

# Supplementary Materials: Reduced Expression of a Novel Midgut Trypsin Gene Involved in Protoxin Activation Correlates with Cry1Ac Resistance in a Laboratory-Selected Strain of *Plutella xylostella* (L.)

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| Genes               | PxTryp<br>p_SPc1 | HaTryp<br>p_SPc | OtTryp<br>p_SPc3 | DmTryp<br>p_SPc | AmTryp<br>p_SPc7 | BtTryp<br>_SPc1 | ZnTryp<br>p_SPc | SgTryp<br>p_SPc | PhcTryp<br>p_SPc2 |
|---------------------|------------------|-----------------|------------------|-----------------|------------------|-----------------|-----------------|-----------------|-------------------|
| PxTryp<br>_SPc1     | 100%             |                 |                  |                 |                  |                 |                 |                 |                   |
| HaTryp<br>_SPc      | 52%              | 100%            |                  |                 |                  |                 |                 |                 |                   |
| OtTryp<br>_SPc3     | 35%              | 28%             | 100%             |                 |                  |                 |                 |                 |                   |
| DmTryp<br>p_SPc     | 26%              | 26%             | 41%              | 100%            |                  |                 |                 |                 |                   |
| AmTryp<br>p_SPc7    | 29%              | 24%             | 30%              | 28%             | 100%             |                 |                 |                 |                   |
| BtTryp<br>_SPc1     | 26%              | 23%             | 27%              | 27%             | 29%              | 100%            |                 |                 |                   |
| ZnTryp<br>_SPc      | 32%              | 26%             | 47%              | 39%             | 33%              | 31%             | 100%            |                 |                   |
| SgTryp<br>_SPc      | 20%              | 25%             | 32%              | 25%             | 25%              | 18%             | 29%             | 100%            |                   |
| PhcTryp<br>p_SPc2   | 24%              | 20%             | 26%              | 25%             | 29%              | 21%             | 23%             | 17%             | 100%              |
| Sequence similarity |                  |                 |                  |                 |                  |                 |                 |                 |                   |
|                     | <10%             | >10%            | >20%             | >30%            | >40%             | >50%            | 100%            |                 |                   |

**Figure S1.** Pairwise comparisons of protein sequence identities among nine trypsin orthologs of PxTryp\_SPc1 from different insect species. Abbreviations: PxTryp\_SPc1 (*Plutella xylostella*, MN422356), HaTryp\_SPc1 (*Helicoverpa armigera*, ABU98624), OtTryp\_SPc3 (*Onthophagus taurus*, XP\_022900611), DmTryp\_SPc (*Drosophila melanogaster*, NP\_001285772), AmTryp\_SPc7 (*Apis mellifera*, XP\_623564), BtTryp\_SPc1 (*Bemisia tabaci*, XP\_018896298), ZnTryp\_SPc (*Zootermopsis nevadensis*, XP\_021914447), SgTryp\_SPc (*Schistocerca gregaria*, CAA70820) and PhcTryp\_SPc2 (*Pediculus humanus corporis*, AAV48634).

**Table 1.** List of primers used in this study.

| Purpose       | Primer Name | Primer Sequence (5'–3') | T <sub>m</sub> (°C) | PCR Product Size (bp) | Positions (bp) <sup>†</sup> |
|---------------|-------------|-------------------------|---------------------|-----------------------|-----------------------------|
| Gene cloning  | Try1-F      | GGTTACCTGGGCTGTGCTTC    | 65                  | 799                   | -2–766                      |
|               | Try1-R      | CTCATTCGTCGGTTTGGCTT    |                     |                       |                             |
| qPCR analysis | qTry1-F     | GAATGTCAGCACCAGCAGC     | 57                  | 135                   | 217–351                     |

|                                 |              |                              |    |     |        |
|---------------------------------|--------------|------------------------------|----|-----|--------|
| dsRNA<br>synthesis <sup>‡</sup> | qTry1-R      | CAGATGTCGGCGTCGTAG           | 57 | 120 | -      |
|                                 | qL32-F       | CCAATTTACCGCCCTACC           |    |     |        |
|                                 | qL32-R       | TACCCTGTTGTCAATACCTCT        |    |     |        |
|                                 | dsTry1-F     | GGCTGTGCTTCTTCTTACCG         | 58 | 389 | 10–399 |
|                                 | dsTry1-R     | GCTGGATGGCAGGAGTGA           |    |     |        |
|                                 | dsEGFP-F     | T7-<br>CCACAAGTTCAGCGTGTCCG  |    |     |        |
|                                 | dsEGFP-<br>R | T7-<br>AAGTTCACCTTGATGCCGTTT | 58 | 469 | -      |

<sup>†</sup>Positions corresponding to the full-length cDNA sequence (GenBank accession no. MN422356) of the *PxTryp\_Spc1* gene. <sup>‡</sup>T7 RNA polymerase promoter sequence (TAATACGACTCACTATAGGGAGA) was appended to the forward and reverse primers 5' ends to synthesize dsRNA template. The short line “-” denotes Not Available (N/A).