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

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Purpose:

My project aimed to develop a method that will more directly target the integration of marker genes onto balancer chromosomes by combining the simplicity of recombinase-mediated cassette exchange (RMCE), a recently developed integration system, with the well established P-element transposon system developed by Rubin and Spradling (as cited by Bateman et al, 1999). The anticipated outcome will greatly simplify the desired mutation screening process resulting in time, money, and manpower savings. Ultimately, this methodology innovation will significantly facilitate the manipulation of genes and genetic analyses.

Methods and Results:

The task covered this summer was to target the target cassette of the RMCE created in a P-element transposon vector to the desired balancer chromosomes on the III- and X-chromosomes (balancers TM3 and FM7h respectively). Flies containing the three elements of transposon, balancer, and transposase, which is required to move the transposon, were crossed. The flies were then screened for the absence of the markers of the transposase, so the transposon does not move in future crosses, and for the markers of the transposon and balancer together. For the III-chromosome cross, a total of 9052 F2 generation flies were screened. Of these, 156 of these were males with the desired phenotypes. For the X-chromosomes cross, 3260 F3 generation flies were screened. Of these, 19 were bar-eyed males (bar-eyes signaling the presence of the balancer) with the desired phenotypes. These flies from the III- and X-chromosome cross were then back-crossed to determine whether the transposon was indeed on the intended balancer chromosome. 53 backcrosses were made for the III-chromosome flies, and 26 crosses were found to be successful jumps. Of the 19 backcrosses made for the X-chromosome flies, only 4 crosses were found to be successful jumps. These successful flies were crossed and put into stocks.

Another task covered this summer was to molecularly determine the location of the transposons already targeted to the II-chromosome balancer (Cyo) of three strains of flies provided by my advisor using inverse PCR techniques. One transposon was found in a 5'UTR, another found 25 nucleotides upstream of B and E transcripts and in the intron of A,G, and F transcripts, and the third was found in a Doc element of heterochromatin.

Future Directions:

The stocks of flies generated for the III- and X-chromosome balancers will undergo Inverse PCR to determine the location of their respective transposons. The RMCE technique will then be tested on the inserted target P-element transposon cassette by creating donor cassettes created with marker genes that will undergo RMCE.

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