FOUNDATION PLANT SERVICES at UC DAVIS
and the
RUSSELL RANCH NATIONAL COLLECTION

Deborah Golino, Director, FPS

Foundation
Plant
Services

http://fps.ucdavis.edu

Foundation Plant Services

UCDAVIS

Foundation Plant Services:

- Produces, tests, maintains and distributes elite disease-tested plant propagation material
- Provides plant importation and quarantine services, virus testing and elimination
- Coordinates release of UC patented horticultural varieties
- Links researchers, nurseries, and producers

CROP PROGRAMS AT FPS

- Grape
- Strawberry
- Tree
- Rose
- Sweet potato
- Pistachio

CALIFORNIA REGISTRATION & CERTIFICATION PROGRAMS


GRAPES


FRUIT & NUT TREES

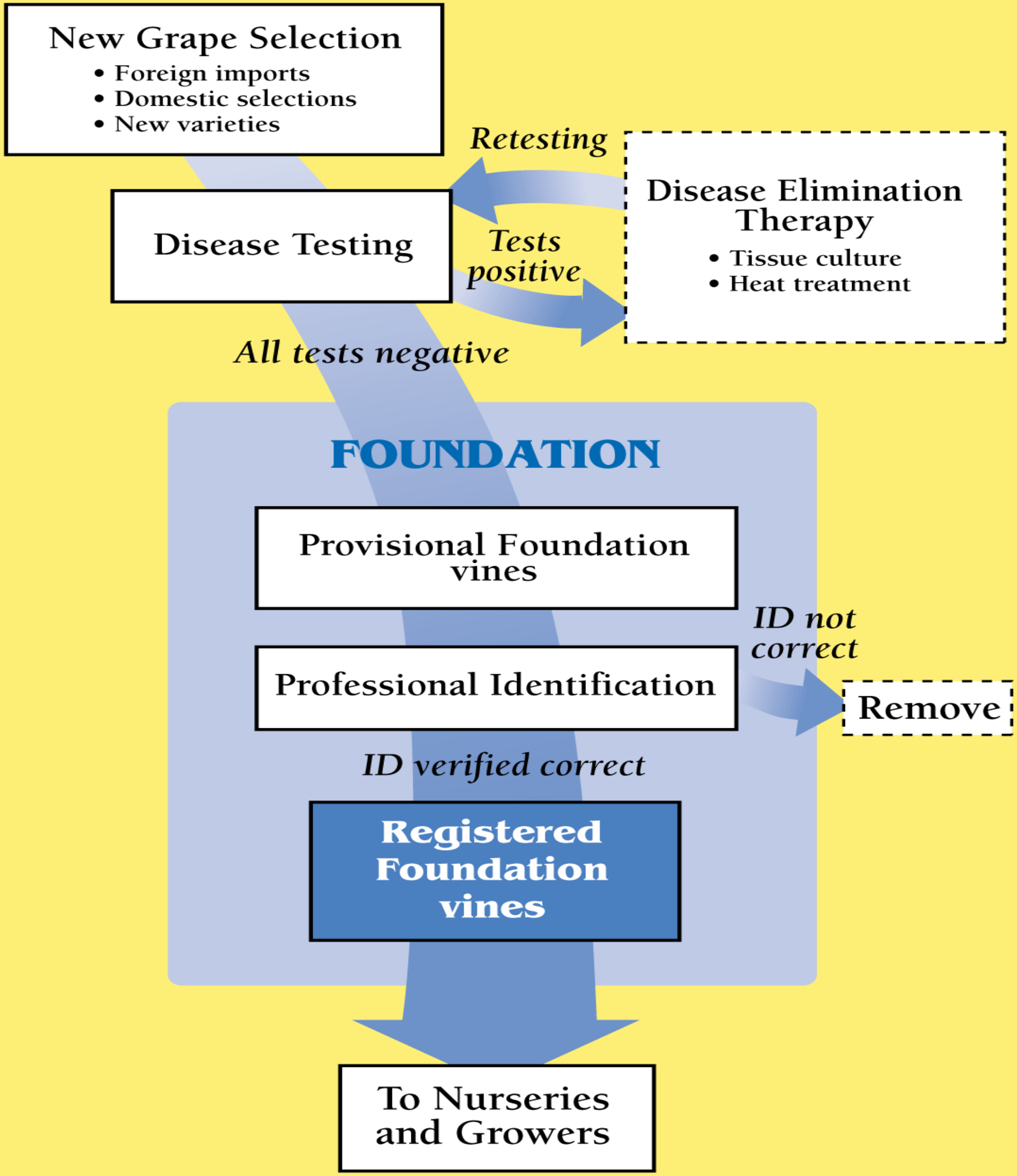

STRAWBERRIES

THE GREAT SEAL OF THE STATE OF CALIFORNIA

THE UNIVERSITY OF CALIFORNIA DAVIS

Why do you need clean plant material?

Grapevine Virus Diseases



New Grape Selection

- Foreign imports
- Domestic selections
- New varieties

Disease Testing

Retesting

Disease Elimination Therapy

- Tissue culture
- Heat treatment

Tests positive

All tests negative

FOUNDATION

Provisional Foundation vines

Professional Identification

ID not correct

Remove

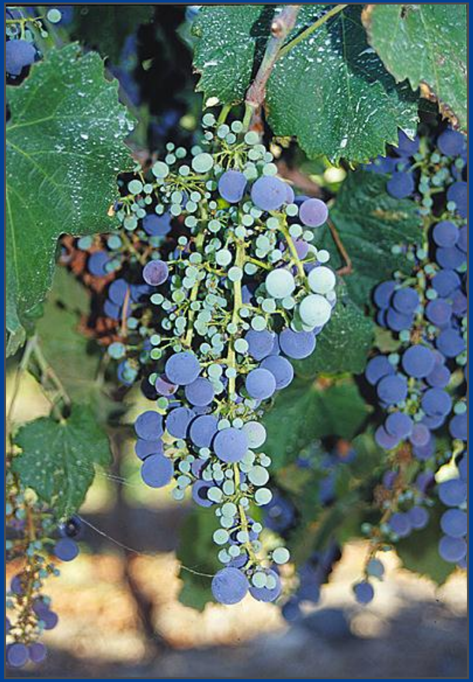
ID verified correct

Registered Foundation vines

To Nurseries and Growers

FPS Target Grapevine Diseases


- Grapevine Degeneration
    - Fanleaf
- Grapevine Decline
    - Tomato Ringspot Virus
- Leafroll
- Rugose Wood Complex
    - Kober Stem Grooving
    - Corky Bark
    - LN33 Stem Grooving
    - Rupestris Stem Pitting
- Fleck
- Minor Viruses

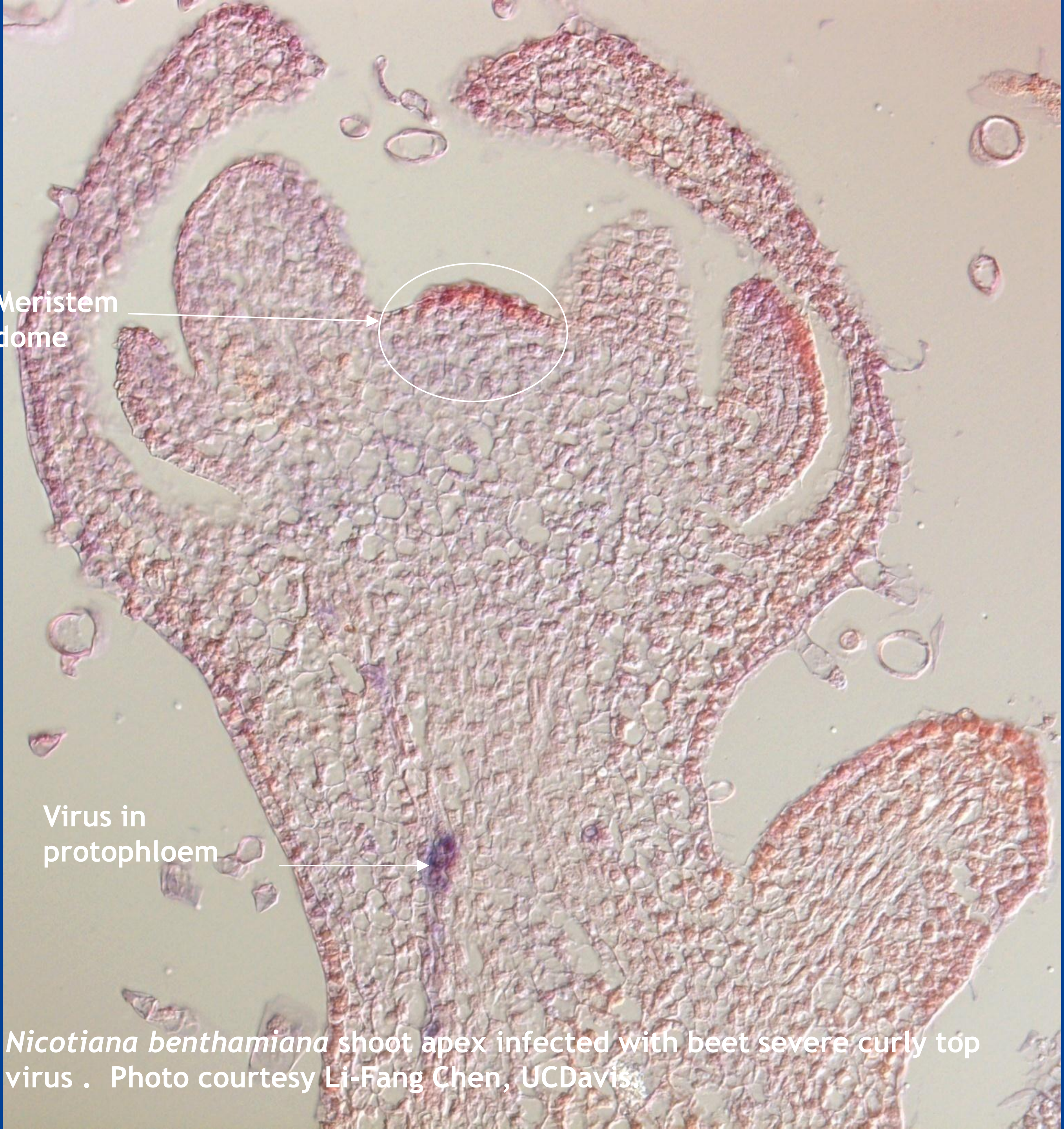



THE DANGERS OF SAMSONITE IMPORTATION

Pierce’s Disease

PPV on nectarine

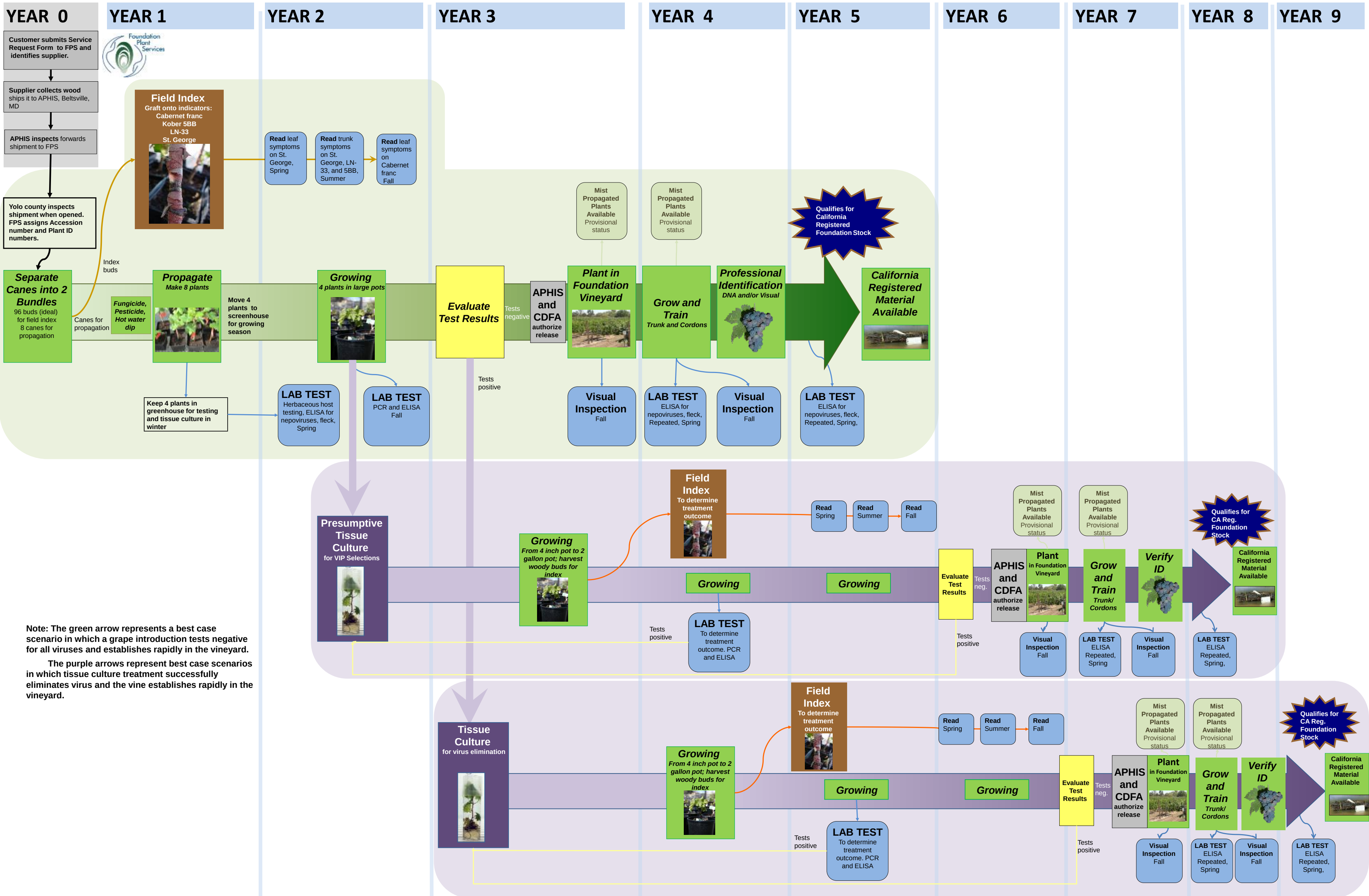
MICROSHOOT TIP CULTURE



Nicotiana benthamiana shoot apex infected with beet severe curly top virus . Photo courtesy Li-Fang Chen, UCDavis

Process Description: Grapevine Importation through Foundation Plant Services, UC Davis (Simplified)

Document # FPS2012-01 S. Sim Revised March 6, 2012



YEAR 0

YEAR 1

YEAR 2

YEAR 3

YEAR 4

YEAR 5

YEAR 6

YEAR 7

YEAR 8

YEAR 9

Customer submits Service Request Form to FPS and identifies supplier.

Supplier collects wood ships it to APHIS, Beltsville, MD

APHIS inspects forwards shipment to FPS

Yolo county inspects shipment when opened. FPS assigns Accession number and Plant ID numbers.

Separate Canes into 2 Bundles
96 buds (ideal) for field index
8 canes for propagation

Index buds

Canes for propagation

Fungicide, Pesticide, Hot water dip

Field Index
Graft onto indicators:
Cabernet franc
Kober 5BB
LN-33
St. George

Read leaf symptoms on St. George, Spring

Read trunk symptoms on St. George, LN-33, and 5BB, Summer

Read leaf symptoms on Cabernet franc Fall

Propagate
Make 8 plants

Move 4 plants to screenhouse for growing season

Keep 4 plants in greenhouse for testing and tissue culture in winter

LAB TEST
Herbaceous host testing, ELISA for nepoviruses, fleck, Spring

Growing
4 plants in large pots

LAB TEST
PCR and ELISA
Fall

Evaluate Test Results

Tests negative

Tests positive

APHIS and CDFA
authorize release

Mist Propagated Plants Available
Provisional status

Plant in Foundation Vineyard

Visual Inspection
Fall

Mist Propagated Plants Available
Provisional status

Grow and Train
Trunk and Cordons

LAB TEST
ELISA for nepoviruses, fleck, Repeated, Spring

Visual Inspection
Fall

Professional Identification
DNA and/or Visual

Qualifies for California Registered Foundation Stock

LAB TEST
ELISA for nepoviruses, fleck, Repeated, Spring,

California Registered Material Available

Presumptive Tissue Culture
for VIP Selections

Growing
From 4 inch pot to 2 gallon pot; harvest woody buds for index

Field Index
To determine treatment outcome

Read Spring

Read Summer

Read Fall

Growing

LAB TEST
To determine treatment outcome. PCR and ELISA

Tests positive

Growing

Evaluate Test Results

Tests neg.

Tests positive

APHIS and CDFA
authorize release

Mist Propagated Plants Available
Provisional status

Plant
in Foundation Vineyard

Visual Inspection
Fall

Mist Propagated Plants Available
Provisional status

Grow and Train
Trunk/ Cordons

LAB TEST
ELISA Repeated, Spring

Visual Inspection
Fall

Verify ID

Qualifies for CA Reg. Foundation Stock

LAB TEST
ELISA Repeated, Spring,

California Registered Material Available

Tissue Culture
for virus elimination

Growing
From 4 inch pot to 2 gallon pot; harvest woody buds for index

Field Index
To determine treatment outcome

Read Spring

Read Summer

Read Fall

Growing

LAB TEST
To determine treatment outcome. PCR and ELISA

Tests positive

Growing

Evaluate Test Results

Tests neg.

Tests positive

APHIS and CDFA
authorize release

Mist Propagated Plants Available
Provisional status

Plant
in Foundation Vineyard

Visual Inspection
Fall

Mist Propagated Plants Available
Provisional status

Grow and Train
Trunk/ Cordons

LAB TEST
ELISA Repeated, Spring

Visual Inspection
Fall

Verify ID

Qualifies for CA Reg. Foundation Stock

LAB TEST
ELISA Repeated, Spring,

California Registered Material Available

Note: The green arrow represents a best case scenario in which a grape introduction tests negative for all viruses and establishes rapidly in the vineyard.

The purple arrows represent best case scenarios in which tissue culture treatment successfully eliminates virus and the vine establishes rapidly in the vineyard.

EVOLUTION OF DIAGNOSTICS AT FOUNDATION PLANT SERVICES

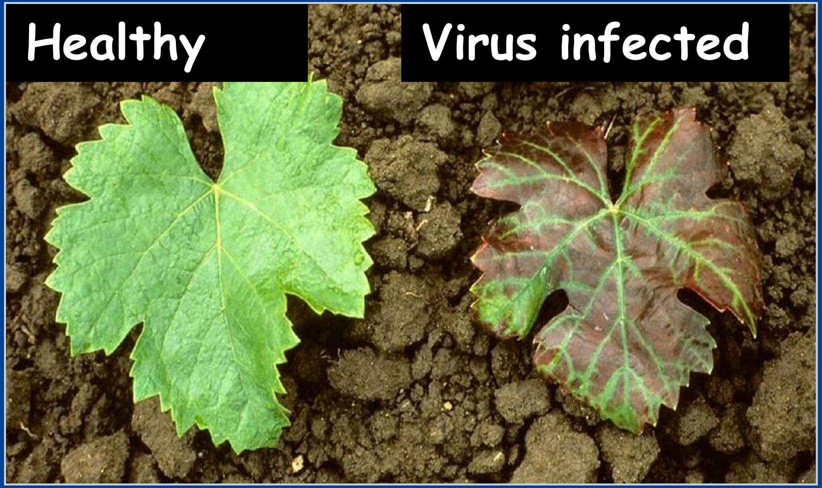
Biological indexing

Herbaceous testing

1. *Chenopodium amaranticolor*
2. *C. quinoa*
3. *Cucumis sativus*
4. *Nicotiana benthamiana*
5. *N. clevelandii*
6. *N. occidentalis*

Biological indexing

Field indexing



Woody indicator	Disease
St. George	Fanleaf, Rupestris Stem Pitting Virus, Fleck Virus
Cabernet franc	Leafroll viruses
LN-33	Corky Bark
Kober 5BB	Stem Grooving

Serological techniques: ELISA

- Since early 1990s

Nepoviruses (GFLV, ToRSV)

Leafrolls

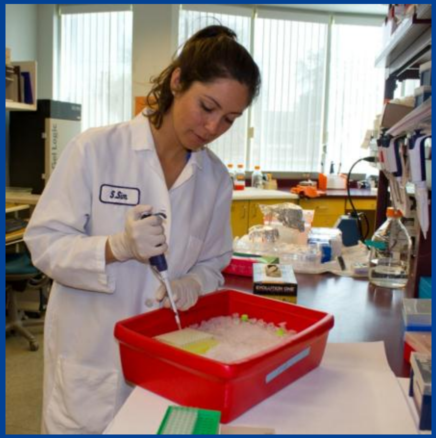
- Yr 2000 added Arabis Mosaic Virus (ArMV)
- Most antibodies are produced at FPS

Molecular techniques

Conventional RT-PCR

- 1993 Fanleaf and Leafrolls
- Currently FPS has developed and validated assays for 34 grapevine pathogens

MagMAX-96



Molecular techniques

qPCR (real-time quantitative PCR)

- Started using in 2001

- Able to run more samples faster than ELISA and conventional PCR

- qPCR is more than 300 times more sensitive than conventional PCR

- Currently is the routine detection method at FPS

Available online at www.sciencedirect.com

ScienceDirect

ELSEVIER

Journal of Virological Methods 141 (2007) 22–29

Journal of Virological Methods

www.elsevier.com/locate/jviromet

Real-time RT-PCR (TaqMan®) assays for the detection of *Grapevine Leafroll associated viruses* 1–5 and 9

Fatima Osman[a], Christian Leutenegger[b], Deborah Golino[a], Adib Rowhani[a,*]

PROTOCOL 2010 TESTS

Group	Pathogen	Symbols	ELISA	qPCR	PCR	Herb. Ind.	Woody Ind.
Nepoviruses	Grapvine fanleaf virus	GFLV	√	√	√	√	St. George
	Tomato ringspot virus	ToRSV	√	√	√	√	
	Tobacco ringspot virus	TRSV		√	√	√	
	Arabis mosaic virus	ArMV	√		√	√	
	Strawberry latent ringspot virus	SLRSV			√	√	
	Blueberry leaf mottle virus	BLMV			√	√	
	Raspberry ringspot virus	RpRSV			√	√	
	Tomato black ring virus	TBRV			√	√	
	Grapevine deformation virus	GDefV			√	√	
	Artichoke Italian latent virus	AILV			√	√	
Closteroviruses	Grapevine leafroll associated virus 1	GLRaV-1	√	√	√		Cab. Franc
	Grapevine leafroll associated virus 2	GLRaV-2	√	√	√		Cab. Franc
	Grapevine leafroll associated virus 2RG	GLRaV-2RG		√	√		
	Grapevine leafroll associated virus 3	GLRaV-3	√	√	√		Cab. Franc
	Grapevine leafroll associated virus 4	GLRaV-4	√ gen.	√	√		Cab. Franc
	Grapevine leafroll associated virus 4 strain 5	GLRaV-5	√ gen.	√	V		Cab. Franc
	Grapevine leafroll associated virus 4 strain 6	GLRaV-6	√ gen.		√		Cab. Franc
	Grapevine leafroll associated virus 7	GLRaV-7		√	√		Cab. Franc
	Grapevine leafroll associated virus 4 strain 9	GLRaV-9	√ gen.	√	√		Cab. Franc
	Grapevine leafroll associated virus 4 strain 10	GLRaV-10		√	√		Cab. Franc
	Grapevine leafroll associated virus 4 strain 11	GLRaV-11			√		Cab. Franc
	Grapevine leafroll-associated virus 4 strain Car.	GLRaCV	√ gen.	√	√		Cab. Franc
Vitiviruses	Grapevine virus A	GVA		√	√		Kober 5BB
	Grapevine virus B	GVB		√	√		
	Grapevine virus D	GVD		√	√		
	Grapevine virus E	GVE		√			
Foveavirus	Grapevine rupestris stem pitting associated virus (all strains)	GRSPaV		√	√		St. G.
Maculavirus	Grapevine fleck virus	GFkV	√	√	√		St. G.
	Grapevine redglobe virus	GRGV			√		
Marafiviruses	Grapevine syrah virus-1	GSyV-1		√	√		
	Grapevine vein feathering virus	GVFV			√		
	Grapevine asteroid mosaic virus	GAMV			√		
Phytoplasma	Universal detection	Phyto			√		
Pierce's Disease	Xylella fastidiosa	PD		√	√		

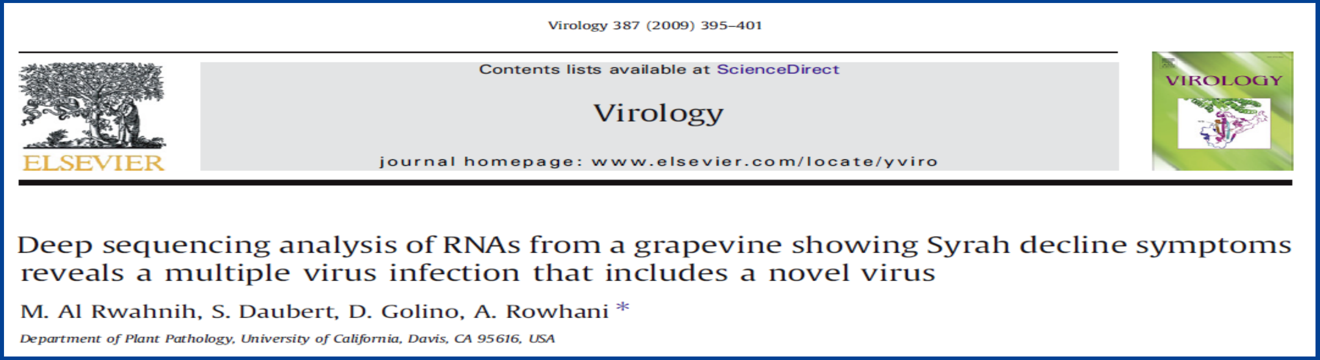
Next Generation Sequencing (NGS)

Why use NGS technologies?

- Rapid, accurate and efficient
- Detects all nucleic acids (RNA and DNA) in an organism
- Known and unknown viruses

Hiseq 2000, Illumina

Genome Sequencer FLX system, Roche



Virology 387 (2009) 395–401

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

Deep sequencing analysis of RNAs from a grapevine showing Syrah decline symptoms reveals a multiple virus infection that includes a novel virus

M. Al Rwahnih, S. Daubert, D. Golino, A. Rowhani *

Department of Plant Pathology, University of California, Davis, CA 95616, USA

NGS is like a FULL BODY SCANNER

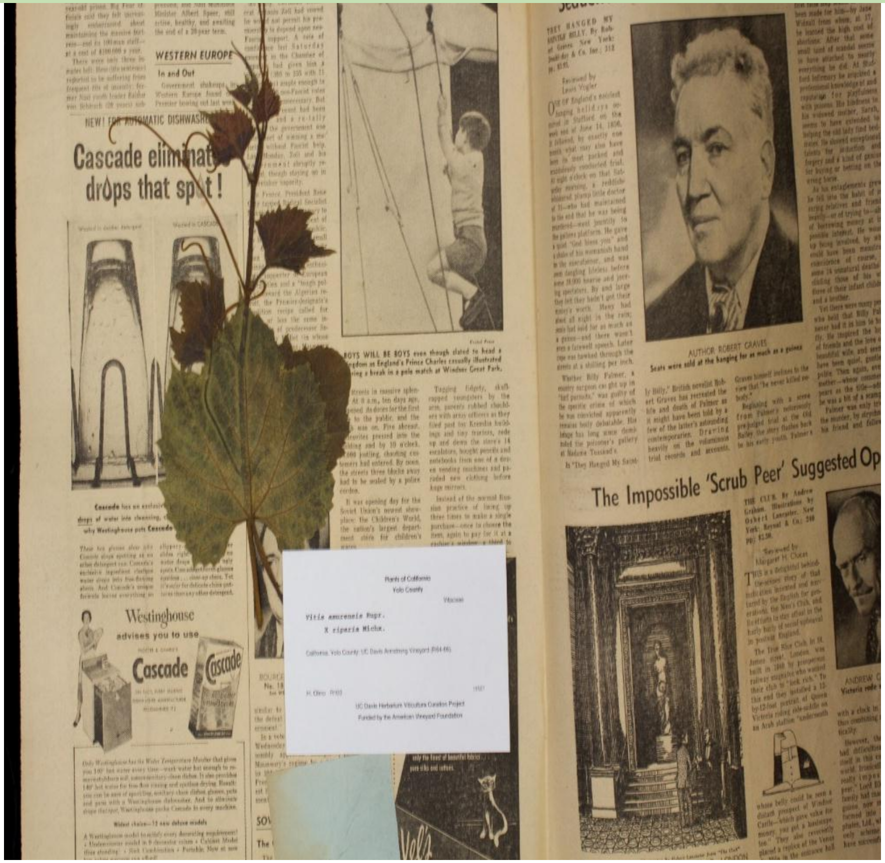
Is Red Blotch a new disease?

UC Davis Center for Plant Diversity/ Herbarium

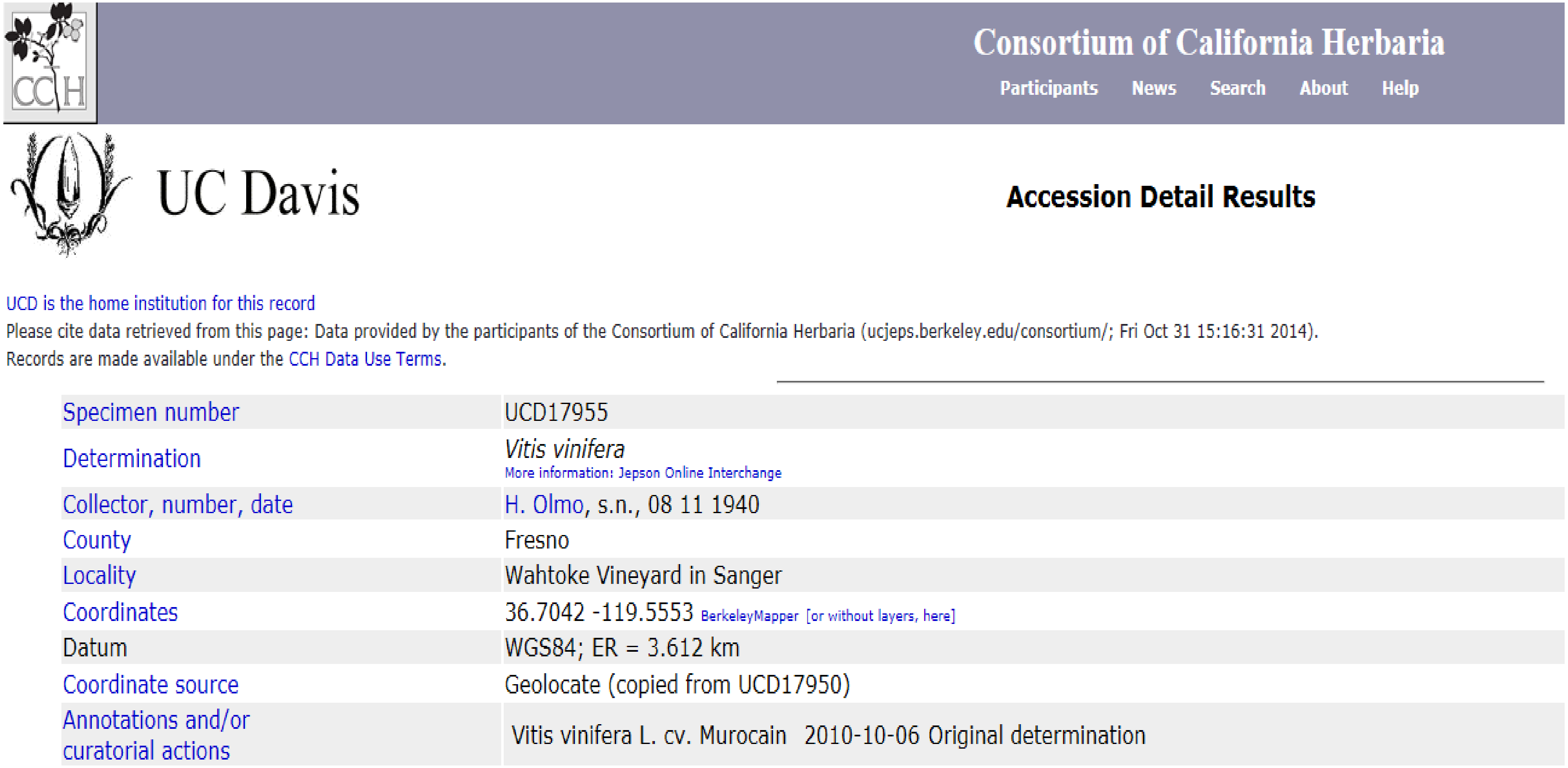
- The Herbarium has over 300,000 dead plants called herbarium specimens.
- Each specimen consists of a flattened and dried plant glued onto an archival paper with a label.
- The label has specimen data: cultivar, place, date and the collector name.



The herbarium has few un-mounted grape samples from Yolo County



Example of a specimen records



Accession Detail Results

UCD is the home institution for this record
Please cite data retrieved from this page: Data provided by the participants of the Consortium of California Herbaria (ucjeps.berkeley.edu/consortium/; Fri Oct 31 15:16:31 2014).
Records are made available under the CCH Data Use Terms.

Specimen number	UCD17955
Determination	*Vitis vinifera* More information: Jepson Online Interchange
Collector, number, date	H. Olmo, s.n., 08 11 1940
County	Fresno
Locality	Wahtoke Vineyard in Sanger
Coordinates	36.7042 -119.5553 BerkeleyMapper [or without layers, here]
Datum	WGS84; ER = 3.612 km
Coordinate source	Geolocate (copied from UCD17950)
Annotations and/or curatorial actions	Vitis vinifera L. cv. Murocain 2010-10-06 Original determination

Sample collection

- Fifty six grapevine specimens were collected (Approximately 0.5 g of leaf/petiole tissue).
- specimens were originally harvested and pressed between 1937-1950.
- Pieces of foil containing each sample were placed in individual Ziplock bags to prevent cross contamination.
- The 56 sample bags were transported to a lab in which research on grapevine had NOT previously been conducted.

Sample collection in the herbarium

List of samples- Group 1: *Vitis* cultivars from Napa or Sonoma County

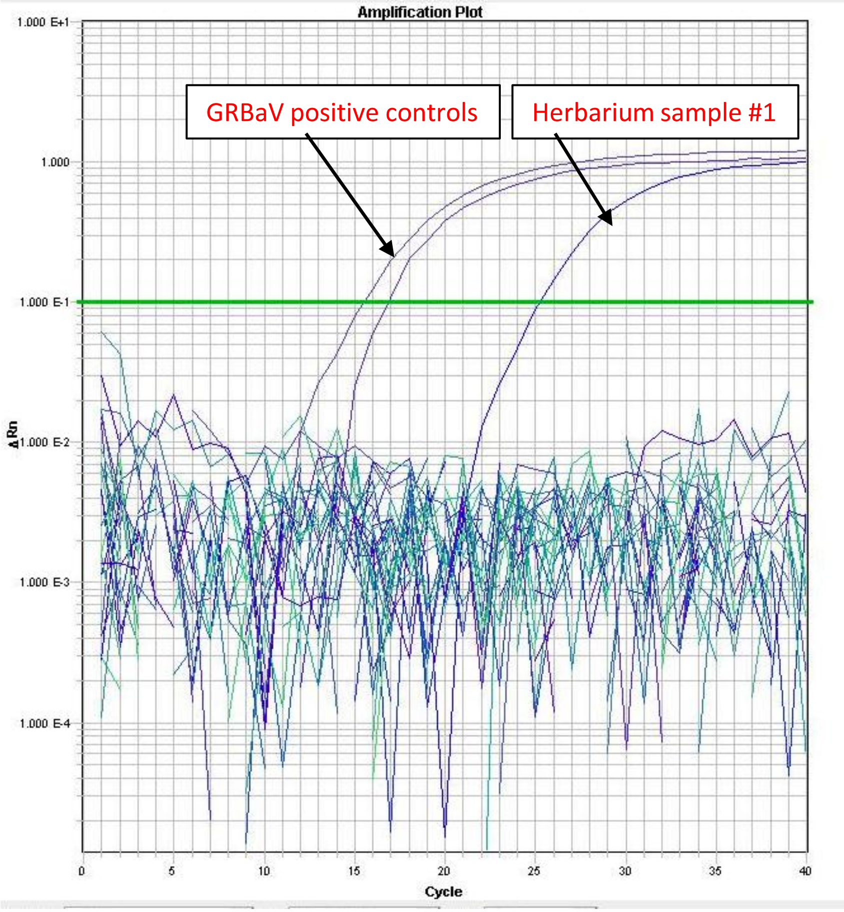
Sample #	Herbarium ID#	Variety/cultivar	Location (County)	Year of collection	Collector	Tissue
1	DAV202170	Vitis vinifera L. cultivar Early Burgundy	Sonoma	1940	H. Olmo	Leaves
2	DAV202866	Vitis vinifera L. cultivar aff. Napa Gamay	Napa	1939-1940	H. Olmo	Leaves
3	DAV202172	Vitis vinifera L. cultivar Petite Bouschet	Sonoma	1937	H. Olmo	Leaves
4	DAV202202	Vitis vinifera L. cultivar Rosetta	Sonoma	1938?	H. Olmo	Leaves and petioles
5	DAV202196	Vitis vinifera L. cultivar Zinfandel	Napa	1935	H. Olmo	Leaves and petioles
6	DAV202174	Vitis vinifera L. cultivar Black Malvoisie	Sonoma	1935	H. Olmo	Leaves and petioles
7	DAV202201	Vitis vinifera L. cultivar Burger	Napa	1935	H. Olmo	Leaves and petioles
8	DAV202171	Vitis vinifera L. cultivar Carignane	Sonoma	1937	H. Olmo	Leaves and petioles
9	DAV202870	Vitis vinifera L. cultivar aff. Merlot	Napa	1939-1940	H. Olmo	Leaves and petioles;
10	DAV202872	Vitis vinifera L. cultivar aff. Gamay	Sonoma	1939-1940	H. Olmo	Leaves and petioles
11	DAV202227	Vitis vinifera L. cultivar Grey Riesling	Sonoma	1938	H. Olmo	Leaves and petioles
12	DAV202860	Vitis vinifera L. cultivar Early Burgundy	Napa	1939-1940	H. Olmo	Leaves and petioles

Precautions to prevent cross-contamination

- **Extractions were conducted in an isolated lab (The Michelmore Lab)**
- **Samples processed with ALL new materials and reagents.**

GRBaV PCR Results

- **All herbarium samples were tested for GRBaV using both conventional and Quantitative PCR assays**
- **Only one sample found to be positive for GRBaV (sample #1)**



Sample #1

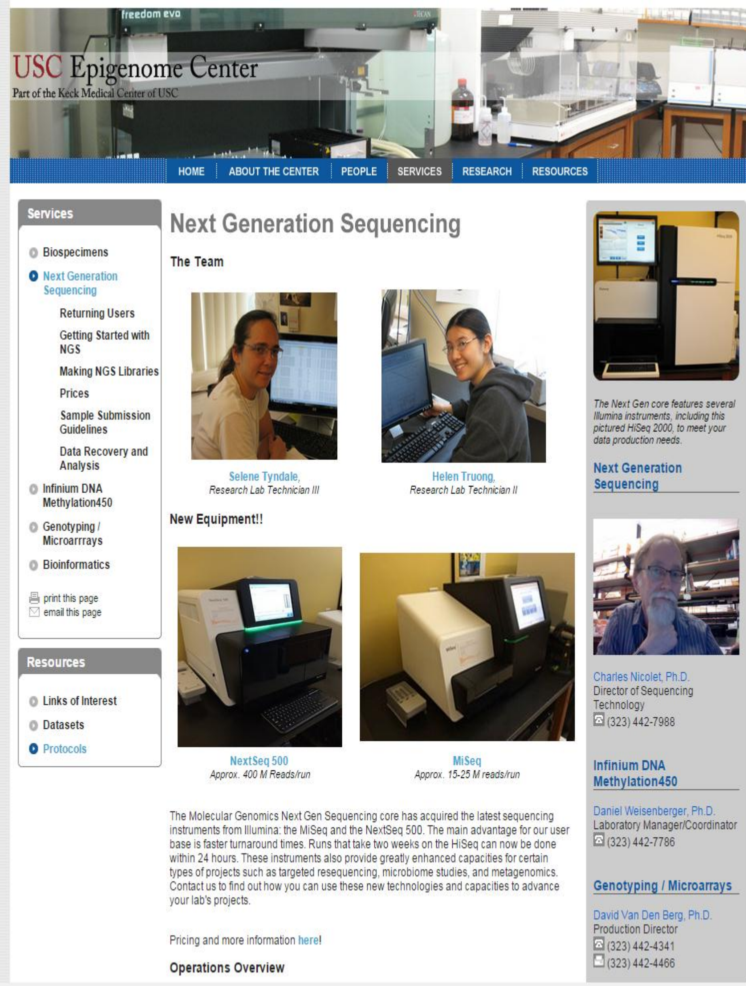
Cultivar: Early Burgundy
Location: Sonoma
Collector: H. Olmo
Year of collection: 1940

What is Next: Genomic analysis

Obtain the full genome sequence of GRBaV herbarium isolate and compare it with the recently sequenced GRBaV isolates.

What is Next: Genomic analysis

- 0.1g of tissue was collected from the original specimen
- Total nucleic acid was extracted in an isolated lab and sent to the sequencing facility at the University of Southern California (They never worked with any grapevine material)
- NGS run generated about 88 million illumina reads
- More than 92,000 reads mapped against the GRBaV genome
- The herbarium isolate shared 99% identity with other genebank isolates.



Next Generation Sequencing

The Team

Selene Tyndale,
Research Lab Technician III

Helen Truong,
Research Lab Technician II

New Equipment!!

NextSeq 500
Approx. 400 M Reads/run

MiSeq
Approx. 15-25 M reads/run

The Molecular Genomics Next Gen Sequencing core has acquired the latest sequencing instruments from Illumina: the MiSeq and the NextSeq 500. The main advantage for our user base is faster turnaround times. Runs that take two weeks on the HiSeq can now be done within 24 hours. These instruments also provide greatly enhanced capacities for certain types of projects such as targeted resequencing, microbiome studies, and metagenomics. Contact us to find out how you can use these new technologies and capacities to advance your lab's projects.

Pricing and more information here!

Operations Overview

The Next Gen core features several Illumina instruments, including this pictured HiSeq 2000, to meet your data production needs.

Next Generation Sequencing

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David Van Den Berg, Ph.D.
Production Director
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Summary of results

The results suggest that GRBaV was present in the Sonoma wine grape growing area for at least 74 years before the virus was identified and correlated to specific symptoms.

Thank you

American Vineyard Foundation
Fruit Tree, Nut Tree and Grapevine Improvement
Advisory Board (IAB)
California Grape Nurseries

Thank you