5. An alternative transfer procedure involves semidry blotting using the same transfer buffer. We currently use the Pharmacia Phast System and Western semidry transfer for all Western blots. The convenience of this system justifies the purchase and running costs.
6. PVDF membrane must first be wetted in methanol for a few minutes and then rinsed in water. If at any point in the procedure the membrane dries out, then the methanol wetting procedure must be repeated.
7. Heat is generated during the transfer. A freezer-pack cooling-pad frozen beforehand at –20°C should be used to cool the buffer during transfer.
8. Antibody concentration must be determined empirically for each application. Frequently, a tradeoff must be made between signal and background. Manipulation of primary antibody concentration and the quantity of material applied originally to the gel will help significantly to achieve a clear signal. As a guide, rabbit polyclonal antisera should be used at 1:100–1:1000 and monoclonal antisera at greater than 1:1000.
9. Incubations with antibody are set up as follows: Strips of parafilm are glued to a flat surface (plastic or glass) creating a hydrophobic surface; the membrane, protein-side up, is placed on top of the parafilm, and 0.3 mL/cm^2 of antibody solution is applied to the membrane; and the solution is covered with a lid to decrease evaporative loss. This method is very convenient and uses minimal amounts of reagents and antisera (1–2 mL for a 3 × 5 cm Phast gel Western blot).
10. Chemiluminesence may alternatively be used for the detection system. In this case, we would use the Amersham ECL kit. Substitute the block and antibody dilution solutions for: 1X PBS, 5% (w/v) nonfat milk powder, 2% (w/v) BSA, and use a secondary antibody labeled with horseradish peroxidase.
11. Quantitation of transgene expression can be estimated crudely by Western blotting or, more quantitatively, by ELISA analysis (*see* Chapter 46). The purified transgene product is used to create a standard curve, which should be prepared in a healthy plant extract to ensure comparability with transgenic samples.

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