Supplementary information

Preloading budding yeast with all-in-one CRISPR/Cas9 vectors for easy and high-efficient genome editing

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This file contains 7 figures and additional materials and methods.

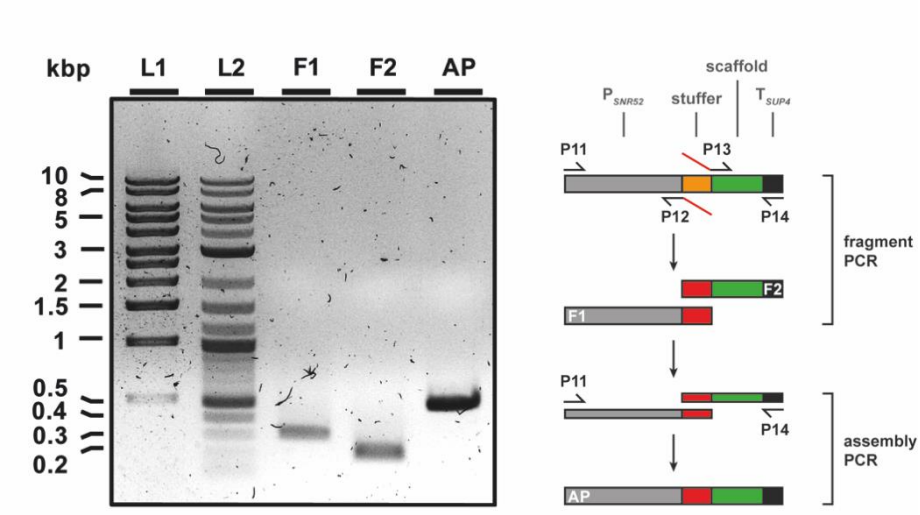

Figure S1. Gel-electrophoretic analysis of assembly PCR intermediates and the final assembly product. At first, both fragments that should be assembled were amplified individually. The *SNR52* promoter containing fragment (F1; 311 bp) was amplified by using primers P11 and P12. The gRNA-scaffold and *SUP4* terminator containing fragment (F2; 211 bp) was amplified by using primers P13 and P14. Overhang primers P12 and P13 introduced the anti-*ADE2* protospacer sequences (red) to the fragments, respectively, that function as overlapping regions in the following assembly PCR step. The assembly product (AP; 502 bp) was amplified with the outer primers P11 and P14. L1 = 1 kbp DNA ladder (Carl Roth); L2 = 2-Log DNA ladder (NEB).

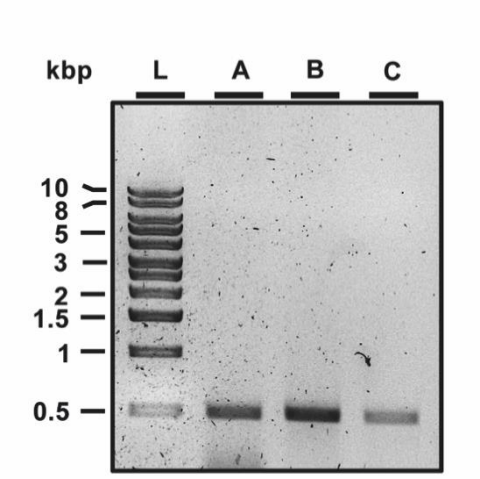

Figure S2. Colony PCR products for confirming proper introduction of anti-*ADE2* protospacer sequence in plasmid pCAS9i analyzed by agarose gel electrophoresis. The anti-*ADE2* protospacer sequence was introduced into PmeI & KpnI cleaved plasmid pCAS9i by method A (one-step *in vivo* HR-mediated assembly of overlapping oligonucleotides P12 and P13; A), by method B (*in vivo* HR-mediated integration of *ADE2* protospacer containing gRNA assembly PCR product; B) or method C (PCR amplification of pCAS9i backbone with primers P12 and P13 and subsequent *in vivo* HR-mediated recircularization of the plasmid; C). Positive transformants yield a PCR product of 508 bp. L = 1 kbp DNA ladder (Carl Roth).

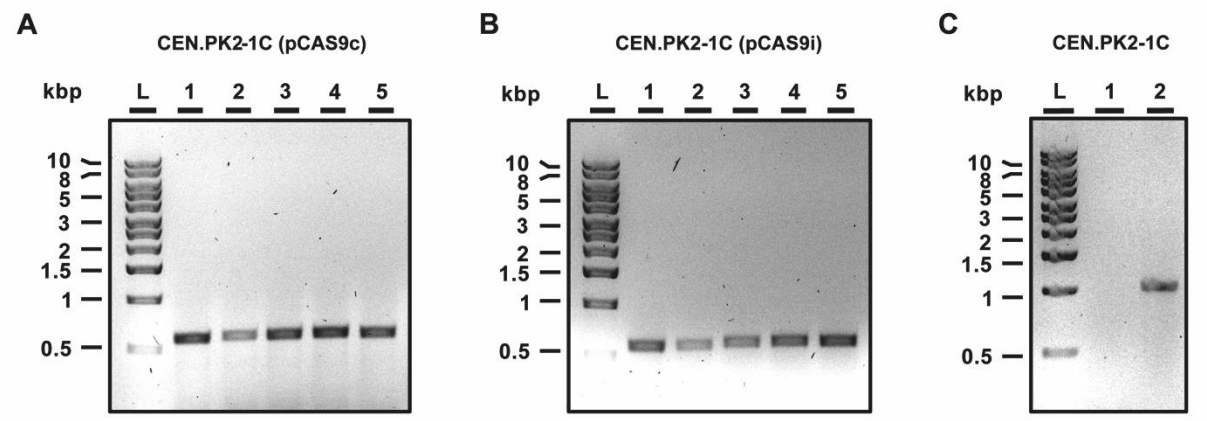

Figure S3. Colony PCR products for confirming the disruption of *ADE2* by integration of loxP-*LEU2*-loxP donor DNA in a CEN.PK2-1C background analyzed by agarose gel electrophoresis. A. Confirmation of pCAS9c-supported donor DNA integration of five randomly selected transformants (1–5). **B.** Confirmation of pCAS9i-supported donor DNA integration of five randomly selected transformants (1–5). Positive transformants yield a PCR product of 598 bp. **C.** PCR-negative control. Primers that were used to confirm the integration of loxP-*LEU2*-loxP into the *ADE2* locus do not yield a PCR product for unmodified CEN.PK2-1C cells (1). The presence and quality of genomic DNA was checked by amplifying a 1 kbp fragment of the *S. cerevisae* genome (2). L = 1 kbp DNA ladder (Carl Roth).

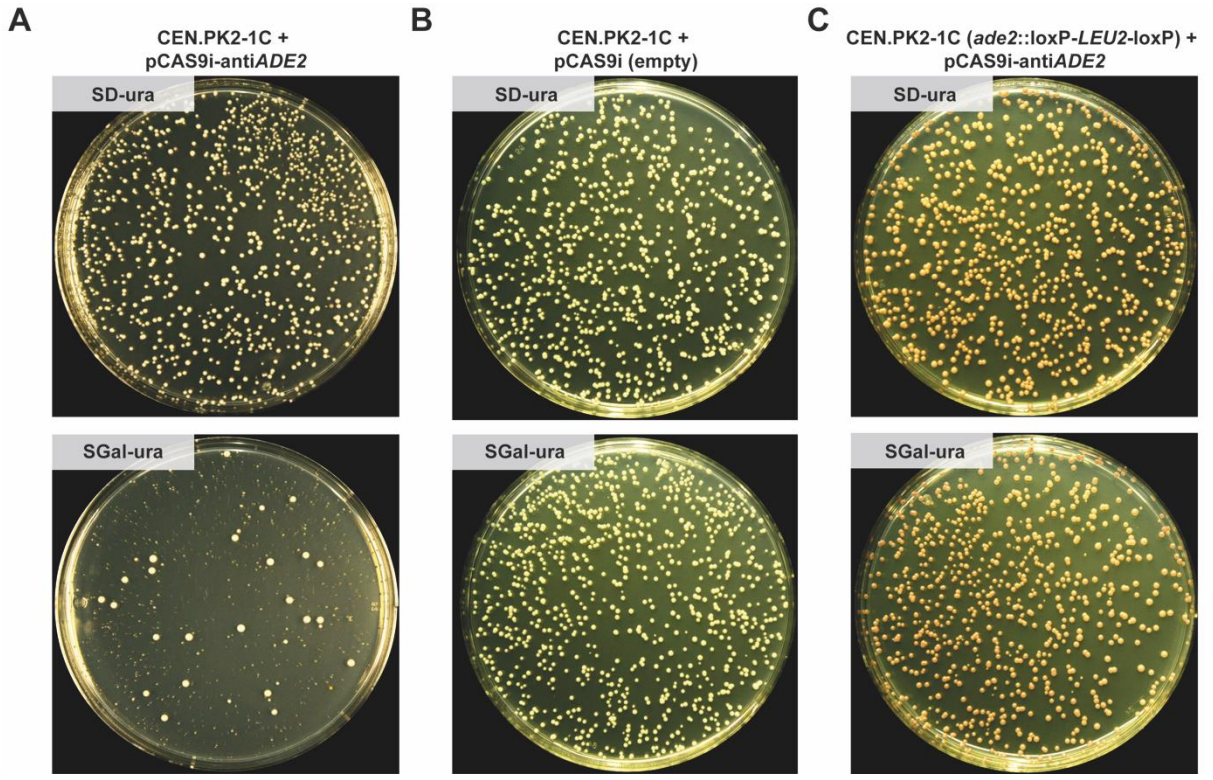

Figure S4. A functional *ADE2*-targeting CRISPR/Cas9 system expressed from pCAS9i is toxic for yeast. Images show representative agar plates. The identical number (100 µl of OD_{600} = 0.002) of pCAS9i-anti*ADE2* harboring CEN.PK2-1C cells was **A.** plated on uracil-free glucose (SD-ura) or galactose (SGal-ura) containing agar media. Glucose containing media (SD-ura) do not promote the expression of Cas9 from P_{GAL1} and cells grew unimpaired. On galactose containing media (SGal-ura) cells express a functional *ADE2*-targeting CRISPR/Cas9 system. Colonies with the same size as on SD media recovered only from a small percentage of plated cells, whereas the majority of cells showed strongly impaired growth. **B.** Reference strains that do not express a functional *ADE2*-targeting gRNA or **C.** that do not provide a genomic protospacer sequence that could be addressed by the gRNA-Cas9 complex grew unaffected on galactose media. Colonies recovered from all plated cells with the same size as on glucose containing media.

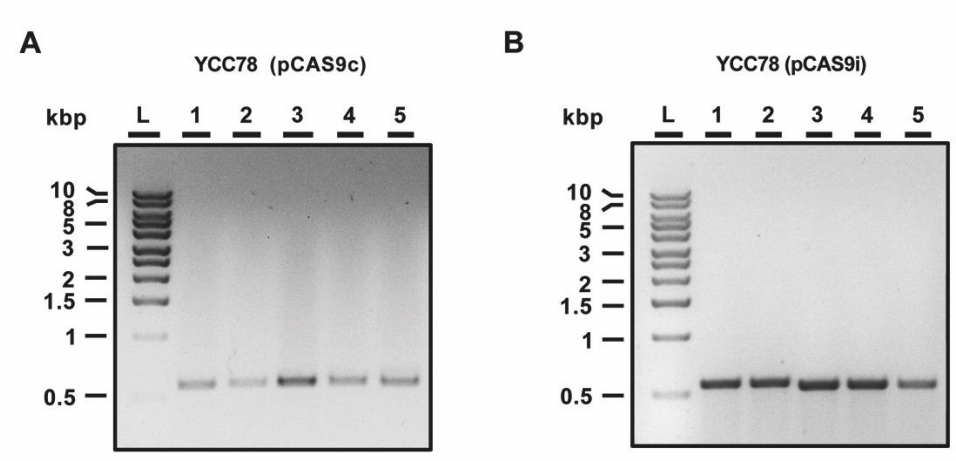

Figure S5. Colony PCR products for confirming the disruption of *ADE8* by integration of loxP-kanMX-loxP donor DNA in an YCC78 background analyzed by agarose gel electrophoresis. A. Confirmation of pCAS9c-supported donor DNA integration of five randomly selected transformants (1–5). **B.** Confirmation of pCAS9i-supported donor DNA integration of five randomly selected transformants (1–5). Positive transformants yield a PCR product of 575 bp. L = 1 kbp DNA ladder (Carl Roth).

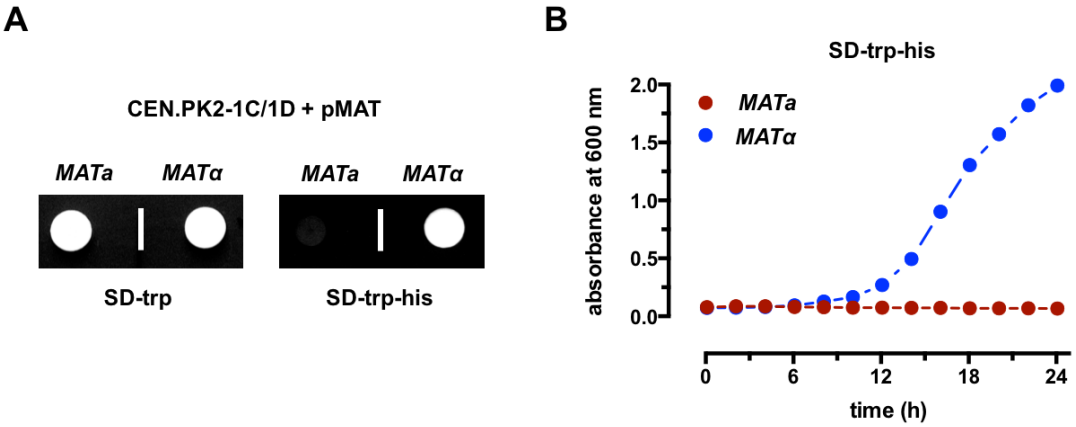

Figure S6. pMAT harboring *MATα* cells functionally express *HIS3*. A. Growth of *MAT*a (CEN.PK2-1C) and *MATα* (CEN.PK2-1D) cells harboring pMAT (*TRP1* marker) was tested on SD-trp-his agar medium. For that, equal amounts of cells (7 µl of a cell suspension with OD_{600} = 1.0) were spotted on the indicated agar medium. *HIS3* is only expressed in *MATα* genomic background and complements for genomically encoded non-functional *his3* of the CEN.PK2 background. **B.** Growth curves of pMAT harboring *MAT*a (CEN.PK2-1C) and *MATα* (CEN.PK2-1D) in SD-trp-his liquid media. Growth was only observed for *MATα* (CEN.PK2-1D) cells.

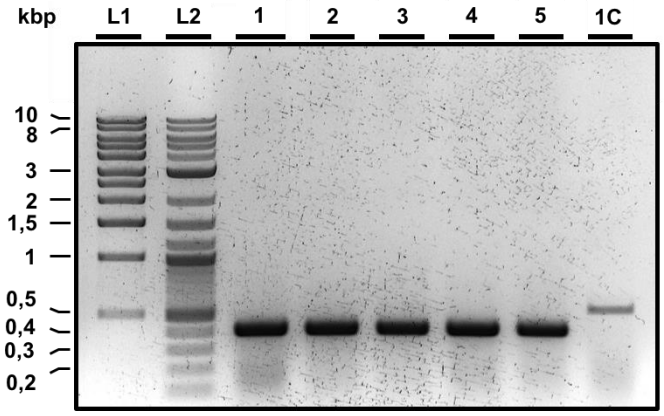

Figure S7. Colony PCR products for confirming CRISPR/Cas9-mediated mating type switching from *MAT*a (CEN.PK2-1C) to *MATα* (CEN.PK2-1D) analyzed by agarose gel electrophoresis. Confirmation of successfully switched mating type of five randomly selected pMAT harboring clones with His^+ phenotype (1–5). Additionally, the initial CEN.PK2-1C strain was tested for its mating type as a control (1C). Three oligonucleotides were used for each PCR simultaneously with two forward primers, either binding in the Ya or Y*α* region and an universal reverse primer binding downstream of the *MAT* locus. *MAT*a DNA as a template generates a 544 bp PCR product, whereas *MAT*α yields a 404 bp PCR product. *HML* and *HMR* DNAs do not work as proper PCR templates. This PCR approach for assessing the mating type of yeast cells was described elsewhere [1]. L1 = 1 kbp DNA ladder (Carl Roth); L2 = 2-Log DNA ladder (NEB).

Additional materials and methods

Plasmid construction and yeast transformation

Transformation of CEN.PK2-1C for recombination-based cloning *in vivo* were routinely carried out by using the Frozen-EZ Yeast Transformation II kit (Zymo Research) according to the manufacturer's instructions.

The 2-micron (2 µ) sequence of plasmid pRS425 was amplified with the primers P1 and P2 and used to replace the CEN/ARS sequence of the BglII/NheI (NEB) cleaved plasmid pCM188 [2] *via* recombination-based cloning resulting in plasmid pCM188(2 µ). The gRNA expression cassette from plasmid p425-SNR52p-gRNA.HIS3-SUP4t (is equivalent to p426-SNR52p-gRNA.CAN1.Y-SUP4t [3] but with pRS426 backbone and anti*HIS3*-gRNA; kindly provided by Dr. Christopher Schneider) was amplified with two individual PCRs (Q5 DNA polymerase, NEB) using overhang primers P3 & P4 (for P_{SNR52}) and P5 & P6 (for gRNA scaffold sequence - T_{SUP4}). The overhang primers were designed in a way that introduces a gRNA stuffer sequence (contains unique KpnI & MssI restriction sites) between P_{SNR52} and the gRNA scaffold sequence and allows for integrating the gRNA expression cassette into AvrI (NEB) cleaved vector pCM188(2µ). Assembly of the universal gRNA cassette and simultaneous integration into pCM188(2µ) were performed by *in vivo* recombination-based cloning. The *URA3* marker-2 µ-universal gRNA cassette from the yielded plasmid was amplified (Q5 DNA polymerase, NEB) with the primers P7 & P8 and introduced into the KpnI & SnaBI cleaved plasmid p414-TEF1p-Cas9-CYC1t [3] by recombination-based cloning with simultaneous removal of the *TRP1* marker and the CEN/ARS sequence. The resulting plasmid was called pCAS9c (constitutive). To generate pCAS9i (inducible), P_{TEF1} from plasmid pCAS9c was replaced by P_{GAL1}. For that, pCAS9c was linearized with SpeI (NEB) and P_{GAL1} amplified (Q5 DNA polymerase, NEB) from plasmid pGREG504 [4] with primers P9 & P10 was introduced by recombination-based cloning. Multiplex plasmids pCAS9cd und pCAS9id (duplex) were generated by introducing a second gRNA expression cassette into NotI-cleaved plasmids pCAS9c and pCAS9i. The gRNA expression cassette was PCR-amplified (Q5 DNA polymerase, NEB) with primers P27 and P28 from plasmid pCAS9c or pCAS9i, respectively.

For generation of plasmid pMAT, the *HIS3* ORF amplified from plasmid pGREG506 with overhang primers P37 and P38 was inserted into SalI cut plasmid pGREG504 [4] by *in vivo* recombination-based cloning. The resulting plasmid was cut with AscI and NotI to remove the *GAL1* promoter which was replaced by the *HO* promoter amplified from yeast genomic

DNA with overhang primers P39 and P40 by yeast *in vivo* cloning. In an equivalent manner, the *ALPHA2* ORF (amplified with primers P41 and P42 from yeast genomic DNA) and P_{TEF1} (amplified with primers P43 and P44 from yeast genomic DNA) were stepwise inserted into plasmid pGREG505 [4]. The resulting plasmid was used as a template to amplify the P_{TEF1}-*ALPHA2*-T_{CYC1} cassette (primers P45 and P46) which was subsequently inserted into AsiSI cleaved vector pGREG504-P_{HO}-*HIS3* by recombination based cloning with simultaneous removal of the loxP-kanMX-loxP cassette. To avoid recombination events between both T_{CYC1} sequences present in the resulting plasmid, T_{CYC1} of the inserted P_{TEF1}-*ALPHA2*-T_{CYC1} cassette was replaced by T_{TEF1}. For that, the plasmid was cut with PacI and T_{TEF1} amplified with overhang primers P47 and P48 from yeast genomic DNA was inserted *via in vivo* cloning.

All cloning products were checked by PCRs using pairs of primers that amplify a DNA fragment containing the artificial integration border that results from the HR event.

PCRs were performed according to the manufacturer's instructions. Primers and oligonucleotides used in this study are listed in **Table S1**.

Colony PCR

Colony PCRs were performed in order to confirm the integration of donor DNA into the respective locus. A pair of primers was used with one primer binding within the genomic locus and the other one within the integrated donor DNA cassette so that a PCR product (loxP-*LEU2*-loxP in *ADE2*: P19 & P20; loxP-kanMX-loxP in *ADE8*: P25 & P26) only appears when the right cassette was integrated into the right locus. For preparing the PCR template, yeast cells were suspended in 30 µl of 0.2% SDS and incubated for 10 min at 95 ℃. 0.5 µl of the cell suspension was used as template for a 25 µl PCR mix. Colony PCRs were conducted with the OneTaq 2X Master Mix with Standard Buffer (NEB) according to the manufacturer's instructions.

Assembly PCR

A full-length gRNA expression cassette that contains an *ADE2* targeting protospacer sequence was constructed via assembly PCR. First, two individual fragments were amplified (Q5 DNA polymerase): P11 & P12 were used to amplify P_{SNR52} (F1; 311 bp) and P13 & P14 were used to amplify the gRNA scaffold – T_{SUP4} sequence (F2; 211 bp) from vector pCAS9c, respectively. P12 & P13 are overhang primers that introduce the 20 bp *ADE2* targeting protospacer sequence to one end of both fragments, respectively, that also functions as overlapping regions for the assembly reaction. F1 and F2 were gel purified (Zymoclean Gel

DNA Recovery Kit, Zymo Research) and 3 µl of each gel purified fragment were used as a template for a second PCR (assembly reaction; Q5 DNA polymerase, NEB) employing primers P11 & P14. The resulting assembly product (AP; 502 bp) was checked via gel electrophoretic analysis.

Table S1. Primers and oligonucleotides used in this study.

Primer	Sequence	Amplicon
P1 (fw)	CACAAATTGCAAAATTTAATTGCTTGCAAAAGGTCACATGCTGTATGATCCAATATCAAAGGAAATGATAGC	2-micron
P2 (rv)	GGAACTCGATTTCTGACTGGGTTGGAAGGCAAGAGAGCCCCGAGCCTGAACGAAGCATCTGTG	2-micron
P3 (fw)	CATGTGACCTTTTGCAAGCAATTAAATTTTGCTGATATCGAATTCGCTGGAGC	P_{SNR52}
P4 (rv)	GGTACCCTCGCAGATGTTGCTGATGTCGTCGTTTAAACGATCATTTATCTTTCACTGCGGAG	P_{SNR52}
P5 (fw)	GACGACATCAGCAACATCTGCGAGGGTACCGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAG	scaffold – T_{SUP4}
P6 (rv)	CGTCGTGACTGGGAAAACCCTGGCGTTACCCAATTAACCCTCACTAAAGGG	scaffold – T_{SUP4}
P7 (fw)	CATTATACTGAAAACCTTGCTTGAGAAGGTTTTGGGACGCTCGAAGGCTTTAATTTGCGGAGGCGTATCACGAGGCCC	*URA3*-2μ-gRNA cassette
P8 (rv)	GAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCGGGAACAAAAGCTGGAGCTC	*URA3*-2μ-gRNA cassette
P9 (fw)	CCCTCACTAAAGGGAACAAAAGCTGGAGCTGTACGGATTAGAAGCCGCC	P_{GAL1}
P10 (rv)	GTACTTCTTGTCCATTTTTCCCGGGGGATCCCGGGGTTTTTTCTCCTTGACG	P_{GAL1}
P11 (fw)	TGATATCGAATTCGCTGGAGC	Assembly PCR; F1
P12 (rv)	TGTGGATAGTCTCTACAATTGATCATTTATCTTTCACTGCGGAG	*ADE2* oligo; F1
P13 (fw)	AATTGTAGAGACTATCCACAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAG	*ADE2* oligo; F2
P14 (rv)	CGCAATTAACCCTCACTAAAGGG	Assembly PCR; F2
P15 (fw)	CGGTTTAGTGTTTTCTTACCCAATTGTAGAGACTATCCCAGCTGAAGCTTCGTACGCTGCAG	loxP-*LEU2*-loxP
P16 (rv)	GCAGGCGCATAACATAAGTCACAAATATTGTCCTTGGCCGCATAGGCCACTAGTGGATCTG	loxP-*LEU2*-loxP
P17 (fw)	GATCAATTGTAGAGACTATCCACA	ColPCR *ADE2* gRNA
P18 (rv)	CTTCGGGGCGAAAACTCTC	ColPCR *ADE2* gRNA
P19 (fw)	CCGGAAGCTTTGGAAGTACTG	ColPCR Integration *ADE2*
P20 (rv)	TCATGGTTCTGCCCCAG	ColPCR Integration *ADE2*
P21 (fw)	GAGAACAAGCCTCTGACGGCGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAG	*ADE8* oligo
P22 (rv)	GCCGTCAGAGGCTTGTTCTCGATCATTTATCTTTCACTGCGGAG	*ADE8* oligo

P23 (fw)	GCATGGCGCAAATGTCAGGACGAGAACAAGCCTCTGACCAGCTGAAGCTTCGTACGCTGCAG	loxP-kanMX-loxP
P24 (rv)	CGACCTCCTCGATGACATAGTGCACCATGCATCCGGGGCCGCATAGGCCACTAGTGGATCTG	loxP-kanMX-loxP
P25 (fw)	GTTTCGATGGTACCACACACGC	ColPCR Integration *ADE8*
P26 (rv)	CGGCCTCGAAACGTGAGTC	ColPCR Integration *ADE8*
P27(fw)	GAGTCATGTAATTAGTTATGTCACGCTCTAGAGCGGCCGCCACCGCTGATATCGAATTCGCTGG	2nd gRNA
P28 (rv)	CCGGGAACAAAAGCTGGAGCTCCACCGCGGTGTTACATGACTCGAAGACATAAAAAAC	2nd gRNA
P29 (fw)	GATACGTTCTCTATGGAGGAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAG	*CAN1* oligo
P30 (rv)	TCCTCCATAGAGAACGTATCGATCATTTATCTTTCACTGCGGAG	*CAN1* oligo
P31 (fw)	CCGACGAGAGTAAATGGCGAGGATACGTTCTCTATGGCAGGGAAGTCATAACACAGTCC	*HIS3*
P32 (fw)	TCTGTACTTCTCCTTCATCTTCATCACCTATGCCATGTTATTAGGTACCGGCCGC	*HIS3*
P33 (fw)	TCTTCTGTTGTTACACTCTCGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAG	*MAT*x oligo
P34 (rv)	GAGAGTGTAACAACAGAAGAGATCATTTATCTTTCACTGCGGAG	*MAT*x oligo
P35 (fw)	CACTCTACAAAACCAAAACCGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAG	*MAT*z oligo
P36 (rv)	GGTTTTGGTTTTGTAGAGTGGATCATTTATCTTTCACTGCGGAG	*MAT*z oligo
P37 (fw)	GTAGGAAAGGCAAAATACTATCAAAATTTTCGGCGCCATGACAGAGCAGAAAGCCC	*HIS3 ORF*
P38 (rv)	GCGTGACATAACTAATTACATGACTCGAGGGCGCCCTACATAAGAACACCTTTGGTGG	*HIS3 ORF*
P39 (fw)	GGGAACAAAAGCTGGAGCTCGTTTAAACGGCGCGCCCATTTTTGTTTCTTTTGGAC	P_{HO}
P40 (rv)	CGCTTTACTAGGGCTTTCTGCTCTGTCATGGCGCCTTTAAAGTATAGATAGAA	P_{HO}
P41 (fw)	GCACCGTTAAGAACCATATCCAAGAATCAAAAATGAATAAAATACCCATTAAAGACCTTTTAAATC	*ALPHA2 ORF*
P42 (rv)	GCGTGACATAACTAATTACATGACTCGAGTCATTCTTTCTTCTTTGCCAGAG	*ALPHA2 ORF*
P43 (fw)	AACAAAAGCTGGAGCTCGTTTAAACGGCGCGCCATAGCTTCAAAATGTTTCTACTCCTT	P_{TEF1}
P44 (rv)	GTGGATTTAAAAGGTCTTTAATGGGTATTTTATTCATAAACTTAGATTAGATTGCTATGCTTTCT	P_{TEF1}
P45 (fw)	GCTTGAGAAGGTTTTGGGACGCTCGAAGGCTTTAATTTGCGCGCGCAATTAACCCTCAC	P_{TEF1}-*ALPHA2*- T_{CYC1}
P46 (rv)	GGGCGAATTGGGTACCCTTAATTAAGACAACCCGCTATACGAAGTTATTAGGTACCGG	P_{TEF1}-*ALPHA2*- T_{CYC1}

P47 (fw)	GGTGAGCCTCTGGCAAAGAAGAAAGAATGACTCGAGACT GACAATAAAAAGATTCTTGTTTTCAAG	T_{TEF1}
P48 (rv)	CGACTCACTATAGGGCGAATTGGGTACCCTTAATTAAGCA GTATAGCGACCAGCATTCAC	T_{TEF1}

Underlined parts of the sequences represent homology regions for recombination based cloning.

References

1. Huxley C, Green ED, Dunham I (1990) Rapid assessment of S. cerevisiae mating type by PCR. Trends Genet 6(8):236.

2. Gari E, Piedrafita L, Aldea M, Herrero E (1997) A set of vectors with a tetracycline-regulatable promoter system for modulated gene expression in Saccharomyces cerevisiae. Yeast 13(9):837-48.

3. DiCarlo JE, Norville JE, Mali P, Rios X, Aach J, et al. (2013) Genome engineering in Saccharomyces cerevisiae using CRISPR-Cas systems. Nucleic Acids Res 41(7):4336-43.

4. Jansen G, Wu C, Schade B, Thomas DY, Whiteway M (2005) Drag&Drop cloning in yeast. Gene 344:43-51.