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# Title: A Suppressor Screen for the Characterization of Genetic Links Regulating Chronological Lifespan in *Saccharomyces cerevisiae*

## Authors and Affiliations:

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# Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes, SC all set**

3. **Interview statements:** Considering the COVID-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

   ☒ Use Interview Statements from previously submitted ASV

4. **Filming location:** Will the filming need to take place in multiple locations? **No, 3 rooms in the same hallway**

**Current Protocol Length**

Number of Steps: 26
Number of Shots: 52, 11 of them SC or LAB MEDIA

# Introduction

**1. Introductory Interview Statements**

*Videographer: Interview statements already filmed and submitted, skip to protocol.*

**REQUIRED:**

1.1. **James Arnone:** Aging is characterized…..

1.1.1. LAB MEDIA: Take 1: blackmagic cam 2_1_2020-03-10_0845_C0000.mov and blackmagic cam1_1_2020-03-12_0621_C0000.mov, same statement different frame. Take 2: blackmagic cam 2_1_2020-03-10_0849_C0001.mov and blackmagic cam1_1_2020-03-12_0625_C0001.mov. *Video Editor: Use the previously uploaded ASV as a reference to edit, some statements can be cut. https://www.jove.com/v/61506/a-suppressor-screen-for-characterization-genetic-links-regulating*

# Protocol

## 2. Identify Potential Genetic Interactions for Screening

2.1. Begin by identifying the genetic backgrounds that results in an abnormally shortened chronological life span, or CLS, in *Saccharomyces cerevisiae* using the *Saccharomyces* Genome Database **[1-TXT]**.

2.1.1. WIDE: Establishing shot of talent at the computer. **TEXT: SGD: https://www.yeastgenome.org**

2.2. Select the **Function** tab from the options on the top of the webpage, then select **Phenotype** followed by **Browse all Phenotypes [1]**.

2.2.1. SCREEN: 1 Sir2 Identification.mp4. 0:25 – 0:33.

2.3. From the **Yeast Phenotype Ontology** options scroll to the **Development** subheading and select **Chronological Lifespan**, found under the **Lifespan** subheading **[1]**.

2.3.1. SCREEN: 1 Sir2 Identification.mp4. 0:33 – 0:50. Chronological Lifespan selected.

2.4. Select the qualifier for **increased** to identify genes with a phenotype that results in an increased chronological lifespan when deleted **[1]**.

2.4.1. SCREEN: 1 Sir2 Identification.mp4. 0:50 – 0:55.

2.5. Identify target genes to screen for genetic interactions that may suppress the phenotype, based on reported or predicted ontology attributes, of the identified mutant **[1]**.

2.5.1. SCREEN: 1 Sir2 Identification.mp4. 0:55 – end. Scroll down target genes.

2.6. *SIR2 (pronounce 'sir-2')* was selected for this demonstration based on the reported CLS phenotype and interactions with autophagy **[1]**.

2.6.1. SCREEN: 2 Sir2 DNA Sequence Take 2.mp4. 0:20 – 0:30.

## 3. Design the Cloning Strategy to Clone *SIR2* into the pRS315 Vector

3.1. Design primers to have a 21 to 22 nucleotide complementarity to the intergenic regions upstream and downstream of the *SIR2 (pronounce 'sir-2')* **[1]**. Ensure that the entire gene, along with the untranslated regions of the mRNA is cloned by mapping those features from the available datasets **[2]**.

3.1.1. SCREEN: 4 PCR Primers.mp4.

3.1.2. SCREEN: 2 Sir2 DNA Sequence Take 2.mp4. 0:55 - 1:00.

3.2. Ensure that the PCR primer design results in forward and reverse primers that have a melting temperature above 53 and below 60 degrees Celsius **[1]**.

3.2.1. LAB MEDIA: Figure 1 A.

3.3. Next, add restriction enzyme digestion target sites to the 5-prime end of each primer that are compatible to the plasmid-cloning vector **[1]**.

3.3.1. LAB MEDIA: Figure 1 A. *Video Editor: Emphasize the red and purple sequences in the FP and RP.*

3.4. Finally, add a four-nucleotide sequence overhang to the 5-prime end of each primer to allow the restriction enzyme to bind and digest the amplicon **[1]**.

3.4.1. LAB MEDIA: Figure 1 A. *Video Editor: Emphasize the grey sequences in the FP and RP.*

3.5. After the oligonucleotides are commercially synthesized, resuspend them by centrifuging in a tabletop microfuge at maximum speed for 4 minutes **[1]**, then add TE solution to make a stock concentration of 100 micromolar **[2]**. Store the stock at -20 degrees Celsius and dilute 10 X for use in PCR applications **[3]**.

3.5.1. Talent centrifuging the oligos.

3.5.2. Talent adding TE solution to the oligos.

3.5.3. Talent putting the stock in the freezer.

3.6. To produce an amplicon that is suitable for cloning, utilize a high-fidelity PCR polymerase to avoid the unintentional generation of mutations into the sequence being amplified **[1]**.

3.6.1. Talent adding the polymerase to the PCR master mix.

3.7. After performing PCR **[1]**, verify the product on a 1% TAE-agarose gel **[2]**. A 2.5 kilobase DNA fragment should be visible **[3]**.

3.7.1. Talent programming the thermocycler.

3.7.2. Talent loading product on a gel.

3.7.3. LAB MEDIA: Figure 2. *Video Editor: Emphasize the lanes with the + template.*

3.8. To insert the *SIR2* gene into the pRS315 plasmid vector, perform a digestion and ligation reaction as described in the text manuscript **[1]**. Then, proceed with transformation of the ligation product into *E. coli* **[2]**.

3.8.1. Talent putting together reagents for the restriction digestion.

3.8.2. Talent taking the ligation product out of the incubator.

3.9. Thaw a 50-microliter tube of frozen, competent *E. coli* cells on ice **[1]** and immediately add 15 microliters of the ligation reaction **[2]**. Flick the tube several times **[3]**, then immediately place it on ice and incubate for 30 minutes **[4]**.

3.9.1. Talent putting frozen cells on ice.

3.9.2. Talent adding ligation reaction to the cells.

3.9.3. Talent flicking the tube.

3.9.4. Talent placing the tube on ice.

3.10. Heat-shock the cells for 20 seconds in a water bath at exactly 42 degrees Celsius **[1]**, and immediately return the tubes to ice for a 2-minute incubation **[2]**. Add 450 microliters of room temperature recovery media to each transformation reaction **[3]** and incubate for 60 minutes at 37 degrees Celsius with shaking **[4]**.

3.10.1. Talent putting the cells in a water bath.

3.10.2. Talent putting the tubes on ice.

3.10.3. Talent adding recovery media to a tube.

3.10.4. Talent putting the tubes in the incubator and starting the shaking.

3.11. For each transformation reaction, make a 1 to 10 dilution of cells **[1]**. Using sterile technique, plate 150 microliters of the undiluted cells and the 1 to 10 dilutions onto LB-ampicillin plates **[2]**. Incubate the plates at 37 degrees Celsius overnight **[3]**.

3.11.1. Talent making a dilution.

3.11.2. Talent plating the cells.

3.11.3. Talent putting the plates in the incubator and closing the door.

3.12. On the next day, inoculate the potential transformants into 5 milliliters of LB with ampicillin and grow them overnight **[1]**.

3.12.1. Talent inoculating a colony into the LB.

## 4. Transform the Vector into *atg1Δ* and Wild Type Yeast Strains

4.1. Pellet 15 milliliters of wild type and *atg1*-null yeast cells grown to early to mid-log phase **[1-TXT]**. Decant the supernatant **[2]**, then resuspend the pellet in 1 milliliter of sterile double distilled water **[2]** and transfer the contents to a 1.7-milliliter microfuge tube **[4]**.

4.1.1. Talent putting a tube with yeast in the centrifuge and closing the lid. **TEXT: 3 minutes at > 800 *x g* at room temperature**

4.1.2. Talent decanting the supernatant.

4.1.3. Talent resuspending the pellet.

4.1.4. Talent transferring the cells to a microfuge tube.

4.2. Repeat the centrifugation, then remove the supernatant and re-suspend the cells in 250 microliters of 100 millimolar lithium acetate with gentle pipetting **[1]**. Split the

cells into separate microfuge tubes, using 50 microliters of the cell-lithium acetate mix per transformation **[2]**.

4.2.1. Talent removing water from centrifuged cells and adding lithium acetate.

4.2.2. Talent splitting the cells into different tubes.

4.3. Set up a transformation mix as described in the text manuscript **[1]**, then add 5 microliters of the appropriate plasmid for each transformation **[2]**. Vortex the tubes to mix thoroughly **[3]**.

4.3.1. Talent preparing a transformation mix.

4.3.2. Talent adding plasmid to a transformation tube.

4.3.3. Talent vortexing a tube.

4.4. Incubate the samples at 30 degrees Celsius for 45 minutes **[1]**, then heat shock them at 42 degrees Celsius for 10 minutes **[2]**.

4.4.1. Talent putting the tubes in the incubator.

4.4.2. Talent heat shocking the samples.

4.5. Pellet the cells **[1-TXT]** and carefully remove the transformation mix **[2]**, then add 300 microliters of sterile water **[3]**. Pellet cells and carefully remove the water. Resuspend the samples in 200 microliters of sterile water **[4]**.

4.5.1. Talent putting the tubes in the centrifuge and closing the lid. **TEXT: 3 minutes at > 800 *x g* at room temperature**

4.5.2. Talent removing the transformation mix.

4.5.3. Talent adding water to the cells.

4.5.4. Talent removing supernatant and resuspending the cells in water.

4.6. Set up 1 to 10 and 1 to 100 dilutions for each transformed strain of yeast **[1]**. Using sterile technique, plate 150 microliters from each sample onto SC-leucine plates to select for the plasmids **[2]**. Incubate the plates at 30 degrees Celsius for 48 to 72 hours **[3]**.

4.6.1. Talent setting up dilutions.

4.6.2. Talent plating the cells.

4.6.3. Talent putting the plates in the incubator and closing the door.

## 5. Determine the Chronological Life Span to Test for Shortened CLS Phenotype Suppression

5.1. Test the effect of overexpression of the putative suppressor on CLS by determining the number of colony-forming units that remain as a function of time **[1]**.

5.1.1. Talent taking a plate out of the incubator.

5.2. Inoculate a single colony of the strain into SC-leucine media **[1]** and grow it at 30 degrees Celsius for 72 hours with shaking **[2]**.

5.2.1. Talent inoculating a colony into the media.

5.2.2. Talent putting the media in the incubator and closing the door.

5.3. Dilute an aliquot of the culture, so that the result is a uniform number of cells in a 150-microliter volume of sterile water **[1]**. Plate the culture using sterile technique **[2]** and grow it at 30 degrees Celsius for 72 hours **[3]**.

5.3.1. Talent diluting the culture.

5.3.2. Talent plating the cells.

5.3.3. Talent putting the plate in the incubator and closing the door.

# Results

**6. Results: Chronological Lifespan of *atg1Δ+pRS315-SIR2***

6.1. After *SIR2* was PCR amplified with the designed primers, the products were visualized using agarose gel electrophoresis and compared to a no template control reaction **[1]**.

6.1.1. LAB MEDIA: Figure 2.

6.2. The pRS315 plasmid was purified from *E. coli* and visualized by agarose gel electrophoresis **[1]**. Plasmid prep from transformant number 2 was selected for cloning, which had a concentration of 256 nanograms per microliter **[2]**.

6.2.1. LAB MEDIA: Figure 3.

6.2.2. LAB MEDIA: Figure 3. *Video Editor: Emphasize the lane with miniprep #2.*

6.3. Double digestion with HindIII *(pronounce 'hind-3')* and SacII *(pronounce 'sac-2')* was used to screen the transformants for the *pRS315-SIR2* vector **[1]**. The vector was then transformed into both wild-type and the *atg1 (spell out 'A-T-G-1')* mutant to generate the strains used for chronological lifespan, or CLS, characterization **[1]**.

6.3.1. LAB MEDIA: Figure 4.

6.4. The relative copy number was determined by qPCR **[1]** and it was found that the *pRS315-SIR2* vector increases *SIR2* copy number in a wild type yeast background **[2]**.

6.4.1. LAB MEDIA: Figure 5.

6.4.2. LAB MEDIA: Figure 5. *Video Editor: Emphasize the SIR2 bar.*

6.5. The chronological lifespan of *atg1Δ+pRS315-SIR2 (spell out 'A-T-G-1-mutant-P-R-S-315-sir-2')* was compared to an isogenic strain of yeast containing an empty vector. The aging cultures were plated at consistent, equivalent dilutions and were grown for 72 hours at 30 degrees Celsius prior to imaging and quantification **[1]**.

6.5.1. LAB MEDIA: Figure 6 A.

6.6. There was no statistically significant effect of the *SIR2* construct on the CLS in the *atg1-mutant* background **[1]**. Additionally, *SIR2* overexpression actually produced a decrease in the CLS compared to the empty vector control in the wild-type background **[2]**.

6.6.1. LAB MEDIA: Figure 6 B. *Video Editor: Emphasize the two blue lines.*

6.6.2. LAB MEDIA: Figure 6 B. *Video Editor: Emphasize the two brown lines.*

# Conclusion

**7. Conclusion Interview Statements**

7.1. **James Arnone:** Be sure to plate the same dilution at every ……..

7.1.1. LAB MEDIA: blackmagic cam 2_1_2020-03-10_0919_C0016.mov or blackmagic cam1_1_2020-03-12_0655_C0016.mov. *Suggested B-roll: 5.3.2.*