

**CINVESTAV**



**Senasica**

# **Evaluación de la viabilidad de CLa en cítricos infectados con HLB**

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# Huanglongbing en México

- Enfermedad en cítricos causada por la bacteria Candidatus *Liberibacter asiaticus*, que habita los tejidos vasculares de los cítricos y es transmitida por el insecto vector *Diaphorina citri*.

# Síntomas de HLB



Material de Colima y Sinaloa.

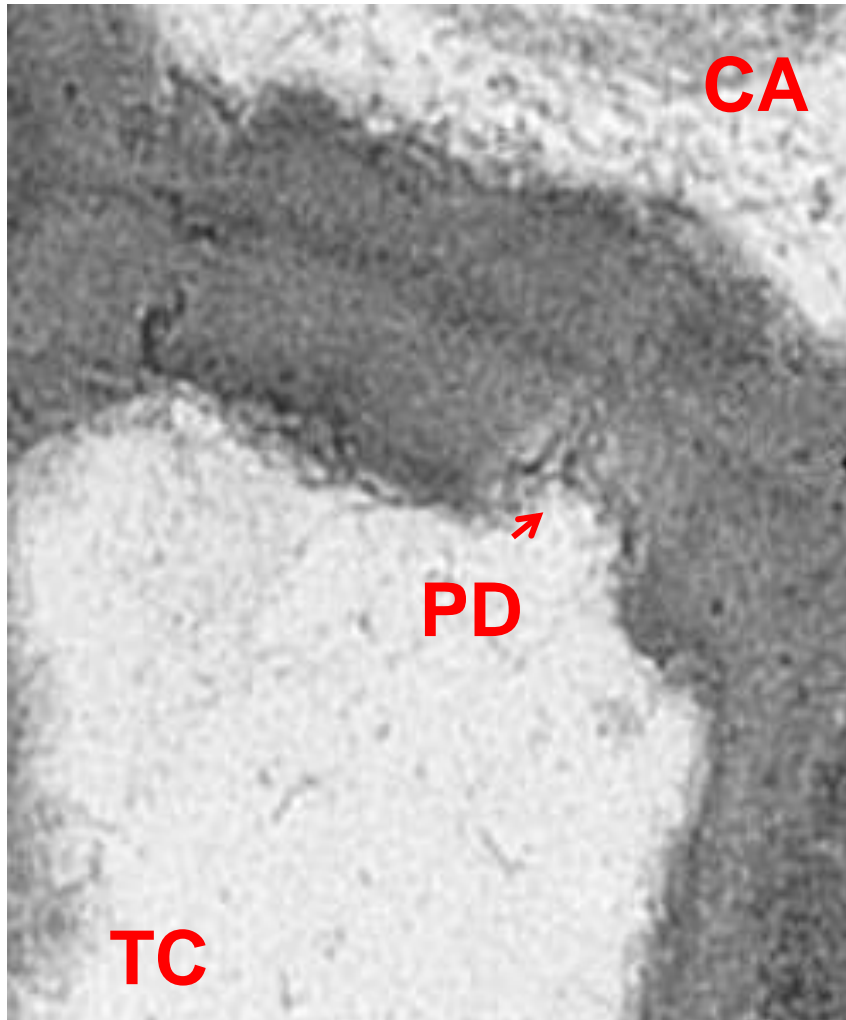






HLB es causado por la bacteria CLa que no es cultivable in vitro.

SANA



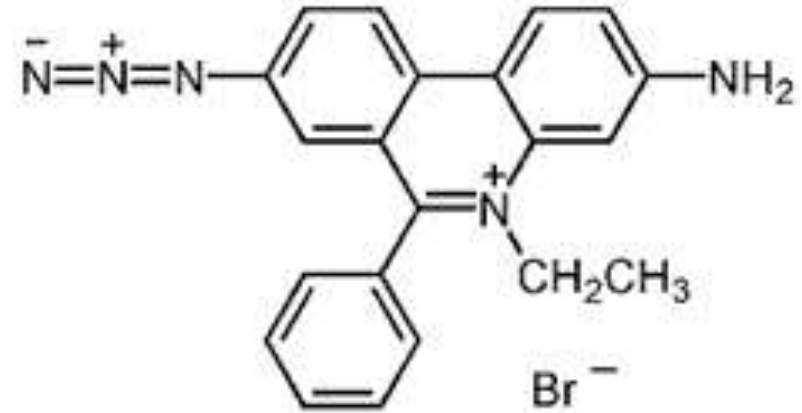
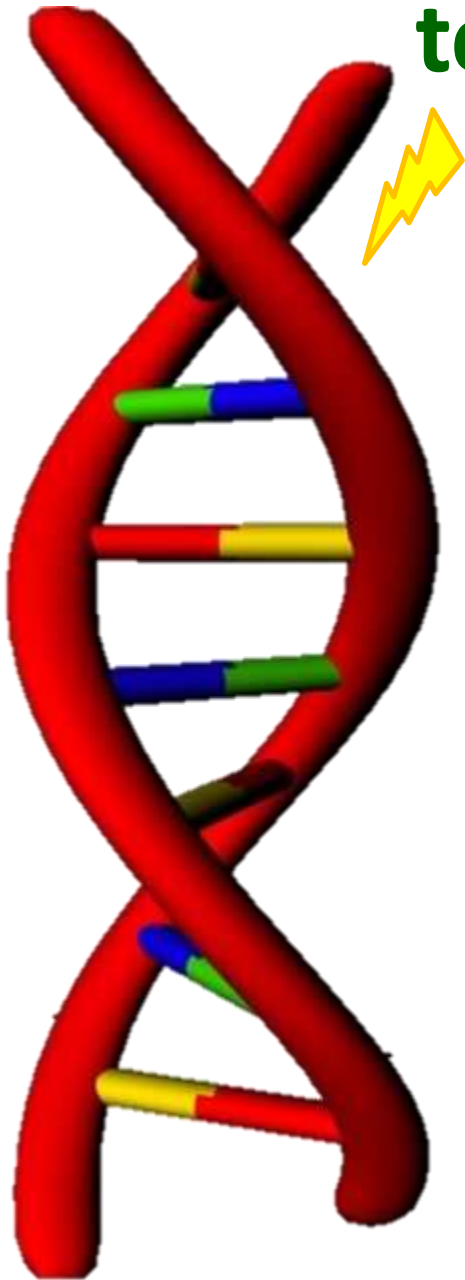
ENFERMA



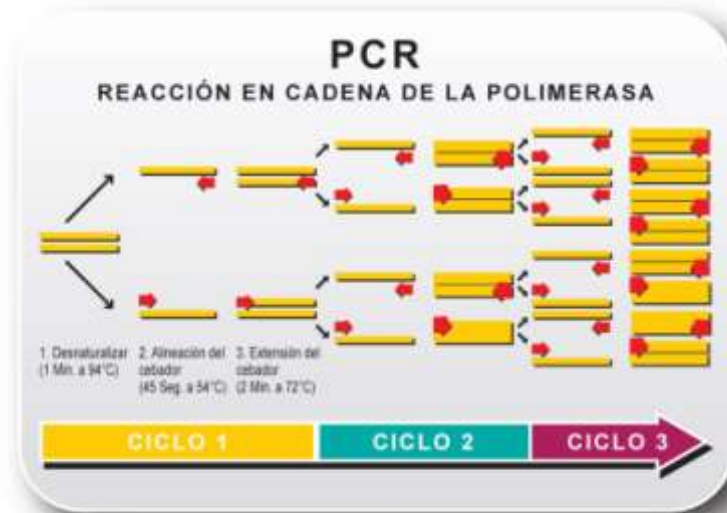
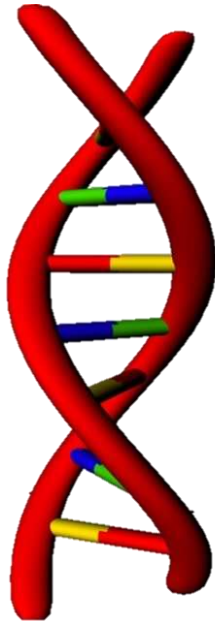
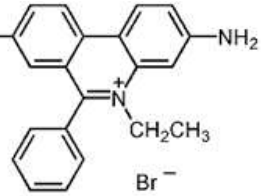
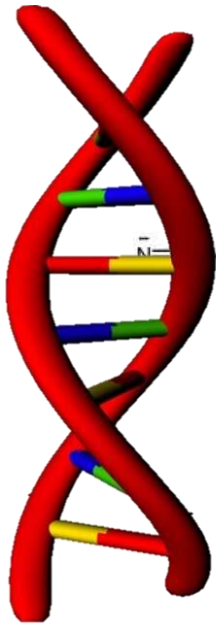
Strategic Planning for the Florida Citrus Industry:  
Addressing Citrus Greening (2010).

**¿Cómo cuantificar la viabilidad de una bacteria no cultivable?**

# Evaluación de viabilidad de CLa por la técnica de EMA PCR

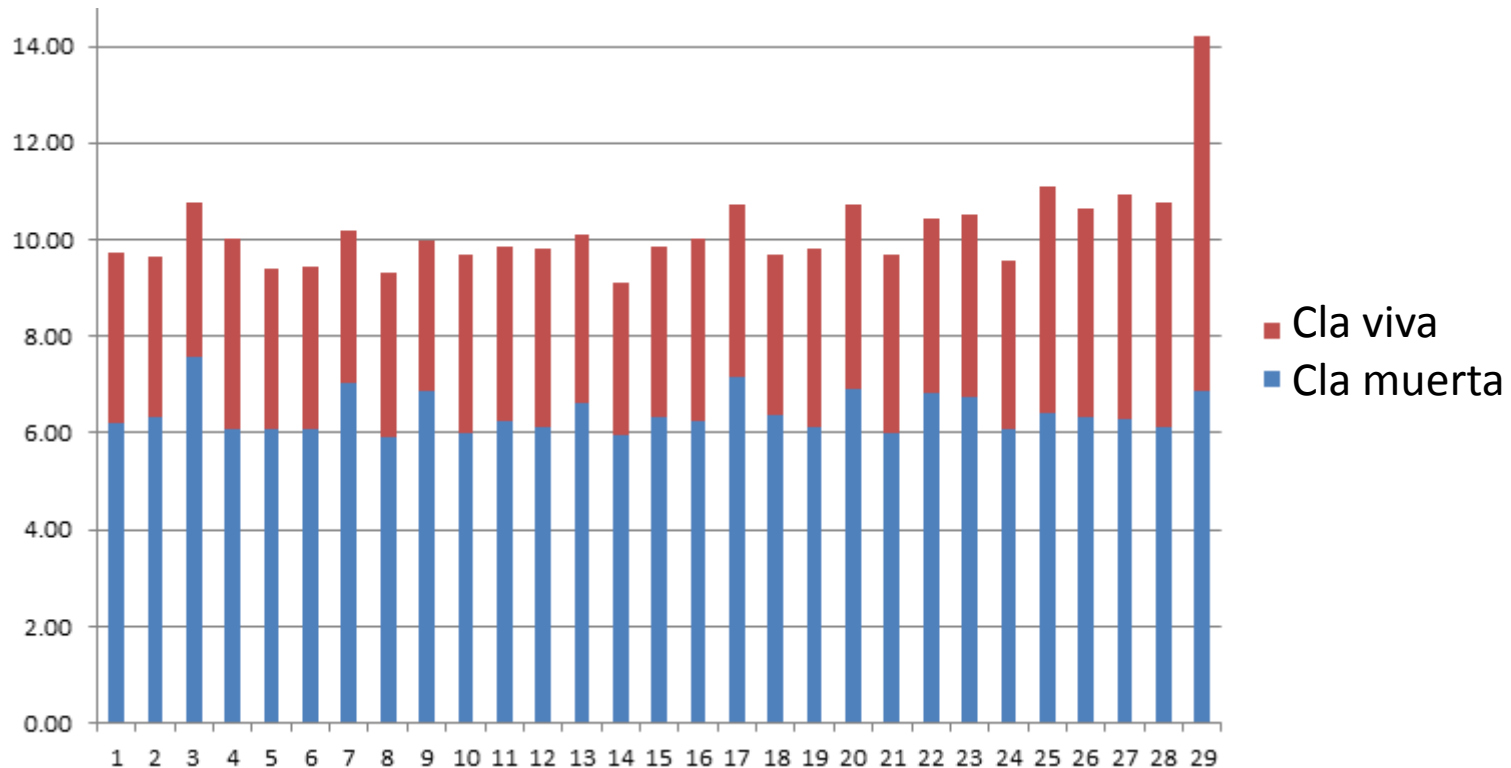


# EMA-qPCR

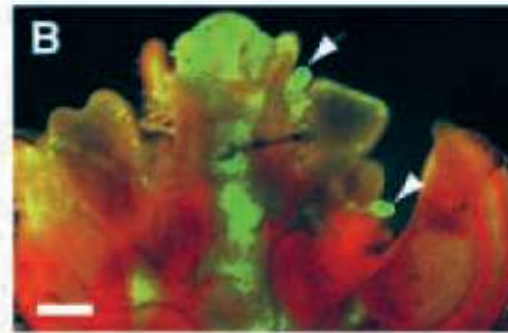
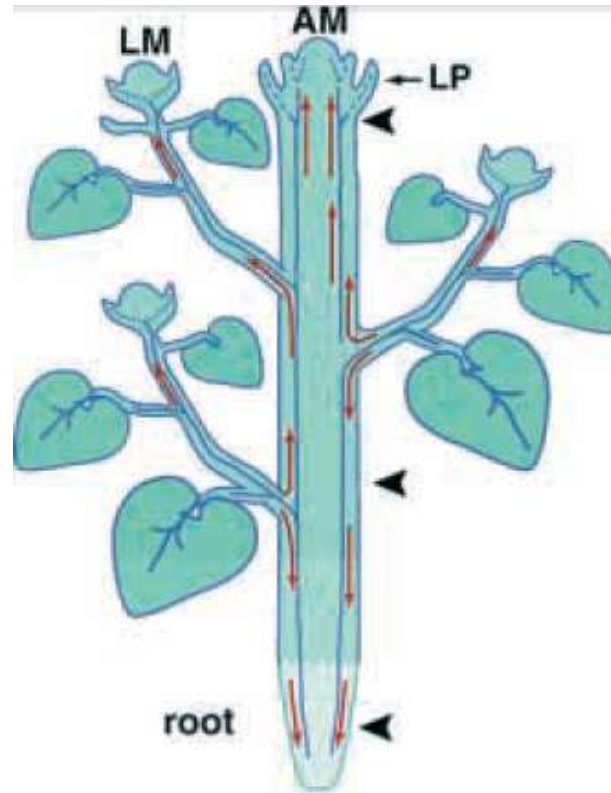




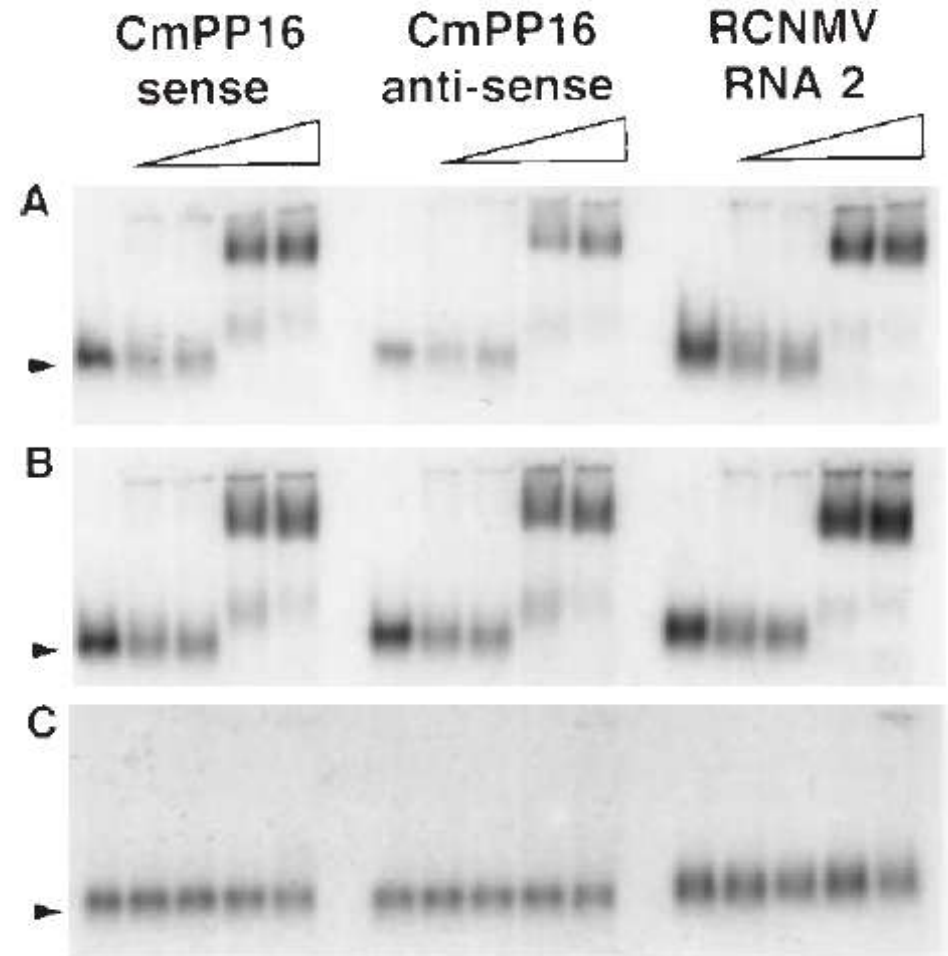
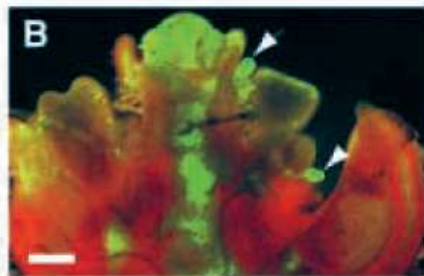
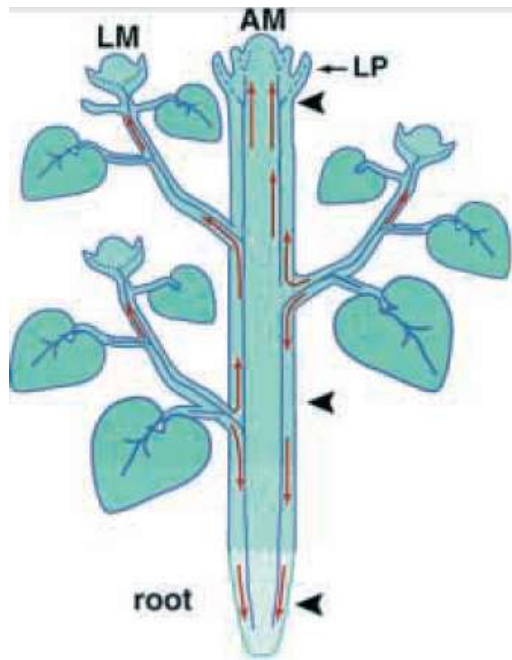
# Detección de CLa por EMA PCR tiempo real en plantas infectadas por HLB



# Complejos Ribonucleoprotéicos son estables en el tejido vascular

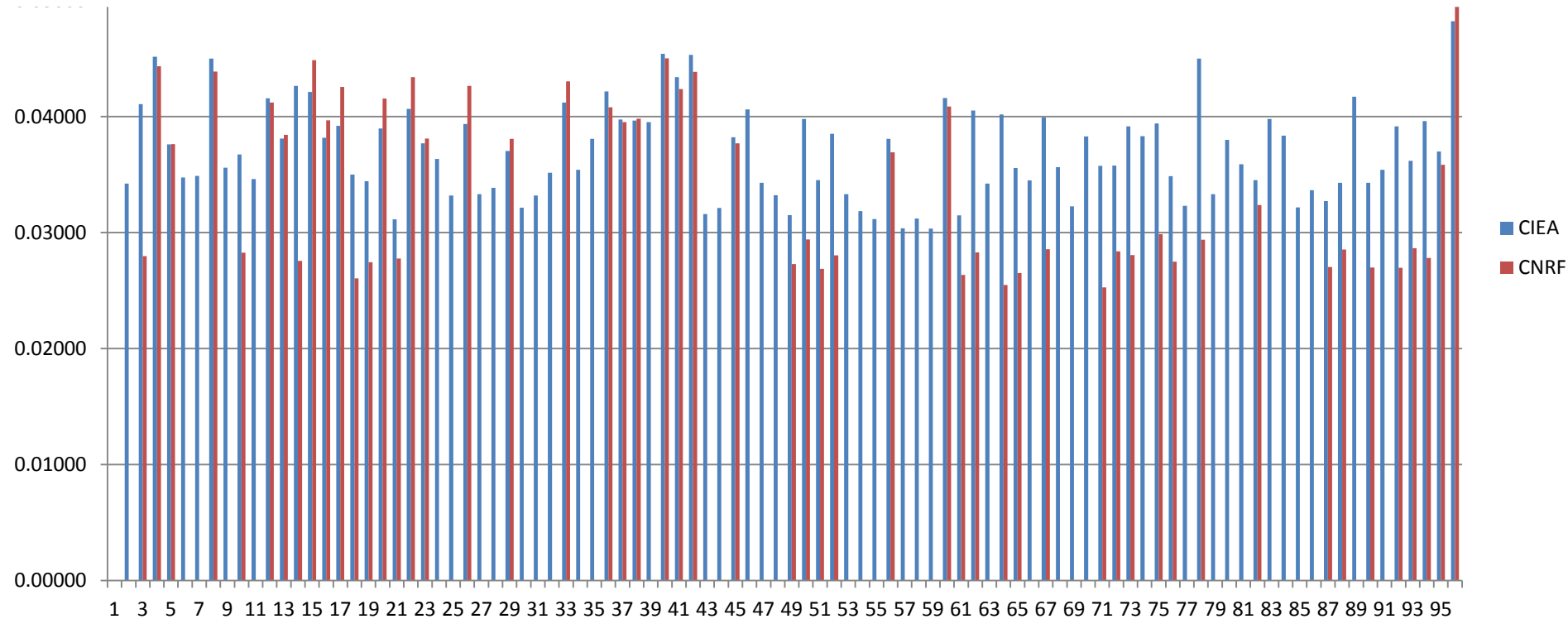


# DNA y RNA son estables en el tejido vascular





# Detección por PCR tiempo real de CLa



**CLa 16S**

5' -GTTTGATCCTGGCTCAGAACGAACGC-3'

5' -TAGGGCGTATACGGTATTAGCACACG-3'

FAM-ACGGGTGAGTAACGCGTAGGAATC-HBQ1

**Citrus COX**

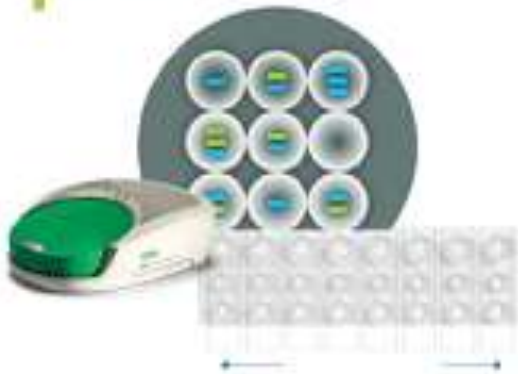
5' -GTATGCCACGTCGCATTCCAGA-3'

5' -GCCAAAAGTCTAAGGGCATTTC-3'

HEX-ATCCAGATGCTTACGCTGG-BHQ2

# Detección de CLa por ddPCR

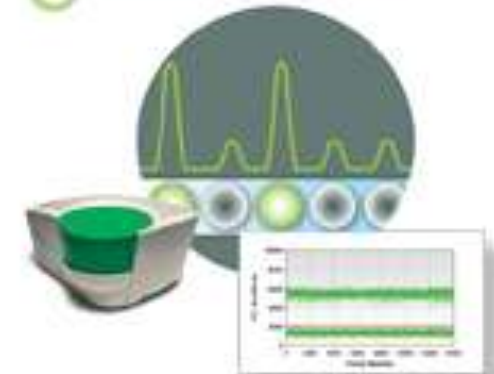
1 Generate droplets



2 Perform PCR with EvaGreen or hydrolysis probes



3 Read and analyze results



Multiplex

CLa 16S

5' -GTTTGATCCTGGCTCAGAACGAACGC-3'  
5' -TAGGGCGTATACGGTATTAGCACACG-3'  
FAM-ACGGGTGAGTAACGCGTAGGAATC-HBQ1

Citrus COX

5' -GTATGCCACGTCGCATTCCAGA-3'  
5' -GCCAAAAGTCTAAGGGCATTTC-3'  
VIC-ATCCAGATGCTTACGCTGG-BHQ2

FAM

VIC

FAM VIC

FAM versus

FAM Concentration

VIC Concentration

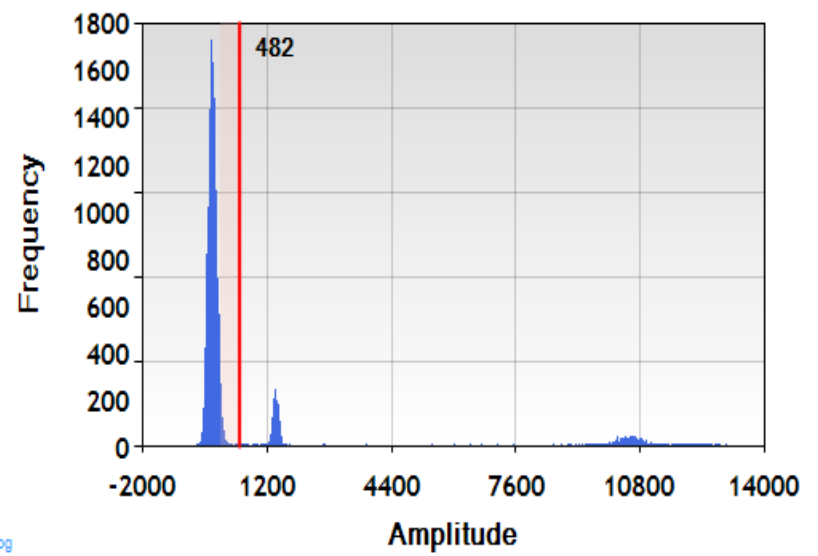
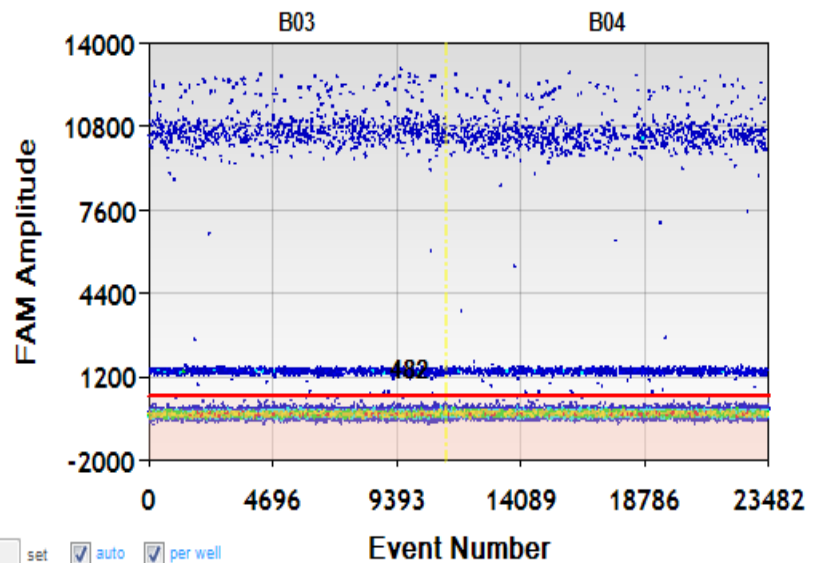
FAM VIC Concentration

Events

Options

Conc:175 FAM Pos:3452 Neg:20029 Quality 98

FAM Histogram - B03 Auto Threshold



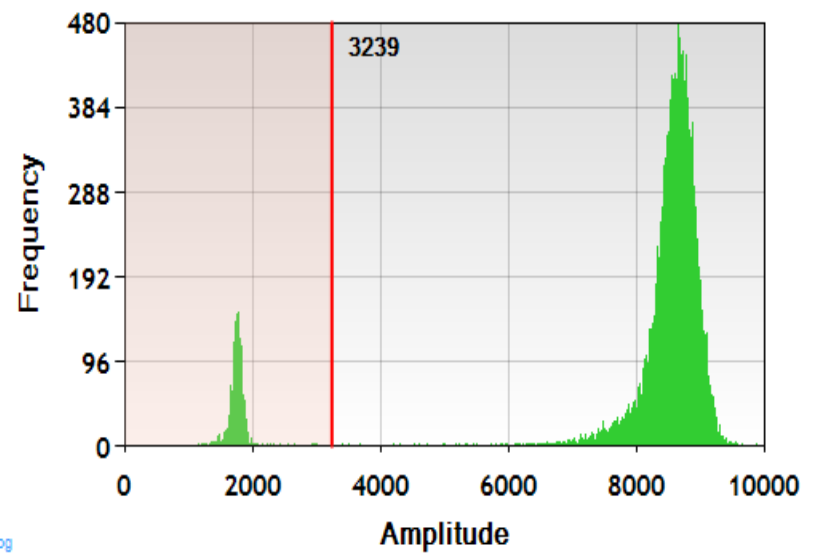
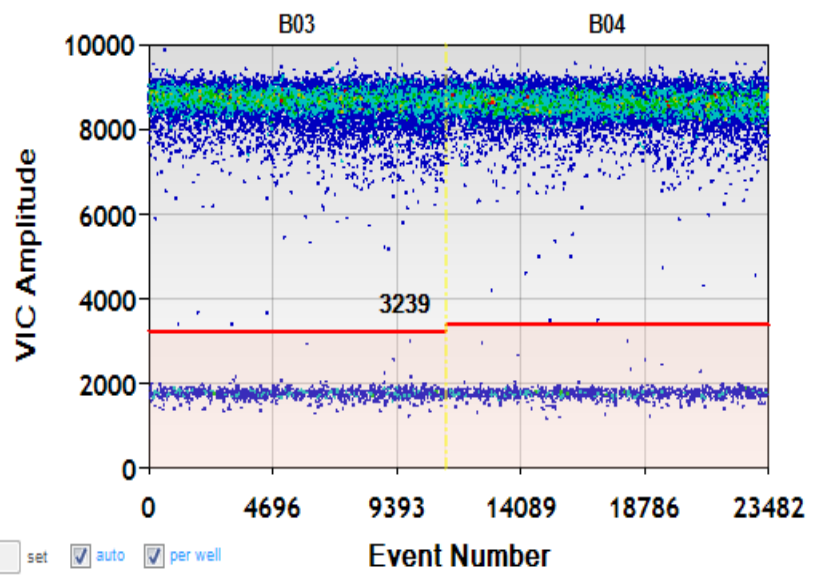
482 set  auto  per well

log

Options

Conc:2710 VIC Pos:21492 Neg:1989 Quality 95

VIC Histogram - B03 Auto Threshold



3239 set  auto  per well

log

CLa

COX



FAM

VIC

FAM VIC

FAM VIC versus

FAM Concentration

VIC Concentration

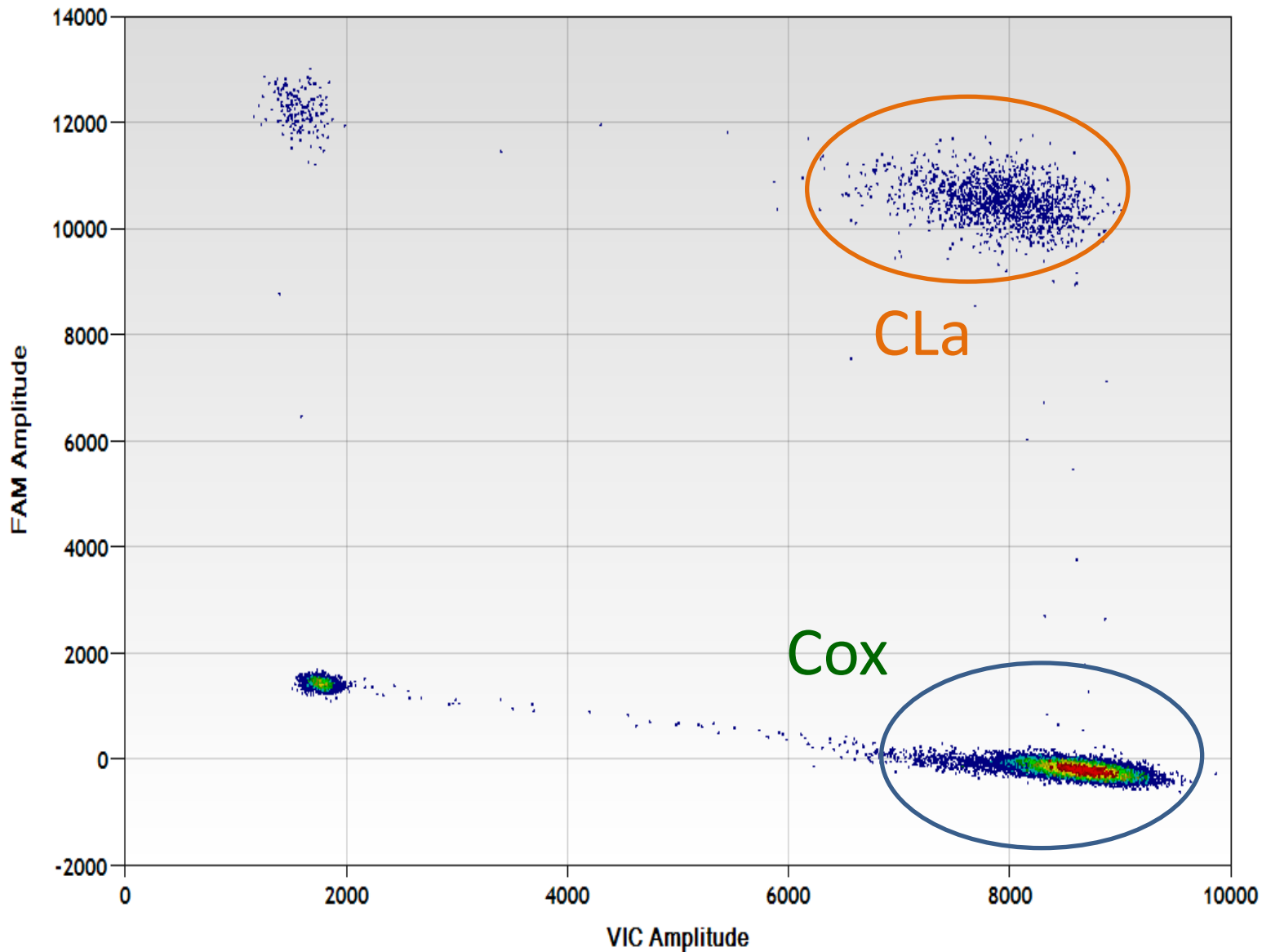
FAM VIC Concentration

Events

Options

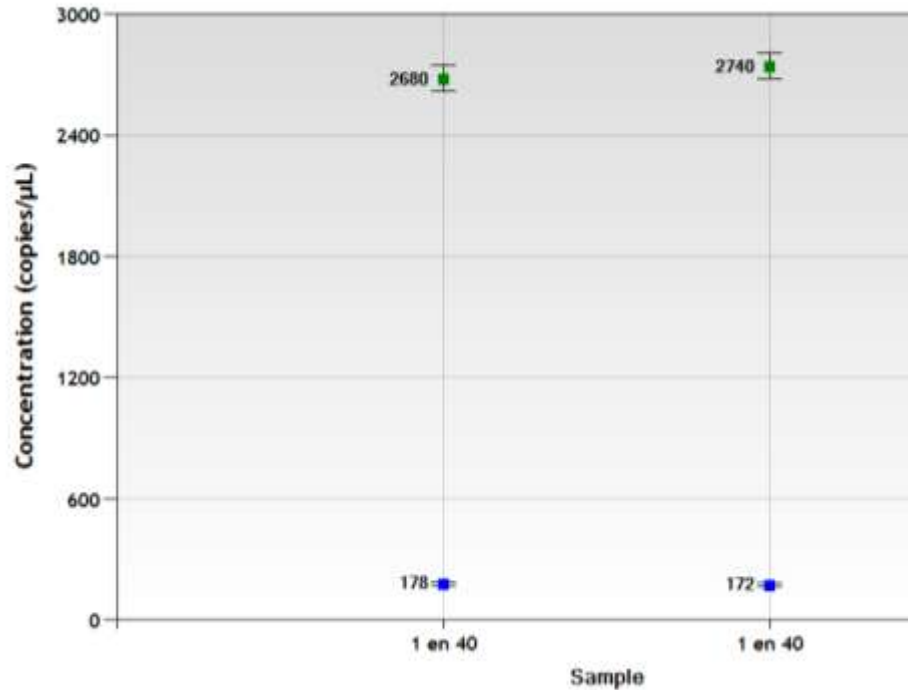
Quality FAM:98 VIC:95

FAM+VIC+:1463 FAM+VIC-:1989 FAM-VIC+:20029 FAM-VIC-:0

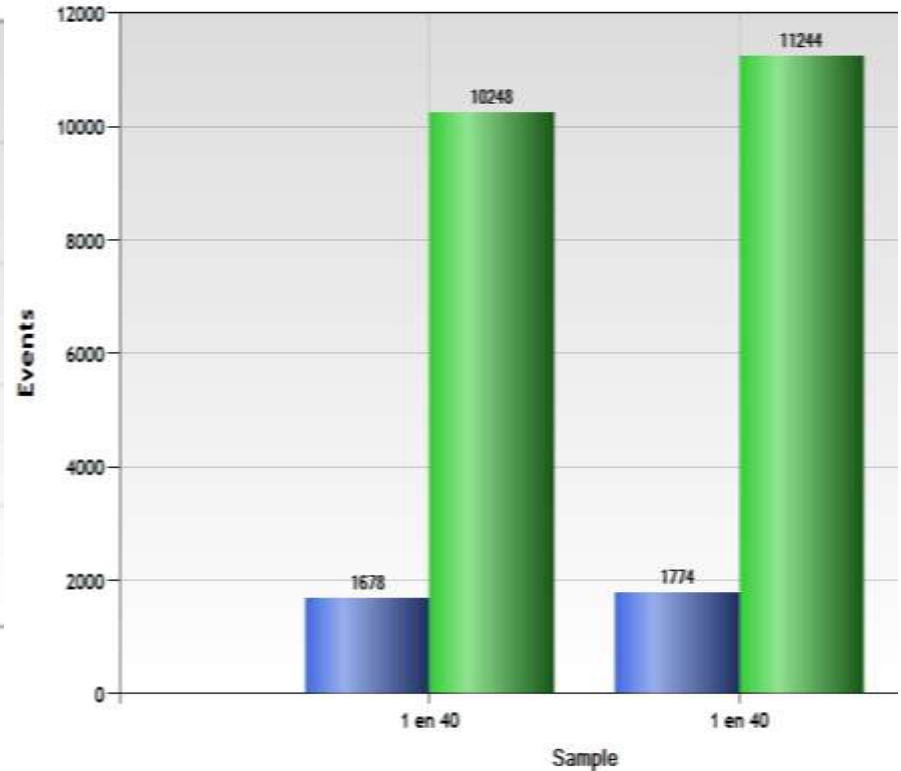


# Cuantificación absoluta de CLa

ddPCR Concentration



FAM Pos:3452 VIC Pos:21492



3452 copias de CLa y 21492 copias de COX

# Conclusiones

## HLB

- Se cuenta con métodos sensibles para cuantificar CLa viable.  
*EMA-PCR, PCR tiempo real, ddPCR.*
- Se identificaron dos haplotipos de CLa.



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