

ROSBREED

Enabling marker-assisted breeding in Rosaceae

New Age of DNA Marker Discovery in the Rosaceae

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RosBREED- Vision

Integration of modern genomics tools with traditional breeding approaches will transform crop improvement in

Rosaceae, significantly improving profitability and sustainability of U.S. rosaceous crop industries & contribute to the increased consumption and enjoyment of these fruit, nut and floral products.

Amy Iezzoni, Michigan State Univ

Cameron Peace, WA State Univ

4 yrs

\$7.2M federal

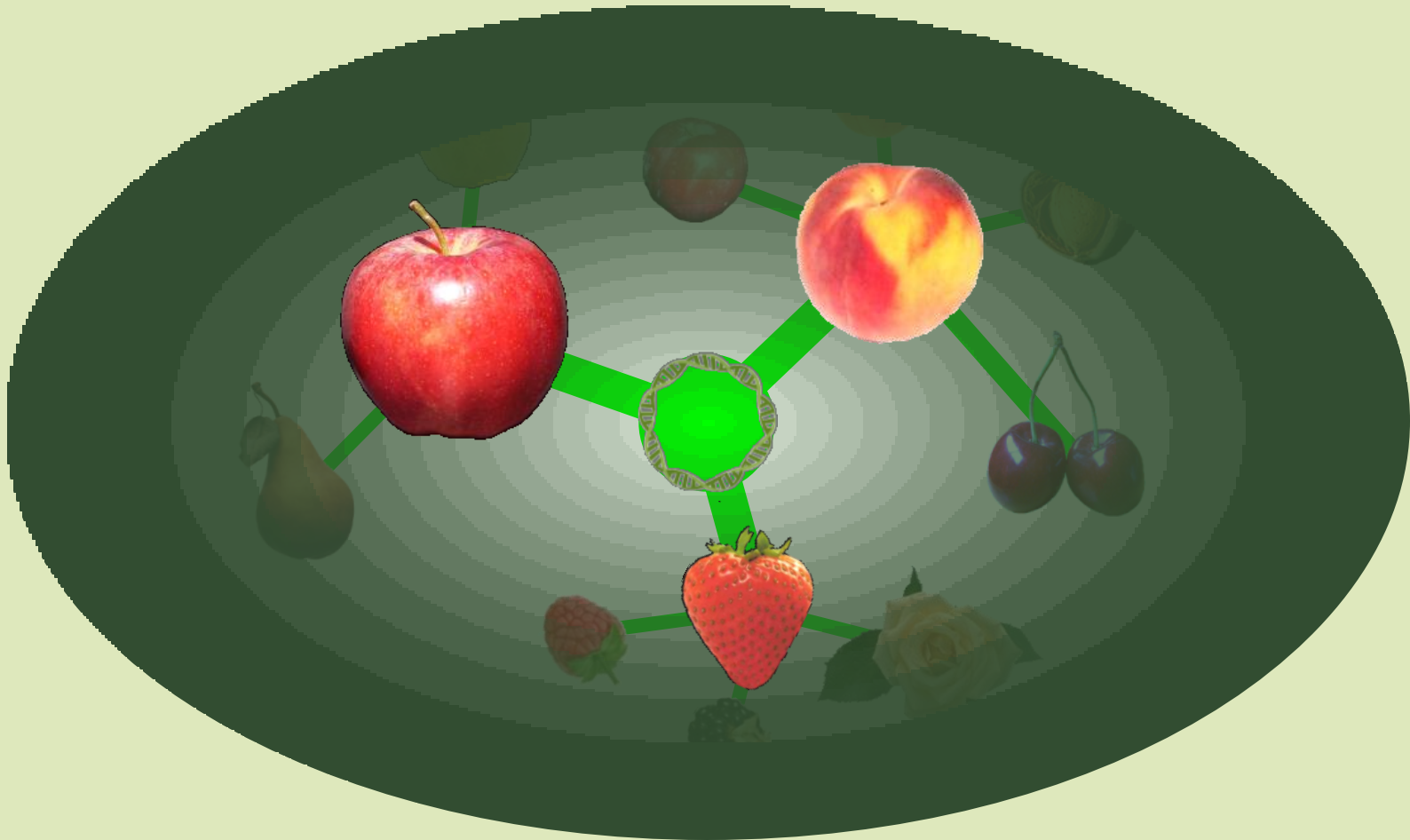
\$7.2M matching



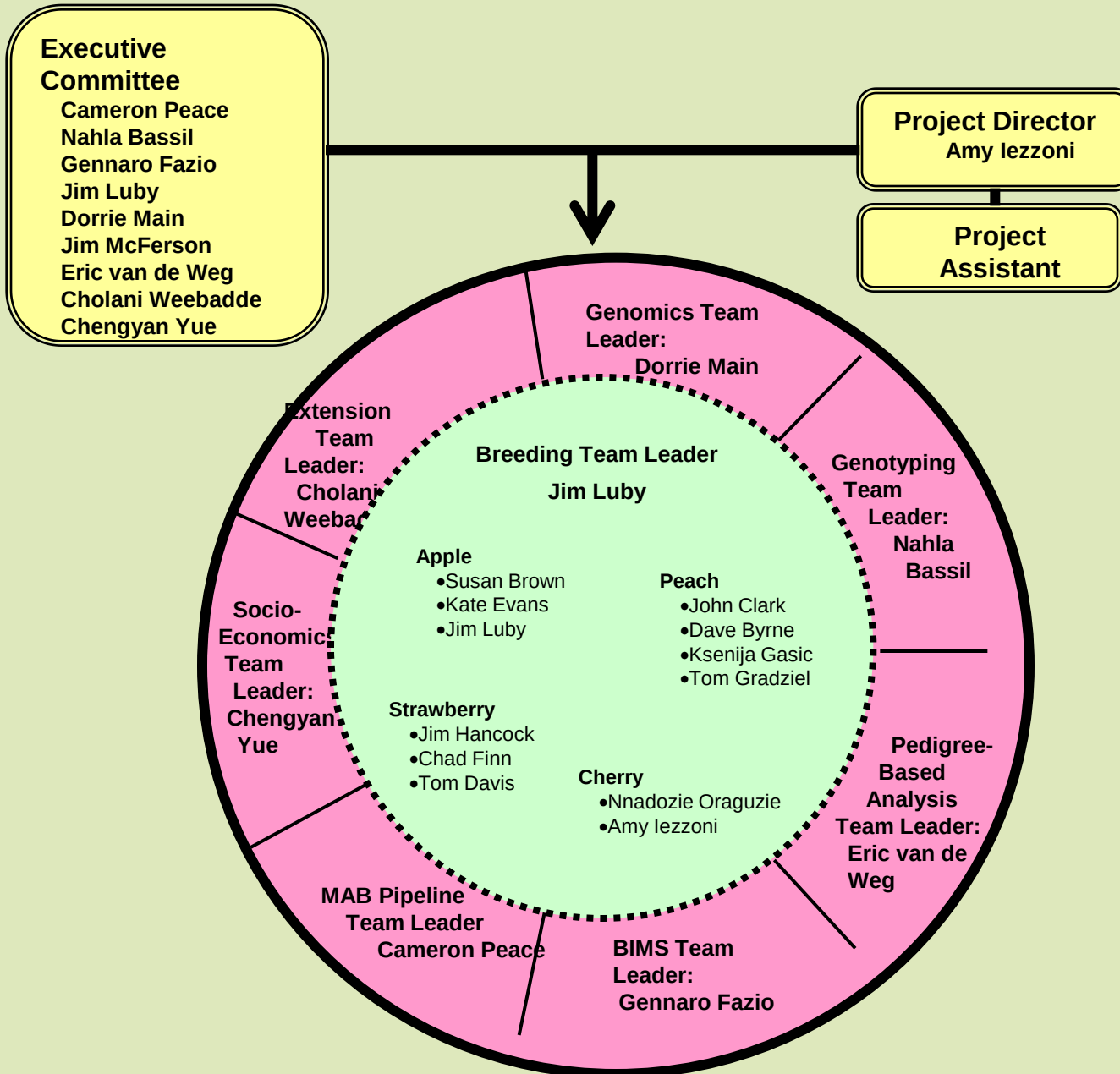
This project is supported
by the Specialty Crops
Research Initiative of
USDA's National Institute of
Food and Agriculture

Five Crops

Apple, strawberry, peach and sweet & tart cherry:
Focus on Fruit quality



RosBREED Organization



RosBREEDs International Partners.



Plant Research Intl., NL
East Malling Research, UK
INRA – Bordeaux, Avignon & Angers, France
CRA-FRU, Rome, Italy

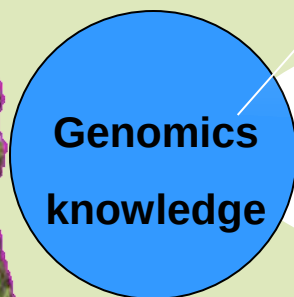
IGA, Udine, Italy
Andres Bello University, Chile
ARC, SA
Plant & Food Research, NZ

RosBREED bridges this chasm

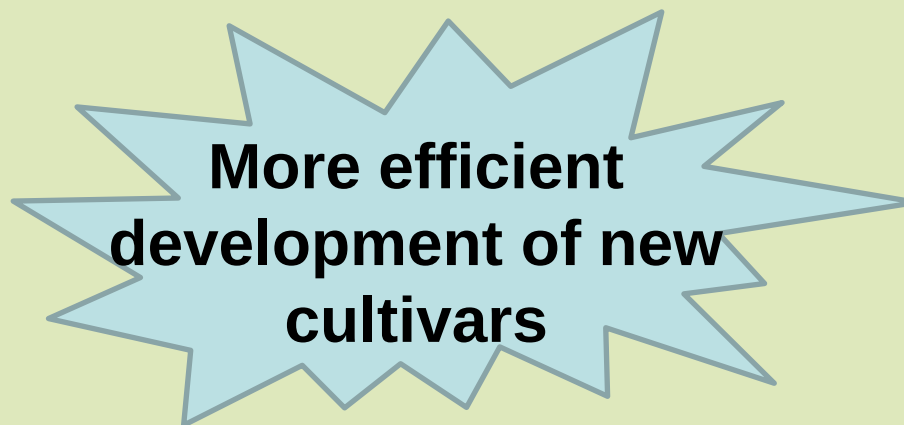
Genomics
Resources



Genomics
Research



Breeding
Programs



Overview of RosBREED Deliverables

Market trait values assessed from

- * Breeders
- * Market Intermediaries
- * Producers
- * Consumers

Germplasm data sets

- Crop Reference Sets
- Breeding Pedigree Popns
- * Plant material
- * Phenotypic data
- * Genotypic data

Enabling technologies

- * Statistical software
PediMap, FlexQTL
- * Genotyping platforms
- * MAB Pipeline



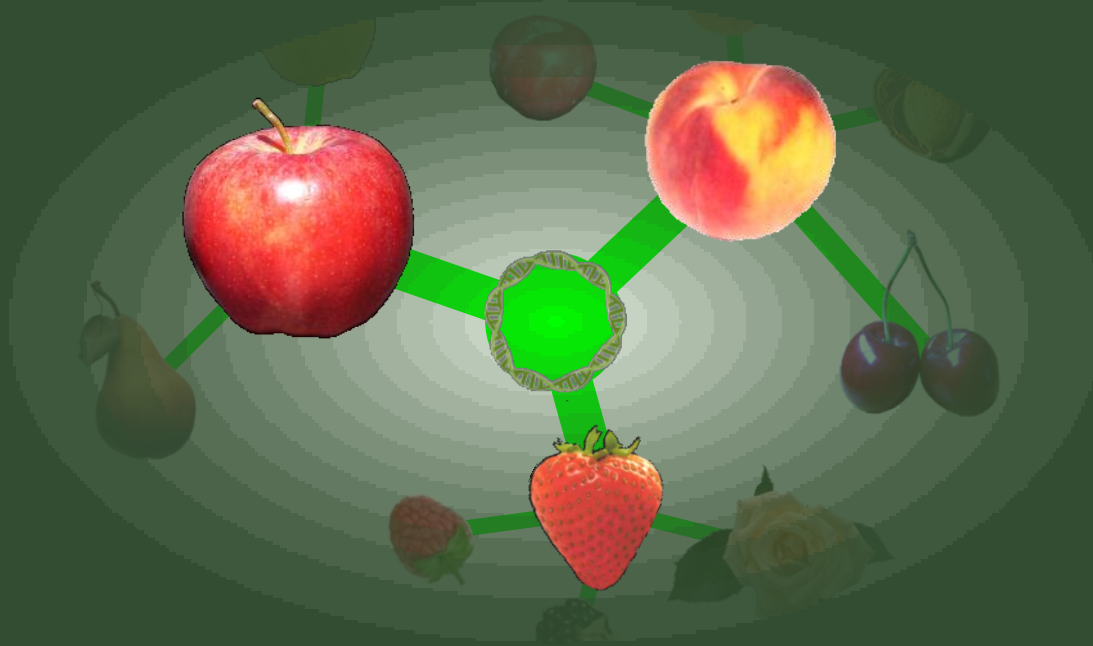


DNA Markers Developing

High throughput
SNP genome
scans
(SNP Consortium)



Markers for MAB



Markers Testing for MAB

Fast-Tracked Pipelining in 2011 for Fruit Quality

Peach

1. Texture: *endoPG* *F-M* locus
2. Flavor: sweetness & acidity QTL



Cherry

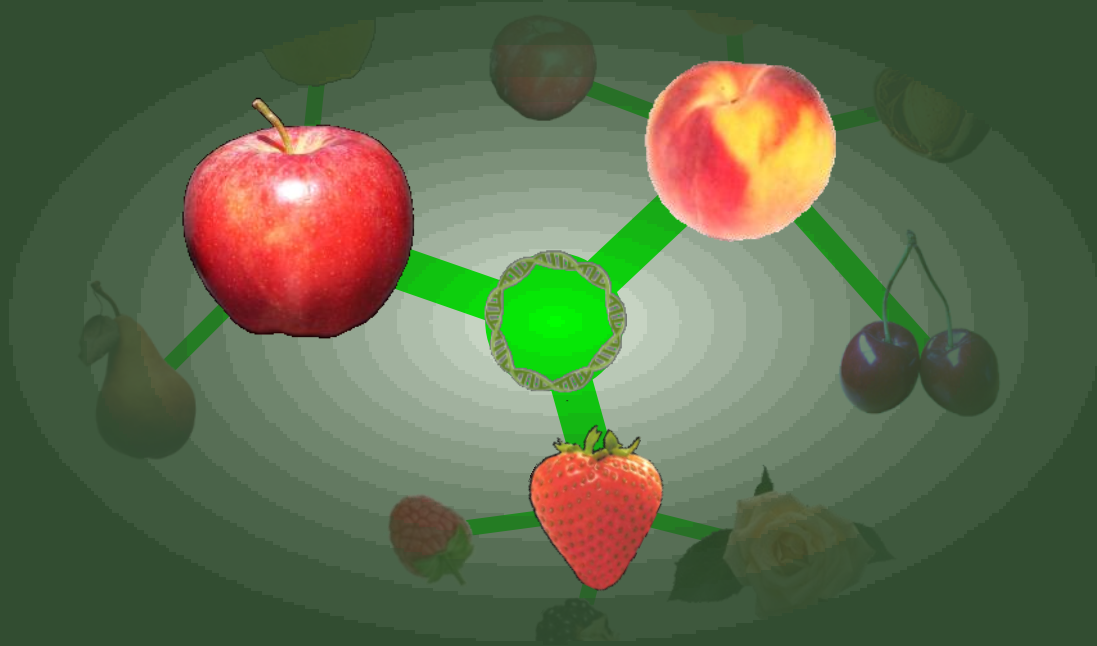
1. Fruit quality: fruit size and firmness QTL
2. Flavor: acidity QTL



Apple

1. Flavor and Texture: acidity, crispness, juiciness QTL
2. Texture: Firmness QTL





High Throughput SNP Genome Scans

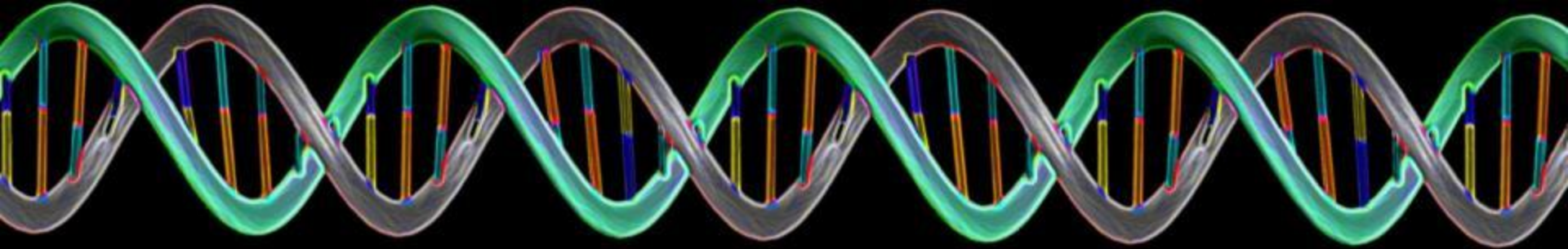


Image courtesy of NSF

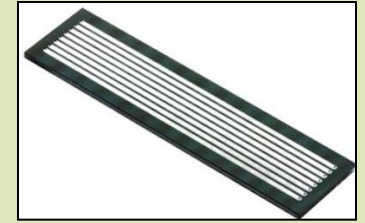
Reference Genomes Now Available

Apple, peach & diploid strawberry genome



Images courtesy of www.beakandskiff.com, www.ehow.com, Jim Hancock

Infinium Chip Development: Apple, Peach and Cherry



Re-sequencing of
SNP Detection Panel:

Illumina

Jasper Rees, ARC, SA
David Chagne, PFR, NZ

Ignazio Verde, Italy
Pere Arus, Spain

30		57		16		8	
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Alignment to reference genome: **apple, peach**

Chromosome	Position	RefBase	Best Base	2ndBestBase	SequenceDepth
scaffold_1	167	A	G	A	32
scaffold_1	6537	T	C	T	17
scaffold_1	6730	A	T	A	24
scaffold_1	7335	G	T	G	26
scaffold_1	7839	A	G	A	28

SNP detection and filtering: **SOAP, CLC BIO**

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Infinium Chip Development: Apple, Peach and Cherry, cont.

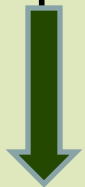
SNP validation: **GoldenGate**

384 SNPs

480 DNA samples



SNP Consortium Community
chooses parameters for Infinium
chip: **SNP Summit**

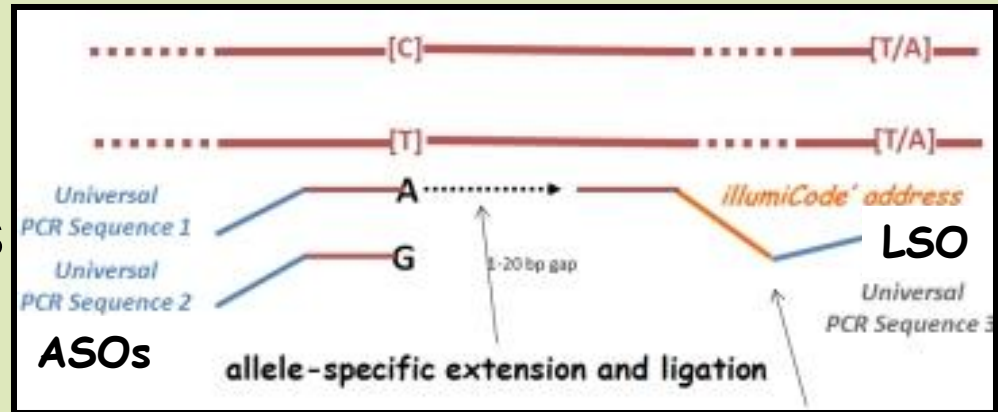


Final filtering

Infinium chips ordered

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Criteria for SNP Validation Using GoldenGate Assay

- Equidistant on each scaffold
144, 96, 96, 48 in apple, peach, sweet and tart cherry
- Location in genome
(40%: genes; 20%: introns; 20% promoter; 20% intergenic)
- 20% accession-specific
- Zoomed-in region



Overview of GoldenGate Validation Results

Crop	Failed	Monomorphic	Polymorphic	Total
Apple	46	25	73	144
Peach	25	20	51	96
Sweet Cherry	38	30	28	96
Tart Cherry	13	20	15	48
Total	122	95	167	384



Cindy Lawley, Illumina



Umesh Rosyara, MSU

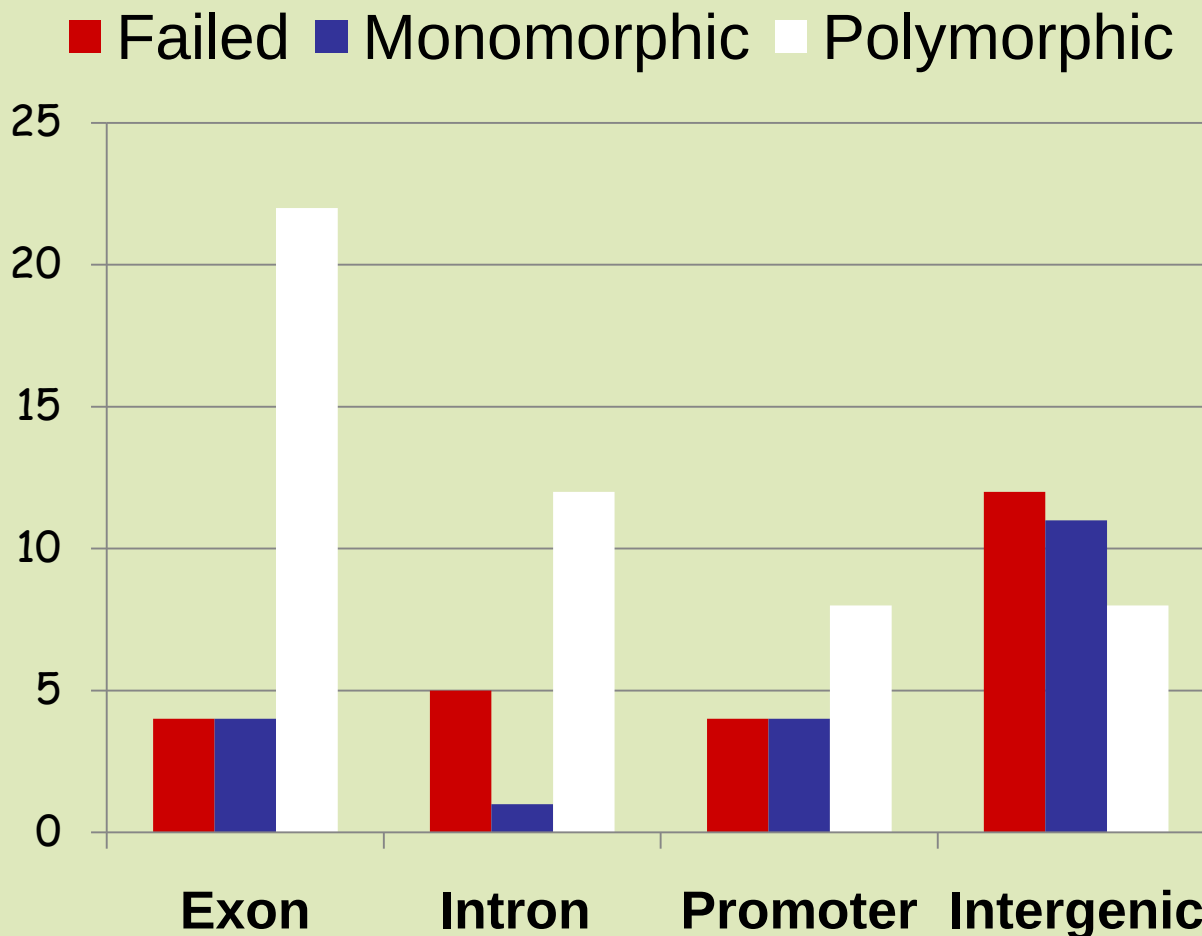
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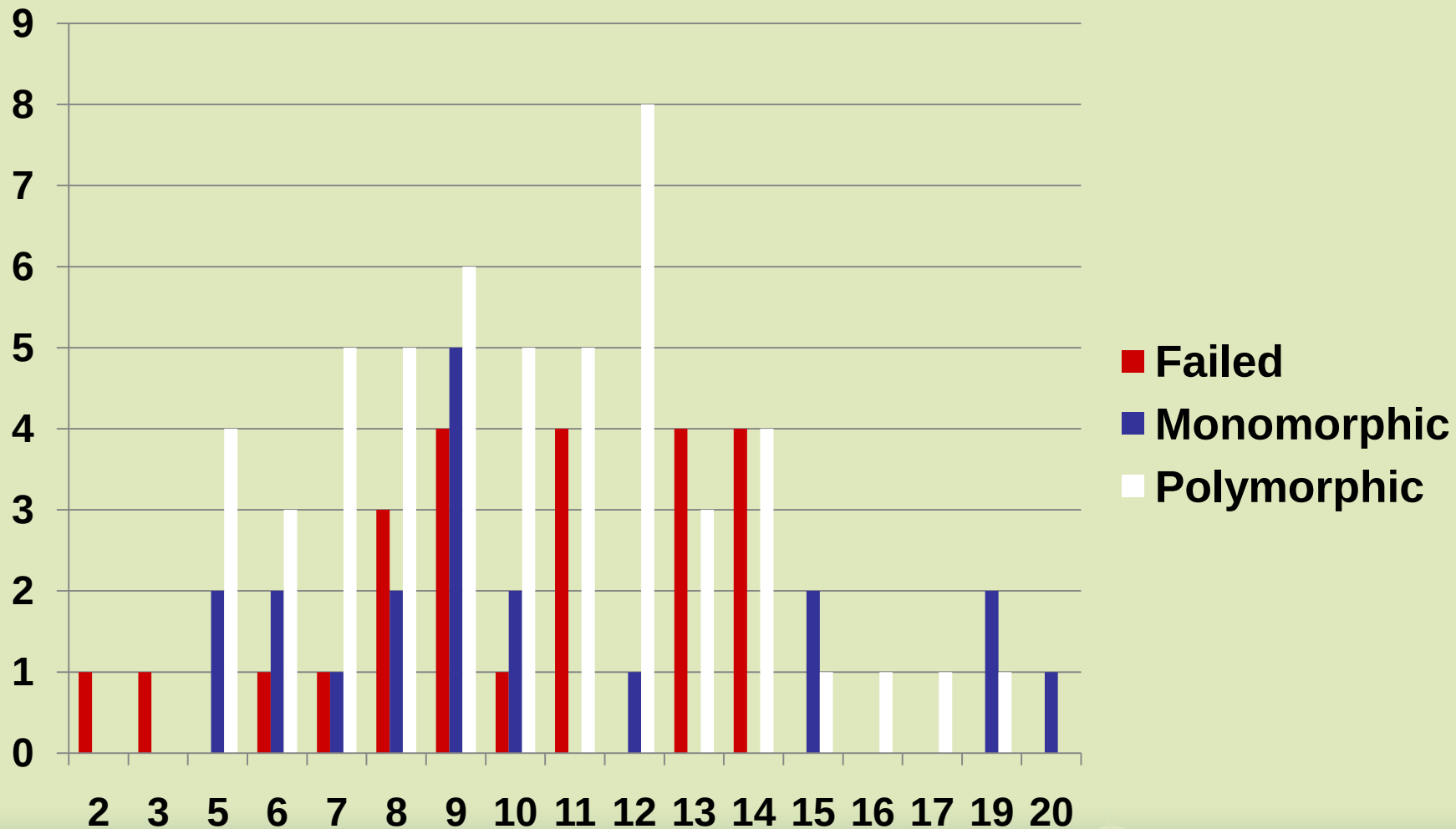


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Effect of SNP Location (Peach)



Effect of Number of Peach Accessions Containing SNP data



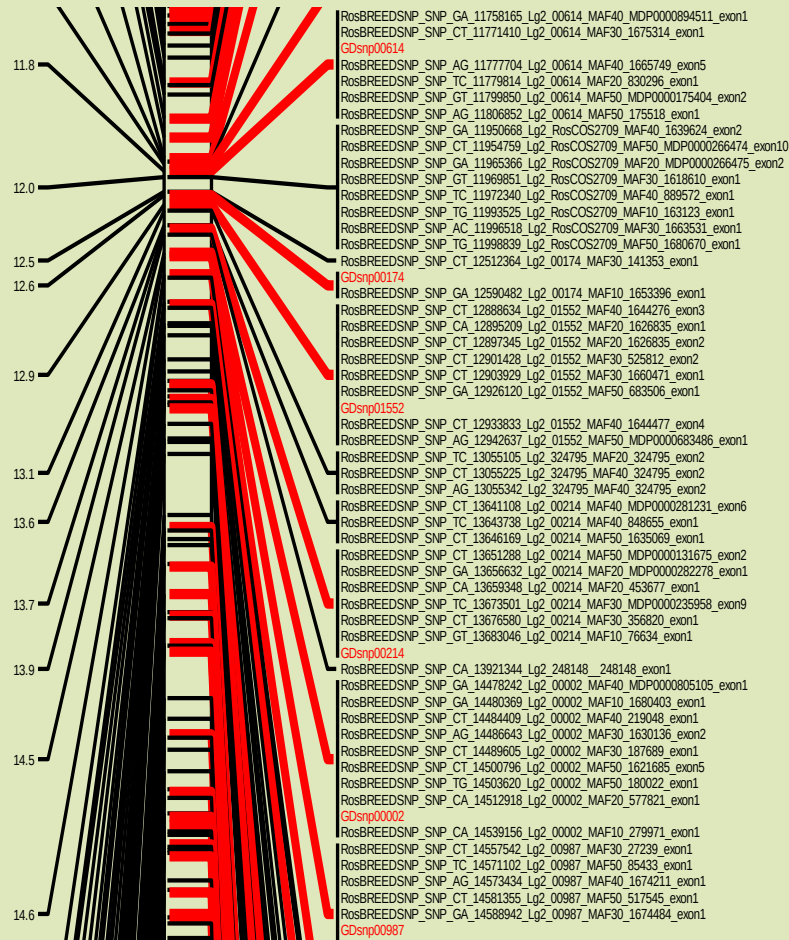
9K Infinium Apple Chip



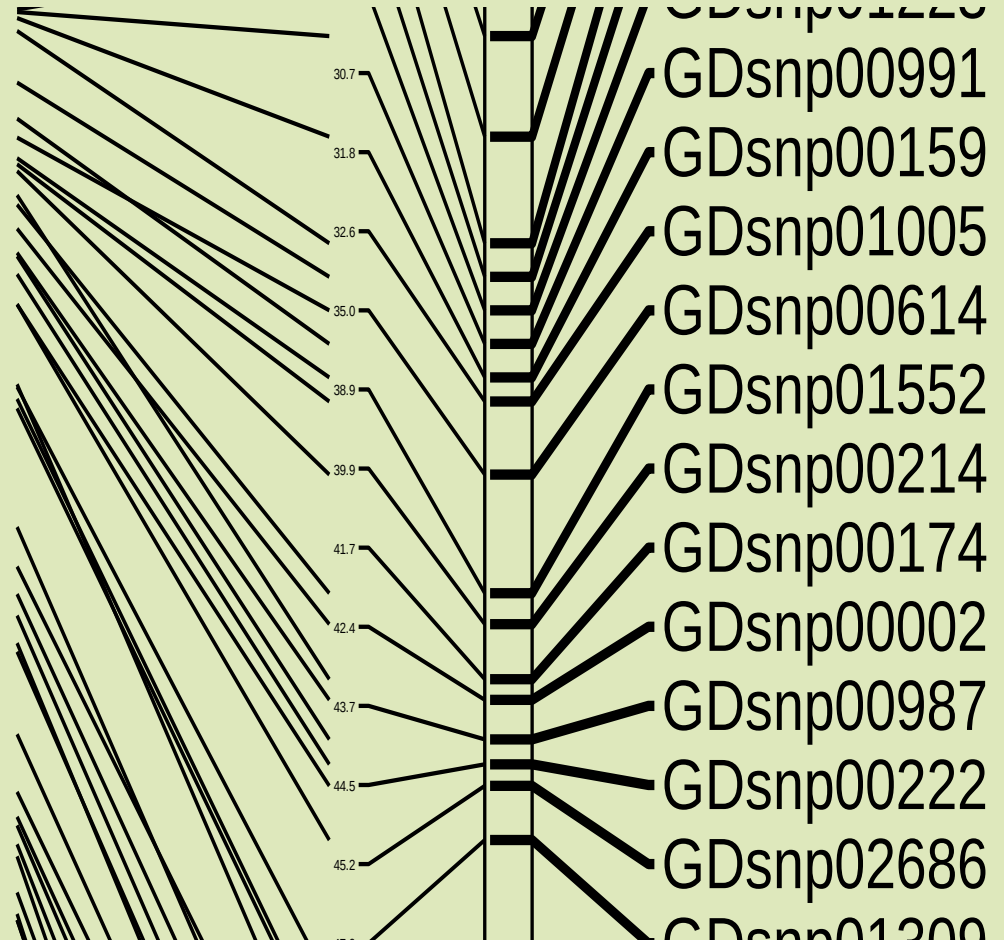
- 🍏 Exonic SNPs based on GD gene prediction and ESTs
- 🍏 Exclude Infinium I assay needing two probes (no C/G and A/T transversions)
- 🍏 Evenly spread loci ('focal points') on 'Golden Delicious' genetic map (1343 cM)
 - Clusters of SNPs in 200kb regions around GD_SNPs (max 10 SNPs per cluster) and ranging in minor allele frequency (MAF: 0.1, 0.2, 0.3, 0.4):
6,009
 - Fill in gaps between GD_SNPs using RosCOS (**128**), candidate genes (**1,091**), GG-validated (74), GD_SNPs (**693**)
- 🍏 Pear SNPs: IASMA SNPLex-validated (**69**), and in pear sequences aligning to apple TF (**1,064**)

Close up (LG2)

Genome sequence



Genetic map



9K Infinium Peach Chip



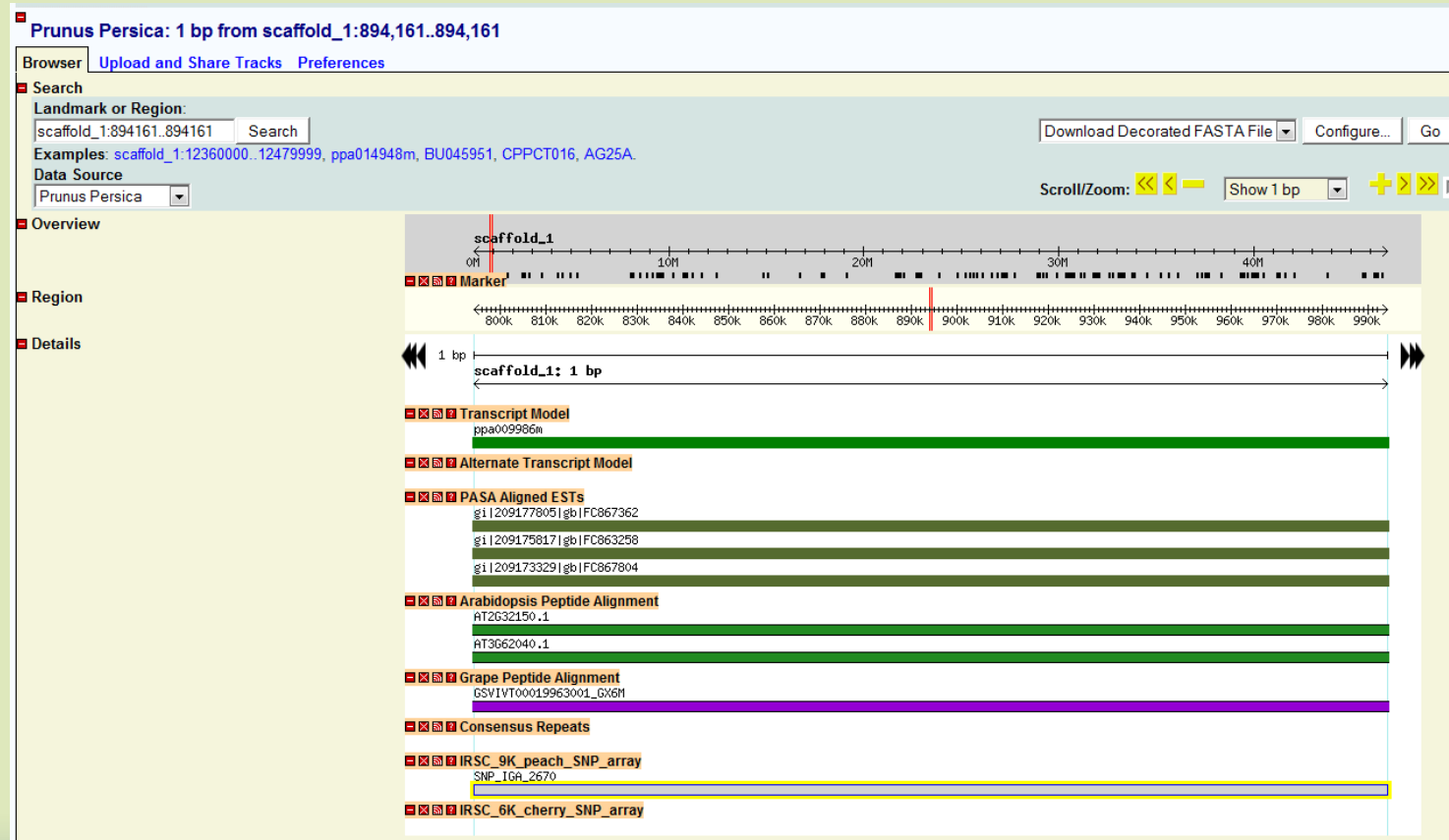
- 🍑 Exonic SNPs
- 🍑 Exclude Infinium I
- 🍑 Evenly spread loci around sequestered SNPs consisting of:
RosCOS (225), candidate genes (30), mapped (87) and GG-validated (45) SNPs.

Peach SNP Array: Downloadable excel file with direct link to GDR peach GBrowse

1	This file contains the SNPs selected for inclusion on the International Rosaceae SNP Consortium (IRSC) Peach SNP Infinium Array that is currently in production					
2						
3	SNP Name	SNP Type	Scaffold	Position	Gbrowse Link	Flanking Sequence
4	SNP_IGA_679	T/C	scaffold_1	94646	SNP_IGA_679	CACTATTGTTAGCAGCCTGCAAGAATGCATTTTG/
5	SNP_IGA_1003	A/G	scaffold_1	149552	SNP_IGA_1003	TGAAAATTTGAATTTTGTAGCTCTTGATAAAATTAA/
6	SNP_IGA_1034	A/G	scaffold_1	155117	SNP_IGA_1034	TAATTTGTGGTGCTTGTGAGCTTTCAGAAAGATC
7	SNP_IGA_1103	A/C	scaffold_1	186183	SNP_IGA_1103	GAAGGAAATGTTGGAGGGTGGTGCCAAATTCCT
8	SNP_IGA_1129	A/G	scaffold_1	209703	SNP_IGA_1129	TTGTGTTTATTCTATGTAATGTATGGTACTGTTGA
9	SNP_IGA_1135	A/G	scaffold_1	217942	SNP_IGA_1135	TAAACCTATTGCACGCATTTCTCATGTACTAGTA
10	SNP_IGA_1432	A/C	scaffold_1	346295	SNP_IGA_1432	CCCAATTAACGAAAAAGGTTTTCCACATGTTGA/
11	SNP_IGA_1455	T/C	scaffold_1	365034	SNP_IGA_1455	CAGTGCAAAACGCCTAAACATGCACCCAGTTCC/
12	SNP_IGA_1640	A/C	scaffold_1	444438	SNP_IGA_1640	ATCTCCTCCTGAATATGGTCCAATTCTATGGCTCT
13	RosCOS1201-071	T/G	scaffold_1	450381	RosCOS1201-071	TTATTTTATCTAGTTACTTCGCATTTTCGTTACTCAA/

6K Infinium Cherry Chip

- Exonic SNPs
- Exclude Infinium I
- Sweet Cherry (4,180), tart cherry (1,551), RosCOS (227), candidate genes (2)



Cherry SNP
Chip:
Downloadable
excel file with
direct link to
GDR peach
GBrowse



Infinium Chips will be used via Pedigree-Based Analysis to identify and validate marker-locus-trait associations for **application in breeding**





RoSBREED's

MARKER-ASSISTED BREEDING PIPELINE

*SOCIO-
ECONOMICS
INFORMATION*

*DNA
INFORMATION*

P
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1. Choose valuable, impactful targets
2. Choose efficient genetic screening technologies and service providers
3. Adapt reported genetic tests to local genetic screening approach
4. Validate genetic tests across crop
5. Assess utility of genetic tests for breeding germplasm
6. Inform parent selection and crossing
7. Identify efficient seedling selection schemes
8. Trial seedling selection schemes

1

2

3

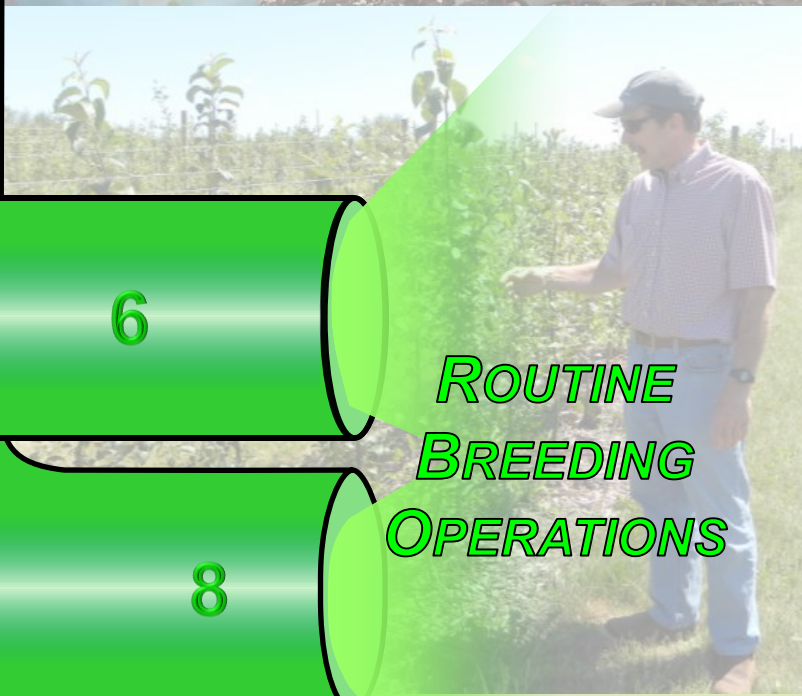
4

5

7

6

8



*ROUTINE
BREEDING
OPERATIONS*

To Achieve Our Dream

Ultra-crisp tasty apples
sweet juicy peaches
flavorful cherries
luscious strawberries

Consistent quality, available & affordable

Enjoyed by consumers, regularly

Sustainably produced throughout the U.S. and worldwide





Sequencing and Bioinformatic Support

OSU

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Intuitive Genomics

Doug Bryant
Todd Mockler

intuitivegenomics

GDR, WSU

Stephen Flitkin



MSU

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IGA, Italy

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Rutgers

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PFR, NZ

Ross Crowhurst



Illumina

Mark Hansen



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Questions?

