCCTG GATTACGACC AATCGCCCGC CGGCTGCCGG GACGCCCTGC TGCAACTTAC CTCCGGGATG

dTri10P-D_S
Downstream HR
HSVtk Tri10
4501 GTCCAGACCC ACGTCACCAC CCCCGGCTCC ATACCGACGA TATGCGACCT GGCGCGCACG TTTGCCCGGG AGATGGGGGA GGCTAACTGA CATGGATTTC
hphtkC2_AS

Downstream HR
Tri10
dTri10P-D_S
4601 CCAAAGCCTA GACAAGTCCG AGAGACGAGC CTGTTGATGT ACTACCTAGA CGTCGTGTTT CCTCTGCAAT GCATCAACCC AAACAACAAT TGTCTGGGAA

Tri10
Downstream HR
4701 AGAGAGAGTG GCTGTTGACT ATACTGACCT CTGCGCGGCC TACGTACTAT GCCACATTGT GCATGTCGCT CCTCTATAAA GAATCGCTTT CAAGCCCTTG

Tri10
Downstream HR
4801 CAGATCTGAA CAGGCGATGG TATGGAAGAG AGAGAAGACA TACTACTACA TTCTTGCACT CCAGGAGTCT CAGAAGCTGC TGGGTGGGCT CGACAAGACA

Tri10
Downstream HR
4901 TTTGGCATCA CAAGGCTGAA AGGTACCGTC GTTGCCCTTG CTTGCATGCT ACAGCTTATC AGTTTTGAGG TAAGACGAAT CCACCATTGT TTCGATGCTC

Tri10
Downstream HR
5001 GATGTCGATG CTCGATATCC GATCTACGAT TATCGTTGGT CACTAACAAA TTAAAATAGT CTTCGCACCT AAGCAGGGGA GATTGGCGCG TTCACCTCCA

Tri10
Downstream HR
5101 TGCGGCCAAC ATACTCATTC CTGTCTTGGT TGAGGGATGG TCCACAGCTT TGCAATCAGG CCCCCCAGCC ACCTCCATAT GGTGCGAGCT GGATGAATCA

Tri10
Downstream HR
5201 CACTTCGGCT CGACTGAAGA TCAAACCTCT TTGAGCTTCG AATACGTCGG AGCTTTGAGA TTCCTGTCAA ACTCACTCGC CGCAGTCGGC ATCCTGTCTT

Tri10
Downstream HR
5301 GCATATCTAT TGGCCCATCA GCACCATTTG AAGATTACGG CCATCTCCTG GACCAGCCAG GCCTTATACA GCTGGACGAG GTGCTGGGGT GCAGGAATTG

Tri10
Downstream HR
5401 GACCATGTTG ACTATTCTCG AAGTGGGTAA GCTGGATCGT TGGAAGCGAC AGGAGCAAGA ACATAATCGC TTGAGCCTAA AGACGCTCGC TAGGCGCGCC

Tri10
Downstream HR
5501 ATGATGATTG AGGATATGTT GTCAGACGAG CTACAAAGGC TACCGACAGA CGAGACGCTT CCAGACCTCA TCACTCAGAT TTACGCCGCC TCTATCATGA

Tri10
Downstream HR
5601 CGTATCTGCA TACAGTAGTT TCCGGACTCA ATCCCAACCT TTCAGAGGTT CAGGATAGTG TGGCCGGGAC GCTTCAATTG TTGGAGAGGC TCCCAAATCT

Tri10
Downstream HR
5701 TGAAGCTGTC ACGAGCGTTA CTTGGCCTCT AGCTGTCACA GGATGCATGG CCTCAGAAAG TCATAAGGAC TTTTTCAGAA ATACTCTGAG GTCGTATGAG

Tri10
Downstream HR
5801 GCGACATTCA GCTCCTTAAA AAAGTATGAC GGAACTCTTC AGGTCTTGGA AGACGCTTGG AAGAGAAGAG AGATAGATAC AGAGTCTCCA ATGAGATGGG

Tri10
Downstream HR
5901 AAGACTTGAC GGATCACCAT GGGCTTCCAG TGCTACTTTG GTAGGGATGG TATCGGCGCA GAGACTACGG ATACCGATAC CCGAAGTCTT TCTTCTGCAT

Downstream HR
6001 CAGCTACCCG ATACATGTAA GTTTGATGCT CACTTACAGA TGATGCATGC AGGGAAGTTT TATTAGATGT TATGGGTTCG ACTCATTTTG GCGTTTAAAC

Downstream HR
6101 AGGGATTGGC ATGATGCATG ATTTAAATGG AATAGAAGAG CGGAAAACGT TTCCCATGTA ACTTGAATGA TGGACTTTCA ATATATATTT CTAGTATACT

Downstream HR
6201 TTTTTAACCG TGAACCGCAT CTTCTCCATC TATCCAACCA ATCGTGCTGA AAGCAGTCTG GAACCATGGT GGGCGAATCA GTGCTGTGTC CCTTGATTGT

Vb_S
Downstream HR
6301 CTCGACATGG CTTGACATTG TTTAGCCCGT ACGACAAACG ATATTGATCA TAGTGAAGGA GTGTTGATTT CAAGCGAGGG GATTTGTCGA AGTTCGGGGT
dTri10P-D_AS

Vb_S
6401 ACCGAGCTCG AATTCGTAAT CATGGTCATA GCTGTTTCCT GTGTGAAATT GTTATCCGCT CACAATTCCA CACAACATAC GAGCCGGAAG CATAAAGTGT
dTri10P-D_AS

6501 AAAGCCTGGG GTGCCTAATG AGTGAGCTAA CTCACATTAA TTGCGTTGCG CTCACTGCCC GCTTTCCAGT CGGGAAACCT GTCGTGCCAG CTGCATTAAT
6601 GAATCGGCCA ACGCGCGGGG AGAGGCGGTT TGCGTATTGG GCGCTCTTCC GCTTCCTCGC TCACTGACTC GCTGCGCTCG GTCGTTCGGC TGCGGCGAGC
6701 GGTATCAGCT CACTCAAAGG CGGTAATACG GTTATCCACA GAATCAGGGG ATAACGCAGG AAAGAACATG TGAGCAAAAG GCCAGCAAAA GGCCAGGAAC
6801 CGTAAAAAGG CCGCGTTGCT GGCGTTTTTC CATAGGCTCC GCCCCCCTGA CGAGCATCAC AAAAATCGAC GCTCAAGTCA GAGGTGGCGA AACCCGACAG
6901 GACTATAAAG ATACCAGGCG TTTCCCCCTG GAAGCTCCCT CGTGCGCTCT CCTGTTCCGA CCCTGCCGCT TACCGGATAC CTGTCCGCCT TTCTCCCTTC
7001 GGGAAGCGTG GCGCTTTCTC ATAGCTCACG CTGTAGGTAT CTCAGTTCGG TGTAGGTCGT TCGCTCCAAG CTGGGCTGTG TGCACGAACC CCCCGTTCAG
7101 CCCGACCGCT GCGCCTTATC CGGTAACTAT CGTCTTGAGT CCAACCCGGT AAGACACGAC TTATCGCCAC TGGCAGCAGC CACTGGTAAC AGGATTAGCA
7201 GAGCGAGGTA TGTAGGCGGT GCTACAGAGT TCTTGAAGTG GTGGCCTAAC TACGGCTACA CTAGAAGGAC AGTATTTGGT ATCTGCGCTC TGCTGAAGCC
7301 AGTTACCTTC GGAAAAAGAG TTGGTAGCTC TTGATCCGGC AAACAAACCA CCGCTGGTAG CGGTGGTTTT TTTGTTTGCA AGCAGCAGAT TACGCGCAGA

7401 AAAAAAGGAT CTCAAGAAGA TCCTTTGATC TTTTCTACGG GGTCTGACGC TCAGTGGAAC GAAAACTCAC GTTAAGGGAT TTTGGTCATG AGATTATCAA

7501 AAAGGATCTT CACCTAGATC CTTTTAAATT AAAAATGAAG TTTTAAATCA ATCTAAAGTA TATATGAGTA AACTTGGTCT GACAGTTACC AATGCTTAAT

AhdI bla

7601 CAGTGAGGCA CCTATCTCAG CGATCTGTCT ATTTCGTTCA TCCATAGTTG CCTGACTCCC CGTCGTGTAG ATAACTACGA TACGGGAGGG CTTACCATCT

bla

7701 GGCCCCAGTG CTGCAATGAT ACCGCGAGAC CCACGCTCAC CGGCTCCAGA TTTATCAGCA ATAAACCAGC CAGCCGGAAG GGCCGAGCGC AGAAGTGGTC

bla

7801 CTGCAACTTT ATCCGCCTCC ATCCAGTCTA TTAATTGTTG CCGGGAAGCT AGAGTAAGTA GTTCGCCAGT TAATAGTTTG CGCAACGTTG TTGCCATTGC

bla

7901 TACAGGCATC GTGGTGTCAC GCTCGTCGTT TGGTATGGCT TCATTCAGCT CCGGTTCCCA ACGATCAAGG CGAGTTACAT GATCCCCCAT GTTGTGCAAA

bla

8001 AAAGCGGTTA GCTCCTTCGG TCCTCCGATC GTTGTCAGAA GTAAGTTGGC CGCAGTGTTA TCACTCATGG TTATGGCAGC ACTGCATAAT TCTCTTACTG

bla

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bla

8201 AATACGGGAT AATACCGCGC CACATAGCAG AACTTTAAAA GTGCTCATCA TTGGAAAACG TTCTTCGGGG CGAAAACTCT CAAGGATCTT ACCGCTGTTG

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bla

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bla

8501 CGGATACATA TTTGAATGTA TTTAGAAAAA TAAACAAATA GGGGTTCCGC GCACATTTCC CCGAAAAGTG CCACCTGACG TCTAAGAAAC CATTATTATC

8601 ATGACATTAA CCTATAAAAA TAGGCGTATC ACGAGGCCCT TTCGTCTCGC GCGTTTCGGT GATGACGGTG AAAACCTCTG ACACATGCAG CTCCCGGAGA

8701 CGGTCACAGC TTGTCTGTAA GCGGATGCCG GGAGCAGACA AGCCCGTCAG GGCGCGTCAG CGGGTGTTGG CGGGTGTCGG GGCTGGCTTA ACTATGCGGC

8801 ATCAGAGCAG ATTGTACTGA GAGTGCACCA TATGCGGTGT GAAATACCGC ACAGATGCGT AAGGAGAAAA TACCGCATCA GGCGCCATTC GCCATTCAGG

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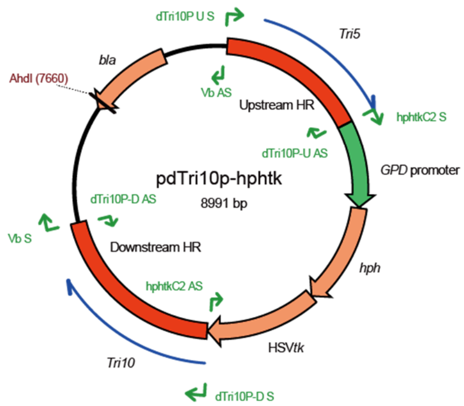

Tri5 partial CDS

SbfI Upstream HR

SwTri10P-U_S

1 GGGTAACGCC AGGGTTTTCC CAGTCACGAC GTTGTAAAAC GACGGCCAGT GCCAAGCTTG CATGCCTGCA GGTCGACTCT AGAGCAGTAC AACTTTGGAG

Vb_AS

Tri5 partial CDS

Upstream HR

SwTri10P-U_S

101 GATTCCCAGG ATCTGATGAC TACCCTCAAT TCCTTCGTCG TATGAATGGT TTGGGTCATT GTGTTGGGGC TTCTCTATGG CCCAAGGACC TGTTTGATGA

Upstream HR

Tri5 partial CDS

201 GCGGAAGCAT TTCCTTGAAA TCACGTCAGC CGTTGCTCAG ATGGAGAACT GGATGGTTTG GGTCAATGAT CTCATGTCAT TCTACAAGGA ATTCGACGAT

Upstream HR

Tri5 partial CDS

301 GAGCGTGACC AAATCAGTCT GGTCAAGAAC TTTGTCACCT GCCATGAGAT CACTCTGGAT GAAGCTTTGG AGAAGCTCAC CCAGGAAACC CTACACTCGT

Upstream HR

Tri5 partial CDS

401 CTAAGCAGAT GGTTGCTGTC TTCTCGGACA AGGACCCTCA GGTGATGGAC ACGATTGAGT GTTTCATGCA TGGCTACGTC ACGTGGCACT TGTGCGACGC

Upstream HR

Tri5 partial CDS

501 TCGATACCGC CTCCATGAGA TTTATGAAAA GGTCAAGGAT CAGGATACAG AGGACGCCAA GAAGTTCTGC AAGTTCTTTG AGCAGGCGGC CAATGTCGGC

Upstream HR

Tri5 partial CDS

601 GCCGTTGCAC CCTCGGAGTG GGCTTATCCA CAAGTTGCAC AACTGGCAAA CGTTCGGGCC AAGGACGATG TGAAGGAGGC TCAGAAGCCC ATCCTAAGTT

Tri10 promoter

Upstream HR

Tri5 partial CDS

701 CAATTGAGCT AGTGGAGTAA CCGAAGGCGA GTTTGGAAGT ATGTTTTGCG GGTACGGATA CTCGTTTGGA GAATGGTGGT CTGTTATAAT GATTACAAAT

Tri10 promoter

801 AGTTCGGTCG TGTTTTGTTA GAATGAACAG TTGAACAAGG ATAATTACTT CGGAATAGGC AGTTGAAACT GAATGTCTGT ACGTAACCTG AGCCTGTAAC

Tri10 promoter

901 CATTTCCCAC TTGAGTGCAG GCTTTTGCGT AACCAAGTCT GTACACCCGT CGGTGCGACA GGGCTACCCC CAACCCTGCA ACTGCATCTG CAGCTGCAGC

Tri10 promoter

1001 TGGCAGACTG GTAGACTGGC GCTACGAACT GTAGTGCGAT GCGGGAATCT TGTACCCGCT CGGAGGTTGG AGGGAGCTGG CACATTCTCT AGACCCGCGA

Tri10 promoter

1101 ATTGATCTTC AAAGCGCTTG CGTTTTGTCC AGATCAGTGA CCATACCCTT GCCTTTTCGC ACCACCCAAA CGTCCACTGA ACGAGGCGTA CAGAAACCAC

Tri10 promoter
1201 ACAAGATAAG GTTTGATGCC TGCTTGAGCA CTATGAGGGA CACGACACTT CTGTAAAACT CTATCCTTGC ATTATATTGT AACATCGTTT AACTTCTCCA

gpd10P_S
GPD promoter
Tri10 promoter
1301 CGCCCATTCC TTCCCGTCTT TCCCCCAACT TCAATTGTAT GCCAACCAAC AATCATCAAA TTATCATTAT TATTGTCGTT AGTCATCCCG GTGACTCTTT
JTri10P_AS

GPD promoter
gpd10P_S
1401 CTGGCATGCG GAGAGACGGA CGGACGCAGA GAGAAGGGCT GAGTAATAAG CGCCACTGCG CCAGACAGCT CTGGCGGCTC TGAGGTGCAG TGGATGATTA

GPD promoter
1501 TTAATCCGGG ACCGGCCGCC CCTCCGCCCC GAAGTGGAAA GGCTGGTGTG CCCCTCGTTG ACCAAGAATC TATTGCATCA TCGGAGAATA TGGAGCTTCA

GPD promoter
1601 TCGAATCACC GGCAGTAAGC GAAGGAGAAT GTGAAGCCAG GGGTGTATAG CCGTCGGCGA AATAGCATGC CATTAACCTA GGTACAGAAG TCCAATTGCT

GPD promoter
1701 TCCGATCTGG TAAAAGATTC ACGAGATAGT ACCTTCTCCG AAGTAGGTAG AGCGAGTACC CGGCGCGTAA GCTCCCTAAT TGGCCCATCC GGCATCTGTA

GPD promoter
1801 GGGCGTCCAA ATATCGTGCC TCTCCTGCTT TGCCCGGTGT ATGAAACCGG AAAGGCCGCT CAGGAGCTGG CCAGCGGCGC AGACCGGGAA CACAAGCTGG

GPD promoter
1901 CAGTCGACCC ATCCGGTGCT CTGCACTCGA CCTGCTGAGG TCCCTCAGTC CCTGGTAGGC AGCTTTGCCC CGTCTGTCCG CCCGGTGTGT CGGCGGGGTT

GPD promoter
2001 GACAAGGTCG TTGCGTCAGT CCAACATTTG TTGCCATATT TTCCTGCTCT CCCCACCAGC TGCTCTTTTC TTTTCTCTTT CTTTTCCCAT CTTCAGTATA

GPD promoter
2101 TTCATCTTCC CATCCAAGAA CCTTTATTTC CCCTAAGTAA GTACTTTGCT ACATCCATAC TCCATCCTTC CCATCCCTTA TTCCTTTGAA CCTTTCAGTT

Downstream HR
GPD promoter
Tri10
JTri10P_S
2201 CGAGCTTTCC CACTTCATCG CAGCTTGACT AACAGCTACC CCGCTTGAGA TGGATTTCCC AAAGCCTAGA CAAGTCCGAG AGACGAGCCT GTTGATGTAC
gpd10P_AS

Tri10
Downstream HR
2301 TACCTAGACG TCGTGTTTCC TCTGCAATGC ATCAACCCAA ACAACAATTG TCTGGGAAAG AGAGAGTGGC TGTTGACTAT ACTGACCTCT GCGCGGCCTA

Tri10
Downstream HR
2401 CGTACTATGC CACATTGTGC ATGTCGCTCC TCTATAAAGA ATCGCTTTCA AGCCCTTGCA GATCTGAACA GGCGATGGTA TGGAAGAGAG AGAAGACATA

Tri10
Downstream HR
2501 CTACTACATT CTTGCACTCC AGGAGTCTCA GAAGCTGCTG GGTGGGCTCG ACAAGACATT TGGCATCACA AGGCTGAAAG GTACCGTCGT TGCCCTTGCT

Tri10
Downstream HR
2601 TGCATGCTAC AGCTTATCAG TTTTGAGGTA AGACGAATCC ACCATTGTTT CGATGCTCGA TGTCGATGCT CGATATCCGA TCTACGATTA TCGTTGGTCA

Tri10
Downstream HR
2701 CTAACAAATT AAAATAGTCT TCGCACCTAA GCAGGGGAGA TTGGCGCGTT CACCTCCATG CGGCCAACAT ACTCATTCCT GTCTTGGTTG AGGGATGGTC

Tri10
Downstream HR
2801 CACAGCTTTG CAATCAGGCC CCCCAGCCAC CTCCATATGG TGCGAGCTGG ATGAATCACA CTTCGGCTCG ACTGAAGATC AAACCTCTTT GAGCTTCGAA

Tri10
Downstream HR
2901 TACGTCGGAG CTTTGAGATT CCTGTCAAAC TCACTCGCCG CAGTCGGCAT CCTGTCTTGC ATATCTATTG GCCCATCAGC ACCATTTGAA GATTACGGCC

Tri10
Downstream HR
3001 ATCTCCTGGA CCAGCCAGGC CTTATACAGC TGGACGAGGT GCTGGGGTGC AGGAATTGGA CCATGTTGAC TATTCTCGAA GTGGGTAAGC TGGATCGTTG

Tri10
Downstream HR
3101 GAAGCGACAG GAGCAAGAAC ATAATCGCTT GAGCCTAAAG ACGCTCGCTA GGCGCGCCAT GATGATTGAG GATATGTTGT CAGACGAGCT ACAAAGGCTA

Tri10
Downstream HR
3201 CCGACAGACG AGACGCTTCC AGACCTCATC ACTCAGATTT ACGCCGCCTC TATCATGACG TATCTGCATA CAGTAGTTTC CGGACTCAAT CCCAACCTTT

Tri10
Downstream HR
3301 CAGAGGTTCA GGATAGTGTG GCCGGGACGC TTCAATTGTT GGAGAGGCTC CCAAATCTTG AAGCTGTCAC GAGCGTTACT TGGCCTCTAG CTGTCACAGG

Tri10
Downstream HR
3401 ATGCATGGCC TCAGAAAGTC ATAAGGACTT TTTCAGAAAT ACTCTGAGGT CGTATGAGGC GACATTCAGC TCCTTAAAAA AGTATGACGG AACTCTTCAG

Tri10
Downstream HR
3501 GTCTTGGAAG ACGCTTGGAA GAGAAGAGAG ATAGATACAG AGTCTCCAAT GAGATGGGAA GACTTGACGG ATCACCATGG GCTTCCAGTG CTACTTTGGT

Tri10
Downstream HR
3601 AGGGATGGTA TCGGCGCAGA GACTACGGAT ACCGATACCC GAAGTCTTTC TTCTGCATCA GCTACCCGAT ACATGTAAGT TTGATGCTCA CTTACAGATG

Downstream HR
3701 ATGCATGCAG GGAAGTTTTA TTAGATGTTA TGGGTTCGAC TCATTTTGGC GTTTAAACAG GGATTGGCAT GATGCATGAT TTAAATGGAA TAGAAGAGCG

Downstream HR
3801 GAAAACGTTT CCCATGTAAC TTGAATGATG GACTTTCAAT ATATATTTCT AGTATACTTT TTTAACCGTG AACCGCATCT TCTCCATCTA TCCAACCAAT

Downstream HR
3901 CGTGCTGAAA GCAGTCTGGA ACCATGGTGG GCGAATCAGT GCTGTGTCCC TTGATTGTCT CGACATGGCT TGACATTGTT TAGCCCGTAC GACAAACGAT

Downstream HR
Vb_S
4001 ATTGATCATA GTGAAGGAGT GTTGATTTCA AGCGAGGGGA TTTGTCGAAG TTCGGGGTAC CGAGCTCGAA TTCGTAATCA TGGTCATAGC TGTTTCCTGT
dTri10P-D_AS

4101 GTGAAATTGT TATCCGCTCA CAATTCCACA CAACATACGA GCCGGAAGCA TAAAGTGTAA AGCCTGGGGT GCCTAATGAG TGAGCTAACT CACATTAATT
4201 GCGTTGCGCT CACTGCCCGC TTTCCAGTCG GGAAACCTGT CGTGCCAGCT GCATTAATGA ATCGGCCAAC GCGCGGGGAG AGGCGGTTTG CGTATTGGGC

4301 GCTCTTCCGC TTCCTCGCTC ACTGACTCGC TGCGCTCGGT CGTTCGGCTG CGGCGAGCGG TATCAGCTCA CTCAAAGGCG GTAATACGGT TATCCACAGA
4401 ATCAGGGGAT AACGCAGGAA AGAACATGTG AGCAAAAGGC CAGCAAAAGG CCAGGAACCG TAAAAAGGCC GCGTTGCTGG CGTTTTTCCA TAGGCTCCGC
4501 CCCCCTGACG AGCATCACAA AAATCGACGC TCAAGTCAGA GGTGGCGAAA CCCGACAGGA CTATAAAGAT ACCAGGCGTT TCCCCCTGGA AGCTCCCTCG
4601 TGCGCTCTCC TGTTCCGACC CTGCCGCTTA CCGGATACCT GTCCGCCTTT CTCCCTTCGG GAAGCGTGGC GCTTTCTCAT AGCTCACGCT GTAGGTATCT
4701 CAGTTCGGTG TAGGTCGTTC GCTCCAAGCT GGGCTGTGTG CACGAACCCC CCGTTCAGCC CGACCGCTGC GCCTTATCCG GTAACTATCG TCTTGAGTCC
4801 AACCCGGTAA GACACGACTT ATCGCCACTG GCAGCAGCCA CTGGTAACAG GATTAGCAGA GCGAGGTATG TAGGCGGTGC TACAGAGTTC TTGAAGTGGT
4901 GGCCTAACTA CGGCTACACT AGAAGGACAG TATTTGGTAT CTGCGCTCTG CTGAAGCCAG TTACCTTCGG AAAAAGAGTT GGTAGCTCTT GATCCGGCAA
5001 ACAAACCACC GCTGGTAGCG GTGGTTTTTT TGTTTGCAAG CAGCAGATTA CGCGCAGAAA AAAAGGATCT CAAGAAGATC CTTTGATCTT TTCTACGGGG
5101 TCTGACGCTC AGTGGAACGA AAACTCACGT TAAGGGATTT TGGTCATGAG ATTATCAAAA AGGATCTTCA CCTAGATCCT TTTAAATTAA AAATGAAGTT
5201 TTAAATCAAT CTAAAGTATA TATGAGTAAA CTTGGTCTGA CAGTTACCAA TGCTTAATCA GTGAGGCACC TATCTCAGCG ATCTGTCTAT TTCGTTCATC

bla

5301 CATAGTTGCC TGACTCCCCG TCGTGTAGAT AACTACGATA CGGGAGGGCT TACCATCTGG CCCCAGTGCT GCAATGATAC CGCGAGACCC ACGCTCACCG

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5401 GCTCCAGATT TATCAGCAAT AAACCAGCCA GCCGGAAGGG CCGAGCGCAG AAGTGGTCCT GCAACTTTAT CCGCCTCCAT CCAGTCTATT AATTGTTGCC

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5601 ATTCAGCTCC GGTTCCCAAC GATCAAGGCG AGTTACATGA TCCCCCATGT TGTGCAAAAA AGCGGTTAGC TCCTTCGGTC CTCCGATCGT TGTCAGAAGT

bla

5701 AAGTTGGCCG CAGTGTTATC ACTCATGGTT ATGGCAGCAC TGCATAATTC TCTTACTGTC ATGCCATCCG TAAGATGCTT TTCTGTGACT GGTGAGTACT

bla

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bla

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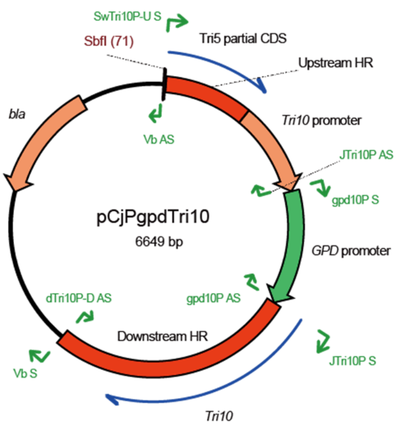

Upstream HR
Tri5 CDS partial
SbfI
SwTri10P-U_S

1 GGGTAACGCC AGGGTTTTCC CAGTCACGAC GTTGTAAAAC GACGGCCAGT GCCAAGCTTG CATGCCTGCA GGTCGACTCT AGAGCAGTAC AACTTTGGAG

Vb_AS

Upstream HR
Tri5 CDS partial
SwTri10P-U_S

101 GATTCCCAGG ATCTGATGAC TACCCTCAAT TCCTTCGTCG TATGAATGGT TTGGGTCATT GTGTTGGGGC TTCTCTATGG CCCAAGGACC TGTTTGATGA

Tri5 CDS partial
Upstream HR

201 GCGGAAGCAT TTCCTTGAAA TCACGTCAGC CGTTGCTCAG ATGGAGAACT GGATGGTTTG GGTCAATGAT CTCATGTCAT TCTACAAGGA ATTCGACGAT

Tri5 CDS partial
Upstream HR

301 GAGCGTGACC AAATCAGTCT GGTCAAGAAC TTTGTCACCT GCCATGAGAT CACTCTGGAT GAAGCTTTGG AGAAGCTCAC CCAGGAAACC CTACACTCGT

Tri5 CDS partial
Upstream HR

401 CTAAGCAGAT GGTTGCTGTC TTCTCGGACA AGGACCCTCA GGTGATGGAC ACGATTGAGT GTTTCATGCA TGGCTACGTC ACGTGGCACT TGTGCGACGC

Tri5 CDS partial
Upstream HR

501 TCGATACCGC CTCCATGAGA TTTATGAAAA GGTCAAGGAT CAGGATACAG AGGACGCCAA GAAGTTCTGC AAGTTCTTTG AGCAGGCGGC CAATGTCGGC

Tri5 CDS partial
Upstream HR

601 GCCGTTGCAC CCTCGGAGTG GGCTTATCCA CAAGTTGCAC AACTGGCAAA CGTTCGGGCC AAGGACGATG TGAAGGAGGC TCAGAAGCCC ATCCTAAGTT

Tri5 CDS partial
Tri10 promoter
Upstream HR
701 CAATTGAGCT AGTGGAGTAA CCGAAGGCGA GTTTGGAAGT ATGTTTTGCG GGTACGGATA CTCGTTTGGA GAATGGTGGT CTGTTATAAT GATTACAAAT

Tri10 promoter
801 AGTTCGGTCG TGTTTTGTTA GAATGAACAG TTGAACAAGG ATAATTACTT CGGAATAGGC AGTTGAAACT GAATGTCTGT ACGTAACCTG AGCCTGTAAC

Tri10 promoter
901 CATTTCCCAC TTGAGTGCAG GCTTTTGCGT AACCAAGTCT GTACACCCGT CGGTGCGACA GGGCTACCCC CAACCCTGCA ACTGCATCTG CAGCTGCAGC

Tri10 promoter
1001 TGGCAGACTG GTAGACTGGC GCTACGAACT GTAGTGCGAT GCGGGAATCT TGTACCCGCT CGGAGGTTGG AGGGAGCTGG CACATTCTCT AGACCCGCGA

Tri10 promoter
1101 ATTGATCTTC AAAGCGCTTG CGTTTTGTCC AGATCAGTGA CCATACCCTT GCCTTTTCGC ACCACCCAAA CGTCCACTGA ACGAGGCGTA CAGAAACCAC

Tri10 promoter
1201 ACAAGATAAG GTTTGATGCC TGCTTGAGCA CTATGAGGGA CACGACACTT CTGTAAAACT CTATCCTTGC ATTATATTGT AACATCGTTT AACTTCTCCA

tef10P_S
TEF promoter
Tri10 promoter
1301 CGCCCATTCC TTCCCGTCTT TCCCCCAACT TCAATTGTAT GCCAACCAAC AATCATCAAA TTATCATTAT TATTGTCGTT AGTCATCTAG CGACTTAGAG
JTri10P_AS

TEF promoter
tef10P_S
1401 TGCACGCTCA AAAGATACCC CTAACTTCTG ATTTGATAGC ATTTGTATAT GGCATATAGT ACGCCTGAAG CATAAAAAAA AAAACAGAAT TATTATAGTT

TEF promoter
1501 TATTAATACG AGACAGCAGA ATCACCGCCC AAGTTAAGCC TTTGTGCTGA TCATGCTCTC GAACGGGCCA AGTTCGGGAA AAGCAAAGGA GCGTTTAGTG

TEF promoter
1601 AGGGGCAATT TGACTCACCT CCCAGGCAAC AGATGAGGGG GGCAAAAAGA AAGAAATTTT CGTGAGTCAA TATGGATTCC GAGCATCATT TTCTTGCGGT

TEF promoter
1701 CTATCTTGCT ACGTATGTTG ATCTTGACGC TGTGGATCAA GCAACGCCAC TCGCTCGCTC CATCGCAGGC TGGTCGCAGA CAAATTAAAA GGCGGCAAAC

TEF promoter
1801 TCGTACAGCC GCGGGGTTGT CCGCTGCAAA GTACAGAGTG ATAAAAGCCG CCATGCGACC ATCAACGCGT TGATGCCCAG CTTTTTCGAT CCGAGAATCC

TEF promoter
1901 ACCGTAGAGG CGATAGCAAG TAAAGAAAAG CTAAACAAAA AAAAATTTCT GCCCCTAAGC CATGAAAACG AGATGGGGTG GAGCAGAACC AAGGAAAGAG

TEF promoter
2001 TCGCGCTGGG CTGCCGTTCC GGAAGGTGTT GTAAAGGCTC GACGCCCAAG GTGGGAGTCT AGGAGAAGAA TTTGCATCGG GAGTGGGGCG GGTTACCCCT

TEF promoter
2101 CCATATCCAA TGACAGATAT CTACCAGCCA AGGGTTTGAG CCCGCCCGCT TAGTCGTCGT CCTCGCTTGC CCCTCCATAA AAGGATTTCC CCTCCCCCTC

TEF promoter
2201 CCACAAAATT TTCTTTCCCT TCCTCTCCTT GTCCGCTTCA GTACGTATAT CTTCCCTTCC CTCGCTTCTC TCCTCCATCC TTCTTTCATC CATCTCCTGC

Downstream HR
Tri10
JTri10_S
TEF promoter
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tef10P_AS

Tri10
JTri10_S
Downstream HR
2401 TTCCCAAAGC CTAGACAAGT CCGAGAGACG AGCCTGTTGA TGTACTACCT AGACGTCGTG TTTCCTCTGC AATGCATCAA CCCAAACAAC AATTGTCTGG
tef10P_AS

Tri10
Downstream HR
2501 GAAAGAGAGA GTGGCTGTTG ACTATACTGA CCTCTGCGCG GCCTACGTAC TATGCCACAT TGTGCATGTC GCTCCTCTAT AAAGAATCGC TTTCAAGCCC

Tri10
Downstream HR
2601 TTGCAGATCT GAACAGGCGA TGGTATGGAA GAGAGAGAAG ACATACTACT ACATTCTTGC ACTCCAGGAG TCTCAGAAGC TGCTGGGTGG GCTCGACAAG

Tri10
Downstream HR
2701 ACATTTGGCA TCACAAGGCT GAAAGGTACC GTCGTTGCCC TTGCTTGCAT GCTACAGCTT ATCAGTTTTG AGGTAAGACG AATCCACCAT TGTTTCGATG

Tri10
Downstream HR
2801 CTCGATGTCG ATGCTCGATA TCCGATCTAC GATTATCGTT GGTCACTAAC AAATTAAAAT AGTCTTCGCA CCTAAGCAGG GGAGATTGGC GCGTTCACCT

Tri10
Downstream HR
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Tri10
Downstream HR
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Tri10
Downstream HR
3101 CTTGCATATC TATTGGCCCA TCAGCACCAT TTGAAGATTA CGGCCATCTC CTGGACCAGC CAGGCCTTAT ACAGCTGGAC GAGGTGCTGG GGTGCAGGAA

Tri10
Downstream HR
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Tri10
Downstream HR
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Downstream HR
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Tri10
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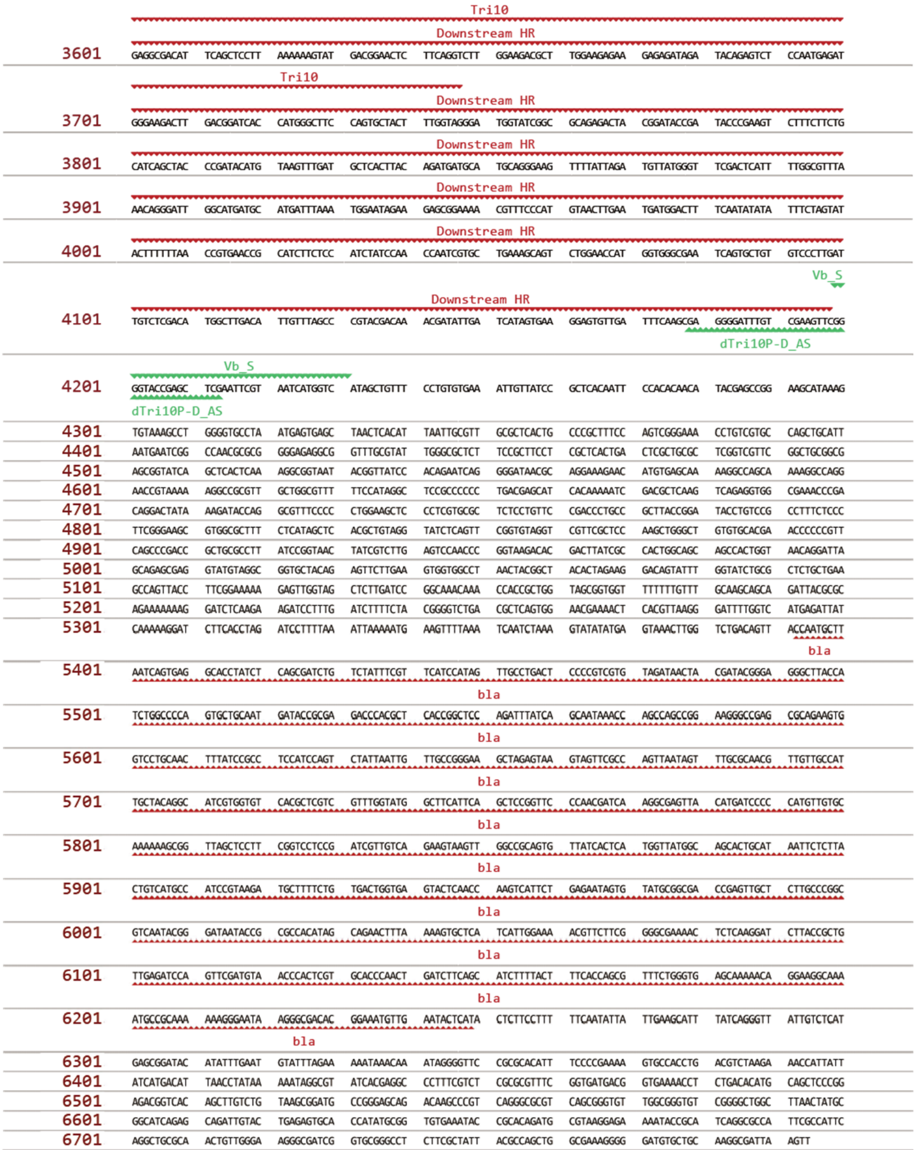
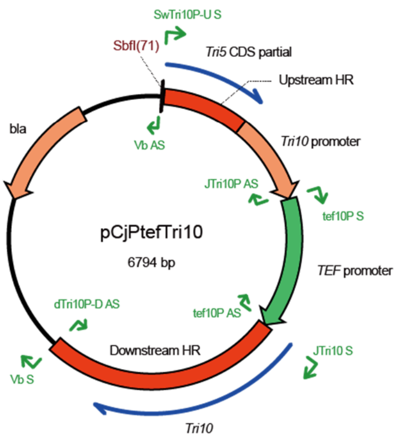
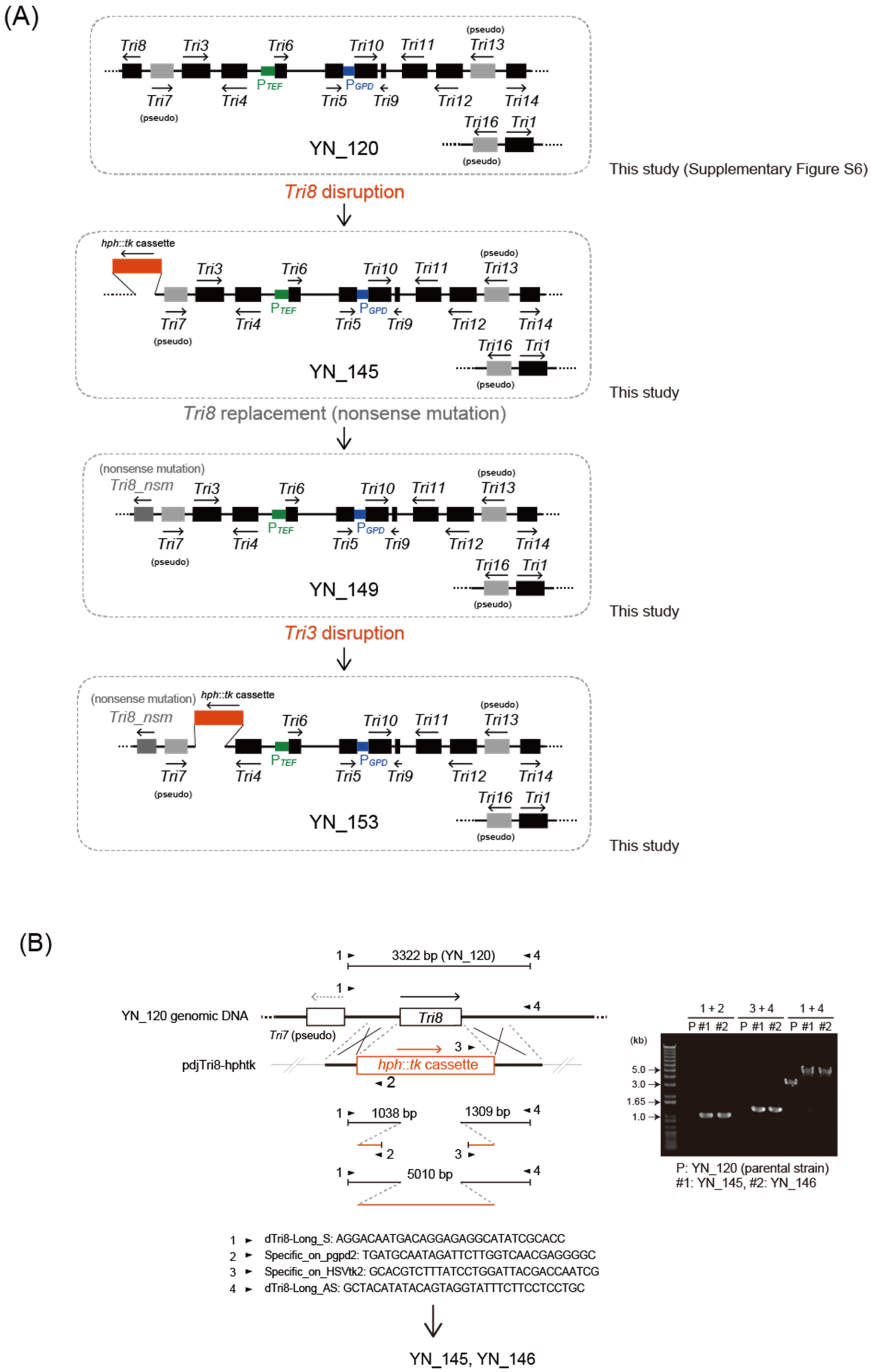
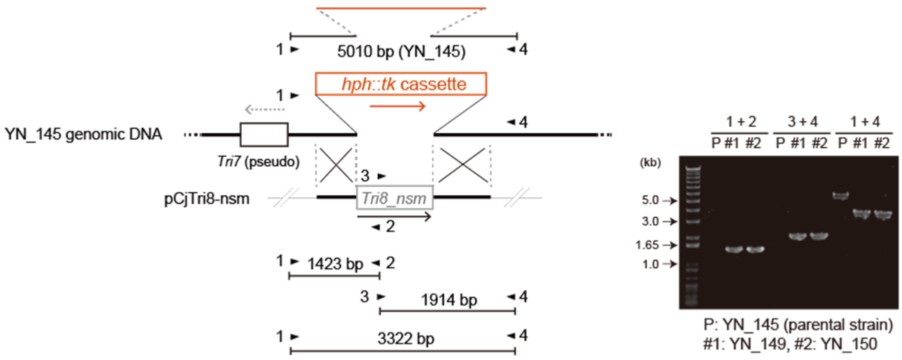




Supplementary Figure S6. Trichothecene production of transgenic *F. graminearum* strains with constitutive overexpression of *Tri6* and/or *Tri10*. (A) Pedigree of transformants with multiple genetic manipulations for the overproduction of 15-ADON. The previously described strain Δ*Tri6* tk (YN_001) contains a hygromycin B phosphotransferase gene translationally fused to a herpes simplex virus thymidine kinase gene (*hph::tk*) [9, 10]. This strain was used to generate a marker-free *Tri6*-overexpressor strain YN_004 (Δ*Tri6* / P_{TEF}::*Tri6*) by conditional negative selection against the *hph::tk* cassette with 2′-deoxy-5-fluorouridine (5-FdU). In strain YN_004, a strong *Aspergillus nidulans TEF1α* promoter fragment [9] is inserted upstream of the *Tri6* coding region. The promoter disruptants YN_112 (Δ*Tri6* / P_{TEF}::*Tri6*, ΔP_{Tri10} / P_{GPD}::*hph::tk*) and YN_109 (ΔP_{Tri10} / P_{GPD}::*hph::tk*) were obtained from YN_004 and the WT strains, respectively, by positive selection against *hph::tk* with hygromycin B. Marker-free transformants YN_120 (Δ*Tri6* / P_{TEF}::*Tri6*, ΔP_{Tri10} / P_{GPD}) and YN_116 (ΔP_{Tri10} / P_{Tri10} + P_{TEF}) were obtained from YN_112 and YN_109, respectively, by the negative selection. (B) Generation of strains YN_120 and YN_116. YN_004 was created from YN_001 following transformation with pCjTefTri6, where 5-FdU resistant transformants with double crossover homologous recombination were negatively selected against *hph::tk*. YN_112 and YN_109 were created from YN_004 and the WT strains, respectively, by positive selection with hygromycin B following transformation with pdTri10p-hphtk. YN_120 and YN_116 were created by negative selection with 5-FdU, respectively, following transformation of YN_112 with pCjPgpdTri10 and YN_109 with pCjPtefTri10. (C) Production of trichothecenes by the transgenic strains. The WT (JCM 9873), YN_004, YN_116, and YN_120 strains were cultured on 100 mL of YS_60 (0.1% [w/v] Bacto™ yeast extract, 6% [w/v] sucrose) and YG_60 (0.1% [w/v] Bacto™ yeast extract, 6% [w/v] glucose) medium in a 300 mL-Erlenmeyer flask with gyratory shaking at 25ºC. In contrast to the WT and *Tri6*-overexpressor YN_004 strains, YN_116 produced 15-ADON on YG_60 medium that does not contain sucrose. The 15-ADON production was further enhanced in the culture of YN_120, in which both *Tri6* and *Tri10* were constitutively overexpressed. YN_120 also produced 15-ADON on YG medium (0.5% [w/v] Bacto™ yeast extract, 2% [w/v] glucose) that contains a lower amount of glucose and a higher amount of yeast extract. (D) Sequences and maps of disruption and replacement vectors.

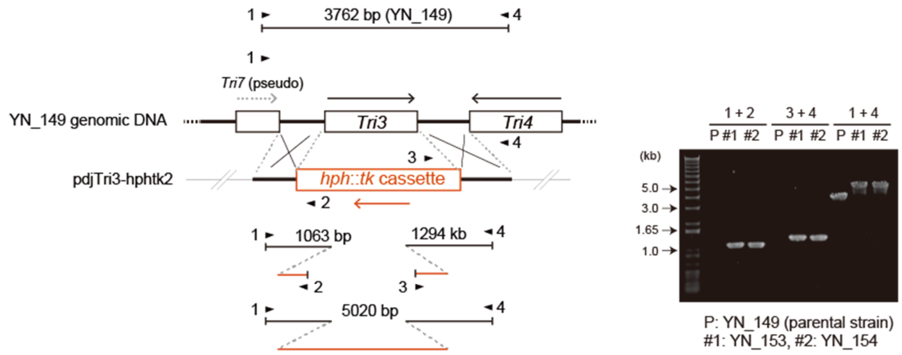

(A)
Tri8
Tri3
Tri6
Tri10
Tri11
(pseudo)
Tri13
Tri7
(pseudo)
Tri4
PTEF
Tri5
PGPD
Tri9
Tri12
Tri14
Tri16
Tri1
YN_120
This study (Supplementary Figure S6)
Tri8 disruption
hph::tk cassette
YN_145
This study
Tri8 replacement (nonsense mutation)
(nonsense mutation)
Tri8_nsm
YN_149
This study
Tri3 disruption
YN_153
This study
(B)
3322 bp (YN_120)
YN_120 genomic DNA
Tri7 (pseudo)
pdjTri8-hphtk
1038 bp
1309 bp
5010 bp
1 ► dTri8-Long_S: AGGACAATGACAGGAGAGGCATATCGCACC
2 ► Specific_on_pgpd2: TGATGCAATAGATTCTTGGTCAACGAGGGGC
3 ► Specific_on_HSVtk2: GCACGTCTTTATCCTGGATTACGACCAATCG
4 ► dTri8-Long_AS: GCTACATATACAGTAGGTATTTCTTCCTCCTGC
YN_145, YN_146
1+2
3+4
1+4
P #1 #2
(kb)
5.0
3.0
1.65
1.0
P: YN_120 (parental strain)
#1: YN_145, #2: YN_146



1 ► dTri8-Long_S: AGGACAATGACAGGAGAGGCATATCGCACC
2 ► Tri8nsm_AS: GTGTTGGTTTAGTTGAGGCCCAGACCAAGCG
3 ► Tri8nsm_S: CAACTAAACCAACACGCCCAGTGTTGC
4 ► dTri8-Long_AS: GCTACATATACAGTAGGTATTTCTTCCTCCTGC

↓

YN149, YN150

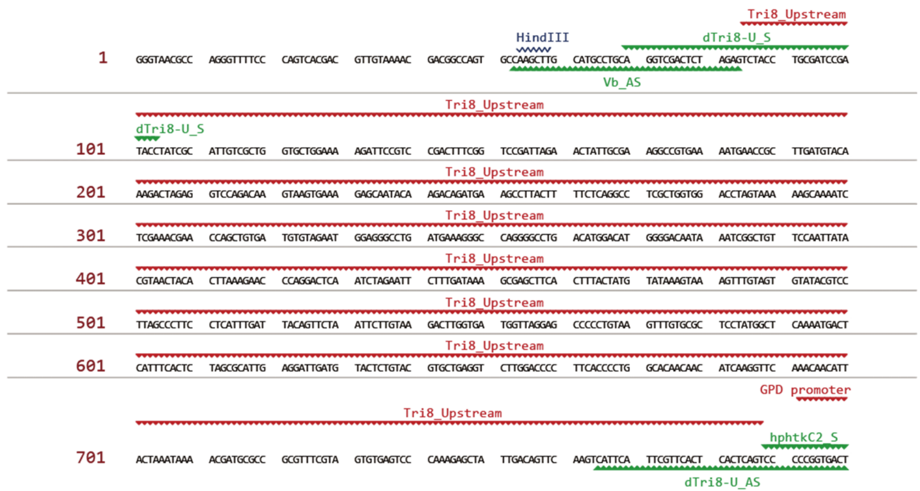


1 ► dTri3-Long_S: GAGCTTTGTGAACACCATTGGCGTCGACAC
2 ► Specific_on_HSVtk2: GCACGTCTTTATCCTGGATTACGACCAATCG
3 ► Specific_on_pgpd2: TGATGCAATAGATTCTTGGTCAACGAGGGGC
4 ► dTri3-Long_AS: GCGAGCTTACGACATTGAGCTTTATGACACC

↓

YN_153, YN_154

(C)



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dTri8-U_AS

GPD promoter
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GPD promoter
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GPD promoter
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GPD promoter
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GPD promoter
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GPD promoter
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GPD promoter
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GPD promoter hph
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hph
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hph
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hph
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hph
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hph
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hph
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HSVtk
hph
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HSVtk
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HSVtk
dTri8-D_S
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hphtkC2_AS

HSVtk Tri8_Downstream
dTri8-D_S
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Tri8_Downstream
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Tri8_Downstream
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Tri8_Downstream
Vb_S
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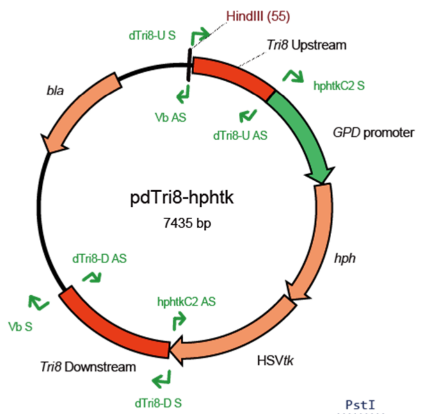

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Vb_AS

Tri8_Upstream
dTri8-U_S
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Tri8_Upstream
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Tri8_Upstream
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Tri8-nsm
Tri8_Upstream
+3 M A L D
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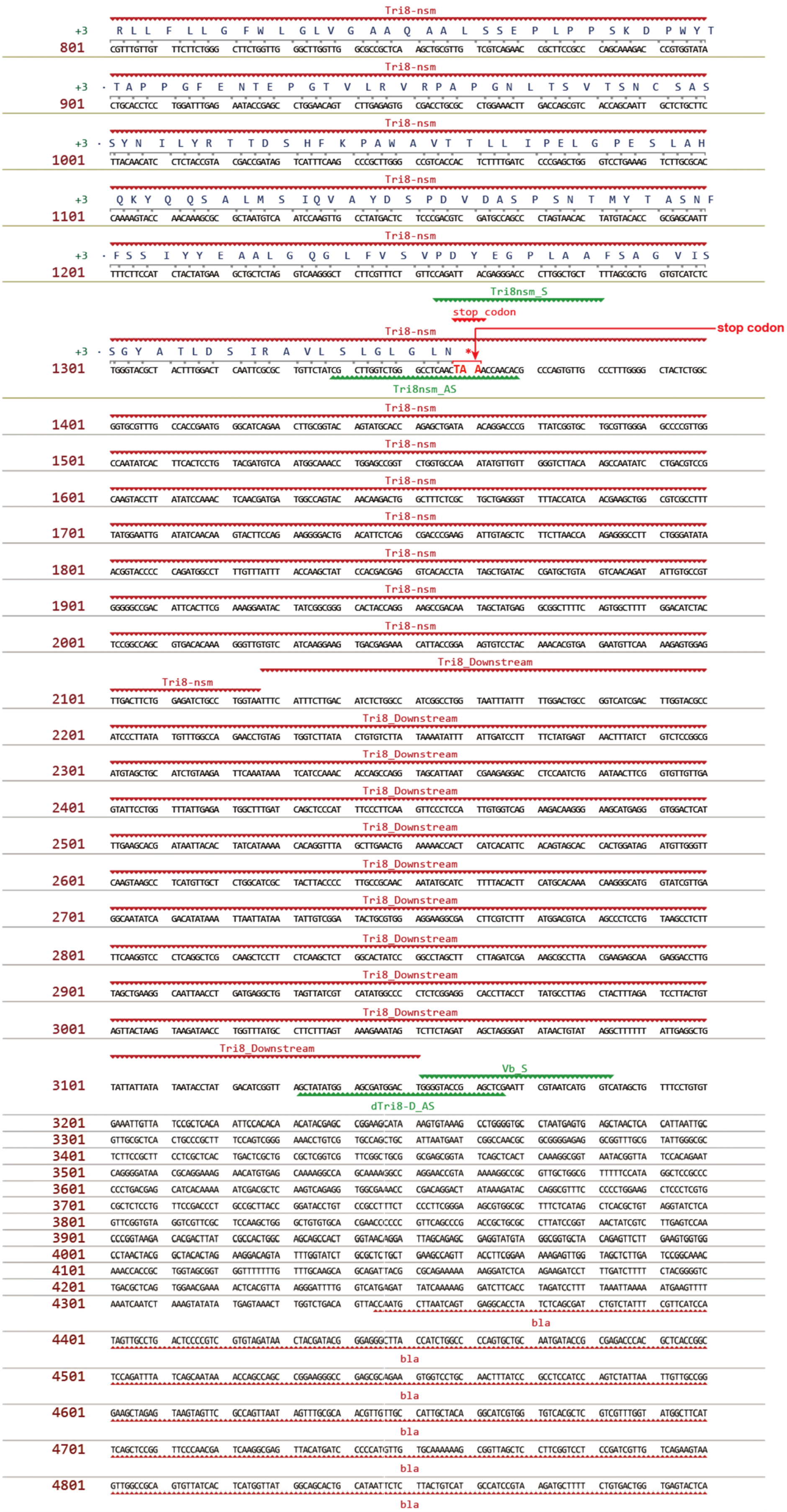
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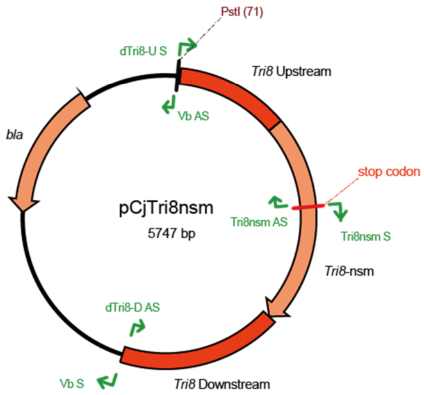

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Vb_AS

Tri3_Upstream
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Tri3_Upstream
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Tri3_Upstream
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Tri3_Upstream
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Tri3_Upstream
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Tri3_Upstream
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Tri3_Upstream
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hphtkC2_AS
Tri3_Upstream
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dTri3-U_AS2
HSVtk

hphtkC2_AS
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HSVtk

1001 GCGTCCCGGC AGCCGGCGGG CGATTGGTCG TAATCCAGGA TAAAGACGTG CATGGAACGG AGGCGTTTGG CCAAGACGTC CAAGGCCCAG GCAAACACGT
HSVtk

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HSVtk

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HSVtk

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hph

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hph

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hph

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hph

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dTri3-D_S2
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Tri3_Downstream
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Vb_S
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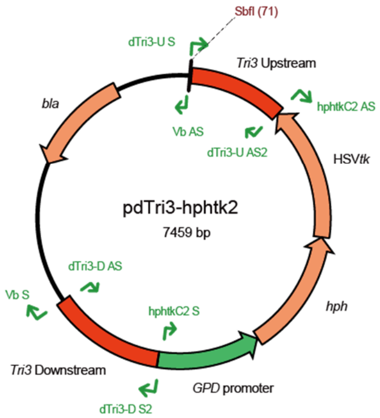

Supplementary Figure S7. Transgenic *F. graminearum* strains used for the investigation of the effect of *Tri6* and *Tri10* overexpression on 15-deCAL accumulation. (A) Pedigree of transformants with multiple genetic manipulations for the generation of a *Tri3*-disrupted strain, YN_153, with overexpression of *Tri6* and *Tri10*. The gene disruptants, YN_145 (Δ*Tri6* / P_{TEF}::*Tri6*, ΔP_{Tri10} / P_{GPD}, Δ*Tri8* / P_{GPD}::*hph::tk*) and YN_153 (Δ*Tri6* / P_{TEF}::*Tri6*, ΔP_{Tri10} / P_{GPD}, Δ*Tri8* / *Tri8_nsm*, Δ*Tri3* / P_{GPD}::*hph::tk*), were obtained from the marker-free transformants YN_120 (**Supplementary Figure S6**) and YN_149 (Δ*Tri6* / P_{TEF}::*Tri6*, ΔP_{Tri10} / P_{GPD}, Δ*Tri8* / *Tri8_nsm*), respectively, by positive selection. YN_149 was created from YN_145 by negative selection. (B) Generation of strain YN_153. YN_145 was created from YN_120 by positive selection with hygromycin B following transformation with pdjTri8-hphtk. YN_149 was created from YN_145 by negative selection with 5-FdU following transformation with pCjTri8-nsm. YN153 was created from YN_149 by positive selection with hygromycin B after transformation with pdjTri3-hphtk2. (C) Sequences and maps of disruption and replacement vectors.

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