Plating Bacteriophage M13

Protocole à analyser : construire un organigramme, expliquer la pression de sélection sur le plasmide F, expliquer l'intérêt de l'IPTG et du X-gal. Puis mettre en œuvre. Ce mode opératoire est repris avec quelques modifications de « Molecular cloning, Cold Spring Harbor Manual, 2° edition ».

MATERIALS

Buffers and Solutions

IPTG 100x solution (sterile filtration, 2,38 % w/v)
X-gal 100x solution (2% w/v in dimethylsulfoxyde)

Media

- LB agar plates containing tetracycline (10µg/mL). These plates are needed only if a tetracycline-resistant strain of *E. coli*, such as XL1-Blue is used to propagate the virus.
- LB or YT medium top agar or agarose containing 5 mM $MgCl_2$. The addition of Mg^{2+} (5 mM) to media is reported to improve the yield of bacteriophage M13 cultures infected at low multiplicity.

  Or, when using E. *coli* strains that carry a deletion of the proline biosynthetic operon *(Δ[Lac-proAB)* in the bacterial chromosome and the complementing *proAB genes* on the F' plasmid (JM109), use supplemented M9 minimal medium.

Vectors and Bacterial Strains

- Bacteriophage M13 stock in LB or YT medium or M13 plaque in 1 mL of LB or YT medium.

  Medium from a fully- grown liquid culture of bacteria infected with bacteriophage Ml 3 contains between 10^{10} and 10^{12} pfu/ml. A bacteriophage M13 plaque contains between 10^6 and 10^8 pfu.
- Suitable *E. coli F'* strain, prepared as a master culture (JM109 *: supE, Δ[Lac-proAB], hsdR17, recA1 / F'traΔ36, proAB+, lacI^q, lacZΔM15 or* XL1-Blue : *supE+, lac-, hsdR17, recA1 / F' proAB+, lacI^q, lacZΔM15, Tn10(tet^R)* for example).

Special Equipment

Heating block or water bath preset to 47°C and ice-water bath.

METHOD

1. Streak a master culture of a bacterial strain carrying an F' plasmid onto either a supplemented minimal (M9) agar plate (JM109) or an LB plate containing tetracycline (XLI-Blue). Incubate the plate for 24-36 hours at 37°C.

2. To prepare plating bacteria, inoculate 5 ml of LB or YT medium in a 20-ml sterile culture tube with a single, well-isolated colony picked from the agar plate prepared in Step 1. Agitate the culture for 6-8 hours at 37°C in a rotary shaker. Chill the culture in an ice bath for 20 minutes and then store it at 4°C. These plating bacteria can be stored for periods of up to 1 week at 4°C.

Note : IMPORTANT , DO NOT grow the cells to saturation, as this will increase the risk of losing the pili encoded by the F' plasmid.

3. Prepare sterile tubes containing 3 ml of melted LB or YT medium top agar or agarose, supplemented with 5 mM $MgCl_2$. Allow the tubes to equilibrate to 47°C in a heating block or water bath. Add 40 µl of 2% X-gal solution and 40 µl of 2,4% IPTG solution to each of the tubes containing top agar.

4. Label a series of sterile tubes according to the dilution factor and amount of bacteriophage stock to be added (please see Step 5), and deliver 100 µl of plating bacteria from Step 2 into each of these tubes.

5. Prepare tenfold serial dilutions (10^{-6} to 10^{-9} for example) of the bacteriophage stock in LB or YT medium. Dispense 10 µl or 100 µl of each dilution to be assayed into a sterile tube containing plating bacteria from Step 4. 1 Mix the bacteriophage particles with the bacterial culture by vortexing gently.

Unlike bacteriophage lambda, M 13 adsorbs rapidly to bacteria; there is thus no need to incubate the plating bacteria with the bacteriophage suspension before adding top agar.

6. Immediately, repeat the addition of top agar with X-gal and IPTG for each tube of infected culture prepared in Step 5 :

- pour the contents of one of these tubes into one of the infected cultures ;
- mix the culture with the agar/agarose by gently vortexing for 3 seconds, and then pour the mixture onto a labeled plate containing LB or YT agar medium supplemented with 5 mM $MgCl_2$ and equilibrated to room temperature;
- Swirl the plate gently to ensure an even distribution of bacteria and top agar.

  Work quickly so that the top agar spreads over the entire surface of the agar before it sets.

7. Replace the lids on the plates and allow the top agar/agarose to harden for 5 minutes at room temperature. Wipe excess condensation off the lids with Kimwipes. Invert the plates and incubate them at 37°C.

Pale blue plaques begin to appear after 4 hours. The color gradually intensifies as the plaques enlarge. Development of both plaques and color is complete after 8-12 hours of incubation. The blue color will intensify further if the plates are placed for several hours at 4°C or examined against a canary yellow background.