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Genes Large or Small, P[acman] Places Them All

A new technique for inserting large genes or collections of genes into precise locations on the chromosome may enable researchers to overcome some of the challenges they face in trying to pin down a gene's function. The researchers said their tool, which they call P[acman], is now available to researchers who can use the method to help them understand the function of genes in basic biology or disease.

The scientists, led by Howard Hughes Medical Institute investigator Hugo J. Bellen, developed the technique in the fruitfly *Drosophila melanogaster*, an organism widely used in genetic research. Although the tool was developed in *Drosophila*, the researchers said that P[acman] can also be used to insert genes in mammalian chromosomes.

Bellen and his colleagues published their findings November 30, 2006, in *Science Express*, which provides rapid online publication of select articles from the journal *Science*. Bellen is at the Baylor College of Medicine (BCM); other coauthors include Koen J. T. Venken and Yuchun He of BCM and Roger Hoskins of Lawrence Berkeley National Laboratory.

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In exploring gene function, researchers often alter a gene of interest through mutation and then insert the mutant gene into the genome of a model organism such as a fruitfly to begin to determine the gene's function. One widely used technique for inserting such genes involves incorporating the mutant gene into P-element vectors which are used to reproduce the mutant gene and insert it into the *Drosophila* genome.

However, said Bellen, fruitfly biologists have long had difficulties in reproducing, or cloning, large DNA fragments—including genes or

collections of genes. Furthermore, they have also faced challenges in trying to manipulate those large fragments effectively and insert them at a specific point into the genome.

“Because these DNA fragments integrate at random into the genome, there is a major problem with comparing different mutations in the same gene,” said Bellen. “There are positional effects, in that two inserted genes may be expressed at different levels or even in different tissues.”

To overcome these problems -- and to more easily manipulate the DNA -- the paper's first author, Koen Venken, modified a bacterial artificial chromosome (BAC) tailored for manipulating and reproducing a wider range of DNA fragments. This BAC exists only as a few copies in the bacteria, meaning that it can be efficiently manipulated to insert DNA fragments. But the BAC can also be triggered to produce large numbers of copies for DNA sequencing and insertion into fly genomes.

Venken applied a method called “recombineering” to the fruitfly genes to retrieve specific large DNA fragments and insert them into the new BACs. The method commandeers DNA repair machinery that can be introduced in the bacteria to manipulate the DNA fragments. Previously, recombineering technology had mostly been used by mouse geneticists

Venken then used a recently developed methodology based on an enzyme called ϕ C31 integrase to integrate the BAC-carried gene fragment to specific docking sites in the fruitfly chromosome. Thus, the researchers named their technique “P/ ϕ C31 artificial chromosome for manipulation,” or P[acman].

“With these docking sites, you can integrate a piece of DNA at specific places, so if you have five different mutations you can compare them very effectively, because they are always integrated within the same site and are subject to the same position effects,” said Bellen. Such precision can greatly reduce the number of experiments needed for a given gene, he noted. Instead of generating many different versions of the same transgenic animal to ensure that position effects were not influencing a mutant gene's function, researchers using P[acman] need only generate one transgenic animal to compare to another.

While P[acman] offers major advantages for studying large genes and gene collections “we use it for small genes as well, because it is so much easier and faster to manipulate and mutate the piece of DNA in bacteria,” noted Bellen.

Bellen said that P[acman] should be applicable to mammals, such as mice, a species widely used in genetic studies. The recombineering technology was developed by mouse geneticists in collaboration with bacterial geneticists to facilitate the creation of modified BAC transgenic mice, he pointed out, and

other researchers are already working to optimize the phiC31 integrase for use in mammalian cells.

The researchers are also creating libraries of the new BAC vectors containing large pieces of genomic fly DNA. Thus, researchers could readily obtain specific genes already inserted into the BACs for their studies. Finally, Bellen and his colleagues will seek to automate P[acman], using robotic manipulators to speed up the application of the technique to individual genetic studies.

Bellen said the mutant flies used in the technique are available to researchers from the Bloomington Stock Center and the new BACs from the Drosophila Genome Research Center < <http://dgrc.cgb.indiana.edu> />.