LAMP: field based detection of sweetpotato viruses

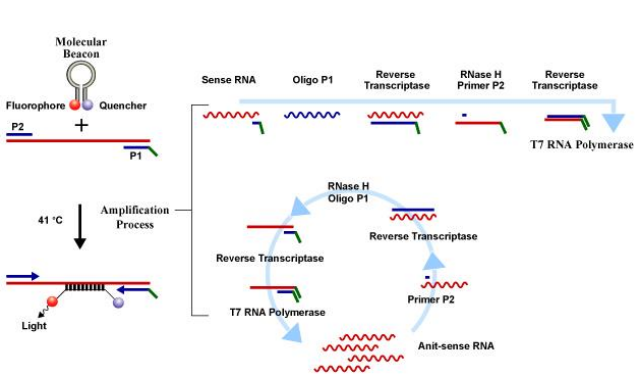
December 7, 2016

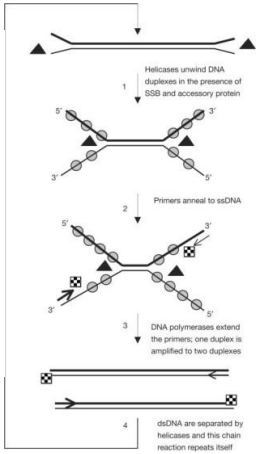
Sweetpotato diagnostics

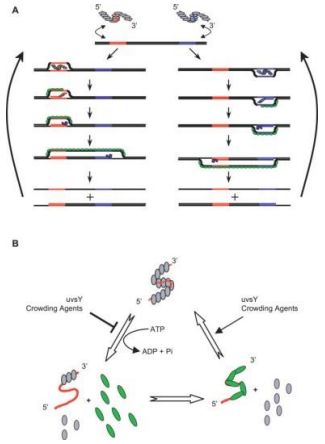
- NCM ELISA kits available for 10 viruses → require lab
- Could be converted to lateral flow device format for field use
- However, most viruses occur in very low titre when infecting sweetpotato by themselves, making results directly from sweetpotato unreliable
- Sensitive methods include PCR, several 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

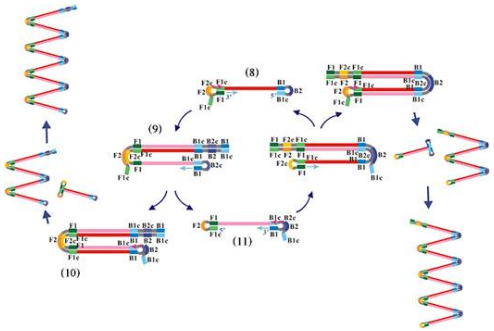
Isothermal amplification methods: Overcoming many of the limitations of PCR-based methods (cost and complexity of thermal cycling equipment)

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



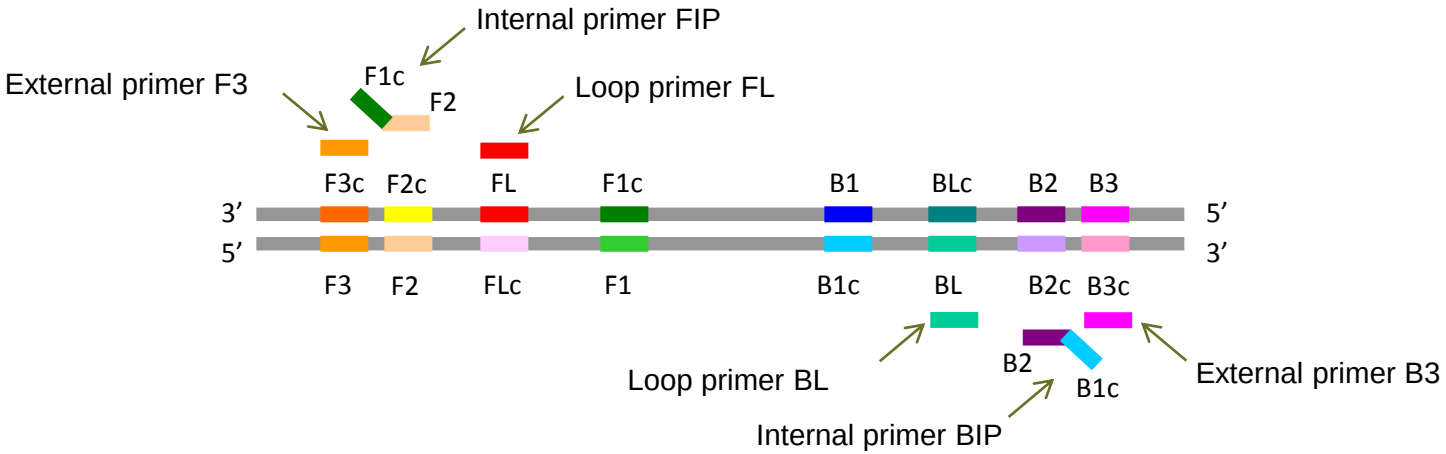






Towards sensitive molecular field detection kits: LAMP

- 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

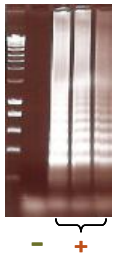
DNA/RNA extraction

Alcaline PEG extraction: few minutes

LAMP

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

agarose gel electrophoresis

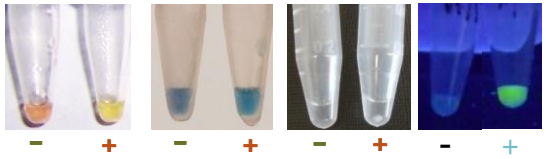


method development / optimisation

in the lab

Improving non-instrumented detection methods

colour change/ precipitation

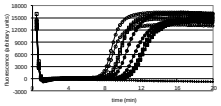


LFD detection

multiplex detection

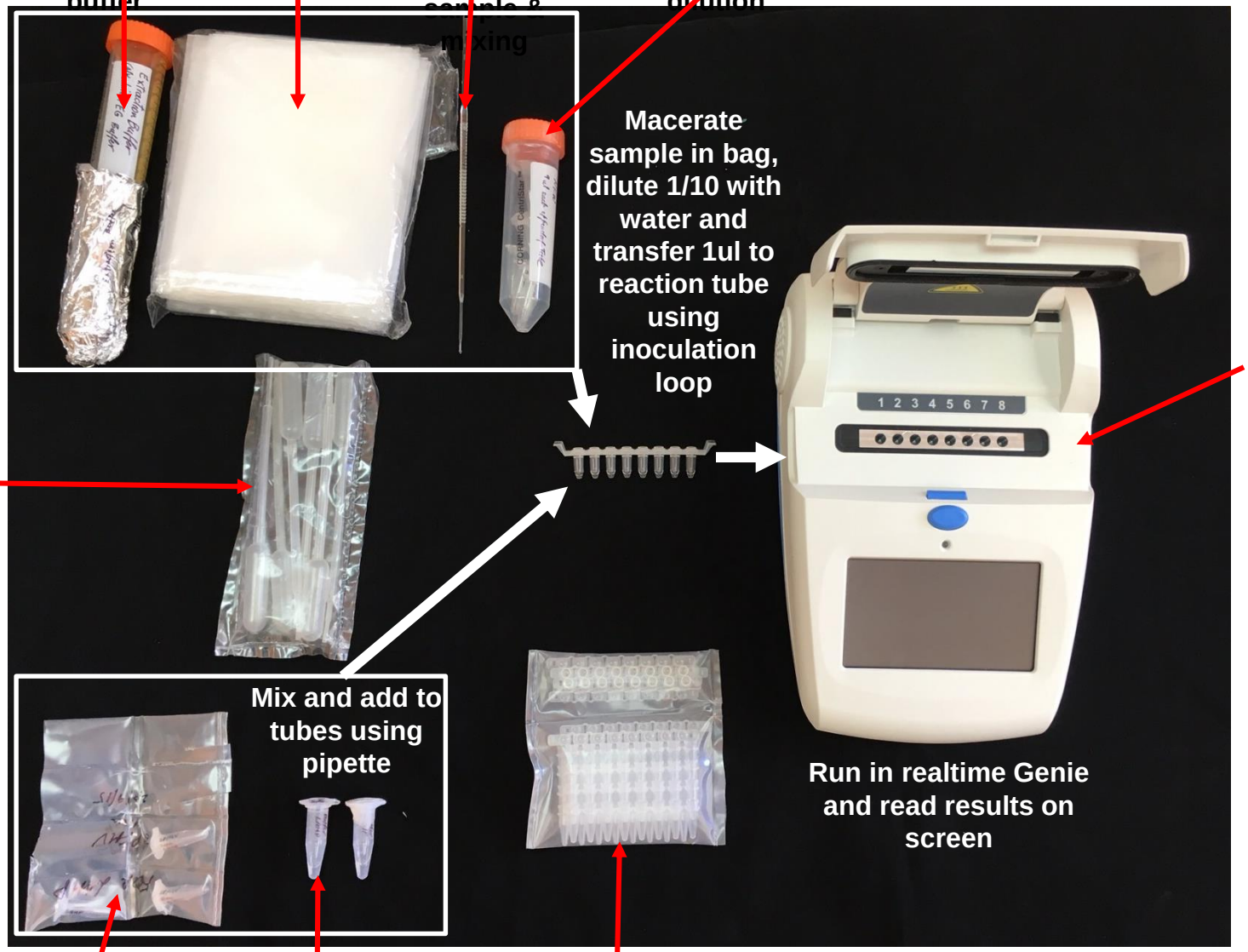
in the field

real-time: Genie

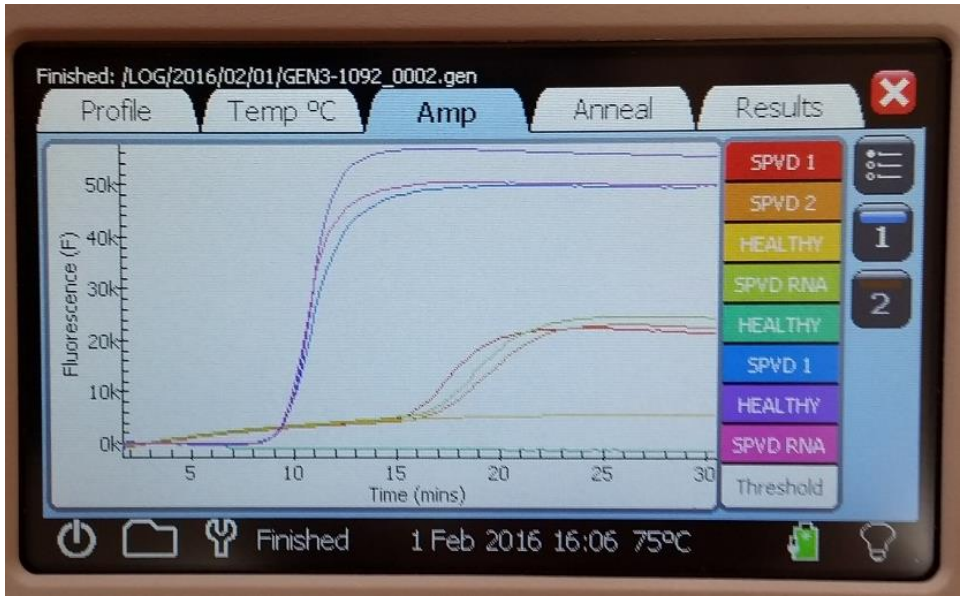


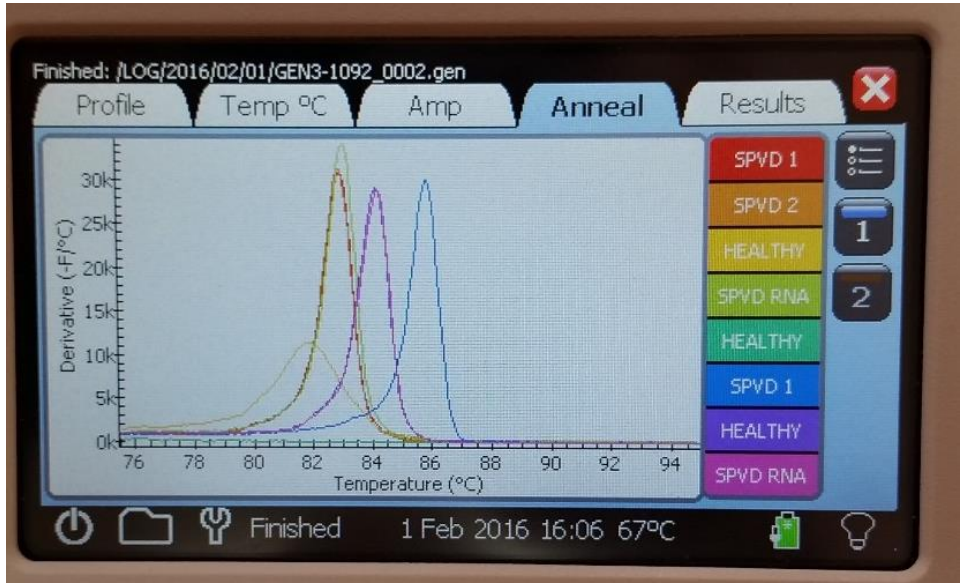
(semi)-quantitative real-time

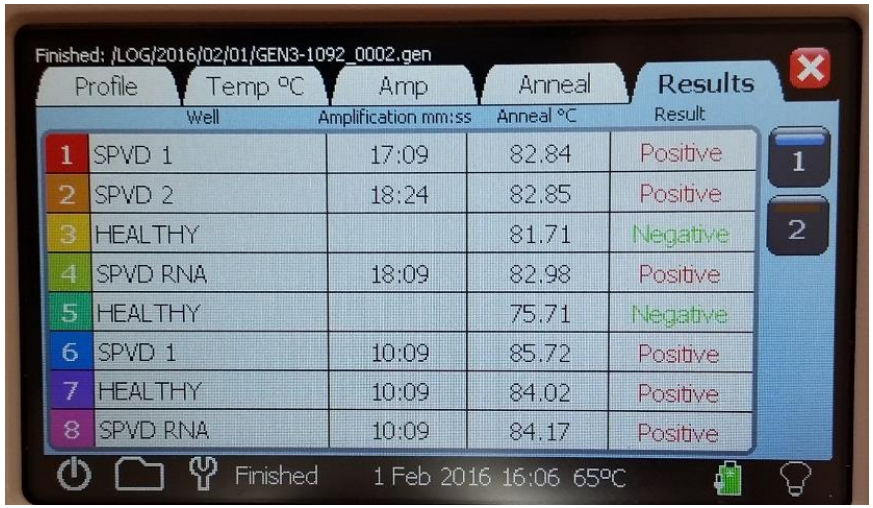
Prototype LAMP field kit



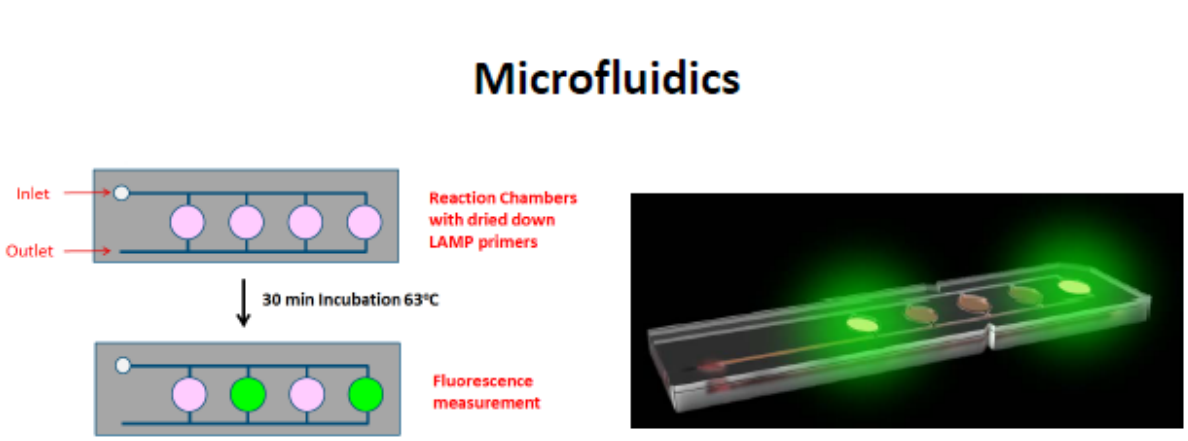
Genie output







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



Microfluidics allow multiple reactions combined with detection *(PRI)*

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

CIP is a member of CGIAR

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