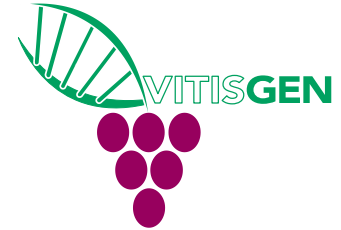


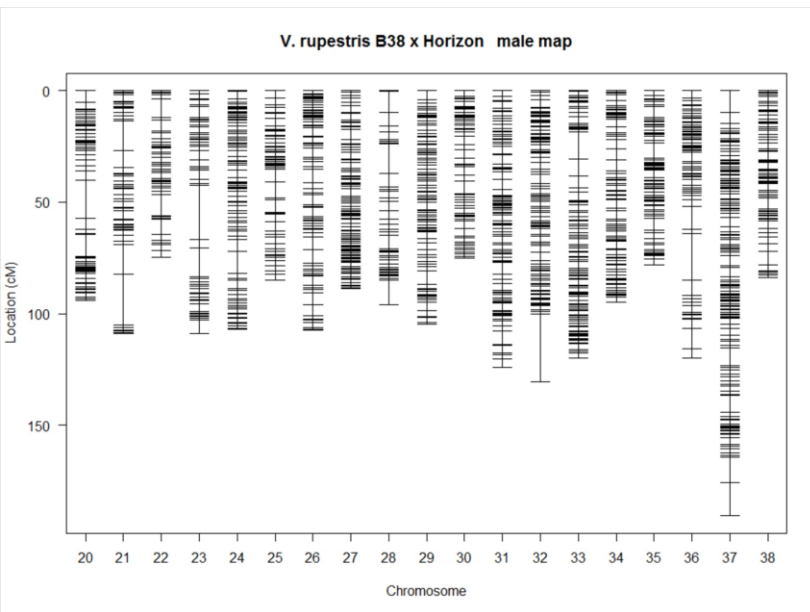


# Grapevine Breeding in the United States: Perspectives from the *VitisGen* Project



Bruce I. Reisch<sup>1</sup>, Lance Cadle-Davidson<sup>2</sup>, Elizabeth Demmings<sup>1</sup> and the VitisGen Team.

<sup>1</sup>Cornell University, and <sup>2</sup>USDA ARS-GGRU Geneva, New York, USA



USDA-NIFA Specialty Crop Research Initiative  
Project Number 2011-51181-30635

# “Accelerating grape cultivar improvement via phenotyping centers and next generation markers”



*<http://www.VitisGen.org>*



- Overall project goal: *To address industry needs via application of new technology to grape cultivar development*
- A 5-year project funded by the USDA-National Institute of Food and Agriculture (NIFA) Specialty Crop Research Initiative **AND** the grape industry
- \$4.5 million, 5 year project
- Began September, 2011
- Collaboration between 11 research institutions with oversight from an industry advisory panel



# “Accelerating grape cultivar improvement via phenotyping centers and next generation markers”

## *VitisGen* primary objectives

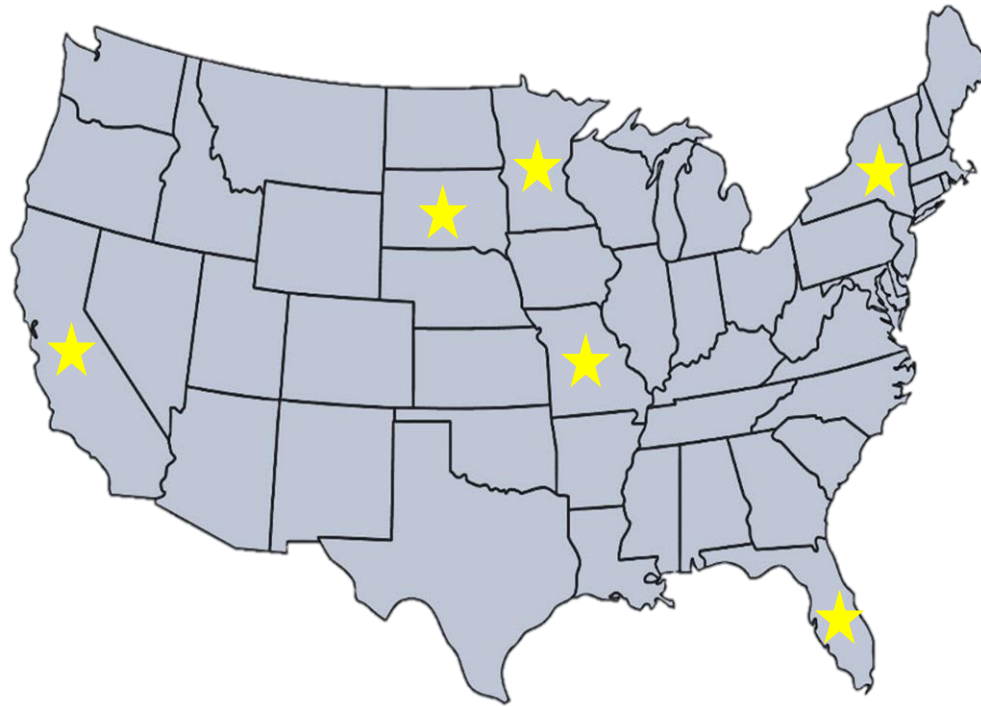


1. Identify top priority traits and document their value
2. **Develop more than 30 new DNA markers linked with traits**
3. **Apply markers to accelerate cultivar improvement**
4. Enhance communication between industry, researchers, and consumers; develop educational resources in breeding and genetics





# *Vitis*Gen unites breeding programs nationwide



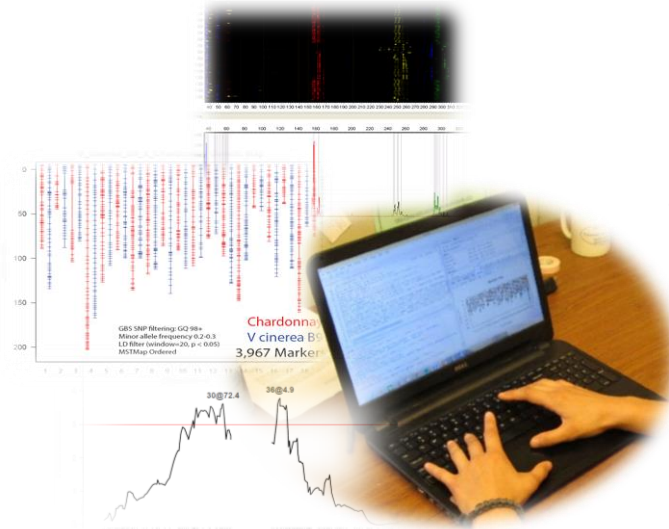
# Coordinated effort leads to marker development and application



## Breeding



## Genetics



## Trait evaluation





# Three trait evaluation centers



## Fruit Quality



- For example:
  - tannin extractability
  - off-odorants
  - organic acids
  - anthocyanin

## Powdery Mildew Resistance



- For example:
  - count data
  - hyphal transects
  - visual ratings

## Low Temperature Responses



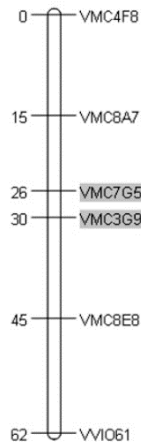
- For example:
  - differential thermal analysis
  - chilling fulfillment

- Receive samples for analysis and phenotype samples using standardized protocols

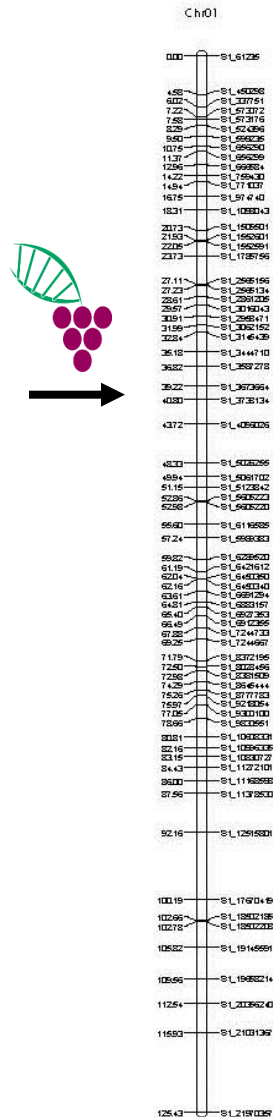
# VitisGen high-resolution genetic maps



SSRs



GBS



- Heterozygous mapping strategy (HetMappS) for high-resolution genotyping-by-sequencing (GBS) markers

Hyma, Barba *et al.*, *PLoS One* 2015 10(8): e0134880  
doi: 10.1371/journal.pone.0134880

- Next generation mapping of enological traits in an  $F_2$  interspecific grapevine hybrid family

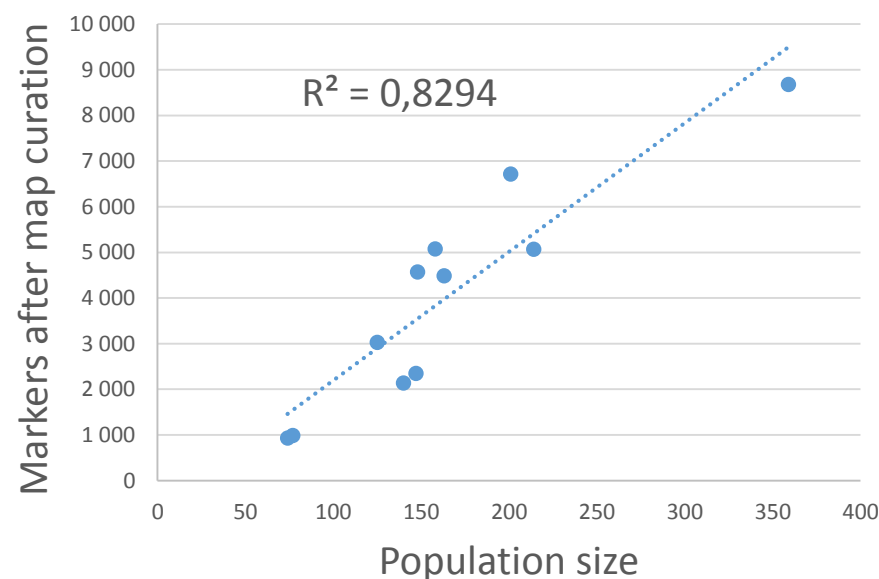
Yang *et al.*, *PLoS One* 2016 11(3): e0149560  
doi: 10.1371/journal.pone.0149560



# Dense *VitisGen* genetic maps in 16 families



VitisGen Core Populations †	No. individuals	No. SNP markers after build	No. markers after map curation
Riesling x Cabernet Sauvignon	140	91,278	2,133
<i>V. riparia</i> 37 x Seyval F2	424	291,453	1,449 *
<i>V. rupestris</i> B38 x Horizon	214	219,737	5,065
Horizon x Illinois 547-1	359	295,944	8,678
Horizon x <i>V. cinerea</i> B9	158	209,055	5,074
Chardonnay x <i>V. cinerea</i> B9	148	185,049	4,571
C87-41 x B82-43	74	91,419	931
B37-28 x C56-11	336	96,140	1,160
C81-227 x Y315-43-04	284	156,127	1,357 *
A90-37 x C45-64	77	101,429	988
MN 1264 x MN 1214	147	192,995	2,345
MN 1264 x MN 1246	125	151,456	3,024
Regent x MN 1307	108	155,409	1,589 *
MN 1264 x MN 1302	117	152,434	1,703 *
<i>V. rupestris</i> 'Wichita Refuge' x PC000138-10	201	216,016	6,717
Norton x Cabernet Sauvignon	163	172,505	4,486
588224 x Cabernet Sauvignon	209	226,069	

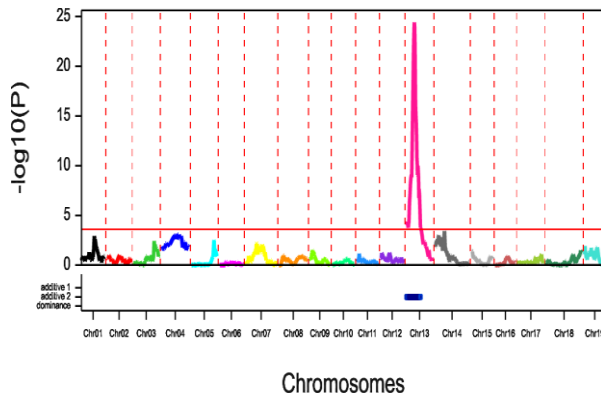


\* integrated map



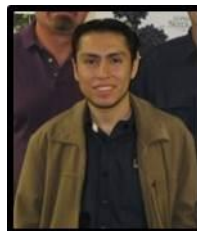
# QTL analyses lead to marker-trait associations

Powdery mildew phenotyping  
center data



>70 marker-trait associations  
identified =

- 24 for fruit chemistry
- 18 for pest and disease resistance
- 16 for trichomes and predatory mites
- 6 for low temperature response
- 13 for leaf morphology



Jonathan  
Fresnedo



Shanshan  
Yang

# The unexpected benefits of local phenotyping

# Local trait evaluation



- Characterization of 100+ unique traits within breeding programs



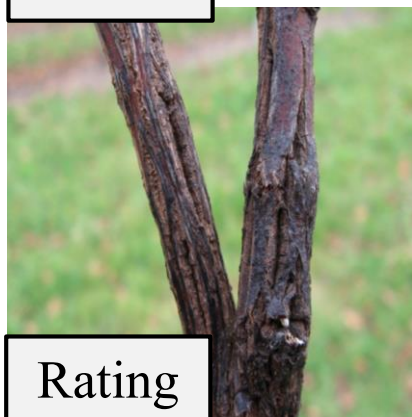
# Discovery of two new loci for phomopsis resistance



Rating  
“1”



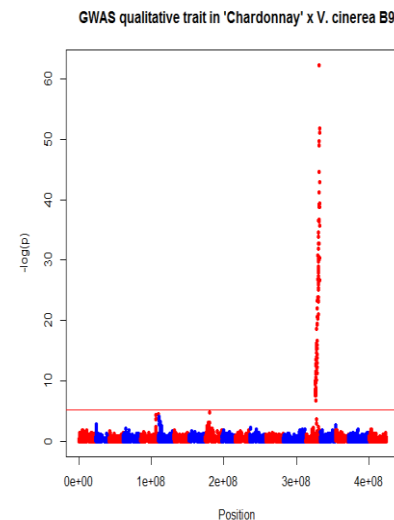
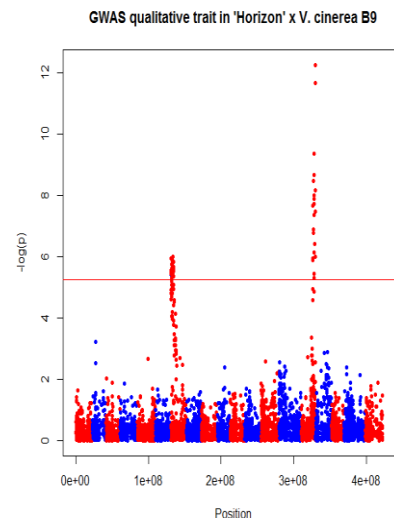
Rating  
“2”



Rating  
“3”

‘Horizon’ x *V. cinerea*  
7,634 SNPs: **chr7** and **chr15**

‘Chardonnay’ x *V. cinerea*  
8,806 SNPs: **chr 15**



Steve Luce



Paola Barba



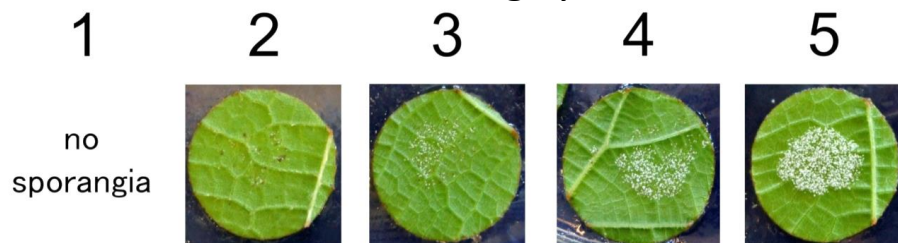
Barba et al. (submitted)

# QTL for downy mildew sporulation (replicated between years)

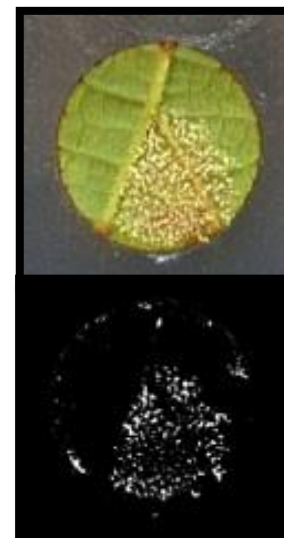


Chromosome	Heterozygous Parent	% Variance Explained
7	<b>'Horizon'</b> x <i>V. cinerea</i> B9; <b><i>V. rupestris</i> B38</b> x <b>'Horizon'</b>	9.0
9	<i>V. rupestris</i> B38 x <b>'Horizon'</b>	10.5
11	<i>V. rupestris</i> B38 x <b>'Horizon'</b>	9.4
14	<b><i>V. rupestris</i> B38</b> x <b>'Horizon'</b>	10.2
18	<i>V. rupestris</i> B38 x <b>'Horizon'</b>	13.1

Manual rating system:



Computer  
vision:



Acknowledgment:  
Konstantin Divilov



Understanding powdery mildew  
resistance; durability; new loci



# PM Precision Phenotyping

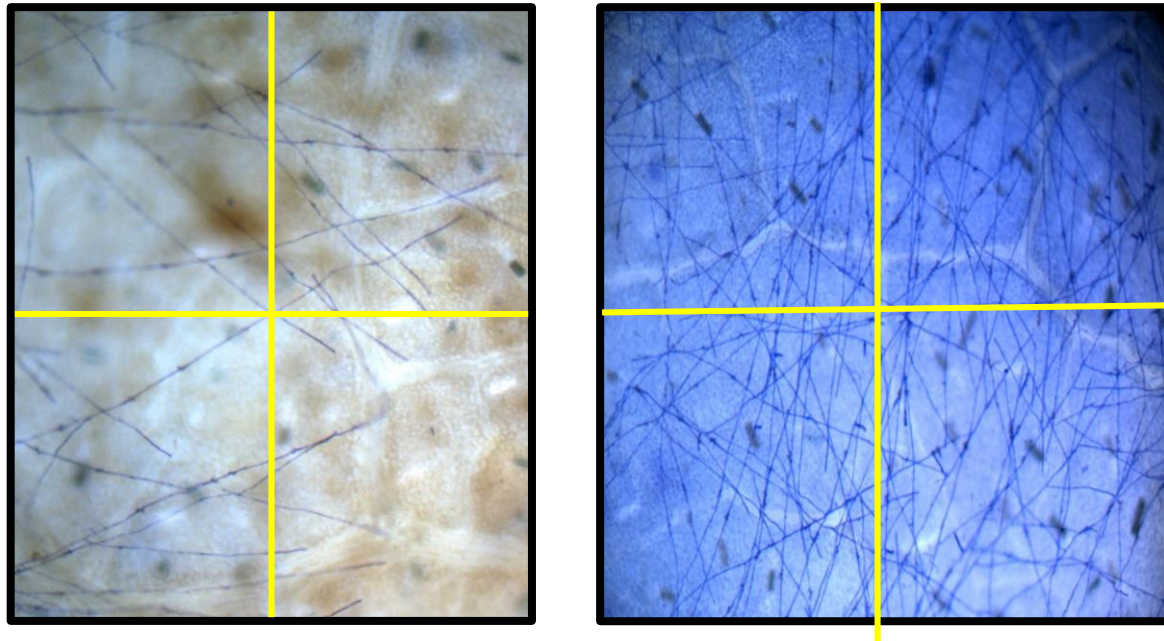
## Laboratory approach



Plan A: Standard 48 hpi quantification of penetration and colony success rates

Plan B: Evaluate colonization and sporulation at 8-9 dpi  
Challenge = how to quantify colonization objectively?

Hyphal Transects



➤ Working well for identifying QTL

Cadle-Davidson et al., 2016 Phytopathology 106 (10):

1159-1169 <http://dx.doi.org/10.1094/PHYTO-02-16-0080-FI>

# Vineyard approach vs. laboratory approach



## QTL analysis with field ratings

Date	Linkage Group	LOD	R <sup>2</sup>
June 2014	13	7.39	18.4
June 2015	13	6.43	18.9
Aug. 2015	NA	n.s.	NA

Mapping family: C81-227 x Y215-43-04

## QTL analysis with hyphal transect data

Date	Linkage Group	LOD	R <sup>2</sup>
Sept. 2012	13	8.3	27.1
July 2013	13	15.3	46.2
May 2014	13	21.5	55.9
June 2014	13	17.2	54.9

Mapping family: C81-227 x Y215-43-04

- Identified the *REN1* locus on linkage group 13
- Precision phenotyping yields QTLs that are more significant and explain a higher percent of the phenotypic variance (R<sup>2</sup>)

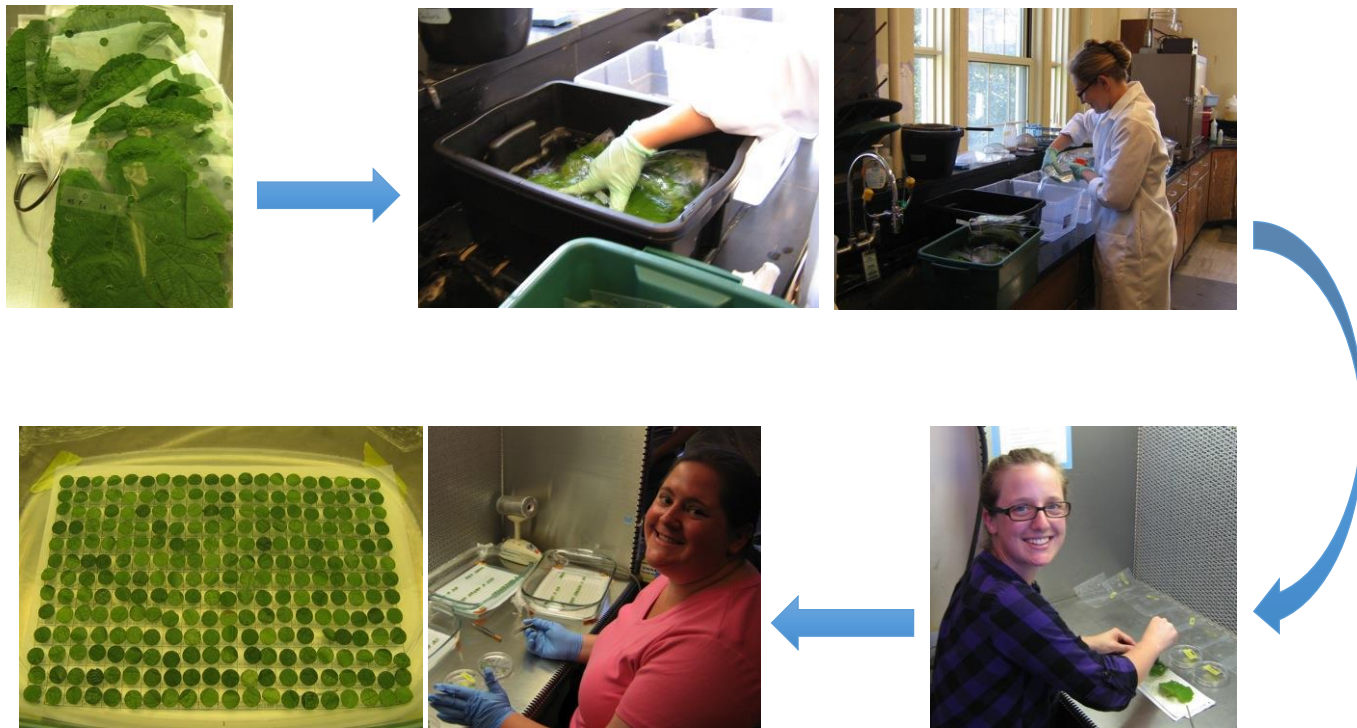
# PM Precision Phenotyping

## Laboratory approach



Hypothesis: Field evaluation works for qualitative resistance, but need tighter control for minor and moderate quantitative resistance analysis.

Solution: Breeders ship leaves to a phenotyping center for processing surface sterilized 1-cm leaf discs.



Up to 260 leaf discs on agar are inoculated with a single isolate of powdery mildew.

All treatments within one environment, novel opportunity for replicate blocks -> increased statistical power.



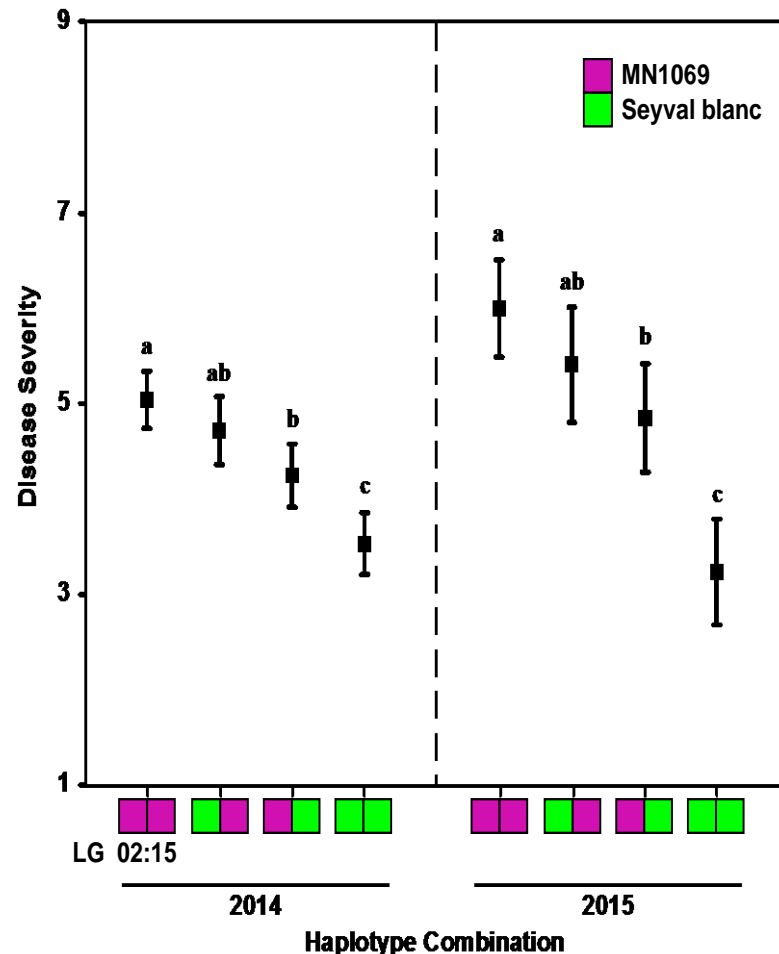
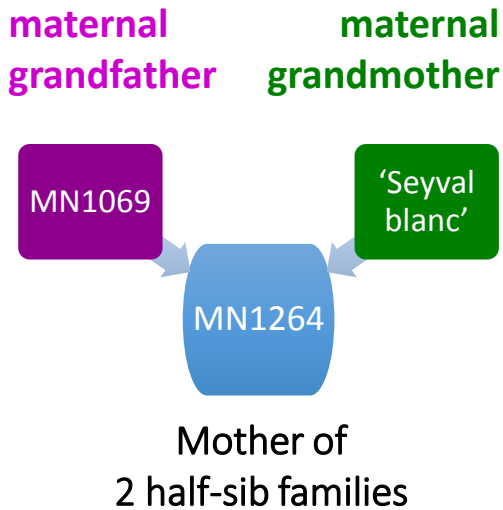
# Race specificity and durability

Locus	Chr	<i>Vitis</i> spp. source	Race-specific	Protect <i>RUN1</i> ?	Reference
<b>REN1</b>	13	<i>vinifera</i>	Yes	Unknown	Theor Appl Genet 116:427–438
<b>REN2</b>	14	<i>cinerea</i>	Yes	Maybe	Phytopathology 105:1104-1113
<b>REN4</b>	18	<i>romanetii</i>	No	Yes	Phytopathology 101: 502-508
<b>RUN1</b>	12	<i>rotundifolia</i>	Yes	n/a	Vitis 50:173-175 Phytopathology 105:1104-1113
<b>RUN1.2a</b>	12	<i>rotundifolia</i>	Yes	No	Phytopathology 105:1104-1113
<b>RUN1.2b</b>	12	<i>rotundifolia</i>	Yes	No	Phytopathology 105:1104-1113
<b>RUN2.1</b>	18	<i>rotundifolia</i>	Yes	Maybe	Phytopathology 102:83-93 Phytopathology 105:1104-1113
<b>RUN2.2</b>	18	<i>rotundifolia</i>	Yes	No	Phytopathology 105:1104-1113
	?	<i>aestivalis</i>	Yes	Unknown	Phytopathology 102:83-93
	7?	<i>rupestris</i>	Yes	Maybe	Phytopathology 105:1097-1103

- Don't blindly pyramid and assume improved durability!
- *V. rupestris* B38, *Run2.1*, and *Ren2* may provide some minor protection of *Run1*.
- Need to find more sources and continue testing combinations

Acknowledgments: Bruce Reisch, David Ramming, Craig Ledbetter, Andy Walker, Summaira Riaz, Pal Kozma, David Gadoury, Bob Seem, Ian Dry, Angela Feechan, Michael Milgroom, Joe Smilanick, Molly Cadle-Davidson, Marianna Kocsis, Paola Barba, Omer Frenkel, Marin Brewer, Raj Majumdar, Siraprapa Mahanil, Sara Lagerholm, Michelle Schaub, Anna Nowogrodzki, Hema Kasinathan, Mary Jean Welser, Paige Appleton, Wei Zhang, Nancy Consolie, Jackie Lillis, Erin Galarneau, Franka Gabler, and others

# Haplotype analysis of PM field data



Novel identification of *REN10* on LG02

- Independently validated in PM Center
- Reduces colonization (Hyphal Transect)
- Reduces sporulation

Explains 25-40% of phenotypic variance



Soon Li Teh, J. Fresnedo-Ramírez, M. Clark, Q. Sun, L. Cadle-Davidson, J. Luby. 2016. Molecular Breeding (in press)

Fruit quality:  
positive and negative



# Marker for Fruit Chemistry (w/ Yang, Fresnedo, Sacks et al.)



Compound classes	# of putative QTLs
Anthocyanins	8
Organic acids	4
Herbaceous aromas (MPs, C6 aldehydes and alcohols)	4
Sugars, yeast assimilable nitrogen	7
Juice protein	1
<b>TOTAL</b>	<b>24</b>

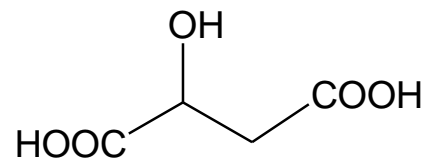
Data used in:

Cadle-Davidson, et al. (2016) "A next-generation marker genotyping platform (AmpSeq) in heterozygous crops: a case study for marker assisted selection in grapevine." *Horticultural Research*, **3** doi:10.1038/hortres.2016.2

Yang, Fresnedo-Ramirez et al. (2016) "Next generation mapping of enological traits in an F<sub>2</sub> interspecific grapevine hybrid family' ." *PLOS One*, 11(3): e0149560. doi:10.1371/journal.pone.0149560

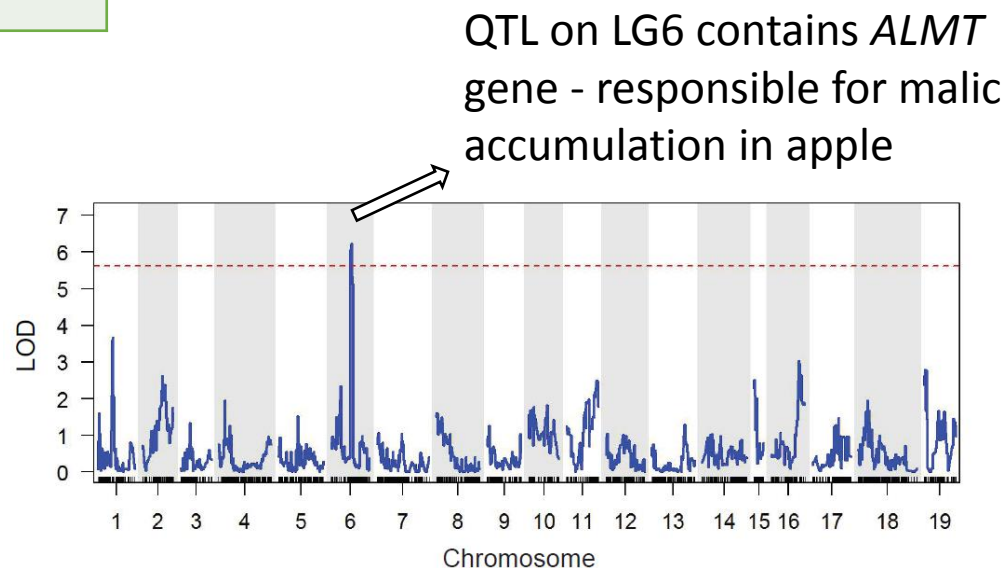
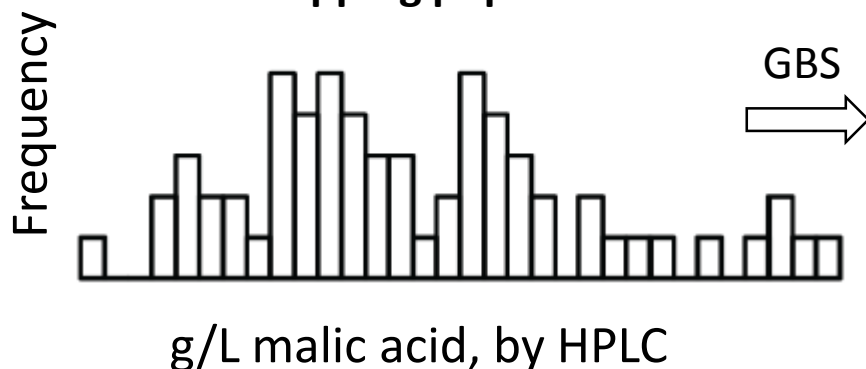
# A major challenge with wild *Vitis*— excess **sourness** from excessive malic acid

Source	Typical titratable acidity (g/L)
<i>V. vinifera</i>	5-7
Wild <i>Vitis</i>	15-40
Lemon juice	30-50
Cranberry juice	15-25



← *Mostly malic acid*

**Mapping population**



# Accelerating grape cultivar improvement



- ✓ Marker validation of SSR and AmpSeq markers
- ✓ Marker-assisted breeding on more than 15,000 seedlings

M.A.B. @ Cornell	2013	2014	2015	2016
No. seedlings:	2,595	4,829	3,848	3,755
Percent retained:	50.1%	40.1%	33.5%	26.4%
No. seedlings after nursery selection	591 (22%)	619 (13%)	508 (13%)	568 (15%)







13.0204 – marginally acceptable

13.0214 – best population



# AmpSeq for MASS



Progeny	Resistance	REN2 haploblock								RUN1 tag	REN4 tag	Note
a	s	T	T	A	C	C	T	C	T	0	1	None
b	s	Y	Y	A	C	C	T	C	T	0	0	None
c	s	Y	Y	R	M	M	K	Y	Y	0	0	Ren2
d	r	T	T	A	C	C	T	C	T	479	0	Run1
e	r	T	T	A	C	C	T	C	T	0	522	Ren4
f	r	T	T	A	C	C	T	Y	Y	0	302	Ren4
g	r	Y	Y	R	M	M	K	Y	Y	0	746	Ren2 + Ren4
h	r	Y	Y	R	M	M	K	Y	Y	497	0	Run1 + Ren2
i	r	T	T	A	C	C	T	C	T	576	601	Run1 + Ren4
j	r	T	T	A	C	C	T	C	T	468	319	Run1 + Ren4
k	r	T	T	A	C	C	T	C	T	287	458	Run1 + Ren4
l	r	Y	Y	R	M	M	K	Y	Y	270	577	Run1+ Ren2+ Ren4 (Stacked)
m	r	T	Y	R	M	M	K	Y	Y	233	713	Run1+ Ren2+ Ren4 (Stacked)
n	r	Y	Y	R	M	M	K	Y	Y	208	702	Run1+ Ren2+ Ren4 (Stacked)

✧ **Advantages:** reliability, flexibility, high-throughput, cost- effectiveness, ease-of-automation and speed

✧ A next-generation marker genotyping platform (AmpSeq) in heterozygous crops: a case study for marker-assisted selection in grapevine Yang *et al.*, *Hort. Res.* 2016 3, 16002, doi:10.1038/hortres.2016.2

# VitisGen Technology

Decrease cost



## GBS vs SNPChip for marker development

- 10- to 300-fold **increase** in marker density over Vitis9kSNP Array
- 10-fold **decrease** in marker cost per seedling = \$15
  - About \$0.00005 to \$0.00015 per marker

## AmpSeq vs SSRs for marker application

- 50-fold **increase** in multiplexing over SSRs
- Cost per sample similar to SSR = \$4-6
  - About \$0.02 per marker
- Discovering/genotyping SNPs at same time as SSR size alleles

# ***VitisGen* begins to “bear fruit”**



**NY12.0107.**

***“PM and DM resistant”***



**NY12.0118.**

***“PM and DM resistant”***





# NY06.0514.06

*“Run1/Ren2/Rpv1 Hybrid”*

- Excellent resistance to PM, DM, Bunch Rot
- Moderate resistance to Black Rot
- Moderately winter hardy (1<sup>o</sup> bud kill ~ -26 C)
- Midseason bud break
- Grafting may be needed ...
- Wine: fruity: blackberry, plum, cherry; slightly herbaceous: green pepper; good body, medium tannin; chocolate notes





# Summary of Accomplishments

- Developed pipelines and protocols for sample processing and data analysis for GBS and SSR markers
- 2.6M SNPs identified using a diverse collection of 16,609 vines
- Medium- to high-resolution genetic maps developed for 16 core families
- >70 Marker-trait associations identified
- MAS on over 20,000 seedlings
  - More and better markers: tools for breeders → *precision* breeding
  - Gene stacking – e.g. multiple loci for PM resistance
  - Larger populations can be planted and culled effectively in year one
- Developed AmpSeq: rapid and inexpensive method to detect GBS or gene-specific markers
- GBS markers: multitude of future uses in genetic studies



# The *Vitis*Gen team



## **Executive Committee:**

Bruce Reisch  
Lance Cadle-Davidson  
Anne Fennell  
Hans Walter-Peterson  
Julian Alston

## **Project Manager:**

Elizabeth Takacs

## **Genetics Team:**

Lance Cadle-Davidson  
Qi Sun  
Ed Buckler  
Jason Londo  
Peter Schweitzer  
Chris Owens  
Sean Myles  
Genomic Diversity Facility  
Cornell Core Laboratories



## **Funding:**

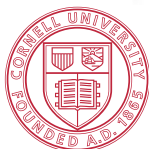
USDA-NIFA Specialty Crop Research Initiative  
Project Number 2011-51181-30635



United States Department of Agriculture  
National Institute of Food and Agriculture



E&J Gallo Winery



Cornell University

## **Trait Evaluation Team:**

Anne Fennell  
Gavin Sacks  
Anna-Katharine Mansfield  
Lance Cadle-Davidson  
David Gadoury  
Bob Seem  
Wayne Wilcox  
Cornell Wine Analysis Lab

## **Breeding Team:**

Bruce Reisch  
Lance Cadle-Davidson  
Anne Fennell  
Chin-Feng Hwang  
Craig Ledbetter  
Chris Owens  
Andy Walker  
Gan-Yuan Zhong  
Matthew Clark

## **Extension and Outreach Team:**

Hans Walter-Peterson  
Eric Stafne  
Jim Wolpert

## **Trait Economics Team:**

Julian Alston  
Scott Davidson  
Jayson Lusk

# The *Vitis*Gen team



## Advisory Panel:

Jean-Mari Peltier, NGWI  
Nick Dokoozlian, Gallo  
Dennis Rak, NY  
Jerry Lohr, CA  
Jack Okamoto, ARS  
Kathleen Nave, Table  
Grape Commission, CA  
Richard Smith, CA  
Andrew Zaninovitch, CA  
John Martini, NY  
Franka Gabler, CTGC, CA  
Peter Hofher, MO  
Tom Burr, Cornell U., NY  
Peter Cousins, Gallo  
Daniel Bosch,  
Constellation, CA  
Ed Buckler, ARS, NY



## Funding:

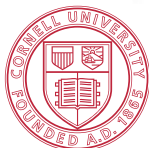
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Project Number 2011-51181-30635



United States Department of Agriculture  
National Institute of Food and Agriculture



E&J Gallo Winery



Cornell University

## Students/Postdocs/Technical Assistants:

Shanshan Yang  
Jonathan Fresnedo  
Soon Li Teh  
Paola Barba  
Katie Hyma  
Linda Cote  
Jacqueline Lillis  
Michelle Schaub  
Anna Nowogrodzki  
Hema Kasinathan  
Konstantin Divilov  
Steve Luce  
Mike Colizzi  
Connor Fortin  
Anne Repka  
Alex Isby  
Lindsay Springer  
Li-Ling Chen  
Surya Sapkota  
David Manns  
Elizabeth Burzynski  
Imelda Ryona