Session 14

Research Presentation

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Mitigation of Hop Latent Viroid (HLVd) in Australia using biotechnology tools

ARC RESEARCH HUB FOR MEDICINAL AGRICULTURE

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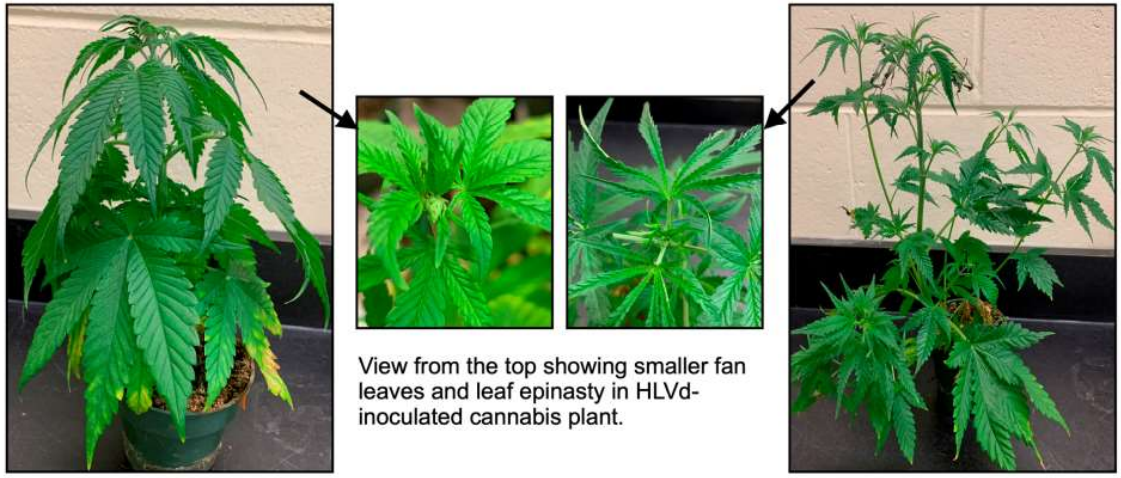
ACannabis - 13 March 2024

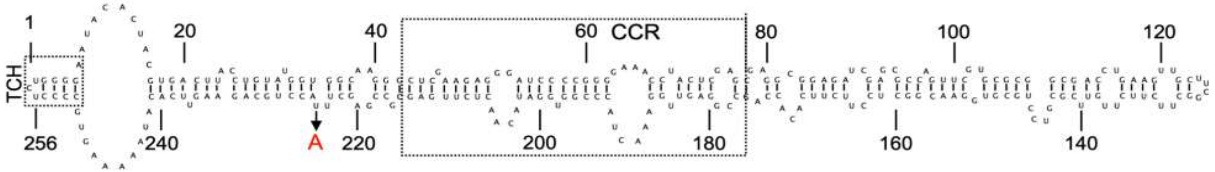
Australian Government
Australian Research Council

LA TROBE UNIVERSITY

Hop Latent Viroid (HLVd)

- 256 nucleotide, noncoding RNA pathogen endemic to hop; cannabis is a non-host
- Symptoms and disease severity are genotype-dependent – “duds” or “dudding disease”
- In 2019, ~90% of Californian cannabis plants tested positive for HLVd
- Potential US$ 4B losses p.a.





https://medicinalgenomics.com/hop-latent-viroid-in-cannabis/

Adkar-Purushothama *et al*, 2023
Viruses 15

HLVd in Cannabis: Problems and Solutions

Problems:

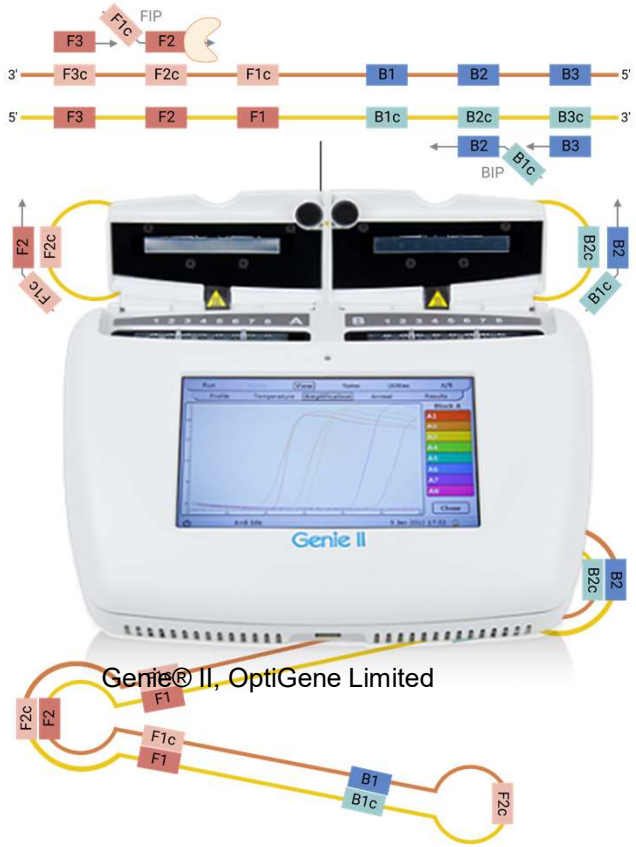
- Infection can affect yield through reduced trichome density, looser flower buds, and up to 50% reduction in terpene and cannabinoid content
- Plants can be asymptotic until very late in their growth cycle
- Resistant cultivars can still be a source of contamination.
- Propagates through tools and machinery or through imported and infected cuttings, flowers, seeds (or even pollen)
- Now has a foothold in Australia

Solutions:

- Prevention through molecular testing and quarantining of imported (and infected) plant material
- Thermotherapy (hot or cold) can reduce the pathogen titre/load
- Immediate destruction of plants with visible symptoms to reduce spread
- Chemical sterilisation of tools (*e.g.* secateurs) between each plant (labour-intensive, harsh on equipment, GMP issues)
- Culturing of plant tissue without vasculature can produce viroid-free propagules. Explants can be tested for viroid presence and be propagated to rid the tissue of pathogens

Loop-mediated isothermal amplification (LAMP)

- Rapid nucleic acid amplification technique
  - Time to result < 20 minutes
- Uses 4-6 primers to target a gene of interest
  - At different regions
- Constant amplification temperature
  - Only requires a heat source to perform
- Tolerates range of samples with minimal preparation
- Simplistic result output
  - Turbidity
  - Intercalating dyes
  - Real-time fluorescent readers



Genie® II, OptiGene Limited

Image created with BioRender

LAMP vs PCR-based assay

	LAMP	qPCR	PCR
Time to result	**≤20 min**	~90 min	~2-3 hrs
Sample prep	**Minimal prep**	Stringent prep	Stringent prep
Field-deployable?	**Yes**	Yes^	No
Real-time results?	**Yes**	Yes^	No
Accuracy	**Very high (≥95%)**	Very high (≥95%)	High (~80%)
Cost	**$**	$$$	$$$

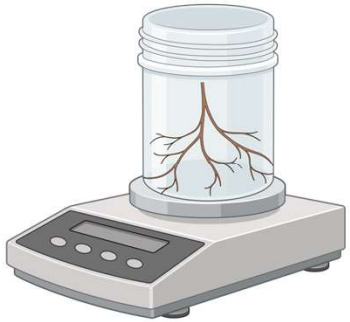
^ Requires specialist equipment

LAMP sample preparation

Example: RNA extraction from *Cannabis sativa* for detection of Hops Latent viroid (HLVd)

Assay developed by: Alexandra Knox (PhD candidate) and Professor Travis Beddoe – Agriculture BioSolutions

1

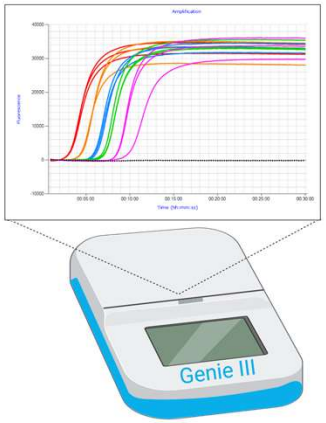


Weigh ~100-300 mg
of wet root

2

Homogenise in
1:100 (w/v) of PBST

3

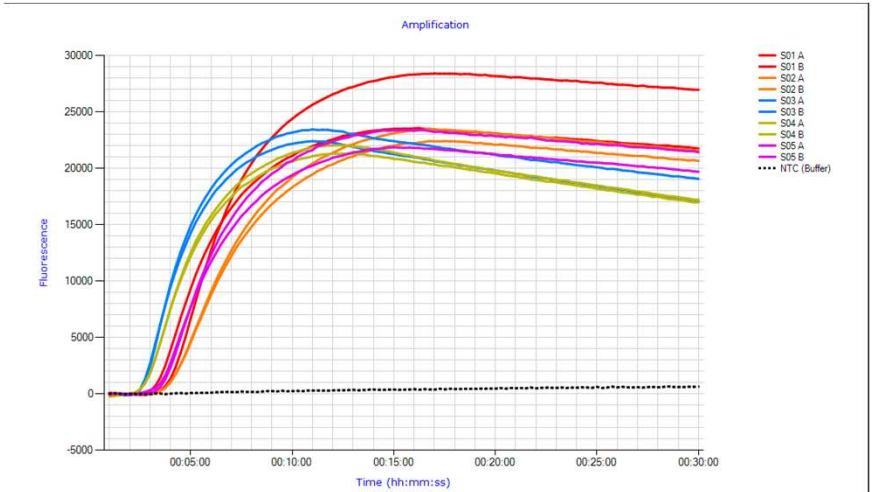


5 µl crude sample
into assay

LAMP Genie II results output

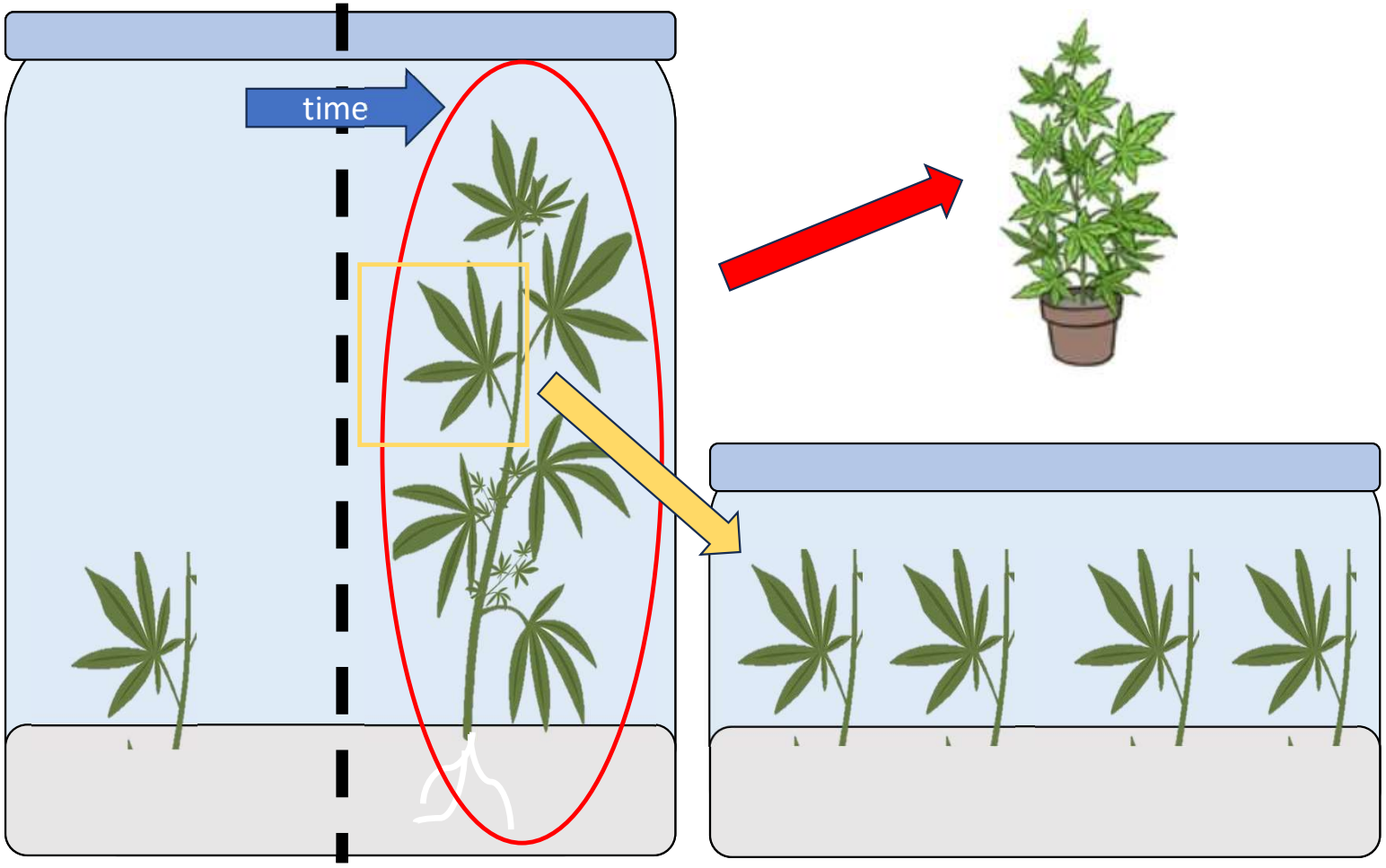
Example: RNA detection from *Cannabis sativa*

RNA extraction reference assay (18S rRNA)



Sample	Tp 1 (mm:ss)	Tp 2 (mm:ss)
S01	04:59	04:21
S02	05:14	05:14
S03	03:36	03:34
S04	03:48	03:51
S05	04:27	04:40

Introduction to Tissue Culture (TC)



Tissue Culture: Pros and Cons

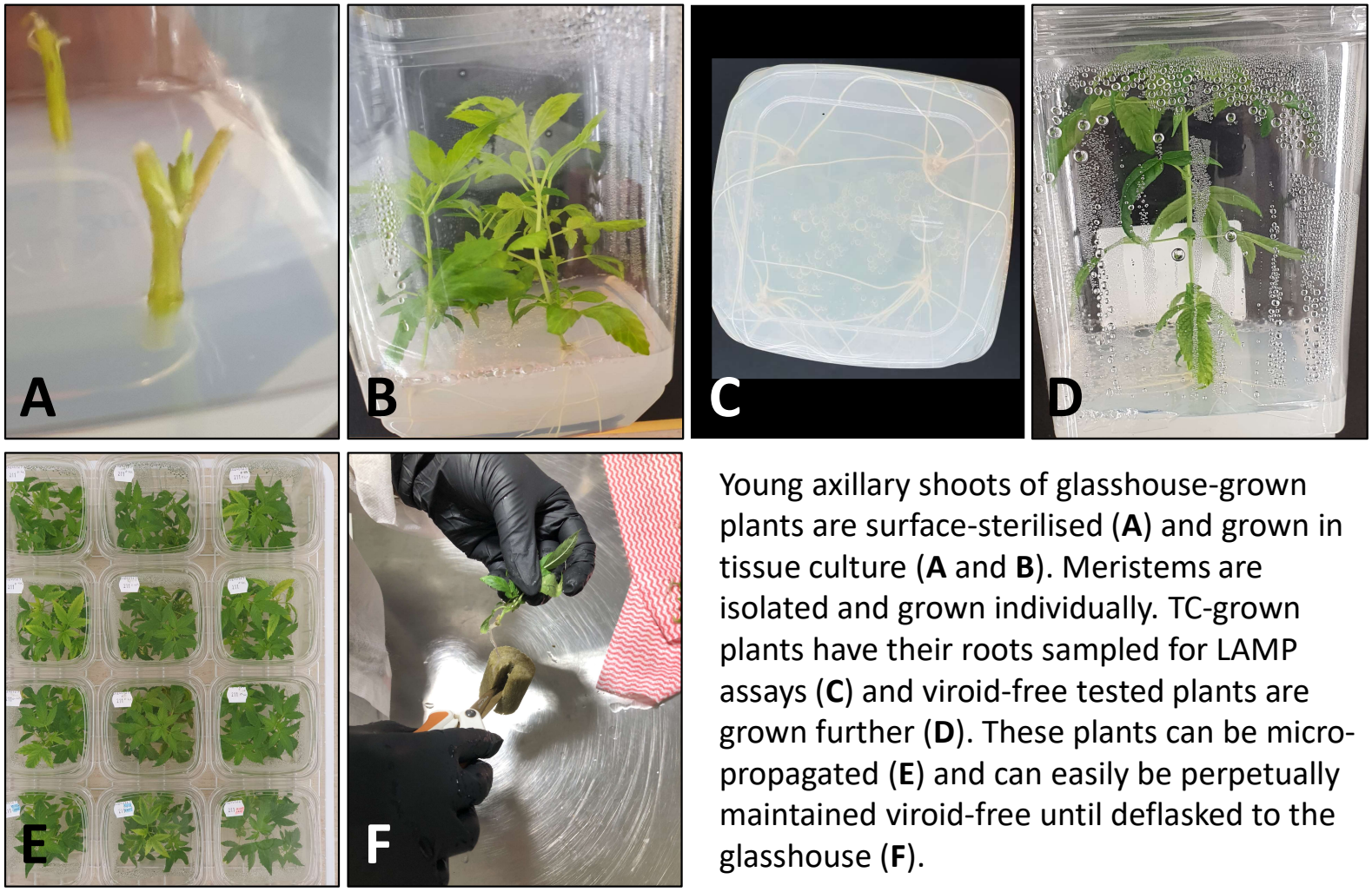
Advantages:

- Perpetual propagation of disease-free plant material
- Can produce large number of plants in a limited amount of space and time
- Safekeeping of genetic diversity
- Low maintenance costs (energy, water, space and labour)
- Cannabis plants grown *in vitro* are true-to-type when compared to traditional propagation method of vegetative propagation (harvest index, cannabinoid content in production plants; number of cuttings produced by mother plants)

Disadvantages:

- Requires a specialised facility and equipment
- Requires trained personnel
- Plant growth in tissue culture conditions seems to be cultivar-dependent

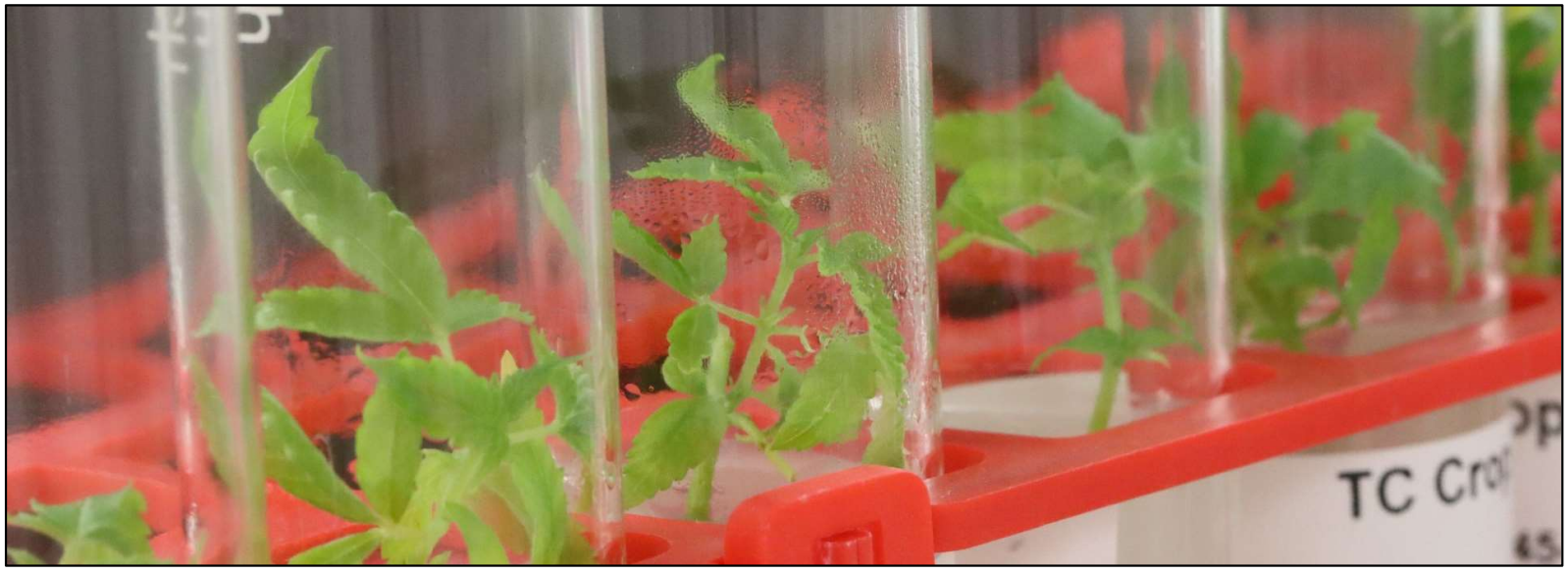
Establishing Viroid-free Crops in TC



Young axillary shoots of glasshouse-grown plants are surface-sterilised (**A**) and grown in tissue culture (**A** and **B**). Meristems are isolated and grown individually. TC-grown plants have their roots sampled for LAMP assays (**C**) and viroid-free tested plants are grown further (**D**). These plants can be micro-propagated (**E**) and can easily be perpetually maintained viroid-free until deflasked to the glasshouse (**F**).

Conclusion and Perspectives

- Tissue culture and LAMP assay detection of HLVd can future-proof the cannabis industry, in combination with multilayer management (a regular testing regime and good hygiene practices)
- Molecular diagnostics tools applicable to cannabis aren't restricted to HLVd - other pathogens can be rapidly detected in a timely and cost-effective manner
  - e.g. Powdery mildew (*Golovinomyces* spp.), *Fusarium* and *Pythium* root and crown rot, etc



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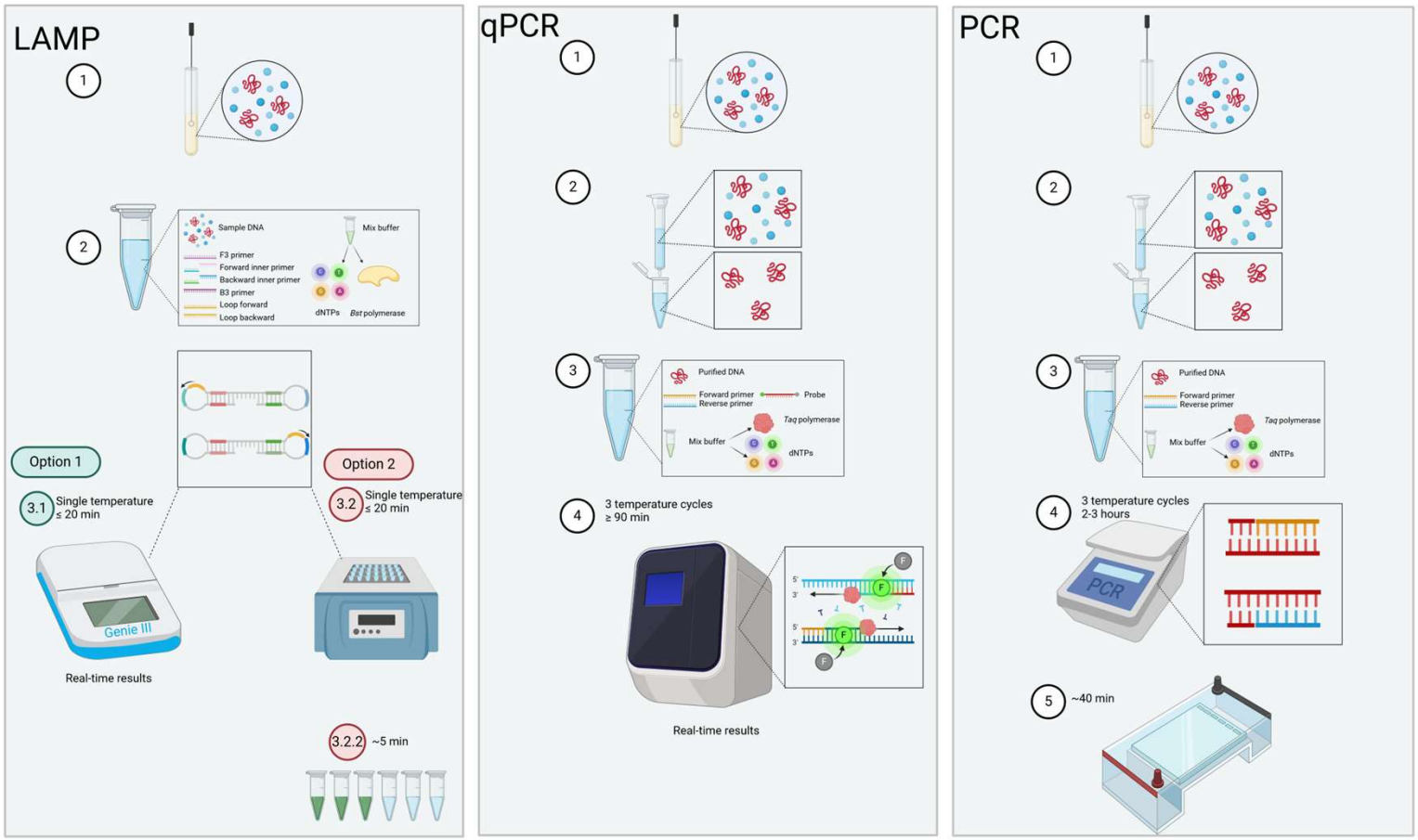
A Xerox Company

LAMP vs PCR-based: Workflow comparison



Knox & Beddoe, 2021 *Animals 11*