

BME

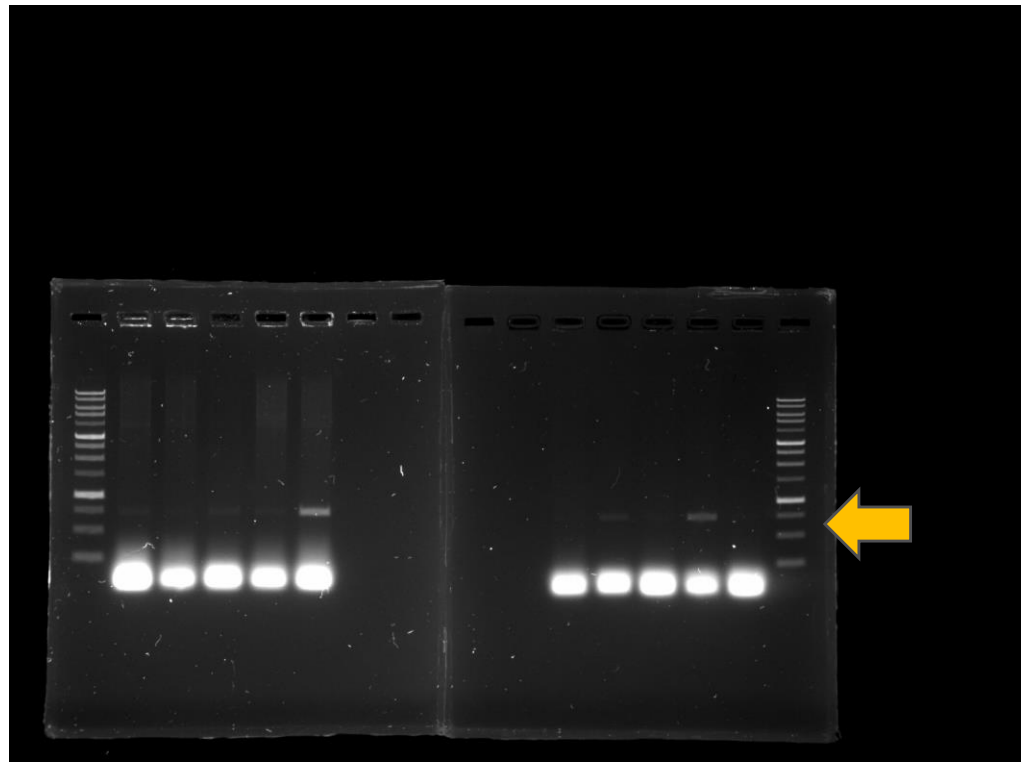
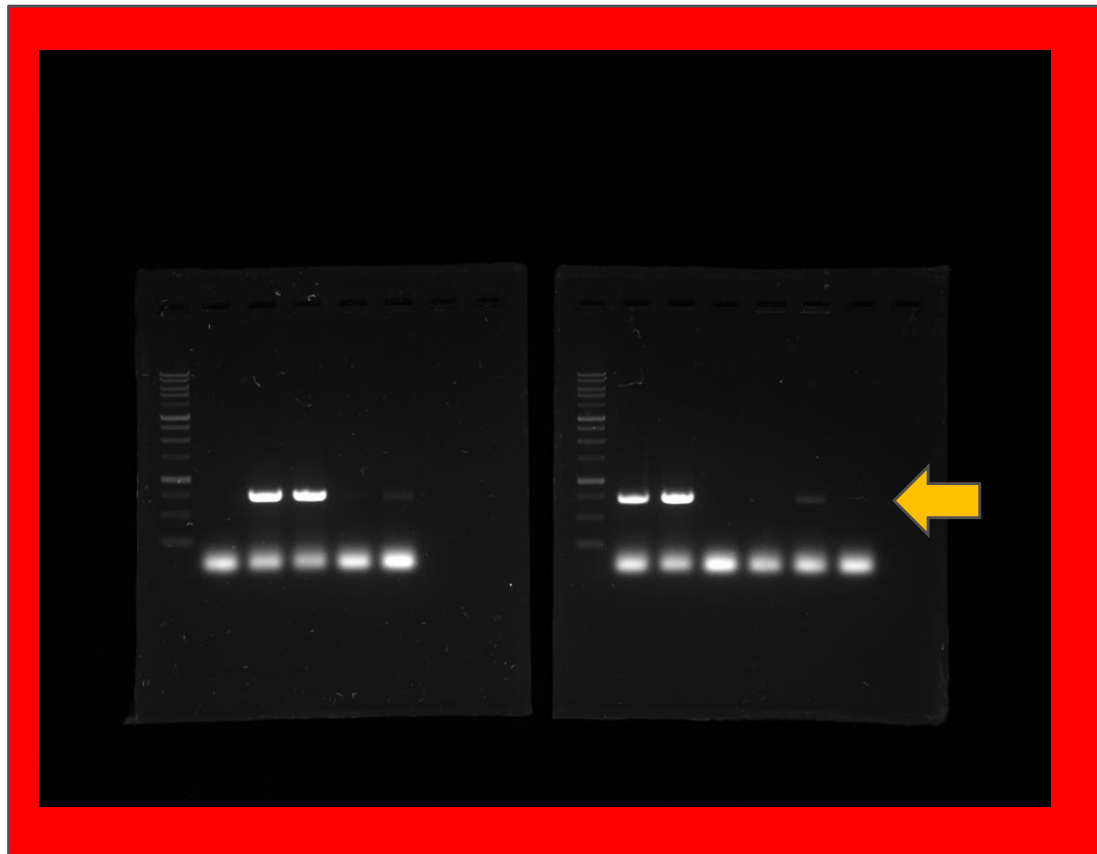
Molecular Biology
Experiment

Protein induction

SKKU BME

3rd grade, 2nd semester

Last week results



Today

E. coli
seeding

Protein
induction
by IPTG

E. coli prep.

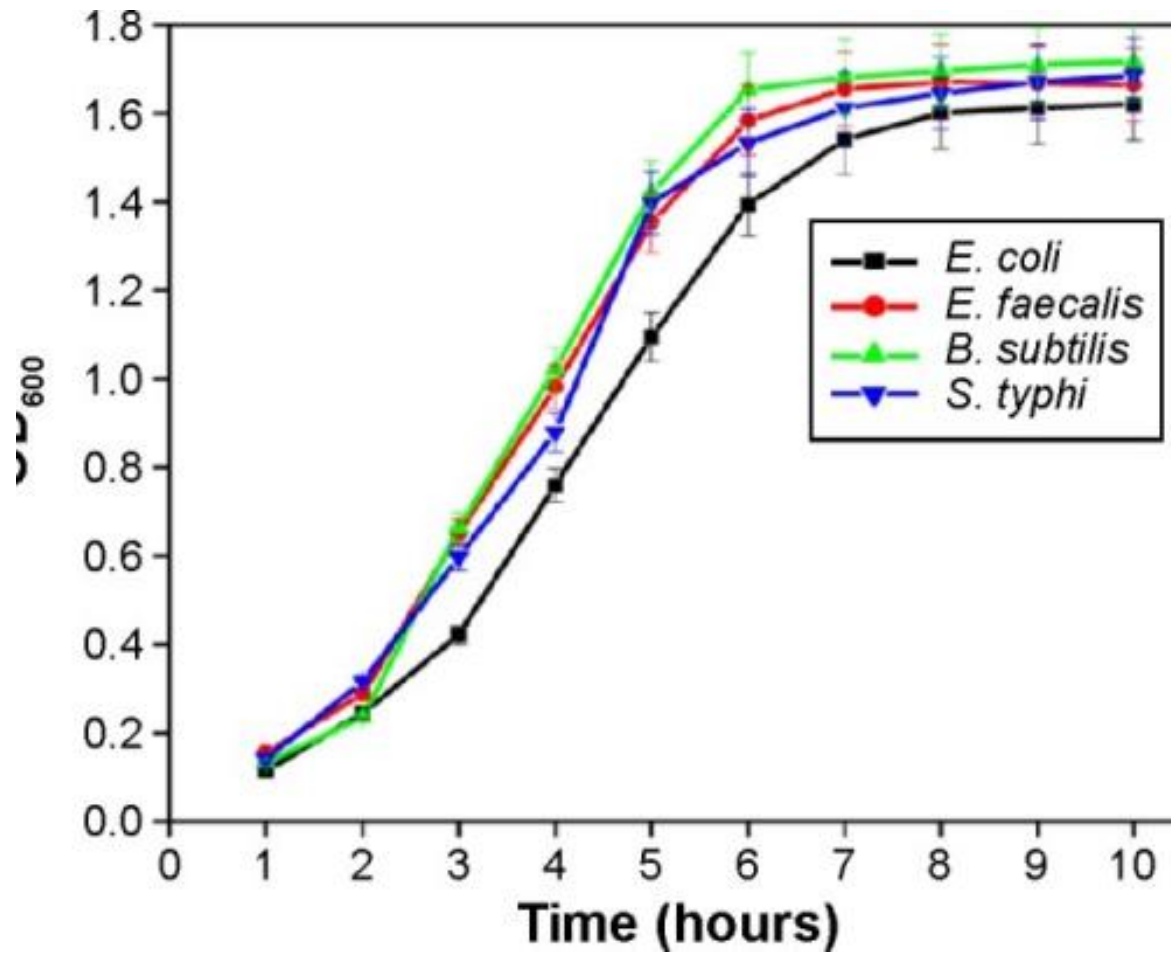
Today

- E. coli growth curve
- Protein induction
- E. coli prep.

E.coli growth curve

E. coli growth curve

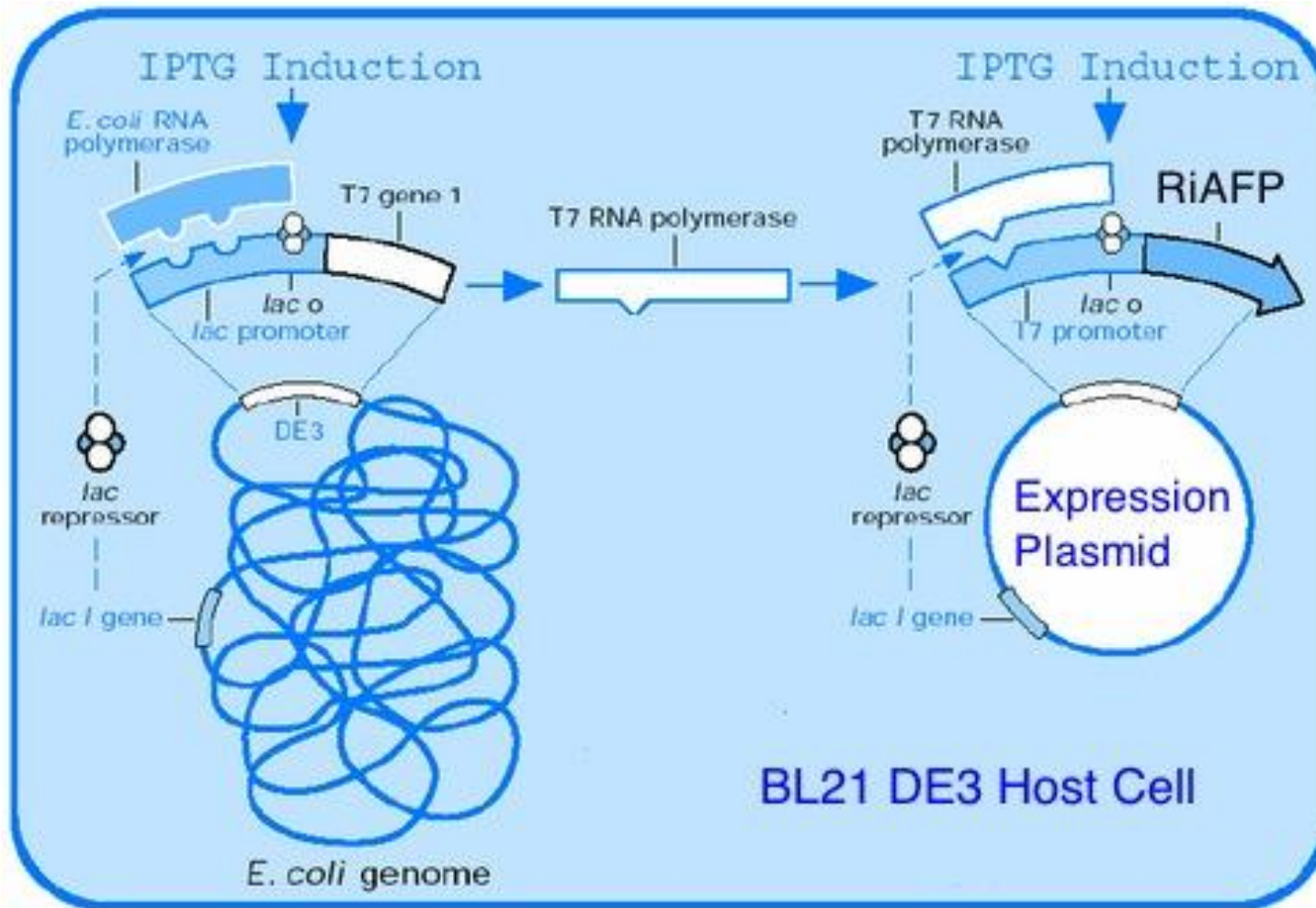
- OD600 is an abbreviation indicating the absorbance, or **optical density**, of a sample measured at a wavelength of 600 nm. Also seen written as "O.D. 600," "o.d. 600," "OD₆₀₀" etc. It is a common method for estimating the concentration of **bacterial** or other cells in a liquid.



E. coli growth curve

- Check the O.D. at each time point and draw the growth curve.

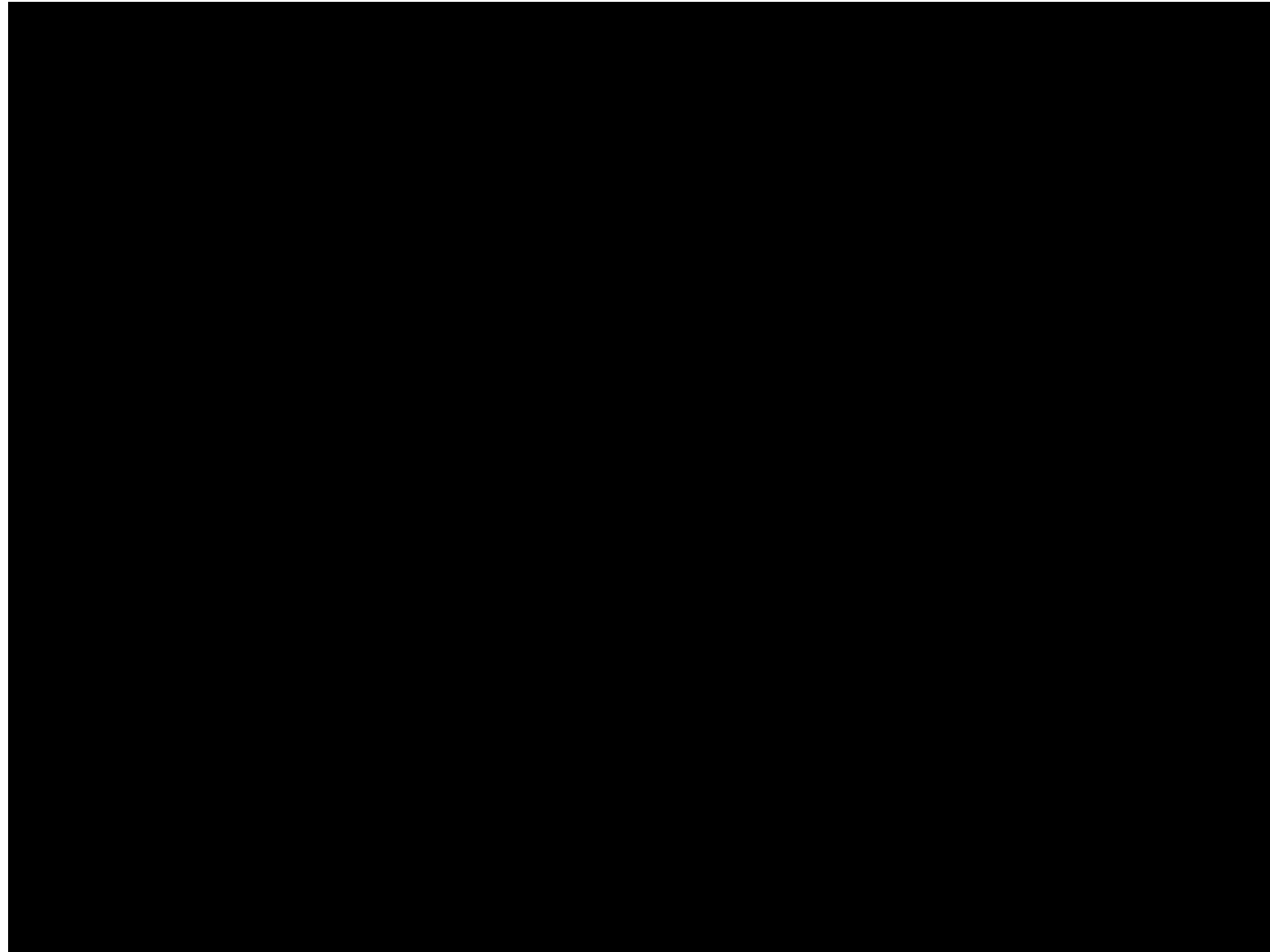
IPTG induction



IPTG induction

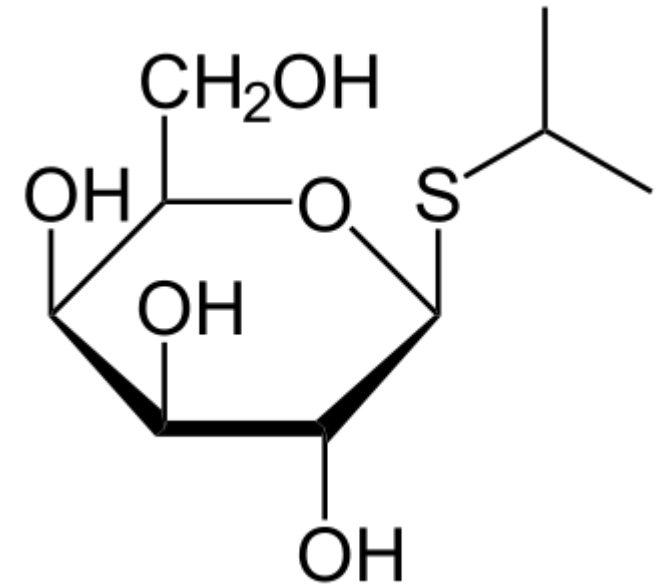
- Mechanism of IPTG induction
- Lac promoter
- T7 promoter
- Lac O
- Lac repressor
- Lac I gene

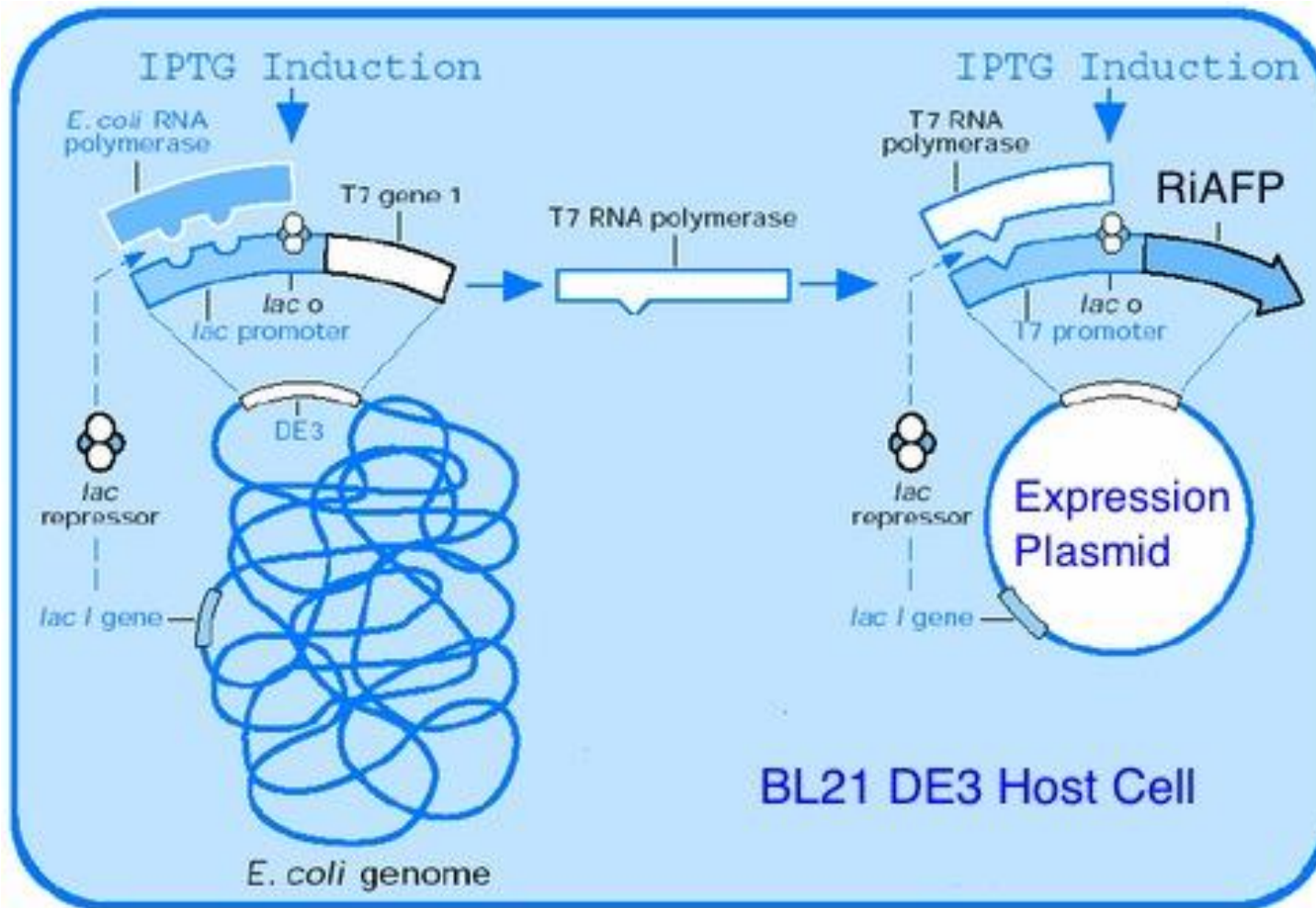
Lac promoter in E.coli



IPTG

- Isopropyl β -D-1-thiogalactopyranoside (IPTG) is a molecular biology reagent. This compound is *a molecular mimic of allolactose*, a lactose metabolite that triggers transcription of the lac operon, and it is therefore used to induce protein expression where the gene is under the control of the lac operator.
- IPTG, unlike allolactose, is *not hydrolyzable by β -galactosidase*.





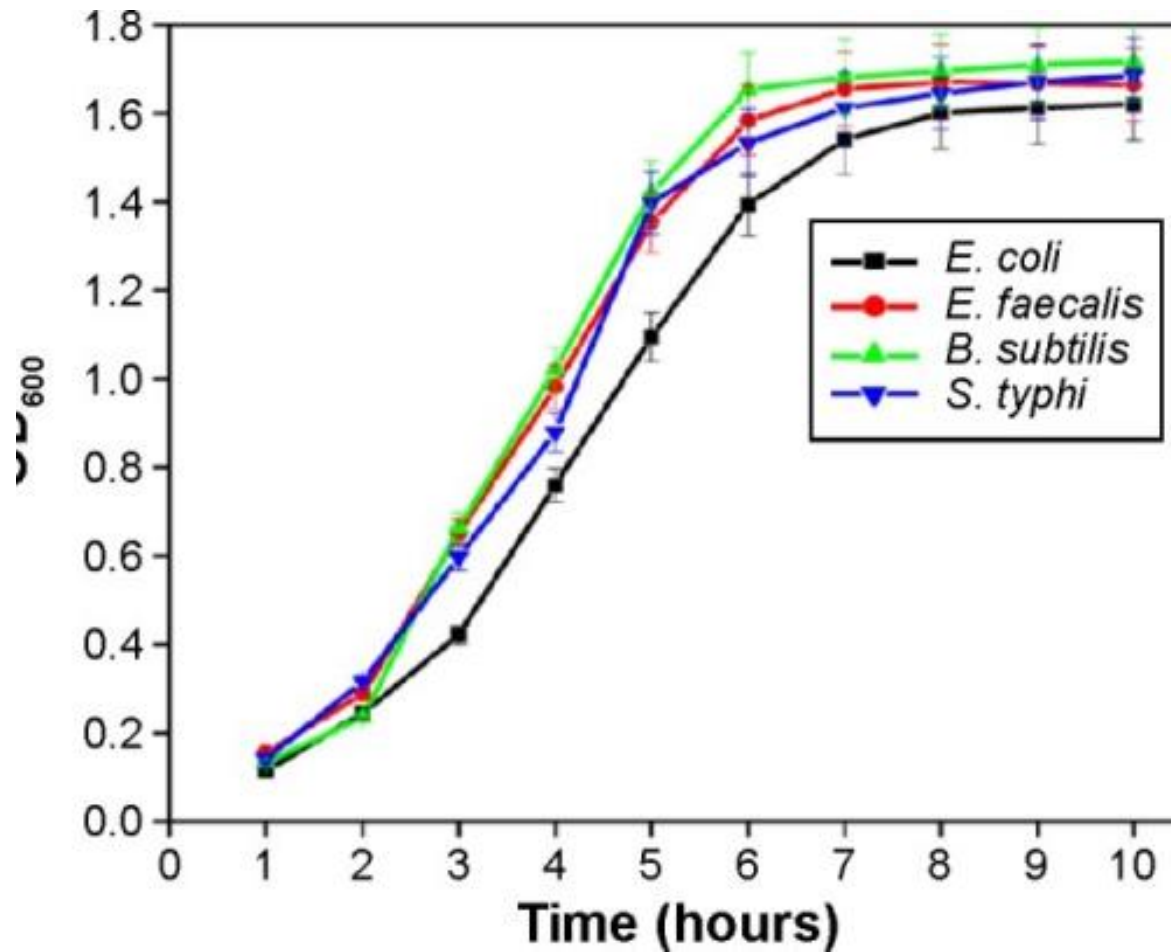
IPTG induction

- Mechanism of IPTG induction
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Protein induction protocol

- Transform expression plasmid into BL21(DE3). Plate on antibiotic selection plates and incubate overnight at 37°C.
- Resuspend a single colony in 5 ml liquid culture with antibiotic.
- Incubate at 37°C until OD₆₀₀ reaches 0.4–0.8.
- Before the induction, make a non-induction control sample.
- Induce with 4 or 40 µl of a 100 mM stock of IPTG (final concentration of 40 or 400 µM) and induce for 3 to 5 hours at 37°C.

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- Check for expression either by Coomassie stained protein gel, Western Blot or activity assay. Check expression in both the total cell extract (soluble + insoluble) and the soluble fraction only.
*If a fraction of the target protein is insoluble, repeat expression at a lower temperature (15 to 30°C) or test expression in Lemo21(DE3) ([NEB #C2528](#)).
 - For large scale, inoculate 1 L of liquid medium (with antibiotic) with a freshly grown colony or 10 ml of freshly grown culture. Incubate at 37°C until OD₆₀₀ reaches 0.4–0.8. Add 40 or 400 µM IPTG and express protein using optimal time/temperature determined in a small scale trial.



E. coli growth curve

- Check the O.D. at each time point and draw the growth curve.
- *Two groups of cultures*
 - *1. E.coli without IPTG*
 - *2. E.coli with IPTG*

Home work

- Find the mechanism of acrylamide gel formation. (See this site: <http://elte.prompt.hu/sites/default/files/tananyagok/IntroductionToPracticalBiochemistry/ch07s03.html>)
- What is the polymerization chain reaction?
- Find roles of TEMED and Ammonium persulfate in SDS PAGE gel.