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Improved Gal4 screening kit for large-scale generation of enhancer-trap strains.

Yoshihara, Motojiro,¹ and Kei Ito². ¹Center for Learning and Memory, Massachusetts Institute of Technology, Cambridge, MA 02139 USA; ²National Institute for Basic Biology, Myodaiji, Okazaki, 444-8585 Aichi, Japan.

The Gal4 enhancer-trap system, devised by Brand and Perrimon (1993), is a powerful tool for generating P-element insertion mutants, expression drivers and cell-specific marker strains. However, the original set of lines suffered from a low mobilization frequency of the Gal4-carrying transposon. The Gal4 system requires mating Gal4-containing strains with a UAS-linked reporter strain for detecting the enhancer activity. This makes large-scale generation and screening of Gal4 enhancer-trap lines much more laborious than the previous *pLacZ*-based enhancer-trap system.

To perform more efficient large scale screening, we have made the following improvements to the original system:

(1) We moved the location of the Gal4 source insertion from the original X chromosome onto the second (*CyO*) chromosome, so that we can also screen mutants on the X-chromosome. We also performed a screening of the Gal4 source strains to select the one with the highest mobilization frequency.

(2) We made *CyO* and *TM6* balancer chromosomes with a UAS-*lacZ* reporter construct for convenient detection of Gal4 expression by X-gal staining (no need for additional crossing with a reporter line). We chose UAS-*lacZ* rather than UAS-GFP as a reporter, since UAS-GFP sometimes fails to detect Gal4 activity in early embryos. To make Gal4 insertion (with the *white*⁺ marker) easily discernible, *white*⁺ was excised out of the UAS-*lacZ* insertion.

(3) We isogenized all genetic backgrounds.

Making an efficient Gal4 strain for the jumping start point:

We mobilized the Gal4 vector, pGawB (Brand and Perrimon, 1993), from the X-chromosome by crossing to the $\Delta 2-3$ strain (Robertson *et al.*, 1988), obtaining 31 second *CyO* chromosomes with pGawB insertions. We tested the jumping frequency of each strain by crossing to the $\Delta 2-3$ strain (Table 1). We examined the rate of excision from the *CyO* chromosome by counting F2 with curly wings and white eyes (*Cy*⁻, *w*⁻), and examined the rate of transposition to other chromosomes by counting F2 with straight wings and red eyes (*Cy*⁺, *w*⁺). The five lines with the highest efficiency are listed in Table 1. Out of 31 strains examined, strain 238-048 was the most efficient. The pGawB vector in 238-048 is efficiently excised (generating many *Cy*⁻ and *w*⁻ F2s) and efficiently transposed (generating many *Cy*⁺ and *w*⁺ F2s). Using this strain it is feasible to isolate newly transposed insertions from every vial of the initial crossing.

Making UAS-lacZ balancer chromosomes lacking the white⁺ marker:

We mobilized the UAS-*lacZ* reporter element, pUAST-*lacZ* (Brand and Perrimon, 1993) to *CyO* and *TM6* balancer chromosomes by crossing to the $\Delta 2-3$ strain. Isolated were six *CyO* and nine

Figure 1 (next page). Mating scheme used for the large-scale screening.

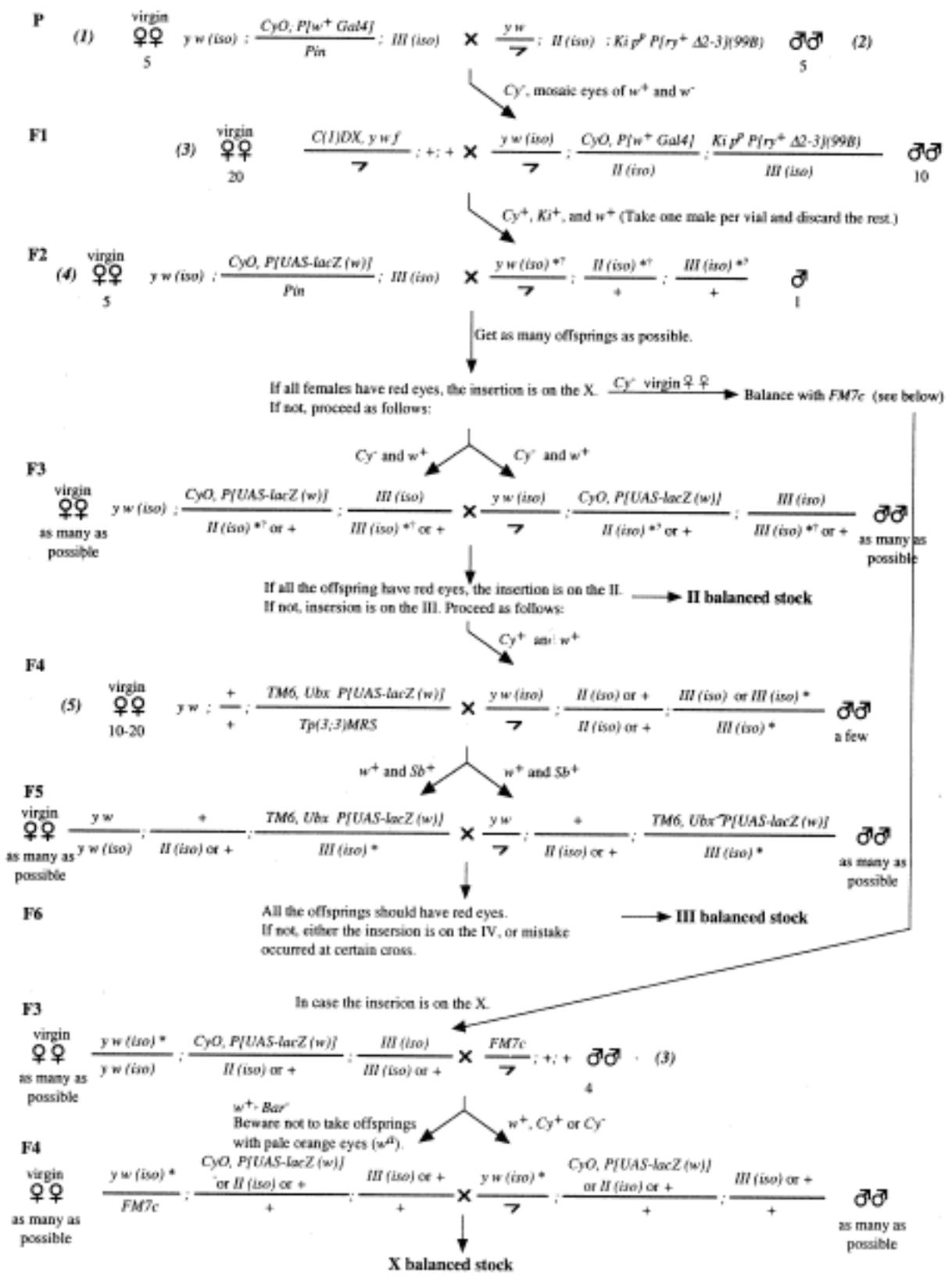


Table 1. Frequency of excision and transposition of the candidate strains.

Candidate strain	Average number of Cy ⁺ and w ⁺ F2 (transposed)	Average number of Cy ⁺ and w ⁺ F2 (excised)	Average number of Cy ⁺ and w ⁺ F2 (most are non-excised)
217-038	7.4	84.6	257.8
238-022	12.2	72.6	202.2
238-048	26.6	209.8	152.6
520-009	7.0	71.2	187.2
520-021	6.4	46.0	247.0

The respective Gal4 source line candidates, carrying pGawB on CyO were crossed with the P-transposase strain $\Delta 2-3$. Ten F1 males were mated to 20 C(1)DX virgin females in a single vial. Average number of F2 offspring from five such vials are show above.

tested by crossing them to the original Gal4 strain of Brand and Perrimon, which shows characteristic expression pattern (spots in each segment). The line “37” for the second balancer and “23” for the third showed the clearest expression pattern driven by Gal4.

To remove the *white*⁺ marker from the two selected balancer strains, we then mated them with the $\Delta 2-3$ strain for imprecise excision. From the second balancer line “37” we isolated 20 *CyO* chromosomes that lacked the *white*⁺ marker. The 20 lines, each carrying imprecisely excised pUAS-*lacZ*, were crossed with Gal4 lines and subjected to X-gal staining. We found two strains that still showed normal UAS-*lacZ* activity. One of the two lines, termed “*CyUW14*”, was used for our screening as a standard second chromosome balancer. From the third balancer line “23”, we isolated 30 *TM6* chromosomes lacking the *white*⁺ marker. Among them two showed normal UAS-*lacZ* activity. We chose one of them, named as “*TM6UW23-1*”, for our screening as a standard third balancer.

Isogenization of the chromosomes to be mutated:

To avoid mistakenly picking up background mutations, we isogenized all the chromosomes to be mutated. Six standard strains were established as isogenized strains using *FM7c*, *CyO*, and *TM6*. Each strain has the X chromosomes derived from a single chromosome with *yellow* and *white*, and second and third chromosomes derived from a single Canton-S second and third chromosome, respectively. The six strains were tested with regard to viability, mating, and flight behavior. The healthiest strain, named “iso5”, was used for the isogenization of the necessary chromosomes.

Via this approach we established the following five strains that can be used as a “screening kit”. The kit is available from the Bloomington Stock Center and the National Institute of Genetics, Japan. A typical mating scheme using this kit is shown in Figure 1. Numbers in parenthesis indicate strains shown below.

- (1) 238-048
y w (iso); CyO, pGawB/Pin; III (iso)
- (2) +; *II (iso); Ki p^p P[ry+ $\Delta 2-3$](99B)*
- (3) C(1)DX/FM7c
- (4) *CyUW14*
y w (iso); CyUW14/Pin; III (iso)
- (5) *TM6UW23-1*
y w (non-iso); TM6UW23-1/Tp(3;3)MRS

TM6 chromosomes carrying UAS-*lacZ*. We then tested the response of UAS-linked *lacZ* to Gal4 activity by X-gal staining. Out of the 15 lines, three showed characteristic *lacZ* expression patterns even without any Gal4 activity. This is likely because the UAS-*lacZ* element trapped certain nearby enhancers. The Gal4 responses of the rest of the lines were

(Isogenized X, second, and third chromosomes are noted as *y w (iso)*, *II (iso)*, and *III (iso)*, respectively.)

This kit will be useful for various kinds of Gal4 screening. It is especially effective for lethal or sterile mutant screening, since the balancers with UAS-*lacZ* always remain in the stocks, making the mutant lines readily stainable for *lacZ*. If homozygotes are healthy the balancers might be segregated out from stocks relatively quickly (sometimes within less than ten generations).

Using this kit, an attempt to generate a large set of Gal4 enhancer-trap strains was performed between 1997 and 1999. Eight fly research groups in Japan participated to form the "NP consortium" and each generated about 500 lines, resulting in a collection of more than 4000 lines. (In some cases conventional balancers were used instead of the ones described here.) The groups that participated were: T. Aigaki (Tokyo Metropolitan University), S. Goto and S. Hayashi (National Institute of Genetics), K. Ito (ERATO, JST; currently National Institute for Basic Biology), H. Nakagoshi (PRESTO, JST; currently Okayama University), and F. Matsuzaki (National Institute of Neuroscience; currently Tohoku University), T. Tanimura (Kyushu University), R. Ueda (Mitsubishi-Kasei Institute of Life Sciences), T. Uemura (Kyoto University), and M. Yoshihara (Gunma University; currently Massachusetts Institute of Technology). The insertion site of each line is being examined by S. Goto and S. Hayashi. The collection, after removal of the duplicated stocks, is to become publicly available once a distribution facility is established.

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