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## Endogenous Promoters enable efficient CRISPR/Cas9 in a Tilapia Cell Line Model

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## **Abstract**

Cell cultures are effective supplemental models to study specific biochemical pathways used for environmental adaption in animals. They enable isolation from system influence and facilitate control of the extracellular environment. The recent rise of CRISPR/Cas9 gene editing has provided an additional powerful tool to aid in these studies. For work focusing on fish species many fish cell lines now exist. However, conventional tools and methods for implementing CRISPR/Cas9 gene targeting in these cells doesn't always provide sufficient results as seen in other animal cell models. A tilapia brain cell line (OmB) developed in our lab has proven useful for gene expression studies. Previous work with multiple conventional methods attempting to apply CRISPR/Cas9 failed to indicate genomic alteration at the targeted sites. Here we present a plasmid vector based system utilizing both Polymerase II and III tilapia endogenous promoters that enables proficient gene targeting. This system demonstrated efficient cleavage in most target sites attempted with efficiency as high as 80%. These tools will allow generation of knockout cell lines for gene targeting studies in tilapia and other phylogenetically related fish species.

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