

March 10, 2022

Bernadette Juarez
APHIS Deputy Administrator
Biotechnology Regulatory Services

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By ajdrummond for BRS Document Control Officer at 3:20 pm, Mar 10, 2022

Dr. Juarez,

With this letter we respectfully request a Regulatory Status Review from USDA-APHIS's Biotechnology Regulatory Services (BRS) for the following Plant Trait Mechanism of Action: *Thlaspi arvense* L. (pennycress; field pennycress) CRISPR/Cas9 generated mutant lines, featuring disruption of both *fatty acid elongation 1 (FAE1)* and *transparent testa 8 (TT8)*. In response to each of five previously submitted AIR letters¹, BRS deregulated pennycress lines harboring disruptions in *FAE1* and/or *TT8* genes, concluding that: "...your gene-edited pennycress lines are not themselves plant pests...Additionally, pennycress is not listed as a Federal noxious weed pursuant to 7 CFR part 360 and USDA has no reason to believe that the intended phenotypes of your two pennycress lines would increase the weediness of pennycress." This RSR requests evaluation of pennycress with mutations in the same genes that result in the same low erucic acid and lower fiber phenotypes previously generated and deregulated.

1. Information about Requestor

First Name: Traci

Last Name: Hagedorn

Position: Consultant

Organization Name (if applicable): Hjelle Advisors, LLC on behalf of CoverCress Inc.

Contact information (choose one or both)

Telephone: 3123992803

Email address: thagedorn@hjelleadvisors.com

2. Does the request contain Confidential Business Information (CBI)?

No, this RSR request does not contain CBI.

3. Description of the comparator plant:

Scientific name (genus, species): *Thlaspi arvense*

¹ Illinois State University, Loss of *FAE1* function – USDA response August 8, 2018; Illinois State University, Loss of *TT8* function – USDA response April 9, 2019; Cover Cress, Inc. Loss of *FAE1*, *TT8*, *AOP2* – USDA response January 29, 2020; CoverCress, Inc. Loss of *FAE1*, *TT8*, *AOP2* – USDA response May 7, 2020; CoverCress, Inc. Loss of *TT8*, *AOP2* – USDA response August 31, 2020

Common Name: pennycress; field pennycress; CoverCress™

Subspecies / Cultivar / Breeding Line:

CoverCress™ Whole Grain that has been produced through gene edits resulting in the low-erucic acid, lower-fiber phenotype. CoverCress, Inc. has developed through selection and breeding (not gene-editing) several elite germplasm lines that have optimized agronomic characteristics. These lines to date include B3, B28, B48, B74, 2032, 1718SPRX:012-B-B-30, 1718SPRX:012-B-B-39, 182025-B-B-4, 183002-B-14, 183002-B-6, 183011-B-5, 183056-B-29, and 183059-B-20. It is envisioned that the lines will undergo late stage editing to produce the low-erucic acid, lower-fiber phenotype within germplasm that is already optimized for commercial production. The description and data provided within this RSR are from edits to the B3 line.

4. Genotype of the modified plant (if genetic material is not inserted into the genome):

Nature of modification(s):

Mutations were introduced into pennycress cultivar B3 using a CRISPR/SpCas9 DNA construct designed to target genomic edits to the *FAE1* and *TT8* genes. This transgene construct was delivered to B3 using a disabled *Agrobacterium tumefaciens* strain (GV3101) and a standard floral dip transformation method. When integrated into the plant genome, the expressed *Streptococcus pyogenes* CRISPR-associated protein 9 (SpCas9) endonuclease was guided to two unique, targeted loci (the *FAE1* and *TT8* genes). At these locations, the SpCas9 endonuclease catalyzed double-stranded DNA breaks, which were then repaired by the plant's error-prone endogenous non-homologous end joining (NHEJ DNA) repair mechanisms, resulting in heritable mutations at the targeted loci.

The design pipeline has two steps to ensure the gRNA is specific to the gene and there is no off-targeting. First, an online gRNA design tool that has an inbuilt pennycress genome is used to identify gRNA target sites and provide a list of putative off-target sites.

Furthermore, a blast search is performed with a candidate protospacer sequence against the pennycress genome to avoid the use of a protospacer with potential off-targets.

DsRED fluorescent protein from *Discosoma* was included in the plasmid to confer red fluorescence in plants that successfully took up the plasmid introduced by *A. tumefaciens*. Presence of the edits in T₁ plants was confirmed through visualization of red fluorescent protein under a light system and confirmatory PCR screening of a fragment of the T-DNA. Seed from the progeny T₂ generation was screened for segregants that did not have the transgene as indicated by lack of red fluorescence. One batch of plants isolated from the non-detect T₂ progeny of a single transformed B3 pennycress plant contained mutations in both *FAE1* and *TT8* resulting in a low erucic and lower fiber phenotype (referred to as B3WG).

Resulting progeny in the T₃ generation were screened again for negative presence of DsRED and Cas as well as homozygous edits to *FAE1* and *TT8*. Three types of plants were identified. B3 WG.1 harbors a single A insertion in the *FAE1* gene and a single G insertion in the *TT8* gene. B3 WG.2 harbors a single A insertion in the *FAE1* gene and a single base

deletion in the *TT8* gene. B3 WG.3 harbors a single A insertion in the *FAE1* gene and a 2bp deletion in the *TT8* gene. In all three iterations, B3WG plants produced yellow seed (a marker for lower fiber) and low accumulation of erucic acid in seeds. The B3 WG.1 and B3 WG.2 plants were taken forward for subsequent characterization and seed bulk up.

Sequence and Comparison of each Modification

FAE1

The top line of the following sequence comparison is the unmodified sequence of *FAE1*. One representative modified gene sequence of *FAE1* is included for comparison (B3 WG.1). One site in the gene is edited and includes a single base pair insertion, highlighted in red. The full gene sequences and sequence comparison are included in Appendix 1.

```
Ta_FAE1          GCCTTCACCGTTTTTCGGTTTGGCTCTCTACATCGTAA-CCCGGCCCAAACCGGTTTACCT
Ta_fae1_mut1    GCCTTCACCGTTTTTCGGTTTGGCTCTCTACATCGTAACCCGGCCCAAACCGGTTTACCT
*****
```

TT8

The top line of the following sequence comparison is the unmodified sequence of *TT8*. Two representative modified gene sequences of *TT8* are included for comparison. One site in the gene is edited; one modified sequence includes a single G deletion (B3 WG.2) and one modified sequence includes a single G insertion (B3 WG.1). The edits are highlighted in red. The full gene sequences and sequence comparison are included in Appendix 1.

```
Ta_TT8_Wt       GGGAGAATGGATACTACAACGGTGCAATAAAG-ACGAGGAAGACAACCTCAGCCGGCGGAA
Ta_TT8_Mut2    GGGAGAATGGATACTACAACGGTGCAATAAA-ACGAGGAAGACAACCTCAGCCGGCGGAA
Ta_TT8_Mut1    GGGAGAATGGATACTACAACGGTGCAATAAAGACGAGGAAGACAACCTCAGCCGGCGGAA
*****
```

5. Description of new trait

Intended trait: low erucic acid and lower fiber seeds

Intended phenotype: yellow seed (as a marker for lower fiber) and low accumulation of erucic acid in seeds

Erucic acid in seed oil in homozygous *fae1* mutants is consistently <0.1% of total fatty acids, compared with >35% of total fatty acids in wild-type pennycress seeds. The fatty acid profile of homozygous *fae1* mutants is comprised predominantly of oleic acid (C18:1), linoleic acid (C18:2) and linolenic acid (C18:3), which have known nutritional and energy value.

In homozygous *TT8* mutants, the seed coats of pennycress are light yellow colored in contrast to the naturally dark seeds produced by wild-type pennycress, signifying the absence or reduction of condensed tannins in the seed coat. These seeds contain lower levels of undigestible fiber, and thusly, higher metabolizable energy for animal feed.

In a composition study of several light-colored pennycress mutants versus 95 wild type pennycress accessions harvested at various locations across the USA, NIR spectroscopy

analysis revealed that the light-colored pennycress contained 10-19.7% Acid Detergent Fiber (ADF) and 13.1-24.1% Neutral Detergent Fiber (NDF), while the dark-colored pennycress contained 20.8-37.9% ADF and 26.3-35.1% NDF. Additional composition studies consistently show a 25-37% reduction in various fiber components relative to the dark wild type seed (Ulmasov et al. 2020).

Description of the MOA:

Targeted changes in Field Pennycress composition to produce CoverCress™

Field pennycress contains high levels of oil (~25-30%) that makes it a desirable ultra-low carbon fuel feedstock.

In addition to this primary value for fuel, the seed, meal and/or oil could provide an energy source for animal feeds. The utility of pennycress for this use, however, is limited by that fact that the that oil contains >35% erucic acid. Erucic acid is a 22-carbon monounsaturated acid that is absorbed, distributed and metabolized like other fatty acids involving primarily metabolism via mitochondrial beta-oxidation and, to a lesser extent, peroxisomal beta-oxidation. Like other longer-chain fatty acids, the rate of mitochondrial beta oxidation is comparatively lower for erucic acid; however, elevated erucic acid levels induce liver peroxisomal oxidation pathways as a mechanism of compensation. Interest in the safety of erucic acid occurred when results of studies in rats associated the dietary intake of high doses of erucic acid with myocardial lipidosis and heart lesions. Oilseed rape conventionally contains similarly high levels of erucic acid. Low erucic acid varieties were identified and marketed as canola, which have been shown to be safe for inclusion in animal feed.

Reduction in erucic acid is achieved through disruption of Fatty Acid Elongation 1 (*FAE1*) (Figure 2), resulting in higher levels of oleic (18:1) (Qiu et al. 2006). It is through this same mechanism that erucic acid levels are lowered in pennycress (McGinn et al. 2019). The edit to *FAE1* causes a frameshift which results in a premature stop codon and a truncated, non-functional protein.

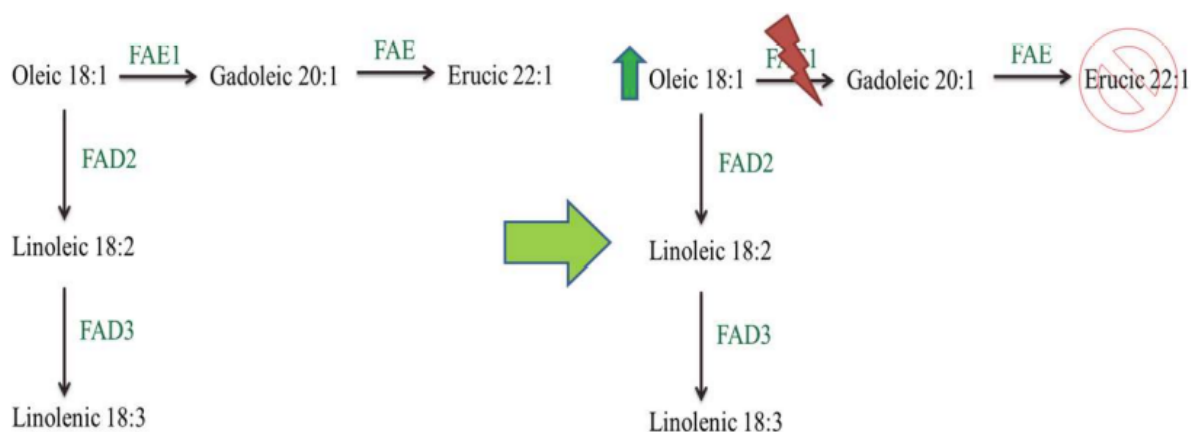


Figure 2: Fatty Acid Pathway to Reduce Erucic Acid Levels

Field Pennycress is also high in fiber (level is variable, but Acid Detergent Fraction values of up to 40% mass on a dry weight basis have been observed), which can impact digestibility as a feed ingredient.

The production of seed coat fiber was first characterized in the model plant *Arabidopsis*. *Arabidopsis* seed coats derive their brown color from the accumulation of proanthocyanidins (PAs), a class of flavonoid chemicals (polymerized flavan-3-ols, or condensed tannins) that protect against a variety of biotic and abiotic stresses and help maintain seed dormancy and viability (Debeaujon et al. 2003). PAs start out as colorless epicatechin compounds until they are transported to the vacuole where they are polymerized and oxidized as the seed desiccates. In *Arabidopsis*, PAs are only produced in a narrowly defined cell layer in the endothelium of the seed, and *TTG1*, *TT8/bHLH042*, and *TT2/MYB123* and have been demonstrated as being the three main regulators of PA biosynthesis in seed coat (Baudry et al. 2004; Lepiniec et al. 2006). Gonzalez et al. (2009) described how the *TTG1* works in a complex with a particular combination of MYB class and bHLH class transcription factors to regulate epidermal development of the seed coat (Figure 3).

Loss-of-function mutants in these genes exhibit the transparent “testa” phenotype as a result of low levels of oxidized PAs in the seed coat. The transparent testa phenotype has been observed in brassicas, including canola, and is characterized by yellow seeds that have more oil because of the resulting thinner seed coat and larger embryo (Abraham and Bhatia 1986). Meal from these brassicas have also been shown to be useful in animal feed because of the relatively lower fiber and higher metabolizable energy (Slominski et al. 1994 and 1999; Simbaya et al. 1995). Similarly, the transparent testa phenotype was observed with loss-of-function mutations in orthologs of these genes in pennycress, resulting in reduced fiber content (Chopra et al. 2018). In CoverCress™ the *TT8* gene was edited for loss of function. The edit to *TT8* causes a frameshift which results in a premature stop codon and a truncated, non-functional protein.

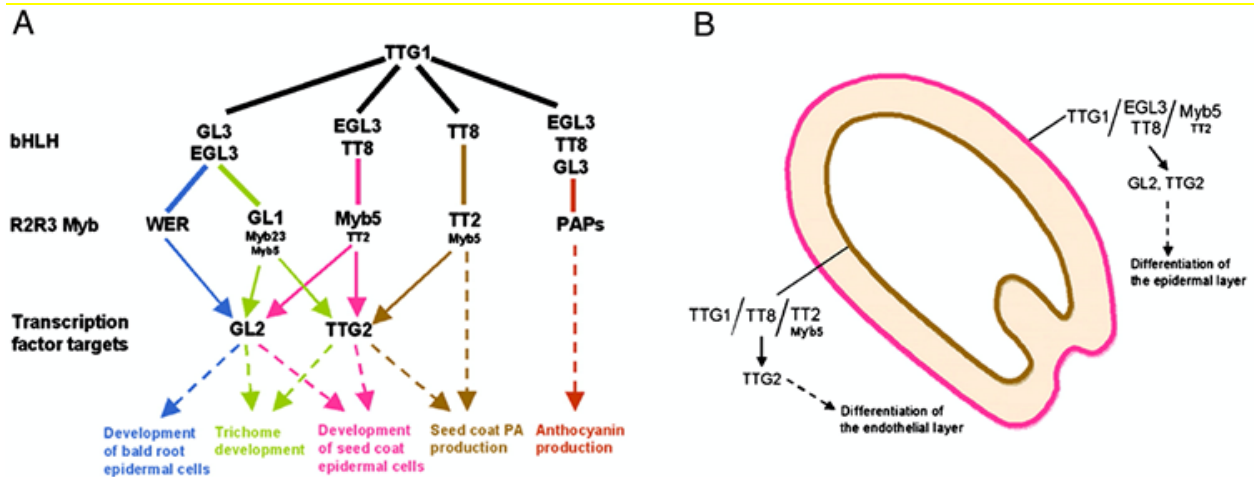


Figure 3: Models for the regulation of TTG-1 dependent pathways in seed coat development. (A) A regulatory network for the positive control of TTG1-dependent epidermal cell fates. Solid lines indicate interactions between members of a complex. Solid arrows indicate direct regulation of *GL2* or *TTG2* targets. Dashed arrows indicate a multi-step differentiation pathway. Colored lines and arrows indicate specific regulator combinations and the pathway controlled. Text size in the case of the MYBs indicates their relative contributions to cell fate regulation. (B) A regulatory model for the differentiation of the seed coat outer and inner layers specified by specific TTG1-dependent transcriptional complexes. Text size in the case of MYB5 and TT2 MYBs indicates their relative contributions to the development of the outer and inner testa layers (Figure from Gonzalez et al. 2009).

In conclusion, CoverCress™ is currently being developed as a new cash cover crop that could be grown between the fall harvest and spring establishment of traditional crops in temperate regions. Gene editing of the *FAE1* and *TT8* genes has been demonstrated to cause loss of function in these two genes with a resulting low-erucic acid and lower-fiber phenotype. The mode of action for loss of gene function in producing this phenotype is well-understood. There is no evidence that this phenotype would enhance the weediness of pennycress and in fact, may reduce seed dormancy, a major factor in the weediness of this plant. Based on this information, we request USDA's review of the PTMOA and confirmation that the two traits of low erucic and lower fiber, produced through editing for loss of function of *FAE1* and *TT8*, when produced by simultaneous or sequential editing of pennycress are not subject to regulation under Part 340.

References

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Slominski, B. A., L. D. Campbell, and W. Guenter (1994) Carbohydrates and dietary fiber components of yellow and brown-seeded canola. *J. Agric. Food Chem.* 42: 704–707

Ulmasov, T., G. Hartnell, J. C. Sedbrook, M. D. Marks, R. Chopra, and M. Esfahanian (2020) Low fiber pennycress meal and methods of making (U.S. Patent No. 10,709,151). U.S. Patent and Trademark Office.

Appendix 1 – Full Sequences and Sequence Comparisons

Unmodified and Modified Sequences of *FAE1* and *TT8*

FAE1 Wild Type, unmodified sequence

TaFAE1 WT CDS;

ATGACGTC CGTTAACGTTAAGCTCCTTTACCATTACGTCATCACCAACTTTTTCAACC
 TTTGCTTCTTCCCGTTAGCGGCGATCGTTGCCGAAAAGCCTCTCGGCTTACCACAA
 ACGATCTTCACCACTTCTACTATTCCTATCTCCAACACAACCTAATAACCATATCTCT
 ACTCTTTGCCTTCACCGTTTTTCGGTT**TGGCTCTCTACATCGTAACC**CGGCCCAAACCG
 GTTTACCTCGTTGACCATTCCCTGCTACCTTCCACCATCGCATCTTAGAAGCAGTATCT
 CTAAGGTCATGGATATCTTCTATCAAGTAAGATTAGCCGATCCTTTACGGAACGCGG
 CAAGCGATGATTCGTCCTGGCTTGATTTCTTGAGGAAGATTCAGGAGCGGTCTGGTC
 TAGGCGATGAAACCCACGGCCCCGAGGGACTGCTTCAGGTCCCTCCACGGAAGACT
 TTTGCCGCGGCGCGTGAAGAAACAGAGCAAGTGATCATCGGTGCGCTCGAAAACT
 ATTCGAGAACACCAAAGTTAACCCTAAAGAGATTGGTATACTTGTGGTGAAGTCAA
 GCATGTTTAATCCGACTCCTTCGCTCTCGGCGATGGTTGTTAATACTTTCAAGCTCCG
 AAGCAACATCAGAAGCTTTAATCTTGGAGGAATGGGTTGTAGTGCCGGCGTTATAGC
 CATTGATCTGGCTAAGGACTTGTTGCATGTCCATAAAAACACTTATGCTCTTGTGGT
 GAGCACAGAGAACATCACTTACAACATTTATGCTGGTGATAACAGATCCATGATGGT
 TTCGAATTGCTTGTTCGGTGTGGTGGGGCCGCGATTTTGCTCTCCAACAAGCCGAG
 GGACCGGAGACGGTCCAAGTACCAGCTACTTCACACGGTTCGGACGCATAACCGGAG
 CTGACGACAAGTCTTCCGATGTGTGCAACAAGAAGACGACGAGAGCGGTAAAACC
 GGGGTGTGTTTGTCCAAGGACATAACCGGTGTTGCCGGGAGAAGTGTTCAGAAAAA
 CATAACAACATTGGGTCCGTTGGTTCTTCTTTTAGCGAGAAATTTCTTTTTTTCGTT
 ACCTTCATCGCCAAGAACTCTTTAAAGACAAGATCAAACATTACTACGTCCCGGAT
 TTCAAGCTTGCTATCGACCATTTTTGTATTTCATGCCGGAGGCAGAGCCGTGATCGAT
 GTGCTACAGAAGAAGTCTAGGTCTATTGCCGATCGATGTGGAGGCATCTAGGTCAACG
 TTACATAGATTTGGGAACACTTCGCTAGCTCAATTTGGTATGAATTGGCGTACATA
 GAGGCAAAAGGAAGGATGAAGAGAGGGAACAAAGTTTGGCAGATTGCTTTAGGGT
 CAGGGTTTAAGTGTAATAGTGCGGTTTGGGTGGCTCTACGCAATGTCAAGGCTTCGA
 CAAATAGTCCTTGGGAACATTGCATTGATAGATATCCAGATGCAATTGATTCTGATT
 CGGGTAAGTCAGAGACTCGTGTCCAAAACGGTCGGTCCTAA

FAE1 Modified Sequence, Representative Sequence

TaFAE1 Mutant1 CDS;

ATGACGTC CGTTAACGTTAAGCTCCTTTACCATTACGTCATCACCAACTTTTTCAACC
 TTTGCTTCTTCCCGTTAGCGGCGATCGTTGCCGAAAAGCCTCTCGGCTTACCACAA
 ACGATCTTCACCACTTCTACTATTCCTATCTCCAACACAACCTAATAACCATATCTCT
 ACTCTTTGCCTTCACCGTTTTTCGGTT**TGGCTCTCTACATCGTAA(A)CC**CGGCCCAAAC
 CGGTTTACCTCGTTGACCATTCCCTGCTACCTTCCACCATCGCATCTTAGAAGCAGTAT
 CTCTAAGGTCATGGATATCTTCTATCAAGTAAGATTAGCCGATCCTTTACGGAACGC
 GGCAAGCGATGATTCGTCCTGGCTTGATTTCTTGAGGAAGATTCAGGAGCGGTCTGG
 TCTAGGCGATGAAACCCACGGCCCCGAGGGACTGCTTCAGGTCCCTCCACGGAAGA
 CTTTTGCCGCGGCGCGTGAAGAAACAGAGCAAGTGATCATCGGTGCGCTCGAAAAA
 CTATTCGAGAACACCAAAGTTAACCCTAAAGAGATTGGTATACTTGTGGTGAAGTCA

gaaaatctataaaatTTTTatgaatagACACACACATGGACATGATGAATCTAATGGAGGAGGGTGGAACTATTCTCAGACAGTATCAACACTTCTTATGTCACAACCCACGAGTCTTTTTTCAGATTCAGTTTCCACATCTTCTTACATCCAATCATCATTGCCACATGGAAGGCTGATAATTTTAAAGAGCATCAGCGAGTGGAACTAAATCGACGTCGTCGTCGCAATGGATGCTCAAACACATAATCTTGAGAGTTCTTTACTCCACGACCACACTAAAGAAAAGAGGCTGCCTCGAGAAGAGCTTAATCACGTGGTGGCAGAGCGCCGCAGGAGAGAGAAGCTGATGAGAGATTCATAACACTGAGATCATTGGTTCCCTTTGTGACCAAGATGGATAAAGTCTCAATTCTTGAGACACCATCAACTACGTAAACCATCTTCGAAATAGGGTCCAAGAGCTGGAGACTAATCATCACGAACAAAACATAAGCGGATGCGTAGCTGTAAGGGA AAAACGTGGGAAGAGGTCGTTGAGGTTTCCATCATAGAGAGTGATGTTTTGTTAGAGATGAGATGCGAGTACCGAGATGGTCTATTGCTCGACATCCTTCAGGTTCTTAAGGAACATGGTATAGAGACTACTGCAGTTCATACCGCGGTGAACGAGCGTGATTCGAGGCCGAGATAAGGGCTATGGTGAGAGGGAAGAAACCAAGCATTGCTGAGGTCAAAGAGCCATCCATCAAACACTATATCCAATATTAACACTATAG

Sequence Comparisons of the unmodified and modified genes

FAE1

The top lines of the following sequence comparison are the unmodified sequence of *FAE1*. One representative modified gene sequence of *FAE1* is included for comparison (B3 WG.1). One site in the gene is edited and includes a single base pair insertion, highlighted in red.

Ta_FAE1	ATGACGTCCGTTAACGTTAAGCTCCTTTACCATTACGTATCACCAACTTTTTCAACCTT
Ta_fael_mut1	ATGACGTCCGTTAACGTTAAGCTCCTTTACCATTACGTATCACCAACTTTTTCAACCTT *****
Ta_FAE1	TGCTTCTTCCCCTTAGCGGCGATCGTTGCCGAAAAGCCTCTCGGCTTACCACAAACGAT
Ta_fael_mut1	TGCTTCTTCCCCTTAGCGGCGATCGTTGCCGAAAAGCCTCTCGGCTTACCACAAACGAT *****
Ta_FAE1	CTTCACCACTTCTACTATTCCATCTCCAACACAACCTAATAACCATATCTCTACTCTTT
Ta_fael_mut1	CTTCACCACTTCTACTATTCCATCTCCAACACAACCTAATAACCATATCTCTACTCTTT *****
Ta_FAE1	GCCTTCACCGTTTTTCGGTTTTGGCTCTCTACATCGTAA-CCCGGCCAAACCGGTTTACCT
Ta_fael_mut1	GCCTTCACCGTTTTTCGGTTTTGGCTCTCTACATCGTAA C CCCGGCCAAACCGGTTTACCT *****
Ta_FAE1	CGTTGACCATTCTGCTACCTTCCACCATCGCATCTTAGAAGCAGTATCTCTAAGGTCAT
Ta_fael_mut1	CGTTGACCATTCTGCTACCTTCCACCATCGCATCTTAGAAGCAGTATCTCTAAGGTCAT *****
Ta_FAE1	GGATATCTTCTATCAAGTAAGATTAGCCGATCCTTTACGGAACGCGGCAAGCGATGATTC
Ta_fael_mut1	GGATATCTTCTATCAAGTAAGATTAGCCGATCCTTTACGGAACGCGGCAAGCGATGATTC *****
Ta_FAE1	GTCCTGGCTTGATTCTTGGAGGAAGATTAGGAGCGGTCTGGTCTAGGCGATGAAACCA
Ta_fael_mut1	GTCCTGGCTTGATTCTTGGAGGAAGATTAGGAGCGGTCTGGTCTAGGCGATGAAACCA *****
Ta_FAE1	CGGCCCCGAGGGACTGCTTCAGGTCCCTCCACGGAAGACTTTTGCCGCGGCGGTGAAGA
Ta_fael_mut1	CGGCCCCGAGGGACTGCTTCAGGTCCCTCCACGGAAGACTTTTGCCGCGGCGGTGAAGA *****

Ta_FAE1 AACAGAGCAAGTGATCATCGGTGCGCTCGAAAACTATTTCGAGAACACCAAAGTTAACCC
Ta_fael_mut1 AACAGAGCAAGTGATCATCGGTGCGCTCGAAAACTATTTCGAGAACACCAAAGTTAACCC

Ta_FAE1 TAAAGAGATTGGTATACTTGTGGTGAAGCAAGCATGTTTAAATCCGACTCCTTCGCTCTC
Ta_fael_mut1 TAAAGAGATTGGTATACTTGTGGTGAAGCAAGCATGTTTAAATCCGACTCCTTCGCTCTC

Ta_FAE1 GGCGATGGTTGTTAATACTTTCAAGCTCCGAAGCAACATCAGAAGCTTTAATCTTGAGG
Ta_fael_mut1 GGCGATGGTTGTTAATACTTTCAAGCTCCGAAGCAACATCAGAAGCTTTAATCTTGAGG

Ta_FAE1 AATGGGTGTAGTGCCGGCGTTATAGCCATTGATCTGGCTAAGGACTTGTTCATGTCCA
Ta_fael_mut1 AATGGGTGTAGTGCCGGCGTTATAGCCATTGATCTGGCTAAGGACTTGTTCATGTCCA

Ta_FAE1 TAAAAACACTTATGCTCTTGTGGTGAAGCAAGCATGTTTAAATCCGACTCCTTCGCTCTC
Ta_fael_mut1 TAAAAACACTTATGCTCTTGTGGTGAAGCAAGCATGTTTAAATCCGACTCCTTCGCTCTC

Ta_FAE1 TGATAACAGATCCATGATGGTTTCGAATTGCTTGTTCGGTGGGGCCGCGATTTT
Ta_fael_mut1 TGATAACAGATCCATGATGGTTTCGAATTGCTTGTTCGGTGGGGCCGCGATTTT

Ta_FAE1 GCTCTCCAACAAGCCGAGGGACCGGAGACGGTCCAAGTACCAGTACTTCACACGGTTCCG
Ta_fael_mut1 GCTCTCCAACAAGCCGAGGGACCGGAGACGGTCCAAGTACCAGTACTTCACACGGTTCCG

Ta_FAE1 GACGCATACCGGAGCTGACGACAAGTCTTTCCGATGTGTGCAACAAGAAGACGACGAGAG
Ta_fael_mut1 GACGCATACCGGAGCTGACGACAAGTCTTTCCGATGTGTGCAACAAGAAGACGACGAGAG

Ta_FAE1 CGGTAAAACCGGGTGTGTTTGTCCAAGGACATAACCGGTGTTGCCGGGAGAACTGTTCA
Ta_fael_mut1 CGGTAAAACCGGGTGTGTTTGTCCAAGGACATAACCGGTGTTGCCGGGAGAACTGTTCA

Ta_FAE1 GAAAAACATAACAACATTGGGTCCGTTGGTTCTTCCTTTTAGCGAGAAATTTCTTTTTT
Ta_fael_mut1 GAAAAACATAACAACATTGGGTCCGTTGGTTCTTCCTTTTAGCGAGAAATTTCTTTTTT

Ta_FAE1 CGTTACCTTCATCGCCAAGAACTCTTTAAAGACAAGATCAAACATTACTACGTCCTCCGGA
Ta_fael_mut1 CGTTACCTTCATCGCCAAGAACTCTTTAAAGACAAGATCAAACATTACTACGTCCTCCGGA

Ta_FAE1 TTTCAAGCTTGCTATCGACCATTTTGTATTTCATGCCGGAGGCAGAGCCGTGATCGATGT
Ta_fael_mut1 TTTCAAGCTTGCTATCGACCATTTTGTATTTCATGCCGGAGGCAGAGCCGTGATCGATGT

Ta_FAE1 GCTACAGAAGAACTTAGGTCTATTGCCGATCGATGTGGAGGCATCTAGGTCAACGTTACA
Ta_fael_mut1 GCTACAGAAGAACTTAGGTCTATTGCCGATCGATGTGGAGGCATCTAGGTCAACGTTACA

Ta_FAE1 TAGATTTGGGAACACTTCGTCTAGCTCAATTTGGTATGAATTGGCGTACATAGAGGCAAA
Ta_fael_mut1 TAGATTTGGGAACACTTCGTCTAGCTCAATTTGGTATGAATTGGCGTACATAGAGGCAAA

Ta_FAE1 AGGAAGGATGAAGAGAGGGAACAAAGTTTGGCAGATTGCTTTAGGGTCAGGGTTAAGTG
Ta_fael_mut1 AGGAAGGATGAAGAGAGGGAACAAAGTTTGGCAGATTGCTTTAGGGTCAGGGTTAAGTG

Ta_FAE1 TAATAGTGCGGTTTGGGTGGCTCTACGCAATGTCAAGGCTTCGACAAATAGTCCTTGGGA
Ta_fael_mut1 TAATAGTGCGGTTTGGGTGGCTCTACGCAATGTCAAGGCTTCGACAAATAGTCCTTGGGA

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*****
Ta_FAE1      ACATTGCATTGATAGATATCCAGATGCAATTGATTCTGATTCCGGGTAAGTCAGAGACTCG
Ta_fae1_mut1 ACATTGCATTGATAGATATCCAGATGCAATTGATTCTGATTCCGGGTAAGTCAGAGACTCG
*****

Ta_FAE1      TGTCCAAAACGGTCGGTCCTAA
Ta_fae1_mut1 TGTCCAAAACGGTCGGTCCTAA
*****

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TT8

The top lines of the following sequence comparison are the unmodified sequence of *TT8*. Two representative modified gene sequences of *TT8* are included for comparison. One site in the gene is edited; one modified sequence includes a single G deletion (B3 WG.2) and one modified sequence includes a single G insertion (B3 WG.1). The edits are highlighted in red.

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Ta_TT8_Wt      ATGGATGAATCAAGTATTTTTACGGCAGAGAAAGTGATCGGAGCTGAGAAAAGAGAGCTT
Ta_TT8_Mut2    ATGGATGAATCAAGTATTTTTACGGCAGAGAAAGTGATCGGAGCTGAGAAAAGAGAGCTT
Ta_TT8_Mut1    ATGGATGAATCAAGTATTTTTACGGCAGAGAAAGTGATCGGAGCTGAGAAAAGAGAGCTT
*****

Ta_TT8_Wt      CAAGGGCTGCTTAAGGCGGCGGTGCAATCTGTGGAGTGGACTTATAGTCTCTTCTGGCAA
Ta_TT8_Mut2    CAAGGGCTGCTTAAGGCGGCGGTGCAATCTGTGGAGTGGACTTATAGTCTCTTCTGGCAA
Ta_TT8_Mut1    CAAGGGCTGCTTAAGGCGGCGGTGCAATCTGTGGAGTGGACTTATAGTCTCTTCTGGCAA
*****

Ta_TT8_Wt      CTTTGTCTCAACAAAGGTTCTTTTTTTTTTTTTAATAAATTTTCATCGATCTCTCACAAATA
Ta_TT8_Mut2    CTTTGTCTCAACAAAGGTTCTTTTTTTTTTTTTAATAAATTTTCATCGATCTCTCACAAATA
Ta_TT8_Mut1    CTTTGTCTCAACAAAGGTTCTTTTTTTTTTTTTAATAAATTTTCATCGATCTCTCACAAATA
*****

Ta_TT8_Wt      AAAACCCTAAATTTTATATCATTTATTATTATATGTTAACTACATAAATTATCAGTATTT
Ta_TT8_Mut2    AAAACCCTAAATTTTATATCATTTATTATTATATGTTAACTACATAAATTATCAGTATTT
Ta_TT8_Mut1    AAAACCCTAAATTTTATATCATTTATTATTATATGTTAACTACATAAATTATCAGTATTT
*****

Ta_TT8_Wt      TAACCGTCCATGTGCTTTATTTGGTTCCATTTCTGTCTCATATTTTACTTGAGGTTTCAGA
Ta_TT8_Mut2    TAACCGTCCATGTGCTTTATTTGGTTCCATTTCTGTCTCATATTTTACTTGAGGTTTCAGA
Ta_TT8_Mut1    TAACCGTCCATGTGCTTTATTTGGTTCCATTTCTGTCTCATATTTTACTTGAGGTTTCAGA
*****

Ta_TT8_Wt      CTGCCGAGCACATCTCTCGTTTGTCTCGAATCTGTGAGACTTTTTTCGTTTATTGGCACTT
Ta_TT8_Mut2    CTGCCGAGCACATCTCTCGTTTGTCTCGAATCTGTGAGACTTTTTTCGTTTATTGGCACTT
Ta_TT8_Mut1    CTGCCGAGCACATCTCTCGTTTGTCTCGAATCTGTGAGACTTTTTTCGTTTATTGGCACTT
*****

Ta_TT8_Wt      CTGTGTCAATTGAGTTATACTGAAGTAATTATATGTTTAAATGAATTAGGGTTTTGCTGT
Ta_TT8_Mut2    CTGTGTCAATTGAGTTATACTGAAGTAATTATATGTTTAAATGAATTAGGGTTTTGCTGT
Ta_TT8_Mut1    CTGTGTCAATTGAGTTATACTGAAGTAATTATATGTTTAAATGAATTAGGGTTTTGCTGT
*****

Ta_TT8_Wt      GGGAGAATGGATACTACAACGGTGAATAAAG-ACGAGGAAGACAACACTCAGCCGGCGGAA
Ta_TT8_Mut2    GGGAGAATGGATACTACAACGGTGAATAAAG-ACGAGGAAGACAACACTCAGCCGGCGGAA
Ta_TT8_Mut1    GGGAGAATGGATACTACAACGGTGAATAAAG-ACGAGGAAGACAACACTCAGCCGGCGGAA
*****

Ta_TT8_Wt      GTGACGGCGGAAGAGGCTGCGTTAGAGAGGAGTCAGCAGCTAAGGGAACCTTACGAGGCC

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Ta_TT8_Wt TAATTTTCTGTCTTTATTTTCTTGGATGCTCCTTCTACATCGGTCGGTTCCTTAATGGTT
Ta_TT8_Mut2 TAATTTTCTGTCTTTATTTTCTTGGATGCTCCTTCTACATCGGTCGGTTCCTTAATGGTT
Ta_TT8_Mut1 TAATTTTCTGTCTTTATTTTCTTGGATGCTCCTTCTACATCGGTCGGTTCCTTAATGGTT

Ta_TT8_Wt AGAGTGCCAAAATCCAGGTAAACGTTGTCTTTATTGATTAATTTCTAATTTTGAGTAATAT
Ta_TT8_Mut2 AGAGTGCCAAAATCCAGGTAAACGTTGTCTTTATTGATTAATTTCTAATTTTGAGTAATAT
Ta_TT8_Mut1 AGAGTGCCAAAATCCAGGTAAACGTTGTCTTTATTGATTAATTTCTAATTTTGAGTAATAT

Ta_TT8_Wt TTTACATTTATTTTACATGTTTGAAAATTGTTTTGTGATAAAAAAAAAAGCAGACAGTGGT
Ta_TT8_Mut2 TTTACATTTATTTTACATGTTTGAAAATTGTTTTGTGATAAAAAAAAAAGCAGACAGTGGT
Ta_TT8_Mut1 TTTACATTTATTTTACATGTTTGAAAATTGTTTTGTGATAAAAAAAAAAGCAGACAGTGGT

Ta_TT8_Wt TTGCATTCCCATGCTTGATGGCGTTGTGGAAC TAGGCACAACGAACAAGGTACGGCGTAG
Ta_TT8_Mut2 TTGCATTCCCATGCTTGATGGCGTTGTGGAAC TAGGCACAACGAACAAGGTACGGCGTAG
Ta_TT8_Mut1 TTGCATTCCCATGCTTGATGGCGTTGTGGAAC TAGGCACAACGAACAAGGTACGGCGTAG

Ta_TT8_Wt TTATCTTTTTATATATGCATAACCAAATGGTTAAGAAAAGGTTAGAAGAGAAATAGATC
Ta_TT8_Mut2 TTATCTTTTTATATATGCATAACCAAATGGTTAAGAAAAGGTTAGAAGAGAAATAGATC
Ta_TT8_Mut1 TTATCTTTTTATATATGCATAACCAAATGGTTAAGAAAAGGTTAGAAGAGAAATAGATC

Ta_TT8_Wt ATGCTTAAGTTTTATCAGTTAAATTA AAAATGTA AAAATAAGATATTATGTTTCATTAATA
Ta_TT8_Mut2 ATGCTTAAGTTTTATCAGTTAAATTA AAAATGTA AAAATAAGATATTATGTTTCATTAATA
Ta_TT8_Mut1 ATGCTTAAGTTTTATCAGTTAAATTA AAAATGTA AAAATAAGATATTATGTTTCATTAATA

Ta_TT8_Wt ATGTATAGTCCCTGTAGTTAAAAAAAAGAATAAAAATATTTAACCATTTGAAGTCATAAT
Ta_TT8_Mut2 ATGTATAGTCCCTGTAGTTAAAAAAAAGAATAAAAATATTTAACCATTTGAAGTCATAAT
Ta_TT8_Mut1 ATGTATAGTCCCTGTAGTTAAAAAAAAGAATAAAAATATTTAACCATTTGAAGTCATAAT

Ta_TT8_Wt ATAAAAATATTGTTTTTGGAGATAGTACATAATTCTCACAATAAAAAAATAACAAAGGG
Ta_TT8_Mut2 ATAAAAATATTGTTTTTGGAGATAGTACATAATTCTCACAATAAAAAAATAACAAAGGG
Ta_TT8_Mut1 ATAAAAATATTGTTTTTGGAGATAGTACATAATTCTCACAATAAAAAAATAACAAAGGG

Ta_TT8_Wt ATGATTAAGGGAAGGAGTTGGATACATGTTGTTTGTCTGTGTGTGTGAAGGTA AAAAGAAAG
Ta_TT8_Mut2 ATGATTAAGGGAAGGAGTTGGATACATGTTGTTTGTCTGTGTGTGTGAAGGTA AAAAGAAAG
Ta_TT8_Mut1 ATGATTAAGGGAAGGAGTTGGATACATGTTGTTTGTCTGTGTGTGTGAAGGTA AAAAGAAAG

Ta_TT8_Wt ATATAGCGTTTGTGAGCTCATAAAGAGTTTTTTCCATAACCACCCCAAGTCAAACCCAA
Ta_TT8_Mut2 ATATAGCGTTTGTGAGCTCATAAAGAGTTTTTTCCATAACCACCCCAAGTCAAACCCAA
Ta_TT8_Mut1 ATATAGCGTTTGTGAGCTCATAAAGAGTTTTTTCCATAACCACCCCAAGTCAAACCCAA

Ta_TT8_Wt AAGCTGCTCTTTCGAACTCCATCAACGAAGAGCACGAAGAAGACGAAGAACAAGAAG
Ta_TT8_Mut2 AAGCTGCTCTTTCGAACTCCATCAACGAAGAGCACGAAGAAGACGAAGAACAAGAAG
Ta_TT8_Mut1 AAGCTGCTCTTTCGAACTCCATCAACGAAGAGCACGAAGAAGACGAAGAACAAGAAG

Ta_TT8_Wt AAGAAGAAGAAGAAGTAGAAGAAGAAATGACAATGTCAGAGGAGATAAGGCTTGGCT
Ta_TT8_Mut2 AAGAAGAAGAAGAAGTAGAAGAAGAAATGACAATGTCAGAGGAGATAAGGCTTGGCT
Ta_TT8_Mut1 AAGAAGAAGAAGAAGTAGAAGAAGAAATGACAATGTCAGAGGAGATAAGGCTTGGCT

Ta_TT8_Wt CTCCTGATGATGATGACGTCTCCAATCAA AACCTACTCTCTGATTTCCATGTAGAATCAA
Ta_TT8_Mut2 CTCCTGATGATGATGACGTCTCCAATCAA AACCTACTCTCTGATTTCCATGTAGAATCAA

Ta_TT8_Mut1 CTCCTGATGATGATGACGTCTCCAATCAAACCTACTCTCTGATTTCATGTAGAATCAA

Ta_TT8_Wt CCCACACTTTAGGTATACACTTATACATTAATTAGTTAACGATATCATTACACGTATCT
Ta_TT8_Mut2 CCCACACTTTAGGTATACACTTATACATTAATTAGTTAACGATATCATTACACGTATCT
Ta_TT8_Mut1 CCCACACTTTAGGTATACACTTATACATTAATTAGTTAACGATATCATTACACGTATCT

Ta_TT8_Wt ATTTATTTTGTTAACAAGAAATTTAAAAATATTCGCCATTTCTTTGTTATGTCTAAAGAA
Ta_TT8_Mut2 ATTTATTTTGTTAACAAGAAATTTAAAAATATTCGCCATTTCTTTGTTATGTCTAAAGAA
Ta_TT8_Mut1 ATTTATTTTGTTAACAAGAAATTTAAAAATATTCGCCATTTCTTTGTTATGTCTAAAGAA

Ta_TT8_Wt AATCTATAAAATTTTATGAATAGACACACACATGGACATGATGAATCTAATGGAGGAGGG
Ta_TT8_Mut2 AATCTATAAAATTTTATGAATAGACACACACATGGACATGATGAATCTAATGGAGGAGGG
Ta_TT8_Mut1 AATCTATAAAATTTTATGAATAGACACACACATGGACATGATGAATCTAATGGAGGAGGG

Ta_TT8_Wt TGGAACTATTCTCAGACAGTATCAACACTTCTTATGTCACAACCCACGAGTCTTTTTTC
Ta_TT8_Mut2 TGGAACTATTCTCAGACAGTATCAACACTTCTTATGTCACAACCCACGAGTCTTTTTTC
Ta_TT8_Mut1 TGGAACTATTCTCAGACAGTATCAACACTTCTTATGTCACAACCCACGAGTCTTTTTTC

Ta_TT8_Wt AGATTCAGTTTCCACATCTTCTTACATCCAATCATCATTTGCCACATGGAAGGCTGATAA
Ta_TT8_Mut2 AGATTCAGTTTCCACATCTTCTTACATCCAATCATCATTTGCCACATGGAAGGCTGATAA
Ta_TT8_Mut1 AGATTCAGTTTCCACATCTTCTTACATCCAATCATCATTTGCCACATGGAAGGCTGATAA

Ta_TT8_Wt TTTTAAAGAGCATCAGCGAGTGGAACTAAATCGACGTCGTCGTCGCAATGGATGCTCAA
Ta_TT8_Mut2 TTTTAAAGAGCATCAGCGAGTGGAACTAAATCGACGTCGTCGTCGCAATGGATGCTCAA
Ta_TT8_Mut1 TTTTAAAGAGCATCAGCGAGTGGAACTAAATCGACGTCGTCGTCGCAATGGATGCTCAA

Ta_TT8_Wt ACACATAATCTTGAGAGTTCCCTTTACTCCACGACCACACTAAAGAAAAGAGGCTGCCTCG
Ta_TT8_Mut2 ACACATAATCTTGAGAGTTCCCTTTACTCCACGACCACACTAAAGAAAAGAGGCTGCCTCG
Ta_TT8_Mut1 ACACATAATCTTGAGAGTTCCCTTTACTCCACGACCACACTAAAGAAAAGAGGCTGCCTCG

Ta_TT8_Wt AGAAGAGCTTAATCACGTGGTGGCAGAGCGCCGAGGAGAGAGAAGCTGAATGAGAGATT
Ta_TT8_Mut2 AGAAGAGCTTAATCACGTGGTGGCAGAGCGCCGAGGAGAGAGAAGCTGAATGAGAGATT
Ta_TT8_Mut1 AGAAGAGCTTAATCACGTGGTGGCAGAGCGCCGAGGAGAGAGAAGCTGAATGAGAGATT

Ta_TT8_Wt CATAACACTGAGATCATTGGTTCCCTTTGTGACCAAGATGGATAAAGTCTCAATTCTTGG
Ta_TT8_Mut2 CATAACACTGAGATCATTGGTTCCCTTTGTGACCAAGATGGATAAAGTCTCAATTCTTGG
Ta_TT8_Mut1 CATAACACTGAGATCATTGGTTCCCTTTGTGACCAAGATGGATAAAGTCTCAATTCTTGG

Ta_TT8_Wt AGACACCATCAACTACGTAAACCATCTTCGAAATAGGGTCCAAGAGCTGGAGACTAATCA
Ta_TT8_Mut2 AGACACCATCAACTACGTAAACCATCTTCGAAATAGGGTCCAAGAGCTGGAGACTAATCA
Ta_TT8_Mut1 AGACACCATCAACTACGTAAACCATCTTCGAAATAGGGTCCAAGAGCTGGAGACTAATCA

Ta_TT8_Wt TCACGAACAAAAACATAAGCGGATGCGTAGCTGTAAGGGAAAAACGTGGGAAGAGGTCGT
Ta_TT8_Mut2 TCACGAACAAAAACATAAGCGGATGCGTAGCTGTAAGGGAAAAACGTGGGAAGAGGTCGT
Ta_TT8_Mut1 TCACGAACAAAAACATAAGCGGATGCGTAGCTGTAAGGGAAAAACGTGGGAAGAGGTCGT

Ta_TT8_Wt TGAGGTTTCCATCATAGAGAGTGTATGTTTTGTTAGAGATGAGATGCGAGTACCGAGATGG
Ta_TT8_Mut2 TGAGGTTTCCATCATAGAGAGTGTATGTTTTGTTAGAGATGAGATGCGAGTACCGAGATGG
Ta_TT8_Mut1 TGAGGTTTCCATCATAGAGAGTGTATGTTTTGTTAGAGATGAGATGCGAGTACCGAGATGG

Ta_TT8_Wt TCTATTGCTCGACATCCTTCAGGTTCTTAAGGAACATGGTATAGAGACTACTGCAGTTCA
Ta_TT8_Mut2 TCTATTGCTCGACATCCTTCAGGTTCTTAAGGAACATGGTATAGAGACTACTGCAGTTCA
Ta_TT8_Mut1 TCTATTGCTCGACATCCTTCAGGTTCTTAAGGAACATGGTATAGAGACTACTGCAGTTCA

Ta_TT8_Wt TACCGCGGTGAACGAGCGTGATTTTCGAGGCCGAGATAAGGGCTATGGTGAGAGGGAAGAA
Ta_TT8_Mut2 TACCGCGGTGAACGAGCGTGATTTTCGAGGCCGAGATAAGGGCTATGGTGAGAGGGAAGAA
Ta_TT8_Mut1 TACCGCGGTGAACGAGCGTGATTTTCGAGGCCGAGATAAGGGCTATGGTGAGAGGGAAGAA

Ta_TT8_Wt ACCAAGCATTGCTGAGGTCAAAGAGCCATCCATCAAACATATCCAATATTTAACTATA
Ta_TT8_Mut2 ACCAAGCATTGCTGAGGTCAAAGAGCCATCCATCAAACATATCCAATATTTAACTATA
Ta_TT8_Mut1 ACCAAGCATTGCTGAGGTCAAAGAGCCATCCATCAAACATATCCAATATTTAACTATA

Ta_TT8_Wt G
Ta_TT8_Mut2 G
Ta_TT8_Mut1 G