DOI: 10.1534/genetics.107.081075

Note

The Hermes Transposon of *Musca domestica* Is an Efficient Tool for the Mutagenesis of *Schizosaccharomyces pombe*

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Manuscript received August 24, 2007
Accepted for publication September 24, 2007

ABSTRACT

Currently, no transposon-based method for the mutagenesis of *Schizosaccharomyces pombe* exists. We have developed such a system based on the introduction of the hermes transposon from the housefly into *S. pombe*. This system efficiently disrupts open reading frames and allows the insertion sites to be readily identified.

THE fission yeast *Schizosaccharomyces pombe* is a model organism that has been studied widely to address questions of cell biology, cell cycle, and DNA repair. The study of gene function in model organisms benefits greatly from the application of transposable elements as tools for mutagenesis. Transposon mutagenesis creates mutations that can be readily identified because they are linked to transposon sequence. Such systems are well established and used in many model organisms including bacteria, *Saccharomyces cerevisiae*, *Drosophila melanogaster*, *Caenorhabditis elegans*, and the mouse (Smith *et al.* 1996; Ross-Macdonald *et al.* 1999; Bellen *et al.* 2004; Ding *et al.* 2005; Dupuy *et al.* 2005; Williams *et al.* 2005). However, no transposon system for the mutagenesis of *S. pombe* exists. Initial attempts to use the retrotransposon Tf1 of *S. pombe* as a mutagen were unsuccessful because insertions occur primarily upstream of open reading frames (ORFs) (Behrens *et al.* 2000; Singleton and Levin 2002).

To develop a method for the transposon-based mutagenesis of *S. pombe*, we tested the DNA transposon hermes from the housefly (*Musca domestica*) for activity in *S. pombe*. Hermes uses a single transposase enzyme that binds to the terminal inverted repeats (TIRs), excises the transposon, and integrates the transposon DNA into new a location (Guimond *et al.* 2003; Zhou *et al.* 2004).

To test hermes for transposition activity in *S. pombe*, we incorporated the TIRs and sequence encoding the transposase into separate plasmids that were both introduced into the *S. pombe* strain YHL912 (*h-*, *leu1*-32, *ura4*-294; Levin 1995) (Figure 1). The expression plasmid contained the transposase gene and the donor plasmid contained a source of the transposon DNA flanked by the TIRs (Abrams *et al.* 1986). To test various levels of transposase expression for transposition activity, we used the *nmt1* promoter in the context of the pREP3X, pREP41X, and pREP81X expression vectors (Figure 1A). These vectors provide three levels of transcription and can also be repressed in the presence of thiamine. pREP3X provides the highest level of transcript, while levels drop ~61-fold with pREP41X and an additional 17-fold with pREP81X (Basi *et al.* 1993; Forsburg 1993). Our initial experiments suggested that levels of transposition were very high, and as a result additional expression plasmids were built using T317A, a mutation in the transposase that lowers activity (P. Bafuma and N. Craig, unpublished results).

To detect insertions in the genome, we cloned the *kanMX6* cassette between the TIRs of the donor plasmid (Figure 1B) (Bahler *et al.* 1998). This gene caused cells with an insertion to be resistant to G418. To provide an alternative selection, a similar plasmid was constructed with *nat*, a gene that gives cells resistance to nourseothricin (Figure 1C) (Sato *et al.* 2005). An additional donor plasmid was constructed with a p15A bacterial origin of replication in the transposon so that sites of integration could be readily isolated in bacteria (Figure 1D). All plasmids are described in supplemental Table S1 at http://www.genetics.org/supplemental/.

To measure the expression of hermes transposase, we performed immunoblots that were probed with an

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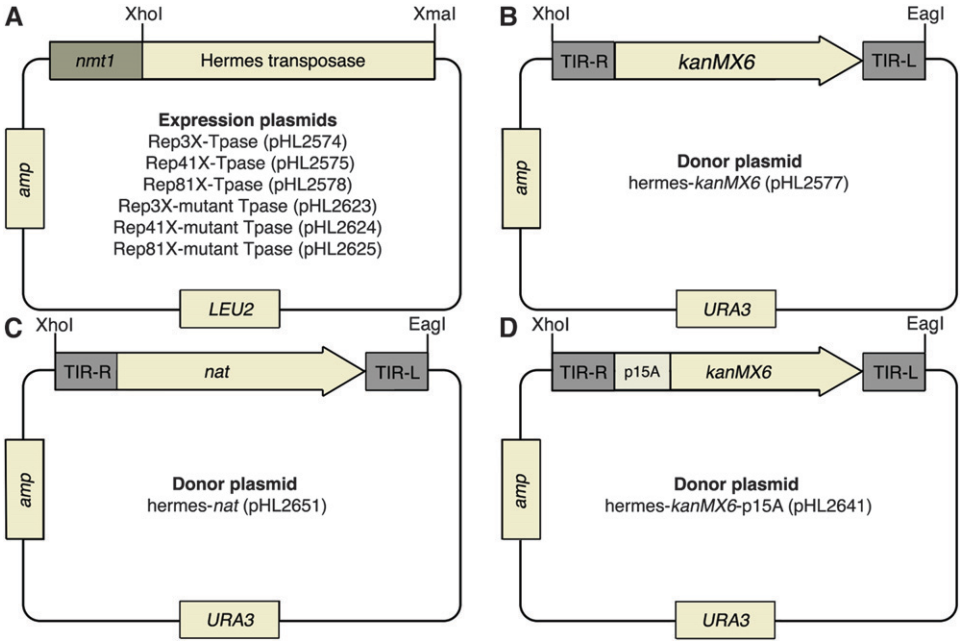

Figure 1.—The donor and expression plasmids for hermes transposition in *S. pombe*. (A) Six expression plasmids were built to control and vary the expression of the hermes transposase. Variation is achieved by using three strengths of the *nmt1* promoter as well as a T317A mutant transposase. The *Xho*I–*Xma*I fragment containing the transposase was 1844 bp. (B) The donor plasmids provide the source of transposon DNA flanked by the TIRs. Here the *kanMX6* cassette was cloned between the TIRs, giving cells with insertions resistance to the drug G418. The *Xho*I–*Eag*I fragment containing *kanMX6* and the TIRs was 2782 bp. (C) Another version of the donor plasmid was constructed using *nat*, a gene that gives cells with insertions resistance to nourseothricin. The *Xho*I–*Eag*I fragment containing *nat* was 2548 bp. (D) This donor plasmid contains a p15A bacterial origin of replication, allowing for insertions to be readily isolated in bacteria. The *Xho*I–*Eag*I fragment containing p15A and *kanMX6* was 3333 bp.

antibody specific for the transposase (N. Craig, unpublished results). Figure 2A shows there was a correlation between transposase expression and promoter strength, with pREP3X producing the highest levels of transposase and pREP81X producing the lowest.

To measure levels of transposition produced by the expression and donor plasmids, we performed a qualitative transposition assay. Three factors influenced the level of transposition: strength of promoter, the T317A mutation, and the p15A ori in the donor plasmid (Figure 2B). As expression went from high (REP3X) to low (REP81X) transposition decreased for the donor with p15A (Figure 2B, right) and the donor that lacked the ori (Figure 2B, left). The T317A mutation caused a modest reduction of transposition, while the p15A ori lowered transposition levels substantially. Although we have no information about why the addition of p15A lowered transposition levels, the increased size of the transposon may have reduced the efficiency of transposition.

To test whether the cells with resistance to G418 actually contained inserts, and to measure the number of insertions, DNA blots were performed. Following induction for transposition, cells resistant to G418 were obtained and single colonies were isolated. Genomic DNA derived from the isolated colonies was digested with *Eco*RI and blotted. Because the *Eco*RI site was at the extreme end of *kanMX6* and the blot was probed with *kanMX6*, each copy of *kanMX6* produced a single band. The blots showed that all of the G418-resistant strains had at least one hermes insertion. The first blot (Figure 3A, top left) includes strains that resulted from the expression of transposase from REP3X-Tpase and REP41X-Tpase. Seven of these eight stains contained a single insertion, while one pREP3X strain contained two insertions. The remaining 37 lanes in the DNA blots were derived from strains that resulted from the expression of transposase from REP81X-Tpase, and 12 of these (32%) contained multiple bands indicating two or more insertions. It was unclear why the cells expressing the most transposase produced a higher percentage of strains with single insertions. The small number of strains containing REP3X-Tpase and REP41X-Tpase did not allow us to draw any conclusions about this observation.

To test whether the insertions were due to transposase-mediated integration and not other forms of recombination, we analyzed 26 hermes insertions for the 8-bp target-site duplications (TSD) that result from transposase-mediated insertion (Guimond *et al.* 2003). Integration sites were determined by using inverse PCR on genomic DNA digested with *Eco*RI and ligated into circles (Figure 3B). All oligonucleotides used are described in supplemental Table S2 at http://www.genetics.org/supplemental/. TSDs were found for each of the insertions and we created a sequence logo showing the preference for bases at each position (supplemental Table S3 and Figure 4A). The strong preference for T at position 2, for A at position 7, and the other preferences all closely matched what was observed previously for the insertion sites of hermes in *D. melanogaster* (Guimond *et al.* 2003). These data indicate that all the insertions of hermes tested resulted from *bona fide* integration catalyzed by the transposase.

To observe any preferences of hermes when integrating in the genome, we mapped the positions of the 26 insertions relative to ORFs (supplemental Table S3 at http://www.genetics.org/supplemental/). Insertions that occurred outside of ORFs were given a position on the

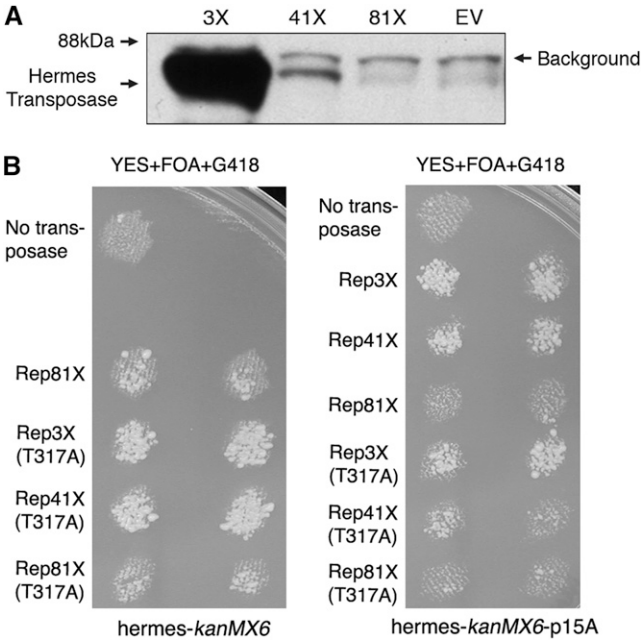

Figure 2.—The expression and activity of hermes transposase in *S. pombe*. (A) The immunoblot of extracts showed the expression of YHL912 containing the transposase-expressing plasmids REP3X-Tpase (pHL2574), REP41X-Tpase (pHL2575), and REP81X-Tpase (pHL2578) compared to the empty vector Rep3 (pHL423). A whole-cell protein extract was obtained using a bead-beater protocol. Cells were grown in EMM minus leucine to a final OD of 1.0 and 40 OD units of cells were pelleted. After protein extraction, 12 μg of protein was subjected to electrophoresis on a 10–20% Tris–glycine gel (Invitrogen, San Diego) and transferred to a membrane. The membrane was probed overnight at room temperature with an antitransposase antibody at a concentration of 1:1000. The antibody was polyclonal and was raised in rabbit (X. Li, and N. Craig, unpublished results). The band-labeled background was independent of transposon expression. (B) This patch assay qualitatively measures the levels of transposition. Cells were initially grown as patches on EMM −ura −leu −B1 for 2 days to induce transposase expression. These patches were then replica printed to plates containing EMM −leu + FOA + B1 to select for cells lacking the donor plasmid. Patches from these plates were printed to plates with YES + FOA + G418 to detect cells with insertions. (Left) Strains with the donor plasmid hermes–*kanMX6* (pHL2577). (Right) Strains with the donor plasmid hermes–*kanMX6*-p15A (pHL2641). Each row has two patches of YHL912 containing the expression plasmids Rep81X Tpase (pHL2578), Rep3X mutant Tpase (pHL2623), Rep41X mutant Tpase (pHL2624), and Rep81X mutant Tpase (pHL2625) (left) and Rep3X Tpase (pHL2574), Rep41X Tpase (pHL2575), Rep81X Tpase (pHL2578), Rep3X mutant Tpase (pHL2623), Rep41X mutant Tpase (pHL2624), and Rep81X mutant Tpase (pHL2625) (right). The control strains were YHL912 with hermes–*kanMX6* (pHL2577) and the empty expression vector pHL423.

basis of their proximity to either the 5′-end or the 3′-end of the ORF, depending on which was closest. We observed no preference for hermes to integrate into intergenic regions *vs.* ORFs as seen by the wide distribution of insertion sites in Figure 4B. Hermes did insert at various locations within ORFs, indicating that it is able to cause gene disruptions in *S. pombe*. Of the 26 insertions mapped, 14 (54%) occurred in open reading frames. This percentage correlates well with the portion of the genome that encodes protein (57.5%), indicating that hermes did not discriminate between coding sequence and intergenic regions (Wood *et al.* 2002). This result is in stark contrast to the previously reported integration preference of Tf1 for regions upstream of ORFs (Kelly and Levin 2005). Hermes clearly relies on a different mechanism of target recognition than Tf1.

Hermes has the ability to disrupt open reading frames in *S. pombe*. However, to use hermes as a tool requires a quantitative understanding of the level of transposition. To more accurately measure the level of transposition of the hermes system, we conducted a quantitative assay. The assay allowed us to calculate the number of transposition events per generation using a strain containing wild-type transposase under the control of the REP81X-Tpase expression vector and the donor plasmid hermes–*kanMX6*. Three transformants were induced for transposition in liquid EMM media, and cell density measurements were taken before and after growth to calculate the number of generations. After cultures reached an OD_{600} of ~5, they were diluted and new cultures were started. This prevented the cells from entering stationary phase. In all, a series of four sequential cultures were used for each strain tested. Cells containing the donor plasmid are already resistant to G418 due to the plasmid. Thus, only cells lacking the URA3-containing donor plasmid with its *kanMX6* can be tested for the resistance to G418 caused by hermes integration. To measure transposition levels at each time point, cells were plated onto EMM + FOA (to determine the number of cells that had lost the donor plasmid) and YES + FOA + G418 (to determine the number of cells that had lost the donor plasmid and also contained a hermes insertion). The fraction of cells on YES + FOA + G418 divided by those on EMM + FOA gave us the transposition frequency. We found that all three transformants had similar transposition frequencies per cell generation (Figure 5). After ~25 cell generations, we found that 1.5–2.75% of the cells contained insertions. This rate of transposition is ideal for generating mutation libraries because it produces a substantial number of cells with insertions while keeping the frequency of double insertions to a minimum.

We tested whether this library of insertion events contained the expected number of cells with disruptions. Cells with mutations in *ade6* or *ade7* were identified by the formation of red colonies on agar plates that contained yeast extract. Of 106,000 cells screened, 7 produced red colonies. We expected ~19 red colonies by taking the total number of base pairs in *ade6* and *ade7* (2559 bp) divided by the size of the *pombe* genome (14.1 Mb). This assumed that all disruptions would cause a phenotype. Since this is unlikely to be true, our prediction of 19 colonies was an overestimate. Thus, the number of *ade*− colonies that we observed was consistent with randomly distributed integration. To determine which

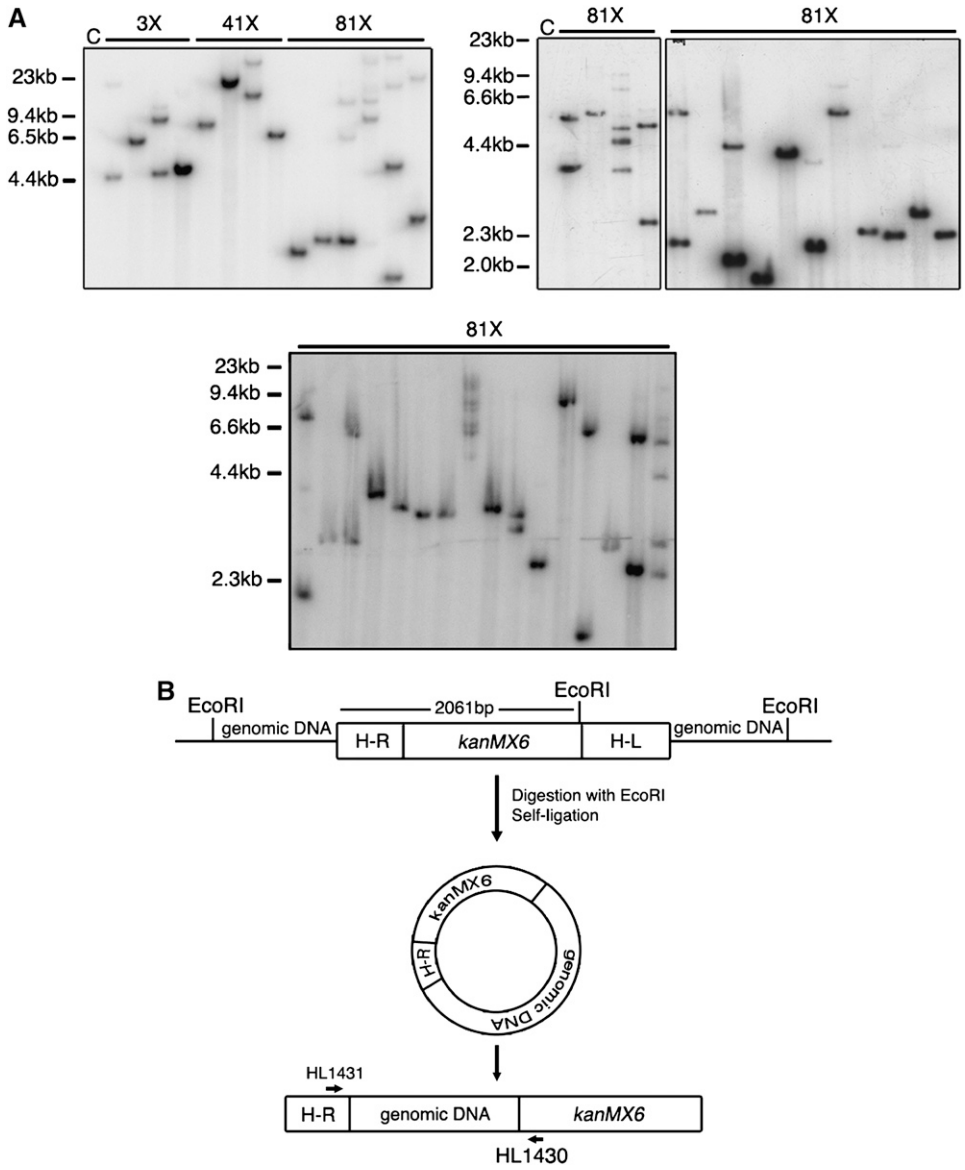

FIGURE 3.—The DNA blots of strains with insertions of hermes. (A) Independent patches of YHL912 containing the donor hermes–*kanMX6* (pHL2577) were induced for the expression of hermes transposase with Rep3X Tpase (pHL2574), Rep41X Tpase (pHL2575), or Rep81X Tpase (pHL2578). Genomic DNA from FOA/G418-resistant strains was digested with *Eco*RI and separated by agarose gel and then transferred to a nitrocellulose membrane and probed with a radiolabeled *neo* fragment. Individual bands indicate insertions of the hermes transposon. The expression vector used to generate the strains is shown at the top of each blot. Lanes labeled "C" contained DNA from the parental strain YHL912. (B) The method for inverse PCR used to identify the position of the insertions is shown. Two micrograms of genomic DNA was digested with *Eco*RI, phenol extracted, and diluted to 1 ng/μl. The dilution was ligated using T4 DNA ligase at 18° overnight. The resulting material was ethanol precipitated and 100 ng of circularized DNA was amplified by PCR. The arrows indicate the PCR primers used to amplify the genomic DNA adjacent to hermes right (HL1430, 5′-GCCT CGACATCATCTGCCC out from *kanMX6* and HL1431, 5′-CTCTAG CGGTGATCTTAACATC out from hermes right). The junction with hermes left was amplified using a primer in hermes left (HL1893, 5′-ACCCGAGTGTCGATGAATCAA TGAA) and one in the flanking genomic DNA as identified by the inverse PCR.

genes were disrupted by the insertions, we mated each of the seven strains with tester strains defective for *ade6* or *ade7*. Selection of the mating mixture for adenine prototrophy revealed that five of the strains had insertions in *ade6* and two had insertions in *ade7*. That the majority of strains were disrupted in *ade6* is consistent with the size of the *ade6* open reading frame being substantially larger than that of *ade7*. Mating of the *ade*− strains

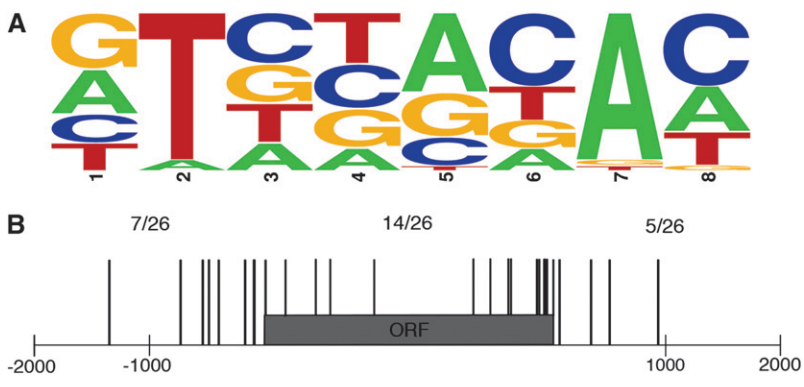

FIGURE 4.—The insertion-site preferences of hermes. (A) A sequence logo was generated for the 26 insertions identified from YHL912 containing the donor hermes–*kanMX6* (pHL2577) and Rep81X Tpase (pHL2578). Nucleotide preferences within the target-site duplication are shown for each of the eight positions. The most preferred nucleotide is shown at the top of each column and the least preferred at the bottom. The height of each nucleotide correlates with its frequency. (B) Insertion sites for 26 strains were mapped relative to their position in or near an ORF. Positions in the ORF were determined by calculating the distance as a percentage within each individual ORF. Positions in the 5′ or 3′ region were determined on the basis of whether the insertion was closest to the 5′- or the 3′-end of an ORF.

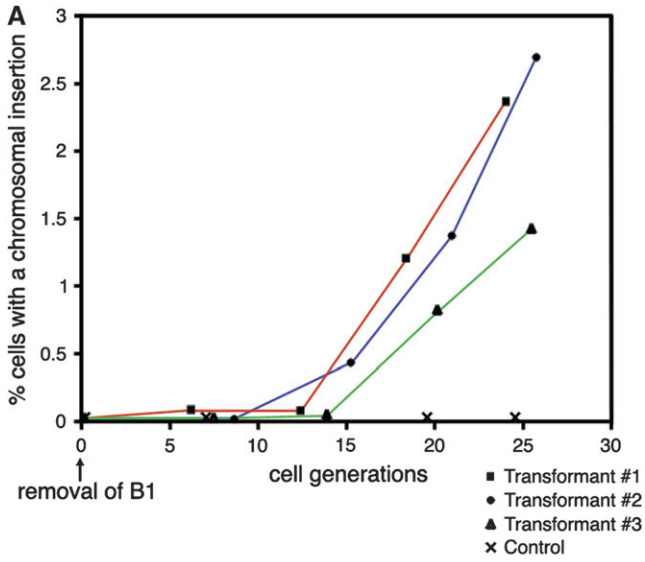

FIGURE 5.—Quantitative measures of insertion rates. (A) Levels of transposition of three transformants of YHL912 containing the REP81X-Tpase plasmid (pHL2578) and the hermes–kan donor (pHL2577). The percentage of cells with chromosomal insertions is shown on the *y*-axis and the total number of cell generations on the *x*-axis. Growth was carried out in a series of four liquid cultures, each starting at OD 0.05, which were diluted when the OD was ~5.0. One culture of transformant 1 reached OD 7.6. The number of colonies on a YES + FOA + G418 plate divided by the number of colonies on an EMM + FOA plate gave the transposition frequency. The control strain with an empty pREP3 expression plasmid (pHL423) shows no transposition. (B) Transposition rates per generation were calculated by determining the slope of the line between the third and final data points for each transformant.

with *ade*+ strains and the analysis of the spores demonstrated that each of the *ade*− alleles was linked to a transposon insertion.

In summary, the integration of hermes indicates that this system is well suited for the insertional mutagenesis of *S. pombe*. Our system utilizes the DNA transposon hermes, which can insert into ORFs and cause gene disruptions. We constructed six expression plasmids and three donor plasmids. We quantified the transposition frequency for REP81X-Tpase (pHL2578) with donor hermes–*kanMX6* (pHL2577) and found this combination to be ideal for mutagenesis. The reduced activity of hermes with the p15A origin of replication and our observation that this version tended to cause deletions (data not shown) indicates that the best plasmids for mutagenesis are the REP81X-Tpase (pHL2578) and the *kanMX6* donor (pHL2577). To generate a library of hermes insertions, we recommend that cells be freshly transformed with the plasmids and that single transformants be grown in liquid cultures as was done above to quantify the transposition rate. Although we did not quantify the activity of the hermes–nat donor (pHL2651), qualitative assays indicated that it, too, is suitable for mutagenesis. The high level of transposon activity that hermes exhibited in *S. pombe* suggests the intriguing possibility that hermes may function broadly in a variety of other organisms.

We thank Gerry Smith for helpful discussions about the quantitative transposition assay and adenine mutagenesis. This research was supported by the Intramural Research Program of the National Institutes of Health from the National Institute of Child Health and Human Development.

LITERATURE CITED

ABRAMS, E., L. NEIGEBORN and M. CARLSON, 1986 Molecular analysis of *SNF2* and *SNF5*, genes required for expression of glucose-repressible genes in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **6:** 3643–3651.

BAHLER, J., J. Q. WU, M. S. LONGTINE, N. G. SHAH, A. MCKENZIE *et al.*, 1998 Heterologous modules for efficient and versatile PCR-based gene targeting in Schizosaccharomyces pombe. Yeast **14:** 943–951.

BASI, G., E. SCHMID and K. MAUNDRELL, 1993 TATA box mutations in the Schizosaccharomyces pombe nmt1 promoter affect transcription efficiency but not the transcription start point or thiamine repressibility. Gene **123:** 131–136.

BEHRENS, R., J. HAYLES and P. NURSE, 2000 Fission yeast retrotransposon Tf1 integration is targeted to 5′ ends of open reading frames. Nucleic Acids Res. **28:** 4709–4716.

BELLEN, H. J., R. W. LEVIS, G. C. LIAO, Y. C. HE, J. W. CARLSON *et al.*, 2004 The BDGP gene disruption project: single transposon insertions associated with 40% of Drosophila genes. Genetics **167:** 761–781.

DING, S., X. H. WU, G. LI, M. HAN, Y. ZHUANG *et al.*, 2005 Efficient transposition of the piggyBac resource (PB) transposon in mammalian cells and mice. Cell **122:** 473–483.

DUPUY, A. J., K. AKAGI, D. A. LARGAESPADA, N. G. COPELAND and N. A. JENKINS, 2005 Mammalian mutagenesis using a highly mobile somatic Sleeping Beauty transposon system. Nature **436:** 221–226.

FORSBURG, S. L., 1993 Comparison of Schizosaccharomyces pombe expression systems. Nucleic Acids Res. **21:** 2955–2956.

GUIMOND, N., D. K. BIDESHI, A. C. PINKERTON, P. W. ATKINSON and D. A. O'BROCHTA, 2003 Patterns of hermes transposition in Drosophila melanogaster. Mol. Genet. Genomics **268:** 779–790.

KELLY, F. D., and H. L. LEVIN, 2005 The evolution of transposons in Schizosaccharomyces pombe. Cytogenet. Genome Res. **110:** 566–574.

LEVIN, H. L., 1995 A novel mechanism of self-primed reverse transcription defines a new family of retroelements. Mol. Cell. Biol. **15:** 3310–3317.

ROSS-MACDONALD, P., P. S. R. COELHO, T. ROEMER, S. AGARWAL, A. KUMAR *et al.*, 1999 Large-scale analysis of the yeast genome by transposon tagging and gene disruption. Nature **402:** 413–418.

SATO, M., S. DHUT and T. TODA, 2005 New drug-resistant cassettes for gene disruption and epitope tagging in Schizosaccharomyces pombe. Yeast **22:** 583–591.

SINGLETON, T. L., and H. L. LEVIN, 2002 A long terminal repeat retrotransposon of fission yeast has strong preferences for specific sites of insertion. Eukaryot. Cell **1:** 44–55.

SMITH, V., K. N. CHOU, D. LASHKARI, D. BOTSTEIN and P. O. BROWN, 1996 Functional analysis of the genes of yeast chromosome V by genetic footprinting. Science **274:** 2069–2074.

WILLIAMS, D. C., T. BOULIN, A. F. RUAUD, E. M. JORGENSEN and J. L. BESSEREAU, 2005 Characterization of Mos1-mediated mutagenesis in *Caenorhabditis elegans*: a method for the rapid identification of mutated genes. Genetics **169:** 1779–1785.

WOOD, V., R. GWILLIAM, M. A. RAJANDREAM, M. LYNE, R. LYNE *et al.*, 2002 The genome sequence of Schizosaccharomyces pombe. Nature **415:** 871–880.

ZHOU, L. Q., R. MITRA, P. W. ATKINSON, A. B. HICKMAN, F. DYDA *et al.*, 2004 Transposition of hAT elements links transposable elements and V(D)J recombination. Nature **432:** 995–1001.

Communicating editor: D. VOYTAS