Spring 2018

NMD Gene Screen

Abigail Chua
Washington University in St. Louis

Toward a Better Understanding of...

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Abigail Chua

Mentor: Abigael Cheruiyot

Nonsense mediated mRNA decay (NMD) is a surveillance pathway that regulates the number of cellular RNAs and, therefore, gene expression. The pathway recognizes premature termination codons (PTCs) in order to start degradation of RNAs encoding potentially harmful proteins. Considering the overall mechanism of NMD, we are trying to figure out the genes that have affiliation with the pathway. Though there are several genes that have already been identified in inactivation of NMD, there are still many that have not been discovered yet. With this data, we hope to include it in several cancer therapeutic strategies.

We will be using the You Lab's NMD fluorescence (U2OS) reporter cell line to measure the intensity in NMD knockout. A second reporter via a tethering system was tested as well to test genes involved with the spliceosome complex, however, due to several insufficient outcomes in our tethering experiments, we have decided not to use genes associated with the spliceosome complex. The genes that we predict to be associated with NMD will then be quantified using Western Blot analysis. Our main focus will be those genes encoding proteins from the U2:SF3B complex and U2:SF3A complex as fold change values via guide RNAs of these two complexes look promising where fold changes greater than 1.5 are considered significant.