Protocol 7

Analysis of Recombinant Bacteriophage M13 Clones

SEVERAL METHODS ARE USED TO ANALYZE THE SIZE AND ORIENTATION of foreign DNA sequences carried in M13 recombinants.

- screening *lac*$^-$ (white) bacteriophage M13 plaques by hybridization (please see the panel on **ALTERNATIVE PROCOTOL: SCREENING BACTERIOPHAGE M13 PLAQUES BY HYBRIDIZATION** at the end of this protocol)
- analysis of *lac*$^-$ plaques by PCR (please see Chapter 8, Protocol 7)
- analysis of small-scale RF DNA preparations (Protocol 3) by restriction enzyme digestion, gel electrophoresis, and Southern hybridization
- electrophoretic analysis of the size of single-stranded DNA in putative recombinant clones (this protocol)

In this protocol, recombinant bacteriophage M13 clones carrying sequences of foreign DNA longer than 200–300 nucleotides are detected by gel electrophoresis of single-stranded DNA released from infected bacteria into the surrounding medium.

MATERIALS

CAUTION: Please see Appendix 12 for appropriate handling of materials marked with <!>.

Buffers and Solutions

Please see Appendix 1 for components of stock solutions, buffers, and reagents. Dilute stock solutions to the appropriate concentrations.

SDS (2% w/v)
20x SSC
Sucrose gel-loading buffer

Gels

Agarose gel (0.7%) cast in 0.5x TBE, containing 0.5 μg/ml ethidium bromide <!>
Please see Step 4.

Nucleic Acids and Oligonucleotides

Single-stranded recombinant bacteriophage M13 DNA
Choose previously characterized recombinants that carry foreign sequences of known size to use as positive controls during gel electrophoresis. Please see Step 4 note.

Special Equipment

Water bath preset to 65°C

Additional Reagents

Step 1 of this protocol requires the reagents listed in Protocol 2 of this chapter.
Step 7 of this protocol may require the reagents listed in Chapter 2, Protocols 21 and 22.

Vectors and Bacterial Strains

Bacteriophage M13 recombinants plaques in top agarose
Prepared as described in Protocol 6 of this chapter.
Bacteriophage M13 nonrecombinant vector, grown as well-isolated plaques in top agarose
Prepared as described in Protocol 1 of this chapter.
E. coli *F′ strain*
For a listing of strains suitable for the propagation of bacteriophage M13, please see Table 3-2 in the introduction to this chapter.

METHOD

1. Prepare stocks of putative recombinant bacteriophages from single plaques, grown in an appropriate F′ host, as described in Protocol 2.

   As controls, prepare stocks of several nonrecombinant bacteriophages (picked from well-isolated dark blue plaques).

2. Use a micropipettor with a sterile tip to transfer 20 µl of each of the supernatants into a fresh microfuge tube. Store the remainder of the supernatants at 4°C until needed.

3. To each 20-µl aliquot of supernatant, add 1 µl of 2% SDS. Tap the sides of the tubes to mix the contents, and then incubate the tubes for 5 minutes at 65°C.

4. To each tube, add 5 µl of sucrose gel-loading buffer. Again mix the contents of the tubes by tapping and then analyze each sample by electrophoresis through an 0.7% agarose gel. Run the gel at 5 V/cm. As positive controls, use single-stranded DNA preparations of previously characterized M13 recombinants that carry foreign sequences of known size.

   Electrophoresis at low voltage eliminates problems associated with salt fronts created in the gel by the large volume of sample.

5. When the bromophenol blue has traveled the full length of the gel, photograph the DNA under UV illumination.

6. Compare the electrophoretic mobilities of the single-stranded DNAs liberated from the putative recombinants with those of the DNAs liberated from the control nonrecombinant bacteriophages.

   The single-stranded DNAs of recombinants carrying sequences of foreign DNA longer than 200–300 nucleotides migrate slightly more slowly than empty vector through 0.7% agarose gels. Once recombinants of the desired size have been identified, single-stranded DNAs can be prepared from supernatants stored at 4°C (Step 2).

7. If necessary, confirm the presence of foreign DNA sequences by transferring single-stranded DNAs from the gel to a nitrocellulose or nylon membrane (please see Chapter 2, Protocol 21)

and hybridizing to an appropriate radiolabeled probe (please see Chapter 2, Protocol 22). Soak the gel in 10 volumes of 20x SSC for 45 minutes, and then transfer the DNA directly to the membrane.

There is no need to denature the DNA by soaking the gel in alkali.

Southern blotting using oligonucleotide probes is particularly useful in identifying recombinants carrying different strands of a target DNA.

ALTERNATIVE PROTOCOL: SCREENING BACTERIOPHAGE M13 PLAQUES BY HYBRIDIZATION

Bacteriophage M13 plaques can be screened by hybridization to ^{32}P-labeled probes by following essentially the same methods devised for screening bacteriophage λ.

Method

1. Transfer the bacteriophage DNA to a nitrocellulose or nylon filter as described in Chapter 2, Protocol 21.
2. After removing the filter from the surface of the agar or agarose, allow it to dry (DNA side up) at room temperature.

   The single-stranded bacteriophage M13 DNA transferred to the filter does not need to be denatured with alkali.
3. Bake the filter under vacuum for 2 hours at 80°C or autoclave for 3 minutes, or, in the case of nylon filters, expose to UV irradiation to fix the DNA to the filter (please see Chapter 2, Protocol 21).
4. Hybridize the immobilized DNAs to an appropriate ^{32}P-labeled DNA probe as described in Chapter 2, Protocol 22.

   Double-stranded DNA probes will hybridize to all M13 recombinants that carry the target sequence irrespective of the orientation of the segment of foreign DNA within the vector. Single-stranded probes will hybridize only to recombinants that carry complementary sequences attached to the (+) strand of M13 bacteriophage DNA.

   If the filter is treated with alkali, as in conventional Benton-Davis screening of plaques, double-stranded M13 RF DNA released from the infected bacteria will be denatured. Both the (+) and (–) strands of M13 recombinants therefore become available for hybridization. Since the amount of (+) strand attached to the filter is much greater than the amount of RF DNA, single-stranded probes complementary to the (+) strand will generate a much stronger hybridization signal than probes complementary to the (–) strand. A weak hybridization signal with a single-stranded probe usually results from hybridization of the probe to denatured M13 RF DNA in plaques that contain the insert in the opposite orientation. This difference in intensity of hybridization has been used as a method to assay the orientation of cloned inserts (Picken 1990).