

S1 Table. Potential off-targets in the genome of *Helicoverpa zea*. Seed sequences (12 nucleotides upstream of the protospacer adjacent motif) of the target site 1 (GCTCTGATCGAG) and target site 2 (CCGTGAGTCGTG) of the TO gene were used to search genome of *H. zea* to identify potential off-targets that had three or less mismatches with the seed sequences of the targets and ten or less total mismatches. Primer pairs designed to PCR amplify and sequence off-target sequences are shown next to the selected sequences. Seed sequence region is shown in blue text and nucleotides not matching the target sequences are shown in lowercase text. PAM sequences are shown in bold black text. A lower case “c” at the end of the start position indicates an off-target sequence on the complementary strand of a scaffold. N/A: primers were not designed to these off-targets.

| gRNA target site | Genomic Scaffold # | Off-target start position | Off target sequence | PAM | Total mismatch # | Forward PCR Primer (5' to 3') | Reverse PCR primer (5' to 3') | Amplicon size (bp) |
|------------------|--------------------|---------------------------|-------------------------------|-----|------------------|-------------------------------|-------------------------------|--------------------|
| 1 | 1430 | 28347 | AgaTgaac atTCTGATCGAG | CGG | 8 | AAACCACACTTAGTTGAGAGGCA | TGCCCCAGTTAAATCTTCGA | 413 |
| 2 | 185 | 258131 | TcgctgAg tCGTGAGTCGTG | AGG | 8 | AAATTAAGTGATGCTGCCGTAAG | TGGTGAAACGGTACATTTAGC | 567 |
| 2 | 1210 | 80173 | GGaTttgt aCGTGAGTCGTG | AGG | 7 | GTGAATGTATCTGGGACGGTTC | GATGACGACGGACGAGGTCT | 1172 |
| 2 | 1570 | 50187 | GtaaaAtga CGTGAGTCGTG | AGG | 7 | TGAGTAATAACAAGGAAACCGC | ATTGGTTTAGCTTTCGGCGT | 416 |
| 1 | 522 | 165033 | AgtTAgc AGCTCTGcCtGAG | TGG | 6 | N/A | N/A | N/A |
| 1 | 1430 | 18518 | TTCTAtct GtTCTGcaCGAG | TGG | 7 | N/A | N/A | N/A |
| 2 | 40 | 528393c | TGTgaAAC CCGTGAcTgGTA | CGG | 6 | N/A | N/A | N/A |
| 2 | 40 | 548156c | Atacatt CCGTGAcTcaTa | TGG | 9 | N/A | N/A | N/A |
| 2 | 180 | 97132c | CaTcccAg gaGTGAGTCGTG | AGG | 8 | N/A | N/A | N/A |
| 2 | 185 | 19054 | TaTacAtt CtGTGAGTCcaG | CGG | 9 | N/A | N/A | N/A |
| 2 | 2020 | 29321 | AGgaaAtaa aaGTGAGTCGTG | AGG | 7 | N/A | N/A | N/A |
| 2 | 2020 | 34742c | cccTcgct CCtgGgGTCGTG | CGG | 9 | N/A | N/A | N/A |