Lecture 3 - E. coli and Plasmids (AMG text pp. 31-42)
August 28, 2000

E. coli strains have been developed that are optimized for recombinant DNA applications.

Where did E. coli K-12 come from and why was it chosen as the bacterial strain for molecular genetics?

Is E. coli K-12 the same bacterial strain that causes fatal cases of food poisoning? What is the difference?

The lac operon of E. coli is a very useful system in applied molecular genetics. The lac operon of E. coli has been modified to permit inducer-mediated regulation of gene expression.

LacI<sup>q</sup> is an over-expressing strain of E. coli that makes large amounts of lac repressor protein (off is really OFF). By introducing a recombinant plasmid that contains a gene of interest inserted downstream of the lac promoter, in this case, the lac UV5 promoter which is super activated by RNA polymerase and CAP, it is possible to induce expression of the cloned gene by adding isopropylthiogalactoside to the culture.

What is IPTG and how does it work to regulate gene expression in this system?

Lac repressor is a DNA binding protein with a Kd for non-specific DNA sequences in the micromolar range. What accounts for the highly specific repression of lac-regulated genes considering that Lac repressor binds non-specific DNA so well (micromolar Kd)?

A second modification of the lac operon for use in molecular genetics, is its application to cloning experiments based on a phenomenon called "alpha complementation".

How would the presence of blue or white bacterial colonies on an agar plate be used to distinguish between bacteria containing functional alpha β-galactosidase polypeptide, and those which express defective alpha protein (or no alpha protein at all)?

What is X-gal and how is it used in this assay?

Three general biochemical process have been genetically eliminated in E.coli K-12 strains used for cloning:

1. Bacterial restriction modification systems have been removed.
2. DNA recombination systems are modified to prevent rearrangements.
3. Endonuclease activity has been mutated to increase plasmid yields.

Restriction modification systems have been removed from E. coli K-12 strains because they will interfere with the replication of foreign DNA in bacterial cells. The bacteria will degrade foreign DNA the same way bacteriophage DNA is degraded as a defense against invasion!

The EcoK restriction system encodes proteins that degrade foreign DNA that is not properly methylated a the sequence 5’-AAC-(N)5-GTGC-3’ which would occur in cloned DNA.
The second type of modification system is that of the mcrA/mcrB/mrr complex which degrades foreign DNA that is not properly methylated, such as methylated DNA obtained from mouse and human cells which contains CpG methylated DNA.

*Can the same strain of E. coli K-12 be used for all molecular genetic applications?*

*Why might this not be such a great idea and instead use different strains for different applications?*

*What might also explain the availability of so many different “optimal” E. coli K-12 strains for cloning?*

**Plasmid Biology**
Three types of naturally occurring bacterial plasmids have been exploited for molecular genetic applications;

- *virulence plasmids* encoding bacterial toxins such as colicins (ColE1 plasmid ori is in cloning vectors),

- *conjugation plasmids* (F plasmid is used to carry lacI gene and binding protein for M13 phage), and drug

- *resistance plasmids* (R plasmids have been a source of antibiotic resistance genes).

*How are antibiotic resistance genes used in applied molecular genetics?*

*Can you think of any reason why an ampicillin-resistant E. coli K-12 colony might not contain a copy of the recombinant plasmid (you isolated DNA from amp-resistant cells and found no plasmid)?*

**Cloning vectors** are DNA molecules that are used to "transport" cloned sequences between biological hosts and the test tube (e.g., from bacterial cells to test tubes to plant cells).

Cloning vectors share **four common properties:**

1. Ability to promote **autonomous replication**.

2. Contain a **genetic marker** (usually dominant) for selection.

3. **Unique restriction sites** to facilitate cloning of insert DNA.

4. **Minimum** amount of nonessential DNA to optimize cloning.

The most useful cloning vectors are obtained from commercial sources, such as Stratagene Cloning Systems, Promega, Invitrogen.