Dec 05, 2025

E3 decoy library high-throughput yeast two-hybrid screening assay

Journal of Experimental Botany

DOI
https://dx.doi.org/10.17504/protocols.io.dm6gpm72pgzp/v1

Chen-An Chen[1], Yi-Tsung Tu[1], Chin-Mei Lee[1]

[1]Institute of Plant Biology, National Taiwan University, Taipei, 106319, Taiwan

Chen-An Chen

OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.dm6gpm72pgzp/v1

External link: https://doi.org/10.1093/jxb/eraf536

Protocol Citation: Chen-An Chen, Yi-Tsung Tu, Chin-Mei Lee 2025. E3 decoy library high-throughput yeast two-hybrid screening assay. **protocols.io** https://dx.doi.org/10.17504/protocols.io.dm6gpm72pgzp/v1

Manuscript citation:
Tu Y, Chen C, Gendron JM, Lee C (2025) Dissecting ubiquitin-mediated circadian clock regulation in Arabidopsis via high-throughput yeast screening. Journal of experimental botany (). doi: 10.1093/jxb/eraf536

Protocol status: Working
We use this protocol and it's working

Created: December 04, 2025

Last Modified: December 05, 2025

Protocol Integer ID: 234213

Keywords: E3 ligase, ubiquitination, yeast two-hybrid, protein-protein interaction, Arabidopsis, circadian clock regulation in arabidopsi, arabidopsis e3 ubiquitin, mediated circadian clock regulation, arabidopsi, throughput yeast screen, e3 decoy library, throughput yeast, yeast, potential protein, decoy library, protein interaction

Funders Acknowledgements:
MOST
Grant ID: MOST-109-2311-B-002-030-MY2
NSTC
Grant ID: NSTC-111-2311-B-002-012-MY3
NTU
Grant ID: NTU-CC-112L891804
NTU
Grant ID: NTU-CC-114L895003
MOST
Grant ID: MOST-110-2811-B-002-523-
NSTC
Grant ID: NSTC-113-2811-B-002-122-
NTU
Grant ID: NTU-CC-111L4000

Disclaimer

Abstract

This protocol describes a yeast two-hybrid (Y2H) assay designed to systematically screen for potential protein–protein interactions using the Arabidopsis E3 ubiquitin ligase decoy library. It serves as a detailed methodological supplement to *"Dissecting ubiquitin-mediated circadian clock regulation in Arabidopsis via high-throughput yeast screens"* by Tu and Chen (2026) (link).

Materials

Yeast strains and vectors

1. The opposite mating type of *Saccharomyces cerevisiae* strains AH109 and Y187.
2. The yeast two-hybrid vector *pDEST22* serves as the bait vector carrying the gene of interest.

Equipment and consumables

Shaking incubator, cell density meter, 96-well PCR plate, adhesive film for 96-well PCR plate, reagent reservoir, 8-channel pipette, sterile glass culture tube

Media recipes

1 YPD(A) medium

1.1

	Components	Amounts
	Difco peptone	20g
	Yeast extract	10g
	Dextrose	20g
	Adenine (A)	0.20g
	Total	1L
	Adjust the pH to 6.5	

2 SD medium

2.1

	Components	Amounts
	Yeast nitrogen base without amino acids	6.7g
	Dextrose	20g
	Amino acid drop-out mix	1.5g
	Agar (for plates only)	20g
	Total	1L
	Adjust the pH to 5.8	

3 Amino acid drop-out mix

3.1

	Reagent (g)	-Leu	-Trp	-Leu-Trp	-Leu-Trp-His	Brand (catalog no.)
	Adenine	1	0.5	2	0.5	Bioshop (ADA001.25)
	Alanine	4	2	8	2	Panreac (A3690)
	Arginine	4	2	8	2	Panreac (A3675)
	Asparagine	4	2	8	2	Panreac (A3720)
	Aspartic acid	4	2	8	2	Sigma (A-7219)
	Cysteine	4	2	8	2	Panreac (A1703)
	Glutamine	4	2	8	2	Sigma (G3126)
	Glutamic acid	4	2	8	2	ACROS (G0059)
	Glycine	4	2	8	2	Sigma (G-8790)
	Histidine	4	2	8	0	Panreac (A3733)
	Inositol	4	2	8	2	Sigma (I-7508)
	Isoleucine	4	2	8	2	Bioshop (ISO910)

	Reagent (g)	-Leu	-Trp	-Leu-Trp	-Leu-Trp-His	Brand (catalog no.)
	Leucine	0	10	0	0	Sigma (L-1512)
	Lysine	4	2	8	2	Bioshop (LYS101.100)
	Methionine	4	2	8	2	Panreac (A1340,0100)
	Phenylalanine	4	2	8	2	Scharlau (FE0180)
	Proline	4	2	8	2	Scharlau (PR0055)
	Serine	4	2	8	2	SIGMA (S5386)
	Threonine	4	2	8	2	Panreac (A3969)
	Tryptophan	4	0	0	0	Sigma (T-8941)
	Tyrosine	4	2	8	2	Scharlau (TI0325)
	Uracil	4	2	8	2	Sigma (U-1128)
	Valine	4	2	8	2	Scharlau (VA0055)

Yeast strains and vectors

4 1. The opposite mating type of *Saccharomyces cerevisiae* strains AH109 and Y187.

5 2. The yeast two-hybrid vector *pDEST22* serves as the bait vector carrying the gene of interest.

Equipment and consumables

6 Shaking incubator, cell density meter, 96-well PCR plate, adhesive film for 96-well PCR plate, reagent reservoir, 8-channel pipette, sterile glass culture tube

Procedure

7 **Transformation**

7.1 Transform *pDEST32-E3 decoy* into yeast AH109 strain and culture in 3~5mL SD-Leu liquid medium at 30°C for one day.

7.2 Transform *pDEST22-bait* into yeast Y187 strain and culture in 3~5mL SD-Trp liquid medium at 30°C for one day.

8 **Prepare the E3 decoy yeast into 96-well plate**

8.1 Add 50µl *pDEST32-E3 decoy* bacterial liquid culture to 100µl SD-Leu liquid medium per well in 96-well plate.

8.2 Seal film carefully and ensure that no bacterial liquid is attracted to the film by static electricity.

8.3 Incubate at 30°C overnight.

8.4 (optional) Drop the bacterial culture on SD-Leu agar plate (2.5µl/drop) and incubate at 30°C for two days. This step allows the E3-decoy yeast cultures to be directly inoculated into liquid medium from the colonies in the next experiment.

9 **Mate the E3 decoy strain and the bait strain**

9.1 Mix the *pDEST32-E3 decoy/pDEST32* culture and the *pDEST22-bait/pDEST22* culture in a new 96-well plate. Each well should contain: 30µl *pDEST22-bait/pDEST22* culture, 30µl *pDEST32-E3 decoy/pDEST32* culture, and 100µl YPDA medium. Notice:

9.2 Notice: Centrifuge the *pDEST32–E3 decoy* 96-well plate at 2000 rpm for 2 minutes before opening it to avoid cross-contamination between wells.

9.3 Notice: Pre-mixing the *pDEST22-bait/pDEST22* culture with YPDA medium prior to dispensing into the 96-well plate.

9.4 Seal film carefully and ensure that no bacterial liquid is attracted to the film by static electricity.

9.5 Incubate at 30°C overnight.

9.6 Drop 2.5 µl/well mating bacterial culture on SD-Leu-Trp and SD-Leu-Trp-His agar plate.

9.7 Check the plate and take photos every day, paying attention on day 3 (for SD-Leu-Trp), and on day 4 and 5 (for SD-Leu-Trp-His).

9.8 Identify the potential E3 candidates by comparing with those of the control plate (*pDEST32-E3 decoy + pDEST22*).

10 **Y2H assay with serial dilution after high-throughput screening**

10.1 Culture liquid yeast (*pDEST22/pDEST22-bait/pDEST32/pDEST32-E3 decoy*) in glass tubes, separately. Incubate at 30°C overnight.

10.2 Mixing 100µl *pDEST22/pDEST22-bait* and 100µl *pDEST32/pDEST32-E3 decoy* in 3ml YPDA medium for mating. Incubate at 30°C overnight with shaking at 200 rpm.

10.3 Take 10–20 µl of the culture and streak for single colonies. Incubate at 30 °C for two days.

10.4 Pick a single colony and culture into SD-Leu-Trp liquid medium. Incubate at 30°C overnight with shaking at 200 rpm.

10.5 Adjust OD_{600} to 0.3 with SD-Leu-Trp liquid medium as 1x concentration. Prepare the 10x and 100x dilutions with SD-Leu-Trp liquid medium. Mix thoroughly by pipetting to ensure homogeneity before transferring culture to the respective dilutions.

10.6 Dropping 2.5 µl/well liquid yeast onto SD-Leu-Trp and SD-Leu-Trp-His agar plates.

10.7 Check the plate and take photos every day, especially on day 3 (for SD-Leu-Trp), and on day 4 and 5 (for SD-Leu-Trp-His).