Protein-Protein Interaction-Yeast two Hybrid System

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Research Article

Abstract: Many proteins recognize and bind to other proteins**.** The total of all protein-protein interactions is sometimes referred to as the protein interactome by those enthusiastic about "omics" terminology. Mass screening of such interactions has proved possible by means of the "Two hybrid" system. It is assumed that the binding of a novel protein to one that is well characterized may provide some hint as the function. Two hybrid analyses depend on the modular structure of transcriptional activator proteins. Many of these proteins contain two factor domains -DNA Binding Domain (DBD) and Activation Domain (AD). The DBD recognizes a specific sequence in the DNA upstream of a promoter and the AD stimulates transcription by binding to RNA polymerase. Provided that the two domains interact, they will activate transcription. The whole experiment is based on this principle of Yeast two Hybrid system.

Keywords*:* DBD-DNA Binding Domain, AD-Activation Domain

Introduction

Two hybrid system is based on the modular structure of transcriptional activator proteins. Many of these proteins contain two factor domains -DNA Binding Domain (DBD) and Activation Domain (AD). The DBD recognizes a specific sequence in the DNA upstream of a promoter and the AD stimulates transcription by binding to RNA Polymerase. Provided that the two domains interact, they will activate transcription. It is not usually necessary for the two domains to be covalently joined to form a single protein.

In the two hybrid system, both the DBD and the AD are fused to two other proteins (X and Y). These two hybrid proteins are referred to as "bait" and"prey" respectively.The bait and prey fusion plasmids are transformed into yeast cells of different mating types. This results in two sets of approximately 6000 transformants. All possible matings are carried out between the two sets using a laboratory robot to manipulate the colonies. When the two yeast mate, the diploid cell will have a bait plasmid and a prey plasmid.

If the two fusion proteins X and Y interact, the reporter gene is switched on. In Yeast HIS3 or URA3 gene are usually used. If the reporter gene is not activated, the yeast strain cannot grow unless provided with histidine or uracil respectively. If the reporter gene is turned on, the cells can grow on medium without histidine or uracil. Thus the diploid cells from the 6000 X 6000 matings are selected on medium lacking the chosen nutrient. Only those combinations where proteins X and Y interact yield viable colonies.

Materials and Methods

YPAD agar plate, petriplate, nylon or nitrocellulose membrane, master plate containing yeas, 125mm Whatman filter papers, X gal solution, liquid nitrogen.

Z-buffer

-NaH2PO4.7H2O-1.61g
-NaH2PO4.H2O-0.48g
-KCl-0.07g
-MgSO4.7H2O-0.01g
-Water-upto 100ml
-Ph-7.0

Preparation of X-Gal

10mg of X-Gal is dissolved in 100ul of DMF(N,N-dimethyl formamide)

X-Gal Solution

100ul X-Gal in DMF**;** 60ul 2-mercaptoethanol and 10ml Z buffer were combined.

Procedure

1.From the master plate containing Yeast, replica plate the patches placed directly onto a membrane (nylon or nitrocellulose) that has been placed on the surface of a YPAD agar plate.

2.Two round 125mm whatman filter papers in a 15-cm petriplate.Saturate with about 5ml of the X-Gal solution were stacked. Air bubbles were removed.

3.Using forceps, carefully removed were the membrane from the surface of the YPAD plate. Completely immersed the membrane werein liquid nitrogen for 20-30sec.

4.The frozen membrane was placed on top of the soaked whatman filters colony side up. Air bubbles were removed. Tiping the plates slightly,the excess X-gal solution was removed.

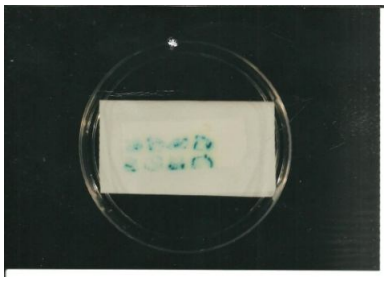
5.Covering the plates, they were incubated at 37degree Celsius. The plates were placed at a slight angle, so excess X-gal solution does not accumulate on the membrane.
6.The appearance of blue colourwas monitored over a 24 h. period. The final results were scored at 24h.

Principle

The protein coding sequence of the bait protein is cloned into a vector containing the DNA binding sequence (DBD-X (bait) fusion). The protein coding sequence of the prey protein is cloned into a vector that contains sequences for transcription activation (AD-Y (prey) fusion). Both vectors must also contain the necessary elements for growth and protein expression in yeast. The recombinant vectors are introduced into the appropriate yeast strain. Only if proteins X and Y physically interact with one another are the DBD and AD brought together to reconstitute a functionally active factor that binds to **Principle of the yeast two hybrid system.**The protein coding sequence of the bait protein is cloned into a vector containingthe DNA binding sequence (DBD-X (bait) fusion). The protein coding sequence of the prey protein is cloned into a vector that containssequences for transcription activation (AD-Y (prey) fusion). Both vectors must also contain the necessary elements for growth and proteinexpression in yeast. The recombinant vectors are introduced into the appropriate yeast strain. Only if proteins X and Y physically interactwith one another are the DBD and AD brought together to reconstitute a functionally active factor that binds to upstream specific sequencesof the reporter gene and activates expression.(Cell Biol Educ. 2002 Spring-Summer; 1: 43–62).

Result

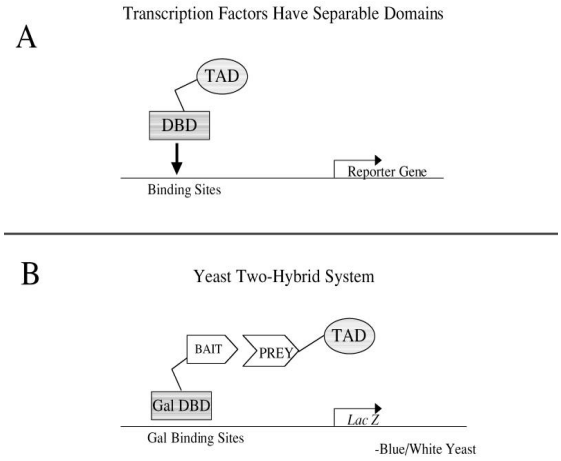
After 24h the appearance of blue colour is observed confirming protein-protein interaction.



Appearance of blue colony confirms protein-protein interaction(yeast two hybrid system).

Discussion

Thus the appearance of blue colony shows that protein-protein interaction has taken place. To identify all possible interaction has taken place. To identify all possible interactions usingthe 2-hybrid system, haploid upstream specific sequencesof the reporter gene and activates expression.



alfa yeast are transformed with a bait library, and are transformed with the prey library. When the 2 yeast types are mated with each other,the diploid cells will each contain a single bait fusion protein and a single prey fusion protein. If the two proteins interact, they activate the reporter gene, which turns the yeast cells blue when grown on X-Gal media(lacz gene from *E.coli*). This process can be done for all 6,000 yeast proteins using automated techniques.

Precautions

1.From the master plate containing Yeast replica plate, the patches formed directly onto a membrane (nylon or nitrocellulose) that has been placed on the surface of YPAD agar plate should be done carefully.
2.Two round 125mm whatman papers should be stacked in a 15cm petriplate and saturated with about 15ml of X-gal solution carefully, so that all the air bubbles are removed.
3.Using forceps, carefully remove the membrane from the surface of the YPAD plate and completely immerse the membrane in liquid nitrogen for 20-30 seconds.

References

1. Diversity in Genetic In Vivo Methods for Protein-Protein Interaction Studies: from the Yeast Two-Hybrid System to the Mammalian Split-Luciferase System Microbiol. Mol. Biol. Rev., 76 (2) 331-382, 2012.
2. Using the Two-Hybrid Screen in the Classroom Laboratory Daniel P. Odom and Martha J. GrosselCellBiol Educ., Spring-Summer; 1: 43–62, 2002.
3. A yeast two -hybrid system reconstituting substrate recognition of the von Hippel-Lindau tumor suppressor protein Nucleic Acids Res., 35 (21) e142, 2007.

4. FANCC, FANCE, and FANCD2 Form a Ternary Complex Essential to the Integrity of the Fanconi Anemia DNA Damage Response PathwayJ. Biol. Chem., 280 (43) 36118-36125, 2005.
5. The von Hippel-Lindau Tumor Suppressor Protein: Roles in Cancer and Oxygen Sensing Cold Spring HarbSymp Quant Biol., 70 (0) 159-166, 2005.
6. The In vitro and In vivo Effects of Re-Expressing Methylated von Hippel-Lindau Tumor Suppressor Gene in Clear Cell Renal Carcinoma with 5-Aza-2'-deoxycytidineClin. Cancer Res., 10 (20) 7011-7021, 2004.
7. Identification of Elongin C and Skp1 Sequences That Determine Cullin Selection J. Biol. Chem., 279 (41) 43019-43026, 2004.
8. CUL-2 and ZYG-11 promote meiotic anaphase II and the proper placement of the anterior-posterior axis in C. elegans Development, 131 (15) 3513-3525, 2004.