a

1) 14 z-stacks

2) single frame z-project

3) n time points → single particle tracking, **MSD**

4) projection around t-axis → kymograph over acquisition time

0 → n

b

t-ura4<ori, Rtf1 ON

5 µm

a b

3 6 9 12 15

18 21 24 27 30

a b

3 6 9 12 15

18 21 24 27 30

c

0 → 30   0 → 30 Time in min

a   b

5 µm

← estimated colocalization time

d

RFB ON   RFB OFF   no RFB

0 → 30   0 → 30   0 → 30 Time in min

1   5 µm   — 1 min

2

3

4

5

6

7

8

9

10

Supplementary Figure 1: Acquisition and analysis of kymographs over time (related to Fig. 1).

a Scheme of time-lapse movies acquisition and processing. (1) 14-optical slices collected over chosen time frame. (2) z-projection at each time interval. (3) 2D movie of n frames which can be subjected to single particle tracking. (4) projection over time-axis movie from point (3) generation of kymograph over acquisition time.

b Example of a 30 min processed 2D movie mounted to investigate co-localization between the *LacO*-marked RFB (red) and the nuclear periphery (Npp106-GFP).

c Example of co-localization time estimation based on kymographs constructed from movies obtained from b. Briefly, every 10 s 3D-image stacks were collected for GFP and mCherry channels. After generating a projection around the z-axis, subsequently all frames of the resulting 2D-movie were projected horizontally over the time-axis to build a kymograph for each fluorescent channel (see panel

d Kymographs used to assign co-localization time in indicated conditions. Numbers correspond cell # presented on Fig. 1f.

Supplementary Figure 2: Analysis of RFB relocation in G2 cells (related to Fig. 2, 4, 5 and 6).

Co-localization events in G2-phase in indicated conditions and strains, as described on Fig. 1 b-c. Two-hundred cells were analyzed for each strain and condition.
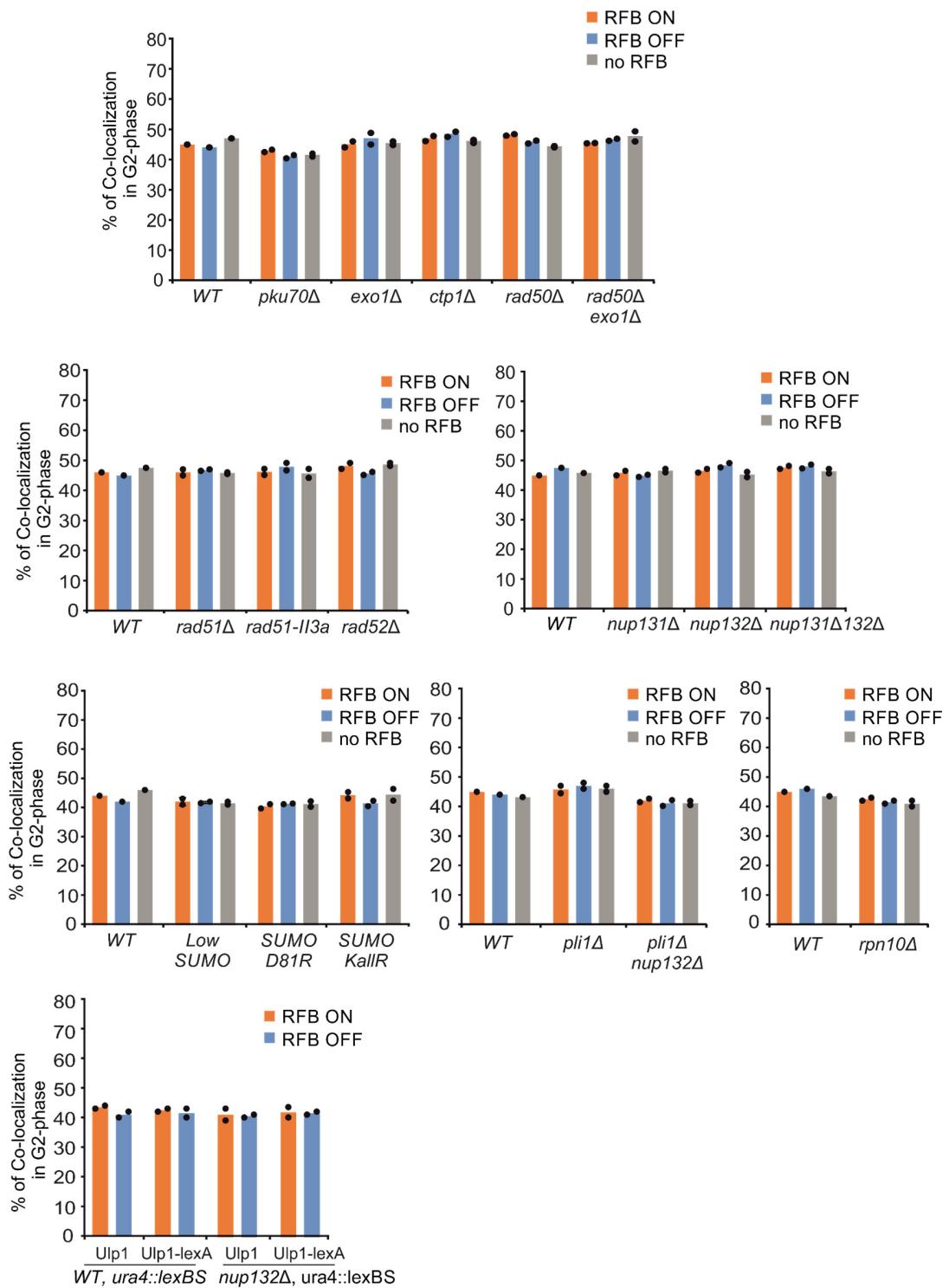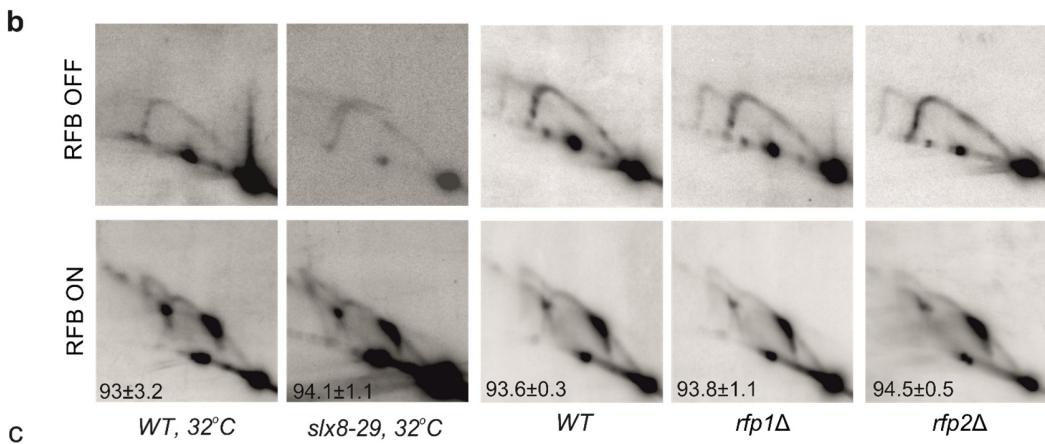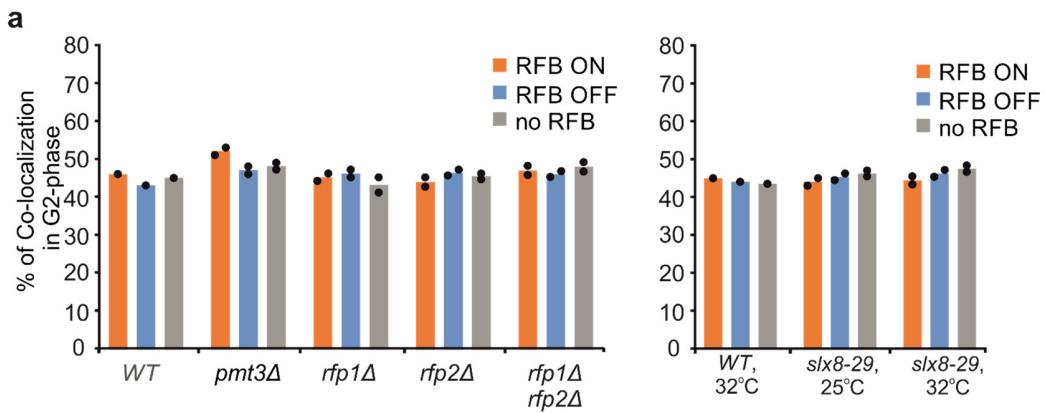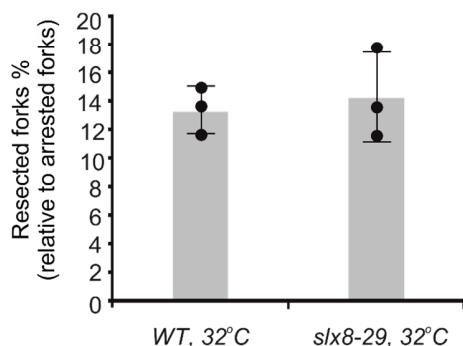
Supplementary Figure 3: Requirement of SUMO and STUBL in RDR and RFB relocation (related to Fig. 3).

a Co-localization events in in G2-phase cells in indicated conditions and strains. The *slx8-29* strains were grown at permissive (25°C) and restrictive (32°C) temperature. Dots represent values obtained from two independent biological experiments. For each set of data, *WT* strain was analyzed alongside. Two-hundred cells were analyzed for each strain and condition.

b Representative RI analysis in indicated strains and conditions as described on Fig. 3. The *ura4* gene was used as probe. Numbers indicate the % of forks blocked by the RFB ± SD.

c Quantification of resected forks. Values are means of 3 independent biological replicates ± SD.

a

5 µm

nup131Δ   *nup132Δ*   *nup131Δ132Δ*

b

DIC

GFP

merge

5 µm

WT   *nup131Δ132Δ*

d

% of mRNA export defect

WT *nup131Δ* *nup132Δ* *nup131Δ132Δ* *pli1Δ132Δ* *WT, 42°C*

c

nuclear/cytoplasm signal (a.u.)

n = 40 n = 40   n = 40 n = 40

RFB: ON OFF   ON OFF

WT   *nup131Δ132Δ*

e

polyA

DAPI

5 µm

WT   *nup131Δ*   *nup132Δ*   *nup131Δ132Δ*   *pli1Δ nup132Δ*   *WT, 42°C*
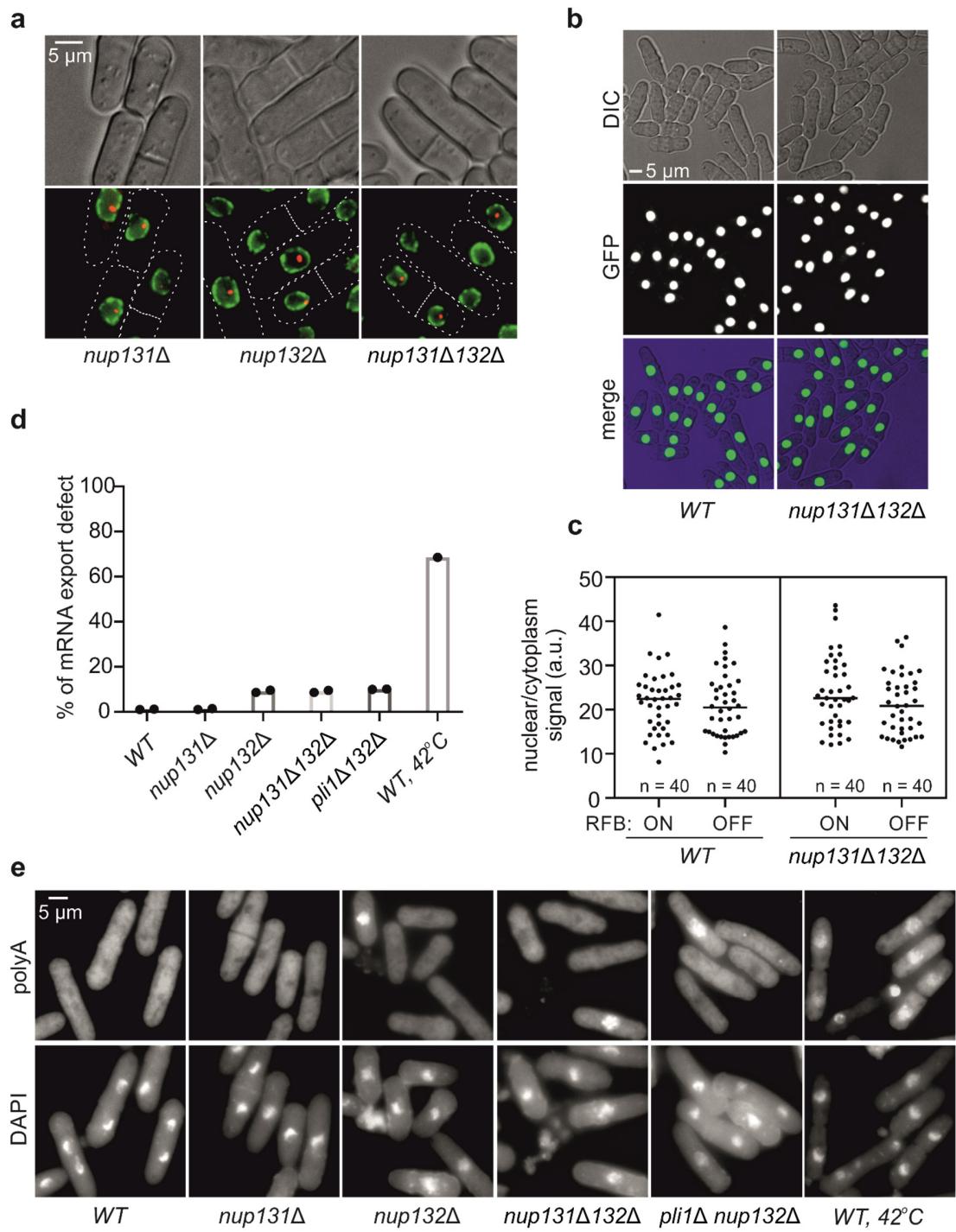
Supplementary Figure 4: Macromolecular transport in the absence of Nup132 and Nup131 (related to Fig. 4).

a Representative images of the nuclear periphery stained by Npp106-GFP (Green) and of the *LacO*-marked RFB (red) in indicated strains.

b Protein import-export was examined in the strains expressing stably LacI-NLS-GFP without *LacO* arrays integrated into the genome. Representative images obtained for *WT* and *nup131Δ nup132Δ* are shown.

c Quantification of panel b. Fluorescence intensity was measured in the nucleus and cytoplasm using ImageJ on constant volume squares to calculate ratio nuclear/cytoplasmic (N/C) signal. Each dot represents single cell ratio N/C. Bars show median N/C ratio for each group of 40 cells (RFB OFF, RFB ON).

d Percentage of cells showing mRNA export defects, as scored by the nuclear accumulation of the polyA signal in the indicated strains. *WT* heat shocked at $42^{o}C$ for 30 min in water-bath was used as a positive control for mRNA export defect in one experiment. At least 200 cells were analyzed for each strain and condition. Dots correspond to values obtained from two independent biological replicates.

e Representative images, used for panel d, of subcellular localization of poly(A)+RNA examined by FISH with Cy3-oligo-dT$_{50}$ probe in indicated strains. DAPI staining shows the position of nucleus within each cell.
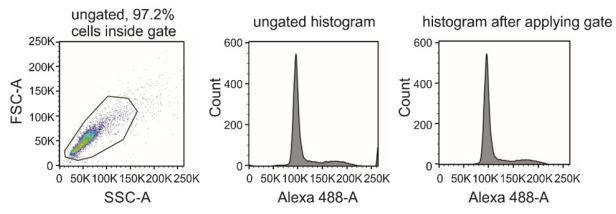
General gating strategy utilized in flow cytometry experiments to analyze DNA content in cells

a
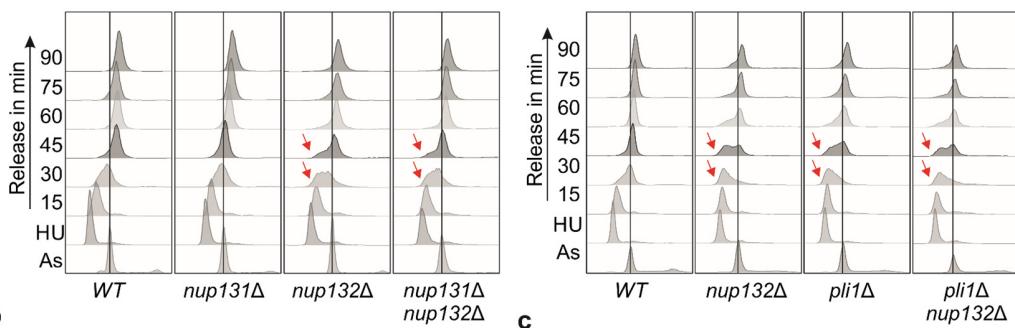
b   **c**
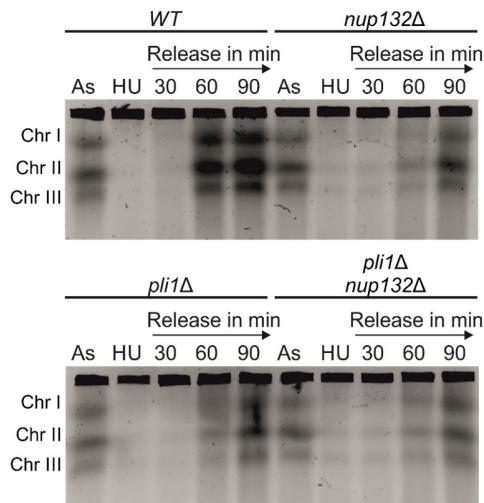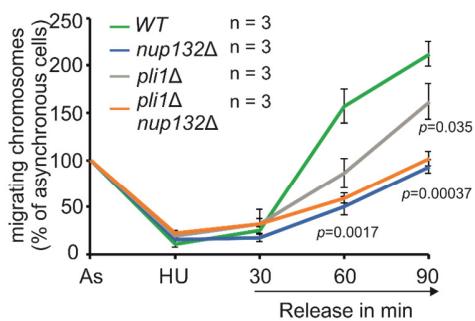
Supplementary Figure 5: Nup132 and Pli1 promote recovery from HU-stalled forks (related to Fig. 4, 5 and 7).

a Flow cytometry analysis of indicated strains in indicated conditions. Logarithmically growing cells were exposed to 20 mM HU for 4 hours and then released into fresh, HU-free, rich medium YES at 30°C to monitor S-phase progression at indicated time after release.

b Analysis of chromosome by pulse field gel electrophoresis (PFGE) in the above mentioned conditions. Representative images of chromosomes migration during PFGE in indicated strains and conditions.

c Quantification of % of chromosomes migrating into the gel after release from HU block. *p* value was calculated by two-sided t-test.

a *t>ura4<ori*

RFB OFF or ON

5-FOA^R colonies

PCR screening:
ura4 deletion
ura4 mutation
Translocation

b main replication direction

Chr II

Chr III

main replication direction

c *t>ura4<ori*

RFB OFF — RFB ON

wt

nup131Δ

nup132Δ

nup131Δ132Δ

d

Mutagenesis rate	*ura4* deletion rate	Translocation rate
RFB OFF / RFB ON	RFB OFF / RFB ON	RFB OFF / RFB ON

Mutagenesis rate panel:
event/cell/division×10^-7

p=0.0054
p=0.019
p=0.0069

WT   nup131Δ   nup132Δ   nup131Δ nup132Δ

Supplementary Figure 6: Role of Nup131 and Nup132 in RFB-induced genome instability (related to Fig 4).

a Diagram of the *t>ura4<ori* construct contacting two *RTS1*-RFB (purple bars) in inverted repeat orientation at both side of the *ura4⁺* gene (in green). After several generations upon fork arrest (RFB ON) or not (RFB OFF), the genetic loss of *ura4⁺* was selected on 5FOA-containing media. Independent 5FOA^R colonies were analyzed by PCR to identify *ura4* deletion, *ura4* mutation and translocation.

b Diagram of the *t>ura4<ori* construct containing two *RTS1*-RFB (purple bars) in inverted repeat orientation at both sides of the *ura4⁺* gene (in green) on chromosome III and of the chromosome II harboring the *RTS1* sequence at its natural location, near the *mat1* locus. Primers used for amplifying 1 kb *ura4* fragment and the 650 bp *rng3* fragment are represented in green and red, respectively. Primers used for amplifying translocation junction (1.2 kb) are depicted in orange on chromosome II (TL II) and black on chromosome III (TL III).

c Example of PCR-based screening to detect deletion, mutation and translocation events in indicated strains and conditions. The *rng3* PCR product was used as a control of positive PCR amplification together with the TLII-TLIII primers to detect translocation junctions. Strains harboring a deletion of the *ura4* gene (*ura4-D18*), or wild type *ura4⁺*, or stable translocations were used as controls of PCR amplification.

d Rate of mutagenesis (left panel), deletion (middle) and translocation (right) in indicated strains and conditions. Each dot correspond to one value from 21 independent cultures. Values are median ± 95 % confidence interval. *p* value was calculated using the two-sided Mann & Whitney U test.

Table S1: Strains used in this study

Strain number	Mating type	Genotype	Reference
SL1524	h-	npp106-GFP:Nat arg3::mCherry-LacI nmt41:rtf1:sup35 ade6-704 leu1-32 t-LacO 7,9Kb:Kan:ura4$^+$<ori (uraR)	this study
KK47	h-	npp106-GFP:Nat arg3::mCherry-LacI nmt41:rtf1:sup35 ade6-704 leu1-32 t-LacO 7,9Kb:Kan:ura4$^+$-ori (ura4+, no RTS1)	this study
AS90	h-	npp106-GFP:Nat arg3::psv40:GFP-LacI nmt41:rtf1:sup35 ade6-704 leu1-32 LacO7.9Kb:Kan:ura4+<ori	this study
KK434	h-	npp106-GFP:Nat nmt41:rtf1:sup35 ade6-704 leu1-32 t-ura4-SD20<ori (uraR)	this study
KK327	h+	man1-GFP:Nat nmt41:rtf1:sup35 ade6-704 leu1-32 t-ura4-SD20<ori (uraR)	this study
KK481	h-	sad1-GFP nmt41:rtf1:sup35 ade6-704 leu1-32 t-ura4-SD20<ori (uraR)	this study
KK1296	h-	pku70::Hygro npp106-GFP:Nat arg3::mCherry-LacI nmt41:rtf1:sup35 ade6-704 leu1-32 t-LacO 7,9Kb:Kan:ura4$^+$<ori (uraR)	this study
KK1313	h+	pku70::Hygro npp106-GFP:Nat arg3::mCherry-LacI nmt41:rtf1:sup35 ade6-704 leu1-32 t-LacO 7,9Kb:Kan:ura4$^+$-ori (ura4, no RTS1)	this study
KK43	h+	exo1::Nat npp106-GFP:Nat arg3::mCherry-LacI nmt41:rtf1:sup35 ade6-704 leu1-32 t-LacO 7,9Kb:Kan:ura4$^+$<ori (uraR)	this study
KK85	h-	exo1::Nat npp106-GFP:Nat arg3::mCherry-LacI nmt41:rtf1:sup35 ade6-704 leu1-32 t-LacO 7,9Kb:Kan:ura4$^+$-ori (ura4, no RTS1)	this study
KK507	h+	ctp1::Hygro npp106-GFP:Nat arg3::mCherry-LacI nmt41:rtf1:sup35 ade6-704 leu1-32 t-LacO 7,9Kb:Kan:ura4$^+$<ori (uraR)	this study
KK509	h+	ctp1::Hygro npp106-GFP:Nat arg3::mCherry-LacI nmt41:rtf1:sup35 ade6-704 leu1-32 t-LacO 7,9Kb:Kan:ura4$^+$-ori (ura4, no RTS1)	this study
KK143	h-	rad50::Kan npp106-GFP:Nat arg3::mCherry-LacI nmt41:rtf1:sup35 ade6-704 leu1-32 t-LacO 7,9Kb:Kan:ura4$^+$<ori (uraR)	this study
KK144	h+	rad50::Kan npp106-GFP:Nat arg3::mCherry-LacI nmt41:rtf1:sup35 ade6-704 leu1-32 t-LacO 7,9Kb:Kan:ura4$^+$-ori (ura4, no RTS1)	this study
KK147	h-	exo1::Nat rad50::Kan npp106-GFP:Nat arg3::mCherry-LacI nmt41:rtf1:sup35 ade6-704 leu1-32 t-LacO 7,9Kb:Kan:ura4$^+$<ori (uraR)	this study
KK150	h+	exo1::Nat rad50::Kan npp106-GFP:Nat arg3::mCherry-LacI nmt41:rtf1:sup35 ade6-704 leu1-32 t-LacO 7,9Kb:Kan:ura4$^+$-ori (ura4, no RTS1)	this study
KK170	h+	rad51::Kan npp106-GFP:Nat arg3::mCherry-LacI nmt41:rtf1:sup35 ade6-704 leu1-32 t-LacO 7,9Kb:Kan:ura4$^+$<ori (uraR)	this study
KK172	h-	rad51::Kan npp106-GFP:Nat arg3::mCherry-LacI nmt41:rtf1:sup35 ade6-704 leu1-32 t-LacO 7,9Kb:Kan:ura4$^+$-ori (ura4, no RTS1)	this study
KK526	h-	loxP:rad51 R152A-R324A- K334A:LoxM3 (rad51-3A) npp106-GFP:Nat arg3::mCherry-LacI nmt41:rtf1:sup35 ade6-704 leu1-32 t-LacO 7,9Kb:Kan:ura4$^+$<ori (uraR)	this study
KK529	h+	loxP:rad51 R152A-R324A- K334A:LoxM3 (rad51-3A) npp106-GFP:Nat arg3::mCherry-LacI nmt41:rtf1:sup35 ade6-704 leu1-32 t-LacO 7,9Kb:Kan:ura4$^+$-ori (ura4, no RTS1)	this study

KK59	*h-*	*rad52::Kan npp106-GFP:Nat arg3::mCherry-LacI nmt41:rtf1:sup35 ade6-704 leu1-32 t-LacO 7,9Kb:Kan:ura4$^+$<ori (uraR)*	this study
KK58	*h-*	*rad52::Kan npp106-GFP:Nat arg3::mCherry-LacI nmt41:rtf1:sup35 ade6-704 leu1-32 t-LacO 7,9Kb:Kan:ura4$^+$-ori (ura4, no RTS1)*	this study
KK1281	*h-*	*pku70::Hygro npp106-GFP:Nat nmt41:rtf1:sup35 ade6-704 leu1-32 t-ura4-SD20<ori (uraR)*	this study
KK1292	*h-*	*exo1::Nat npp106-GFP:Nat nmt41:rtf1:sup35 ade6-704 leu1-32 t-ura4-SD20<ori (uraR)*	this study
KK1278	*h+*	*rad50::Kan npp106-GFP:Nat nmt41:rtf1:sup35 ade6-704 leu1-32 t-ura4-SD20<ori (uraR)*	this study
KK1287	*h-*	*loxP:rad51 R152A-R324A- K334A:LoxM3 (rad51-3A) npp106-GFP:Nat nmt41:rtf1:sup35 ade6-704 leu1-32 t-ura4-SD20<ori (uraR)*	this study
YC6	*h-*	*t-ura4-SD20-ori nmt41:rtf1:sup35 ade6-704 leu1-32*	7
YC13	*h-*	*t-ura4-SD20<ori nmt41:rtf1:sup35 ade6-704 leu1-32*	7
SL1409	*h-*	*slx8-29:Hygro npp106-GFP:Nat arg3::mCherry-lacI rtf1:nmt41:sup35 t-lacO7,9kb:kan-ura4+<ori ade6-704 leu1-32*	this study
KK92	*h+*	*slx8-29-Hygro npp106-GFP:Nat arg3::mCherry-LacI nmt41:rtf1:sup35 ade6-704 leu1-32 t-LacO 7,9Kb:Kan:ura4$^+$-ori (ura+, no RTS1)*	this study
KK105	*h+*	*pmt3::Hygro npp106-GFP:Nat arg3::mCherry-LacI nmt41:rtf1:sup35 ade6-704 leu1-32 t-LacO 7,9Kb:Kan:ura4$^+$<ori (uraR)*	this study
KK464	*h-*	*npp106-GFP:Nat arg3::mCherry-LacI nmt41:rtf1:sup35 ade6-704 leu1-32 his3D1 t-LacO 7,9Kb:Kan:ura4$^+$<ori (uraR)*	this study
KK460	*h+*	*rfp1::his3 npp106-GFP:Nat arg3::mCherry-LacI nmt41:rtf1:sup35 ade6-704 leu1-32 his3D1 t-LacO 7,9Kb:Kan:ura4$^+$<ori (uraR)*	this study
KK463	*h+*	*rfp2::Hygro npp106-GFP:Nat arg3::mCherry-LacI nmt41:rtf1:sup35 ade6-704 leu1-32 his3D1 t-LacO 7,9Kb:Kan:ura4$^+$<ori (uraR)*	this study
KK465	*h-*	*rfp1::his3 rfp2::Hygro npp106-GFP:Nat arg3::mCherry-LacI nmt41:rtf1:sup35 ade6-704 leu1-32 his3D1 t-LacO 7,9Kb:Kan:ura4$^+$<ori (uraR)*	this study
KK662	*h+*	*npp106-GFP:Nat arg3::mCherry-LacI nmt41:rtf1:sup35 ade6-704 leu1-32 his3D1 t-LacO 7,9Kb:Kan:ura4$^+$-ori (ura4, no RTS1)*	this study
KK663	*h-*	*rfp1::his3 npp106-GFP:Nat arg3::mCherry-LacI nmt41:rtf1:sup35 ade6-704 leu1-32 his3D1 t-LacO 7,9Kb:Kan:ura4$^+$-ori (ura4, no RTS1)*	this study
KK164	*h-*	*rfp2::Hygro npp106-GFP:Nat arg3::mCherry-LacI nmt41:rtf1:sup35 ade6-704 leu1-32 t-LacO 7,9Kb:Kan:ura4$^+$-ori (ura4, no RTS1)*	this study
KK661	*h+*	*rfp1::his3 rfp2::Hygro npp106-GFP:Nat arg3::mCherry-LacI nmt41:rtf1:sup35 ade6-704 leu1-32 his3D1 t-LacO 7,9Kb:Kan:ura4$^+$-ori (ura4, no RTS1)*	this study
KK1018	*h+*	*slx8-29-Hygro npp106-GFP:Nat arg3::psv40:GFP-LacI nmt41:rtf1:sup35 ade6-704 leu1-32 LacO7.9kb:Kan:ura4+<ori*	this study
YC297	*h+*	slx8-29:Hygro nmt41:rtf1:sup35 ade6-704 leu1-32 t-ura4-SD20-ori (ura4+, no RTS1)	this study
YC301	*h+*	slx8-29:Hygro nmt41:rtf1:sup35 ade6-704 leu1-32 t-ura4-SD20<ori (uraR)	this study
KK110	*h+*	*nup131::Hygro npp106-GFP:Nat arg3::mCherry-LacI nmt41:rtf1:sup35 ade6-704 leu1-32 t-LacO 7,9Kb:Kan:ura4$^+$<ori (uraR)*	this study

KK112	h-	nup131::Hygro npp106-GFP:Nat arg3::mCherry-LacI nmt41:rtf1:sup35 ade6-704 leu1-32 t-LacO 7,9Kb:Kan:ura4+-ori (ura4, no RTS1)	this study
KK114	h-	nup132::Hygro npp106-GFP:Nat arg3::mCherry-LacI nmt41:rtf1:sup35 ade6-704 leu1-32 t-LacO 7,9Kb:Kan:ura4+<ori (uraR)	this study
KK116	h+	nup132::Hygro npp106-GFP:Nat arg3::mCherry-LacI nmt41:rtf1:sup35 ade6-704 leu1-32 t-LacO 7,9Kb:Kan:ura4+-ori (ura4, no RTS1)	this study
KK118	h+	nup131::Hygro nup132::Hygro npp106-GFP:Nat arg3::mCherry-LacI nmt41:rtf1:sup35 ade6-704 leu1-32 t-LacO 7,9Kb:Kan:ura4+<ori (uraR)	this study
KK119	h-	nup131::Hygro nup132::Hygro npp106-GFP:Nat arg3::mCherry-LacI nmt41:rtf1:sup35 ade6-704 leu1-32 t-LacO 7,9Kb:Kan:ura4+-ori (ura4, no RTS1)	this study
KK261	h+	nup131::Hygro nmt41:rtf1:sup35 ade6-704 leu1-32 t-ura4+-ori (ura4, no RTS1)	this study
KK266	h-	nup132::Nat nmt41:rtf1:sup35 ade6-704 leu1-32 t-ura4+-ori (ura4, no RTS1)	this study
KK271	h-	nup131::Hygro nup132::Nat nmt41:rtf1:sup35 ade6-704 leu1-32 t-ura4+-ori (ura4, no RTS1)	this study
KK222	h-	nup131::Hygro nmt41:rtf1:sup35 ade6-704 leu1-32 t-ura4+<ori (uraR)	this study
KK227	h+	nup132::Nat nmt41:rtf1:sup35 ade6-704 leu1-32 t-ura4+<ori (uraR)	this study
KK232	h+	nup131::Hygro nup132::Nat nmt41:rtf1:sup35 ade6-704 leu1-32 t-ura4+<ori (uraR)	this study
KK1254	h+	nup132::Nat npp106-GFP:Nat nmt41:rtf1:sup35 ade6-704 leu1-32 t-ura4-SD20<ori (uraR)	this study
KK440	h-	nup131::Hygro nup132::Nat npp106-GFP:Nat nmt41:rtf1:sup35 ade6-704 leu1-32 t-ura4-SD20<ori (uraR)	this study
KK531	h-	nmt41:rtf1:sup35 ade6-704 leu1-32 t>ura4+<ori (RuraR)	this study
KK535	h-	nup131::Hygro nmt41:rtf1:sup35 ade6-704 leu1-32 t>ura4+<ori (RuraR)	this study
KK539	h-	nup132::Nat nmt41:rtf1:sup35 ade6-704 leu1-32 t>ura4+<ori (RuraR)	this study
KK543	h-	nup131::Hygro nup132::Nat nmt41:rtf1:sup35 ade6-704 leu1-32 t>ura4+<ori (RuraR)	this study
KK810	h+	arg3::psv40:GFP-LacI nmt41:rtf1:sup35 ade6-704 leu1-32 t-ura4-SD20<ori (uraR)	this study
KK814	h+	nup131::Hygro nup132::Hygro arg3::psv40:GFP-LacI nmt41:rtf1:sup35 ade6-704 leu1-32 t-ura4-SD20<ori (uraR)	this study
KK936	h+	pli1::Hygro nmt41:rtf1:sup35 ade6-704 leu1-32 t-ura4-SD20<ori (uraR)	this study
KK957	h-	pli1::Hygro nmt41:rtf1:sup35 ade6-704 leu1-32 t-ura4-SD20-ori (ura4, no RTS1)	this study
KK940	h-	pli1::Hygro nup132::Nat nmt41:rtf1:sup35 ade6-704 leu1-32 t-ura4-SD20<ori (uraR)	this study
KK965	h+	pli1::Hygro nup132::Nat nmt41:rtf1:sup35 ade6-704 leu1-32 t-ura4-SD20-ori (ura4, no RTS1)	this study
KK154	h-	pli1::Hygro npp106-GFP:Nat arg3::mCherry-LacI nmt41:rtf1:sup35 ade6-704 leu1-32 t-LacO 7,9Kb:Kan:ura4+<ori (uraR)	this study
KK156	h+	pli1::Hygro npp106-GFP:Nat arg3::mCherry-LacI nmt41:rtf1:sup35 ade6-704 leu1-32 t-LacO 7,9Kb:Kan:ura4+-ori (ura4, no RTS1)	this study
KK1086	h+	pli1::Hygro nup132::Hygro npp106-GFP:Nat arg3::mCherry-LacI nmt41:rtf1:sup35 ade6-704 leu1-32 t-LacO 7,9Kb:Kan:ura4+<ori (uraR)	this study

KK1176	h-	pli1::Hygro nup132::Hygro npp106-GFP:Nat arg3::mCherry-LacI nmt41:rtf1:sup35 ade6-704 leu1-32 t-LacO 7,9Kb:Kan:ura4+-ori (ura4, no RTS1)	this study
KK1100	h-	pli1::Hygro npp106-GFP:Nat arg3::psv40:GFP-LacI nmt41:rtf1:sup35 ade6-704 leu1-32 t-LacO 7,9Kb:Kan:ura4+<ori (uraR)	this study
KK1256	h-	pli1::Hygro npp106-GFP:Nat nmt41:rtf1:sup35 ade6-704 leu1-32 t-ura4-SD20<ori (uraR)	this study
KK1258	h+	pli1::Hygro nup132::Nat npp106-GFP:Nat nmt41:rtf1:sup35 ade6-704 leu1-32 t-ura4-SD20<ori (uraR)	this study
KK1029	h-	pli1::Hygro slx8-29:Hygro nmt41:rtf1:sup35 ade6-704 leu1-32 t-ura4+<ori (uraR)	this study
KK1032	h+	pli1::Hygro slx8-29:Hygro nmt41:rtf1:sup35 ade6-704 leu1-32 t-ura4-SD20-ori (ura4, no RTS1)	this study
KK1157	h-	Low SUMO (tetO7-TATACYC1 pmt3:Kan) npp106-GFP:Nat arg3::mCherry-LacI nmt41:rtf1:sup35 ade6-704 leu1-32 t-LacO 7,9Kb:Kan:ura4+<ori (uraR)	this study
KK1159	h-	Low SUMO (tetO7-TATACYC1 pmt3:Kan) npp106-GFP:Nat arg3::mCherry-LacI nmt41:rtf1:sup35 ade6-704 leu1-32 t-LacO 7,9Kb:Kan:ura4+-ori (ura4, no RTS1)	this study
KK1161	h+	SUMO-D81R (pmt3D81R) npp106-GFP:Nat arg3::mCherry-LacI nmt41:rtf1:sup35 ade6-704 leu1-32 t-LacO 7,9Kb:Kan:ura4+<ori (uraR)	this study
KK1164	h+	SUMO-D81R (pmt3D81R) npp106-GFP:Nat arg3::mCherry-LacI nmt41:rtf1:sup35 ade6-704 leu1-32 t-LacO 7,9Kb:Kan:ura4+-ori (ura4, no RTS1)	this study
KK1051	h-	Low SUMO (tetO7-TATACYC1 pmt3:Kan) nmt41:rtf1:sup35 ade6-704 leu1-32 t-ura4-SD20<ori (uraR)	this study
KK1046	h-	Low SUMO (tetO7-TATACYC1 pmt3:Kan) nmt41:rtf1:sup35 ade6-704 leu1-32 t-ura4-SD20-ori (ura4, noRTS1)	this study
KK1052	h-	Low SUMO (tetO7-TATACYC1 pmt3:Kan) nup132::Nat nmt41:rtf1:sup35 ade6-704 leu1-32 t-ura4-SD20<ori (uraR)	this study
KK1045	h-	Low SUMO (tetO7-TATACYC1 pmt3:Kan) nup132::Nat nmt41:rtf1:sup35 ade6-704 leu1-32 t-ura4-SD20-ori (ura4, noRTS1)	this study
KK1067	h+	SUMO-D81R (pmt3D81R) nmt41:rtf1:sup35 ade6-704 leu1-32 t-ura4-SD20<ori (uraR)	this study
KK1069	h+	SUMO-D81R (pmt3D81R) nup132::Nat nmt41:rtf1:sup35 ade6-704 leu1-32 t-ura4-SD20<ori (uraR)	this study
KK1075	h-	SUMO-D81R (pmt3D81R) nmt41:rtf1:sup35 ade6-704 leu1-32 t-ura4-SD20-ori (ura4, noRTS1)	this study
KK1076	h-	nup132::Nat SUMO-D81R (pmt3D81R) nmt41:rtf1:sup35 ade6-704 leu1-32 t-ura4-SD20-ori (ura4, noRTS1)	this study
KK1350	h-	SUMO-KallR (pmt3-KallR) npp106-GFP:Nat arg3::mCherry-LacI nmt41:rtf1:sup35 ade6-704 leu1-32 t-LacO 7,9Kb:Kan:ura4+<ori (uraR)	this study
KK1353	h-	SUMO-KallR (pmt3-KallR) npp106-GFP:Nat arg3::mCherry-LacI nmt41:rtf1:sup35 ade6-704 leu1-32 t-LacO 7,9Kb:Kan:ura4+-ori (ura4, no RTS1)	this study
KK1340	h+	SUMO-KallR (pmt3-KallR) nmt41:rtf1:sup35 ade6-704 leu1-32 t-ura4-SD20<ori (uraR)	this study
KK1351	h-	SUMO-KallR (pmt3-KallR) nmt41:rtf1:sup35 ade6-704 leu1-32 t-ura4-SD20-ori (ura4, noRTS1)	this study
KK1345	h+	nup132::Nat SUMO-KallR (pmt3-KallR) nmt41:rtf1:sup35 ade6-704 leu1-32 t-ura4-SD20<ori (uraR)	this study

KK1352	h-	nup132::Nat SUMO-KallR (pmt3-KallR) nmt41:rtf1:sup35 ade6-704 leu1-32 t-ura4-SD20-ori (ura4, noRTS1)	this study
KK1151	h+	rpn10::Hygro npp106-GFP:Nat arg3::mCherry-LacI nmt41:rtf1:sup35 ade6-704 leu1-32 t-LacO 7,9Kb:Kan:ura4+<ori (uraR)	this study
KK1154	h-	rpn10::Hygro npp106-GFP:Nat arg3::mCherry-LacI nmt41:rtf1:sup35 ade6-704 leu1-32 t-LacO 7,9Kb:Kan:ura4+-ori (ura4, no RTS1)	this study
KK1079	h-	rpn10::Hygro nmt41:rtf1:sup35 ade6-704 leu1-32 t-ura4-SD20<ori (uraR)	this study
KK1081	h+	rpn10::Hygro nmt41:rtf1:sup35 ade6-704 leu1-32 t-ura4-SD20-ori (ura4, noRTS1)	this study
KK1082	h+	rpn10::Hygro nup132::Nat nmt41:rtf1:sup35 ade6-704 leu1-32 t-ura4-SD20<ori (uraR)	this study
KK1085	h-	rpn10::Hygro nup132::Nat nmt41:rtf1:sup35 ade6-704 leu1-32 t-ura4-SD20-ori (ura4, noRTS1)	this study
KK1047	h-	pnmt81-ulp1:Kan nmt41:rtf1:sup35 ade6-704 leu1-32 t-ura4-SD20<ori (uraR)	this study
KK1043	h+	pnmt81-ulp1:Kan nmt41:rtf1:sup35 ade6-704 leu1-32 t-ura4-SD20-ori (ura4, no RTS1)	this study
KK1049	h-	pnmt81-ulp1:Kan nup132::Nat nmt41:rtf1:sup35 ade6-704 leu1-32 t-ura4-SD20<ori (uraR)	this study
KK1044	h-	pnmt81-ulp1:Kan nup132::Nat nmt41:rtf1:sup35 ade6-704 leu1-32 t-ura4-SD20-ori (ura4, no RTS1)	this study
KK1192	h+	npp106-GFP:Nat arg3::mCherry-LacI nmt41:rtf1:sup35 ade6-704 leu1-32 t-LacO 7,9Kb:Kan:ura4::LexBS<ori (uraR)	this study
KK1193	h-	ulp1-lexA:Hygro npp106-GFP:Nat arg3::mCherry-LacI nmt41:rtf1:sup35 ade6-704 leu1-32 t-LacO 7,9Kb:Kan:ura4::LexBS<ori (uraR)	this study
KK1194	h+	nup132::Hygro npp106-GFP:Nat arg3::mCherry-LacI nmt41:rtf1:sup35 ade6-704 leu1-32 t-LacO 7,9Kb:Kan:ura4::LexBS<ori (uraR)	this study
KK1195	h-	nup132::Hygro ulp1-lexA:Hygro npp106-GFP:Nat arg3::mCherry-LacI nmt41:rtf1:sup35 ade6-704 leu1-32 t-LacO 7,9Kb:Kan:ura4::LexBS<ori (uraR)	this study
KK1122	h-	nmt41:rtf1:sup35 ade6-704 leu1-32 t-Kan-lexBS:ura4-SD20<ori (uraR)	this study
KK1121	h-	ulp1-lexA:Hygro nmt41:rtf1:sup35 ade6-704 leu1-32 t-ura4-SD20<ori (uraR)	this study
KK1131	h-	ulp1-lexA:Hygro nmt41:rtf1:sup35 ade6-704 leu1-32 t-Kan-lexBS:ura4-SD20<ori (uraR)	this study
KK1139	h-	nup132::Nat nmt41:rtf1:sup35 ade6-704 leu1-32 t-Kan-lexBS:ura4-SD20<ori (uraR)	this study
KK1137	h-	nup132::Nat ulp1-lexA:Hygro nmt41:rtf1:sup35 ade6-704 leu1-32 t-ura4-SD20<ori (uraR)	this study
KK1141	h-	nup132::Nat ulp1-lexA :Hygro nmt41:rtf1:sup35 ade6-704 leu1-32 t-Kan-lexBS:ura4-SD20<ori (uraR)	this study
KK697	h-	ulp1::Kan nmt41:rtf1:sup35 ade6-704 leu1-32 t-ura4-SD20<ori (uraR)	this study
KK442	h-	ssb3-YFP:Nat nmt41:rtf1:sup35 ade6-704 leu1-32 t-ura4-SD20<ori (uraR)	this study
KK1097	h-	pli1::Hygro ssb3-YFP:Nat nmt41:rtf1:sup35 ade6-704 leu1-32 t-ura4+<ori (uraR)	this study

Table S2: primers used in this study

Name	Distance (bp) from the *RTS1*-RFB position	Sequence (5'-3')	Experiment
L5F	-110	AGGGCATTAAGGCTTATTTACAGA	ChIP (all experiments)
L5R		TCACGTTTAATTTCAAACATCCA	ChIP (all experiments)
L3F	110	TTTAAATCAAATCTTCCATGCG	ChIP (all experiments)
L3R		TGTACCCATGAGCAAACTGC	ChIP (all experiments)
L400F	400	ATCTGACATGGCATTCCTCA	ChIP (all experiments)
L400R		GATGCCAGACCGTAATGACA	ChIP (all experiments)
L600F	600	CCATTGACTAGGAGGACTTTGAG	ChIP RPA
L600R		CCCTGGCGGTTGTAGTTAGT	ChIP RPA
L900F	900	AACGGTTGTAGAAGACGAGCA	ChIP RPA
L900R		TGTAAGCACACCTTCAATGTATCA	ChIP RPA
L1400F	1400	AACATCGGTGACCTCGTTCT	ChIP RPA
L1400R		CTCTTCGCTCCAAGCGTTAT	ChIP RPA
L2200F	2200	AAGGCAAGAAACGCTGAGAC	ChIP RPA
L2200R		GGCATGCATACTACCCGATAA	ChIP RPA
L3000F	3000	TCGTACGTACTGCATCGCTATAA	ChIP RPA
L3000R		TTCATCCTACCTTCTAGTTCGGTAA	ChIP RPA
II50F	Control locus on ChrII	CACCGCAGTTCTACGTATCCT	ChIP (all experiments)
II50R		CGATGTAACGGTATGCGGTA	ChIP (all experiments)
CenF	centromere (King et al 2008), cnt - center region	CAACCGTTGCAACTTACATCAGCA	ChIP Man1 and Sad1
CenR		CCGGTCGCCAAATAGCAATGAGAT	ChIP Man1 and Sad1
Lo5	ura4	GTTTAACTATGCTTCGTCGG	PCR screening (deletion/mutation)
Up5		AAAACAAACGCAAACAAGGC	PCR screening (deletion/mutation)
Rng3Lo	rng3	AAGGACTGCGTTCTTCTAGC	PCR screening efficiency control
Rng3Up		TGAATCCTCCGTTCAGTAGG	PCR screening efficiency control
TLII	TLII/TLIII	TTTCCTTTCACGGCTAACCC	PCR screening translocation
TLIII		TGTACCCATGAGCAAACTGC	PCR screening translocation
ade6-23	Control locus on ChrIII	GGCTGCCTCTACCATCATTC	ChIP (all experiments)
ade6-25		TTAAGCTGAGCTGCCAAGGT	ChIP (all experiments)
Ura4-1F	Within *ura4+* in proximity to lexBS; 600 bp away from RFB	GACTCCACGACCAACAATGA	ChIP lexA
Ura4-1R		CTGGTATCGGCTTGGATGTT	ChIP lexA