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# **Detection of pathogens in plants: New technologies for old challenges**

Petr Karlovsky

# Diagnosis of plant disease

<b>Host plant</b>	Pathogen			
<b>Symptoms</b>	Phenotype			Geno- type
	Morpho- logy	Physio- logy	Protein	Nucleic acid

# Detection of pathogens

<b>Host plant</b>	<b>Pathogen</b>			
<b>Symptoms</b>	<b>Phenotype</b>			<b>Geno- type</b>
	<b>Morpho- logy</b>	<b>Physio- logy</b>	<b>Protein</b>	<b>Nucleic acid</b>

# Molecular diagnostics

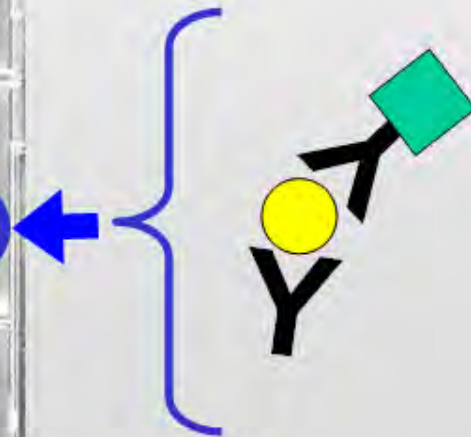
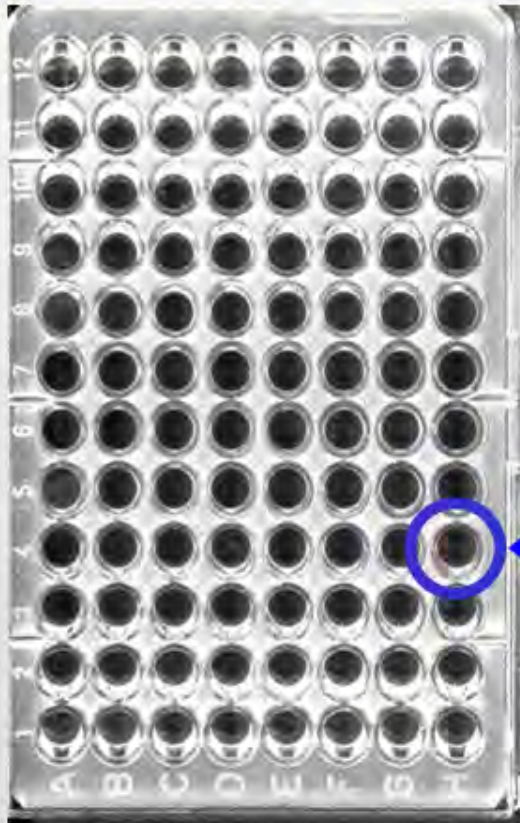
<b>Host plant</b>	<b>Pathogen</b>			
<b>Symptoms</b>	<b>Phenotype</b>			<b>Geno- type</b>
	<b>Morpho- logy</b>	<b>Physio- logy</b>	<b>Protein</b>	<b>Nucleic acid</b>

# Detection of plant pathogens

- 1970th - ELISA
- 1980th - Nucleic acid hybridization
- 1990th - PCR
- 2000th - On-chip laboratory  
Lateral-flow immunoassay
- 20XX - Remote sensing?

# Immunochemical detection

**Microtiter plate**

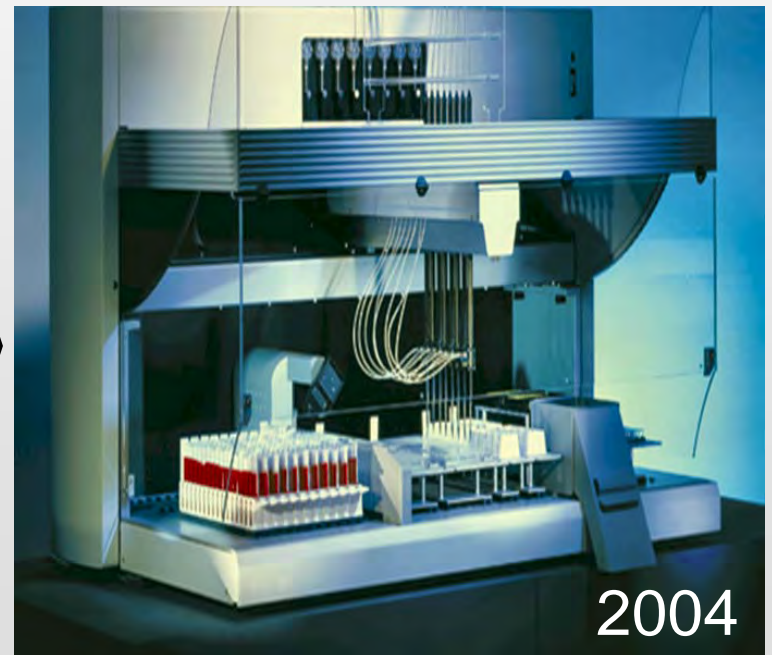
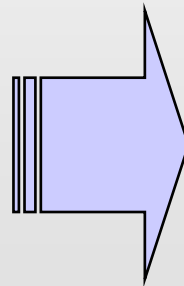




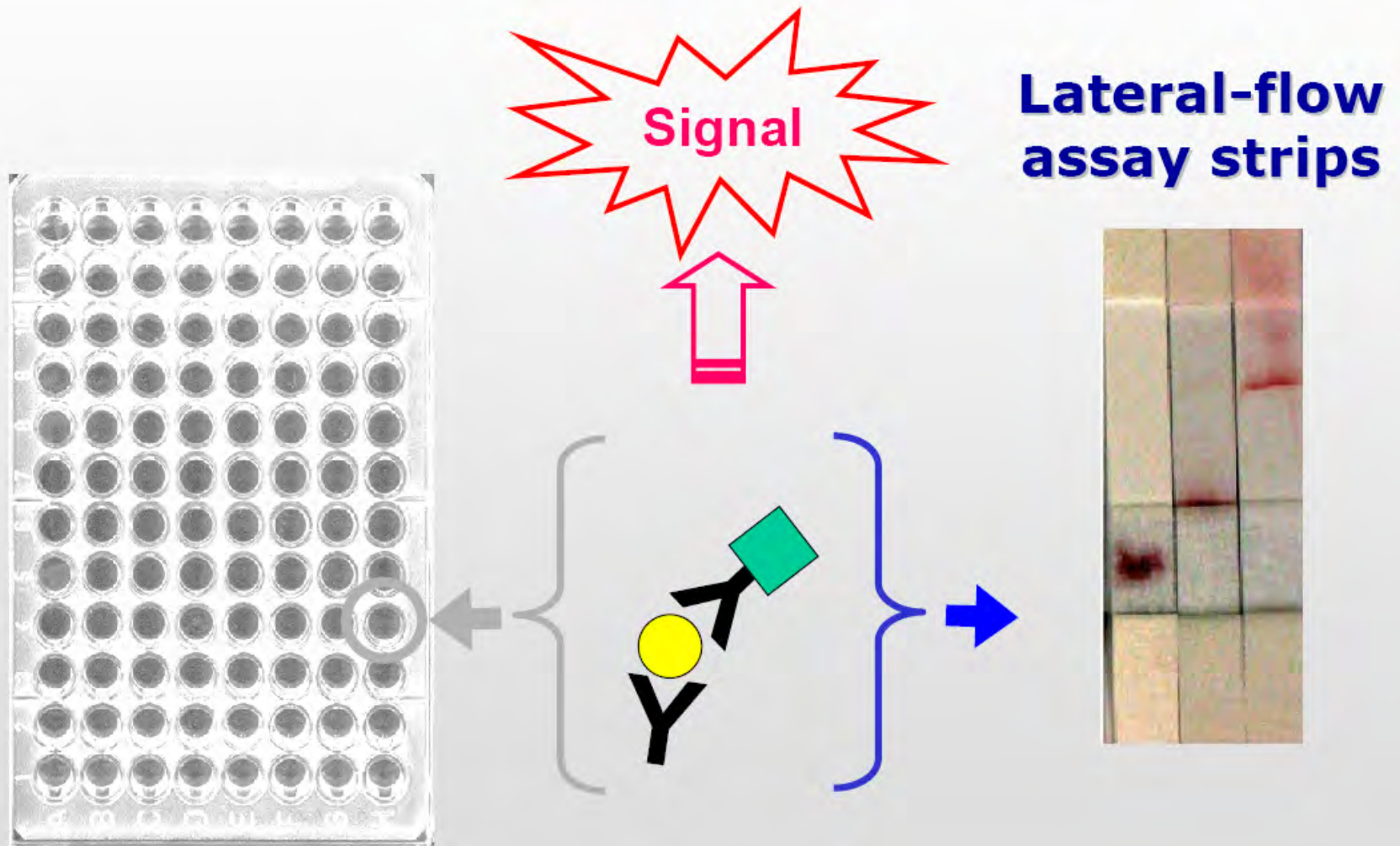
1976:  
Microtiter plate assay for  
plant pathogens by ELISA



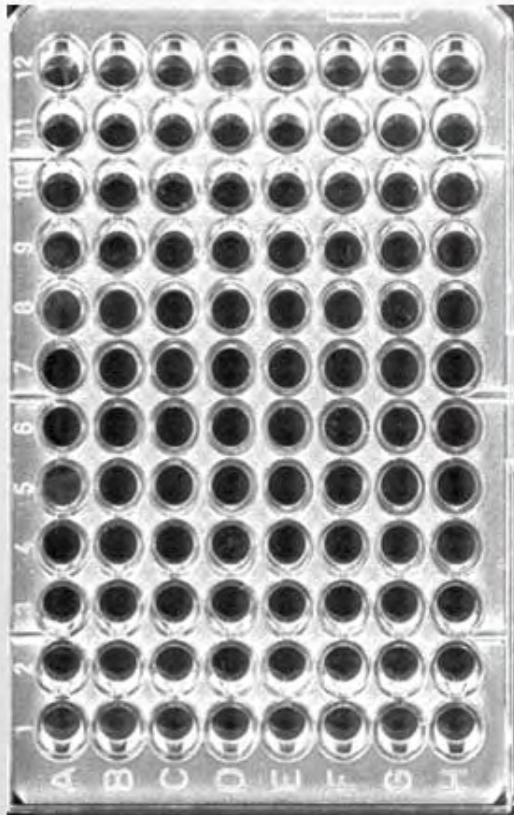
1976:  
Microtiter plate assay for  
plant pathogens by ELISA



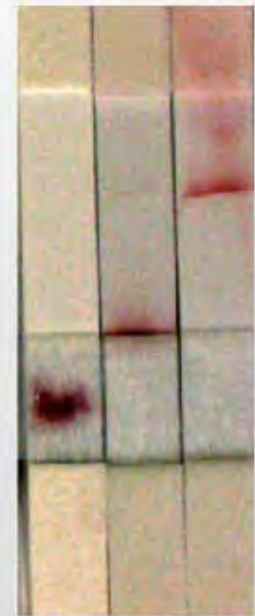
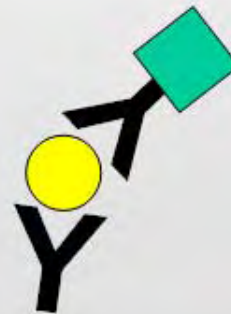
# Immunochemical detection



# Immunochemical detection



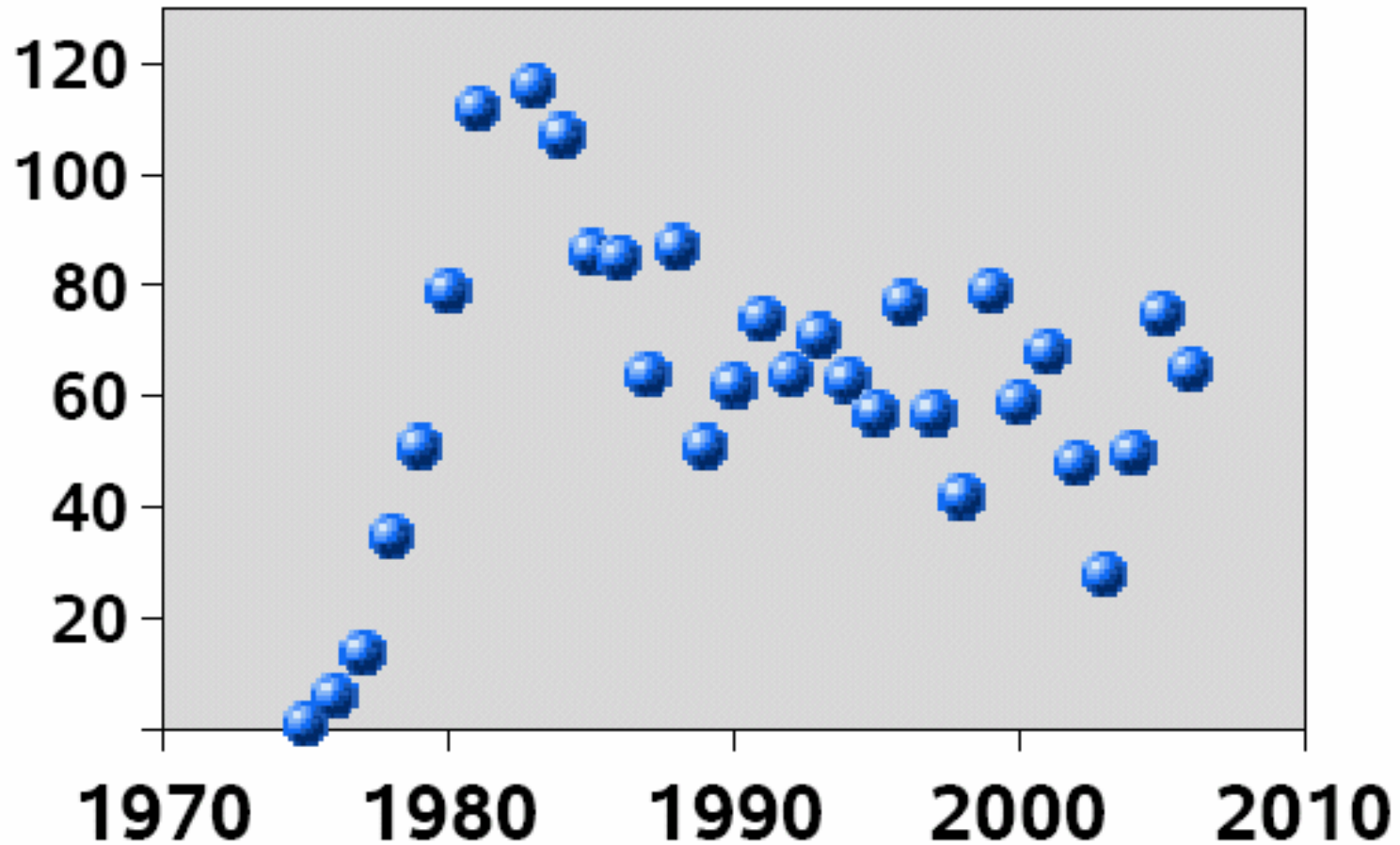
**Screening  
for antibodies**



# Immunochemical detection

- Limited control of specificity
- Limited sensitivity
- Economical
- Easy to use
- Chemically diverse targets
- /■ Relies on phenotypic characters

# ELISA: publication record



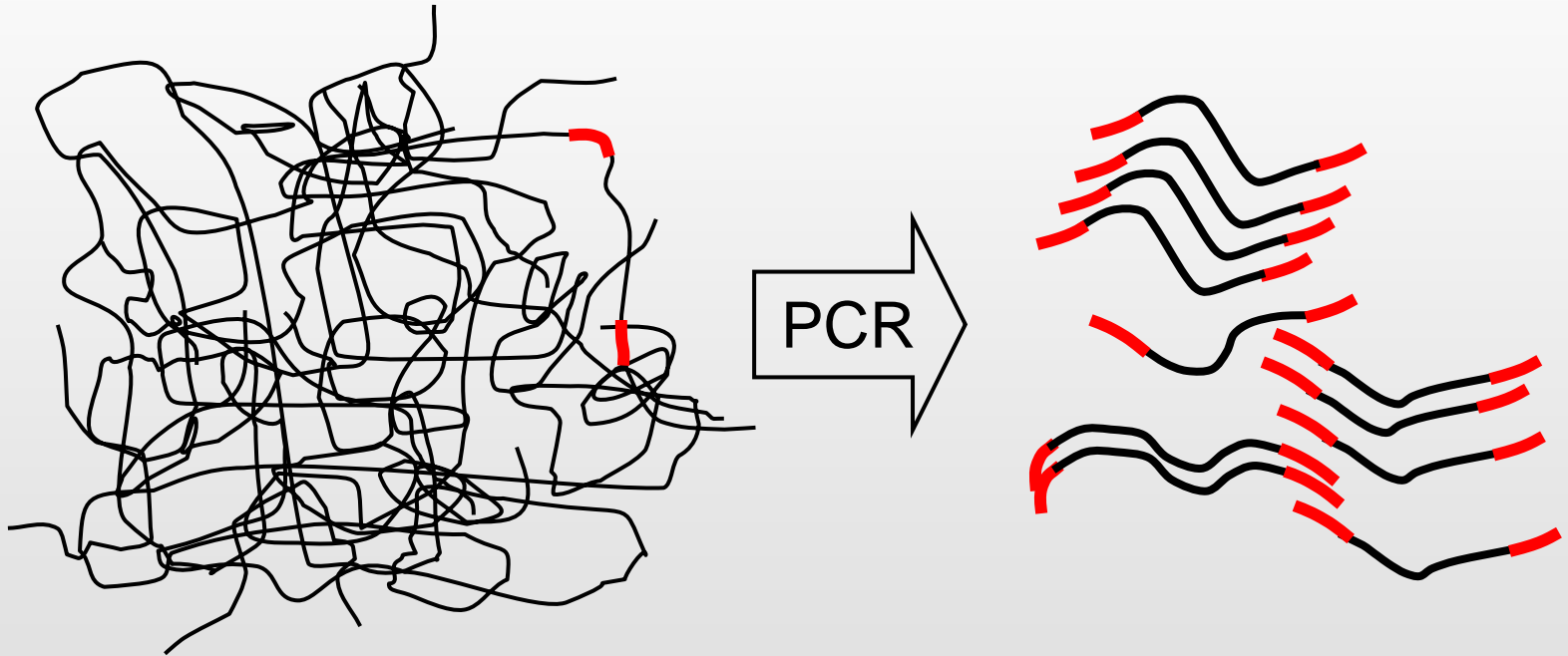
# Nucleic acids in pathogen detection

## ■ Hybridization

- ▶ Dot-hybridization
- ▶ Allele Specific Oligonucleotides (ASO)
- ▶ DNA microarrays

## ■ Polymerase chain reaction

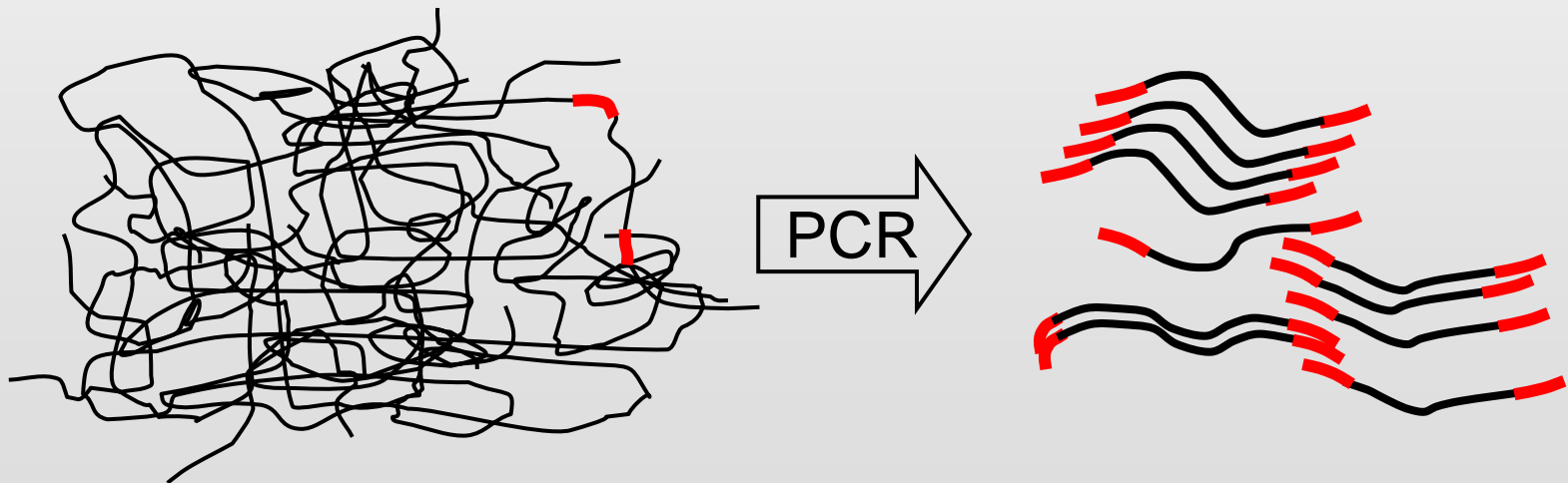
# Polymerase chain reaction



# Polymerase chain reaction

Discrimination of dead vs. living cells:

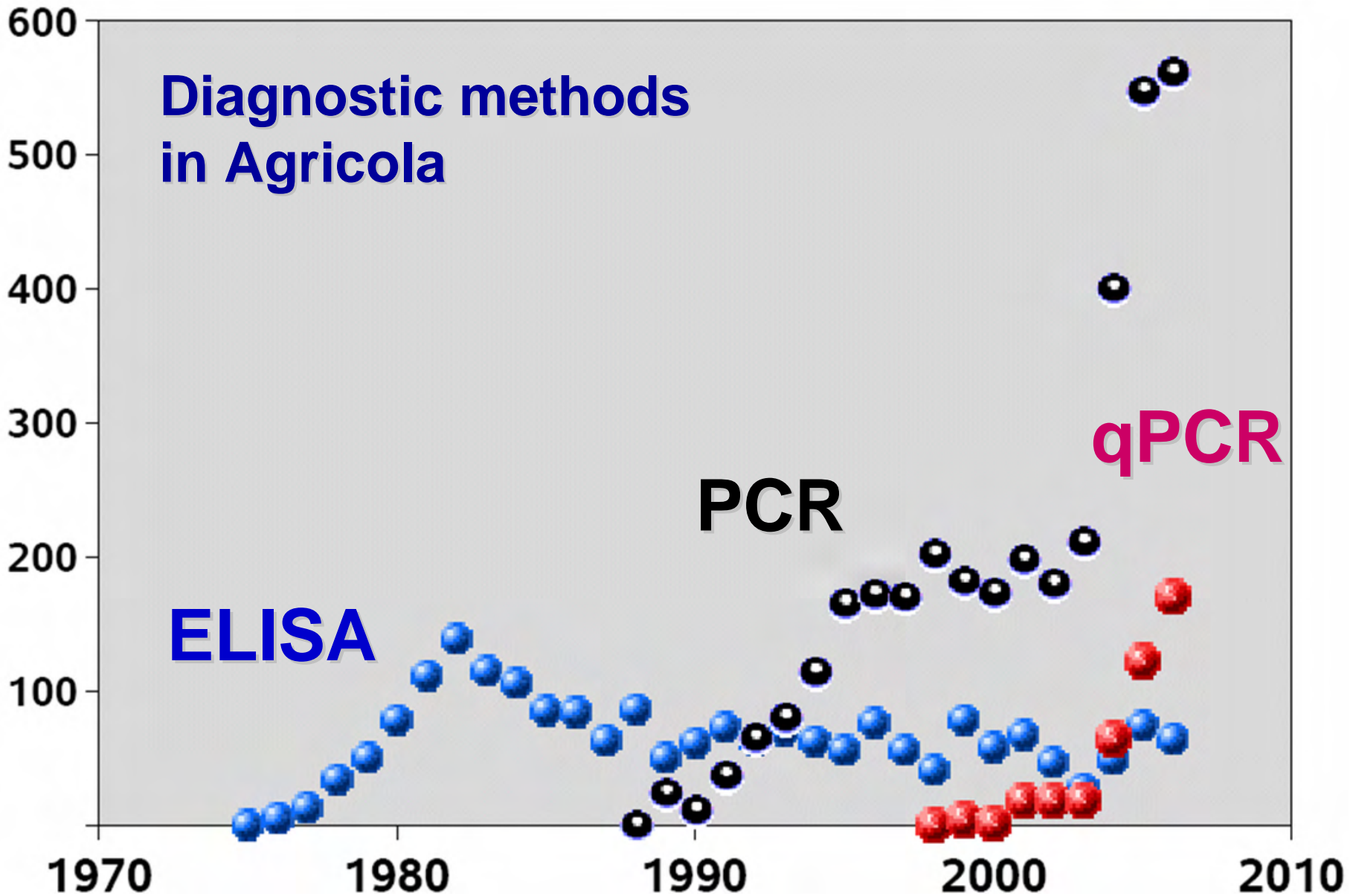
- Bio-PCR
- Ethidium Monoazide



# PCR

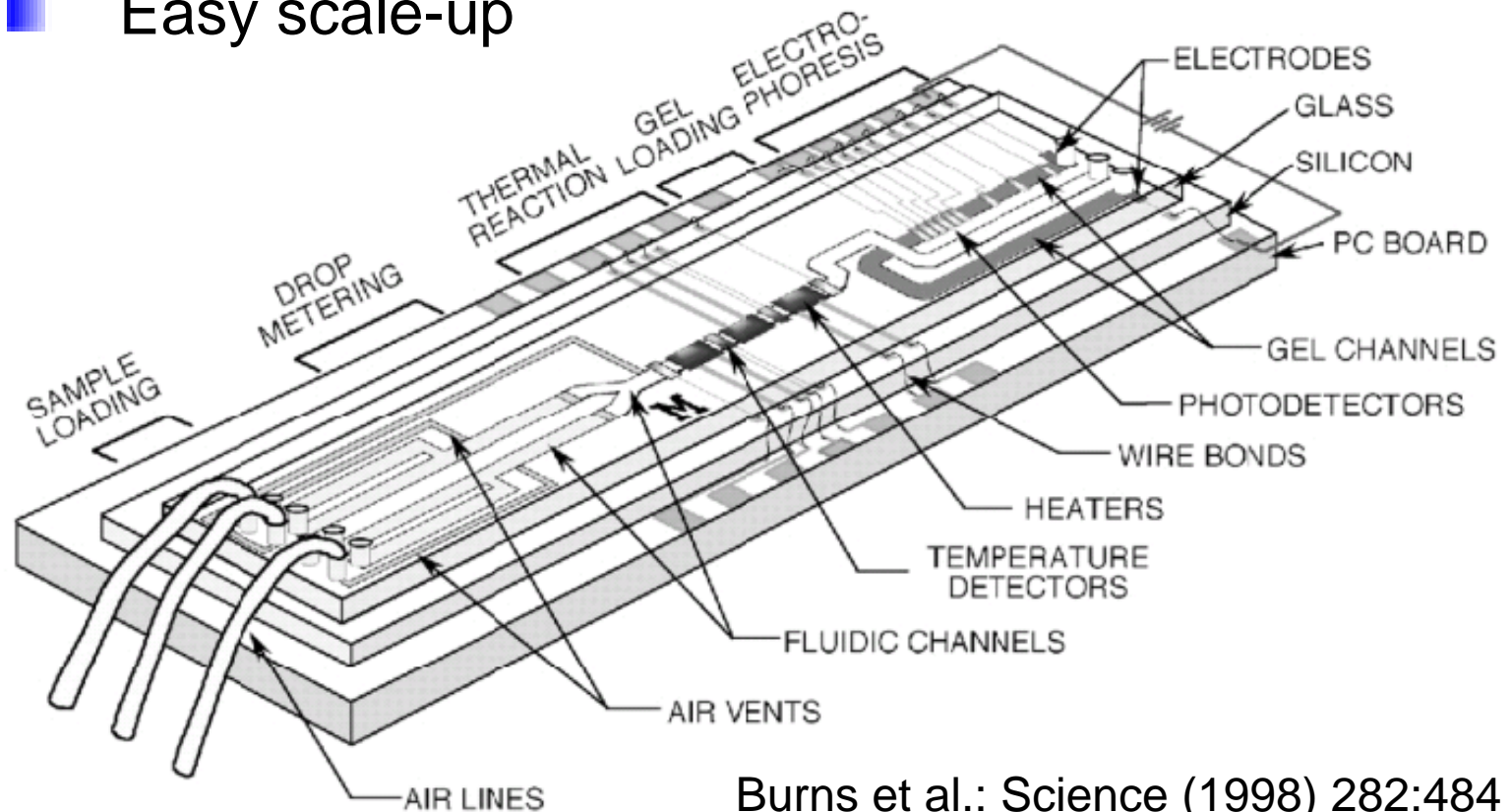
- Full control of specificity
- Ultimate sensitivity
- Qualitative (end-point)  
vs.
- Quantitative (real-time)

# Diagnostic methods in Agricola



# Lab-on-Chip

- Low costs of reagents and labor
- Increased speed
- Easy scale-up



Burns et al.: Science (1998) 282:484

# Lab-on-Chip technologies

- Cycling
  - ▶ Temperature control
  - ▶ Flow-through
- Detection
  - ▶ Fluorescence
  - ▶ Electrochemistry
- Capillary electrophoresis



STMicroelectronic

# Inspiration for plant pathogen detection

- Past: medical diagnostics
  - ▶ ELISA
  - ▶ PCR, DNA-microarrays
  - ▶ Lab-on-Chip, Lateral-Flow Immunoassay

# Inspiration for plant pathogen detection

## ■ Past: medical diagnostics

- ▶ ELISA
- ▶ PCR, DNA-microarrays
- ▶ Lab-on-Chip

## ■ Future: military & anti-terrorism technology

- ▶ On-site detection (PortCheck)
- ▶ Remote sensing

# Remote pathogen detection

- Optical sensors
  - ▶ Fluorescence
  - ▶ Reflectance
  - ▶ (Raman scattering ?)

# Remote pathogen detection

## ■ Optical sensors

- ▶ Fluorescence
- ▶ Reflectance
- ▶ (Raman scattering ?)

## ■ Mass spectrometry

- ▶ VOC collection & enrichment
- ▶ MRM detection



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