**Lab: Serial dilution, mutation rates, and linear regression**

**Learning objectives:**

1. Be able to use serial dilution to determine cell density.
2. Be able to calculate CFUs
3. Understand the concept of linear regression analysis (R2 and p-value).

**Background**

For this exercise, you will use the following yeast strain from the previous lab.

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| --- | --- |
| Strain | Genotype |
| DBY1394 | Haploid, his4-539(am) ade2 |

In the genotypes, genes in upper case are wildtype ones, and those in low-case are mutant ones. The *ade2* mutant can display red pigment on YPD plates.

**Day 1:**

1. Prepare 2ml YPD in 10 ml white falcon tubes (Use 1ml pipette)
2. Pick single red colony of DBY1394 using a 200ul tip with pipette.
3. Add the cells to the 2ml YPD liquid, mix well by pipetting the cells up and down.
4. Label the name of strain, your initials, and date on the tube.
5. Leave the tubes at 30C shaker.

**Day 2:**

1. Measure the OD values of your sample. (The instructor will pool the samples first).
2. Do 10-fold serial dilution of the yeast culture
   1. Make 6 eppendorf tubes of 0.9ml water, label as ‘10x’, ‘100x’, ‘1000x’, ‘10^4x’, ‘10^5x’, and ‘10^6x’.
   2. Add 0.9 ml water (or YPD) to each tube.
   3. Transfer 0.1ml of culture from the original tube to the next dilution, mix well, and then transfer to the next dilution.
3. Label the names of strain, your initial and data on edge of 6 YPD plates.
4. Add ~4 glass beads to each plate.
5. Add 100ul of each yeast dilution to the plate.
6. Shake the plate horizontally in several directions to evenly spread the cells on plate.
7. Pour the glass beads back to the collection flask.
8. Seal the plate with parafilm, leave it upside-down in 30C incubator for 2 days.

**Day 3:**

1. Count the number of red and white colonies in each plate.
2. Input your results to the GoogleDoc class spreadsheet.
3. The instructor will show you how to use linear regression to analyze the entire results. A software call ‘R’ will be used. ***You are encouraged to bring your laptop to the class***.

**Report (Your report should be based on the entire class, written in WORD and submitted to WebCT).**

1. Calculate the mean and standard deviation of the mutation rates.
2. Calculate the CFU per ml for all the samples.
3. Plot OD ~ CFU concentration, apply linear regression, and discuss the results.
4. Plot mutation rate ~ OD, apply linear regression, and discuss the results.

**Materials**

* Yeast cultures at various OD values
* A set of pipetman (1000ul, 200ul, 20ul)
* Tips (1000ul, 200ul, 20ul)
* One vortexer
* One rack for eppendorf tubes
* Six 1.5ml eppendorf tubes
* Four YPD plates
* One maker pen
* Bunsen burners
* Sterile Glass beads
* Beads recollection flasks and funnels
* Sterile distilled H2O
* 70% ethanol bottle
* Water bottle
* One hemocytometer
* Cover slips
* One microscope
* One hand tally counter
* One spectrometer for the entire class (Different spectrometers may give different readings.)