Improved pathogen management in crops using rapid in-field diagnostics

Catherine Harrison

Septoria the disease

- *Mycosphaerella graminicola* (*Septoria tritici*, leaf blotch) is the most common foliar disease of wheat in Europe.
- Most fungicides used on wheat are targeted against its control.

Economic losses due to leaf blotch

- Up to 16% of the global harvest is lost to plant diseases.
- In the UK, foliar diseases of wheat account for losses of up to 12%.
- Septoria tritici residual yield losses £43-53 million p.a. UK
- Estimated than £82million p.a. UK is targeted against its control.

Azole resistance

- Azoles are the primary control method in the UK.
- CYP51 gene is important in the biosynthesis of a fungal cell membrane component.
- 33 known mutations on the CYP51 gene.

Aim of project

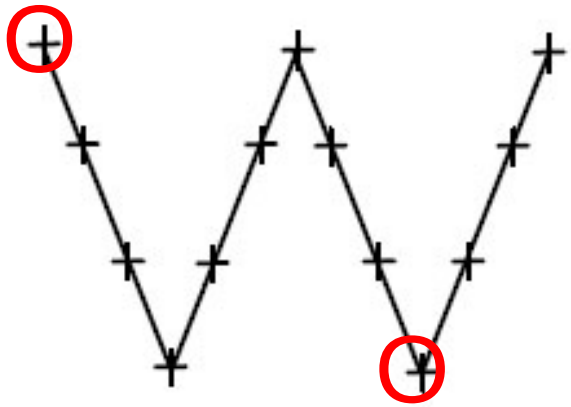
- To develop a rapid, in-field test to detect fungicide resistant genotypes.
- To enhance decision making, achieving effective disease control and more responsible use of chemicals in crops.
- The tests will be deployed on a hand held platform with automated result calling.

Septoria population evaluation

- Evaluate the population structure of pathogens to enable effective sampling in the field.
- How common are the known mutations.
- How variable are populations:
  - From farm to farm
  - From field to field
  - Within a field

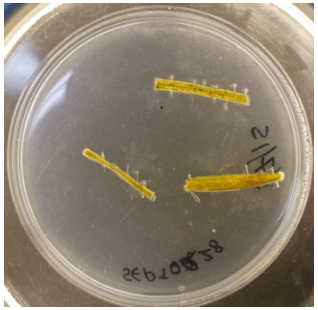
Septoria sampling plan

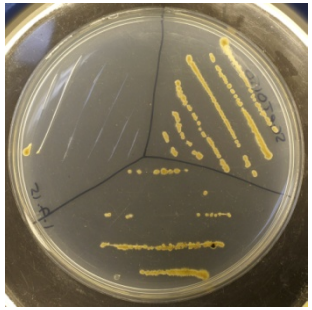
- To establish a sampling strategy
- 4 Farms
- 3 fields per farm
- 20 samples from each field using a W grid
- 2 intensive sampling points

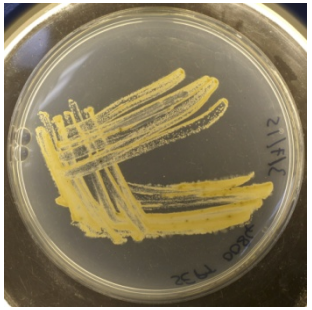


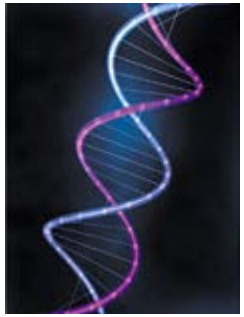
- Total 2160 samples

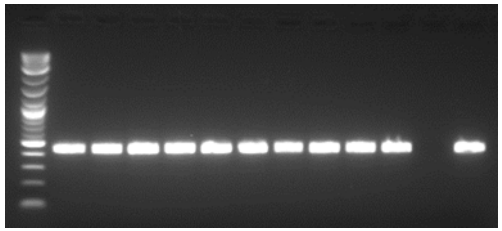
Septoria sequencing process

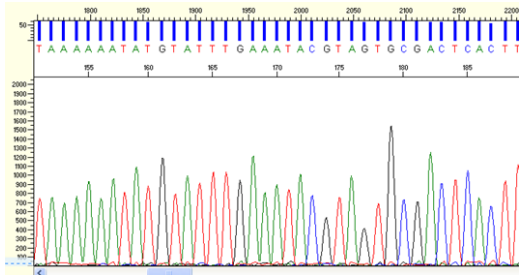


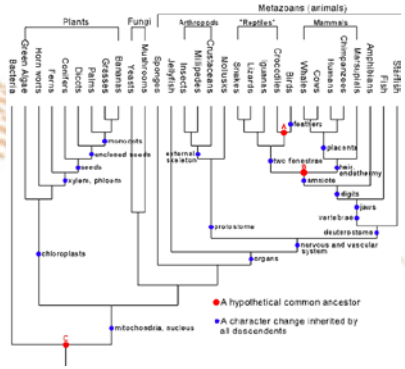




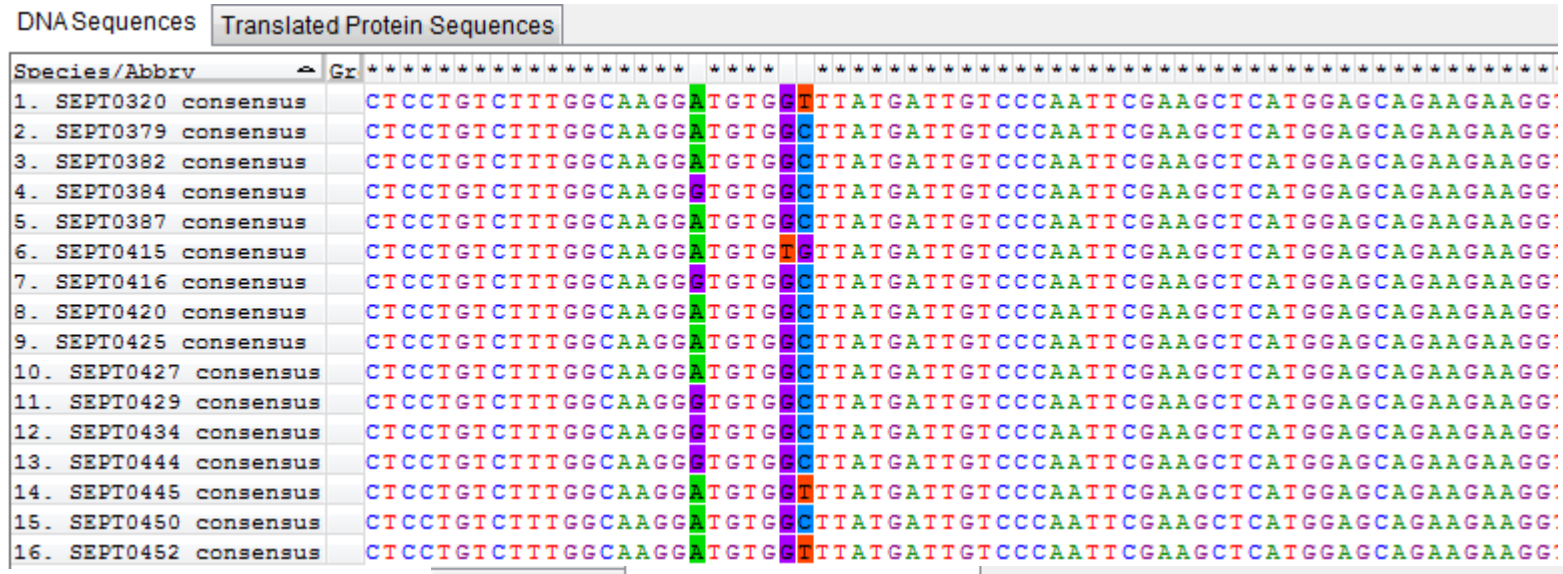


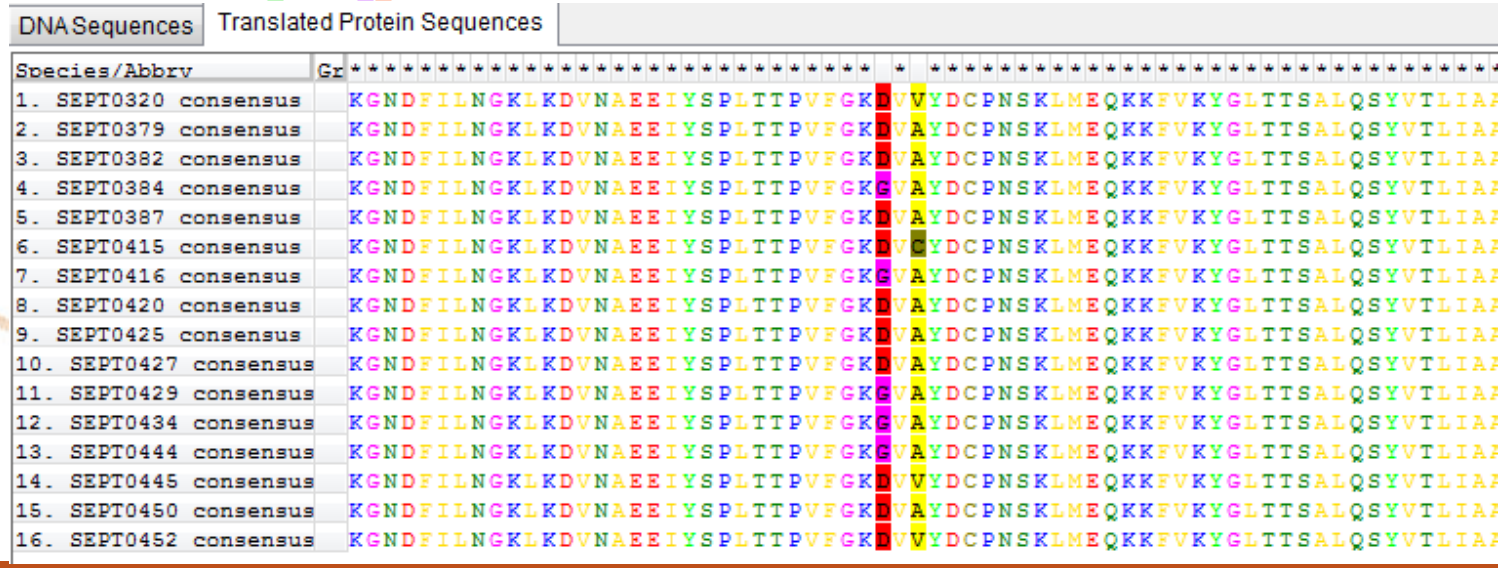






Sequence variation

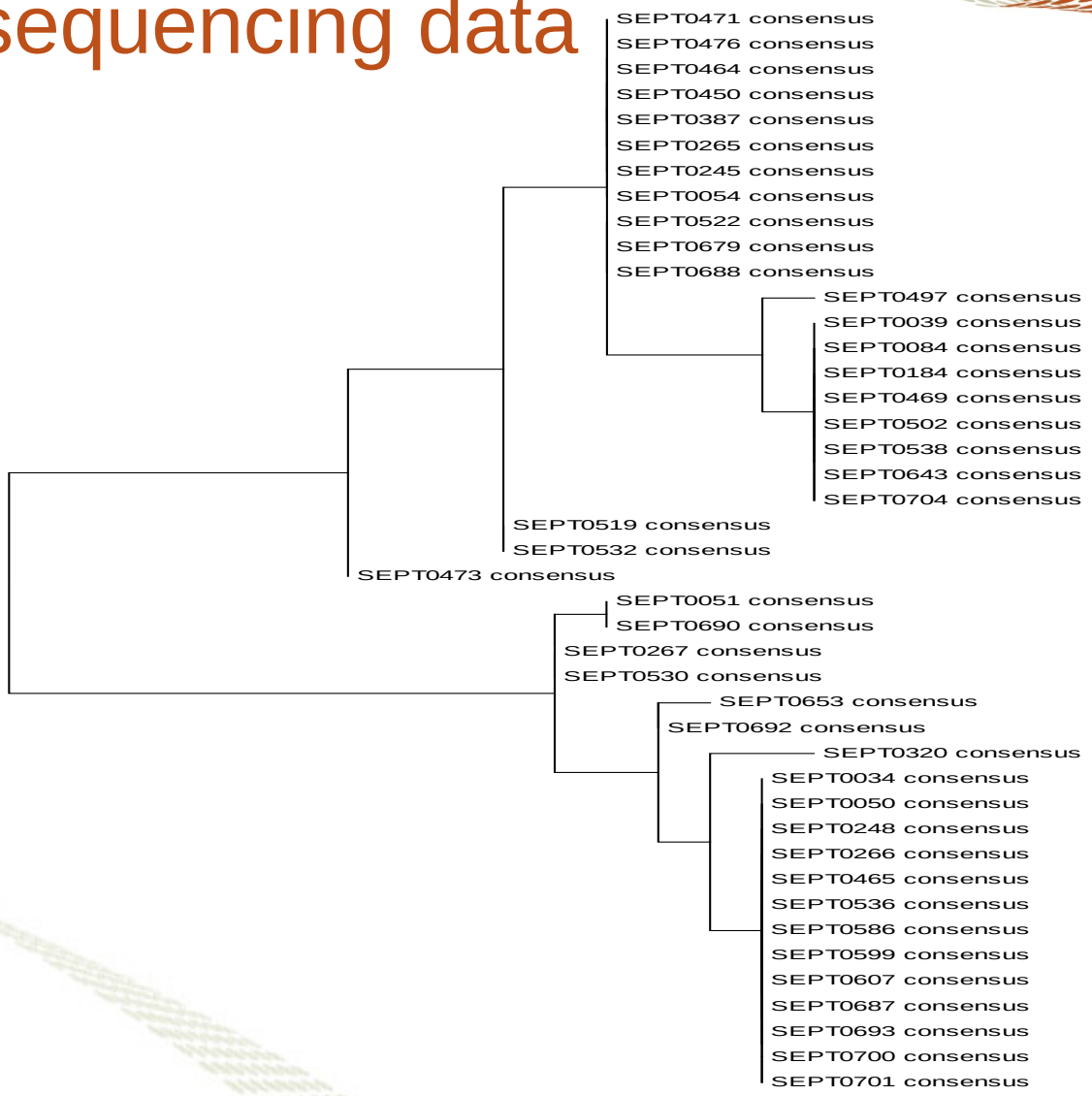




Mutation combinations found

	50	134	136	188	379	381	461	459-461	% isolated
Wild type	L	D	V	S	A	I	Y		
Type 1	S	G	A	S	A	V	H		47.23
Type 2	S	D	A	N	G	V	Y	deletion	24.52
Type 3	S	D	V	N	A	V	Y	deletion	18.87
Type 4	S	D	A	S	A	V	H		5.65
Type 5	S	D	A	N	A	V	Y	deletion	3.73

Septoria sequencing data



Importance of mutations found

- D134G – Causes reduced Azole sensitivity in combination with changes at 459-461
- V136A – Causes reduced Azole sensitivity in combination with changes at 459-461
- A379G – Found in resistant strains in combination with 381
- I381V– Causes reduced Azole sensitivity – in combination with changes at 459-461

Importance of mutations found

- Y461H – Found in resistant strains in combination with other changes
- 459-461- deletion causes azole sensitivity
- 50 and 188 – No effect on azole sensitivity

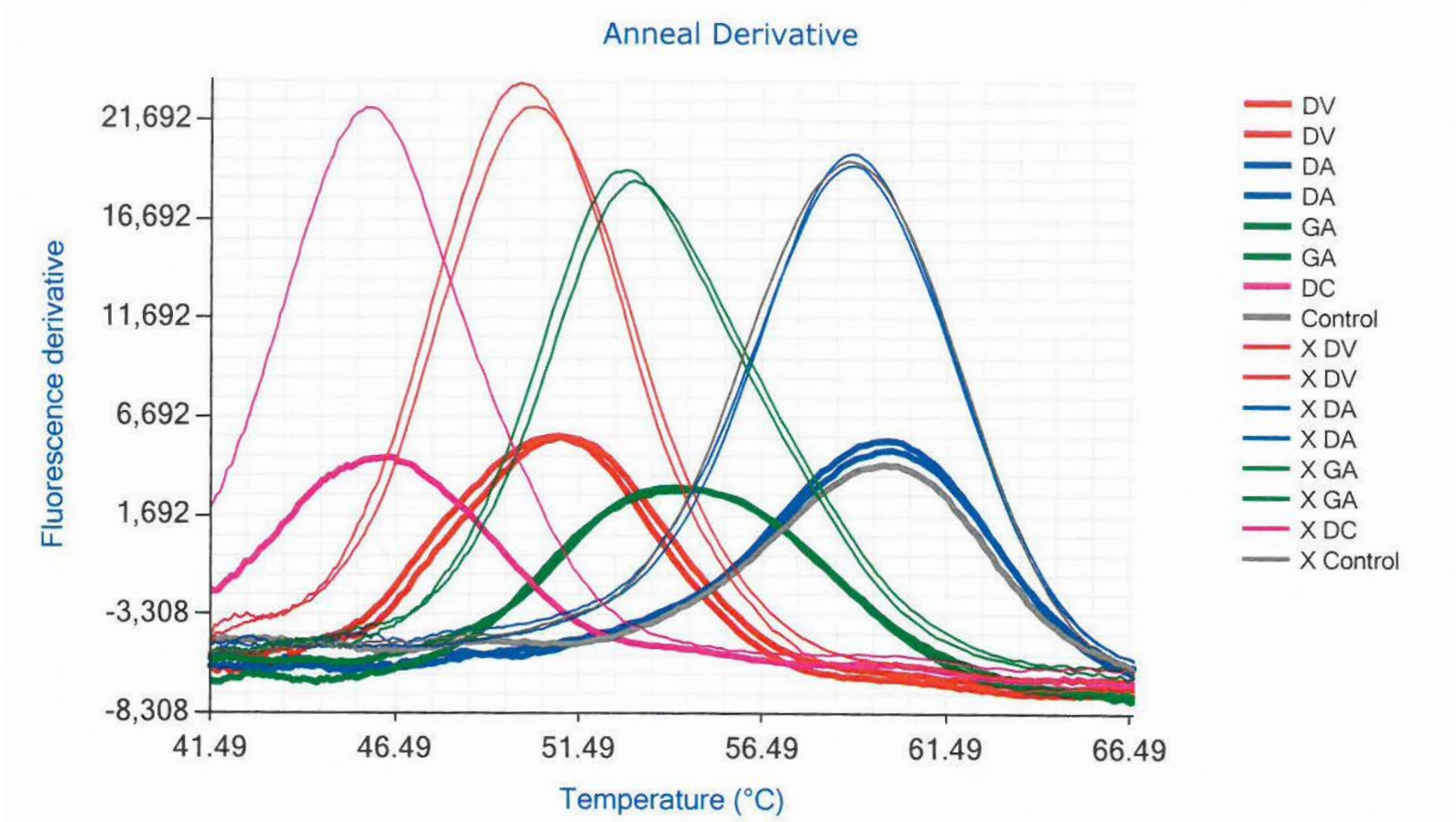
The assay requirements

- The assay is a DNA based LAMP assay
- Must be able to detect SNPs (single nucleotide polymorphisms).
- Ideally be a multiplex reaction.
- Must be rapid (less than 20 minutes)
- Must be able to be performed in the field.

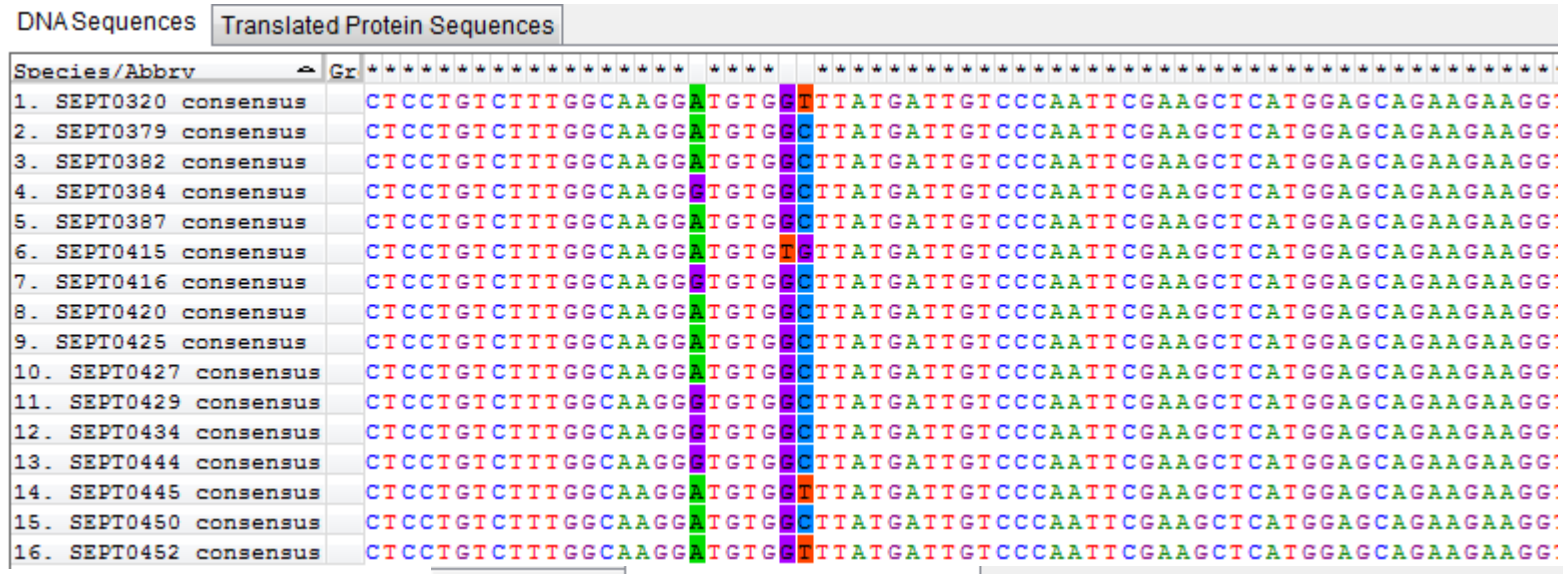
New developments required

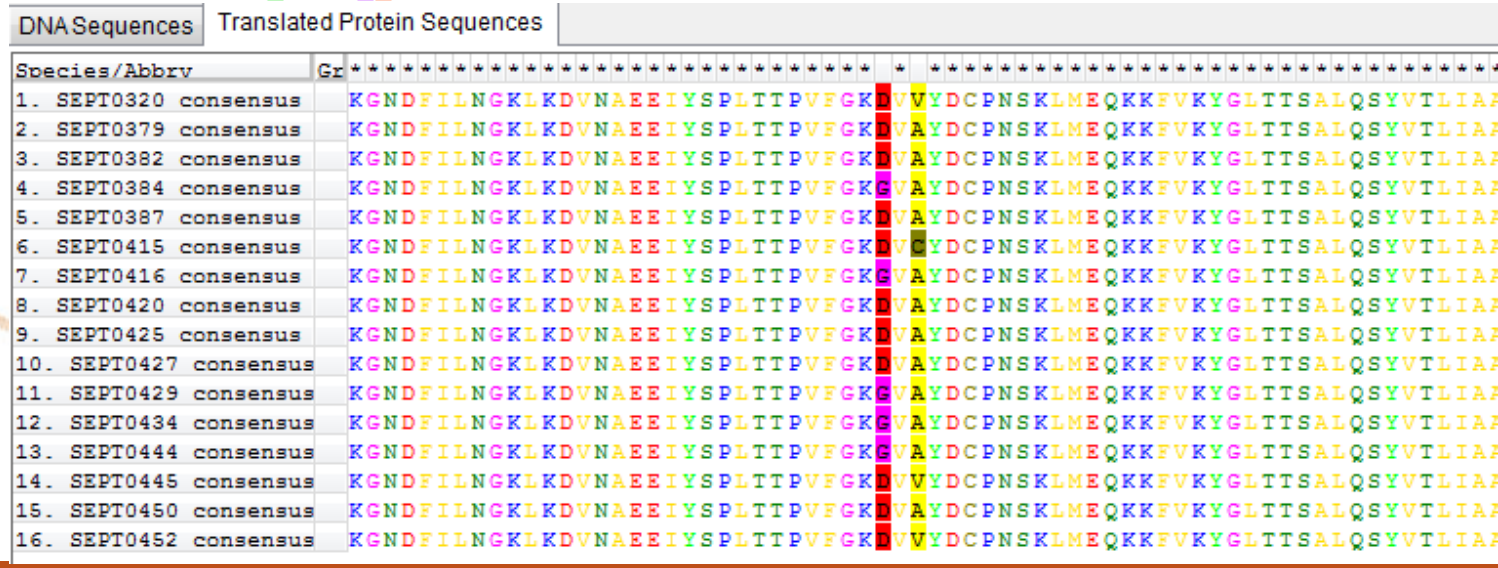
- To develop new probe methods to enable SNP detection and multiplexing. (Fera and GeneSys Biotech)
- Improved LAMP chemistry/reagents (GeneSys Biotech).
- To develop improved software for LAMP probe assays (OptiSense).

Initial method comparison

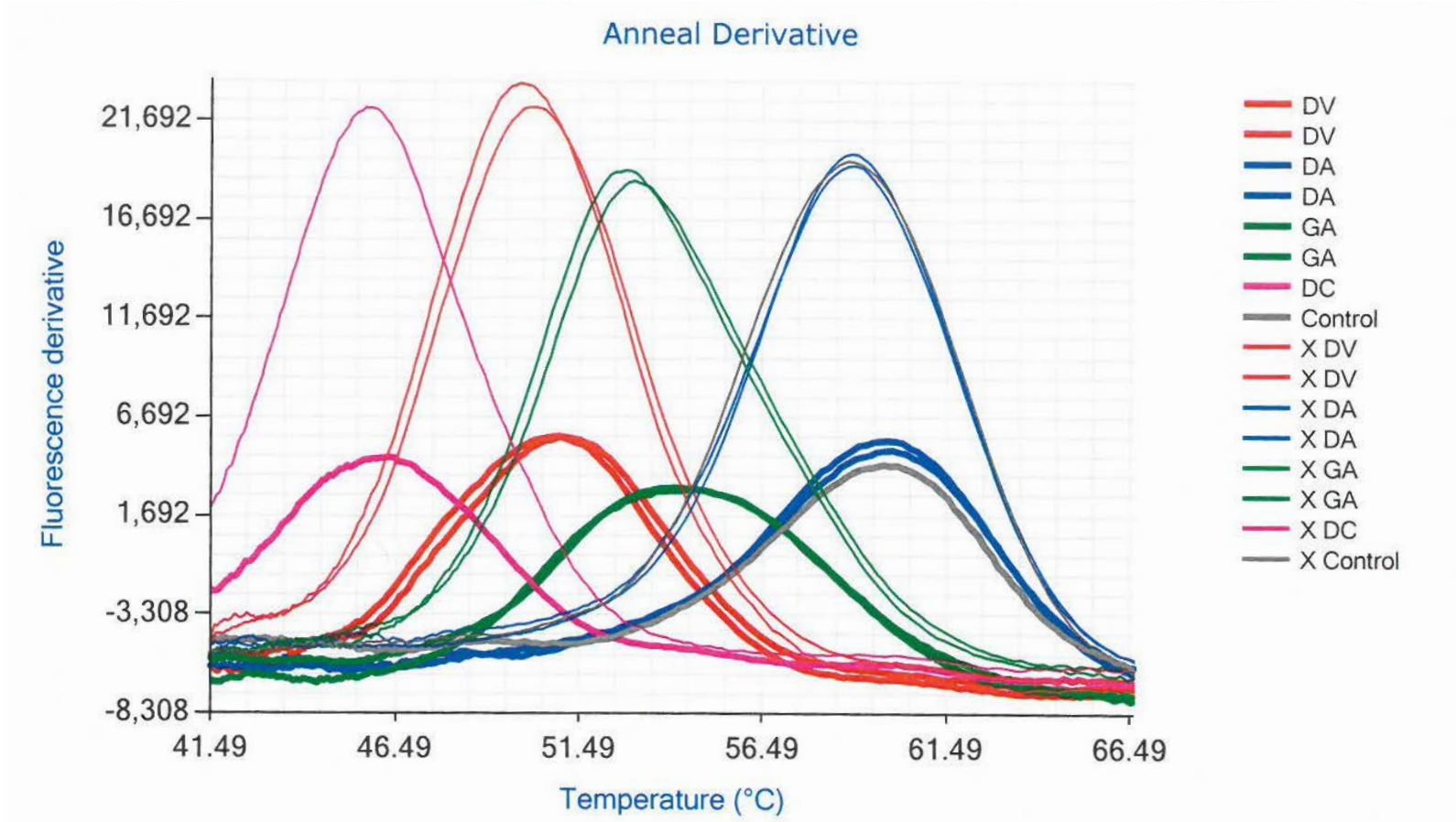


Sequence variation



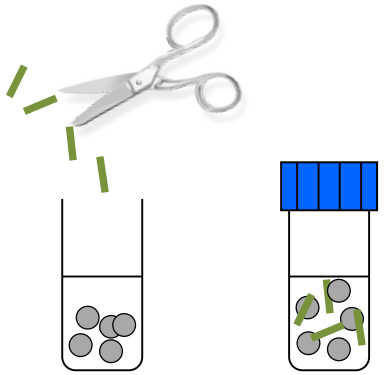


Initial method comparison



LAMP for detection of *Septoria tritici*

1. Preparation of samples

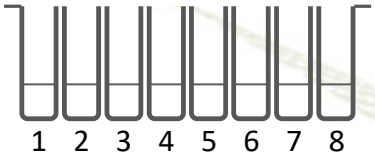


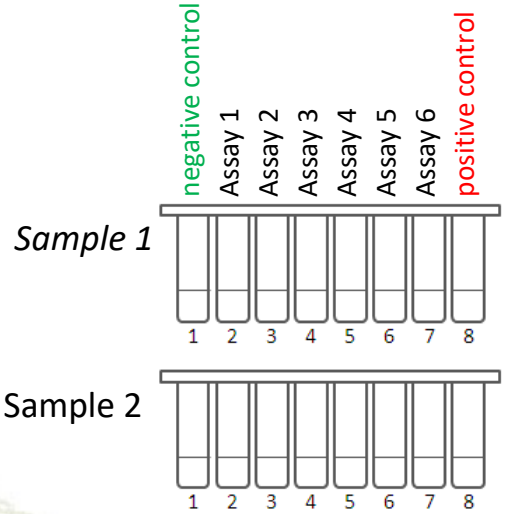
Shake for 1 minute

Dilute 1 in 10

2. Preparation of test strips

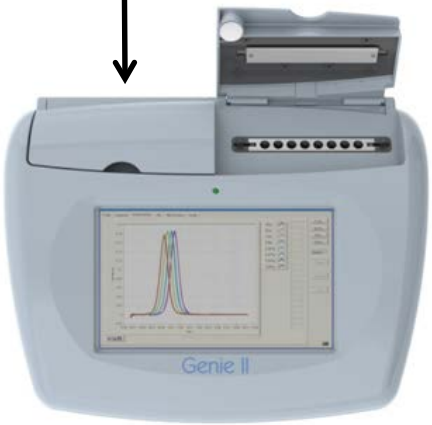
Transfer sample into LAMP reactions





Block A: Sample 1

Block B: Sample 2



Acknowledgments

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  - Agrii
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