3. Wash the filters five times in TBST, 5 min for each wash with gentle agitation (*see* **Note 4**).
4. Incubate the filters for 90 min at room temperature, with gentle agitation, in an appropriate dilution of primary-CP antibody (*see* **Note 5**), diluted in fresh blocking buffer.
5. Wash the filters five times in TBST, 5 min for each wash with gentle agitation (*see* **Note 4**).
6. Incubate the filters with secondary antibody conjugated with a suitable detection system (*see* **Note 6**) (e.g., alkaline phosphatase) for 90 min at room temperature with gentle agitation.
7. Wash filters as in **step 5**.
8. Develop the filters using a suitable detection system, e.g., color detection or chemiluminesence (*see* **Note 6**).

3.3. Western Blot Analysis of Expressed Fusion Proteins

1. Grow antibody-positive colonies overnight in liquid broth at 37°C in the presence of 5 mmol/L IPTG.
2. Harvest cells (0.5 mL) by microcentrifungation and resuspend in SDS-PAGE loading buffer (50–100 μL) and boil for 2 min.
3. Spin briefly in a microcentrifuge to remove cell debris and remove supernatant to a fresh tube.
4. Boil sample for 2 min prior to separation on 12.5% polyacrylamide gels, Western blotting onto a suitable membrane and detection of bands as described above (*see also* Chapter 45).

4. Notes

1. An alternative to using sterile loops is to use autoclaved pipet tips, using a fresh tip after each duplicate streak has been completed.
2. We have observed that good signals can be obtained using no pretreatment of the filters. However, best signals are obtained when the colonies are treated to disrupt them, such as bug-busting buffer or by treating the colonies as described for nucleic acid probes.
3. Gentle agitation can be achieved using a shaking platform, a shaking incubator (set at room temperature), a rotating platform, or gentle shaking of the container by hand every 5–10 min.
4. If multiple filters are screened at the same time, ensure that the filters do not stick together during incubation and shaking.
5. A typical dilution to use for good-quality polyclonal antisera would be a 1:500 or 1:1000 dilution.
6. Commercial antibodies are available that are coupled to enzymes such as horseradish peroxidase or alkaline phosphatase that react with specific determinants on primary antibodies (species specific). These primary–secondary complexes can then be detected using chromogenic substrates, available from a wide range of commercial sources, which also provide clear instruction protocol booklets (e.g., Stratagene, Amersham, Boehringer Mannheim, Promega, and Pharmacia).