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.