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Using CRISPR/Cas9 Gene Editing to Remove *twist2* to Determine its Role During Somite Development in Zebrafish

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Using CRISPR/Cas9 Gene Editing to Remove *twist2* to Determine its Role During Somite Development in Zebrafish

Caleb Holdener

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Embryonic Mesoderm is a precursor to various types of tissues, such as the somatic mesoderm. Somites are a segmented tissue that gives rise to the sclerotome in vertebrates. This sclerotome further develops into the axial skeleton (Stickney, Barresi, and Devoto 2000). Somites are easily observable under low power microscopy during fish development. In fish, great strides have been made in studying the developmental fate of somites (Stoiber, Haslett, and Sanger 1999; Stickney, Barresi, and Devoto 2000). Zebrafish have been studied in many labs due to their similarities with higher vertebrates. These advantages include their inexpensive cost of care, external fertilization, high fecundity, embryonic transparency, and quick organ development (Nusslein-Volhard, Dahm 2002; Hoon 2009).

Both vertebrate and invertebrate genomes contain a family of genes known as the Twist genes. These genes are essential for embryonic development and survival and have been shown to have expression in sclerotome development and formation (Germanguz et al. 2007). Twist genes play an important role in embryonic research due to their ability as transcription factors to activate and repress other genes. By utilizing CRISPR/Cas9 editing in the zebrafish, it is possible to further study the role that these genes play during development (Ansai and Kinoshita 2014). Genetic compensation can occur if a portion of a gene remains after editing, which complicates studies to remove a gene's function (El-Brolosy and Stainier 2017). By attempting to fully knock out Twist2, genetic compensation will hopefully be avoided, and further research on Twist2's impact on sclerotome development can occur.