

Supplementary Materials for **Multiplexed gene control reveals rapid mRNA turnover**

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Other Supplementary Material for this manuscript includes the following:
(available at advances.sciencemag.org/cgi/content/full/3/7/e1700006/DC1)

- data file S1 (Microsoft Excel format). C_t values for one of the replicate experiments of pooled strains in the MGC experiment.
- data file S2 (Microsoft Excel format). Time courses in the replicate measurements.
- data file S3 (Microsoft Excel format). mRNA (relative) levels and decay rates.
- data file S4 (Microsoft Excel format). Time courses for *YJR139C* and *YDR032C* mRNAs.

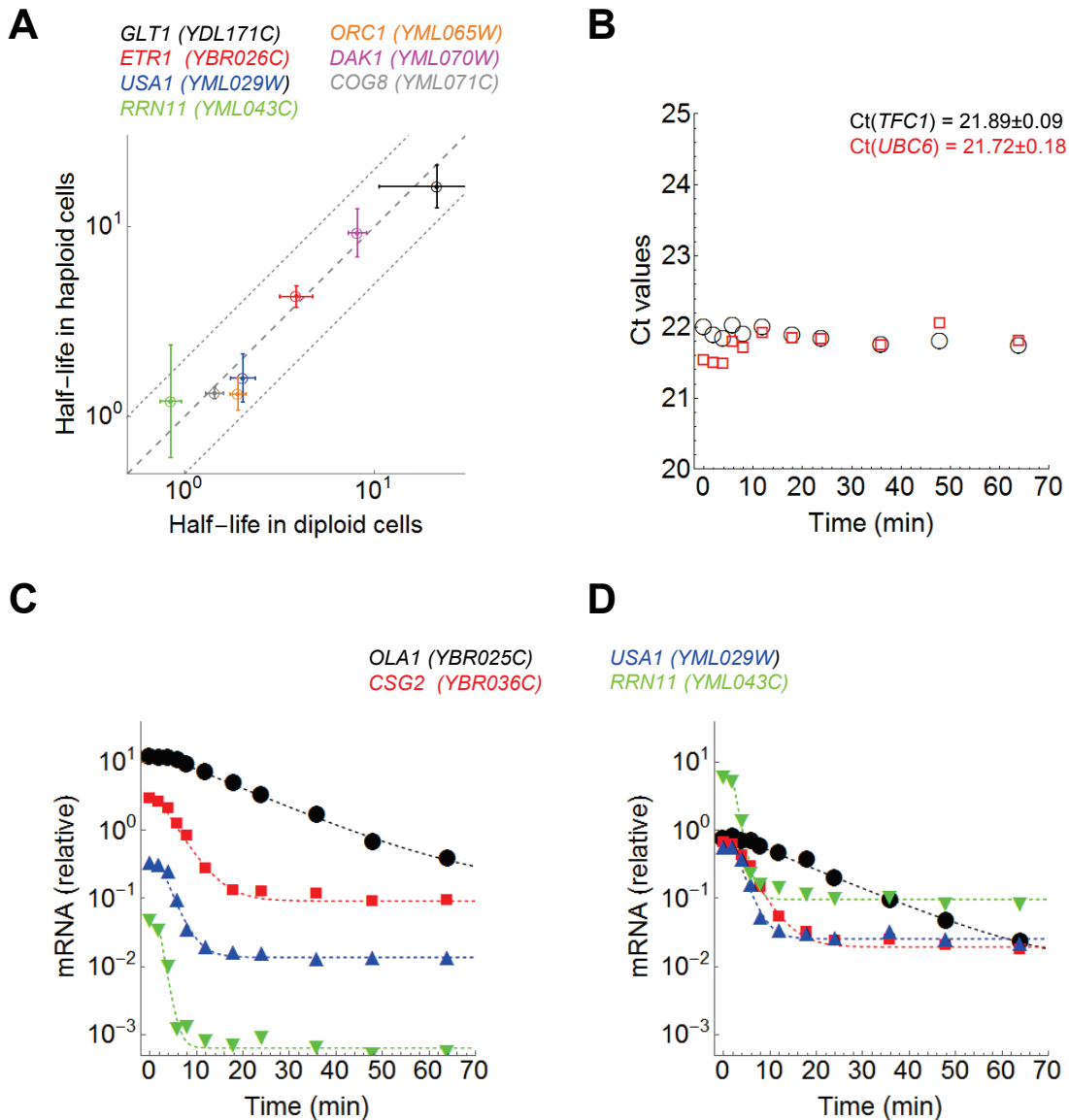


fig. S1. The cell ploidy and the choice of reference mRNAs have no impact on measured half-lives. (A) Half-lives of mRNAs in haploid and diploid cells measured by MGC. The gray dashed line indicates the expected equality of the two half-lives and the dotted line a two-fold deviation from the equality. (B) The Ct values of *TFC1* and *UBC6* during the time course display low variability ($CV_{TFC1} = 0.4\%$ and $CV_{UBC6} = 0.8\%$). (C) mRNAs decay time courses with *TFC1* and *UBC6* used as gene of reference. The following half-lives were fitted: *YBR025C* (*OLA1*): 11.5, *YBR036C* (*CSG2*): 2.6 min, *YML029W* (*USA1*): 1.7 and *YML043C* (*RRN11*): 0.7 min. (D) mRNA decay time courses with the respective endogenous mRNA levels used as reference. The following half-lives were fitted: *YBR025C* (*OLA1*): 10.5 min, *YBR036C* (*CSG2*): 2.5 min, *YML029W* (*USA1*): 1.5 and *YML043C* (*RRN11*): 0.8 min.

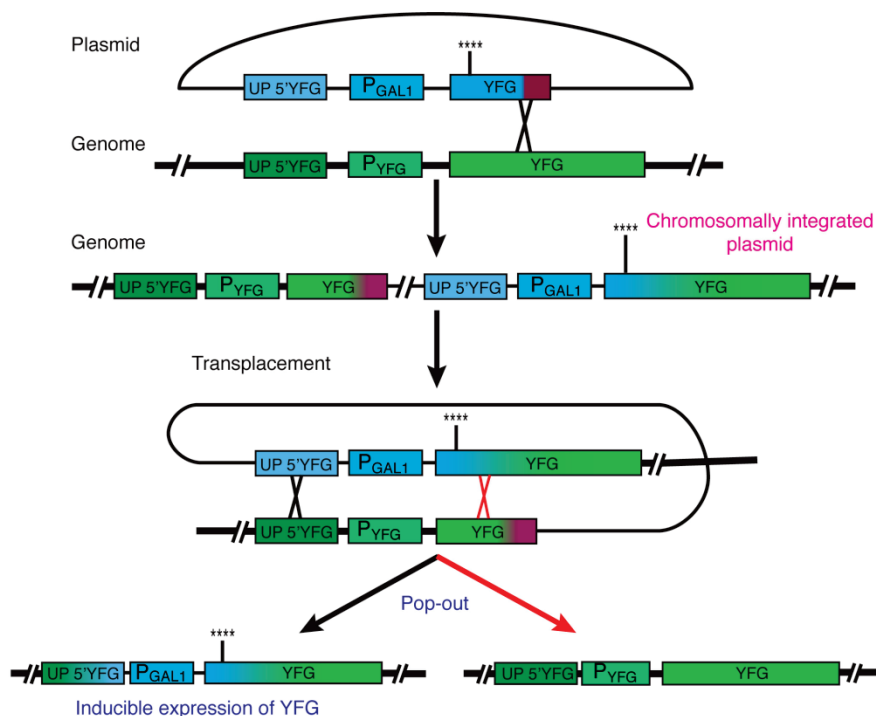
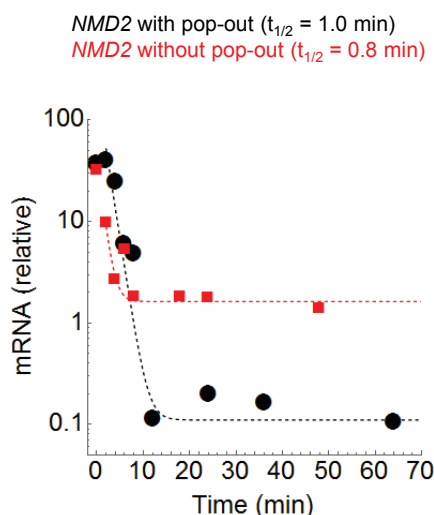
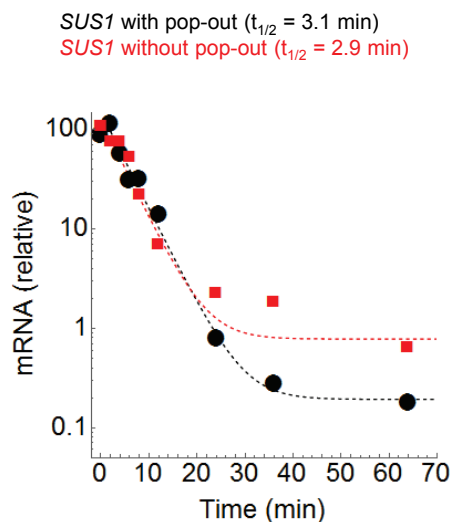
A**B****C**

fig. S2. Pop-out of the chromosomally integrated plasmid is dispensable for half-life estimation of mature mRNA. The half-lives of mRNAs (\pm standard error of the fitting) were determined as described in Bonde *et al.* (A) After homologous recombination, the plasmid is integrated into the chromosome and the promoter of the gene is replaced. The plasmid can be popped out by transplacement, so that only the replaced promoter (P_{GAL1}) remains in the chromosome. Negative selection on FOA plates permits identification of strains with popped-out plasmid (blue text). (B) Time course of mRNA decay of *SUS1* with (blue) and without (red) popped-out plasmid. (C) Time course of mRNA decay of *NMD2* with (blue) and without (red) popped-out of the plasmid.

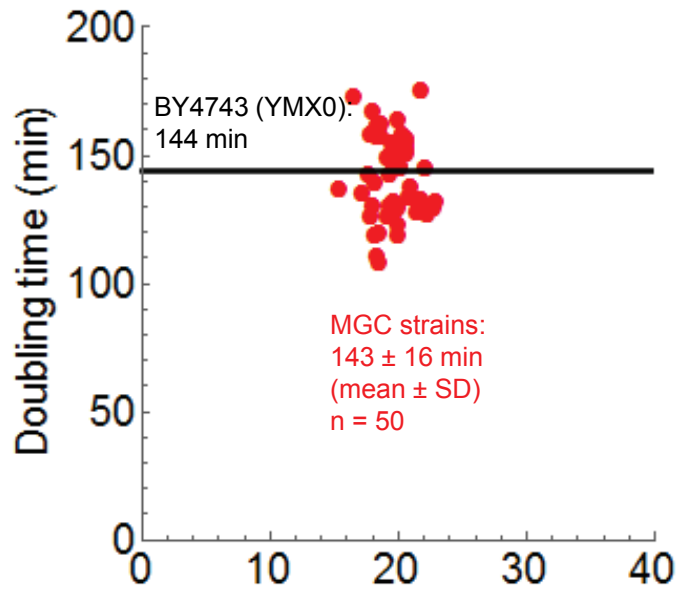


fig. S3. The doubling time of the MGC strains (red dots) is similar to that of the parent diploid strain (black line). The doubling times were measured in synthetic complete (SC) medium (2% raffinose, 0.005% glucose).

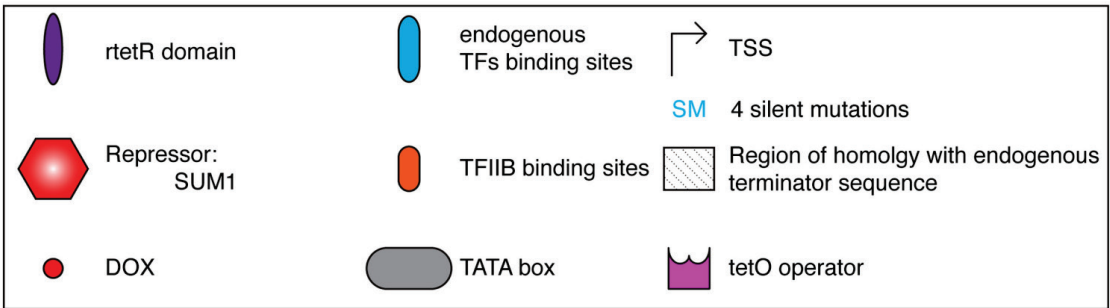
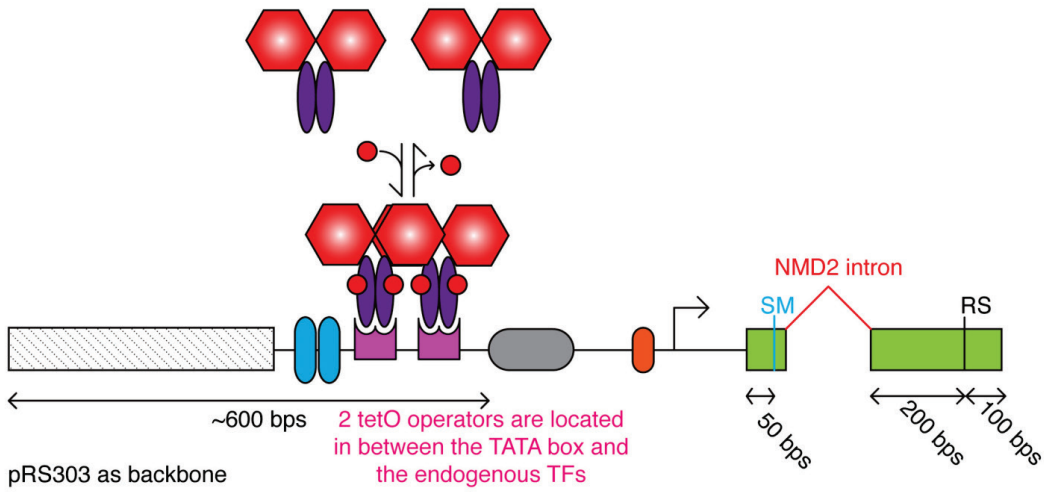


fig. S4. Insertional promoter strategy.

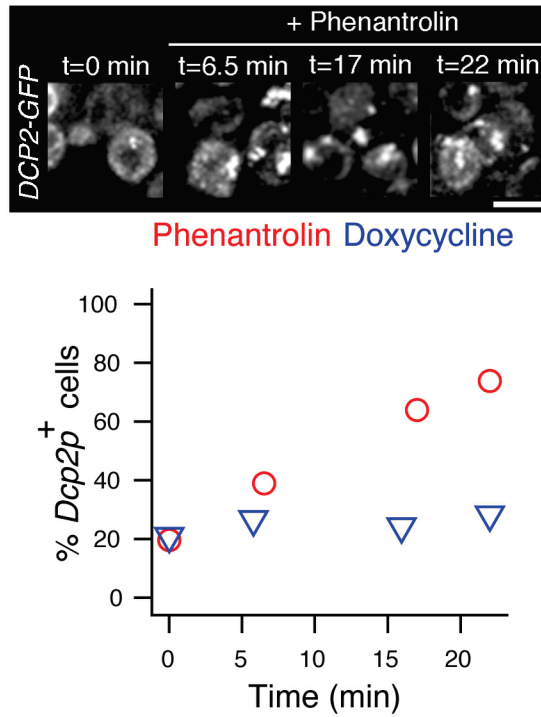


fig. S5. Phenanthroline increases the number of P bodies. Top. Representative images of *DCP2-GFP* cells before and after addition of phenanthroline to the media); scale bar: 2 μ m. Bottom. Changes in the percentage of *Dcp2p* positive cells after addition of phenanthroline (red) or doxycycline to the media (blue).

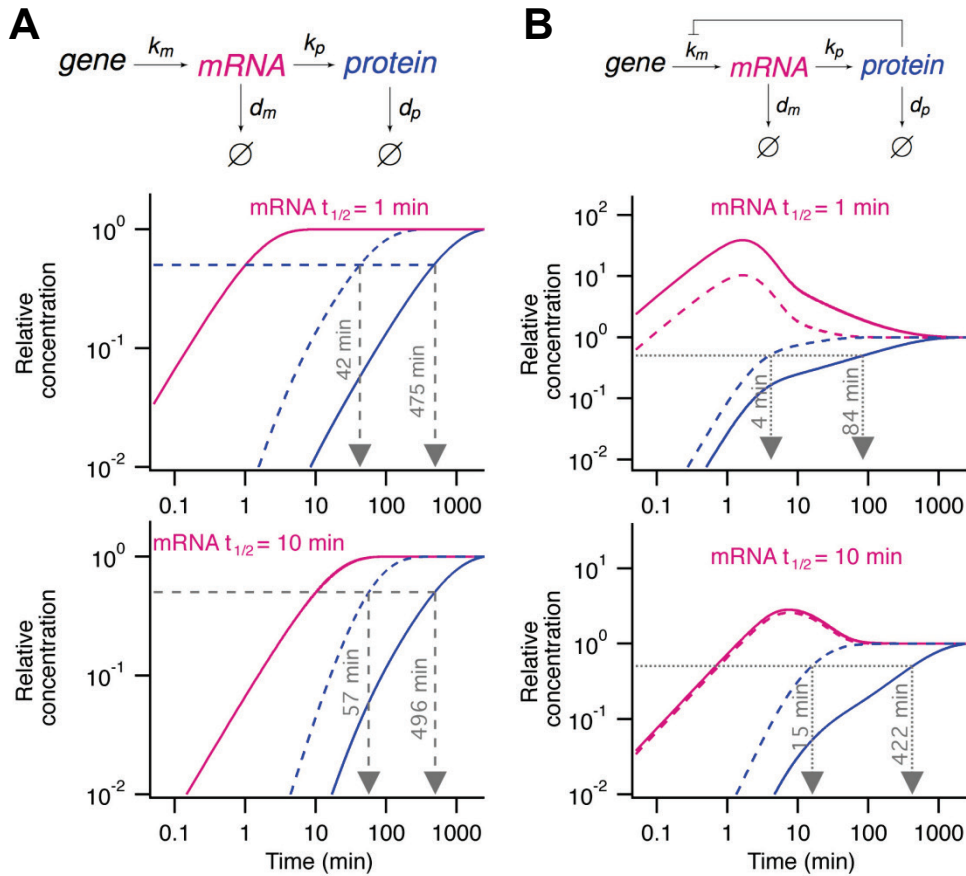
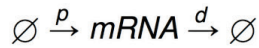
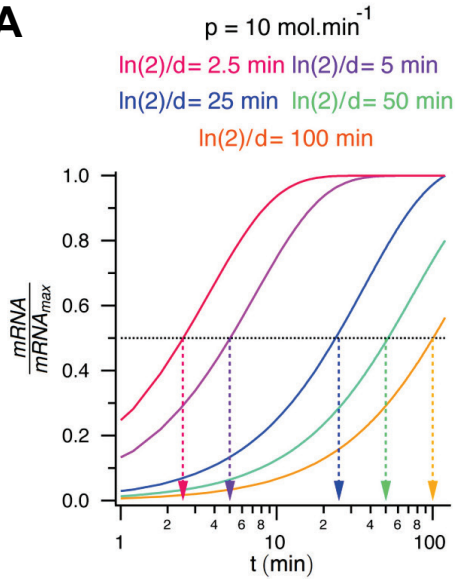


fig. S6. mRNA half-life has a major impact on response time of a protein in a negative feedback loop. (A) The response time of the protein as a function of mRNA half-life ($t_{1/2}=1$ and 10 min in the middle and bottom panels, respectively) when the protein half-life is 40 min (dashed blue line) or 8 hours (plain blue line). Parameter values are the same as described for the feedback loop without Hill function. (B) The response time of negative feedback loops as a function of mRNA half-life ($t_{1/2}=1$ and 10 min in the top and bottom panels, respectively) when the protein half-life is 40 min (dashed blue line) or 8 hours (plain blue line). The mRNA and protein concentrations are normalized to their steady state level. The response time is defined as the time necessary to reach half of the steady-state level (grey arrow). For the model of the negative feedback loop, the following parameters were used: *mRNA* is transcribed at $k_m = 10$ nM.min⁻¹ (top) and 1 nM.min⁻¹ (bottom), and degraded at $d_m = 0.69$ min⁻¹ (top) and 0.069 min⁻¹ (bottom) to reflect half-lives of 1 and 10 min, respectively. *mRNA* is translated into *protein* at $k_p = 100$ nM.min⁻¹, which is degraded at rate $d_p = 0.017$ min⁻¹ (blue dashed line) or $d_p = 0.0014$ min⁻¹ (blue plain line). The response of the promoter to the repressor protein is described by a Hill-type function with $K = 10$ nM, $n = 2$.



A



B

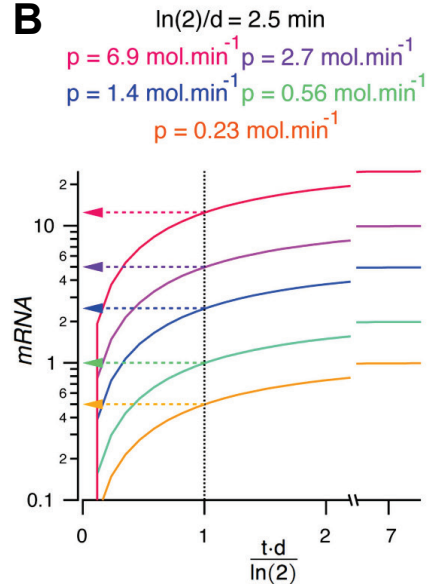


fig. S7. Degradation rates and not synthesis rates affect the response time. Both these rates determine the steady-state mRNA level ($mRNA_{max}$). **(A)** The time needed to reach half of $mRNA_{max}$ (response time) solely depends on the decay rate, d , of the mRNA. In other words, the shorter the mRNA half-life is, the faster the mRNA reaches its steady state level. **(B)** In contrast the synthesis rate, p , does not influence the response time of gene expression upon induction.

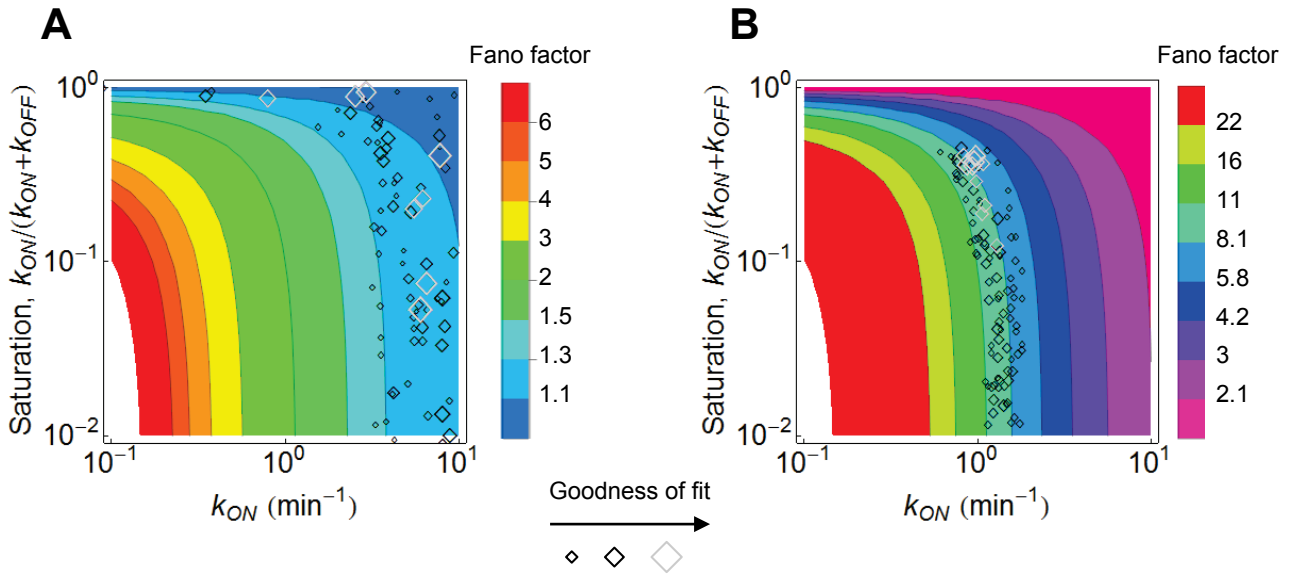


fig. S8. Parameter estimation of the two state-model to fit the *USA1* RNA copy number distributions. The size of symbol (diamond) is inversely proportional to the SSE (sum of squared residuals) and thus, reflects the goodness of the fit in the two-dimensional parameter space. The ten best fits are denoted by gray color. The Fano factor of the distribution is closely related to the goodness of the fit. The Fano factor is the ratio of the variance to the mean, and the Poisson distribution has a Fano factor equal to 1. The value of the decay rate constant is taken from the MGC experiment. The contour plot shows the Fano factor as a function of the k_{on} and saturation, $k_{ON}/(k_{ON} + k_{OFF})$. k_{Tr} was calculated to match the mean of the measured mRNA copy number. The random parameter values were obtained as follows. First, the logarithms of k_{ON} and k_{Tr} were randomly generated from a uniform distribution, between 0.001 and 10 min⁻¹, and between $0.83 \langle mRNA \rangle k_{dec}$ and 1200, respectively. $\langle mRNA \rangle$ is the mean value of the measured distribution. Last, k_{OFF} was randomly generated so that the predicted Fano factor was in the 2.25fold range around the Fano factor of the experimental distribution. **(A)** *USA1* (endogenous promoter). The Fano factor of the experimental distribution is 1.3 (see table S7), which indicates it is similar to a Poisson distribution. **(B)** *USA1* (substitutional promoter).

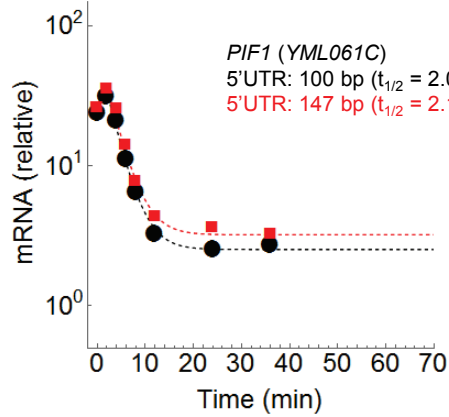


fig. S9. Impact of 5'UTR length on the decay profiles of mRNAs with varying 5'UTR lengths (right). The fitted half-lives (\pm standard error of the fitting) are indicated at the top of the panels. The length of 5'UTR of *YML061C* is 63 bp in the Nagalakshmi data set and 140 ± 7 bp in the Pelechano data set. The constructs with the two different cloned 5'UTR lengths (100 and 147 bp) yielded almost identical steady-state level and decay rates.

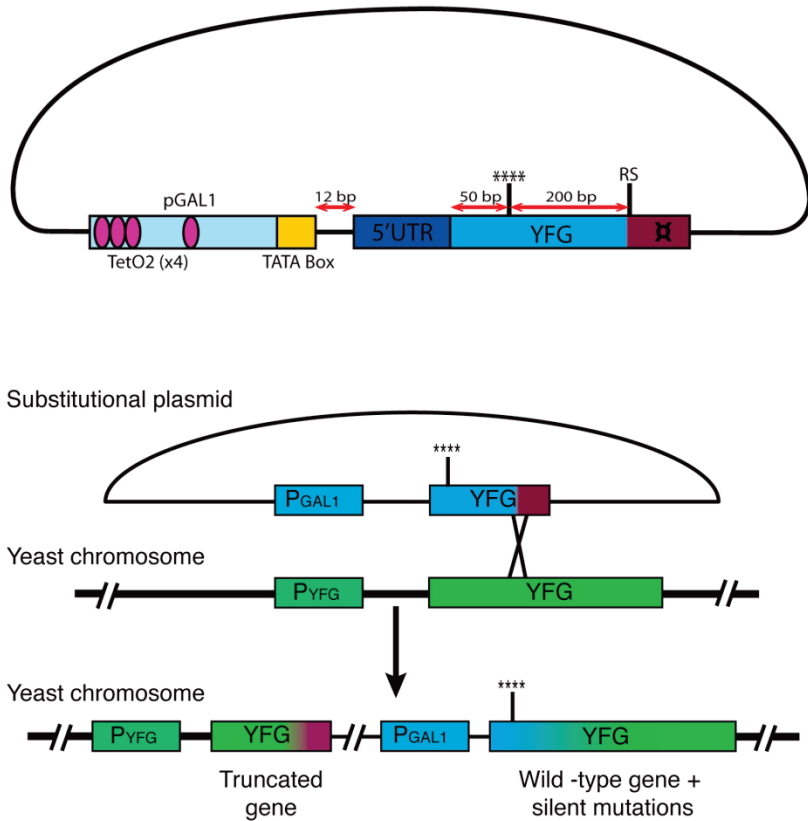


fig. S10. Design of the plasmids with the substitutable promoters and the strategy for chromosomal integration.

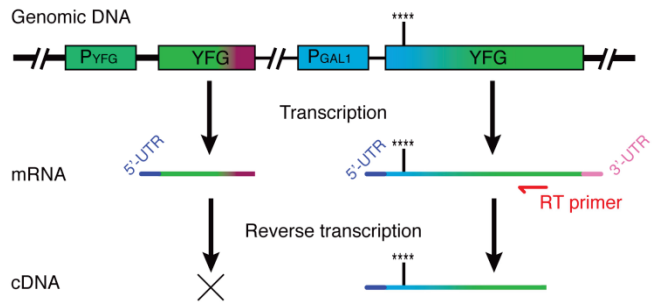


fig. S11. Reverse transcription strategy.

table S1. Plasmid list. tetO2 refers to the version 2 of the *tet* operator.

Common name	Systematic name	Plasmid number	Plasmid description
tTA	-	pMG013	pRS305-BstZI-P _{CLN3} -XbaI-tTA-BamHI
rTetR.SUM1	-	pSV_238	pRS303-SalI-P _{tet2} .rTetR.SUM1.Tact1-NotI
rTetR.SSN6	-	pSV_237	pRS303-SalI-P _{tet2} .rTetR.SSN6.Tact1-NotI
<i>OLA1</i>	YBR025C	pSV_228	pRS306-KpnI-Fig1-XhoI-Tgal7-AvrII-[tetO2]4inP _{gal1} -BglII-5'UTR_YBR025C_4silmut_RS_BamHI-EcoRI-Ter-NotI
<i>ETR1</i>	YBR026C	pSV_229	pRS306-KpnI-Fig1-XhoI-Tgal7-AvrII-[tetO2]4inP _{gal1} -BglII-5'UTR_YBR026C_4silmut_RS_BamHI-EcoRI-Ter-NotI
<i>HMT1</i>	YBR034C	pSV_232	pRS306-KpnI-Fig1-XhoI-Tgal7-AvrII-[tetO2]4inP _{gal1} -BglII-5'UTR_YBR034C_4silmut_RS_BamHI-EcoRI-Ter-NotI
<i>CSG2</i>	YBR036C	pSV_233	pRS306-KpnI-Fig1-XhoI-Tgal7-AvrII-[tetO2]4inP _{gal1} -BglII-5'UTR_YBR036C_4silmut_RS_BamHI-SphI-Ter-NotI
<i>ZTA1</i>	YBR046C	pSV_235	pRS306-KpnI-Fig1-XhoI-Tgal7-AvrII-[tetO2]4inP _{gal1} -BamHI-5'UTR_YBR046C_4silmut_RS_MfeI-SphI-Ter-NotI
<i>REG2</i>	YBR050C	pSV_236	pRS306-KpnI-Fig1-XhoI-Tgal7-AvrII-[tetO2]4inP _{gal1} -BglII-5'UTR_YBR050C_4silmut_RS_BamHI-EcoRI-Ter-NotI
<i>RFS1</i>	YBR052C	pCP001	pRS306-KpnI-Fig1-XhoI-Tgal7-AvrII-[tetO2]4inP _{gal1} -BglII-5'UTR_YBR052C_4silmut_RS_BamHI-EcoRI-Ter-NotI
<i>NRG2</i>	YBR066C	pCP002	pRS306-KpnI-Fig1-XhoI-Tgal7-AvrII-[tetO2]4inP _{gal1} -BglII-5'UTR_YBR066C_4silmut_RS_BamHI-EcoRI-Ter-NotI
<i>MIS1</i>	YBR084W	pCP003	pRS306-KpnI-Fig1-XhoI-Tgal7-AvrII-[tetO2]4inP _{gal1} -BglII-5'UTR_YBR084W_4silmut_RS_BamHI-SphI-Ter-NotI
<i>PGK1</i>	YCR012W	pEC006	pRS306-KpnI-Fig1-XhoI-Tgal7-AvrII-[tetO2]4inP _{gal1} -SphI-5'UTR_YCR012W_4silmut_RS_MfeI-EcoRI-Ter-NotI
<i>GLT1</i>	YDL171C	pSV_225	pRS306K-pnI-Fig1-XhoI-Tgal7-AvrII-[tetO2]4inP _{gal1} -BamHI-5'UTR_GLT1_4silmut_RS_SpeI-ClaI-Ter-NotI
<i>GLT1</i>	YDL171C	pSV_248	pRS306-BamHI-UP-GLT1-TFs-[tetO2]2-TATA_GLT1_4silmut_NMD2intron_RS_SpeI-NotI
<i>SUC2</i>	YIL162W	pSV_224	pRS306-KpnI-Fig1-XhoI-Tgal7-AvrII-[tetO2]4inP _{gal1} -BglII-5'UTR_SUC2_4silmut_RS_BamHI-EcoRI-Ter-NotI
<i>GAL2</i>	YLR081W	pSV_222	pRS306-KpnI-Fig1-XhoI-Tgal7-AvrII-[tetO2]4inP _{gal1} -BglII-5'UTR_GAL2_4silmut_RS_ClaI-EcoRI-Ter-NotI
<i>USA1</i>	YML029W	pSM001	pRS306-KpnI-Fig1-XhoI-Tgal7-AvrII-[tetO2]4inP _{gal1} -BglII-5'UTR_YML029W_4silmut_RS_ClaI-EcoRI-Ter-NotI
<i>USA1</i>	YML029W	pSV_243	pRS306-SpeI-UP-YML029W-TFs-[tetO2]2-TATA_YML029W_4silmut_NMD2intron_RS_BglII-NotI
<i>USA1</i>	YML029W	pSV_247	pRS306-SpeI-UP-YML029W-TFs-[tetO2]2-TATA_YML029W_4silmut_RS_BglII-NotI
<i>NDC1</i>	YML031W	pSV_203	pRS306-KpnI-Fig1-XhoI-Tgal7-AvrII-[tetO2]4inP _{gal1} -BglII-5'UTR_YML031W_4silmut_RS_BamHI-EcoRI-Ter-NotI
<i>RAD52</i>	YML032C	pSV_213	pRS306-KpnI-Fig1-XhoI-Tgal7-AvrII-[tetO2]4inP _{gal1} -BglII-5'UTR_YML032C_4silmut_RS_BamHI-EcoRI-Ter-NotI
<i>RRN11</i>	YML043C	pSM002	pRS306-KpnI-Fig1-XhoI-Tgal7-AvrII-[tetO2]4inP _{gal1} -BglII-5'UTR_YML043C_4silmut_RS_MfeI-EcoRI-Ter-NotI
<i>PRP39</i>	YML046W	pSM003	pRS306-KpnI-Fig1-XhoI-Tgal7-AvrII-[tetO2]4inP _{gal1} -BglII-5'UTR_YML046W_4silmut_RS_MfeI-EcoRI-Ter-NotI
<i>GSF2</i>	YML048W	pSV_209	pRS306-KpnI-Fig1-XhoI-Tgal7-AvrII-[tetO2]4inPgal1-BglII-5'UTR_YML048W_4silmut_RS_BamHI-EcoRI-Ter-NotI
<i>AIM32</i>	YML050W	pSV_266	pRS306KpnI-Fig1-XhoI-Tgal7-AvrII-[tetO2]4inPgal1-BglII-5'UTR_YML050W_4silmut_RS_BamHI-EcoRI-Ter
<i>GAL80</i>	YML051W	pSV_267	pRS306KpnI-Fig1-XhoI-Tgal7-AvrII-[tetO2]4inPgal1-BamHI-5'UTR_YML051W_4silmut_RS_SpeI-SphI-Ter-NotI

Common name	Systematic name	Plasmid number	Plasmid description
<i>CYB2</i>	YML054C	pEC007	pRS306-KpnI-Fig1-XhoI-Tgal7-AvrII-[tetO2]4inP _{gal1} -BglII-5'UTR_YML054C_4silmut_RS_MfeI-EcoRI-Ter-NotI
<i>NTE1</i>	YML059C	pSV_268	pRS306KpnI-Fig1-XhoI-Tgal7-AvrII-[tetO2]4inPgal1-BamHI-5'UTR_YML059C_4silmut_RS_BglII-SpeI-Ter-NotI
<i>OGG1</i>	YML060W	pSV_205	pRS306-KpnI-Fig1-XhoI-Tgal7-AvrII-[tetO2]4inP _{gal1} -BglII-5'UTR_YML060W_4silmut_RS_BamHI-EcoRI-Ter-NotI
<i>PIF1</i>	YML061C	pEC009	pRS306-KpnI-Fig1-XhoI-Tgal7-AvrII-[tetO2]4inP _{gal1} -BglII-5'UTR_YML061C_4silmut_RS_PmeI-EcoRI-Ter-NotI
<i>MFT1</i>	YML062C	pSV_210	pRS306-KpnI-Fig1-XhoI-Tgal7-AvrII-[tetO2]4inP _{gal1} -BglII-5'UTR_YML062C_4silmut_RS_BamHI-EcoRI-Ter-NotI
<i>ORC1</i>	YML065W	pSM004	pRS306-KpnI-Fig1-XhoI-Tgal7-AvrII-[tetO2]4inP _{gal1} -BglII-5'UTR_YML065W_4silmut_RS_MfeI-EcoRI-Ter-NotI
<i>ERV41</i>	YML067C	pSV_219	pRS306-KpnI-Fig1-XhoI-Tgal7-AvrII-[tetO2]4inP _{gal1} -BglII-5'UTR_YML067C_4silmut_RS_BamHI-EcoRI-Ter-NotI
<i>DAK1</i>	YML070W	pSV_207	pRS306-KpnI-Fig1-XhoI-Tgal7-AvrII-[tetO2]4inP _{gal1} -BglII-5'UTR_YML070W_4silmut_RS_BamHI-EcoRI-Ter-NotI
<i>COG8</i>	YML071C	pEC005	pRS306-KpnI-Fig1-XhoI-Tgal7-AvrII-[tetO2]4inP _{gal1} -BglII-5'UTR_YML071C_4silmut_RS_MfeI-EcoRI-Ter-NotI
<i>WAR1</i>	YML076C	pSV_218	pRS306-KpnI-Fig1-XhoI-Tgal7-AvrII-[tetO2]4inP _{gal1} -BamHI-5'UTR_YML076C_4silmut_RS_SpeI-SphI-Ter-NotI
<i>DUS1</i>	YML080W	pSM005	pRS306-KpnI-Fig1-XhoI-Tgal7-AvrII-[tetO2]4inP _{gal1} -BglII-5'UTR_YML080W_4silmut_RS_MfeI-EcoRI-Ter-NotI
<i>TDA9</i>	YML081W	pSV_307	pRS306-KpnI-Fig1-XhoI-Tgal7-AvrII-[tetO2]4inP _{gal1} -BglII-5'UTR_YML081W_4silmut_RS_BamHI-SphI-Ter-NotI
<i>ALO1</i>	YML086C	pSV_217	pRS306-KpnI-Fig1-XhoI-Tgal7-AvrII-[tetO2]4inP _{gal1} -BamHI-5'UTR_YML086C_4silmut_RS_SpeI-SphI-Ter-NotI
<i>AIM33</i>	YML087C	pSV_269	pRS306KpnI-Fig1-XhoI-Tgal7-AvrII-[tetO2]4inPgal1-BglII-5'UTR_YML087C_4silmut_RS_SpeI(WT)-EcoRI-Ter-NotI
<i>UFO1</i>	YML088W	pSV_208	pRS306-KpnI-Fig1-XhoI-Tgal7-AvrII-[tetO2]4inP _{gal1} -BglII-5'UTR_YML088W_4silmut_RS_BamHI-EcoRI-Ter-NotI
<i>RPM2</i>	YML091C	pSV_270	pRS306KpnI-Fig1-XhoI-Tgal7-AvrII-[tetO2]4inPgal1-EcoRI-5'UTR_YML091C_4silmut_RS_SpeI-SphI-Ter-NotI
<i>UTP14</i>	YML093W	pSV_206	pRS306-KpnI-Fig1-XhoI-Tgal7-AvrII-[tetO2]4inP _{gal1} -BglII-5'UTR_YML093W_4silmut_RS_BamHI-SphI-Ter-NotI
<i>YML096W</i>	YML096W	pSV_271	pRS306KpnI-Fig1-XhoI-Tgal7-AvrII-[tetO2]4inPgal1-BglII-5'UTR_YML096W_4silmut_RS_BamHI-EcoRI-Ter-NotI
<i>VPS9</i>	YML097C	pSV_215	pRS306-KpnI-Fig1-XhoI-Tgal7-AvrII-[tetO2]4inP _{gal1} -BglII-5'UTR_YML097C_4silmut_RS_BamHI-EcoRI-Ter-NotI
<i>ARG81</i>	YML099C	pSV_214	pRS306-KpnI-Fig1-XhoI-Tgal7-AvrII-[tetO2]4inP _{gal1} -BglII-5'UTR_YML099C_4silmut_RS_BamHI-EcoRI-Ter-NotI
<i>TSL1</i>	YML100W	pSV_272	pRS306KpnI-Fig1-XhoI-Tgal7-AvrII-[tetO2]4inPgal1-BglII-5'UTR_YML100W_4silmut_RS_BamHI-EcoRI-Ter-NotI
<i>CAC2</i>	YML102W	pEC008	pRS306-KpnI-Fig1-XhoI-Tgal7-AvrII-[tetO2]4inP _{gal1} -BglII-5'UTR_YML102W_4silmut_RS_MfeI-EcoRI-Ter-NotI
<i>NUP188</i>	YML103C	pSV_273	pRS306KpnI-Fig1-XhoI-Tgal7-AvrII-[tetO2]4inPgal1-BamHI-5'UTR_YML103C_4silmut_RS_SpeI-SphI-Ter-NotI
<i>PML39</i>	YML107C	pSV_274	pRS306KpnI-Fig1-XhoI-Tgal7-AvrII-[tetO2]4inPgal1-BamHI-5'UTR_YML107C_4silmut_RS_SpeI-SphI-Ter-NotI
<i>TAF8</i>	YML114C	pEC003	pRS306-KpnI-Fig1-XhoI-Tgal7-AvrII-[tetO2]4inP _{gal1} -BglII-5'UTR(100bp)_YML114C_4silmut_RS_BseRI-SphI-Ter-NotI
<i>TAF8</i>	YML114C	pEC004	pRS306-KpnI-Fig1-XhoI-Tgal7-AvrII-[tetO2]4inP _{gal1} -BglII-5'UTR(181bp)_YML114C_4silmut_RS_BseRI-SphI-Ter-NotI
<i>VAN1</i>	YML115C	pSV_211	pRS306-KpnI-Fig1-XhoI-Tgal7-AvrII-[tetO2]4inP _{gal1} -BglII-5'UTR_YML115C_4silmut_RS_BamHI-EcoRI-Ter-NotI

Common name	Systematic name	Plasmid number	Plasmid description
<i>ATRI</i>	YML116W	pSM006	pRS306-KpnI-Fig1-XhoI-Tgal7-AvrII-[tetO2]4inP _{gal1} -BglII-5'UTR_YML116W_4silmut_RS_MfeI-EcoRI-Ter-NotI
<i>PHO84</i>	YML123C	pSV_212	pRS306-KpnI-Fig1-XhoI-Tgal7-AvrII-[tetO2]4inP _{gal1} -BglII-5'UTR_YML123C_4silmut_RS_BamHI-EcoRI-Ter-NotI
<i>RSC9</i>	YML127W	pSV_204	pRS306-KpnI-Fig1-XhoI-Tgal7-AvrII-[tetO2]4inP _{gal1} -BglII-5'UTR_YML127W_4silmut_RS_BamHI-EcoRI-Ter-NotI
<i>ERO1</i>	YML130C	pSM007	pRS306-KpnI-Fig1-XhoI-Tgal7-AvrII-[tetO2]4inP _{gal1} -BglII-5'UTR_YML130C_4silmut_RS_MfeI-EcoRI-Ter-NotI
<i>ADH2</i>	YMR303C	pSV_226	pRS306-KpnI-Fig1-XhoI-Tgal7-AvrII-[tetO2]4inP _{gal1} -BglII-5'UTR_ADH2_4silmut_RS_BamHI-EcoRI-Ter-NotI

table S2. Strain list.

Strain number		Common name	Systematic name	Ploidy	Silent mutations	Promoter	Marker
YMX0			4x tTA	H	BY4741	-	-LEU
YMX00			4x tTA	D	BY4743	-	-LEU
YMX3	YMX4	<i>OLA1</i>	YBR025C	D	+	Substitutional	-LEU -URA
YMX5	YMX6	<i>ETR1</i>	YBR026C	D	+	Substitutional	-LEU -URA
YMX151	YMX152	<i>HMT1</i>	YBR034C	D	+	Substitutional	-LEU -URA
YMX9	YMX10	<i>CSG2</i>	YBR036C	D	+	Substitutional	-LEU -URA
YMX153	YMX154	<i>ZTA1</i>	YBR046C	D	+	Substitutional	-LEU -URA
YMX13	YMX14	<i>REG2</i>	YBR050C	D	+	Substitutional	-LEU -URA
YMX155	YMX156	<i>RFS1</i>	YBR052C	D	+	Substitutional	-LEU -URA
YMX17	YMX18	<i>NRG2</i>	YBR066C	D	+	Substitutional	-LEU -URA
YMX157	YMX158	<i>MIS1</i>	YBR084W	D	+	Substitutional	-LEU -URA
YMX159	YMX160	<i>PGK1</i>	YCR012W	D	+	Substitutional	-LEU -URA
YMX161	YMX162	<i>GLT1</i>	YDL171C	D	+	Substitutional	-LEU -URA
YMX163	YMX164	<i>SUC2</i>	YIL162W	D	+	Substitutional	-LEU -URA
YMX27	YMX28	<i>GAL2</i>	YLR081W	D	+	Substitutional	-LEU -URA
YMX29	YMX30	<i>USA1</i>	YML029W	D	+	Substitutional	-LEU -URA
YMX31	YMX32	<i>NDC1</i>	YML031W	D	+	Substitutional	-LEU -URA
YMX33	YMX34	<i>RAD52</i>	YML032C	D	+	Substitutional	-LEU -URA
YMX35	YMX36	<i>RRN11</i>	YML043C	D	+	Substitutional	-LEU -URA
YMX165	YMX166	<i>PRP39</i>	YML046W	D	+	Substitutional	-LEU -URA
YMX39	YMX40	<i>GSF2</i>	YML048W	D	+	Substitutional	-LEU -URA
YMX41	YMX42	<i>CYB2</i>	YML054C	D	+	Substitutional	-LEU -URA
YMX43	YMX44	<i>OGG1</i>	YML060W	D	+	Substitutional	-LEU -URA
YMX173	YMX174	<i>PIF1</i>	YML061C	D	+	Substitutional	-LEU -URA
YMX175	YMX176	<i>MFT1</i>	YML062C	D	+	Substitutional	-LEU -URA
YMX49	YMX50	<i>ORC1</i>	YML065W	D	+	Substitutional	-LEU -URA
YMX51	YMX52	<i>ERV41</i>	YML067C	D	+	Substitutional	-LEU -URA
YMX53	YMX54	<i>DAK1</i>	YML070W	D	+	Substitutional	-LEU -URA
YMX55	YMX56	<i>COG8</i>	YML071C	D	+	Substitutional	-LEU -URA
YMX57	YMX58	<i>WAR1</i>	YML076C	D	+	Substitutional	-LEU -URA
YMX59	YMX60	<i>DUS1</i>	YML080W	D	+	Substitutional	-LEU -URA
YMX61	YMX62	<i>TDA9</i>	YML081W	D	+	Substitutional	-LEU -URA
YMX63	YMX64	<i>ALO1</i>	YML086C	D	+	Substitutional	-LEU -URA
YMX177	YMX178	<i>UFO1</i>	YML088W	D	+	Substitutional	-LEU -URA
YMX67	YMX68	<i>UTP14</i>	YML093W	D	+	Substitutional	-LEU -URA
YMX69	YMX70	<i>VPS9</i>	YML097C	D	+	Substitutional	-LEU -URA
YMX71	YMX72	<i>ARG81</i>	YML099C	D	+	Substitutional	-LEU -URA
YMX73	YMX74	<i>CAC2</i>	YML102W	D	+	Substitutional	-LEU -URA
YMX75	YMX76	<i>VAN1</i>	YML115C	D	+	Substitutional	-LEU -URA
YMX77	YMX78	<i>ATR1</i>	YML116W	D	+	Substitutional	-LEU -URA

Strain number		Common name	Systematic name	Ploidy	Silent mutations	Promoter	Marker
YMX79	YMX80	<i>PHO84</i>	YML123C	D	+	Substitutional	-LEU -URA
YMX81	YMX82	<i>RSC9</i>	YML127W	D	+	Substitutional	-LEU -URA
YMX179	YMX180	<i>ERO1</i>	YML130C	D	+	Substitutional	-LEU -URA
YMX181	YMX182	<i>ADH2</i>	YMR303C	D	+	Substitutional	-LEU -URA
YMX87	YMX88	<i>GAL1</i>	YBR020W	D	+	Substitutional	-LEU -URA
YMX89	YMX90	<i>USA1</i>	YML029W	H	+	Substitutional	-LEU -URA
YMX91	YMX92	<i>ORC1</i>	YML065W	H	-	Substitutional	-LEU -URA
YMX93	YMX94	<i>DAK1</i>	YML070W	H	+	Substitutional	-LEU -URA
YMX97	YMX98	<i>ORC1</i>	YML065W	H	+	Substitutional	-LEU -URA
YMX107	YMX108	<i>USA1</i>	YML029W	H	+	Substitutional	-LEU -URA
YMX109	YMX110	<i>USA1</i>	YML029W	H	-	Substitutional	-LEU -URA
YMX111	YMX112	<i>RRN11</i>	YML043C	H	+	Substitutional	-LEU -URA
YMX113	YMX114	<i>RRN11</i>	YML043C	H	-	Substitutional	-LEU -URA
YMX115	YMX116	<i>COG8</i>	YML071C	H	+	Substitutional	-LEU -URA
YMX117	YMX118	<i>COG8</i>	YML071C	H	-	Substitutional	-LEU -URA
YMX119	YMX120	<i>DAK1</i>	YML070W	H	-	Substitutional	-LEU -URA
YMX121	YMX122	<i>GAL80</i>	YML051W	D	+	Substitutional	-LEU -URA
YMX123	YMX124	<i>AIM33</i>	YML087C	D	+	Substitutional	-LEU, -HIS
YMX125	YMX126	<i>YML096W</i>	YML096W	D	+	Substitutional	-LEU, -HIS
YMX127	YMX128	<i>TSL1</i>	YML100W	D	+	Substitutional	-LEU -URA
YMX129	YMX130	<i>NUP188</i>	YML103C	D	+	Substitutional	-LEU -URA
YMX131	YMX132	<i>PML39</i>	YML107C	D	+	Substitutional	-LEU -URA
YMX101	YMX102	<i>USA1</i>	YML029W	H	+	Insertional	-LEU, -HIS
YMX103	YMX104	<i>USA1</i>	YML029W	H	+, no intron	Insertional	-LEU, -HIS
YMX105	YMX106	<i>GLT1</i>	YDL171C	H	+	Insertional	-LEU, -HIS
YMX133	YMX134	<i>AIM32</i>	YML050W	D	+	Substitutional	-LEU -URA
YMX135	YMX136	<i>NTE1</i>	YML059C	D	+	Substitutional	-LEU -URA
YMX137	YMX138	<i>RPM2</i>	YML091C	D	+	Substitutional	-LEU -URA

table S3. Cloned 5'UTR lengths of genes.

Systematic name	Common name	5'-UTR (bp)
YBR025C	<i>OLA1</i>	132
YBR026C	<i>ETR1</i>	100
YBR034C	<i>HMT1</i>	100
YBR036C	<i>CSG2</i>	100
YBR046C	<i>ZTA1</i>	100
YBR050C	<i>REG2</i>	187
YBR052C	<i>RFS1</i>	100
YBR066C	<i>NRG2</i>	142
YBR084W	<i>MIS1</i>	103
YCR012W	<i>PGK1</i>	100
YDL171C	<i>GLT1</i>	110
YIL162W	<i>SUC2</i>	100
YLR081W	<i>GAL2</i>	100
YML029W	<i>USA1</i>	100
YML031W	<i>NDC1</i>	100
YML032C	<i>RAD52</i>	100
YML043C	<i>RRN11</i>	164
YML046W	<i>PRP39</i>	100
YML048W	<i>GSF2</i>	100
YML050W	<i>AIM32</i>	100
YML051W	<i>GAL80</i>	100
YML054C	<i>CYB2</i>	100
YML059C	<i>NTE1</i>	100
YML060W	<i>OGG1</i>	100
YML061C	<i>PIF1</i>	100
YML062C	<i>MFT1</i>	100
YML065W	<i>ORC1</i>	100
YML067C	<i>ERV41</i>	100
YML070W	<i>DAK1</i>	100
YML071C	<i>COG8</i>	109
YML076C	<i>WAR1</i>	100
YML080W	<i>DUS1</i>	126
YML081W	<i>TDA9</i>	143
YML086C	<i>ALO1</i>	159
YML087C	<i>AIM33</i>	100
YML088W	<i>UFO1</i>	100
YML091C	<i>RPM2</i>	264
YML093W	<i>UTP14</i>	100

Systematic name	Common name	5'-UTR (bp)
YML096W	YML096W	100
YML097C	<i>VPS9</i>	100
YML099C	<i>ARG81</i>	100
YML100W	TSL1	100
YML102W	<i>CAC2</i>	100
YML103C	NUP188	100
YML107C	PLM39	100
YML115C	<i>VAN1</i>	100
YML116W	<i>ATR1</i>	101
YML123C	<i>PHO84</i>	100
YML127W	<i>RSC9</i>	100
YML130C	<i>ERO1</i>	106
YMR303C	<i>ADH2</i>	100

table S4. qPCR primers detecting marked mRNAs and their amplification efficiencies.

Systematic name	Common name	Forward primer (5' to 3')	Reverse primer (5' to 3')	Eff. (%)
YBR025C	<i>OLA1</i>	TCCAGGAAACAATTTAAAA GCCGG	GTGATGGCTTGGAAA AAGGTAGAC	1
YBR026C	<i>ETR1</i>	CCAAACATTTTAAGTCGCTC ATCTATTC	TTTTCACTGACAAAAC CTTGGTACA	1.09
YBR034C	<i>HMT1</i>	GCTAAGTGAGAGTGAGCAA CA	CGAACAGTATCTTGAA GCATCTCT	0.96
YBR036C	<i>CSG2</i>	ACGTGATTCAAACCTAAGTG CTTATCT	GTCGCCGTTAGTTTCA GGC	1
YBR046C	<i>ZTA1</i>	TGATGAAATTGGAGGTTAT GACGTAATC	TGATTAGTAACTCTTC CTCCGAAATCG	0.95
YBR050C	<i>REG2</i>	GAAAGTGTGTGAGAATGAC TTTGAGTA	TTTACTACTCTCCTCC TCTTCTGGA	1.12
YBR052C	<i>RFS1</i>	CCACATTGGCTGAGAACGA AAAA	TGGCACTTGGAAAATC TCAGCC	0.87
YBR066C	<i>NRG2</i>	TTTCAGGCATAAGAGTTATC TGGGAGG	AGAAAGTGCAGATTT CCCATCA	0.92
YBR084W	<i>MIS1</i>	TCGCCTACAGTACACGCTTC TC	TGGCTTTTTCTCGAAT AGATTGAGCA	1.06
YCR012W	<i>PGK1</i>	CATAAGGGTCGATTTCAAC GTCC	GCAAAGCAGCAACAA TTCTTTGG	1
YDL171C	<i>GLT1</i>	GAATTAGAAAAGAAAGCAT GCCAGTG	TATAGTGCCTCCTTCG TAAGCTTC	0.98
YIL162W	<i>SUC2</i>	TGACTAATGATACAAGCGA TAGACCTT	CCATTTGGGTCATTCA TCCAGC	0.97
YLR081W	<i>GAL2</i>	CTGGCGAGGATGTCATCTCT TC	TTAGAATACTTTTGAG ATTGTGCGC	0.99
YML029W	<i>USA1</i>	ATGGTCAAGCGAAATAGAC CTAATC	TATATTGAAGTAGTCG CCCAACCG	0.92
YML031W	<i>NDC1</i>	CGTACCATACCATATTCAGT GATGTG	AACCTCGTAACTAAAT GGTTGAATCG	1.21
YML032C	<i>RAD52</i>	TTCGGTAACCATTCGGAAG ATATTCA	ATACTCAGGTCCTAAT TTCTTGCCA	0.98
YML043C	<i>RRN11</i>	GCTCAAAGAAGAAAATTGA AATACCAATAC	TCTGTGGTAGTAGATT TCTTGGAAATTC	1.05
YML046W	<i>PRP39</i>	CTGATGCGCTACGTGGATT G	CCAGTCTAAACCACG ATATGCTTG	1.12
YML048W	<i>GSF2</i>	TTCAAGTCTCTAACGAAGA CACCA	CCGTCTTGAAGGGA AAATCTTC	0.78
YML050W	<i>AIM32</i>	CTTCCATCATAGTTTTAAGC ACATTAGC	GCGTTTTATTTCTTGGC AATAACAGTTTG	0.84
YML051W	<i>GAL80</i>	GTATGGCGGGGTAATGTGT CTT	ATTAGGGTGGGTTTCG TCGG	1.08
YML054C	<i>CYB2</i>	TACGGTTCAACGGTACCTA AATCC	AGTCCATGACTGTGTG CGTTTT	1.22
YML059C	<i>NTE1</i>	TTGCATGCGTTCAATGAATT GCA	AAGGCTATTCTTGGTG TTTTGGC	1.04
YML060W	<i>OGG1</i>	CTAGCTAACGTCTTCAGGC TG	TGCTGTACTGGTTTAA CTTTTCATCC	0.89
YML061C	<i>PIF1</i>	ACCTAGGAGACCTTTTATCT GTAGT	CGATAGTTTGGCGTGC GATA	0.95

Systematic name	Common name	Forward primer (5' to 3')	Reverse primer (5' to 3')	Eff. (%)
YML062C	<i>MFT1</i>	CACTACAGCGAGGTTGACA CT	CCTGTCAGCTTGTTA CTTTCC	0.98
YML065W	<i>ORC1</i>	AACGTTGAAGGATTTACAG GGTTG	TCCATCGATTATGTTA CCTTGTTTCATC	1.07
YML067C	<i>ERV41</i>	GTACAAAAAAGTCGACC AAGGGT	ACCAAACCTCGGTCCAT GCTATAAA	0.93
YML070W	<i>DAK1</i>	TCTCAAAGGCTTCGCGCTA G	CGGAATCGGTCTTTCT GAAGAGA	1.1
YML071C	<i>COG8</i>	CAAAAGCGCTTGAGCCTTG	TCAAGGCTCAAGCGCT TTTG	1.09
YML076C	<i>WAR1</i>	GACTGAACCTGCCCTGAGT AG	TGACGTCCAGTTAGTT TTTGCATC	1.16
YML080W	<i>DUS1</i>	GACTGAACCTGCCCTGAGT AG	GTCAAAAAGCTGACG TCCAGTTAG	1.16
YML081W	<i>TDA9</i>	ATCCTCCATTACCTCTGTTG ATAGA	TGGGGATCGGTAGTA CTTGTATT	1.13
YML086C	<i>ALO1</i>	GGGCCGGAATATACTCAGC TAAA	AACTCGACAACCTCAT CAATTGAAC	0.89
YML087C	<i>AIM33</i>	CCGTTTTATCCTTTCTGCAC AGG	CCACGATTCTACCATT TTGCGTT	1
YML088W	<i>UFO1</i>	CCCGAAATCCTGATAAATA TATTCTCACAC	TAGAAAGTTCTTGCAA CGTGAACAA	0.97
YML091C	<i>RPM2</i>	CCGCTCAGAAAAAGACAGC AAC	CAAACCTTGGTTTTGC CTTAGATACT	0.97
YML093W	<i>UTP14</i>	TTTTGGACGCATTACAGCTT GC	CTTTGTGATTGTCGC TGGAGTT	1.1
YML096W	YML096 W	TGATGAATTGATTGAGTTTC CAGAAGG	TGTTGAAAATCGACG ATTCATTGGT	1.04
YML097C	<i>VPS9</i>	TTCAGCCAGTTAGAGCAAG CA	CCGCTGCCCTATTAGG ATCATC	0.89
YML099C	<i>ARG81</i>	AAAGACGTTACAGGCTGC T	GGATGCCGAAGATCA CACTTAAC	0.97
YML100W	<i>TSL1</i>	AATTCGAGCTAGATACGTC ACTCC	CATAGCCTGGATGTTC ACGAGA	1.1
YML102W	<i>CAC2</i>	TCCAAAAGAATAGCGCAAA TGACA	ATCCTAACCTTGTTAT CACCACCA	0.96
YML103C	<i>NUP188</i>	CCTTCACTCATGTAGCAAAC TTCAT	AATTGCTTGGCATCCA CAGC	0.97
YML107C	<i>PLM39</i>	TTCGCTGGATAAAAACACG AAACT	CCATTTGGTAATAAGT CGCTCTCC	1
YML114C	<i>TAF8</i>	AGTTAAACTCCTTAAACATC TGCATTTCAA	AGTGTTAAATTGTGCA GGTTCCGA	0.99
YML115C	<i>VAN1</i>	CAACGGTCTTAGTCTGCCA TT	CTGAGCGTTTGTCTT GATGTTG	0.92
YML116W	<i>ATR1</i>	ATGAGAACGAAACTGAGCT ACCTG	GCTGTTGCTGTAAAG ATTCTCCG	1.3
YML123C	<i>PHO84</i>	GTCTTCATAAGGAGCATCT AACCGA	AGCAAAATCATTCAA ATGGTTGTGG	0.88
YML127W	<i>RSC9</i>	AGGCTCCGGCGACTAGCT	ATTAGACTGTAATCGA GACACTTTAATAGGGT T	1.06
YML130C	<i>ERO1</i>	CTGCTACATCTAATAATAGC TACATCGC	GTCGACCTTACAAAA GTGAGTGTC	1.06
YMR303C	<i>ADH2</i>	CAACGGCAAATTAGAACAC AAGGA	CAGACACCAGAGTAC TTGACGT	1.21

table S5. qPCR primers detecting endogenous mRNAs with their efficiencies.

Systematic name	Common name	Forward primer (5' to 3')	Reverse primer (5' to 3')	Eff. (%)
YBR123C	<i>TFC1</i>	GCGGTATTGACAGCAG GTTCAA	CCATCCAGTTAGTTCATTCCG CCTTA	1.08
YER100W	<i>UBC6</i>	ACCATCAGAAGAAGA CATTAGCAAGA	TCATCACCTGTATTTGCCGC AT	0.93
YBR018C	<i>GAL7</i>	CTAGCCATTCCCATAG ACGTTACAA	TTGACCTAACCAAGGTCTTT TAGCTC	1.01
YBR020W	<i>GAL1</i>	ACCTGAGTTCAATTCT AGCGCAA	GCGATCTAGCAACAAAATC CGGTTTA	1.00
YBR025C	<i>OLA1</i>	TCCAGGTAATAACTTG AAAGCCGG	GTGATGGCTTGAAAAAGG TAGAC	0.96
YBR026C	<i>ETR1</i>	CCAAGCACTTCAAATC GCTCATCTATTC	TTTTACTGACAAAACCTTG GTACA	0.85
YBR034C	<i>HMT1</i>	GCTAAGTGAAAGCGA ACAGCA	CGAACAGTATCTTGAAGCA TCTCT	0.87
YBR036C	<i>CSG2</i>	ACGTGATTCAAACAAA ATGTTTGTCT	GTCGCCGTTAGTTTCAGGC	0.98
YBR046C	<i>ZTA1</i>	TGATGAAATTGGTGGA TACGATGTAATC	TGATGAAATTGGAGGTTAT GACGTAATC	0.92
YBR050C	<i>REG2</i>	GAAAGTGTGTGAGAAT GACTTTGAGTA	TTTACTACTTTTCTTCTCCT CTGGA	0.77
YBR052C	<i>RFS1</i>	TTGCCACATTAGCAGA AAATGAAAAAAA	CCACATTGGCTGAGAACGA AAAA	0.86
YBR066C	<i>NRG2</i>	AGATAGGAAATGTGA ATTGCCATCAAT	TTTCAGGCATAAGAGTTAT CTGGGAGG	0.92
YBR084W	<i>MIS1</i>	TCGCCAACTGTGCATG CTTCTC	TGGCTTTTCTCGAATAGAT TGAGCA	1.06
YCR012W	<i>PGK1</i>	CATCAGAGTTGACTTC AACGTCC	GCAAAGCAGCAACAATTCT TTGG	0.86
YDL171C	<i>GLT1</i>	GAATTAGAAAAGAAA GCATGCCAGTG	AATTGTCCACCTTCGTAA GCTTC	1.04
YIL162W	<i>SUC2</i>	TGACAAACGAAACTA GCGATAGACCTT	CCATTTGGGTCATTTCATCCA GC	1.02
YLR081W	<i>GAL2</i>	TGACAAACGAAACTA GCGATAGACCTT	CCATTTGGGTCATTTCATCCA GC	0.82
YML029W	<i>USA1</i>	ATGGTCAAGCGAGATT GATCTTATC	TATATTGAAGTAGTCGCC AACCG	0.97
YML031W	<i>NDC1</i>	CGTACCATACCATCTT TAGCGACGTG	AACCTCGTAACTAAATGGT TGAATCG	0.96
YML032C	<i>RAD52</i>	TTCGGTAACCATTCCG AGGACATACA	ATACTCAGGTCTAATTTCT TGTCCTA	0.82
YML043C	<i>RRN11</i>	GCTCAAAGAAGAAAG TTAAAGTATCAATAC	TCTGTGGTAGTAGATTTCTT GGAAATTC	1.04
YML046W	<i>PRP39</i>	CTGATGCCCTTCGAGG TTTG	CCAGTCTAAACCACGATAT GCTTG	1.16
YML048W	<i>GSF2</i>	TTCAGGTGTCAAATGA AGACACCA	CCGTCTTGAAGGGAAAAT CTTC	0.79
YML050W	<i>AIM32</i>	CTCCATCATAGCTTC AAACATATTAGC	GCGTTTATTTCTTGGAATA ACAGTTTG	0.83
YML051W	<i>GAL80</i>	CCATAAGAGTCGGATT CGTCGG	ATTAGGGTGGGTTTCGTCG G	1.14
YML054C	<i>CYB2</i>	CTGGTGAAGACGTGAT CTCTTCA	TTAGAATACTTTTGAGATTG TGCGC	1.11
YML059C	<i>NTE1</i>	TTGCATGCGTTCAATG AATTGCA	AAGACTGTTTTTCGTGTTTT GGC	1.10

Systematic name	Common name	Forward primer (5' to 3')	Reverse primer (5' to 3')	Eff.
YML060W	<i>OGG1</i>	CTAGCAAATGTGCTAC AGGCTG	TGCTGTACTGGTTTAACTTT TCATCC	0.99
YML061C	<i>PIF1</i>	TACGGTTCTACCGTTC CAAAATCC	CGATAGTTTGGCGTGCGAT A	1.11
YML062C	<i>MFT1</i>	CACTACAGTGAAGTAG ATACT	CCTGTCAGCTTGGTTACTTT TCC	0.96
YML065W	<i>ORC1</i>	AACGTTGAAGGATTTA CAGGGTTG	GATGAGCAGGGAAATATAA TCGATGGA	0.95
YML067C	<i>ERV41</i>	GTACAAGAAGAAATC CACCAAGGGT	ACCAAACCTCGGTCCATGCT ATAAA	0.90
YML070W	<i>DAK1</i>	TCTCAAAGGGTTTGCC CTTG	CGGAATCGGTCTTTCTGAA GAGA	0.96
YML071C	<i>COG8</i>	CAAAAACGGTTAAGTC TTG	GGAACAGCCCTAGATGAAA AATAAC	0.99
YML076C	<i>WAR1</i>	AATGATAATTCAAAGA CCGACC	TGGCCTTATCTATGCATGG GAC	0.99
YML080W	<i>DUS1</i>	ACCAAGAAGGCCATTT ATCTGTAGT	CGATAGTTTGGCGTGCGAT A	1.08
YML081W	<i>TDA9</i>	ATCCTCCACTATCTAT GCTGATAGA	TGGGGATCGGTAGTACTTG TATT	1
YML086C	<i>ALO1</i>	GGGCCGGAATTTATTC TGCAAAA	AACTCGACAACCTCATCAA TTGAAC	0.92
YML087C	<i>AIM33</i>	TCCGTTTTATCCATTTT GTACTGGATTG	CCACGATTCTACCATTTTGC GTT	1
YML088W	<i>UFO1</i>	CCCGAAATCCTGATAA ACATCTTTTCTCAC	TAGAAAGTTCTTGCAACGT GAACAA	0.81
YML091C	<i>RPM2</i>	CCGCTCAAAGAAAA CTGCAAC	CAAACCTTGGTTTTGCCTTA GATACT	0.90
YML093W	<i>UTP14</i>	TTTTGGATGCTTTGCA ACTTGC	CTTTTGTGATTGTGCTGGA GTT	0.98
YML096W	YML096 W	TGATGAGTTAATCGAA TTTCCAGAAGG	TGTTGAAAATCGACGATTC ATTGGT	0.95
YML097C	<i>VPS9</i>	TTCAGCCAATTGGAAC AGGCA	CCGCTGCCCTATTAGGATC ATC	1.02
YML099C	<i>ARG81</i>	AAAGACGTTTACTGGG TGTT	GGATGCCGAAGATCACACT TAAC	0.92
YML100W	<i>TSL1</i>	AATTCGAGCTTGACAC CTCTCTC	CATAGCCTGGATGTTACAG AGA	1.05
YML102W	<i>CAC2</i>	ATCCTCCACTATCTAT GCTGATAGA	TGGGGATCGGTAGTACTTG TATT	0.91
YML103C	<i>NUP188</i>	CCTTCACTCATGTTGC TAATTTTATGAAC	AATTGCTTGGCATCCACAG C	1
YML107C	<i>PLM39</i>	TTCGCTGGACAAGAAT ACCAAAC	CCATTTGGTAATAAGTCGC TCTCC	1.34
YML114C	<i>TAF8</i>	TCCCGTAGTTGAAATT CTAAAGAAAACA	AGTGTTAAATTGTGCAGGT TCCGA	0.98
YML115C	<i>VAN1</i>	CAATGGACTAAGCCTG CCATT	CTGAGCGTTTGTCTTGATG TTG	0.817
YML116W	<i>ATR1</i>	ATGAGAACGAAACTG AGCTACCTG	AGTGTTAAATTGTGCAGGT TCCGA	1.25
YML123C	<i>PHO84</i>	GTCTTCATAAAGAACA CCTTACCGA	AGCAAATCATTCAAATGG TTGTGG	0.86
YML127W	<i>RSC9</i>	CACCCGCCACAAGCTC C	ATTAGACTGTAATCGAGAC ACTTTAATAGGGTT	1.06
YML130C	<i>ERO1</i>	CTGCAACTTCAAACAA TAGCTACATCGC	GTCGACCTTACAAAAGTGA GTGTC	1.06
YMR303C	<i>ADH2</i>	CAACGGCAAGTTGGA GCATAAGGA	CAGACACCAGACTTGA CGT	1.09

table S6. Cross-reaction of primers to detect the marked mRNA with the endogenous mRNAs. Differences between the Ct values (Δ Ct) obtained with primers matching the marked and endogenous mRNAs. Δ Ct is shown for the examined mRNAs at the standard annealing temperature of the qPCR, 60°C. If Δ Ct(60°C) < 5, the Δ Ct was also measured at 68°C. The template for the qPCR is cDNA reverse transcribed from endogenous (wt) mRNAs.

Systematic name	Common name	Δ Ct (60°C)	Δ Ct (68°C)	Systematic name	Common name	Δ Ct (60°C)	Δ Ct (68°C)
YBR025C	<i>OLA1</i>	10.71		YML065W	<i>ORC1</i>	7.87	
YBR026C	<i>ETR1</i>	3.08	13.26	YML067C	<i>ERV41</i>	9.08	
YBR034C	<i>HMT1</i>	13.80		YML070W	<i>DAK1</i>	14.79	
YBR036C	<i>CSG2</i>	5.69		YML071C	<i>COG8</i>	14.75	
YBR046C	<i>ZTA1</i>	8.61		YML076C	<i>WAR1</i>	5.75	
YBR050C	<i>REG2</i>	9.89		YML080W	<i>DUS1</i>	12.63	
YBR052C	<i>RFS1</i>	3.28	11.30	YML086C	<i>ALO1</i>	13.39	
YBR066C	<i>NRG2</i>	8.83		YML087C	<i>AIM33</i>	22.45	
YBR084W	<i>MIS1</i>	5.29		YML088W	<i>UFO1</i>	8.69	
YCR012W	<i>PGK1</i>	7.29		YML091C	<i>RPM2</i>	13.80	
YDL171C	<i>GLT1</i>	0.62	7.57	YML093W	<i>UTP14</i>	15.39	
YIL162W	<i>SUC2</i>	2.29	8.41	YML096W	<i>YML096W</i>	10.24	
YLR081W	<i>GAL2</i>	4.24		YML097C	<i>VPS9</i>	14.19	
YML029W	<i>USA1</i>	20.57		YML099C	<i>ARG81</i>	15.65	
YML031W	<i>NDC1</i>	5.54		YML100W	<i>TSL1</i>	11.79	
YML032C	<i>RAD52</i>	11.71		YML102W	<i>CAC2</i>	7.87	
YML043C	<i>RRN11</i>	11.36		YML103C	<i>NUP188</i>	27.28	
YML046W	<i>PRP39</i>	8.85		YML107C	<i>PML39</i>	14.31	
YML048W	<i>GSF2</i>	8.12		YML114C	<i>TAF8</i>	7.33	
YML050W	<i>AIM32</i>	9.05		YML115C	<i>VANI</i>	5.42	
YML051W	<i>GAL80</i>	8.69		YML116W	<i>ATR1</i>	12.34	
YML054C	<i>CYB2</i>	8.47		YML123C	<i>PHO84</i>	8.73	
YML059C	<i>NTE1</i>	5.26		YML127W	<i>RSC9</i>	23.96	
YML060W	<i>OGG1</i>	15.59		YML130C	<i>ERO1</i>	0.46	6.75
YML061C	<i>PIF1</i>	8.43		YMR303C	<i>ADH2</i>	12.01	
YML062C	<i>MFT1</i>	18.53					

table S7. Mean, Fano factor, and CV of the RNA molecule copy number distributions.

Gene common name	Endogenous Promoter			Substitutional promoter		
	Mean	Fano factor	CV	Mean	Fano factor	CV
<i>USA1</i>	3.35	1.31	0.62	33.08	8.23	0.50
<i>COG8</i>	3.27	1.40	0.65	35.84	10.89	0.55
<i>UTP14</i>	4.73	1.77	0.61	36.25	9.87	0.52
<i>PIF1</i>	3.19	2.80	0.94	35.86	23.12	0.80
<i>DAK1</i>	8.69	2.44	0.53	89.05	30.49	0.59