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Inactivation of a proteorhodopsin like gene in *Aurantiochytrium* by double homologous recombination

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Aurantiochytrium limacinum, a marine heterotroph which belongs to a crude oil-degrading class of protists called the Labyrinthulomycetes, is one of a variety of thraustochytrids known to produce zoospores that respond to chemical cues, as well as light, but the mechanisms by which they do so are unknown. While many papers have been published concerning how zoospores across different taxa respond to light, little research has been done in investigating the mechanism of light sensitivity of *Aurantiochytrium limacinum*, which plays an important role in the carbon cycle by decomposing crude oil, tarballs, and other non-living organic matter. Since rhodopsins can be involved in phototaxis, we hypothesized that the gene 7690 in *Aurantiochytrium*, which encodes a protein with similarity to rhodopsins, serves as a photoreceptor for zoospore phototaxis. By attempting to knock out the 7690 genes through double homologous recombination, this research aimed to determine whether the 7690 protein is required for phototaxis of the zoospores. We extracted, purified and restriction digested a plasmid containing the antibiotic resistance cassette we call 'GZG' (made of promoter and terminator regions of the *Aurantiochytrium* GAPDH gene surrounding, and driving the expression of, the sh ble gene, which encodes resistance to zeocin) in between DNA from upstream and downstream of the 7690 genes, and introduced that whole construct into *Aurantiochytrium* by electroporation. After testing 6 different isolates using colony PCR to search for double homologous recombinants, we identified a singular potential knockout which can be useful in investigating whether the 7690 genes is required for phototaxis.

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