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Standard Guide for Identification of Bacteriophage Lambda (λ) or Its DNA[1]

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INTRODUCTION

This guide is intended to determine the identification of bacteriophage lambda or its DNA. The objective is to describe laboratory characterization procedures that are sufficient to verify that a biological preparation believed to contain lambda or lambda DNA for use in any step of a biotechnology process actually does contain this bacteriophage or its DNA.

This guide assumes a basic knowledge of virology and molecular biology.

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1. Scope

1.1 This guide covers the procedures for identifying bacteriophage lambda used in biotechnology.

1.2 There are hundreds of lambda variants that can be used for biotechnology. These lambda variants are derived from wild type lambda and differ in genome size and genotype.

1.3 If the bacteriophage lambda is to be used to construct a recombinant molecule, then the same criteria as prescribed in Section 5 should be used to characterize the newly made DNA.

2. Referenced Documents

2.1 *ASTM Standards:*

E 1873 Guide for Detection of Nucleic Acid Sequences by the Polymerase Chain Reaction Technique[2]

3. Terminology

~~2~~3.1 *Definitions:*

~~2~~3.1.1 *bacteriophage*—a virus that infects bacteria.

~~2~~3.1.2 *induction*—the relief of repression of transcription of lysogenic phage genes encoding the functions for lytic growth, so that the phage will grow lytically.

~~2~~3.1.3 *lysogen*—a bacterial strain that has a phage stably maintained. In the case of lambda, the phage is integrated into the host genome. The integrated phage is called a prophage.

~~2~~3.1.4 *multiplicity of infection*—the ratio of infecting phage to host bacteria.

~~2~~3.1.5 *temperate bacteriophage*—a bacteriophage that can grow lytically, killing the host, or can exist stably in the host.

~~2~~3.1.6 *vector*—a fragment of DNA usually containing an origin of replication that is engineered to accept a foreign piece of DNA.

~~2~~3.1.7 *wild type*—the naturally occurring, original isolate.

~~3~~4. General Information

~~3~~4.1 Bacteriophage lambda is a temperate bacteriophage with an icosahedral head about 50 nm in diameter. There is a single, non-contractile tail about 150 nm long, ending in a single tail fiber.[3]

~~3~~4.2 The genome of lambda consists of a single molecule of linear double-stranded DNA with a length of about 49 kilobase pairs for wild type lambda. The ends of the genome are cohesive; DNA molecule is terminated by single-stranded regions of complementary base sequence allowing circularization of a molecule. The sequence of the entire phage genome has been determined.[3]

~~[2] Hendrix, R., Roberts, J., Stahl, F., and Weisberg, R., *Lambda II*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1983.~~

[2] *Annual Book of ASTM Standards*, Vol. 11.05.

~~[3] Maniatis, T., Fritsch, E., and Sambrook,~~

[3] Hendrix, R., Roberts, J., Stahl, F., and Weisberg, R., ~~*Molecular Cloning*~~*Lambda II, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1982~~3.

34.3 The naturally preferred host is *Escherichia coli* K12. The wild type phage makes turbid plaques. Many variants, however, have mutations in the *cI* gene encoding repressor. These variants produce clear plaques.[3]

34.4 Bacteriophage lambda are used primarily as DNA vectors for cloning DNA fragments. These vectors have been engineered to accept easily the foreign DNA. The DNA sequences of many vectors have been altered from the wild type, that is, whole (nonessential) regions have been deleted. Wild type lambda DNA, when cut with restriction enzymes, is used also as molecular weight markers in polyacrylamide or agarose gel electrophoresis.

4. [3] Mice transgenic for bacteriophage lambda have been constructed to enable mutation detection in the mouse genome.[4,5]

5. Bacteriophage Growth and Purification

45.1 Phage can be grown by any one of a number of published protocols,[3] as follows:

45.1.1 Phage can be grown lytically by infecting a host at a multiplicity of infection of usually less than one. Infection requires magnesium (Mg^{++}). The culture is grown until lysis is evident (cell debris will be seen in the culture), usually several hours. Chloroform is added to kill remaining unlysed cells and the bacterial debris is centrifuged out. The phage remains in the supernatant fraction.

45.1.2 Phage can be grown by inducing a phage lysogen. The more widely used lambda cloning vectors carry *cI* temperature sensitive (ts) mutations so that induction of the lysogen occurs by raising the temperature of the culture. The culture is grown after induction until lysis is evident.

45.2 Many phage vectors have a mutation in the *S* gene that encodes a protein necessary for lysis of the host. Such mutant phage will not lyse the host. Often such phages have a temperature sensitive repressor gene and exist as lysogens. The phage can be induced by raising the temperature and, 90 min later, collecting the cells by centrifugation. The supernatant fraction can be discarded, as it contains no phage. The cells are resuspended in a small volume and lysed by the addition of chloroform.

45.3 It is important that contaminating host (*E. coli*) DNA be removed from the preparation by treatment with DNase prior to isolating lambda DNA. Phage must be maintained in 10 m*M* Mg^{++} to maintain stability of virions. Phage particles can be concentrated by polyethylene glycol precipitation. If viable phage are desired, for purposes other than for DNA extraction, concentration should not be more than 50-fold, and resuspension, after precipitation, should be gentle.

56. Characterization

56.1 Inasmuch as the uses of lambda are almost exclusively for its DNA, characterization of the DNA by restriction enzyme analysis is the criterion for judging uncontaminated, pure lambda. Before characterization, one should know the expected restriction enzyme sites in the particular lambda variant.

56.2 Once purified virions are obtained, DNA can be extracted using a number of protocols, all of which involve denaturation of the phage proteins.[3] Phage DNA can be collected and concentrated by ethanol precipitation. To avoid shearing of phage DNA, preparations should be mixed gently.

56.3 Restriction enzyme analysis of DNA shall be accomplished following any one of a number of published protocols or references to protocols.[6]

56.4 The companies that supply the enzymes provide protocols or references to protocols. It is important to note that the phage DNA should be heated at 65°C for 5 min, after restriction enzyme incubation, to denature the cohesive ends.

6.5 The presence and identification of lambda DNA can be accomplished by polymerase chain reaction.

6.5.1 For general information on detection of DNA by PCR see Guide E 1873.

6.5.2 Primers for the detection of bacteriophage lambda should be chosen based on the reason for detection. Since a bacteriophage lambda genome integrated into E. coli will split in the attachment region (att), care should be taken to avoid primers from the att region.

7. Keywords

7.1 bacteriophage; cloning vector; lambda; PCR; polymerase chain reaction; recombinant DNA

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[4] Gossen, J.A., De Leeuw, W.J.F., Tan, C.H.T., Zwarthoff, E.C., Berends, F., Lohman, P.H.M., Knook, K.L. and Vijg, J. Proc. Natl. Acad. Sci. USA, Vol. 86, 1989, pp.7971–7975.

[5] Kohler, S.W., Provost, G.S., Fieck, A., Krezt, P.L., Bullock, W.O., Sorge, J.A., Putman, D.L., and Short, J.M. Proc. Natl. Acad. Sci. USA, Vol. 88, 1991, pp. 7958–7962.

[6] Sambrook, J., Fritsch, E.F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual, Second Edition*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.