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Review Article

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

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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.

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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 *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,

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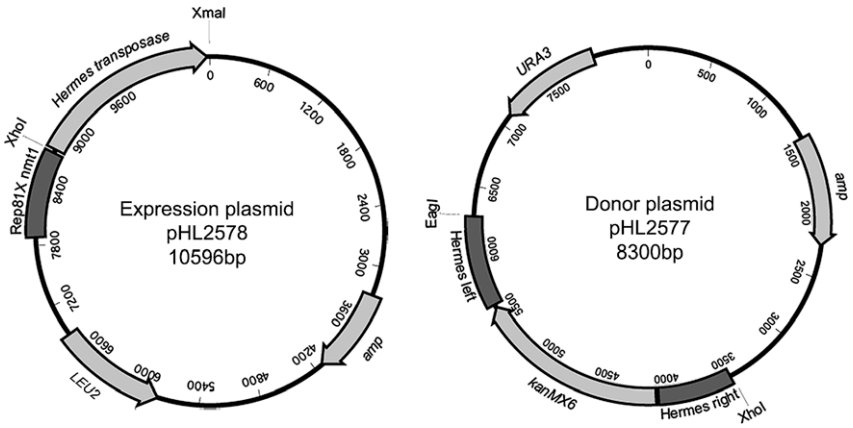

Fig. 1. *Hermes* mutagenesis plasmids. The expression plasmid (pHL2578) contains the transposase (Tpase) driven by the *nmt1* promoter. Restriction sites used to clone in the transposase are shown. This plasmid contains *S. cerevisiae LEU2* for selection in *S. pombe*. The donor plasmid (pHL2577) contains 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.

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).

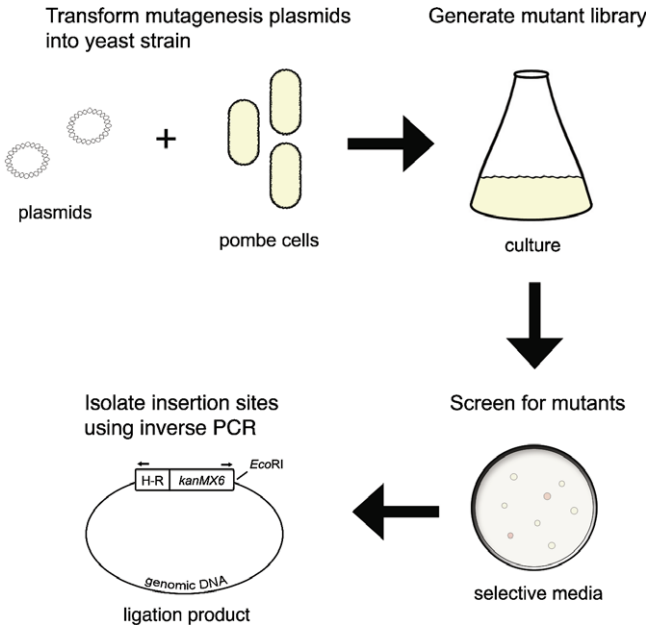

Fig. 2. Overview of method. The donor and expression plasmids are sequentially introduced into a strain of *S. pombe* using lithium acetate transformation. Strains containing plasmids are grown in liquid EMM medium lacking leucine and uracil to select for the plasmids. Cultures are grown in a series 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. See Table 3 for primer sequences.

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/l 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). See Tables 1 and 2 Thiamine at a concentration of 10 μM is also added to the media to repress the *nmt1* promoter.

3.1.3 When colonies from the transformation are ~1 mm, 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 ~1 mm, 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 μM. 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.

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