CRISPR-Mediated Knockout of FRY in Normal Breast Cells

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The Fry gene is highly conserved, being identified within several eukaryotic organisms, ranging from yeast to Homo sapiens. Fry protein and its orthologues have been found to have diverse functions having implications in microtubule acetylation, epidermal morphogenesis, dendritic branching, and mitotic chromosomal alignment. In previous studies, Fry has been classified as a mammary carcinoma susceptibility (Msc) gene using Quantitative Trait Locus mapping. Partial FRY knock-down studies have shown changed cellular morphology, adhesion, and polarity. Currently, there are no known studies that have a FRY knockout model. We aim to create a CRISPR-mediated FRY knock-out cell line by targeting exon 2 of the FRY gene in the normal mammalian breast cell line, MCF-12A. In this study, we designed CRISPR/Cas9 plasmids along with additional homology donor plasmids to excise exon 2 of the FRY gene via Homology-Direct Repair (HDR). The homology donor plasmids were constructed using the Gibson Assembly method. The MCF12A cells were transfected, selected using antibiotics, and validated using PCR and DNA sequencing.