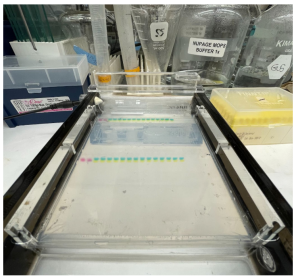
DEVELOPING A PLATFORM TO STUDY THE PATHOGENIC MECHANISMS OF NF1 MISSENSE MUTATIONS



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Neurofibromatosis type 1 (NF1) is an autosomal dominant condition. One of the most serious manifestations of NF1 is the growth of tumors on the nerve sheath. To date, over 3,000 pathogenic mutations have been identified in the *NF1* gene, which encodes the protein neurofibromin. Missense mutations (MMs) account for ~38% of these pathogenic mutations and result in an amino acid substitution as a result of a single base pair mutation. The pathogenic mechanisms for many MMs have not yet been determined. We hypothesize that MMs disrupt neurofibromin's protein-protein interactions. To test that, prime editing was used to add a FLAG tag to N-term of *NF1* in immortalized human Schwann cells (hSCs). Created FLAG-NF1 hSC will allow the use of immunoprecipitation (IP) and mass spectrometry (MS) to identify MM-dysregulated neurofibromin protein interactors when MMs are modeled. To confirm the successful integration of the FLAG tag into *NF1* hSC lines, the potential FLAG-NF1 hSCs were screened via polymerase chain reaction (PCR) for the flag tag insert and then sent for sequencing. Positively identified clones were analyzed via T-vector cloning. Further, sequencing identified NF1 FLAG-tagged homozygous and heterozygous cell lines. lastly, FLAG-antibody immunoblotting did not detect FLAG-tagged neurofibromin, indicating an inability to use FLAG-IP MS. Next steps will use different FLAG-antibodies, as well as testing other protein tags. Overall, this project will develop a platform to further study pathogenic NF1 MMs.

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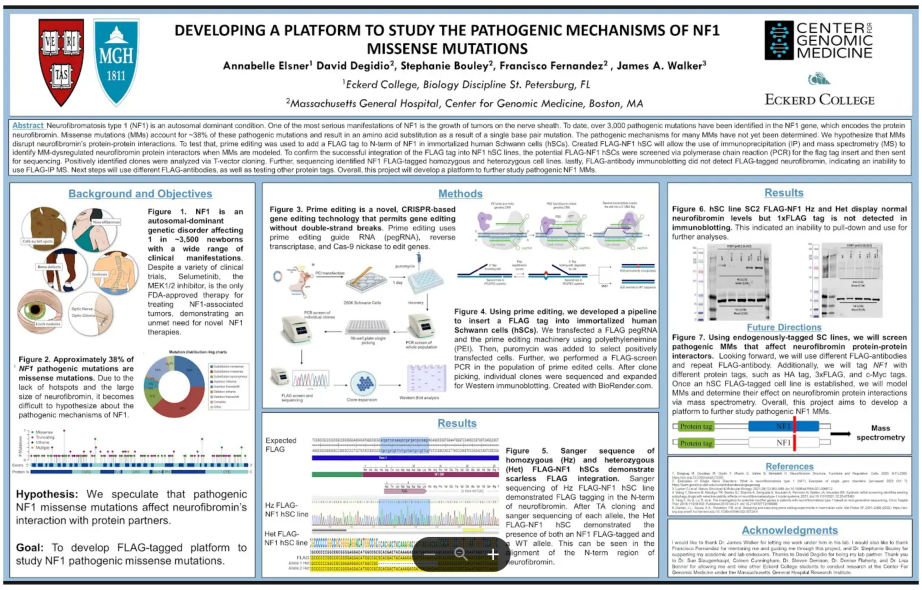

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