

Primers	Sequence (5'→3')	Purpose
SgRNAseq_F	CGTCTTCTCTTTGAAAAGATA	Sequencing of sgRNAs in pGS
SgRNAseq_R	TTGAAGTCCGTTTATTAAGTT	Sequencing of sgRNAs in pGS
01_F	GATCGCACACGGTGTGGTGGGCCC	Hybridization of a dsOligo containing a sgRNA sequence targeting the URA3 gene (KO)
01_R	AAACGGGCCCACCACACCGTGTGC	Hybridization of a dsOligo containing a sgRNA sequence targeting the URA3 gene (KO)
URA3KO_F	CAGAATAGCAGAATGGGCAGACATTACGAATGC ACACGGTGTGGTGGGCTGATCA	Hybridization of the donor DNA for the URA3 gene KO
URA3KO_R	CTTCTTCCGCCGCTGCTTCAAACCGCTAACAA TATGATCAGCCCACCACACCGT	Hybridization of the donor DNA for the URA3 gene KO
02_F	GATCGAAGTAACAAAGGAACCTAG	Hybridization of a dsOligo containing a sgRNA sequence targeting the URA3 gene (KI)
02_R	AAACCTAGGTTCTTTGTTACTTC	Hybridization of a dsOligo containing a sgRNA sequence targeting the URA3 gene (KI)
URA3KI60_F	TGCCCAGTATTCTTAACCCA	Amplification of the URA3 gene plus 60bp homologies
URA3KI60_R	TTAAATTGAAGCTCTAATTTGTGA	Amplification of the URA3 gene plus 60bp homologies
URA3KI1000_F	TCATAAAATTGATAAGGAGAATCC	Amplification of the URA3 gene plus 1Kb homologies
URA3KI1000_R	GTTATCAGATATTATCAGGTGG	Amplification of the URA3 gene plus 1Kb homologies
URAKOver_F	CTGCCAAGCTATTTAATATCA	Confirmation of URA3 gene KO
URAKOKIver_R	AAGTAACAGTATTTTACGGGG	Confirmation of URA3 gene KO and KI
URAKIver_F	AAAAGGATTAAAGATGCTAAGAG	Confirmation of URA3 gene KI
LEU_F	GATCGTTTTGTTAGGTGCTGTGGG	Hybridization of a dsOligo containing a sgRNA sequence targeting LEU2
LEU_R	AAACCCACAGCACCTAACAAAAC	Hybridization of a dsOligo containing a sgRNA sequence targeting LEU2
XR_F	GTTTCGACGGATTCTAGAACTAGTGGATCCATG CCTTCTATTAAGTTG	Amplification of Sc. stipitis xylose reductase (xyl1)
XR_R	TCGAATTCCTGCAGCCCGGGGATCCTTAGACG AAGATAGGAATC	Amplification of Sc. stipitis xylose reductase (xyl1)
P425_F	GCTGAAATATACGGGTTCCC	Amplification of the XR expressing cassette in p425
P425_R	CTGGTAGGTTAGATCCCAGG	Amplification of the XR expressing cassette in p425
29_F	GATCCTTACATGTTTGGCACGCAG	Hybridization of a dsOligo containing a sgRNA sequence for pGS.29
29_R	AAACCTGCGTGCCAAACATGTAAG	Hybridization of a dsOligo containing a sgRNA sequence for pGS.29
30_F	GATCCTTACATGTTTGGCACGTAG	Hybridization of a dsOligo containing a sgRNA sequence for pGS.29
30_R	AAACCTACGTGCCAAACATGTAAG	Hybridization of a dsOligo containing a sgRNA sequence for pGS.30