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Supplemental Data

Retrotransposon Tf1 Is Targeted to Pol II Promoters by Transcription Activators

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SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Plasmid constructions and cell growth- The construction of pHL414-2 has been described previously (Levin et al., 1993). To generate pHL2541, a precise deletion of the beta-lactamase gene (*bla*) of pHL414-2 was created by replacing the Bst API-Mlu I fragment with a product of fusion PCR. The primers used for this and all other constructs are described in the supplementary material (Table S1). The plasmids produced and the strains of *S. pombe* containing the plasmids are listed in Tables S2 and S3, respectively. To create the target plasmid with *bub1-ade6*, pHL2410, a 1.2 kb Sph I fragment encoding thymidine kinase was removed from pNR210 (a gift of N. Rhind and P. Russell, Scripps Research Institute, La Jolla, CA). *LEU2d* of pESP1 (Stratagene, La Jolla, CA) was then inserted into the resulting plasmid as a PCR fragment into the Sph I and Xma I sites. The deletions of pHL2410 were created using fusion PCR products and the oligonucleotides used are indicated in supplementary Table S2. To create deletion constructs B, E, F, G, H, I, J, K, and M, fusion PCR products were inserted into the Sbf I and Bmg BI sites of pHL2410. Deletion C was created by inserting a fusion PCR product into the Sbf I and Spe I sites of pHL2410. Deletion D was generated by inserting a fusion PCR product into the Bmg BI and Spe I sites of pHL2410. Deletion construct L (pHL2653) was created by inserting the 173 bp PCR product containing the target window into the Xma I and Spe I

sites of pHL2410. The target plasmid with *fbp1*, pHL2543, was made by first destroying the Hind III site in pHL1288 (Balasundaram et al., 1999) that is at the 3' end of *LEU2*. The resulting plasmid, pHL2542, has a unique Hind III site into which was inserted the 3.2 kb Hind III fragment of pAV06 (Vassarotti and Friesen, 1985) that contains *fbp1*. The product of this ligation, pHL2543, has *fbp1* oriented so that it is transcribed towards *LEU2*. The single and six-nucleotide substitutions in UAS1 of the *fbp1* promoter were generated according to the protocol of QuickChange II Site-Directed Mutagenesis kit (STRATAGENE, La Jolla, CA). The mutagenic primers containing the 6 nt mutation used were HL1907 and HL1908.

The target plasmid with *nup124*, pHL1338 was generated previously as a derivative of pHL1288 (Balasundaram et al., 1999). The target plasmids containing *SPCC4F11.03c* and *SPBC365.14c* were pHL2603 and pHL2688, respectively, and were constructed by inserting PCR products into the Sbf I and Spe I sites of pHL2410. The plasmid that expressed TAP-IN, pHL2512, was produced first by PCR amplifying IN coding sequence using primers HL1248 and HL1249. The product was inserted into the Nde I and Bam HI sites of pREP1-NTAP (Tasto et al., 2001). Then the sequence encoding TAP and IN was amplified with primers HL1262 and HL1263 to add an NLS at the N-terminus of TAP-IN. This product was inserted into the Pac I and Bsr GI sites of the TAP-IN plasmid to generate pHL2512.

The *aft1* gene was deleted from YHL1101 using the drug-resistance marker, *nat1*, encoding nourseothricin acetyltransferase (Sato et al., 2005). A fragment of DNA containing the *nat1* gene flanked by 80 nt of sequence adjacent to *atf1* was created by PCR using pCR2.1-*nat* (pHL2621) as the template and HL1574 and HL1575 as the

primers. To induce homologous recombination between *atf1* and the *nat1*-containing fragment, 5 µg of the *nat1* DNA was introduced to YHL1101 by lithium acetate transformation. NAT resistant colonies were isolated on YES plates containing NAT (nourseothricin, commercial name; clon NAT, WERNER BioAgents, Germany) (100 µg/ml). DNA blots and PCR were performed to confirm the single gene deletion.

Selective media contained Edinburgh minimal media (EMM) (Moreno et al., 1991) and 2 g of dropout mix similar to that of Rose *et al* (Rose et al., 1990) except adenine levels were increased to a final concentration of 250 µg/ml. A liter of rich media, YES, contained 5 g Difco yeast extract, 30 g glucose, and 2 g of dropout powder. When indicated, 10 µM vitamin B1 was added to repress the *nmt1* promoter. EMM 5-FOA plates contained 1 mg/ml 5-Fluoroorotic acid (#F5050, United States Biologicals, Swampscott, MA). YES FOA/G418 contained 500 µg/ml (corrected for purity) of Geneticin (#11811-031, Life Technologies, Rockville, MD) and 1 mg/ml 5-FOA. All strains of *S. pombe* were YHL1101 (h+, *ura4-D18, leu1-32, ade6-M210*) transformed by lithium acetate with various plasmids.

Target plasmid assay- Target plasmids were introduced into *S. pombe* by transformation with lithium acetate followed by selection on EMM medium lacking leucine. These strains were then transformed with the Tf1 plasmid by selecting on EMM lacking leucine and uracil. Strains were arranged on EMM-leu-ura+B1 plates in two cm^2 patches. Following 48 hrs of incubation these plates were replica printed to EMM-leu-ura-B1 to induce transposition. After four days of incubation the strains were printed to EMM-leu+FOA+B1 to select against the Tf1 donor plasmid. Following two days of incubation

the plates were again printed to EMM-leu+FOA+B1. To select for transposition events, the strains were then printed to EMM-leu+FOA+G418. After two days growth the patches were washed in 0.5 ml dH_2O and resuspended in 0.5 ml citrate phosphate sorbitol buffer with zymolase 100T (50 mM Na_2HPO_4, 50 mM Citric acid, 1.2 M Sorbitol, [buffer adjusted to pH5.6] 2 mg/ml Zymolase 100T from Seikagaku) and incubated at 37 °C for 1 hr. After the incubation, the cells were resuspended in 100 µl TE (10 mM Tris-HCl pH 7.9, 1 mM EDTA). Twelve microliters of 10% SDS was added and the cells were incubated at 65 °C for 5 min. Then 33 µl of 5 M potassium acetate was added, the samples were mixed well and placed on ice for 30 min. The cells were then centrifuged at 4 °C for 10 min at 14,000 rpm in a minifuge. The supernatants were ethanol precipitated and the pellets were resuspended in 100 µl of 1X TE. These samples were phenol extracted and ethanol precipitated. This material was then electroporated into bacteria. Construct A (pHL2410) was assayed with pHL414-2 as its source of Tf1-*neo* while the remainder of the deletion constructs were assayed with pHL2541. The insertions in construct A and the plasmid with *fbp1* were derived from three independent yeast transformants. Each one gave similar patterns of integration. For the remainder of the constructs the inserts were also isolated from independent transformants.

RNA blots- Forty ml of cells harvested at OD_{600}=1.0 were resuspended in 400 µl of buffer AE (50 mM NaOAc, pH 5.3, and 10 mM EDTA) and 40 µl of 10% SDS. The resuspended cells were transferred to a 2 ml tube containing 400 µl of phenol equilibrated with water, and 400 µl of acid-washed glass-beads (0.4 mm diameter) and they were disrupted in a Mini-Beadbeater-8 (Biospec Products, Bartlesville, OK) for a total of five

min in cycles of 30 sec on, 30 sec off. The cell lysates were cooled at -80 °C for 10 min before centrifugation for 5 min to separate the aqueous phase from the cell debris, phenol and beads. Phenol-chloroform extraction was repeated until the interface was no longer white. Ten µg of total RNA was loaded on a 1.0% formamide denaturing gel. After the RNA was transferred to a nylon membrane, it was hybridized with PCR generated probes specific for *ade6* or *bub1*. The primers are listed in Table S1. The actin probe was produced from a one kb Eco RI-Bam HI fragment of pHL859-1. The RNAs prepared for Fig. 2B and 2C were derived from strains YHL9318-YHL9321, YHL9323, YHL9325, YHL9326, and YHL9328. For each deletion plasmid two yeast transformants were analyzed and found to produce similar amounts of *ade6* and *bub1* mRNA. The levels of RNA from the separate transformants were quantified with phosphoimaging and averaged. For Fig. 4, The RNAs were derived from YHL9435 through YHL9440. Four separate transformants of each plasmid were included on the RNA blot and the levels of signal were averaged.

Micrococcal nuclease mapping- Cultures of YHL8964 and YHL9085 were grown to a final OD_{600} of 0.5-0.7. One gram of cells (two 500 ml cultures) was washed three times with 250 ml sterile water, resuspended in 2 ml of preincubation buffer (0.7 M beta-mercaptoethanol, 3 mM EDTA, 20 mM Tris-HCl pH 8.0) and agitated at 30 °C for 10 min. After a wash in 5 ml 1 M Sorbitol, the cells were resuspended in 2.5 ml of zymolyase solution without zymolyase (37.5 mM Tris-HCl (pH 7.5), 0.75 M Sorbitol, 1.25% Glucose, 6.25 mM EDTA). Once resuspended, 2.5 ml of zymolyase solution with zymolyase (0.25% Zymolyase-100T from Seikagaku) was added and the cells were

agitated at 30 °C for 20 min. Following centrifugation, the pellets were washed with 1 M Sorbitol and resuspended in 7 ml of lysis buffer (18% Ficoll 400, 10 mM KH_2P0_4, 10 mM K_2HPO_4, 1 mM $MgCl_2$, 0.25 mM EGTA, 0.25 mM EDTA, and 1 mM PMSF). After centrifugation in a SS34 rotor (Sorvall) at 13,000 rpm at 4 °C for 40 min the pellets were resuspended in 8 ml buffer A (10 mM Tris-HCl (pH 8.0), 150 mM NaCl, 5 mM KCl, 1 mM EDTA (pH 8.0), 1 mM PMSF) until no particles were visible. Five µl of 1 M $CaCl_2$ was added to 1 ml aliquots of the resuspension and each aliquot was digested for 5 min at 37 °C with different amounts of 10 U/µl MNase that achieved final concentrations of 0, 20, 30, 50, 225 U/ml. The reactions were subsequently stopped by the addition of 20 mM EDTA and 1% SDS (final concentrations) and incubated with 40 µg Proteinase K (Merck) at 50 °C overnight. After centrifugation, 1 ml of supernatant was phenol/$CHCl_3$ extracted and digested with a final concentration of 0.16 mg/ml RNase A at 37 °C for two hrs. Two additional extractions with phenol/$CHCl_3$ were performed followed by a $CHCl_3$ extraction and ethanol precipitation. For YHL8964 and YHL9085 600 ng of the DNA were digested with Eco RI. After digestion, the DNAs were ethanol precipitated, loaded on a 1% TBE agarose gel (25 cm long). As a control for sequence preferences of MNase, 600 ng of naked DNA treated with increasing amounts of MNase (see below for preparation) was included on the gel. Electrophoresis ran in the cold room at 80 V for 24 hrs in 1X TBE. The gel was then transferred to GeneScreen plus membrane and hybridized. The *bub1-ade6* sequence was probed with a 200 bp PCR product generated with the primers HL1317 and HL1318.

Two µg of naked DNA from YHL8964 (for Fig. 2D) was digested with 0.2, 0.4, and 2.0 units of MNase for 5 min at 37 °C. After 5 min, the reactions were stopped with 4

μl of 0.5 M EDTA. All three concentrations of digests were pooled together and extracted with phenol/chloroform and ethanol precipitated. This material was included on the gel containing MNase digests of the chromatin fractions.

Preparation of naked DNA for MNase digestion- Cells of YHL8964 were inoculated in EMM –ura, -leu +B1 at an OD of 0.05 and grown for ~36-40 hrs until they reached OD=8.0. One hundred ml of cells were resuspended in 2.5 ml of SP1 (1.2 M Sorbitol, 50 mM Citric acid monohydrate, 50 mM $Na_2HPO_4*7H_2O$, 40 mM EDTA with 5 M NaOH to make pH 5.6) containing 15 mg zymolyase. The cells were incubated at 37 °C for 2 hrs or until cells became dark under a phase-contrast microscope. The spheroplasted cells were pelleted by spinning at 3000 rpm for 5 min in a minifuge. The pellets were resuspended in 7.5 ml of 5X TE and 0.75 ml of 10% SDS was added followed by an incubation at room temperature for 1 hr. Then the mixture was incubated at 65 °C for 5 min. After transfer to 14 ml Falcon snap cap tubes 2.5 ml of 5 M KOAc was added and the samples were incubated at room temperature for 30 min. To remove debris the mixtures were centrifuged at 5000 rpm for 15 min in a SS34 rotor. The supernatant was pipetted into a new tube and 10 ml of ice-cold isopropanol was added. After incubation on ice for 5 min the mixture was centrifuged at 8000 rpm (SS34) for 10 min to pellet the nucleic acids. The pellet was resuspended in 3 ml 5X TE and RNase A was added to a final concentration of 100 μg/ml. After an incubation of 1 hr at 37 °C, 0.1 ml 10% SDS was added followed by the addition of Proteinase K to final concentration of 50 μg/ml. The samples were incubated overnight at 50 °C. Then 3 ml of equilibrated phenol was added and the samples were mixed gently and thoroughly. After centrifugation at 5000

rpm (SS34) for 5 min the phenol extraction was repeated. Then the samples were extracted with 3 ml of phenol/$CHCl_3$/isoamyl alcohol. The DNA was then precipitated by adding 1/10 volume 5 M NaCl and 2.5 volumes 100% ethanol. After centrifugation at 8000 rpm (SS34) for 10 min, the pellets were washed using 2 ml 70% ethanol and air-dried. The DNA was resuspended in 100 µl 1X TE. Ten microliters were run on a 1% gel to determine concentration.

Coimmunoprecipitation- YHL1101 (wild type) or SPJ152 (*atf1*-FLAG::KanMx6) was transformed with pHL2512, a TAP-IN expression plasmid. Ten milliters of each transformant was harvested at OD 1.0-1.5 and extracted with a bead beater in 200 µl of NP-40 buffer (50mM Tris-Cl pH8.0, 150mM NaCl, 10% Glycerol, 1mM EDTA, 1mM DTT, 0.2mM PMSF, 0.1% NP-40, Complete protease inhibitor [Roche]) and 100 µl of glass beads. Five hundred micrograms of extracted proteins were immunoprecipitated with 30 µl of prewashed anti-FLAG M2 agarose (Sigma). After an incubation of two hours at 4.0 °C the precipitate was washed six times in 500 µl of the NP-40 buffer. The protein bound to the beads were eluted in 35 µl of 2x sample buffer with a 30 min incubation at 37˚C.

Chromatin immunoprecipitation- ChIP was performed as described in (Noma et al., 2001). For this experiment, the strain SPJ-152 was chosen, in which *atf1* was tagged with FLAG in the genome. The recruitment of Atf1p was tested using anti-FLAG antibody. The primer sets used were: for *ade6*, HL1201 and HL2061; for *fbp1*, HL2058 and HL2059; and for *act*, HL1446 and HL2063. To calculate the relative enrichment of Atf1p

in the promoters of *ade6* and *fbp1*, *ade6* or *bub1* signal intensity was normalized by actin signal and then, this ChIP signal was normalized further by the signal intensity from the whole cell extract.

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Table S1: Oligonucleotides used in construction of plasmids.

Purpose	Oligonucleotide
Deletion of amp from pHL414-2 by fusion PCR. Is upstream of the *Mlu*I site in pHL414-2.	HL1303: CCAAGGACGTACAAAATAGAAAAC
Deletion of amp from pHL414-2 by fusion PCR. Is downstream of the *Bst*API site in pHL414-2.	HL1304: CACGTAGCGATAGCGGAGTG
Deletion of amp from pHL414-2 by fusion PCR. Is top strand primer at the end of amp.	HL1305: CTGTCAGACCAAGTTTACTC
Deletion of amp from pHL414-2 by fusion PCR. Is bottom strand primer at eh beginning of amp with complementarity to opposite end of amp.	HL1306: GTATATATGAGTAAACTTGGTCTGACAGATTGAAGCATTTATCAGGGTTATTG
Construction of deletions in pHL2410. Primer for fusion PCR. (Construct B, C, E, F, G, H, I, J, K)	HL1191: TACTTGTTACCCATCATTGAA
Construction of deletions in pHL2410. Primer for fusion PCR. (Construct D)	HL1192: TCGCTTAAACATCAAATGCAT
Construction of deletions in pHL2410. Primer for fusion PCR. (Construct B, E, F, G, H, I, J, K)	HL1193: CTTCTTAGACAGTTCAACAA
Construction of deletions in pHL2410. Primer for fusion PCR. (Construct C, D)	HL1194: CACAGGAAACAGCTATGACC
Construction of deletions in pHL2410. Primer for fusion PCR. (Construct B)	HL1195: TTAATGATGTTCGTCATCTTCTCATACAG
Construction of deletions in pHL2410. Primer for fusion PCR. (Construct B)	HL1196: AGCTGTATGAGAAGATGACGAACATCATTAATAGTGATACGCACATTGAAACATGGACGACTTTTAA
Construction of deletions in pHL2410. Primer for fusion PCR. (Construct E, F, H)	HL1197: ATGTCCGATTGGCGGCTTACAGAAAATGTAC
Construction of deletions in pHL2410. Primer for fusion PCR. (Construct H)	HL1198: GTACATTTTCTGTAAGCCGCCAATCGGACATTAAGGTATAACGACAACAA ACGTTGCTTTATAT
Construction of deletions in pHL2410. Primer for fusion PCR. (Construct G, I)	HL1199: ATACTGCACCAGGCTGGATTTCGTTTACCTC
Construction of deletions in pHL2410. Primer for fusion PCR. (Construct I)	HL1200: GAGGTAAACGAAATCCAGCCTGGTGCAGTATATAAACTCCGCACTAACTCACTACAATAAACAACT
Construction of deletions in pHL2410. Primer for fusion PCR (Construct J), and for ChIP assay (*ade6*)	HL1201: TTATTTGAGTTTTGCATCGTATTAATTCTGT
Construction of deletions in pHL2410. Primer for fusion PCR. (Construct J)	HL1202: ACAGAATTAATACGATGCAAAACTCAAATAAATGAGCGAAAAACAGGTTGTAGGGATCC
Construction of deletions in pHL2410. Primer for fusion PCR. (Construct C)	HL1203: TATTCGATGAAGTATGTATATACCTTGGCAG
Construction of deletions in pHL2410. Primer for fusion PCR. (Construct C)	HL1204: CTGCCAAGGTATATACATACTTCATCGAATATGTATTATCATGGGTTCGGATTCTGATTTAAGCAA
Construction of deletions in pHL2410. Primer for fusion PCR. (Construct D)	HL1208: CCAACAACAGGTGATTCGACAGGATCTTTGA
Construction of deletions in pHL2410. Primer for fusion PCR. (Construct D)	HL1209: TCAAAGATCCTGTCGAATCACCTGTTGTTGGTTAGTTCGACCATAGACATAACTGTTAAATGATTTTG
Construction of deletions in pHL2410. Primer for fusion PCR. (Construct E)	HL1214: GTACATTTTCTGTAAGCCGCCAATCGGACATATGAGCGAAAAACAGGTTGTAGGGATCC
Construction of deletions in pHL2410. Primer for fusion PCR. (Construct F)	HL1215: GTACATTTTCTGTAAGCCGCCAATCGGACATATAAAGTCCGCACTAACTCACTACAATAAACAACT
Construction of deletions in pHL2410. Primer for fusion PCR. (Construct G)	HL1534: GAGGTAAACGAAATCCAGCCTGGTGCAGTATATGAGCGAAAAACAGGTTGTAGGGATCC
Construction of deletions in pHL2410. Primer for fusion PCR. (Construct K)	HL1535: GAGTTTATTATTTGAGTTTTGCATCGTATT
Construction of deletions in pHL2410. Primer for fusion PCR. (Construct K)	HL1536: AATACGATGCAAAACTCAAATAATAAACTCATGAGCGAAAAACAGGTTGTAGGGATCC

Construction of construct L in pHL2410. Forward primer containing *Spe*I at the end. (Construct L)	HL1490: GACTAGTCTTATTTGAGTTTTGCATCGT
Construction of construct L in pHL2410. Reverse primer containing *Xma*I at the end. (Construct L)	HL1491: TCCCCCCGGGGGGATAAGGTATAACGACAACAAA
Construction of deletions in pHL2410. Primer for fusion PCR. (Construct M)	HL1899: GTACATTTTCTGTAAGCCGCCAATCGGACATAACTCAAATAATAAACTCCGCACTAACTCACTACA
Construction of deletions in pHL2410 with Tf1 (L). Primer for fusion PCR. (Construct M-L)	HL1695: CAGAAGTCGGGCCCAAACAAC
Construction of deletions in pHL2410 with Tf1 (L). Primer for fusion PCR. (Construct M-L)	HL1812: GTACATTTTCTGTAAGCCGCCAATCGGACATTGTTAGCTACGCAGTTTGGTATTTG
Construction of deletions in pHL2410 with Tf1 (R). Primer for fusion PCR. (Construct M-R)	HL1840: ATCATGTCCGATTGGCGGCTTACAGAAAATGTAC
Construction of deletions in pHL2410 with Tf1 (R). Primer for fusion PCR. (Construct M-R)	HL1841: GTACATTTTCTGTAAGCCGCCAATCGGACATGATTGTCAGCAATACTACACTACGCTATG
Construction of deletions in pHL2410 with Tf1 (R). Primer for fusion PCR. (Construct M-R)	HL1842: GCTTCCATTGTAAGACTTTGTCTATATTTTCTTG
Construction of pHL2603. Primer for amplifying *SPCC4F11.03c* fragment.	HL1482: GGACTAGTGCATGCATTGGTGTTTATAACAAAGGC
Construction of pHL2603. Primer for amplifying *SPCC4F11.03c* fragment.	HL1483: GAAGACGTCCTGCAGGTAGTGTGAACAGGCAACTTCG
Construction of pHL2688. Primer for amplifying *SPBC365.14c* fragment.	HL1484: GGACTAGTGTGCTTGACTTCTAATCTTCGGC
Construction of pHL2688. Primer for amplifying *SPBC365.14c* fragment.	HL1485: GATATCCTGCAGGGACATGATAAACGCTCCCTTCCTCG
Construction of pHL2693, *fbp1* UAS1-1nt mutation	HL1349: TTCCCAATCATCTACCTCACAAAGCATTA
Construction of pHL2693, *fbp1* UAS1-1nt mutation	HL1350: CTAATGCTTTGTGAGGTAGATGATTGGGAA
Construction of pHL2709, *fbp1* UAS1-6nt mutation	HL1907: CCTTTAGCACTTCCCAATCATGATGGAGACAAAGCATTAGTACTCATCATC
Construction of pHL2709, *fbp1* UAS1-6nt mutation	HL1908: GATGATGAGTACTAATGCTTTGTCTCCATCATGATTGGGAAGTGCTAAAGG
Construction of *atf1* deletion cassette.	HL1574: GCTCCAATGGAGGGGCCGGATCTGTCGCTAATGCGAATCCAGCTGACCAATCCGATGGTGTAACTCCGTCT TTCGTAGGCCGGATCCCCGGGTTAATTAA
Construction of *atf1* deletion cassette.	HL1575: CCGCTTCCGATGACACTTGTTGCAACAGCAGCCGAATTACTCTTGGCCACTGGGCAGTCTTTATGAGCAATC AATAAGGGAATTCGAGCTCGTTTAAAC
Primer for IN amplification	HL1248: AACATTCTAATTCTACATATGACAGATGATTTTAAAAACCAAG
Primer for IN amplification	HL1249: TTCAATCAATTAGGATCCGTTCTCAGATATTTAGATTATTG
Construction of NLS-TAP-IN.	HL1262: AACATTCTAATTCTATTAATTAAATGGCGCCTAAGAAGAAGCGTAAGGTTGTAAAAGCTGATGCGCAACAA AATAAC
Construction of NLS-TAP-IN.	HL1263: AGTAAATTCAACAATTTTGTATCATTCG
ChIP analysis. Primer for testing *ade6* region.	HL2061: CCTGGTGCAGTATAAGGTATAACG
ChIP analysis. Primer for testing *fbp1* region.	HL2058: CCTGGATGACCAGTCATTATAG
ChIP analysis. Primer for testing *fbp1* region.	HL2059: CATGGCTTATACAGCTAAATG
ChIP analysis. Primer for testing *act* region.	HL1446: GAAATCGCAGCGTTGGTTAT
ChIP analysis. Primer for testing *act* region.	HL2063: GAATAGCAACATAAAAGGCAGGTGC
Primer for *ade6* probe in nucleosomal array analysis.	HL1317: CCTCGAGACGTACGGTGCGC
Primer for *ade6* probe in nucleosomal array analysis.	HL1318: CAAAATGCTTGATGATGCTATTCAG
Primer for *fbp1* probe in nucleosomal array analysis.	HL1444: AAGCTTTACCTTTAAGAATTG
Primer for *fbp1* probe in nucleosomal array analysis.	HL1445: GTGGAATTTTCACTAAGCTAC
Primer for *ade6* probe in Northern blot analysis.	HL1448: GTGAGCACATTGATGCATCA
Primer for *ade6* probe in Northern blot analysis.	HL1449: GGCAGCATCTTTCATCTTGC

Primer for *bub1* probe in Northern blot analysis.	HL1479: ATGTCCGATTGGCGGCTTAC
Primer for *bub1* probe in Northern blot analysis.	HL1480: CCTATGCCTTTCGATGCCAA

Table S2: Construction of plasmids by fusion PCR.

Plasmid; Description	Half and fusion products	Half product	Half product	Half and fusion products
pHL2541; Deletion amp from pHL414-2	HL1303	HL1305	HL1306	HL1304
pHL2544; Deletion B	HL1191	HL1195	HL1196	HL1193
pHL2550; Deletion C	HL1191	HL1203	HL1204	HL1194
pHL2457; Deletion D	HL1192	HL1208	HL1209	HL1194
pHL2548; Deletion E	HL1191	HL1197	HL1214	HL1193
pHL2549; Deletion F	HL1191	HL1197	HL1215	HL1193
pHL2583; Deletion G	HL1191	HL1199	HL1534	HL1193
pHL2545; Deletion H	HL1191	HL1197	HL1198	HL1193
pHL2546; Deletion I	HL1191	HL1199	HL1200	HL1193
pHL2547; Deletion J	HL1191	HL1201	HL1202	HL1193
pHL2581; Deletion K, template was Del H.	HL1191	HL1535	HL1536	HL1193
pHL2686; Deletion M	HL1191	HL1197	HL1899	HL1193
pHL2674; Deletion M-L, template was A-L	HL1191	HL1197	HL1812	HL1695
pHL2687; Deletion M-R, template was A-R	HL1191	HL1840	HL1841	HL1842

Table S3: Strains of *S. pombe*

Yeast strains[1]	Tf1 expression plasmid	Target plasmid
YHL8964	pHL414-2	pHL2410 (WT *bub1-ade6*, Construct A)
YHL9326	pHL2541	pHL2410 (WT *bub1-ade6*, Construct A)
YHL9321	pHL2541	pHL2548 (Construct E)
YHL9325	pHL2541	pHL2583 (Construct G)
YHL9318	pHL2541	pHL2545 (Construct H)
YHL9319	pHL2541	pHL2546 (Construct I)
YHL9320	pHL2541	pHL2547 (Construct J)
YHL9323	pHL2541	pHL2581 (Construct K)
YHL9192	pHL2541	pHL2543 (WT *fbp1*)
YHL9085	pHL414-2	pSP1[2] (Empty vector)
YHL9151	pHL2541	pSP1[2] (Empty vector)
YHL9328	pHL2541	pSP1[2] (Empty vector)
YHL9435-1, ~ 3.		pHL2410 (WT *bub1-ade6*, Construct A)
YHL9441		pSP1[2] (Empty vector)
YHL9436-1, ~ 4.		pHL2698 (Construct A-L; Construct A with Tf1 in the left orientation)
YHL9437-1, ~ 4.		pHL2699 (Construct A-R; Construct A with Tf1 in the right orientation)
YHL9438-1, ~ 4.		pHL2686 (Construct M)
YHL9439-1, ~ 4.		pHL2674 (Construct M-L; Construct M with Tf1 in the

		left orientation)
YHL9440-1, ~ 4.		pHL2687 (Construct M-R; Construct M with Tf1 in the right orientation)
YHL9442-1, ~ 2.		pHL2547 (Construct J)
YHL9443-1, ~ 4.		pHL2700 (Construct J-L; Construct J with Tf1 in the left orientation)
YHL9444-1, ~ 4.		pHL2701 (Construct J-R; Construct J with Tf1 in the right orientation)
YHL9526		h^+, *ura4-D18, leu1-32, ade6-M210, atf1::nat1*
SPJ152		h90, *ura4-D18, leu1-32, ade6-M216, atf1-Flag*::*KanMx6*

[1] All plasmids were introduced into YHL1101 (h^+, *ura4-D18, leu1-32, ade6-M210*)

[2] Cottarel, G., Beach, D., and Deuschle, U. 1993. Two new multi-purpose multicopy *Schizosaccharomyces pombe* shuttle vectors, pSP1 and pSP2. *Curr Genet* **23** (5-6); 547-548

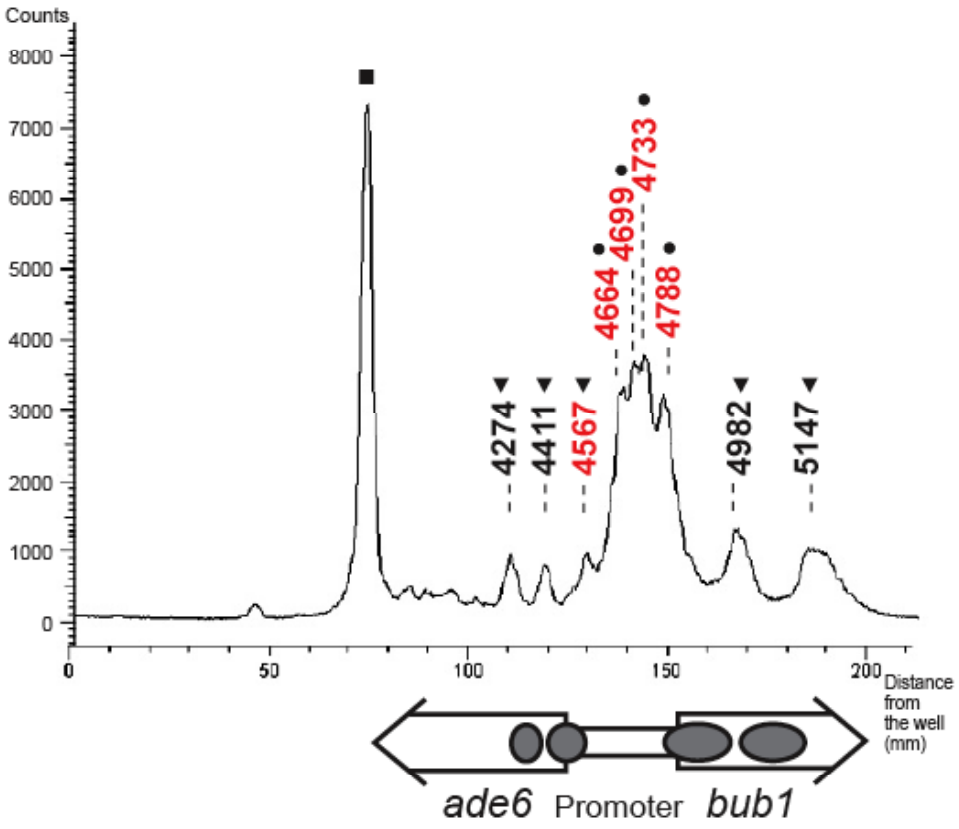

Fig. S1. The positions of MNase cleavages in *bub1-ade6.* The lane from the WT sample (Fig. 2D) treated with 20 units/ml MNase is shown in profile using ImageQuant TL software. The plasmid coordinates for each peak are shown. Coordinates within the intergenic sequence are shown in red.

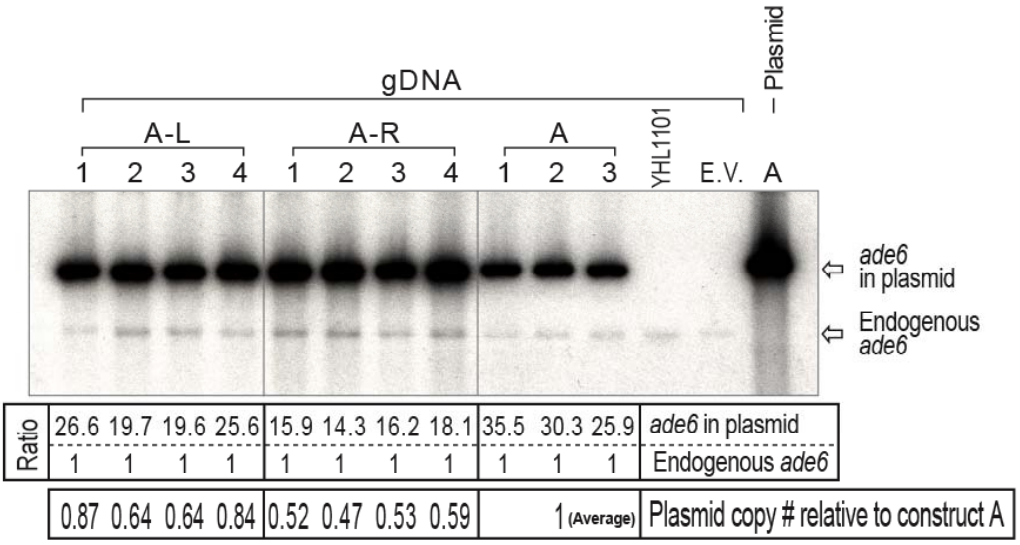

Fig. S2. The determination of plasmid copy numbers in each transformant containing the construct A series. The genomic DNAs from transformants harboring construct A, A-L, or A-R were digested by Bam HI and Sac I and were hybridized with the *ade6* specific probe. The sizes of the *ade6* fragments from the target plasmid and from the genomic DNA are 4.7kb and 2.6kb, respectively. The intensities of the *ade6* fragments were determined by phosphoimaging and the differences of the amounts loaded were adjusted by normalizing to the amounts of endogenous *ade6*. The copy numbers of the construct A-L and construct A-R target plasmid were normalized by the average value of 3 individual strains with construct A.

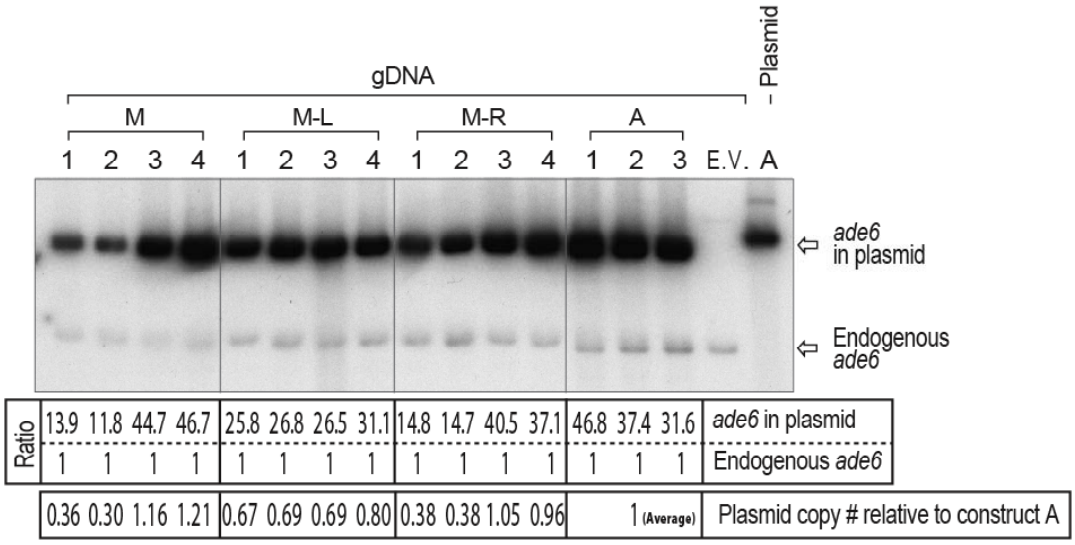

Fig. S3. The determination of plasmid copy numbers in each transformant containing the construct M series. The genomic DNAs from transformants harboring construct M, M-L, M-R, or A were digested by Bam HI and Sac I and were hybridized with the *ade6* specific probe. The sizes of the *ade6* fragments from the target plasmid and from the genomic DNA are 4.7kb and 2.6kb, respectively. The intensities of the *ade6* fragment were calculated by phosphoimaging as described in Fig. S2.

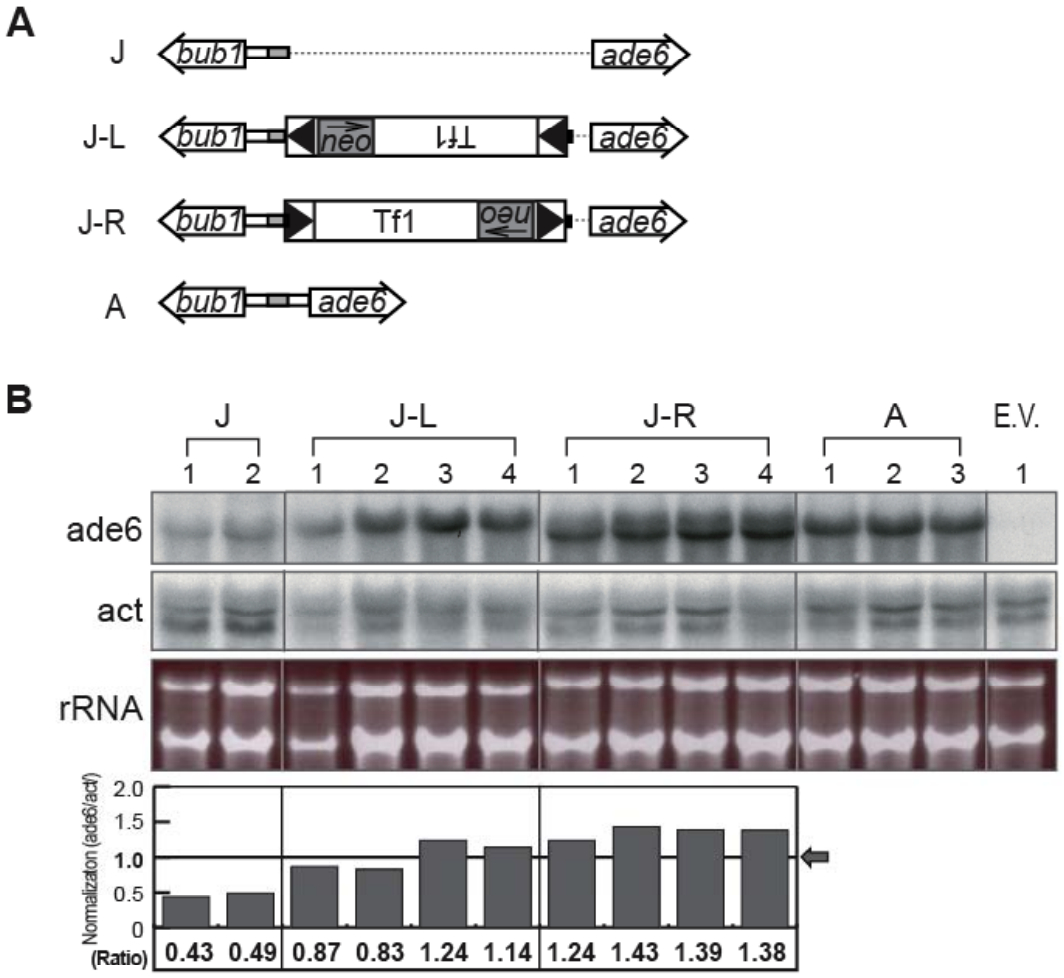

Fig. S4. The insertion of Tf1 in construct J repairs the damaged promoter. A. Diagrams of construct J, construct J with an insertion of Tf1-*neo* in the left orientation at position 4619 (J-L), construct J with an insertion of Tf1-*neo* in the right orientation at position 4619 (J-R), and construct A are shown. J-L and J-R are the resulting products of the targeting assay performed with a construct J target plasmid. B. The mRNA levels of *ade6* and actin were determined in cells (YHL9442-YHL9444, YHL9435, and YHL9441) containing the constructs shown in A by RNA blot analysis. The rRNA levels are shown as stained with ethidium bromide. The amounts of mRNA were quantified by phosphoimaging and each

ade6 mRNA level was normalized to actin mRNA. The normalized *ade6* mRNA levels of construct J, J-L, and J-R were normalized further to the *ade6* level of construct A. The average value of *ade6* for construct A was set to 1.0 (arrow). The individual *ade6* mRNA values normalized are shown in the graph below.