

## 2. Materials

### 2.1. Library Plating

1. Isopropyl  $\beta$ -D-thiogalactopyranoside (IPTG) 1M stock in water. Store at  $-20^{\circ}\text{C}$ .
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  $37^{\circ}\text{C}$ .
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  $37^{\circ}\text{C}$ .

### 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*).