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Yeast two-hybrid interaction assay

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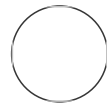
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COMMENTS 0



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ABSTRACT

Protein-protein interaction can be tested using yeast two-hybrid assays. The bait protein is fused to the Gal4 DNA-binding domain (BD) and the prey is fused to the Gal4 transcriptional activation domain (AD) are both expressed in the same yeast cell. Activation of the reporter genes occurs only if bait and prey interact.

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Yeast Two-Hybrid interaction assay

From Matchmaker Gold Yeast Two-hybrid system (Clontech/Takara Bio):

Vectors and yeast cell lines: plasmids pGADT7 (Prey) and Y187 (Leucine auxotrophy); plasmid pGBKT7 (Bait) and Y2HGold (Tryptophan auxotrophy)

Clone your genes in both vectors.

For DNA sequencing:

on pGADT7 and pGBKT7 use T7 promoter primer 5'-TAATACGACTCACTATAGGG .

on pGADT7 use primer 5'-AGATGGTGCACGATGCACAG

on pGBKT7 use primer 5'-TTTTTCGTTTTAAACCTAAGAGTC

1. Transformation of haploids :

The day before :

Plate Y187 (*MAT α*) and Y2HGold (*MAT α*) yeast cell lines on YPDA. The plates must be generously covered.

The day of transformation:

- For each transformation (include the No DNA transformation control), take a sterile loop-full of yeast cells from overnight growth on a plate, resuspend in 1 ml sterile H₂O (in 1.5 ml tube) by vortexing.

- Spin 30 sec, 16000g, RT

- Remove the supernatant

- Add to the pellet in the tube without pipetting and in the following order :

Component, in	Concentration	Volume	Comment
PEG 3500	50% w/v in H ₂ O	240 ul	
LiAc	1 M in H ₂ O	36 ul	
Boiled Salmon	2 mg/mL in H ₂ O	50 ul	boiled 5-10 min and place on the ice
Plasmid DNA		1-5 ug in 34 uL H ₂ O	Alternatively, use 15uL of a miniprep

- Mix by vortexing.
- Incubate at 42°C for 2-3 hours.
- Centrifuge 30 sec, 16000g and discard the supernatant (transformation mix).
- Resuspend the yeast cell pellet in 200ul sterile H₂O and plate 20ul and ≈180ul on SC–Leucine (SC-L, for pGADT7 vectors) and SC–Tryptophan (SC-W, for pGBKT7 vectors).
- Incubate at 30°C for 3-4 days and pick 4 colonies.
- Make frozen stocks.

2. Toxicity test.

Both the prey and bait need to be tested for toxicity before the interaction test.

The growth of your “constructs” need to be compared to the growth of the cells transformed by the empty pGADT7 or pGBKT7 vectors using the drop test (see below).

3. Drop test on 120x120 plates (ref 688102, GBO):

WARNING: Humidity will spread your drops, so you really need to dry your plates thoroughly before performing the drop test.

- In 10 mL SC-W or SC-L liquid culture put a loop of yeast cells from a single haploid colony and grow ON, 30°C, 250 rpm.
- Next day, count your cells (Mallassez cell or OD_{600nm} : one OD unit = 10⁷ cells/ml).
- Pellet 10⁸ cells by spinning 5 min, 3000 rpm using a benchtop centrifuge.
- Resuspend the pellet in 1 ml sterile H₂O. Cells are at 1.10⁸ cells/ml.
- Make 1 mL dilutions to 10⁷-10⁶-10⁵-10⁴-10³ cells/ml in sterile water
- Load by 10 ul drops your 6 dilutions onto a plate: Your first drop contains 10⁶ cells and the last drop 10 cells.
- Incubate at 30°C, 3 to 7 days.

4. Autoactivation test.

The pGBKT7 haploid transformed cells are plated on the interaction test media (SC-W-H, and SC-W-Ade, and/or +aureobasidine, and/or +X-a-Gal) to assess their autoactivating properties by drop test. The growth of your “constructs” need to be compared to the absence of growth of the cells transformed by the empty pGBKT7 vector.

Do a drop test as described above.

5. Yeast mating for diploids

- For each haploid *MATa* and *MATalpha* and using the tip of a fresh cone, take a small swipe and mix together in a 50 mL tube containing 500 ul 2x YPDA medium at 30°, 250 rpm, O/N or all day. For 2x YPD : add adenine (2x final) to YPD medium.

To select the diploids:

- Centrifuge (3-5 min at 3000 rpm), remove the supernatant
- Wash the pellet with 1 ml of H₂O
- Centrifuge (3-5 min at 3000 rpm), remove the supernatant
- Add 1 ml of water
- On three separate plates, spread 100 ul, 10 ul (add 10 ul to 90 ul H₂O), and remaining (spin, resuspend in 200ul H₂O) on SC-L-W plates.

- After 2-3 days, you can collect 2-4 colonies.

6. Interaction assay

Do a drop test with your diploids, with the positive and negative controls.

- The positive control is pGBKT7-53 (which encodes the Gal4 DNA-BD fused with murine p53) and pGADT7-T (encodes the Gal4 AD fused with SV40 large T-antigen). Since p53 and large T-antigen are known to interact in a yeast two-hybrid assay, diploid cells will grow on interaction test media.

- The negative control is pGBKT7-Lam (which encodes the Gal4 BD fused with lamin) and pGADT7-T. The diploid cells will not grow on interaction test media.

- Load your 10 ul drops (see 3. Drop test) on the chosen interaction medium depending on the stringency:

SC-W-L (growth control)

SC-W-L-H

SC-W-L-A

SC-W-L+X-aGal

SC-W-L+Aur-A

SC-W-L+ X-a Gal+Aur-A

- incubate at 30°C for 3-7 days and compare growth with controls.

Media, recipes

SC medium

component	1L	4x400mL	4L
YNB (w/o ammonium sulfate) (BD, #233520)	1.7g	2.7g	6.8g
Ammonium sulfate (Euromedex, #2019)	5g	8g	20g
CSM (-L, -H, -W, -Ade, -Ura) (MP, #4550-122)	0.59g	0.944g	2.36g
Dextrose (D-+Glucose) (Euromedex, #UG305)	20g	32g	80g

SC composition

- Aliquot in 400ml bottles and autoclave 110°, 30 min and store at RT.

- To pour 120x120 plates, add 10g agar agar (euromedex ref 1330-D) for 400ml, autoclave and pour the plates.

YPD-A

Component	4L	4x400mL
Yeast extract (Euromedex, #Uy2010)	40 g	16 g
Bactopeptone (BD, #211677)	40 g	16 g
Dextrose (Euromedex, #UG3050)	80 g	32 g

YPD composition

- Aliquot in 400ml bottles and autoclave at 110°C, 30 min.

-->Add Adenine (100x stock, 4°C) before use

- To pour 120x120 plates, add 10g agar agar (euromedex ref 1330-D) for 400 ml, autoclave, add adenine (1x from 100x stock) and pour the plates.

Amino acid and nucleotide stocks in H₂O ; autoclave 110°C, 30 min

A	Reference	Concentration	Comment
Aureobasidine-A	clontech 63046	100ul of stock	stock : 500 ug/ml in ethanol (stored at 4
L-Leucine (100 X)	Sigma L-8000	10 g/L	
Uracil (100 X)	Sigma U-0750	2 g/L	0.8 g uracil + 1.68 g bicarbonate (NaHC
L-Histidine (100 X)	Sigma H-8000	2 g/L	
Adenine (100 X)	sigma A-8626	4 g/L	2 g for 450 ml H ₂ O (hot H ₂ O) + 2.08 ml
L-Tryptophan (100 X)	Sigma T0254	5 g/L	
X-alpha-Galactose	Euromedex EU	800 ul of stock	stock : 20 mg/ml in Dimethyl Formamid

Amino acids and nucleotides stocks

Glycerol stocks

Store your yeast cell stocks at -80°C in 1 ml of 15% glycerol/85% appropriate SC medium.

On day 1, plate your cells (cover the totality of the plate). ON.

On day 2, add a big loop of cells to 1 ml of 15% glycerol/85% appropriate medium, vortex, freeze on dry ice and store at -80°C.

