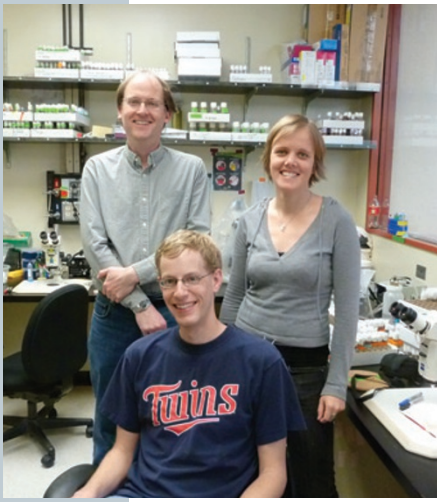
THE AUTHOR FILE

Thomas Clandinin

A new genetic construct enhances enhancer traps.

For scientists studying vision, a common experiment is watching flies watch movies of animated bars or dots. When he arrived at Stanford University 9 years ago, Thomas Clandinin wanted to unravel how flies sense and respond to motion, so he decided to reversibly inactivate neurons in the brain and look for defects in their behavior, such as staying still or walking in unexpected directions during the movie. He generated fly lines with neurons that could be turned off, but so many different neurons were affected that it was hard to link odd behaviors to any particular set of neurons. In his quest for a method to target neurons more specifically, Clandinin ended up creating a way to make genetic tools more generally applicable.



Thomas Clandinin (left) and coauthors Daryl Gohl (center) and Marion Silies.

A common way to manipulate fly genomes is to create insertions called 'enhancer traps' and breed flies that carry them with flies carrying reporter genes. To make an enhancer trap, a genetic construct with a minimal promoter and (usually) the yeast-derived transcription factor *Gal4* is inserted into the genome at random; *Gal4* is expressed only if it happens to be near an endogenous enhancer or regulatory element. As tissues in which expression occurs vary depending on the site of insertion and the construct used, researchers must screen the resultant flies to look for desired expression patterns.

Often, however, too many cell types express the transcription factor, and thus additional manipulations are needed. Researchers can, for example, create another set of flies that combine cell-specific promoters with a transcription factor inhibitor (such as *Gal80*) and then breed the insects, hoping that expression in undesired cell types will be subtracted out in the progeny. It is a hope that did not quite come through in Clandinin's case. "We did the experiments, got the enhancer traps and realized it wasn't possible to go anywhere after that," he says.

Many different sorts of genetic tools can be used in enhancer traps, but until now, using a new genetic strategy meant starting from scratch, creating a completely new set of flies, then screening their expression patterns and hoping for the best. To target just the right set of neurons the right way, Clandinin and his postdoc Daryl Gohl developed new genetic constructs and prepared to generate enhancer traps from them. Then Gohl pointed out that going through with those plans would mean microinjecting potentially hundreds of plasmids into fly embryos at a cost of about $200 for each different plasmid. "I had a brief panic when I realized what we were in for," recalls Clandinin.

Gohl and Clandinin went into the fly room and began drawing out possibilities on the whiteboard, eventually coming up with the genetic platform reported in this issue of *Nature Methods*, a pipeline that allows replacing a particular enhancer trap with any other sequence. They called it integrase swappable *in vivo* targeting element, or InSITE. The specifics are complicated, involving three recombinases and a set of mobile elements. The outcome, however, is simple. With the InSITE system, if a fly line with the desired expression pattern carries the wrong genetic tool, the right tool can be swapped in with just a few simple crosses, without injecting any plasmids or transforming any flies. "It was the perfect combination of postdoc laziness and principal investigator cheapness that inspired the swappable technology," jokes Clandinin.

> "Adapting your toolkit to the new thing that will be built next year will now be trivial." —Thomas Clandinin

The resource describes a collection of InSITE enhancer trap lines and swap lines that have been deposited with the Bloomington Drosophila Stock Center Library at Indiana University; plasmids into which most genetic tools can be inserted have been deposited with the nonprofit repository Addgene. At least four other fly geneticists have already applied the system in their laboratories, says Clandinin. "This is a living system. If a new transcription factor comes out, we'll be able to add it to the InSITE system very easily." In fact, researchers do not even need to start with an InSITE enhancer trap line, says Clandinin, even a simple piece of DNA will do. "Adapting your toolkit to the new thing that will be built next year will now be trivial."

Others may continue improving the InSITE system, but Clandinin is ready to put it to work. "We're going to use it to do the experiment I hoped to do 9 years ago," he says.

Monya Baker

Gohl, D.M. *et al.* A versatile *in vivo* system for directed dissection of gene expression patterns. *Nat. Methods* **8**, 231–237 (2011).