GENOMICS

Tinkering with the fly genome

A new strategy provides a leap forward in making transgenic flies.

One of the most important items in the biologist's toolbox is the ability to modify an organism's genome. Like construction workers, biologists can either tear out genetic information from the genome of an animal or put in additional genetic information. And fortunately, the tools biologists have at their disposal to manipulate genomes keep getting bigger and better.

The fruit fly *Drosophila melanogaster* is one model system frequently used in research, in fields ranging from neurobiology to developmental biology. By manipulating the fly genome, through adding or removing genes, biologists can understand the function of specific genes and what traits they control. Although it is already possible to genetically manipulate flies, present methods for doing so have several limitations. When it comes to adding a specific gene to the fly genome, it is difficult to add large genes or to control where the gene inserts. Recently, a team of scientists led by Hugo Bellen at the Baylor College of Medicine developed a new system, called P[acman], to overcome these problems (Venken *et al.*, 2006).

The standard approach for generating a transgenic fly involves first engineering a desired piece of DNA (usually containing a specific gene under study) and then injecting that DNA into a fly embryo. To develop a better platform for generating transgenic flies, Bellen and colleagues incorporated several recently developed technologies into this standard approach. To facilitate the DNA engineering step, the scientists turned to recombineering, a method that allows one to rapidly combine large pieces of DNA directly through homologous recombination in genetically modified bacteria, rather than using more traditional and laborious subcloning methods. The scientists also used a recently developed plasmid system (Wild *et al.*, 2002) that allowed them to maintain the plasmid DNA in low quantities when manipulating it in the bacteria, but also to amplify it before injection. This is important because the DNA is more stable in the bacteria in low quantities, but larger amounts are needed for injection.

The scientists also used a recently developed system for inserting the DNA into the fly genome. This system, developed by Michelle Calos of Stanford University, makes use of an integrase enzyme (originally from a bacteriophage), which is injected into the fly along with the DNA to be inserted (Groth *et al.*, 2004). Using specific recognition sequences that are added to the insert DNA when it is engineered and complimentary recognition sequences that are added to the fly genome, the integrase enzyme is able to precisely insert an engineered piece of DNA into the fly genome, always in the same location.

This combined approach was much more robust than previous strategies to generate transgenic flies. Because genes are predictably inserted into the same spot every time an experiment is performed, potential differences in gene expression that could result when genes insert randomly are not a concern. Additionally, this approach can be used to manipulate large chunks of DNA, which is an important advantage because some genes, or gene clusters, are quite big. According to Koen Venken, a graduate student in Bellen's lab who helped develop P[acman], the size of DNA that can be inserted is about four times larger than what was routinely possible in the past. Although the group's initial work focused on using this system to insert additional genes into the genome, Venken points out that this approach could be applied in other ways, including "to tag proteins with biochemical tags, to allow immunoprecipitations or to determine the expression pattern of any gene by integrating an enhanced green fluorescent protein".

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RESEARCH PAPERS

Groth A.C., *et al.* Construction of transgenic Drosophila by using the site-specific integrase from phage phiC31. *Genetics* **166**, 1775–1782 (2004).

Venken, K.J.T. *et al.* P[acman]: a BAC transgenic platform for targeted insertion of large DNA fragments in *D. melanogaster*. *Science* **314**, 1747–1751 (2006).

Wild, J. *et al.* Conditionally amplifiable BACs: Switching from single-copy to high-copy vectors and genomic clones. *Genome Res.* **12**, 1434–1444 (2002).