Translation Regulation with Artificial Introns

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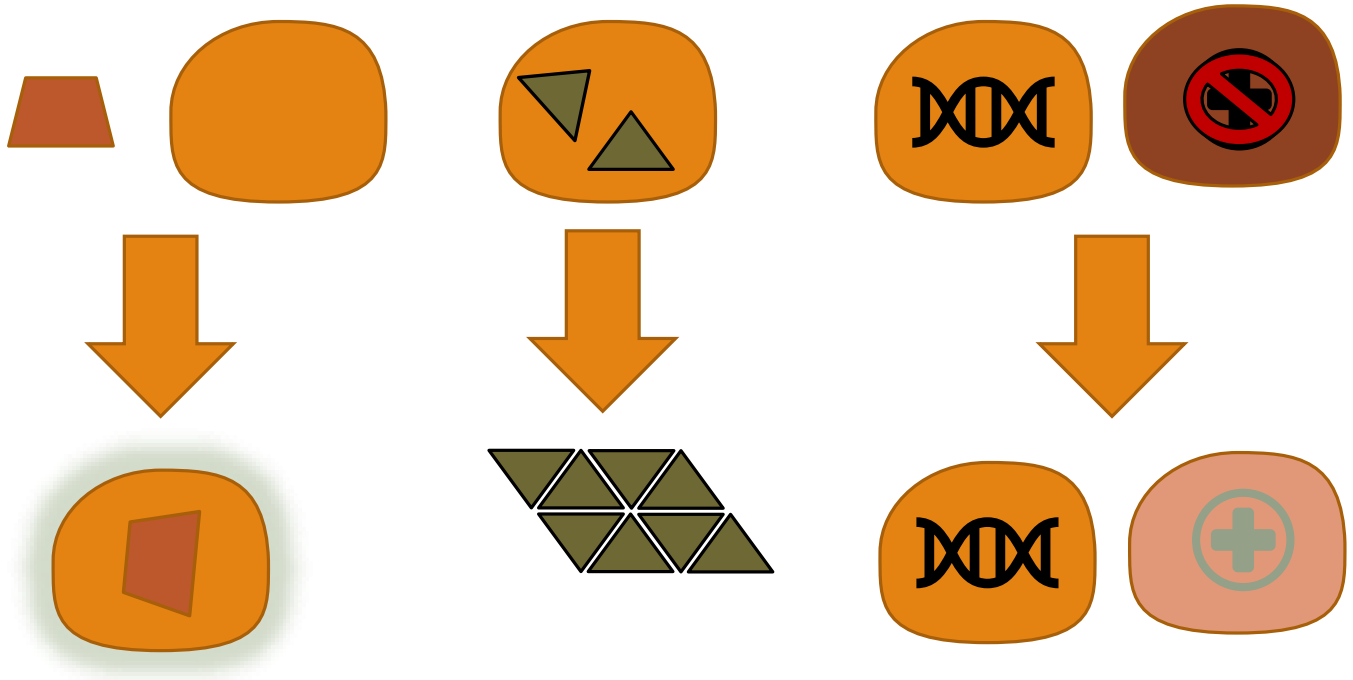
What is Synthetic Biology?

Design and control organisms

- Biosensors
- Compound and material production
- Therapies

Cellular Factories

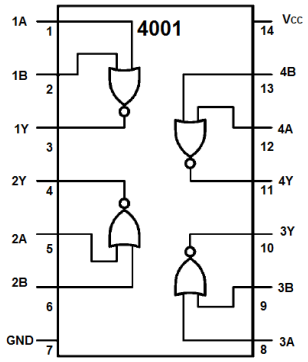


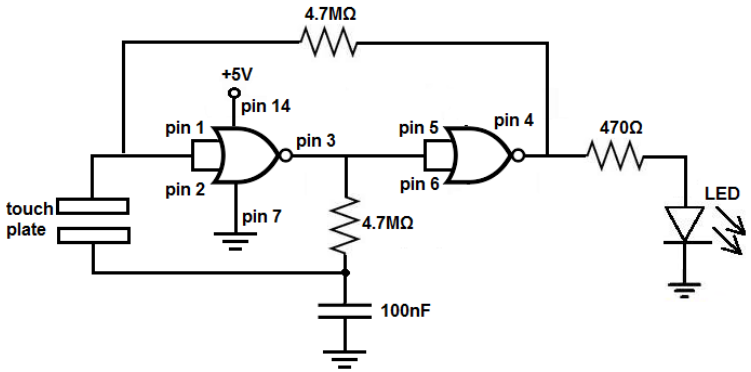
Synthetic Biology & Electrical Engineering

Integrated circuits are essential for the development of modern computing

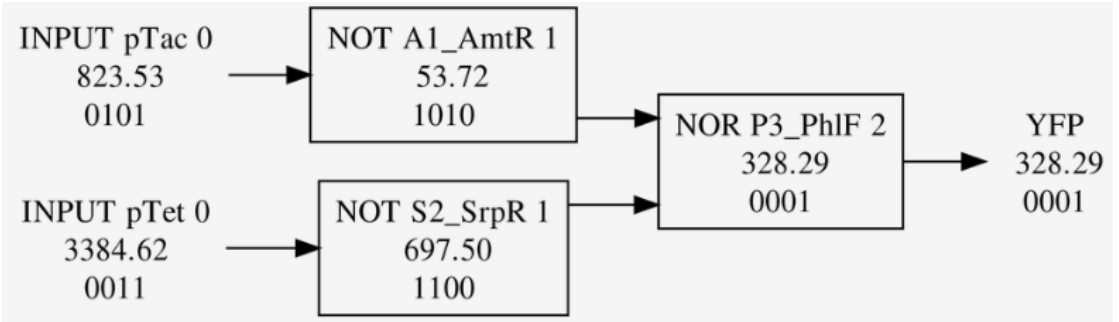
Combination of genetic components to form complex gene circuits provide new solutions

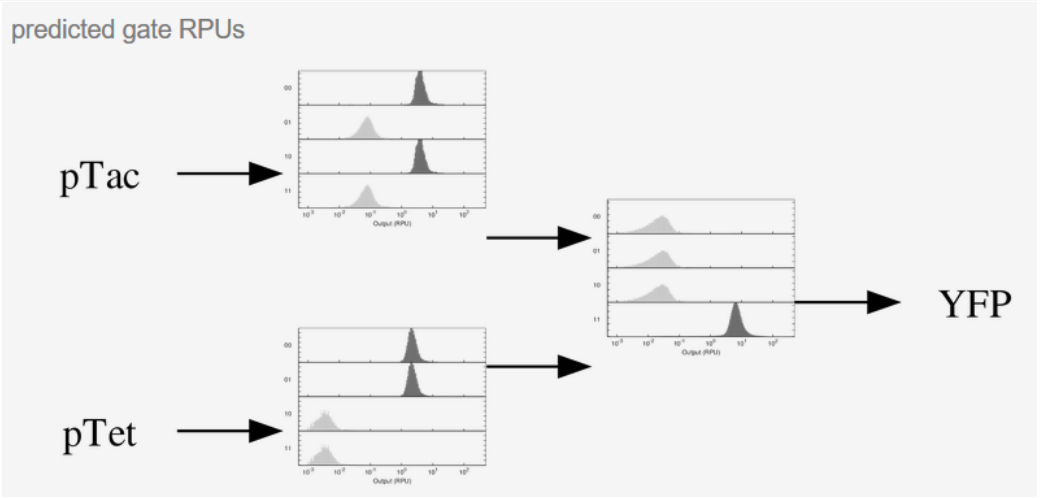
Program biological systems to make decisions based on their environment and stimulus





(*How to Build a Touch On-Off Circuit with a 4001 NOR Gate Chip*)





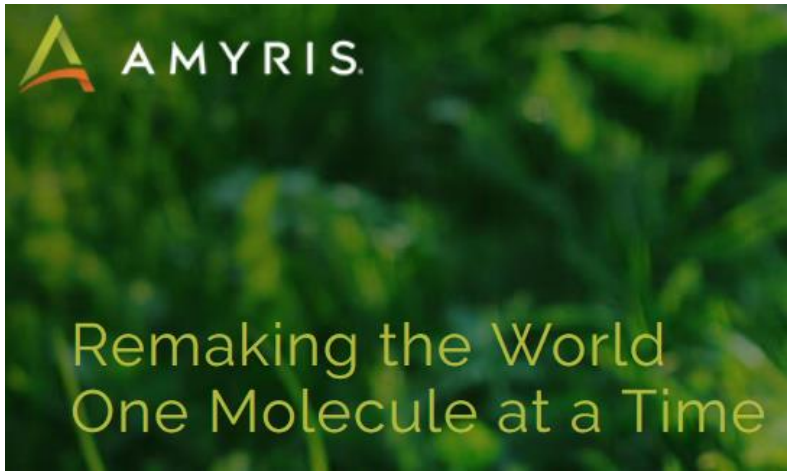
(*Cello*)

Synthetic Biology Advantages

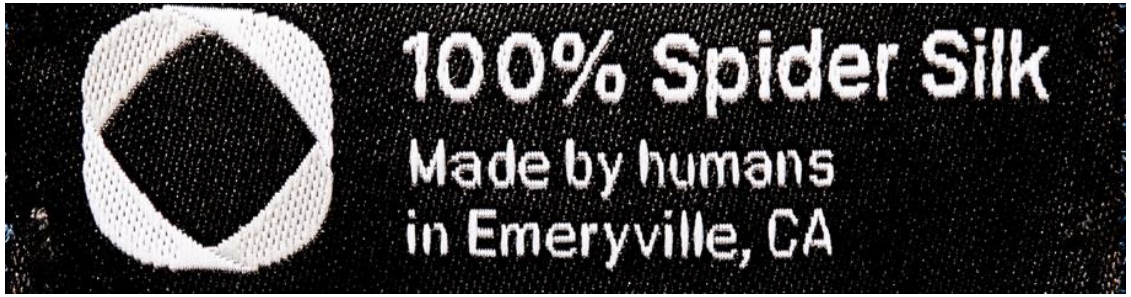
Ability to utilize chemistry and create compounds

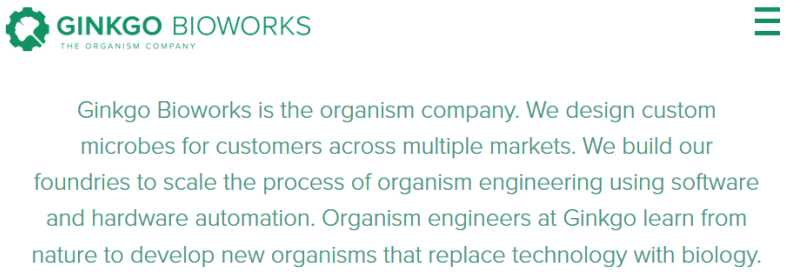
Directly interact with chemical stimuli

Ability to multiply and allow for scaling



Bolt Threads is harnessing proteins found in nature to create fibers and fabrics with both practical and revolutionary uses.





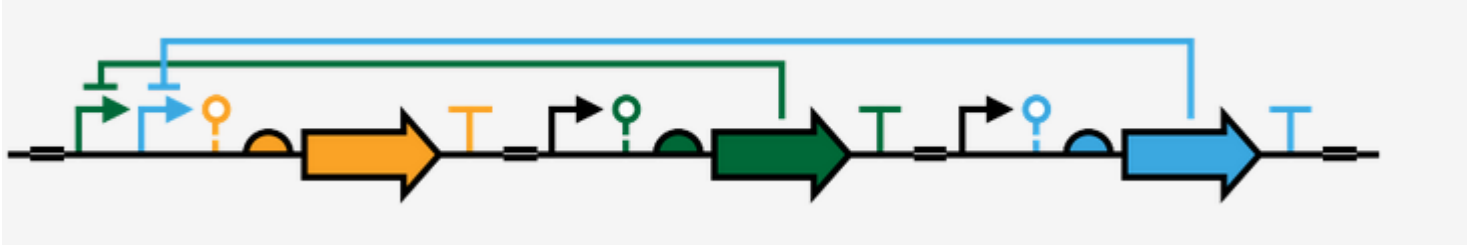
(*Amyris*) (*Bolt Threads*) (“The Organism Company”) (“Senti Biosciences”)

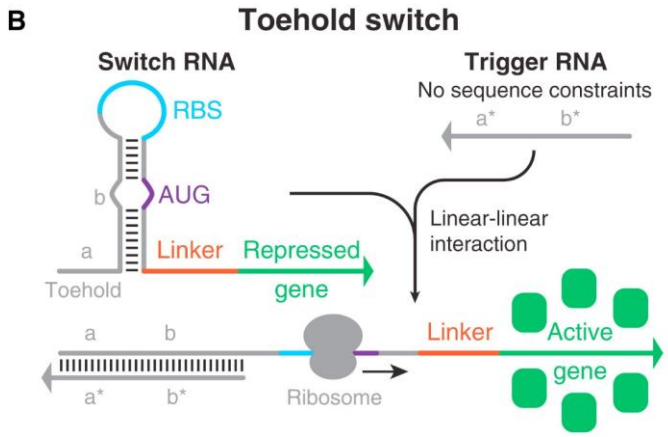
Project Inspiration: Gene Regulation Parts

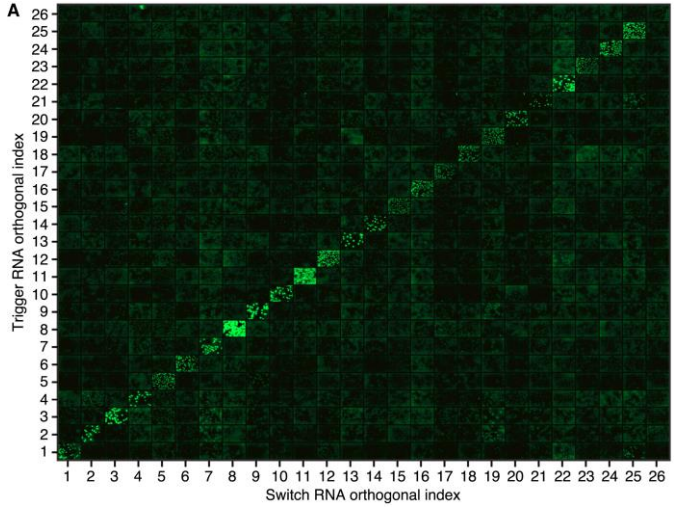
Gene regulation parts are essential for creating genetic circuits

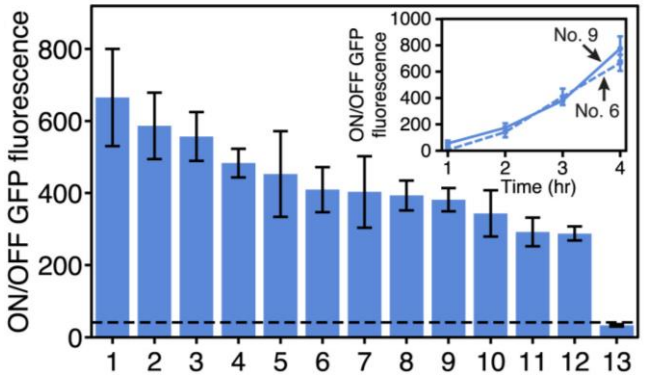
- Modular
- High Specificity
- High Dynamic Range

Toehold switch









(Green et al., 2014) (*Cello*)

Artificial Intron Demonstrates Directional Specific Splicing

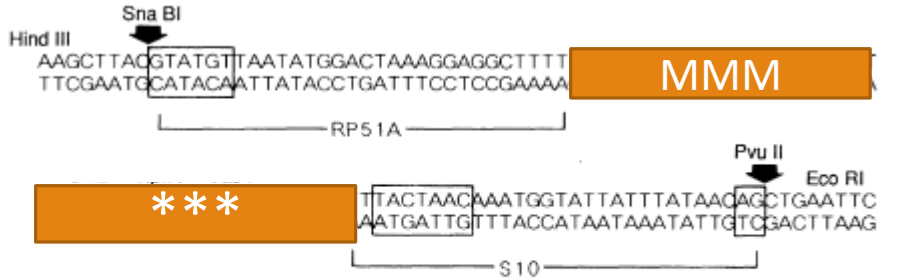
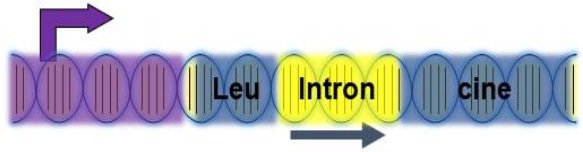
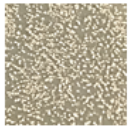
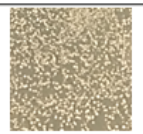
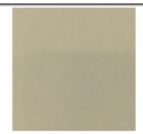


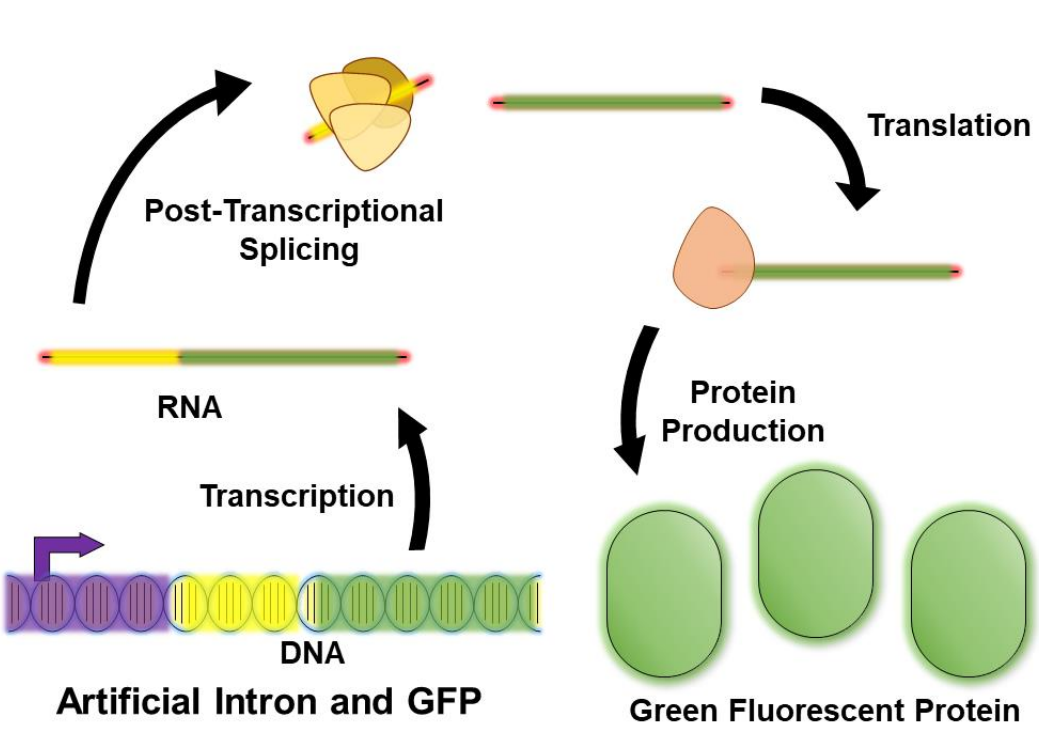
Fig. 1. Structure of the artificial intron. The 5′ end fragment and the 3′ end fragment were chemically synthesized, and the polylinker fragment (from Pst I to Sac I) was obtained from plasmid pUC18. The consensus sequences are shown in boxes. This intron was cloned in the Hind III–Eco RI site of pUC18 to generate pUC-AI. The 5′ end fragment of the yeast *RP51A* intron and the 3′ end fragment of the *S10* intron are indicated.

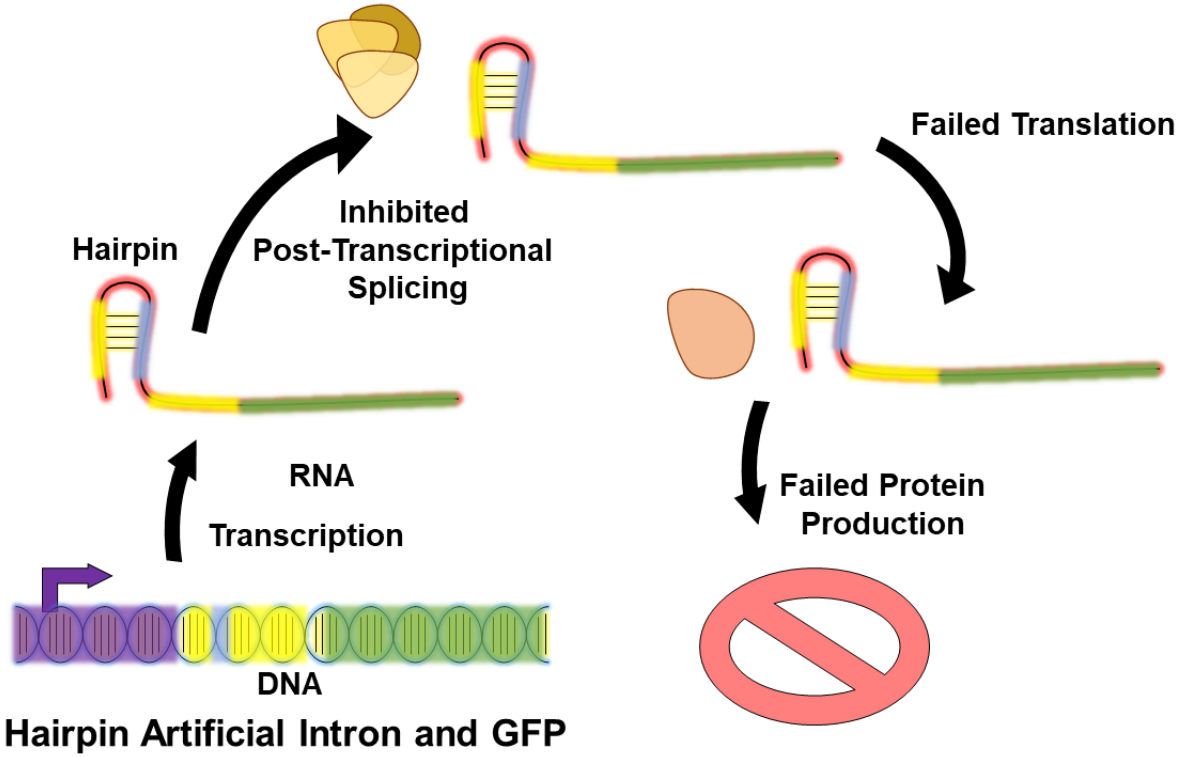
(Yoshimatsu and Nagawa, 1989)



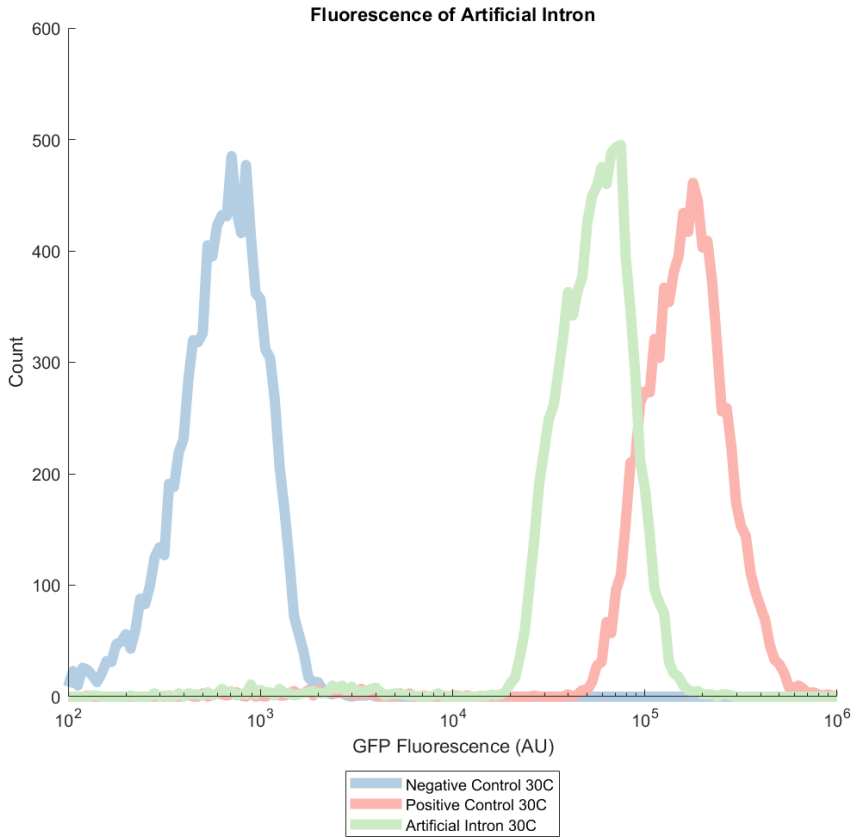
 **Intron 1 (Forward)** (Leucine Production = Successful Splicing)	 **Intron 2 (Backward)** (No Leucine Production = Unsuccessful Splicing)
 **Control +Leu2** (Leucine Production= Survival)	 **Control –Leu2** (No Leucine Production = No Survival)

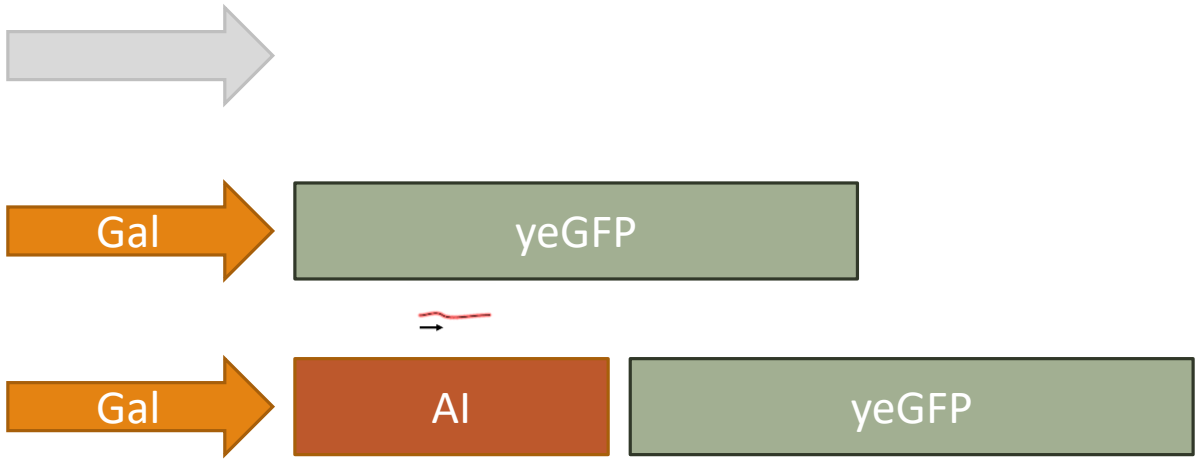
Hairpins Designed to Inhibit Splice Site Recognition





Intron Splicing Inhibition with Hairpins





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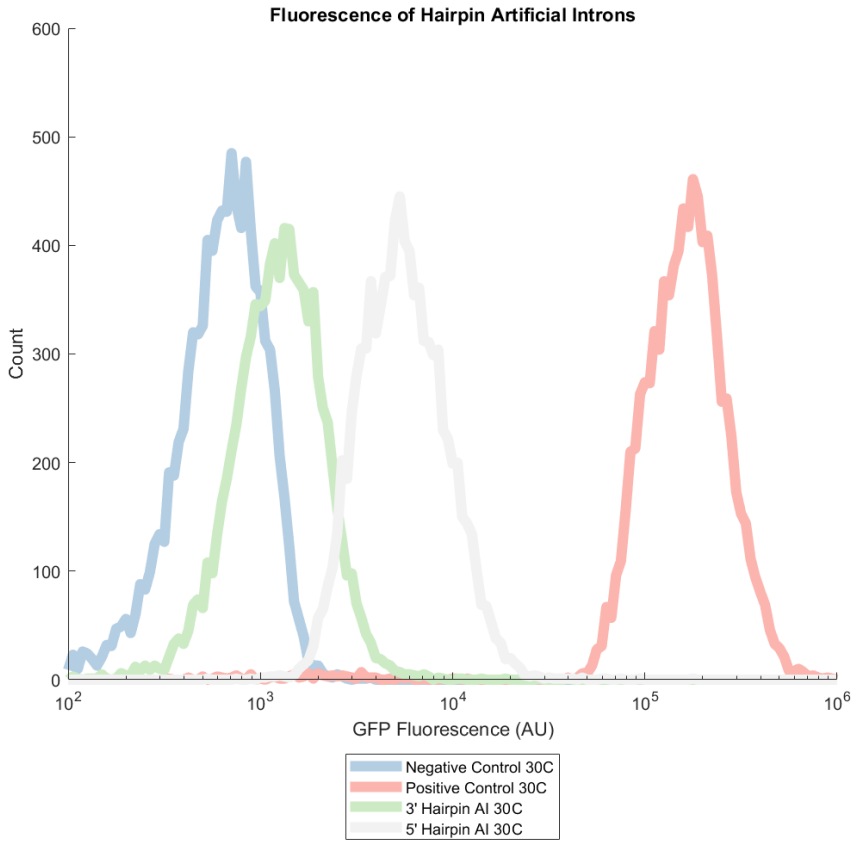
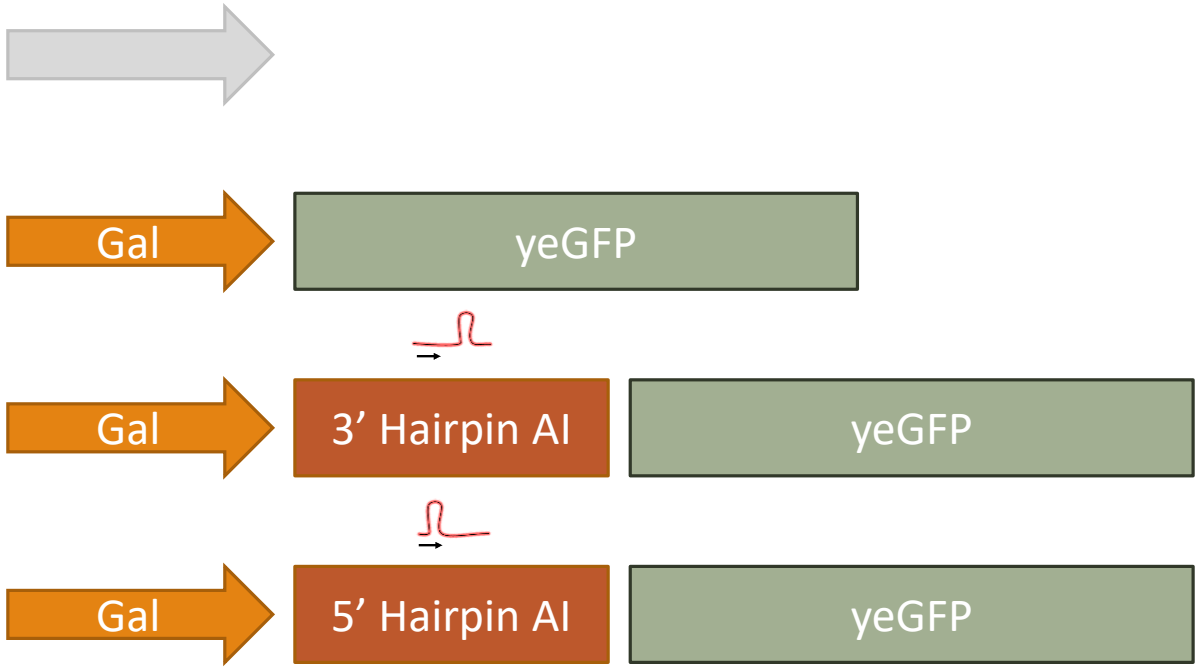
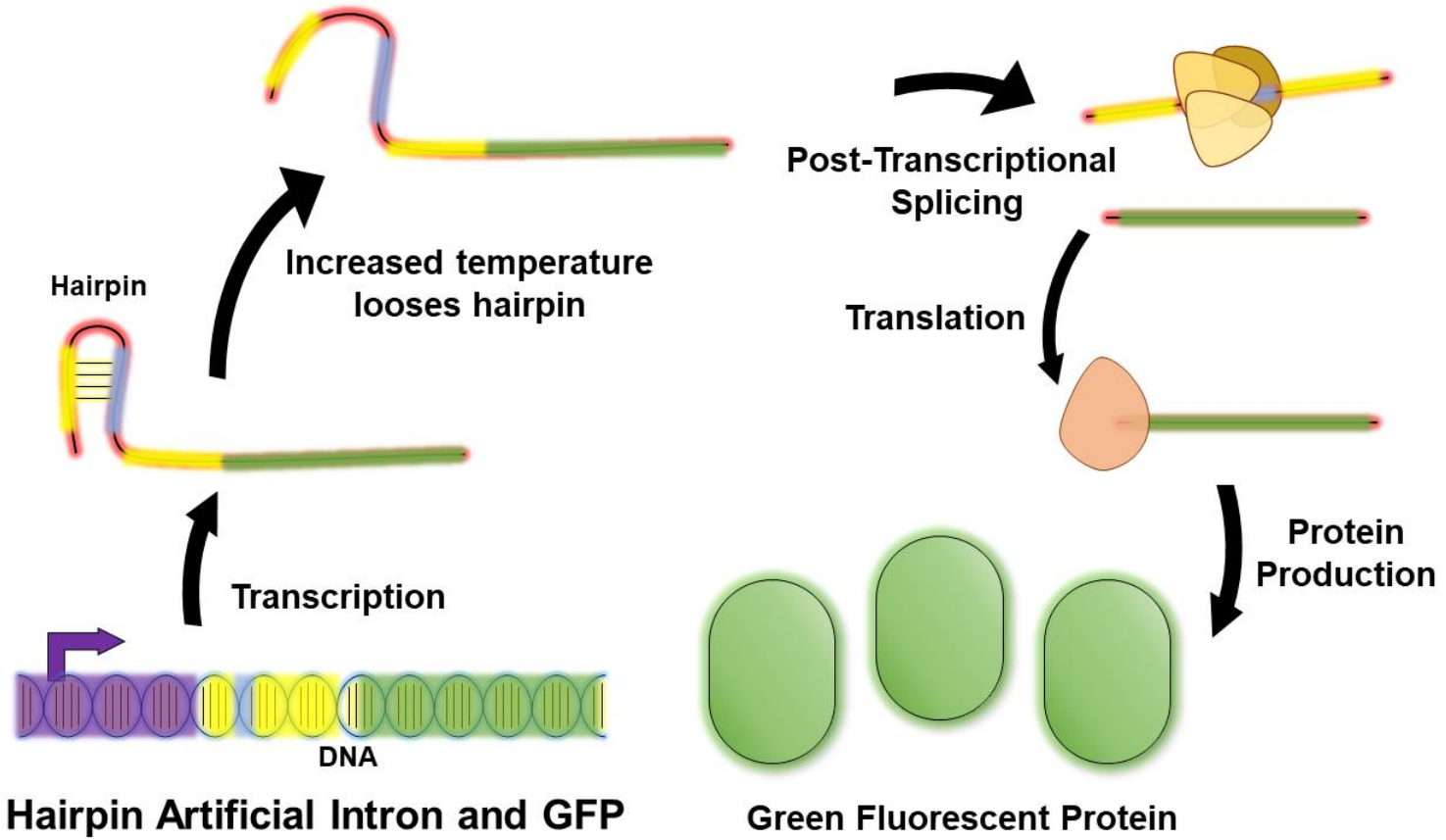




Temperature Sensitivity of Intron Splicing Inhibition



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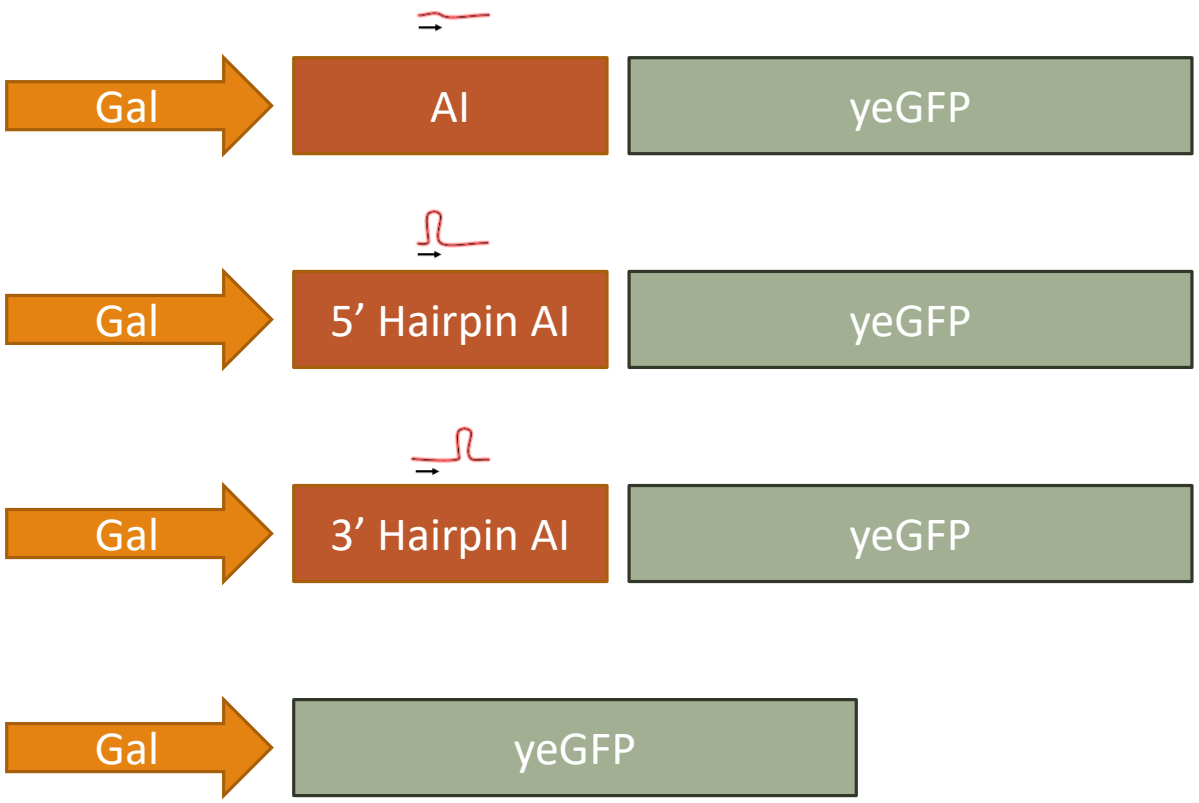
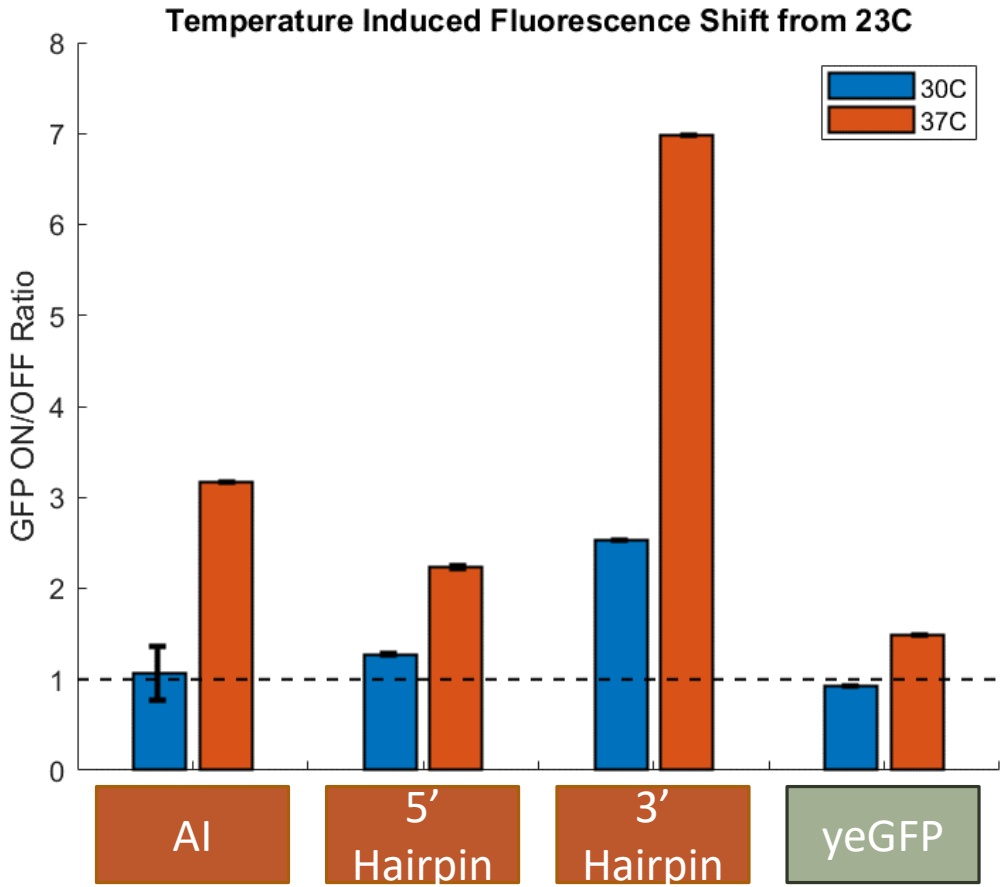
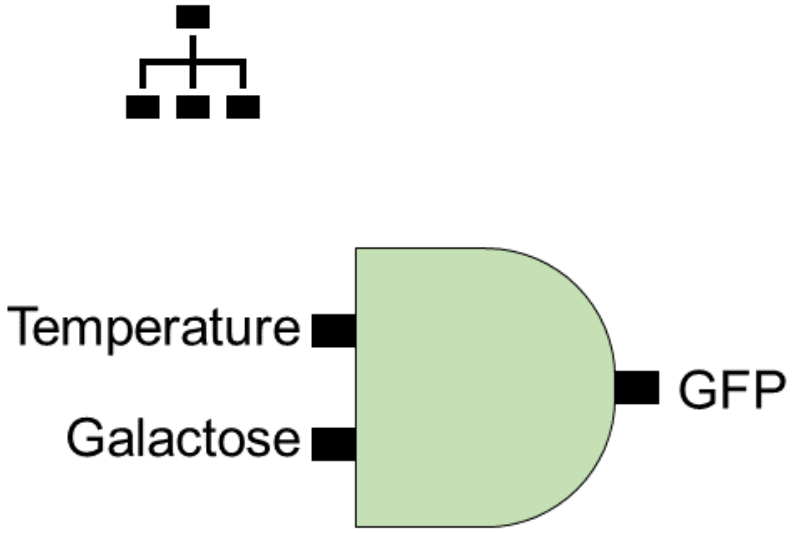
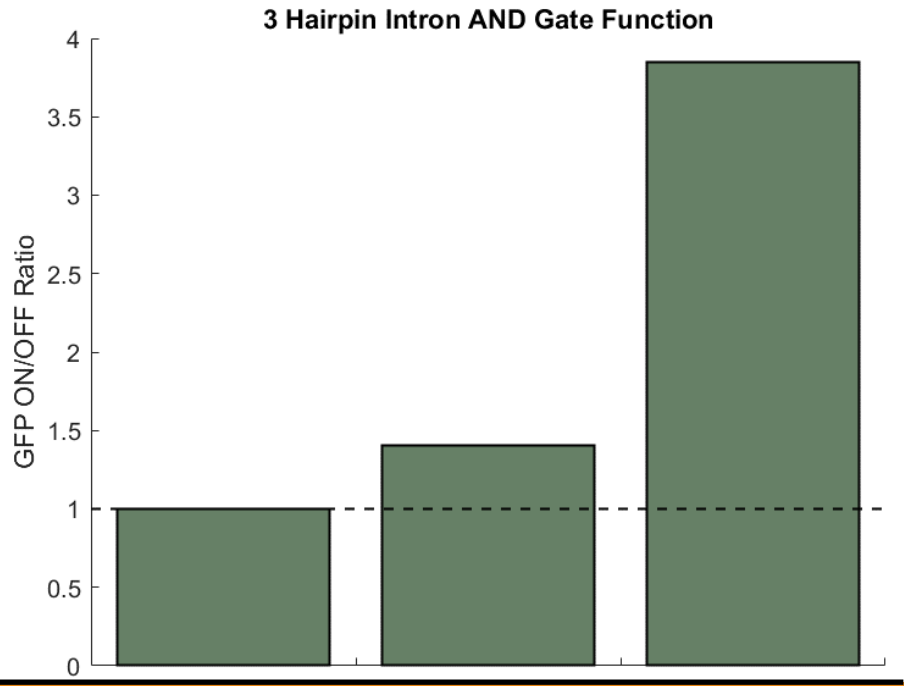




*AI = Artificial Intron

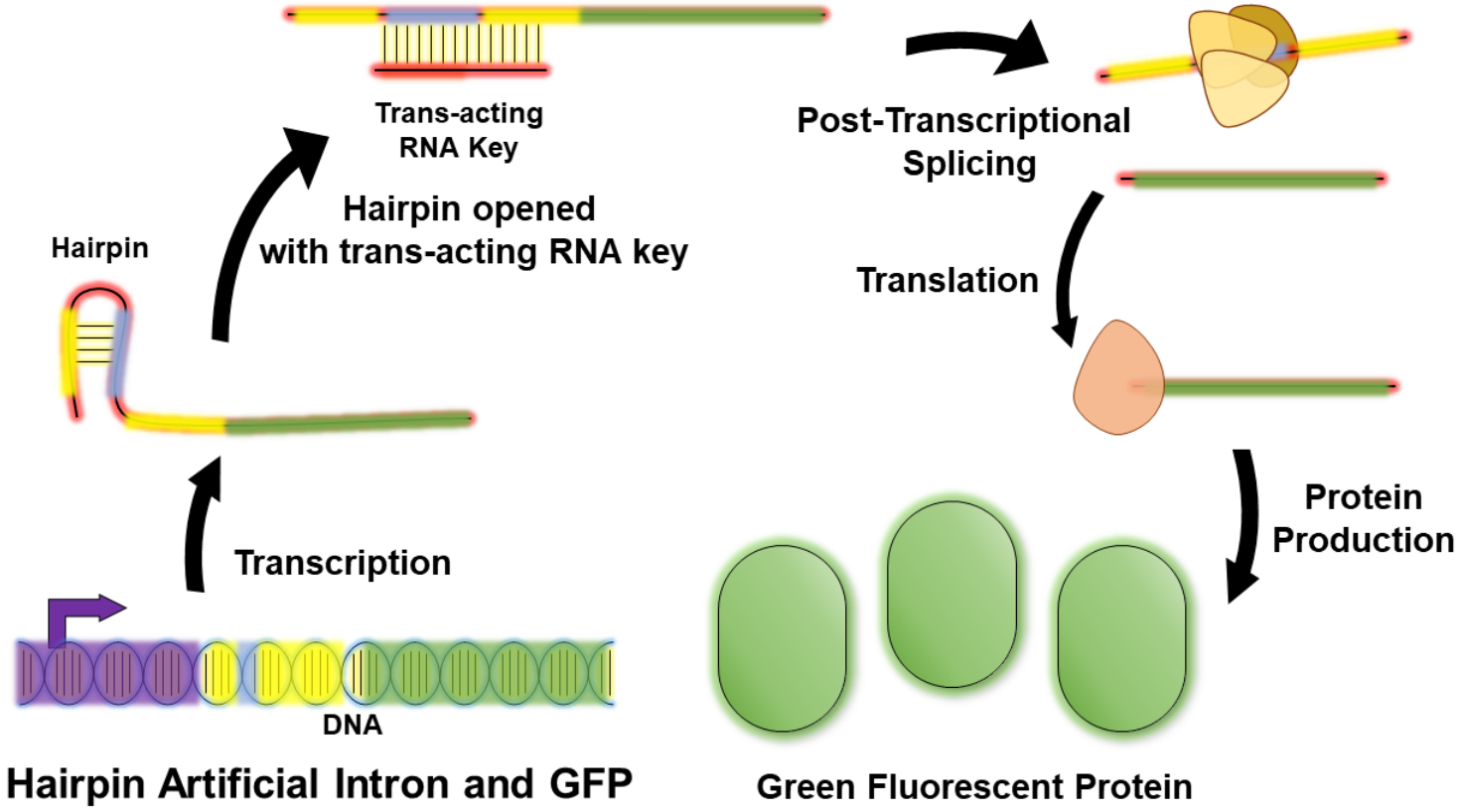
Logic AND Gate Functionality





Input			
Temperature	-	-	+
Galactose	-	+	+

Future Steps: *trans*-acting RNAs to control intron splicing



Acknowledgements

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