

## **Microbiology: The Effect of Environmental Mechanical Forces on the Growth of Walled Cells**

**Research question:** To what extent does the environmental mechanical force (EMF) affect the growth of *Saccharomyces cerevisiae* (measured by yeast colony size) on stiffness conditioned potato dextrose agar (PDA) media plates containing different agar over a 3-day-period?

**Biology Extended Essay**

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## Chapter I: Introduction

It is well known that life activities are a complex network system of chemical reactions and physical variables, which are modulated by the interaction of the organism with its environments, such as temperature, moisture, mechanical forces and so on. It has been written in the IB Biology textbook that the responses of organisms to external temporary stimuli is one of the characteristics of life and that, generally, organisms exhibit transient stress behaviors in response to the stimuli. All multicellular organisms, such as human beings, animals or plants can sense and respond to the heat, cold, touch or pressure. In 2021, the Nobel Prize in Physiology and Medicine was awarded jointly to Drs. David Julius and Ardem Patapoutian, whose finding filled the gap in our understanding of the complex interaction between organism and their environment, for their groundbreaking discoveries about temperature and touch receptors (The Nobel Prize). In their investigation, unsurprisingly, temperature plays a crucial role in life activity by influencing the activation of enzymes (protein) that promote metabolism of organisms at the desired optimum temperature, but the mechanical forces and touch mentioned that affect the cells or organisms piqued my interest to explore more. It has also been shown from the literature that external forces on human bodies arise from mechanical actions such as blood flow, trauma, or the loading of bones, while abnormal mechanical forces also come from inflammation or the growth of solid tumors or cancers. During body's metabolism, variable external mechanical forces that cells constantly perceive are critical for sculpting cells or tissues into their functional structure (G. Paci and Y. Mao, Seminars in Cell and developmental Biology 120 (2021) 160-170).

However, there are few studies related to the effect of environmental mechanical forces (EMF) on unicellular organisms or walled cells (e. g. yeast) that were able to grow under natural conditions on surfaces with different hardness (rocks or biotic surface) while interacting with different mechanical forces. As common matrix used for grow yeast in the laboratory, potato dextrose agar (PDA) media allows creamy or white yeast colonies to be easily observed. Through speculation, different EMF might affect the growth activity of yeast. In order to test this hypothesis, yeasts (*Saccharomyces cerevisiae*) are cultivated on PDA plates made of different concentrations of agar which helps adjust the hardness and elasticity of the culture matrix, and the growth capacity of yeasts are measured by their colonies size using ImageJ Software. This investigation will help us better understand the effects of surrounding environmental mechanical forces (EMF) on the growth of walled cells.

## **1.1 Research Question**

*To what extent does the environmental mechanical force (EMF) affect the growth of *Saccharomyces cerevisiae* (measured by yeast colony size) on stiffness conditioned potato dextrose agar (PDA) media plates containing different agar over a 3-day-period?*

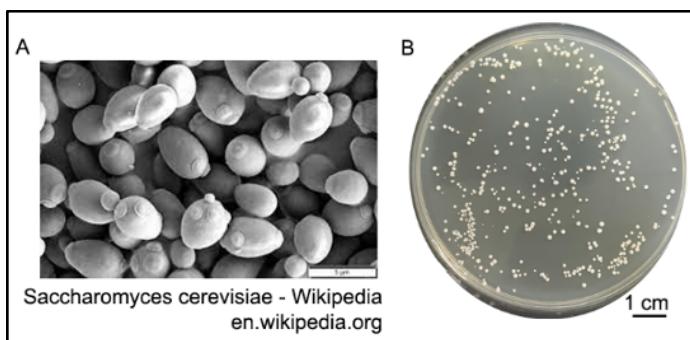
In order to answer this question, the effect of mechanical forces on *S. cerevisiae* will be determined through changing the culture matrix stiffness with different agar concentration and measuring the growth rate of yeast by its colony size on the conditioned matrix.

## **1.2 Background**

Beside chemical signals, cell behavior is also guided by the mechanical properties of the cells and their surrounding environments (Bayir, et al. 2019). It is evident that environmental

mechanical forces (EMF) can profoundly affect cell growth and also its physiological behaviors, as body cells can sense and transduce external mechanical inputs into biochemical and electrical signals, which influence processes such as cell proliferation, adhesion and migration (Bayir, et al. 2019). Unicellular fungi grow as clusters adhering to biotic surfaces (e. g. plant or animal hosts) or abiotic surfaces (rocks, medical devices, etc.), thus forming a functional community (Mishra. et al. 2022). A stiffness change in the natural habitat can pose various mechanical forces to these organisms, so some fungus have developed to adapt to the surrounding environment (Mishra. et al. 2022). For instance, studies have suggested that organisms as simple as *S. cerevisiae* (Fig. 1) have also evolved complex signaling networks to sense and respond to intrinsic and extrinsic mechanical forces even though they can oppose mechanical force by their rigid cell walls (Mishra. et al. 2022). Different matrix stiffness might also stimulate *S. cerevisiae* in a way that affects their function, proliferation as well as migration through altering mechanical forces.

**Fig 1.** *Saccharomyces cerevisiae*. **A.** Electron micrograph of *S. cerevisiae*, extracted from [en.wikipedia.org](https://en.wikipedia.org) **B.** The image of *S. cerevisiae* on the PDA plate.



Agar is commonly used for solidifying the microbiological culture media, as it can be easily melted at higher temperature and has no nutritive value for many kinds of microorganisms. One

of the interesting nonlinear mechanical behaviors when shear is applied is strain stiffening, observed only in a few polysaccharide gels such as in agar gels, and if the individual fibrils are stretched beyond the Hookean limit, they start to stiffen. (Macromolecules). Thus, agar concentrations are correlated with different hardness or mechanical forces of the agar matrix. Notably, the concentration of glucose in the agar has a negligible effect on radial growth rate (Kamath & Bungay, 1988). Hence, agar concentration is manipulated to alter mechanical forces (IV) of the agar matrix.

Similar to multicellular organisms, unicellular eukaryotes such as *S. cerevisiae* are also exposed to a myriad of physical forces, including compressive, tensile, and shear forces in their natural habitat (Mishra et al. 2022). *S. cerevisiae* is a small single cell with a doubling time at 27–30°C of 1.25–2 h and importantly can be cultured easily, which permits their rapid production and maintenance of multiple strains at low cost (Stewart, n. d). Colonies on nutrient agar show linear increases in diameter and height with time throughout most of the growth cycle (Kamath & Bungay, 1988). Additionally, Jones & Gray (19781, b, 1979) has observed that mature colonies show an exponential increase in the diameter with time in a constant nutrient concentration at the colony-gel interface. Thus, yeasts' growth can be quantified by measuring the size of yeast colonies (area of rounded shape) and *S. cerevisiae* can be used as a good model organism to study the effects of EMF on walled cells' growth by measuring the size of yeast colonies (area of rounded shape).

### **1.3 Hypothesis**

As yeast cells come across higher external forces from the harder PDA plate resulting from higher concentration of agar, the yeast might destabilize and reorganize away from the obstacle

(Mishra et al. 2022), and a hypoosmotic shock promoted by stronger mechanical forces is likely to result in a transient inhibition of cell-wall growth (Rojas & Huang, 2018). The hypothesis is that the yeast growth will be inhibited, shown as smaller size of colonies, when they are cultivated on harder PDA media made with higher concentration of agar.

#### **1.4 Variables**

##### **1. Independent variable (IV)**

- Mechanical forces (stiffness) of PDA plates changed by agar concentrations (1.5 %, 2 %, 2.5%, 3%, 3.5%)

##### **2. Dependent variable (DV)**

- Growth size (area) of colonies (Measured by ImageJ Software, NIH)
- Growth rate of colonies (%)

##### **3. Controlled variable (CV)**

Controlled variables	How to control	Effect on the result
<b>Condition of incubation (Temperature &amp; Time)</b>	All the yeast grown on PDA media are set in the same incubator at 27°C for 3 days.  Referred to International Baccalaureate guidelines, the incubation temperature of the Petri dishes was kept at 27°C.	<i>S. cerevisiae</i> is a small single cell with a doubling time of 27-30°C of 1.25–2 h (Stewart). Optimal temperature and growing time ensure yeast colonies survive so the data can be collected.
<b>PDA media (Before adding agar)</b>	It can be controlled through following the same recipe to make the potato dextrose agar media for each trial, under the same	Same PDA media without adding agar will guarantee yeast colonies not affected by different nutrient levels provided. Colonies

	<p>conditions. In each trial, 50g diced potatoes are measured by the same weight scale and 500 cm<sup>3</sup> H<sub>2</sub>O measured by a glass measuring cylinder.</p>	<p>on nutrient agar show linear increases in diameter and height with time throughout most of the growth cycle (Kamath &amp; Bungay, 1988).</p>
<b>Sterilization techniques</b>	<p>The PDA media and the apparatus (spreader, beaker and plate) are autoclaved with a pressure cooker. The operating branch and some apparatuses are sterilized regularly by hypochlorite or ethanol. Once the contamination occurs, all contaminants need to be destroyed at high temperatures.</p>	<p>Bacterial contamination occurring will jeopardize the experiments as other contaminated bacteria or fungus in the PAD plates will affect the yeast growth or kill the yeast.</p>
<b>Quantity of yeast (<i>S. cerevisiae</i>; brand name: RedMan)</b>	<p>0.5 g <i>S. cerevisiae</i> are activated with 100 cm<sup>3</sup> hot water (80°C), and wait 45 mins. Then the solution is diluted in a ratio of 1:1000 by first adding 1 cm<sup>3</sup> solution to 99 cm<sup>3</sup> water, and then adding extra 99 cm<sup>3</sup> water. Finally, 100 µmol yeast was applied to the PDA plate by a micropipette.</p>	<p>The same amount of <i>S. cerevisiae</i> applied to the plate can guarantee that the change in yeast colony area or size is due to the changing agar concentration instead of yeast quantity difference.</p>

## Chapter II: Investigation

### 2.1 Material and Apparatus

Measurement equipment	General apparatus	Materials
1 × Texture profile analysis 1 × Micropipette $\pm 0.1 \mu\text{mol}$ 1 × Weight scale $\pm 0.1\text{g}$ 5 × Beaker ( $500 \text{ cm}^3$ ) 2 × Glass measuring cylinder ( $250 \text{ cm}^3$ ) $\pm 2\text{cm}^3$	1 × Spreader 50 × Pipette tip 1 × Pressure cooker 50 × Plate dish (15 × 100 mm) 1 × Masking tape 1 × Paraffin 1 × Knife 1 × Marker pen 1 × hard plastic board 1 × Phone with camera 1 × Alcohol lamp	200 g Agar 1000 g Saccharomyces cerevisiae 5000 cm <sup>3</sup> Water 100 g Dextrose 250 g Potato

### 2.2 Methodology

#### 1. Make solid Potato Dextrose Agar (PDA) media for growing *S. cerevisiae*.

- a. Prepare 50g potatoes measured by a weight scale, and cut them into dices. (Fig.2-A)
- b. Add the diced potato to a beaker ( $250 \text{ cm}^3$ ).
- c. Add  $500 \text{ cm}^3$  water measured by a glass measuring cylinder ( $250 \text{ cm}^3$ ) into the beaker.
- d. Boil the diced potatoes on a heater for 30 mins until softened. (Stirring it during the process)
- e. Filter to obtain its extract to a beaker ( $250 \text{ cm}^3$ ).
- f. Add water to the total volume of  $500 \text{ cm}^3$  and mix well.
- g. Sterilization.

1. Take some tin foil and wrap your plates
  2. Place them into a pressure cooker and set the maximum temperature. (Fig.2-B)
  3. Add 7.5 g agars (for 1.5% concentration) and 5g dextrose while shaking the beaker.
  4. Repeat step (g-2) for 15 mins.
  5. Remove the beaker at the temperature of 60°C, and leave it for 5mins.
  6. Frame the lips for final sterilization.
  7. Dispense 20 - 25 cm<sup>3</sup> portions into sterile 15 × 100 mm per dishes (25-30 µl) and hit the plate with a frame to remove bubbles. (Avoid melting the media).
  8. Leave the plate for 20 mins till it cools.
  9. Flipped the plates.
- i. Cover the plate and label it as a form of “concentration of agar + trial”. (Fig.2-C)

## **2. Repeat step 1-2 for 8 trials**

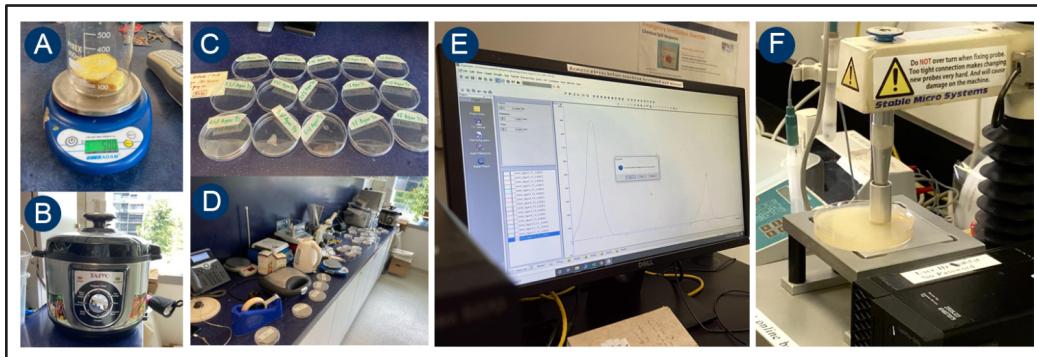
## **3. Repeat step 1-2 for 8 trials but change agar concentration in h(3) to 2.0%, 2.5%, 3.0%, 3.5% by changing agar mass to 10g, 12.5g, 15g, 17.5g respectively.**

**Table 1.** Potato dextrose agar (PDA) media recipe

Agar concentration (%)	Agar mass (g) +/- 0.1g	Total volume (cm <sup>3</sup> ) +/- 2cm <sup>3</sup>	Dextrose mass (g) +/- 0.1g
1.5	7.5	500	5
2	10	500	5
2.5	12.5	500	5
3	15	500	5
3.5	17.5	500	5

**Fig. 2 A-D.** The process of making potato dextrose agar(PDA) media for yeast cultivation. **E-F.**

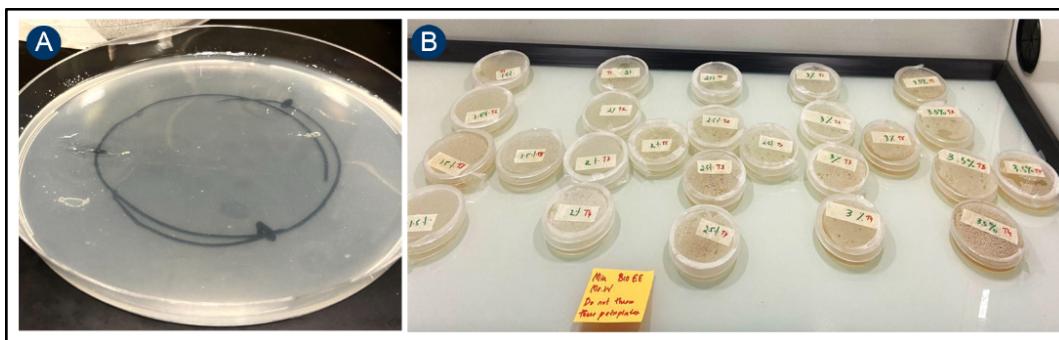
Using Texture Analysis Profile to collect data of mechanical forces, hardness and resilience.



4. Put these plates in the refrigerator for 3 hours to cool them down.
5. Measure the atomic forces in PDA plates by Texture profile analysis. (Fig. 2-F)
6. Sketch the graph of agar concentration (X-axis) and culture stiffness measured by Texture Analysis Profile (Y-axis). (They were measured in University Laboratory)
  - a. Draw a circle briefly on the bottom of the plate. (Fig. 3-A)
  - b. The Texture Analysis Profile is conducted using a TA-XT2i Texture Analysis Profile (stable Micro System, Surrey, UK) according to the AIB Standard Procedure (2011) with modification.
  - c. The agar plate sample is subjected to a double compression by an SP20 probe (20mm diameter cylindrical probe) at a speed of 2 mm/s to a distance of 2mm.
  - d. Conduct 3 replicates for each concentration of agar in the plate.
  - e. Collect the data. (Fig. 2-E)
6. Plating the *S. cerevisiae* on conditioned PDA media for another 5 trials. (Fig. 3-B)
  - a. Activate 0.5g yeast with 100 cm<sup>3</sup> hot water (80°C) for 45 mins.
  - b. Dilute the yeast solution in a ratio of 1:100 by adding 1 cm<sup>3</sup> solution to 99 cm<sup>3</sup> water.
  - c. Repeat step(6-b).

- d. Use a micropipette to apply 100  $\mu\text{mL}$  *S. cerevisiae* (1:1000) to the plate.
- e. Eject the pipette tips.
- f. Sterile the spreader with an alcohol lamp.
- g. Gently spread the yeast culture around the plate with the spreader.
  - Tilt the plate and take your spreader at 90° to the plate.
  - Start at the bottom and very gently swipe it back and forth from the top to the bottom of the plate.

**Fig.3 A.** Draw a circle on the bottom of the plates and select three random spots on it for data collection in PDA media with different agar concentrations. **B.** Culturing yeast colonies on PDA media.



## 7. After 3 days, take photos and measure yeast colony size by ImageJ Software and get the result data.

- a. Open the ImageJ Software and open a file of the image.
- a. Make a copy of the image using Image > Duplicate.
- b. Leave one copy as-is for now, and use your other copy to create a binary image.
- c. Use Process > Binary > Make Binary to create a binary image
- d. When particles are merged together, the Process > Binary > Watershed accurately cuts them apart by adding a 1 Pixel thick line where it feels the division should be.

- e. Click on the Binary image to select it, then go to Analyze > Analyze Particles. (Data are in Appendix B, C, D, E, F, G)

### **Risk assessment:**

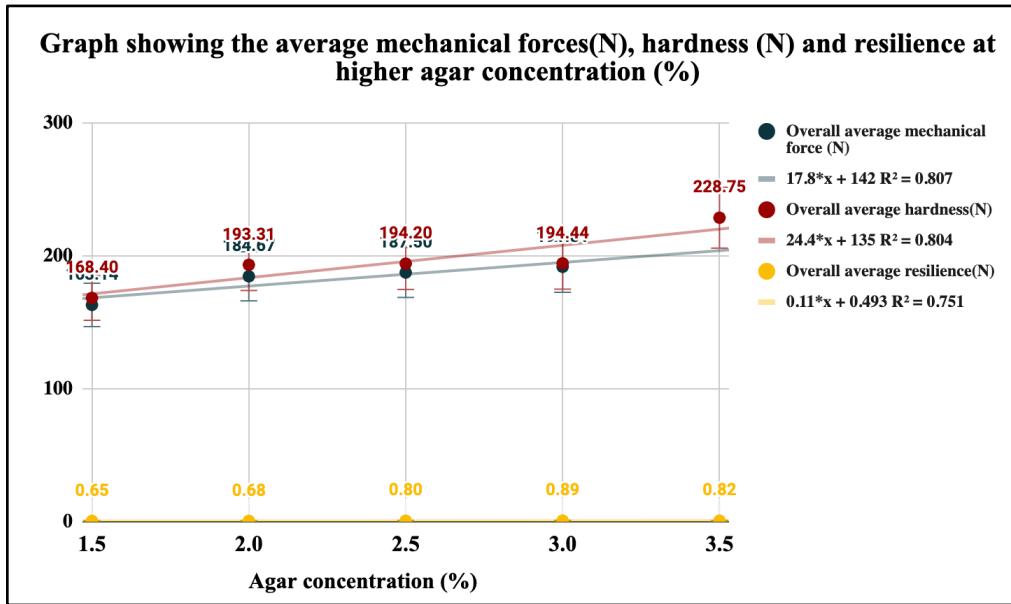
1. Prevent cutting fingers when using a knife.
2. When working in such close proximity to a burner to maintain a zone of sterility there is a risk of burns. Allow the heated apparatus (heater, pressure cooker, alcohol lamp. etc) to cool before handing it, and keep a safe distance between the heated apparatus before it cools.
3. Cleaning up the table after the experiment prevents others ingesting yeasts accidentally, which might hurt digestion.
4. Once bacterial contamination occurs during yeast cultivation, contaminants have to be destroyed to prevent cutaneous mycoses if contacting it . When destroying the contaminated objects, remember to wash hands carefully and wipe the apparatus with hypochlorite and ethanol.

### **2.3 Result Data (IV):**

***Processed Table. 1. Showing average mechanical forces(N), hardness(N), resilience(N) in PDA media with agar concentration of 1.5 % 2.0%, 2.5%, 3.0%, 3.5% (N : Newton).***

Agar concentration(%)	Overall average mechanical force (N)	Overall average hardness(N)	Overall average resilience(N)
1.5	163.14	168.40	0.65
2	184.67	193.31	0.68
2.5	187.50	194.20	0.8
3	191.81	194.44	0.89
3.5	193.73	228.75	0.82

**Graph 1.**



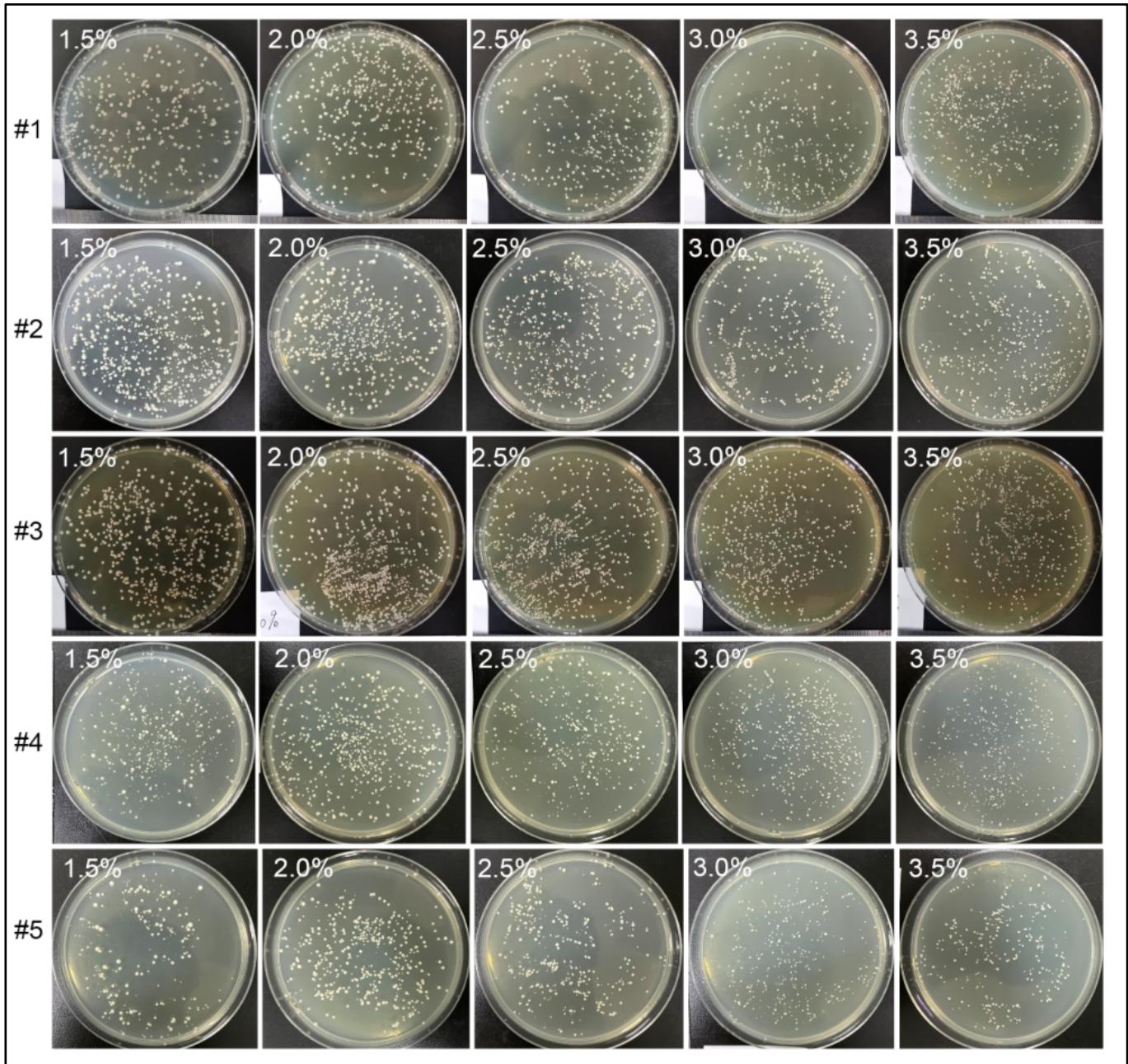
**The hardness of PDA media positively correlates with the concentration of agar**

Conditioned PDA media plates were prepared with potato infusion, dextrose and 1.5%, 2.0%, 2.5%, 3.0% or 3.5% agar, named 1.5%, 2.0%, 2.5%, 3.0% or 3.0% PDA media respectively. After cooling and solidification, the hardness of conditioned PDA media plates were measured using Texture analyzers (Ta.XT2i, Stable Micro Systems) by collecting three different spots on the PDA media and calculating the average.

## **2.4 Result Data(DV):**

### **Qualitative**

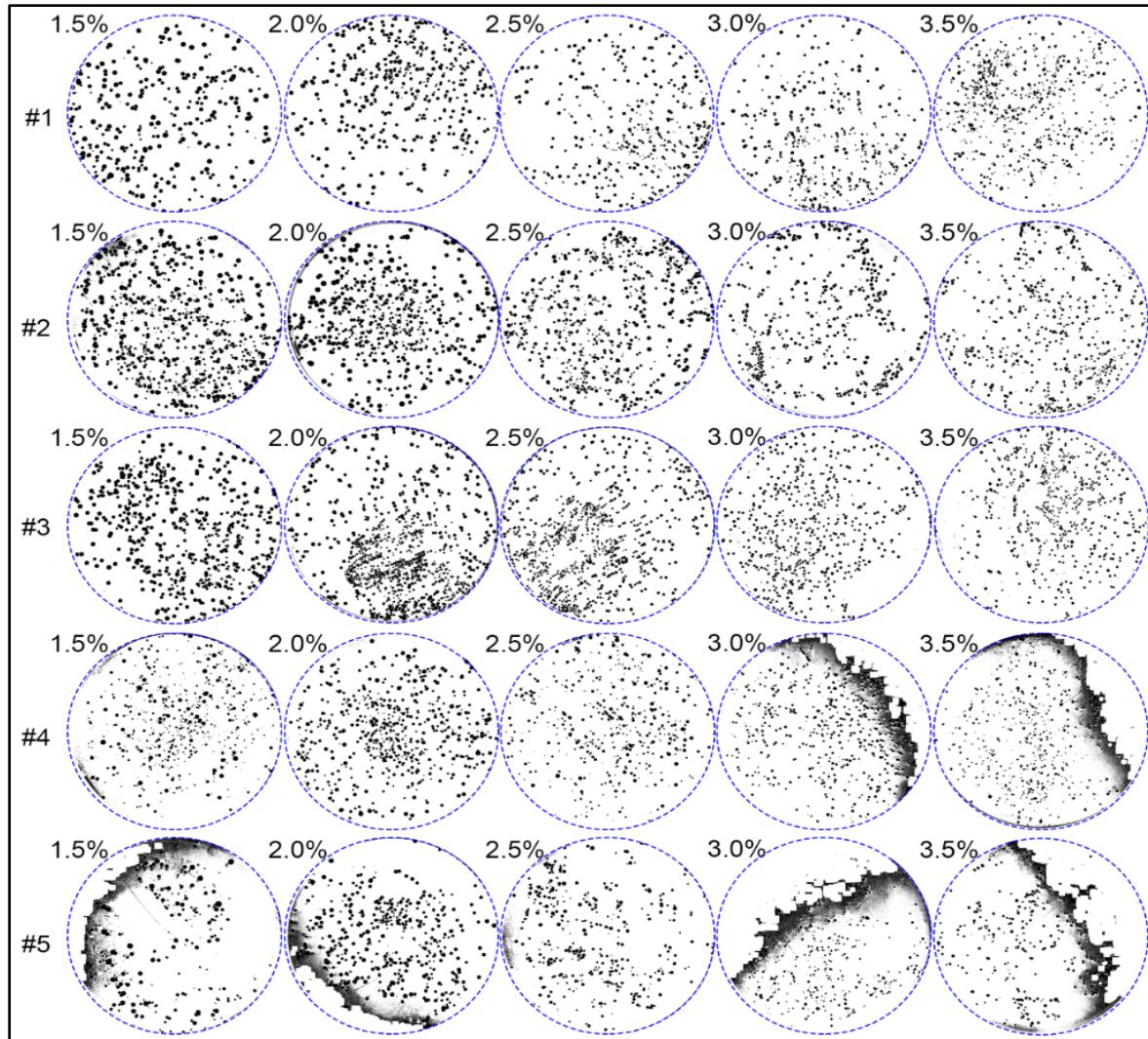
**Fig 4.** The represent image of *S. cerevisiae* colonies cultured on PDA media with agar concentrations of 1.5%, 2.0%, 2.5%, 3.0% and 3.5%.



### The growth rate of *S. cerevisiae* decreased in harder PDA media (e. g. Trial 1)

To determine the effect of environmental mechanical forces changed by surface stiffness on the growth of *S. cerevisiae*, the yeasts were cultured in solid 1.5%, 2.0%, 2.5% or 3.0% PDA media for another three days at the temperature of 27 °C to form colonies and then each colony size was measured. To better elaborate, Figure 4 depicts that the *S. cerevisiae* colonies exhibit a **larger round** pattern distribution on 1.5 % and 2.0% PDA media, **smaller round** pattern distribution on 2.5%, 3.0% and 3.5% PDA media, and **irregular shape** pattern distribution in harder 3.0% and 3.5% PDA media.

**Fig. 5.** Binary versions of the scanned images of *S. cerevisiae* colonies on PDA media with agar concentrations of 1.5%, 2.0%, 2.5%, 3.0% and 3.5% for 5 replicated trials.



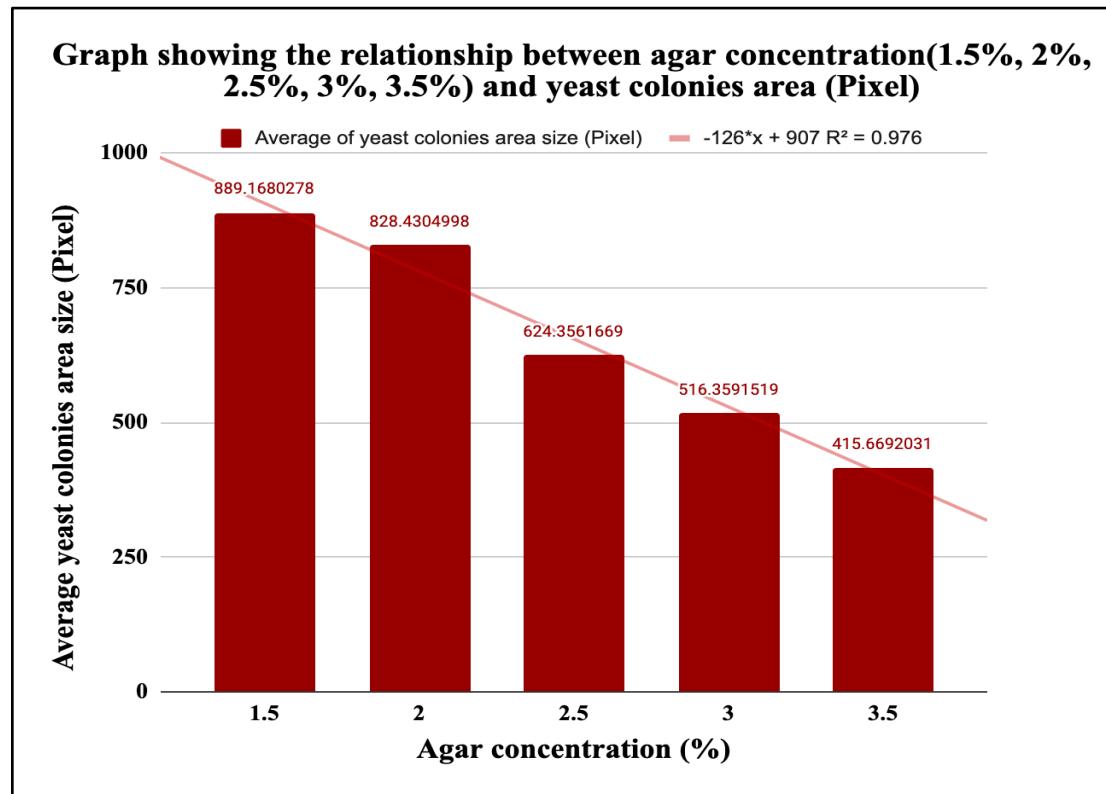
In Figure 5, Processed Table 2 and Graph 2, after processing with ImageJ Software, the average areas of yeasts' colonies grown on stiffer plates (with higher agar concentrations) was significantly smaller than those grown on softer plates (with lower agar concentrations).

## Quantitative

*Processed Table 2 showing the relationship between agar concentration (1.5%, 2%, 2.5%, 3%, 3.5%) and yeast colonies area (Pixel).*

Agar concentration (%)	Average of total area of yeast colonies (Pixel)						Standard Deviation (SD)
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Average	
1.5	889.26	1,253.42	1,331.68	549.63	421.85	889.17	406.87
2	672.28	1,151.39	790.06	965.50	562.92	828.43	234.22
2.5	438.51	1,063.12	565.13	674.51	380.51	624.36	270.43
3	365.09	803.80	667.80	374.27	370.85	516.36	206.03
3.5	239.63	734.03	420.23	295.94	388.52	415.67	191.99

*Graph 2.*



**Processed Table 3** showing average showing the relationship between agar concentration (1.5%, 2%, 2.5%, 3%, 3.5%) and yeast growth rate (%).

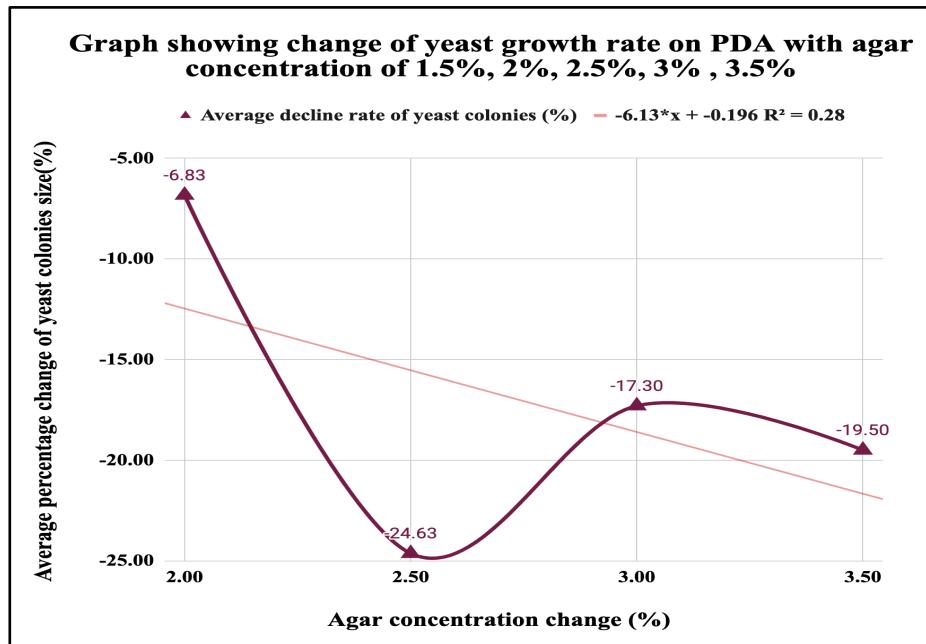
Initial agar concentration (%)	Final agar concentration (%)	Change of growth rate in yeast colonies (%)
1.50	2.00	-6.83
2.00	2.50	-24.63
2.50	3.00	-17.30
3.00	3.50	-19.50

In order to put these changes in yeast growth into perspective, the mean percentage change of yeast growth size was calculated by the following formula, and the data were indicated by the processed Table 3 and Graph 3.

*Change of Yeast growth rate =*

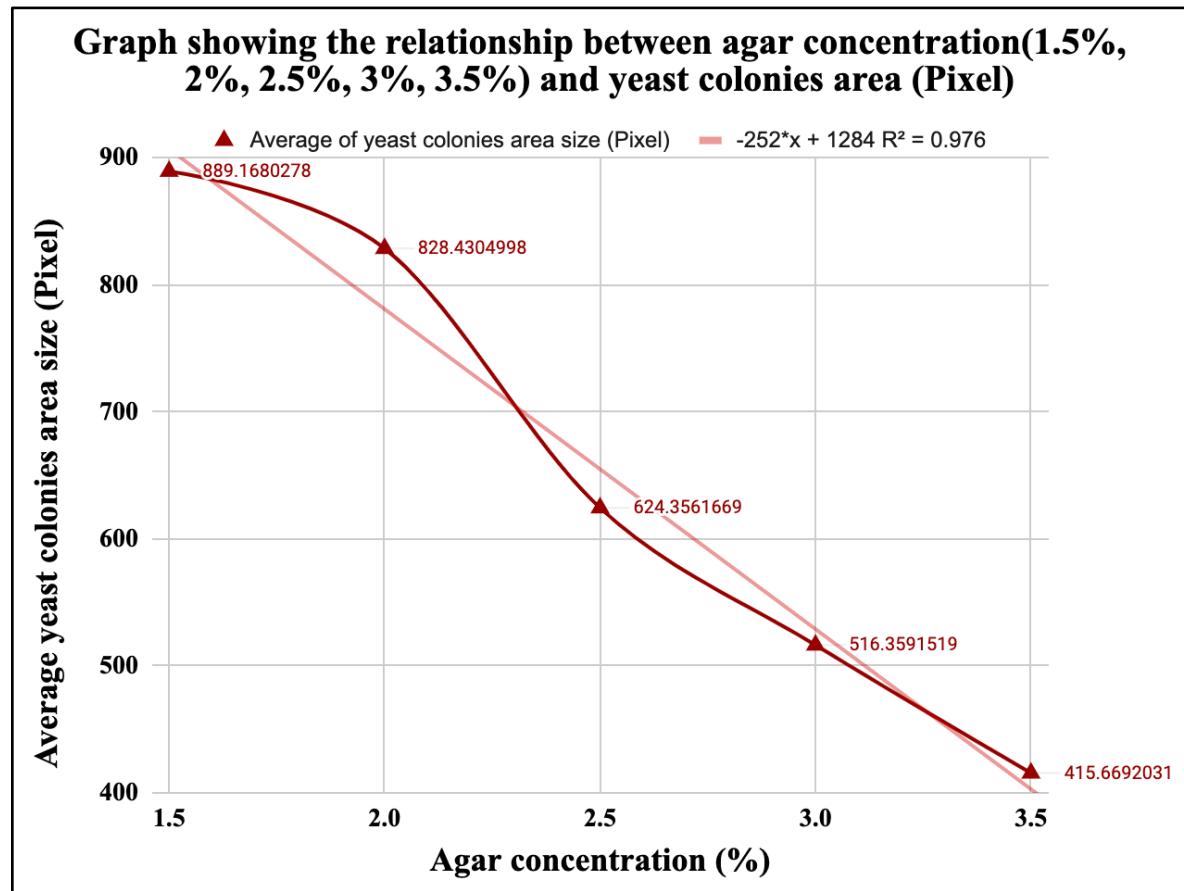
$$\frac{\text{The Change of Yeast colony area after each increase of } 0.5\% \text{ agar concentration in PDA media}}{\text{Yeast growth area in previous PDA media}} \times 100\%$$

**Graph 3.**



Graph 3 demonstrates that there is a negatively downward trend between agar concentration and yeast area growth rate. However, the yeast growth rate change was the most (-24.63%) when agar concentration is within the range of 2% to 2.5%. Later, the percentage change of yeast colony area or size slightly increased to -17.30% when agar concentration increases from 2.5% to 3%.

**Graph 4.**



In Graph 4, as agar concentration increases from 1.5% to 3.5%, the yeast colony size decrease from 889.168 pixel to 414.668 pixel, which illustrates a negative correlation between the average yeast colony size and agar concentration.

## Chapter III: Discussion

### **4.1 Conclusion**

In conclusion, stronger environmental mechanical forces inhibit *S. cerevisiae*'s growth on harder matrix of the PDA media made with higher concentration of the agar. To elaborate, both Graph 1 and processed Table 1 showed that the mechanical properties of PDA media including resilience (N), hardness (N) and mechanical forces (N) positively correlated with higher agar concentration (%). From 1.5% to 3.5% agar concentration, the mechanical forces increased from 163.14N to 193.73N, indicating that higher agar concentration (%) resulted in harder or stronger mechanical force of PDA media. Specifically, both Figure 4 and 5 showed that a harder matrix medium or stronger mechanical force inhibits the yeast growth shown as smaller colonies supporting the hypothesis.

The qualitative data producing in-depth analysis of specific patterns from the observation identifies rich data that can lead to further research. As Graph 2(quantitative data) illustrates that the *S. cerevisiae* colony's size (pixel) was smaller when higher agar concentration was present, the relative area size (pixel) of the yeast colonies quantified by ImageJ Software provided the numerical measurement, and concluded that weaker mechanical forces actually foster yeasts' growth. It's evident that larger round distribution patterns of *S. cerevisiae* colonies grow on the softer 1.5% and 2% PDA media, and the smaller round colonies distribute on the harder 2.5%, 3% or 3.5% PDA media (where yeasts grow the worst). Graph 4 indicates that yeasts' development and growth capacity was negatively correlated with the environment's stiffness and surrounding mechanical force, in a way supporting the hypothesis that EMF suppresses the growth of walled

cells such as yeast, as the average colony area present a downward trend on the third day when increasing agar concentration from 1.5% to 3.5%.

Additionally, the coordinate (2.50, -24.63) shown in Graph 3 implies that yeasts grew the worst when in range of 2% to 2.5% agar concentration due to the lowest average area change rate, then it continues to decrease on PDA media with agar concentration as well as mechanical forces increasing. In Graph 2 and 3, stronger environmental mechanical forces produced by higher agar concentration will eventually curb the growth of walled cells such as *S. cerevisiae*, and its size decrease most in growth status occurs when agar concentration presents in the range of 2% to 2.5%. The hypothesis is accepted due to the negative correlation between yeasts colony size and agar concentration, which can also be linked to the research. Accordingly, Rojas and Huang (2018) reported that a hypoosmotic shock promoted by increasing mechanical forces will increase the turgor pressure and membrane tension, leading to transient inhibition of cell-wall growth via electrical depolarization. Moreover, it also clear to explain why 1.5 % to 2 % agar concentration is always and typically used to cultivate yeasts in the solid culture media, which is always helps to determine the colony characteristics of the isolates (such as colony morphology, hemolysis, pigment production, etc.) (Tankeshwar). Beside the laboratory, the yeasts are exposed to various EMF in their natural habitat, so Mishra (2022) mentioned that “yeast have evolved complex signaling networks to sense and respond to intrinsic and extrinsic mechanical forces”, which could be counted as an adaptive evolution. Consequently, this investigation did help us better understand the effects of sustained environmental mechanical forces on the growth of walled cells such as unicellular fungi which are exposed to a myriad of mechanical cues in their natural habitats.

The standard deviation of the raw data is calculated as it measures how far the data is from the mean, and it gives an insight of whether the data in one condition is spread out or not. As Graph 1 demonstrates, the high standard deviation (191.99 to 406.87) of the average mechanical forces reflects the large amount of variation in the data group, indicating that mechanical forces in the same agar concentration is significantly different, which is likely to be accounted for the unavoidable evaporation of matrix and its unequal thickness. Nonetheless, the hypothesis is still accepted as data of each trial is within the same range, illustrating an accurate and general trend. The investigation briefly and fundamentally builds the understanding and relationship between the interactions between walled cells and their environment.

#### **4.2 Evaluation of Procedure**

The experimental procedure is concise and easy to follow due to the simple methodology and uncomplicated materials required. It is also easy to operate due to the available access to required apparatus in university labs and the notion that it can be executed with materials, as the Texture Analysis Profile has a high force sensor accuracy, which is defined as the smallest amount of force that can be applied to the sensor body required to cause a linear and repeatable variation in the voltage output (FUTEK). Besides, ImageJ Software elaborates the data of colony sizes clearly and more accurately, and supports the relationship through the quantitative data which helps observe the growing status of yeast colonies easier. The errors are minimized by repeating trials 10 times and calculating the standard deviation of raw data, to ensure the validity and reliability of the experiment. This investigation showed that mechanical forces changed by agar concentration, which simulates the environmental mechanical forces generated by different surfaces in yeasts' habitat, is likely to curb the growth of microbes such as yeast. The hypothesis

is accepted that when yeast is cultured on stiffer PDA media made with a higher range of agar concentration.

Nevertheless, strict requirements for handling the process of yeast cultures can be an issue whereas it is not a case for this investigation. As the major challenge, contamination of yeast culture is usually caused by an airborne route or by the contaminated flask or plate surface. In order to avoid it, the apparatus had to be carefully autoclaved by being sprayed or wiped with hypochlorite and ethanol, and contaminated plates and objects had to be disposed of immediately. Secondly, data collected from the experiment relies on a large proportion of human input which in turn creates a larger capacity of human error, and the inconsistency of data resulted in high standard deviation (Table 1 & 2). It can be addressed by increasing the trial of each concentration from 10 times to 100 times and then calculating its average. To maximize the forces sensor accuracy, the number of sites selected in the line of different circular diameters drawn on the plate bottoms can increase from 3 to 10 to increase data validity and precision, and then they can be measured by the Texture Analysis Profile (Figure 3-A). Thirdly, the high background presented in 1.5%, 2%, 3%, 3.5% in replicated 5 (#5) and 3%, 3.5% in replicated 4 (#4) (Figure 5) caused by the uneven matrix thickness or photography will result in inaccurate identification of yeast colonies, which is measured by the ImageJ Software. It causes less accurate area data, so it is acceptable to cook the potato dextrose agar media for a longer time and set a uniform thickness of each plate to prevent uneven matrix, or photography in a location with abundant light. In addition, the systematic errors caused by the physical operation might happen in the calculated uncertainty such as hand-eye coordination (measuring cylinder, beakers, weight scale) that would result in inconsistent data recording, so it can be improved by

expanding the number of trials and/or changing the initial yeast number in the culture to test how environmental mechanical forces affect the yeasts' growth.

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## Appendices

**Appendix A:** Raw Table 1 shows average mechanical forces(N), hardness(N), resilience(N) with PDA media agar concentration of 1.5 % 2.0%, 2.5%, 3.0%, 3.5%. (gravity : g=9.806 65 m s<sup>-2</sup>)

Batch	Deepness (mm)	Spot	Mechanical force (g)	Hardness (g)	Resilience
1.5% Agar T1	2	1	1452.861	1477.538	0.709
	2	2	1576.81	1670.585	0.667
	2	3	1619.141	1705.799	0.664
1.5% Agar T2	2	1	1671.79	1763.452	0.633
	2	2	1640.272	1735.194	0.643
	2	3	1538.807	1620.139	0.672
1.5% Agar T3	2	1	1664.869	1770.247	0.639
	2	2	1673.19	1771.946	0.641
	2	3	1845.003	1969.136	0.606
2% Agar T1	2	1	1783.653	1915.075	
	2	2	1873.479	2067.915	
	2	3	1856.653	2013.648	
2% Agar T2	2	1	1834.638	1873.49	0.743
	2	2	1770.534	1860.004	0.734
	2	3	1932.143	2070.739	0.69
2% Agar T3	2	1	1841.468	1943.58	0.634
	2	2	1848.722	2103.141	0.646
	2	3	1878.805	2033.4	0.633
2.5% Agar T1	2	1	1739.97	2123.78	0.426
	2	2	1809.3	2033.48	0.33
2.5% Agar T2	2	1	1973.5	2049.9	0.284
	2	2	1845.5	1993.4	0.226
2.5% Agar T3	2	1	1982.69	1983.4	0.751
	2	2	1892.89	2209.97	0.696
	2	3	1946.97	1895.8	0.639
3% Agar T1	2	1	1745.891	1938.4	
	2	2	1573.941	1622.205	0.894
	2	3	2134.5	1839.48	0.976
3% Agar T2	2	1	2190.327	2267.952	0.842
	2	2	2242.287	2319.12	
	2	3	2180.352	2251.883	0.852
3 % Agar T3	2	1	1591.881	1623.743	0.881
	2	2	1993.49	1983.39	0.906
	2	3	1610.383	1653.322	0.889
3.5% Agar T1	2	1	1393.52	1939.49	0.775
	2	2	1667.486	1711.813	
	2	3	1556.276	1711.813	0.897
3.5% agar T2	2	1	2179.342	2261.811	0.844
	2	2	2334.5	1938.134	0.759
	2	3	2492.827	2632.501	0.743
	2	4	2898.226	3109.04	0.689
3.5% Agar T3	2	1	1592.833	1930.39	0.848
	2	2	1893.59	2993.48	0.899
	2	3	1903.62	2844.39	0.815

**Appendix B:** Raw Table 2 shows the yeast colony area on PDA with agar concentration of 1.5%, 2%, 2.5%, 3%, 3.5% (Trial 1).

Saccharomyces cerevisiae's colonies area on PDA with agar concentration of 1.5%, 2%, 2.5%, 3%, 3.5% (Trial 1)					
Colony label	Colony area on PDA with 1.5% agar concentration (Pixel)	Colony area on PDA with 2% agar concentration (Pixel)	Colony area on PDA with 2.5% agar concentration (Pixel)	Colony area on PDA with 3% agar concentration (Pixel)	Colony area on PDA with 3.5% agar concentration (Pixel)
1	2836	1556	1289	1182	961
2	2472	1521	1113	1166	930
3	2369	1472	1109	1156	927
4	1979	1458	1101	1080	924
5	1972	1406	1082	1074	918
6	1885	1395	1052	1056	906
7	1880	1367	1040	1051	901
8	1865	1355	1028	1017	880
9	1829	1305	1011	995	880
10	1817	1283	1010	991	872
11	1812	1272	1010	951	868
12	1805	1248	1002	944	822
13	1803	1238	961	940	812
14	1767	1230	952	940	810
15	1730	1213	942	902	809
16	1727	1210	940	901	798
17	1721	1188	929	901	795
18	1712	1185	922	894	772
19	1710	1166	918	892	751
20	1707	1161	911	878	736
21	1691	1153	910	871	734
22	1668	1151	904	869	731
23	1656	1150	901	837	728
24	1653	1145	893	827	723
25	1642	1139	892	823	720
26	1641	1134	886	820	713
27	1610	1115	884	814	704
28	1597	1111	882	813	702
29	1579	1109	879	811	701
30	1571	1109	877	809	696
31	1568	1101	875	804	695
32	1557	1096	873	801	692
33	1554	1093	872	799	691
34	1552	1090	872	794	690
35	1551	1087	868	790	686
36	1550	1078	867	784	681
37	1535	1077	867	781	677
38	1534	1073	855	776	676
39	1527	1073	850	776	676
40	1524	1072	849	772	674
41	1517	1068	844	770	673
42	1490	1067	844	769	669
43	1476	1066	842	760	664
44	1462	1066	840	760	664
45	1456	1063	832	758	652
46	1448	1059	831	757	652
47	1447	1055	830	748	650
48	1444	1051	827	748	644
49	1442	1051	824	743	644
50	1436	1047	824	732	641
51	1428	1045	821	723	641
52	1427	1042	816	719	639
53	1415	1039	816	716	635
54	1384	1038	814	715	633
55	1380	1035	813	712	632
56	1373	1034	809	712	630

57	1371	1033	806	711	627
58	1367	1022	805	710	620
59	1356	1022	805	710	610
60	1342	1019	803	710	601
61	1342	1012	802	709	591
62	1324	1010	785	709	591
63	1322	1008	783	706	587
64	1306	1006	778	702	587
65	1302	1004	777	692	583
66	1297	1002	768	690	582
67	1286	1001	767	680	582
68	1271	996	746	677	580
69	1263	985	758	666	577
70	1262	983	758	663	575
71	1255	981	756	649	571
72	1245	977	753	640	563
73	1242	975	752	644	559
74	1234	974	745	644	559
75	1231	963	739	637	559
76	1231	961	736	634	559
77	1223	960	735	634	551
78	1225	958	729	634	549
79	1224	957	717	627	544
80	1223	954	715	620	541
81	1189	953	709	621	532
82	1188	952	705	621	531
83	1181	951	704	621	531
84	1180	946	703	619	537
85	1161	944	701	615	516
86	1159	941	699	615	510
87	1156	936	698	593	504
88	1152	931	691	597	501
89	1152	925	686	582	500
90	1149	923	682	582	499
91	1147	921	680	579	493
92	1144	921	677	578	493
93	1118	918	669	573	494
94	1115	917	668	576	493
95	1123	915	668	576	491
96	1117	913	665	576	486
97	1115	912	662	574	480
98	1115	910	659	566	479
99	1113	901	655	565	474
100	1097	900	655	560	475
101	1096	900	652	557	466
102	1088	900	648	548	459
103	1088	897	644	545	455
104	1085	896	636	543	452
105	1084	895	636	542	452
106	1077	892	636	540	448
107	1069	890	635	539	448
108	1066	890	632	538	443
109	1064	887	626	537	440
110	1060	885	621	536	440
111	1043	880	621	534	439
112	1038	879	621	533	438
113	1037	879	620	529	438
114	1034	872	618	527	434
115	1032	870	615	526	433

116	1025	869	612	520	431
117	1024	862	611	520	429
118	1024	855	610	517	429
119	1021	853	607	514	428
120	1013	850	604	514	426
121	1010	849	602	511	426
122	1007	849	602	509	425
123	1002	844	601	508	423
124	980	843	597	507	422
125	978	842	593	506	419
126	972	838	588	506	419
127	970	838	580	505	418
128	965	835	576	505	417
129	964	831	575	505	417
130	959	827	569	494	415
131	955	826	567	493	415
132	951	826	566	489	414
133	947	825	562	487	413
134	941	817	557	482	413
135	941	816	556	480	411
136	930	815	555	475	410
137	923	815	554	468	410
138	909	814	552	466	407
139	902	807	549	462	407
140	892	805	549	461	405
141	892	804	548	459	403
142	889	802	548	459	403
143	887	796	545	455	403
144	882	792	541	455	404
145	882	791	533	455	401
146	877	788	532	450	399
147	867	784	532	447	397
148	865	783	525	447	396
149	864	788	523	445	396
150	862	787	521	445	390
151	861	786	516	441	389
152	860	785	511	440	389
153	859	784	504	438	388
154	852	783	500	437	387
155	847	782	499	436	387
156	845	781	499	434	387
157	837	780	496	433	385
158	837	779	495	427	385
159	835	777	493	423	384
160	830	777	493	423	383
161	817	775	492	424	382
162	812	775	491	423	380
163	812	771	491	417	380
164	809	771	490	417	380
165	804	771	488	411	379
166	801	769	487	410	377
167	793	769	485	409	377
168	793	765	483	409	377
169	782	764	481	408	377
170	780	763	479	407	376
171	779	760	478	399	375
172	778	757	477	396	374
173	775	750	474	395	364
174	775	746	472	390	361

2

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175	770	742	466	389	359
176	762	741	464	385	357
177	761	741	461	385	357
178	757	739	461	382	353
179	756	735	456	382	351
180	756	734	454	381	350
181	754	732	454	379	347
182	753	731	455	378	346
183	751	730	452	376	344
184	749	728	449	376	343
185	744	722	448	373	341
186	741	722	445	372	341
187	733	721	435	372	340
188	727	720	429	371	339
189	722	720	427	371	338
190	722	717	426	370	335
191	717	716	423	363	336
192	711	711	421	363	335
193	710	708	421	363	335
194	703	707	420	362	333
195	703	706	417	371	332
196	698	704	414	359	330
197	697	703	411	359	328
198	697	702	409	359	328
199	697	700	401	358	326
200	693	697	381	353	323
201	691	696	400	356	323
202	682	695	394	355	324
203	678	694	392	351	322
204	674	687	391	347	321
205	672	685	390	346	320
206	671	682	388	342	317
207	671	682	386	342	317
208	669	681	383	335	314
209	657	678	383	333	309
210	640	676	380	333	309
211	637	676	376	330	308
212	636	675	377	329	308
213	635	675	376	329	307
214	634	673	374	327	304
215	634	670	372	327	303
216	631	667	372	326	301
217	631	665	371	326	292
218	622	664	370	325	291
219	621	662	370	323	290
220	613	661	368	324	298
221	612	660	352	323	298
222	611	660	352	297	297
223	607	657	351	316	297
224	596	657	346	313	295
225	593	652	345	311	294
226	591	651	340	310	292
227	583	650	337	309	292
228	579	650	337	308	291
229	578	642	336	308	291
230	577	642	332	307	291
231	575	642	327	304	291
232	574	640	325	303	290
233	574	639	321	303	290

234	569	637	320	302	289
235	569	636	319	301	287
236	568	634	318	300	286
237	564	628	316	298	286
238	550	622	316	295	286
239	550	617	315	294	285
240	547	611	315	294	285
241	538	611	313	293	284
242	532	609	306	290	284
243	517	619	306	288	283
244	517	614	302	287	282
245	506	618	298	284	282
246	504	617	297	283	281
247	501	614	297	282	280
248	498	613	292	276	277
249	498	612	292	276	276
250	487	601	283	273	277
251	477	601	282	273	277
252	465	598	280		

293	91	513	197	210	242
294	65	512	197	208	241
295	23	509	195	207	240
296	11	508	195	204	240
297	9	508	190	195	239
298	7	501	186	194	238
299	6	493	185	194	237
300		484	183	193	237
301		482	183	191	237
302		474	182	190	234
303		471	182	190	234
304		470	179	190	234
305		461	178	188	232
306		457	178	187	232
307		457	177	183	231
308		456	171	180	230
309		455	170	179	230
310		455	165	179	229
311		454	163	176	229
312		453	159	174	229
313		450	156	174	228
314		447	156	171	228
315		443	153	170	228
316		442	151	169	228
317		440	151	162	227
318		437	149	162	227
319		428	146	161	226
320		420	142	158	225
321		419	141	157	224
322		414	139	156	223
323		410	137	149	223
324		410	134	148	223
325		408	133	148	223
326		408	125	148	222
327		404	124	147	221
328		399	123	144	221
329		394	121	142	221
330		390	119	139	220
331		389	116	139	220
332		384	113	138	220
333		384	113	138	220
334		382	112	137	220
335		374	104	137	218
336		372	101	137	217
337		371	101	136	216
338		370	101	135	214
339		364	99	134	214
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341		359	98	132	214
342		359	96	131	214
343		358	93	127	214
344		345	92	125	212
345		344	89	124	211
346		331	81	123	211
347		328	76	123	210
348		319	75	121	210
349		311	75	121	210
350		310	71	119	208
351		304	71	119	207

352		292	68	119	207
353		289	66	119	206
354		283	63	115	206
355		278	61	114	206
356		273	60	115	205
357		268	57	112	204
358		265	55	111	204
359		256	55	110	203
360		250	54	108	203
361		248	51	105	203
362		248	48	104	202
363		246	45	104	201
364		245	45	97	201
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372		198	31	90	195
373		198	31	88	194
374		185	29	86	194
375		181	28	84	193
376		164	28	83	193
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380		140	23	77	190
381		140	22	76	189
382		135	21	75	189
383		128	21	74	189
384		120	21	74	188
385		113	21	73	188
386		88	21	70	188
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388		72	15	69	188
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393		38	11	59	185
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395		25	10	56	184
396		20	10	55	184
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405		7	7	41	180
406		7	6	40	179
407		5	5	39	176
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409		5	5	31	174
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437		7	152		115
438		7	151		114
439		7	151		113
440		6	150		112
441		150			111
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443		149			109
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**Appendix C:** Raw Table 3 shows the yeast colony area on PDA with agar concentration of 1.5%, 2%, 2.5%, 3%, 3.5% (Trial 2).

<b>Saccharomyces cerevisiae's colonies area on PDA with agar concentration of 1.5%, 2%, 2.5%, 3%, 3.5% (Trial 2)</b>					
<b>Colony label</b>	<b>Colony area on PDA with 1.5% agar concentration (Pixel)</b>	<b>Colony area on PDA with 2% agar concentration (Pixel)</b>	<b>Colony area on PDA with 2.5% agar concentration (Pixel)</b>	<b>Colony area on PDA with 3% agar concentration (Pixel)</b>	<b>Colony area on PDA with 3.5% agar concentration (Pixel)</b>
1	8769	6926	4051	3327	3448
2	6028	5554	4003	3179	3179
3	5731	5418	3856	3166	3038
4	5446	5351	3437	3118	2570
5	5300	5012	3407	2768	2469
6	5121	4937	3348	2697	2394
7	4560	4760	3305	2495	2301
8	4466	4658	3279	2469	2256
9	4341	4602	3272	2445	2218
10	4332	4581	3132	2405	2014
11	4325	4525	3069	2403	1989
12	4324	4405	3048	2356	1954
13	4264	4373	3012	2339	1946
14	4159	4373	2987	2301	1935
15	4145	4328	2979	2297	1905
16	4142	4308	2967	2256	1892
17	4080	4294	2959	2255	1873
18	4015	4259	2942	2200	1870
19	4007	4239	2898	2195	1858
20	3976	4164	2866	2164	1841
21	3947	4161	2813	2163	1837
22	3933	4127	2805	2147	1832
23	3905	4069	2798	2088	1832
24	3878	4053	2780	2086	1805
25	3874	4018	2705	2055	1781
26	3854	4006	2696	2033	1780
27	3847	3878	2681	2005	1778
28	3826	3859	2676	2004	1778
29	3825	3840	2675	1996	1766
30	3806	3800	2615	1992	1759
31	3798	3791	2606	1989	1741
32	3767	3764	2575	1977	1719
33	3764	3758	2549	1966	1714
34	3751	3757	2532	1953	1711
35	3648	3736	2527	1944	1705
36	3646	3721	2526	1935	1699
37	3636	3695	2523	1929	1689
38	3611	3685	2502	1907	1678
39	3601	3624	2474	1892	1674
40	3574	3591	2431	1877	1668
41	3546	3581	2418	1843	1666
42	3538	3503	2406	1841	1665
43	3529	3472	2398	1837	1653
44	3505	3467	2372	1833	1653
45	3484	3464	2368	1825	1645
46	3455	3439	2367	1825	1641
47	3442	3423	2360	1818	1630

48	3430	3391	2355	1818	1627
49	3430	3375	2348	1807	1619
50	3422	3319	2327	1805	1618
51	3411	3313	2286	1780	1611
52	3388	3300	2266	1759	1574
53	3346	3295	2242	1752	1564
54	3338	3274	2241	1745	1563
55	3296	3262	2232	1740	1562
56	3282	3241	2226	1738	1560
57	3273	3158	2219	1734	1554
58	3257	3142	2206	1723	1553
59	3250	3133	2203	1719	1552
60	3217	3132	2198	1717	1526
61	3176	3126	2184	1714	1515
62	3166	3107	2177	1712	1513
63	3162	3106	2176	1711	1489
64	3148	3077	2154	1705	1489
65	3140	3074	2148	1704	1486
66	3109	3065	2145	1699	1485
67	3101	3034	2143	1679	1485
68	3083	3020	2139	1674	1480
69	3074	3016	2138	1666	1473
70	3064	3014	2125	1658	1472
71	3063	3013	2124	1654	1471
72	3056	2997	2123	1653	1471
73	3048	2985	2111	1649	1469
74	3040	2986	2110	1644	1450
75	3028	2966	2105	1641	1443
76	3025	2952	2095	1634	1441
77	3023	2931	2088	1634	1440
78	3018	2907	2050	1630	1440
79	3003	2896	2047	1629	1439
80	2992	2891	2043	1619	1439
81	2988	2856	2040	1619	1438
82	2963	2846	2037	1618	1434
83	2949	2837	2032	1612	1432
84	2943	2830	2020	1611	1425
85	2918	2824	2020	1608	1422
86	2899	2823	2018	1598	1421
87	2888	2821	2014	1595	1420
88	2883	2819	2012	1581	1413
89	2875	2801	1999	1580	1408
90	2866	2777	1997	1574	1408
91	2864	2776	1990	1563	1406
92	2851	2770	1985	1559	1404
93	2848	2762	1976	1548	1402
94	2841	2755	1958	1547	1401
95	2819	2752	1953	1545	1397
96	2811	2752	1949	1541	1392
97	2795	2749	1940	1533	1389
98	2793	2732	1934	1532	1381
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150	2410	2269	1631	1332	1223
151	2407	2253	1624	1329	1222
152	2406	2248	1624	1326	1221
153	2396	2240	1621	1325	1221
154	2373	2238	1604	1321	1220
155	2364	2231	1603	1313	1220
156	2358	2221	1602	1310	1218
157	2353	2214	1600	1300	1211
158	2352	2214	1597	1298	1210
159	2347	2194	1588	1296	1210
160	2343	2193	1585	1291	1210
161	2342	2193	1571	1287	1203
162	2341	2188	1568	1285	1202
163	2338	2186	1565	1278	1200
164	2335	2183	1544	1277	1197
165	2316	2178	1542	1276	1188
166	2315	2164	1538	1266	1188
167	2301	2156	1536	1261	1182
168	2300	2136	1535	1259	1182
169	2299	2129	1531	1259	1182
170	2297	2124	1530	1253	1181
171	2271	2123	1526	1252	1175
172	2270	2117	1526	1162	1162
173	2248	2115	1525	1250	1160
174	2241	2114	1513	1244	1160
175	2237	2092	1511	1240	1159
176	2226	2092	1510	1237	1159
177	2220	2091	1508	1230	1157
178	2218	2078	1494	1229	1153
179	2200	2059	1493	1226	1153
180	2189	2059	1487	1225	1153
181	2184	2053	1483	1223	1151
182	2180	2049	1483	1218	1150
183	2173	2031	1470	1216	1144
184	2162	2023	1468	1214	1141
185	2161	2018	1467	1210	1134
186	2153	1997	1466	1208	1134
187	2152	1989	1466	1201	1128
188	2151	1984	1457	1200	1128
189	2150	1980	1454	1198	1124
190	2144	1968	1453	1184	1123
191	2142	1967	1452	1182	1122
192	2140	1966	1451	1182	1122
193	2135	1961	1448	1179	1121
194	2134	1943	1436	1179	1119
195	2123	1942	1431	1175	1117
196	2108	1937	1429	1175	1115
197	2105	1935	1429	1174	1109
198	2104	1935	1427	1167	1109
199	2091	1932	1426	1164	1109
200	2089	1918	1423	1158	1108
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253	1717	1649	1248	983	1010	304	1424	1460	1083	829	917
254	1709	1647	1244	979	1006	305	1416	1456	1082	828	916
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256	1697	1643	1233	969	1004	307	1414	1447	1076	823	915
257	1692	1635	1230	968	1002	308	1413	1444	1075	822	911
258	1680	1635	1228	963	998	309	1409	1441	1075	818	910
259	1673	1622	1227	963	993	310	1407	1435	1064	811	908
260	1652	1620	1224	960	991	311	1407	1433	1062	811	908
261	1648	1617	1221	948	991	312	1404	1431	1062	810	908
262	1643	1606	1215	947	989	313	1403	1430	1048	805	908
263	1636	1603	1212	941	987	314	1403	1428	1047	804	906
264	1626	1602	1211	930	985	315	1394	1424	1037	798	902
265	1597	1602	1209	927	983	316	1391	1423	1037	798	899
266	1580	1600	1208	927	982	317	1387	1422	1030	796	895
267	1578	1593	1203	925	982	318	1385	1420	1029	791	894
268	1569	1591	1199	924	981	319	1382	1417	1025	788	892
269	1567	1585	1193	921	980	320	1379	1416	1022	786	890
270	1566	1577	1189	920	980	321	1379	1410	1018	785	888
271	1561	1575	1189	917	977	322	1374	1409	1010	785	887
272	1559	1564	1189	915	971	323	1372	1403	1008	780	886
273	1556	1563	1187	914	971	324	1370	1396	1000	773	885
274	1554	1558	1182	912	969	325	1369	1395	1000	770	884
275	1543	1554	1180	910	968	326	1366	1394	1000	768	882
276	1542	1551	1178	906	962	327	1360	1384	996	768	881
277	1541	1551	1174	898	962	328	1360	1383	986	761	880
278	1531	1550	1173	895	960	329	1351	1376	981	761	877
279	1530	1550	1172	892	959	330	1345	1369	981	761	876
280	1526	1542	1168	888	957	331	1341	1364	977	758	872
281	1522	1542	1151	880	954	332	1339	1359	969	758	871
282	1520	1540	1147	880	949	333	1339	1355	969	756	870
283	1517	1525	1145	876	947	334	1336	1350	966	753	868
284	1514	1523	1145	875	947	335	1326	1347	965	751	865
285	1509	1516	1144	874	946	336	1323	1343	954	745	863
286	1496	1516	1141	873	945	337	1315	1343	950	743	859
287	1493	1513	1138	871	945	338	1311	1333	944	742	858
288	1491	1512	1138	870	941	339	1311	1333	941	742	855
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290	1475	1505	1134	868	938	341	1308	1332	932	738	852
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292	1456	1498	1131	863	936	343	1294	1322	926	734	849
293	1454	1493	1122	862	934	344	1293	1310	926	733	847
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295	1452	1484	1119	858	932	346	1289	1303	914	729	845
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298	1444	1478	1104	850	927	349	1269	1293	912	715	839
299	1444	1472	1102	848	926	350	1261	1291	909	715	837
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302	1432	1472	1096	831	920	353	1255	1275	897	700	833

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507	750	664	510	278	632
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509	746	657	508	271	630
510	744	656	507	271	626
511	740	654	502	270	626
512	725	651	500	269	623
513	724	650	494	266	618
514	700	649	493	263	616
515	694	645	492	261	616
516	691	642	491	259	615
517	687	641	491	257	614
518	686	636	486	257	613
519	684	634	481	256	613
520	679	631	481	251	611
521	676	622	481	251	609
522	674	613	479	250	607
523	672	611	475	249	606
524	669	608	474	249	606
525	662	608	467	246	606
526	660	605	466	245	604
527	660	602	461	244	604
528	657	602	460	243	600
529	655	594	458	236	600
530	654	591	452	236	599
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533	652	588	448	230	597
534	652	586	446	228	595
535	652	583	443	225	594
536	650	580	443	219	594
537	649	578	441	211	593
538	642	576	438	209	591
539	634	575	436	209	591
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542	624	564	431	204	588
543	622	563	429	204	588
544	620	562	426	200	587
545	617	560	426	198	586
546	612	558	423	197	582
547	606	557	422	195	579
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550	596	548	417	189	579
551	592	546	409	186	576
552	590	545	407	186	574
553	588	543	407	184	573
554	587	539	402	178	572
555	587	539	401	175	571
556	586	538	396	174	571
557	581	535	387	173	571

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560	580	528	383	168	561
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562	568	524	381	165	558
563	561	521	375	164	558
564	556	517	374	164	554
565	554	516	367	164	549
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567	553	512	364	161	547
568	549	508	364	160	546
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572	538	497	353	156	543
573	537	497	351	156	542
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577	522	485	343	151	540
578	520	484	342	148	540
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582	509	478	319	142	535
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585	501	473	312	134	530
586	495	471	307	133	530
587	493	470	304	132	530
588	493	470	302	131	530
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591	488	462	292	122	525
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593	487	461	285	115	518
594	485	455	280	114	516
595	481	455	265	113	516
596	476	455	263	112	516
597	473	454	262	110	515
598	473	454	257	104	514
599	467	454	246	100	512
600	466	446	241	99	508
601	463	440	234	96	508
602	462	440	234	88	508
603	455	437	230	87	503
604	454	432	229	86	503
605	452	429	228	86	503
606	452	426	224	85	502
607	451	422	222	85	502
608	450	418	217	81	501

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611	445	412	207	76	496
612	443	410	205	75	494
613	441	409	203	74	493
614	441	405	200	74	487
615	440	404	199	73	484
616	437	401	196	73	482
617	436	400	185	72	481
618	430	400	177	71	481
619	424	399	175	71	479
620	415	398	172	65	477
621	403	396	171	61	476
622	402	395	170	56	475
623	402	394	170	51	475
624	401	393	170	51	473
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626	400	388	165	50	472
627	399	388	160	48	463
628	398	385	160	47	462
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630	395	385	156	45	461
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632	394	381	147	45	457
633	392	381	144	44	456
634	385	380	141	43	454
635	381	379	138	43	454
636	380	375	137	39	453
637	380	373	136	39	452
638	378	367	134	32	452
639	373	367	134	31	451
640	368	367	133	30	451
641	368	363	132	30	447
642	366	362	127	30	447
643	363	362	123	28	447
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646	360	359	119	24	439
647	359	356	119	24	438
648	358	356	115	23	438
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651	350	355	111	22	434
652	346	354	110	21	434
653	346	353	104	21	432
654	344	352	101	21	432
655	342	350	100	20	431
656	340	349	99	20	431
657	339	349	99	20	429
658	337	348	96	20	429
659	336	347	92	19	428

660	333	347	91	18	426
661	332	344	87	18	425
662	332	343	85	18	424
663	332	342	85	17	418
664	331	340	83	17	418
665	330	338	83	17	416
666	330	337	80	17	416
667	328	336	79	17	416
668	327	336	76	16	415
669	325	335	76	16	413
670	323	334	68	16	413
671	323	334	65	15	412
672	322	330	62	15	412
673	321	327	60	14	411
674	320	325	59	14	410
675	319	325	59	14	408
676	319	325	59	14	407
677	318	324	59	14	400
678	317	324	57	14	398
679	316	322	55	14	395
680	314	322	53	13	394
681	313	319	52	13	390
682	313	314	52	13	390
683	313	313	51	13	389
684	310	313	51	13	388
685	308	312	50	13	385
686	305	311	47	12	384
687	303	310	47	12	383
688	299	309	46	12	381
689	296	308	45	12	379
690	295	307	44	12	379
691	294	302	43	12	379
692	292	301	40	12	378
693	289	297	39	12	375
694	284	296	39	11	375
695	281	295	36	11	373
696	280	295	35	10	366
697	280	294	35	10	365
698	279	294	34	10	364
699	278	293	34	10	364
700	277	293	363		
701	277	291	362		
702	274	291	361		
703	273	290	361		
704	270	287	360		
705	269	284	359		
706	268	283	358		
707	268	280	357		
708	267	280	354		
709	263	280	353		
710	259	279	353		

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712	258	274	274		351
713	258	271	271		350
714	257	270	270		349
715	253	270	270		349
716	252	269	269		346
717	251	269	269		346
718	247	269	269		343
719	246	268	268		343
720	243	268	268		343
721	241	267	267		341
722	240	267	267		341
723	239	267	267		340
724	238	267	267		339
725	237	266	266		336
726	235	266	266		336
727	234	265	265		335
728	234	264	264		334
729	233	264	264		332
730	230	264	264		330
731	229	263	263		329
732	224	262	262		325
733	223	261	261		324
734	222	260	260		324
735	222	259	259		324
736	222	259	259		322
737	221	259	259		321
738	217	258	258		319
739	216	256	256		314
740	213	256	256		312
741	211	255	255		309
742	211	253	253		309
743	210	249	249		306
744	210	248	248		305
745	209	248	248		304
746	208	247	247		304
747	207	247	247		300
748	207	246	246		298
749	205	245	245		297
750	204	244	244		297
751	202	244	244		293
752	201	243	243		293
753	201	242	242		293
754	200	242	242		291
755	199	242	242		290
756	198	241	241		285
757	195	241	241		284
758	195	239	239		280
759	195	238	238		279
760	194	238	238		279
761	193	238	238		278

762	191	236		278	813	142	192		224
763	190	236		278	814	142	192		225
764	189	233		277	815	142	192		225
765	189	233		274	816	141	191		219
766	189	232		274	817	141	190		218
767	187	231		271	818	139	190		216
768	186	231		267	819	138	190		212
769	184	230		265	820	137	189		211
770	184	229		264	821	137	188		211
771	184	228		264	822	137	188		210
772	183	225		264	823	137	187		210
773	182	225		263	824	137	187		209
774	182	225		261	825	137	186		209
775	182	224		260	826	136	186		209
776	181	222		258	827	136	184		207
777	179	222		257	828	136	183		206
778	178	220		257	829	134	183		200
779	177	216		254	830	133	182		198
780	177	216		254	831	133	181		195
781	176	215		253	832	132	181		195
782	175	214		253	833	132	179		195
783	175	213		251	834	132	179		194
784	172	212		251	835	132	177		194
785	171	212		250	836	131	177		193
786	171	212		250	837	131	177		192
787	170	211		249	838	130	175		191
788	170	209		249	839	129	175		191
789	170	207		246	840	128	173		191
790	170	206		246	841	128	172		189
791	170	205		245	842	127	171		189
792	167	204		244	843	126	171		189
793	166	204		244	844	125	171		186
794	163	202		243	845	125	169		186
795	161	202		243	846	124	169		185
796	157	202		242	847	123	169		185
797	157	201		241	848	123	167		184
798	155	201		240	849	122	167		184
799	154	201		238	850	122	166		182
800	154	200		236	851	122	166		179
801	152	199		236	852	122	165		176
802	151	199		236	853	121	165		175
803	150	199		234	854	121	164		175
804	149	198		232	855	120	162		174
805	149	197		231	856	119	162		174
806	148	196		230	857	118	161		172
807	147	195		230	858	117	161		172
808	145	195		230	859	117	161		171
809	145	194		229	860	117	161		171
810	145	194		225	861	116	161		169
811	144	193		225	862	116	161		167
812	143	193		224	863	115	159		165

16

864	114	159		164
865	114	159		164
866	114	158		162
867	113	158		160
868	113	154		160
869	113	154		160
870	112	153		157
871	112	152		157
872	111	151		156
873	110	151		154
874	110	150		153
875	110	148		151
876	108	147		149
877	108	147		147
878	107	146		146
879	107	146		146
880	107	145		145
881	106	144		144
882	106	143		144
883	106	142		144
884	105	141		142
885	104	140		142
886	104	138		141
887	103	137		139
888	102	135		138
889	101	135		137
890	101	135		136
891	100	134		135
892	100	133		135
893		133		133
894		132		133
895		132		131
896		131		130
897		130		130
898		130		124
899		130		124
900		130		121
901		129		116
902		129		116
903		129		115
904		129		115
905		129		115
906		127		114
907		127		114
908		126		113
909		126		111
910		125		110
911		125		110
912		125		110
913		124		109
914		123		108

915		120		107
916		119		104
917		117		101
918		116		100
919		116		96
920		114		96
921		114		96
922		114		93
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928		108		87
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931		107		84
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933		107		84
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936		103		81
937		103		80
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939		101		79
940		101		78
941		101		76
942		100		76
943		100		74
944		100		74
945		100		73
946		98		73
947		98		72
948		97		70
949		97		70
950		96		67
951		96		64
952		95		63
953		95		62
954		95		62
955		94		62
956		93		62
957		93		60

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**Appendix D:** Raw Table 4 shows the yeast colony area on PDA with agar concentration of 1.5%, 2%, 2.5%, 3%, 3.5% (Trial 3).

<b>Saccharomyces cerevisiae's colonies area on PDA with agar concentration of 1.5%, 2%, 2.5%, 3%, 3.5% (Trial 3)</b>					
<b>Colony label</b>	<b>Colony area on PDA with 1.5% agar concentration (Pixel)</b>	<b>Colony area on PDA with 2% agar concentration (Pixel)</b>	<b>Colony area on PDA with 2.5% agar concentration (Pixel)</b>	<b>Colony area on PDA with 3% agar concentration (Pixel)</b>	<b>Colony area on PDA with 3.5% agar concentration (Pixel)</b>
1	4465	3501	2643	2011	2071
2	4465	3501	2643	2011	2071
3	4318	3349	2516	1985	1928
4	4199	3264	2285	1917	1881
5	3898	3223	2218	1889	1876
6	3675	3092	2172	1826	1728
7	3623	3062	2134	1714	1692
8	3534	2983	2075	1681	1664
9	3471	2868	2007	1672	1633
10	3458	2856	2001	1627	1606
11	3427	2809	1986	1588	1603
12	3360	2790	1985	1540	1596
13	3359	2775	1975	1538	1579
14	3325	2662	1958	1535	1569
15	3322	2657	1867	1533	1519
16	3300	2656	1698	1515	1506
17	3238	2648	1697	1514	1483
18	3229	2624	1694	1501	1469
19	3186	2595	1676	1490	1450
20	3164	2552	1671	1474	1421
21	3149	2524	1669	1473	1420
22	3145	2519	1667	1441	1417
23	3120	2507	1663	1420	1409
24	3111	2482	1634	1416	1392
25	3105	2479	1633	1415	1388
26	3057	2467	1631	1413	1384
27	3048	2446	1626	1412	1364
28	3041	2441	1614	1406	1358
29	3039	2426	1589	1403	1345
30	2994	2422	1588	1387	1326
31	2974	2414	1581	1371	1323
32	2946	2406	1575	1369	1320
33	2946	2403	1567	1368	1316
34	2941	2385	1564	1366	1311
35	2906	2368	1562	1352	1310
36	2905	2367	1559	1349	1306
37	2904	2362	1551	1348	1302
38	2892	2357	1547	1342	1285
39	2885	2356	1544	1339	1285
40	2879	2347	1542	1338	1284
41	2875	2339	1533	1336	1280
42	2872	2327	1519	1335	1280
43	2869	2315	1516	1334	1275
44	2868	2304	1508	1329	1274
45	2829	2294	1503	1328	1272
46	2824	2292	1502	1316	1272
47	2823	2288	1494	1305	1256
48	2814	2287	1485	1305	1255

49	2813	2281	1484	1294	1244		100	2433	1939	1326	1176	939
50	2808	2277	1482	1292	1229		101	2429	1932	1326	1174	936
51	2799	2275	1481	1288	1228		102	2422	1929	1321	1171	933
52	2770	2265	1480	1289	1225		103	2415	1903	1321	1162	933
53	2752	2262	1469	1285	1225		104	2414	1902	1320	1161	929
54	2749	2261	1462	1285	1225		105	2412	1897	1320	1159	927
55	2748	2247	1462	1284	1220		106	2411	1883	1319	1158	915
56	2742	2238	1458	1283	1209		107	2395	1881	1312	1155	914
57	2733	2224	1458	1281	1209		108	2392	1872	1297	1155	911
58	2718	2221	1458	1281	1209		109	2389	1867	1292	1151	910
59	2716	2220	1457	1280	1209		110	2389	1866	1291	1151	909
60	2696	2219	1452	1278	1167		111	2379	1864	1289	1149	907
61	2886	2211	1443	1276	1165		112	2375	1864	1284	1148	907
62	2676	2207	1434	1276	1153		113	2373	1863	1283	1148	903
63	2643	2205	1431	1276	1153		114	2372	1862	1282	1148	903
64	2640	2145	1430	1275	1159		115	2371	1861	1281	1146	900
65	2632	2145	1429	1273	1150		116	2371	1860	1279	1143	900
66	2627	2144	1420	1270	1156		117	2366	1859	1272	1141	895
67	2589	2143	1416	1268	1121		118	2352	1854	1271	1139	893
68	2586	2141	1416	1265	1114		119	2351	1847	1271	1139	891
69	2567	2126	1416	1260	1113		120	2348	1845	1269	1137	889
70	2546	2124	1412	1238	1095		121	2347	1845	1267	1137	889
71	2546	2123	1411	1234	1094		122	2338	1836	1266	1136	883
72	2541	2109	1410	1228	1093		123	2325	1835	1265	1133	883
73	2536	2099	1406	1227	1091		124	2324	1831	1260	1131	883
74	2522	2078	1405	1225	1091		125	2321	1823	1260	1137	876
75	2517	2068	1404	1225	1090		126	2319	1823	1258	1124	875
76	2508	2066	1401	1222	1079		127	2316	1820	1251	1122	874
77	2506	2061	1399	1221	1074		128	2314	1815	1250	1119	874
78	2506	2051	1397	1220	1066		129	2312	1810	1249	1118	874
79	2504	2046	1390	1218	1056		130	2303	1808	1245	1118	872
80	2500	2045	1386	1217	1044		131	2301	1803	1243	1114	870
81	2499	2043	1381	1217	1028		132	2300	1799	1240	1111	870
82	2498	2029	1379	1215	1036		133	2299	1791	1234	1108	868
83	2496	2024	1375	1215	1018		134	2297	1784	1227	1102	868
84	2479	2024	1374	1215	1018		135	2290	1777	1226	1099	867
85	2471	2018	1374	1211	1015		136	2286	1771	1223	1098	866
86	2469	2008	1372	1210	1011		137	2272	1765	1222	1098	865
87	2468	1996	1369	1194	1003		138	2271	1764	1220	1097	863
88	2466	1990	1364	1191	988		139	2268	1764	1220	1094	863
89	2462	1987	1360	1191	974		140	2257	1759	1217	1093	861
90	2459	1980	1356	1188	969		141	2253	1752	1215	1093	859
91	2459	1980	1354	1188	967		142	2249	1751	1211	1093	858
92	2457	1975	1354	1185	964		143	2248	1750	1208	1092	856
93	2456	1972	1351	1183	957		144	2244	1748	1208	1091	855
94	2452	1963	1350	1183	955		145	2243	1737	1207	1091	855
95	2448	1959	1349	1182	944		146	2232	1735	1206	1085	853
96	2445	1958	1336	1181	942		147	2227	1731	1205	1083	852
97	2445	1958	1333	1180	941		148	2223	1728	1200	1081	852
98	2440	1949	1331	1179	941		149	2206	1727	1200	1076	851
99	2437	1946	1330	1176	939		150	2189	1723	1199	1075	849

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151	2172	1721	1192	1075	847		202	1993	1519	1050	986	755
152	2162	1718	1191	1073	845		203	1982	1506	1040	985	755
153	2161	1716	1190	1073	845		204	1981	1505	1039	983	754
154	2159	1707	1189	1072	842		205	1979	1503	1038	983	754
155	2153	1706	1188	1059	839		206	1975	1501	1037	979	750
156	2150	1696	1184	1058	835		207	1975	1494	1035	978	748
157	2147	1690	1184	1052	835		208	1973	1493	1031	972	747
158	2145	1687	1183	1051	834		209	1968	1477	1031	972	745
159	2138	1686	1181	1050	834		210	1967	1475	1030	971	745
160	2137	1685	1179	1050	832		211	1965	1471	1028	971	745
161	2136	1675	1178	1050	832		212	1958	1465	1027	970	743
162	2136	1667	1178	1050	831		213	1955	1453	1020	969	740
163	2134	1662	1177	1046	831		214	1949	1447	1018	969	738
164	2129	1655	1165	1042	830		215	1945	1438	1012	968	737
165	2112	1644	1160	1041	829		216	1937	1436	1011	967	736
166	2112	1642	1156	1039	825		217	1934	1432	1006	966	736
167	2103	1641	1153	1037	822		218	1932	1432	1006	964	735
168	2102	1640	1150	1034	821		219	1927	1432	1003	963	735
169	2097	1639	1149	1033	820		220	1926	1426	999	961	734
170	2095	1636	1147	1033	815		221	1925	1426	997	961	733
171	2094	1634	1141	1032	813		222	1919	1424	997	960	732
172	2093	1631	1135	1030	810		223	1917	1421	996	959	731
173	2091	1629	1132	1029	809		224	1902	1419	993	958	731
174	2082	1629	1132	1029	808		225	1894	1418	992	958	731
175	2075	1628	1131	1028	803		226	1893	1410	991	957	729
176	2074	1627	1125	1026	800		227	1884	1405	991	955	729
177	2073	1627	1125	1024	800		228	1884	1400	989	955	729
178	2071	1626	1124	1022	799		229	1881	1392	986	954	728
179	2071	1615	1119	1020	798		230	1879	1391	985	952	726
180	2069	1607	1106	1020	798		231	1873	1387	985	952	725
181	2069	1601	1104	1019	797		232	1870	1385	983	951	725
182	2066	1599	1104	1019	790		233	1868	1377	982	950	725
183	2060	1598	1102	1017	789		234	1866	1375	980	950	722
184	2058	1595	1090	1017	788		235	1861	1370	979	946	722
185	2058	1594	1088	1014	788		236	1858	1368	978	945	721
186	2054	1593	1086	1011	785		237	1857	1363	978	944	721
187	2050	1592	1080	1010	781		238	1853	1360	977	942	721
188	2048	1576	1080	1008	780		239	1852	1357	976	942	720
189	2047	1575	1079	1007	780		240	1852	1354	971	942	720
190	2045	1575	1078	1005	780		241	1843	1353	970	938	717
191	2040	1572	1075	1004	773		242	1843	1351	964	936	717
192	2031	1569	1075	1001	773		243	1840	1346	963	932	717
193	2027	1565	1070	999	772		244	1835	1329	961	931	716
194	2025	1563	1070	997	771		245	1833	1324	961	928	715
195	2018	1539	1069	994	769		246	1829	1320	960	9	

253	1816	1304	943	915	703		304	1639	1159	852	842	643
254	1814	1304	942	912	697		305	1629	1157	851	842	643
255	1813	1302	938	911	696		306	1629	1156	850	842	642
256	1812	1298	956	911	695		307	1604	1150	846	842	641
257	1810	1296	955	911	693		308	1602	1150	855	847	640
258	1809	1294	953	909	692		309	1599	1149	842	840	640
259	1804	1289	933	908	692		310	1599	1136	842	839	640
260	1794	1285	932	903	692		311	1597	1134	839	838	637
261	1793	1285	930	903	691		312	1594	1131	839	838	637
262	1792	1284	934	902	690		313	1589	1128	838	837	637
263	1790	1281	926	899	689		314	1587	1127	837	836	636
264	1790	1275	919	899	689		315	1583	1127	836	835	635
265	1789	1273	919	899	689		316	1583	1116	830	832	634
266	1782	1270	917	898	687		317	1578	1115	829	832	631
267	1781	1262	917	893	686		318	1576	1114	825	830	631
268	1772	1261	917	893	685		319	1571	1114	822	829	627
269	1770	1260	915	893	685		320	1566	1110	821	829	626
270	1767	1257	915	891	685		321	1564	1110	817	829	625
271	1767	1257	914	891	684		322	1564	1106	814	828	624
272	1766	1257	913	891	682		323	1556	1098	814	828	623
273	1765	1252	913	889	680		324	1546	1096	814	827	623
274	1764	1251	908	887	679		325	1546	1094	812	826	622
275	1763	1236	908	885	678		326	1544	1093	810	825	621
276	1761	1235	904	883	676		327	1536	1093	809	825	620
277	1757	1234	904	881	675		328	1536	1092	806	824	620
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928			341	244		115
929			339	243		114
930			338	243		113
931			338	241		113
932			334	241		113
933			334	241		113
934			334	241		113
935			333	240		112
936			332	239		112
937			331	236		112
938			331	235		112
939			330	235		112
940			328	234		112
941			326	233		111
942			324	233		111
943			321	232		111
944			320	231		110
945			319	231		110
946			319	231		110
947			319	231		110
948			318	230		110
949			317	229		109
950			314	229		109
951			314	228		109
952			314	228		109
953			313	228		108
954			312	228		107
955			311	228		107
956			311	227		106
957			310	225		106
958			307	223		105
959			307	223		104
960			306	223		104
961			306	222		104
962			305	222		104
963			305	221		103
964			304	221		102
965			303	221		102
966			303	221		102

967	303	221	99
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969	301	219	99
970	301	218	98
971	299	218	97
972	296	218	96
973	296	217	95
974	294	217	95
975	294	216	95
976	293	216	94
977	293	216	93
978	291	215	93
979	290	214	93
980	286	209	92
981	286	208	92
982	286	208	91
983	285	206	90
984	284	205	90
985	284	204	87
986	284	204	87
987	284	204	86
988	283	204	86
989	283	203	85
990	282	202	85
991	281	201	85
992	281	201	84
993	279	200	84
994	278	200	84
995	277	199	83
996	276	196	83
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1000	269	192	82
1001	269	192	81
1002	269	191	80
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1004	268	191	80
1005	267	191	80
1006	266	190	79
1007	266	189	79
1008	266	188	78
1009	263	187	78
1010	263	187	77
1011	263	187	77
1012	260	187	76
1013	259	185	76
1014	259	184	76
1015	259	183	75
1016	258	182	75
1017	257	182	75

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1019			257	180		74
1020			255	180		74
1021			255	179		73
1022			254	177		73
1023			251	176		73
1024			251	176		73
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1029			249	174		71
1030			249	174		71
1031			248	173		70
1032			246	172		70
1033			246	169		69
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1035			245	168		68
1036			243	167		68
1037			243	166		68
1038			241	166		67
1039			241	165		66
1040			241	164		65
1041			239	163		64
1042			239	162		64
1043			238	162		63
1044			237	161		62
1045			237	160		62
1046			236	160		61
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1052			232	155		60
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1056			231	154		59
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1059			226	154		58
1060			226	153		57
1061			225	153		57
1062			224	152		57
1063			224	152		57
1064			224	151		57
1065			223	150		56
1066			222	150		56
1067			221	150		55
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1070	219	149	54	1121		186	128	43
1071	218	148	53	1122		186	128	43
1072	218	148	53	1123		185	128	43
1073	218	147	53	1124		185	127	43
1074	215	146	52	1125		184	127	41
1075	215	146	52	1126		184	127	41
1076	215	146	52	1127		184	127	41
1077	215	146	52	1128		183	126	40
1078	214	145	52	1129		181	126	40
1079	212	145	51	1130		180	126	40
1080	211	145	51	1131		180	126	40
1081	211	145	51	1132		179	125	40
1082	210	144	51	1133		176	125	40
1083	210	144	51	1134		176	124	39
1084	210	144	51	1135		176	124	39
1085	208	144	51	1136		176	124	39
1086	207	143	50	1137		175	123	39
1087	207	143	50	1138		174	123	39
1088	207	143	50	1139		174	122	39
1089	207	142	50	1140		173	122	39
1090	206	142	50	1141		173	122	39
1091	205	141	49	1142		172	122	39
1092	204	141	49	1143		172	122	38
1093	204	141	49	1144		171	121	38
1094	203	140	49	1145		169	121	38
1095	203	140	49	1146		169	120	38
1096	203	139	48	1147		168	119	38
1097	202	139	48	1148		166	119	37
1098	202	138	48	1149		166	119	37
1099	200	138	48	1150		166	119	37
1100	200	138	48	1151		165	118	37
1101	200	137	48	1152		165	118	37
1102	198	137	47	1153		165	117	37
1103	197	136	47	1154		164	117	37
1104	196	135	47	1155		164	116	36
1105	196	135	47	1156		164	116	36
1106	196	135	46	1157		164	116	35
1107	196	134	46	1158		163	115	35
1108	194	134	46	1159		163	114	35
1109	193	134	46	1160		163	114	35
1110	193	134	46	1161		162	114	34
1111	192	134	45	1162		162	114	34
1112	192	133	45	1163		162	114	34
1113	192	132	45	1164		161	113	34
1114	191	131	45	1165		160	113	33
1115	190	131	44	1166		160	113	33
1116	188	130	44	1167		160	113	33
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1176		156	108		32		1227		130		93		25
1177		155	108		32		1228		130		93		25
1178		155	107		32		1229		129		92		25
1179		155	107		32		1230		128		92		25
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1183		153	105		31		1234		127		91		24
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1185		152	105		31		1236		127		89		24
1186		152	104		31		1237		126		89		24
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1188		151	103		30		1239		126		89		23
1189		150	103		30		1240		125		88		23
1190		150	102		30		1241		125		87		23
1191		150	102		30		1242		125		87		23
1192		149	102		30		1243		125		87		23
1193		149	101		29		1244		124		86		23
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1197		145	100		29		1248		123		85		23
1198		143	100		29		1249		123		85		23
1199		142	100		29		1250		123		84		22
1200		140	100		28		1251		122		84		22
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1209		138	96		27		1260		118		82		22
1210		138	96		27		1261		118		81		22
1211		137	96		26		1262		117		81		22
1212		137	96		26		1263		117		81		21
1213		136	96		26		1264		117		81		
1214		136	96		26		1265		116		80		
1215		135	96		26		1266		116		80		
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1219		132	94		26		1270		114		77		
1220		132	94		25		1271		114		77		
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1310		90	66		
1311		90	66		
1312		90	66		
1313		90	65		
1314		90	65		
1315		90	65		

**Appendix E:** Raw table3 showing the yeast colony area on PDA with agar concentration of 1.5%, 2%, 2.5%, 3%, 3.5% (Trial 4).

<b>Saccharomyces cerevisiae's colonies area on PDA with agar concentration of 1.5%, 2%, 2.5%, 3%, 3.5% (Trial 4)</b>					
<b>Colony label</b>	<b>Colony area on PDA with 1.5% agar concentration (Pixel)</b>	<b>Colony area on PDA with 2% agar concentration (Pixel)</b>	<b>Colony area on PDA with 2.5% agar concentration (Pixel)</b>	<b>Colony area on PDA with 3% agar concentration (Pixel)</b>	<b>Colony area on PDA with 3.5% agar concentration (Pixel)</b>
1	4676	3883	3201	2617	2235
2	4566	3502	3006	2354	2117
3	4427	3224	2878	2236	2114
4	3990	3110	2835	2232	2110
5	3852	3087	2741	2044	1958
6	3818	3068	2487	2006	1939
7	3619	3057	2474	1962	1916
8	3536	3041	2468	1949	1880
9	3349	2993	2412	1887	1806
10	3314	2939	2386	1872	1794
11	3297	2837	2310	1871	1786
12	3162	2821	2298	1824	1742
13	3086	2820	2293	1813	1731
14	3072	2804	2201	1786	1620
15	2951	2793	2182	1774	1610
16	2908	2739	2169	1767	1609
17	2848	2679	2100	1764	1592
18	2833	2660	2100	1755	1570
19	2821	2657	2089	1721	1553
20	2775	2657	2016	1680	1472
21	2748	2655	2012	1668	1459
22	2542	2653	2002	1663	1440
23	2515	2613	1995	1661	1439
24	2485	2609	1983	1659	1427
25	2482	2596	1979	1636	1420
26	2471	2553	1974	1634	1405
27	2389	2546	1954	1620	1357
28	2366	2514	1904	1614	1350
29	2301	2507	1903	1607	1340
30	2250	2498	1896	1598	1319
31	2248	2495	1881	1586	1294
32	2240	2486	1858	1531	1235
33	2240	2485	1853	1529	1223
34	2234	2469	1831	1510	1213
35	2220	2440	1824	1501	1199
36	2218	2430	1820	1459	1195
37	2141	2416	1811	1450	1187
38	2141	2357	1792	1444	1187
39	2125	2340	1780	1439	1177
40	2112	2328	1747	1432	1176
41	2097	2319	1735	1425	1161
42	2082	2290	1734	1418	1135
43	2018	2286	1714	1415	1135
44	2014	2281	1710	1405	1129
45	2010	2267	1708	1404	1125
46	1967	2262	1688	1402	1113
47	1930	2256	1683	1395	1112
48	1900	2246	1621	1391	1108
49	1899	2231	1590	1391	1081
50	1891	2225	1574	1381	1078

51	1861	2211	1558	1375	1072	105	1270	1723	1194	1146	867
52	1853	2206	1549	1374	1069	106	1262	1714	1193	1142	866
53	1850	2180	1530	1372	1068	107	1255	1709	1188	1138	862
54	1849	2162	1528	1371	1066	108	1254	1698	1183	1136	861
55	1839	2159	1528	1363	1051	109	1250	1697	1183	1135	860
56	1832	2159	1528	1361	1043	110	1235	1696	1182	1133	858
57	1806	2156	1518	1346	1040	111	1233	1695	1175	1130	851
58	1789	2147	1512	1340	1040	112	1230	1681	1173	1130	848
59	1781	2116	1490	1339	1039	113	1216	1679	1171	1122	846
60	1762	2115	1482	1329	1034	114	1216	1676	1161	1121	839
61	1724	2100	1481	1322	1023	115	1212	1675	1160	1121	839
62	1697	2091	1480	1318	1014	116	1203	1651	1157	1121	824
63	1678	2090	1478	1316	1014	117	1178	1650	1152	1112	822
64	1675	2086	1477	1301	1011	118	1168	1648	1149	1108	822
65	1662	2083	1472	1301	1007	119	1160	1641	1147	1106	819
66	1660	2071	1467	1300	1006	120	1158	1641	1147	1101	818
67	1651	2063	1465	1300	1004	121	1151	1640	1146	1101	812
68	1650	2053	1464	1295	993	122	1146	1637	1129	1100	809
69	1644	2047	1408	1287	991	123	1139	1635	1129	1099	808
70	1640	2039	1402	1286	976	124	1135	1633	1121	1099	807
71	1638	2031	1401	1282	976	125	1127	1632	1119	1092	803
72	1630	2031	1388	1278	968	126	1127	1632	1116	1090	803
73	1619	2031	1380	1274	967	127	1124	1626	1116	1089	800
74	1599	2027	1368	1267	964	128	1121	1624	1108	1086	799
75	1593	2017	1363	1264	963	129	1118	1622	1084	1085	791
76	1573	2003	1352	1264	962	130	1112	1616	1081	1082	784
77	1569	1996	1342	1268	962	131	1111	1608	1084	1081	774
78	1527	1989	1308	1254	950	132	1105	1601	1090	1075	767
79	1526	1952	1303	1252	949	133	1103	1600	1074	1072	766
80	1507	1949	1301	1249	948	134	1101	1600	1074	1071	759
81	1499	1938	1300	1245	942	135	1099	1596	1067	1070	758
82	1497	1898	1296	1243	940	136	1087	1595	1066	1070	757
83	1496	1886	1295	1216	931	137	1085	1585	1064	1069	755
84	1461	1858	1294	1215	930	138	1067	1583	1062	1063	752
85	1457	1852	1290	1215	927	139	1065	1574	1058	1061	750
86	1450	1844	1290	1209	921	140	1063	1568	1050	1057	741
87	1439	1841	1287	1208	917	141	1063	1564	1049	1057	738
88	1434	1835	1280	1196	917	142	1057	1554	1047	1052	738
89	1394	1835	1265	1196	910	143	1049	1546	1036	1051	737
90	1368	1825	1261	1192	910	144	1038	1542	1029	1046	732
91	1355	1819	1261	1182	909	145	1037	1532	1024	1046	728
92	1353	1800	1257	1178	897	146	1031	1530	1023	1037	725
93	1347	1796	1254	1178	895	147	1022	1529	1021	1036	724
94	1338	1788	1251	1172	889	148	1019	1524	1017	1028	724
95	1334	1780	1248	1170	889	149	1017	1521	1007	1028	724
96	1331	1772	1238	1167	888	150	1015	1510	1006	1028	723
97	1316	1771	1236	1166	888	151	993	1510	1002	1025	721
98	1315	1769	1226	1165	880	152	992	1510	999	1023	721
99	1306	1752	1222	1164	874	153	991	1506	998	1023	711
100	1289	1740	1218	1160	872	154	982	1504	992	1023	709
101	1283	1740	1215	1153	871	155	973	1499	985	1016	709
102	1279	1730	1199	1151	869	156	972	1495	975	1015	708
103	1273	1725	1198	1149	868	157	967	1494	964	1013	707
104	1270	1722	1194	1147	867	158	954	1492	958	1012	705

2

3

159	953	1488	957	1010	701	213	754	1312	736	909	636
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161	946	1485	948	1004	694	215	753	1308	726	901	634
162	945	1484	938	1004	692	216	749	1304	725	896	632
163	942	1483	936	999	692	217	748	1294	722	894	632
164	938	1473	930	996	690	218	748	1293	721	894	631
165	936	1462	924	996	690	219	744	1286	717	894	630
166	920	1456	922	994	689	220	737	1287	716	892	629
167	918	1454	919	993	689	221	735	1274	715	890	627
168	911	1451	918	986	689	222	732	1271	710	889	626
169	904	1450	914	981	689	223	732	1268	709	889	625
170	903	1446	912	978	689	224	730	1267	706	888	625
171	901	1446	901	973	688	225	728	1264	704	888	624
172	897	1445	897	973	686	226	728	1266	701	887	624
173	894	1433	896	972	686	227	724	1244	701	885	624
174	884	1432	893	968	684	228	724	1243	700	881	621
175	884	1431	892	966	680	229	721	1241	700	881	620
176	883	1429	882	961	679	230	717	1234	699	879	617
177	875	1429	880	960	673	231	710	1231	695	879	616
178	875	1426	879	960	672	232	709	1221	695	875	613
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181	849	1406	844	957	667	235	695	1215	681	872	609
182	830	1404	843	957	666	236	694	1209	679	871	608
183	828	1403	842	955	666	237	692	1207	679	871	608
184	826	1403	842	954	665	238	690	1206	678	871	607
185	824	1402	839	953	665	239	689	1205	674	870	606
186	819	1401	831	952	664	240	687	1199	671	867	605
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189	809	1384	820	949	662	243	679	1176	648	863	604
190	807	1381	819	949	662	244	678	1171	641	860	604
191	806	1381	818	946	662	245	677	1171	635	858	604
192	803	1371	815	944	661	246	672	1170	635	856	602
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597	227	268	62	412	303	651	192	148	343	273
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14

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16

17

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968	58			171	165			1022				158	153

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1075				143	144			1129				134	136
1076				143	144			1130				133	136

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1776			74	74
1777			74	73

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**Appendix F:** Raw Table 4 shows the yeast colony area on PDA with agar concentration of 1.5%, 2%, 2.5%, 3%, 3.5% (Trial 5).

<b>Saccharomyces cerevisiae's colonies area on PDA with agar concentration of 1.5%, 2%, 2.5%, 3%, 3.5% (Trial 5)</b>					
<b>Colony label</b>	<b>Colony area on PDA with 1.5% agar concentration (Pixel)</b>	<b>Colony area on PDA with 2% agar concentration (Pixel)</b>	<b>Colony area on PDA with 2.5% agar concentration (Pixel)</b>	<b>Colony area on PDA with 3% agar concentration (Pixel)</b>	<b>Colony area on PDA with 3.5% agar concentration (Pixel)</b>
1	4718	3909	3125	2945	2364
2	4675	3906	2757	2482	2272
3	4593	3873	2740	2458	2258
4	4348	3822	2650	2424	2196
5	4262	3693	2643	2411	2063
6	4228	3505	2606	2404	2043
7	4039	3298	2558	2280	2034
8	3900	3274	2467	2166	2033
9	3881	3183	2461	2005	1908
10	3748	3160	2397	1974	1897
11	3562	3158	2349	1963	1846
12	3363	3114	2332	1959	1820
13	3349	3053	2291	1949	1807
14	3160	3025	2228	1936	1737
15	3112	3012	2189	1933	1732
16	3074	2999	2154	1913	1707
17	3059	2977	2151	1788	1698
18	3044	2970	2109	1734	1687
19	3017	2918	2102	1722	1684
20	2986	2918	2075	1695	1681
21	2933	2915	2063	1693	1675
22	2917	2908	2063	1677	1647
23	2904	2889	2062	1675	1628
24	2901	2854	2049	1568	1619
25	2752	2844	2028	1562	1619
26	2752	2768	2017	1554	1619
27	2741	2748	1985	1552	1593
28	2735	2699	1976	1552	1589
29	2727	2694	1965	1526	1572
30	2702	2690	1963	1506	1568
31	2690	2684	1962	1493	1562
32	2671	2667	1954	1488	1543
33	2635	2664	1917	1479	1517
34	2579	2593	1892	1477	1514
35	2535	2581	1870	1447	1509
36	2505	2569	1869	1427	1464
37	2502	2563	1868	1415	1455
38	2501	2519	1822	1405	1434
39	2491	2519	1806	1395	1403
40	2476	2512	1799	1367	1398
41	2465	2490	1788	1321	1395
42	2433	2485	1786	1319	1390
43	2389	2481	1766	1308	1388
44	2386	2479	1758	1303	1380
45	2382	2471	1756	1299	1379
46	2380	2467	1745	1292	1373
47	2361	2461	1742	1287	1370
48	2349	2458	1726	1257	1347

49	2334	2451	1721	1242	1340
50	2315	2387	1695	1238	1338
51	2242	2386	1682	1236	1338
52	2236	2369	1678	1229	1336
53	2198	2347	1675	1216	1334
54	2197	2344	1666	1202	1328
55	2188	2331	1663	1200	1319
56	2180	2318	1634	1194	1319
57	2157	2317	1634	1190	1316
58	2155	2312	1611	1182	1312
59	2140	2306	1610	1178	1308
60	2120	2299	1604	1171	1298
61	2115	2296	1597	1167	1291
62	2101	2284	1588	1164	1287
63	2095	2281	1583	1161	1281
64	2079	2259	1574	1160	1278
65	2038	2251	1573	1159	1266
66	2032	2243	1566	1145	1260
67	2028	2222	1566	1139	1251
68	2008	2221	1563	1131	1251
69	1976	2212	1554	1131	1247
70	1935	2201	1550	1128	1246
71	1912	2199	1531	1122	1244
72	1889	2190	1525	1120	1225
73	1878	2185	1513	1118	1220
74	1834	2170	1508	1107	1219
75	1826	2168	1500	1100	1218
76	1783	2166	1500	1095	1217
77	1781	2163	1498	1089	1212
78	1778	2154	1494	1076	1211
79	1771	2151	1487	1074	1209
80	1764	2147	1487	1074	1208
81	1764	2146	1475	1071	1187
82	1754	2144	1470	1061	1182
83	1737	2142	1469	1055	1172
84	1717	2128	1467	1043	1170
85	1699	2104	1461	1042	1166
86	1684	2104	1457	1037	1162
87	1678	2102	1453	1036	1153
88	1668	2095	1448	1035	1152
89	1664	2079	1442	1021	1143
90	1664	2077	1431	1020	1140
91	1659	2075	1431	999	1140
92	1650	2069	1423	996	1138
93	1650	2063	1410	993	1136
94	1644	2054	1400	985	1129
95	1644	2036	1380	973	1129
96	1628	2025	1378	971	1124
97	1626	2022	1377	969	1118
98	1607	2007	1372	967	1115
99	1571	1999	1367	965	1111

100	1568	1993	1362	961	1109
101	1551	1989	1360	960	1109
102	1547	1978	1349	952	1103
103	1545	1965	1340	944	1101
104	1530	1949	1330	944	1101
105	1524	1944	1328	943	1094
106	1521	1940	1325	942	1093
107	1515	1933	1324	937	1092
108	1511	1930	1324	936	1091
109	1500	1927	1311	934	1089
110	1497	1924	1292	933	1089
111	1485	1917	1284	928	1086
112	1453	1913	1283	922	1081
113	1451	1904	1283	921	1079
114	1446	1902	1280	917	1078
115	1444	1897	1275	911	1077
116	1439	1894	1259	907	1077
117	1406	1892	1256	902	1060
118	1381	1884	1254	897	1050
119	1376	1881	1254	881	1047
120	1371	1879	1254	877	1042
121	1364	1858	1250	871	1039
122	1361	1858	1237	868	1037
123	1360	1854	1236	864	1035
124	1345	1854	1232	862	1032
125	1320	1851	1231	859	1030
126	1306	1848	1220	853	1026
127	1290	1847	1219	851	1023
128	1287	1842	1214	848	1023
129	1273	1836	1214	848	1022
130	1262	1828	1210	846	1022
131	1251	1821	1193	841	1021
132	1245	1820	1191	841	1015
133	1229	1817	1191	838	1015
134	1226	1805	1190	834	1014
135	1216	1803	1189	830	1009
136	1187	1794	1188	827	1009
137	1158	1792	1183	823	1004
138	1158	1784	1174	823	1004
139	1144	1784	1172	813	998
140	1121	1781	1171	813	994
141	1094	1772	1163	812	991
142	1084	1764	1162	812	989
143	1071	1746	1156	807	988
144	1057	1745	1145	807	984
145	1045	1738	1143	806	984
146	1035	1735	1142	806	983
147	1033	1730	1131	805	983
148	1031	1729	1128	802	975
149	1027	1728	1128	799	972
150	1024	1727	1125	799	971

2

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152	998	1722	1123	796	970
153	992	1721	1122	793	969
154	986	1718	1120	792	966
155	975	1712	1117	788	965
156	970	1706	1117	788	964
157	970	1705	1113	785	963
158	967	1704	1109	785	961
159	959	1701	1097	784	961
160	944	1698	1094	783	960
161	941	1697	1089	779	958
162	940	1688	1087	776	957
163	930	1688	1087	770	954
164	929	1674	1084	765	951
165	925	1670	1083	765	951
166	924	1667	1083	764	948
167	921	1662	1081	763	941
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169	902	1649	1079	757	937
170	896	1641	1078	756	933
171	894	1640	1076	754	925
172	894	1638	1074	744	921
173	890	1632	1071	740	919
174	888	1627	1066	739	919
175	873	1614	1055	737	910
176	871	1614	1050	736	910
177	868	1610	1048	733	909
178	866	1610	1044	732	909
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181	859	1594	1041	727	903
182	857	1590	1040	726	902
183	856	1588	1038	726	900
184	854	1587	1035	723	897
185	846	1587	1033	723	896
186	844	1585	1030	722	894
187	823	1558	1025	721	889
188	816	1556	1021	719	889
189	815	1551	1016	719	889
190	809	1548	1007	717	883
191	809	1538	1001	716	881
192	809	1536	994	715	881
193	807	1528	990	713	880
194	801	1526	988	711	875
195	799	1522	984	711	868
196	783	1498	984	703	863
197	781	1493	983	702	857
198	779	1493	982	701	857
199	778	1491	982	698	856
200	777	1490	977	698	856
201	770	1487	972	695	855

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203	762	1483	967	691	851
204	757	1478	966	688	848
205	755	1477	964	683	847
206	751	1468	961	683	842
207	744	1462	956	681	842
208	743	1459	954	681	832
209	742	1458	950	680	829
210	741	1456	946	679	828
211	735	1443	945	679	828
212	728	1441	944	678	826
213	726	1440	932	677	825
214	724	1436	930	673	824
215	719	1418	926	672	823
216	716	1409	926	667	819
217	715	1407	922	663	816
218	715	1406	920	662	814
219	712	1405	911	662	813
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221	707	1387	906	661	807
222	707	1381	904	655	802
223	692	1381	896	655	802
224	689	1380	892	653	801
225	689	1373	890	651	798
226	684	1373	889	647	796
227	680	1365	881	643	792
228	680	1364	880	642	791
229	676	1353	874	642	787
230	675	1352	874	641	785
231	656	1350	873	639	785
232	655	1344	872	639	784
233	648	1340	869	639	781
234	647	1339	868	639	780
235	645	1338	855	634	

355	415	825	500	494	590
356	413	824	499	494	589
357	412	818	495	493	586
358	410	813	495	493	586
359	409	811	494	492	585
360	407	808	494	490	585
361	405	807	493	487	582
362	404	801	491	486	582
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364	403	789	486	485	575
365	401	787	483	483	574
366	401	787	481	482	572
367	398	784	481	481	572
368	396	763	480	481	571
369	395	762	473	481	566
370	395	756	469	480	563
371	394	753	468	479	562
372	394	751	467	479	558
373	394	750	465	478	556
374	393	749	465	477	554
375	393	746	463	477	554
376	392	745	459	471	552
377	391	741	458	470	550
378	391	741	457	470	549
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380	390	728	454	469	547
381	390	726	451	469	541
382	388	722	450	469	539
383	387	722	450	467	539
384	386	720	445	467	537
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387	381	711	439	466	531
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389	378	702	433	466	530
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393	372	690	425	461	525
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395	369	682	424	460	520
396	368	682	421	460	519
397	366	676	420	457	518
398	364	673	418	454	513
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401	361	662	412	451	511
402	359	660	408	451	510
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417	348	619	382	439	493
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421	346	603	379	437	486
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423	343	599	374	436	483
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427	339	591	368	436	480
428	339	590	368	435	478
429	338	581	366	434	476
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433	337	573	362	433	474
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436	334	568	359	431	469
437	334	568	358	429	468
438	333	564	357	429	466
439	332	553	355	429	461
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441	330	551	353	429	460
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8

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279	523	1126	696	579	703
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281	518	1117	693	576	699
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6

7

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673	225	273	120	284	269	724	207	245	95	263	246
674	225	273	119	283	268	725	207	245	94	263	245
675	224	271	118	283	268	726	207	245	94	263	245
676	224	271	118	281	267	727	207	244	93	262	244
677	222	271	118	281	267	728	206	243	93	261	243
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681	221	268	115	279	266	732	205	238	92	256	239
682	221	268	115	278	265	733	204	238	92	256	239
683	221	267	113	278	264	734	204	237	91	255	238
684	221	267	112	278	263	735	203	236	91	255	238
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686	220	266	111	277	262	737	203	234	89	255	236
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688	220	265	109	277	261	739	202	234	88	253	235
689	219	265	109	276	261	740	202	234	88	253	235
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692	219	261	106	274	259	743	201	231	88	253	234
693	219	261	106	274	259	744	201	231	88	253	234
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