



Tabula Rasa: Decoupling genetic information from medium

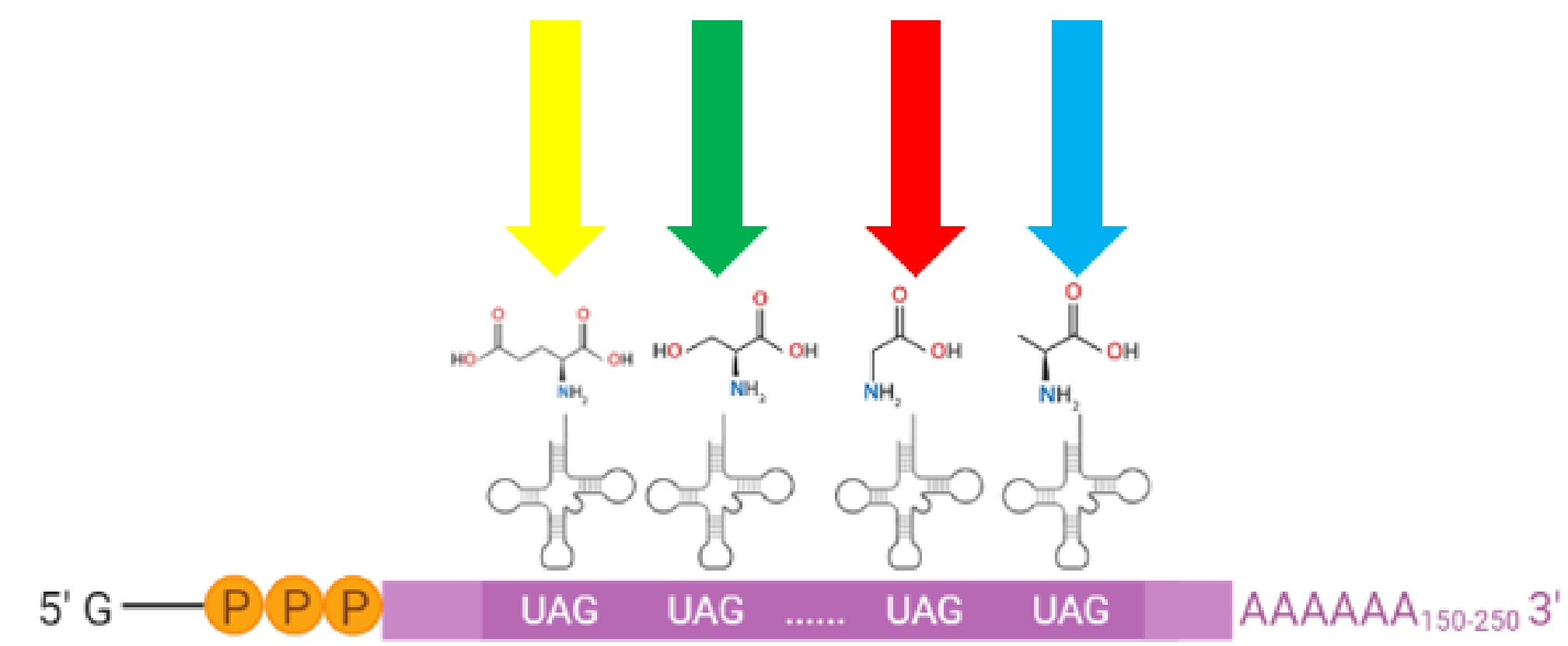


Chengyuan (Wesley) Wu, Adam Sychla & Michael Smanski
Biotechnology Institute, University of Minnesota-Twin Cities

Abstract

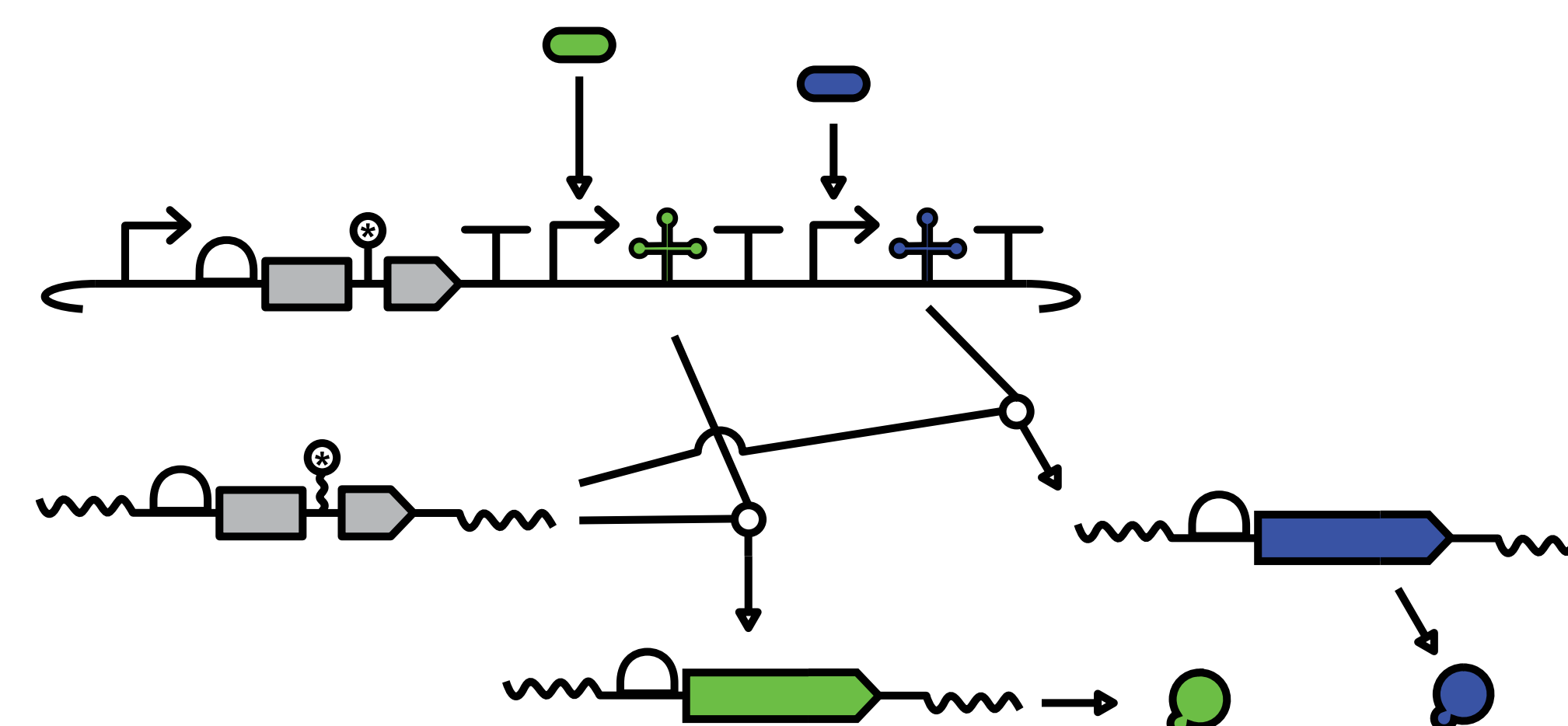
tRNAs are indispensable participants in the translation process. On each tRNA, there is a three-base-pair anticodon that binds with the codon on mRNA, such that only one corresponding amino acid can be added to the peptide chain each time. In synthetic biology, control over the final product generally occurs at the nucleic acid coding level while the translation process is held constant. In this project, we utilized tRNA biology to create a system where translation patterns can be changed in a controllable, inducible manner, decoupling the product from the genetic code. To achieve this goal, we are building a gene circuit composed of a constitutively expressed nonsense mutated GFP, one induced tyrosine amber suppressor tRNA, and one induced histidine amber suppressor tRNA. Currently, we realized a transformation from a mutated GFP Y66X to BFP (GFP Y66H) dependent on a co-expressed histidine suppressor tRNA. This proof of concept demonstrates that plasmid-delivered tRNAs are processed, involved in the translation, and can rescue the fluorescent protein.

Figure 2. Controlled tRNA expression can decouple translation from genetically encoded information



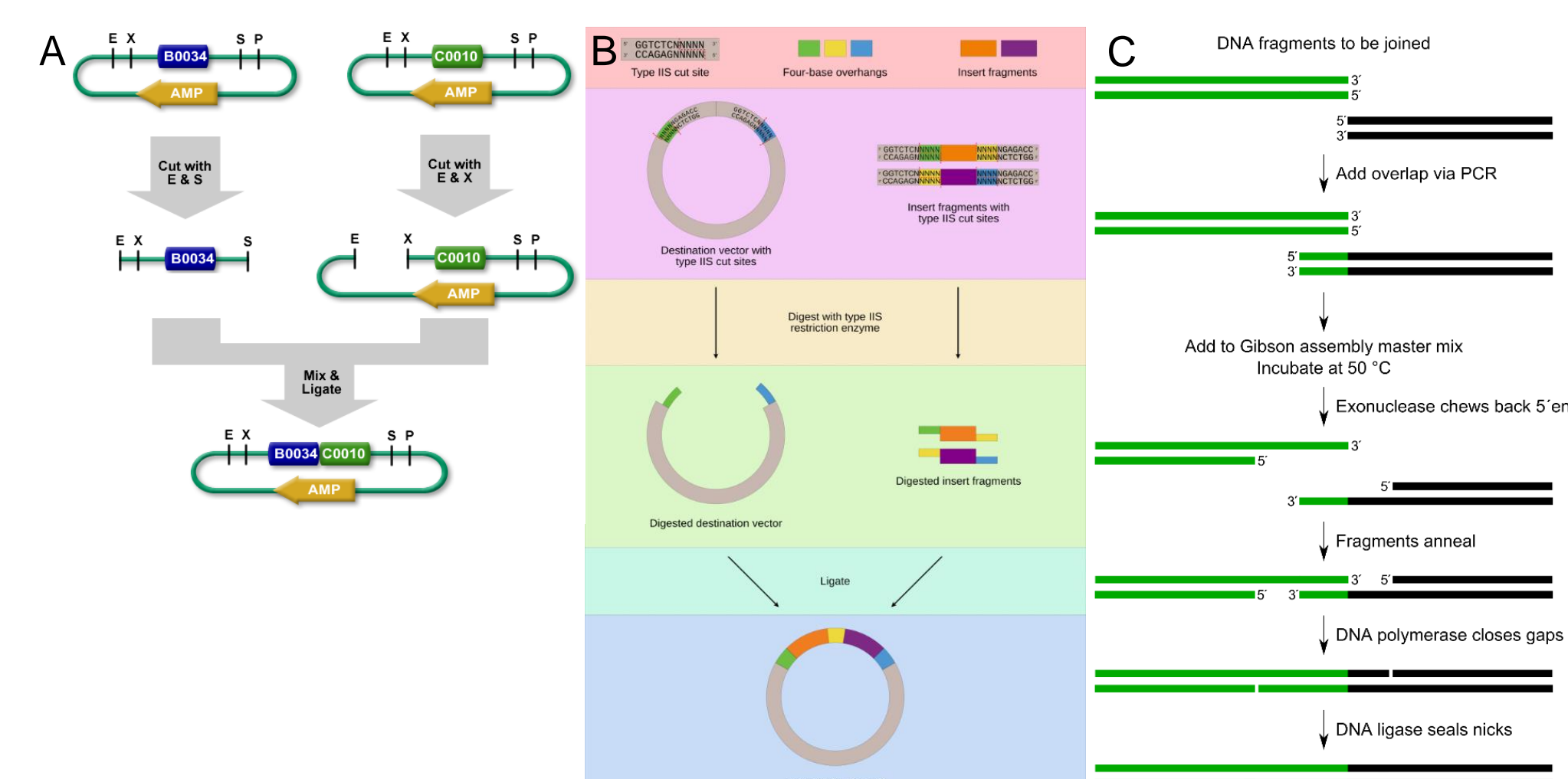
In translation, protein product is determined by the encoding sequence based on a near-universally conserved genetic code. We seek to decouple coding from protein sequence by controlling the expression of synthetic tRNAs. As the cellular tRNA profile changes, so do the peptide output by a given mRNA sequence. This can be done in response to light, chemical, or other environmental cues.

Figure 3. Switching tRNAs enables the change of peptide without variation in gene sequence



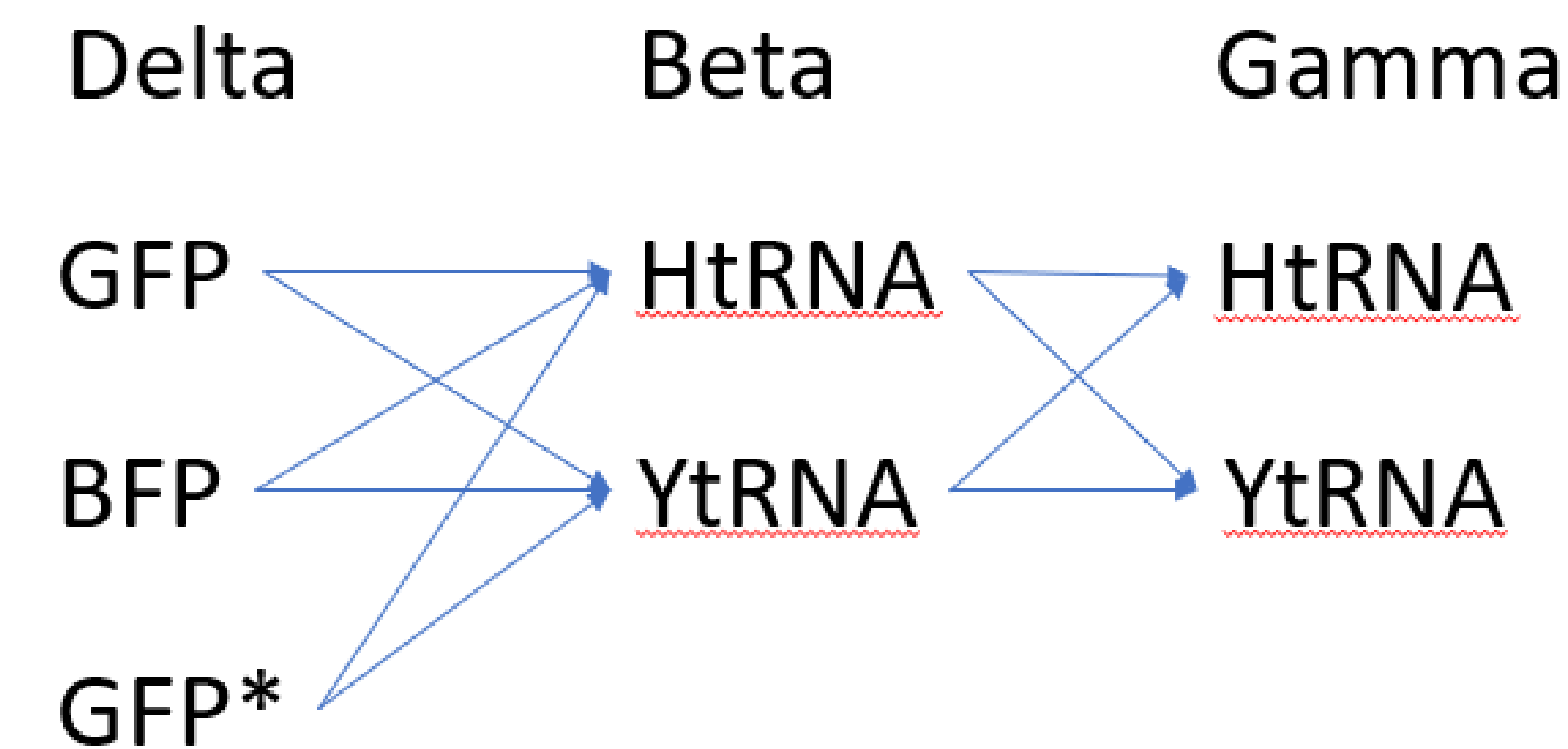
The gene circuit is composed of a constitutive expressed GFP* (GFP Y66X) and two induced suppressor tRNAs, charged with either tyrosine (YtRNA) or histidine (HtRNA). This allows translation into GFP or BFP (GFP Y66H), respectively.

Figure 4. DNA assembly strategies differ in advantages and weakness



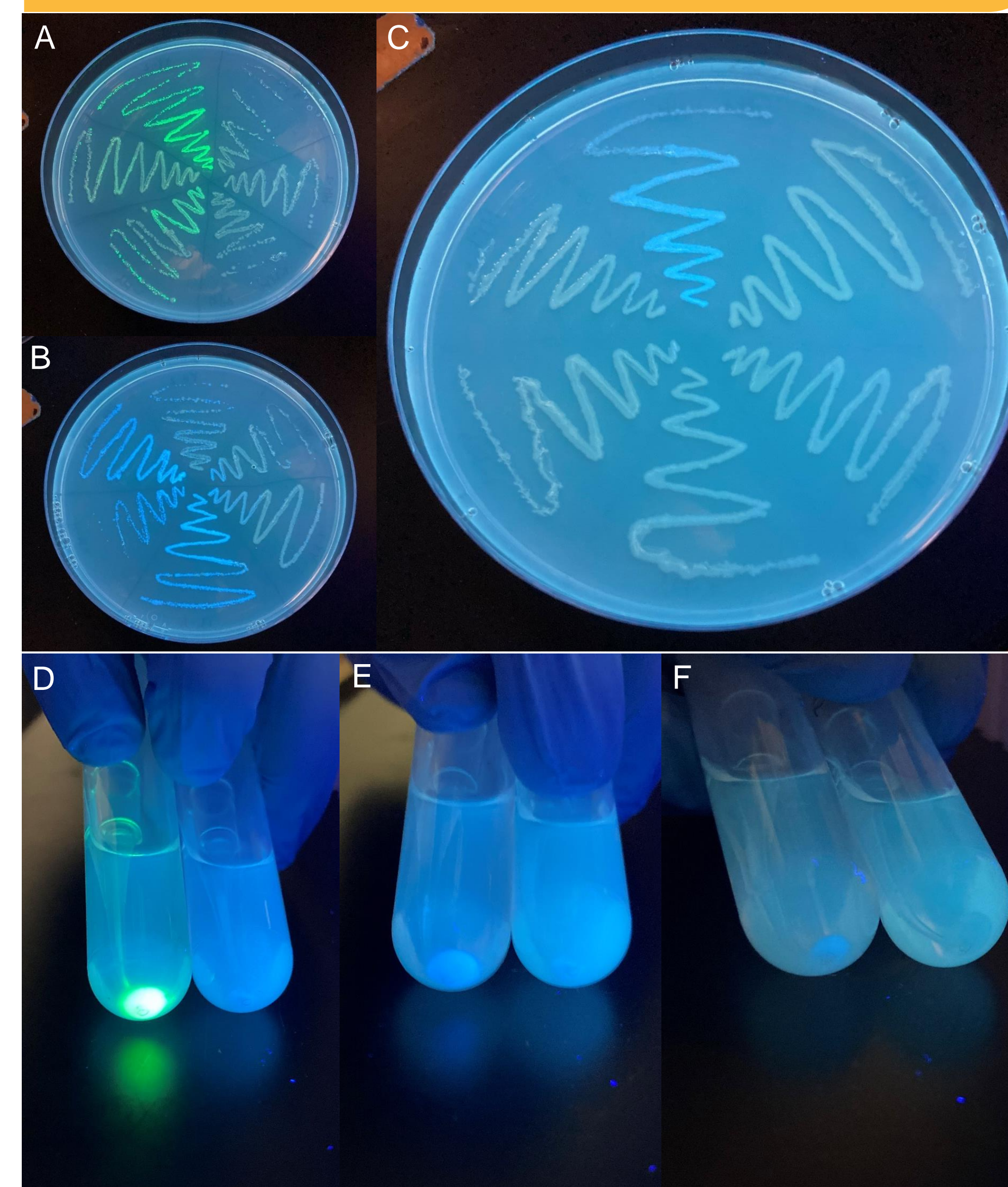
A) Traditional cloning utilizes Type II restriction enzymes.⁴ B) Golden Gate cloning uses Type IIS restriction enzymes that cut outside their recognition site to allow a one-pot combinatorial assembly.⁵ C) Gibson (Isothermal) assembly is a one-pot reaction that depends on extending from short homologies.⁶

Figure 5. The final gene circuits are composed of one fluorescence protein and two tRNAs, assembled combinatorially



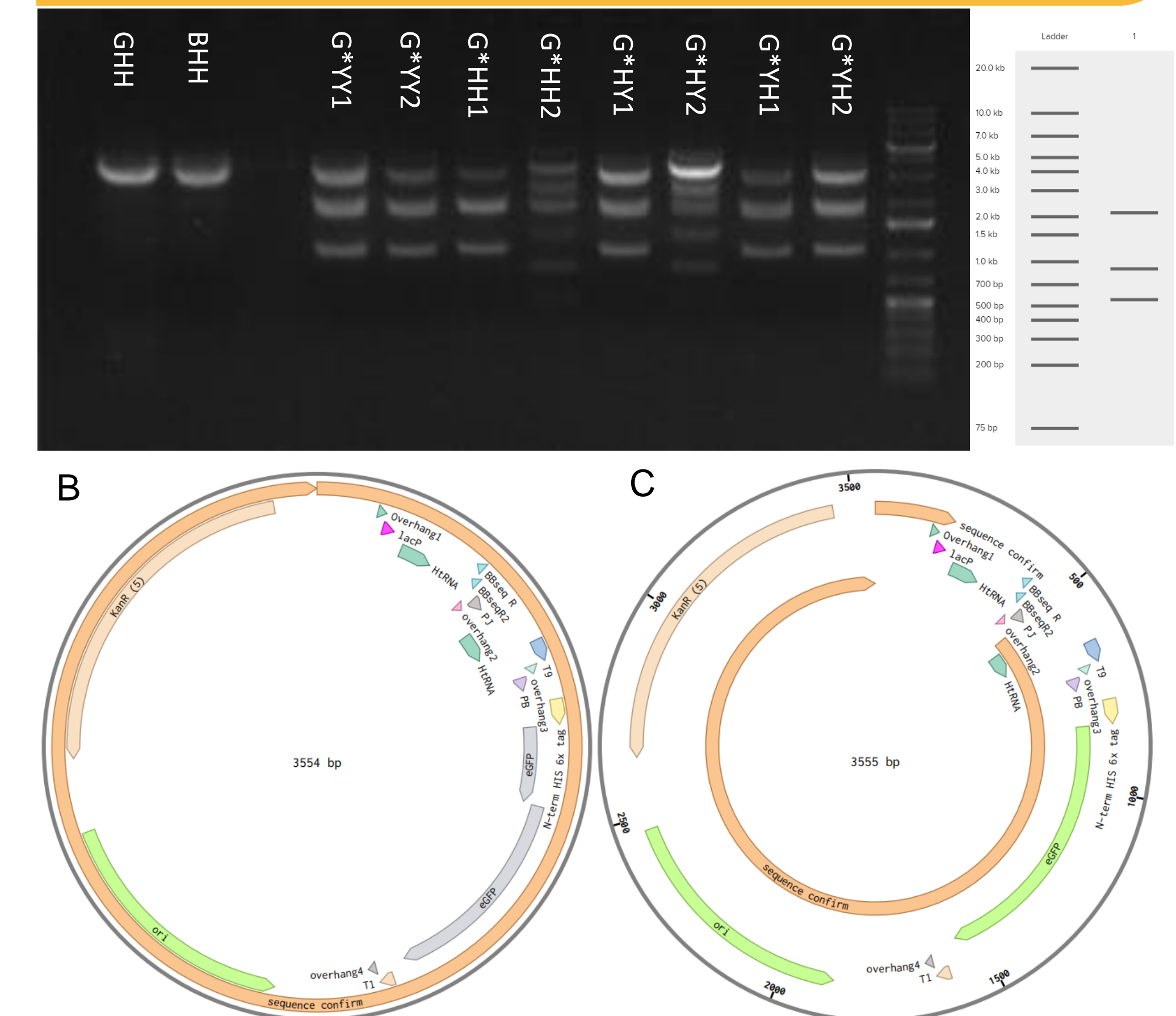
We assembled GFP, BFP (GFP Y66H), and GFP* (GFP Y66X) together with two tRNAs. These were done combinatorially such that each protein was expressed in tandem with either two copies of a tRNA or with one copy of each. Segments on each plasmid were assembled by Golden Gate or Gibson assembly.

Figure 6. Suppressor tRNAs rescue the nonsense mutation in GFP Y66X



Colonies of assembled constructs were illuminated with 365 nm ultraviolet. A) GFP assembled bacteria had strong green fluorescence at left three streaks, B) BFP assembled bacteria had strong blue fluorescence at left two streaks and bottom streak, C) GFP* assembled bacteria had light blue fluorescence at central up streak. Bacteria cultures of single colonies were illuminated with 365 nm ultraviolet. Right tubes are negative controls. D) GFP assembly culture showed strong green fluorescence, E) BFP assembly culture showed strong blue fluorescence, F) GFP* assembly culture showed light blue fluorescence.

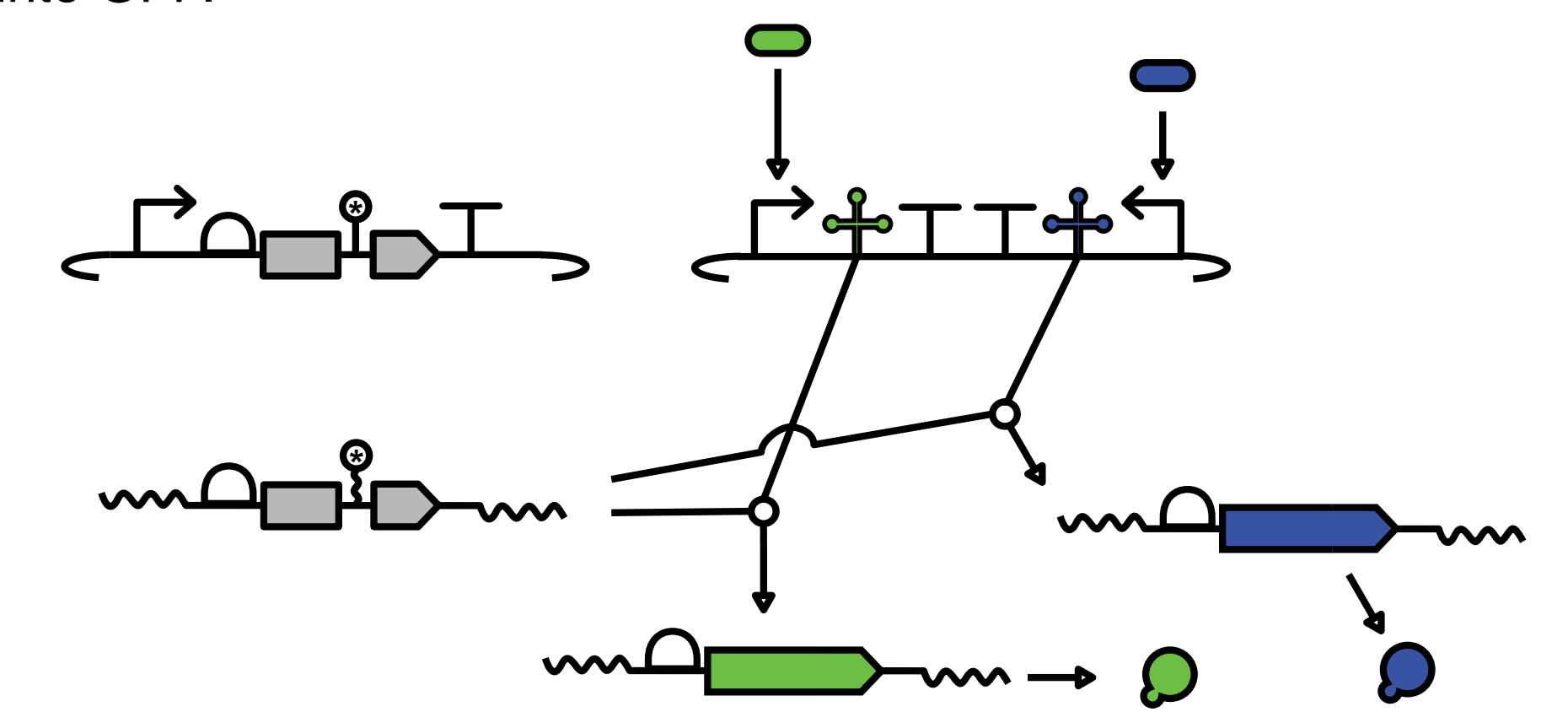
Figure 7. Assembled plasmids are not stably maintained in E. coli



A) Plasmids derived from the reculture of glycerol stock were digested with AvrII and XmaI (G: GFP; B: BFP; G*: mutated GFP; Y: tyrosine suppressor tRNA; H: Histidine suppressor tRNA). It is expected to see three bands that were 554 bp, 886 bp, and 2113 bp. B) G*HH2 and G*HY2 were sent for whole plasmid sequencing. They were found to have the complete sequence of the plasmid. Incorrect bands should be not fully digested. C) Other plasmids were sequenced with a primer on fluorescence protein and were found to lose the first suppressor tRNA and second promoter sequence. It was probably due to the homologous sequences on plasmids.

Conclusions and Future Plans

- External suppressor HtRNA added amino acid at amber stop codon and allowed the translation kept on.
- Current genetic circuits are not stable in this strain
- We will assemble new circuit designs for increased stability.
- We are going to demonstrate that YtRNA can also rescue GFP* into GFP.



Acknowledgement

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References

1. Chin, J. W. (2011). The EMBO Journal, 30(12), 2312-2324
2. Neidhardt, F., & Curtiss, R. (1996). Washington, D.C.: ASM Press.
3. Schäfer, J. (1993). Cold Spring Harbor Laboratory Press (1922).
4. Brown, J. (2006, August 6). Retrieved from <http://parts.igem.org/Help:Assembly>
5. Golden Gate Cloning. (2021, July 06). Retrieved from https://en.wikipedia.org/wiki/Golden_Gate_Cloning#/media/File:Golden_Gate_assembly.svg
6. Gibson assembly. (2021, January 02). Retrieved from https://en.wikipedia.org/wiki/Gibson_assembly