12. Place the membrane in a sealable plastic bag (moisten nitrocellulose with 2X SSC first). Seal the bag very close to the membrane on three sides, but leave 3–4 cm of bag on the open end. This extra space will allow the bag to be opened and resealed several times as solutions are removed or added.
13. Add a generous volume of prehybridization solution (10–20 mL, depending on the blot size) and seal the bag close to the cut edge, excluding as many bubbles as possible.
14. Prehybridize at 65°C for at least 4 h (overnight, if background has been a problem).
15. Cut the end off the plastic bag and squeeze the prehybridization solution out. Rolling a pipet over the bag will get most of it out. Seal the bag close to the edge and then cut a corner off to add the hybridization mix through.
16. Prepare the hybridization solution:
    a. Radiolabel the hybridization probe using any of the commercially available kits (e.g., nick translation, random prime, and so on).
    b. Use a minimum volume of hybridization solution (5–6 mL is usually adequate for an 11 × 14 cm membrane; use less if possible).
    c. Just prior to use, add 2 μg denatured, sheared salmon sperm DNA/mL.
    d. Denature the hybridization probe (*see* **Note 12**) and add it to the hybridization solution.
17. Add the hybridization solution to the bag and seal the bag as close to the membrane as possible, being careful to eliminate all the bubbles. Two blots, placed back to back, can be hybridized in the same bag.
18. Hybridize overnight at 65°C, preferably in a shaking water bath or on a rocker, to promote movement of the solution in the bag.
19. Transfer the membrane from the bag and wash sequentially with ~200 mL each of:
    a. 6X wash mix, 65°C, 30 min;
    b. 0.3X wash mix, 65°C, 30 min;
    c. 0.3X wash mix, 65°C, 30 min; and
    d. 2X SSC briefly at room temp to rinse SDS away.
20. Place the blot between two layers of plastic wrap, and, if the blot will be reprobed later, care should be taken to keep the membrane moist (*see* **Note 13**).
21. Expose to imaging film.

3.5. Interpretation of Results

3.5.1. Interpretation of PCR Results

Figure 1 shows a variety of PCR results that were obtained using the above techniques. Details of the reactions are described in the figure legend. Panels A and B show very clean, predictable, and easy to interpret results; those in Panel C are less clear-cut, but quite common.

In panel A, the negative control lanes (–, 6 and 7) lack amplification products when the green fluorescent protein (GFP) primers were used, and the posi-