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1. One need was to confirm that the saRNAs were replicative and to test their expression dynamic over time. Another major need for RNA therapeutics in general was to increase the duration of expression. mRNAs express for about 48 hours before they are completely degraded in cells. This limitation has made RNA therapy largely ineffective for cellular programming or protein replacement. A third need was to characterize the mechanisms by which cells detect and mount an immune response to saRNAs so that these mechanisms can be repressed during saRNA therapy to reduce side effects and maximize expression.
2. The major constraints in this project were limited time, the use of highly novel workflows, working through failures, and engagement with biological systems not yet fully characterized.
3. One engineering standard that was critical to our project was to perform a cellulose chromatography protocol on our RNA after in vitro transcription which removes contaminant dsRNA. dsRNA is highly immunogenic and thus reduces the effectiveness of delivered RNA and increases the severity of side effects. Another important engineering standard we employed was to perform whole-plasmid sequencing on all our plasmids prior to running in vitro transcription to ensure sequence fidelity.
4. RACER RNAs, which self-amplify and circularize, may be highly relevant in a multitude of applications. One such application is in vivo CAR-T therapy in which these RNAs would be used to transiently express CARs in T cells in vivo to combat cancer. Another potentially important application would be using RACERs to express proteins that increase metabolism in adipose tissue, thus burning fat and treating obesity.
5. In our design group, we effectively delineated tasks to one another and each worked on our own side projects. This allowed us to be highly efficient and to explore more ideas simultaneously. Another effective measure was weekly meetings with our mentor, which allowed us to consolidate our results and figure out the best path forward.
6. My biggest motivation was the prospect of creating something that is both novel and useful. I think RNA therapeutics, in general, have enormous potential, but I know that currently they are limited. So I was excited each day to try and overcome these limitations with creativity. To overcome challenges, I simply tried to stay present, focus on the best path forward, and I tried to stay neutral whether results were good or bad.
7. The most innovative idea in this project is the use of these inducible cleavage elements which allow for circularization of the subgenomic RNAs. Another innovative idea was to include the spacer between the IRES and the subgenomic promoter which rescued the replicative ability of our system.