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Molecular plant disease diagnostics

- Fungal diseases reduce crop yield (quality and quantity)
- Rapid and precise detection and ID of causal pathogen(s)
- Implement appropriate recommendations for disease management

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Molecular plant disease diagnostics

- DNA-based tools
- High-throughput
- Data (sequences) in fungal repository platforms
 - Share/compare in NCBI

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Molecular plant disease diagnostics

- Koch's postulate - isolation and culturing, reinoculation, microscopy, etc.
 - Coupled with molecular tools can provide more informative results
- (More costly – equipment, reagents, etc.)

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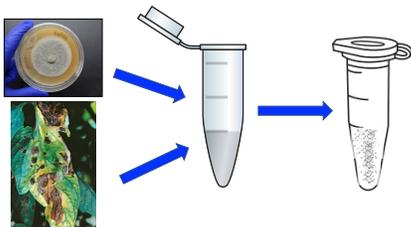
Commonly used tools

- PCR-based assays
- Isothermal amplification based methods
- Post amplification techniques
- Protein-based assays
- Next-Generation sequencing

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DNA extraction

- Fungal **DNA** template -> basic starting material when using any of the molecular tools for disease diagnosis



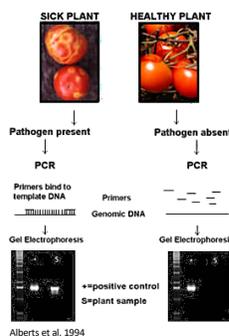
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PCR-based assays

- Polymerase Chain Reaction
- Exponential amplification of fungal gene (marker) through the following repeated steps
 - Denaturation
 - Annealing
 - Elongation
- Primer pair specific for the pathogen

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PCR-based assays



- Presence of band in gel indicate fungal detection
- qPCR variant -> quantify PCR amplicon
- Confirmation
 - Sent off for Sanger sequencing
 - BLAST search in NCBI

Alberts et al. 1994

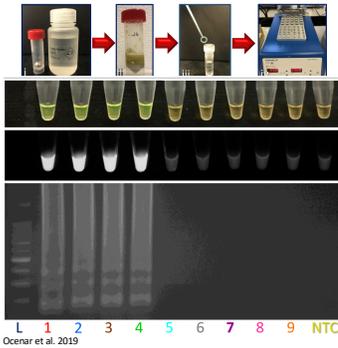
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Isothermal amplification based methods

- Loop-Mediated Amplification (LAMP)
- Employ DNA polymerase, 4 specific primers
 - 6 distinct sequences on DNA template
- Visualize amplification through reaction turbidity, dyes, gel electrophoresis

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Isothermal amplification based methods



Ocenar et al. 2019

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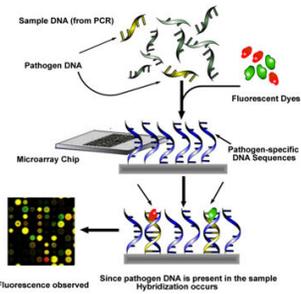
Post amplification techniques

- DNA Microarray
 - Pathogen-specific DNA sequences immobilized onto a solid surface
 - amplified by PCR
 - labeled with fluorescent dyes
 - hybridized to the array

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Post amplification techniques

- DNA Microarray



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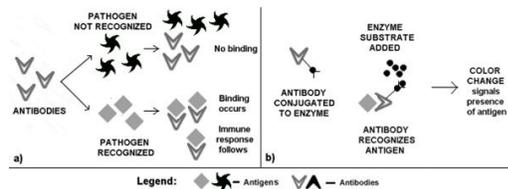
Protein-based assays

- Interaction of antibody and antigen
- Trigger immune response
- Kits contain antibody able to recognize proteins of pathogen or diseased plant

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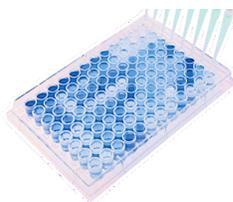
Protein-based assays

- ELISA (enzyme-linked immunosorbent assay)



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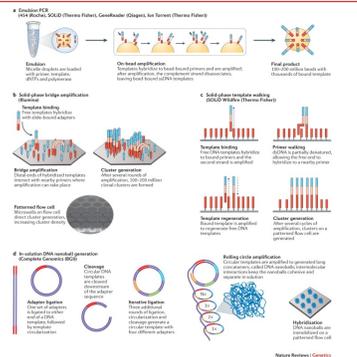
Protein-based assays



- ELISA (enzyme-linked immunosorbent assay)
- Color change in the reaction indicate pathogen detection

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Next-Generation sequencing



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Summary

- Early detection (before symptoms appear)
- Sensitivity and cost effective
- Implement appropriate recommendations for disease management

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