Development of probes and platforms for the detection and identification of captured fungal spores

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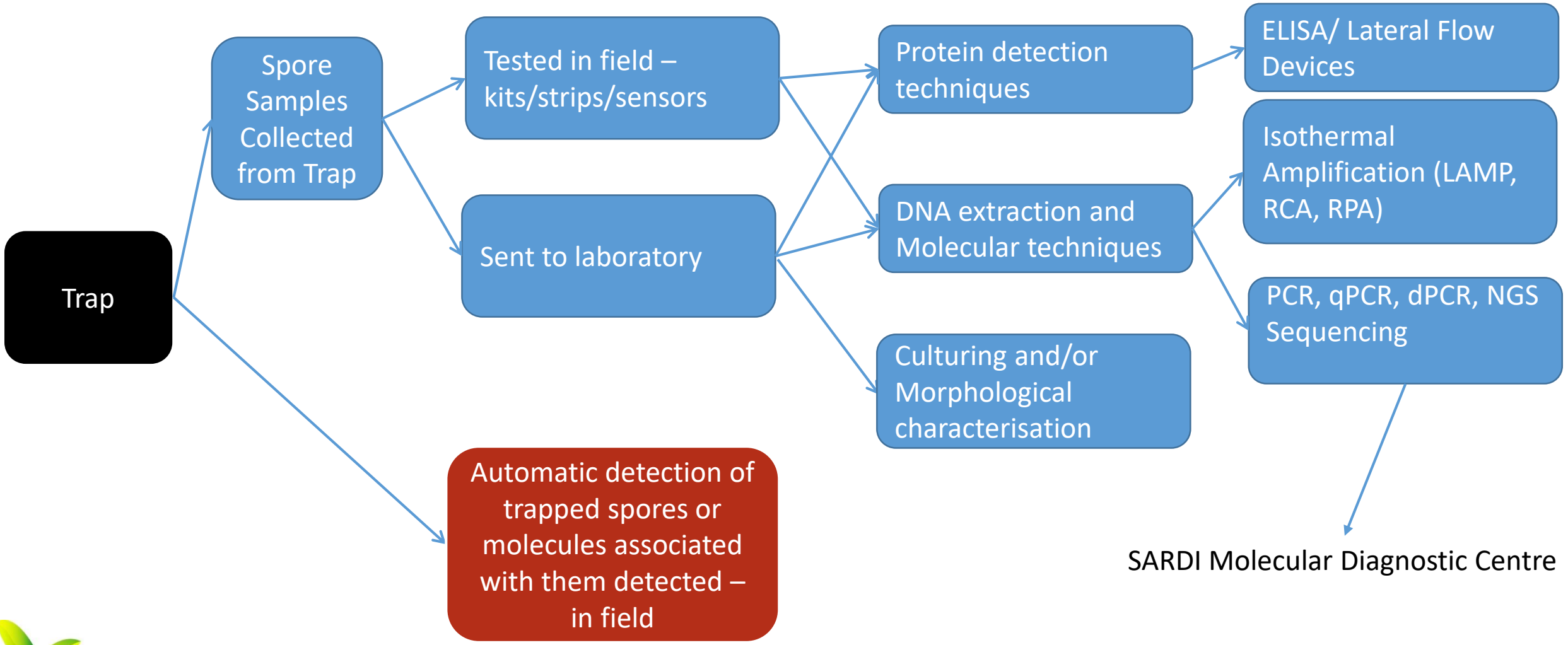
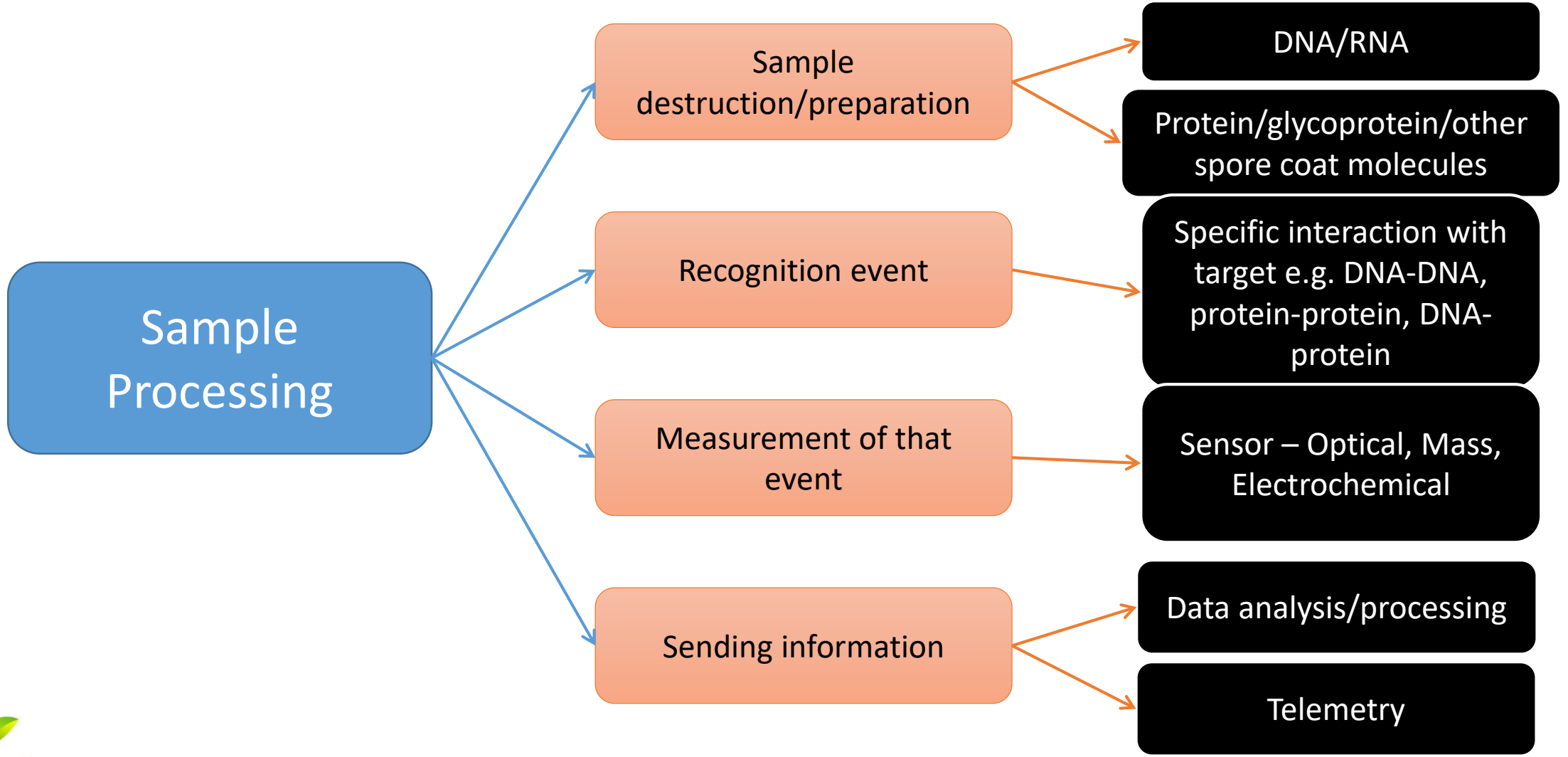
Processing of surveillance samples



Investigating how to make diagnostic tests for autonomous in-field use – components and considerations



Investigating how to make diagnostic tests for autonomous in-field use - key output

Protein/glycoprotein/other spore coat molecules

Specific interaction with target e.g. DNA-DNA, protein-protein, DNA-protein

Prob… …ent

Aptamers

Detection of semi-intact spore captured in solution
- *Puccinia striiformis f. sp. tritici (Pst)*
(Wheat Stripe Rust)

Sensor – Optical

Data processing

Platform Development

Dr Linh Nguyen

IPAS
Institute for Photonics and Advanced Sensing

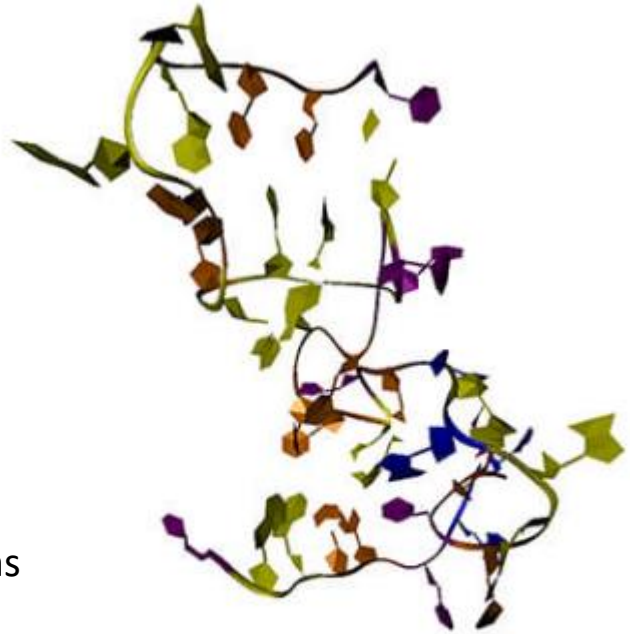
Fiber Optic Sensor to sensitively monitor interactions taking place
-Fiber Optic Sensor is spliced directly into normal optical fibres
- Can be functionalised to carry any probes

What are Aptamers?

Coined from the Greek word *aptus* “to fit”

ssDNA or RNA molecules – tertiary structure allows specific binding

Origins: a PhD study into the mRNA translational operator motif of the DNA polymerase of bacteriophage T4 – mutations produce hairpin with the same affinity to regulator protein



Structure of aptamer developed by Base Pair Biotechnologies to IL-7

Selected *in vitro* to chosen target

Chemically synthesised = lower cost for commercial production

Increased stability for transport, storage and field use

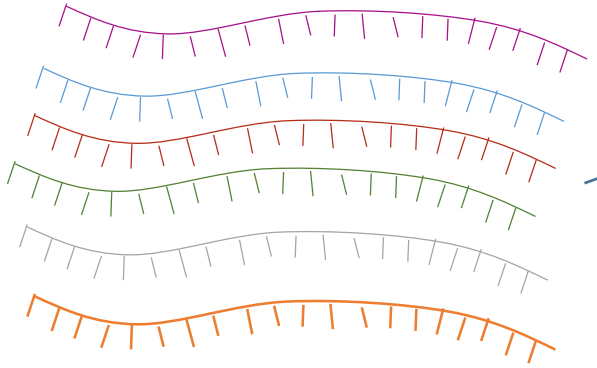
https://www.basepairbio.com/

Aptamer Selection – SELEX

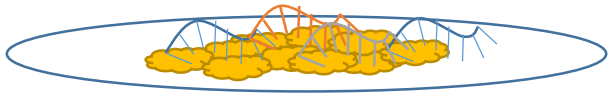
Systematic Evolution of Ligands by Exponential enrichment

Cell SELEX (Sefeh *et al.* 2010)
Single Selection Protocol (Liu *et al*. 2012)

Initial library 10^{15} Sequences

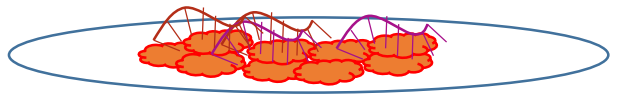


ssDNA library



Counter-selection (non-target)

Unbound sequences removed

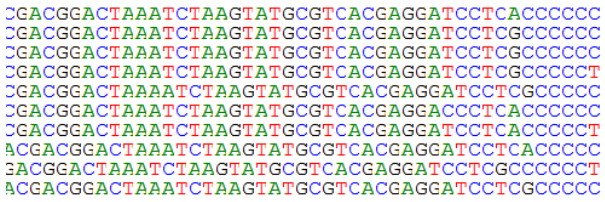


Selection (target)

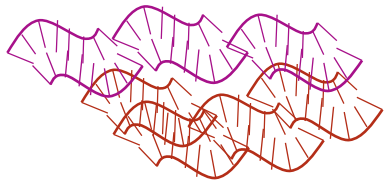
Elution

Amplification

ssDNA purification



NGS Sequencing



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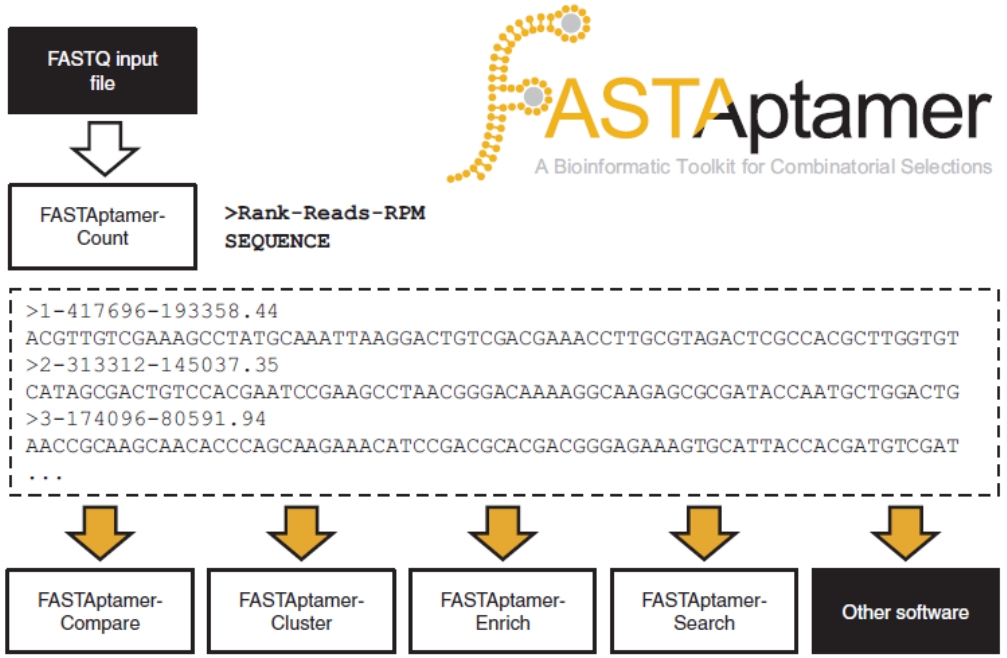
biosecurity **built on science**

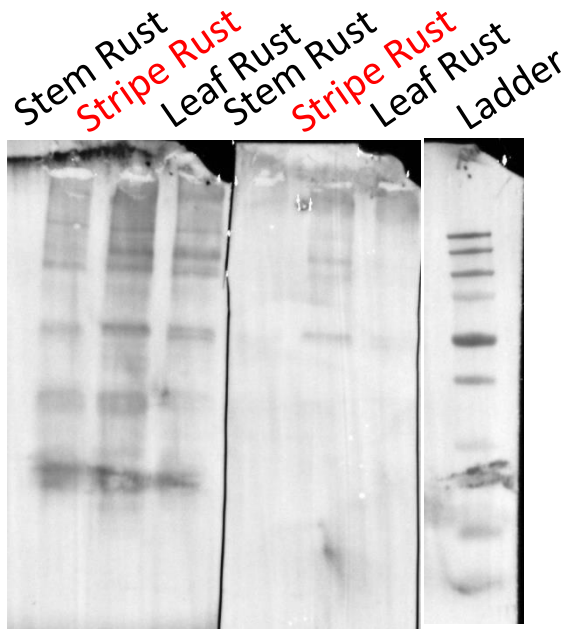
Aptamer Selection – NGS Analysis

Pipelines –

- FastAptamer (Alam *et al.* 2015) – based on sequence frequency and enrichment over cycles
- APTANI (Caroli *et al.* 2016) – based on a common structural motif within binding aptamers in a selected library
- APTATRACE (Dao 2016) – similar to APTANI but doesn't work on the assumption only a single motif is responsible for binding



Stripe Rust Aptamer Development

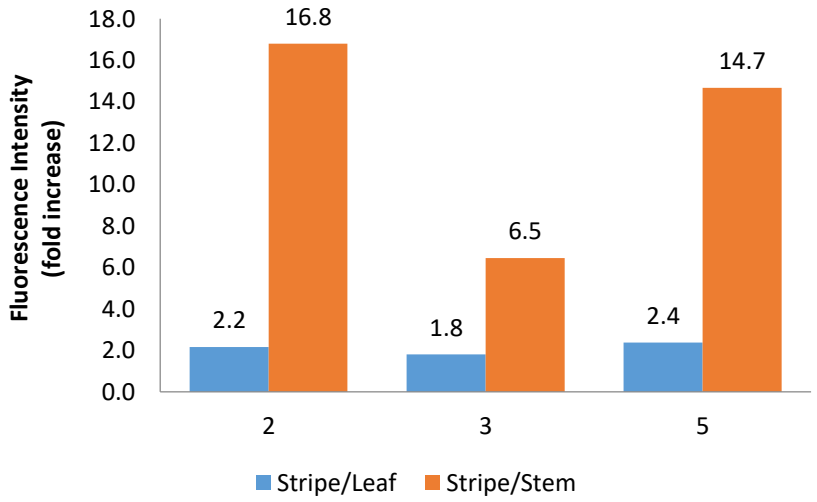


FAM Apt-25-37-3&5

FAM Apt-25-37-1&2

AP-conjugated anti-FITC Ab, developed with NBT and BCIP

	Single-selection protocol		Multi-selection protocol		
Name	Reads (x)	RPM (x)	Reads (y)	RPM (y)	Enrichment (y/x)
25-37-2	1	3.81	900	20.24	5.31
25-37-3	1	3.81	802	18.04	4.73
25-37-4	1	3.81	750	16.87	4.43
25-37-5	1	3.81	614	13.81	3.62
25-37-6	1	3.81	530	11.92	3.13
25-37-7	1	3.81	280	6.3	1.65
25-37-8	1	3.81	268	6.03	1.58



More non-specific binding with Leaf Rust (Puccinia tritcina) than Stem Rust (Puccinia graminia f. sp. Tritici) which was used as counter-selector

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First Selection Results

- Non-specific Binding
- Low Sensitivity – as shown by SPR
- Developed against total protein extract

Aptamer Second Selection – Enriched Library

Enriched starting library

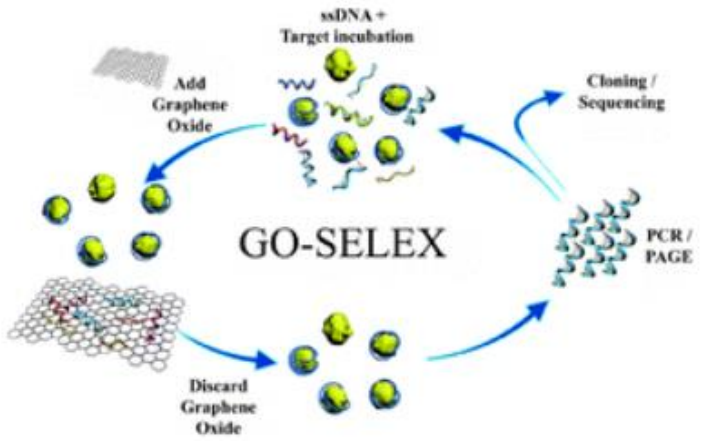
Single Selection

Multi-Round Selection

GO-SELEX

NGS Analysis

Surface Solubilised Targets



Park *et al*. 2012

Aptamer Development – Outcomes, Difficulties and Opportunities

- Feasibility of Approach – both probe and platform development – do they offer qualities that provide value over other techniques?

- Protocols to apply strategy to rust spores and potentially other targets

- Difficulties include:
  - Efficient purification of ssDNA – protocol change using asymmetric PCR (Zhang *et al.* 2015)
  - Analysis – HPC requirements especially for structural analysis of files containing millions of reads – Amazon Web Server
  - Immobilisation strategies can be problematic, we believe we may lose sensitivity as we move further from the fibre: solution interface

- Opportunites
  - Alternative targets – e.g. Viral and bacterial targets for aptamer development
  - Alternative platforms for aptamers (low tech) – lateral flow
  - Alternative uses for RI measurements for platform

- For more information, please email Kelly.Hill3@sa.gov.au