

Supplementary Information

Development of a comprehensive set of tools for genome engineering in a cold- and thermo-tolerant *Kluyveromyces marxianus* yeast strain

Yumiko Nambu-Nishida^{1,2}, Keiji Nishida³, Tomohisa Hasunuma³, Akihiko Kondo^{2,3,4,*}

¹Technology Research Association of Highly Efficient Gene Design (TRAHED), 7-1-49 Minatojimaminamimachi, Chuo-ku, Kobe 650-0047, Japan

²Department of Chemical Science and Engineering, Graduate School of Engineering, Kobe University, 1-1 Rokkodai-cho, Nada-ku, Kobe, Hyogo 657-8501, Japan

³Graduate School of Science, Technology and Innovation, Kobe University, 1-1 Rokkodai-cho, Nada-ku, Kobe, Hyogo 657-8501, Japan

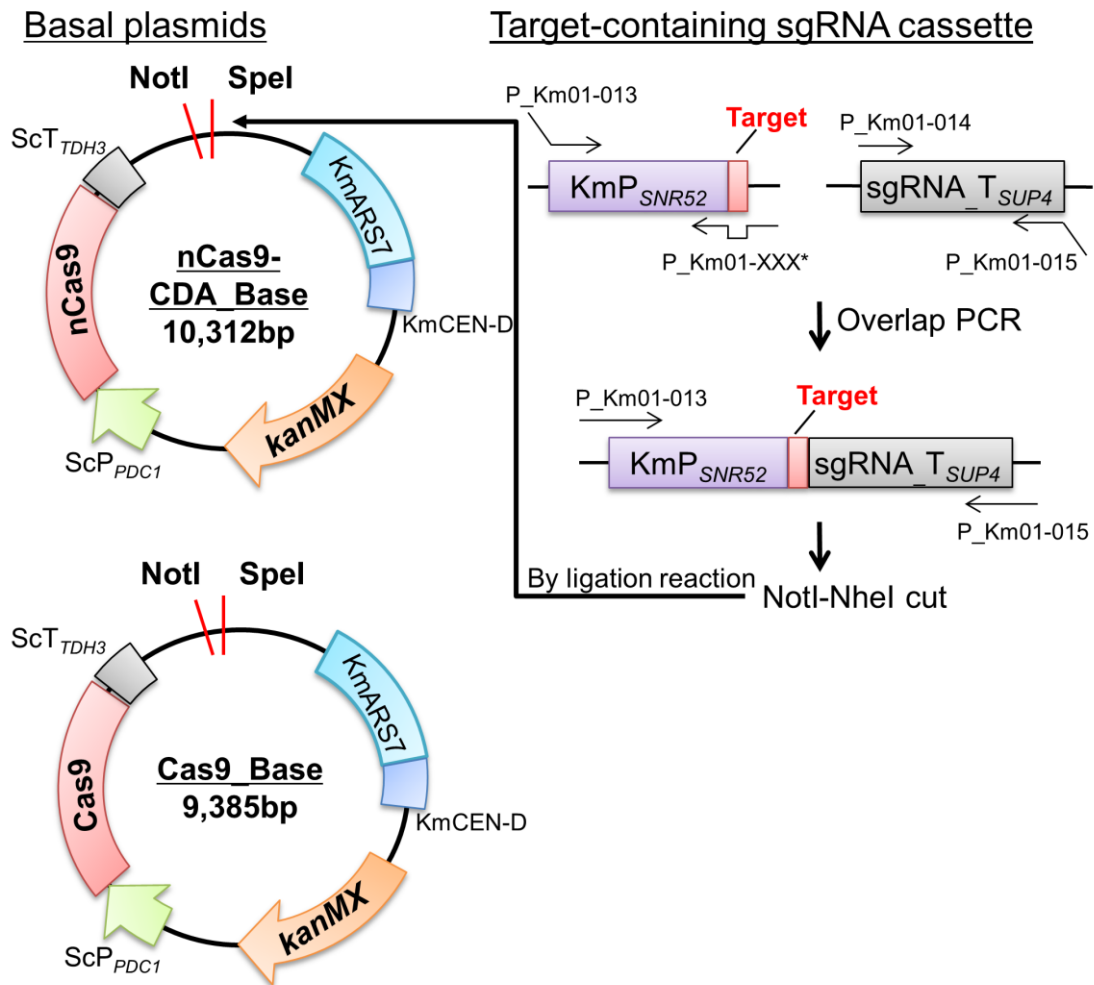
⁴ Biomass Engineering Program, RIKEN, 1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama, Kanagawa 230-0045, Japan

*akondo@kobe-u.ac.jp

AAGCTAGCTCTAGATGCCGCAAATAGGGCAGGCGATATACAAGGCGGTATACAGGGCGGTATACAAGGCG
TATGCCAGACGGTATGCCTGGAGGCATTGTCAGGAGGCATTGTCAGGAGGCATTGCCGGCGAAATCGCC
AGCTTGAGTATGGCCCCAGAGGGAAGAAAAACAGTTACCCAGGTGTGTGCCCTGTATTACCCTGACTT
TGAAATGTACGCGAAGTACACTTCGTGCAGGGTGATGATGCTCGTATCAGGTGGACTTGGCGGACACGGT
ACGGTACGGTCCCCTTTTTTATTTTTCTTTTGGGAATGAGAAGGCCACGTGACGTTACCCTGGCCCTG
TGTTTCCCCCTCGCATCTTGTACTGCCTTAGGACCCCTCGTAGTGTAGCGGTCCGGGCCCCAGATTGC
GATGTGTTTCGAGAAAAACAACAAAAAATAAGTTGAAAAGCGCGGATCAGGCGGTCCGCCCGGAGTGC
GCGCGCTGATTCTCGCCTGACAGGGCGGGATTCGCAACGTCCGCAGTTCGAATCNNNNNNNNNNNNNNN
NNNNNGTTTTAGAGCTAGAAATAGCAAGTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCAC
CGAGTCGGTGGTGCTTTTTTGTTTTTTATCACTAGTATGCGGCCGCGAATTCAGG

Supplementary Figure S1 - Sequence of target-containing sgRNA cassette.

“N” indicates target specific sequence. This sequence indicates as follows: Purple: KmP_{SNR52}, Gray: sgRNA, Green: Sup4 terminator.



Supplementary Figure S2 - Construction of genome-editing plasmids.

Constructed basal plasmids nCas9-CDA_Base and Cas9_Base are shown on the left. Target-containing sgRNA cassettes were constructed by overlap PCR using target sequence-containing primers (P_Km01-XXX, where XXX was a 3-digit number as shown in the figure) and then inserted into the basal plasmids at NotI and SpeI/NheI restriction enzyme sites.

AAAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAACTTGGTCTGACAGTTACCAATGCTTAAT
CAGTGAGGCACCTATCTCAGCGATCTGTCTATTTGTTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAG
ATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGAACCACGCTCAC
CGGCTCCAGATTTATCAGCAATAAACAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCTGCAACTTT
ATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTG
CGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTACGCTCGTCGTTTGGTATGGCTTCATTCAGCT
CCGGTCCCAACGATCAAGGCGAGTTACATGATCCCCATGTTGTGCAAAAAGCGGTTAGCTCCTTCGG
TCCTCCGATCGTTGTGAGAAGTAAGTTGGCCGAGTGTATCACTCATGGTTATGGCAGCACTGCATAAT
TCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTACTCAACCAAGTCATTCTGAG
AATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGCGTCAATACGGGATAATACCGCGCCACATAGCAG
AACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACCTCAAGGATCTTACCGCTGTTG
AGATCCAGTTCGATGTAACCCACTCGTGCACCAACTGATCTTCAGCATCTTTACTTTCACCAGCGTTT
CTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAGGAATAAGGGCGACACGGAAATGTTGAAT
ACTCATACTCTTCTTTTTCAATATTATTGAAGCATTATCAGGGTATTGTCTCATGAGCGGATACATA
TTTGAATGTATTTAGAAAAATAAACAAATAGGGTTCCGCGTTAATTAAT TGAGATAAGCACACTGCACC
CATACTTCTTAAAAACGTAGCTTCCAGTTTTTGGTGGTTCGGCTTCTTCCCGATTCCGCCCGCTAA
ACGCATATTTTTGTTGCCTGGTGGCATTGCAAAATGCATAACCTATGCATTTAAAGATTATGTATGCT
CTTCTGACTTTTCGTGTGATGAGGCTCGTGGAAAAATGAATAATTTATGAATTTGAGAACAATTTGTG
TTGTTACGGTATTTTACTATGGAATAATCAATCAATTGAGGATTTTATGCAAATATCGTTTGAATATTTT
TCCGACCTTTGAGTACTTTTCTTATAATTGCATAATATTGTCCGCTGCCCTTTTTCTGTTAGACGGT
GTCTTGATCTACTTGCTATCGTTCAACACCACCTATTTTCTAACTATTTTTTTTTTAGCTCATTGAAT
CAGCTTATGGTATGGCACATTTTTGCATAAACCTAGCTGCCTCGTTGAACATAGGAAAAAAAAATATA
TAAACAAGGCTCTTCACTCTCCTTGCAATCAGATTTGGGTTTGTCCCTTTATTTTCATATTTCTTGTC
ATATTCTTTCTCAATTATTATTTTCTACTCATAACCTCAGCAAAATAACACAGTCAAATCAATCAAAG
CTAGCGAAATGGCACCGAAGAAGAAGCGTAAAGTCGGAATCCACGGAGTTCCTGCGGCAATGGACAAGAA
GTACTCCATTGGGCTCGATATCGGCACAAACAGCGTCGGTGGGCCGTCATTACGGACGAGTACAAGGTG
CCGAGCAAAAATTCAAAGTTCTGGGCAATACCGATCGCCACAGCATAAAGAAGAACCTCATTGGCGCCC
TCCTGTTGACTCCGGGGAGACGGCCGAAGCCACGCGCTCAAAGAACAGCACGGCGCAGATATACCCG
CAGAAAGAATCGGATCTGCTACCTGCAGGAGATCTTTAGTAATGAGATGGCTAAGGTGGATGACTCTTC
TTCCATAGGCTGGAGGAGTCCTTTTTGGTGGAGGAGGATAAAAAGCACGAGCGCCACCCAATCTTTGGCA
ATATCGTGGACGAGGTGGCGTACCATGAAAAGTACCCAACCATATATCATCTGAGGAAGAAGCTTGTAGA
CAGTACTGATAAGGCTGACTTGCGGTTGATCTATCTCGCGCTGGCGCATATGATCAAATTTCCGGGACAC

TTCTCATCGAGGGGGACCTGAACCCAGACAACAGCGATGTCGACAACTCTTTATCCAACGGTTCAGA
CTTACAATCAGCTTTTTCGAAGAGAACCCGATCAACGCATCCGGAGTTGACGCCAAAGCAATCCTGAGCGG
TAGGCTGTCCAAATCCCGGGGCTCGAAAACCTCATCGCACAGCTCCCTGGGGAGAAGAAGAACGGCCTG
TTTGGTAATCTTATCGCCCTGTCACTCGGGCTGACCCCAACTTTAAATCTAACTTCGACCTGGCCGAAG
ATGCCAAGCTTCAACTGAGCAAAGACACCTACGATGATGATCTCGACAATCTGCTGGCCAGATCGGCCA
CCAGTACGCAGACCTTTTTTTGGCGGCAAAGAACCTGTCAGACGCCATTCTGCTGAGTGATATTCTGCGA
GTGAACACGGAGATCACCAAAGCTCCGCTGAGCGCTAGTATGATCAAGCGCTATGATGAGCACCACCAAG
ACTTGACTTTGCTGAAGGCCCTTGTGAGACAGCAACTGCCTGAGAAGTACAAGGAAATTTTCTCGATCA
GTCTAAAAATGGCTACGCCGGATACATTGACGGCGGAGCAAGCCAGGAGGAATTTACAAATTTATTAAG
CCCATCTTGGAAAAATGGACGGCACCGAGGAGCTGCTGGTAAAGCTTAACAGAGAAGATCTGTTGCGCA
AACAGCGCACTTTCGACAATGGAAGCATCCCCACCAGATTCACCTGGGCGAACTGCACGCTATCCTCAG
GCGGCAAGAGGATTTCTACCCCTTTTGAAGATAACAGGAAAAGATTGAGAAAATCCTCACATTTGCG
ATACCCTACTATGTAGGCCCCCTCGCCCGGGGAAATTCAGATTCGCGTGGATGACTCGCAAATCAGAAG
AGACCATCACTCCCTGGAACCTCGAGGAAGTCGTGGATAAGGGGGCCTCTGCCAGTCTTCATCGAAAG
GATGACTAACTTTGATAAAAAATCTGCCTAACGAAAAGGTGCTTCCTAACACTCTCTGCTGTACGAGTAC
TTCACAGTTTATAACGAGCTCACCAAGGTCAAATACGTCACAGAAGGGATGAGAAAGCCAGCATTCTGT
CTGGAGAGCAGAAGAAAGCTATCGTGGACCTCCTCTTCAAGACGAACCGGAAAGTTACCGTGAAACAGCT
CAAAGAAGACTATTTCAAAAAGATTGAATGTTTCGACTCTGTTGAAATCAGCGGAGTGGAGGATCGCTTC
AACGCATCCCTGGGAACGTATCACGATCTCCTGAAAATCATTAAAGACAAGGACTTCCTGGACAATGAGG
AGAACGAGGACATTCTTGAGGACATTGTCCTCACCCTTACGTTGTTTGAAGATAGGGAGATGATTGAAGA
ACGCTTGAAAACCTACGCTCATCTCTTCGACGACAAAGTCATGAAACAGCTCAAGAGGGCGCGATATACA
GGATGGGGGCGGCTGTCAAGAAAACCTGATCAATGGGATCCGAGACAAGCAGAGTGAAAGACAATCCTGG
ATTTTCTTAAGTCCGATGGATTTGCCAACCGGAACTTCATGCAGTTGATCCATGATGACTCTCTCACCTT
TAAGGAGGACATCCAGAAAGCACAAGTTTCTGGCCAGGGGACAGTCTTACGAGCACATCGCTAATCTT
GCAGGTAGCCCAGCTATCAAAAAGGGAATACTGCAGACCGTTAAGGTCGTGGATGAACTCGTCAAAGTAA
TGGGAAGGCATAAGCCCGAGAATATCGTTATCGAGATGGCCCGAGAGAACCAAACCTACCCAGAAGGGACA
GAAGAACAGTAGGGAAAGGATGAAGAGGATTGAAGAGGGTATAAAGAAGCTGGGGTCCCAAATCCTTAAG
GAACACCCAGTTGAAAACACCCAGCTTCAGAATGAGAAGCTCTACCTGTACTACCTGCAGAACGGCAGGG
ACATGTACGTGGATCAGGAACTGGACATCAATCGGCTCTCCGACTACGACGTGGATCATATCGTGCCCA
GTCTTTTCTCAAAGATGATTCTATTGATAATAAAGTGTGACAAGATCCGATAAAAAATAGAGGGAAGAGT
GATAACGTCCCCTCAGAAGAAGTTGTCAAGAAAATGAAAATTATTGGCGGCAGCTGCTGAACGCCAAAC
TGATCACACAACGGAAAGTTCGATAATCTGACTAAGGCTGAACGAGGTGGCCTGTCTGAGTTGGATAAAGC

CGGCTTCATCAAAAGGCAGCTTGTGAGACACGCCAGATCACCAAGCACGTGGCCCAAATTCTCGATTCA
CGCATGAACACCAAGTACGATGAAAATGACAACTGATTCGAGAGGTGAAAGTTACTCTGAAGTCTA
AGCTGGTCTCAGATTTAGAAAAGGACTTTCAGTTTTATAAGGTGAGAGAGATCAACAATTACCACCATGC
GCATGATGCCTACCTGAATGCAGTGGTAGGCACTGCACTTATCAAAAAATATCCCAAGCTTGAATCTGAA
TTTGTTTACGGAGACTATAAAGTGTACGATGTTAGGAAAATGATCGCAAAGTCTGAGCAGGAAATAGGCA
AGGCCACCGCTAAGTACTTCTTTTACAGCAATATTATGAATTTTTCAAGACCGAGATTACACTGGCCAA
TGGAGAGATTCGGAAGCGACCACTTATCGAAACAAACGGAGAAACAGGAGAAATCGTGTGGGACAAGGGT
AGGGATTTTCGCGACAGTCCGGAAGGTCCTGTCCATGCCGCAGGTGAACATCGTTAAAAAGACCGAAGTAC
AGACCGGAGGCTTCTCCAAGGAAAAGTATCCTCCCGAAAAGGAACAGCGACAAGCTGATCGCACGCAAAAA
AGATTGGGACCCCAAGAAATACGGCGGATTCGATTCTCCTACAGTCGTTACAGTGTACTGGTTGTGGCC
AAAGTGGAGAAAAGGAAGTCTAAAAACTCAAAGCGTCAAGGAACTGCTGGGCATCACAATCATGGAGC
GATCAAGCTTCGAAAAAACCCCATCGACTTCTCGAGGCCGAAAGGATATAAAGAGGTCAAAAAAGACCT
CATCATTAAAGCTTCCCAAGTACTCTCTTTGAGCTTGAAAACGGCCGAAACGAATGCTCGCTAGTGCG
GGCGAGCTGCAGAAAAGGTAACGAGCTGGCACTGCCCTCTAAATACGTTAATTTCTGTATCTGGCCAGCC
ACTATGAAAAGCTCAAAGGGTCTCCCGAAGATAATGAGCAGAAGCAGCTGTTCTGGAACAACACAAACA
CTACCTTGATGAGATCATCGAGCAAATAAGCGAATTCTCCAAAAGAGTGATCCTCGCCGACGCTAACCTC
GATAAGGTGCTTTCTGCTTACAATAAGCACAGGGATAAGCCATCAGGGAGCAGGCAGAAAACATTATCC
ACTTGTTTACTCTGACCAACTTGGGCGCGCTGCAGCCTCAAGTACTTCGACACCACCATAGACAGAAA
GCGGTACACCTCTACAAAAGGAGGTCCTGGACGCCACACTGATTCATCAGTCAATTACGGGGCTCTATGAA
ACAAGAATCGACCTCTCTCAGCTCGGTGGAGACAGCAGGGCTGACCCCAAGAAGAAGAGGAAGGTGGCAT
GCATCCAGGCTAGCGTGAATTTACTTTAAATCTTGCAATTAATAAATTTTCTTTTTATAGCTTTATGAC
TTAGTTTCAATTTATATACTATTTAATGACATTTTCGATTCATTGATTGAAAGCTTTGTGTTTTTTCTT
GATGCGCTATTGCATTGTTCTTGTCTTTTTCGCCACATGTAATATCTGTAGTAGATACCTGATACATTGT
GGATGCTGAGTGAAATTTTAGTTAATAATGGAGGCGCTCTTAATAATTTGGGGATATTGGCTTTTTTT
TTAAAGTTTACAAATGAATTTTTTCCGCCAGGATGCGGCCGCTCTAGACTAGTCCTAGGGGATCCGTCAC
CCGGCCAGCGGAATTCAGGGATGATCTTGAGAAGTTCTTAGAGTCTTACGAGGGAACAGAAGATTTGGAA
CCAGCCAAAGCTGCTATAGCGGAAGCAGATATTTTGCTAAGTAAATGATTAATAATTAATATGTGGAA
ATACATTAATCTTTTTATATTTTTGCAGTTCGTTGTCGCTATAATTTATAGTCATCTCGTTAGTTCAAAC
AAGACTTCTGAAGTGAAAACCACTTTCAGTCTTCAAATAAAAAATGAAAATCAGTGAAGAAGGTAA
CGACTTCATGTTATATATGAATTGAATAGTAATGAAAATAACCAAAAACAGCTCAACAGAAAACAAACAA
AATACGTTAAGACCTGAACTCCTAGCAGAACCATAACTGCCAAATATTTATTATCTGTGGAGATCTTATA
TTCTAAAACCAAAAAAATAAATTAAGTAAAAAGAAGATGTTCTAACTGAGGTTCGAACTCAGGA

CCTTTGCCGTGTGAAGGCAACGTGATAGCCACTACACTATTAGAACTACCTTATGGGAAAAGAAAATA
GAGTACAAGTGAATGGTAAGATCTGTGACCTTTTCTAAACACTTAATTCCATATAGACAGTCCACCC
ACCATAAGGTCACAATTATAATGTCTTTAGAAGACCACTGTCGTTTCATCATCTTCTAAGCCCTCTCT
AAAGCGGCATATTTCCGTAATTTGTTCTTCTTTGCACAGGCACGTGAGATGACTCCGATTATCCCACAT
GCATATTTAGCCTCTCTAGGGGCTCGAGCTATAGCAAGTCAAGGAAAGAAAAGTATTATGATCTGGTCA
CGTGATAAAAATTTATTAATTTTAAAACTATATAATTTATTATTTTTATTTTAAAGTTTAAAGTAATT
TTAATAGTATTCTATATTTTAAATAAACATACTTTAAAAATTTATTTAATAATTTATTATTTTTAAATAC
AATGTTTTTATTTAAAAACAAAATTATAAGTTAAAAAGTTGTTCCGAAAGTAAAATATATTTTATAGGACG
TCGACATGGAGGCCAGAATACCCTCCTTGACAGTCTTGACGTGCGCAGCTCAGGGGCATGATGTGACTG
TCGCCGTACATTTAGCCCATACATCCCCATGTATAATCATTGTCATCCATACATTTTGATGGCCGCACG
GCGGAAGCAAAAATTACGGCTCCTCGCTGCAGACCTGCGAGCAGGAAACGCTCCCCTCACAGACGCGT
TGAATTGTCCCACGCCGCCCTGTAGAGAAATATAAAAGGTTAGGATTTGCCACTGAGGTTCTTCTT
TCATATACTTCCTTTTAAAACTTGCTAGGATACAGTTCTCACATCACATCCGAACATAAACAACCATGG
GTAAGGAAAAGACTCACGTTTTGAGGCCGCGATTAAATCCAACATGGATGCTGATTTATATGGGTATAA
ATGGGCTCGCGATAATGTGGGCAATCAGGTGCGACAATCTATCGATTGTATGGGAAGCCCGATGCGCCA
GAGTTGTTTCTGAAACATGGCAAAGGTAGCGTTGCCAATGATGTTACAGATGAGATGGTCAGACTAACT
GGCTGACGGAATTTATGCCTCTTCCGACCATCAAGCATTTTATCCGTAATCCTGATGATGCATGGTTACT
CACCCTGCGATCCCCGGCAAAACAGCATTCCAGGTATTAGAAGAATATCCTGATTCAGGTGAAAATATT
GTTGATGCGCTGGCAGTGTTCCTGCGCCGTTGCATTGATTCTGTTTGTAAATTGCTTTTAAACAGCG
ATCGCGTATTTGCTCTCGCTCAGGCGCAATCACGAATGAATAACGGTTTGGTTGATGCGAGTGATTTGA
TGACGAGCGTAATGGCTGGCCTGTTGAACAAGTCTGAAAAGAAATGCATAAGCTTTTGCATTCTCACCG
GATTCAGTCGTCACCTCATGGTGATTTCTCACTTGATAACCTTATTTTTGACGAGGGGAAATTAATAGGTT
GTATTGATGTTGGACGAGTCGGAATCGCAGACCGATACCAGGATCTTGCCATCCTATGGAAGTGCCTCGG
TGAGTTTTCTCCTTCATTACAGAAACGGCTTTTTTCAAAAATATGGTATTGATAATCCTGATATGAATAAA
TTGCAGTTTCATTTGATGCTCGATGAGTTTTTCTAATCAGTACTGACAATAAAAAGATTCTTGTTTTCAA
GAACTTGTCATTTGTATAGTTTTTTTATATTGTAGTTGTTCTATTTTAAATCAAATGTTAGCGTGATTTAT
ATTTTTTTTTCGCTCGACATCATCTGCCAGATGCGAAGTTAAGTGCGCAGAAAGTAAATATCATGCGTCA
ATCGTATGTGAATGCTGGTCGCTATACTGCTAGGCCGGCCATAGGATAACAGGGTAATGTTAACCAGG
TTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCC
GACAGGACTATAAAGATACCAGGGTTTTCCCCCTGGAAGCTCCCTCGTGCCTCTCCTGTTCCGACCCTG
CCGTTACCGGATACCTGTCCGCCTTTCTCCCTCGGGAAGCGTGGCGCTTTCATAGCTCACGCTGTA
GGTATCTCAGTTCGGTGTAGGTCGTTCCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTACGCCGA

CCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCA
GCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGC
CTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAA
AAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTTGTTTGAAGCAG
CAGATTACGGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGTCTGACGCTCAGT
GGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTT
AAATT

Supplementary Figure S3 - Sequence of Cas9_Base plasmid.

Light green: ScP_{PDC1}, Red: Cas9 protein, Gray: ScT_{TDH3}, Cyan: KmARS7, Green:
KmCEN-D, Yellow: *kanMX*

AAAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAACTTGGTCTGACAGTTACCAATGCTTAAT
CAGTGAGGCACCTATCTCAGCGATCTGTCTATTTGTTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAG
ATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGAACCACGCTCAC
CGGCTCCAGATTTATCAGCAATAAACAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCTGCAACTTT
ATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTG
CGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTACGCTCGTCGTTTGGTATGGCTTCATTCAGCT
CCGGTCCCAACGATCAAGGCGAGTTACATGATCCCCATGTTGTGCAAAAAGCGGTTAGCTCCTTCGG
TCCTCCGATCGTTGTGAGAAGTAAGTTGGCCGAGTGTATCACTCATGGTTATGGCAGCACTGCATAAT
TCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTACTCAACCAAGTCATTCTGAG
AATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGCGTCAATACGGGATAATACCGCGCCACATAGCAG
AACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTGGGGCGAAAACCTCAAGGATCTTACCGCTGTTG
AGATCCAGTTCGATGTAACCCACTCGTGCACCAACTGATCTTCAGCATCTTTACTTTCACCAGCGTTT
CTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAGGAATAAGGGCGACACGGAAATGTTGAAT
ACTCATACTCTTCTTTTTCAATATTATTGAAGCATTATCAGGGTATTGTCTCATGAGCGGATACATA
TTTGAATGTATTTAGAAAAATAAACAAATAGGGTTCCGCGTAAATTAATTGAGATAAGCACACTGCACC
CATACTTCTTAAAAACGTAGCTTCCAGTTTTTGGTGGTTCGGCTTCTTCCCGATTCCGCCCGCTAA
ACGCATATTTTGTGCTGGTGGCATTGCAAAATGCATAACCTATGCATTTAAAGATTATGTATGCT
CTTCTGACTTTTCGTGTGATGAGGCTCGTGGAAAAATGAATAATTTATGAATTTGAGAACAATTTGTG
TTGTTACGGTATTTTACTATGGAATAATCAATCAATTGAGGATTTTATGCAAATATCGTTTGAATATTTT
TCCGACCTTTGAGTACTTTTCTTATAATTGCATAATATTGTCCGCTGCCCTTTTTCTGTTAGACGGT
GTCTTGATCTACTTGCTATCGTTCAACACCACCTATTTTCTAACTATTTTTTTTTTAGCTCATTGAAT
CAGCTTATGGTATGGCACATTTTGCATAAACCTAGCTGCCTCGTTGAACATAGGAAAAAAAAATATA
TAAACAAGGCTCTTCACTCTCCTTGAATCAGATTTGGGTTTGTCCCTTTATTTTCATATTTCTTGTC
ATATTCTTTCTCAATTATTATTTTCTACTCATAACCTCAGCAAAATAACACAGTCAAATCAATCAAAG
CTAGCGAAATGGCACCGAAGAAGAAGCGTAAAGTCGGAATCCACGGAGTTCCTGCGGCAATGGACAAGAA
GTACTCCATTGGGCTCGCTATCGGCACAAACAGCGTCGGTGGGCCGTCATTACGGACGAGTACAAGGTG
CCGAGCAAAAATTCAAAGTTCTGGGCAATACCGATCGCCACAGCATAAAGAAGAACCTCATTGGCGCC
TCCTGTTGACTCCGGGGAGACGGCCGAAGCCACGCGCTCAAAGAACAGCACGGCGCAGATATACCCG
CAGAAAGAATCGGATCTGCTACCTGCAGGAGATCTTTAGTAATGAGATGGCTAAGGTGGATGACTCTTC
TTCCATAGGCTGGAGGAGTCCTTTTTGGTGGAGGAGGATAAAAAGCACGAGCGCCACCCAATCTTTGGCA
ATATCGTGGACGAGGTGGCGTACCATGAAAAGTACCAACCATATATCATCTGAGGAAGAAGCTTGTAGA
CAGTACTGATAAGGCTGACTTGCAGTTGATCTATCTCGCGCTGGCGCATATGATCAAATTTCCGGGACAC

TTCTCATCGAGGGGGACCTGAACCCAGACAACAGCGATGTCGACAACTCTTTATCCAACGGTTCAGAC
CTTACAATCAGCTTTTTCGAAGAGAACCCGATCAACGCATCCGGAGTTGACGCCAAAGCAATCCTGAGCGC
TAGGCTGTCCAAATCCCGGGGCTCGAAAACCTCATCGCACAGCTCCCTGGGGAGAAGAAGAACGGCCTG
TTTGGTAATCTTATCGCCCTGTCACTCGGGCTGACCCCAACTTTAAATCTAACTTCGACCTGGCCGAAG
ATGCCAAGCTTCAACTGAGCAAAGACACCTACGATGATGATCTCGACAATCTGCTGGCCAGATCGGCCA
CCAGTACGCAGACCTTTTTTTGGCGGCAAAGAACCTGTCAGACGCCATTCTGCTGAGTGATATTCTGCGA
GTGAACACGGAGATCACCAAAGCTCCGCTGAGCGCTAGTATGATCAAGCGCTATGATGAGCACCACCAAG
ACTTGACTTTGCTGAAGGCCCTTGTGAGACAGCAACTGCCTGAGAAGTACAAGGAAATTTTCTTCGATCA
GTCTAAAAATGGCTACGCCGGATACATTGACGGCGGAGCAAGCCAGGAGGAATTTACAAATTTATTAAG
CCCATCTTGAAAAAATGGACGGCACCGAGGAGCTGCTGGTAAAGCTTAACAGAGAAGATCTGTTGCGCA
AACAGCGCACTTTCGACAATGGAAGCATCCCCACCAGATTCACCTGGGCGAACTGCACGCTATCCTCAG
GCGGCAAGAGGATTTCTACCCCTTTTTGAAAGATAACAGGAAAAGATTGAGAAAATCCTCACATTTGCG
ATACCCTACTATGTAGGCCCCCTCGCCCGGGGAAATTCAGATTCGCGTGGATGACTCGCAAATCAGAAG
AGACCATCACTCCCTGGAACCTCGAGGAAGTCGTGGATAAGGGGGCCTCTGCCAGTCTTCATCGAAAG
GATGACTAACTTTGATAAAAAATCTGCCTAACGAAAAGGTGCTTCCTAAACACTCTCTGCTGTACGAGTAC
TTCACAGTTTATAACGAGCTCACCAAGGTCAAATACGTCACAGAAGGGATGAGAAAGCCAGCATTCTGT
CTGGAGAGCAGAAGAAAGCTATCGTGGACCTCCTCTTCAAGACGAACCGGAAAGTTACCGTGAAACAGCT
CAAAGAAGACTATTTCAAAAAGATTGAATGTTTCGACTCTGTTGAAATCAGCGGAGTGGAGGATCGCTTC
AACGCATCCCTGGGAACGTATCACGATCTCCTGAAAATCATTAAAGACAAGGACTTCCTGGACAATGAGG
AGAACGAGGACATTCTTGAGGACATTGTCCTCACCCCTACGTTGTTTGAAGATAGGGAGATGATTGAAGA
ACGCTTGAAAACCTACGCTCATCTCTTCGACGACAAAGTCATGAAACAGCTCAAGAGGGCGCGATATACA
GGATGGGGGGCGGCTGTCAAGAAAACCTGATCAATGGGATCCGAGACAAGCAGAGTGAAAGACAATCCTGG
ATTTTCTTAAGTCCGATGGATTTGCCAACCGGAACTTCATGCAGTTGATCCATGATGACTCTCTCACCTT
TAAGGAGGACATCCAGAAAGCACAAGTTTCTGGCCAGGGGACAGTCTTCACGAGCACATCGCTAATCTT
GCAGGTAGCCCAGCTATCAAAAAGGGAATACTGCAGACCGTTAAGGTCGTGGATGAACTCGTCAAAGTAA
TGGGAAGGCATAAGCCCGAGAATATCGTTATCGAGATGGCCCGAGAGAACCAAACTACCCAGAAGGGACA
GAAGAACAGTAGGGAAAGGATGAAGAGGATTGAAGAGGGTATAAAGAAGTGGGGTCCCAAATCCTTAAG
GAACACCCAGTTGAAAACACCCAGCTTCAGAATGAGAAGCTCTACCTGTACTACCTGCAGAACGGCAGGG
ACATGTACGTGGATCAGGAACTGGACATCAATCGGCTCTCCGACTACGACGTGGATCATATCGTGCCCA
GTCTTTTCTCAAAGATGATTCTATTGATAATAAAGTGTGACAAGATCCGATAAAAAATAGAGGGAAGAGT
GATAACGTCCCCTCAGAAGAAGTTGTCAAGAAAATGAAAAATTATTGGCGGCAGCTGCTGAACGCCAAAC
TGATCACACAACGGAAGTTCGATAATCTGACTAAGGCTGAACGAGGTGGCCTGTCTGAGTTGGATAAAGC

CGGCTTCATCAAAAGGCAGCTTGTTGAGACACGCCAGATCACCAAGCACGTGGCCCAAATTCTCGATTCA
CGCATGAACACCAAGTACGATGAAAATGACAACTGATTCGAGAGGTGAAAGTTACTCTGAAGTCTA
AGCTGGTCTCAGATTTAGAAAAGGACTTTCAGTTTTATAAGGTGAGAGAGATCAACAATTACCACCATGC
GCATGATGCCTACCTGAATGCAGTGGTAGGCACTGCACTTATCAAAAAATATCCCAAGCTTGAATCTGAA
TTTGTTTACGGAGACTATAAAGTGTACGATGTTAGGAAAATGATCGCAAAGTCTGAGCAGGAAATAGGCA
AGGCCACCGCTAAGTACTTCTTTTACAGCAATATTATGAATTTTTCAAGACCGAGATTACACTGGCCAA
TGGAGAGATTCGGAAGCGACCACTTATCGAAACAAACGGAGAAACAGGAGAAATCGTGTGGGACAAGGGT
AGGGATTTGCGGACAGTCCGGAAGGTCCTGTCCATGCCGCAGGTGAACATCGTTAAAAGACCGAAGTAC
AGACCGGAGGCTTCTCCAAGGAAAAGTATCCTCCCGAAAAGGAACAGCGACAAGCTGATCGCACGCAAAAA
AGATTGGGACCCCAAGAAATACGGCGGATTCGATTCTCCTACAGTCGTTACAGTGTACTGGTTGTGGCC
AAAGTGGAGAAAAGGGAAGTCTAAAAACTCAAAGCGTCAAGGAACTGCTGGGCATCACAATCATGGAGC
GATCAAGCTTCGAAAAAACCCCATCGACTTTCGAGGCGAAAGGATATAAAGAGGTCAAAAAAGACCT
CATCATTAAAGCTTCCCAAGTACTCTCTTTGAGCTTGAAAACGGCCGAAACGAATGCTCGCTAGTGCG
GGCGAGCTGCAGAAAGGTAACGAGCTGGCACTGCCCTCTAAATACGTTAATTTCTGTATCTGGCCAGCC
ACTATGAAAAGCTCAAAGGGTCTCCCGAAGATAATGAGCAGAAGCAGCTGTTCTGGAACAACACAAACA
CTACCTTGATGAGATCATCGAGCAAATAAGCGAATTCTCCAAAAGAGTGATCCTCGCCGACGCTAACCTC
GATAAGGTGCTTTCTGCTTACAATAAGCACAGGGATAAGCCCATCAGGGAGCAGGCAGAAAACATTATCC
ACTTGTTTACTCTGACCAACTTGGGCGCGCTGCAGCCTCAAGTACTTCGACACCACCATAGACAGAAA
GCGGTACACCTCTACAAAGGAGGTCCTGGACGCCACACTGATTCATCAGTCAATTACGGGGCTCTATGAA
ACAAGAATCGACCTCTCTCAGCTCGGTGGAGACAGCAGGGCTGACCCCAAGAAGAAGAGGAAGGTGGGTG
GAGGAGGTACCGGCGGTGGAGGCTCAGCAGAATACGTACGAGCTCTGTTGACTTCAATGGGAATGACGA
GGAGGATCTCCCCTTAAGAAGGGCGATATTCTCCGCATCAGAGATAAGCCCGAAGAACAATGGTGGAA
GCCGAGGATAGCGAAGGGAAAAGGGGCATGATTCTGGTGCCATATGTGGAGAAATATTCCGGTGACTACA
AAGACCATGATGGGGATTACAAAGACCAGACATCGACTACAAAGACGACGACGATAAATCAGGGATGAC
AGACGCCGAGTACGTGCGCATTATGAGAACTGGATATTTACACCTTCAAGAAGCAGTTCTTCAACAAC
AAGAAATCTGTGTCACACCGCTGCTACGTGCTGTTTGTGAGTTGAAGCGAAGGGGGCGAAGAAGGGCTTGT
TTTGGGGCTATGCCGTCAACAAGCCCCAAAGTGGCACCGAGAGAGGAATACACGCTGAGATATTCAGTAT
CCGAAAAGGTGGAAGAGTATCTTCGGGATAATCCTGGGCAGTTTACGATCAACTGGTATTCCAGCTGGAGT
CCTTGCGCTGATTGTGCCGAGAAAATTCTGGAATGGTATAATCAGGAACTTCGGGGAAACGGGCACACAT
TGAAAATCTGGGCCTGCAAGCTGTACTACGAGAAGAATGCCCGAACCAGATAGGACTCTGGAATCTGAG
GGACAATGGTGTAGGCCTGAACGTGATGGTTTCCGAGCACTATCAGTGTGTCGGAAGATTTTCATCCAA
AGCTCTCATAACCAGCTCAATGAAAACCGCTGGTTGGAGAAAACACTGAAACGTGCGGAGAAGCGGAGAT

CCGAGCTGAGCATCATGATCCAGGTCAAGATTCTGCATACCACTAAGTCTCCAGCCGTTGGTCCCAAGAA
GAAAAGAAAAGTCGCATGCATCCAGGCTAGCGTGAATTTACTTTAAATCTTGCATTTAATAAATTTTCT
TTTTATAGCTTTATGACTTAGTTTCAATTTATATACTATTTAATGACATTTTCGATTCATTGATTGAAA
GCTTTGTGTTTTTCTTGATGCGCTATTGCATTGTTCTTGTCTTTTTCGCCACATGTAATATCTGTAGTA
GATACCTGATACATTGTGGATGCTGAGTGAATTTTAGTTAATAATGGAGGCGCTCTTAATAATTTGGG
GATATTGGCTTTTTTTTTTAAAGTTTACAAATGAATTTTTCCGCCAGGATGCGGCCGCTCTAGACTAGT
CCTAGGGGATCCGTCACCCGGCCAGCGGAATTCAGGGATGATCTTGAGAAGTTCTTAGAGTCTTACGAGG
GAACAGAAGATTTGGAACCAGCCAAAGCTGCTATAGCGGAAGCAGATATTTGCTAAGTAAATGATTAA
TAATTAATATGTGAAAACATTAATCTTTTTATATTTTGCAGTTCGTTGTCGCTATAATTTATAGTC
ATCTCGTTAGTTCAAACAAGACTTCTTGAAGTAAAACCACTTTCAGTCTTCAAACATAAAATGAAAAT
CAGTGGAAGAAGGTAAACGACTTCATGTTATATATGAATTGAATAGTAATGGAAATAACCAAAAACAGCT
CAACAGAAAACAAAACAAAATACGTTAAGACCTGAACTCCTAGCAGAACCATAACTGCCAAATATTTATTA
TCTGTGGAGATCTTATATTCTAAAACCAAAAAAATAAECTTAAAAGTAAAAAGAAGATGTTCTAACT
GAGGTTCGAACTCAGGACCTTTGCCGTGTGAAGGCAACGTGATAGCCACTACACTATTAGAACTACCTTA
TGGGAAAAAGAAAAATAGAGTACAACAGAAATGGTAAGATCTGTGACCTTTCTAAACACTTAATTCAT
ATAGACAGTTCACCCACCATAAGGTCACAATTATAATGTCTTTAGAAGACCACTGTCGTTTCATCATCT
TCCTAAGCCCTCTCTCTAAAGCGGCATATTTCCGTAATTTGTTCTTTGCACAGGCACGTGAGATGAC
TCCGATTATTCCACATGCATATTTAGCCTCTCTAGGGGCTCGAGCTATAGCAAGTCAAGGAAAGAAAA
CTATTATGATCTGGTCACGTGTATAAAAATTTATTAATTTTAAACTATATAATTTATTTTTTTATTT
TAAAGTTTAAAGTAATTTAATAGTATTCTATATTTTAAATAAACATACTTTAAAATTTATTTAATAAT
TTATTTTTTTAAATACAATGTTTTATTTAAAACAAAATTATAAGTTAAAAGTTGTTCCGAAAGTAAA
ATATATTTTATAGGACGTCGACATGGAGGCCAGAATACCCTCCTTGACAGTCTTGACGTGCGCAGCTCA
GGGGCATGATGTGACTGTCGCCCCGTACATTTAGCCCATACATCCCATGTATAATCATTTGCATCCATAC
ATTTTGATGGCCGACGGCGGAAGCAAAAATTACGGCTCCTCGCTGCAGACCTGCGAGCAGGGAAACGC
TCCCCTCACAGACGCGTTGAATTGTCCCACGCCGCGCCCCTGTAGAGAAATATAAAAGGTTAGGATTTG
CCTAGAGGTTCTTCTTTTATATACTTCTTTTTAAAATCTTGCTAGGATACAGTCTCACATCACATCCG
AACATAAACAACCATGGGTAAAGAAAAGACTCACGTTTCGAGGCCGCGATTAATTTCCAACATGGATGCT
GATTTATATGGGTATAAATGGGCTCGCGATAATGTCGGGCAATCAGGTGCGACAATCTATCGATTGTATG
GGAAGCCCGATGCGCCAGAGTTGTTTCTGAAACATGGCAAAGGTAGCGTTGCCAATGATGTTACAGATGA
GATGGTCAGACTAACTGGCTGACGGAATTTATGCCTCTCCGACCATCAAGCATTTTATCCGTACTCCT
GATGATGCATGGTACTCACCCTGCGATCCCCGGCAAAACAGCATTCCAGGTATTAGAAGAATATCCTG
ATTCAGGTGAAAATATTGTTGATGCGCTGGCAGTGTTCCTGCGCCGTTGCATTTCGATTCCTGTTGTAA

TTGTCCTTTTAAACAGCGATCGCGTATTTTCGTCTCGCTCAGGCGCAATCACGAATGAATAACGGTTTGGTT
GATGCGAGTGATTTTATGATGACGAGCGTAATGGCTGGCCTGTTGAACAAGTCTGAAAGAAATGCATAAGC
TTTTGCCATTCTCACCGGATTCAGTCGTCACTCATGGTGATTTCTCACTTGATAACCTTATTTTTGACGA
GGGAAATTAATAGGTTGTATTGATGTTGGACGAGTCGGAATCGCAGACCGATACCAGGATCTTGCCATC
CTATGGAAGTGCCTCGGTGAGTTTTCTCCTTCATTACAGAAACGGCTTTTTCAAAAATATGGTATTGATA
ATCCTGATATGAATAAATTGCAGTTTCATTGATGCTCGATGAGTTTTCTAATCAGTACTGACAATAAA
AAGATTCTTGTTCAGAACTTGCATTTGTATAGTTTTTTTATATTGTAGTTGTTCTATTTTAATCAA
ATGTTAGCGTGATTTATTTTTTTTTCGCCTCGACATCATCTGCCAGATGCGAAGTTAAGTGCAGCAA
AGTAATATCATGCGTCAATCGTATGTGAATGCTGGTCGCTATACTGCCTAGGCCGGCCATAGGGATAACA
GGGTAATGTTAACCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAATCGACGCTCAAGT
CAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCTGGAAGCTCCCTCGTGGCT
CTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCTTTCTCCCTTCGGAAGCGTGGCGCTTC
TCATAGCTCAGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCCGCTCCAAGCTGGGCTGTGTGCAGAA
CCCCCGTTCAGCCGACCGCTGGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACAGG
ACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGA
GTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAG
CCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTT
TTTTTGTTCGAAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTAC
GGGGTCTGACGCTCAGTGGAACGAAAACCTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATC
TTCACCTAGATCCTTTTAAATT

Supplementary Figure S4 - Plasmid nCas9-CDA_Base sequences.

Light green: ScP_{PDC1}, Red: nCas9 protein, Gray: ScT_{TDH3}, Cyan: KmARS7, Green:
KmCEN-D, Yellow: *kanMX*

AGCGCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCATTAATGCAGCTGGCACGACAGGTT
TCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAATGTGAGTTAGCTCACTCATTAGGCACCCAG
GCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGTGGAATTGTGAGCGGATAACAATTTACACAGGAA
ACAGCTATGACCATGATTACGCCAAGCTCAGAATTAACCCTCACTAAAGGGACTAGTCCTGCAGGTTTAA
ACGAATTCGCCCTTCAGAGAGTCTGCAACACCGATTTATCGGTTTGCAGGTAACACAATACCGATTACTT
GGTGTCTGAGGAGATCTCAATTCTCTCTTTTTCTTCAACTTTATTTTCTTGGTATGCTTCTTGTGCAT
TCGCCTATTAATTTATATTAGCGATTCTTATGACATCGGCCATCACTGAGTATAGTGAGTAGTAGTGTT
GATATTTGAAATCTATTATAGATAAGTTCCTTTACATATCGTGCAGCACTTGTATGTGAAGTATTAGT
AGCTATATTGGCTATATACAGAGAGAAAAGCTATAACTATTTATATCATAACTGTTTGTATAACTTAACGA
CCCATGCTTTGGTTCAAAGCAGCGTTTTGCTCTTGGAAACGTTGTCCAACACGTTCACTATGTTTCAAGA
ACTTTTCTGAGCATTTCATTATGCAGCTTTGCTCCTTGAAGTTAGCTTAGCAGAGGTAAAGTCGTTGAC
ACAGTCACTGAAACATCTTTGACCAAGTTGGAGTATAGACGCATGAAGTCCTTCATTTGCTTTTGTTC
ACAATTCTTTGAACTCTTGTGTTCTTACCATTTAGTTGATCCATTTTTAACTGTGTATTTGATTTTT
TTGTTCTAGCAATCGACGGTCCAAGACTCAATAAAAATTGACTCTTGTATCTTTCTTCACTAGTAGTA
AAGCAGAAAAGTCTAGCAGAAAATGTGTATCTTTATAACGGTCAATGGTCTAAGAAGCAAGGTTTGGTTCT
TCGCACTTGTATTAACCTCTTTTTCTTGGTTGTGAAATTCACATAATGTACTTCACCAAAAAACAAAA
AAAAAAAAAAAAAAAAAAAAATTTTCGGTGCAAAAAACAGCTTCTAGCACGTGACTAACTTGTAGCGTATA
CAATGTGCGCAATGCCATTACACTCTAACATCTCTGTATAGTTTATATAAACGATCAACTTGTAACTT
CTGATCCGAGTACACTCGAACCTCTGCTTGTCTCAGTATAGCGACCAGCATTACATACGATTGACGCA
TGATATTACTTTCTGCGCACTTAACTTCGCATCTGGGCAGATGATGTGAGGCGAAAAAATATAAATC
ACGCTAACATTTGATTAATAAGAACAACTACAATATAAAAAACTATACAAATGACAAGTTCTTGAAAA
CAAGAATCTTTTTATTGTCAGTACTGATCATGAGATGCCTGCAAGCAATTCGTTCTGTATCAGGCGCAGG
AGCGTCCCGTCCGGGTCGACCAAAGCGGCCATCGTGCCTCCCCTCCTGCAGTTCGGGGGCATGGATGC
GCGGATAGCCGCTGCTGGTTTCTGGATGCCGACGGATTTGCACTGCCGGTAGAACTCCGCGAGGTGCTC
CAGCCTCAGGCAGCAGCTGAACCAACTCGCGAGGGGATCGAGCCCGGGTGGGCGAAGAACTCCAGCATG
AGATCCCCGCGCTGGAGGATCATCCAGCCGGCGTCCCGGAAAACGATTCCGAAGCCCAACCTTTCATAGA
AGGCGGCGGTGGGATCGAAATCTCGTGATGGCAGGTTGGGCGTCGCTTGGTCCGTCATACCCATGCCGGT
ACCGGATCCCTTGTACAGCTCGTCCATGCCGAGAGTGATCCCGCGGCGGTCACGAACTCCAGCAGGACC
ATGTGATCGCGCTTCTCGTTGGGGTCTTTGCTCAGGGCGGACTGGGTGCTCAGGTAGTGTTGTGCGGCA
GCAGCACGGGGCCGTGCGCGATGGGGTGTCTGCTGGTAGTGGTCCGGCAGCTGCACGCTGCCGTCTC
GATGTTGTGGCGGATCTTGAAGTTCACCTTGATGCCGTTCTTCTGCTTGTGCGCCATGATATAGACGTTG
TGGCTGTTGTAGTTGTAAGTCCAGCTTGTGCCCCAGGATGTTGCCGTCCTCCTTGAAGTCGATGCCCTCA

GCTCGATGCGGTTACCAGGGTGTGCGCCTCGAACTTCACCTCGGCGCGGGTCTTGTAGTTGCCGTCGTC
CTTGAAGAAGATGGTGCCTCCTGGACGTAGCCTTCGGGCATGGCGGACTTGAAGAAGTCGTGCTGCTTC
ATGTGGTGGGGTAGCGGCTGAAGCACTGCACGCCGTAGGTCAGGGTGGTCACGAGGGTGGGCCAGGGCA
CGGGCAGCTTGCCGGTGGTGCAGATGAACTTCAGGGTCAGCTTGCCGTAGGTGGCATCGCCCTCGCCCTC
GCGGACACGCTGAACTTGTGGCCGTTTACGTCGCCGTCCAGCTCGACCAGGATGGGCACCACCCCGGTG
AACAGCTCCTCGCCCTTGCTCACCATGCTAGCTTTGATTGATTGACTGTGTTATTTGCGTGAGGTTAT
GAGTAGAAAAATAATAATTGAGAAAAGGAATATGACAAGAAATAGAAAATAAAGGGAACAAACCCAAATCT
GATTGCAAGGAGAGTGAAAGAGCCTTGTTTATATATTTTTTTTTTCTATGTTCAACGAGGACAGCTAGGT
TTATGCAAAAATGTGCCATCACCATAAGCTGATTCAAATGAGCTAAAAAAAAAATAGTTAGAAAATAAGG
TGGTGTGAACGATAGCAAGTAGATCAAGACACCGTCTAACAGAAAAAGGGCAGCGGACAATATTATGC
AATTATGAAGAAAAGTACTCAAAGGGTCGGAAAAATATTCAAACGATATTTGCATAAAATCCTCAATTGA
TTGATTATTCCATAGTAAAAACCGTAACAACACAAAATTGTTCTCAAATTCATAAATTATTCATTTTT
CCACGAGCCTCATCACACGAAAAGTCAGAAGAGCATACATAATCTTTAAATGCATAGGTTATGCATTTT
GCAAAATGCCACCAGGCAACAAAAATATGCGTTTTAGCGGGCGGAATCGGGAAGGAAGCCGGAACCACAAA
AACTGGAAGCTACGTTTTTAAGGAAGGTATGGGTGCAGTGTGCTTATCTCAAACCTTTGATTAGTTTATGG
GCTAGCTTATGATACTTCCTCTTGCTGTTCCAGTTTACAAATGATTTAACGAACCTTCTACACTTTTACG
GTAGTATTTCTTGTGGTGTAGGCTCAGATTCAATATGACTGCTAATCAGTAAAATTAATGGAACA
GCCAGACACACAGCCTTTGAAAAATGTTAAAAACGATCACATATATGTAGTCACGTGGTTAGTGCATAGA
GTCAGGCGATGGTGGTTAGTCCCTAGCCAGAGCCATTCCCCTCTTGTTCGAAATGCGATGACTGAATG
GGGAAGAAGCTCTTAGTATTTCTGTAGTTTTTTTTGCGATTTCTATTGTTGCCTGCCCCGACTTTCTT
TTACCTTTTGTAGTACAACATTAATTATAATGCATAATAGATCTAGAAGCGTATTGTGATGAGCATCC
TTGAAATAAGAAAGGAAGTCACAGGTACAGTAAAAAAGAGCCGTGACAGGCACCTAATTTAGTTTTG
ATTGAGGTATAATGAGCTCAGAAGACCAAGATGCTCTAAAAGTAAAGCAGAATGGTCTTTCCAATCAGAA
TTCGTCACCTTGTGGTGCCTTAACTGCTGGTGAAGATCTATGGTTTATCAGATGACTTCATTCTATATG
AGAACTCCACTAAAACTGTTTCGACCGCCAGATTTGACTATACGCATTATATTCGAGTACTGTTGACTG
GGAGTGATGATACAAATAAGGATTCTAAGGTACGACGTTCAAATATAAGTTTTGGAGTCCCAAATATAC
ATACTACTTCGAAAATTCCTCCATTGGAATAGTTACTAAGGCACTAAATAAGTATGGGTGGAAGTTATT
CCTGATAGAATATTGCCTCCTTTGGTGGCAAATTCCTTGCTGGGGTAGTTCTTTATACCACATACTTGA
CTACCTGAATAATTTCCAGTCAGATCAGTGGAGCCAGTTTTCAACACAATTTGTTTGACTACTTAAG
GTAAGGGCGAATTCGCGGCCGCTAAATTCAATTCGCCCTATAGTGAGTCGTATTACAATTCAGTGGCCGT
CGTTTTACAACGTCGTGACTGGGAAAACCTGGCGTTACCCAACCTAATCGCCTTGCAGCACATCCCCCT
TTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTATACG

TACGGCAGTTTAAGGTTTACACCTATAAAAAGAGAGAGCCGTTATCGTCTGTTTGTGGATGTACAGAGTGA
TATTATTGACACGCCGGGGGCGACGGATGGTGATCCCCCTGGCCAGTGCACGTCTGCTGTGAGATAAAGTC
TCCCGTGAACCTTACCCGGTGGTGCATATCGGGGATGAAAGCTGGCGCATGATGACCACCGATATGGCCA
GTGTGCCGGTCTCCGTTATCGGGGAAGAAGTGGCTGATCTCAGCCACCGCGAAAATGACATCAAAAACGC
CATTAACTGATGTTCTGGGGAATATAAATGTCAGGCATGAGATTATCAAAAAGGATCTTCACCTAGATC
CTTTTCACGTAGAAAAGCCAGTCCGCAGAAAACGGTGCTGACCCCGGATGAATGTCAGCTACTGGGCTATCT
GGACAAGGGAAAACGCAAGCGCAAAGAGAAAAGCAGGTAGCTTGCAGTGGGCTTACATGGCGATAGCTAGA
CTGGGCGGTTTTATGGACAGCAAGCGAACCAGGAATTGCCAGCTGGGGCGCCCTCTGGTAAGGTTGGGAAG
CCCTGCAAAGTAAACTGGATGGCTTTCTTGCCGCAAGGATCTGATGGCGCAGGGGATCAAGCTCTGATC
AAGAGACAGGATGAGGATCGTTTCGCATGATTGAACAAGATGGATTGCACGCAGGTTCTCCGGCCGCTTG
GGTGGAGAGGCTATTCGGCTATGACTGGGCACAACAGACAATCGGCTGCTCTGATGCCGCCGTGTTCCGG
CTGTGACGCGCAGGGGCGCCCGTTCTTTTTGTCAAGACCGACCTGTCCGGTGCCCTGAATGAACTGCAAG
ACGAGGCAGCGCGGCTATCGTGGCTGGCCACGACGGGCGTTCCTTGCGCAGCTGTGCTCGACGTTGTCAC
TGAAGCGGGAAGGGACTGGCTGCTATTGGGGCAAGTGCCGGGGCAGGATCTCCTGTCATCTCACCTTGCT
CCTGCCGAGAAAAGTATCCATCATGGCTGATGCAATGCGGCGGCTGCATACGCTTGATCCGGCTACCTGCC
CATTGACCACCAAGCGAAAACATCGCATCGAGCGAGCACGTACTCGGATGGAAGCCGGTCTTGTCGATCA
GGATGATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCCGAACTGTTCCGCCAGGCTCAAGGCGAGCATG
CCCGACGGCGAGGATCTCGTCTGACCCATGGCGATGCCTGCTTGCCGAATATCATGGTGGAAAATGGCC
GCTTTTCTGGATTCATCGACTGTGGCCGGCTGGGTGTGGCGGACCGCTATCAGGACATAGCGTTGGCTAC
CCGTGATATTGCTGAAGAGCTTGGCGGCGAATGGGCTGACCGCTTCCTCGTGCTTTACGGTATCGCCGT
CCCGATTGCGAGCGCATCGCCTTCTATCGCCTTCTTGACGAGTTCTTCTGAATTATTAACGCTTACAATT
TCCTGATGCGGTATTTTCTCCTTACGCATCTGTGCGGTATTTACACCCGCATCAGGTGGCACTTTTCCGG
GAAATGTGCGCGGAACCCCTATTTGTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGATT
ATCAAAAAGGATCTTCACCTAGATCCTTTAAATTA AAAATGAAGTTTTAAATCAATCTAAAGTATATAT
GAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTT
GTTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCC
CAGTGCTGCAATGATACCGGAGAGACCCAGCTCACCAGCTCCAGATTTATCAGCAATAAACCAGCCAGCC
GGAAGGGCCGAGCGCAGAAGTGGTCCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGG
AAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGGCGAACGTTGTTGCCATTGCTACAGGCATCGTGGT
GTCACGCTCGTCTTTGGTATGGCTTCATTGAGCTCCGGTCCCAACGATCAAGGCGAGTTACATGATCC
CCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCCTCCGATCGTTGTCAGAAGTAAGTTGGCCGCAG
TGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTC

TGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCG
GCGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTT
CGGGGCGAAAACCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGACCCAA
CTGATCTTCAGCATCTTTTACTTTACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCA
AAAAAGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTCCTTTTTCAATATTATTGAAGCA
TTTATCAGGGTTATTGTCTCATGACCAAAATCCCTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCC
CGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAA
AAACCACCGCTACCAGCGGTGGTTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAAGT
GCTTCAGCAGAGCGCAGATACCAAATACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAA
CTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAG
TCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTGGGGCTGAACGGGGG
GTTTCGTGCACACAGCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATG
AGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGA
GAGCGCAGGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTGGGGTTTCGCCACCTCT
GACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGCGGAGCCTATGGAAAACGCCAGCAACGCGGC
CTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCTGCGTTATCCCCTGATTCT
GTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGAGCCGAACGACCGAGCGCAGCG
AGTCAGTGAGCGAGGAAGCGGAAG

Supplementary Figure S5 - Plasmid TOPO/F7 sequences.

Yellow: bleomycin gene, **Cyan**: GFP coding sequence, **Green**: ScP_{PDC1}

Supplementary Table S1 - Primers used in this study

Primer name	Sequence (5'-3')	Purpose
Universal primers for sgRNA cassette construction		
P_Km01_013(pKmSNR52_Fw)	aagctagctctagaTGCCGCAAATAGGGCAGG	amplification of P _{KmSNR52} region
P_Km01_014(tracerRNA_Fw)	GTTTTAGAGCTAGAAATAGCAAG	amplification of tracrRNA_T _{sup4} fragment
P_Km01_015(tracerRNA_Rv)	cctgaattcGCGGCCGCatactagtCATAAAAAACAAAA AAAGCACCACC	amplification of tracrRNA_T _{sup4} fragment
Target-specific primers		
P_Km01_016(single1(NEJ1)_Fw)	CTAGCTCTAAAACCTTATTATCAGTATTTGTAG ATTCGAACTGCGGACGTTG	amplification of target <i>NEJ1</i>
P_Km01_017(single2(DNL4)_Fw)	CTAGCTCTAAAACTCTCCAGAGTTTAAATGGTT GATTCGAACTGCGGACGTTG	amplification of target <i>Dnl4</i>
P_Km01_018(deletion1(Ura3)_Fw)	CTAGCTCTAAAACCCTTTGATTAGTTTATGGGCG ATTCGAACTGCGGACGTTG	amplification of target Ura3_Fw
P_Km01_019(deletion2(Ura3)_Rv)	CTAGCTCTAAAACAGAACAAGCAGAGGTTCTGA GGATTCGAACTGCGGACGTTG	amplification of target Ura3_Rv
P_Km01_059(deletion2(Sed1)_Fw)	CTAGCTCTAAAACCTGGAATAGAACTGGATGTG GATTCGAACTGCGGACGTTG	amplification of target <i>Sed1-2</i>

Primer pairs for insert donor DNA

P_Km01_039(Ura3(5)_Fw)	ACCTTAAGTAGTCAAACAAATTGTG	amplification of GFP and bleomycin expression donor DNA containing length of 1000 bp <i>Ura3</i> homologous sequence (donor DNA-I)
P_Km01_046(Ura3(3)_Rv)	CAGAGAGTCTGCAACACCGA	
Ura3_up_100F	TGAATCTGAGCTCATCACCAAC	amplification of GFP and bleomycin expression donor DNA containing length of 122/109 bp <i>Ura3</i> homologous sequence (donor DNA-II)
Ura3_down100_R	GTATACAATGTGCGCAATGCC	
P_Km01_052(Ble_GFP_50bp_Rv)	TGGAACAGCAAGAGGAAGTATC	amplification of GFP expression donor DNA containing length of 50 bp <i>Ura3</i> homologous sequence (donor DNA-III)
P_Km01_053(GFP_50bp_Fw)	GATCAACTTGTA ACTTCTGATCCGAGTACACTC GAACCTCTGCTTGTCTgatccctgtacagctcgtc	
P_Km01_060(GFP_Sed1_Fw)	TATTCGTGGTACCAGTTTTATTTGCATCATCTGC ATTTGCTGCTTTCTCTatggtagcaagggcga	amplification of GFP codin region donor DNA containing length of 50 bp <i>Sed1</i> homologous sequence (donor DNA-IV)
P_Km01_061(GFP_Sed1_Rv)	gaaGATGGGGCAACTGAGGTCACtACTGGAATAG AACTGGATGTGTCGTTaccctgtacagctcgtcca	
P_Km01_052(Ble_GFP_50bp_Rv)	TGGAACAGCAAGAGGAAGTATC	amplification of P_{PDC1} region donor DNA containing length of 50 bp homologous sequence (donor DNA-V)
P_Km01_064(pPDC1_Fw)	atgggcaccaccccggtgaa	
P_Km01_065(EGFP_Rv)	GCTAGCatggtagcaaggg	amplification of GFP coding region donor

P_Km01_066(EGFP_Fw)	AACTATACAAATGACAAGTTCTTGAAAACAAG AATCTTTTTATTGTCAGTgatccctgtacagctcgtc	DNA containing length of 50 bp homologous sequence (donor DNA-VI)
P_Km01_051(Ble_GFP_50bp_Fw)	GATCAACTTGTA ACTTCTGATCCG	amplification of T _{TEF1} region donor DNA containing length of 50 bp homologous sequence (donor DNA-VII)
P_Km01_067(TEF1t_Rv)	ACTGACAATAAAAAGATTCTTGT	

Red indicates each target sequence.