

AUTHENTICATION OF KEY BIOLOGICAL OR CHEMICAL RESOURCES

Human Cell Lines: The human normal muscle cell lines were originally generated by Dr. Alexander and extensively characterized for their ability to proliferate and the expression level of specific muscle factors (Alexander *et al.*, *Sk. Musc.*, 2011). These cells were aliquoted, and cryogenically stored in liquid nitrogen at low serial passage numbers. To ensure for correct sample validity, the uninfected (or mock infected) normal cells will be tested for myogenicity using standard myogenic differentiation (switch to low serum) and proliferation (cell counts) assays. The human muscle cells containing the FKRP (L276I) CRISPR-induced mutation will be screened several times by standard Sanger sequencing for the FKRP L276I genetic mutation, prior to any experimental procedures.

Zebrafish Lines: The zebrafish LGMD2I and WWS/MEB models will be screened via standard PCR genotyping for both the transgene (IRES-eGFP reporter), and the specific human FKRP mutant protein (western blot analysis using human-specific FKRP antibody on zebrafish protein lysates). Breeders will be validated by PCR genotyping prior to experimentation. Additional secondary screening of “hit” lead compounds will allow for additional screening and confirmation of expression of the hFKRP transgene via both western blot and PCR genotyping analyses.

Chemicals and Libraries for Drug Screening: The calcium signaling chemical library will be purchased from Otava chemicals. Any “hit” compounds will be validated in secondary, double blinded (genotype and drug), screens in the LGMD2I and WWS/MEB zebrafish models. We will also use pentetic acid (Sigma-Aldrich) (a compound that has shown significant efficacy in ameliorating muscle degeneration in several zebrafish models) as a positive control and positive control for all secondary zebrafish screens. These steps will ensure for validity of lead “hit” targets identified from the zebrafish screens, and reduce any bias the experimenter might have.