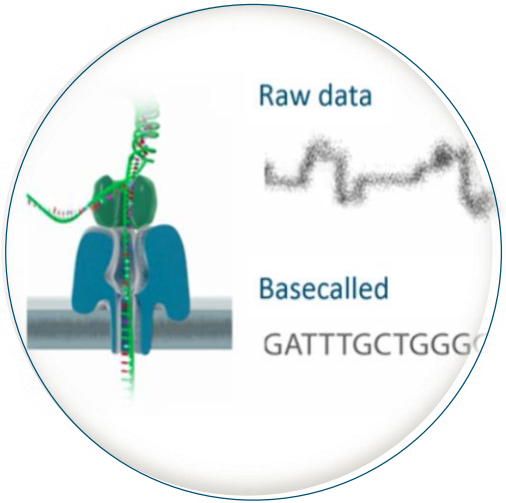
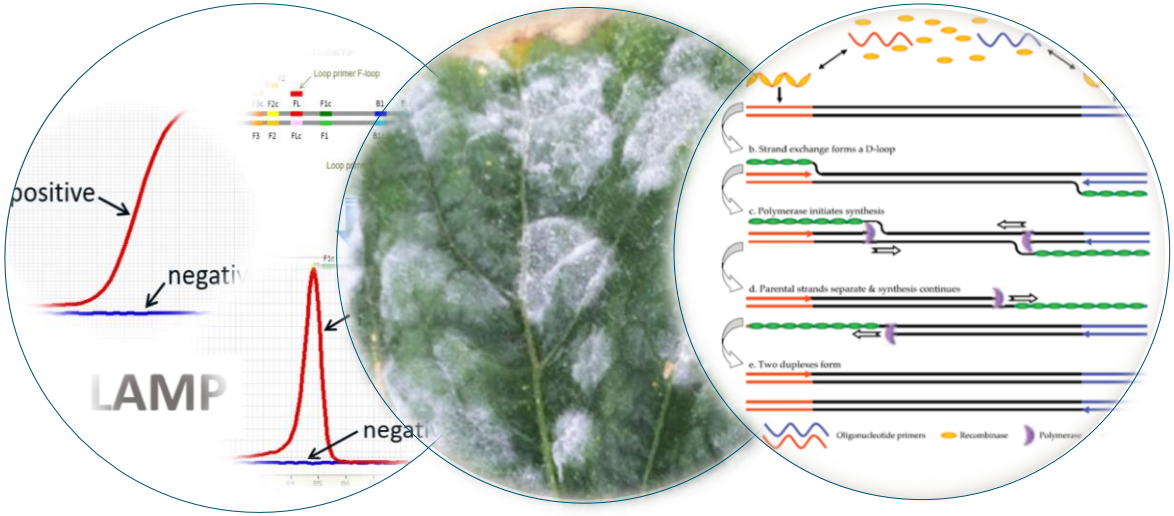
Development of a rapid on-site test for the detection and characterisation of Alternaria spp on potato

Cor Schoen & Bert Evenhuis





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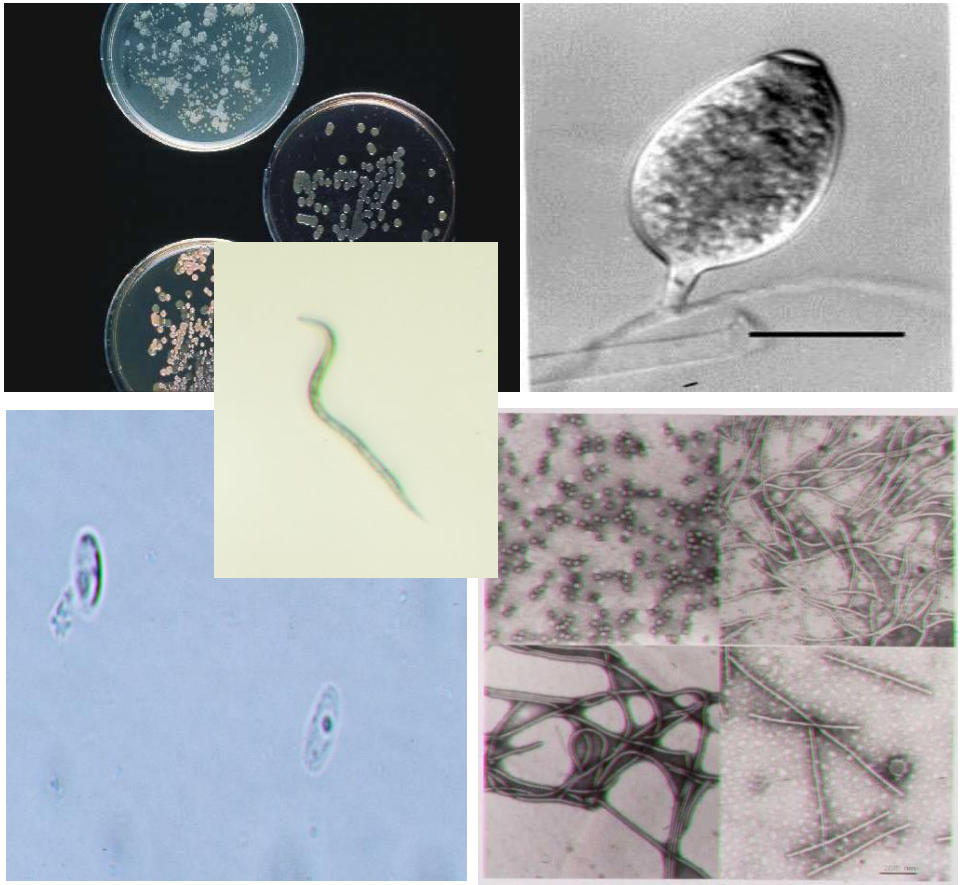
Private Public Partnership

Detection

Plant pathogens

- Bacteria
- Viruses / viroids
- Nematodes
- Fungi (i.e. Alternaria spp)
- Insects
- Phytoplasms
- Oomycetes



On-Site Confirmation and Monitoring

Correct and rapid diagnosis of plant material directly at the production site is required to secure production of healthy plant material

Symptoms: - for instance tiny black spots not clear whether it is Alternaria
- can vary depending on the circumstances and Alternaria spp.

The development of a rapid and sensitive method for identification of pathogens directly is therefore helpfull

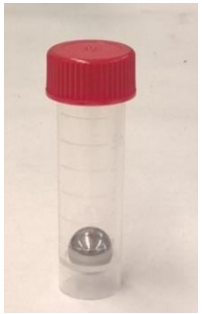
This can lead to more rapid or later interventions - cost savings
- reduction of yield loss

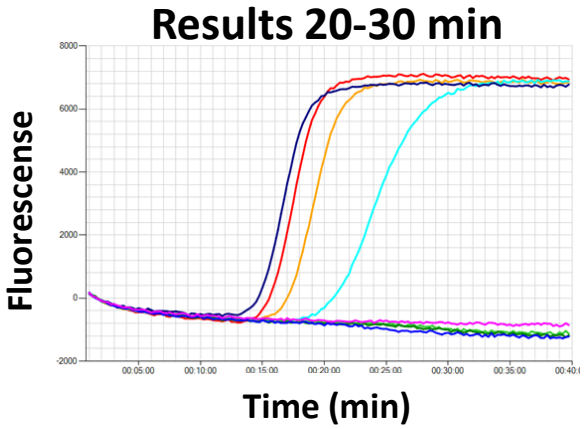
Why LAMP?

- Short reaction time (approximately 30 minutes), making quick decisions possible
- Sensitivity and specificity are similar to lab-based methods (real-time PCR)
- No temperature cycles = simple equipment
- Tolerance for inhibitors = crude extraction methods
- Simple extraction methods reduce the chance of errors
- High amplification efficiency simplifies interpretation of results (clear +/-)
- Most important it can tell *A. solani* or not

How does it work

Plant pathogen amplification in crude plant extracts





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Conclusions, on-site detection

- Different LAMP plant pathogenic kits have been developed
- LAMP - sensitive, specific DNA amplification method
- Genie - easy platform for performing LAMP tests

Bottlenecks on-site LAMP

- Single LAMP assays work well, but what to do if the test result is negative
- Symptoms not always clear, circumstances vary greatly
- Situations where it is not clear which pathogen or characteristic is of importance
- Some targets have very variable genomic sequences
- -> requires multiplex LAMP

  Disadvantage: difficult to produce, not flexible and relative expensive

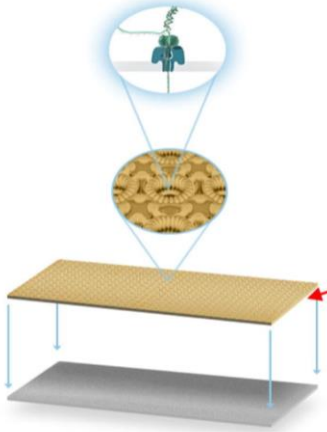
MinION next gen amplicon sequencing

Development of a rapid on-site diagnostic method used away from high-end laboratories, by combining (isothermal target) amplification and next gen amplicon sequencing of different specific plant pathogen DNA areas → MinION

- Widely applicable
- Fast
- Easy to implement 'on-site'
- Minimum / simple equipment
- Little in-depth bioinformatics knowledge is necessary

MinION sequencing

Flowcell

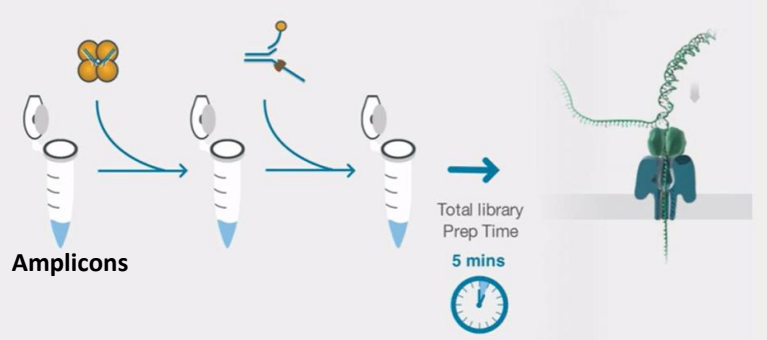


Flowcell

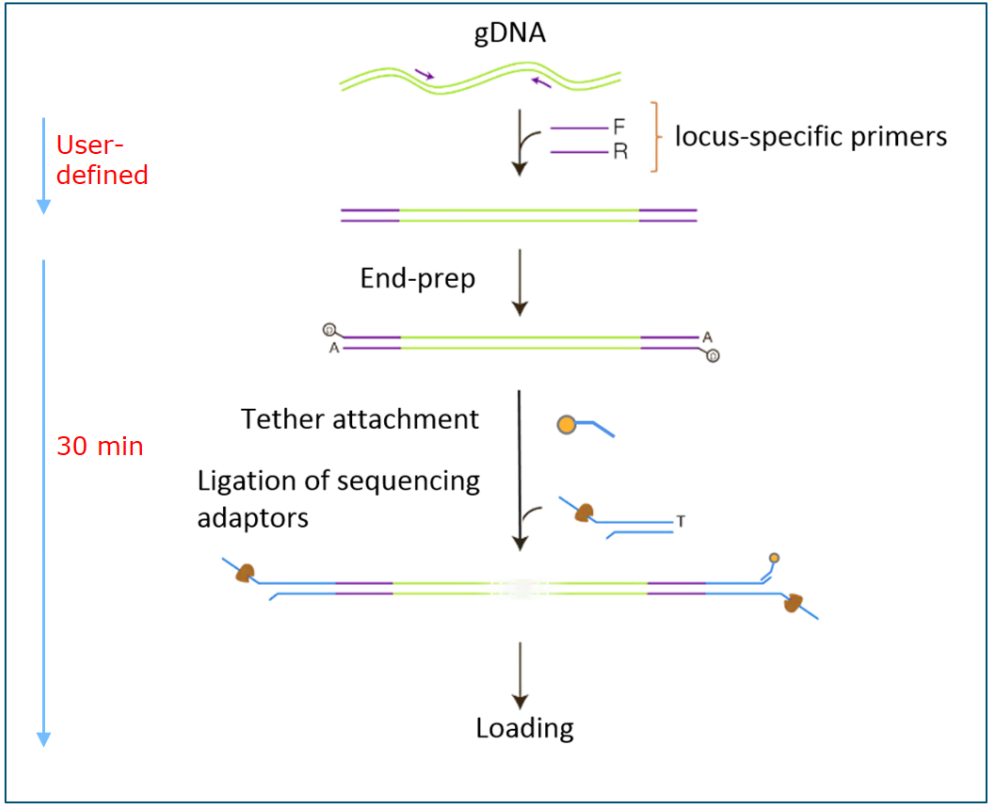
MinION device

USB port

MinION sequencing



1D Amplicon by ligation



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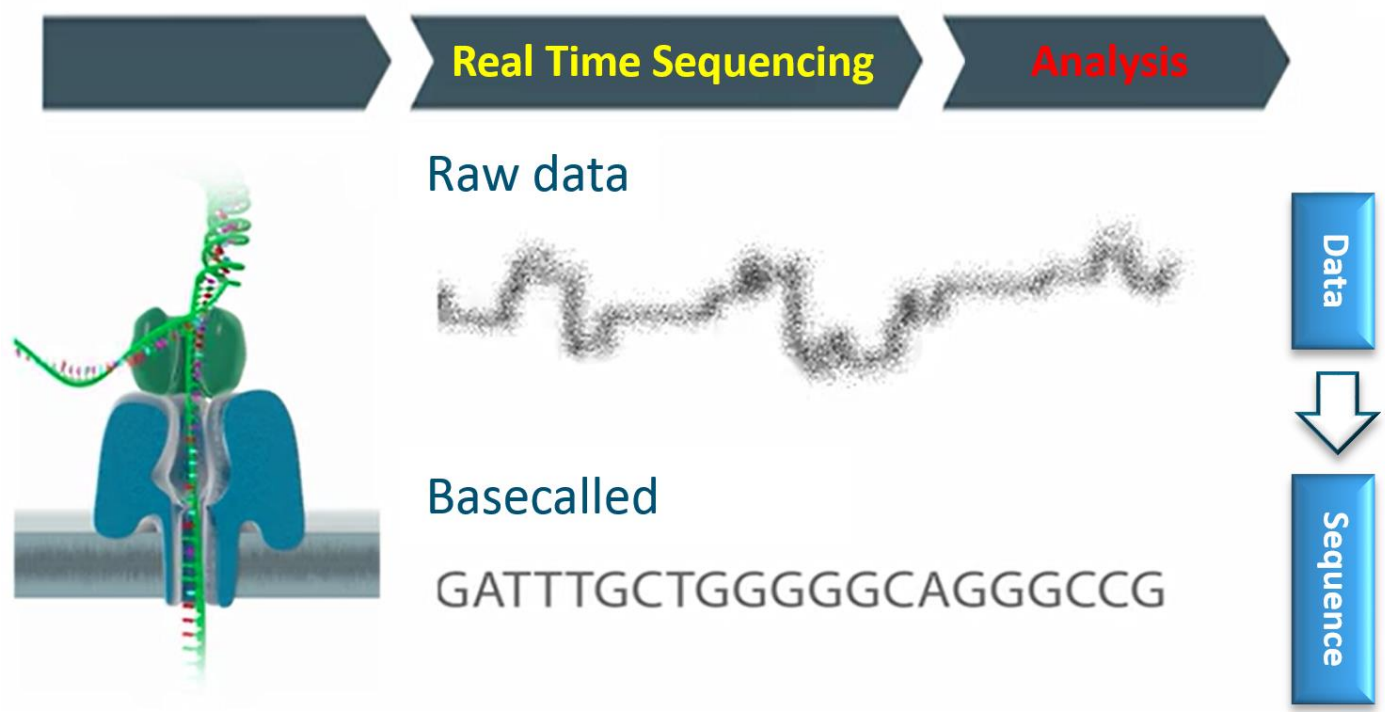
MinION sequencing



Fungal resistance

Sequencing of the SDHI complex (3 genes) is used to demonstrate *Alternaria solani/alternata* fungicide resistance identification in symptomatic potato leaf

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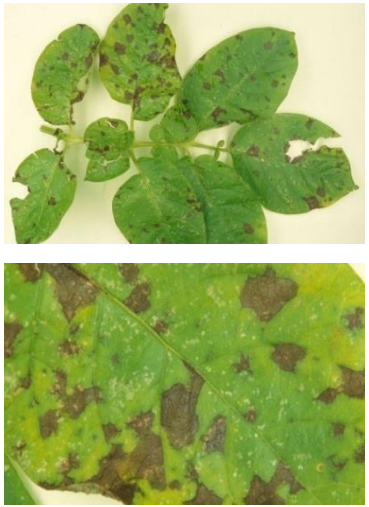

Symptomatic potato leaf

Multilocus detection

AsSdhB	CAC	
	CGC 278 (Arg)	**H278R** solani
	TAC 278 (Tyr)	**H278Y** solani
AsSdhC	CAC	
	CGC 134 (Arg)	**H134R**
AsSdhD	CAC	
	CGC 133 (Arg)	**H133R**
	GAA (GLUTAMIC ACID)	**D123E**

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Plant pathogen amplification in crude plant extracts

Fungal resistance genes

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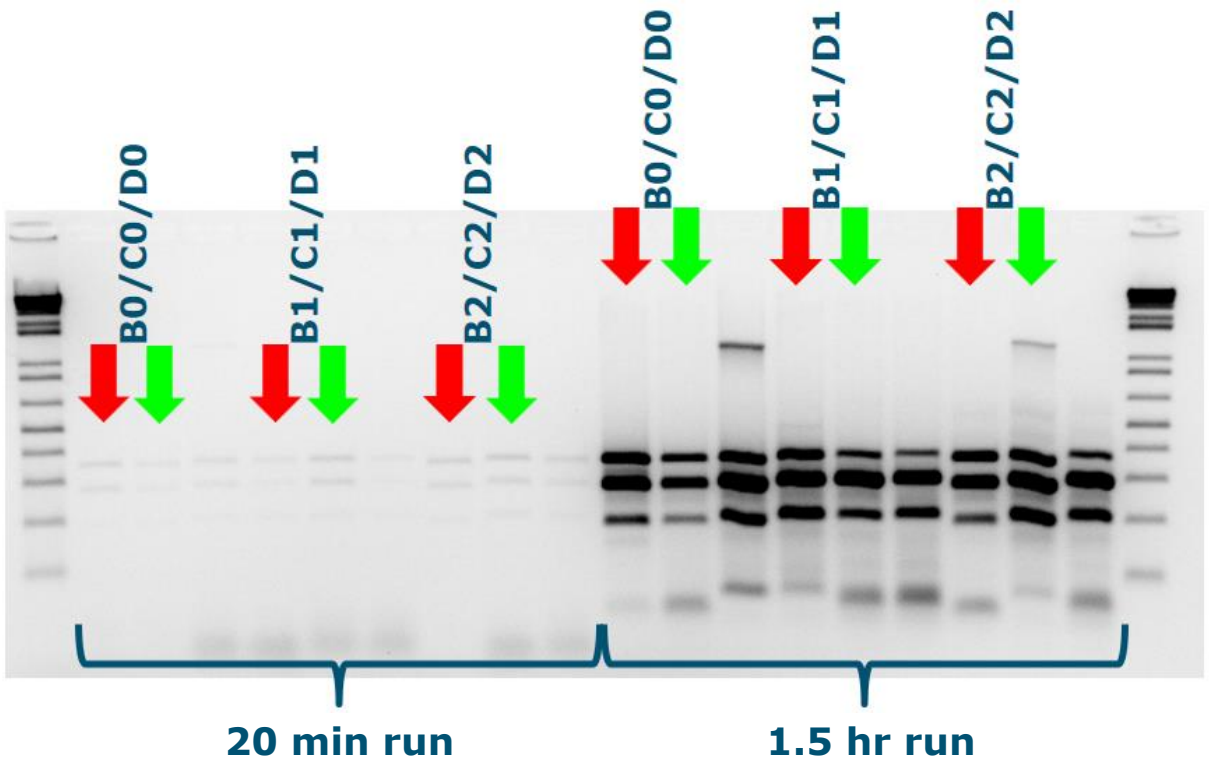
Plant pathogen amplification in crude plant extracts

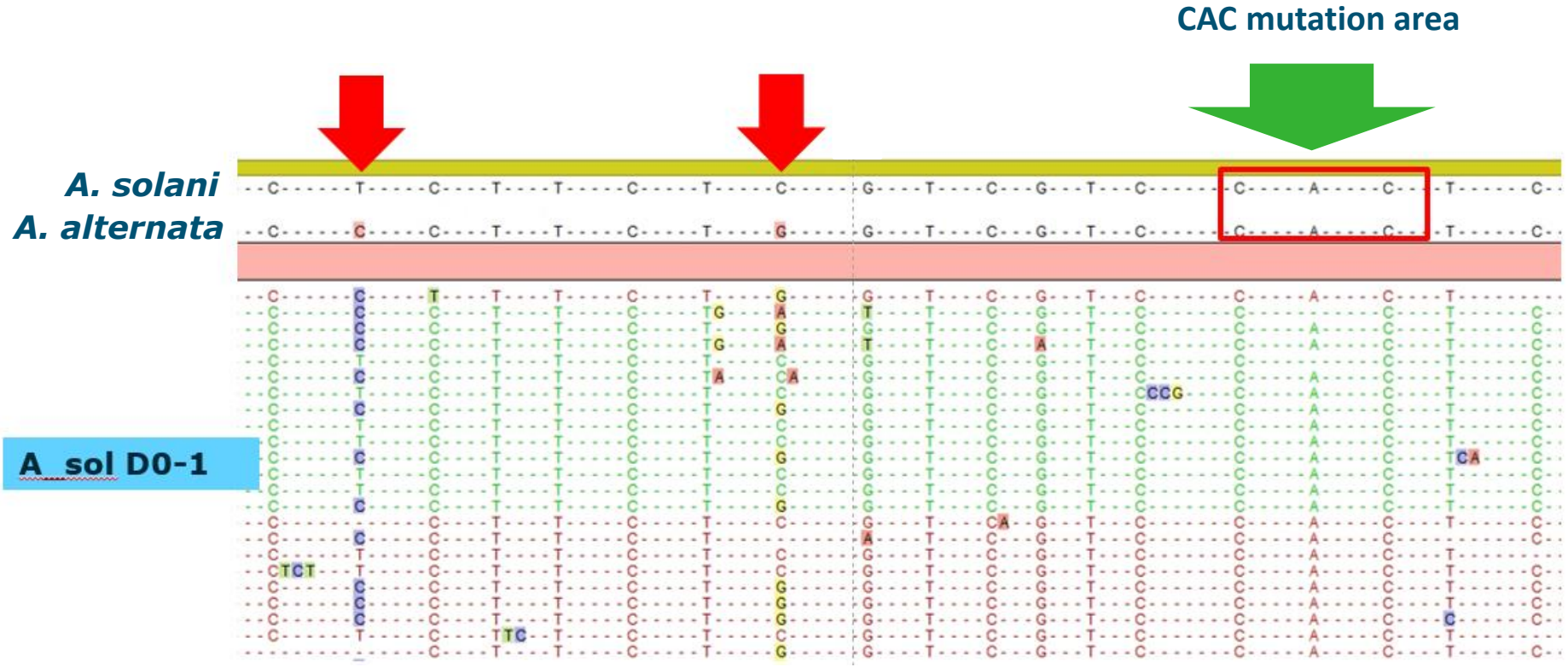
Alternaria solani

B0/C0/D0
B1/C1/D1
B2/C2/D2

Alternaria alternata

B0/C0/D0
B1/C1/D1
B2/C2/D2





MinION sequencing of the SdhD complex can be be used for *Alternaria solani/alternata f*ungicide resistance identification.

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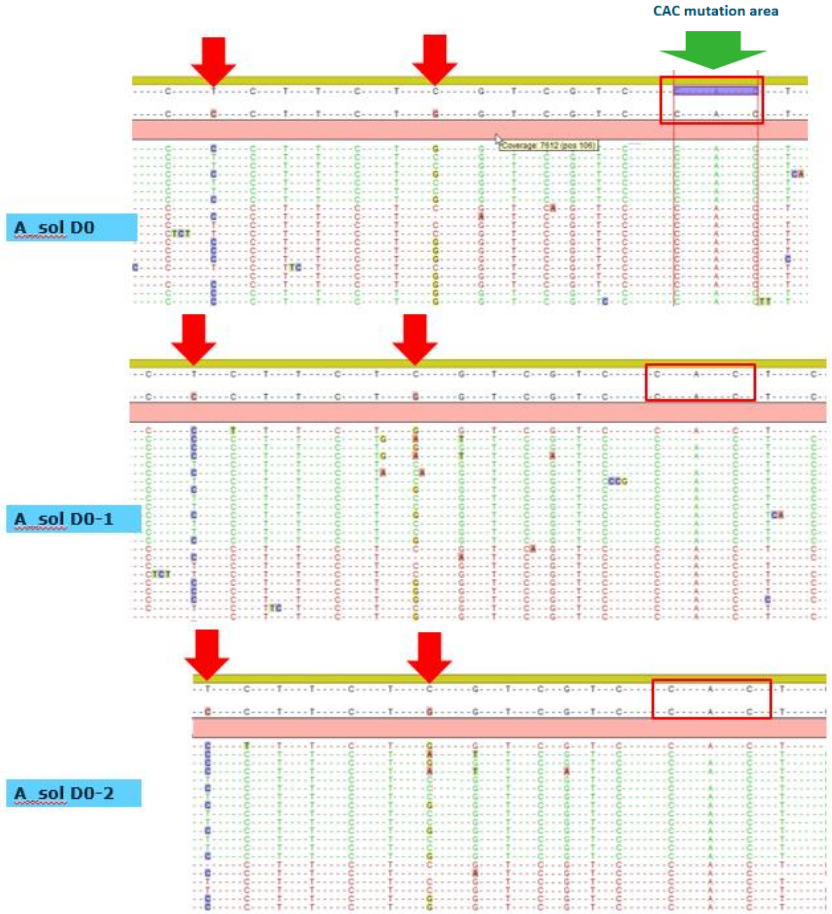
- *A. alternata and A. solani can be* identified
- Different *Alternaria* samples can be discriminated:
  - B0, B1 andB2
  - C0, C1 and C2
  - D0, D1 and D2
- Fungal resistance B, C and D genes can be discriminated



Conclusions MinION barcode sequencing (1)

- RPA amplification of different targets is possible
- Within 30 minutes 100.000 reads
- Flow-cells can be re-used ≥ 2 times
- Different amplicons within one sample can be identified with MinION
- Internal barcoding with RPA is possible

Conclusions MinION barcode sequencing (2)

- Oxford Nanopore sequencing platform combined with a simple extraction amplification system can provide a rapid way for initial diagnosis of plant diseases
- The process of diagnosis compared to the current diagnostic methods is significant shortened
- The method can be performed in the laboratory or ‘field conditions’ and analysis of the results for diagnostics does not necessitate deep knowledge in bioinformatics tools
- This opens the possibility for taxonomic identification of the disease from symptomatic plant samples in real-time by the WIMP (what’s in my pot) workflow

Acknowledgements

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