Adaptive evolution lab protocol

Materials needed:
mCherry expressing HB101 strain on an Amp plate
white HB101 strain on am Amp plate
sterile microfuge tubes and 15ml conical tubes
LB amp plates and LB broth

**Set up done by the teacher**

1. Grow up two overnight cultures of mCherry and white E. coli strains.
	1. Use a sterile loop to pick one colony into a conical tube containing 3 ml LB Amp.
	2. Incubate overnight at 370C. If a shaking waterbath or equivalent piece of equipment is available, shake the culture tubes as they grow, otherwise incubate without shaking.
	3. The next day, the bacterial concentrations should be about 10^8 cells per ml.
2. Start the natural selection time course.
	1. T = 0. We want a culture that is ~104 cfu/ml and contains 97% cherry and 3% white. In order to get this ratio and concentration, we need to add about 100 times more cherry than white to a flask with 100 ml LB amp.
	2. Vortex or rack the overnight culture then dilute cherry 1 to 10 by adding 100 microliters of cherry overnight into a microfuge tube containing 900 microliters of LB. Then add 100 microliters of this dilution to the 100 ml competition flask.
	3. Vortex or rack the overnight culture then dilute the white overnight 1 to 100 by adding 10 microliters of white overnight into a microfuge tube containing 990 microliters of LB. Add only 10 microliters of this white dilution into the competition flask.
	4. Shake competition flask then immediately remove 30 ml of culture and chill at 40C in a flask labeled “T0”. This will be the T0 culture for students to use.
	5. T = 8 hr. Remove 30 ml and put at 40C in a flask labeled “T8”. This will be the T8 culture for students to use.
	6. T = 24 hr. Remove 30 ml and put at 40C in a flask labeled “T24”. This will be the T24 culture for students to use.

**Experiments for the students**

1. Each student team should collect 250 ul of T0, T8 and T24 samples and 9 LB Amp plates.
2. Serially dilute the samples to find the concentration of bacteria and the ratio of cherry to white bacteria.
	1. The students should make 1/10, 1/100, and 1/1000 fold dilutions for each time point as follows. Take 3 microfuge tubes and 3 LB Amp plates and label them “1/10” “1/100” and “1/1000” respectively. Add 900 microliters of LB into each tube.
	2. Vortex the T0 sample then add 100 micro liters of the collected sample into the “1/10” tube. Vortex the tube or rack it to mix up the bacteria. Pipette 100 microliters of the “1/10” dilution onto the “1/10” plate and spread using a sterile spreader or inoculation loop. Repeat by adding 100 microliters of the “1/10” tube to the “1/100” tube and spreading it onto the “1/100” plate. Repeat again for the “1/1000” tube and plate.
	3. Repeat for T8 and T24 samples.
3. Let dilution plates incubate 24 hrs at 37 degrees, then another 24 hours at 4 degrees.
4. Select the proper plate to count. The highest accuracy can be attained from a plate with anywhere )plate is the most reasonable and then count the colonies.
5. Calculate the concentration of each strain at each of the time points. The number of colony forming units per milliliter in the competition tube can be calculated as follows:

**# of colonies on the plate\_\_\_\_\_** \* **1/dilution written on the plate\_\_\_\_\_\_** \* **10 = CFU’s/ml \_\_\_\_\_\_**

Ex: 174 colonies on the “1/100” plate would give 174,000 CFUs/ml in the starting concentration.

**174** X (**1/“1/100” = 100)** X **10** = **174** X **1000**

1. Calculate the doubling time of each strain of bacteria by comparing the T0, T8 and T24 cultures as follows:

The doubling time between T0 and T8 is: T8 CFUs/ml = T0 CFUs/ml \* 2^X where “X” is the number of doublings in 8 hours.

Solving for X shows that X = log(T8/T0)/log(2). The doubling time in hours is therefore 24/X.

Ex: The white bacteria increased from 1,400 CFUs/ml to 198,000 CFUs/ml in 8 hours

X = log(198,000/1,400)/log(2) = 7.14 doublings in 8 hours, for a doubling time of 1.12 hours or 67n minutes.

1. Calculate the same doubling time for T8 to T24 and T0 to T24. Average the three numbers to obtain your final doubling time.

**Data Table:**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Time point** | **Strain** | **# of Colonies** | **Plate dilution** | **CFUs/ml** |
| **T0** | **Cherry** | **X** | **X 10** | **=** |
| **T0** | **White** | **X** | **X 10** | **=** |
| **T8** | **Cherry** | **X** | **X 10** | **=** |
| **T8** | **White** | **X** | **X 10** | **=** |
| **T24** | **Cherry** | **X** | **X 10** | **=** |
| **T24** | **White** | **X** | **X 10** | **=** |

The expected doubling time of white is 70 minutes.

The expected doubling time of mCherry is 110 minutes. The frequency of white/total should increase from 3% to 45% over 24 hours.