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Standard Guide for Identification of Bacteriophage M13 or Its DNA[1]

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INTRODUCTION

This guide covers the identification of bacteriophage M13 or its DNA and was developed by Task Group E48.02.03 on viruses. The objective is to describe laboratory characterization procedures that would be sufficient to verify that a biological preparation believed to contain M13 (or M13 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 microbiology and molecular biology.

1. Scope

1.1 This guide covers the identification of bacteriophage M13 used in biotechnology.

1.2 There are many variants of M13 that have been developed specifically for cloning technology. These variants have foreign DNA inserted into the M13 genome, causing the M13 to differ in size and genotype.

1.3 If the M13 is to be used to construct a recombinant molecule, then the criteria described in Section 6 should be used to characterize the newly made DNA.

2. Terminology

2.1 *Definitions:*

2.1.1 *alpha complementation*—the ability of a short amino-terminal fragment (alpha fragment) of β-galactosidase to form a functional complex with the carboxyl terminal fragment (omega fragment).

2.1.2 *F*—an F factor that contains a portion of the *E. coli* genome.

2.1.3 *F factor*—an episome of *E. coli*. Encoded on it are the functions necessary to produce an F pilus.

2.1.4 *F pilus*—a protrusion on *E. coli* that is necessary for mating. The F pilus also contains the receptor for phage M13.

2.1.5 *multiple cloning site*—DNA that contains several contiguous restriction enzyme recognition sites; also called a polylinker.

3. Significance and Use

3.1 This guide is intended for use in a biotechnology laboratory when the need arises to identify a preparation containing M13 bacteriophage or DNA.

4. Background Information about M13 Bacteriophage

4.1 M13 is a filamentous bacteriophage that infects male (F^+F or Hfr) *Escherichia coli*. The phage particles contain circular single-stranded DNA, 6407 nucleotides in length, coated with the protein product of the M13 gene 8.[2]

4.2 The phage attaches to a receptor at the end of the F pilus. The infecting single-stranded DNA (+ strand) replicates in the cell: a complementary (−) strand is synthesized, resulting in a double-stranded, replicative form (RF). Using the RF as a template, both strands can be replicated, increasing the copy number of the RF to about 20 to 40 per cell. Late in infection (+) strands are preferentially produced and packaged into phage particles for export from the cell.[2]

4.3 M13 infection is not lethal to *Escherichia coli*. Bacteriophage DNA is continually replicated and packaged, causing a decrease in the growth rate of the host. The "plaque" seen upon infection by M13 is the result of an area of decreased growth rate, not actually cell killing.[2]

4.4 M13 has extensive sequence homology to bacteriophages f1 and fd, differing in only a few bases.[2]

4.5 M13 is used in biotechnology primarily as a vector into which foreign DNA can be cloned. Commonly used M13 variants are the M13 mp series. These M13 bacteriophages have a portion of the *E. coli* lac operon with a multiple cloning site within a truncated *lacZ* gene.

4.5.1 A portion of the *E. coli* lac operon containing the 3-prime end of the *lacI* gene, the lac promoter and operator, and the alpha complementation portion of the *lacZ* gene is

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[2] Rasched, I., and Oberer, E., Ff coliphages, Structural and Functional Relationships, Microbiological Reviews, Vol 50, 1986, pp. 401–427.

inserted near the origin of replication of the M13.[3,4,5]

5. Bacteriophage Growth and Purification

5.1 Bacteriophage M13 can be grown by any of a number of published protocols.[4,5]

5.2 Depending upon whether single-stranded (+) strand DNA in phage particles or RF DNA is desired, different protocols would be used.

5.2.1 The (+) strand DNA is exported as phage particles from cells infected with M13 phage. Cells from a single plaque are grown with vigorous aeration for 5 to 8 h. Longer times will result in fewer phage particles. The bacterial cells are centrifuged out and the bacteriophage remain in the supernatant fraction. The phage particles are usually precipitated and concentrated using polyethylene glycol. If the DNA is desired, the protein coat is disrupted using phenol.

5.2.2 Double-stranded RF DNA is isolated from cells that have been grown overnight. RF DNA can be isolated using a protocol for plasmid DNA purification.

6. Characterization Criteria

6.1 Since the uses of M13 are almost exclusively for its DNA, characterization of double-stranded RF DNA by restriction enzyme analysis or of the single-stranded phage DNA by DNA sequencing should be the criterion for determination.

6.1.1 RF DNA analysis using restriction enzymes shall be accomplished using any one of a number of protocols.[4,5]

6.1.2 Sequencing the single-stranded virion DNA can be accomplished using the Sanger dideoxy chain termination method.[4,5]

6.1.3 There are a number of variants of M13 that have been genetically engineered for use as cloning vectors. These variants differ from one another usually in orientation and content of the multiple cloning site. One variant can be distinguished from another by either restriction enzyme analysis of the RF DNA or DNA sequencing. Sequencing the multiple cloning site region is sufficient for discrimination among the variants.

7. Keywords

7.1 bacteriophage; cloning vector; DNA sequencing; M13; recombinant DNA

[3] Messing, J., Cloning in M13 Phage or How to Use Biology At Its Best, Gene Vol 100, 1991, pp. 3–12.

[4] Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Smith, J. A., Seidman, J. G., and Struhl, K., eds., Current Protocols in Molecular Biology, Greene Publishing Associates and Wiley-Interscience, New York, NY, 1989.

[5] Sambrook, J., Fritsch, E. F., and Maniatis, T., Molecular Cloning, A Laboratory Manual, 2nd edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.