Yeast Two-Hybrid Procedure

1. Generate a GAL4 DNA-BD fusion by cloning the gene of interest in frame with the GAL4 DNA binding domain of pGBKT7.
2. Transform AH109 with bait plasmid, detect bait expression and test for autoactivation and cell toxicity.
3. Mate the pretransformed library strain with the bait strain.
4. Restreak colonies that can grow on TDO plates to QDO plates.
5. Restreak positive clones to single colonies on QDO/+X-a-Gal plates.
6. Rescue plasmid DNA from positive yeast clones.
7. Identify and isolate AD/library plasmids from E. coli transformants.
8. Cotransform DNA-BD/bait and AD/library plasmids into AH109.
9. Repeat Step 5.
10. Select true positive clones and sequence cDNA inserts.

Protocol: Transformation of Yeast Cells

1. Materials:

· Stock solution

50%PEG4000: prepare with sterile deionized H_2O

10×TE buffer: 0.1 M Tris·Cl, 10 mM EDTA, pH 8.0, autoclave

10×LiAc: 1 M LiAc, adjust to pH 8.0 via diluted HAc

· DMSO

· ssDNA

· Mixture(350 μl)

	1X
LiAc(1M)	36 μl
10XTE	24 μl
ssDNA(10 mg/ml)	5 μl
PEG4000	240 μl
D.W.	35 μl
dsDNA	10 μl

2. Pick one colony of yeast cells in 2 ml YPD, incubate at 30℃ overnight with shaking at 200 rpm
3. Centrifuge at 10，000 rpm for 1 min, decant
4. Resuspend the pellet with 1 ml H_2O
5. Add 800 ml LiAc (0.1 M)
6. Wash once with LiAc, decant and resolve in 450 μl LiAc(0.1 M)
7. Centrifuge at 10，000 rpm for 1 min, decant
8. Add 350 μl Mixture and 5μl DNA(up to 5 μg)
9. Vortex vigorously to suspend cells completely
10. Add 40 μl DMSO, vortex briefly
11. 42℃ Water bath for 20 min
12. Chill the cells on ice for 3 min
13. Short spin at top speed for 30 s
14. Add 100-150 ml H_2O , spread or streak cells on 100-cm plates

Protocol: Yeast Plasmid DNA Minipreparation

1. Materials:

Sol. .A

1 M Sorbitol

100 mM Na Citrate, pH7.0

10 mM $MgCl_2$

0.6 mg/ml Zymolyase or Lyticase (Add Zymolyase fresh)

1% β-mercaptoethanol

Sol.B

0.5%SDS

100 mM Tris, pH8.0

50 mM EDTA

5 M KAc

70%EtOH

2. Grow 2 ml culture to late log phase
3. Centrifuge 3000 rpm for 1min
4. Resuspend pellet in 0.2 ml Sol. A, mix gently
5. Heat at 70℃ for 20 min, let cool to RT
6. Add 0.8 ml Sol. B, mix gently
7. Heat at 70℃ for 20 min ,let cool at RT
8. Add 0.2 ml 5 M KAc
9. Incubate on ice briefly
10. Spin in microfuge for 5 min
11. Remove 750 ul supernatant ,add 1 vol. isopropanol
12. Incubate at RT for 5 min, and short spin at top speed for 30 s
13. Resuspend in 10 ul H_2O

Protocol: Control Mating Protocol

AH109 pretransformed with pGBKT7-53, and Y187 pretransformed with pTD1-1 or pGADT7-T, are provided with every Matchmaker Pretransformed Library as a positive control. To familiarize yourself with the procedures and expected resµlts of a two-hybrid assay, use these strains to perform a control mating before you begin screening the library.

1. Materials:
   - SD/-Trp Agar plates
   - SD/-Leu Agar plates
   - SD/-Ade/-His/-Leu/-Trp/X-a-Gal agar plates
   - 2xYPDA medium
   - YPD liquid medium + 25% glycerol (Freezing Medium)
   - AH109[pGBKT7-53] (supplied)
   - Y187[pTD1-1 or pGADT7-T] (supplied)
   - Y187[pGBKT7]
2. Streak the provided control strains from the glycerol stocks on the selection media indicated below:
   - AH109[pGBKT7-53] SD/-Trp Agar
   - Y187[pTD1-1 or pGADT7-T] SD/-Leu Agar
   - Negative control: AH109[pGADT7] SD/-Trp Agar
3. Grow at 30℃ for 3 days.
4. Pick one 2-3 mm colony of each type for use with this small scale mating procedure and mate the following.
   - Positive Control: AH109[pGADT7-53] and Y187[pTD1-1 or pGADT7-T]
   - Negative Control: AH109[pGBKT7] and Y187[pGADT7]
5. Place both colonies in a single 1.5 ml centrifµge tube containing 500 µl of 2xYPDA and vortex to mix.
6. Incubate with shaking at 200 rpm at 30℃ overnight [20-24 hr].
7. From the mated cµlture (0.5 ml) spread 100 µl of 1/10, 1/100 and 1/1,000 dilutions on each of the following agar plates. Incubate plates (colony side

down) at 30℃ for 3-5 days.

· SD/-Trp

· SD/-Leu

· SD/-Leu/-Trp (DDO)

· SD/-Leu/-Trp/-Ade/-His/X-a-Gal (QDO + X-α-Gal):

8. Expected resµlts after 3-5 days:

Positive controls:

· Similar number of colonies on DDO and QDO agar plates

· Colonies on QDO + X-α-Gal are blue

· Negative control: colonies on DDO, no colonies on QDO agar plates.

9. Pick healthy 2 mm colonies from DDO plates, restreak onto fresh DDO plates, and incubate at 30℃ for 3-4 days.

· Short-term storage (< 4 weeks): Seal with Parafilm and store at 30℃.

· Long-term storage: Scoop a large healthy colony and fµlly resuspend in 500 µl of YPD + 25% glycerol. Store at –30℃.

Protocol: Mating

1. Materials:

- Pretransformed Library
- Bait construct transformed into AH109 on SD/-Trp
- 2xYPDA liquid medium
- 0.5xYPDA liquid medium
- YPD liquid medium + 25% glycerol (Freezing Medium)
- The following SD agar plates

  SD/-Trp 5-10 100 mm plates)

  SD/-Leu (5-10 100 mm plates)

  SD/-Leu/-Trp (5-10 100 mm plates)

  SD/-Ade/-His/-Leu/-Trp/X-a-Gal (50-55 150 mm plates)
- Kanamycin sulfate (50 mg/ml)

2. Prepare a concentrated overnight culture of the bait strain (AH109 [pGBKT7+Bait]) as follows:
   A. Inoculate one fresh, large (2－3 mm) colony of the bait strain into 50 ml of SD/-Trp liquid medium.
   B. Incubate shaking (250－270 rpm) at 30℃ until the OD_{600} reaches 0.8 (16－20 hr).
   C. Centrifuge to pellet the cells (1,000 g for 5 min), discard the supernatant.
   D. Resuspend the pellet to a cell density of >1x108 cells per ml in SD/-Trp (4－5 ml).
3. Combine the Library Strain with the Bait Strain as follows:
   A. Thaw a 1 ml aliquot of the library strain in a room temperature water bath. Remove 10 μl for titering on 100 mm SD/-Leu agar plates.
   B. Combine the 1 ml of library strain with the 5 ml bait strain in a sterile 2 L flask.
   C. Add 45 ml of 2xYPDA liquid medium (with 50 μg/ml kanamycin).
   D. Rinse cells from the library vial twice with 1 ml 2xYPDA and add to the 2 L flask.
4. Incubate at 30° C for 20－24 hr, slowly shaking (30－50 rpm).
5. After 20 hr, check a drop of the culture under a phase contrast microscope (40X). If zygotes are present, continue to Step 6, if not, allow mating to continue, incubate for an additional 4 hr.
6. Centrifuge to pellet the cells (1,000 g for 10 min).
7. Meanwhile rinse the 2L flask twice with 50 ml 0.5xYPDA (with 50 μg/ml kanamycin), combine the rinses and use this to resuspend the pelleted cells.
8. Centrifuge to pellet the cells (1,000 g for 10 min) and discard the supernatant.
9. Resuspend all pelleteted cells in 10 ml of 0.5xYPDA/Kan liquid medium. Measure the total volume of cells + medium.
10. From the mated culture spread 100 μl of 1/10, 1/100, 1/1,000, 1/10,000 dilutions on each of the following 100mm agar plates and incubate at 30° C

for 3－5 days.

- SD/-Trp
- SD/-Leu
- SD/-Leu/-Trp (DDO)

11. Plate the remainder of the culture, 200 μl per 150 mm on SD/-Ade/-His/-Leu/-Trp/X-a-Gal (QDO+X-a-Gal) agar plate (50－55 plates). Incubate at 30℃ for 3－8 days.
12. Calculate the number of screened clones (diploids) by counting the colonies from the SD/-Leu/-Trp (DDO) plates after 3－5 days.
    - Number of Screened Clones = cfu/ml of diploids x resuspension volume (ml)
    - It is imperative that at least 1 million diploids are screened, using less than this will result in less chance of detecting genuine interactions on QDO.
13. Determine the mating efficiency.
    A. Measure viabilities
        - No. of cfu/ml on SD/–Leu = viability of the Prey Library
        - No. of cfu/ml on SD/–Trp = viability of Bait
        - No. of cfu/ml on SD/–Leu/–Trp = viability of diploids
    B. Calculate Mating Efficiency (percentage of diploids):
        - No. of cfu/ml of diploids x 100/ No. of cfu/ml of limiting partner = % Diploids
14. Further analyze all the positive interactions to identify duplicates and to verify that the interactions are genuine.

Yeast Media Recipes

A. Rich Media

YPDA Liquid (1 L)	
Reagent	**Amount**
YPD	50 g
L-Adenine Hemisulphate	15 ml of 0.2% stock solution
Deionized water	Up to 1 L
Adjust pH to 6.5 if necessary, then autoclave.	

YPDA Agar (1 L)	
Reagent	**Amount**
YPD agar	70 g
L-adenine hemisulphate	15 ml of 0.2% stock solution
Deionized water	Up to 1 L
Adjust pH to 6.5 if necessary, then autoclave.	

2X YPDA Liquid (1 L)	
Reagent	**Amount**
YPD	100 g
L-adenine hemisulphate	15 ml of 0.2% stock solution
Deionized water	Up to 1 L
Adjust pH to 6.5 if necessary, then autoclave.	

0.5 X YPDA Liquid (1 L)	
Reagent	**Amount**
YPD	25 g
L-adenine hemisulphate	15 ml of 0.2% stock solution
Deionized water	Up to 1 L
Adjust pH to 6.5 if necessary, then autoclave.	

B. Single Dropout Media

SD/-Trp Liquid (1 L)	
Reagent	**Amount**
Minimal SD Base	26.7 g
-Trp DO Supplement	0.74 g
Deionized water	Up to 1 L
Adjust pH to 5.8, then autoclave. Store at 4° C in subdued light.	

SD/-Trp Agar (1 L)	
Reagent	**Amount**
Minimal SD Agar Base	46.7 g
-Trp DO supplement	0.74 g
Deionized water	Up to 1 L
Adjust pH to 5.8, then autoclave. Store at 4° C in subdued light	

SD/-Leu Liquid (1 L)	
Reagent	**Amount**
Minimal SD Base	26.7 g
-Leu DO Supplement	0.69 g
Deionized water	Up to 1 L
Adjust pH to 5.8, then autoclave. Store at 4° C in subdued light.	

SD/-Leu Agar (1 L)	
Reagent	**Amount**
Minimal SD Agar Base	46.7 g
-Leu DO supplement	0.69 g
Deionized water	Up to 1 L
Adjust pH to 5.8, then autoclave. Store at 4° C in subdued light	

C. Double Dropout (DDO) Media

SD/-Leu/-Trp Liquid (1 L)	
Reagent	**Amount**
Minimal SD Base	26.7 g
-Leu/-Trp DO Supplement	0.64 g
Deionized water	Up to 1 L
Adjust pH to 5.8, then autoclave. Store at 4° C in subdued light.	

SD/-Leu/-Trp Agar (1 L)	
Reagent	**Amount**
Minimal SD Agar Base	46.7 g
-Leu/-Trp DO Supplement	0.64 g
Deionized water	Up to 1 L
Adjust pH to 5.8, then autoclave. Store at 4° C in subdued light	