Designation: E 1285 – 89 (Reapproved 2000)

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

This standard is issued under the fixed designation E 1285; 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. Terminology

2.1 *Definitions:*

2.1.1 *bacteriophage*—a virus that infects bacteria.

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

2.1.3 *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.

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

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

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

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

3. General Information

3.1 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.[2]

3.2 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.[2]

3.3 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.[2]

3.4 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.[2]

4. Bacteriophage Growth and Purification

4.1 Phage can be grown by any one of a number of published protocols,[2] as follows:

4.1.1 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.

4.1.2 Phage can be grown by inducing a phage lysogen. The more widely used lambda cloning vectors carry *cI* temperature

[1] This guide is under the jurisdiction of ASTM Committee E48 on Biotechnology and is the direct responsibility of Subcommittee E48.02 on Characterization and Identification of Biological Systems.

Current edition approved Feb. 24, 1989. Published April 1989.

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