

Chapter 21

Yeast Two-Hybrid Screens: Improvement of Array-Based Screening Results by N- and C-terminally Tagged Fusion Proteins

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Abstract

Matrix-based yeast two-hybrid screens are an alternative to library-based screens. Recent improvements of matrix screens (also called array screens), use various pooling strategies as well as novel vectors that increase its efficiency while decreasing the false-negative rate, thus increasing reliability. In this chapter, we describe a screening strategy that systematically combines N- and C-terminal fusion proteins using a recently developed vector system.

Key words: Yeast two-hybrid, Protein–protein interactions, Permuted fusion tags

1. Introduction

1.1. The Yeast Two-Hybrid System

The yeast two-hybrid (Y2H) method was originally developed by Stanley Fields and is a genetic method to detect binary protein–protein interactions (PPIs) (1). It exploits the modularity of eukaryotic transcription factors and the ease of genetic engineering of the yeast, *Saccharomyces cerevisiae*, to monitor PPIs. A bait protein is fused to the DNA-binding domain (DBD) and a prey protein is fused to the activation domain (AD) of a transcriptional activator, often the yeast Gal4 protein. The term “two-hybrid” is based on these two chimeric proteins. The bait and prey fusions are co-expressed in yeast and upon physical interaction between the bait and prey protein, the functional transcription factor (TF) is reconstituted. This results in the activation of a reporter gene, which allows either growth under selective conditions or produces a color or fluorescence signal (auxotrophic yeast strain, lacZ, or GFP reporter gene).

1.2. Matrix-Based Yeast Two-Hybrid Screens

In a matrix-screen, the possible combinations of open reading frames (ORFs) are systematically examined by performing direct mating of a set of baits with a set of preys expressed in opposite yeast mating types. This has two major advantages compared to the library screen approach:

1. Each prey is arrayed on an individual position. Thus, the interacting prey can be simply identified by the matrix position and additional identification steps of the interacting prey by a colony PCR and sequencing reactions are obsolete.
2. Array screens can be automated by using a replication robot.

1.3. Combining N- and C-terminally Fused Test Domains

Yeast two-hybrid screens do not generate complete protein interactomes. As for any other detection method, it is almost impossible to detect all physiologically occurring interactants of every screened bait protein. Apart from effects that originate in the heterogenic yeast expression system, e.g., due to a lack of posttranslational modifications, false-negative interactions can be partly traced back to steric hindrance effects due to the used fusion tags. They can prevent physical interactions by covering the respective interaction sites or preventing subsequent transcriptional activation.

Most Y2H vector systems use N-terminally fused test domains, but this can avoid any interactions which involve regions around the N-terminus of these proteins. Thus, we developed C-terminal fusions of the DNA-binding and activation domains and also tested pairwise combinations of N- and C-terminal fusions (2, 3). Stellberger et al. (2) tested all pairwise interactions among the ~70 ORFs of the Varicella Zoster Virus using both N- and C-terminal vectors as well as combinations thereof (Fig. 1). About ~20,000 individual Y2H tests resulted in 182 NN, 90 NC, 151 CN, and 146 CC interactions (Fig. 2). Overlaps between screens ranged from 17% (NC–CN) to 43% (CN–CC). Performing four screens (i.e., permutations) instead of one resulted in about twice as many PPIs, and thus fewer false-negatives. Different vector combinations show unique, as well as overlapping PPI-data, supporting the impact of steric hindrance and the need of free termini for a sub-fraction of PPIs (Fig. 3).

2. Materials

2.1. Yeast-Rich Media

1. 1 YEPD liquid medium: 10 g yeast extract, 20 g peptone, and 20 g glucose. Make up to 1 L with sterile water and autoclave.
2. YEPD solid medium: 10 g yeast extract, 20 g peptone, 20 g glucose, and 16 g agar. Make up to 1 L with sterile water and autoclave. After autoclaving cool media to ~60°C, add 4 ml of 1% adenine solution [1% in 0.1 M NaOH (see Note 1)]. Pour

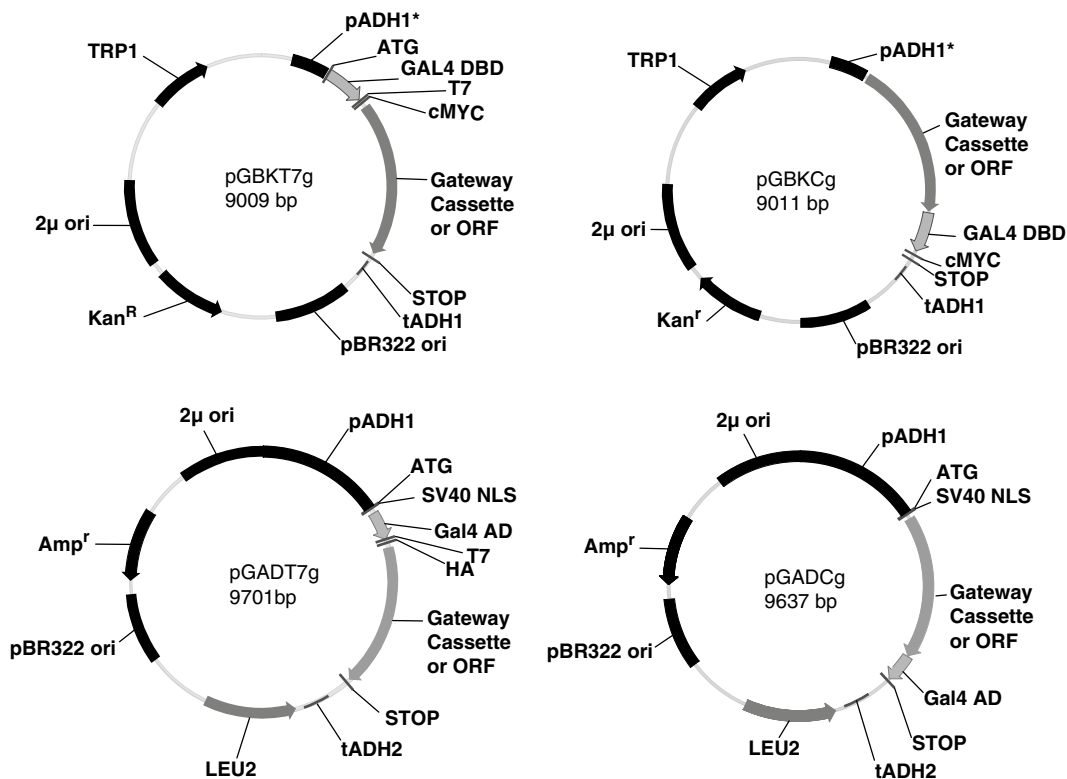


Fig. 1. Vectors described in this study including their parental vectors. pGBKT7g and pGADT7g generate N-terminal fusions of DNA-binding (DBD) and activation domain (AD) fusions, respectively. The new vectors pGBKCg and pGADCg fuse DBD and AD at the C-terminus of inserted ORFs. Note that both pGBK-vectors use a truncated version of the ADH promoter (indicated by *asterisk*) which may reduce expression levels and thus interaction signals (8, 9).

about 40 ml into sterile 1-well plates in a clean bench and let them solidify (see Note 2).

2.2. Yeast-Selective Media

1. Dropout mix (-His, -Leu, and -Trp): 1 g methionine, 1 g arginine, 2.5 g phenylalanine, 3 g lysine, 3 g tyrosine, 4 g isoleucine, 5 g glutamic acid, 5 g aspartic acid, 7.5 g valine, 10 g threonine, 20 g serine, 1 g adenine, and 1 g uracil. Mix all components and store under dry conditions at room temperature.
2. Medium concentrate (5×): 8.5 g yeast nitrogen base, 25 g ammonium sulfate, 100 g glucose, and 7 g dropout mix. Make up to 1 L with water and sterile filter. Store at 4°C (see Notes 3 and 4).
3. Amino acid stock solutions (see Note 1): 4 g/L histidine, 7.2 g/L leucine, and 4.8 g/L tryptophan. Each amino acid dissolved in water and sterile filtrated.
4. 3-amino-triazole (3-AT) stock solution: 0.5 M. Sterile filtrate (see Note 1).
5. For 1 L of minimal medium autoclave 16 g of agar in 800 ml of water, cool the medium to ~60°C, and then add 200 ml 5×

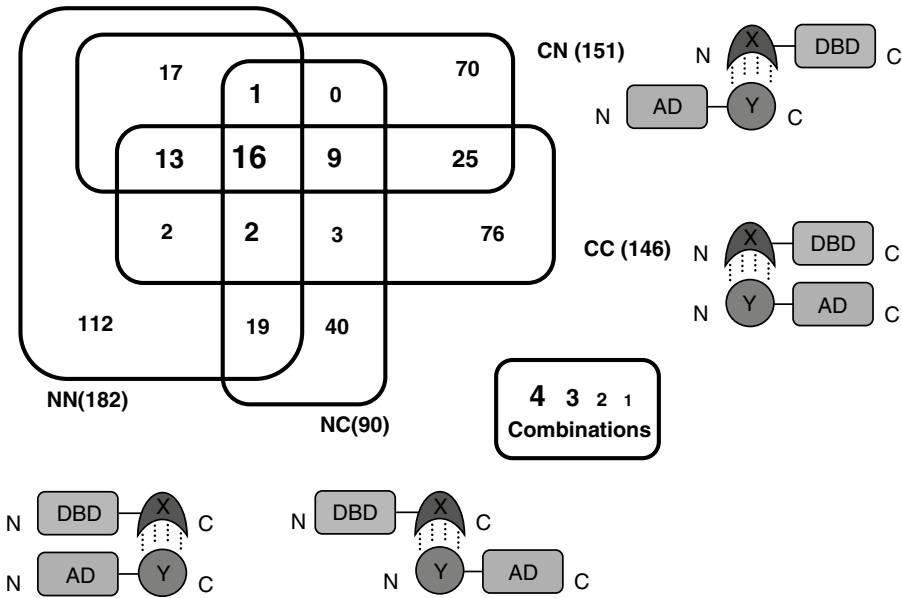


Fig. 2. Overlaps between tag-topology combinations. This *Venn diagram* shows the distribution of 405 VZV interactions found in either one or more tag-topologies. One hundred and eighty two interactions were detected in the traditional screen (NN); 90, 151 and 146 PPIs were additionally identified in the new combinatorial screens (NC, CN, and CC, respectively). The differences in the tag topologies are sketched next to the corresponding topology abbreviations. The number of overlaps between different screens is indicated by the thickness of numbers decreasing from 4 to 1. Modified after ref. 2.

medium concentrate and mix. Pour ca. 40 ml into each sterile Omnitray plate under sterile hood and let them solidify (see Note 2). Depending on the required selective plates you have to add the missing amino acids or 3-AT. Liquid minimal media can be prepared without adding agar. Corresponding amino acids are added from the amino acid stock solutions as follows (see Note 5).

6. Selection of baits (-Trp plates): 8.3 ml leucine and 8.3 ml histidine.
7. Selection of preys (-Leu plates): 8.3 ml tryptophan and 8.3 ml histidine.
8. Selection of diploids (-Leu-Trp plates): 8.3 ml histidine.
9. Readout medium (-Leu-Trp-His plates): add 3-AT from 0.5 M stock solution as needed for screening self-activating baits.

2.3. Yeast Transformation

1. Carrier DNA (salmon sperm DNA): dissolve 7.75 mg/ml salmon sperm DNA in water and store at -20°C following a 15 min 121°C autoclave cycle.
2. 96 PEG solution (100 ml): mix 45.6 g PEG, 6.1 ml of 2 M LiOAc (lithium acetate), 1.14 ml of 1 M Tris-HCl pH 7.5, and

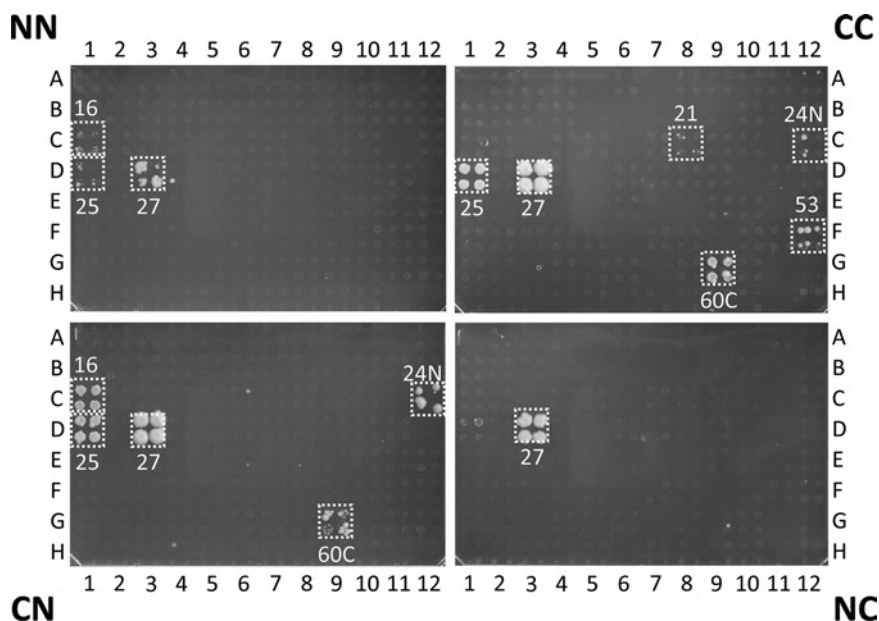


Fig. 3. Different vector combinations detect common, as well as different PPIs. Y2H screens of the four different vector combinations showing the differences on 25 mM 3AT. The same bait, ORF24N (Uniprot accession P09280, 238 N-terminal amino acids) was used as bait with N- and C-terminally fused DNA-binding and activation domains and screened against a whole-genome array of Varicella Zoster Virus (VZV). The N-terminal bait and prey constructs (in pGBKT7g, pGADT7g, NN) show different interaction patterns compared to the C-terminal constructs cloned into pGBKCg and pGADCg (CC) as well crosswise combinations thereof (NC and CN). Preys are indicated by their *ORF number*, e.g., the prey ORF27 is a subunit of the VZV nuclear egress complex together with ORF24 and was described in HSV-1 (10). Note that *N* and *C* labels near yeast colonies indicate N- and C-terminal protein fragments, not AD or DBD fusions (e.g., 60 C is a C-terminal domain of ORF60).

232 μ l 0.5 M EDTA; make up to 100 ml with sterile water and autoclave. Store 96 PEG solution at room temperature.

3. CT110: mix 20.73 ml 96PEG, 0.58 ml boiled salmon sperm DNA (boil frozen salmon sperm DNA at 95°C for 5 min) and 2.62 ml DMSO. Add DMSO last and mix quickly after adding by shaking vigorously and vortex for 30 s (see Note 6).

2.4. Screen Procedure, Retests, and Bait Self-activation Test

1. 96-Well microtiter plates, round bottom.
2. 1-Well plates.
3. Bleach solution (20%): dilute a 12% sodium hypochlorite solution 1:5 with water (see Note 7).
4. 95% ethanol solution, industrial.
5. Autoclaved water.
6. Replication tool or robot, 96- and 384-pinning tool.
7. 1% (*w/v*) adenine solution (1% in 0.1 M NaOH), sterile filtrate.
8. YEPD and selective media as liquids and agar plates as described (see Subheading 2.1).

2.5. Vectors

1. Bait plasmids: pGBKT7g (4) and pGBKCg (2).
2. Prey plasmids: pGADT7g (4) and pGADCg (2).

Any other vectors can be used as long as they are compatible with each other and the yeast strains.

2.6. Yeast Strains

1. AH109: genotype (MAT a, trp 1-901, leu2-3, 112, ura3-52, his3-200, Δ gal4, Δ gal80, LYS2: GAL1_{UAS}-GAL1_{TATA}-HIS3, GAL2_{UAS}-GAL2_{TATA}-ADE2, URA3: MEL1_{UAS}-MEL1_{TATA}-lacZ) (5, 6).
2. Y187: genotype (MAT α , ura3-52, his3-200, ade2-101, trp1-901, leu2-3, 112, Δ gal4, met, Δ gal80, URA3: GAL1_{UAS}-GAL1_{TATA}-lacZ) (7).

3. Methods

The following protocols describe the Y2H assay with the HIS3 reporter and the pGBKT7g/pGADT7g vector system. The protocols are applicable for the combinations with the pGBKCg/pGADCg system and others as well. However, if using other vector systems, different yeast and *E. coli* selection markers have to be considered during the selection steps and the selection media have to be exchanged.

Each Y2H screen may be repeated four times using the two different bait- and two different prey arrays:

1. pGBKT7g-pGADT7g (NN).
2. pGBKT7g-pGADCg (NC).
3. pGBKCg-pGADT7g (CN).
4. pGBKCg-pGADCg (CC).

3.1. Yeast Transformation for Bait and Prey Construction

This protocol is suitable for 100 yeast transformations and may be scaled up or down as needed. Selection of the transformed yeast cells requires leucine or tryptophan-free media (“-Leu” or “-Trp,” depending on the selective marker on the plasmid). Moreover, at least one of the haploid strains must contain a two-hybrid reporter gene under GAL4 control.

1. Inoculate 50 ml YEPD liquid medium with ~200 μ l liquid stock of yeast strains (e.g., AH109, Y187 or any other appropriate yeast strain; use Y187 strains for preys and AH109 for baits or vice versa) in a 250-ml flask and grow overnight with shaking at 30°C (minimum 15 h and max. 24 h).
2. Spin down cells in 50-ml conical tube (3,000 \times g, 5 min at room temperature), pour off supernatant and dissolve the pellet by adding 2 ml LiOAc (0.1 M) and transfer resuspended yeast to two 1.5-ml microfuge tubes. Spin out yeast and resuspend in a total volume of 1.8 ml LiOAc (0.1 M).

3. Prepare CT110 solution.
4. Add all the competent yeast cells prepared above and mix vigorously by hand or by vortexing for 1 min. Immediately pipette 245 μ l into each of 96 wells of a 96-well plate.
5. Add 50–100 ng of plasmid and positive control (e.g., empty vector) and negative control (only CT110). Seal the 96-well plate with plastic or aluminum tape and vortex for 4 min.
6. Incubate at 42°C for 30 min.
7. Spin the 96-well plate for 10 min at 1,500 g; discard the supernatant and aspirate with eight channel wand or by tapping on cotton napkin for couple of times. Add 150 μ l of sterile water to all 96 wells, resuspend and plate cells on selective agar plates (e.g., standard Petri dishes) with -Leu for pGADT7g/pGADCg or -Trp for pGBKT7g/pGBKCg.
8. Incubate plates at 30°C for 3 days. After 2 days, the colonies start to appear; pick colonies after 3 days.
9. Rearray baits and preys in 96-well plates. Grow them up again for 1–2 days in -Leu- or -Trp-liquid minimal medium at 30°C (see Note 8).
10. The bait and prey plate can now be used to make a couple of copies on selective agar medium, to backup the arrays as glycerol (25%) stocks for -80°C long-term storage, and to use the baits directly for the self-activation test (see below). For plate storage at 4°C, it is recommended to have haploids rather on minimal agar medium than on YEPD medium since loss of plasmids can occur on nonselective medium.

3.2. Bait Self-activation Test

The aim of this test is to measure the background reporter activity (here: HIS3) of bait proteins in the absence of an interacting prey protein. This measurement is used for choosing the selection conditions used during the interaction screen and can be achieved by mating individual bait strain with a single prey strain that carries the empty prey plasmid. Ninety-six individual bait activation tests can be carried out on one plate simultaneously.

1. Load a 96-well plate (round bottom) with ~200 μ l YEPD liquid medium.
2. Inoculate plate with baits by replicating the 96-format bait plate from solid medium into the destination plate by using a sterile 96-pinning tool (see Subheading 3.3.1 for sterilization details).
3. Inoculate the yeast strain Y187 which carries the empty prey vector in 30–50 ml YEPD liquid medium.
4. Grow yeast for ~18 h at 30°C (it is not necessary to shake the 96-well plate, whereas shaking of the prey strain in a flask is recommended).

5. Pellet yeast by centrifugation for 10 min at $1,500 \times g$; discard the supernatant; and aspirate with eight channel wand or by tapping on cotton napkin for a couple of times.
6. Use 96-replication tool to pin baits from 96-well source plate onto a YEPD single-well agar plate as quadruplicates.
7. Pour the yeast strain with the empty prey vector into a single-well plate.
8. Use 384-replication tool to pin yeast onto the YEPD single-well agar plate that harbors the baits already.
9. Mating occurs at 30°C for 1 to max. 2 days.
10. Replicate from mating plate on -Leu-Trp agar single-well plates to select diploids.
11. Incubate for 2–3 days at 30°C .
12. Pin diploids on -Leu-Trp-His agar medium in single-well plates with different concentrations of 3-AT (e.g., 0, 1, 2, 4, 8, ..., 128 mM).
13. Select yeast for about 7 days at 30°C .
14. Determine minimal-inhibitory concentration of 3-AT which is needed for a single bait to suppress self-activation growth for use in the interaction screen.

3.3. Yeast Two-Hybrid Screen

3.3.1. Preparations

1. Sterilization steps: sterilize the pinning tool by dipping the pins into a 20% bleach solution for 20 s, sterile water for 1 s, 95% ethanol for 20 s, and sterile water again for 1 s. Repeat this sterilization after each transfer (see Note 9).
2. Prepare prey array for screening: use the sterile replicator to transfer the yeast prey array (e.g., 384 format) from selective plates to single-well plates containing solid YEPD medium and grow the array overnight in a 30°C incubator (max. 24 h) (see Note 10). Ideally, the template prey array should be kept on selective plates.
3. Prepare bait liquid culture (DBD fusion-expressing yeast strain): inoculate 20–30 ml of liquid YEPD medium in a 50-ml conical flask with a bait strain from plates with selective medium and grow in a 30°C shaker for 18–22 h.

3.3.2. Mating Procedure

1. Add a corresponding volume adenine from a 1% adenine stock solution to a final concentration of 0.004% into the bait liquid culture. This step is recommended to obtain a higher mating efficiency (see Note 11).
2. Pour the overnight liquid bait culture into a sterile 1-well plate. Dip the sterilized pins of the pin-replicator [thick pins (diameter >1 mm) should be used to pin baits] into the bait liquid culture and place directly onto a fresh 1-well plate containing solid YEPD media. Repeat with the required number of plates

and allow the yeast to dry onto the plates for ca. 10 min (see Note 12).

3. Pick up the prey array yeast colonies with sterilized pins [thin pins (≤ 1 mm diameter) should be used, see Note 13] and transfer them directly onto the baits pinned onto the YEPD plate so that each of the 384 bait spots per plate receives different prey yeast cells (i.e., a different AD fusion protein).
4. Incubate 1–2 days at 30°C to allow mating. Mating will take place in <15 h, but a longer period is recommended (max. 2 days) because some baits strains show poor mating efficiency.

3.3.3. Selection of Diploids

1. Transfer the colonies from YEPD mating plates to single-well plates containing -Leu-Trp medium using the sterilized pinning tool (thin pins should be used in this step).
2. Grow for 2–3 days at 30°C until the colonies are >1 mm in diameter (see Note 14).

3.3.4. Interaction Selection

1. Transfer the colonies from -Leu-Trp plates to a single-well plate containing solid -His-Leu-Trp agar, using the sterilized pinning tool. If the baits are self-activating, they have to be transferred to -His-Leu-Trp with the specific concentration of 3-AT which was determined in the self-activation assay (see Subheading 3.2). Incubate at 30°C for 6–10 days (see Note 15).
2. Score the interactions by looking for growing colonies that are significantly above background by size and that are present as duplicate (or quadruplicate) colonies. Scoring can be done manually or using automated image analysis procedures. When using image analysis, care must be taken not to score contaminated colonies as positives.

3.4. Retests

Testing for reproducibility of interactions greatly increases the reliability of the interaction data. This protocol is used for retesting interaction pairs detected in a Yeast two-hybrid screen.

1. Rearray bait and prey strains or positively tested prey pool of each interaction pair to be tested in 96-well microtiter plates. Use an individual 96-well plate for the baits, as well as for the preys. For each retested interaction, fill one well of the bait plate and one corresponding well of the prey plate with ~200 μ l YEPD.
2. For each retested interaction, inoculate the bait strain into a well of the 96-well bait plate and the prey strain at the corresponding position of the 96-well prey plate. For example, bait “X” is transferred at positions B1, B2, and B3 of the bait plate.

The preys to be tested are arrayed into B1 (prey 1), B2 (prey 2), and the prey strain that carries the empty prey vector into B3 of the prey plate. The B3 test position is the control that helps to verify the background/self-activation.

3. Incubate the plates over night at 30°C.
4. Spin the bait and prey plates for 10 min at 1,500 × *g*.
5. Discard the supernatant and aspirate with eight channel wand or by tapping on cotton napkin a couple of times.
6. Pin baits with a sterile 96-pinning tool on -Trp and preys on -Leu selective agar medium as quadruplicates.
7. Allow baits and preys to grow at 30°C for 2–3 days.
8. Mating: first, transfer baits with a sterile 384-pinning tool on YEPD mating plates and second, transfer preys onto baits.

The rest of the procedure can be done according to the screening protocol. For interaction retesting diploids are pinned on -Leu-Trp-His selective media plates with different concentration of 3-AT. The control test position has to be compared to bait self-activation background signals. Reproducible interactions should show up on different concentrations of 3-AT, whereas the activation control test position indicates clearly no colony growth (see Note 16).

4. Notes

1. Stock solutions can be stored up to 6 months at 4°C. Alternatively, the stock solutions can be frozen as aliquots at -20°C for long time storage.
2. 1-Well plates are available from NUNC (Thermo Fisher Scientific). Prepared agar plates should be stored for 1–2 days with closed lid under a sterile hood before use. Fresh solidified media is often wet and cannot be used directly.
3. Medium concentrate can be stored at 4°C up to 6 months.
4. Some components of the medium concentrate (e.g., amino acids) are not well soluble in water. The solution has to be stirred before the filtration step for up to 5 h until all components are dissolved. Heating is not recommended because of the heat sensitivity of amino acids.
5. Selection media may differ due to the used Y2H expression vector system and have to be adapted. For instance, in the pDEST32/pDEST22 system the selection markers for baits and preys are interchanged (baits are selected on -Leu and preys on -Trp) while selection of pGBKT7g/pGBKCG baits is

done on -Trp. pGADT7g/pGADCg preys must be selected on -Leu medium.

6. CT110 has to be prepared freshly before yeast transfection and should not be stored.
7. Sodium hypochlorite solution is not very stable and has to be freshly prepared. Alternatively, other disinfection solutions with a bleaching effect can be used. We do not recommend to use a final concentrations higher than 2.4% since the steel pins of the replication tool might stain.
8. Yeast on agar medium can be stored for ~2 months at 4°C. The plates should be sealed with a sealing film to avoid drying-out. Baits and preys should be stored on the corresponding selective media since loss of plasmids can occur on nonselective medium.
9. Sterilization steps have to be established for the robotic system and sterilization solutions that are used. For instance, the minimal time required for sterilization should be tested in advance since this will speed up the whole screen. However, it must be ensured that no cross-contamination occurs.
10. The needed baits and prey arrays can also be used for the mating procedure when grown on/in selective medium. To our knowledge this does not influence the mating efficiency much but we recommend using YEPD medium since yeast grows faster and higher cell numbers can be achieved.
11. Adenine achieves a higher mating efficiency. Many yeast strains (e.g., AH109 and Y187) are deficient in synthesizing adenine since they can carry an additional adenine selection marker.
12. After transfer from the liquid culture allow the plates to dry for 10–30 min. The positions should be dry when the preys are copied onto the bait spots. Also the plate should be checked if enough bait cells were transferred. Reasonable amounts were transferred when each spot occurs cloudy. This is critical for a good mating efficiency.
13. Thick pins can be used as well. We use thin pins since more replication steps can be done from a single source plate. If only a replication tool with thick pins is available more prey array plates have to be prepared since only a couple of transfer steps can be done because of source plate depletion.
14. This step is an essential control step because only diploid cells containing the Leu2 and Trp1 marker on the prey and bait vectors will grow on this medium. It also leads to an amplification of diploid cells, which increases the efficiency of the next selection step.
15. We normally score interactions after 7 days. But the plates should be examined every day. Most two-hybrid positive

colonies appear within 3–5 days, but occasionally positive interactions can be observed later. Very small colonies are usually designated as background; however, there is no absolute measure to distinguish between the background and real positives. When there are many (i.e., >30) large colonies per array of 6,000 positions, we consider these baits as “random” activators. In this case, the screen should be repeated to ensure that these positives are reproducible (unless the screen is done already in duplicate or quadruplicate).

16. Pinning the retest onto readout medium with various concentrations 3-AT can be used to semi-quantify interactions. This helps, e.g., to distinguish between “strong” and “weak” signals and might also help to separate spurious ones.

Acknowledgments

This work was supported by the German Government via BMBF (Zoonosis Network, Consortium on ecology and pathogenesis of SARS, project code 01KIO701) to A.v.B, by a grant of the Baden-Württemberg Stiftung (Germany) to P.U., R.H. and T.S., and by a grant from the European Union (HEALTH-F3-2009-223101) to P.U.

References

1. Fields, S., and Song, O. (1989) A novel genetic system to detect protein–protein interactions. *Nature* **340**, 245–6.
2. Stellberger, T., Hauser, R., Baiker, A., Pothineni, V. R., Haas, J., and Uetz, P. (2010) Improving the yeast two-hybrid system with permuted fusions proteins: the Varicella Zoster Virus interactome. *Proteome Sci* **8**, 8.
3. Chen, Y. C., Rajagopala, S. V., Stellberger, T., and Uetz, P. (2010) Exhaustive benchmarking of the yeast two-hybrid system. *Nat Methods* **7**, 667–8.
4. Uetz, P., Dong, Y. A., Zeretzke, C., Atzler, C., Baiker, A., Berger, B., Rajagopala, S. V., Roupelieva, M., Rose, D., Fossum, E., and Haas, J. (2006) Herpesviral protein networks and their interaction with the human proteome. *Science* **311**, 239–42.
5. James, P. (2001) Yeast two-hybrid vectors and strains. *Methods Mol Biol* **177**, 41–84.
6. James, P., Halladay, J., and Craig, E. A. (1996) Genomic libraries and a host strain designed for highly efficient two-hybrid selection in yeast. *Genetics* **144**, 1425–36.
7. Harper, J. W., Adami, G. R., Wei, N., Keyomarsi, K., and Elledge, S. J. (1993) The p21 Cdk-interacting protein Cip1 is a potent inhibitor of G1 cyclin-dependent kinases. *Cell* **75**, 805–16.
8. Bartel, P., Chien, C. T., Sternglanz, R., and Fields, S. (1993) Elimination of false positives that arise in using the two-hybrid system. *Biotechniques* **14**, 920–4.
9. Legrain, P., Dokhelar, M. C., and Transy, C. (1994) Detection of protein–protein interactions using different vectors in the two-hybrid system. *Nucleic Acids Res* **22**, 3241–2.
10. Ye, G. J., Vaughan, K. T., Vallee, R. B., and Roizman, B. (2000) The herpes simplex virus 1 U(L)34 protein interacts with a cytoplasmic dynein intermediate chain and targets nuclear membrane. *J Virol* **74**, 1355–63.