SELEX-sequencing

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Oxidative stress damages proteins, lipids, and DNA and thus contributes to diseases such as cancer and neurodegenerative disease. The transcription factor Nrf2 is a master regulator of the response to oxidative stress. In the presence of stress, Nrf2 trans-locates into the nucleus and binds a consensus DNA sequence (the antioxidant response element, ARE) that leads to up regulation of detoxification genes.

The Slattery lab has published that Nrf2 target genes are differentially responsive to Nrf2 activity; the differences between these responses is correlated with ARE motif quality (Lacher et al. 2015). Some Nrf2 target genes are regulated by a perfect ARE (TGCTGAGTCAT; strong binding), while most others contain various combinations of mismatches in the ARE (weaker binding). My recent work in the Slattery lab supports the hypothesis that not all AREs are equivalent. Perfect AREs respond strongly to small increases in Nrf2 and are switch-like in responding to stress; imperfect AREs respond to Nrf2 activity in a linear manner. This data suggested that subtle changes to the ARE sequence can have a significant impact on Nrf2 binding.

The degree to which Nrf2 mediates gene expression is regulated by the strength of the ARE motif; however the rules governing Nrf2:ARE interactions are not well defined. Interdependencies between positions exist (e.g., G in position 7 is only preferred when position 6 is A). Consequently, my previous research using

in vivo Luciferase reporter assays and *in vitro* electro-mobility shift assays (EMSA) will not lead to a comprehensive understanding of how the positional variations in ARE binding motif translates to Nrf2 binding and gene expression changes.

My UROP this semester supported further investigation of the binding preferences through SELEX-sequencing, systematic evolution of ligands by exponential enrichment coupled with DNA sequencing. DNA libraries containing a randomized region (n^{16} possibilities- all potential ARE combinations represented 100 times) flanked by defined regions are used to bind the TF interest. DNA bound by the complex is then separated from unbound DNA using EMSA and the bound DNA is then amplified by PCR, sequenced, and used for subsequent rounds of DNA binding and selection. This high through-put method produces a **c**omprehensive view of Nrf2:ARE binding preferences once sequencing is complete. Figure 1 (below) is a visual to aid in the explanation of the elaborate method.

Due to the complexity of SELEX-seq, black and white results are not constructible. The interdependencies are still being discovered and the favorable nucleotides are being researched. However, my objectives have been met on the basis of further exploring the Nrf2:ARE binding affinities. Not only have I been able to broaden my knowledge of SELEX-seq, but also better my lab skills in all of the techniques that are included in this method, including PCR, EMSA, and statistical analysis.

Moreover, my UROP this semester has provided me the opportunity to obtain high-quality lab experience and help me come to terms with my love for science. I hope to continue my research in my future endeavors after my undergraduate studies, in either graduate or professional school. I am so appreciative of all of the support and assistance from the entire UROP faculty with my semester project.

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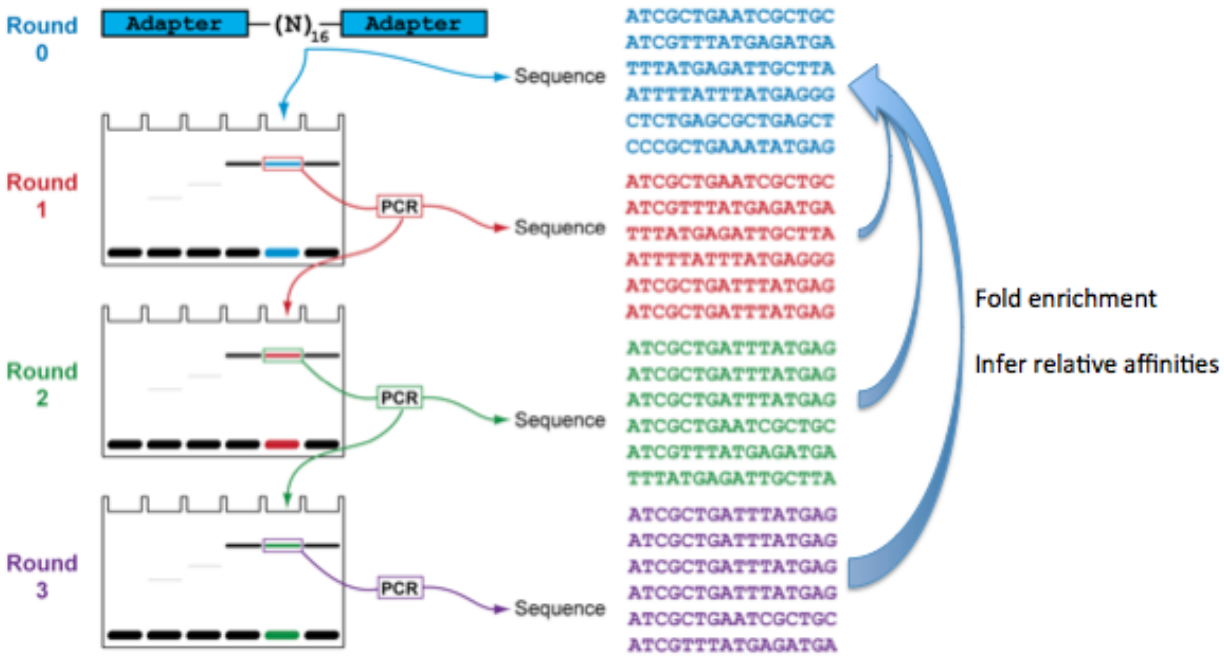

Figure 1. SELEX-sequencing.

Lacher, S.E., Lee, J.S., Wang, X., Campbell, M.R., Bell, D.A. and Slattery, M. 2015. Beyond antioxidant genes in the ancient Nrf2 regulatory network. Free radical biology & medicine 88, 452-465.