

**I. DEREPRESSING AND HARVESTING CELLS**

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[http://biology.clc.uc.edu/fankhauser/Labs/Genetics/Lac\\_Operon\\_Derepression/Lac\\_Operon\\_Induction.htm](http://biology.clc.uc.edu/fankhauser/Labs/Genetics/Lac_Operon_Derepression/Lac_Operon_Induction.htm)

This is an experiment to demonstrate that the lactose operon in *E. coli* is repressed when grown on glucose, but can be induced to produce lactose metabolizing enzymes when grown on lactose. The experiment will consist of three steps:

- 1) **Groups of 4 students** will transfer *E. coli* cells which were grown overnight in 0.1 %glucose medium to a 1% lactose medium. Each group will withdraw aliquots of cells every 20 minutes.
- 2) **The group of 4** will read the  $A_{660}$  of the sample, add toluene, shake and place on ice.
- 3) **Teams of 2 students wwill perform the assay** to determine the specific activity of  $\beta$  galactosidase in the toluenized samples (see the next page):

**EQUIPMENT FOR CLASS:**

37°C shaking incubator  
clinical table top centrifuge, with angle head  
5x spectrophotometers with 2 cuvettes in rack  
4x sterile plugged 250 mL flask  
10x 16 x 150 mm test tubes  
200x 13x100 mm test tubes (35 for 4 students)

**SUPPLIES PER CLASS:**

60 mL *E coli* B, grown ON in CSHA + 0.1%glu  
200 mL sterile CSHA medium + 1.0 % lactose  
5 mL micropipettor and sterile tips  
toluene  
parafilm

**Per group of four:** Label five tubes 13 x 100 mm culture aliquot tubes for time of aliquot:

T-0, T-20, T-40, T-60 and T-80. (# = minutes grown in lactose)

1. Grow *E. coli* B over night with shaking in 50 mL CSHA minimal medium + 0.1%.
2. **Spin down cells** in a 16 x 150 mm tubes, 10 mL each, in a clinical centrifuge at setting of 4 for 10 min. Decant as much of liquid off as possible (remove glucose medium), save pellets.
3. **Resuspend each pellet in 10 mL CSHA + 1% lactose. Read  $A_{660}$ .** Calculate how to prepare at least 25mL/team of four of a dilution of the cells in CSHA + 1% lactose to yield  $A_{660}$  of 0.200 in sterile plugged 250 mL flask. (Show your math.)
4. **Withdraw T-0, begin incubation with aeration:** Transfer **7 mL** for each team of four from the culture flask into a cuvette. Place culture flask back in 37°C shaking incubator. Start a timer to measure the time the culture has grown in lactose medium. Then immediately read and record the  $A_{660}$  of the T-0 aliquot, transfer to the 13x100 tube labeled T-0. Immediately add a drop or two of toluene, cover with a double layer of parafilm, shake well, *place on ice*.
5. **Withdraw T-20** after 20 minutes: transfer **7 mL** per each team of four into a cuvette. Read and record its  $A_{660}$ , transfer to the 13x100 tube T-20. Add a drop or two of toluene, cover with parafilm, shake well, *place on ice*.
6. **Withdraw T-40** after 20 minutes more (40 minutes total): transfer **3 mL** for each team of four into a cuvette. Read and record its  $A_{660}$ , transfer to the 13x100 tube T-40. Add a drop or two of toluene, cover with parafilm, shake well, *place on ice*.
7. **Withdraw T- 60** after 20 minutes more (60 minutes total): transfer **3 mL** for each team of four into a cuvette. Read and record its  $A_{660}$ , transfer to the 13x100 tube T-60. Add a drop or two of toluene, cover with parafilm, shake well, *place on ice*.
8. **Withdraw T- 80** after 20 minutes more (80 minutes total): transfer **3 mL** for each team of four into a cuvette. Read and record its  $A_{660}$ , transfer to the 13x100 tube T-60. Add a drop or two of toluene, cover with parafilm, shake well, *place on ice*.
9. **Perform assay of  $\beta$  galactosidase in each of the samples.**  
(See protocol  $\beta$  galactosidase assay in toluenized cells.)