

**Lecture 4 - Transformation and Phage** (AMG text pp. 42-55)  
August 30, 2000

The two primary methods of **bacterial transformation** are the **CaCl<sub>2</sub>/Heat Shock** chemical transformation method, and **Electroporation**. Both methods work well, although electroporation is more efficient. The molecular mechanism of bacterial transformation by these methods is not completely understood.

Chemical transformation utilizes CaCl<sub>2</sub> and heat to stimulate DNA uptake. This method is simple and convenient but transformation efficiencies are relatively low.

DNA transformation by **electroporation** is done by delivering a short electric pulse to cells that are suspended in low ionic strength solution in a special cuvette. Transformation frequencies using electroporation are **10<sup>2</sup> - 10<sup>3</sup> higher** than with CaCl<sub>2</sub>.

*What is the chemical property of biomembranes that prevents exogenous DNA from diffusing into cells?*

*What are some plausible explanations for how DNA transformation by chemicals and electroporation might work?*

**Lambda phage** is a bacterial virus that infects E. coli, and depending on early events (and genetics), can either multiply within cells leading to **cell lysis**, or the viral DNA can integrate into the bacterial genome in a process called **lysogeny**.

If **lambda gene transcription** is *activated*, then lytic infection occurs leading to phage release and infection of nearby E. coli cells. Alternatively, if **lambda gene transcription** is *repressed*, then the infection is lysogenic resulting in lambda DNA integration into the E. coli genome. Applied molecular genetic methods used in the lab are designed to take advantage of cell lysis.

The **plaque formation assay** can be used to isolate pure strains of lambda phage by using a low multiplicity of infection (*MOI*). Bacteriophage plaques are physical areas on a petri plate where the bacteria have all lysed due to multiple rounds of infection by a single clonal phage. Each plaque contains about *1 million virus particles* derived from a single infection event. The viral particles in a single plaque are therefore clonally related (same DNA sequence).

*What does it mean to "titer a phage stock" and how is it done?*

*Why are the host bacteria grown in media containing the sugar maltose prior to infection?*

Sometimes it is desirable to purify large amounts of phage for the purpose of isolating recombinant DNA from a clonal isolate. One method to do this is to infect a **liquid culture** of E. coli with phage stock and then harvest the lysate 4-8 hours later. The bacterial debris (lysed cells) is removed by low speed centrifugation and then **polyethylene glycol (PEG)** is added to the supernatant and the phage are then pelleted by high speed ultracentrifugation.

*Why does it matter how much phage stock you add to the bacterial liquid culture, doesn't it all just mix around and lead to 100% infection eventually?*

*What is the biochemical explanation for adding PEG to the phage suspension?*

*Once you had obtained a highly concentrated stock of purified phage, how would you separate the recombinant phage DNA away from the phage head and tail proteins?*

Two important **technical hurdles** had to be overcome before lambda vectors could be utilized as cloning reagents for genomic and cDNA libraries.

1. In vitro lambda phage **packaging extracts** were developed and made available commercially (see Stratagene Gigapack packaging extracts).

2. Genetic strategies were developed to **increase the yield of recombinant phage** in DNA libraries to make up for the low ratio of insert to vector DNA used in the initial ligation reactions.

The first of these genetic strategies was developed for **cDNA libraries** and the second for **genomic DNA libraries**. Note that the total length of lambda DNA that can be efficiently packaged is a crucial determinant in this protein-driven process with a maximum limit of 52 kb (vector + insert).

**Insertional cloning into the cI gene** of the lambda-gt10 cDNA cloning vector (DNA inserts of ~1-5 kb) can be selected in hfl (high frequency of lysogeny) mutant strains of E. coli. In hflA strains of E. coli, expression of the lambda cII gene is elevated, resulting in transcriptional induction of the lambda cI repressor gene which promotes lysogeny. Disruption of the lambda cI coding sequence by DNA insertion into the unique EcoRI site of the lambda gt10 cDNA cloning vector, blocks the lysogenic pathway **leading to cell lysis and plaque formation**.

**Replacement of  $\lambda$  DNA** containing the *red* and *gam* genes in the EMBL3 genomic DNA cloning vector with BamHI compatible DNA inserts of ~10-20 kb, permits lytic growth of recombinant phage in E. coli strains containing the P2 bacteriophage lysogen.

*Does lambda DNA packaging require any enzymes or ATP hydrolysis?*

*Why is it important to use low ratios of insert DNA to vector DNA in the construction of lambda DNA libraries, wouldn't it just be easier to increase the ratio to decrease the number of "empty vectors" in the library stock?*

**M13 filamentous phage** have a single strand genome that exists temporarily inside infected E. coli cells as a double strand plasmid. M13 phage are budded off of an infected cell and single strand DNA can be purified for use in **DNA sequencing or in vitro mutagenesis**. Initially M13 phage vectors required a working knowledge of phage biology and was primarily used for creating single strand DNA molecules for DNA sequencing. Fortunately, M13-derived cloning vectors called "**phagemids**" have been developed which take advantage of M13 replication to produce single strand molecules, but can be propagated as conventional ColE1-based replicating double strand plasmids.

**Bluescript KS+** is an example of a phagemid cloning vector. Often times, phagemid vectors are used to prepare single strand DNA for in vitro mutagenesis protocols using oligonucleotide primers (chapter 3).

Infection of *E. coli* F<sup>+</sup> cells containing phagemid DNA with replication-deficient M13 helper phage results in the packaging of single strand phagemid DNA. The **orientation of the M13 replication origin** (+ or -), relative to the insert DNA, in the phagemid vector, determines which strand of the insert DNA (**coding or non-coding**) will be contained in the packaged phage.

Since the bacterial sex pilus proteins are encoded on the **F' plasmid**, and the pilus is required for M13 phage attachment, *E. coli* strains have been developed that include a transposon encoded antibiotic resistance gene on the F' plasmid (tetr or kanr). Rolling circle replication and phage packaging by helper virus proteins result in the production of recombinant M13 phage.

*Why is a helper phage required to produce single strand DNA from an M13 phagemid?*

*What does the orientation-dependence of the M13 ori tell you about filamentous phage replication and how is this different than lambda phage replication?*

**High level expression of recombinant proteins in bacteria** is a common process in Biotechnology. The production of human insulin in bacteria is one such example. Another example is the production of plant calreticulin protein in bacteria as a means to generate anti-calreticulin antibodies in rabbits.

Regulated expression of genes in *E. coli* using the **pET vector system**. The two essential components of this T7 bacteriophage RNA polymerase-based expression system are the pET plasmid containing the coding sequence of a DNA insert downstream of the **T7 promoter** (Tpr), and an *E. coli* strain containing a genomic copy of the **T7 RNA polymerase** (T7 pol) gene under the control of the lac UV5 promoter.

Also shown in this example is a **T7 lysozyme** encoding plasmid containing the chloramphenicol-resistance gene (cat), which provides a means to inhibit the small amount of T7 RNA polymerase that is expressed in the absence of IPTG. High level expression of the histidine-tagged calreticulin protein is obtained by blocking lacI repressor function with the addition of IPTG to the media.

Homework 2 - "My pET Wabbit eats Weeds"