Thermoregulatory Translational Control of Heat Shock Protein 20 and Other Genes By Novel RNA Thermometers

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Introduction

RNA thermometers are functional RNAs that melt with an increase in temperature, allowing for access to the ribosomal binding site.

Two of our samples were upstream of the gene to produce heat shock protein 20 (HSP20). HSP20 prevents denaturation at high temperatures.

Materials and methods

We used a GFP/RFP reporter assay to measure fluorescence before and after heat shocking our samples.

First, we created our plasmid with our sequence of interest inserted between GFP and RFP and cloned it into our samples.

Then, we measured fluorescence on our plate reader, heat shocked our samples, and then measured fluorescence again.

Results

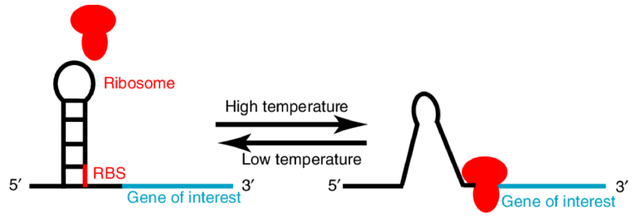

Figure 1. Representation of a RNA thermometer melting due to an increase in temperature, allowing for the ribosome to bind to the ribosomal binding site (RBS), also known as the Shine-Dalgarno sequence (SD). This then allows for the ribosome to produce the gene of interest connected to the RNA thermometer.

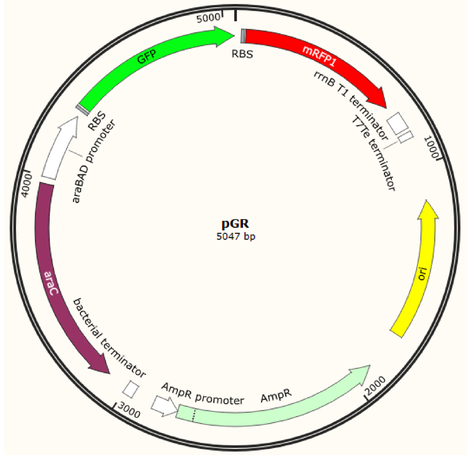

Figure 1. Plasmid design for testing RNA thermometers using a GFP and RFP reporter system.

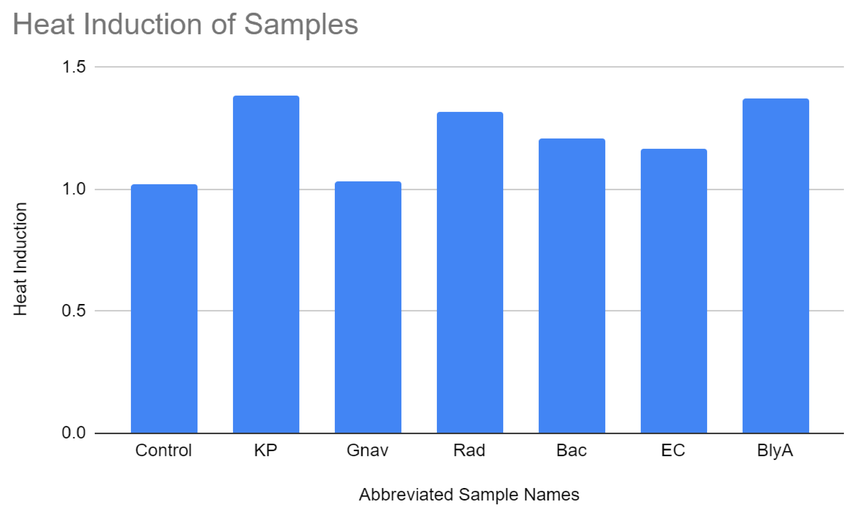

Figure 1. Average heat induction of samples based on fluorescence production of GFP and RFP before and after heat shock. Control is a negative control and BlyA is a positive control. N=6 for all samples except BlyA, which is n=4.

Conclusions

The two samples that showed the highest heat induction folds were *Klebsiella Pneumoniae* (KP) and *Radicin* (Rad), which were both upstream of HSP20.

We still need to do more testing in the future to work out the protocol since the average heat induction of BlyA is around 4-fold.

Literature cited

Krajewski, S.S., Nagel, M. and Narberhaus, F., 2013. Short ROSE-like RNA thermometers control IbpA synthesis in Pseudomonas species. PloS one, 8(5), p.e65168.

Török, Z., Goloubinoff, P., Horváth, I., Tsvetkova, N.M., Glatz, A., Balogh, G., Varvasovszki, V., Los, D.A., Vierling, E., Crowe, J.H. and Vígh, L., 2001. Synechocystis HSP17 is an amphitropic protein that stabilizes heat-stressed membranes and binds denatured proteins for subsequent chaperone-mediated refolding. Proceedings of the National Academy of Sciences, 98(6), pp.3098-3103.

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Further information

Please **email me** at ktenhoff@callutheran.edu if you have a question or comment.