Lecture 11

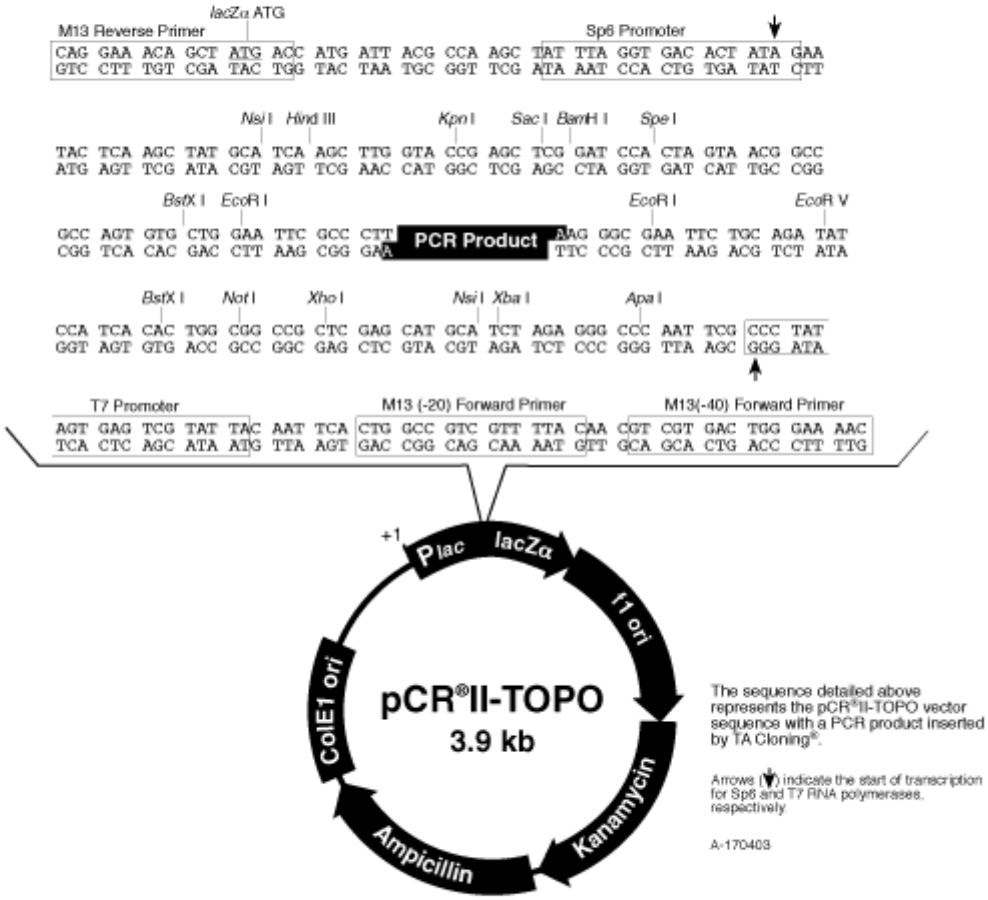
The lac operon

It's time to talk about the lactose operon, and why it keeps popping up in our experiments.

Remember this? Do you remember when we talked about this vector, pCRII-TOPO?

Comments for pCR®II-TOPO
3950 nucleotides

LacZα gene: bases 1-588
M13 Reverse priming site: bases 205-221
Sp6 promoter: bases 239-256
Multiple Cloning Site: bases 269-399
T7 promoter: bases 406-425
M13 (-20) Forward priming site: bases 433-448
M13 (-40) Forward priming site: bases 453-468
f1 origin: bases 590-1004
Kanamycin resistance ORF: bases 1338-2132
Ampicillin resistance ORF: bases 2150-3010
ColE1 origin: bases 3155-3828



The sequence detailed above represents the pCR®II-TOPO vector sequence with a PCR product inserted by TA Cloning®.

Arrows (↓) indicate the start of transcription for Sp6 and T7 RNA polymerases, respectively.

A-170403

The sequence of pCR®II-TOPO has been compiled from information in sequence databases, published sequences, and other sources. Portions of this vector have not yet been completely sequenced. If you suspect an error in the sequence, please contact Invitrogen's Technical Services Department at 800-955-6288.

U.S. Headquarters
Tel: 1-800-955-6288
Fax: 1-760-603-7201

European Headquarters
Tel: +31 (0) 594 515 175
Fax: +31 (0) 594 515 312

http://www.invitrogen.com/content/vectors/pcriitopo_map.pdf

Now we're ready to talk about a part that we overlooked previously - the portion of the lacZ gene that is included.

The lacZ gene codes for beta galactosidase, the first gene in the lac operon. By including it in the vector sequence, and plating plasmid-containing cells on media containing IPTG and the colorimetric substrate X-gal, we can do a little "trick" to find out which cells have plasmids containing insertions.

- In the pCRII-TOPO plasmid, the cloning site is actually within the lacZ sequence. If we succeed in cloning a piece of DNA into that site, the lacZ gene is interrupted and usually non-functional (Note that the multiple cloning site starts at nt. 269, right in the middle of the lacZ-alpha gene. That means that if anything is cloned into this plasmid, the lacZ-alpha gene will be interrupted.)
- If we offer cells a substrate (X-gal) that beta galactosidase can turn into an insoluble blue dye, we can see where our beta galactosidase activity resides. A blue color means beta-gal enzyme is present, while no color indicates it is not present.

There are really two principal ways in which parts of the lac operon are used in molecular biology, and this is very important to understand.

1. The lac Z gene may be used as a simple "reporter" gene, separated from its natural promoter. For example, we might put the lac Z gene in front of a mammalian promoter, transfect the DNA into mammalian cells, and then fix and stain a tissue with X-gal. The cells that turn blue must have the beta galactosidase enzyme, the product of the lac Z gene, which could only have been expressed from the donated promoter. Ergo - the promoter was active. Actually, there are controls that are needed to make this brave statement, but you get the idea. We used only the coding sequence for the bacterial enzyme as a reporter, and it has nothing to do with the lac promoter in this scenario.

2. We may use the lac promoter to drive or regulate expression of any gene in an appropriate prokaryotic cell (e.g. E. coli, where it comes from). That is, lac Z may be nowhere in the picture. We've just taken the regulatory machinery and are using it without the downstream genes.

We need to talk a bit more about each of these approaches, but first, we need to solidify your understanding of the lac operon.

Blue-white screening and the *lac* operon.

Many plasmids make use of bits and pieces of the lac operon, which you may have learned about in your genetics class. If you "lac knowledge" in this area, you might want to consult a few of these fine on-line sources:

Study resources

- Intermediate Genetics-North Dakota State. This site provides an overview of the lac operon, and this important table.

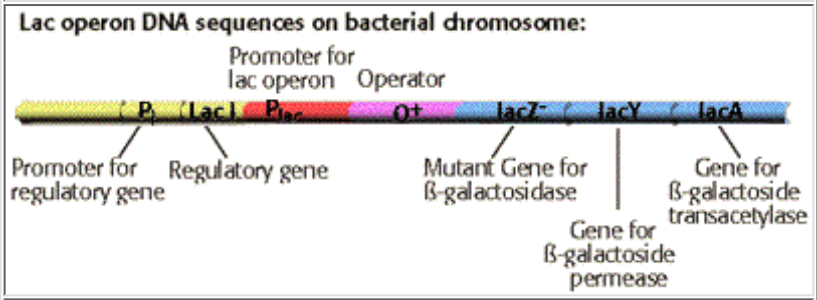
Mutant *lac* gene	Mutant Phenotype
I$^-$	constitutive expression because the operator is never closed
O$^-$	constitutive expression because the repressor protein can not bind
P$^-$	no expression of the operon because RNA polymerase cannot bind
lac Z$^-$	no glucose or galactose production from lactose
lac Y$^-$	no induction because lactose will not be taken into the cell

You should absorb the information well enough to be able to understand each row in the table, and explain the effect of the mutation at a molecular level.

Already know it all?

Test your knowledge at The Biology Project - University of Arizona

The *lac* operon



The Biology Project, University of Arizona

http://www.biology.arizona.edu/molecular_bio/problem_sets/mol_genetics_of_prokaryotes/07t.html

Many plasmids carry a portion of the lacZ gene (sometimes called lacZ-alpha), which when expressed in a cell can complement a partially deleted genomic allele (lacZ-delta-M15) and between the two parts form a functional beta galactosidase enzyme.

Here's the picture:

By plating the colonies in the presence of isopropyl beta-D-thiogalactoside (**IPTG**) and the color-changing substrate 5- bromo- 4- chloro- 3- indolyl- beta- D- galacto- (arentyougladyoutookorganic)- pyranoside (**X-gal**), one can obtain the following color test:

State of the plasmid...	Intact lacZ-alpha gene	Interrupted lacZ-alpha
The cause?	No insertion at ligation	Insertion
State of the X-gal...	Cleaved by lacZ protein	No cleavage
Color of colony on plate?	**BLUE**	WHITE

The reason this works is that the IPTG de-represses the lac operon, by binding to the lac repressor (the lac I gene product), preventing it from binding to the operator. The lacZ-alpha fragment is therefore transcribed and translated, and forms a functional beta-galactosidase enzyme. The substrate X-gal, when cleaved, leaves a water-insoluble blue product that marks the colonies. A blue color indicates an intact lacZ-alpha gene, and that implies that no interruption of the gene took place during cloning.

This method is called "blue-white screening" (or sometimes as a misnomer "blue-white selection"), and it allows one to identify which colonies contain plasmids with inserted sequences, based solely on their lack of blue color.

An exam question on blue-white screening

from James Cook University, North Queensland

12. Students have carried out an experiment in which they have constructed recombinant plasmids. The plasmids have the blue/white selection system. Two ligations were performed and all transformations were carried out with 200 ng of plasmid DNA and in one case also with the addition of 200 ng of desirable DNA

unrestricted plasmid	restricted and dephosphorylated plasmid	restricted and dephosphorylated plasmid, ligated	restricted and dephosphorylated plasmid, ligated with desired DNA fragment
200 blue colonies	180 blue colonies; 2 white colonies	210 blue colonies; 1 white colony	195 blue colonies; 12 white colonies

fragment. After transforming E.coli JM101 with the DNA mixtures, 100 ul aliquots were plated out on 2YT agar plates containing 150 ug/ml of ampicillin, as well as appropriate quantities of X-gal and IPTG. After overnight incubation at 37 ºC the plates were examined. The results from their experiments are listed in the table above. Please comment on these results. (10 mins)

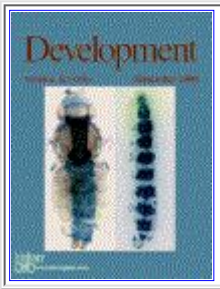
Our use of the lac operon in lab

The blue-white screening method is only one use of the lac operon. The beta galactosidase enzyme is often used as a "reporter gene" for expression in recombinant tissues.

For example:

beta galactosidase assay kit from Specialty Media, and from Gene Therapy Systems

Differential staining of transgenic mice (brain example from Tsien et al. and liver example from Lodish lab) or transgenic fly brains.



Development 127 (18)

The cover shows an adult fly and a larva of Drosophila of genotype pnr-Gal4/UAS-lacZ stained with X-gal. The expression domain of pnr appears in blue and defines a dorsal region in most of the body segments of both the larva and the adult insect. For further details see article by M. Calleja, H. Herranz, C. Estella, J. Castal, P. Lawrence, P. Simpson and G. Morata, in this issue, Development 127, 3971-3980.
http://dev.biologists.org/

Development 127 (12)

The cover shows the exorbital lobe of the mouse lacrimal gland at the day of birth. The epithelial component of the gland expresses a lacZ reporter based on the Pax6 gene and, as a consequence, labels blue with the lacZ substrate X-gal. The gland was excised from the mesenchyme underlying the skin and flattened under a coverslip after labeling. The iterative branching and terminal acini of the developing structure are clearly visible. The gland is surrounded by unlabeled cells of mesenchymal origin. For further details, see the article by Makarenkova, H. P., Ito, M., Govindarajan, V., Faber, S. C., Sun, L., McMahon, G., Overbeek, P. A. and Lang, R. A. in this issue, Development 127, 2563-2572.
http://dev.biologists.org/

A quite different part, put to work.

Now that we've talked about how to make pretty pictures using X-gal and expressed beta galactosidase, lets turn our attention to a slightly different use of the lac operon. That is, using ONLY the lac promoter/operator region, and not the beta galactosidase

Here's an example of an application

> Secretion of Mouse-Metallothionein by Engineered E. coli Cells

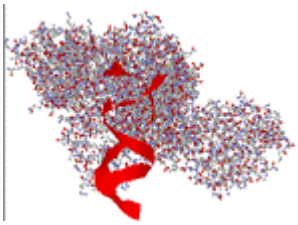
Here's a general discussion of some types of expression systems. We'll have much more about this later in the course.

Is it actually useful to know all this information?

> Why yes - if you want to talk to the "bikini princess", understanding the lac operon and modern expression systems will be critical!

Get it on Protein Explorer

The PDB code for lac repressor co-crystalized with DNA is 1EFA



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