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The *Hermes* transposon of *Musca domestica* and its use as a mutagen of *Schizosaccharomyces pombe*

Jung M. Park, Adam G. Everetts, and Henry L. Levin¹

Section on Eukaryotic Transposable Elements, Laboratory of Gene Regulation and Development, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20892, USA

Abstract

Transposon mutagenesis allows for the discovery and characterization of genes by creating mutations that can be easily mapped and sequenced. Moreover, this method allows for a relatively unbiased approach to isolating genes of interest. Recently, a system of transposon based mutagenesis for *Schizosaccharomyces pombe* became available. This mutagenesis relies on *Hermes*, a DNA transposon from the house fly that readily integrates into the chromosomes of *S. pombe*. The *Hermes* system is distinct from the retrotransposons of *S. pombe* because it efficiently integrates into open reading frames. To mutagenize *S. pombe*, cells are transformed with a plasmid that contains a drug resistance marker flanked by the terminal inverted repeats of *Hermes*. The *Hermes* transposase expressed from a second plasmid excises the resistance marker with the inverted repeats and inserts this DNA into chromosomal sites. After *S. pombe* with these two plasmids grow 25 generations, approximately 2% of the cells contain insertions. Of the cells with insertions, 68% contain single integration events. The protocols listed here provide the detailed information necessary to mutagenize a strain of interest, screen for specific phenotypes, and sequence the positions of insertion.

Keywords

Transposon; *Hermes*; mutagenesis; *Schizosaccharomyces pombe*

1. Introduction

Transposon mutagenesis is an important tool used in many model organisms to screen for genes involved in a variety of processes (1–6). Unfortunately, in the yeasts the endogenous transposons possess unique targeting mechanisms that direct integration to specific sites in the host chromosomes (7–9). As a result, these transposons do not actively target open reading frames (ORFs) and can not function as efficient mutagens. To overcome this problem, we have tested an exogenous DNA transposon called *Hermes* from the house fly (10). In the yeast *Schizosaccharomyces pombe*, *Hermes* has high transposition activity and in 54% (14 of 26) of insertions, open reading frames are disrupted (10). Although the proportion of inserts that disrupt ORFs may vary from experiment to experiment, we have been able to generate insertion mutations in specific genes with frequencies expected for unbiased integration (10). The

¹Corresponding Author: Henry Levin, National Institutes of Health, Building 18T, Room 106, Bethesda, MD 20892-5431, Tel: 301-402-4281, Fax: 301-496-4491, henry_levin@nih.gov.

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Hermes system can be used in virtually any strain background, making it more versatile than screening a set of deletions. The ease of creating a mutant library and identifying the insertion sites via inverse PCR also make it desirable. That some of these mutations result in hypomorphic alleles of essential genes is another valuable feature that distinguishes insertional mutagenesis from deletion sets.

The *Hermes* transposon when mobilized from a plasmid and grown in *S. pombe* for 25 generations produces insertions in approximately 2% of the cells (10). About 70% of the strains contain a single integration of *Hermes*. When 106,000 colonies with integration events are screened for disruptions of genes required for adenine biosynthesis, five disruptions of *ade6* and two of *ade7* can be isolated (10).

2. Plasmids constructed for induction of *Hermes* transposition

The mutagenesis system available with *Hermes* requires that two plasmids be introduced into the strain of *S. pombe* chosen for mutagenesis (10). One plasmid contains the transposase gene driven by the Rep81X *nmt1* promoter. This plasmid also contains *LEU2* to select for its presence in *S. pombe* (Fig. 1, pHL2578). While the Rep41X version of the *nmt1* promoter allows for higher levels of expression and greater numbers of cells with insertions, the Rep81X version tends to avoid fluctuations in the proportion of the cells that have insertions (Evertts and Levin, unpublished). The second plasmid introduced into *S. pombe* contains the donor sequence, which consists of *kanMX6* flanked by the inverted terminal repeats, *Hermes* right and *Hermes* left. The donor plasmid is marked with *URA3*, to allow for selection in *S. pombe* (Fig. 1, pHL2577). Transposition occurs when the transposase (Tpase) cleaves the *Hermes* right and left sequences in the donor plasmid and inserts this fragment containing *kanMX6* into chromosomal sequences. Cells with copies of *kanMX6* inserted into *S. pombe* chromosomes are selected on medium containing G418. The direct selection for integration is made possible because we also include 5-fluoroorotic acid (5-FOA) in the medium. 5-FOA selects against cells containing the donor plasmid with the original copy of *kanMX6*. Using this system of two plasmids, extensive libraries of transposon insertions can be generated, the mutant strains can be screened for phenotypes, and positions of the insertion events can be sequenced, all with a relatively simple set of procedures (Fig. 2).

3. Method

3.1 Generating a library of insertions in *S. pombe* using *Hermes*

3.1.1 Select a strain of *S. pombe* that is Ura⁻ and Leu⁻ and suitable for detecting your desired phenotype.

3.1.2 Introduce the plasmids containing the donor and expression cassettes separately into your strain of *S. pombe* using lithium acetate transformation (11). We transform in the donor plasmid first, followed by a second transformation to introduce the expression plasmid. These sequential transformations help to avoid recombination between plasmids. It is extremely useful to also create a strain that does not express the transposase as a negative control. For this we transform in an empty expression vector (Rep81X) in place of the expression plasmid (12). Following the second transformation, plate cells onto Edinburgh minimal media (EMM) lacking uracil and leucine. The EMM we use is the standard mixture (11) except it also contains 2 gm/liter dropout powder (an equal weight mix of the 19 standard amino acids, leaving out leucine, plus 2.5 times more adenine than the amino acids). Thiamine at a concentration of 10 μ M is also added to the media to repress the *nmt1* promoter.

3.1.3 When colonies from the transformation are ~1mm, streak onto fresh media to isolate single colonies.

3.1.4 To prevent *Hermes* from disrupting genes in the starter stain we are careful to minimize the propagation of cells with the transposon plasmids. We recommend that freshly transformed colonies be immediately cultured for mutagenesis. We typically do not maintain frozen stocks of these strains. This is because residual expression of *nmt1* causes *Hermes* transposition even when cells are grown in the presence of thiamine.

3.1.5 When purified colonies are ~1mm, inoculate one colony from each strain into a 5 ml starter culture of EMM lacking uracil and leucine and add thiamine to a final concentration of 10 μ M. For this and all the following steps below do the same with the control strain lacking the transposase. This will help you confirm that the 5-FOA and G418 plates are functioning correctly.

3.1.6 Incubate the starter cultures at 32°C ~16 hrs overnight in a rolling drum.

3.1.7 Next morning wash the cells from the starter culture four times in 50 ml of EMM lacking uracil and leucine to remove thiamine. With these cells inoculate a 50 ml culture of EMM lacking uracil, leucine, and thiamine, at an initial OD_{600nm} of 0.05. To monitor the transposition rates, we record the initial OD_{600nm}, referred to here as OD_I, for each culture.

3.1.8 Grow the cultures shaking in a 32°C incubator to densities at least as high as OD_{600nm} 2.0 but no higher than OD_{600nm} 5.0.

3.1.9 Measure the OD_{600nm} and record as OD_F; where F stands for final.

3.1.10 Use both OD_{600nm} measurements from 3.1.7 and 3.1.9 to calculate the number of cell generations during this growth stage. To do this, use the equation $n = [\ln(\text{OD}_F / \text{OD}_I)] / 0.693$; where n is the number of cell generations and $\ln$ is the natural log.

3.1.11 After measuring the OD_F of the previous cultures use these cells to inoculate new 50 ml cultures of EMM lacking uracil, leucine, and thiamine, at an initial OD_{600nm} of 0.05. Again, record the exact OD_{600nm} measurement obtained at the start of growth as OD_I.

3.1.12 As before grow the cultures to densities at least as high as OD_{600nm} 2.0 but no higher than OD_{600nm} 5.0.

3.1.13 Measure the OD_{600nm} and record as OD_F. Use the equation in step 3.1.10 to calculate the number of cell generations for this second culture.

3.1.14 Repeat steps 3.1.8 – 3.1.13, two to three additional times until the cells reach a cumulative number of approximately 25 generations and have reached the desired proportion with insertions (See below for calculation).

3.2 Estimating the fraction of cells with insertions

3.2.1 You can assay cells from each of the sequential cultures to monitor the increase in transposition events relative to generation number (Fig. 3). The cultures to be tested are used to create a series of five 10-fold dilutions starting with undiluted cells (OD 5 corresponds to 10⁸ cells/ml) and finishing with a concentration of approximately 10⁴ cells/ml.

3.2.2 Spread 100 μ l from the three most diluted cultures onto plates containing EMM and dropout mixture with leucine (250 μ g/ml), uracil (50 μ g/ml), 5-FOA (1mg/ml), and 10 μ M thiamine (See recipes).

3.2.3 Spread 100 μ l from the three least diluted cultures onto plates containing YES with 5-FOA (1 mg/ml) and G418 (500 μ g/ml). This formulation is described in the recipe section below.

3.2.4 Incubate these plates at 32°C for 3 days or until colonies form and can be counted. Count the number of colonies on each plate. The number of colonies on EMM media with 5-FOA represents the number of cells, out of the total plated, that have lost the donor plasmid. Typically, this is about 1% to 5%. The number of colonies on the plates with YES 5-FOA+G418 represents the number of cells, out of the total plated, which have lost the donor plasmid and have a genomic insertion. A calculation of the transposition frequency can be made by dividing the number of colonies on YES 5-FOA+G418 by the number of colonies on EMM 5-FOA. Because G418 is not active in minimal media, we use YES with G418. In experiments reported previously, calculations were made after 25 cell generations and the percentage of cells with insertions was between 1.5% and 2.75%. You may use this as a guide with the understanding that your background strain and experimental conditions may lead to a slightly different frequency.

3.3 Screening the library for mutants of interest

3.3.1 We suggest two options for screening the cells for phenotypes. Choose the option that best fits your desired phenotype.

3.3.2 Option #1. Resuspend the final 50 ml cultures in 500 mL of EMM+leu+ura+FOA+thiamine to a final OD of 0.25 in order to select against the donor plasmid. Let these cells grow ~20–24 hours. Next, dilute the EMM+leu+ura+FOA+thiamine cultures down to OD 0.5 in 500 mL of YES+FOA+G418 and incubate to isolate the cells with insertions. Grow the cultures for 24 hrs to an OD_{600nm} of no higher than 5.0. The majority of cells that remain will lack the donor plasmid and also contain a transposon integration. This can be checked by plating a sample of cells onto YES, YES+G418, and EMM lacking uracil. The cells can be plated onto selective media or screened for phenotypes.

3.3.3 Option #2. Spread cells from the final 50 ml culture onto plates containing YES+FOA+G418 to reveal the cells with insertions that have also lost the donor plasmid. Use a dilution calculated to provide optimal colony numbers for your particular screen or selection. Incubate plates for 3 days or until colonies are one to two mm in diameter. Replica-print the plates onto media to screen or select for your specific phenotype. Use Figure 3 to approximate the transposition frequency of your culture using the cell generations calculated. We recommend doing a DNA blot to measure the number of insertions in the isolated mutants. Mutants with two or more insertions can be pursued with the understanding that the phenotype of interest is likely linked to just one of the insertions.

3.4 Testing the mutagenesis system

3.4.1 To test the mutagenesis system in your strain background, we recommend doing a simple screen for strains deficient in adenine biosynthesis. A disruption in the genes *ade6* or *ade7* will result in red colonies on media with a low concentration of adenine. This screen requires simple yeast extract (YE) media (See recipes). YE has a concentration of adenine that allows Ade⁻ strains to grow, but also turn red, so that mutants can be identified and analyzed. This test screen is useful because it requires the generation of a mutant library, screening the library for a phenotype, and confirming that the insertions are linked to the phenotype.

3.4.2 Select a strain of *S. pombe* that is Leu⁻, Ura⁻ and Ade⁺.

3.4.3 Grow the strain from a frozen stock on YE media for 3 days or until colonies form. Note the color of the colonies. White indicates that your strain is fully Ade⁺.

3.4.4 Introduce the plasmids containing the donor and expression cassettes into your strain of *S. pombe* using lithium acetate transformation (11). When transforming in the

expression plasmid, introduce separately the empty plasmid that lacks the transposase to serve as a negative control. For these transformations use EMM media that contains thiamine at a concentration of 10 μ M to inhibit T_pase expression.

3.4.5 Follow steps 3.1.3 – 3.1.14 to generate a library of *Hermes* insertions. Store cells from the final culture at 4°C or freeze at –80°C in 15% glycerol if necessary.

3.4.6 Plate a dilution series of 10⁸ cells/ml to 10⁴ cells/ml from the final 50 ml culture onto YE+FOA+G418. This medium will allow any cell to grow that has lost the mutagenesis plasmid and has a genomic insertion. Allow the plates to incubate at 32°C for 3 days or until colonies form. Count the number of colonies on each plate and identify the dilution corresponding to ~1000 colonies/plate.

3.4.7 Use the dilution obtained in 3.5.6 and plate cells at this concentration onto ~75 plates containing YE+FOA+G418. You can adjust the number of plates depending on your dilution. Aim for 75,000 colonies.

3.4.8 Incubate plates at 32°C for 3 days or until colonies form and can be counted.

3.4.9 Count the number of colonies on 3 plates. Average the number of colonies between the 3 plates and multiply by 75 to estimate the total cells screened.

3.4.10 Visually inspect each plate for the growth of red colonies.

3.4.11 Pick any red colonies and streak on YE for singles.

3.4.12 Pick a single colony from each red strain and make a patch on YES media.

3.4.13 Incubate the plate at 32°C for 2 days.

3.4.14 Replica-print to EMM-ade, EMM-ura, and YES+FOA+G418. No growth on EMM-ade suggests a mutation in *ade6* or *ade7*. No growth on EMM-ura suggests the absence of the donor plasmid, and growth on YES+FOA+G418 suggests a genomic insertion.

3.4.15 Insertions in *ade6* and *ade7* can be confirmed by mating the mutant candidates with tester strains containing an *ade6* or *ade7* mutation and testing the Ade phenotypes of the resulting spores. An absence of Ade⁺ spores indicates that the transposon generated mutation is linked to the Ade allele of the tester strain.

3.5 Isolating insertion sites using inverse PCR

3.5.1 This procedure identifies the insertion site adjacent to the right end of the transposon. To determine the genomic sequence flanking the left end of the transposon, we use PCR with individual primers predicted to anneal in the genomic DNA with a primer that anneals to the left end of *Hermes* (HL1893).

3.5.2 Colony purify any mutants of interest on YES and test colonies on EMM lacking uracil and leucine to guarantee loss of the plasmids. Isolate the genomic DNA from these cells using any standard technique. We use a zymolyase digestion method. Twenty-five milliliter cultures are grown to OD 8.0, pelleted at 3000 RPM for 5 minutes, and resuspended in 2.5 mL of Sp1 (1.2 M sorbitol, 50 mM citric acid monohydrate, and 50 mM Na₂HPO₄*7H₂O, 40 mM EDTA, pH 5.6) containing 15 mg Zymolyase 100T (Use zymolase from Seikagaku). Cells are incubated at 37°C for 1–2 hours in a rolling drum. The cells are pelleted and resuspended in 7.5 mL 5X TE and 1% SDS. Cells are incubated at 25°C for one hour before adding 2.5 mL of 5M KOAc. Cells are incubated on ice for 30 min and centrifuged for 15 minutes at 5000 RPM. 12.5 ml of cold isopropanol is added to the supernatant and incubated on ice for 5 minutes, then centrifuged at 8000 RPM for 10 minutes. Nucleic acids are resuspended in 3 mL 5X TE and incubated at 37°C for one to two hours in the presence of 100 ug/mL RNase A. Three phenol extractions and one

phenol/chloroform/isoamyl alcohol extraction are performed to remove proteins. The nucleic acids are precipitated using 1/10 volume of 5M NaCl and 2.5 volumes of 100% ethanol. The pellet is washed with 2 ml of cold 70% ethanol, air dried, and resuspended in 100 µl of 1X TE.

3.5.3 Digest 2 µg of genomic DNA in a volume of 40 µl with 20 units of *EcoRI* (NEB) for 16 hours at 37°C. Alternatively, if *EcoRI* is too near or far from the insert site, *SacI* can be used with the same set of PCR primers.

3.5.4 Phenol/chloroform extract the digested DNA and dilute to a concentration of 1 ng/µl to promote intramolecular ligation.

3.5.5 Ligate the diluted DNA in a volume of 400 µl using 1 unit/µl of T4 DNA ligase (Invitrogen) at 18°C for 16 hrs.

3.5.6 Use 100 ng of ligation product in a PCR with primers HL1430 and HL1431.

3.5.7 Run the PCR products on an agarose gel and gel purify the single bands using a standard gel purification kit. We use a Qiagen gel extraction kit.

3.5.8 Clone each fragment into a vector such as pCR2.1 (Invitrogen) that can be used to sequence the insert.

3.6 Conclusions

The high level of Hermes integration in exogenous hosts provides a unique opportunity to apply the highly valued technique of transposon-mediated mutagenesis to study the genes of *S. pombe*. The reagents necessary for these experiments are readily available from our laboratory.

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Figure 1.

Hermes mutagenesis plasmids. The expression plasmid (pHL2578) contains the transposase (Tpase) driven by the *nmt1* promoter. Restriction sites used to clone in the transposase are shown. This plasmid contains *S. cerevisiae LEU2* for selection in *S. pombe*. The donor plasmid (pHL2577) contains the inverted repeats flanking *kanMX6*. Restriction sites used to clone in this fragment are shown. The plasmid contains *S. cerevisiae URA3* for selection in *S. pombe*. The *URA3* gene is also used to select for cells that have lost the donor plasmid when testing for chromosomal insertions.

Figure 2.

Overview of method. The donor and expression plasmids are sequentially introduced into a strain of *S. pombe* using lithium acetate transformation. Strains containing plasmids are grown in liquid EMM medium lacking leucine and uracil to select for the plasmids. Cultures are grown in a series of flasks for a total of approximately 25 cell generations in order to produce a library of *Hermes* insertions. The cells are screened for a phenotype using plate medium specific to the individual screen. The method of inverse PCR is shown. Genomic DNA is digested with the endonuclease *EcoRI* and then ligated into a circle. The DNA is purified and used in a PCR to amplify a region of *Hermes* right and its flanking genomic DNA. The PCR product is run on an agarose gel and purified before being sequenced.

Figure 3.

Quantitative transposition assay. The graph shows the transposition frequency of 3 transformants of strain YHL912 transformed with the expression plasmid (pHL2578) and the donor plasmid (pHL2577). The x-axis shows the number of cell generations and the y-axis shows the percentage of cells that have chromosomal insertions. The transposition frequency increases as the number of cell generations increases. Transposition is measured by plating serially diluted cells onto EMM+FOA (to determine the number of cells that have lost the donor plasmid) and YES+FOA+G418 (to determine the number of cells that have lost the donor plasmid and have a genomic insertion). After 25 cell generations, transformants 1 and 2 have transposition frequencies of approximately 2.5%, while transformant 3's transposition frequency is approximately 1.5%. This figure has been reproduced with the permission of *Genetics* (10).

Table 1

growth media for fission yeast

Edinburgh minimal media (per liter)
3 g potassium hydrogen phthalate
4.13 g sodium phosphate dibasic heptahydrate ($\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$)
5 g ammonium chloride (NH_4Cl)
20 g glucose
2 g dropout powder (see YES)
20 ml 50X salt stock (see table 2)
1 ml 1000X vitamin stock (see table 2)
0.1 ml 10,000X mineral stock (see table 2)
EMM is typically made without leucine or uracil, or thiamine. These supplements are added as needed from sterile liquid stocks to final concentrations of 225 mg/l, 225 mg/l, and 10 μM respectively. We make 2X stocks of 250 ml of EMM, as well as 4% agar stocks of 250 ml. When making plates we heat the agar until it is entirely liquid and mix it with 2X EMM to create 500 ml of 1X EMM with 2% agar. 5-FOA is added at a concentration of 1 mg/ml with selecting for cells that have lost the URA3 containing plasmid. We add 5-FOA to 2X EMM and mix until the powder has dissolved. Do not heat 5-FOA above 40°C. Plates are made by mixing this medium with 4% molten agar.
YE (per liter)
30 g glucose
5 g yeast extract
YES (per liter)
30 g glucose
5 g yeast extract
2 g dropout powder (an equal weight mix of all amino acids plus 2.5 times more adenine than the amino acids)
When making plates we heat the agar until it is entirely liquid and mix it with 2X YES to create 500 ml of 1X YES with 2% agar. 5-FOA is added at a concentration of 1 mg/ml when selecting for cells that have lost the URA3 containing plasmid. We add 5-FOA to 2X YES and mix until the powder has dissolved. Do not heat 5-FOA above 40°C. G418 is added at a concentration of 500ug/mL. Plates are made by mixing this media with equal volumes of 4% molten agar.

Table 2

stock solutions for making EMM

50X salt stock (per liter)
52.5 g $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$
0.735 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$
50 g KCl
2 g NaCl
1000X vitamin stock (per liter)
1 g pantothenic acid
10 g nicotinic acid
10 g inositol
10 mg biotin
10000X mineral stock (per liter)
5 g boric acid
4 g MnSO_4
4 g $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$
2 g $\text{FeCl}_2 \cdot 6\text{H}_2\text{O}$
0.4 g molybdic acid
1 g KI
0.4 g $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$
10 g citric acid

Table 3

Oligonucleotides

Oligonucleotide	Sequence (5' to 3')	Use
HL1430	GCCTCGACATCATCTGCCC	5' Primer in <i>kanMX6</i> for inverse PCR
HL1431	CTCTAGCGGTGATCTTAACATC	3' Primer in hermes right for inverse PCR
HL1893	ACCCGAGTGTGCGATGAATCAATGAA	In hermes left to PCR amplify junction with genomic DNA