

# Typing of Citrus tristeza virus strains by plate hybridization with a panel of probes

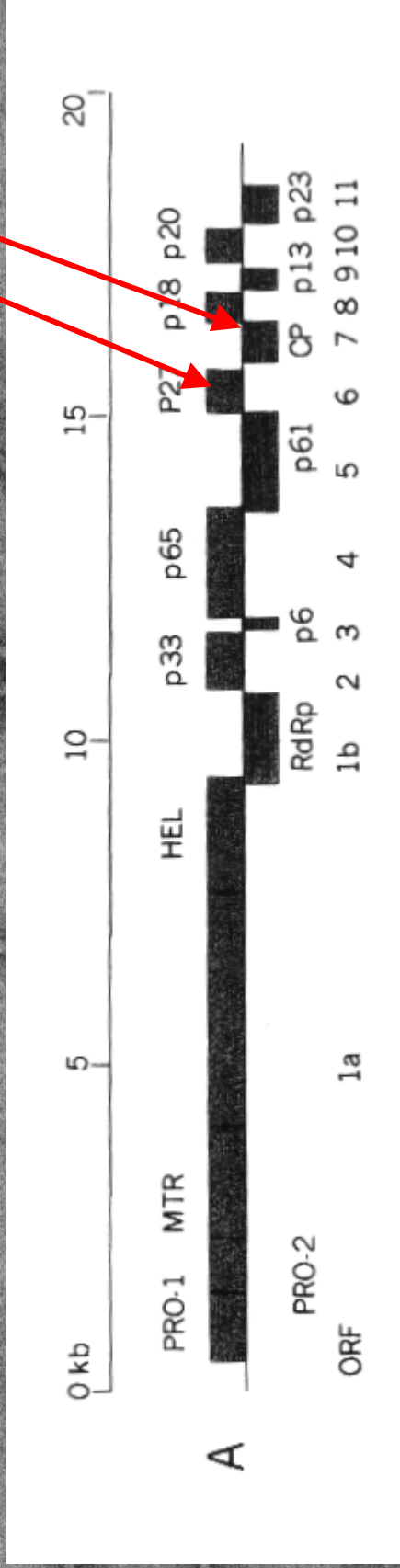
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<http://www.ecoport.org/resources/slide> shows







# CTV Diagnosis

## Objectives

Detection → High sensitivity, large spectrum  
Typing → Strain discrimination

## Problems

High volume of samples  
Low technical skill

Methods that:

are user-friendly  
are cheap  
require low degree of  
manipulation

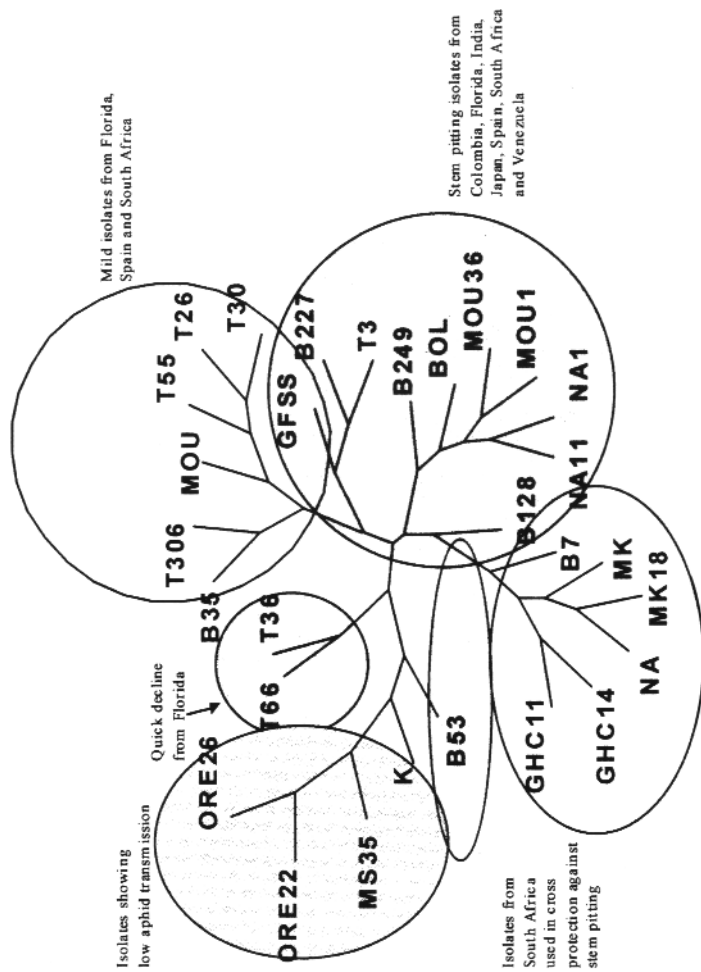


Fig. 3. An unrooted tree based on the coat protein gene sequences of several CTV isolates showing the association of different groups with biological activity. The tree was constructed using the neighbor-joining method from the PHYLIP package (evolution.genetics.washington.edu/phylip.html) and TreeView (42) computer programs.



Decline on  
sour orange

Gr 1

Gr 2

Gr 3a

Gr 3b

Gr 4

Gr 5

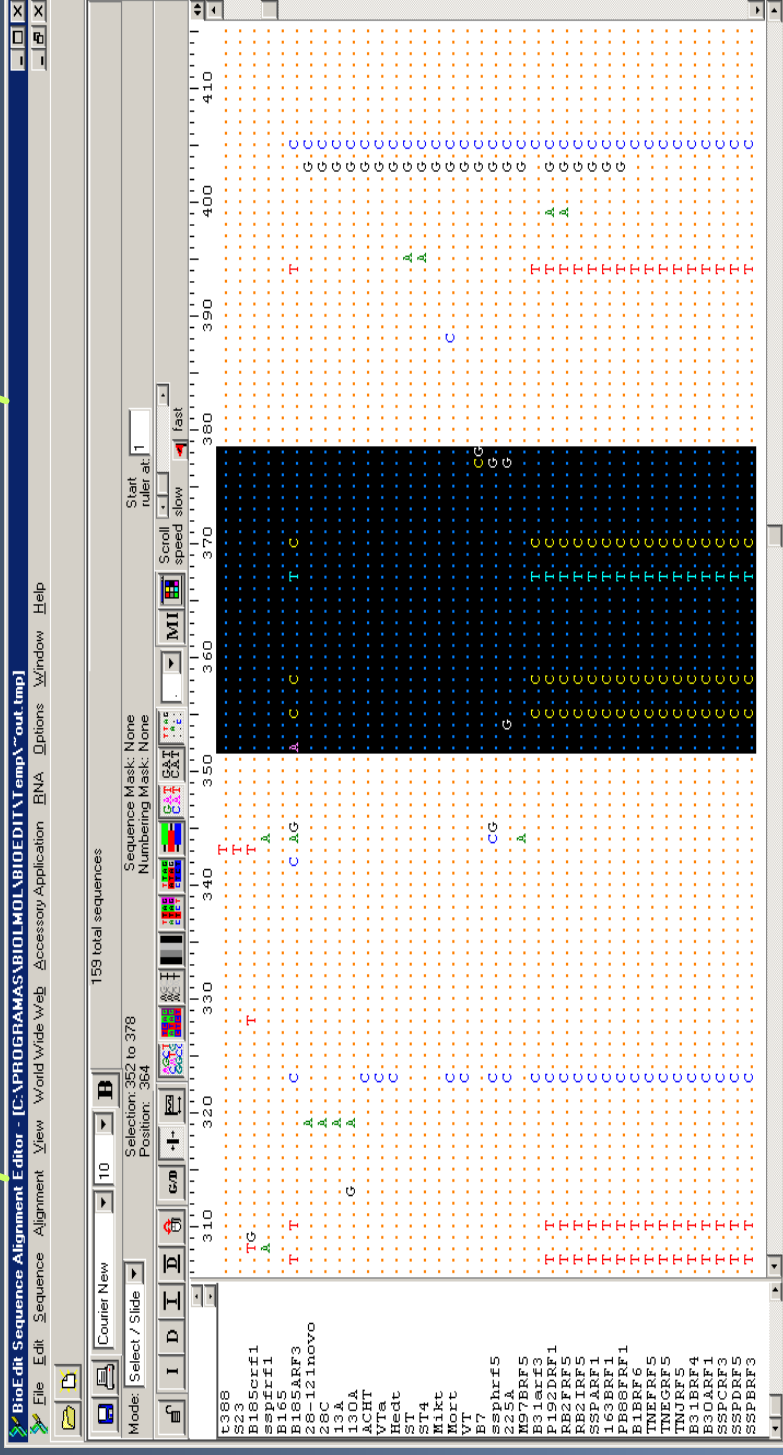
Gr M

Stem-pitting,  
reduction of fruit size  
even on tolerant rootstocks



Note: according to sequence data all groups except  
group M should react with MCa13

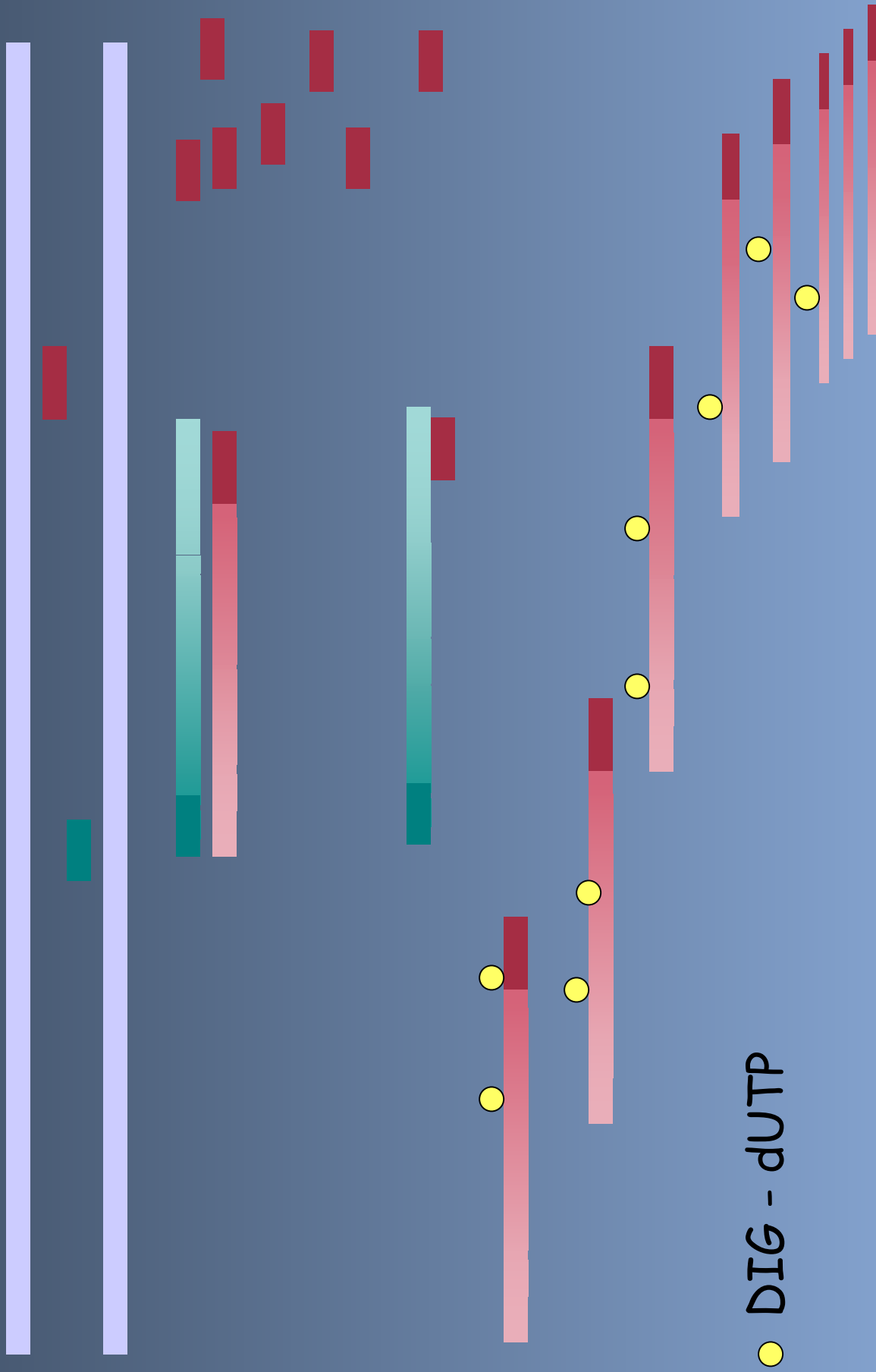
# Design of probes to identify groups in naive asymmetric PCR ELISA assay



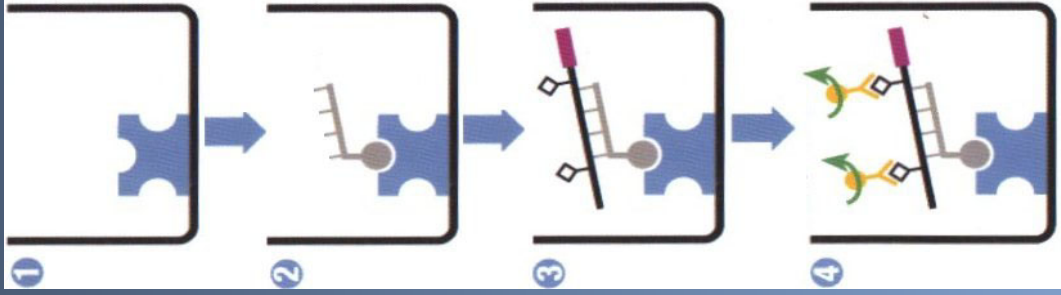
Regions for probe design should preferably have 100% homology to only one group  
 Pattern of parsimonious mismatches should be similar within each group up to 3 mismatches  
 Minimize number of "strange" parsimonious patterns  
 Discard "strange sequences"  
 Non- parsimonious mismatches not considered.

Trimming of probes for similar  $T_m$   
 Maximize effect of mismatches on  $T_m$  (metcalc)

# Asymmetric PCR



# Asymmetric PCR ELISA



Coat ELISA plate with Streptavidin

wash

Add the biotinylated capture probe

wash

Add the asymmetric RT-PCR Dig labelled product,  
hybridise.

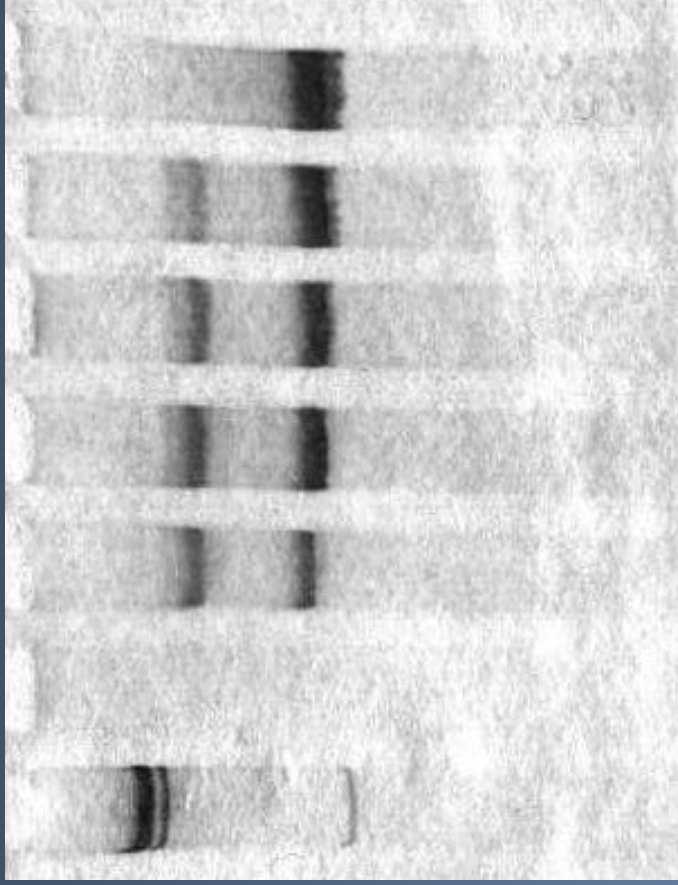
wash

Add the anti-DIG-f(ab)'2 alkaline  
phosphatase conjugate

wash

Add substrate. Absorbance readings at 405 nm.

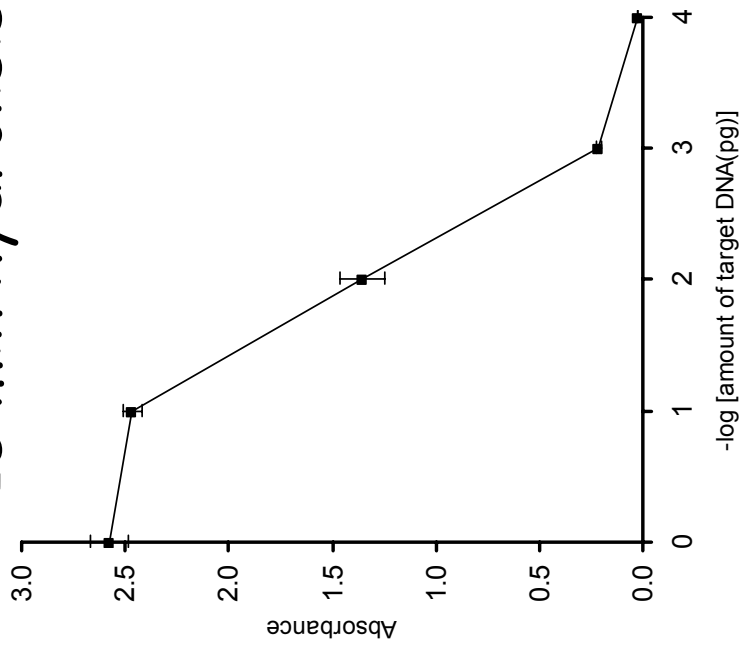
M C- 25:1 10:1 10:1 5:1 1:1



ss-DNA

ds-DNA

# 15 min hydrolysis

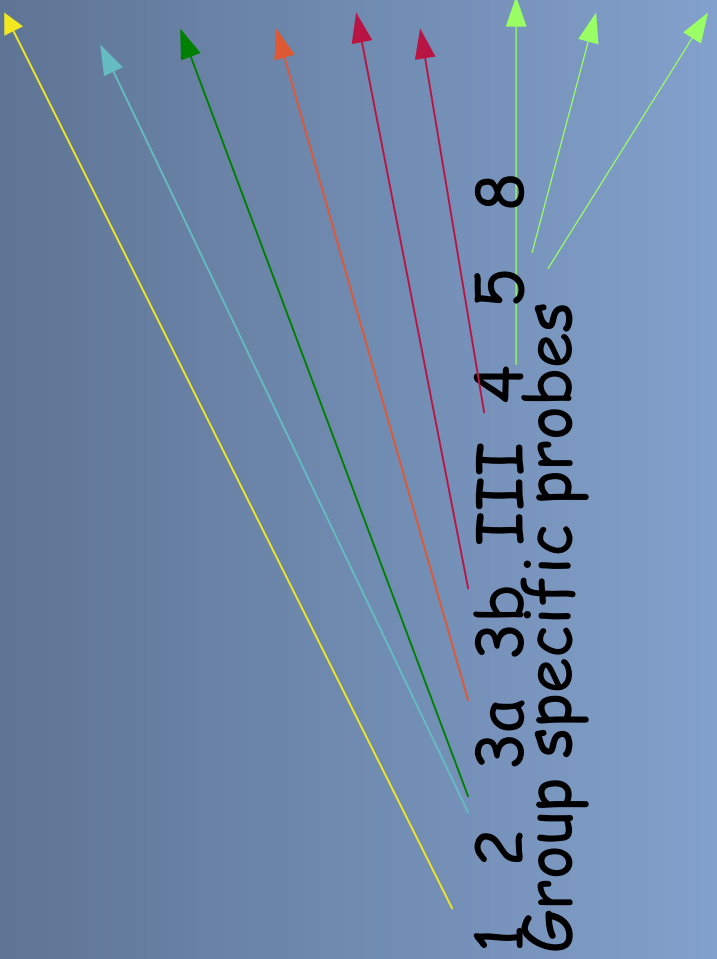
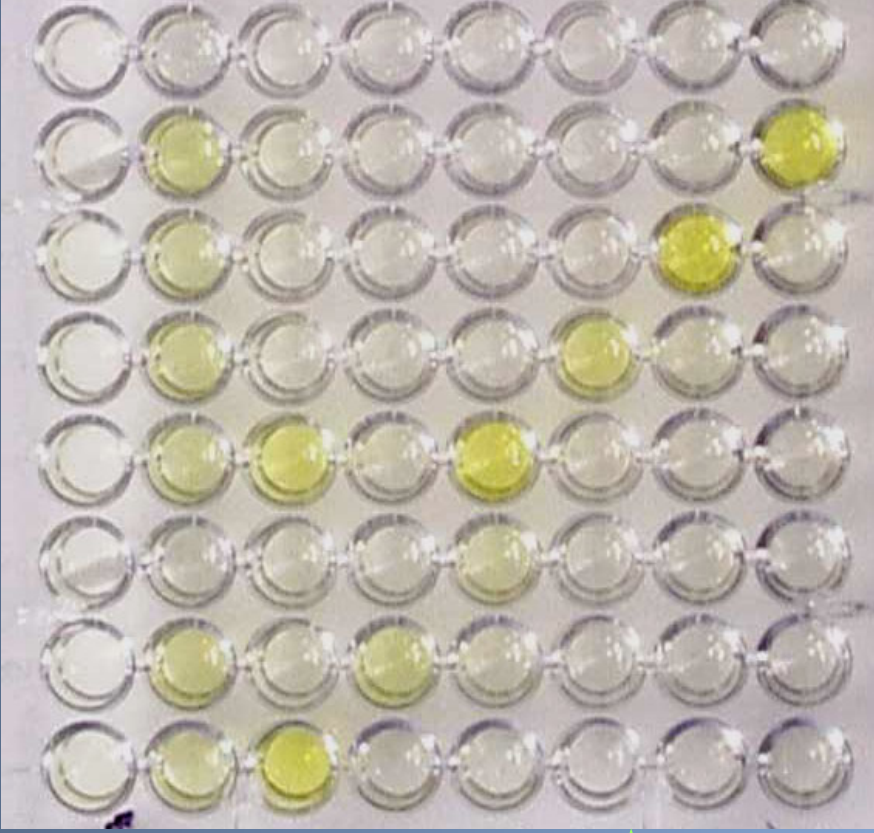


IC-RT/asymmetric PCR

Positive ?

Asymmetric re-amplification

Samples to be tested



# Pattern of reaction (initial rate) of characteristic strains from each group

probe / group	GR 1	GR2	GR3a	GR3b	Gr4	Gr5	Gr M
B1	0.466	0.006	0.006	0.006	0.007	0.007	0.007
B2	0.004	0.155	0.005	0.005	0.005	0.007	0.005
B3a	0.305	0.013	0.24	0.012	0.108	0.006	0.208
B3b	0.041	0.004	0.004	0.276	0.018	0.063	0.169
III	0.004	0.006	0.016	0.462	0.007	0.005	0.006
B4	0.004	0.004	0.011	0.006	0.472	0.008	0.028
B5	0.005	0.004	0.004	0.004	0.005	0.31	0.006
B8	0.235	0.008	0.005	0.016	0.005	0.016	0.543

Each group has a unique pattern that is not proportional to the patterns of the other groups.

# Unknown isolates - initial rate of reaction

Probe / sample	p1	p2	p3	p4	p5	p6	p7	p8	p9	p10	p11	C-	
B1	0,007	0,004	0,003	0,006	0,003	0,004	0,004	0,004	0,005	0,004	0,004	0,005	0,004
B2	0,14	0,187	0,003	0,164	0,009	0,006	0,006	0,004	0,259	0,005	0,006	0,005	0,004
B3a	0,249	0,006	0,005	0,004	0,004	0,004	0,004	0,092	0,007	0,005	0,008	0,006	0,004
B3b	0,004	0	0,002	0	0,215	0,089	0,003	0,003	0,001	0,062	0,005	0,002	0
III	0,147	0,004	0,077	0,004	0,176	0,004	0,004	0,004	0,009	0,004	0,006	0,005	0,006
B4	0,006	0,005	0,006	0,006	0,004	0,008	0,317	0,005	0,005	0,006	0,005	0,006	0,005
B5	0,005	0,003	0,003	0,004	0,16	0,004	0,004	0,003	0,003	0,004	0,006	0,006	0,006
B8	0,016	0,021	0,004	0,025	0,009	0,353	0,005	0,027	0,241	0,088	0,031	0,013	

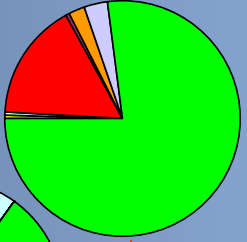
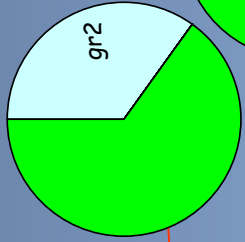
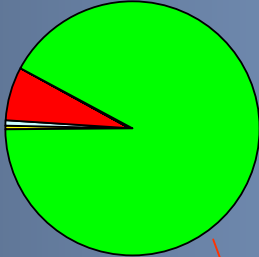
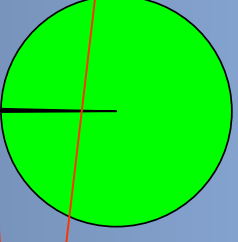
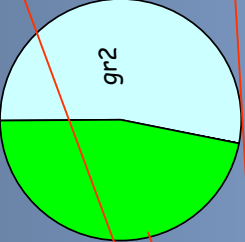
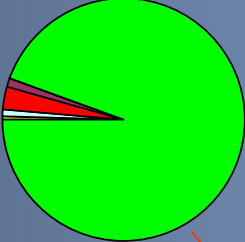
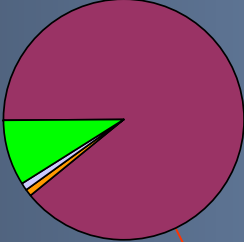
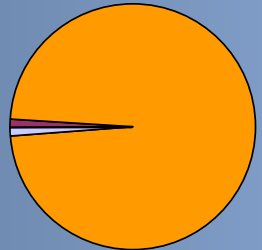
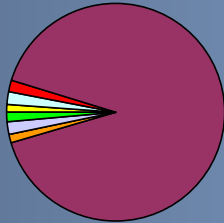
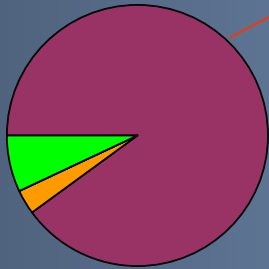
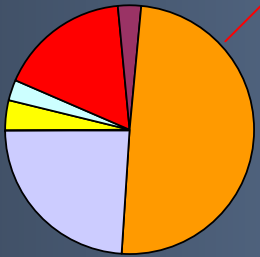
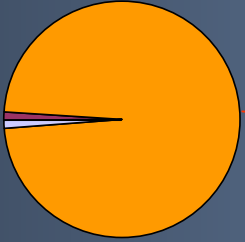
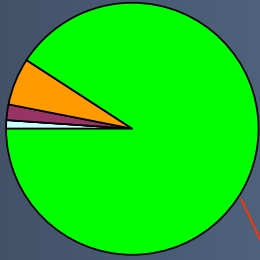
- It is assumed that the signal of an isolate results from the sum of the signals of the groups composing that isolate, according to their respective patterns, multiplied by the relative amount of DNA of that group.
- Successive iterations enable to estimate the relative proportion of strains composing an isolate that best fit to the existing patterns.

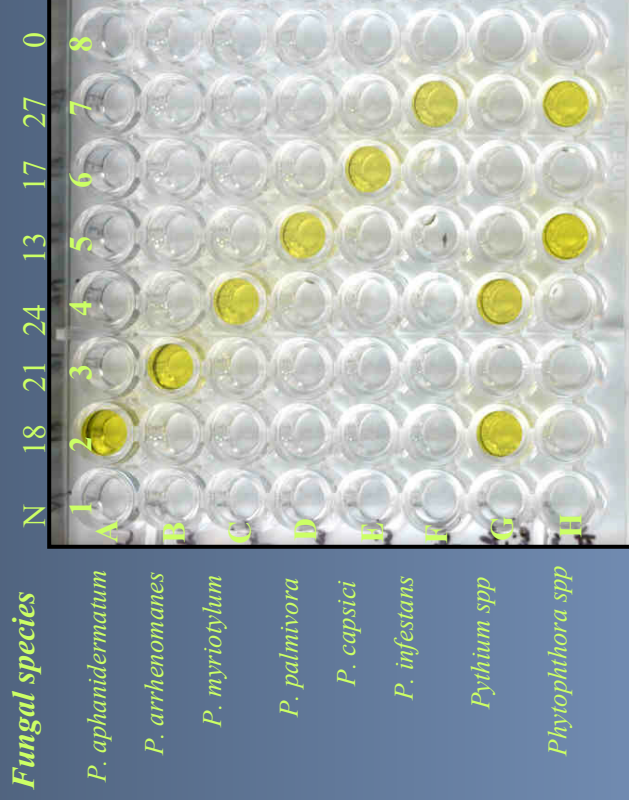
## Strain composition (%)

Resultados	p1	p2	p3	p4	p5	p6	p7	p8	p9	p10	p11
Gr 1	0	0	2	0	0	0	0	0	0	0	2
Gr 2	41	99	6	98	2	0	0	99	0	12	24
Gr 3a	46	0	1	0	0	0	11	0	0	0	0
Gr 3b	13	0	82	0	42	0	0	0	0	5	8
Gr 4	0	0	5	0	0	0	89	0	0	0	5
Gr 5	0	0	4	0	56	0	0	0	0	1	16
Gr M	0	1	0	2	0	100	0	1	98	74	44

The system is not closed: bad fittings are detected and new groups may be added if necessary.

Gr 1  
 Gr 2  
 Gr 3a  
 Gr 3b  
 Gr 4  
 Gr 5  
 Gr M





PCR ELISA reactions of the DIG-labeled amplified DNA of the ITS1 regions of three species of *Pythium* (*P. aphanidermatum*, *P. arrhenomanes* and *P. myriotylum*) and three species of *Phytophthora* (*P. palmivora*, *P. capsici* and *P. infestans*) and mixtures of the DNAs of two *Pythium* species [*P. aphanidermatum* and *P. myriotylum*] and two *Phytophthora* species [*P. palmivora* and *P. infestans*] with their homologous and heterologous capture probes. The DNA samples were placed horizontally in rows A through H. The capture probe numbers are shown above the plate. They were placed vertically in columns 2 through 7. Column N received neither capture probe nor DIG-labeled DNA. Column 0 received the DIG-labeled DNAs, but no capture probes.

With the contribution of:

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