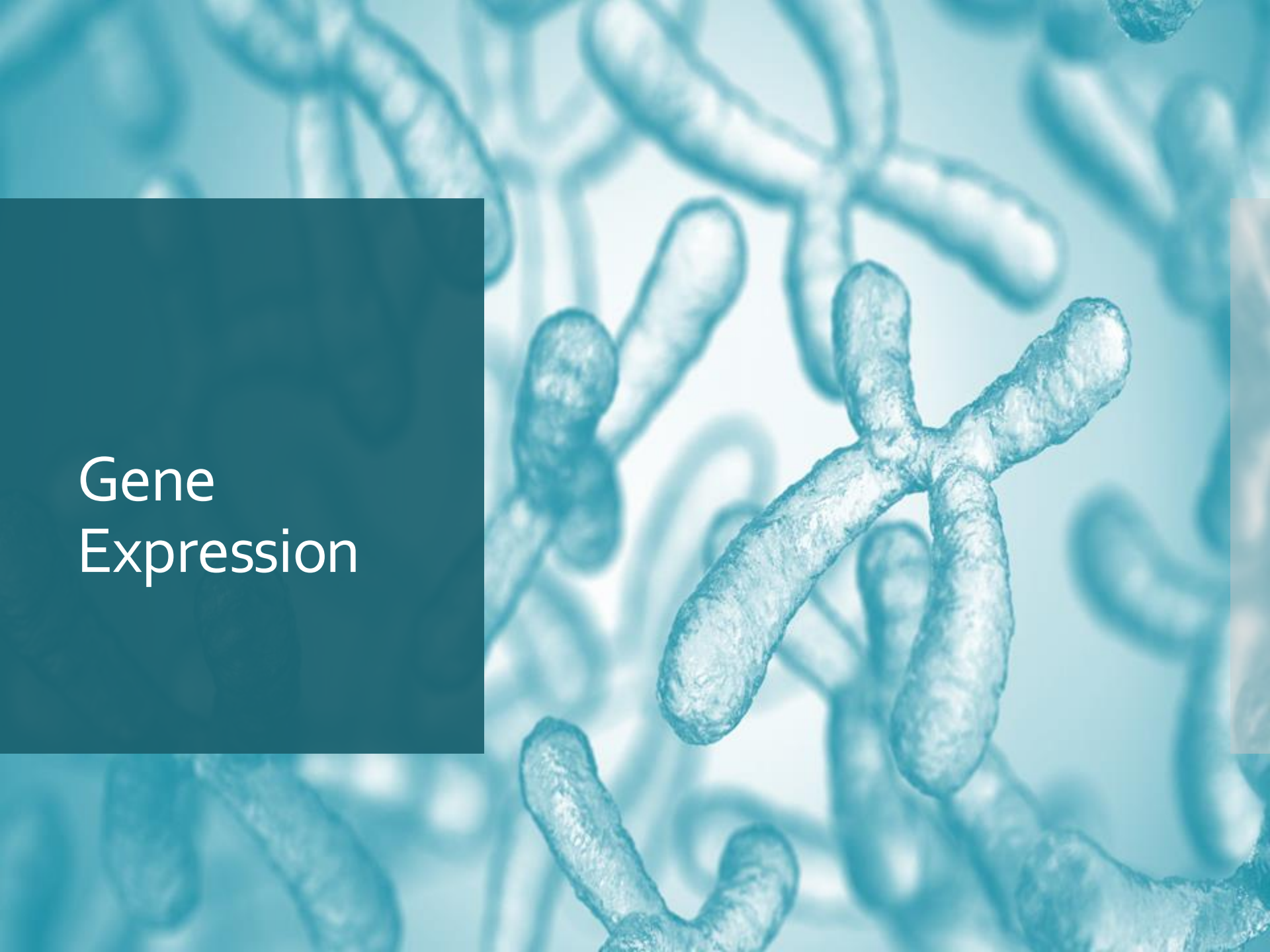
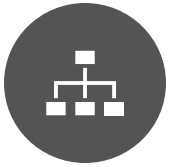

Gene
Expression

Objectives

Describe the concept of gene expression.

Describe how prokaryotic gene regulation occurs at the level of transcription.



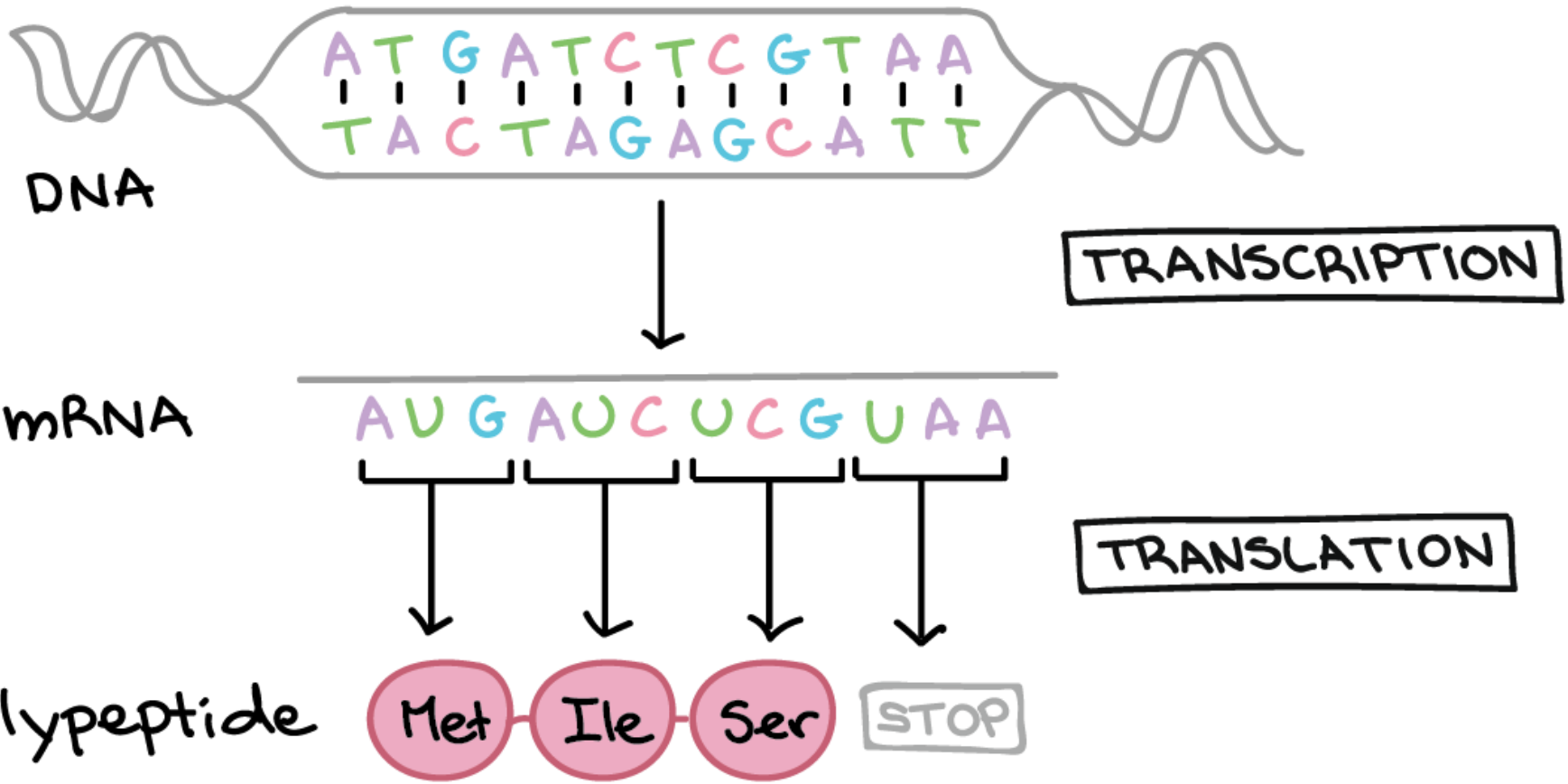
Explain the structure and function of an inducible operon.

Describe how an inducer (allolactose) and the lac repressor ensure the expression of the lac operon only at appropriate times.

Describe how glucose influences lac operon function.

Analyze the effect of inducers and repressors on *lac* gene expression.

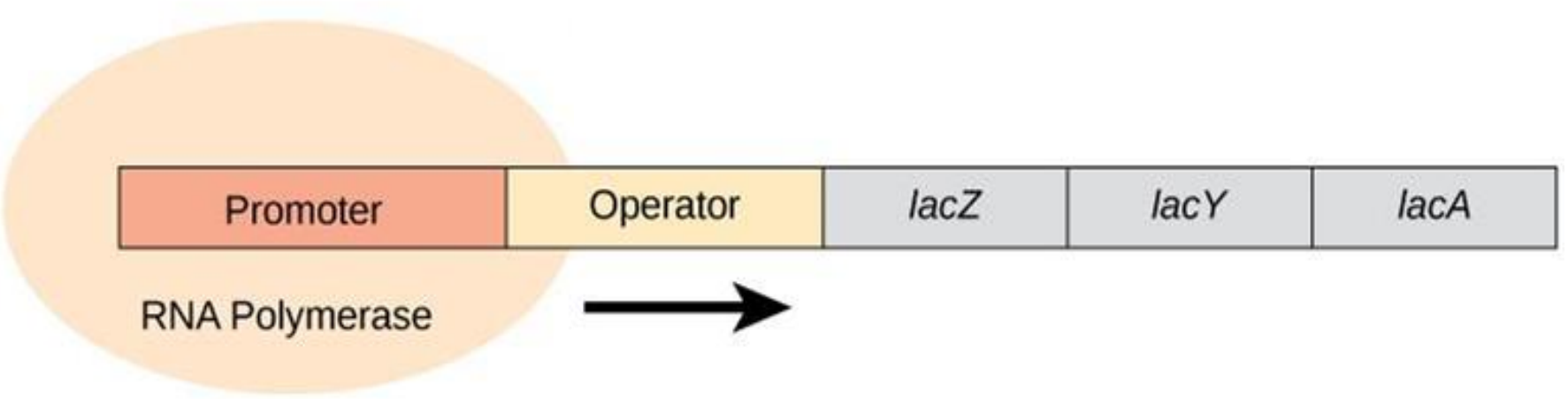
THE CENTRAL DOGMA



What is Gene Expression?

Operons

- The DNA of prokaryotes (bacteria) is organized into a circular chromosome, supercoiled within the nucleoid region of the cell cytoplasm.
- Proteins that are involved in the same biochemical pathway, are encoded together in blocks called operons.
- For example, all of the genes needed to use lactose as an energy source are coded next to each other in the lactose (or lac) operon, and transcribed into a single mRNA.



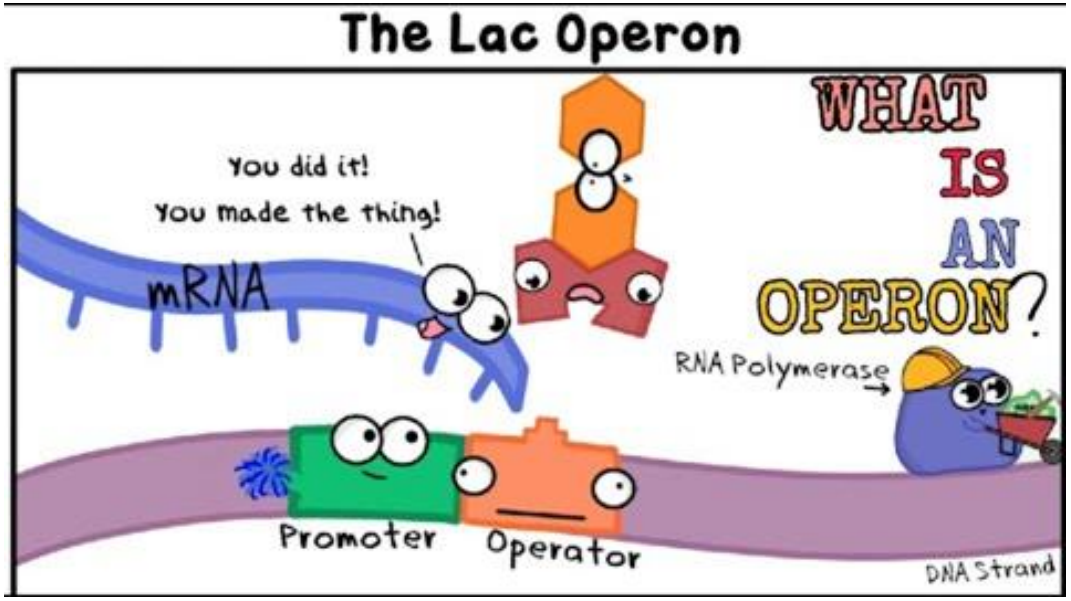
RNA polymerase- needed to start transcription and produce mRNA

Promoter-sequence of DNA that the polymerase binds

Operator-part of the DNA that repressor can bind to (on/off switch)

Repressor-binding of the repressor blocks RNA polymerase and prevents the production of mRNA and so no production of proteins.

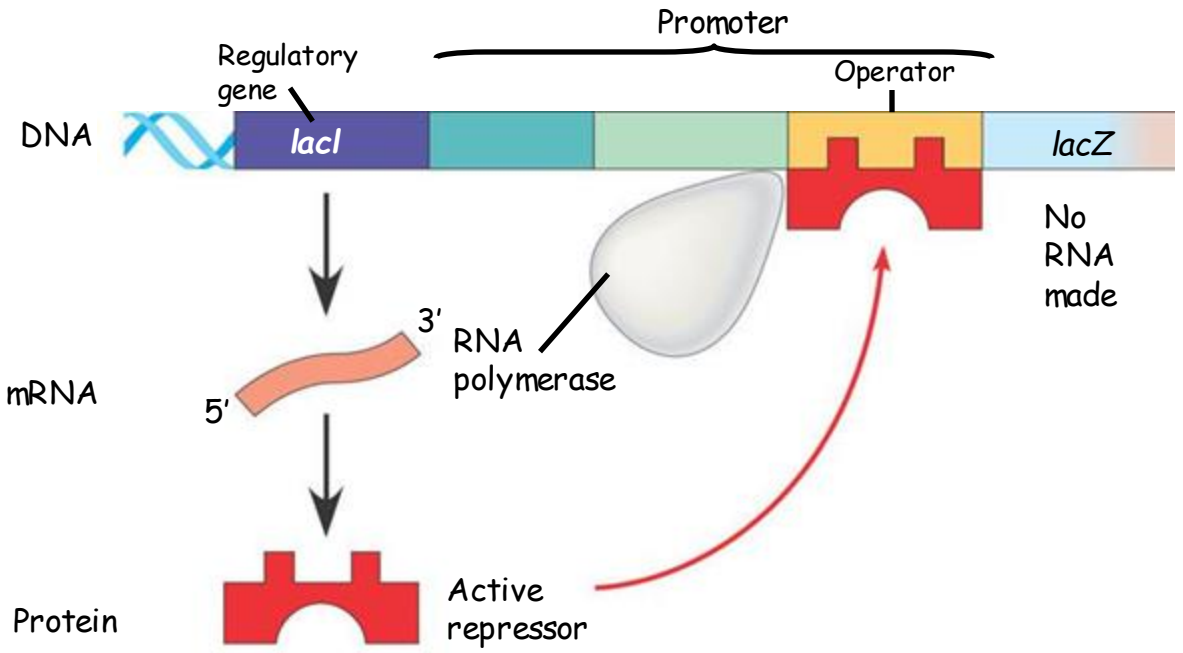
3 lactose utilizations genes (lacZ, lacY, lacA).



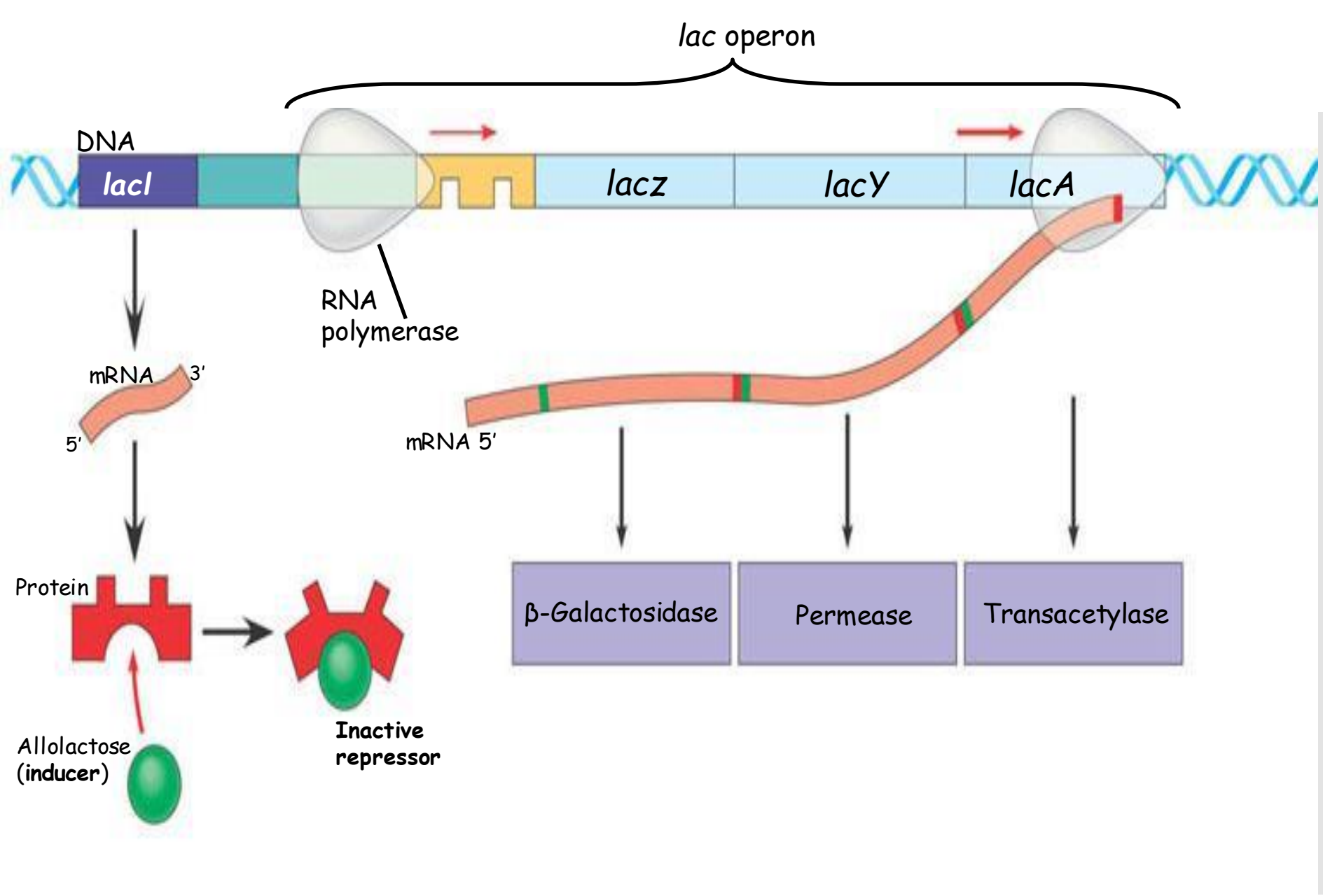
- The *lac* **operon**: regulated synthesis of inducible enzymes

The Inducible Operon: The *lac* operon

https://www.youtube.com/watch?v=AVuj0q4mKa8



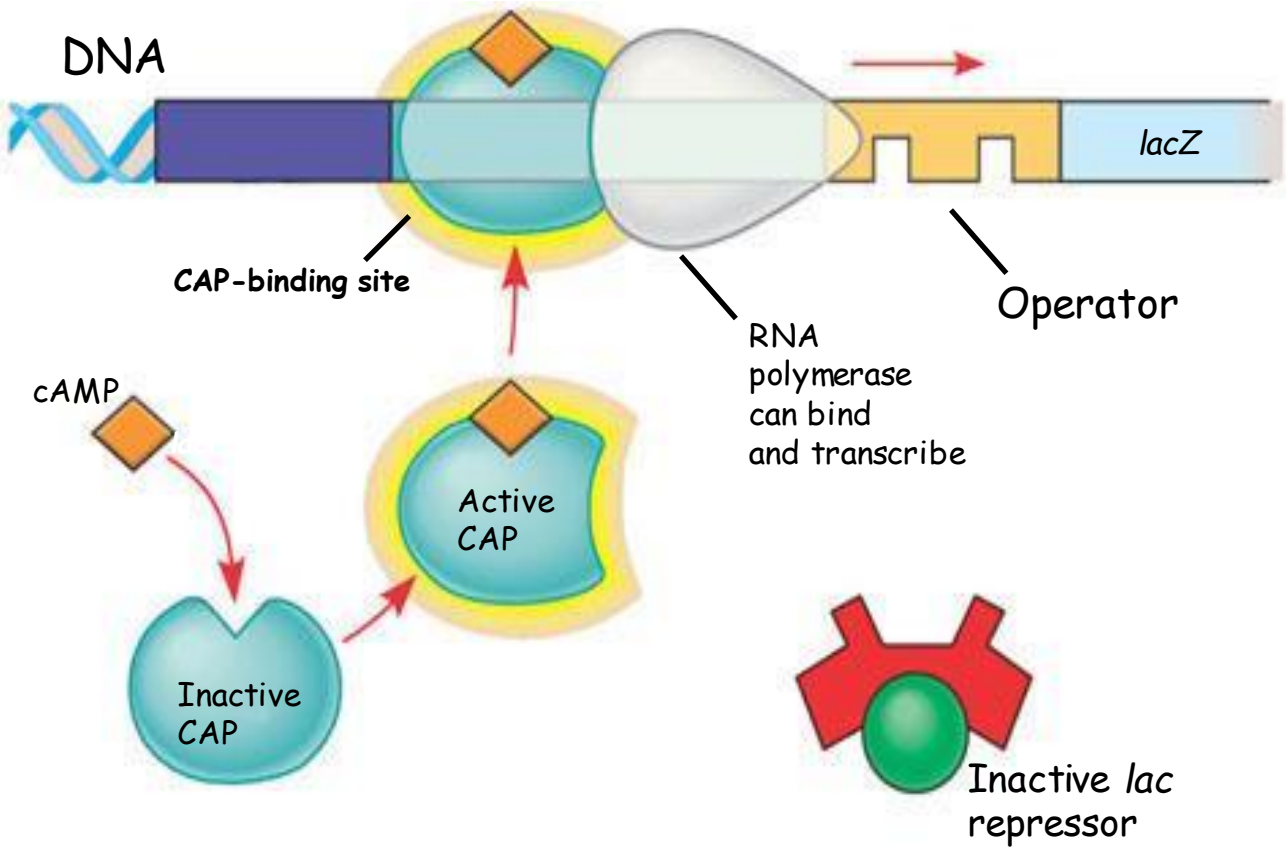
(a) Lactose absent, repressor active, operon off. The *lac* repressor is innately active, and in the absence of lactose it switches off the operon by binding to the operator.



(b) **Lactose present, repressor inactive, operon on.** Allolactose, an isomer of lactose, derepresses the operon by inactivating the repressor. In this way, the enzymes for lactose utilization are induced.

In *E. coli*, when glucose, a preferred food source, is scarce

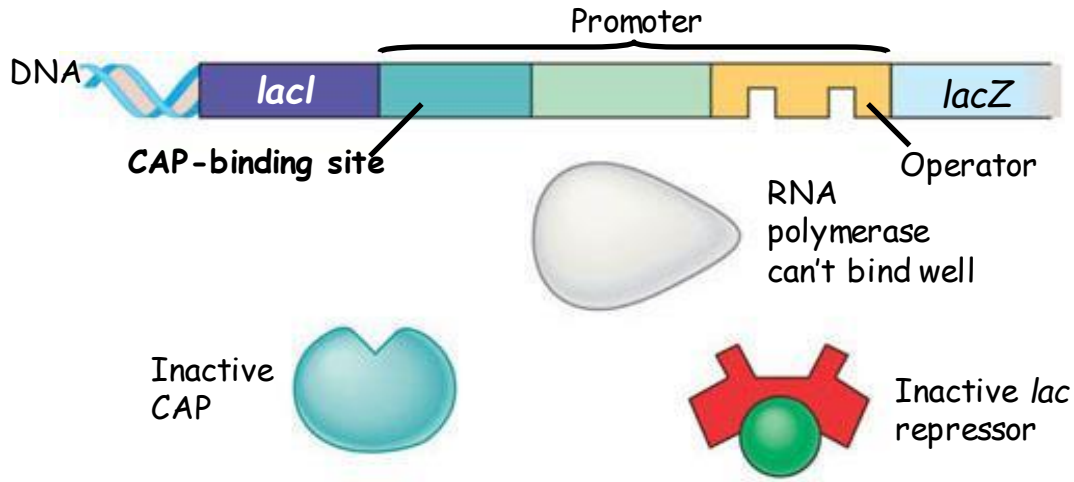
The *lac* operon is activated by the binding of active CAP (CAP-cAMP), which helps RNA polymerase bind



Lactose present, glucose scarce (cAMP level high): abundant *lac* mRNA synthesized.

When glucose levels in *E. coli* are high

CAP detaches from the *lac* operon, turning it down, even if lactose is present



(b)

Lactose present, glucose present (cAMP level low): little *lac* mRNA synthesized.

Our System

- **β-Galactosidase (β-Gal) is an enzyme** produced by Escherichia coli (E. coli) that catalyzes the hydrolysis of the disaccharide lactose to the monosaccharides galactose and glucose (metabolized by cells).
- Although lactose is the natural substrate for β-gal, β-gal will hydrolyze other molecules that have the relevant bond in the same place as lactose (other β-galactosides).
- **Ortho-nitrophenyl-β-galactoside (ONPG), is cleaved by β-gal into galactose and o-nitrophenol**
- ONPG and galactose are colorless, but **ortho-nitrophenol is a bright yellow with an absorption wavelength of 420 nm.**

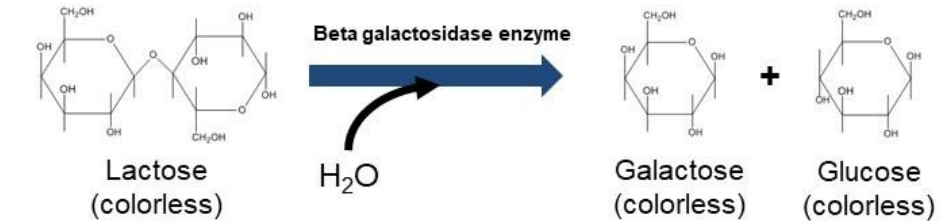


Fig 1a.

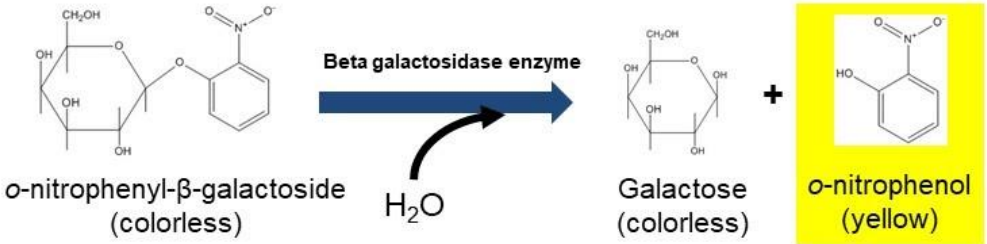


Fig 1b.

Safety

Gloves, Goggles, Closed-toe shoes

All pipet tips should be ejected into the tip waste bin on your bench.

All ONPG/Z Buffer should be poured into the waste container on the side bench. Remember to put the lid back on when you are done.

Rinse your glass cuvettes in the sink and **dispose of them in the biohazard trash can**

Gloves and paper towel go in the regular trash!

Do not move the spectrophotometer

1

Activity 1: Determining the optimal growth conditions for *LacZ* expression.

2

Activity 2: What are the effects of adding sugars to the β-galactosidase assay

What are we checking today?

How to label tubes for Activity 1 and 2

Activity 1

- One set of plastic tubes, one set of cuvettes (sample labels)

- 1a , 1b , 1c
- 2a , 2b , 2c
- 3a , 3b , 3c
- 4a , 4b , 4c
- Blank

Activity 2

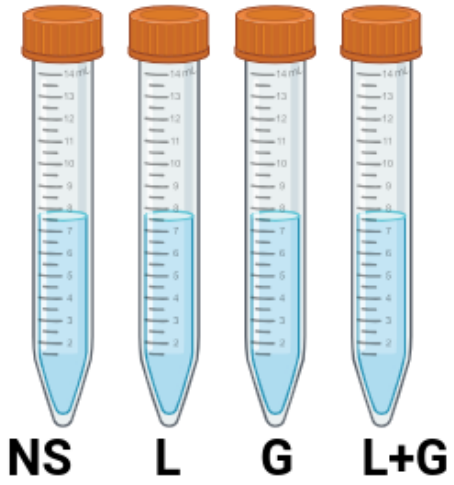
Plastic tubes:

- Rxn 1 T1
- Rxn 1 T2
- Rxn 2 T1
- Rxn 2 T2
- Rxn 3 T1
- Rxn 3 T2
- Rxn 4 T1
- Rxn 4 T2

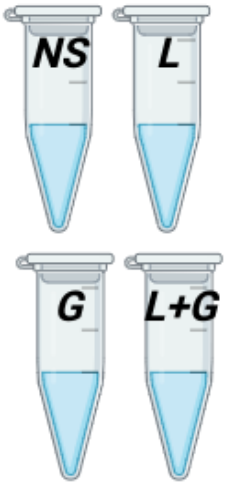
Cuvettes:

- Rxn 1
- Rxn 2
- Rxn 3
- Rxn 4

Activity 1



200ul E.coli + 200ul water

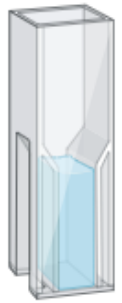


Lysis

12 tubes

Blank

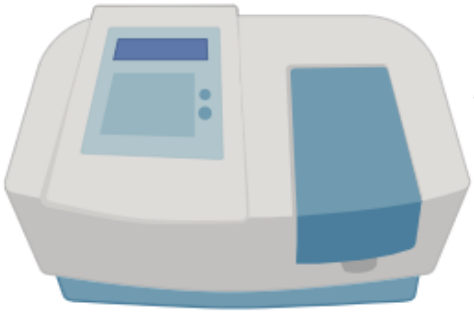
+ ONPG



(Z buffer)

(lysis tubes)

13 cuvettes



Dilute the four E. coli culture conditions (200uL of E. coli + 200uL of distilled water) in microcentrifuge tubes. Close cap and mix well.

Label 13 microcentrifuge tubes per reactions in table 1

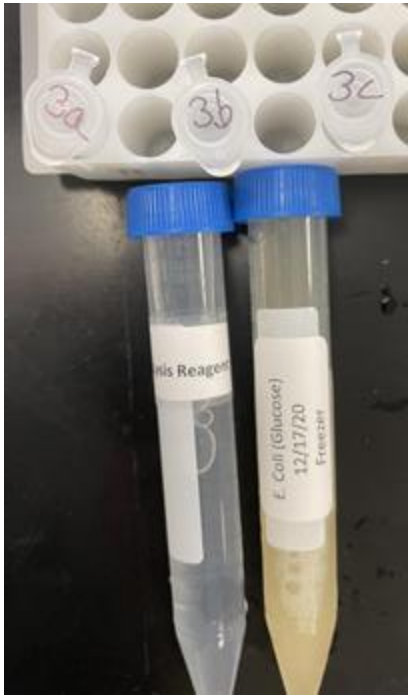
- Add appropriate vol of E coli (or TSB for the blank), then add lysis reagent.
- Cap and mix tubes
- Incubate at room temp for 10 min, then place on ice

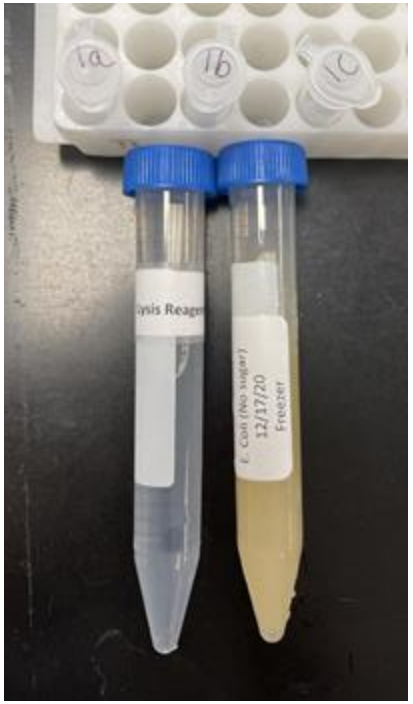
Lab 13 cuvettes per reactions in table 1

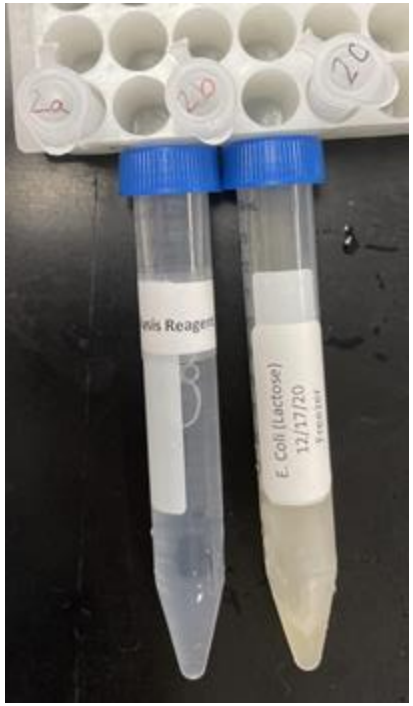
- Add Z buffer to each cuvette (as calculated in table 1)

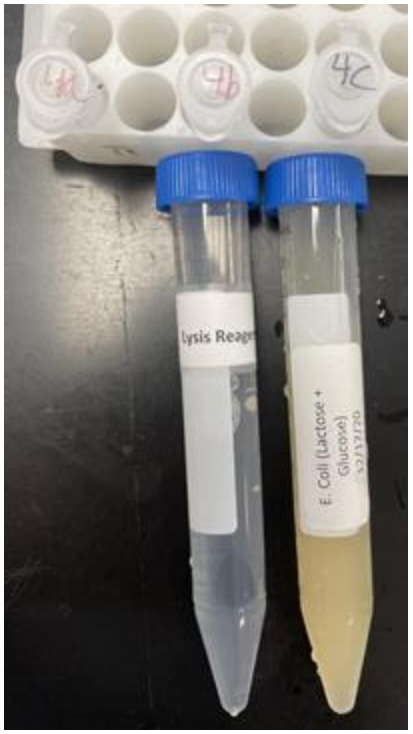
Add contents of lysis tubes to cuvettes

- Add vol of ONPG per table 1, START TIMER COUNTING UP, record time that ONPG was added to each tube.
- Stop reaction when color matches post-it note yellow. Add 1mL of 1M sodium carbonate, record stop time


3a
3b
3c
E. Coli (Glucose)
12/17/20
Freezer


1a
1b
1c
E. Coli (No sugar)
12/17/20
Freezer


2a
2b
2c
E. Coli (Lactose)
12/17/20


4a
4b
4c
E. Coli (Lactose +
Glucose)

Activity 1 continued …

Take absorbance measures at A_{420} for each sample. Be sure to blank instrument first

Calculate B-gal activity, record in table 2

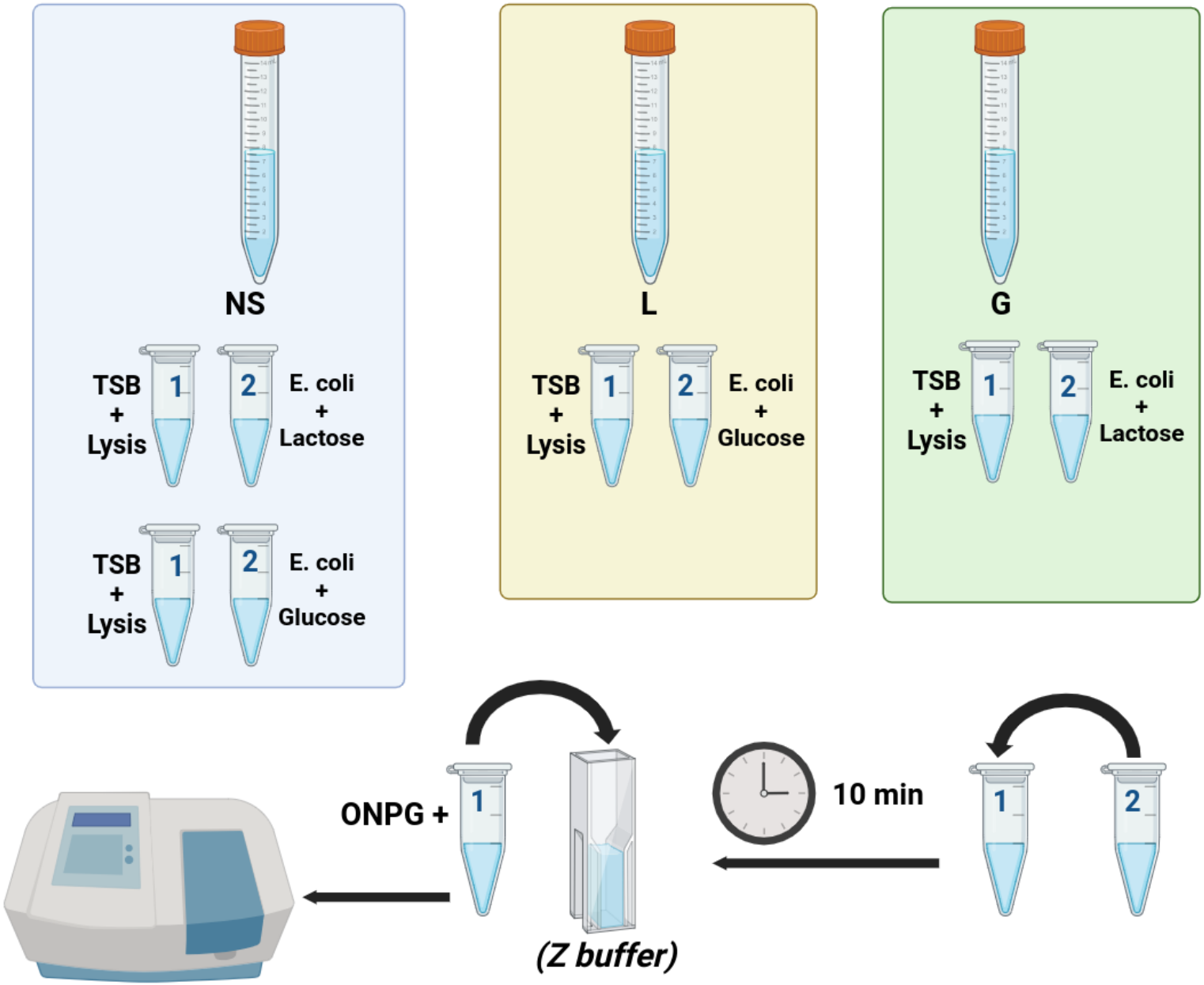
Refer to canvas "Lab activity: Calculating enzyme activity" for info on calculations

(Absorbance/Elapsed Time)x1000

Table 1

Culture Conditions	Rxn #	Lysis Reagent	*E. coli* Culture Volume	Unused media for differences in enzyme	Z Buffer to bring the total to 1mL	Vol. ONPG (4 mg/mL) for 0.8 mg
No sugars *E. coli* culture	1a	10 µL	10 µL	90 µL	690uL	200uL
	1b	10 µL	20 µL	80 µL	690uL	200uL
	1c	10 µL	100 µL	0	690uL	200uL
Lactose *E. coli* culture	2a	10 µL	10 µL	90 µL	690uL	200uL
	2b	10 µL	20 µL	80 µL	690uL	200uL
	2c	10 µL	100 µL	0	690uL	200uL
Glucose *E. coli* culture	3a	10 µL	10 µL	90 µL	690uL	200uL
	3b	10 µL	20 µL	80 µL	690uL	200uL
	3c	10 µL	100 µL	0	690uL	200uL
Glucose + Lactose *E. coli culture*	4a	10 µL	10 µL	90 µL	690uL	200uL
	4b	10 µL	20 µL	80 µL	690uL	200uL
	4c	10 µL	100 µL	0	690uL	200uL
BLANK	Blank	10 µL	0 µL	100 µL	690uL	200uL

Activity 2


NS
TSB + Lysis
1
2
E. coli + Lactose
TSB + Lysis
1
2
E. coli + Glucose
L
TSB + Lysis
1
2
E. coli + Glucose
G
TSB + Lysis
1
2
E. coli + Lactose
ONPG +
1
(Z buffer)
10 min
1
2

Label 8 microcentrifuge tubes according to table 3.1 (2 tubes/rxn)

- In Tube 1 of rxn, add TSB & lysis reagent.
- In Tube 2 of rxn, add culture & sugar.

Take 10uL of Tube 2 Content & add that to Tube 1

- Incubate for 10 min. At room temp. After 10 min., place the tubes in ice.

Label 4 cuvettes according to table 3.1

- Add Z buffer as calculated in Table 3.1, add contents of lysis tubes
- Add vol of ONPG per Table 3.1, START TIMER COUNTING UP, record time that ONPG was added to each tube.
- Stop reaction when color matches post-it note yellow. Add 1mL of 1M sodium carbonate, record stop time

Activity 2 continued …

Take absorbance measures at A_{420} for each sample. Be sure to blank instrument first

Calculate B-gal activity, record in table 2

Refer to canvas "Lab activity: Calculating enzyme activity" for info on calculations

(Absorbance/Elapsed Time)x1000

Table 3.1

		Tube 1				
Culture Conditions	Rxn #	Fresh TSB for differences in enzyme vol.	Lysis Reagent	*E. coli* volume from **Tube 2**	Z buffer to bring to 1 mL total	Vol. ONPG (4 mg/mL) for 0.8 mg
No sugars *E. coli* culture + 20% Lactose						
	1	40 µL	5 µL	10 µL	745µL	200µL
Glucose *E. coli* culture + 20% Lactose						
	2	40 µL	5 µL	10 µL	745µL	200µL
No sugars *E. coli* culture + 20% Glucose						
	3	40 µL	5 µL	10 µL	745µL	200µL
Lactose *E. coli* culture + 20% Glucose						
	4	40 µL	5 µL	10 µL	745µL	200µL
BLANK		50µL	5µL		745µL	200µL