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Applying High Throughput Deep Mutational Scanning (DMS) to Study Transcription Factors (Tfs) Implicated in the Biology of Substance Use Disorders (SUD)

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Several transcription factors (TFs) are implicated in substance use disorders (SUD), notably in acute response to use and in lasting transcriptional programs that reinforce further use. How genetic variation confers resilience or susceptibility is not well understood. Whether such variation is found in non-coding regulatory regions or coding sequences, it is likely to be rare and may be undetected by GWAS thus far. For TFs, this variation could reside in the highly structured DNA binding domains or in disordered regions where the conformational plasticity likely conveys regulatory plasticity across different cell types and substance use states. Our lab uses Deep Mutational Scanning (DMS) to functionally assess thousands of TF coding variants in parallel.

DMS libraries mutate each residue to all 19 alternative amino acids. Activity of the mutated TFs is assessed as change in transcriptional reporter expression and quantified by Sort-seq. We are generating and testing reporters for EGR1, ELK1, and RUNX2, using known reporters as controls for dynamic range of TF activity reporter expression. Our system integrates the transcriptional reporter and variant of interest in the same “Combo” plasmid, enabling multiplexing of several genes in the same experiment. Additionally, we are testing both lentiviral (random) and landing pad (single allele specific integration) systems.

The results will provide an experimental look-up table for rare coding variant function, as such variants are identified by larger and more diverse GWAS. Additionally, our results can resolve critical residues in the DNA binding domains, perhaps providing nucleotide-level evidence of non-coding regulatory mutation tolerance.