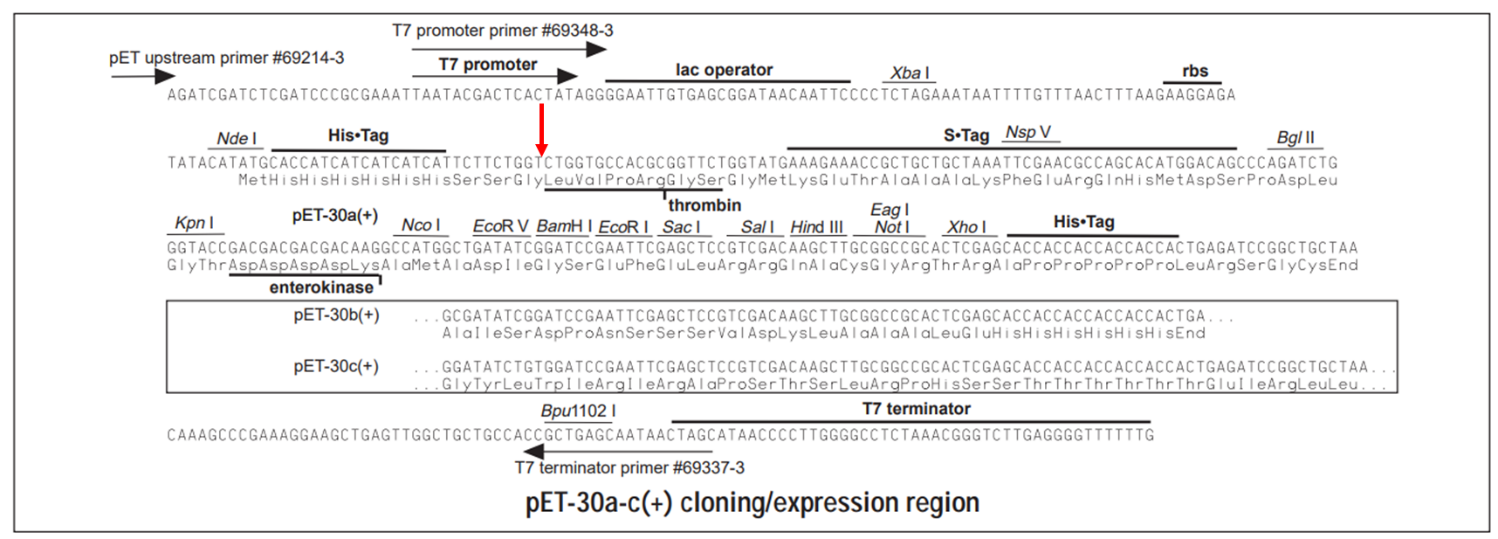
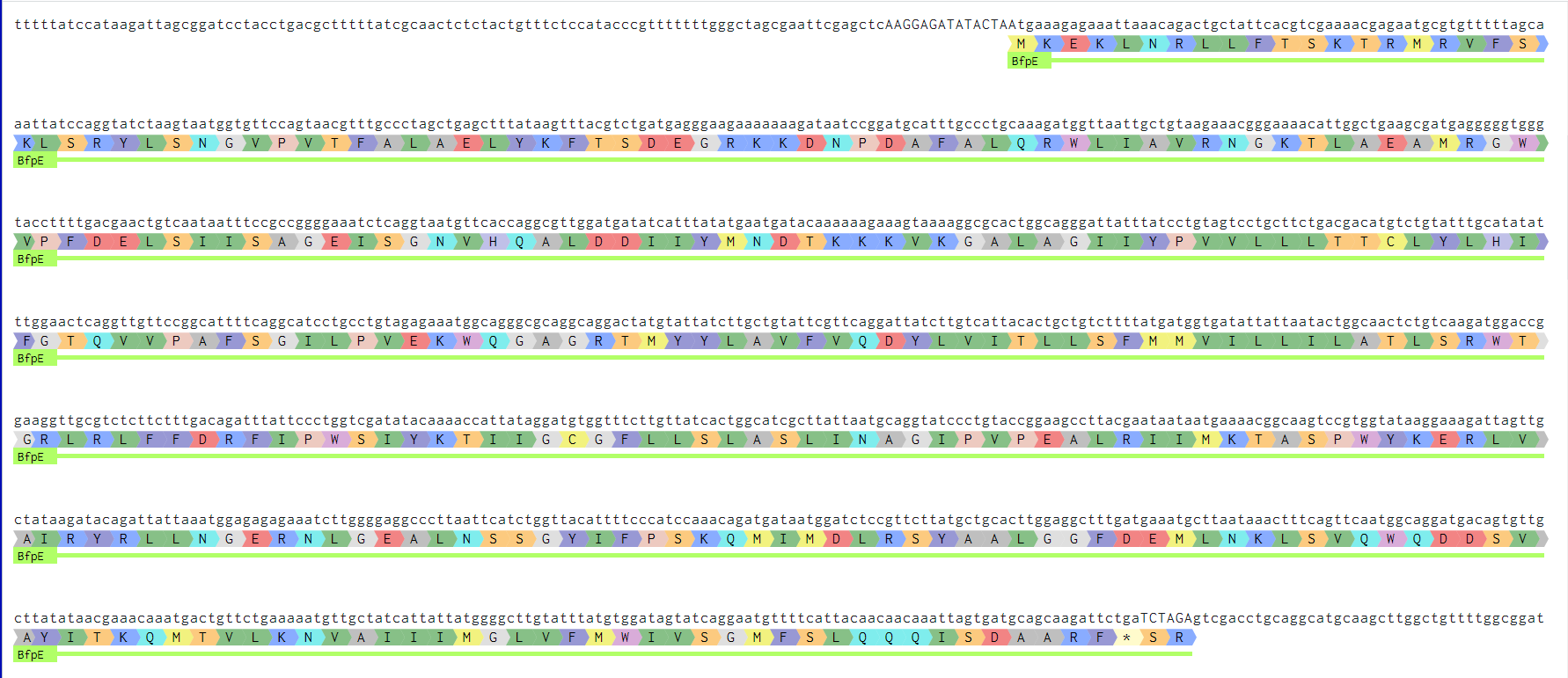
**Class 1: fast cloning to create a chimera using a vector DNA and an insert sequence.**

**a)**



b)



**Fig S1: a)** Example vector map. Red arrow indicates insertion site, just after glycine. b) Example sequence of insert gene, here BfpE highlighted in green, M to \*.

Vector part 1 sequence (>40bp): sequences from left side of insertion point.

AGATCGATCTCGATCCCGCGAAATTAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATTCCCCTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATATACATATGCACCATCATCATCATCATTCTTCTGGT

Vector part 2 sequence (>40bp): sequences from right side of insertion point.

CTGGTGCCACGCGGTTCTGGTATGAAAGAAACCGCTGCTGCTAAATTCGAACGCCAGCACATGGACAGCCCAGATCTGGGTACCGACGACGACGACAAGGCCATGGCTGATATCGGATCCGAATTCGAGCTCCGTCGACAAGCTTGCGGC

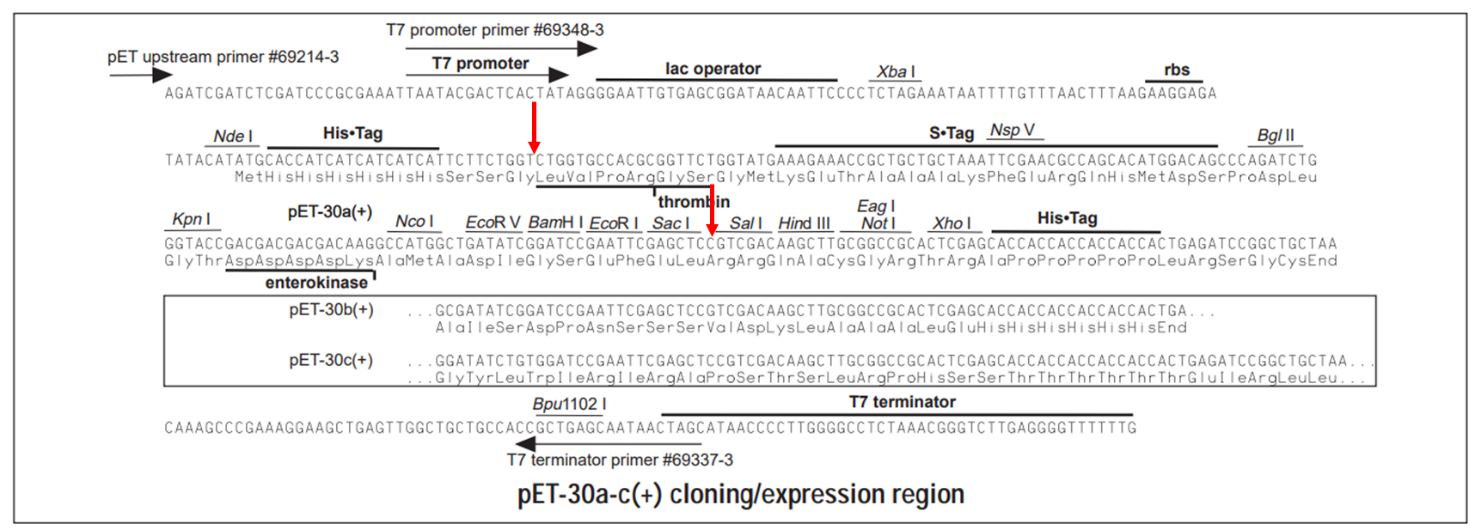
Insert DNA sequence:

atgaaagagaaattaaacagactgctattcacgtcgaaaacgagaatgcgtgtttttagcaaattatccaggtatctaagtaatggtgttccagtaacgtttgccctagctgagctttataagtttacgtctgatgagggaagaaaaaaagataatccggatgcatttgccctgcaaagatggttaattgctgtaagaaacgggaaaacattggctgaagcgatgagggggtgggtaccttttgacgaactgtcaataatttccgccggggaaatctcaggtaatgttcaccaggcgttggatgatatcatttatatgaatgatacaaaaaagaaagtaaaaggcgcactggcagggattatttatcctgtagtcctgcttctgacgacatgtctgtatttgcatatatttggaactcaggttgttccggcattttcaggcatcctgcctgtagagaaatggcagggcgcaggcaggactatgtattatcttgctgtattcgttcaggattatcttgtcattacactgctgtcttttatgatggtgatattattaatactggcaactctgtcaagatggaccggaaggttgcgtctcttctttgacagatttattccctggtcgatatacaaaaccattataggatgtggtttcttgttatcactggcatcgcttattaatgcaggtatccctgtaccggaagccttacgaataataatgaaaacggcaagtccgtggtataaggaaagattagttgctataagatacagattattaaatggagagagaaatcttggggaggcccttaattcatctggttacattttcccatccaaacagatgataatggatctccgttcttatgctgcacttggaggctttgatgaaatgcttaataaactttcagttcaatggcaggatgacagtgttgcttatataacgaaacaaatgactgttctgaaaaatgttgctatcattattatggggcttgtatttatgtggatagtatcaggaatgttttcattacaacaacaaattagtgatgcagcaagattctga

Organize input sequence for primer designing as; Vector part 1+ Insert + Vector part 2:

AGATCGATCTCGATCCCGCGAAATTAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATTCCCCTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATATACATATGCACCATCATCATCATCATTCTTCTGGT **+** atgaaagagaaattaaacagactgctattcacgtcgaaaacgagaatgcgtgtttttagcaaattatccaggtatctaagtaatggtgttccagtaacgtttgccctagctgagctttataagtttacgtctgatgagggaagaaaaaaagataatccggatgcatttgccctgcaaagatggttaattgctgtaagaaacgggaaaacattggctgaagcgatgagggggtgggtaccttttgacgaactgtcaataatttccgccggggaaatctcaggtaatgttcaccaggcgttggatgatatcatttatatgaatgatacaaaaaagaaagtaaaaggcgcactggcagggattatttatcctgtagtcctgcttctgacgacatgtctgtatttgcatatatttggaactcaggttgttccggcattttcaggcatcctgcctgtagagaaatggcagggcgcaggcaggactatgtattatcttgctgtattcgttcaggattatcttgtcattacactgctgtcttttatgatggtgatattattaatactggcaactctgtcaagatggaccggaaggttgcgtctcttctttgacagatttattccctggtcgatatacaaaaccattataggatgtggtttcttgttatcactggcatcgcttattaatgcaggtatccctgtaccggaagccttacgaataataatgaaaacggcaagtccgtggtataaggaaagattagttgctataagatacagattattaaatggagagagaaatcttggggaggcccttaattcatctggttacattttcccatccaaacagatgataatggatctccgttcttatgctgcacttggaggctttgatgaaatgcttaataaactttcagttcaatggcaggatgacagtgttgcttatataacgaaacaaatgactgttctgaaaaatgttgctatcattattatggggcttgtatttatgtggatagtatcaggaatgttttcattacaacaacaaattagtgatgcagcaagattctga **+** CTGGTGCCACGCGGTTCTGGTATGAAAGAAACCGCTGCTGCTAAATTCGAACGCCAGCACATGGACAGCCCAGATCTGGGTACCGACGACGACGACAAGGCCATGGCTGATATCGGATCCGAATTCGAGCTCCGTCGACAAGCTTGCGGC

**Class 1: fast cloning to create a chimera using a vector DNA and an insert sequence after deleting a certain region from the vector DNA.**

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**Fig S2.** Example to insertion by deleting some region of vector. Here, we want to insert the same BfpE at pointed arrow to keep the His tag and end he sequence by *Sal*I restriction site.

Vector part 1 sequence (>40bp): sequences from left side of the first insertion point.

AGATCGATCTCGATCCCGCGAAATTAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATTCCCCTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATATACATATGCACCATCATCATCATCATTCTTCTGGT

Vector part 2 sequence (>40bp): sequences from right side of the second insertion point.

GTCGACAAGCTTGCGGCCGCACTCGAGCACCACCACCACCACCACTGAGATCCGGCTGCTAA

**Insert DNA: Same as above.**

Organize input sequence for primer designing as; Vector part 1+ Insert + Vector part 2:

AGATCGATCTCGATCCCGCGAAATTAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATTCCCCTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATATACATATGCACCATCATCATCATCATTCTTCTGGT **+** atgaaagagaaattaaacagactgctattcacgtcgaaaacgagaatgcgtgtttttagcaaattatccaggtatctaagtaatggtgttccagtaacgtttgccctagctgagctttataagtttacgtctgatgagggaagaaaaaaagataatccggatgcatttgccctgcaaagatggttaattgctgtaagaaacgggaaaacattggctgaagcgatgagggggtgggtaccttttgacgaactgtcaataatttccgccggggaaatctcaggtaatgttcaccaggcgttggatgatatcatttatatgaatgatacaaaaaagaaagtaaaaggcgcactggcagggattatttatcctgtagtcctgcttctgacgacatgtctgtatttgcatatatttggaactcaggttgttccggcattttcaggcatcctgcctgtagagaaatggcagggcgcaggcaggactatgtattatcttgctgtattcgttcaggattatcttgtcattacactgctgtcttttatgatggtgatattattaatactggcaactctgtcaagatggaccggaaggttgcgtctcttctttgacagatttattccctggtcgatatacaaaaccattataggatgtggtttcttgttatcactggcatcgcttattaatgcaggtatccctgtaccggaagccttacgaataataatgaaaacggcaagtccgtggtataaggaaagattagttgctataagatacagattattaaatggagagagaaatcttggggaggcccttaattcatctggttacattttcccatccaaacagatgataatggatctccgttcttatgctgcacttggaggctttgatgaaatgcttaataaactttcagttcaatggcaggatgacagtgttgcttatataacgaaacaaatgactgttctgaaaaatgttgctatcattattatggggcttgtatttatgtggatagtatcaggaatgttttcattacaacaacaaattagtgatgcagcaagattctga **+** GTCGACAAGCTTGCGGCCGCACTCGAGCACCACCACCACCACCACTGAGATCCGGCTGCTAA

**Class 2: Fast cloning to create a deletion, insertion, or combination of the two.**

1. **Deletion.**



Fig 3S. I this example we want to delete the gray highlighted DNA sequence which is a transmembrane domain.

Input sequence format for this deletion will be:

ATGAAAGAGAAATTAAACAGACTGCTATTCACGTCGAAAACGAGAATGCGTGTTTTTAGCAAATTATCCAGGTATCTAAGTAATGGTGTTCCAGTAACGTTTGCCCTAGCTGAGCTTTATAAGTTTACGTCTGATGAGGGAAGAAAAAAAGATAATCCGGATGCATTTGCCCTGCAAAGATGGTTAATTGCTGTAAGAAACGGGAAAACATTGGCTGAAGCGATGAGGGGGTGGGTACCTTTTGACGAACTGTCAATAATTTCCGCCGGGGAAATCTCAGGTAATGTTCACCAGGCGTTGGATGATATCATTTATATGAATGATACAAAAAAGAAAGTAAAAGGC \*

GCACTGGCAGGGATTATTTATCCTGTAGTCCTGCTTCTGACGACATGTCTGTATTTGCATATATTTGGAACTCAGGTTGTTCCGGCATTTTCAGGCATCCTGCCTGTAGAGAAATGGCAGGGCGCAGGCAGGACTATGTATTATCTTGCTGTATTCGTTCAGGATTATCTTGTCATTACACTGCTGTCTTTTATGATGGTG \*

ATATTATTAATACTGGCAACTCTGTCAAGATGGACCGGAAGGTTGCGTCTCTTCTTTGACAGATTTATTCCCTGGTCGATATACAAAACCATTATAGGATGTGGTTTCTTGTTATCACTGGCATCGCTTATTAATGCAGGTATCCCTGTACCGGAAGCCTTACGAATAATAATGAAAACGGCAAGTCCGTGGTATAAGGAAAGATTAGTTGCTATAAGATACAGATTATTAAATGGAGAGAGAAATCTTGGGGAGGCCCTTAATTCATCTGGTTACATTTTCCCATCCAAACAGATGATAATGGATCTCCGTTCTTATGCTGCACTTGGAGGCTTTGATGAAATGCTTAATAAACTTTCAGTTCAATGGCAGGATGACAGTGTTGCTTATATAACGAAACAAATGACTGTTCTGAAAAATGTTGCTATCATTATTATGGGGCTTGTATTTATGTGGATAGTATCAGGAATGTTTTCATTACAACAACAAATTAGTGATGCAGCAAGATTCTGA \*

The gray is deletion sequence marked my \* symbol. The end of sequence also mut get marked by \* symbol. Note: FastCloneAssist take care of gaps by itself.

1. **Insertion of a sequence**



Fig 4S: Let assume we want to insert a small fluorophore tag (FLAsH tag) at the red arrow just after Gly.

The input sequence format for this class 2 primer designing will be.

ATGAAAGAGAAATTAAACAGACTGCTATTCACGTCGAAAACGAGAATGCGTGTTTTTAGCAAATTATCCAGGTATCTAAGTAATGGTGTTCCAGTAACGTTTGCCCTAGCTGAGCTTTATAAGTTTACGTCTGATGAGGGAAGAAAAAAAGATAATCCGGATGCATTTGCCCTGCAAAGATGGTTAATTGCTGTAAGAAACGGGAAAACATTGGCTGAAGCGATGAGGGGGTGGGTACCTTTTGACGAACTGTCAATAATTTCCGCCGGGGAAATCTCAGGTAATGTTCACCAGGCGTTGGATGATATCATTTATATGAATGATACAAAAAAGAAAGTAAAAGGC\*\*ATATTATTAATACTGGCAACTCTGTCAAGATGGACCGGAAGGTTGCGTCTCTTCTTTGACAGATTTATTCCCTGGTCGATATACAAAACCATTATAGGATGTGGTTTCTTGTTATCACTGGCATCGCTTATTAATGCAGGTATCCCTGTACCGGAAGCCTTACGAATAATAATGAAAACGGCAAGTCCGTGGTATAAGGAAAGATTAGTTGCTATAAGATACAGATTATTAAATGGAGAGAGAAATCTTGGGGAGGCCCTTAATTCATCTGGTTACATTTTCCCATCCAAACAGATGATAATGGATCTCCGTTCTTATGCTGCACTTGGAGGCTTTGATGAAATGCTTAATAAACTTTCAGTTCAATGGCAGGATGACAGTGTTGCTTATATAACGAAACAAATGACTGTTCTGAAAAATGTTGCTATCATTATTATGGGGCTTGTATTTATGTGGATAGTATCAGGAATGTTTTCATTACAACAACAAATTAGTGATGCAGCAAGATTCTGA\* TGTTGCCCGGGCTGCTGT

Note: The gray highlighted sequence is DNA sequence for FLAsH-tag (CCPGCC).

1. **Deletion and Insertion of a sequence**

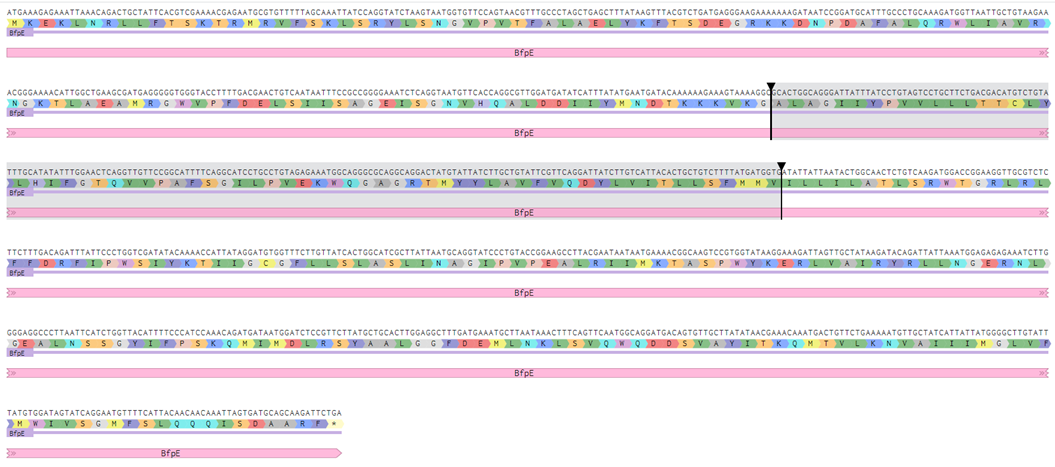


Fig S5: Lets assume here we want to delete the gray highlighted sequence and insert a FLAsH tag sequence there. The input sequence format will be.

ATGAAAGAGAAATTAAACAGACTGCTATTCACGTCGAAAACGAGAATGCGTGTTTTTAGCAAATTATCCAGGTATCTAAGTAATGGTGTTCCAGTAACGTTTGCCCTAGCTGAGCTTTATAAGTTTACGTCTGATGAGGGAAGAAAAAAAGATAATCCGGATGCATTTGCCCTGCAAAGATGGTTAATTGCTGTAAGAAACGGGAAAACATTGGCTGAAGCGATGAGGGGGTGGGTACCTTTTGACGAACTGTCAATAATTTCCGCCGGGGAAATCTCAGGTAATGTTCACCAGGCGTTGGATGATATCATTTATATGAATGATACAAAAAAGAAAGTAAAAGGC \*

GCACTGGCAGGGATTATTTATCCTGTAGTCCTGCTTCTGACGACATGTCTGTATTTGCATATATTTGGAACTCAGGTTGTTCCGGCATTTTCAGGCATCCTGCCTGTAGAGAAATGGCAGGGCGCAGGCAGGACTATGTATTATCTTGCTGTATTCGTTCAGGATTATCTTGTCATTACACTGCTGTCTTTTATGATGGTG \*

ATATTATTAATACTGGCAACTCTGTCAAGATGGACCGGAAGGTTGCGTCTCTTCTTTGACAGATTTATTCCCTGGTCGATATACAAAACCATTATAGGATGTGGTTTCTTGTTATCACTGGCATCGCTTATTAATGCAGGTATCCCTGTACCGGAAGCCTTACGAATAATAATGAAAACGGCAAGTCCGTGGTATAAGGAAAGATTAGTTGCTATAAGATACAGATTATTAAATGGAGAGAGAAATCTTGGGGAGGCCCTTAATTCATCTGGTTACATTTTCCCATCCAAACAGATGATAATGGATCTCCGTTCTTATGCTGCACTTGGAGGCTTTGATGAAATGCTTAATAAACTTTCAGTTCAATGGCAGGATGACAGTGTTGCTTATATAACGAAACAAATGACTGTTCTGAAAAATGTTGCTATCATTATTATGGGGCTTGTATTTATGTGGATAGTATCAGGAATGTTTTCATTACAACAACAAATTAGTGATGCAGCAAGATTCTGA \* TGTTGCCCGGGCTGCTGT