A method for fast and high efficiency multiplex genome editing using CRISPR/Cas9 in *Drosophila*

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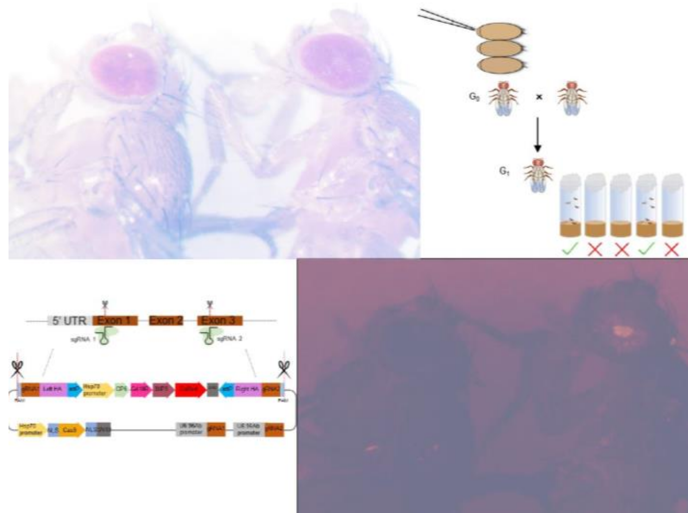

The use of CRISPR/Cas9 has expedited genome editing in a wide variety of organisms. This technique has also been applied in *Drosophila melanogaster* to generate indels or introduce specific sequences using homology-directed repair (HDR). However, the efficiency of HDR mediated CRISPR/Cas9 mutagenesis in *Drosophila melanogaster* is low. The current approaches for achieving efficient CRISPR/Cas9 mutagenesis in *Drosophila* require the use of transgenic Cas9-expressing embryos. As the genetic background of the fly may potentially interfere with downstream behavioral analysis, the cur rent approaches present a major obstacle in the study of genes that regulate behavior in *Drosophila*. We describe two strategies for fast and efficient CRISPR-mediated genome engineering in *Drosophila*. Our methods to mutagenize specific loci are independent of genetic backgrounds and do not require the use of Cas9-expressing transgenic flies. The first strategy uses sgRNA/Cas9 expression vectors in combination with an HDR donor plasmid that allows insertion of a fluorescent marker within the gene of interest. The introduction of the fluorescent marker disrupts the function of the gene and allows easy visible screening of the mutagenic event. The second strategy makes use of the GoldenBraid (GB) system to assemble a single plasmid that comprises all of the CRISPR components. This strategy eliminates time-consuming traditional cloning as it involves the use of a set of plasmids that incorporate multipartite assemblies of standard DNA parts that carry the Cas9 encoding gene, a selection cassette, an exchangeable donor cassette and tandem gRNAs in a binary fashion to build a multi-functional plasmid. The donor DNA and tandem gRNAs are synthesized and easily exchangeable making this method highly adaptable to any desired genome modification. These strategies can be used to edit genomes of any strains or species amenable to injections, and can therefore be used to characterize the function of genes and regulatory elements that modulate complex behaviors, even if they depend on specific genetic backgrounds. Our collection of DNA assemblies and GB plasmids will be a valuable resource for researchers to study gene function in any strain or species.

Speaker Bio:

I grew up in India in a town called Nashik, also known as the 'wine capital of India'. I moved to Mumbai after high school to study at St. Xavier's college. I received my bachelor's degree in Biochemistry from Mumbai University and ranked 5th in the final University examination. During my undergraduate studies, I was intrigued by gene regulation mechanisms and decided to pursue studies in Genetics. I received a scholarship from the Department of Biotechnology, Govt. of India for my master's degree in Molecular and Human Genetics from Banaras Hindu University, India. I did my PhD in Biochemistry at the Medical College of Wisconsin, USA. During my PhD, I worked on identifying mechanisms of oncogene-induced senescence using cancer cell lines. I published my first paper in a record-breaking speed and was the first student to graduate from my class. I then moved to the Baylor College of Medicine for my postdoctoral studies. I was intrigued by the complexity that underlies behavior and so I transitioned to genetics and neuroscience for my postdoc.

While I was a student, I was actively involved with the Graduate Student Council and I am currently serving as the President of the Postdoctoral Association at the Baylor College of Medicine.

Topic areas: Methods, Technology, & Resources, Molecular & Cellular Genetics, Gene Expression