

APPLIED MICROBIOLOGY AND BIOTECHNOLOGY

CRISPR/Cas9-based toolkit for rapid marker recycling and combinatorial libraries in *Komagataella phaffii*

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Table S1. Plasmids list

Name	Description	Source	GenBank
pGS124	<i>P_{GAP}-MEL1-T_{AOX1}</i>	This study	OR763072
pGS170	<i>KpHis4</i> upstream and downstream homologous arms	This study	OR763072
pGS178	used for single-crossover to knock out <i>Ku70</i>	This study	OR763070
pGS188	empty gRNA plasmid with an <i>SpCas9</i> expression cassette	This study	OR298115
pGS188-KpDNL4	gRNA plasmid targeting DNA ligase IV with an <i>SpCas9</i> expression cassette	This study	OR763068
pGS188-KanMX	gRNA plasmid targeting KanMX with an <i>SpCas9</i> expression cassette	This study	OR763069
pGS188-KpHis4	gRNA plasmid targeting KpHis4 with an <i>SpCas9</i> expression cassette	This study	OR763067
pGS327	empty gRNA plasmid, no <i>SpCas9</i> expression cassette, derived from pGS188	This study	OR298114
pCrtE	<i>P_{ADH2}-crtE-T_{DAS1}</i>	This study	OR763076
pCrtI	<i>P_{GAP}-crtI-T_{DAS2}</i>	This study	OR763075
PCrtYB-1	<i>P_{FBP1}-crtYB-T_{FBA1}</i>	This study	OR763073
pCrtYB	<i>P_{FBP1}-crtYB^{W61R}-T_{FBA1}</i>	This study	OR763074
ptHMG1	<i>P_{FBA1}-tHMG1-T_{AOX1}</i>	This study	OR763077
pGS287	<i>P_{GAP}-ERG10-T_{PMP20}</i>	This study	OR763066
pGS288	<i>P_{PGI1}-ERG8-T_{TPI1}</i>	This study	OR763065
pGS289	<i>P_{PGD}-ERG12-T_{CAT1}</i>	This study	OR763064
pGS290	<i>P_{TAL1}-MVD1-T_{FDH1}</i>	This study	OR763063
pGS291	<i>P_{MSR1c3}-ERG20-T_{ADH2}</i>	This study	OR763062

pGS292	<i>P_{GPM1}-mERG13-T_{FLD1}</i>	This study	OR763061
pGS293	<i>P_{TPI1}-mIDI1-T_{Gcw14}</i>	This study	OR763060
pGS332	gRNA expression cassette for targeting <i>KpHis4</i> derived from pGS327	This study	OR763059
pGS492	gRNA expression cassette for targeting <i>KpADH900</i> derived from pGS327	This study	OR763058
pGS575	gRNA expression cassette for targeting <i>KpADH900-2</i> derived from pGS327	This study	OR763055
pGS566	gRNA expression cassette for targeting <i>II-4</i> derived from pGS327	This study	OR763057
pGS567	gRNA expression cassette for targeting <i>II-5</i> derived from pGS327	This study	OR763056
pGS568	gRNA expression cassette for targeting <i>II-6</i> derived from pGS327	This study	OR763054

Table S2. Strains list

Strain	Description	Source
CBS7435	<i>Komagataella phaffii</i> Wild type	Purchased from Invitrogen
<i>Kp6</i>	CBS7435 <i>Ku70</i> Δ :: <i>KanMX</i>	This study
<i>Kp9</i>	CBS7435 <i>DNL4</i> Δ <i>Ku70</i> Δ :: <i>KanMX</i>	This study
<i>Kp12</i>	CBS7435 <i>DNL4</i> Δ <i>Ku70</i> Δ :: <i>P_{HXT1}-SpCas9-T_{DAS1}</i>	This study
<i>zw69</i>	CBS7435 <i>DNL4</i> Δ <i>Ku70</i> Δ :: <i>P_{HXT1}-SpCas9-T_{DAS1}</i> <i>ADH900</i> :: <i>P_{GAP}-crtI-T_{DAS2}-P_{FBP1}-crtYB-T_{FBA1}-P_{FBA1}-tHMG1-T_{AOX1}-P_{ADH2}-crtE-T_{DAS1}-P_{GAP}-ERG10-T_{PMP20}-P_{PGI1}-ERG8-T_{TPI1}-P_{PGD1}-ERG12-T_{CAT1}-P_{TAL1}-mMVD1-T_{FDH1}-P_{MSR1c3}-ERG20-T_{ADH2}-P_{GPM1}-mERG13-T_{FLD1}-P_{TPI1}-mIDI1-T_{Gcw14}</i>	This study
<i>Zw106</i>	CBS7435 <i>DNL4</i> Δ <i>Ku70</i> Δ :: <i>P_{HXT1}-SpCas9-T_{DAS1}</i> <i>ADH900</i> :: <i>P_{GAP}-crtI-T_{DAS2}-P_{GAP}-tHMG1-T_{AOX1}-P_{GAP}-crtE-T_{DAS1}-P_{GAP}-crtYB^{W61R}-T_{PMP20}</i>	This study
<i>zw107</i>	CBS7435 <i>DNL4</i> Δ <i>Ku70</i> Δ :: <i>P_{HXT1}-SpCas9-T_{DAS1}</i> <i>ADH900</i> :: <i>P_{GAP}-crtI-T_{DAS2}-P_{GAP}-tHMG1-T_{AOX1}-P_{GAP}-crtE-T_{DAS1}-P_{GAP}-crtYB^{W61R}-T_{PMP20}</i>	This study
<i>Zw109</i>	CBS7435 <i>DNL4</i> Δ <i>Ku70</i> Δ :: <i>P_{HXT1}-SpCas9-T_{DAS1}</i> <i>ADH900</i> :: <i>P_{GAP}-crtI-T_{DAS2}-P_{FBP1}-crtYB-T_{FBA1}-P_{FBA1}-tHMG1-T_{AOX1}-P_{ADH2}-crtE-T_{DAS1}-P_{GAP}-ERG10-T_{PMP20}-P_{PGI1}-ERG8-T_{TPI1}-P_{PGD1}-ERG12-T_{CAT1}-P_{TAL1}-mMVD1-T_{FDH1}-P_{MSR1c3}-ERG20-T_{ADH2}-P_{GPM1}-mERG13-T_{FLD1}-P_{TPI1}-mIDI1-T_{Gcw14}</i>	This study

Table S3. Primer list

Name	sequence	Application
KpDNL4-DHA-F	AGTGGATCAATGCGAAGGATAGGGCTGGGT GATGATCTACCAATC	used for knock out <i>KpDNL4</i>
KpDNL4-DHA-R	GACGAGGACTTCGCTAACCGTCACG	
KpDNL4-UHA-F	ACGCGCGCGTGGTAGAAGTTTATCT	
KpDNL4-UHA-R	ATCCTTCGCATTGATCCACTGTTGGAGCGC ATTCTATTCTCATAC	
kpHis4-UHA-F	GCAAAGTTGGTAGATGTGACTTCCACT	

kpHis4-UHA-R-pGAP	GGACGAGGACACCAAGACATTTCTACAAAA ACTGGGAAAGGAGCTTCTAACACAGG	used for integration of <i>MEL1</i> at KpHis4 locus
PGAP-F-KpHis4-UHA	CCTGTGTTAGAAGCTCCTTTCCCAGTTTTTG TAGAAATGTCTTGGTGTCTCCTCGTCC	
TAOX1-R-KpHis4-DHA	GACTACACCAGGGCAAGTCTCAACTTTTCA CTTAATCTTCTGTACTCTGA	
KpHis4-DHA-F-TAOX1	TCAGAGTACAGAAGATTAAGTGAAAAGTTGA GACTTGCCCTGGTGTAGTC	
KpHis4-DHA-R1	TTAAATAAGTCCCAGTTTCTCCATACGAACC	
kpKu70-DHA-F	AATACATCCAGTTCAAGTTACCTAAACAAAT CAAAGCCTCCAAAAAGGCAGATCTCATCG	used for integration of <i>SpCas9</i> at <i>KpKu70</i> locus
KpKu70-DHA-R	CGTTAATCTACAGTTAGCAGAGTGC	
KpKu70-UHA-F	CCAGATGTAATCACCGCCGCTGAAT	
kpKu70-UHA-R	ACTCCCAAAGTGTCAAGTACACAAGGGTCTCC TCACTGACATTCTCTAAGATGATCTGGAGC	
pHTX1-F-Ku70-DHA	CGATGAGATCTGCCTTTTTGGAGGCTTTGAT TTGTTTAGGTAAGTTGAAGTGGATGTATT	
TDAS1-R-KpKu70	GATCATCTTAGAGAATGTCAAGTGAAGGAGAC CCTTGTGACTGACACTTTGGGAGTCCCTAT	used for multi- fragment integration
KpADH900-DHA-F-TPMP20	AGACACCAGGTCATTGAACTAACGACAAGG TTAAGACGGTGGCTACTACCATC	
KpADH900-DHA-R	ATTATTGACTATACACTTAATCGGCAGATAA GAAAATCTTG	
KpADH900-UHA-F	TCAAATGTAACATTGGAATGATTCGATTTGA	
KpADH900-UHA-R-pGAP	TGGACGAGGACACCAAGACATTTCTACAAA AAGGGTGAGGAACGTTCTCAACCATA	
pGAP-F-ADH900-UHA	CAATATGGTTGAGAACGTTCTCACCCTTTT TGTAAGAAATGTCTTGGTGTCTCCTCGT	
pGAP-F-TAOX1	CTCTTCAGAGTACAGAAGATTAAGTGAAATT TTTGTAGAAATGTCTTGGTGTCTCCTCGTCC	
pGAP-F-TDAS1	GGACTCCCAAAGTGTCAAGTACACAAGGGTCT TTTTGTAGAAATGTCTTGGTGTCTCCTCGTCC	
pGAP-F-TDAS2	AAAGGAGGAACCAAGTTTACGAACCCCGTCT TTTTGTAGAAATGTCTTGGTGTCTCCTCGTCC	
TAOX1-R-pGAP	GACGAGGACACCAAGACATTTCTACAAAAAT TTCACCTTAATCTTCTGTACTCTGAAGAG	
TDAS1-R-pGAP	GGACGAGGACACCAAGACATTTCTACAAAA AGACCCTTGTGACTGACACTTTGGGAGTCC	
TDAS2-R-pGAP	GGACGAGGACACCAAGACATTTCTACAAAA AGACGGGGTTTCGTAACTGGTTCCTCCTTT	
TPMP20-R-KpADH900-DHA	GCAGATGGTAGTAGCCACCGTCTTAACCTT GTCGTTAGTTCAATGACCTGGTG	
pGAP-F-TPMP20	TCCAGACACCAGGTCATTGAACTAACGACA AGTTTTTGTAGAAATGTCTTGGTGTCTCCTCG	
TPMP20-R-pGAP	CGAGGACACCAAGACATTTCTACAAAAACTT GTCGTTAGTTCAATGACCTGGTGTCTGGA	
KpHis4-DHA-F-TGcw14	GACTAGTATATACGCATCCTTCCCGTCGTTA GTTGAGACTTGCCCTGGTGTAGTC	
pADH2-F-TAOX1	TCCTCTTCAGAGTACAGAAGATTAAGTGAAA CGCAGCGTTTTCTGACGGTACTAGA	
pFBA1-F-TFBA1	GCTCACAGCGTACACATCACCCTCATTTCT CTATTGAACGGcttgaaatttgg	
pFBP1-F-TDAS2	AGGAACCAAGTTTACGAACCCCGTCTGCTCA AACGAGTGGAGAGGGGAAATC	
pGPM1-F-TADH2	ATCTAGCGACTCTGGCAGATAAGATATCAAT ACCTTGGGTTATTAGTAGTGTCCG	
pMSR1c3-F-TFDH1	gtttctccttcaaactTCTCACCTCCTTTTAGTGCAG TTGCGCCGTGATTC	

pPGD1-F-TTPI1	CTGTATCTGTAACCCAAGGGGTGGATTATG GTAGaatcatcaattggaatGACCCTATCG	
pPGI1-F-TPMP20	ACCAGGTCATTGAACTAACGACAAGTAGGT CACCTGTGTTACTAGATGTC	
pTAL1-F-TCAT1	TCGAaacatcatcagaaaaCTAAACCAGAGATCG TGTTTTGATTAAAGATTGCTGCTAC	
pTPI1-F-TFLD1	TCGCGGTATTGATCGACGCTCTgtgaaTCAA CGAGACACTCTTCCGTCAGT	
TADH2-R-pGPM1	ACTACTAATAACCCAAGGTATTGATATCTTA TCTGCCAGAGTCGCTAGATctgga	
TAOX1-R-pADH2	TACCGTCAGAAAACGCTGCGTTTCACTTAAT CTTCTGTACTCTGAAGAGGAGTGG	
TCAT1-R-pTAL1	AATCTTAATCAAAACACGATCTCTGGTTTAGt tttctgatgatgttTCGATCATCG	
TDAS2-R-pFBP1	GATTTCCCTCTCCACTCGTTTGAGCAGACG GGGTTCTGTAACCTGGTTCCT	
TFBA1-R-pFBA1	ccaaatttcaagCCGTTCAATAGAGAAATGAGTG GTGATGTGTACGCTGTGAGC	
TFDH1-R-pMSR1c3	AATCACGGCGCAACTGCACTAAAAGGAGGT GAGAagtttgaaggagaaactC	
TFLD1-R-pTPI1	TGACGGAAGAGTGTCTCGTTGAttcacAGAG CGTCGATCAATACCGCGAAG	
TGcw14-R-KpHis4-DHA	GACTACACCAGGGCAAGTCTCAACTAACGA CGGGAAGGATGCGTATATAC	
TPMP20-R-pPGI1	GACATCTAGTAACACAGGTGACCTACTTGTC GTTAGTTCAATGACCTGGT	
TTPI1-R-pPGD1	attccaattgatgattCTACCATAATCCACCCCTTGG GTTACAGATACAG	
KpADH900-DHA-F-TGcw14	GACTAGTATATACGCATCCTTCCCGTCGTTG TTAAGACGGTGGCTACTACCATCT	
TGcw14-R-KpADH900-DHA	TCCGCAGATGGTAGTAGCCACCGTCTTAAC AACGACGGGAAGGATGCGTATATAC	
KpADH900-DHA-2-F-KpADH900-UHA	ATATGGTTGAGAACGTTCCCTCACCCGGATC CTGGATTTACAACGCAAGACCTATGGA	Large DNA fragment knockout
KpADH900-DHA-2-R	ATAGCCATGGTTGAGTCCAATCTATGTG	
KpADH900-UHA-R-KpADH900-DAH2	ATCCATAGGTCTTGAGTTGTAAATCCAGGA TCCGGGTGAGGAACGTTCTCAACCATA	
CrtE-F	ATGGATTACGCGAACATCCTCACAGC	Construction of yeast libraries
CrtI-F	ATGGGAAAAGAACAAGATCAGG	
CrtYB-F	ATGACGGCTCTCGCATATTACC	
DAS2TT-R	GACGGGGTTCGTAAACTGGTTCCTC	
FBA1TT-R	ATGAGTGGTGATGTGTACGCTGTGAGC	
KpADH900-UHA-R	GGGTGAGGAACGTTCTCAACCATA	
pGAP-F-CrtE	GCTGTGAGGATGTTGCGGTAATCCATCTGT GTTTTGATAGttgttcaattgattg	
pGAP-F-TAOX1-2	tttgatgttttcttgGTATTTCCCACTCCTCTTCAGA GTACAGAAGATTAAGTGAAG	
pGAP-F-TDAS2-2	TTTTTTGACCAATAAATGGAACAGGAAGGAA AGGAGGAACCAAGTTTACGAACCCCGTC	
pGAP-F-TFBA1-2	ATAACACTTTGTTGTGAGACAGTAATAAAAA GCTCACAGCGTACACATCACCACATCAT	
pGAP-F2-KpADH900-UHA	GACTCTTGCGCAGGCCGTCGTACGATAAGG GCAATATGGTTGAGAACGTTCTCACCC	
pGAP-R-CrtI	CCTGATCTTGTTCTTTTCCCATcTGTGTTTTG ATAGttgttcaattgattg	
pGAP-R-CrtE-2	TATCATCCTGAGGAGTAACTCGAGTGGAA TTGCTGTGAGGATGTTGCGGTAATCCAT	

pGAP-R-Ctrl-2	CGATACCACATCCCACGATGATAGCTGTGG GTTTATCCTGATCTTGTCTTTTCCCAT
pGAP-R-CrtYB	GGTAATATGCGAGAGCCGTCATcTGTGTTTT GATAGttgttcaattgattg
pGAP-R-CrtYB-2	GACCAAGAATTGGGAGAGTATAGATCAGAT GGATCTGGTAATATGCGAGAGCCGTCAT
pGAP-R-tHMG1	GACTTCAGTTTTACCAATTGGTCCATcTGT GTTTTGATAGttgttcaattgattg
pGAP-R-tHMG1-2	TTTGTACAGGAGCAGTAAAAGACTTCTTGGT GACTTCAGTTTTACCAATTGGTCCAT
TAOX1-R	TTTCACTTAATCTTCTGTACTCTGAAGAGGA GTGG
tHMG1-F	ATGGACCAATTGGTGAAAACCTGAAGTC
pTEF1-F-KpADH900- UHA	TATGGTTGAGAACGTTCTCACCATAACTG TCGCCTCTTTTATCTGC
pTEF1-F-TAOX1	CTCTTCAGAGTACAGAAGATTAAGTGAAAAT AACTGTCGCCTCTTTTATCTGC
pTEF1-F-TDAS2	GAGGAACCAAGTTTACGAACCCCGTCATAAC TGTCGCCTCTTTTATCTGC
pTEF1-F-TFBA1	GCTCACAGCGTACACATCACCCTCATATAA CTGTCGCCTCTTTTATCTGC
pTEF1-R-Ctrl	CCTGATCTTGTTCTTTTCCCATcGTTGGCGA ATAACTAAAATGTATGTAGTGA _g
pTEF1-R-CrtE	GCTGTGAGGATGTTGCGTAATCCATCGTT GGCGAATAACTAAAATGTATGTAGTGA _g
pTEF1-R-CrtYB	GGTAATATGCGAGAGCCGTCATcGTTGGCG AATAACTAAAATGTATGTAGTGA _g
pTEF1-R-tHMG1	GACTTCAGTTTTACCAATTGGTCCATcGTT GGCGAATAACTAAAATGTATGTAGTGA _g
pPGI1-F-KpADH900- UHA	TATGGTTGAGAACGTTCTCACCCTAGGTC ACCTGTGTTACTAGATGTCGGATG
pPGI1-F-TAOX1	CCTCTTCAGAGTACAGAAGATTAAGTGAAAT AGGTCACCTGTGTTACTAGATGTCGGATG
pPGI1-F-TDAS2	GAGGAACCAAGTTTACGAACCCCGTCTAGGT CACCTGTGTTACTAGATGTCGGATG
pPGI1-F-TFBA1	GCTCACAGCGTACACATCACCCTCATTAG GTCACCTGTGTTACTAGATGTCGGATG
pPGI1-R-Ctrl	CCTGATCTTGTTCTTTTCCCATcTTTATCAAT TGGTTGGAGTTGAATTATTCCGGAAG
pPGI1-R-CrtE	GCTGTGAGGATGTTGCGTAATCCATCTTTA TCAATTGTTGGAGTTGAATTATTCCGGAAG
pPGI1-R-CrtYB	GGTAATATGCGAGAGCCGTCATcTTTATCAA TTGGTTGGAGTTGAATTATTCCGGAAG
pPGI1-R-tHMG1	GACTTCAGTTTTACCAATTGGTCCATcTTTA TCAATTGTTGGAGTTGAATTATTCCGGAAG

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Table S4. Gene list and sequence

***SpCas9* DNA sequence.**

ATGGACAAGAAGTACTCCATTGGGCTCGATATCGGCACAAACAGCGTCGGCTGGGC
CGTCATTACGGACGAGTACAAGGTGCCGAGCAAAAAATTCAAAGTTCTGGGCAATAC
CGATCGCCACAGCATAAAGAAGAACCCTCATTGGCGCCCTCCTGTTGACTCCGGGG
AGACGGCCGAAGCCACGCGGCTCAAAGAAGACGACGCGCAGATATACCCGCAG
AAAGAATCGGATCTGCTACCTGCAGGAGATCTTTAGTAATGAGATGGCTAAGGTGGAT
GACTCTTTCTTCCATAGGCTGGAGGAGTCCTTTTTGGTGGAGGAGGATAAAAAGCAC
GAGCGCCACCCAATCTTTGGCAATATCGTGGACGAGGTGGCGTACCATGAAAAGTAC
CCAACCATATATCATCTGAGGAAGAAGCTTGTAGACAGTACTGATAAGGCTGACTTGC
GGTTGATCTATCTCGCGCTGGCGCATATGATCAAATTCGGGGACACTTCCTCATCGA

GGGGGACCTGAACCCAGACAACAGCGATGTGACAAACTCTTTATCCAACCTGGTTCA
GACTTACAATCAGCTTTTTCGAAGAGAACCCGATCAACGCATCCGGAGTTGACGCCAA
AGCAATCCTGAGCGCTAGGCTGTCCAAATCCCGGCGGCTCGAAAACCTCATCGCAC
AGCTCCCTGGGGAGAAGAAGAACGGCCTGTTTGGTAATCTTATCGCCCTGTCACTCG
GGCTGACCCCCAACTTTAAATCTAACTTCGACCTGGCCGAAGATGCCAAGCTTCAAC
TGAGCAAAGACACCTACGATGATGATCTCGACAATCTGCTGGCCCAGATCGGCGACC
AGTACGCAGACCTTTTTTTGGCGGCAAAGAACCTGTCAGACGCCATTCTGCTGAGTG
ATATTCTGCGAGTGAACACGGAGATCACCAAAGCTCCGCTGAGCGCTAGTATGATCA
AGCGCTATGATGAGCACCACCAAGACTTGACTTTGCTGAAGGCCCTTGTGAGACAG
CAACTGCCTGAGAAGTACAAGGAAATTTTCTTCGATCAGTCTAAAAATGGCTACGCCG
GATACATTGACGGCGGAGCAAGCCAGGAGGAATTTTACAAATTTATTAAGCCCATCTT
GGAAAAAATGGACGGCACCGAGGAGCTGCTGGTAAAGCTTAACAGAGAAGATCTGT
TGCGCAAACAGCGCACTTTTCGACAATGGAAGCATCCCCACCAGATTCACCTGGGC
GAACTGCACGCTATCCTCAGGCGGCAAGAGGATTTCTACCCCTTTTTTAAAAGATAAC
AGGGAAAAGATTGAGAAAATCCTCACATTTTCGGATACCCTACTATGTAGGCCCCCTCG
CCCGGGGAAATTCCAGATTCGCGTGGATGACTCGCAAATCAGAAGAAACCATCACTC
CCTGGAACCTTCGAGGAAGTCGTGGATAAGGGGGCCTCTGCCAGTCCTTCATCGAA
AGGATGACTAACTTTGATAAAAATCTGCCTAACGAAAAGGTGCTTCCTAAACACTCTC
TGCTGTACGAGTACTTCACAGTTTATAACGAGCTCACCAAGGTCAAATACGTCACAGA
AGGGATGAGAAAGCCAGCATTCTGTCTGGAGAGCAGAAGAAAGCTATCGTGGACC
TCCTCTTCAAGACGAACCGGAAAGTTACCGTGAAACAGCTCAAAGAAGACTATTTCA
AAAAGATTGAATGTTTCGACTCTGTTGAAATCAGCGGAGTGGAGGATCGCTTCAACG
CATCCCTGGGAACGTATCACGATCTCCTGAAAATCATTAAAGACAAGGACTTCCTGGA
CAATGAGGAGAACGAGGACATTCTTGAGGACATTGTCCTACCCCTTACGTTGTTTGA
AGATAGGGAGATGATTGAAGAACGCTTGAAAACCTTACGCTCATCTCTTCGACGACAA
AGTCATGAAACAGCTCAAGAGGCGCCGATATACAGGATGGGGGCGGCTGTCAAGAA
AACTGATCAATGGGATCCGAGACAAGCAGAGTGGAAAGACAATCCTGGATTTTCTTA
AGTCCGATGGATTTGCCAACCGGAACTTCATGCAGTTGATCCATGATGACTCTCTCA
CCTTTAAGGAGGACATCCAGAAAGCACAAGTTTCTGGCCAGGGGGACAGTCTTCAC
GAGCACATCGCTAATCTTGCAAGGTAGCCCAGCTATCAAAAAGGGAATACTGCAGACC
GTTAAGGTCTGTTGATGAACCTGTCAAAAGTAATGGGAAGGCATAAGCCCGAGAATATC
GTTATCGAGATGGCCCGAGAGAACCAAACCTACCCAGAAGGGACAGAAGAACAGTAG
GGAAAGGATGAAGAGGATTGAAGAGGGTATAAAAGAACTGGGGTCCCAAATCCTTAA
GGAACACCCAGTTGAAAACACCCAGCTTCAGAATGAGAAGCTCTACCTGTACTACCT
GCAGAACGGCAGGGACATGTACGTGGATCAGGAACCTGGACATCAATCGGCTCTCCG
ACTACGACGTGGATCATATCGTGCCCCAGTCTTTTCTCAAAGATGATTCTATTGATAAT
AAAGTGTGACAAGATCCGATAAAAATAGAGGGAAGAGTGATAACGTCCCTCAGAA
GAAGTTGTCAAGAAAATGAAAAATTATTGGCGGCAGCTGCTGAACGCCAAACTGATC
ACACAACGGAAGTTCGATAATCTGACTAAGGCTGAACGAGGTGGCCTGTCTGAGTTG
GATAAAGCCGGCTTCATCAAAGGCAGCTTGTTGAGACACGCCAGATCACCAAGCAC
GTGGCCCAAATTCTCGATTCACGCATGAACACCAAGTACGATGAAAATGACAACTGA
TTCGAGAGGTGAAAGTTATTACTCTGAAGTCTAAGCTGGTTTCAGATTTTCAAAAGGA
CTTTCAGTTTTATAAGGTGAGAGAGATCAACAATTACCACCATGCGCATGATGCCTAC
CTGAATGCAGTGGTAGGCACTGCACTTATCAAAAAATATCCCAAGCTTGAATCTGAAT
TTGTTTACGGAGACTATAAAGTGACGATGTTAGGAAAATGATCGCAAAGTCTGAGCA
GGAAATAGGCAAGGCCACCGCTAAGTACTTCTTTTACAGCAATATTATGAATTTTTTCA
AGACCGAGATTACACTGGCCAATGGAGAGATTCGGAAGCGACCACTTATCGAAACAA
ACGGAGAAACAGGAGAAATCGTGTGGGACAAGGGTAGGGATTTGCGGACAGTCCG
GAAGGTCCTGTCCATGCCGCAGGTGAACATCGTTAAAAAGACCGAAGTACAGACCG
GAGGCTTCTCCAAGGAAAGTATCCTCCCGAAAAGGAACAGCGACAAGCTGATCGCA
CGAAAAAAGATTGGGACCCCAAGAAATACGGCGGATTCGATTCTCCTACAGTCGCT
TACAGTGTACTGGTTGTGGCCAAAGTGGAGAAAGGGAAGTCTAAAAAACTCAAAAGC
GTCAAGGAACTGCTGGGCATCACAAATCATGGAGCGATCAAGCTTCGAAAAAAACCCC
ATCGACTTTCTCGAGGCGAAAAGGATATAAAGAGGTCAAAAAAGACCTCATCATTAAAGC
TTCCCAAGTACTCTCTCTTTGAGCTTGAAAACGGCCGGAACGAATGCTCGCTAGTG
CGGGCGAGCTGCAGAAAGGTAACGAGCTGGCACTGCCCTCTAAATACGTTAATTTCT
TGTATCTGGCCAGCCACTATGAAAAGCTCAAAGGGTCCCCCGAAGATAATGAGCAGA

AGCAGCTGTTTCGTGGAACAACACAAACACTACCTTGATGAGATCATCGAGCAAATAA
GCGAATTCTCCAAAAGAGTGATCCTCGCCGACGCTAACCTCGATAAGGTGCTTTCTG
CTTACAATAAGCACAGGGATAAGCCCATCAGGGAGCAGGCAGAAAACATTATCCACT
TGTTTACTCTGACCAACTTGGGCGCGCCTGCAGCCTTCAAGTACTTCGACACCACCA
TAGACAGAAAGCGGTACACCTCTACAAAGGAGGTCCTGGACGCCACACTGATTCATC
AGTCAATTACGGGGCTCTATGAAACAAGAATCGACCTCTCTCAGCTCGGTGGAGATC
CAAAGAAGAAAAGAAAAGTTTAA

The blue bases encode the *SpCas9* protein sequence, the red bases encode the nuclear localization signal peptide *SV40*, and the black encodes the stop codon

GFP DNA sequence.

ATGAGTAAAGGAGAAGAAGAACTTTTCACTGGAGTTGTCCCAATTCTTGTTGAATTAGATG
GTGATGTTAATGGGCACAAATTTTCTGTCACTGGAGAGGGTGAAGGTGATGCTACAT
ACGGAAAGCTTACCCTTAAATTTATTTGCACTACTGGAAACTACCAAGTTCCATGGCC
AACACTTGTCACTACTTTCTCTTATGGTGTTCAATGCTTTTCCCGTTATCCGGATCATA
TGAAACGGCATGACTTTTTCAAGAGTGCCATGCCCGAAGGTTATGTACAGGAACGCA
CTATATCTTTCAAAGATGACGGGAAGTACAAGACGCGTGCTGAAGTCAAGTTTGAAG
GTGATACCCTTGTTAATCGTATCGAGTTAAAGGTATTGATTTTAAAGAAGATGGAAAC
ATTCTCGGACACAACTCGAGTACAAGTATACTCACACAATGTATACATCACGGCAG
ACAAACAAAAGAATGGAATCAAAGCTAACTTCAAAATTCGCCACAACATTGAAGATGG
CTCAGTTCAACTAGCAGACCATTATCAACAAAATACTCCAATTGGCGATGGCCCTGTC
CTTTTACCAGACAACCATTACCTGTGACACAATCTGCCCTTTCGAAAGATCCCAACG
AAAAGCGTGACCACATGGTCCTTCTTGAGTTTGTAACTGCTGCTGGGATTACACATG
GCATGGATGAACATACAAATAG

CrtI DNA sequence.

ATGGGAAAAGAACAAGATCAGGATAAACCACAGCTATCATCGTGGGATGTGGTATC
GGTGGAATCGCCACTGCCGCTCGTCTTGCTAAAGAAGGTTTCCAGGTCACGGTGTT
CGAGAAGAACGACTACTCCGGAGGTCGATGCTCTTTAATCGAGCGAGATGGTTATCG
ATTCGATCAGGGGGCCAGTTTGCTGCTCTTGCCAGATCTCTCCAAGCAGACATTCTGA
AGATTTGGGAGAGAAGATGGAAGATTGGGTCGATCTCATCAAGTGTGAACCCAACTA
TGTTTGCCACTTCCACGATGAAGAGACTTTCCTCTTTCAACCGACATGGCGTTGCT
CAAGCGGGAAGTCGAGCGTTTTGAAGGCAAAGATGGATTTGATCGGTTCTTGTCGTT
TATCCAAGAAGCCCACAGACATTACGAGCTTGCTGTCGTTTACGTCCTGCAGAAGAA
CTTCCCTGGCTTCGCAGCATTCTTACGGCTACAGTTTATTGGCCAAATCCTGGCTCTT
CACCCCTTCGAGTCTATCTGGACAAGAGTTTGTGATATTTCAAGACCGACAGATTAC
GAAGAGTCTTCTCGTTTGCAGTGATGTACATGGGTCAAAGCCCATACAGTGCGCCCG
GAACATATTCTTGCTCCAATACACCGAATTGACCGAGGGCATCTGGTATCCGAGAG
GAGGCTTTTGGCAGGTTCTTAATACTCTTCTTCAGATCATCAAGCGCAACAATCCCTC
AGCCAAGTTCAATTTCAACGCTCCAGTTTCCAGGTTCTTCTCTCTCCTGCCAAGGA
CCGAGCGACTGGTGTTGCACTTGAATCCGGCGAGGAACATCACGCCGATGTTGTGA
TTGTCAATGCTGACCTCGTTTACGCCTCCGAGCACTTGATTCCTGACGATGCCAGAA
ACAAGATTGGCCAAGTGGGTGAAGTCAAGAGAAGTTGGTGGGCTGACTTAGTTGGT
GGAAAGAAGCTCAAGGGAAGTTGCAGTAGTTTGAGCTTCTACTGGAGCATGGACCG
AATCGTGGACGGTCTGGGCGGACACAATATCTTCTTGCCGAGGACTTCAAGGGAT
CATTCGACACAATCTTCGAGGAGTTGGGTCTCCAGCCGATCCTTCCTTTTACGTGA
ACGTTCCCTCGCGAATCGATCCTTCTGCCGCTCCCGAAGGCAAAGATGCTATCGTCA
TTCTTGTCGCGTGTGGCCATATCGACGCTTCAACCCCTCAAGATTACAACAAGCTTG
TTGCTCGGGCAATGAAGTTTGTGATCCACACGCTTTCCGCCAAGCTTGGACTTCCCG
ACTTTGAAAAAATGATTGTGGCAGAGAAGGTTACAGATGCTCCCTCTTGGGAGAAAG
AATCAACCTCAAGGACGGAAGCATCTTGGGACTGGCTCACAACCTTTATGCAAGTTC
TTGGTTTCAGGCCGAGCACCAGACATCCCAAGTATGACAAGTTGTTCTTTGTCGGGG
CTTCGACTCATCCCGGAAGTGGGTTCCCATCGTCTTGGCTGGAGCCAAGTTAACT
GCCAACCAAGTTCTCGAATCCTTTGACCGATCCCCAGCTCCAGATCCCAATATGTCA
CTCTCCGTACCATATGGAACCTCTCAAATCAAATGGAACGGGTATCGATTCTCAGG
TCCAGCTGAAGTTCATGGATTTGGAGAGATGGGTATACCTTTTGGTATTGTTGATTGG
GGCCGTGATCGCTCGATCCGTTGGTGTTCTTGCTTTCTGA

CrtYB DNA sequence.

ATGACGGCTCTCGCATATTACCAGATCCATCTGATCTATACTCTCCCAATTCTTGGTCT
TCTCGGTCTGCTCACTTCCCCGATTTTGACAAAATTTGACATCTACAAAATATCGATCC
TCGTATTTATTGCGTTTAGTGCAACCACACCATGGGACTCATGGATCATCAGAAATGG
CGCATGGACATATCCATCAGCGGAGAGTGGCCAAGGCGTGTTTGAACGTTTCTAGA
TGTTCCATATGAAGAGTACGCTTTCTTTGTCAATTCAAACCGTAATCACCGGCTTGGTC
TACGTCTTGGCAACTAGGCACCTTCTCCCATCTCTCGCGCTTCCCAAGACTAGATCG
TCCGCCCTTTCTCTCGCGCTCAAGGCGCTCATCCCTCTGCCCATTATCTACCTATTTA
CCGCTCACCCCAGCCCATCGCCCGACCCGCTCGTGACAGATCACTACTTCTACATG
CGGGCACTCTCCTTACTCATCACCCCACCTACCATGCTCTTGGCAGCATTATCAGGC
GAATATGCTTTCGATTGGAAAAGTGCCCGAGCAAAGTCAACTATTGCAGCAATCATGA
TCCCGACGGTGTATCTGATTTGGGTAGATTATGTTGCTGTCTGGTCAAGACTCTTGGTC
GATCAACGATGAGAAGATTGTAGGGTGGAGGCTTGGAGGTGTACTACCCATTGAGGA
AGCTATGTTCTTCTTACTGACGAATCTAATGATTGTTCTGGGTCTGTCTGCCTGCGAT
CATACTCAGGCCCTATACCTGCTACACGGTCTGAACCTATTTATGGCAACAAAAAGATGC
CATCTTCATTTCCCTCATTACACCGCCTGTGCTCTCCCTGTTTTTTAGCAGCCGACC
ATACTCTTCTCAGCCAAAACGTGACTTGGAAGTGGCAGTCAAGTTGTTGGAGGAAAA
GAGCCGGAGCTTTTTTTGTTGCCTCGGCTGGATTTCTAGCGAAGTTAGGGAGAGGC
TGTTTGGACTATACGCATTCTGCCGGGTGACTGATGATCTTATCGACTCTCCTGAAGT
ATCTTCCAACCCGCATGCCACAATTGACATGGTCTCCGATTTTCTTACCCTACTATTTG
GGCCCCCGCTACACCCTTCGCAACCTGACAAGATCCTTTCTTCGCCTTTACTTCCTC
CTTCGCACCCTTCCCGACCCACGGGAATGTATCCCCTCCCGCCTCCTCCTTCGCTCT
CGCCTGCCGAGCTCGTTCAATTCTTACCAGAAAGGGTTCCCGTTCAATACCATTTG
CCTTCAGGTTGCTCGCTAAGTTGCAAGGGCTGATCCCTCGATACCCACTCGACGAAC
TCCTTAGAGGATACACCACTGATCTTATCTTTCTTTATCGACAGAGGCAGTCCAGGC
TCGGAAGACGCCTATCGAGACCACAGCTGACTTGCTGGACTATGGTCTATGTGTAGC
AGGCTCAGTCGCCGAGCTATTGGTCTATGTCTCTTGGGCAAGTGCACCAAGTCAGGT
CCCTGCCACCATAGAAGAAAGAGAAGCTGTGTTAGTGGCAAGCCGAGAGATGGGAA
CTGCCCTTCAGTTGGTGAACATTGCTAGGGACATTAAAGGGGACGCAACAGAAGGG
AGATTTTACCTACCACTCTCATTCTTTGGTCTTCGGGATGAATCAAAGCTTGCATCC
CGACTGATTGGACGGAACCTCGGCCTCAAGATTTCGACAAACTCCTCAGTCTATCTC
CTTCGTCCACATTACCATCTTCAAACGCCTCAGAAAGCTTCCGGTTTCAATGGAAGA
CGTACTCGCTTCCATTAGTCGCCTACGCAGAGGATCTTGCCAAACATTCTTATAAGGG
AATTGACCGACTTCCTACCGAGGTTCAAGCGGGAATGCGAGCGGCTTGC GCGAGCT
ACCTACTGATCGGCCGAGAGATCAAAGTCGTTTGGAAAGGAGACGTCGGAGAGAGA
AGGACAGTTGCCGGATGGAGGAGAGTACGGAAAGTCTTGAGTGTGGTCATGAGCG
GATGGGAAGGGCAGTAA

***CrtYB*^{W61R} DNA sequence.**

ATGACGGCTCTCGCATATTACCAGATCCATCTGATCTATACTCTCCCAATTCTTGGTCT
TCTCGGTCTGCTCACTTCCCCGATTTTGACAAAATTTGACATCTACAAAATATCGATCC
TCGTATTTATTGCGTTTAGTGCAACCACACCATGGGACTCATGGATCATCAGAAATGG
CGCAAGAACATATCCATCAGCGGAGAGTGGCCAAGGCGTGTTTGAACGTTTCTAGA
TGTTCCATATGAAGAGTACGCTTTCTTTGTCAATTCAAACCGTAATCACCGGCTTGGTC
TACGTCTTGGCAACTAGGCACCTTCTCCCATCTCTCGCGCTTCCCAAGACTAGATCG
TCCGCCCTTTCTCTCGCGCTCAAGGCGCTCATCCCTCTGCCCATTATCTACCTATTTA
CCGCTCACCCCAGCCCATCGCCCGACCCGCTCGTGACAGATCACTACTTCTACATG
CGGGCACTCTCCTTACTCATCACCCCACCTACCATGCTCTTGGCAGCATTATCAGGC
GAATATGCTTTCGATTGGAAAAGTGCCCGAGCAAAGTCAACTATTGCAGCAATCATGA
TCCCGACGGTGTATCTGATTTGGGTAGATTATGTTGCTGTCTGGTCAAGACTCTTGGTC
GATCAACGATGAGAAGATTGTAGGGTGGAGGCTTGGAGGTGTACTACCCATTGAGGA
AGCTATGTTCTTCTTACTGACGAATCTAATGATTGTTCTGGGTCTGTCTGCCTGCGAT
CATACTCAGGCCCTATACCTGCTACACGGTCTGAACCTATTTATGGCAACAAAAAGATGC
CATCTTCATTTCCCTCATTACACCGCCTGTGCTCTCCCTGTTTTTTAGCAGCCGACC
ATACTCTTCTCAGCCAAAACGTGACTTGGAAGTGGCAGTCAAGTTGTTGGAGGAAAA
GAGCCGGAGCTTTTTTTGTTGCCTCGGCTGGATTTCTAGCGAAGTTAGGGAGAGGC
TGTTTGGACTATACGCATTCTGCCGGGTGACTGATGATCTTATCGACTCTCCTGAAGT
ATCTTCCAACCCGCATGCCACAATTGACATGGTCTCCGATTTTCTTACCCTACTATTTG
GGCCCCCGCTACACCCTTCGCAACCTGACAAGATCCTTTCTTCGCCTTTACTTCCTC

CTTCGCACCCTTCCCGACCCACGGGAATGTATCCCCTCCCGCCTCCTCCTTCGCTCT
 CGCCTGCCGAGCTCGTTCAATTCTTACCGAAAGGGTTCCCGTTCAATACCATTTTCG
 CCTTCAGGTTGCTCGCTAAGTTGCAAGGGCTGATCCCTCGATACCCACTCGACGAAC
 TCCTTAGAGGATACACCACTGATCTTATCTTTCTTTATCGACAGAGGCAGTCCAGGC
 TCGGAAGACGCCTATCGAGACCACAGCTGACTTGCTGGACTATGGTCTATGTGTAGC
 AGGCTCAGTCGCCGAGCTATTGGTCTATGTCTCTTGGGCAAGTGCACCAAGTCAGGT
 CCCTGCCACCATAGAAGAAAGAGAAGCTGTGTTAGTGGCAAGCCGAGAGATGGGAA
 CTGCCCTTCAGTTGGTGAACATTGCTAGGGACATTAAAGGGGACGCAACAGAAGGG
 AGATTTTACCTACCACTCTCATTCTTTGGTCTTCGGGATGAATCAAAGCTTGCGATCC
 CGACTGATTGGACGGAACCTCGGCCTCAAGATTTGACAAACTCCTCAGTCTATCTC
 CTTTCGTCCACATTACCATCTTCAAACGCCTCAGAAAGCTTCCGGTTCAATGGAAGA
 CGTACTCGCTTCCATTAGTCGCCTACGCAGAGGATCTTGCCAAACATTCTTATAAGGG
 AATTGACCGACTTCCTACCGAGGTTCAAGCGGGAATGCGAGCGGCTTGCGCGAGCT
 ACCTACTGATCGGCCGAGAGATCAAAGTCGTTTGGAAGGAGACGTCGGAGAGAGA
 AGGACAGTTGCCGGATGGAGGAGAGTACGGAAAGTCTTGAGTGTGGTCATGAGCG
 GATGGGAAGGGCAGTAA

CrtE DNA sequence.

ATGGATTACGCGAACATCCTCACAGCAATTCCACTCGAGTTTACTCCTCAGGATGATA
 TCGTGCTCCTTGAACCGTATCACTACCTAGGAAAGAACCCTGGAAAAGAAATTCGAT
 CACAACATCATCGAGGCTTTCAACTATTGGTTGGATGTCAAGAAGGAGGATCTCGAGG
 TCATCCAGAACGTTGTTGGCATGCTACATACCGCTAGCTTATTAATGGACGATGTGGA
 GGATTTCATCGGTCCTCAGGCGTGGGTGCCTGTGGCCCATCTAATTTACGGGATTCC
 GCAGACAATAAACTGCAAACTACGTCTACTTTCTGGCTTATCAAGAGATCTTCAAG
 CTTTCGCCCAACACCGATACCCATGCCTGTAATTCCTCCTTCATCTGCTTCGTTCAAT
 CATCCGTCTCCTCTGCATCCTCCTCCTCCTCGGCCTCGTCTGAAAACGGGGGCACG
 TCAACTCCTAATTCGCAGATTCCGTTCTCGAAAGATACGTATCTTGATAAAGTGATCAC
 AGACGAGATGCTTTCCCTCCATAGAGGGCAAGGCCTGGAGCTATTCTGGAGAGATAG
 TCTGACGTGTCCTAGCGAAGAGGAATATGTGAAAATGGTTCTTGGAAGACGGGAGG
 TTTGTTCCGTATAGCGGTCAGATTGATGATGGCAAAGTCAGAATGTGACATAGACTTT
 GTCCAGCTTGTCAACTTGATCTCAATATACTTCCAGATCAGGGATGACTATATGAACCT
 TCAGTCTTCTGAGTATGCCATAATAAGAATTTTGCAGAGGACCTCACAGAAGGAAAA
 TTCAGTTTTCCCACTATCCACTCGATTTCATGCCAACCCCTCATCGAGACTCGTCATCA
 ATACGTTGCAGAAGAAATCGACCTCTCCTGAGATCCTTCACCACTGTGTAACTACAT
 GCGCACAGAAACCCACTCATTGCAATATACTCAGGAAGTCCTCAACACCTTGTGAGG
 TGCACTCGAGAGAGAACTAGGAAGGCTTCAAGGAGAGTTTCGCAGAAGCTAACTCAA
 AGATTGATCTTGGAGACGTAGAGTCGGAAGGAAGAACGGGGAAGAACGTCAAATTG
 GAAGCGATCCTGAAAAGCTAGCCGATATCCCTCTGTGA

tHMG1 DNA sequence.

ATGGACCAATTGGTGAAAACCTGAAGTCACCAAGAAGTCTTTTACTGCTCCTGTACAAA
 AGGCTTCTACACCAGTTTTAAACCAATAAAACAGTCATTTCTGGATCGAAAGTCAAAAG
 TTTATCATCTGCGCAATCGAGCTCATCAGGACCTTCATCATCTAGTGAGGAAGATGAT
 TCCCGCGATATTGAAAGCTTGGATAAGAAAATACGTCCTTTAGAAGAATTAGAAGCATT
 ATTAAGTAGTGGAATAACAAAACAAATTGAAGAACAAAGAGGTCGCTGCCTTGTTATT
 CACGGTAAGTTACCTTTGTACGCTTTGGAGAAAAAATTAGGTGATACTACGAGAGCGG
 TTGCGGTACGTAGGAAGGCTCTTTCAATTTTGGCAGAAGCTCCTGTATTAGCATCTGA
 TCGTTTACCATATAAAAATTATGACTACGACCGCGTATTTGGCGCTTGTTGTGAAAATG
 TTATAGGTTACATGCCTTTGCCCGTTGGTGTTATAGGCCCTTGTTATCGATGGTACA
 TCTTATCATATACCAATGGCAACTACAGAGGGTTGTTTGGTAGCTTCTGCCATGCGTG
 GCTGTAAGGCAATCAATGCTGGCGGTGGTGCAACAACTGTTTTAACTAAGGATGGTA
 TGACAAGAGGCCAGTAGTCCGTTTCCCAACTTTGAAAAGATCTGGTGCCTGTAAGA
 TATGGTTAGACTCAGAAGAGGGACAAAACGCAATTAAGGCTTTTAACTCTACATC
 AAGATTTGCACGTCTGCAACATATTCAAACCTTGCTAGCAGGAGATTTACTCTTCATGA
 GATTTAGAACAACACTACTGGTGACGCAATGGGTATGAATATGATTTCTAAAGGTGTCGA
 ATACTCATTAAAGCAAATGGTAGAAGAGTATGGCTGGGAAGATATGGAGGTTGTCTCC
 GTTTCTGGTAACTACTGTACCGACAAAAAACAGCTGCCATCACTGGATCGAAGGT
 CGTGGTAAGAGTGTGCTCGCAGAAGCTACTATTCCTGGTGATGTTGTCAGAAAAGTG

TTAAAAAGTGATGTTTCCGCATTGGTTGAGTTGAACATTGCTAAGAATTTGGTTGGAT
CTGCAATGGCTGGGTCTGTTGGTGGATTAAACGCACATGCAGCTAATTTAGTGACAG
CTGTTTTCTTGGCATTAGGACAAGATCCTGCACAAAATGTTGAAAGTTCCAACGTAT
AACATTGATGAAAGAAGTGGACGGTGATTTGAGAATTTCCGTATCCATGCCATCCATC
GAAGTAGGTACCATCGGTGGTGGTACTGTTCTAGAACCACAAGGTGCCATGTTGGAC
TTATTAGGTGTAAGAGGCCCGCATGCTACCGCTCCTGGTACCAACGCACGTCAATTA
GCAAGAATAGTTGCCTGTGCCGTCTTGGCAGGTGAATTATCCTTATGTGCTGCCCTA
GCAGCCGGCCATTTGGTTCAAAGTCATATGACCCACAACAGGAAACCTGCTGAACCA
ACAAAACCTAACAATTTGGACGCCACTGATATAAATCGTTTGAAAGATGGGTCCGTCA
CCTGCATTAAATCCTAA

***ERG10* DNA sequence.**

ATGTCTCAGAACGTTTACATTGTATCGACTGCCAGAACCCCAATTGGTTCATTCCAGG
GTTCTCTATCCTCCAAGACAGCAGTGGAATTGGGTGCTGTTGCTTTAAAGGCGCCT
TGGCTAAGGTTCCAGAATTGGATGCATCCAAGGATTTTGACGAAATTATTTTGGTAA
CGTTCTTTCTGCCAATTTGGGCCAAGCTCCGGCCAGACAAGTTGCTTTGGCTGCCG
GTTTGAGTAATCATATCGTTGCAAGCACAGTTAACAAGGTCTGTGCATCCGCTATGAA
GGCAATCATTTTGGGTGCTCAATCCATCAAATGTGGTAATGCTGATGTTGTCGTAGCT
GGTGGTTGTGAATCTATGACTAACGCACCATACTACATGCCAGCAGCCCGTGCGGGT
GCCAAATTTGGCCAAACTGTTCTTGTTGATGGTGTGCGAAAGAGATGGGTTGAACGAT
GCGTACGATGGTCTAGCCATGGGTGTACACGCAGAAAAGTGTGCCCGTGATTGGGAT
ATTACTAGAGAACAACAAGACAATTTTGCCATCGAATCCTACCAAAAATCTCAAAAATC
TCAAAGGAAGGTAAATTCGACAATGAAATTGTACCTGTTACCATTAAAGGGATTTAGAG
GTAAGCCTGATACTCAAGTCACGAAGGACGAGGAACCTGCTAGATTACACGTTGAAA
AATTGAGATCTGCAAGGACTGTTTTCCAAAAAGAAAACGGTACTGTTACTGCCGCTAA
CGTTTCTCAATCAACGATGGTGCTGCAGCCGTCATCTTGTTTTCCGAAAAAGTTTT
GAAGGAAAAGAATTTGAAGCCTTTGGCTATTATCAAAGGTTGGGGTGAGGCCGCTCA
TCAACCAGCTGATTTTACATGGGCTCCATCTCTTGCAAGTTCCAAAGGCTTTGAAACAT
GCTGGCATCGAAGACATCAATTCTGTTGATTACTTTGAATTCAATGAAGCCTTTTCGG
TTGTCGGTTTGGTGAACACTAAGATTTTGAAGCTAGACCCATCTAAGGTTAATGTATAT
GGTGGTGCTGTTGCTCTAGGTCACCCATTGGGTTGTTCTGGTGCTAGAGTGGTTGTT
ACACTGCTATCCATCTTACAGCAAGAAGGAGGTAAGATCGGTGTTGCCGCCATTTGTA
ATGGTGGTGGTGGTGCTTCCTCTATTGTCATTGAAAAGATATGA

***ERG8* DNA sequence.**

ATGTCAGAGTTGAGAGCCTTCAGTGCCCCAGGGAAAGCGTTACTAGCTGGTGGATAT
TTAGTTTTAGATACAAAATATGAAGCATTTGTAGTCGGATTATCGGCAAGAATGCATGC
TGTAGCCCATCCTTACGGTTCATTGCAAGGGTCTGATAAGTTTGAAGTGCGTGTGAA
AAGTAAACAATTTAAAGATGGGGAGTGGCTGTACCATATAAGTCCTAAAAGTGGCTTC
ATTCCTGTTTCGATAGGCGGATCTAAGAACCCTTTCATTGAAAAAGTTATCGCTAACGT
ATTTAGCTACTTTAAACCTAACATGGACGACTACTGCAATAGAACTTGTTGTTATTG
ATATTTTCTCTGATGATGCCTACCATTCTCAGGAGGATAGCGTTACCGAACATCGTGG
CAACAGAAGATTGAGTTTTTCATTGCGACAGAATTGAAGAAGTTCCCAAAACAGGGCT
GGGCTCCTCGGCAGGTTTAGTCACAGTTTTAACTACAGCTTTGGCCTCCTTTTTTGTA
TCGGACCTGGAAAATAATGTAGACAAATATAGAGAAGTTATTCATAATTTAGCACAAGT
TGCTCATTGTCAAGCTCAGGGTAAAATTGGAAGCGGGTTTGATGTAGCGGCGGCAG
CATATGGATCTATCAGATATAGAAGATTCCCACCCGCATTAATCTCTAATTTGCCAGATA
TTGGAAGTGCTACTTACGGCAGTAACTGGCGCATTGGTTGATGAAGAAGACTGGA
ATATTACGATTAAAAGTAACCATTTACCTTCGGGATTAACCTTTATGGATGGGCGATATTA
AGAATGGTTCAGAAACAGTAAACTGGTCCAGAAGGTAAAAAATTGGTATGATTGCGCA
TATGCCAGAAAGCTTGAAAATATATACAGAACTCGATCATGCAAATCTAGATTTATGGA
TGGACTATCTAACTAGATCGCTTACACGAGACTCATGACGATTACAGCGATCAGATAT
TTGAGTCTCTTGAGAGGAATGACTGTACCTGTCAAAGTATCCTGAAATCACAGAAGT
TAGAGATGCAGTTGCCACAATTAGACGTTCTTTAGAAAAATAACTAAAGAATCTGGT
GCCGATATCGAACCTCCCGTACAACTAGCTTATTGGATGATTGCCAGACCTTAAAAG
GAGTTCTTACTTGCTTAATACCTGGTGCTGGTGGTTATGACGCCATTGCAGTGATTAC
TAAGCAAGATGTTGATCTTAGGGCTCAAACCGCTAATGACAAAAGATTTTCTAAGGTT
CAATGGCTGGATGTAACCTCAGGCTGACTGGGGTGTTAGGAAAGAAAAAGATCCGGA

AACTTATCTTGATAAAATAA

ERG12 DNA sequence.

ATGTCATTACCGTTCTTAACTTCTGCACCGGGAAAGGTTATTATTTTTGGTGAACACTC
TGCTGTGTACAACAAGCCTGCCGTCGCTGCTAGTGTGTCTGCGTTGAGAACCCTACCT
GCTAATAAGCGAGTCATCTGCACCAGATACTATTGAATTGGAATCCCGGACATTAGC
TTAATCATAAGTGGTCCATCAATGATTTCAATGCCATCACCGAGGATCAAGTAAACTC
CAAAAATTGGCCAAGGCTCAACAAGCCACCGATGGCTTGTCTCAGGAACCTCGTTAG
TCTTTTGGATCCGTTGTTAGCTCAACTATCCGAATCCTTCCACTACCATGCAGCGTTTT
GTTTCCTGTATATGTTTGTTCCTATGCCCCCATGCCAAGAATATTAAGTTTTCTTTAA
AGTCTACTTTACCCATCGGTGCTGGGTGGGCTCAAGCGCCTCTATTTCTGTATCACT
GGCCTTAGCTATGGCCTACTTGGGGGGGTTAATAGGATCTAATGACTTGGAAAAGCT
GTCAGAAAACGATAAGCATATAGTGAATCAATGGGCCTTCATAGGTGAAAAGTGATT
CACGGTACCCCTTCAGGAATAGATAACGCTGTGGCCACTTATGGTAATGCCCTGCTAT
TTGAAAAGACTCACATAATGGAACAATAAACACAAACAATTTTAAGTTCTTAGATGATT
TCCAGCCATTCCAATGATCCTAACCTATACTAGAATTCCAAGGTCTACAAAAGATCTT
GTTGCTCGCGTTCGTGTGTTGGTCACCGAGAAATTTCTGAAGTTATGAAGCCAATT
CTAGATGCCATGGGTGAATGTGCCCTACAAGGCTTAGAGATCATGACTAAGTTAAGTA
AATGTAAAGGCACCGATGACGAGGCTGTAGAACTAATAATGAACTGTATGAACAAC
ATTGGAATTGATAAGAATAAATCATGGACTGCTTGTCTCAATCGGTGTTTCTCATCCTG
GATTAGAATTATTAATAAATCTGAGCGATGATTTGAGAATTGGCTCCACAAAACCTACC
GGTGCTGGTGGCGGCGGTTGCTCTTTGACTTTGTTACGAAGAGACATTACTCAAGAG
CAAATTGACAGCTTCAAAAAGAAATTGCAAGATGATTTTAGTTACGAGACATTTGAAAC
AGACTTGGGTGGGACTGGCTGCTGTTTGTAAAGCGCAAAAAATTTGAATAAAGATCTT
AAAATCAAATCCCTAGTATTCCAATTATTTGAAAATAAACTACCACAAAGCAACAATT
GACGATCTATTATTGCCAGGAAACACGAATTTACCATGGACTTCATAA

mMVD1 DNA sequence.

ATGACCGTTTACACAGCATCCGTTACCGCACCCGTCAACATCGCAACCCTTAAGTATT
GGGGGAAAAGGGACACGAAGTTGAATCTGCCACCAATTTCGTCCATATCAGTGACTT
TATCGCAAGATGACCTCAGAACGTTGACCTCTGCGGCTACTGCACCTGAGTTTGAAC
GCGACACTTTGTGGTTAAATGGAGAACCACACAGCATCGACAATGAAAGAACTCAAA
ATTGTCTGCGCGACCTACGCCAATTAAGAAAGGAAATGGAATCGAAGGACGCCTCAT
TGCCACATTATCTCAATGGAACTCCACATTGTCTCCGAAAATAACTTTCTACAGCA
GCTGGTTTAGCTTCCTCCGCTGCTGGCTTTGCTGCATTGGTTTCTGCAATTGCTAAGT
TATACCAATTACCACAGTCAACTTCAGAAATATCTAGAATAGCAAGAAAGGGGTCTGG
TTCAGCTTGTAGATCGTTGTTTGGCGGATACGTGGCCTGGGAAATGGGAAAAGCTGA
AGATGGTCATGATTCCATGGCAGTACAAATCGCAGACAGCTCTGACTGGCCTCAGAT
GAAAGCTTGTGTCTAGTTGTCAGCGATATTAATAAGGATGTGAGTTCCACTCAGGGT
ATGCAATTGACCGTGGCAACCTCCGAATTTAAAGAAAGAAATTGAACATGTCGTAC
CAAAGAGATTTGAAGTCATGCGTAAAGCCATTGTTGAAAAAGATTTGCCACCTTTGC
AAAGGAAACAATGATGGATTCCAACCTTTCCATGCCACATGTTTGGACTCTTTCCCT
CCAATATTCTACATGAATGACACTTCCAAGCGTATCATCAGTTGGTGCCACACCATTA
TCAGTTTTACGGAGAAACAATCGTTGCATACACGTTTGATGCAGGTCCAAATGCTGTG
TTGTACTACTTAGCTGAAAATGAGTCGAAACTCTTTGCATTTATCTATAAATTGTTTGGC
TCTGTTCTGGATGGGACAAGAAATTTACTACTGAGCAGCTTGAGGCTTTCAACCATC
AATTTGAATCATCTAACTTTACTGCACGTGAATTGGATCTTGAGTTGCAAAAGGATGTT
GCCAGAGTGATTTTAACTCAAGTCGGTTCAGGCCCAAGAAACAACGAATCTTTG
ATTGACGCAAAGACTGGTCTACCAAAGGAATAA

ERG20 DNA sequence.

ATGGCTTCAGAAAAAGAAATTAGGAGAGAGAGATTCTTGAACGTTTTCCCTAAATTAG
TAGAGGAATTGAACGCATCGCTTTTGGCTTACGGTATGCCTAAGGAAGCATGTGACT
GGTATGCCCACTCATTGAACTACAACACTCCAGGCGGTAAGCTAAATAGAGGTTTGTG
CGTTGTGGACACGTATGCTATTCTCTCAACAAGACCGTTGAACAATTGGGGCAAGA
AGAATACGAAAAGGTTGCCATTCTAGGTTGGTGCATTGAGTTGTTGCAGGCTTACTTC
TTGGTCGCCGATGATATGATGGACAAGTCCATTACCAGAAGAGGCCAACCATGTTGG
TACAAGGTTCTGAAGTTGGGGAAATTGCCATCAATGACGCATTGATGTTAGAGGCT
GCTATCTACAAGCTTTTGAATCTCACTTCAGAAACGAAAAATACTACATAGATATCAC

CGAATTGTTCCATGAGGTCACCTTCCAAACCGAATTGGGCCAATTGATGGACTTAATC
ACTGCACCTGAAGACAAAGTCGACTTGAGTAAGTTCTCCCTAAAGAAGCACTCCTTC
ATAGTTACTTTCAAGACTGCTTACTATTCTTTCTACTTGCCTGTCGCATTGGCCATGTA
CGTTGCCGGTATCACGGATGAAAAGGATTTGAAACAAGCCAGAGATGTCTTGATTCC
ATTGGGTGAATACTTCCAAATTCAAGATGACTACTTAGACTGCTTCGGTACCCAGAA
CAGATCGGTAAGATCGGTACAGATATCCAAGATAACAAATGTTCTTGGGTAATCAACA
AGGCATTGGAAC TTGCTTCCGCAGAACAAAGAAAGACTTTAGACGAAAATTACGGTA
AGAAGGACTCAGTCGCAGAAGCCAAATGCAAAAAGATTTTCAATGACTTGAAAATTGA
ACAGCTATACCACGAATATGAAGAGTCTATTGCCAAGGATTTGAAGGCCAAAATTTCT
CAGGTCGATGAGTCTCGTGGCTTCAAAGCTGATGTCTTAAGTGCCTTCTGAACAAA
GTTTACAAGAGAAGCAAA TAG

***mERG13* DNA sequence.**

ATGAAACTCTCAACTAACTTTGTTGGTGTGGTATTAAAGGAAGACTTAGGCCGCAAA
AGCAACAACAATTACACAATACAACTTGCAAATGACTGAACTAAAAAACAAAAGAC
CGCTGAACAAAAAACAGACCTCAAAATGTCGGTATTAAAGGTATCCAAATTTACATC
CCAATCAATGTGTCAACCAATCTGAGCTAGAGAAATTTGATGGCGTTTCTCAAGGTA
AATACACAATTGGTCTGGGCCAAACCAACATGTCTTTTGTCAATGACAGAGAAGATAT
CTACTCGATGTCCCTAACTGTTTTGTCTAAGTTGATCAAGAGTTACAACATCGACACC
AACAAAATTGGTAGATTAGAAGTCGGTACTGAAACTCTGATTGACAAGTCCAAGTCTG
TCAAGTCTGTCTTGATGCAATTGTTTGGTGAAAACACTGACGTCGAAGGTATTGACAC
GCTTAATGCCTGTTACGGTGGTACCAACGCGTTGTTCAACTCTTTGAACTGGATTGAA
TCTAACGCATGGGATGGTAGAGATGCCATTGTAGTTTGCGGTGATATTGCCATCTACG
ATAAGGGTGCCGCAAGACCAACCGGTGGTGCCGGTACTGTTGCTATGTGGATCGGT
CCTGATGCTCCAATTGTATTTGACTCTGTAAGAGCTTCTTACATGGAACACGCCTACG
ATTTTTACAAGCCAGATTTACAGCGCAATATCCTTACGTCGATGGTCATTTTTCTTA
ACTTGTTACGTCAAGGCTCTTGATCAAGTTTACAAGAGTTATTCCAAGAAGGCTATTTT
TAAAGGGTTGGTTAGCGATCCCGCTGGTTCGGATGCTTTGAACGTTTTGAAATATTTT
GACTACAACGTTTTCCATGTTCCAACCTGTAAATTGGTCACAAAATCATACGGTAGATT
ACTATATAACGATTTTCAAGGCCAATCCTCAATTGTTCCCAGAAGTTGACGCCGAATTA
GCTACTCGCGATTATGACGAATCTTTAACCGATAAGAACATTGAAAAAACTTTTTGTAA
TGTTGCTAAGCCATTCCACAAAGAGAGAGTTGCCCAATCTTTGATTGTTCCAACAAAC
ACAGGTAACATGTACACCGCATCTGTTTATGCCGCCTTTGCATCTCTATTAACATATGT
TGGATCTGACGACTTACAAGGCAAGCGTGTTGGTTTATTTTCTTACGGTTCCGGTTTA
GCTGCATCTCTATATTCTTGCAAATTGTTGGTGACGTCCAACATATTATCAAGGAATT
AGATATTACTAACAAATTAGCCAAGAGAATCACCGAACTCCAAAGGATTACGAAGCT
GCCATCGAATTGAGAGAAAATGCCCATTTGAAGAAGAACTTCAAACCTCAAGGTTCC
ATTGAGCATTGCAAAGTGGTGTCTTACTACTTGACCAACATCGATGACAAATTTAGAA
GATCTTACGATGTTAAAAAATAA

***mIDI1* DNA sequence.**

ATGACTGCCGACAACAATAGTATGCCCCATGGTGCAGTATCTAGTTACGCCAAATTAG
TGCAAAACCAAACACCTGAAGACATTTTGAAGAGTTTCCTGAAATTATTCCATTACA
ACAAAGACCTAATACCCGATCTAGTGAAACGTCAAATGACGAAAGCGGAGAAACATG
TTTTTCTGGTCATGATGAGGAGCAAATTAAGTTAATGAATGAAAATTGTATTGTTTTG
ATTGGGACGATAATGCTATTGGTGCCGGTACCAAGAAAGTTTGTCAATTAATGGAAAAT
ATTGAAAAGGGTTTACTACATCGTGCATTCTCCGTCTTTATTTTCAATGAACAAGGTGA
ATTACTTTTACAACAAAGAGCCACTGAAAAATAACTTTCCCTGATCTTTGGACTAACA
CATGCTGCTCTCATCCACTATGTATTGATGACGAATTAGGTTTGAAGGGTAAGCTAGA
CGATAAGATTAAGGGCGCTATTACTGCGGCGGTGAGAAAAGTAGATCATGAATTAGGT
ATTCCAGAAGATGAACTAAGACAAGGGGTAAAGTTTCACTTTTTAAACAGAATCCATTA
CATGGCACCAAGCAATGAACCATGGGGTGAACATGAAATTGATTACATCCTATTTTATA
AGATCAACGCTAAAGAAAACCTTGACTGTCAACCCAAACGTCAATGAAGTTAGAGACTT
CAAATGGGTTTTACCAAATGATTTGAAAACATGTTTGCTGACCCAAGTTACAAGTTTA
CGCCTTGGTTTAAAGATTATTTGCGAGAATTACTTATTCAACTGGTGGGAGCAATTAGAT
GACCTTTCTGAAGTGGAATGACAGGCAAATTCATAGAATGCTATAA

***MEL1* DNA sequence.**

atgtttgctttctactttctaccgcatgcatcagtttgaagggcggttttgggggtgtctccgagttacaatggccttgggtctactcc

CTACATGCGCACAGAAACCCACTCATTCTGAATATACTCAGGAAGTCCTCAACACCTTG
TCAGGTGCACTCGAGAGAGAACTAGGAAGGCTTCAAGGAGAGTTTCGCAGAAGCTAA
CTCAAAGATTGATCTTGGAGACGTAGAGTCGGAAGGAAGAACGGGGAAGAACGTCA
AATTGGAAGCGATCCTGAAAAAGCTAGCCGATATCCCTCTGT^{agc}ACGGGAAGTCTTT
ACAGTTTTAGTTAGGAGCCCTTATATATGACAGTAATGCTAGTACGTTTTGTTTTGTTA
ATTAATAACTTAGTTTATGTTAGCCTAGTATAGACTCCATCAATTTTTTTTGTATTACGT
AAGCCGCGATGATAATATCTGATGAAAAATTCCTATCAGAAAATAATTTATCAAAAGTTT
CATGCGATATGAGACTAAGTAGAATAGGGACTCCCCAAAGTGTCAGTCACAAGGGTC
GCCTCCAAAAAGGCAGATCTCATCG

The promoter P_{ADH2} was marked yellow, *CrtE* was marked blue, T_{DAS1} was marked green, and the underlined parts were the flanking homologous sequences used for assembly

fragment F2

GCTCACAGCGTACACATCACCACCTCATTTCTCTATTGAACGGcttqaaatttqgaaaccagat
gaaaaataaaaaggaatggaagaagaatgAGAAAAGGATAATTAATCTTTGGTTTAGCTAAATTCTT
CATTGCACTTTGACCTTAAAGGGGCTGATTTAAGGTTATGCCGgggaagaagaatagcGC
GATGAGCAAAGTCGATGCCTAAAGGAGTGTTTTGCTACCTCATTTAAGAAGAGAATA
GGACGTGCATCCAGCGATGCGTGCTAGGACAAAGAACCGCACTTGGCGGGTACAAA
CCTGACGTCAATTCCTGATATTATTGACATTTGAGCTGACCAATTAAGGTGCCCATCCA
CAATAGCCACCTGGATAGCGGAATGCACCCCCATTGAGTTGATCAAACCTACCATTTTG
CTTATACCTCAAGTTAATGTTGAACTACCATTCTTCACATGCTCCTCCTAGATCCCCTG
TCCCCTTTCTCCCCCTCTTTCATCCTTTAATTTGCATTTCTTGACGGTCTTCTATCCCT
AGAAAGTTTGGAACGCCTGCTATATGGTTAGGACACGACTGACTAGCTAtaaaattttcaga
ccagactctttctcttcttaaCGCAAATTTAACAGGCAGACAACAACATAGGAAAGAATCACCATA
TAGGTTGGACTCTTTACAGACGTCCTTGGCCGTTGACCATGGTGGTACAGTTGTCCA
AGTTCTACAAGTTTGTCTGAAGAATGAAGTTATTGGTCTTGGGTGCAGCTTTCATCT
GTTTCGATTTATTTCGGCTAAGAGTTTACCATTGTGTGCTCGTATGGGGAAGGGTGCAA
GGATCAGTAATACAGTCGAACCTGGAGTATCTACCATAGTGGGGATACAATGTAGTTTA
TCTGTTATCTCGATTGTTCTTAATTAAGgttttctttgatccTCTTCTAGTCCACACCTCCTAGAT
GACATTCGAGCTGCCTGGATTGGATGCCTAGGTTTATTGCCTAGTTCAATACAATTTCG
TGCGGGCTACAGTAGAAGGCCCTTACATAATCCGGAAGCATGGTCCCCCACCAAAT
TGAGAGCTTTTTTCAGCCTTCACTGGTGGTATCATTTTCGGGAGATAATAAGGTTTCGA
TTGGGAATTCCCACCAGAGAACACTATAGAGGGACCAAGCTGATGCTAGCCTGACAT
CCCCAAAGCACACTTCGTAATTGAAAACCGTTACCTCTAGCACACTGTCCAGACTAC
CCCCGTCAAAAAAACGCTCTTTTTCTCGACTAATTGAGTCTTCAACTCATCCCGTCTC
TGCCGAATTACTTGAATTCATTTACACCTCCGTTGCTTACGTACTCTCACCGGTCTC
CGGTGTACATGGATCCGCTATTGCCAGATATTTCTCATACAACAATCACCAGATCAAG
GTCGTGAACGGACCAATGGCATCCAGAGCAATCCTGAACAGATAGGGGTCCGGGCT
GTATAAAGTGAAATAACGTGACTTGAACCAGCAACTATGTCCCAGTTGTGCTACACTT
AACACGCGATTACCCCGGAGCTCACCAGGCCTCTTCCCCCTCTCATTGGAACCCCTC
CTAGCGCTTCGAAATAATGGCTGCGTACTATTTAACTGGTGCCAGTTCCCGCTGACAA
TatcctttttctctcccCTTAGTTCCCCACATATCAATTGAACATATTTTTTACACAgATGGACCA
ATTGGTGAAAACCTGAAGTCACCAAGAAGTCTTTTACTGCTCCTGTACAAAAGGCTTCT
ACACCAGTTTTTAACCAATAAAACAGTCATTTCTGGATCGAAAAGTCAAAAGTTTATCATC
TGCGAATCGAGCTCATCAGGACCTTCATCATCTAGTGAGGAAGATGATTCGCGCA
TATTGAAAGCTTGGATAAGAAAATACGTCTTTAGAGAATTAGAAGCATTATTAAGTA
GTGGAAATACAAAACAATTGAAGAACAAGAGGTCGCTGCCTTGGTTATTCACGGTA
AGTTACCTTTGTACGCTTTGGAGAAAAAATTAGGTGATACTACGAGAGCGGTTGCGGT
ACGTAGGAAGGCTCTTTCAATTTTGGCAGAAGCTCCTGTATTAGCATCTGATCGTTTA
CCATATAAAAATTATGACTACGACCGCGTATTTGGCGCTTGTGTGAAAATGTTATAGG
TTACATGCCTTTGCCCGTTGGTGTTATAGGCCCTTGGTTATCGATGGTACATCTTATC
ATATACCAATGGCAACTACAGAGGGTTGTTTGGTAGCTTCTGCCATGCGTGGCTGTAA
GGCAATCAATGCTGGCGGTGGTGCAACAACCTGTTTTAACTAAGGATGGTATGACAAG
AGGCCCAGTAGTCCGTTTCCCAACTTTGAAAAGATCTGGTGCCTGTAAGATATGGTTA
GACTCAGAAGAGGGACAAAACGCAATTAAGAAAGCTTTTAACTCTACATCAAGATTTG
CACGTCTGCAACATATTCAAACCTTGCTAGCAGGAGATTTACTCTTCATGAGATTTAGA
ACAACTACTGGTGACGCAATGGGTATGAATATGATTTCTAAAGGTGTGCAATACTCATT
AAAGCAAATGGTAGAAGAGTATGGCTGGGAAGATATGGAGGTTGTCTCCGTTTCTGG

TAACTACTGTACCGACAAAAAACAGCTGCCATCAACTGGATCGAAGGTCGTGGTAA
 GAGTGTCTGTCGCAGAAGCTACTATTCTGGTGATGTTGTCAGAAAAGTGTTAAAAAG
 TGATGTTTCCGCATTGGTTGAGTTGAACATTGCTAAGAATTTGGTTGGATCTGCAATG
 GCTGGGTCTGTTGGTGGATTAAACGCACATGCAGCTAATTTAGTGACAGCTGTTTTCT
 TGGCATTAGGACAAGATCCTGCACAAAATGTTGAAAGTTCCAAGTGTATAACATTGAT
 GAAAGAAGTGGACGGTGATTTGAGAATTTCCGTATCCATGCCATCCATCGAAGTAGGT
 ACCATCGGTGGTGGTACTGTTCTAGAACCACAAGGTGCCATGTTGGACTTATTAGGT
 GTAAGAGGCCCCGCATGCTACCGCTCCTGGTACCAACGCACGTCAATTAGCAAGAATA
 GTTGCCTGTGCCGTCTTGGCAGGTGAATTATCCTTATGTGCTGCCCTAGCAGCCGGC
 CATTGTTCAAAGTCATATGACCCACAACAGGAAACCTGCTGAACCAACAAAACCTA
 ACAATTTGGACGCCACTGATATAAATCGTTTGAAAGATGGGTCCGTACCTGCATTAA
 ATCCTAAtagcTCAAGAGGATGTCAGAATGCCATTTGCCTGAGAGATGCAGGCTTCATT
 TTTGATTACTTTTTTATTTGTAACCTATATAGTATAGGatTTTTgtcattttgttcttctcGTACGAGC
 TTGCTCCTGATCAGCCTATCTCGCAGCTGATGAATATCTTGTGGTAGGGGTTTGGGAA
 AATCATTTCGAGtttgatgttttcttgGTATTTCCACTCCTCTTCAGAGTACAGAAGATTAAAGT
 GAAACGCAGCGTTTTCTGACGGTA

The promoter P_{FBA1} was marked yellow, $tHMG1$ was marked blue, T_{AOX1} was marked green, and the underlined parts were the flanking homologous sequences used for assembly.

fragment F3

AGGAACCAGTTTACGAACCCCGTCTGCTCAAACGAGTGGAGAGGGAAATCGATTCT
 AGCAGTTAAATCAATGCTGGAAAATATTCGAGATTACCTAATCGGATCTGGAACTTACT
 TCGACCTGACATTTTCTTGCCTGGGGAGCCACGATCGATTATGTAATCAAGAATATGG
 ACAGAGGGAAACAGATTTAGCTGTCAAAGCCCCAAGAGAAGCTACCGATCAATGGAT
 GCGGATAGATAAAGAAAAgtcctttttttcattaGCCATCCGAGTTGTCCAATCAAATGTCTGC
 CTGCTACGCTGGAGAGGAATCACGCGTGTTAACATTTCGGATTGTGCGCTAAAATAA
 GCCTATTACCTACACAGTAAAACCCGGGGGGGTGCTTTGGTATCAATGACCCCGGGAT
 TTTATCCACcagttttttctttctggCAAGAGTGCATTGCATCCCCGTACAAATAGTAGCAACCT
 CCACAAGAGGAATCCCCTATGAGCGAGAAGTCCATAGTAATACCCCGCGGAAAAGA
 GATATTTTGTTCCTGTTGCCCTTGAACCTTCAGTTTCCCCCATCAGTTTATATAGTAG
 CCGGGTTCCCAATCTCTAGCCCTTCTTTCCTCCTATTTCAATCCTCTCTTCTTACGTTA
 TCTTACATTAGCgATGATGACGGCTCTCGCATATTACCAGATCCATCTGATCTATACTCT
 CCCAATTCTTGGTCTTCTCGGTCTGCTCACTTCCCCGATTTTGACAAAATTTGACATC
 TACAAAATATCGATCCTCGTATTTATTGCGTTTAGTGCAACCACACCATGGGACTCATG
 GATCATCAGAAATGGCGCATGGACATATCCATCAGCGGAGAGTGGCCAAGGCGTGTT
 TGGAACGTTTCTAGATGTTCCATATGAAGAGTACGCTTTCTTTGTCAATCAAACCGTAA
 TCACCGGCTTGGTCTACGTCTTGGCACTAGGCACCTTCTCCCATCTCTCGCGCTTC
 CCAAGACTAGATCGTCCGCCCTTCTCTCGCGCTCAAGGCGCTCATCCCTCTGCCCA
 TTATCTACCTATTTACCGCTCACCCCGACCCATCGCCCGACCCGCTCGTGACAGATC
 ACTACTTCTACATGCGGGCACTCTCCTTACTCATCACCCACCTACCATGCTCTTGGC
 AGCATTATCAGGCGAATATGCTTTCGATTGGAAAAGTGGCCGAGCAAAGTCAACTATT
 GCAGCAATCATGATCCCGACGGTGTATCTGATTTGGGTAGATTATGTTGCTGTGCGGT
 AAGACTCTTGGTCGATCAACGATGAGAAGATTGTAGGGTGGAGGCTTGGAGGTGTA
 CTACCCATTGAGGAAGCTATGTTCTTCTTACTGACGAATCTAATGATTGTTCTGGGTCT
 GTCTGCCTGCGATCATACTCAGGCCCTATACCTGCTACACGGTTCGAACCTATTTATGGC
 AACAAAAAGATGCCATCTTCATTTCCCCTCATTACACCGCCTGTGCTCTCCCTGTTTT
 TTAGCAGCCGACCATACTCTTCTCAGCCAAAACGTGACTTGGAAGTGGCAGTCAAGT
 TGTGGAGGAAAAGAGCCGGAGCTTTTTTGTGCTCGGCTGGATTTCCTAGCGAA
 GTTAGGGAGAGGCTGGTTGGACTATACGCATTCTGCCGGGTGACTGATGATCTTATC
 GACTCTCCTGAAGTATCTTCCAACCCGCATGCCACAATTGACATGGTCTCCGATTTTC
 TTACCCTACTATTTGGGCCCCCGCTACACCCTTCGCAACCTGACAAGATCCTTTCTTC
 GCCTTTACTTCTCCTTCGCACCCTTCCCGACCCACGGGAATGTATCCCTCCCGCC
 TCCTCCTTCGCTCTCGCCTGCCGAGCTCGTTCAATTCCTTACCGAAAGGGTTCCCGT
 TCAATACCATTTTCGCTTCAGGTTGCTCGCTAAGTTGCAAGGGCTGATCCCTCGATAC
 CCACTCGACGAACCTTAGAGGATACACCACTGATCTTATCTTTCCTTTATCGACAG
 AGGCAGTCCAGGCTCGGAAGACGCCTATCGAGACCACAGCTGACTTGCTGGACTAT
 GGTCTATGTGTAGCAGGCTCAGTCGCCGAGCTATTGGTCTATGTCTCTTGGGCAAGT

GCACCAAGTCAGGTCCCTGCCACCATAGAAGAAAGAGAAGCTGTGTTAGTGGCAAAG
 CCGAGAGATGGGAACTGCCCTTCAGTTGGTGAACATTGCTAGGGACATTAAAGGGG
 ACGCAACAGAAGGGAGATTTTACCTACCACTCTCATTCTTTGGTCTTCGGGATGAATC
 AAAGCTTGCGATCCCGACTGATTGGACGGAACCTCGGCCTCAAGATTTGACAAACT
 CCTCAGTCTATCTCCTTCGTCCACATTACCATTCTCAAACGCCTCAGAAAGCTTCGG
 TTCGAATGGAAGACGTACTCGCTTCATTAGTCGCCTACGCAGAGGATCTTGCCAAA
 CATTCTTATAAGGGAATTGACCGACTTCCTACCGAGGTTCAAGCGGGAATGCGAGCG
 GCTTGCGCGAGCTACCTACTGATCGGCCGAGAGATCAAAGTCGTTTGGAAGGAGA
 CGTCGGAGAGAGAAGGACAGTTGCCGGATGGAGGAGAGTACGGAAAGTCTTGAGT
 GTGGTCATGAGCGGATGGGAAGGGCAGTAAtagcGTAGTAGTAGTAGCAGAGTAT
 CTACAGTGGTGTGTATAATGTATATGAGTGTACTTACCAACCAAATTCGGTTTAGTATTT
 CGTCGACGATGATGTAGTACGAGTCCTTCGCGAATCCGTTACTCTCAAGACGGGGAA
 AAAAAACGACGAAAATGACCAACTTACTCAACTAAGCAAACCTCAAGAAACATAACAC
 TTTGTTGTGAGACAGTAATAAAAAAGCTCACAGCGTACACATCACCCTCATTTCTCTA
 TTGAACGGcttgaaatttg

The promoter P_{FBP1} was marked yellow, $CrtYB$ was marked blue, T_{FBA1} was marked green, and the underlined parts were the flanking homologous sequences used for assembly.

fragment F4

GATCATCTTAGAGAATGTCAGTGAGGATTTTTGTAGAAATGTCTTGGTGTCTCGTC
 CAATCAGGTAGCCATCTCTGAAATATCTGGCTCCGTTGCAACTCCGAACGACCTGCT
 GGCAACGTAAAATTCTCCGGGGTAAAACCTAAATGTGGAGTAATGGAACCAGAAACAT
 CTCTTCCCTTCTCTCTCCTTCCACCGCCCGTTACCGTCCCTAGGAAATTTTACTCTGC
 TGGAGAGCTTCTTCTACGGCCCCCTTGACAGCAATGCTCTTCCAGCATTACGTTGCG
 GGTAAAACGGAGGTCGTGTACCCGACCTAGCAGCCCAGGGATGGAAAAGTCCCGG
 CCGTCGCTGGCAATAATAGCGGGCGGACGCATGTCATGAGATTATTGGAAACCACCA
 GAATCGAATATAAAGGCGAACACCTTTCCCAATTTTGGTTTCTCCTGACCCAAAGAC
 TTTAAATTTAATTTATTTGTCCCTAtttcaatcaattgaacaaCTATCAAAACACAgatATGGGAA
 AAGAACAAGATCAGGATAAACCCACAGCTATCATCGTGGGATGTGGTATCGGTGGAA
 TCGCCACTGCCGCTCGTCTTGCTAAAGAAGGTTTCCAGGTCACGGTGTTTCGAGAAG
 AACGACTACTCCGGAGGTCGATGCTCTTTAATCGAGCGAGATGGTTATCGATTGATC
 AGGGGCCAGTTTGCTGCTCTTGCCAGATCTCTCCAAGCAGACATTGGAAGATTTGG
 GAGAGAAGATGGAAGATTGGGTCGATCTCATCAAGTGTGAACCCAACTATGTTTGCC
 ACTTCCACGATGAAGAGACTTTCCTCTTTCAACCGACATGGCGTTGCTCAAGCGGG
 AAGTCGAGCGTTTTGAAGGCAAAGATGGATTGATCGGTTCTTGTCGTTTATCCAAGA
 AGCCACAGACATTACGAGCTTGCTGTCTGTTACGTCCTGCAGAAGAACTTCCCTG
 GCTTCGCAGCATTCTTACGGCTACAGTTCATTGGCCAAATCCTGGCTCTTACCCCTT
 CGAGTCTATCTGGACAAGAGTTTGTGATATTTCAAGACCGACAGATTACGAAGAGTC
 TTCTCGTTTGCAGTGATGTACATGGGTCAAAGCCCATACAGTGCGCCCGGAACATATT
 CCTTGCTCCAATACACCGAATTGACCGAGGGCATCTGGTATCCGAGAGGAGGCTTTT
 GGCAGGTTTCTAATACTCTTCTTCAGATCATCAAGCGCAACAATCCCTCAGCCAAGTT
 CAATTTCAACGCTCCAGTTTCCCAGGTTCTTCTCTCTCCTGCCAAGGACCGAGCGAC
 TGGTGTTTCGACTTGAATCCGGCGAGGAACATCACGCCGATGTTGTGATTGTCAATGC
 TGACCTCGTTTACGCCTCCGAGCACTTGATTCTGACGATGCCAGAAACAAGATTGG
 CCAACTGGGTGAAGTCAAGAGAAGTTGGTGGGCTGACTTAGTTGGTGGAAAGAAGC
 TCAAGGGAAGTTGCAGTAGTTTGAGCTTCTACTGGAGCATGGACCGAATCGTGGAC
 GGTCTGGGCGGACACAATATCTTCTTGCCGAGGACTTCAAGGGATCATTCGACACA
 ATCTTCGAGGAGTTGGGTCTCCAGCCGATCCTTCTTTTACGTGAACGTTCCCTCG
 CGAATCGATCCTTCTGCCGCTCCCGAAGGCAAAGATGCTATCGTCATTCTTGTCGG
 TGTGGCCATATCGACGCTTCGAACCTCAAGATTACAACAAGCTTGTTGCTCGGGCA
 ATGAAGTTTGTGATCCACACGCTTTCGCCAAGCTTGGACTTCCCGACTTTGAAAAA
 ATGATTGTGGCAGAGAAGGTTACGATGCTCCCTCTTGGGAGAAAGAATTCAACCTC
 AAGGACGGAAGCATCTTGGGACTGGCTCACAACTTTATGCAAGTTCTTGGTTTCAGG
 CCGAGCACCAAGACATCCCAAGTATGACAAGTTGTTCTTTGTCGGGGCTTCGACTCAT
 CCCGGAAGTGGGGTTCCCATCGTCTTGGCTGGAGCCAAGTTAACTGCCAACCAAGT
 TCTCGAATCCTTTGACCGATCCCCAGCTCCAGATCCCAATATGTCACTCTCCGTACCA
 TATGGAAAACCTCTCAAATCAAATGGAACGGGTATCGATTCTCAGGTCCAGCTGAAGT

TCATGGATTGGAGAGATGGGTATACCTTTTGGTATTGTTGATTGGGGCCGTGATCGC
TCGATCCGTTGGTGTCTTCTGCTTTCTGA^{tagc}GTAGATTGGCCACTAACGGGTAGTA
GTTGTGTAAGTCTATTaaatttgattttgtTTATGGATGATCATCGTAGTGGCTATCTGTTTACC
TGTAGGACATCCTAGGGTGGGATGGTGTATGACACCCCTCAATCTTCAGATGCAAC
ACTATGTGGTAGGTCATTGACATAAGGTTTAGGAAAGACCTGTTTTTTGACCAATAAAT
GGAACAGGAAGGAAAGGAGGAACCAGTTTACGAACCCCGTCGCTCAAACGAGTG
GAGAGGGAAATC

The promoter P_{GAP} was marked yellow, $CrtI$ was marked blue, T_{DAS2} was marked green, and the underlined parts were the flanking homologous sequences used for assembly.

fragment F5

GCACCTCTGCTAACTGTAGATTAACGGCGCTCGACAACCCTTAATTCGAGATAAGCT
GGGGGAACATTTCGCGAAAAATGAAACAAGTCGGCTGTTATAGTATATTATTATAATATT
GAAAGATCTCAAAAGACTACTTATTTTTGAATGAACCAAGTATGAAATCAACCTATTTG
GGGTTGACCAAAATAAGTAAATATTAATTGTCTGAaagcacagatgcttcgttCGCAATGTTTCTA
CTCCTTTTTTACTCTTCCAGATTTTCTCGGACTCCGCGCATCGCCGTACCACTTCAAA
ACACCCAAGCACAGCATACTAAATTTTCCCTCTTCTTCTCTAGGGTGTCTGTTAATTA
CCCGTACTAAAGGTTTGGAAAAAGAAAAAGGGACCGCCTCGTTTCTTTTTCTTCGTC
GAAAAAGGCAATAAAAAATTTTATCACGTTTCTTTTTCTTGAAATTTTTTTTTTAGTTTT
TTTCTCTTTCAGTGACCTCCATTGATATTTAAGTTAATAAACGGTCTTCAATTTCTCAAG
TTTCAGTTTCATTTTTCTTGTTCTATTACAACTTTTTTTACTTCTTGTTTATTAGAAAGAA
AGCATAGCAATCTAATCTAAGGGGCGGTGTTGACAATTAATCATCGGCATAGTATATCG
GCATAGTATAATACGACAAGGTGAGGAACATAACCATGGGTAAGGAAAAGACTCACGT
TTCGAGGCCGCGATTAAATTCACATGGATGCTGATTATATGGGTATAAATGGGCTC
GCGATAATGTCGGGCAATCAGGTGCGACAATCTATCGATTGTATGGGAAGCCCGATG
CGCCAGAGTTGTTTCTGAAACATGGCAAAGGTAGCGTTGCCAATGATGTTACAGATG
AGATGGTCAGACTAACTGGCTGACGGAATTTATGCCTCTTCCGACCATCAAGCATTT
TATCCGTAATCCTGATGATGCATGGTTACTCACCCTGCGATCCCCGGCAAAACAGC
ATTCCAGGTATTAGAAGAATATCCTGATTGAGGTGAAAAATTTGTTGATGCGCTGGCA
GTGTTTCTGCGCCGGTTGCATTCGATTCTGTTTGTATTGTCCTTTTAAACAGCGATC
GCGTATTTGCGCTCGCTCAGGCGCAATCACGAATGAATAACGGTTTGGTTGATGCGA
GTGATTTTGTGACGAGCGTAATGGCTGGCCTGTTGAACAAGTCTGGAAAGAAATGC
ATAAGCTTTTGCCATTCTCACCGGATTGAGTCGTCACCTCATGGTGATTTCTCACTTGAT
AACCTTATTTTTGACGAGGGGAAATTAATAGGTTGTATTGATGTTGGACGAGTCGGAA
TCGCAGACCGATACAGGATCTTGCCATCCTATGGAACGCTCGGTGAGTTTTCTC
CTTCATTACAGAAACGGCTTTTTTCAAAAATATGGTATTGATAATCCTGATATGAATAAT
TGCAGTTTCATTTGATGCTCGATGAGTTTTTCTAACACGTCCGACGGCGGCCACGG
GTCCAGGCCTCGGAGATCCGTCCCCCTTTTCTTTGTCGATA^TCATGTAATTAGTTA
TGTCACGCTTACATTACGCGCTCCCCCACATCCGCTCTAACCGAAAAGGAAGGAG
TTAGACAACCTGAAGTCTAGGTCCCTATTTATTTTTTATAGTTATGTTAGTATTAAGAA
CGTTATTTATATTTCAAATTTTTCTTTTTTTCTGTACAGACGCGTGACGCATGTAACA
TTATACTGAAAACCTTGCTTGAGAAGGTTTTGGGACGCTCGAAGGCTTTAATTTGCTT
GAGATCCTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACC
AGCGGTGGTTTGTGTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACGG
CTTCAGCAGAGCGCAGATACCAATACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCAC
CACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAG
TGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTGGACTCAAGACGATAG
TTACCGGATAAGGCGCAGCGGTGCGGCTGAACGGGGGGTTCGTGCACACAGCCCA
GCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAA
GCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGT
CGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATA
GTCCTGTGCGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAG
GGGGGCGGAGCCTATGGAAAAGCTGGAGCTGGCCTTGTCAGATGTAATCACCG
CCGCTGAA

The promoter P_{TEF1} was marked yellow, promoter P_{EM7} was marked purple, $KanMX$ was marked blue, T_{CYC1} was marked green, Ori was marked red, $PARS1$ was marked brown and the underlined parts were the flanking homologous sequences used for assembly.

fragment F6
<u>ATGGAAAAAGCTGGAGCTGGCCTTGTCCAGATGTAATCACGCCGCTGAATGCCT</u> <u>GCCTGGGTAGACCCACAGAAATTCGTTGCAAATGTCGCCAATTCTCTAAGGGATCA</u> <u>TGGCACCTTGAGTTACTGGCTTTATAC</u>AgaaccaattttcagaacgAAAGGGCCAATCAGGT TTACAACAAATTCACCTACGGGTCTGACTACCTGGGCCCATATTGGGATCCTGGCCG TACACATTTTCAGAAATCATTTAAAGGAGTTGAATCACATCTTACTGGATAGCGAGCTTT TTGACGAAGTGAAAATTTCTAATTTTAAACAAGAGGAAGGGGTCAAAAACGGAGATAT CTTATACTtgaaaaagagatgACAATCAgtgatttcatcaatttgtATCTAGTTGGCCTTCTGTGTTTT CGTGGAAGCAGCAACGAGGAAAGGAGGGTATCCTAGATGATTTTTACAACGAACCTGA ACGACTGCTTTGAGGGGGGTAACATGAAAGTAATATGGAACCTCGTCTAGTATTTGC CAGGAGGAAGCAAAGGGTTGTATAGGCTTTAGTACTTATAGAGGAAACGGGGTTACG TGCAAGCGCGCATGCCTGAGCTTTGAGGGGGGGGACTTTCAcatcttcttctcaCACTTA GCCCTAACACAGAGAATAATAAAAAGCATTGCAAGATGAGTGTTGTGTCAGCAAGCAATA CGACATCCACGAAGGCATTATCTTTGTAATTGAATTGACCCCGGAGCTTCACGCGCC GGCTTCAGAAGGGAAATCTCAGCTCCAGATCATCTTAGAGAATGTCAGTGAGGATTT <u>TTGTAGAAATGTCTTGGTGTCTCGTCC</u> The upstream homologous arm of <i>KpKu70</i> was marked gray and the underlined parts were the flanking homologous sequences used for assembly.
fragment F7
<u>TCCCAAAGTGTCAGTCACAAGGGTCCGCTCCAAAAAGGCAGATCTCATCGCCAAC</u> <u>ATTGACAAGTACTTTTCAG</u>CagaagttcaaagagaCTAAGGCCTGATTCTGTTCCTTACTTT TTCTCGCAACGTGTTTTTTTCCACCACATTGCCTATGTTGTAATGCAATGCAGATG CTGGCCCAAGTTTTTGACGATTCTCGAAAATTGGCATTTCGTCGATGCCATTGGCCAA ACTGAAAATTCAAGACAAAATAGATTGGATTTTATCTGCAACGTCTTCCACCTACACAA CCACTCTACAACTTCAGACAAACATGTTTATAAAAGCAGCTACTAGATCCAAAATGAC AAGTTCGTTATTCTCTACTACGTTTGTGTTGGCATTGTTGGTGGCTAGCAACAAC CTCTTGCCATGTCCTGTTGACCACTCTATGAATAACGAGACTCCGCAAGAATTGAAAC CATTGCAGGCTGAATCTTCTACTAGAAAGTTGAACTCTTCCGCTTAAGTCAAATAAAA CTACTGACACAGATGATGCACAGAAACAACGGATCACGCTCTTGACTGATTAGTCCC GTCATTTTGGttctcattttctcacaGTCACCTATCAATGTATGATCACCTGGAAGGATTTCCCT ACGAtacttcaaacttttACTTGATAATATTACTCATTATGGCTCAGGAATGCAGACTGCCTGA TTCAAGACGCTGCTCTTCTTATTTAACACTTGTACACTAACCCCATGGAAGCCAGGGA AGGGAATAACCATCTCTCTGGTAATAAATCGGTCTTTATTTATGCATAGAAAAGGAATC TATTATATTTTCGTTTCAATTT<u>GGCACTCTGCTAACTGTAGATTAACGGCGCTCGACAACC</u> <u>CTTAATTTCGAGA</u> The downstream homologous arm of <i>KpKu70</i> was marked gray and the underlined parts were the flanking homologous sequences used for assembly.

26

27 Table S6. Guide RNA sequence (5'→3')

Target locus	Sequence (5'→3')
KpHis4	ATGGCTCTCTTAACGTTATC
KpADH900	CGTTCTTACCACCGTACACG
II-4	CCTAAATACTACCTAAACAG
II-5	aactttgaaacaaaagaagg
II-6	CCAATATAGGATTGAACTCG
KanMX	TTACTCACCCTGCGATCCC
crtE	GGGAGGTTTGTTCGTATAG
DNA ligase IV	GGCGGGAAACGTTACGCAGC
KpADH900-2	TGTGAAGAGATTATCGCCCC

28

29 Table S7. Plasmid sequence

pGS188 (5'→3', 9907bp)

ATTTAAATGACCCTTGTGACTGACACTTTGGGAGTCCCTATTCTACTTAGT
CTCATATCGCATGAACTTTTGATAAATTATTTTCTGATAGGAATTTTTCATC
AGATATTATCATCGCGGCTTACGTAATAACAAAAAAATTGATGGAGTCTAT
ACTAGGCTAACATAAACTAAGTTATTAATTAACAAAAACAAACGTA TAGC
ATTACTGTCATATATAAGGGCTCCTAACTAAAAGTGTAAAGACTTCCCGTCT
GAGTTAAACTTTTCTTTTCTTCTTTGGATCTCCACCGAGCTGAGAGAGGTC
GATTCTTGTTTCATAGAGCCCCGTAATTGACTGATGAATCAGTGTGGCGTC
CAGGACCTCCTTTGTAGAGGTGTACCGCTTTCTGTCTATGGTGGTGTCTGA
AGTACTTGAAGGCTGCAGGCGCGCCCAAGTTGGTCAGAGTAAACAAGTG
GATAATGTTTTCTGCCTGCTCCCTGATGGGCTTATCCCTGTGCTTATTGTA
AGCAGAAAGCACCTTATCGAGGTTAGCGTCGGCGAGGATCACTCTTTTG
GAGAATTCGCTTATTTGCTCGATGATCTCATCAAGGTAGTGTGTTGT
TCCACGAACAGCTGCTTCTGCTCATTATCTTCGGGGGACCCTTTGAGCTT
TTCATAGTGGCTGGCCAGATACAAGAAATTAACGTATTTAGAGGGCAGTGC
CAGCTCGTTACCTTTCTGCAGCTCGCCCGCACTAGCGAGCATTTCGTTTCC
GGCCGTTTTCAAGCTCAAAGAGAGAGTACTTGGGAAGCTTAATGATGAGG
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 ATAAATATACTATAACAGCCGACTTGTTTCATTTTCGCGAATGTTCCCCCAG
 C T T A T C T C G A

Annotation:

the sequence (9-258 bp) is **DAS1 terminator** (3'→5');
 the sequence (264-287 bp) is **SV40 (nuclear location signal)** (3'→5');
 the sequence (288-4391 bp) is **SpCas9** (3'→5');
 the sequence (4405-4954 bp) is **bidirectional promoter HTX1**;
 the sequence (4955-4997 bp) is **Hammerhead ribozyme** (5'→3');
 the sequence (5005-6032 bp) is **RFP expression cassette** (5'→3');
 the sequence (6040-6115 bp) is **sgRNA Scaffold** (5'→3');
 the sequence (6116-6187 bp) is **HDV ribozyme** (5'→3');

the sequence (6188-6434 bp) is **AOX1 terminator** (5'→3');
the sequence (6441-6926 bp) is **GAP promoter** (5'→3');
the sequence (6928-7719 bp) is **KpURA3** (5'→3');
the sequence (7720-7966 bp) is **AOX1 terminator** (5'→3');
the sequence (7986-8574 bp) is **Ori** (3'→5');
the sequence (8575-8822 bp) is **CYC1 terminator** (3'→5');
the sequence (8888-9262 bp) is **BleoR (encoding bleomycin Resistance Protein)** (3'→5');
the sequence (9281-9328 bp) is **EM7 promoter** (3'→5');
the sequence (9336-9722 bp) is **TEF1 promoter** (3'→5');
the sequence (9744-9907 bp) is **PARS1** (3'→5');

pGS327 (5'→3', 5838bp)

GCGaacgaagcatctgtgcttTCGACAATTAATATTTACTTATTTTGGTCAACCCCAAATAGGTT
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 G A G T C C G A G A A A A T C T G G A A G A G T A A A A A G G A G T A G A A C A T T

Annotation:

the sequence (22-185 bp) is **PARS1** (3'→5');

the sequence (211-457 bp) is **AOX1 terminator** (3'→5');

the sequence (458-529 bp) is **HDV ribozyme** (3'→5');
the sequence (530-605 bp) is **sgRNA Scaffold** (3'→5');
the sequence (613-1640 bp) is **RFP expression cassette** (3'→5');
the sequence (1648-1718 bp) is **tRNA^{Gly}** (3'→5');
the sequence (1719-2141 bp) is **promoter of *KpTEF1*** (3'→5');
the sequence (2188-2673 bp) is **GAP promoter** (5'→3');
the sequence (2675-3391 bp) is **GFP** (5'→3');
the sequence (3392-3641 bp) is **DAS2 terminator** (5'→3');
the sequence (3667-4255 bp) is **Ori** (5'→3');
the sequence (4256-4503 bp) is **CYC1 terminator** (3'→5');
the sequence (4569-5378 bp) is **kanMX** (3'→5');
the sequence (5397-5444 bp) is **EM7 promoter** (3'→5');
the sequence (5452-5838) is **TEF1 promoter** (3'→5').

pZW1 (5'→3', 5838bp)

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Annotation:

the sequence (15-401 bp) is **TEF1 promoter** (5'→3');
the sequence (409-456 bp) is **EM7 promoter** (5'→3');
the sequence (475-1284 bp) is **kanMX** (5'→3');
the sequence (1350-1597 bp) is **CYC1 terminator** (5'→3');
the sequence (1598-2186 bp) is **Ori** (5'→3');
the sequence (2206-3001bp) is **Ku70 UHA (the upstream homology arm of Ku70)** (5'→3');
the sequence (3003-3488 bp) is **GAP promoter** (5'→3');
the sequence (3493-5241 bp) is **CrtI** (5'→3');
the sequence (5246-5495 bp) is **DAS2 terminator** (5'→3');
the sequence (5496-6133 bp) is **FBP1 promoter** (5'→3');
the sequence (6138-8159 bp) is **CrtYB** (5'→3');
the sequence (8164-8413 bp) is **FBA1 terminator** (5'→3');
the sequence (8414-10098 bp) is **FBA1 promoter** (5'→3');
the sequence (10100-11677 bp) is **tHMG1** (5'→3');
the sequence (11682-11929 bp) is **AOX1 terminator** (5'→3');
the sequence (11930-13141 bp) is **ADH2 promoter** (5'→3');
the sequence (13143-14271 bp) is **CrtE** (5'→3');
the sequence (14275-14524 bp) is **DAS1 terminator** (5'→3');
the sequence (14525-15374bp) is **Ku70 DHA (the downstream homology arm of Ku70)** (5'→3');
the sequence (15394-15557 bp) is **PARS1** (3'→5').

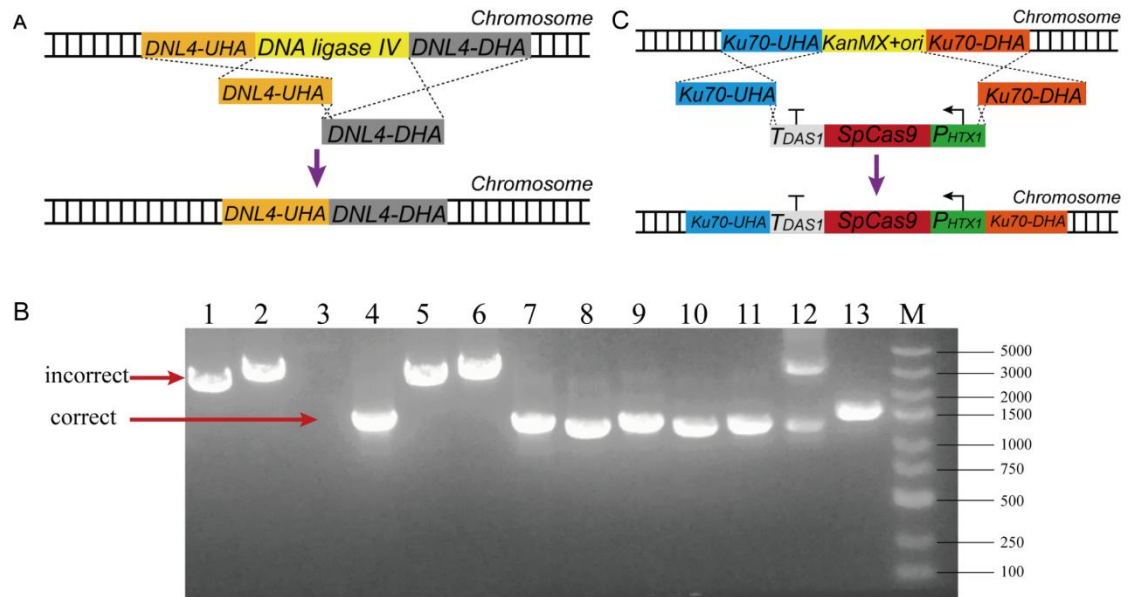


Figure S1. Knockout of *DNA ligase IV* and integration of *SpCas9*. (A) was the design of knockout *DNA ligase IV*, and (B) was a picture of agarose gel electrophoresis validation for the knockout *DNA ligase IV*. (C) The design of the *SpCas9* expression cassette integrated at the *Ku70* locus.

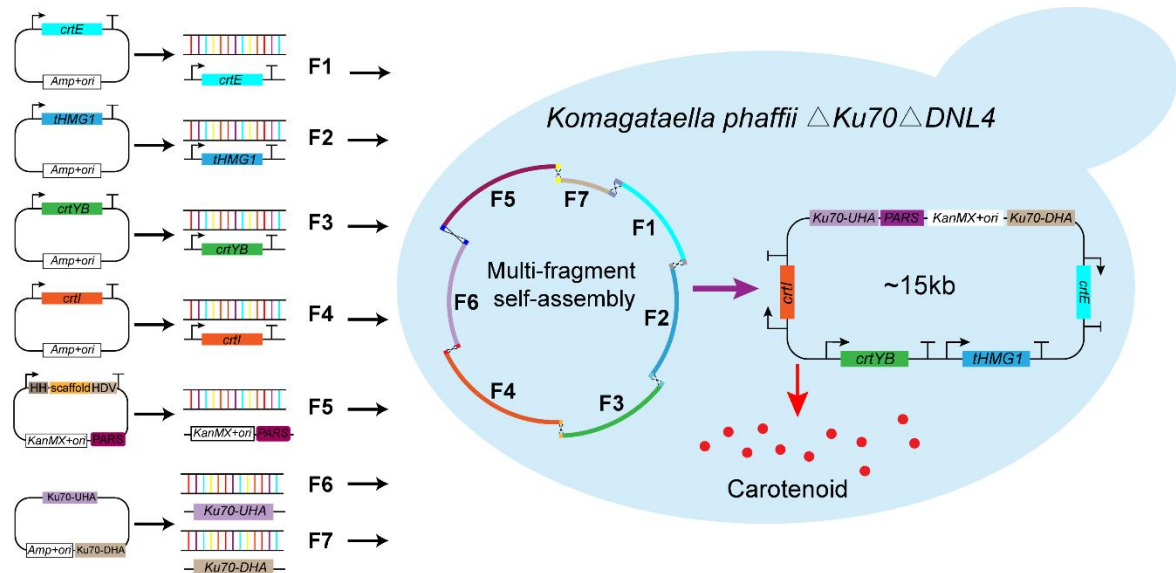


Figure S2. Design of multiple fragments spontaneously assembled intracellularly into a complete plasmid.

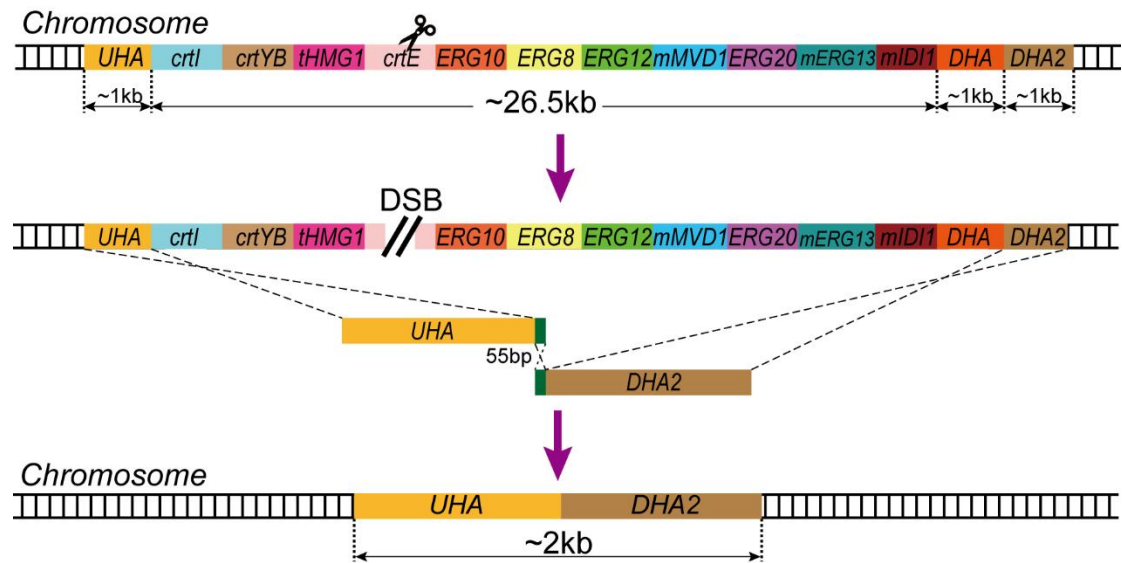


Figure S3. Design of single gRNA plasmid-mediated knockout of a large DNA fragment. The gRNA targeting crtE was designed to cleave the chromosome to form a single DSB, and simultaneously the upstream and downstream homologous arms around 1000bp were provided as repair templates for the knockout of the large DNA fragment.

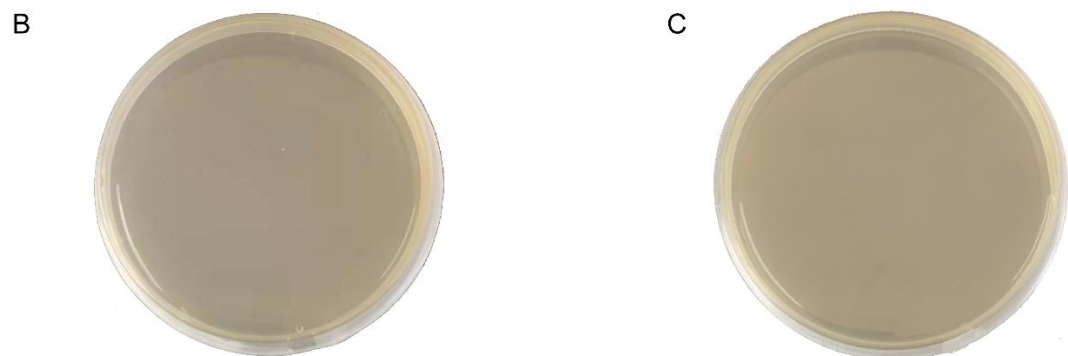
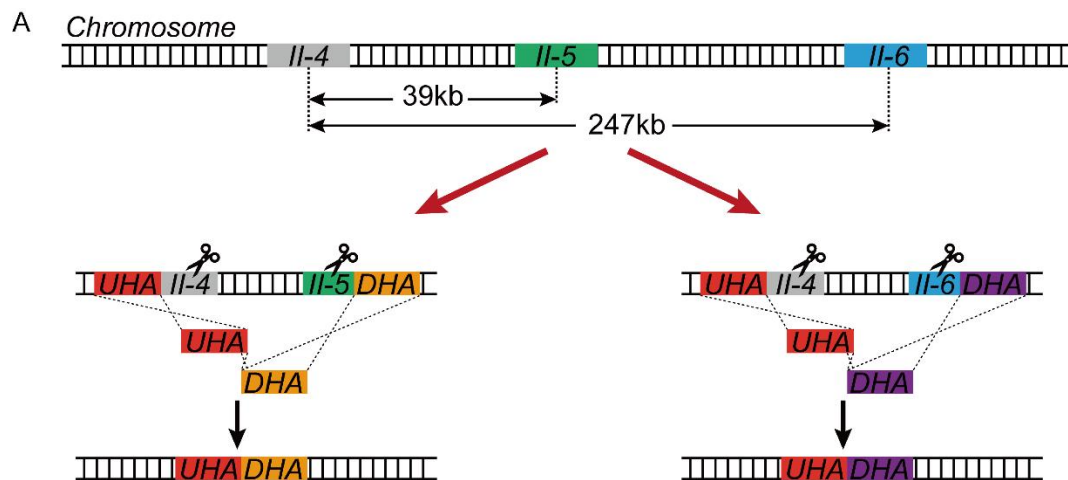


Figure S4. Design and results of double gRNA plasmid-mediated knockout of

54 larger DNA fragments. (a) Design of double gRNA plasmid-mediated knockout
55 of larger DNA fragments. (b) and (c) were the experiment results of (a), and
56 no colonies grew on the plates.
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59 Figure S5. Plate image of yeast combinatorial library. Obviously, it could be
60 observed that colonies of different color grades appeared on the plate.
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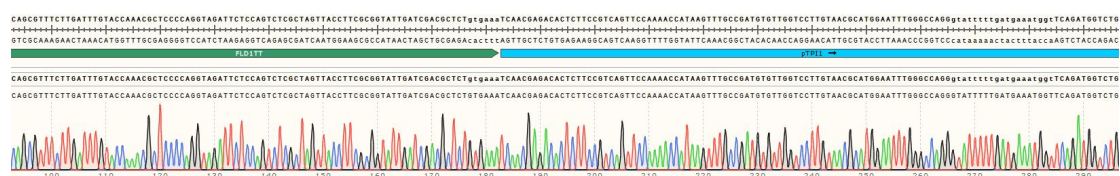
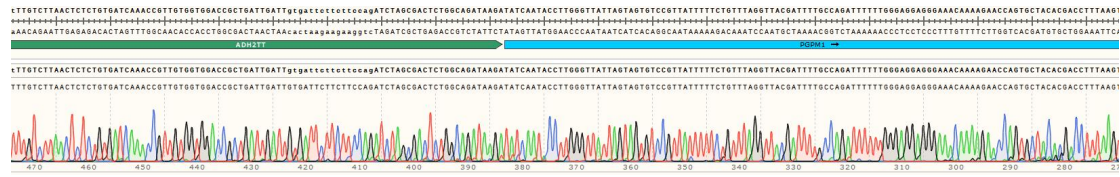
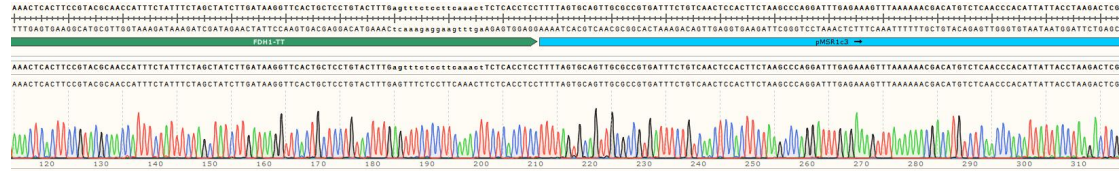
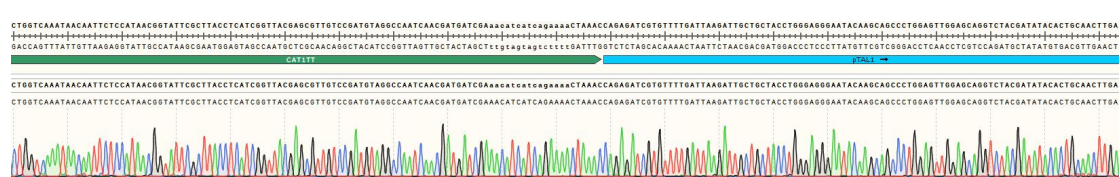
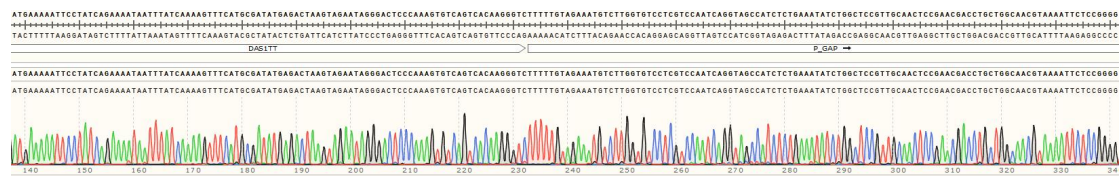
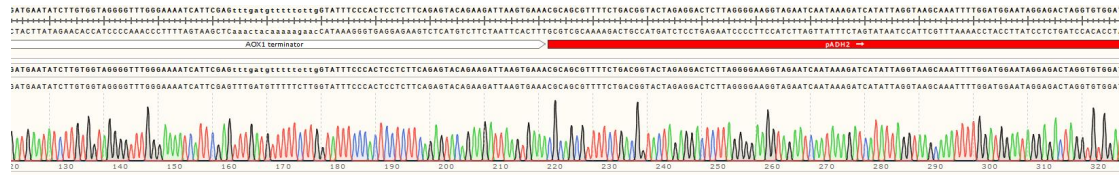
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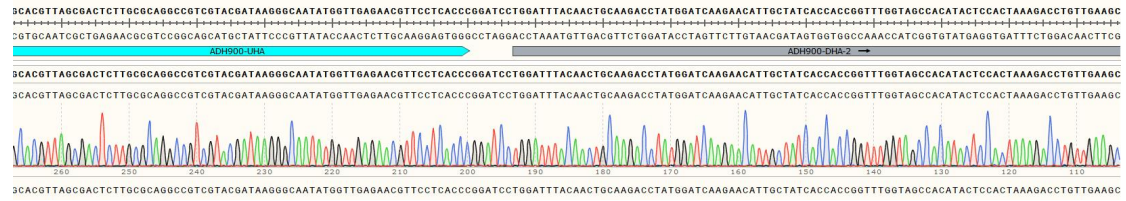


Figure S9. Sequencing results of large DNA fragment knockout by introducing two DSBs.

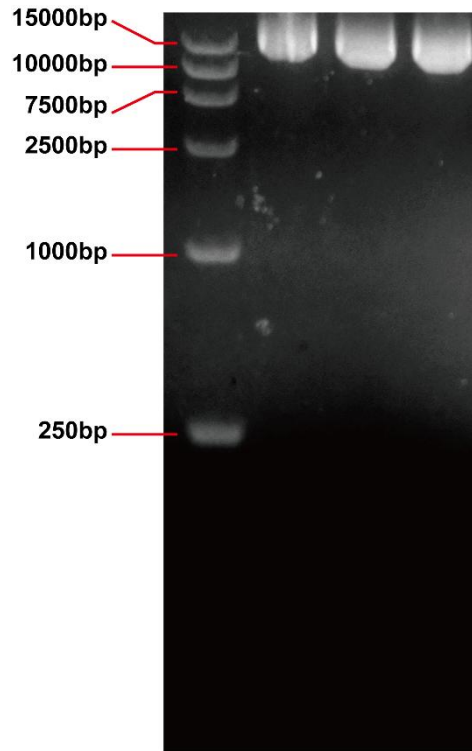
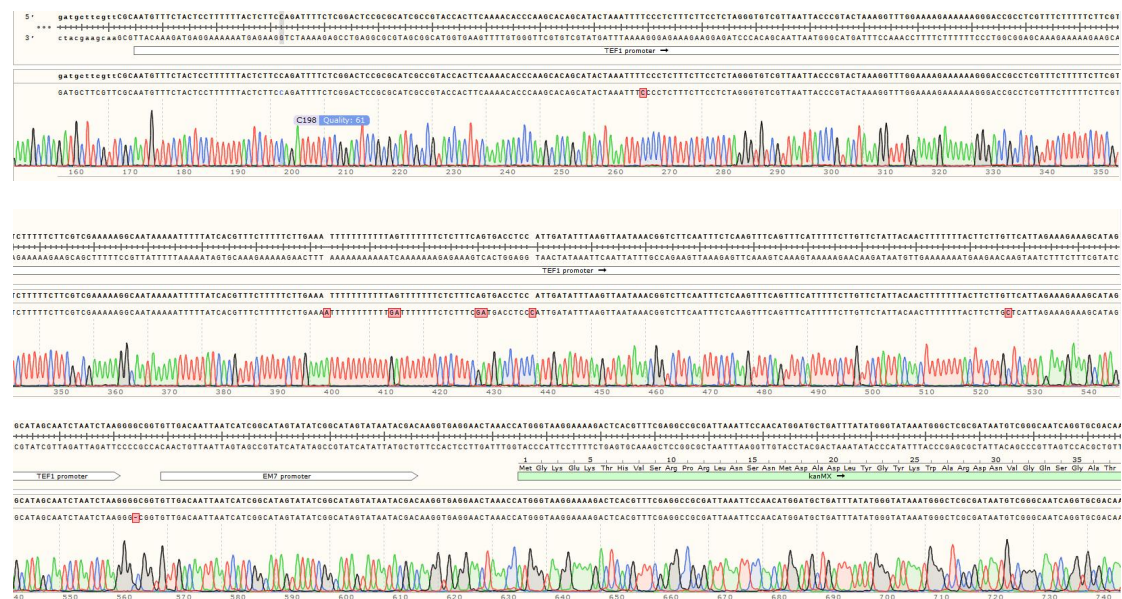
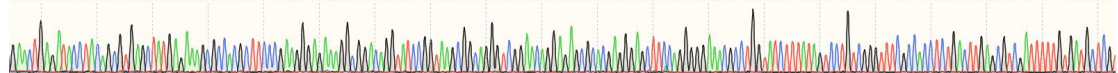
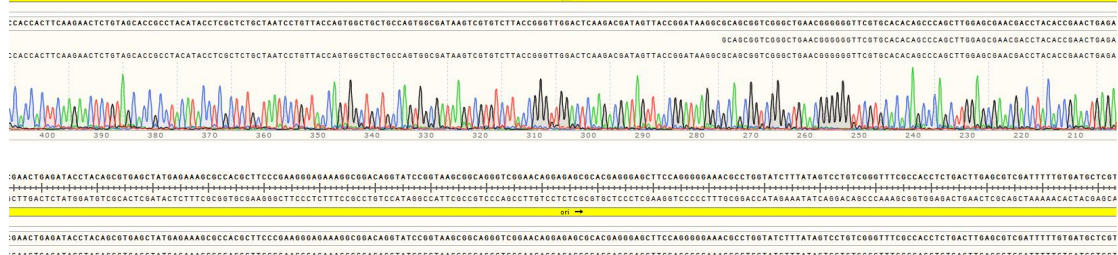
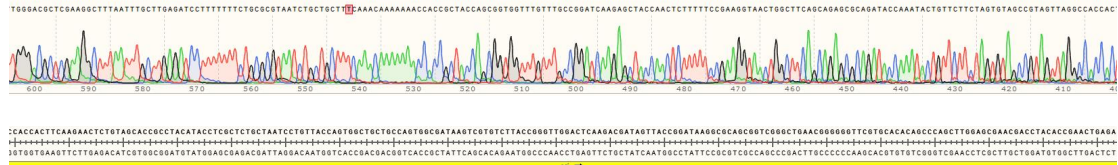
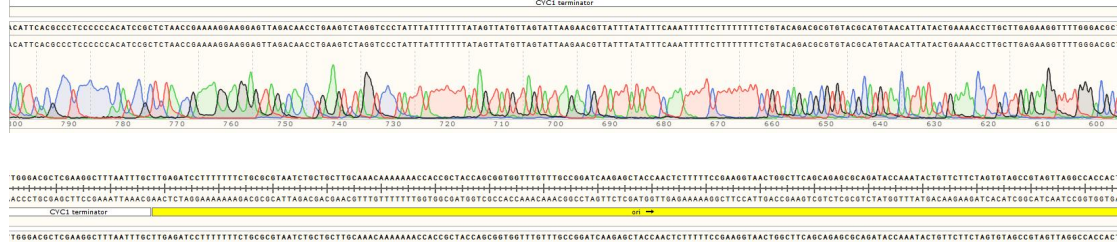
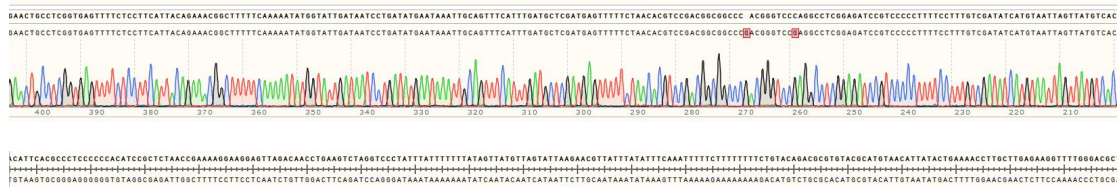
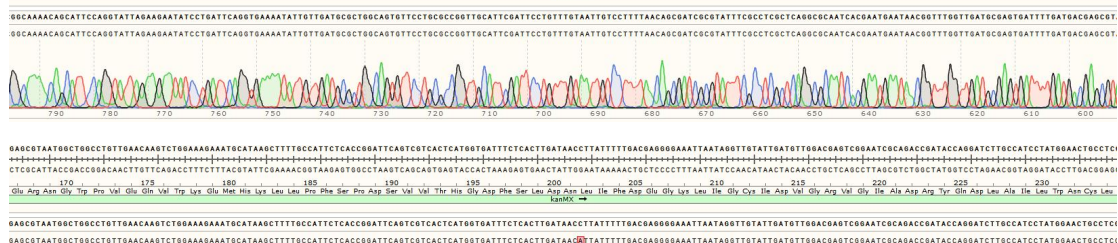


Figure S10. Gel electrophoresis result of plasmid pZW1





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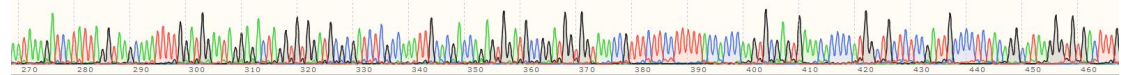
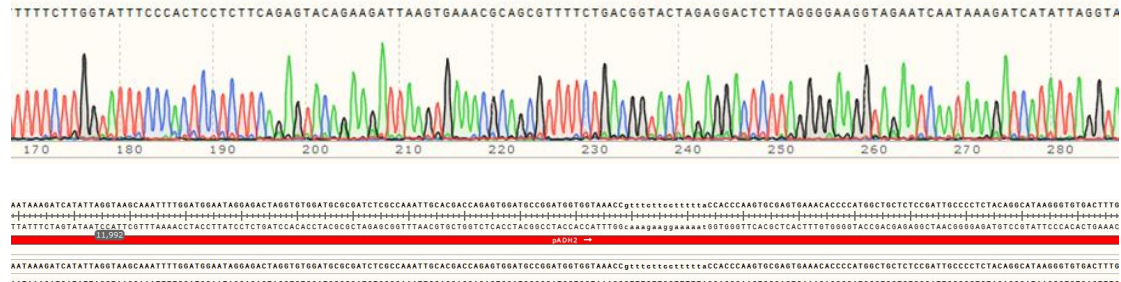
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350 340 330 320 310 300 290 280 270 260 250 240 230 220 210 200 190 180 170 160

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CTE →

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 His Thr Ala Ser Leu Leu Met Asp Asp Val Glu Asp Ser Val Leu Arg Arg Gly Ser Pro Val Ala His Leu Ile Tyr Gly Ile Pro Gln Thr Ile Asn Thr Ala Asn Val Tyr Tyr Leu Phe Ala Tyr Gln Gly Ile Phe Leu Asp Arg Pro Thr Ile Pro Met Pro Ile Val Pro Pro Ser Ser Ala Ser

[illegible]

180 190 200 210 220 230 240 250 260 270 280 290 300 310 320 330 340 350 360 370

TTGCGCTCAATCATCCGCTCTCTGCACTCTCTCTCGCGCTCGTCTGAAACGGGGACGTCGCACTCTCAATTGCGACATTCGTTCTGCAAAAGACGATGATCTGATAAGGTATGACACGACGAGATGCTTCTCTCCATGAAGGCAACGCTTGAGCATATCTTGAGGAGATGATCTGACGTCTCAAC

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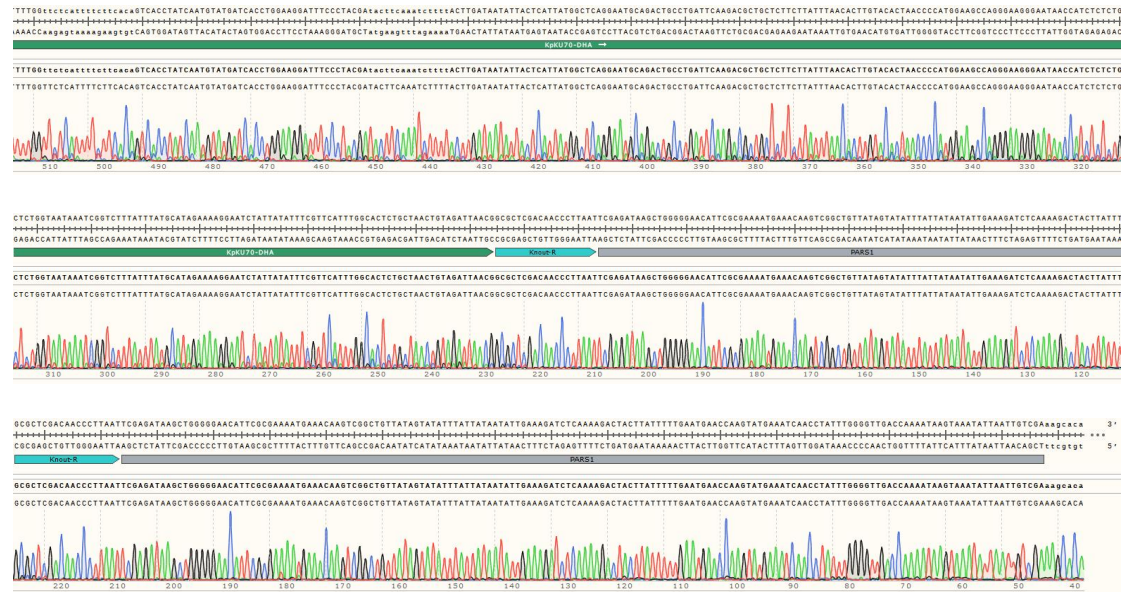


Figure S11. Complete sequencing results results of multi-fragment self-assembly into the plasmid pZW1.