2.1. Yeast-2-Hybrid

Y2H was first developed in the late 1980s\textsuperscript{11} as a generalizable and highly sensitive method to screen for interactions among binary pairs of proteins and is still frequently used both as a first pass screening tool and for genome-scale exploratory studies today.\textsuperscript{16–18} Despite several design variations since its inception which have resulted in improved assay efficiency,\textsuperscript{19–22} the basic principle of the Y2H assay remains the same. That being that Y2H takes advantage of the fact that the process of transcriptional activation (and thus expression of a suitable reporter gene) depends on the tethering of two distinct protein domains to a target promoter: first, a DNA-binding domain (BD) that binds to the upstream DNA element and, second, an activation domain (AD) that binds to the reporter gene.

Figure 1. Basic overview of low- and high-resolution interaction surveying. (A) Low-resolution surveying (left) tends to begin with interaction data (typically in binary format), creates a cohesive interaction network map, and then assigns proteins to complexes through application of a clustering algorithm. This method, while often not as accurate as high-resolution mapping of macrocomplexes, can generally be applied to entire genomes. High-resolution surveying (right) is focused on determining conclusively the nature of protein associations, specifically through determination of associating secondary structure configurations. (B) Relative representation of interaction survey methods in the GRID database. The majority of representative interactions are the result of affinity capture-MS methods such as tandem affinity purification (TAP). The remaining majority are the result of yeast-2-hybrid studies.