Gene induction: β–galactosidase in E. coli

The purpose of this activity is to:

- measure the activity of β-galactosidase in a culture of *E. coli* that has been exposed to lactose
- compare the β-galactosidase activity of the above culture with a culture of the same strain of *E. coli* that has not been exposed to lactose, and to a solution of extracted enzyme.

Procedure

SAFETY:

- Although this microorganism is not harmful, you should follow good microbiological practice when working with it. Avoid spillages and put pipettes immediately into disinfectant after use.
- Avoid getting any methylbenzene on your skin, work in a well-ventilated area and do not inhale the vapour unnecessarily. If you feel dizzy, let your teacher know.
- Avoid skin contact with the enzyme solution.

<include diagrams © Nuffield 1986>

Investigation

a Disinfect the working area with the disinfectant provided.
b Set out and label 5 test tubes, 1 to 5.
c Add 0.1 cm^3 of *E. coli* in nutrient broth (that has **not** been induced with lactose) to tube 1. Dispose of the pipette you have used into the container labelled 'waste'.
d Add 0.1 cm^3 of *E. coli* in nutrient broth (that **has** been induced with lactose) to tube 2. Dispose of the pipette you have used into the container labelled 'waste'.
e Add 0.1 cm^3 of β-galactosidase enzyme solution to tube 3. Avoid skin contact with this solution.
f Add 0.1 cm^3 of nutrient broth with lactose to tube 4.
g Add nothing extra to tube 5.
h Add a drop of methylbenzene to each tube. This kills the cells and disrupts the cell membranes which will allow the ONPG and enzymes to meet. Cover with parafilm or a stopper and shake to mix the contents thoroughly. Avoid skin contact with this chemical.
i In a fume cupboard, use a hairdryer to evaporate the methylbenzene. It is less dense than water and appears as a greasy film on the surface. When it has all evaporated, you can proceed to the next step (see safety note above).
j Add 2 cm^3 of ONPG in Z-buffer to all five tubes.
k Transfer to a water bath at 37 °C, and record the time.
l Measure the depth of colour in the samples (either transmission or absorbance) at 420 nm (or 440 nm) at 10 minute intervals until there is no further change.
m Plot a graph of depth of colour against time. (The value will decrease with time if you are measuring transmission or increase if you are measuring absorbance.)

QUESTIONS

1 Draw up a table to show clearly what you have put in each tube.

2 What is the purpose of tubes 4 and 5 in the procedure?

3 Describe your results.

4 What do the results tell you about the effect of lactose on this strain of *E. coli*