Development of a Novel Method to Discover DNA:Protein Interaction Partners

P-01.1-09

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Regulatory functions of certain DNA elements, be it linear or adopting higher-order structures like DNA-quadruplexes, are often achieved in concert with proteins that bind to them, either via stable or transient interactions. Conventional methods for detection of protein interaction partners of known DNA are in general suitable for the detection of strong and stable interactions, however, weak and transient ones are often overlooked. To be able to detect all types of interactions, we set on to develop a novel approach for DNA:protein interaction partners discovery based on a three-component system that enables enzyme-catalyzed proximity labeling of proteins with biotin. It can be used either *in vivo* or *in vitro*, with an appropriate source of proteins added. The three components are (1) a synthetic nucleotide fragment F-DNA, (2) chimeric protein Tus–TurboID, which contains a mutant biotin ligase, and (3) chimeric protein *GAL4*(1–147)–sfGFP, which serves as the internal control of the system. F-DNA is the core element of the system and consists of a DNA sequence of interest fused to two other protein binding sites, one for each of the chimeric proteins; the components of the system and target interaction partners are therefore colocalized. Upon biotin addition biotin ligase Tus–TurboID biotinylates *GAL4*(1–147)–sfGFP and target interaction partners of the DNA of interest. Biotinylated proteins can be easily isolated and analyzed. To characterize the system and optimize the method, we first constructed an *in vitro* system involving a known DNA:protein interaction pair, namely GADD(DNA):p53(protein). After preparation of purified Tus–TurboID, *GAL4*(1–147)–sfGFP, and F-DNA we confirmed that chimeric proteins colocalize with F-DNA, using electrophoretic mobility shift assay. We also tested some of the reaction conditions during the biotinylation step, however, to make the method applicable, there is still a need for further optimization.