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APÉNDICE A

AGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGC
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GATGGTGATGTAAGTTTGTCACAAATCAGTGAGGAATTGAGCCATGCTCCAACAAAGAAATTCCCTGCAAGGGTGTTTCTCAAGATTGA
TATAGACAACTTACCAAGTGCTGTTTGCTCTAGGTGTAAGTTGAATATTGCAGGTAATCGATCTGTGAGATATGCTGGGTTTGCGAGCA
GTTTTCAAACCAAGCAAAAGTTGTCCCCTGCTGTTGGTGCAACTCCTGAATCCCTGATGCCATTGCTGGAAACCAATCAGAAGATTGAA
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*ACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGC*CAGCTGGCGTAATAGCGAAGAGGCCCGCACC
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GGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCCTGCGTTA
TCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAGCCGAACGACCGAGCGCAGCGAGTCAGT
GAGCGAGGAAGCGGAAG

Figura 17. Secuencia del vector TOPO PCR®4 más la secuencia del fragmento de 600 pb clonado. CAGCTG: sitio de corte de la enzima, *TTAACCCTCACTAAAGGGAC*: secuencia del promotor de la RNA polimerasa T3, letras azules: secuencia del fragmento de 600 pb, *letra en cursiva*: región que se transcribe.

Fragmentos:
a:2618 pb
b: 985 pb es el que contiene el fragmento clonado de 600 pb
c: 593 pb
d: 360 pb

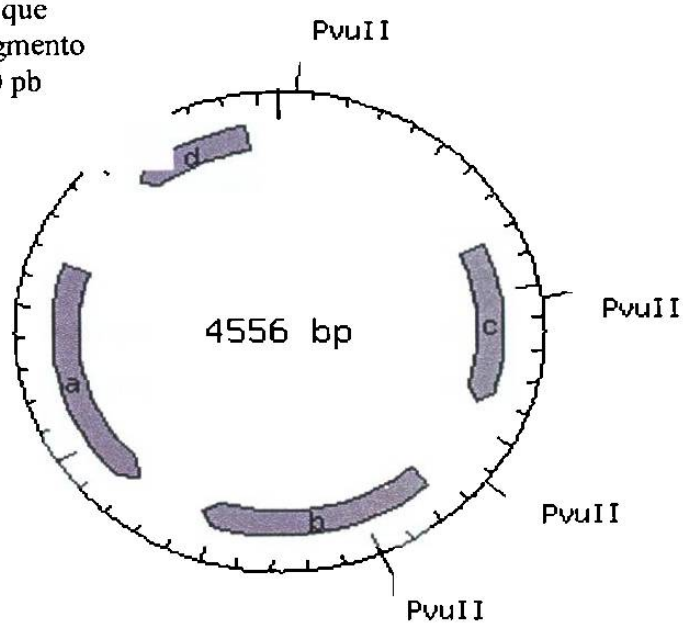

Figura 18. Simulación de la digestión del plásmido con la enzima *Pvu* II, con el programa NEB Cutter V2.0

Tabla XVII
Mezcla de reacción para transcripción *in vitro*

Componente	volumen
Buffer 5X para transcripción	20 µL
DTT 100 mM	10 µL
Inhibidor de ribonucleasas RNAsin	100 u
rATP, rGTP, rUTP y rCTP (2.5 mM) preparados por mezclar cada uno de los cuatro rNTP´s de concentración 10 mM	20 µL
DNA plasmido linealizado	2-5 µg
RNA polimerasa T3	40 u
Agua libre de nucleasa a un volumen final	100 µL

esta mezcla se incuba 1 h a 37 °C

APÉNDICE B

GCTTCCTGGAAAAGCTGATGACAAAAGGAAAGTTCCTGAATTTGTCAAGTCAAAACTGT
ATGATGGTGACGTGAGTTTGTCACAAATCAGTGAGGAGTTGAGCCATGCTCCAACCAAG
AAATTCCCTGCAAGGGTATTTCTCAAGATTGATATTGATAACTTG**CCAAGTGCTGTTTG**
CTCCAGATGCAAACTGAATATTGCTGGAAATCGATCTGTGAGGTATGCCAGCTTTGCAA
GCAGCTTTCAAACAAAGCAAAAGTTGTCCCCTGCTGTTGGTGCAACTCCTGAGTCCTTG
ATGCCTTTACTAGAAACAAACCAGAAGATTGAGAAGGCAATTGCAATTAGGGATTTCCT
AAAAACGATGGAAGGTCAATGGAAAAACCAGAAGCGACTCCACCCCTTATCTGATGAGA
AACCGACAATCAAGAACTTCACCTTGAAGCTAACATGCGCTATAATC**TACAGCCTCACT**
CCAGATGGAAGAACTGACATGGCAGAGAGGATAATATCCGACAATAATAAGGGGTTTCA
GAATGATAGGAACTTTTTTGGTGATGGTGAAGGGCCAATAAGGACATGGAGTGTGTTGA
CAAAACCAGA

Figura 19. Secuencia reportada por Barthe, *et al.*, 1998 (no. de acceso en banco de genes AFO36338). L, secuencias consenso en 22 secuencias reportadas en GenBank.

APÉNDICE C

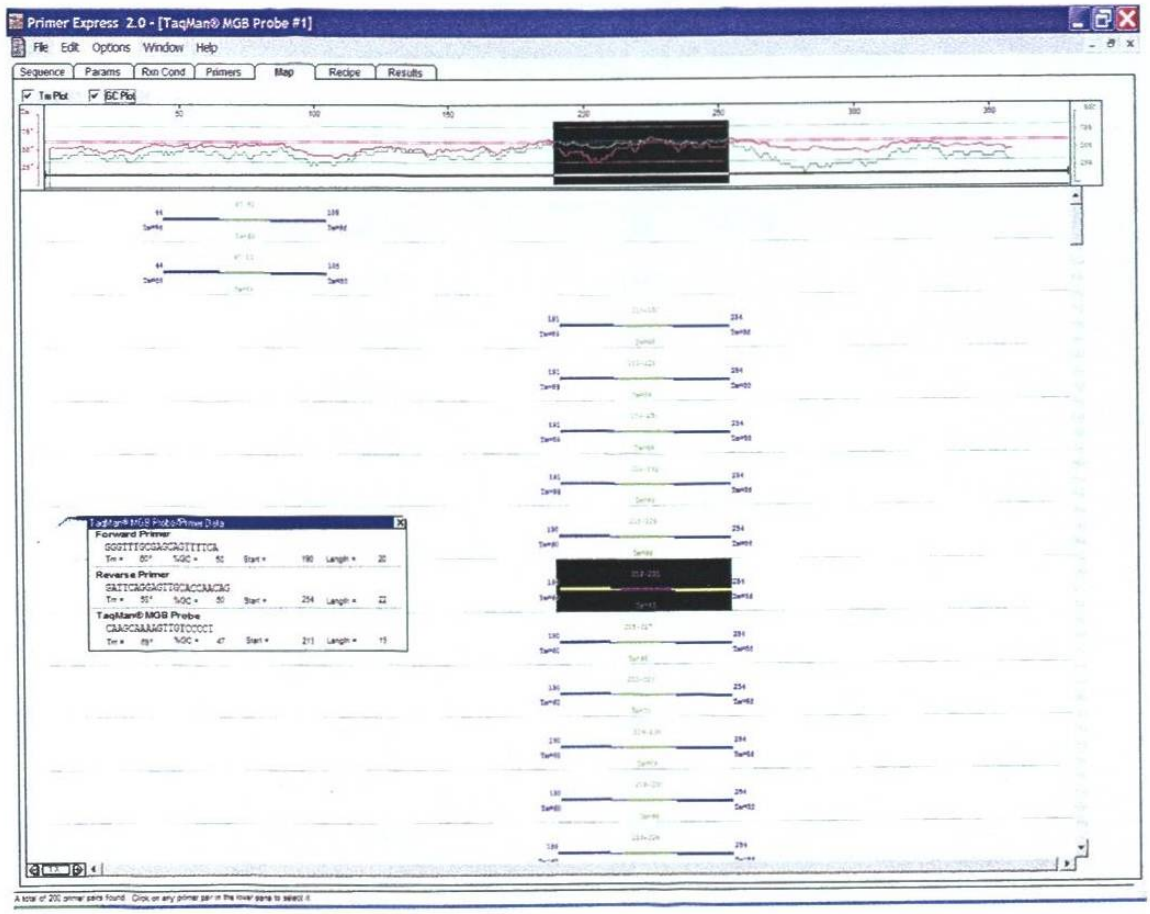

Figura 20. Resultado del análisis de la secuencia de 559 bases por medio del programa Pimers express ™ para la selección de un conjunto de primers y sonda para RT-PCR tiempo real

APÉNDICE D

Tabla XVIII
Similaridad de la secuencia de 79 bases que se amplifica en RT-PCR tiempo real, con otras secuencias reportadas en el banco de genes

No. de acceso	% de similaridad	
AY194918	100	79/79
AY194917	100	
AY194908	100	
AY194905	100	
AY194904	100	
AY194916	98	78/79
AY194915	98	
AY194914	98	
AY194913	98	
AY194912	98	
AY194911	98	
AY194909	98	
AY194907	98	
AY194900	98	
AY194906	97	77/79
AY194903	97	
AY194902	97	
AY194901	97	
AM159538	97	
AY654894	97	
AFO36926	93	74/79
AFO69855	92	66/71
AFO36338	92	

RESUMEN CURRICULAR

Gabriela Barragán Valencia

Candidata para el Grado de

Doctora en Ciencias con Especialidad en Biotecnología

Tesis: "Implementación de un método cuantitativo para la detección del virus psorosis de cítricos y su evaluación en muestras de campo"

Campo de estudio: Biotecnología Agrícola

Datos personales: Nacida en Cd. Juarez Chihuahua el 10 de julio de 1973. Hija de Carlota Valencia Hernández e Ignacio Barragán García.

Educación: Egresada del Instituto Tecnológico de Durango. Títulos obtenidos: Ingeniero Químico y Maestra en Ciencias en Ingeniería Bioquímica.

Experiencia Profesional: Asesora pedagógica y Coordinadora académica en el Consejo Nacional de Fomento Educativo, Delegación Durango; de enero del 2000 a enero del 2002.

La Asociación Mexicana de Microbiología A.C.

otorga la presente

CONSTANCIA

a:

V.G. Barragán, L.M.A. Morales, Z.I. Quintero y O.G. Alvarez

Por su trabajo titulado **"OBTENCIÓN DE UN ESTÁNDAR DE CONCENTRACIÓN DE CDNA DEL VIRUS PSOROSIS DE CÍTRICOS COMO CONTROL DE CUANTIFICACIÓN PARA PCR EN TIEMPO REAL"** presentado en el XXXV Congreso Nacional de Microbiología, realizado del 3 al 6 de abril de 2006 en Oaxtepec, Mor., México

Dra. Esperanza Martínez Romero
Presidenta de la Mesa Directiva 2004-2006

LA SOCIEDAD ESTUDIANTIL DE BIOTECNOLOGÍA Y GENÓMICA

SEBioGen

Otorga el presente

Reconocimiento a:

Gabriela Barragán Valencia

Por su participación en la exposición de carteles con el trabajo "Relaciones filogenéticas del virus psorosis de cítricos con base a la secuencia del gen que codifica para la proteína de la cápside" dentro del marco del evento:

1er Congreso Internacional de Biotecnología y Genómica
SEBioGen

genobiotec

Realizado en la Biblioteca Universitaria Raúl Rangel Frías, Monterrey, N.L.
del 30 de Mayo al 1 de Junio de 2007

Dr. Juan Manuel Alcocer González
Director Facultad de Ciencias Biológicas

Humberto Antonio Salazar Sesatty
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Cd. Guadalupe, N. L., a 11 de diciembre del 2006

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Estado de México.

Asunto: Envío de contribución

Por este medio me permito someter a la consideración del Comité Editorial la contribución como artículo para la sección de protección vegetal: DETECCIÓN CUANTITATIVA DEL VIRUS PSOROSIS DE CÍTRICOS MEDIANTE RT-PCR TIEMPO REAL.

Sin otro particular por el momento, le envío un cordial saludo

ATENTAMENTE

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ccp. Dra. Genoveva Alvarez Ojeda. Investigadora del INIFAP.
ccp. Dra. María de los Angeles Peña del Río. Investigadora del INIFAP.

DETECCIÓN CUANTITATIVA DEL VIRUS PSOROSIS DE CÍTRICOS MEDIANTE RT-PCR TIEMPO REAL

QUANTITATIVE DIAGNOSIS OF CITRUS PSOROSIS VIRUS BY REAL TIME RT-PCR

Gabriela Barragán-Valencia[1], Alberto Morales-Loredo[2*], Maria Genoveva Álvarez-Ojeda[2], María de los Angeles Peña del Río[2], Isela Quintero-Zapata[1]

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RESUMEN

La RT-PCR tiempo real es una alternativa para la detección del virus psorosis de cítricos (CPsV), brinda la oportunidad de evaluar carga viral que puede ser un indicador útil en el estudio de la psorosis. En este trabajo se implementó un protocolo de RT-PCR tiempo real para la detección cuantitativa del CPsV, para lo que se diseñaron un conjunto de iniciadores y sonda Taqman con base en una secuencia de 559 nt del gen de la proteína de la cápside del CPsV procedente de tejido infectado de un árbol de campo*. En el desarrollo de la curva estándar se obtuvo un $R^2 = 0.998$ de la cantidad de ARN molde con respecto a los valores de C_T y una eficiencia de amplificación de 94.5%. La detección del virus por medio del protocolo implementado en la planta de invernadero fue consistente. En la detección en muestras de campo se obtuvo un 40% de sensibilidad. Se cuantificó carga viral en muestras de la planta de invernadero y en muestras procedentes de cuatro árboles de campo.

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* En este trabajo se obtuvieron dos secuencias de un fragmento de 559 nt y se reportaron en NCBI. Los números de acceso de estas secuencias son: EF618547 y EF618548.