**Lab: Serial dilution, cell count, and linear regression**

**Learning objectives:**

1. Be able to use serial dilution to determine cell density.
2. Be able to generate and use standard curve.
3. Be able to use hemocytometer to determine cell density.
4. Be able to calculate CFUs
5. 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 | Description |
| SGU57 | A yeast wild isolate, diploid. |

**Preparation by the instructor:**

1. Prepare 20ml YPD in 100 ml flasks
2. Pick single red colony of SGU57 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.
6. Next day, make sure cultures are in different stages of growth.

**Day 1 for students:**

1. Measure the OD values of the yeast cultures.
2. Do 10-fold serial dilution of the yeast culture in 6 eppendorf tubes
   1. Prepare 6 tubes , 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 date on edge of 5 YPD plates, excluding the most concentrated ones.
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.
9. Use hemocytometer to estimate the cell density of ‘10x’, ‘100x’, ‘1000x’ diluted cultures. A tally counter should be used.

**Day 2 for students:**

1. Count the number colonies in each plate.
2. Input your results to the GoogleDoc class spreadsheet.
3. Use linear regression to analyze the entire results. ***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 SpelELearn site).**

1. Calculate the CFU per ul, cell densities for all the groups.
2. Estimate the standard deviation for cell number per OD unit, CFU per OD unit.
3. Plot CFU ~ OD concentration, apply linear regression, and discuss the results.
4. Plot CFU ~ cell densities estimated by hemocytometer, apply linear regression, and discuss the results.

**Materials**

* Yeast cultures at various OD values
* A set of pipetman (1000ul, 200ul)
* Tips (1000ul, 200ul)
* 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.)