Supplemental Figures

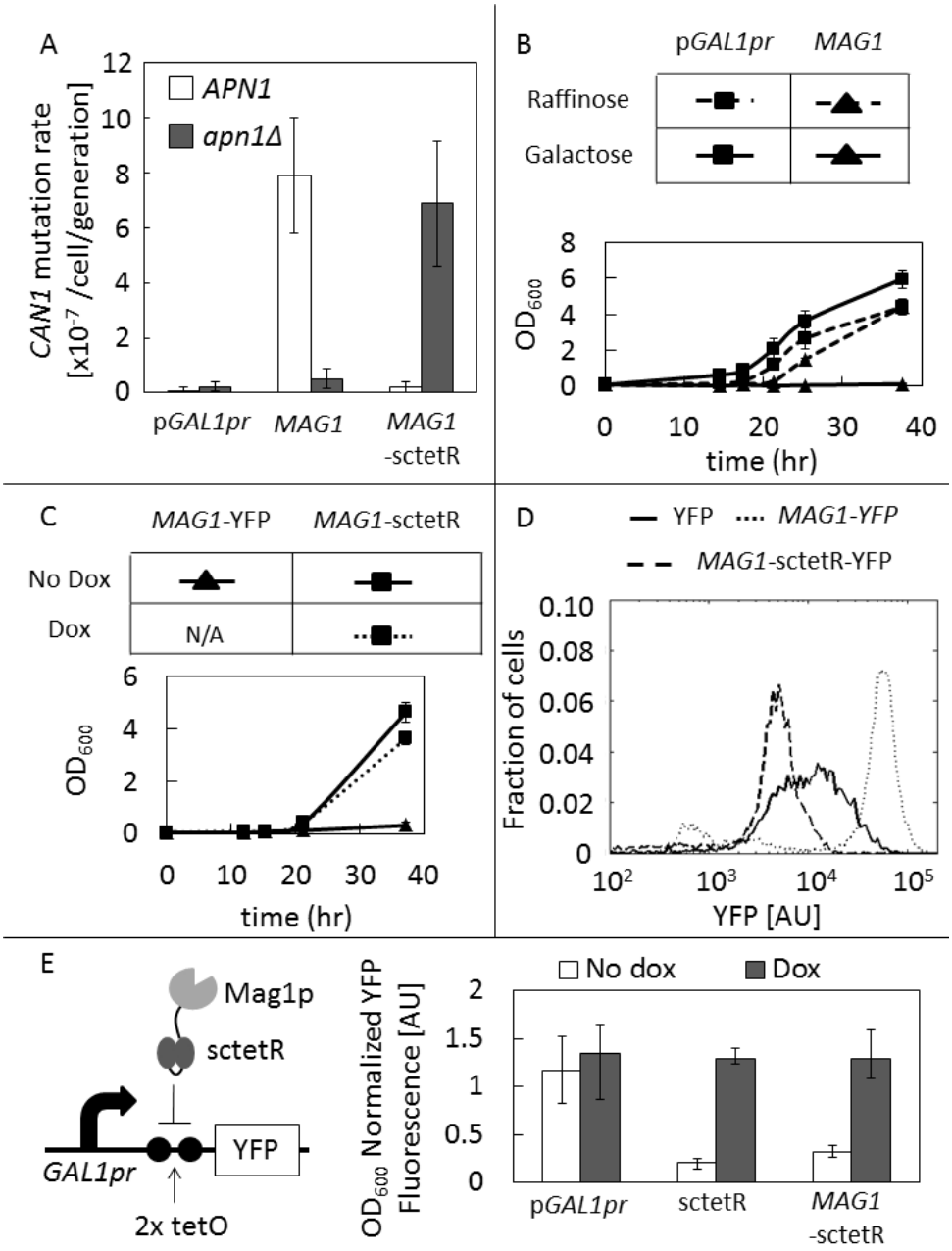

Figure S1: The mutator protein *MAG1* and DNA binding domain sctetR are functional and retain function when fused. (A) *MAG1* overexpression in WT cells leads to an increase in the background mutation rate at *CAN1* as compared to an empty vector (p*GAL1pr*). The *MAG1-sctetR* fusion has decreased but significant mutator activity, as evidenced by the increased mutation rate in an *apn1Δ*. Surprisingly, *MAG1* overexpression in an *apn1Δ* does not lead to a measurable increase in the mutation rate. This is (B) due to a severe growth defect, which is (C) relieved specifically upon fusion to sctetR and does not depend on sctetR's ability to bind DNA (+dox growth curve). (D) The reduction in mutator function upon fusion to sctetR could in part be due to decreased expression levels as measured by flow cytometry on YFP-tagged mutators. (E) The *MAG*-sctetR fusion protein retains the ability to bind tetO as measured by fluorescence knockdown from a tet-repressible promoter in WT cells. Error bars on mutation rate represent 95% c.i. Error bars on fluorescence knockdown and growth represent the range of values observed in triplicate experiments.

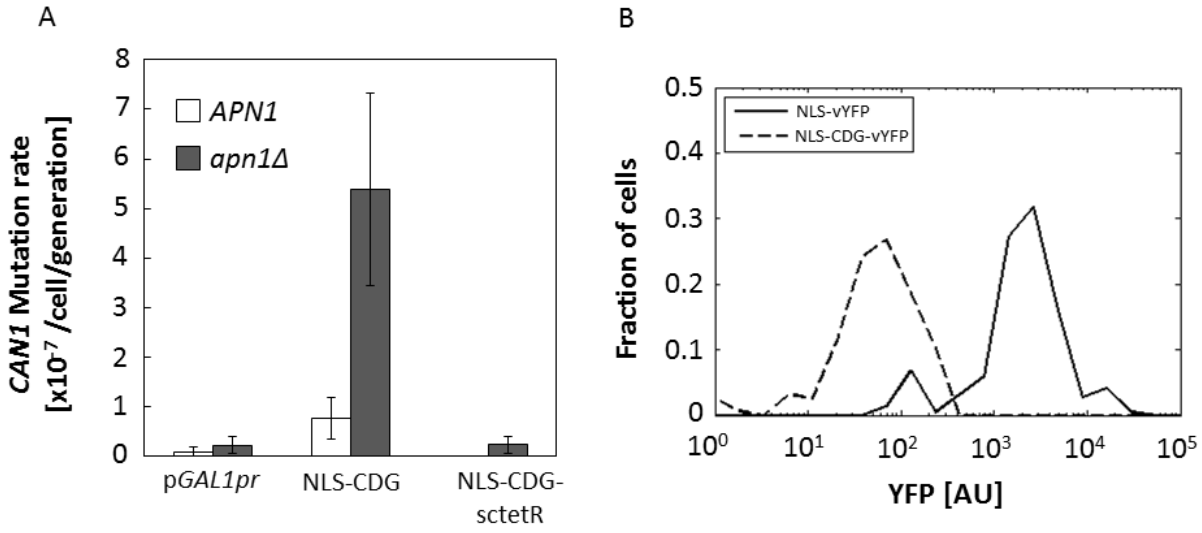

Figure S2: **CDG-sctetR does not retain ability to mutate DNA.** As alluded to in the main text, we also (A) CDG but not CDG-sctetR increases the background mutation rate in *apn1Δ* as compared to an empty vector (p*GAL1pr*). (B) Expression of a nuclear localization signal (NLS)-tagged CDG-vYFP fusion was measured by fluorescence microscopy. Histograms represent cellular autofluorescence-subtracted YFP expression in arbitrary units (AU) as measured by fluorescence microscopy. Expression of CDG-YFP is significantly lower than both NLS-vYFP and *MAG1*-vYFP (Fig. S2C), possibly explaining its lack of activity. Error bars represent 95% c.i.

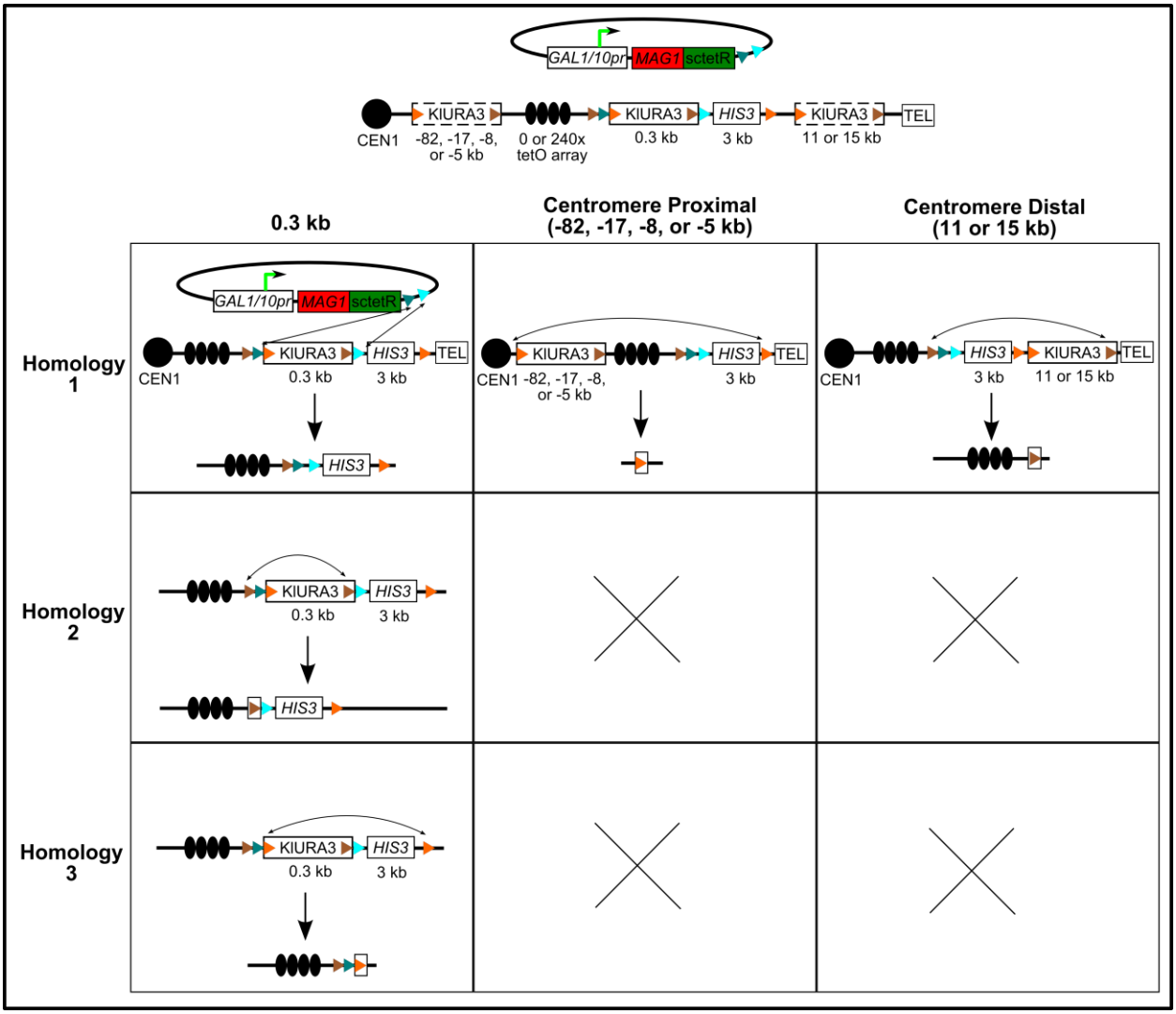

Figure S3: Inter and intra-chromosomal repetitive homologous sequences lead to deletions. Various repetitive homologous sequences introduced during strain construction—17 bp (brown), 18 bp (orange), 201 bp (dark cyan), 430 bp (light cyan)—can mediate different HR-dependent deletions of the mutation rate marker *KlURA3* depending on its position. At 0.3 kb there are three possible deletions, only one of which leads to simultaneous deletion of the *HIS3* marker. At all other positions, there is only one possible deletion, and it always results in simultaneous deletion of the *HIS3* marker.

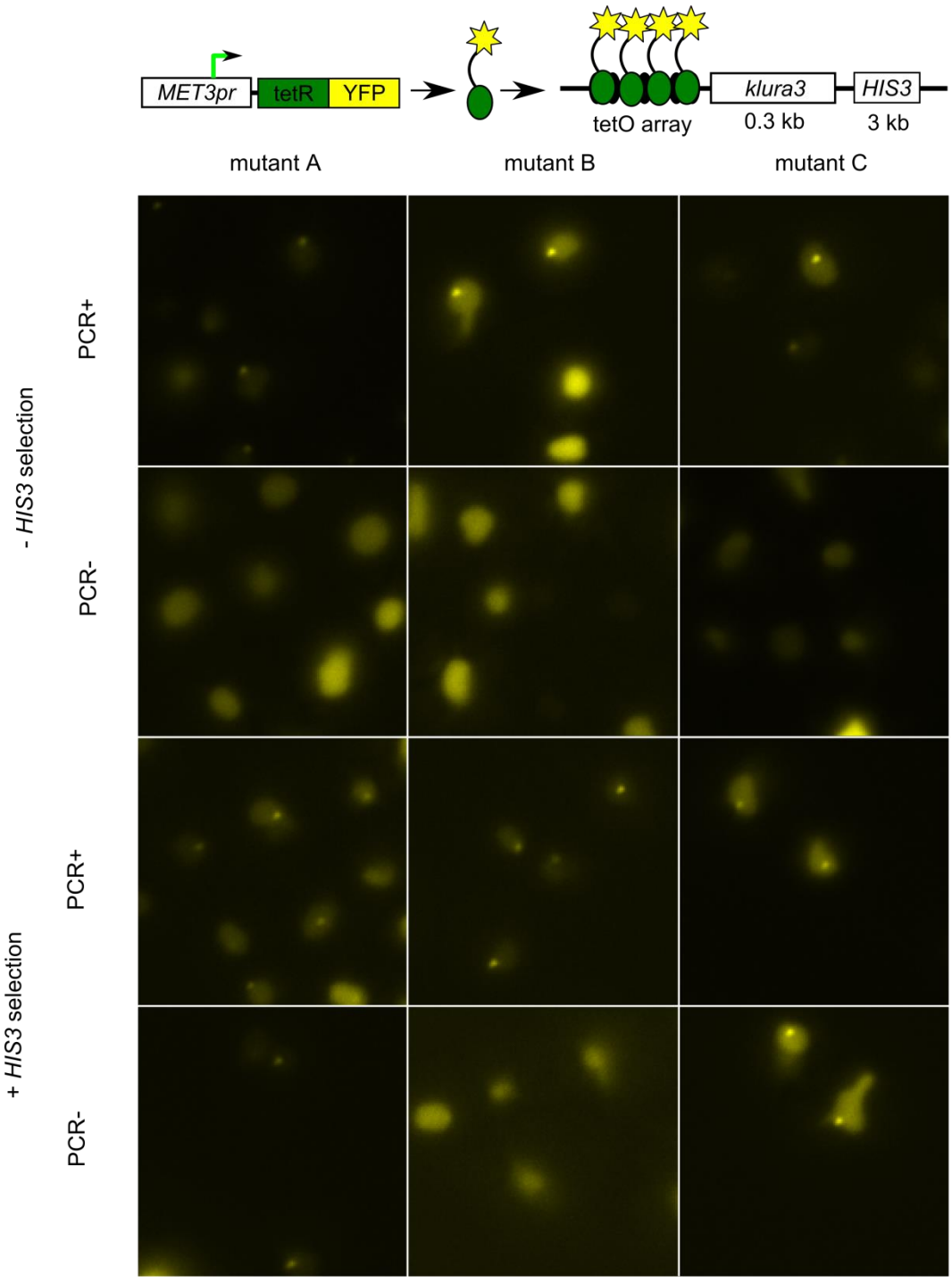

Figure S4: Localization of tetR-YFP and YFP foci observation confirms 240x tetO array presence in point mutants. Transformation of a plasmid delivering a methionine-inducible fusion of tetR to YFP shows that PCR+ mutants created in the absence of selection for *HIS3* retain the array while PCR- mutants do not. Under selection, all PCR+ and most PCR- mutants retain the array, consistent with a *KlURA3* deletion by repetitive homology that preserves the *HIS3* marker (see Fig. S4).

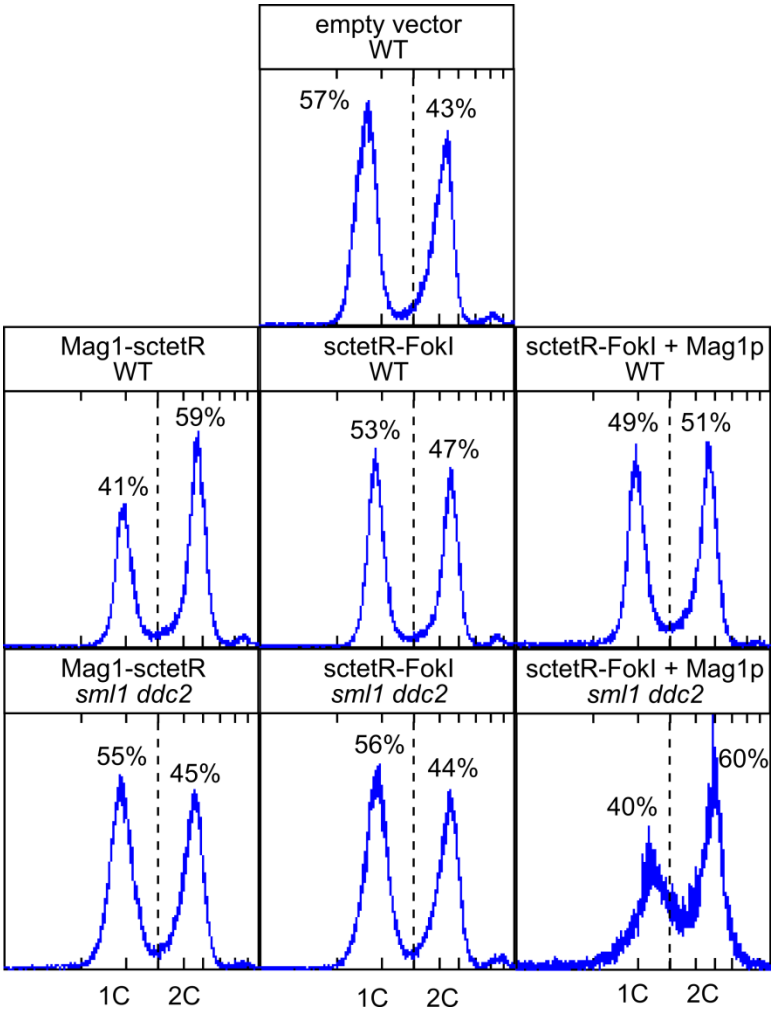

Figure S5: Cell cycle distributions show importance of DNA damage checkpoint activation in DSB repair fate. Compared to sctetR-FokI, Mag1-sctetR expression increases the fraction of cells with 2C DNA content as determined by flow cytometric analysis of exponentially growing cells stained with SYTOX green. This increase is indicative of the DNA damage checkpoint activation because it is eliminated in checkpoint-deficient (*sml1 ddc2*) strains. Co-expression of Mag1p causes increased checkpoint activation as compared to expression of sctetR-FokI alone. Checkpoint-deficient strains co-expressing Mag1p grow significantly slower than other strains, explaining why the number of cells with 2C DNA content increases in this case.

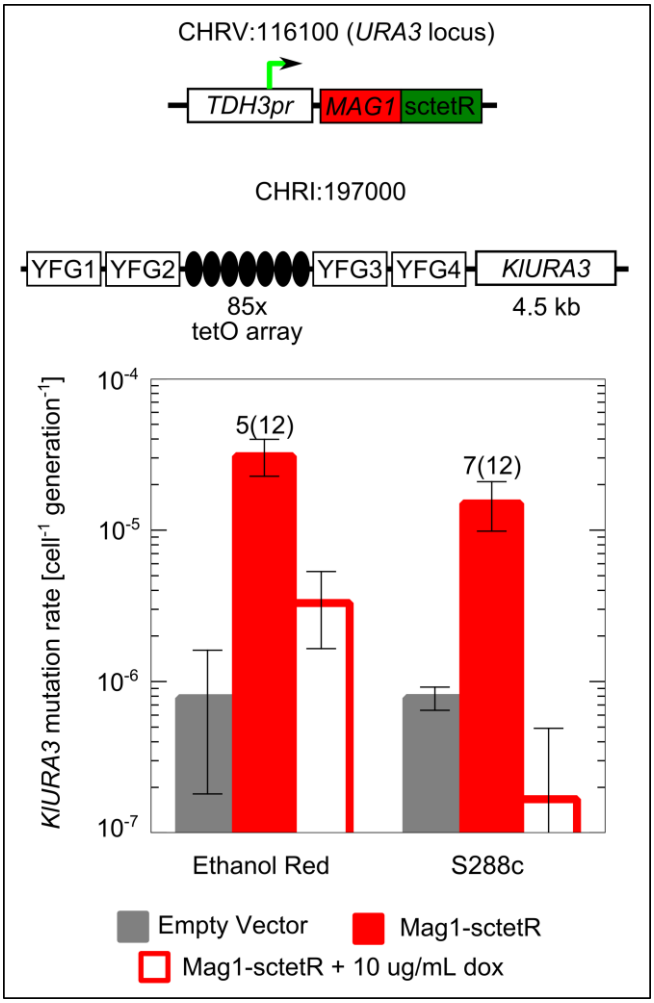

Figure S6: Deployment of TaGTEAM in application scenarios. Other strains and expression scenarios also support targeted point mutations by TaGTEAM. Constitutive expression of Mag1-sctetR from a strong, commonly used promoter allows targeted mutagenesis in a variety of carbon sources in prototrophic lab (S288c) and industrial (Ethanol Red) strains of yeast. The mutator and target genes were inserted without the addition of markers. Numbers refer to PCR+(total) mutants. Error bars represent 95% c.i.

Supplemental Tables

Table S1: Strain List

Name	Parent	Genetic change	Integrating plasmid/ PCR primers	Usage notes
NY0003	N/A	*MATa ade2-1 trp1-1 can1-100 leu2-3,112 his 3-11,5 ura3* GAL+	N/A	W303 base strain, confirmed to be *RAD5* using the protocol recommended by the SGD community wiki (http://wiki.yeastgenome.org/index.php/CommunityW303.html).
NY0339	NY0003	*can1-100Δ::KlURA3*	primers 1 and 2	
NY0343	NY0339	*klura3Δ::CAN1*	primers 3 and 4	
NY0378	NY0343	*apn1Δ::*KanR	primers 5 and 6	
NY0389	NY0343	CHRI197000::pNB0537	Integration of pNB0537	
NY0526	NY0389	CHRI180000::*KlURA3*	primers 7 and 8	centromeric side distance dependence
NY0542	NY0389	CHRI209000::*KlURA3*	primers 15 and 16	telomeric side distance dependence
NY0543	NY0389	CHRI213000::*KlURA3*	primers 17 and 18	telomeric side distance dependence
NY0544	NY0389	CHRI189000::*KlURA3*	primers 9 and 10	centromeric side distance dependence
NY0545	NY0389	CHRI192000::*KlURA3*	primers 11 and 12	centromeric side distance dependence
NY0554	NY0389	pNB0537::*KlURA3*	primers 13 and 14	240x array targeted mutagenesis test strain
NY0612	NY0339	*ade2-1Δ::CgTRP1*	primers 19 and 20	clean delete of entire *ade2* cassette
NY0619	NY0624	*his3-11,5::*pNB0603	Integration of pNB0603	plasmid targeted mutagenesis test strain
NY0620	NY0624	*his3-11,5::*pRS303	Integration of pRS303	plasmid targeted mutagenesis test strain empty vector control
NY0737	NY0544	*exo1Δ*::KanR	Primers 21 and 22	
NY0739	NY0554	*exo1Δ*::KanR	Primers 21 and 22	
NY0763	NY0343	CHRI197000::pNB0673	Integration of pNB0673	
NY0775	NY0544	*sgs1Δ::CgTRP1*	Primers 23 and 24	
NY0777	NY0554	*sgs1Δ::CgTRP1*	Primers 23 and 24	
NY0873	NY0763	pNB0673::*KlURA3*	primers 13 and 14	0x array test strain
NY0874	NY0389	CHRI118000::*KlURA3*	Primers 25 and 26	centromeric side distance dependence
NY0883	NY0554	*RAD52*::*RAD52*-CFP-KanR	Primers 27 and 28	240x array Rad52-CFP strain
NY0885	NY0873	*RAD52*::*RAD52*-CFP-KanR	Primers 27 and 28	no array Rad52-CFP strain
NY0894	NY0873	*rev3Δ::CgTRP1*	Primers 29 and 30	
NY0896	NY0873	*rad52Δ::CgTRP1*	Primers 31 and 32	
NY0901	NY0544	*rev3Δ::CgTRP1*	Primers 29 and 30	
NY0903	NY0544	*rad52Δ::CgTRP1*	Primers 31 and 32	
NY0909	NY0554	*rev3Δ::CgTRP1*	Primers 29 and 30	
NY0911	NY0554	*rad52Δ::CgTRP1*	Primers 31 and 32	
NY0923	NY0873	*exo1Δ*::KanR	Primers 21 and 22	
NY0924	NY0873	*sgs1Δ::CgTRP1*	Primers 23 and 24	
NY0927	NY0343	CHRI197000::pNB0775	Integration of pNB0775	85x no homology test strain
NY0931	NY0737	*sgs1Δ::CgTRP1*	Primers 23 and 24	

NY0932	NY0739	*sgs1Δ*::*CgTRP1*	Primers 23 and 24	
NY0951	NY0554	*sml1Δ*::*CgTRP1*	Primers 33 and 34	
NY0971	NY0951	*ddc2Δ*::KanR	Primers 35 and 36	
NY0973	N/A	Ethanol Red *MATA/α*	N/A	Kind gift of K. Verstrepen
NY0977	NY0973	Ethanol Red *MATα*	N/A	Sporulation of NY0973
NY1005	N/A	S288c	N/A	FY5 from Fink lab at MIT
NY1009	NY1005	*URA3*::*ura3*	Primers 37 and 38	
NY1010	NY1005	*ura3Δ*::*TDH3pr*-Mag1-sctetR	Primers 37 and 38	
NY1014	NY0977	*URA3*::*ura3*	Primers 37 and 38	
NY1015	NY0977	*ura3Δ*::*TDH3pr*-Mag1-sctetR	Primers 37 and 38	
NY1066	NY1009	CHRI197000::pNB0849	integration of pNB0849	S288c empty vector control strain
NY1068	NY1014	CHRI197000::pNB0849	integration of pNB0849	Ethanol Red empty vector control strain
NY1077	NY1010	CHRI197000::pNB0849	integration of pNB0849	S288c test strain
NY1113	NY1015	CHRI197000::pNB0849	integration of pNB0849	Ethanol Red test strain

- Yeast transformations were performed using the method in (48).
- *CgTRP1* refers to the copy of the *TRP1* gene from *Candida glabrata*, used here to prevent recombination at the native *TRP1* locus.
- All distances on chromosome I correspond to positions in the reference sequence (S288C background). W303 differs significantly in this region from the reference sequence, and primers were designed using the known W303 sequence (49).
(http://www.sanger.ac.uk/research/projects/genomeinformatics/sgrp.html). Distances were confirmed by PCR (primers 42, 48, and 105-108) from one position to the next.
- Clean delete means deletion of the promoter, ORF, and terminator of a gene so as to remove any possible homology for marker recombination during fluctuation analysis.

Table S2: Plasmid List

Name	Cloning Method	Backbone	Insert(s)	Insert PCR primers	Addgene deposited	Usage notes
Plasmids used						
pLAU44	Kind gift of D. Sherrat (50)					
pRS4D1	Kind gift of J. Collins (51)					
pCDG	Kind gift of B. Demple (32)					
pYES-MAG	Kind gift of L. Samson (29)					
pWH610(B+sB)	Kind gift of W. Hillen (30)					
Plasmids constructed and used						
pNB0298	Ligation	PRS415 (XhoI/BamHI)	*GAL1pr* (XhoI/BamHI)	64 and 65	no	p*GAL1pr*
pNB0435	Ligation	pNB0298 (SpeI/SacI)	NLS-CDG (SpeI/SacI)	66 and 67	no	
pNB0437	Ligation	pNB0298 (SpeI/SacI)	*MAG1* (SpeI/SacI)	68 and 69	no	
pNB0441	Ligation	pNB0435 (SalI/SacI)	*ACT1t*(SalI/SacI)	70 and 71	no	
pNB0443	Ligation	pNB0437 (SalI/SacI)	*ACT1t*(SalI/SacI)	70 and 71	no	
pNB0449	Ligation	pNB0441 (NgoMIV/XhoI)	none (blunted)	N/A	no	NLS-CDG
pNB0450	Ligation	pNB0443 (NgoMIV/XhoI)	none (blunted)	N/A	no	*MAG1*
pNB0451	Ligation	pRS4D1 (NotI/SacI)	none (blunted)	N/A	no	sctetR binding test by fluorescence knockdown
pNB0461	Gap repair	pNB0449 (SalI/NotI)	sctetR	72 and 73	no	NLS-CDG-sctetR
pNB0470	Gap repair	pNB0450 (SpeI/SalI)	sctetR	74 and 73	no	sctetR
pNB0471	Gap repair	pNB0450 (SalI/NotI)	vYFP	75 and 76	no	*MAG1*-vYFP
pNB0472	Gap repair	pNB0450 (SalI/NotI)	sctetR	77 and 73	yes	plasmid *MAG1*-sctetR
pNB0473	Gap repair	pNB0449 (SalI/NotI)	vYFP	78 and 76	no	NLS-CDG-vYFP
pNB0476	Gap repair	pNB0450 (SpeI/SalI)	vYFP	79 and 76	no	NLS-vYFP
pNB0602	Gap repair	pNB0450 (SalI/NotI)	sctetR-cYFP	91 and 90	no	*MAG1*-sctetR-cYFP
pNB0603	Ligation	PRS303 (XhoI/SacI)	pNB0298 (XhoI/SpeI) and pNB0472 (SpeI/SacI)	N/A	yes	integrated *MAG1*-sctetR
pNB0537	Ligation	pLAU44 (NotI/XbaI)	CHRI 5' homology (AscI/XbaI) and CHRI 3' homology (NotI/AscI)	93 to 96	yes	integrated 240x tetO array
pNB0568	Ligation	pBS (NotI/XbaI)	pNB0537 (Not1/XbaI)	N/A	no	
pNB0586	Ligation	pRS316 (XbaI/XhoI)	240x teto array (XbaI/XhoI)	N/A	yes	plasmid 240x tetO array
pNB0640	Ligation	pNB0586 (XhoI)	*ade2-1* cassette (XhoI)	97 and 98	no	plasmid 240x tetO array w/*ade2-1*
pNB0653	Ligation	pBS (ApaI/HindIII)	*KlURA3* cassette	99 and 100	no	
pNB0663	Ligation	pNB0450 (BamHI/SalI)	FokI (19) (BamHI/XhoI)	N/A	no	
pNB0665	Gap repair	pNB0663 (BamHI)	sctetR	88 and 103	yes	sctetR-FOKI
pNB0673	Ligation	pNB0537 (XhoI/XbaI)	none (blunted)	N/A	no	integrated 0x tetO array
pNB0763	Ligation	pBS (EcoRI/XmaI)	pNB0537 (EcoRI/XmaI)			
pNB0773	Ligation	pNB0763 (NotI/XbaI)	pNB0568 (NotI/XbaI)	N/A	no	
pNB0775	Ligation	pNB0773 (NgoMIV/HindIII)	pNB0653 (NgoMIV/HindIII)	N/A	yes	integrated 85x array w/o homology
pNB0784	Ligation	pNB0298 (XhoI/SacI)	pNB0298 (XhoI/AgeI) and	N/A	no	

			pNB0665 (AgeI/SacI)			
pNB0785	Gap repair	pNB0784 (XhoI)	*MAG1-ACT1t*	101 and 102	no	coexpression of sctetR-FOKI and *MAG1*
pNB0841	Ligation	pRS306 (NdeI/NcoI)	fragment of *URA3*	104 and 105	no	
pNB0843	Ligation	pNB0841 (NheI/AscI)	*TDH3pr*-Mag1-sctetR-*ACT1t*	106 and 107	yes	integration of *TDH3*pr-Mag1-sctetR to delete *URA3*
pNB0844	Ligation	pNB0298 (XhoI/SacI)	*TDH3pr* (XhoI/XbaI) and pNB0472 (SpeI/SacI)	N/A	no	centromeric plasmid with *TDH3*pr-Mag1-sctetR
pNB0849	Ligation	pNB0775	*GCN5* cassette (SpeI/PstI), SPT15 cassette (PstI/NheI), SPT3 cassette (EcoRI/BsiWI), *TAF12pr-TAF12-CYC1t* (BsiWI/HindIII)	108 to 117	yes	integration of 85x array with genes of interest and *KlURA3* at 4.5 kb, in this case genes are for gTME.
* Cassette means promoter, ORF, and terminator						

Table S3: Primer List

	Name	Sequence	Template
Integrating primers			
1	CgCan1KO(+)	tcttcagacttcttaactcctgtaaaaacaaaaaaaaaaaaaggcatagc CACAGGAAAC AGCTATGACC	*KlURA3* on pBluescript
2	CgCan1KO(-)	agaatgcgaaatggcgtggaaatgtgatcaaaggtaataaaacgtcatat GTTGTAAAAC GACGGCCAGT	"
3	CAN1insv2(+)	GGTTGCGAACAGAGTAAACCGAATCAGGG	*CAN1*
4	CAN1insv2(-)	GCTTCTACTCCGTCTGCTTTCTTTTCGGG	"
5	APN1KO-Kanv2(+)	ATGCCTTCGACACCTAGCTTTGTTAGATCTGCTGTCTCGAAATACAAATT GATCTGTTTAGCTTGCCTCGTCCC	pNB0132
6	APN1KO-Kanv2(-)	TTATTCTTTCTTAGTCTTCCTCTTCTTTGTCATTTGTGACAAGATATCAT AAACTGGATGGCGGCGGTTAG	"
7	URA-17kb(+)	GTTAGTTAGTTACTGTTAGGACGCTTCGGCGAGCTGATGTCTGACTTCTC CACTATAGGGCGAATTGGGTAC	*KlURA3* on pBluescript
8	URA-17kb(-)	TTACGGCCATTATCAGCGGTAAAACACCCAAGGTGTTGACTAAGTGATGG AAAGGGAACAAAAGCTGGAGC	"
9	URA-8kb(+)	agatttccaagcaagcttttagtggaaatcatcgcgcgcaagccagcggt CACTATAGGGCGAATTGGGTAC	*KlURA3* on pBluescript
10	URA-8kb(-)	TCCGCACGTCCTACGTTTAGAAAGTAACGATGCCAATCTTCATCACGGTA AAAGGGAACAAAAGCTGGAGC	"
11	URA-5kb(+)	TTTGGAAGTGACTGGCGCCGCCGCTGGCTACTATAATAGCAGCGACTGTA CACTATAGGGCGAATTGGGTAC	*KlURA3* on pBluescript
12	URA-5kb(-)	TTGGTGCACGTTCGCTCGGCGAGTAAAAGAGGTAATCCAAACGACGGGAT AAAGGGAACAAAAGCTGGAGC	"
13	URAsfm(+)	actgcccgctttccagtcgggaaacctgtcgtgccagctgcattaatgaa CACTATAGGGCGAATTGGGTAC	*KlURA3* on pBluescript
14	URAsfm(-)	GCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTA AAAGGGAACAAAAGCTGGAGC	"
15	URA3kbv3(+)	atcgaaataaaatgctgtatcacgggcgattattccatggcgaaatgagg CACTATAGGGCGAATTGGGTAC	*KlURA3* on pBluescript
16	URA3kbv4(-)	GGTGTTAGATACGGATGTGAAAGGGCGATAAGACATTTGGAAGTTAATGA AAAGGGAACAAAAGCTGGAGC	"
17	URA11kbv2(+)	gcagtctttacacttctggcactaattaatgtggcctcaggagccacaga CACTATAGGGCGAATTGGGTAC	*KlURA3* on pBluescript
18	URA11kb(-)	GAATACTGGTAAAAATTTATATTCATCCCACTTTTCCTCTGGCCTGCTGG AAAGGGAACAAAAGCTGGAGC	"
19	CgKO-ADE2(+)	gcgcactaccagtatatcatctcatttccgtaaataccaaatgtattata CACAGGAAAC AGCTATGACC	*CgTRP1* on pBluescript
20	CgKO-ADE2(-)	ATTGAGCCGCCTTATATGAACTGTATCGAAACGTTATTTTTTTAATCGCA GTTGTAAAAC GACGGCCAGT	"
21	PrKO-EXO1(+)	ATGGGTATCC AAGGTCTTCT TCCTCAGTTA AAGCCCATAC AGAATCCAGT GATCTGTTTAGCTTGCCTCGTCCC	pNB0132
22	PrKO-EXO1(-)	TTTATAAACAAATTGGGAAAGCAAGGAGATAGATCTGACTGCCGGCCGAG AAACTGGATGGCGGCGGTTAG	"
23	CgKO-SGS1(+)	ATGGTGACGA AGCCGTCACA TAACTTAAGA AGGGAGCACA AATGGTTAAA CACAGGAAAC AGCTATGACC	*CgTRP1* on pBluescript
24	CgKO-SGS1(-)	TCACTTTCTTCCTCTGTAGTGACCTCGGTAATTTCTAAAACCTCGTCTCC GTTGTAAAAC GACGGCCAGT	"
25	URA75kb(+)	ATAGCTAGGT AATTTTAATC TGGGGAGAGA AATGGTGAAC TTTTTTCAAT CACTATAGGGCGAATTGGGTAC	*KlURA3* on pBluescript
26	URA75kb(-)	CTGAAATTGAAGCAGCACCACAAGATATCAATCAACAACCGAATCAATAA AAAGGGAACAAAAGCTGGAGC	"
27	r52-FPfuse(+)	GAGAAGTTGGAAGACCAAAGATCAATCCCCTGCATGCACGCAAGCCTACT TCTAAAGGTGAAGAATTATTCACTGG	pNB0263

28	r52-FPfuse(-)	AGTAATAAATAATGATGCAAATTTTTTATTTGTTTCGGCCAGGAAGCGTT TTAGTATCGAATCGACAGCAG	"
29	REV3KO(+)	ATGTCGAGGG AGTCGAACGA CACAATACAG AGCGATACGG TTAGATCATC CACAGGAAAC AGCTATGACC	*CgTRP1* on pBluescript
30	REV3KO(-)	TTTGAACAGATTGATTATCTCTCAAGTATCTTTCTGCTTTGACACGAGAG GTTGTAAAAC GACGGCCAGT	"
31	RAD52KO(+)	GGAGGTTGCC AAGAACTGCT GAAGGTTCTG GTGGCTTTGG TGTGTTGTTG CACAGGAAAC AGCTATGACC	*CgTRP1* on pBluescript
32	RAD52KO(-)	AGTAATAAATAATGATGCAAATTTTTTATTTGTTTCGGCCAGGAAGCGTT GTTGTAAAAC GACGGCCAGT	"
33	CgKO-sml1(+)	GATCTTACGG TCTCACTAAC CTCTCTTCAA CTGCTCAATA ATTTCCCGCT CACAGGAAAC AGCTATGACC	*CgTRP1* on pBluescript
34	CgKO-sml1(-)	CAGAACTAGTGGGAAATGGAAAGAGAAAAGAAAAGAGTATGAAAGGAACT GTTGTAAAAC GACGGCCAGT	"
35	PrKO-ddc2(+)	CACGAAACGT CAACACAATC ATCAAACTCT TTTGCATATT TCTATTATAG GATCTGTTTAGCTTGCCTCGTCCC	pNB0132
36	PrKO-ddc2(-)	TCTTTCCTAAAACGAAAATAATATAAATTATATATAGTTAATATTAAGCA AAACTGGATGGCGGCGGTTAG	"
37	U3KO(+)	GGAGCACAGACTTAGATTGG	pNB0841 or pNB0843
38	U3KO(-)	CTTTGTCGCTCTTCGCAATGTC	"
check primers			
39	Cgchk(-)	GGTCATAGCTGTTTCCTGTG	changes marked with *KlURA3* or *CgTRP1*
40	apn1KOchk(+)	GCGGC CAAGAAGGAA CCGATTCACG	deletion of *APN1*
41	met25pchk(-)	CGAGGCAAGCTAAACAGATC	changes marked with KanR
42	URA197chk(-)	GTACCCAATTCGCCCTATAGTG	*KlURA3* insertions
43	URA17kbchk(+)	GACTGGGAAGTTCTGTCGTAG	*KlURA3* at -17kb
44	URA8kbchk(+)	CTCAGGAAAATTACTGGCGAAGG	*KlURA3* at -8kb
45	URA5kbchk(+)	CGCATCTTCAAACGGCAGCAAG	*KlURA3* at -5kb
46	URAsfmchk(+)	cccagcttttgttccctttagtg	*KlURA3* inside pNB0537
47	URA3kbchkv2(+)	GTCATTGAGATATGATAGCCTGTTCC	*KlURA3* at 11kb
48	URA197chk(+)	GCTCCAGCTTTTGTTCCCTTT	*KlURA3* insertions
49	URA11kbchkV2(-)	ATGTGCCTGATGAACTAACACAAGG	*KlURA3* at 15kb
50	URA0kbckv2(+)	TTCGAAAGCTCTATCATATGGC	*KlURA3* at CHRI197000
51	ADE2KOchk(+)	CGCATCTGTTCCTCTATCTTC	deletion of *ade2-1*
52	CAN1KOchk(+)	gcttagcatttgccgttgg	deletion of *can1-100*
53	RAD52KOchk(+)	ACTAAATGGTTGAATCGGGTC	deletion of *RAD52*
54	CHRIinschV2(+)	TTCACTACACCTCGGACATGGATTTG	integration of pNB0537 and pNB0639
55	CHRIinschk(-)	CCCTATCAGTGATAGAGAGACGGACG	integration of pNB0537
56	URA75kbchk(+)	GAGGAAAAGATTCATCAACTGGC	*KlURA3* at -82kb
57	PrKO-EXO1chk(+)	CTGAGGTTGACTACTACGAGC	Deletion of *EXO1*
58	CgKO-SGS1chk(+)	GAAATGCGAAATGTGAAGGAAGAG	Deletion of *SGS1*
59	REV3KOchk(+)	GACGAGTGCAGTGCGTCTAG	Deletion of *REV3*
60	sml1kochk(+)	ATGTTTAGACCTCGTACATAGG	Deletion of *SML1*
61	ddc2kochk(+)	AAGAGTCAGACAGGCTCGC	Deletion of *DDC2*
62	U3KOchk(+)	TGCGAGGCATATTTATGGTGAAG	Deletion of *URA3* with *TDH3pr*-Mag1-sctetR
63	U3KOGPDchk(-)	GGCAGTATTGATAATGATAAACTCG	"
plasmid construction primers			
64	XhoI-GAL1(+)	GCGGCCTCGAGCAAAAATTCTTACTT	*GAL1*pr
65	BamHI-GAL1(-)	GCGGCGGATCCGTTTTTTCTCCTTGACG	"
66	SpeI-CDG(+)	ccgcgactagtaacaaa ATGCCGAAAAAAAAACGCAAAGTG TTTGGAGAGAGCTGGAAGAAGC	CDG
67	SacI-CDG(-)	atattgagctcgttcatgtgcggcgcctaagttctgtcgacttatta CAGCTCCTTCCAGTCAATGG	"
68	SpeI-MAG(+)	ccgcgactagtaacaaa ATGAAACTAAAAAGGGAGTATGATG	*MAG1*

69	SacI-MAG(-)	atattgagctcgttcatgtgcggcgcctaagttctgtcgactta TTAGGATTTCACGAAATTTTCTTC	"
70	SalI-ACT1UTR(+)	ataatgtcgacgttcatgtgcggccgc TCTGCTTTTGTGCGCGTATG	*ACT1t*
71	SacI-ACT1UTR(-)	cggcggagctc AATTTTTGAAATTTTCGTAGAAAAGGG	"
72	CDG-(sc)tetR(+)	GGCAAGAAGC CCATTGACTG GAAGGAGCTG GTC GAC GGT GCT GGT TTA ATT AAC tctagattagataaaagtaaag	sctetR
73	MUT-(sc)tetR(-)	GGTACATACATAAACATACGCGCACAAAAGCAGA ttatta GTCGCCGCTTTCGCACTTTAG	"
74	sctetR-GAL(+)	ATACTTTAAC GTCAAGGAGA AAAAACTATA AACAAA ATGCCGAAAAAAAAAACGCAAAGTG tctagattagataaaagtaaag	"
75	MAG-YFP(+)	AT GAAGGCAGAA GAAAATTTCG TGAAATCC GTC GAC GGT GCT GGT TTA ATT AAC TCTAAAGGTGAAGAATTATTCACTGG	vYFP
76	ACT1t-YFP(-)	GGTACATACATAAACATACGCGCACAAAAGCAGA TTATTA TTTGTACAATTCATCCATACCATGG	"
77	MAG-(sc)tetR(+)	AT GAAGGCAGAA GAAAATTTCG TGAAATCC GTC GAC GGT GCT GGT TTA ATT AAC tctagattagataaaagtaaag	sctetR
78	CDG-YFP(+)	GGCAAGAAGC CCATTGACTG GAAGGAGCTG GTC GAC GGT GCT GGT TTA ATT AAC TCTAAAGGTGAAGAATTATTCACTGG	vYFP
79	Gal-YFP(+)	ATACTTTAAC GTCAAGGAGA AAAAACTATA AACAAA ATGCCGAAAAAAAAAACGCAAAGTG TCTAAAGGTGAAGAATTATTCACTGG	"
80	AscI-chr1up(+)	tatgcggcggcgcgcc ATTTTGACATATACTGATATGGACCTC	CHRI:197000 5' homology
81	XbaI-chr1up(-)	gcggctctaga TTCAGATATGAGGCCATAAATGGAG	"
82	Not1-chr1ins25(+)	GTGGT GCGGCCGC TTTCAAGTAG TTCACAAAGA	CHRI:197000 3' homology
83	Asc1-chr1ins23(-)	ATAAT GGCGCGCC CAATCGCTGG GAATGAGCAA	"
84	XhoIA-ade2(+)	gaggactcgagcctagg AAGCTTTTGACCAGGTTATTATAAAAG	*ade2-1* cassette
85	XhoI-ade2(-)	gaggactcgag CAGGTAATTATTCCTTGCTTCTTG	"
86	ApaI-KlU(+)	tatta gggccc ggagacaatc	*KlURA3* on pBluescript
87	Hind3-KlU(-)	gagga aagctt GCTTATCGCAATGGTTGTAATGG	"
88	GAL10-MAG1(+)	TGATTATTAAACTTCTTTGCGTCCATCCAAAAAAAAAGTAAGAATTTTTG gctagc aacaaa ATGAAACTAAAAAGGGAGTATGATG	*MAG1*
89	pBS-ACT1(-)	tgcgcaactgttgggaagggcgatcggtgcgggcctcttcgctattacgc cccggg AATTTTTGAAATTTTCGTAGAAAAGGG	"
90	sctetR-FOKI(-)	CTTCTCCTCCAGCTCGCTCTTCACCAGCTG GTTAATTAAACCAGCACCGTCGAC GTCGCCGCTTTCGCACTTTAG	sctetR
91	NdeI-U3f(+)	gagga catatg gcggccgc TAGTGTTGAAGAAACATGAAATTGCC	pRS316
92	NcoI-U3f(-)	gagga CCATGG GGCGCGCC actagt GCTAGC ATAACTTCGTATAATGTATGCTATACGAAGTTAT AAAAATCAGTCAAGATATCCAC	"
93	NheI-GPDMS(+)	gagga gctagc CGAGTTTATCATTATCAATACTGCC	pNB0844
94	PciI-GPDMS(-)	gagga acatgt ggcgcgcc ATAACTTCGTATAATGTATGCTATACGAAGTTAT AATTTTTGAAATTTTCGTAGAAAAGGG	"
95	SpeIGCN5(+)	agaag actagt tcttaaacacttatgggcagc	genomic DNA
96	PstIGCN5(-)	gcggc CTGCAG ATATAGTTACATAAAGGTAAATACCAACG	"
97	PstISPT15(+)	gagga ctgcag gaatttgtacttctttcgaaatcg	"
98	NheISPT15(-)	GCGGC gctagc ttaattaa ATAAACATCTTATTATAAAACATTGATATATAAATATAG	"
99	EcoRISPT3(+)	gagga gaattc GATGTTCGGTTACATGTCTTAG	"
100	BsiWISPT3(-)	gagga cgtacg cacgcaatttttttaatcactgagttc	"
101	BsiWITAF12(+)	gagga cgtacg GTTCTCTCGTTGATACTTTTAGCC	"
102	Hind3TAF12(-)	cgccg aagctt ggtcat gctagc ttatttttttgtattcaacgat gcaacattgtttccattgttttttg	"
103	NheICYC1t(+)	gagga gctagc CATGTAATTAGTTATGTCACGC	"
104	Hind3CYC1t(-)	GCGGC aagctt TAAAGCCTTCGAGCGTCCC	"
primers to confirm distances of markers from the 240x array on the telomeric side			
105	46451(+)	tggtcaactcaacgattcttagg	genomic DNA
106	46241(-)	CACTATAGCTTGCTGTATGTCTC	"
107	42459(+)	GAGAAATTGGCTACTTAGGAAGAG	"
108	42393(-)	GCTGAATACGATATGGACTAGAG	"

sequencing primers			
109	KlU-seq1(+)	cgttcatggtgacacttttagc	“
110	KlU-seq2(+)	CATCAAATGGTGGTTATTCGTGG	“
111	KlU-seq1(-)	GTAAGATGAAGTTGAAGTAGTGTTGC	“
112	KlU-seq2(-)	CTCTTTTTCGATGATGTAGTTTCTGG	“
* Cassette means promoter, ORF, and terminator			