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twist1b Knockout Generation in Zebrafish Using CRISPR-Cas9

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Twist1b Knockout in Zebrafish

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The Twist gene is found in numerous organisms, including humans, mice and zebrafish (Yeo et al., 2009). It is a vital gene in development and is needed for the organism to survive (Yeo et al., 2009). Specifically, the Twist gene family aids in specialization and differentiation of mesodermal cells, telling somites, segmented blocks of mesoderm, what to become (Germanguz et al., 2007). Over time, the Twist gene has duplicated into paralogs, each having differences in their functions (Germanguz et al., 2007). Twist1b is a protein coding gene that is a paralog to the human Twist1 gene (ZFIN, 2020). In zebrafish, it starts to be expressed at ~6 hours post-fertilization (Germanguz et al., 2007). Previous research has attempted to knock out Twist1b completely, but these attempts failed due to the mutated gene's capability to activate genetic compensation (El-Brolosy et al., 2019). This means that even when the majority of Twist1b is taken out through techniques like CRISPR, the gene can maintain function through protein feedback loops (El-Brolosy et al., 2019). The goal of this study is to see what will happen during the development of the sclerotome, a subcompartment of the somite, when *twist1b* is completely knocked out, which has yet to be done. The CRISPR-cas9 system was used and two guide RNAs were created, using gRNA design from the CHOPCHOP website. The gRNAs were then amplified, transcribed, and then microinjected into developing zebrafish embryos. These studies will inform us on what role(s) Twist1b is playing during sclerotome development.