

Lawrence Zhang
*miRNA and Cancer, Phase II: Constructing a Bidirectional Cassette to Identify miRNA
Regulators*

Abnormal miRNA levels can lead to the development of cancers. Consequently, miRNA therapy has been developed to offset the effects of abnormal miRNA levels in cancer cells. However, the current model of this therapy cannot regulate the exact amount of miRNA activity within cells. Thus, the purpose of this project is to construct a cassette that identifies unique gene regulators of certain miRNAs to better regulate miRNA activity.

The cassette is comprised of a bidirectional promoter and two different luciferase genes: eYFP and mCherry. Attached to the 3'UTR of the mCherry are two miRNA let-7 binding sites. This allows let-7 miRNAs to attach to these sites and suppress mCherry expression. So, cells transfected with this cassette should exhibit visibly lower levels of mCherry in comparison to eYFP.

After the cassette was constructed, restriction endonuclease digestion verified the location of the components of the cassette and sequencing confirmed the validity of the let-7 binding sites. Fluorescence microscopy results indicate, as expected, dramatic lower mCherry activity compared to eYFP activity.

This cassette can then be used to identify miRNA regulators. Random mutations will be introduced into cells with this cassette. Any mutations to miRNA regulators will cause abnormal miRNA levels, resulting in varying mCherry activity. Cells with these mutations can then be isolated with fluorescence activated cell sorting. Inverse PCR can then be performed to identify these mutated miRNA regulators. By identifying these regulators, miRNA therapy will be more accurate, serving as a better and safer treatment of cancers.