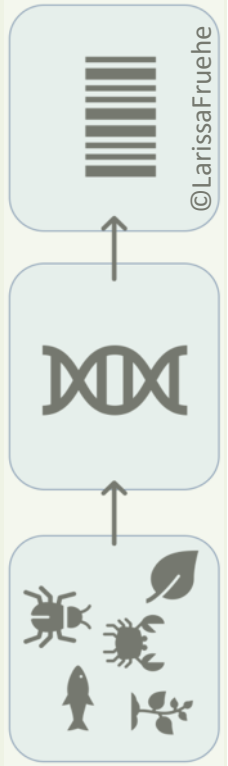

Molecular Diagnostics:
Barcoding and LAMP

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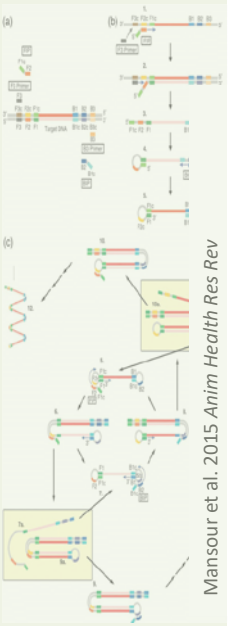
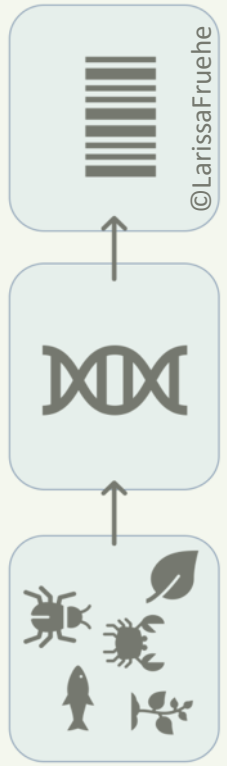
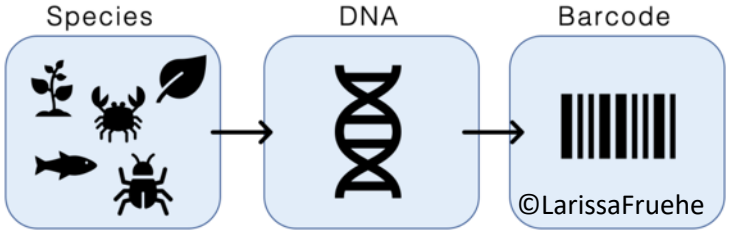

Mansour et al. 2015 *Anim Health Res Rev*

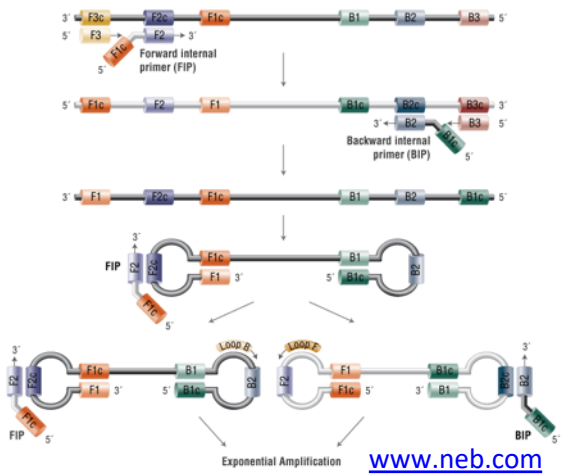
2nd ASEAN Diagnosticians Forum
Bangkok, Thailand
20-21 August 2019



Overview

- Barcoding
  - Definition
  - Pros and Cons
  - Applications
- LAMP
  - Definition
  - Pros and Cons
  - Applications
- Summary





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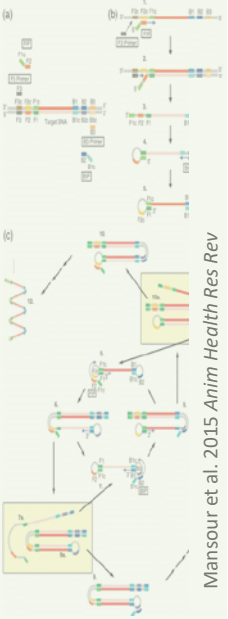
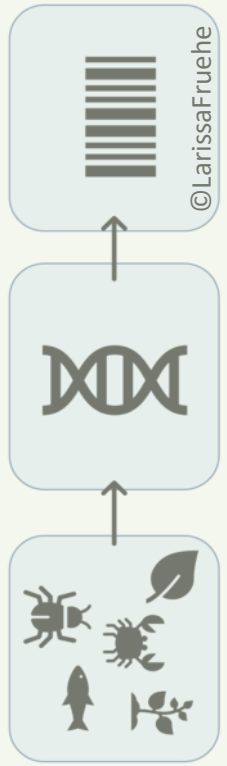
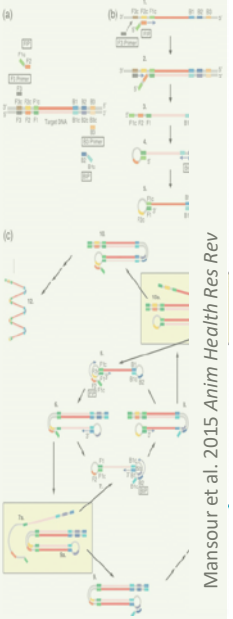


Mansour et al. 2015 *Anim Health Res Rev*



Barcoding – *Definition*

- Identification of species based on short section of DNA from specific gene(s).
  - Comparison with a reference library/database, allow DNA sequence to identify organism to species level.
  - E.g. barcode in supermarket.



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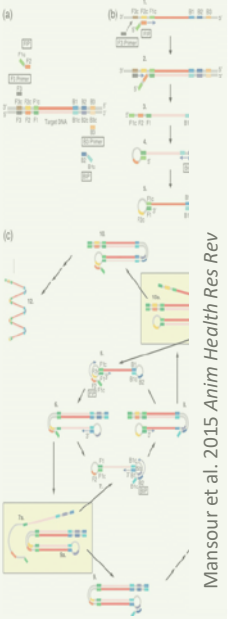
Barcoding – *Pros* (diagnostics)

- Identify unknown organism faster and more accurately than traditional morphology/biology based taxonomy.
  - Greater taxonomic resolution (identification to species level)
  - E.g. plants can be identified in absence of flower or fruit;
  - E.g. fungi can be identified in absence of or where morphological characters overlaps.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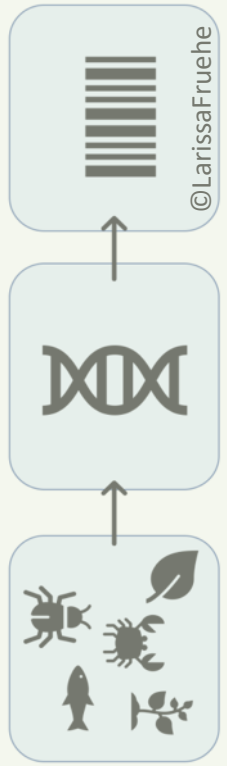
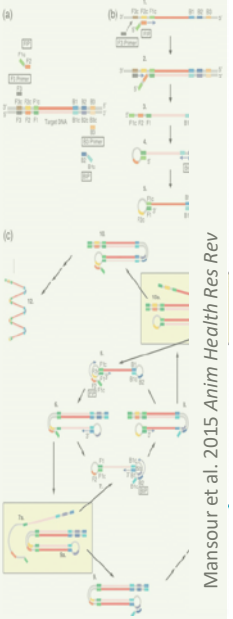


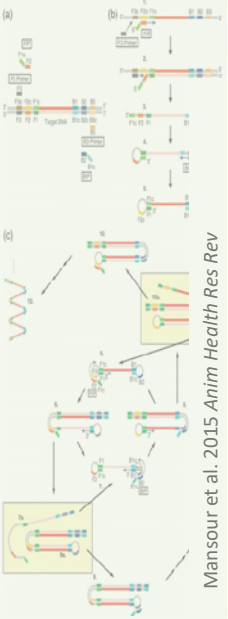
Barcoding – *Pros* (diagnostics)

- Lower cost and faster than traditional taxonomic training
  - Molecular biology and bioinformatics skills are applicable across all living organisms.

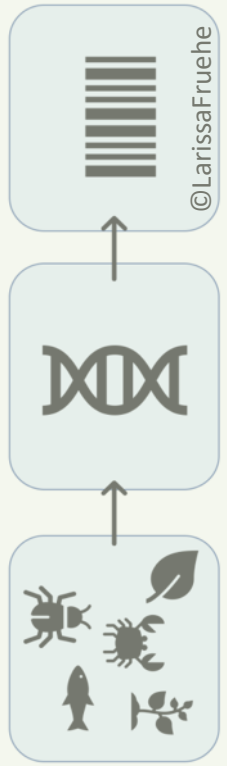

Mansour et al. 2015 *Anim Health Res Rev*

Barcoding – *Cons* (diagnostics)

- Lack of complete / reliable barcode reference libraries
  - GenBank®: NIH genetic sequence database
    - Publicly available
    - Shares data with DDBJ (Japan) and ENA (EU)
    - All genetic information: DNA and RNA.
    - Lack of metadata, sequencing error.
  - Barcode of Life Data Systems (BOLD)
    - Acquire, store, analyse, publish DNA barcode records.
    - Combines molecular, morphology, distribution data.
    - Publicly available; includes data not published in GenBank.
    - Mainly animals/insects focussed.

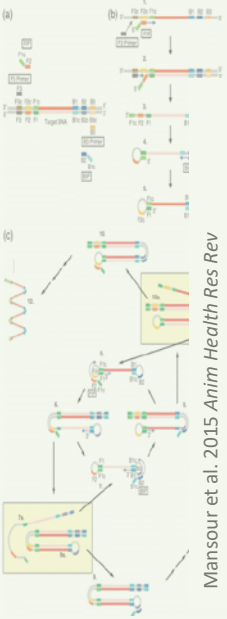


Mansour et al. 2015 *Anim Health Res Rev*



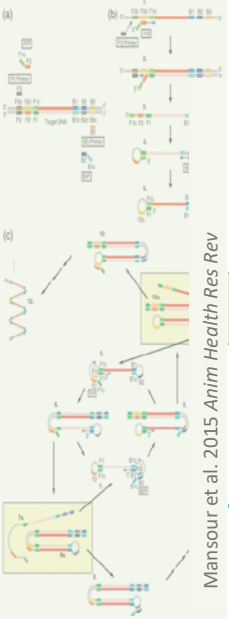
Barcoding – *Cons* (diagnostics)

- Mismatch/mistake between morphological and barcode based identification.
  - Species not present in reference databases will not be identified.
  - DNA sequences linked to incorrect name will lead to incorrect identification → perpetuate incorrect identification.
- Detection of 'species' by barcode does not mean living organism is present.
  - E.g. soil with *Phytophthora* barcode vs. lupin baiting


Mansour et al. 2015 *Anim Health Res Rev*

Barcoding – *Diagnostic Applications*

- Current and increased use in diagnostic protocols for regulated pests.
  - Diagnostic protocols; e.g. IPPC, NAPPO, EPPO.
  - EPPO PM7/129(1) DNA barcoding as an identification tool for a number of regulated pests.
    - Reference database: https://qbank.eppo.int/
  - IDphy: molecular and morphological identification of *Phytophthora* based on the types.
    - http://idtools.org/id/phytophthora/index.php
    - Identification Technology Program, part of USDA APHIS PPQ division.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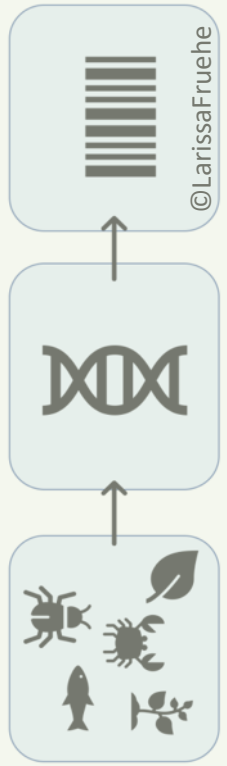
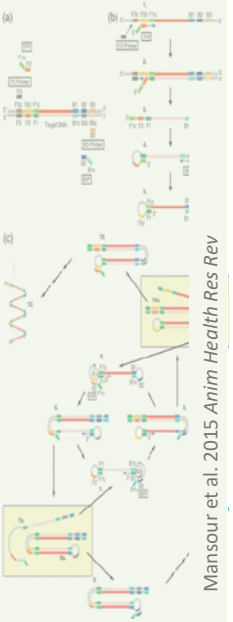
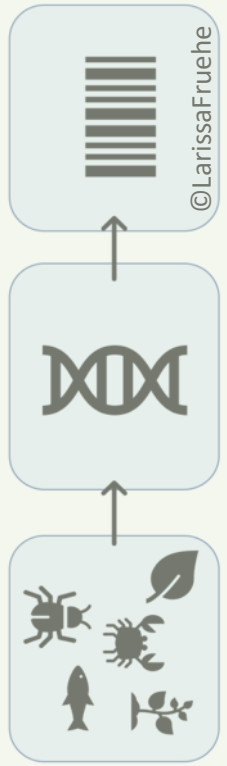


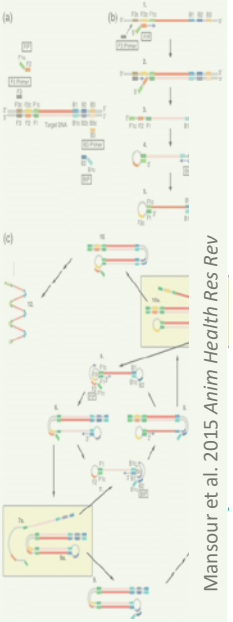
LAMP – *Definition*

- Loop-mediated <u>isothermal amplification</u>
  - Amplification of target DNA sequence at a constant temperature.

- Notomi et al. 2000 *Nucleic Acids Research* 28(12) e63.

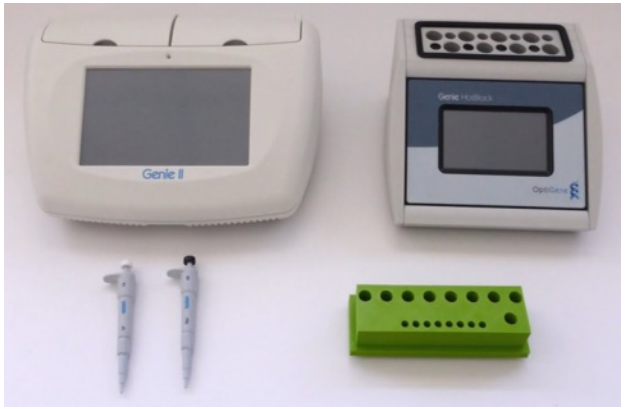


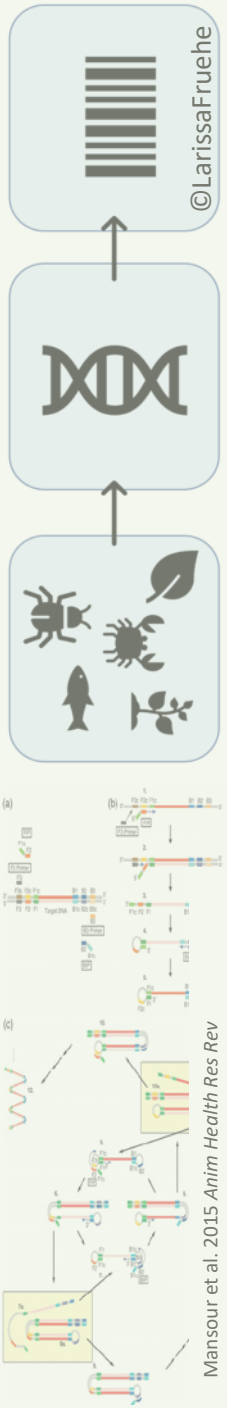




LAMP – *Pros* (diagnostics)

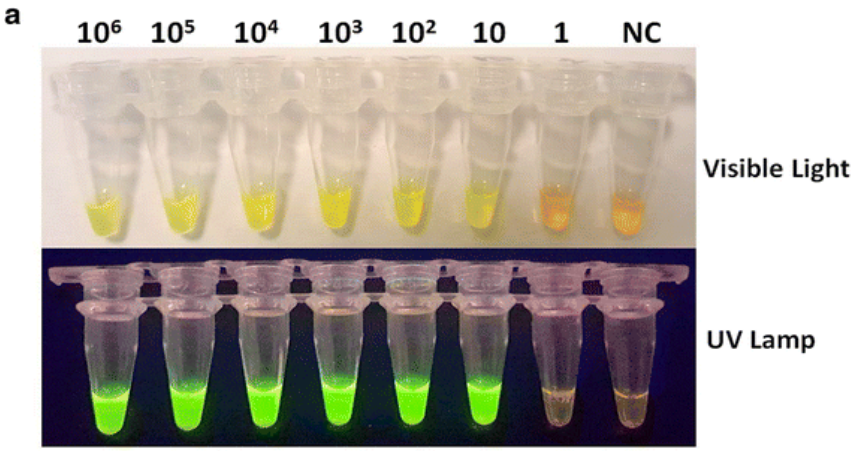
- Cheaper and more robust equipment and reagents than conventional and real-time PCR.
  - Commercial kits are fast (30-60 mins) and easy to use (any skill level)
  - Potential for on-site / field applications.





LAMP – *Pros* (diagnostics)

- Use of multiple primers that targets multiple distinct regions of DNA increases specificity.
- Results can be visualised by eye.

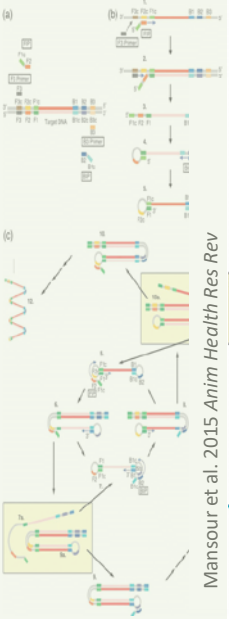


Bentaleb et al. 2016 *BMC* 16:517

Mansour et al. 2015 *Anim Health Res Rev*

LAMP – *Cons* (diagnostics)

- Specific diagnostic application.
  - Unsuitable for unknown organism.
- Test design limitations (less versatile than PCR).
  - Primer design constraints.
    - Difficult to design by “eye.”
    - Use of 4 or 6 primers limits choice in target site.
  - DNA sequence to target.
    - Conserved region vs highly variable
    - Cross-reactions in genetically close species.
    - Degenerate primers may cause non-specific amplification.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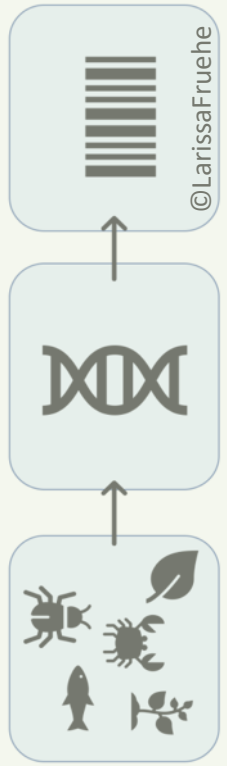
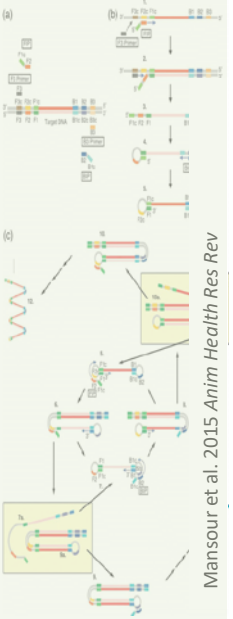


LAMP – *Cons* (diagnostics)

- Changing taxonomy?
  - E.g. Can *Phyllosticta citricarpa* LAMP test distinguish *P. paracitricarpa*.
- Emerging / evolving pathogens?
  - E.g. LAMP tests for *Xylella fastidiosa* subsp. *morus* and *sandyi*, but what about subsp. *fastidiosa*, *multiplex*, *pauca*?
- 'Positive' detection does not mean presence of living organism.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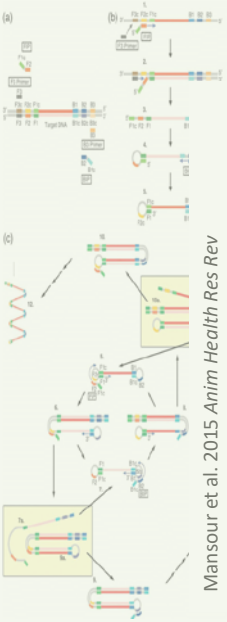
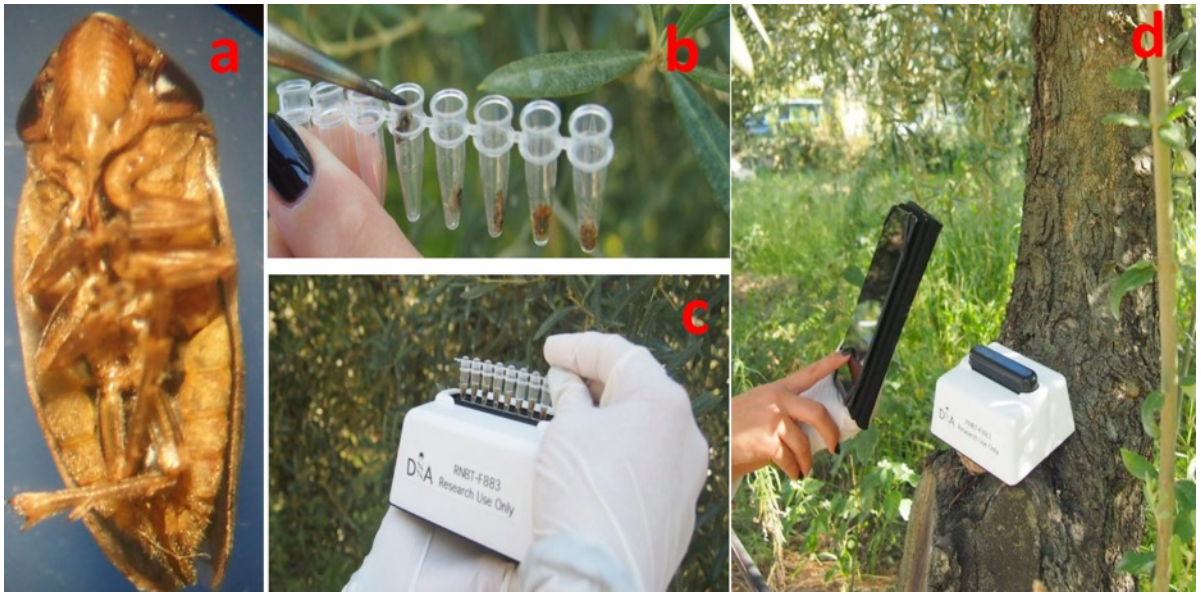
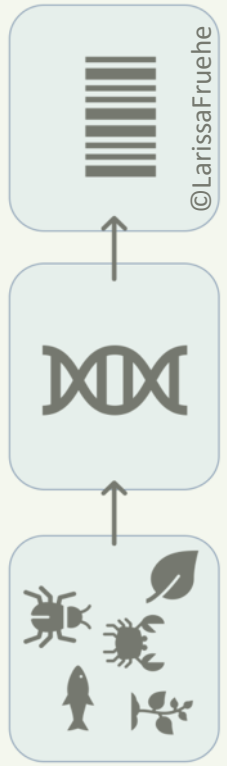
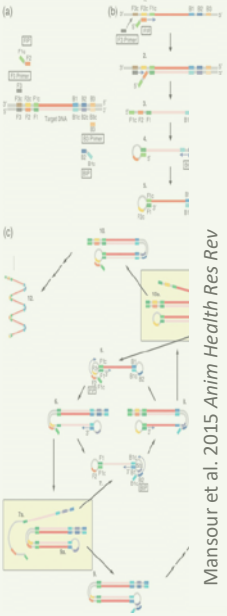


LAMP – *Diagnostic Applications*

- Yaseen et al. 2015: On-site detection of *X. fastidiosa* in host plants and vectors.
  - In-field testing of plants and vectors for *X. fastidiosa*.
  - Compared with conventional PCR and ELISA.

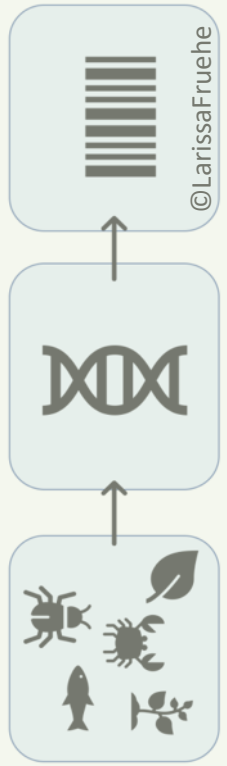






LAMP – *Diagnostic Applications*

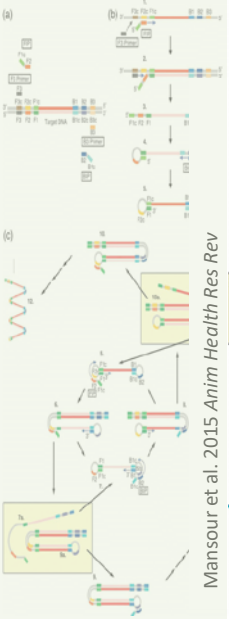
- Blaser et al. 2019: from lab to point of entry – LAMP-based test to detect quarantine insect species
  - Targeting *Bemisia tabaci*, *Thrips palmi*, and *Bactrocera* (*B. dorsalis* complex) and *Zeugodacus* at the Swiss borders.
  - Tests developed, tested and validated under laboratory conditions → transferred to plant health inspectors with minimal training at Zurich Airport.
  - LAMP results cross-validated by DNA barcoding.
  - DNA extract from negative result sent to lab for barcoding (enables update of LAMP assay).



Summary – Barcoding & LAMP

- Tools for different applications.
  - Barcoding = general/routine identifications
  - LAMP = surveillance

- Complementary tools.
  - LAMP tests up-to-date with barcoding.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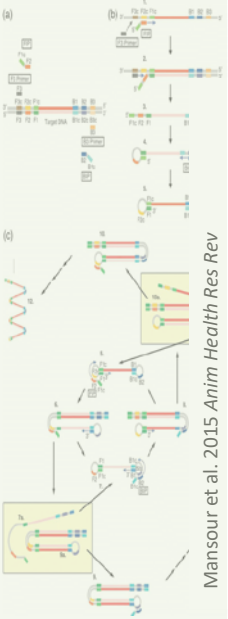

Acknowledgements

- AANZFTA Economic Cooperation Work Programme
- Delegates
- Hosts

Questions