Creation of an Eastern Oyster With an Easily Modifiable Genome

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Climate change threatens oyster populations around the world. Rising ocean temperatures lead to severe challenges. As such, there is a clear need for research into ways to protect endogenous oyster populations from an array of issues. For this project, I focused on Crassostrea virginica (Eastern oyster). I hypothesized that it would be possible to generate an easily modifiable transgenic oyster of use in characterizing the phenotypic effects associated with overexpressing any gene. To examine this, I collected gametes from locally harvested Eastern oysters then used these to generate embryos in a controlled environment. ~3 hours after fertilization, embryos were transfected with a plasmid containing the gene for green fluorescent protein (GFP) preceded by an oyster-competent promoter. Importantly, both sides of the promoter-driven GFP cassette were flanked by two separate sequences: an outer pair facilitating initial insertion into the Eastern oyster genome and an inner pair of FRT (Flippase Recognition Target) sequences designed to allow highly efficient, targeted replacement of GFP with any desired gene. Successful generation of a transgenic Eastern oyster through insertion of the GFP cassette was confirmed with PCR and observation of GFP. In conclusion, my findings support my hypothesis that it would be possible to generate an easily modifiable transgenic oyster. Geographically distinct oyster populations face diverse challenges driven by regional climate, stressors, and human activity. The oyster created in this project represents an easily manipulatable organism where GFP loss serves as an indicator of successful, targeted insertion of any desired gene into the Eastern oyster genome.

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