Identification and Analysis of Recombinants

IN SITU HYBRIDIZATION OF BACTERIOPHAGE λ PLAQUES

After a collection of recombinant bacteriophages has been prepared, it is necessary to identify and isolate specific desired recombinants from the population. The most commonly used method involves screening of bacteriophage plaques by hybridization with ^{32}P-labeled DNA probes (Benton and Davis 1977). Bacteriophages are plated at an appropriate density, and an imprint of the pattern of plaques is obtained by gently layering a nitrocellulose filter (or nylon membrane) onto the surface of the top agarose. Bacteriophage particles and DNA are transferred to the filter by capillary action in an exact replica of the pattern of plaques. After denaturation with alkali, the DNA is irreversibly bonded to the filter by baking and is then hybridized to the ^{32}P-labeled probe. Excess probe is washed away, and the filters are then exposed to autoradiographic film. Hybridizing plaques, identified by aligning the film with the original agar plate, are picked for further analysis. This method is particularly valuable for identifying small numbers of recombinant bacteriophages that carry sequences of interest in complex cDNA or genomic DNA libraries (Sim et al. 1979).

To screen a library of mammalian DNA (genome complexity $3 \cdot 10^9$ bp), several hundred thousand recombinant plaques must be examined. Table 2.6 gives the maximum number of plaques that can be screened in culture dishes of different sizes. In the following example, the volumes given are suitable for screening approximately 50,000 plaques in a 150-mm petri dish.

TABLE 2.6 Numbers of Plaques in Culture Dishes of Various Sizes

Size of petri dish (mm)	Total area (cm^2)	Volume of bottom agar (ml)	Volume of indicator bacteria (ml)	Volume of top agarose (ml)	Maximum number of plaques/dish
90	63.9	30	0.1	2.5	15,000
150	176.7	80	0.3	6.5	50,000