4. A sensitive method for the detection of gene expression. Detection of gene expression depends on the nature of the insert itself. For example, if a reporter gene has been used in order to study the effects that a viral leader sequence has on gene expression, then the reporter gene product (GUS, CAT, LUC) is easily screened using its appropriate substrate ***(5–8)***; or if a gene encoding for a protein to which an antiserum exists, then this antiserum can be used to detect the expression of that protein.

This chapter describes PEG-mediated DNA uptake into tobacco protoplasts and the subsequent detection of coat protein (CP) gene expression by Western blotting.

2. Materials

2.1. Protoplast Isolation and Transformation

1. Mature tobacco plants (mature leaves are easier to peel; *see* **Note 1**).
2. Narrow-tipped tweezers, 64-μm Nitex sieve, parafilm, 9- and 5-cm Petri dishes.
3. Hemocytometer.
4. Protoplasting solution: 0.2 g/L macerozyme (Sigma, Dorset, UK), 1.0 g/L cellulase (Sigma), 80 μg/L mannitol, 20 g/L sucrose, 2.35 g/L MS salts, pH to 5.6 with 0.1*M* NaOH. The protoplasting solution was made up in 50-mL amounts, filter-sterilized and stored frozen.
5. Protoplast wash solution: 80 g/L mannitol, 2.35 g/L MS salts, pH to 5.6 with 0.1*M* NaOH. The protoplast wash solution was made up in 50-mL amounts, filter-sterilized and stored frozen.
6. 21% Sucrose solution, filter-sterilized, and stored at room temperature.
7. PEG solution: 250 g/L PEG 6000, 23.6 g/L $Ca(NO_3)_2 \cdot 4H_2O$, 82 g/L mannitol, 3.9 g/L MES, pH to 6.0 with 0.1*M* NaOH. This solution is made up in a 50-mL amount, filter-sterilized, then aliquoted into 1.5-mL microcentrifuge tubes and stored at –20°C.
8. 0.275*M* Calcium nitrate solution: 65 g/L $Ca(NO_3)_2 \cdot 4H_2O$ and 2.0 g/L MES, pH to 6.0 with 0.1*M* NaOH, and autoclaved.
9. Protoplast recovery medium: 80 g/L mannitol, 20 g/L sucrose, 2.35 g/L MS salts, pH to 5.6 with 0.1*M* NaOH, filter-sterilized and stored frozen.
10. Fluorescein diacetate (FDA) stock solution: 2 mg/mL FDA prepared in acetone and stored in the fridge.

2.2. Electrophoresis and Western Blotting

The techniques of polyacrylamide gel electrophoresis (PAGE) and Western blotting are described elsewhere in this book.

3. Methods

3.1. Protoplast Isolation

1. The lower epidermis of a *Nicotiana tabacum* leaf is removed by peeling with a pair of tweezers (*see* **Note 1**).