Virtual Yeast Adaptation Lab

Experimental Packet

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Contents

Overview

The Virtual Yeast Experiment was developed to be an effective learning tool for statistics students. The virtual labs are interactive simulations that enable students to perform experiments, create their own testable hypothesis and collect data upon which they can perform statistical analyses. It aims to be a fun and unique way for students to integrate statistical concepts with research experiments.

Background

The budding yeast *Saccharomyces cerevisiae* has been widely used in the fermentation and brewing industries. In industrial production processes using yeast, cells are usually exposed to some environmental changes such as an increase in osmotic pressure or accumulation of ethanol and/or carbon dioxide[1]. Upon encountering such environmental changes, cells dynamically change their complex biological networks consisting of gene expression, protein expression and/or interaction, and metabolic flow[1]. Since the environmental changes (stress) affect the yield and productivity, the yeast strains that can grow well under stress conditions are highly useful for industrial production[1,2].

Yeasts are fungal organisms that can reproduce both sexually and asexually. Yeast primarily reproduce asexually by an asymmetric division process called budding where offspring arise from a single parent, and inherit the genes of that parent only[3]. The offspring will be the exact genetic copies of the parent. A colony is defined as a visible mass of microorganisms all originating from a single mother cell, therefore a yeast colony constitutes a clone of yeast daughter cells from a single yeast parent cell. Fitness is the rate of this growth or cell division.

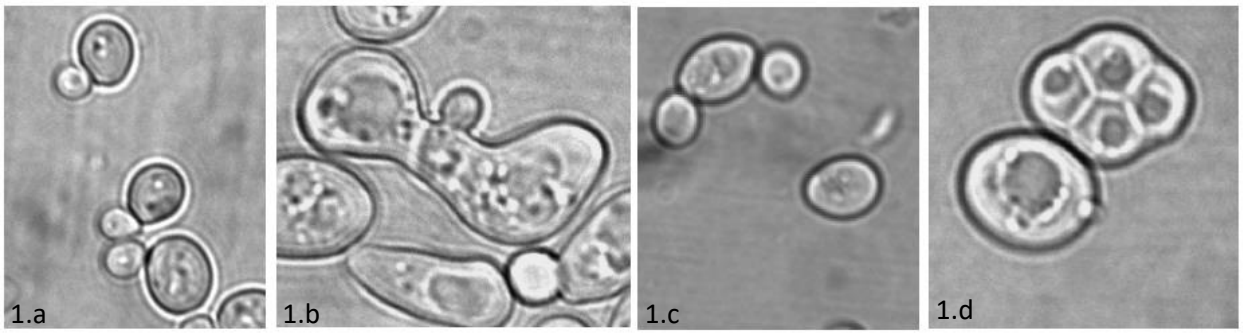


Figure 1. Reproductive cycle of yeast. Asexual budding in haploid yeast cells creating genetically identical daughter cells (Fig 1.a) Haploid cells are capable of mating with other haploid cells to produce a diploid cell. This process begins with the fusion of the cytoplasm, and then the haploid cells become fertilized and become a diploid zygote (Fig 1.b). These diploid cells (Fig 1.c) can undergo meiosis to produce four haploid spores (Fig 1.d). Finally, these haploids can then undergo germination and become haploid cells again

In this experiment, we looked at adaption to yeast *Saccharomyces cerevisiae* to salt stress. Yeast is a good model system to study salt stress tolerance, because it contains several highly conserved pathways that mediate the salt stress response[2]. Here, we will look at how well yeast adapts to salt stress (1M NaCl) by the size of the colonies formed and the fitness of the yeast strains.

Materials

1. Yeast Strain, SCE13
2. Yeast growth media, YPD
3. Salt, 1M NaCl
4. Agar Plates with YPD and NaCl
5. Test tubes
6. 96-well Microtiter Plate

Method

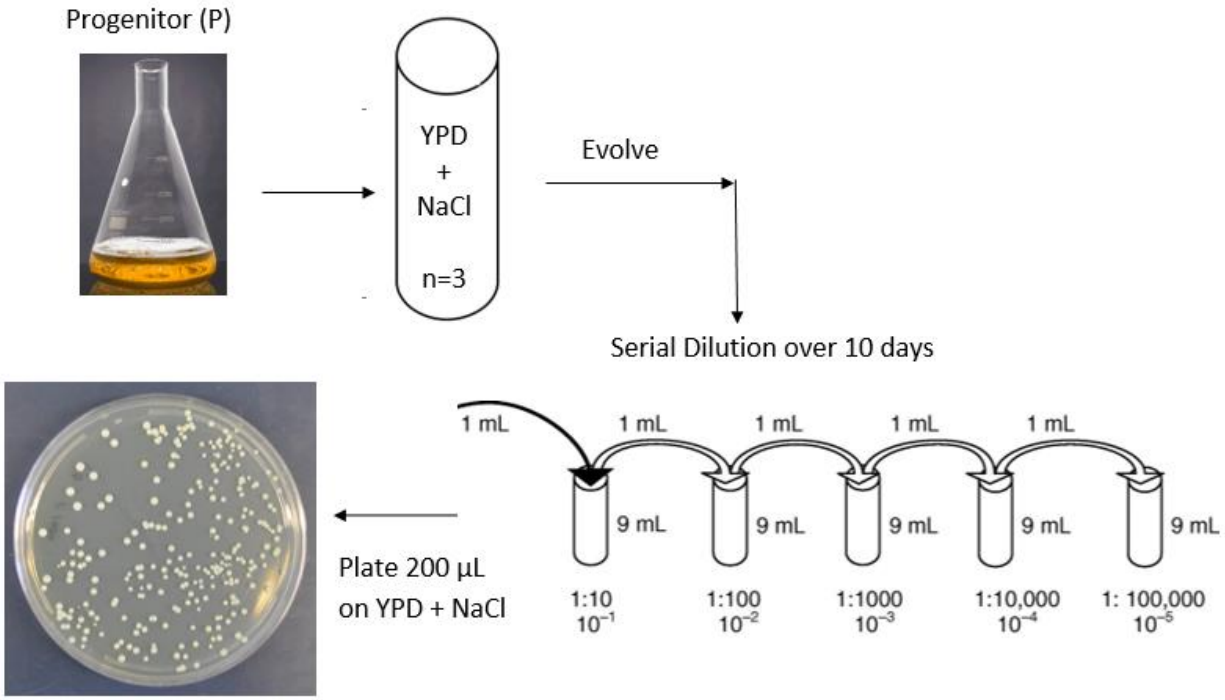


Figure 2. Flowchart of Experimental Method

Day 1- Setting up the Experiment

Pipette 100µL of yeast strain, SCE13 into 3 test tubes, to create three populations named P1-T0, P2-T0 and P3-T0, containing 10mL YPD and 1M NaCl and incubate at 30°C for two days.

Day 2- Performing First Serial Dilution

The first serial dilution was performed. Three test tubes labelled P1-T1, P2-T1 and P3-T1 were set up containing 10mL YPD and 1M NaCl. 100µL of solution was removed from P1-T0, P2-T0 and P3-T0 and added to P1-T1, P2-T1 and P3-T1 respectively, and incubated at 30°C

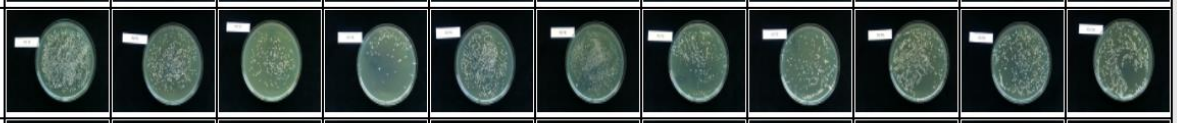
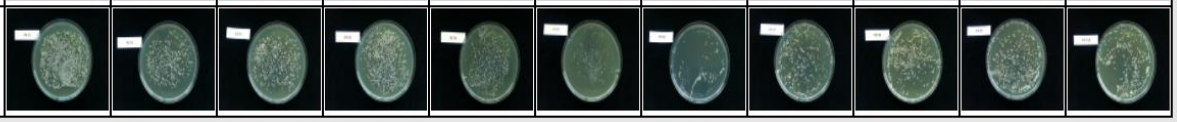
Day 3 to 11 - Continue with serial dilutions

In total 10 serial dilutions were performed.

Day 12 – Plating on Agar Plates

On the agar plates containing YPD and 1M NaCl, 200µL of 10^5 dilutions were plated from each serial dilution tubes (T0-T10) for all populations (P1, P2, P3). These plates were incubated at 30°C for two days. See Table 1.

Table 1. Yeast NaCl Agar Plates for 6 Populations across 10 serial dilution tranfers

Population/Transfer No.	T0	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
P1											
P2											
P3											

Day 14 – Photographing the agar plates

All the plates were photographed.
5 images were taken for each plate for all the populations from T0, T1, T5 and T10.
These images were taken in order to view the yeast colonies more clearly.

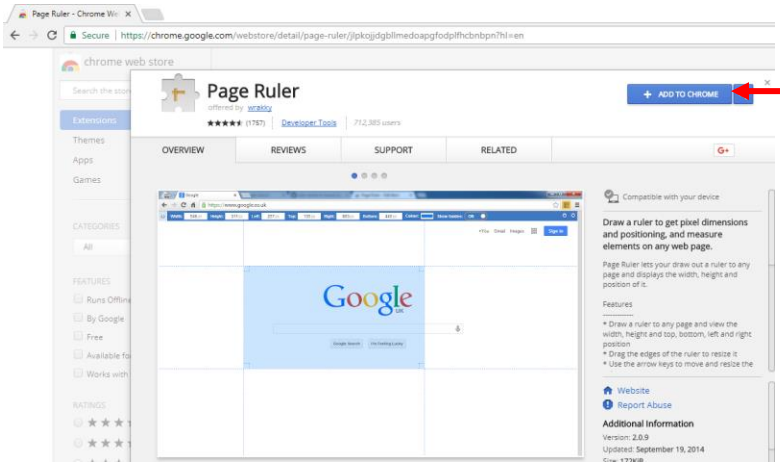
Using the Virtual Experiment

Now that you know how the experiment was conducted, you can begin data collection and analyses in just a few easy steps.

Before we begin you will need to add two extensions to your Google Chrome browser

1. Page Ruler

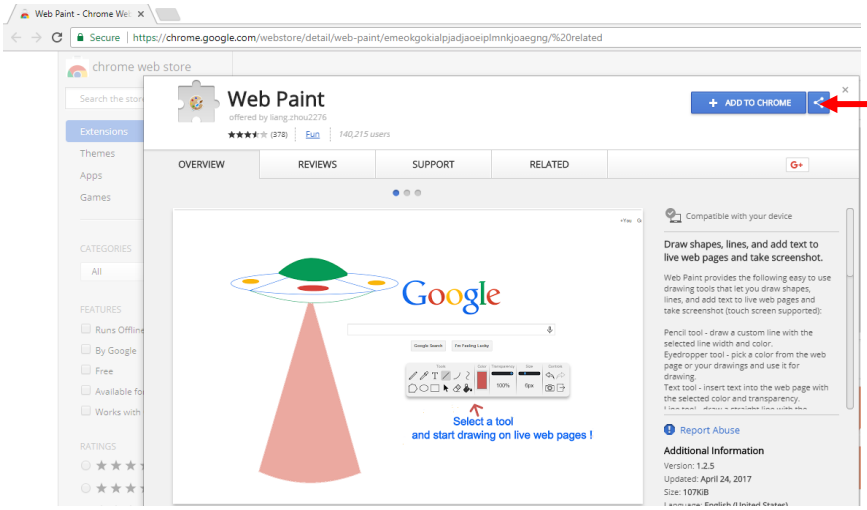
https://chrome.google.com/webstore/detail/page-ruler/jlpkojjdgbllmedoapgfodplfhcbnbpn?hl=en



Click on ADD TO CHROME – Follow the on-screen instructions to add Web Paint Extension

2. Web Paint

https://chrome.google.com/webstore/detail/web-paint/emeokgokialpjadjaoeiplmnkjoaegng/related



Click on ADD TO CHROME – Follow the on-screen instructions to add Web Paint Extension

Once you have added the extensions, you are all ready to begin collecting the data.

A) Measuring Colony Size

To do this

1. Go to Colony Size Agar Plates Sections
   There are 3 Tables in this section organized by Yeast Population.

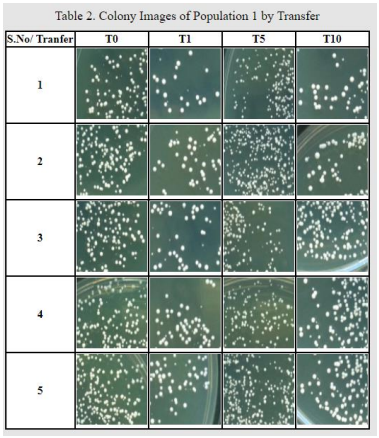


Table 2. Colony Images of Population 1 by Transfer

S.No/ Tranfer	T0	T1	T5	T10
1				
2				
3				
4				
5				

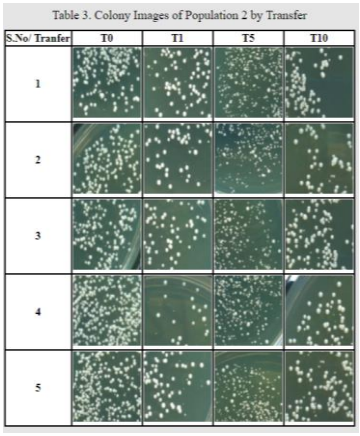


Table 3. Colony Images of Population 2 by Transfer

S.No/ Tranfer	T0	T1	T5	T10
1				
2				
3				
4				
5				

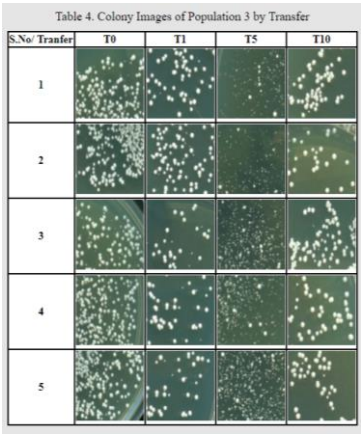


Table 4. Colony Images of Population 3 by Transfer

S.No/ Tranfer	T0	T1	T5	T10
1				
2				
3				
4				
5				

2. Select a Population to begin collecting data.
   Here in this example we will use Population 1.

Measure yeast colony size from T1 (Transfer 1).

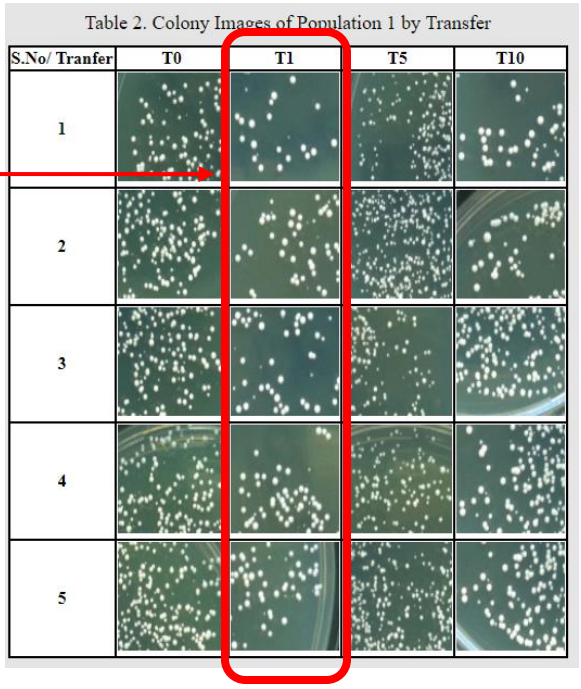


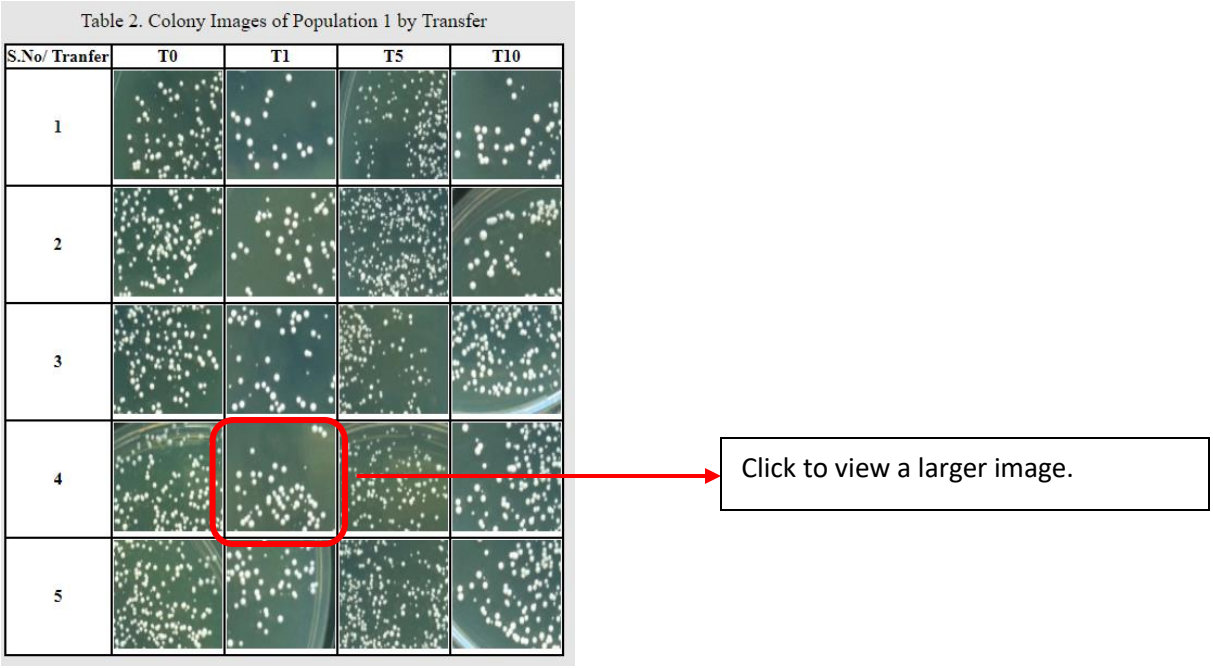
Table 2. Colony Images of Population 1 by Transfer

S.No/ Tranfer	T0	T1	T5	T10
1				
2				
3				
4				
5				

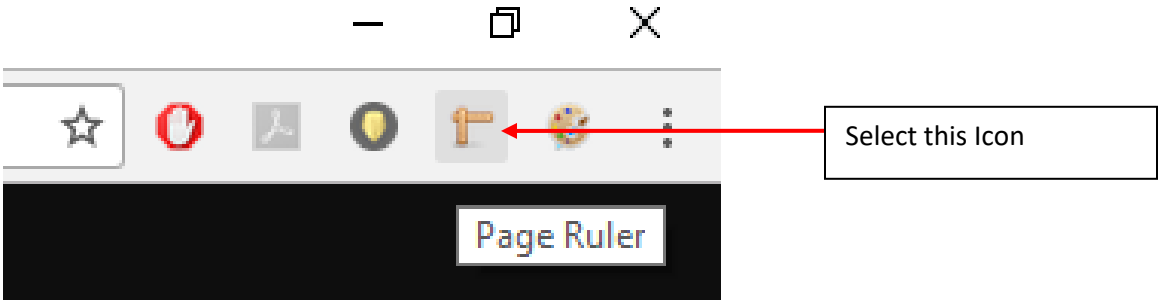
The images in the Table have been organized by Transfer Number- T0, T1, T5, T10.

Each Transfer has a set of 5 images of the Agar Plate

3. Next click on the Thumbnail image to view a larger version of the image.



4. Now select the Page Ruler Option on the top right-hand corner of your screen to open the ruler.



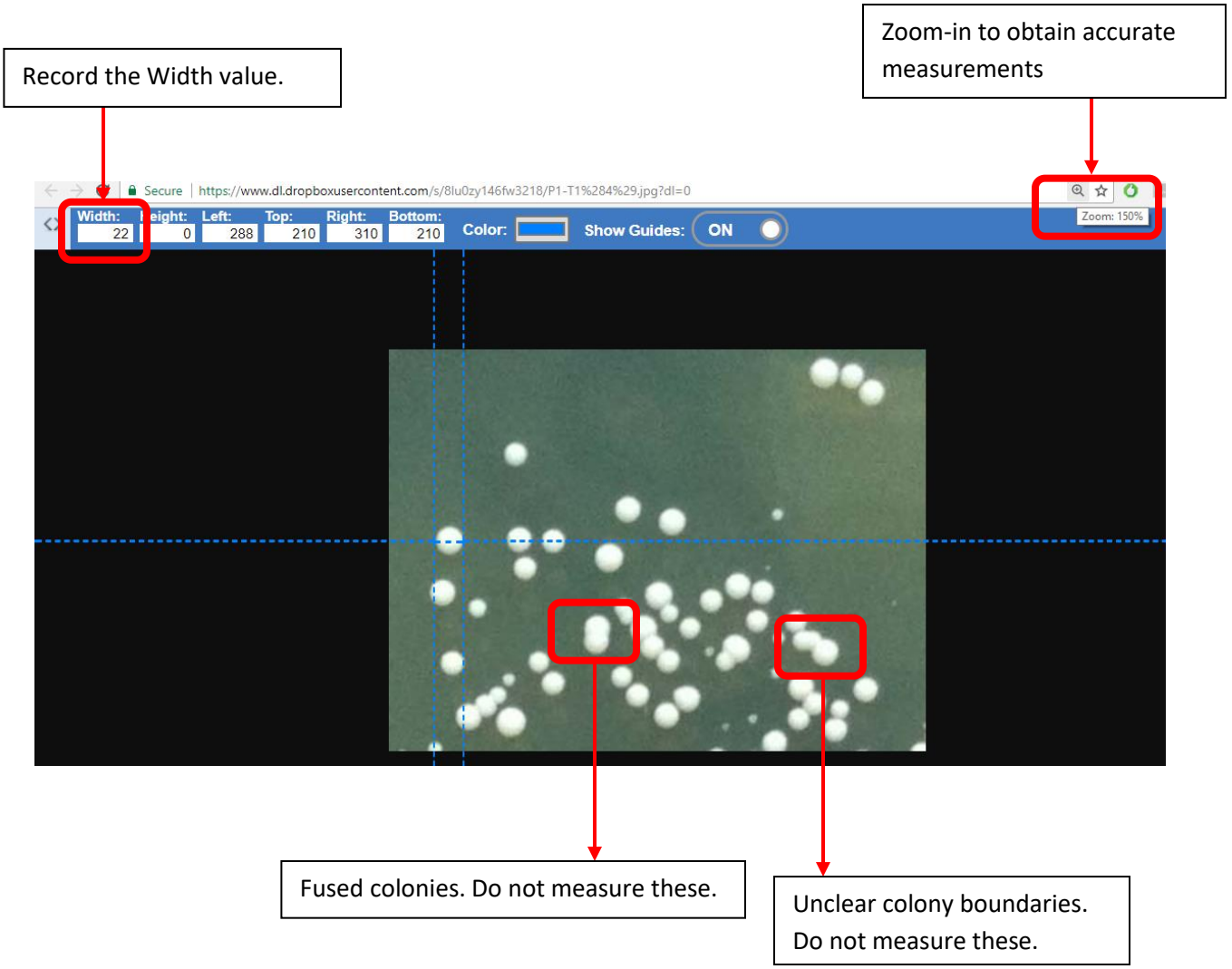
5. This will open the Page Ruler.

   To measure the colony size, click on one end of the colony and then drag the cursor to the other end of the colony. Read the measurement in the Width box. This is the diameter of the colony. Record this measurement. The units are pixels (px).

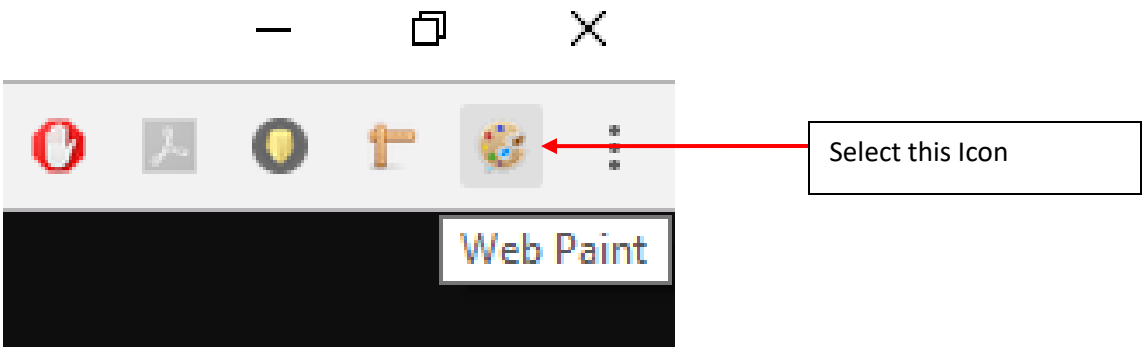
   Note: You can Zoom-in to view the image better. Zooming-in on an image does not change diameter of the colony when using the Page Ruler. Feel free to zoom in to take accurate measurements of the colonies.

   Note: You can only measure single, stand-alone colonies. Yeast can undergo budding whereby a cell grows and divides into two daughter cells which are seen as fused colonies in the image. Do not measure these colonies, along with colonies that do not have a defined outline since we

cannot accurately measure the diameter of these colonies. We only want to measure the diameter of single colonies.



6. Once you have measured the colony, click the Web Paint Option on the top right-hand corner of your screen.



7. Using the Web Paint cross out the colonies you have already measured.

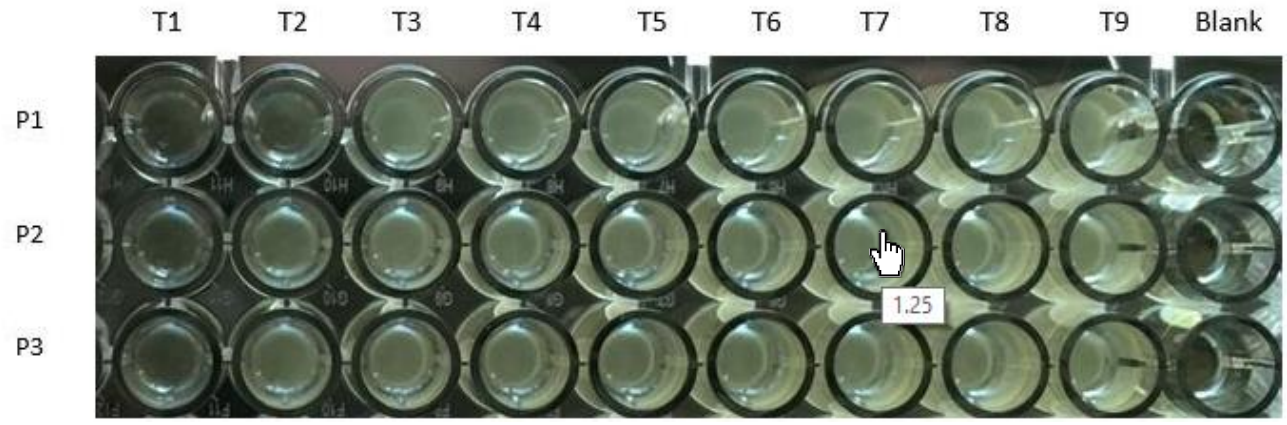
8. Now you are ready to begin measuring colony sizes!

   Note: Remember to measure all the valid colonies in an image.

B) Fitness Test

The Fitness Test image provides the optical density values of the different Populations- P1, P2, P3 over 9 transfers, T1 to T9.

To obtain optical density values, simply hover the cursor over a well. Record the value.



Refer to Figure 1. from webpage. Image shows fitness test results. 1µl of each population evolving in high salt into high salt medium in 96 well plates. Incubated 2 days at 30°C.

Acknowledgements

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References

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2. Dhar, R., Sägesser, R., Weikert, C., Yuan, J. and Wagner, A. (2011), Adaptation of *Saccharomyces cerevisiae* to saline stress through laboratory evolution. *Journal of Evolutionary Biology*, 24(5), 1135–1153.

3. Hartwell, L. H. (1974). Saccharomyces cerevisiae cell cycle. *Bacteriological Reviews*, *38*(2), 164–198.