

The Insertion Green Monster (iGM) Method for Expression of Multiple Exogenous Genes in Yeast

Vyacheslav M. Labunskyy,^{*,1} Yo Suzuki,^{†,1} Timothy J. Hanly,[†] Ayako Murao,[†] Frederick P. Roth,^{‡,§,2} and Vadim N. Gladyshev^{*,2}

*Division of Genetics, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts 02115, [†]Department of Synthetic Biology and Bioenergy, J. Craig Venter Institute, La Jolla, California 92037, [‡]Donnelly Centre and Departments of Molecular Genetics and Computer Science, University of Toronto, Toronto, Ontario MSS 3E1, Canada, and [§]Lunenfeld-Tanenbaum Research Institute, Mt. Sinai Hospital, Toronto, Ontario M5T 3L9, Canada

¹These authors contributed equally to this work.

²Corresponding authors: University of Toronto, Donnelly Centre, 160 College Street, Toronto, Ontario M5S 3E1, Canada. E-mail: fritz.roth@utoronto.ca; Division of Genetics, Department of Medicine, Brigham and Women's Hospital, 77 Avenue Louis Pasteur, Boston, MA 02115. E-mail: vgladyshev@rics.bwh.harvard.edu

DOI: 10.1534/g3.114.010868

	Vbal claning junction	sites	Homology to the KanMYA case	otto	
	Xhol Nhel Secil	SnaBl Sex41	Sec/	Peci Asci B	
		00001	0007		80
->>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>	+ + +	Y . Y	T ACCONTRACTOR	TANK TANGE	I I
progmost map			t cgACGGATCCCGGGT		
	Homology to the KanMX4 casset	te	100		Sall/Xhol cloning junction
		100 I	120 I	140	180
pYOGM081 map		ACCCGGCCAGCGAC	ATGGAGGCCCAGAATAC	CCTCCTTGACAGTCTTG	ACGTGCG tcgagc t
	URA3 rescuing region	1			
		180 I	200 I	220 I	240 I
pYOGM081 map	tttcaa <mark>TTCAATTCATC1</mark>	TTTTTTTTTTGTT	CTITITITIGATICCGG	STTTCTTTGAAATTTTTT	TGATTCGGTAATCT
	URA3 rescuing region				
		260 I	280 I	300	320 I
pYOGM081 map	CCGAGCAGAAGGAAGAAG	GAAGGAAGGAGCAC	AGACTTAGATTGGTATA	TATACGCATATGTGGTG	TTGAAGAAACATGA
	URA3 rescuing region				
		340	360	380	400
pYOGM081 map	AATTGCCCAGTATTCTT	ACCCAACTGCACAG	AACAAAAACCTGCAGGA	AACGAAGATAAATCATG	TCGAAAGCTACATA
	URA3 rescuing region				
		420	440	460	480
pYOGM081 map	TAAGGAACGTGCTGCTAC	TCATCCTAGTCCTG	TTGCTGCCAAGCTATTI	AATATCATGCACGAAAA	GCAAACAAACTTGT
	URA3 rescuing region				
		500	520	540	560
pYOGM081 map	GTGCTTCATTGGATGTTC	GTACCACCAAGGAA	TTACTGGAGTTAGTTG	AGCATTAGGTCCCAAAA	TTTGTTTACTAAAA
	URA3 rescuing region				
		580	600	620	640
pYOGM081 map	ACACATGTGGATATCTTC	ACTGATTTTTCCAT	GGAGGGCACAGTTAAGC	CGCTAAAGGCATTATCC	GCCAAGTACAATTT
,	I IPA3 rescuing region				
	Crows rescaling region	660	680	700	720
nYOGM081 man	TTTACTCTTCGAAGACAC		I	I	TGTATACAGAATAG
proontorinap		A A A A A A A A A A A A A A A A A A A		in denot here to cooo	
	URA3 rescuing region	740	760	780	800
aVOCH081 man	CACANTEGECACACATT		CICCICCCCCCCCCCC	TETTACCECTICAAGE	
progmost map	CAGAATGGGCAGACATTA		GIGGIGGGCCCAGGIAI	TGTTAGCGGTTTGAAGC	AGGCGGCGGAAGAA
	URA3 rescuing region	Stul			
	URA3 rescuing region	820 820	840 I	860 I	880 I
pYOGM081 map	GTAACAAAGGAACCTAG		840 I AGCAGAATTGTCATGC/	860 I AAGGGCTCCCTAGCTACT	880 I GGAGAATATACTAA
pYOGM081 map	URA3 rescuing region GTAACAAAGGAACCTAG/ URA3 rescuing region		840 I AGCAGAATTGTCATGC/	860 I AAGGGCTCCCTAGCTACT	880 I GGAGAATATACTAA
pYOGM081 map	URA3 rescuing region GTAACAAAGGAACCTAGA URA3 rescuing region		840 I AGCAGAATTGTCATGC/ 920 I	860 AAGGGCTCCCTAGCTACT 940	880 GGAGAATATACTAA 960
pYOGM081 map pYOGM081 map	URA3 rescuing region GTAACAAAGGAACCTAG/ URA3 rescuing region GGGTACTGTTGACATTGC	STUU REA AGGACCTTTTGATGTT 900 CGAAGAGCGACAAAG	840 I AGCAGAATIGICATGC/ 820 I ATTTIGITATCGGCTTI	BED AAGGGCTCCCTAGCTACT P40 FATTGCTCAAAGAGACAT	880 I GGAGAATATACTAA 980 I GGGTGGAAGAGATG
pYOGM081 map pYOGM081 map	URA3 rescuing region GTAACAAAGGAACCTAG/ URA3 rescuing region GGGTACTGTTGACATTGC	STUT 825 AGGCCTTTTGATGTT 900 I GAAGAGCGACAAAG	840 I AGCAGAATTGTCATGC/ 220 I ATTTTGTTATCGGCTT1	800 AAGGGGCTCCCTAGCTACT 940 IATTGCTCAAAGAGACAT	880 J GGAGAATATACTAA 980 J GGGTGGAAGAGATG
pYOGM081 map pYOGM081 map	URA3 rescuing region GTAACAAAGGAACCTAG/ URA3 rescuing region GGGTACTGTTGACATTGC URA3 rescuing region	B25 B25 B26 B26 B26 B26 B26 B26 B26 B26	840 IAGCAGAATTGTCATGC/ 920 IATTTTGTTATCGGCTT1 1,000	800 1 AAGGGGCTCCCTAGCTACT 940 1 ATTGCTCAAAGAGACAT 1,020	880 J GGAGAATATACTAA 980 J GGGTGGAAGAGATG 1,040
pYOGM081 map pYOGM081 map pYOGM081 map	URA3 rescuing region GTAACAAAGGAACCTAGA URA3 rescuing region GGGTACTGTTGACATTGC URA3 rescuing region AAGGTTACGATTGGTTG	STUI 824 17 17 17 17 17 17 17 17 17 17	840 AGCAGAATTGTCATGC/ 920 I ATTITGTTATCGGCTTT 1,000 I GTGGGTTTAGATGACA/	860 I AAGGGGCTCCCTAGCTACT 940 I ATTGCTCAAAGAGACAT 1,020 I AGGGAGACGCATTGGGTC	880 J GGAGAATATACTAA 950 J GGGTGGAAGAGAGATG 1,040 J AACAGTATAGAACC
pYOGM081 map pYOGM081 map pYOGM081 map	URA3 rescuing region GTAACAAAGGAACCTAG/ URA3 rescuing region URA3 rescuing region AAGGTTACGATTGGTTG/ URA3 rescuing region	STUJ 824 17 17 17 17 17 17 17 17 17 17	840 I AGCAGAATTGTCATGC/ 920 I ATTTTGTTATCGGCTTT 1,000 I GTGGGTTTAGATGACA/	860 I AAGGGGCTCCCTAGCTACT 940 I ATTGCTCAAAGAGACAT 1,020 I AGGGGAGACGCATTGGGTC	BBD J GGAGAATATACTAA 960 J GGGTGGAAGAGAGATG 1,040 J AACAGTATAGAACC
pYOGM081 map pYOGM081 map pYOGM081 map	URA3 rescuing region GTAACAAAGGAACCTAG/ URA3 rescuing region GGGTACTGTTGACATTGC URA3 rescuing region AAGGTTACGATTGGTTG/ URA3 rescuing region	STUI 824 AGGCCTTTTGATGTT 900 IGGAAGAGCGACAAAG 980 I ATTATGACACCCGGT 1,060	840 I AGCAGAATTGTCATGC/ 920 I ATTTTGTTATCGGCTTT 1,000 GTGGGTTTAGATGACA/ 1,080	860 I AAGGGGTCCCTAGCTACT 940 I FATTGCTCAAAGAGACAT 1,020 I AGGGGAGACGCATTGGGTC 1,100	880 I GGAGAATATACTAA 990 I GGGTGGAAGAGAGATG 1,040 I AACAGTATAGAACC 1,120
pYOGM081 map pYOGM081 map pYOGM081 map	URA3 rescuing region GTAACAAAGGAACCTAG/ URA3 rescuing region GGGTACTGTTGACATTGC URA3 rescuing region AAGGTTACGATTGGTTG/ URA3 rescuing region GTGGATGATGTGGTCTC	STULL BC BC BC BC BC BC BC BC BC BC	840 AGCAGAATTGTCATGC/ 920 1 ATTTTGTTATCGGCTTT 1,000 GTGGGTTTAGATGACA/ 1,080 TATTATTGTTGGAAGAG	860 I AAGGGGCTCCCTAGCTACT 940 I FATTGCTCAAAGAGACAT 1,020 I AGGGGAGACGCATTGGGTC 1,100 I GGACTATTTGCAAAGGGA	BBO GGAGAATATACTAA 960 I GGGTGGAAGAGAGAT 1,040 I AACAGTATAGAACC 1,120 I AGGGGATGCTAAGGT
pYOGM081 map pYOGM081 map pYOGM081 map	URA3 rescuing region GTAACAAAGGAACCTAG/ URA3 rescuing region GGGTACTGTTGACATTGC URA3 rescuing region AAGGTTACGATTGGTTG/ URA3 rescuing region GTGGATGATGTGGTCTCT	980 I AGGECTTTTTGATGTT GAAGAGCGACAAAG 980 I ATTATGACACCCGGT 1,060 I ACAGGATCTGACAT	840 AGCAGAATTGTCATGC/ 920 ATTTTGTTATCGGCTTT 1,000 GTGGGTTTAGATGACA/ 1,080 TATTATTGTTGGAAGAG	860 AAGGGGCTCCCTAGCTACT 940 1 CATTGCTCAAAGAGAGACAT 1,020 AGGGGAGACGCATTGGGTC 1,100 5GACTATTTGCAAAGGGA	BBD GGAGAATATACTAA 960 I GGGTGGAAGAGATG 1,040 I AACAGTATAGAACC 1,120 AGGGATGCTAAGGT
pYOGM081 map pYOGM081 map pYOGM081 map pYOGM081 map	URA3 rescuing region GTAACAAAGGAACCTAG/ URA3 rescuing region GGGTACTGTTGACATTGC URA3 rescuing region GTGGATGATGGTGGTCTC1 URA3 rescuing region	STULI 825 SOO I GAAGAGCGACAAAG 980 I ATTATGACACCCGGT 1,060 TACCAGGATCTGACAT 1,140	840 I AGCAGAATTGTCATGC/ 920 ATTTTGTTATCGGCTTT 1,000 GTGGGTTTAGATGACA/ 1,080 TATTATTGTTGGAAGAG	890 AAGGGGCTCCCTAGCTACT 940 I ATTGCTCAAAGAGACAT 1,020 AGGGGAGACGCATTGGGTC 1,100 5GACTATTTGCAAAGGGA 1,180	880 J GGAGAATATACTAA 980 J GGGTGGAAGAGATG 1,040 J AACAGTATAGAACC 1,120 AGGGATGCTAAGGT 1,200
pYOGM081 map pYOGM081 map pYOGM081 map pYOGM081 map	URA3 rescuing region GTAACAAAGGAACCTAG/ URA3 rescuing region GGGTACTGTTGACATTGC URA3 rescuing region AAGGTTACGATTGGTTG/ URA3 rescuing region GTGGATGATGTGGTCTCT URA3 rescuing region AGAGGGTGAACGTTACAC	800 I GAAGAGCGACAAAG 980 ATTATGACACCCGGT 1,060 I TACAGGATCTGACAT 1,140 GAAAAAGCAGGCTGGG	840 AGCAGAATTGTCATGC/ 820 ATTTTGTTATCGGCTTT 1,000 I GTGGGTTTAGATGACA/ 1,080 TATTATTGTTGGAAGAG/ 1,180 1,180 1,180	890 AAGGGGCTCCCTAGCTACT 940 I ATTGCTCAAAGAGACAT 1,00 I GGACTATTTGCAAAGGGA 1,180 1,180	880 J GGAGAATATACTAA 990 J GGGTGGAAGAGATG 1,00 AACAGTATAGAACC 1,120 AGGGATGCTAAGGT 1,200 1,200 AAAAAACTGTATAT
pYOGM081 map pYOGM081 map pYOGM081 map pYOGM081 map	URA3 rescuing region GTAACAAAGGAACCTAG/ URA3 rescuing region GGGTACTGTTGACATTGC URA3 rescuing region AAGGTTACGATTGGTTG/ URA3 rescuing region GTGGATGATGTGGTCTC1 URA3 rescuing region AGAGGGTGAACGTTACAC	800 I GAAGAGCGACAAAG 800 ATTATGACACCCGGT 1.060 I GAAGAGGATCTGACAT 1,140 GAAAAGCAGGCTGGG	840 AGCAGAATTGTCATGC/ 920 ATTTTGTTATCGGCTTT 1,000 GTGGGTTTAGATGACA/ 1,080 TATTATTGTTGGAAGAG/ 1.180 1.190 1.190	800 AAGGGGCTCCCTAGCTACT 940 1 ATTGCTCAAAGAGACAT 1,00 1,100 5GACTATTTGCAAAGGGA 1,180 1,180 1,180 3 3 3 3 3 3 3 3 3 3 3 3 3	BBD J GGAGAATATACTAA 960 J GGGTGGAAGAGAGATG 1,040 AACAGTATAGAACC 1,120 J AGGGGATGCTAAGGT 1,200 J AAAAACTGTATTAT 320//Xbol cloning lunction
pYOGM081 map pYOGM081 map pYOGM081 map pYOGM081 map	URA3 rescuing region GTAACAAAGGAACCTAG/ URA3 rescuing region GGGTACTGTTGACATTGC URA3 rescuing region AAGGTTACGATTGGTTG/ URA3 rescuing region AGAGGTGAAGGTGACGTTACAG	900 IGAAGAGCGACAAAG 980 ITTATGACACCCGGT 1.060 IACAGGATCTGACAT 1,140 GAAAAGCAGGCTGGG	840 AGCAGAATTGTCATGC/ 920 ATTITGTTATCGGCTTT 1,000 GTGGGTTTAGATGACA/ 1,080 TATTATTGTTGGAAGACA/ 1,180 AAGCATATTGAGAAG/	890 AAGGGGCTCCCTAGCTACT 940 IATTGCTCAAAGAGACAT 1.020 AGGGGAGACGCAITGGGTC 1.100 3GACTATTTGCAAAGGGA 1.180 ATGCGGGCCAGCAAAACTA [⁸	BBD J GGAGAATATACTAA 960 J GGGTGGAAGAGAGATG 1,040 J AACAGTATAGAACC 1,120 J AGGGATGCTAAGGT 1,200 AAAAACTGTATTAT 301/Xhol cloning junction i, ADH1 terminator
pYOGM081 map pYOGM081 map pYOGM081 map pYOGM081 map	URA3 rescuing region GTAACAAAGGAACCTAG/ URA3 rescuing region GGGTACTGTTGACATTGC URA3 rescuing region GTGGATGATGGTTGGTTG/ URA3 rescuing region AGAGGGTGAACGTTACAC URA3 rescuing region	STULI BCO SCOL	840 AGCAGAATTGTCATGC/ 920 I ATTITGTTATCGGCTTT 1,000 GTGGGTTTAGAGAGACA/ 1,080 TATTATTGTTGGAAGACA/ 1,160 1,160 1,240	860 AAGGGGCTCCCTAGCTACT 940 FATTGCTCAAAGAGACAT 1,020 AGGGGAGACGCATTGGGTC 1,100 SGACTATTTGCAAAGGGA 1,180 ATGCGGCCAGCAAAACTA	BBD GGAGAATATACTAA 960 I GGGTGGAAGAGATG 1,040 I AACAGTATAGAACC 1,120 I AGGGATGCTAAGGT 1,200 I AAAAAACTGTATTAT 3all/Xhol cloning Junction ADH1 terminator 1,280 I
pYOGM081 map pYOGM081 map pYOGM081 map pYOGM081 map pYOGM081 map	URA3 rescuing region GTAACAAAGGAACCTAGA URA3 rescuing region GGGTACTGTTGACATTGC URA3 rescuing region GTGGATGATGTGGTTCT URA3 rescuing region AGAGGGTGAACGTTACAC URA3 rescuing region AGAGGGTGAACGTTACAC	STULL BCO BCO I GAAGAGCGACAAAG BEO I I,060 I ACAGGATCTGACAT 1,140 I GAAAAAGCAGGCTGGG 1,220 I TAAACTCACAAAATT	840 AGCAGAATTGTCATGC/ 820 ATTTTGTATCGGCTTT 1,000 GTGGGTTTAGATGACA/ 1,080 TATTATTGTTGGAAGAC 1,180 TATTATTGTTGGAAGAC 1,180 AAGCATATTTGAGAAG/ 1,240 1240 AGAGCTTCAATTTAA	B60 AAGGGGCTCCCTAGCTACT 940 IATTGCTCAAAGAGACAT 1,020 IAGGGGAGACGCATTGGGTC 1,100 IATGCGGCCAGCAAAAGGGA 1,180 ATGCGGCCAGCAAAACTA 1,260 I TATATCAGTTATTACCC	BBD GGAGAATATACTAA 960 1 GGGTGGAAGAGATG 1,040 1 AACAGTATAGAACC 1,120 1,200 1
рYOGM081 map рYOGM081 map рYOGM081 map рYOGM081 map рYOGM081 map	URA3 rescuing region GTAACAAAGGAACCTAG/ URA3 rescuing region GGGTACTGTTGACATTGC URA3 rescuing region AAGGTTACGATTGGTTG/ URA3 rescuing region AGAGGGTGAACGTTACAG URA3 rescuing region AGAGGGTGAACGTTACAG	STULI B25 B26 B26 B27 B27 B27 B27 B27 B27 B27 B27 B27 B27	840 AGCAGAATTGTCATGC/ 920 ATTTTGTTATCGGCTTT 1,000 GTGGGTTTAGATGACA/ 1,080 1,160 AAGCATATTTGAGAAGA(1,160 AAGCATATTTGAGAAGA(1,240 1,240 1,240	860 AAGGGGCTCCCTAGCTACT 940 1 CATTGCTCAAAGGACAT 1,020 AGGGGAGACGCATTGGGTC 1,100 5 GACTATTTGCAAAGGGA 1,180 ATGCGGCCAGCAAAACTA 1,280	GGAGAATATACTAA 960 1 GGGTGGAAGAGATG 1,040 1 AACAGTATAGAACC 1,120 AAGGGATGCTAAGGT 1,200 AAAAACTGTATTAT Sall/Xhol cloning junction 1,200 1,200 AAAAACTGTATTAT Sall/Xhol cloning junction 1,200 1,200 AAAAACTGTATTAT
pYOGM081 map pYOGM081 map pYOGM081 map pYOGM081 map pYOGM081 map	URA3 rescuing region GTAACAAAGGAACCTAG/ URA3 rescuing region GGGTACTGTTGACATTGC URA3 rescuing region AAGGTTACGATTGGTTG/ URA3 rescuing region AGAGGGTGAACGTTACAG URA3 rescuing region AGAGGGTGAACGTTACAG URA3 rescuing region AAGTAAATGCATGTATAG	STULI B25 B26 B27 B27 B27 B27 B27 B27 B27 B27	840 A GC A G A A T I G T C A T G C / 820 A T T T T G T T A T C G G C T T I 1,000 G T G G G T T T A G A T G A C A / 1,080 T A T T A T T G T T G G A A G A (1,160 A A G C A T A T T T G A G A A G / 1,240	890 AAGGGGCTCCCTAGCTACT 940 (ATTGCTCAAAGAGACAT 1,020 AGGGGAGACGCATTGGGTC 1,100 5GACTATTTGCAAAGGGA 1,180 1,180 1,280 1,280 (ATATCAGTTATTACCC) 1,240	BBD GGAGAATATACTAA 960 1 GGGTGGAAGAGATG 1,040 AACAGTATAGAACC 1,120 1,120 AGGGGATGCTAAGGT 1,200 1
рYOGM081 map рYOGM081 map рYOGM081 map рYOGM081 map рYOGM081 map	URA3 rescuing region GTAACAAAGGAACCTAG/ URA3 rescuing region GGGTACTGTTGACATTGC URA3 rescuing region AAGGTTACGATTGGTTG/ URA3 rescuing region AGAGGGTGAACGTTACAC URA3 rescuing region AGAGGGTGAACGTTACAC URA3 rescuing region AAGTAAATGCATGTATAC	980 I GAAGAGCGACAAAG 980 ATTATGACACCCGGT 1,060 I TACAGGATCTGACAT 1,140 I GAAAAGCAGGCTGGG 1,220 I TACACTCACAAATT 1,300 ATTTCTTATGACTT	840 AGCAGAATTGTCATGC/ 820 ATTTTGTTATCGGCTTT 1,000 I GTGGGTTTAGATGACA/ 1,080 TATTATTGTTGGAAGAA 1,180 AAGCATATTTGAGAAGA/ 1,240 AGAGCTTCAATTTAA 1,320 ATGATTTTATTATTA/	890 AAGGGGCTCCCTAGCTACT 940 ATTGCTCAAAGAGACAT 1,200 3GGGGAGACGCATTGGGTC 1,100 3GACTATTTGCAAAGGGA 1,180 ATGCGGCCAGCAAAACTA 1,280 1,260 1,2	BBD GGAGAATATACTAA 990 GGGTGGAAGAGATG 1,040 AACAGTATAGAACC 1,120 1,120 1,120 1,200 1
рYOGM081 map рYOGM081 map рYOGM081 map рYOGM081 map рYOGM081 map рYOGM081 map	URA3 rescuing region GTAACAAAGGAACCTAG/ URA3 rescuing region GGGTACTGTTGACATTGC URA3 rescuing region AAGGTTACGATTGGTTG/ URA3 rescuing region AGAGGGTGAACGTTACAC URA3 rescuing region AGAGGGTGAACGTTACAC URA3 rescuing region AGAGGGTGAACGTTACAC URA3 rescuing region AGAGGGTGAACGTTACAC URA3 rescuing region AGAGGGTGAACGTTACAC	800 I GAAGAGCGACAAAG 900 I GAAGAGCGACAAAG 900 ATTATGACACCCGGT 1,060 1,140 I GAAAAAGCAGGCTGGG 1,220 1,220 1,20 1,20 1,300 ATTTCTTATGATTT	840 AGCAGAATIGICATGC/ 920 ATTITGITATCGGCTTT 1,000 TATTATIGITAGATGACA/ 1,080 TATTATIGITGGAAGAG/ 1,180 AAGCATATITGAGAAG/ 1,240 AGAGCTTCAATTTAA 1,320 ATGATITITATTATTA/	890 AAGGGGCTCCCTAGCTACT 940 IATTGCTCAAAGAGACAT 1,00 3GGGGAGACGCATTGGGTC 1,100 1,100 1,100 1,180 ATGCGGCCAGCAAAACTA 1,280 1,260 1,240 AATAAGTTATAAAAAAAAA	BBD GGAGAATATACTAA 990 GGGTGGAAGAGATG 1,040 AACAGTATAGAACC 1,120 1 AGGGATGCTAAGAT 1,200 1 AGGGATGCTAAGGT 1,200 1,2
pYOGM081 map pYOGM081 map pYOGM081 map pYOGM081 map pYOGM081 map pYOGM081 map	URA3 rescuing region GTAACAAAGGAACCTAG/ URA3 rescuing region GGGTACTGTTGACATTGG URA3 rescuing region GTGGATGATGGTTGG URA3 rescuing region AGAGGGTGAACGTTACAG URA3 rescuing region AGAGGGTGAACGTTACAG ADH1 terminator CCACTTCTAAATAAGCG/ ADH1 terminator	800 100 100 100 100 100 100 100	840 AGCAGAATTGTCATGC/ 920 ATTTTGTTATCGGCTTT 1,000 GTGGGTTTAGATGACA/ 1,080 TATTATTGTTGGAAGAAG/ 1,180 TATTATTGTTGGAAGAAG/ 1,240 AGGAGCTTCAATTTAATTA/ 1,220 ATGATTTTATTATTA/ 1,400	800 AAGGGGCTCCCTAGCTACT 940 ATTGCTCAAAGAGACAT 1,00 3GGGAGACGCATTGGGTC 1,100 3GACTATTTGCAAAGGGA 1,180 ATGCGGCCAGCAAAACTA 1,280 ATATCAGTTATACCC 1,340 ATAAGTTATAAAAAAAA	BBD GGAGAATATACTAA GGGTGGAAGAGATG 1.400 I AACAGTATAGAACC 1.120 I AGGGATGCTAAGGT 1.200 I AGGGATGCTAAGGT 1.200 I AACAGTATACAAG 1.200 I AGGGATGCTAAGGT 1.200 I AGGGATGCTAAGGT 1.200 I AACAGTGTATACAAA 1.200 I AGGGAGAAGAGAGAGAGAGAGAGAGAGAGAGAGAGAG
рYOGM081 map рYOGM081 map рYOGM081 map рYOGM081 map рYOGM081 map рYOGM081 map	URA3 rescuing region GTAACAAAGGAACCTAG/ URA3 rescuing region GGGTACTGTTGACATTGG URA3 rescuing region GTGGATGATGGTGGTCC1 URA3 rescuing region AGAGGGTGAACGTTACAG URA3 rescuing region AGAGGGTGAACGTTACAG ADH1 terminator CCACTTCTAAATAAGCG/ IADH1 terminator	STULI BCO GGC CTTTTGATGTT BCO GAAGAGCGACAAAG BEO I I.GEO I I.GEO I I.GEO I I.ACAGGATCTGACAT I.140 GAAAAAGCAGGCTGGG I.220 TAAAACTCACAAATT I.300 I I.380 SGTTTTAAAACGAAA	840 AGCAGAATTGTCATGC/ 820 ATTTTGTATCGGCTTT 1,000 TATTATGTTAGATGACA/ 1,080 TATTATTGTTGGAAGAC 1,180 AAGCATATTTGAGAAGA 1,240 AGAGCTTCCAATTTAA 1,320 ATGATTTTTATTATTA/ 1,400 ATTCTTGAGTCTTGAGT/	860 AAGGGGCTCCCTAGCTACT 940 TATTGCTCAAAGAGACAT 1,020 AGGGGAGACGCATTGGGTC 1,100 GGACTATTTGCAAAGGGA 1,180 ATGCGGCCAGCAAAACTA 1,260 TATATCAGTTATTACCC 1,240 AATAAGTTATAAAAAAAA 1,420 AACTCTTTCCTGTAGGTC	BBD GGAGAATATACTAA BC GGGTGGAAGAGATG 1,040 I AACAGTATAGAACC 1,120 I AGGGATGCTAAGGT 1,200 I AAAAAACTGTATAGAACC 1,120 I AAAAAACTGTATAGA 1,200 I AAAAAACTGTATATA 1,200 I AAAAAACTGTATAGAGT 1,200 I AAAAAACTGTATAGAAC 1,200 I AAAAAACTGTATACAAA 1,200 I AAAAAACTGTATACAAA 1,360 I AAAGTGTATACAAAA
рYOGM081 map рYOGM081 map рYOGM081 map рYOGM081 map рYOGM081 map рYOGM081 map	URA3 rescuing region GTAACAAAGGAACCTAGA URA3 rescuing region GGGTACTGTTGACATTGC URA3 rescuing region AAGGTTACGATTGGTTGA URA3 rescuing region AGAGGGTGAACGTTACAC URA3 rescuing region AGAGGGTGAACGTTACAC URA3 rescuing region AGAGGGTGAACGTTACAC URA3 rescuing region AGAGGGTGAACGTTACAC URA3 rescuing region AGAGGGTGAACGTTACAC ADH1 terminator TTTTAAAGTGACTCTTAC	STULI BCO GAAGAGCGACAAAG BEO I ATTATGACACCCGGT 1.060 IACAGGATCTGACAT 1.140 SAAAAAGCAGGCTGGG 1.220 I TAAACTCACAAATT 1.300 AATTTCTTATGATTT 1.380 SGTTTTAAAAACGAAA	840 AGCAGAATIGTCATGC/ 920 ATTITGTATCGGCTTT 1,000 TATTATIGTAGAAGACA/ 1,000 TATTATIGTGGAAGACA/ 1,160 AAGCATATITGAGAAGAC 1,160 AAGCATATITGAGAAGAC 1,240 AGGAGCTTCAATITAATTA/ 1,240 AGGAGCTTCAATITAATTA/ 1,400 ATTCTTGTTCTTGAGT/	800 AAGGGGCTCCCTAGCTACT 940 1 CATTGCTCAAAGAGACAT 1,020 3 3 3 3 3 3 3 3 3 3 3 3 3	BBD GGAGAATATACTAA 900 1 GGGTGGAAGAGATG 1,040 1 AACAGTATAGAACC 1,120 AACAGTATAGAACC 1,120 AACAGTGTATAGAACC 1,120 AAAAACTGTATAGA 1,120 AAAAACTGTATATA 3all/Xhol cloning Junction 1,280 AAAAAACTGTATTAT 3all/Xhol cloning Junction 1,280 1,280 AAAAACTGTATACAAA 1,280 AAAAACTGTATACAAAA
рYOGM081 map рYOGM081 map рYOGM081 map рYOGM081 map рYOGM081 map рYOGM081 map рYOGM081 map	URA3 rescuing region GTAACAAAGGAACCTAGA URA3 rescuing region GGGTACTGTTGACATTGC URA3 rescuing region AAGGTTACGATTGGTTGA URA3 rescuing region AGAGGGTGAACGTTACAG URA3 rescuing region AGAGGGTGAACGTTACAG AGAGGTAAATGCATGTATAG ADH1 terminator TTTTAAAGTGACTCTTAG ADH1 terminator	STULI B25 B26 B27 B27 B27 B27 B27 B27 B27 B27	840 A GC A G A A T I G T C A T G C / 920 A T T T T G T T A T C G G C T T 1 1,000 T A T T A T G T G G A A G A C A / 1,160 A A G C A T A T T G A G A A G A C / 1,160 1,160 A A G C A T A T T T G A G A A G / 1,240 A G A G C T C A A T T T A A T A / 1,320 A T G A T T T T A T T A T T A / 1,400 A T T C T T G T C T T G A G T / 1,400 1,400	800 AAGGGGCTCCCTAGCTACT 940 CATTGCTCAAAGAGACAT 1,000 GGGGGAGACGCATTGGGTC 1,100 GGACTATTTGCAAAGGGA 1,180 ATGCGGCCAGCAAAACTA 1,280 ATGCGGCCAGCAAAACTA 1,280 ATATCAGTTATACCCG 1,240 ATATAAGTTATAAAAAAAA 1,420 AACTCTTTCCTGTAGGTC Two copies of tetO	GGAGAATATACTAA 900 1 GGGTGGAAGAGATG 1,040 1 AACAGTATAGAACC 1,120 AACAGTATAGAACC 1,120 1,20
рYOGM081 map рYOGM081 map рYOGM081 map рYOGM081 map рYOGM081 map рYOGM081 map рYOGM081 map	URA3 rescuing region GTAACAAAGGAACCTAGA URA3 rescuing region GGGTACTGTTGACATTGC URA3 rescuing region AAGGTTACGATTGGTTG7 URA3 rescuing region AGAGGGTGAACGTTACAG URA3 rescuing region AGAGGGTGAACGTTACAG URA3 rescuing region AGAGGGTGAACGTTACAG ADH1 terminator CCACTTCTAAATAAGCGA	825 800 1 500 1 500 1 500 1 500 1 507 507 507 507 507 507 507 507	840 A GC A G A A T I G T C A T G C / 820 A T T T T G T T A T C G G C T T 1 1,000 G T G G G T T T A G A T G A C A / 1,080 T A T T A T T G T T G G A A G A G 1,160 A A G C A T A T T T G A G A A G A 1,240 1,240 A G A G C T C A A T T T A A T A / 1,320 A T G A T T T T T T A T T A T T A / 1,400 A T T C T T G T C T T G A G T / 1,480 1,160 1,480 1,160 1,480	B90 AAGGGGCTCCCTAGCTACT 940 (ATTGCTCAAAGAGACAT 1,020 AGGGGAGACGCATTGGGTC 1,100 5GACTATTTGCAAAGGGA 1,180 1,180 ATGCGGCCAGCAAAACTA 1,280 1,280 1,140 AATAAGTTATACCC 1,240 AATAAGTTATAAAAAAA 1,420 AACTCTTTCCTGTAGGTC 1,500	BBD GGAGAATATACTAA 900 1 GGGTGGAAGAGATG 1,040 AACAGTGTATAGAACC 1,120 1,200 1,
рYOGM081 map рYOGM081 map рYOGM081 map рYOGM081 map рYOGM081 map рYOGM081 map рYOGM081 map	URA3 rescuing region GTAACAAAGGAACCTAG/ URA3 rescuing region GGGTACTGTTGACATTGC URA3 rescuing region AAGGTTACGATTGGTTG/ URA3 rescuing region AGAGGGTGAACGTTACAG URA3 rescuing region AGAGGGTGAACGTTACAG URA3 rescuing region AAGTAAATGCATGATATAG ADH1 terminator CCACTTCTAAATAAGCG/ ADH1 terminator TTTTAAAGTGACTCTTAG ADH1 terminator	STULI B25 B20 I GAAGAGCCTTTTGATGTT B00 I GAAGAGCGACAAAG 980 ATTATGACACCCGGT 1,060 I 1,060 I 1,140 I 1,220 TAAACTCACAAATT 1,300 I 1,300 GTTTTTATGACACGAAA 1,460 I CTCTTATTGACCACA	840 AGCAGAATIGTCATGC/ 820 ATTITGTATCGGCTTT 1,000 GTGGGTITAGATGACA/ 1,080 TATTATIGTTGGAAGAAG/ 1,160 AAGCATATTTGAGAAG/ 1,240 AGAGCTTCAATTTAA 1,200 ATGATTTTTATTATTA/ 1,400 ATTCTTGTTCTTGAGT/ 1,460 CCTCTACCGGCAGATC/	B90 AAGGGGCTCCCTAGCTACT 940 (ATTGCTCAAAGAGACAT 1,200 3GGGGAGACGCATTGGGTC 1,100 3GACTATTTGCAAAGGGA 1,180 ATGCGGCCAGCAAAACTA 1,280 1,240 AATAAGTTATAACAAAA 1,420 AACTCTTTCCTGTAGGTC 1,60 1,60 1,60 1,60 1,60 1,60 1,60 1,60	BBD GGAGAATATACTAA 900 I GGGTGGAAGAGATG 1,040 AACAGTGTATAGAACC 1,120 I AACAGTATAGAACC 1,120 I AAGGGATGCTAAGGT 1,200 I AAGGGATGCTAAGGT 1,200 I AAGGGATGCTAAGGT 1,200 I AAGGGATGCTAAGGAG I AGGGTTGCTTTCTCA
рYOGM081 map рYOGM081 map рYOGM081 map рYOGM081 map рYOGM081 map рYOGM081 map рYOGM081 map	URA3 rescuing region GGTACTGTTGACATTGG URA3 rescuing region AAGGTTACGATTGGCATTGG URA3 rescuing region GTGGATGATGGTGGTCC URA3 rescuing region AGAGGGTGAACGTTACAG URA3 rescuing region AAGTAAATGCATGTATAG ADH1 terminator CCACTTCTAAATAAGCG/ ADH1 terminator TTTTAAAGTGACTCTTAG IDH1 terminator GGTATAGCATGAGGTCGG Two copies of tetO	STULI SCOL	840 AGCAGAATIGICATGC/ 920 ATTITGITATCGGCTTT 1,000 TATTATIGITAGATGACA/ 1,180 AAGCATATITGAGAAGA/ 1,180 AAGCATATITGAGAAG/ 1,240 AGAGCTICAATITAA 1,240 AGAGCTICAATITAA 1,240 AGAGCTICAATITAA 1,240 AGAGCTICAATITAA 1,240 AGAGCTICAATITAA 1,240 AGAGCTICAATITAA 1,240 AGAGCTICAATITAA 1,240 AGAGCTICAATITAA 1,240 AGAGCTICAATITAA 1,240 AGAGCTICAATITAA 1,240 AGAGCTICAATITAA 1,240 AGAGCTICAATITAA 1,240 AGAGCTICAATITAA 1,240 AGAGCTICAATITAA 1,240 AGAGCTICAATITAA 1,240 AGAGCTICAATITAA 1,240 AGAGCTICAATITAA 1,240 ATGATITICATIGICAA 1,240 AGAGCTICAATITAA 1,480 AGAGCTICAAGAGCAA	B90 AAGGGGCTCCCTAGCTACT 940 ATTGCTCAAAGAGACAT 1,200 AGGGGAGACGCATTGGGTC 1,100 3GACTATTTGCAAAGGGA 1,180 ATGCGGCCAGCAAAACTA 1,280 1,260 1,240 AATAAGTTATAACAAA 1,220 AATAAGTTATAAAAAAAA 1,220 AATAAGTTATAAAAAAAA 1,220 AATAACTCTTCCTGTAGGTC Two copies of telO 1,500 AATTCCTCGATCCCTATC	GGAGAATATACTAA GGAGAATATACTAA GGGTGGAAGAGATG 1,040 1 AACAGTATAGAACC 1,120 1,120 AGGGATGCTAAGGT 1,200 AACAACTGTATTAT 1,200 AACAGTATAGAGAGT 1,200
рYOGM081 map рYOGM081 map рYOGM081 map рYOGM081 map рYOGM081 map рYOGM081 map рYOGM081 map	URA3 rescuing region GGTACTGTTGACATTGG URA3 rescuing region AAGGTACTGTTGACATTGG URA3 rescuing region GTGGATGATGGTGGTCTC URA3 rescuing region AGAGGGTGAACGTTACAG URA3 rescuing region AAGTAAATGCATGTATAG ADH1 terminator CCACTTCTAAATAAGCG IADH1 terminator TTTTAAAGTGACTCTTAG ADH1 terminator TTTTAAAGTGACTCTTAG IADH1 terminator GGTATAGCATGAGGTCGG Two copies of tetO	800 G A A G A G C G A C A A A G 800 1 G A A G A G C G A C A A A G 800 1 1,060 1,140	840 AGCAGAATTGTCATGC/ 820 ATTTTGTATCGGCTTT 1,000 GTGGGTTTAGATGACA/ 1,080 TATTATTGTTGGAAGAC 1,180 TATTATTGTTGGAAGAC 1,240 AGGAGCTTCAATTTAA 1,240 AGGAGCTTCAATTTAA 1,240 AGGAGCTTCAATTTAA 1,240 ATGATTTTTATTATTA/ 1,400 ATTCTTGTTCTTGAGT/ 1,460 CCTCTACCGGCAGATC/ <u>S80//</u> 1,560	BEO AAGGGGCTCCCTAGCTACT P40 TATTGCTCAAAGGAACAT 1,020 AGGGGAGACGCATTGGGTC 1,100 GGACTATTTGCAAAGGGA 1,180 ATGCGGCCAGCAAAACTA 1,180 ATGCGGCCAGCAAAACTA 1,260 TATATCAGTTATTACCC 1,240 AATAAGTTATAAAAAAAA 1,420 AATTACTTTCCTGTAGGTC 1,260 AATTCCTCGATCCCTATC	BBD GGAGAATATACTAA 900 GGGTGGAAGAGATG 1,040 AACAGTATAGAACC 1,120 AACAGTATAGAACC 1,120 AGGGATGCTAAGGT 1,200 AAAAACTGTATAGA 1,120 AACAGTATAGAAGT 1,200 1,120 1,20 1

Figure S1 Sequence of the pYOGM081 plasmid containing the iGM gene insertion module. In a version of the plasmid not containing the HA-tag (pTJH001), nucleotides 4,954–5,101 are removed and replaced with a stop codon (TAA) and a *Hind*III site (Materials and Methods). In plasmids containing the *ADH1* (pTJH002), *TEF* (pTJH003), and *CUP1* (pTJH004) promoters for driving the expression of a gene introduced between the Gateway cloning sites, nucleotides 2,755–3,232 are replaced with the respective sequences shown below (Materials and Methods).

	TATA region of CYC1			
	1,620	1,/	640 1	1,660 1,680 1,680 1
pYOGM081 map	TGTGCTCTGTATGTATATAAA	ACTCTTGTTTTCTTCTTT	τστοταλαταττοτττοστι	TATACATTAGGTCCTTTGTAGC
	TATA region of CYC1			GFP coding sequence
	1,700	1,3	720 1	1,760
pYOGM081 map	ΑΤΑΑΑΤΤΑCΤΑΤΑCΤΤCΤΑΤΑ	GACACGCAAACACAAATA	CACACACTAAATTACCGGA1	CAATTCGGGGGATCCATGAGT
	GFP coding sequence			
	1,780 	1,/	800 1	1,820 1,840
pYOGM081 map	AAAGGAGAAGAACTTTTCACT	GGAGTGGTCCCAGTTCTT	GTTGAATTAGATGGCGATGI	TAATGGGCAAAAATTCTCTGT
	GFP coding sequence			
	1,860 	1,	560 1	1,900 1,920 I I
pYOGM081 map	CAGTGGAGAGGGTGAAGGTGA	TGCAACATACGGAAAACT	TACCCTTAATTTTATTTGC	ACTACTGGGAAGCTACCTGTTC
	GFP coding sequence			
	1,940	1,1	960	1,980 2,000 I I
pYOGM081 map	CATGGCCAACACTTGTCACTA	CTTTCTCTTATGGTGTTC	AATGCTTCTCAAGATACCC/	AGATCATATGAAACAGCATGAC
	GFP coding sequence			
	2,020	2,1	040 : 1	2,060 2,080
pYOGM081 map	TTTTTCAAGAGTGCCATGCCC	GAAGGTTATGTACAGGAA	Α G A A C T A T A T T T T A C A A A G A	ATGACGGGAACTACAAGACACG
	GFP coding sequence			
	2,100	2,	120 I	2,140 2,160 I I
pYOGM081 map	TGCTGAAGTCAAGTTTGAAGG	TGATACCCTTGTTAATAG	AATCGAGTTAAAAGGTATTC	<u>SATTTTAAAGAAGATGGAAACA</u>
	GFP coding sequence			
	2,160	2,:	200 : 1	2,220 2,240
pYOGM081 map	TTCTTGGACACAAAATGGAAT	ACAACTATAACTCACATA	ATGTATACATCATGGGAGAG	CAAACCAAAGAATGGCATCAAA
	GFP coding sequence			
	2,260	2,:	280 1	2,326 2,326
pYOGM081 map	GTTAACTTCAAAATTAGACAC	AACATTAAAGATGGAAGCO	GTTCAATTAGCAGACCATT/	ATCAACAAAATACTCCAATTGG
	GFP coding sequence			
	2,340	2,	360 1	2,380 2,400
pYOGM081 map	CGATGGCCCTGTCCTTTTACC	AGACAACCATTACCTGTC	CACACAATCIGCCCTTTCC/	AAAGATCCCAACGAAAAGAGAGAG
	GFP coding sequence			
	2,420	2,	440 :: 1	2,460 2,480 1 I
pYOGM081 map	ATCACATGATCCTTCTTGAGT	TTGTAACAGCTGCTAGGA	T T AC AC AT GGC AT GG AT GA /	ACTATACAAA taaGGGCCCTGC
	CYC1 terminator			
	2,500	2,1	520 1	2,540 2,560
pYOGM081 map	AGGAGGGCCGCATCATGTAAT	TAGTTATGTCACGCTTAC	ATTCACGCCCTCCCCCACA	ATCCGCTCTAACCGAAAAGGAA
	CYC1 terminator			
	2,560	2,1	800	2,620 2,640
pYOGM081 map	GGAGTTAGACAACCTGAAGTC	TAGGTCCCTATTTATTTT	TTTATAGTTATGTTAGTATI	TAAGAACGTTATTTATATTTCA
	CYC1 terminator			
	2,660	2,	880	2,720
pYOGM081 map	AATTTTCTTTTTTCTGTA	CAGACGCGTGTACGCATG	ΤΑΑCΑΤΤΑΤΑCΙGAAAACCΙ	IGCIIGAGAAGGIIIIGGGAC
	CVC1 terminator	HindIII/Stul cloning junction	promoter	
	2,740	2.1	760	2,780 2,800
pYOGM081 map	I GCTCGAAGGCTTTAATTTGCG	GCCAAGCT c c tGTACGGA	I TTAGAAGCCGCCGAGCGGG	
	GAL 1-10 promoter			
	2,820	2,	640	2,860 2,880
pYOGM081 map	TCTCCTCCGTGCGTCCTCGTC	TTCACCGGTCGCGTTCCT	I GAAACGCAGATGTGCCTCGC	GCCGCACTGCTCCGAACAATA
15 37.0	GAL1-10 promoter			
	2,900	2,	920	2,940 2,960
pYOGM081 map	AAGATTCTACAATACTAGCTT	TTATGGTTATGAAGAGGA	AAAATTGGCAGTAACCTGGC	CCCACAAACCTTCAAATTAAC
8	GAL1-10 promoter			
	2,960	3,	000	3,020 3,040
pYOGM081 map	GAATCAAATTAACAACCATAG	GATGATAATGCGATTAGT	TITTTAGCCTTATTTCTGGC	GTAATTAATCAGCGAAGCGAT
8 56 66 B	GAL1-10 promoter			
	3,060	3,	080	3,100 3,120
pYOGM081 map	GATTTTTGATCTATTAACAGA	TATATAAATGGAAAAGCT	GCATAACCACTTTAACTAA	ACTITCAACATITICAGITIG
	GAL1-10 promoter			
	3,140	3,	160	3,180 3,200
nYOGM081 man		CATAAAAGTATCAACAAA	AAATTGTTAATATACCTCT/	

	GAL1-10 promoter		Gateway cloning site		
		3,220	3,240 I	3,260 I	3,280 I
pYOGM081 map	AAACCCCGGATTCTAGAA	ACTAGTGGATCCCCCATC	ACAAGTTTGTACAAAAA	AGCTGAACGAGAAACGT	AAAATGATATA
	Gateway cloning site				
		3,300 	3,320	3,340 I	3,360
pYOGM081 map	AATATCAATATATTAAAT	TAGATTTTGCATAAAAA	ACAGACTACATAATACT	GTAAAACACAACATATC	CAGTCACTATG
		3,380	3,400	3,420	3,440
pYOGM081 map	GCGGCCGCATTAGGCACC	CCAGGCTTTACACTTTA	TGCTTCCGGCTCGTATA	ATGTGTGGATTTTGAGT	TAGGATCCGTC
		3,460	3.480	3,500	3,520
nYOGM081 man	GAGATTTTCAGGAGCTAA			ACCACCGTTGATATATC	
proontoormap	and all the coorder and			AccAccorrowin Aran	cenniodenie
		3,540	3,560	3,580	3,600
pYOGM081 map	GTAAAGAACATTTTGAGG	GCATTTCAGTCAGTTGCT	CAATGTACCTATAACCA	GACCGTTCAGCTGGATA	TTACGGCCTTT
		3,620	3,640	3,660	3,680
pYOGM081 map	TTAAAGACCGTAAAGAAA	I \AATAAGCACAAGTTTTA	TCCGGCCTTTATTCACA	TTCTTGCCCGCCTGATG	AATGCTCATCC
	[EcoB/]				
		3,700	3,720	3,740	3,760
oYOGM081 map	GGAATTCCGTATGGCAAT		TGATATGGGATAGTGTT		
preemeerinap	<u> </u>				
		3,780 I	3,800 I	3,820 I	3,840 I
pYOGM081 map	AAACTGAAACGTTTTCAT	CGCTCTGGAGTGAATAC	CACGACGATTTCCGGCA	GTTTCTACACATATATT	CGCAAGATGTG
		3,860	3,880	3,900	3,920
pYOGM081 map	GCGTGTTACGGTGAAAAC	CTGGCCTATTTCCCTAA	AGGGTTTATTGAGAATA	TGTTTTTCGTCTCAGCC	AATCCCTGGGT
		3,940 	3,960	3,980 	4,000 I
pYOGM081 map	GAGTTTCACCAGTTTTGA	ATTTAAACGTGGCCAATA	TGGACAACTTCTTCGCC	CCCGTTTTCACCATGGG	CAAATATTATA
		4,020	4,040	4,060	4,080
pYOGM081 map	CGCAAGGCGACAAGGTGC	TGATGCCGCTGGCGATT	CAGGTTCATCATGCCGT	CTGTGATGGCTTCCATG	TCGGCAGAATG
		4.100	4.120	4.140	4,160
oYOGM081 map			GGGCGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG		
proontoormap	er i har gaar i hennene	A 180	400	4990	4240

proGM081 map	ATAACAGTATGCGTATTT	GCGCGCTGATTITIGCG	GTATAAGAATATATACT	GATAIGTATACCCGAAG	
		4,260	4,280	4,300	4,320 I
pYOGM081 map	GAGGTATGCTATGAAGCA	GCGTATTACAGTGACAG	TTGACAGCGACAGCTAT	CAGTTGCTCAAGGCATA	TATGATGTCAA
		4,340	4,360	4,380	4,400
pYOGM081 map	TATCTCCGGTCTGGTAAG	I GC AC AAC C AT GC AG AAT G	AAGCCCGTCGTCTGCGT	GCCGAACGCTGGAAAGC	GGAAAATCAGG
				cod	B coding sequence
		4,420	4,440	4,480	4,480
pYOGM081 map	AAGGGATGGCTGAGGTCG	GCCCGGTTTATTGAAATG	AACGGCTCTTTTGCTGA	CGAGAACAGGGGCTGGT	GAAATGCAGTT
	ccdB coding sequence				
		4,500	4,520	4,540	4,560
pYOGM081 map	TAAGGTTTACACCTATAA	AAGAGAGAGCCGTTATC	GTCTGTTTGTGGATGTA	CAGAGTGATATTATTGA	CACGCCCGGGC
	ccdB coding sequence				
		4,580	4,600	4,620	4,640
pYOGM081 map	GACGGATGGTGATCCCCC	TGGCCAGTGCACGTCTG	CTGTCAGATAAAGTCTC	CCGTGAACTTTACCCGG	TGGTGCATATC
	ccdB coding sequence				
	Change and substantial strategies of a strategies	4,660	4,680	4,700	4,720
pYOGM081 map	GGGGATGAAAGCTGGCGC	ATGATGACCACCGATAT	GGCCAGTGTGCCGGTCT	CCGTTATCGGGGGAAGAAG	STGGCTGATCT
	ccdB coding sequence				
		4,740	4,760	4,780	4,800 I
pYOGM081 map	CAGCCACCGCGAAAATGA	CATCAAAAACGCCATTA	ACCTGATGTTCTGGGGA	ATATAAATGTCAGGCTC	CCTTATACACA
	Sall	Gateway cloning site			
	Ţ	4,820	4,840	4,860	4,880
pYOGM081 map	GCCAGTCTGCAGGTCGAC	CATAGTGACTGGATATG	TTGTGTTTTTACAGTATT	ATGTAGTCTGTTTTTTA	IGCAAAATCTA
	Gateway cloning site				EcoRI
	1999 - 1997 - 1997 - 1997 - 1997 - 1997 - 1997 - 1997 - 1997 - 1997 - 1997 - 1997 - 1997 - 1997 - 1997 - 1997 -	4,900	4,920	4,940	4,960
pYOGM081 map	ATTTAATATATIGATATT	TATATCATTTTACGTTT	CICGTICAGCTITCITG	TACAAAGTGGTGATGGG	TGCAGGAATT
	HindIII	HA tao			
		4,980	5,000	5,020	5,040
pYOGM081 map		GAATGTACCCATACGAT	GTTCCTGACTATGCGGG	CTATCCCTATGACGTCC	CGGACTATGCA
	ΔΔ				



	pBluescript SK+ backbone			
		6,420	6,440 I	6,460 6,480
pYOGM081 map	CGGAAAAAGAGTTGGTA	GCTCTTGATCCGGCAAACA	AACCACCGCTGGTAGCGGTGG	TTTTTTTGTTTGCAAGCAGCAGA
	pBluescript SK+ backbone			
		6,500	6,520 I	6,540 6,560
pYOGM081 map	TTACGCGCAGAAAAAAA	GGATCTCAAGAAGATCCTTT	IGATCTTTTCTACGGGGTCTG	ACGCTCAGTGGAACGAAAACTCA
	pBluescript SK+ backbone			
		6,580	6,600 I	6,620 6,640
pYOGM081 map	CGTTAAGGGATTTTGGT	CATGAGATTATCAAAAAGGA	ATCTTCACCTAGATCCTTTTA	AATTAAAAATGAAGTTTTAAATC
	pBluescript SK+ backbone			
		6,660 I	6,680 I	6,700 6,720
pYOGM081 map	AATCTAAAGTATATATG	AGTAAACTTGGTCTGACAG	TTACCAATGCTTAATCAGTGA	GGCACCTATCTCAGCGATCTGTC
	pBluescript SK+ backbone			
		6,740 I	6,760 I	6,780 6,800
pYOGM081 map	TATTTCGTTCATCCATA	GTTGCCTGACTCCCCGTCGT	IGTAGATAACTACGATACGGG	AGGGCTTACCATCTGGCCCCAGT
	pBluescript SK+ backbone			
		6,820	6,840 I	6,860 6,680
pYOGM081 map	GCTGCAATGATACCGCG	AGACCCACGCTCACCGGCT	CCAGATTTATCAGCAATAAAC	CAGCCAGCCGGAAGGGCCGAGCG
	pBluescript SK+ backbone			
		6,900	6,920	6,940 6,960
pYOGM081 map	CAGAAGTGGTCCTGCAA	CTTTATCCGCCTCCATCCAC	STCTATTAATTGTTGCCGGGA	AGCTAGAGTAAGTAGTTCGCCAG
	pBluescript SK+ backbone			
		6,980	7,000	7,020 7,040
pYOGM081 map	TTAATAGTTTGCGCAAC	GTTGTTGCCATTGCTACAGO	SCATCGTGGTGTCACGCTCGT	CGTTTGGTATGGCTTCATTCAGC
	pBluescript SK+ backbone			
		7,060	7,080	7,100 7,120
pYOGM081 map	TCCGGTTCCCAACGATC	AAGGCGAGTTACATGATCCC	CCATGTTGTGCAAAAAAGCG	GTTAGCTCCTTCGGTCCTCCGAT
	pBluescript SK+ backbone			
		7,140	7,160	7,180 7,200
pYOGM081 map	CGTTGTCAGAAGTAAGT	TGGCCGCAGTGTTATCACTO	CATGGTTATGGCAGCACTGCA	TAATTCTCTTACTGTCATGCCAT
	pBluescript SK+ backbone			
		7,220	7,240	7,260 7,280
pYOGM081 map	CCGTAAGATGCTTTTCT	GTGACTGGTGAGTACTCAAC	CAAGTCATTCTGAGAATAGT	GTATGCGGCGACCGAGTTGCTCT
	pBluescript SK+ backbone			
		7,300	7,320	7,340 7,360
pYOGM081 map	TGCCCGGCGTCAATACG	GGATAATACCGCGCCACATA	AGCAGAACTTTAAAAGTGCTC	ATCATTGGAAAACGTTCTTCGGG
	pBluescript SK+ backbone			
		7,380	7.400	7,420 7,440
pYOGM081 map	GCGAAAACTCTCAAGGA	TCTTACCGCTGTTGAGATCO	AGTTCGATGTAACCCACTCG	TGCACCCAACTGATCTTCAGCAT
	pBluescript SK+ backbone			
		7,460	7,480	7,500 7,520
pYOGM081 map	CTTTTACTTTCACCAGC	GTTTCTGGGTGAGCAAAAAG	AGGAAGGCAAAATGCCGCAA	AAAAGGGAATAAGGGCGACACGG
	pBluescript SK+ backbone			
		7,540	7,560	7,580 7,600
pYOGM081 map	AAATGTTGAATACTCAT	ACTCTTCCTTTTTCAATATT	ATTGAAGCATTTATCAGGGT	TATTGTCTCATGAGCGGATACAT
	pBluescript SK+ backbone			
		7,620	7,640	7,660 7,680
pYOGM081 map	ATTTGAATGTATTTAGA	AAAATAAACAAATAGGGGTT	CCGCGCACATTTCCCCGAAA	AGTGCCACCTAAATTGTAAGCGT
	pBluescript SK+ backbone			
		7,700	7,720	7,740 7,760
pYOGM081 map	ΤΑΑΤΑΤΤΤΤΓΓΤΑΑΑΑΤ	TCGCGTTAAATTTTTGTTAA	ATCAGCTCATTTTTTAACCA	ATAGGCCGAAATCGGCAAAATCC
	pBluescript SK+ backbone			
		7.780	7,800	7.820 7.840
pYOGM081 map	CTTATAAATCAAAAGAA	TAGACCGAGATAGGGTTGAG	STGTTGTTCCAGTTTGGAACA	AGAGTCCACTATTAAAGAACGTG
	pBluescript SK+ backbone			
		7,860	7,880	7,900 7,920
pYOGM081 map	GACTCCAACGTCAAAGG	GCGAAAAACCGTCTATCAGG	GCGATGGCCCACTACGTGAA	CCATCACCCTAATCAAGTTTTT
	pBluescript SK+ backbone			
		7,940	7,960	7,980 8,000
pYOGM081 map	GGGGTCGAGGTGCCGTA	AAGCACTAAATCGGAACCCI	AAAGGGAGCCCCCGATTTAG	AGCTTGACGGGGAAAGCCGGCGA

	pBluescript SK+ backbone				
		8,020	8,040	8,060 8,06	50
pYOGM081 map	ACGTGGCGAGAAAGGAA	GGAAGAAAGCGAAAGGAGCG	GGCGCTAGGGCGCTGGCAAG	STGTAGCGGTCACGCTGCGCGT;	A
	pBluescript SK+ backbone				
		8,100	8,120	8,140 8,16	90 1
pYOGM081 map	ACCACCACACCCGCCGC	GCTTAATGCGCCGCTACAGGG	GCGCGTCCCATTCGCCATTC	AGGCTGCGCAACTGTTGGGAAG	ŝ
	pBluescript SK+ backbone				
		8,180	8,200	8,220 8,24	io i
pYOGM081 map	GCGATCGGTGCGGGCCT	TTCGCTATTACGCCAGCTGG	CGAAAGGGGGGATGTGCTGC	AAGGCGATTAAGTTGGGTAACG	Ċ
	pBluescript SK+ backbone				
		8,260	8,280 I	8,300 8,32	20 I
pYOGM081 map	CAGGGTTTTCCCAGTCA	CGACGTTGTAAAACGACGGCC	AGTGAGCGCGCGTAATACG	ACTCACTATAGGGCGAATTGGG	ŕ
	script SK+ backbone				
pYOGM081 map	ACCGGGCCCCCC				

ADH1 promoter

	20	40 I	60 I	80 I
ADH1 promoter	ACAATATGGACTTCCTCTTTTCTGGCAA	CCAAACCCATACATCGGGATTC	CTATAATACCTTCGTTGGTC	TCCCTAACAT
	100	120	140	160
ADH1 promoter	GTAGGTGGCGGAGGGGGGGAGATATACAATA	GAACAGATACCAGACAAGACAT	AATGGGCTAAACAAGACTAG	ACCAATTACA
	180	200	220	240
ADH1 promoter	CTGCCTCATTGATGGTGGTACATAACGA	ACTAATACTGTAGCCCTAGACT	TGATAGCCATCATCATATCO	GAAGTTTCACT
	260	280 I	300	320
ADH1 promoter	ACCCTTTTTCCATTTGCCATCTATTGAA	GTAATAATAGGCGCATGCAACT	тсттттстттттттттстт	тстстстссс
	340	360	360	400
ADH1 promoter	CCGTTGTTGTCTCACCATATCCGCAATG	ACAAAAAATGATGGAAGACGG	GTGTACTAAAGGAAAAAATI	AACGACAAAG
	420	440	460	480
ADH1 promoter	ACAGCACCAACAGATGTCGTTGTTCCAG	AGCTGATGAGGGGTATCTCGAA	GCACACGAAACTTTTTCCTT	CCTTCATTCA
	500	520	540	560
ADH1 promoter	CGCACACTACTCTCTAATGAGCAACGGT	ATACGGCCTTCCTTCCAGTTAC	TTGAATTTGAAATAAAAAAA	AGTTTGCTGT
	580	eço	620	640
ADH1 promoter	CTTGCTATCAAGTATAAATAGACCTGCA	ATTATTAATCTTTTGTTTCCTC	GTCATTGTTCTCGTTCCCTT	тсттссттот
	eeo	680	700	
ADH1 promoter	TTCTTTTTCTGCACAATATTTCAAGCTA	TACCAAGCATACAATCAACTAT	CTCATATACA	

TEF promoter

	20	40	60	80
TEF promoter	GACATGGAGGCCCAGAATACCCTCCTTGAC	AGTCTTGACGTGCGCAGCT	CAGGGGCATGATGTGACTGTC	SCCCGTACAT
	100	120	140	160
TEF promoter	TTAGCCCATACATCCCCATGTATAATCATT	IGCATCCATACATTTTGAT	GGCCGCACGCGCGAAGCAAAA	ATTACGGCT
	180 I	200	220	240
TEF promoter	CCTCGCTGCAGACCTGCGAGCAGGGAAACG	TCCCCTCACAGACGCGTT	GAATTGTCCCCACGCCGCGCCC	CTGTAGAGA
	260 I	280	300	320
TEF promoter	AATATAAAAGGTTAGGATTTGCCACTGAGG	TTCTTCTTTCATATACTTC	CTTTTAAAATCTTGCTAGGATA	ACAGTTCTCA
	340 I			
TEF promoter	CATCACATCCGAACATAAACAACC			

CUP1 promoter

	20	40	60	80
CUP1 promoter	AACTTCAACGATTTCTATGATGCATTTT	TAATTAGTAAGCCGATCCCAT	TACCGACATTTGGGCGCTAT	ACGTGCATAT
	100	120	140	160
CUP1 promoter	GTTCATGTATGTATCTGTATTTAAAACAC	TTTTGTATTATTTTTCCTCAT	ATATGTGTATAGGTTTATAC	GGATGATTTA
	180	200	220	240
CUP1 promoter	ATTATTACTTCACCACCCTTTATTTCAG	GCTGATATCTTAGCCTTGTTAG	TAGTTAGAAAAAGACATTTT	TGCTGTCAGT
	260	280	300	320
CUP1 promoter	CACTGTCAAGAGATTCTTTTGCTGGCATT	TCTTCTAGAAGCAAAAAGAG	GATGCGTCTTTTCCGCTGAA	CCGTTCCAGC
	340	360	380	400
CUP1 promoter	AAAAAAGACTACCAACGCAATATGGATTG	TCAGAATCATATAAAAGAGA	AGCAAATAACTCCTTGTCTTG	TATCAATTGC
	420	440	460	460
CUP1 promoter	ATTATAATATCTTCTTGTTAGTGCAATAT	CATATAGAAGTCATCGAAAT	AG AT AT T A AG A A A A A A C A A A C T	GTACAATCAA
	500			
CUP1 promoter	TCAATCAATCATCACATAAA			



Figure S2 Integration of the metazoan Sec biosynthesis and insertion genes into the yeast genome. Panels show read coverage of exogenous genes introduced using iGM method. Coverage density maps were generated by aligning reads obtained by sequencing the whole genome of the iGM11 mutant to the sequences of the introduced exogenous genes. The entire ORF of the gene beginning from the ATG start codon and ending at the stop codon were used for alignment. For SPS2, which is a selenoprotein, its ORF together with 3'-UTR flanking region containing a SECIS element was used to generate the coverage map.



Figure S3 Sequencing of the whole genome confirms deletion of the yeast genes. Panels show read coverage of endogenous genes that were replaced by inserted genes. Coverage density maps were generated by aligning reads obtained by sequencing the whole genome of the iGM11 mutant to the sequences of the deleted genes together with 250 bp 5'-UTR and 250 bp 3'-UTR flanking regions.



Figure S4 Expression analysis of the exogenous genes introduced into the yeast genome using iGM method. Expression of proteins carrying HA-tag upon culture of cells in the presence of galactose was detected in individual ProMonster strains and a strain containing all of the 11 gene insertions (iGM11) by Western blotting with HA-tag specific antibodies. Expected sizes of proteins are shown on the right.

 Table S1
 PCR primers used for generating a universal gene insertion module.

Primer sequence	Template	
Fragment 1		
1st round		
5'-CAGAAGCTT(HindIII)TCAC 5'-TAGAGCTC(SacI)GCTAGCI GAGCTC(SacI)GTTTTCGACAC	GTACTGACAATAAAAAGATTC-3' (NheI)GGCGCGCCAGATCT(BgIII) C-3'	Template for the first round of PCR: pAG25 (Goldstein and McCusker 1999)
2nd round		
5'-CAGAAGCTT(HindIII)TCAC 5'-GGAATTC(EcoRI)ACCAGG A(PacI)TACGTA(SnaBI)GAGC	To extend the downstream end, the product of the first round of PCR was used as a template for the second round of PCR	
Fragment 2		
5'-CCGCTCGAG(Xhol)CTAGC CCAGGT(SexAl)GAGCTC(Sac 5'-CCCAAGCTT(HindIII)GGG, AAGACTGTCAAGG-3'	:(Nhel)CGCGG(SacII)TACGTA(SnaBI) I)GACGGATCCCCGGGTTAA-3′ ATATCGCGTCGAC(SalI)GCACGTC	pAG25 (Goldstein and McCusker 1999)
Fragment 3		
5'-CCGCTCGAG(Xhol)CTTTT(5'-CCCAAGCTT(HindIII)GGG/ AACTGATATAAT-3'	pYOGM057 with the URA3 sequence derived from pRS316 (Sikorski and Hieter 1989)	
Fragment 4		
PCR was not used for this fra	agment	
Fragment 5		
5'-GAAGGCCT(Stul)GTACGG	ATTAGAAGCCGCCGAG-3'	pAG416GAL-ccdB-HA
5'-GAAGGCCT(Stul)GACTCG	AGTTAGCACTGAGC-3′	(Alberti <i>et al.</i> 2007)
HA-less construct		¥9.014004
HAIess-HindIII_F 5'-GACI		pYOGM081
Construct with the ADH1 n		
ADH1pr Ewd		BV4741 genomic DNA
ADH1pr_Rev	5'-GCTTTTTTGTACAAACTTGTGATTGTATATGAGATAGTTGATTG-3'	BI474I genomic DNA
ADH1pr pYOGM081 Fwd	5'-CTATCTCATATACAATCACAAGTTTGTACAAAAAAGCTGAACG-3'	pYOGM081
ADH1pr pYOGM081 Rev	5'-GAGGAAGTCCATATTGTACAGGAGCTTGGCCGCAAATTAAAG-3'	P
Construct with the TEF prov	noter	
TEFpr_Fwd TEFpr_Rev	5'-TGCGGCCAAGCTCCTGTGACATGGAGGCCCAGAATACCCTC-3' 5'-GCTTTTTGTACAAACTTGTGATGGTTGTTTATGTTCGGATGTG-3'	pFA6a-kanMX4 (Wach <i>et al.</i> 1994)
TEFpr pYOGM081 Fwd	5'-CCGAACATAAACAACCATCACAAGTTTGTACAAAAAAGCTGAAC-3'	pYOGM081
TEFpr_pYOGM081_Rev	5'-CTGGGCCTCCATGTCACAGGAGCTTGGCCGCAAATTAAAGCC-3'	
Construct with the CUP1 pr	omoter	
CUP1pr_Fwd 5'-GCTT	TAATTTGCGGCCAAGCTCCTGTAACTTCAACGATTTCTATGATGC-3'	BY4741 genomic DNA
CUP1pr_Rev 5'-CAGC	TTTTTTGTACAAACTTGTGATTGTATGTGATGATGATTG-3'	
CUP1pr_pYOGM081_Fwd	5'-CAATCATCACATAAAATCACAAGTTTGTACAAAAAAGC TGAACGAGAAACG-3'	pYOGM081
CUP1pr_pYOGM081_Rev	5'-CATAGAAATCGTTGAAGTTACAGGAGCTTGGCCGCAAA TTAAAGCCTTC-3'	

Deleted ORF	Gene insertion	Primer sequence
YKL069W	TRSP	5'-TAGCGACAGAGTGGTTCAATTC-3'
		5'-TCAATTTGGCGAACAGGGAATG-3'
YER042W	PSTK	5'-AGTGTTGCAGAATCGAGAAGAG-3'
		5'-TCATAAATAAGGGCACGTACAC-3'
YOL118C	SECS	5'-ACACATACCAGGATGCTTCTTC-3'
		5'-GCTGACTAATTTGAAGCTATCG-3'
YLR123C	SPS1	5'-ACAGCCAGAATCATAGACAAAC-3'
		5'-TTCAGCTGATGTGCCATGTAAC-3'
YER108C	SPS2	5'-GATGTTAAGTCTTTTGCGGCAG-3'
		5'-AAAGTCGTTGCTGTGAAAATGG-3'
YCL033C	SBP2	5'-AAGAATCCTTGGAGGCTTCAAC-3'
		5'-GTCCACGATCTCAAACCCTTTC-3'
YKR012C	SBP2L	5'-TTACACAACGCAAACTACGTAC-3'
		5'-TTAGGACCATCTTGCATTTGAG-3'
YDL242W	EEFSEC	5'-TCAAGCGTTATGTCTTCGACAC-3'
		5'-GTTTCGATATTCGCACATTTGC-3'
YGL109W	SECP43	5'-ACAAGGAGTTCATGGAACAGAG-3'
		5'-CAACTAAAGAGTACAACTGTCC-3'
YFR057W	RPL30	5'-CTCTGACATCATTAGAAGCATG-3'
		5'-AGATAACTCTGAACTGTGCATC-3'
YDL227C	cSPS2	5'-CTCTGTTCCCTCTCATATTTAC-3'
		5'-CTACTCCAGCATTCTAGTTAAG-3'

 Table S2
 List of genotyping primers used in this study.

SUPPLEMENTARY REFERENCES

Alberti, S., A. D. Gitler, and S. Lindquist, 2007 A suite of Gateway cloning vectors for high-throughput genetic analysis in Saccharomyces cerevisiae. Yeast 24: 913-919.

Goldstein, A. L., and J. H. McCusker, 1999 Three new dominant drug resistance cassettes for gene disruption in Saccharomyces cerevisiae. Yeast 15: 1541-1553.

Sikorski, R. S., and P. Hieter, 1989 A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in Saccharomyces cerevisiae. Genetics 122: 19-27.

Wach, A., A. Brachat, R. Pohlmann, and P. Philippsen, 1994 New heterologous modules for classical or PCR-based gene disruptions in Saccharomyces cerevisiae. Yeast 10: 1793-1808.