

ELECTRONIC BIOCHIP FOR THE SIMULTANEOUS DETECTION AND CHARACTERIZATION OF POTATO VIRUSES

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Microelectronic DNA chips is an emerging technology that can be of great interest for diagnosis. Using electricity, this new generation of microarrays is more accurate, faster and more flexible than other available technologies. The NanoChip® Electronic Microarray, is a silicon chip that contains 100 test sites laid out in a geometric grid. Each test site can be controlled electronically from the computer. An streptavidine coated layer on the chip acts as the interface between the electrically active surface and the biological test environment. DNA or RNA molecules can be rapidly moved and concentrated to the designated test sites on the chip. We have developed an electronic chip able to detect specifically and simultaneously several potato viruses (PVY, PVX and PLRV) as well as different PVY variants (PVY^{NNTN} and PVY^O).



100-site test array. Each test site is electronically connected to the Nanochip system by a platinum wire.

The microchip is similar to that used in many computers and enables extremely precise control of each individual test site.



The chip contains platinum wires which are connected to a computer controller once the chip is inserted into the Workstation.

MATERIAL AND METHODS

Virus isolates:

Isolates from infected potatoes kindly supplied by Dr. J. Legorburu from Neiker, Vitoria, Spain, were used as controls. Namely, PVX (isolate RP), PLRV (isolate A), PVY^O (isolate Palogán), PVY^N (isolate Hertha) and PVY^{NNTN} (isolate ML02).

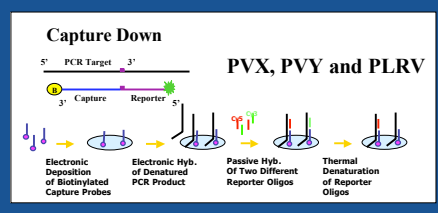
Viral target preparation:

Viral RNA isolation was performed using the RNeasy Plant Mini Kit, according to the manufacturer's protocol (Qiagen). Briefly, plant extract (200µl) was mixed with RLT buffer (350µl) and lysate applied to QIAshredder spin column, recovering the flow-through. Subsequently, ethanol 100% (0.5 vol.) was added to the lysate and the mixture applied to RNeasy column. After washing with RW1 buffer and RPE buffer, RNA was finally eluted with 50µl of Rnase-free water.

CAPTURE DOWN FOR SIMULTANEOUS DETECTION OF PVX, PVY AND PLRV

Multiplex RT-PCR amplification for PVX, PVY and PLRV:

The cocktail amplification consisted of 10 mM Tris-HCl, pH 8.8, 2.5 mM MgCl₂, 0.3% Triton X-100 (v/v), 0.3µM PVX primers, 0.6µM PVY primers, 1µM PLRV primers, 0.4mM dNTPs, 10 units of AMV-RT (Promega), 10 units of Taq DNA polymerase (Promega) and 5µl of sample RNA in a total volume of 25µl. Synthesis of cDNA was performed at 42°C for 45 min, and the amplification process was carried out for 40 cycles under the following conditions: 94°C for 1 min, 55°C for 1 min and 72°C for 2 min.



PVY

Capture probe
5' Biotine-TCC TCG GTG GTG TGC CTC TCT GTG T 3'

Reporter probe
5' TCC TCT TGT GTA C 3'- Cy5

PVX

Capture probe
5' Biotine-TGT GCC ATA GTG TCT GTG GGC ACC T 3'

Reporter probe
5' TCA TGT CCT TCC A 3'- Cy5

PLRV

Capture probe
5' Biotine-TTT GTA AAC ACG AAT GTC TCG CTT G 3'

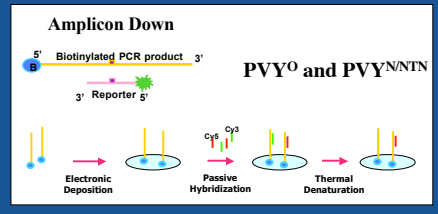
Reporter probe
5' AGC CTC GTC CTC G 3'- Cy5

PVX primers (Nie & Singh, 2001): 5' TAG CAC AAC ACA GGC CAG AC 3' and 5' GGC AGC ATT CAT TTC AGC TTC 3'
 PLRV primers (Nie & Singh, 2001): 5' CGC GCT AAC AGA GTT CAG CC 3' and 5' GCA ATG GGG GTC CAA CTC AT 3'
 PVY primers: PVY nucleotide sequences were retrieved from NCBI's integrated databases, GenBank, EMBL and DDBJ. Advanced BLAST 2.0 (Altschul et al., 1997) was used for alignment. Oligo software was used to design the following primers, 5' CAT CAC GAA CAC CAG TGA GGG CTA 3' and 5' TCA CAT GTT CTT GAC TCC AAG TAG 3'

AMPLICON DOWN FOR CHARACTERIZATION OF PVY ISOLATES

RT-PCR amplification for a PVY region that allows the characterization of isolates (PVY^O and PVY^{NNTN}):

The cocktail amplification consisted of 10 mM Tris-HCl, pH 8.8, 2.5 mM MgCl₂, 0.3% Triton X-100 (v/v), 1µM PVY primers, 0.25mM dNTPs, 5 units of AMV-RT (Promega), 5 units of Taq DNA polymerase (Promega) and 5µl of sample RNA in a total volume of 25µl. Synthesis of cDNA was performed at 42°C for 45 min, and the amplification process was carried out for 40 cycles under the following conditions: 94°C for 30 sec, 55°C for 30 sec and 72°C for 1 min.



PVY^O

Reporter probe
5' Cy5-TGC AAA AGT CCA AC 3'

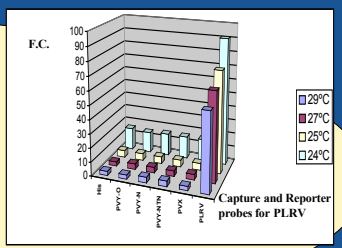
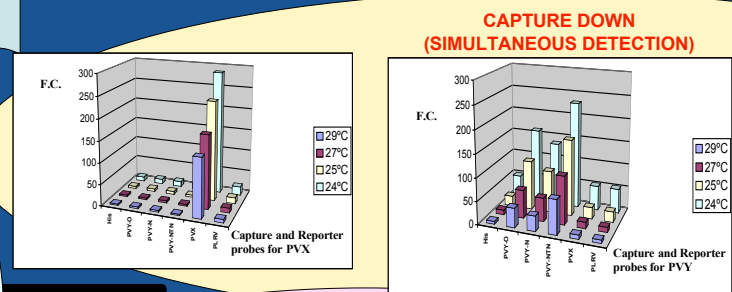
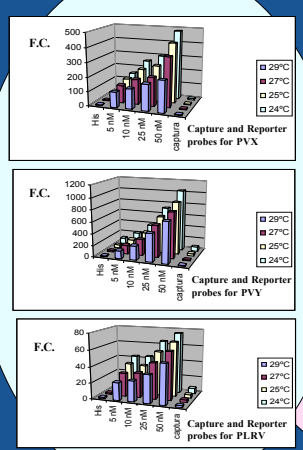
PVY^{NNTN}

Reporter probe
5' Cy5- CGA ATA GTT GCA AAC 3'

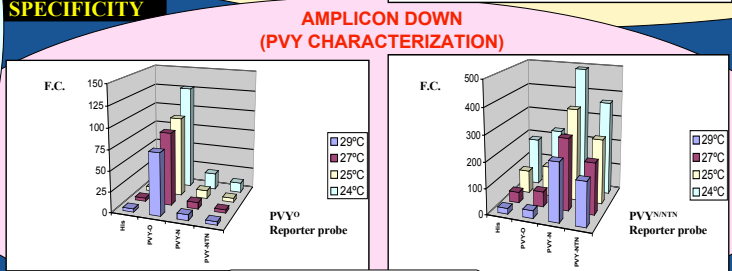
PVY primers (Nie & Singh, 2002): 5'-Biotine- CGC AAA AAC ACT CAC AAA AGC 3' and 5' CAT TTG TGC CCA ATT GCC 3'

RESULTS

SENSITIVITY



SPECIFICITY



CONCLUSIONS

- Tests performed on target oligos and on PCR amplicons showed a detection limit around 1nM. Nanochip technology required around 10⁸-10¹⁰ molecules of amplified targets.
- The specificity is not a limiting factor. Nanochip technology was able to detect and characterize the main potato viruses, successfully.

References
 Nie, X., Singh, R.P. 2001. Journal of Virological Methods 91, 37-49.
 Nie, X., Singh, R.P. 2002. Journal of Virological Methods 104, 41-54.
 Altschul et al., 1997. Nucleic Acids Research 25, 3389-3402.

His: Histidine buffer. Nontarget control 5nM, 10nM, 25 nM and 50 nM; Dilutions of target
 Capture: Capture probes. Nontarget control
 F.C.: Fluorescence counts

His: Histidine buffer. Nontarget control
 PVY-O, PVY-N, PVY-NTN, PVX, PLRV: Positive controls
 F.C.: Fluorescence counts