

Supplemental Material:

A Stable, Autonomously Replicating Plasmid Vector Containing *Pichia pastoris* Centromeric DNA

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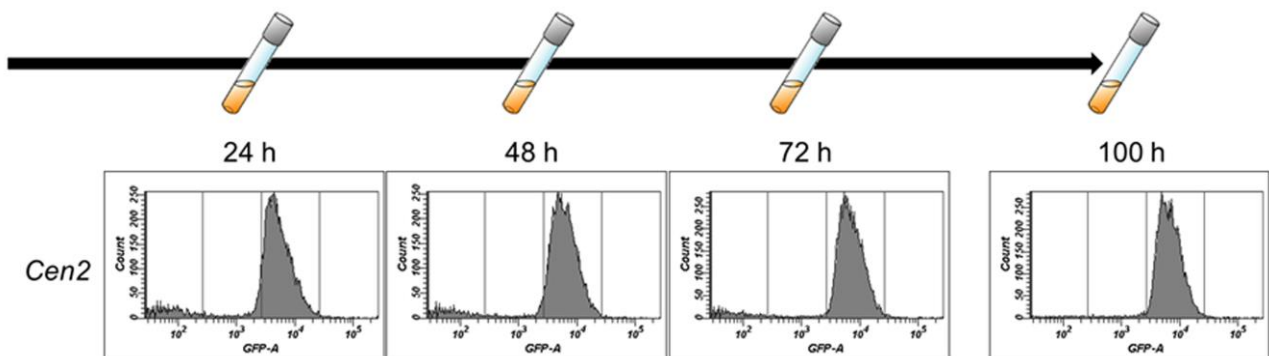


Figure S1. Evaluation of autonomously replicating activity and plasmid retention in *P. pastoris*. CBS7435 harboring pUC19-Cen2-EGFP (*Cen2*) was grown in BMGY medium with 200 $\mu\text{g}/\text{mL}$ Zeocin for 100 h. The GFP expression level of each cell was measured by FCM, and the histogram data from 10,000 cells per strain are shown. The vertical axis indicates the number of cells, and the horizontal axis indicates the green fluorescence signal (GFP-A). For each strain, three different transformants were analyzed, and one typical histogram is displayed.

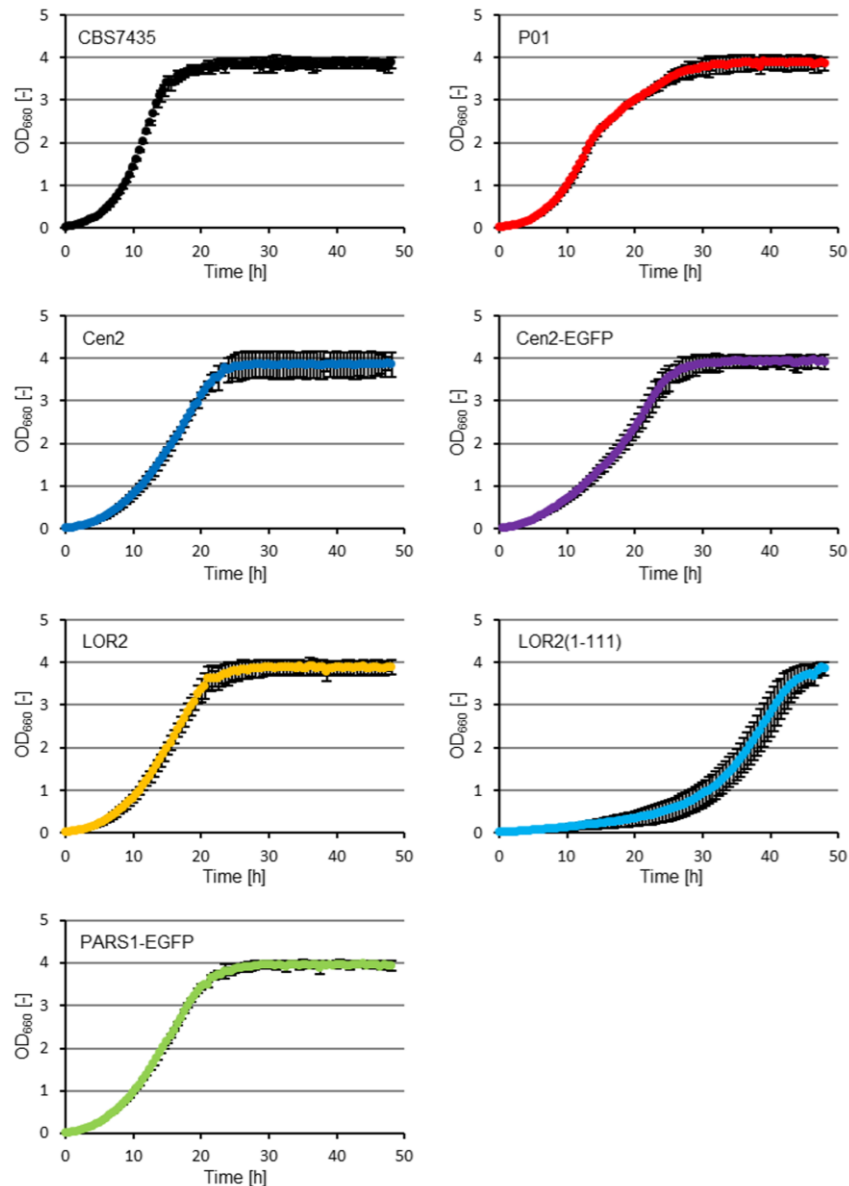


Figure S2. Measurement of cell growth. The strain CBS7435 was grown in BMGY medium without Zeocin for 48 h. The strain P01 and CBS7435 harboring pUC19-Cen2 (Cen2), pUC19-Cen2-EGFP (Cen2-EGFP), pUC19-LOR2 (LOR2), pUC19-LOR2(1-111) (LOR2(1-111)) and pUC19-PARS1-EGFP (PARS1-EGFP) were grown in BMGY medium with 200 $\mu\text{g}/\text{mL}$ Zeocin for 48 h. Cell growth was automatically monitored every 30 min in L-shaped test tubes by using a Bio-photorecorder. Error bars represent the standard deviation from three independent transformants ($n = 3$).

Table S1. Putative centromeric DNA sequences.

Chromosome	Accession number	Length (bp)	Length of putative centromere region (bp)
1	FR839628.1	2,894,792	5,354
2	FR839629.1	2,396,129	6,655
3	FR839630.1	2,263,199	6,181
4	FR839631.1	1,825,687	6,229

Chromosome	Left Outer Repeat (LOR) position	Central Core (CC) position	Right Outer Repeat (ROR) position
1	1,401,537 - 1,403,528	1,403,529 - 1,404,898	1,404,899 - 1,406,890
2	843,845 - 846,543	846,544 - 847,800	847,801 - 850,499
3	34,397 - 37,045	37,046 - 37,928	37,929 - 40,577
4	78,869 - 81,427	81,428 - 82,538	82,539 - 85,097

Table S2. Sequence list.

LOR2 (2,699 bp)

5'-CCAATCAAACAAGGTGACTTGCGCGAAGCAATGATTTGTGGATGGGCTGCGGTATGGCAGCATAA
CAATGCAACGCTATTTTCAGAAATTGTAAAGTGTAAGGAAATATTC AACCCATAAAGAATCTACCGA
AACGTAGGATAAATAATTTCCACAGAGTACACTTTTGGTTTTTATGGACGGCGTTGAGTTGCACAGAT
GATGGAATATTGTGATAAAATACGCTATATAATTTGGAGACCCTAAGATCAAGTGGACTAGGTCGATT
GCGTATATTAGGGTATTAAAGACTTACTTAATTCTATAAGAGCTCGGCAAGCTATATTCTCAGTTCCTT
CGCAGTCGACCTTCAGTGCCAAAACCTTTAAAGTGGACGGGTGTTTTCTAAAGATTGCTATTGTTCA
ATTGCCCGGTTTTTTAACCGCTGAAAACCAAGTGGCAAGAGTGGACTACGTGCGCATTGCAATTTGTAT
AATCCATAATAATTTGTTCCACGGCCTTAAGTTATTGTACTGTTTGCTCAATTTGAAGCGGAATATCC
GTCCTAGTCTCGAATCCTTATTTAAGTACCGGTATCATTTGCATATCCTCTCAGCCCTCAAACGCCCAT
TCTTTATAGTAATGGTTATGTATTAATCGACTACGATGATTGACTCAGGGAGGCACGAATAGTATAATTT
GTAACCTTGCAAGTGGGAAGCTAATTTCCGGTGTCTTGAAAGACGAGGTTAAAAATTTGAACCCAAAC
CGATTTTACCGGAGATCCTACCTCAAATTAGAACAACCTTTAAAGCTTTCAAGGAATTGAAATTCATC
TTTATAGACCGTAATCTTTGAAGAGTAATCAATGTAAGAGCAAGTTCTAAAGAGAGGATCCAAATT
CCTTCTGATAGCAATTGTAAGGAGCTGCAAAGTTCAACATAATAATTTGCGATAGTAACCGTCCCTACGG
ATACTTAAATCGGTATCAAAGAGTGACCAATCCTGAATCAGTGTAGTGCAAAAAGAAATAACGATGA
TAAACGAATCAAGATTCTGATGAGTTGTCCAAGACGGGGCTATCATAATATTTAAAAAATAGAACCTA
CTAATTTAACAAGGTAAATCTCATAGATTTAAGTTTCACGACTAGCTTAGCTACCACTCAAATAAGCTC
CCGCTTGTTTCATTCGGCTGGGGTCTCCAATCTCCATCTCCAATGGATATTTTTTTTTTTAAGTGGAGAGA
GATGTTTCAGTAAACTGTGAGTTCAGTTGTCAGAAGCAACCAATATTTACCATGGAGACGAATAAGCA
GATATTATATACTCTATTTTTACAGCCCTTTTGTATAATCCTCAGGGATGGTTGGCGCTTCCCTGTGTTT
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AACTTTGCCCAACTAAATACTTTATCTGAATGAATCATTAAGAACACGACAAGTTCAGTATGTTGC
TTGGAGCCTCCGATTCACAGTCTTGTAAGTAGTGCGTGTTTTCAAATATTTGCATTAGCAATCCCTCCA
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TCGAAATGGTTAACTCCTGCTTAGTTTCATTTAAAAAATGAATAGAGAAGTAGATACAGAATACTTTG
TAAGACTGCTTACATGAATGATGCCAGAGAGTGTGCCGAAAGATCTACATGCTACTAAATTTACTATC
AATAGGGATAAAATGTATTAGGCTACCTACTATGCCGTATTGGAAAGGCGAAGTGCTTTGTATGCAAG
GGATTGGGGCATAGGCAAACGGATCTATGACAAGAGATACGAAATTACTTCTGTTACCCACTCTGCTC
CTACCAAACCAATATATCTGTGACCAAAAATATCATTTGCTTGTGCTCCACATAGCGGCCAGGCTC
AAAGAGAACACTAGATCTGCTTTCAGACATTACCGCGTGCAAATATGACAAGTCACTAGGTGATGAA
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CTTGAATAGTCAAATGCTGAGTTCATTTTAAAGTGAATATTTTTACCGAAGTCGATTTTAGCCCAT
AGTCAAACAACAGAAGAGAAAAGACAAACCTTCTTTTTCATGACACATGAAACAATCACAAAAAT
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AGTTCCAGAGCATGATGTTAGGGAAACTTACAGGGATGATGTGGGCCACAGCATAATATCAACAATA
CCTAACAATCGGTATTTAATTCATAGATATTCATACCAATTTCTCCACGATGAGAAGGGGCGACTCCTC
TTCTTGGACGTTCTGAAAATACAGGTACGCTCACGTCAGTGCCTGAGCACATACAGCGACGTTAGTA
TCAATCTTCTGGGACATCTCTAATGCTCTAAAATGAAGAAAAAGT-3'

CC2 (1,257 bp)

5'-AAAATTAGTATCAGTTGGCCATGCGACCAAGCCTGTTGGAACGCAAAGCATATCCGAATTATGGT
TGGA AATTACCTTCGACCTTCAAACACTAAACTCTTCACAGGGCTATTATTCCAGCTATTGCAGAACT
TCATACGAATTTGGGCTTGCTAAAAATTGGCAGGATCGTTCTTCAATAACTGAATACAGTATTGTACCT
ATTCAAGCAGTTAGTCCATACTTAGGGCCTTCCTAGCCTGTCCAGTCTACCATTAACGAAAGTTCTGA
GCTGTGTCTATCCAAATTAATTTGTGTGAGAACCTACGTAACCACTATAAGTCTCATAACGTTTTTTGAA
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GTCTTTACCAGGCCGAATCCTGTAGTTAAAATAATTTCTATTTGGACAACCTAAGGGTATCCCCTCA
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CTGTGTAATGCTCGCTGGTGTAGTAGCATAAGTCATTCCAATGGTAACTACCAAGCTTGTAAATAACC
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TTCGATAATGTTTTGGTCTAGGTTTCGTGCATGAAATTTTTTCTTCATCCAGACTATCTCTGCGCTGCAC
TGTTACATAGCCAACCTGATACTGATTC-3'

ROR2 (2,699 bp)

5'-ACTTTTTCTTCATTTTAGAGCATTAGAGATGTCCCAGGAAGATTGATACTAACGTCGCTGTATGTGC
TCAGGCACTGACGTGAGCGTACCTGTATTTTTCAGAACGTCCAAGAAGAGGAGTCGCCCCCTTCTCATC
GTGGAGAATTGGTATTGAATATCTATGAATTAATAACCGATTGTTAGGTATTGTTGATATTATGCTGTGG
CCCACATCATCCCTGTAAGTTTCCCTAACATCATGCTCTGGAACCTATAACGTATGGGTAGAACCTTGATC
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CCCCGTCCTGGACAACATCATCAGAATCTTGATTGTTTATCATCGTTATTTCTTTTTGCACTACACTGAT
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TTATAGAATTAAGTAAAGTCTTTAA**C**ACCCTAATATACGCAATCGACCTAGTCCACTTGATCTTAGGGTC
TCCAAAATTATATAGCGTATTTTATCACAAATTCATCATCTGTGCAACTCAACGCCGTCCATAAAAA
CCAAAAGTGTACTCTGTGGAAATTTATCTACGTTTCGGTAGATTCTTTATAGGGTTGAATATTTCC
TTTACACTTTACAATTTCTGAAATAGCGTTGCATTGTTATGCTGCCATACCGCAGCCCATCCACAAATC
ATTGCTTCGCGCAAGTCACCTTGTTTGATTGG-3'

PARS1 (164 bp)

5'-TCGAGATAAGCTGGGGGAACATTCGCGAAAATGAAACAAGTCGGCTGTTATAGTATATTTATTATAA
TATTGAAAGATCTCAAAGACTACTTATTTTTGAATGAACCAAGTATGAAATCAACCTATTTGGGGTT
GACCAAATAAGTAAATATTAATTGTCTGA-3'

Gray background; LOR2(1-111)

Bold red font; the different nucleotide between the LOR2 and ROR2.