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

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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 Gal-4 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 *Δ2-3* 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 *Δ2-3* 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 *Δ2-3* strain. Isolated were six *CyO* and nine *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 response of the rest of the lines were 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 *white*$^+$ marker from the two selected balancer strains, we then mated them with the *Δ2-3* 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 pUAST-*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 *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 Inst. 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$^+$ Δ2-3](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

(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 Univ.), S. Goto and S. Hayashi (National Inst. of Genetics), K. Ito (ERATO, JST; currently National Inst. for Basic Biology), H. Nakagoshi (PRESTO, JST; currently Okayama Univ.) and F. Matsuzaki (National Inst. of Neuroscience; currently Tohoku Univ.), T. Tanimura (Kyushu Univ.), R. Ueda (Mitsubishi-Kasei Inst. of Life Sciences), T. Uemura (Kyoto Univ.), and M. Yoshihara (Gunma Univ.; currently Massachusetts Inst. 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.

Acknowledgments

This work has been done in MY's former affiliation, Institute for Behavioral Science in Gunma University, and KI's former affiliation, ERATO Yamamoto Behavior Genes Project, JST. We thank Drs. Yoshiaki Kidokoro at Gunma Univ. and Daisuke Yamamoto at ERATO for their generosity. We are obliged to Ms. Masako Terada and Fumiko Sekiguchi at Gunma Univ. and Wakae Awano at ERATO for their excellent technical assistance, and Dr. Ryu Ueda at Mitsubishi-Kasei Inst. and members of the NP consortium for useful advice. We also thank Dr. J. Troy Littleton at Massachusetts Inst. of Technology for critically reading the manuscript. This work was supported by a grant-in-aid from Ministry of Education, Science, Sports and Culture of Japan to MY and the operational budget of the ERATO Yamamoto Behavior Genes Project, JST.

References

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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 *Δ2-3.* 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 shown above.

FIGURE LEGEND

Figure 1: Mating scheme used for the large-scale screening.