

## File S2

Chimeric guide RNA (cgRNA) for targeting (A) *inositol oxygenase (inox)* and (B) *phytoene desaturase (pds)* genes of wheat, and (C) *pds* gene of *Nicotiana benthamiana*. (i) cgRNA for protospacer 1, (ii) cgRNA for protospacer 2 and (iii) cgRNA for targeting both protospacer 1 and 2. Target region is given in blue font and guide RNA scaffold in red font. Restriction site SpeI (highlighted in yellow) was used for ligation of the two cgRNAs.

### (A)

#### (i)

NNNNNAGACGTACGAGTTTGTGCAGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTA  
GTCCGTTATCAACTTGAAAAAGTGGCACCAGTCGGTGCTTTTNNNNNNNN

#### (ii)

NNNNNCAAGACGGAGATGAGCATCTGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTA  
GTCCGTTATCAACTTGAAAAAGTGGCACCAGTCGGTGCTTTTNNNNNNNN

#### (iii)

NNNNNAGACGTACGAGTTTGTGCAGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTA  
GTCCGTTATCAACTTGAAAAAGTGGCACCAGTCGGTGCTTTACTAGTNNNNCAAGACGGAG  
ATGAGCATCTGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTT  
GAAAAAGTGGCACCAGTCGGTGCTTTTNNNNNNN

**(B)**

**(i)**

NNNNN**TTTGCCATGCCAAACAAACC**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTA  
GTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTNNNNNNNN

**(ii)**

NNNNN**GGCGCCCTTAAATGGAGTGT**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTA  
GTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTNNNNNNN

**(C)**

**(i)**

NNNNNGCTTTTCCCTGATGAAATTTGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTA  
GTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTNNNNNNNN

**(ii)**

NNNNNATCATGTTGTCAAACCTCCAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTA  
GTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTNNNNNNNN