EXERCISE

7

Protein Interaction Analysis in Yeast

Background

The yeast two-hybrid system is a way to analyze protein-protein interactions in vivo. The system is based on the observation that many eukaryotic transcription factors consist of 2 separable domains: one domain binds a specific DNA sequence, and the other activates transcription. DNA-binding and activation domains from different transcription factors often retain function when combined. The two-hybrid system uses transcription of a reporter gene in yeast to assay the interaction between 2 proteins, one of which is fused to an activator domain, and the other is fused to a DNA-binding domain. If the 2 proteins in question interact, they will bring together the DNA-binding and the transcription–activation domains, and transcription of the reporter gene will occur. If the 2 proteins do not interact, transcription of the reporter gene will not occur, because although the DNA-binding domain can find its DNA target, it requires the activation domain to stimulate transcription.

Two uses of yeast two-hybrid screening are common. First, the system is used to screen for unknown genes encoding proteins that interact with a protein encoded by a known gene. Second, the approach is used to determine whether 2 known proteins interact and to study molecular aspects of the interaction.

You will use the yeast two-hybrid system to determine whether 2 *Agrobacterium* virulence proteins, VirE1 and VirE2, interact. The *virE2* gene is fused to the gene encoding the DNA-binding domain of the repressor protein LexA. The *virE1* gene is fused to the gene encoding the activation domain of the yeast transcription factor Gal4. If VirE1 and VirE2 proteins interact in vivo, the interaction will tether the Gal4 activation domain to a LexA binding site (operator) located upstream from a *lacZ* reporter gene. If *lacZ* is induced, the resulting β-galactosidase will turn

the yeast blue in the presence of the chromogenic substrate X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactoside).

You will use a yeast strain that contains the *lacZ* reporter gene. The *virE1* and *virE2* gene fusions reside in separate plasmids. You will transform plasmid DNAs into yeast. As controls, transform with no plasmid, and with the plasmids containing the DNA-binding and activation domains alone, without the fused proteins.

Plasmid	**Contains**
pAD	activation domain of Gal4
pAD–E1	activation domain of Gal4 fused to *virE1*
pBD	DNA-binding domain of LexA
pBD–E2	DNA-binding domain of LexA fused to *virE2*

Steps of the experiment are

A. Yeast transformation

B. Filter β-galactosidase assay

Notes

A. YEAST TRANSFORMATION

Procedure

(TAs do this part)

1. Grow yeast to 10^7 cells/mL in YEPD (1 optical density [OD] at 600 nm = 2×10^7 cells/mL).

(Students start here)

2. Prepare:
   a. Four 1.5-mL tubes, each containing 50 μg denatured salmon sperm DNA (10 μg/μL) and plasmid DNAs.

Tube	Plasmid DNA (0.1 μg/μL)
#1	0.5 μg pBD–E2, 0.5 μg pAD
#2	0.5 μg pBD, 0.5 μg pAD–E1
#3	0.5 μg pBD–E2, 0.5 μg pAD–E1
#4	no plasmid

   b. LITE (100 mM lithium acetate, pH 7.0; 10 mM Tris, pH 7.4; 1 mM EDTA)

   c. PEG–LITE

   d. Selection plates (YNB plus 2% galactose, 30 μg/mL leucine)

3. Harvest 50 mL yeast; centrifuge for 5 minutes, maximum speed in a bench-top centrifuge.

4. Wash yeast twice with TE (10 mM Tris, pH 7.0, 1 mM EDTA).To wash, add a small volume of TE, vortex to resuspend, add TE to 50 mL, and spin.

5. Resuspend yeast in LITE to 10^9/mL (i.e., 0.5 mL if a 50-mL culture was used).

6. Add 100 μL yeast suspension to DNA, mix.

7. Add 0.6 mL PEG–LITE, mix.
8. Shake 30 minutes at 30°C.
9. Heat shock for 15 minutes at 42°C.
10. Spread 100 μL on selection plates (larger volumes reduce transformation efficiency). Incubate 28° C.
    - expect 1000 to 2000 colonies per dish
    - if scoring transformation efficiency, spread 10 μL cells diluted with 90 μL PEG–LITE
    - for a simple plasmid transformation, use 0.5 μg plasmid
    - for a library transformation, use 2.5 μg
    - scale up by using proportionately more DNA, cells, LITE and PEG–LITE

Solutions for Yeast Transformation

50% PEG: Add 25 g polyethylene glycol (molecular weight, 3350) to 50-mL Falcon tube, add sterile water to 50 mL

LITE:
10 mL 1 *M* lithium acetate
10 mL 10× TE, pH 7.0
80 mL sterile water

PEG–LITE:
5 mL 1 *M* lithium acetate
5 mL 10× TE, pH 7.0
40 mL 50% PEG

1 *M* lithium acetate (filter sterilize)

10× TE, pH 7.0: 100 mM Tris, pH 7.0; 10 mM EDTA, pH 8.0 (filter sterilize)

Salmon sperm DNA (10 mg/mL): Purchased, or can be prepared as follows:

Combine 100 mg salmon sperm DNA with 10 mL distilled water. Sonicate 5 minutes at maximum setting.

Extract with 1 volume TE-saturated phenol, then with 1 volume 50:50 (v/v) TE-saturated phenol:$CHCl_3$, then with 1 volume $CHCl_3$. Precipitate with 1 mL 3 *M* sodium acetate and 25 mL ethanol. Wash pellet with 70% ethanol, dissolve in 10 mL sterile distilled water. Store aliquots at −20°C. Before use, boil 5 minutes and chill in ice water.

YNB–galactose agar:
850 mL distilled water
6.7 g yeast nitrogen base (YNB) without amino acids (Difco)
2 g dropout powder
0.1 g NaOH
20 g agar (Difco Bacto agar)

Autoclave, then add:
Galactose to 2% (filter sterilized)
Raffinose to 1% (filter sterilized)

For these experiments, dropout powder should contain adenine (2.5 g), L-arginine (1.2 g), L-aspartic acid (6 g), L-glutamic acid (6 g), L-isoleucine (1.8 g), L-leucine (3.6 g), L-lysine (1.8 g), L-methionine (1.2 g), L-phenylalanine (3 g), L-serine (22.5 g), L-threonine (12 g), L-tyrosine (1.8 g), and L-valine (9 g).

YEPD broth:
900 mL distilled water
10 g yeast extract (Difco)
20 g peptone (Difco)
0.1 g NaOH

Autoclave, then add 100 ml sterile 20% glucose

Notes

B. FILTER β-GALACTOSIDASE ASSAY

Procedure

1. Replica plate onto filter paper:
   a. Fasten a stack of two 12-cm Whatman filters to replica plating block
   b. Place culture upside down on filter and press down firmly
   c. Sharply, lift culture dish from filter (colonies should transfer completely to filter)
2. Trim filter so that it will fit in petri dish lid. Leave tag for label.
3. Freeze filter in liquid nitrogen for 1 minute; thaw for 1 minute.
4. Add 2 mL LacZ–BME buffer and 50 μL 40 mg/mL X-gal to petri lid.
5. Lay 9-cm filter onto puddle of LacZ–BME–X-gal.
6. Lay replica filter, **colony side up,** onto 9-cm filter.
7. Incubate at 30°C.
8. Note color development at 10, 20, 60, and 120 minutes (β-galactosidase-positive colonies will turn blue).

Results

Record the color at the specified times:

Input Plasmids	Color				
	10 min	20 min	30 min	60 min	120 min
pBD–E2 + pAD					
pBD + pAD–E1					
pBD–E2 + pAD–E1					

Solutions for Filter β-Galactosidase Assay

LacZ buffer (store at 20°C)

0.06 *M* $Na_2HPO_4 \cdot 7\ H_2O$	16.1 g/L (8.5 g/L if anhydrous is used)
0.04 *M* $NaH_2PO_4 \cdot H_2O$	5.5 g/L
10 mM KCl	0.75 g/L
2 mM $MgSO_4$ (anhydrous)	0.24 g/L
	Add water to 0.9 L; adjust pH to 7.0; and add water to 1 L

LacZ–BME (make fresh before use)
50 mL LacZ buffer
137 μL β-mercaptoethanol (13 *M* stock)

40 mg/mL X-gal (store at −20°C; TA will supply)
200 mg; dissolve in 5 mL DMSO

EXERCISE 7. STUDY QUESTIONS

1. What is the purpose of each control strain that you tested in this experiment?

2. If you use the yeast two-hybrid protein interaction trap to identify unknown genes encoding proteins that interact with a protein encoded by a known gene, how can you test whether the protein–protein interaction is genuine?

Notes