

LAMP: field based detection of sweetpotato viruses

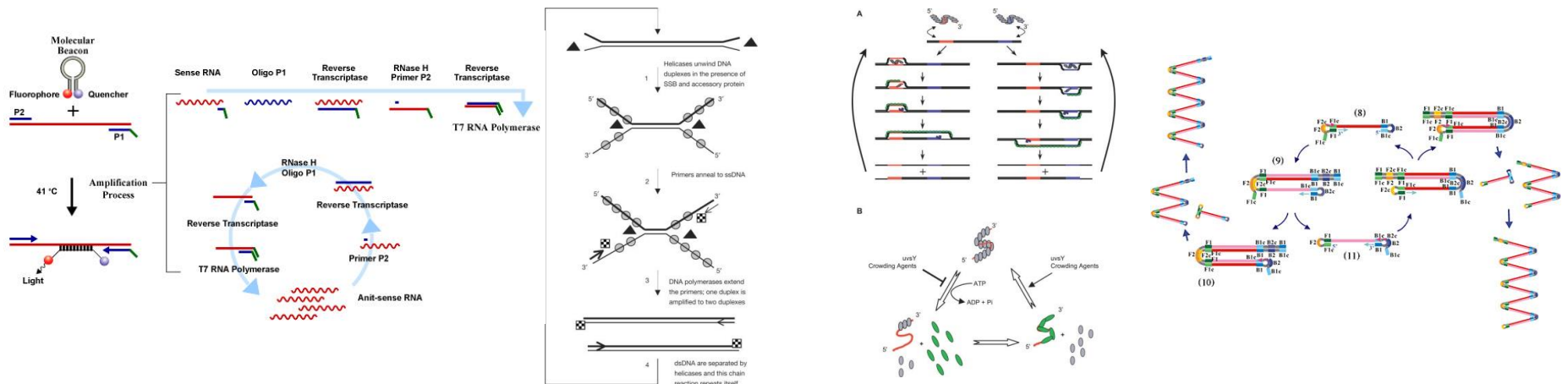
December 7, 2016

Sweetpotato diagnostics

- NCM ELISA kits available for 10 viruses → require lab
- Could be converted to lateral flow device format for field use
- However, most viruses occur in very low titre when infecting sweetpotato by themselves, making results directly from sweetpotato unreliable
- Sensitive methods include PCR, several assays are available for most viruses, but they require lab conditions & complex equipment → unsuitable for field
- There is a clear need for sensitive field diagnostic methods

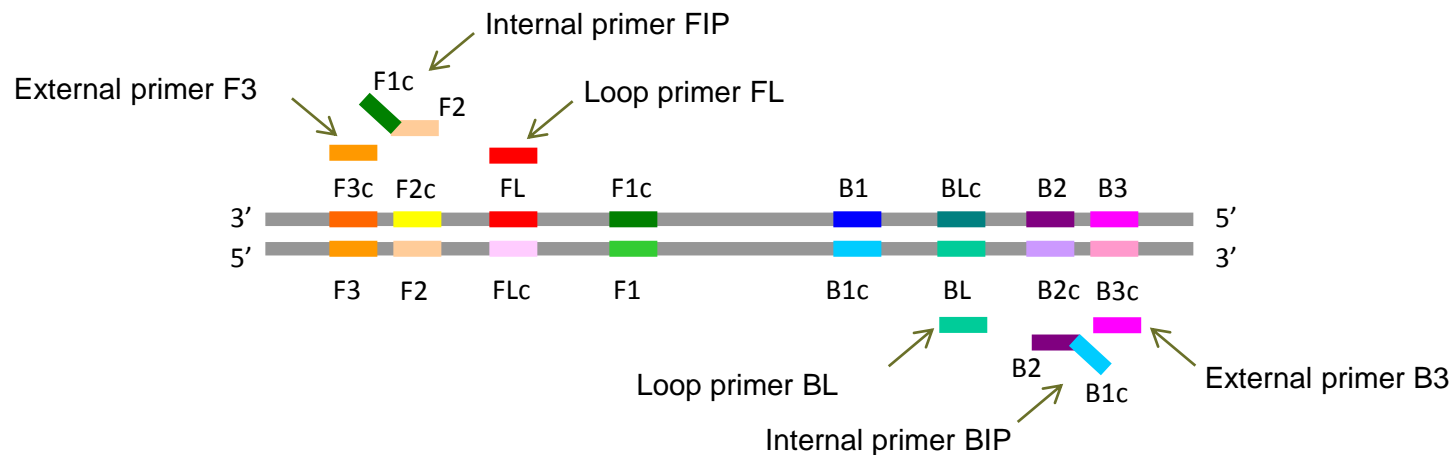
Isothermal amplification methods: Overcoming many of the limitations of PCR-based methods (cost and complexity of thermal cycling equipment)

- Helicase Dependent Amplification (HDA) 65°C <30m
- Recombinase Polymerase Amplification (RPA) 37-42°C <30m
- Nucleic acid sequence based amplification (NASBA) 41°C >1h
- Isothermal and Chimeric primer-initiated Amplification of Nucleic acids (ICAN) 55°C > 1h
- Loop-mediated isothermal AMPLification (LAMP) 65°C <30m

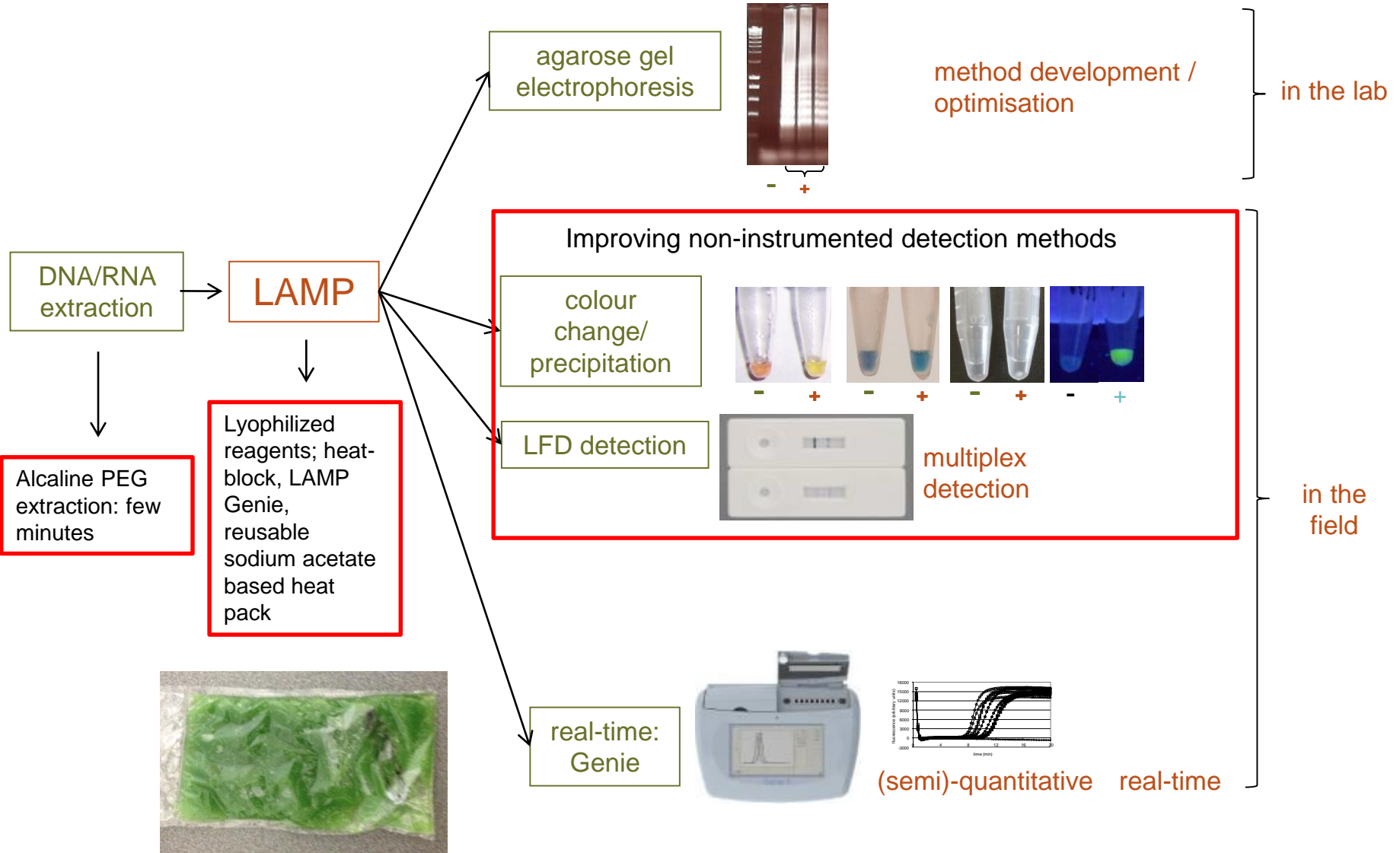


Towards sensitive molecular field detection kits: LAMP

- Functions at a single temperature (no complicated and expensive thermal cycling equipment required)
- Enzymes more robust to contaminants & can be lyophilized (robustness for field conditions)
- 10-30 minute reaction time & more sensitive even than PCR



<https://www.youtube.com/watch?v=ZXq756u1msE>



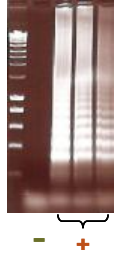
DNA/RNA extraction

LAMP

Alkaline PEG extraction: few minutes

Lyophilized reagents; heat-block, LAMP Genie, reusable sodium acetate based heat pack

agarose gel electrophoresis



method development / optimisation

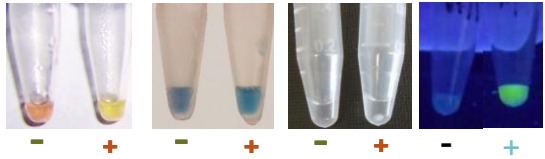
in the lab

Improving non-instrumented detection methods

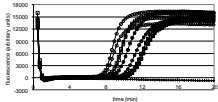
colour change/precipitation

LFD detection

multiplex detection



real-time: Genie



(semi)-quantitative real-time

in the field

Prototype LAMP field kit

Alkaline PEG extraction buffer

Sample maceration bags

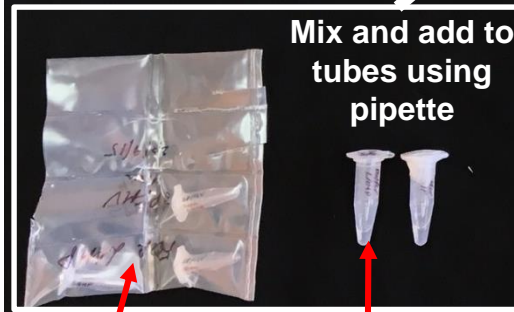
Discardable inoculation loop for transferring sample

Tube with water for sample dilution



Macerate sample in bag, dilute 1/10 with water and transfer 1ul to reaction tube using inoculation loop

Discardable pipette for adding extraction buffer & reaction buffer



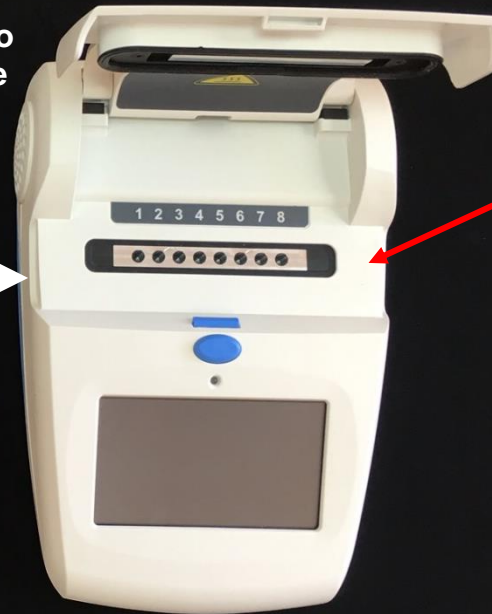
Mix and add to tubes using pipette



Lyophilized LAMP reagents: primers & enzyme

LAMP buffer

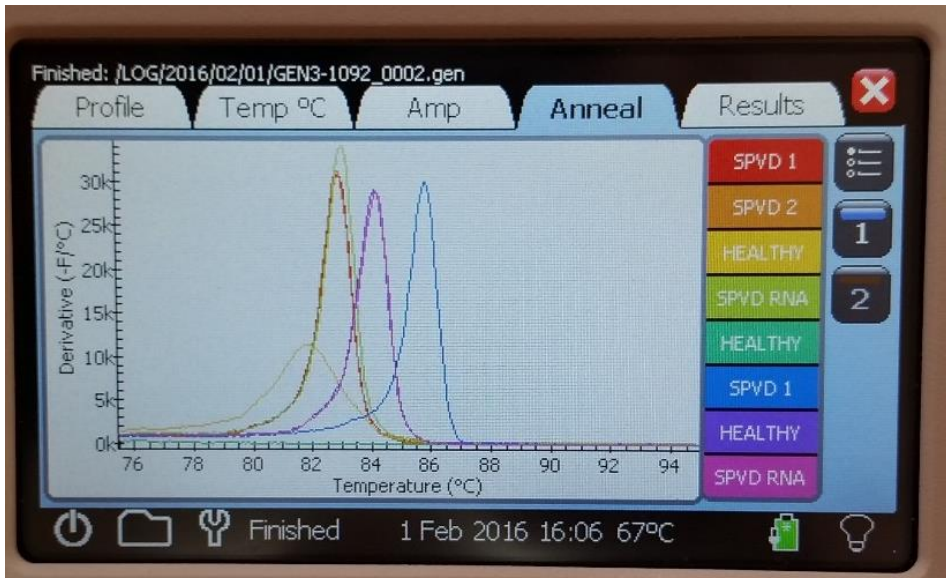
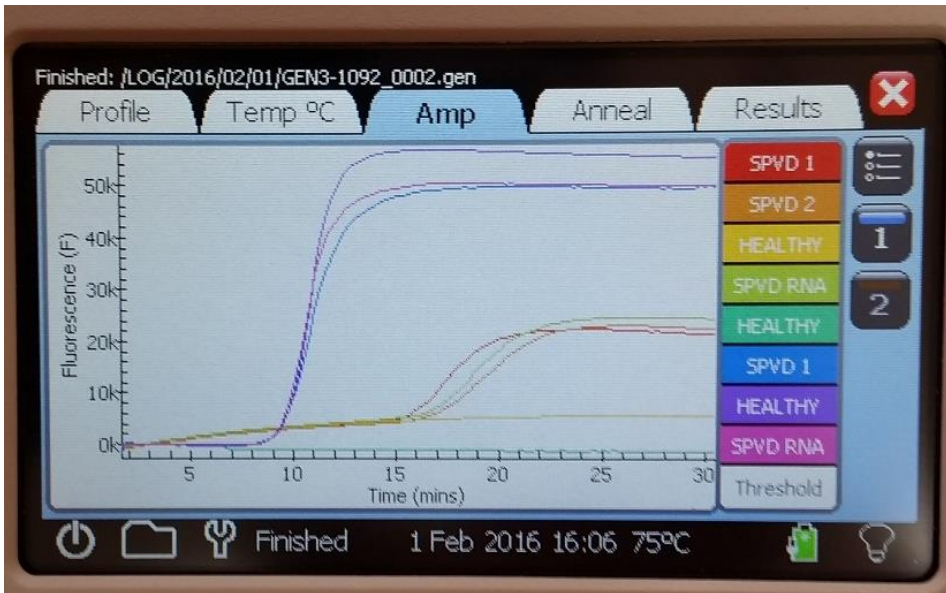
Reaction tubes



Realtime Genie

Run in realtime Genie and read results on screen

Genie output



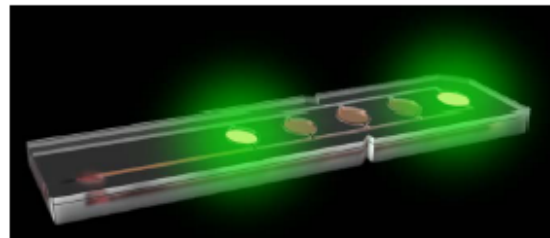
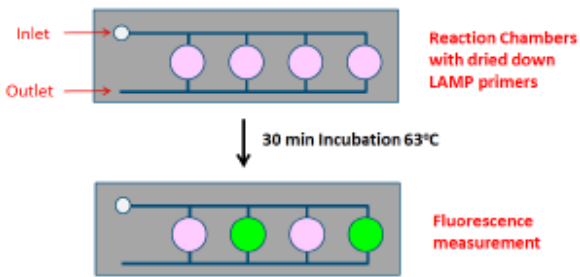
Finished: /LOG/2016/02/01/GEN3-1092_0002.gen

Well	Profile	Temp °C	Amp	Anneal	Results
			Amplification mm:ss	Anneal °C	Result
1	SPVD 1		17:09	82.84	Positive
2	SPVD 2		18:24	82.85	Positive
3	HEALTHY			81.71	Negative
4	SPVD RNA		18:09	82.98	Positive
5	HEALTHY			75.71	Negative
6	SPVD 1		10:09	85.72	Positive
7	HEALTHY		10:09	84.02	Positive
8	SPVD RNA		10:09	84.17	Positive

Finished 1 Feb 2016 16:06 65°C

In the future: microfluidic LAMP (next generation phytosanitation project)

Microfluidics



Microfluidics allow multiple reactions combined with detection (PRI)



User Interface via Android app for user friendly data collection



Electronic Manifold holds chip with integrated temp. control and detection



The International Potato Center (known by its Spanish acronym CIP) is a research-for-development organization with a focus on potato, sweetpotato, and Andean roots and tubers. CIP is dedicated to delivering sustainable science-based solutions to the pressing world issues of hunger, poverty, gender equity, climate change and the preservation of our Earth's fragile biodiversity and natural resources.

www.cipotato.org



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