RNA Broccoli Aptamer to track transcription rates in vitro

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Abstract

Regulation of gene expression is essential to understanding cellular structure and function. We seek to study the effects of histone post translational modifications (PTMs) on transcription rate using an engineered DNA sequence containing *C. elegans* promoter elements and nucleosome positioning DNA sequences, followed by an RNA aptamer sequence that will fluoresce upon full transcription. DNA in eukaryotic nuclei is organized into nucleosomes, each composed of an eight-histone protein core wrapped ~1.7 times by approximately 147 base pairs of DNA. PTMs to histone tails are known to regulate nucleosome structure and by extension gene expression; however, the residual effect of PTMs on transcription rate is poorly understood. Our approach will allow us to detect the transcription rate of reconstituted chromatin *in vitro*, comparing histones with PTMs to those without. Use the Broccoli aptamer, a derivative of the well-known Spinach aptamer, will allow for detection of transcription rates in common qPCR instruments. These methods can further be used to discern if multiple PTMs on a single histone are synergistic or nullify each other, as well as measuring the role of histone variants on transcription rate, ultimately allowing for more precise manipulation of transcriptional output.

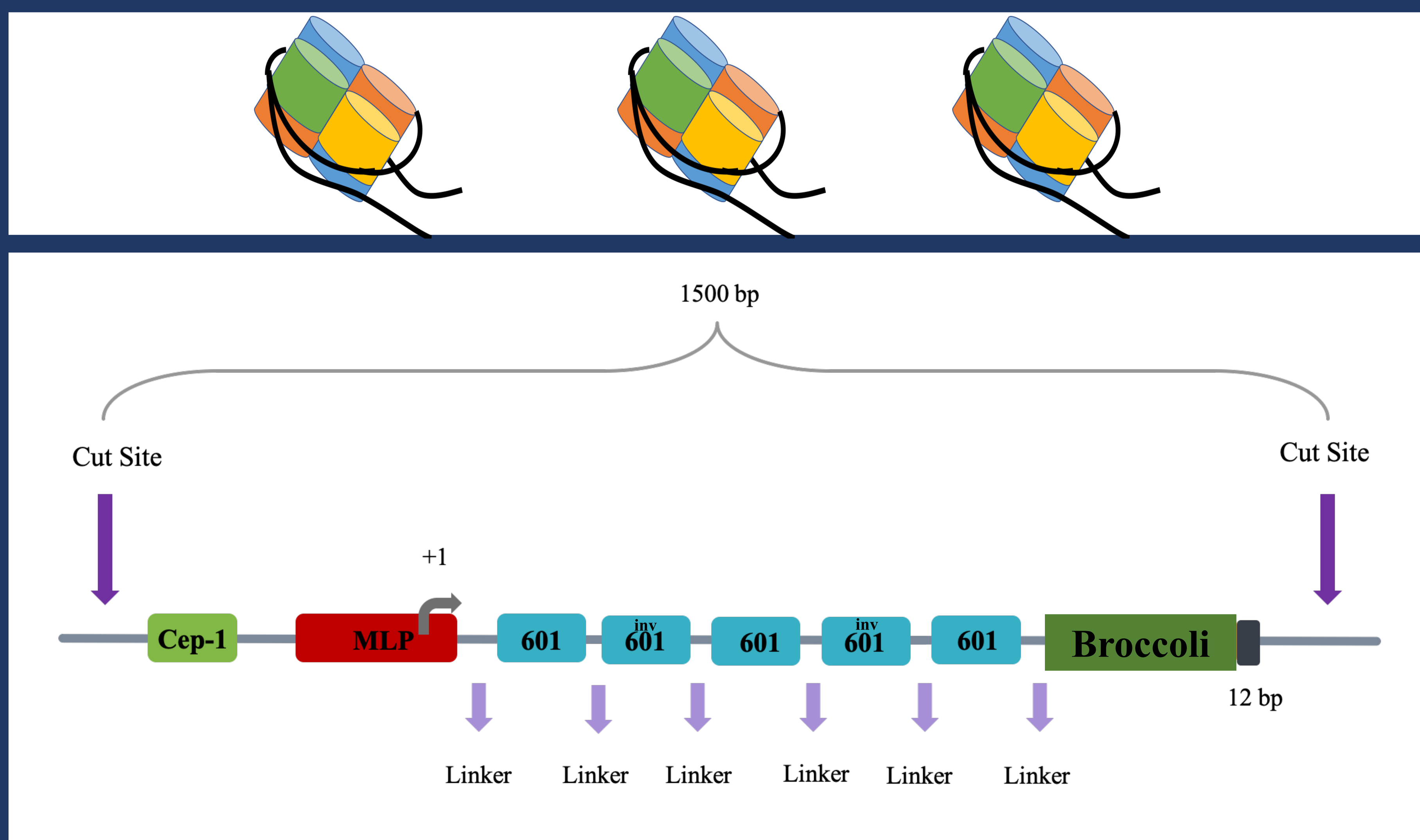

Figure 1: Background. A. Depiction of histone octamer/nucleosome core. Each nucleosome is composed of two tetramers, each with an H2A, H2B, H3, and H4 histone. There are approximately 147 bp of DNA wrapped about 1.7 times around each octamer. B. Depiction of organization of broccoli DNA construct, with 601 sequences in alternating orientation for optimal chromatin structure.

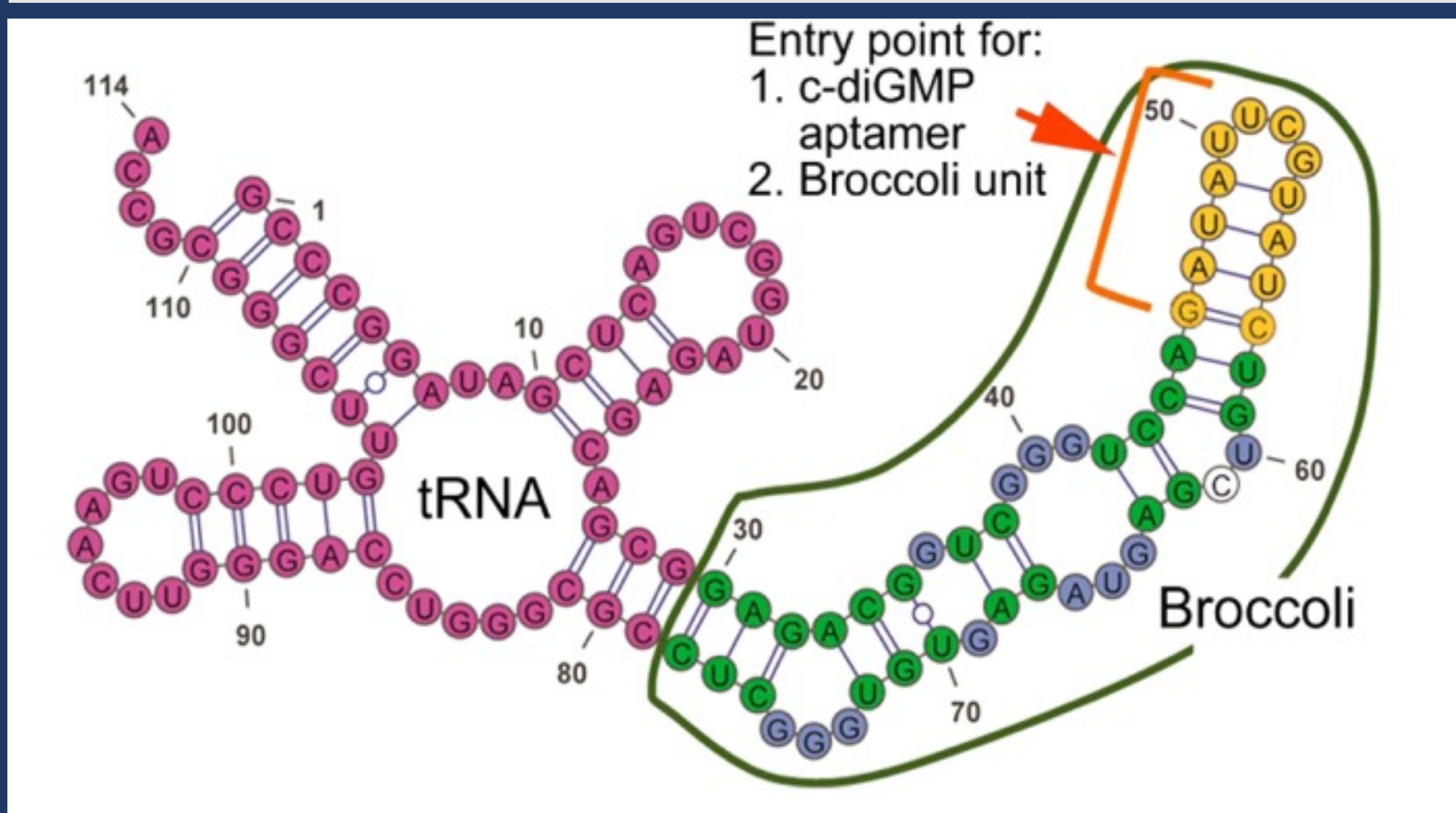

Figure 2: Broccoli structure as reported by Filonov, et. al.[1] Here, broccoli is depicted attached to a tRNA scaffold for increased stability. For our purposes this scaffold would be unnecessary.

REFERENCES:
1. Filonov, G. S., Moon, J. D., Svensen, N., & Jaffrey, S. R. (2014). Broccoli: rapid selection of an RNA mimic of green fluorescent protein by fluorescence-based selection and directed evolution. *Journal of the American Chemical Society*, *136*(46), 16299–16308. https://doi.org/10.1021/ja508478x
2. Okuda, M., Fourmy, D., & Yoshizawa, S. (2017). Use of Baby Spinach and Broccoli for imaging of structured cellular RNAs. *Nucleic acids research*, *45*(3), 1404–1415. https://doi.org/10.1093/nar/gkw794
3. Trachman, R. J., 3rd, Demeshkina, N. A., Lau, M., Panchapakesan, S., Jeng, S., Unrau, P. J., & Ferré-D'Amaré, A. R. (2017). Structural basis for high-affinity fluorophore binding and activation by RNA Mango. *Nature chemical biology*, *13*(7), 807–813. https://doi.org/10.1038/nchembio.2392

Aptamer:	Mango	Broccoli	Spinach-2
Thermostability:	-	More heat stable than spinach 2 at temperatures below 100°C	Begins degradation at 25°C
Kd:	-	360	560
Fluorophore:	TO (thiazole orange)	DFHBI	DFHBI
Fluorescence:	High	Higher fluorescence than spinach for low Mg concentrations	-
Salt Dependence:	No	No	Yes- high potassium dependence
Mg Dependence:	1 mM $MgCl_2$ required in spectroscopy	Max fluorescence at 300 microM, but not required	Max fluorescence at 1 mM
In vitro/in vivo:	In vitro	Both	In vitro
Wavelength Excitation/Emission	510/535 nm	472/507 nm	482/505 nm

Figure 3: Comparison of properties of various viable aptamers.

Ligation Troubleshoot:

As we began efforts to ligate the inverted 601 sequence and the broccoli sequence into the appropriate vector (TOPO pCR4) we have had trouble having successful ligations. To address these issues we focused our energy on troubleshooting the inverted 601 ligation by:

- Doing multiple trials with different ligases (NEB quick ligase/T4 ligase, ThermoFischer T4 ligase)
- Performing pre-ligation restriction digestions of insert and plasmid with both restriction enzymes present at once, as well as doing sequential digestions with enzyme-specific buffers
- Increased the concentration of the TOPO pCR4 vector by doing a midi plasmid prep, as opposed to a mini plasmid prep

We are hopeful that a recent ligation, scheduled to be completed March 24 will be successful, at which point we will turn our attention to completing the broccoli ligation.