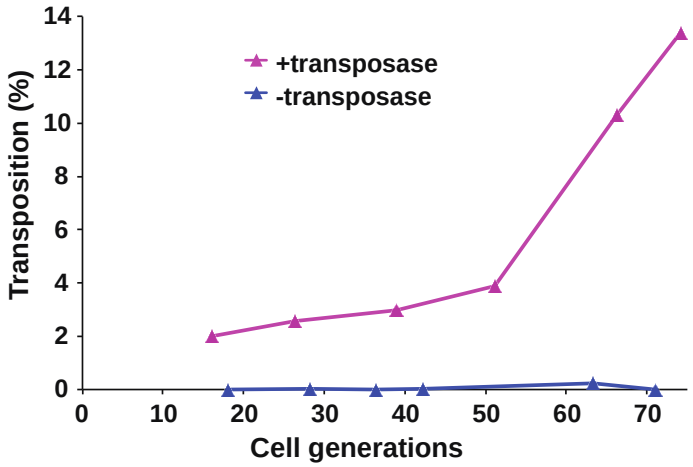

Figure S1 The percentage of cells with an insertion was measured during prolonged growth in liquid culture. After 74 generations 13.4% of the cells expressing the transposase contained an insertion (Pink). Cells in a control culture did not express the transposase (Blue).

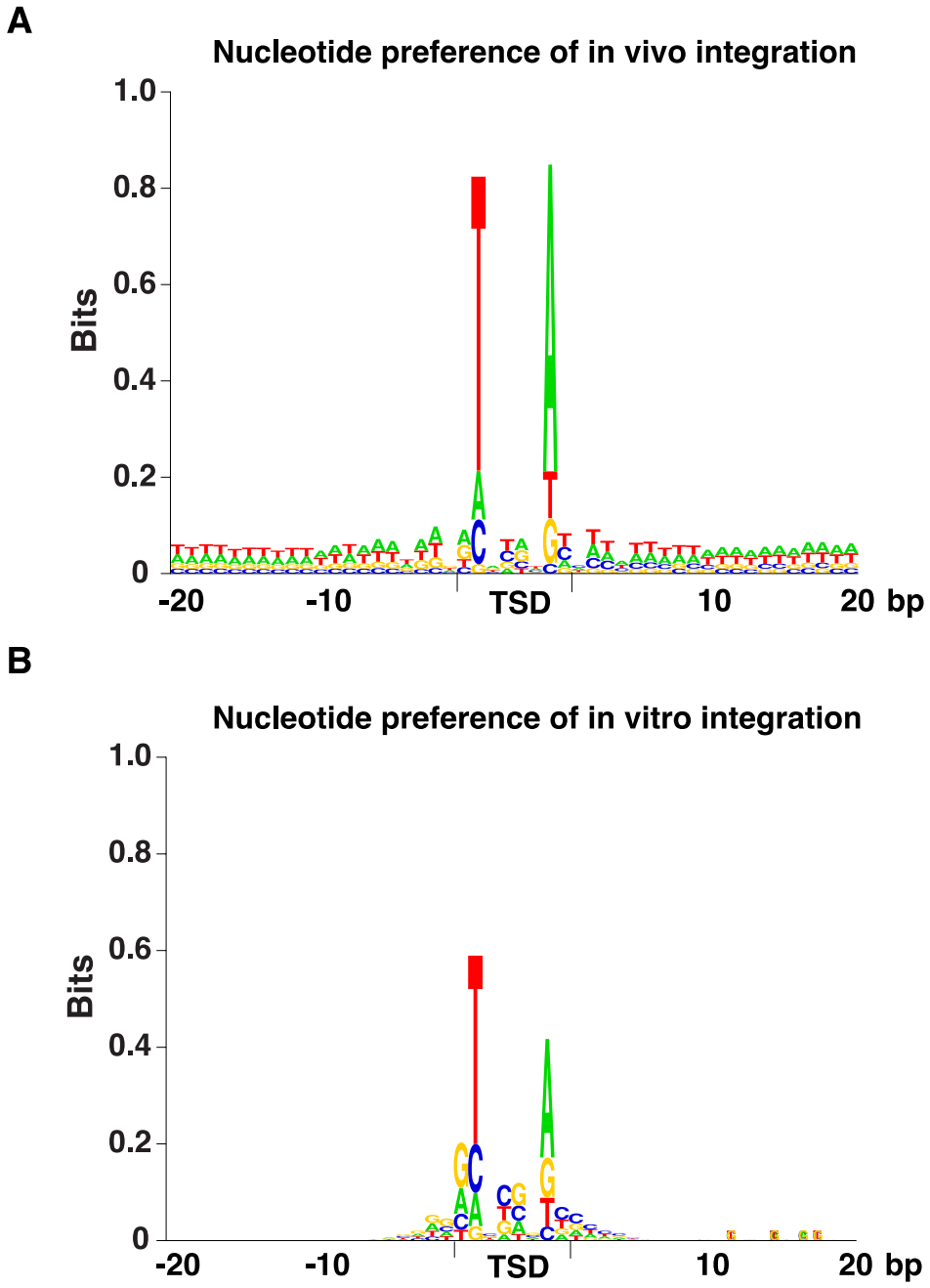

Figure S2 The average nucleotide frequencies flanking the insertion sites were determined. All Hermes insertions were aligned and the nucleotide patterns were analyzed by sequence logo created by a program written in Visual Basic based on an existing algorithm (Schneider and Stephens, 1990). The positions of the eight nucleotides at the target sites that are duplicated during integration are indicated by TSD. The nucleotide preferences for the *in vivo* integration sites are shown in (A) and the *in vitro* integration sites are shown in (B).

Schneider, T. D., and Stephens, R. M., 1990 Sequence logos: a new way to display consensus sequences. Nucleic Acids Res 18:6097-6100.

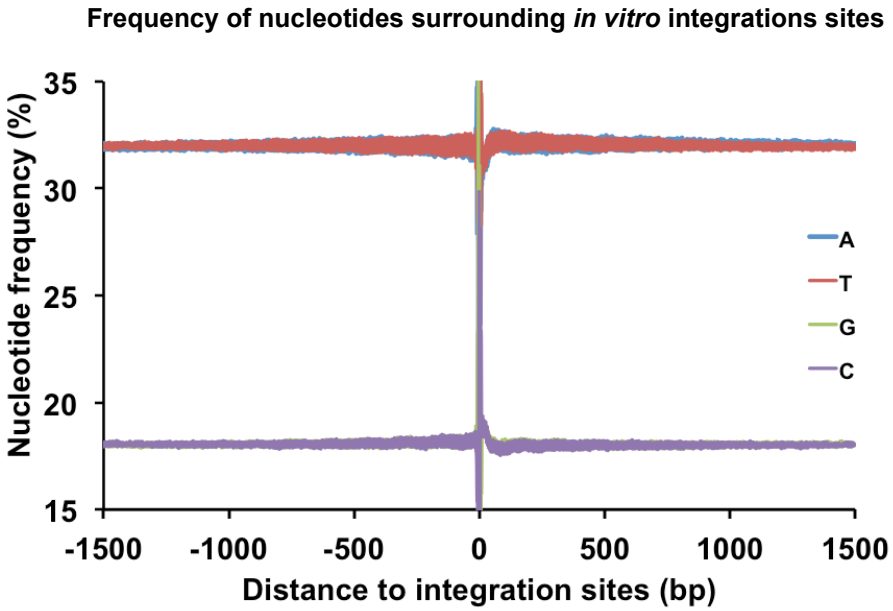

Figure S3 The average nucleotide frequencies were determined for the positions on either side of the *in vitro* insertion sites. The insertion sites were aligned and the nucleotide frequencies were calculated for a window that extended 1.5 kb on both sides.

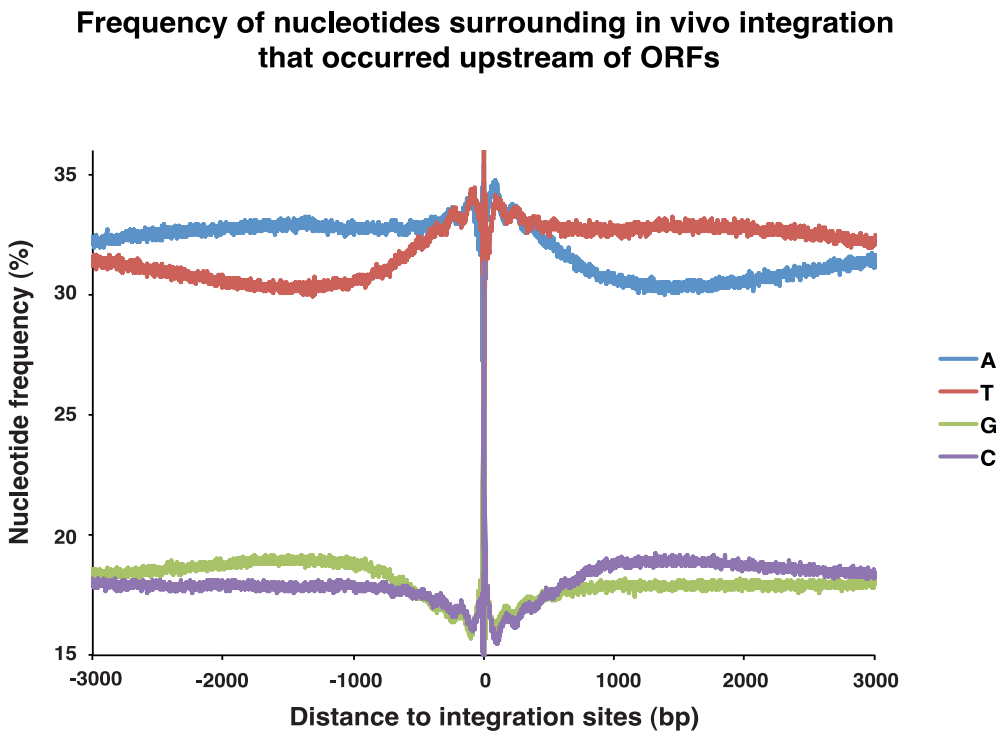

Figure S4 The average nucleotide frequencies were determined for the positions on either side of the *in vivo* insertion sites that occurred upstream of ORFs. The insertion sites were aligned and the nucleotide frequencies were calculated for a window that extended 3.0 kb on both sides.

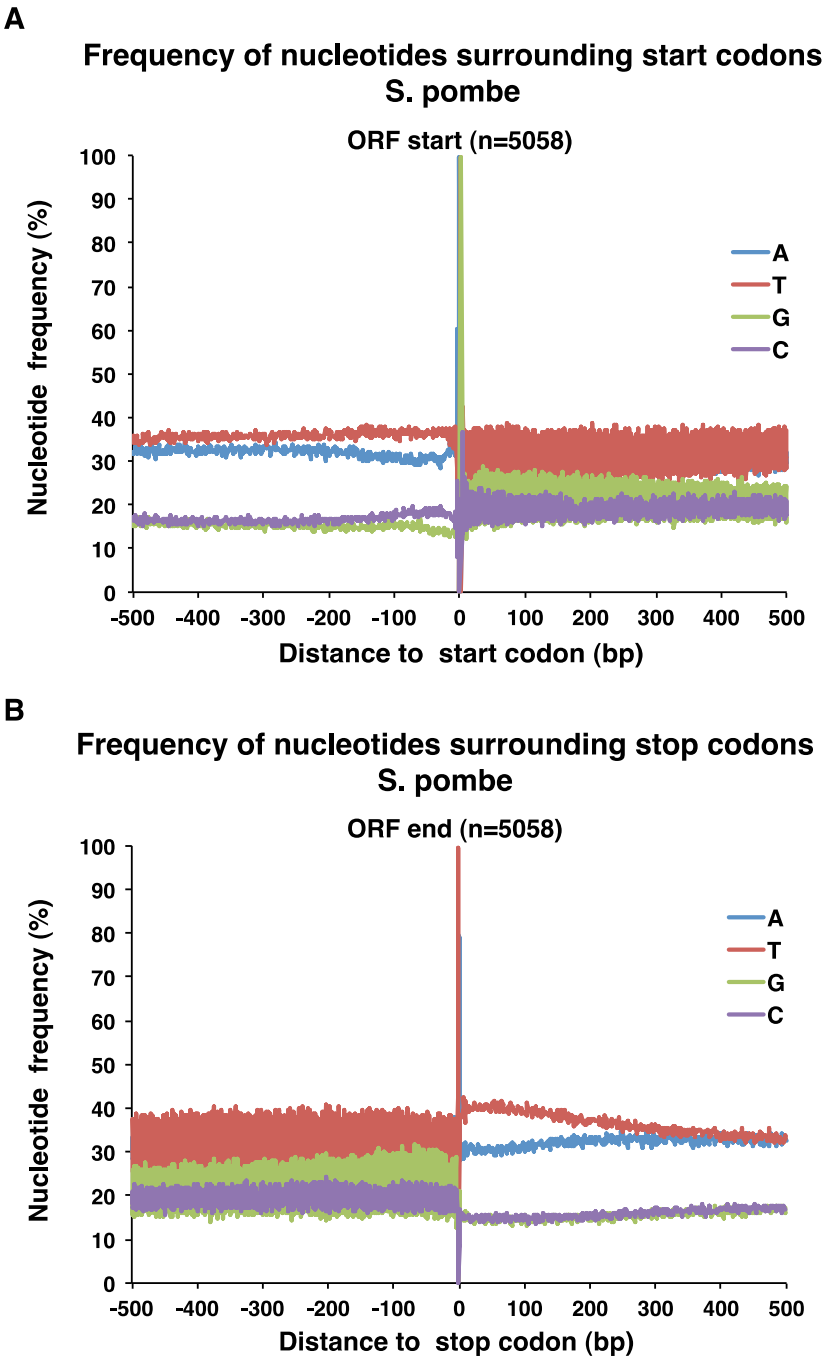

Figure S5 The average nucleotide frequencies upstream and downstream of ORFs were determined. A. The ATGs of the *S. pombe* ORFs were aligned and the nucleotide frequencies upstream of the ORFs were plotted. B. The stop codons of the *S. pombe* ORFs were aligned and the nucleotides frequencies downstream were plotted.

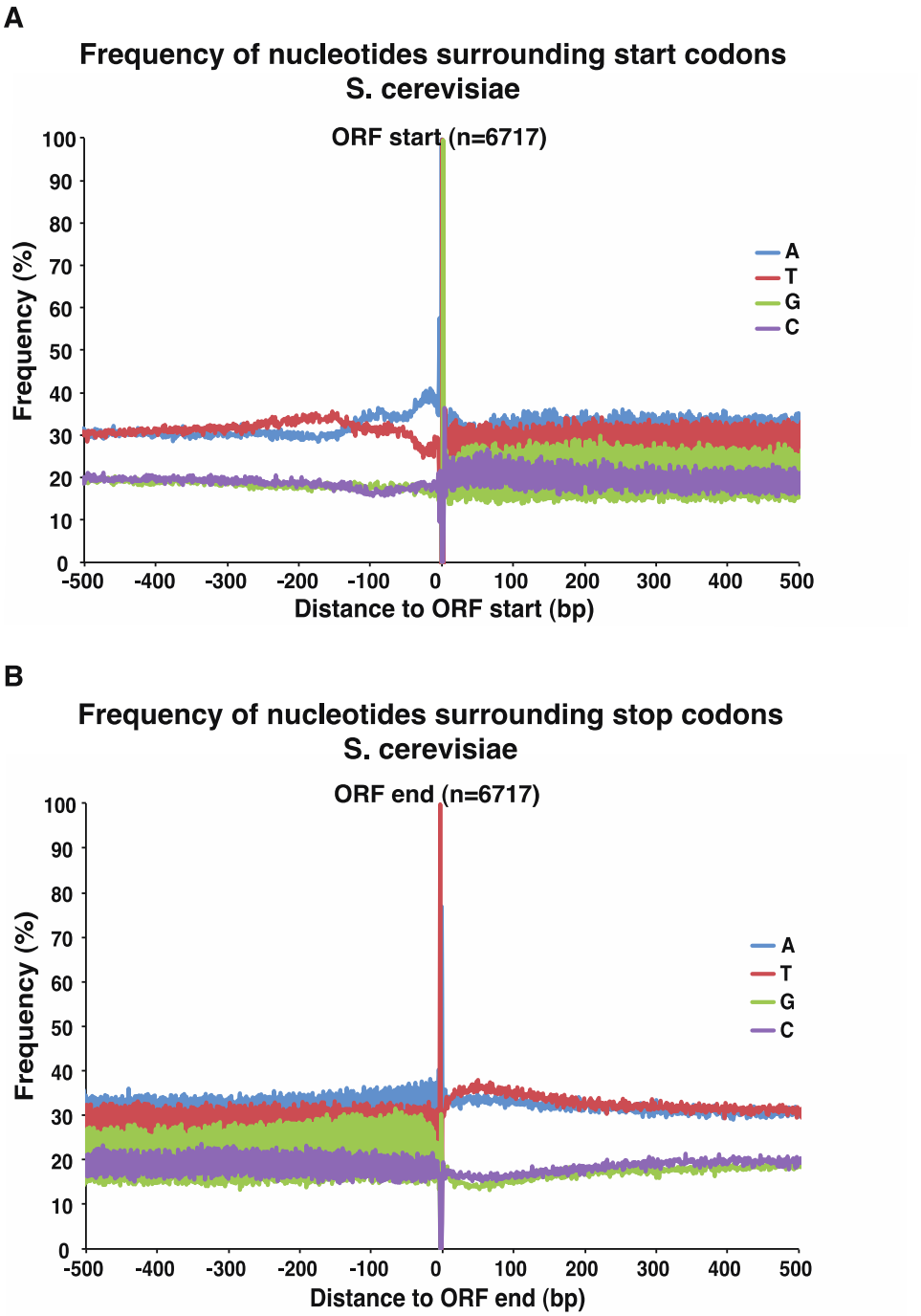

Figure S6 The average nucleotide frequencies upstream and downstream of ORFs in *Saccharomyces cerevisiae* were determined. A. The ATGs of the *S. cerevisiae* ORFs were aligned and the nucleotide frequencies upstream of the ORFs were plotted. B. The stop codons of the *S. cerevisiae* ORFs were aligned and the nucleotides frequencies downstream were plotted.

File S1

Materials and Methods

Plasmid constructs

The donor plasmid (pHL2577) and expression plasmid (pHL2578) have been previously described and are listed in Table S6. The Ura-marked donor plasmid consists of the KanMX6 gene cloned between the Hermes TIRs. The Leu-marked expression plasmid contains the Hermes transposase gene driven by the Rep81x nmt1 promoter.

The plasmid pHL2806 was created by combining the HindIII fragment of pHL2578 containing LEU2 with the HindIII fragment of pON177 in order to replace the URA3 marker of pON177 with LEU2.

To generate the plasmids for the essentiality test, the DNA fragments encoding mmf1, mrpl16, mrpl19, and SPBC2d10.08c were amplified by PCR using genomic DNA from YHL912 as a template and the primers in Table S7. Each PCR reaction consisted of 5 µL 10X Pfx amplification buffer, 1.5 µL 10 mM dNTPs, 1 µL 50 mM MgSO4, 1.5 µL primer mix (10 µM each), 100 ng template DNA, 0.4 µL Platinum Pfx DNA polymerase (2.5 U/µL), and water to 50 µL. The thermocycling conditions were as follows: 94ºC 2 min, 25-35x [94ºC 15 sec, 55ºC 30 sec, 68ºC for 1 minute per kb], 72ºC 10 min, hold 4ºC. The products were purified on Qiagen PCR purification columns and cloned into pCR□2.1-TOPO (Invitrogen) following manufacturer's instructions. The constructs were then digested with SalI and XhoI (NEB) (SPBC2d10.08c) or with XhoI and SacI (NEB) (mmf1, mrpl16, and mrpl19) to isolate the PCR gene inserts. The gene inserts were then cloned into pHL2612 to generate the deletion plasmids listed in Table S6.

Generating a library of Hermes insertions

A single colony from YHL9609 was used to inoculate a 5 mL starter culture of EMM-Leu-Ura+B1 that was grown for 16 hours at 32ºC and ~200 rpm. The culture was then pelleted at 3,000 rpm for 5 minutes and washed three times with 50 mL EMM-Leu-Ura-B1 to remove any traces of thiamine. The pellet was resuspended in 2 ml of the same media and the OD600 of the culture was measured. These measurements were used to calculate the volume of cells needed to inoculate 50 ml EMM-Leu-Ura-B1 at OD600=0.05. The absence of thiamine from this culture induced transcription of the Hermes transposase enzyme and initiated transposition. This culture was grown to OD600 =5, and was used to inoculate a new series of cultures at OD600=0.05. The serial passaged cultures were continued until the percent of cells with integration reached 13%, or about 80 generations of cell division. In all, it took a series of 12 sequential cultures for the strain to reach this point.

The final 50 ml culture was used to inoculate 500 ml of EMM+Leu+Ura+B1+FOA at OD600=0.25 to select against the donor plasmid. The cultures were grown for 4 days until an OD600 of 3 was reached. The EMM+FOA culture was pelleted, washed, and used to inoculate 500 mL YES+FOA+G418 at OD600=0.5 to select cells with Hermes insertions. The YES+FOA+G418 culture was grown for 48 hours to an OD600 of 10.

To measure transposition frequencies over the course of serial passaging, cells from cultures that reached OD600=5 were used to make a series of five 10-fold dilutions, starting at 10^8 cells/ml and ending with 10^4 cells/ml. From the three most dilute cultures, 100 µl of cells were spread on EMM+Leu+Ura+FOA+B1 plates. The same volume from the three least dilute cultures was spread on YES+FOA+G418 plates. Transposition frequency was calculated by dividing the number of colonies on EMM+Leu+Ura+FOA+B1 by the number of colonies on YES+FOA+G418.

To confirm that the majority of the cells lacked the donor plasmid and contained a Hermes integration event following growth in YES+FOA+G418 cultures, cells from the final YES+FOA+G418 cultures were plated as above, but on EMM complete and EMM-Ura. The percentage of cells retaining the donor plasmid was calculated by dividing the number of colonies on EMM -Ura by the number of colonies on EMM complete.

The protocol that follows below describes methods to prepare Hermes insertion libraries for high-throughput Illumina sequencing.

Genomic DNA extraction

The final 500 ml YES+FOA+G418 liquid culture was spun down at 3,000 rpm for 5 minutes. The pellet of cells was resuspended in 35 ml of Sp1 (1.2 M sorbitol, 50 mM citric acid monohydrate, 50 mM Na2HPO4*7H20, and 40 mM EDTA, pH 5.6) containing 105 mg Zymolyase 100T (Seikagaku) previously dissolved. Cells were incubated at 37ºC for 1-2 hours with gentle shaking. The cells were pelleted and resuspended in 105 mL of 5X TE and 1% SDS. Cells were incubated at 25ºC for 1 hour and then at 65ºC for five minutes. After this point, 35 ml of 5M KOAc was added to the mixture, and the cells were incubated on ice for 30 minutes. The cells were centrifuged for 15 min at 4200 rpm in an SS34 rotor, and an equal volume of ice-cold isopropanol was added to the supernatant. The mixture was placed at -20ºC overnight. The mixture was then centrifuged at 8000 rpm in an SS34 rotor for 10 minutes. The DNA pellet was resuspended in 21 ml of 5X TE, and RNaseA (Qiagen) was added to a final concentration of 100 µg/ml. The solution was incubated at 37ºC for 1-2 hours. Three phenol extractions and one phenol/chloroform/isoamyl alcohol extraction were performed to remove proteins. The nucleic acids were precipitated using 1/10 volume of 5 M NaCl and 2.5 volumes of 100% ethanol. The pellet was washed with 14 ml 70% ethanol, air dried, and resuspended in 300 µL 1X TE. The yield was approximately 100 µg.

Restriction endonuclease digestion

Genomic DNA was digested with MseI in a series of 6 duplicate digests. Each digest consisted of 1-2 µg DNA, 10 µl 10X NEB Buffer #2, 1 µL 100X BSA (NEB), 3 µl MseI (10U/µl, NEB), and water to 100 µl. The digests were incubated at 37ºC for 16 hours, followed by purification of each digest on a Qiagen PCR purification column. DNA was serially eluted from each column with a total of 150 µl EB.

Linker ligation

This step ligates MseI compatible ends of digested genomic DNA to linkers containing MseI restriction-site overhangs. Linkers were prepared by mixing equal amounts of HL1870 and HL1871 at a concentration of 10 µM in PCR buffer. For linker annealing, the mixture was denatured for 1 minute at 95ºC in a PCR machine, and the temperature was decreased to 80ºC for 7 minutes, and continued decrease by 10ºC every 7 minutes until 20ºC. The mixture was stored at -20ºC when not in use.

The linkers were ligated to MseI-digested DNA in a reaction consisting of 125 µl of purified MseI digest product, 40 µl 5X Ligase buffer, 35 µl annealed linker oligonucleotides (10 µM), and 5 µl T4 DNA Ligase (1U/µl). The reaction mixture was divided in 20 µl aliquots among 10 tubes, which were incubated at 18ºC for 16 hours, and stored at -20ºC.

PCR amplification of Hermes insertion sites

This step uses the linker-ligated genomic DNA fragments as templates for PCR amplification of genomic sequences flanking Hermes integration sites. A series of 40 PCR reactions were carried out in a 96-well plate. Each reaction consisted of 8 µl linker ligation template, 5 µl 10X PCR buffer, 1 µl dNTPs (10 mM), 1 µl LTR primer (10 µM, HL2215), 1 µl linker primer (10 µM, HL2216), 1 µl 50X Titanium Taq polymerase (Clontech), and water to 50 µl. Amplification was performed with the following cycling conditions: 94ºC 1 min, 6x [94ºC 15 sec, 65ºC 30 sec, 72ºC 30 sec], 24x [94ºC 15 sec, 60ºC 30 sec, 72ºC 30 sec], 72ºC 10 min, hold 4ºC.

Gel extraction and purification

Following amplification, PCR reactions were pooled and purified on 6 Qiagen PCR purification columns. The DNA was eluted in 400 µl EB and the entire volume of eluate was loaded in 1 lane (tape together multiple wells to create one large lane) of a 2% low melting point agarose, 1X TAE gel and run a few cm at 80V, next to a 100 bp DNA size standard for size reference. The DNA from 150-500 bp was excised with a clean razor blade and extracted using the Qiagen Gel purification kit following manufacturer's instructions. The DNA was eluted with 500 µL EB and extracted with one phenol extraction, one phenol:chloroform:isoamyl alcohol extraction, and one final chloroform extraction, using a 1:1 volume for each extraction. The products were purified on one Qiagen PCR purification column and eluted in 70 µl TE. DNA was quantified with a Quant-iT PicoGreen Fluorescence kit for dsDNA (Invitrogen).

Illumina sequencing

The DNA samples were sequenced by the Illumina Fast Track Sequencing Service in Hayward, California. The sequence reads were 50 nt in length. The raw sequences for the *in vitro* and *in vivo* inserts were deposited in the SRA database of Genbank with accession number SRA043841.1.

Genomic resources

All genomic resource data of *S. pombe* used in this study was downloaded from the Wellcome Trust Sanger Institute (ftp://ftp.sanger.ac.uk/pub/yeast/pombe/). The chromosome contigs are the Aug. 2007 version. The CDS coordinates are the Feb. 2009 version. The UTR data was extracted from the .embl files of 20110204 version.

Mapping Hermes integration sites on the genome of S. pombe.

Sequence reads from Illumina were screened for those containing Hermes left end. Then the Hermes sequences were trimmed. The trimmed sequences were aligned to the S. pombe genome using the NCBI BLAST software (blastall) on a local computer. The BLAST results were filtered to collect matches with genomic sequence that started from the first nucleotide after the Hermes end and with identities greater than or equal to 95% and expect (e) values less than or equal to 0.05. Then, of the matches that met these criteria, the one with the highest bit score was used to obtain the coordinates for the unique insertion sites. Sequences that were found to have the same insertion coordinate and the same orientation, were considered to be duplicate reads, and were counted as only one independent integration event.

Matched random control (MRC)

For each Hermes insertion site, the distance between the integration site and the responsible MseI site (d) was calculated. Then another MseI site coordinate (m) was randomly chosen from the S. pombe genome. Then m+d or m-d was taken as an MRC site. To "add" or "subtract" was also randomly determined. Thus, the MRC dataset has the same size as the experimental integration dataset and matches the distances to MseI sites.

General bioinformatic analysis and programming

The scripts for screening the raw sequences, filtering the BLAST outputs, extracting features from .embl files, determining the locations of Hermes insertion according to chromosomal features, generating MRC and other analyses were written in Perl or Ruby programming languages.

Nucleosome DNA preparation and sequencing

Nucleosomal DNA was prepared as described previously (YAMANE *et al.* 2011), and DNA samples were sequenced by using the Illumina sequencing protocol. Bowtie (LANGMEAD *et al.* 2009) was used to map the Illumina sequencing reads, trimmed to 25 bp of high quality reads, against the reference genome allowing for 2 mismatches. The mapped data was filtered to remove all sequences that mapped to more than a single location. The end positions of the reads were aligned relative to the center of the nucleosome by shifting the plus strands reads by +73 bp and the minus strand reads by -73 bp. The final nucleosome maps were produced by applying gaussian smoothing to the raw data in order to reduce noise. More detailed methodology will be described in a forthcoming manuscript on the genome wide mapping of nucleosomes in S. pombe.

Essentiality Test

To test the viability of four representative Bioneer strains heterozygous for deletions in nuclear-encoded mitochondrial genes designated as essential, we performed a plasmid shuffle assay. Following transformation with both the plasmids encoding the deleted genes and pHL2806 (needed to allow sporulation), the diploid strains carrying the plasmid with the deleted genes were sporulated by inoculating them at OD600=0.05 in 200 µl of low nitrogen media, and incubating them for 3 days at 25ºC in a rotating wheel. Low nitrogen media is identical to EMM media, except with 1g/l glutamic acid in place of NH4Cl. The cells were then pelleted and resuspended in water to OD600=0.1, and 20 µl of a 10-fold diluted stock of glusulase (PerkinElmer) was added to the mixture. The mixtures were allowed to incubate overnight at 32ºC in a rotating wheel. The cells were then pelleted, resuspended in 300 µl of 30% ethanol, and incubated for 30 minutes at 25ºC. Subsequently, the cells were pelleted, resuspended in 100 µl of water, plated on YES+G418 to select for haploid cells containing the KanMX6 deletion cassette, and grown for 3 days at 32ºC.

Gene essentiality was tested on solid media by patch assays and drop assays. For each patch assay, 15 cells from the spore germination plates were colony purified on YES+G418 plates and then patched to YES master plates that were grown at 32ºC for 2 days. These master plates were replica printed to YES+FOA plates (to select against the deletion plasmid) and grown at either 32ºC or 37ºC for 46 hours.

For each drop assay, a 1 ml stock of cells at OD= 0.5 was used to create a series of 5-fold dilutions. From each dilution, 10 µl of cells was plated on YES and PM+FOA plates and grown at 32ºC or 37ºC for 2-3 days.

Table S1

ORFs designated by the consortium to be essential but had high integration densities.

Gene	Gene function	integration/kb/million insertions
SPBC21C3.10c	5-amino-6-(5-phosphoribosylamino) uracil reductase	161.5
tim10 SPAC222.03c	Tim9-Tim10 complex subunit Tim10 (predicted)	130.2
mrpl16 SPBC1105.03c	mitochondrial ribosomal protein subunit L16	128.4
SPBP8B7.05c	carbonic anhydrase (predicted)	124.5
SPBC2D10.08c	mitochondrial ribosomal protein subunit Yml6	123.5
mrpl31 SPCC16A11.11	mitochondrial ribosomal protein subunit L31	111.6
ste11 aff1 stex SPBC32C12.0	transcription factor Ste11	108.4
SPAC1486.07c mrpl19	mitochondrial ribosomal protein subunit L19	102.0
SPCC1672.01	histidinol-phosphatase (predicted)	100.8
SPCC2H8.04	sequence orphan	99.4
met9 met5 SPAC56F8.10	methylenetetrahydrofolate reductase Met9	94.9
SPAC17H9.07	signal recognition particle subunit Srp21 (predicted)	94.6
tpx1 SPCC576.03c	thioredoxin peroxidase Tpx1	86.2
vph2 SPCC757.10	endoplasmic reticulum membrane involved in assembly of the V-ATPase	84.5
ups1 ups SPAC31G5.08	uroporphyrinogen-III synthase Ups1	80.7
mmf1 pmf1 SPBC2G2.04c	YjgF family protein Mmf1	79.4
grx4 SPBC26H8.06	glutaredoxin Grx4	79.3
SPAC30C2.03	sequence orphan	78.9
SPBC2A9.10	Bin3 family	78.4
SPAC12B10.02c	sequence orphan	78.4
mrp10 SPAC24C9.13c	mitochondrial ribosomal protein subunit Mrp10	78.0
SPBC3B9.14c mrpl3	mitochondrial ribosomal protein subunit L3	77.3
SPCC1682.09c	guanine nucleotide transporter	74.1
SPAC1002.16c	nicotinic acid plasma membrane transporter (predicted)	74.0
SPAC688.09	mitochondrial pyrimidine nucletide transporter (predicted)	70.9
SPBC577.09	ERCC-8 DNA repair homolog (predicted)	70.3
SPAC17G8.02	uridine ribohydrolase (predicted)	69.8
SPAC31G5.06	mitochondrial protein	69.8
dea2 SPBC1198.02	adenine deaminase Dea2	67.8
SPCC63.10c	dolichol kinase (predicted)	67.4
SPAC24C9.06c	aconitate hydratase	66.5
SPBC354.06 mrps16	mitochondrial ribosomal protein subunit S16	65.5
SPBP4H10.15	aconitate hydratase/mitochondrial ribosomal protein subunit L49, fusion protein	64.9
bet5 SPAC688.15 SPAC3G9.1	TRAPP complex subunit Bet5 (predicted)	64.6
usp103 yhc1 SPBP35G2.09	U1 snRNP-associated protein Usp103 (predicted)	63.6
crk1 mop1 mcs6 SPBC19F8.(	cyclin-dependent kinase activating kinase Crk1	63.3
SPAPB17E12.09	sequence orphan	63.2
mrp20 SPAC31A2.08	mitochondrial ribosomal protein subunit L23 (predicted)	62.8
fta3 SPBP8B7.12c sma3	Sim4 and Mal2 associated (4 and 2 associated) protein 3	62.8
SPBC887.07 mrpl38	mitochondrial ribosomal protein subunit L38	62.7
SPBC2F12.10	mitochondrial ribosomal protein subunit L35	62.3
SPCC4B3.09c	mitochondrial ribosomal protein subunit L12	62.1
SPAPB24D3.06c	DUF1749 family protein	61.3
SPBC119.18	mitochondrial distribution and morphology protein Mdm35 (predicted)	61.2
SPBC17G9.13c	sequence orphan	61.1
SPAC2F7.15 rsm24	mitochondrial ribosomal protein subunit S24 (predicted)	60.9
SPBC16H5.15	conserved fungal protein	60.9
SPCC1393.11	mitochondrial ribosomal protein subunit L20	60.7
sum2 SPBC800.09	G2/M transition checkpoint protein Sum2	60.6
alr2 SPBC359.02	alanine racemase Alr2	59.8
SPAC3A12.19	mitochondrial ribosomal protein subunit L27	59.4

Table S2 The levels of integration for each ORF of the genome

Table S2 is available for download at http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.113.152744/-/DC1.

Table S3

Test of deletion strains version 2.0 for presence of ORFs designated by the consortium to be nonessential.

chromosome	gene	start	end	strand	Number of integrations	Density (integration/kb /million insertions)	Gene description	bands in colony PCR	bands with the same primers in wt.	ORF detected on Southern blot
chr1	rpl36a SPAC15E1.03 rpl42	3722509	3723019	+	0	0.00	60S ribosomal protein L36/L42	2	2	√
chr1	ubi1 SPAC11G7.04	3969009	3969395	+	0	0.00	ribosomal-ubiquitin fusion protein Ubi1	1	2	
chr1	SPAC186.06	5542419	5542973	+	0	0.00	human MAWBP homolog	0	2	
chr2	smd3 SPBC19C2.14	1702182	1702628	+	0	0.00	Sm snRNP core protein Smd3	2	2	
chr2	SPBC16A3.16	4267784	4268090	-	0	0.00	mitochondrial inner membrane protein	0	2	
chr3	SPCC14G10.03c ump1	748037	748530	-	0	0.00	proteasome maturation factor Ump1	1	2	
chr3	SPCC162.01c	1587869	1588603	+	0	0.00	RNA-binding protein	2	2	
chr1	rhp54 rad54 SPAC15A10.03c	3680587	3683145	-	1	1.08	Rad54 homolog Rhp54	0	2	
chr2	SPBC577.06c	758408	764229	-	4	1.91	phosphatidylinositol kinase	2	2	
chr2	tif213 SPBC17G9.09	2187712	2189052	+	1	2.07	translation initiation factor eIF2 gamma subunit	2	2	√
chr2	arp5 SPBC365.10	2512667	2514887	+	2	2.50	actin-like protein Arp5	0	2	
chr1	fas1 SPAC926.09c	3900367	3906588	-	6	2.67	fatty acid synthase beta subunit Fas1	2	2	
chr1	SPAC186.03	5535198	5536280	+	1	2.56	L-asparaginase	1	1	
chr1	nup184 SPAP27G11.10c	1625076	1629841	-	6	3.49	nucleoporin Nup184	1	1	
chr1	spt6 SPAC694.07c SPAC1F7.01c	4212706	4216803	-	6	4.06	transcription elongation factor Spt6	2	2	√
chr3	bgs4 orb11 cwg1 SPCC1840.02c	2249279	2255146	-	9	4.25	1,3-beta-glucan synthase subunit Bgs4	2	2	
chr2	gpd1 SPBC215.05	4035814	4036971	+	2	4.79	glycerol-3-phosphate dehydrogenase Gpd1	0	1	
chr3	tif6 SPCC1919.09	2223154	2224257	+	2	5.03	translation initiation factor eIF6	2	2	√
chr1	pub1 SPAC11G7.02	3963323	3965870	+	5	5.44	ubiquitin-protein ligase E3	1	2	
chr2	atp16 SPBC13E7.04	3046873	3047355	+	1	5.74	F1-ATPase delta subunit	2	2	
chr2	SPBC1539.08 arf6	4375857	4376810	+	2	5.82	ADP-ribosylation factor, Arf family	1	2	
chr2	SPBC27B12.08	1335724	1341821	+	13	5.91	AP-1 accessory protein	0	1	
chr1	arp42 arp4 SPAC23D3.09	4353826	4355118	-	3	6.44	SWI/SNF and RSC complex subunit Arp42	0	2	
chr1	SPAC869.04	5511137	5512369	-	3	6.75	formamidase-like protein	1	2	
chr2	ypt7 SPBC405.04c	3147478	3148230	-	2	7.37	GTPase Ypt7	1	1	
chr1	vps28 SPAC1B3.07c	4940690	4941436	-	2	7.43	ESCRT I complex subunit Vps28	0	2	
chr3	SPCC5E4.10c	651622	651994	-	1	7.44	sequence orphan	0	2	
chr2	wis1 spc2 smf2 SPBC409.07c	1143967	1145784	-	5	7.63	MAP kinase kinase Wis1	2	2	
chr1	ies6 SPAC222.04c	950298	950651	-	1	7.84	chromatin remodeling complex subunit Ies6	2	2	
chr1	SPAC664.13	1729505	1729858	+	1	7.84	sequence orphan	2	2	
chr2	SPBPB10D8.01	81295	82953	+	5	8.36	cysteine transporter	0	2	
chr3	rpt4 SPCC1682.16	405612	407251	+	5	8.46	19S proteasome regulatory subunit Rpt4	2	2	√
chr2	sec63 SPBC36B7.03	2387093	2389038	+	6	8.55	ER protein translocation subcomplex subunit Sec63	2	2	√
chr1	SPAC20G8.09c	1415542	1418750	-	10	8.64	N-acetyltransferase Nat10	2	2	
chr2	mcs4 SPBC887.10	3557274	3558842	+	5	8.84	two-component response regulator	0	2	
chr2	SPBC9B6.07	1824562	1825497	+	3	8.89	nucleolar protein Nop52 family	0	2	
chr1	plc1 SPAC22F8.11	4807786	4810485	+	9	9.25	phosphoinositide phospholipase C Plc1	2	2	
chr3	ekc1 SPCC777.16c SPCC663.01c	1626547	1629120	-	16	17.24	protein phosphatase regulatory subunit Ekc1	2	2	
chr1	mcl1 slr3 SPAPB1E7.02c	3283195	3285745	-	16	17.40	DNA polymerase alpha accessory factor Mcl1	0	2	
chr2	swi5 SPBC409.03	1138056	1138530	+	3	17.52	Swi5 protein	1	1	
chr3	pof3 SPCC338.16	1345394	1347127	-	11	17.60	F-box protein Pof3	0	2	
chr1	SPAC6F6.03c	2734109	2736126	-	13	17.87	ribosome export GTPase	2	2	
chr3	SPCC1919.04	2211103	2211873	+	5	17.99	sequence orphan	1	2	
chr1	SPAC16C9.05 cph1	799550	800764	+	8	18.26	PHD finger containing protein	2	2	
chr2	SPBC119.12	740275	741480	+	8	18.40	Golgi matrix protein	0	2	
chr2	lsd1 SPBC146.09c swm1 saf110	1016000	1019002	-	20	18.47	histone demethylase SWIRM1	2	2	
chr2	nfs1 SPBC21D10.11c	2422769	2424265	+	10	18.53	iron-sulfur cluster assembly protein Nfs1	2	2	
chr1	pzh1 SPAC57A7.08	1527964	1529511	-	14	25.09	serine/threonine protein phosphatase Pzh1	0	2	
chr3	set1 SPCC306.04c	412765	415527	-	25	25.10	histone lysine methyltransferase Set1	0	1	
chr3	set9 SPCC4B3.12	1154281	1155606	-	12	25.10	histone lysine methyltransferase Set9	2	2	
chr2	rad3 SPBC216.05	904365	911525	+	65	25.18	ATR checkpoint kinase	0	2	
chr2	pdb1 SPBC30D10.13c	3070543	3071643	+	10	25.19	pyruvate dehydrogenase e1 component beta subunit Pdb1	2	2	
chr1	pds5 SPAC110.02	1912042	1915659	+	33	25.30	cohesin-associated protein Pds5	0	2	
chr1	cit1 SPAC6C3.04	2328675	2330096	+	13	25.36	citrate synthase	2	2	
chr3	ccr4 SPCC31H12.08c SPCC5E4.02c	637461	639533	-	19	25.42	CCR4-Not complex subunit Ccr4	0	2	
chr2	hip3 SPBC31F10.14c hir3	3777778	3782670	-	45	25.51	HIRA interacting protein Hip3	0	2	
chr2	SPBC1685.04	502873	503850	+	9	25.53	sequence orphan	0	1	
chr2	SPBC30D10.14	3067996	3068745	-	9	33.29	dienelactone hydrolase family	0	2	
chr3	SPCC645.13	1258634	1260799	+	26	33.30	transcription elongation regulator	0	2	
chr3	SPCC569.07	2415649	2417061	-	17	33.37	aromatic aminotransferase	0	2	
chr1	atp2 SPAC222.12c	968783	970360	-	19	33.40	F1-ATPase beta subunit	2	2	
chr2	SPBC1A4.05	1984516	1986671	+	26	33.45	sequence orphan	1	2	
chr1	spo15 SPAC1F3.06c	625430	631303	-	71	33.53	sporulation protein Spo15	0	2	
chr1	fhl1 SPAC1142.08 SPAC8C9.01	3639988	3642219	+	27	33.55	fork head transcription factor Fhl1	0	2	
chr2	amo1 SPBC15D4.10c	3028705	3030192	-	18	33.55	nuclear rim protein Amo1	1	2	
chr1	hem2 SPAC1805.06c	2783941	2784930	-	12	33.62	porphobilinogen synthase Hem2	2	2	
chr2	vps13a SPBC31F10.18c SPBC21C3.01c	3789386	3798703	-	113	33.64	chorein homolog	0	2	
chr1	gmh3 SPAC22E12.06c	5029000	5029998	-	15	41.65	alpha-1,2-galactosyltransferase Gmh3	0	2	
chr3	SPCC1840.07c	2270637	2271635	-	15	41.65	phosphoprotein phosphatase	0	2	
chr3	SPCC622.19 jmj4 mug149	1438375	1439839	+	22	41.65	Jmj4 protein	0	2	
chr1	SPAC24C9.05c mug70	3045121	3047313	-	33	41.74	conserved protein (fungal and plant)	0	2	
chr1	SPAC16E8.17c	3532418	3533413	-	15	41.77	succinate-CoA ligase	0	1	
chr1	SPAC31A2.12	412084	413874	+	27	41.82	arrestin/PY protein 1	0	2	
chr1	msh3 swi4 SPAC8F11.03	2855931	2858980	+	46	41.83	MutS protein homolog 3	0	2	
chr2	SPBC1773.16c	318693	320480	-	27	41.89	transcription factor	0	2	
chr3	SPCC1620.12c	2167799	2169586	-	27	41.89	GTPase activating protein	0	2	
chr1	idp1 SPAC6G10.08	3231599	3232855	+	19	41.93	isocitrate dehydrogenase Idp1	1	2	

Table S4 Chromosome coordinates of Hermes insertions

Table S4 is available for download at http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.113.152744/-/DC1.

Table S5 Strains of *S. pombe* used in this study

Yeast strain	Genotype	Reference
YHL#10046	FY243, h-cdc19-P1 ura4-D18 leu1-32 ade6-M210	Gift of Susan Forsburg
YHL240	h+ ura4-294::neo-ura4+	This study
YHL912	h- leu1-32 ura4-294	(*1*)
YHL9176	YHL912 with pHL423 and pHL2577	
YHL9451	YHL912 with pHL2577	This study
YHL9609	YHL 9451 with pHL2578	This study
YHL9799	yBN146 with pHL2808	This study
YHL9800	yBN147 with pHL 2809	This study
YHL9801	yBN148 with pHL2810	This study
YHL10044	yBN149 with pHL2825	This study
YHL10045	YHL10046 with pSLF124	
yBN24	h- ade6-M210/ade6-M216 ura4-D18 leu1-32 SPBC21C3.09c::KanMX4/ SPBC21C3.09c	Bioneer Corporation collection BG_3108
yBN146	h+/h+ ade6-M210/ade6-M216 ura4-D18/ura4-D18 leu1-32/leu1-32 mmf1::KanMX4/mmf1	Bioneer Corporation collection BG_3366
yBN147	h+/h+ ade6-M210/ade6-M216 ura4-D18/ura4-D18 leu1-32/leu1-32 mrpl16::KanMX4/mrpl16	Bioneer Corporation collection BG_2355
yBN148	h+/h+ ade6-M210/ade6-M216 ura4-D18/ura4-D18 leu1-32/leu1-32 mrpl19::KanMX4/mrpl19	Bioneer Corporation collection BG_0301
yBN149	h+/h+ ade6-M210/ade6-M216 ura4-D18/ura4-D18 leu1-32/leu1-32 SPBC2d10.08c::KanMX4/SPBC2d10.08c	Bioneer Corporation collection BG_3334

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Table S6 Plasmids.

Plasmid	Markers	Description	Reference
pHL423	amp, LEU2	Rep3 *nmt1* promoter, control plasmid lacking Hermes transposase	(*1*)
pHL2577	amp, URA3	Hermes donor with TIR's flanking *kanMX6*	(*2*)
pHL2578	amp, LEU2	rep81X *nmt1* promoter expressing wt Hermes transposase	(*2*)
pHL2612	amp, Ura4	URA4 cloned into pRO322 vector	(*3*)
pHL2806	amp, LEU2	A 2.2 kb Hind III fragment with LEU2 from REP81X was inserted into the 9.0 kb Hind III fragment of PON177{Kim, 2010 #4464}. This plasmid allows sporulation of Bioneer diploids.	this study
pHL2808	amp, Ura4	mmf1 gene PCR cloned into pHL2612	this study
pHL2809	amp, Ura4	mrpl16 gene PCR cloned into pHL2612	this study
pHL2810	amp, Ura4	mrpl19 gene PCR cloned into pHL2612	this study
pHL2825	amp, Ura4	SPBC2d10.08c gene PCR cloned into pHL2612	this study
pSLF124	amp, Ura4	carries genomic clone of cdc19 to complement disruption of cdc19 in FY243	gift of Susan Forsbrug

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3. C. Adams, D. Haldar, R. T. Kamakaka, *Yeast* **22**, 1307 (Dec, 2005).

Table S7 Oligonucleotides.

Name	Sequence; function
HL1870	GTAATACGACTCACTATAGGGCTCCGCTTAAGGGAC; linker primer
HL1871	[phos]TAGTCCCTTAAGCGGAG[AmC7~Q]; linker primer
HL2215	AATGATACGGCGACCACCGAGATCT-CTATGTGGCTTACGTTTGCCTG; PCR primer with P5 tag followed by Hermes seq
HL2216	CAAGCAGAAGACGGCATACGAGCTCTTCCGATCT-GTAATAACGACTCACTATAGGGC' PCR primer with P7 tag followed by linker seq
HL2434	CTATGTGGCTTACGTTTGCCTGTGGCTTGTTG; custom Illumina sequencing primer
HL2550	ATCTCGAGTCCAGGAGCAGATGCAGCAC; PCR primer to clone mmf1
HL2551	ATGAGCTCGGCGCACGAAAATGAAAAGC; PCR primer to clone mmf1
HL2552	ATCTCGAGCAACGGAGCAGAGAGCAGCA; PCR primer to clone mrpl16
HL2553	ATGAGCTCTGAGGCAAGGGGTGGTAACG; PCR primer to clone mrpl16
HL2554	ATCTCGAGTCGTCGTCGTCGTGATCCTC; PCR primer to clone mrpl19
HL2555	AACTCTGCATCCCGGGACAA; PCR primer to clone mrpl19
HL2753	GTCGACAGGTTTCCATGCATTAGCTGGGCA; PCR primer to clone SPBC2d10.08c
HL2754	CTCGAGTGCAGCAATCTGAACGACGGCA; GTCGACAGGTTTCCATGCATTAGCTGGGCA PCR primer to clone SPBC2d10.08c