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Sensitized zebrafish mutant lines for environmental screens generated by CRISPR-Cas9

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Rapid and cost-effective screens for waterborne pollutants that affect the flora and fauna, as well as human health are needed. We propose to develop an up-scalable assay to test watershed contamination using sensitized zebrafish larvae focusing on two environmental toxicological endpoints: (1) the development and the regeneration of a superficial sensory organ, the lateral line (LL), and (2) larval swimming behavior. Using CRISPR-Cas9 technology we generated at least one null allele in 4 different genes. Cannabis receptors 1 and 2 (*cnr1, cnr2*), necessary for proper functioning of the CNS, which will predictably be affected in swimming behaviors. Tnks1bp1 and atf5a involved in development and regeneration of the LL. We expect that in the absence of the respective gene products larvae will exhibit greater susceptibility to waterborne pollutants. To identify alleles for the genes and grow stable mutant lines into the F2 and F3 generation, we systematically genotyped potential heterozygote or homozygote carriers. To do so, we prepare genomic DNA (gDNA) from fin clips of juvenile and adult fish or from whole embryos and larvae. We designed PCR primers to amplify the targeted region in the gene and sequence the amplicons looking for insertions or deletions (INDELs) that will create a translational frame shift and eventually introduce an early stop codon. Three out of the four identified alleles are homozygote viable, meaning that unchallenged animals which are completely lacking the given gene products, are developing into healthy and fertile adults. Both, adult fish and larvae from those 3 lines will be ideal for testing water quality and are expected to display increased sensitivity in the presence of potential contaminants and can therefore serve as sensitized environmental sentinels in up-scalable environmental toxicology screens.

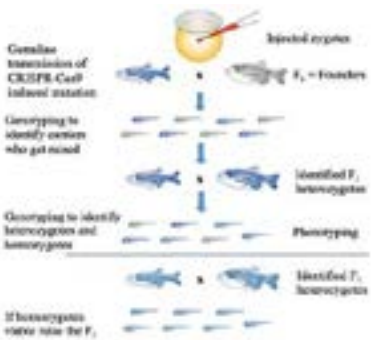

Figure 1: Zebrafish mutagenesis. The mutant lines developed will allow to generate environmental sentinels for toxicology screens.

Biography

Viveca M Vélez Negrón is passionate about environmental neuroscience. Her participation in this project using zebrafish has encouraged her to develop her research skills. Currently, her goal is to study pharmacy and combine it with research to take her career to the next level.

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