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**Title of the project: Mitochondrial transfer in fish embryos**

Mitochondrial homoplasmy is essential for normal development. Conversely, the heteroplasmy of mtDNA, where more than one mtDNA haplotype is present in the same individual, is detrimental to embryogenesis. So the sperm mtDNA is usually delivered to the egg during fertilization and then rapidly eliminated to avoid heteroplasmy. But heteroplasmy is inevitable when implementing *mitochondrial replacement therapy* (MRT) techniques. Heteroplasmy can lead to individual genetic instability, cognitive or behavioral impairment, and diseases, as well as affect the survival of mtDNA. However, we know almost nothing about the dynamics of heteroplasmy within individuals during their lifetime, particularly after the mitochondrial replacement.

In the present study, we will do mitochondrial transfer in zebrafish, which is closer to mammals than any other moder organism and easier to handle. The main objective is to induce mitochondrial DNA heteroplasmy by interspecific transplantation of mitochondria from zebrafish (*Danio rerio*) to giant danio (*Devario aequipinnatus*), aiming to study the dynamics of heterodimers. Thus providing basic information for the extension and improvement of the mitochondrial replacement techniques.

In short, the experiments will be performed as follows. Mitochondria for our study will be isolate form zebrafish eggs. The sample is further purified by Percoll density gradient centrifugation. The protein concentration is then determined using the BCA method. After that, mitohcondria are labeled with the PKH26 Red Fluorescent lipophilic membrane dye. Subsequently, PKH 26 labeled mitochondria will be injected into the giant danio 1-4 cell embryos. Finally, observe the survival and migration of transplanted mitochondria during different developmental stages and tissues of individuals through a series of photos taken every 24 hours. For cryopreservation, Dimethyl sulfoxide (DMSO) will be added to the mitochondria suspension at a concentration of 10% (v/v). The sample is then immediately frozen at -80°C.