MICHIGAN STATE
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Wednesday, May 13, 2019

Dear Editors:

I am pleased to submit the enclosed manuscript to *The Journal of Visualized Experiments* entitled "*Silencing the spark: CRISPR/Cas9 genome editing in weakly electric fish*". This manuscript represents a significant milestone in our ongoing efforts to characterize the genetic mechanisms underlying electric organ evolution in weakly electric fish, which are an important emerging model in understanding the evolution of phenotypic diversity on our planet.

This manuscript outlines a full protocol for performing CRISPR/Cas9 mutagenesis that utilizes endogenous NHEJ repair mechanisms in weakly electric fish. We demonstrate this protocol is equally effective in both the mormyrid species *Brienomyrus brachyistius* and the gymnotiform *Brachyhypopomus gauderio* by using CRISPR/Cas9 to target indels and point mutations in the first exon of the sodium channel gene *scn4aa*. Using this protocol, we obtained embryos from both species and genotyped them to confirm that the predicted mutations in the first exon of the sodium channel *scn4aa* were present. The knock-out success phenotype was confirmed with recordings showing reduced amplitude electric organ discharge amplitudes when compared to uninjected size-matched controls.

Beyond the obvious impacts to the community of researchers who study electric fish, we feel that these results are worthy of publication in your journal, which has a broad readership, for the following reasons:

Methodological: Numerous researchers are interested in the editing the genomes of 'non-model systems', particularly teleost fishes. We hope that the methods outlined here will help other researchers develop protocols in their chosen model systems.

Broad Applicability of the Model System: Electric fish are a long-established model in systems neurobiology, and the development gene manipulation resources for electric fishes should greatly facilitate expansion of this system into developmental, and cell-biology approaches.

Genome to Phenome Studies in a Charismatic Phenotype: Much of the efforts to date connecting genome to phenome have focused on relatively similar phenotypes: chemicals, colors and morphology. To contrast, electric fish communicate using a modality that is invisible to our sensory system. Second, EODs are very simple *behaviors* with well-known anatomical and physiological substrates-- studies on other communication behaviors are lacking primarily because they are vastly more complex. Our ability to connect genotypic variation to a behavioral phenotype will be compelling to a wide swath of biologists.

For these reasons, I trust that the broad readership of *JOVE* will find this manuscript of great interest.

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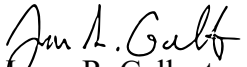
On behalf of myself and my couauthors, I appreciate your time, dedication and care in reviewing this manuscript, and wish you well for the upcoming summer.

Sincerely,



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