Combining Recombinase-Mediated Cassette Exchange (RMCE) with the P-element
Transposon System to Target Marker Genes onto Balancer Chromosomes

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Balancer chromosomes are essential tools that facilitate genetic manipulations by preventing crossing over. In the model organism Drosophila melanogaster, balancers exist for each of the three major chromosomes. All balancers carry one or more marker genes, which make flies carrying the balancer easily identifiable. However, because most markers appear only as adult phenotypes, the identification of embryos and/or larvae that carry a balancer presents a challenge. Several research groups have generated, and continue to develop, new markers that are visible in various tissues and stages of development, among other useful functions. These new markers are frequently constructed in vitro, and must be incorporated onto balancers in living flies. This project seeks to create target insertion sites to integrate marker genes directly onto balancer chromosomes by using Recombinase-Mediated Cassette Exchange (RMCE), a strategy to target insertions of DNA to a defined location in the genome. An RMCE target cassette containing the mini-white gene flanked by recognition sites for the phiC31 integrase and carried on a P-element vector was remobilized and screened for those that insert into each of the three major balancers: multiple insertions of the target cassette on each of the three major balancers were uncovered. I then used inverse-PCR to determine the point of insertion for target cassettes on each balancer: 6 out of the 7 successfully mapped locations are in euchromatic regions that will likely support expression of marker genes. To ensure that the insertions supports suitable levels of transformation by RMCE and that markers for different developmental stages can be expressed at each site, donor cassettes for markers in embryonic, larval, and adult tissue were tested on representative RMCE targets of each balancer. To conclude, I have created several strains that geneticists can use to integrate marker genes more directly onto balancer chromosomes.

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