

Building Interactomes

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The completion of the human genome represents an initial step towards identifying proteins involved in gene regulation, known as the interactome. Several tools have been utilized to explore and identify the different members of the interactome by mapping the complex protein-protein and protein-DNA interactions involved in the regulation of gene expression under normal and abnormal conditions. Our current knowledge of the interactome is being generated utilizing yeast two hybrid system (Y2H), protein microarrays, co-immunoprecipitation, Pull down assays, co-localization, and mass spectrometry. Mapping of the human interactome and other species will advance our knowledge about disease processes and help identify new drugs and drug targets improving the well being of humans.

The yeast two hybrid system

The Y2H technique has been very well utilized since its introduction by Stanley Fields in 1987. The Y2H is a genetic method utilized to identify protein-protein interactions relying on the architecture of trans acting transcriptional activators.

How it works?

The yeast-two hybrid system requires DNA manipulation to study protein-protein interactions. In the first step of the yeast two-hybrid system, the target protein is cloned into the "bait" vector. In this way, the gene encoding the bait protein is placed into a plasmid next to the gene encoding a DNA-binding domain (DBD) from some transcription factor (e.g. LexA), thus generating the DBD-Bait fusion (Ref). If a bait protein has no ability to activate per se the reporter genes in yeast, the yeast cell will not be able to survive on plates lacking histidine and will not turn blue (lacZ-). Separately, a second gene (or a library of cDNAs encoding potential interactors, collectively called "prey"), is cloned in frame adjacent to an activation domain of the Gal4 yeast transcription factor. Since a prey protein has no ability to bind DBD-responsive elements, the yeast cell will again not be able to survive on plates lacking histidine and will not turn blue (lacZ-). If the two proteins fused to the DBD and AD physically interact, they will bring the DBD and the AD close enough together to restore a functional transcription factor. The reporter genes, e.g. a nutritional selection marker (HIS3) or lacZ, are transcribed resulting in histidine prototrophy and blue coloration of yeast cells. Thus, the interaction of two proteins is measured by the reconstitution of a hybrid transcription factor and the consequent activation of a set of specific reporter genes.

Advantages of Y2H

The power of the yeast two-hybrid system lies in its combined approach, where growth selection enables sampling of highly complex cDNA libraries encoding millions of potential binding partners where only those clones which encode an interacting protein survive growth selection are analyzed further using a convenient color assay (e.g. BlueTech). The complexity of such analysis is time consuming and requires expertise, for these reasons the yeast two-hybrid custom services market is expanding and has evolved significantly involving liquid handling robotics for high throughput screening. The versatility of Y2H has allowed for examining single genes at a time resulting in the discovery of several hundreds of novel protein partners. The screening of a cDNA library by Y2H provides information regarding unknown proteins especially the ones of weak or transient interactions making it superior to pull down based assays (immunoprecipitation and affinity chromatography) and Far-Western blotting.

The introduction of an Y2H based kit to identify membrane protein-protein interactions is a major breakthrough in Y2H advancement, making the study of such proteins less challenging.

Mapping of Membrane Protein Interactions by Y2H

The DUALmembrane system kit is provided by Dualsystems Biotech, a Swiss based company. In the DUALmembrane system, the reporter protein is a fusion of the DNA-binding domain of the LexA protein and the Herpes simplex VP16 transactivator. The reporter is fused to the Cub moiety which in turn is fused to an integral membrane protein (collectively called Cub-TF). A transmembrane prey protein is fused to the NubG

moiety. The only requirement is that both Cub and NubG are located on the cytoplasmic face of the membrane. Co-expression of Bait-Cub-TF with a non-interacting Prey-NubG does not lead to the formation of split-ubiquitin nor cleavage by UBPs, resulting in cells that are His³- and lacZ⁻. If the bait and prey interact, Cub and NubG are brought into close proximity, where they will form split-ubiquitin, resulting in cleavage and liberation of the TF reporter. The reporter is now free to enter the nucleus, where it will bind and activate the reporter genes to result in cells that are His⁺ and that turn blue in a β -galactosidase assay. The system is the first genetic screening system for integral membrane proteins and membrane-associated proteins. It also can be utilized as a tool for screening cDNA libraries to find novel proteins interacting with the membrane protein of interest.

It is worth mentioning here the recent work of Serebriiskii et al., (2005), that involves a combined yeast/bacterial two hybrid system. This approach adds a new dimension to Y2H. This combined system will reduce the bait autoactivation phenomena causing false positives and thus reduce lab work required for verifying data. The use of Y2H is not only limited to identify protein-protein interactions, but also for verifying these interactions by performing competitive protein analysis (Ralser et al., 2005)

Y2H system is now considered as key tool for functional genomists because it provides the luxury of identifying protein-protein interaction without the need of antibodies or purified proteins; it is not the only tool for identifying physiological protein partners. These tools include affinity chromatography, immunoprecipitation (Co-IP, ChIP), Far-Western, and mass spectrometry which complements and helps in verifying the results obtained by the Y2H or vice versa.

References:

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