Benchmarks

Bxb1 phage recombinase assists genome engineering in *Drosophila melanogaster*

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Rapid and reliable genome modifications provide the basis for detailed in vivo functional analysis of any genomic entity (gene, regulatory DNA, non-coding RNA, etc). With the advent of CRISPR/Cas9 genome editing technology, manipulation of a particular genomic locus has become a routine undertaking in variety of model organisms, including the fruit fly *Drosophila melanogaster*. To further diversify the available tools for genome engineering, we successfully harnessed the phage recombinase Bxb1 to perform recombinase-mediated cassette exchange (RMCE) in *D. melanogaster*. We demonstrate that Bxb1 possesses highly efficient recombinase activity and could be used alone or in conjunction with other currently available recombinases for creating platforms for cassette exchange of targeted loci.

Site-specific recombinases have revolutionized molecular genetics by allowing researchers to manipulate genomes and gene expression in a highly controlled manner (reviewed in Reference 1). *Drosophila melanogaster* investigators have adopted and greatly benefited from effective and sustained utilization of a variety of site-specific recombinases of diverse origin, such as Flp (2), Cre (3), and Φ C31 (4). More recently, several new site-specific recombinase systems have been shown to function in *Drosophila*, including those based on the Bxb1 (5), KD, B2, B3, and R (6) recombinases, thereby increasing the number of molecular biology tools available for this organism. Here, we present a method utilizing the phage recombinase Bxb1 and its minimal cognate recognition sites (7) to catalyze recombinase-mediated cassette exchange (RMCE) of a Bxb1 attP-primed allele using a germline-

expressed Bxb1 recombinase. Such an allele could undergo a large number of desired cassette replacements for exhaustive in vivo functional characterization of a locus.

As proof-of-principle for this approach, we inserted a vector bearing the selectable marker *hs-neo* [heat-shock-protein-70 promoter-driven neomycin resistance gene (*neo*)] (8) into the fruit fly genome at landing site ZH-51D (9) by Φ C31-mediated integration (4) (Figure 1). We designed the vector in such a way that *hs-neo* was flanked by inverted minimal Bxb1 attP sites (7) and a loxP site immediately preceding this cassette. Landing site ZH-51D allows for the removal of all intervening vector sequences using Cre/loxP-mediated excision (9), which we performed, resulting in a cleaner allele where only the Bxb1 attP-flanked *hs-neo* cassette remained at the locus. We named this allele *BB^{hs-neo}*. Similar Bxb1 attP-primed alleles could be

engineered at loci of interest using CRISPR/Cas9 genome editing technology (10–12).

Next, we generated fly strains that express the phage recombinase Bxb1 in the fly germ line by placing its cDNA under the control of the *vasa* promoter and the *nanos* 3' UTR (*vasa-Bxb1-nos*). We then introduced this construct into landing sites ZH-2A (X), ZH-22A (II), and ZH-86Fa (III) (9) through Φ C31 transgenesis [the construct contains the selectable marker *yellow* (*y+*)]. This construct did not exhibit any toxicity since we have maintained the resulting strains for >3 years without observable adverse effects.

We also created an RMCE vector that contains a *ubiquitin-GFP* (*ubi-GFP*) cassette flanked by inverted minimal Bxb1 attB sites (7) and also contains the selectable marker *white* (*w+*) in the vector backbone (Figure 1). We tested if we could efficiently perform RMCE by injection of 250 ng/ μ l *ubi-GFP* RMCE vector into embryos resulting from crosses of *BB^{hs-neo}* males and *vasa-Bxb1-nos*[ZH-2A], *vasa-Bxb1-nos*[ZH-22A], or *vasa-Bxb1-nos*[ZH-86Fa] females. Two hundred embryos were injected in each case. We raised the resulting larvae to adulthood at 25°C. Approximately 15 males and 15 females hatched from each genotype, and we crossed these G0 parents to *yw* flies.

The Bxb1 recombinase has previously been shown to perform intermolecular recombination in *Drosophila* (5). Our experimental design allowed us to extend these observations by assessing the ability of Bxb1 to perform RMCE in flies. In addition, we could compare the rate of RMCE with the frequency of recombination events between individual Bxb1 attP and attB sites. Because our *ubi-GFP* RMCE vector contains the *w+* marker in the vector backbone, the latter events would result in vector integration and *w+* F1 flies that also fluoresce green as a result of the *ubi-GFP* cassette. If RMCE occurs, the resulting flies would be *ubi-GFP*-expressing and *w-*. Interestingly, in the case of *vasa-Bxb1-nos*[ZH-2A]/+; *BB^{hs-neo}*/+ flies, we detected only RMCE events at a rate of 1.7% (*n* = 600 F1 flies) and no vector integration events. In the case of *BB^{hs-neo}*/+; *vasa-Bxb1-nos*[ZH-86Fa]/+ flies, we observed RMCE at a rate of 4.3% and vector integration events at a rate of 1% (*n*

METHOD SUMMARY

First, a *Drosophila melanogaster* genomic locus is modified in a way that minimal inverted Bxb1 attP sites flank a region of interest. Next, the phage recombinase Bxb1 is expressed in the fly germ line, where it catalyzes efficient recombinase-mediated cassette exchange (RMCE) between the Bxb1 attP-targeted allele and a donor vector containing cognate minimal inverted Bxb1 attB sites.

Figure 1. Schematic representation of the ZH-51D landing site locus on chromosome 2R and its genome engineering using Bxb1-catalyzed recombinase-mediated cassette exchange (RMCE). Dark blue represents a 48 bp minimal Bxb1 attP site (GGTTT-GTCTGGTCAACCAACGCG-GTCTCAGTGGTGACGGTA-CAAACC); light blue represents a 38 bp minimal Bxb1 attB site (GGCTTGTCGAC-GACGGCGGTCTCCGTCGT-CAGGATCAT). Plasmid DNA, maps, and complete vector sequences are available from Addgene (Cambridge, MA) at www.addgene.org. Fly strains generated in this study are available from the Bloomington *Drosophila* Stock Center (Bloomington, IN).

= 600 F1 flies). These differences could be ascribed either to variations in *vasa-Bxb1-nos* expression from landing sites ZH-2A and ZH-86Fa or, possibly, to technical variations in the embryo injections, which often occur between different samples. When each *w+* F1 fly line, resulting from an integration event, was crossed back to the *vasa-Bxb1-nos*[ZH-86Fa] germline integrase strain, intramolecular recombination between the intact attB and attP Bxb1 sites remaining at the locus resulted in completion of the RMCE.

In the previous two experiments, we assessed the rate of Bxb1-driven RMCE as the percentage of GFP-positive F1 flies out of the total number of scored F1 flies. These rates represent the number of RMCE-transformant flies per unit of injected embryos (200 embryos in this case). In order to also investigate the percentage of independent G0 flies that yield Bxb1-driven RMCE, we tested a third source of Bxb1 germline integrase: *vasa-Bxb1-nos*[ZH-22A] inserted on the second chromosome. In this case, we found that 18% (4/22) of G0 *BB^{hs-neo}/vasa-Bxb1-nos*[ZH-22A] flies injected with the *ubi-GFP RMCE* vector produced RMCE progeny. We verified successful RMCE events by PCR and sequencing of independent alleles from each genotype. Since the inverted attP/attB pairs are identical (Figure 1), half of the replaced *ubi-GFP* cassettes were oriented in the forward orientation and half in reverse, as expected. Overall, we conclude that Bxb1-assisted RMCE in *D. melanogaster* occurs at rates that are higher than the RMCE rates reported for the Cre/lox system (13) and lower than those reported for the Φ C31 system (14).

The successful replacement of *hs-neo* with *ubi-GFP* at the ZH-51D locus using the Bxb1 recombinase for RMCE establishes yet another tool that complements the genome engineering repertoire in the fruit fly. We demonstrated that minimal Bxb1 attP/attB sites, which leave very small DNA scars in the genome, are sufficient for potent RMCE. If positioned carefully, these sites are unlikely to interfere with the function of a particular locus of interest. Moreover, combining Bxb1 with other recombination systems, such as Φ C31, Flp, and Cre, has the potential to add versatility to how genomic loci can be manipulated, not only in *D. melanogaster* but also in other model organisms.

Author contributions

R.V. conceived this work, performed the experiments, and wrote the article. R.S.M. edited the article and supported the study.

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Competing interests

The authors declare no competing interests.

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