Supplementary Material

Regulatory Mechanism of Trichothecene Biosynthesis in *Fusarium graminearum*

Maydelene Xiao Xuan Liew[1], Yuichi Nakajima[1], Kazuyuki Maeda[1], Naotsugu Kitamura[1], Makoto Kimura[1,*]

[1]Department of Applied Biosciences, Graduate School of Bioagricultural Sciences, Nagoya University, Furo-cho, Chikusa-ku, Nagoya, Aichi 464-8601, Japan

Correspondence

Makoto Kimura

mkimura@agr.nagoya-u.ac.jp

Supplementary Table 1 | Composition of defined medium used in the study of 15-ADON production in various *F. graminearum* transformant strains.

Defined medium for submerged culture

Element	Concentration
Carbon source	30 g/L
KH_2PO_4	1 g/L
KCl	0.5 g/L
$MgSO_4 \cdot 7H_2O$	0.5 g/L
Trace elements[1]	0.2 mL/L
$FeSO_4 \cdot 7H_2O$	10 mg/L
Nitrogen source [1,2]	5 mM

[1] filter-sterilized.

[2] amino acids mixture or L-Glutamine.

5000 × Trace elements

Element	Concentration
Citric acid	5 g/100 mL
$MnSO_4$	50 mg/100 mL
$ZnSO_4 \cdot 6H_2O$	5 g/100 mL
H_3BO_3	50 mg/100 mL
$Na_2MoO_4 \cdot 2H_2O$	50 mg/100 mL
$CuSO_4 \cdot 5H_2O$	250 mg/100 mL

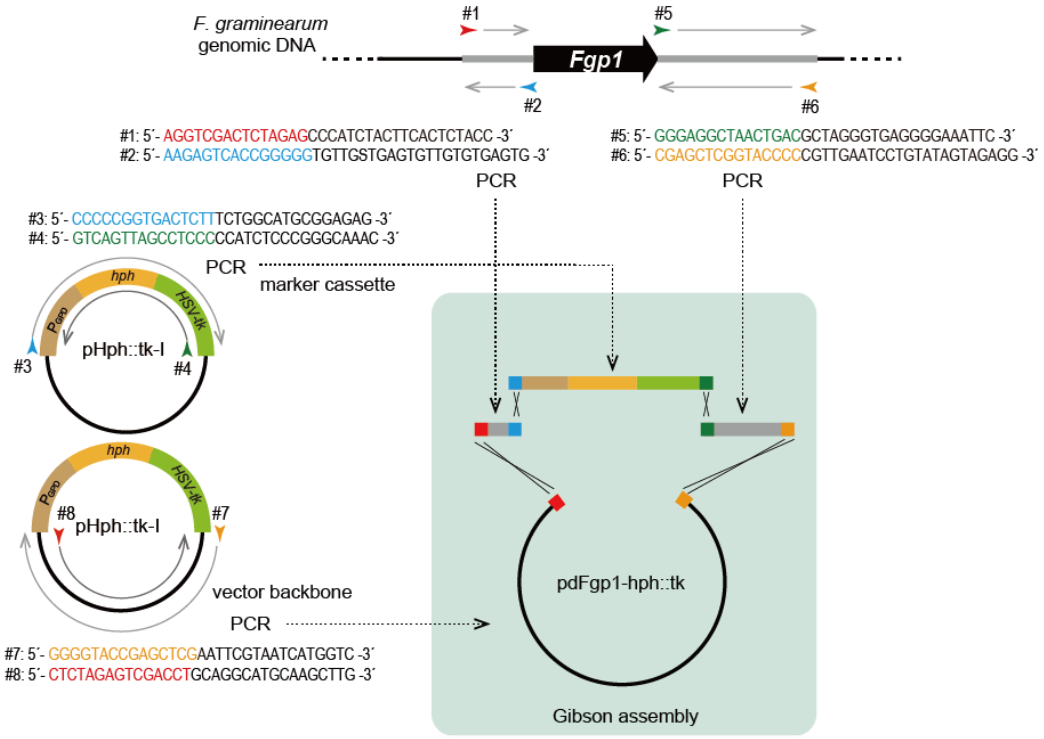
Amino acids mixture

Element	Concentration
L-Glutamic acid	1 mM
L-Glutamine	1.5 mM
L-Leucine	2.5 mM

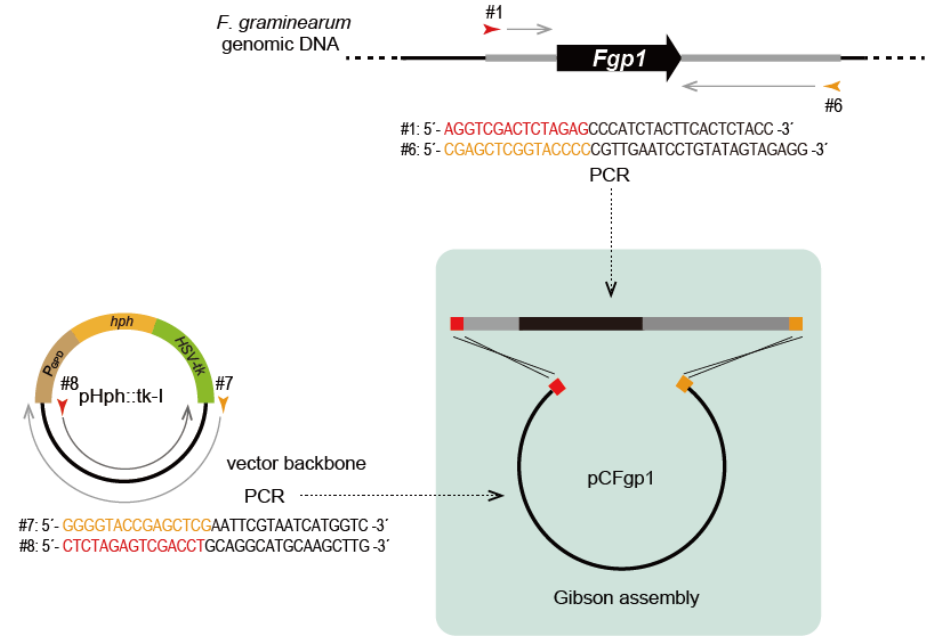
Supplementary Table 2 | List of primers used for qRT-PCR in the study of *Tri10* expression in various *F. graminearum* strains.

Primer name	Sequence (5'-3')	Description
JCM_Tri10_qRT-F	TGTCGCCTCATACGACCTC	Quantitative real-time PCR primers for the analysis of *Tri10* expression
JCM_Tri10_qRT-R	ATGACGGAACTCTTCAGGTCTT	
qRT-Gpd_Fw	CGAAGTTGTCGTTGAGGGAG	Quantitative real-time PCR primers for the analysis of *GPD* expression
qRT-Gpd_Rev	GACAACGAGTGGGGTTACTCC	

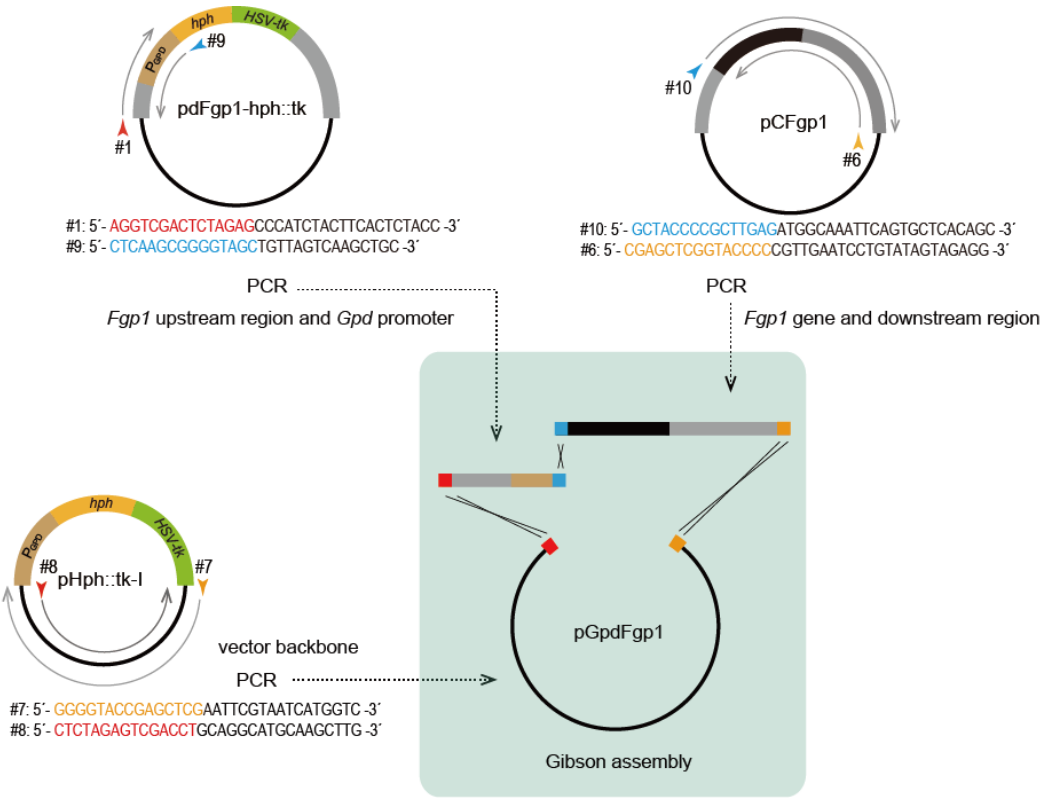
(A)


F. graminearum
genomic DNA
Fgp1
#1
#2
#5
#6
#1: 5′- AGGTCGACTCTAGAGCCCATCTACTTCACTCTACC -3′
#2: 5′- AAGAGTCACCGGGGGTGTTGSTGAGTGTTGTGTGAGTG -3′
#5: 5′- GGGAGGCTAACTGACGCTAGGGTGAGGGGAAATTC -3′
#6: 5′- CGAGCTCGGTACCCCCGTTGAATCCTGTATAGTAGAGG -3′
PCR
PCR
#3: 5′- CCCCCGGTGACTCTTTCTGGCATGCGGAGAG -3′
#4: 5′- GTCAGTTAGCCTCCCCCATCTCCCGGGCAAAC -3′
PCR
marker cassette
hph
HSV-tk
Pgpd
pHph::tk-I
#3
#4
#8
#7
vector backbone
PCR
#7: 5′- GGGGTACCGAGCTCGAATTCGTAATCATGGTC -3′
#8: 5′- CTCTAGAGTCGACCTGCAGGCATGCAAGCTTG -3′
pdFgp1-hph::tk
Gibson assembly

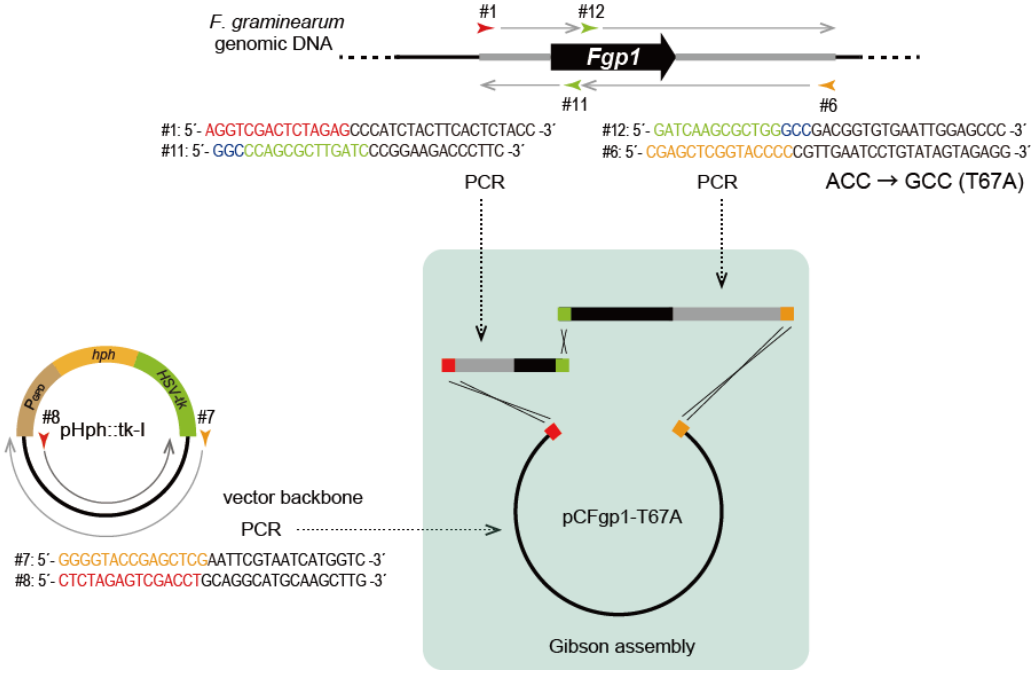
(B)


F. graminearum
genomic DNA
Fgp1
#1
#6
#1: 5′- AGGTCGACTCTAGAGCCCATCTACTTCACTCTACC -3′
#6: 5′- CGAGCTCGGTACCCCCGTTGAATCCTGTATAGTAGAGG -3′
PCR
hph
HSV-tk
Pgpd
#8
pHph::tk-I
#7
vector backbone
PCR
#7: 5′- GGGGTACCGAGCTCGAATTCGTAATCATGGTC -3′
#8: 5′- CTCTAGAGTCGACCTGCAGGCATGCAAGCTTG -3′
pCFgp1
Gibson assembly

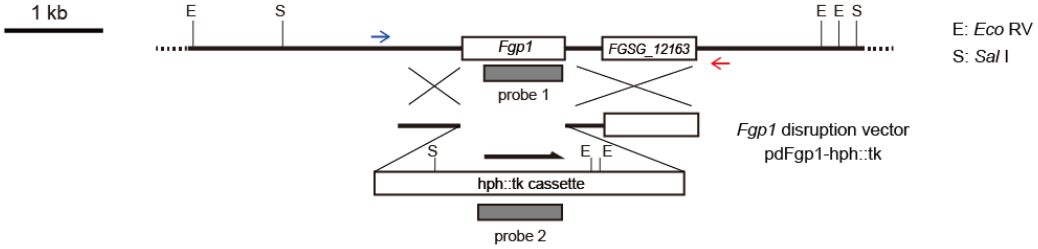
(C)


hph
HSV-tk
Pgpd
#9
pdFgp1-hph::tk
#1
#1: 5′- AGGTCGACTCTAGAGCCCATCTACTTCACTCTACC -3′
#9: 5′- CTCAAGCGGGGTAGCTGTTAGTCAAGCTGC -3′
#10
pCFgp1
#6
#10: 5′- GCTACCCCGCTTGAGATGGCAAATTCAGTGCTCACAGC -3′
#6: 5′- CGAGCTCGGTACCCCCGTTGAATCCTGTATAGTAGAGG -3′
PCR
Fgp1 upstream region and Gpd promoter
PCR
Fgp1 gene and downstream region
pGpdFgp1
Gibson assembly
hph
HSV-tk
Pgpd
#8
pHph::tk-I
#7
vector backbone
PCR
#7: 5′- GGGGTACCGAGCTCGAATTCGTAATCATGGTC -3′
#8: 5′- CTCTAGAGTCGACCTGCAGGCATGCAAGCTTG -3′

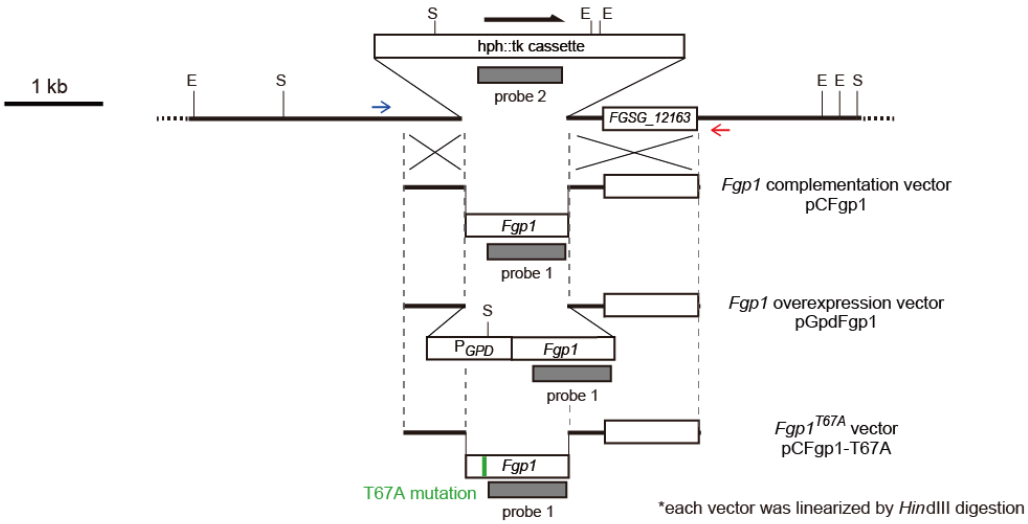
(D)


F. graminearum
genomic DNA
#1
#12
Fgp1
#11
#6
#1: 5′- AGGTCGACTCTAGAGCCCATCTACTTCACTCTACC -3′
#11: 5′- GGCCCAGCGCTTGATCCCGGAAGACCCTTC -3′
#12: 5′- GATCAAGCGCTGGGCCGACGGTGTGAATTGGAGCCC -3′
#6: 5′- CGAGCTCGGTACCCCCGTTGAATCCTGTATAGTAGAGG -3′
PCR
PCR
ACC → GCC (T67A)
hph
HSV-tk
Pgpd
#8
pHph::tk-I
#7
vector backbone
PCR
pCFgp1-T67A
Gibson assembly
#7: 5′- GGGGTACCGAGCTCGAATTCGTAATCATGGTC -3′
#8: 5′- CTCTAGAGTCGACCTGCAGGCATGCAAGCTTG -3′

(E) 1st transformation (positive selection)



2nd transformation (negative selection)

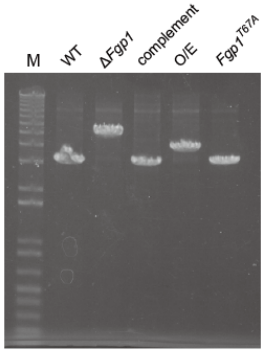


(F)

PCR

→ Fgp1-Long_S:
5'-GGCCTTTCTCAGCTTGACCTAGGGGTCTTC-3'

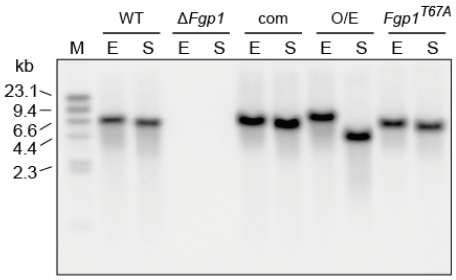
→ Fgp1-Long_AS:
5'-TCCTCGTCTTGTCTTTGTGTTGCTAGCCTG-3'



Correct amplicon size

WT (3,183 bp)
Δ*Fgp1* (5,165 bp)
com (3,183 bp)
O/E (4,045 bp)
Fgp1^{T67A} (3,183 bp)

Southern blot analysis

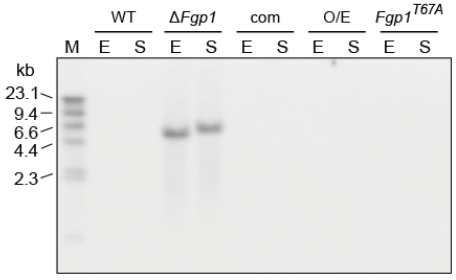


Predicted band sizes

WT (E: 6,253 bp, S: 5,720 bp)
Δ*Fgp1* (E: none, S: none)
com (E: 6,253 bp, S: 5,720 bp)
O/E (E: 7,115 bp, S: 4,282 bp)
Fgp1^{T67A} (E: 6,253 bp, S: 5,720 bp)

probe 1

Primers for probe 1 amplification
Fgp1-probe_F: 5'-GCCGAATCTTGGGCAACTTC-3'
Fgp1-probe_R: 5'-GCCATTTTGGATGGCATGGG-3'



Predicted band sizes

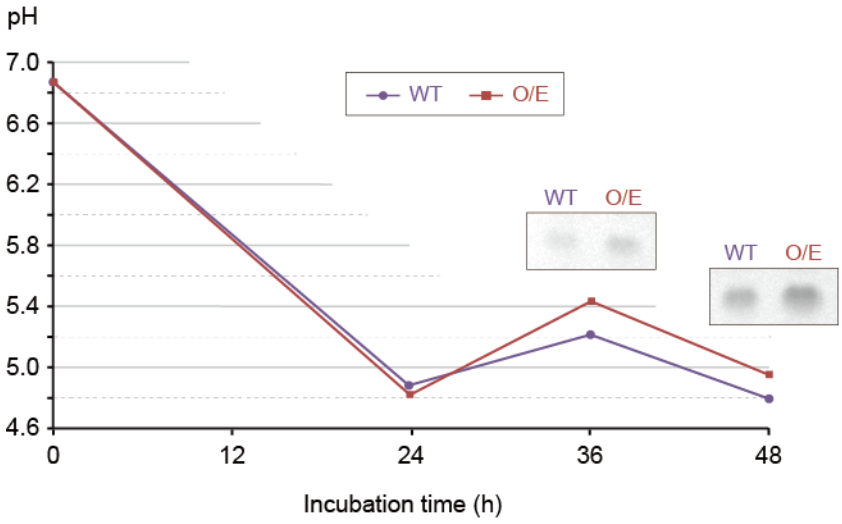
WT (E: none, S: none)
Δ*Fgp1* (E: 4,799 bp, S: 5,399 bp)
com (E: none, S: none)
O/E (E: none, S: none)
Fgp1^{T67A} (E: none, S: none)

probe 2

Primers for probe 2 amplification
hph-PL1: 5'-AGCTGCGCCGATGGTTTCTACAA-3'
hph-PR1: 5'-GGGGCGTCGGTTTCCACTATCG-3'

Supplementary Figure 1 | Construction of homologous recombination vectors and generation of various *Fgp1* transformants. (A) Construction of pdFgp1-hph::tk. Four pairs of PCR primers (primers #1 – #8), with 15 bp overhangs necessary for Gibson Assembly, were designed as shown in the figure. Positive-negative selection marker cassette, containing a glyceraldehyde 3-phosphate dehydrogenase (*GPD*) promoter (from *Aspergillus nidulans* AN8041) fused to *hph::tk*, was amplified from pHph::tk-I (Maeda and Ohsato 2017) with primers #3 × #4. Vector backbone was amplified from the same plasmid with primers #7 × #8. Upstream and downstream regions of *Fgp1* were obtained using genomic DNA as template with primers #1 × #2 and #5 × #6, respectively. The four PCR fragments were connected by Gibson Assembly using the NEBuilder HiFi DNA Assembly Master Mix (New England BioLabs, Ipswich, USA). (B) Construction of pCFgp1. Two pairs of PCR primers (primers #1, #6 − #8), with 15 bp overhangs necessary for Gibson Assembly, were designed as shown in the figure. Vector backbone was amplified from pHph::tk-I with primers #7 × #8, and the region from the upstream of *Fgp1* to its downstream was amplified using genomic DNA as template with primers #1 × #6. The two PCR fragments were assembled by Gibson Assembly. (C) Construction of pGpdFgp1. Three pairs of PCR primers (primers #1, #6 – #10), with 15 bp overhangs necessary for Gibson Assembly, were designed as shown in the figure. Vector backbone was amplified from pHph::tk-I with primers #7 × #8, and the *Fgp1* upstream region and the *GPD* promoter was amplified from pdFgp1-hph::tk with primers #1 × #9. The *Fgp1* gene and its downstream region was amplified from pCFgp1 using primers #10 × #6, and the three PCR fragments were assembled by Gibson Assembly. (D) Construction of pCFgp1-T67A. Three pairs of PCR primers (primers #1, #6– #8, #11– #12), with 15 bp overhangs necessary for Gibson Assembly, were designed as shown in the figure. Primers #11 and #12 contain a single base pair change that would introduce a point mutation into *Fgp1* for the replacement of threonine with alanine at the putative phosphorylation site. Vector backbone was

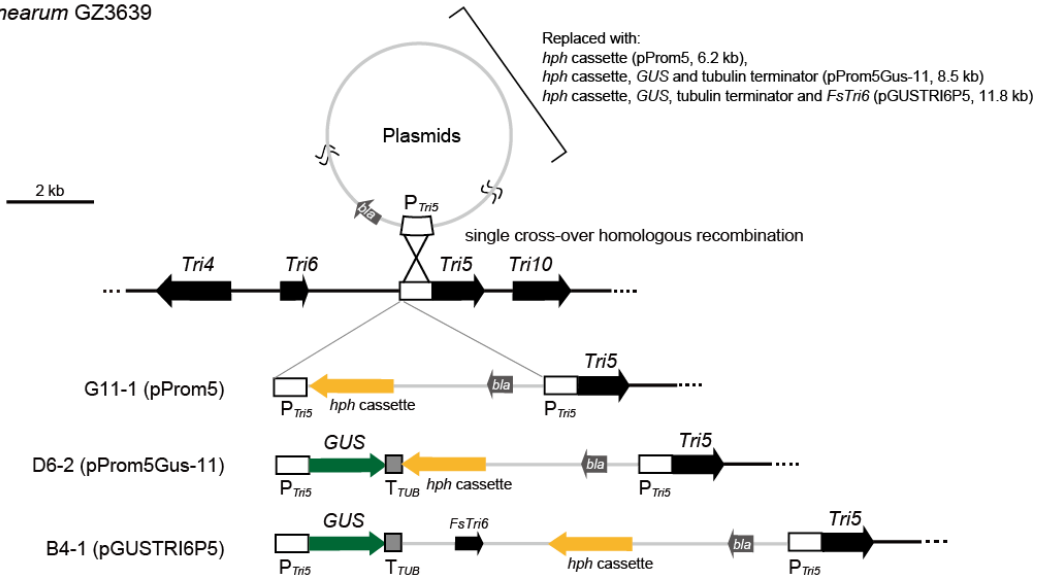
amplified from pHph::tk-I with primers #7 × #8, the region from the upstream of *Fgp1* to the putative phosphorylation site was amplified from the genome with primers #1 × #11, and the remaining region of *Fgp1* and its downstream region was amplified from the genome with primers #12 × #6. The three fragments were then assembled by Gibson Assembly. **(**E) Schematic diagram of the genomic structure of the various *Fgp1* strains generated: deletion strain (Δ*Fgp1*), complemented strain (Δ*Fgp1* + *Fgp1*; com), overexpressor strain (Δ*Fgp1* + P_{GPD}*::Fgp1*; O/E) and phosphorylation site disruptant (Δ*Fgp1* + $Fgp1^{T67A}$; $Fgp1^{T67A}$). (F) PCR and Southern blot verification of the *Fgp1* mutant strains. Expected sizes of amplicons were obtained after PCR with primers (red and blue) located outside of the homologous region (left panel). The *Fgp1* sequence of $Fgp1^{T67A}$ strain was confirmed by DNA sequencing. Southern blot of genomic DNA digested with *Eco*RV and *Sal*I was hybridized with a DIG-labeled probe 1 and probe 2, which was prepared using a PCR DIG Probe Synthesis Kit (Roche Diagnostics GmbH, Mannheim, Germany) and primers described in the figure. Predicted sizes of single bands were detected for the *Eco*RV and *Sal*I digested DNA (right panel).



Supplementary Figure 2 | Toxin production assay of the wild-type (WT) and *Fgp1* overexpressor (O/E) strains, cultured in 30 mL of YS_60 medium (0.1% [w/v] yeast extract, 6% [w/v] sucrose) in a 100-mL Erlenmeyer flask with gyratory shaking (135 rpm) at 25ºC. Fresh conidia were inoculated into the YS_60 medium at a cell density of 1×10^4 conidia/mL. With this inoculum size and 24 h of incubation period, the mycelia are too premature to cause toxin accumulation. 15-ADON was extracted from 500 µL of each medium with ethyl acetate 36 and 48 hr after the inoculation. The insets represent TLC panels of 15-ADON that accumulated in fungal cultures, as detected by UV absorption at 254 nm. The pH profiles of each fungal culture was similar with each other and remained above 4.7, as shown in the graph.

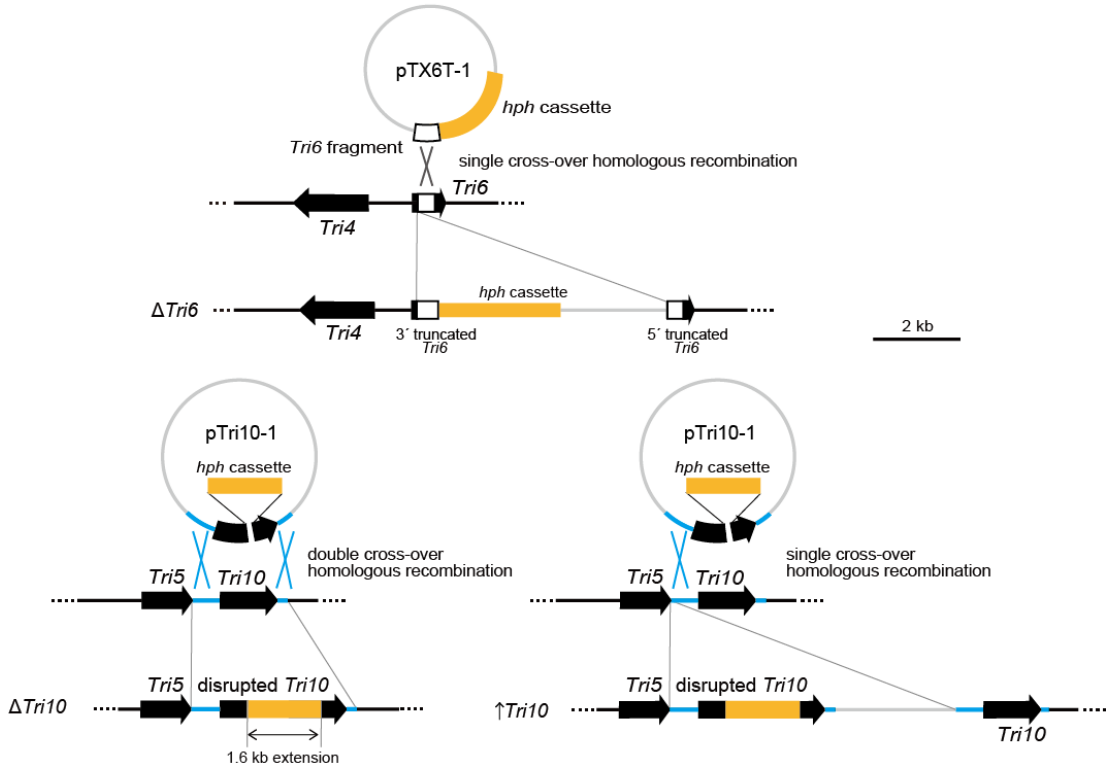
(A)

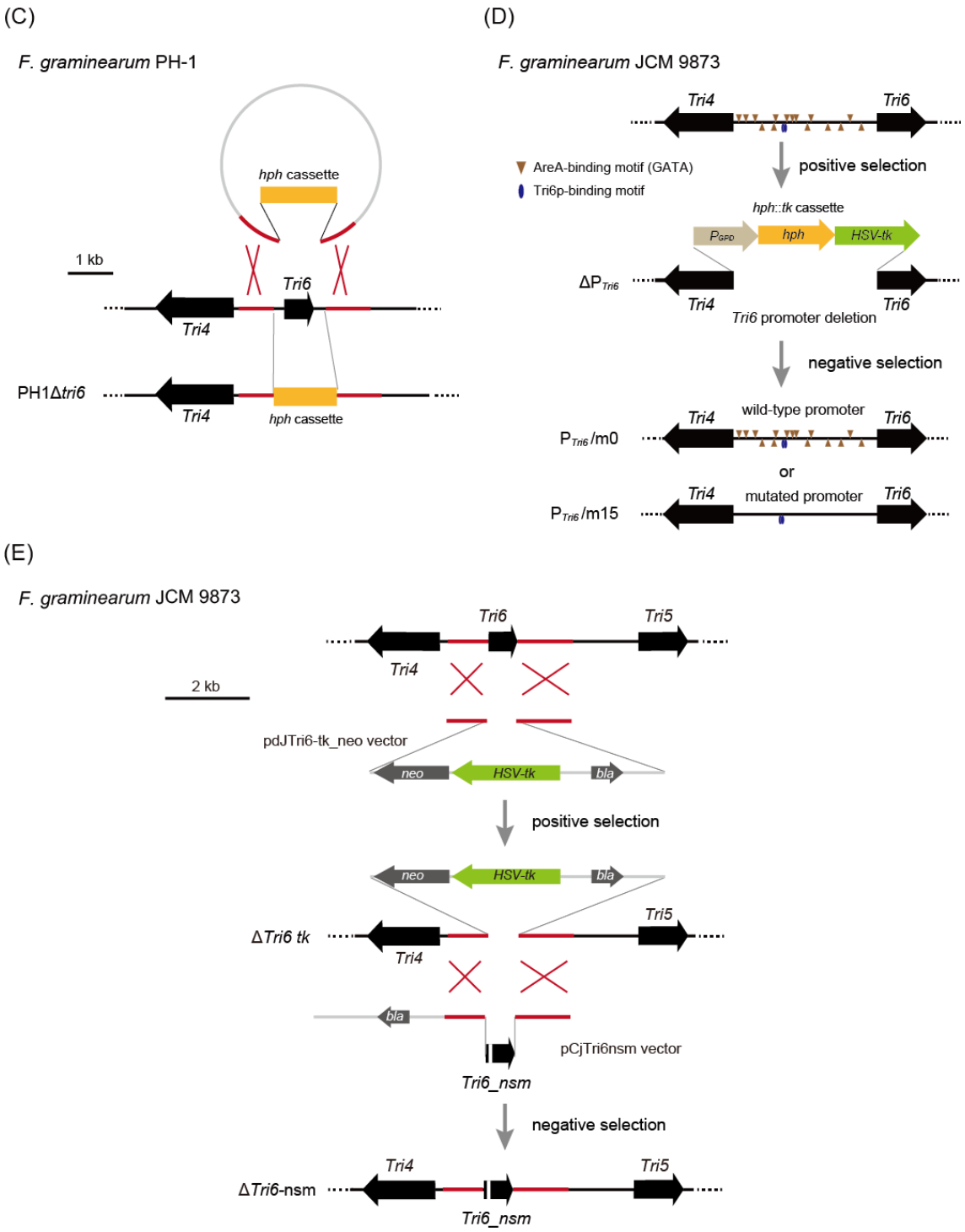
F. graminearum GZ3639



(B)

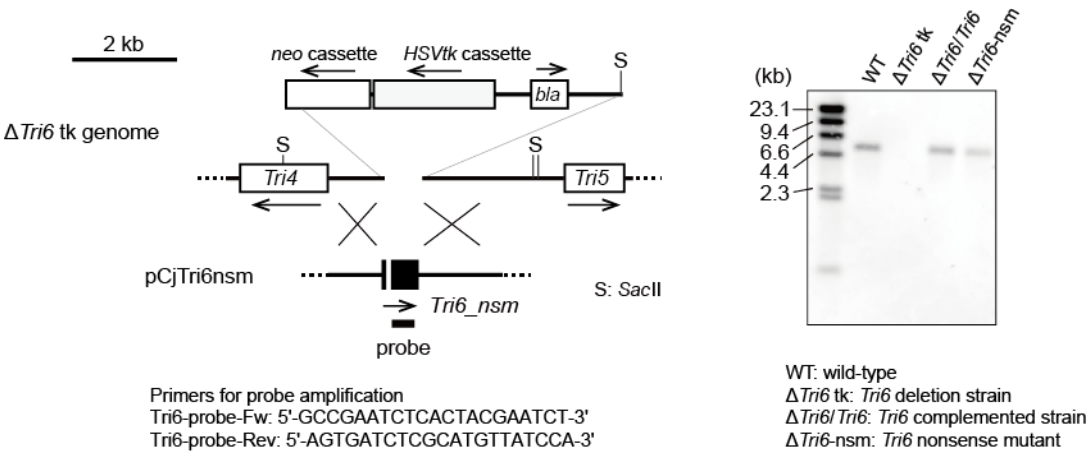
F. sporotrichioides NRRL 3299



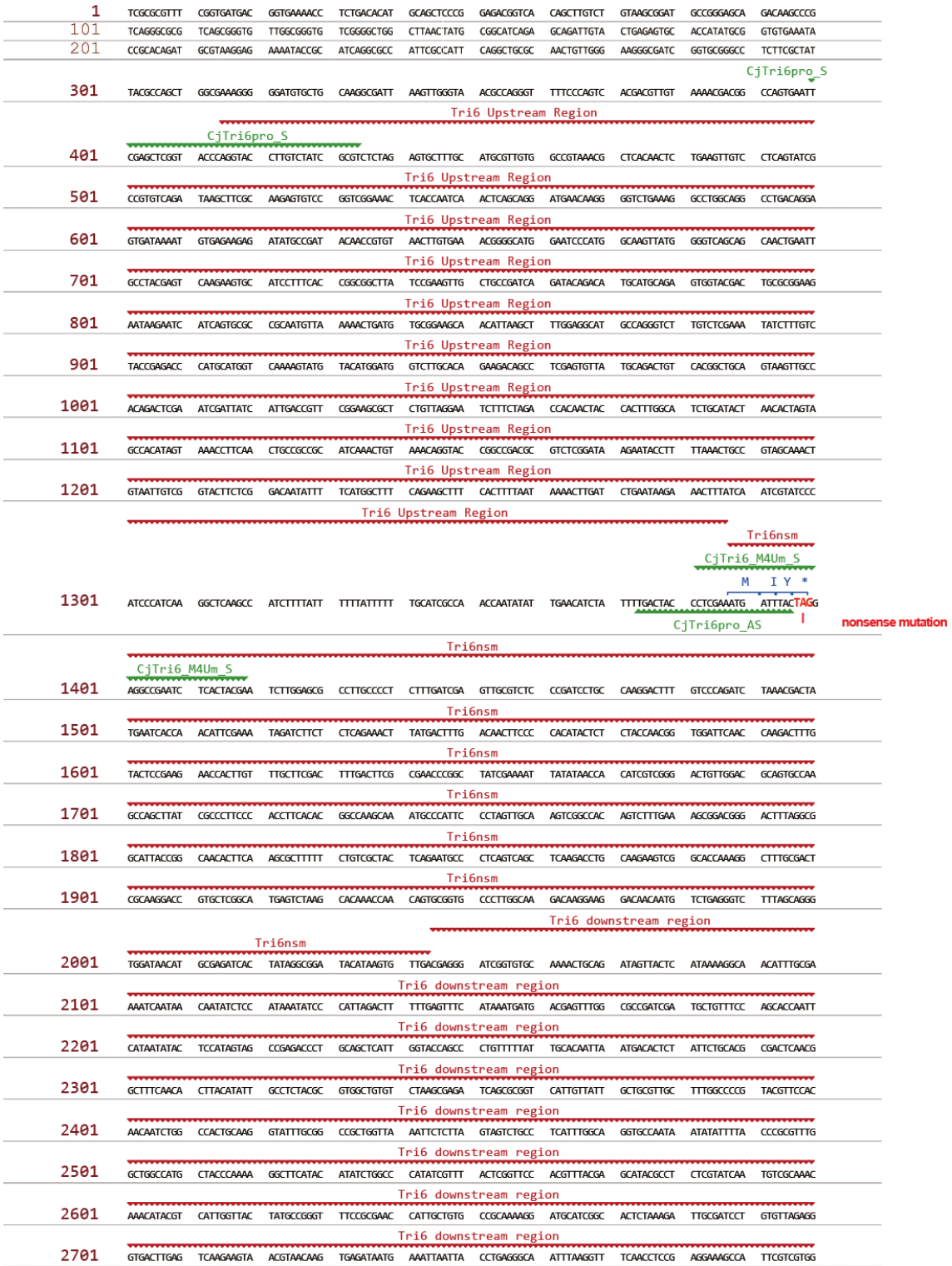


Supplementary Figure 3 | Illustration of genetic transformation of vectors via single or double crossover homologous recombination into the strains presented in Figure 2. (A) Manipulation of the trichothecene gene cluster of *F. graminearum* GZ3639 (Chen et al., 2000). The intergenic region between *Tri6* and *Tri5* is extended by the insertion of the

respective plasmids by a single crossover homologous recombination event. (B) Manipulation of the trichothecene gene cluster of *F. sporotrichioides* strain NRRL 3299 (Proctor et al., 1995; Tag et al., 2001). The core cluster region is extended when the respective plasmids are inserted via a single crossover (for plasmids pTX6T-1 and pTri10-1) or double crossover (for plasmid pTri10-1) homologous recombination events. (C) Manipulation of the trichothecene gene cluster in *F. graminearum* strain NRRL 31084 (PH-1) (Seong et al., 2009). The PH1Δ*tri6* strain was obtained via a double crossover homologous recombination event at upstream and downstream regions flanking *Tri6*, which led to a 0.3 kb extension of the core cluster region. (D) Manipulation of the *Tri6* promoter region in *F. graminearum* strain JCM 9873 (Nakajima et al., 2020). The strains were constructed using a two-step transformation process involving double crossover homologous recombination events, and are marker free with no perturbation within the core cluster region. (E) Manipulation of the trichothecene gene cluster in *F. graminearum* strain JCM 9873. The Δ*Tri6* tk strain was generated using a one-step transformation process (Nakajima et al., 2014), and contains a selection marker cassette replacing the *Tri6* coding region, which leads to 7.0 kb extension in the cluster. The Δ*Tri6*-nsm strain was generated using a two-step transformation process, and contains a dysfunctional copy of *Tri6* gene, which does not lead to perturbation in the cluster region. See **Supplementary Figure 4** for experimental details of the mutant strain construction and confirmation.



Supplementary Figure 4 | Generation of the Δ*Tri6*-nsm mutant strain. The *Tri6* disruption mutant, strain Δ*Tri6* tk (Nakajima et al., 2014), was transformed with pCjTri6nsm (**Supplementary Figure 5**) and a self-cloning strain carrying a mutated *Tri6* (*Tri6_nsm*) was screened by conditional negative selection with 2′-deoxy-5-fluorouridine (5-FdU) (Nakajima et al., 2020). Genomic DNA of a candidate strain, sensitive to G-418 and resistant to 5-FdU, was digested with *Sac*II, and transferred to a Nytran membrane (Cytiva, Tokyo, Japan). The blot was hybridized with a DIG-labeled *Tri6* probe, which was prepared using a PCR DIG Probe Synthesis Kit (Roche Diagnostics GmbH, Mannheim, Germany) and primers described in the figure (left panel). Predicted sizes of single bands were detected for the digested DNA (right panel).


CjTri6pro_S
Tri6 Upstream Region
CjTri6pro_S
Tri6 Upstream Region
Tri6 Upstream Region
Tri6 Upstream Region
Tri6 Upstream Region
Tri6 Upstream Region
Tri6 Upstream Region
Tri6 Upstream Region
Tri6 Upstream Region
Tri6nsm
CjTri6_M4Um_S
M I Y *
CjTri6pro_AS
nonsense mutation
Tri6nsm
CjTri6_M4Um_S
Tri6nsm
Tri6nsm
Tri6nsm
Tri6nsm
Tri6nsm
Tri6 downstream region
Tri6nsm
Tri6 downstream region
Tri6 downstream region
Tri6 downstream region
Tri6 downstream region
Tri6 downstream region
Tri6 downstream region
Tri6 downstream region

Tri6 downstream region

2801 GATATAATCT GCAATAGGGT AGGCTTTGCT GTTTTTTTCG GAGCATTTGT GACTGTTTTG GATTGTGTCT TTGGGGTCCT TTTGCTTTCA AGGCTGGTGA

Tri6 downstream region

2901 GTTTGCAAGG AACGAGTCAG TCACGGCAGG CACTGAGTCA AACACTGTTT CGTAATAATC ATGATTTGTT GTTGCAAAAT TCCAACGAAG CTTTGCGCGG

Tri6 downstream region

3001 TCAGTCAAAT TCGCACACGT CGATACTTTT TGCTGGCATT GGCCCAGTAT TCATCCATGA GTGGCTGAAC CGTAGTTGAT TCTCAAGTCC AACCCTAATA

Tri6 downstream region

3101 GTGCCCGGCG GAATGAGACG TTTTCGGGTG TCTGTAGCCG AGATGTGGAT AGTAACGGTA ACCCTAGTCA AATGAGACGT GGGCAGGGTT CATGGTTGTT

Tri6d ownstream region

3201 GAACCTTGTT CATCAGAATG TTGATGCCGT TGACCTACGG AATACCATCT TTCACATGTA TTTGTTCCCA ACCCACGTGG CTATACCAAC ATCCGCTGTA

Tri6 downstream region BamHI

3301 TATTCATTGG TTGGCTTTAT ATATTGATAC AGGTAATTTC AAGATCGCGA GCGGGAATTT CCTTGTCGGA TCAGAGAGAT GCTCTGGGGA TCCTCTAGAG

CompJTri6ter_AS

3401 TCGACCTGCA GGCATGCAAG CTTGGCGTAA TCATGGTCAT AGCTGTTTCC TGTGTGAAAT TGTTATCCGC TCACAATTCC ACACAACATA CGAGCCGGAA

3501 GCATAAAGTG TAAAGCCTGG GGTGCCTAAT GAGTGAGCTA ACTCACATTA ATTGCGTTGC GCTCACTGCC CGCTTTCCAG TCGGGAAACC TGTCGTGCCA

3601 GCTGCATTAA TGAATCGGCC AACGCGCGGG GAGAGGCGGT TTGCGTATTG GGCGCTCTTC CGCTTCCTCG CTCACTGACT CGCTGCGCTC GGTCGTTCGG

3701 CTGCGGCGAG CGGTATCAGC TCACTCAAAG GCGGTAATAC GGTTATCCAC AGAATCAGGG GATAACGCAG GAAAGAACAT GTGAGCAAAA GGCCAGCAAA

3801 AGGCCAGGAA CCGTAAAAAG GCCGCGTTGC TGGCGTTTTT CCATAGGCTC CGCCCCCCTG ACGAGCATCA CAAAAATCGA CGCTCAAGTC AGAGGTGGCG

3901 AAACCCGACA GGACTATAAA GATACCAGGC GTTTCCCCCT GGAAGCTCCC TCGTGCGCTC TCCTGTTCCG ACCCTGCCGC TTACCGGATA CCTGTCCGCC

4001 TTTCTCCCTT CGGGAAGCGT GGCGCTTTCT CAATGCTCAC GCTGTAGGTA TCTCAGTTCG GTGTAGGTCG TTCGCTCCAA GCTGGGCTGT GTGCACGAAC

4101 CCCCCGTTCA GCCCGACCGC TGCGCCTTAT CCGGTAACTA TCGTCTTGAG TCCAACCCGG TAAGACACGA CTTATCGCCA CTGGCAGCAG CCACTGGTAA

4201 CAGGATTAGC AGAGCGAGGT ATGTAGGCGG TGCTACAGAG TTCTTGAAGT GGTGGCCTAA CTACGGCTAC ACTAGAAGGA CAGTATTTGG TATCTGCGCT

4301 CTGCTGAAGC CAGTTACCTT CGGAAAAAGA GTTGGTAGCT CTTGATCCGG CAAACAAACC ACCGCTGGTA GCGGTGGTTT TTTTGTTTGC AAGCAGCAGA

4401 TTACGCGCAG AAAAAAAGGA TCTCAAGAAG ATCCTTTGAT CTTTTCTACG GGGTCTGACG CTCAGTGGAA CGAAAACTCA CGTTAAGGGA TTTTGGTCAT

4501 GAGATTATCA AAAAGGATCT TCACCTAGAT CCTTTTAAAT TAAAAATGAA GTTTTAAATC AATCTAAAGT ATATATGAGT AAACTTGGTC TGACAGTTAC

bla

4601 CAATGCTTAA TCAGTGAGGC ACCTATCTCA GCGATCTGTC TATTTCGTTC ATCCATAGTT GCCTGACTCC CCGTCGTGTA GATAACTACG ATACGGGAGG

bla

4701 GCTTACCATC TGGCCCCAGT GCTGCAATGA TACCGCGAGA CCCACGCTCA CCGGCTCCAG ATTTATCAGC AATAAACCAG CCAGCCGGAA GGGCCGAGCG

bla

4801 CAGAAGTGGT CCTGCAACTT TATCCGCCTC CATCCAGTCT ATTAATTGTT GCCGGGAAGC TAGAGTAAGT AGTTCGCCAG TTAATAGTTT GCGCAACGTT

bla

4901 GTTGCCATTG CTACAGGCAT CGTGGTGTCA CGCTCGTCGT TTGGTATGGC TTCATTCAGC TCCGGTTCCC AACGATCAAG GCGAGTTACA TGATCCCCCA

bla

5001 TGTTGTGCAA AAAAGCGGTT AGCTCCTTCG GTCCTCCGAT CGTTGTCAGA AGTAAGTTGG CCGCAGTGTT ATCACTCATG GTTATGGCAG CACTGCATAA

bla

5101 TTCTCTTACT GTCATGCCAT CCGTAAGATG CTTTTCTGTG ACTGGTGAGT ACTCAACCAA GTCATTCTGA GAATAGTGTA TGCGGCGACC GAGTTGCTCT

bla

5201 TGCCCGGCGT CAATACGGGA TAATACCGCG CCACATAGCA GAACTTTAAA AGTGCTCATC ATTGGAAAAC GTTCTTCGGG GCGAAAACTC TCAAGGATCT

bla

5301 TACCGCTGTT GAGATCCAGT TCGATGTAAC CCACTCGTGC ACCCAACTGA TCTTCAGCAT CTTTTACTTT CACCAGCGTT TCTGGGTGAG CAAAAACAGG

bla

5401 AAGGCAAAAT GCCGCAAAAA AGGGAATAAG GGCGACACGG AAATGTTGAA TACTCATACT CTTCCTTTTT CAATATTATT GAAGCATTTA TCAGGGTTAT

bla

5501 TGTCTCATGA GCGGATACAT ATTTGAATGT ATTTAGAAAA ATAAACAAAT AGGGGTTCCG CGCACATTTC CCCGAAAAGT GCCACCTGAC GTCTAAGAAA

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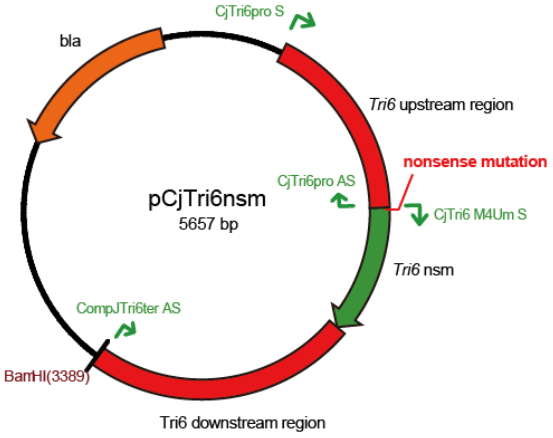
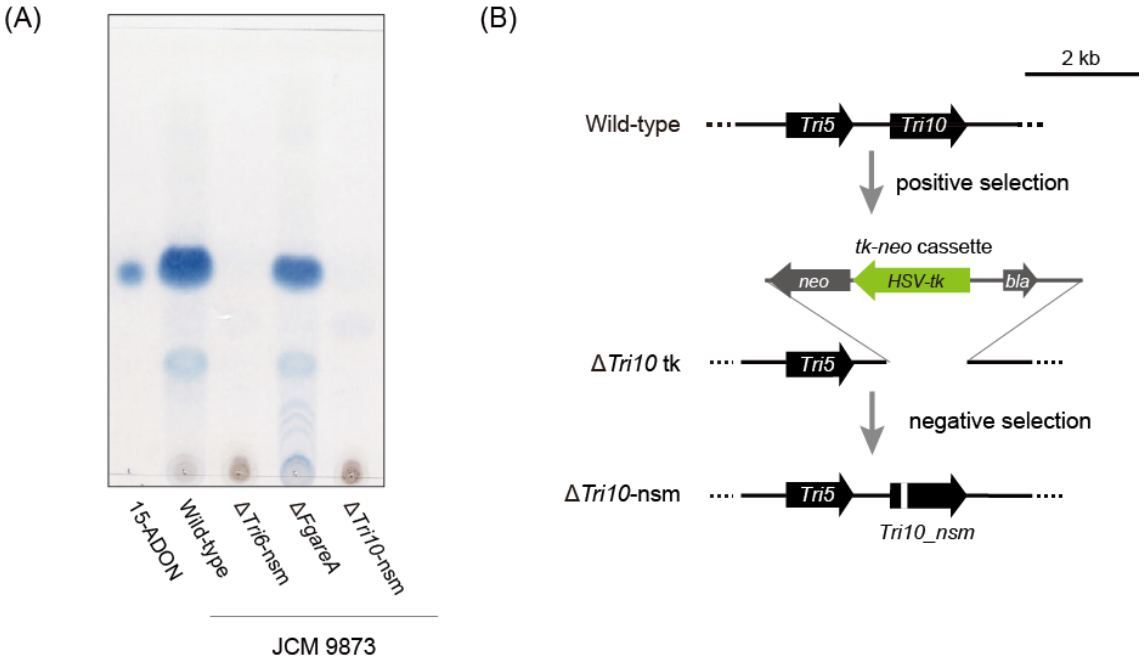
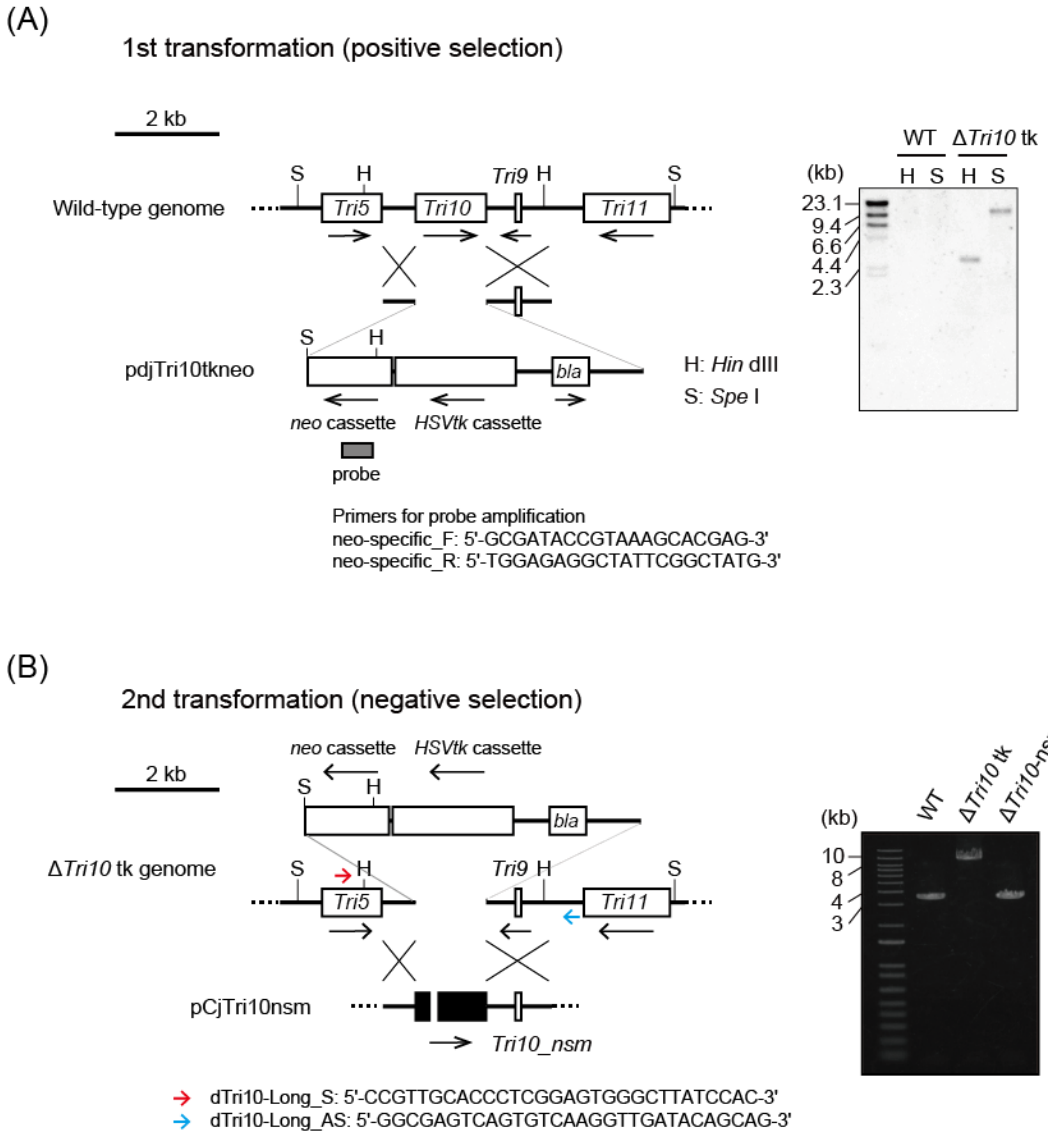


Supplementary Figure 5 | Sequence and structure of pCjTri6nsm. Two pairs of PCR primers (CjTri6pro_S: 5′-TCGAGCTCGGTACCCAGGTACCTTGTCTATCGCGT-3′ and CjTri6pro_AS: 5′-GTAAATCATTTCGAGGGTAGTCA-3′; CjTri6_M4Um_S: 5′-CTCGAAATGATTTACTAGGAGGCCGAATCTCACTACGA-3′ and CompJTri6ter_AS: 5′-CTCTAGAGGATCCCCAGAGCATCTCTCTGATCCGA-3′; sequences overlapping in pUC19 underlined), with 15 bp overhangs necessary for Gibson Assembly and a nonsense mutation for the gene inactivation (doubly underlined), were designed as shown in the figure. The two PCR fragments and *Sma*I-linearized pUC19 were assembled by Gibson Assembly.

Supplementary Figure 6 | Sequence alignment of *Tri10* gene from *F. graminearum* PH-1 (NC_026475 REGION: complement [6646050..6647402]) and *F. sporotrichioides* NRRL 3299 (AF364179 REGION: 2170..3509). Tri6p-binding consensus sequences YNAGGCC on the coding strand (shaded in red) and non-coding strand (boxed in blue) are shown. *F. graminearum* contains four Tri6p-binding consensus sequences while *F. sporotrichioides* contains three.

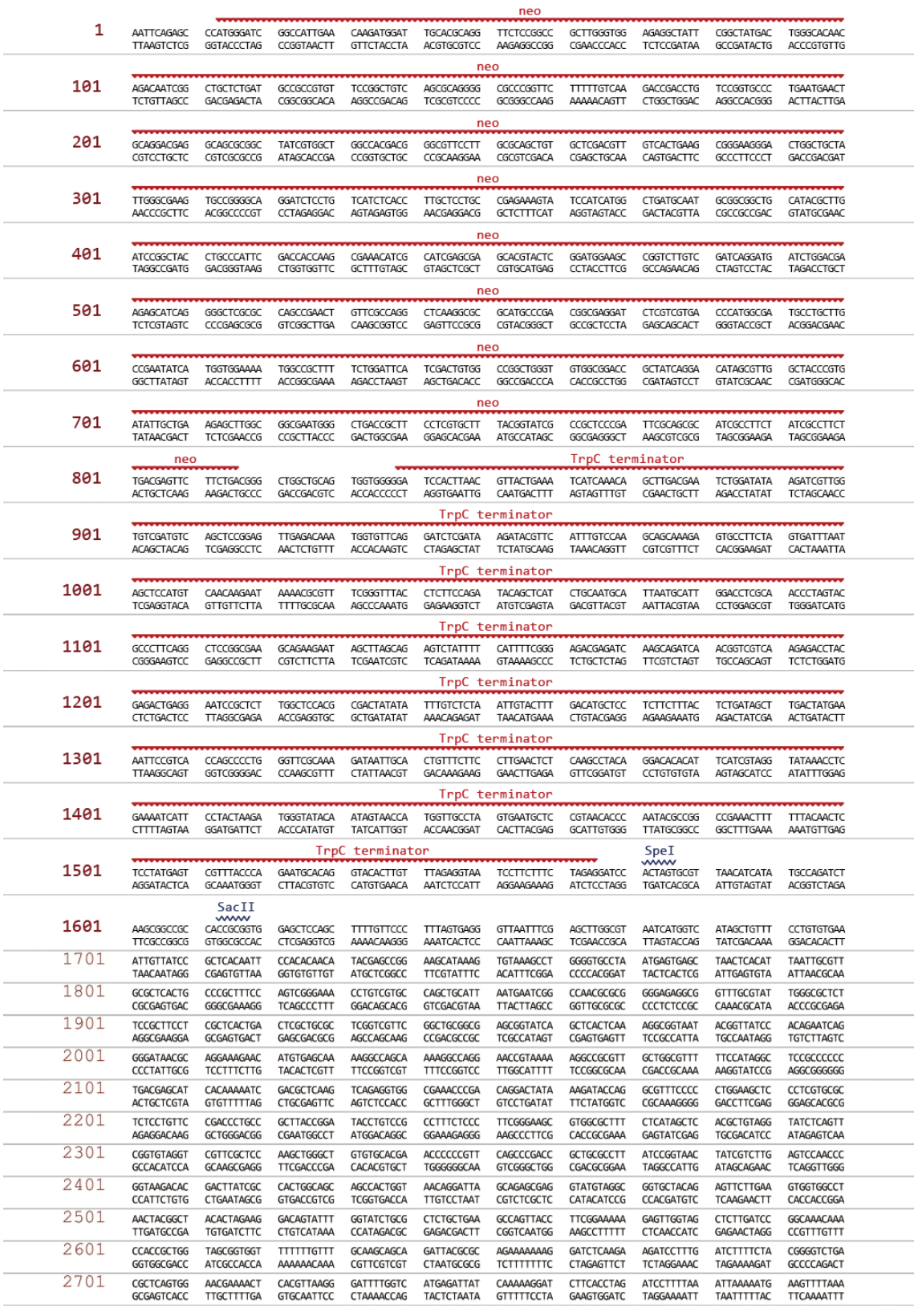


Supplementary Figure 7 | Toxin production assays of the various mutant strains of *F. graminearum* JCM 9873. (A) Each of the four strains analyzed was cultured on the defined media, pH 2.5, with L-glutamine as the nitrogen source and sucrose as the carbon source (**Supplementary Table 1**) for 8 days. The TLC panel shows the spots of 15-ADON extracted from 500 µL of the medium of each culture with ethyl acetate. (B) Schematic representation of the genomic structure Δ*Tri10*-nsm mutant strain. See **Supplementary Figure 8** for experimental details of the mutant strain construction and confirmation. The genomic structure of the Δ*FgareA* strain was described in a previous report (Nakajima et al., 2020). The genomic structure of Δ*Tri6*-nsm is shown in **Supplementary Figure 3E**.



Supplementary Figure 8 | Generation of the Δ*Tri10* tk and Δ*Tri10*-nsm mutant strains. (A) Generation and Southern blot verification of the Δ*Tri10* tk strain. Strain JCM 9873 was transformed with pdjTri10tkneo (**Supplementary Figure 9**) and selected with hygromycin B (left panel). Genomic DNAs of the wild-type (WT) and candidate *Tri10* disruptant were digested with *Hind*III and *Spe*I, and then hybridized with a DIG-labeled probe (*neo*), which was prepared using a PCR DIG Probe Synthesis Kit (Roche Diagnostics GmbH, Mannheim, Germany) and primers described in the figure. Predicted sizes of single bands were detected for the *Hind*III and *Spe*I digested DNA

(right panel). (B) Generation and PCR verification of the Δ*Tri10*-nsm strain. Strain Δ*Tri10* tk was transformed with pCjTri10nsm (**Supplementary Figure 9**) and a candidate strain was obtained by conditional negative selection with 5-FdU (left panel). PCR was performed on the genomic DNAs of the WT, Δ*Tri10* tk, and Δ*Tri10*-nsm strains using primers (red and blue) located outside of the homologous region. Expected sizes of amplicons were obtained (right panel).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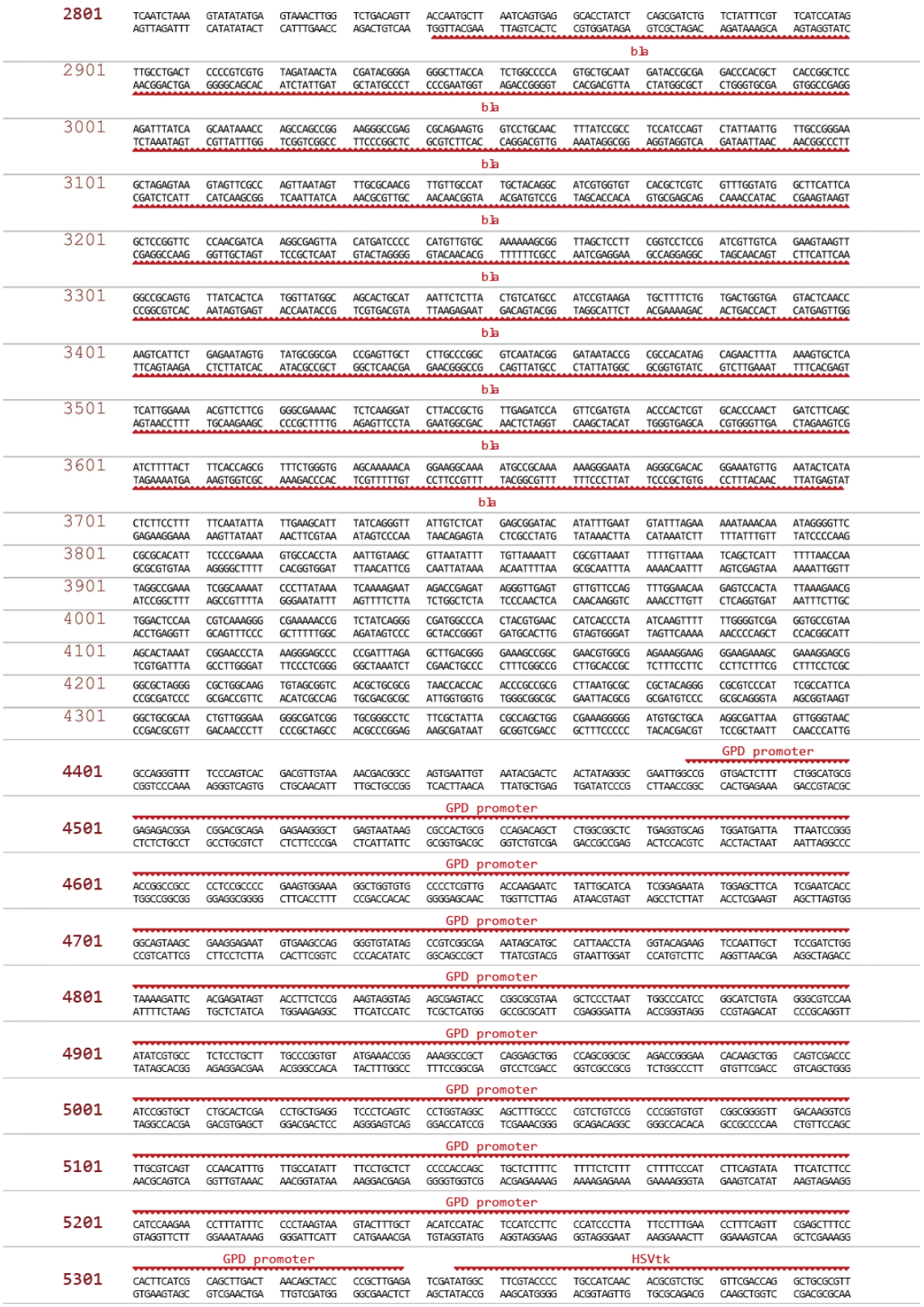
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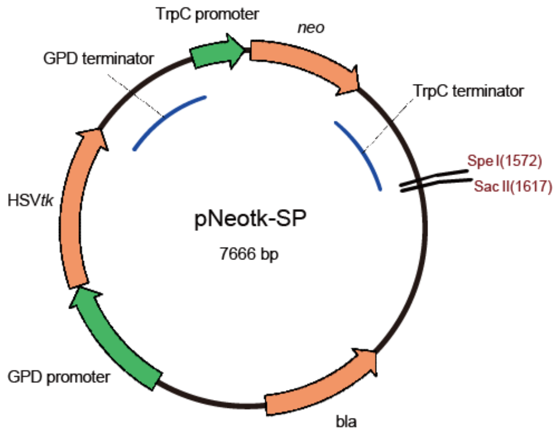

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SpeI

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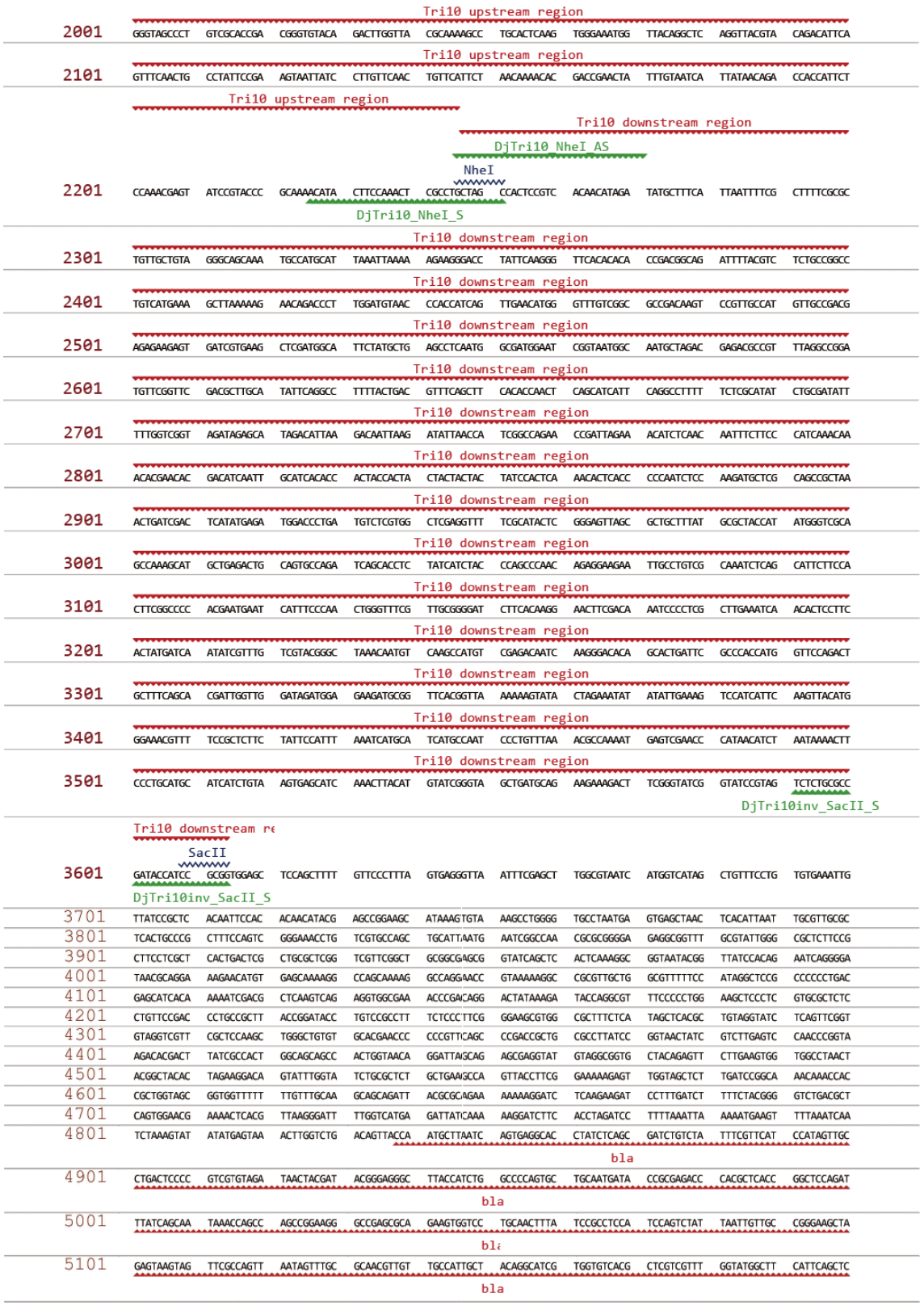
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NheI
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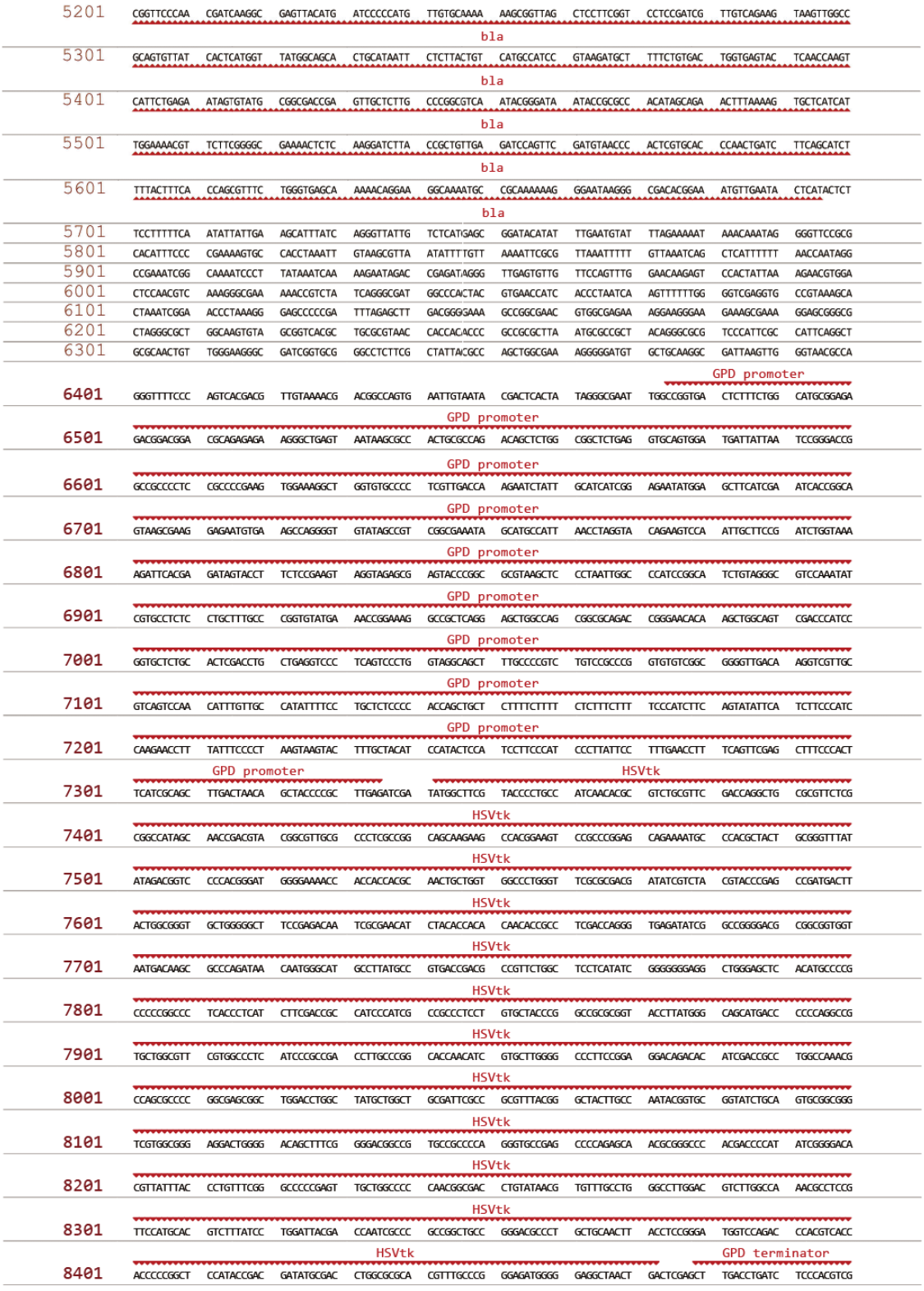
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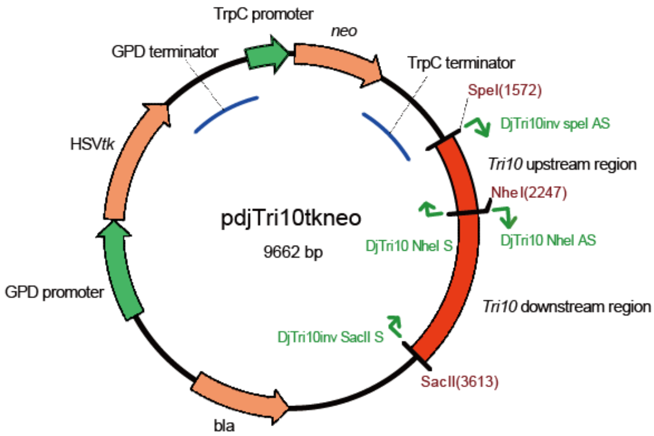

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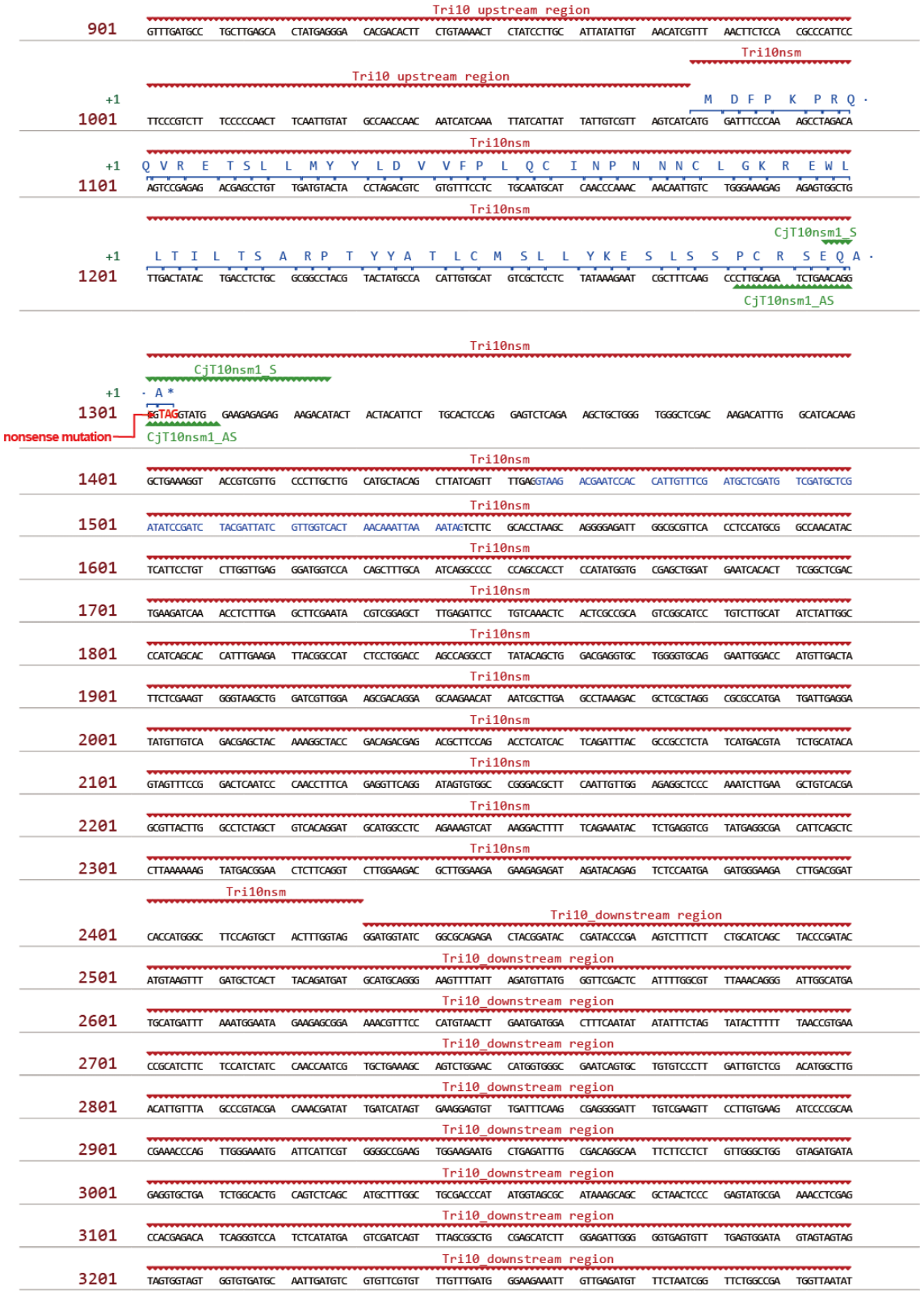
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5001 TTACCAATGC TTAATCAGTG AGGCACCTAT CTCAGCGATC TGTCTATTTC GTTCATCCAT AGTTGCCTGA CTCCCCGTCG TGTAGATAAC TACGATACGG

bla

5101 GAGGGCTTAC CATCTGGCCC CAGTGCTGCA ATGATACCGC GAGACCCACG CTCACCGGCT CCAGATTTAT CAGCAATAAA CCAGCCAGCC GGAAGGGCCG

bla

5201 AGCGCAGAAG TGGTCCTGCA ACTTTATCCG CCTCCATCCA GTCTATTAAT TGTTGCCGGG AAGCTAGAGT AAGTAGTTCG CCAGTTAATA GTTTGCGCAA

bla

5301 CGTTGTTGCC ATTGCTACAG GCATCGTGGT GTCACGCTCG TCGTTTGGTA TGGCTTCATT CAGCTCCGGT TCCCAACGAT CAAGGCGAGT TACATGATCC

bla

5401 CCCATGTTGT GCAAAAAAGC GGTTAGCTCC TTCGGTCCTC CGATCGTTGT CAGAAGTAAG TTGGCCGCAG TGTTATCACT CATGGTTATG GCAGCACTGC

bla

5501 ATAATTCTCT TACTGTCATG CCATCCGTAA GATGCTTTTC TGTGACTGGT GAGTACTCAA CCAAGTCATT CTGAGAATAG TGTATGCGGC GACCGAGTTG

bla

5601 CTCTTGCCCG GCGTCAATAC GGGATAATAC CGCGCCACAT AGCAGAACTT TAAAAGTGCT CATCATTGGA AAACGTTCTT CGGGGCGAAA ACTCTCAAGG

bla

5701 ATCTTACCGC TGTTGAGATC CAGTTCGATG TAACCCACTC GTGCACCCAA CTGATCTTCA GCATCTTTTA CTTTCACCAG CGTTTCTGGG TGAGCAAAAA

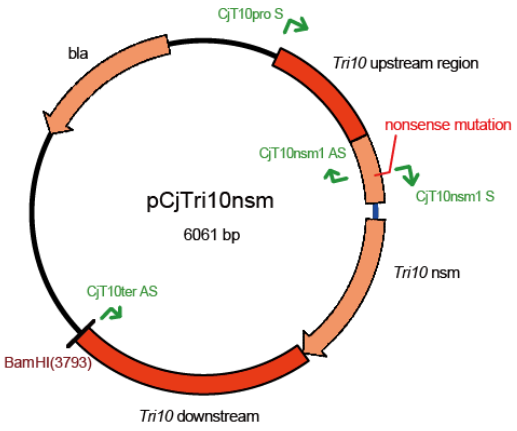
bla

5801 CAGGAAGGCA AAATGCCGCA AAAAAGGGAA TAAGGGCGAC ACGGAAATGT TGAATACTCA TACTCTTCCT TTTTCAATAT TATTGAAGCA TTTATCAGGG

bla

5901 TTATTGTCTC ATGAGCGGAT ACATATTTGA ATGTATTTAG AAAAATAAAC AAATAGGGGT TCCGCGCACA TTTCCCCGAA AAGTGCCACC TGACGTCTAA

6001 GAAACCATTA TTATCATGAC ATTAACCTAT AAAAATAGGC GTATCACGAG GCCCTTTCGT C



Supplementary Figure 9 | Sequences and structures of pNeotk-SP, pdjTri10tkneo, and pCjTri10nsm. For the construction of pdjTri10tkneo, the *Tri10* locus (*Tri10* and its flanking regions) was amplified with primers DjTri10_NheI_S (5′-TATGCTAGCAGGCGAGTTTGGAAGTATGT -3′) and DjTri10_NheI_AS (5′-ATAGCTAGCCACTCCGTCACAACATAGA A -3′) and digested with *Nhe*I (underlined). After self-ligation of the digested fragment, the circular DNA was used as the template for inverse PCR (Akiyama et al., 2000) with outward primers DjTri10inv_SpeI_AS (5′- GCGACTAGTATCCATGATGACTAACGACA -3′) and DjTri10inv_SacII_S (5′- TACCGCGGATGGTATCGGCGCAGAGA -3′) and digested with *Spe*I (doubly underlined) and *Sac*II (wavy underlined). The *Spe*I - *Sac*II fragment was then cloned into the corresponding sites of pNeotk-SP. The resulting vector, pdjTri10tkneo, was used for transformation of the wild-type strain JCM 9873 to generate Δ*Tri10* tk. For the construction of pCjTri10nsm, two pairs of primers (CjT10pro_S: 5′-TCGAGCTCGGTACCCAGGCGAGTTTGGAAGTATGTT -3′ and CjT10nsm1_AS: 5′- CATACCTACGCCTGTTCAGATCTGCAAG -3′; CjT10nsm1_S: 5′- ACAGGCGTAGGTATGGAAGAGAGAGAAGACAT -3′ and CjT10ter_AS: 5′-CTCTAGAGGATCCCCCACTCCGTCACAACATAGATAT -3′; sequences overlapping in pUC19 underlined) and a nonsense mutation for the gene inactivation (doubly underlined), with 15 bp overhangs necessary for Gibson Assembly, were designed as shown in the figure. The two PCR fragments and *Sma*I-linearized pUC19 were assembled by Gibson Assembly. The resulting vector, pCjTri10nsm, was used for transformation of the transgenic strain Δ*Tri10* tk to generate a marker-free self-cloning strain Δ*Tri10*-nsm.

References

Akiyama, K., Watanabe, H., Tsukada, S., and Sasai, H. (2000). A novel method for constructing gene-targeting vectors. *Nucleic Acids Res.* 28, e77-e77. doi:10.1093/nar/28.16.e77

Chen, L., McCormick, S. P., and Hohn, T. M. (2000). Altered regulation of 15-acetyldeoxynivalenol production in *Fusarium graminearum*. *Appl. Environ. Microbiol.* 66, 2062-2065. doi:10.1128/AEM.66.5.2062-2065.2000

Maeda, K., and Ohsato, S. (2017). Molecular genetic characterization of *Fusarium graminearum* genes identified as encoding a precocene II-binding protein. *JSM Mycotoxins* 67, 1-3. doi:10.2520/myco.67-1-3

Nakajima, Y., Akasaka, M., Shiobara, T., Kitou, Y., Maeda, K., Kanamaru, K., et al. (2020). Impact of nitrogen metabolism-associated culture pH changes on regulation of *Fusarium* trichothecene biosynthesis: revision of roles of polyamine agmatine and transcription factor AreA. *Curr. Genet.* 66, 1179-1190. doi:10.1007/s00294-020-01102-x

Nakajima, Y., Tokai, T., Maeda, K., Tanaka, A., Takahashi-Ando, N., Kanamaru, K., et al. (2014). A set of heterologous promoters useful for investigating gene functions in *Fusarium graminearum*. *JSM Mycotoxins* 64, 147-152. doi:10.2520/myco.64.147

Proctor, R. H., Hohn, T. M., McCormick, S. P., and Desjardins, A. E. (1995). *Tri6* encodes an unusual zinc finger protein involved in regulation of trichothecene biosynthesis in *Fusarium sporotrichioides*. *Appl. Environ. Microbiol.* 61, 1923-1930. doi:10.1128/aem.61.5.1923-1930.1995

Seong, K.-Y., Pasquali, M., Zhou, X., Song, J., Hilburn, K., McCormick, S., et al. (2009). Global gene regulation by *Fusarium* transcription factors *Tri6* and *Tri10* reveals adaptations for toxin biosynthesis. *Mol. Microbiol.* 72, 354-367.

doi:10.1111/j.1365-2958.2009.06649.x

Tag, A. G., Garifullina, G. F., Peplow, A. W., Ake, C., Phillips, T. D., Hohn, T. M., et al. (2001). A novel regulatory gene, *Tri10*, controls trichothecene toxin production and gene expression. *Appl. Environ. Microbiol.* 67, 5294-5302. doi:10.1128/AEM.67.11.5294-5302.2001