Quantitative multiplex detection of (plant) pathogens, including *Phytophthora* species, based on PRI-lock probe technology and the OpenArray platform

Phytophthora, Pythium workshop ICPP, August 2008

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Introduction

- Detection and identification in disease management strategies
  - Fast
  - Accurate
  - Sensitive
  - Multiplex
- Current technologies
  - Low level of multiplexing
  - Low throughput
  - Laborious
  - Not always quantitative

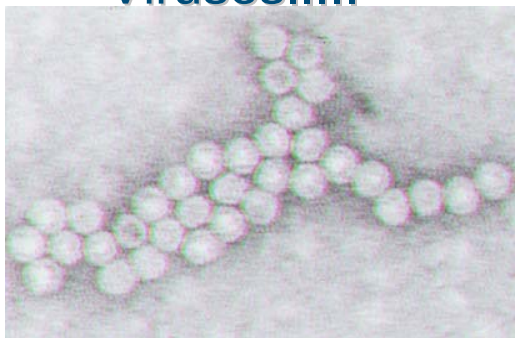
Introduction

- Many targets to be detected simultaneously

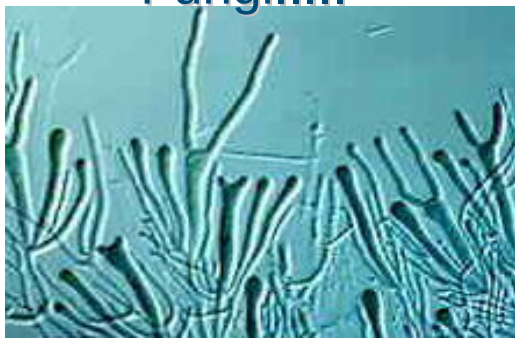
  - Pathogens
    - Fungi
    - Oomycetes
    - Bacteria
    - Nematodes
    - “Viruses”

  - Beneficial microorganisms

Viruses.....



Fungi.....



Nematodes…

Bacteria...

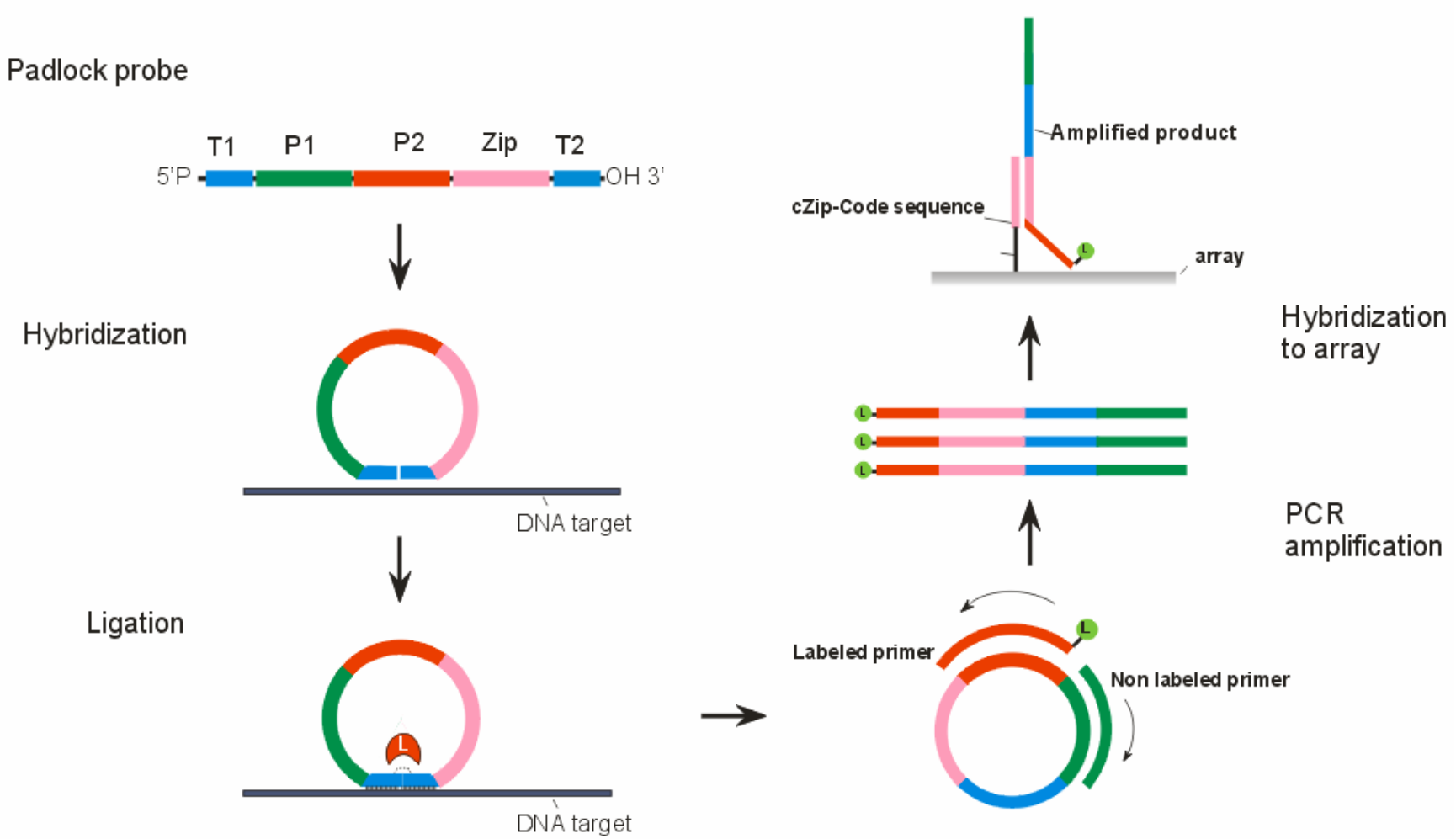
Introduction

- For multiplex detection there is an increasing interest in quantification of the different organisms

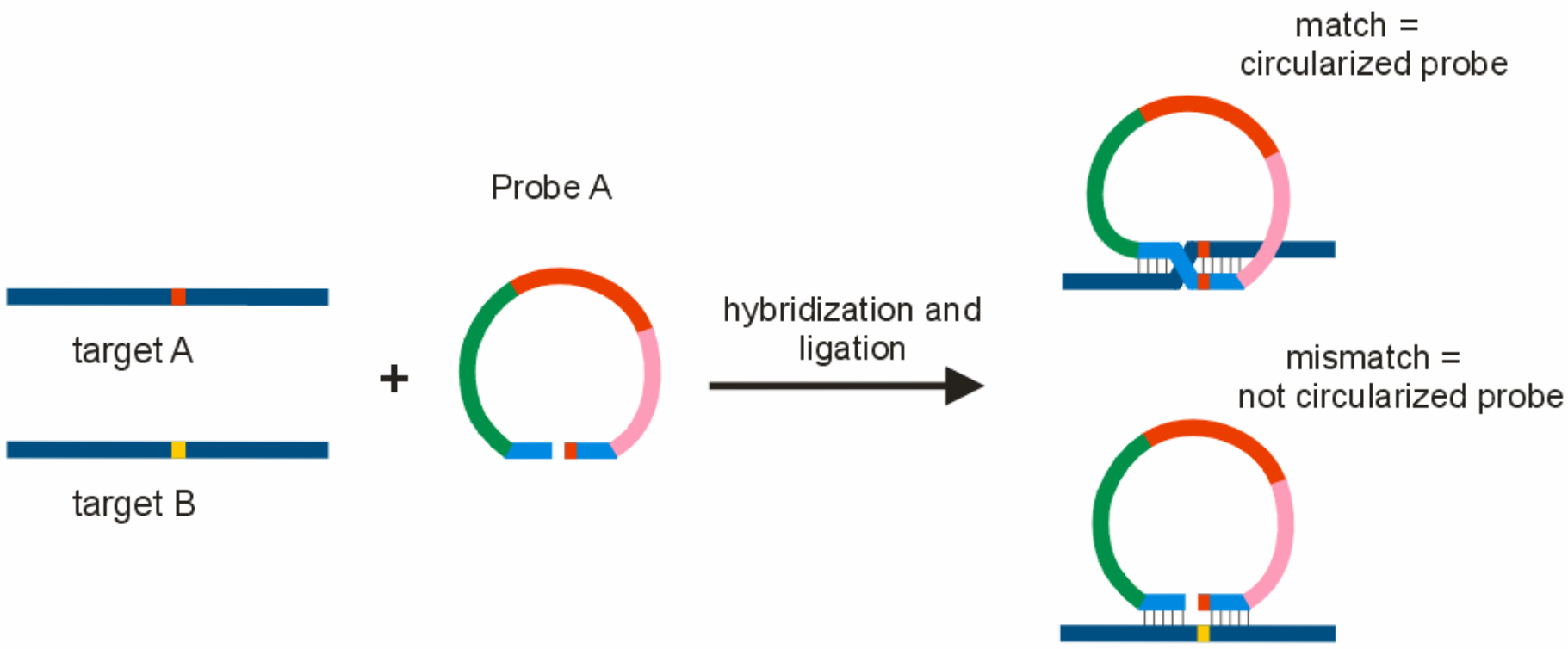
- What are the possibilities for quantitative multiplex detection?
  - Sensitive DNA quantification only with qPCR

- Quantification in PCR for **multiple targets** is theoretically problematic

Padlock Probe Principle

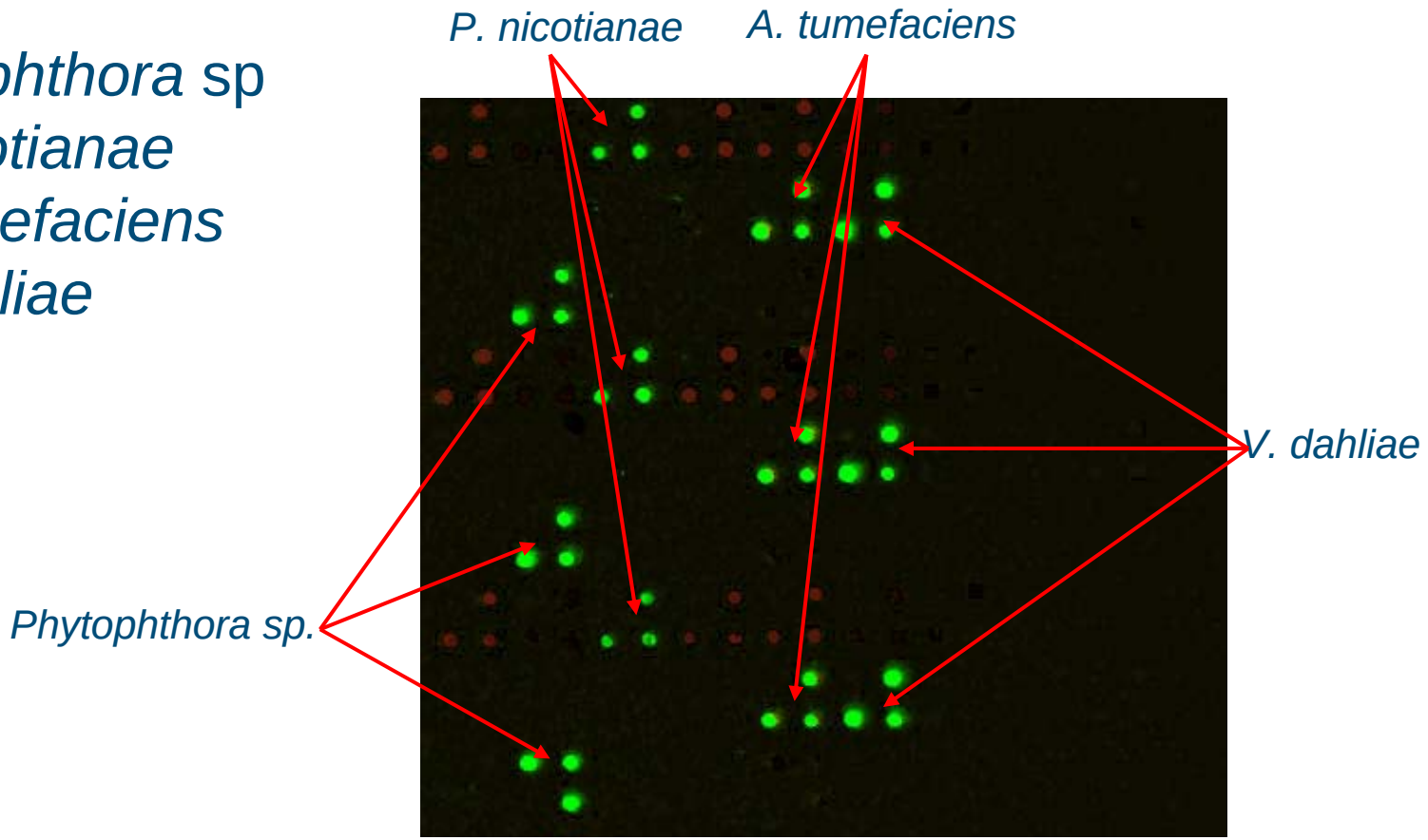


Padlock Probe Principle



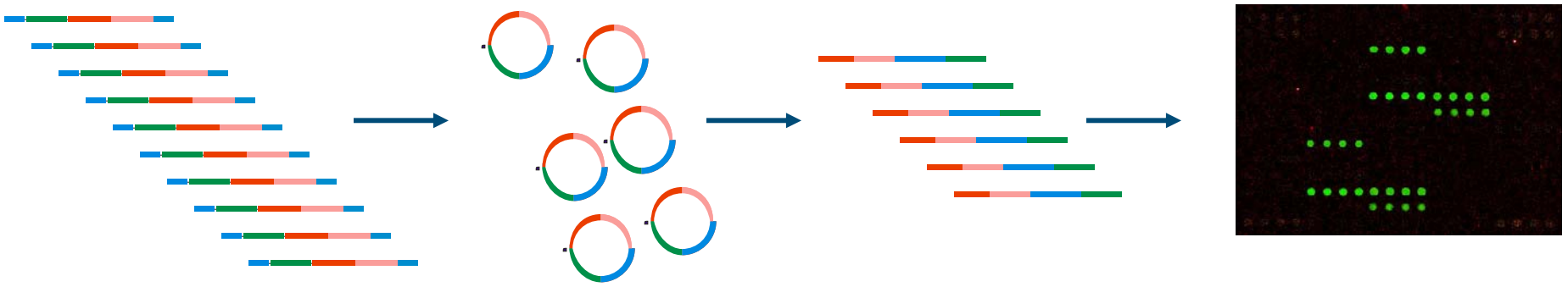
Multiplex detection in recirculation water

- *Phytophthora* sp
- *P. nicotianae*
- *A. tumefaciens*
- *V. dahliae*

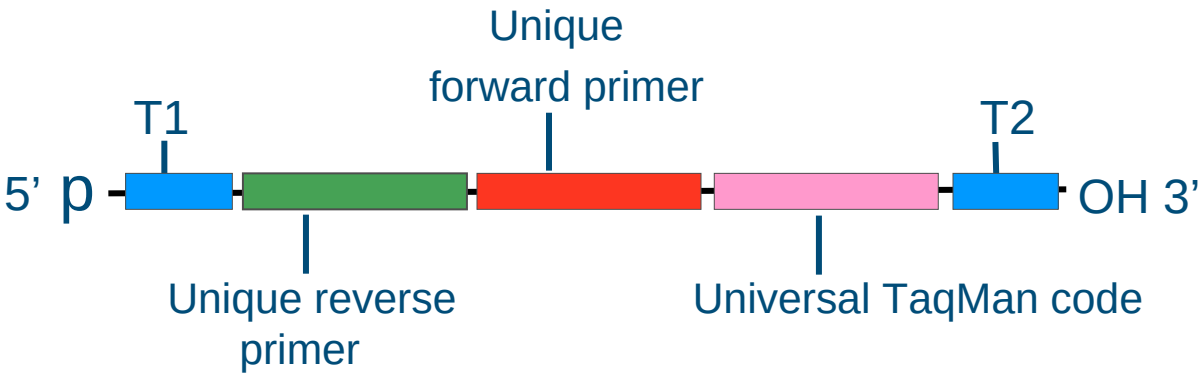


Phytophthora micro-array

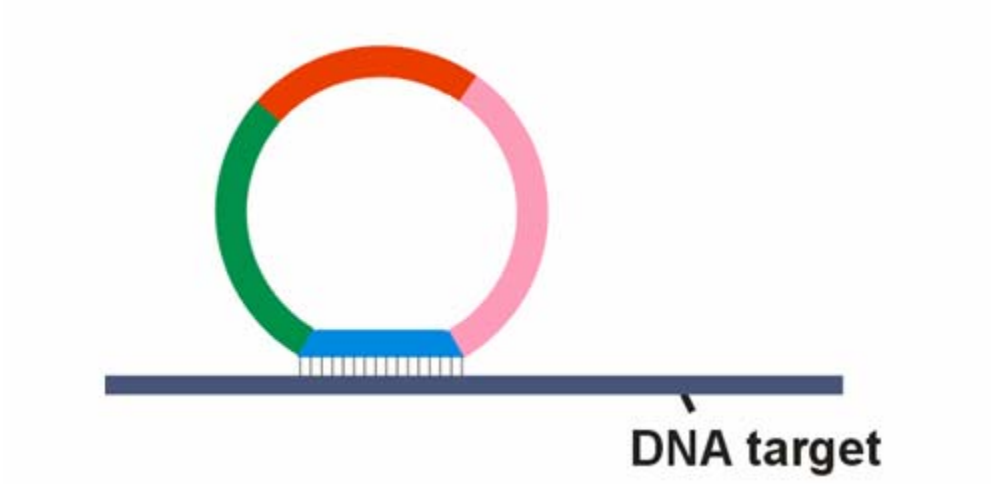
- PLP for *Phytophthora* sp
- PLPs for different *Phytophthora* species
- ligation with mixture of PLPs
- amplification with generic primers
- detection on *Phytophthora* micro-array



PRI-lock Probe Principle



PRI-lock



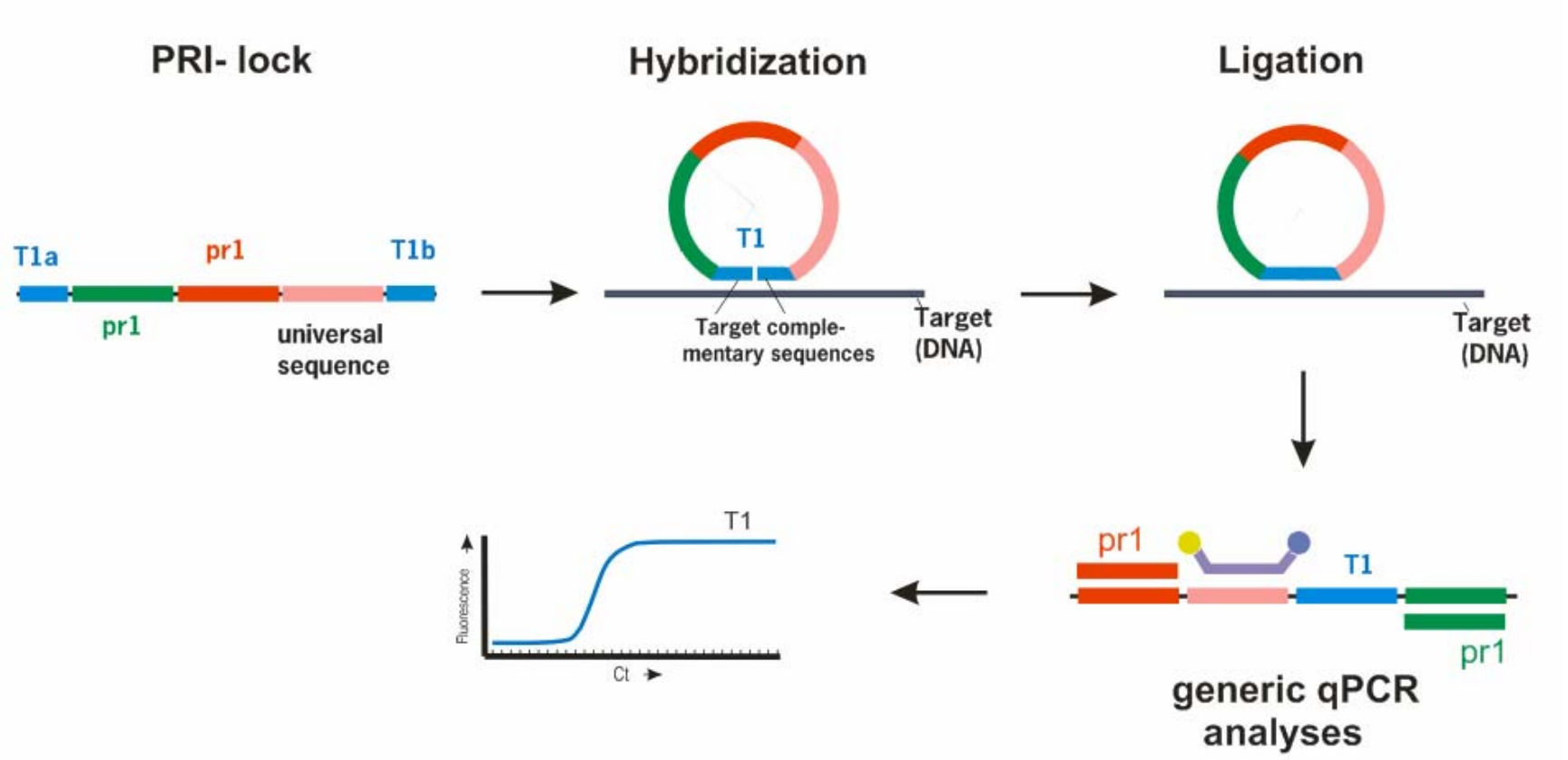
Hybridization

Ligation

Ligation Dependent

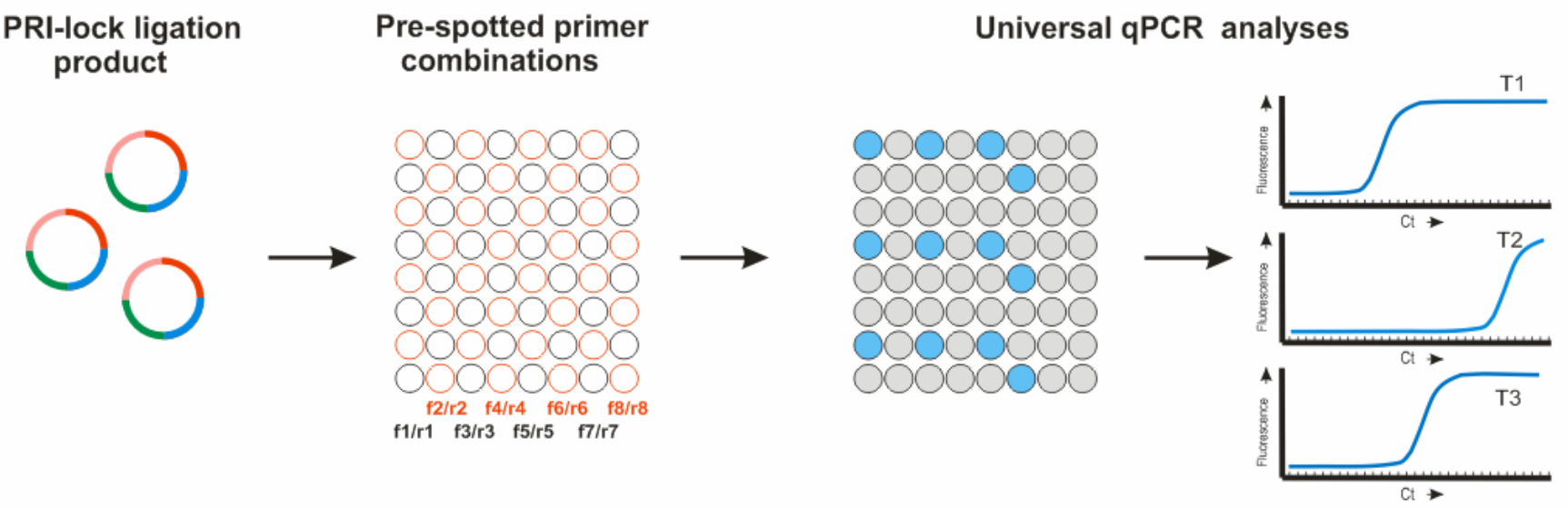
Quantitative Multiplex Target Detection

PRI-lock ligation followed by singleplex amplification



Quantitative Multiplex Target Detection

Multiplex PRI-lock ligation followed by singleplex amplification



Quantitative Multiplex Target Detection

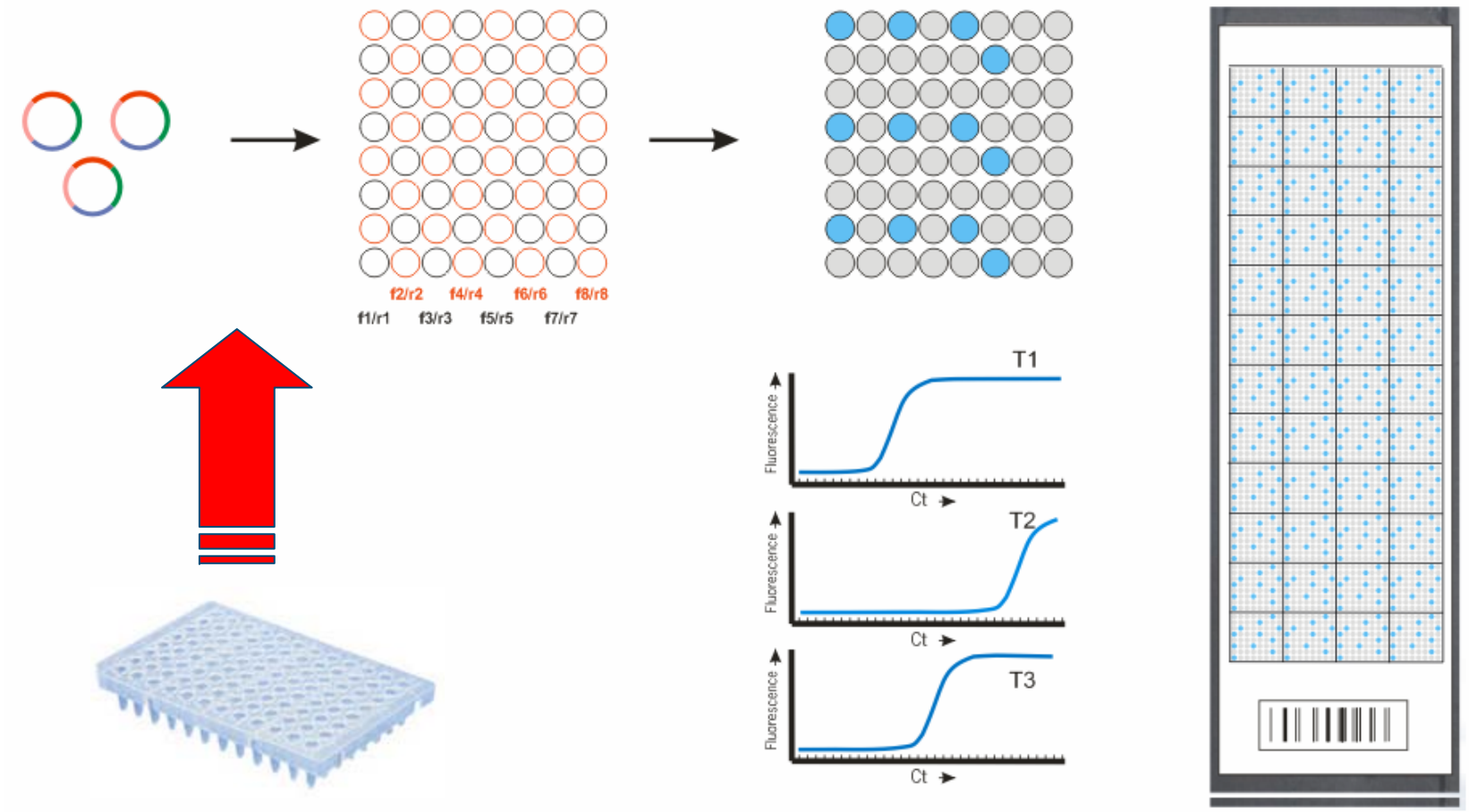
- Testing PRI-lock/primer specificity in a multiplex (96 well) system.

Target template	**PRI-lock probe**	***Myr. ror.* primers**	**All *Phyt*. primers**	***Phyt. inf.* primers**
Myrothecium roridum	*M. roridum*	**21**	40	40
Phytophthora infestans	All *Phytophthora*	40	**21**	40
P. Infestans	*P. infestans*	40	40	**22**
M. roridum	PRI -lock mixture	**21**	40	40
P. infestans	PRI -lock mixture	40	**21**	**22**
M. ror. + P. inf.	PRI -lock mixture	**21**	**21**	**22**
No target	PRI -lock mixture	40	40	40

  - PRI-lock/primer combinations are specific
  - Ct values of the PRI-locks are not influenced by the presence of other templates and PRI-locks in the mixture

Quantitative Multiplex Target Detection

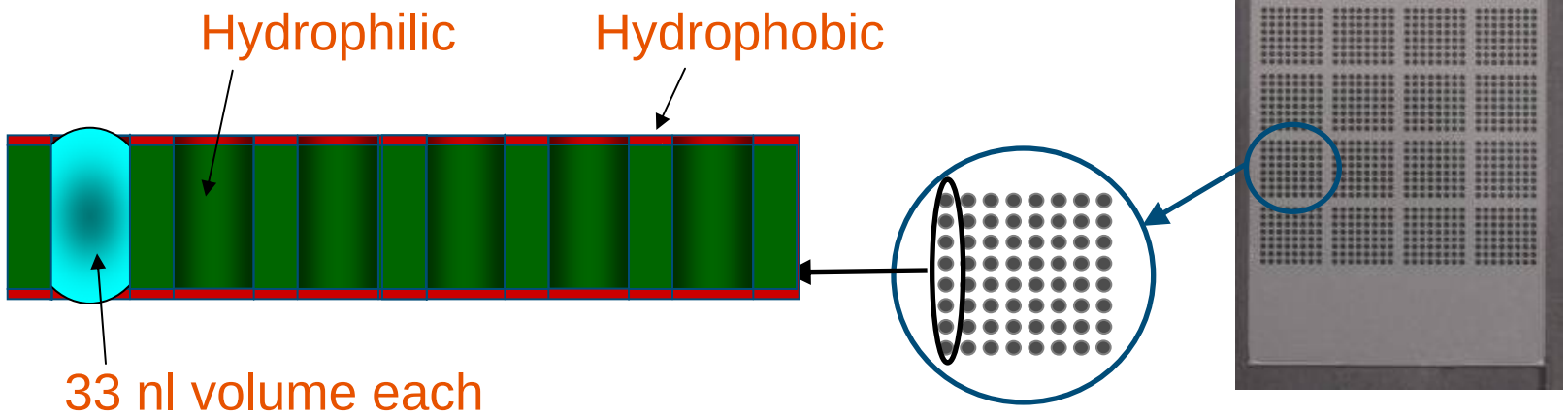
Real-time quantification in a Biotrove ‘PCR array’



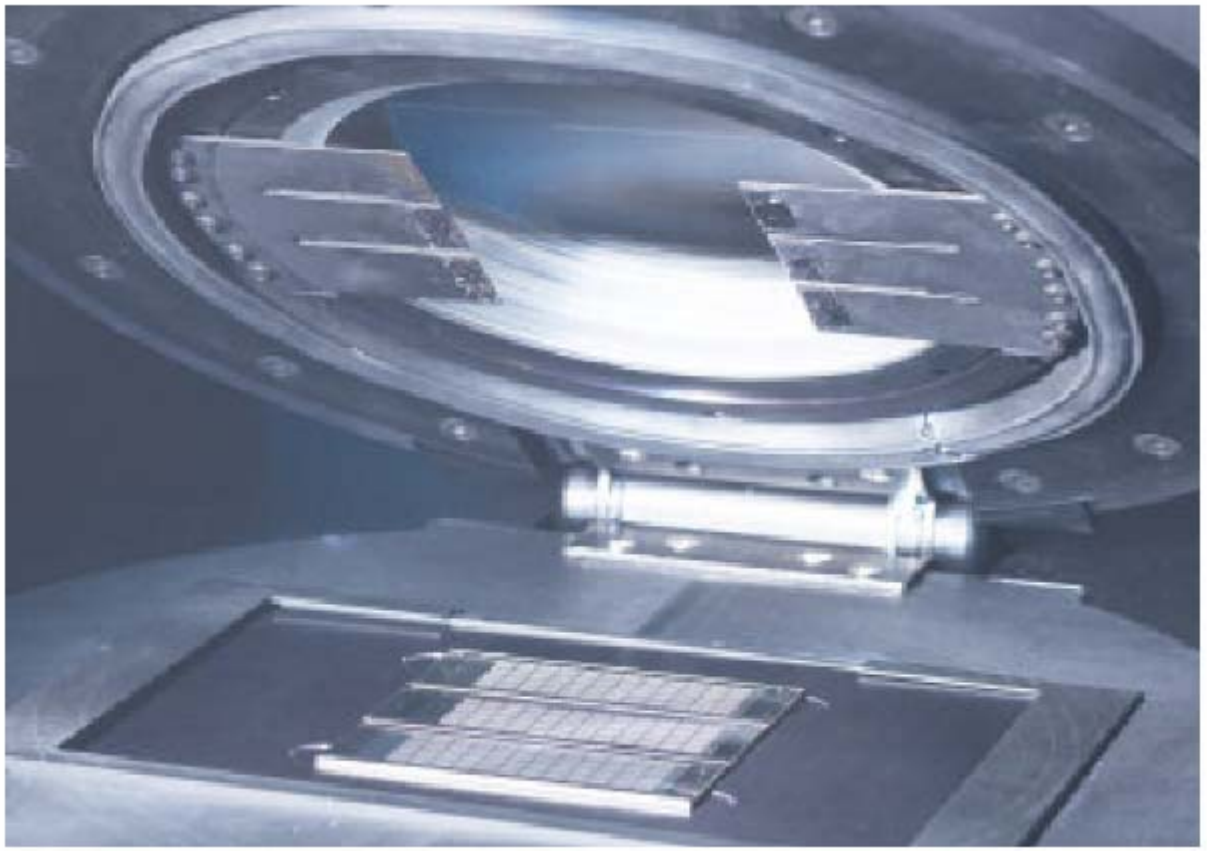
Quantitative Multiplex Target Detection

- 48 subarrays on each plate
- Each subarray consists of 64, 33 nL through-holes in an 8x8 pattern
- Primer pairs spotted in the through-holes
- 3072 reactions in each array
- Loading by capillary action

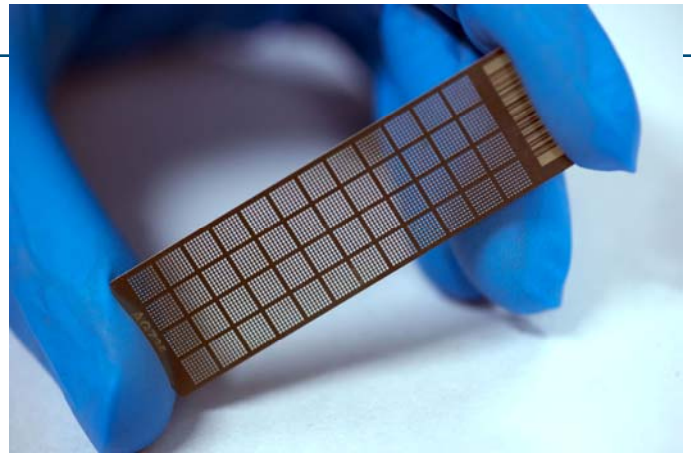
Through-hole cross-section

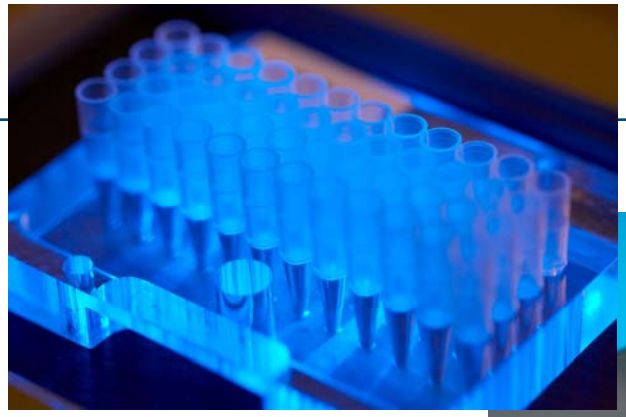


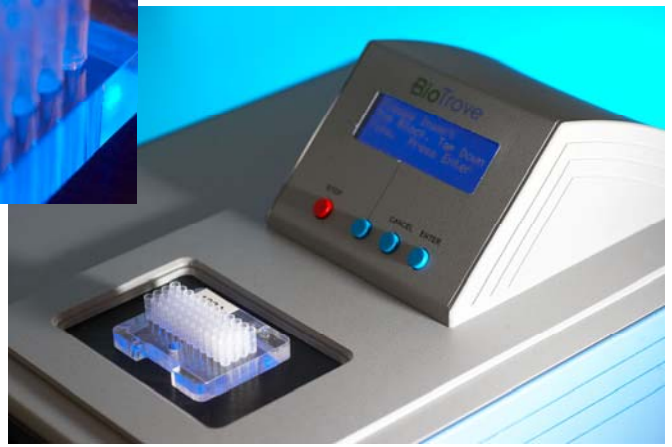
Quantitative Multiplex Target Detection

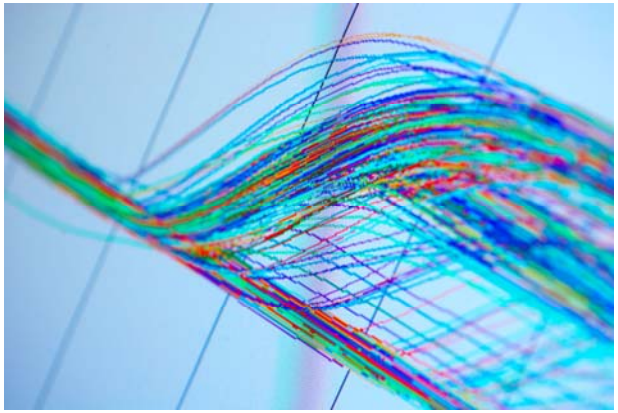


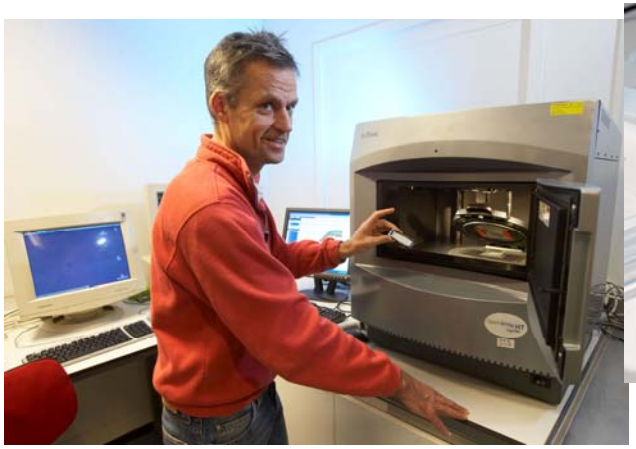
Loading three OpenArray plates to an OpenArray Cycler





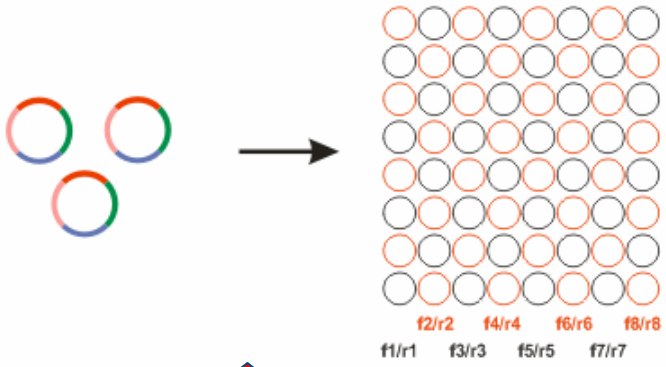

BioTrove

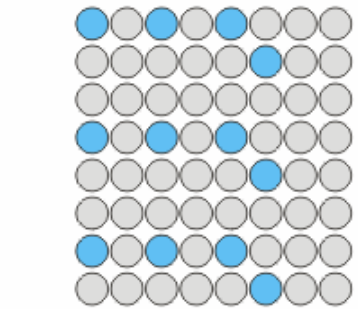


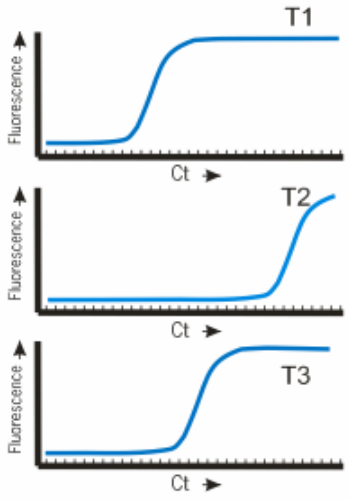


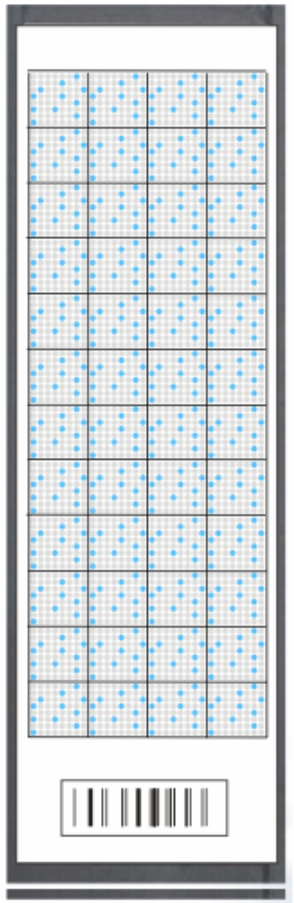
Quantitative Multiplex Target Detection

Real-time quantification in a Biotrove ‘PCR array’









Primer pair	All Phyt	P. inf	M. ror	M. hapla	C. spat	E. car	R. sol 2.2	V. albo/ tri	V. dahl	R. sol 4.1	G. Proteo
Targets											
P. inf/ M. ror	17.2	17.5	18.1	ND	ND	ND	ND	ND	ND	ND	ND
M. hapla/ C. spa/ E. car	ND	ND	ND	19.2	19.4	17.4	ND	ND	ND	ND	19.7
V. dahl/ Rhiz 4-1/ Rhiz 2-2/ V.tri	24.6	ND	ND	ND	ND	ND	17.3	19.4	17.6	19.3	ND
P.inf/ M. hapla/ M. ror/ V. dahl/ V. tri/ E. car	17.0	17.3	18.5	19.3	ND	17.6	ND	19.2	18.1	ND	20.4
MQ	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Quantitative Multiplex Target Detection

- Testing PRI-lock/primer specificity in the Biotrove OpenArray platform

Primer pair	*Phyt spp.*	*P. inf*	*M. ror.*	*M. hap.*	*A.tum.*	*E. car.*	*F. oxy.*	*V. alb./ tri.*	*V. dah.*	*R. sol. 4-1*	*R. sol. 4-2*
Targets											
P. infestans	**16.4**	**15.4**	--	--	--	--	--	--	--	--	--
A.tumefaciens	--	--	--	--	**16.2**	--	--	--	--	--	--
A. tum. / F. oxy. / V. dah.	--	--	--	--	**16.2**	--	**17.7**	--	**15.9**	--	--
P. inf. / M. ror. / M. hap. / A. tum. / E. car. / F. oxy. / V. alb. / V. dah. / R. sol. 4-1 / R. sol. 4-2	**15.9**	**15.0**	**17.0**	**16.9**	**15.9**	**15.4**	**17.3**	**18.0**	**15.8**	**14.4**	**18.8**
Milli Q water	--	--	--	--	--	--	--	--	--	--	--

- PRI-lock/primer combinations are specific
- Ct values of the PRI-locks are not influenced by the presence of other templates in the mixture

Quantitative Multiplex Target Detection

- Advantages:
  - Quantitative
  - High specificity
  - Single and multiplex target detection independent
  - Target recognition and amplification independent
  - Universal TaqMan / SYBR Green PCR conditions
  - High-throughput
  - Low background

- Disadvantages:
  - Low copy numbers of ligated PRI-locks in nanoliter wells

Conclusions

- Working PRI-lock probe multiplex detection system:
  - Currently 30 PRI-lock probes
- Fields of application:
  - Multiplex quantitative target (pathogen) detection
  - Microbial community analysis
  - Multiplex quantitative gene expression analysis
- Instrumentation:
  - Standard real-time PCR machines
  - Nanoliter ‘PCR arrays’ – OpenArray platform (BioTrove)

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  - Jonathan Schimmel
  - Elen Ortenberg

References

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- Van Doorn et al. BMC Genomics, 2007, 8:276

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