

**TRANSCRIPTION FACTOR REGULATION, COFACTOR  
REGULATION, THE DEVELOPMENT OF A LIQUID-BASED  
PROTEIN-BINDING MICROARRAY ASSAY AND ITS  
APPLICATIONS TO BIOMEDICAL RESEARCH**

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## **Abstract**

Transcription factors (TFs) and cofactors (COFs) control which genes are active in a given cell, but the methods for measuring their interactions are expensive, equipment-intensive, and poorly suited for solution-phase biology. Solid-phase protein-binding microarrays (PBMs), the current standard, require custom microarray fabrication, large amounts of purified protein, and fluorescence scanning infrastructure. They also lock TFs and COFs onto a fixed surface, which limits the kinds of interactions that can be observed. This thesis describes the development of a liquid-based PBM: a proximity ligation assay designed to detect TF-COF binding in solution using standard molecular biology equipment.

The assay was built and tested at the Siggers Laboratory at Boston University over seven months, using Lymphoid Enhancer-binding Factor 1 (LEF1) as the model transcription factor. The work involved extensive trial and adjustment of T4 DNA ligase concentration, antibody-oligonucleotide concentration, nuclear extract volume, NaCl levels, and PCR conditions. Several sources of signal noise were identified and addressed along the way, including primer contamination and endogenous ligase activity in the nuclear extract. By late August 2025, the assay was producing consistent, readable ligation bands under the following conditions: 0.5 U/ $\mu$ L T4 DNA ligase, 10 nM antibody-oligonucleotide, and 1  $\mu$ g/ $\mu$ L nuclear extract at corrected salt concentration. Wild-type LEF1 binding sequences produced stronger ligation signals than sequence-disrupted mutant controls, which is the expected result if the assay is detecting real TF-COF interactions rather than background ligation.

The liquid PBM is not yet a replacement for slide-based platforms. But it works, at least at the proof-of-concept level, and it does so with less material and no specialized equipment. That is the contribution: a demonstration that PBM logic can function in solution, and a baseline from which further development can proceed.