

# Supplementary Materials

**Table S1.** The names and accession numbers of other strains used for comparison.

Strain	Accession number
<i>Fusarium oxysporum</i> f. sp. <i>conglutinans</i>	EGU85821.1
<i>Fusarium oxysporum</i> f. sp. <i>raphani</i>	EXL01186.1
<i>Fusarium oxysporum</i> f. sp. <i>lycopersici</i>	XP_018235573.1
<i>Fusarium oxysporum</i> f. sp. <i>vasinfectum</i>	EXM37177.1
<i>Fusarium verticillioides</i>	XP_018750404.1
<i>Fusarium albosuccineum</i>	KAF4458330.1
<i>Fusarium proliferatum</i>	KAG4260530.1
<i>Saccharomyces cerevisiae</i>	<u>DAA11457.1</u>
<i>Saccharomyces cerevisiae</i>	GFP71289.1
<i>Saccharomyces pastorianus</i>	QID88455.1
<i>Zygosaccharomyces bailii</i>	CDH16539.1
<i>Zygosaccharomyces parabailii</i>	AQZ15506.1

**Table S2.** All primers used in the study.

Primers	Sequence(5'-3')	PCR purpose
UpF	CCAGGGACACGTACTAGGGT	Amplify the left
UpR	<u>CTCCAGCCAAG</u> CGGCGATGTAACTTGAAAA	homologous arm of <i>Nto1</i>
DpR	TGAGAAGCCCTAACTACTAAAC	Amplify the right
DpF	<u>GCCGACCGGG</u> TAATGATCGTGGAATCTGAAC	homologous arm of <i>Nto1</i>
HuF	<u>TACATCGCCG</u> CTTGGCTGGAGCTAGTGGAGGT	Amplify the <i>Hyg</i> -cassettes
HdF	CGTTGCAAGACCTGCCTGAA	
HuR	ATGCCTCCGCTCGAAGTA	Amplify the <i>yg</i> -cassettes
HdR	<u>ACGATCATT</u> ACCCGGTCGGCATCTACTCTATTC	
PeF	CGACTGGGCCATCAACAGTAT	Amplify the fragment of <i>Nto1</i>
PeR	TCACCAACGGCTGCAGACT	
HL	CTTGGCTGGAGCTAGTGGAGGT	Amplify the fragment of <i>Hyg</i>
HR	CCCGGTCGGCATCTACTCTATTC	
CupF	AAAGTCCTGGGATTCTATTTATGG	Confirm whether the <i>Hyg</i> gene homologously replaced <i>Nto1</i>
CupR	GACAGACGTCGCGGTGAGTT	
CdpF	TCTGGACCGATGGCTGTGTAG	Confirm whether the <i>Hyg</i> gene homologously replaced <i>Nto1</i>
CdpR	GGGCTGCCTACACTAAATGG	
PrF	GCTGAGTGAGAGATTGAAGGGTTG	Amplify the 523-bp probe for mutants Southern blot
PrR	CCTGGTCTACTGACTGAGTTATGC	
CpF <sup>1</sup>	<u>GACTAGTC</u> ATGCACCACAAGCGTCGGAGT	Amplification <i>Nto1</i> complete sequence
CpR <sup>1</sup>	<u>AAGGGCCCT</u> TGTTCCGTCAAAGCATGGT	
NF	TAGGGCGAATTGGGTAC	Amplify the fragment of <i>Neo</i>
NR	CAGCTCACTGTTACGTC	
PcF	AACACCGCCTGAGTCATCTACG	Amplify the 485-bp probe for complemented strains Southern blot
PcR	CCTCCTTCACACCTGCCTCAAC	
IrF	CATCGGCCACGTCGACTCT	Amplify fragment from elongation factor2 gene
IrR	AGAACCCAGGCGTACTTGAA	

Underlined texts indicate overlapped regions for fusion PCR; <sup>1</sup> Underlined texts indicate introduced digest site.