Developing a low-cost, low-tech assay for identification of commonly grown sweet cherry varieties in California

Principal Investigators:

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California Cherry Board Research Program January 17, 2023

Introduction

- Sweet cherries are delicious and rich in vitamins and mineral nutrients that benefit human health.
- In California, sweet cherries are grown not only for local consumption, but also for shipping to other states in the U.S. and internationally.
- There are several sweet cherry varieties that are commonly grown in California, including Bing, Brooks, Chelan, Coral Champagne, Rainier, and Tulare.
- Cherry breeders have also developed new early-season varieties.

RESEARCH PRIORITIES RELATED TO "POST HARVEST"

For the 2021-22 fiscal year (FY), the California sweet cherry industry identified the following post-harvest-related challenges of greatest priority for addressment through intentionally designed research:

- Post-harvest insect disinfestation: alternatives to methyl bromide as a fumigant for post-harvest disinfestation of sweet cherry intended for export markets
- Sweet cherry varietal identification: a cost-effective bioassay that would permit the identification of a sweet cherry variety using fruit tissue samples

- The goal is to ensure that consumers are informed of the sweet cherry varieties that they purchase from stores.
- These assays could also be used towards varietal identification of scions received at the orchard.

- Identification and certification of plant varieties have become increasingly important and are enabled by advancements in molecular biology and genomics in recent years.
- DNA-based diagnostics has been used in fruit crops such as strawberry, grape, and olive.

Objectives

Objective 1. To identify molecular markers that differ among commonly grown sweet cherry varieties in California.

Objective 2. To establish and validate a low-cost, low-tech assay for identification of commonly grown sweet cherry varieties in California.

Objective 1. To identify molecular markers that differ among commonly grown sweet cherry varieties in California.

- We will identify Single Nucleotide Polymorphisms (SNPs) and Simple Sequence Repeats (SSRs)—the most common DNA sequence variations present in plants—in sweet cherry varieties.
- The molecular marker-based assay will be able to identify the sweet cherry varieties that are considered as important by the California Cherry Board.

Extraction of high-molecular-weight genomic DNA from young leaves of sweet cherry varieties.

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PacBio Single-Molecule Real-Time sequencing (SMRT) library construction

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PacBio SMRT sequencing

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Bioinformatics analysis of sequencing data to identify SNPs and SSRs

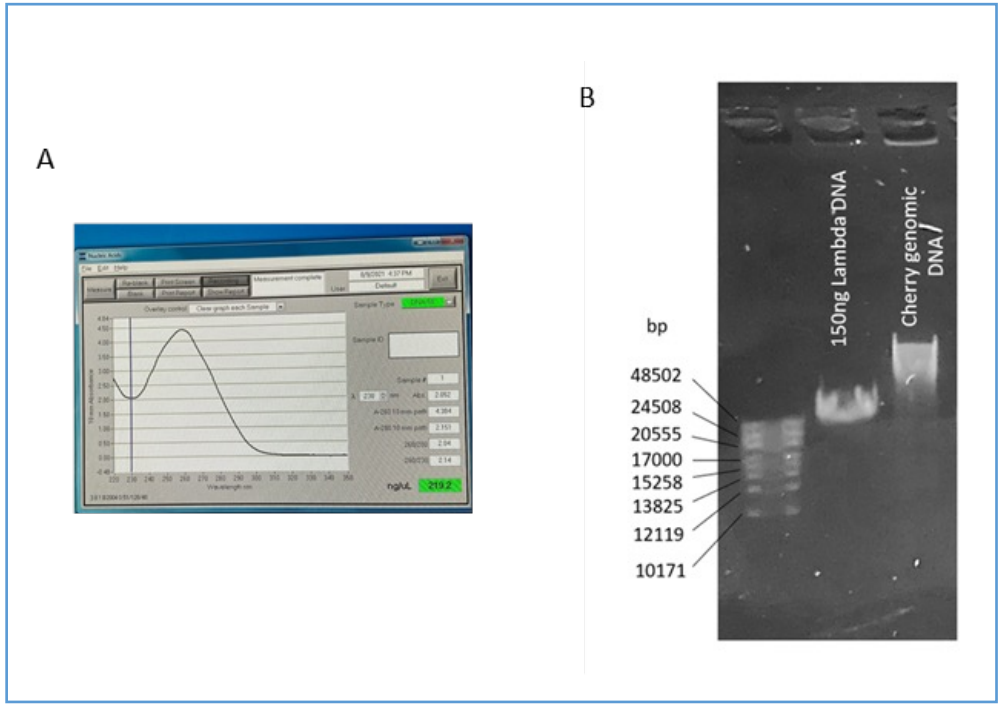
Leaves of 9 sweet cherry varieties were collected from Dave Wilson Nursery

Bing, Coral Champagne, Tulare, Brooks, Royal Tioga, Lapin, Sweet Heart, Royal Hazel, and Black Pearl

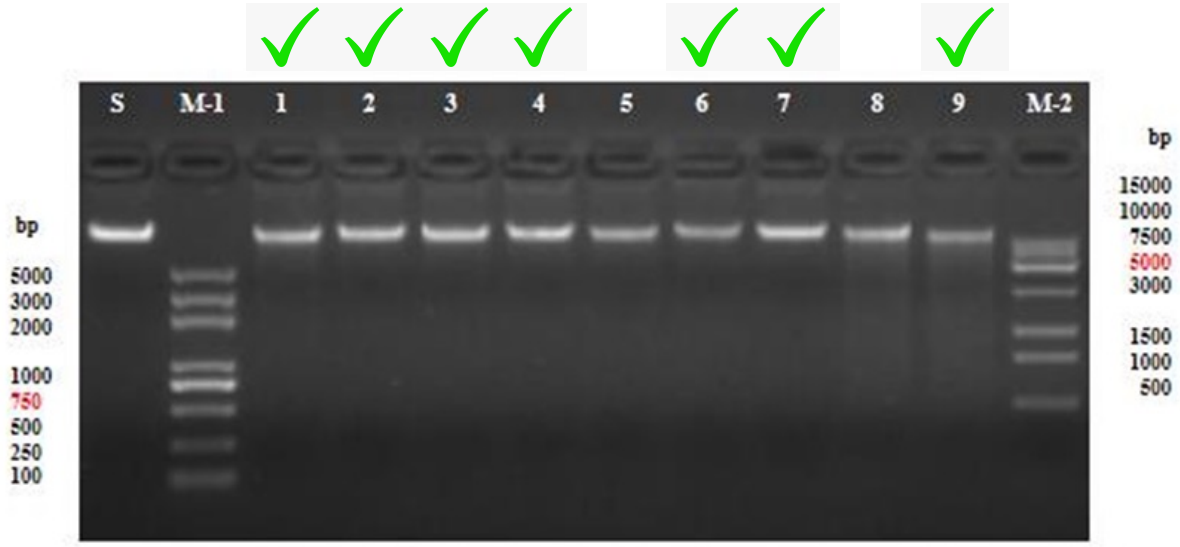
Fruits of 9 sweet cherry varieties were obtained from Morada Produce

Homogenization

High-molecular-weight genomic DNA was extracted from leaves of sweet cherry trees

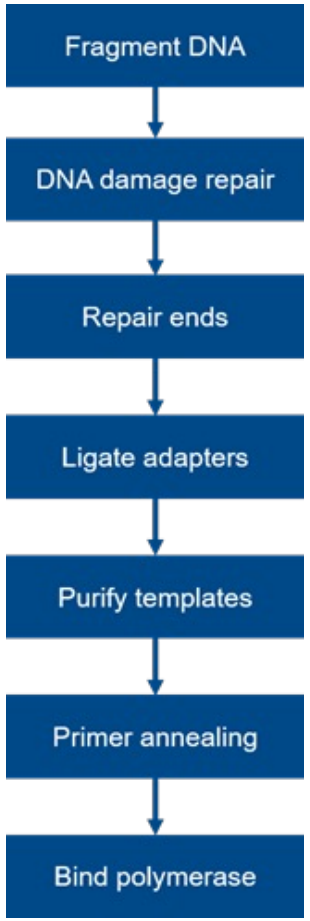


Electrophoresis results of 9 sweet cherry high-molecular-weight genomic DNA samples



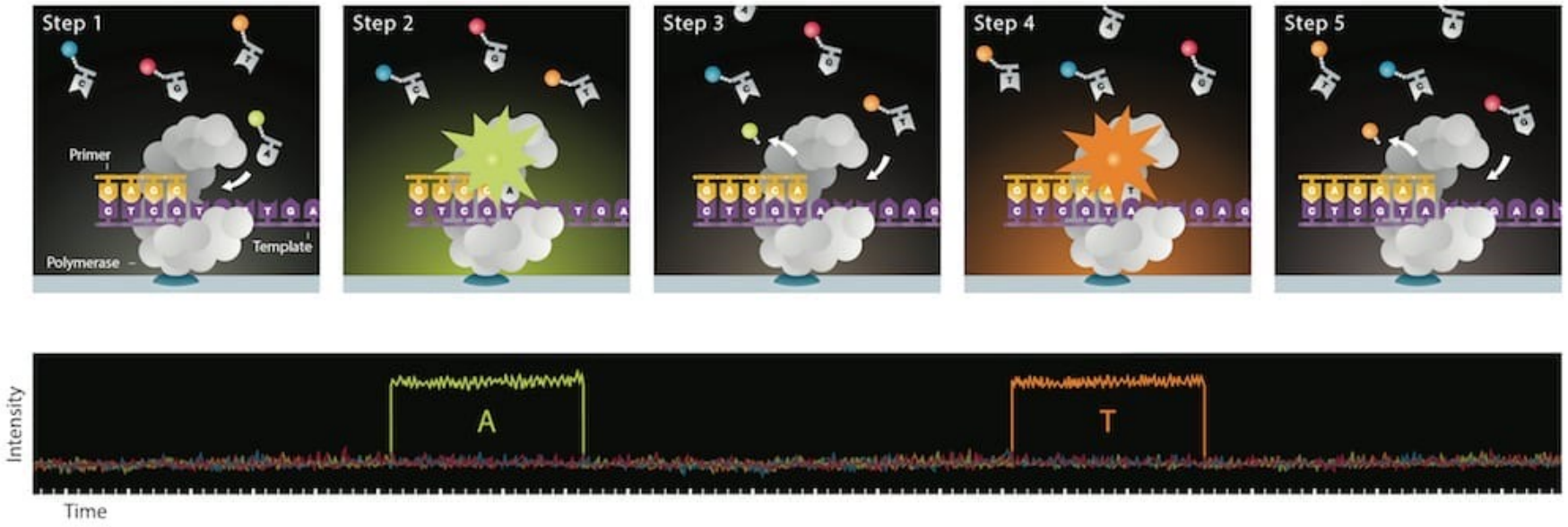
S, standard sample (50 ng); M-1, Trans 15k plus DNA ladder; 1, Coral Champagne; 2, Lapin; 3, Tulare; 4, Black Pearl; 5, Royal Hazel; 6, Royal Tioga; 7, Sweet Heart; 8, Brooks; 9, Bing.

Whole genome SMRT library construction and sequencing



Workflow of library construction.

Sequencing with the PacBio Long-Read Technology, which is a third-generation sequencing technology using single molecule real-time sequencing (SMRT).

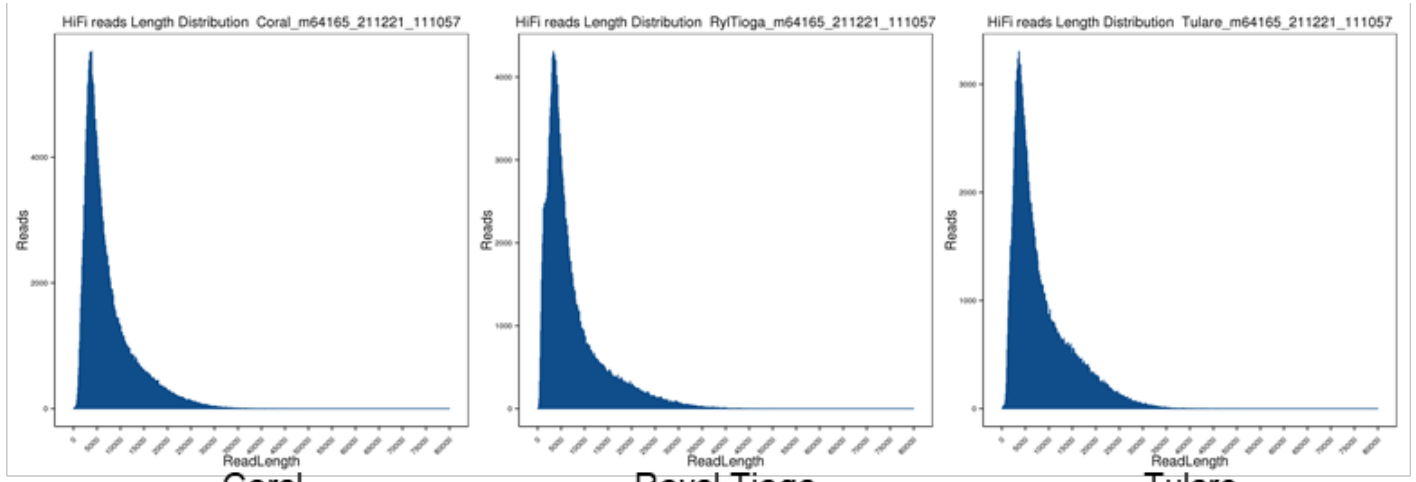


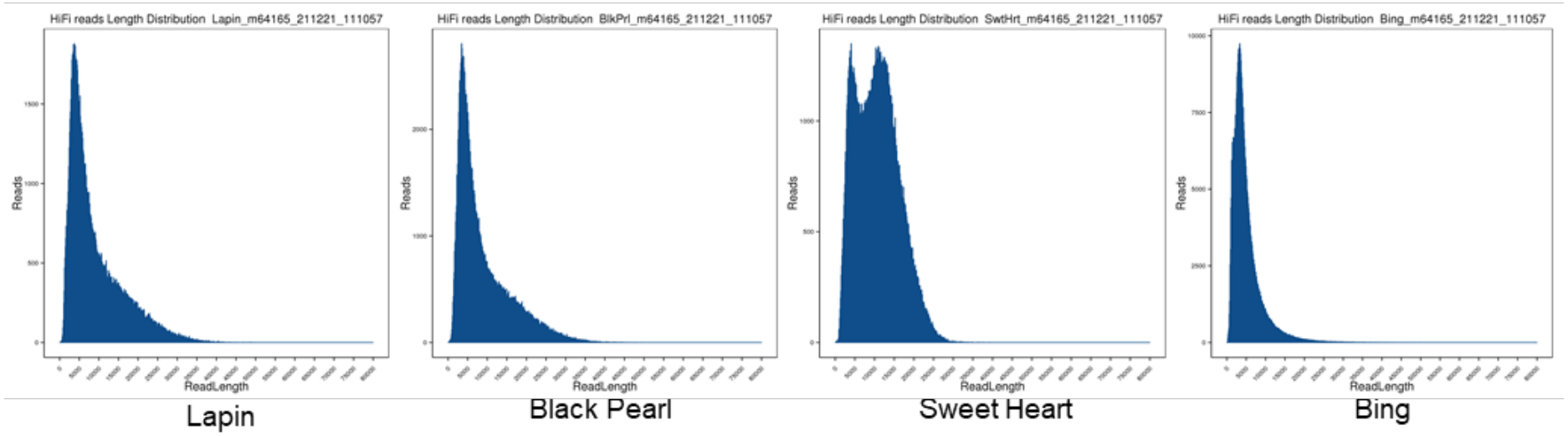
https://clpmag.com/diagnostic-technologies/molecular-diagnostics/smrt-sequencing/

Statistics of high-fidelity (HiFi) reads obtained from PacBio SMRT library sequencing

Sample	HiFi reads bases (bp)	Total bases (G)	HiFi reads number	Average HiFi reads length	N50
Coral Champagne	2574409991	2.57	353471	7283	9621
Royal Tioga	2107358498	2.11	289022	7291	10731
Tulare	1965286170	1.97	236747	8301	11831
Lapin	1351775238	1.35	149658	9032	13261
Black Pearl	1792832010	1.79	201767	8885	13056
Sweet Heart	2050660989	2.05	192601	10647	13301
Bing	2400656345	2.40	482616	4974	6141

HiFi reads length distributions





Mapping of HiFi sequence reads to the reference cherry genome
(var. Satonishiki, produced by Kazusa DNA Research Institute; 2n=16, ~350 Mb)

Seq number	Total length	GC content (%)	Gap rate (%)	N50 length	N90 length
10,148	272,361,615	37.72	9.39	219,566	19,672

Statistics of the reference genome.

Seq number, the total number of the assembled genomic sequences.
Total length, the total length of the assembled genomic sequence.
GC content, the GC content of the reference genome.
Gap rate, the proportion of unknown sequence (N) in the reference genome assembly.
N50 length, the length of scaffold N50, of which 50% of the sequence is higher than this level.
N90 length, the length of scaffold N90, of which 90% of sequence is higher than this level.

Mapping of HiFi sequence reads to the reference cherry genome

Sample	Total	Mapping Rate	Average Depth	Average Coverage	Coverage_10X
Coral Champagne	349849 (100%)	349849 (100.00%)	9.36	92.62%	22.19%
Royal Tioga	284236 (100%)	284236 (100.00%)	7.14	89.85%	7.64%
Tulare	233706 (100%)	233706 (100.00%)	6.92	90.51%	7.33%
Lapin	147888 (100%)	147888 (100.00%)	4.91	86.84%	2.42%
Black Pearl	199582 (100%)	199582 (100.00%)	6.51	90.29%	6.50%
Sweet Heart	190509 (100%)	190509 (100.00%)	7.42	90.84%	8.60%
Bing	474927 (100%)	474927 (100.00%)	8.66	92.59%	14.48%

Detection of sequence variants (I)

Summary of Single Nucleotide Polymorphisms (SNPs) detected in the genomes of 7 sweet cherry varieties

Sample	Upstream	Stop gain	Stop loss	Synonymous SNV	Nonsynonymous SNV	Intronic	Splicing	Downstream	Upstream; Downstream	Intergenic
Coral Champagne	132886	2290	668	46037	76017	162545	712	122632	25140	599750
Royal Tioga	110879	1778	503	37682	61571	140659	563	101873	20295	443468
Tulare	104351	1702	489	34930	57486	129295	537	94692	19940	437983
Lapin	79130	1232	366	26127	41928	97778	413	70443	15338	313491
Black Pearl	102610	1680	483	34915	56636	129242	521	92611	18688	423380
Sweet Heart	107684	1786	502	35869	58338	128013	549	96191	20128	478731
Bing	130323	2338	712	47805	78282	158101	722	122679	24113	605938

Detection of sequence variants (II)

Summary of insertions/deletions (InDels) detected in the genomes of 7 sweet cherry varieties

Sample	Upstream	Stop gain	Stop loss	Intronic	Splicing	Downstream	Upstream; Downstream	Intergenic
Coral Champagne	37774	585	53	48652	277	31720	7107	105951
Royal Tioga	30165	396	37	39319	207	25273	5655	80356
Tulare	28643	392	39	36817	205	23915	5538	77902
Lapin	20705	290	26	25941	149	17077	4135	54103
Black Pearl	28130	399	33	36488	207	23425	5291	75734
Sweet Heart	30048	448	40	36815	238	24736	5635	82262
Bing	33785	542	53	43560	256	29317	6279	98724

InDel refers to the insertion or deletion with length less than 50 bp.

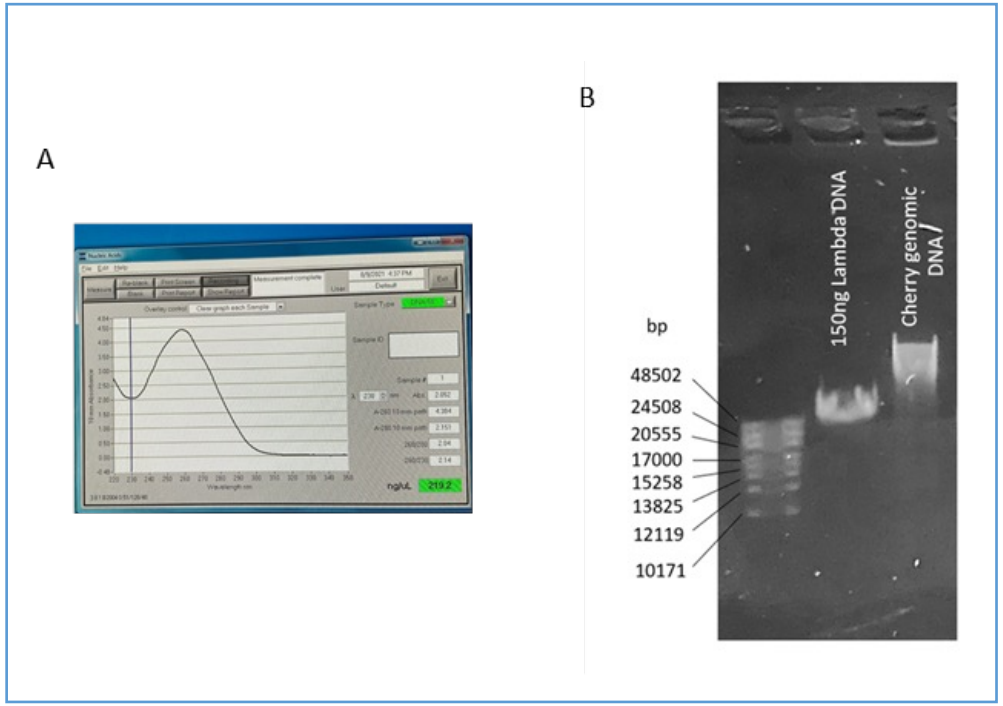
Detection of sequence variants (III)

Summary of structural variants (SVs) detected in the genomes of 7 sweet cherry varieties

Sample	Upstream	Exonic	Downstream	Intronic	Upstream/ Downstream	Intergenic	Splicing	Others	BND	CNV	DEL	DUP	INS	INV	Total
Coral Champagne	5100	4501	4603	5089	1032	15229	48	622	8684	513	15533	945	10489	60	36225
Royal Tioga	4878	4169	4254	4761	956	13654	40	608	6290	418	15292	829	10442	49	33321
Tulare	4646	3977	4185	4505	924	12979	50	618	5838	390	14814	831	9968	43	31885
Lapin	4023	3475	3555	3999	808	11019	42	504	3744	298	13613	623	9113	34	27426
Black Pearl	4456	3976	3947	4469	881	12840	43	592	5756	384	14445	795	9791	33	31205
Sweet Heart	4633	4034	4035	4464	901	13368	44	584	6268	420	14720	833	9776	46	32064
Bing	4902	4241	4389	4875	977	14058	46	615	8064	465	15058	809	9661	46	34104

Structural variants (SVs) are genomic variation with mutations of relatively larger size (>50 bp), including deletions (DEL), duplications (DUP), insertions (INS), inversions (INV), and translocations (BND).

High-molecular-weight genomic DNA was extracted from leaves of sweet cherry trees



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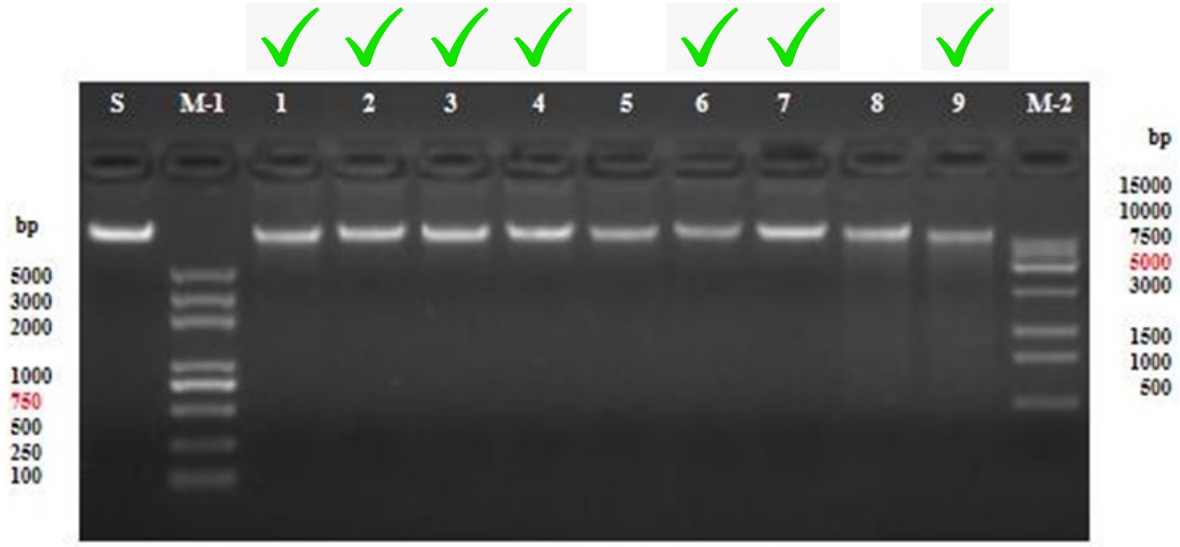

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- Extraction of high-molecular-weight-genomic DNA was repeated multiple times for varieties Royal Hazel and Brooks using both the previously collected and the newly harvested leaves.
- We are using a new method to obtain high-molecular-weight-genomic DNA from Royal Hazel and Brooks.

PLANT DNA ISOLATION KIT

CATALOG NUMBER: 80003

The Bionano Prep™ Plant DNA Isolation Kit provides critical reagents necessary for the isolation of high molecular weight genomic DNA from a variety of plant tissues. We have developed several DNA isolation protocols for plant tissue, depending on the specific needs of your plant of interest. All plant DNA isolation protocols use this same DNA isolation kit.

Review of the Plant Protocol Selection Guide and the associated Plant Database are recommended to decide on the proper protocol for your plant type.

Summary of progress

- We completed high-molecular-weight genomic DNA extraction, whole genome SMRT library construction, and sequencing of 7 sweet cheery varieties.
- We performed bioinformatics analysis on the sequencing data, and identified a large number of DNA sequence variations for the commercial sweet cherry varieties relative to the reference cherry genome.
- Currently, we are preparing high-molecular-weight genomic DNA from Royal Hazel and Brooks and will examine DNA sequence variations in these two varieties.

Our next step

- We will determine the DNA sequence variations that are distinct among all 9 sweet cherry varieties.
- Once the PCR tests using the molecular markers are developed, primers for LAMP (Loop-mediated isothermal amplification) assays will be designed.
- Specificity and sensitivity of detection of the DNA in the leaf, peduncle, and fruit flesh tissues will be evaluated.
- Although sweet cherries are often cross-pollinated, the fleshy fruit tissue is derived from the maternal tissue and could be used for varietal identification.

Questions?

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