



Standard Guide for Identification of Bacteriophage Lambda (λ) or Its DNA¹

This standard is issued under the fixed designation E1285; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ϵ) indicates an editorial change since the last revision or reapproval.

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.

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*:²

E1873 Guide for Detection of Nucleic Acid Sequences by the Polymerase Chain Reaction Technique

3. Terminology

3.1 *Definitions*:

3.1.1 *bacteriophage*—a virus that infects bacteria.

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.

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.

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

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

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

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

4. General Information

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

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

4.3 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

4.4 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

¹ This guide is under the jurisdiction of ASTM Committee E55 on Manufacture of Pharmaceutical Products and is the direct responsibility of Subcommittee E55.04 on General Biopharmaceutical Standards.

Current edition approved Nov. 1, 2006. Published November 2006. Originally in 1989. Last previous edition approved in 2001 as E1285 – 01. DOI: 10.1520/E1285-06.

² For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

³ Hendrix, R., Roberts, J., Stahl, F., and Weisberg, R., *Lambda II*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1983.

used also as molecular weight markers in polyacrylamide or agarose gel electrophoresis.³ Mice transgenic for bacteriophage lambda have been constructed to enable mutation detection in the mouse genome.^{4,5}

5. Bacteriophage Growth and Purification

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

5.1.1 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.

5.1.2 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.

5.2 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.

5.3 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 mM 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.

6. Characterization

6.1 Bacteriophage lambda has various different uses however, 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.

6.2 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.

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

6.4 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 [E1873](#).

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

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., *Proceedings of the National Academy of Sciences of the United States*, 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., *Proceedings of the National Academy of Sciences of the United States*, 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.

ASTM International takes no position respecting the validity of any patent rights asserted in connection with any item mentioned in this standard. Users of this standard are expressly advised that determination of the validity of any such patent rights, and the risk of infringement of such rights, are entirely their own responsibility.

This standard is subject to revision at any time by the responsible technical committee and must be reviewed every five years and if not revised, either reapproved or withdrawn. Your comments are invited either for revision of this standard or for additional standards and should be addressed to ASTM International Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee, which you may attend. If you feel that your comments have not received a fair hearing you should make your views known to the ASTM Committee on Standards, at the address shown below.

This standard is copyrighted by ASTM International, 100 Barr Harbor Drive, PO Box C700, West Conshohocken, PA 19428-2959, United States. Individual reprints (single or multiple copies) of this standard may be obtained by contacting ASTM at the above address or at 610-832-9585 (phone), 610-832-9555 (fax), or service@astm.org (e-mail); or through the ASTM website (www.astm.org). Permission rights to photocopy the standard may also be secured from the ASTM website (www.astm.org/COPYRIGHT/).