Chapter 15

Using Yeast to Identify Coronavirus–Host Protein Interactions

Stuart Weston and Matthew Frieman

Abstract

We have developed a screening system using the yeast *Saccharomyces cerevisiae* to identify eukaryotic genes involved in the replication of mammalian viruses. Yeast come with various advantages, but in the context of coronavirus research and the system outlined here, they are simple and easy to work with and can be used at biosafety level 2. The system involves inducible expression of individual viral proteins and identification of detrimental phenotypes in the yeast. Yeast knockout and overexpression libraries can then be used for genome-wide screening of host proteins that provide a suppressor phenotype. From the yeast hits, a narrowed list of candidate genes can be produced to investigate for roles in viral replication. Since the system only requires expression of viral proteins, it can be used for any current or emerging virus, regardless of biocontainment requirements and ability to culture the virus. In this chapter, we will outline the protocols that can be used to take advantage of *S. cerevisiae* as a tool to advance understanding of how viruses interact with eukaryotic cells.

Key words  Yeast, Suppressor screening, Host–virus interaction, Host factors

1 Introduction

Yeast have a long-standing history of use in cell biology research. The model organisms *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* have been utilized to great effect in developing understanding of higher order eukaryotes owing to a high degree of homology in the context of a simplified genomic system. In the Frieman lab, we have developed a set of protocols that utilize *S. cerevisiae* for the identification of novel eukaryotic factors involved in viral replication. An example of this can be found in the work presented in Weston *et al.* that identified a host factor involved in replication of Middle East respiratory syndrome coronavirus (MERS-CoV) [1]. The system is amenable for use with any other virus through the series of protocols that will be outlined here. Any currently studied or newly emerging coronavirus could potentially be assessed in this system to allow for rapid
identification of host factors involved in viral replication. A major advantage of the yeast system is that it allows for research into viral replication in a biosafety level 2 condition, avoiding potential constraints on certain known and novel coronaviruses. In addition, yeast provide a simple and easy tool with many well-established protocols to aid in understanding of coronavirus replication in a simplified genetic system.

The yeast screening platform utilizes phenotypes induced in *S. cerevisiae* through the overexpression of viral proteins. Our work with MERS-CoV identified four viral genes capable of causing slow growth of the yeast. All other viral systems we have tested (SARS-CoV being another coronavirus) have at least one, if not multiple proteins capable of causing yeast to grow slowly. As will be detailed below, the expression of viral proteins in yeast requires standard cloning procedures with yeast vectors that allow inducible expression. This slow growth phenotype can be leveraged to screen for host factors that may have a role in viral replication. Yeast knockout and overexpression libraries are well established, easily obtainable semi-genome to genome-wide screening platforms that can be used in conjunction with the viral protein induced phenotypes for suppressor screening. A suppressor phenotype in this context is one whereby the knockout or overexpression of a host gene causes a loss of the original slow growth phenotype induced by the viral protein. This suppression is the readout utilized to identify eukaryotic proteins that have a functional genetic interaction with the viral protein of interest. From this point of identifying yeast gene suppressors, mammalian cell culture system can be used to validate the homologous genes using standard techniques (e.g., RNAi/CRISPR) for roles in viral replication.

Throughout this chapter, we will use the example of a slow growth phenotype in the context of a galactose inducible (GAL1) system using uracil (URA)-based selection. However, there are numerous other phenotypes that can be caused by exogenous expression of viral proteins in yeast and used for suppressor screening. Additionally, there are various other inducible expression systems and metabolic selection systems. For the sake of brevity, we will keep the discussion here focused on the system we have developed using MERS-CoV that can be extended to any other virus. As mentioned previously, an advantage of utilizing yeast as an initial screening platform is the wealth of established protocols available. We direct the reader to other sources should a deeper investigation in the way yeast can be used for studying mammalian viruses be desired [2, 3].

One may consider using the protocols provided here as a means to identify genes involved in coronavirus replication that may not be identified in other screening systems. In more complex mammalian cell culture systems, genome wide screens can be biased toward the strongest hits such as entry factors or innate immune response
modulators. The yeast system avoids these issues as it focuses only on a single protein and does not require any viral entry to take place, and yeast do not have an interferon response. Moreover, while yeast share a high degree of genetic homology with mammalian cells, they are significantly simpler with approximately 6000 open reading frames. Therefore, using yeast may allow for the identification of interactions that could be masked in the more complex genetic pathways of mammalian cells. Ultimately, we propose that yeast may be a powerful tool to advance the understanding of coronavirus (and other viral families) replication and identify potential antiviral targets.

2 Materials

2.1 Yeast Strains

1. *S. cerevisiae* strain BY4742 or BY4741 (see Note 1).
2. Yeast knockout library (see Note 2).

2.2 Yeast Culture Media

1. Yeast extract-peptone-dextrose (YEPD or YPD) medium: 1% yeast extract, 2% peptone, and 2% glucose/dextrose. For 1 l dissolve 10 g yeast extract and 20 g peptone in 700 ml ddH2O. Make volume to 900 ml and autoclave for 20 min. Then add 100 ml 20% glucose solution that has been run through a 0.2 μM filter.
2. YPD agar plates: 1% yeast extract, 2% peptone, 2% glucose/dextrose, and 2% agar. For 1 l, prepare as media above with the addition of 20 g agar prior to autoclaving. Add the filtered 20% glucose after autoclaving and pour plates.
3. Casamino acid/yeast nitrogen base (CAA/YNB) yeast minimal medium: 0.17% yeast nitrogen base, 0.5% ammonium sulfate, 0.6% casamino acids, 2% sugar (see Note 3).
4. CAA/YNB agar plates: CAA/YNB medium, 2% agar (see Note 3).
5. 30–50% glycerol solution.

2.3 Plasmid Transformation

2.3.1 Small-Scale and Large-Scale Transformation

1. Plasmids for expression of viral proteins in yeast (see Note 4).
2. Molecular biology grade water.
3. 10× TE: 100 mM Tris–Cl, pH 7.6, 10 mM EDTA.
4. 1 M lithium acetate.
5. 50% polyethylene glycol (PEG).
6. Yeast carrier DNA or salmon sperm DNA (either 10 mg/ml stock or 2 mg/ml stock can be used).
7. Sterile glass tubes.
8. YNB/CAA agar plates.
9. Bench top centrifuge.
10. Microfuge.
11. Water baths or heat blocks set at 30 °C and 42 °C.

2.3.2 Transformation in a 96-Well Format

1. Plasmids for expression of viral proteins in yeast (see Note 4).
2. Molecular biology grade water.
3. 1× TE: 10 mM Tris-Cl, pH 7.6, 1 mM EDTA.
4. 1 M lithium acetate.
5. 50% PEG.
6. 2 mg/ml yeast carrier DNA or salmon sperm DNA.
7. Deep well 96-well plates (1 ml culture volume).
8. 96-well microplate replicator or multichannel pipette.
9. 15 cm YNB/CAA agar plates.
10. Bench-top centrifuge.

2.4 Culturing Yeast

1. Sterile glass tubes.
2. Sterile glass flasks.
3. 96-well plates.
4. 30 °C incubator with a shaker set to 230–270 rpm.
5. An automated plate reader that can incubate at specified temperatures and read OD600 (e.g., BioTeK Synergy HTX) or a spectrophotometer (e.g., a nanodrop).
6. Multichannel pipette.

2.5 NaOH Protein Extraction

1. 0.1 M NaOH.
2. Western blot loading buffer (e.g., NuPAGE loading dye).
3. Beta-mercaptoethanol.

2.6 Genomic DNA Extraction

1. Yeast lysis buffer: 10 mM Tris–Cl, pH 8, 1 mM EDTA, 100 mM NaCl, 1% SDS, 2% Triton-X100.
2. TE buffer: 10 mM Tris–Cl, pH 8, 1 mM EDTA.
3. 1:1 phenol:chloroform.
4. Glass beads.
5. Bead beater homogenizer (e.g., MagNA Lyser).
6. 100% ethanol.
7. 70% ethanol (at 4 °C).
8. RNase.
9. Microfuge.
10. Microfuge tubes.
11. Microfuge tubes suitable for use in bead beater.
3 Methods

In order to take advantage of the yeast system for the identification of eukaryotic factors involved in viral replication, a certain series of steps must be undertaken. Initially, expression plasmids must be produced to exogenously express viral proteins in yeast. The yeast strain and expression plasmids must be chosen to allow for the selection of transformants through auxotrophic selection from the culture media and plates. Following production of expression plasmids for viral proteins of interest through standard cloning procedures (not described here), yeast must be transformed. Successful transformation is best confirmed through western blotting for exogenous proteins along with the auxotrophic selection. Once expression has been confirmed, analysis of the yeast for phenotypes that can be used in suppressor screening must be performed. Here, we will use a discussion of slow growth phenotypes as these are easily analyzed. We direct the reader to other sources for more in-depth discussion of methods for suppressor screening [2, 3]. Once these phenotypes have been identified, library transformations must be performed using knockout libraries, as described here, or overexpression libraries in a large-scale format. These libraries can be screened for suppressor phenotypes, which in the discussion here will be determined by a loss of the slow-growth phenotype. This allows identification of suppressor yeast clones which can be determined as hits once they have been found to have enhanced growth, while maintaining expression of the exogenous viral protein. Finally, genomic extractions and DNA sequencing allow for the identification of the knockout/overexpressed eukaryotic gene that is having a functional genetic interaction with the viral protein. Identification of the mammalian homologs of these suppressor hits will generate a list of candidate genes to investigate for roles in replication of the virus of interest.

3.1 Yeast Culture

1. Yeast stocks can be made by mixing liquid cultured yeast with glycerol to a final concentration of 15–25% and storing at –80 °C. These stocks can be maintained indefinitely (see Note 5).

2. To recover frozen stocks, streak a small amount onto an appropriate agar plate (YPD agar when non-transformed, appropriate dropout for selection of transformed yeast). Incubate this plate at 30 °C until colonies form (2–3 days). Colonies can be picked from these plates for further culture. Seal the plates in parafilm and store at 4 °C, replacing every 1–2 months.

3. Working stock plates can be either those that were struck for single colonies or a single colony can be picked, and a patch culture made by streaking patches and growing for a further 2-3 days at 30 °C (Fig. 1).
4. Pick a colony from the working stock plate and inoculate 5 ml medium in a sterile glass tube. Use YPD to culture non-transformed yeast or appropriate dropout media for transformed yeast. Briefly vortex to disperse the cells from the picked colony in the media. Incubate at 30 °C overnight with agitation. This overnight culture will yield cells in stationary phase (OD600 ~ 1–1.5).

3.2 Culturing Pooled Collections of Yeast Libraries from 96 Well Arrays

1. Thaw each 96-well plate of knockout yeast and replicator pin or multi-channel pipette (5 μl) yeast into 100 μl YPD media in a fresh 96-well plate and grow at 30 °C for 48 h.

2. Combine all wells from each plate and aliquot to make plate pools (1–2 ml aliquots).

3. Media from each plate can be further mixed and aliquoted to make full library pools.

4. Make glycerol stocks for freezing by combining pool libraries with 15–25% final concentration of glycerol and storing at −80 °C.

3.3 Small-Scale Plasmid Transformation

In order to utilize yeast for the study of coronaviruses, it is necessary to transform plasmid constructs into the yeast for exogenous protein expression. Again, for simplicity, only a lithium acetate protocol will be discussed here, but other approaches can be used. We direct the reader to [2, 3] for additional protocols.
1. Transformation is most efficient when cells are in mid-log phase (OD600 ~ 0.4–0.8). The overnight culture described above would therefore yield too much growth. Two approaches can be used to culture cells for transformation; either perform a tenfold dilution series after the initial inoculation of YPD from a single colony. Disperse cells in 10 ml YPD by briefly vortexing, transfer 1 ml of this media into 9 ml, and repeat 4–6 times. Culture all tubes overnight at 30 °C and assess OD600 the following day. Alternatively, culture cells overnight as above, then the following day dilute in fresh YPD to an OD600 around 0.2–0.3 and grow for 3–5 h at 30 °C.

2. Once cells are at the appropriate OD, pellet cells by centrifugation at 1500 × g for 3 min.

3. While cells are being washed (ensuing steps), heat yeast carrier DNA at 95 °C for 5 min then place on ice for at least 5 min prior to use.

4. Resuspend cells in 10 ml molecular biology-grade water.

5. Pellet cells by centrifugation again.

6. Resuspend cells in 10 ml 0.1× TE/0.1 M lithium acetate (diluted in water).

7. Pellet cells by centrifugation again.

8. Resuspend cells in 1/20th of the original growth volume (for a 10 ml culture, this would be 500 μl) of 0.1× TE/0.1 M lithium acetate.

9. Make transformation mixture: mix 50 μg yeast carrier DNA, 50–200 ng of plasmid DNA in a microcentrifuge tube, then add 50 μl of resuspended cells. Finally, add 350 μl PEG solution (40% PEG/1× TE/100 mM lithium acetate, shake to mix thoroughly before and after the addition to transformation mixture).

10. Incubate transformation mixture at 30 °C for 20 min.

11. Incubate mixture at 42 °C for 20 min.

12. Add 700 μl molecular biology-grade water, mix and centrifuge for 3 min at maximum speed in a table-top microcentrifuge.

13. Remove supernatant.

14. Resuspend pellet in 100 μl molecular biology-grade water.

15. Plate out all 100 μl to an agar plate of appropriate dropout media for the selection of transformants. Spread with a sterile glass rod or sterile glass beads.

16. Incubate transformation plate at 30 °C for 48 h.

17. Colonies will form after 2 days if transformation was successful.
18. Pick a colony or colonies and streak for singles onto a fresh agar plate. Incubate for a further 48 h to give a working stock plate that individual colonies can be picked from for experimental use.

3.4 Large-Scale Plasmid Transformation

The above plasmid transformation is best for transforming individual cultures of yeast with individual plasmids. However, it may be desirable to transform in a higher content manner, for example, to transform a yeast library or to transform multiple plasmids into different cultures of yeast. The above protocol can be adapted to this end. Two examples will be given, either transformation of a pooled collection of a yeast library or transformation of libraries in 96-well plates.

3.4.1 Large-Scale Plasmid Transformation of Pooled Yeast Library

1. Grow up overnight culture of pooled collection of a yeast library. 100 μl of glycerol stock into 50 ml of YPD. Incubate overnight with shaking at 230–270 rpm at 30 °C.

2. The following morning put this overnight culture (OD600 ~ 1–1.5) at 4 °C.

3. Produce overnight dilution series cultures (16–18 h culture). Produce 1:10 dilution series for 6–8 dilutions all of 50 ml. Culture with shaking (230–270 rpm) at 30 °C. This approach should generate yeast culture in mid-log phase of growth (OD600 ~ 0.4–0.8) at one of the dilutions.

4. Following the second overnight culture, pick the dilution level that has resulted in the best growth level for mid-log phase growth.

5. Pellet the cells by centrifugation at 1500 × g for 3 min.

6. While cells are being washed (ensuing steps), heat yeast carrier DNA at 95 °C for 5 min, then place on ice for at least 5 min prior to use.

7. Remove supernatant from cells and resuspend with 10 ml molecular biology-grade water.

8. Pellet again and remove supernatant.

9. Resuspend cells in 10 ml 0.1× TE/0.1 M lithium acetate in water.

10. Pellet again and remove supernatant.

11. Resuspend cells in 500 μl of 0.1× TE/0.1 M lithium acetate (a 1/100th of the original culture volume).

12. Make transformation mix in two separate microcentrifuge tubes (values provided are per tube): mix 125 μg yeast carrier DNA and 2.5 μg of plasmid DNA in a microcentrifuge tube, then add 125 μl of yeast cells. Finally add 875 μl of PEG solution (40% PEG/1× TE/100 mM lithium acetate, shake to mix
thoroughly before and after addition to transformation mixture).

13. Incubate transformation mixture tubes at 30 °C for 20 min.
14. Incubate mixtures at 42 °C for 20 min.
15. Add 500 μl molecular biology-grade water and mix.
16. Centrifuge at maximum speed in a bench-top centrifuge for 3 min.
17. Remove supernatant and resuspend each pellet in 1 ml molecular biology grade water. Combine the 1 ml of resuspended cells from each microcentrifuge tube in a falcon tube and make final volume up to 10 ml with further addition of water.
18. Plate all 10 ml out using 200 μl per plate on appropriate dropout and sugar agar plates (~50 plates). For example, URA dropout and glucose-containing plates. Spread with a sterile glass rod or sterile glass beads.
19. Incubate at 30 °C for 48 h.
20. Following incubation, collect colonies and make glycerol stocks: add 3 ml sterile PBS or molecular biology grade water to each plate and swirl/pipette up and down until colonies visibly detach and go into solution. Collect the 3 ml from each plate into a falcon tube(s). Wash each plate with a further 2 ml sterile PBS or molecular biology-grade water and add to the original collections. Centrifuge at 1000 × g for 5 min to pellet the collected cells. Resuspend in a final volume of 20 ml appropriate selection media with sugar. Mix resuspended yeast with glycerol for stocks (as discussed previously). The resuspended yeast can also be directly plated out for suppressor screening (discussed below, also depicted in Fig. 3).

3.4.2 Large-Scale Plasmid Transformation in 96-Well Format

1. Grow up overnight cultures for transformation. Thaw 96 well glycerol stock plates (as supplied with certain libraries purchased from companies such as Dharmacon). Pipette 5 μl or use a microplate replicator to transfer from the glycerol stock into 600 μl YPD media in a 96-deep-well plate.
2. Culture overnight at 30 °C with shaking at 230–270 rpm.
3. Pellet cells by centrifugation of 96-deep-well plate at 1500 × g for 5 min.
4. While cells are being washed (ensuing steps), heat yeast carrier DNA at 95 °C for 5 min, then place on ice for at least 5 min prior to use.
5. Remove YPD media from wells.
6. Resuspend cells in 100 μl water and transfer to a clear plastic 96 well plate.
7. Pellet cells by centrifugation again.
8. Resuspend cells in 96-well plate with 50 μl of transformation mix made in the following way: 1.5 ml 1 M lithium acetate, 2 ml of 2 mg/ml yeast carrier DNA (dilute with 1 × TE if using starting with a high concentration stock), 10 μg plasmid DNA (0.1 μg per well). Make volume to 5 ml with molecular biology grade water. These values are per 96-well plate.
9. Gently vortex plate to disperse cells in the transformation mix.
10. Add 100 μl 50% PEG to the 50 μl transformation mixture in each well.
11. Put on a shaker at 230–270 rpm for 5 min at 30 °C to mix.
12. Incubate for 1 h at 42 °C.
13. Pellet cells by centrifugation at 1500 × g for 10 min.
14. Remove supernatant.
15. Resuspend cells in 50 μl of appropriate selection media with sugar.
16. Transfer the 50 μl of resuspended cells to new 96-deep-well plates containing 600 ul selection media with sugar per well.
17. Incubate at 30 °C for 48 h with shaking at 230–270 rpm.
18. From here, new glycerol stocks of the transformed yeast can be made by mixing cultured yeast with 30% glycerol at a 1:1 ratio and storing at −80 °C. Yeast can also be plated for suppressor screening.

3.5 Protein Extraction

To confirm expression of exogenous protein in the yeast, a protein extraction and western blot is the best approach. Here the NaOH protein extraction is discussed, but as previously, various other approaches can be used (see [2, 3]).

1. Grow an overnight liquid culture of yeast. Pick colonies from the struck singles plate after transformation and grow up in appropriate dropout liquid media with the appropriate induction for expression such as galactose (3–5 ml of culture). Glucose media with the transformed yeast, empty vector transformed yeast, or wild-type yeast grown in YPD can all act as negative controls.
2. Culture overnight at 30 °C to reach stationary phase as described previously (OD600 ~ 1.5).
3. From the overnight culture, take 100–500 μl into a microcentrifuge tube.
4. Pellet yeast cells by centrifugation at 900 × g for 3 min.
5. Remove supernatant and resuspend pellet in 100 μl of 0.1 M NaOH.
6. Incubate for 10 min at RT.
7. Pellet cells by centrifugation as above.
8. Remove supernatant and resuspend pellet in 100 μl 1× western blot buffer with 0.5% beta-mercaptoethanol.
9. Heat sample at 95 °C for 5 min.
10. Centrifuge at maximum speed in a table-top centrifuge for 10 min.
11. Use 10 μl (or more if necessary) of this sample in standard SDS–PAGE/western blotting protocols to confirm expression of exogenous protein.

3.6 Identification of Slow-Growth Phenotypes

As discussed above, there are various yeast phenotypes that can be used for suppressor screening. Here slow growth will be discussed. We direct the reader to [2, 3] for more detailed discussions of alternative screenable phenotypes in yeast. Two methods can be used to identify slow-growing yeast, growth curve analysis, or serial dilution drop cultures.

3.6.1 Growth Curve Analysis

Growth curves are most easily performed with an automated plate reader, capable of measuring OD600, with the ability to incubate at 30 °C. Alternatively OD can be measured using a spectrophotometer. Cultures can be made in either 96-well or 384-well format using clear non-tissue culture-treated plates. This protocol describes a 96-well format, which can be adjusted as necessary for a 384-well format.

1. Make a liquid culture of yeast by picking an individual colony of transformed yeast and culturing in 3–5 ml appropriate selection media with lack of induction of viral gene expression to allow equivalent growth between control and test yeast. For example, if using a GAL1 vector, use raffinose- or glucose-containing media. Culture at 30 °C for 48 h if using raffinose or 24 h if using glucose to reach stationary phase (see Note 6).
2. Dilute this liquid culture to an appropriate level for growth analysis over the next 48 h. A dilution factor of 1:100 to 1:10,000 is usually appropriate as this will give a starting OD600 close to or slightly lower than 0.1. This will allow sufficient growth to be analyzed over the following 48 h period. Dilute the starting culture in appropriate media to induce exogenous gene expression (or inhibit for control—galactose and glucose for the GAL1 system). Make enough culture to plate 100 μl per well of a 96-well plate.
3. Analyze on automated plate reader for 48 h. Set plate reader to incubate at 30 °C. Take OD600 readings at least every 30 min over this period. Prior to reading OD600, have the plate reader shake at ~230 rpm for 30 s to disperse yeast in the culture.
4. Analyze generated growth curves to identify yeast colonies with a growth defect compared to control cells.
3.6.2 Serial Dilution Drop Cultures

This approach involves making serial dilutions of yeast from overnight cultures and plating these onto agar plates to induce expression of the gene of interest (e.g., galactose-containing plates for GAL1 vector). See Fig. 2 for an example image.

1. Grow overnight culture from single transformation colonies (for control vector and gene of interest).

2. Test the OD600 and overnight culture into molecular biology-grade water or culture media to an OD 0.1.

3. Produce a twofold or fivefold dilution series from the original overnight cultures in molecular biology-grade water or culture media (up to 10 should be sufficient).

4. Plate 2–5 μl of the dilution series at even spacing onto an agar plate. This is most easily achieved using 15 cm agar plates and a multi-channel pipette (using a 96-well plate for producing the dilution series) or a 96-well microplate replicator (as was done in Fig. 2).

5. Incubate for 2–3 days until colonies form.

6. Assess growth defects by reduced growth at equivalent dilutions between gene of interest expressing yeast and control vector yeast.

3.7 Suppressor Screening

By this stage, viral genes have been tested for phenotypes that can be used in suppressor screening (e.g., slow growth), and yeast libraries have been transformed with plasmids to express those viral genes. Two formats for library transformation have been...
discussed, either transformation of a pool of the library or transformation of individual wells of an arrayed library. At this stage, the suppressor screen can be performed by plating libraries out on appropriate induction agar plates (e.g., galactose plates for a GAL1 promoter) and looking for faster growing colonies (larger colonies arising from suppression of the slow-growth phenotype). In the case of a pooled library, liquid cultures need to be grown from glycerol stocks (or from the original transformation collection) and spread onto agar plates. For the arrayed libraries, yeast can be multi-channel pipetted or multiple plate replicator plated onto 15 cm agar dishes. These plates can then be grown for 2–3 days and analyzed for fast-growing colonies which will appear as larger colonies (Fig. 3). The below-outlined protocol will discuss the approach for a pooled library, but the same principals apply for screening arrayed libraries.

1. Pick large colonies which are suspected to have a suppressor phenotype. Generate a streak plate on selection plates (e.g., galactose-containing agar plates). Allow the streak plates to grow for 48 h at 30 °C. Multiple isolated colonies from the original large colony can then be tested for higher accuracy.

2. Confirm suppressor phenotype using growth curve analysis or serial drop dilution.

3. Once a suppressor phenotype has been confirmed by growth analysis, confirm expression of the viral protein using NaOH protein extraction and western blotting. If a liquid growth curve was used for analysis, that final culture can be used in the protein extraction protocol. If serial drop dilution was used, colonies can be picked from serial drop dilutions and grown in appropriate induction media (e.g., galactose) to produce a liquid culture from which to extract protein. Alternatively, fresh colonies can be picked from the streak plate and grown up for NaOH protein extraction.

**Fig. 3** Example images of a yeast knockout library transformed with a slow-growth phenotype inducing expression plasmid. On glucose plates, there is no expression of viral protein from the plasmid so all colonies can grow to a similar size. On the galactose plate, suppressors are expected to be the colonies that grow to a larger size.
4. Once colonies have been found that express the viral protein and have enhanced growth rates, a “hit” has been found.

3.8 Identifying “Hit” Genes

In suppressor screening, a hit is defined by a yeast clone that has a suppression of the phenotype in question (e.g., slow growth), while maintaining expression of the viral protein responsible for that phenotype. In this case, the suppression is a result of loss or over-expression of a host gene depending if using a knockout or over-expression library. Each library has its own approach to determining the host gene of interest. Here, we will discuss finding host gene of interest in the knockout library. The original yeast knockout library was designed by homologous recombination of gene cassettes with uniform sequences, with the exception of a unique identifier region. Therefore, by performing a genomic DNA extraction from “hit” yeast clones and using specific primers for PCR amplification and DNA sequencing, the unique region can be found and searched within the database. For further detail, see http://www-sequence.stanford.edu/group/yeast_deletion_project/PCR_strategy.html

3.8.1 Genomic DNA Extraction

1. Make a liquid culture from a single colony to test by overnight growth in glucose-containing media.
2. Centrifuge 1 ml of the overnight cultured yeast in a tube suitable for use in a bead beater homogenizer at 1500 × g for 3 min.
3. Aspirate supernatant and resuspend pellet in 0.2 ml yeast lysis buffer and 0.2 ml phenol:chloroform. Add a roughly similar volume of glass beads.
4. Homogenize cells in bead beater (2 × 1 min at 6000 rpm).
5. Add 0.2 ml 1× TE and vortex briefly.
6. Centrifuge tubes at maximum speed in a microfuge for 5 min.
7. Take the aqueous fraction into a DNase/RNase-free microcentrifuge tube (roughly 0.38 ml). Discard the remaining sample.
8. Add 2× volume (0.76 ml) of 100% ethanol to 1 volume aqueous phase (0.38 ml). Mix thoroughly by vortexing.
9. Centrifuge at maximum speed in a microfuge for 3 min to pellet DNA.
10. Discard supernatant from the pellet.
11. Rinse the pellet with 0.5 ml of 4 °C 70% ethanol. Add slowly to the tube to minimize disruption of pellet. Centrifuge at maximum speed in a microfuge for 1 min.
12. Remove supernatant from the pellet.
13. Allow pellet to air dry.
14. Resuspend DNA pellet in 100 μl TE buffer.
15. RNase treat prior to PCR and subsequent sequencing. Use 25 μl of extracted DNA with an appropriate amount of RNase (depending on supplier).

16. From the extracted genomic DNA, a PCR can be run to amplify the unique identifier region for the knockout collection using “UPTAG” and “DOWNTAG” primers (http://www-sequence.stanford.edu/group/yeast_deletion_project/PCR_strategy.html). DNA sequencing of the PCR product can be performed in any preferred manner (see Note 7).

17. Having identified yeast genes that may have roles in replication of coronaviruses, the mammalian homologs must be identified for further examination. Mammalian homologs can be found either through the yeast genome database (https://www.yeastgenome.org/) or BLAST searches. This provides a subset of genes to assess for replication of coronaviruses in mammalian cell culture through siRNA/CRISPR or other disruptive techniques.

4 Notes

1. The American Type Culture Collection (ATCC) is used as the key general laboratory source of yeast strains for many laboratories. The strains used in the protocols described here are haploid strains (BY4742 and BY4741). A diploid strain (BY4743) is also available combining these two haploid strains [4, 5]. The auxotrophic markers of the strains are: BY4741: MATα his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 and BY4742 MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0. These markers allow for drop-out media to be used for selection. In the system we describe here, the plasmid has the URA3 gene on it for selection; however, alternative auxotrophic marked plasmids are available [6].

2. Yeast libraries are commercially available in arrayed formats with individual yeast clones in 96-well plates (e.g., Horizon Discovery Inc.). These libraries can be combined into pools, which is the main screening strategy described here [7]. Single knockout screening using robotic arraying have also been performed, which will briefly be mentioned [8, 9].

3. To makeCAA/YNB medium, make the following: For 1 l, dissolve 1.7 g yeast nitrogen base and 5 g ammonium sulfate in 450 ml ddH2O in bottle 1 and 6 g casamino acids in 450 ml ddH2O in bottle 2. Autoclave separately, combine, and then add 100 ml 20% sterile sugar solution. Sugar options (depending on the purpose as discussed in the Methods section): 20% glucose, 20% galactose, or 20% raffinose. All are sterile-filtered
through a 0.2 μM filter after dissolving sugar in water. For CAA/YNB agar plates, add 2% agar into bottle 2 prior to autoclaving. Combine bottle 1 and 2, add sugar, and pour in plates.

4. The plasmids used in these protocols are standard yeast overexpression plasmids based on those published in Mumberg et al. [6]. These plasmids can have auxotrophic markers of HIS3, TRP1, LEU2, or URA3 in combination with promoters containing a constitutively expressing promoter (TEF1) or a galactose-inducible promoter (GAL1). The galactose-inducible promoter plasmid system with a URA3 auxotrophic marker is used in the system described here. Similar to mammalian expression plasmids, it is possible to add tags to any of the viral proteins of interest in these yeast expression systems through standard cloning procedures. The galactose-inducible system is regulated by the carbon source and the yeast used for growth. In the presence of 2% glucose, the GAL1 promoter is actively repressed and no transgene is expressed. When yeast carrying the plasmid are grown in 2% galactose, the transgene is expressed to high levels. There is also the option to grow yeast in 2% raffinose which neither activates nor represses expression from the GAL1 promoter.

5. Yeast cultures can be contaminated by other yeast or bacteria. It is not necessary to work in a biosafety hood, but use of sterile equipment and reagents is advisable.

6. When inducing expression of proteins in the GAL1 system that is used here, it is worth noting that cultures may grow slower in galactose- or raffinose- containing media than glucose counterparts as these sugars are utilized less efficiently.

7. Access to DNA sequencing is required for the identification of gene knockouts if using a pooled collection of the yeast libraries rather than arrayed collections. See http://www-sequence.stanford.edu/group/yeast_deletion_project/PCR_strategy.html for information regarding required PCR primers to amplify the tag sequence that is required for the identification of knockout yeast.

References

1. Weston S, Matthews KL, Lent R, Vlk A, Haupt R, Kingsbury T, Frieman MB (2019) A yeast suppressor screen used to identify mammalian SIRT1 as a proviral factor for Middle East respiratory syndrome coronavirus replication. J Virol 93:e00197–e00119
2. Clontech Laboratories Inc. (2009) Yeast protocols handbook. http://www.takara.co.kr/file/manual/pdf/PT3024-1.pdf
3. Smith JS, Burke DJ (2014) Methods in molecular biology: yeast genetics methods and protocols. Springer International Publishing
4. Winston F, Dollard C, Ricupero-Hovasse SL (1995) Construction of a set of convenient saccharomyces cerevisiae strains that are isogenic to S288C. Yeast 11:53–55

5. Brachmann CB, Davies A, Cost GJ, Caputo E, Li J, Hieter P, Boeke JD (1998) Designer deletion strains derived from Saccharomyces cerevisiae S288C: a useful set of strains and plasmids for PCR-mediated gene disruption and other applications. Yeast 14:115–132

6. Mumberg D, Muller R, Funk M (1994) Regulatable promoters of saccharomyces cerevisiae: comparison of transcriptional activity and their use for heterologous expression. Nucleic Acids Res 22:5767–5768

7. Smith AM, Durbic T, Oh J, Urbanus M, Proctor M, Heisler LE, Giaever G, Nislow C (2011) Competitive genomic screens of bar-coded yeast libraries. J Vis Exp 54:2864

8. Scherens B, Goffeau A (2004) The uses of genome-wide yeast mutant collections. Genome Biol 5(7):229

9. Giaever G, Nislow C (2014) The yeast deletion collection: a decade of functional genomics. Genetics 197:451–465