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FLP/FRT and Cre/*lox* recombination technology in *C. elegans*

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ABSTRACT

One of the most powerful aspects of biological inquiry using model organisms is the ability to control gene expression. A holy grail is both temporal and spatial control of the expression of specific gene products – that is, the ability to express or withhold the activity of genes or their products in specific cells at specific times. Ideally such a method would also regulate the precise levels of gene activity, and alterations would be reversible. The related goal of controlled or purposefully randomized expression of visible markers is also tremendously powerful. While not all of these feats have been accomplished in *Caenorhabditis elegans* to date, much progress has been made, and recent technologies put these goals within closer reach. Here, I present published examples of successful two-component site-specific recombination in *C. elegans*. These technologies are based on the principle of controlled intra-molecular excision or inversion of DNA sequences between defined sites, as driven by FLP or Cre recombinases. I discuss several prospects for future applications of this technology.

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1. Introduction

Sauer and Henderson [1,2] adapted the Cre/*lox* system from P1 bacteriophage for use in eukaryotes while Golic and Lindquist [3] harnessed the FLP recombinase from the yeast *Saccharomyces cerevisiae* for use in *Drosophila*. These site-specific recombinases either excise or invert DNA between short target sequences (34 bp), depending on the orientation of the target sequences. Direct repeats lead to excision of the DNA between the targets, leaving one copy of the target sequence as a "scar" (Fig. 1), while inverted target sequences lead to a reversal of the orientation of intervening DNA. One common use of the former arrangement is the removal of DNA bearing a transcriptional stop to direct the expression of a downstream gene, hence the term a "*flp*-out" cassette [4] or "flox"-ed allele (flox being short for "flanked by *lox*") [5]. The research community has taken advantage of the properties of these recombination systems, developing an ever-expanding toolbox that now includes drug-inducible ON–OFF technologies [6], facile manipulation of RNAi (see [7]), and the so-called "brainbow" or "confetti" methods of lineage tracing [8,9] that have been extended into zebrafish [10,11] and *Drosophila* [12,13].

The design of recombination-based gene expression systems must take into account the method by which transgenes are introduced. Historically, the most common and facile method for transgene introduction in *C. elegans* is microinjection of DNA into the germ line which results in the formation of multicopy arrays of introduced DNA. In this case, useful DNA excision or inversion events must confer dominant activity since rearrangement will not necessarily occur on every copy of the transgene. The unpredictable arrangement of genes in arrays can also lead to unexpected recombination products. Relatively new single-copy transgenic technologies such as transposon-mediated transgene targeting (MosSCI) and genome editing via homologous recombination triggered by double-stranded breaks introduced by Mos excision, TALEN, or CRISPR-Cas9 technologies offer additional opportunities for further development of controlled recombinase-mediated gene regulation [36,37].

Here, in to help expedite the use of FLP and Cre recombination in *C. elegans*, I present published examples, together with relevant strategies for plasmid design, *in vivo* methods, cautionary notes, and several future extensions of the technology.

2. Published examples of FLP and Cre mediated recombination in *C. elegans* (summarized in Table 1)

2.1. Demonstration of Cre mediated recombination

Hoier et al. (2000) [14] first used Cre-*loxP* to examine the consequences of loss of *apr-1* post-embryonically in Pn.p cells while avoiding the deleterious effects of its loss during embryogenesis. The authors' strategy (Fig. 2A) was to inactivate a rescuing *apr-1(+)* transgene only in Pn.p cells by excising all but the first intron via

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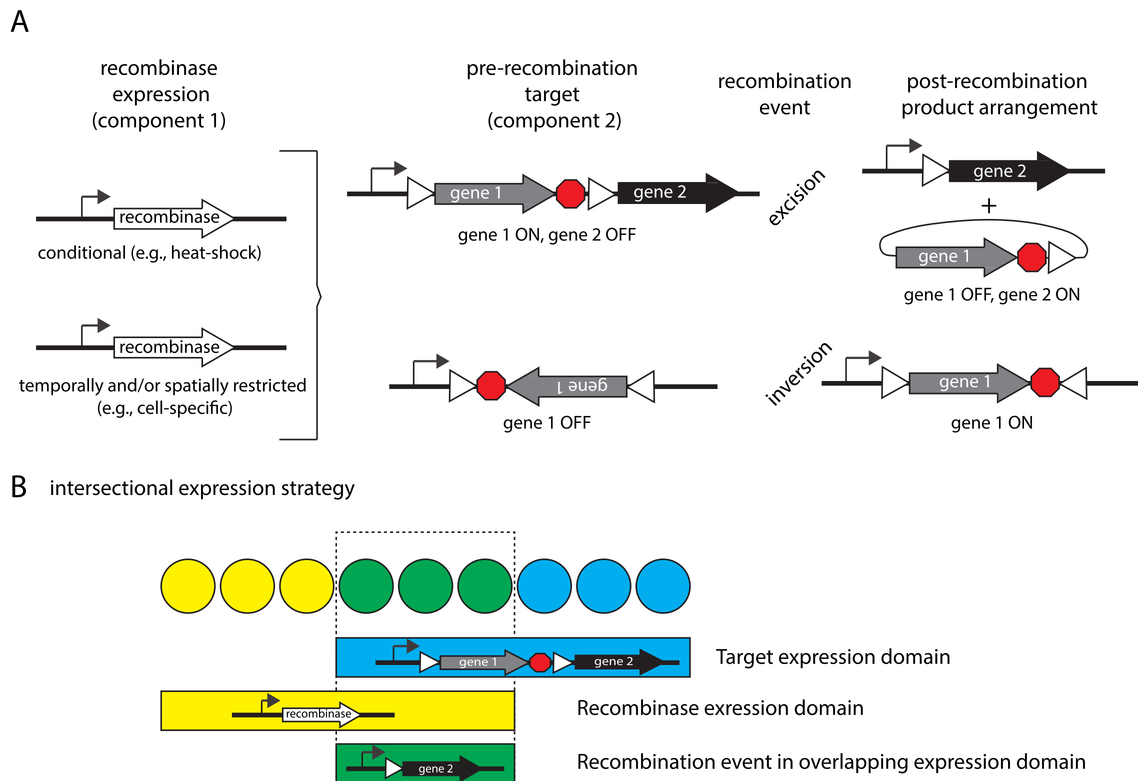

Fig. 1. General strategies for excision, inversion and intersectional applications of Cre and FLP recombinase technologies.

Pn.p-cell-specific expression of Cre. Tissue-specific RNAi was used as a complementary approach and while both methods caused similar phenotypes, in this case the phenotype caused by tissue-specific RNAi was more penetrant. More recently, Cre recombinase-based approaches have been more successful (see below).

2.2. Demonstration of FLP mediated recombination

Two publications in 2008 reported FLP/FRT-based excision systems in *C. elegans*: Davis et al. (2008) [15] and Voutev and Hubbard (2008) [16]. Both demonstrate spatial and temporal control of somatic gene expression, using either heat-shock inducible or spatially-restricted recombinase expression combined with ubiquitous or spatially-restricted target sequences. In each case, transgenes were generated by injection (even if subsequently integrated) and therefore carry the recombinase or target sequences in multiple copies. Both papers provide modular vectors and suggest strategies for designing target constructs by traditional or Gateway cloning (Fig. 2B and C). The plasmids developed for FLP/FRT expression can also be used as templates for Gibson assembly cloning strategies.

2.2.1. Flp-mediated excision to activate gene expression

Davis et al. [15] describes a "FLP-on" cassette design based on the ORFeome and Promoterome resources (Fig. 2B) and its use in controlled reporter or toxin activation. Using heat-shock-driven recombinase and a muscle-specific target transgene bearing an FRT-flanked mCherry-STOP cassette (P*myo-2* > mCherry-STOP > GFP::HIS-11 + unc-54 3′, where ">" or "<", hereafter denotes a target sequence), they demonstrate successful excision of the stop cassette as indicated by expression of the downstream nuclear GFP only after heat-shock. The FLP recombinase was expressed under the control of the heat-shock promoter P*hsp-16.48*. After a 1 h heat shock at 34 °C, nuclear GFP was visible within 3 h and was strong at 15 h (see Table 2 for summary of heat shock conditions). A single excision event within the multicopy array would presumably drive the P*myo-2*-directed expression of GFP::HIS-11 in muscles. Indeed, the authors observed nearly 100% efficiency of FLP-induced recombination (both in terms of % worms and % of muscle cells expressing GFP). However, mCherry expression persisted, indicating that non-recombined copies of the transgene remained in the array.

Next, they inhibited GABA neurotransmission post-developmentally by excision-mediated expression of tetanus toxin after heat-shock induction of FLP recombinase. A high efficiency of excision was indicated by the appearance of characteristic behavioral phenotypes: treated animals displayed the "shrinker Unc" phenotype and were defective for enteric muscle contraction due to loss of GABA transmission in motor neurons.

2.2.2. FLP recombination: single cells, widespread tissue types, cell lineage tracing, dominant-negative and RNAi hairpin strategies

Voutev and Hubbard [16] designed a similar two-part system for excision of transgenic DNA sequences to drive expression of a downstream gene using FLP/FRT. The modular plasmid system is based on expression vectors made available by the Fire lab ("Fire vectors") as well as the Wormgate system and ORFeome clones that allow expression of RNAi-inducing hairpins (Fig. 2C). This paper provides detailed suggestions for plasmid construction and excision monitoring, as well as a supplementary Toolkit with modular components for plasmid construction.

To demonstrate temporal, spatial and combined temporal-spatial control, Voutev and Hubbard employed several strategies. Temporal control was obtained via heat-shock promoter (P*hsp-16.41*::FLP) that excised a GFP stop cassette from an array bearing a ubiquitous promoter (P*pro-1*) to drive *lacZ* expression. Consistent with observations made by Davis et al. [15], expression of *lacZ* following heat-shock induction of FLP recombinase (2 h at

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