

S7 Table. Primers and plasmids used for the construction of all gene deletion fragments except for gene STE3-2.

Gene or region to knockout	Backbone plasmid	Primers use to construct gene deletion fragments				Primers used for mutant confirmation						
		Primers upstream from gene (5'-3')	Amplified fragment (bp)	Primers downstream from gene (5'-3')	Amplified fragment (bp)	Nested primers (5'-3')	Amplified fragment (bp)	Primers (5'-3')	Amplified fragment (bp)	Description		
<i>Ste3-1</i>	pPR2TN	MP066 (Kpn I) - GCGTCCGTTACCGATGGAATGAGAGAG MP067 (EcoR I) - CTGGCGAATTCGTCTCTTTAAGTTC	848	MP064 (Not I) - AGTGACCGCCCGCATGATGCGCGCGACG MP065 (Hind III) - GTTGCAGCTTCAATCTTAGCTGCGCTGG	1128	MP068 - GCGCTGGACAACACGGAGC MP069 - CAGTTTTTCATCAATCCGAACGG	3869	MP029 - CGGTGGCGTCTGCTCGGAC MP030 - ACTCTGATGGCGAAGCAACGGC	836	Amplification of the <i>Ste3-1</i> gene (partial)		
								MP067 - CTGGCGAATTCGTCTCTTTAAGTTC MP092 - TTCCACCATGATATTCGGCAAGCAGG	2220	Upstream flanking region of <i>Ste3-1</i> gene and partial resistance cassette		
								MP091 - AAGATGGATTGCACGCGAGTTCTCC MP065 - GTTGCAGCTTCAATCTTAGCTGCGCTGG	2500	Downstream flanking region of <i>Ste3-1</i> gene and partial resistance cassette		
<i>Hd1</i>	pBS-HYG	MP072 (Cla I) - AGATGATCGATTTTCTATCTGGTGC MP073 (Kpn I) - ATGATGGTACCCTAAGCTGTAGTTTC	775	MP070 (Sac I) - CTGTCGAGCTCAAACCGCTCTCAACC MP071 (Not I) - GGTTTCCGCGCCGTAGATTTCCCTTTTG	913	MP074 - AACGAAAGATGAAGACACCAG MP075 - GTTTCACCAGAACATATCAGG	3508	MP083 - AAAACCTCTCCAAGCGCC MP084 - TCTACATCGGTCTCTCC MP154 - ATGAGATCGTTCAACAGC (paired withMP086)	1002 ²⁵⁸⁰	Amplification of the <i>Hd1</i> gene (partial)		
								MP060 - GTGAAACCCGACGCCAGC MP073 - ATGATGGTACCCTAAGCTGTAGTTTC	1500	Upstream flanking region of <i>Hd1</i> gene and partial resistance cassette		
								MP070 - CTGTCGAGCTCAAACCGCTCTCAACC MP063 - TTTGCCCTCGGACGAGTGTGG	2932	Downstream flanking region of <i>Hd1</i> gene and partial resistance cassette		
<i>Hd2</i>	pPR2TN	MP076 (EcoR I) - GATGGGAATTCCTTCTATCTGGTGCAC MP073 (Kpn I) - ATGATGGTACCCTAAGCTGTAGTTTC	774	MP077 (Not I) - GACGACCGCCCGCTAAACCTTGTGAG MP078 (Hind III) - ACTCCAAGCTTCTCTATGTTGAGAG	629	MP079 - TTTCTATCTGGTGCACAACCTTC MP080 - TATGGTTGAGAGTAATGAGC	3305	MP085 - TGTACAGTTTCAACGAAGC MP086 - GTCTCCGTTCTTTCTCG MP155 - ACATTTTCAATAGGCTGG (paired withMP085)	916 ¹⁵⁹⁵	Amplification of the <i>Hd2</i> gene (partial)		
								MP076 - GATGGGAATTCCTTCTATCTGGTGCAC MP048 - GTACAGTCCGCAACCCGACCGC MP037 - GCGTCCGTTGTGCGACTGTAC MP078 - ACTCCAAGCTTCTCTATGTTGAGAG	2254	Upstream flanking region of <i>Hd2</i> gene and partial resistance cassette		
	pJET1.2+Zeo	MP156 (Bsm I) - TTATTGAATGCTTCTATCTGGTGCAC MP157 (Cla I) - AAAAATCGATCGTAAGCTGTAGTTTC	776	MP158 (Pst I) - TTATACTGCAGTCTCTATGTTGAGAGTAATGAGC MP077 (Not I) - GACGACCGCCCGCTAAACCTTGTGAG	630	MP079 - TTTCTATCTGGTGCACAACCTTC MP080 - TATGGTTGAGAGTAATGAGC	3305	MP085 - TGTACAGTTTCAACGAAGC MP086 - GTCTCCGTTCTTTCTCG	916	Amplification of the <i>Hd2</i> gene (partial)		
								MP049 - AGATAGAACCACACTCGC MP114 - ATAAAAGATCTATGGCCAAGTTGACC MP115 - ATAATCTAGATCAGTCTGCTCCTC MP159 - TTATCTCGGTAACCTGCCCCG	1745	Upstream flanking region of <i>Hd2</i> gene and partial resistance cassette		
<i>Ste3-1/mfa1</i>	pPR2TN	MP087 (Kpn I) - TCATTGGTACCCTGTAAGTCTGCCTG MP088 (EcoR I) - TCACCGAATTCACCTTGCAGCTTGC	1058	MP064 (Not I) - AGTGACCGCCCGCATGATGCGCGCGACG MP065 (Hind III) - GTTGCAGCTTCAATCTTAGCTGCGCTGG	1126	MP068 - GCGCTGGACAACACGGAGC MP089 - GCTTCATTATACATACCTCAGC	4591	MP029 - CGGTGGCGTCTGCTCGGAC MP030 - ACTCTGATGGCGAAGCAACGGC	836	Amplification of the <i>Ste3-1</i> gene (partial)		
								MP100 - TCCATCCTCAACTGATTGC MP101 - AGTGTAAGAAGTCTCGG	357	Amplification of the <i>Mfa1</i> (complete) region		
								MP091 - AAGATGGATTGCACGCGAGTTCTCC MP065 - GTTGCAGCTTCAATCTTAGCTGCGCTGG	2500	Downstream flanking region of <i>Ste3-1</i> gene and partial resistance cassette		
<i>Ste3-2/mfa2</i>	pPR2TN	MP093 (EcoR I) - TTTTCAATTCACCACTTCTCCCG MP094 (Kpn I) - AGAATGGTACCCTGACGCGTTAGC	824	MP095 - AATATAACCGCCCGCTACCTGATACCTTG MP096 - TTCTGAAGCTTAAAGTTCTGATTATCTAGAACCATCC	848	MP097 - CTCTACATAGAAGTGAATGGTCC MP098 - GAGTCTTGTGTTGACGTAACC	3224	MP092 - TTCCACCATGATATTCGGCAAGCAGG MP088 - TCACCGAATTCACCTTGCAGCTTGC	2430	Downstream flanking region of <i>Mfa1</i> gene and partial resistance cassette		
								MP035 - TTATGCATCAACCGCGTCTGGC MP036 - GGACACAGAGGCAACRGTAGTTCC	929	Amplification of the <i>Ste3-2</i> gene (partial)		
								MP102 - TATATCATCTCTCGACCC MP103 - TTCATCTTGCAGACAGC	611	Amplification of the <i>Mfa2</i> (complete) region		
<i>Spo11</i>	pBS-HYG	MP126 (Not I) - TATTTGCGGCGCGTGAATGTTTCAGG MP127 (Sac I) - TATTTGAGCTCTGTTGTCAACCTATGCG	732	MP124 (Kpn I) - TATTTGGTACCCTAAGGAACATGCG MP125 (Cla I) - TTAATATCGATGTCATTTCCCGAAGC	565	MP128 - AAGGAAACATGCGGGACG MP129 - GCTTTGTCAACCTATGCG	4000	MP091 - AAGATGGATTGCACGCGAGTTCTCC MP099 - ACATGCTGTAGGCTTATCGATAGCG MP093 - TTTCAAGATTCACCACTTCTCCCG MP092 - TTCCACCATGATATTCGGCAAGCAGG	2400	Downstream flanking region of <i>Ste3-2</i> gene and partial resistance cassette		
								MP130 - TTAATCTCATCTCTCGGC MP131 - TGACTTGTCCAGTATCTCC	2750	Amplification of the <i>Spo11</i> gene (partial)		
								MP063 - TTTGCCCTCGGACGAGTGTGG MP133 - TTGCCGAGGTTGGTGGATGG	2293	Upstream flanking region of <i>Spo11</i> gene and partial resistance cassette		
<i>Mfa1</i>	pJET1.2+Zeo	MP171 (Pst I) - TTTTCTGCACTAGGTACATCACTCG MP 172 (Not I) - TTTATCGGCGCTCTTACACTTTTGC	1165	MP173 (Cla I) - ATTTTATCGATAGGCAATCAGTTGAGG MP174 (Pme I) - TATTATTTAAACATAAGGCAGTGCAGC	1024	MP175 - ACATCACTCGGTACAGATCC MP176 - ATAAGGCAGTGCAGGAGG	3363	MP132 - TCGTTCCTCAGCCCTAAGACATGC MP060 - GTGAAACCCGACGCCACG	1344	Downstream flanking region of <i>Spo11</i> gene and partial resistance cassette		
								MP100 - TCCATCCTCAACTGATTGC MP101 - AGTGTAAGAAGTCTCGG	357	Amplification of the <i>Mfa1</i> (complete) region		
								MP171 - TTTTCTGCACTAGGTACATCACTCG MP115 - ATAATCTAGATCAGTCTGCTCCTC	1937	Downstream flanking region of <i>Mfa1</i> gene and partial resistance cassette		
<i>Mfa2</i>	pJET1.2+Zeo	MP162 (Pst I) - TTTTCTGCACTAGGTACATCACTCG MP163 (Not I) - TTTTCTGCGCGCTCTTACACTTTTGC	998	MP166 (Cla I) - TTTTATCGATGATATACGAGATCACC MP167 (Bsm I) - TTTTGAATGCAGAACGACTGGAAGAG	1014	MP168 - ATCAGAGGAGTACGATCG MP170 - AGAACGACTGGAAGAGC	3193	MP114 - ATAAAAGATCTATGGCCAAGTTGACC MP174 - TATTAGTTTAAACATAAGGCAGTGCAGC	1794	Upstream flanking region of <i>Mfa1</i> gene and partial resistance cassette		
								MP102 - TATATCATCTCTCGACCC MP103 - TTCATCTTGCAGACAGC	611	Amplification of the <i>Mfa2</i> (complete) region		
								MP162 - TTTTCTGCACTAGGTACATCACTCG MP115 - ATAATCTAGATCAGTCTGCTCCTC	1770	Upstream flanking region of <i>Mfa2</i> gene and partial resistance cassette		
										MP114 - ATAAAAGATCTATGGCCAAGTTGACC MP167 - TTTTGAATGCAGAACGACTGGAAGAG	1784	Downstream flanking region of <i>Mfa2</i> gene and partial resistance cassette