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# Applying high throughput deep mutational scanning to study transcription factors implicated in the biology of Substance Use Disorders

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Deleterious variation in genes coding for transcription factors (TFs) has been implicated in Substance Use Disorders (SUD), although nearly all variants in these genes remain unclassified regarding their effects on TF function. Using deep mutational scanning (DMS), we are scaling up technologies for direct functional assessment of the effects of thousands of genetic variants in parallel. While DMS has been used in characterization of monogenic diseases, it has not yet been applied to SUD.

A comprehensive missense variant library in our TF genes of interest (e.g., *HIVEP2, EGR1, RUNX2*) will be cloned such that every amino acid residue at each position in the protein sequence is mutated to each of the 19 possible alternative amino acids. We will integrate variant libraries and reporters into multiple cell types (N2A, 3T3, and HEK). Activity of the mutated TFs will be assessed as a change of transcriptional reporter expression.

Preliminary data show appropriate doxycycline dose-responsiveness of GFP florescence for transduced HEK cells with two TF constructs. Ongoing work will generate transcriptional activity scores for all possible missense variants these genes.

By applying DMS variant screening to TFs associated with SUD, we will help characterize how altered TF activity relates to the complex biology of SUD. By facilitating parallel investigations of thousands of variants in many TFs, variants will be systematically tested at an increased rate, providing a molecular understanding for the genetic component of SUD and contributing to the future development of rationally designed personalized genetic treatments.