Team 24: Development of Novel Circularizable Self-Amplifying RNAs for Prolonged and Potent Therapeutic Gene Expression

UC San Diego
JACOBS SCHOOL OF ENGINEERING
Bioengineering

Finn McRae, Dyllan Mead, Simon Joseph
Mentors: Michael Tong and Prashant Mali

Background and Motivation

- saRNA and circRNA are two existing technologies designed to tackle the rapid degradation and high dosage requirements of linear mRNA[1].
- saRNAs require less dosage as they code for a RNA Dependent RNA Polymerase (RDRP) that replicates both the gene of interest and entire transcript. Their drawback is that the viral RNAs from which saRNAs are derived are highly immunogenic[1].
- Endogenous systems have far fewer enzymatic tools to degrade circRNA, so their longevity is significantly improved over mRNA. Their drawback is that translation of circRNA is internal ribosome entry site (IRES) dependent instead of CAP dependent and is therefore much lower per circRNA than in traditional mRNA[1].
- **We intend to engineer a system that both circularizes and amplifies to capture the strengths of both saRNA and circRNA while mitigating their weaknesses.**
- If designed properly, our system will be able to express protein at high levels for long periods with minimal transcripts delivered to host systems.

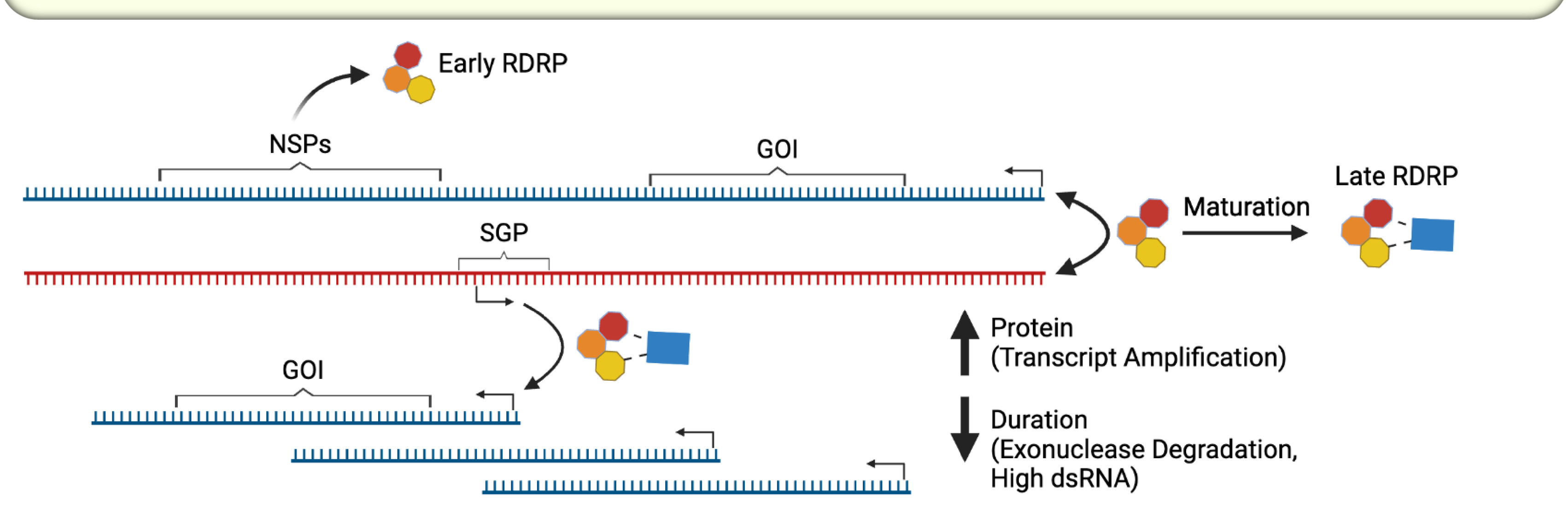


Figure 1. saRNA Mechanism. In saRNA, early NSP complexes including the RDRP genomically copy the "+" strand into the "-" strand. Once the RDRP matures, it recognizes the sub-genomic promoter (SGP) on the "-" strand and copies the GOI downstream of it on the "+" strand[2].

Design Overview

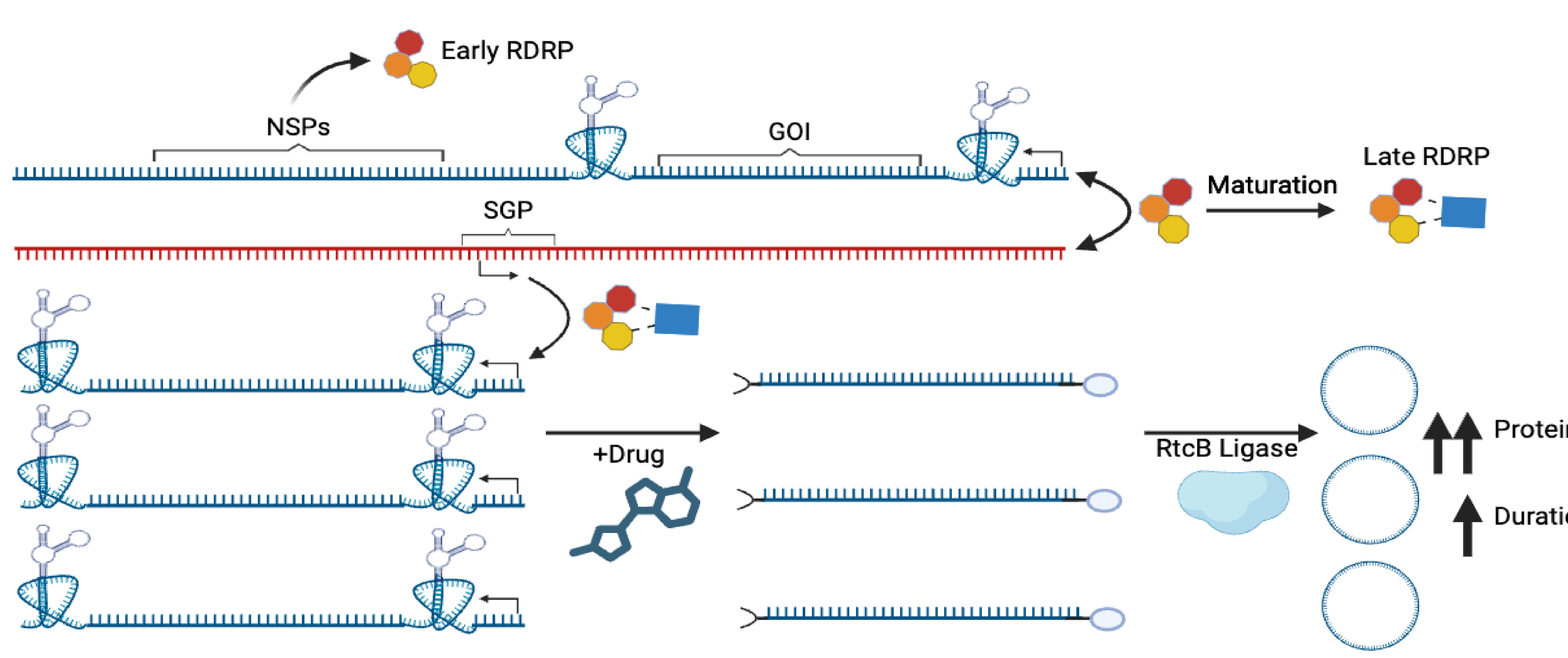


Figure 2. General RACER Mechanism. RACER uses inducible circularizable elements flanking the GOI and downstream of the SGP to couple amplification and circularization. This tunable system allows for circularization at the peak of self-amplifying RNA expression.

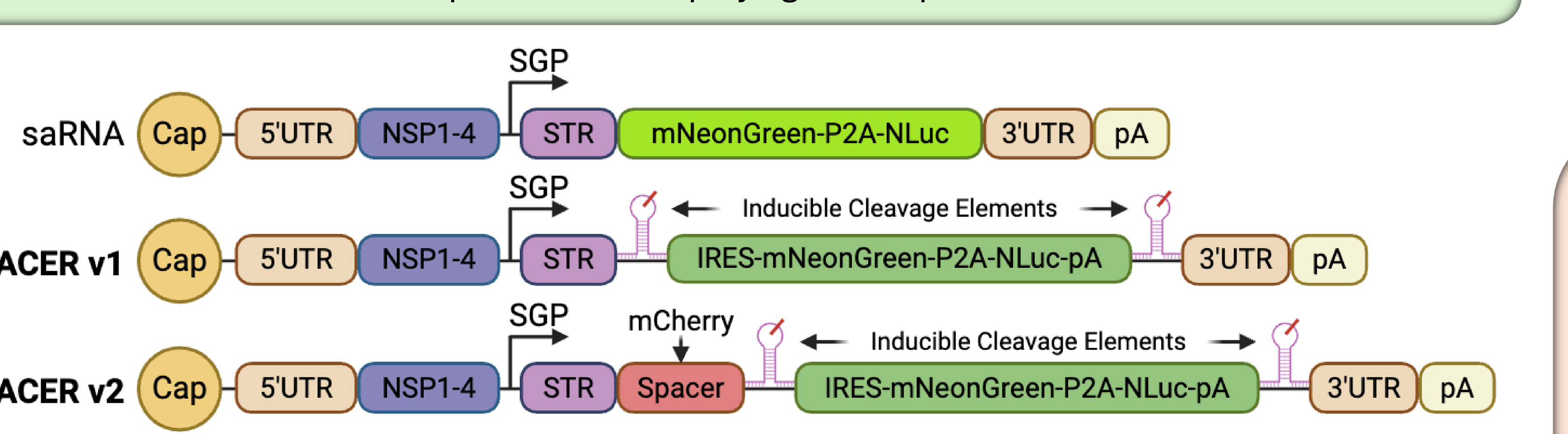
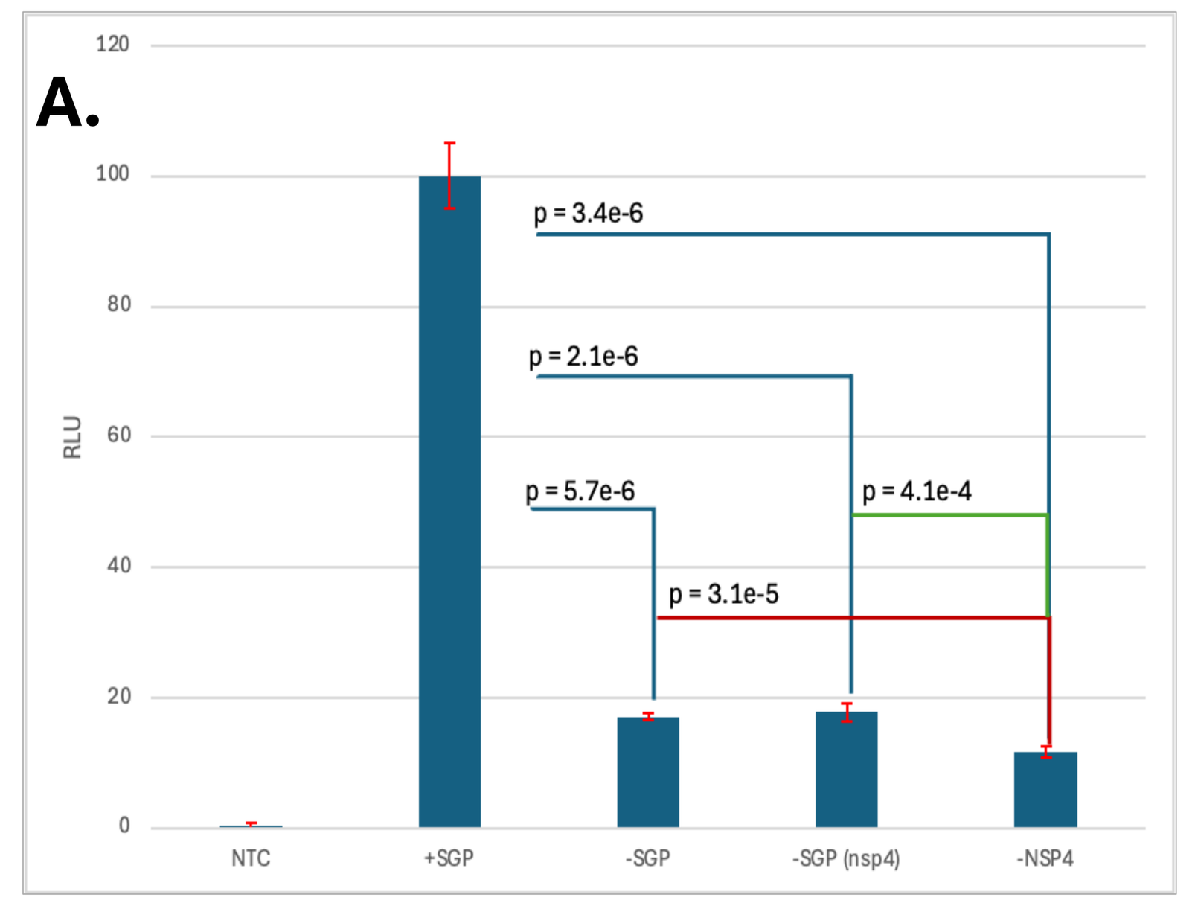
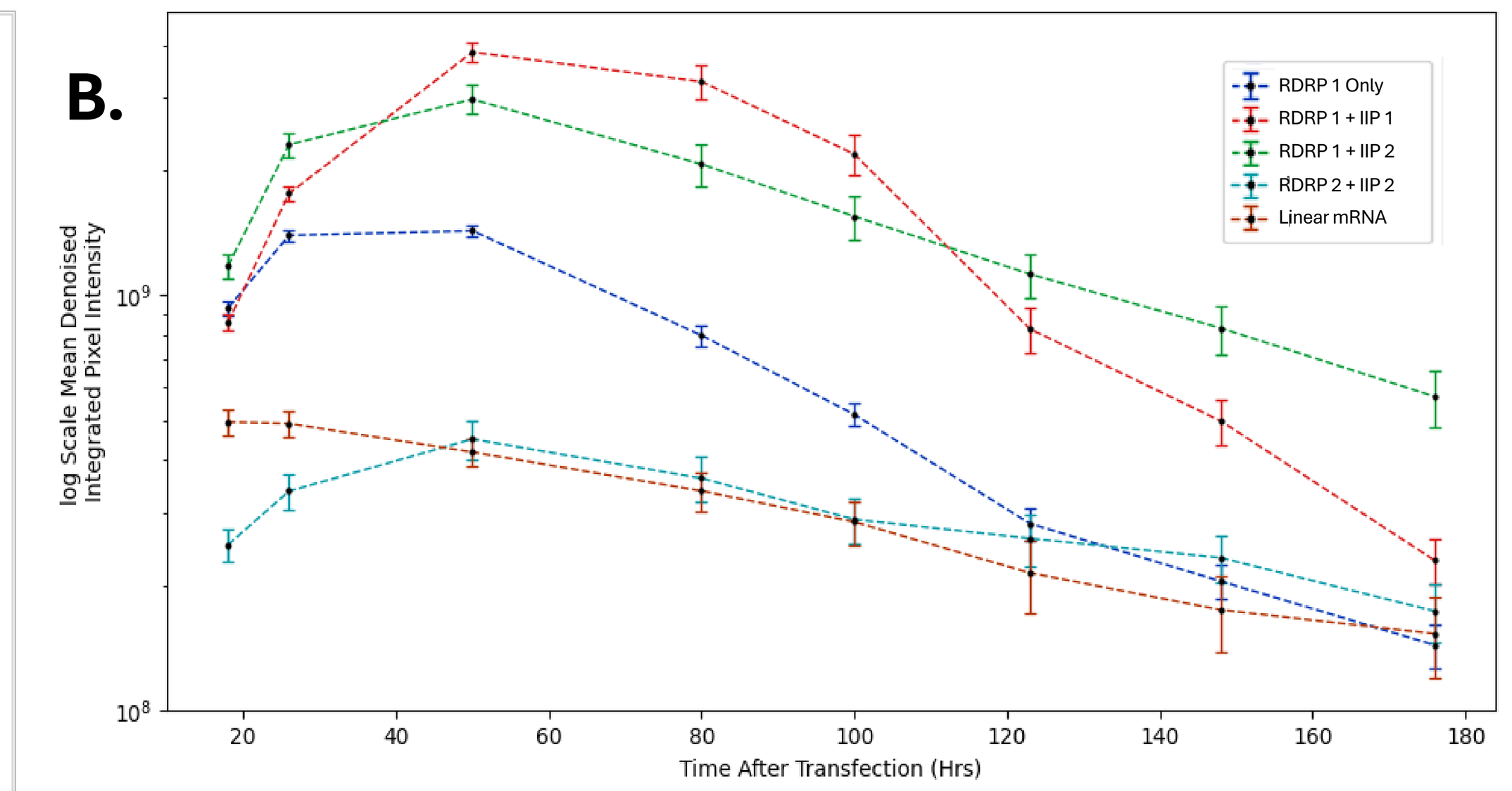


Figure 3. RACER Design Iterations. RACER version 1 did not amplify, so a second iteration was developed to demonstrate circularization and amplification in a combined system.

Results





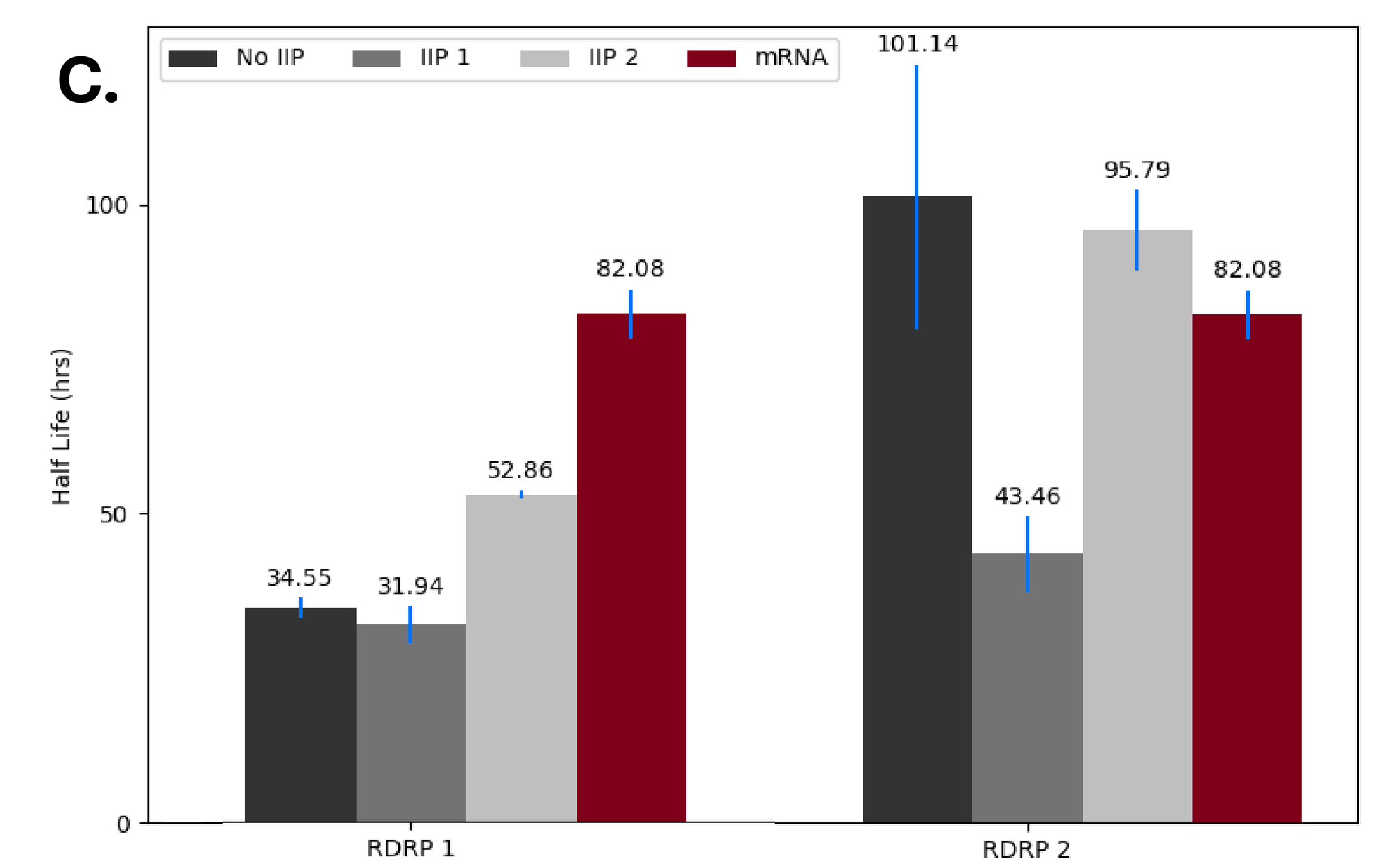
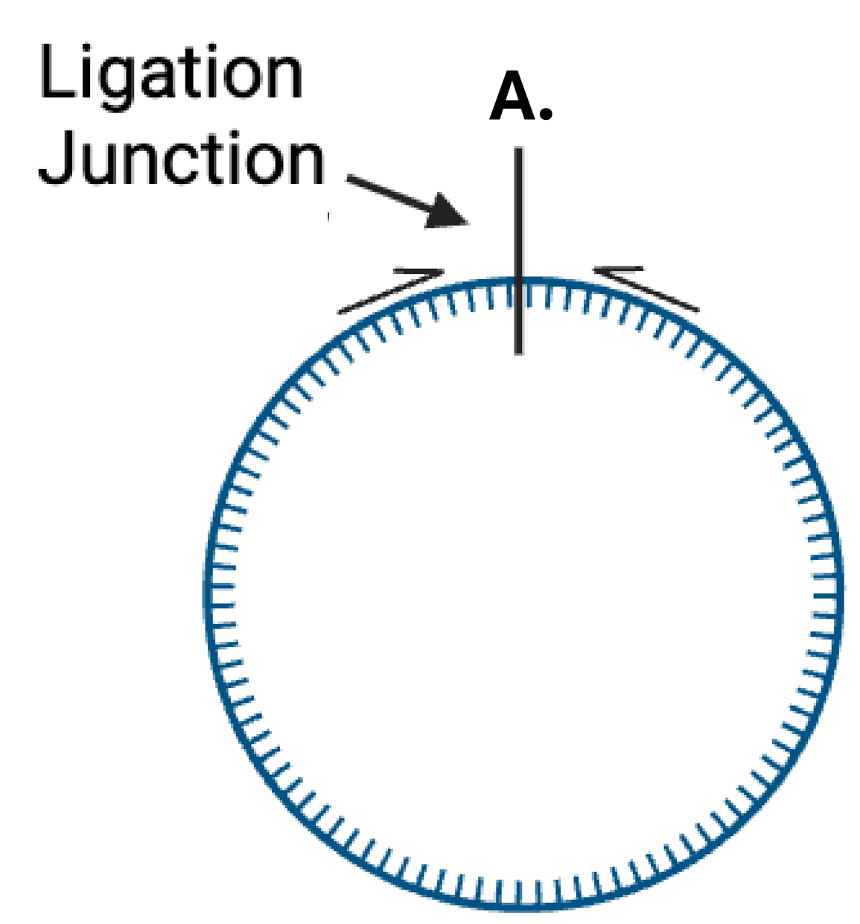


Figure 4. saRNA Structural Elements and Kinetics. **A.** Via qPCR we confirmed that NSP4 and the SGP were necessary for amplification. **B., C.** We identified one RDRP with highly elevated amplification. Further, we found an immune inhibiting peptide that elevates expression further and optimizes pure saRNA half-life. We also confirmed that peak expression is inversely proportional to half-life.



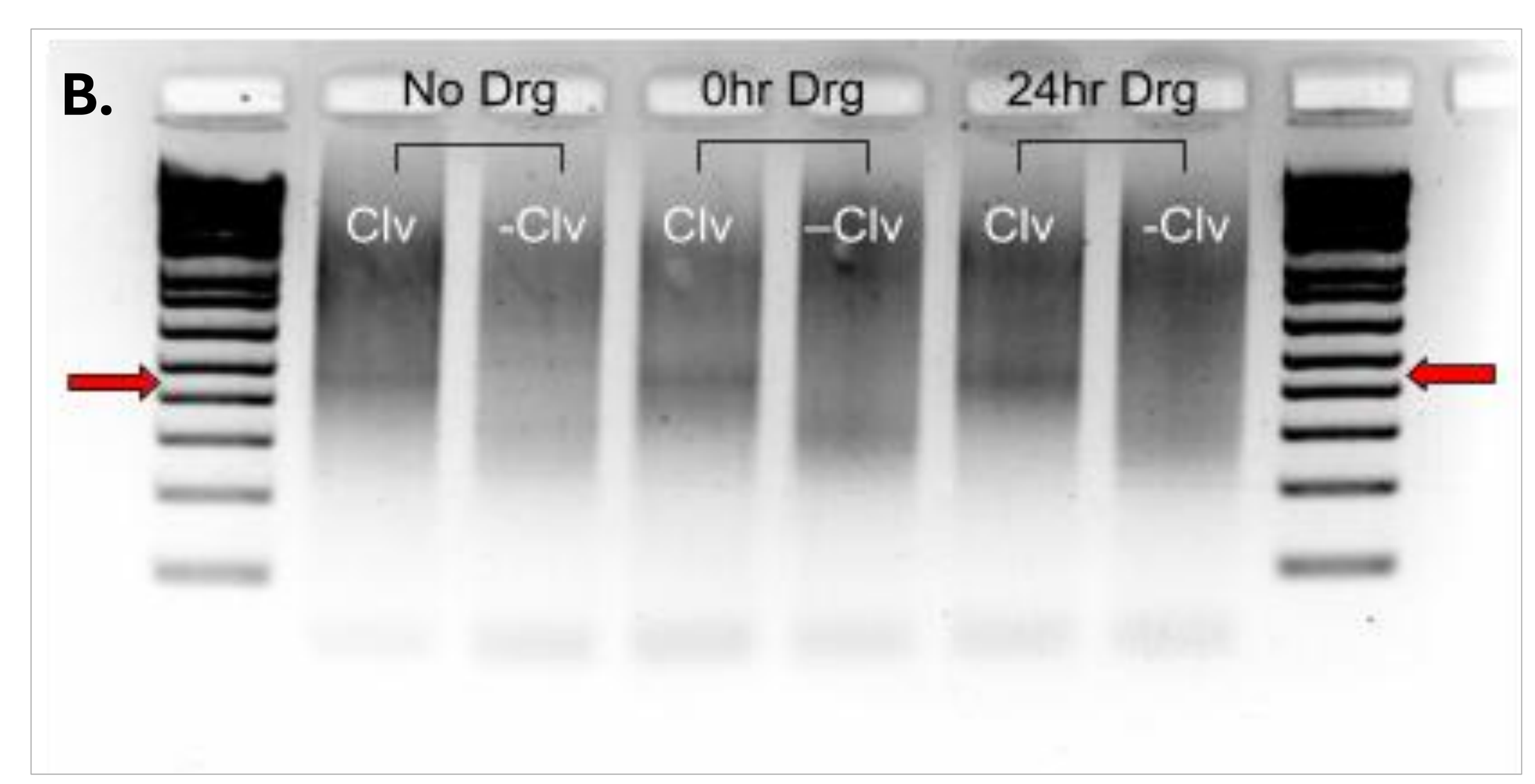
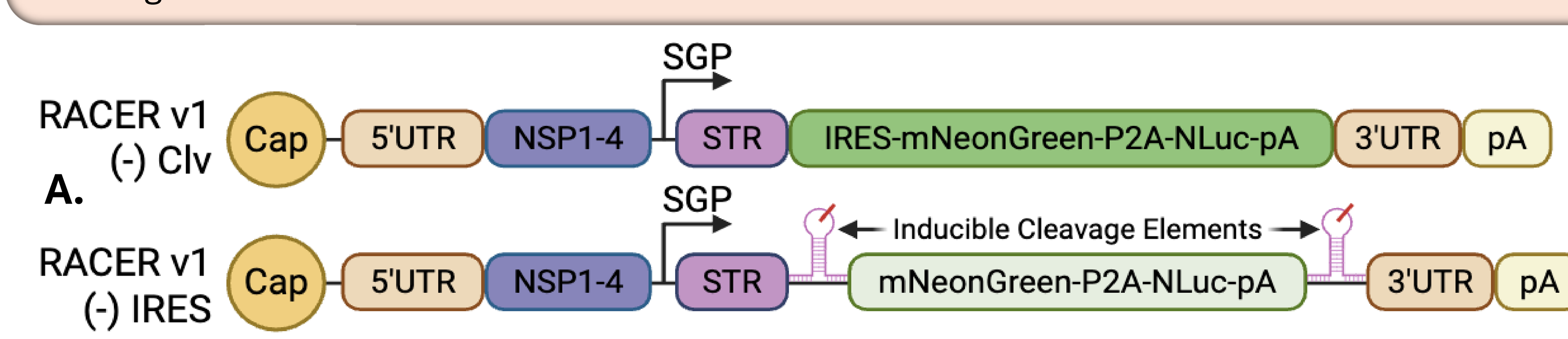
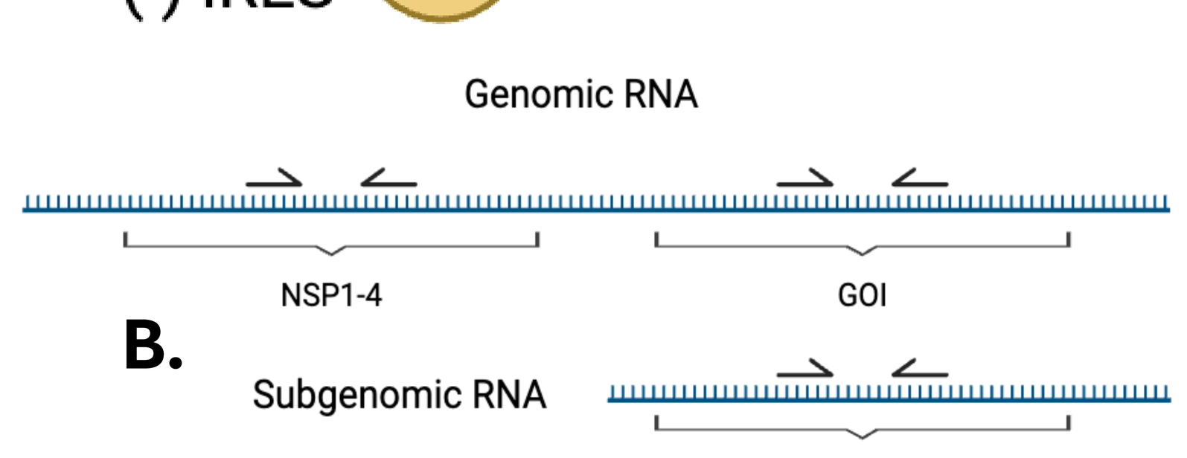


Figure 5. Circularization of RACER RNA validation via cDNA PCR. **A., B.** RNA was taken from transfected cells, reverse transcribed, then amplified with primers that result in amplification across the ligation Junction. The 450 bp band on the gel was expected in the conditions with the cleavage element and not in the conditions without the element.





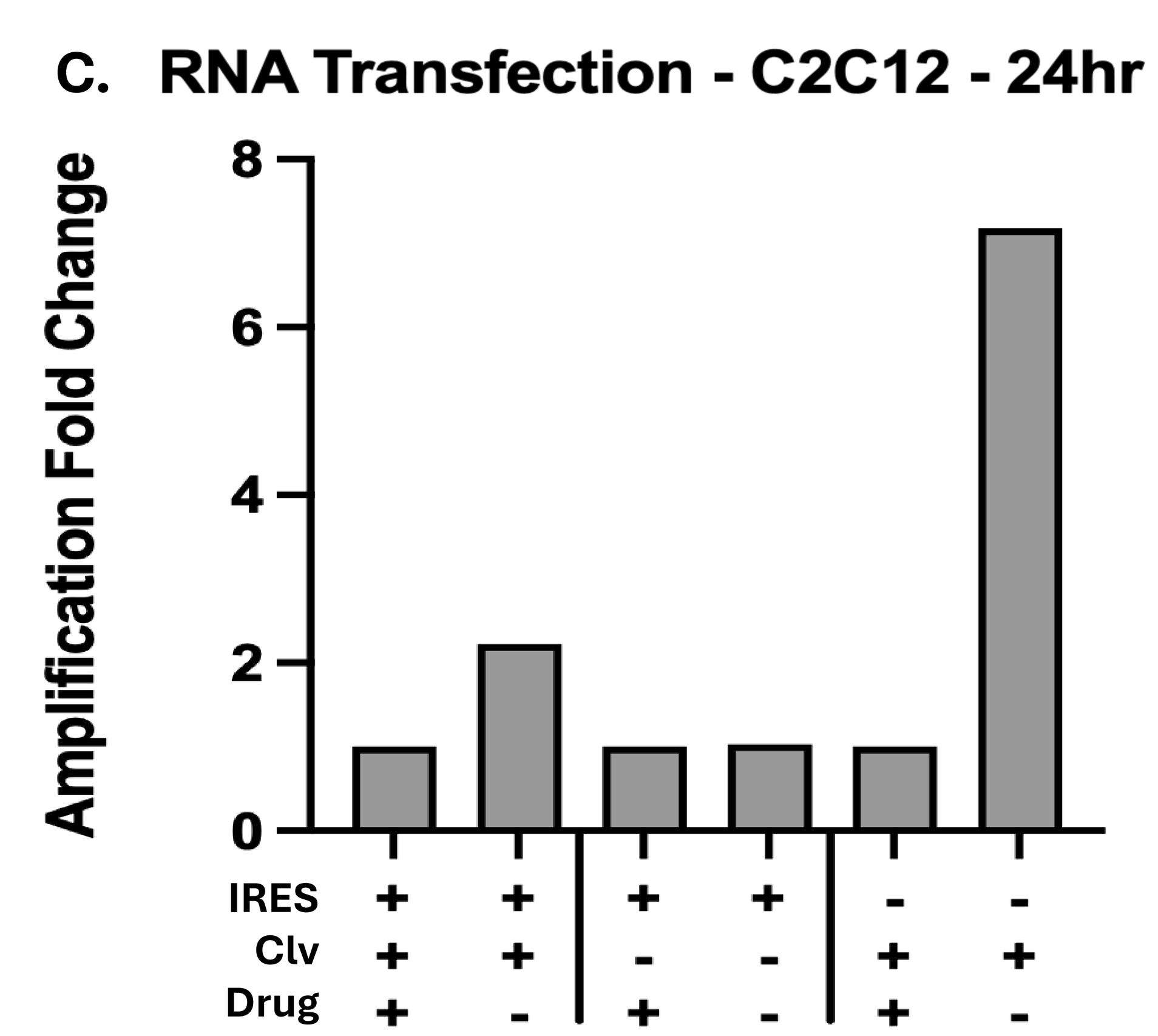


Figure 6. RACER qPCR Analysis. **A., B., C.** we performed qPCR analysis on the amplification of RACER V1 was performed. We observed that only the –IRES trial successfully amplified. This led to the hypothesis that the IRES interfered with SGP binding by NSP-4. An mCherry spacer was thus added in RACER v2 to prevent secondary structure conflict.

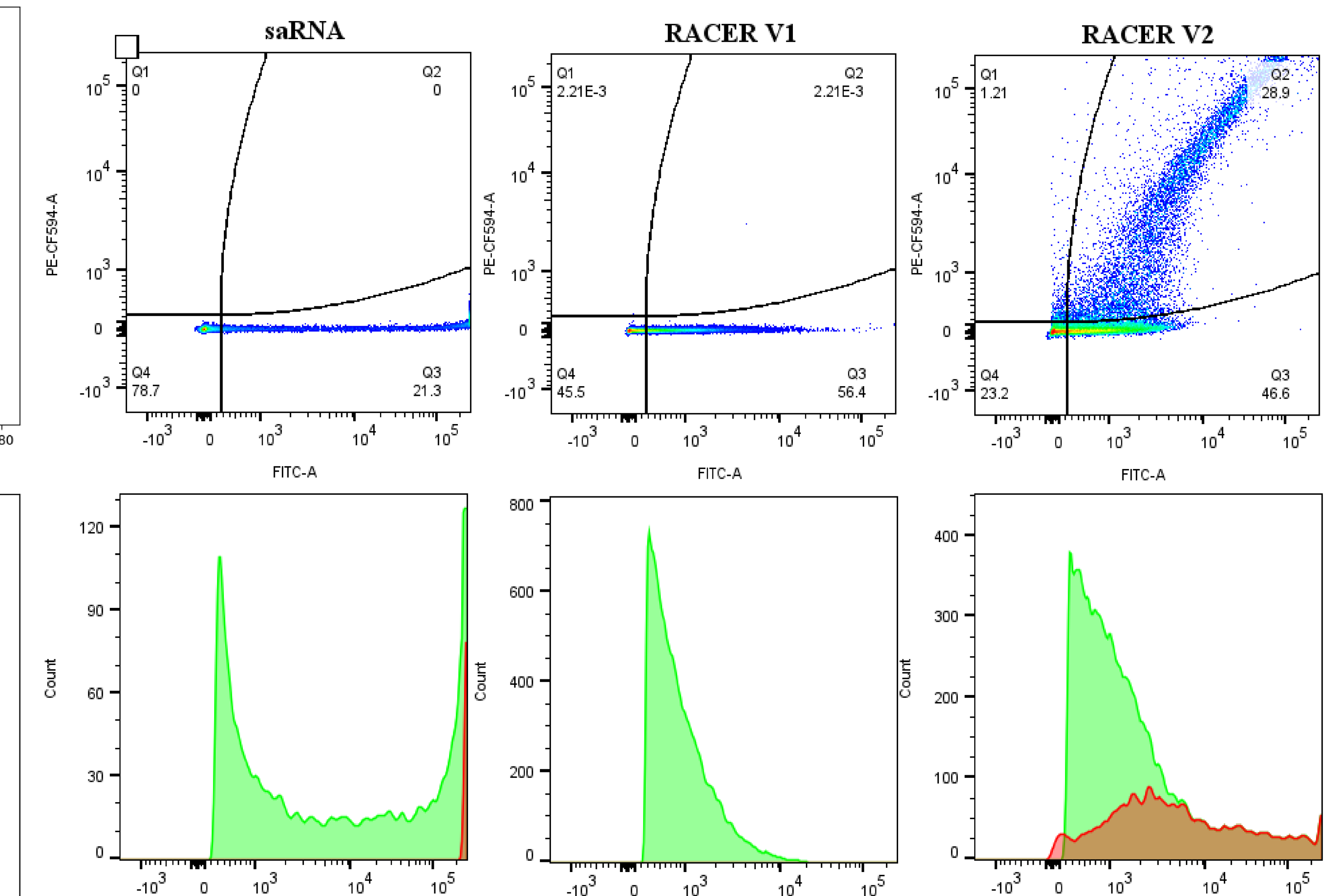
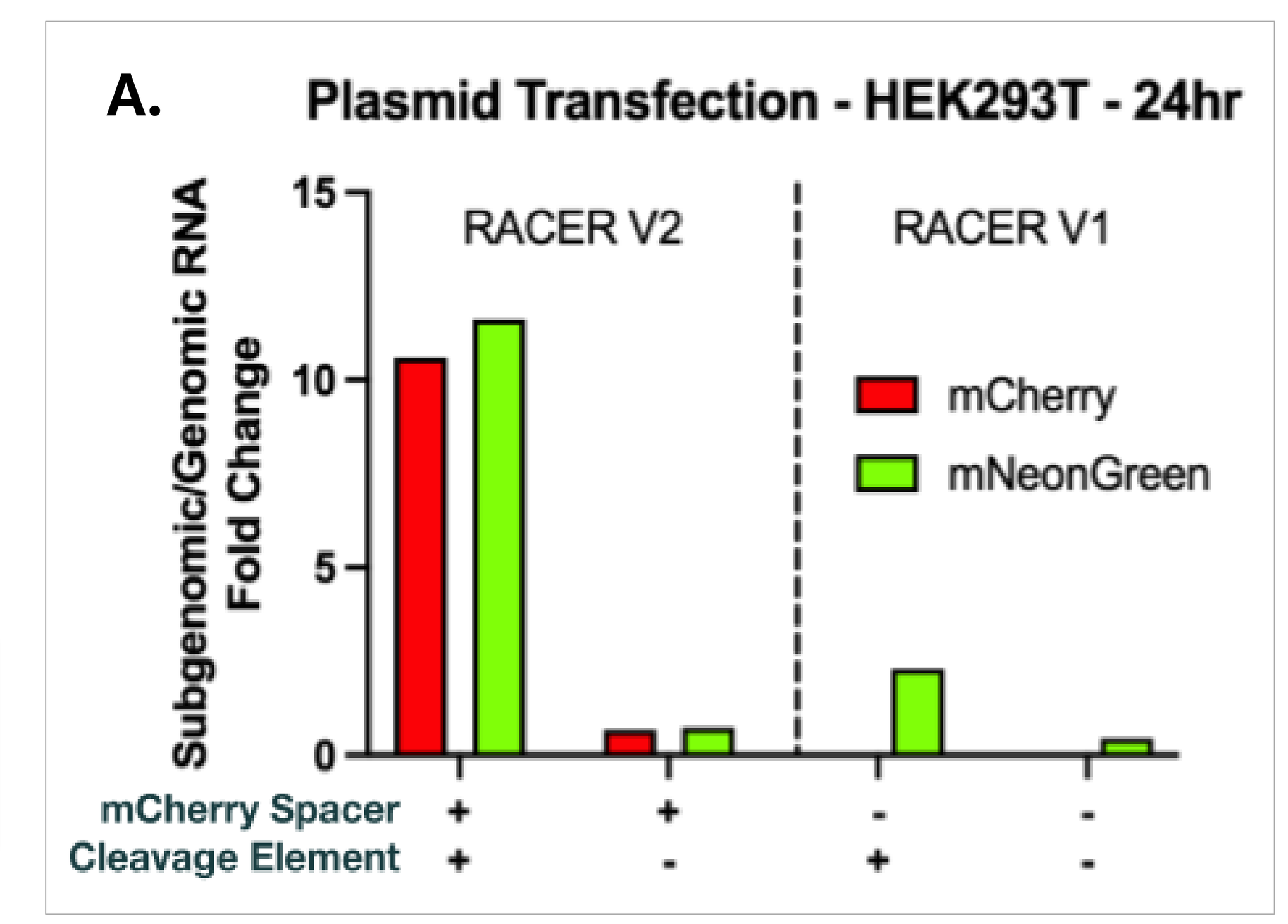


Figure 7. FACS Analysis of Different RACER Versions. FACS was performed on RACER V1 and V2 as well as our basic saRNA construct to further analyze their expression profiles. RACER V2 combines the characteristics of saRNA and RACER V1: showing that it successfully amplifies and circularizes.



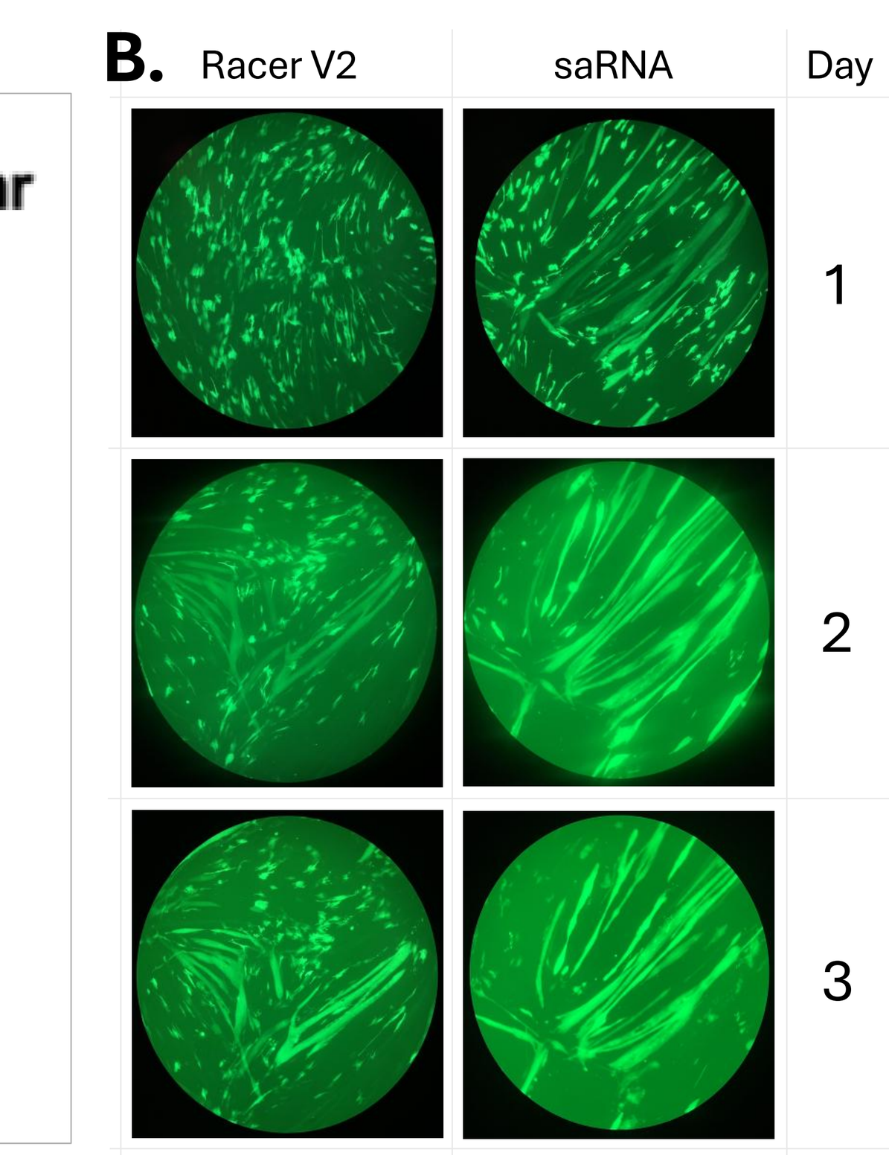


Figure 8. RACER V2 Comparisons. **A.** RACER V2 Demonstrates amplification. **B.** Further, it gets brighter on Day 3: demonstrating circular RNA characteristics.

Conclusions and Future Directions

- **saRNA and circRNA can be successfully combined into a system that expresses protein with the characteristics of both next-gen RNA technologies.**
- Longevity analyses still need to be performed on RACER V2 in C2C12 cells.
- A secreted nano-Luc RACER V2 RNA will be delivered via LNPs in mice to ascertain its behavior in vivo.
- Further immune system RNA-seq and CRISPR KO experiments will be performed to better characterize in-vitro immune responses to saRNA and related proteins.

References

Acknowledgements

bioRender
NIH
THE MALI LAB