Genetic nomenclature

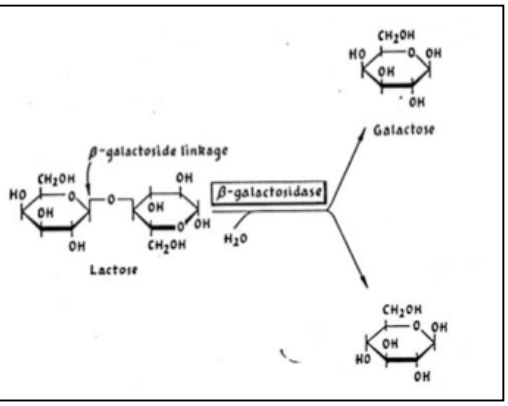
Phenotype: Lac⁻; what's observed

Genotype: *lacZ*⁻ or *lacY*⁻
refers to specific gene defect

Particular mutations = "alleles"
e.g., *lacZ343*

Early knowledge of lactose metabolism:

1. lactose cleaved by cells (=beta-galactosidase)
2. lactose taken into cells (=lactose permease)
3. induction (lactose addition increases lactose cleavage and uptake rates)



Questions answered genetically:

1. How many genes are required for lactose utilization?

2. What is the mechanistic "logic" of induction? (i.e., Does induction involve a positively- or negatively-acting regulatory factor?)

1. How many genes are required for lactose utilization?

A. Isolate hundreds of Lac⁻ mutants (Lac^-_1, Lac^-_2, ...)

B. Determine how many complementation groups are defined by the Lac⁻ mutations.

Lac⁻ mutant isolation:

F^- lac^+ cells

↓

Mutagenize

↓

Plate cells for colonies and screen for mutants

↓

lac^-_1; lac^-_2; etc

B. Determine how many complementation groups (=genes) are defined by the Lac⁻ mutations.

General form of complementation test: Does lac^-_1/lac^-_2 strain grow on lactose or not?

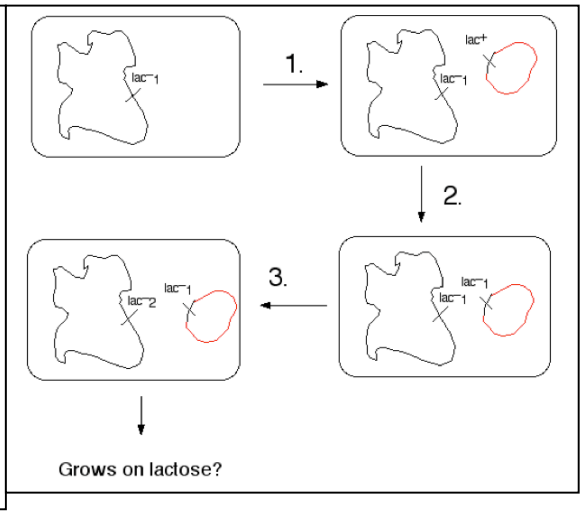
Complementation analysis of lac⁻ mutations

1. Transfer F'lac⁺ into Lac⁻ mutant (e.g., lac^-_1)

2. Allow recombination to take place to transfer mutation (lac^-_1) into F'lac

3. Conjugate F'lac carrying mutation (lac^-_1) into second lac⁻ strain (e.g., lac^-_2)

4. Examine whether F' lac^-_1 / lac^-_2 cells grow on lactose



	F'lac⁻1	F'lac⁻2	F'lac⁻3	F'lac⁻4
lac⁻1	–	–	+	+
lac⁻2	–	–	+	+
lac⁻3	+	+	–	–
lac⁻4	+	+	–	–
lac⁻5	–	–	+	+

Complementation group I= lac⁻ 1, 2, 5
Complementation group II= lac⁻ 3, 4

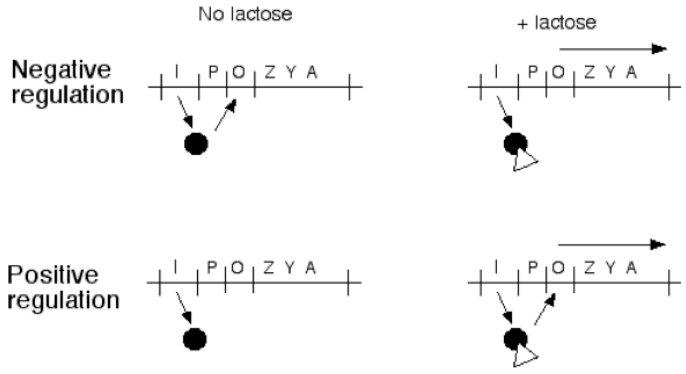
Coding assignments:

One set of mutants were beta-galactosidase–, transport⁺ (= $lacZ^-$ $lacY^+$)

Second set of mutants were beta-galactosidase⁺ transport⁻ (= $lacZ^+$ $lacY^-$)

2. Does induction involve a positively- or negatively-acting factor?

A. Alternative models: Negative vs positive



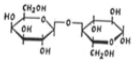
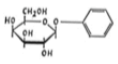
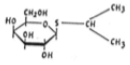
Models distinguished using constitutive mutants

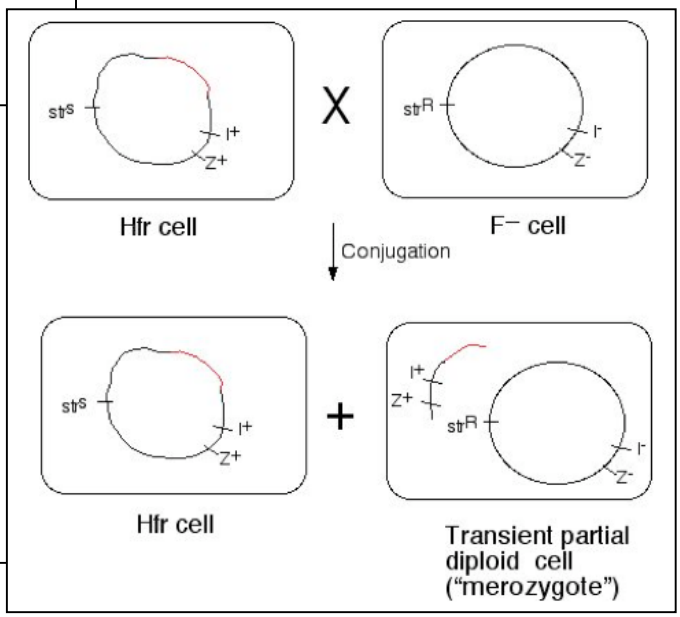
Negative regulation- predicts that the most common class of constitutive mutation would eliminate repressor

Positive regulation- predicts that the most common class of constitutive mutation would permanently activate activator

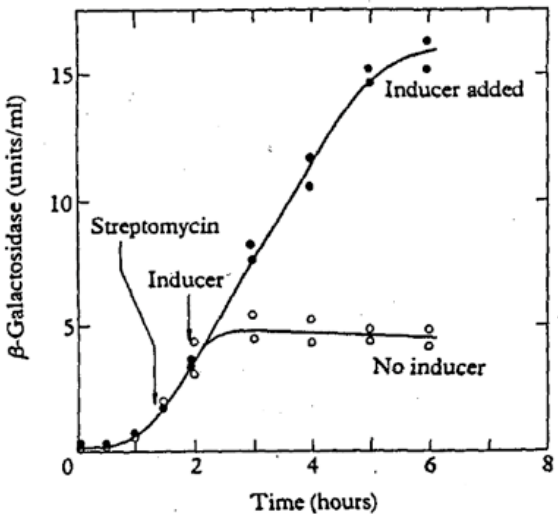
Steps:

1. Isolate constitutive mutants
2. Determine whether such mutations are dominant or recessive to wild-type

Substance	Cleaved by β-galactosidase	Induces *lac* operon transcription
lactose 	+	+
Phenyl-β,D-galactoside (PG) 	+	–
Isopropyl-β,D-thiogalactoside (IPTG) 	–	+



Predictions for positive vs negative regulation (<u>no inducer added</u>)



Why are most common constitutive mutants $lacI^-$ rather than $lacO^-$?

How could one selectively isolate $lacO^-$ mutants?