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

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DOI: 10.1534/g3.114.010868

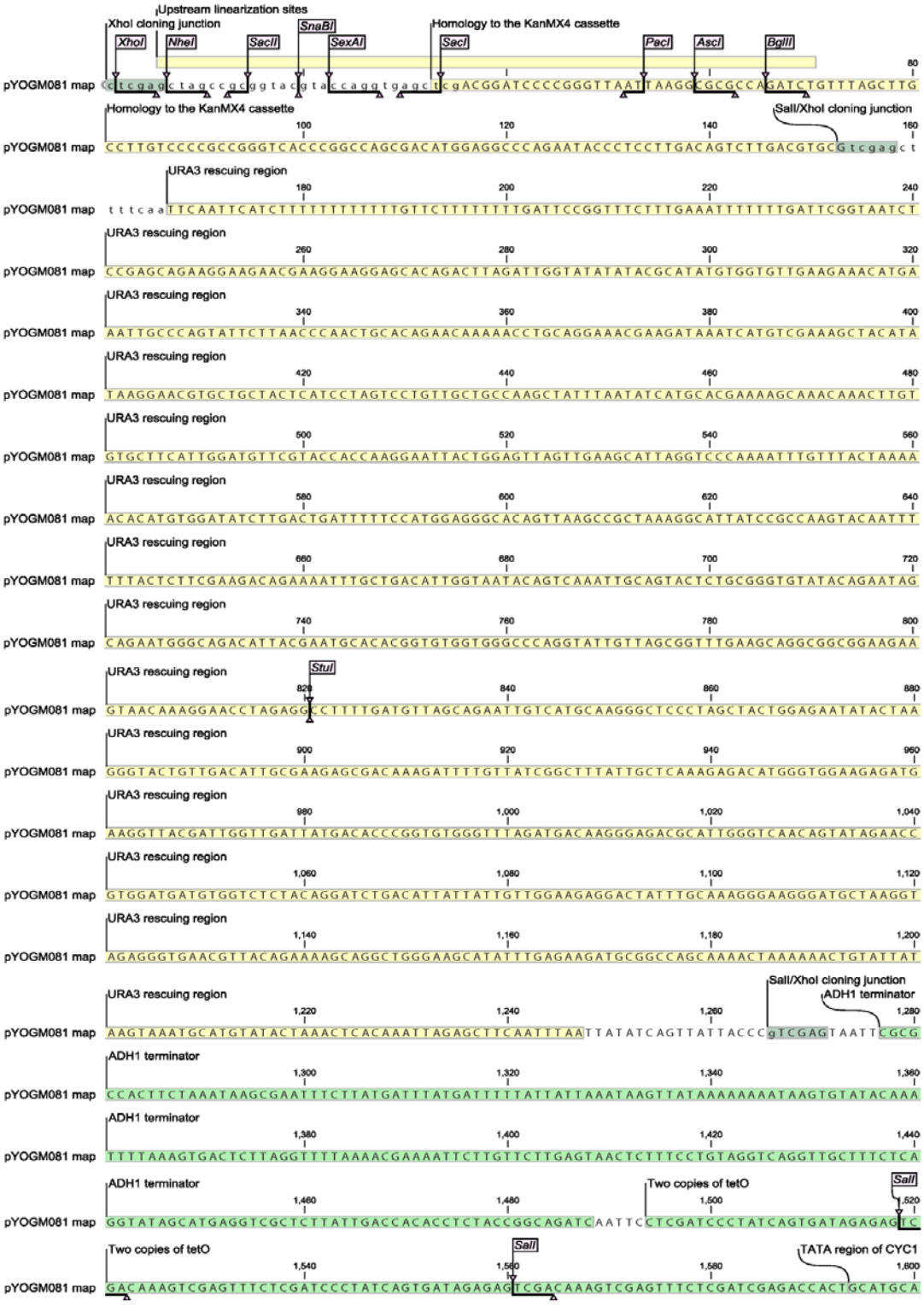


Figure S1 Sequence of the pYOGM081 plasmid containing the iGM gene insertion module. In a version of the plasmid not containing the HA-tag (pTJH001), nucleotides 4,954–5,101 are removed and replaced with a stop codon (TAA) and a *Hin*dIII site (Materials and Methods). In plasmids containing the *ADH1* (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).

Figure S1 (continued).

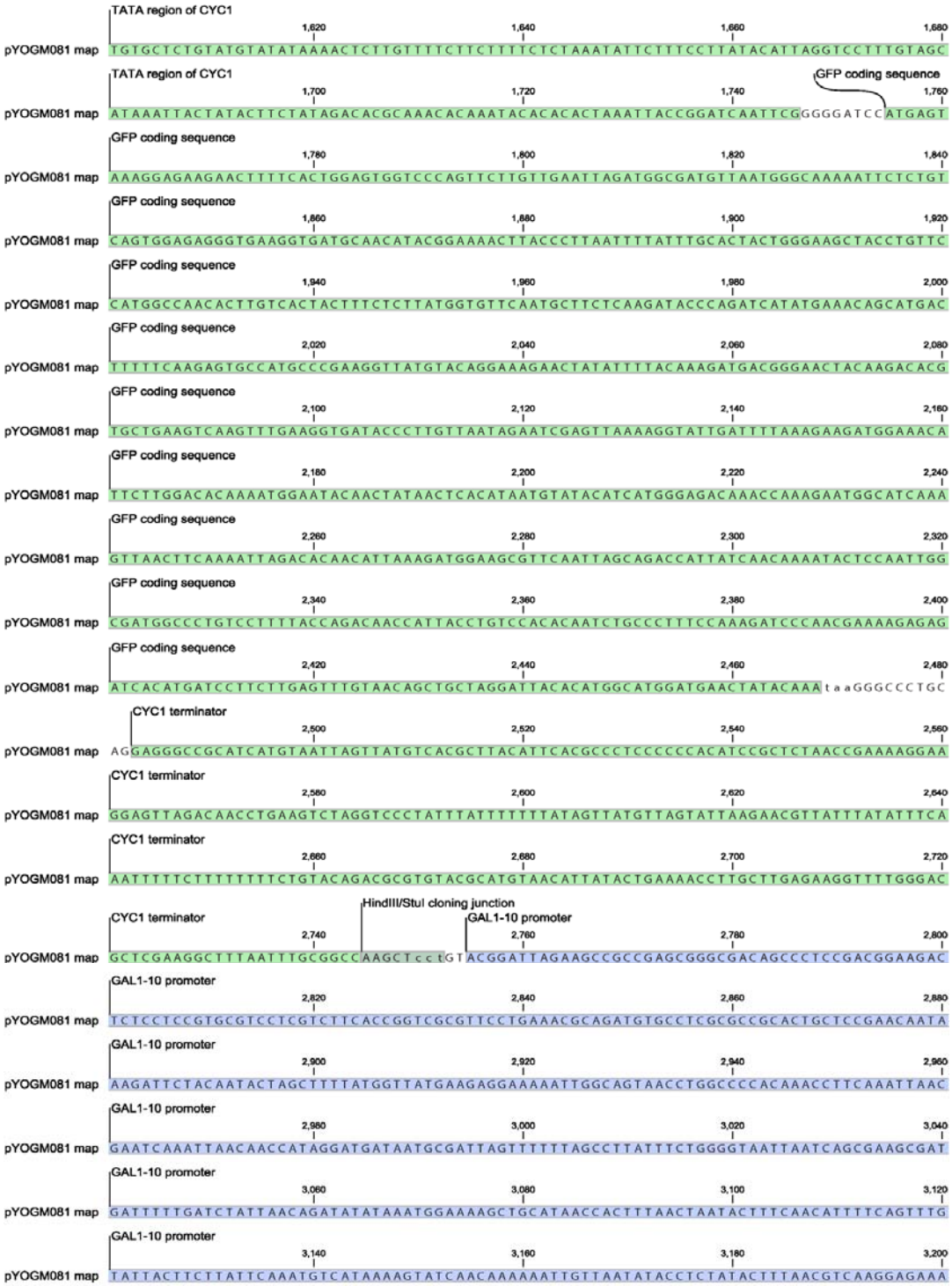


Figure S1 (continued).

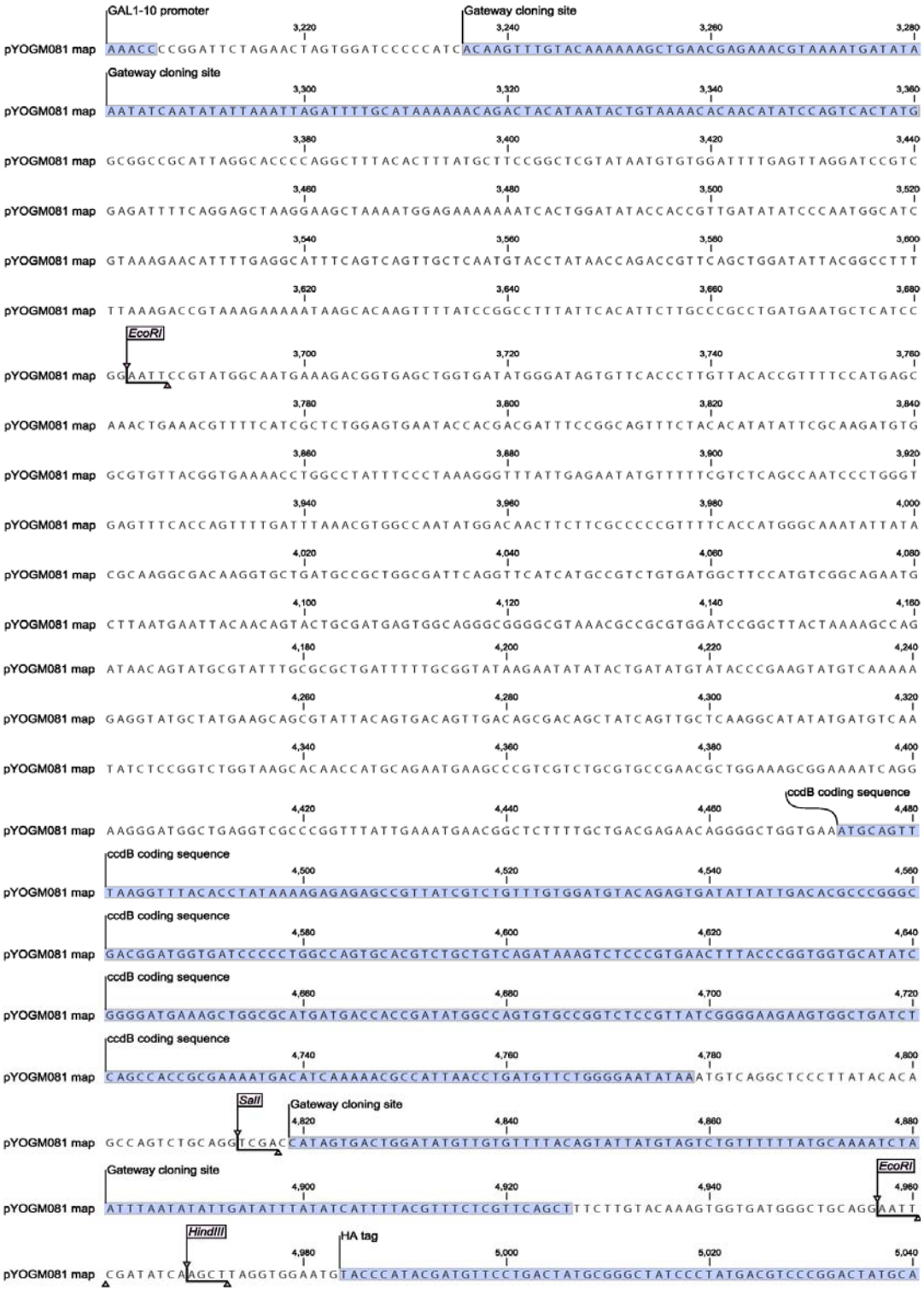
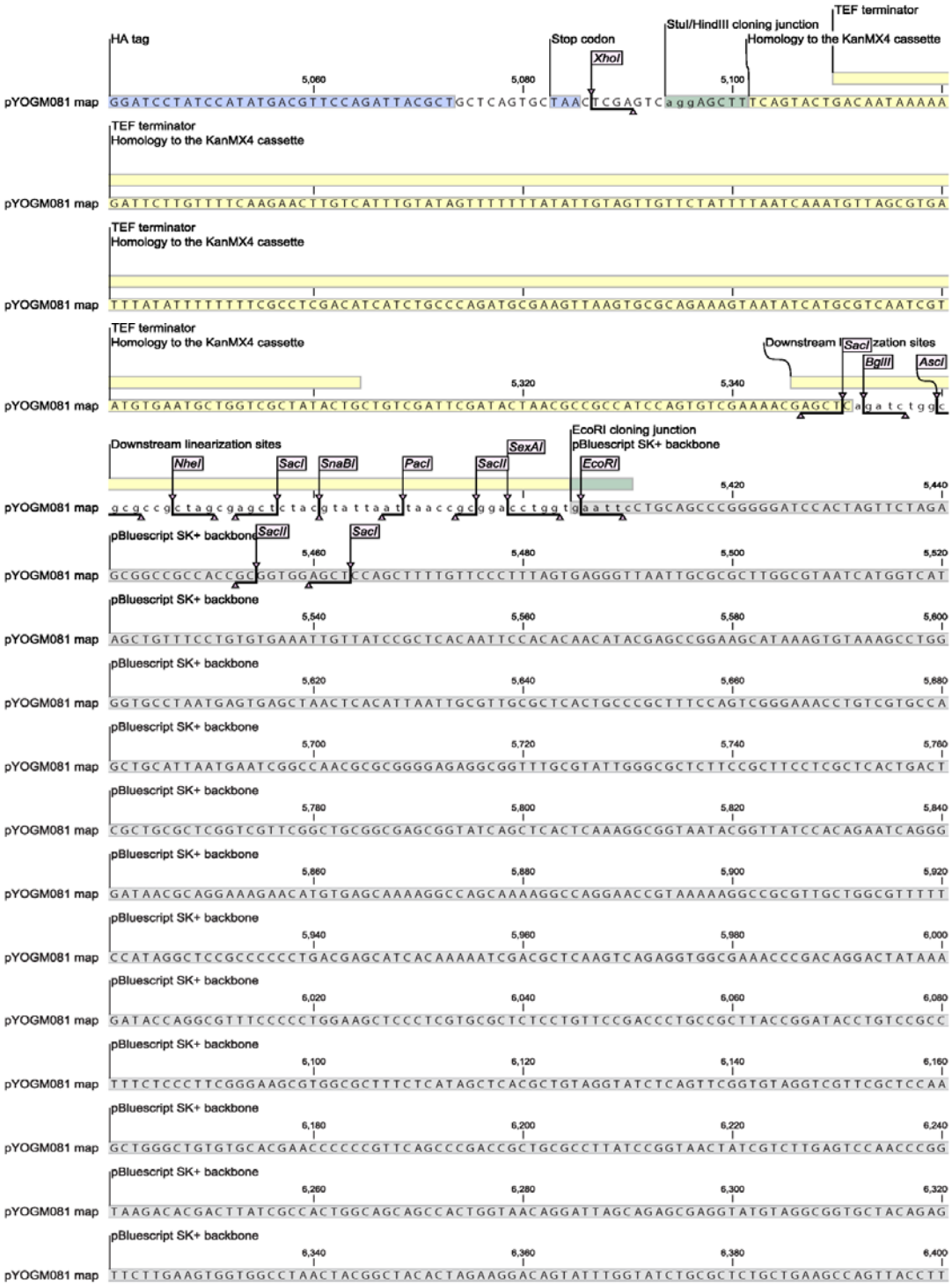


Figure S1 (continued).



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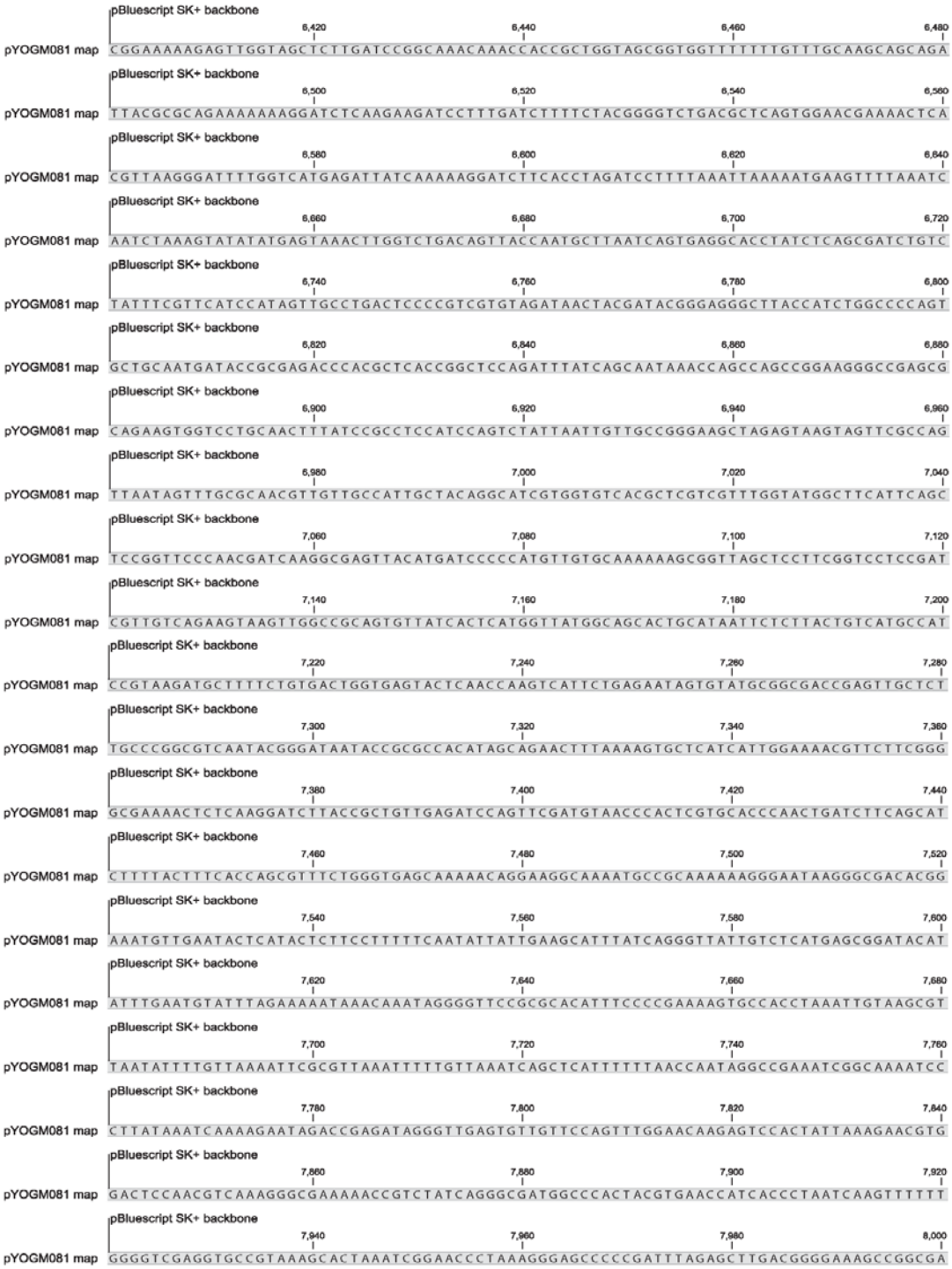
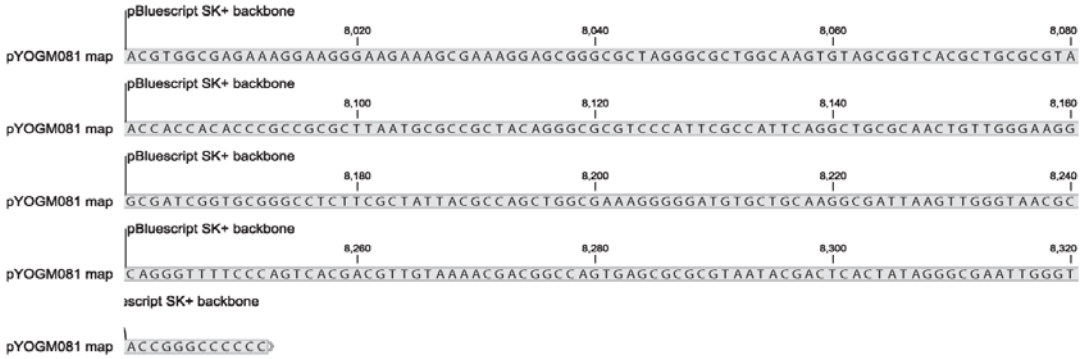
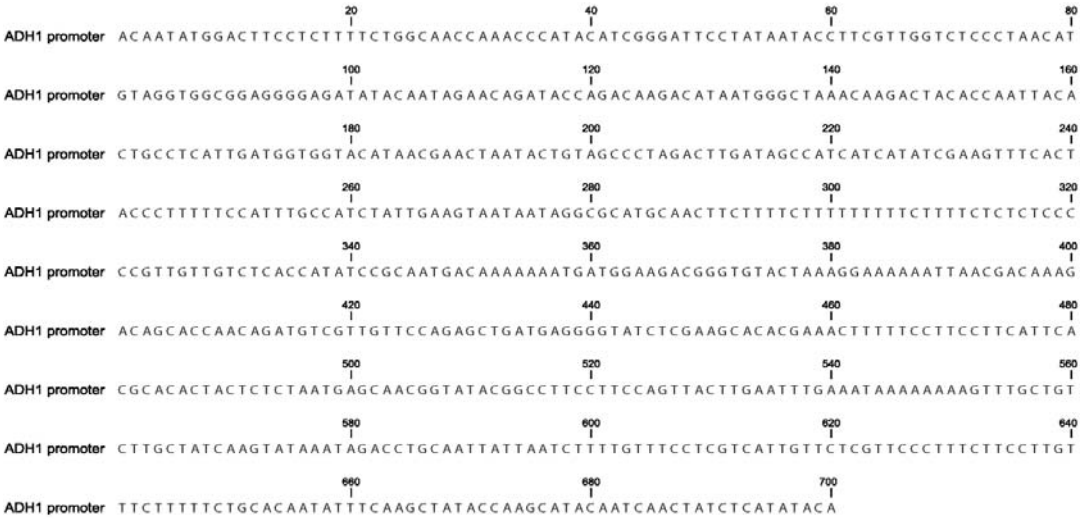


Figure S1 (continued).



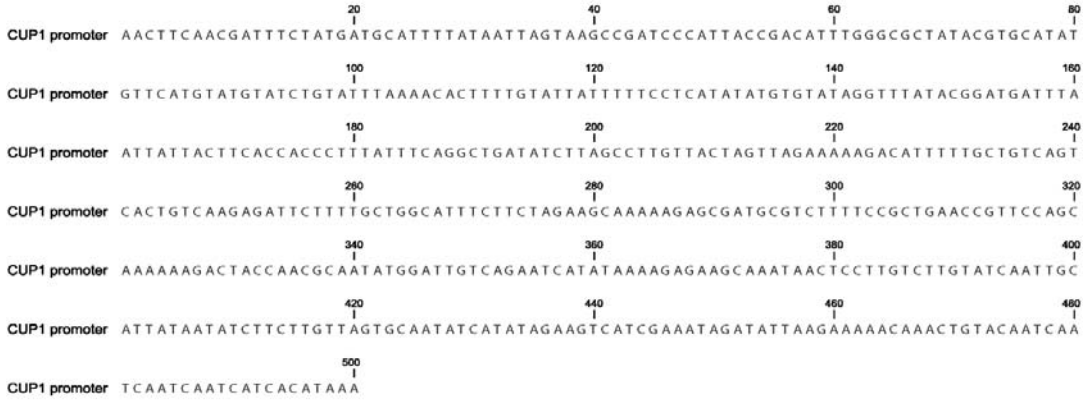
***ADH1* promoter**



***TEF* promoter**

Figure S1 (continued).

***CUP1* promoter**



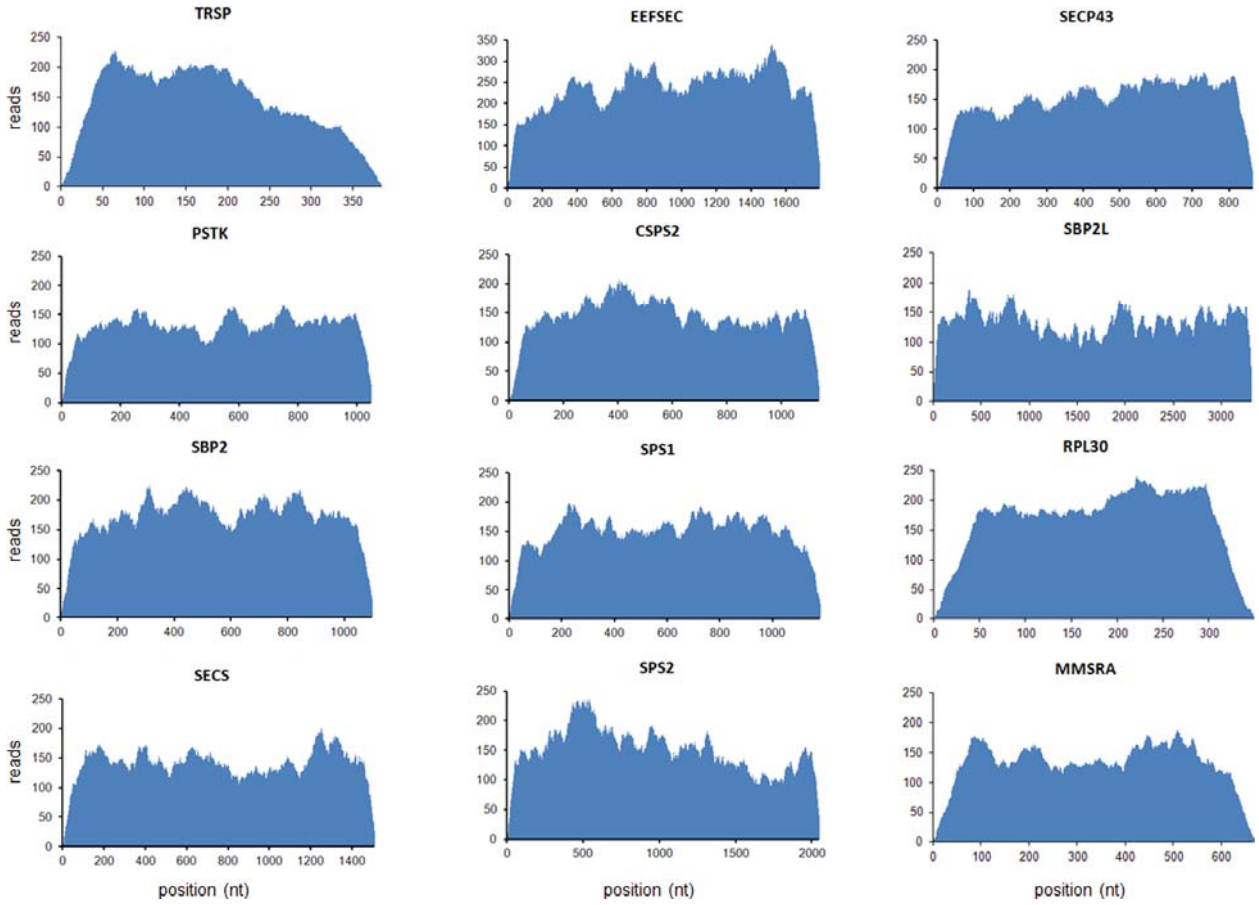


Figure S2 Integration of the metazoan Sec biosynthesis and insertion genes into the yeast genome. Panels show read coverage of exogenous genes introduced using iGM method. Coverage density maps were generated by aligning reads obtained by sequencing the whole genome of the iGM11 mutant to the sequences 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.

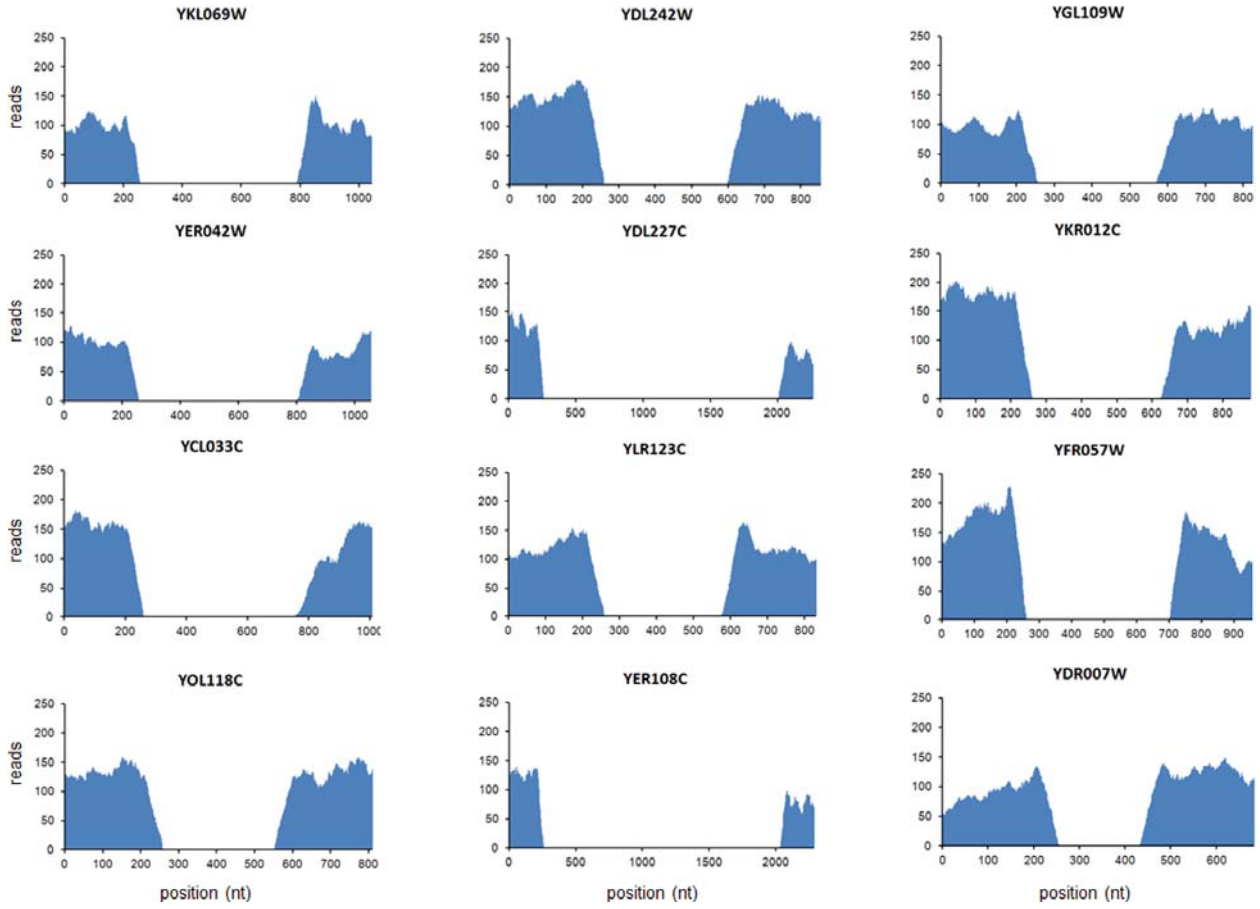


Figure S3 Sequencing of the whole genome confirms deletion of the yeast genes. Panels show read coverage of endogenous genes that were replaced by inserted genes. Coverage density maps were generated by aligning reads obtained by sequencing the whole genome of the iGM11 mutant to the sequences of the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

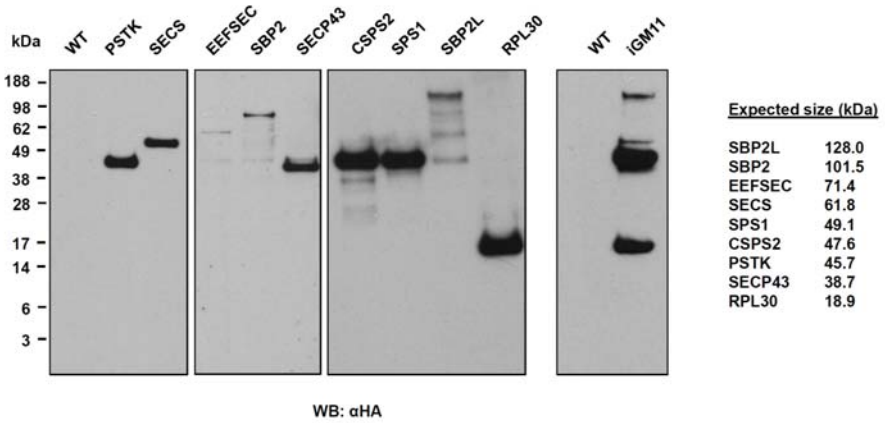


Figure S4 Expression analysis of the exogenous genes introduced into the yeast genome using iGM method. Expression of proteins carrying HA-tag upon culture of cells in the presence of galactose was detected in individual ProMonster strains and a strain containing all of the 11 gene insertions (iGM11) 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)TCAGTACTGACAATAAAAAGATTC-3′ 5′-TAGAGCTC(SacI)GCTAGC(NheI)GGCGCGCCAGATCT(BglII) GAGCTC(SacI)GTTTTCGACAC-3′ **2nd round** 5′-CAGAAGCTT(HindIII)TCAGTACTGACAATAAAAAGATTC-3′ 5′-GGAATTC(EcoRI)ACCAGGT(SexAI)CCGCGG(SacII)TTAATTA A(PacI)TACGTA(SnaBI)GAGCTC(SacI)GCTAG-3′	Template for the first round of PCR: pAG25 (Goldstein and McCusker 1999) 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(XhoI)CTAGC(NheI)CGCGG(SacII)TACGTA(SnaBI) CCAGGT(SexAI)GAGCTC(SacI)GACGGATCCCCGGGTTAA-3′ 5′-CCCAAGCTT(HindIII)GGGATATCGCGTCGAC(SalI)GCACGTC AAGACTGTCAAGG-3′	pAG25 (Goldstein and McCusker 1999)
Fragment 3	
5′-CCGCTCGAG(XhoI)CTTTTCAATTCAATTCATC-3′ 5′-CCCAAGCTT(HindIII)GGGATATCGCGTCGAC(SalI)GGGTAAT AACTGATATAAT-3′	pYOGM057 with the *URA3* sequence derived from pRS316 (Sikorski and Hieter 1989)
Fragment 4	
PCR was not used for this fragment	
Fragment 5	
5′-GAAGGCCT(StuI)GTACGGATTAGAAGCCGCCGAG-3′ 5′-GAAGGCCT(StuI)GACTCGAGTTAGCACTGAGC-3′	pAG416GAL-ccdB-HA (Alberti *et al.* 2007)
HA-less construct	
HAless-HindIII_F 5′-GACTAAGCTT(HindIII)TCAGTACTGACAATAAAAAGATTCTTG-3′ HAless-HindIII_R 5′-GACTAAGCTT(HindIII)TTA(Stop)GCAGCCCATCACCACTTTG-3′	pYOGM081
Construct with the *ADH1* promoter	
ADH1pr_Fwd 5′-TGCGGCCAAGCTCCTGTACAATATGGACTTCCTCTTTTCTG-3′ ADH1pr_Rev 5′-GCTTTTTTGTACAAACTTGTGATTGTATATGAGATAGTTGATTG-3′ ADH1pr_pYOGM081_Fwd 5′-CTATCTCATATACAATCACAAGTTTGTACAAAAAAGCTGAACG-3′ ADH1pr_pYOGM081_Rev 5′-GAGGAAGTCCATATTGTACAGGAGCTTGGCCGCAAATTAAAG-3′	BY4741 genomic DNA pYOGM081
Construct with the *TEF* promoter	
TEFpr_Fwd 5′-TGCGGCCAAGCTCCTGTGACATGGAGGCCCAGAATACCCTC-3′ TEFpr_Rev 5′-GCTTTTTTGTACAAACTTGTGATGGTTGTTTATGTTCGGATGTG-3′ TEFpr_pYOGM081_Fwd 5′-CCGAACATAAACAACCATCACAAGTTTGTACAAAAAAGCTGAAC-3′ TEFpr_pYOGM081_Rev 5′-CTGGGCCTCCATGTCACAGGAGCTTGGCCGCAAATTAAAGCC-3′	pFA6a-kanMX4 (Wach *et al.* 1994) pYOGM081
Construct with the *CUP1* promoter	
CUP1pr_Fwd 5′-GCTTTAATTTGCGGCCAAGCTCCTGTAACTTCAACGATTTCTATGATGC-3′ CUP1pr_Rev 5′-CAGCTTTTTTGTACAAACTTGTGATTTTATGTGATGATTGATTG-3′ CUP1pr_pYOGM081_Fwd 5′-CAATCATCACATAAAATCACAAGTTTGTACAAAAAAGC TGAACGAGAAACG-3′ CUP1pr_pYOGM081_Rev 5′-CATAGAAATCGTTGAAGTTACAGGAGCTTGGCCGCAAA TTAAAGCCTTC-3′	BY4741 genomic DNA pYOGM081

Table S2 List of genotyping primers used in this study.

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′

SUPPLEMENTARY REFERENCES

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