Artículos sobre Técnicas

Alternative procedure for plating M13-transformed *E. coli* cells

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M13, a filamentous DNA phage, generates a functional B-galactosidase enzyme (lac+) forming blue plaques under conditions of IPTG induction and X-gal as enzyme substrate. DNA insertion into any of the cloning sites of M13 disrupts production of the functional B-galactosidase (lac-) and causes the appearance of white plaques.

A standard procedure is to clone the DNA to be sequenced into the M13 vector of choice. The ligated DNA is mixed with competent *E. coli* host cells, incubated on ice for 40 minutes, heat shocked for 2 minutes at 42°C and added directed to 10 μl 100 mM IPTG, 50 μl 2% X-gal, 0,2 ml fresh exponentially growing *E. coli* host cells and 3 ml soft agar (melted at 45°C). After gentle mixing, the solution is plated directly on 2X TY agar plates. The plates are allowed to solidify and incubated at 37°C overnight until plaques are visible which can be scored for the presence of inserts. The insertional inactivation of B-galactosidase activity is pertinent in the identification of recombinants.

We report here an alternative procedure for plating. In the event that the IPTG and X-gal is excluded in the soft agar overlay the plating can be salvaged by making the following modifications. Quickly chill the plates at 0–4°C for 10 minutes to harden the soft agar overlay. Resuspend the 10 μl of 100 mM ITPg and 50 μl 2% X-gal in 100 μl sterile water. Using sterile techniques, the IPTG and X-gal is gently spreaded across the top agar overlay. The small volume of 170 μl will spread evenly and rapidly soak into the surface of the top agar overlay. The plates are then incubated at 37°C until plaques are seen; the color reaction usually taking 12-15 hours. It is our experience that if this modification is done quickly and gently there is minimal distortion to plaque formation and recombinants are easily recognized by their colorless plaques.