A two hybrid test for interaction partners of inner nuclear membrane resident proteins

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Abstract

A guideline and a sample protocol is provided for analysis of protein-protein interactions when at least one protein is a resident of the inner nuclear membrane.

Introduction

The classical two hybrid system \(1) provides an easy means for mapping binary interactions in the global interactomes, screening for new interactors of a protein of interest and testing specific interactions. A major pitfall of this system is the significant probability of false positive and false negative results. These types of errors are especially abundant in the case of integral membrane proteins since their hydrophobic portions tend to demonstrate somewhat nonspecific binding to each other. A common usual way to overcome this problem is to employ protein fragments originated from extramembranous loops. The more complicated split-ubiquitin system was proposed for protein-protein interactions of transmembrane proteins \(2). However, these approaches also have their own limitations, leading to large numbers of false positives and false negatives. Here we provide a guideline and a sample protocol to test for interaction partners of proteins that are natural residents of the inner nuclear membrane and contain at least one nucleoplasmic domain. Most importantly, in the case of these proteins, the full-length polypeptide chain, including intramenbrane segments, can be effectively used as “bait” for the search of protein partners. In our opinion, this approach may produce more reliable data in comparison with that obtained using protein fragments as baits. We have demonstrated usefulness of the classical yeast two hybrid system for membrane proteins containing nucleoplasmic domains as exemplified by identification of protein partners of the BetaM protein \(product of mammalian ATP1B4 gene), which is a natural inner nuclear membrane protein with N-terminus exposed into the intranuclear space \(3).

Reagents

pGBKT7 and pGADT7-RecAB vectors \(Clontech) AH109 yeast strain YPAD medium LiAc buffer \(0.1 M lithium acetate buffered with 10 mM Tris-HCl at pH 7.5), sterile PEG-3350 \(Sigma) Denatured salmon sperm DNA \(2.0 mg/ml) Sterile water SD agar plates -Leu,-Trp plates SD agar plates -Leu,-Trp plates supplemented with α-X-Gal SD agar plates -Leu, -His, -Trp SD agar plates -Ade, -Leu, -His, -Trp supplemented with α-X-Gal

Equipment

42˚C water bath 30˚C incubator shaker

Procedure

Clone your protein of interest in the pGADT7-RecAB vector, and two or more interactor proteins into the pGBKT7 vector using standard procedures in E. coli and proceed to yeast transformation. 1. Inoculate 10 ml of YPAD medium with a single fresh AH109 colony and incubate overnight on a shaker at 30˚C. Dilute the overnight culture with fresh to OD600 of 0.15 and incubate with shaking for approximately 2 h until OD600 is around 0.7. 2. Pellet the cells at 2000 g for 5 min at room temperature, pour off or aspirate the medium, resuspend the cells in 10 ml LiAc buffer and centrifuge again. Resuspend the pellet in 500 µl LiAc. 4. To 50 µl of the suspension of yeast competent cells add 1-3 µg DNA plasmids harboring each of the two interactor partners \(in GBKT7 and GADT7-RecAB), 300 µl sterile 40% w/v PEG-3350 solution in LiAc buffer, 25 µl salmon sperm DNA, and sterile water to 400 µl final volume. Gently mix and incubate at room temperature for 30 min. 5. Heat shock at 42˚C for 15 min. 6. Plate the shocked cells onto the SD -Trp, -Leu selective media and incubate for 2-3 days at 30˚C. 7. Streak several of the grown-up colonies of each cotransfromant on: a) SD -LEU, -HIS, -TRP \(low stringency plates) b) SD -Leu,-Trp plates supplemented with α-X-Gal \(plate for growth-independent test for α-galactosidase) c) SD -Ade, -LEU, -HIS, -TRP supplemented with α-X-Gal \(high stringency plates). 8. Incubate the plates for 3 days at 30˚C and compare growth of your interactor pairs on media with different stringency. If the color of the colonies is ambiguous, incubate the plates at 15˚C for three more days and recheck.

Timing

6-9 days

Anticipated Results

The specificity of interaction may be judged from a comparison of two interaction pairs where one serves as a negative control. Typically, both co-transformants should demonstrate good growth on \(-Leu,-Trp) plates with blue color developed only in the case of the true-positive interaction. Ideally, there may be a significant difference in growth rate of the clones on the low stringency medium \(-LEU, -HIS, -TRP). This is, however, quite rare in the case of membrane proteins, usually unspecific activation of the His gene leads to observable growth. Thus, the most important part is the growth on the high stringency medium \(-Ade, -LEU, -HIS, -TRP, +α-X-gal). The test should be considered positive for a pair of proteins if the contransformant grows on all of the aforementioned media and produce blue color in the presence of α-X-gal, whereas the control pair shows no observable growths on the high stringency medium and no blue pigment.

References

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