**The Lactose Operon in E. coli: Mutant Analysis**

**Part I**

You have isolated a number of independent of beta-galactosidase mutant *E. coli* strains(that is, mutant bacteria that display some non wild-type phenotype in terms of their production of beta-galactosidase, the bacterial enzyme that allows them to digest lactose). For each of your mutants, you assess the production of beta-galactosidase on different media, and report the results in the table below.

*Minimal medium: contains a source of carbon that is neither lactose nor glucose, salts, water, and all the micronutrients necessary for E.coli to grow.*

|  |  |
| --- | --- |
|  | **Production of beta-galactosidase** |
|  | ***Minimal medium (MM)*** | ***MM + IPTG*** | ***MM + IPTG + glucose*** |
| Wild-type | None | Lots | A little |
| Mutant 1 | None | None | None |
| Mutant 2 | Lots | Lots | A little |
| Mutant 3 | Some (not lots, but more than a little) | Lots | A little |
| Mutant 4 | None  | Very, very little | None |
| Mutant 5 | None | A little | A little |
| Mutant 6 | Lots | Lots | A little |

1. Draw a sketch of the lactose operon and of the lac repressor gene.

Should include the *lacZ* gene with its promoter, *lacO* site (at least one), CAP/CRP. For example: see diagram on ppt slide.

1. Based on the data in the table, which mutants could have a mutation in the *lacZ* gene itself? For each of your potential *lacZ* mutants, indicate if it would be a LOF or a GOF mutant.

Mutant 1, maybe Mutant 4 (but Mutant 4 would be hard to explain).

1. Based on the data in the table, which mutants could have a mutation in the *lacO* (the main lac operator site)? For each of your potential *lacO* mutants, indicate if it would be a LOF or a GOF mutant, and briefly explain what it would cause.

Mutant 2, loss of affinity for repressor, Mutant 3, same but less severe, Mutant 6, same as Mutant 2, Mutants1 theoretically (if the *lacO* acquired super high affinity for the repressor) and Mutant 4 (same idea, a bit less severe).

1. Based on the data in the table, which mutants could have a mutation in the *lacI* gene? For each of your potential *lacI* mutants, indicate if it would be a LOF or a GOF mutant, and briefly explain what it would cause.

Maybe Mutant 1: GOF from the perspective of the repressor (*lacI*\_ modern; *i.e.* classical *lacIS*)

Mutant 2 and/or Mutant 6: LOF from the perspective of the repressor (*lacIC* modern; *i.e.* classical *lacI\_)*

Mutant 3: LOF (but only partial) from the perspective of the repressor (*lacIC* modern; *i.e.* classical *lacI\_)*

Mutant 4: GOF from the perspective of the repressor (*lacI*\_ modern; *i.e.* classical *lacIS*)

1. Based on the data in the table, which mutants could have a mutation in the CRP/CAP binding site? For each of your potential CRP/CAP binding site mutants, indicate if it would be a LOF or a GOF mutant, and briefly explain what it would cause.

Mutations that disrupt the CAP/CRP binding site should result in no response to absence of glucose (*i.e.* beta-gal in presence or absence of glucose should stay “very little”):

Mutant 5 fits this description.

In terms of beta-gal production, it would be a LOF (always makes no, or small amounts of beta-gal).

1. Would information about the production of lactose permease (encoded by the *lacY* gene in the lac operon) be of any help to refine your answers to questions a-e? Briefly explain how you would use the information obtained.

If the presence/absence of permease correlates with that of beta-gal, and they vary together, then the mutation in question is most likely regulatory (in the lac promoter, operator, or in the *lacI*, or even in the CRP binding site) or possibly in the *lacY* coding region itself, but is not likely to be in the *lacZ* coding region.

**Part II**

In a subsequent experiment, you add to each of your strains a plasmid (artificial bacterial mini-chromosome) that carries the wild-type *lacI* gene. Then, you assess the production of beta-galactosidase in these strains containing the plasmid. The results are as follows:

|  |  |
| --- | --- |
|  | **Production of beta-galactosidase** |
|  | ***Minimal medium (MM)*** | ***MM + IPTG*** | ***MM + IPTG + glucose*** |
| Wild-type | None | Lots | A little |
| Mutant 1 | None | None | None |
| Mutant 2 | Lots | Lots | A little |
| Mutant 3 | Some (not lots, but more than a little) | Lots | A little |
| Mutant 4 | None  | Very, very little | None |
| Mutant 5 | None | A little | A little |
| Mutant 6 | None | Lots | A little |

1. This information, combined with that in Part I, should be useful to decide where the mutations are in most of the mutants.
	* + Which mutants have mutations in *lacZ*?

Possibly mutant 1 (but it could also have a GOF mutations in the *lacI* gene 🡪 super-repressor), maybe mutant 4 (most likely in the promoter).

These mutants do not get “fixed” by the addition of WT repressor. (Note: it is very difficult, with this information, to distinguish between mutations in the lac promoter and “*lacIS*” mutants (mutants of the lac repressor, that result in a super-repressor not binding to the inducer and never, or almost never falling off the *lacO*. This is because in the presence of both super-repressor and WT repressor… if the super-repressor binds to *lacO*, that’s it-it is not going to “ever” fall off).

* + - Which mutants have mutations in *lacO*?

Mutant 2, mutant 3 (don’t get “fixed” by adding WT repressor).

* + - Which mutants have mutations in *lacI*?

Mutant 6 (when you add WT *lacI*, it has a WT phenotype); also mutant 4 (GOF in the repressor?)

* + - Which mutants have mutations in the CRP/CAP binding site?

Mutant 5

1. What would be the phenotype (in terms of the production of beta-galactosidase on the various media) of each of the mutants, if you added a plasmid carrying a wild-type *lacO* sequence? Briefly explain your answer.

Presence of a plasmid that carries *lacO* should NOT matter. *lacO* can only act in cis, and there is no *lacZ* in cis of the WT *lacO* on the plasmid. So, there will be no production of *lacZ* from the plasmid.

Similarly, the *lacO* on the plasmid won’t affect the expression of the endogenous *lacZ* gene.

One possible effect (that we could imagine, but has not been observed) is that the *lacO* on the plasmid might titrate out some of the lac repressor present in the bacterium, thus potentially causing a slight decrease in the repression of *lacZ* in the absence of inducer (i.e. a little, little bit of beta-gal being produced in the absence of IPTG).