W::Neo: A Novel Dual-Selection Marker for High Efficiency Gene Targeting in *Drosophila*

Wenke Zhou, Juan Huang*, Annie M. Watson, Yang Hong*

Department of Cell Biology and Physiology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, United States of America

**Abstract**

We have recently developed a so-called genomic engineering approach that allows for directed, efficient and versatile modifications of *Drosophila* genome by combining the homologous recombination (HR)-based gene targeting with site-specific DNA integration. In genomic engineering and several similar approaches, a “founder” knock-out line must be generated first through HR-based gene targeting, which can still be a potentially time and resource intensive process. To significantly improve the efficiency and success rate of HR-based gene targeting in *Drosophila*, we have generated a new dual-selection marker termed W::Neo, which is a direct fusion between proteins of eye color marker White (W) and neomycin resistance (Neo). In HR-based gene targeting experiments, mutants carrying W::Neo as the selection marker can be enriched as much as fifty times by taking advantage of the antibiotic selection in *Drosophila* larvae. We have successfully carried out three independent gene targeting experiments using the W::Neo to generate genomic engineering founder knock-out lines in *Drosophila*.

**Introduction**

We have recently developed a new approach termed genomic engineering that combines the gene targeting with phage integrase φC31-mediated DNA integration for the purpose of directed, efficient and versatile modifications of endogenous genomic loci in *Drosophila* [1,2]. Genomic engineering is a two-step process. First, a “founder” knock-out is generated by homologous recombination (HR)-based gene targeting that deletes the target locus and effectively replaces it with a φC31-attP integration site. Second, the target locus can then be modified into virtually any desirable knock-in alleles through φC31-mediated integration of corresponding DNA constructs into the founder line [1,2]. We have also developed an additional integrase system for making sophisticated knock-in mutants by successive and targeted DNA integrations in genomic engineering [3]. Although generating novel knock-in alleles through site-specific DNA integration is extremely efficient compared to the HR-based knock-in/knock-out process, in genomic engineering and several similar approaches [1,2,4,5,6] a founder knock-out line must be first generated through HR-based targeting. In *Drosophila*, the frequency of HR for a given target locus can vary from $10^{-7}$ to $10^{-11}$, i.e. an approximately 10,000-fold difference [2,7]. For target loci that are of $<10^{-4}$ HR frequency, targeting experiments can be highly time and labor intensive. Therefore, more efficient and reliable gene targeting remains crucial for approaches like genomic engineering.

General HR-based gene targeting in *Drosophila* [8,9] requires several rounds of genetic crosses including targeting cross, screening cross, and mapping cross (Figure 1A) [7]. Transgenic flies of targeting construct were first generated to carry the donor DNA as a chromosomal insertion (“P[donor]”) flanked by FRT sites. To initiate the homologous recombination, the donor DNA in P[donor]/hs-FLP hs-I-SceI of targeting cross progeny is excited and linearized by heatshock-induced expression of Flipase (FLP) and the restriction enzyme I-SceI. In screening cross, heatshocked P[donor]/hs-FLP hs-I-SceI targeting females are crossed with w+ males so potential targeting mutant progeny may be recovered based on the dominant w+ marker (i.e. red eye). Mapping cross will be used to further genetically map and confirm the targeting mutants. In order to improve the scalability and throughput in these major genetic crosses, we have in the past developed several measures such as optimizing targeting vectors and hs-FLP hs-I-SceI stocks and introducing a UAS-Rpr negative selection marker (Figure 1A) [7]. These improvements have already yielded a high success rate for a number of targeting experiments [2,7]. Nonetheless, for targeting experiments of $<10^{-4}$ HR frequency, $>10^3$ progeny from screening cross have to be screened visually based on eye color marker w+. This time and labor intensive process directly limits the scale of targeting experiments.

To solve this problem, increasing the HR frequency by the means such as target-specific zinc finger nuclease (ZFN) likely presents one of the most promising approaches [10]. Nonetheless at present target-specific ZFNs or similar nucleases require extensive testing and refining efforts that to large degree may offset the benefits of increased HR frequency [11]. As an alternative, more efficient screening methods can also significantly increase the success rate of gene targeting. To this end, we...
developed an approach to directly enrich the targeting mutants by introducing a dominant selection marker \( \text{Neo} \) in addition to \( w^+ \).

**Results and Discussion**

We took advantage of the well established fact that *Drosophila* larvae are highly sensitive to G418 which is a drug related to neomycin and karamycin, but can be made resistant by the expression of neomycin resistance gene (Neo) \[12\]. By making candidate mutants neomycin-resistant (\( \text{Neo}^+ \)), G418 can be used to directly eliminate the vast number of screening cross progeny carrying no targeting events (Figure 1A). This approach provides several advantages. First, G418 can be easily added to the fly food, thus is fully compatible with the current genetic cross schemes of gene targeting. Second, G418-sensitivity in *Drosophila* larvae is dosage dependent \[12\]. By administrating G418 at pre-determined concentrations it is possible to eliminate a large percentage (e.g., 90%-99%) but not all of the larvae, minimizing the risk of killing \( \text{Neo}^+ \) targeting mutants while at the same time leaving enough number of survival larvae to ensure healthy growing conditions.

Although \( \text{Neo}^+ \) would be an effective marker for enriching targeting mutants, \( w^+ \) is still the most convenient marker for genetic mapping. To incorporate both \( w^+ \) and \( \text{Neo}^+ \) into the targeting mutants, we made a \( \text{white}:\text{Neo} \) (\( W::\text{Neo} \)) gene encoding a chimeric protein in which Neo is directly fused to the C-terminus of \( w^+ \). This design also minimized the size of the \( w^+ \)Neo\(^+\) dual selection marker for potentially more efficient molecular cloning, donor DNA excision and HR. To test the effectiveness of \( W::\text{Neo} \) for being both \( w^+ \) and \( \text{Neo}^+ \), we first generated a pKIKO-WN vector by replacing the \( w^+ \) in an older targeting vector pKIKO \[7\] with \( W::\text{Neo} \). Through standard P-element based transgenesis, we obtained several \( w^+ \)transgenic lines of pKIKO-WN showing that \( W::\text{Neo} \) functioned as a normal \( w^+ \) marker for producing red eye flies (see Figure 2A). We also picked one of the pKIKO-WN lines and confirmed that its \( w^+ \)progeny showed clear resistance to neomycin compared to their \( w^+\text{neo}^- \)siblings carrying no pKIKO-WN (Table S1).

---

**Figure 1. Application of multiple selections in gene targeting.** A. Genetic crosses of ends-out targeting based on the dual positive screening of \( w^+ \) and \( \text{Neo}^+ \) for targeting candidates, together with the negative selection of UAS-Rpr (Rpr\(^+\)) for eliminating false positives. "X": genotypes eliminated or greatly reduced in numbers by G418 selection or UAS-Rpr counter-selection. B. Map of pGX-attP-WN. pGX-attP-WN is a P-element based transforming vector. 5'P and 3'P: 5' and 3' P-element sequences; Amp\(^R\): ampicillin-resistant gene.
To test the effectiveness of W::Neo marker in targeting experiments, we carried out three ends-out (replacement) gene targeting experiments using W::Neo as a dual selection marker (Table 1). We first modified the pRK2-based gene targeting constructs of dArf6 [7] by replacing the w+ with W::Neo. We obtained 16 w+ transgenic donor lines by injecting 1150 embryos. As shown in Table S2, all but three lines showed clear resistance to G418 at 0.20 mg/ml concentration. The three lines that showed reduced resistance to G418 also showed failures in FRT or Cre-mediated excisions and dramatically reduced effectiveness of UAS-Rpr negative selection marker, suggesting these components were likely damaged during transgenic insertion. We picked line #22 (pArf6GX22) to carry out the targeting experiment. Based on the experiments in Figure 2B, we determined that 0.20 mg/ml should be the optimal G418 concentration that eliminates >90% of w+/w+ sibling larvae with apparently no effect on the survival of pArf6GX22/+ larvae.

Using our improved targeting stocks [7], we were able to collect 20,000 targeting females of pArf6GX22/hs-FLP hs-I-SceI from targeting crosses (See Figure 1A). To set up the screening cross, 12,000 targeting females were crossed with w; Gal44-77[w1118 y w; pArf6GX22/TM3, y w; pDscam-N[CG1]/TM3, and y w; pDscam-C[CG1]/TM3. The Gal4 drives the expression of UAS-Rpr marker that eliminates w+; TM3/+ and CyO/+ cross progeny. See Materials and Methods for detail genotypes.

As shown in Table S2, all but three lines showed clear resistance to G418 at 0.20 mg/ml concentration. The three lines that showed reduced resistance to G418 also showed failures in FRT or Cre-mediated excisions and dramatically reduced effectiveness of UAS-Rpr negative selection marker, suggesting these components were likely damaged during transgenic insertion. We picked line #22 (pArf6GX22) to carry out the targeting experiment. Based on the experiments in Figure 2B, we determined that 0.20 mg/ml should be the optimal G418 concentration that eliminates >90% of w+/w+ sibling larvae with apparently no effect on the survival of pArf6GX22/+ larvae.

Using our improved targeting stocks [7], we were able to collect 20,000 targeting females of pArf6GX22/hs-FLP hs-I-SceI from targeting crosses (See Figure 1A). To set up the screening cross, 12,000 targeting females were crossed with w; Gal44-77[w1118 y w; pArf6GX22/TM3, y w; pDscam-N[CG1]/TM3, and y w; pDscam-C[CG1]/TM3. The Gal4 drives the expression of UAS-Rpr marker that eliminates w+; TM3/+ and CyO/+ cross progeny. See Materials and Methods for detail genotypes.

Extrapolating from such data, using W::Neo marker with G418 selection we enriched the targeting mutant frequency from 5/ (7×10^6) to 23/(6.7×10^6), an enrichment of approximately 48 times. The mere 67,000 flies we screened were effectively equivalent to >3×10^6 screening cross progeny without G418 selection.

We then carried out two new targeting experiments against the Dscam locus using the pGX-att-P-WN targeting vector (Figure 1B). Dscam encodes a neuronal adhesion molecule of extraordinary diversity through alternative splicing (Figure 3A) [13]. Based on the genomic engineering approach, we targeted the deletions of exon#4 and #17 to generate two different founder knock-out lines designated as Dscam-N and Dscam-C, respectively. All ten Dscam-N and three Dscam-C transgenic donor lines were resistant to at least 0.25 mg/ml G418 (data not shown). For the donor lines used for carrying out the targeting experiments, pDscam-NGX113 showed G418-resistance similar to pArf6GX22 whereas pDscam-CGX1 appeared to be sensitive to ~0.50 mg/ml G418 (Figure 2B).

Table 1. Design of gene targeting for dArf6, Dscam-N and Dscam-C founder Knock-out lines.

| Target Gene | Target Chromo-some | Exons/mRNA Isoforms | 5’+3’ Arms* (kb) | Targeted gDNA Deletion** (kb) | Genomic Deletion Size (kb) | Protein Deletion/ Full Length (aa) |
|-------------|-------------------|---------------------|----------------|-----------------------------|---------------------------|----------------------------------|
| dArf6       | 2nd               | 3/5                 | 4.5±3.1        | 2R: 11,210,875–11,213,032   | 2.157                     | 175/175                          |
| Dscam-N     | 2nd               | 24/38016            | 5.5±3.2        | 2R: 3249024–3254750         | 5.727                     | 108/2037                         |
| Dscam-C     | 2nd               | 24/38016            | 5.3±3.2        | 2R: 3206840–3214884         | 7.645                     | 439/2037                         |

*: 5’+3’ Arms: the lengths of 5’ and 3’ homology arms in targeting construct.

**: According to Drosophila genome release FB2011.07 at www.flybase.org.

doi:10.1371/journal.pone.0031997.t001
For the Dscam-N targeting, we set up a screening cross using 16,000 targeting females in 800 vials with normal food (i.e. 20 females per vial). After three days we transferred flies to bottles of ~0.20 mg/ml G418 food, at the density of 160 females per bottle. The flies were transferred to new G418 bottles every three to four days. The normal food culture yielded ~1.6 × 10^5 progeny and we recovered 23 targeting candidates (Table 2). In contrast, G418 bottles yielded 3.3 × 10^5 progeny but we recovered 162 targeting mutants. The Dscam-C targeting was carried out similarly. We recovered 3 targeting mutants from 1.9 × 10^5 progeny yielded from normal food, and 3 from ~2.2 × 10^5 progeny yielded from G418 food (Table 2). Based on screening the progeny from normal food, the HR frequency for Dscam-N targeting can be estimated is ~1.4 × 10^-5, while for Dscam-C is ~1.6 × 10^-5. Overall, the enrichment of targeting mutants by G418 selection can be roughly estimated as 34 and 9 times in our Dscam-N and Dscam-C targeting experiments, respectively. These numbers are likely underestimated due to the fact we grew G418 bottles under extremely overcrowded conditions of 160 females per bottle due to constrained incubator space at the time of experiments. As expected, both Dscam-N and Dscam-C mutants are lethal and their lethality can be rescued by integrating back the deleted fragment of gDNA into their corresponding knock-out founder lines (data not shown).

It should be noted that the hsp70 promoter which drives the W::Neo expression in transfection is not transcriptionally insulated and although its expression in eye is boosted with an eye-specific GMR enhancer [7] (Figure 1B) its expression level can still be greatly increased (up to one hundred-fold) by heat-shock [12]. Although we did not carry out heat-shock treatments in screening crosses, it can be easily adapted into the protocol. Finally, for targeting loci that may severely repress hsp70 promoter, we are considering making modified targeting constructs that may feature stronger or insulated promoters.

In summary, we report here the successful applications of a novel w+Neo+ dual selection marker that may effectively enrich the targeting mutants up to fifty times with the help of G418-selection. Our new pGX-attP-WN targeting vector could significantly facilitate the large scale screening experiments, making target loci of <10^-6 HR frequency much more experimentally accessible. Besides gene targeting, the W::Neo marker should be useful in routine Drosophila genetic crosses when both w+ and Neo+ are desirable for selecting a particular genotype. 

Materials and Methods

Fly stocks and genetics

y w/Y; hs-hid; hs-FLP, hs-I-ScI/TM3 e Sb hs-hid ("6935-hid") BL.11412) [13] was used for complementation assays.

**Table 2.** Generation of founder knock-out lines by ends-out targeting.

| Target Gene | G418 (mg/ml) | Targeting Virgins Females | Screening Cross Progeny | Preliminary Candidates | On Target Chr. | Genetically Verified | PCR Verified | HR Frequency (b) |
|-------------|-------------|---------------------------|------------------------|-----------------------|----------------|-------------------|--------------|------------------|
| dArf6       | 0           | 6,000                     | ~7 × 10^3             | 315                   | 30/315         | 5/30d             | 5/5         | ~7 × 10^-6      |
|             | 0.20        | 6,000                     | ~6.7 × 10^4           | 221                   | 43/221         | 23/43d            | 6/6         | ~3.4 × 10^-4    |
| Dscam-N     | 0           | 16,000f                   | ~1.6 × 10^5           | 71                    | 50/71          | 23/50f            | 2/2         | ~1.4 × 10^-4    |
|             | 0.20        | (16,000)f                 | ~3.3 × 10^6           | 557                   | 399/557        | 162/399f          | 5/5         | ~4.9 × 10^-1    |
| Dscam-C     | 0           | 9,400f                    | ~1.9 × 10^5           | 23                    | 11/23          | 3/11f             | 3/3         | ~1.6 × 10^-3    |
|             | 0.20        | (9,400)f                  | ~2.2 × 10^6           | 42                    | 12/42          | 3/12f             | 3/3         | ~1.3 × 10^-4    |

*Total estimated number of screening cross progeny screened in each targeting experiment. Because progeny of multiple vials or bottles were pooled and screened together, we did not register the clonality of the preliminary candidates. We assumed that each targeting mutant obtained was due to a distinct targeting event, based on the low HR frequency observed.

*Since all female candidates were discarded in targeting experiments, the adjusted HR frequency should be twice higher than listed here.

*Screening crosses were set up on the normal food first, then transferred to G418 food after two days.

*A dArf6^{6527 treatment allele generated by P-excision was used for complementation assays [7].

*Null allele of P GP2 Dscam^{att6519} (BL.11412) [13] was used for complementation assays.

doi:10.1371/journal.pone.0031997.t002

New Gene Targeting Marker in Drosophila

...
CyO (BL#5626); w1118; In(2LR)Gla, wg Gla-1/CyO, P{GAL4-twix.G}2.2, P{UAS-2xEGFP}AH2.2 (BL#6662).

DNA Constructs

The W::Neo marker was made by fusing the Neo coding sequence to the C-terminus of W+ in pKIKO vector [7] through overlapping PCR. Cloned W::Neo fragments were sequenced to ensure error-free PCR. pGX-attP-WN was made by replacing the coding sequence of w+ in pGX-attP with W::Neo. Targeting construct of dArf6 was described previously [7]. Molecular cloning of targeting constructs of Dscam-N and Dscam-C was carried out according to the protocols described in Huang et al [7]. Primers used for making targeting constructs are listed in Table S4. We used “cis-analyst” tool at http://www.fruitfly.org/seq_tools/other.html to compare genomic sequences between Drosophila melanogaster and Drosophila pseudoobscura to identify apparently non-conserved non-coding regions for positioning the Qc31-attP and loxP sites in the target locus.

Transgenics and ends-out targeting

All transgenic flies were created using w1118 stocks via the standard P-elements-based transgenic protocol. Most fly cultures and crosses were carried out at room temperature (∼22°C) or 25°C. Ends-out gene targeting and PCR-verification of targeting events were carried out separately and were pooled before loading on the gel. MW: 1kb-plus DNA ladder (from Invitrogen); 5′ and 3′ homology arms of Dscam-N and Dscam-C targeting construct.

doi:10.1371/journal.pone.0031997.g003

Figure 3. Gene targeting of Dscam-N and Dscam-C. A. Targeting design and PCR verification of Dscam-N and Dscam-C founder lines. Boxed are the genomic DNA (gDNA) structure and alternative-splicing patterns of Dscam locus. Dscam locus contains four alternative-splicing exons: 4, 6, 9 and 17 [13]. Green boxes are gDNA regions used for 5′ and 3′ homology arms in the targeting constructs. In the Dscam-N founder knock-out line, a 5.7 kb genomic DNA covering the alternatively spliced exon 4 are deleted. In the Dscam-C founder knock-out line, a 7.6 kb genomic DNA covering the alternatively spliced exon 17 plus all the remaining downstream exons and 3′ UTR are deleted. Dscam-N and Dscam-C founder knock-out lines carrying W::Neo marker are verified by 5′ and 3′ PCRs. 5′ or 3′ PCR is designed with one primer annealing within the W::Neo, while another primer anneals outside the gDNA region used for homology arms (“5′ gDNA” or “3′ gDNA”) in targeting constructs. Thus, only the correct targeting events will yield PCR products of expected size. Dscam-N and Dscam-C founder lines with W::Neo removed are further verified by dPCR-1 and dPCR-2. dPCR-1 is located within, while dPCR-2 spans over, the deleted region of Dscam-N or Dscam-C. B. 5′ and 3′ PCR-1 (red and yellow arrowheads, respectively) results from adults of Dscam-N[med1+/CyO, Dscam-N[med1+/CyO, Dscam-C[med1+/CyO, CyO], Dscam-C[med1+/CyO, CyO], w1118] was used as wild type control. White arrowheads pointing to non-specific PCR products. C. dPCR-1 (yellow arrowhead) and dPCR-2 (red arrowhead) results from embryos of Dscam-N[med1+/CyO, Dscam-C[med1+/CyO, CyO, w1118] removed. Dscam-N[med1+] and Dscam-C[med1+] were balanced on CyO, twi-GAL4, UAS-2xEGFP (“CyO twiGFP”) chromosome so homozygous embryos could be distinguished by the absence of GFP. w1118 was used as the wild type control. For each PCR reaction genomic DNA was prepared by pooling approximately ten embryos together. For each sample, dPCR-1 and dPCR-2 reactions were carried out separately and were pooled before loading on the gel. MW: 1kb-plus DNA ladder (from Invitrogen); 5′ and 3′: the 5′ and 3′ homology arms of Dscam-N and Dscam-C targeting construct.

doi:10.1371/journal.pone.0031997.g003

CyO (BL#5626); w1118; In(2LR)Gln, wg-Casa1/CyO, P[Gala-twix.G]2.2, P[UAS-2xEGFP]AH2.2 (BL#6662).
candidates were carried out as described in Huang et al [7]. Primers used for PCR verifications as shown in Figure 3B,C are listed in Table S4.

G418 treatment and tests

G418 (from Fisher Scientific) was directly added to microwave-melted fly food at ~50°C as described [12]. All G418 concentrations reported here were effective concentrations based on their survival rate in G418 selection is calculated as the percentage rates in Figure 2B, we averaged the survival rates of all CyO/+ and (DOC)

two y w; pKIKO-WN

Cre recombinase. In FLP and lines.

* In each vial, six males of w were grown for five days. Progeny were scored based on WN

# from normal food). Each test was carried out in at least triplicates. To calculate the u+/ survival rates in Figure 2B, we averaged the survival rates of all TM3/+ and CyO/+ cross progeny at a given G418 concentration.

Supporting Information

Table S1 W::Neo marker confers G418 resistance in transgenic flies. * In each vial, six males of y w; pKIKO-WN #/CyO were crossed with six y w; Pn/CyO virgin females and were grown for five days. Progeny were scored based on w marker. In the absence of G418 selection one third of the progeny were expected to be u+/ (i.e. u+/u+ = 50%). ** These experiments were repeated three times. In experiment #2 only two y w; pKIKO-WN #/CyO females were used for each cross, hence the small number of progeny. Avg: Average.

(DOC)

Table S2 G418-resistance test of dArf6 transgenic donor lines. a: Tests were carried out similarly as in Table S1. b, c: hs-FLP and hs-Cre stocks used here constitutively express FLPase or Cre recombinase. In hs-FLP and hs-Cre crosses, constitutively expressed FLPase or Cre will excise the donor DNA, resulting in visible eye color variegation in the cross progeny. The degree of eye color variegation due to the loss of w+ was compared by estimating the percentage of white area in each eye. 0% white area suggests likely at least one of the two FRT or loxP sites were damaged in the transgenic donor DNA insertion. d: Gal42–21 drives UAS-Rpr expression of the transgenic donor DNA that results in 100% lethality in the cross progeny. Significantly reduced lethality suggests either damaged transgenic donor DNA insertion or severe repression of UAS-Rpr expression due to chromosomal location. e: These three lines likely have damaged transgenic donor DNA insertion based on their test results with hs-FLP, hs-Cre and Gal42–21. They also show much higher percentage of u+/ progeny on 0.25 mg/ml G418, presumably due to the fact that reduced G418-resistance in the u+/ progeny allowed better survival of u+/ siblings. f: The transgenic donor line used for targeting. n.d.: Not done Chr.: chromosomal location of the transgenic insertion.

(DOC)

Table S3 Optimal culture density for G418 selection in dArf6 targeting. Crosses were first set up in vials, and were then transferred to G418 bottles after one or two days. It appears that between 20 to 40 females per bottle, the yield of u+ candidates per 100 targeting females remains relatively stable. We decided that crosses of 30 targeting females per bottle appears to be a good compromise between achieving the maximum recovery of u+ candidates and minimizing the number of G418 bottles.

(DOC)

Table S4 Primers used for the generation and verification of Dscam-N and Dscam-C founder knock-lines. * Primers for PCR verifications of dArf6 founder lines were in Huang et al [7]. w+: PCR for verifying founder knock-out lines that contain the u+ marker. w−: PCR for verifying founder lines that had their u+ marker removed by loxP recombination. n/a: not applicable.

(DOC)

Acknowledgments

We are grateful to Dr. Bing Ye, Sige Zou and members of Hong lab for their thoughtful comments on the manuscript.

Author Contributions

Conceived and designed the experiments: YH WZ JH. Performed the experiments: WZ JH AMW YH. Analyzed the data: WZ YH JH. Contributed reagents/materials/analysis tools: WZ JH AMW YH. Wrote the paper: YH WZ.

References

1. Huang J, Zhou W, Dong W, Hong Y (2009) Targeted engineering of the Drosophila genome. Fly (Austin) 3: 274–277.
2. Huang J, Zhou W, Dong W, Watson AM, Hong Y (2009) Directed, efficient, and versatile modifications of the Drosophila genome by genomics engineering. Proc Natl Acad Sci U S A 106: 8284–8289.
3. Huang J, Ghosh P, Hatfull GF, Hong Y (2011) Successive and Targeted DNA Integrations in Drosophila Genome by Bxb1 and phiC31 Integrase. Genetics 189: 391–395.
4. Gao G, McMahon C, Chen J, Rong YS (2008) A powerful method combining homologous recombination and site-specific recombination for targeted mutagenesis in Drosophila. Proc Natl Acad Sci U S A 105: 13999–14004.
5. Choi CM, Vilain N, Langen M, Van Keist S, De Gent N, et al. (2009) Conditional Mutagenesis in Drosophila. Science 324: 54.
6. Gao G, McMahon C, Chen J, Rong YS (2008) A powerful method combining homologous recombination and site-specific recombination for targeted mutagenesis in Drosophila. Proc Natl Acad Sci U S A 105: 13999–14004.
7. Huang J, Ghosh P, Hatfull GF, Hong Y (2011) Successive and Targeted DNA Integrations in Drosophila Genome by Bxb1 and phiC31 Integrase. Genetics 189: 391–395.
8. Gao G, McMahon C, Chen J, Rong YS (2008) A powerful method combining homologous recombination and site-specific recombination for targeted mutagenesis in Drosophila. Proc Natl Acad Sci U S A 105: 13999–14004.
9. Choi CM, Vilain N, Langen M, Van Keist S, De Gent N, et al. (2009) Conditional Mutagenesis in Drosophila. Science 324: 54.
10. Weng R, Chen YW, Bushati N, Chiff E, Cohen SM (2009) Recombinae-Mediated Casette Exchange Provides a Versatile Platform for Gene Targeting: Knockout of miR-181b. Genetics 183: 399–402.
11. Huang J, Zhou W, Watson AM, Jan Y-N, Hong Y (2008) Efficient Ends-Out Gene Targeting In Drosophila. Genetics 180: 703–707.
12. Rong YS, Golic KG (2000) Gene targeting by homologous recombination in Drosophila. Science 288: 2013–2018.
13. Gong WJ, Golic KG (2003) Ends-out, or replacement, gene targeting in Drosophila. Proc Natl Acad Sci U S A 100: 2556–2561.
14. Bennet KJ, Trauman JK, Beaus A, Liu JI, Rutter J, et al. (2008) Efficient gene targeting in Drosophila by direct embryo injection with zinc-finger nucleases. Proc Natl Acad Sci U S A 105: 19821–19826.
15. Ramirez CI, Foley JE, Wright DA, Muller-Lerch F, Rahman SH, et al. (2008) Unexpected failure rates for modular assembly of engineered zinc fingers. Nat Meth 5: 374–375.
16. Steller H, Pirrotta V (1985) The p300 gene encodes a transposable P vector that confers selectable G418 resistance to Drosophila larvae. Embo J 4: 167–171.
17. Schneiter D, Clermen JC, Shu H, Wabry CA, Xiao J, et al. (2008) Drosophila Dscam Is an Axon Guidance Receptor Exhibiting Extraordinary Molecular Diversity. Cell 101: 671–684.
18. Xu T, Rubin GM (1993) Analysis of genetic mosaics in developing and adult Drosophila tissues. Development 117: 1223–1237.