1. Count the number of colonies on each of the plates (plates 1-12).

(table4.G8)

Plate 1: many Plate 2: 1 Plate 3: 3 Plate 4: 5 Plate 5: 550 Plate 6: 56

Plate 7: 44 Plate 8: 91 Plate 9: 6 Plate 10: 6 Plate 11: 21 Plate 12: 6

2. Using the count from Plates 5, and 6, estimate the number of cells plated on each of the plates.

Using this figure, you should be able to estimate the spontaneous mutation frequency. How do you calculate the spontaneous mutation frequency?

What is your estimate of the spontaneous mutation frequency?

We chose plates with 30-300 colonies, as this range is considered statistically significant. Therefore, we selected plate 6 to estimate the number of cells plated on each plate. Plate 6 had a dilution factor of 10^6, with 0.1 ml of undiluted WP2 cell suspension used for each plate.So, the number of cells plated on each of the plates = 5.6×10^7

First, determine the number of spontaneous mutation colonies.then, determine the number of viable cells on each of the plates.In the end, calculate the Spontaneous mutation frequency using the equation below:

Spontaneous mutation frequency: $\frac{colonies\ on\ the\ plate\ 2}{The\ number\ of\ viable\ cells\ on\ the\ plates}$

Spontaneous mutation frequency = $1 / 5.6 \times 10^7 = 1.79 \times 10^{-8}$

3. **Similarly, estimate the apparent spontaneous mutation frequency on the SA2 and SA1 plates (Plates 3 and 4). Why are these only apparent frequencies, and why do they differ from the true frequency estimated from SA3, and from each other?**

Apparent spontaneous mutation frequency of plate 3

$\frac{colonies\ on\ the\ plate\ 3}{The\ number\ of\ cells\ plated\ on\ the\ plates} = 3/5.6*10^7 = 5.36*10^{-8}$

Apparent spontaneous mutation frequency of plate 4

$\frac{colonies\ on\ the\ plate\ 4}{The\ number\ of\ cells\ plated\ on\ the\ plates} = 5/5.6*10^7 = 8.93*10^{-8}$

In plate 1, both plates were supplemented with tryptophan, allowing the WP2 trp^- strain to multiply and divide after plating. During this propagation, spontaneous mutations occurred, leading to the formation of WP2 trp^+ revertants. Therefore, the observed colonies in the medium were a mixture of original WP2 trp^+ revertants in the cell suspension and

newly generated WP2 trp^+ revertants after culture.

In contrast, no tryptophan was added to plate 2. Consequently, the WP2 trp^- strain in the cell suspension could not reproduce after plating. Thus, the colonies observed in plate 2 consisted solely of original WP2 trp^+ revertants, which had undergone spontaneous mutation before culture plating. When calculating the spontaneous frequency, it was assumed that the total number of cells in each medium was the same. However, due to the addition of tryptophan, the quantity of WP2 trp^+ revertants in plates 3 and 4 was higher than in plate 2, leading to a different apparent frequency in plates 3 and 4 compared to plate 2.

The discrepancy in apparent frequency between plates 3 and 4 was attributable to the varying amounts of tryptophan added to each plate, resulting in the production of different quantities of WP2 trp^+ revertants during the propagation process of the WP2 trp^- strain. This discrepancy affected the apparent frequency observed in the two plates.

4. Estimate the mutation frequency induced by MMS. You should compare the mutation frequency with one of the spontaneous frequencies you calculated. From which plate should you determine the spontaneous frequency?

Mutation frequency induced by MMS

$$\frac{colonies\ on\ the\ treated\ plate - colonies\ on\ the\ control\ plate}{The\ number\ of\ viable\ cells\ on\ the\ plates}$$

Plate 7: $\frac{colonies\ on\ the\ plate\ 7 - colonies\ on\ the\ plate\ 9}{The\ number\ of\ viable\ cells\ on\ the\ plates} = (44\text{-}6)\ /5.6*10^{7} = 6.79*10^{-7}$

Plate 8: $\frac{colonies\ on\ the\ plate\ 8 - colonies\ on\ the\ plate\ 9}{The\ number\ of\ viable\ cells\ on\ the\ plates} = (91\text{-}6)\ /5.6*10^{7} = 1.52*10^{-6}$

Plate 10: $\frac{colonies\ on\ the\ plate\ 10 - colonies\ on\ the\ plate\ 4}{The\ number\ of\ viable\ cells\ on\ the\ plates} = (6\text{-}5)\ /5.6*10^{7} = 1.79*10^{-8}$

The mutagenicity of plates 7 and 8 should be compared to the spontaneous mutation frequency in plate 9. A limited amount of tryptophan in these plates allowed only a subset of cells to divide and produce WP2 trp+ revertants. The experimental conditions were controlled, ensuring that all three plates underwent the same experimental method (spot test) and received the same amount of tryptophan. In plates 9, 7, and 8, WP2 trp+ revertants were produced spontaneously, with the existing revertants assumed to be the same. Hence, the mutation frequency induced by MMS should be compared with that of plate 9.Plate 10 should be compared to plate 4, as they share the same tryptophan

concentration and plating method.

5. Estimate the mutation frequency induced by the UV dose of 10 J/m^2. Compare this frequency to that induced by MMS. Which treatment shows the highest induced mutation frequency?

Is it fair to directly compare the two frequencies?

Mutation frequency induced by UV

$$\frac{colonies\ on\ the\ treated\ plate - colonies\ on\ the\ control\ plate}{The\ number\ of\ viable\ cells\ on\ the\ plates}$$

Plate 11: $\frac{colonies\ on\ the\ plate\ 11 - colonies\ on\ the\ plate\ 3}{The\ number\ of\ viable\ cells\ on\ the\ plates}$ = (313-3)/5.6*10^7 = 5.54*10^{-6}

Plate 12: $\frac{colonies\ on\ the\ plate\ 12 - colonies\ on\ the\ plate\ 3}{The\ number\ of\ viable\ cells\ on\ the\ plates}$ = (7-3)/5.6*10^7 = 7.14*10^{-8}

20 seconds of UV treatment with a dose of 10 J/m^2 shows the highest induced mutation frequency

No, it wouldn't be appropriate to directly compare the mutation frequencies between the two treatments. MMS and UV light are distinct in their mechanisms of action—one is chemical, and the other is based on light exposure. While it might be tempting to compare the highest frequency observed in each treatment, it's important to note that the 40-second UV exposure resulted in fewer colonies than plates 7 and 8, despite potentially having a higher mutation frequency. Additionally, plate 10's results are challenging to compare, as it represents a different test setup. Overall, finding a suitable criterion for direct comparison may be challenging in this context.

6. Record the appearance of the MMS spot plates.

Do you think it is valid to count the colonies on this dish? Provide explanations for your answer.

What can you say about the relationship between MMS concentration and the number of mutants?

Clear zones formed around the disc where MMS was dripped inhibited bacterial growth, with no E. coli colonies present within these zones. Most WP2 trp+ revertants grew

scattered around the clear areas, while a few were distributed towards the plate margin. The number of WP2 trp+ revertants in the plate supplemented with 1% MMS was fewer than that in the plate with 2% MMS. In comparison, the water control group showed no clear zones, with only several colonies appearing in a random distribution.

However, this method may be invalid. The observed number of colonies on the plate may not accurately reflect the actual number of colonies obtained through MMS-induced mutation under normal cell conditions. Due to its cytotoxic nature, MMS can kill some E. coli revertants, leading to the formation of clear zones around the disc. While MMS can increase mutation frequency, the partial death of E. coli may decrease the number of spontaneous mutations. Additionally, the location and sizes of colonies are important considerations. As the MMS concentration varies from the center to the margin of the plate, the colony sizes may reflect their viabilities. These factors should be taken into account when studying MMS mutagenesis. Therefore, counting colonies on this plate may not be a valid method.

Furthermore, there appears to be a positive correlation between MMS concentration and the number of mutants, particularly between 1% and 2% MMS concentration (with a linear correlation observed in their log-scale values with a slope of 1.65). However, outside this concentration range, the relationship is uncertain.

7. You are provided with compound Z which is suspected to cause cancer in humans. You test the compound using the WP2 assay (both the spot test and liquid culture test) and find it to be negative (no increase in revertants compared to the control). Another laboratory finds Z induces cancer in rodents.

Can you be sure whether compound Z is a mutagen or non-mutagen?

How might you redesign your experiment to determine whether Z is mutagenic or not?

The WP2 assay is a common method used to assess the mutagenicity of chemicals. However, its limitation lies in its inability to determine whether a single chemical is a mutagen. A pro-mutagen is a chemical that, on its own, cannot induce mutation. Still, with the presence of metabolic activity to activate the pro-mutagen, it can be converted into a mutagen, potentially leading to cancer.

Compound Z is a candidate for being a mutagen. Despite not showing mutagenic effects in the WP2 assay, it has been found to cause cancer in rodents. This discrepancy could be due to metabolic activity.

However, definitive conclusions about compound Z as a pro-mutagen are challenging due to other potential factors affecting the results. To test whether compound Z is mutagenic,

three sets of experimental samples are required. The first set of cells would serve as a control and remain untreated. The second set would be treated with compound Z, incubated, and then observed for normal cell growth. The third set would be treated with compound Z and rat liver extract containing metabolic enzymes.

If compound Z is a mutagen (pro-mutagen), cells in the first and second groups would grow normally, while the third group would exhibit malignant transformation, indicating that compound Z was converted into a mutagen by the rodent's metabolic enzymes, leading to cancer. If all three groups of cells grow normally, compound Z is not a mutagen.

8. Suggest 3 ways in which the sensitivity of the WP2 assay might be increased.

To enhance the sensitivity of the WP2 assay, several strategies can be employed. Firstly, using more sensitive strains such as WP2 uvrA or strains with plasmid pKM101 can improve sensitivity to mutagens like MMS. Additionally, altering cell permeability can make cells more predisposed to mutagens, thus increasing assay sensitivity.

Furthermore, to avoid the adverse effects of high MMS doses, it's crucial to determine appropriate concentration ranges in advance. This can be achieved by setting up more detailed concentration steps for the chemical reagents used in the experiment. Similarly, dividing UV irradiation time into smaller intervals can help identify the most appropriate range of irradiation times.

Maintaining a sterile environment and ensuring proper disinfection of laboratory equipment are essential to prevent contamination and maintain the sensitivity of the assay. Additionally, experiments should be repeated to ensure the reliability of results. Conclusions should be based on the results of at least two independent experiments to improve the sensitivity of the assay.

Lastly, utilizing more suitable software and instruments for result analysis can enhance the sensitivity of the assay. While the spot test may be less sensitive than the treat-and-plate test, choosing the latter method can improve sensitivity if higher sensitivity is required.