DEVELOPING DIAGNOSTICS FOR CROWN GALL DETECTION

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CROWN GALL

Crimson Cabernet
Dean Volenberg

Disease

Prevention

Diagnostics

Disease

- Tumor
- Injured tissues
- Blocks nutrients
- Vine death
- Large economic impact

CROWN GALL

Crimson Cabernet
Dean Volenberg

Disease

Prevention

Diagnostics

Prevention

- Purchasing crown gall-free stock
- Testing the vines before planting
- Treating the vines before planting

Prevention

Selection of new sites

- Previously planted with grapevines?
- Distance from wild grapevines?
- Tested for Crown Gall causing bacteria?

CROWN GALL

Crimson Cabernet
Dean Volenberg

Disease

Prevention

Diagnostics

⊕ Diagnostics

Key to Prevention

A reliable, efficient diagnostic method is necessary

How to test and for what?

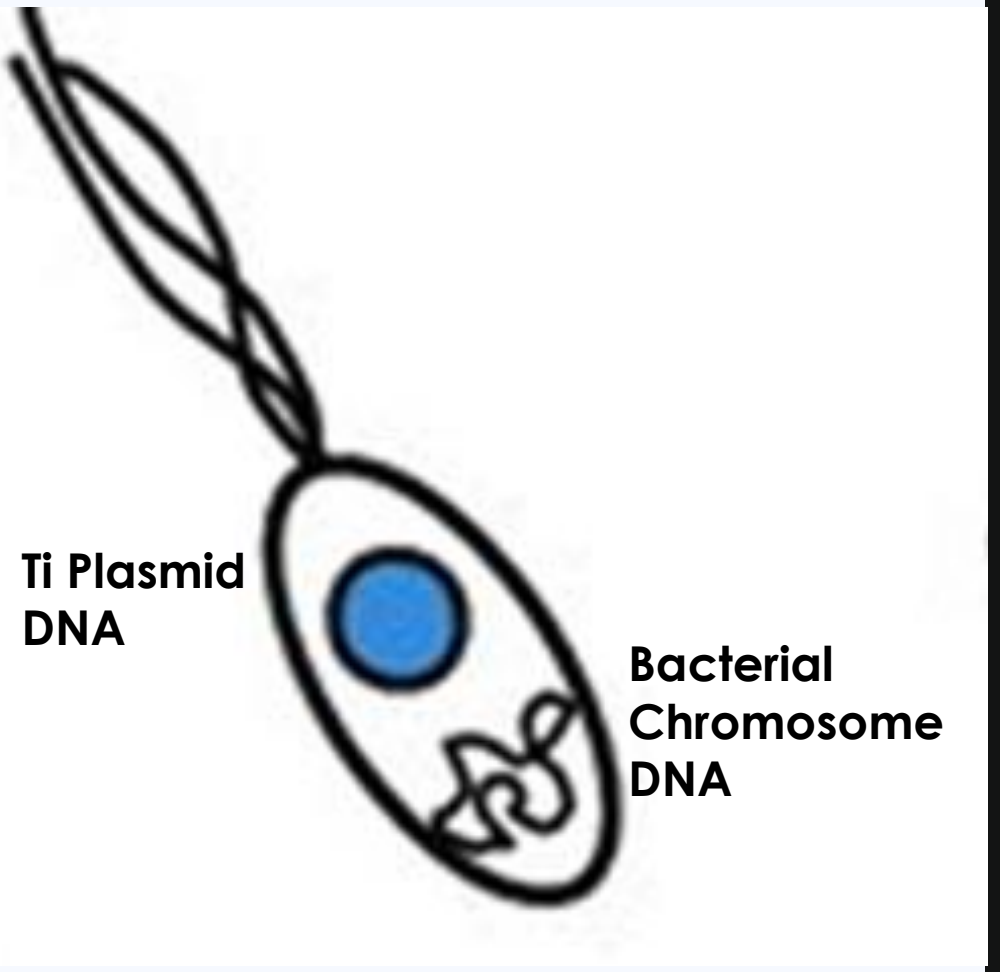
Purify DNA—canes, soil

Run PCR

CAUSAL ORGANISM

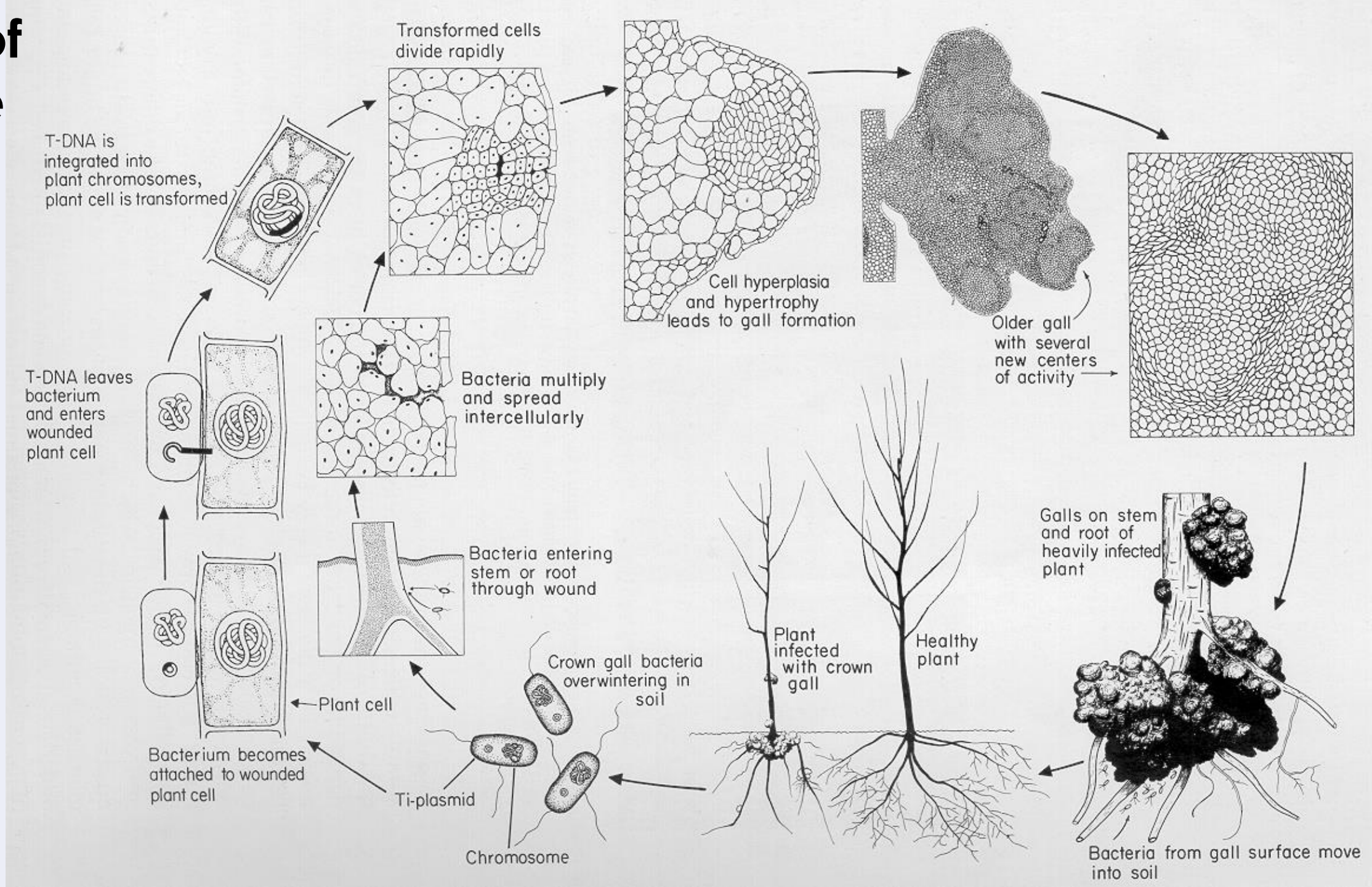
- *Allorhizobum vitis* formerly known as *Agrobacterium vitis*
- Not all *All. vitis* are tumorigenic
- Tumorigenic because of Ti (Tumor Inducing) plasmid

Allorhizobum vitis



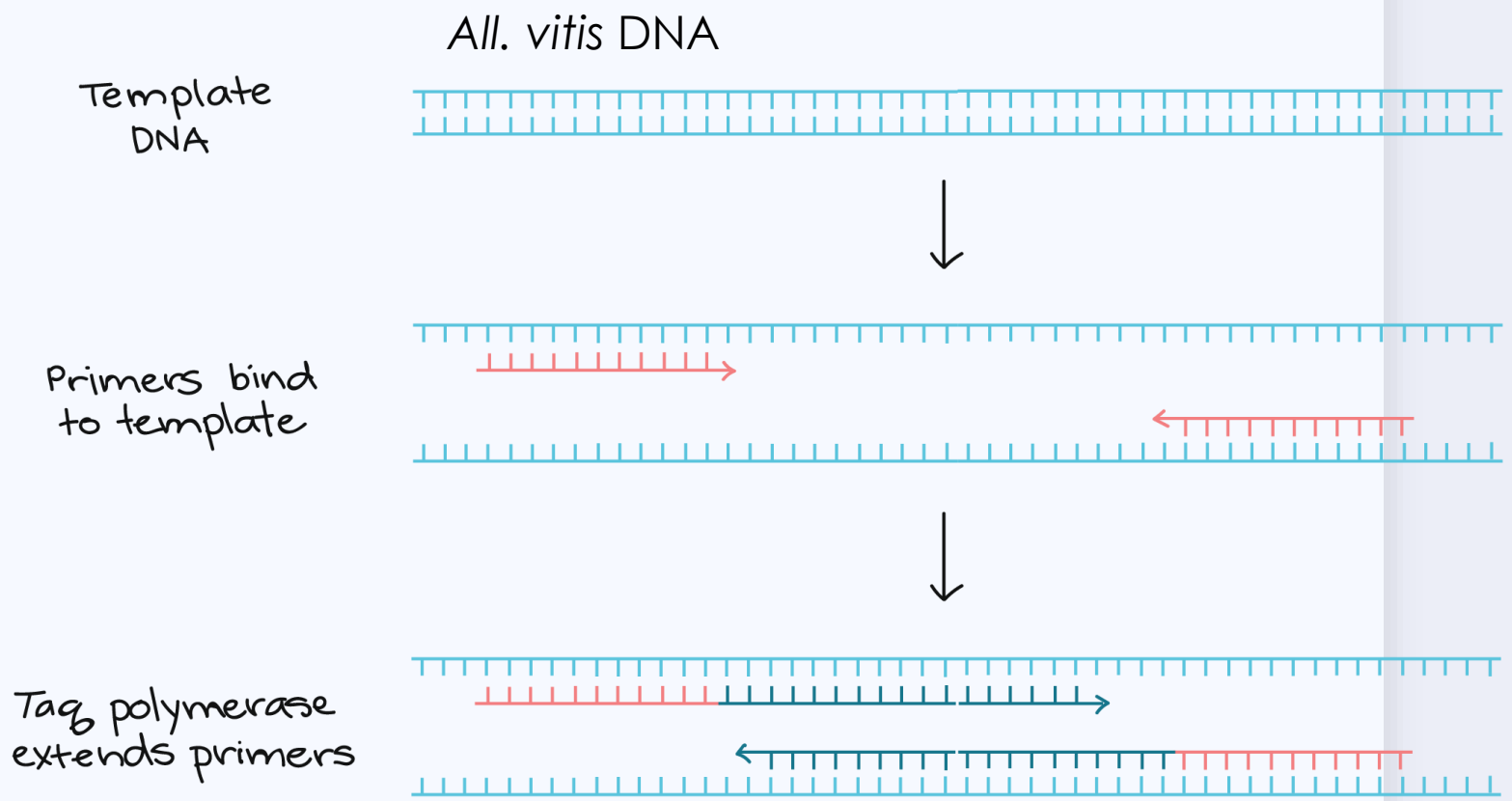
Cycle of Disease

Agrios, 1997



PCR

- Must know some of nucleotide sequence you are looking for
- Primers find that sequence
- Exponential amplification
- Visualized on a gel



Primers Tested for PCR Detection

Ti plasmid

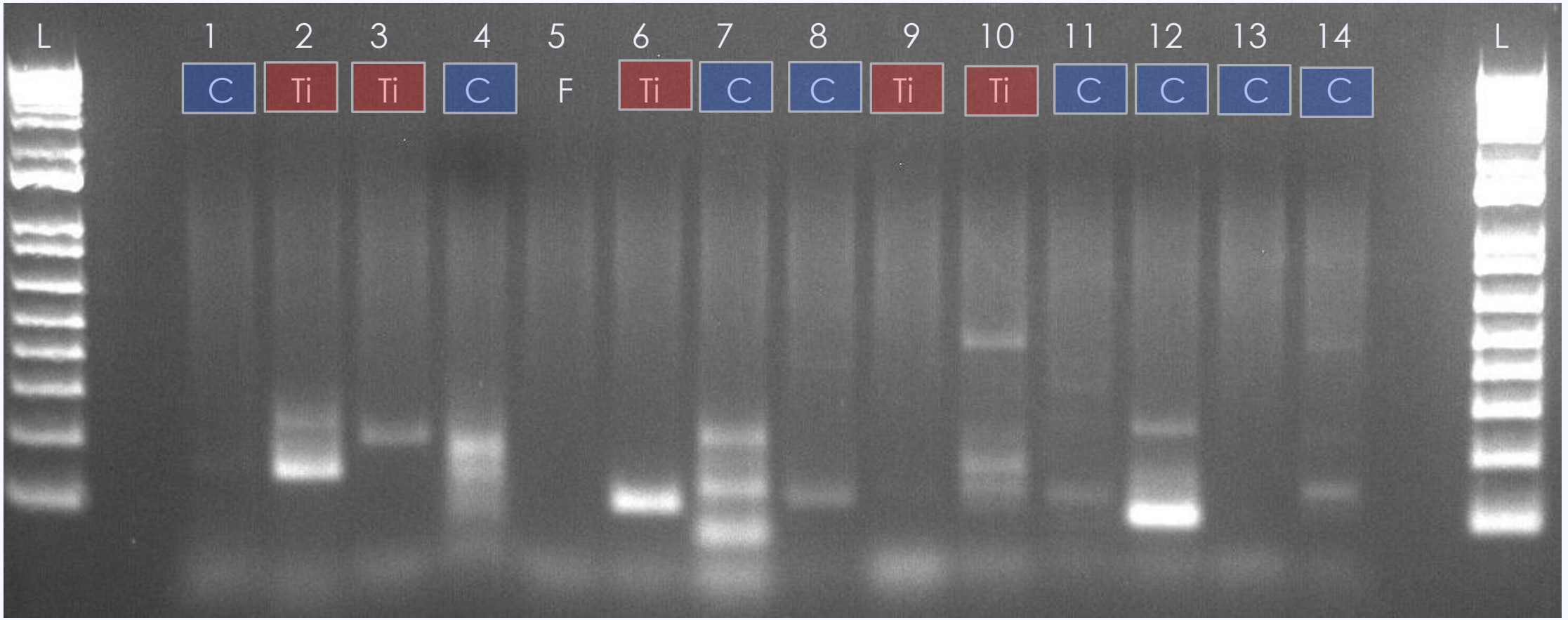
Chromosome

Primer Name	Sequence 5' to 3'	Target	Fragment Length	Use
virD59 F	ATTGGAATATCTGTCCCG	virD2 virulence gene Vitopine	96	pTi Detection
virD59 R	GGCGAGATCGCGGATATT			
virD3cons F	AATCCGGAGGTGATGGT	virD3 virulence gene	149	pTi Detection
virD3cons R	GGCGTCATGTAAGCGTTG			
Avi_1889 F labeled R	TTCTGCATCGACCCGGAAAT	avsI, acyl homoserine lactone synthase	156	Chromosomal Detection
Avi_1889 R labeled F	GCGAAACATCCGCTCCAAAA			
virD2.For1	TTGGAATATCTGTCCCGGAAG	virD2 virulence gene	203	pTi Detection
virD2.Rev1	CTTGTACCAGCAGGGAAGCTTA			
PGF	GGGGCAGGATGCGTTTTTGAG	Polygalacturonase gene of A. vitis CG49	466	Chromosomal Detection
PGR	GACGGCACTGGGGCTAAGGAT			
VirD2S4F	GACCGCAAAACCTGCCAG	virD2 gene of A. vitis S4 vitopine pTi	320	pTi Detection
VirD2S4R	GAGCCTGTATTGACGATGTC			
VirFF	ATGAGAAATTCGAGTTTGCATGATG	virF gene of A. vitis octopine and nopaline	382	pTi Detection
VirFR	TCGTGATGGGTATACGCTACG			
VCF	TTG GAA TAT CTG TCC CGG AAG	virC	414	pTi Detection
VCR	CTT GTA CCA GCA GGG AAG CTT A			
Nested Avi 1889 F	GTAAGCCCCGATGCAAGG	avsI, acyl homoserine lactone synthase	84	Chromosomal Detection
Nested Avi 1889 R	GGACCGATCTTCCAACCAGG			
A.Vit avsi F	ATGAAACAACAGGACGCGACA	avsI, acyl homoserine lactone synthase	185	Chromosomal Detection
A.Vit avsi R	TGCTAAAACAGGATTACGGGTTGGC			

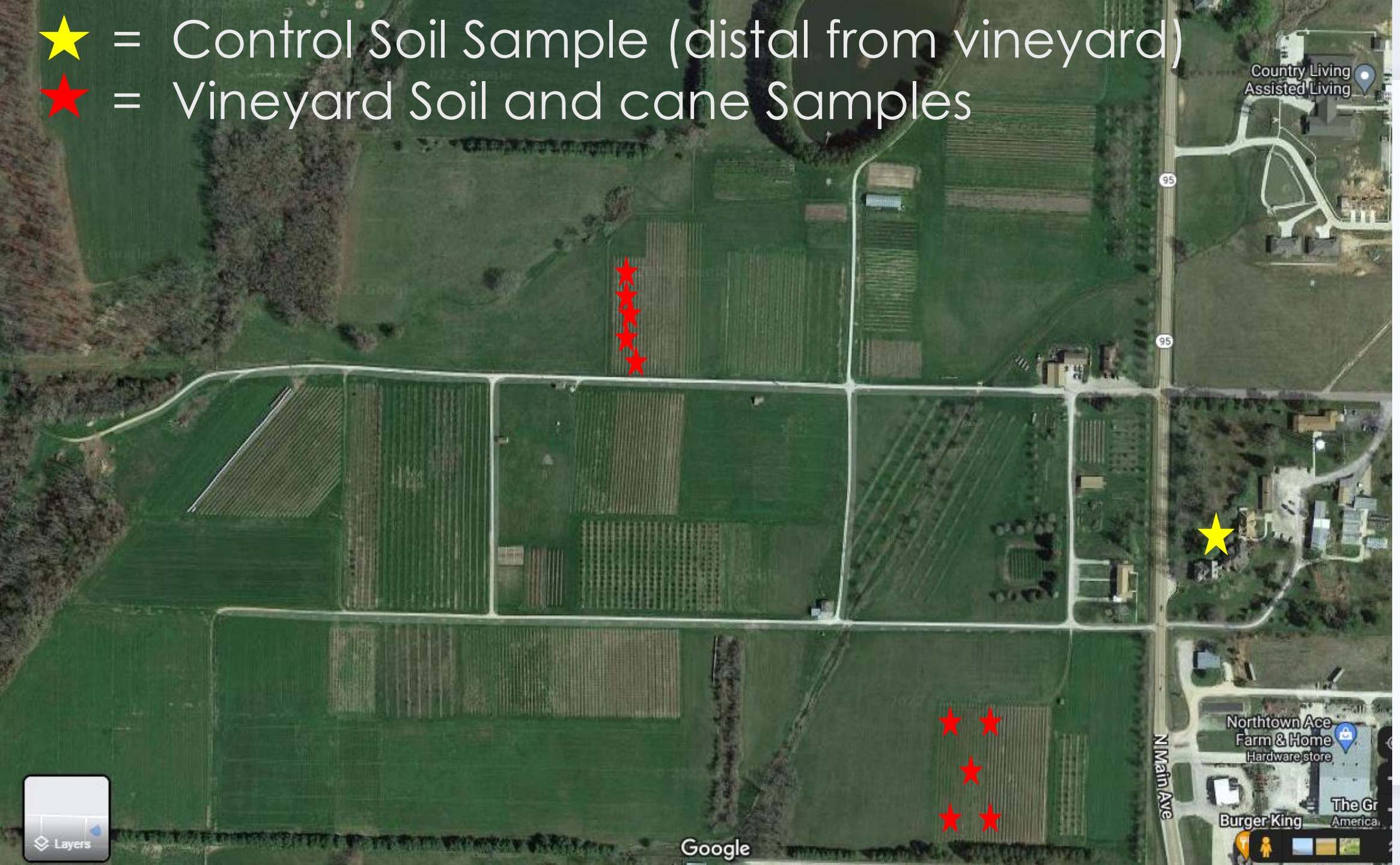
PRIMER TESTS

C=Chromosome
Ti=Ti plasmid F=Fungus

Each lane a different set of primers on the same positive sample.



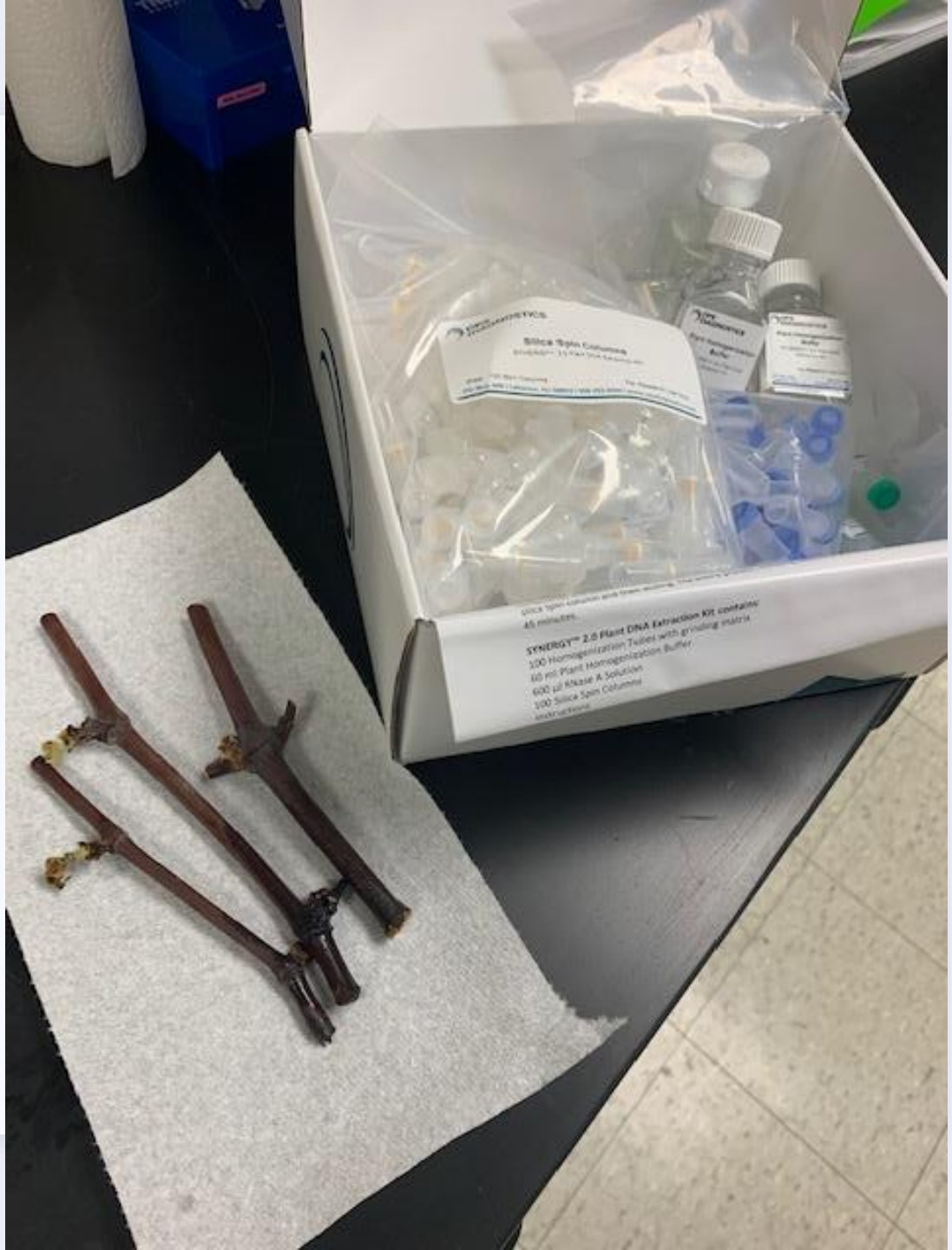
1-AVI1889;156bp 2-VirD3;149bp 3-VirD2;203bp 4-A.vit avsi;185bp 5-F;501bp 6-VirD59;96bp 7-AVInesr;100bp 8-AVInesf;95bp 9-VCF;414bp 10-PGF;466bp 11-7repeat 12-8repeat 13-AVInest; 84bp 14-10repeat


★ = Control Soil Sample (distal from vineyard)
★ = Vineyard Soil and cane Samples
Country Living
Assisted Living
95
95
N Main Ave
Northtown Ace
Farm & Home
Hardware store
Burger King
The Gr
America
Layers
Google

Diagnostics

DNA from Canes

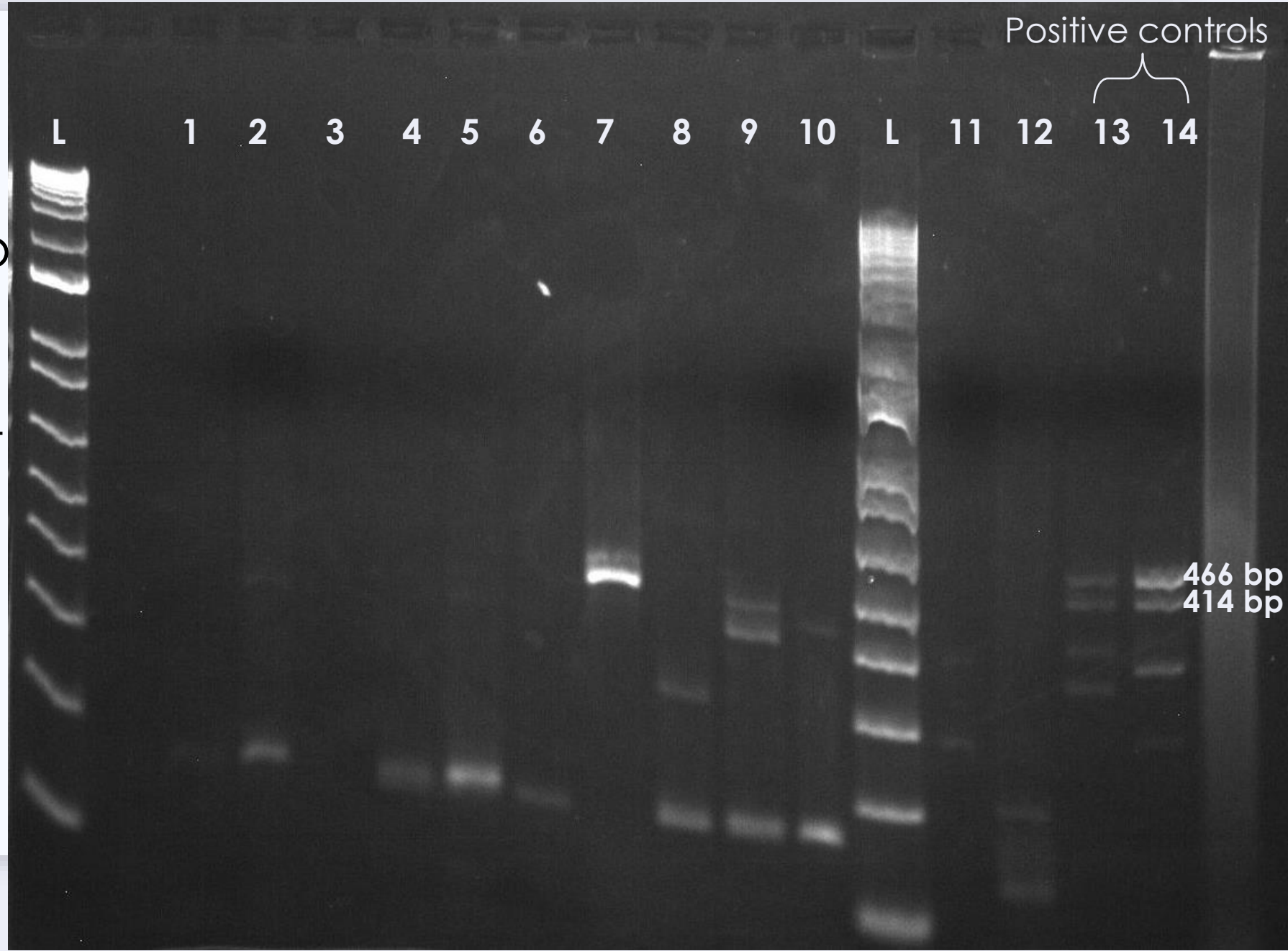
- Shaved 50 mg from each end of cutting (100 mg total)
- Used kit to extract DNA



CANE SAMPLES PCR RESULTS

Chromosome fragment =466 bp

Tumor inducing plasmid fragment =414 bp



SOIL SAMPLING

- Soil taken under dripline
- 20 cm on either side of vine
- Core depth of 8 cm
- Soil Completely Dried
- 0.5 grams starting material

SOIL SAMPLES

CHALLENGES

- Diverse species
- Humic acids
- Heavy metals
- Organic compounds

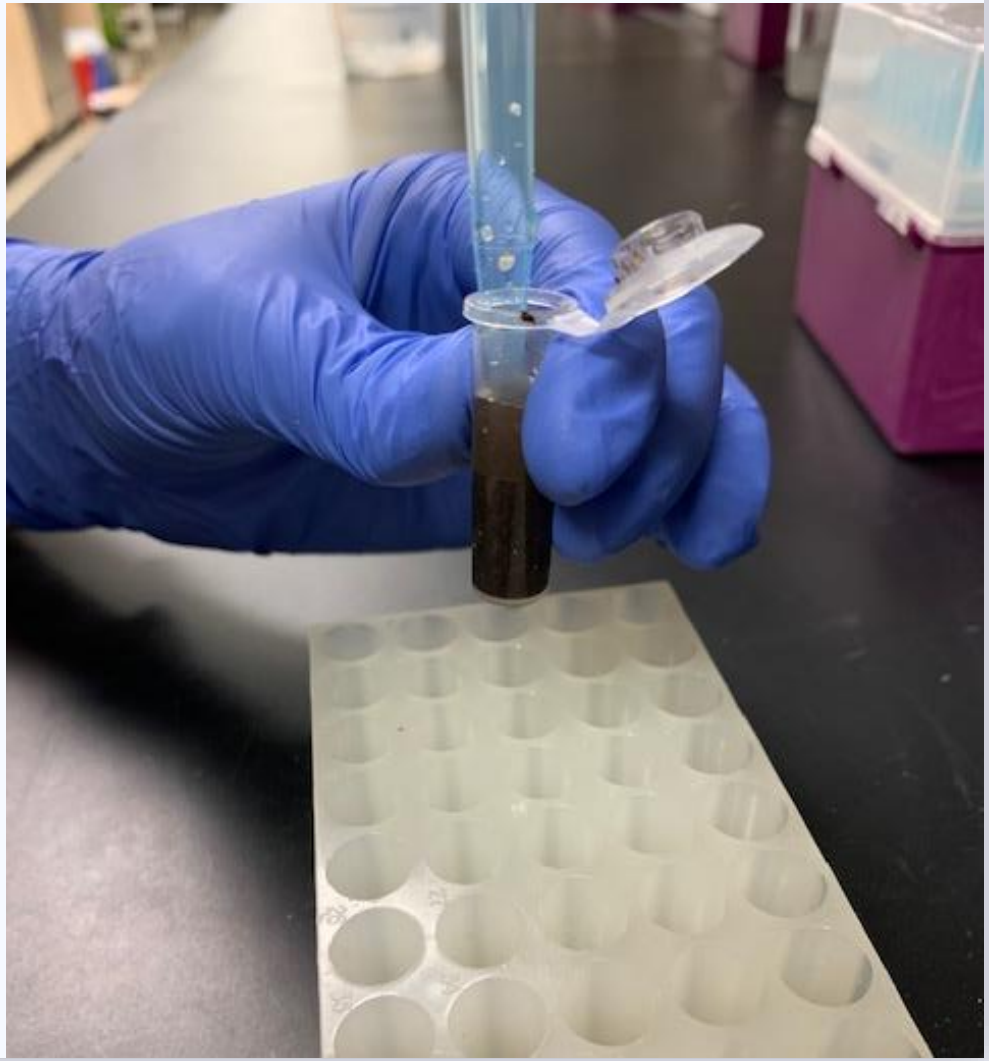
SOLUTIONS

- Specificity of Primers and Nested PCR
- Purification Method
- Dilution of soil DNA to reduce enzyme inhibitors

SOIL DNA

5-STEP PROTOCOL

1. Lyse cells
2. Remove inhibitors
3. Bind DNA to silica column
4. Wash DNA
5. Elute DNA



Mu-DNA: a modular universal DNA extraction method adaptable for a wide range of sample types V.2
Metabarcoding and Metagenomics
Graham S Sellers1,Cristina Di Muri1,Africa Gómez1,Bernd Hänfling1
University of Hull

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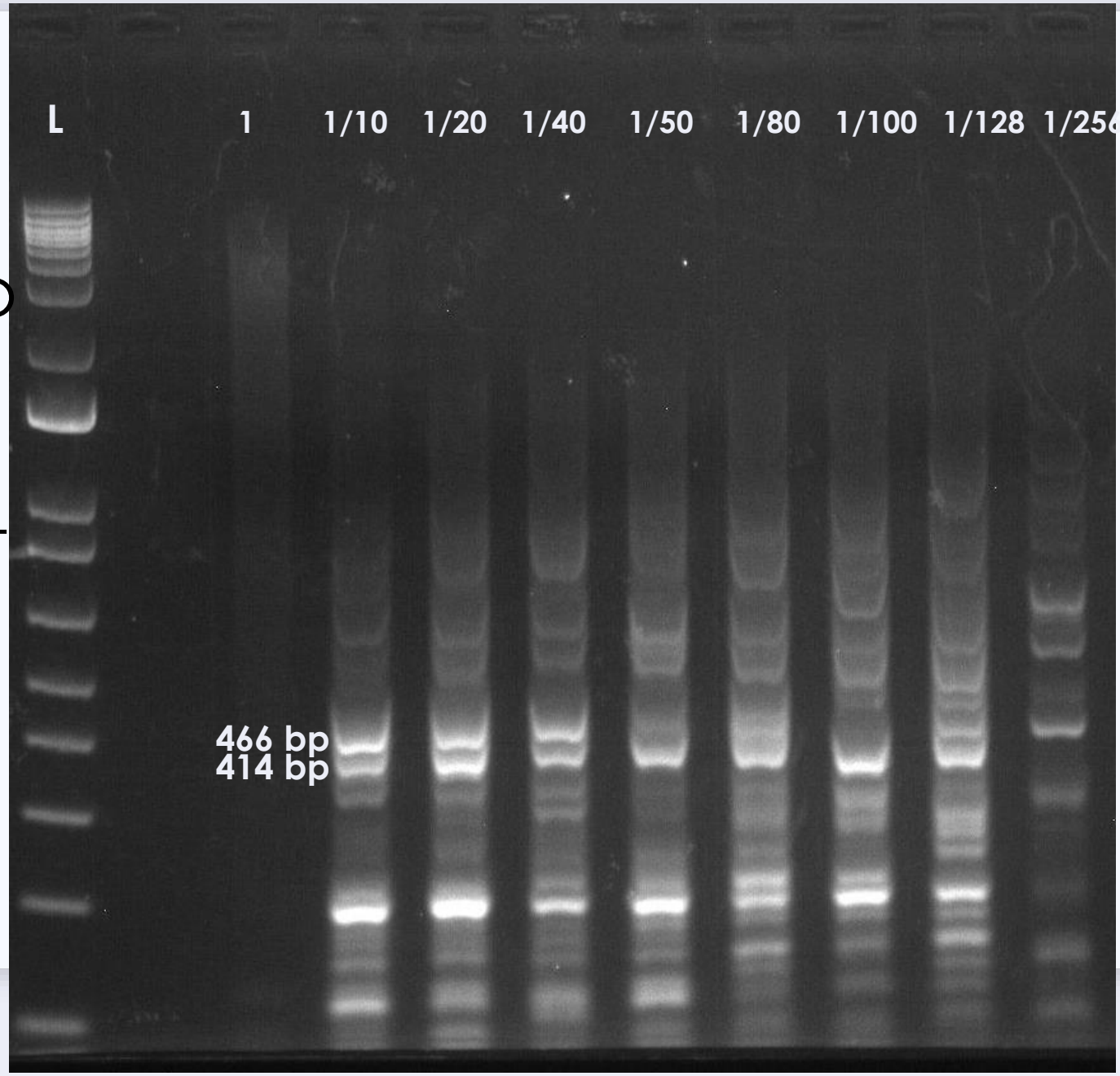
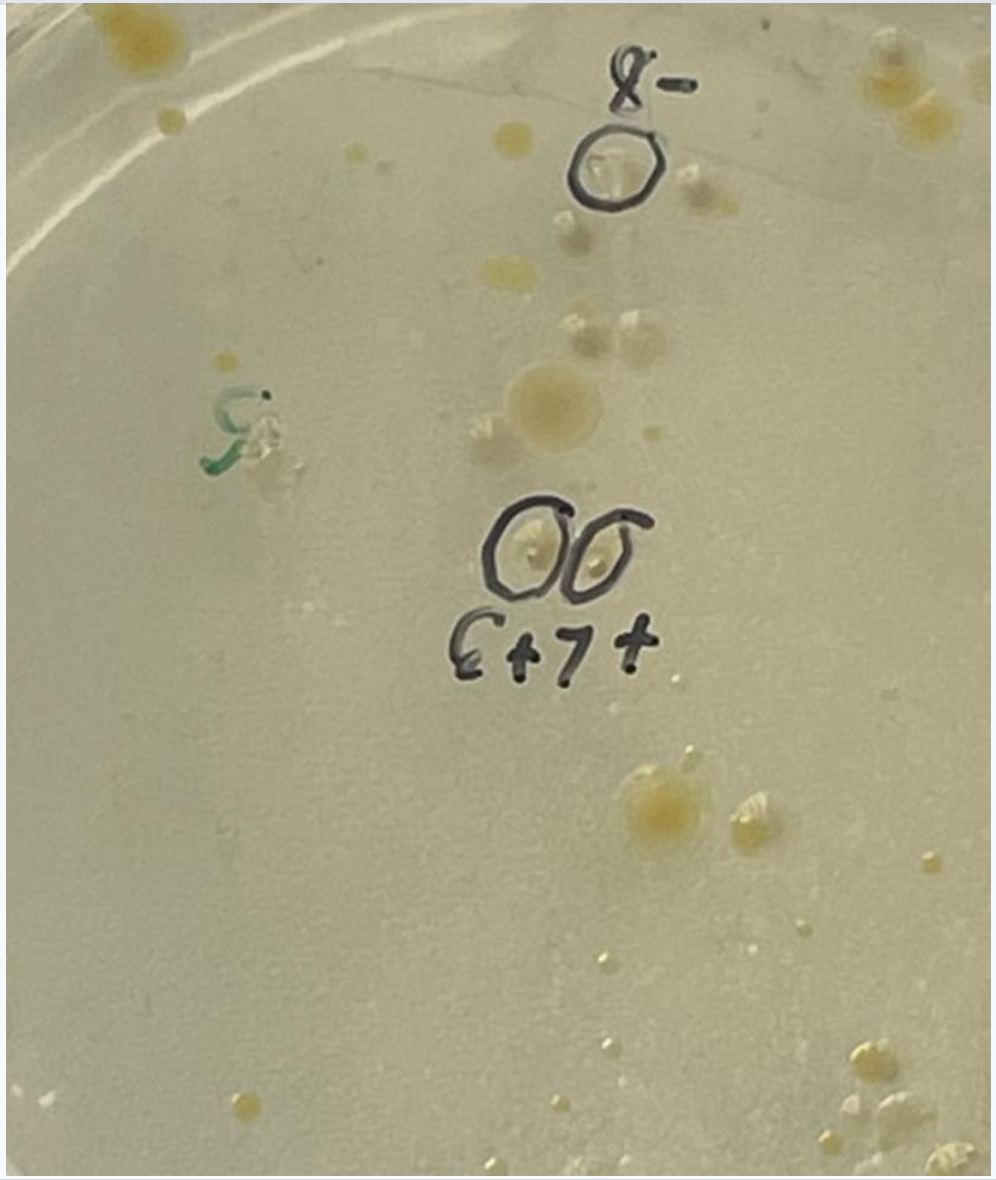


Diagnostics

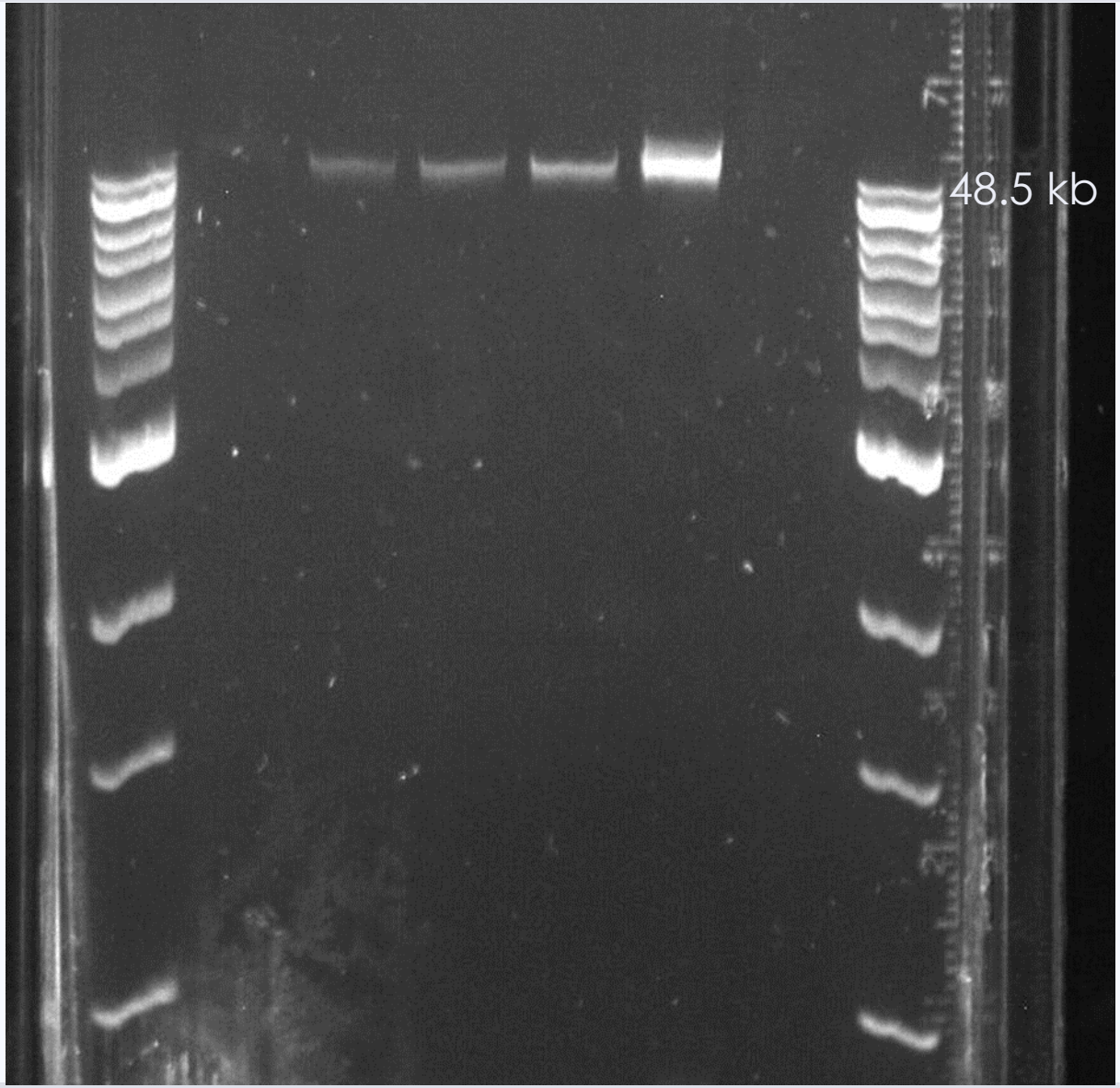
Determining *All. vitis* strains of Missouri

- Scraped gall tissue
- Streaked plate
- Morphological ID



Determining *All. vitis* strains of Missouri

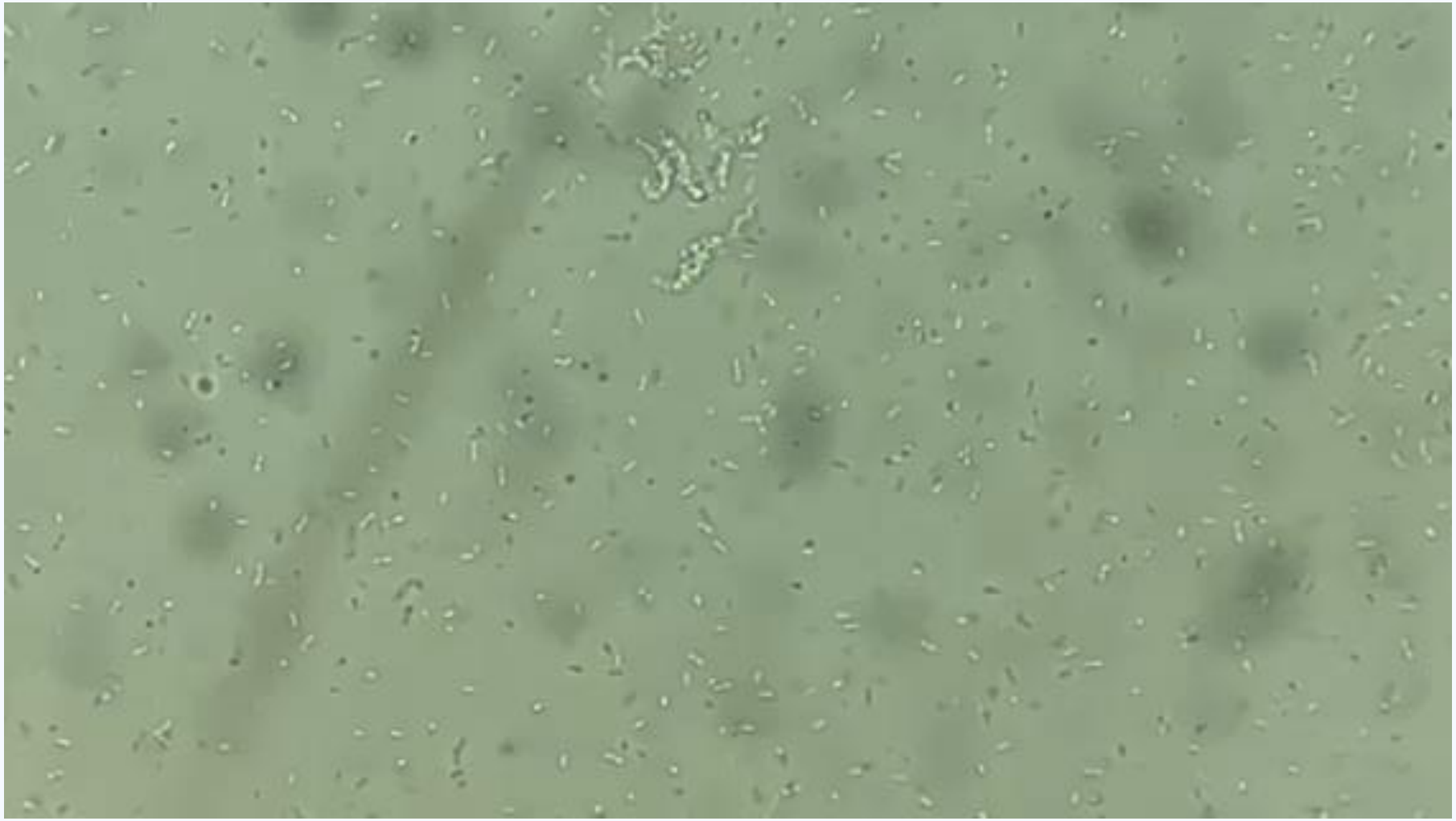
- Grew liquid culture
- Isolated plasmid
- Sent plasmid for next-gen sequencing
- Sequenced plasmid ≈130,000 nucleotides



Summary

- Reliable methods for quality, testable DNA from canes, cuttings, and from soil
- Method to detect *All. vitis* chromosome and Ti plasmid in one PCR
- Isolated and sequenced Ti plasmid from Mountain Grove, MO *All. vitis*

Thank you to Missouri Wine and Grape Board



Questions?