Unit 2: Recombinant DNA Technology

Lecture: M13 Phagemid as cloning vector

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M13 Phage

The bacteriophage known as "M13" forms the basis of cloning systems designed to easily introduce mutations into genes inserted into the phage genome. It also has been used in various "phage display" methodologies and "combinatorial" DNA and peptide libraries.

- M13 is a filamentous bacteriophage which infects *E. coli* host.
- The M13 genome has the following characteristics:
  - Circular *single-stranded* DNA
  - 6400 base pairs long
  - The genome codes for a total of 10 genes (named using Roman numerals I through X)

Refer to the 2nd slide M13 genome map figure provided to you on your e-mail/whatsapp group.
- Gene VII codes for the major structural protein of the bacteriophage particles
- Gene III codes for the minor coat protein
- The gene VII protein forms a tubular array of approx. 2,700 identical subunits surrounding the viral genome
- Approximately five to eight copies of the gene III protein are located at the ends of the filamentous phage (i.e. genome plus gene VII assembly)
- Allows binding to bacterial "sex" pilus
  - Pilus is a bacterial surface structure of *E. coli* which harbor the "F factor" extrachromosomal element

Refer to the 3rd slide lambda phage figure provided to you on your email/whatsapp group
Infection

Single strand genome (designated '+' strand) attached to pilus enters host cell
Major coat protein (gene VIII) stripped off
Minor coat protein (gene III) remains attached
Host components convert single strand (+) genome to double stranded circular DNA (called the replicative or "RF" form)
Transcription begins
Series of promoters
Provides a gradient of transcription such that gene nearest the two transcription terminators are transcribed the most
Two terminators
One at the end of gene VIII
One at the end of gene IV
Transcription of all 10 genes proceeds in same direction

Refer to the 4th slide lambda phage infection and duplication figure provided to you on your email/whatsapp group
Amplification of viral genome
Gene II protein introduces 'nick' in (+) strand
Pol I extends the (+) strand using strand displacement (and the '-' strand as template)
After one trip around the genome the gene II protein nicks again to release a completed (linear) '+' genome
Linear (+) genome is circularized
During first 15-20 minutes of DNA replication the progeny (+) strands are converted to double stranded (RF) form
These serve as additional templates for further transcription
Gene V protein builds up
This is a single stranded DNA binding protein
Prevents conversion of single (+) strand to the RF form
Now get a buildup of circular single stranded (+) DNA (M13 genome)

Refer to the 5<sup>rd</sup> slide lambda phage infection and duplication figure provided to you on your email/whatsapp group
Phage packaging

Major coat protein (Gene VIII) present in *E. coli* membrane

M13 (+) genome, covered in ss binding protein - Gene V protein, move to cell membrane

Gene V protein stripped off and the major coat protein (Gene VIII) covers phage DNA as it is extruded out

Packaging process is therefore *not linked to any size constraint* of the M13 genome

Length of the filamentous phage is determined by size of the DNA in the genome

Inserts of up 42 Kb have been introduced into M13 genome and packaged (7x genome size)

~8 copies of the Gene III protein are attached at the end of the extruded genome

Refer to the 6th slide lambda phage infection and duplication figure provided to you on your email/whatsapp group
Development of M13 into a cloning vector
M13 was developed into a useful cloning vector by inserting the following elements into the genome:

- a gene for the *lac* repressor (*lac I*) protein to allow regulation of the *lac* promoter
- the operator-proximal region of the *lac Z* gene (to allow for a-complementation in a host with operator-proximal deletion of the *lac Z* gene).
- a *lac* promoter upstream of the *lac Z* gene
- a polylinker (multiple cloning site) region inserted several codons into the *lac Z* gene

Refer to the 7th slide lambda phage insertion map and duplication figure provided to you on your email/whatsapp group.
The vectors were named according to the specific polyliner region they contained. The vectors were typically constructed in pairs, with the polyliner regions in opposite orientations.

Refer to the 8th slide lambda phage polyliner map and duplication figure provided to you on your e-mail/whatsapp group.

_Cloning into M13mp vectors_

The RF (double stranded) form of the M13 phage can be isolated and treated just like any other plasmid. The polyliner region can be "opened" using restriction endonucleases appropriate for accepting the fragment of interest. The fragment is ligated into the plink region. The availability of inverse oriented plink's (e.g. mp18, mp19) means that inserted fragments with non-complementary ends can be inserted in either orientation.
Single stranded forms of the phage
The ability to isolate a single stranded form of the phage has advantages in both sequencing and mutagenesis.

- Single stranded DNA template can be read further than double stranded template.
- An efficient mutagenesis method (the "Kunkel" method) was developed using the single stranded form of the phage.
- The M13mp vector with insert is first grown in a mutant *E. coli* host (e.g. CJ236) which would occasionally incorporate uracil into the DNA instead of thymidine.
  - *E. coli* normally synthesizes an enzyme (uracil-N-glycosidase) that removes uracil residues in DNA. However, in *ung* strains, the uracil is not removed.

- The level of uracil mis-incorporation into DNA is enhanced in strains which have a deficiency in dUTPase. This enzyme converts dUTP to dUDP, and therefore, in *dut* strains the levels of dUTP are elevated and enhance misincorporation of dUTP into host DNA.

Refer to the 9th slide lambda phage kunkel method replication figure provided to you on your email/whatsapp group.
- The *E. coli* strain CJ236 has a genotype which includes dut-/ung- features
- A mutagenic primer would be annealed to this single strand template (e.g. to produce a point mutation)
- The primer is extended using the four dNTP's, and is ligated to produce duplex DNA
- The duplex DNA is inserted into a different host *E. coli* (e.g. JM101) which recognizes and excises (degrades) uracil containing DNA (i.e. a strain which is ung+)
- The parent (wild type) strand is preferentially degraded and the mutagenic strand is replicated.
- The phage progeny typically have a high incidence (80-90%) of the desired mutation.

Refer to the 10th slide lambda phage kunkel method replication figure provided to you on your email/whatsapp group
Advantages of modified M13 vectors

1. Facilitate directional cloning by using two different enzymes for cleavage

2. Cloning in dual vectors is advantageous

3. Cloned DNA fragments can be moved between M13mp vectors and pUC vectors with great ease

4. The intergenic region of M13 is now standard fixture of most plasmids

5. Single stranded DNA can be directly isolated from M13 phages