

Table S4 - Plasmids

N.A., not applicable.

Name	Relevant characteristics	Primer(s) used for gRNA	Origin
<b>p414-<i>TEF1p-Cas9-CYC1t</i></b>	CEN6/ARS ampR <i>TEF1p-Cas9-CYC1t</i>	N.A.	(DiCarlo <i>et al.</i> 2013)
<b>pUG-natNT2</b>	ampR <i>AgTEF1p-nat-AgTEF1t</i>	N.A.	(de Kok <i>et al.</i> 2012)
<b>pUG6</b>	ampR <i>AgTEF1p-kanMX-AgTEF1t</i>	N.A.	(Güldener <i>et al.</i> 1996)
<b>pMEL10</b>	2μm ampR <i>KIURA3</i> gRNA-CAN1.Y	N.A.	(Mans <i>et al.</i> 2015)
<b>pMEL11</b>	2μm ampR <i>amdSYM</i> gRNA-CAN1.Y	N.A.	(Mans <i>et al.</i> 2015)
<b>pROS10</b>	2μm ampR <i>URA3</i> gRNA-CAN1.Y gRNA-ADE2.Y	N.A.	(Mans <i>et al.</i> 2015)
<b>pROS11</b>	2μm ampR <i>amdSYM</i> gRNA-CAN1.Y gRNA-ADE2.Y	N.A.	(Mans <i>et al.</i> 2015)
<b>pUDR286</b>	2μm ampR <i>URA3</i> gRNA-TKL2 gRNA-SOL4	9508 & 9503	This study
<b>pUDR287</b>	2μm ampR <i>amdSYM</i> gRNA-GND2	7231	This study
<b>pUDR351</b>	2μm ampR <i>URA3</i> gRNA-CIT3	12537	This study
<b>pUDR353</b>	2μm ampR <i>URA3</i> gRNA-NQM1	12569	This study
<b>pUDR354</b>	2μm ampR <i>URA3</i> gRNA-PYC2 gRNA-SDH1b	12514 & 12521	This study
<b>pUDR355</b>	2μm ampR <i>amdSYM</i> gRNA-SHH3 gRNA-SHH4	9446 & 12529	This study
<b>pUDR376</b>	2μm ampR <i>amdSYM</i> gRNA-X2 (2x)	N.A.	(Wronska <i>et al.</i> 2020)
<b>pUDR458</b>	2μm ampR <i>URA3</i> gRNA-AAC1 gRNA-AAC3	13820 & 13826	This study
<b>pUDR460</b>	2μm ampR <i>amdSYM</i> gRNA-CTP1 gRNA-NDT2	9489 & 13853	This study
<b>pUDR462</b>	2μm ampR <i>amdSYM</i> gRNA-SAL1 gRNA-MPC3	13830 & 13859	This study
<b>pUDR606</b>	2μm ampR <i>amdSYM</i> gRNA-ODC1 gRNA-ODC2	13840 & 15580	This study
<b>pUDR686</b>	2μm ampR <i>URA3</i> gRNA-ODC1 gRNA-CTP1	13840 & 9489	This study
<b>pUDR687</b>	2μm ampR <i>amdSYM</i> gRNA-NDT2 (2x)	13853	This study
<b>pUDR688</b>	2μm ampR <i>amdSYM</i> gRNA-ODC2 (2x)	13846	This study
<b>pUDR722</b>	2μm ampR <i>amdSYM</i> gRNA-FRDS1 (2x)	17280	This study
<b>pUDR723</b>	2μm ampR <i>URA3</i> gRNA-IDP1 (2x)	12285	This study
<b>pUDR724</b>	2μm ampR <i>URA3</i> gRNA-IDP2 (2x)	17287	This study
<b>pUDR725</b>	2μm ampR <i>URA3</i> gRNA-IDP1 gRNA-IDP2	12285 & 17287	This study
<b>pUDR738</b>	2μm ampR <i>URA3</i> gRNA-CTP1 (2x)	9489	This study
<b>pUDR739</b>	2μm ampR <i>URA3</i> gRNA-ALD3 (2x)	17446	This study
<b>pUDR740</b>	2μm ampR <i>amdSYM</i> gRNA-GPD1 gRNA-GPP2	9498	This study
<b>pUDR741</b>	2μm ampR <i>URA3</i> gRNA-GPD1 gRNA-ALD3	7772 & 17446	This study
<b>pUDR742</b>	2μm ampR <i>URA3</i> gRNA-GPP2 gRNA-ALD3	9498 & 17446	This study

DiCarlo JE, Norville JE, Mali P et al. Genome engineering in *Saccharomyces cerevisiae* using CRISPR-Cas systems. Nucleic Acids Res 2013;41: 4336-43.

Mans R, van Rossum HM, Wijsman M et al. CRISPR/Cas9: a molecular Swiss army knife for simultaneous introduction of multiple genetic modifications in *Saccharomyces cerevisiae*. FEMS Yeast Res 2015;15: 1-15.

Wronska AK, Haak MP, Geraats E et al. Exploiting the diversity of *Saccharomycotina* yeasts to engineer biotin-independent growth of *Saccharomyces cerevisiae*. Appl Environ Microbiol 2020;86.

de Kok S, Nijkamp JF, Oud B et al. Laboratory evolution of new lactate transporter genes in a *jen1Δ* mutant of *Saccharomyces cerevisiae* and their identification as *ADY2* alleles by whole-genome resequencing and transcriptome analysis. FEMS Yeast Res 2012;12: 359-74.

Güldener U, Heck S, Fielder T et al. A new efficient gene disruption cassette for repeated use in budding yeast. Nucleic Acids Res 1996;24: 2519-24.