The First Version of the Whole-Genome Sequencing of the Muscadine Grape
(*Muscadinia rotundifolia* cv. Noble)

PRESENTED BY

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Our Muscadine Breeding Program

❖ Currently muscadine grapes is commercially grown in 12 states in the US.

❖ There is a demand for new muscadine cultivars with enhanced fruit/vinification qualities.

❖ Over the years, conventional breeding strategy has resulted in several muscadine cultivars suitable for table and wine markets with improved quality traits.

❖ However, the procedure is lengthy, laborious and expensive, which does not match with the rapid, ever-increasing industry needs.

❖ Our goal is capitalizing on the latest achievements of genomic technologies to develop breeding platform that meets the growing industry demands.

Grapevine Cultivars & Germplasm at CVSFR

Background	Total No. of Vines	No. of Individual Genotypes	No. of Cultivars	Adult [a]	Juvenile [b]
Muscadinia hybrids	3925	2537	51	664	1877
Vitis hybrids	3380	2432	172	589	1843
Muscadinia x *Vitis* (MV)	106	106	-	103	3

a)   3-year old and /or elder; Reproductive Phase.
b)   2-year old and or younger; Growth Phase.

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Grape

Muscadinia Genus (3)

M. rotundifolia
M. munsoniana
M. popenoei

40 chromosomes (*n*=20)

Vineyard-BMP-Supreme (*muscadinia*)-fresh fr...
Vineyard-BMP-Darlene (*muscadinia*)-fresh fru...

Euvitis Genus (14)

V. vinifera
V. labrusca
V. riparia
V. aestivalis
V. rupestris

38 chromosomes (*n*=19)

d-BMP-Stover (*Vitis* hybrid) white wine
Vineyard-BMP-Cynthiana/Norton (*Vitis* hybrid)-red...

Morphological Differences:
Leaves, flower type, tendrils, cluster size, berry size, and age of cutting for propagation.

Muscadinia sp. hold particular agronomical and reproductive traits:

❖ Resistance to most of diseases that limit the worldwide production of *V. vinifera*.

❖ Adapted to poor soil.

❖ Grow well under warm and humid conditions.

❖ Exotic fruit quality traits (berry size, unique flavor spectrum, therapeutic phytochemicals content).

❖ Unique vinification qualities.

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Muscadines have obviously different challenges from those of traditional 'vinifera' grapes

❖ Cold hardiness.
❖ Extended ripening season.
❖ Perfect flower with large berry size.
❖ Fruit quality traits (diversity in berry color & shape, seedlessness, large cluster, thinner skin, dry stem scar, uniform ripening, extended shelf-life).

Male

Female

Perfect

Wet stem scar
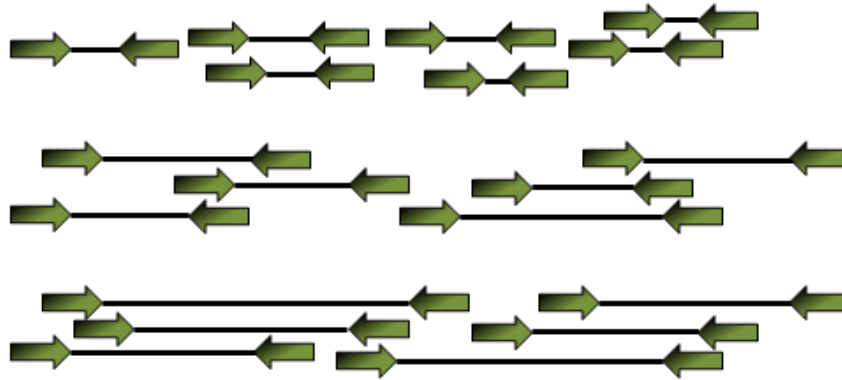
Whole – Genome Sequencing of Muscadine Grape Noble cv.

Main red wine muscadine cultivar released by Nesbitt, 1973.

Thomas (♀) x Tarheel (♂)

- High vigor growth.
- Disease resistant.
- Self-fertile (perfect flower).
- Midseason.
- Uniform ripening.

- Black small berries (3.8 – 4.3 g).
- High yield (9.6 – 10.7 tone/acre).
- Stable purple pigments.
- Suitable for wine and juice production.

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Muscadine Genome Sequencing & Assembly
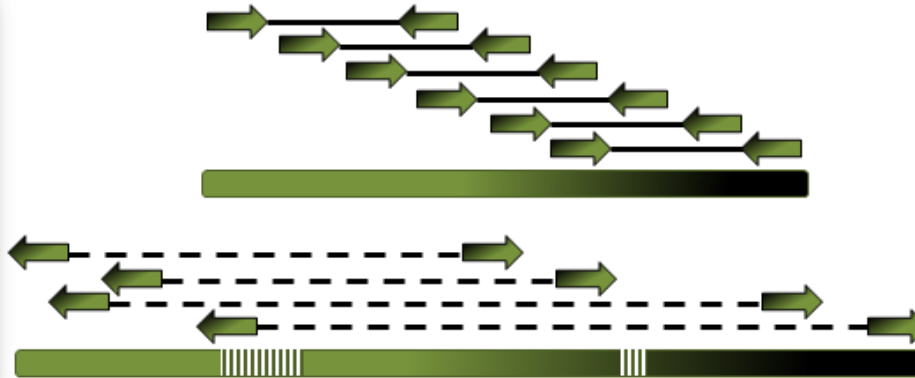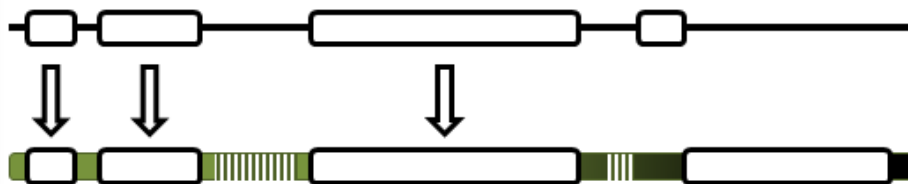
Muscadine Genome Assembly Statistics

Bar chart: Chromosome size (Mbp) vs *V. vinifera* chromosome name. Legend: *V. vinifera* (green), *M. rotundifolia* (purple). X-axis chromosomes: 14, 18, 5, 13, 19, 4, 1, 9, 12, 8, 16, 6, 7, 15, 11, 3, 2, 10, 17, 20.
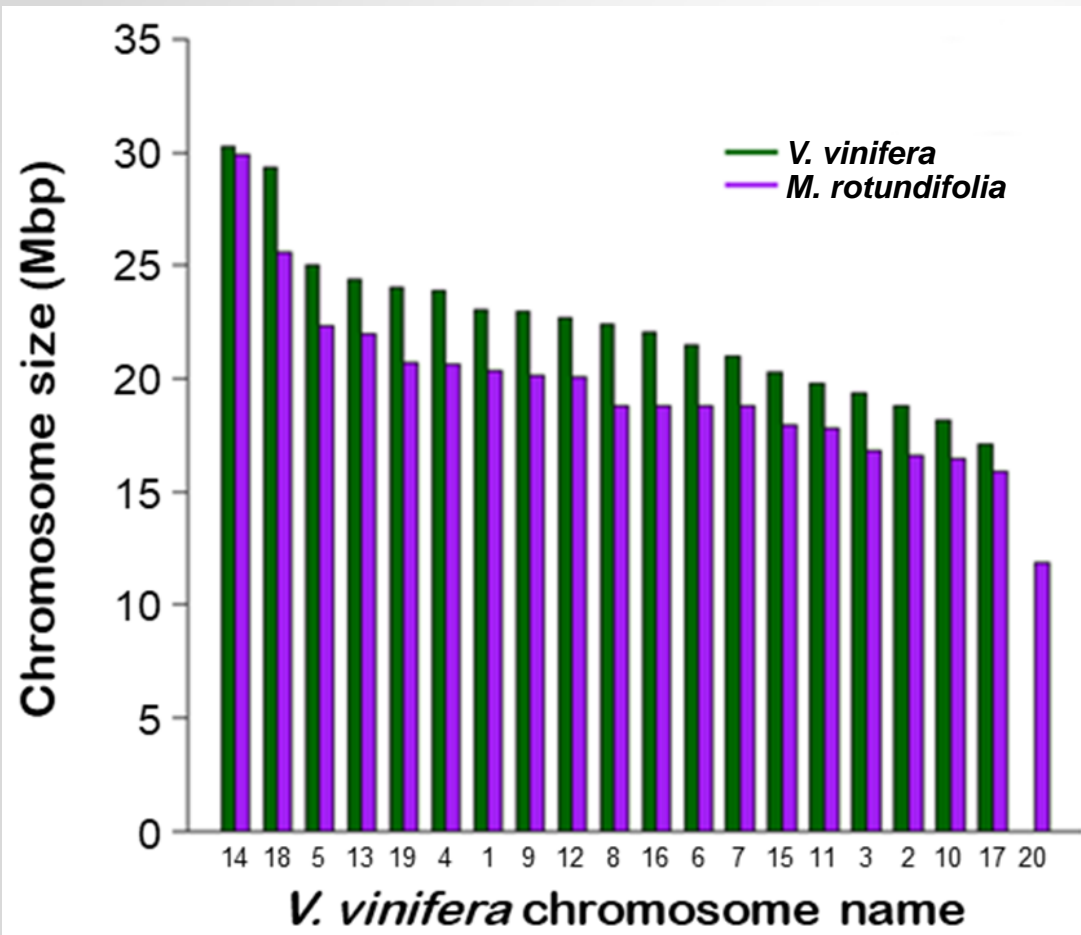
Estimated genome size	**414 Mb (82%)**
Assembly Size	**400.26 Mb (96.6%)**
Contig N50	107 kb
Contig L50	1,011 contigs
Scaffold N50	20.045 Mb
Scaffold L50	9
Scaffold N90	16.486 Mb
Scaffold L90	18
Number of scaffolds	1,629
Heterozygosity	**1.47%**
Unplaced sequence	2.47%
Contig gaps	8,168

Benchmarking Universal Single-Copy Ortholog (BUSCO)

Assessment of muscadine genome assembly and gene set using BUSCOs indicates highly-complete representation of protein-coding genes.

Statistics searching Embryophyta BUSCOs:

1382   Complete BUSCOs (96%).
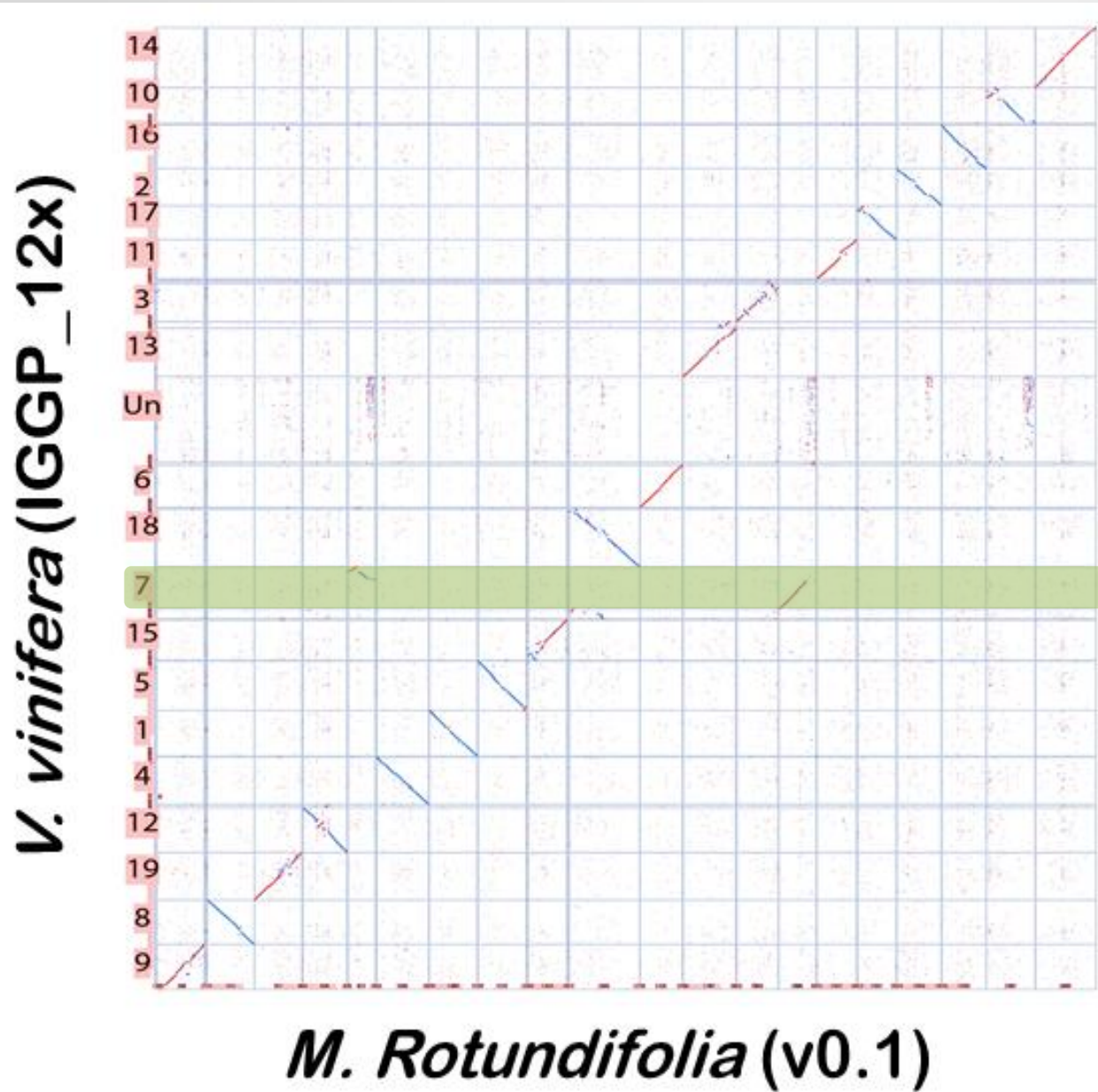
❖ 1339   Complete and single-copy BUSCOs (93%).

❖   43   Complete and duplicated BUSCOs (3%).

17   Fragmented BUSCOs (1.2%).

41   Missing BUSCOs (2.8%).

1440   Total BUSCO groups searched.

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M. rotundifolia and *V. vinifera* display high colinearity

The extra *Muscadinia* chromosome is composed of two large segments homologous to *Vitis* chromosome 7

Characterization of muscadine population

A population of 400 muscadine genotypes (50 standard cultivars and 350 lines) has been carefully selected to ensure diversity and subjected to:

1. **Phenomic Characterization**

❖ Fertility traits (bud fertility, bud fertility coefficient, position of first fruiting bud);

❖ Cluster physical traits (intensity, size, weight, number and weight of berries/cluster);

❖ Berry physical traits (scar pattern, color, size, weight, number and weight of seeds/berry, firmness);

❖ 50 berries traits (berries weight, pomace weight, juice volume);

❖ Yield traits (No. of clusters/vine, yield/vine); and

❖ Berry quality traits (TSS, acidity, pH).

Characterization of muscadine population

2. Phenology traits

- Bud break.
- Green shoot visible.
- Visible inflorescences.
- Partial bloom.
- Full bloom.

- Fruit-set.
- Cluster closure.
- Veraison.
- Post-Veraison.
- Ripening.

## 3. Biotic stress traits

- Leaf roll virus (several strains); and
- Fungal diseases (powdery and downy mildew).

Characterization of muscadine population

4. Metabolomic and sensorial traits

- ❖ Aroma volatile esters production.
- ❖ Total phenolic.
- ❖ Total flavonoid.
- ❖ Antioxidant activity.
- ❖ Antimicrobial activity (*E. coli*).
- ❖ Anticancer activity (Breast cancer cell lines MDA-MB-231 and MDA-MB-468).

Anticancer activity of muscadine extracts using African American breast cancer cell lines
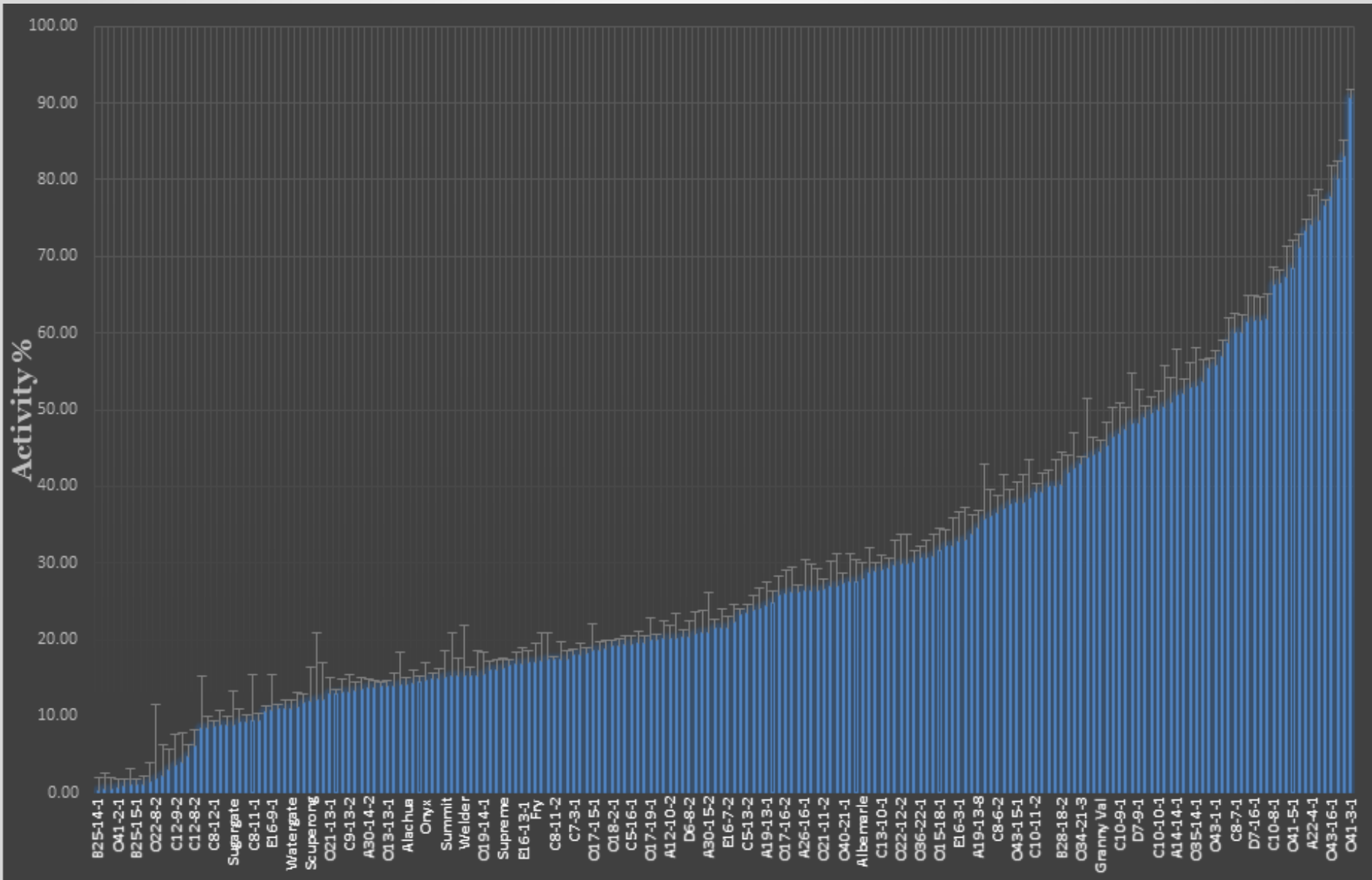
Classification of muscadine genotypes based on their anticancer activity
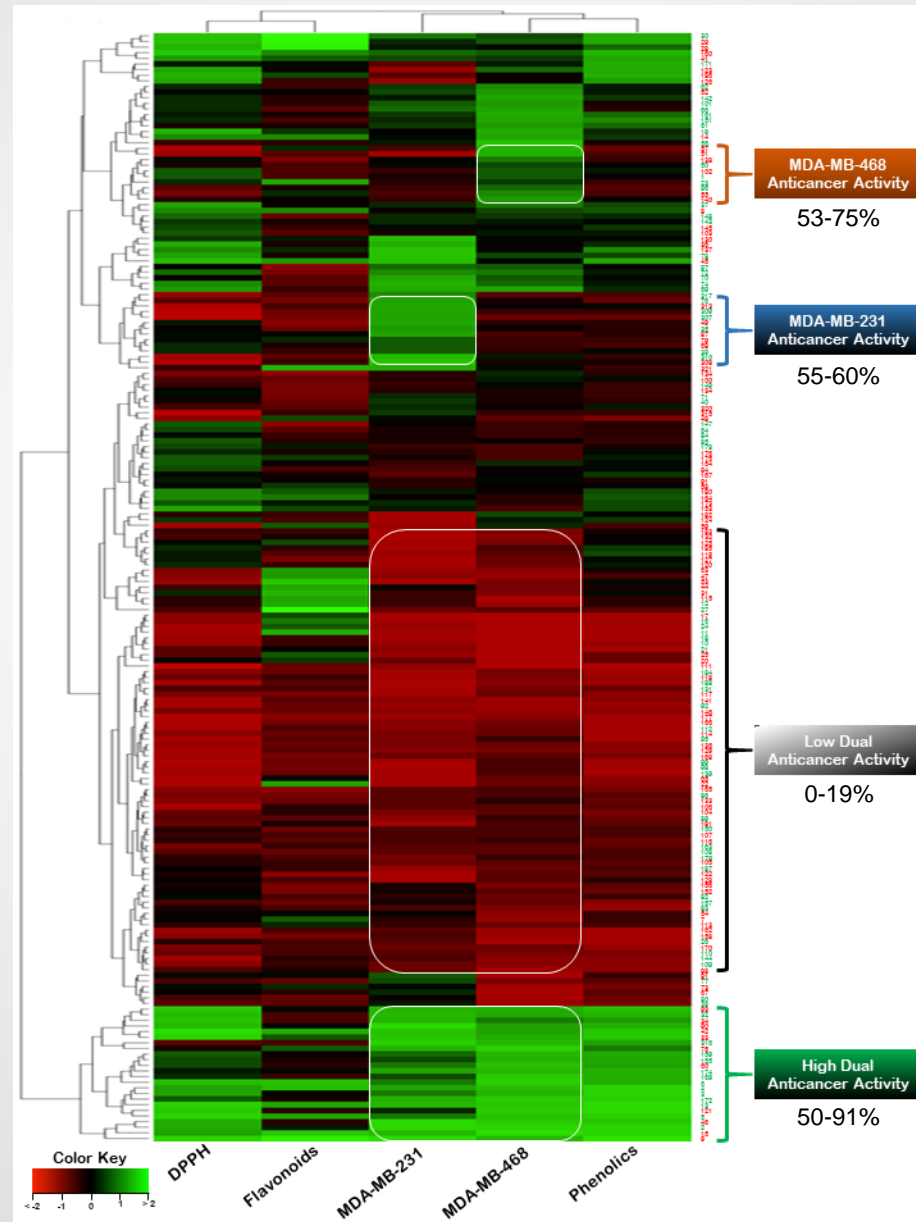
Undergoing

❖ Generating Pacbio Iso-seq and Illumina RNA-seq data on multiple tissues to generate accurate and comprehensive gene predictions.

❖ Performing ATAC-seq (Assay for Transposase-Accessible Chromatin using sequencing) to determine the chromatin accessibility of muscadine genome.

❖ Analyzing of *M. rotundifolia* genes' PAV (presence-absence variation) and their predicted functions compared to *V. vinifera*.

❖ Complete QC analysis and submission to Phytozome.

Acknowledgement

Team Members

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