**Scramble: Inducing the Scramble system**

Your instructor has inoculated a liquid culture of your yeast strain containing the Cre plasmid (pRS413-CreEBD). The plasmid contains the Cre gene (remember that Cre induces the Scramble system) under the control of the estradiol promoter. Adding estradiol to the cells will induce the expression of Cre and therefore the Scramble system.

1. Measure the optical density of the yeast culture, which gives you an estimate of the cell density.
   1. Transfer 1 ml of media to a cuvette. Place in the spectrophotometer and blank the instrument (this only needs to be done once for the class).
   2. Transfer 1 ml of your yeast culture to a cuvette. Place in spectrophotometer and record the optical density.
2. Use the formula C1V1=C2V2 to determine the volume of cells equal to an OD of 0.1

(Concentration (OD) as measured on spec)(Volume to be determined) = (0.1) (25 ml)

Volume to be determined = \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ ml

1. Into a sterile flask, add:
   1. The volume of cells that you calculated above
   2. 25 ml of liquid media
   3. 5 ul of beta-estradiol (1 uM final concentration).
2. Incubate the culture with shaking for 2-2.5 hours. (The cells are Scrambling now!)
3. Transfer 100 ul of the cell culture to a microcentrifuge tube.
4. Spin down the cells for 1 min at full speed.
5. Remove the supernatant (liquid).
6. Resuspend the culture in 1 ml sterile water by pipetting up and down.
7. Repeat steps 6-8 two more times, ending with the cells resuspended in 1 ml sterile water.
8. Onto your two plates, add 150 ul water. Into this drop, add 50 ul of the cells. Spread the cells on the plates with glass beads.
9. Discard beads and when the liquid has absorbed into the plates, incubate the plates upside down at 30C for 2 days.