EXPERIMENT 7

TRANSFORMATION of GENE of INTEREST INTO BACTERIA

&

SELECTION of BACTERIAL CLONES

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INTRODUCTION

Plasmid is circular double stranded DNA. It is distinguishing from the chromosomal DNA in bacterial cells. The length of plasmids vary from 100s to 1000s of base pairs which replicate in the bacteria division. The process of division provides that gene copies which are plasmids and chromosoamal DNAs are transferred to the next generation of bacterial cells. It is used in cloning, gene manipulation and transfering by the research community [1].

Regarding the importance of using plasmids in research, bacteria are used to hold certain genes by using antibiotics resistance genes [2]. Additionally, plasmids are important regarding they can be copied with the gene of interest, in bacteria replication. Moreover, as plasmid structure is circular, this eases the process of ligation and restriction. Also, It is beneficial that plasmids are able to persist for a long time period which increases the gene of interest's replication [2].

Plasmid elements include origin of replication that indicates the start of replication with the help of transcriptional proteins. Antibiotic resistance genes function in the selection of plasmid containing bacteria. Multiple cloning site functions as the restriction sites where the DNA is inserted to. Promoter region functions for obtaining target gene transcription. Selectable markers are the regions which contain the antibiotics resistance genes which will be used to select bacteria. Primer binding site is a short single stranded DNA sequence which functions as a starting point for PCR amplification [2].

There is a need of inserting DNA and plasmid inside a bacteria which can be achieved by using chemical methodologies which are chemically induced competent cell generation, and with physical methods like transformation via heart shock and electroporation for making cell permeable. Considering the fact that DNA is hydrophilic, it is needless to use chemical methods [3].

E.Coli is utilized in DNA amplification due to the fact that It maintains recombinase activity for constructing double stranded DNA fragments of various sizes. Additionally, E.Coli provides stable DNA in cloning for in vivo experimentation [4].

The aim of the experiment was to prepare a significantly large copy amount of identical targeted gene by utilizing plasmids and bacteria as a host. Also, the experiment aims the selection of bacterial clone.

MATERIALS AND METHODS

Competent cells were used for cloning. Ampicillin was used due to the antibiotic property in order to select the bacteria which have the plasmids. Agar plate was used in order to culture bacteria. DNA was used in adding as genetic material. Incubator was utilized for making the growth process of bacterial cell lines possible. Water bath was utilized for incubation. Ice bath was utilized for maintenance of living organism properties by preserving temperature low. LB liquid was used as a medium for the growth of bacteria which consisted of: tryptone for basic nutrients of bacteria, yeast extract for providing vitamins, amino acids and trace elements, NaCl for providing isotonic environment and agar for solidifying agent.

Ampicillin is utilized as a penicillin class antibacterial in situations such as infections. Ampicillin has properties called penetrating gram-positive and gram-negative bacteria. In addition, ampicillin possesses a role in the transpeptidase enzyme inhibition such that bacteria generate the cell wall.

To start the methods of the experiment, the NEB Stable Competent E. coli cells were taken from -80^{o}C temperature and they were put within ice for preserving their temperature low. The agar plate was waited at room temperature for It to get into the room temperature. 50 ul of cells were mixed gently by putting to the tube. Later, 2ul DNA containing 100 ng of plasmid DNA was placed into tube via micropipette. This mixture in the tube was put without shaking the tube and lightly flicking the tube for DNA and competent cells to blend. The tube was put into ice for 30 minutes. Following, the tube was put into the water bath of 42^{o}C for doing heat shock method. The tube was waited in water bath for 30 seconds and kept in ice bath for 5 minutes for physically opening of cell wall of bacteria with the shock affect. Then, 950 ul of LB media in room temperature was supplemented to the bacteria and the tube was put to incubator at 37^{o}C for 90 minutes. Tube is shaken horizontally by using two hands simultaneously rotating and shaking the tube at 250 rpm. Then, plates are warm selected. 50 ul of cells were spread onto the selection plate and left at incubator of 37^{o}C overnight.

RESULTS

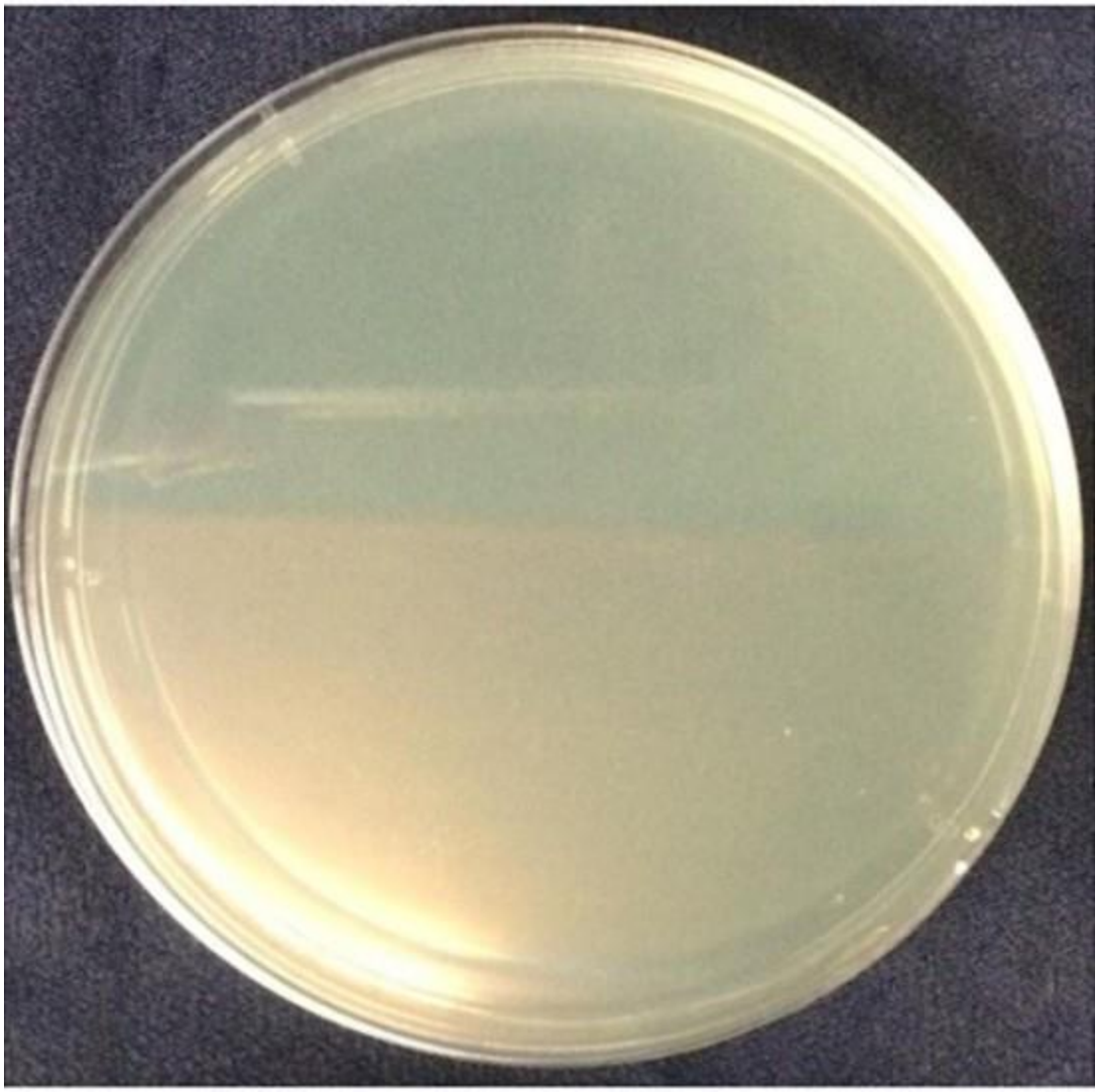

Figure 1: Depiction of Negative Control Agar plate

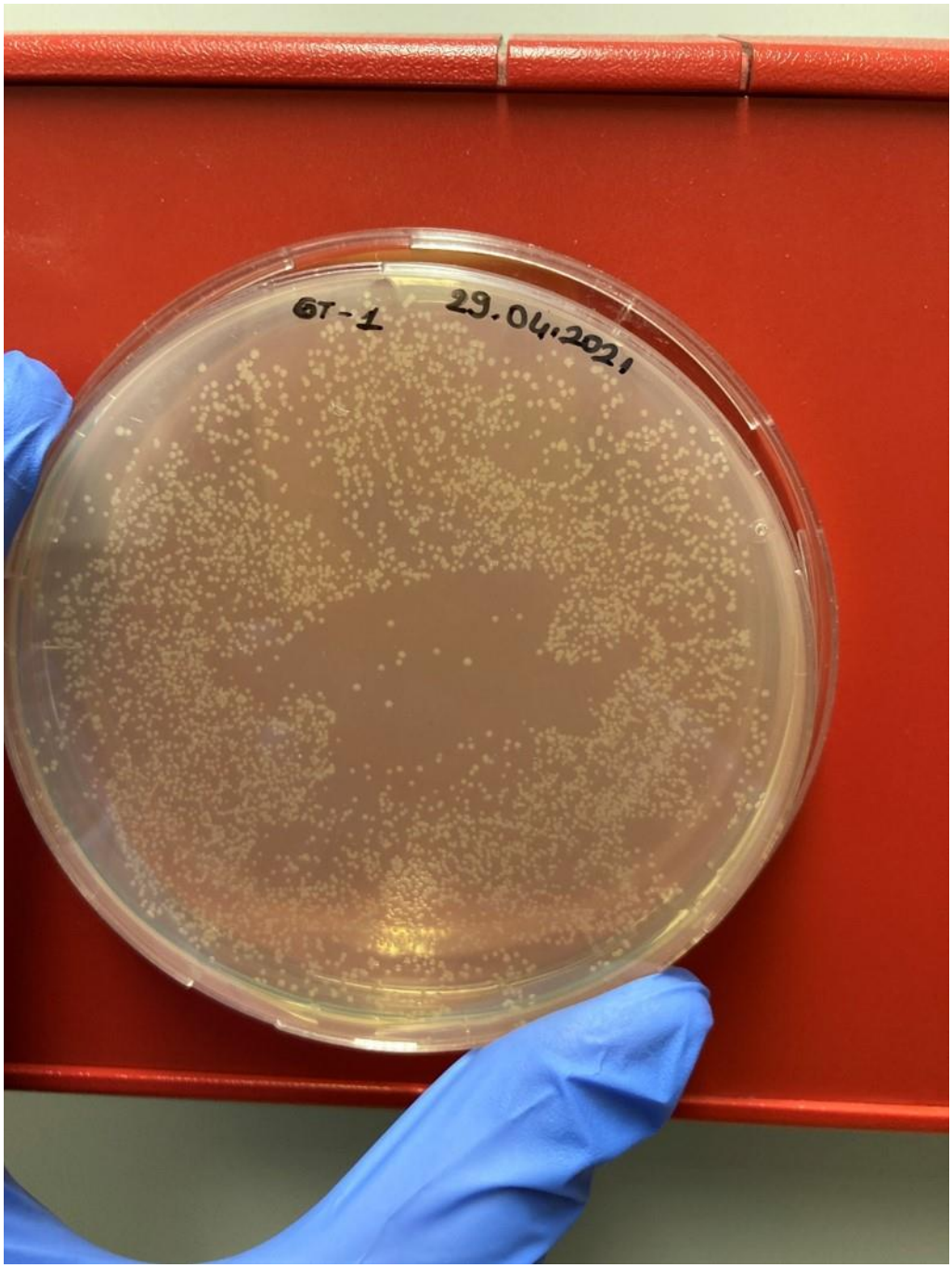

Figure 2: Depiction of GT-1's Agar plate

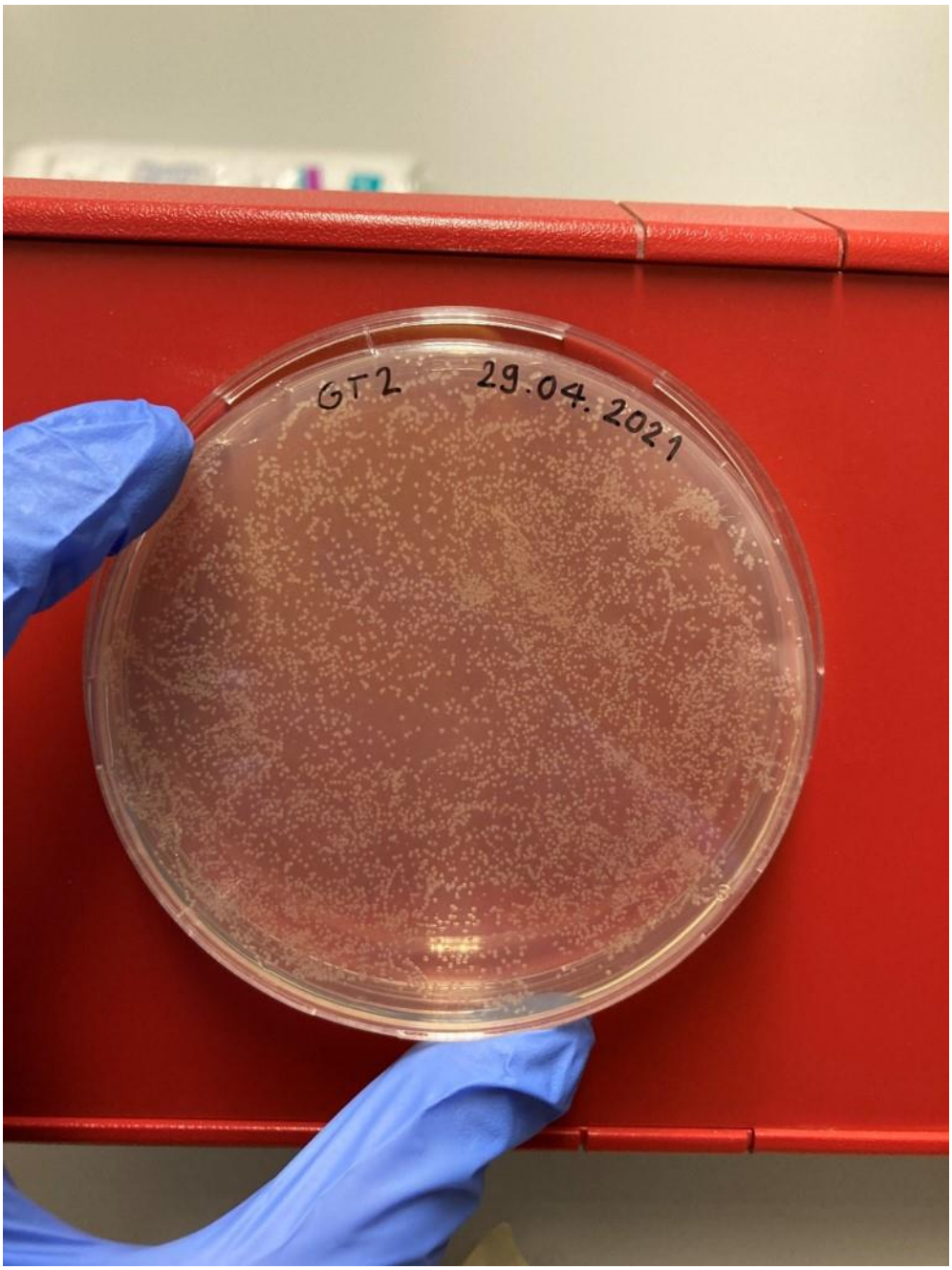

Figure 3: Depiction of GT-2's Agar plate

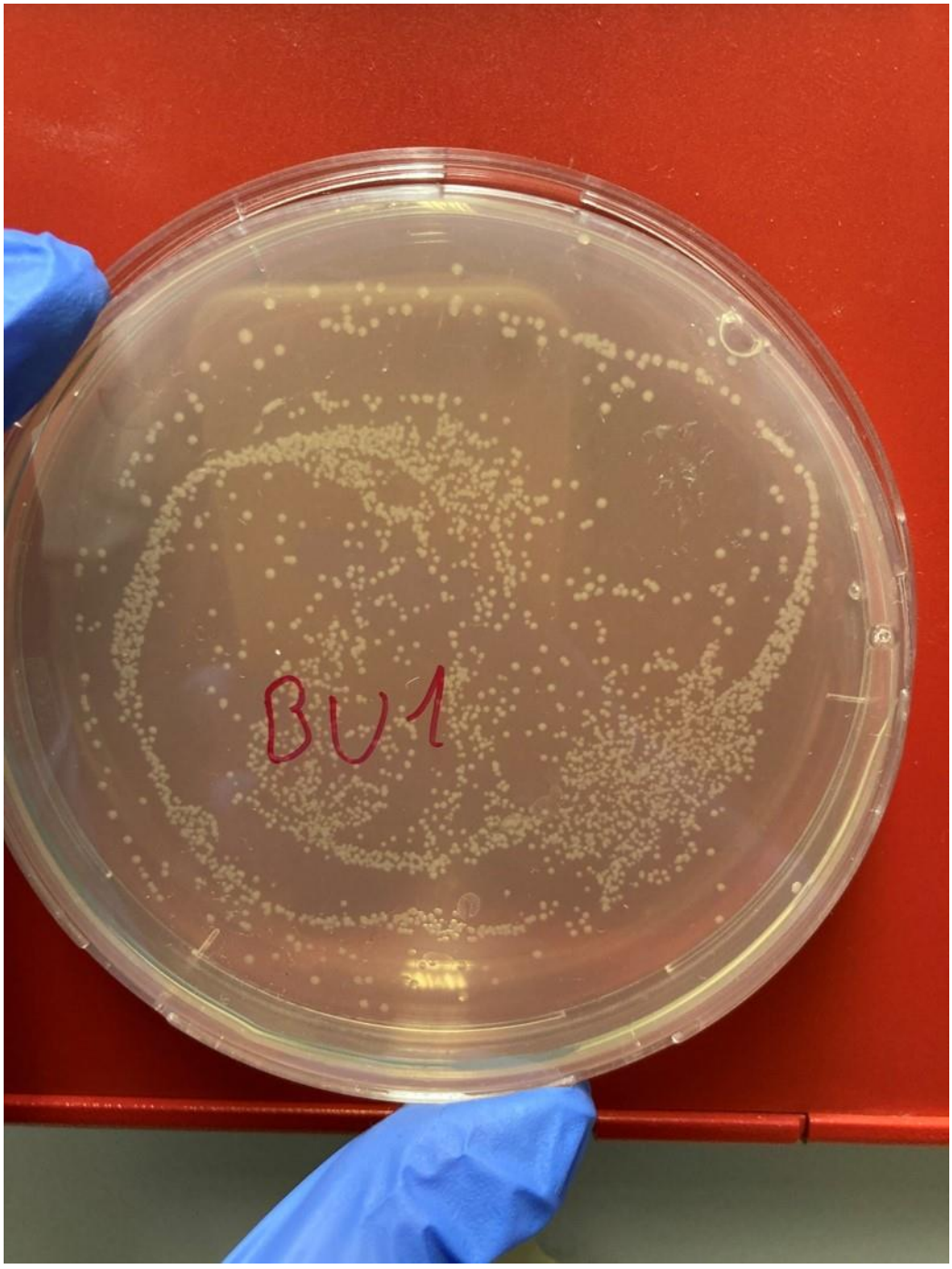

Figure 4: Depiction of BU-1's Agar plate

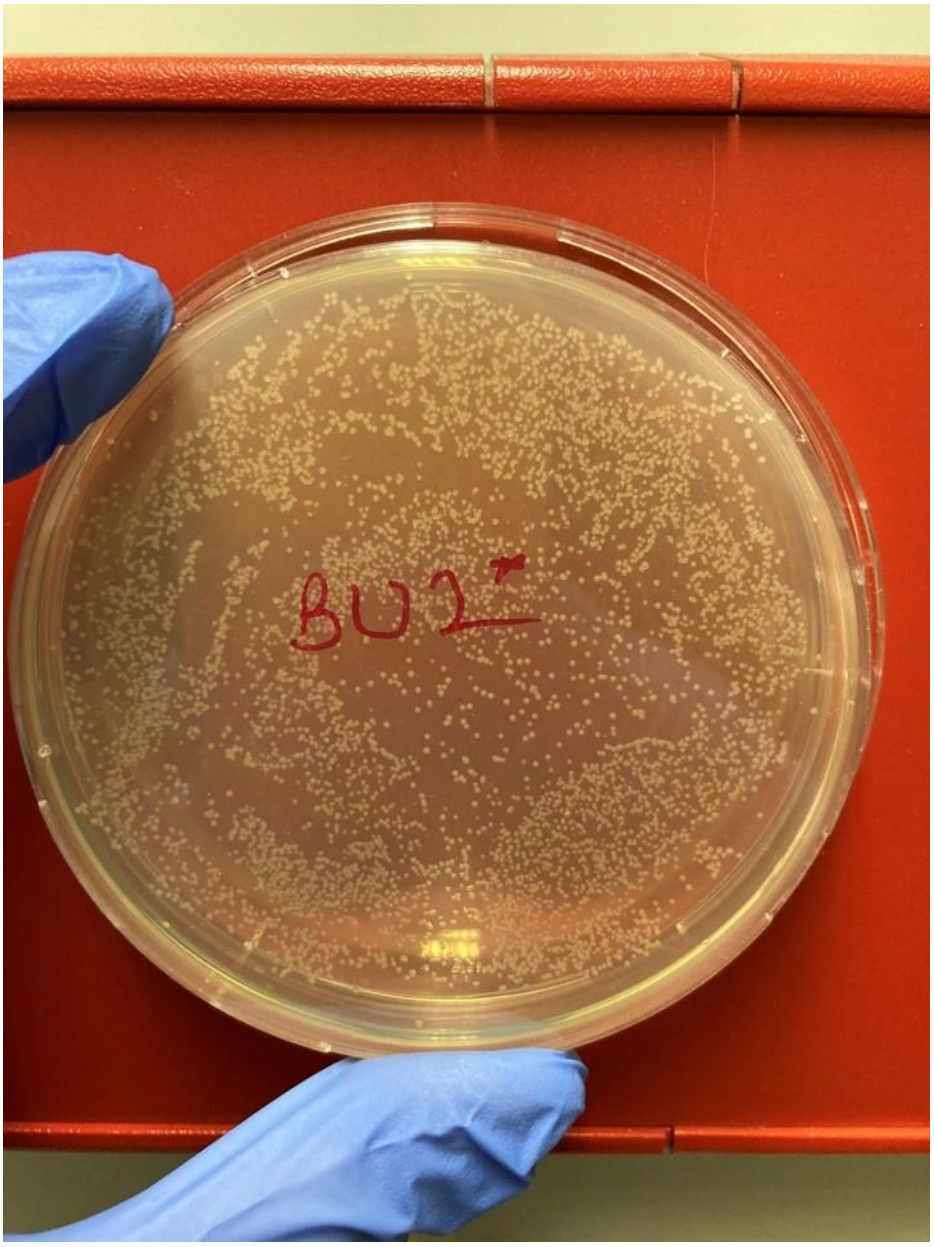

Figure 5: Depiction of BU-2's Agar plate

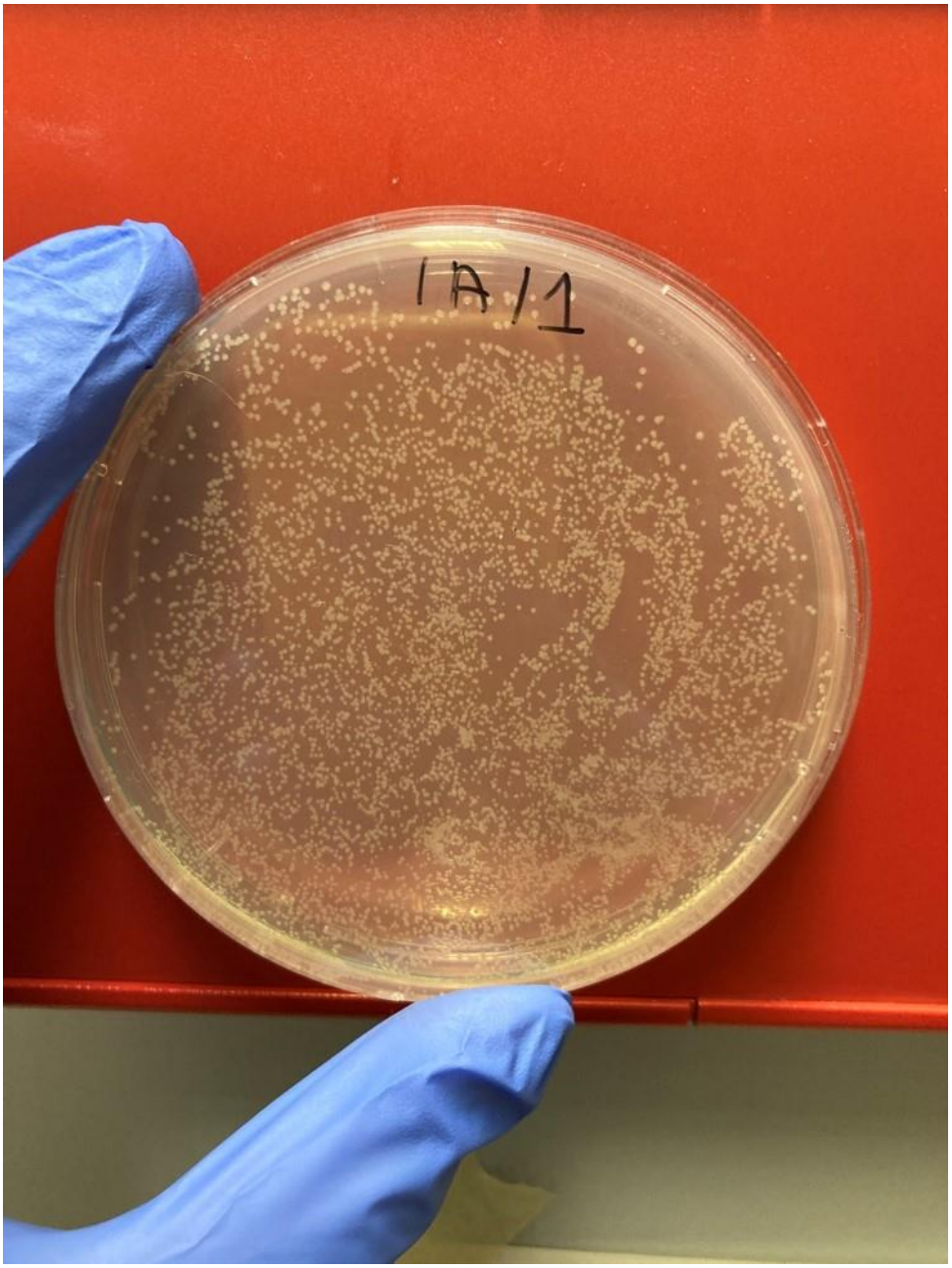

Figure 6: Depiction of IA-1's Agar plate

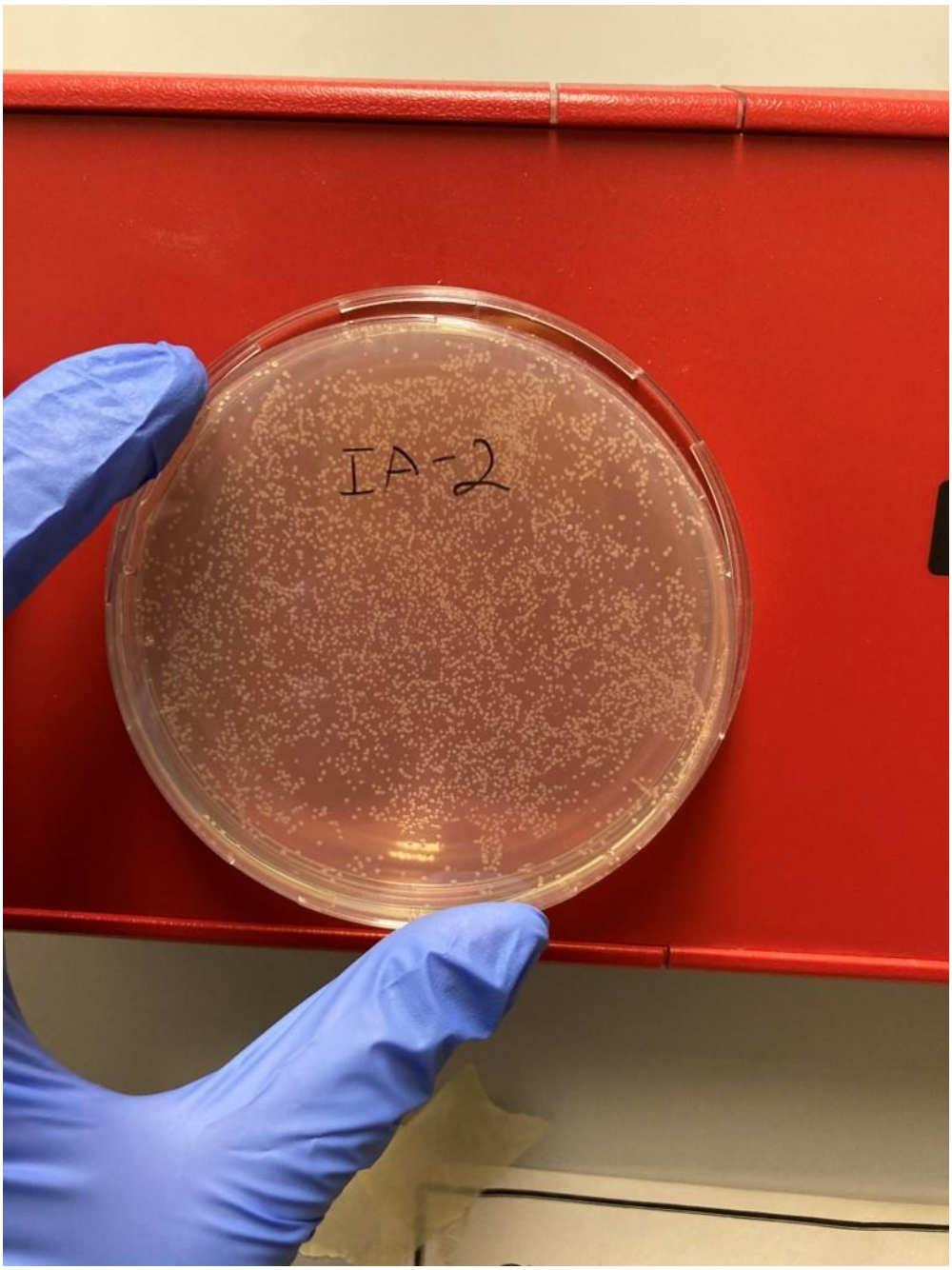

Figure 7: Depiction of IA-2's Agar plate

Figure 8: Depiction of YC-1's Agar plate

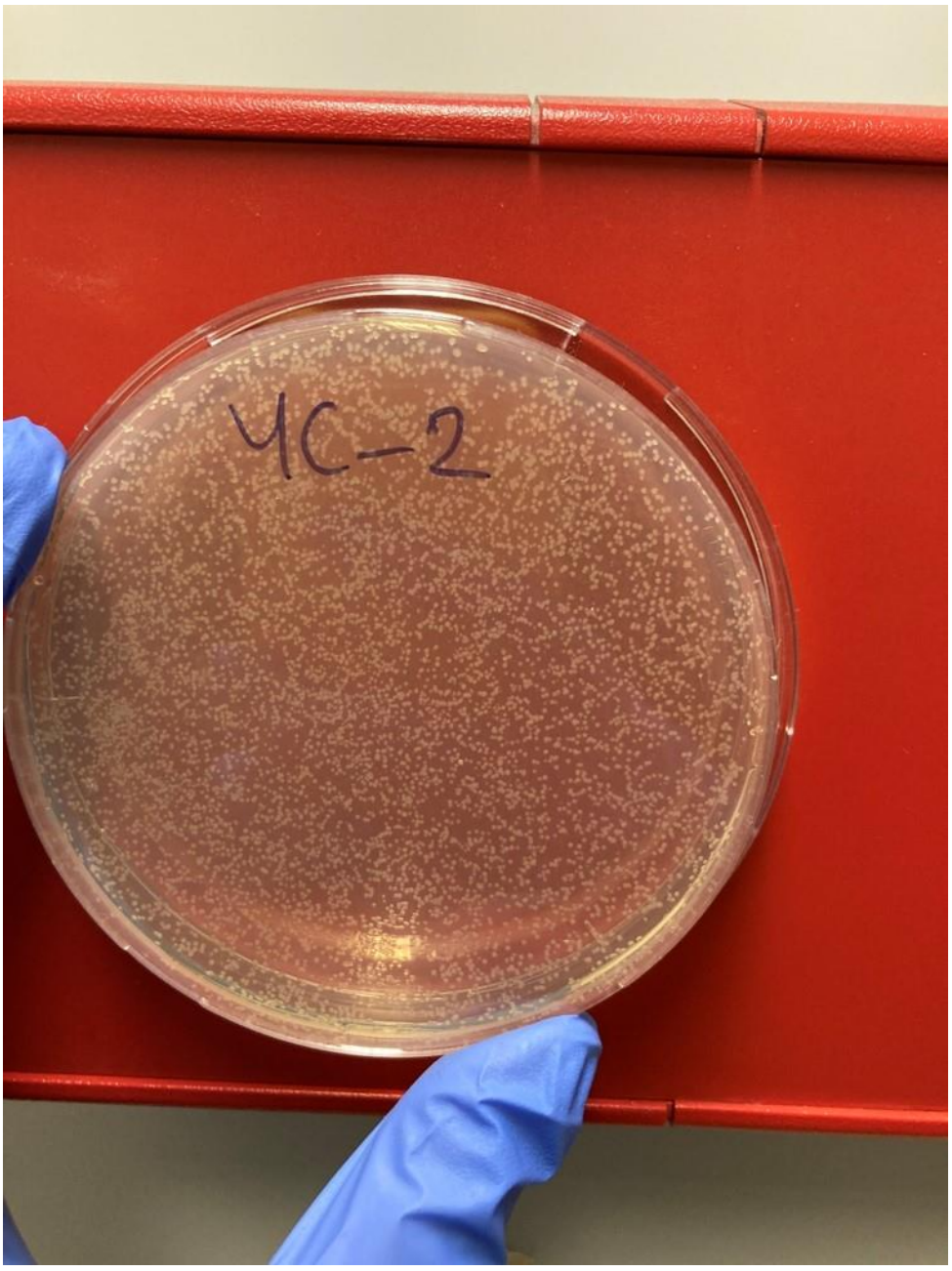

Figure 9: Depiction of YC-2's Agar plate

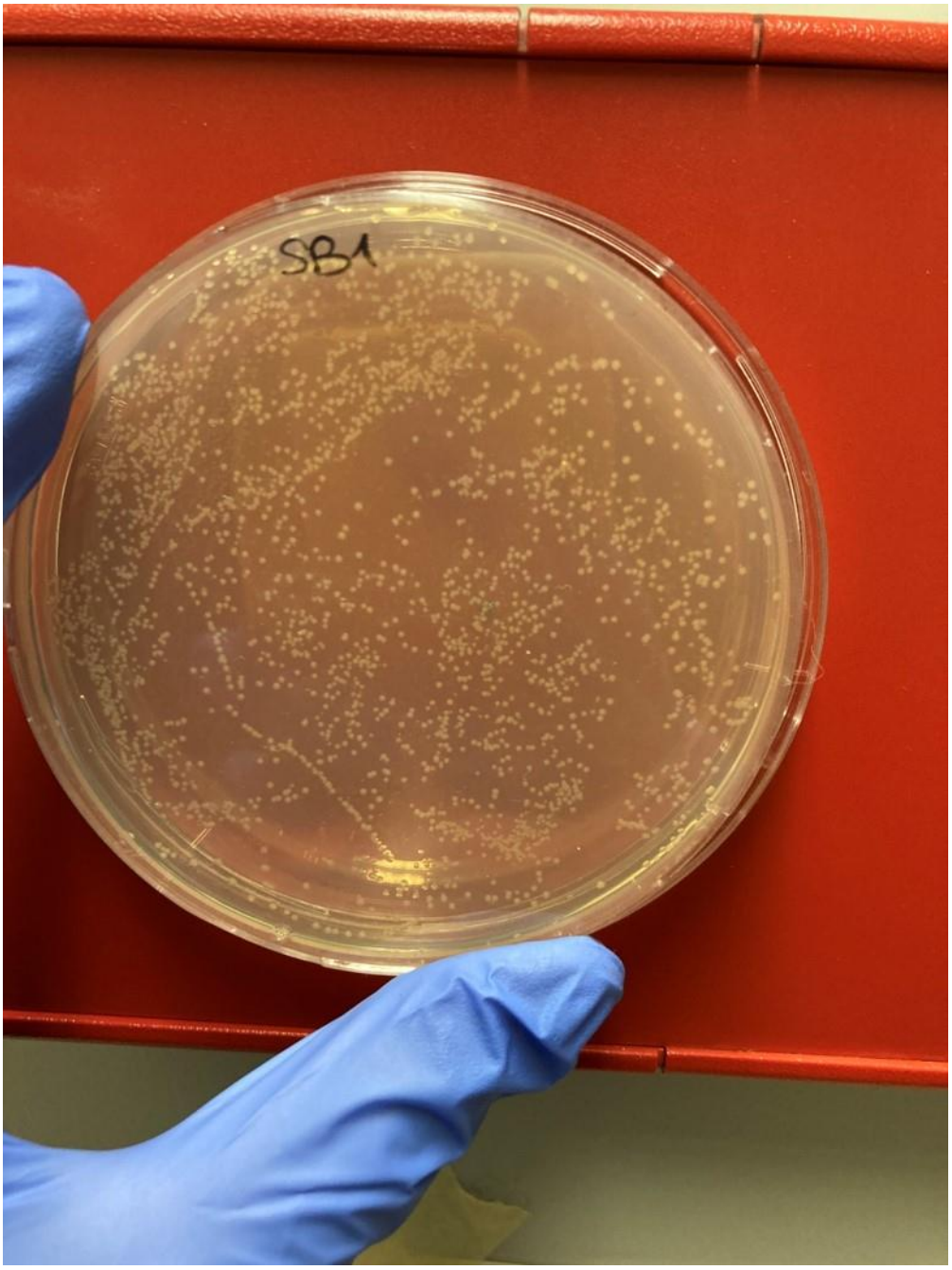

Figure 10: Depiction of SB-1's Agar plate

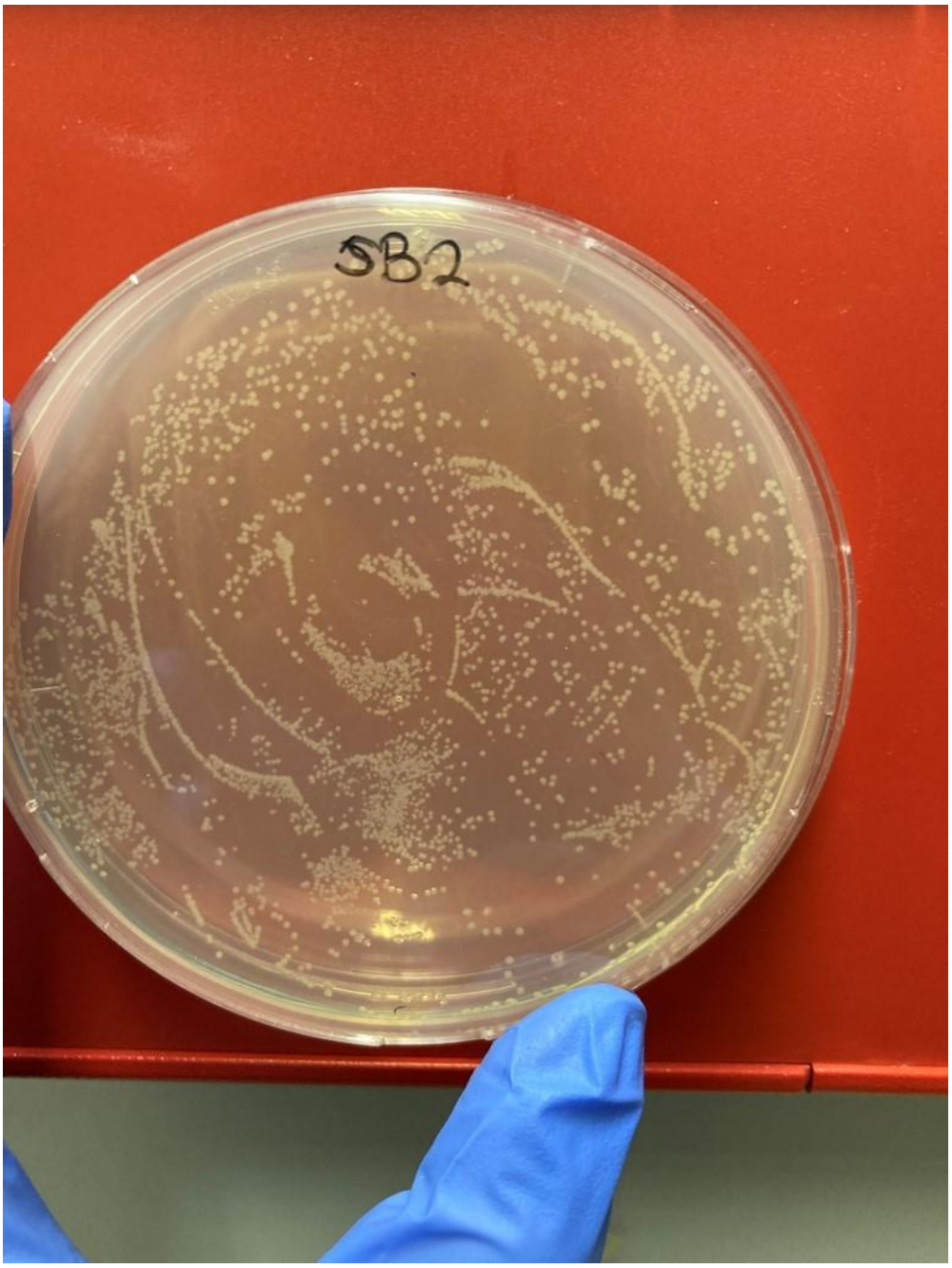

Figure 11: Depiction of SB-2's Agar plate

DISCUSSION

We can observe that each plates' colony of bacteria are successfully. However, in Figure 4 and Figure 11's agar plates, the colonies are closer to each other which will make It harder to take each colony separately when selecting the white, distant and round colonies on the plate, that are found on lower density areas mostly.

Colony densities in Figure 3 and Figure 9 are higher than the other agar plates. We see a higher density and a more equal distribution of colonies in Figure 3 and Figure 9, this can be due to human mistakes that provided contamination which led to the death of some colonies, and colony densities got lower for those agar plates. Another reason could be working far from the burner which is also another way to provide contamination and lower colony densities.

We can see that Figure 2, Figure 4 and Figure 11' plates have distinct unequal distribution of colonies than other agar plates'. This could be due to unequal spreading of cells to the plate which resulted these stripy shapes in colonies.

Also, we can see less turbidity level in Figure 8 than other agar plates. The turbidity level is increased by the bacteria, however due to the small slit on the agar plate, the turbidity level of Figure 8 might have gotten lower.

Survival of bacteria depends on whether they have ampicillin resistance genes or not. If they do, the ampicillin resistance gene will aid them in their survival against antibiotics. Hence, it can be concluded that the bacteria that contain plasmids must have that gene. We can see that no bacteria colony formed on the negative control plate in Figure 1, due to this fact. In Figure 1, bacteria without plasmid were spread.

If digested DNA fragment concentration used in the experiment were lower, this could result in formation of no colony on the plate. This could be solved by using a higher concentration of DNA fragments. If there occurred an error in the transformation of targeted plasmid this would result in a situation where ampicillin resistance gene would not be replicated and bacteria could not survive and form colonies, in which we would not be able to observe colonies on the agar plate. This could be solved by redoing the transformation as there is nothing else to be done. If the experimented far from the burner, then contamination could have occurred, in this case the bacteria density would get lower and we would observe less colonies in such a case. This could be solved by re-doing separating cells to another plate.

REFERENCES

[1] “Plasmid,” *Genome.gov*. [Online]. Available: https://www.genome.gov/genetics-glossary/Plasmid. [Accessed: 06-May-2021].

[2] “Plasmids,” *Plasmids - an overview | ScienceDirect Topics*. [Online]. Available: https://www.sciencedirect.com/topics/neuroscience/plasmids. [Accessed: 06-May-2021].

[3] M. Rahimzadeh, M. Sadeghizadeh, F. Najafi, S. Arab, and H. Mobasheri, “Impact of heat shock step on bacterial transformation efficiency,” *Molecular biology research communications*, Dec-2016. [Online]. Available: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5326489/. [Accessed: 06-May-2021].

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