

Developing electroporation-based delivery of Cas9 RNPs to coral endosymbionts

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Corals engage in symbiosis with a group of photosynthetic dinoflagellates in the family *Symbiodiniaceae*. Although they are of critical importance to corals around the world, these microalgae have been challenging subjects for the application of genome editing techniques. We are developing methods to perform genome editing in *Symbiodiniaceae* species to facilitate research on these organisms using classical genetic techniques. In this work, we will use electroporation to deliver RNPs to the coral endosymbiont. Electroporation is a technique in which short electric pulses are delivered to cells in order to create temporary holes in the membrane. Through these pores, material that can not normally permeate the membrane can be introduced, such as Cas9 proteins complexed with single guide RNAs. We will screen for mutagenesis by targeting *URA3* with Cas9; loss-of-function mutations in this gene are tolerant of 5-Fluoroorotic Acid (5FOA), a compound normally toxic to cells. This enables a convenient screen for mutants and represents a first step in the development of a reverse genetic toolkit for *Symbiodiniaceae*.