Directed Colony Hybridization Using Agarose Gel

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Direct colony hybridization on agarose gel plate was established for the identification of recombinant plasmids. The hybridization of the probe to nucleic acids on dried gel without transferring to solid supports was more effective and simpler than hybridization of such probes to materials immobilized on filters such as nitrocellulose or nylon. D-cycloserine in overlaying agarose was essential for releasing the nucleic acids from colony.

There are many effective methods for hybridizing radioactive probes to nucleic acids on solid supports such as nitrocellulose (3) and nylon filters (1, 3) or on agarose gel (4). However, hybridization on agarose gel has been limited to the use of intact DNA. Here we describe a direct colony hybridization on agarose gel for screening a plasmid library. This method is less tedious and less expensive than previously used methods. In addition, it requires less time and reduces background noise.

Individual colonies (Escherichia coli) containing recombinant plasmids under study were transferred with a sterile toothpick to both AGL (0.7% agarose [Sigma, Co., USA]; 0.5% Bacto tryptone; 0.2% NaCl; 0.2% yeast extract; 80 μg of ampicillin/ml) and LB (1% Bacto tryptone; 0.5% NaCl and 0.5% yeast extract [pH 7.3]; 2% Bacto agar; 80 μg of ampicillin/ml) plate. AGL plate was incubated for 3 or 4 hours at 37°C and then stored at 4°C for 1 hour. This plate was overlaid by pouring 3 ml of melted agarose (0.7%) containing 1 mg of D-cycloserine (Sigma, Co., USA). Then both AGL and LB plate were incubated overnight at 37°C. The entire content of the AGL plate was transferred onto a 3 MM Whatman paper and vacuum-dried for 30 min at 40°C while the master plate (LB) was stored at 4°C in an inverted position for later picking of positives. Before drying, a flake of colored or white paper was inserted into a corner of semisolid agar using a holder as the direction marker of the agarose gel. The dried gel film was soaked in a denaturation solution (0.5 M NaOH; 1.5 M NaCl) for 15 min, and then in a neutralization solution (0.5 M Tris-HCl [pH 8.0]; 1.5 M NaCl) for 15 min at room temperature. The gel film was prehybridized in a hybridization solution (50% formamide; 5X SSPE; 2X Denhardt's reagent; 0.1% SDS; 100 μg of denatured salmon sperm DNA/ml) for 1~2 hours at 42°C and then hybridized in a hybridization solution containing α-32P labelled probe for 16~24 hours at 42°C. This film was wa-

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Fig. 1 The colony hybridization of amylolytic gene of Schwanniomyces occidentalis and Candida tsukubaensis using direct agarose gel film. Panel A (colonies from genomic library) and C (colonies containing gene clone or only plasmid) were hybridized with 2.0 kb of α-32P labelled Schwanniomyces α-amylase gene as probe (2 x 10⁶~1 x 10⁷ cpm/μg DNA). Panel B (colonies from genomic library) and D (colonies containing gene clone or only plasmid) were also hybridized with 0.8 kb of Candida tsukubaensis glucoamylase gene as probe (4 x 10⁷~3 x 10⁸ cpm/μg DNA). All probes were constructed with primer extension method (3). Arrows indicate positive colonies. These positive colonies were reconfirmed by Southern blot.
shed with distilled water (200–300 ml) for 10 min at room temperature and then with 1X SSC, 0.1% SDS (200–300 ml) at 42°C for 30–60 min. After drying the gel film on 3MM paper in the air at room temperature, it was exposed to an X-ray film for 6–12 hours at −70°C with an intensifying screen. The inhibition of cell wall biosynthesis by D-cycloserine may help releasing nucleic acids from each colony. This was confirmed by staining of the gel film with ethidium bromide solution (0.5 μg of ethidium bromide/ml). A careful transfer of the colonies from LB plate (containing appropriate antibiotics) to AGL plate, and the sterilization of agarose solution for 10 min at 10 lb was found to be essential in obtaining best result.

The amylolytic genes from Schwanniomyces occidentalis and Candida tsukubaensis were used to test agarose gel hybridization (Fig. 1).

REFERENCES


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