Yeast two-hybrid interaction assay

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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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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.

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'-TTTTCGTTTTAAAACCTAAGAGTC

1.Transformation of haploids :

The day before :
Plate Y187 (*MATalpha*) and Y2HGold (*MATa*) 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 H_2O (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 order:	Concentration	Volume	Comment
PEG 3500	50% w/v in H2O	240 ul	
LiAc	1 M in H2O	36 ul	
Boiled Salmon sperm carrier DNA	2 mg/mL in H2O	50 ul	boiled 5-10 min and place on the ice, vortex before pipetting
Plasmid DNA		1-5 ug in 34 uL H2O final.	Alternatively, use 15uL of a miniprep diluted with 19uL H2O.

- 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.

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- 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 = 10^7 cells/ml).
- Pellet 10^8 cells by spinning 5 min, 3000 rpm using a benchtop centrifuge.
- Resuspend the pellet in 1 ml sterile H_2O. Cells are at 1.10^8 cells/ml.
- Make 1 mL dilutions to 10^7-10^6-10^5-10^4-10^3 cells/ml in sterile water
- Load by 10 ul drops your 6 dilutions onto a plate: Your first drop contains 10^6 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 H_2O
- 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 H_2O) 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

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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, #UG3050)	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 H_2O ; autoclave 110°C, 30 min

A	Reference	Concentration / dilution	Comment
Aureobasidine-A	clontech 630466	100ul of stock solution in 400 mL	stock : 500 ug/ml in ethanol (stored at 4°C). Short life.
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 (NaHCO3) for 400 ml H2O (hot H2O)
L-Histidine (100 X)	Sigma H-8000	2 g/L	
Adenine (100 X)	sigma A-8626	4 g/L	2 g for 450 ml H2O (hot H2O) + 2.08 ml HCl 12N
L-Tryptophan (100 X)	Sigma T0254	5 g/L	
X-alpha-Galactose	Euromedex EU0012	800 ul of stock solution for 400 mL	stock : 20 mg/ml in Dimethyl Formamide, -20°C

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.

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