

2. Materials

2.1. Library Plating

1. Isopropyl β -D-thiogalactopyranoside (IPTG) 1M stock in water. Store at -20°C .
2. Suitable bacterial host.
3. Nitrocellulose filters (S&S nitrocellulose BA85).
4. Suitable solid-growth-media plates (containing the appropriate antibiotic for the vector in use).

2.2. Screening for Plasmids Expressing CP

1. Bug-busting buffer: 2X SSC containing 0.5% sodium dodecyl sulfate (SDS).
2. Tris-buffered saline (TBS): 50 mM Tris-HCl, pH 7.5, 200 mM NaCl.
3. Blocking buffer: 5% dried milk powder in TBS.
4. TBST: TBS containing 0.05% Tween-20.
5. High titer polyclonal primary antibody or suitable cocktail of monoclonal antisera.
6. Suitable secondary conjugated antibody for detection.
7. Suitable radiochemical or chromogenic reagent of your choice.

2.3. Western Blot Analysis of Expressed Fusion Proteins

1. SDS-PAGE loading buffer: 2% SDS (w/v), 5% mercaptoethanol (v/v), 10 glycerol (v/v) in 0.125 M Tris-HCl, pH 6.8.

3. Methods

3.1. Library Plating

1. Transform cDNA library into a suitable bacterial host and spread out on plates at a suitable density to visualize single colonies and incubate overnight at 37°C .
2. Draw and number a grid on a Petri-dish-sized nitrocellulose circle, soak in 10 mM IPTG, and allow to air-dry before carefully placing it onto the surface of a solid media plate.
3. Draw and number a similar grid on the base (not the lid) of another solid media plate.
4. Pick individual colonies from the original cDNA library plate described in **step 1**; streak onto a numbered position on the nitrocellulose grid; and streak onto the same position on the plain agar master plate, using a sterile loop (*see Note 1*).
5. When a suitable number of colonies have been streaked out onto both plates, invert the plates and incubate overnight at 37°C .

3.2. Screening for Plasmids Expressing CP

1. Remove filters and place colony-side up on filter paper (e.g., Whatman 3MM paper), presoaked in Bug-busting buffer, for 2–3 min (*see Note 2*). Remove and immediately go to **step 2**.
2. Incubate the filters in blocking buffer overnight at room temperature with gentle agitation (*see Note 3*).