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Meeting Abstract

P29-7 Sat Jan 2 **Efficient CRISPR/Cas9 gene editing in a tilapia cell line model using endogenous promoters** *Hamar, JC*; Kültz, D; University of California, Davis; University of California, Davis*
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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 the extracellular environment. For work focusing on fish species many representative cell lines now exist, including a tilapia brain cell line (OmB) developed in our lab. CRISPR/Cas9 gene editing is an additional tool aiding these studies by allowing manipulation of specific genetic loci and evaluating their causal relationship between phenotypes of interest. However, established CRISPR/Cas9 gene targeting tools and methods often have not functioned as efficiently in fish cells as seen in other animal cell models such as mammalian cell lines, consistent with our initial attempts to apply CRISPR/Cas9 in OmB cells that failed to indicate genomic alteration at the targeted sites. Poor expression of heterologous promoters in OmB cells was hypothesized to be a primary cause for this occurrence so we constructed a custom plasmid vector based system utilizing tilapia endogenous promoters (EF1 alpha to express Cas9 and a U6 to express gRNAs). This system demonstrated substantial editing of most target sites attempted with mutational efficiency as high 80%. This work specifically highlighted the importance of phylogenetic proximity in selection of a polymerase III promoter for gRNA expression as commonly used interspecies U6 promoters (human and zebrafish) yielded no detectable gene editing when applied in this system with a common gRNA target sequence. These new tools will allow generation of knockout cell lines for gene targeting studies in tilapia and other phylogenetically close fish species. This study was funded by a grant from NSF (IOS-1656371) and BARD (IS-4800-15 R).