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Tracking Transcription Factors: Validating the Brd4-HypB Calling Card System in vitro

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Tracking Transcription Factors: Validating the Brd4-HypB Calling Card System *in vitro*

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Transcription factors play critical roles in the development of nearly all cell types. While methods currently employed to study transcription factor binding events are limited to only tagging transcription factors bound to the DNA when transcription was halted, a new technology known as the calling card system is able to exhaustively record the memory of transcription factor binding events. Calling card technology harnesses the functionality of genetic transposable elements to identify the targets of DNA binding proteins. When a transposase is fused with a transcription factor, the transposon enzyme will deposit its genetic cargo close to the tagged transcription factor's binding site. Thus, one can track transcription factor binding by looking for transposon insertions.

The purpose of this research project was to assess the functionality of the Brd4-HypBase calling card construct. To create this construct, the general transcription factor Brd4 was fused with a HyperpiggyBac (HypB) transposase and a DHFR degradation domain (DD), all of which was put under the control of a Tet-On promoter. This construction, in theory, would allow for the tight regulation, both transcriptionally and post-translationally, of the Brd4-HypB construct using doxycycline (Dox) and trimethoprim (Tmp), respectively. After culturing primary astrocytes from neonatal mice carrying the TetOn-Brd4-HypB-DHFRDD allele, qPCR and Western blot analyses were performed on cells treated with one of the four combinations of Dox and Tmp to compare the amounts of Brd4-HypB mRNA and stabilized Brd4-HypB protein in treated vs. untreated cells. The qPCR results indicated no significant difference between the amounts of Brd4-HypB mRNA in cells treated with Dox compared to untreated cells, indicating a possible issue with the promoter. Similarly, the Western blot results indicated no significant difference between the amount of Brd4-HypB protein detected in cells treated with Tmp vs. untreated cells.