# Cloning and Expression of a Bacteriophage DNA Ligase for molecular cloning

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**Introduction**

During the last 50 years, major advances in molecular biology and biotechnology were attributed to the discovery of enzymes that allow molecular cloning of important genes. One of these enzymes that has been widely acknowledged for its role in the development of biotechnology is the T4 DNA ligase. This enzyme joins the break in the DNA backbone structure by creating a phosphodiester bond between 5' PO4 and 3' OH ends, in an ATP dependent multi step reaction (3-5). It functions in vivo in the replication and recombination of phage DNA (1,3). Moreover, living organisms use the activity of the DNA ligase in the process of genome duplication, genes rearrangement, and several other tasks including proof editing and repair of genomic DNA. Biotechnologists use these characteristics of DNA ligase to clone genes of interest involved in numerous aspects of medical, animal and plant research. Due to its role in modern DNA recombinant technology, there is a high demand on DNA ligase to allow the ligation of target DNA inserts into a chosen vector as part of DNA cloning technology. The aim of this project is to isolate a bacteriophage from which the DNA ligase gene will be cloned and expressed. The overall goal is to clone a bacteriophage DNA ligase and make it available locally for researchers. Following expression, the enzyme will be tested for activity, and quality control tests will be carried out to compare it with commercially available ligases.

**Materials and Methods**

The work will be carried out in three stages; the 1$^{st}$ stage includes the isolation of bacterial strain-specific phage, the 2$^{nd}$ includes the cloning of DNA ligase gene from the bacteriophage genome, and the 3$^{rd}$ stage will include gene expression, enzyme purification and quality tests of the DNA ligase.

**Bacteriophage isolation**: bacteriophages present where bacteria are present. We isolated bacteriophages specific for *DH5α* strain of *E. coli* from a wastewater sample obtained from Hebron South sewage system. The process involved a pre-incubation of the wastewater sample with *DH5α* for enrichment and amplification of the bacteriophage population. Then, a plating technique was used to detect a strain specific bacteriophage for *DH5α* from the enriched sample. The plating technique "plaque assay" involves seeding a "lawn" of host bacteria with a small volume of sample containing phage. When phage seeded on the lawn infect and lyse the host cells, they produce clear zones which called plaques as a result of cell lysis, these plaques indicate the presence of bacteriophage which will be used for DNA isolation (1).

**Cloning and expression of gene encoding the DNA ligase**: The T4 DNA ligase cDNA will be amplified based on sequence homology with other phage ligases and subsequently cloned using commercially available gene cloning system. Following verification by sequencing, the cDNA will be subcloned in expression vector to facilitate enzyme purification.

**Results**

A *DH5α* specific bacteriophage has been successfully isolated from wastewater sample collected from Hebron south. To determine if the phage is specific to *DH5α*, we have isolated native *E.coli* bacterial cells using EMB media that is used to distinguish *E.coli* in green color from a pool of other bacteria. After the addition of bacteriophage enrichment culture to two different plates, one of them containing isolated *E.coli*, and the other plate containing *DH5α*;

the lysis appeared only in the plate of *DH5α* and no cell lysis was observed in the plate of *E.coli* an indication of the specificity of the bacteriophage to *DH5α* strain. A 1.5 Kb cDNA corresponding to the expected size of the T4 DNA ligase was amplified and cloned in a cloning vector. Work is underway to verify the gene and subclone it in an expression vector to produce the enzyme. This step will be followed by enzyme activity tests and quality control assays.

**References**

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