

File S1

Partial sequence of (A) *inositol oxygenase (inox)* and (B) *phytoene desaturase (pds)* genes of wheat, and (C) *pds* gene of *Nicotiana benthamiana* used to decide the target for modification by CRISPR-Cas System. Targeted regions are shown in blue font and PAM in red. Primer used for amplification of gene after editing is shown in green.

(A)

Forward primer →

GCTCGACGGCGGCTTCACCGTGCCGGACTCCAACGCCTTCGGCCACACCTT **CAGGGACTACGACGCGGAG**TCGGA BASE PAIRS
CGAGCTGCCGCCGAAGTGGCACGGCCTGAGGTTGCGGAAGCCGGTGTGGAAGTCCCTGATGCTGCGCCTCAGCCT 1 TO 75

Protospacer 1

GCGGAAGAAGACGGTGGAGGAGTTCTACAGCGTGAACCACATCAACC **AGACGTACGAGTTTGTGCAG**CGGATGCG BASE PAIRS
CGCCTTCTTCTGCCACCTCCTCAAGATGTCGCACTTGGTGTAGTTGGTCTGCATGCTCAAACACGTTCGCCTACGC 76 TO 150

Protospacer 2

GGACGCATACGGGCGGCTGGA **CAAGACGGAGATGAGCATCTGGG**AGTGCATCGAGCTGCTCAACGAGTTCATCGA BASE PAIRS
CCTGCGTATGCCC GCCGACCTGTTCTGCCTCTACTCGTAGACCCTCACGTAGCTCGACGAGTTGCTCAAGTAGCT 151 TO 225

CGACAGTGACCCCGACCTGGACATGCCGCAGATCGAGCACCTCCTCCAAACCGCCGAGGCCATCCGCAAGGACTA BASE PAIRS
GCTGTCACTGGGGCTGGACCTGTACGGCGTCTAGCTCGTGGAGGAG **GTTTGGCGGCTCCGGTAGG**CGTTCCTGAT 226 TO 300

← Reverse primer

Yellow highlighted region is the site for BsgI used for digestion of amplified DNA.

(B)

Forward primer

Protospacer 1

GCTGAGCTTGGTATTAGTGATCGCTTGCAATGGAAGGAACACTCCATGATATTTGCCATGCCAAACAAACCAGGA BASE PAIRS
CGACTCGAACCATAATCACTAGCGAACGTTACCTTCCTTGTGAGGTACTATAAACGGTACGGTTTGTTTGGTCCT 1 TO 75

Protospacer 2

GAATACAGCCGTTTTGATTTTCCAGAGACTTTGCCGGCGCCCTTAAATGGAGTGTGGCCATACTGAAAAACAAT BASE PAIRS
CTTATGTCGGCAAACTAAAAGGTCTCTGAAACGGCCGCGGGAATTTACCTCACACCCGGTATGACTTTTTGTTA 76 TO 150

GAAATGCTTACTTGGCCGGAGAAGGTGAAG BASE PAIRS
CTTTACGAATGAACCGGCCTCTTCCACTTC 151 TO 180

Reverse primer

(C)

Forward primer

GAATTGGTTTTTGCACCTGCAGAAGAGTGGATAAATCGCAGTGACTCTGAAATTATTGATGCTACAATGAAGGAA BASE PAIRS
CTTAACCAAAAACGTGGACGTCTTCTCACCTATTTAGCGTCACTGAGACTTTAATAACTACGATGTTACTTCCTT 1 TO 75

Protospacer 1

Protospacer 2

CTAGCAAAGCTTTTCCCTGATGAAATTTGGCAGATCAGAGCAAAGCAAATATTGAAGTATCATGTTGTCAA BASE PAIRS
GATCGTTTCGAAAAGGGACTACTTTAAAGCCGTCTAGTCTCGTTTCGTTTTTATAACTTCATAGTACAACAGTTT 76 TO 150

ACTCCAAGTCTGTTTATAAACTGTGCCAGGTTGTGAACCCTGCCGGCCCTTGCAAAGATCCCCTATAGAGGGG BASE PAIRS
TGAGGTTCCAGACAAATATTTTGACACGGTCCAACACTTGGGACGGCCGGGAACGTTTCTAGGGGATATCTCCCC 151 TO 225

Reverse primer