molecular weights of unknown complexes. In addition, it prevents contamination of the EMSA running buffer with a radioactive probe and reduces aqueous waste. This method can be modified with various acrylamide percentages in the retaining and running layers to optimally isolate the desired range of proteins.

REFERENCES

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Efficient and Rapid Procedure for Blue-White Screening of Recombinant Bacterial Clones

Many plasmid cloning vectors allow blue-white screening for recombinant bacterial clones by α-complementation (5). The regulatory sequences and part of the coding information of the β-galactosidase (β-gal) gene (LacZ) carry embedded the multiple cloning site (MCS) of the plasmid vector without interrupting the reading frame for LacZ. In bacterial host cells that express the carboxy-terminal part of β-gal, the two fragments associate to yield an enzymatically active protein.

As an indicator for galactosidase activity, the chromogenic substrate X-gal is used (2) and usually added at 40–100 µM concentration in agar plates producing blue colonies in the case of Lac+ bacteria. Insertion of a fragment of foreign DNA into the MCS almost invariably leads to the production of an amino-terminal fragment that is incapable of α-complementation resulting in a white colony.

The development of this simple color test has greatly simplified the identification of recombinants with plasmid vectors of this type. However, X-gal is expensive and decomposes over time in pre-poured and stored agar plates due to light sensitivity. Costs can be partially reduced by spreading a concentrated solution of X-gal on the surface of a pre-made agar plate instead of incorporating the sugar throughout the entire volume of the agar medium (4). However, because of the low volatility of the solvent dimethylformamide (DMF) it can take up to 3 or 4 h before the plates are usable for spreading the bacteria (4).

Here, I present an easy, rapid and minimal-cost procedure for blue-white screening with X-gal. It is performed on the grown plates, which allows for a preselection of plates that are worth assaying for β-gal activity. The protocol requires a minimal amount of hands-on time, and results are readily available within minutes.

Agar plates are prepared from LB media (4), supplemented with 1.2% bacto-agar (Becton Dickinson Microbiology Systems, Sparks, MD, USA) and 100 µg/mL ampicillin (Sigma, St. Louis, MO, USA). Preparation of competent bacteria and DNA transfections follow standard protocols (1,4). The agar plates to be analyzed with the overnight grown bacterial colonies are removed from the 37°C incubator. An X-gal (Ambion, Austin, TX, USA) stock solution of 20 mg/mL in DMF is diluted to the desired concentration with sterile distilled water (see Table 1) and applied to the plate surface by spraying as shown in Figure 1. Approx-

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approximately 500 µL of X-gal solution released as a fine spray mist by an adjustable dispenser (Figure 1) suffice to cover the 10 cm agar plate with a thin film of fluid. Subsequently, the plates are returned to the 37°C incubator with their covers slightly open to ease evaporation of the liquid.

Table 1 shows the results of a titration experiment determining the required incubation times with various X-gal concentrations. A 4:1 mixture of pBluescript® II SK vector (Stratagene, La Jolla, CA, USA) without insert and the same vector with a 200 bp insert of foreign DNA was used to transfect DH5α™ bacterial cells (Life Technologies, Gaithersburg, MD, USA) by the calcium co-precipitation method (1). As early as 4 min after X-gal application (1280 µg/mL), intense blue-stained colonies are readily discernible from uncolored Lac− bacterial clones. Various E. coli strains were successfully tested with this procedure, i.e., DH5α, DH10B™ (Life Technologies), Epicurian Coli® XL1-Blue and Epicurian Coli SURE® (both from Stratagene). No significant difference in staining behavior was observed. In general, the higher the growth rate of an E. coli strain, the faster the staining is complete. We also tested the E. coli strain DH10Bac™ (Life Technologies) that harbors a baculovirus shuttle vector (bacmid) used for the generation of recombinant baculovirus by homologue recombination as part of the Bac-to-Bac™ Baculovirus Expression System (Life Technologies).

To induce homologue recombination in DH10Bac, the competent cells were transfected with the plasmid transfer vector pFASTBAC™ 1 (Life Technologies) in which the sequences to be expressed in insect cells were inserted. Usually, it takes 24–48 h after transformation and plating of the bacteria until blue-white selection for recombinants is possible (3). With overnight incubated plates (20 h) of such a recombination, spraying using an X-gal concentration of 640 µg/mL allowed analysis of recombinants after 2 h, which added up to only 22 h after transformation. In fact, with all bacteria tested, the total time from transformation of the DNA until the blue-staining of colonies is visible was reduced always by at least 20%–30% using the spraying method as compared to the conventional method with 80 µg/mL X-gal incorporated in the agar medium. This is probably due to the freshness of the X-gal solution as well as to the higher local concentration and the availability of the sugar substrate upon spraying.

For most bacterial strains, the substrate solution can also be poured onto the surface and tilting until it is equally distributed. However, a larger amount of X-gal solution is required (1 mL for 10 cm plates), and the danger of dislodging single colonies and cross-contamination between clones is high.

Taken together, this screening protocol substantially reduces the consumption of X-gal. With 40–100 µg/mL of X-gal usually used in agar plates and an average of 35 mL agar medium per 10 cm plate, the total amount of substrate within each plate is 1400–3500 µg. Approximately 10–20× less X-gal is necessary when the spraying procedure is applied using 320 µg/mL X-gal solution with the results available after 20 min.

Even more substrate can be saved through prescreening of the grown agar plates and by using a lower concentrated X-gal solution (see Table 1). Furthermore, the analysis is more reliable because the substrate is always fresh and it saves time over common protocols, especially with slow-growing E. coli strains like DH10Bac. The ease and minimal hands-on time of the spraying procedure has made it the screening method of choice in our laboratory.

REFERENCES

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