

**Regulatory Mechanism of Trichothecene Biosynthesis in
*Fusarium graminearum***

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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 ₂ PO ₄	1 g/L
KCl	0.5 g/L
MgSO ₄ ·7H ₂ O	0.5 g/L
Trace elements ¹	0.2 mL/L
FeSO ₄ ·7H ₂ O	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 ·6H ₂ O	5 g/100 mL
H ₃ BO ₃	50 mg/100 mL
Na ₂ MoO ₄ ·2H ₂ O	50 mg/100 mL
CuSO ₄ ·5H ₂ O	250 mg/100 mL

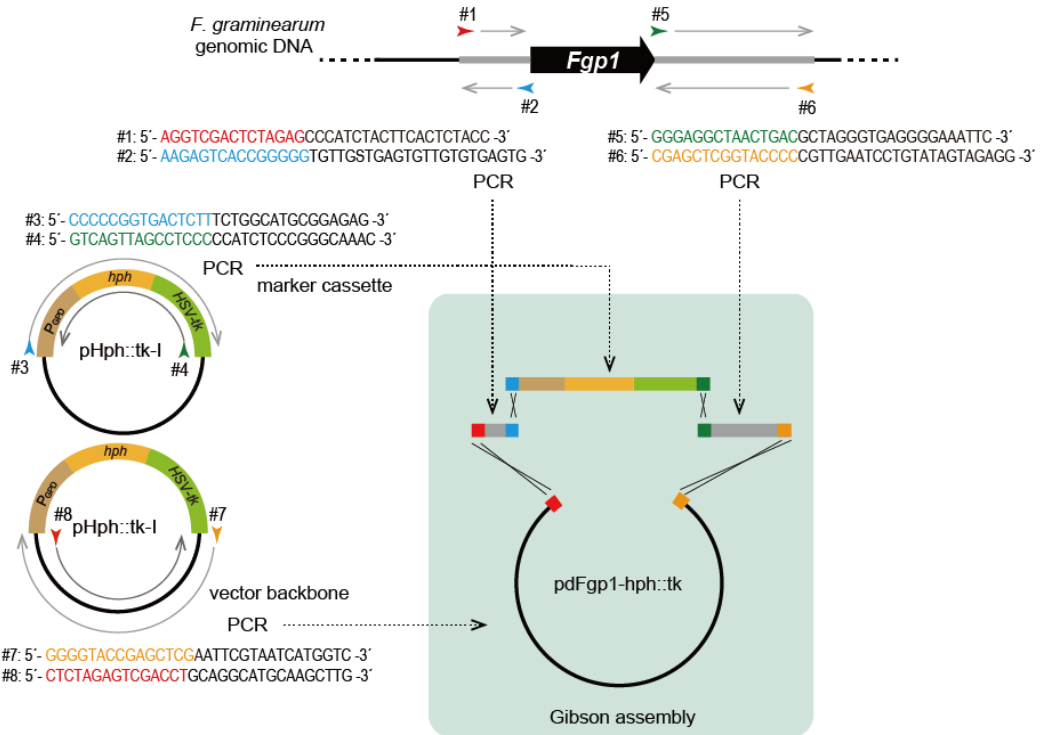
Amino acids mixture

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

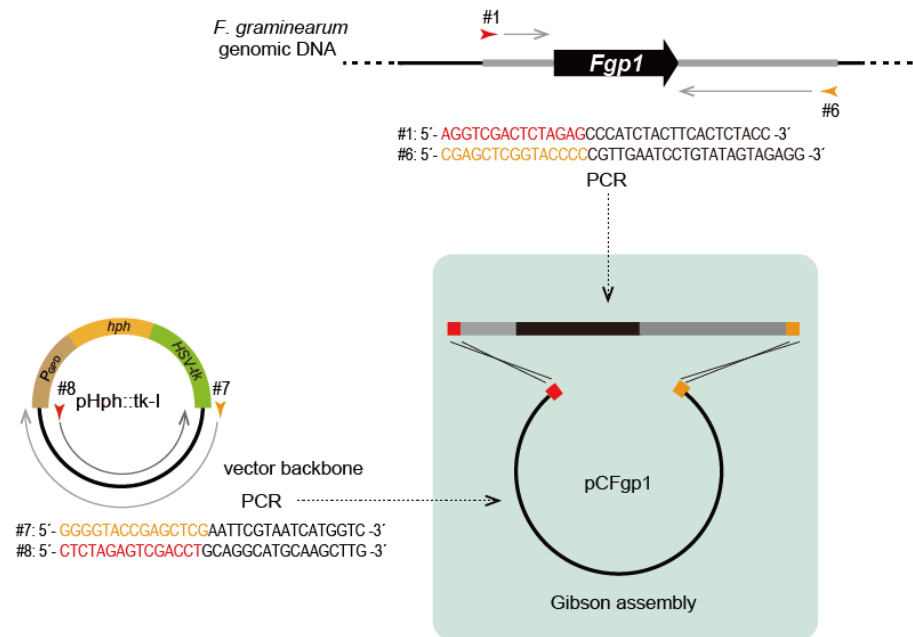
Supplementary Table 2 | List of primers used for qRT-PCR in the study of *Tri10* expression in various *F. graminearum* strains.

Primer name	Sequence (5'-3')	Description
JCM_Tri10_qRT-F	TGTCGCCTCATACGACCTC	Quantitative real-time PCR primers for the analysis of <i>Tri10</i> expression
JCM_Tri10_qRT-R	ATGACGGA ACTCTTCAGGTCTT	
qRT-Gpd_Fw	CGAAGTTGTCGTTGAGGGAG	Quantitative real-time PCR primers for the analysis of <i>GPD</i> expression
qRT-Gpd_Rev	GACAACGAGTG GGGTTACTCC	

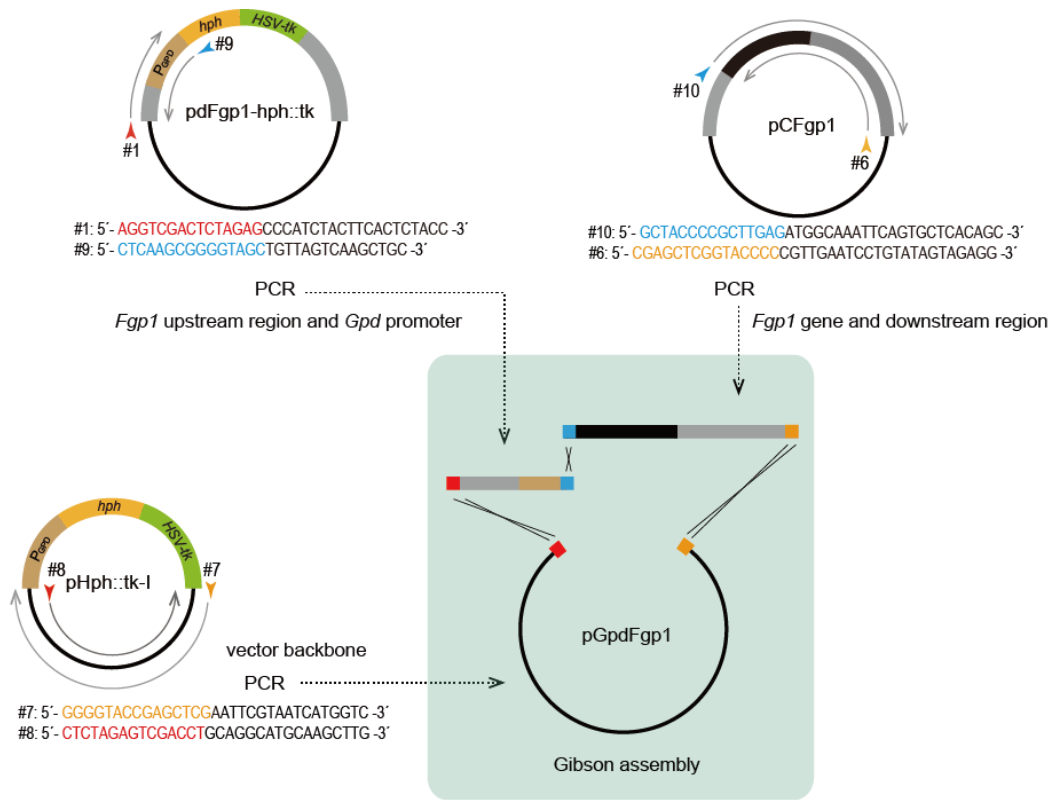
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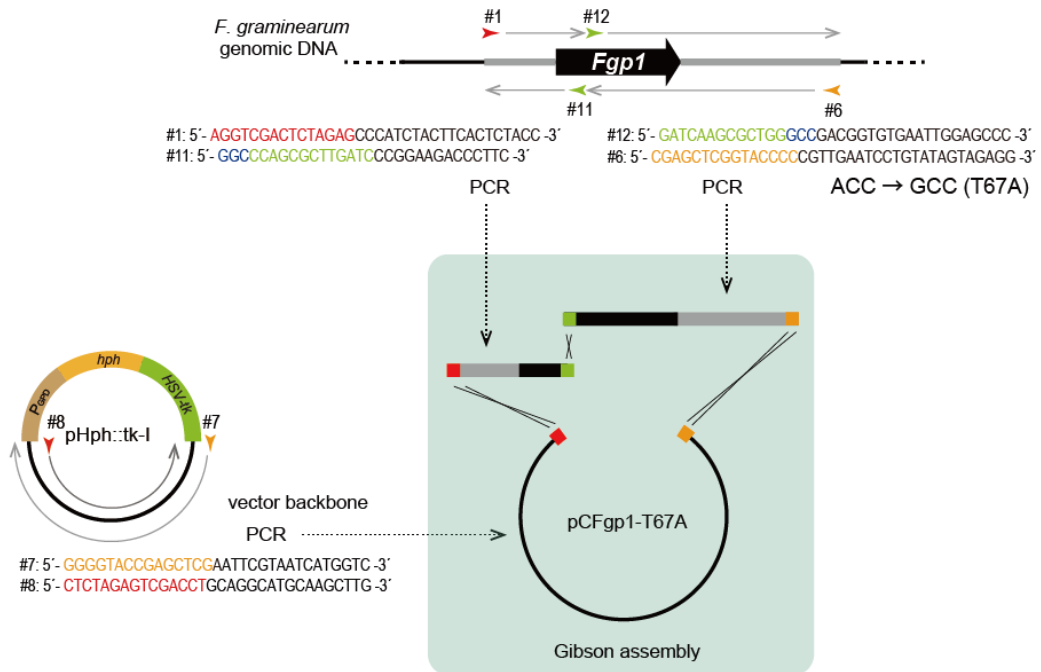
(B)



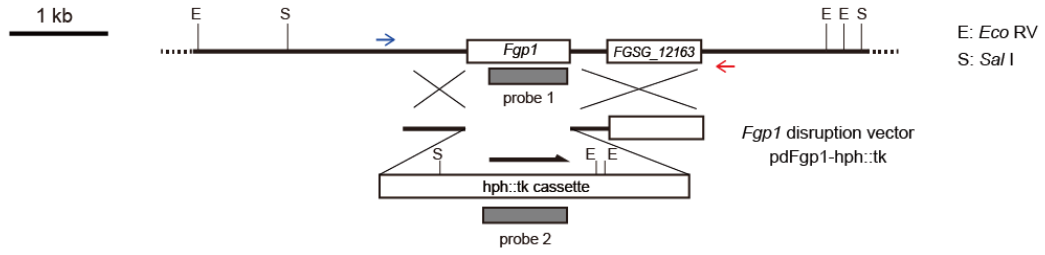
(C)



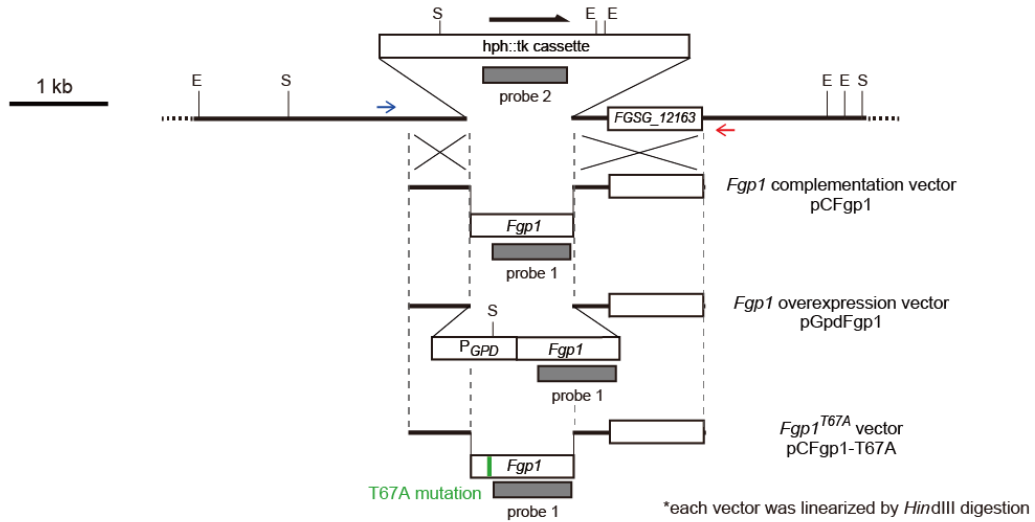
(D)



(E) 1st transformation (positive selection)



2nd transformation (negative selection)

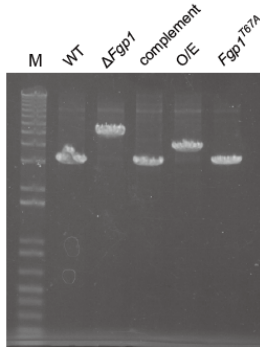


(F)

PCR

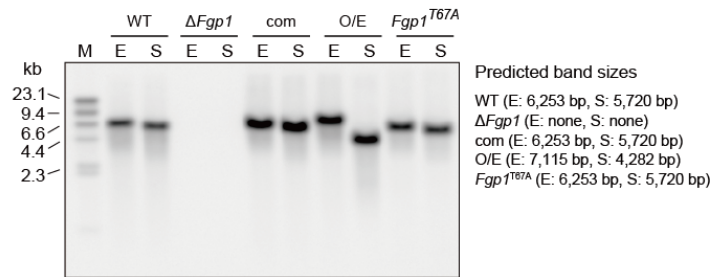
→ *Fgp1*-Long_S:
5'-GGCCTTTCTCAGCTTGACCTAGGGGTCTTC-3'

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

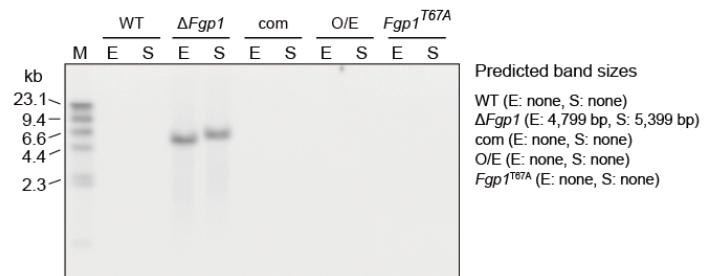


Correct amplicon size
WT (3,183 bp)
 $\Delta Fgp1$ (5,165 bp)
com (3,183 bp)
O/E (4,045 bp)
Fgp1^{T67A} (3,183 bp)

Southern blot analysis



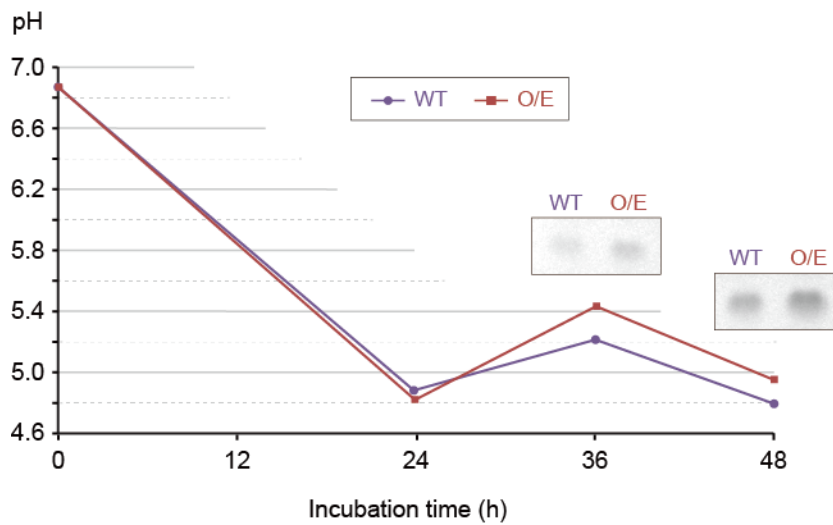
probe 1 Primers for probe 1 amplification
Fgp1-probe_F: 5'-GCCGAATCTTGGGCAACTTC-3'
Fgp1-probe_R: 5'-GCCATTTGGATGGCATGGG-3'



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

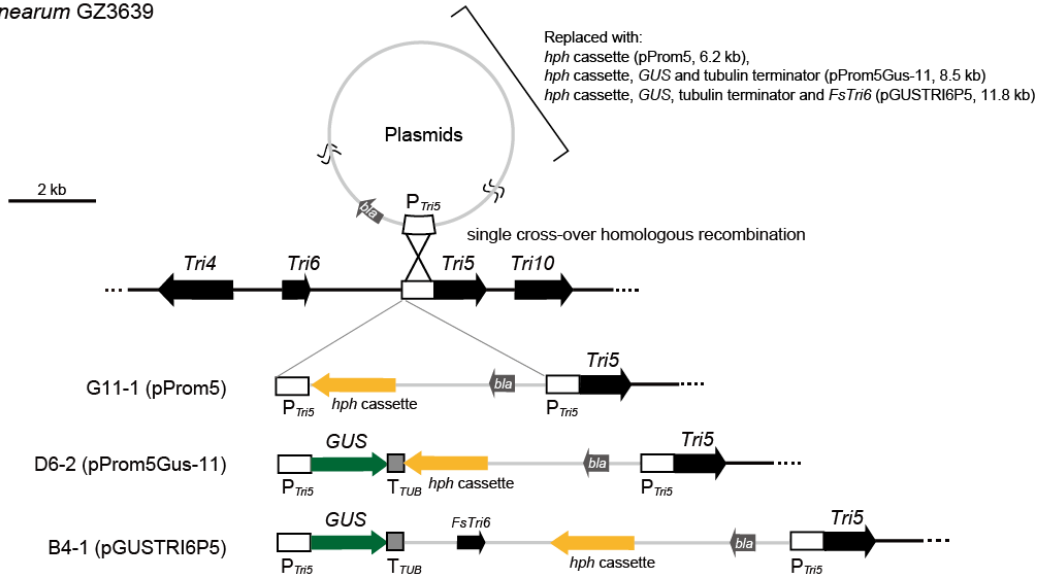
amplified from pHph::tk-I with primers #7 × #8, the region from the upstream of *FgpI* to the putative phosphorylation site was amplified from the genome with primers #1 × #11, and the remaining region of *FgpI* and its downstream region was amplified from the genome with primers #12 × #6. The three fragments were then assembled by Gibson Assembly. (E) Schematic diagram of the genomic structure of the various *FgpI* strains generated: deletion strain ($\Delta FgpI$), complemented strain ($\Delta FgpI + FgpI$; com), overexpressor strain ($\Delta FgpI + P_{GPD}::FgpI$; O/E) and phosphorylation site disruptant ($\Delta FgpI + FgpI^{T67A}$; $FgpI^{T67A}$). (F) PCR and Southern blot verification of the *FgpI* mutant strains. Expected sizes of amplicons were obtained after PCR with primers (red and blue) located outside of the homologous region (left panel). The *FgpI* sequence of *FgpI*^{T67A} strain was confirmed by DNA sequencing. Southern blot of genomic DNA digested with *EcoRV* and *SalI* 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 *EcoRV* and *SalI* digested DNA (right panel).



Supplementary Figure 2 | Toxin production assay of the wild-type (WT) and *FgpI* 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 25°C. 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 μ L 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.

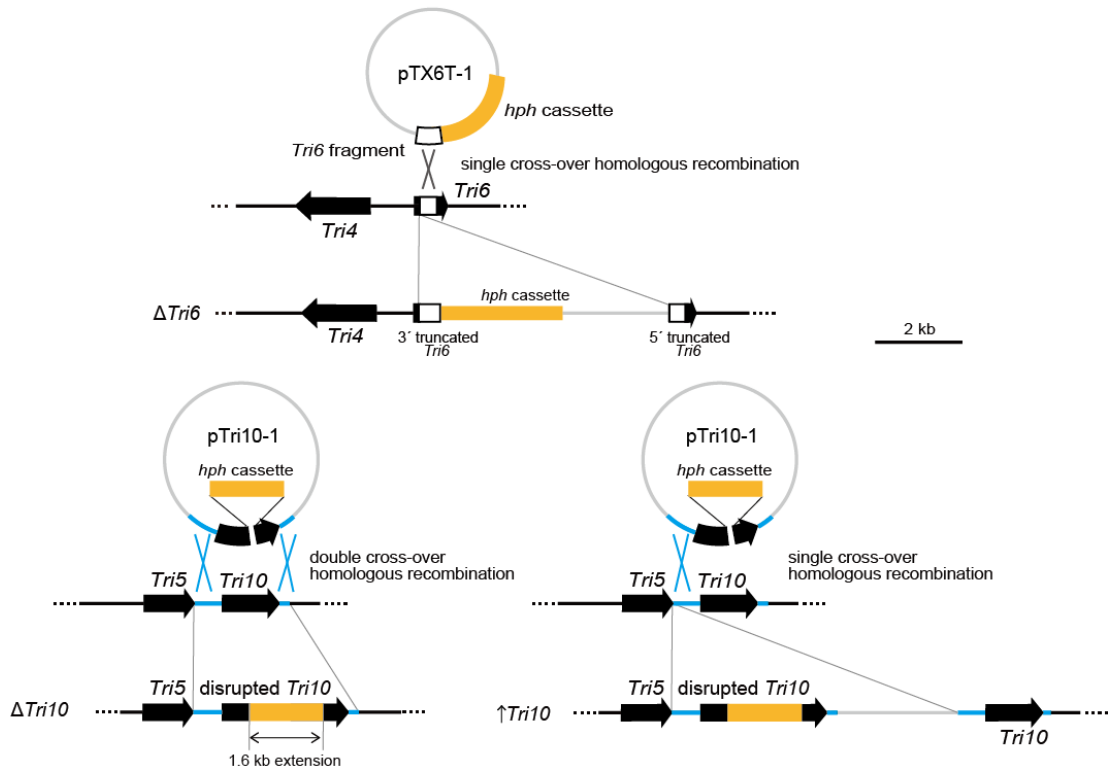
(A)

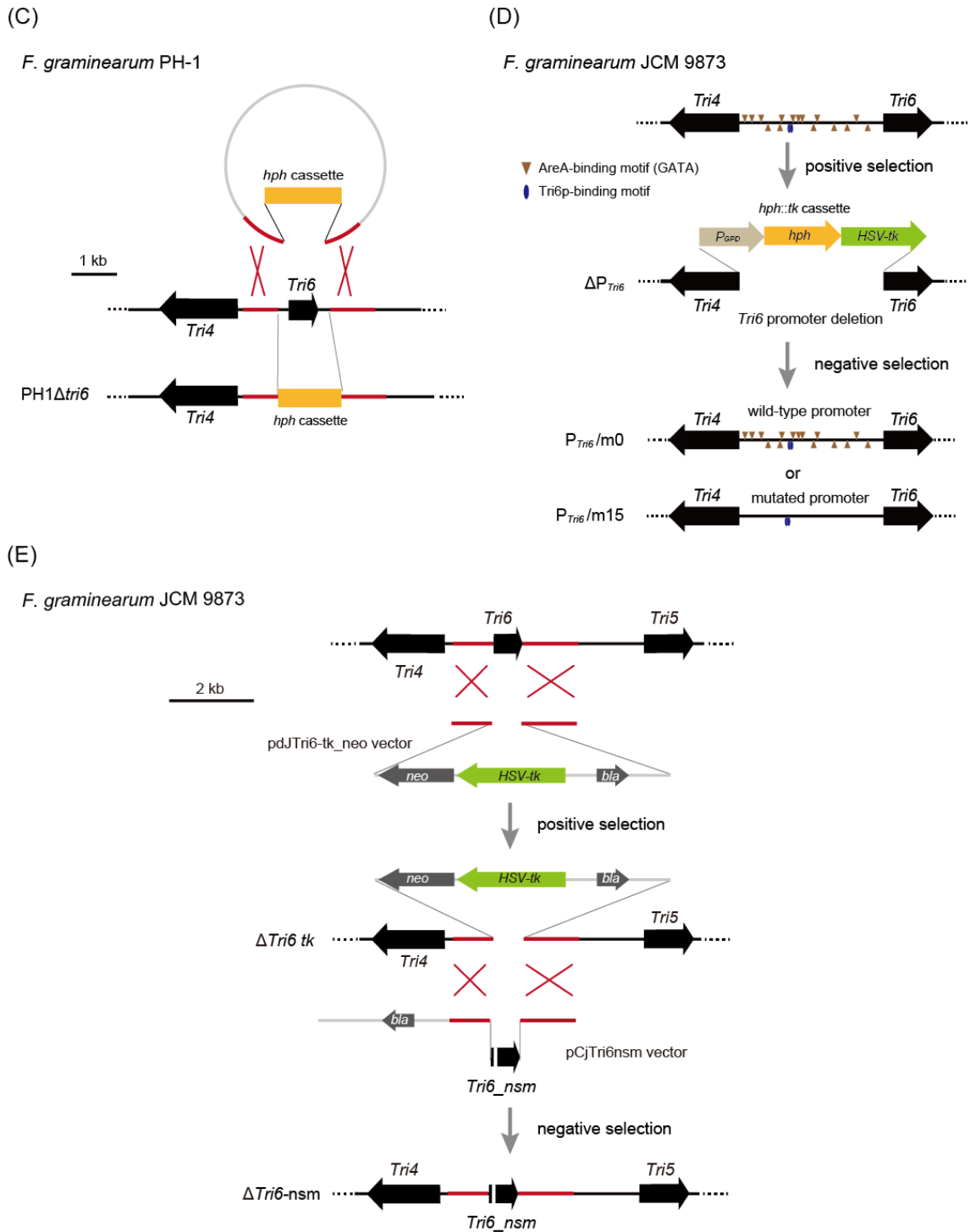
F. graminearum GZ3639



(B)

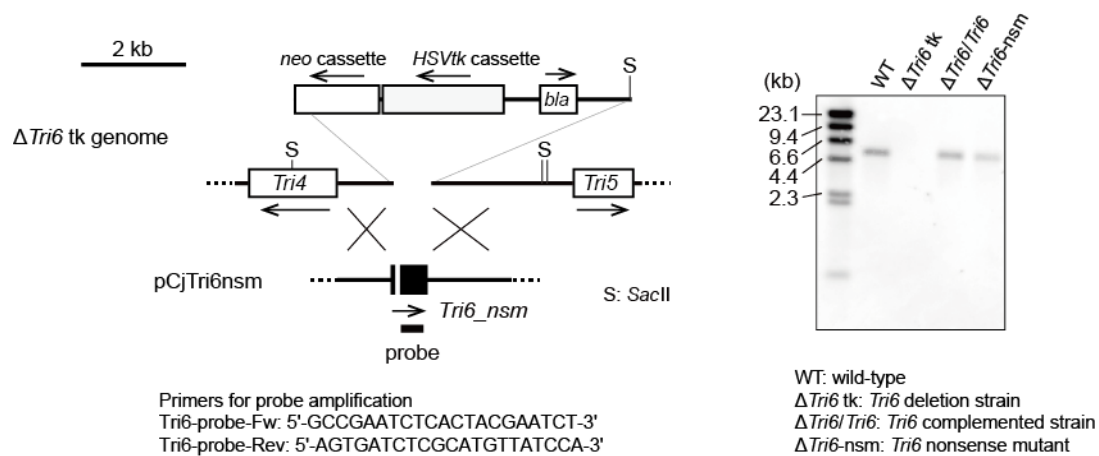
F. sporotrichioides NRRL 3299





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

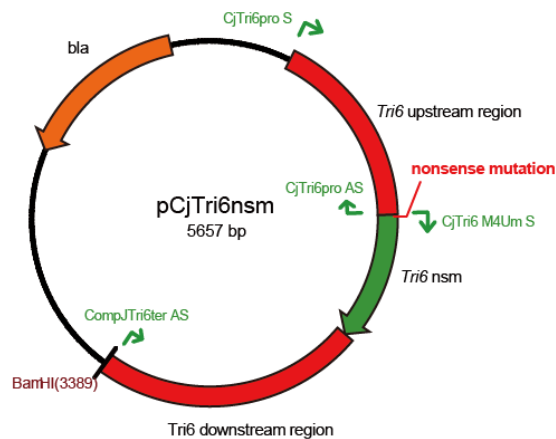
respective plasmids by a single crossover homologous recombination event. (B) Manipulation of the trichothecene gene cluster of *F. sporotrichioides* strain NRRL 3299 (Proctor et al., 1995; Tag et al., 2001). The core cluster region is extended when the respective plasmids are inserted via a single crossover (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 PH1 Δ *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 $\Delta Tri6$ -nsm mutant strain. The *Tri6* disruption mutant, strain $\Delta Tri6$ 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 *SacII*, 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).

1	TCGCCGTTT	CGGTGATGAC	GGTGA AAC	TCTGACACAT	GCASCCTCCG	GAGACGGTCA	CAGCTTGTCT	GTAAGCGGAT	GCCGGGAGCA	GACAAGCCCG
101	TCAGGGCGCG	TCAGCGGGTG	TTGGCGGGTG	TCGGGGCTGG	CTTAAC TATG	CGGCATCAGA	GCAGATTGTA	CTGAGAGTGC	ACCATATGGG	GTGTGAATA
201	CCGCACAGAT	CGGTAAGGAG	AAAATACC	ATCAGGCGCC	ATTCGCCATT	CAGGCTGCGC	AACTGTTGGG	AAGGGCGATC	GGTCCGGGCC	TCTTCGCTAT
										CjTri6pro_5
301	TACGCCAGCT	GGCGAAAGGG	GGATGTGCTG	CAAGSGGATT	AAGTTGGGTA	ACGCCAGGGT	TTTCCAGTC	ACGACGTTGT	AAAACGACGG	CCAAGTAATT
										Tri6 Upstream Region
										CjTri6pro_5
401	CGACTCGGT	ACCCAGGTAC	CTTGTCTATC	GGTCTCTAG	AGTGCCTTGC	ATGCGTGTG	GCCGTAACG	CTCACAACTC	TGAAGTTGTC	CTCAGTATCG
										Tri6 Upstream Region
501	CCGTGTGAGA	TAAGCTTCGC	AAGAGTGTCC	GGTCGGAAAC	TCACCAATCA	ACTCAGCAGG	ATGAACAAGG	GGTCTGAAAG	GCCCTGGCAGG	CCTGACAGGA
										Tri6 Upstream Region
601	GTGATAAAT	GTGAGAAGAG	ATATGCCGAT	ACAACCGTGT	AACCTTGTAA	ACGGGGCATG	GAATCCCATG	GCAAGTTATG	GGGTACAGAG	CAACTGAATT
										Tri6 Upstream Region
701	GCCTACGAGT	CAAGAAGTGC	ATCCTTTTAC	CGCGGCTTA	TCCGAAGTTG	CTGCCGATCA	GATACAGACA	TGCATGCAGA	GTGGTACAGC	TGCCGGGAAG
										Tri6 Upstream Region
801	AATAAGAATC	ATCAGTGC	CGCAATGTTA	AAAAGTGTG	TGCGGAAGCA	ACATTAAGCT	TTGGAGCAT	GCCAGGGTCT	TGCTCGAAA	TATCTTGTTC
										Tri6 Upstream Region
901	TACCAGACC	CATGATGTT	CAAAAGTATG	TACATGGATT	GTCTTGACA	GAAAGCAGCC	TGAGTGTGTA	TGCAGACTGT	CACGCTGCA	GTAAGTTGCC
										Tri6 Upstream Region
1001	ACAGACTCGA	ATCGATTATC	ATTGACCGTT	CGGAAGCGCT	CTGTTAGGAA	TCTTCTAGA	CCACAACACTAC	CACCTTGGCA	TCTGCATCT	AACACTAGTA
										Tri6 Upstream Region
1101	GCCACATAGT	AAACCTTCAA	CTGCGCCCGC	ATCAACTGT	AAACAGTAC	CGGCCGACGC	GTCTCGSATA	AGAATACCTT	TTAACTGCC	GTAGCAAACT
										Tri6 Upstream Region
1201	GTAATTGTG	GTACTTCTG	GACAATATT	TCATGGCTTT	CAGAAGCTTT	CACCTTTAAT	AAAAGTGTG	CTGATAAGA	AACCTTATCA	ATCGTATCCC
										Tri6 Upstream Region
										Tri6nsm
										CjTri6_M4Um_5
										M I Y *
1301	ATCCCATCAA	GGCTCAAGCC	ATCTTTTATT	TTTTTTTTT	TGCATCGCCA	ACCAATATAT	TGAACACTCA	TTTTGACTAC	CCTCGAAATG	ATTACTAGG
										CjTri6pro_AS
										nonsense mutation
										Tri6nsm
										CjTri6_M4Um_5
1401	AGGCCGAATC	TCACTACGAA	TCTTGGAGCG	CCTTGCCCTC	CTTTGATCGA	GTTCGCTCTC	CCGATCCTGC	CAAGGACTTT	GTCCCGATGC	TAAAGACTA
										Tri6nsm
1501	TGATACACA	ACATTGGAAA	TAGACTTTCT	CTCAGAAACT	TATGACTTTG	ACAACCTCCC	CACATACTCT	CTACCAACGG	TGGATTCAAC	CAAGACTTTG
										Tri6nsm
1601	TACTCCGAG	AACCACTTGT	TTGCTTGCAC	TTTACTTTGG	CGAACCCGCG	TATCGAAAAT	TATATAACCA	CATCGTCGGG	ACTGTTGGAC	GCAGTGCCAA
										Tri6nsm
1701	GCCAGCTTAT	CGCCCTTCCC	ACCTCACAC	GGCCAAGCAA	ATGCCATTTC	CCTAGTTGCA	AGTCGCCCAC	AGTCTTTGAA	AGCCGACGGG	ACTTTAGGGG
										Tri6nsm
1801	GCATTACCGG	CAACACTTCA	AGCGCTTTTT	CTGTGCTAC	TCAGAATGCC	CTCAGTCAGC	TCAAGACTGT	CAAGAAGTGG	GCACCAAGGG	CTTTGCGACT
										Tri6nsm
1901	CGCAAGACC	GTGCTCGGCA	TGAGTCTAAG	CACAACCAA	CAGTGGCGTG	CCCTTGGCAA	GACAAGGAAG	GACAACAATG	TCTGAGGGTC	TTTAGCAGGG
										Tri6 downstream region
										Tri6nsm
2001	TGGATAACAT	GCGAGATCAC	TATAGCCGGA	TACATAAGTG	TTGACGAGGG	ATCGGTGTGC	AAAAGTGCAG	ATAGTTACTC	ATAAAGGCA	ACATTTGCGA
										Tri6 downstream region
2101	AAATCAATA	CAATATCTCC	ATAAATATCC	CATTAGACTT	TTTGAATTTC	ATAAATGATG	ACGAGTTTGG	CGCCGATCGA	TGCTGTTTCC	AGCACCAATT
										Tri6 downstream region
2201	CATAATATAC	TCCATAGTAG	CCGAGACCCCT	GCAGCTCATT	GGTACCAGCC	CTGTTTTTAT	TGCACAATTA	ATGACACTCT	ATCTGCAAG	CGACTCAAGG
										Tri6 downstream region
2301	GCTTCAACA	CTTACATATT	GCCTCTACGC	GTGGCTGTGT	CTAAGCGAGA	TCAGCGCGGT	CATTGTTAAT	GCTGCGTTGC	TTTGGCCCCG	TACGTTCCAC
										Tri6 downstream region
2401	AACAATCTGG	CCACTSCAAG	GTATTTGGGG	CCGCTGGTTA	AATCTCTTA	GTAGTCTGCC	TCATTTGGCA	GGTSCCAATA	ATATATTTTA	CCCCGGTTTG
										Tri6 downstream region
2501	GCTGGCCATG	CTACCCAAAA	GGCTTCATAC	ATATCTGGCC	CATATCGTTT	ACTCGGTTCC	ACGTTTACGA	GCATACGCCT	CTCGTATCAA	TGTGSCAAAC
										Tri6 downstream region
2601	AAACATAGCT	CATTGGTTAC	TATGCCGGGT	TTCCGCGAAC	CATTGCTGTG	CCGCAAAAGG	ATGCATCGGC	ACTCTAAAGA	TTGGCATCTC	GTGTTAGAGG
										Tri6 downstream region
2701	GTGACTTGG	TCAAGAAGTA	ACGTAACAAG	TGAGATAATG	AAATTAATTA	CCTGAGGGCA	ATTTAAGGTT	TCAACCTCCG	AGGAAAGCCA	TTGCTCGTGG

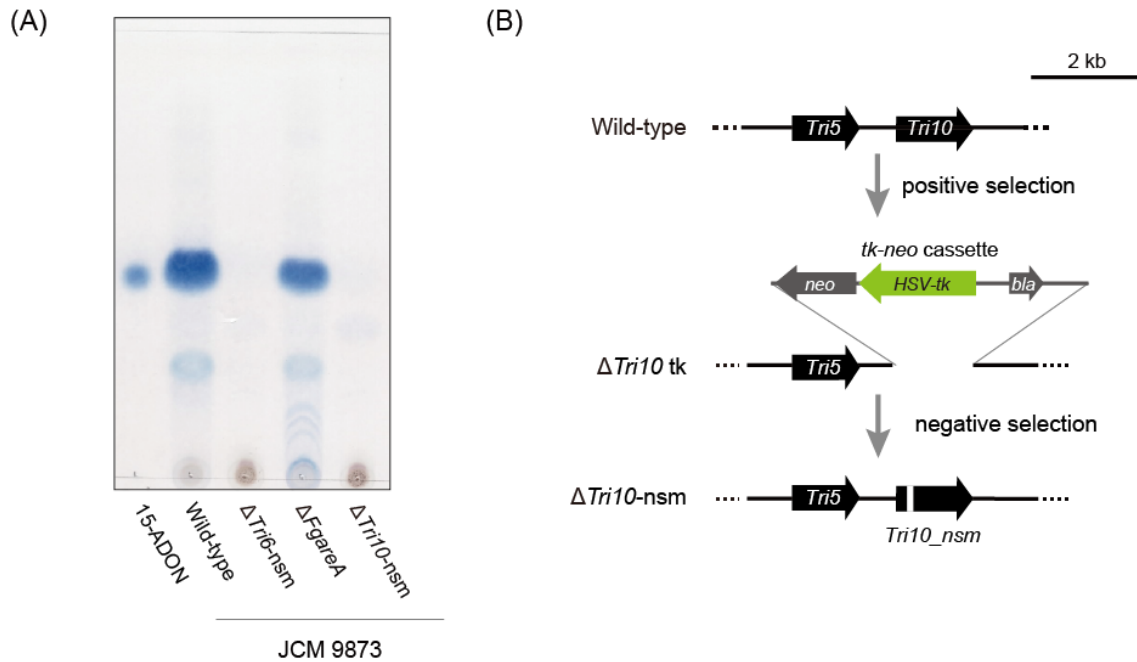
	Tri6 downstream region									
2801	GATATAATCT	GCAATAGGGT	AGGCTTTGCT	GTTTTTTTCG	GAGCATTGT	GACTGTTTTG	GATTGTGTCT	TTGGGGTCTT	TTTGTCTTCA	AGGCTGTGTA
	Tri6 downstream region									
2901	GTTTCAAGG	AACGAGTCAG	TCACGGCAGG	CACTGAGTCA	AACACTGTTT	CGTAATAATC	ATGATTGTGT	GTTGCAAAAT	TCCAACGAAG	CTTTGCCGCG
	Tri6 downstream region									
3001	TCAGTCAAT	TCGCACACGT	CGATACTTTT	TGCTGGCATT	GGCCAGTAT	TCATCCATGA	GTGGCTGAAC	CGTAGTTGAT	TCCTAAGTCC	AACCCTAATA
	Tri6 downstream region									
3101	GTGCCCGCG	GAATGAGACG	TTTTCGGGTG	TCTGTAGCCG	AGATGTGGAT	AGTAACGGTA	ACCTAGTCA	AATGAGACGT	GGCAGGGTT	CATGGTTGT
	Tri6 downstream region									
3201	GAACCTTGT	CATCAAGATG	TTGATGCCGT	TGACCTACGG	AATACCATCT	TTACATGTA	TTTGTTCCTA	ACCCACGTGG	CTATACCAAC	ATCCCGTGA
	Tri6 downstream region									
3301	TATTCATTGG	TTGGCTTTAT	ATATTGATC	AGTAAATTC	AAGATCGCGA	GCGGGAATTT	CCTTGTCCGA	TCAGAGAGAT	GCTCTGGGGA	TCCTCTAGAG
	CompJTri6ter_AS									
3401	TCGACTGCA	GGCATGCAAG	CTTGGCGTAA	TCATGGTCAT	AGCTGTITCC	TGTGTGAAAT	TGTTATCCGC	TCACAAATCC	ACACAACATA	CGAGCCGGAA
3501	GCATAAAGT	TAAAGCCTGG	GGTGCCTAAT	GAGTGAGCTA	ACTCACATTA	ATTGCGTTGC	GCTCACTGCC	CGCTTCCAG	TCGGGAAACC	TGTCGTGCCA
3601	GCTGCATTAA	TGAATCGGCC	AACCGCGGG	GAGAGCGGGT	TTGCGTATTG	GGCGCTCTTC	CGCTTCCTCG	CTCACTGACT	CGCTGCGCTC	GGTCTTCGG
3701	CTGGGCGCAG	CGGTATCAGC	TCACTCAAAG	GCGGTAATAC	GGTTATCCAC	AGAATCAGGG	GATAACGCAG	GAAGAAGCAT	GTGAGCAAAA	GGCCAGCAAA
3801	AGGCCAGGAA	CCGTAAAAAG	GCCGCGTTGC	TGGCGTTTTT	CGATAGSCTC	CGCCCCCTTG	ACGAGCATCA	CAAAAAATCGA	CGCTCAAGTC	AGAGGTGGCG
3901	AAACCCGACA	GGACTATAAA	GATACCAGGC	GTTTCCCTTC	GGAACTCCCT	TCGTGCGCTC	TCCTGTTCCG	ACCCTGCCGC	TTACCGGATA	CGTGTCCGCC
4001	TTTCTCCCTT	CGGGAAGCGT	GGCGCTTTCT	CAATGCTCAC	GCTGTAGSCT	TCTCAGTCCG	GTGTAGTCCG	TTCTGCTCAA	GCTGCGCTGT	GTGCACGAAC
4101	CCCCCGTTCA	GCCCGACCGC	TGCGCTTAT	CCGGTAACTA	TCGTCTTGAG	TCCAACCCGG	TAAGACACGA	CTTATGCCA	CTGGCAGCAG	CCACTGTGTA
4201	CAGGATTAGC	AGAGCAGAGT	ATGTAGSCTC	TGCTACAGAG	TTCTTGAAGT	GGTGGCCTAA	CTACGCTTAC	ACTAGAAGGA	CAGTATTGG	TATCTGGGCT
4301	CTGCTGAAGC	CAGTTACCTT	CGAAAAAAGA	GTTGGTAGCT	CTTGATCCGG	CAAAACAACC	ACCCGTGGTA	GCGGTGGTTT	TTTTGTTTGC	AAGCAGCAGA
4401	TTACGCGCAG	AAAAAAGGA	TCTCAAGAAG	ATCCTTTGAT	CTTTTCTACG	GGGTCTGACG	CTCAGTGAA	CGAAAACTCA	CGTTAAGGGA	TTTTGGTCAT
4501	GAGATTATCA	AAAAGGATCT	TCACCTAGAT	CCTTTTAAAT	TAAAAATGAA	GTTTTAAATC	AATCTAAAGT	ATATATGAGT	AAACTTGGTC	TGACAGTTAC
	bla									
4601	CAATGCTTAA	TCAGTAGGCG	ACCTATCTCA	GCGATCTGTC	TATTCTGTC	ATCCATAGTT	GCTGACTCC	CCGTGCTGTA	GATAACTAGC	ATACGGGAGG
	bla									
4701	GCTTACCATC	TGCCCCAGT	GCTGCAATGA	TACCGCAGGA	CCCACGCTCA	CCGCTCCAG	ATTTATCAGC	AATAAACAG	CCAGCCGGAA	GGCCGAGCG
	bla									
4801	CAGAAGTGGT	CCTGCAACTT	TATCCGCTC	CATCCAGTCT	ATTAATTGTT	GCCGGGAAGC	TAGAGTAAGT	AGTTCGCCAG	TTAATAGTTT	GGCAACGTT
	bla									
4901	GTTGCCATTG	CTACAGGACT	CGTGGTGTCA	CGCTCGTCTG	TTGGTATGGC	TTCAATCAGC	TCCGGTCCC	AACGATCAAG	GCGAGTTACA	TGATCCCCCA
	bla									
5001	TGTTGTGCAA	AAAAGCGGTT	AGCTCCCTCG	GTCCTCCGAT	CGTTGTGAGA	AGTAAGTTGG	CCGAGTGT	ATCACTCATG	GTTATGGCAG	CACTGCATAA
	bla									
5101	TTCTTACT	GTCATGCCAT	CGTAAGATG	CTTTTCTGTG	ACTGGTGTG	ACTCAACAA	GTCACTTGA	GAATAGTGT	TGCGGCGACC	GAGTTGCTCT
	bla									
5201	TGCCCCGCT	CAATACGGGA	TAATACCGCG	CCACATAGCA	GAACCTTAAA	AGTCTCATC	ATTTGAAAA	GTTCTCCGG	GCGAAAACTC	TCAAGGATCT
	bla									
5301	TACCGCTGTT	GAGATCCAGT	TCGATGTAAC	CCACTCTGTC	ACCCAACGTA	TCTTCAGCAT	CTTTACTTT	CACCAAGCTT	TCTGGTGTG	CAAAAAAGG
	bla									
5401	AAGCAAAAT	GCCGCAAAA	AGGAATAAG	GCCGACACGG	AAATGTGAA	TACTCATACT	CTTCTTTTT	CAATATTAT	GAAGCATTTA	TCAGGGTTAT
	bla									
5501	TGCTCATGA	GCGGATACAT	ATTTGAATG	ATTTAGAAAA	ATAAACAAAT	AGGGGTTCCG	GCGACATTTC	CCGAAAAAGT	GCCACCTGAC	GTCTAAGAAA
5601	CCATTATTAT	CATGACATTA	ACCTATAAAA	ATAGGCGTAT	CACGAGSCCC	TTTCGTC				



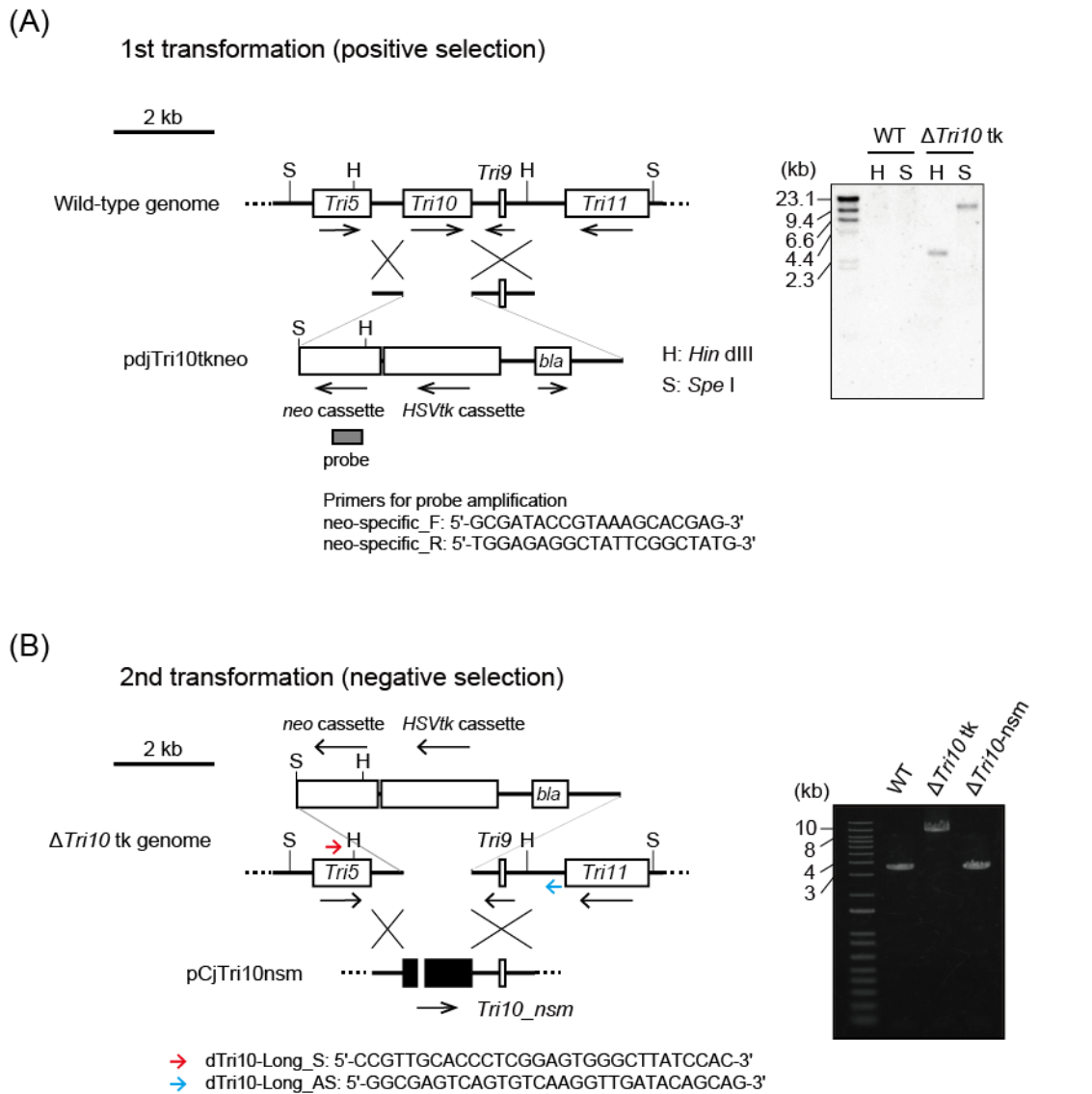
Supplementary Figure 5 | Sequence and structure of pCjTri6nsm. Two pairs of PCR primers (CjTri6pro_S: 5'-TCGAGCTCGGTACCCAGGTACCTTGTCTATCGCGT-3' and CjTri6pro_AS: 5'-GTAAATCATTTTCGAGGGTAGTCA-3'; CjTri6_M4Um_S: 5'-CTCGAAATGATTTACTAGGAGGCCGAATCTCACTACGA-3' and CompJTri6ter_AS: 5'-CTCTAGAGGATCCCCAGAGCATCTCTCTGATCCGA-3'; 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.

	<i>Tri10</i> start codon →		
<i>F. graminearum</i>	ATGGATTCCCAAAGCCTAGACAAGTCCGAGAGACGAGCCTGTTGATGTACTACCTAGACGTGCTGTTTCTCTGCAATG	80	
<i>F. sporotrichioides</i>	ATCGAATTTCGGAAGCCGAGACAGTTCCAGAGAGACGAGTCTGTTGATGTACTACCTGGACGTGCTGTTTCTCTGCAATA	80	
<i>F. graminearum</i>	CATCAACCCAAACAACAATTGTCTGGGAAAGAGAGAGTGGCTGTTGACTATACTGACCTCTGCGCGGCCTACGTACTATG	160	
<i>F. sporotrichioides</i>	CATTTCCACCAAACAACAATTGTCTGGGGAAAAGAGAGTGGTGTGTTGACTATACTAACTTCTGCTCGCCCTACATACTATG	160	
<i>F. graminearum</i>	CCACATTGTGCATGTCGCTCCTCTATAAAGAATCGCTTTCAGCCCTTGCAGATCTGAACAGGCGATGTTATGGAAGAGA	240	
<i>F. sporotrichioides</i>	CAACATTGTGCCTGCGCCTCCTTTATAAAGAGTCTCTTCAACCTCGTGCAGAAAGTGAACAAACATAGTATGGAAGAGG	240	
<i>F. graminearum</i>	GAGAAGACATACTACTACATTTCTGCACTCCAGGAGTCTCAGAAGCTGCTGGGTGGGCTCGACAAGACATTTGGCATCAC	320	
<i>F. sporotrichioides</i>	GAAAAGAGCTACTACTACATTTCTGCGCTCCAGGAGTCTCAAAAGCTTGGGTGGGCTTAAACAAGACCTTTGGTATCAC	320	
<i>F. graminearum</i>	AAGGCTGAAAGGTACCGTCGTTGCCCTTGCCTGTCATGCTACAGCTTATCAGTTTTGAGGTAAGACGAATCCACCATTGTT	400	
<i>F. sporotrichioides</i>	AAGGCTGAAAGGGACGGTCGTTGCCCTCGCTGTCATGCTTACAGTTATCGGGTTTGAAGTAAGACGAATCCACCATTGACT	400	
<i>F. graminearum</i>	TCGATGCTCGATGTCGATGCTCGATATCCGATCTACGATTATCGTTGGTCACTAACAAAATAAAAATAGTCTTCGCACCTA	480	
<i>F. sporotrichioides</i>	A-----CGATGTTCAATACCAGATGTATAATTATTGTTGGCGACTAACGCATTGCGACAGTCTTCGCACCTG	467	
<i>F. graminearum</i>	AGCAGGGGAGATTGGCGGTTCCCTCCATGCGGCCAACATACTACTATTCTGTCTTGGTTGAGGGATGGTCCACAGCTTT	560	
<i>F. sporotrichioides</i>	AGTAGGGGAGATTGGCGTGTTCACCTCCTTGTCTGCCAACACACTATTCTGTGTTGGCCGAGGGTTGGTCCACAGCTTT	547	
<i>F. graminearum</i>	GCAATCAGGCCCCCCAGCCACCTCCATATGGTGCAGCTGGATGAATCACACTTCGGCTCGATGAAGATCAAACTCTTT	640	
<i>F. sporotrichioides</i>	GCAGTCAGGCCCCCCAGCCACTTCCATATGGTGTGAGTTAGACGAATCGGACTTTGATTCAATCGACGATCAGACCTCTT	627	
<i>F. graminearum</i>	TGAGCTTCGAATACGTCGGAGCTTTGAGATTCCTGTCAAACCTCACTCGCCGAGTCGGCATCCTGTCTTGCATATCTATT	720	
<i>F. sporotrichioides</i>	TGAGCTTCGAATATCTCGAGCTTTGAGATTCCTGTGCAACTCCTTGGCGAAAATCGGCATCTTATCTTGCATATCTGTT	707	
<i>F. graminearum</i>	GGCCCATCAGCACCATTGGAAGATTACGGCCATCTCTGGACCAGCCAGGCCCTTATACAGCTGGACGAGGTGCTGGGGTG	800	
<i>F. sporotrichioides</i>	GGTCCAGCAGCGCCATTGGAAGACTATGGTCACCTCTGGACCAGCCAGGCCCTGATACAGCTGGAAGAGGTACTGGGGTG	787	
<i>F. graminearum</i>	CAGGAATTGGACCATGTTGACTATTCTCGAAGTGGGTAAGCTGGATCGTTGGAAGCGACAGGAGCAAGAACATAATCGCT	880	
<i>F. sporotrichioides</i>	CAAGAACTGGGCCATGCTGACTATTCTTGAAGTGGGTAAGCTGGACAGGTGGAAGCGCCAGGAGCAGGAACATAACCGTT	867	
<i>F. graminearum</i>	TGAGCCTAAAGACGCTCGCTAGGCGCGCCATGATGATTGAGGATATGTTGTCAGACGAGCTACAAAGGCTACCGACAGAC	960	
<i>F. sporotrichioides</i>	TGAGCCTGAAGACACTTGCTATGCGCGCAATGATTATAGAGGATATGTTGACAGACGAACTACAAAACCTCCGACAAGC	947	
<i>F. graminearum</i>	GAGACGCTTCCAGACCTCATCACTCAGATTTACGCCGCTCTATCATGACGATCTGCATACAGTAGTTTCCGGACTCAA	1040	
<i>F. sporotrichioides</i>	GAGACGCTACCGGATCTGATCACCACATTTACGCCGCTCTATCGCGACATACCTGCATACAGTAGTTTCCAGACTGAA	1027	
<i>F. graminearum</i>	TCCCAACCTTTTCAGAGGTTCCAGGATAGTGTGGCCGGGACGCTTCAATTGTTGGAGAGGCTCCCAAATCTTGAAGCTGTCA	1120	
<i>F. sporotrichioides</i>	TCCCAACCTTTTCAGAGGTTCCAGGATAGCGTGTGCCAACAATATTATTGTTGGAGAGGCTCCAGACTTGAAGCTGTCCG	1107	
<i>F. graminearum</i>	CGAGCGTTACTTGGCCTTAGCTGTACAGGATGCATGGCCTCAGAAAGTCATAAGGACTTTTTTCAGAAATACTCTGAGG	1200	
<i>F. sporotrichioides</i>	CGAGCGTTACTTGGCCTTTGGCTGTACGGGGTGTATGGCTTCAGAAAGTCATAAGGACTTTTTTCAGAAATACTCTGAGG	1187	
<i>F. graminearum</i>	TCGTATGAGGCGACATTCAGCTCCTTAAAAAAGTATGACGGAACCTTTCAGGCTTGGAAAGACGCTTGGAAAGAGAAGAGA	1280	
<i>F. sporotrichioides</i>	TCTTATGAAGCGACATTCAGCTCGTTAAAGAAGTACGACGGAGTCTTTCAGGCTTGGAAAGATGCTTGGAAAGAAAAGAGA	1267	
<i>F. graminearum</i>	GATAGATACAGAGTCTCCAATGAGATGGGAAGACTTGACGGATCACCATGGGCTTCCAGTGTACTTTGGTAG	1353	
<i>F. sporotrichioides</i>	GGTAGATACAGAGTCTCCAATGAGTGGGAGGATTTGATGGATCACCATGGGCTTCCAGTGTCTCTTTCTAA	1340	

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 μ L of the medium of each culture with ethyl acetate. (B) Schematic representation of the genomic structure $\Delta Tri10$ -nsm mutant strain. See **Supplementary Figure 8** for experimental details of the mutant strain construction and confirmation. The genomic structure of the $\Delta FgareA$ strain was described in a previous report (Nakajima et al., 2020). The genomic structure of $\Delta Tri6$ -nsm is shown in **Supplementary Figure 3E**.



Supplementary Figure 8 | Generation of the Δ Tri10 tk and Δ Tri10-nsm mutant strains.

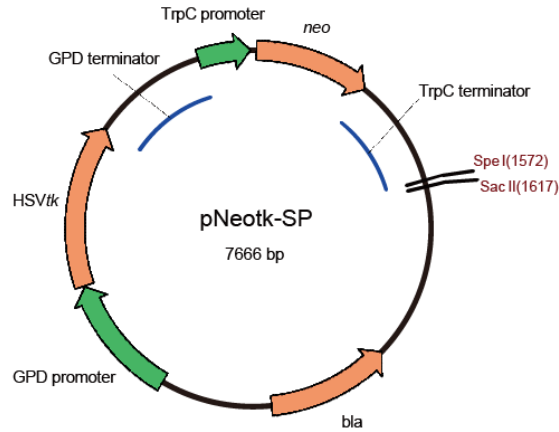
(A) Generation and Southern blot verification of the Δ Tri10 tk strain. Strain JCM 9873 was transformed with pdjTri10tkneo (**Supplementary Figure 9**) and selected with hygromycin B (left panel). Genomic DNAs of the wild-type (WT) and candidate *Tri10* disruptant were digested with *Hind*III and *Spe*I, 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 $\Delta Tri10$ -nsm strain. Strain $\Delta Tri10$ 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, $\Delta Tri10$ tk, and $\Delta Tri10$ -nsm strains using primers (red and blue) located outside of the homologous region. Expected sizes of amplicons were obtained (right panel).

1	neo									
	AATTAGAGC TTAAGTCTCG	CCATGGGATC GATACCCCTAG	GCCATTGAA CCGGTAACCT	CAAGATGGAT GTTCTACCTA	TGCACGCAGG ACGTGCGTCC	TTCTCCGGCC AAGAGGCCGG	GCTTGGGTGG CGAACCCACC	AGAGGCTATT TCTCCGATAA	CGGCTATGAC GCGGATACTG	TGGGCACAA ACCCGTTGTT
101	neo									
	AGACAATCGG TCTGTTAGCC	CTGCTCTGAT GACGAGACTA	GCCGCGGTGT CGCGGGCACA	TCCGCTGTCT AGGCCGACAG	AGCSCAGGGG TCCGCTCCCC	CGCCCGGTTT GCGGGCCAAG	TTTTTGTCAA AAAAACAGTT	GACCGACTGT CTGGCTGGAC	TCCGCTGCCG AGGCCACGGG	TGATGAAGT ACTTACTTTA
201	neo									
	GCAGGACGAG CGTCTCTGCT	GCAGCGCGGC CGTCCGCGCG	TATCGTGGCT ATASCACCGA	GGCCACGACG CCGGTCTGTC	GGGTTCTCTT CCGCAAGGAA	GCGCAGCTGT CGGTCTGACA	GCTCGACGTT CGAGCTGCAA	GTCAGTGAAG CAGTGACTTC	CGGGAAGGGA GCCTTCCCT	CTGGCTGCTA GACCGACGAT
301	neo									
	TTGGCGAAG AACCCGCTTC	TGCCGGGCA ACGGCCCCGT	GGATCTCTGT CTTAGAGSAC	TCATCTCACG AGTAGAGTGG	TTGCTCTGCG AACGAGSACG	CGAGAAAGTA GCTCTTTTCA	TCCATCATGG AGTAGTACC	CTGATGCAAT GACTAGTTTA	CGGCGCGGTG CGCCCGCAGC	CATACGCTTG GTATGCGAAC
401	neo									
	ATCCGGCTAC TAGGCGGATG	CTGCCATTTC GACGGGTAAG	GACCAACAAG CTGGGTGTTT	CGAAAGATCG GCTTTGTAGC	CATCGAGCGA GTAGCTCGCT	GCACGTACTC GTGCTATGAG	GGATGGAAGC CCTACTCTCG	CGGCTTTGTC GCCAGAACAG	GATCGAGTGT CTAGCTCTAC	ATCTGGACGA TAGACTGCTT
501	neo									
	AGAGCATCAG TCTGATGATC	GGGCTCGCG CCCGAGCGCG	CAGCGAACT GTCGGCTTGA	GTTGCGCAGG CAAGCGTCTG	CTCAAGGCGC GAGTCCGCGC	GCATGCCCGA CGTACGGGCT	CGGCGAGGAT GCCGCTCCTA	CTCGCTGGA GAGCAGCACT	CCCATGGCGA GGTACCCTG	TGCTCTCTTG ACGAGCAAGC
601	neo									
	CGAATATCA GGCTTATAGT	TGGTGGAAAA ACCACCTTTT	TGCCCGCTTT ACCAGCGAAA	TCTGGATTCA AGACTAAAGT	TGCACTGTGG AGCTGACACC	CCGGCTGGGT GGCCGACCCA	GTGGCCGACC CACCGCTGCG	GCTATCAGSA CGATAGTCTT	CATAGCGTTG GTATCGCAAC	GCTACCCGTT CGATGGCAC
701	neo									
	ATATTGCTGA TATAAGACT	AGAGCTTGGC TCTCGAACCG	GGCAATGGG CCGCTTACCC	CTGACCGCTT GACTGGCGAA	CCTCGTGGTT GGAGCAGCAA	TACGGTATCG ATGCCATAGC	CGGCTCCCGA GGCGAGGGCT	TTGCGAGCGC AAGCGTCCGC	ATCGGCTTCT TAGCGGAAGA	ATGCGCTTCT TAGCGGAAGA
801	neo					TrpC terminator				
	TGACGAGTTC ACTGCTCAAG	TTCTGACGGG AAGACTGCCC	CTGGCTGAGC GACCGACGTC	TGGTGGGGGA ACCACCCCTC	TCCACTTAAC AGTGAAGTTG	GTTACTGAAA CAATGACTTT	TCATCAACA AGTAGTTTGT	GCTTACGAAA CGAACTGCTT	TCTGTATATA AGACTATAT	AGATGTTGG TCTAGCAACC
901	TrpC terminator									
	TGTGATGTC ACAGTACAG	AGCTCCGGAG TCGAGSCTTC	TGAGACAACA AACTCTGTTT	TGGTGTTCAG ACCACAAGTC	GATCTCGATA CTAGAGCTAT	AGATAGTTC TCTATGCAAG	ATTTGTCCAA TAACAGGTT	CGAGCAAGA CGTGTCTTCT	GTGCTTCTA CACGSAAGAT	GTGATTTAAT CACTAAATTA
1001	TrpC terminator									
	AGCTCCATGT TCGAGTACA	CAACAAGAAT GTTGTTCTTA	AAAACGCTT TTTTGCGCAA	TGGGTTTTAC AGCCCAAATG	CTCTCCAGA GAGAAGGTCT	TACAGTCAAT ATGTCGAGTA	CTGCAATGCA GAGGTTACGT	TTAATGCATT AATTACGTAA	GGACTTCGCA CCTGGAGCGT	ACCCTAGTAC TGGGATCATG
1101	TrpC terminator									
	GCCCTTCAGG CGGAAAGTCC	CTCCGGCGAA GAGGCGGCTT	GCAGAAAGAT GCTCTTCTTA	AGCTTAGCAG TCGAATGCTC	AGCTATTTTT TCGATAAAAA	CATTTTCGGG GTAAAAGCCC	AGACGAGATC TCTGCTCTAG	AAGCAGATCA TTGCTCTAGT	ACGGCTGCA TGCCAGCAGT	AGAGACTTAC TCTCTGGATG
1201	TrpC terminator									
	GAGACTGAGG CTCTGACTCC	AATTCGCTCT TTAGCGGAGA	TGGTCCACG ACCAGGTGTC	CGACTATATA GCTGATATAT	TTTGTCTCTA AAACAGAGAT	ATTGTACTTT TAACATGAAA	GACATGCTCC CTGTACGAGG	TCTTCTTTAC AGAAGSAAATG	TCTGATAGCT AGACTATCGA	TGACTATGAA ACTGATACTT
1301	TrpC terminator									
	AATTCCGTCA TTAAGSAGT	CCAGCCCTGG GTCGGGGAC	GGTTCGAAA CCAAGCGTTT	GATAATTGCA CTATTAACGT	CTGTTTCTC GACAAGAAGG	CTTGAACCTC GAATCTGAGA	CAAGCTACA GTTCCGATGT	GGACACACAT CCTGTGTGTA	TCATCGTAGG AGTAGCATCC	TATAAACCTC ATATTTGGAG
1401	TrpC terminator									
	GAAATCATT CTTTTAGTAA	CCTACTAAGA GGATGATTCT	TGGTATACA ACCCATATGT	ATAGTAACCA TATCATTGGT	TGGTGGCTA ACCAACGGAT	GTGAATGCTC CACTTACGAG	GTAACACCC GCATTTGGGG	AATAGCCGG TTATGCGGCC	CGSAACTTT GGCTTTGAAA	TTTCAACCTC AAATGTTGAG
1501	TrpC terminator					SpeI				
	TCCTATGAGT AGSATACTCA	CGTTTACCCA GCAAAATGGT	GAATGCACAG CTTAGGTGTC	GTACACTGTG CATGTGAACA	TTAGAGTAA AATCTCCATT	TCCTCTTTTC AGSAAAGAA	TAGAGSAGT ATCTCTTAGG	ACTAGTCGCT TGATCACGCA	TAACATCAT ATTGTAGTAT	TGCCAGATCT ACGGTCTAGA
1601	SacII									
	AAGCGGCCG TTGCGCGCG	CACCCGCGTG GTGCGCCAC	GAGCTCCAGC CTCGAGTCTG	TTTTGTCCCC AAAACAAGGG	TTTAGTGAGG AAATCACTCC	GTTAATTTCG CAATTAAGC	AGCTTGGCGT TCGAACCGCA	AATCATGCTC TTAGTACCAG	ATAGCTGTTT TATCGACAAA	CCTGTGTGAA GGACACACTT
1701	ATTGTTATCC TAACAATAGG	GCTCACAATT CGAGTGTAA	CCACACAACA GGTGTGTTGT	TACGAGCCGG ATGCTCGGCC	AAGCATAAAG TTGTAATTTT	TGTAAGCCT ACATTTCCGA	GGGGTGCTA CCCCACGGAT	ATGAGTGAGC TACTCACTCG	TAACTACAT ATTGAGTGT	TAATGCGTT ATTAACCGAA
1801	GGCTCACTG CGCGAGTGAC	CCCGCTTCC GGCGAAAGG	AGTCGGGAAA TCAGCCCTTT	CCTGTCTGTC GGACGACAG	CAGCTGCATT GTGACGTAA	AATGAATCGG TTACTTAGCC	CAACGCGCG GGTTCGCGC	GGGAGAGCG CCCTCTCCG	GTTTGGCAT CAAACGCATA	TGGCGCTCT ACCCGCGAGA
1901	TCCGCTTCT AGCGAAGGA	CGCTCACTGA GAGGAGTACT	CTCGTCTGCG GAGCGACGCG	TGGTCTGTC AGCCAGCAAG	GGCTCGGCG CGAGCGCCG	AGCGGATACA TCGCGATAGT	GCTCACTCAA TCCGCGATG	AGCGGTAAT TCCGCGATTA	ACGGTTATCC TGCCATAGG	ACAGAACTAG TGCTTAGTCT
2001	GGGATAACGC CCCTATTGCG	AGGAAAGAAC TCCCTTTCTG	ATGTGAGCAA TACACTGTTT	AAGCCAGCA TTCCGGTCTG	AAAGCCAGG TTTCCGGTCC	AACCGTAAAA TTGGCATTTT	AGGCCGCGTT TCCGGCGCAA	GCTGGCGTT CGACCGCAA	TTCCATAGG AAGGATCCG	TCCGCCCCC AGCGGGGGG
2101	TGACGAGCAT ACTGCTGTA	CACAAAATC GTGTTTTAG	GACGCTCAAG CTGCGAGTTC	TCAGAGTGG AGTCTCACCC	CGAAACCGA GCTTTGGGCT	CAGGACTATA GTCTGATAT	AAGATACCG TTCTATGGTC	CGGTTTTCCC CGCAAGGGG	CCTGGAAGTC GACCTTCGAG	CCTGTCGCG GGAGCACGCG
2201	TCTCTGTTC AGAGGACAAG	CGACCTGCCC GCTGGACGG	GCTTACCAGA CGAATGGCTT	TACCTGTCG ATGGACAGCG	CCTTCTCCC GGAAAGAGGG	TTCCGGAAGC AAGCCCTTCG	GTGGCGCTT CACCCGAAA	CTCATAGCTC GAGTATCGAG	ACGCTGAGG TGCACATCC	TATCTCAGT ATAGAGTCAA
2301	CGGTGTAGT GCCACATCA	CGTTCGCTCC GCAAGCGAGG	AAGCTGGCT TTCGACCCGA	GTGTGCACGA CACAGTGTCT	ACCCCGCTT TGGGGGCAA	CAGCCGACC GTCCGGTGG	GCTGCGCTT CGACCGGAA	ATCCGTAAC TAGGCCATTG	TATGCTGTG ATAGCAGAAG	AGTCCAACTC TCAGGTTGGG
2401	GGTAAGACAC CCATTCTGTG	GACCTTATCG CTGAATAGCG	CAGTGGACG GTGACGCTCG	AGCCACTGGT TCGGTGACCA	AAACAGGATTA TTTGCTAAT	GCAGAGCGAG CGTCTCGCT	GTATGTAGCC CATACATCG	GGTGCTACAG CCACGATGTC	AGTCTTGA TCAAGAACTT	GTGGTGGCT CACCAACCGA
2501	AACACTGGCT TTGATGCCGA	ACACTAGAAG TGTGATCTTC	GACGATTTT CTGTATAAA	GGTATCTGCG CCATAGAGCG	CTCTGCTGAA GAGACGACTT	CGCAGTTACC CGGTCAATGG	TTCCGAAAA AAGCTTTTTT	GAGTGGTGA CTCAACATC	CTCTTGAAC GAGAAGTGG	GGCAACAAA CCGTTTGT
2601	CCACCGCTGG GGTGGCGACC	TAGCGGTGGT ATCCGCAACA	TTTTTTGTTT AAAAAACAAA	GCAAGCAGCA CGTTCGCTCG	GATTACGCG CTAATGCGCG	AGAAAAAAG TCTTTTTTTC	GATCTCAAGA CTAGAGTTCT	AGATCTTTG TCTAGAAAC	ATCTTTTCA TAGAAAAAGT	CGGGCTTGA GCCCAAGACT
2701	CGCTCAGTGG GCGAGTCAAC	AACGAAAAC TTGCTTTTGA	CAGCTAAGG GTGCAATTCC	GATTTTGGT CTAAAACAG	ATAGAGTAT TACTTAATA	CAAAAAGAT GTTTTCTTA	CTTCACTAG GAAGTGGATC	ATCTTTTAA TAGAAAAAT	ATTAATAAG TAATTTTAC	AAGTTTTAA TTCAAAAT

2801	TCAATCTAAA AGTTAGATTT	GTATATATGA CATATATACT	GTAACCTTGG CATTTTGAACC	TCTGACAGTT AGACTGTCAA	ACCAATGCTT TSGTTACGAA	AATCAGTGA TTAGTCACTC	GCACCTATCT CGTGGATAGA	CAGCGATCTG GTCGCTAGAC	TCTATTTCTG AGATAAAGCA	TCATCCATAG AGTAGGTATC
b1a										
2901	TTGCTGACT AACGGACTGA	CCCCGCTGG GGGGCAGCAC	TAGATAACTA ATCTATTGAT	CGATACGGGA GCTATGCCCT	GGGCTTACCA CCCAGATGGT	TCTGGCCCCA AGACCGGGGT	GTGCTGCAAT CACGACGTTA	GATACCGCGA CTATGGCGCT	GACCCAGGCT CTGGGTGCGA	CACCCGGCTCC GTGGCCGAGG
b1a										
3001	AGATTTATCA TCTAAATAGT	GCAATAAAC CGTTATTGG	AGCCAGCCGG TCGGTCCGCC	AAGGGCCGAG TTCCGGCTCT	CGCAGAAGTG GCGTCTTCA	GTCTGCAAC CAGGACGTTG	TTTATCCGCC AAATAGGCGG	TCCATCCAGT AGTATGGTCA	CTATTAATTG GATAATTAAC	TTGCCGGGAA AACGGCCCTT
b1a										
3101	GCTAGAGTAA CGATCTCATT	GTAGTCTGCG CATCAAGCGG	AGTTAATAGT TCAATTATCA	TTGCGCAACG AACGCGTTGC	TTGTGGCCAT AACACCGSTA	TGCTACAGGC ACGATGTCCG	ATCGTGGTGT TAGCACACACA	CACGCTCGTC GTGCGAGCAG	GTTTGGTATG CAAACCATAC	GCTTCATTCA CGAAGTAAGT
b1a										
3201	GCTCCGGTTC CGGGCCCAAG	CCAACGATCA GGTTGCTAGT	AGGCGAGTTA TCCGCTCAAT	CATGATCCCC GTACTAGGGG	CATGTTGTGC GTACAACAGC	AAAAAAGCGG TTTTTTCGCC	TTAGTCCCTT AATCGAGGAA	CGGTCTCCCG GCCAGGAGGC	ATCGTGTGCA TAGCAACAGT	GAAGTAAGTT CTTCACTTCA
b1a										
3301	GGCCGCAAGT CGGGCTCAC	TTATCACTCA AATAGTGAGT	TGTTTATGGC ACCAATACCC	AGCACTGCAT TCGTGACGTA	AATTCTCTTA TTAAGAGAA	CTGTCACTCC GACAGTACGG	ATCCGTAAGA TAGGCATTCT	TGCTTTTCTG ACGAAAAGAC	TGACTGGTGA ACTGACCACT	GTACTCAACC CATGAGTTGG
b1a										
3401	AAGTCACTCT TTCAAGTAA	GAGAATAGTG CTCTTATCAC	TATCGCGGCA ATACGCCCTT	CCGAGTGTCT GGCTCAACGA	CTTGCCCGCG GAACGGCCCG	GTCAATACGG CAGTTATGCC	GATAATACGG CTATTATGCG	CGCCACATAG GCGGTGTATC	CAGAAGTTTA GCTTTGAAAT	AAAGTCTCA TTTACAGAGT
b1a										
3501	TCATTGGAAA AGTAACCTTT	ACGTTCTTCG TGCAGAAGC	GGGCGAAAAC CCCCCTTTTG	TCTCAAGGAT AGAGTTCTTA	CTTACCCTG GAATGGCCCA	TTGAGATCCA AACTCTAGGT	GTTCGATGTA CAAGCTACAT	ACCCACTCGT TGGGTGAGCA	GCACCCAAC CGTGGTGTGA	GATCTTCAGC CTAGAAGTCC
b1a										
3601	ATCTTTTACT TAGAAAATGA	TTCAACAGCG AAGTGGTCCG	TTTCTGGGTG AAAGACCAC	AGCAAAAACA TCGTTTTTGT	GAAAGGCAAA CCTTCCGTTT	ATGCCGCAAA TACGGCGTTC	AAAGGGAATA TTTCCCTTAT	AGGGCGACAC TCCCGCTGTG	GGAAATGTTG CCTTTACAAC	AATACTCATA TTATGAGTAT
b1a										
3701	CTCTCCCTT GAGAGGAAA	TTCAATATTA AAGTTATAAT	TTGAAGCATT AACTTCGTAA	TATCAGGGTT ATAGTCCCAA	ATTGTCTCAT TAACAGAGTA	GAGCGGATAC CTCGCTATG	ATATTGAAAT TATAAECTTA	GTATTTAGAA CATAAATCTT	AAATAAACAA TTTATTGTGT	ATAGGGTTC TATCCCCAAG
3801	CGCCGACATT CGCGGTGTA	TCCCCGAAAA AGGGGCTTTT	GTGCCACCTA CACGGTGGAT	AATTGTAAGC TTAACATTCT	GTAAATATTT CAATTATAAA	TGTTAAATTT ACAATTTTAA	CGCGTAAAT GCGCAATTTA	TTTTGTAAAA AAAACAATTT	TCAGCTCATT AGTCGAGTAA	TTTTAACCAA AAAATTTGTT
3901	TAGGCGGAAA ATCCGGCTTT	TCGGCAAAAT AGCCGTTTTA	CCCTTATAAA GGGAATATTT	TCAAAAGAAT AGTTTTCTTA	AGACCGAGAT TCTGGCTCTA	AGGGTTGAGT TCCCAACTCA	GTGTTCCAG CAACAAGGTC	TTTGGACAAA AAACTTGTGT	GAGTCCACTA CTCAGGTGAT	TTAAGAAGC AATTTCTTGC
4001	TGGACTCAA ACCTGAGGTT	CGTCAAAGGG AGGCTTTCCC	CGAAAAACCG GCTTTTTGGC	TCTATCAGGG AGATAGTCCC	CGATGGCCCA GCTACCAGGT	CTACGTGAAC GATGCACTTG	CATCACCCCTA GTAGTGGGAT	ATCAAGTTTT TAGTCAAAA	TTGGGGTCGA AAACCCAGCT	GGTCCGTAA CCACGGCATT
4101	AGCACTAAT TCGTGATTTA	TCGAAACCTA GCCTTGGGAT	AAGGGAGCCC TTCCCTCGGG	CGGATTTAGA GGCTAAATCT	CGTTGACGGG CGAAGTCCCC	GAAAGCCGGC CTTTCCGGCG	GAACTGGCG CTTGACCCG	AGAAAGAGC TCTTCCCTTC	GGAAAGAGC CCTTCTTCG	GAAAGAGCG CTTCTCCG
4201	GGCGTAAGC CCGATGCC	CGCTGGCAAG CGACCGTTCC	TGTAGCGGTC ACATCCGCA	ACGCTGCGCG TGGCAGCGCG	TAACCAACAC ATTGGTGGTG	ACCCGCGCG TGGCGGCGCG	CTTAATGCG GAAATACGCG	CGCTACAGGG GCGCAGGGTA	CGGCTCCCAT GCGGAGGGTA	TGCGCATTCA AGCGGTAAGT
4301	GGCTGCGCAA CCGACGCTTT	CTGTTGGGAA GACAACCTTT	GGGCGATCG CCCGTAGCC	TGCGGGCTC ACGCCCCGAG	TTGCTATTA AAGCGATAAT	CGCCAGCTGG GCGGTGACCC	CGAAGGGGG GCTTTCCCC	ATGTGCTGCA TACACGAGCT	AGGCGATTA TCCGCTAAT	GTGGTGAAC CAACCCATTG
GPD promoter										
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GPD promoter										
4501	GAGAGACGGA CTCTGCTCT	CGGACGCGA GCCTGCTCT	GAGAAGSSCT CTCTCCCGA	GAGTAATAAG CTCATTATTC	CGCCACTGCG GCGGTGACCG	CCAGACAGCT GGTCTGCGA	CTGGCGCTC GACCGCCGAG	TGAGGTGCA ACTCCAGTCT	TGAGTATTA ACCTACTAAT	TTAATCCGGG AATTAGGCC
GPD promoter										
4601	ACCGSCGCC TGGCCGCGG	CCTCCGCCCC GGAGGCGGGG	GAAGTGGAAA CTTCACTTTT	GGCTGGTGTG CCGACACAC	CCCTCGTTTG GGGAGCAAC	ACCAAGATTC TGGTCTTAG	TATTGATCA ATAACGTAGT	TCGAGAGATA AGCCTCTTAT	TGAGCTTCA ACCTCGAAGT	TGGAATACC AGCTTAGTGG
GPD promoter										
4701	GGCAGTAAGC ATTTCTTAAG	GAAGGAGAA CTTCTCTTA	GTGAAGCCAG CACTTCGGTC	GGGTGTATAG CCCACATATC	CGTCCGCGA GGCAGCCGCT	AATAGCATGC TTATGCTACG	CATTAACCTA GTAATTGGAT	GGTACAGAA CCATGTCTCT	TCCAATTGCT AGGTTAACGA	TCCGACTCTG AGGCTAGACC
GPD promoter										
4801	TAAAGATTTC ATTTCTTAAG	ACGAGATAGT TGCTCTATCA	ACCTTCTCCG TGAAGAGGCG	AAGTAGGTAG TTCATCCATC	AGCGAGTACC TGCCTCATGG	CGGCGGTAA GCCGCGCATT	GCTCCCTAAT CGAGGGATTA	TGGCCATCC ACCGGGTAGG	GGCATCTGTA CCGTAGACAT	GGCGTCCAA CCCGAGGTT
GPD promoter										
4901	ATATCGTGCC TATAGCACGG	TCTCTGCTT AGAGGACGAA	TGCCCCGTGT ACGGGCAACA	ATGAACCGG TACTTTGGCC	AAAGCCCTCT TTTCCGCGGA	CAGSAGCTGG GTCCTCGACC	CCAGCAGGCG GGTCCGCGCG	AGACCCGGAA TCTGGCCCTT	CACAAGCTGG GTGTTCGACC	CAGTCCACC GTCAGCTGGG
GPD promoter										
5001	ATCCGCTGCT TAGGCCACGA	CTGCACTCGA GACGTGAGCT	CCTGCTGAGG GGACGACTCC	TCCCTCAGTC AGGGAGTCA	CCTGGTAGGC GGACCATCCG	AGCTTTGCCC TCGAAACGGG	CGTCTGTCCG GCAGACAGGC	CCCGGTGTGT GGGCGACACA	CGGCGGGGTT GCCGCCCAA	GACAAGGTCG CTGTTCCAGC
GPD promoter										
5101	TTGGTCACT AACGCACTCA	CCAACATTTG GGTTGTAAAC	TTGCCATATT AACGCTATAA	TTCTGCTCT AAGGACGAGA	CCCCACCAGC GGGGTGGTGG	TGCTCTTTTC ACGAGAAAAG	TTTTCTCTTT AAAAGAGAAA	CTTTTCCCAT GAAAAGGGTA	CTTCAGTATA GAAGTCATAT	TTGATCTTCC AAGTASAGG
GPD promoter										
5201	CATCCAAGAA GTAGGTTCTT	CTTTATTTTC GGAAATAAAG	CCCTAAGTAA GGGATTCATT	GTACTTTGCT CATGAAACGA	ACATCCATAC TGTAGGTATG	TCCATCTTTC AGGTAGGAA	CCATCCCTTA GGTAGGGAA	TTCTTTGAA AAGGAAACTT	CTTTTCAATT GGAAAGTCAA	CGAGCTTCC GCTCGAAGG
GPD promoter										
5301	CACCTCATCG GTGAAGTAGC	CAGCTGACT GTCGAACGTA	AACAGCTACC TTGTGATGG	CGGCTGAGA GGCGAACTCT	TCGATATGCG AGCTATACCG	TTGCTACCCC AAGCATGGGG	TGCCATCAAC ACGGTAGTTG	ACGGTCTGCG TGCGCAGACG	GTTCGACCAG CAAGCTGGTC	GCTGCGGTT CGACGCGCAA
HSVtk										

	HSVtk									
5401	CTGCGGCCA GAGCGCCGT	TAGCAACCGA ATCGTTGGCT	CGTACGGCGT GCATGCCCA	TGCGCCCTCG ACGCGGGAGC	CGGCGACGAA GGCCGTGTT	GAAGCCACGG CTTCGGTGC	AAGTCCGCC TTCAGGCGGG	GGAGCAGAAA CCTCGTCTTT	ATGCCACAGC TACGGTGGG	TACTGCCGGT ATGACGCCCA
	HSVtk									
5501	TTATATAGC AATATATCTG	GSTCCCCAG CCAGGGGTG	GSATGGGGAA CCTACCCCTT	AACCACCACC TTGGTGGTGG	ACGCAACTGC TGCGTTGAGC	TGGTGGCCCT ACCACCGGGA	GGSTTCGCG CCCAAAGCGG	GACGATATCG CTGCTATAGC	TCTACGTACC AGATGCATGG	CGAGCCGATG GCTCGGCTAC
	HSVtk									
5601	ACTTACTGC TGAATGACCG	GGGTGCTGG CCCACGACCC	GGCTCCGAG CGAAAGGCTC	ACAATCGGA TGTAGCGCT	ACATCTACAC TGTAGATGTG	CACACAACAC GTGTGTTGTG	CGCTCGACC GCGAGGCTGG	AGGGTGAAT TCCCACCTCTA	ATCGGCCGG TAGCCGGCCC	GACGCGGCG CTGCGCCGCC
	HSVtk									
5701	TGTAATGAC ACCACTACTG	AAGCCGCCAG TTGCGGGTGC	ATAACAATGG TATTGTTACC	GCATGCCTTA GCTACGSAAT	TGCGGTGACC ACGGCACTGG	GACGCGGTTT CTGCGGCAAG	TGGCTCTCA ACCGAGGAGT	TATCGGGGG ATAGCCCCCC	GAGGCTGGGA CTCCGACCCT	GCTCACATGC CGAGTGTACG
	HSVtk									
5801	CGCGCCCGC GGCGGGGGC	GCCCTCACCC CGGAGTGGG	TCATCTTGA AGTAAAGGCT	CGCCATCCC GCGGTAGAGG	ATCGCCGCC TAGCGGGGG	TCCTGTGCTA AGGACACGAA	CCCGCCCGC GGCCCGGCG	CGGTACCTTA GCCATGGAAT	TGGGCAGCAT ACCCTGCTGA	GACCCCGAG CTGGGGGTC
	HSVtk									
5901	GCGTGTCTG CGCACGACC	CGTTCGTGG GCAAGCACC	CCTCATCCG GGAGTAGGGC	CGACCTTGC GGCTGGAACG	CGGCGACAA GGCCGTGGTT	CATCGTCTT GTAGCACGAA	GGGGCCCTT CCCCGGGAA	CGAGGACAG GCCTCTGTCT	ACACATCGAC TGTGTAGCTG	CGCTGGCA GCGGACCGGT
	HSVtk									
6001	AAGCGCAGG TTGCGGTGCG	CCCCGGCAG GGGGCCGCTC	CGGCTGGACC GCCGACTGG	TGGTATGCT ACCGATACGA	GGCTGCGATT CGAGCTCTAA	CGCGCGGTTT GGCGCGCAAA	ACGGGCTACT TGGCCGATGA	TGGCAATAG ACGGTTATGC	GTGGGTATC CACGCAATAG	TGCGTGGCG ACGTCACGCC
	HSVtk									
6101	CGGTGTGG GCCAGCACC	CGGGAGGACT GCCCTCTGGA	GGGAGACGCT CCCTCTGCGA	TTGCGGAGC AAGCCCTGTC	GCCTGCGCG CGGCGCGGCG	CCCAGGGTGC GGGTCCGACG	CGAGCCCCG GCTCGGGGTC	AGCAACCGG TCGTTGCGCC	GCCCAGACC CGGGTGTGG	CCATATCGG GGTATAGCCC
	HSVtk									
6201	GACAGTTAT CTGTGCAATA	TTACCCTGTT AATGGGACAA	TGCGGCCCCC AGCCCGGGGG	GAGTTGCTGG CTCAACGACC	CCCCCAACGG GGGGTGTGCC	CGACCTGTAT GCTGGACATA	AACGTGTTTG TTGCACAAAC	CCTGGGCTT GGACCCGGAA	GGAGCTCTTG CTGTCAGAAC	GCCAAAGCC CGGTTTGGCG
	HSVtk									
6301	TCGGTTCCAT AGSCAAGSTA	GCACGTCTTT CGTGCAAGAA	ATCCTGGATT TAGSACCTAA	ACGACCAATC TGCTGGTTAG	GCCCGCCGCG CGGGCGGCGG	TGCCGGGAGC ACGSCCCTGC	CCCTGCTGCA GGGACGACTG	ACTTACTCCC TGAATGGAAG	GGATGTTCC CCCTACCAGG	ASACCAAGT TCTGGSTGCA
	HSVtk									
6401	CACCACCCC GTGGTGGGG	GGCTCCATAC CCGAGGTATG	CGACGATATG GCTGCTATAC	CGACCTGGCG GCTGGACCGC	CGCAGTTTTG GCGTGCAAAC	CCCCGGGAGT GGCCCTCTA	GGGGGAGGCT CCCCCTCCGA	AACTGACTCG TTGACTGAGC	AGCTTGACT TCGAACCTGA	GATCTCCCAC CTAGAGGGTG
	GPD terminator									
6501	GTGCGACAAG CAGGTGTTC	GTGATGGCA CAGTACCGT	AGCGTAGAC TGCAGTCTG	TGCGACCAAC ACGCTGGTTG	ACCTTGGAA TGGACACTTG	ACGGGCTCAA TGCAGCAGTT	CGGCGCGTT GCCCGGCAA	CCGCTGCTC GGCAGCAGA	CGGAGACTG GCCTCTGAGC	GAAGCCTGT CTTCGGACGA
	GPD terminator									
6601	TTTCAATAT AAAGTATTA	GAACAACACC CTTTGTTGG	CATGGGGGAA GTACCCCTT	TTATGAAAG AATACCTTC	GCAATGAACC GTTACTTGG	AAAAAACA TTTTGTTGA	AAAAAGGGC TTTTTCCC	AGCGAAGAA TGCTTCTTT	AAGTCGTA TTCAACGATT	CGTCAACAG GCAGTGTTC
	GPD terminator									
6701	ACTATTGGG TGATAAGCC	CCATGACAAC GTAAGTGTG	CAAGGCTTGG GTTCCGAACC	GTGCGCGCG CAGCCGCGGC	TGGGAGTGT ACCCCTCACA	GGCTAGTGC CCGGATCAAG	AGTGGTAGTT TCACCATCAA	AGCTAGTAT TCGATGCATA	GCAGCTCTA CGCTGAGGAT	ATAAATAACA TATTTATTGT
	GPD terminator									
6801	AAAAAATCAG TTTTTATGTC	ATTAATAGAG TAATTTACTC	GACCACTTA CTGGTGAAT	GTAGTACTAT CATCATGATA	AGACGAAGTC TCTGCTCAG	ATCGAAGTAC TAGCTTGAAT	AGGGCATCTG TCCCCTAGAC	TGCATTTTGT ACGTAATAACA	TGTGAACCCG ACACTTGGCG	TTCAAATATC AAGTTTATAG
	GPD terminator									
6901	AAAAATATA TTTTAGTAT	ACTCGGACT TGSAGCTGA	TGGCTGGAT ACCGACTAC	GTCAAATTA CAGTTAAGT	TCGGTGTATA AGGCACATAT	CACACATCT GTGTGAAGA	CGCACCTTGT GCGTGSAAAC	GAAGCAGCA CTTGTGCGGT	GCGTGGAC CGGCAAGCTG	GCAGTTTAT GCTCAAAGTA
	GPD terminator									
7001	CAGGCTCTG GTCCGAAGAC	AAAAAGGAA TTTTCTCCT	TTAGAAAAA AATCTTTTT	AGTATCTGT TCCATAGACA	AATTAGCAGT TTAATCGTCA	GCAGACCATG CGTCTGGTAC	TAATGTAATG ATTACATTAC	AATACGATCC TTATGCTAGG	GACAAGCTCC CTGTTCGAGG	ATTATTGAAG TAATAACTTC
	GPD terminator									
7101	CATTATCAG GTAATAGTC	GGTTATTGC CCAATAACAG	TCATGAGCG AGTACTCGCC	ATACATATTT TATGTATAAA	GAATGATTT CTTACATAAA	AGAAAAATA TCTTTTTATT	ACAAATAGGG TGTTTTATCC	GTTCCGCGCA CAAGGCGCGT	CATTTCCCG GTAAGGGGC	AAAAGTCCA TTTTACGGT
	GPD terminator									
7201	CCTGACGCT GGACTGCAGA	AAGAAACCAT TTCTTTGGTA	TATTATCATG ATAATAGTAC	ACATTAACCT TGTAATTGGA	ATAAAAATAG TATTTTTATC	CGCATCAGG CGCATAGTGC	AGGCCCTTTC TCCGGGAAAG	GTCTTCAAGA CAGAAGTCTC	ATTGTGCAGA TAACAGCTGT	GAAGATGATA CTTCTACTAT
	TrpC promoter									
7301	TTGAAGGAGC AACTCTCTG	ACTTTTTGG TGAAAAACCC	CTTGGCTGSA GAACCGACTC	GCTAGTGGAG CGATCACCTC	GTCAACAATG CAGTTGTTAC	AATGCTATT TTACGGATAA	TTGGTTTATG AAACAAATCA	CGTCCAGGCG GCAAGTCCGC	GTGAGCACAA CACTCGTGT	AATTTGTGTC TTAAACACAG
	TrpC promoter									
7401	GTTTGACAAG CAAACTGTTC	ATGGTTCATT TACCAAGTAA	TAGGCAACTG ATCCGTTGAC	GTGAGATCAG CAGTCTAGTC	CCCCACTTGT GGGGTGAACA	AGCAGTAGCG TCGTCATCGC	GCGCCGCTCG CGCCGCGAGC	AAGTGTGACT TTCACACTGA	CTTATTAGCA GAATAATCGT	GACAGGAAAG CTGCTCTTGC
	TrpC promoter									
7501	AGSACATTAT TCCGTGAATA	TATCATCTGC ATAGTAGAGG	TGCTTGGTGC ACGAAACACG	ACGATAACCT TGCTATTGAA	GGTGGTTTTG CCACGCAAAC	TCAAGCAAGG AGTTCGTTCC	TAAGTGAACG ATTCACTTGC	ACCCGGTCA TGGGCGAGTA	ACCTTCTTAA TGSAAAGATT	GTTGCCCCCT CAAGCGGGAA
	TrpC promoter									
7601	CCTCCCTTTA GGAGGGAAAT	TTTCAGATT AAAGTCTAAG	AATCTGACTT TTAGACTGAA	ACCTATTCTA TGGATAAGAT	CCAAGCATC GGTTCGTAG	GATAAGCTTG CTAATCGAAC	ATATCG TATAGC			

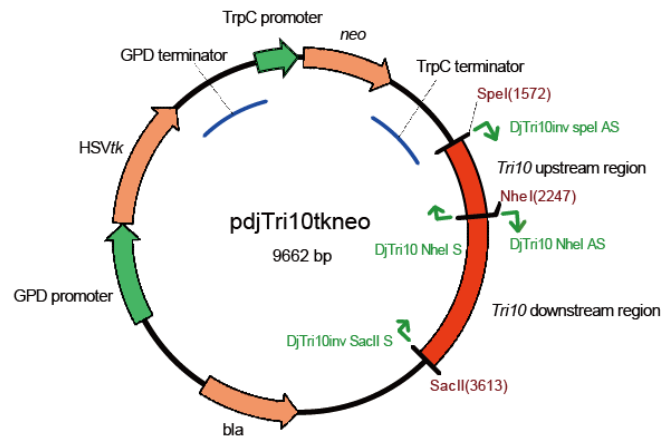


1	AATTCAGAGC	CCATGGGATC	GSCCATTGAA	CAAGATGGAT	TGCACGCGAG	TTCTCCGGCC	GCTTGGGTGG	AGAGSCTATT	CGGCTATGAC	TGGGCACAAC
101	AGACAATCGG	CTGCTCTGAT	GCCGCCCTGT	TCCGGCTGTG	AGCGCAGGGG	CGCCCGGTTC	TTTTTGTCAA	GACCGACCTG	TCCGGTGGCC	TGAATGAACT
201	GCAGGACGAG	GCAGCGCGGC	TATCGTGCT	GGCCACGACG	GGGCTTCTT	GCGCAGCTGT	GCTCGACGTT	GTCACCTGAAG	CGGGAGGGGA	CTGGCTGCTA
301	TTGGGCGAAG	TGCCGGGGCA	GGATCTCCTG	TCATCTCACC	TTGCTCTGCG	CGAGAAAGTA	TCCATCATGG	CTGATGCAAT	GCGGCGGCTG	CATACGCTTG
401	ATCCGGCTAC	CTGCCCAATC	GACCACCAAG	CGAAACATCG	CATCGAGCGA	GCACGTACTC	GGATGGGAAG	CGGTCTTGTC	GATCAGGATG	ATCTGGACGA
501	AGAGCATCAG	GGGCTCGCGC	CAGCCGAACT	GTTCCGCCAG	CTCAAGGCGC	GCATGCCCGA	CGGCGAGGAT	CTCGTCTGA	CCCATGGCGA	TGCTGCTTGG
601	CCGAATATCA	TGGTGGAAAA	TGGCCGCTTT	TCTGGATTCA	TCGACTGTGG	CCGCTGGGGT	GTGGCGGACC	GCTATCAGGA	CATAGCGTTG	GCTACCCGTTG
701	ATATTGCTGA	AGAGCTTGGC	GGCGAATGGG	CTGACCCTTT	CCTGCTGCTT	TACGGTATCG	CCGCTCCCGA	TTGCGAGCGC	ATCGCCTTCT	ATCGCCTTCT
801	TGACGAGTTC	TTCTGACGGG	CTGGCTGCAG	TGGTGGGGGA	TCCACTTAAC	GTTACTGAAA	TCATCAAAAC	GCTTGACGAA	TCTGGATATA	AGATGTTGG
901	TGTCGATGTC	AGTCCGGAG	TTGAGACAAA	TGGTGTTCAG	GATCTCGATA	AGATACGTTT	ATTTGTCCAA	GCAGCAAAGA	GTGCTTCTA	GTGATTTAAT
1001	AGCTCCATGT	CAACAAGAAT	AAAACCGSTT	TCGGSTTTAC	CTCTTCCAGA	TACAGCTCAT	CTGCAATGCA	TTAATGCATT	GGACCTCGCA	ACCTAGTAG
1101	GCCCTTCAGG	CTCCGGCGAA	GCAGAAGAAT	AGCTTAGCAG	AGTCTATTTT	CATTTTCGGG	AGACGAGATC	AAGCAGATCA	ACGGTCTGCA	AGAGACTTAC
1201	GAGACTGAGG	AATCCGCTCT	TGGCTCCAGG	CGACTATATA	TTTGTCTCTA	ATTGTACTTT	GACATGCTCC	TCTTCTTTAC	TCTGATAGCT	TGACTATGAA
1301	AATTCGGTCA	CCAGCCCTTG	GSTTCCGAAA	GATAATTGCA	CTGTTTCTTC	CTTGAACCTT	CAASCTTACA	GGACACACAT	TCATCGTAGG	TATAAACCTC
1401	GAAAATCATT	CCTACTAAGA	TGGGTATACA	ATAGTAACCA	TGGTGCCTA	GTGAATGCTC	CGTAACACCC	AATACGCCGG	CCGAAACTTT	TTTACAACCTC
1501	TCCTATGAGT	CGTTTACCBA	GAATGCACAG	GTACACTTGT	TTAGAGGTAA	TCCTTCTTTC	TAGAGGATCC	ACTAGTATCC	ATGATGACTA	ACGACAATAA
1601	TAATGATAAT	TTGATGATTG	TTGGTTGGCA	TACAATTGAA	GTTGGGGGAA	AGACGGGGAAG	GAATGGGCGT	GGAGAAGTTA	AACGATGTTA	CAATATAATG
1701	CAAGGATAGA	GTTTTACAGA	AGTGTCTGTG	CCCTCATAGT	GCTCAAGCAG	GCATCAAAAC	TTATCTTGTG	TGGTTTCTGT	ACGCCCTGTT	CAGTGGACGT
1801	TTGGTGGTGG	CGAAAAGGCA	AGGGTATGGT	CACTGATCTG	GACAAAACGC	AAGCGCTTTG	AAGATCAATT	CGCGGGTCTA	GAGAATGTGC	CAGCTCCCTC
1901	CAACTCCGGA	GCGGGTACAA	GATTCGCCGA	TCGCACTACA	GTTGATAGCG	CCAGTCTACC	AGTCTGCCAG	CTGACGCTGC	AGATGCAATT	GCAGGGTGG

2001	Tri10 upstream region									
	GGGTAGCCCT	GTGCGACCGA	CGGGTGTACA	GACTTGGTTA	CGCAAAAGCC	TGCACCTCAAG	TGGGAAATGG	TTACAGGCTC	AGGTTACGTA	CAGACATTCA
2101	Tri10 upstream region									
	GTTTCAACTG	CCTATTCGGA	AGTAATTATC	CTTGTTC AAC	TGTTTCACTT	AACA AAAACAC	GACCGA ACTA	TTTGTAA TCA	TTATAACAGA	CCACCATTCT
2201	Tri10 upstream region									
	CCAAACAGT	ATCCGTACCC	GCAAAACATA	CTTCCAACT	CGCCTGCTAG	CCACTCCGTC	ACAACATAGA	TATGCTTTCA	TTAATTTTGG	CTTTTCGCGC
	DjTri10_NheI_5									
	Tri10 downstream region									
2301	TGTTGCTGTA	GGG CAGCAA	TGCCATGCAT	TAAATTA AAA	AGAAGGGACC	TATTC AAGGG	TTACACACAC	CCGACGGCAG	ATTTTACGTC	TCTGCCG6CC
2401	Tri10 downstream region									
	TGTCATGAAA	GCTTAAAAAG	AACAGACCTT	TGGATGTAAC	CCACCATCAG	TTGAACATGG	GTTTGTCCGC	GCCGACAAGT	CCGTTGCCAT	GTTCGCCGACG
2501	Tri10 downstream region									
	AGAGAAGAGT	GATCGTGAAG	CTCGATGGCA	TTCTATGCTG	AGCCTCAATG	GCGTATGGAAT	CGTAATGGC	AATGCTAGAC	GAGACGCCGT	TTAGCCCGGA
2601	Tri10 downstream region									
	TGTTGCGTTC	GACGCTTGCA	TATTCAGGCC	TTTTACTGAC	GTTTCAGCTT	CACACCAACT	CAGCATCATT	CAGGCCTTTT	TCTCGCATAT	CTGCGATATT
2701	Tri10 downstream region									
	TTTGTGCGT	AGATAGASCA	TAGACATTAA	GACAATTAAG	ATATTAACCA	TCGGCCAGAA	CCGATTAGAA	ACATCTCAAC	AATTTCTTCC	CATCAAAACA
2801	Tri10 downstream region									
	ACACGAACAC	GACATCAATT	GCATCACACC	ACTACCACTA	CTACTACTAC	TATCCACTCA	AACACTCACC	CCCAATCTCC	AAGATGCTCG	CAGCCGCTAA
2901	Tri10 downstream region									
	ACTGATGCAC	TCATATGAGA	TGGACCCTGA	TGTCTCGTGG	CTCAGGTTT	TGCGATACTC	GGGAGTTAGC	GCTGCTTTAT	GCCTACCAT	ATGGTCCGCA
3001	Tri10 downstream region									
	GCCAAAGCAT	GCTGAGACTG	CAGTGCCAGA	TCAGCACCTC	TATCATCTAC	CCAGCCCAAC	AGAGGAAGAA	TTGCCGTGCG	CAAATCTCAG	CATTTCTCCA
3101	Tri10 downstream region									
	CTTCGGCCCC	ACGAATGAAT	CATTTCCCAA	CTGGGTTTGG	TTGGGGGAT	CTTCACAAGG	AACTTCGACA	AATCCCTCG	CTTGAATCA	ACACTCTTC
3201	Tri10 downstream region									
	ACTATGATCA	ATATCGTTTG	TCGTACGGGC	TAAACAATGT	CAGCCATGT	CGAGCAATC	AAGGGACACA	GCACTGATT	GCCCACCATG	GTTCCAGACT
3301	Tri10 downstream region									
	GCTTTCAGCA	CGATTGGTTG	GATAGATGGA	GAAGATCGGG	TTACCGGTTA	AAAAAGTATA	CTAGAAATAT	ATATTGAAG	TCCATCATTC	AAGTTACATG
3401	Tri10 downstream region									
	GGAAAGTTT	TCGCTCTTC	TATTCATTT	AAATCATGCA	TCATGCCAAT	CCCTGTTTAA	ACGCCAAAAT	GAGTGAACC	CATAACATCT	AATAAAACTT
3501	Tri10 downstream region									
	CCCTGCATGC	ATCATCTGTA	AGTGAGCATC	AAACTACAT	GTATCGGGTA	GCTGATGCAG	AAGAAAGACT	TCGGGTATCG	GTATCCGTAG	TCTCTGCGCC
	DjTri10inv_SacII_5									
3601	Tri10 downstream region									
	GATACATCC	CGGGTGAGC	TCCAGCTTTT	GTTCCCTTTA	GTGAGGGTTA	ATTTGAGCT	TGGCGTAATC	ATGGTCATAG	CTGTTTCTG	TGTGAATTTG
	DjTri10inv_SacII_5									
3701	TTATCCGCTC	ACAATTCAC	ACAACATAG	AGCCGGAAGC	ATAAAGTGA	AAGCCTGGGG	TGCCTAATGA	GTGAGCTAAC	TCACATTAAT	TGCTTGC
3801	TCACTGCCCG	CTTTCAGTC	GGGAAACCTG	TCGTGCCAGC	TGCATTAA TG	AATCGGCCAA	CGCGCGGGGA	GAGCGGTTT	GCATATTGGG	CGCTCTCCG
3901	CTTCTCGCT	CAC TGACTCG	CTGCGCTCGG	TCGTTCCGGT	CGGGCAGCGC	GTATCAGCTC	ACTCAAAGGC	GGTAATACGG	TTATCCACAG	AATCAGGGGA
4001	TAACG CAGGA	AAGAATCATGT	GAGCAAAAGG	CCAGCAAAG	GCCAGGAACC	GTAAAAAGC	CGCGTTGCTG	GCCTTTTTCC	ATAGCTCCG	CCCCCTGAC
4101	GAGCATCACA	AAAATCGACG	CTCAAGTCAG	AGGTGGCGAA	ACCCGACAGG	ACTATAAAGA	TACCAGGCGT	TTCCCTCTGG	AAGCTCCCTC	GTGCGCTCTC
4201	CTGTTCCGAC	CCTGCCGCTT	ACCGGATACC	TGTCCGCTT	TCTCCCTTCC	GGAAAGCTGG	CGCTTTCTCA	TAGCTCACGC	TGTAGTATC	TCAGTTCGGT
4301	GTAGTCTGTT	CGCTCCAAGC	TGGCTGTGT	GCACGAACCC	CCGTTTACAG	CCGACCGCTG	CGCTTATCC	GGTAACATC	GTCTTGAGTC	CAACCCGGTA
4401	AGACAGACT	TATCGCCACT	GGCAGCAGCC	ACTGTAAACA	GGATTAISCAG	AGCGAGGAT	GTAGGCGGTG	CTACAGAGTT	CTTGAAGTGG	TGGCCTAACT
4501	ACGCTACAC	TAGAAGGACA	GTATTTGGTA	TCTGCGCTCT	GCTGAAGSCA	GTTACTCTCG	GAAAAGAGT	TGGTAGCTCT	TGATCCGGCA	AACAACCCAC
4601	CGCTGGTAGC	GGTGGTTTTT	TTGTTTGC AA	GCAGCAGATT	ACGGCAGAA	AAAAAGGATC	TCAAGAAGAT	CCTTTGATCT	TTTCTACGGC	GTCTGACGCT
4701	CAGTGGAAAGC	AAAACTCAGC	TTAAGGGATT	TTGCTCATGA	GATTATCAAA	AAGGATCTTC	ACCTAGATCC	TTTTAAATTA	AAAATGAAGT	TTTAAATCAA
4801	TCTAAAGTAT	ATATAGTAA	ACTTGGTCTG	ACAGTTACCA	ATGCTTAATC	AGTGAGGCAC	CTATCTCAGC	GATCTGTCTA	TTTCTTTCAT	CCATAGTTGC
4901	bla									
	CTGACTCCCC	GTGCTGTAGA	TAAC TACGAT	ACGGGAGGGC	TTACCATCTG	GCCCCAGTGC	TGCAATGATA	CCGCGAGACC	CACGCTCACC	GGCTCCAGAT
5001	bla									
	TTATCAGCAA	TAAAC CAGCC	AGCCGG AAGG	GCCGAGCGCA	GAAGTGTGCC	TGCAACTTTA	TCCGCTTCCA	TCCAGTCTAT	TAATTTGTGC	CGGGAAGCTA
5101	bla									
	GAGTAAGTAG	TTCCAGGATT	AATAGTTTGC	GCAACGTTGT	TGCCATTGCT	ACAGGCATCG	TGGTGTCAAG	CTGCTGTTT	GGTATGGCTT	CATTGACGTC

5201	CGGTCCCAA	CGATCAAGGC	GAGTTACATG	ATCCCCATG	TTGTGCAAAA	AAGCGGTTAG	CTCCTTCGGT	CCTCCGATCG	TTGTGAGAAG	TAAGTTGGCC
	bla									
5301	GCAGTGTAT	CACTCATGGT	TATGGCAGCA	CTGCATAATT	CTCTACTGT	CATGCCATCC	GTAAGATGCT	TTTCTGTGAC	TGGTGAGTAC	TCAACCAAGT
	bla									
5401	CATTCTGAGA	ATAGTGTATG	CGGCACCGCA	GTTGCTCTTG	CCCGCGTCA	ATACGGGATA	ATACCGCGCC	ACATAGCAGA	ACTTTAAAAG	TGCTCATCAT
	bla									
5501	TGGAAAACGT	TCTTCGGGGC	GAACACTCTC	AAGGATCTTA	CCGCTGTGGA	GATCCAGTTC	GATGTAACCC	ACTCGTGAC	CCAAGTATC	TTCAGCATCT
	bla									
5601	TTTACTTTCA	CCAGCGTTTC	TGGGTGAGCA	AAACAGGAA	GGCAAAATGC	CGCAAAAAAG	GGAATAAGGG	CGACACGAA	ATGTTGAATA	CTCATACTCT
	bla									
5701	TCCTTTTTCA	ATATTATTGA	AGCATTATC	AGGGTTATTG	TCTCATGAGC	GGATACATAT	TTGAATGTAT	TTAGAAAAAT	AAACAAATAG	GGTTCGCGC
5801	CACATTTCCC	CGAAAGTGC	CACCTAAATT	GTAAGCGTTA	ATATTTTGTT	AAAAATCGCG	TTAAATTTTT	GTAAATCAG	CTCATTTTTT	AACCAATAGG
5901	CCGAAATCGG	CAAAATCCCT	TATAAATCAA	AAGAATAGAC	CGAGATAGGG	TTGAGTGTG	TTCCAGTTTG	GAACAAGAT	CCACTATTAA	AGAAGCTGGA
6001	CTCCAACGTC	AAAGGGCGAA	AAACCGTCTA	TCAGGGCGAT	GGCCCACTAC	GTGAACCATC	ACCTTAATCA	AGTTTTTTGG	GGTGAGGTG	CCGTAAAGCA
6101	CTAAATCGGA	ACCTTAAAGG	GAGCCCCGA	TTTAGAGCTT	GACGGGGAAA	GCCGCGAAC	GTGGCGAGAA	AGGAAGGAA	GAAAGCGAA	GGAGCGGGC
6201	CTAGGGCGCT	GGCAAGTGT	GCGTACAGC	TGCGGTAAAC	CACCACACCC	GCCGCGTTA	ATGCGCCGCT	ACAGGGCGCG	TCCATTTCG	CATTCAAGGT
6301	GCGCAACTGT	TGGGAAGGGC	GATCGGTGCG	GGCCTCTCG	CTATTACGCG	AGCTGGCGAA	AGGGGATGT	GCTGCAAGC	GATTAAGTTG	GGTAAACCGCA
	GPD promoter									
6401	GGGTTTTCCC	AGTCACAGCG	TTGTAAAAGC	ACGGCCAGTG	AATTGTAATA	CGACTACTA	TAGGGCGAAT	TGGCCGGTGA	CTCTTCTGG	CATGCGGAGA
	GPD promoter									
6501	GACGCGGGA	CGCAGAGAGA	AGGSGCTGAGT	AATAAGCGCC	ACTGCGCCAG	ACAGCTCTGG	CGGCTCTGAG	GTGCAAGTGA	TGATTATTA	TCCGGGACCG
	GPD promoter									
6601	GCCGCCCTC	CGCCCCGAGG	TGGAAAAGCT	GSTGTGCCCC	TCGTGACCA	AGAATCTATT	GCATCATCGG	AGAATATGGA	GCTTCATCGA	ATCACCGGCA
	GPD promoter									
6701	GTAAGCGAAG	GAGAAATGTA	AGCCAGGGGT	GTATAGCGT	CGGCGAATA	GCATGCCATT	AACCTAGSTA	CAGAAGTCCA	ATTGCTCCG	ATCTGATAAA
	GPD promoter									
6801	AGATTACGA	GATAGTACT	TCTCCGAAGT	AGTAGAGCG	AGTACCGGCG	GCGTAAGCTC	CCTAATTGGC	CCATCCGSCA	TCTGTAGGGC	GTCCAAATAT
	GPD promoter									
6901	CGTCCCTCTC	CTGCTTTGCC	CGGTGTATGA	AACCGGAAG	GCCGCTCAGG	AGCTGGCCAG	CGGCGGAGAC	CGGGAAACA	AGCTGGCAGT	CGACCCATCC
	GPD promoter									
7001	GGTGCTCTGC	ACTCGACTTG	CTGAGTCCC	TCAGTCCCTG	GTAGGCAAGT	TTGCCCGCTC	TGTCCCGCCG	GTGTGTGCGC	GGGGTTGACA	AGTCTGTGTC
	GPD promoter									
7101	GTAGTCCAA	CATTTGTTGC	CATATTTTCC	TGCTCTCCCC	ACCAGCTGCT	CTTTTCTTTT	CTCTTCTTTT	TCCCATTCTC	AGTATATTCA	TCTTCCATC
	GPD promoter									
7201	CAAGAACCCT	TATTTCCCTT	AAGTAAGTAC	TTTGCTACAT	CCATACTCCA	TCCTTCCCAT	CCCTTATTCC	TTTGAACCTT	TCAGTTCGAG	CTTTCCACT
	GPD promoter					HSVtk				
7301	TCATCGAGC	TTGACTAACA	GCTACCCCGC	TTGAGATCGA	TATGCTCTGG	TACCCTGCCC	ATCAACACGC	GTCTGCGTTC	GACCAGGCTG	CGGTTCTCG
	HSVtk									
7401	CGCCCATAGC	AACCGACGTA	CGGCGTTGCG	CCCTCGCCGG	CAGCAAGAG	CCACGGAAAT	CGCCCGGAG	CAGAAAATGC	CCACGCTACT	CGGGTTTAT
	HSVtk									
7501	ATAGACGTC	CCACCGGAT	GGGAAAAACC	ACCACACGCG	AACTGCTGGT	GGCCCTGGGT	TCGCGCGAGC	ATATCGTCTA	CGTACCCGAG	CCGATGACTT
	HSVtk									
7601	ACTGCGGGT	GCTGGGGGCT	TCCGAGACAA	TCGCGAACAT	CTACACACA	CAACACCGCC	TCGACCGGGG	TGAGATATCG	GCCGGGGAGC	CGGCGTGGT
	HSVtk									
7701	AATGACAAGC	GCCAGATAA	CAATGGGCAT	GCCTTATGCC	GTGACCGAGC	CGTCTTGGC	TCCTCATATC	GGGGGGGAGG	CTGGGAGCTC	ACATGCCCCG
	HSVtk									
7801	CCCCCGCCC	TCACCTCAT	CTTCGACCGC	CATCCATCG	CGGCTCTCT	GTGTACCCG	GCCGCGCGGT	ACCTTATGGG	CAGCATGACC	CCCCAGGCGC
	HSVtk									
7901	TGCTGCGTT	CGTGGCCCTC	ATCCCGCCGA	CCTTGCCCGG	CACCAACATC	GTGCTTGGGG	CCCTTCCGGA	GGACAGACAC	ATCGACCGCC	TGGCCAAACG
	HSVtk									
8001	CCAGCGCCCC	GGCGAGCGGC	TGGACTTGGC	TATGCTGGCT	GCGATTGCCC	GCGTTACGG	GCTACTTGGC	AATACGGTGC	GSTATCTGCA	GTGCGGCGGG
	HSVtk									
8101	TCGTGCGGG	AGGACTGGGG	ACAGCTTTGG	GGGACGCGCG	TGCGGCGCCG	CCCCAGAGCA	ACGCGGGCCC	ACGACCCCAT	ATCGGGGACA	
	HSVtk									
8201	CGTTATTAC	CCTGTTTCGG	GCCCCGAGT	TGCTGCCCCC	CAACGCGCAC	CTGTATAACG	TGTTTGGCTG	GGCTTTGGAC	GTCTTGGCCA	AACGCTCCG
	HSVtk									
8301	TTCCATGAC	GTCTTTATCC	TGGATTACGA	CCAATCGCCC	GCCGCTGCC	GGGACGCCCT	GCTGCAACTT	ACCTCCGGGA	TGGTCCAGAC	CCAGTCCACC
	HSVtk					GPD terminator				
8401	ACCCCCGCT	CCATACCGAC	GATATCGGAC	CTGGCGGCA	CGTTTCCCGC	GGAGATGGGG	GAGGTAACCT	GACTCGAGCT	TGACTGATC	TCCCACCTCG

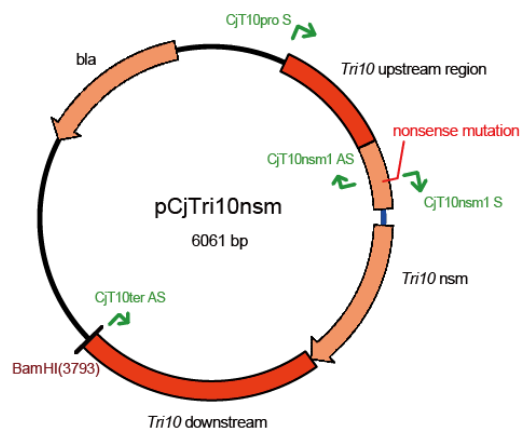
8501	GPD terminator									
	CACAGGTCG	ATGGCAACGC	CTAGACTGCG	ACCAACACCT	TGGAACACGG	CGTCAACGGC	GCCGTTCCEG	TCGTCTCGGA	GACTGCGAAG	CCTGCTTTTC
8601	GPD terminator									
	AATAATGAAA	CAAACCCATG	GGGGAAATTAT	GGAAAGGCAA	TGAACCAAAA	CAAACATAAA	AGGGGACGCG	AAGAAAAAGT	CGGTAACGTC	ACAACGACTA
8701	GPD terminator									
	TTGCGGCCAT	GACAACCAAG	GCTTGGGTCG	GCGCCGTGGG	GAGTGTGGCC	TAGTGCAGTG	GTAGTTAGCT	ACGTATCGGA	CTCTAATAA	ATAACAAAA
8801	GPD terminator									
	AATCAGATTA	AATGAGGACC	ACCTTAGTAG	TACTATAGAC	GAGTCATCG	AACTACAGGG	CATCTGTGCA	TTTTGTGTG	AACCCGTCA	AATATCAAAA
8901	GPD terminator									
	TCATAAACCT	GCGACTTGGC	TGGATGTGCA	AATTCATCCG	TGTATACACA	CATTCTCGCA	CCTTGTGAAG	CAGCCAGCCG	TTGCACGACG	TTTCATCAGG
9001	GPD terminator									
	CTCTGAAAA	GAGGAATTAG	AAAAAAAGST	ATCTGTAATT	AGCAGTGCAG	ACCATGTAAT	GTAATGAATA	CGATCCGACA	AGCTCCATTA	TTGAAGCATT
9101	GPD terminator									
	TATCAGGGTT	ATTGTCTCAT	GAGCGGATAC	ATATTTGAAT	GTATTTAGAA	AAATAAACAA	ATAGGGGTTT	CAGCGACATT	TCCCCGAAAA	GTGCCACCTG
9201	GPD terminator					TrpC promoter				
	ACGTCTAAGA	AACCATTATT	ATCATGACAT	TAACCTATAA	AAATAGGCGT	ATCAGAGGCG	CCTTCTGTCT	TCAAGAATTG	TGCAGAGAAG	ATGATATTGA
9301	TrpC promoter									
	AGSAGCACTT	TTTGGCTTGG	GCTGGAGCTA	GTGGAGSTCA	ACAATGAATG	CCTATTTTGG	TTTAGTGTCT	CAGGCGGTGA	GCACAAAATT	TGTGTCTTTT
9401	TrpC promoter									
	GACAAGATGG	TTCATTTAGG	CAACTGTGCA	GATCAGCCCC	ACTTGTAGCA	GTAGCGGCGG	CGCTCGAAGT	GTGACTCTTA	TTAGCAGACA	GGAACGAGGA
9501	TrpC promoter									
	CATTATTATC	ATCTGCTGCT	TGGTGCACGA	TAACTTGGTG	CGTTTGTCAA	GCAAGSTAAG	TGAACGACCC	GGTCATACCT	TCTTAAGTTC	GCCTTCTCTC
9601	TrpC promoter									
	CCTTATTTC	AGATTCAATC	TGACTTACCT	ATTCTACCCA	AGCATCGATA	AGCTTGATAT	CG			



1	TCGCGCGTTT	CGGTGATGAC	GGTGAAAACC	TCTGACACAT	GCAGCTCCCG	GAGACGSTCA	CAGCTTGTCT	GTAAGCGGAT	GCCGGGAGCA	GACAAGCCCG
101	TCAGGGCGCG	TCAGCGGGTG	TTGGCGGGTG	TCGGGGCTGG	CTTAACATG	CGGCATCAGA	GCAGATTGTA	CTGAGAGTGC	ACCATATGCG	GTGTGAAATA
201	CCGCACAGAT	CGCTAAGGAG	AAAAATACCG	ATCAGGCGCC	ATTGCCATT	CAGGCTGCGC	AACGTGTTGG	AAGGGCGATC	GGTGCGGGCC	TCTTCGCTAT
301	TACGCCAGCT	GGCGAAAAGG	GGATGTGCTG	CAAGSCGATT	AAGTTGGSTA	ACGCCAGGGT	TTTCCAGTC	ACGACGTGTG	AAAACGACGG	CCAGTGAATT
	CjT10pro S									
401	Tri10 upstream region									
	CGAGCTCGGT	ACCCAGGCGA	GTTTGGAAAT	ATGTTTTGCG	GGTACGGATA	CTCGTTTGA	GAATGGTGGT	CTGTTATAAT	GATTACAAT	AGTTGGGTGG
501	Tri10 upstream region									
	TGTTTTGTTA	GAATGAACAG	TTGAACAAGG	ATAATTACTT	CGGAATAGGC	AGTTGAAACT	GAATGCTGTG	ACGTAACCTG	AGCCTGTAAC	CATTTCCAC
601	Tri10 upstream region									
	TTGAGTGCAG	GCITTTGCGT	AAACAAGTCT	GTACACCGT	CGGTGCGACA	GGGCTACCCC	CAACCCTGCA	ACTGCATCTG	CAGCTGCAGC	TGGCAGACTG
701	Tri10 upstream region									
	GTAGACTGCG	GCTACGAACT	GTAGTGCAT	GCGGGAATCT	TGTACCCGCT	CGGAGTTTGG	AGGGAGCTGG	CACATTTCT	AGACCCGCGA	ATTGACTTTC
801	Tri10 upstream region									
	AAAGCGCTTG	CGTTTTGTCC	AGATCAGTGA	CCATACCCCT	GCCTTTTGC	ACCACCCAAA	CGTCCACTGA	ACGAGGCGTA	CAGAAACAC	ACAAGATAAG

		Tri10 upstream region									
901		GTTCGATGCC	TGCTTGAGCA	CTATGAGGGA	CAGCAGACT	CTGTAAAATC	CTATCCTTGC	ATTATATTGT	AACATCGTTC	AACTCTCCCA	CGCCATCTCC
		Tri10 upstream region									
	+1									M D F P K P R Q	
1001		TTCCCGTCTT	TCCCCCAACT	TCAATTGTAT	GCCAAACAA	AATCACTAAA	TTATCATTAT	TATTGTCTGT	AGTCATCATG	GATTTCCCAA	AGCCTAGACA
		Tri10nsm									
	+1	Q V R E T S L L M Y Y L D V V F P L Q C I N P N N N C L G K R E W L									
1101		AGTCCGAGAG	ACGAGCCTGT	TGATGTAATA	CCTAGACGTC	GTGTTCTCTC	TGCAATGCAT	CAACCCAAAC	AACAATTGTC	TGGGAAAGAG	AGAGTGGCTG
		Tri10nsm									
	+1	L T I L T S A R P T Y Y A T L C M S L L Y K E S L S S P C R S E Q A									
1201		TTGACTATAC	TGACCTCTGC	GCGGCTCAG	TACTATGCCA	CATTGTGCAT	GTCGCTCTCC	TATAAAGAA	CGCTTCAAG	CCCTTGCAGA	TCTGAACAGG
		CjT10nsm1_S									
		CjT10nsm1_AS									
		Tri10nsm									
	+1	A *									
1301		GGTAGGATG	GAAGAGAGAG	AAGACATACT	ACTACATTCT	TGCACCTCAG	GAGTCTCAGA	AGTCTCTGGG	TGGGCTCGAC	AAGACATTTG	GCATCACAAG
		CjT10nsm1_AS									
		Tri10nsm									
1401		GCTGAAAGGT	ACCCTGCTTG	CCCTTGCCTG	CATGCTACAG	CTTATCAGTT	TTGAGGTAAG	ACGAATCCAC	CATTGTTTCG	ATGCTCGATG	TCGATGCTCG
		Tri10nsm									
1501		ATATCCGATC	TACGATTATC	GTTGGTCACT	AACAATATA	AATAGTCTTC	GCACTAAGC	AGGGGAGATT	GCGCGTTCA	CCTCCATGCG	GCCCAACATC
		Tri10nsm									
1601		TCATTCCTGT	CTTGGTTGAG	GGATGGTCCA	CAGCTTTGCA	ATCAGGCCCC	CCAGCCACCT	CCATATGGTG	CGAGCTGGAT	GAATCACACT	TCGGCTCGAC
		Tri10nsm									
1701		TGAAGATCAA	ACCTCTTTGA	GCTTCGAATA	CGTCGGAGCT	TTGAGATTCC	TGTCAAACCT	ACTCGCCGCA	GTCGGCATCC	TGTCTTGCAT	ATCTATTGGC
		Tri10nsm									
1801		CCATCAGCAC	CATTTGAAGA	TTACGGCCAT	CTCCTGACC	AGCCAGGCTT	TATACAGCTG	GACGAGGTGC	TGGGGTCGAG	GAATTGGACC	ATGTTGACTA
		Tri10nsm									
1901		TTCTCGAAGT	GGGTAAGCTG	GATCGTTGGA	AGCGACAGGA	GCAAGAATAT	AATCGCTTGA	GCCTAAAGAC	GCTCGCTAAG	CGCGCCATGA	TGATTTGAGGA
		Tri10nsm									
2001		TATGTTGTCA	GACGAGCTAC	AAAGGCTACC	GACAGACGAG	ACGCTTCCAG	ACCTCATCAC	TCAGATTTAC	GCCGCTCTA	TCATGACGTA	TCTGCATACA
		Tri10nsm									
2101		GTAGTTCCG	GACTCAATCC	CAACCTTTCA	GAGGTTGAG	ATAGTGGCC	CGGGACGCTT	CAATTGTTGG	AGAGGCTCCC	AAATCTTGAA	GCTGTACAGA
		Tri10nsm									
2201		GCGTACCTG	GCCTCAGCT	GTCACAGGAT	GCATGGCTC	AGAAAGTCAT	AAGGACTTTT	TCAGAAATAC	TCTGAGTCTG	TATGAGCGCA	CATTGAGCTC
		Tri10nsm									
2301		CTTAAAAAAG	TATGACGGA	CTCTCAGGT	CTTGGAAAG	GCTTGGAAAG	GAAGAGAGAT	AGATACAGAG	TCTCAATGA	GATGGGAAGA	CTTGACGAT
		Tri10nsm									
		Tri10 downstream region									
2401		CACCATGGGC	TTCCAGTGCT	ACTTTGGTAG	GGATGGTATC	GCGCGAGAGA	CTACGGATAC	CGATACCCGA	AGTCTTCTT	CTGCATCAGC	TACCCGATAC
		Tri10 downstream region									
2501		ATGTAAGTTT	GATGCTCACT	TACAGATGAT	GCATGACAGG	AAGTTTATTT	AGATGTTATG	GTTTCGACTC	ATTTTGGCGT	TTAAACAGGG	ATTGGCATGA
		Tri10 downstream region									
2601		TGCATGATTT	AAATGGAATA	GAAGAGCGGA	AAACGTTTCC	CATGTAACCT	GAATGATGGA	CTTTCAATAT	ATATTTCTAG	TATACTTTTT	TAACCGTGAA
		Tri10 downstream region									
2701		CCGCATCTTC	TCCATCTATC	CAACCAATCG	TGCTGAAAGC	AGTCTGGAAC	CATGGTGGGC	GAATCAGTGC	TGTGTCCCTT	GATTGTCTCG	ACATGGCTTG
		Tri10 downstream region									
2801		ACATTGTTTA	GCCCCAGCA	CAAACGATAT	TGATCATAGT	GAAGGAGTGT	TGATTTCAAG	CGAGGGGATT	TGTGGAAGTT	CCTTGTGAAG	ATCCCCGCAA
		Tri10 downstream region									
2901		CGAAACCCAG	TTGGGAAATG	ATTCAATCGT	GGGGCCGAA	TGGGAAGATG	CTGAGATTTG	CGACAGGCAA	TTCTTCTCT	GTTGGGCTGG	GTAGATGATA
		Tri10 downstream region									
3001		GAGGTGCTGA	TCTGGCACTG	CAGTCTCAGC	ATGCTTTGGC	TGCGACCCAT	ATGGTAGCGC	ATAAAGCAGC	GCTAACTCCC	GAGTATGCGA	AAACCTCGAG
		Tri10 downstream region									
3101		CCACGAGACA	TCAGGGTCCA	TCTCATATGA	GTCGATCAGT	TTAGCGGCTG	CGAGCATCTT	GGAGATTGGG	GSTGAGTGT	TGAGTGSATA	GTAGTAGTAG
		Tri10 downstream region									
3201		TAGTGTAGT	GGTGTGATGC	AATTGATGTC	GTGTTCTGTT	TTGTTTATG	GGAAAGAAAT	GTTGAGATGT	TTCTAATCGG	TTCTGGCCGA	TGGTTAATAT

	Tri10 downstream region									
3301	CCTAATGTC	TTAATGCTA	TGCTCTATCT	ACCGACCAA	AATATCGCAG	ATATGCGAGA	AAAAGSCTG	AATGATGCTG	AGTTGTGTG	ANGCTGAAC
	Tri10 downstream region									
3401	GTCAATAAA	GGCCTGAATA	TGCAAGCGTC	GAACCGAACA	TCCGGCCTAA	ACGGCGTCTC	GTCATGCAAT	GCCATTACCG	ATCCCATCGC	CATTGAGGCT
	Tri10 downstream region									
3501	CAGCATAGAA	TGCCATCGAG	CTTCACGATC	ACTCTTCTCT	CGTCGGCAAC	ATGGCAACGG	ACTTGTCCGC	GCCGACAAC	CCATGTTCAA	CTGATGTGG
	Tri10 downstream region									
3601	GTTACATCCA	AGGGTCTGTT	CTTTTTAAGC	TTTCATGACA	GGCCGGCAGA	GACGTAAAT	CTGCCGTCCG	TGTGTGTGAA	CCCTGAATA	GGTCCCTCT
	Tri10 downstream region									
3701	TTTTAATTTA	ATGCATGSCA	TTTGCTGCC	TACAGCAACA	GCSCGAAAG	CGAAAAATTA	TGAAAGCATA	TCTATGTTGT	GACGGAGTGG	BamHI GGATCCTCT
	CjT10ter_AS									
3801	AGAGTGCACC	TGCAGGCATG	CAAGCTTGGC	GTAATCATGG	TCATAGCTGT	TTCTGTGTG	AAATTGTTAT	CCGCTCACA	TTCCACACA	CATACGAGCC
	CjT10ter_AS									
3901	GGAAGCATAA	AGTGTAAAGC	CTGGGTGCC	TAATGAGTGA	GCTAACAC	ATTAATTGCG	TTGCGCTCAC	TGCCCGCTT	CCAGTCGGGA	AACCTGTCGT
4001	GCCAGCTGCA	TTAATGAATC	GGCCAACGCG	CGGGGAGAGG	CGGTTTGCCT	ATTGGGCGCT	CTTCCGCTTC	CTCGCTCACT	GACTCGCTGC	GCTCGGTGCT
4101	TCGGCTGCGG	CGAGCGGTAT	CAGCTCACTC	AAAGCGGTA	ATACGGTTAT	CCACAGAATC	AGGGGATAAC	GCAGAAAGA	ACATGTGAGC	AAAAGCCAG
4201	CAAAAGGCCA	GGAAACGTAA	AAAGCCGCG	TTGCTGGCGT	TTTTCCATAG	GCTCCGCCCC	CCTGACGAGC	ATCACAAAA	TCGACGCTCA	AGTCAGAGGT
4301	GGCAGAACCC	GACAGGACTA	TAAAGTACC	AGCGTTTCC	CCCTGGAAAGC	TCCCTCGTGC	GCTCTCCTGT	TCCGACCCCTG	CCGTTTACCG	GATACCTGTC
4401	CGCCTTTCTC	CTTCGGGAA	CGTGGCGCT	TTCTCAATGC	TCACGCTGTA	GGTATCTCAG	TTGCGGTGAG	GTCGTCGCT	CCAAGCTGGG	CTGTGTGAC
4501	GAACCCCGCG	TTACGCCGA	CCGCTGCGCC	TTATCCGTA	ACTATGCTCT	TGAGTCAAC	CCGTAANGAC	ACGACTTATC	GCCACTGGCA	GCAGCCACTG
4601	GTAACAGSAT	TAGCAGAGCG	AGGTATGTAG	GCGGTCTAC	AGAGTCTTG	AAGTGTGGC	CTAACTACGG	CTACACTAGA	AGGACGATAT	TTGGTATCTG
4701	CGCTCTGCTG	AAGCAGTTA	CCTTCGAAA	AAGAGTTGGT	AGCTCTTGAT	CCGGCAAAAC	AACACCCTCT	GGTAGCGGTG	GTTTTTTTGT	TTGCAAGCAG
4801	CAGATTACGC	GCAGAAAAA	AGGATCTCAA	GAAGATCCTT	TGATCTTTTC	TACGGGGTCT	GACGCTCAGT	GGAAAGAAAA	CTCAGGTTAA	GGGATTTTGG
4901	TCATGAGATT	ATCAAAAAAG	ATCTTCACTT	AGATCCITTT	AAATTAAAAA	TGAAGTTTTA	AATCAATCTA	AAGTATATAT	GAGTAAACTT	GGTCTGACAG
5001	TTACCAATGC	TTAATCAGTG	AGGCACCTAT	CTCAGGATC	TGCTATTTT	GTTCACTCAT	AGTTGCTGTA	CTCCCGCTCG	TGTAGATAAC	TACGATACGG
	bla									
5101	GAGGGCTTAC	CATCTGCCCC	CAGTCTGCA	ATGATACCGC	GAGACCACCG	CTCACCGGCT	CCAGATTTAT	CAGCAATAAA	CCAGCCAGCC	GGAAAGGCCG
	bla									
5201	AGCGCAGAAG	TGTCCTGCA	ACTTTATCCG	CCTCCATCCA	GTCATTAAT	TGTTGCCGGG	AAGTAGAGT	AAGTAGTTCG	CCAGTAAATA	GTTTGCAGAA
	bla									
5301	CGTTGTGCC	ATTGCTACAG	GCATCTGGT	GTCAGCTCG	TGTTTTGTA	TGGCTTATT	CAGCTCCGCT	TCCAACGAT	CAAGCGAGT	TACATGATCC
	bla									
5401	CCCATGTTGT	GCAAAAAAGC	GTTTAGCTCC	TTGCTCTC	CGATCGTGT	CAGAAGTAAG	TTGGCCGAG	TGTTATCACT	CATGTTTATG	GCAGCACTGC
	bla									
5501	ATAATCTCT	TACTGTCATG	CCATCCGTAA	GATGCTTTTC	TGTGAGTGT	GAGTACTCAA	CCAAGTCAT	CTGAGAATAG	TGATGCGGC	GACCGAGTTG
	bla									
5601	CTCTTGCCG	GCCTCAATAC	GGGATAATAC	CGCCACAT	AGCAGAACTT	TAAAAGTCT	CATCATTGGA	AAAGCTTCT	CGGGGCAAA	ACTCTCAAGG
	bla									
5701	ATCTTACCG	TGTTGAGATC	CAGTTCGATG	TAACCCACTC	GTGCACCAA	CTGATCTTCA	GCATCTTTTA	CTTTCACAG	CGTTTCTGGG	TGAGCAAAAA
	bla									
5801	CAGGAAGGCA	AAATGCCGCA	AAAAAGGAA	TAAGGGCGAC	ACGAAATGT	TGAATACTCA	TACTCTTCT	TTTTCAATAT	TATTGAAGCA	TTTATCAGGG
	bla									
5901	TTATTGCTC	ATGAGCGGAT	ACATATTTGA	ATGTATTTAG	AAAAATAAAC	AAATAGGGGT	TCCGCGACA	TTTCCCGAA	AAGTCCACC	TGACGTCTAA
6001	GAAACCATTA	TTATCATGAC	ATTAACCTAT	AAAAATAGGC	GTATCACGAG	GCCCTTCGT	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'-ATAGCTAGCCACTCCGTCACAACATAGAA -3') and digested with *NheI* (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 *SpeI* (doubly underlined) and *SacII* (wavy underlined). The *SpeI* - *SacII* 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 $\Delta Tri10$ tk. For the construction of pCjTri10nsm, two pairs of primers (CjT10pro_S: 5'-TCGAGCTCGGTACCCAGGCGAGTTTGGAAGTATGTT -3' and CjT10nsm1_AS: 5'- CATACCTACGCCTGTTCAGATCTGCAAG -3'; CjT10nsm1_S: 5'- ACAGGCGTAGGTATGGAAGAGAGAGAAAGACAT -3' and CjT10ter_AS: 5'- CTCTAGAGGATCCCCACTCCGTCACAACATAGATAT -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 *SmaI*-linearized pUC19 were assembled by Gibson Assembly. The resulting vector, pCjTri10nsm, was used for transformation of the transgenic strain $\Delta Tri10$ tk to generate a marker-free self-cloning strain $\Delta Tri10$ -nsm.

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