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Reconfiguring gene traps for new tasks using iTRAC

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We recently developed integrase-mediated trap conversion (iTRAC) as a means of exploiting gene traps to create new genetic tools, such as new markers for imaging, drivers for gene expression and landing sites for gene and chromosome engineering. The principle of iTRAC is simple: primary gene traps are generated with transposon vectors carrying ϕ*C31* integrase docking sites, which are subsequently utilized to integrate different constructs into trapped loci. Thus, iTRAC allows us to reconfigure selected traps for new purposes. Two features make iTRAC an attractive approach for Drosophila research. First, its versatility permits the exploitation of gene traps in an open-ended way, for applications that were not envisaged during the primary trapping screen. Second, iTRAC is readily transferable to new species and provides a means for developing complex genetic tools in Drosophilids that lack the facility of *Drosophila melanogaster* genetics.

Keywords: iTRAC, gene trapping, site-specific integration, gene-trap conversion, ϕ*C31* integrase, new model organisms, Drosophilids

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Introduction

In the era of forward genetics, gene trapping (enhancer-trapping and exon-trapping) occupied a central spot in the effort to identify new genes of interest and to generate cell-, tissue- and stage-specific markers for developmental biology.[1-6] The same approach was also used to produce diverse drivers for gene expression, based on GAL4/UAS and other expression systems,[7,8] which are used to express a variety of transgenes in a given pattern. One of the intrinsic limitations of gene trapping is that a given trap, once generated, cannot be easily exploited for different applications. For example, a GAL4 trap could in principle be converted into an alternative (e.g., LexA) expression driver,[9] but the process is laborious and rarely implemented in practice. Moreover, this type of trap conversion would not be feasible in species that are not as genetically tractable as *Drosophila melanogaster*.

With the rise of reverse genetics, gene trapping screens are gradually giving way to more targeted approaches for tagging genes and for generating new drivers for gene expression in Drosophila.[10-13] These approaches are based on gene targeting using homologous recombination[14,15] or artificial nucleases designed to target specific sites in the genome.[16,17] Site-specific integration using the bacteriophage ϕ*C31* integrase has also been developed as a means of integrating constructs into specific loci carrying the *attP* integrase recognition site.[18] The ϕ*C31* integrase system is now widely used for efficient integration of diverse constructs into well-characterized landing sites in the genome (reviewed in ref. 19–22).

We recently developed an approach named iTRAC (integrase mediated trap conversion), which combines the explorative powers of unbiased (random) gene trapping screens with the potential to introduce new constructs into selected trapped loci[23] (**Fig. 1**). iTRAC was conceived as a method to overcome the intrinsic limitation in the use of gene traps mentioned above: the fact that traps are generated by random insertion into the genome and, once generated, cannot be re-created with new trapping constructs. This means that, although new technologies and experimental needs emerge, the use of existing traps is locked and

cannot be easily adapted to these needs. We designed iTRAC as a means of reconfiguring traps for new purposes, in an open-ended fashion. Similar approaches have been developed by others, aiming to achieve flexibility in generating new drivers, gene fusions and mutagenic insertions.[24-26]

iTRAC is easy to implement in organisms with limited genetic resources (e.g., lacking genetic markers, balancer chromosomes or a sequenced genome)—indeed, we originally applied iTRAC in the crustacean *Parhyale hawaiensis*.[23] This feature makes it attractive for routine use not only in *Drosophila melanogaster*, but also in Drosophilids that lack established genetic tools.

	random insertion	targeted insertion
specific use	Conventional enhancer- and exon-trapping (refs. 1-4)	Gene-targeting, zinc-finger nucleases (refs. 14-17)
open-ended use	iTRAC, InSITE, MiMIC, G-MARET (refs. 23-26)	SIRT, IMAGO, genomic engineering (refs. 11-13)

Figure 1. Transgenic approaches for generating markers, expression drivers, gene tags and mutants in Drosophila. Approaches are grouped according to two criteria: (A) whether they rely on random or targeted insertions and (B) whether they employ transgenes designed for a specific purpose, or ones that are efficiently reconfigured for multiple tasks. In the latter case, the ϕ*C31* integrase system allows transgene insertions to be exploited for new purposes in an open-ended way. iTRAC involves screening random insertions for gene traps and allows these to be adapted for diverse applications.

How iTRAC Works

The iTRAC approach consists of two phases, which are depicted in **Figure 2**. First, a gene-trapping screen is performed, generating random insertions of a primary trapping construct into the genome and selecting traps based on the expression of a fluorescent reporter. The *Minos* transposon that we selected as our trapping vector integrates randomly in the genome, with practically no hotspots or coldspots, avoiding the insertional biases seen with P and piggyBac vectors.[27,28] The trapping vector carries a transformation marker (not shown in **Fig. 2**), a trapping construct (in our case a Parhyale splice acceptor site followed by the coding sequence of DsRed), and the ϕ*C31* docking site.[23] In our initial configuration the docking site consists of a single *attP* site located before the splice acceptor of the trapping construct, but other arrangements, such as double *attP* sites flanking the trapping construct, would also be effective (see below).

Once the gene-trapping screen is complete and traps have been recovered based on expression of the fluorescent reporter, those primary traps can serve as a platform for developing new tools. New genetic constructs implementing new tasks can be integrated into the trapped locus at any point, by injecting the transgenic lines carrying the *attP*-bearing primary trap with new constructs carrying the cognate *attB* site, together with the ϕ*C31* integrase[23,25,26] (**Fig. 2**). Independent experiments can be used to bring diverse effector constructs into the locus, such as reporters for new imaging applications, expression drivers, mutagenic constructs or chromosome tags, as discussed in the next section.

Different experimental configurations can be used to bring effector constructs into the trapped locus, either adding to or replacing the primary trap. In its simplest form, iTRAC employs a single *attP* docking site to integrate an entire plasmid carrying an effector and a single *attB*.[23] If primary traps have been created with double *attP* sites flanking the trapping construct, the primary trapping construct can be cleanly replaced by a new effector construct using recombinase-mediated cassette exchange.[20,26] Schemes also exist for removing parts of the primary trapping construct by making use of other recombinase systems, such as Cre/lox,[24,25] or by bringing in additional inverted repeats of the *Minos* element and mobilizing parts of the resulting compound transposon.

Both phases of iTRAC can be implemented by straightforward screens for gain or loss of visible fluorescent markers. ϕ*C31*-mediated integration is already routinely used, with efficiencies that match those of conventional transposon-mediated transformation.[21,23]

Endless Applications of iTRAC

The most appealing feature of iTRAC is its versatility: by allowing new effector constructs to be designed and implemented after the primary traps have been generated, it turns these traps into a hugely adaptable resource. The range of possible effector constructs is endless. We list here some of the applications envisaged at present:

(a) One of the most direct applications is the expression of new reporters or effector molecules for different types of imaging or optogenetics approaches. Coupling these reporters/effectors to specific gene traps without needing to involve GAL4 expression drivers, will free the use of GAL4/UAS for additional experimental manipulations. This approach may prove to be particularly fruitful in developing optogenetics tools, where the effectors are conditionally switched on by light. Optogenetics—the manipulation of cellular processes by light[29]—appear set to revolutionize neurobiology and cell biology in the coming years.

(b) Another immediate application of iTRAC will be to generate drivers for gene expression, based on different types of binary expression systems driven by GAL4, LexA, tTA or QF.[7,24,30-33] The InSITE vector system, which employs the same approach as iTRAC, was designed specifically for this purpose.[25]

(c) iTRAC provides continued access to a trapped locus, which may be exploited for genetic manipulation of the trapped gene. With exon trapping, iTRAC could be used to generate knock-outs (by inserting a strong transcriptional terminator[26]), or to create dominant negative alleles and gene fusions.

(d) Transposon insertions are often used as landmarks for chromosome

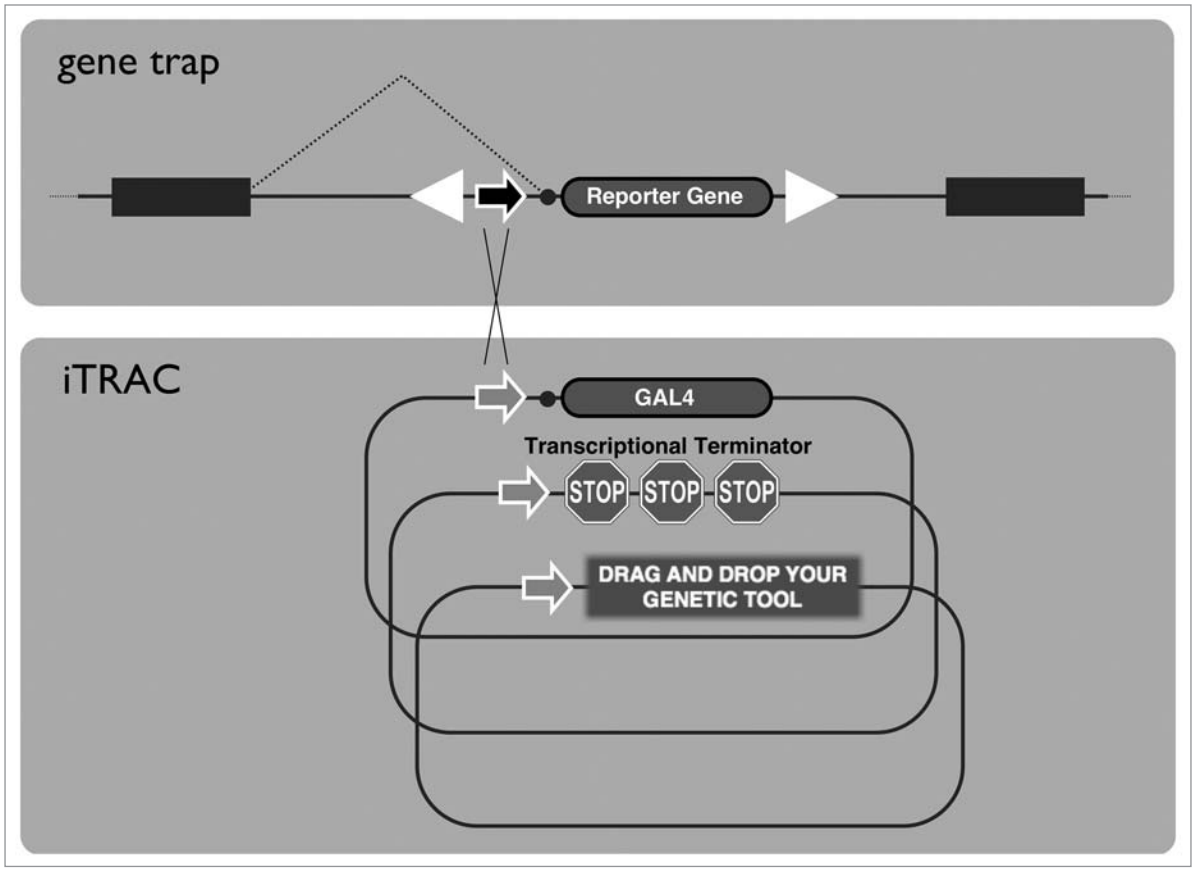

Figure 2. Schematic overview of iTRAC. Gene traps are generated with a *Minos* transposon vector (inverted repeats shown with white arrowheads) carrying a trapping cassette with a visible reporter gene. Subsequently, selected gene traps can be exploited to generate new genetic tools (different reporters, GAL4 drivers, knockdowns, gene fusions, etc.,) by integrating new effector constructs into the trapped locus. This is achieved using the ϕ*C31* integrase and cognate *attP* and *attB* sites present in the original trapping construct and in the effector constructs, respectively (black and gray arrows). Different configurations and possible uses of iTRAC are described in the text.

tagging or engineering. iTRAC allows us to use traps as flexible multipurpose tags. For example, fluorescent tagging of chromosomal regions can be used to monitor their position within the nucleus, in live cells;[34] equally, introduction of recombinase sites (FRT or loxP) can convert these into breakpoints for chromosome engineering.[35]

Most importantly, as experimental needs and technologies evolve, iTRAC will allow existing gene traps to be exploited for new tasks that are not yet envisaged.

iTRAC in Different Species

Another appealing feature of iTRAC is ease-of-use and transferability to new species—in fact, our initial aim in designing iTRAC was to facilitate the development of genetic tools in non-model organisms.[23]

iTRAC is easily transferable for two reasons. First, the genetic elements and tools that were used to build iTRAC are known to work in diverse species. These include the *Minos* transposon vector, which is widely used in arthropods, but also in Ciona and in mammalian cells,[28,36-40] the *3xP3-DsRed* (or *3xP3-EGFP*) transformation marker used in diverse insects and a crustacean,[39,41] and the ϕ*C31* system, which has been applied in arthropods, vertebrates and plants.[18,42-45] Enhancer-trapping core promoter elements are not, in our experience, transferable across divergent species, but exon-trapping splice acceptors are likely to be so. The crustacean splice-acceptor that we used in Parhyale is also effective in Drosophila, and likely to be functional in wide range of species.[23]

Second, iTRAC is portable to new species because it is easy to use. A primary trapping screen using fluorescent proteins as transformation and trapping markers, and subsequent trap conversion involving loss of the trapping marker and/or integration of a new one, provide straightforward ways for selecting and converting traps even in species that lack the elaborate toolkit and genetic resources of *Drosophila melanogaster*. iTRAC thus offers a simple and versatile approach for generating new tools in any species where we possess basic transformation and breeding techniques, among flies and beyond.

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