Evaluating microRNA Binding Using Luciferase Constructs

Matthew Anderson,[1,2] Melanie Winkle, Ph.D.[1] George Calin, M.D., Ph.D.[1]

1. Department of Translational Molecular Pathology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA.

2. Biology Department, Hillsdale College, Hillsdale, MI, USA.

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Background

- Sepsis is a severe and life-threatening condition that results from systemic immune dysregulation
- miR-93-5p is upregulated in early-stage sepsis and is proposed as a potential targeted therapeutic

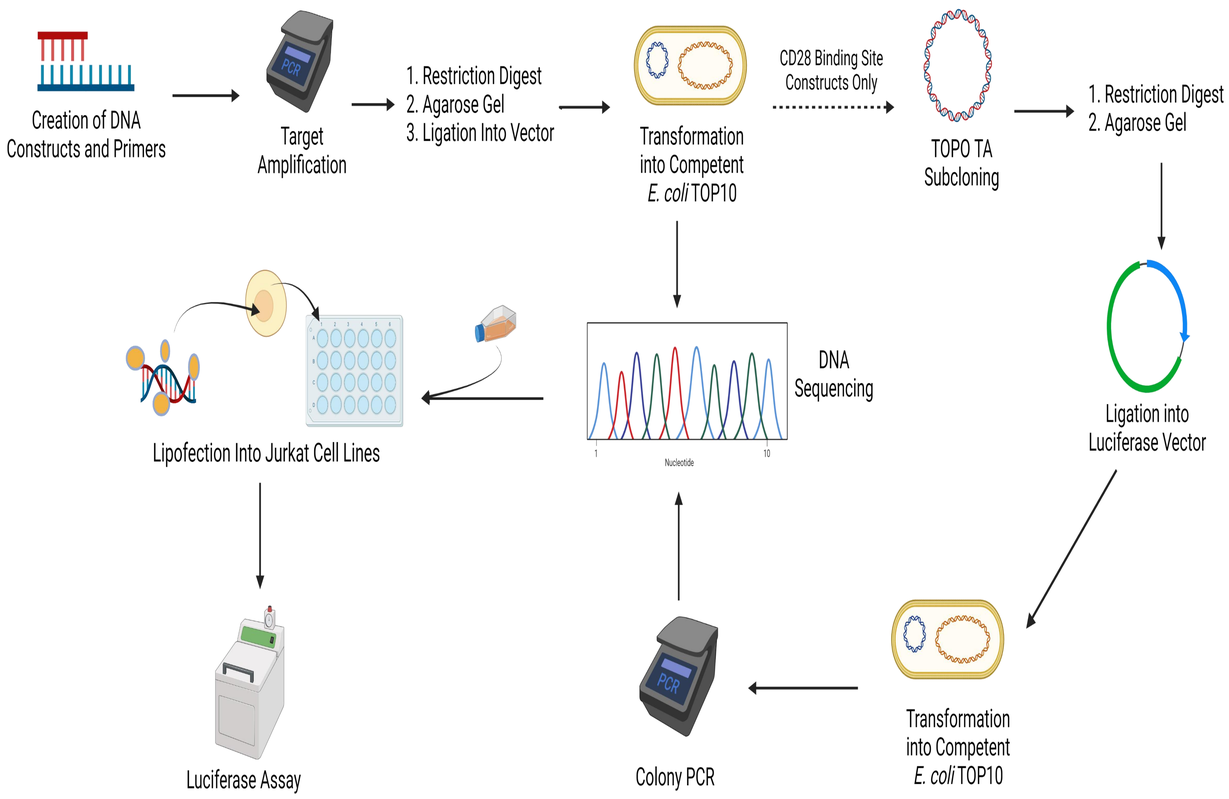
Aims

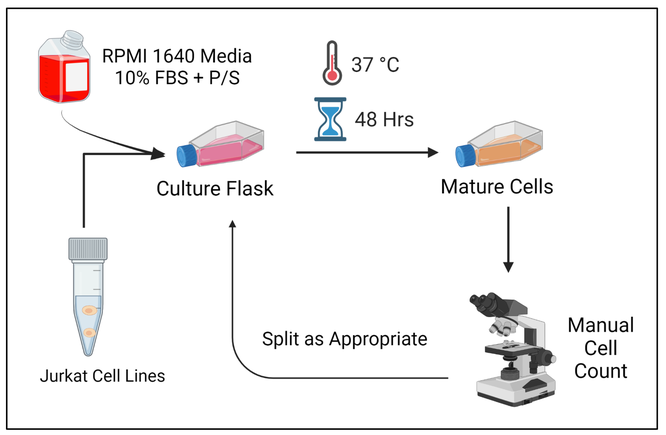
- The luciferase assay can be used to evaluate miRNA binding by comparing florescence levels between miRNA knockouts and controls

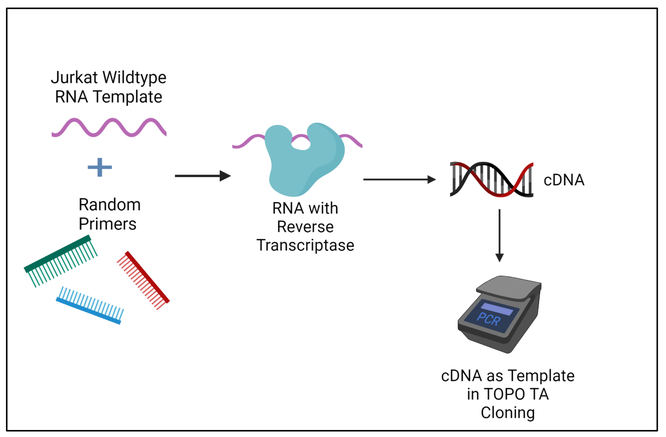
Hypothesis

- We cloned a complementary miR-93-5p sponge construct for use with the luciferase assay in evaluating small molecule inhibitors of miR-93-5p function
- We also cloned luciferase constructs containing predicted miR-93-5p binding sites present on the CD28 gene to evaluate miRNA binding

Methods







Results

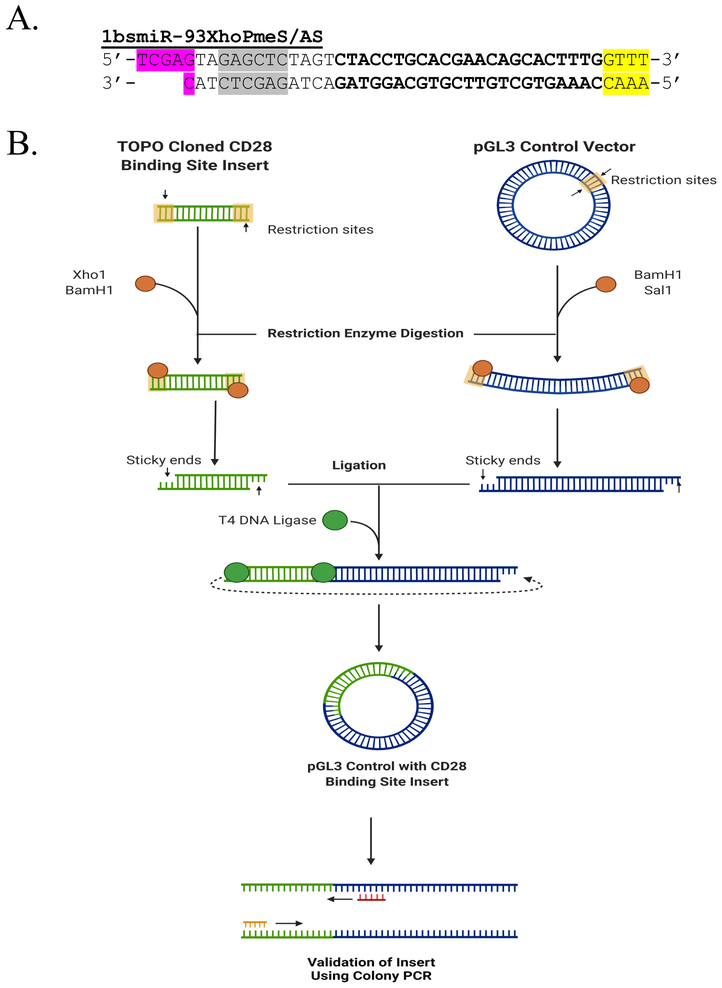


Fig 1. Construct and Subcloning Design
A) Complementary miR-93-5p sponge construct
B) Schematic of the subcloning procedure for CD28 binding site inserts into the luciferase vector

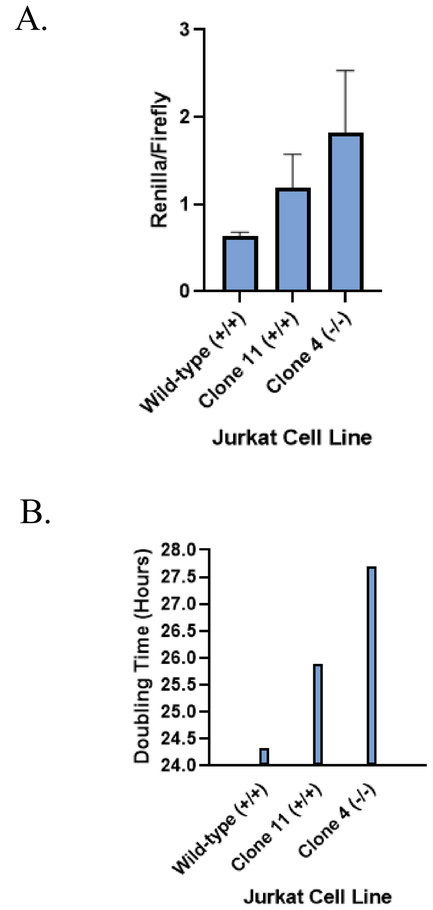


Fig 2. Dual Luciferase Reporter Assay Results Post-Transfection with the miR-93-5p Sponge
A) Renilla/Firefly luciferase values across Jurkat cell lines. (n=3, SEM shown)
B) Average doubling time of Jurkat cells over several passages (n=6)

Conclusions

- Pilot results suggest that the luciferase assay can be used with Jurkat suspension cells, however there are low levels of transfection across all cell lines which require optimization
- As expected, the Jurkat miR-93-5p knockout expressed lower levels of firefly luciferase activity compared to the wild-type and control lines
- Preliminary results suggest that the luciferase assay can be used to evaluate miR-93-5p binding using luciferase constructs, however additional studies are required to confirm and optimize the efficacy

Future Work

- Optimization of the transfection process using electroporation, lipofection, or a combination of the two
- Following colony PCR validation of the presence of the CD28 insert post ligation, the CD28 constructs will be sent for DNA sequencing and eventual transfection into the Jurkat cell lines

Acknowledgements

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References

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2. Kluiver J, Slezak-Prochazka I, Smigielska-Czepiel K, Halsema N, Kroesen BJ, van den Berg A. Generation of miRNA sponge constructs. *Methods*. 2012;58(2):113-117.

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