

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.



Frequency of nucleotides surrounding in vitro integrations sites

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: 94°C 2 min, 25-35x [94°C 15 sec, 55°C 30 sec, 68°C for 1 minute per kb], 72°C 10 min, hold 4°C. The products were purified on Qiagen PCR purification columns and cloned into pCRI2.1-TOPO (Invitrogen) following manufacturer's instructions. The constructs were then digested with Sall and Xhol (NEB) (SPBC2d10.08c) or with Xhol and Sacl (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 32°C 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 10⁸ cells/ml and ending with 10⁴ 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 highthroughput 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 37°C 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 25°C for 1 hour and then at 65°C 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 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 37°C 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 NACI 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 Msel 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 Msel (10U/µl, NEB), and water to 100 µl. The digests were incubated at 37°C 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 Msel compatible ends of digested genomic DNA to linkers containing Msel restriction-site overhangs. Linkers were prepared by mixing equal amounts of HL1870 and HL1871 at a concentration of 10 μ M in PCR buffer. For linker annealing, the mixture was denatured for 1 minute at 95°C in a PCR machine, and the temperature was decreased to 80°C for 7 minutes, and continued decrease by 10°C every 7 minutes until 20°C. The mixture was stored at -20°C when not in use.

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

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 μ l linker ligation template, 5 μ l 10X PCR buffer, 1 μ l dNTPs (10 mM), 1 μ l LTR primer (10 μ M, HL2215), 1 μ l linker primer (10 μ M, HL2216), 1 μ l 50X Titanium Taq polymerase (Clontech), and water to 50 μ l. Amplification was performed with the following cycling conditions: 94°C 1 min, 6x [94°C 15 sec, 65°C 30 sec, 72°C 30 sec], 72°C 10 min, hold 4°C.

Gel extraction and purification

Following amplification, PCR reactions were pooled and purified on 6 Qiagen PCR purification columns. The DNA was eluted in 400 μ I 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 μ L 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 μ I 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 Msel site (d) was calculated. Then another Msel 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 Msel 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 25°C 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 32°C in a rotating wheel. The cells were then pelleted, resuspended in 300 µl of 30% ethanol, and incubated for 30 minutes at 25°C. 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 32°C.

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 32°C for 2 days. These master plates were replica printed to YES+FOA plates (to select against the deletion plasmid) and grown at either 32°C or 37°C 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 μ l of cells was plated on YES and PM+FOA plates and grown at 32°C or 37°C 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.

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

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chrl SPECGB8.07 182452 182456 182456 182577 18257 18257 18257 18257 182577 <th< td=""><td>chr2</td><td>mcs4 SPBC887.10</td><td>3557274</td><td>3558842 +</td><td>5</td><td>8.84</td><td>two-component response regulator</td><td>0</td><td>2</td><td>2</td></th<>	chr2	mcs4 SPBC887.10	3557274	3558842 +	5	8.84	two-component response regulator	0	2	2
ch1 ebc1 SPAC2298.11 480776 4810485 + 9 43.2 probabinosidia phospholeside pho	chr2	SPBC9B6.07	1824562	1825497 +	3	8.89	nucleolar protein Nop52 family	0	2	2
Back Back <th< td=""><td>chr1</td><td>plc1 SPAC22F8.11</td><td>4807786</td><td>4810485 +</td><td>9</td><td>9.25</td><td>phosphoinositide phospholipase C PIc1</td><td>2</td><td>2</td><td>2</td></th<>	chr1	plc1 SPAC22F8.11	4807786	4810485 +	9	9.25	phosphoinositide phospholipase C PIc1	2	2	2
Initis Status Status<	chr3	eKC1 SPCC///.16C SPCC663.01C	1020547	1629120 -	16	17.24	protein phosphatase regulatory subunit EKCI	2	2	2
chrd pp03 SPCC393.16 1347127 1 17.0 17.00	chr2	swi5 SPBC409 03	1138056	3285745 - 1138530 ⊥	10	17.40	Swi5 protein	1	4	
chrl SPACeF5.036 2734109 2734128 - 13 17.97 plosene expont GTPase 2 2 chrl SPACeF5.036 cph1 7950 800764 - 6 12.2 2 chrl SPACeF5.036 cph1 7950 800764 - 8 12.6 PhD Inger containing protein 2 2 chrl Ist1 SPEC140.05 sewm1 sat10 101600 10902 - 20 14.7 Phot SPACeF5.03 2 2 chrl pt1 SPACS7A7.08 13276 1228151 - 14 25.0 erine/threorine protein phosphatase Pb11 0 2 2 chrl pt1 SPACS7A7.08 13278 15525 - 2 2.5.0 16 1600 kpise methyltransferase Sel1 0 2 <td< td=""><td>chr3</td><td>pof3 SPCC338.16</td><td>1345394</td><td>1347127 -</td><td>11</td><td>17.60</td><td>F-box protein Pof3</td><td>0</td><td></td><td>2</td></td<>	chr3	pof3 SPCC338.16	1345394	1347127 -	11	17.60	F-box protein Pof3	0		2
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chrl SPAC160.08 cph1 79007 74007	chr3	SPCC1919.04	2211103	2211873 +	5	17.99	sequence orphan	1	2	2
ch:2 SPBC119:12 744275 741480 * <td>chr1</td> <td>SPAC16C9.05 cph1</td> <td>799550</td> <td>800764 +</td> <td>8</td> <td>18.26</td> <td>PHD finger containing protein</td> <td>2</td> <td>2</td> <td>2</td>	chr1	SPAC16C9.05 cph1	799550	800764 +	8	18.26	PHD finger containing protein	2	2	2
ch2 isd1 SPBC104.06e swm1 sat110 101000 101000 2 2 2 ch1 sp11 SPAC2T01011c 224226 10 15.51 100-sufur cluster assembly protein Nts1 0 2 ch1 sp11 SPAC2T0.04 127976 15527 25 25.01 histone lysine methyltransferase Set1 0 1 ch3 set1 SPC236.04c 11756 - 12 25.10 histone lysine methyltransferase Set1 0 2 ch47 set3 SPBC216.05 904365 911555 - 12 25.10 histone lysine methyltransferase Set1 0 2 ch12 pd51 SPBC3010.13c 307643 3071643 4 10 25.10 fNT checkpoint Kinase 0 2 2 ch14 cit1 SPAC3C3.04 23267 23096 4 32.30 citrate synthase 2 2 2 ch14 cit1 SPAC3C3.04 23267 23096 4 32.30 citrate synthase 2 2 ch2 hp2G1850.01.13c 50350 4 9 25.51 kiRA interacting protein Ntg3 2	chr2	SPBC119.12	740275	741480 +	8	18.40	Golgi matrix protein	0	2	2
chrl phil s PEQLUIU.11C 242/09 24/09 24/09 24/09 24/09 24/09 24/09 24/09 24/09 24/09 24/09 24/09 24/09 24/09 24/09 24/09 24/09 <	chr2	Isd1 SPBC146.09c swm1 saf110	1016000	1019002 -	20	18.47	histone demethylase SWIRM1	2	2	2
child get1 6PC Contention 41277 14278 1417 1416070 15060 12 2101 1416070 15060 211 1416070 15060 211 1416070 15060 212 210 1416070 15060 212 21 1417 <td>chr2</td> <td>nts1 SPBC21D10.11C</td> <td>2422769</td> <td>2424265 +</td> <td>10</td> <td>18.53</td> <td>Iron-sultur cluster assembly protein NIST</td> <td>2</td> <td>-</td> <td>2</td>	chr2	nts1 SPBC21D10.11C	2422769	2424265 +	10	18.53	Iron-sultur cluster assembly protein NIST	2	-	2
brid seld SPCC483.12 115428 115596 - 12 25.00 histone lysine methyltransferase Sel3 2 2 chr2 rdd SPBC216.05 90486 911555 + 0 25.10 ATR checkpoint kinase 0 2 chr1 pdt SPBC3010.13C 307043 3071643 + 10 25.00 chr3 pdt septed dehydrogenase e1 component beta subunit Pdb1 2 2 chr1 pdt SPBC3101.012C 1912042 191555 + 3 25.30 chrate synthase 2 2 chr1 thr3 PACC5414.02C 637461 63933 - 9 25.53 sequence orphan 0 2 chr2 SPBC3001.014 306799 308674 - 9 33.01 transcription elongation regulator 0 2 chr3 SPCC545.13 125864 247079 - 45 33.01 transcription elongation regulator 0 2 chr1 sp15 SPAC1F3.05 198451 198671 - 26 33.01 transcription elongation regulator 0 2 2	chr3	set1 SPCC306 04c	412765	415527 -	25	25.09	histone lysine methyltransferase Set1	0	4	
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chrl pds SPAC110.02 1912042 <td>chr2</td> <td>pdb1 SPBC30D10.13c</td> <td>3070543</td> <td>3071643 +</td> <td>10</td> <td>25.19</td> <td>pyruvate dehydrogenase e1 component beta subunit Pdb1</td> <td>2</td> <td>2</td> <td>2</td>	chr2	pdb1 SPBC30D10.13c	3070543	3071643 +	10	25.19	pyruvate dehydrogenase e1 component beta subunit Pdb1	2	2	2
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Chrl2 SP BC30D10.14 306794 3068745 - 9 3.29 direnleatore hydrolase family 0 2 chr3 SPCC685.13 1258634 1260799 + 26 3.30 transcription elongation regulator 0 2 chr3 SPCC689.07 241564 1260799 + 26 3.30 transcription elongation regulator 0 2 chr1 atp2 SPAC222.12c 968783 970360 - 19 340 F1-ATPase beta subunit 2 2 chr1 sp15 SPAC1F3.06c 625430 631303 - 71 33.53 sporulation protein Sp015 0 2 chr1 sp15 SPAC1F3.06c 625430 639398 642219 + 27 35.5 fork head transcription factor Fh11 0 2 2 chr1 sp15 SPAC1F3.06c 627430 303998 642219 + 27 35.5 fork head transcription factor Fh11 0 2 2 chr2 amo1 SPBC15D4.10c 302870 303192 - 113 364 chorein homolog 0	chr2	SPBC1685 0/	502873	503850 +	45	20.01	RIRA Interacting protein hips	0		2
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Chrl Amol 97-BC 1804-100 3026703 3027067 3027067 30270677 30270677 30270677 3027077 3026711 3027313 333 41.74 conserved protein fungal and plant) 0 2 2 chr1 SPAC3142.12 41307313 - 15 41.77 succinate-CoA ligase 0 1 2 chr1 SPAC3142.12 413083 41.77 succinat	chr1	INIT SPAC1142.08 SPAC809.01	3039988	3042219 +	27	33.55	fork nead transcription factor Fni i	0	2	2
Inclusion for the formation of the	chr1	hem2 SPAC1805 06c	2783941	2784930 -	10	33.55	nornhohilinggen synthase Hem?	2	4	
chr1 gmh3 SPAC22E12.06c 502900 502998 - 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 SPAC2429.05c mug70 304512 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 285900 + 46 41.83 Mut5 protein homolog 3 0 2 chr2 SPEC1773.16c 318693 320480 - 27 41.89 transcription factor 0 2 chr3 SPCC1620.12c	chr2	vps13a SPBC31F10.18c SPBC21C3.01c	3789386	3798703 -	113	33.64	chorein homolog	0		2
chr3 SPCC1840.07c 2270637 2271635 - 15 41.65 phosphorotein phosphatase 0 2 chr3 SPCC622.19 jm/4 mug149 1438375 143939 + 22 41.65 dm/4 protein 0 2 chr1 SPAC24C9.05c mug70 304512 304731 - 33 41.74 conserved protein (fungal and plant) 0 2 chr1 SPAC16E8.17c 3532418 3533413 - 15 41.77 succinate-CoA ligase 0 1 chr1 SPAC16E8.17c 3532418 3533413 - 15 41.77 succinate-CoA ligase 0 1 chr1 SPAC16E8.17c 3532418 3533413 + 27 41.82 arrestin/PY protein 1 0 2 chr1 msh3 swi4 SPAC8F11.03 2855931 285890 + 46 41.83 Mut5 protein homolog 3 0 2 chr2 SPEC1773.16c 318693 320480 - 27 41.89 transcription factor 0 2 chr3 SPC1620.12c 216779 <td< td=""><td>chr1</td><td>gmh3 SPAC22E12.06c</td><td>5029000</td><td>5029998 -</td><td>15</td><td>41.65</td><td>alpha-1,2-galactosyltransferase Gmh3</td><td>0</td><td></td><td>2</td></td<>	chr1	gmh3 SPAC22E12.06c	5029000	5029998 -	15	41.65	alpha-1,2-galactosyltransferase Gmh3	0		2
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chrl idp1 SPAC6G10.08 3221599 3232855 + 19 41.93 isocitrate dehydrogenase ldp1 1 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 ldp1	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.

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	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

Table S5 Strains of S. pombe used in this study

1. H. L. Levin, *Mol Cell Biol* **15**, 3310 (1995).

Table S6 Plasmids.

Plasmid	Markers	Description	Reference
pHL423	amp, LEU2	Rep3 <i>nmt1</i> promoter, control plasmid lacking Hermes transposase	(1)
pHL2577	amp, URA3	Hermes donor with TIR's flanking <i>kanMX6</i>	(2)
pHL2578	amp, LEU2	rep81X <i>nmt1</i> 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

1. K. Maundrell, Gene **123**, 127 (1993).

2. A. G. Evertts, C. Plymire, N. L. Craig, H. L. Levin, *Genetics* 177, 2519 (Dec, 2007).

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