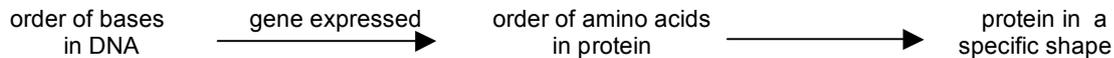
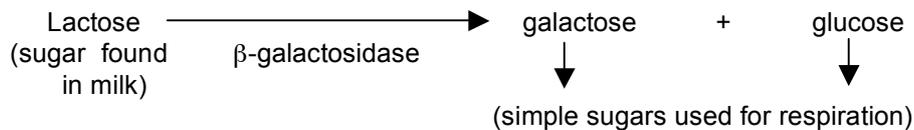


## Introduction

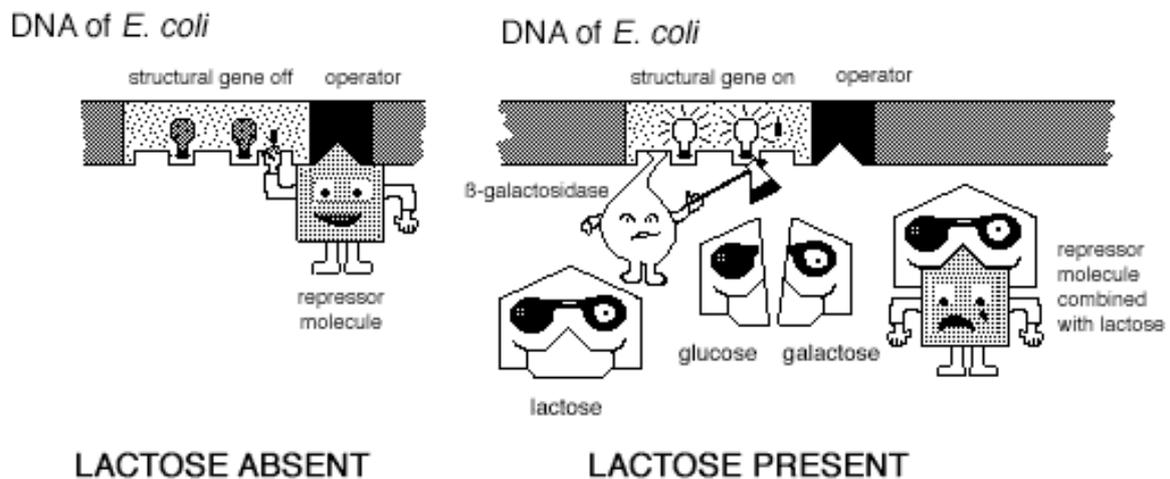
Genes are composed of DNA. The order of bases in the DNA is a code for the order of amino acids in a protein. When the gene is expressed and synthesises the protein it codes for, the protein is a specific shape (due to the order of its amino acids) and can thus carry out a specific function e.g. the protein may be an enzyme which catalyses just one chemical reaction.



Protein synthesis is an energy-requiring process. However, some enzymes are not required all the time and energy can thus be saved by living organisms if they can control their genes and only switch them on when the enzyme they code for is required. e.g. *Escherichia coli* (*E. coli*) is a bacterium which can break down the sugar lactose to glucose and galactose. To use lactose the *E. coli* must first activate the appropriate gene which will then synthesise the enzyme  $\beta$ -galactosidase.



Lactose is found in milk but not in many other environments. It would, therefore, be a waste of energy if the gene for  $\beta$ -galactosidase was permanently switched on. It would be more efficient if the *E. coli* could switch the gene on only when its enzyme was required. Two scientists, Jacob & Monod put forward a hypothesis to explain how organisms can switch genes on and off. The Jacob-Monod Hypothesis is best explained by your teacher and reading Torrance "Higher Biology" pg. 141 - 142.



ONPG is a colourless synthetic compound which can also be broken down by  $\beta$ -galactosidase into galactose and a yellow compound.



The intensity of the yellow formed can thus be an indication of the activity of the enzyme  $\beta$ -galactosidase.

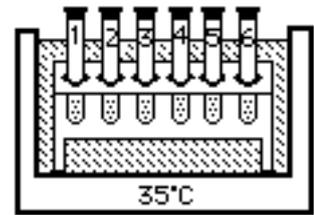
Unit 'Control and Regulation (H): Genetic control'  
Title 'Induction of the lac operon in *E. coli*'

**Collect**

- 6 test tubes & rack
- marker pen
- 0.1 M sodium phosphate solution (at least 30 cm<sup>3</sup>)
- 5 or 10 cm<sup>3</sup> disposable syringe
- 24 hr. culture of *E. coli* in nutrient broth
- 24 hr. culture of *E. coli* in nutrient broth with lactose
- 2 x 1 cm<sup>3</sup> sterile pipettes with filler
- methylbenzene (toluene)
- dropper
- parafilm or stoppers for test tubes
- β-galactosidase
- ONPG solution
- disinfectant
- disposable gloves

## The experiment

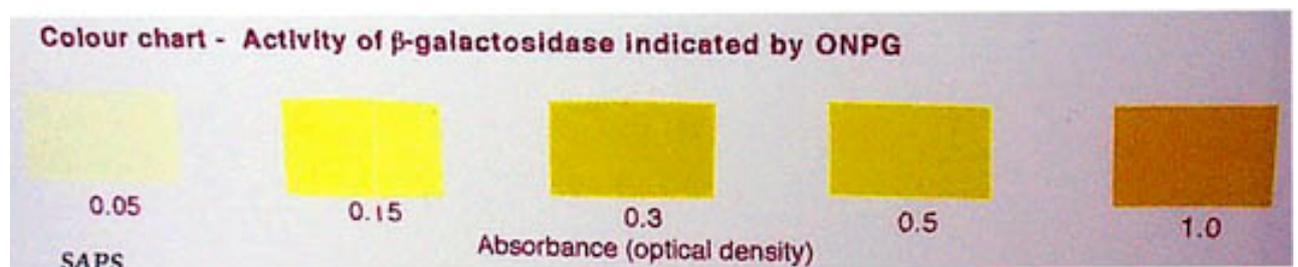
1. Wipe down the bench with disinfectant.
2. Label the test tubes 1 to 6.
3. Add 5cm<sup>3</sup> sodium phosphate solution to each test tube.
4. Add 5 drops of methylbenzene to each test tube. This speeds up the formation of any yellow colour. (CARE! This chemical is harmful and highly flammable.)
5. Using aseptic technique, transfer 1 cm<sup>3</sup> of the *E.coli* in **nutrient broth** to test tubes 1 & 2. (CARE! This strain of *E. coli* is safe but avoid spillages and immediately put pipette in disinfectant after use.)
6. Again using aseptic technique transfer 1 cm<sup>3</sup> of the *E. coli* in **nutrient broth with lactose** to test tubes 3 & 4.
7. Add 1 cm<sup>3</sup> of the enzyme β-galactosidase to test tubes 5 & 6.
8. Finally add 1 cm<sup>3</sup> of the ONPG solution to test tubes 1, 3 and 5 and 0.05 g of lactose powder to both test tubes 1 and 2.
9. Cover each test tube with parafilm or a stopper and shake to help distribute the ONPG.
10. Put all test tubes in a water bath set at 35 °C.



## Results

If a colorimeter is available, the intensity of the yellow colour formed can be measured every 10 minutes, using the appropriate even-numbered test-tube to zero the colorimeter each time.

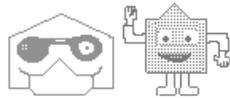
If obtaining quantitative results, plot the % transmission against time for those tubes turning yellow.



## Student Notes

1.

Term	Function
1. repressor molecule	This, when present, combines with the repressor molecule, thus allowing the operator to switch on the structural gene.
2. inducer	This is made by the regulator so it can combine with the operator and prevent it switching on the structural gene.
3. structural gene	Can be switched off by the repressor molecule. When repressor molecule is combined with inducer, it is switched on and activates the structural gene.
4. regulator	Makes the repressor molecule.
5. operator	When activated this makes a specific protein.



Rewrite the above, selecting the appropriate function for each term.

2. Why is it important that each enzyme has a different shape?
3. What determines the shape of an enzyme? (2 part answer required)
4. Why is it useful for organisms to be able to switch genes on and off when required?
5. Explain:
  - (i) the formation of the yellow compound in test-tube 5.
  - (ii) the slower formation of the yellow compound in test-tube 3.
  - (iii) the difference in speed of formation of the yellow compound in test-tubes 1 & 3.

## Possible Project Work

1. Does ONPG in nutrient agar cause the structural gene to be switched on?
2. What concentration of lactose in nutrient agar is required to switch on the structural gene?
3. Do any other sugars have the same effect as lactose?
4. What happens when the pH of the sodium phosphate solution is changed?
5. Does the rate of ONPG breakdown decrease as the lactose concentration increases?
6. Do other micro-organisms behave in the same way as *E. coli*?

## References

Cappuccino J.G. and Sherman N. (1983)., *Microbiology, A Laboratory Manual*, Addison/Wesley ISBN 0201 111 608.

Acknowledgements to M. Smith (Dollar Academy), M. Hamilton (SSERC) and J. Rennie (George Watson's College).

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## Technical Notes

Sodium phosphate ( $\text{Na}_2\text{HPO}_4$  dibasic, anhydrous) to provide an alkaline pH.

24 hr culture of *E. coli* in nutrient broth with lactose: 0.1 g lactose in 10 cm<sup>3</sup> nutrient broth.

*E. coli* (K12 strain) - from Philip Harris Education (tel. 0845 120 4520), Cat. H50650/8 about £6.

*Kluyveromyces lactis* (*K. lactis*) is an alternative organism to *E. coli*. Results are comparable to *E. coli* if 2 cm<sup>3</sup> of a 4-5 day old culture are added to the test-tubes.

*K. lactis* - from NCBE, Science and Technology Centre, Earley Gate, University of Reading, Whiteknights, READING, RG6 6BZ. Tel: 0118 987 3743 Fax: 0118 975 0140. Slope culture about £6.00.

$\beta$ -galactosidase can be diluted 10x with distilled water for the experiment.

$\beta$ -galactosidase - from NCBE, Science and Technology Centre, Earley Gate, University of Reading, Whiteknights, READING, RG6 6BZ. Tel: 0118 987 3743 Fax: 0118 975 0140. 100 cm<sup>3</sup> about £12.

ONPG (o-Nitrophenyl  $\beta$ -D-Galactopyranoside) solution:

0.04 g ONPG in 10 cm<sup>3</sup> 0.1 M sodium phosphate.

This solution will remain active for one week if stored in a refrigerator.

ONPG - from Sigma Aldrich (tel. 0800 44 77 88), Cat. N1127, 500mg about £6.50, 1g about £100.