TECHNICAL SHEET No. 17

Virus Detection: *Potato Virus X* (PVX)

Method: Immunocapture RT-PCR

General

Virus detected: PVX from potato leaves.
General method: Immunoicapture RT-PCR, PCR-ELISA.
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Developed by

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Introduction

For animation of RT-PCR (need shockwave downloaded):
http://www.bio.davidson.edu/courses/Immunology/Flash/RT_PCR.html

For WEB information on PVX see:
http://www.im.ac.cn/vide/descr651.htm
http://life.bio2.edu/ICTVdB/56010018.htm

Information on RT-PCR-ELISA can be found in Shamloul and Hadidi (1999), Shamloul et al. (2002) and at the Web site:
http://biochem.boehringer-annheim.com/prod_inf/manuals/pcr_man/Chapter10/CHAP10-Seite254.htm

Materials and Methods

Immunocapture

1. Add 200 µl of anti-PVX antibody (diluted 1:1000 in coating buffer) to each ELISA plate well or to PCR tube and incubate at 37^{o}C for 3 h. We used a commercial antibody (Bioreba) and an anti-PVX antibody, a gift from Prof. Gad Loebenstein.
2. Empty the ELISA well/ PCR tube and wash 3 times with TBST.
3. Homogenize 25 mg of plant leaf or tuber with 5 ml of suitable extraction buffer.
4. Add 200 µl of homogenate to the coated ELISA well/PCR tube and incubate for 18h at 4^{o}C.
5. Empty the ELISA well/ PCR tube and wash 3 times with TBST.
6. Dry the ELISA well/ PCR tube, add 5 to10 µl ddH2O and heat at 70 ^{o}C for 15 min.

RT-PCR

a) cRNA synthesis

1. To 5 µl IC add 8 µl ddH_2O and 1 µl primer 1(100 pmoles); incubate at 70 ^{o}C for 10 min, followed by 30 min in and ice bath.
2. Add 1 µl of each dNTPs (25 mM each), 4 µl reverse transcriptase 5 x buffer, 1 µl AMV reverse transcriptase (Promega); incubate at 42 ^{o}C for 1h.

3. Heat for 10 min at 90°C; adjust the volume to 50 µl with ddH_2O

b) PCR. The following two primers allowed amplifying a 713-bp fragment from the plasmid pGEM/PVX which contains the cloned virus.

R823: 5'ATGTCAGCACCAGCTAGCAC3'
C382: 5'TTATGGTGGTGGTGAAGTGAC3'

1. The PCR reaction contains 5 µl from the reverse transcriptase reaction, 0.25 µl 25mM dNTPs, 1 µl each primers 1, 2 and 3 (100 pmoles), 2.5 µl Taq 10 x buffer and 1 unit *Taq* polymerase; add ddH_2O to a final volume of 25 µl.
2. Cycling: One cycle: 95°C for 3 min, 50°C for 2 min, 72°C for 2 min. Thirty cycles: 95°C for 1min, 55°C for 1min, 72°C for 1 min. One additional cycle: 72°C for 10 min.
3. Analysis of reaction products: Subject the reaction products to 1% agarose gel electrophoresis.

Results

The figure shows the detection of PVX by immunocapture RT-PCR using three primers. The RNA was extracted from infected *Nicotiana glutinosa*.

M: molecular weight markers; 1-3: RT-PCR; 1'-3': IC-RT-PCR; P: cloned virus