BENCHMARKS

Benchmarks are brief communications that describe helpful hints, shortcuts, techniques or substantive modifications of existing methods.

Device for the Simultaneous Screening of Bacteriophage λ Clones

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Sophisticated automated devices that facilitate the screening and isolation of yeast artificial chromosomes (YACs), cosmids and bacteriophage

clones in high density arrays are being used extensively in the Human Genome Program and other large-scale sequencing projects (2). However, such devices are not available in most small or mid-size laboratories, where, after primary screening of genomic and cDNA libraries, the primary positive clones should be manually rescreened several times to confirm, purify and isolate the true positives. To assist in this process

Figure 1. Schematic diagram of the assembly of the screening frame. The different parts (A, B and C) are glued together at the small folds at both ends of each B and C pieces.

we have constructed a device comprising a large culture plate and a plastic frame designed to divide the culture surface into eight equal triangular areas (Figure 1). This system allows the simultaneous screening of eight clones using a single sheet of nitrocellulose (NC) paper of up to 22 × 22 cm that fits into a standard hybridization oven.

In the primary screening and subsequent purification rounds, we use 600-cm² square bioassay plates (243 × 243 × 18 mm; Nunc, Roskilde, Denmark). These plates can be employed many times after being carefully washed with absolute ethanol and/or gas-sterilized. The plastic frame is easily made with two pieces of rigid plastic (306 × 18 × 1.5 mm) maintained in a crossed position by four stretchers of a semi-rigid plastic (230 × 18 × 0.2 mm), which are also used for the interior divisions (see Figure 1). Both materials can be found in local hardware stores, and it takes only a few minutes to put them together with an appropriate glue. The final product is a robust frame that can be sterilized by a 5-min irradiation in standard shortwave UV transilluminators and can be used indefinitely.

The device is used as follows: Molten agar is poured into the square plates (350 mL of 1.5% agar/plate, ca. 1 cm deep). When the agar becomes solid, the frame is set deeply into the

agar bed of the culture plate. The phages are then seeded by mixing the components of a standard infection (0.1 mL of an overnight *Escherichia coli* culture plus the appropriate number of plaque-forming units) with 3.5 mL of molten agar or agarose (top layer) and pouring the mixture into the triangular areas created. The frame should protrude several millimeters over the agar bed. This design, together with some care in the seeding of the phages, prevents cross-contamination and spills. When the plaques are grown, the frame is carefully removed and the plate is overlaid with a single piece of NC paper, which is asymmetrically pierced and further processed in the standard way (1). Figure 2 shows a typical screening performed with our device.

The main advantage of this device is the reduction in labor required for re-screening primary positive clones, since only a single plate and a single filter are used for every eight clones, as compared to the eight plates and the same number of filters needed when using standard methods. This system is easy to use and can be scaled up. We routinely process up to six plates (i.e., 48 clones) at once; and after almost two years of continuous use by many different people in our laboratory, we have not detected any contamination derived from the use of the frame. We have also tried other configurations of the frame, but found this one to be the most convenient in terms of simplicity, cost and labor involved in its manufacture.

REFERENCES

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Figure 2. Result of a typical screening using the device described. A single nitrocellulose sheet (22 × 22 cm) was used to analyze eight bacteriophage clones isolated in the primary screening (from which only two were confirmed as positive). Note the wide diagonal separations (caused by part "A" of the screening frame) and the lack of contamination and spills in the eight areas created.