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# Additional Paper

# Transposable elements as tools for genomics and genetics in *Drosophila*

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## Abstract

The *P-element* has been the workhorse of *Drosophila* genetics since it was developed as a tool for transgenesis in 1982; the subsequent development of a variety of systems based on the transposon have provided a range of powerful and flexible tools for genetics and genomics applications. *P-element* insertions are frequently used as starting-points for generating chromosomal deletions to remove flanking genes, either by screening for imprecise excision events or by selecting for male recombination events. Elements that utilise the yeast FLP/FLP recombination target (FRT) site-specific recombination system have been widely used to generate molecularly marked mitotic clones for mosaic analysis, extending the reach of this powerful genetic tool to virtually all areas of developmental biology. *P-elements* are still widely used as traditional mutagenesis reagents and form the backbone of projects aimed at generating insertions in every predicted gene in the fly genome. In addition, vectors based on the FLP/FRT system are being used for genome-wide applications, including the development of molecularly-mapped deletion and duplication kits. In addition to these 'traditional' genetic approaches, a variety of engineered elements have been developed for a wide range of transgenic applications, including enhancer trapping, gene-tagging, targeted misexpression, RNA interference (RNAi) delivery and homologous recombination/gene replacement. To complement the use of *P-elements*, alternative transposon vectors have been developed. The most widely used of these are the lepidopteran element *piggyBac* and a *Drosophila hydei* transposon, *Minos*. In total, a range of transposon vectors offers the *Drosophila* biologist considerable flexibility and sophistication in manipulating the genome of the fly and has allowed rapid advances in all areas of developmental biology and genome science.

***Keywords:*** Drosophila, *mutagenesis,* P-elements, *transposons, RS elements, transgenesis*

## WHY THE FLY?

As the emphasis of genomic research moves from high-throughput genome sequencing towards the functional or 'post-genomic' era, so new tools and techniques are being employed to unravel what exactly the millions of basepairs actually do. For multicellular organisms, complete sequences (at least in draft form) are now available for *Homo sapiens*,[1] *Arabidopsis thaliana*,[2] *Caenorhabditis elegans*,[3] *Drosophila melanogaster*,[4] *Anopheles gambiae*[5] and *Fugu rubripes*.[6] With even more genomes in the pipeline, we will soon have a pretty good idea of the basic gene content of many organisms. Gene function and the way in which genes interact to build organisms, however, remains as elusive as ever: enter the model organism and the old workhorse, genetics.

As a model organism, *Drosophila* offers many advantages for post-genomics; these include a small genome size, relatively easy husbandry and, most importantly, a range of very sophisticated genetic tools. Although *Drosophila* is many millions of

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years removed from humans in evolutionary terms, there are still many similarities (bilateral symmetry, a nervous system and complex behaviours, to name but a few) and many of the genes in the fruit fly, such as those involved in setting out a body plan[7] or in tumour formation,[8] have orthologous genes in more complex animals. With over 70 per cent of the 1,378 human disease genes defined by Online Mendelian Inheritance in Man (OMIM) as having one or more orthologues/paralogues in the *Drosophila* genome,[9] it is hoped that studies in *Drosophila* can act as a Rosetta Stone for unlocking the secrets of how human embryonic development progresses and how, with the use of fly models, diseases such as Alzheimer's and Parkinson's are caused.[10,11]

***Drosophila* is a genetically trackable system for studying human disease**

## CLASSICAL GENETICS

*Drosophila* has been the focus of genetic studies for almost 100 years and has made many important contributions to our understanding of heredity and the action of genes. A variety of methods are available for generating heritable changes in the genome and these can be broadly divided into three categories: chemical agents causing single base changes; ionising radiation generating chromosomal aberrations; and transposons, which cause mutations by insertional inactivation. The most widely-used transposon today, and the subject of much of this review, is the *P-element*.

## *P-ELEMENT* BIOLOGY

*P-elements* are thought to have entered the *D. melanogaster* population less than 100 years ago by horizontal transfer from another *Drosophila* species and they have since spread to most wild and laboratory populations. First recognised as factors in P strains that caused hybrid dysgenesis (a syndrome that includes mutation, sterility and male recombination) in crosses between male P strains and female M strains,[12] the *P-element* has since become the most widely-used tool for studying gene function in the fly.[13,14] Broadly speaking, *P-elements* can be divided into two groups: autonomous elements, which encode their own source of the Transposase needed for mobilisation, and non-autonomous elements that need an external source of Transposase in order to move. Non-autonomous elements occur either as natural mutations, produced as a result of internal deletion, or in the form of the engineered constructs widely used in the laboratory.

**Mobile DNA elements provide a range of tools for manipulating the genome**

The wild-type autonomous *P-element* is 2.9 kb in size and contains a four-exon *transposase* gene and a number of inverted repeats (Figure 1). In order to transpose, all *P-elements* must have intact 31-basepair perfect inverse terminal repeats and 11-basepair subterminal inverted repeats; the repeats are the site of action of the Transposase. *P-element* transposition is naturally restricted to the germ line because the splicing of the intron between exons 2 and 3 of the *transposase* is inhibited in somatic cells by a splicing repressor protein.[15,16] In the soma, the splicing of the remaining three exons results in the production of a truncated Transposase protein that acts as a repressor of *P-element* mobility.[17] This truncated repressor is also responsible for the fact that, in wild type strains, *P-element* mobility is restricted to crosses between M strain females and P strain males, since P strain females pass on the repressor protein through the cytoplasm of their eggs.[18,19] Once this was understood, it was relatively easy to engineer the *transposase* gene, by deleting the regulated intron, to produce a Transposase source, Δ2-3, that will function in any tissue of the fly.[20]

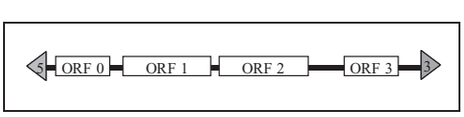



**Figure 1:** The basic structure of an autonomous *P-element*. The element consists of four open reading frames (ORF) and 5′ and 3′ inverse terminal repeat ends (shown as triangles 5 and 3), which are required for transposition

## USES OF *P-ELEMENTS*

The use of *P-elements* for transgenesis was first developed by Rubin and Spradling,[21] who restored wild type function to *rosy* mutant flies by injecting a *P-element* containing a functional *rosy* gene into *Drosophila* embryos and recovering rescued flies among the progeny of the injected individuals.[22] Since then, *P-elements* have been widely adapted and modified to provide a range of functional tools for biologists; they can be used for gene tagging, gene disruption, chromosome engineering and inducible gene expression. In essence, all that is required to generate a transposable construct is a pair of suitable ends that can be acted upon by a source of Transposase supplied *in trans*. Generally, Transposase is supplied by co-injecting the construct with an element that produces Transposase but cannot itself move because it has damaged ends ('wings-clipped' elements), or by introducing the construct into an embryo that carries an autosomal copy of the Δ2-3 Transposase source.

### Gene disruption

**Molecularly tagged mutations accelerate genetic mapping**

One of the early uses for *P-elements* was in large-scale mutagenesis screens, the major advantage over traditional chemical or radiation methods being that mutants were molecularly tagged by virtue of the *P-element* sequence. First attempts used naturally-occurring chromosomes containing many non-autonomous elements but this quickly gave way to a more refined strategy using single engineered elements.[23] Once injected into an embryo and incorporated into the genome, a *P-element* construct can be easily mobilised using a separate source of Transposase, creating many lines with a single element inserted randomly in the genome. Elements that transpose into genes may disrupt their function producing visible or lethal phenotypes. A variety of engineered constructs is available, each containing a selectable marker gene — generally the eye colour markers *white*, *pP{CaSpeR}* or *rosy*, *pP{Car20}*. Other markers such as neomycin resistance, however, have also been employed (for example *pP{hsneo}*). These vectors contain a variety of unique restriction sites, allowing the introduction of cloned DNA back into the fly.

The most useful elements used today contain *Escherichia coli* plasmid sequences that allow easy cloning of genomic DNA flanking the insertion site (ie *pP{lacW}* or *pP{PZ}*). An alternative to plasmid rescue for mapping insertion sites, and a method generally applicable to almost all elements, is to sequence the product of an inverse polymerase chain reaction (PCR) primed from either end of the *P-element*.[24] Over the past dozen or so years, various projects have generated collections of *P-element* mutations and today 4,216 of the 14,000 or so predicted genes have associated *P-element* insertions and, in total, 9,161 different *P-element* alleles are listed in FlyBase.[25]

Mutagenesis efforts have culminated in the Gene Disruption Project,[26,27] a consolidated effort to mutate every gene in the *Drosophila* genome with a *P-element*. Initial results were promising, with a lethal screen hitting over a quarter of the 3,600 or so genes needed for viability.[28] However, whether the goal of obtaining an insertion in every gene is achievable with *P-elements* is a matter of some debate, the major problem being that of *P-element* insertion bias. *P-elements* preferentially transpose into the 5′ region of genes[29] and have a bias toward a particular sequence motif[15] as well as exhibiting a preference for inserting near existing *P-elements*. In addition to this, there is a well-documented preference for some genes, which are hit at extremely high frequency (such as *escargot* or *singed*) — so called 'hot spots' — and a distinct dislike of other genes, so called 'cold spots', which have to date never been hit. These considerations greatly increase the number of insertions theoretically needed for full genome coverage and only time will tell if the approach will be successful. Alternatives transposons for use in mutagenesis are discussed below.

**Exploring gene regulation with reporter systems is easy and efficient**

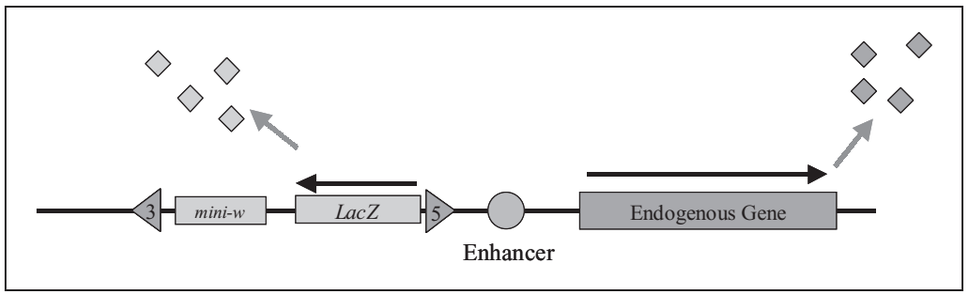



**Figure 2:** Enhancer trapping. A *P-element* construct containing a transformation marker, in this case a functional copy of the *white* gene (*mini-w*), and a *LacZ* reporter gene driven by a weak basal promoter inserts near a gene. An endogenous enhancer (grey circle) may then control the expression of the *LacZ* reporter in a similar pattern to the endogenous gene (black arrows). *P-element* ends are shown as triangles (5 and 3), and gene products are shown as squares

## *Enhancer* trapping

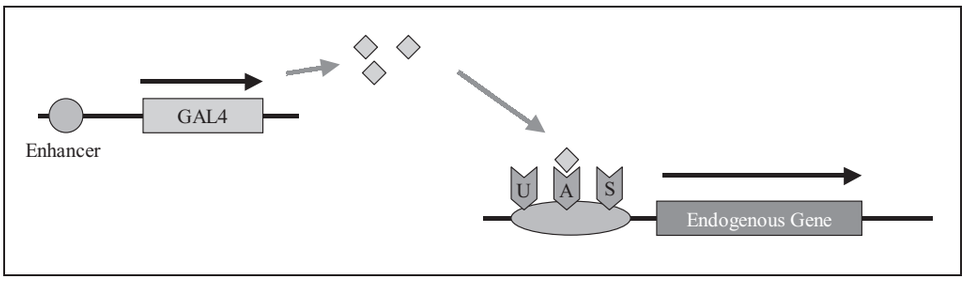
In addition to phenotypic screening, *P-elements* also can be used to study the pattern and timing of gene expression by enhancer trapping (Figure 2).[30,31] Here, *P-element* constructs carrying a reporter gene linked to a weak basal promoter are randomly mobilised in the genome. Elements that insert near an endogenous genomic enhancer may activate the weak basal promoter and express the reporter gene under the control of that enhancer. In some instances, the reporter expression is identical to the pattern of the endogenous gene and in other cases, a subset of expression domains are detected. Commonly, the *E. coli β-galactosidase* gene is used as a reporter, since detection by the colour change of a chromogenic substrate is an easy and cheap assay. More recently, green fluorescent protein (GFP) or luciferase reporters have been employed, allowing real-time imaging by fluorescence microscopy. Enhancer trap screens have generated a range of expression markers that are used extensively in developmental biology; for example, the $P\{ry^{+t7.2}=PZ\}wg^{02657}$ insertion is a widely-used marker for *wingless* expression. An extensive and searchable catalogue of over 3,700 enhancer trap lines is available online at the FlyView web server (http://pbio07.uni-muenster.de/).[32]

**Regulated transgene expression is a powerful tool for studying gene function**

Two main elements have been used in large-scale screens: $pP\{PZ\}$[30] and $pP\{LacW\}$,[33] which differ by the selectable marker employed (*ry* and *w*, respectively). In addition to these, specialised constructs have been developed for studying particular biological processes such as eye development[34] or brain anatomy.[35] While in some cases the insertions can disrupt the expression or function of the target gene, in many cases there is no detectable phenotype associated with the insertion, making functional inferences about the trapped gene less straightforward.

## The GAL4-UAS system

One widely-used variant of the enhancer trap strategy is the GAL4-UAS system developed by Brand and Perrimon.[36] This binary system utilises enhancer trapping with a construct carrying the *Saccharomyces cerevisiae* transcriptional activator, GAL4, as a reporter gene. Expression of GAL4 has no apparent consequences for the fly, even when expressed at relatively high levels. Enhancer traps can be monitored by detecting the GAL4 protein but, more importantly, the activity of the GAL4 protein as a transcription factor can be detected by monitoring the expression of a second reporter gene under the control of a GAL4 responsive promoter, or upstream activation sequence (UAS) (Figure 3). On the one hand, reporter




**The GAL4-UAS system has revolutionised the study of developmental biology**

**Figure 3:** GAL4-activated gene expression. In the GAL4-UAS system, a construct containing the *GAL4* gene is inserted randomly in the genome. As with the enhancer trap strategy shown in Figure 2, it may come under the influence of a genomic enhancer and express *GAL4* in a pattern dictated by the enhancer. The GAL4 protein can then act at any UAS sites in the genome to activate expression of a gene of interest. Two scenarios are possible; in the first, a gene of interest is introduced into the genome in a *P-element* construct carrying UAS sites. In the second, a set of UAS sites in a *P-element* (an EP-element) are mobilised at random in the genome; if they insert in the vicinity of an endogenous gene, GAL4 can be used to activate the expression of that gene

genes such as *LacZ* or *GFP* can be used to visualise the expression pattern of the enhancer. On the other hand, and far more importantly, any gene placed downstream of the UAS sequences in a construct can be activated by the GAL4 protein, be it a transcription factor, a signalling molecule or a cytoskeletal component. It is fair to say that the GAL4-UAS system has revolutionised the way in which biological processes in the fly are studied, since the consequences of ectopic or over-expression of any gene of interest can now be studied. Since its development, the system has been utilised in hundreds of published papers dealing with almost every aspect of biology. With many hundreds of GAL4 enhancer trap lines available, along with constructs driving GAL4 expression with defined promoter sequences, it is possible to express a gene of interest at almost any stage of development and in any tissue. Readers should note that the Sept–Oct, 2002 issue of the journal *Genesis* was devoted to the GAL4-UAS system and contains descriptions of many GAL4 lines and UAS constructs.[37]

**Genome-wide misexpression screens help in functionally categorising genes**

The GAL4-UAS system is extremely useful for directing the misexpression of genes; however, the original method requires that individual transgenic lines be established for any gene of interest. To overcome this limitation, and allow 'genome-wide' screens for identifying genes that can affect particular developmental processes, Rorth *et al.* developed the EP-element.[38,39] The EP system (Figure 3) consists of an element bearing a set of UAS sequences that, when mobilised, act as a transposable enhancer by inserting GAL4-responsive elements at random in the genome. If an EP-element inserts near a gene, then introducing a source of GAL4 protein can induce the expression of that gene, either via a GAL4 enhancer trap line or via GAL4 driven by a specific promoter. Using the EP-element, a number of large-scale dominant overexpression screens and dominant suppression screens have identified genes involved in a range of developmental processes ranging from axon guidance[40] to cuticle formation.[41]

## Gene trapping

The dual tag gene trap vector, pGT (Figure 4), was designed to address the problem that not all enhancer traps produce mutations in the target gene.[42] The construct consists of a promoter-less *GAL4* reporter gene with a splice acceptor sequence at its 5′ end and a *mini-w* gene controlled by an eye promoter but lacking a polyadenylation signal. The *mini-w* is terminated at its 3′ end by a splice donor

**Progress in gene trapping techniques will greatly facilitate cell biology in the fly**

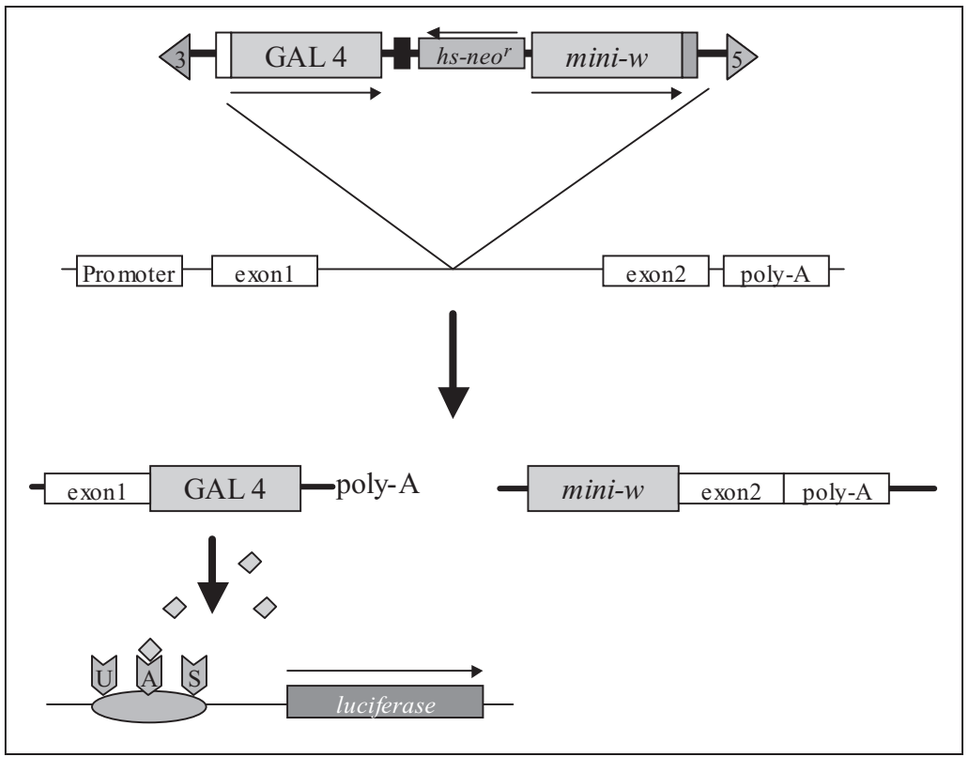



**Figure 4:** Dual-tagged gene trapping. The pGT1 vector consists of a *GAL4* gene with a splice acceptor site at its 5′ end (empty block) and an *hsp*70 terminator at its 3′ end (solid black block). A *mini-white* (*mini-w*) gene without a polyadenylation signal has a splice donor site at its 3′ end (solid grey block). A *hs-neo*$^r$ marker may be used as a primary selectable marker. Upon insertion into a gene, two fusion transcripts are produced — one containing the 5′ part of the gene and *GAL4*, and the other the 3′ portion of the gene and *mini-w*. The resulting GAL4 protein may then act on a UAS enhancer, driving expression of a reporter gene, in this case *luciferase*. Functional (ie polyadenylated) *white* mRNA, and hence eye pigmentation, can only be produced if the *mini-w* gene splices onto an exon containing a polyadenylation site

sequence and, lacking a functional polyadenylation site, it will only be expressed if it is inserted into a gene and spliced onto a downstream exon(s) possessing a polyadenylation signal. Similarly, the *GAL4* reporter can only be expressed as a transcriptional fusion if it is inserted downstream from the first exon of the endogenous gene. Production of GAL4 protein can be assayed by activation of a reporter gene, such as *luciferase*, under the control of *GAL4* regulatory sequences. pGT-bearing lines can, therefore, be screened for eye colour as well as for GAL4 activity and, if both selections are employed, it is almost certain that the element disrupts a gene. An additional benefit of the system is that, since *GAL4* is expressed as a transcriptional fusion, it should directly match the expression of the host gene, greatly facilitating studies into gene function.

A second strategy for gene trapping has recently been described.[43] In this scheme, a construct carrying a *GFP* reporter gene flanked by splice acceptor and splice donor sites is mobilised and gene fusions detected by screening for *GFP* expression. While the efficiency of gene trapping screens is low compared with screens using other *P-elements*, it is amenable to automation by utilising fluorescence-based sorters to select positive lines from large populations. Gene trapping will undoubtedly become more widely used in the near future.

## Genome manipulation

As well as providing a mechanism for mutating or manipulating the expression of individual genes, *P-elements* are extensively employed as tools for generating genome rearrangements such as deletions and inversions. In this role, a number of powerful systems are being developed that suggest we may soon be able to manipulate the *Drosophila* genome with the ease and accuracy with which simpler genomes, such as *S. cerevisiae*, can be handled. Such developments will be vital if we are to understand how the genome is dynamically deployed during growth and development.

**Controlling site-specific recombination can manipulate the genome with precision**

## Site-specific recombination using FRT sites

The recombination system of the *S. cerevisiae* 2 μ plasmid[44] consists of a gene encoding a recombinase (FLP), which acts on recombination sequences referred to as FLP recombination target (FRT) sites. The system has been transferred into *Drosophila* via *P-elements* with FLP under the control of an inducible *hsp70* heat shock promoter and elements bearing FRT sites.[45] FLP activity on DNA flanked by appropriately-oriented FRT sites results in the excision of the DNA between the sites, creating a circular DNA molecule with one FRT site.[46] If there are more FRT sites in the genome, then further rounds of FLP-induced recombination may reintroduce the circularised DNA back into the chromosome at the new location;[47] any recombination events that occur in the germ line will then be passed on to the progeny. The system can also be used to promote recombination between elements located *in trans* (see below).

**Cell lineage studies at single cell resolution with the FLP/FRT system**

The FLP/FRT system has been used in a variety of ways in *Drosophila*. Using the system to produce genetic mosaics (clones of cells homozygous for a particular mutation) gives far greater flexibility and control than the traditional X-ray-induced mitotic recombination method.[48] A set of chromosomes containing FRT sites close to the centromere on each arm of the chromosome has been developed. Using these chromosomes in mosaic experiments simply requires recombining the mutation of interest onto the FRT-containing chromosome. In general, an FRT chromosome carrying a mutation is placed *in trans* to an FRT chromosome carrying a reporter gene, such as *GFP* or *βGal*, driven by a ubiquitous promoter. Clones of cells that are homozygous for the mutation are then readily identified by the loss of the marker gene. A method for lineage tracing single neurons with FLP/FRT, MARCM (**M**osaic **A**nalysis with a **R**epressible **C**ell **M**arker), is becoming increasingly popular among neurobiologists, since it allows single neuron resolution in anatomical studies. It can also be used to generate a marked cell carrying a particular homozygous mutation.[49] This technique relies on gaining expression of a marker gene rather than the loss of marker expression described above. To carry out a MARCM analysis, a *P-element* bearing the yeast *GAL80* gene driven by a ubiquitous *tubulin* promoter (*GAL80* is a repressor of *GAL4*-mediated gene activation) is engineered to be distal to an FRT site and *in trans* to an FRT-bearing chromosome carrying a mutant of interest. Other chromosomes carry a *GAL4* driver, a UAS-driven marker gene and a heat-shock inducible source of FLP recombinase. In heterozygous animals, expression of *GAL80* prevents *GAL4* activation of the marker gene. FLP-induced recombination results in clones of cells that are homozygous for the mutation of interest and have lost the *GAL80* repressor. Therefore, homozygous mutant clones are easily identified, since they express the marker in a background of cells that do not.

As described below, the FLP/FRT system can also be used to generate gross chromosomal rearrangements and is becoming an increasingly powerful method for manipulating the genome.[50] Along with *Drosophila*, the FLP/FRT

system has also been successfully applied to mouse embryonic stem cells[51] and plant cells,[52,53] indicating its broad utility.

## Creating deficiencies with *P-elements*

**A *P-element* insert is a start point for further genetic analysis**

Before exploring the use of the FLP/FRT system, we should mention that almost any *P-element* can be useful for generating deletions of flanking DNA.[54,55] When a *P-element* is excised from a chromosome, a double-stranded break (DSB) is created;[56] the DSB is a staggered cut and leaves at least 33 nucleotides of single-stranded DNA.[57] The DSB can be closed by homology-directed repair, using either the sister chromatid or the homologous chromosome as a template,[56] or by non-homologous repair where each end of the DSB is simply ligated together. If the ends of the DSB are degraded before repair, a deletion of the genetic material will occur, an event known as imprecise excision.[58] The frequency of imprecise excisions is approximately 1 per cent of excision events. Deletion sizes can range from a few basepairs to several kilobases. Much larger deletions can be created using hybrid element insertion (HEI).[59] HEI occurs between *P-elements* that are *in trans* on sister chromatids. Instead of excising one whole *P-element* at the 3′ and 5′ ends, transposase can sometimes excise the 3′ end of the element on one chromatid and the 5′ end of the element on the other. The 'free ends' of the elements are then integrated back into the genome near to the original excision events, potentially causing a deletion or duplication of the material between the elements.[60] Deletions are readily detected by looking for recombination of flanking markers in the male germ line. (For some reason that is not really understood, but is extremely useful for geneticists, there is no recombination in male *Drosophila*.)[61]

**A combination of transposon mutagenesis and site-specific recombination can create custom deletions mapped with nucleotide resolution**

When generating deletions using HEI, it can be difficult to create a deletion of a specific size or in a precisely-defined region, as, by its very nature, the reinsertion event will occur in an unpredictable site. It would be very useful to be able to generate precisely-defined deletions, accurately mapped on the genome, with molecularly-described endpoints. Such a resource could be used to generate a deficiency kit, a set of deletions covering much of the genome. Over the years, the *Drosophila* community has collected a deficiency kit in an *ad hoc* fashion, using individual deletions as they have been generated; however, the existing kit is extremely heterogeneous and the molecular structure of each deletion is generally very poorly defined (many are only mapped cytologically). Deficiency kits are extremely valuable tools for performing crude, genome-wide screens for enhancers or suppressors of a particular phenotype or for scanning the genome for regions that give specific phenotypes when deleted. Once identified by a deficiency, the region of the genome can be studied in detail to identify the particular gene responsible for the phenotype. Unfortunately, due to heterogeneity and poor molecular definition in the current deficiency kit, the journey from a genetic interaction to an identified DNA sequence can be lengthy.

In order to generate a new, or second generation, deficiency kit, the European DrosDel Consortium ( http://www.drosdel.org.uk/) is making use of a system devised by Golic and Golic, based on FLP/FRT.[50] The chromosomal forms of two elements, *P{RS3}* and *P{RS5}*, each contain a *white* gene construct ($w^{hs}$); they differ by the position of FRT sites located in an intron of the *w* gene and at the 5′ or 3′ ends (shown as arrows in Figure 5). The activity of FLP recombinase on each of these elements produces a chromosomal remnant form of each element, *P{RS3r}* or *P{RS5r}*, each containing a non-functional part of the *w* gene and a single FRT site. A second FLP-mediated recombination between a pair of the remnant *RS3r* and *RS5r* elements will result in a reconstituted functional *w* gene. If the two elements are *in trans* and in the correct orientation with respect to each other, the FLP-mediated

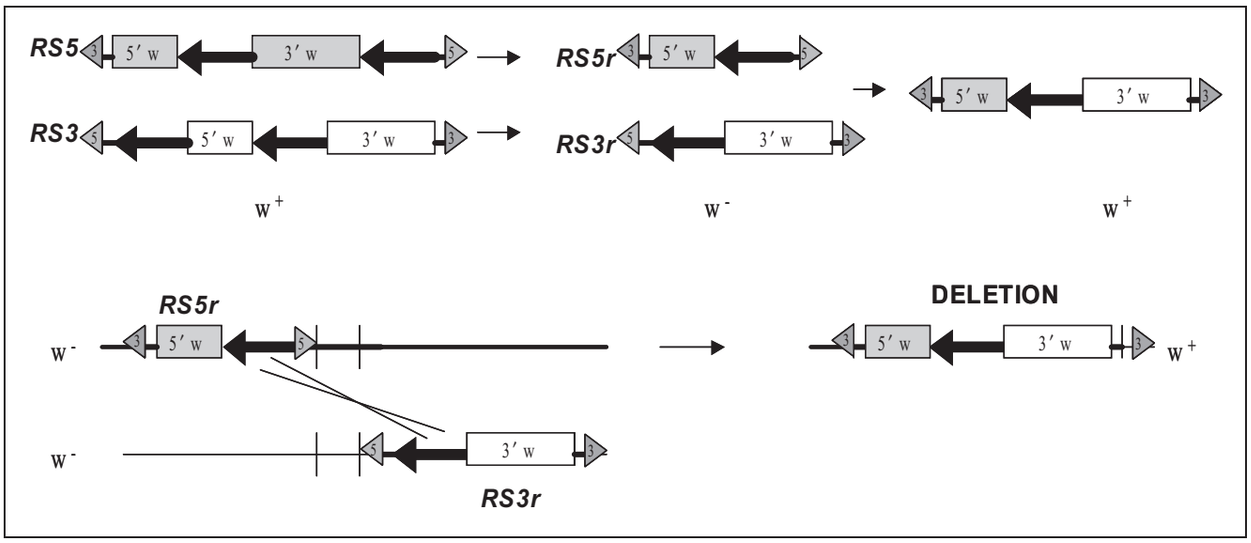



**Figure 5:** Creating genomic deletions with RS elements. The P{*RS3*} and P{*RS5*} elements contain a functional *white* gene (split into two parts) and two FRT sites (shown as thick arrows in the *P-element*). The elements differ in the position of the FRT sites in the element. Internal recombination between the FRT sites mediated by FLP recombinase produces the remnant form of the elements, P{*RS3r*} and P{*RS5r*}; each now has a non-functional half of the *white* gene and a single FRT site. If a P{*RS3r*} and a P{*RS5r*} are arranged *in trans* at different locations on homologous chromosomes and they are in the orientation shown, a FLP-mediated recombination between them produces a reconstituted *P-element* with a functional *white* gene. The intervening genomic DNA is deleted

**Finally, the *Drosophila* field has a system for targeted gene knockout**

recombination will generate a deletion of the chromosome material between the sites. If the elements are close enough together (in our hands they can be up to 1 Mb apart) and the recombination occurs in the germ line, then red-eyed progeny (from the reconstituted *w* gene) will carry a chromosomal deficiency of defined length. If the starting RS elements have been mapped onto the genome by sequencing, the endpoints of the deletion are precisely known. The goal of the DrosDel project is to produce approximately 0.5 Mb deletions with 0.167 Mb overlaps covering as much of the genome as possible (estimated to be ~650 deletions for full genome coverage). In addition to generating a kit, all of the mapped elements will be available to the community to allow the design and generation of custom deletions of a required size in regions of interest. To date, over 2,700 RS elements have been mapped, and over 7,500 deletions of between 1 basepair and 1 Mb can potentially be produced. The functional genomics company, Exelixis, is reported to have used a similar strategy for generating deletions based on *piggyBac* transposons; however, these stocks are not in the public domain.

## Gene targeting

For many years, the bane of the research life of the Drosophilist (apart, of course, from devastating mite infestations) has been the inability to generate targeted gene knockouts or replacements. While such techniques are commonplace in yeast or mammalian systems, the ability to do this in flies has been sadly lacking. Fortunately, this now appears to be resolved with the recent development of a targeted gene replacement strategy by Rong and Golic.[62] In this scheme, a *P-element* vector containing the gene (or fragment of a gene) of interest is constructed, and engineered to incorporate a recognition site for the *I-SceI* restriction enzyme. The vector also contains a marker gene and two FRT sites (Figure 6). Fly lines carrying the targeting

**In principle, every gene in the fly genome can now be mutated or tagged**

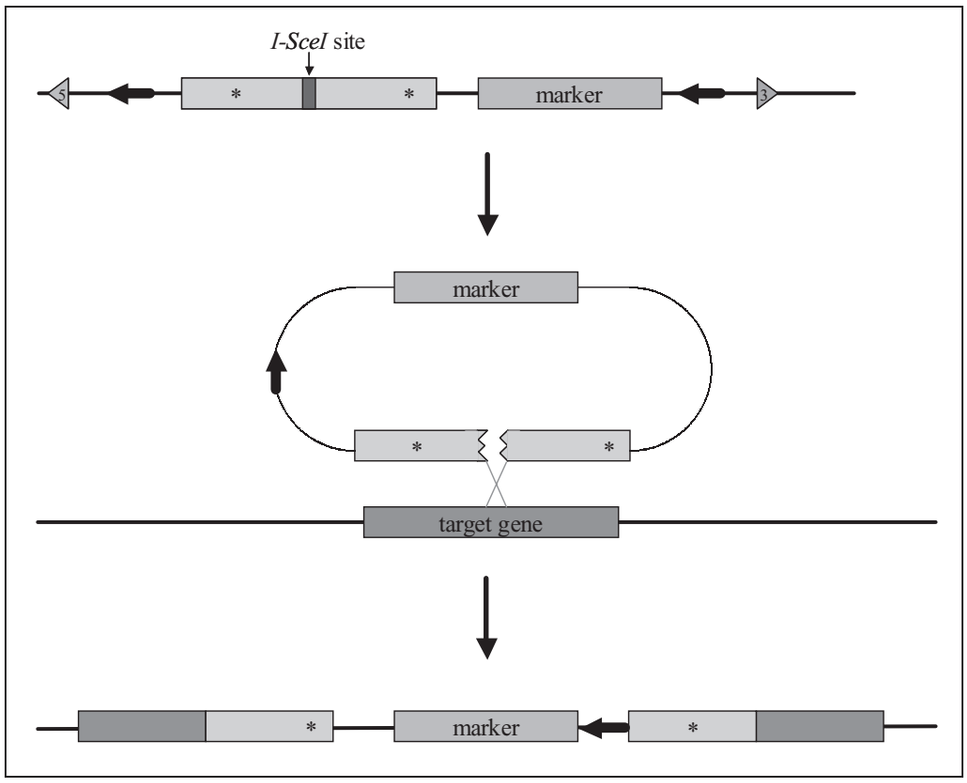



**Figure 6:** Ends-in gene targeting. A *P-element* targeting vector consists of two FRT sites (shown as black arrows within the *P-element*), a marker gene and an engineered gene of interest (shown in light grey). The gene contains a recognition site for the restriction endonuclease *I-SceI* and has been engineered such that the gene also contains two mutations, generally termination codons on each side of the *I-SceI* site (marked by the asterisk). The targeting construct is introduced into the genome by conventional germ line transformation. Once in the genome, FLP-induced recombination excises the gene and the marker forming an extra chromosomal circle. The circle is then cut by *I-SceI* to produce a linear molecule. The homologous recombination system will then recombine the mutated DNA from the targeting construct with the endogenous gene to generate a duplication consisting of half the target gene and half the endogenous gene. Since the targeting construct DNA contains mutations, both of the duplicated copies will be mutated. Clearly, some knowledge of the biochemistry of the protein of interest would allow the creation of useful mutations such as those with a temperature-sensitive phenotype.

construct are crossed with lines expressing both FLP recombinase and the *I-SceI* enzyme under the control of a heat shock promoter. Upon induction of the FLP, a recombination between the two FRT sites in the targeting construct creates a circular extrachromosomal fragment. The circle is then cut by *I-SceI* to yield a linear molecule that is incorporated into the genome via homologous recombination, resulting in a duplication of the target gene. Each of the duplicates consists partly of the original target and partly of the introduced fragment; therefore, to successfully mutate a gene, two mutations must be introduced into the targeting construct, one on each side of the *I-SceI* site. The *I-SceI* site is usually engineered into an intron in the targeting construct; however, it has been observed that in this location it is sometimes removed as a result of the DSB and gap repair mode of recombination.[62,63] A modification to the system incorporating a second restriction enzyme, *I-CreI*, allows the resolution of the duplicated gene into a single mutated

copy. This modified method provides a route for replacing a given locus with a mutated copy, leaving no exogenous DNA (other than the actual mutation) in the genome and, therefore, has considerable potential as a tool for detailed functional analysis of any gene.[64] Despite some initial concerns about the exact mechanisms of gene targeting and subsequent DSB repair,[65] the technique has been successful with several genes[62,64,66] and will undoubtedly become more widespread in the near future.

**Inducible RNAi is the tool of choice for functional analysis of genes identified in microarray screens**

## RNAi

Before we leave *P-elements*, we should briefly mention the use of transposons as vectors for inducible RNAi. First developed in *C. elegans*, RNAi allows the silencing of a gene without having to mutate the endogenous copy.[67] Injection of double-stranded (ds) RNA into an embryo or transfecting a tissue culture cell can result in almost complete and specific silencing of target gene expression.[68] The exact mechanism of RNAi is still not fully understood, but it involves the cleavage of the injected dsRNA into ~25 basepair fragments.[69] These fragments then act as a 'degradation template' for the host gene mRNA.[70] The problems associated with RNAi in *Drosophila* when induced by injection into embryos are that the effect is not stably inherited, and that silencing a gene early in development can hinder the analysis of its effects later in development.[71] One solution to this was suggested by Kennerdell and Carthew,[72] who developed a *P-element* construct consisting of a dyad symmetrical *LacZ* sequence separated by a 5-basepair linker. When the dsRNA was induced using the GAL4-UAS system, *LacZ* production from a normal UAS-*LacZ* construct was very much reduced compared to controls. Since then, similar systems for inducing dsRNA expression with the GAL4-UAS system have been developed in a number of labs.[73–75] The advantage of these approaches is that by using a transgene it is heritable and that a gene can be silenced at any stage or in any tissue where a suitable GAL4 driver is available. The disadvantage, however, is that any construct created will be specific to the gene being studied, and it is therefore not applicable for a general genome scan. Even though the gene targeting method described above will be used more extensively in the future, the ability to control when a gene is inactivated is still a powerful tool and therefore inducible RNAi is likely to be of widespread utility.

**Avoiding the insertion bias of *P-elements*, other transposons will generate complete genome coverage of inserts**

## ALTERNATIVE TRANSPOSABLE ELEMENTS

As we have shown, although *P-elements* are immensely powerful and flexible tools for genetic analysis, they do suffer from certain limitations. Chief among these is the insertion site specificity, which makes it difficult to generate insertions in some regions of the genome. There is, therefore, a need for alternative transposable elements with either a different pattern of site specificity or with no specificity. Two such transposons that have been adapted for use in *Drosophila* are the *piggyBac* and *Minos* elements.

The *piggyBac* is a lepidopteran-derived element of the TTAA-specific Class II short inverted repeat family[76] identified in the cabbage looper, *Trichoplusia ni*. It is 2,476 bp in length, contains a single ORF encoding a 68 kDa transposase and is bounded by 13 bp terminal inverted repeats.[77,78] The element inserts specifically at TTAA sites, which are duplicated upon insertion[77] and are crucial to proper excision.[79] *piggyBac* excises cleanly from the original site of insertion and to date no examples of imprecise excision have been reported.[80] While this may limit their use in generating deletions, anecdotal evidence indicates that insertion in the genome is essentially random with respect to sequence, and genes are targeted as frequently as flanking DNA. In addition to their utility in *Drosophila*, *piggyBac* vectors have been introduced into, and successfully transposed in, a number of different species, including the medfly, *Ceratitis*

*capitata*, the mosquito, *Aedes aegypti*, and the flour beetle, *Tribolium castaneum*,[81–83] suggesting that it may be used as a 'universal' insect transformation vector. The use of *piggyBac* elements is on the increase within the *Drosophila* community and it is likely that collections of insertions will be available in the near future.

A second transposon, the *Minos* element, has also been introduced and mobilised in *D. melanogaster*. *Minos* is a Tc-1/mariner-like element isolated from *Drosophila hydei*. The element is 1.8 kb in length, contains two ORFs, is bounded by 255-basepair perfect inverted repeats and inserts at TA dinucleotides.[84,85] Remarkably, *Minos* has been shown to mobilise in a diverse range of organisms, including *A. aegypti*, the butterfly *Spodoptera frugiperda*,[86] and even the mouse,[87,88] suggesting that it may become a multi-phyla vector for transgenesis. It is, however, necessary introduce a note of caution; there are potential dangers associated with cross-species transfer of elements. After all, the *P-element* was introduced into *D. melanogaster* less than 100 years ago and is now present in nearly all wild populations. Introduction of new elements may involve a similar fate for the species, with hitherto unseen consequences.

In closing, transposable elements provide extremely flexible tools for manipulating the genome of *Drosophila* and, over the past few years, a range of extremely sophisticated systems has been developed that allows great versatility when manipulating individual genes or large regions of the genome. Given that engineered transposons have only been around for a relatively short period of time (20 years), the authors imagine that their utility will increase in the coming years. The authors apologise to those workers whom we have not cited due to space constraints. We direct readers interested in *P-element* biology to the excellent review by Engels[89] and note that details on transposon constructs and vectors are accessible via the FlyBase web site.[25]

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