Video Article

Mating-based Overexpression Library Screening in Yeast

Elliott Hayden[1], Shuzhen Chen[1], Abagail Chumley[1], Quan Zhong[1], Shulin Ju[1]

[1]Department of Biological Sciences, Wright State University

Correspondence to: Shulin Ju at shulin.ju@wright.edu

URL: https://www.jove.com/video/57978
DOI: doi:10.3791/57978

Keywords: Genetics, Issue 137, Yeast, mating, high-throughput, over-expression screening, neurodegeneration, protein misfolding

Date Published: 7/6/2018

Citation: Hayden, E., Chen, S., Chumley, A., Zhong, Q., Ju, S. Mating-based Overexpression Library Screening in Yeast. *J. Vis. Exp.* (137), e57978, doi:10.3791/57978 (2018).

Abstract

Budding yeast has been widely used as a model in studying proteins associated with human diseases. Genome-wide genetic screening is a powerful tool commonly used in yeast studies. The expression of a number of neurodegenerative disease-associated proteins in yeast causes cytotoxicity and aggregate formation, recapitulating findings seen in patients with these disorders. Here, we describe a method for screening a yeast model of the Amyotrophic Lateral Sclerosis-associated protein FUS for modifiers of its toxicity. Instead of using transformation, this new screening platform relies on the mating of yeast to introduce an arrayed library of plasmids into the yeast model. The mating method has two clear advantages: first, it is highly efficient; second, the pre-transformed arrayed library of plasmids can be stored for long-term as a glycerol stock, and quickly applied to other screens without the labor-intensive step of transformation into the yeast model each time. We demonstrate how this method can successfully be used to screen for genes that modify the toxicity of FUS.

Video Link

The video component of this article can be found at https://www.jove.com/video/57978/

Introduction

The budding yeast *Saccharomyces cerevisiae* has been widely used in basic scientific research[1] to understand cellular processes directly related to human diseases. Moreover, it has been used as a model organism for studying human disease-associated proteins, such as those linked to the most common neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, Huntington's disease, and Amyotrophic Lateral Sclerosis (ALS)[2]. An advantage of the yeast model is the ease with which a genome-wide screen can be performed to identify cellular pathways related to the toxicity of disease-related proteins, thus giving insight into the mechanism of their toxicity. One such screen is called an overexpression library screen, in which each of the 5,500 yeast genes in an arrayed library is transformed into a yeast model to identify which genes can modify toxicity when overexpressed. This screening method has been successfully applied in the yeast models of multiple neurodegenerative disease-associated proteins, including huntingtin for Huntington's disease[3], α-synuclein for Parkinson's disease[4,5], Aβ for Alzheimer's disease[6], and FUS and TDP-43 for ALS[7,8,9]. While it is usually done in a high-throughput manner[10], the most labor-intensive step of the screen is individually transforming 5,500 yeast genes from an arrayed library. This step must be performed each time the screening is repeated, and whenever a newly established yeast model needs to be studied. It is important to find a more efficient way to accomplish this task.

Yeast cells can stably exist in both haploid and diploid forms. There are two opposite mating types of haploid cells, mating type a and α. Haploid cells of each mating type produce and secrete their own specific mating pheromone, to which only the opposite mating type cells respond. This allows mating between a and α cells to produce stable diploid cells, a/α. This process is spontaneous and highly efficient[11]. We can take advantage of this unique life cycle of *S. cerevisiae* to introduce the plasmid library. More specifically, each gene in the arrayed plasmid library will be transformed into haploid cells of one mating type, *i.e.*, α cell. These cells containing the library genes will then be stored in glycerol stock in an arrayed 96-well format. For each yeast model that needs to be screened, yeast cells containing the library genes can be thawed from the glycerol stock, and the screening can be done through mating with the yeast model of interest in the opposite mating type, *i.e.*, mating type a. This idea of using mating to bring together two genes into yeast is not new. It has been successfully applied in the high-throughput yeast two-hybrid screening, in which a bait construct (*i.e.*, Gal4-DNA-binding domain fusions) in one mating type is brought together through mating with a prey construct from an arrayed library[12]. However, this strategy has never been applied in overexpression library screenings, which have always used traditional transformation methods.

Our laboratory previously established a yeast model of the ALS-associated protein FUS[7]. Through overexpression library screening using the transformation method we discovered five yeast genes (*ECM32, NAM8, SBP1, SKO1*, and *VHR1*) that rescue toxicity of FUS when overexpressed. These findings were independently confirmed with a similar study by another group[8]. hUPF1, a human homolog of *ECM32*, was later shown to suppress toxicity in primary neuronal cells[13] and in an animal model of ALS[14] as well. Using these five genes as proof of principle, we demonstrate that all five genes similarly rescue FUS toxicity when they are introduced into the FUS yeast model by mating. Since yeast cells containing the library genes can be stored permanently in glycerol stock and revived whenever needed, this mating-based method will remove the time-consuming step of transformation each time the library needs to be screened against. Since mating is highly efficient with no plasmid

Copyright © 2018 Journal of Visualized Experiments

transformation involved, this strategy also significantly decreases the cost associated with purification and transformation of a large plasmid library. We will successfully apply this method to a library screening against yeast model of FUS.

The procedure for mating-based screening is briefly described in **Figure 1**. Initially, the arrayed plasmid library is transformed into a haploid yeast strain of mating type α using a high-throughput yeast transformation protocol in which each well of a 96-well plate contains yeast transformed with a specific library plasmid. This collection of transformed yeast is saved as a glycerol stock that can be thawed and revived for use later on. The yeast model of interest, in this case FUS toxicity, must be generated in a haploid yeast strain with the opposite mating type (mating type a). In a high-throughput manner using sterile 96-pin replicators, the FUS strain and yeast strains containing the plasmid library are transferred to 96-well plates containing rich media and allowed to mate. Following mating, a small volume from each well of the mating culture is transferred to 96-well plates containing synthetic dropout media in which only diploid yeast containing both the FUS and library genes can grow. A robotic spotting machine is then used to transfer yeast culture from each well onto agar plates where the expression of FUS and the library genes is induced. Additionally, yeast culture is spotted to control agar plates where FUS and the library genes are not expressed. Following growth on agar plates, genes that rescue or exacerbate FUS toxicity will be identified.

Protocol

NOTE: The protocol described here is designed for screening library plasmids contained in ten 96-well plates but can be scaled up or down accordingly. The protocol needs to be repeated to complete the whole library screening. Usually, screening against 10 plates of library genes each time can be comfortably handled by 1 person.

1. Preparation for 96-well Yeast Transformation

NOTE: This step is done as previously described[7,10].

1. Aliquot 5 µL (about 50–100 ng) of plasmid DNA from an arrayed plasmid library into each well of a round-bottom 96-well plate.
   NOTE: An example of such a library would be a yeast gene overexpression library[10].
2. Inoculate 150 mL of yeast peptone dextrose (YPD) media in a 500 mL flask with a colony (2–3 mm diameter) of the haploid yeast strain W303α. Incubate them overnight at 30 °C with shaking (200 rpm).
3. The following morning, measure the OD_{600} of the overnight culture and dilute the yeast culture to OD_{600} = 0.1 in (up to) 2 L of YPD. Incubate it at 30 °C with shaking (200 rpm) for ~5 h until the culture reaches OD_{600} = 0.4–0.6.

2. Yeast Transformation

1. Harvest the yeast culture by filling 8 sterile 250 mL centrifuge bottles and centrifuging them at room temperature at 3,000 x *g* for 10 min. Pour off the supernatant without disrupting the pellet.
   NOTE: Carry out all centrifugation steps at room temperature.
2. Wash the yeast with sterile-distilled H_2O. For this, add 100 mL of sterile H_2O to each centrifuge bottle and vortex it to resuspend the cell pellet. Combine the washed cells into 2 bottles and centrifuge them at 3,000 x *g* for 5 min. Pour off the supernatant.
3. Wash the cells in each bottle in 100 mL of 0.1M LiOAc/1XTE (100 mM LiOAc; 10 mM Tris, pH 8.0; 1 mM EDTA) and centrifuge them at 3,000 x *g* for 5 min. Pour off the supernatant.
4. While centrifuging, boil 5 mL of salmon sperm DNA (10 mg/mL) at 100 °C using a block heater for 3 min and then cool it on ice.
5. Resuspend the cell pellet in 25 mL of 0.1M LiOAc/1XTE in each bottle, combine the resuspended cells and transfer them to a 150 mL flask. Add 5 mL of the pre-cooled salmon sperm DNA and incubate it at 30 °C with shaking (225 rpm) for 30 min.
6. Pour the cell mixture into a sterile disposable reagent reservoir and, using a multichannel pipette, transfer 35 µL of the cell mixture to each well of the round-bottom 96-well plate containing the library plasmid DNA. Vortex the 96-well plates using a plate vortexer for 1 min at 1,000 rpm. Incubate the plates for 30 min at 30 °C without shaking.
   NOTE: Do not stack the plates, so the heat can transfer more efficiently. We have found that vortexing the 96-well plates at 1,000 rpm does not cause liquid to spill out of the wells, but a safe vortex speed should be tested before performing the step.
7. In a flask, prepare 200 mL of the transformation buffer containing a final concentration of 40% PEG3350, 10% DMSO, and 0.1M LiOAc. Prepare the transformation buffer immediately before use and mix it thoroughly by shaking.
8. Remove 96-well plates from the 30 °C incubator and mix them for 30 s at 1,000 rpm using the plate vortexer. Add 125 µL of the transformation buffer to each well and then vortex the plates for 1 min at 1,000 rpm.
9. Incubate the plates at 30 °C for 30 min and then heat shock the yeast by placing the plates in a 42 °C incubator for 15 min.
   NOTE: Do not stack the plates.
10. Centrifuge the plates for 5 min at 3,000 x *g*. Remove the transformation buffer from the wells by inverting the plates over a waste bin and forcefully dumping the buffer from the plates. Quickly blot the inverted plates on a clean paper towel to remove any liquid on the top of the plates.
11. Rinse the cells by adding to the plates 200 µL of minimal dropout medium corresponding to the selectable marker on the library plasmid.
    NOTE: We used a synthetic Ura- medium.
12. Centrifuge the plates for 5 min at 3,000 x *g* and remove the supernatant over a waste bin as in step 2.10.
13. Add 160 µL of minimal Ura-medium containing 2% glucose. Vortex the plates for 1 min at 1,000 rpm and incubate them at 30 °C for 48 h without shaking. After 48 h, note that a pellet of transformed yeast is visible at the bottom of each well.
14. Using a liquid dispenser, add 100 µL of Ura-media containing glucose into each well of a new set of 96-well plates.
15. Vortex the plates containing the transformed yeast (from step 2.13) at 1,000 rpm for 30 s. Using a sterile plastic 96-pin replicator, place the pins into the wells containing the transformed yeast and then inoculate them into the corresponding wells of the new plates filled with media. Incubate the new plates at 30 °C for 24 h.
    NOTE: If working with 10 plates, the medium can also be dispensed manually using multi-channel pipettes.

Copyright © 2018 Journal of Visualized Experiments

16. After 24 h, a pellet of yeast should be visible in the bottom of each well containing successfully transformed yeast. To save these yeast strains as glycerol stocks, add 50 µL of 50% glycerol to each well, vortex the plates for 30 s at 1,000 rpm, seal the plates with sealing tape and freeze them at -80 °C.
NOTE: The above steps only need to be performed once. Future screenings of different yeast models against the same library disposable reagent reservoir can start from the glycerol stocks and proceed immediately from the steps below.

3. Mating Between Cells Containing Library Genes and Query Yeast

1. To revive the library strains from the glycerol stock, take the 96-well plates out of the -80 °C freezer, remove the sealing tape, and let the yeast thaw at room temperature for approximately 30 min.
2. Once the yeast has thawed, use a sterile plastic 96-pin replicator to inoculate the glycerol stocks to 160 µL of fresh Ura-media containing 2% glucose in 96-well plates and incubate them at 30 °C for 24 h. Immediately after the library strains are used, seal the plates with sealing tape, and return them to the -80 °C freezer.
3. On the same day the library strains (W303α) are thawed, inoculate the query yeast strain (here, FUS in W303a) to 50 mL of YPD and grow it overnight at 30 °C with shaking at 250 rpm.
4. The next morning, pour the query yeast strain to a sterile disposable reagent reservoir, and aliquot 160 µL of the query strain to each well of a 96-well plate using a multichannel pipette.
5. Using the liquid dispenser, dispense 160 µL of YPD media into each well of ten 96-well plates. Use these plates later for mating the query yeast strain with the library yeast.
6. Briefly vortex the 96-well plate containing the query strain and then use a sterile 96-pin replicator to transfer the query strain to the YPD plates.
7. Briefly vortex the library strain plates and, for each library strain plate, use a new sterile 96-pin replicator to transfer the library strains to the YPD plates that have been inoculated with the query strain.
NOTE: Cells in glycerol lose their viability after multiple thaw-freeze cycles. Returning the library strains to the long-term storage (-80 °C freezer) as soon as possible will keep the library in good quality, and ready for future use. Properly maintained, the glycerol stock can be frozen and thawed at least 10 times. We also strongly recommend keeping a working copy and backup copies of the glycerol stock. When the working stock does not work, immediately make a new working copy from the backup copy.
8. Incubate the YPD plates at 30 °C for 24 h. Note that a pellet of yeast will be visible in the bottom of each well after 24 h.
9. Fill 96-well plates with a minimal dropout medium containing 2% raffinose, corresponding to the selectable markers on the plasmid in the query strain as well as on the library plasmid (*e.g.*, here Ura-His-).
10. Use a sterile 96-pin replicator to transfer yeast from the YPD mating cultures to the selective media. Incubate the 96-well plates at 30 °C for 48 h; only yeast cells that have mated and formed diploid cells and, therefore, contain both the query plasmid and the library plasmid will be able to grow in this media. After 2 days, observe that a pellet is visible on the bottom of the wells.

4. Spotting Assay

1. **After 48 h of growth in the raffinose-containing selective media, spot the yeast on agar plates.**
    1. Prepare 2 sets of Ura-His- dropout plates containing 2% agar using clear polystyrene plates, one containing 2% galactose and the other containing 2% glucose.
    2. Vortex the 96-well plates for 1 min at 1,000 rpm, then spot the yeast to the Ura-His- dropout plates containing 2% galactose and 2% agar (FUS and library genes are induced) and to the Ura-His- dropout plates containing 2% glucose and 2% agar (FUS and library genes repressed) using a robotic spotting machine, by which the culture in each well is spotted onto the agar plates in quadruplicate (*i.e.*, the culture in 1 well is spotted to 4 spots on the agar plate).
    3. After spotting, let the agar plates dry, and then place them upside down in a 30 °C incubator. Photograph the agar plates every 24 h to record a faster/more yeast growth in which the toxicity to the query strain is rescued or to record a slower/less growth in which the toxicity to the query strain is exacerbated. Incubate the plates for 4 days.
    NOTE: The culture in each well can be spotted on agar plates 1-to-1, as shown in a previous transformation-based method[10]. However, the 1-to-4 spotting here (which can be conveniently set up using the robotic spotting machine) significantly increases the robustness of the assay by reducing the number of false positives. Positive hits are only considered when all 4 colonies from the same well show a similar phenotype.

Representative Results

The ALS-associated protein FUS, an RNA/DNA binding protein, was previously studied in haploid yeast[7,8]. Genetic screening using the transformation-based method discovered several yeast genes that suppress FUS toxicity. The human homolog of one of the yeast genes was later demonstrated to be effective at suppressing toxicity in a primary neuronal cell and rat model of ALS[13]. Here, we are using the same yeast model to show that overexpression library screening can be performed by mating as effectively as by transformation.

FUS is toxic to both haploid and diploid yeast cells
The previous yeast model of FUS and subsequent overexpression library screening was performed in the haploid cell background. For the mating-based method to work, FUS toxicity needs to be demonstrated in diploid yeast. To do this, we mated the FUS yeast model in w303a (mating type a) with w303α transformed with an empty vector (mating type α). As indicated in **Figure 2**, although not as strong as in haploid yeast, FUS toxicity is evident in diploid yeast.

Copyright © 2018 Journal of Visualized Experiments

Suppression genes previously identified work in diploid yeast
As a proof of principle for the mating-based method, we tested the five genes previously identified from the transformation-based method. Mating was used to introduce each of the five genes into the haploid yeast model of FUS (in W303a), and their ability to rescue the toxicity of FUS in the subsequent diploid yeast was tested (W303a/α). As shown in **Figure 3**, all five genes rescue the toxicity of FUS in diploid yeast, indicating that the mating method was effective.

A pilot screening of 940 genes (arrayed on ten 96-well plates)
Following the protocols described above, we applied the mating-based method to an overexpression library screening of 940 genes. **Figure 4** displays a picture of one representative plate. As indicated on the right side of the figure (FUS and library genes expressed), FUS was toxic to diploid yeast. The library gene indicated by the green square rescued FUS toxicity while that indicated by the red square enhanced toxicity.

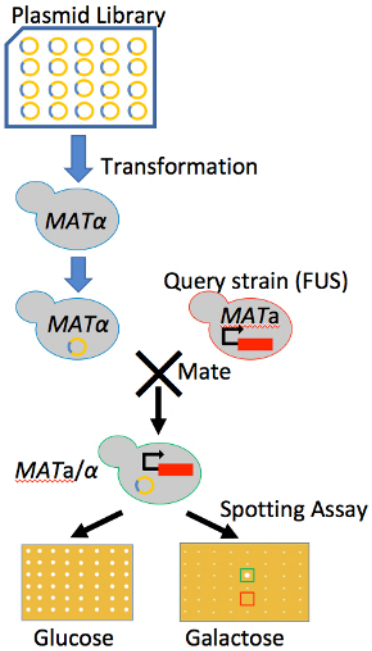

Figure 1: Diagram for screening yeast models of protein toxicity using mating. A plasmid library (under control of the GAL1 promoter which is highly induced in the presence of galactose) is transformed into a haploid yeast strain (MATα) using a high-throughput transformation protocol. This collection of yeast, transformed with the library plasmids, is stored as a glycerol stock at -80 °C, and is revived when needed to mate with the haploid yeast model of protein toxicity (in our case, one copy of FUS integrated at the HIS3 locus, GAL1 promoter, MATa). Diploid yeast containing the library plasmid and toxic protein (FUS) are selected and spotted to glucose (FUS and library gene 'off') and galactose (FUS and library gene 'on') agar plates. The growth of the yeast was followed to identify genes that rescue or exacerbate the toxicity of FUS. The green square indicates an example of a suppressor gene that rescues FUS toxicity, and the red square indicates an example of an enhancer gene that exacerbates the FUS toxicity when overexpressed. Please click here to view a larger version of this figure.

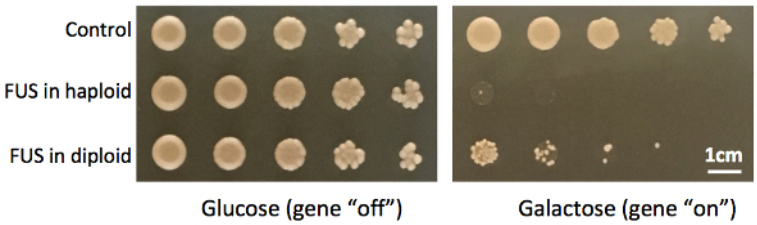

Figure 2: FUS is toxic to both haploid and diploid yeast cells. Haploid yeast (w303 MATa) transformed with pRS303Gal1-FUS were either transformed with an empty plasmid or mated with yeast of the opposite mating type (w303 MATα) transformed with the same empty plasmid to generate a diploid yeast strain. These yeast strains along with a control strain were then 5x serially diluted (from left to right) and spotted to Ura-His-Glucose medium (left, FUS expression repressed) and Ura-His-Galactose medium (right, FUS expression induced). The picture was taken after 2 days of growth at 30 °C. Nearly identical growth of the haploid and diploid control strain was observed, so only the haploid control strain was shown. Please click here to view a larger version of this figure.

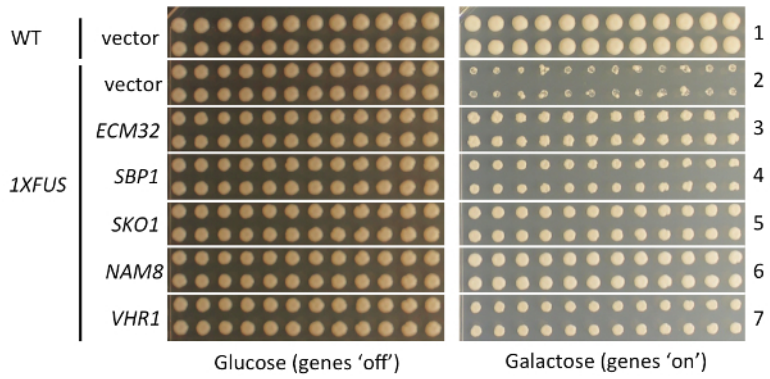

Figure 3: Five yeast genes (*ECM32, NAM8, SBP1, SKO1*, and *VHR1*) rescue FUS toxicity through the mating-based method. Plasmids containing previously identified yeast genes that suppress FUS toxicity were transformed into a haploid yeast strain (w303 MATα). These yeast were then mated with haploid yeast of the opposite mating type (w303 MATa) transformed with a FUS expression plasmid. Diploid yeast containing FUS and either an empty vector or one of the five suppression genes was selected and spotted in replicates on agar plates containing glucose (genes 'off') and galactose (genes 'on'). (1) shows a control yeast strain transformed with two empty vectors. (2) shows the diploid FUS yeast strain with an empty vector where the expression of FUS is very toxic. (3–7) show diploid yeast expressing FUS as well as a suppression gene that can rescue FUS toxicity. Each number (1–7) shows two rows of twelve identical replicates. The picture, representing three independent experiments, was taken after 3 days of growth at 30 °C. Please click here to view a larger version of this figure.

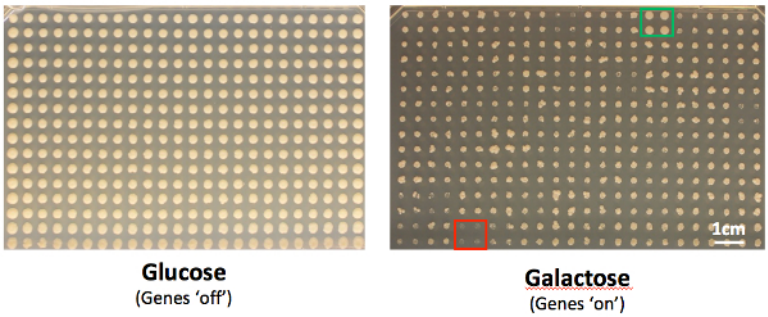

Figure 4: Library screening for genes that rescue or exacerbate FUS toxicity. Haploid yeast containing FUS was mated with haploid yeast containing the library genes. After mating, diploid cells containing both FUS and a library gene were selected and then spotted to glucose (FUS and library genes 'off') and galactose agar plates (FUS and library genes 'on') in quadruplicate. On the galactose plate, in which FUS and the library gene were expressed, most of the yeast were unable to grow well. This indicates that FUS is toxic and most library genes were unable to rescue toxicity. The green square demonstrates an example of a library gene that suppresses FUS toxicity and allows the yeast to form colonies. The red square indicates an example of a library gene that exacerbates FUS toxicity. The plates shown here are representative of 10 plates of library genes that were screened against. The picture was taken after 3 days of growth at 30 °C. Please click here to view a larger version of this figure.

Discussion

Here, we describe a protocol to perform a plasmid overexpression screen in yeast using mating to introduce the plasmid library into the yeast model. Using this approach, multiple yeast models of neurodegenerative disease protein toxicity can be screened using the same collection of yeast transformed with a plasmid library. The laborious process of transformation only needs to be performed once, after which highly efficient yeast mating is used to introduce the plasmid library into the query strain. This protocol does rely on the use of robotic equipment to dispense media and spot yeast cultures onto agar plates. While the protocol can be performed without the use of robotic equipment, it will be more time consuming. This method was successfully used to screen for genes that can modify the toxicity of FUS.

We observed that FUS is slightly less toxic in the diploid yeast background. This is most likely due to gene copy number and differences in growth rate of diploid yeast. Unless the phenotype that is being studied is mating type or ploidy-dependent, the growth phenotype of the toxicity is consistent in haploid and diploid yeast. Because of this, the mating-based method is expected to work widely in many yeast models of various growth phenotypes. Nonetheless, the phenotype of the yeast model should be verified to make sure that it is still present in the diploid background before this screening method is performed. This method can be used to study many different phenotypes in yeast and is not limited to the study of neurodegenerative protein toxicity. In addition, any plasmid library containing yeast expression vectors can be used.

After performing the screen, there are a number of verification assays that will help ensure the identified hits are specific to the yeast model being screened. Enhancers of toxicity should be tested in yeast without co-expressing the disease protein of interest. Enhancers causing toxicity independent of the disease protein of interest should be eliminated from further study. It is important to consider whether the suppressors of toxicity are affecting expression of the disease protein by affecting the Gal1 promoter. Any suppressors affecting expression from the Gal1 promoter should be eliminated from further study.

Yeast strains containing the library plasmid are permanently stored in glycerol stock and can be quickly revived when needed so that the mating-based method may be easily applied to other yeast models in which the same library genes need to be screened against. The efficiency of the

mating-based method becomes obvious when the same type of screening is used to remove false positives or when multiple different yeast models need to be studied. We have successfully used this method to screen a yeast model of TDP-43, another protein linked to ALS.

Disclosures

The authors have nothing to disclose.

Acknowledgements

We are thankful for the thoughtful discussions with members of the Ju laboratory and Zhong laboratory, and the financial support from the Wright State University.

References

1. Dujon, B. A., Louis, E. J. Genome diversity and evolution in the budding yeasts (Saccharomycotina). *Genetics.* **206** (2), 717-750 (2017).
2. Khurana, V., Lindquist, S. Modelling neurodegeneration in *Saccharomyces cerevisiae*.: why cook with baker's yeast? *Nature Reviews Neuroscience.* **11** (6), 436-449 (2010).
3. Willingham, S., Outeiro, T. F., DeVit, M. J., Lindquist, S. L., Muchowski, P. J. Yeast genes that enhance the toxicity of a mutant huntingtin fragment or alpha-synuclein. *Science.* **302** (5651), 1769-1772 (2003).
4. Outeiro, T. F., Lindquist, S. Yeast cells provide insight into alpha-synuclein biology and pathobiology. *Science.* **302** (5651), 1772-1775 (2003).
5. Cooper, A. A. *et al.* Alpha-synuclein blocks ER-Golgi traffic and Rab1 rescues neuron loss in Parkinson's models. *Science.* **313** (5785), 324-328 (2006).
6. Treusch, S. *et al.* Functional links between Abeta toxicity, endocytic trafficking, and Alzheimer's disease risk factors in yeast. *Science.* **334** (6060), 1241-1245 (2011).
7. Ju, S. *et al.* A yeast model of FUS/TLS-dependent cytotoxicity. *PLoS Biology.* **9** (4), e1001052 (2011).
8. Sun, Z. *et al.* Molecular determinants and genetic modifiers of aggregation and toxicity for the ALS disease protein FUS/TLS. *PLoS Biology.* **9** (4), e1000614 (2011).
9. Johnson, B. S., McCaffery, J. M., Lindquist, S., Gitler, A. D. A yeast TDP-43 proteinopathy model: Exploring the molecular determinants of TDP-43 aggregation and cellular toxicity. *Proceedings of the National Academy of Sciences of the United States of America.* **105** (17), 6439-6444 (2008).
10. Fleming, M. S., Gitler, A. D. High-throughput yeast plasmid overexpression screen. *Journal of Visualized Experiments.* (53), e2836 (2011).
11. Herskowitz, I. Life cycle of the budding yeast *Saccharomyces cerevisiae*. *Microbiological Reviews.* **52** (4), 536-553, (1988).
12. Suter, B., Auerbach, D., Stagljar, I. Yeast-based functional genomics and proteomics technologies: the first 15 years and beyond. *Biotechniques.* **40** (5), 625-644 (2006).
13. Barmada, S. J. *et al.* Amelioration of toxicity in neuronal models of amyotrophic lateral sclerosis by hUPF1. *Proceedings of the National Academy of Sciences of the United States of America.* **112** (25), 7821-7826 (2015).
14. Jackson, K. L. *et al.* Preservation of forelimb function by UPF1 gene therapy in a rat model of TDP-43-induced motor paralysis. *Gene Therapy.* **22** (1), 20-28 (2015).

Copyright © 2018 Journal of Visualized Experiments