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 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
P
(1) virgin ♀♀
y w (iso) : Cy O, P[w+ Gal4] /
Pin : III (iso) × y w
 fullName
H (iso) : K p P [ry+ Δ2-3] (99B)
5

F1
(3) virgin ♀♀
C(t)OX, y w f ; + × y w (iso) : Cy O, P[w+ Gal4] /
H (iso) : K p P [ry+ Δ2-3] (99B)
20
 fullName
Cy +, K p +, and w + (Take one male per vial and discard the rest.)

F2
(4) virgin ♀♀
y w (iso) : Cy O, P[UAS-lacZ (w)] /
Pin : III (iso) × y w (iso) ×7
 fullName
H (iso) or + ; III (iso) ×7
4
 fullName
Get as many offsprings as possible.

If all females have red eyes, the insertion is on the X. Cy + virgin ♀ Balance with FM7c (see below)
If not, proceed as follows:

F3
virgin ♀♀
y w (iso) : Cy O, P[UAS-lacZ (w)] /
H (iso) ×7 or + ; III (iso) ×7 or +
 fullName
Cy + and w +

If all the offspring have red eyes, the insertion is on the II. Proceed as follows:

F4
(5) virgin ♀♀
y w ; + : TM6, Ubx P[UAS-lacZ (w)] /
T [3,3]MRS ; III (iso) ×7
 fullName
Cy + and Sb +

F5
virgin ♀♀
y w (iso) ; H (iso) or + ; III (iso) ×7
 fullName
As many as possible

F6
As many as possible

All the offsprings should have red eyes.
If not, either the insertion is on the IV, or mistake occurred at certain cross.

F3
virgin ♀♀
y w (iso) ×7 ; Cy O, P[UAS-lacZ (w)] /
H (iso) or + ; III (iso) ×7
 fullName
FM7c ; w +, Bar +

F4
virgin ♀♀
y w (iso) ×7 or H (iso) or + ; III (iso) or +
 fullName
Cy O, P[UAS-lacZ (w)]

X balanced stock
**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 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 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 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); K1 p7 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-isoz); 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 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.

Acknowledgments: The 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 University and Daisuke Yamamoto at ERATO for their generosity. We are obliged to Ms. Masako Terada and Fumiko Sekiguchi at Gunma University and Wakae Awano at ERATO for their excellent technical assistance, and Dr. Ryu Ueda at Mitsubishi-Kasei Institute and members of the NP consortium for useful advice. We also thank Dr. J. Troy Littleton at Massachusetts Institute 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.