



# **DNAble™ Field Test Kit for Rapid Detection of *Clavibacter michiganensis* subsp. *michiganensis* in Tomato Tissue**

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# Features of *DNABLE™ QuickStick* Technology



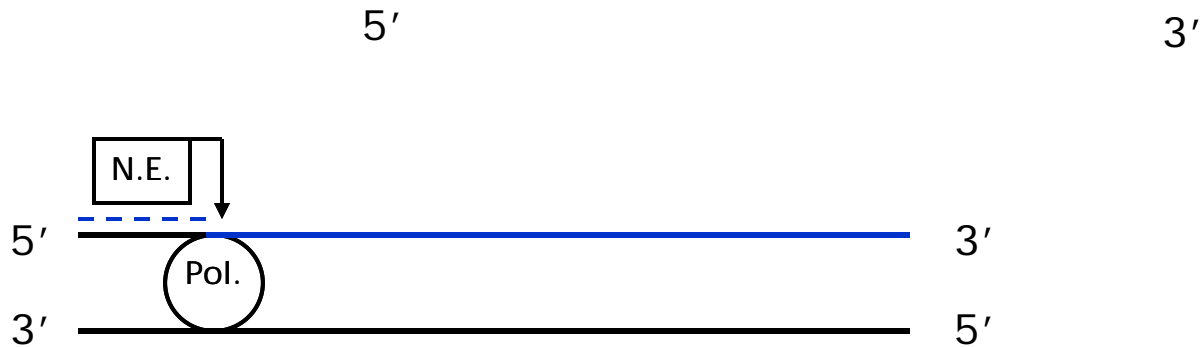
- ❑ Easy to use
- ❑ Reagents are stable (20-25°C)
- ❑ Simple field adaptable sample preparation
- ❑ Rapid qualitative results (< 20 minutes)
- ❑ Nucleic acid amplification platform
  - ❑ NEAR Technology
  - ❑ Lateral Flow Detection
  - ❑ Specificity down to a single nucleotide base
  - ❑ Sensitive detection of DNA from a crude matrix (no purification needed)
- ❑ Complete validated kits



# NEAR Technology



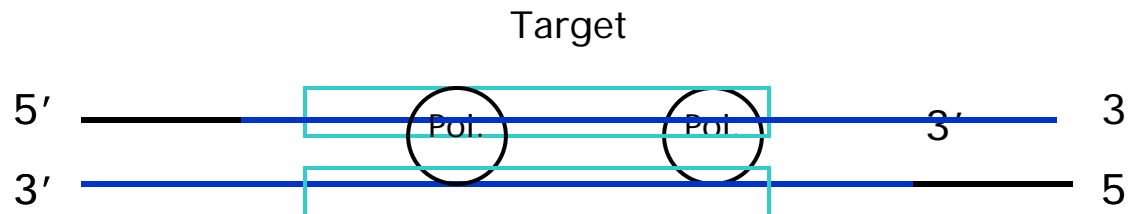
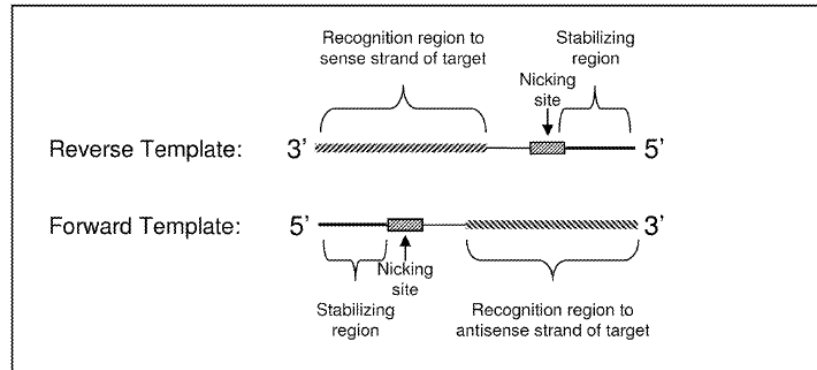
## Genome Fragmentation



# NEAR Technology



## Primer Anneal and Extension



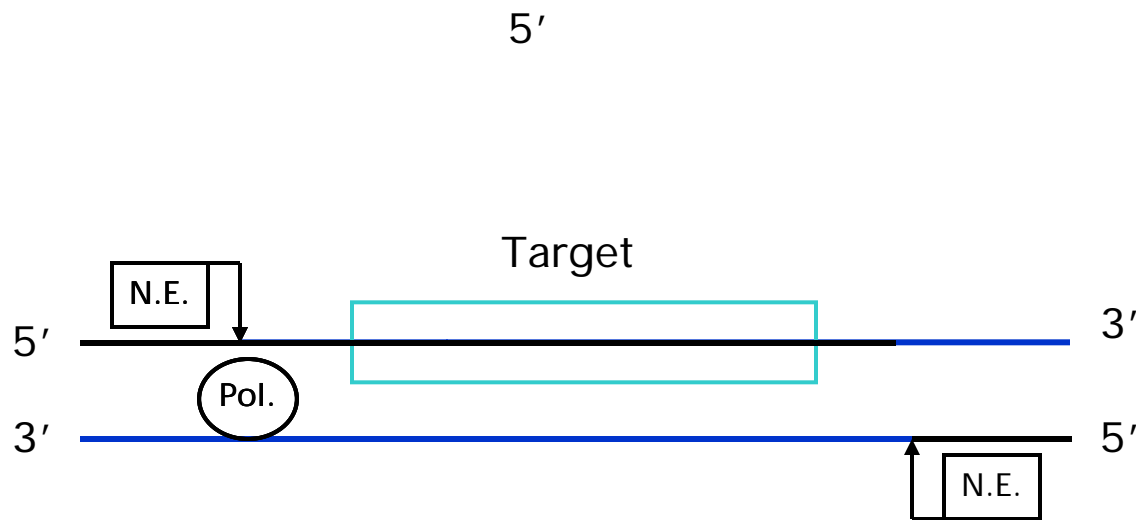
Complement

**Amplification Duplex**

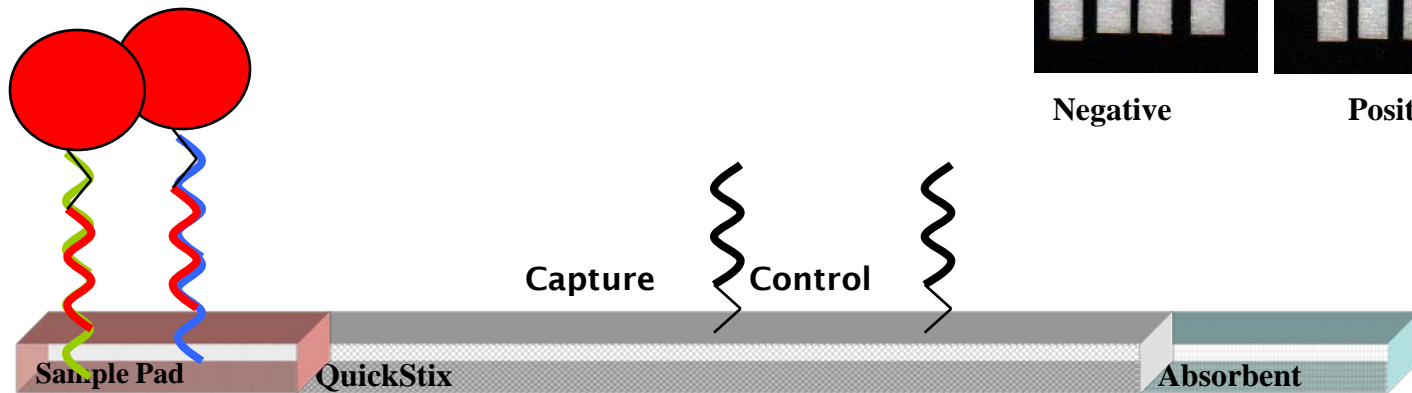
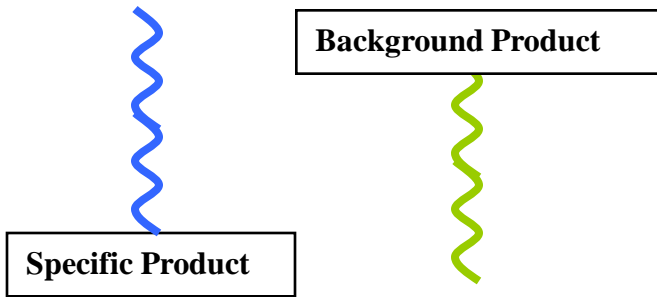
# NEAR Technology



## Rapid Amplification of Duplex



# Lateral Flow (DNA)



# Crude Sample Matrices



**Sample direct from Leaf Tissue or Stem**

***No DNA purification requirement***





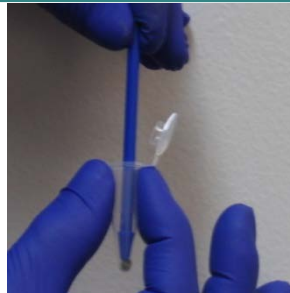
## Tomato Tissue Analysis Sample Procedure



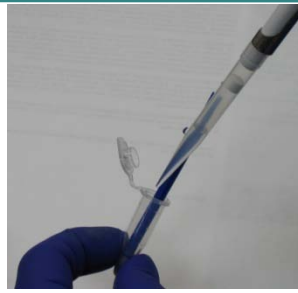
Sample plant tissue



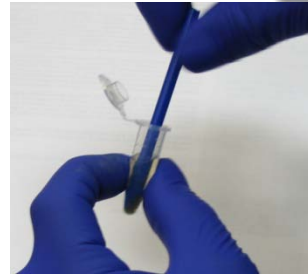
Eject sample into extraction tube



Macerate with pestle



Add 0.5mL extraction buffer



Macerate with pestle



Using the tube and cap interface of a 0.5mL micro-centrifuge tube to collect stem or leaf punch.



Transfer to a 1.5 mL micro-centrifuge tube and grind for 20 sec.



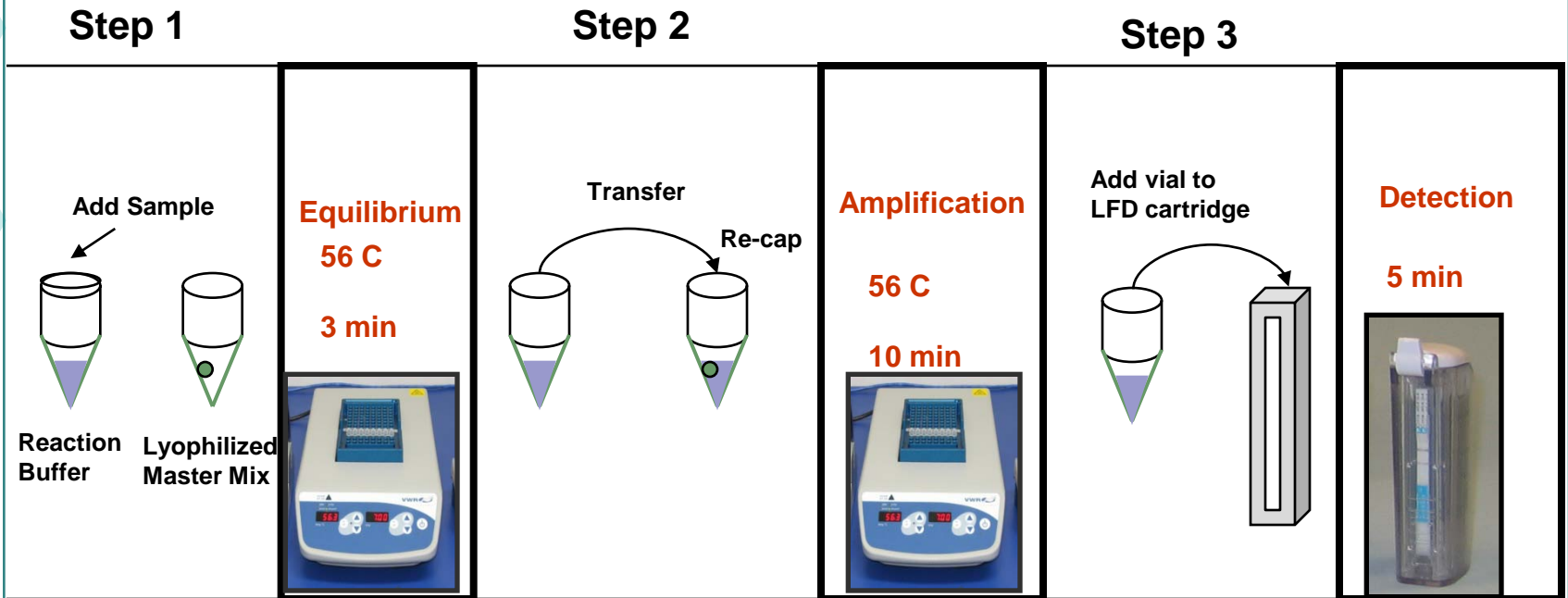
Add 1mL extraction buffer and grind for 20 sec.

Assay 10 uL

*Demonstrated results in Laboratory Infected Samples and Naturally occurring Greenhouse Infected Samples*



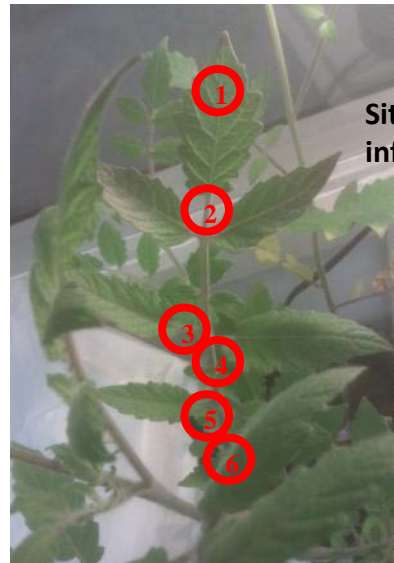
# Assay Procedure



- Heat block
- Strip in closed cartridge; visual detection
- Traditional test and control lines on strip within 2 minutes

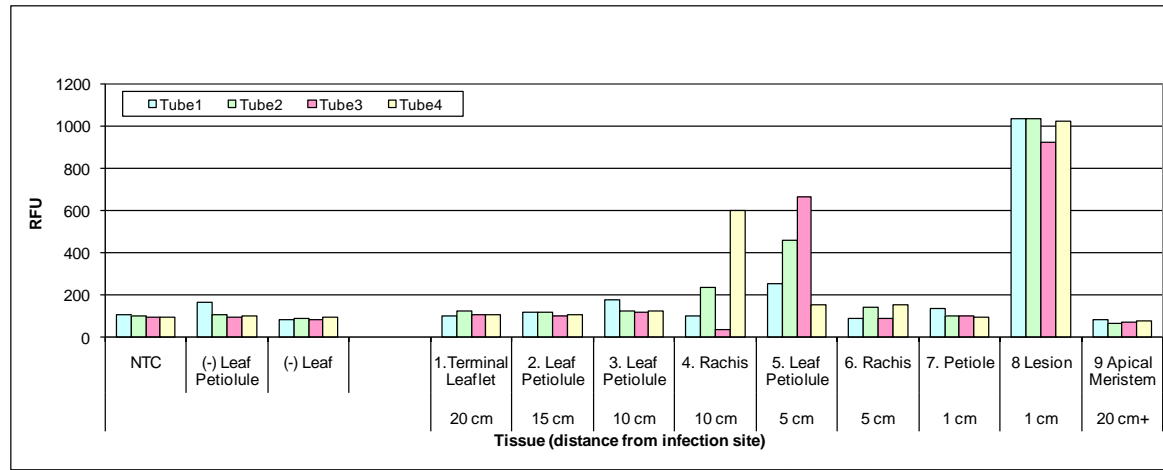
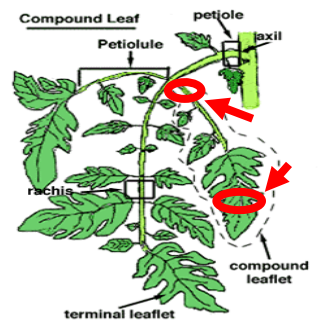


# Laboratory Infected Tomato Tissue Testing



- The plant was inoculated by injection of *Cmm* into the stem
- The plant became weakly symptomatic 16 days post infection
- Test results on this day were positive at a lesion (1 cm), Leaf Petiole (5 cm) and Rachis (10 cm) as shown below

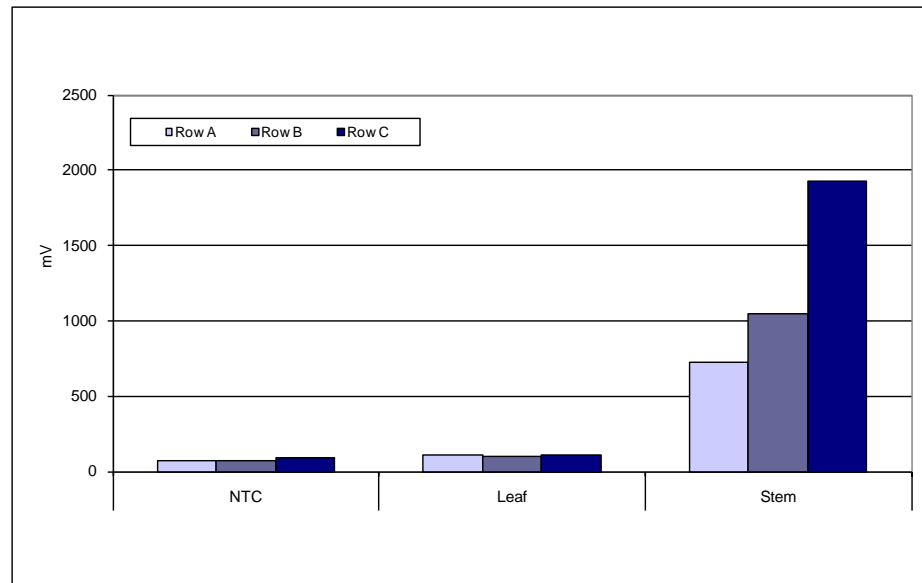
## Primary sampling locations



# Greenhouse Infected Tomato Tissue Testing



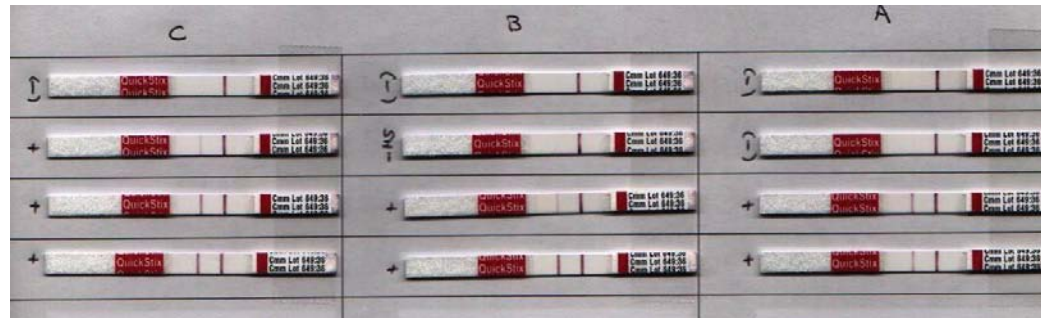
- Mature plant showing symptoms of *Cmm* infection was harvested
- Analysis of samples from a leaf (using leaf procedure) and from a lesion containing stem (using stem procedure) was conducted
- **Test result of the stem was positive.**



# Cmm Assay- Sensitivity Analysis



- Sensitivity analysis achieved by a titration of bacterial suspensions in a tomato tissue matrix
- Able to detect consistently at a concentration of  $1 \times 10^5$  CFU/ml in 1ml of crude sample matrix
- Testing 10ul of crude sample results in consistent detection of 1000 CFU/rxn

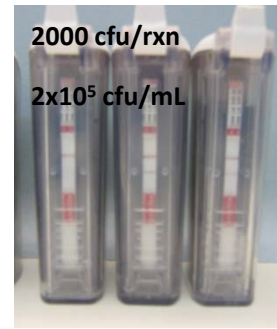
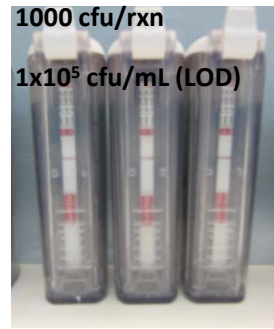
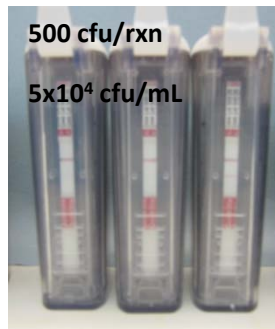
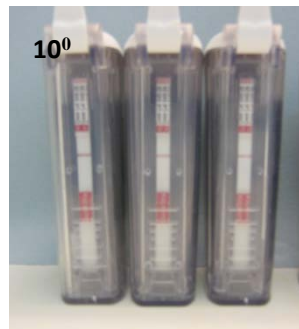


$10^0$  – Negative control

500 cfu/rxn ( $5 \times 10^4$  cfu/ml)

1000 cfu/rxn ( $1 \times 10^5$  cfu/ml)

2000 cfu/rxn ( $2 \times 10^5$  cfu/ml)





# Cmm Assay- Specificity Analysis



<b>Genetically diverse panel of <i>Clavibacter michiganensis</i> subsp. <i>michiganensis</i> with international origin</b>	<b># Isolates</b>	<b># Positive Detection</b>	<b># Negative Detection</b>
	94	94	0

Sample	Result
<i>Pseudomonas corrugata</i>	Negative
<i>Pectobacterium carotovorum</i> subs. <i>Carotaovorum</i>	Negative
<i>Xanthomonas vesicatoria</i>	Negative
<i>Xanthomonas vesicatoria</i> strain SV 25	Negative
<i>Xanthomonas axonopodis</i>	Negative
<i>Microbacterium paraxydans</i>	Negative
<i>Fusarium oxysporum</i>	Negative
<i>Verticillium dahliae</i>	Negative
<i>Ocrobacterum grignonense</i>	Negative
<i>Ocrobacterum tritici</i>	Negative

Results show specific detection of *Cmm* isolates



\*Representative data of out-group panel

## Summary



- DNABLE™ Field Test Kit for Detection of *Clavibacter michiganensis* subsp. *michiganensis* in Tomato Tissue
  - Amplification direct from a crude sample with no purification
  - Exceptional Speed
  - Portability
  - Superior diagnostic tools for the industry
  - Utilization of a new technology
  - Give **same-day results, on-site**
  
- Currently being evaluated in a field setting to verify the product performance
  
- Following this verification of product specifications, product to be release for validation with our collaborating Life Science Companies







*Robust assay system allowing for PCR quality results  
direct from the crude sample matrix and in a non-  
laboratory environment*

*For more information regarding DNABLE™ Products*

*Contact:*

*[dnable@envirologix.com](mailto:dnable@envirologix.com)*