

Table S1. Primers used for cloning.

RemoveFor	GCAGATATCCAGCACAGTGGCGGCCGCTCGAGTCTAGAGGGCCCTTCGAATGAGTTTAAACCCGCTGATCTGATAA CAACAGTGTAGATG
RemoveRev	CATCTACACTGTTGTTATCAGATCAGCGGGTTTAAACTCATTTCGAAGGGCCCTCTAGACTCGAGCGGCCGCCACTG TGCTGGATATCTGC
CipA1For	CGGCCGCTCGAGAATGCAACACCGACCAAGGGAGC
CipA3Rev	GCGGGTTTAAACTCACGTAGAATCGAGACCGAGGAGAGGGTTAGGGATAGGCTTACCTTCGAAGGGCCATTTCGAA TCATCTGTCCGGTGTGTTACAGG
CipA3For	CGGCCGCTCGAGGTATCGGCCGCCACAATGACAGTTCGAG
egl2For2	GAGAGAAGCTCATCATCACCATCACCATGGTCAGCAGACTGTCTGGGGCCA
egl2Rev2	GTAGAGGACCCACCTCCTCCAGATCCCCATGGCTTTCTTGCGAGACACG
cbh2For	GCAGAAGGCTCTTTGGACAAGAGAGAAGCTCGGCCGGATTATAAAGATGACGATGACAAACAAGCTTGCTCAAGCG TCTGGGGCCAATG
cbh2Rev	GTAGAGGACCCACCTCCTCCAGATCCCCATGGCAGGAACGATGGGTTTGCCTTTGTGAGAAGC
bgl1For3	GTTTTATTTCGCAGCATCCTCCGCATTAGCTGCAGGTGAACAAAACTCATCTCAGAAGAGGATCTGGATGAACTGG CGTTCTCTCCTCCATTCTATCCC
bgl1Rev3	CGTCGCCATTTACATCACCGTAAACAACCTGAGGAGAACCTCCACCGCCACTACCCCCGGGTTGCACCTTCGGGAG CGCTGCGTGAAGGGGC
BsaXIFor	GACCACACCTTACCAGCATGACTAGCCAGCTTTTGTTCCT
BsaXIRevRevised	GGAGAGCGCACGAGGGAGCT
GAL10For	CTGATTAATTACCCAGAAATAAGGCTAAAAAACTAATCGCATTATCATC
GAL10RevRevised	CAAGACAATCAAAACCTTCATTTATATTGAATTTTCAAAAATTCTTACTTTTTTTTTTGG
PreproForRevised	CCAAAAAAAAGTAAGAATTTTTGAAAATTCATATAAATGAAGGTTTTGATTGTCTTG
PreproRev	CCTAGGTACGATTTGTCATCGTCATCTTTATAATCCGGCCGAGCTTCTCTCTTGTC
DR1For	GATTATAAAGATGACGATGACAAATACGTACCTAGGAAAGAAGAACATGTGATCATCCAGGCCG
DR1Rev	CCATATGGGACCCACCTCCTCCAGATCCGAGCTCCTTGCTCTGTGCAGATTCAGATCG
C-mycFor	GAGCTCGGATCTGGAGGAGGTGGGTCCCATATGGAACAAAACTCATCTCAGAAGAGGATCTGTGATGGACTTCTT CGCCAGAGGTTTGGTCAAGTCTC
ADH1Rev	AGGGAACAAAAGCTGGCTAGTCATGCCGGTAGAGGTGTGGTC
AGA2For	GGCAGTAACCTGGCCCCACAAACCTTCAAATGAACG
pYD1Rev	GTCGATTTTGTACATCTACAC
9E-docStFor	CCATGGGGATCTGGAGGAGGTGGGTCCCTCTACTAAATTATACGGCGACGTC

9E-docStRev	GACTTGACCAAACCTCTGGCGAAGAAGTCCATTAGTTCTTGTACGGCAATGTA
G1A2For1	GCGATTAGTTTTTTAGCCTTATTTCTGGGG
G1A2Rev1	ACCTGCAGCTAATGCGGAGGATGCTGCGAATAAACTGCAGTAAAAATTGAAGGAAATCTCATGGTTTTTTCTCCT TGACGTTAAAGTATAGAGG
G1A2For2	CCTCAGGTTGTTTACGGTGATGTAAATGGCGACG
G1A2Rev2	GAGATCCGCTTATTTAGAAGTGTCGAATTCCTAATAAGGTAGGTGGGGTATGCTCTTTATC
G1A2For3	GAATTCGACACTTCTAAATAAGCGGATCTC
G1A2Rev3	GTAATACGACTCACTATAGGGCGAATTGGGCTCGAGGGCATGCGAAGGAAAATG
G10A1For1	GTACAGATCCCGACCCATTTGC
G10A1Rev1	CGCTTAACTGCTCATTGCTATATTGAAGTACGGATTAGAAGCCGCCGAGCGGGTGACAGC
G10A1For2	GTACTTCAATATAGCAATGAGCAGTTAAGCG
G10A1Rev2	ACCATGGTGTGGTGTATGATGAGCTTCTCTCTTGTCCAAAGAGCC
G10A1For3	CCATGGGGATCTGGAGGAGGTGGG
G10A1Rev3	CTAAAGGGAACAAAAGCTGGCTAGTCATGCCGGTAGAGGTGTGGTC
G10A1For4	GACCACACCTCTACCGGCATGACTAGCCAGCTTTTGTTCCTTTAG
G10A1Rev4	CCTGGTATCTTTATAGTCCTG
BGL1-ctrl-For	GGGCCCCCCTCGAGGTGACGGTATCGATACGGATTAGAAGCCGCCGAGCGGGTGACAG
BGL1-ctrl-Rev	CAATTAACCCTCACTAAAGGGAACAAAAGCTGGAGCTCCTCGAGGGCATGCGAAGGAAAATGAGAAATATCG
EGII-ctrl-For	GGGCCCCCCTCGAGGTGACGGTATCGATATCGCTTCGCTGATTAATTACCCAG
EGII-ctrl-Rev	CAATTAACCCTCACTAAAGGGAACAAAAGCTGGAGCTCCATGCCGGTAGAGGTGTGGTCAATAAGAGC

Table S2. PCR fragment sets for plasmid construction using the DNA assembler method (1).

Template	Primer pairs	PCR product
pYD1	BsaXIFor/BsaXIRevRevised	F0
Yeast genomic DNA	GAL10For/GAL10RevRevised	F1
pRSGAL	PreproForRevised/PreproRev	F2
NW1	DR1For/DR1Rev	F3
Yeast genomic DNA	C-mycFor/ADH1Rev	F4
pYD1	AGA2For/pYD1Rev	F5
<i>T. reesei</i> cDNA	cbh2For/cbh2Rev	FLAG-CBHII
<i>C. thermocellum</i> genomic DNA	9E-docStFor/9E-docStRev	docS
pYD1	G1A2For1/G1A2Rev1	G1A2-HR1
<i>A. aculeatus</i> cDNA	bgl1For3 / bgl1Rev3	c-Myc-BGL1
<i>C. thermocellum</i> gDNA	G1A2For2/G1A2Rev2	docAt
Yeast genomic DNA	G1A2For3/G1A2Rev3	ADH2
pYD1	G10A1For1/ G10A1Rev1	G10A1-HR1
pRS425-CBHII	G10A1For2/ G10A1Rev2	G10A1-HR2
pRS425-CBHII	G10A1For3/ G10A1Rev3	G10A1-HR3
pYD1	G10A1For4/ G10A1Rev4	G10A1-HR4
<i>T. reesei</i> cDNA	egl2For2/ egl2Rev2	HisG-EGII
pRS425-CBHII-BGL1	BGL1-ctrl-For/BGL1-ctrl-Rev	BGL1-ctrl
pYD1-R1-EGII	EGII-ctrl-For/EGII-ctrl-Rev	EGII-ctrl

1. **Shao, Z., H. Zhao, and H. Zhao.** 2009. DNA assembler, an *in vivo* genetic method for rapid construction of biochemical pathways. *Nucleic Acids Res* **37**:e16.